THE UNIVERSITY OF HULL


Synthesis of Water-Soluble Porphyrin-Dendron Conjugates for Targeted
Photodynamic Therapy


being a Thesis submitted for the Degree of

Doctor of Philosophy in the University of Hull


by


Francesca Bryden, MChem (Hons)


October 2013

# Abstract

The targeting of photosensitisers with tumour-associated biomolecules is widely used for improved photosensitiser tumour localisation during photodynamic therapy, allowing fewer side effects in comparison to conventional cancer treatments. In particular, conjugation to antibody fragments allows exploitation of their high affinity towards tumour-associated antigens; however current methods of conjugating porphyrins to antibody fragments represent a compromise between high binding ratios and good stoichiometric and site-specific control. The work presented herein addresses this problem through the synthesis of porphyrin-dendron conjugates and their attachment at the interchain disulfide bridge of antibody fragments, allowing improved binding ratios while maintaining good structural control.

Synthesis of a range of click-functionalised porphyrins and dendrons bearing complimentary peripheral functionalities was carried out, followed by click conjugation of these structures under microwave irradiation to produce a range of lipophilic and hydrophilic porphyrin-dendron conjugates with between two and four peripheral porphyrins. Photophysical evaluation demonstrated retention of UV-vis and fluorescent character of porphyrins after conjugation, with some quenching of UV-vis absorption observed due to the close proximity of the porphyrins. Singlet oxygen quantum yields showed some quenching in all conjugates, with more sterically hindered systems showing the greatest reduction in SOQY in comparison to control porphyrins.

Conjugation of porphyrins to a HER2-targeted Herceptin™ Fab fragment was carried out through pre-conjugation of an alkyne-dibromomaleimide heterobifunctional linker to the Fab fragment, with two examples of cationic porphyrins conjugated *via* a click chemistry strategy to yield conjugates with precise 1:1 stoichiometry. Preliminary cytotoxicity studies of the targeted photosensitisers showed that both conjugates exhibited limited dark toxicity, and excellent cell killing in the HER2+ BT-474 cell line.

Successful focal-point deprotection and azide functionalisation was carried out on a single porphyrin-dendron conjugate, with a successful model click reaction to an alkyne-functionalised sugar displaying the possibility of bioconjugation of porphyrin-dendron conjugates *via* a click methodology.

# Acknowledgements

# Abbreviations

ALA: 5-Aminoleuvinic acid

$Boc_2O$: Di-tert-butyl-dicarbonate

BOP: Benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate

BNCT: Boron neutron capture therapy

CuAAC: Copper-catalysed azide-alkyne cycloaddition

Cy5: Cyanine dye 5

DBU: 1,8-Diazabicyclo[5.4.0]undec-7-ene

DBM: Dibromomaleimide

DCAD: Di-*p*-chlorobenzyl azodicarboxylate

DCM: Dichloromethane

DDQ: 2,3-Dichloro-5,6-dicyanobenzoquinone

DFT: Density functional theory

DIAD: Di-isopropyl azodicarboxylate

DLC: Discotic liquid crystal

DMEAD: Di-2-methoxyethyl azodicarboxylate

DMF: *N,N*-dimethylformamide

DMSO: Dimethylsulfoxide

DNA: Deoxyribonucleic acid

DSSC: Dye-sensitised solar cells

ED-B: Extra domain B

EGF: Epidermal growth factor

EGFR: Epidermal growth factor receptor

EPR: Enhanced permeation retention

Fab: Fragment antigen binding

GLUT: Glucose transporting proteins

GPC: Gel permeation chromatography

HER2: Human epidermal growth factor receptor 2

HMPA: Hexamethylphosphoramide

HOMO: Highest occupied molecular orbital

HpD: Haematoporphyrin derivative

HPLC: High performance liquid chromatography

HSA: Human serum albumin

IC: Internal conversion

IR: Infrared

IRR: Irradiated

ISA: Imidazole-1-sulfonyl azide

ISC: Intersystem crossing

MAb: Monoclonal antibody

MeOH: Methanol

MS: Mass spectrometry

m-THPC: *Meta*-tetra(hydroxyphenyl)chlorin

NCS: Isothiocyanate

NHS: *N*-hydroxysuccinimide

NI: Non-irradiated

NIR: Near infrared

NMP: *N*-Methylpyrrolidone

NMR: Nuclear magnetic resonance

PAMAM: Poly(amidoamine)

PDT: Photodynamic therapy

PEG: Poly(ethylene glycol)

PIC: Photoimmunoconjugate

PP*a:* Pyropheophorbide-a

PPI: Poly(propylene imine)

PpIX: Protoporphyrin IX

PS: Photosensitiser

PyBOP: Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate

ROS: Reactive oxygen species

RT: Room temperature

RuAAC: Ruthenium-catalysed azide-alkyne cycloaddition

scFv: Single chain fragment variable

SIP: Small immunoprotein

SOQY: Singlet oxygen quantum yield

SWNT: Single-walled nanotubes

TAA: Tumour associated antigen

TAMRA: 6-Carboxy-tetramethylrhodamine

TBAC: Tetrabutylammonium chloride

TBAI: Tetrabutylammonium iodide

TBTA: Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine

TCEP: Tris(2-carboxyethyl)phosphine

TFA: Trifluoroacetic acid

THF: Tetrahydrofuran

TLC: Thin layer chromatography

TMS: Tetramethylsilane

TPP: Tetraphenylporphyrin

TPPS: Tetraphenylporphyrin sulfonate

TPyP: Tetrapyridylporphyrin

TSA: Tumour specific antigen

VEGF: Vascular endothelial growth factor

# COSHH statement

All experiments were carried out in accordance with the University of Hull's Health and Safety guidelines. A full COSHH and risk assessment was carried out for each new experiment, signed by the undertaking student, supervisor, (Prof. R.W. Boyle) and the departmental safety officer (Dr T. McCreedy) before any practical work started. The COSHH forms carry the reference numbers FB01-FB37.

# Table of contents

# Table of figures, tables and schemes

## Figures

## Tables

# Schemes

# 1. Literature review

## 1.1. Photodynamic therapy

Photodynamic therapy (PDT) is a promising clinical alternative for the treatment of many cancers and non-neoplastic conditions, which works *via* the action of a treatment agent known as a photosensitiser (PS); typically a large, highly-conjugated molecule which is capable of producing cytotoxic reactive oxygen species (ROS) in the presence of molecular oxygen and light of a specific wavelength.

Although some surface tumours can be treated through topical application of photosensitisers such as ALA, in most cases cancer treatment utilising PDT begins with systemic administration of the photosensitiser drug. This is then followed by a time period in which the photosensitiser preferentially accumulates in the tumour tissue, usually as a result of passive processes associated with the unusual morphology of the tumour tissue.[1] Selective irradiation of the target tissue with light can then be carried out, producing ROS *in situ*, which leads to tissue damage and tumour destruction.

The combination of preferential localisation of the photosensitiser in tumour tissue and selective irradiation of the target area leads to "dual selectivity", resulting in the cytotoxic effects being effectively limited to the target tissue. As a result, PDT displays numerous advantages over conventional cancer treatments such as chemotherapy, radiotherapy and surgery. Unlike these alternatives, treatment with PDT is relatively non-invasive, leading to little or no scarring, and exhibits few negative side effects.[2]

## 1.2. Mode of action

In order for the generation of ROS to take place in the target tissue, irradiation of the photosensitiser with visible or near-infra red (NIR) light must first take place. Before irradiation occurs, the photosensitiser exists in its ground state ($S_0$), with the two electrons of the HOMO existing in a paired state. Upon irradiation with photons of the correct energy, one of the paired electrons in the HOMO is promoted into a higher energy orbital (Figure 1), forming an excited singlet state ($S_1$). The lifetime of this excited state is extremely short ($10^{-12}$-$10^{-9}$s), with the energy lost *via* the emission of light of longer wavelength, known as fluorescence ($S_1 \rightarrow S_0$ transition), or *via* non-

radiative processes known as internal conversion (IC), before reaction with cell substrates can occur.
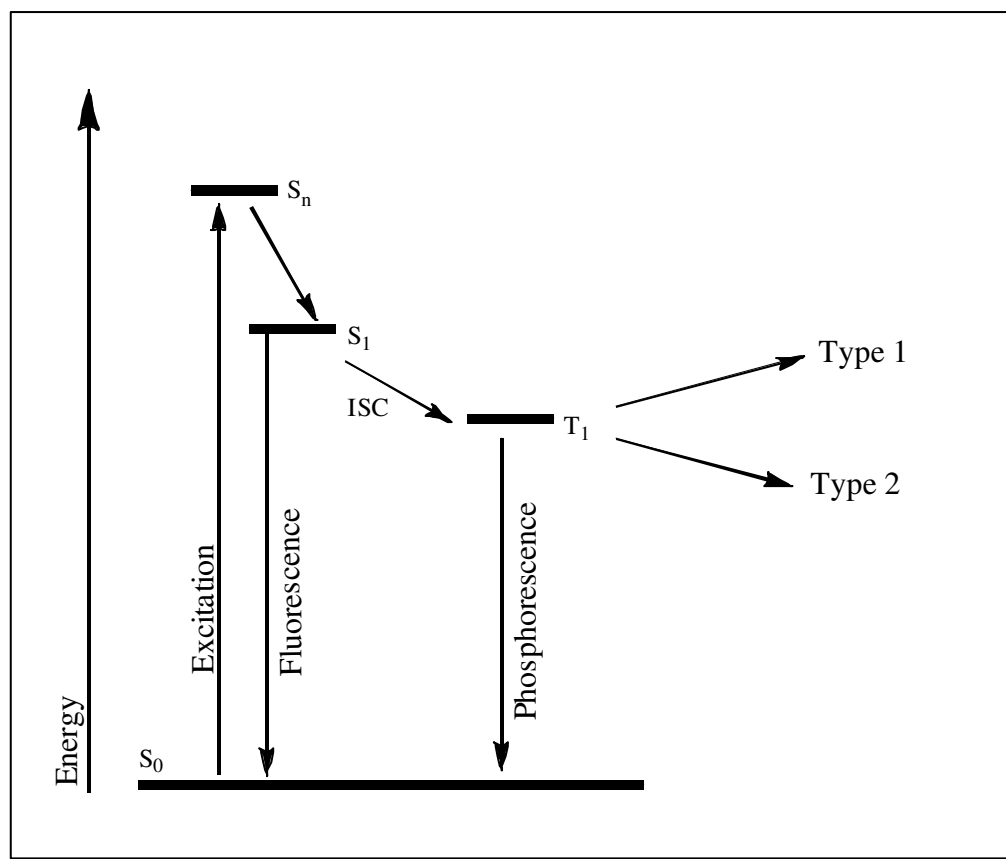


*Figure 1: Jablonski diagram showing the electronic processes involved after irradiation of the photosensitiser with light.*

Alternatively, the molecule can lose some energy *via* intersystem crossing (ISC), undergoing spin inversion of the excited electron to form an excited triplet state ($T_1$). While the photosensitiser can again undergo loss of energy *via* a radiative process, known as phosphorescence, the transition from the excited triplet state to the singlet ground state ($T_1 \rightarrow S_0$) is formally spin forbidden. As a result, the $T_1$ excited state has a considerably longer lifetime than the $S_1$ state ($10^{-3}$-$10^{-1}$s), increasing the probability of reactions with other cell components occurring.[3, 4] Reactions of the excited triplet state photosensitiser with ground state (triplet) oxygen to form reactive oxygen species can also occur, *via* two distinct mechanisms.

In the Type I, or free radical, mechanism, the photosensitiser reacts directly with cell substrates and molecular oxygen, resulting in a transfer of electrons and the production of a number of radical charged species (Table 1). The generated radicals can then react with molecular oxygen, due to its diradical character, producing a range of reactive

oxygen species, including hydrogen peroxide, and hydroxyl and peroxyl radicals.[3] Further reaction of these highly reactive ROS with cellular components leads to significant oxidative damage (Figure 2), ultimately leading to cell death.

| Type 1 processes | Type 2 processes |
|---|---|
| $PS^* + X \rightarrow PS^{\bullet-} + X^{\bullet+}$ <br> $PS^{\bullet-} + O_2 \rightarrow PS + O_2^{\bullet-}$ <br> $PS^* + O_2 \rightarrow PS^{\bullet+} + O_2^{\bullet-}$ <br> $2O_2^{\bullet-} + 2H^+ \rightarrow {}^3O_2 + H_2O_2$ <br> ${}^3O_2^{\bullet-} + Fe^{3+} \rightarrow {}^3O_2 + Fe^{2+}$ <br> $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^-$ <br> $O_2^{\bullet-} + OH^{\bullet} \rightarrow O_2 + OH$ <br> $PS^* + X\text{-}H \rightarrow PS\text{-}H^{\bullet} + X^{\bullet}$ <br> $X^{\bullet} + O_2 \rightarrow RO_2^{\bullet}$ <br> $RO_2^{\bullet} + R\text{-}H \rightarrow RO_2H + R^{\bullet}$ | ${}^3PS + {}^3O_2 \rightarrow {}^1PS + {}^1O_2$ <br> ${}^1O_2 + X \rightarrow \text{Oxidative damage}$ |

*Table 1: type 1 and type 2 processes, where PS is the photosensitiser molecule and X is any sensitive cell substrate.*

In contrast, the Type II mechanism occurs *via* a direct reaction between the excited photosensitiser and ground state oxygen. This triplet-triplet reaction is possible due to the fact that triplet oxygen ($^3O_2$), with two unpaired, spin aligned electrons, is the ground state of oxygen, and can react directly with the excited photosensitiser to generate two forms of singlet oxygen ($^1\Delta_g$ and $^1\Sigma_g$). $^1\Sigma_g$ is the higher energy singlet state (157 kJ mol$^{-1}$ $_>$ $^3\Sigma_g$),[5] with two spin paired electrons in different orbitals, and as a result is an extremely short lived state (<0.33 ms in methanol).[5] In contrast, $^1\Delta_g$ has two spin paired electrons in the same orbital, and is therefore lower in energy (94 kJ mol$^{-1}$ $_>$ $^3\Sigma_g$)[5] and has a detectable lifetime in water. As a result, it is this excited singlet oxygen state which is the cause of Type II oxidative damage and cell death in the body.[6]

Despite its longer lifetime, the $^1\Delta_g$ singlet oxygen species is still highly reactive, and can cause significant oxidative damage to cellular components. However, unlike the ROS produced during the Type I mechanism, singlet oxygen has a short lifetime (~3 μs)[7] and limited radius of action[8] (~100 nm) in cells, effectively limiting any cytotoxic action to the target tissue only.
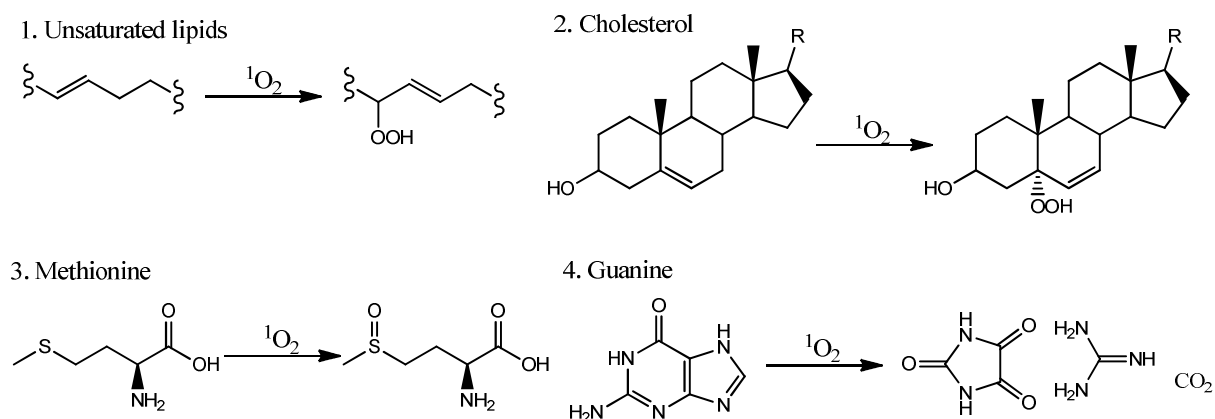
*Figure 2: Examples of oxidative damage of cell components caused by the release of singlet oxygen into the cell.[9]*

Most photosensitisers produce reactive oxygen species by a mixture of both Type I and Type II processes, with the ratio of the two mechanisms dependent on the type of photosensitiser utilised, and concentrations of both cell substrates and molecular oxygen. Regardless of the mechanism by which the cell damage occurs, the reaction of the photosensitiser returns it to the $S_0$ ground state, allowing the excitation process to be repeated. This process will continue until the PS is damaged through photobleaching; a process in which the photosensitiser is modified or fragmented by the ROS produced, leading to loss of photosensitiser activity.[10]

### 1.1.1. Methods of cell death

The oxidative damage caused by the ROS can lead to target tissue death in three ways; direct cell death, anti-vascular effects, and immune effects, all of which play a role in the overall cytotoxic activity of the photosensitiser.

Direct cell death occurs as a result of oxidative damage caused directly to the target cells, and can occur *via* either apoptosis or necrosis. Although most photosensitisers are capable of causing direct cell death *via* either mechanism, a complex set of both internal factors, such as cellular metabolic state, cell cycle phase,[11] and cell type,[12] or external factors such as photosensitiser and light dose,[12] and time between injection and irradiation,[13] contribute to the mode of cell death in each case.

Apoptosis is most often associated with photosensitisers which locate in organelles such as the mitochondria, and is best described as "programmed" cell death. Apoptotic cell death is mediated by chemical pathways, and characterised by slower disintegration of the organelles and plasma membrane[12] (Figure 3), leading to loss of cell content *via* the formation of blebs which can be easily removed by phagocytosis.[11]

In contrast, necrosis, or "accidental" cell death often results after severe cell damage, and in the case of PDT is often associated with both high light and photosensitiser doses[14] and photosensitisers which preferentially localise in cell membranes.[11] Necrotic cell death is a rapid, disorganised cell death, characteristically showing organelle and membrane disintegration, and sudden release of the cell contents.[12]
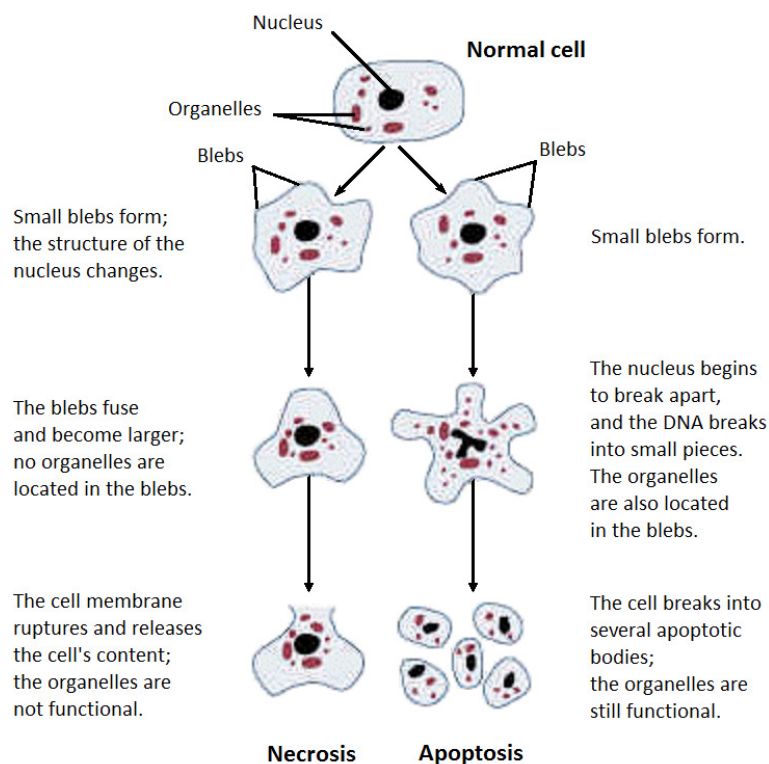
*Figure 3: Flow chart detailing the different processes occurring as part of apoptosis and necrosis. In particular, apoptosis is associated with bleb formation and retention of organelles, whereas necrosis involves early loss of cell membrane function and release.[15]*

In contrast, vascular and immune cell death effects occur as a result of processes initiated by photosensitiser action rather than direct ROS effects upon the tumour cells. Although direct cell death mechanisms are responsible for the majority of tumour cell death directly following irradiation, complete tumour eradication is not usually possible

without the action of both vascular and immune system effects.

The immune effect begins with initiation of inflammation by direct cell death of the target tissue, which leads to the recruitment of immune cells to the area. These immune cells can then target and eradicate tumour cells not directly affected by photosensitiser action.[11] In contrast, vascular effects result from damage to non-neoplastic cells, in which the photosensitiser is targeted towards the characteristic neovasculature of tumour tissue. Damage to this tissue leads to clotting and irreversible destruction of the tumour vasculature, and loss of blood supply to the target tissue. Vascular effects are often the only way to obtain effective cell killing in large solid tumours,[16] and drug-resistant tumours.[17]

## 1.1.2. Photosensitisers

To date, many different molecules have been evaluated for their effectiveness as potential photosensitisers, with the majority being based around large, organic macrocycles with highly conjugated systems of multiple bonds, allowing for effective absorption of visible light. In the evaluation of these photosensitisers, a number of criteria have been developed which characterise an "ideal" photosensitiser. Although to date, no one photosensitiser has been produced which fulfils all of these criteria, a good photosensitiser will display desirable parameters for as many variables as possible, in particular five key characteristics:

1. Preferential accumulation in the target tissue over the surrounding tissues.
2. A quantum yield of greater than 0.5 for both the triplet state ($\Phi T$) and singlet oxygen ($\Phi\Delta$).
3. An absorption band with a high ($>20,000$ - $30,000$ $M^{-1}cm^{-1}$) extinction coefficient between 630-850 nm.[18] Light below this wavelength range shows poor tissue penetration, making irradiation of the entire tumour difficult, whereas light of a higher wavelength has greater tissue penetration, but insufficient energy to convert oxygen from the triplet to singlet state.
4. Little or no toxicity in the absence of irradiating light ("dark toxicity").
5. The active substance is pure and well-characterised, with a fixed composition.

Although these five criteria are key to the development of any new photosensitiser, a number of additional characteristics are also highly desirable, including a simple and

high yielding synthesis, good water solubility to allow ease of administration, and limited stability in the body to avoid prolonged skin sensitisation after treatment.

### 1.1.2.1.    1st generation photosensitisers

The first photosensitisers explored clinically were all derivatives of haematoporphyrin, an endogenous porphyrin photosensitiser produced from the acid hydrolysis of the blood component haemoglobin. Although haematoporphyrin does act as a photosensitiser, its poor localisation in tumours[19] means that it is unsuitable for clinical use.
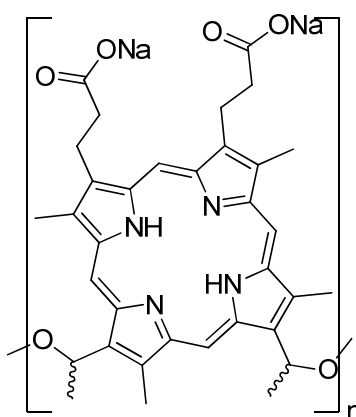


*Figure 4: Structure of HpD. The product resulting from the acidification and neutralisation of haematoporphyrin contains a complex mixture of monomers, dimers and oligomers.*

A process of acidification, filtration and subsequent neutralisation of haematoporphyrin produces haematoporphyrin derivative (HpD) (Figure 4), a photosensitiser first described in 1961 by Lipson and Schwartz, who utilised it as fluorescent diagnostic tool for tumour tissue during surgery.[9]  However, the highly reactive pseudobenzylic hydroxyl groups on the porphyrin skeleton mean that a number of intermolecular reactions can take place to form ether, ester and carbon-carbon linkages, and as a result HpD is utilised as a complex mixture of dimers and oligomers rather than a pure compound.[1]

Partial purification can be utilised to isolate the oligomer-fraction of this mixture, which shows greater photodynamic activity than other isolated fractions, and has been licensed as Porfimer sodium (tradename Photofrin®). Regulatory approval of Porfimer sodium was first granted in 1993,[20] and it has subsequently received approvals in over forty

countries for a wide range of malignant diseases, including oesophageal cancer, lung cancer, microinvasive endobronchial cancer, gastric cancers, bladder cancer and cervical dysplasia.[21]

However, despite its widespread clinical usage, Porfimer sodium displays less than ideal properties as a PDT agent. Although Porfimer sodium is a purified fraction of HpD, it still exists as a mixture of over 60 porphyrin-containing components.[10] This complex composition limits reproducibility between batches of Porfimer sodium, and makes it difficult to definitively identify the active component of the photosensitiser.

In addition, although Porfimer sodium shows improved uptake into tumour tissue in comparison to haematoporphyrin, accumulation is still less than 3%.[1] Of the remainder of the photosensitiser administrated to the patient, a significant proportion accumulates in the skin, leading to skin photosensitivity lasting for 1-2 months.[10] The slow accumulation of Porfimer sodium in tumours also means that a delay of 48-72 hours between injection and irradiation is necessary,[2] requiring the patient to be admitted to hospital for several days during treatment.

Finally, and most significantly, Porfimer sodium has only one, poorly absorbing (1170 $M^{-1}cm^{-1}$)[20] Q-band on its spectrum in the red region, at 630 nm.[9] At this wavelength, light penetration of skin is still relatively poor (not exceeding 1-2 mm[22]), limiting the size and location of tumours which can be treated.

### 1.1.2.2.    2nd generation photosensitisers

Due to the limitations of both HpD and Porfimer sodium as photosensitisers, a number of second-generation photosensitisers have been developed, with the vast majority based around a modified tetrapyrrolic skeleton (Figure 5).[18] These macrocycles present a number of advantages over haematoporphyrin derivatives, most notably that many exist as chemically pure compounds. The structural flexibility of the tetrapyrroles also allows them to be easily modified through both substitution and metallation to alter water solubility, extinction coefficients, positioning of Q-bands and photophysical properties.
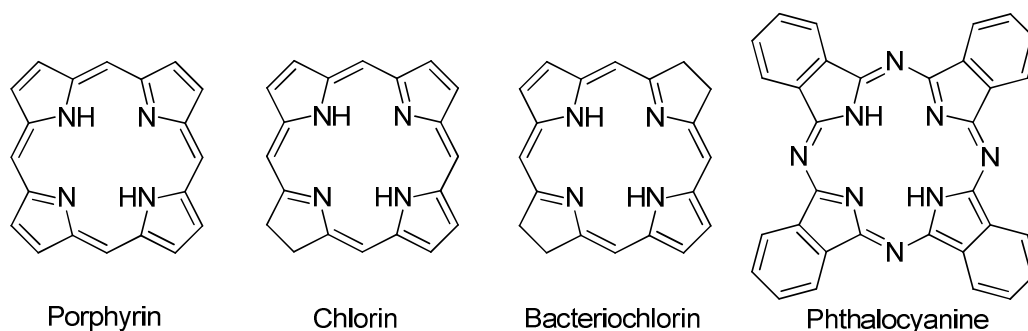
*Figure 5: The skeleton structures of the four most common macrocyclic photosensitisers used in PDT.*

Porphyrins show a strong Soret absorption band at 400 nm, and weaker Q-bands at higher wavelengths, with the highest wavelength Q-band generally occurring between 620 and 640 nm,[1] and are the most commonly utilised of these photosensitiser molecules due to their oxidative stability and ease of synthesis. Although functionalisation of porphyrin *meso*-positions or metallation is possible, and can increase water solubility and singlet oxygen yields, modification in this way has a limited effect on the positioning of the highest wavelength Q-band.[20] In contrast, while the tetrapyrrolic structure of chlorins and bacteriochlorins is very similar to that of porphyrins, the reduction of one or two pyrrolic double bonds respectively increases the wavelength of the most intense Q-band (650-670 nm for chlorins and 730-800 nm for bacteriochlorins[20]), and its absorption coefficient, without reducing quantum yields of singlet oxygen and the triplet state.

Phthalocyanines are another example of the effect of extending the aromatic system of these macrocycles, utilising the addition of further aryl rings to produce longer - wavelength Q-bands (670-780 nm[20]) with very high (>100,000 $M^{-1}cm^{-1}$) extinction coefficients. However, this strategy also increases the lipophilicity of phthalocyanines, making them difficult to administer as drugs without the use of carrier systems,[23] or further modification such as sulfonation to increase water solubility.

To date, a number of 2nd generation tetrapyrroles have been approved for clinical use around the world. These include m-THPC (Foscan®) for treatment of head and neck cancer, 5-aminoleuvinic acid (ALA, Levulan®), which is approved for treatment of actinic keratoses, and its methyl ester (Metvix®), for treatment of actinic keratoses and basal cell carcinoma.[21]

### 1.1.2.3. 3rd generation photosensitisers

Despite the clinical successes of second generation photosensitisers, none of the photosensitiser molecules developed to date can be described as optimised in terms of the five key characteristics discussed previously. In particular, all second generation photosensitisers display a relatively poor selective accumulation in the target tissue, with most showing maximum tumour: non-neoplastic tissue ratios of below 4.0.[24] As a result, significant accumulations of the photosensitiser in non-target tissues can occur, leading to side effects such as generalised skin photosensitivity.[25]

The poor selectivity for tumour tissues of most second-generation photosensitisers is due to the fact that their accumulation is due to passive processes as a result of the unusual morphology of the tumour tissue alone. The most significant mechanism of uptake is the "enhanced permeation and retention" effect (EPR), in which the rapidly growing tumour tissue has created an environment in which large molecules can more easily enter and remain in the tissue. The rapid growth of new blood vessels to supply the tumour tissue leads to capillaries with a poor structure (Figure 6), allowing photosensitiser molecules to permeate the tissue, and the lack of lymphatic drainage associated with tumour tissue means that these large molecules are retained for longer in tumour tissue than in non-neoplastic tissue.[26]
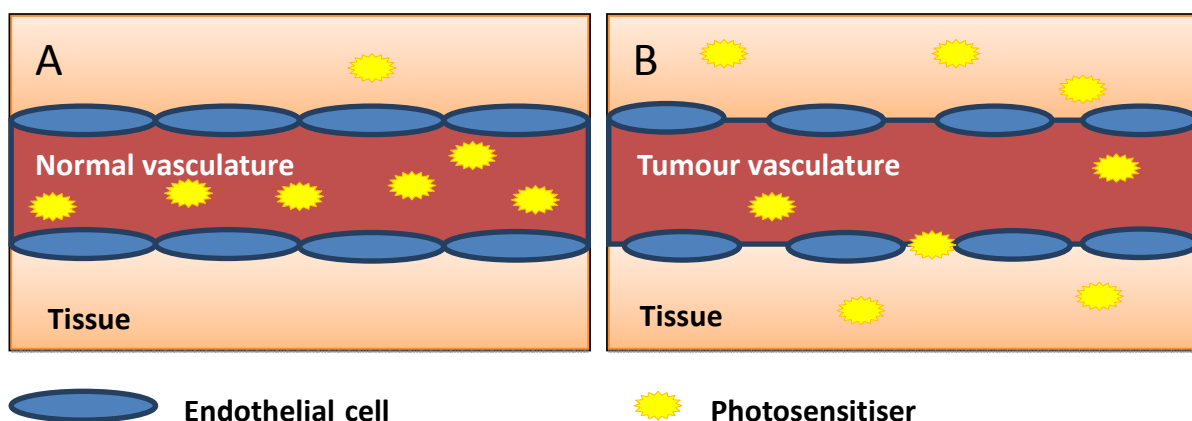


*Figure 6: Diagram showing the tightly-arranged capillary endothelial cells in normal vasculature (A) and the poorly arranged endothelial cells in tumour neovasculature (B). The larger gaps in the endothelium makes it leaky and allows the passage of larger molecules into the tumour tissue.[26]*

In contrast, "third generation" photosensitisers aim to enhance the selectivity of

photosensitisers for target tissue by utilising active targeting. Due to the rapid growth and proliferation of neoplastic cells, many naturally-occurring receptors are overexpressed on their surface. These include receptors for a range of proteins, carbohydrates, and antibodies, leading to an increased uptake of these biomolecules by the tumour tissue. Conjugation of a photosensitising moiety to a biomolecule associated with one of these overexpressed receptors (Figure 7) allows active targeting of the tumour tissue through use of the biomolecule as a targeting group for the photosensitiser.
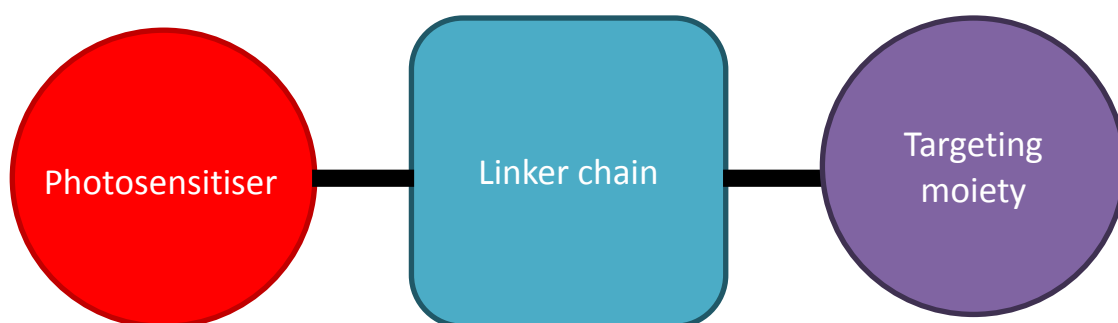


*Figure 7: Schematic representation of 3rd generation photosensitiser. The photosensitiser unit utilised can be any 2nd generation tetrapyrrolic or other photosensitising group, while the targeting moiety can be selected from a range of receptor specific biomolecules, including proteins and antibodies. A linker chain may be used to join these two groups, with biocompatible chains such as PEG and poly-L-lysine widely utilised.*

As a result, a vast array of biological molecules including antibodies, antibody fragments, proteins, peptides and carbohydrates are available for use as targeting moieties. All of these targeting groups display very distinct properties depending on the biomolecule, the receptor, and the nature of their interaction.

### 1.1.3. Biological targeting moieties

#### 1.1.3.1. Proteins

Proteins, in particular those present naturally in human serum, are an obvious choice for use as a targeting biomolecule due to the fact that unconjugated photosensitisers naturally interact with a range of serum proteins when injected into the human body. While these interactions are generally non-covalent, the size and type of protein the photosensitiser interacts with has a significant impact on the mode of transportation, the

site of cell localisation and the potential for internalisation of the photosensitiser.[24]

In addition, as serum components are generally vital for the rapid proliferation of tumour cells, overexpression of receptors for many types of serum proteins, in particular lipoproteins, is observed on the surface of tumour tissues.[27] As many serum proteins are internalised in the target cells, either through non-specific internalisation, or through the action of protein associated receptors,[28] conjugation to these proteins also increases the likelihood of the photosensitiser causing damage to key cell components. As a result, serum proteins such as lipoproteins and albumin, along with a number of other endogenous proteins represent an attractive option as both non-covalently and covalently-attached transportation modalities for photosensitisers.
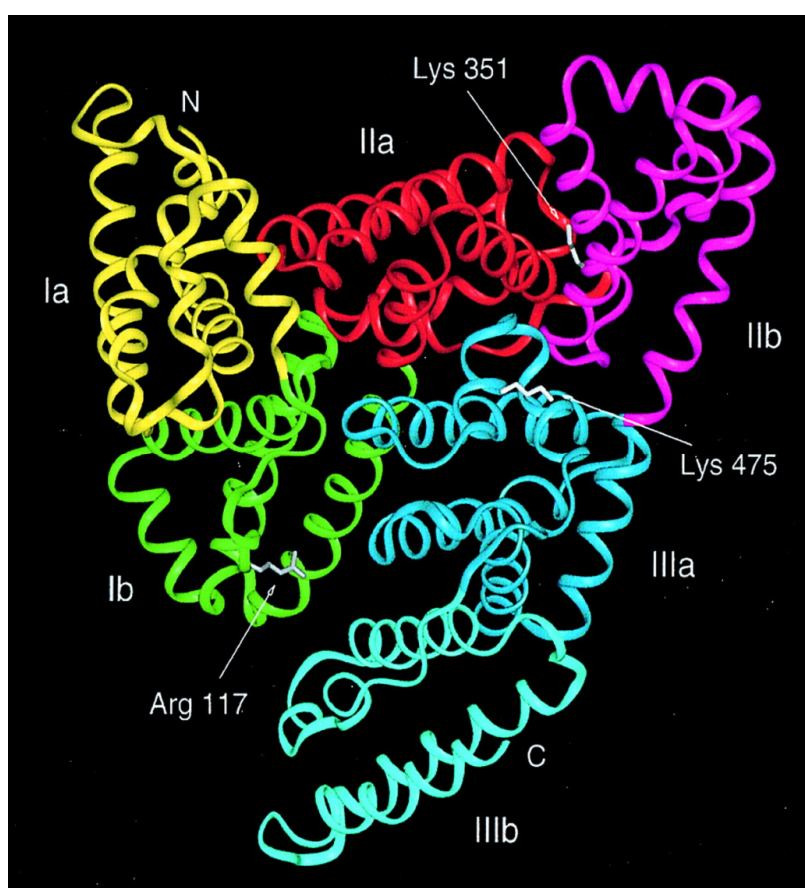


*Figure 8: The crystal structure of human serum albumin (HSA)[29] shows the large, highly folded structure typical of serum proteins. The large number of binding sites present can act as both an advantage and a disadvantage in the use of these biomolecules*

However, proteins also show a number of limitations as targeting moieties; while the large size of serum proteins (>70 kDa in many cases) theoretically allows for high binding ratios of photosensitiser:protein to be achieved[30] as a result of numerous surface

amino acid residues,  the complex 3D folding of the protein means that many of these sites are unavailable for conjugation,[18] and large numbers of identical residues available on the protein means that control of binding ratios and binding site is difficult to achieve.[18] Additionally, while covalent bonds can be formed between the protein and the photosensitiser, there is also a tendency for the photosensitiser molecules to aggregate, and to form non-covalent bonds with the protein.[30] Upon injection into the body, these photosensitiser molecules can then redistribute themselves to other serum proteins, leading to uptake into non-target tissues.

### 1.1.3.2.    Peptides

Peptides and proteins are related classes of biomolecule, with both comprising a structure of multiple amino acids joined by peptide bonds. The differentiating factor between the two is size, with anything above 50 amino acids being classed as a protein, while anything below this size is usually considered to be a peptide.[31] The smaller size of peptides means that they often lack the complex folding and 3D structure of proteins, however their reduced complexity does allow for simple peptides to be obtained synthetically rather than derived from natural sources.

The small size of peptides relative to other biomolecules allows them to easily permeate cell membranes; they both accumulate and clear rapidly from the body, and as targeting moieties these properties limit negative side effects such as generalised photosensitivity.[31] Additionally, the ability to produce synthetic peptides means that they can be modified relatively easily, allowing the production of novel peptides with high affinity and specificity, and increased ease of conjugation to photosensitisers.

However, the small size and lack of 3D structure of peptides means that they are particularly sensitive to endo- and exo-peptidases present naturally in the body,[31] and can be easily degraded before reaching their intended target. Their limited size increases the risk of photosensitiser binding to areas vital for receptor recognition,[32] and also often limits loading ratios to just 1:1.[33, 34]

### 1.1.3.3.    Carbohydrates

Overexpression of glucose transporting proteins (GLUT) and β-galactose receptors on

the surface of many cancer cells is common due to the increased energy requirement of the rapidly proliferating cells.[35] For this reason, these and other saccharides represent attractive targets for conjugation to photosensitisers. As well as acting as selective targeting moieties, carbohydrate groups can also increase the overall hydrophilicity of the photosensitiser conjugate,[32] allowing greater ease of administration, and increase uptake into highly sensitive regions of the cell such as the mitochondria.[36]

However, the small size of mono- and disaccharides means that loading ratios are low, with 1:1 conjugates representing best-case scenarios, and optimum uptake and phototoxicity requiring three saccharide units per photosensitiser.[37] Additionally, there are few reported cases of increased target cell uptake due the targeting action of the saccharide units. The selectivity of this targeting method is limited by the large numbers of GLUT and other transport proteins present on normal cells, and the increased uptake of carbohydrate conjugated photosensitisers into tumour cells in many cases is due to the increased water solubility alone.[35]

### 1.1.3.4.  *Antibodies and antibody fragments*

Antibodies and antibody fragments are often considered to represent the ideal example of a targeting moiety; the specific nature of the interaction between each antibody and its associated antigen receptor[31] means that using a tumour specific antigen (TSA) as a targeting group allows prevention of all non-specific uptake of the photosensitiser. However, in practice the presence of TSAs is limited to experimentally induced tumours only,[38] with spontaneous tumours displaying overexpression of tumour associated antigens (TAA) only. While TAAs are also expressed on normal cell surfaces, cytotoxic effects can be limited to target tissue only if other tissues expressing the TAA are inaccessible to the antibody, or if the cells are not exposed to daylight or light during treatment.

The ability of antibodies to be internalised into the cell by their associated antigen is also a significant advantage to this type of targeting group; typically internalisation of the antibody also allows for internalisation of the photosensitiser, allowing for greater oxidative damage to cellular components. Additionally, bioconjugation to antibodies and antibody fragments is facile and well-understood due to the widespread use of antibodies as targeting agents for a plethora of drugs in the literature. As a result, a

number of simple reactions are known which proceed rapidly under mild conditions,[31] allowing conjugation of porphyrins to MAbs in typical loading ratios of 20:1.[39]

However, use of whole monoclonal antibodies as targeting groups does have numerous disadvantages. MAbs have a typical molecular weight of around 150 kDa and a complex 3D structure, leading to problems including poor tumour penetration, lack of internalisation[30] and long residence time in the blood.[38] The large size of the MAb also means that a large number of surface amino acid residues are present, allowing extremely large conjugation ratios, and associated problems including reduction in solubility,[40] loss of specific binding affinity for the target antigen,[41] and creation of non-stoichiometric conjugates.

Due to their smaller size, and ability to be bioengineered,[42] antibody fragments represent a far more promising targeting moiety. A range of antibody fragment types exist with molecular weights ranging from 25-50 kDa,[38] however in all cases the complete antigen binding region is maintained,[43] allowing the retention of the binding specificity while increasing tumour surface penetration and accumulation.[38] However, the smaller size of these fragments does limit loading ratios, with conjugation of large numbers of photosensitiser molecules leading to partial loss of activity and water-solubility of the fragment.[31] Despite these disadvantages, a number of studies to date have demonstrated the versatility and targeting potential of antibody fragments.

### 1.1.4.  Antibody fragments as targeting moieties

Although, as previously stated, numerous conjugation methods exist in the literature for bioconjugation to antibodies and antibody fragments, the vast majority of the work aiming to conjugate antibody fragments to photosensitisers utilises the lysine residues of the antibody fragment (Figure 9) as binding sites.
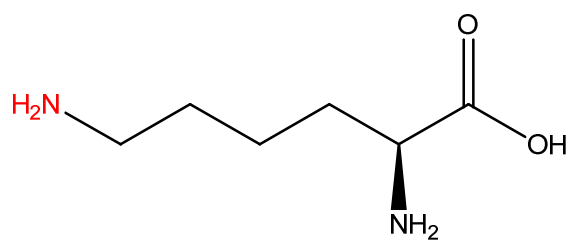


*Figure 9: Structure of L-lysine. The amine functional group present in the lysine residue*

15

*(highlighted in red) is suitable for conjugation with a number of amine-reactive groups.*

Lysine residues are common on the surface of many monoclonal antibodies, and are particularly appealing as they offer an amine binding site, allowing considerable synthetic flexibility in the choice of conjugation method. In particular, isothiocyanate (NCS)[39] groups and peptide-coupling reagents, including *N*-hydroxysuccinimide (NHS)[44] groups are commonly used for conjugation with porphyrins. These amine-reactive groups are simple to synthesise, and react rapidly and in a high-yielding fashion to form bonds suitable for use in biological systems.

The first example of coupling of an NCS-functionalised porphyrin to an antibody fragment was explored by Staneloudi *et al.*,[45] with a scFv conjugated to two water-soluble porphyrins *via* lysine residues to produce a range of conjugates. Photosensitiser: antibody conjugation ratios of 5, 10 and 20:1 were achieved, although ratios greater than 20:1 led to a loss of antigen selectivity. Cytotoxicity studies of the 5:1 PIC showed increased accumulation in target tissues, and selective cytotoxicity for target cell lines only. However, the low binding ratios studied limited the photosensitiser concentrations which could be investigated, with 74% of target cells remaining viable after irradiation.[45]

Conjugation of the photosensitiser tin (IV) chlorin *e6* and the fluorescent dye Cy5 *via* NHS-mediated peptide coupling to the L19 scFv by Birchler *et al.*[46] has also been attempted in the literature. Conjugation of Cy5 with the scFv obtained ratios of 1.5:1, considerably lower than ratios obtained for other scFv fragments. Despite this, use of this vascular-targeted PIC for the treatment of ocular neovascularisation in mice yielded good results. Cy5-L19 conjugates allowed visualisation of newly-formed blood vessels in the eye, while the PIC showed selective coagulation of angiogenic blood vessels only after irradiation, causing no damage to the iris, sclera or other pre-existing blood vessels the eyes.[46]

Fabbrini *et al.*[47] also used an NHS-ester of tin (IV) chlorin *e6* to conjugate to L19 antibody fragments. In this work, conjugation was attempted to both the SIP and scFv fragments of this antibody, with binding ratios of 2.01:1 and 2.12:1 obtained for the two PICs respectively, with a small amount of non-conjugated photosensitiser present in each. The localisation and activity of the two conjugates was explored, with both

conjugates localising selectively in the vasculature of three small-tumour models. The SIP conjugate was found to be significantly more effective as a PDT treatment agent, although both PICs were shown to be more effective than the free photosensitiser. The effectiveness *in vivo* of the SIP conjugate was also determined, with it showing a 50% tumour size reduction in a single treatment, and long-term tumour size control with repeated treatments.

Higher conjugation ratios were obtained by Palumbo *et al.*,[48] who conjugated an NHS functionalised, water-soluble porphyrin to the larger SIP fragment of the L19 antibody *via* peptide coupling. Binding ratios of 3:1 were obtained on each monomeric structure, giving an overall binding ratio of 6:1 on the homodimeric antibody fragment. The effectiveness of this conjugate was trialled on two murine cancer models, showing in both cases that the L19-SIP preferentially accumulated in the neovasculature of the cancer, leading to vascular shutdown, and complete, long term eradication of the neoplastic tissue through triggering of an immune response. No repeated treatment was required in order to lead to a complete cure of the cancer.

Considerably larger coupling ratios were obtained *via* peptide coupling utilising a carbodiimide coupling reagent by Bhatti *et al.*,[49,50] who utilised the spacing of the 13 surface lysine residues present on the anti-HER2 C6.5 scFv to give conjugation ratio of 8-10:1 with pyropheophorbide-*a* (PP*a*) and Verteporfin. While these large conjugation ratios were considerably higher than those achieved for other antibody fragments trialled, this figure was highly variable, and included a large percentage (30%) of non-covalently bound photosensitiser.[49]

Subsequent work by the same group achieved improved conjugation ratios of PP*a* and Verteporfin (~14.1:1) with a maximum non-covalent contribution of 18%, resulting in a covalently bound photosensitiser: scFv ratio of approximately 11:1 for both PICs.[50] In both cases, while the free photosensitisers displayed uptake in both HER2 positive and control cell lines, the PP*a* PIC showed selective uptake in HER2 lines only. However, although conjugation improved uptake speed and retention time of Verteporfin, control cell lines also showed considerable uptake of this photosensitiser, with this non-selective uptake attributed to the large proportion of non-conjugated photosensitiser present.[49, 50]

However, despite the widespread usage of the lysine residue as an excellent bioconjugation handle for the synthesis of photoimmunoconjugates, the presence of these residues in large numbers on the surface of most antibody fragments also has disadvantages. Firstly, the large numbers of lysine residues means that many porphyrins can bind to a single antibody fragment, altering solubility and internalisation properties of the fragment, and leading to the synthesis of conjugates with averaged molecular weights rather than stoichiometric binding ratios. Additionally, the location of the lysine residues means the placement of the photosensitiser molecules on the antibody fragment is difficult to control, leading to self-quenching of the photosensitiser as a result of the close proximity of numerous porphyrins, and loss of antigen specificity as a result of binding to the active site of antibody fragment. As a result, a rise in alternative methods of bioconjugation has been observed in the literature.

One such alternative allows the site specific and stoichiometric conjugation of a single photosensitiser molecule to an antibody fragment through modification of the fragment itself. This approach was used by Hussain *et al*., with the attachment of a 20 kDa protein SNAP tag to an anti-EGFR scFV allowing the efficient and facile bioconjugation of a chlorin *e6* photosensitiser bearing a benzylguanine functionality, with a precisely controlled 1:1 conjugation ratio.[51] The effectiveness of this targeted photosensitiser was then trialled using a range of four EGFR+ cell lines, with the PIC causing cell death in the target cell lines only, while the free chlorin *e6* led to cell death in both EGFR+ and control cell lines. However, synthesis of photoimmunoconjugates in this way has several disadvantages; firstly, it requires the time consuming modification of both the photosensitiser and the antibody fragment to create a bioorthogonal conjugation site. Secondly, the conjugation ratio of this method is limited to 1:1 only, with multiple addition of the large SNAP tag to the antibody fragment having the potential to significantly alter the properties of the scFv.

An alternative and less time-consuming approach is conjugation to an alternative amino acid residue which is also present on the surface of antibody fragments, but which is less numerous than lysine, and is not found in the active site of antibody fragments. Cystine is a dimeric amino acid, consisting of two cysteine amino acids joined *via* thiol residues to create a disulfide bridge. Although these disulfide bridges are common in antibody fragments, only interchain disulfide bridges are available for functionalisation. Reduction of these interchain disulfide bridges produces two thiol groups, which can

then be functionalised by photosensitiser moieties bearing thiol-reactive groups. Although this approach is less common than linking to lysine residues, the limited number of cystine residues in any antibody fragment allows more specific photosensitiser conjugation, and a smaller range of binding ratios.

The work of Hasan's group[52-55] utilised a reduced disulfide bridge (Figure 10) as a binding site on the OC125 F(ab')2 fragment for the production of PIC aimed at ovarian cancers.

Conjugation of chlorin *e6* to the F(ab')2 *via* either a cationic or anionic poly-L-lysine linker was utilised to produce charged conjugates with conjugation ratios of 15-20:1, and the effects of the charge upon cell localisation and uptake studied.[53] Uptake of both PICs was shown to be due to active endocytosis, with the cationic conjugate showing 17 times greater uptake than the anionic conjugate, although uptake of the free chlorin was found to be greater than either PIC. Despite this, the cationic PIC was shown to have the lowest cell survival fraction, which was attributed to its localisation in the cell lysosomes.

Further study of these PICs *in vivo* confirmed the effectiveness of the cationic conjugate.[52] Photosensitiser delivery to target organs was found to be 2.5-80 times greater for the cationic conjugate compared to the anionic PIC, with specific values dependent on the site of the tumour. The cationic PIC also showed good target tissue accumulation ratios, even for organs such as the intestines, in which high levels of photosensitiser are normally observed. This selectivity was poorer for both the anionic conjugate and the free photosensitiser.
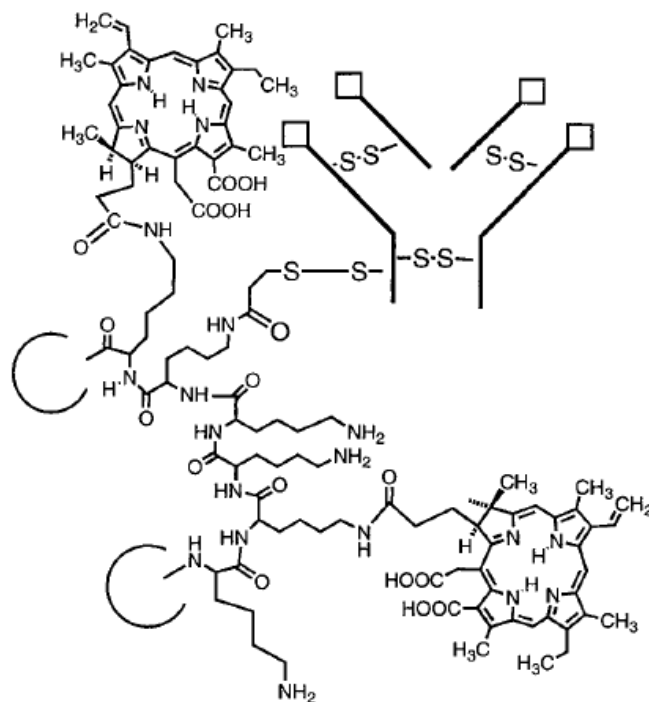
*Figure 10: Structure of the cationic antibody conjugate. The disulfide bridge utilised as a conjugation site can clearly be seen on the antibody fragment.*[54]

These results were confirmed in the *in vivo* treatment of ovarian cancer cell lines in mice,[54] with the effectiveness of the cationic PIC attributed to internalisation due to either its positive charge or lysosomal degradation improving the properties of the photosensitiser. The cationic PIC showed the best survival rates, with a 90% loss of tumour mass after irradiation, and a mean survival time of 44.1 days. However, despite these positive results, rapid regrowth of the tumour from surviving cells was found to be a significant problem.

A potential solution to this regrowth was explored in the treatment of ovarian and breast cancer cell lines in mice with combined approach of the cationic PIC and cisplatin therapy. While the effects of the two treatments singularly were variable dependent on cell line, a synergistic effect was observed with the combined treatment towards all cell lines, even in those displaying platinum resistance.[55]

An alternative to the use of commercial polymeric linker chains such as poly-L-lysine was explored by Lu *et al.*[56] Conjugation to the disulfide bridge of the OV-TL 16 antibody Fab' was carried out to a maleimide functionalised monomer, while conjugation of the commercial photosensitiser *Me₆* was carried out on another monomer. Polymerisation of these and a third spacer monomer was carried out to produce a

HPMA polymer bearing both the antibody fragment and the photosensitiser in an average 1:11 antibody: porphyrin ratio. Biological evaluation of the targeted conjugate showed more effective tumour destruction and slowed regrowth in comparison to a porphyrin-polymer conjugate, however complete tumour eradication was not observed in either case. However, although this method allows for facile synthesis of a porphyrin functionalised polymer, it also allows for a high degree of variability of both the structure and stoichiometry of the targeted photosensitiser, with some conjugates containing more than one antibody fragment.

While conjugation of photosensitisers to fragments of the L19 anti-neovascular antibody has been carried out *via* lysine residues,[46-48] the conjugates produced to date do not allow for creation of targeted photosensitisers with good control over the binding site and conjugation stoichiometry. The SIP format of this antibody contains an interchain disulfide bridge (Figure 11) which represents an excellent target for site specific conjugation of two thiol-reactive photosensitisers.
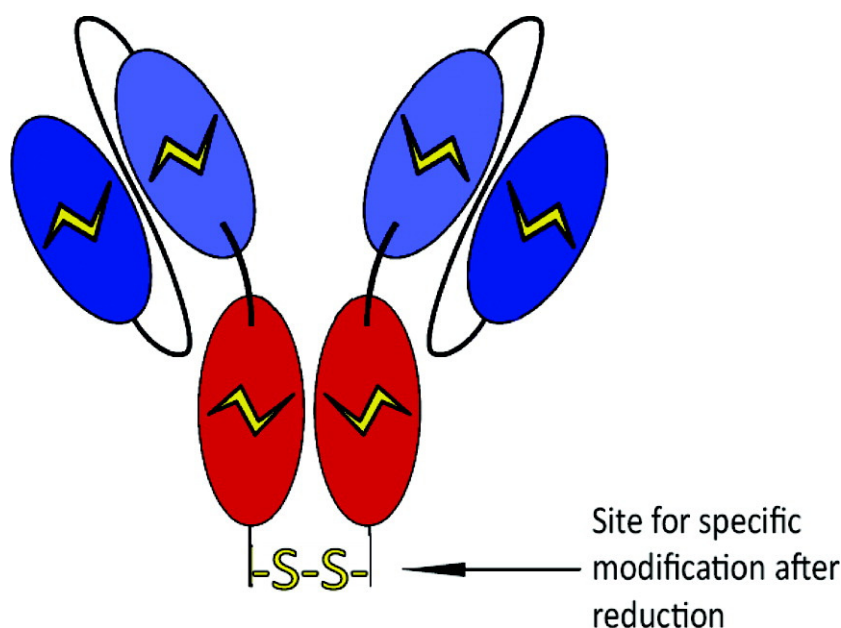


*Figure 11: Schematic representation of the structure of the L19-SIP, showing the disulfide bridge binding site.*[57]

Despite the specific number of binding sites on the L19 antibody, binding ratios of greater than 2:1 can be observed due to non-covalent photosensitiser binding, or conjugation to other binding sites, in particular to lysine residues. In the work of Alonso *et al.*,[57] a water soluble porphyrin was functionalised with the thiol-reactive maleimide functionality, and conjugated to the L19 SIP disulfide bridge. Conjugation of the

porphyrin in ratios of exactly 2:1 was confirmed by MS, with the highly selective maleimide group showing no reactivity towards the amine functionality on lysine residues in competition with thiol groups, limiting binding to these sites. Non-covalent binding was also not observed due to the use of a cationic photosensitiser.

Although the binding ratios were confirmed by MS, the absorption spectrum of the photosensitisers suggested binding ratios of lower than this (0.76-1.75:1), which was attributed to quenching resulting from the close proximity of the two thiol binding sites. Polyethylene glycol linker chains between the antibody and photosensitisers were found to reduce quenching and improve photosensitiser activity as a result of reduced steric hindrance.

The use of disulfide bridges as bioconjugation sites offers significant improvements in the control of both the site of conjugation and the conjugation stoichiometry of the photosensitiser in comparison to conjugation *via* lysine residues. Despite this, a significant drawback of the disulfide bridge conjugation method is the low conjugation ratios, which are limited by the small number of interchain disulfide bridges present on a typical antibody fragment. Two methods to increase these binding ratios have been attempted in the literature; conjugation of numerous photosensitiser moieties to a single antibody fragment *via* use of a commercial polymer chain as a linker, and conjugation of both the photosensitiser and antibody fragment to monomers and subsequent growth of a polymeric linker chain. While both of these methods produce significant increases in binding ratios, the associated loss of stoichiometric control due to the variable molecular weight of polymers and varying number of conjugation sites is highly undesirable. A far more promising alternative is the use of dendritic structures to create porphyrin-dendron conjugates suitable for use as part of a 3rd generation photosensitiser.

## 1.2. Dendrons and porphyrin-dendron conjugates

### 1.2.1. Dendrimers and dendrons

The first example of the synthesis of a dendritic structure was carried out by Vögtle *et al.*[58] in 1978, who described the product as a "cascade molecule", referring to the cascading increase in the number of branches as the size increased. These dendrimers were synthesised by Michael addition of acrylonitrile to a primary amine followed by

reduction of the cyano group to an amine. Repetition of these two steps was carried out to create a multiply branched structure.
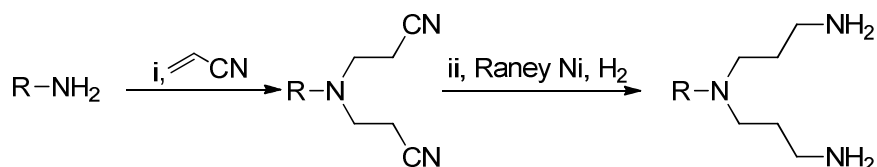


*Figure 12: Synthesis of the "cascade molecule", now often referred to as a PPI dendron. Subsequent repetition of steps i and ii can be utilised to create higher generation dendrons.*

The word dendron was first used to describe these types of molecules by Tomalia *et al.*[59] in 1986, and comes from the Greek word meaning "tree", which describes the regular, repetitive branching pattern that these molecules adopt (Figure 13). Unlike hyperbranched polymers which can be highly polydisperse, the synthesis of both dendrimers and dendrons is carried out in a way which allows excellent structural control and produces highly monodisperse structures. Hyperbranched polymers are generally constructed *via* the addition of monomers in a single step, leading to the repeated addition of the monomer to the growing polymer chain, with this lack of control over monomer addition generally leading to the synthesis of a highly polydisperse structure with irregular branching.

In contrast, dendritic structures (Figure 13) are constructed by the repetition of at least two steps, an addition step followed by a modification step which prevents the repeated addition of the starting material in an uncontrolled fashion. This method of synthesis produces two very different types of binding sites available on the structure; the single central group which is termed the focal point, and the multiple groups present on the outer surface of the structure termed the peripheral groups.
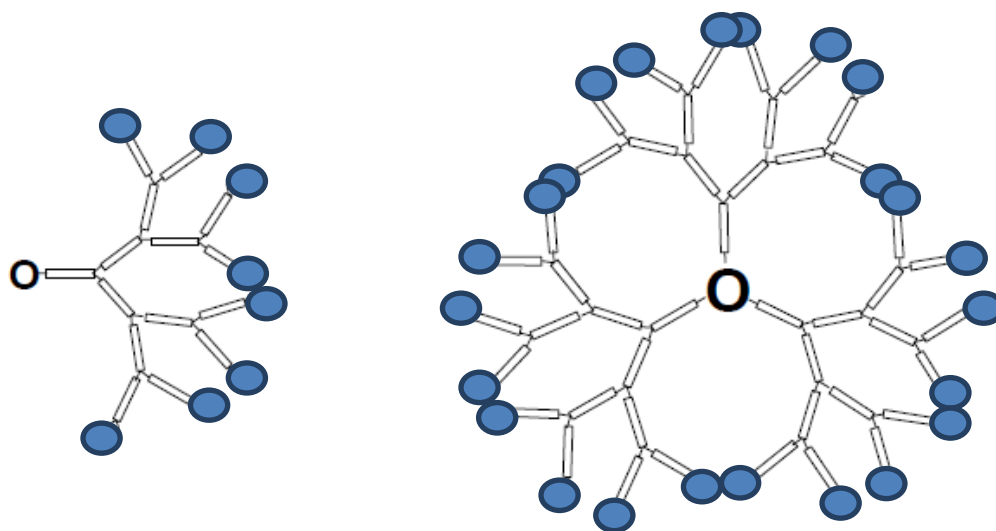
*Figure 13: Structures of dendrimers and dendrons. Dendrimers (right) are synthetic spherical macromolecules, with a regular, highly branched structure. Dendrons (left) are 2D branches of a dendrimer, originating from a single focal functional group (O), while both structures terminate in numerous peripheral groups (blue circles).*

Since the synthesis of the first dendritic structures the types of dendrimer have increased exponentially, with the dendrimers generally named by the bond formed upon the addition step of the synthesis. Dendrimers are also classified by generation, with all dendrimers beginning at G=0, and the presence of each new level of branching representing an increase of one generation.

The stepwise synthesis of dendritic structures means that they are generally highly monodisperse, with the nature of the synthesis allowing excellent control over the growth, size and structure of the dendron. A symmetrical starting material can be used to yield a circular or spherical shape dendrimer, with the dendrimer growing out from a central focal point equally in all directions to create a 3D spherical structure, while an asymmetric starting material yields a wedge-shape 2D dendron which grows out in one direction only, leaving the focal point exposed and suitable for later functionalisation. Although the structures of dendrimers and dendrons are very different, both are easy to modify and are generally amphiphilic, with many dendrimers showing greatly improved solubility in comparison to their linear analogues.[60] While both dendrimers and dendrons display numerous binding sites on the periphery of the structure, generally conjugation at the focal point is limited to dendrons only.[61]

The properties of dendrimers make them ideal for biological,[62] gene transfection,[63] and

drug delivery[64] applications, in particular in the encapsulation of drug molecules to improve solubility and uptake properties. Although dendrons are less useful in the encapsulation of molecules, they are widely used in many applications, as they allow multiple conjugations to a single site. For this reason, they represent excellent multiplier groups in the synthesis of targeted photosensitisers. Three types of dendron were selected for synthesis in this work; poly(amidoamine) (PAMAM) dendrons, tris dendrons and aryl ether dendrons.

### 1.2.1.1.  PAMAM dendrons

Poly(amidoamine) (PAMAM) dendrimers are named for the amide bonds joining the branching points of the dendrimer and the amine functionalities at the periphery of the structure. These dendrimers were first developed in 1985 by Tomalia, who described them as "starburst polymers" referring to their star-like shape at higher generations, and were the first dendrimers to be commercially synthesised[62] due to their structural flexibility and ease of synthesis. The first synthesis of a PAMAM dendrimer used an ammonia core, with growth carried out through stepwise addition of methyl acrylate and ethylenediamine, however in practice ethylenediamine or any other symmetrical diamine molecule can be used as the core of the dendrimer. Alternatively, a heterobifunctional monoamine core can be used to yield an asymmetric PAMAM dendron.
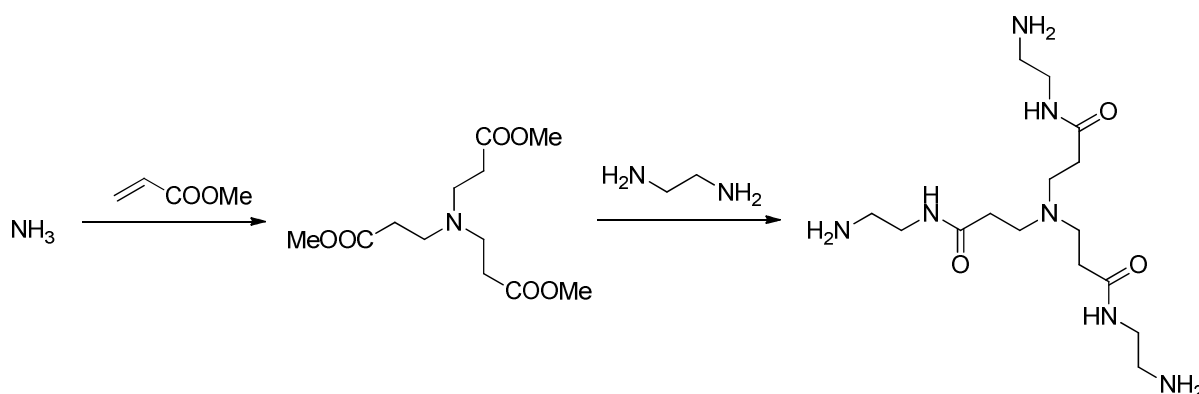


Figure 14: synthesis of a PAMAM dendron. Stepwise addition can be repeated up to 10th generation until "starburst effect" occurs, in which the peripheral groups become too closely packed to allow further reaction.[60]

PAMAM dendrons can be synthesised as either ester-terminated half generation dendrons (G=-0.5 onwards) or amine-terminated full generation dendrons (G=0 onwards) (Figure 14), allowing the structure to be modified to yield almost any

peripheral functional groups.[65] PAMAM dendrimers are highly biocompatible, non-immunogenic, hydrophilic and easily modifiable[61] and as a result are widely used for biological, biomimicry, gene transfection and drug delivery applications.[62]

In contrast, there is currently relatively little published literature regarding the synthesis and utilisation of PAMAM dendrons. However, the results which have been published to date highlight the broad utility of these versatile molecules.
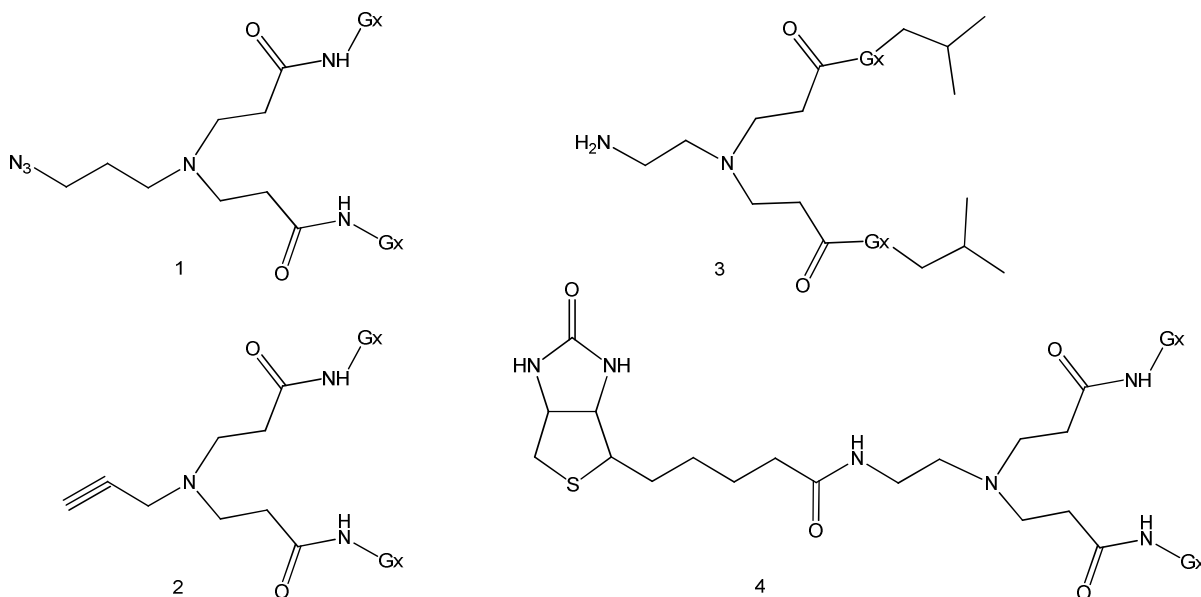


*Figure 15: Focal-point functionalised dendrons 1-4. Gx indicates repeated generations of PAMAM dendrimer growth, with x indicating the generation number. For 1, x=0.5-4.5, 2, x=0.5-3.5, 3, x=2.5, 4, x=0.5-3.5.*

In part, this versatility is due to the nature of the PAMAM dendron synthesis, which theoretically allows dendrons to be constructed from the addition of methyl acrylate to any monoamine. For example (Figure 15), starting materials containing azide[66, 67] (**1**) and alkyne[68] (**2**) functionalities have been utilised to produce dendrimers with the corresponding functionality at the focal point, which could be subsequently conjugated *via* click chemistry to produce both symmetric and asymmetric dendrimers.[69] Utilisation of a mono-protected diamine starting material (**3**) allowed the synthesis of a similarly asymmetric structure, which was deprotected and used to form asymmetric dendrimers *via* a convergent synthesis method.[70] Conjugation of a diamine reagent to biotin (**4**) was also utilised to create a dendron capable of binding to avidin at the focal point.[71]

Synthesis of similarly asymmetric PAMAM dendrons has also been carried out for use in a wide range of applications. In particular, solid-supported dendrons have been the focus of much research, with the synthesis of dendrons conjugated to both silicon wafers[72] and silica gel,[73] allowing them to be utilised in a variety of solid-supported applications, including as amphiphilic adsorbents in water purification.[74] However, solution-phase PAMAM dendrons have also been utilised in a range of both biological applications, including use as confocal imaging agents,[75] gene transfer agents,[76] and physical chemistry applications in light harvesting antenna[77, 78] and Pd(0) nanoparticle stabilisation.[79]

### 1.2.1.2.  Tris dendrons

In contrast to the wide range of amine-functionalised reagents utilised for PAMAM dendron synthesis, the functional variability of tris dendrons derives from a range of modifications to a common starting material; tris(hydroxymethyl)aminomethane (tris). The first use of tris in dendrimer chemistry was by Newkome *et al*,[80] who synthesised the first example of an arborol in 1985. These dendrimer-like structures are named as a result of the tree-like structure in combination with the multiple alcohol peripheral groups on their surface. However, due to the asymmetric nature of tris, the molecule generated is not symmetrical and therefore is not a true dendrimer, although a dendrimer-like structure can be produced at higher generations.
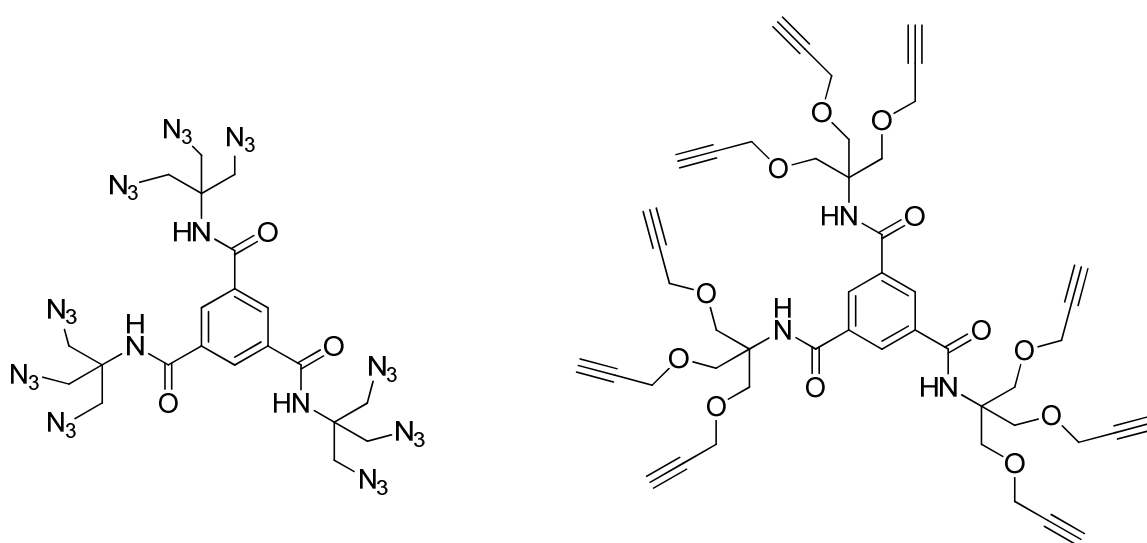


*Figure 16: Structures of tris-containing dendrimers synthesised by Chabre et al., with each containing nine peripheral click functionalities.*

27

Subsequent work by Chabre *et al.*[81] has successfully used tris to create a range of symmetrical dendrons bearing both azide and alkyne terminal functionalities (Figure 16). Initially, tris molecules were peripheralised with click functionalities, and subsequent conjugation of the amine focal point to the core molecule trimesoyl chloride allowed the synthesis of dendrimers containing nine peripheral groups. Click conjugation of sugars to the periphery of these molecules was then used to synthesise multivalent glycoconjugates for antibacterial applications.

Despite the limited use of tris in the synthesis of dendrimers, this molecule has been widely used in the literature to synthesise a range of three-armed dendrons, created *via* modification of the three alcohol groups to form the dendron periphery, and modification of the single amine to form the dendron focal point. Due to the versatility of both of these functional groups, a range of dendrons have been synthesised (Figure 17) showing different functionalities at these two positions for two key applications; carbohydrate conjugation, and functionalisation for use in further synthesis.
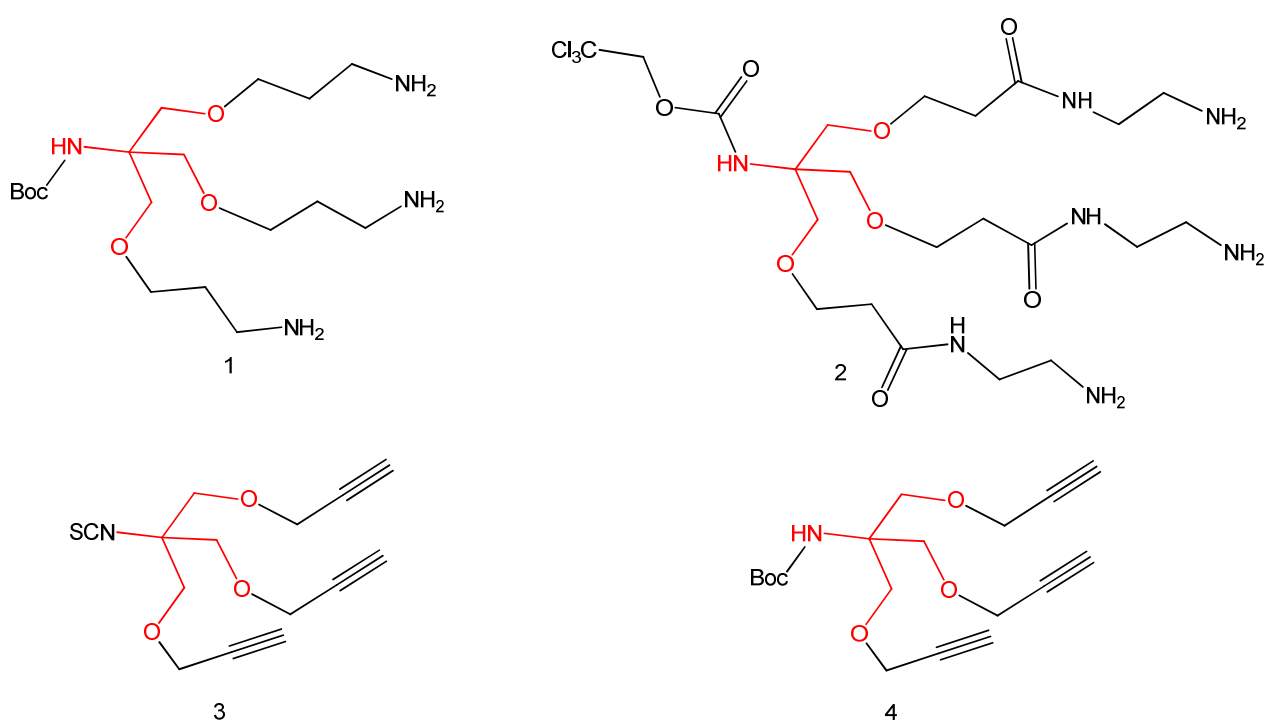


*Figure 17: Examples of amine (1,2) and alkyne (3,4) peripheral functionalisation of the tris dendron skeleton (highlighted in red). A range of focal point functionalisations are also seen to allow further conjugation of the dendron.*

In particular, tris dendrons are used to conjugate three carbohydrate moieties to a single core group in order to create a "cluster glycoside effect"; an enhanced binding potency of multivalent carbohydrate clusters in comparison to their monovalent counterparts.[82] A range of synthetic strategies were utilised by Miller *et al*. to conjugate three sugars to tris dendrons bearing peripheral amines (**1,2**), followed by conjugation to the amine focal point conjugation to a fluorescent label.[83]

Conjugation of azide-functionalised mannose derivatives to alkyne-functionalised tris dendrons is a strategy utilised by two groups. Schlick *et al*. (**3**) carried out this reaction followed by functionalising the tris focal point with an NCS group, allowing conjugation to a multifunctionalised PAMAM dendrimer,[84] while Chabre *et al*. utilised a similar strategy for mannose conjugation to tris-dendrimers (**4**), which could then be reacted with thiol-functionalised central units to create dendrimer-like structures bearing 12 or 18 sugars.[85]

Other applications of tris dendrons utilise these versatile linker groups in a range of ways, including conjugation to resins to create cleavable and non-cleavable multipliers for various small ligands,[86] use as fluorinated tags or as fluorinated dendrimer building blocks,[87] or in the synthesis of multiply-functionalised macromolecules for biological and materials applications.[88]

### *1.2.1.3.   Aryl ether dendrons*

The first synthesis of aryl ether dendrons was published by the group of Frechet in 1990,[89] with these molecules often being referred to as Frechet dendrons as a result. The name aryl ether dendrons originates from the fact that these dendrons have aryl branching points joined by ether linkages, allowing for a high level of structural flexibility due to the fact that each generation can be constructed with either two or three branching points on each aryl ring.

Synthesis of aryl ether dendrons is generally carried out through a Williamson ether synthesis between a benzylic bromide and a phenolic alcohol. Subsequent bromination of a benzylic alcohol reforms a benzylic bromine, allowing formation of the next generation. This synthesis differs from most dendrons found in the literature due to the fact that it is convergent rather than divergent in nature, with the synthesis beginning

from the groups which will form the periphery of the dendron and working inwards towards the focal point. This leads to highly reproducible and homogenous structures showing few imperfections even at high generations, with the convergent synthesis method ensuring complete functionalisation with the desired peripheral groups in all cases. This synthesis method also allows for facile and versatile focal point modification, allowing attachment to a number of structures and leading to widespread use in the literature.
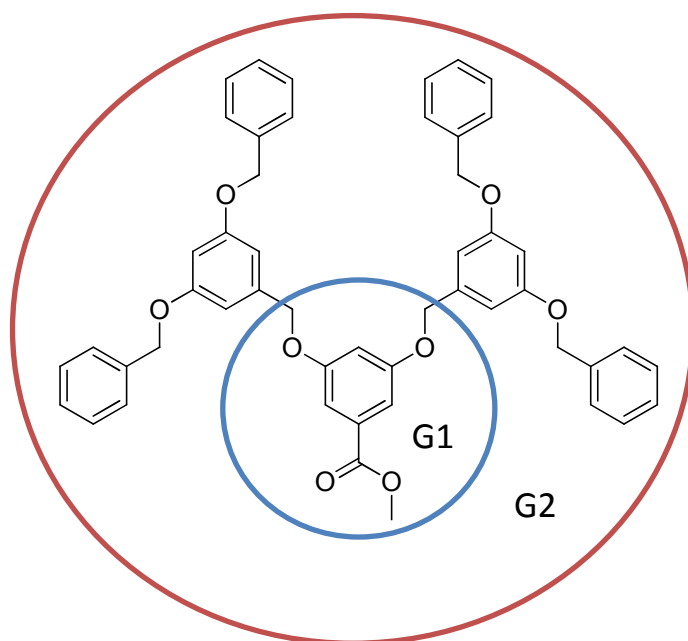


*Figure 18: Structure of the aryl ether dendron showing the structure of the G1 and G2 dendrons.*

The first published synthesis of aryl ether dendrons contained only structures with peripheral benzyl ether functionalities (Figure 18), meaning that further functionalisation of the periphery was difficult. However, since this time, many variations of this synthesis have been published showing the structural diversity which can be achieved on the peripheral phenolic groups. This structural flexibility allows the modification of aryl ether dendrons to be utilised in a wide variety of applications (Figure 19).
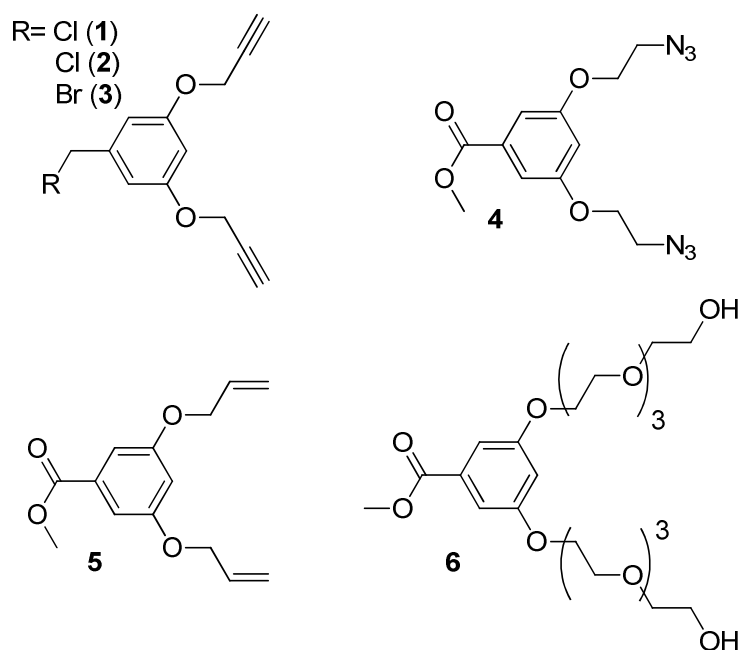
*Figure 19: Examples of the structural variability of the two-armed aryl ether skeleton.*

The peripheral phenolic functionalities are particularly suited to modification for click chemistry, and in particular the reaction with propargyl bromide to form the corresponding alkyne ether has been used by many groups. Alkyne functionalised dendron (**1**) has been used to conjugate to azide functionalised sugars in the synthesis of glycoclusters for evaluation of Alzheimer's related amyloid peptides,[90] with the focal point chlorine later converted to an azide for further click reaction. A similar strategy using (**2**) and higher generation structures with identical functionalisation was also used by Rajakumar *et al.*, with click chemistry utilised to peripherally functionalise the dendron with benzothiazole groups for light-harvesting applications in DSSC.[91] In contrast, the bromine functionalised dendron (**3**) and a three-armed analogue were click functionalised with carborane, for applications in boron neutron capture therapy (BNCT) for treatment of cancers.[92]

While click functionalisation using propargyl bromide is facile and well-understood, use of the complementary azide functional group to peripherally decorate aryl ether dendrons has also been carried out to produce dendron (**4**) and higher generation dendrons and dendrimers. Subsequent click reactions with alkyne functionalised sugars were used to create modified glycodendrimers as potential anti-bacterial drugs for use in treatment of chronic lung infections in cystic fibrosis sufferers.[93]

However, while click functionalisation of these dendrons has received a large amount of

attention in the literature, numerous strategies have also been published which do not rely on use of alkyne or azide groups. Elmer *et al*. functionalised a $G_1$ aryl ether dendron with allyl groups (**5**) with the dendron subsequently cross-linked to produce "cored dendrimers" suitable for drug delivery applications,[94] while Zhao *et al*. attached PEG chains to the dendron periphery to create a dendron (**6**) which was used to produce a pretzelane macromolecule.[95]

The ease of functionalisation of aryl ether dendrons at both the periphery and focal point of the structure means that the reactive and physical properties of these molecules can be easily modified, leading to the widespread use of these dendrons. In particular, a number of recent publications have exploited the aromatic properties of these structures, with use in self assembling fluorescent gels[96,97], light harvesting systems[98] and fluorescent Cu(II) and NO detection systems.[99] However, the versatility of these molecules has also been exploited in other areas of chemistry, with aryl ether structures used in the synthesis of multiresponsive self-assembly gels,[100] perfluoronated macromolecular host structures,[101] and ligands for palladium(0) catalysts.[102]

### 1.2.2. Porphyrin dendron conjugates

Incorporation of porphyrins into dendrimers was first attempted in 1993,[103] with many early papers describing the non-covalent encapsulation of tetrapyrroles in the dendrimer cavity. While this first example of a dendritic porphyrin structure was due to non-covalent interactions, much of the subsequent literature has explored the covalent conjugation of porphyrins and dendritic structures. While conjugation to PAMAM dendrons is often ideal for biological applications due to the biocompatibility and solubilising properties of these dendrons, aryl ether dendrons are also widely used due to their highly conjugated system providing beneficial electronic properties in many light harvesting and nanotechnology applications.

Due to the nature of the dendron structure, there are three ways in which porphyrins and dendrons can be conjugated; most commonly utilised is the conjugation of dendron focal points to the *meso* positions of the porphyrin, allowing encapsulation of the porphyrin into a dendrimer structure. The amphiphilic nature of these conjugates makes them particularly suitable for biochemistry applications, including *meso*

functionalisation of multi-porphyrin arrays as mimics of coenzyme B12,[104] and covalent encapsulation of porphyrins to act as probes for molecular oxygen[105-107] and pH[108] in biological systems.

The electronic and structural properties of the conjugated dendrons also make these type of conjugates ideal for a number of other applications, with highly conjugated dendrons used to create structures for use in light harvesting applications,[109, 110] photocatalysis and photovoltaic cells[111] and artificial photosynthesis systems.[112, 113] The rigid, sterically hindered nature of the dendrimer also facilitates use in many applications, with dendrimers utilised in synthesis of sterically-hindered oxidation catalysts using manganese porphyrins,[114] to increase the solubility of zinc porphyrins for use in porphyrin "wires",[115] and to alter the photophysical properties of porphyrins for use as ligands for luminescent erbium conjugates.[116]

While complete encapsulation of the porphyrin in a dendrimer-like structure is common, conjugation of porphyrins to the focal point of dendrons can also be carried out in lower conjugation ratio to create non-spherical structures. There are several examples of this in the literature; Kimata *et al*. used a 1:1 porphyrin-dendron ratios in order to create "free-radical equivalents" with the dendron utilised as a fifth-ligand for the rhodium core of a metalloporphyrin.[117] Similarly, Sengupta *et al*.[118] also used the conjugation of a single dendron to a β-position of a chlorin structure, with the structure of the dendron allowing attachment of multiple alkyl chains to create a discotic liquid crystal (DLC) structure.

The second method of conjugation is through the attachment of multiple porphyrins to the periphery of the dendron structure, leaving the focal point free to attach to another structure. While this method has been used on dendrimer structures frequently in the literature, conjugation of porphyrins to the periphery of dendrons is far less frequently utilised. To date, only one area of research has utilised porphyrin dendron-conjugates in this way, synthesising dendrons functionalised with either two porphyrins[119] or one porphyrin and one phthalocyanine.[120] These dendrons were then conjugated to single-walled nanotubes (SWNT), allowing covalent functionalisation of SWNT with multiple porphyrins without disturbing the π-system of the nanotubes with excessive surface binding.
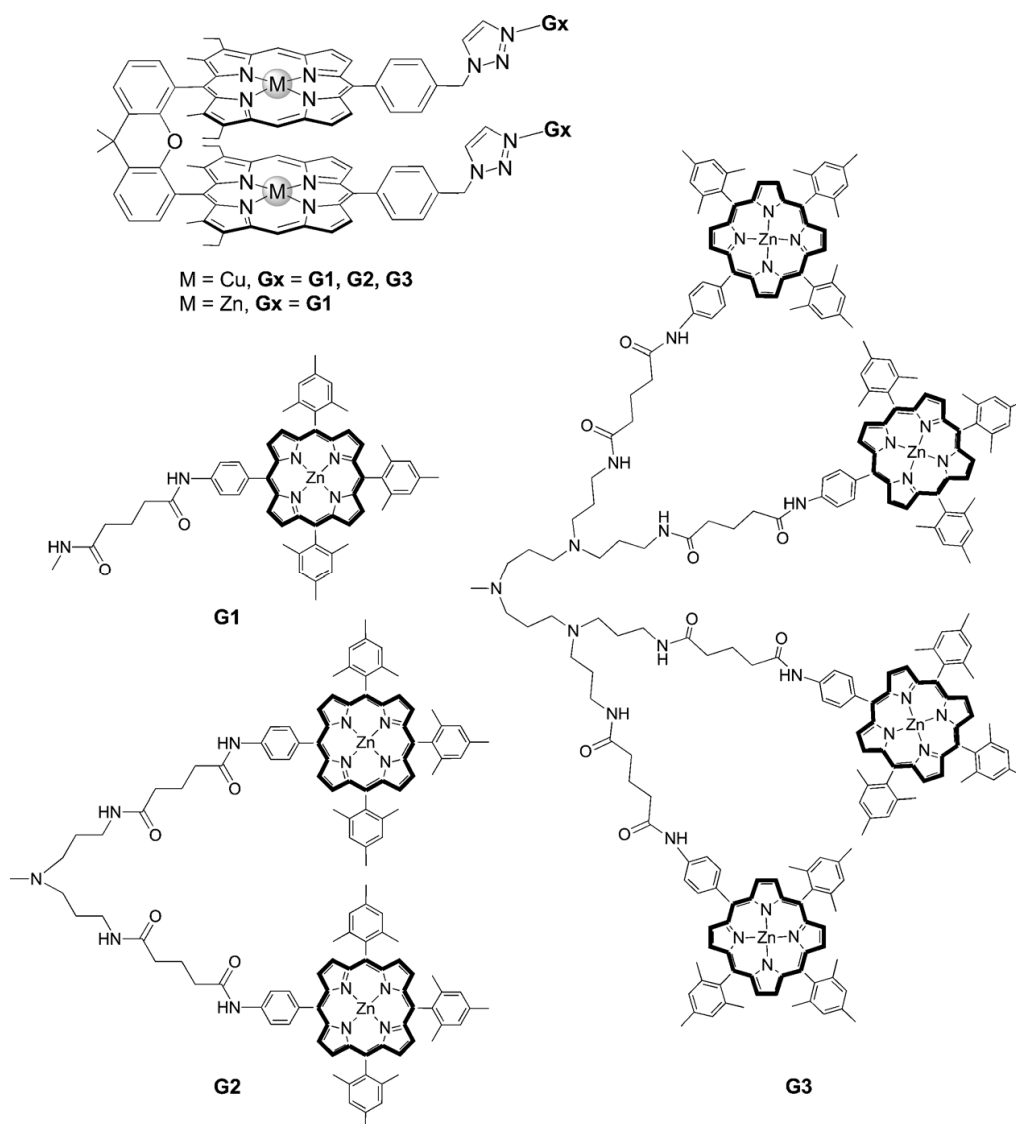
*Figure 20: A combination of two central porphyrins (top left) each conjugated to the focal point of one dendron, and multiple porphyrins conjugated to the periphery of these dendrons (G1-G3) are utilised in order to create a complex structure for use as a light-harvesting device.[121]*

The third method of conjugation of dendrons and porphyrins is a combination of the above two methods to produce a conjugate bearing porphyrins both on the periphery and the focal point of the dendron. Dendron-porphyrin conjugates have previously been utilised to model natural light-harvesting devices, however the work of Brégier *et al.* is the first to conjugate porphyrins at both the periphery and focal point of a dendron (Figure 20) for this application.[121] Further work on these doubly-conjugated systems has subsequently been carried out by Harvey *et al.*[122] who produced similar structures, however the use of rigid rather than flexible linker chains in the dendron allowed for optimal structural positioning of the donor and acceptor, and easier computer modelling of the system.

## 1.2.3. Dendrons in photosensitiser applications

To date, conjugation of porphyrins and other tetrapyrrolic molecules to dendritic
structures has also been utilised to some extent to produce photosensitisers for PDT, in
the majority of cases with the aim of increasing the amphiphilicity of hydrophobic
photosensitisers.[123-125] However, additional advantages of dendrimer conjugation
include reduced skin photosensitivity due to the increased photosensitiser size and
increased tumour accumulation due to the enhanced permeation-retention effect.[103] The
ability of higher-generation dendrimers to non-covalently encapsulate molecules has
also been used to create combined PDT and chemotherapy systems. Click conjugation
of PAMAM dendrons to the *meso* positions of a azide porphyrin was used by Ma *et
al.*[126] to create a porphyrin-centred dendron which was then used to encapsulate the anti-
cancer drug doxorubicin, to create a combined drug which was shown to be more
effective than either PDT or chemotherapy alone.

However, despite the widespread usage of dendrimers and dendrons conjugated to
porphyrins, the use of a dendron unit as linker group in a 3rd generation photosensitiser
is a relatively new area. To date, by far the most common use of dendrons in this way is
in glycodendrimers; dendrons with at least one targeting carbohydrate molecule
conjugated to the periphery, and a single photosensitiser unit at the focal point of the
dendron.
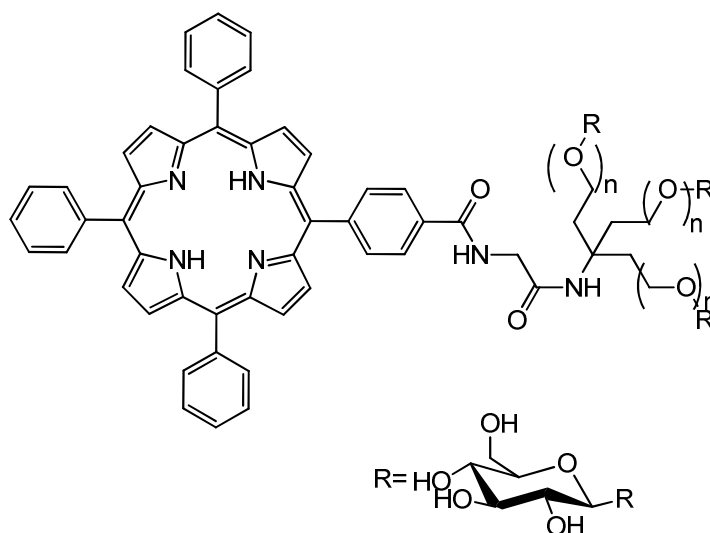


*Figure 21: Example structure of a glycodendrimer, with a central porphyrin moiety, and
one glycodendrimer unit with three peripheral glucose molecules attached.*

Conjugation of photosensitisers to glycodendrimers (Figure 21) is desirable for a

35

number of reasons; these highly hydrophilic, clustered carbohydrate-targeting moieties increase the amphiphilicity of the conjugate, and can allow the targeting of lectin-type receptors overexpressed on tumour cells,[127] without significantly altering the electronic spectra of the photosensitiser. To date, a number of glycodendrimer-porphyrin conjugates have been synthesised, containing a range of linker chain lengths[128] and dendron sizes. Most commonly three carbohydrate targeting groups are utilised per photosensitiser,[127, 128] although conjugates have been synthesised with as many as 12 per photosensitiser unit.[129]

Although glycodendrimers represent a promising targeting molecule for 3rd generation PDT, the use of dendrons to increase the loading ratios of porphyrin molecules onto other targeting moieties, such as proteins, antibodies or antibody residues is an area not currently well explored.

The work of Morosini *et al*.[130] is the first to date which aims to conjugate multiple photosensitiser units to a targeting moiety *via* PAMAM dendrons. This group utilised both monoprotected ethylene diamine starting material, and the more amphiphilic Boc-protected 2,2-(ethylenedioxy)-bis-ethylamine starting material to produce both lipophilic and amphiphilic PAMAM dendrimers. Although synthetic and porphyrin conjugation yields of the lipophilic dendrimers were poor, synthesis and conjugation of the water-soluble dendrimers proceeded well. Photophysical properties of the porphyrin photosensitiser utilised were found to be unaffected by the attachment of dendrons, however conjugation to a targeting group was not attempted.

## 1.3. Click chemistry

Click chemistry is best thought of as a "chemical philosophy" and was first proposed by Sharpless *et al*. in 2001. This philosophy aimed to change the field of drug discovery by producing a "chemical toolbox" of modular chemical building blocks which could be reacted together in well-understood reactions under a diverse range of conditions, allowing the creation of large and complex molecules through the conjugation of these smaller units.[131] These well-understood, robust reactions were termed "click reactions".

In order to be termed a click reaction, Sharpless outlined a number of conditions which the reaction must fulfil, including being facile, wide in scope, high yielding with few or

no side products, simple to purify, tolerant to water and oxygen, tolerant to changes in solvent, and utilising readily available starting materials.[131]
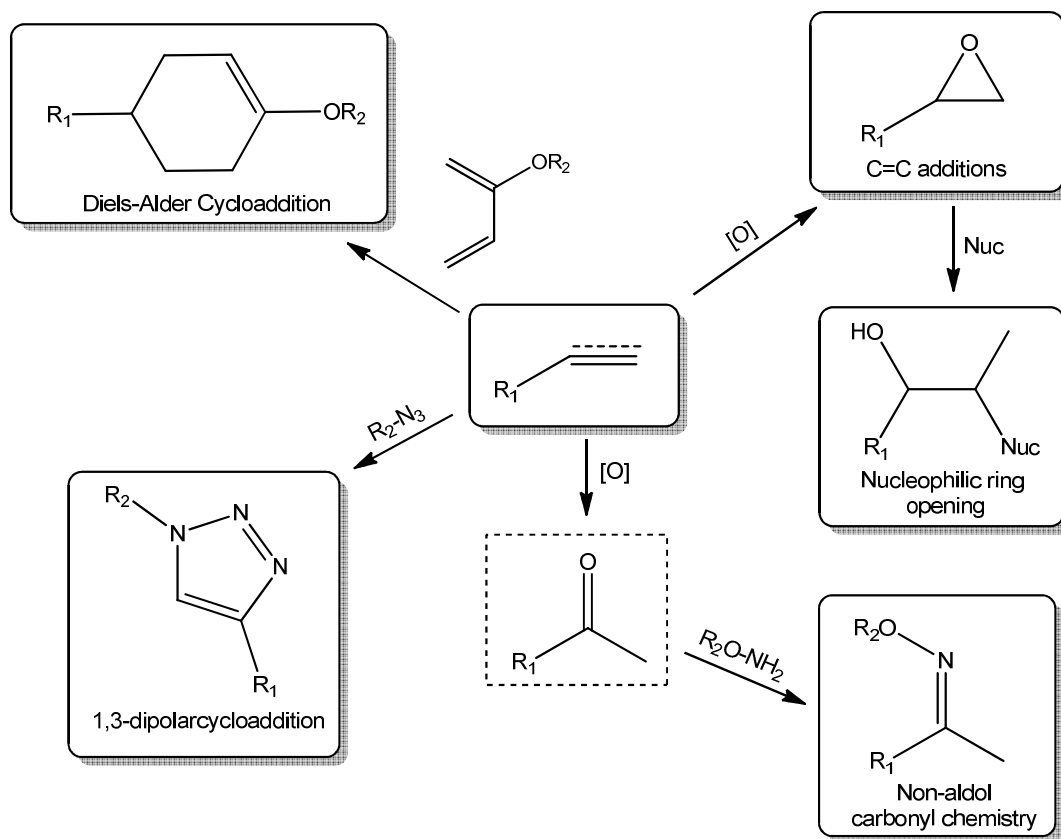


*Figure 22: A number of reactions have been proposed as possible click reactions, most of which originate from simple alkene and alkyne starting materials.[132]*

Although a number of reactions have been proposed as potential click reactions (Figure 22), in many cases the reaction fails to live up to this rather idealised vision of synthetic chemistry. For example, many nucleophilic ring opening reactions are susceptible to water, and the Diels-Alder reaction can show poor stereoselectivity. However, the Huisgen 1,3-dipolar cycloaddition is an example of a true click reaction (Figure 23).
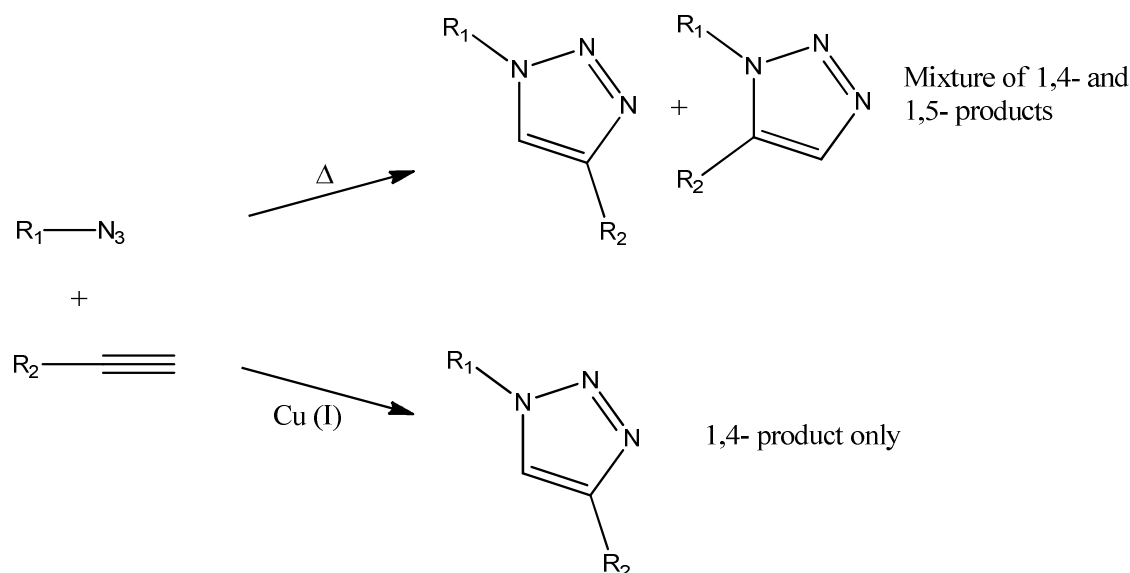
*Figure 23: Schematic showing the products of the thermal and copper-catalysed reactions between azide and alkyne products. A mixture of the 1,4- and 1,5-disubsituted isomers is obtained via the thermal reaction, while the copper-catalysed reaction is regiospecific for the 1,4-isomer only.*

The Huisgen 1,3-dipolar cycloaddition utilises the reaction between azide and alkyne functional groups to produce a 1,2,3-triazole linkage. Both azides and alkynes represent ideal starting materials for a click reaction for a number of reasons; they are essentially inert in biological systems, showing no reaction with either water or oxygen,[133] are easy to synthesise and are tolerant of a wide range of reaction conditions and solvents.[132] Additionally, the resulting triazole linkage is a rigid, stable linkage which has similar steric and electronic properties to the amide bond, but is significantly more chemically stable, in particular showing complete resistance to hydrolytic cleavage.

Under normal conditions this reaction requires high temperatures (> 100 °C) to force it to completion, and yields the product as a mixture of the 1,4- and 1,5-disubsituted triazole. However, in 2002 the use of a copper (I) catalyst to dramatically alter the nature of this reaction was reported independently by the groups of Sharpless[134] and Meldal.[135] The addition of the copper (I) catalyst significantly alters both the mechanism and the nature of the reaction, leading to a stepwise reaction which is both significantly ($10^7$ times)[136] faster than the uncatalysed reaction, and highly stereoselective, leading to the 1,4-regioisomer only.

The copper-catalysed azide-alkyne click (CuAAC) reaction has become so ubiquitous and well-recognised that "click reaction" is often utilised synonymously with CuAAC.

The popularity of this reaction is due to both its robustness and its flexibility, with the reaction able to accommodate a range of catalysts, solvents, starting materials and conditions. There are also few limitations regarding the nature of the azide and the alkyne reagents utilised, with both electronic and steric changes having limited impact. To date, the only significant requirement for the starting materials in the copper-catalysed reaction is that only terminal alkynes can be utilised. In contrast, the ruthenium-catalysed reaction (RuAAC) operates *via* a different mechanism, allowing reactions between internal alkynes and azides[137] although in the case of the RuAAC, the 1,5-subsituted triazole is the only product.
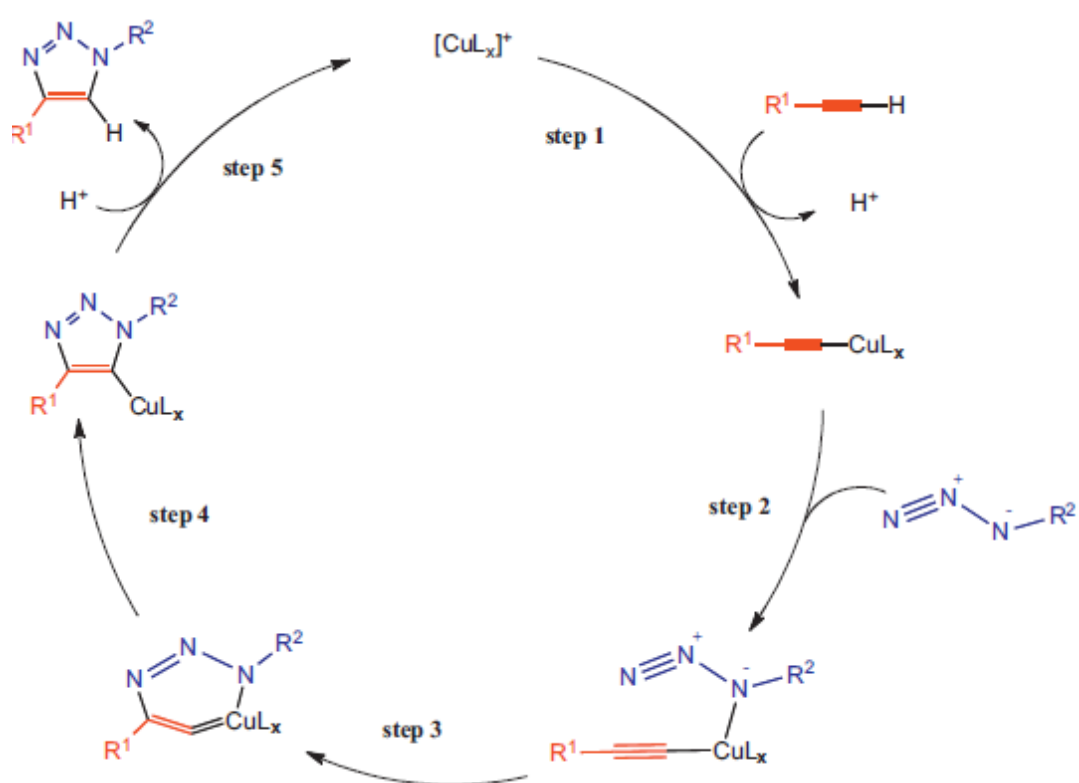


*Figure 24: Proposed mechanism of the formation of the 1,4-disubstituted triazole ring from an alkyne and azide, based on density functional theory (DFT) calculations.*

Although the mechanism of the CuAAC is a source of some debate, it is widely recognised that this reaction does not occur *via* the concerted 1,3-cycloaddition mechanism of the uncatalysed reaction. It is known that the reaction is 2$^{nd}$ order with respect to copper, and previous work has suggested a bimetallic reaction mechanism in which the alkyne and azide molecules coordinate to different copper centres.[138] More recently, a new mechanism has been proposed (Figure 24) which shows conjugation of both the azide and the alkyne to the same copper centre, with the second copper atom

interacting with the alkyne to positively influence the formation of the C-N bond.[138]

Although a relatively short amount of time has passed since the introduction of the philosophy of click chemistry, applications of click reactions, and in particular CuAAC, have grown significantly since then. Although this reaction is almost universally utilised, drug discovery,[139] medicinal chemistry[140] and polymer chemistry[141] have seen a particular growth in the use of click chemistry. These areas represent a good match to click chemistry due to the fact that this reaction is high-yielding, works well in polar solvents, and produces polar, biologically stable bonds.

## 1.4. Microwave reactions

### 1.4.1. Microwave heating and microwave effects

The use of microwave heating in organic synthesis was first published in 1986, with early work utilising domestic microwave ovens with no pressure or temperature control. The first dedicated chemical microwave reactors with heat and pressure control were developed during the mid-1990s, and have steadily replaced the domestic microwave oven in the laboratory.[142] To date, the use of microwave heating has been shown to be highly versatile, with recent reviews highlighting the application of microwave heating to almost any reaction which can be carried out under conventional heating.[142, 143]

In comparison to conventional (convection) heating, microwave heating shows several advantages, including reduced reaction times, improved yields and reduced occurance of side-reactions. However, commercial microwave reactors operate at a frequency of 2.45 GHz, and as a consequence the energy of the waves is too low to cleave bonds. Therefore, unlike UV irradiation, any changes to reaction outcome are not as a result of microwave absorption by reactants. While numerous papers have suggested that the highly effective nature of microwave heating can be attributed to orientation of dipolar molecules and intermediates in line with the dielectric field to alter reaction activation energy,[144] this is a highly controversial theory, and more recent papers have suggested that these non-thermal effects can be replicated by simple convection heating,[145] and by heating in microwave-absorbing silicon carbide vessels.[146]
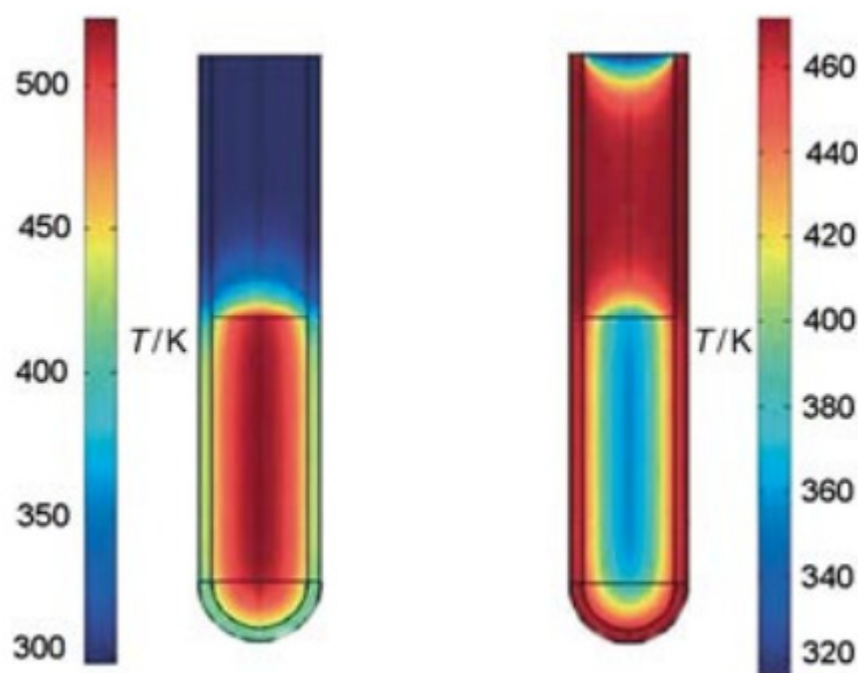
*Figure 25: Thermal image of reaction vessels heated by microwave heating (left) and convection heating (right). Conventional heating leads to rapid heating of the vessel walls, while the reaction mixture is largely unheated. Conversely, the use of microwave-transparent reaction vessels allows rapid bulk-heating by microwaves leading to a heated reaction mixture without vessel heating.[147]*

As a result, most of the effectiveness of the microwave reaction can be attributed to thermal effects. In conventional heating, a heat source such as a hotplate warms the reaction vessel, which then gradually heats the reaction mixture *via* production of convection currents. This is slow, inefficient, and highly dependent on the heat conductivity of the reaction vessel. In comparison, microwave heating operates *via* dielectric heating, with molecules with a dipole moment gaining heat through the absorption of microwave radiation.[144] Use of a microwave-transparent vessel therefore allows simultaneous heating of all parts of the reaction mixture (bulk heating) without heating of the reaction vessel walls (Figure 25). These thermal effects allow efficient heating of strongly microwave-absorbing reaction components, leading to superheating solvents under pressure, and specific heating of heterogeneous catalysts,[144] increasing the overall rate of reaction.

### 1.4.2. Microwave heating in click chemistry

Although the original philosophy of click reactions aimed to produce reactions which

would proceed to completion without the need for intensification, the use of microwave heating in the CuAAC has been widely reported in the literature. In particular, microwave heating is utilised when reaction times are longer than optimal, and is generally considered to reduce reaction times from days to hours, and from hours to minutes.[148]

As with many reactions, microwave heating of click reactions offers advantages of increased yields and reduced side reactions. Additionally, it has been shown that microwave heating, unlike convection heating of the CuAAC, does not lead to production of the 1,5-disubstituted product, even with heating above 100 °C.[149] Use of microwave heating for the CuAAC reaction was first described in 2004,[149] and has since been utilised for a range of starting materials, in particular large, sterically hindered molecules such as peptides, macromolecules, oligonucleotides, carbohydrates,[147] but has also been used for CuAAC reactions involving both aromatic and aliphatic small molecules.[148]

Use of microwave heating for porphyrin-click reactions has numerous advantages; in particular, the positioning of the click moiety directly onto the porphyrin skeleton followed by attempted conjugation to large molecules leads to high steric hindrance, and poor reaction times. For this reason, conjugation of lipophilic porphyrins to large biological molecules using microwave heating has been reported several times in the literature. Synthesis of porphyrin-sugar conjugates[150, 151] with microwave heating showed many advantages, giving significantly reduced reaction times and high yields, while conjugation of lipophilic porphyrins to a triethylene glycol linker chain for conjugation to VEGF-like peptide has also been reported, with short reaction times and essentially quantitative yields.[152] Despite this, use of microwave click for porphyrins is very limited in the literature, and to date no examples of microwave-heated porphyrin-dendron conjugate synthesis, or microwave click conjugation of hydrophilic porphyrins have been reported.

## 1.5. Conclusion

In conclusion, photodynamic therapy represents a highly promising treatment modality for a number of neoplastic and non-neoplastic conditions, particularly if the selectivity and tumour:normal tissue accumulation ratio of photosensitisers can be improved

through the use of biomolecules as targeting moieties. Of the targeting moieties studied to date, antibody fragments represent an attractive target as they combine the highly specific nature of the antibody-antigen interaction and simple bioconjugation methods of antibodies with the increased tumour surface penetration and accumulation of smaller molecules.

Conjugation to reduced interchain disulfide bridges rather than lysine residues on antibody fragments allows greater control of the conjugation ratios of $3^{rd}$ generation photosensitisers, producing well-defined structures highly desirable for clinical PDT. Use of these disulfide bridge binding sites in combination with dendron linker groups to create porphyrin-dendron conjugates allows increased conjugation ratios without the loss of this structural control.

The synthesis of porphyrin-dendron conjugates can be improved significantly by incorporation of the facile, high-yielding CuAAC reaction to allow conjugation between a range of dendrons and $AB_3$ functionalised porphyrins. Microwave heating represents an ideal method to improve yields, reduce reaction times and minimise the formation of by-products during these reactions, particularly the 1,5-disubstituted triazole regioisomer which can be produced as a result of conventional heating of the CuAAC reaction.

## 1.6. Research Aims

The aim of this work is to synthesise and evaluate a range of hydrophilic porphyrin-dendron conjugates, and to conjugate these structures to antibody fragments *via* interchain disulfide bridges. This strategy will allow the synthesis of targeted photosensitisers with improved photosensitiser binding ratios, without loss of the site specificity and precise stoichiometry afforded by use of the disulfide bridge as a binding site.

A range of $AB_3$ type porphyrins bearing a single protected amine functionality and multiple latent water-solubilising functionalities will be synthesised *via* an Alder-Longo mixed condensation reaction. Synthetic pathways will be devised to convert these to water-soluble porphyrins bearing a conjugation handle suitable for use in mild, low-temperature click reactions. Synthesis of a range of PAMAM, tris and aryl ether type

dendrons will be carried out, with all bearing between two and four peripheral click functionalities complementary to those displayed by the synthesised porphyrins.
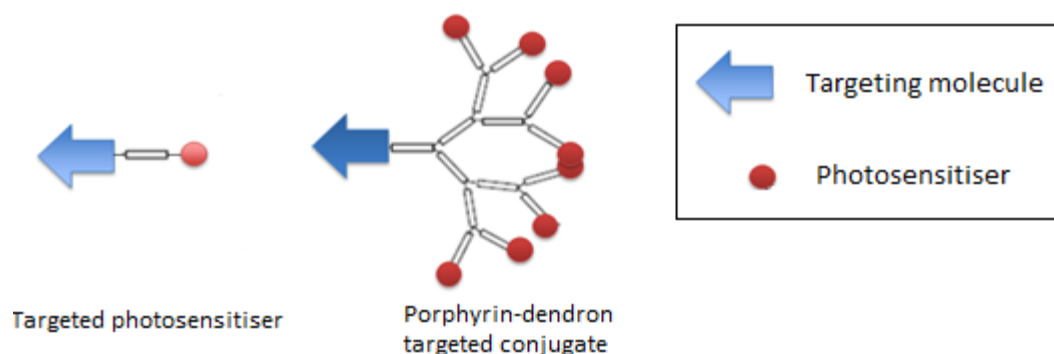


*Figure 26*:  *Representation of the photosensitiser loading of conventional targeted photosensitiser, and the porphyrin-dendron targeted conjugate.*

Click conjugation of both a model lipophilic porphyrin and hydrophilic porphyrins to all synthesised dendrons will be carried out, to produce a range of dendron structures displaying full porphyrin peripheralisation (Figure 26). Evaluation of these porphyrin-dendron conjugates will be carried out to assess photophysical characteristics including fluorescence and UV-vis absorption.

Development of a bioconjugation strategy will be carried out, allowing attachment of single porphyrins and porphyrin-dendron conjugates to interchain disulfide bridges present on Fab fragments *via* a dibromomaleimide functionality. Biological evaluation of the synthesised conjugates will then be conducted in order to assess the structures as targeted photosensitisers.

## 2. Synthesis of "clickable porphyrins"

### 2.1. Introduction

The aim of this section is the synthesis of a range of porphyrins suitable for conjugation *via* the CuAAC click reaction, with the eventual aim of conjugating these porphyrins to dendrons and antibody fragments in order to produce a range of third-generation photosensitisers. In order for the porphyrins to be suitable for this application, they need to fulfil three specific criteria; firstly, they must possess a functional group suitable for conjugation *via* the CuAAC reaction; either an azide or terminal alkyne functionality. Secondly, they must be converted from the free-base porphyrin to the zinc derivative prior to click conjugation to prevent metallation with the copper catalyst during the reaction. Finally, they must be water-soluble, or have the potential to be converted to a hydrophilic derivative through further functionalisation.

### 2.2. Porphyrin synthesis

In order to synthesise a range of porphyrins which fulfil the outlined criteria, functionalisation of the porphine skeleton is required. Although functionalisation of porphyrins with a range of groups can be achieved through both *meso-* or β-substitution (Figure 27), only *meso*-substituted porphyrins will be explored in this work due to their well-characterised nature and comparatively facile synthesis.
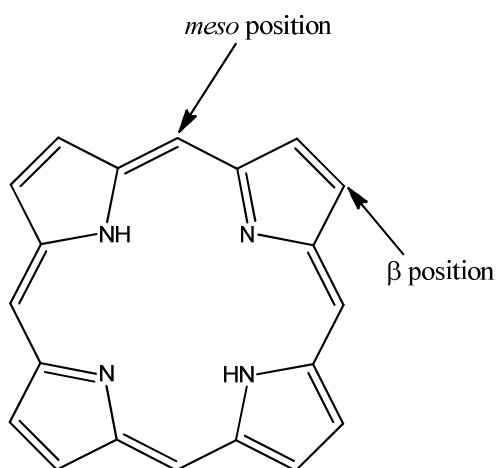


*Figure 27: Porphyrin skeleton showing the location of the meso- and β-positions. The porphyrin skeleton possesses four sites for meso-substitution and eight for β-substitution.*

Synthesis of tetra *meso*-substituted porphyrins is generally carried out by reaction of

pyrrole with aldehydes bearing the desired functionalities (Figure 28) to produce a porphyrinogen ring structure. This structure is then oxidised to produce the desired porphyrin. However, the nature of this synthesis means that the porphyrin produced is only one component of a complex mixture of both linear and cyclic pyrrolic structures, derived from oxidation and termination reactions occurring at each step of the porphyrin synthesis. As a result, yields of even simple porphyrins are relatively poor.
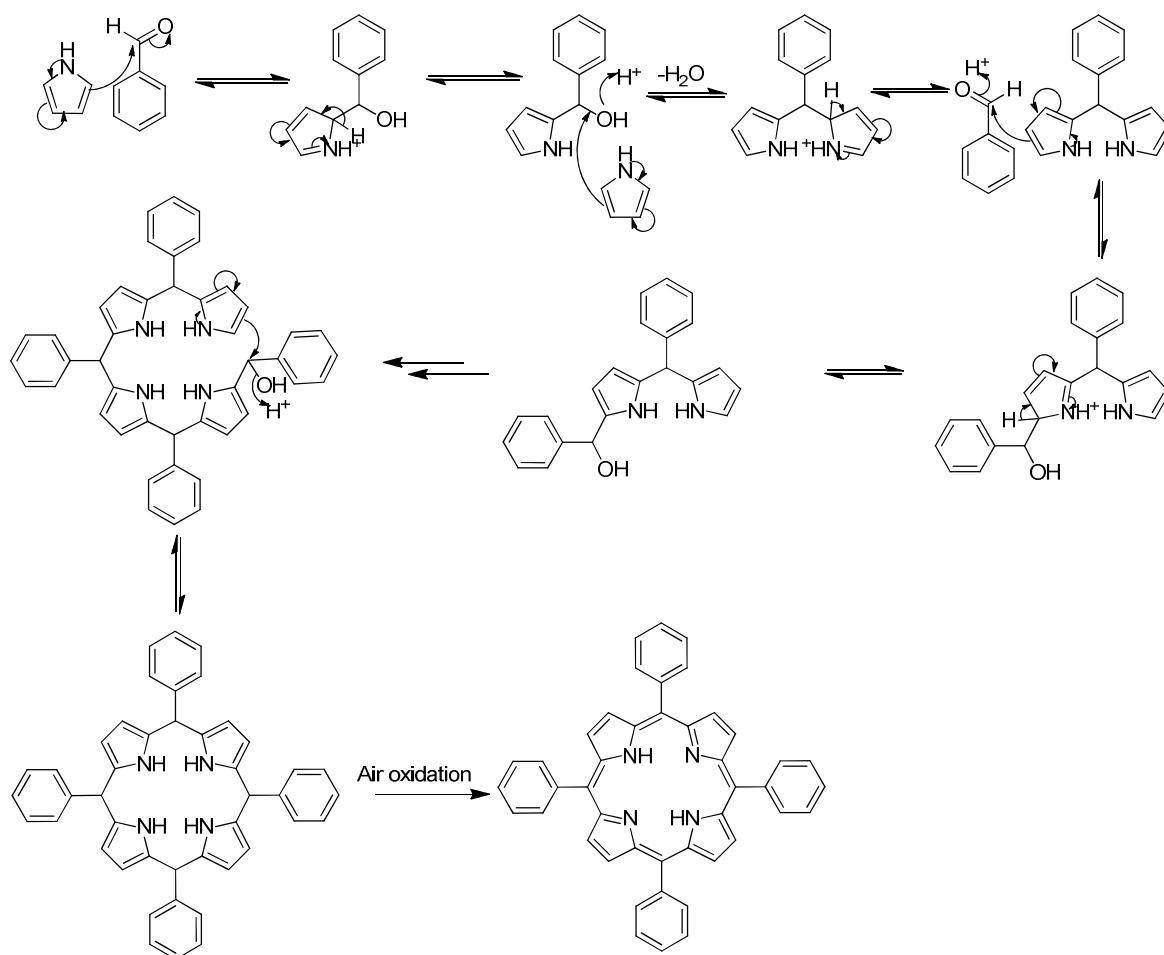


*Figure 28: Synthesis of tetraphenylporphyrin via the Adler-Longo synthesis.*

To date, three major synthetic methods for the production of *meso*-substituted porphyrins have been developed and are widely utilised. The Rothemund reaction (Table 2) was described in 1935,[153] and was the first synthesis of tetraphenylporphyrin to be developed. This method is characterised by the use of high temperature and pressure to increase the yield of the thermally stable porphyrin skeleton in a highly concentrated system. Although several modifications, such as the addition of zinc salts,[154] have been suggested in order to improve reaction yields, the major disadvantage of this method is that it gives very poor yields, while the forcing conditions utilised

limit the scope of the method to a few highly robust aldehydes.

The Adler-Longo method was first developed in the 1967,[155] and is an example of a modified Rothmund synthesis. This method utilises an acidic solvent which also acts as catalyst, and operates at atmospheric pressure, allowing facile air oxidation of porphyrinogen to the desired porphyrin product. As a result, reaction times are considerably shorter, and the milder reaction conditions allow a far greater range of functionalised aldehydes to be utilised. Despite this, the high temperatures and highly acidic conditions mean that use of air-, acid- or thermally-sensitive aldehydes is severely limited.

In contrast, the Lindsey method was developed in 1987[156] as a mild synthesis which allows the use of a wide range of sensitive aldehydes. This method is carried out at room temperature under an inert atmosphere, with addition of a weakly acidic catalyst such as boron trifluoride etherate, and an oxidant such as DDQ. Although use of these extremely mild conditions dramatically increases the scope of porphyrins which can be synthesised, the low reactant concentration required limits the scalability of the reaction.

| Synthesis method | Rothemund | Adler-Longo | Lindsey |
|---|---|---|---|
| Reaction conditions | 150 °C, 24 hours, high pressure | 141 °C, 30 min | RT, 1 hour, under $N_2$ |
| Reaction solvent | Pyridine | Propionic/acetic acid | Dichloromethane |
| Concentration | >3 M | 0.2-0.05 M | $10^{-2}$ M |
| Additives | - | - | DDQ, $BF_3$ |
| Yields | <10% | ~20% | 35-50% |
| Scope | Few highly robust aldehydes can be used | Greater range of aldehydes can be used, must be stable towards oxygen, heat and acid. | Wide range of air-, acid- and heat-sensitive aldehydes can be used. |

Table 2: Outline of the main features of the three most common meso-substituted porphyrin synthetic methods.[156, 157]

Throughout this work, the Adler-Longo method will be used to synthesise all starting porphyrins. Although the Lindsey method has been utilised previously in the literature

for direct synthesis of porphyrins with azide and alkyne functionalities, the Adler method is a well-known and facile reaction, requiring no inert atmosphere or additional reagents. Although the harsh conditions of the Adler-Longo method prevent the use of aldehydes bearing unprotected alkyne or azide functionalities, synthesis and subsequent derivatisation of porphyrins bearing more robust functional groups can be utilised to create porphyrins bearing clickable groups.

## 2.3. Synthesis of asymmetric porphyrin species

In order to synthesise a range of porphyrins with both hydrophilic properties and a click-appropriate moiety, the synthesis of asymmetric porphyrin species is required, allowing the synthesis of a porphyrin bearing one *meso* click functionality and three *meso* water-solubilising functionalities.

Two methods of producing asymmetrically *meso*-substituted porphyrins are widely utilised in the literature. The simplest method is the modification of a symmetrical porphyrin such as TPP at a single *meso* position to produce an asymmetric derivative. While this method of synthesis is still utilised for the synthesis of several porphyrins, in general it is limited by the reactions which can be carried out on the *meso* phenyl groups, and can only be utilised to produce a very limited number of porphyrins. Additionally, while the initial synthesis of a symmetrical porphyrin is relatively high yielding and allows for simpler purification, further modification is generally statistical in nature, leading to poor yields and requiring column chromatography to separate the product from the crude material.

An alternative to this is the direct synthesis of asymmetric porphyrins from two or more aldehyde starting materials, using a mixed aldehyde condensation version of one of the above porphyrin synthesis methods. Mixed condensations utilise a mixture of aldehydes to produce porphyrins bearing more than one type of functional group across the four *meso* positions. Most commonly, a mixture of two aldehydes in a ratio of 3:1 is utilised to create $AB_3$ porphyrins.
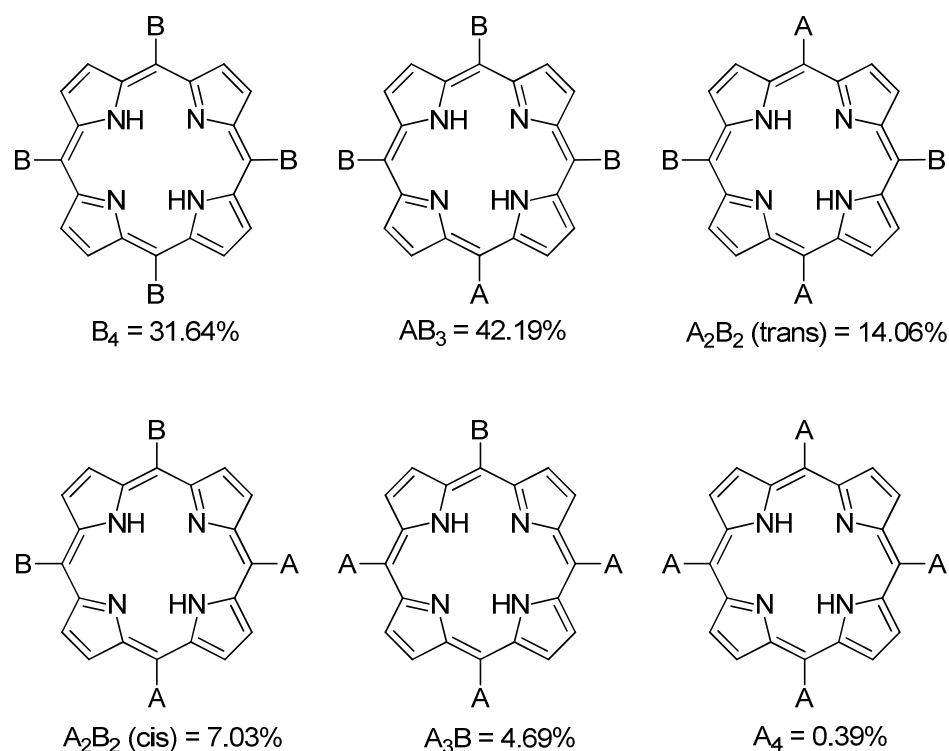
*Figure 29: Theoretical relative abundances of porphyrins produced in a synthesis using a 3:1 ratio of aldehydes A and B. Practically, these abundances vary based on the relative reactivities of the two aldehydes.[158]*

Synthesis of asymmetric porphyrins in this way has numerous advantages; most notably, that synthesis is not limited to functionalisation of a phenyl ring, and can produce a far greater range of functionalities, including modifications to both the A and B positions. Despite this, synthesis is relatively simple and can be carried out without modification to standard porphyrin synthesis conditions.

However, while the synthesis is comparatively facile, the reaction produces a range of six possible porphyrin products (Figure 29). The production of a statistical mixture of porphyrins, in combination with the high yields of other pyrrolic, non-porphyrin products results in the need for column chromatography to purify the reaction mixture, and correspondingly low yields of the desired porphyrin.

## 2.4. Porphyrin click functionalisation

Since the introduction of the CuAAC reaction as the definitive example of a click reaction, the functionalisation of compounds with azide and alkyne groups has seen a meteoric rise in popularity. This is particularly true in the field of porphyrin chemistry which lends itself well to use of the CuAAC reaction for a number of reasons; the

insensitivity to steric hindrance, mild reaction conditions and the flexibility of solvent choice of the CuAAC reaction means that it is ideally suited to the conjugation of bulky and often lipophilic porphyrins to a range of substrates, in particular biomolecules which may be highly water-soluble and sensitive to harsh reaction conditions.

Since the publication of the first porphyrin click paper in 2006,[159] numerous methods have been developed for the attachment of both azide and alkyne functionalities to tetrapyrrolic structures through a number of different strategies (see Dumoulin *et al.* for a comprehensive review[160]).

While strategies do exist for the symmetrical functionalisation of porphyrins with azides[161] and alkynes,[162, 163] functionalisation in this way has several disadvantages; the symmetrical structures limit the opportunities for water-solubilisation of the porphyrin, and selective click functionalisation of just one azide or alkyne group is difficult due to the high yielding nature of click reaction. As a result, a number of strategies exist for mono-functionalisation to produce both alkyne and azide porphyrins.

Alkyne porphyrins were the first click-functionalised porphyrins to be developed due to their utility in a range of other coupling reactions, and are far more prevalent in the literature as a result. Synthesis of asymmetrical alkyne porphyrins has been carried out in three ways in the literature; addition of an alkyne linker chain to a previously functionalised porphyrin, with commercially available linkers such as propargyl bromide,[164] propargyl alcohol,[165] and propargylic acid[166] most commonly used; synthesis directly from alkyne-functionalised aldehydes; or addition of acetylene to halide-functionalised porphyrins *via* Sonogashira couplings.[167]

Although the synthesis of azide-functionalised porphyrins is less common, several synthetic methods are still found in the literature. Direct nucleophilic substitution of alkyl halide functionalised porphyrins with sodium azide can be carried out at high temperatures,[161] while the use of a strong base allows decoration of alcohol porphyrins with azide linker chains.[168] However, in general functionalisation of porphyrins with azide groups is carried out through the transformation of an aryl amine to an azide *via* one of two methods. The most commonly used of these methods is diazotization, which utilises sodium nitrite in an acidic solvent to form a diazonium salt intermediate, followed by immediate reaction with sodium azide. This method has been shown to be

rapid and effective, and can been carried out either directly on the *meso* position[169] of a porphyrin, or on an aryl amine attached to this position.[151] However, the use of an acidic solvent creates harsh reaction conditions which are unsuitable for some functionalities, and the reaction must be maintained below 5 ˚C in order to avoid degradation of the diazonium salt intermediate.

An alternative to the diazotization method is the use of a diazotransfer reagents such as azidotrimethylsilane.[170] While this is a less commonly utilised method, it allows for use of milder reaction conditions, and previously a reported example showed both similar yields and greater ease of use in comparison to the diazotization of the same porphyrin.

While the numerous methods for click functionalisation of porphyrins allow for a high degree of flexibility in the design of a synthetic strategy, regardless of the method of functionalisation, the metallation of the free-base porphyrin prior to undergoing the click reaction is an essential step. Metallation in this way prevents insertion of the copper catalyst into the free-base porphyrin cavity, which requires forcing conditions to remove, and necessitates the use of an excess of the catalyst. While other metals can be used, metallation with zinc is ubiquitous due to ease of both insertion and removal. Additionally, the $d^{10}$ $Zn^{2+}$ ion allows the porphyrin to retain fluorescent properties and singlet oxygen yields, and facile NMR analysis is still possible on the resulting product.

## 2.5. Azide-functionalised porphyrins

### 2.5.1. Synthesis of lipophilic porphyrins

#### 2.5.1.1. *Synthesis of a lipophilic azido porphyrin*

The initial aim of this work was the synthesis of a simple, lipophilic azide porphyrin molecule for use as a model in further reactions. Use of a model porphyrin in this way allows the optimisation of reactions without the use of large quantities of water soluble porphyrins, which are complex and time consuming to produce. In order to act as a good model porphyrin, several attributes are desirable; the synthesis of the porphyrin must be rapid, facile, and easily scalable, the structure of the porphyrin must allow for facile column chromatography on normal-phase silica, and the porphyrin must show good reactivity in a simple click reaction.

The first model azide porphyrin synthesised was **5**, a functionalised tetraphenylporphyrin (TPP) bearing an aryl azide, which possesses several advantages as a synthetic target. Firstly, the product can be synthesised *via* asymmetric functionalisation of TPP, removing the need for a more complex and lower yielding mixed condensation synthesis. Secondly, the proposed synthetic pathway utilises facile transformations, and has been previously utilised in the literature to synthesise this molecule.[151]
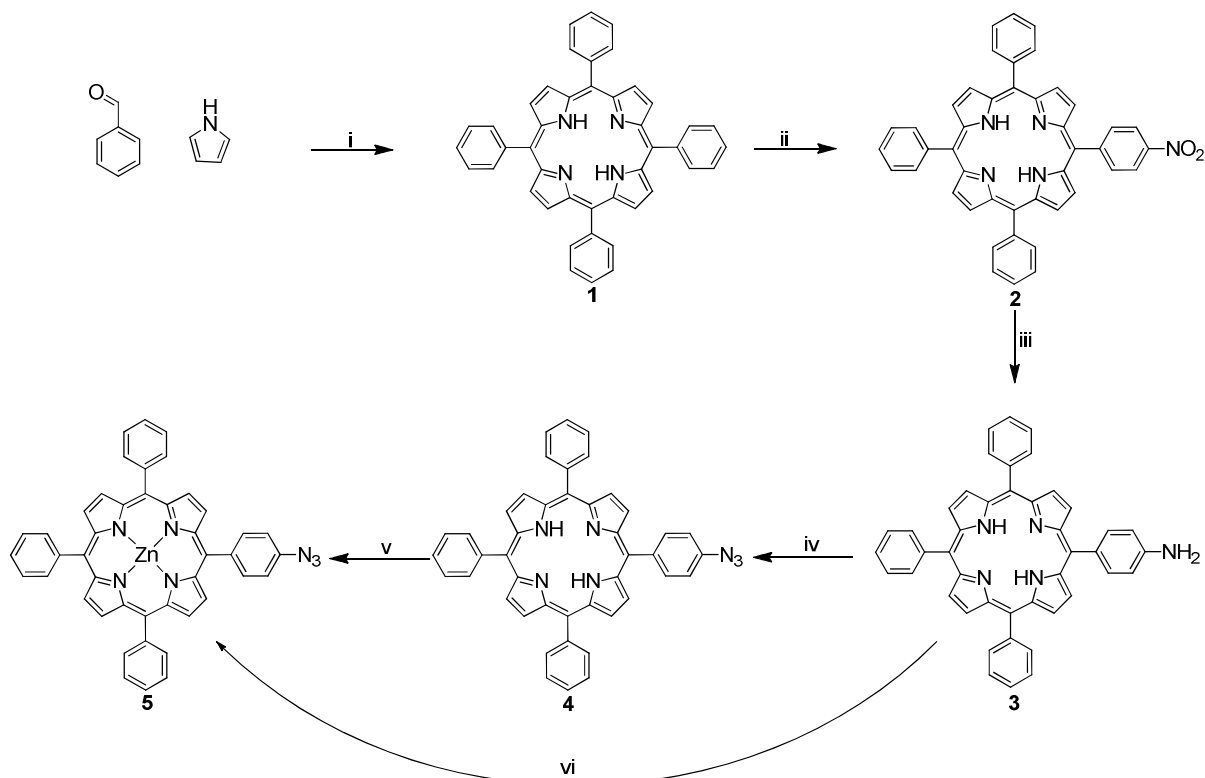


*Scheme 1: Reagents and conditions: (i) propionic acid, reflux, 3 hours, (ii) nitronium tetrafluoroborate in sulfolane, rt, 8 hours, (iii) tin (II) chloride, HCl$_{(aq)}$, 60 °C, 1 hour, (iv) sodium nitrite, sodium azide, 0 °C, 1 hour, (v) zinc acetate, rt, 1 hour, (vi) imidazole-1-sulfonyl azide hydrogensulfate, zinc acetate, triethylamine, rt, 17 hours.*

Synthesis of **1** was carried out under conventional Adler-Longo synthesis conditions to obtain the product in a 32% yield, comparable with that observed in the literature. Conventionally, synthesis of **2** is carried out under highly acidic conditions with sodium nitrite as the nitrating agent, to rapidly produce a statistical mixture of mono-, di-, tri- and tetra-nitrated derivatives. Although careful control of reaction duration can lead to preferential formation of the mono-nitrated product, a mixture is always produced and yields suffer as a result.

An alternative synthesis as described by Smith *et al.*[171] utilises the milder nitrating agent, nitronium tetrafluoroborate in sulfolane to nitrate in a highly selective manner, with the authors reporting near-quantitative yields of the mono-nitrated product and no evidence of multiply-nitrated products. Using this method produced **2** in 81% yield, with the remainder of the mixture being unreacted TPP and di-nitrated porphyrins. This yield is somewhat lower than the literature value of 94%,[171] with the loss of yield attributed to the need to add significantly larger quantities of nitronium tetrafluoroborate than suggested in the literature in order to force the reaction to completion. However, this yield is still significantly higher than that achieved under conventional conditions[172] and this method also offers advantages of increased ease of synthesis and purification.

Synthesis of porphyrins **3**-**5** was initially carried out utilising the method developed by Severac *et al.*[169] At each stage, the yield of the desired porphyrin was greater than 90%, with porphyrin identity confirmed by NMR, MS and UV-Vis. Synthesis of **5** was also attempted directly from **3** utilising the diazotransfer reagent imidazole-1-sulfonyl azide hydrogensulfonate (ISA). This reagent was developed as a safe alternative to trifluoromethanesulfonyl azide,[173, 174] and can be utilised to transform both aromatic and aliphatic amines to azides under mild conditions. Although this reagent is commonly utilised with a Cu (II) catalyst, use of other metal salts including zinc has been trialled.

Utilising an excess of the zinc (II) catalyst allowed a one-pot metallation and conversion of the amine to the azide group to produce **5** in 93% yield, higher than yields reported for this reaction on other aromatic molecules.[173] Analysis of the product by MS, NMR and UV-vis confirmed this product to be identical to previous batches of **5**. For the synthesis of **5** there is no clear advantage for either method; diazotization utilises cheaper reagents and is faster, while the diazotransfer method is simpler, milder and is a one-step process. However, for acid-sensitive porphyrins, use of the diazotransfer reagent represents a mild and facile method of azide synthesis.

The second model porphyrin synthesised was **8**, a lipophilic porphyrin bearing three phenyl groups similar to **5**, and a benzylic azide functionality. While the synthesis of this porphyrin is unknown in the literature, it also offers several advantages as a model porphyrin. Firstly, the reaction scheme is shorter than that of **5**, and does not include the time-consuming statistical modification of TPP, allowing more rapid production of large quantities of the porphyrin. Secondly, unlike the synthesis of **5**, the synthesis of **8** uses

relatively mild reaction conditions, and would be more suitable for a wider range of functionalities. Finally, the use of a benzylic azide may reduce any steric hindrance occurring in an aryl azide porphyrin.
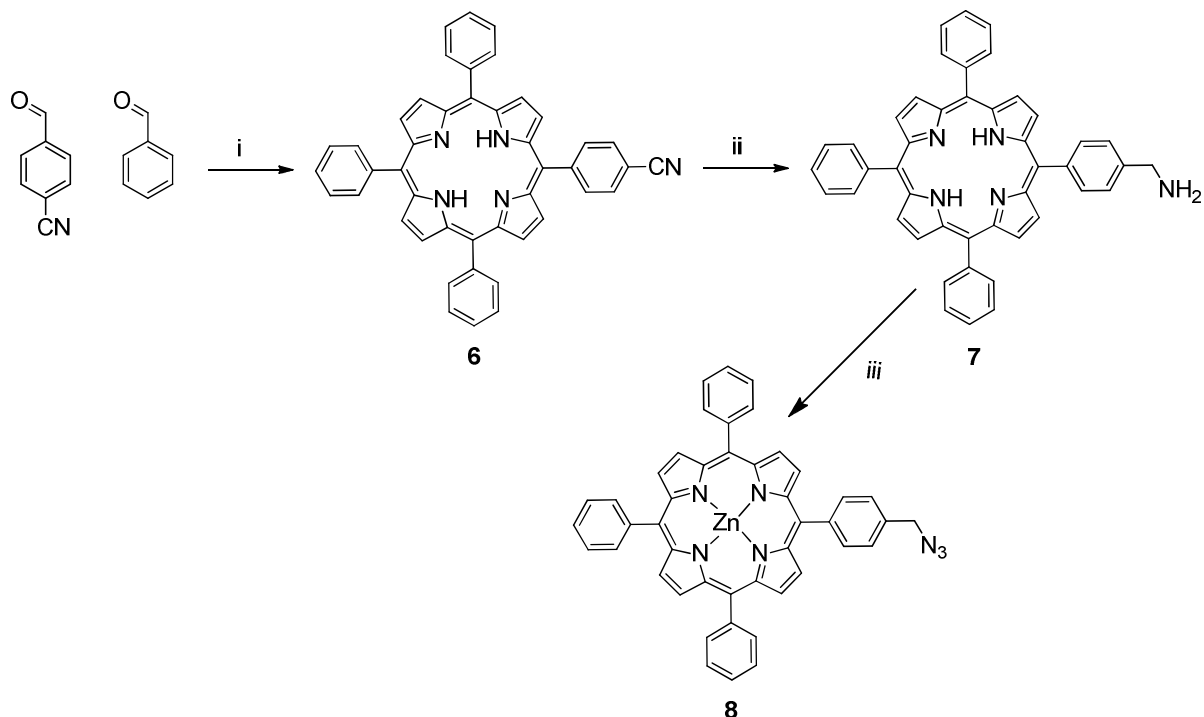


*Scheme 2: Reagents and conditions: (i) propionic acid, reflux, 3 hours, (ii) LiAlH$_4$, rt, 17 hours, (iii) imidazole-1-sulfonyl azide hydrogensulfate, zinc acetate, triethylamine, rt, 3 hours.*

Synthesis of **6** was carried out *via* the Adler-Longo crossed condensation of benzaldehyde and 4-cyanobenzaldehyde to give the desired product in a yield of 10%, which while low for many reactions, is excellent for a porphyrin synthesis of this type. Reduction of the nitrile functionality was then carried out utilising lithium aluminium hydride, with the product obtained in a relatively poor yield of 67%. While the reaction showed excellent yields by TLC, the loss of product at this stage was due to the formation of a large quantity of a sticky precipitate upon quenching of the LiAlH$_4$. Large quantities of porphyrin were trapped inside this precipitate, and although filtration and multiple washing steps were attempted, a large percentage of the porphyrin remained unrecovered at this stage.

The conversion of the benzyl amine **7** to the azide product **8** was again carried out utilising the diazo-transfer reagent ISA. Due to the highly reactive nature of the benzylic amine, the reaction proceeded rapidly to give the product in excellent yield (92%), with

the identity of the product confirmed by NMR, MS and UV-vis.

The reactivity of porphyrins **5** and **8** was then examined utilising a model click reaction with the alkyne, phenylacetylene. Under microwave irradiation, both model porphyrins showed very high yields of the target compound (> 90%), with no formation of by-products observed. However, the reaction time observed for **8** was considerably longer than that observed for **5**, taking 3 hours to reach completion by TLC rather than the 1 hour observed for **5** under identical conditions. As the synthesis of both porphyrins was facile, with no large advantage in yield in either case, porphyrin **5** was selected as the model porphyrin for use throughout the remainder of this work due to its greater reactivity in the click reaction.

## 2.5.1.2.    *Synthesis of a range of lipophilic porphyrins*

As the use of a diazo-transfer reagent was shown to be highly effective in the synthesis of azide porphyrins from substituted tetraphenylporphyrin species, this method was then utilised to synthesise a range of other lipophilic porphyrins. While these lipophilic porphyrins do not fulfil the water-solubility criteria outlined for use in third generation photosensitisers, all of the synthesised lipophilic porphyrins possessed reactive functionalities which could be utilised to engender water solubility to these porphyrins either before or after a click reaction. Examples of reactions of this type include saponification of ester groups,[175] reaction of amines, alcohols or thiols with pentafluoro porphyrins,[176] cleavage of ethers to form alcohols,[177] and use of palladium-catalysed coupling reactions on halide-functionalised porphyrins.[178]

Initially, a range of AB$_3$ porphyrins was synthesised, all bearing a single *p*-acetamidophenyl group acting as a protected aryl amine, and three substituted phenyl rings bearing functionalities which could be modified through a range of well-known reactions, as outlined above. Synthesis of acetamido porphyrins **9a-g** was carried out successfully *via* the Adler-Longo reaction, with all yields in the acceptable range for an asymmetrical porphyrin synthesis of this type.
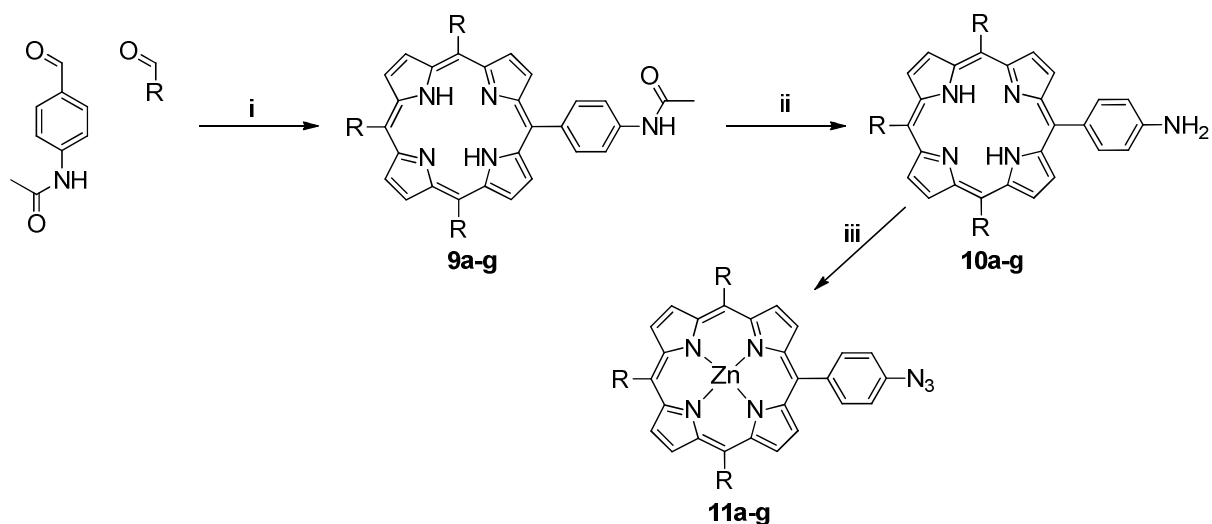
*Scheme 3: Reagents and conditions: (i) propionic acid, reflux (ii) HCl$_{(aq)}$, reflux (iii) imidazole-1-sulfonyl azide hydrogensulfate, zinc acetate, triethylamine, rt, 17 hours.*

| Structure | R= | Yield 9 (%) | Yield 10 (%) | Yield 11 (%) |
|-----------|-----|-------------|--------------|--------------|
| a |  | 7.9 | 98.9 | 86.4 |
| b |  | 6.6 | 96.1 | 91.4 |
| c |  | 7.8 | 94.7 | 80.3 |
| d |  | 4.7 | 90.1 | 87.6 |
| e |  | 7.7 | 66.6 | 83.9 |
| f |  | 8.8 | 63.9 | 84.9 |
| g |  | 6.0 | 60.3 | 88.4 |

*Table 3: Structures and yields of porphyrins **9a-g** to **11a-g**.*

Hydrolysis of the acetamido group to form the free amine was carried out in a mixture of HCl$_{(aq)}$ and TFA due to the poor solubility of the lipophilic porphyrins in HCl$_{(aq)}$ alone. Compounds **10a-g** were produced successfully, with yields ranging from acceptable to excellent. The reduced yields of **10e** and **10f** were attributed to the poor

solubility of these porphyrins in DCM, with some of the product lost during an aqueous washing step. The poor yield of **10g** is consistent with that found in the literature,[179] and is as a result of the degradation of this light-sensitive porphyrin on silica during column chromatography purification.

The conversion of porphyrins **10a-g** to porphyrins **11a-g** was carried out with ISA and a zinc catalyst, utilising the method developed for the synthesis of **5**. In all cases, synthesis of the product was complete after 24 hours at RT, with all products generated in excellent yields (Table 3). The identity of all porphyrins was confirmed by NMR and MS, and the presence of the zinc confirmed by UV-vis. The click reactivity of this range of porphyrins was tested with small scale microwave click reactions using phenylacetylene yielding the desired product in all cases, as confirmed by MS.

### 2.5.2.   Synthesis of hydrophilic porphyrins

While the synthesis of a range of lipophilic porphyrins bearing latent water-solubilising functionalities allows for creation of lipophilic conjugates and subsequent derivatisation to engender water solubility, this synthetic method does have limitations. Firstly, click reactions incorporating lipophilic porphyrins require the use of organic solvents and highly forcing conditions, which makes conjugation to sensitive and water-soluble biomolecules difficult. Secondly, many reactions which could be utilised to functionalise these porphyrins require highly forcing conditions which may not be suitable for the attached biomolecule or dendron. Thirdly, many of these derivatisation reactions do not reach completion, and will generate large numbers of by-products when utilised on porphyrin-dendron conjugates, making purification and characterisation difficult.

For these reasons, the synthesis of a range of porphyrins which exhibit water-solubility at the point of click conjugation was attempted. A range of water solubilisation techniques for tetrapyrrolic structures exist in the literature, and can be subdivided based on whether the resulting hydrophilic porphyrin is anionic, cationic or uncharged. In order to produce a wide range of water-soluble porphyrin molecules, the synthesis of hydrophilic porphyrins *via* all three water-solubilisation routes was attempted.

### 2.5.2.1. Synthesis of a sulfonated porphyrin

While several methods exist for the synthesis of anionic porphyrins, including the addition of carboxylic acid and phosphate groups to the porphyrin, sulfonation is a facile and widely-utilised method of engendering water solubility in porphyrins, and particularly for the highly lipophilic phthalocyanines. Although the reaction conditions for sulfonation are relatively harsh (heating for extended periods of time in concentrated sulfuric acid), this method is favoured in the literature as sulfonation of highly hydrophobic, but simple to synthesise substituted tetraphenylporphyrin derivatives allows the cheap and rapid synthesis of water-soluble porphyrins without the need for mixed condensation synthetic methods.
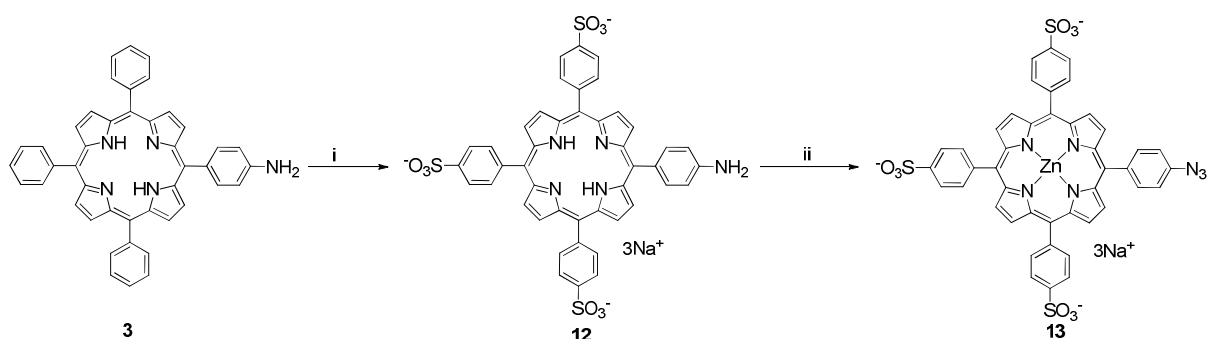


Scheme 4: Reagents and conditions: (i) sulfuric acid, 110 ˚C, 48 hours, (ii) imidazole-1-sulfonyl azide hydrogensulfate, zinc acetate, triethylamine, rt, 17 hours.

Although sulfonation of the triphenyl azide functionalised porphyrin **4** is theoretically possible, this method was not attempted for several reasons. Firstly, examples of aryl azide functionalities decomposing in the presence of strong acids are noted in the literature,[180] and any decomposition of the azide functionality during sulfonation would lead to the synthesis of by-products which would be difficult to remove. Secondly, while the synthesis of sulfonated porphyrins is very simple, subsequent neutralisation and separation of most sulfonated porphyrins from the sulfuric acid and water-soluble sulfate salts produced is difficult. While numerous complex workup procedures have been developed in an attempt to solve this problem,[181-183] often the only practical solution to obtain a pure sample of the porphyrin is dialysis, which is time-consuming and expensive.

Use of starting material **3** provides a simple solution to both of these problems; the amine functionality is extremely stable, and resistant to the forcing conditions required

for sulfonation. Additionally, the sulfonation of **3** also protonates the amine group, leading to the production of a water-insoluble zwitterionic structure. This fact has been utilised in literature synthesis of the trisulfonated amino porphyrin (TPPS-NH$_2$)[184] to create a facile workup procedure for this porphyrin, with removal of the zwitterion from an aqueous solution by filtration, and subsequent neutralisation to generate a water-soluble species.

Synthesis of **12** was carried out according to a literature synthesis and workup[184] to generate the ammonium salt in high yield. However, it was found that after isolation of the sample, rapid loss of ammonia occurred to regenerate the poorly soluble protonated derivative, making further reaction and characterisation difficult. For this reason, cation substitution was attempted in order to generate the more stable sodium salt. Synthesis of the sodium salt was initially trialled utilising a cation exchange resin, and while the sodium salt was successfully generated, transfer of impurities from the resin to the product resulted in poor characterisation and a highly impure product. As a result, cation exchange was carried out using triethylamine in water to generate an organic-soluble porphyrin, followed by subsequent generation of the sodium salt utilising sodium iodide in acetone.

Synthesis of azide porphyrin **13** was then carried out utilising ISA as described previously, with the use of methanol as a solvent. The reaction proceeded rapidly to generate the desired product, and the crude was purified by reverse phase column chromatography to generate the pure product in excellent yield, with the identity of this porphyrin confirmed by NMR and MS.

### *2.5.2.2.   Synthesis of a cationic azido porphyrin*

The synthesis of cationic derivatives is an alternative method of water-solubilisation of porphyrins, which generally involves the synthesis of porphyrins bearing *meso* pyridyl functionalities, followed by methylation to generate the quaternary pyridinium salt. Although synthesis and purification of these porphyrins is often more complex than the sulfonation of meso phenyl porphyrins to yield anionic derivatives, the high dark toxicity of sulfonated porphyrins limits their use as therapeutic agents,[185] and as a result the use of cationic porphyrins has gained popularity.

In order to create a cationic, water-soluble porphyrin, a 3:1 mixed condensation of 4-pyridinecarboxaldehyde and 4-acetamidobenzaldehyde was utilised to create an $AB_3$ porphyrin bearing three pyridyl groups and an *p*-acetamidophenyl functionality.
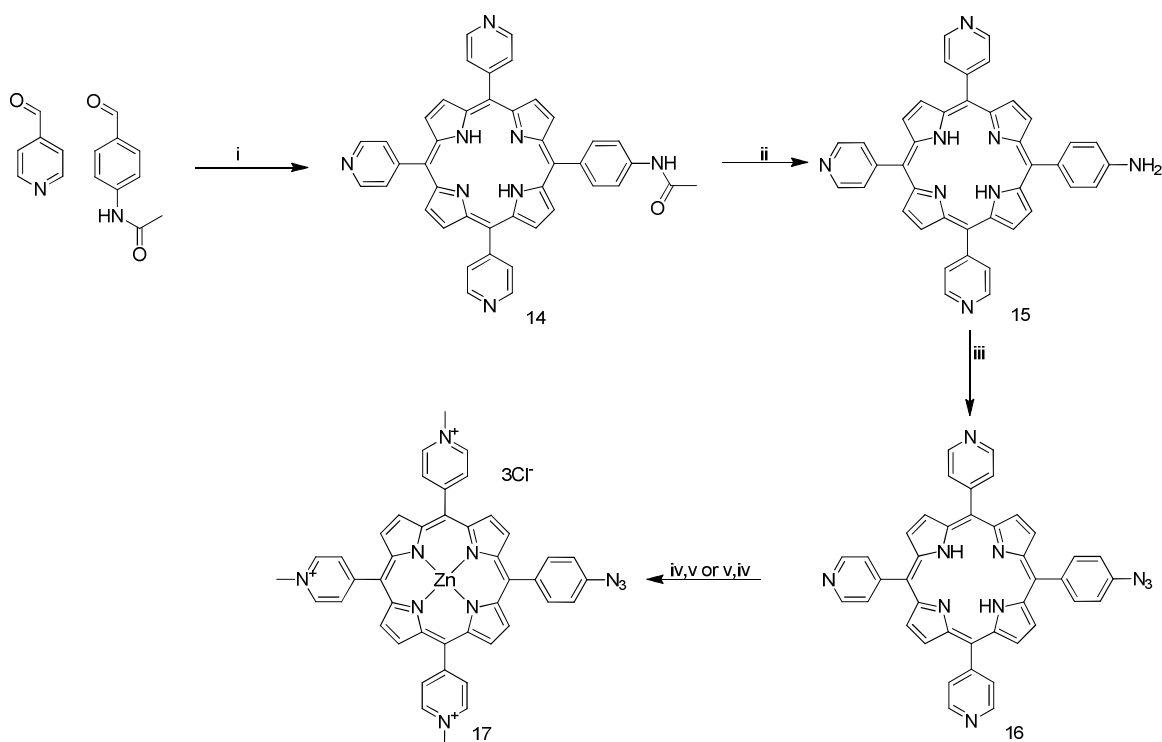


*Scheme 5: Reagents and conditions: (i) pyrrole, propionic acid, reflux, 2 hours, (ii) hydrochloric acid$_{(aq)}$, reflux, 3 hours, (iii) sodium nitrite, sodium azide, 0 °C, 1 hour, (iv) zinc acetate, rt, 1 hour, (v) methyl iodide, 40 °C, 17 hours.*

The synthesis of **14** was carried out according to a literature procedure[177] to obtain the *p*-acetamidophenyl-functionalised tri-pyridyl porphyrin (**14**) in 5.5% yield, with the product confirmed by NMR. The yield obtained is somewhat higher than the literature value of 3.9%,[177] which can be explained by the incorporation of a sodium bicarbonate washing step in the workup procedure. This process removes any residual propionic acid, and can increase yields by neutralising any acidified porphyrins, which can otherwise be lost during column chromatography.

Conversion of the acetamido functionality to an amine was carried out in refluxing $HCl_{(aq)}$ to give **15** in 90% yield, with the NMR showing complete loss of the acetamido functional group. Subsequent conversion of the amine to the azide was carried out *via* the diazotization method to yield **16** in a 96% yield, comparable with the yields obtained for **4**.

As the conversion of **16** to **17** is a two-step process, involving the methylation of pyridyl groups to engender hydrophilicity and metallation of the porphyrin core, there were two possible ways in which the synthesis could proceed. Initially, metallation of the neutral porphyrin was attempted first as this allowed synthesis and workup to be carried out as for metallation of **5**. The porphyrin was reacted with zinc (II) acetate in a DCM:methanol solvent mixture, with the reaction monitored by the appearance of a green spot on the TLC, at a lower $R_f$ than the free-base porphyrin. Although the workup proceeded as expected, subsequent attempts to dissolve the porphyrin in any solvent were unsuccessful, preventing further characterisation.

The formation of an insoluble porphyrin species can be attributed to the polymerisation of the desired product *via* the zinc centre (Figure 30). The formation of such pyridyl porphyrin polymers is described in the literature,[186, 187] and is as a result of the arrangement of the four nitrogen atoms in the porphyrin core. Although zinc can exist as either a four- or six-coordinate structure, it preferentially adopts a four-coordinate tetrahedral conformation. However, the four core amines in the porphyrin are locked into a square planar configuration and when acting as ligands for the zinc core leave two metal orbitals available for bonding. As a result, the pyridyl nitrogen lone pair from another porphyrin molecule can act as a fifth ligand for the zinc centre, leading to the formation of dimeric, oligomeric and polymeric porphyrin species.[186]
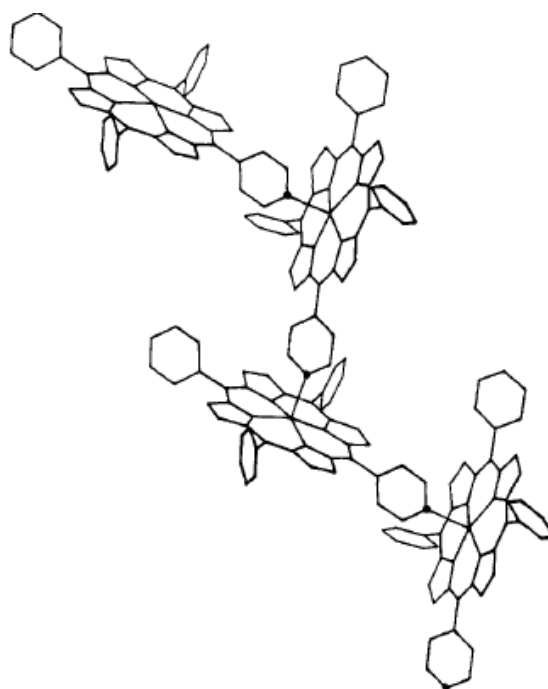
*Figure 30: Schematic showing the polymeric structure formed between zinc pyridyl porphyrins.[187] The formation of five-coordinate zinc species over octahedral six-coordinate species can be attributed to steric hindrance rather than preference for the five-coordinate geometry.*

As the metallated porphyrin species was too insoluble to allow methylation of the pyridyl rings, synthesis of **17** was then attempted *via* methylation followed by metallation. Methylation of **16** was carried out with methyl iodide in DMF, and the product was obtained in 91% yield, with complete methylation confirmed by NMR and MS. Metallation with zinc (II) acetate was then carried out in water, utilising a ammonium hexafluorophosphate/TBAC workup[188] to remove all non-porphyrin by-products and exchange the iodide counter-anions for chloride ions. The metallation was carried out in a 90% yield, with the insertion of the zinc confirmed by MS and characteristic changes to the UV-vis spectrum.

Synthesis of **17** was also attempted utilising diazo transfer reagent ISA in order to compare the practicality of the two methods. As a previous synthesis of **17** had demonstrated that metallation of the pyridyl porphyrin before methylation led to formation of an insoluble mixture, formation of the zinc azide was carried out on the cationic porphyrin.
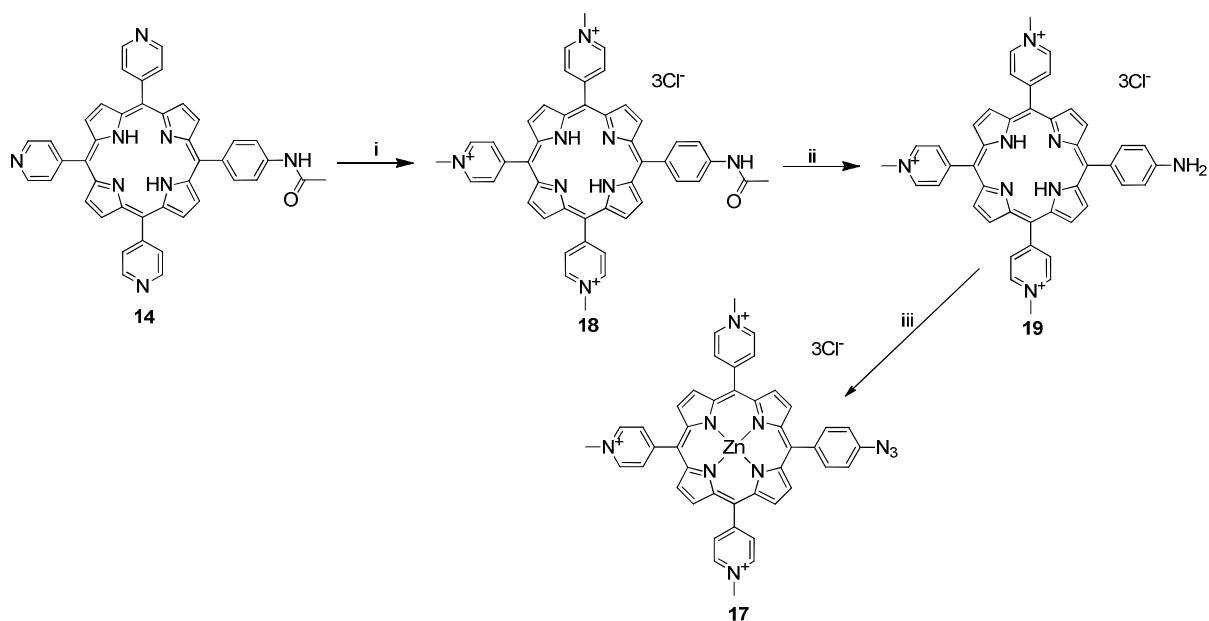
*Scheme 6: Reagents and conditions: (i) methyl iodide, 40 °C, 17 hours. (ii) hydrochloric acid, reflux, 3 hours, (iii) imidazole-1-sulfonyl azide hydrogensulfate, zinc acetate, triethylamine, rt, 17 hours.*

Synthesis of **19** through the reaction of porphyrin **15** with methyl iodide would lead to methylation of the primary amine in preference to the pyridyl groups, yielding the unreactive quaternary amine, and as a result methylation of porphyrins bearing protected amines has been carried out in the literature.[189] For this reason, methylation was carried out on the protected amino porphyrin **14** using methyl iodide, followed by formation of the chloride salt through an ammonium hexafluorophosphate/TBAC workup, with the desired product obtained in excellent yield. Deprotection of the acetamido functionality to produce **19** was carried out in refluxing hydrochloric acid, with NMR showing complete conversion to the amine derivative after 3 hours, with no formation of by-products.

Finally, the diazotization reaction was carried out as previously described for **13**. However, in contrast to the synthesis of the anionic azide, synthesis of **17** utilising this method was very slow, taking 48 hours for the reaction to reach completion. In addition, a number of by-products and a significant proportion of unreacted starting material were observed, leading to lengthy purification by reverse phase column chromatography and a moderate (67%) yield. The poor yield and long reaction time can be attributed to the poor reactivity of the aryl amine in this electron deficient system. Due to the poor yield of the final step of this synthetic method, and the difficulty of purifying the cationic

product, production of the azide *via* diazotization was selected as the preferred method for synthesis of **17**.

### 2.5.2.3.  *Synthesis of a cationic porphyrin bearing a linker chain*

Although the diazotization of an aromatic amine functionalised porphyrin represents a facile method of attaching an azide functionality to a porphyrin, it can only be utilised to introduce an azide directly on to the porphyrin structure. Synthesis of a porphyrin bearing an azide conjugated *via* a linker group allows control of the distance between the porphyrin and the attached dendron. This can then be utilised to reduce problems arising from the close proximity of two or more porphyrins, including steric effects and quenching. For this reason, synthesis of an amine-reactive porphyrin, and subsequent conjugation to an amine-functionalised linker chain was attempted.
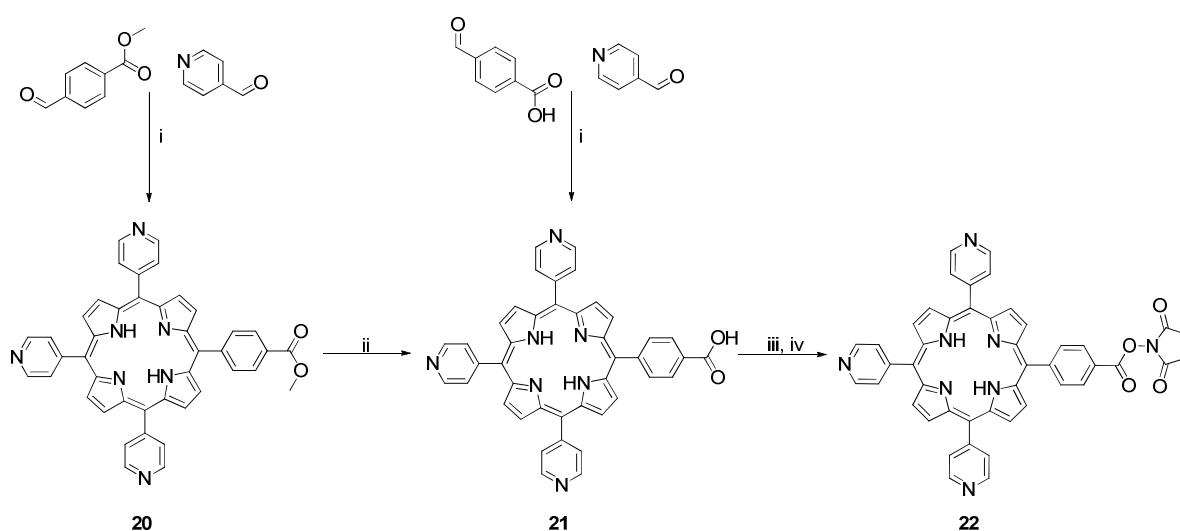


*Scheme 7: Reagents and conditions: (i) pyrrole, propionic acid, reflux, 2 hours, (ii) lithium hydroxide, 50 °C, 24 hours, (iii) thionyl chloride, 50 °C, 30 min, (iv) N-hydroxysuccinimide, 50 °C, 3 hours.*

Synthesis of **21** was initially attempted by a mixed aldehyde condensation utilising a 3:1 ratio of 4-pyridinecarboxaldehyde and 4-carboxybenzaldehyde to form the AB$_3$ porphyrin bearing a single carboxylic acid moiety.  While yields comparable to literature values were obtained,[190] repeated synthesis of this porphyrin highlighted that this figure was highly variable, with yields of less than 1% obtained several times. This problem is likely to be as a result of the reactivity of 4-carboxybenzaldehyde; large yields of TPyP during these syntheses indicate that this aldehyde may be poorly

reactive, and the presence of a carboxylic acid on the porphyrin means that a basic workup cannot be utilised to neutralise acidified porphyrins before column chromatography, leading to additional loss of product.

For this reason, synthesis of **20** from 4-pyridinecarboxaldehyde and methyl 4-formylbenzoate was attempted utilising a literature method.[191] The AB$_3$ porphyrin bearing a methyl ester instead of the carboxylic acid was synthesised in yields of 5.3%, consistent with those observed in the literature. Saponification of the ester was then carried out with potassium hydroxide in good yield (76%), with NMR confirming complete saponification after 24 hours. While the overall yield from these two steps was comparable with the direct synthesis of **21**, importantly this method was highly reproducible, with consistent yields obtained upon repeated synthesis.

Synthesis of **22** was carried out in a two-step, one-pot reaction. Initially, porphyrin **21** was reacted with thionyl chloride to yield the acid chloride porphyrin, with subsequent addition of *N*-hydroxysuccinimide to produce the desired porphyrin in excellent yield (92%), comparable to those obtained in the literature.[190]

Synthesis and subsequent conjugation of a linker chain bearing an amine and an azide functionality was then attempted, utilising a PEG linker chain as the starting material in order to allow increased amphiphilic character of the final product. While bifunctional derivatives of heptaethyleneglycol and longer chains bearing the required amine and azide functionalities are available commercially, the prohibitive cost of these linker chains means they are unsuitable for large scale synthesis. For this reason, synthesis of the linker chain was attempted from functionalised triethylene glycol derivatives, which allow synthesis of a linker chain of a reasonable size without high cost. Initially, synthesis of the linker chain was attempted from an asymmetrical triethylene glycol derivative 2-(2-(2-chloroethoxy)ethoxy)ethanol *via* a four-step synthetic pathway.
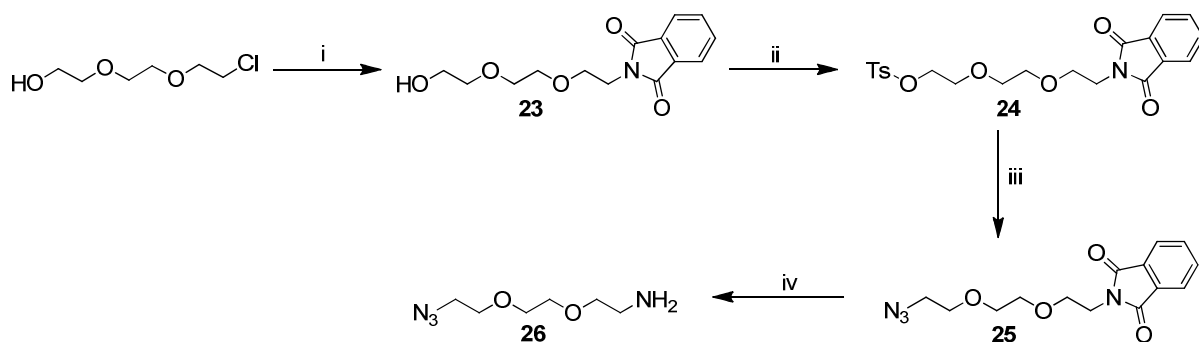
*Scheme 8: Reagents and conditions: (i) potassium phthalimide, 100 °C, 17 hours, (ii) tosyl chloride, triethylamine, rt, 17 hours, (iii) sodium azide, reflux, 48 hours, (iv) hydrazine monohydrate, 25 °C, 17 hours.*

Synthesis of **23** was carried out using the Gabriel synthesis according to a literature method,[192] using substitution of the chloride group for a phthalimide moiety to obtain **23** in near-quantitative yields, with the identity of the product confirmed by NMR and MS. Conversion of the alcohol to a sulfonate ester was then carried out *via* addition of tosyl chloride, producing **24** in a 61% yield, comparable with yields seen in the literature.[193] Synthesis of **25** was then carried out *via* nucleophilic substitution of the tosyl group utilising sodium azide, achieving a moderate yield of 71%. This value is lower than values observed in the literature,[192] which can be attributed to loss of some product during aqueous workup to remove any unreacted sodium azide. As **25** is relatively hydrophilic, some loss of the product into the water washings is inevitable; however this workup procedure was deemed necessary due to the high toxicity and potential shock sensitivity of the residual sodium azide in the crude reaction product.

Finally, conversion of the phthalimide group to a primary amine was carried out with hydrazine monohydrate according to the Ing-Manske modification of the Gabriel synthesis, to obtain **26** in 55% yield. Again, the poor yield of this step can be attributed to loss of the amphiphilic chain during aqueous workup, however attempted purification of this product *via* column chromatography led to similarly poor yields due to adhesion of the highly polar chain to the silica.

Losses of product at each synthetic step mean that the overall yield of this synthesis is just 24%, which coupled with the long reaction times means that synthesis of gram quantities of **26** *via* this method is time-consuming and expensive. For this reason, synthesis of this product was attempted *via* an alternative synthetic pathway.
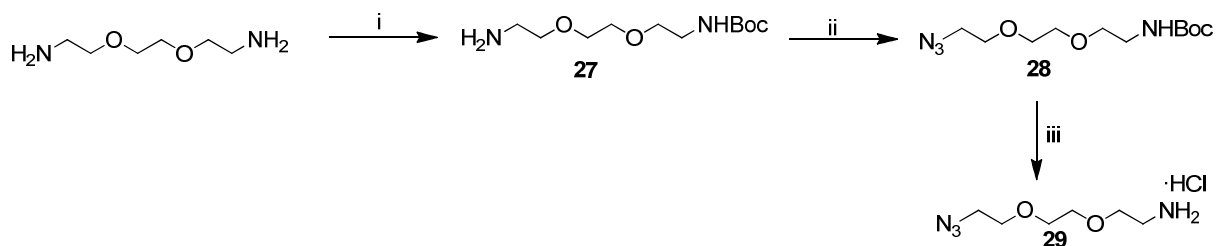
*Scheme 9: Reagents and conditions: (i) Boc₂O, 0-25 °C, 17 hours, (ii) imidazole-1-sulfonyl azide hydrochloride, rt, 17 hours, (iii) HCl in dioxane, rt, 17 hours.*

This synthesis utilises the diamine starting material 2,2′-(ethylenedioxy)diethylamine in conjunction with the diazo-transfer reagent ISA to produce the mono-azide chain. Although synthesis of **29** directly from the diamine chain is known in the literature, it inevitably leads to the synthesis of the diazide by-product, a shock-sensitive and potentially explosive material.

For safety reasons, synthesis was therefore attempted *via* mono-protection of the diamine chain with Boc₂O to produce **27**. As this synthesis will always produce a statistical mixture of the unprotected, mono-protected and di-protected species, yields are low if a 1:1 ratio of Boc₂O and the diamine are utilised. For this reason, a large excess of the diamine chain was utilised to produce **27** in a 91% yield, comparable to the near-quantitative yields obtained in the literature.[194-196]

Subsequent conversion of **27** to the mono-azide **28** was carried out utilising ISA and a copper (II) sulfate catalyst. **28** was produced in a 87% yield, with the synthesis of the azide confirmed initially by a TLC colorimetric test[197] and subsequently by NMR and MS. The Boc-protected chain was then deprotected with HCl in dioxane (4 M), to yield the HCl salt **29** in 89% yield, with NMR analysis showing loss of the characteristic Boc peak. Synthesis of the desired product *via* this pathway was achieved in just three synthetic steps, with an overall yield of 70%, and for this reason this synthetic method was selected as the preferred method of generating large quantities of **29**.

Conjugation of **29** to amine-reactive porphyrin **22** was then attempted *via* a four-step synthetic pathway.
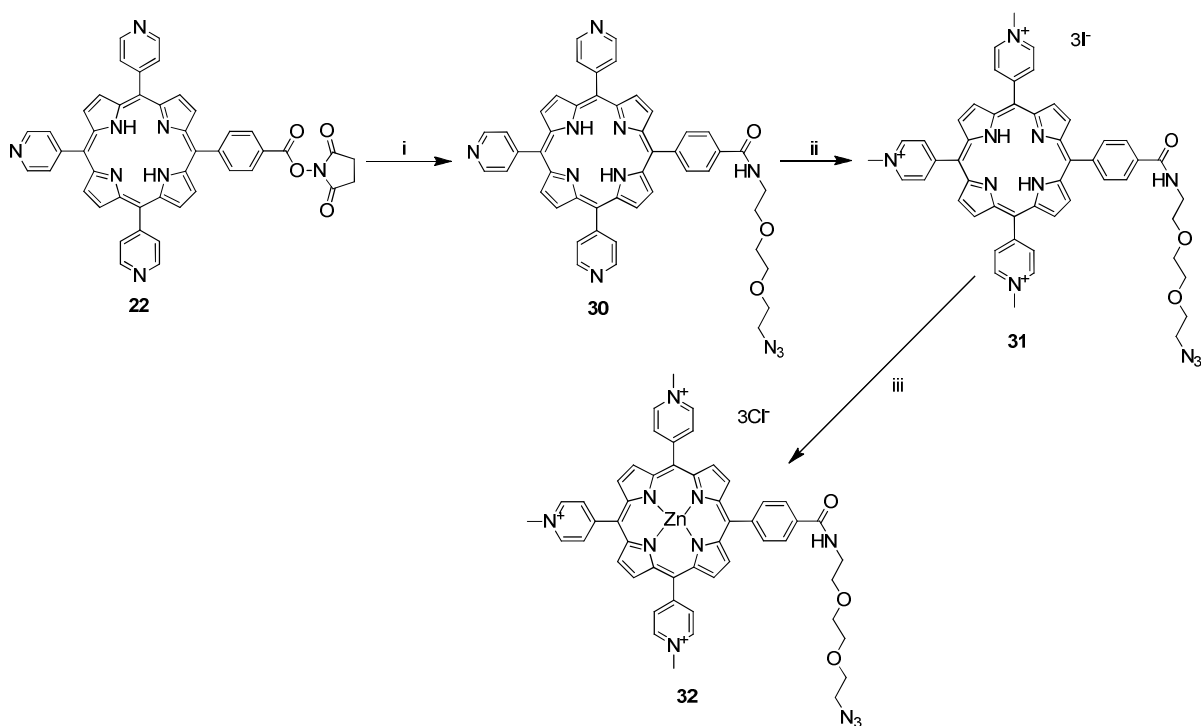
*Scheme 10: Reagents and conditions: (i) **29**, potassium carbonate, 40 °C, 48 hours, (ii) methyl iodide, 40 °C, 17 hours, (iii) zinc acetate, rt, 1 hour.*

Conjugation of porphyrin **22** to linker chain **29** was carried out utilising conditions from the synthesis of an analogous porphyrin as a starting point for this method.[57] Consumption of the starting material was monitored by TLC, and it was found that the reaction was complete after only 48 hours, rather than 5 days as noted in the literature, possibly due to the shorter length of the linker chain used. Workup was carried out by precipitation of the lipophilic porphyrin from the reaction mixture with water, followed by filtration and column chromatography to give the desired product in 82% yield.

Both the methylation and metallation steps to synthesise **31** and **32** respectively proceeded well, with reaction and workup conditions utilised as for **17**. Both synthetic steps yielded the desired product in excellent yields, and the identity of both products was confirmed by NMR, MS and UV-vis.

### 2.5.2.4.   Synthesis of hexahydroxy azido porphyrin

Although methylation and sulfonation of porphyrins to form charged species is an excellent method of creating highly hydrophilic porphyrins, a number of methods are available in the literature for the synthesis of uncharged, hydrophilic porphyrins. In

particular, synthesis of tetrapyrroles functionalised with numerous highly polar, uncharged groups such as amines and alcohols is a widely utilised method, used to create photosensitisers such as the clinically approved drug Foscan (m-THPC). In particular, the hydroxyl group is an excellent water-solubilising moiety, showing more limited reactivity *in vivo* in comparison to amine functionalities, while still allowing for good water solubilisation.

Porphyrins bearing methoxy functionalities as latent water-solubilising groups represent an ideal synthetic target for the production of uncharged hydrophilic porphyrins. While the methoxy ether is lipophilic enough to allow for initial purification steps to be carried out utilising normal phase column chromatography, simple cleavage of the methyl group utilising BBr$_3$ exposes the highly hydrophilic hydroxyl groups. While synthesis of porphyrins bearing one methoxy functionality per meso group is common, use of a dimethoxy aldehyde offers increased hydrophilicity, particularly for AB$_3$-type porphyrins.
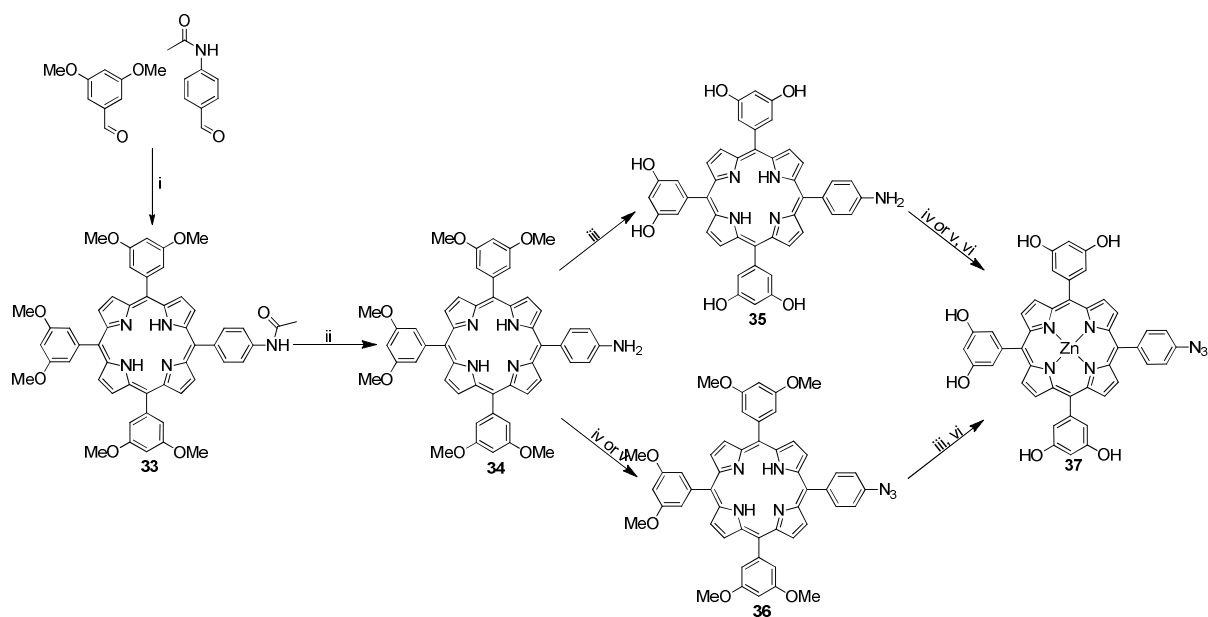


*Scheme 11: Reagents and conditions: (i) pyrrole, propionic acid, reflux, 2 hours, (ii) hydrochloric acid, reflux, 2 hours, (iii) boron tribromide, rt, 17 hours, (iv), imidazole-1-sulfonyl azide hydrogensulfate, zinc acetate, potassium carbonate, rt, 17 hours (v) sodium nitrite, sodium azide, 0 °C, 1 hour, (vi) zinc acetate, rt, 1 hour.*

Synthesis of an AB$_3$ porphyrin bearing a protected amine functionality and three dimethoxy functionalities was therefore attempted, utilising a mixed aldehyde condensation between 3,5-dimethoxybenzaldehyde and 4-acetamidobenzaldehyde.

Synthesis of **33** was carried out according to a literature procedure[177] and proceeded well to give the product in a moderate (6.4%) yield. This yield is lower than observed in the literature and is probably due to loss of product during purification; as many of the non-porphyrin by-products were insoluble in methanol, they could not be removed by methanol:DCM precipitation. The crude mixture therefore required purification by multiple column chromatography steps, and consequentially loss of some product occurred during this process. Conversion of the acetamido to the amine **34** was carried out by refluxing in $HCl_{(aq)}$ to obtain **34** in good yield, with complete removal of the acetamido group confirmed by NMR.

As the conversion of **34** to **37** is a two-step process, involving the deprotection of the methyl ether functionalities to engender hydrophilicity and conversion of the aryl amine to an azide, there were two possible ways in which the synthesis could proceed. Deciding on a synthetic strategy was further complicated by the fact that synthesis of the azide could be attempted either by diazotization with sodium azide in TFA, or by reaction with the diazo-transfer reagent ISA.

Initially, synthesis of **37** was attempted *via* the synthesis of **35**, as this process represents a reaction already known in the literature.[177] The deprotection was carried out using boron tribromide in DCM, to obtain **35** in 60% yield. This result is lower than expected, and can be attributed to the fact the porphyrin was observed to have precipitated from the reaction mixture before the reaction was complete, leading to the formation of substantial quantities of porphyrins still bearing some methoxy groups. The observed precipitation is likely to be as a result of the relatively poor solubility of the amphiphilic, partially-deprotected porphyrins in the reaction solvent, and substitution for a solvent such as DMF may lead to increased yields.

Synthesis of the azido porphyrin **37** from **35** was then attempted *via* diazotization with sodium nitrite and sodium azide, however after workup the reaction TLC showed a mixture of multiple porphyrins had been produced. Synthesis of this large number of porphyrins was attributed to the harsh reaction conditions, possibly as a result of nitration of the porphyrin β-positions. The synthesis of **37** from **35** was then repeated, utilising the diazo-transfer compound imidazole-1-sulfonyl azide hydrochloride and a zinc (II) acetate catalyst as discussed previously. Although the product of this reaction

was a single porphyrin, NMR data was inconclusive and MS data suggested that a multiply-azide functionalised product was the result. From this result, it seemed likely that multiple substitution of azide functionalities onto the alcohol groups was occurring, although this reaction has not been reported in the literature.

For this reason, synthesis of the target porphyrin was then attempted *via* conversion of **34** to the azide-functionalised **36,** followed by deprotection of the methyl ether groups. Synthesis of **36** was initially trialled utilising the diazotization conditions, however again the production of multiple porphyrins was observed. This suggests that reaction with the porphyrin ring itself rather than interaction with the hydroxyl groups could be the reason for the formation of these multiple porphyrins under diazotization conditions. However, synthesis of the azide group utilising imidazole-1-sulfonyl azide hydrochloride and a zinc catalyst produced the zinc azide porphyrin (**36Zn**) in excellent yields, as confirmed by NMR and MS.

Synthesis of **37** from **36** was attempted by deprotection of the methyl ether functionalities utilising $BBr_3$, followed by metallation to reinsert the zinc removed during workup of the deprotection step. Although NMR suggested loss of all ether groups, a trial click reaction was unsuccessful and MS showed no azide functionality present on the porphyrin. A similar problem has been noted in the literature, with large excesses of boron tribromide leading to the loss of an azide functionality of L-azidohomoalanine,[198] and it was assumed that a similar process was occurring during the deprotection step of **36**

At this point, it was decided that all methods to convert the amine functionality of this porphyrin to an azide had been exhausted. For this reason, conjugation of the hexahydroxy porphyrin to azide linker group **29** was trialled as a viable alternative for the synthesis of an azide-functionalised hexahydroxy porphyrin.
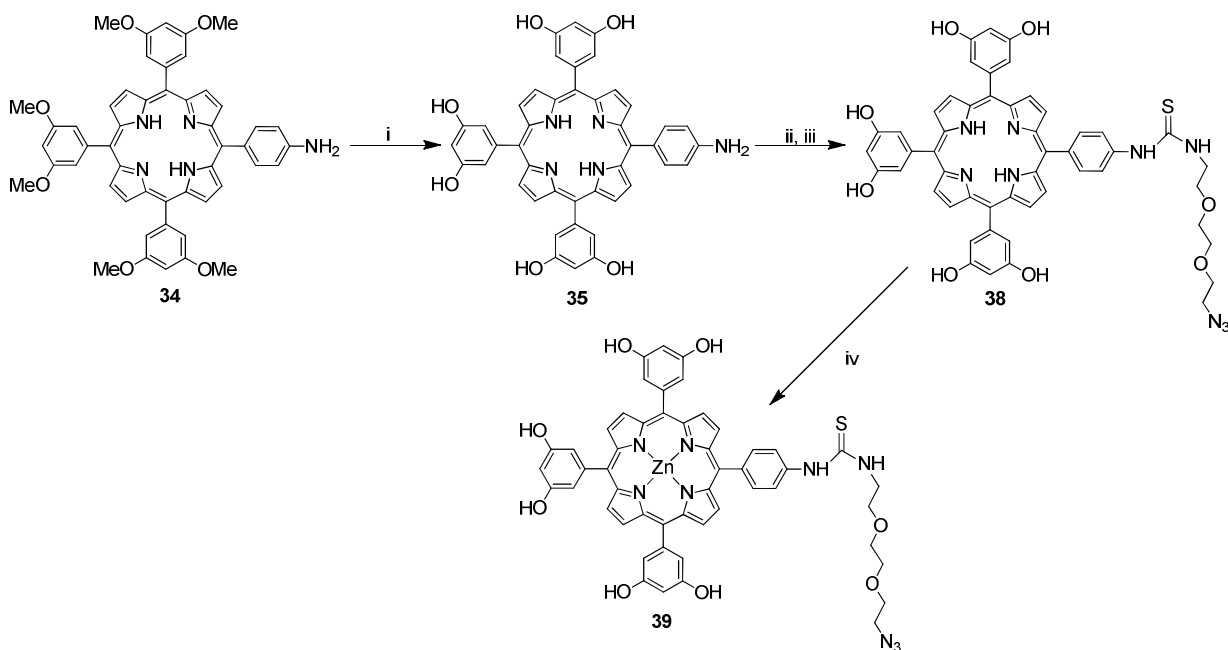
*Scheme 12: Reagents and conditions: (i) boron tribromide, rt, 17 hours, (ii) thiocarbonyldi-2-pyridone, 0 °C, 10 min, (iii) 22, triethylamine, rt, 1 hour, (vi) zinc acetate, rt, 1 hour.*

Conjugation of **35** to **29** was carried out as a two-step procedure; formation of the isothiocyanate group was achieved *via* reaction with thiocarbonyldi-2-pyridone, followed by precipitation of the product and immediate reaction with **29**. Although synthesis of the NCS porphyrin has been reported in the literature,[177] use of the literature reaction conditions led to the formation of multiple products, which was attributed to reaction between the hydroxyl groups and thiocarbonyldi-2-pyridone. For this reason, the reaction was carried out for 5 minutes at 0 °C, and the crude product used immediately in the next step. Synthesis of **38** was carried out in good yield, with the product characterised by NMR and MS. Subsequent metallation of **38** was carried out with zinc (II) acetate in methanol, and porphyrin **39** was obtained in high yield with the presence of the zinc confirmed by MS and UV-vis.

## 2.6. Alkyne-functionalised porphyrins

Although the synthesis of a range of both lipophilic and hydrophilic azide-functionalised porphyrins was carried out successfully, the synthesis of a water-soluble porphyrin bearing an alkyne functionality was also attempted. The synthesis of porphyrins bearing both azide and alkyne functionalities was considered important in order to allow increased synthetic flexibility during the synthesis of porphyrin-dendron

conjugates. A number of problems are associated with the CuAAC reaction, in particular lack of reactivity when utilising very electron deficient azides or very electron rich alkynes, and occurrence of alkyne homocoupling side reactions such as Glaser couplings.[199] Increased structural flexibility when carrying out large numbers of these reactions allows for modification of a synthetic scheme if such problems arise.

As previously discussed, although direct synthesis of a porphyrin bearing an alkyne group is possible, the high cost of protected alkyne aldehydes such as 4-trimethylsilylbenzaldehyde means that synthesis of these porphyrins on a large scale is prohibitively expensive. The conjugation of alkyne functionalised chains to porphyrins bearing suitable reactive functionalities is a widely utilised alternative synthetic method, and avoids the need for use of expensive starting materials. In addition, conjugation of NHS-ester functionalised porphyrin **22** to a chain bearing amine and alkyne functionalities represents a facile way to synthesise an alkyne-functionalised porphyrin without the need for generation of additional starting materials. Although, as previously discussed, a number of alkyne linker chains are commercially available, these are generally small, lipophilic molecules. In order to maintain the water-soluble characteristic of the porphyrin, conjugation of a longer, amphiphilic alkyne chain was attempted.
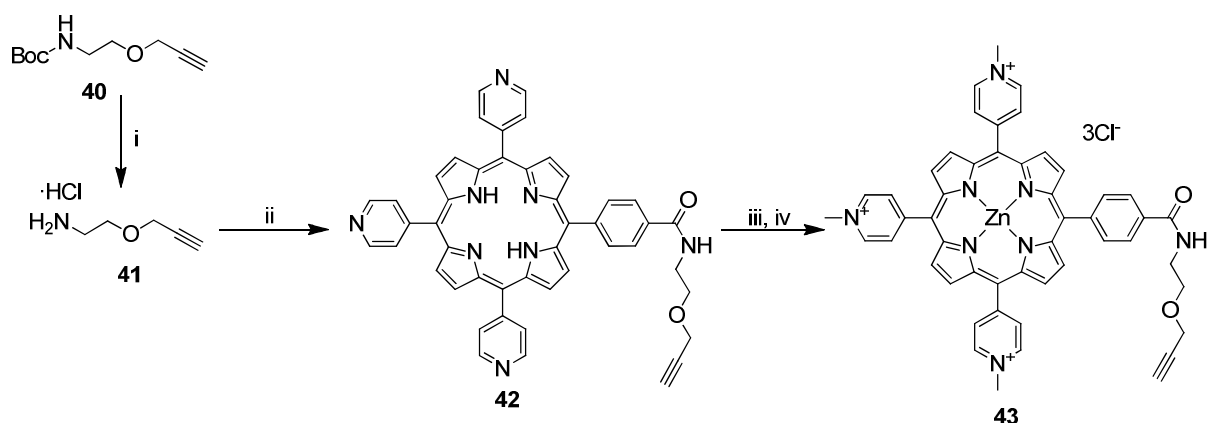


*Scheme 13: Reagents and conditions: (i) HCl in dioxane, rt, 17 hour, (ii) **22**, potassium carbonate, 40 °C, 48 hours, (iii) methyl iodide, 40 °C, 17 hours, (iv) zinc acetate, rt, 1 hour.*

Synthesis of **41** was carried out by Boc-deprotection of **40** using HCl in dioxane (4 M) to yield the HCl salt as a waxy solid in a 90% yield, with NMR confirming the complete loss of the Boc peak. Conjugation of **41** to **22** was then attempted utilising the method

previously optimised for **30** to obtain the product **42** in a 60% yield, comparable to analogous reactions found in the literature,[57] but lower than obtained for the synthesis of **30**. It was also observed that in addition to the synthesis of **42**, a large quantity of carboxylic acid functionalised porphyrin **21** was produced as a by-product during this reaction. Generally, generation of this porphyrin is observed in these reactions through the hydrolysis of **22** due to excess water in the system, and therefore the presence of this porphyrin was attributed to insufficiently anhydrous reaction conditions. Removal of the acid by-product was carried out easily *via* column chromatography to yield a pure sample of the desired product, as confirmed by NMR.

Synthesis of **43** was then attempted *via* methylation and subsequent metallation of **42** using conditions previously outlined, and appeared to proceed well and with high yield. However, although the presence of the desired product was confirmed by MS, NMR data showed that the product was a mixture of two porphyrins, with the major component being the carboxylic acid porphyrin **44** (Figure 31).
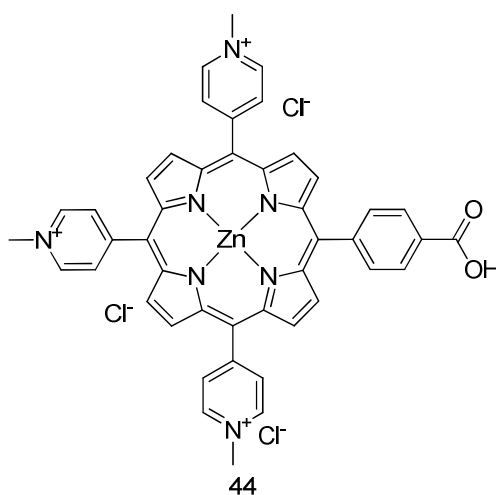


*Figure 31: Degradation of **43** through loss of the attached alkyne chain leads to the formation of the carboxylic acid derivative.*

These results suggest that formation of both acidic porphyrins as by-products was occurring as a result of degradation of porphyrins **42** and **43** through hydrolytic cleavage of the amide bond, a process which generally requires high temperatures and a strongly acidic or basic catalyst. Despite this, stability testing showed that both porphyrins **42** and **43** were unstable when dissolved in methanol at room temperature, and gradually underwent loss of the conjugated linker chain to form the carboxylic acid derivative porphyrins, with the increase in the by-product clearly visible on TLC. There is no precedent for this reaction in the literature and as a result it is difficult to suggest a

possible mechanism or cause for this process.

However, the successful synthesis of the analogous porphyrin **30** suggested that this loss of an amide-conjugated linker chain is not a universal process, and may be highly dependent on either the alkyne functionality or the length of the chain. For this reason, synthesis and conjugation of alkyne-functionalised chains with chain length increased by one unit was attempted.
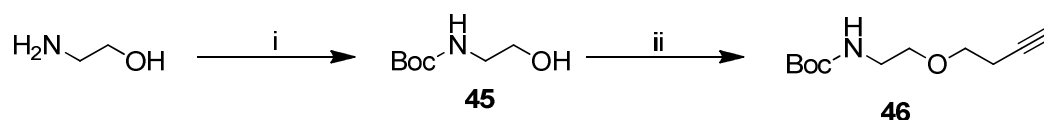


*Scheme 14: Reagents and conditions: (i) Boc$_2$O, rt, 17 hours, (ii) 4-bromo-1-butyne, sodium iodide, TBAI, potassium hydroxide, 70 °C, 72 hours.*

Synthesis of an 8-membered chain was initially attempted using the amine starting material ethanolamine. Boc-protection of this amine proceeded well, although in moderate yields, to produce a sample of **45** as confirmed by NMR. Synthesis of **46** was then attempted *via* the Williamson ether synthesis; conjugation of 4-bromo-1-butyne to the alcohol utilising potassium hydroxide as a base. Although this reaction has been shown in the literature to proceed well between **45** and the shorter chain analogue propargyl bromide,[200] in this case no reaction was observed, even after heating for extended periods of time.
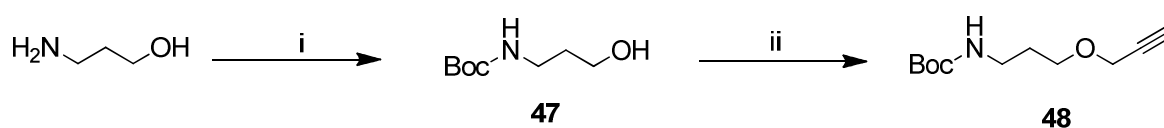


*Scheme 15: Reagents and conditions: (i) Boc$_2$O, rt, 17 hours, (ii) propargyl bromide, sodium iodide, TBAI, potassium hydroxide, rt, 24 hours.*

For this reason, synthesis of an 8-membered chain was then attempted from the longer amine starting material, 3-aminopropanol. The initial Boc protection step proceeded well and **47** was obtained in near-quantitative yield, with the identity of the product confirmed by NMR. Synthesis of **48** was then attempted *via* the Williamson ether synthesis, to produce the desired product in 54% yield.

75
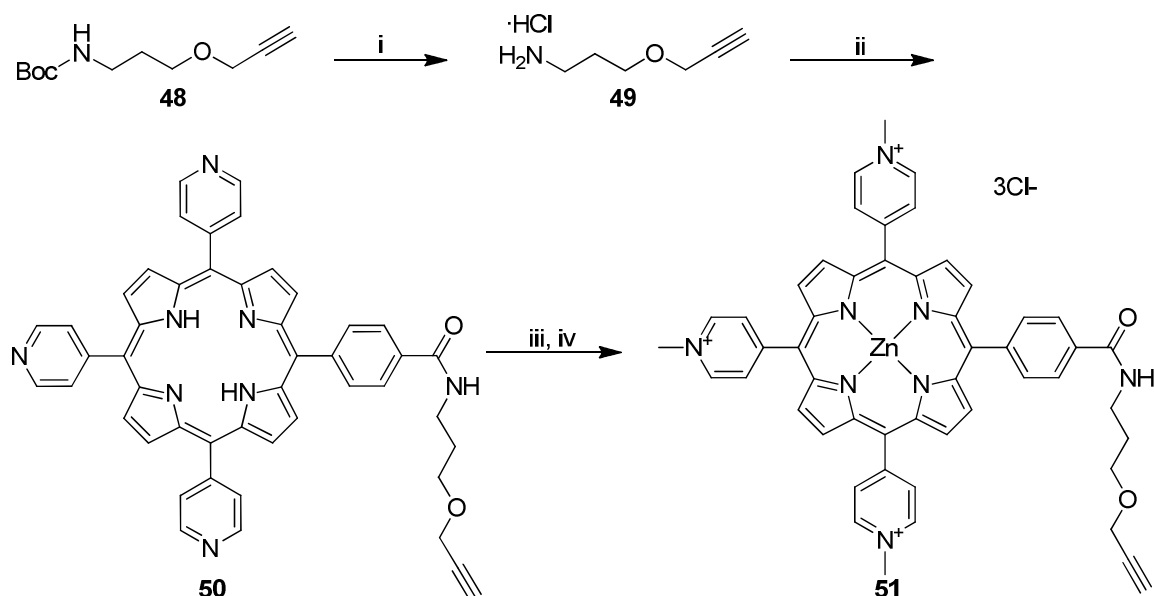
*Scheme 16: Reagents and conditions: (i) HCl in dioxane, rt, 17 hour, (ii) 22, potassium carbonate, 40 °C, 48 hours, (iii) methyl iodide, 40 °C, 17 hours, (iv) zinc acetate, rt, 1 hour.*

Conjugation of this linker chain to NHS-functionalised porphyrin **22** was then attempted**.** Initial deprotection of **48** was again carried out utilising HCl in dioxane to produce the HCl salt in excellent yield. Conjugation of **49** to **22** was then carried out utilising identical conditions to the synthesis of **30**. Although the yield of this reaction was again moderate (60%), in this case no significant production of the carboxylic acid porphyrin by-product was observed.  The identity of **50** was confirmed by MS and NMR, with stability testing in methanol showing no hydrolysis of the amide bond even after 1 week at room temperature. Methylation and metallation of **50** was carried out as previously described to yield **51** in good yields (80% overall), with NMR and MS confirming the presence of the alkyne-functionalised porphyrin only.

This result confirms that the loss of the linker chain at the amide functionality is not a problem which affects all linker chains conjugated to this porphyrin, and that the loss of the chain in this way does not appear to be caused by the terminal alkyne present. However, the length of the linker chain does appear to play a part in this reaction, with an increase in the chain length of just one unit being sufficient to prevent this hydrolysis from occurring. Further work with non-alkyne linker chains could be utilised to determine whether this hydrolysis affects porphyrins bearing any chain of this length, or whether this effect is specific to **43** only.

## 2.7. Conclusions

Initially, the synthesis of a range of lipophilic, azide-functionalised porphyrins was carried out, including synthesis of a model azide-functionalised porphyrin for use in optimisation of click reactions, and the production of azide porphyrins through the use of diazo-transfer reagent, which could be converted to hydrophilic derivatives through future functionalisation.

Synthesis of a range of hydrophilic click-functionalised porphyrins was also carried out successfully, with cationic, anionic and neutral water-soluble porphyrins produced through several synthetic strategies. The use of click-functionalised linker chains to produce both azide and alkyne functionalised porphyrins was also carried out, allowing for greater synthetic flexibility and control of steric effects during subsequent reactions.

# 3. Synthesis of "clickable" dendron

## 3.1.  Introduction

The aim of this section is the synthesis of click-functionalised dendrons, and the subsequent conjugation of these dendrons to porphyrins bearing complimentary functionalities, with the eventual aim of synthesising porphyrin-dendron conjugates for use in antibody-targeted photodynamic therapy.

In order for the synthesised porphyrin-dendron conjugates to be suitable for use as targeted photosensitisers, they must fulfil several criteria. Firstly, they must be water-soluble, to avoid aggregation and non-covalent conjugation to the antibody fragment. Secondly, they must contain two or more porphyrins conjugated to the dendron linker group to allow for increased porphyrin loading on the antibody structure. Thirdly, they must be of a known, stoichiometric composition to allow the synthesis of targeted photosensitisers of a known structure. Finally, they must contain a single bioorthogonal functional group suitable for conjugation to antibody fragments *via* interchain disulfide bridges.

## 3.2.  Synthesis of dendrons

Although a number of different methods have been developed for the synthesis of both dendrons and dendrimers, all of these are based on either a convergent or divergent strategy, or a combination of these two methods.

The divergent synthesis of dendrimers (Figure 32) begins with a core structure, functionalised with two or more reactive coupling points. These points then react with a second molecule bearing a single active, unprotected functional group, and one or more protected groups. Coupling between these produces a branching structure peripheralised with the protected functional groups. A second step, which may be a reduction, deprotection or other activation of the protected functional groups, is carried out to give the branching structure peripheralised with active, unprotected groups. These steps can be repeated on the synthesised structure to grow the dendrimer out from the central focal point, with each repetition growing the structure by one generation.
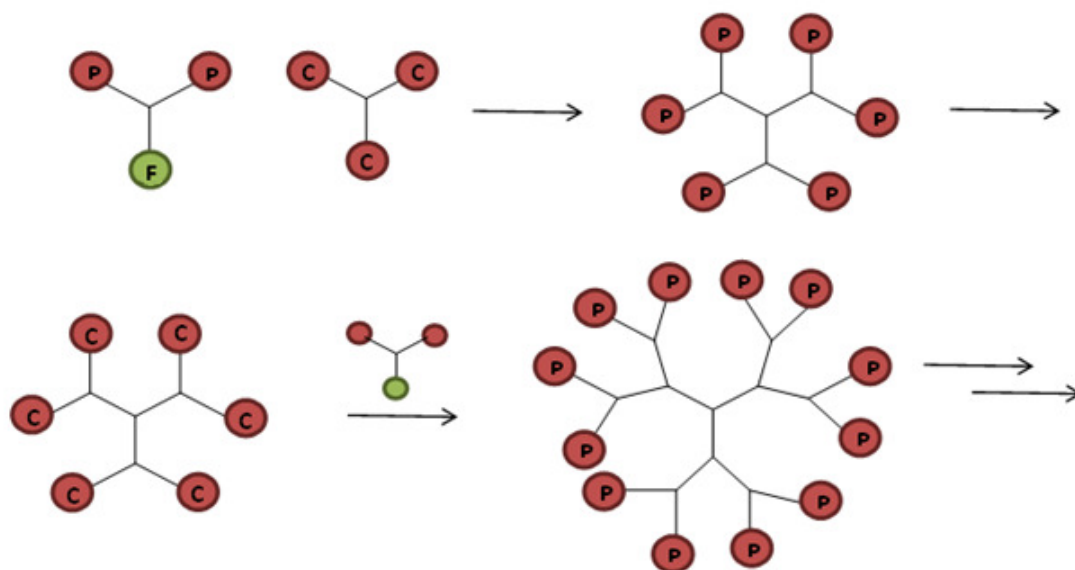
*Figure 32: Divergent synthesis of dendrimers, with the structure growing outwards from a central focal point to the periphery. C= coupling point. F= active, unprotected functional group, P= protected, inactive functional group.*

In contrast, the convergent synthetic method (Figure 33) grows the dendrimer structure from the periphery inwards towards a central focal point. This synthesis begins from a structure bearing a single active, unprotected site and two or more protected groups. This is then reacted with a molecule bearing two coupling points and a different protected group, to produce a branched structure bearing different protected groups on both the periphery and the focal point. Transformation of the protected focal point to a reactive site is then carried out, allowing repetition of the two previous steps to grow the dendrimer inwards from central focal point, with each repetition increasing the size by one generation. Synthesis in this way produces a dendron structure, which can finally be reacted with a core molecule bearing two or more reactive sites to create a symmetrical, 3D dendrimer structure.
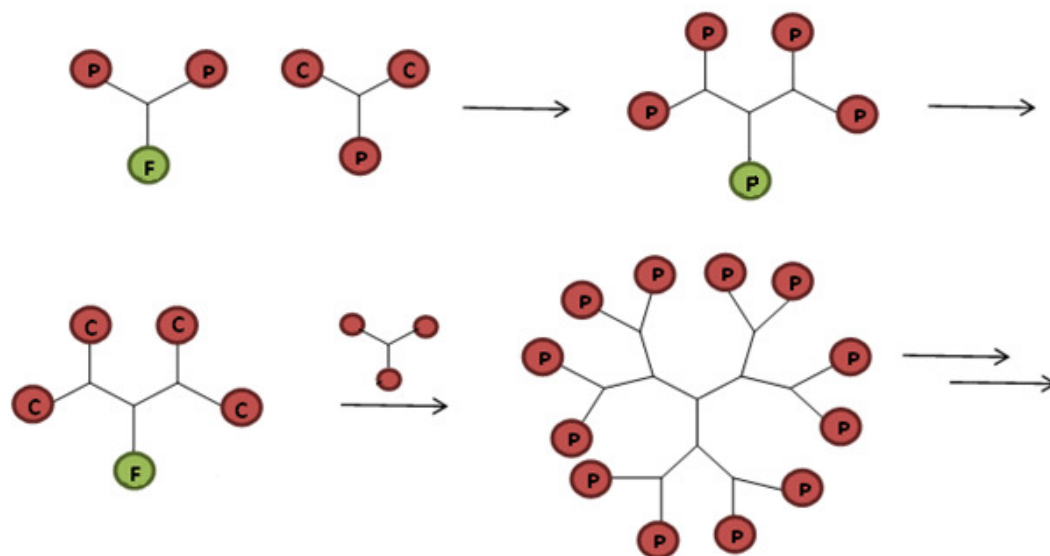
*Figure 33: Convergent synthesis of dendrimers, with the structure growing inwards from the periphery to the core of the molecule. C= coupling point. F= active, unprotected functional group, P= protected, inactive functional group.*

In order to produce porphyrin-dendron conjugates which are suitable for use as targeted 3rd generation photosensitisers, the dendrons synthesised in this work need to fulfil a range of specific criteria; more than one peripheral click functionalisation, a single amine functionality at the focal point, suitable for subsequent derivatisation and conjugation to antibody fragments, and amphiphilic or hydrophilic character. For this reason, three types of dendron were selected for synthesis in this work; PAMAM, tris, and aryl ether dendrons.

As previously discussed, the synthesis of all PAMAM dendrimers and dendrons is based on the Michael addition of methyl acrylate to an amine, followed by subsequent addition of an amine to the resulting ester peripheral groups (Figure 34). Although the standard synthesis conditions produce a symmetrical dendrimer with either amine or ester peripheral groups, the synthesis can be easily modified. Use of a heterobifunctional amine-functionalised starting material allows for the synthesis of unsymmetrical dendrons, while the use of either an alternative acrylate molecule or methyl acrylate and a heterobifunctional amine chain can be used to alter the peripheral functionalities of the dendron. In this work, use of a heterobifunctional triethylene glycol chain will be used to create an amphiphilic unsymmetrical dendron structure, while a range of strategies will be attempted to functionalise the periphery with both azide and alkyne groups.
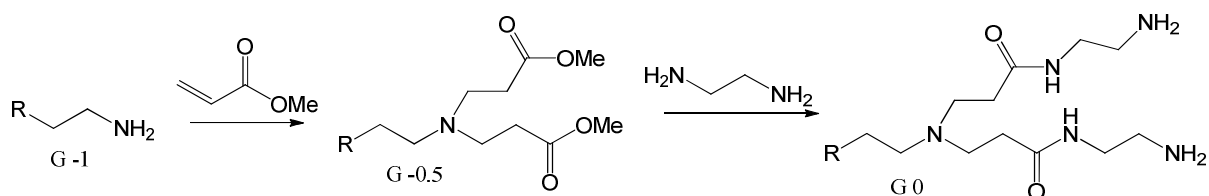
*Figure 34: Synthetic scheme for the production of PAMAM dendron from generation -1 (G -1) to generation 0 (G 0).*

Due to the fact that all tris dendrons are based on the core molecule tris(hydroxymethyl)aminomethane (tris), these molecules offer less structural flexibility than PAMAM dendrons. As a result, any structural variation must be created through modification of the focal point amine and the peripheral alcohols. In this work, protection of the amine with a Boc group will be carried out, followed by functionalisation of the alcohol groups with alkynes (Figure 35).
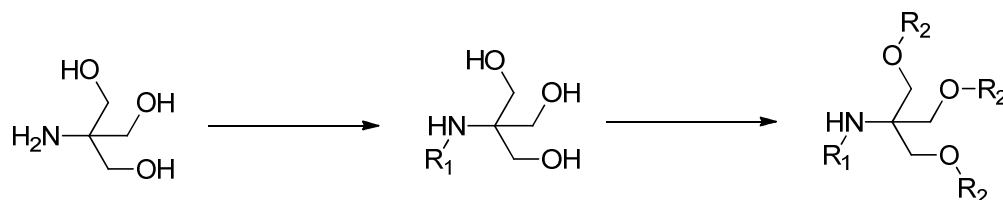


*Figure 35: Synthesis of a G1 tris dendron structure.*

Although the conventional synthesis of aryl ether dendrons uses modifications to the structure 5-(hydroxymethyl)benzene-1,3-diol, alternative syntheses in the literature have utilised a range of structures with two or three phenol groups and a ester focal point. Use of these alternative starting materials allows a large variation in both the number of peripheral groups and the focal point functionalisation. In this work, a Williamson ether synthesis is utilised to functionalise the alcohol groups with alkyne groups on a range of structures, followed by conjugation of a triethyleneglycol chain to the focal point in order to increase amphiphilic character while providing a functionalisable amine focal point (Figure 36).
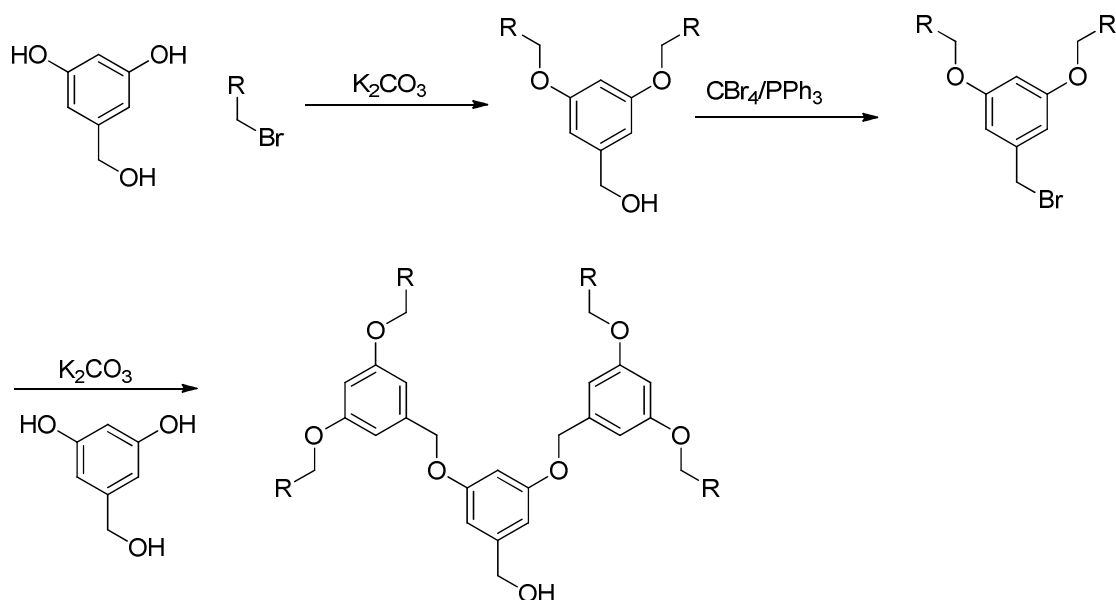
*Figure 36: Synthesis of G1 and G2 aryl ether dendron structures.*

## 3.3. Click functionalisation of dendrons

As with the use of the CuAAC reaction in the field of porphyrin chemistry, its use in dendrimer and dendron chemistry has expanded considerably in recent years. Dendritic chemistry and click chemistry in many ways represent an ideal pairing, with the mild, high yielding nature of click chemistry meaning that multiple functionalisation of dendrimers occurs rapidly and in high yields, leading to insignificant quantities of partially functionalised structures, of particular importance in higher generations. In addition, the insensitivity of CuAAC to the nature of other functional groups in the reactants allows conjugation of dendrimers to a variety of substrates.[141]

The CuAAC reaction has been utilised in two key areas of dendritic chemistry; the synthesis of dendrons and dendrimers, and the functionalisation of dendritic structures. To date, the divergent synthesis of both dendrons and dendrimers *via* formation of triazole linkages has been shown,[201, 202] but is relatively uncommon. Far more widely observed is the use of the click reaction to construct dendrimers convergently from azide-and alkyne-functionalised dendrons[66-69] to form asymmetric[69] or symmetrical dendrimers. The peripheral functionalisation of dendrons and dendrimers with alkyne or azide groups, and subsequent click conjugation of these groups is also an area well-documented in the literature. In particular, functionalisation of aryl ether type dendrons,[203, 204] tris-type dendrons,[84, 85] and PAMAM dendrons[205] has been recorded.

Click functionalisation of dendrons and dendrimers has been reported in the literature in many different ways, with the type of reaction used largely dependent on the nature of the dendritic structure. While focal point click functionalisation of PAMAM dendrons is common,[66-69] click functionalisation of the periphery of these molecules has not been seen in the literature. This is likely as a result of the fact that conventional PAMAM synthesis produces dendrons peripherally functionalised with either ester or amine functionalities, which are not readily converted to click functionalities.

In contrast, the synthesis of both tris and aryl ether dendrons produces dendrons bearing peripheral alcohol groups, which allows facile functionalisation with both azide and alkyne functionalities through a range of nucleophilic subsitution reactions. Williamson ether synthesis utilising propargyl bromide is a facile method of converting the terminal alcohol groups to alkynes, and requires only a mild base such as potassium carbonate[206, 207] or potassium hydroxide[204] in the case of aryl ether and tris dendrons respectively. In contrast, azide functionalised dendrons are generally synthesised through the conversion of the alcohol groups to good leaving groups, followed by a nucleophilic substitution with sodium azide. This reaction has been carried out on both aryl ether and tris dendrons using halides[202, 208] as the leaving group.

## 3.4.  Synthesis of bis dendrons

The synthesis of dendrons bearing two peripheral click functionalities was attempted first due to the simplicity of producing first generation dendrons in comparison to the larger, higher-generation structures with increased numbers of peripheral sites. As for the earlier synthesis of porphyrins, synthesis of dendrons bearing both azide and alkyne functionalities was attempted in order to increase synthetic flexibility, however as the majority of porphyrins synthesised were functionalised with azide groups, alkyne dendrons were the focus of this work.

The mono-protected amino chain **20** was utilised as the starting material for all di-dendrons synthesised for several reasons. Firstly, the triethylene glycol-based chain is amphiphilic and cheap to synthesise, and the amine functionality lends itself well to numerous methods of synthesising asymmetric PAMAM-like dendrons. Although synthesis of similar dendrons bearing amino peripheral functionalisation has been seen

in the literature,[130] to date, synthesis of PAMAM-like dendrons bearing click peripheral functionalities has not been published.

### 3.4.1. Synthesis of methyl acrylate PAMAM dendrons

Initially, synthesis of amine-functionalised $G_0$ PAMAM dendrons was attempted *via* the addition of methyl acrylate and ethylene diamine to **20**, as described by Morosini *et al*.[130] Although this synthesis generates amine-peripheral functionalisation on the dendron, further functionalisation of the dendron could be used to allow click-functionalisation.
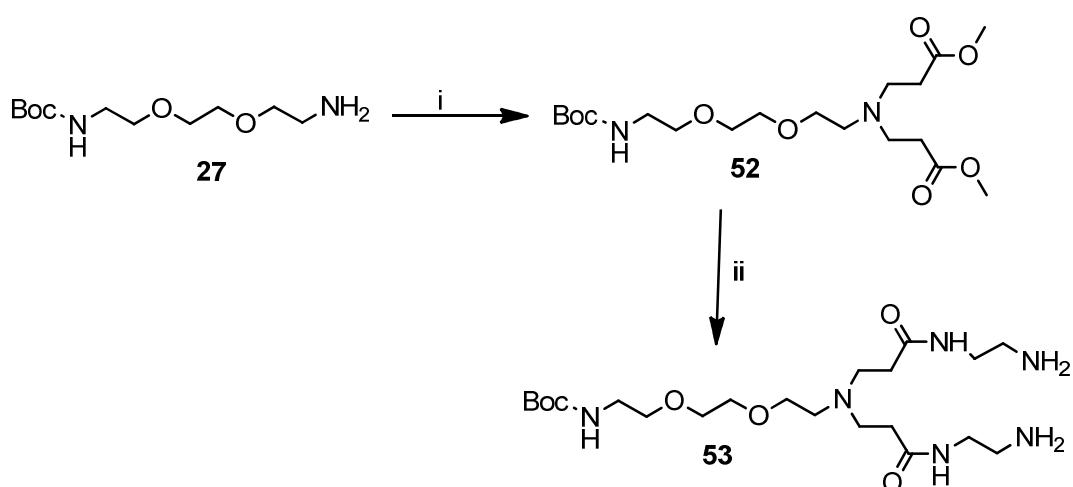


*Scheme 17 : Reagents and conditions: (i) methyl acrylate, rt, 96 hours (ii) ethylene diamine, -40 °C-rt, 48 hours.*

Synthesis of **52** was carried out by addition of a large excess of methyl acrylate to mono-protected amine **27**. After stirring for 96 hours, purification of the crude product by silica plug yielded the product in an 85% yield, higher than obtained in the literature (63%),[130] with the identity of the product confirmed by NMR and MS. However, subsequent addition of ethylenediamine to yield **53** was not successful, with NMR and GPC analysis of the product showing the presence of multiple species. In particular, the large excess of ethylenediamine required was extremely difficult to remove, even utilising azeotropic distillation to remove the majority of the starting material.
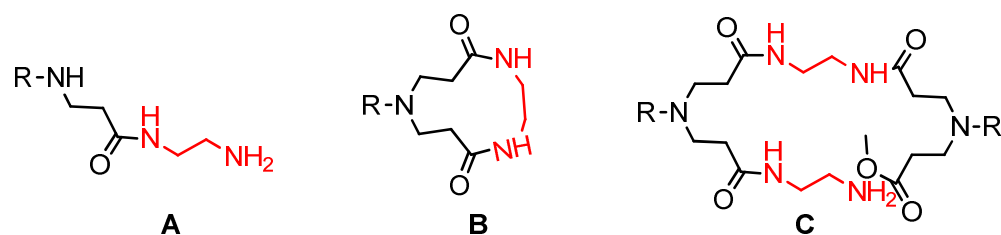
*Figure 37: Defect-producing processes which occur during the addition of ethylene diamine (highlighted in red) to G-0.5 PAMAM structures. A=loss of arm, B=cyclisation, C=dimerisation. Combination of these processes results in the synthesis of a large number of by-products.*

Problems of this nature are not uncommon in the synthesis of PAMAM dendrimers and the generation of multiple unwanted by-products is known, arising from the double-addition of a diamine species to one or more dendrons in several ways, or loss of a methyl acrylate arm before addition (Figure 37). Purification of PAMAM dendrimers is usually undertaken utilising gel electrophoresis or size-exclusion chromatography, however these methods were not possible on the much smaller dendron structure. Additionally, purification of this complex mixture by column chromatography was not possible due to the highly polar nature of both the product and the synthesised by-products.

For this reason, synthesis of PAMAM-type dendrons was then attempted from addition of several mono-amine species to **52**. Use of a mono-amine species has several advantages over the use of ethylene diamine; use of amines bearing azides or alkynes allows for the synthesis of click-functionalised dendrons without the need for further modification, and reduces the number of potential by-products by preventing multiple additions to the same chain.
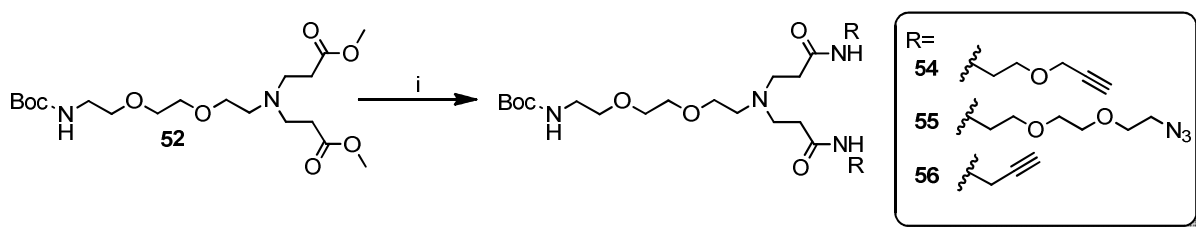


*Scheme 18: Synthesis of functionalised PAMAM dendrons.*

Initially, conjugation of previously synthesised azide and alkyne amines **29** and **49** to G-$_{0.5}$ PAMAM dendron **52** was attempted. In each case, a small excess of the amino chain

(2-2.5 times) was utilised, and moderate heating employed after no reaction was observed at ambient temperature, however in both cases, after 10 days no formation of the product was observed. The lack of product formation can be attributed to the small excess of the amine used; unmodified ester functionalities are poorly reactive towards amines, and as a result conventional PAMAM synthesis often uses greater than 100 times excess of the amine. However, the time and cost involved in the synthesis of **29** and **49** mean that use of large excesses would be highly impractical and time-consuming, and for this reason no further attempts to synthesise these products were made.

Synthesis of **56** was then attempted *via* the addition of commercially available propargylamine to **52**, utilising a larger (10 times) excess of the amine and high temperature to force the reaction to completion. Despite this, after 5 days no product formation or consumption of starting materials was observed. The high cost of propargylamine relative to ethylenediamine, combined with the difficulty of removing a very large excess of this reagent from the crude product meant that a synthesis utilising larger excesses of propargylamine was not attempted.

### 3.4.2.   Synthesis of other acrylate and acrylamide PAMAM-dendrons

Conventional PAMAM synthesis uses addition of methyl acrylate to amine-functionalised chains in order to create ester- and amine- terminated dendrons which require additional transformations in order to create peripheral click functionalisation. An alternative method of generating these click-functionalised dendrons is the use of other acrylate and acrylamide starting materials to produce azide- and alkyne-terminated dendrons without the need for further derivatisation.



*Figure 38: Structure of acrylate (left) and acrylamide (right) molecules.*

The synthesis of both acrylates and acrylamides is facile and well described in the literature, with the conjugation of alcohol- or amine-functionalised chains to acryloyl

chloride used to create acrylates and acrylamides respectively (Figure 38).
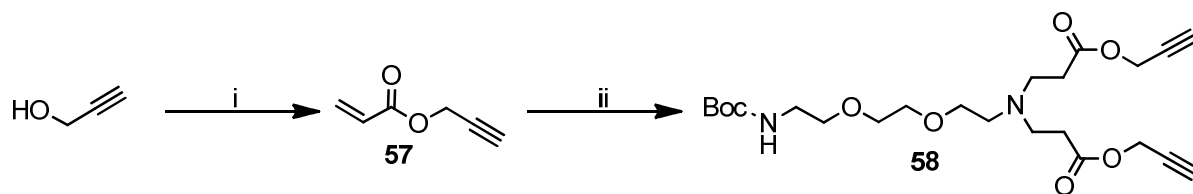


*Scheme 19: Reagents and conditions: (i) acryloyl chloride, triethylamine, 0 °C-rt, 30 min, (ii) 27, rt, 96 hours.*

Synthesis of the alkyne-functionalised propargyl acrylate was attempted first, as this synthesis is well known in the literature, and is easy to carry out with commercially available reagents. Synthesis of **57** was carried out according to a literature method,[209] and the product was obtained in 78% yield, with the identity of the product confirmed by NMR and MS. Conjugation of **57** to **27** was then attempted utilising conditions used to synthesise **58**; a five day stir in methanol at room temperature. While the reaction appeared to proceed well on TLC and was complete after two days, MS and NMR showed that none of dendron **58** had been formed, with the methyl ester dendron **52** being the only product. The formation of **52** during this reaction can be attributed to a transesterification process occurring between **57** and the methanol solvent, which would replace the alkyne chain with a methyl group. Reaction between the formed methyl acrylate and **27** would lead to synthesis of **52** alone.

Synthesis of **58** was then carried out using THF as the reaction solvent, with the desired product obtained in a 79% yield. Although yields obtained were similar to that obtained for an analogous PAMAM dendron, it was noted that reaction times were considerably longer for the addition of **57** in comparison to the addition of methyl acrylate. The slower rate of reaction was attributed to the increased steric hindrance of the larger alkyne chain in comparison to methyl acrylate, increasing the time required for appreciable addition of the second acrylate molecule.

87
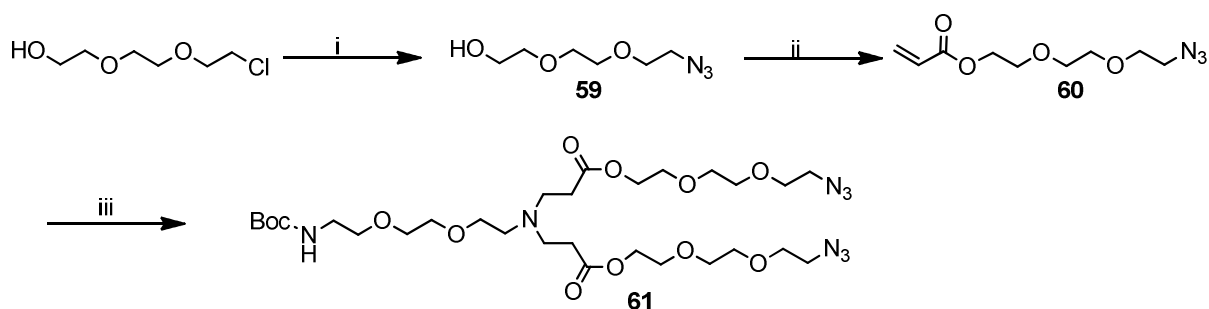
*Scheme 20: Reagents and conditions: (i) sodium azide, 100 °C, 48 hours, (ii) acryloyl chloride, triethylamine, 0 °C-rt, 30 min, (iii), **27**, rt, 10 days.*

Synthesis of an azide-terminated acrylate was also attempted. As short azide-functionalised chains are not available commercially due to the safety risk of low molecular-weight azides, a longer triethylene glycol azide chain was synthesised through nucleophilic substitution on 2-(2-(2-chloroethoxy)ethoxy)ethanol with sodium azide, with **59** obtained in near-quantitative yield. Conjugation of **59** to acryloyl chloride was carried out as for **57**, with the acrylate **60** obtained in a moderate yield (66%), due to loss of the relatively hydrophilic product during aqueous workup.

Michael addition of **27** to **60** was then carried out utilising conditions as optimised for the synthesis of **55**. However, after stirring at room temperature for 10 days TLC analysis showed a substantial quantity of starting material **27** was still present, and isolation and NMR of the only product formed showed formation of the single-armed analogue only, derived from a single Michael addition reaction on **27**. The lack of the formation of the desired product was again attributed to the increasing steric hindrance of the system in comparison with both dendrons **52** and **58**, reducing the rate of reaction between the one-armed product and the acrylate starting material to the point where the formation of the two-armed product occurred too slowly to be detected over a 10 day time period.

### 3.4.2.1. *Synthesis of amide-containing dendrons.*

Following the successful synthesis of dendron **58**, synthesis of PAMAM-like dendrons from an analogous acrylamide starting material was also attempted. Although acrylate and acrylamide molecules are structurally very similar, substitution of the ester for an amide in the dendron is desirable from a biological perspective. This is due to the fact

88

that acrylate molecules, as with any ester, can be efficiently cleaved by the large number of esterases present in the body, whereas amide bonds show greater biological stability. Due to the previous unsuccessful synthesis of azide acrylates, and the poor commercial availability of azide chains, synthesis was attempted utilising alkyne derivatives only.
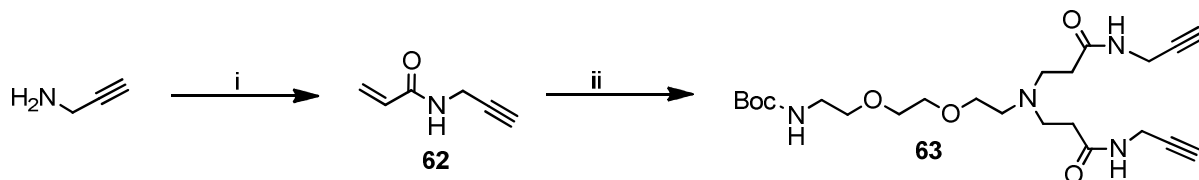


*Scheme 21: Reagents and conditions: (i) acryloyl chloride, triethylamine, 0 °C-rt, 1 hour, (ii) 27, 50 °C, 96 hours.*

Synthesis of propargyl acrylamide **62** was carried out according to a literature synthesis[210] from propargylamine and acryloyl chloride to obtain the product in a 66% yield. Conjugation of **62** to **27** was initially trialled using reaction conditions optimised for **58**, however after 48 hours no product formation was observed. Synthesis of **63** was then attempted using conditions shown in the literature to be highly favourable for synthesis of branched molecules of this type,[211] however after 96 hours no reaction was observed.

For this reason, synthesis of the amine-containing dendron was then attempted using non-acrylate starting materials. Initially, bromoacetyl bromide was selected for this synthesis as this dibrominated compound has a large difference in the reactivity of the two bromine functionalities, which allows regiospecific addition of an amine to the acyl bromide. Addition of the formed mono-brominated species to chain **27** can then be carried out *via* an $S_N2$ –type reaction rather than a Michael addition.
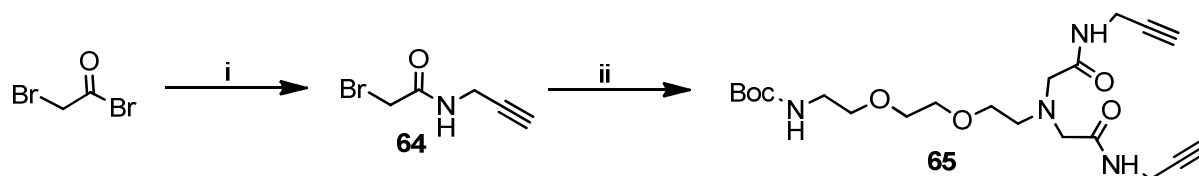


*Scheme 22: Reagents and conditions: (i) propargylamine, rt, 1 hour, (ii) DBU, 27, rt, 17 hours.*

Synthesis of **64** was carried out according to a literature procedure[212] and the desired product obtained in a near quantitative yield, with the identity of the product confirmed

by NMR. Subsequent conjugation of **64** to **27** was carried out in acetonitrile with DBU acting as a base, with TLC showing no further reaction after 17 hours. However, NMR showed that the mono-substituted chain was the only product, with no formation of the disubstituted dendron. Increased reaction times and use of an excess of **64** also showed no appreciable formation of the disubstituted species.

This result is unexpected due to the fact that alkylation of amines generally proceeds to completion due to the increased reactivity of secondary amines in comparison to primary amines. It would therefore be expected that the second addition of **64** would occur more rapidly than the first. A proposed explanation for the lack of any disubstituted product is the fact that this product has only one carbon between the amine and the attached carbonyl group. Conjugation of two molecules of **64** to the same amine of **27** therefore leads to two electron-dense carbonyl groups in very close proximity, which is likely to be disfavoured due to both electronic and steric factors.

Synthesis of an amide-containing PAMAM dendron was then attempted by addition of a commercially available mono-amine, ethanolamine, to the G-0.5 PAMAM dendron **52**. Although previous attempts at synthesis of this nature were unsuccessful, ethanolamine offers numerous advantages over other amines already trialled. Primarily, as ethanolamine contains only one amine functionality, the synthesis of multiple by-products observed when utilising ethylene diamine will not occur. Secondly, the resulting alcohol-functionalised dendron can easily be converted to an alkyne dendron *via* a Williamson ether synthesis utilising propargyl bromide. Finally, ethanolamine is cheap in comparison to many other amines, and can be removed relatively easily utilising azeotropic distillation, meaning that a large excess can be used without problems.
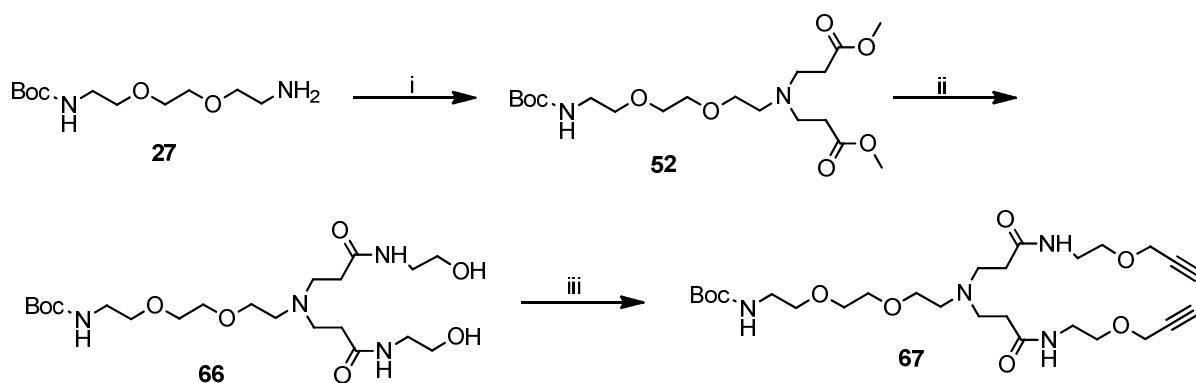
*Scheme 23: Reagents and conditions: (i) methyl acrylate, rt, 48 hours, (ii) ethanolamine, rt, 5 days, (iii) propargyl bromide, sodium iodide, TBAI, potassium hydroxide, 40 °C, 24 hours.*

Synthesis of **66** was carried out by addition of a large excess (>50 times) of ethanolamine to **52**, followed by stirring for 5 days at room temperature. Removal of the large excess of residual ethanolamine was carried out by azeotropic distillation with chlorobenzene to give **66** in a 93% yield, with NMR showing no residual ethanolamine or chlorobenzene present. The structure of the product was confirmed by IR, with the spectrum showing clear peaks for the amide and alcohol functionalities, confirming the successful addition of the ethanolamine at the amine rather than the alcohol position. Subsequent addition of propargyl bromide proceeded well under mild conditions, with the product **67** obtained in good yield (80%) after purification by column chromatography.

## 3.5. Synthesis of tris dendron

The aim of this section was the synthesis of a three-armed dendron bearing peripheral alkyne functionalities, from the starting material tris(hydroxymethyl)aminomethane. Two syntheses of tri-alkyne dendrons from a tris skeleton have been published,[84, 85] both of which utilise Boc protection of the amine functional group followed by Williamson ether synthesis with propargyl bromide to form three alkyne functionalities on the periphery of the molecule. In this work the method of Chabre *et al.*[85] was utilised, which offers a mild, high yielding synthesis without the need for harsh bases such as sodium hydride or corresponding low-temperature reaction conditions.

91
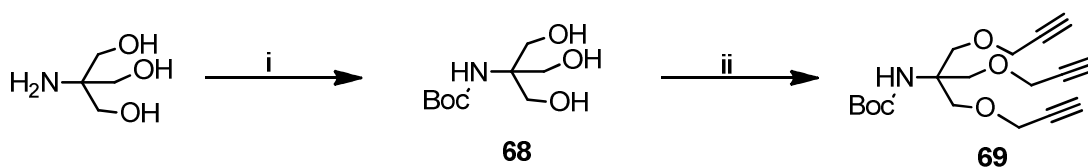
*Scheme 24: Reagents and conditions: (i) Boc$_2$O, rt, 17 hours, (ii) propargyl bromide, 35 °C, 24 hours.*

Synthesis of **68** was carried out by addition of Boc$_2$O in dioxane:water with sodium hydroxide as the base. This reaction proceeded in a 96% yield with the identity of **68** confirmed by NMR. Alkylation of the alcohol groups was then carried out *via* the Williamson ether synthesis, to obtain product **69** in a 40% yield, with the remainder of the reaction components being a number of multiply-alkylated species. This yield is somewhat lower than literature value of 66%, however this difference can largely be attributed to the fact that this is a non-optimised yield from a single reaction. NMR confirmed the identity of **69**, which was synthesised in a 38.4% yield overall.

## 3.6. Synthesis of aryl ether dendrons

Following successful synthesis of dendrons bearing both two and three alkyne functionalities, synthesis of a range of aryl-ether type dendrons was also attempted, utilising a synthesis modified from the literature method developed by Branderhorst *et al.*[213] Use of aryl ether dendrons has several advantages over the dendrons synthesised previously in this work; firstly, these dendrons are synthesised using the strong and biologically stable ether linkages, limiting the probability of cleavage in the body. Secondly, growth of these dendrons can be carried out very simply to produce dendrons bearing large numbers of alkyne functionalities, whereas the structures of the tris and PAMAM-type dendrons effectively limit the amount of alkyne functionalisation. However, unlike the previously synthesised dendrons, the aryl ether dendrons are lipophilic, and the focal point is generally functionalised with either a bromo or ester functionality. As a result, all of the synthesised dendrons were conjugated to the triethylene glycol chain **27**, which both increased the amphiphilic character and allowed attachment of an amine functional group to allow later functionalisation.
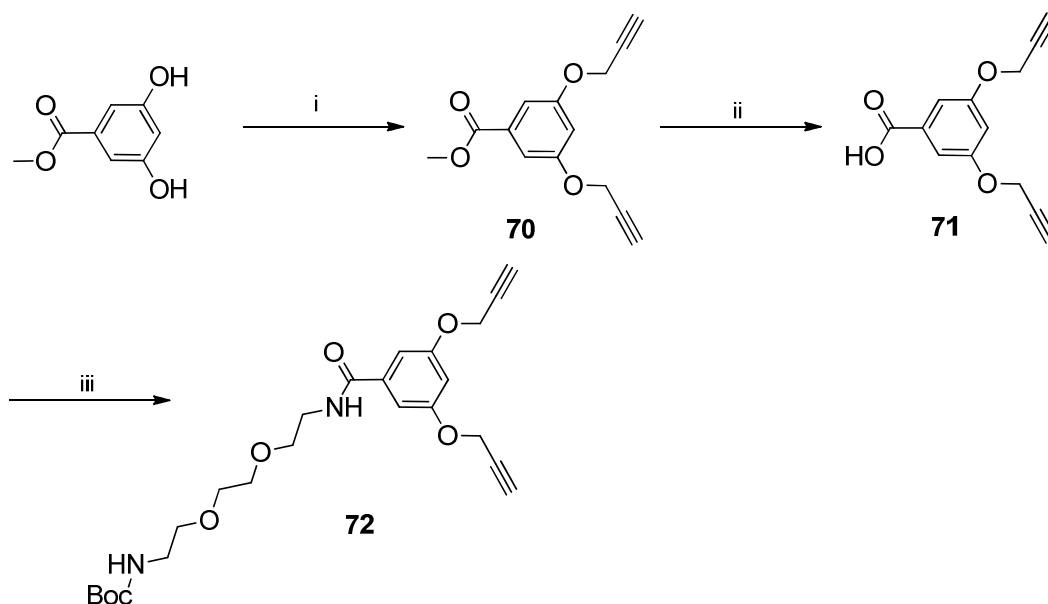
*Scheme 25: Reagents and conditions: (i) propargyl bromide, K$_2$CO$_3$, reflux, 17 hours, (ii) MeOH, H$_2$O, NaOH, rt, 5 hours, (iii) **27**, PyBOP, rt, 17 hours.*

Conversion of the commercially available starting material methyl-3,5-dihydroxybenzoate to **71** was carried out in two steps according to general literature synthesis methods. Both steps proceeded well, with excellent yields and no need for purification other than a simple aqueous workup, to produce **71** in an overall 80% yield, with the identity of **70** and **71** confirmed by NMR and MS. Conjugation of **71** to **27** was carried out using a method similar to that seen in the literature,[213] however PyBOP was used as the coupling reagent instead of BOP to avoid the formation of carcinogenic by-products. **72** was synthesised in excellent yield (83%) after column purification.

An identical synthesis method was then used to produce **75** from methyl-3,4,5-trihydroxybenzoate. As seen for **72**, all steps were carried out in good yield, with the product identity confirmed with NMR and MS at each step, and the final product **75** was produced in an overall 57% yield.
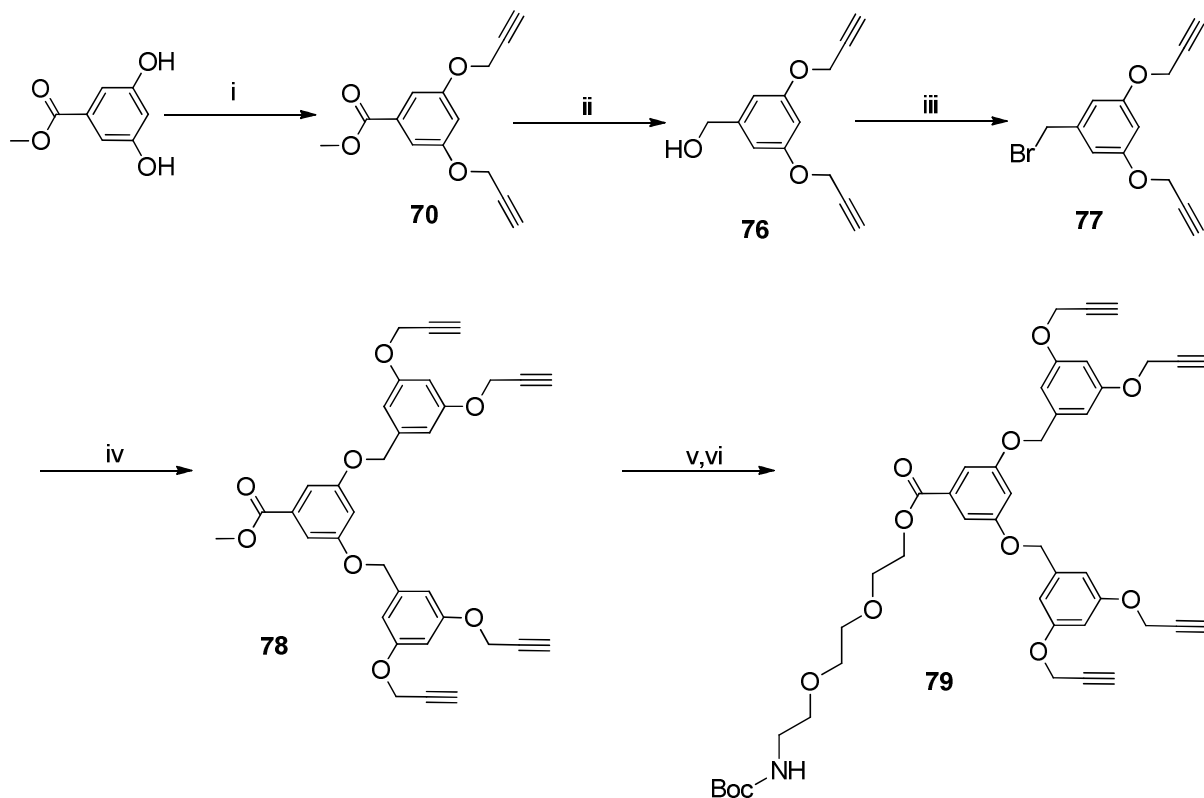


*Scheme 27: Reagents and conditions: (i) propargyl bromide, K₂CO₃, reflux, 17 hours, (ii) LiAlH₄, rt, 17 hours, 6 hours, (iii) phosphorus tribromide, rt, 17 hours, (iv) methyl-3,5-dihydroxybenzoate, K₂CO₃, reflux, 17 hours, (v) MeOH, H₂O, NaOH, rt, 5 hours, (vi) 27, PyBOP, rt, 17 hours.*

The synthesis of a 2nd generation aryl ether dendron was then attempted according to a literature method to produce a dendron bearing four peripheral alkyne groups. Conversion of the previously synthesised dendron **70** to the molecule bearing a benzylic alcohol was carried out using lithium aluminium hydride, to produce the desired product in 95% yield. Subsequent reaction of the alcohol with phosphorus tribromide yielded the bromide in an excellent yield (90%), with the conversion confirmed by characteristic changes to the NMR and MS data. Finally, synthesis of **78** was achieved

through the Williamson ether synthesis between the commercially available methyl-3,5-dihydroxybenzoate and **77** to produce the tetra-alkyne structure in good yield (78%) after column chromatography. Synthesis of structure **79** was then carried out in a two-step process as for structure **75**, with the desired product obtained in 87% overall yield, significantly better than that obtained in previous literature synthesis.[213]

The successful synthesis of both first and second generation aryl ether dendrons bearing 2, 3 and 4 alkyne functionalities suggests that dendron structures of this type can be generated with far larger quantities of click functionalities on the peripheral sites, with the only limitations being the increased lipophilicity of the higher generation structures, and the difficulty of ensuring full porphyrin functionalisation of structures bearing larger numbers of alkynes.

## 3.7.  Synthesis of porphyrin-dendron conjugates

To date, synthesis of both a range of azide-functionalised porphyrins and a range of alkyne-functionalised dendrons bearing between 2 and 4 alkyne groups has been carried out successfully. The aim of this section is therefore the combination of the two clickable "building blocks" to synthesise a range of porphyrin-dendron conjugates suitable for use as targeted photosensitisers.
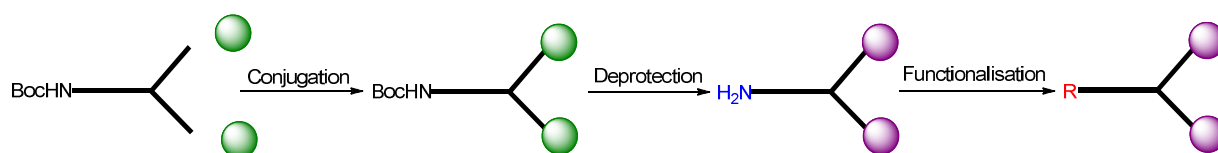


*Figure 39: Schematic outlining the synthetic method of all porphyrin-dendron conjugates. Zinc-protected porphyrins are represented by green spheres while free-base porphyrins are shown as purple.*

Although reaction conditions required were variable depending on the nature of both the dendron and the porphyrin, a generic synthetic pathway for the synthesis of all porphyrin-dendron conjugates was devised (Figure 39). Initially, functionalisation of the dendron with the porphyrin will be carried out by a click reaction, followed by a deprotection step to remove both the Boc-protecting group and the zinc in the porphyrin cavity. Finally, functionalisation of the deprotected amino group will be carried out to

95

allow bioorthogonal conjugation with an antibody bearing a disulfide bridge.

### 3.7.1. Synthesis of TPP-porphyrin conjugates

Although the eventual aim of this work is the synthesis of water-soluble porphyrin-dendron conjugates, initially conjugation was attempted using model porphyrin **5** to create a range of lipophilic porphyrin-dendron conjugates, followed by test deprotection to ensure this could be carried out successfully without degradation of the porphyrin or dendron. Synthesis of conjugates using a model porphyrin in this way ensured that the dendrons could be successfully clicked and deprotected without the use of cationic porphyrins, while the lipophilic conjugates synthesised could be purified by column chromatography. In addition, the click conjugation of this porphyrin to sugars[151], and the conjugation of similar lipophilic porphyrins to aryl ether dendrons[119] are both known in the literature, allowing the development of a click methodology from existing reactions.

### 3.7.1.1. Synthesis of bis porphyrin-dendrons
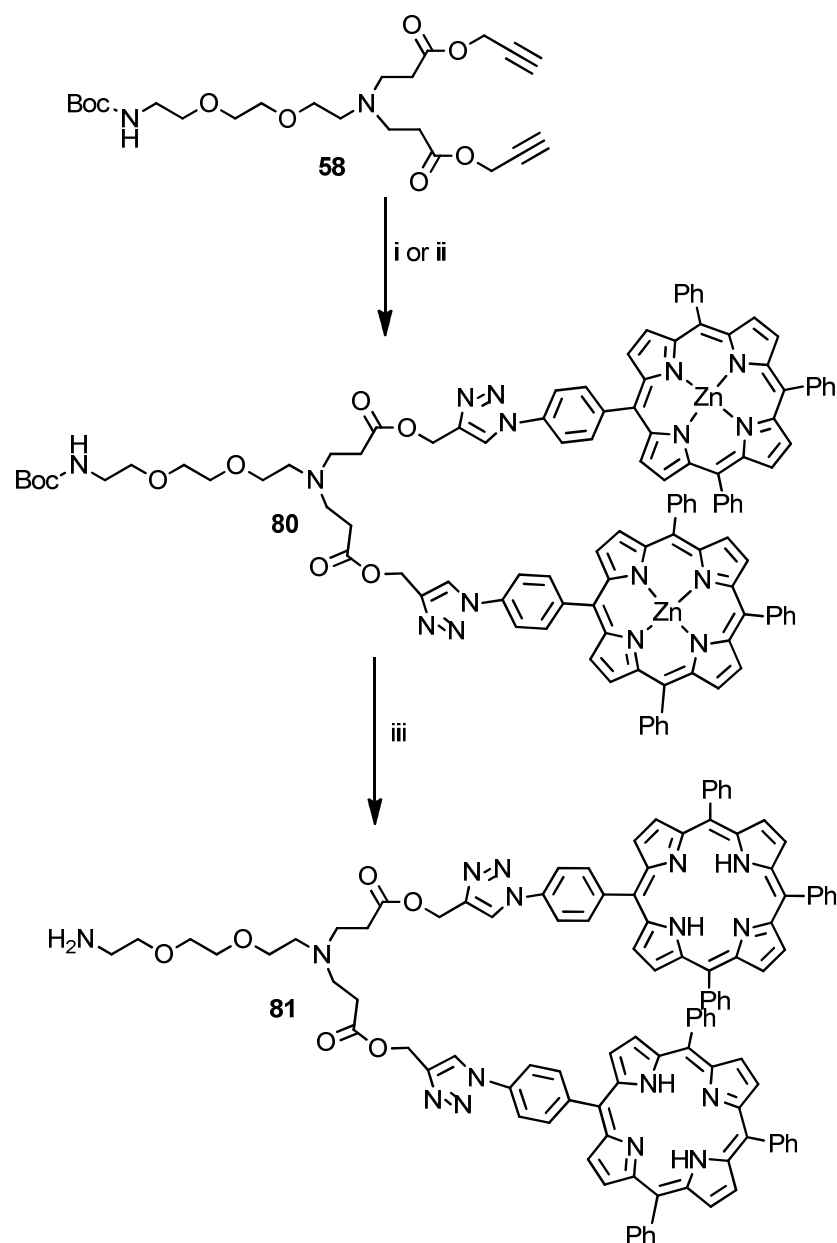


*Scheme 28: Reagents and conditions: (i)* **5**, *copper (II) sulfate pentahydrate, sodium ascorbate, 80 °C (MW), 1 hour. (ii) trifluoroacetic acid, rt, 4 hours.*

Initially, the conjugation between **5** and **58** was attempted in a DCM:water solvent mixture with a copper (II) sulfate and sodium ascorbate catalyst. While these biphasic reaction conditions are widely used in the literature, no reaction was observed after heating to reflux for 3 days, with the lack of reaction attributed to the low reflux temperature of DCM. As a result, use of a toluene:water solvent system was then attempted, with the desired product **80** obtained after heating to reflux for four days. However, even after this time **80** was obtained in low yield (33%), with a large quantity of unreacted porphyrin remaining. The poor yield obtained, despite the intensification of

the reaction conditions, can be attributed to the high steric effects of attaching two large porphyrins onto a small molecule, and also the use of the biphasic solvent system, which results in the reaction occurring only at the interface of the two solvents.

Microwave heating of this reaction was then attempted and was shown to improve both reaction times and yield, with heating to 80 °C for one hour producing a 46% yield of **80**, with the identity of the product confirmed by NMR and MS. As observed for the convection heating reaction, a significant quantity of unreacted **5** was observed following reaction completion, however further heating did not lead to an increased yield. Deprotection of **80** was then carried out by stirring in TFA at room temperature, followed by basic workup to give **81** in a 90% yield. The product was characterised by $^1$H-NMR showing characteristic loss of the intense Boc peak, and a UV-vis spectrum showing four Q-bands consistent with a free-base porphyrin.
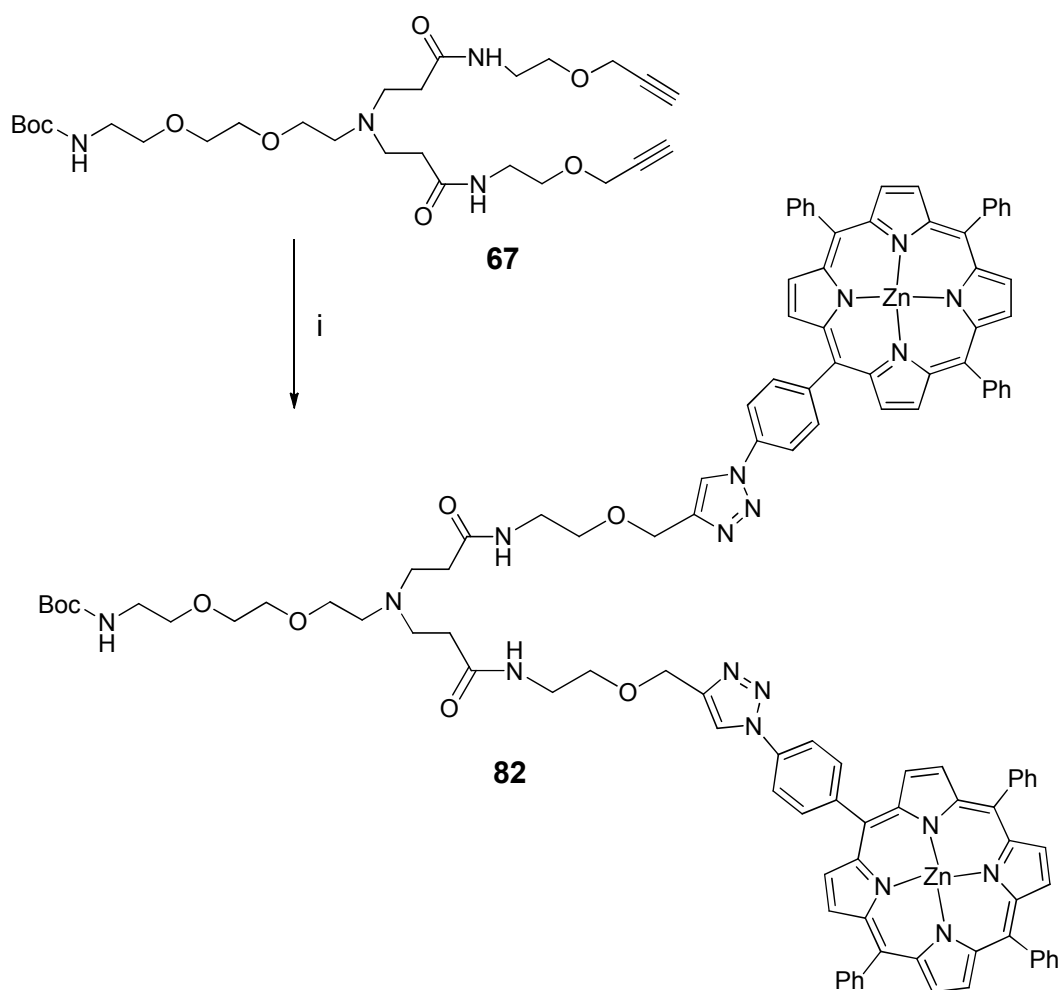


*Scheme 29: Reagents and conditions: (i) 5, copper (II) sulfate pentahydrate, sodium ascorbate, 80 °C (MW), 2 hours.*

Due to the poor solubility of dendron **67** in toluene, synthesis of porphyrin-dendron conjugate **82** was carried out in a solvent mixture of 1:1 THF:water, under microwave heating to 80°C. The reaction time was considerably longer than that observed for the synthesis of **80** and a small quantity of unreacted starting material **5** was observed after the reaction had finished, however the product was obtained in considerably improved (65%) yield with the structure confirmed by NMR and MS. In both cases, the unreacted porphyrin **5** and the moderate yields observed were attributed to competing homocoupling side reactions occurring between the alkyne-functionalised dendrons, a problem previously observed in the literature.[133]

### *3.7.1.2.    Synthesis of tris porphyrin-dendron*
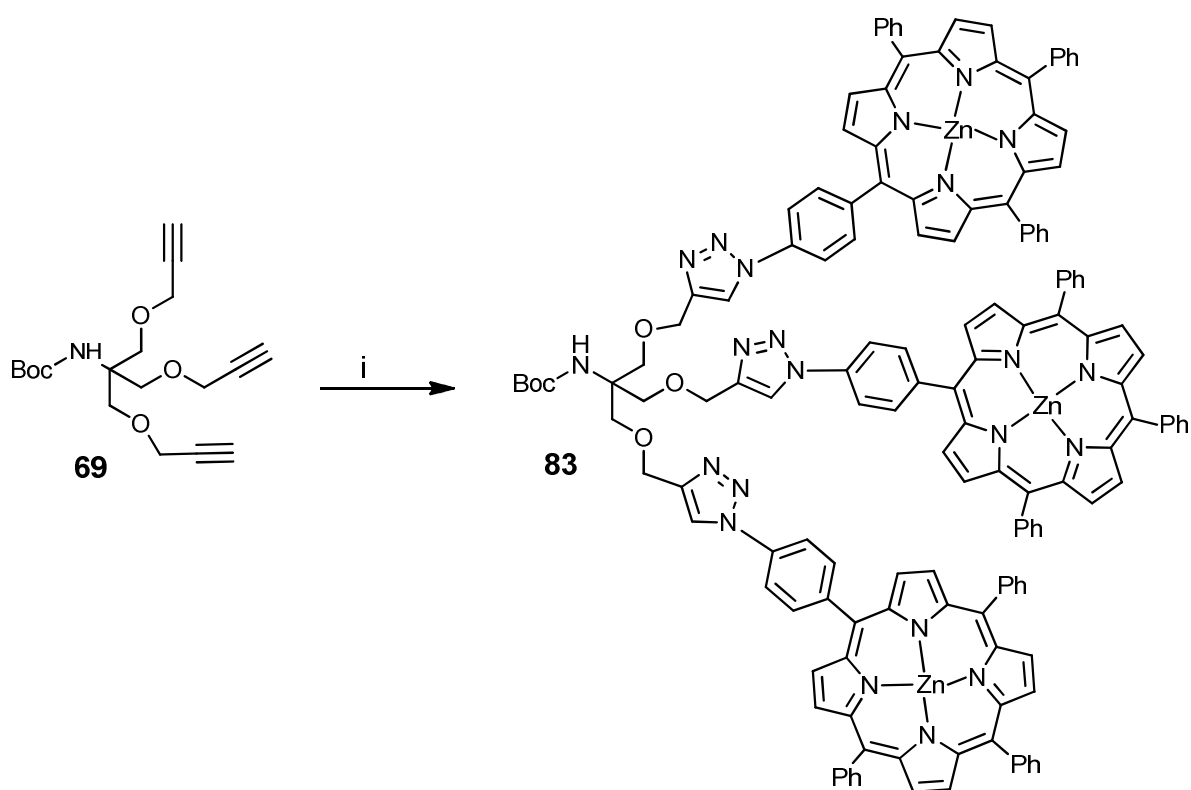


*Scheme 30: Reagents and conditions: (i) **5**, copper (II) sulfate pentahydrate, sodium ascorbate, 80 °C (MW), 3 hours.*

As for the synthesis of **80**, conjugation of porphyrin **5** to dendron **69** was carried out using a biphasic toluene:water solvent system under microwave heating, to yield **83** in a moderate 56% yield, as confirmed by NMR and MS. Again, highly forcing conditions were required in order to synthesise the desired product, with the increased reaction

time in comparison to the synthesis of **81** being attributed to the increased steric hindrance of addition of three rather than two porphyrins to the dendron skeleton.

### 3.7.1.3. *Synthesis of aryl ether porphyrin-dendrons*

Due to the limited solubility of the aryl ether dendrons in toluene:water, all of the conjugation reactions using these dendrons were carried out in a THF:water solvent system according to the conditions developed for product **82**.
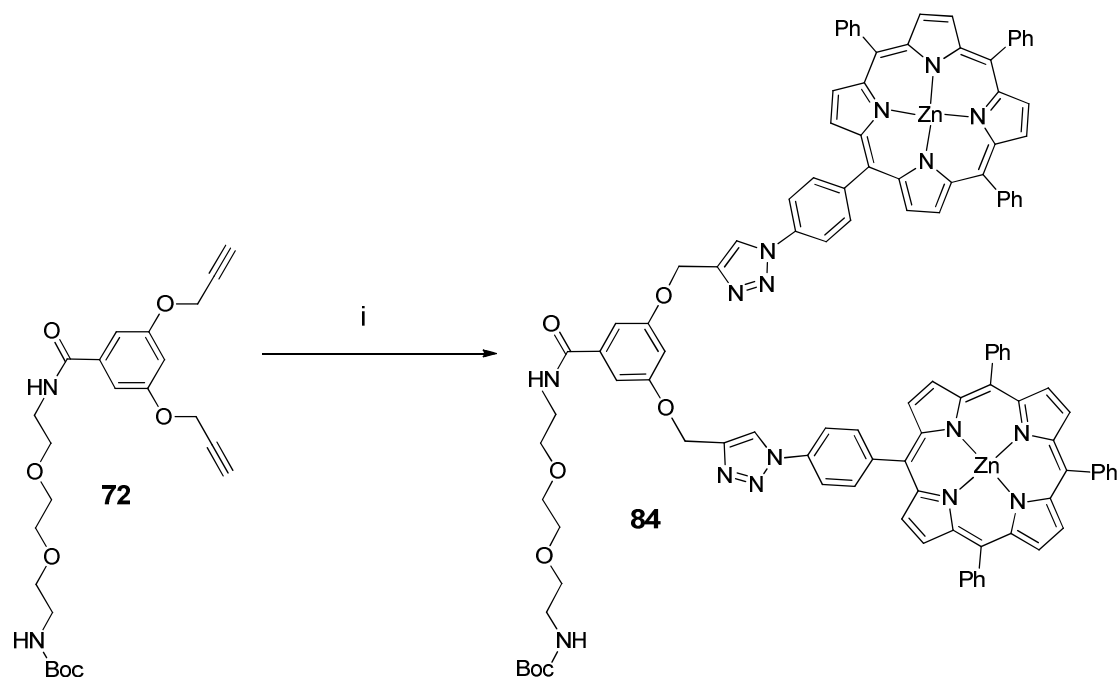


*Scheme 31: Reagents and conditions: (i) 5, copper (II) sulfate pentahydrate, sodium ascorbate, 80 °C (MW), 60 minutes.*

Synthesis of **84** proceeded rapidly and in excellent yield (91%), with only a small amount of residual starting material remaining. The reason for the far higher yield of this conjugate in comparison to **80** and **82** is unknown, however it could be attributed to reduced steric hindrance of the system allowing a more rapid reaction between dendron **72** and porphyrin **5** generating the desired product more rapidly and reducing effect of competing homocoupling reactions.

Synthesis of tris porphyrin conjugate **86** was carried out under identical reaction conditions, with the reaction proceeding more slowly and with only moderate yield (49%), with the conjugation of all three porphyrins confirmed by NMR and MS. Again, the long reaction time and poor yield can be attributed to increased steric hindrance of the system and formation of unwanted by-products. Deprotection of **86** was then carried out in TFA overnight, followed by an aqueous basic workup to yield **87** in excellent

yield, with the loss of the Boc group and zinc metallation accompanied by characteristic changes in both the NMR and UV-vis spectra.
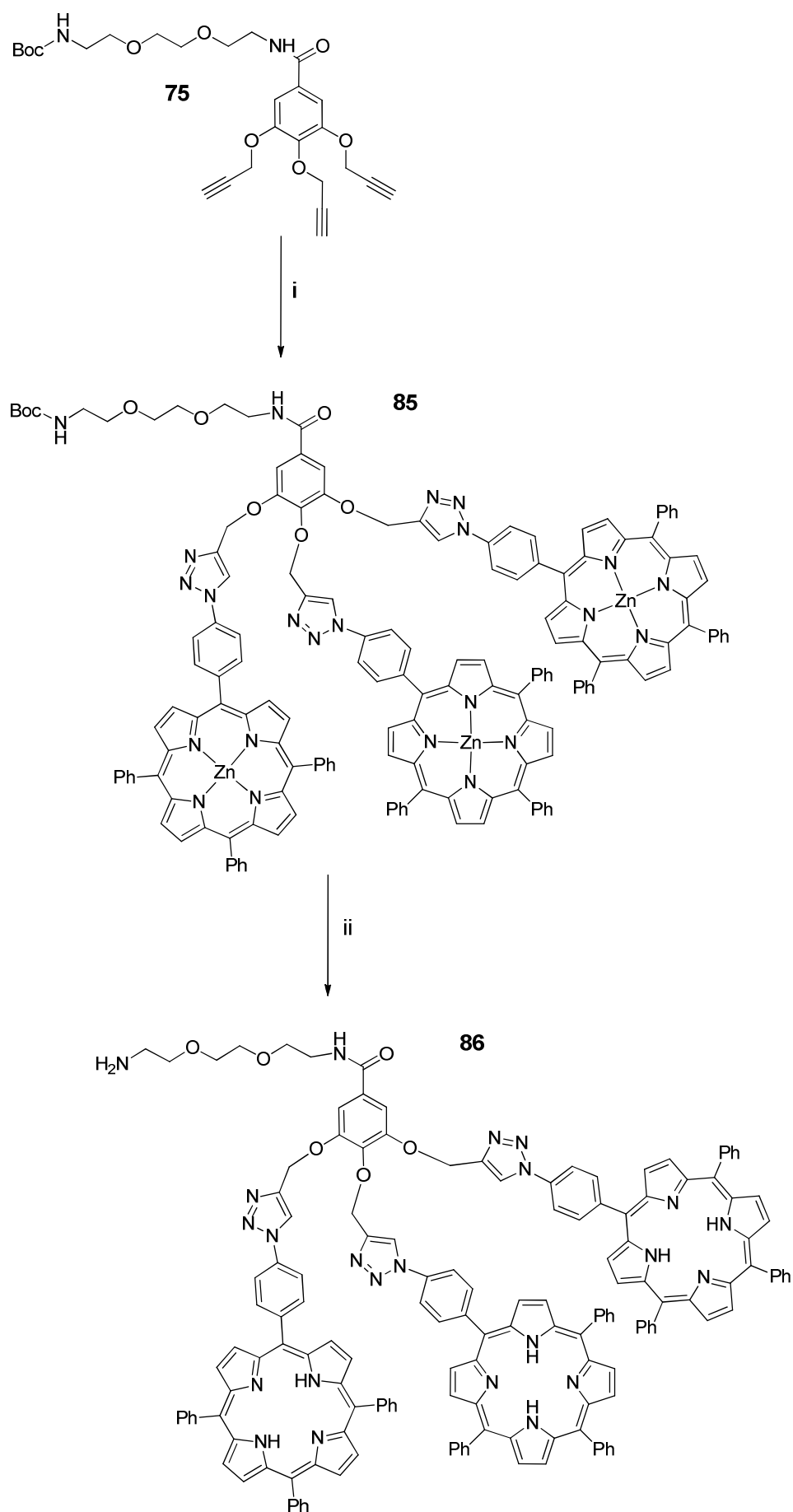
*Scheme 32: Reagents and conditions: (i)* **5**, *copper (II) sulfate pentahydrate, sodium ascorbate, 80 °C (MW), 100 minutes, (ii) TFA, rt, 17 hours.*

Synthesis of **87** was then carried out under the same conditions, with the reaction again taking around 100 minutes to reach completion by TLC. After purification by column chromatography, the product was obtained in moderate yield (52%) with the conjugation of all four porphyrin molecules confirmed by NMR and MS.
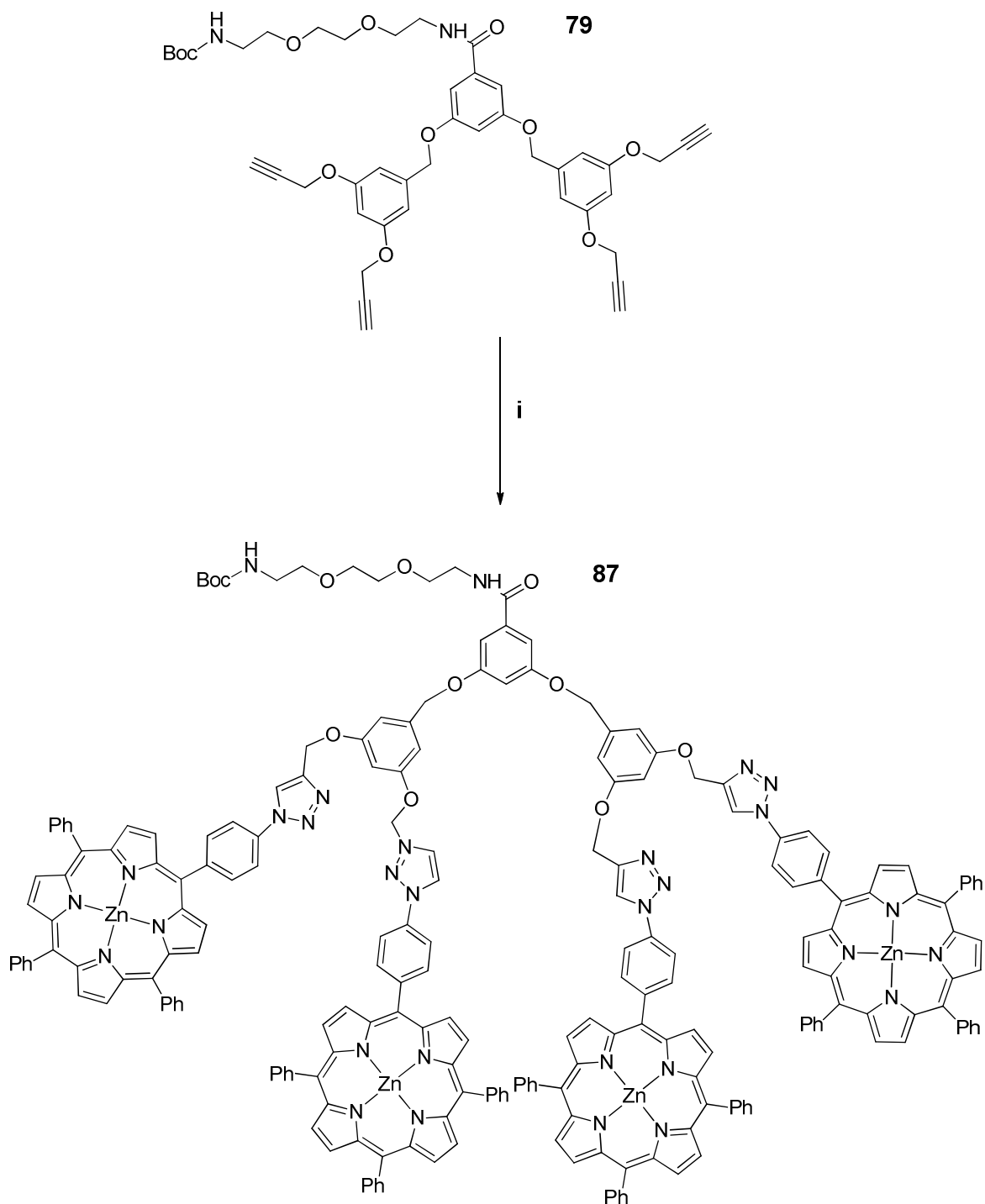


*Scheme 33: Reagents and conditions: (i) **5**, copper (II) sulfate pentahydrate, sodium ascorbate, 80 °C (MW), 100 minutes.*

UV-vis and fluorescence spectroscopy was carried out on all structures synthesised, with both spectra for all compounds showing retention of the characteristic bands of porphyrin **5** (Figure 40), confirming retention of the photophysical characteristics of the porphyrin despite close proximity to both the dendron and other porphyrins.
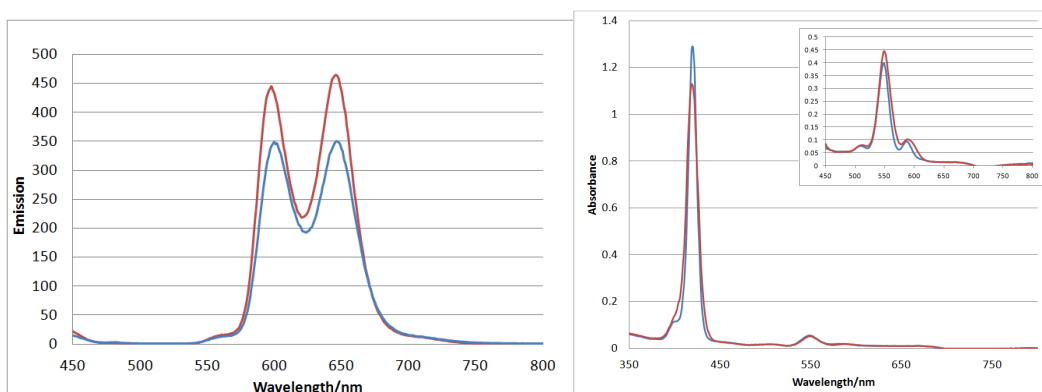


*Figure 40: Graphs showing fluorescence (left) and UV-vis (right) spectra of porphyrin **5** (red) and a representative example of a porphyrin-dendron conjugate (blue).*

Although structures showed the expected stoichiometry as confirmed by both NMR and MS, spectrophotometric determination of the porphyrin loading was in most cases lower than shown *via* other methods (Table 4). A possible cause for this loss of UV-visible absorbance and fluorescence could be due to the dendron structure forcing the porphyrins into close proximity, leading to quenching of the excited state. This is supported by the fact that highly strained systems such as **84** show considerable loss of UV-vis activity, while less strained systems such as **82** have spectrophotometric stoichiometry comparable to that determined by NMR.

| Structure | Stoichiometry determined by NMR | Stoichiometry determined by UV-vis absorption |
|---|---|---|
| **80** | 2 | 1.67 |
| **82** | 2 | 2.06 |
| **83** | 2 | 1.71 |
| **84** | 3 | 2.27 |
| **85** | 3 | 2.61 |
| **87** | 4 | 3.02 |

*Table 4: Calculation of dendron stoichiometry using NMR and UV-vis data. While all of the NMR and MS data shows the formation of the stoichiometric dendron structures, the reduced stoichiometry indicated by the UV-vis data suggests reduced UV-visible absorption due to steric hindrance.*

### 3.7.2. Synthesis of water soluble porphyrin-dendron conjugates

As the conjugation of a lipophilic porphyrin to all of the synthesised dendrons was carried out successfully, synthesis of hydrophilic conjugates using a water-soluble porphyrin was then attempted. Although a range of water soluble porphyrins had previously been synthesised, conjugation was attempted using the cationic azide porphyrins only, for several reasons. Firstly, the previous synthesis of porphyrins containing porphyrin **5** highlighted possible steric hindrance problems on some of the dendrons, leading to reduced yields and quenching between the porphyrins. As cationic azide porphyrins had been synthesised both with the azide directly on the porphyrin and *via* a linker chain, this allowed for synthetic flexibility to reduce the interactions between the porphyrins. Additionally, both the anionic and neutral porphyrins are less than ideal for use *in vivo*, giving high dark toxicity and a significant proportion of non-covalent bonding to antibody fragments respectively. As a result, use of these porphyrins could lead to issues with later biological testing, and was therefore not attempted.

#### *3.7.2.1. Synthesis of bis-cationic dendrons*

Initially, the synthesis of **88** was attempted *via* a simple room temperature stir, however after 48 hours no reaction was observed. Mild heating to approximately 40 °C overnight led to some reaction, however the reaction did not reach completion and the formation of several by-products was observed. For this reason, the synthesis was attempted using microwave heating in a $^t$BuOH:water solvent mixture, with the reaction showing complete consumption of the starting material after 1 hour at 40 °C, and NMR confirming the formation of the diporphyrin species. Attempts to increase reaction speed by increasing temperature were unsuccessful due to the formation of copper-porphyrin species as a result of trans-metallation of the zinc-protected porphyrin. However, addition of TBTA as a ligand for the Cu$^I$ catalyst led to a large increase in the rate of reaction, with the product formed in an 80% yield after just 20 minutes.
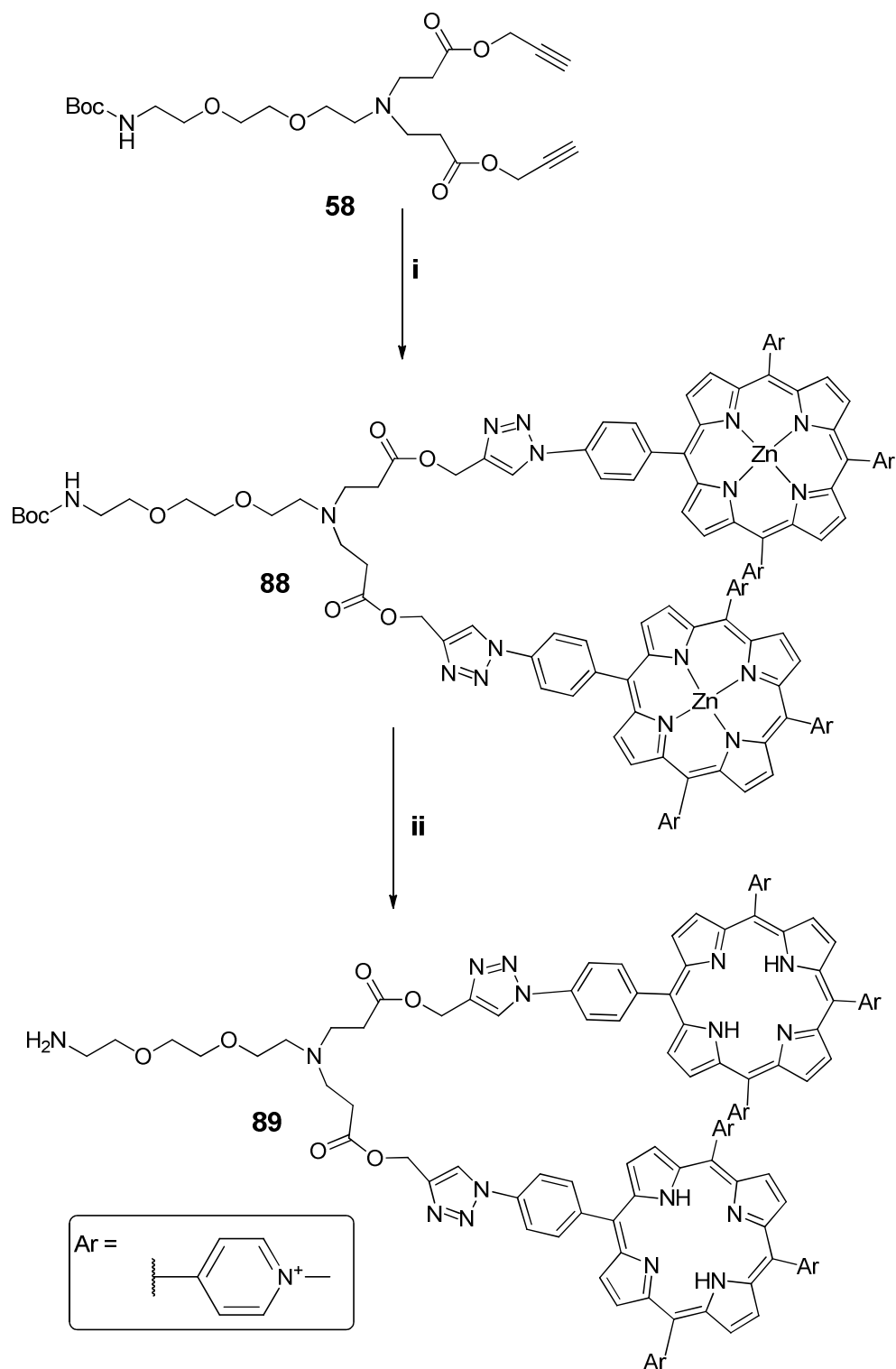
*Scheme 34: Reagents and conditions: (i) **17**, copper (II) sulfate pentahydrate, sodium ascorbate, TBTA, 40 °C (MW), 20 min, (ii) TFA, RT, 4 hours.*

Synthesis of **89** was then attempted by deprotection of **88** with TFA for 1 hour at rt., with TLC analysis after this time showing complete consumption of **88**. However, while some formation of the desired product was observed, the product of this reaction was a mixture of two products. The formation of the second product was attributed to acid-

catalysed degradation of the conjugate.
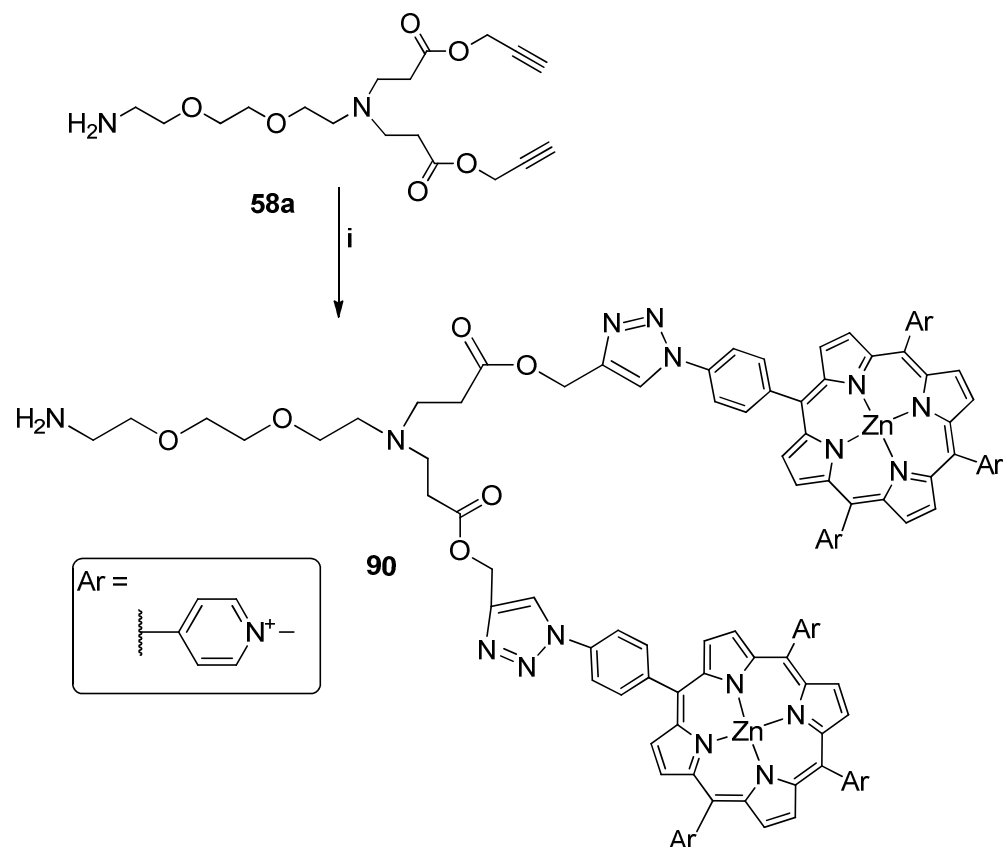


*Scheme 35:  Reagents and conditions: (i) **17**, copper (II) sulfate pentahydrate, sodium ascorbate, TBTA, 40 °C (MW), 20 min .*

For this reason, synthesis of the deprotected porphyrin-dendron conjugate was attempted through the conjugation of porphyrin **17** to a dendron bearing the free amine. Synthesis of deprotected PAMAM dendron **58a** was carried out through a room temperature stir in TFA overnight, with the NMR showing the loss of the characteristic Boc peak. Conjugation of **58a** to porphyrin **17** was then carried out as for the synthesis of **88**. After 20 minutes of microwave irradiation, HPLC analysis showed a product peak at the expected $R_f$ of 12.80 minutes, however a peak at 11.70 minutes corresponded to the $R_f$ value of the previously observed impurity, and comprised approximately 25% of the mixture. The quantity of this by-product porphyrin could be increased by simply leaving products **88** or **90** in solution at rt for extended periods of time, and after 24 hours was the sole species in the reaction mixture. NMR of the pure sample of this by-product identified it as a fragment of the dendron species (Figure 41).
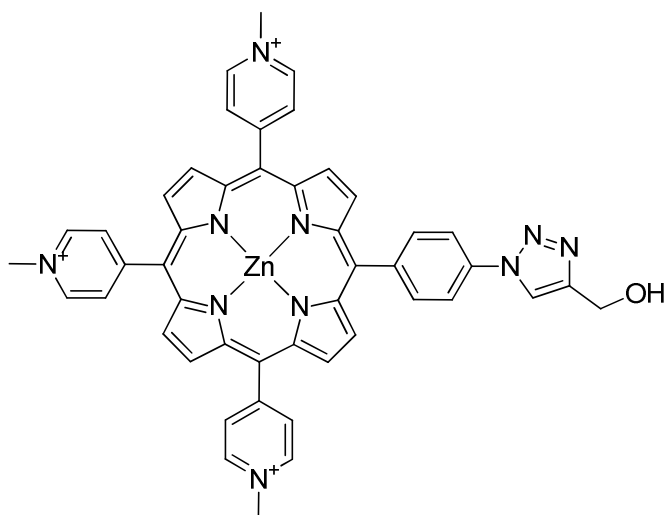
*Figure 41: By-product formed from the degradation of cationic porphyrin-dendron species **88** and **90**.*

The formation of this product is clearly the result of the hydrolysis of the ester of the dendron into the component acid and alcohol fragments, however the reason for this hydrolysis is not easily understood. Although the dendron only showed hydrolysis when in solution, hydrolysis of esters by water alone is generally a very slow process, and usually requires catalysis from either an acid or a base. In addition, both dendron **58** and porphyrin-dendron conjugate **80** exhibited no cleavage of this ester bond, suggesting that this cleavage cannot simply be due to either inherent reactivity of the dendron or simple steric effects of having two porphyrins attached to this molecule. One possible explanation for this process is that proximity of two highly charged porphyrins in **88** and **90** decreases the stability of the molecule to the point where hydrolysis by water alone is possible. As a result, it is clear that synthesis of a biologically stable cationic porphyrin-dendron conjugate from dendron **58** is unlikely, and for this reason no further synthesis was attempted on this molecule.

As the ester bond in dendron **58** was shown to exhibit poor hydrolytic stability when conjugated to cationic porphyrins, synthesis of bis cationic dendrons was then attempted from dendron **67**, which contains stronger amide bonds rather than esters.
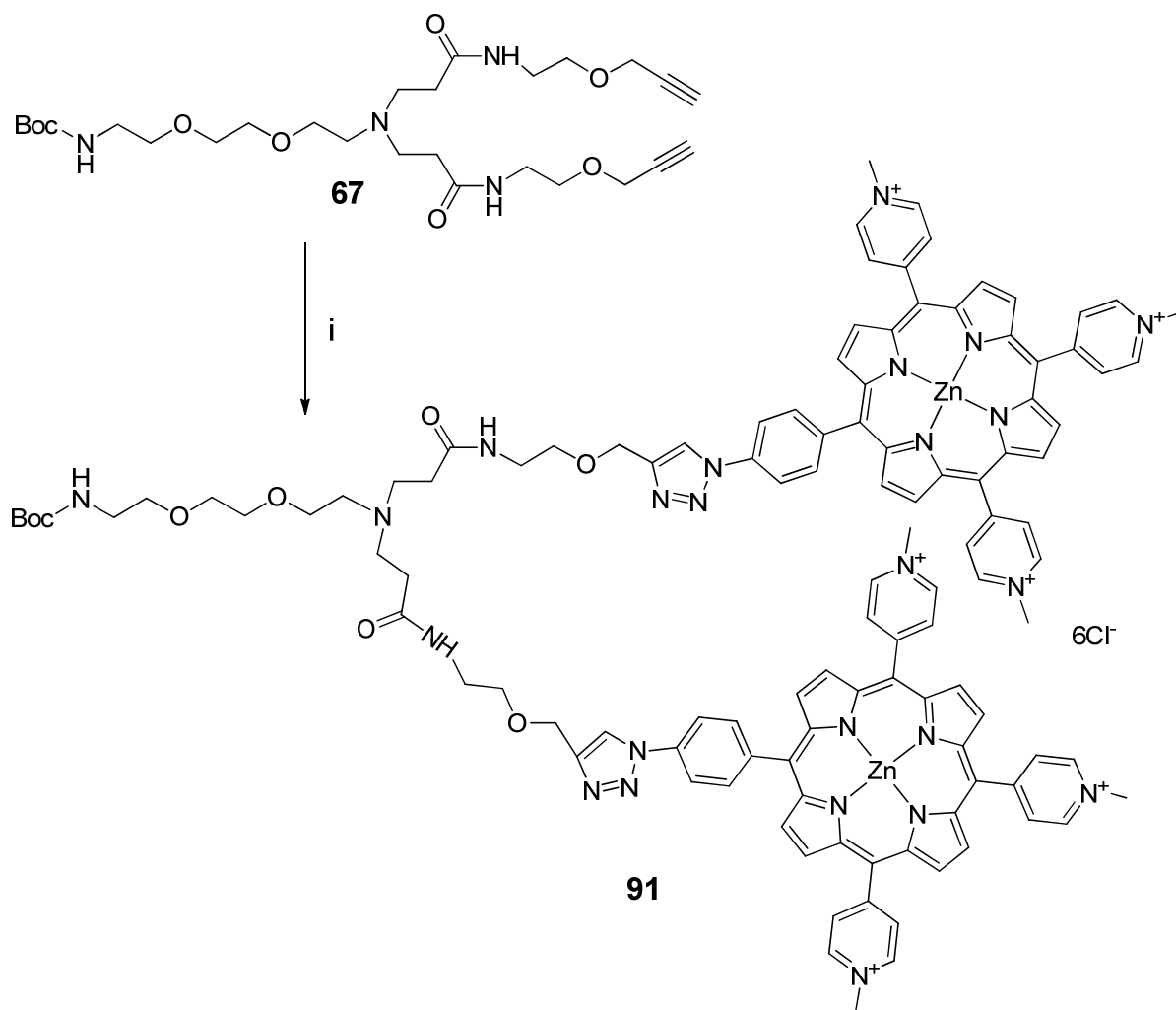
*Scheme 36: Reagents and conditions: (i)* **17**, *copper (II) sulfate pentahydrate, sodium ascorbate, TBTA, 45 °C (MW), 90 min.*

Conjugation of **17** to dendron **67** was again carried out under microwave irradiation, with the reaction mixture heated to 45 °C with no sign of transmetallation of the copper catalyst. After 90 minutes, complete consumption of the porphyrin starting material was observed by TLC, with the identity of the desired product confirmed by NMR. Unlike the synthesis of the analogous lipophilic conjugate **82**, no residual starting porphyrin was observed upon completion of the reaction, and the desired product was obtained in excellent yield. The difference in the two reactions is likely to be as a result of the water-solubility of the porphyrin **17**, which allows the reaction to proceed without the need for a biphasic reaction mixture. As a result, the reaction occurs at lower temperature and more rapidly, reducing the presence of side products produced through competing homocoupling reactions.

The stability of the synthesised conjugate **91** in solution was also trialled. However, unlike conjugate **88**, the formation of degradation products was not observed during an acidic deprotection of the Boc group, and the dendron showed no degradation in aqueous solution for 48 hours both before and after deprotection. This confirms that the degradation of conjugates **88** and **90** was as a result of the cleavage of the ester bond, a process which does not occur with the more hydrolytically stable amide bond of **91**.
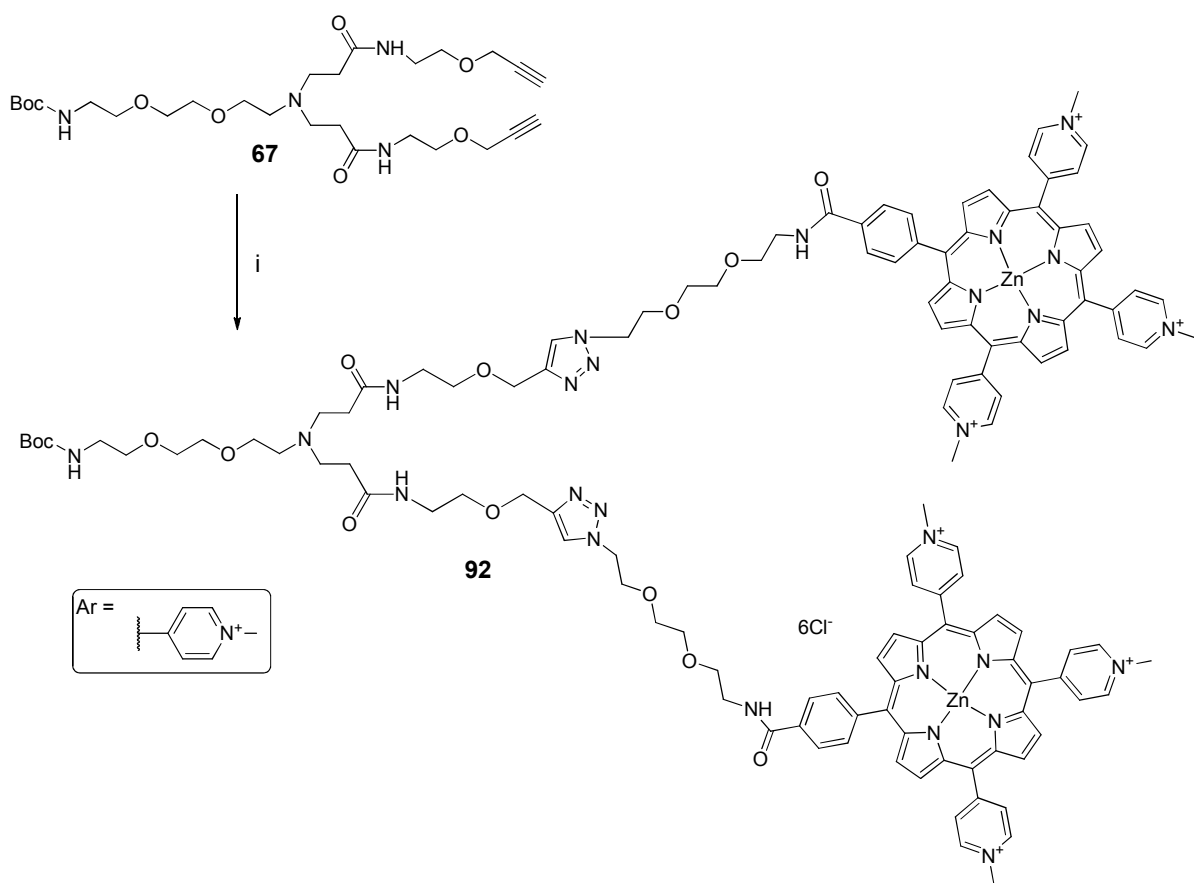


*Scheme 37: Reagents and conditions: (i) **32**, copper (II) sulfate pentahydrate, sodium ascorbate, TBTA, 45 °C (MW), 25 min.*

Synthesis of **92** was carried out under identical reaction conditions as **91**, with the synthesis of the conjugate proceeding much more rapidly than the synthesis of **91**. This increased speed of reaction can be attributed to the linker chain on the porphyrin leading to reduced steric hindrance in the system and increased reaction speeds. As for the synthesis of **91**, the synthesis of **92** proceed in far better yield than that seen for the lipophilic porphyrin-dendron conjugates, with the identity of the porphyrin confirmed by NMR.

### 3.7.2.2. Synthesis of tri-cationic porphyrin-dendron.
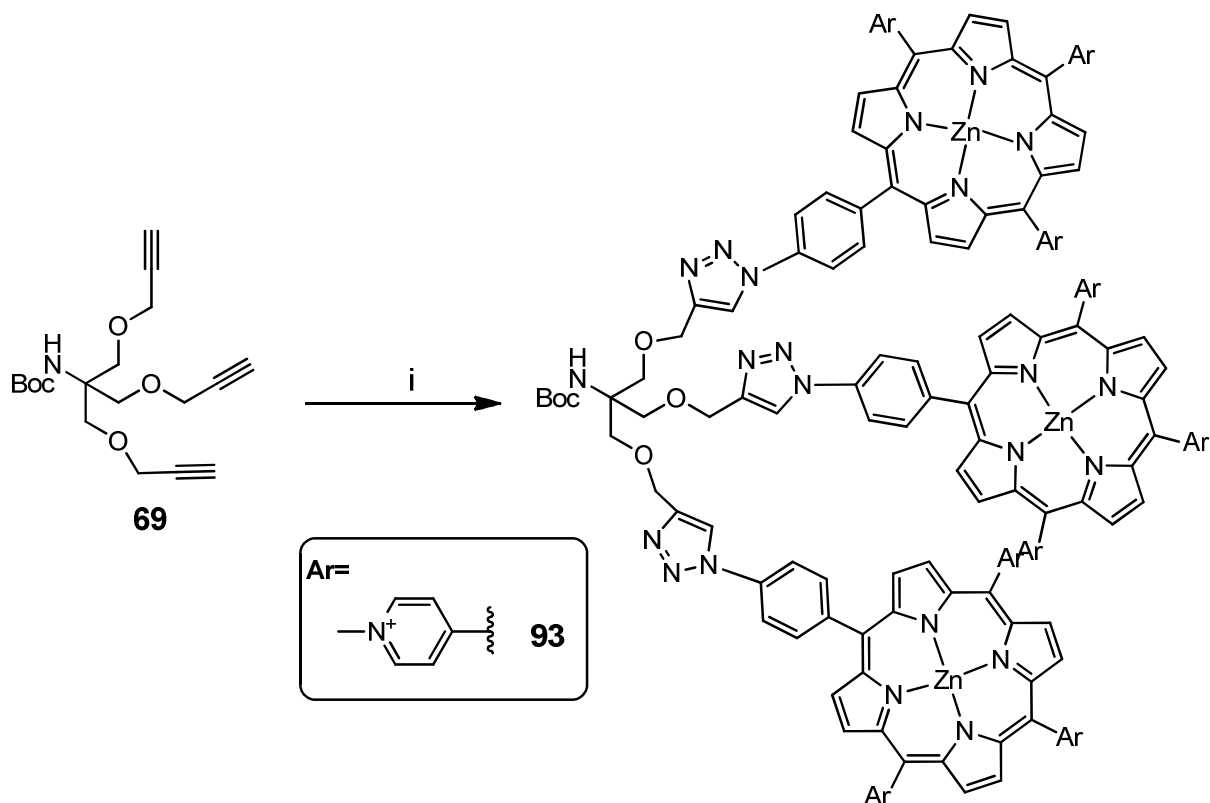


Scheme 38: Reagents and conditions: (i) **17**, copper (II) sulfate pentahydrate, sodium ascorbate, TBTA, 45 °C (MW), 5 hours.

The conjugation of **17** to dendron **69** was carried out under click conditions optimised from previous conjugations. However, after heating to 45 °C for 5 hours, HPLC analysis of the crude mixture showed large quantities of residual porphyrin starting material as well as several minimal impurities, and a large peak at 9.42 min. NMR analysis of this mixture showed that the only product was the dendron with a single porphyrin attached, with no production of the bis or tris porphyrin species observed. Conjugation of the single porphyrin only was assumed to be as a result of the close proximity of the three peripheral alkyne groups, and the resulting high steric hindrance preventing the conjugation of more than one porphyrin. As a result, no further conjugation was attempted to dendron **69**.

### 3.7.2.3. Synthesis of aryl ether dendrons
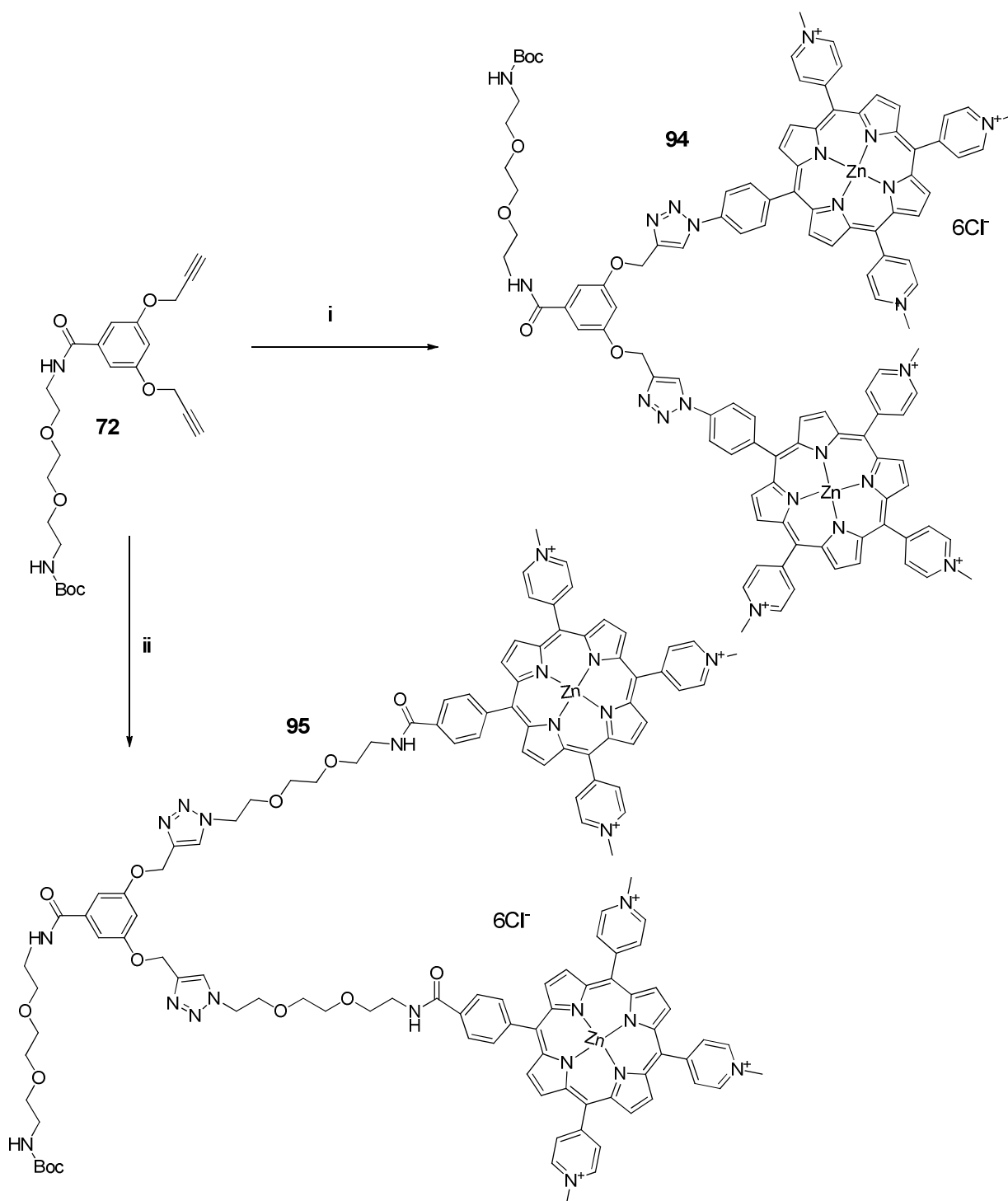


Scheme 39: Reagents and conditions: (i) **17**, copper (II) sulfate pentahydrate, sodium ascorbate, TBTA, 45 °C (MW), 30 minutes, (ii) **32**, copper (II) sulfate pentahydrate, sodium ascorbate, TBTA, 45 °C (MW), 25 minutes.

Conjugation of both porphyrins **17** and **32** to the di-alkyne aryl ether dendron **72** was again carried out under microwave irradiation at 45 °C, with both reactions proceeding rapidly and in excellent yield. NMR confirmed the structure of both **94** and **95**, with the

successful conjugation of two porphyrins in both cases.
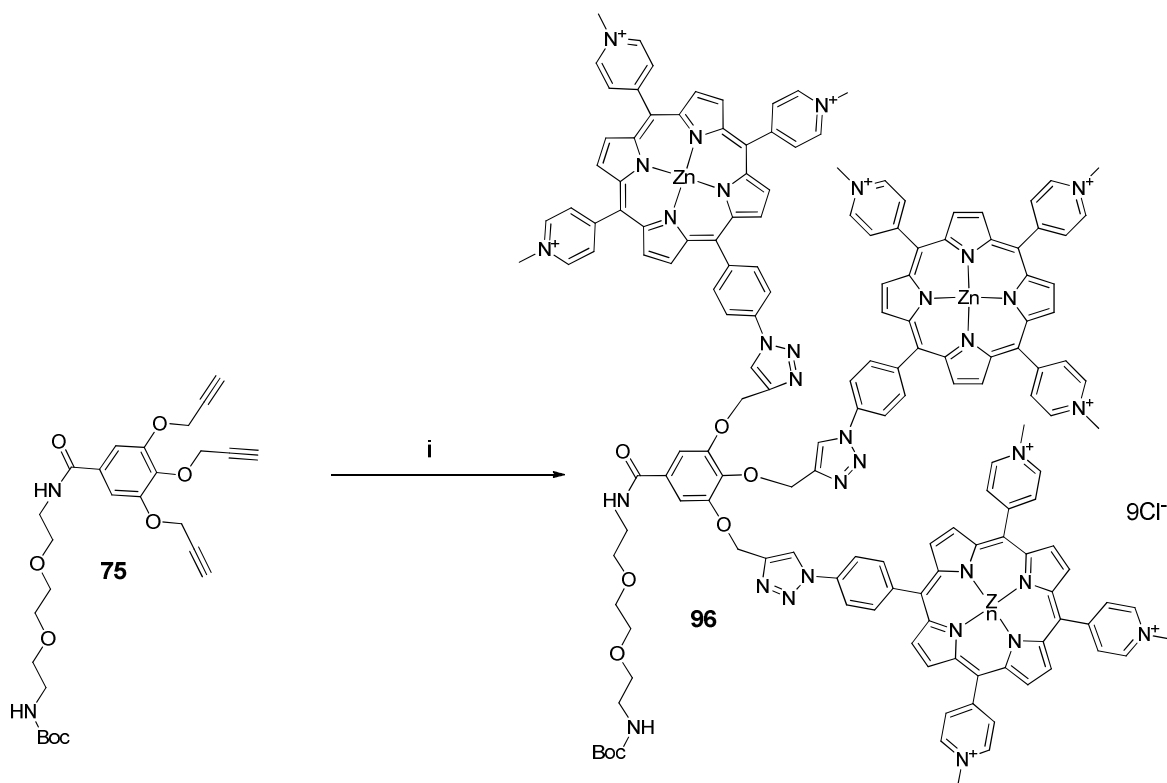


*Scheme 40: Reagents and conditions: (i) **17**, copper (II) sulfate pentahydrate, sodium ascorbate, TBTA, 45 °C (MW), 5 hours.*

However, in comparison conjugation of porphyrin **17** to dendron **75** was considerably slower. Monitoring of the reaction by HPLC showed the formation of two products after 3 hours microwave irradiation, with retention times of 9.20 and 11.50 minutes. Addition of more **17** and further heating led to conversion of the product at 9.20 minutes to the product at 11.50 minutes, yielding a pure sample of this product. However, NMR analysis showed the only product to be the bis porphyrin structure, with no formation of the desired product **96**. As seen for the synthesis of **93**, the long reaction time and lack of formation of the tri substituted species can be attributed to the high steric hindrance of the system, however central aromatic ring of **96** reduces this steric hindrance and allows conjugation of a second porphyrin in comparison to **93**. It is assumed that conjugation of two porphyrins occurs at the 3 and 5 positions of the aryl ring, allowing no room for the conjugation of the third porphyrin at the 4 position.
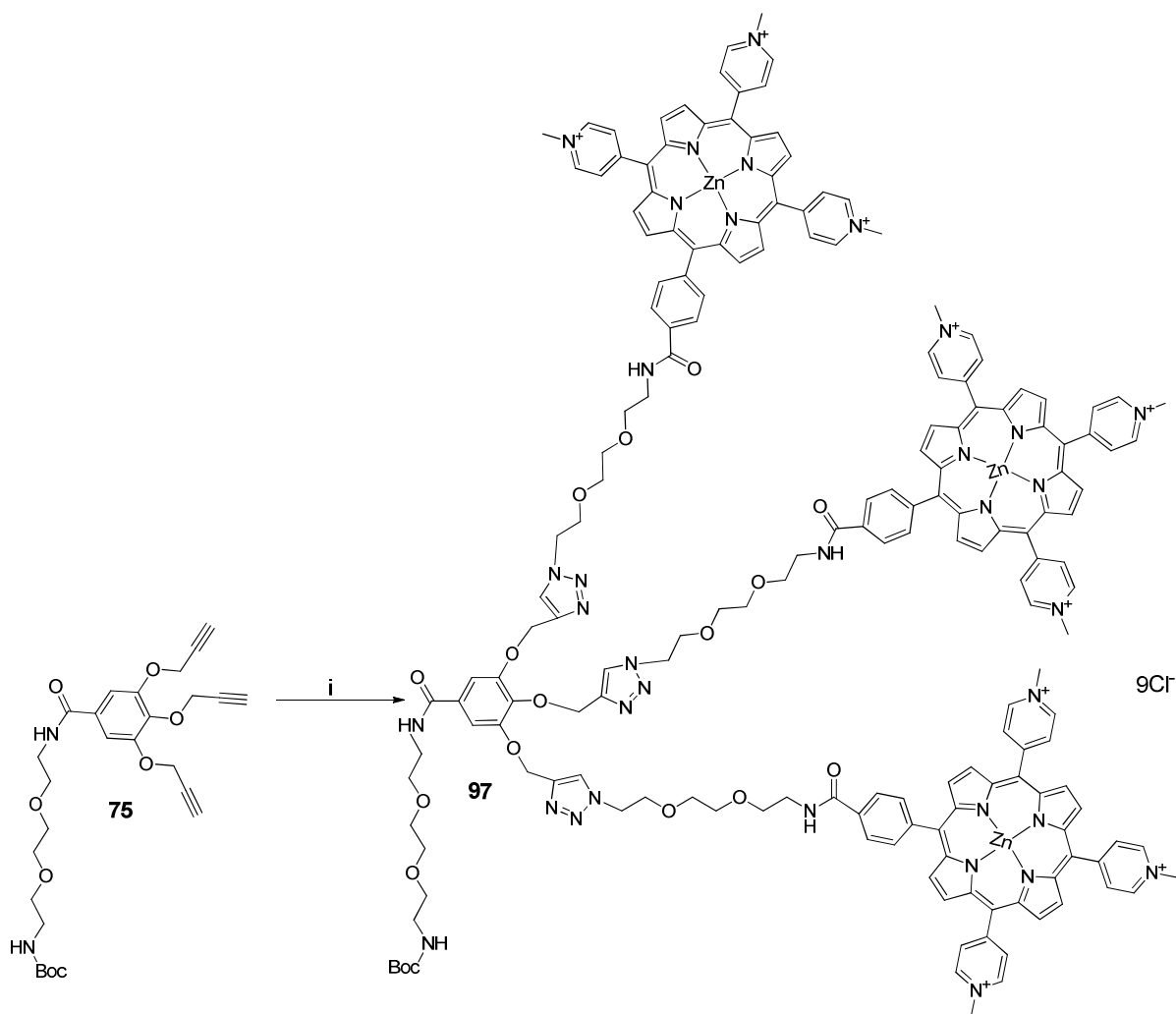
*Scheme 41: Reagents and conditions: (i) 32, copper (II) sulfate pentahydrate, sodium ascorbate, TBTA, 45 °C (MW), 1 hour.*

For this reason, conjugation to **75** was then attempted using porphyrin **32**, with the aim of using the linker chain to reduce steric hindrance and allow conjugation of 3 porphyrins to the dendron. As expected, the synthesis of **97** was considerably faster than the synthesis of **96**, with the HPLC showing complete consumption of the starting material after 1 hour. NMR confirmed the conjugation of three porphyrins to the dendron structure, with the desired product obtained in excellent yield.
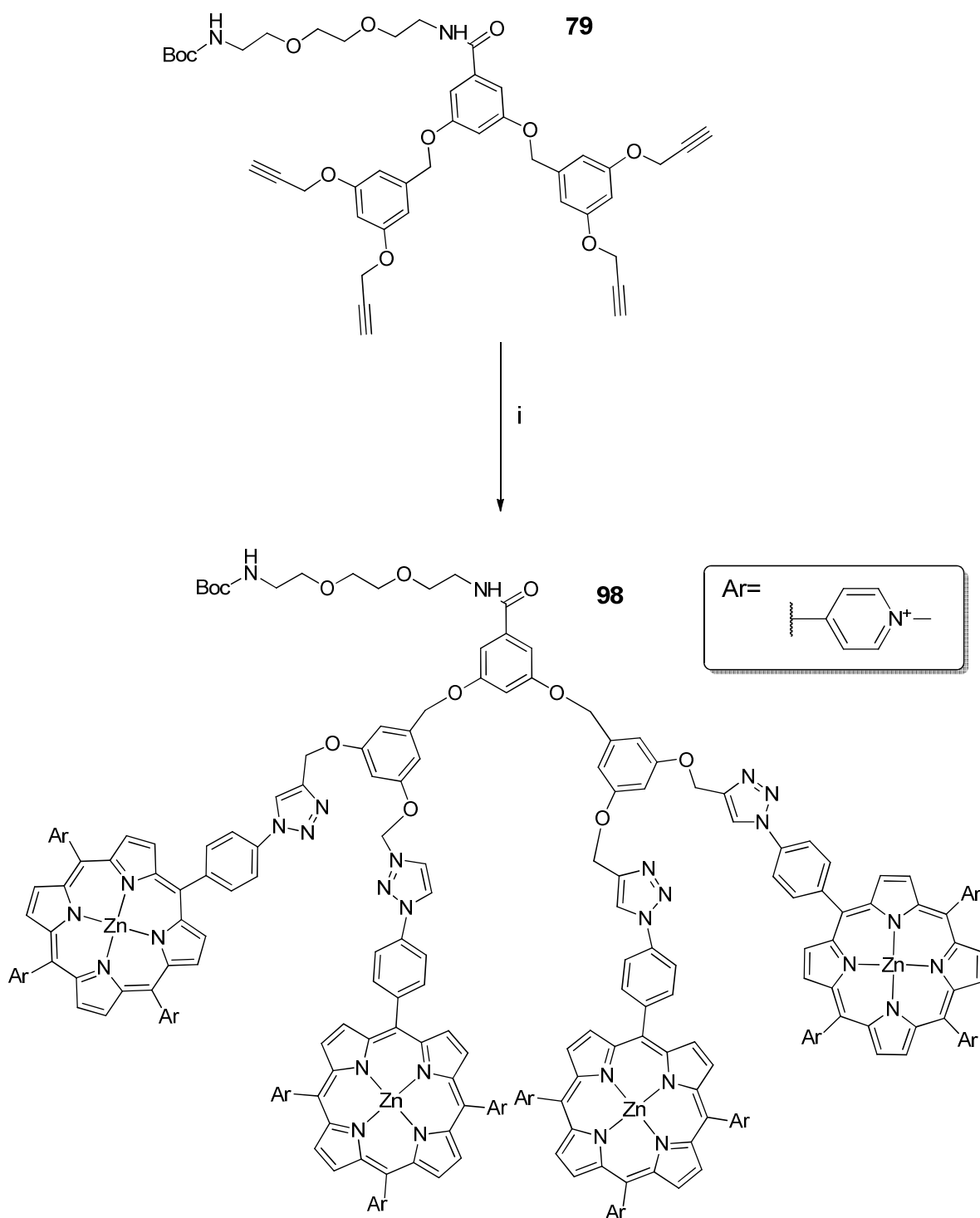
*Scheme 42: Reagents and conditions: (i) 17, copper (II) sulfate pentahydrate, sodium ascorbate, TBTA, 45 °C (MW), 5 hours.*

Synthesis of the tetraporphyrin dendron conjugate **98** was initially attempted through the conjugation of **17** to dendron **79** under identical conditions to the previous porphyrin dendron conjugates. However, due to the poor solubility of dendron **79** in *t*-butanol, no reaction was observed after 1 hour heating to 45 °C. Synthesis was then attempted in a THF:water solvent system, with HPLC analysis showing formation of multiple products

after 30 minutes. Extended heating of the mixture showed formation of a single product by HPLC, however NMR analysis of the product revealed formation of the tri-substituted dendron only. As for the synthesis of **96**, the lack of the desired product was attributed to the close proximity of the porphyrins on the structure and the resulting high steric hindrance.
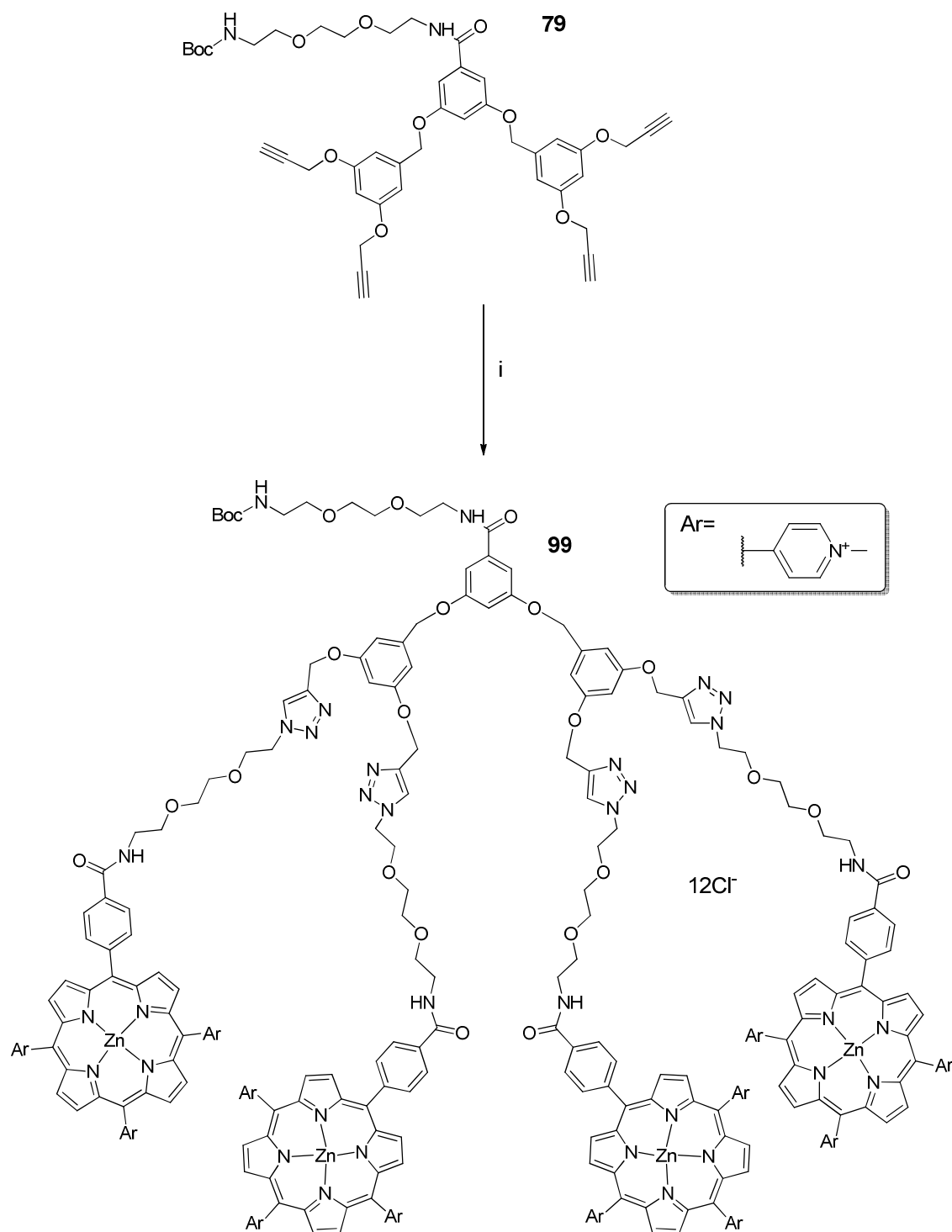


*Scheme 43: Reagents and conditions: (i) **17**, copper (II) sulfate pentahydrate, sodium ascorbate, TBTA, 45 °C (MW), 90 minutes.*

For this reason, conjugation of **79** to the porphyrin **32** was attempted under identical conditions. As for the synthesis of **97**, use of this linker chain bearing azide porphyrin led to significantly reduced reaction times and formation of the desired product, as confirmed by NMR. However, synthesis of **99** did require increased reaction times in comparison to the synthesis of all other porphyrin-dendron conjugates using porphyrin **32**, and led to poorer yields with a considerable quantity of the porphyrin starting material remaining at the completion of the reaction. This can be attributed to the increasing steric hindrance of the system as the number of porphyrins increases, which could be reduced by the use of a longer linker chain on the porphyrin. This would allow synthesis of structures bearing four porphyrins more rapidly and with improved yield, and could also allow for the synthesis of dendron structures with greater than four porphyrins conjugated to the periphery.

### 3.7.3. Photophysical characteristics of cationic conjugates

As for the lipophilic dendron conjugates, the photophysical characteristics of the cationic dendrons were examined (Figure 42), and in both cases the fluorescence and the UV-vis data of all dendrons was found to be highly similar to the unconjugated porphyrin despite the high steric hindrance in the system.
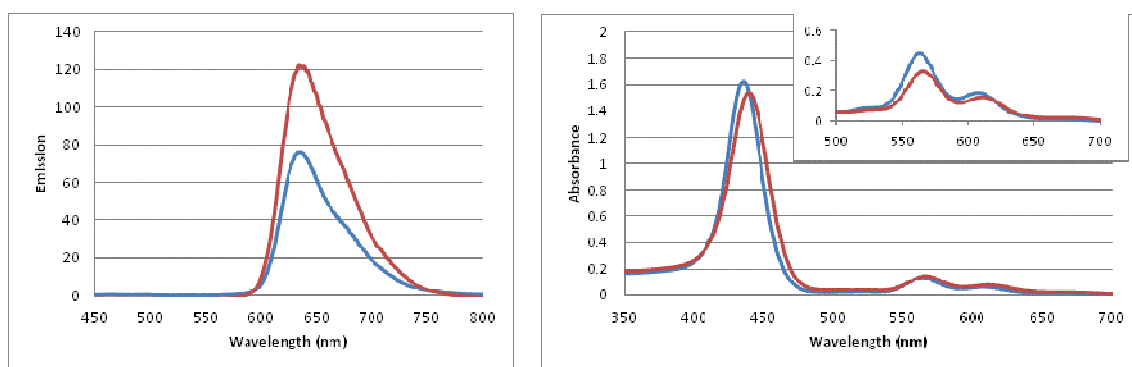


*Figure 42: Graphs showing fluorescence (left) and UV-vis (right) spectra of porphyrin 17 (red) and a representative example of a porphyrin-dendron conjugate (blue).*

Spectrophotometric determination of the porphyrin loading was then carried out and the values obtained compared to those obtained through NMR (Table 5). In all cases, as seen for the lipophilic dendrons, the calculated spectrophotometric values were lower than those obtained *via* NMR integration, as a result to quenching of the porphyrin excited state due to the close proximity of the porphyrins. This is supported by the fact

that for all dendrons bearing two porphyrins, the spectrophotometrically determined stoichiometry was higher for structure containing the PEG-spacer functionalised porphyrin **17** and the longer-armed PAMAM dendron **67**, both of which would be expected to be associated with lower steric hindrance.

| Structure | Stoichiometry determined by NMR | Stoichiometry determined by UV-vis absorption |
|:---:|:---:|:---:|
| **91** | 2 | 1.43 |
| **92** | 2 | 1.49 |
| **94** | 2 | 1.01 |
| **95** | 2 | 1.36 |
| **97** | 3 | 1.92 |
| **99** | 4 | 3.48 |

*Table 5: Calculation of dendron stoichiometry using NMR and UV-vis data. As for table 1, while stoichiometry as determined by NMR shows the correct number of porphyrins attached in each case, the steric hindrance of the system leads to reduced UV-vis absorption values.*

In particular, the lowest UV-vis calculated values were obtained for dendrons **94** and **97**, suggesting that both are highly sterically hindered systems. This is unsurprising, as conjugate **94** contains the most sterically hindered porphyrin and dendron structures, and conjugate **97** contains the three alkyne arms in close proximity on a single aryl ring. In contrast, conjugate **99** shows far less quenching of the porphyrins in comparison to **97**, likely as a result of the greater distance between the four porphyrins on the larger second generation structure. As a result, future work could attempt to reduce steric hindrance in the three porphyrin conjugate through synthesis of a second generation aryl ether structure bearing three alkyne functionalities, or through use of a longer alkyne-functionalised chain instead of propargyl bromide to create the dendron arms. Alternatively, synthesis of a porphyrin bearing a longer PEG chain would allow for reduction of steric hindrance in all of the conjugates, and would allow for calculated spectrophotometric data to more closely mirror the NMR values.

Following the successful characterisation of all porphyrin-dendrons by UV-vis and fluorescence, singlet oxygen quantum yield (SOQY) data was obtained by Geraldine Rosser (Durham University). Data was collected for all synthesised cationic porphyrin-dendron conjugates and two control porphyrins **17a** and **32a** (Figure 43), synthesised

from the click conjugation of porphyrins **17** and **32** to propargyl alcohol, allowing evaluation of the effect of the triazole linkage on the SOQY.
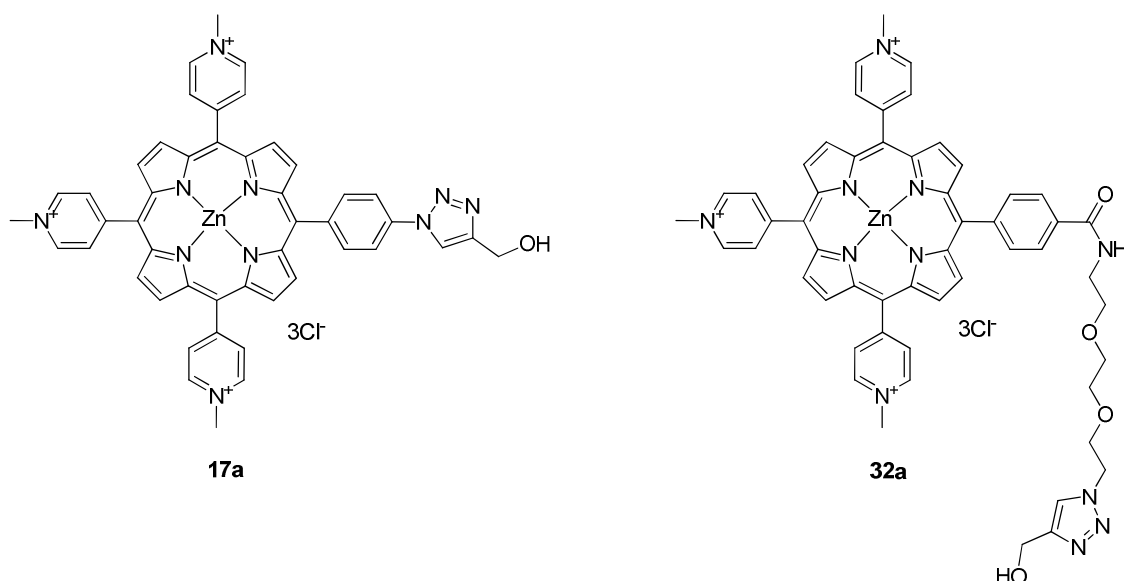


*Figure 43: Structures of comparison compounds **17a** and **32a**.*

Singlet oxygen quantum yield data was collected for all structures at 355 nm in $D_2O$, with the results normalised against absorption of the conjugates at this wavelength.

| Sample | Singlet oxygen quantum yield | Conjugate:parent porphyrin UV-vis absorption ratio | Conjugate potential singlet oxygen yield |
|--------|------------------------------|---------------------------------------------------|------------------------------------------|
| **17a** | 0.47 | 1 | 0.47 |
| **32a** | 0.86 | 1 | 0.86 |
| **91** | 0.27 | 2.09 | 0.56 |
| **92** | 0.36 | 2.25 | 0.81 |
| **94** | 0.21 | 1.41 | 0.29 |
| **95** | 0.27 | 1.98 | 0.53 |
| **97** | 0.28 | 2.68 | 0.75 |
| **99** | 0.28 | 4.30 | 1.20 |

*Table 6: Measured singlet oxygen quantum yields and calculated UV-vis absorption ratios and potential singlet oxygen yields for synthesised porphyrin-dendron conjugates.*

In general, it can be seen that the SOQY values are highest for the control porphyrins

**17a** and **32a**, with the result obtained for **32a** considerably higher than that of **17a**. These results are unsurprising; the close proximity of the porphyrins to each other in all of the porphyrin-dendron conjugates will lead to quenching and reduction of singlet oxygen quantum yield in comparison to the sterically unhindered single porphyrins. The reduced SOQY of **17a** in comparison to **32a** suggests that the proximity of the triazole ring in some way reduces the quantum yield, possibly as a result of quenching of the porphyrin.

While all synthesised porphyrin-dendron conjugates show reduced SOQY in comparison to the control porphyrins, a considerable variation between different conjugates is still observed. In general, it can be seen that reducing steric effects through the use of porphyrins bearing linker chains leads to a higher SOQY, with the result for **92** being considerably higher than the result obtained for **91,** and similarly the result obtained for **95** being higher than the result obtained for **94.** In addition, bis porphyrin conjugates synthesised from aryl ether dendron **72** (**94** and **95**) in comparison to the PAMAM dendron **67** (**91** and **92**) show poorer SOQY in both cases, a fact which can be attributed to the shorter arm length of the aryl-ether dendron and corresponding increased steric hindrance.

Both of the larger conjugates **97** and **99** display lower SOQY than the bis dendron **92**, which is expected due to the increased steric hindrance caused by additional porphyrins and the use of the aryl ether rather than PAMAM structure. However, it is surprising to note that the results obtained from **97** and **99** are similar to the results obtained from **94** and **95**, suggesting that the steric hindrance in these systems is not significantly higher than that observed utilising a bis aryl ether dendron. In particular, it is of note that the results for both **97** and **99** are identical, despite the presence of four porphyrins on **99**. This result suggests, similar to the UV-vis data, that the use of the higher generation dendron allows for significantly reduced steric hindrance, supporting the hypothesis that a second-generation structure bearing 3 porphyrins might give improved photophysical and biological results in comparison to **97**.

Overall, this data highlights the importance of controlling steric hindrance factors in these structures, with the addition of a linker chain to the porphyrin significantly improving the photophysical properties of the conjugate. However, despite the use of

porphyrin **32**, a steric hindrance effect is observed in both UV-vis and SOQY results, suggesting that the length of this linker chain is not optimised and a porphyrin-dendron conjugate with improved photophysical characteristics could be synthesised utilising a porphyrin with a longer linker chain. While theoretically the linker chain length could be increased until the porphyrins no longer interacted more than individual porphyrins in solution, the theoretical improvement needs to be evaluated against the cost and synthetic complexity of utilising such a long chain.

Although all synthesised porphyrin-dendron conjugates show reduced SOQY when compared to the control porphyrins, this does not mean that a single porphyrin would necessarily produce a larger absolute quantity of singlet oxygen than a porphyrin-dendron conjugate as part of a targeted photosensitiser. This is because the SOQY is defined as the quantity of singlet oxygen produced per quanta of light absorbed, however significantly larger amounts of light will be absorbed by porphyrin-dendron conjugates compared to single porphyrins due to their increased absorptivity values. As a result, while the porphyrin-dendron conjugates will produce less singlet oxygen per quanta of absorbed light, the increased absorption of light may mean that they produce more singlet oxygen overall.

For this reason, the ε value was calculated for control porphyrins **17a** and **32a** and all of the synthesised dendrons. This value was then multiplied by SOQY to give an indication of the quantity of singlet oxygen which could be produced by a fixed quantity of a targeted photosensitiser containing one of these porphyrin-containing structures.

These results suggest that dendrons **91** and **92** have the potential to produce the same or greater absolute amounts of singlet oxygen than the corresponding control porphyrin, suggesting that they should therefore show improved cytotoxic effects in comparison to single porphyrins when utilised in targeted photosensitisers. In contrast, both dendrons **94** and **95** show values lower than those obtained for the corresponding control porphyrin, suggesting that the steric hindrance which limits both the SOQY and the absorbance may lead to poorer cell killing effects *in vivo*. Similarly, **97** shows particularly poor results, with a potential absolute singlet oxygen production below that of the control porphyrin despite the 3 attached porphyrins. As previously suggested, this result can be attributed to the extremely high steric hindrance in the system potentially limiting the effectiveness of this porphyrin-dendron conjugate as part of a targeted

photosensitiser. In contrast, results obtained for **99** are more promising, with a potential singlet oxygen production of around 1.5 times higher than the control porphyrin, suggesting that 2$^{nd}$ generation aryl ether structures may be more effective as multiplier groups than 1$^{st}$ generation structures.

## 3.8.  Conclusion

To date, a range of dendrons including PAMAM, tris and aryl ether type dendrons have been synthesised, all bearing between 2 and 4 alkyne peripheral groups, and a protected amine focal point. Conjugation of both lipophilic and hydrophilic porphyrins to the peripheral groups of all synthesised dendrons has been attempted using microwave irradiation. In all cases, click conjugation of lipophilic porphyrin **5** to the synthesised dendrons was carried out successfully, with subsequent UV-vis and fluorescent data showing the retention of the photophysical characteristics of the porphyrin, but with loss of some UV-vis absorbance suggesting steric hindrance in the system and subsequent quenching of the porphyrin.

Conjugation of cationic porphyrins to all dendrons was attempted, and while synthesis of all bis-porphyrin dendrons was successful, conjugation of greater than two porphyrins was only possible utilising the PEG linker chain functionalised porphyrin **32**, a fact which was attributed to the high steric hindrance of the system. The photophysical properties of all synthesised cationic porphyrin-dendron conjugates were evaluated, including UV-vis, fluorescence and singlet oxygen quantum yield measurements. The results obtained showed some quenching effects due to steric hindrance in all systems, with PAMAM and 2$^{nd}$ generation aryl ether dendrons utilising porphyrin **32** shown to have the most favourable properties, with the high steric hindrance of the other conjugates limiting their effectiveness as potential targeted photosensitisers.

Future work could allow the synthesis of porphyrins with a longer linker chain, to allow reduction of steric hindrance in the system and quenching of porphyrins. Synthesis of higher generations of dendrons with greater numbers of peripheral click functionalities could also be carried out, in particular the synthesis of higher generations of PAMAM dendrons, allowing the conjugation of greater number of porphyrins without the high lipophilicity of the higher generation aryl ether dendrons.

# 4. Conjugation to antibody fragments

## 4.1. Introduction

Following the successful synthesis of a range of water-soluble porphyrins and porphyrin-dendron conjugates, the aim of this chapter is the conjugation of these photosensitisers to antibody fragments in order to create targeted photosensitisers suitable for use in photodynamic therapy. Conjugation will initially be attempted between single porphyrins and the interchain disulfide bridge of a HER2-targeted antibody Fab fragment, followed by subsequent functionalisation of porphyrin-dendron conjugates, and their conjugation to the antibody fragment, allowing increased binding ratios on the targeted photosensitisers.

## 4.2. Conjugation to disulfide bridges

Due to their highly nucleophilic character, cysteine thiol residues present in antibody fragments are ideal targets for conjugation of a range of drugs, including photosensitisers. While these residues are occasionally found as free thiol groups, the nucleophilic and highly reactive nature of these groups means that they will readily dimerise with other thiol residues to form both intrachain and interchain disulfide bridges. These disulfide bridges perform a key role in maintaining the structure of many antibodies and antibody fragments, increasing both the thermal and chemical stability of the antibody, helping to form and maintain the 3D structure, and increasing structural rigidity.[214]

While single cysteine residues can be introduced into antibodies and antibody fragments through genetic engineering, these lone thiol groups are highly reactive, and often lead to the formation of dimeric antibody structures.[215] An alternative to this is the reduction of the naturally occurring disulfide bridges in the antibody fragment utilising reducing agents such as TCEP. Although intrachain disulfide bridges are defined as "solvent inaccessible" and are generally buried inside the complex structure of the antibody, the interchain disulfide bridges present between chains of the antibody are easily accessible and can be reduced to generate two free thiol groups. These groups are ideal conjugation targets for photosensitiser molecules as their low natural abundance in most antibody fragments[216] allows for good stoichiometric control, and the fact that the

interchain disulfide bridges are located away from the binding site of the antibody fragment means that binding is unlikely to effect antibody-receptor binding.

To date, numerous methods are available in the literature allowing conjugation to reduced disulfide bridges, which can be divided into two main categories: use of haloacetyl functionalities, in particular iodoacetamido groups, and the use of maleimide-based functionalities.

The iodoacetamido functional group is one of a larger class of haloacetamides, and is the most reactive example of this group, showing good selectivity for thiol functionalities at physiological pH.[217] In comparison to the maleimide functional groups, iodoacetamido functionalities show good resistance to nucleophilic attack by amine residues, reducing instances of hydrolysis and cross linking.[218] However, reactions with a large number of other residues are possible outside of the physiological pH range, including mono or di alkylation of amine residues at above pH 8,[219] conjugation to carboxylic acids [219] and reaction with other sulfur–containing, poorly reactive residues such as methionine in the absence of reduced disulfide bridges.[217]

Alternatively, several methods have been developed allowing conjugation to the reduced disulfide bridge using maleimides and related brominated functionalities (Figure 44), with these groups allowing for facile conjugation to disulfide bridges and easy functionalisation of the nitrogen of the ring to allow attachment of a wide variety of structures.
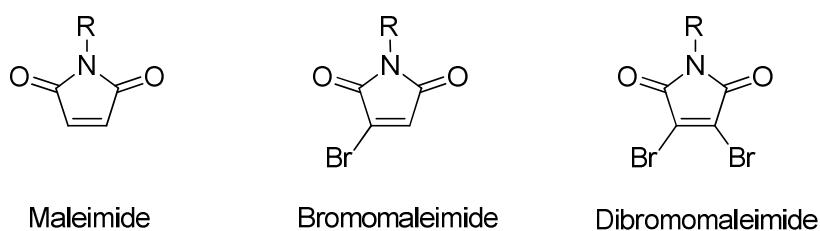


*Figure 44: Structures of the maleimide, bromomaleimide and dibromomaleimide functional groups.*

The most commonly utilised of these structures for attachment to the disulfide bridge is the maleimide functionality. Conjugation of this group to disulfide bridges occurs *via* the addition of a thiol group, leading to loss of the maleimide double bond, and possible

conjugation ratios of 2:1. This method allows irreversible conjugation of the maleimide to the disulfide bridge, and is highly selective for thiol groups only in a narrow pH range. However, outside of the pH range 6.5-7.5, reaction with amine lysine residues occurs in competition with the thiol residues, leading to loss of selectivity. In addition, reaction with maleimides leads to loss of the bridging character of the disulfide bridge, which can lead to structural changes in the antibody fragment, reduced stability[220] and loss of effector functions.[221]

The addition of a single bromine to the maleimide structure forms the bromomaleimide functionality, which also allows conjugation of a single thiol group to the ring structure. However, unlike the maleimide, reaction of the bromomaleimide occurs through a nucleophilic substitution of the bromine, allowing retention of the double bond on the ring structure. Reaction of this double bond with a second thiol group can then be carried out in a similar fashion to the maleimide. As with the maleimide, the bromomaleimide shows a pH dependent selectivity for thiols over amine residues, however unlike the maleimide the bond formation has been shown to be reversible, with cleavage of the thiol-bromomaleimide bond demonstrated both with TCEP[216] and naturally in cells,[222] with the starting antibody fragment reformed in both cases.

The addition of two bromines at the carbons of the double bond of the maleimide structure forms the dibromomaleimide. As for the conjugation of the bromomaleimide structure, conjugation to thiol functionalities is carried out through nucleophilic substitution of the bromines with the thiol group. Due to the position of the two bromine functionalities, nucleophilic substitution by both thiol groups of a disulfide bridge is possible, allowing the replacement of the disulfide bridge with a rigid carbon-carbon bridge. Bridging of the disulfide bridge in this way allows retention of structure and function of the antibody,[215] and allows a 1:1 dibromomaleimide:disulfide bridge conjugation ratio. As observed for the bromomaleimide structure, the dibromomaleimide also shows excellent selectivity for thiols groups over amines at physiological pH, and shows cleavage to reform the starting antibody fragment in cells.[222]
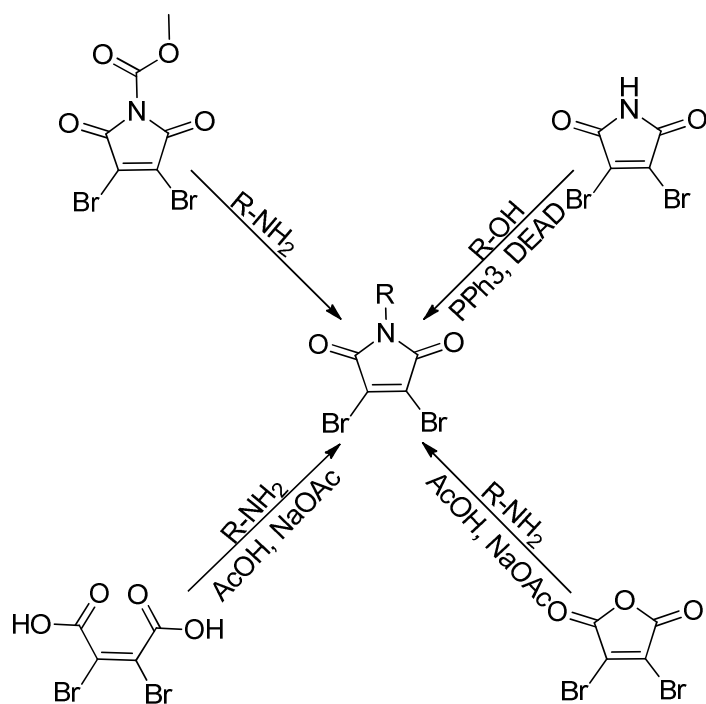
*Figure 45: Schematic displaying the literature methods of dibromomaleimide attachment.*

Conjugation of the dibromomaleimide ring to a wide variety of functionalities has been carried out in the literature *via* several methods (Figure 45). The first method developed used the nucleophilic substitution of molecules bearing aliphatic halides with the amine of the dibromomaleimide ring,[223, 224] however this method requires the formation of a halide-substituted substrate in order to work, and often gives poor yields. Use of the Mitsonobu reaction has also been utilised extensively to conjugate maleimide to alcohol-functionalised chains, [225-227] and has been used in a single example to conjugate a dibromomaleimide to an alcohol-terminated PEG chain.[228] While use of the Mitsunobu reaction allows simple conjugation to alcohol functionalities, use of the DEAD/DIAD reagent can be hazardous, and both the azodicarbonyl reagent and triphenylphosphine oxide can be difficult to remove from the crude product.

Alternatively, more modern methods make use of a range of reagents to generate the dibromomaleimide ring on a primary amine-functionalised starting material. Most commonly, the addition of the dibromomaleimide ring is carried out in refluxing acetic acid utilising either dibromomaleic anhydride[216] or 3,4-dibromomaleic acid[229] as the source of the dibromomaleimide ring, with the high temperatures and acidic conditions making this method unsuitable for thermally or hydrolytically sensitive structures. As a result, a more recent method uses a *N*-methoxylcarbonyldibromomaleimide structure to

126

produce dibromomaleimides from primary amines under mild reaction conditions,[230] with the improved leaving group allowing facile and rapid attachment to a greater range of structures.

## 4.3. HER2 targeting

The Human Epidermal Growth Factor Receptor 2 (HER2) is part of the epidermal growth factor (EGF) family of transmembrane glycoprotein receptors.[231] HER2 is over-expressed in many cancers, in particular in around 25-30% of all cases of breast cancer,[232] and is associated with poorer prognosis, metastasis and cancer recurrence, with a causal link with tumour aggressiveness suggested.[232]



*Figure 46: Herceptin antibody Fab structure*

While no natural antibodies for HER2 exist, Herceptin™ (Figure 46) is a commercially available recombinant monoclonal antibody developed to target the HER2 receptor, and is currently licensed as a treatment for HER2+ breast cancers. Despite this, drug resistance is common, and less than half of patients with HER2+ breast cancers respond to this treatment,[233] even when given in combination with chemotherapy.[234] In particular, use of Herceptin™ to treat metastatic tumours is generally unsuccessful, with many metastatic tumours showing very different HER2 expression in comparison to the primary tumour.[235] In addition, many serious side effects have been recorded, [232] with cardiac dysfunction observed in up to 27% of cases.[233]

An alternative to the use of Herceptin™ as an anti-cancer drug is the use of this antibody and associated fragments as targeting groups as part of a third generation photosensitiser, allowing exploitation of the highly specific antibody-receptor interaction while avoiding many of the issues of Herceptin™ as a drug. To date, numerous examples of conjugation of therapeutic and diagnostic structures to the lysine residues of Herceptin™ have been published, with recent examples targeting indium-labelled macrocyclic structures,[236] tubulin-targeting anti-cancer dolastatin derivatives[237] and gene delivery polymeric structures[238] to HER2 positive cancers with promising results. Despite this, only a single example of HER2 targeted photodynamic therapy using Herceptin as a targeting group has been published to date.[239] In this work, Rosenblum *et al.* conjugated three Rhodamine-based photosensitisers (TAMRA, Rhodamine-B and Rhodamine-6G) to Herceptin™ and showed targeted cell death in HER2+ cells in comparison to HER2 negative control cell lines utilising two out of the three targeted photosensitisers.
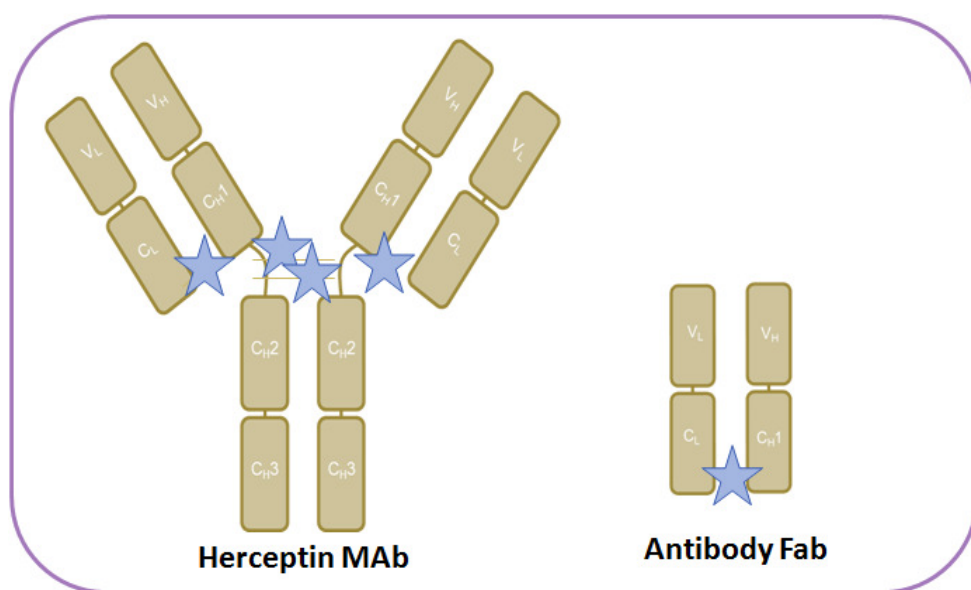


*Figure 47: Structures of the Herceptin MAb and Fab, with the position of the interchain disulfide bridges indicated with stars.*

In addition to the numerous examples of conjugation to Herceptin™ and antibody fragments *via* the lysine residues, conjugation to both the Herceptin™ MAb and the smaller Fab fragment is also possible through the interchain disulfide bridges present on both structures; while the MAb has four interchain disulfide bridges, the Fab contains just a single example (Figure 47), allowing for greater control over the site and

128

stoichiometry of binding.[240] In this work, conjugation to the Fab fragment will be carried out through conjugation to the disulfide bridge *via* the dibromomaleimide functional group.

## 4.4. Conjugation of a single porphyrin

### 4.4.1. Conjugation of a dibromomaleimide to a single porphyrin

As previously discussed, the initial aim of this work was the conjugation of a single porphyrin to a Herceptin™ antibody fragment *via* use of the dibromomaleimide functionality. Initially, this synthesis was attempted through the direct attachment of the dibromomaleimide onto the porphyrin *via* the nitrogen of the dibromomaleimide ring.
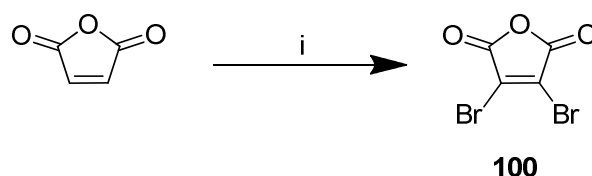


*Scheme 44: Reagents and conditions: (i) bromine, aluminium chloride, 130 °C, 17 hours.*

Initially, synthesis of the dibromomaleic anhydride **100** was carried out through direct bromination of maleic anhydride. This reaction was carried out according to a literature method,[241] using an aluminium chloride catalyst, with the product obtained in excellent yield (94%) after heating overnight. The identity of the product was confirmed by $^{13}$C - NMR and melting point, with both values found to be in agreement with the literature values.
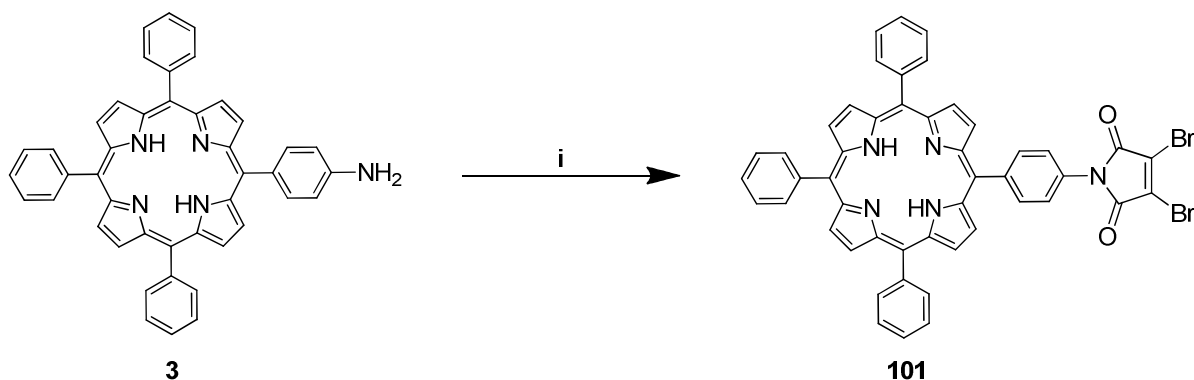


*Scheme 45: Reagents and conditions: (i) **100**, acetic acid, 120 °C, 5 hours.*

Conjugation of **100** to amine-functionalised porphyrins was then attempted using a literature method [216, 241] with the starting materials heated to reflux in acetic acid. Initially, this method was trialled on the lipophilic amine porphyrin **3** as a model reaction, with the desired porphyrin obtained in a relatively poor yield (39%). The reason for this poor yield is unknown, but could be attributed to the relatively harsh reaction conditions required.
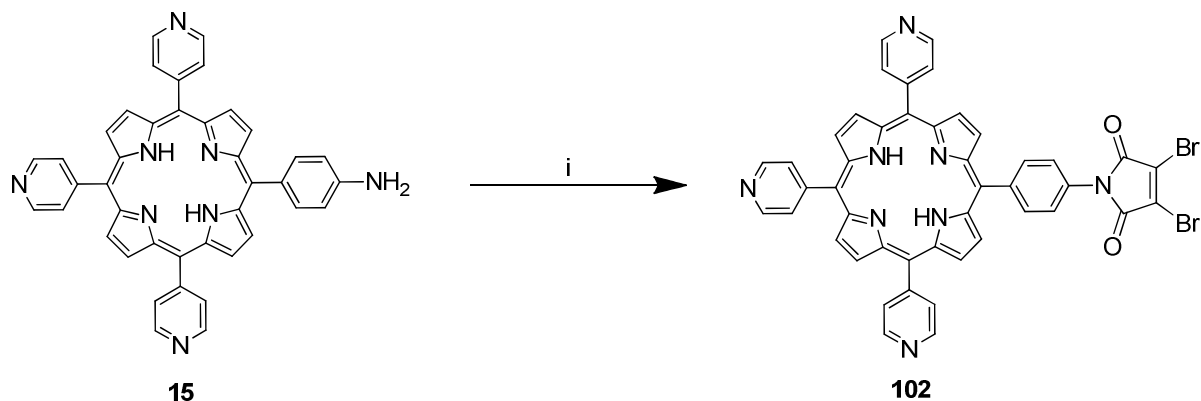


*Scheme 46: Reagents and conditions: (i) **100**, acetic acid, 120 °C, 5 hours or **100**, sodium acetate, 100 °C, 25.5 hours.*

The reaction conditions utilised for the synthesis of **101** were then trialled on the amine-functionalised pyridyl porphyrin **15**. TLC monitoring of this reaction showed the formation of a small quantity of two porphyrins with very similar $R_f$ values, as well as the formation of a large quantity of a black, insoluble material. The formation of these unexpected by-products was attributed to the harsh reaction conditions, and as a result no further attempts to synthesise dibromomaleimide porphyrins using this method were carried out. The reaction was then repeated with conditions found in the literature,[57] however despite the lower reaction temperature TLC analysis still showed formation of two porphyrins and a large quantity of insoluble black material.
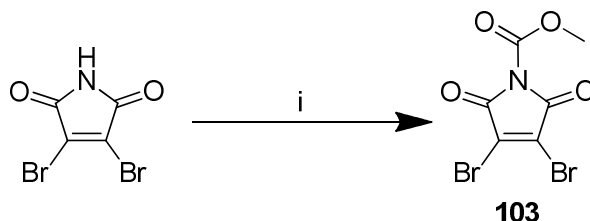


*Scheme 47: Reagents and conditions: (i) methyl chloroformate, 4-methylmorpholine, rt, 20 mins.*

For this reason, attachment of amine-functionalised porphyrins to the nitrogen of the dibromomaleimide ring was then attempted using the dibromomaleimide species **103**. Synthesis of **103** was initially carried out according to a literature method,[230] with dibromomaleimide reacted with methyl chloroformate in the presence of a base to generate the product in excellent yield (92%).
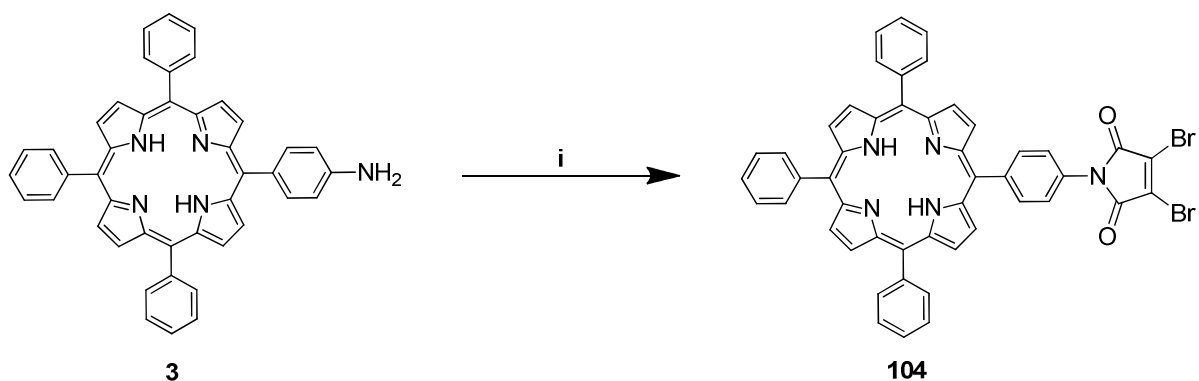


*Scheme 48: Reagents and conditions: (i) **103**, rt, 17 hours.*

The reaction of **103** with the model porphyrin **3** was then carried out. In contrast to the reaction with **100**, the formation of **104** proceeded rapidly and in excellent yield under mild conditions, with identity of the product confirmed by NMR and MS.
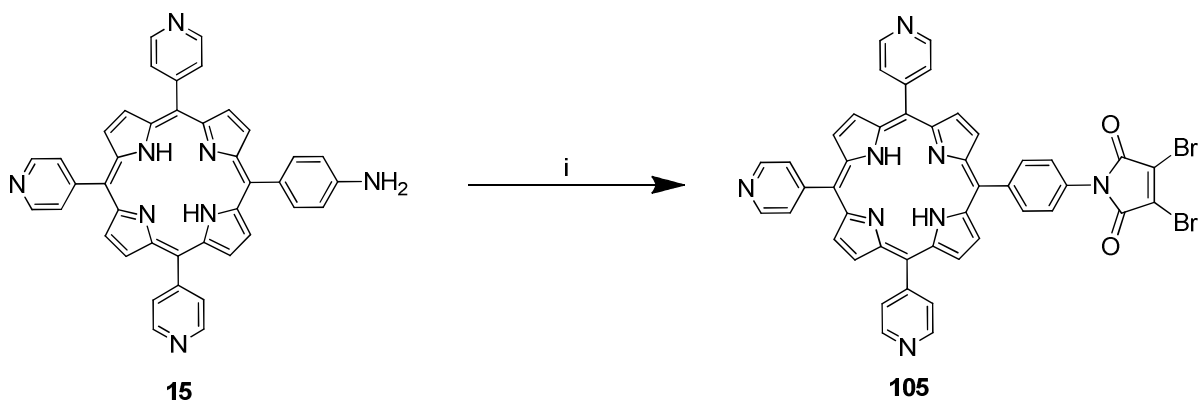


*Scheme 49: Reagents and conditions: (i) **103**, rt, 17 hours.*

Reaction of **103** with **15** was then attempted under similar conditions, with DMF used as a solvent instead of DCM due to the poorer solubility of **15**. After 24 hours at room temperature, TLC analysis showed formation of a small quantity of a single porphyrin,

131

as well as the formation of the black, insoluble material as seen for the previous synthesis of **102**. The formation of this by-product in both cases suggests that this insoluble material is not formed due to harsh reaction conditions, and is instead as a result of the porphyrin reacting directly with the dibromomaleimide ring itself. A possible explanation is that this by-product is formed through the reaction of the nitrogen of the pyridyl ring with the dibromomaleimide ring, resulting in the formation of insoluble dimeric and oligomeric species.
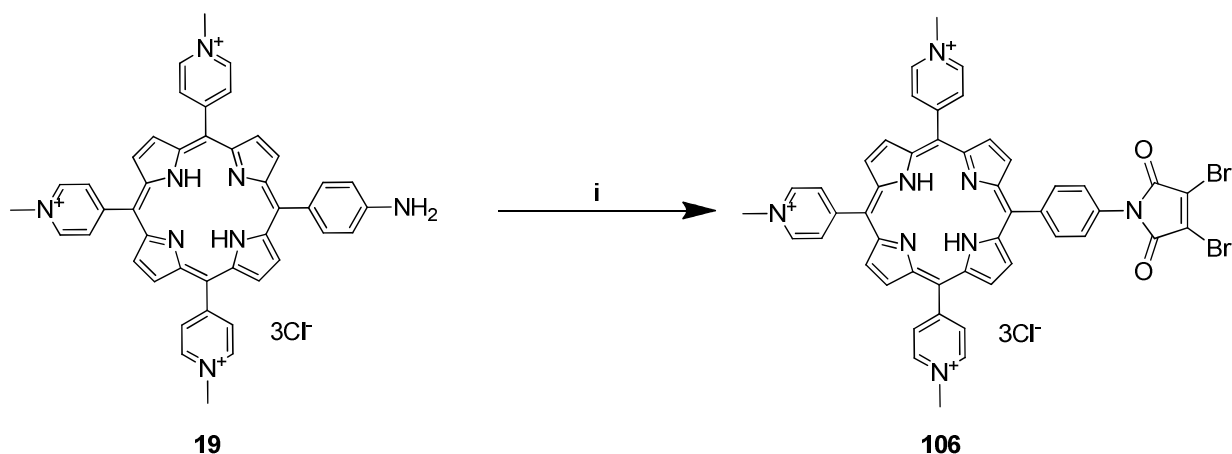


*Scheme 50: Reagents and conditions: (i)* **103***, rt, 17 hours.*

For this reason, attachment of the dibromomaleimide to the water-soluble porphyrin **19** was then attempted, with no formation of the insoluble by-product observed after 24 hours. The lack of this by-product suggests that its formation is as a result of reaction between the pyridyl groups and the dibromomaleimide ring, with the methylation of these pyridyl groups preventing the reaction from taking place. However, HPLC monitoring of this reaction showed a large quantity of unreacted starting material, and the formation of a small amount of two porphyrin species. The poor conversion in this reaction was attributed to the limited reactivity of the amine on this porphyrin, as noted in the previous reaction of **19** with ISA hydrogen sulfate. Additionally, the synthesis of two porphyrins was attributed to the use of methanol as a solvent, which could lead to nucleophilic attack of the solvent molecules at the carbonyl carbons of the ring, leading to ring-opening of the dibromomaleimide, forming both the desired product and the ring-opened analogue. The reaction was repeated in DMSO and DMF, and while a single product was obtained in both cases, the extremely poor yield (< 10%) of the desired porphyrin in both cases meant that purification was not attempted.
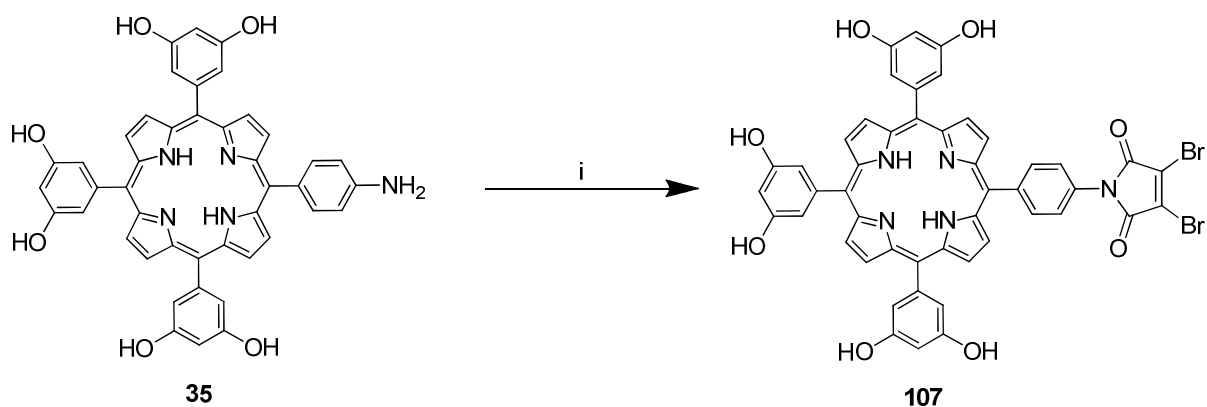
*Scheme 51: Reagents and conditions: (i)* ***103****, rt, 17 hours.*

For this reason, conjugation of the dibromomaleimide ring was then attempted using the hexahydroxy porphyrin **35**. Reaction with this porphyrin was attempted as it shows solubility in both water and organic solvents, and has no pyridyl groups to react with dibromomaleimide, while the amine shows improved reactivity over that of porphyrin **19**. The reaction was carried out under same conditions as for the synthesis of **105**, with TLC analysis after 24 hours showing a single product. However, $^{13}$C NMR analysis showed multiple peaks in the carbonyl region, suggesting the presence of multiple dibromomaleimide rings on each porphyrin. As previous attempts to synthesise the azide porphyrin **37** from **35** showed reaction of the phenolic alcohol groups, it is likely that these multiple signals are as a result of a similar process, with both the amine and the alcohol functionalities of **35** reacting with the dibromomaleimide ring.

### 4.4.2. Synthesis of a dibromomaleimide linker chain

As all attempts to synthesise a water-soluble porphyrin with a dibromomaleimide group attached *via* direct conjugation to a primary amine were unsuccessful, an alternative synthesis method was attempted. As a number of azide-functionalised porphyrins had been successfully synthesised during chapter 2, synthesis of heterobifunctional linkers bearing alkyne and dibromomaleimide functionalities was attempted, allowing click conjugation of the chain to the porphyrin either before or after conjugation of the linker chain to an antibody fragment.
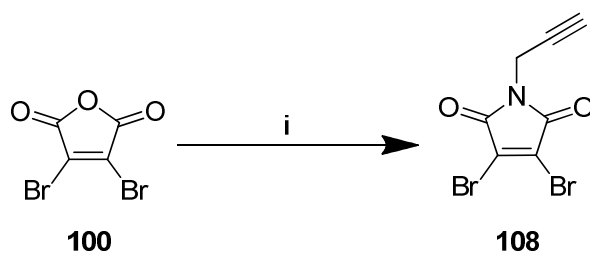
*Scheme 52: Reagents and conditions: (i) propargyl amine, 120 °C, 17 hours.*

Initially, synthesis of a short linker chain was attempted *via* the reaction of dibromomaleimide anhydride **100** and the commercially available propargylamine. The desired product was obtained in a moderate yield (37%), comparable with yields obtained in the literature using similar methods,[223] and was well characterised by NMR and MS. Synthesis of a longer heterobifunctional linker was then attempted in order to reduce any potential steric hindrance problems. In all cases a PEG linker chain was utilised as starting material in order to provide good amphiphilic character to the final structure. While a number of longer heterobifunctional PEG chains are available with good oligomeric purity, the price of these structures is often prohibitively high, and for this reason synthesis was initially attempted using the shorter tetra- and triethylene glycol type structures.
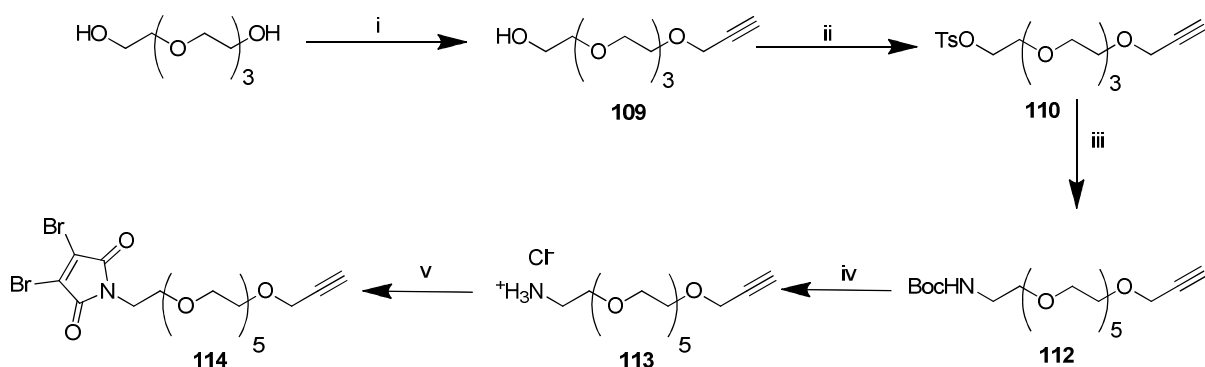


*Scheme 53: Reagents and conditions: (i) propargyl bromide, sodium hydride, 0 °C-rt, 17 hours, (ii) tosyl chloride, triethylamine, rt, 17 hours, (iii) **111**, sodium hydride, 0 °C-rt, 17 hours, (iv) HCl in dioxane, rt, 17 hours, (v) **103**, triethylamine, rt, 17 hours.*

Initially, monofunctionalisation of the symmetrical tetraethylene glycol chain was carried out using propargyl bromide according to a literature method.[242] The desired product was obtained in a 38% yield, with synthesis of some of the dialkyne and the tetraethylene glycol starting material also present in the crude reaction mixture. The yield was lower than expected from the literature value (65%), which could be

attributed to the fact that this is an unoptimised yield from a single reaction, and could be improved with measures such as improved cooling of the reaction, slower addition of propargyl bromide, or optimisation of reaction stoichiometry. Tosylation of the second alcohol functionality was then carried out with tosyl chloride to produce **110** in a 90% yield, similar to that obtained in the literature.[242]

Conjugation of **110** and Boc-protected amine **111** was then carried out *via* the Williamson ether synthesis, with sodium hydride used to deprotonate the alcohol. The desired product **112** was obtained in good yield (69%) and was characterised by NMR and MS. Deprotection of **112** was then carried out using HCl in dioxane, with the HCl salt obtained in 92% after stirring at room temperature for 17 hours. Synthesis of **114** was then attempted through reaction with **103**, with TLC analysis after 17 hours at room temperature showing no reaction occurring. Initially, it was assumed that this was as a result of the starting material **113** being in the form of an HCl salt, and addition of triethylamine was then attempted in order to generate the neutral amine. However, after 17 hours at room temperature, again no reaction was observed.
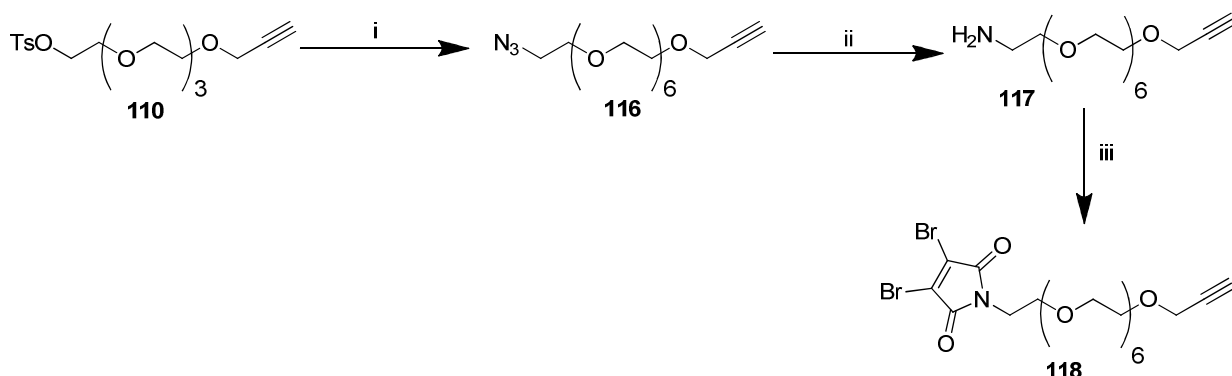


*Scheme 54: Reagents and conditions: (i) **115**, sodium hydride, 0 °C-rt, 17 hours, (ii) PBu₃, rt, 1 hour, (iii) **103**, triethylamine, rt, 17 hours.*

As a result, synthesis of the amine-functionalised PEG chain was then attempted *via* an alternative method in order to eliminate any effect that either the salt or the triethylamine had on the reaction. Tosylate-functionalised chain **110** was reacted with azide chain **115** in the presence of sodium hydride to generate **116** in very good yield (81%), with the identity of the product confirmed by NMR and MS. Subsequent conversion of the azide to the amine-functionalised chain **117** was originally trialled *via* the Staudinger reaction using triphenylphosphine, however the reaction was slow, and

after stirring at rt overnight a significant proportion of unreacted **116** was observed by TLC. For this reason, the reaction was repeated using the more reactive phosphine analogue tributylphosphine. The reaction proceeded far more rapidly using tributylphosphine, and after 1 hour at rt the reaction was shown to be complete by TLC. However, removal of the unreacted tributylphosphine and phosphorus-containing by-products was difficult as the highly polar product **117** could not be purified either by an aqueous workup or by column chromatography.

As a result, NMR analysis of the pure product **117** could not be carried out. However, use of a colometric ninhydrin TLC test showed the presence of the amine functional group on the product, and MS analysis of the crude product confirmed the formation of **117**. For this reason, synthesis of **118** from the reaction with the crude mixture and **103** was attempted. However, as observed for the synthesis of **114**, TLC analysis after 17 hours at room temperature showed no consumption of either starting material. As the addition of **103** to both amine functionalised chains was unsuccessful, the lack of reactivity cannot be attributed to the presence of the HCl salt or the use of triethylamine. For this reason, it was hypothesised that the lack of reactivity of both chains was due to their length and the structure the chain adopts in apolar solvents. The coiling and folding of long PEG chains around free water molecules has been previously observed in the literature,[243] and it is possible that a similar method could allow the more lipophilic alkyne end of the chain to form a coiled structure around the more polar amine in the apolar reaction solvent, preventing the reaction from occurring. While use of a more polar solvent such as methanol or water would theoretically prevent the coiling effect, these solvents are incompatible with the water-sensitive dibromomaleimide ring.
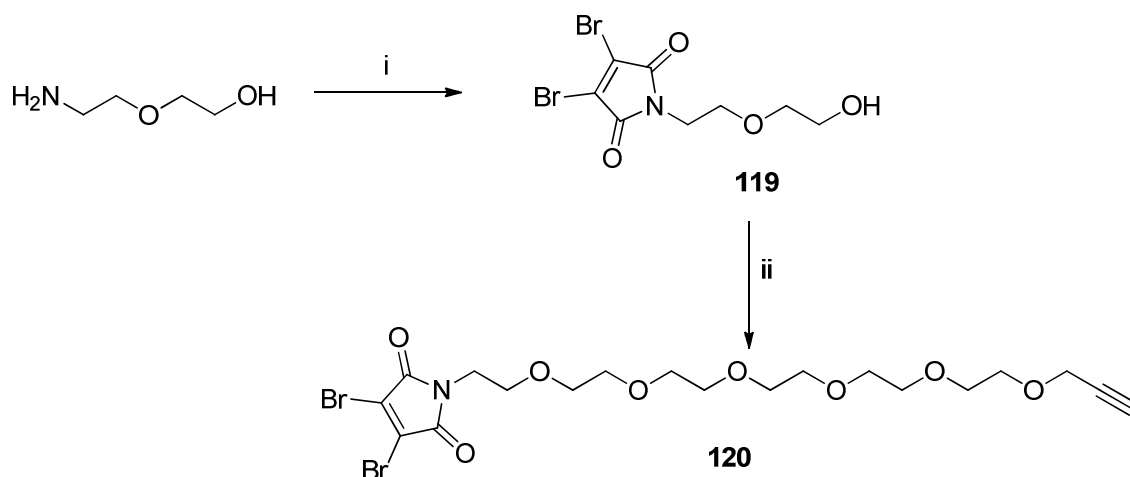
*Scheme 55: Reagents and conditions: (i) **103**, rt, 17 hours, (ii) **110**, sodium hydride, 0 °C-rt, 17 hours.*

In order to produce a chain with the dibromomaleimide ring attached, conjugation of **103** to the chain was attempted before the Williamson ether synthesis step to generate the long PEG chain. Initially, conjugation of **103** to the short diethylene glycol derivative was carried out to produce **119** in an 88% yield, with attachment of the ring confirmed by the presence of the characteristic peaks on $^{13}$C NMR. Conjugation of this chain to **110** was then carried out utilising sodium hydride as a base, with TLC analysis after 17 hours showing complete consumption of starting material **119**. However, after purification by column chromatography the only product was a small quantity of the methyl ether of **110**, which was assumed to have formed during the methanol quenching of the sodium hydride. As no residual **119** was present in the reaction mixture, it was assumed that degradation of the dibromomaleimide ring had taken place in the harsh reaction conditions.
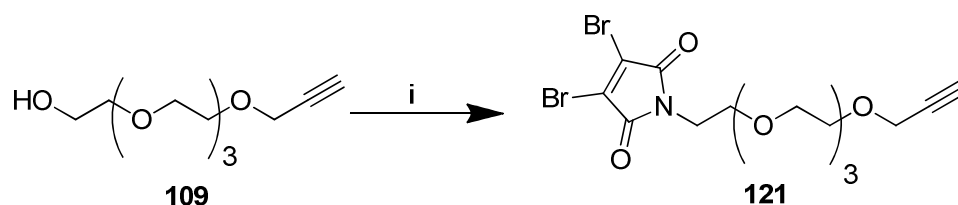


*Scheme 56: Reagents and conditions: (i) PPh₃, DIAD/DMEAD/DCAD, -78 °C-rt, 17 hours.*

As all previous methods of functionalising a long PEG chain with **103** were unsuccessful, synthesis of a long heterobiofunctional alkyne-dibromomaleimide was

then attempted *via* the Mitsunobu reaction. Initially, functionalisation of the monoalkyne tetraethylene glycol **109** was attempted using standard reaction conditions; with premixing of the triphenylphosphine and DIAD starting materials at -78 °C to pre-form the reactive betaine intermediate. After 17 hours, TLC monitoring showed complete consumption of the starting materials, and formation of a new product. Purification was then attempted *via* column chromatography; however removal of the reduced DIAD species was not possible due to the highly similar $R_f$ values of the product and the impurity. This is a widely recognised problem in the literature, with a number of papers aiming to develop alternatives to both the DIAD and triphenylphosphine reagents which can be removed easily from the product after completion of the reaction.[244-247]

The first of these DIAD alternatives trialled was di-*p*-chlorobenzyl azodicarboxylate (DCAD), a DIAD alternative designed to be insoluble in DCM, allowing removal by filtration from DCM reaction mixtures, and also with a low $R_f$ value to allow facile column removal.[245] However, while synthesis of **121** was carried out successfully and purification from the DCAD was possible by filtration and subsequent column chromatography, the method gave poor yield and was not reproducible, requiring repeated column chromatography to remove all of the reduced DCAD species from the product. This can be attributed to the fact that the product **121** also had relatively poor solubility in DCM, with large batches precipitating out during the filtration stages. As a result, multiple filtration steps resulted in loss of large quantities of the product while a single filtration step produced a mixture of **121** and the reduced DCAD species which could not be removed easily by column chromatography due to the highly similar $R_f$ values of the two species.

As a result, synthesis utilising the DIAD alternative di-2-methoxyethyl azodicarboxylate (DMEAD) was then attempted. DMEAD was developed with the aim of producing a water-soluble DIAD alternative which could be removed by water washing, and which also had a low $R_f$ to allow facile removal by column chromatography.[246] Synthesis carried out using DMEAD again gave excellent conversion after 17 hours, with the combination of an aqueous workup followed by column chromatography producing the desired product **121** in good yield (62%), with NMR confirming the complete removal of the reduced DMEAD species.
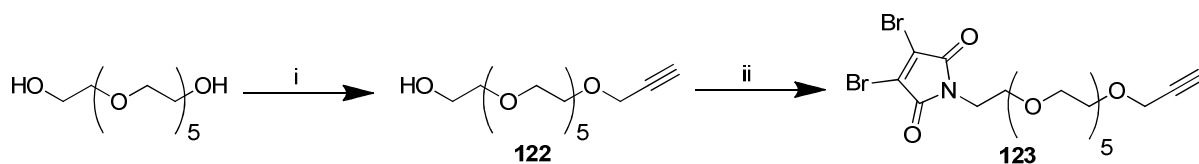
*Scheme 57: Reagents and conditions: PPh₃, DMEAD, -78 °C-rt, 17 hours.*

The synthesis of the longer hexaethylene glycol analogue of **121** was then attempted utilising the methods developed previously. Attachment of a single alkyne chain to the hexaethylene glycol starting material was carried out *via* the Williamson ether synthesis, with the desired product obtained in 42% yield with the 1:1 conjugation of propargyl bromide confirmed by NMR and MS. Attachment of the dibromomaleimide ring to **122** was then attempted *via* reaction conditions optimised for **121**, however after 17 hours no product formation was observed. The lack of the formation of product **123** again supports the hypothesis that it is the length of the PEG chain which limits the reactivity, with the shorter tetraethylene glycol chain reacting readily, while the coiling of **122** around the polar alcohol functionality preventing conjugation to the dibromomaleimide. As a result of the difficulty of finding a solvent suitable for both the longer PEG chain and the dibromomaleimide ring, no further attempts to synthesise a longer analogue were carried out.

### 4.4.3. Conjugation of dibromomaleimide linker chains

Following the successful synthesis of two heterobiofunctional linker chains bearing both an alkyne and a dibromomaleimide functionality, conjugation of an antibody fragment and porphyrin *via* these linker chains was then attempted. Due to the nature of the chain, two methods were available for the conjugation; click conjugation to the porphyrin could be carried out first, followed by attachment of the dibromomaleimide to the antibody fragment. Alternatively, conjugation of the chain to the antibody fragment *via* the dibromomaleimide could be carried out followed by click conjugation to the porphyrin.

Initially, click conjugation to the porphyrin as the first step was selected, as this method allowed for NMR confirmation of the attachment of the porphyrin to the linker chain.
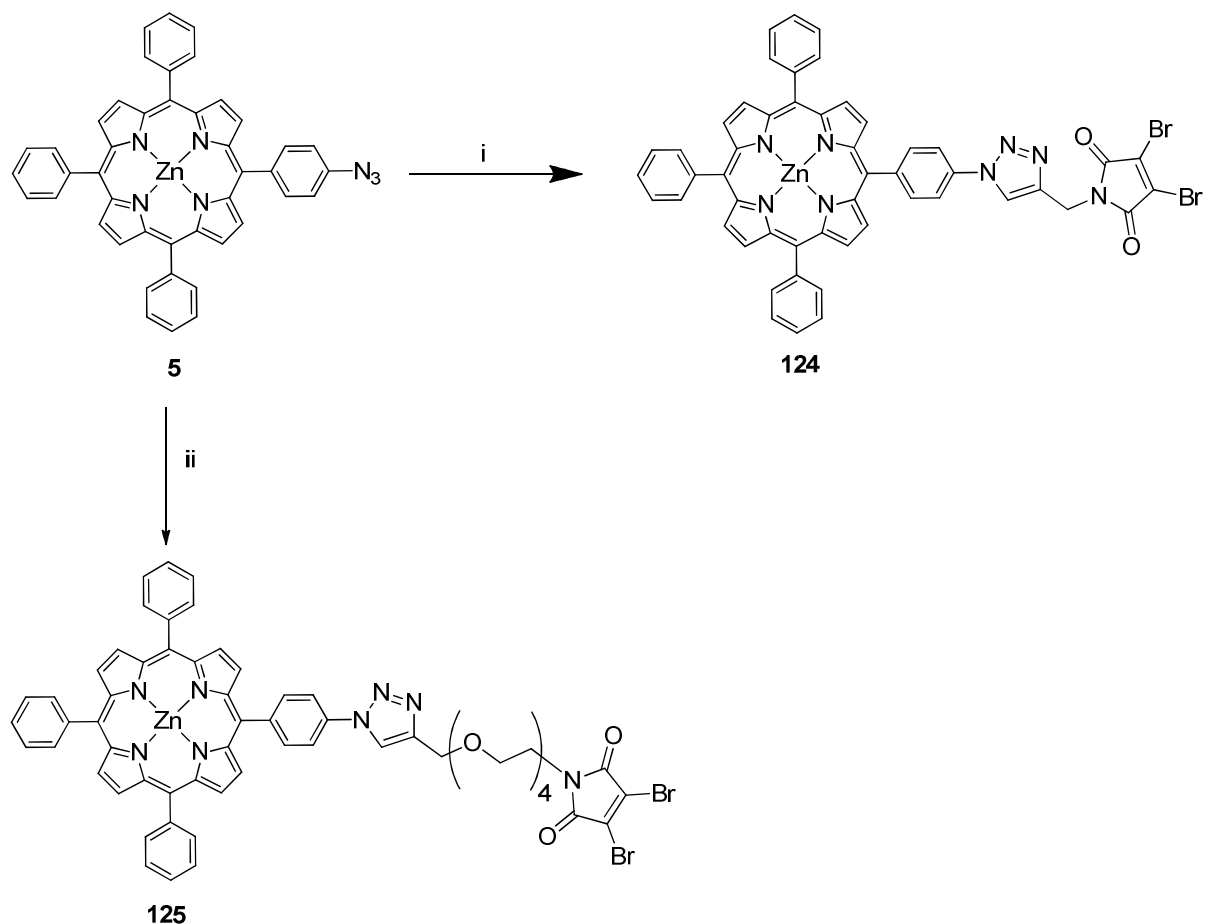
Scheme 58: Reagents and conditions: (i) **108**, copper (II) sulfate pentahydrate, sodium ascorbate, 80 °C (MW), 2 hours, (ii) **121**, copper (II) sulfate pentahydrate, sodium ascorbate, 90 °C (MW), 40 mins.

Click conjugation of the two linker chains was initially trialled on the model porphyrin **5** under microwave heating in order to increase the rate of reaction. In both cases the reaction proceeded rapidly and in good yield, with the identity of the final product confirmed by NMR and MS. Minimal formation of the ring-opened product was observed in both cases, despite the use of a small amount of water in the solvent mixture, however a large excess of both chains was required in order to force the reaction to completion. As a result it was hypothesised that hydrolysis of the dibromomaleimide chains was occurring, with these ring-opened structures being more soluble in the water layer of the biphasic system than the ring-closed chains. As a result, reaction between the lipophilic porphyrin **5** and the ring closed chain in the organic layer occurred more rapidly, with the resulting product mixture containing only a small proportion of the ring-opened dibromomaleimide-porphyrin conjugate.
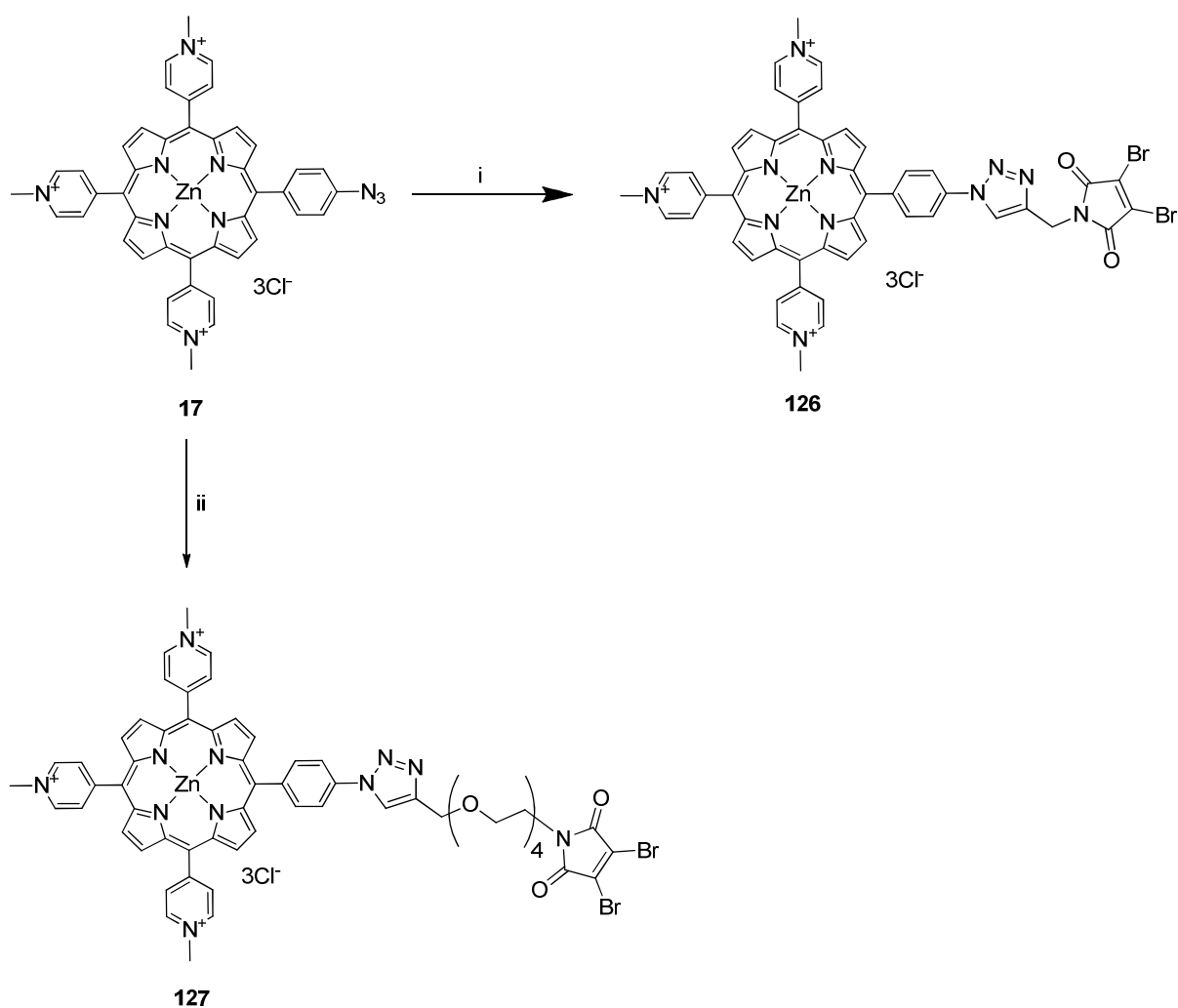
*Scheme 59: Reagents and conditions: (i) **108**, copper (II) sulfate pentahydrate, sodium ascorbate, 45 ˚C (MW), 75 mins, (ii) **121**, copper (II) sulfate pentahydrate, sodium ascorbate, 45 ˚C (MW), 40 mins.*

Conjugation of the two dibromomaleimide chains to the water soluble azide porphyrin **17** was then attempted using conditions optimised for the porphyrin-dendron reaction, with the addition of a large excess of the linker chain as for the reaction with **5**. Reaction with the short linker chain **108** was monitored by HPLC and showed formation of a single product in good (70%) yield on a reactions using less than 10 mg porphyrins, with reactions on a larger scale showing yields of less than 10%. Heating for prolonged periods of time led to the formation of a second product with an $R_f$ consistent with the product **126**, however in all cases yields of this product were very low (> 5%). These results suggest that the majority of the porphyrin product formed was the ring-opened species, with the $R_f$ of this major component supporting this theory. In contrast, only a small quantity of the desired product **126** was synthesised in all cases after extended heating. As for the synthesis of **124**, this suggests that a large quantity of

the linker chain **108** was hydrolysed by the large quantity of the water present, with the water soluble porphyrin in this case reacting with the more water-soluble ring-opened chain in preference to the more lipophilic ring-closed structure.

Similarly, synthesis of **127** under identical conditions showed formation of two porphyrin species in poor yield by HPLC analysis, and no formation of the expected product. In order to eliminate the use of water to prevent hydrolysis, the reaction was repeated using DMSO and NMP as reaction solvents. In both cases, a large number of peaks were observed on HPLC, suggesting that degradation of both the porphyrin and dibromomaleimide species occurs in these solvents.

In both cases, combination of the water-reactive dibromomaleimide linker chain and the water-soluble porphyrin and catalyst lead to difficulties in the selection of a suitable reaction solvent. For this reason, an alternative conjugation strategy was decided upon, initially reacting the water-reactive dibromomaleimide with the reduced disulfide bridge of the antibody fragment, followed by click conjugation of the porphyrin.

### 4.4.4.  Pre-conjugation of linker chains to antibody fragment

As previous attempts to produce a dibromomaleimide functionalised porphyrin through click chemistry was unsuccessful, conjugation to antibodies was then attempted through pre-conjugation of the heterobifunctional linker chain to the antibody fragment. This methodology allows for reaction of the reactive dibromomaleimide functionality first and therefore minimises the risk of hydrolysis of the linker chain during click conjugation of the porphyrin.

Initially, cleavage of the interchain disulfide bridge of the HER2 antibody Fab fragment was carried out at 37 °C in borate buffer, with TCEP as the reducing agent. MS analysis after 90 minutes showed cleavage of the Fab fragment into the light and heavy chain components (Figure 48) as expected, with no unreacted Fab observed.
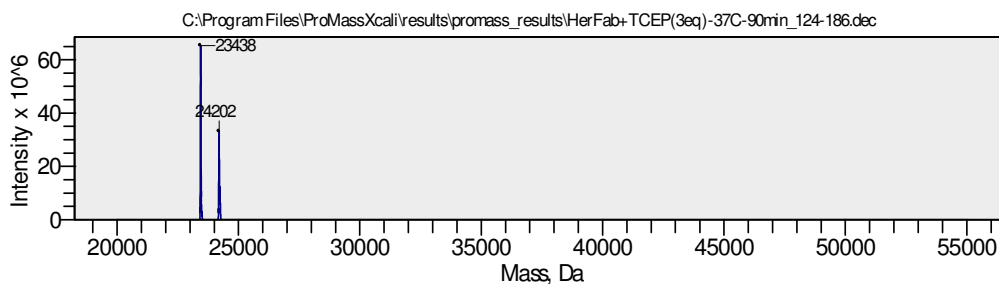
*Figure 48: Deconvoluted mass spectrometry result of cleaved Fab fragment.*

Subsequent re-bridging of the disulfide bridge utilising heterobifunctional linker **108** was then carried out at room temperature for 40 minutes, with mass spectrometry (Figure 49) showing complete consumption of the light and heavy chain components to produce the single Fab fragment bridged with the alkyne linker chain.
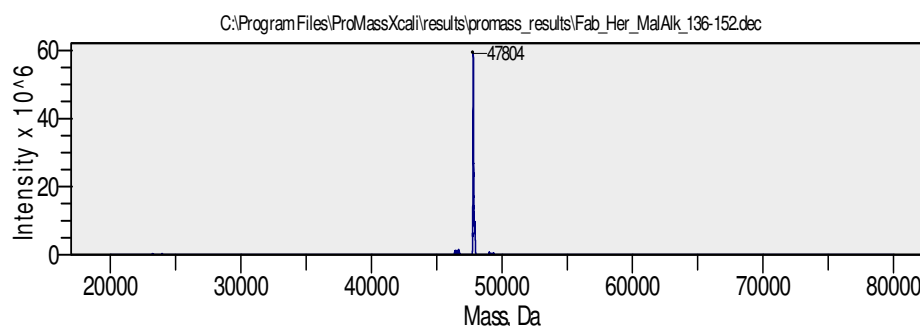
*Figure 49: Deconvoluted mass spectrometry results for the HER2 Fab fragment conjugated to the heterobifunctional linker chain.*

Click conjugation of synthesised cationic porphyrins **17** and **32** to the alkyne-functionalised Fab fragment was then attempted in PBS buffer, with copper (II) sulfate and sodium ascorbate used as the catalyst system. After 1 hour, the reaction was complete, with the complete functionalisation of the antibody fragment confirmed by mass spectrometry (Figure 50). In both cases a 1:1 stoichiometric porphyrin:Fab fragment binding ratio was observed, with no formation of multiply-loaded antibody species observed.
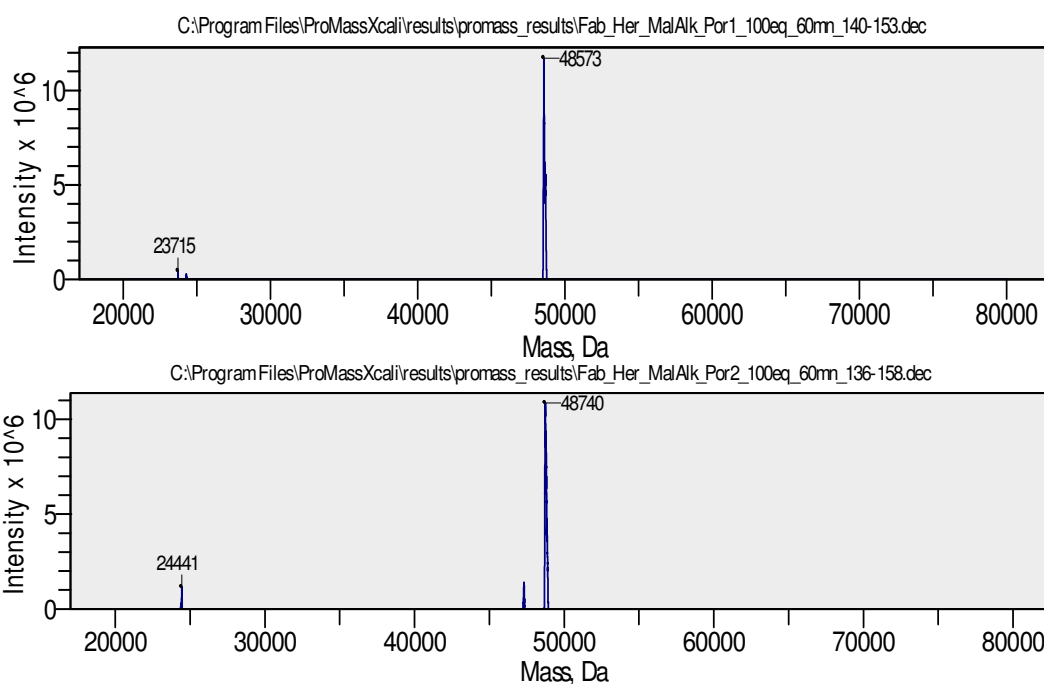
*Figure 50: Mass spectrometry results showing the HER2 Fab fragment conjugated to porphyrins **17** (top) and **32** (bottom) via a click chemistry strategy.*

Bridging and subsequent click functionalisation of the Fab fragment was also demonstrated utilising SDS-PAGE (Figure 51), with the resulting gel showing the expected antibody fragment in all cases, with no unbridged antibody or non-covalently bound porphyrin observed.
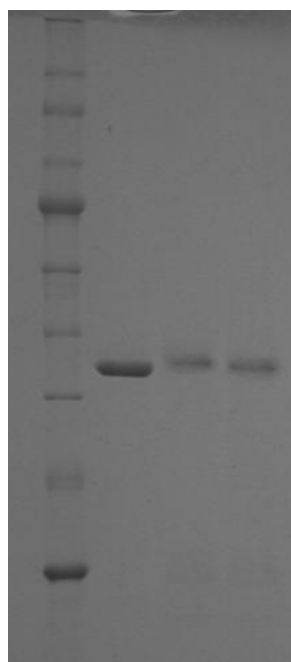


*Figure 51: SDS-PAGE of synthesised Fab conjugates. Left to right shows the antibody ladder, alkyne bridged Fab fragment, Fab-**17** conjugate and Fab-**32** conjugate.*

144

### 4.4.5.  Biological evaluation of targeted photosensitisers

Following the successful synthesis of two targeted photosensitisers bearing a single cationic porphyrin on a HER2-targeted Fab fragment, biological evaluation of these conjugates was then carried out.

In order to evaluate these conjugates as potential photosensitisers, preliminary cytotoxicity assays were carried out by Huguette Savoie. For each of the conjugates Fab-**17** and Fab-**32**, evaluation of cell killing effect was carried out using two cell lines, the HER2+ BT-474 breast cancer cell line and the HER2- control cell line MDA-MB-468. In each case, the assays were carried out with light irradiation of 20 J/cm$^2$ and photosensitiser doses of 12.375 µM for Fab-**32** and 8.53µM for Fab-**17** respectively.

| # : IRR = 0.056 NI = 0.057 | | | corrected reading (-#) | | % cell survival | | % standard deviation | |
|---|---|---|---|---|---|---|---|---|
| | IRR | NI | IRR | NI | IRR | NI | IRR | NI |
| BT cells only | 0.814 | 0.832 | 0.758 | 0.775 | **100.0** | **100.0** | 16.3 | 8.0 |
| BT + Fab-32 | 0.079 | 0.981 | 0.023 | 0.924 | **3.1** | **119.2** | 0.05 | 6.5 |
| BT + Fab-17 | 0.0835 | 1.091 | 0.0275 | 1.034 | **3.6** | **133.5** | 0.05 | 4.4 |
| MDA cells only | * | * | * | * | * | * | * | * |
| MDA + Fab-32 | * | * | * | * | * | * | * | * |
| MDA + Fab-17 | * | * | * | * | * | * | * | * |

*Table 7: table of preliminary cytotoxicity assay results; (\*=results pending).*

The results of these preliminary assays (Table 7) are promising for both targeted photosensitisers. In both cases, the dark control displays little or no cell death, suggesting no dark toxicity for either of the conjugates. This result is somewhat unexpected from the literature, with previous work finding cationic porphyrins exhibiting some dark toxicity,[248] however it is extremely desirable in order to limit damage to healthy tissue.

In contrast to the non-irradiated control, excellent cytotoxicity was observed for both targeted photosensitisers in the HER2+ cell line, with only 3% cell survival observed in both cases.  This demonstrates that both synthesised conjugates are capable of highly effective cell killing in the presence of light and also display low dark toxicity,

suggesting that they are ideal candidates for further evaluation as photosensitisers. Pending results from the HER2- cell line MDA-MB-468 will allow for confirmation that the Herceptin Fab fragment allows targeting of the photosensitisers, limiting cytotoxic effect of the conjugates to HER2+ target cells only.

## 4.5. Conjugating dendrons to antibody fragments

Following the successful synthesis and preliminary biological evaluation of targeted photosensitisers bearing a single porphyrin, functionalisation of a porphyrin-dendron conjugate for antibody conjugation was then attempted. As all conjugates synthesised possess a Boc-protected amine at the dendron focal point, the aim of this section was the deprotection of the amine, followed by functionalisation of the amine with a reactive handle suitable for conjugation to antibody fragments. Two methods are possible for the conjugation of the conjugate in this way; direct functionalisation of the amine with a dibromomaleimide group to allow direct conjugation to a disulfide bridge, or azide functionalisation of the dendron focal point followed by click conjugation to a linker-chain functionalised Fab fragment as previously described.

Conjugate **94** was used as a model for all synthesised dendrons due to the ease of synthesis of dendron **72** and porphyrin **17** and the facile conjugation of the two structures, however it is assumed that these synthetic methods could be applied to all synthesised conjugates.
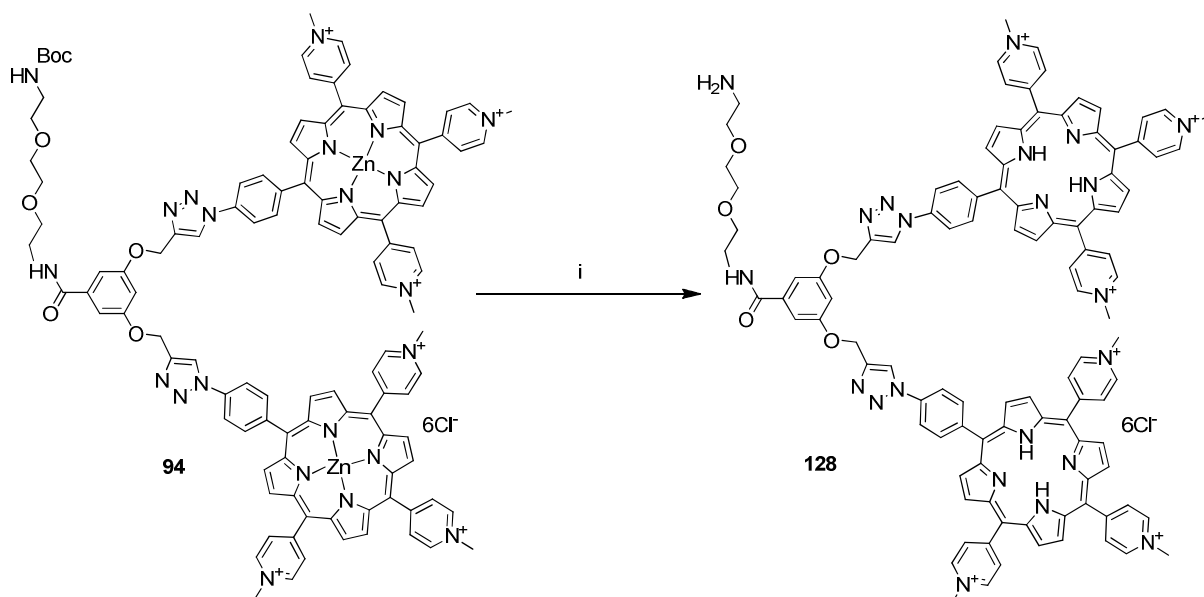
*Scheme 60: Reagents and conditions:(i) Quadrapure BZA, HCl$_{(aq)}$, rt, 4 hours.*

Deprotection of **94** to form **128** was carried out using mildly acidic conditions of dilute HCl$_{(aq)}$ at room temperature, with the TLC showing the complete consumption of the starting material after 3 hours. Neutralisation of the acidic species was then carried out with sodium hydrogen carbonate solution, however the resulting neutralised porphyrin was a characteristic red colour, suggesting copper insertion into the central cavity of the porphyrin. The metallation of the porphyrin was attributed to residual copper present, suggesting that the workup carried out after synthesis of **94** was not sufficient to remove all traces of copper after the click reaction.

For this reason, removal of all residual copper was then carried out using a metal-chelating resin Quadrapure BZA, with the aqueous sample of **94** shaken with the resin overnight. Subsequent deprotection and neutralisation was carried out to obtain the product **128** in good yield, with ${}^1$H NMR analysis showing the characteristic loss of the Boc peak. HPLC analysis showed a single peak, however UV-vis characterisation suggested a mixture of the zinc and free-base porphyrins. As the acidic conditions should fully remove the complexed zinc, it is suggested that some residual zinc remains in the reaction mixture after precipitation, and following the neutralisation of the mixture, any residual zinc is recomplexed by the free porphyrin species. The single peak observed by HPLC can be attributed to the fact that many metallated porphyrins, in particular zinc species, are indistinguishable from the free-base porphyrins by retention time.

Despite the successful synthesis of **128**, alternative methods of synthesising amine-functionalised conjugates were also trialled, for several reasons. Firstly, as previously mentioned the deprotection yields a mixture of the zinc and free-base products. Secondly, the copper removal step and the deprotection are time consuming, with the entire workup taking around two days to complete. Thirdly, the workup of the deprotected product is extremely time-consuming, and incomplete neutralisation of the acid can result in reaction of the ammonium hexafluorophosphate and loss of the product. Finally, the reaction is only moderately yielding, with around 20% of the product lost during reaction and workup. For this reason, synthesis was then attempted through deprotection of dendron **72** followed by subsequent click conjugation of porphyrin **17**. This method allows synthesis of dendron **130** without the need for deprotection of the conjugate, and also allows retention of the zinc in the porphyrins for subsequent click reactions.
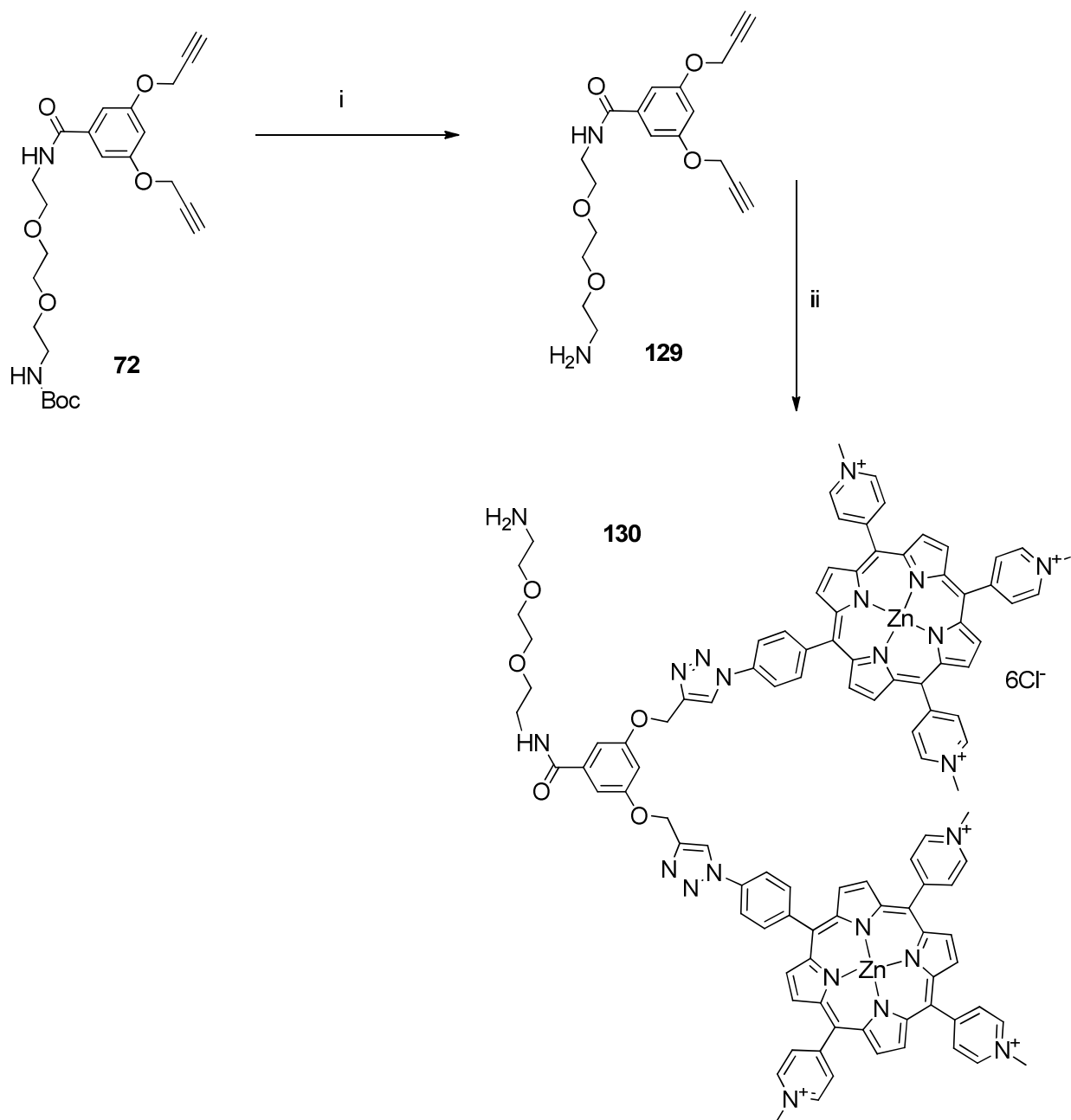
*Scheme 61: Reagents and conditions: (i) HCl in dioxane, rt, 17 hours, (ii) 17, copper (II) sulfate pentahydrate, sodium ascorbate, 45 ˚C (MW), 30 mins.*

Deprotection of dendron **72** was carried out in HCl in dioxane at rt, with NMR showing complete loss of the characteristic Boc peak after overnight stirring. Subsequent click reaction with porphyrin **17** was then carried out using identical conditions for the synthesis of conjugate **94**, with TLC analysis confirming the consumption of all starting material after 30 minutes heating. NMR analysis confirmed the click conjugation of two porphyrins to the dendron to yield **130** in 72% yield.
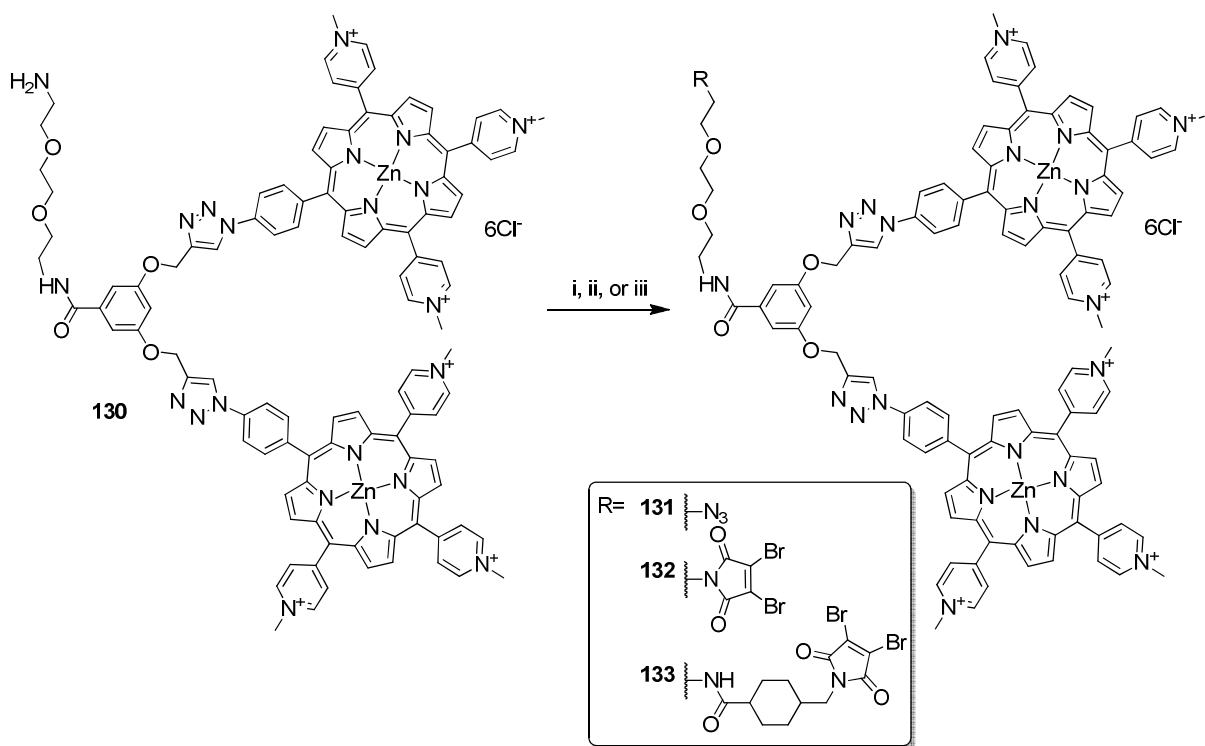
*Scheme 62: Reagents and conditions: (i) ISA hydrogen sulfate, potassium carbonate, copper (II) sulfate, rt, 17 hours, (ii) **103**, rt, 17 hours, (iii) **134**, potassium carbonate, rt, 17 hours.*

Functionalisation of the focal point of dendron **130** was then attempted *via* two methods, firstly the transformation of the primary amine to the azide using the diazo transfer reagent imidazole-1-sulfonyl azide hydrogensulfate, and secondly the functionalisation of the primary amine with **103** to form the dibromomaleimide. In both cases, TLC analysis after 24 hours showed no reaction had occurred, with extended reaction times also leading to no appreciable formation of products **131** or **132**.
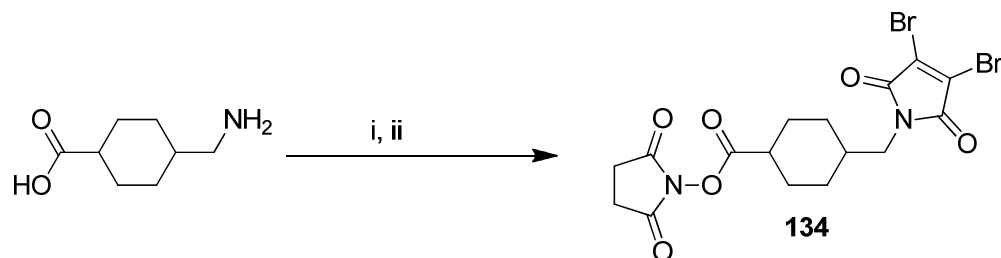


*Scheme 63: Reagents and conditions: (i) **103**, rt, 17 hours, (ii) DCC, N-hydroxysuccinimide, rt, 17 hours.*

Functionalisation of the focal point was then attempted using an alternative method, using a synthesised SMCC-analogue, **134**. SMCC is a commercially-available reagent

which is widely utilised to attach maleimide functionalities to a range of structures, with the NHS-ester allowing for facile functionalisation of primary amines, and the cyclohexane ring allowing for greater water stability of the maleimide structure. Synthesis of **134** was carried out using the commercially available trans-4-(aminomethyl)cyclohexanecarboxylic acid as a starting material in a two-step process. Initially, functionalisation of the amine was carried out using **103**, followed by activation of the ester with *N*-hydroxysuccinimide according to a literature method.[249] Both steps of the method were carried out successfully, with the desired product **134** obtained in an overall 46% yield, which while low is consistent with yields of SMCC obtained in the literature.[249]

Subsequent reaction of the SMCC-analogue **134** with conjugate **130** was then attempted in THF:water, with TLC analysis after 24 hours showing no reaction. Stirring for 48 hours at room temperature showed formation of a small amount of two products, with HPLC analysis showing around 90% residual starting material. Increasing the reaction time further did not lead to any appreciable increase in product formation. The two products formed were assumed to be the product **133** and the hydrolysed, ring-opened product, formed as a result of extended stirring in water. While the cyclohexyl ring does increase the hydrolytic stability of the maleimide ring in SMCC, extended periods of time in aqueous environments can still lead to hydrolysis of the structure, and it is assumed that this is also the case for the dibromomaleimide analogue **134**. For this reason, extended reaction times and heating to drive the reaction to completion were not attempted.
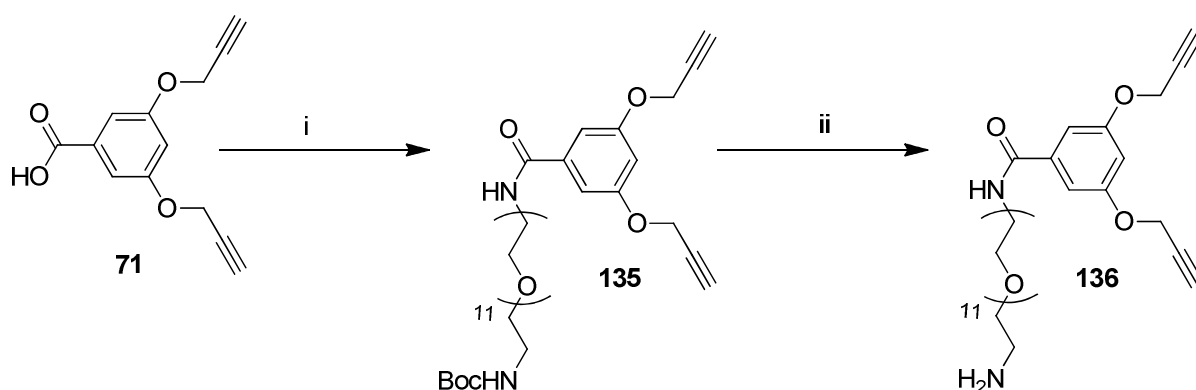


*Scheme 64: Reagents and conditions: (i) O-(2-Aminoethyl)-O'-[2-(Boc-amino)ethyl] decaethylene glycol, PyBOP, rt, 17 hours, (ii) HCl in dioxane, rt, 17 hours.*

As the functionalisation of the primary amine of conjugate **130** was unsuccessful in all

three methods attempted, the lack of reactivity of the amine was attributed to steric hindrance, with the large, bulky porphyrin structures preventing reaction of the amine at the end of a relatively short triethylene glycol chain. In order to allow functionalisation of the porphyrin-dendron conjugate focal point, synthesis of a dendron bearing a longer PEG chain was then attempted.

Conjugation of the commercially available heterobifunctional chain *O*-(2-Aminoethyl)-*O'*-[2-(Boc-amino)ethyl] decaethylene glycol to dendron **71** was carried out according to the method developed for the synthesis of **72**. Following overnight stirring with PyBOP, the desired product **135** was obtained in 81% yield, similar to that obtained for the triethylene glycol analogue **72**. Successful synthesis was confirmed by NMR and MS. Removal of the Boc group was then carried out using HCl in dioxane at room temperature overnight, with NMR analysis confirming the loss of the Boc peak to produce the desired product in excellent (96%) yield.
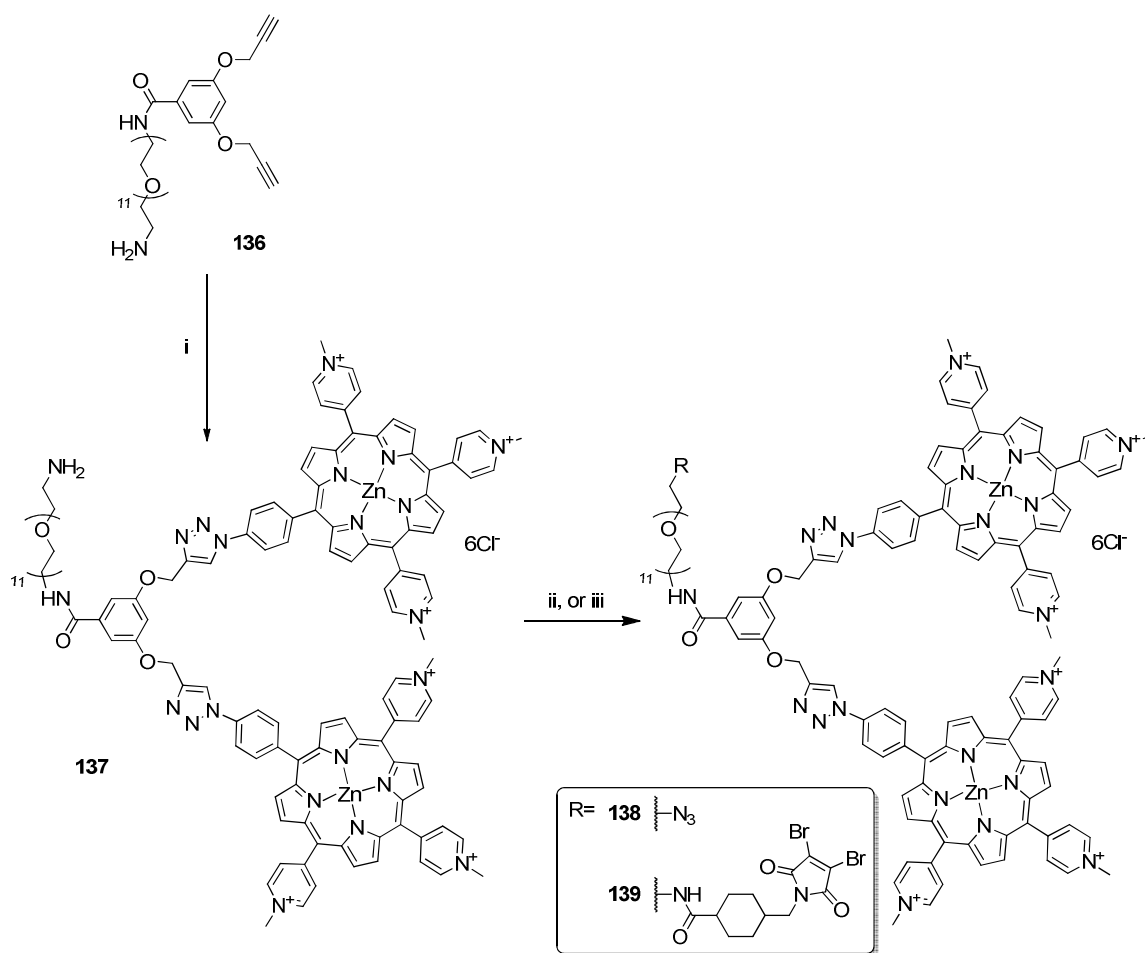


*Scheme 65: Reagents and conditions: (i)* **17**, *copper (II) sulfate pentahydrate, sodium ascorbate, 45 ˚C (MW), 60 mins, (ii) ISA hydrogen sulfate, potassium carbonate, copper (II) sulfate, rt, 17 hours, (iii)* **134**, *potassium carbonate, rt, 17 hours.*

152

Click conjugation of porphyrin **17** to the extended dendron **136** was then carried out using conditions as for previous conjugations, with TLC analysis showing complete consumption of the porphyrin starting material after 1 hour of microwave heating. NMR analysis confirmed the conjugation of two porphyrins to the structure, with the product obtained in good yield. Synthesis of **138** was carried out using identical conditions as for the synthesis of **131**, with HPLC analysis after 17 hours showing complete consumption of the starting material, and the formation of a single product. NMR characterisation confirmed the identity of the product, obtained in an 80% yield.

Synthesis of **139** was then attempted using identical conditions as for the synthesis of **133**, however after 48 hours HPLC analysis showed formation of 2 products and over 80% of the starting material still present. For this reason, synthesis was carried out with DMSO as the solvent, with the HPLC after 17 hours showing formation of a single product in around 40% yield, with longer reaction times having no significant effect on product yield.
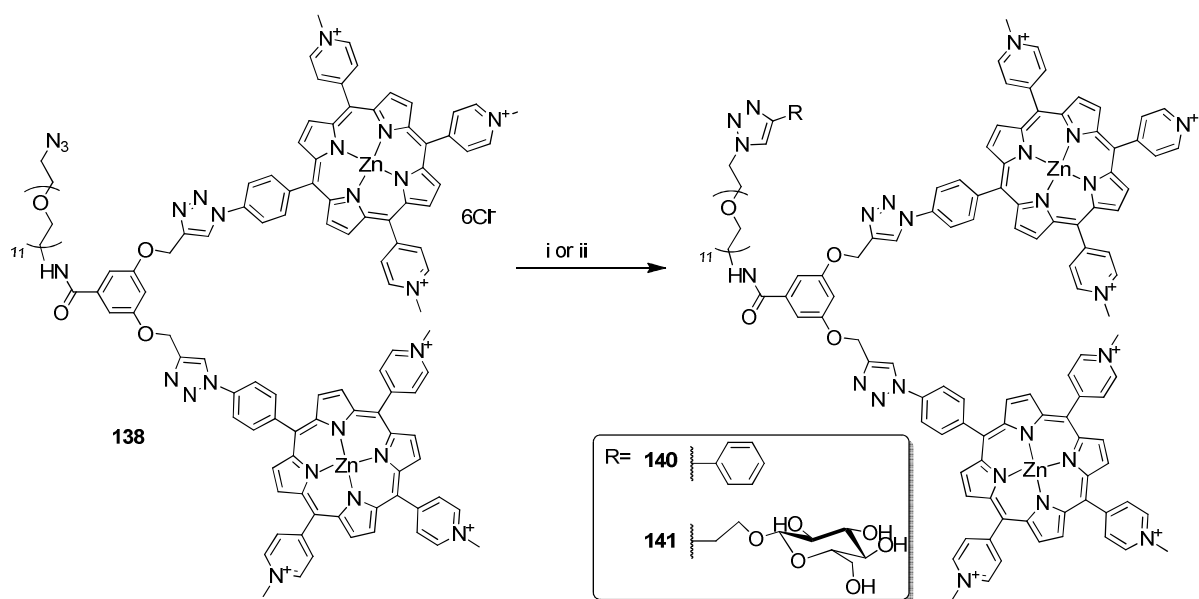


*Scheme 66: Reagents and conditions: (i) phenylacetylene, copper (II) sulfate pentahydrate, sodium ascorbate, 45 ˚C (MW), 3 hours, (ii) propargyl glucose, copper (II) sulfate pentahydrate, sodium ascorbate, rt, 17 hours.*

Click conjugation of conjugate **138** was then attempted, initially using phenylacetylene as a simple model alkyne. However, after microwave heating for 3 hours, TLC analysis showed no formation of the desired product. The lack of reactivity was attributed to the

high lipophilicity of phenylacetylene, with the addition of the alkyne to the THF:water solvent system leading to the formation of a biphasic mixture. While a THF:water solvent system has been used successfully in click reactions previously, generally these solvents are miscible and therefore allow the reaction to happen in the bulk solvent rather than at the solvent interface. In contrast, use of the biphasic solvent system toluene:water for click reactions has previously been shown to require higher temperatures and prolonged heating.

For this reason, click conjugation of **138** was then attempted using the click-functionalised sugar propargyl glucose. In addition to being highly water-soluble and therefore allowing the reaction to take place in water, click functionalised sugars have previously been used in the literature[151] as targeting groups for porphyrin photosensitisers, representing ideal biomolecules for use in these model bioconjugation reactions.

Synthesis of **141** was carried out in water with a copper (II) sulfate/sodium ascorbate catalyst system. After 5 hours, HPLC analysis showed the partial consumption of the starting material, and formation of a single peak at lower $R_f$, consistent with the addition of the more hydrophilic sugar functionality. Increased reaction time showed an increase of the product peak, with a 50:50 mixture of the starting material and product obtained after 17 hours reaction time. While these results suggest the successful click conjugation of conjugate **138** to a targeting biomolecule, it is clear that further reaction optimisation or development of a purification methodology would be required in order to obtain a pure sample of **141** for analysis.

### 4.6. Conclusions

A number of synthetic strategies were attempted in order to functionalise porphyrins with a dibromomaleimide group. While use of activated dibromomaleimide **103** and heterobifunctional linker chain **108** allowed functionalisation of amine and azide substituted tetraphenylporphyrins respectively, all attempts to transfer these reactions to a range of water-soluble porphyrins were unsuccessful. For this reason, synthesis of HER2 targeted photosensitisers was then attempted through the pre-functionalisation of Herceptin™ Fab fragments with heterobifunctional linker chain **108**. Alkyne functionalisation of the Fab fragment followed by click conjugation of two cationic porphyrins was carried out successfully to produce two targeted photosensitisers.

Preliminary cytotoxicity studies of the targeted photosensitisers Fab-**17** and Fab-**32** showed that both conjugates exhibited limited dark toxicity, and excellent cell killing in the HER2+ BT-474 cell line. However, full cytoxicity studies of these conjugates would be required to confirm that the cell killing is selective for the HER2+ cell line only, and to explore the cytotoxicity of these targeted photosensitisers at a range of concentrations.

Following the successful bioconjugation of single porphyrins to Herceptin™ Fab fragments, bioconjugation of previously synthesised porphyrin-dendron conjugates was then attempted. While deprotection of the protected amine at the dendron focal point both before and after porphyrin conjugation was carried out successfully, subsequent functionalisation of this amine *via* a number of methods was unsuccessful, a fact which was attributed to the high steric hindrance of the system. As a result, synthesis of an analogous dendron bearing a longer PEG chain at focal point was carried out, with functionalisation of the corresponding porphyrin-dendron conjugate with azide carried out in high yield. Model click reaction of the azide focal point to a click-functionalised sugar was carried out in moderate yield, with further optimisation of the reaction required in order to allow isolation of the product.

Following the successful work outlined in this chapter, further work could include the optimisation of the click reaction of **138** to biomolecules, followed by conjugation to the alkyne-functionalised Fab fragment. Photophysical and biological evaluation of this HER2 targeted porphyrin-dendron conjugate would allow for assessment of its suitability as a therapeutic agent.

# 5. Conclusions and Further Work

## 5.1. Conclusions

During the course of this work, the synthesis of a wide range of porphyrins was attempted, with the aim of producing structures with a single click functionality, zinc insertion into the central cavity, and the potential for water-solubilisation. Initially, synthesis of a range of lipophilic azide porphyrins was carried out, including an azide-functionalised tetraphenylporphyrin derivative utilised as a model porphyrin, and a number of porphyrins bearing latent water-solubilising functionalities. Synthesis of water-soluble clickable porphyrins was also carried out, with cationic, anionic and neutral water-soluble porphyrins successfully synthesised bearing azide functionalities both directly on a meso aryl ring and attached *via* a triethylene glycol linker chain. Synthesis of a single example of an alkyne functionalised porphyrin was also carried out in order to allow for structural flexibility.

Modification of existing dendron synthetic methods was carried out in order to produce a range of dendrons bearing protected amine focal points and between two and four peripheral click functionalities. Synthesis of azide-peripheralised dendrons was unsuccessful in all cases, however alkyne peripheral functionalisation was carried out to produce two examples of bis-alkyne PAMAM dendrons, an alkyne tris-type dendron, and three aryl ether dendrons bearing two, three and four peripheral alkynes.

Click conjugation of lipophilic porphyrin **5** was carried out with all dendrons as a test reaction, with successful synthesis of the desired porphyrin-dendron conjugates confirmed by NMR and MS in all cases. Following this, conjugation of cationic porphyrins **17** and **32** was attempted to all dendrons. Cationic porphyrin attachment to dendron **58** lead to cleavage of the ester bonds of the conjugate as a result of the high steric hindrance and relatively weak ester bonds, however successful conjugation of both cationic porphyrins to all other bis-alkyne dendrons was carried out successfully. High steric hindrance prevented full peripheralisation of all other dendrons with porphyrin **17**, however use of the linker chain functionalised porphyrin **32** allowed successful conjugation to dendrons **75** and **79** to yield conjugates bearing three and four porphyrins respectively.

156

Photophysical evaluation of all synthesised cationic porphyrin-dendron was carried out, including UV-vis, fluorescence and singlet oxygen quantum yield measurements. Both the UV-vis and SOQY results obtained showed some quenching effects due to steric hindrance in all systems, in particular in conjugate **97**. In contrast, PAMAM and 2$^{nd}$ generation aryl ether dendrons were shown to have the most favourable properties, with the potential absolute singlet oxygen production of all of these conjugates calculated to be greater than or equal to that of the control porphyrin.

Following the successful functionalisation of a lipophilic porphyrin with a dibromomaleimide ring, a number of methods were evaluated for the direct attachment of a dibromomaleimide ring to a range of water-soluble porphyrin species. In all cases, attachment of the dibromomaleimide was unsuccessful or extremely poorly yielding. As a result, synthesis of heterobifunctional linkers bearing both alkyne and dibromomaleimide functionalities was carried out.

Although click conjugation of these linker chains directly to cationic porphyrins was unsuccessful, pre-conjugation of an example of these linker chains to a Herceptin™ Fab fragment was carried out successfully, allowing click conjugation of two examples of cationic porphyrins. Preliminary biological evaluation of targeted photosensitisers Fab-**17** and Fab-**32** was carried out, with cytotoxicity assays showing low dark toxicity and excellent cell killing in the HER2+ cell line following irradiation with light for both conjugates.

Following successful attachment of single porphyrins to antibody fragments, functionalisation of a representative porphyrin-dendron was then trialled. Deprotection of the Boc-protected amine focal point was carried out successfully both pre- and post-click conjugation to porphyrins, however the pre-deprotection strategy was preferred due to the improved yields and reduced workup complexity. A number of different functionalisation methods were trialled on the free amine, however the high steric hindrance of the system led to limited functionalisation. Synthesis of an analogue bearing an increased length PEG chain at the focal point allowed for successful azide functionalisation, with a room-temperature model bioconjugation to an alkyne-functionalised sugar displays the potential for multiply-loading targeting biomolecules utilising this click conjugation strategy.

## 5.2. Overall achievements in relation to the field

This work has achieved a number of advances relative to the fields of porphyrin chemistry, porphyrin-dendron conjugation and the synthesis and evaluation of targeted photosensitisers suitable for use in photodynamic therapy.

In relation to porphyrin chemistry, this work has expanded upon the known synthetic methods for the synthesis of click functionalised porphyrins. Firstly, use and modification of existing azide-synthesis methods was utilised to produce water-soluble click-functionalised porphyrins not previously seen in the literature. Secondly, the development of a new method for the synthesis of zinc-azido porphyrins was carried out, utilising ISA hydrogen sulfate and zinc (II) acetate to generate a range of lipophilic and hydrophilic azide porphyrins in a mild, facile, one-pot synthesis.

The synthesis of porphyrin-dendron conjugates is a relatively unexplored area in the literature, with few examples of dendrons peripherally functionalised with porphyrins published. A number of novel click-functionalised dendrons have been synthesised, including the first examples of alkyne peripheralised PAMAM-type structures, and the modification of existing aryl ether dendron syntheses. In addition, this work includes the extensive development of a porphyrin-dendron click strategy, producing a wide range of lipophilic porphyrin-dendron structures and the first examples of cationic porphyrins conjugated to dendrons in this way.

Finally, this work also makes an important advance in the field of bioconjugation of porphyrins to antibody fragments, with the development of a novel conjugation strategy utilising alkyne pre-functionalisation of the antibody. Utilising this method, synthesis and biological evaluation of the first examples of photosensitisers conjugated to antibody fragments *via* the dibromomaleimide linker group was carried out.

## 5.3. Further work

In the short term, a range of further work could be carried out in this project. Firstly, full cytotoxicity assays of both of the synthesised targeted photosensitisers Fab-**17** and Fab-**32** could be carried out, demonstrating their selectivity through the use of a HER2- cell line, and comparing this to a free-photosensitiser control in both cell lines. Additionally,

evaluation of the cytotoxicity of both conjugates at a range of concentrations would allow for comparison to other targeted photosensitisers in the literature.

Following this, conjugation of azide-functionalised conjugate **138** to an alkyne-functionalised Fab fragment could be carried out, allowing the successful synthesis of an antibody fragment targeted porphyrin-dendron. Photophysical and biological evaluation of this conjugate would then allow confirmation of its potential for use as a 3$^{rd}$ generation photosensitiser for photodynamic therapy. In addition, synthesis of longer PEG-chain analogues of all synthesised dendrons could be carried out, followed by conjugation to water-soluble porphyrins to create a range of conjugatable porphyrin-dendron species. Bioconjugation and subsequent biological evaluation of these structures would allow evaluation of the impact of the use of different porphyrins, dendrons and loading ratios on the effectiveness of these structures as photosensitisers.

In the long term, the results gained from photophysical and biological evaluation of a range of porphyrin-dendron structures could be utilised to guide the direction of this research. Results indicating greater loading ratios showing more effective cytotoxic activity would show the need for synthesis of higher-generation dendrons with greater alkyne peripheralisation, while a preference for the more hydrophilic PAMAM-type conjugates over the aryl ether dendron structures would suggest a need for further development of second- and third-generation structures of this type, or the possibility of a mixed-type dendron structure. A comparison of the bis porphyrin dendrons utilising porphyrin **17** and **32** would allow the evaluation of the impact of steric effects on the cytotoxicity of these structures, with synthesis of porphyrins and dendrons bearing extended PEG linker chains allowing a solution to this problem.

In addition, alternative bioconjugation strategies could be utilised on the synthesised porphyrin-dendron structures to allow their exploitation in a number of different photodynamic therapy applications. Alkyne functionalisation at the disulfide bridge of antibody fragments targeted to other tumour-associated antigens would allow treatment of a wide range of HER2-negative cancers. In particular, use of antibody fragments targeting neovasculature, such as L19 would allow for the synthesis of a photosensitiser capable of targeting a wide range of tumours. Alternatively, use of targeting moieties such as peptides or carbohydrates would allow the exploitation of alternative tumour-targeting methods, exploiting properties such as reduced residence time and the

increased possibility for structural modification.

Alternatively, the development of other procedures for the conjugation of porphyrin-dendron conjugates and antibody fragment would allow for these conjugates to be utilised in a number of different applications. In particular, development of a click methodology utilising the strained, copper-free click reaction would allow for pre-administration of the antibody fragment, followed by subsequent *in vivo* attachment of azide-functionalised porphyrin-dendron conjugates. Such biologically-compatible conjugation methods allow for use as dual PET imaging-treatment modalities while avoiding the problem of the long half-life of the antibody fragment in the bloodstream, utilising gallium-68 metallated porphyrins to carry out PET imaging of tumours.

Finally, as the CuAAC reaction is widespread throughout organic chemistry, use of the conjugatable porphyrin-dendron species could be applied to a number of areas in which increased loading of porphyrins onto structures is desirable. In particular, applications in polymer synthesis, loading of nanoparticles and surface coating of materials could all be attempted utilising these structures.

# 6. Experimental

## 6.1. General information

$^1$H NMR and $^{13}$C NMR were obtained using a Jeol JNMLA400 spectrometer at 400 MHz for $^1$H NMR and 100.5MHz for $^{13}$C NMR, and referenced against standard internal TMS or proton resonances arising from incomplete deuteration of the NMR solvent. Splitting patterns are designated as s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), m (multiplet) and br s (broad singlet). MS data was obtained from the EPSRC National Mass Spectrometry Service, Swansea and Mass Spectrometry Centre, University of York. Melting points were obtained on a Stuart SMP10 melting point apparatus without correction. UV-vis spectra were measured on a Varian Cary 50 Bio UV-vis spectrophotometer. Fluorescence spectra were measured on a Varian Cary Eclipse Fluorescence spectrophotometer.

High performance liquid chromatography (HPLC) analysis was carried out on a Jasco system equipped with a Jasco PU-1580 dual pump, Jasco MD-1515 multiwavelength detector and a Gemini-NX C18 column (100A, 150x 4.6 mm), using acetonitrile (0.1% TFA) (solvent B) and water (0.1% TFA) (solvent A) as the mobile phase. All microwave reactions were conducted using a CEM Benchmate microwave reactor, at 150W unless otherwise stated. All programmes used maximum stirring, maximum pressure of 200 bar, and 2 minute heating steps. Reaction temperatures were monitored using an external IR temperature probe and carried out in a 10ml sealed reaction vessel.

All reagents involved in the synthesis were reagent grade, purchased from Alfa Aesar and Sigma Aldrich, and used as received. Dry pyridine was used as purchased, all other dry solvents were obtained from drying solvents over molecular sieves according to the method of Williams *et al*[250]. All other reagents were used as purchased. Bulk solvent was removed by rotary evaporation under reduced pressure and trace solvent was removed by a vacuum oven. Reactions were run at room temperature (rt) unless otherwise stated. All reactions were monitored by TLC using Fluka Analytical 0.2 mm layer thickness 60 Å pore silica gel plates with or without UV indicator (F-254). Silica gel (Fluorochem LC60A 35–70 $\mu$m) was used for normal phase column chromatography, C18 silica gel (Fluka Analytical Silica Gel 90 C18) was used for reverse phase column chromatography.

Material **40** was synthesised and kindly provided by Dr F. Giuntini, University of Hull.

Propargyl glucose was kindly provided by Dr E.M Scanlan, Trinity College Dublin.

**Tetraphenylporphyrin (1)**
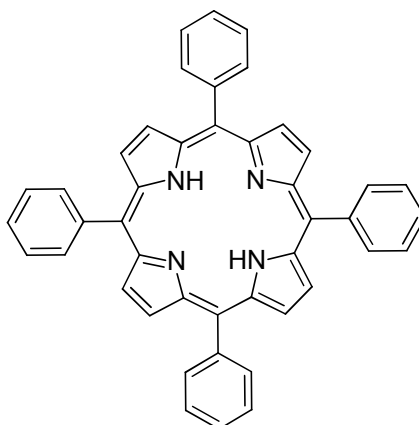


To a stirred solution of benzaldehyde (8.48 g, 80 mmol) in refluxing propionic acid (500 ml) was added pyrrole (5.54 ml, 80 mmol) dropwise and the mixture stirred under reflux for 3 hour, protected from light. The reaction was cooled to room temperature and the solvent removed under reduced pressure. The crude was precipitated from methanol to yield the product as a purple solid (3.94 g, 32.1%)

$^1$H-NMR(CDCl$_3$): δ 7.79 (m, 12H, 5,10,15,20-m,p-Ph), 8.25 (m, 8H, 5,10,15,20-o-Ph), 8.88 (m,8H, βH). $^{13}$C-NMR (CDCl$_3$): δ 120.22, 126.82 (β-C), 127.86, 134.76 (β-C), 142.33.

**5-[4-Nitrophenyl]-10,15,20-triphenylporphyrin (2)[171]**
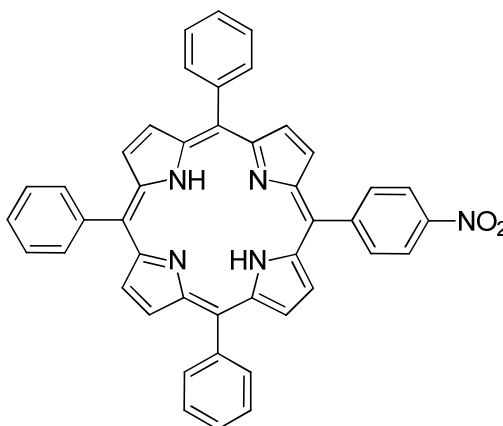


A solution of tetraphenylporphyrin (0.96 g, 1.51 mmol) in dichloromethane (180 ml) was purged with nitrogen for 10 minutes, followed by the addition of nitronium tetrafluoroborate in sulfolane (2.74 ml, 1.37 mmol) dropwise over a period of 30 minutes. The mixture was stirred at room temperature for 45 minutes, followed by the addition of nitronium tetrafluoroborate in sulfolane (2.74 ml, 1.37 mmol) dropwise over a period of 30 minutes. The mixture was stirred at room temperature for 45 minutes, followed by the addition of nitronium tetrafluoroborate in sulfolane (1.0 ml, 0.5 mmol) dropwise over a period of 10 minutes. The mixture was stirred at room temperature for 10 minutes, followed by the addition of nitronium tetrafluoroborate in sulfolane (1.0 ml, 0.5 mmol) dropwise over a period of 10 minutes. The organic layer was washed with water (500 ml), dried ($Na_2SO_4$) and the solvent removed under reduced pressure. The crude was redissolved in acetone, and the porphyrin precipitated with addition of water and filtered. The product was purified by column chromatography (silica, 1:1 hexane:DCM) and precipitated from MeOH over DCM to yield the product as a purple solid (810 mg, 81.4%).

$R_f$ = 0.23 (silica, 50% hexane:dichloromethane). UV-vis (DCM): $\lambda$max, nm 419, 450, 517, 552, 581. $\varepsilon$ (419 nm)= 292504$M^{-1}cm^{-1}$. $^1$H-NMR(CDCl$_3$): $\delta$ -2.78 (s, 2H,- N*H*), 7.78 (m, 9H, 10,15,20-m,p-Ph), 8.22 (m, 6H, 10,15,20-o-Ph), 8.39 (d, 2H, J= 8.13 Hz, 5-m-Ph), 8.62 (d, 2H, J= 8.16 Hz, 5-o-Ph), 8.73 (m, 2H, βH), 8.89 (m,6H, βH). $^{13}$C-NMR (CDCl$_3$): $\delta$ 116.60, 120.66, 121.06, 121.83, 126.75 (β-C), 127.76, 127.87, 134.53 (β-C),135.12, 141.88, 141.91, 147.70. MS: (ESI) m/z 659.2 (100[M + H]+),

## 5-[4-Aminophenyl]-10,15,20-triphenylporphyrin (3) [169]
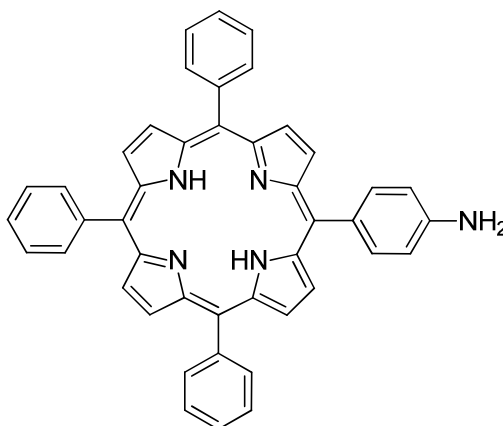


To a solution of 5-[4-nitrophenyl]-10,15,20-triphenylporphyrin (400 mg, 0.607 mmol) in $HCl_{(aq)}$ (36%, 150 ml) was added tin (II) chloride dihydrate (411 mg, 1.82 mmol) and the mixture stirred at 60 ˚C under $N_2$ for 1 hour. The solvent was removed under reduced pressure and the residue was dissolved in DCM/TEA (9:1, 200 ml) and stirred for 10 minutes at room temperature. The solution was washed with water (3 x 200 ml) and the organic layer dried ($Na_2SO_4$). The solvent was removed under reduced pressure, and the crude precipitated from MeOH over DCM to yield the product as a purple solid (378 mg, 98.9%).

*$R_f$ = 0.40 (silica, dichloromethane). UV-vis (DCM): λmax, nm 420, 454, 515, 554, 594. ε (420 nm)= 266896 $M^{-1}cm^{-1}$. $^1$H-NMR(CDCl$_3$): δ 6.95 (m, 2H, 5-m-Ph), 7.72 (m, 9H, 10,15,20-m,p-Ph), 7.96 (m, 2H, 5-o-Ph), 8.22 (m, 6H, 10,15,20-o-Ph) , 8.73-8.93 (m, 8H, βH). $^{13}$C-NMR (CDCl$_3$): δ 113.56, 119.84, 120.06, 120.96, 126.77 (β-C), 127.74, 132.48, 134.67 (β-C), 135.78, 142.33, 142.38, 146.13. MS: (ESI) m/z 629.3 (100[M + H]+),*

**5-[4-Azidophenyl]-10,15,20-triphenylporphyrin (4)** [169]
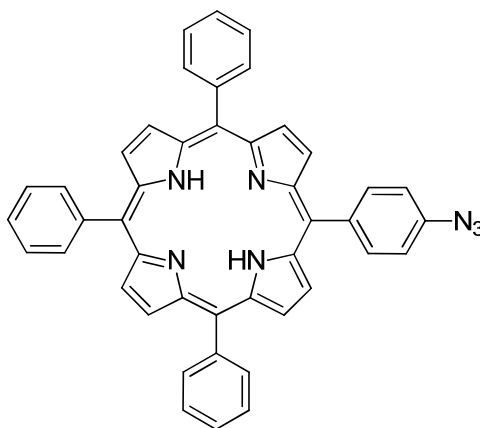


To a stirred solution of 5-[4-aminophenyl]-10,15,20-triphenylporphyrin (200 mg, 0.315 mmol) in TFA (4 ml) at 0 °C was added dropwise a solution of sodium nitrite (44 mg, 0.63mmol) in water. The mixture was stirred for 15 minutes at 0 °C. A solution of sodium azide (83 mg, 1.26 mmol) in water was added dropwise and the reaction mixture stirred at 0 °C for 1 hour. The mixture was diluted with water and saturated sodium bicarbonate solution added until the colour changed from green to purple. The aqueous mixture was extracted with dichloromethane, the organic layer dried ($Na_2SO_4$) and the solvent removed under reduced pressure. The crude was precipitated from MeOH over DCM to yield the product as a purple solid (193 mg, 93.7%).

*$R_f$ = 0.34 (silica, 50% hexane:dichloromethane). UV-vis (DCM): λmax, nm 420, 450, 515, 552, 593. ε (420 nm)= 393878 $M^{-1}cm^{-1}$. $^1$H-NMR(CDCl$_3$): δ -2.78 (s, 2H,- N<u>H</u>), 7.43 (d, 2H, J= 8.16 Hz, 5-m-Ph), 7.77 (m, 9H, 10,15,20-m,p-Ph), 8.22 (m, 8H, 5,10,15,20-o-Ph), 8.90 (m, 8H, βH). $^{13}$C-NMR (CDCl$_3$): δ 117.43, 120.25, 126.69 (β-C), 127.74, 134.54 (β-C), 135.70, 139.67, 140.04, 142.09. MS: (ESI) m/z 655.2 (100[M + H]+),*

**Zinc 5-[4-azidophenyl]-10,15,20-triphenylporphyrin (5)[151]**



To a stirred solution of 5-[4-azidophenyl]-10,15,20-triphenylporphyrin (159 mg, 0.243 mmol) in dichloromethane (30 ml) was added zinc acetate (159 mg, 0.867 mmol) in methanol (1 ml). The mixture was stirred at room temperature for 1 hour, and the solvent removed under reduced pressure. The crude was precipitated from MeOH over DCM to yield the product as a deep purple solid (150 mg, 86.1%).

*$R_f$ = 0.30 (silica, 50% hexane:dichloromethane). UV-vis (DCM): λmax, nm 420, 548, 586. ε (420 nm)= 624657 $M^{-1}cm^{-1}$. $^1$H-NMR(CDCl$_3$): δ 7.25 (d, 2H, J= 8.40 Hz, 5-m-Ph), 7.77 (m, 9H, 10,15,20-m,p-Ph), 8.22 (m, 8H, 5,10,15,20-o-Ph), 8.87 (m, 8H, βH). $^{13}$C-NMR (CDCl$_3$): δ 117.19, 119.27, 121.34, 126.52 (β-C), 127.48, 131.59, 132.01, 132.10, 134.42 (β-C),135.52, 142.81, 150.07, 150.17, 150.26. MS: (MALDI) m/z 718(100[M +H]+), HRMS: calcd. for C$_{44}$H$_{28}$N$_7$Zn: 718.16922 found 718.17529.*
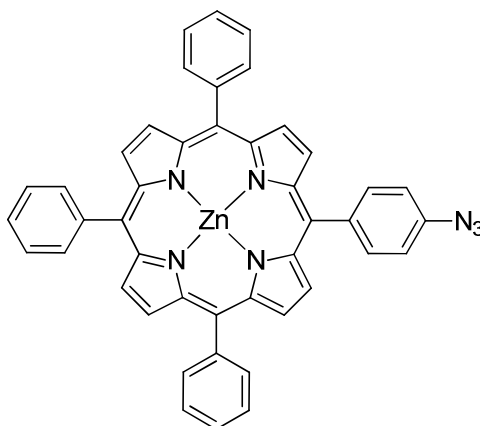
**Zinc 5-[4-azidophenyl]-10,15,20-triphenylporphyrin (5)**
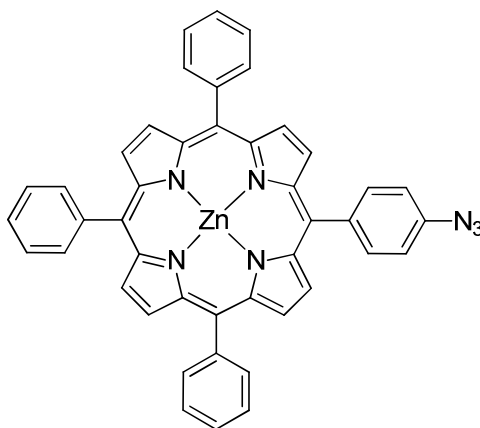


To a stirred solution of 5-[4-aminophenyl]-10,15,20-triphenylporphyrin (50 mg, 0.079 mmol in DCM:methanol (3:2, 10 ml) was added zinc acetate (25 mg, 0.14 mmol), and triethylamine (25 mg, 0.250 mmol). Imidazole-1-sulfonyl azide hydrogen sulfate (24 mg, 0.090 mmol) was added, and the mixture stirred at rt for 17 hours. Water was added, and the mixture filtered. The residue was precipitated from MeOH over DCM to yield the product as a purple solid (53 mg, 93.6%).

*$R_f$ = 0.30 (silica, 50% hexane:dichloromethane). UV-vis (DCM): λmax, nm 420, 548, 586. ε (420 nm)= 624657 $M^{-1}cm^{-1}$. $^1$H-NMR(CDCl$_3$): δ 7.25 (d, 2H, J= 8.41 Hz, 5-m-Ph), 7.77 (m, 9H, 10,15,20-m,p-Ph), 8.22 (m, 8H, 5,10,15,20-o-Ph), 8.87 (m, 8H, βH). $^{13}$C-NMR (CDCl$_3$): δ 117.19, 126.52 (β-C), 127.48, 131.59, 132.10 (β-C), 135.52, 142.81, 150.66, 150.17, 150.26. MS: (MALDI) m/z 718(100[M +H]+), HRMS: calcd. for C$_{44}$H$_{28}$N$_7$Zn: 718.16922 found 718.17529.*

**5-[4-Cyanophenyl]-10,15,20-triphenyl porphyrin (6)**
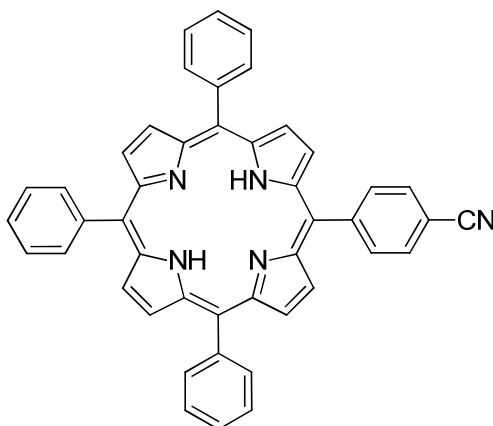


To a stirred solution of 4-cyanobenzaldehyde (1.40 g, 10.7 mmol) in refluxing propionic acid (250 ml) was added benzaldehyde (2.70 g, 25.5 mmol). Pyrrole (2.5 ml, 36 mmol) was added dropwise and the mixture stirred under reflux for 1 hour, protected from light. The reaction was cooled to room temperature and the solvent removed under reduced pressure. The crude was purified by column chromatography (silica, 1:4 hexane:DCM), and precipitated from MeOH over DCM to yield the product as a purple solid (580 mg, 10.1%).

*UV-vis (DCM): λmax, nm 418, 515, 551, 589, 647. ε (418 nm)= 652282 $M^{-1}cm^{-1}$. $^{1}H$-NMR(CDCl$_3$): δ 7.76 (m, 9H, 10,15,20-m,p-Ar), 8.06 (d, 2H, J= 8.16 Hz, 5-m-Ar), 8.21 (m, 6H, 10,15,20-o-Ar), 8.33 (d, 2H, J= 7.92 Hz, 5-o-Ar), 8.72-8.89 (m, 8H, βH). $^{13}C$-NMR (CDCl$_3$): δ 111.80, 117.11, 119.02, 120.58, 120.97, 126.72 (β-C),126.75, 127.85, 130.50, 134.53 (β-C), 134.98, 141.88, 147.21. MS: (MALDI) m/z 639 (100[M ]+).*

## 5-[4-Aminomethylphenyl]-10,15,20-triphenyl porphyrin (7)
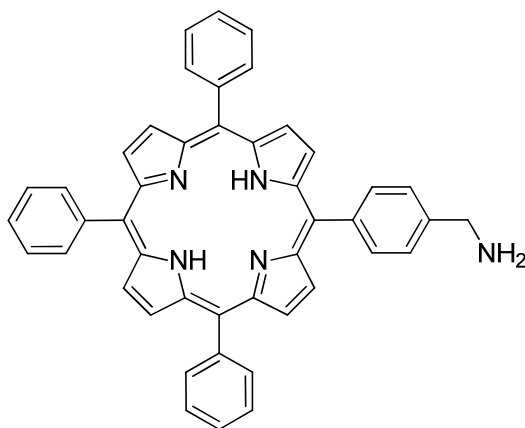


To a stirred suspension of lithium aluminium hydride (80 mg, 2.10 mmol) in dry THF (50 ml) was added dropwise a solution of 5-[4-cyanophenyl]-10,15,20-triphenyl porphyrin (236 mg, 0.370 mmol), and the mixture stirred overnight at rt under $N_2$. The mixture was quenched with NaOH, extracted with DCM and the solvent removed under reduced pressure. The crude was purified by column chromatography (silica, 0-5% MeOH:DCM) and precipitated from MeOH over DCM to yield the product as a purple solid (159 mg, 66.8%).

*UV-vis (DCM): λmax, nm 418, 514, 552, 594, 646. ε (418 nm)= 339913 $M^{-1}cm^{-1}$. $^1$H-NMR(CDCl$_3$): δ 4.25 (s, 2H, CH$_2$), 7.74 (m, 11H, 10,15,20-m,p-Ar, 5-m-Ar), 8.18 (m, 8H, 5,10,15,20-o-Ar), 8.83 (m, 8H, βH). $^{13}$C-NMR (CDCl$_3$): δ 46.40 (CH$_2$) 119.99, 120.11, 125.41, 126.66 (β-C), 127.69, 134.54 (β-C),137.75, 140.65, 142.16. MS: (ESI) m/z 644 (100[M + H]+), HRMS: calcd. for C$_{45}$H$_{34}$N$_5$: 644.2809 found 644.2800.*

**Zinc 5-[4-azidomethylphenyl]-10,15,20-triphenyl porphyrin (8)**
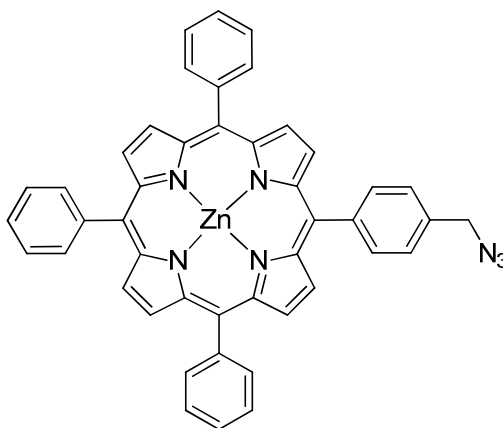


To a stirred solution of 5-[4-aminomethylphenyl]-10,15,20-triphenyl porphyrin (25.7 mg, 0.040 mmol) in DCM:methanol (3:2, 10 ml) was added triethylamine (25 mg, 0.250 mmol) and zinc acetate (25 mg, 0.135 mmol). Imidazole-1-sulfonyl azide hydrogen sulfate (12 mg, 0.045 mmol) was added and the mixture stirred for 3 hours at rt. The solvent was removed under reduced pressure and the crude purified by column chromatography (silica, DCM). The product was precipitated from MeOH over DCM to yield the product as a purple solid (26.9 mg, 91.9%).

*UV-vis (DCM): λmax, nm 419, 548, 587. ε (419 nm)= 678173 $M^{-1}cm^{-1}$. $^1$H-NMR(CDCl$_3$): δ 4.14 (s, 2H, C$\underline{H}_2$),7.35 (d, 2H, J= 7.76 Hz, 5-m-Ar) 7.74 (m, 9H, 10,15,20-m,p-Ar), 8.15 (d, 2H, J=7.72 Hz, 5-o-H), 8.23 (m, 6H, 10,15,20-o-Ar), 8.85-8.98 (m, 8H, βH). $^{13}$C-NMR (CDCl$_3$): δ 54.28 (C$\underline{H}_2$), 120.15, 121.19, 121.26, 126.13, 126.54 (β-C), 127.50, 131.75, 132.04, 132.11, 134.42 (β-C),134.63, 142.78, 142.87, 150.00, 150.21, 150.26. MS: (MALDI) m/z 731 (100[M ]+), HRMS: calcd. for C$_{45}$H$_{29}$N$_7$Zn: 731.17704 found 731.17786.*

## 5-[4-Acetamidophenyl]-10,15,20-tri-(4-carbomethoxyphenyl)porphyrin (9a)[175]
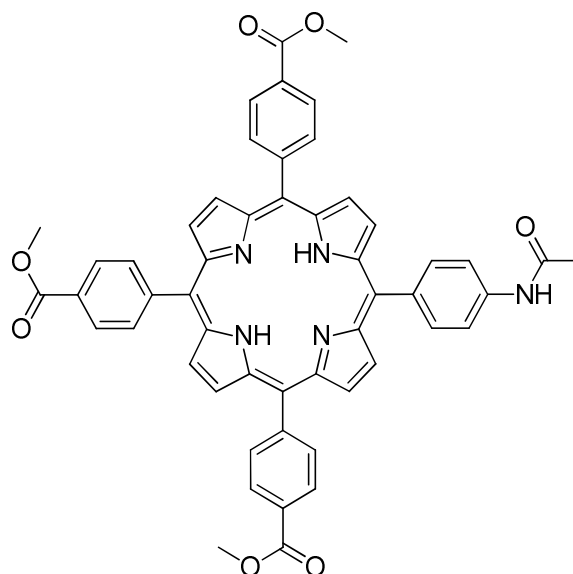


To a stirred solution of 4-acetamidobenzaldehyde (1.76 g, 10.7 mmol) in refluxing propionic acid (250 ml) was added methyl-4-formylbenzoate (4.19 g, 25.5 mmol). Pyrrole (2.5 ml, 36 mmol) was added dropwise and the mixture stirred under reflux for 1 hour, protected from light. The reaction was cooled to room temperature and the solvent removed under reduced pressure. The crude was purified by column chromatography (silica, 1.5% MeOH:DCM), and precipitated from MeOH over DCM to yield the product as a purple solid (604 mg, 7.93%).

*UV-vis (DCM): λmax, nm 421, 515, 552, 592, 648. ε (421 nm)= 526464 $M^{-1}cm^{-1}$.*
*$^{1}$H-NMR(CDCl$_3$): δ 2.33 (s, 3H, O=C-C$\underline{H}_3$), 4.11 (s, 9H,O-CH$_3$), 7.55 (s, 1H, N$\underline{H}$), 7.91 (d, 2H, J=8.36 Hz,5-m-Ar ), 8.17 (d, 2H, J=8.36 Hz, 5-o-Ar), 8.28 (m, 6H, 10,15,20-m-Ar), 8.44 (m, 6H, 10,15,20-o-Ar), 8.78-8.89 (m, 8H, β-H). $^{13}$C-NMR (CDCl$_3$): δ 24.86 (O=C-C$\underline{H}_3$), 52.45 (O-C$\underline{H}_3$),118.03, 119.18, 120.38, 127.94 (β-C), 129.68, 134.51 (β-C), 135.08, 137.63, 137.95, 146.73, 167.26 (O=$\underline{C}$-O-CH$_3$), 168.58 (O=$\underline{C}$-NH). MS: (MALDI) m/z 845 (100[M ]+).*

**5-[4-Acetamidophenyl]-10,15,20-tri-(4-ethoxyphenyl)porphyrin (9b)**



To a stirred solution of 4-acetamidobenzaldehyde (1.76 g, 10.7 mmol) in refluxing propionic acid (250 ml) was added 4-ethoxybenzaldehyde (3.82 g, 25.5 mmol). Pyrrole (2.5 ml, 36 mmol) was added dropwise and the mixture stirred under reflux for 1 hour, protected from light. The reaction was cooled to room temperature and the solvent removed under reduced pressure. The crude was purified by column chromatography (silica, 1.5% MeOH:DCM), and precipitated from MeOH over DCM to yield the product as a purple solid (480 mg, 6.64%).

*UV-vis (DCM): λmax, nm 422, 518, 557, 595, 651. ε (422 nm)= 556396 $M^{-1}cm^{-1}$. $^1$H-NMR(CDCl$_3$): δ 1.62 (t, 9H, J= 6.92 Hz, CH$_2$-C$\underline{H}_3$), 2.35 (s, 3H, O=C-C$\underline{H}_3$), 4.32 (m, 6H, C$\underline{H}_2$-CH$_3$), 7.25 (m, 6H, 10,15,20-m-Ar), 7.48 (s, 1H, N$\underline{H}$), 7.86 (d, 2H, J= 8.16 Hz, 5-m-Ar), 8.09 (m, 6H, 10,15,20-o-Ar), 8.13 (d, 2H, J=8.16 Hz, 5-o-Ar), 8.84 (m, 8H, βH). $^{13}$C-NMR (CDCl$_3$): δ 14.95 (C$\underline{H}_3$-CH$_2$), 24.60 (O=C-C$\underline{H}_3$),63.72 (CH$_3$-C$\underline{H}_2$), 112.65 (β-C), 114.49, 114.94, 114.98, 117.93, 119.02, 119.87, 120.09, 129.54, 130.25, 134.40, 135.07, 135.58 (β-C), 137.50, 138.28, 158.75, 168.70 ($\underline{C}$=O). MS: (ESI) m/z 804 (100[M + H]+), HRMS: calcd. for C$_{52}$H$_{46}$N$_5$O$_4$:804.3544 found 804.3546.*

**5-[4-Acetamidophenyl]-10,15,20-tri-(3-methoxyphenyl)porphyrin (9c)**
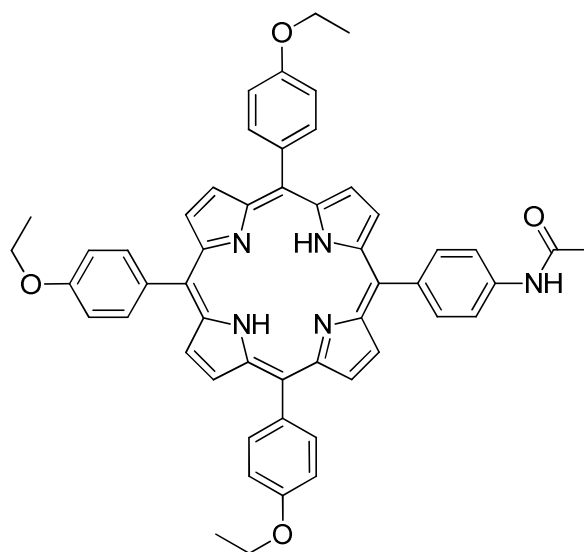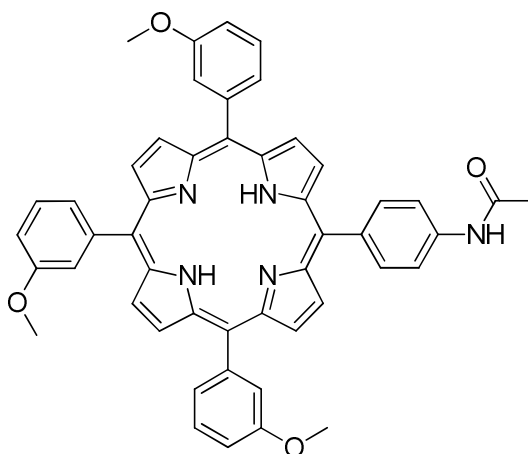


To a stirred solution of 4-acetamidobenzaldehyde (1.76 g, 10.7 mmol) in refluxing propionic acid (250 ml) was added 3-methoxybenzaldehyde (3.10 ml, 25.5 mmol). Pyrrole (2.5 ml, 36 mmol) was added dropwise and the mixture stirred under reflux for 1 hour, protected from light. The reaction was cooled to room temperature and the solvent removed under reduced pressure. The crude was purified by column chromatography (silica, 1% MeOH:DCM), and precipitated from MeOH over DCM to yield the product as a purple solid (535 mg, 7.81%).

*UV-vis (DCM): λmax, nm 420, 515, 552, 593, 649. ε (420 nm)= 523059 $M^{-1}cm^{-1}$. $^{1}H$-NMR(CDCl$_3$): δ 2.31 (s, 3H, C$\underline{H}_3$-C=O), 3.96 (s, 9H, O-CH$_3$), 7.31 (m, 3H, 10,15,20-p-Ar),7.45 (s, 1H, N$\underline{H}$), 7.63 (m, 3H, 10,15,20-m-Ar), 7.80 (m, 8H ,10,15,20-o-H, 5-m-Ar), 8.13 (d, 2H, J= 8.16 Hz, 5-o-Ar), 8.83-8.97 (m, 8H, βH)). $^{13}C$-NMR (CDCl$_3$): δ 24.82 (O=C-$\underline{C}$H$_3$), 55.48 (O-C$\underline{H}_3$), 113.55, 117.94, 119.80, 119.87, 120.42, 127.48 (β-C), 127.65 (β-C), 135.05, 137.56, 138.05, 143.42, 157.92, 168.54 ($\underline{C}$=O). MS: (ESI) m/z 762 (100[M + H]+), HRMS: calcd. for C$_{49}$H$_{40}$N$_5$O$_4$: 762.3075 found 762.3082.*

**5-[4-Acetamidophenyl]-10,15,20-tri-(4-chlorophenyl)porphyrin (9d)**
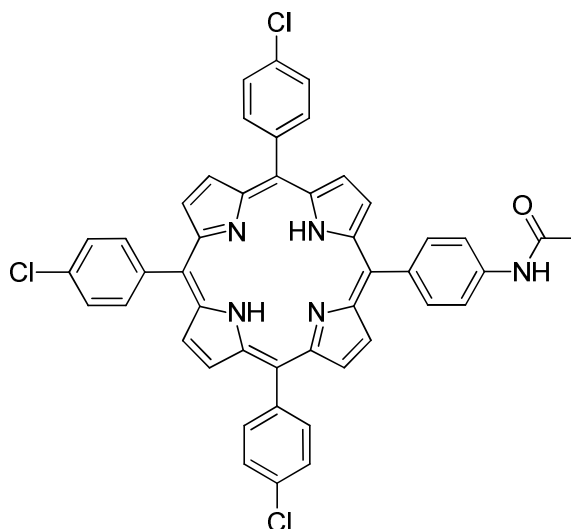


To a stirred solution of 4-acetamidobenzaldehyde (3.52 g, 21.4 mmol) in refluxing propionic acid (500 ml) was added 4-chlorobenzaldehyde (3.52 g, 51 mmol). Pyrrole (5 ml, 72 mmol) was added dropwise and the mixture stirred under reflux for 1 hour, protected from light. The reaction was cooled to room temperature and the solvent removed under reduced pressure. The crude was purified by column chromatography (silica, 1% MeOH:DCM), and precipitated from MeOH over DCM to yield the product as a purple solid (660 mg, 4.74%).

*UV-vis (DCM): λmax, nm 419, 515, 552, 590, 648. ε (419 nm)= 632246 $M^{-1}cm^{-1}$. $^{1}H$-NMR(CDCl$_3$): δ 2.36 (s, 3H, O=C-C$\underline{H}_3$), 7.52 (s, 1H, N$\underline{H}$), 7.72 (m, 6H, 10,15,20-m-Ar),7.89 (d, 2H, J= 8.36 Hz, 5-m-Ar), 8.14 (m, 8H, 5,10,15,20-o-Ar) 8.81-8.89 (m, 8H, β-H). $^{13}C$-NMR (CDCl$_3$): δ 24.82 (O=C-C$\underline{H}_3$),117.80, 118.02,118.50, 118.91, 127.00 (β-C), 134.31, 135.09,135.48 (β-C), 137.69, 140.42, 168.68 (O=$\underline{C}$-NH). MS: (ESI) m/z 776 (100[M+H ]+), HRMS: calcd. for C$_{46}$H$_{31}$N$_5$O$_1$Cl$_3$: 774.1589 found 774.1585.*

**5-[4-Acetamidophenyl]-10,15,20-tri-(4-bromophenyl)porphyrin (9e)**



To a stirred solution of 4-acetamidobenzaldehyde (1.76 g, 10.7 mmol) in refluxing propionic acid (250 ml) was added 4-bromobenzaldehyde (4.72 g, 25.5 mmol). Pyrrole (2.5 ml, 36 mmol) was added dropwise and the mixture stirred under reflux for 1 hour, protected from light. The reaction was cooled to room temperature and the solvent removed under reduced pressure. The crude was purified by column chromatography (silica, 1% MeOH:DCM), and precipitated from MeOH over DCM to yield the product as a purple solid (630 mg, 7.73%).

*UV-vis (DCM): λmax, nm 420, 516, 551, 590, 649.  ε (420 nm)= 452546 M$^{-1}$cm$^{-1}$. $^{1}$H-NMR(CDCl$_3$): δ 2.36 (s, 3H, O=C-C$\underline{H}_3$), 7.52 (s, 1H, N$\underline{H}$), 7.88 (m, 8H, 5,10,15,20-m-Ar), 8.05 (m, 6H, 10,15,20-o-Ar), 8.16 (d, 2H, J=8.16  Hz, 5-o-Ar),  8.80-8.87 (m, 8H, β-H).  $^{13}$C-NMR (CDCl$_3$): δ 24.87 (O=C-$\underline{C}$H$_3$),118.03, 118.83, 120.15, 122.56, 129.94 (β-C),135.09, 135.82 (β-C), 137.76, 140.90,  168.56 (O=$\underline{C}$-NH). MS: (ESI) m/z 910 (100[M + H]+), HRMS: calcd. for C$_{46}$H$_{30}$Br$_3$N$_5$O$_1$: 906.0073 found 906.0073.*
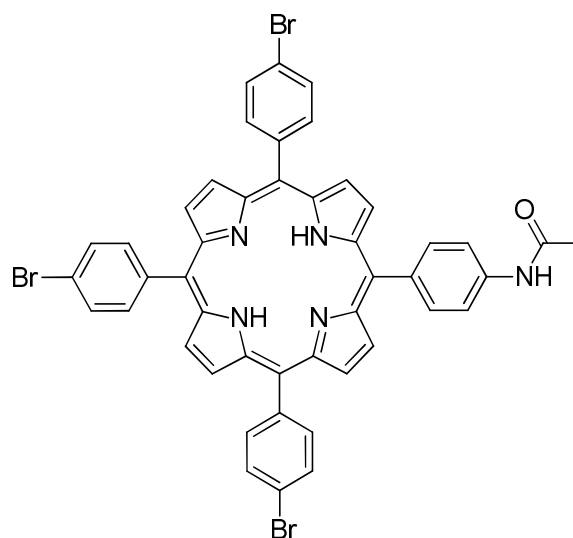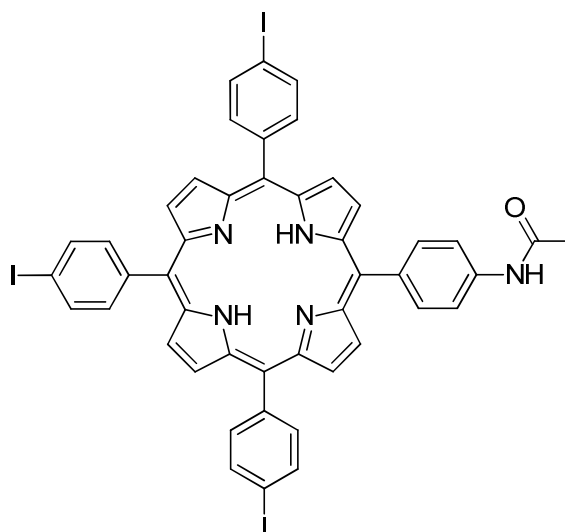
**5-[4-Acetamidophenyl]-10,15,20-tri-(4-iodophenyl)porphyrin (9f)**



To a stirred solution of 4-acetamidobenzaldehyde (1.76 g, 10.7 mmol) in refluxing propionic acid (250 ml) was added 4-iodobenzaldehyde (5.92 g, 25.5 mmol). Pyrrole (2.5 ml, 36 mmol) was added dropwise and the mixture stirred under reflux for 1 hour, protected from light. The reaction was cooled to room temperature and the solvent removed under reduced pressure. The crude was purified by column chromatography (silica, 1% MeOH:DCM), and precipitated from MeOH over DCM to yield the product as a purple solid (829 mg, 8.78%).

*UV-vis (DCM): λmax, nm 421, 516, 553, 591, 649. ε (421 nm)= 507361 $M^{-1}cm^{-1}$. $^1$H-NMR(CDCl$_3$): δ 2.36 (s, 3H, O=C-CH$_3$), 7.52 (s, 1H, NH), 7.91 (m, 8H, 5,10,15,20-m-Ar), 8.08 (m, 8H, 5,10,15,20-o-Ar), 8.81-8.87 (m, 8H, β-H). $^{13}$C-NMR (CDCl$_3$): δ 24.88 (O=C-CH$_3$),118.03, 118.83, 118.93, 120.21,135.67, 135.89 (β-C), 135.92 (β-C),136.11, 139.00, 141.51, 168.53 (O=C-NH). MS: (ESI) m/z 1049 (100[M + H]+), HRMS: calcd. for C$_{46}$H$_{31}$N$_5$O$_1$I$_3$:1049.9657 found 1049.9665.*

**5-[4-Nitrophenyl]-10,15,20-tri-(2,3,4,5,6-pentafluorophenyl) porphyrin (9g)**
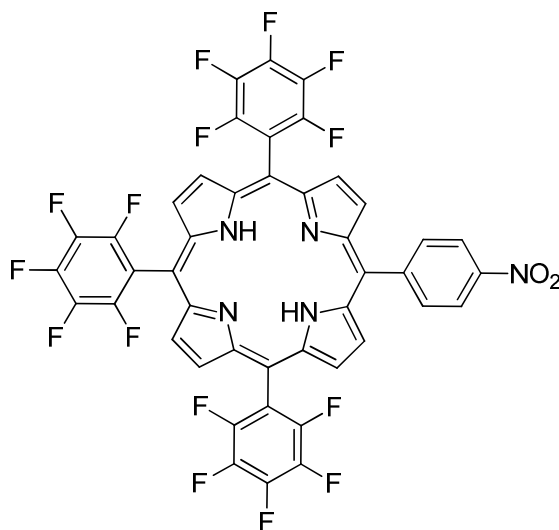


To a stirred solution of 4-nitrobenzaldehyde (1.62 g, 10.7 mmol) in refluxing propionic acid (250 ml) was added 2,3,4,5,6-pentafluorobenzaldehyde (4.98 g, 25.5 mmol). Pyrrole (2.5 ml, 36 mmol) was added dropwise and the mixture stirred under reflux for 1 hour, protected from light. The reaction was cooled to room temperature and the solvent removed under reduced pressure. The crude was purified by column chromatography (silica, 1-3% MeOH:DCM), and precipitated from MeOH over DCM to yield the product as a purple solid (4809i mg, 6.08%).

*UV-vis (DCM): λmax, nm 414, 509, 637. ε (414 nm)= 332299 $M^{-1}cm^{-1}$. $^{1}$H-NMR(CDCl$_3$): δ 8.40 (m, 2H, 5-m-Ar), 8.67 (m, 2H, 5-o-Ar), 8.86-8.92 (m, 8H, βH). $^{13}$C-NMR (CDCl$_3$): δ 102.52, 103.42, 115.46, 115.69, 119.76, 120.35, 122.10 (β-C), 135.06 (β-C), 136.33, 138.80, 143.52, 145.25, 147.74, 148.13. MS: (MALDI) m/z 929 (100[M ]+), HRMS: calcd. for $C_{44}H_{14}N_5F_{15}O_2$: 929.09025 found 929.08468.*

# 5-[4-Aminophenyl]-10,15,20-tri-(4-carbomethoxyphenyl)porphyrin (10a)[175]
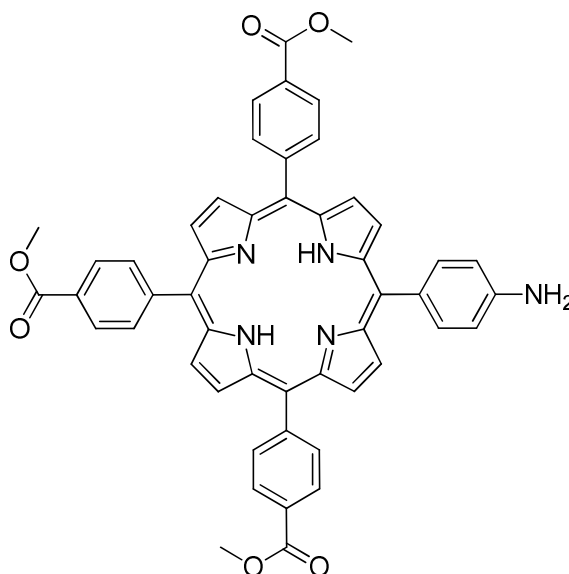


5-[4-Acetamidophenyl]-10,15,20-tri-(4-carbomethoxyphenyl)porphyrin (200 mg, 0.236 mmol) was dissolved in $HCl_{(aq)}$ (18%, 50 ml) and heated to reflux for 3 hours. The solvent was removed under reduced pressure and the residue redissolved in methanol (50 ml). Sulfuric acid (10 ml) was added, and the mixture heated to 60 °C for 48 hours. The solvent was removed under reduced pressure and the residue redissolved in triethylamine:DCM (1:9). The organic layer was washed with water, dried ($MgSO_4$) and the solvent removed under reduced pressure. The product was purified by column chromatography (silica, 1% MeOH:DCM) and precipitated from MeOH over DCM to yield the product as a purple solid (187.4 mg, 98.9%).

*UV-vis (DCM): λmax, nm 422, 517, 556, 590, 651. ε (422 nm)= 406598 $M^{-1}cm^{-1}$. $^1H$-NMR(CDCl$_3$): δ 4.10 (s, 9H,O-CH$_3$), 7.02 (d, 2H, J=8.16 Hz,5-m-Ar ), 7.94 (d, 2H, J= 7.96 Hz, 5-o-Ar), 8.29 (m, 6H, 10,15,20-m-Ar), 8.41 (m, 6H, 10,15,20-o-Ar), 8.77-8.97 (m, 8H, β-H). $^{13}C$-NMR (CDCl$_3$): δ 52.42 (O-$\underline{C}H_3$),113.34, 118.54, 118.98, 121.80, 127.90 (β-C), 127.93,129.59, 131.85 (β-C), 134.51, 135.68, 146.79, 146.85, 167.28 (O=$\underline{C}$-O-CH$_3$). MS: (ESI) m/z 732 (100[M + H]+), HRMS: calcd. for $C_{44}H_{29}N_5Cl_3$: 732.1483 found 732.1478.*

**5-[4-Aminophenyl]-10,15,20-tri-(4-ethoxyphenyl)porphyrin (10b)**



A solution of 5-[4-acetamidophenyl]-10,15,20-tri-(4-ethoxyphenyl)porphyrin (90 mg, 0.112 mmol) and $HCl_{(aq)}$ (18%, 100 ml) in trifluoroacetic acid (15 ml) was heated to reflux for 17 hours. The mixture was cooled to rt and the solvent removed under reduced pressure. The residue was redissolved in triethylamine:DCM (1:9), washed with water and the organic layer dried ($MgSO_4$). The solvent was removed under reduced pressure and the product purified by column chromatography (1% MeOH:DCM). The crude was precipitated from MeOH over DCM to yield the product as a purple solid (82 mg, 96.1%).

*UV-vis (DCM): λmax, nm 424, 519, 560, 595, 653. ε (424 nm)= 591875 $M^{-1}cm^{-1}$. $^1$H-NMR(CDCl$_3$): δ 1.62 (m, 9H, CH$_2$-C$\underline{H}_3$), 4.31 (m, 6H, C$\underline{H}_2$-CH$_3$) 4.67 (s, 2H, N$\underline{H}_2$) 7.25 (m, 6H, 10,15,20-m-Ar), 7.64 (d, 2H, J= 7.52 Hz, 5-m-Ar), 8.09 (m, 6H, 10,15,20-o-Ar), 8.22 (d, 2H, J=7.56 Hz, 5-o-Ar), 8.84-8.93 (m, 8H, βH). $^{13}$C-NMR (CDCl$_3$): δ 15.06 ($\underline{C}$H$_3$-CH$_2$), 63.70 (CH$_3$-C$\underline{H}_2$), 112.62 (β-C),113.42, 119.49, 119.67, 120.49, 132.48, 134.55, 135.58 (β-C), 135.67, 145.94, 158.70. MS: (ESI) m/z 784 (100[M + Na]+), HRMS: calcd. for C$_{50}$H$_{43}$N$_5$O$_3$Na: 784.32581 found 784.32392.*
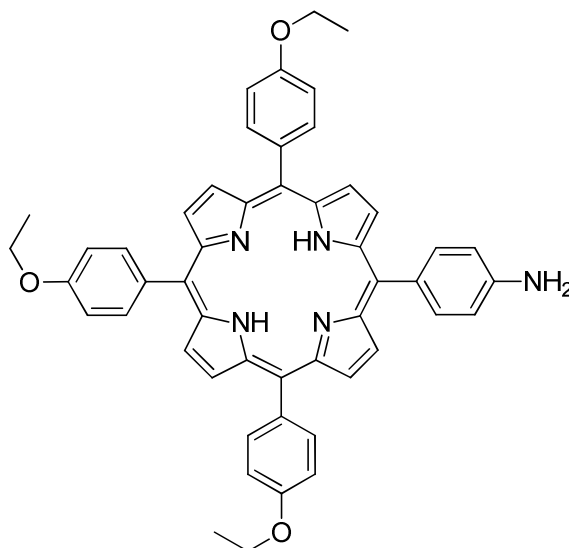
**5-[4-Aminophenyl]-10,15,20-tri-(3-methoxyphenyl)porphyrin (10c)**
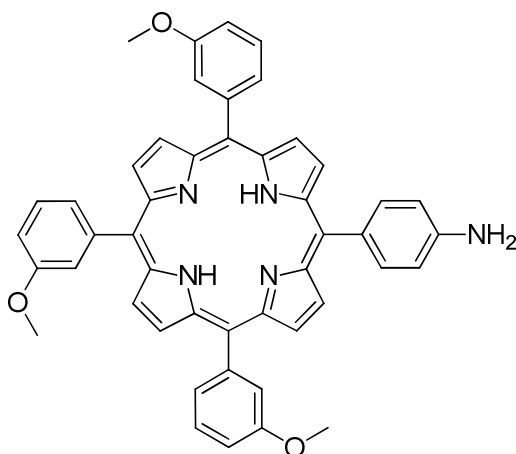


A solution of 5-[4-acetamidophenyl]-10,15,20-tri-(3-methoxyphenyl)porphyrin (200 mg, 0.263 mmol) and $HCl_{(aq)}$ (18%, 100 ml) in trifluoroacetic acid (20 ml) was heated to reflux for 3 hours. The mixture was cooled to rt and the solvent removed under reduced pressure. The residue was redissolved in triethylamine:DCM (1:9), washed with water and the organic layer dried ($MgSO_4$). The solvent was removed under reduced pressure and the product purified by column chromatography (silica, 1% MeOH:DCM) and the product precipitated from MeOH over DCM (179 mg, 94.7%).

*UV-vis (DCM): λmax, nm 421, 517,553, 591, 648. ε (421 nm)= 585771$M^{-1}cm^{-1}$.*
*$^1$H-NMR(CDCl$_3$): δ 3.96 (s, 11H, N$\underline{H}_2$, O-CH$_3$), 7.02 (d, 2H, J= 8.36 Hz, 5-m-Ar), 7.33 (m, 3H, 10,15,20-p-Ar), 7.58 (m, 3H, 10,15,20-m-Ar), 7.80 (m, 6H ,10,15,20-o-H), 7.97 (d, 2H, J= 8.16 Hz, 5-o-Ar), 8.87-8.93 (m, 8H, βH)). $^{13}$C-NMR (CDCl$_3$): δ 55.48 (O-C$\underline{H}_3$), 113.44, 113.52, 119.39, 119.69, 120.42, 120.91, 127.45 (β-C), 127.67 (β-C), 132.34, 135.67, 143.51, 143.58, 146.00, 157.91. MS: (ESI) m/z 720 (100[M + H]+), HRMS: calcd. for C$_{47}$H$_{38}$N$_5$O$_3$: 720.2969 found 720.2963.*

**5-[4-Aminophenyl]-10,15,20-tri-(4-chlorophenyl)porphyrin (10d)**



A solution of 5-[4-acetamidophenyl]-10,15,20-tri-(4-chlorophenyl)porphyrin (100 mg, 0.129 mmol) in $HCl_{(aq)}$ (18%, 100 ml) was heated to reflux for 3 hours. The mixture was cooled to rt and the solvent removed under reduced pressure. The residue was redissolved in triethylamine:DCM (1:9), washed with water and the organic layer dried ($MgSO_4$). The solvent was removed under reduced pressure and the product purified by column chromatography (silica, DCM). The crude was precipitated from MeOH over DCM to yield the product as a purple solid (85 mg, 90.1%).

*UV-vis (DCM): λmax, nm 421, 517, 555, 593, 649. ε (421 nm)= 523760 $M^{-1}cm^{-1}$. $^1H$-NMR(CDCl$_3$): δ 4.04 (s, 2H, N$\underline{H}_2$), 7.06 (d, 2H, J= 8.36 Hz, 5-m-Ar), 7.74 (m, 6H, 10,15,20-m-Ar), 7.98 (d, 2H, J= 8.16, 5-m-Ar), 8.14 (m, 6H, 10,15,20-o-Ar) 8.80-8.96 (m, 8H, β-H). $^{13}C$-NMR (CDCl$_3$): δ 113.49, 118.29, 118.65, 121.51, 126.97, 127.00 (β-C), 132.03, 134.25, 135.51 (β-C), 137.73, 140.50, 140.58, 146.20. MS: (ESI) m/z 732 (100[M + H]+), HRMS: calcd. for $C_{44}H_{29}N_5Cl_3$: 732.1483 found 732.1478.*
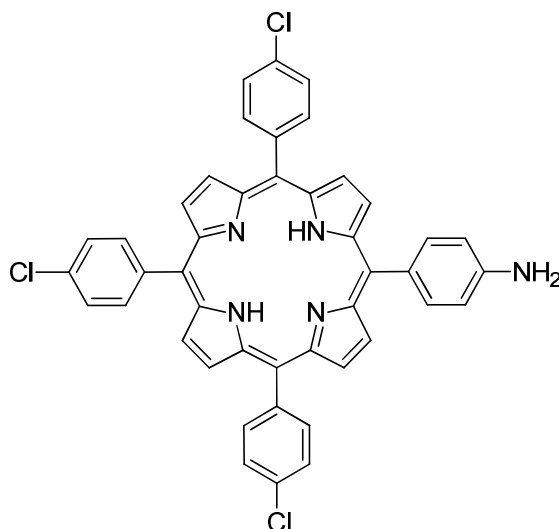
**5-[4-Aminophenyl]-10,15,20-tri-(4-bromophenyl)porphyrin (10e)**



A solution of 5-[4-acetamidophenyl]-10,15,20-tri-(4-bromophenyl)porphyrin (200 mg, 0.221 mmol) and $HCl_{(aq)}$ (18%, 100 ml) in trifluoroacetic acid (30 ml) was heated to reflux for 3 hours. The mixture was cooled to rt and the solvent removed under reduced pressure. The residue was redissolved in triethylamine:DCM (1:9), washed with water and the organic layer dried ($MgSO_4$). The solvent was removed under reduced pressure and the product purified by column chromatography (silica, DCM). The crude was precipitated from MeOH over DCM to yield the product as a purple solid (127 mg, 66.6%).

*UV-vis (DCM): λmax, nm 421, 517, 555, 594, 650.  ε (421 nm)= 467713$M^{-1}cm^{-1}$. $^1H$-NMR(CDCl$_3$): δ 4.03 (s, 2H, N$\underline{H}_2$), 7.06 (d, 2H, J= 8.16 Hz, 5-m-Ar), 7.87 (m, 6H, 10,15,20-m-Ar), 7.97 (d, 2H, J= 8.16 Hz, 5-o-Ar), 8.07 (m, 6H, 10,15,20-o-Ar),  8.80-8.96 (m, 8H, β-H).  $^{13}C$-NMR (CDCl$_3$): δ 113.49, 118.27, 118.65, 121.56, 122.50, 129.91 (β-C), 129.95, 132.01, 135.73, 135.84 (β-C), 140.98, 141.06, 146.21.  MS: (ESI) m/z 867 (100[M + H]+), HRMS: calcd. for $C_{44}H_{29}Br_3N_5$: 863.9968 found 863.9965.*
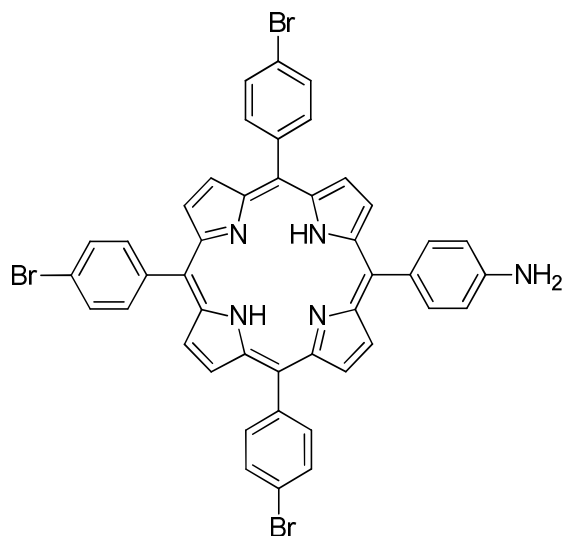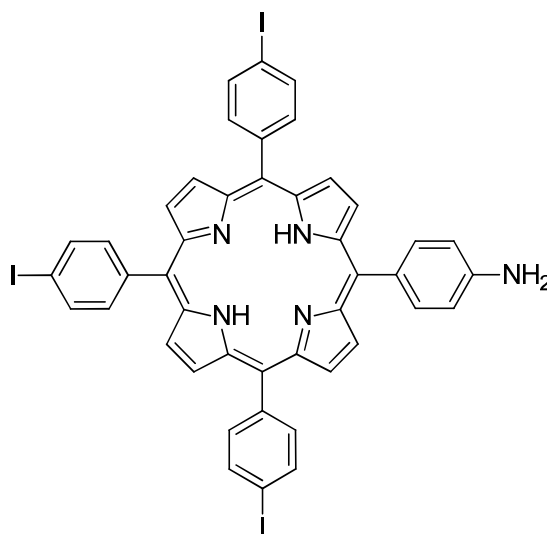
**5-[4-Aminophenyl]-10,15,20-tri-(4-iodophenyl)porphyrin (10f)**



A solution of 5-[4-acetamidophenyl]-10,15,20-tri-(4-iodophenyl)porphyrin (200 mg, 0.191 mmol) and $HCl_{(aq)}$ (18%, 100 ml) in trifluoroacetic acid (30 ml) was heated to reflux for 3 hours. The mixture was cooled to rt and the solvent removed under reduced pressure. The residue was redissolved in triethylamine:DCM (1:9), washed with water and the organic layer dried ($MgSO_4$). The solvent was removed under reduced pressure and the product purified by column chromatography (silica, DCM). The crude was precipitated from MeOH over DCM to yield the product as a purple solid (123 mg, 63.9%).

*UV-vis (DCM): λmax, nm 422, 518, 554, 594, 649. ε (422 nm)= 473087$M^{-1}cm^{-1}$. $^1$H-NMR(CDCl$_3$): δ 4.03 (s, 2H, N$\underline{H}_2$), 7.06 (d, 2H, J= 8.16 Hz, 5-m-Ar), 7.87 (m, 8H, 10,15,20-m-Ar, 5-m-Ar), 8.08 (m, 6H, 10,15,20-o-Ar) 8.80-8.96 (m, 8H, β-H). $^{13}$C-NMR (CDCl$_3$): δ 94.18, 113.48, 118.35, 118.73, 121.54, 132.00, 135.72, 135.85, 135.89 (β-C), 136.14 (β-C), 141.58, 141.65, 146.19. MS: (ESI) m/z 1007 (100[M + H]+), HRMS: calcd. for C$_{44}$H$_{28}$I$_3$N$_5$: 1007.9552 found 1007.9544.*

**5-[4-Aminophenyl]-10,15,20-tri-(2,3,4,5,6-pentafluorophenyl) porphyrin (10g)[179]**
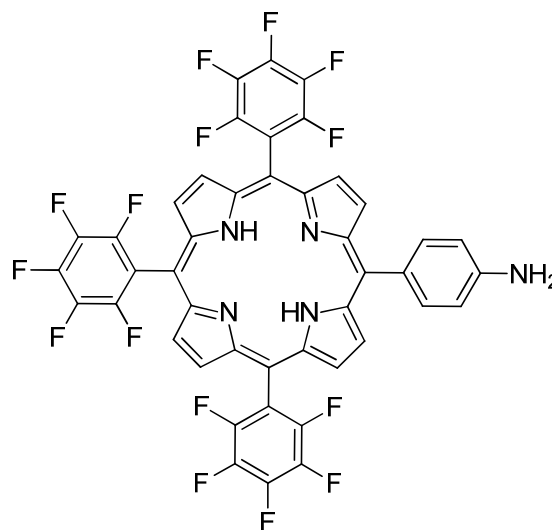


To a solution of 5-[4-Nitrophenyl]-10,15,20-tri-(2,3,4,5,6-pentafluorophenyl) porphyrin (345 mg, 0.369 mmol) in DCM (10 ml) was added tin (II) chloride dihydrate (1.67 g, 7.65 mmol) and HCl$_{(aq)}$:diethyl ether (1 M, 15 ml) and the mixture stirred at rt for 17 hours. The mixture was poured into ice-cold water and the product extracted with DCM (100 ml). The organic layer was neutralised with sat. NaHCO$_3$ solution and washed with water. The organic layer was dried (MgSO$_4$) and the solvent removed under reduced pressure. The crude was purified by column chromatography (silica, DCM) and precipitated from MeOH over DCM to yield the product as a purple solid (200 mg, 60.3%).

*UV-vis (DCM): λmax, nm 417, 513, 591. ε (417 nm)= 215211 $M^{-1}cm^{-1}$. $^1$H-NMR(CDCl$_3$): δ 4.06 (s, 2H, NH$_2$), 7.07 (d, 2H, J= 8.16 Hz, 5-m-Ar), 7.97 (d, 2H, J=8.16 Hz, 5-m-Ar), 8.80-9.07 (m, 8H, βH). $^{13}$C-NMR (CDCl$_3$): δ 101.37, 102.81, 113.56 (β-C), 116.09, 124.20, 130.99, 135.91 (β-C), 136.25, 138.78, 140.95, 143.54, 145.33, 146.61, 147.74. MS: (MALDI) m/z 899 (100[M ]+), HRMS: calcd. for C$_{44}$H$_{16}$N$_5$F$_{15}$: 899.11607 found 899.11546.*

**Zinc 5-[4-azidophenyl]-10,15,20- tri-(4-carbomethoxyphenyl)porphyrin (11a)**
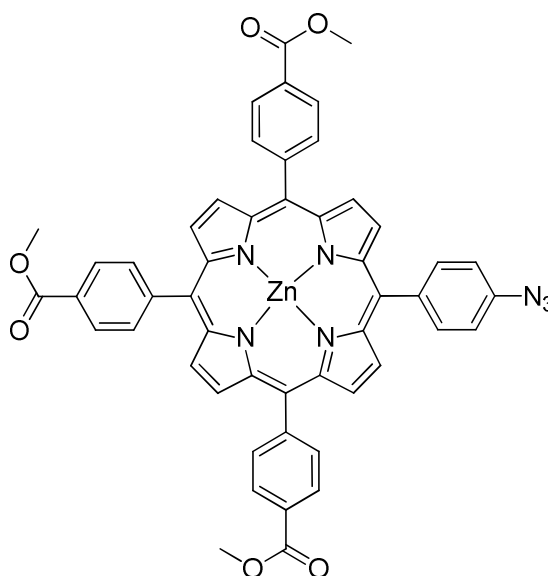


To a stirred solution of 5-[4-aminophenyl]-10,15,20-tri(4-carbomethoxyphenyl)porphyrin (64.3 mg, 0.079 mmol) in DCM:methanol (3:2, 5 ml) was added zinc acetate (50 mg, 0.270 mmol), and triethylamine (50 mg, 0.500 mmol). Imidazole-1-sulfonyl azide hydrogen sulfate (24 mg, 0.090 mmol) was added, and the mixture stirred at rt for 17 hours. The solvent was removed under reduced pressure and the crude purified by column chromatography (silica, 0.5% MeOH:DCM). The crude was precipitated from MeOH over DCM to yield the product as a purple solid (60.8 mg, 86.4%).

*UV-vis (DCM): λmax, nm 422, 549, 590. ε (422 nm)= 691271 $M^{-1}cm^{-1}$. $^{1}H$-NMR (CDCl$_{3}$): δ 4.11 (s, 9H,O-CH$_{3}$), 7.42 (m, 2H, 5-m-Ar ), 8.19 (m, 2H, 5-o-Ar), 8.30 (m, 6H, 10,15,20-m-Ar), 8.42 (m, 6H, 10,15,20-o-Ar), 8.87 (m, 8H, β-H). $^{13}C$-NMR (CDCl$_{3}$): δ 52.37 (O-$\underline{C}$H$_{3}$),117.14, 119.47, 119.68, 120.10,127.61 (β-C), 129.09, 131.56, 131.66, 131.88, 134.49 (β-C), 135.56, 139.53, 139.76, 148.01, 149.49, 149.57, 150.11, 167.55 (O=$\underline{C}$-O-CH$_{3}$). MS: (ESI) m/z 892 (100[M + H]+), HRMS: calcd. for C$_{50}$H$_{34}$N$_{7}$O$_{6}$Zn: 892.1857 found 892.1862.*

**Zinc 5-[4-azidophenyl]-10,15,20-tri-(4-ethoxyphenyl)porphyrin (11b)**



To a stirred solution of 5-[4-aminophenyl]-10,15,20-tri(3-methoxyphenyl)porphyrin (60.1 mg, 0.079 mmol) in DCM:methanol (3:2, 10 ml) was added zinc acetate (50 mg, 0.27 mmol), and triethylamine (50 mg, 0.500 mmol). Imidazole-1-sulfonyl azide hydrogen sulfate (24 mg, 0.090 mmol) was added, and the mixture stirred at rt for 17 hours. The solvent was removed under reduced pressure and the crude purified by column chromatography (silica, DCM). The crude was precipitated from MeOH over DCM to yield the product as a purple solid (61.3 mg, 91.4%).

*UV-vis (DCM): λmax, nm 423, 551, 591. ε (423 nm)= 700336 $M^{-1}cm^{-1}$. $^1H$-NMR(CDCl$_3$): δ 1.61 (m, 9H, CH$_2$-C$\underline{H}_3$), 4.31 (m, 6H, C$\underline{H}_2$-CH$_3$), 7.23 (m, 6H, 10,15,20-m-Ar), 7.35 (d, 2H, J= 8.36 Hz, 5-m-Ar), 8.08 (m, 6H, 10,15,20-o-Ar), 8.17 (d, 2H, J=8.36 Hz, 5-o-Ar), 8.89-8.98 (m, 8H, βH). $^{13}C$-NMR (CDCl$_3$): δ 15.07 ($\underline{C}H_3$-CH$_2$), 63.71 (CH$_3$-C$\underline{H}_2$), 112.55 (β-C),116.10, 117.22, 119.47, 120.97, 128.76, 131.45, 131.97, 132.03, 132.13, 134.98, 135.39 (β-C), 135.52, 139.40, 139.68, 149.99, 150.49, 150.56, 158.61. MS: (MALDI) m/z 849 (100[M ]+), HRMS: calcd. for C$_{50}$H$_{39}$N$_7$O$_3$Zn: 849.24004 found 849.24329.*
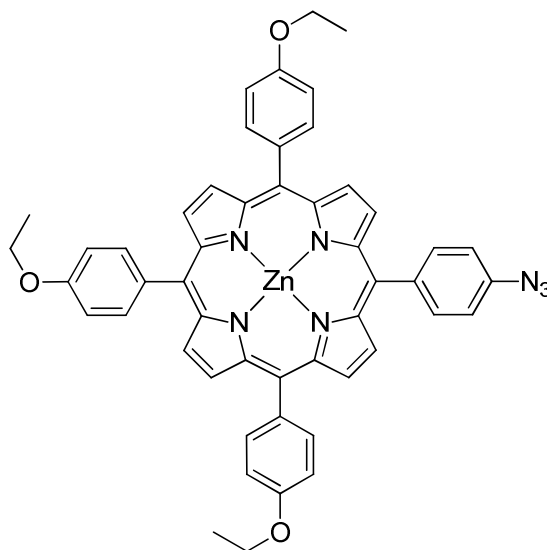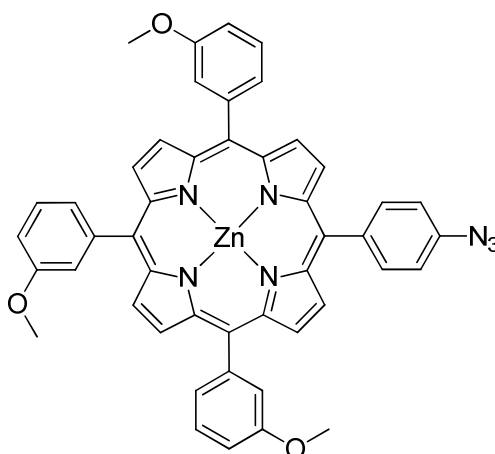
**Zinc 5-[4-azidophenyl]-10,15,20-tri-(3-methoxyphenyl)porphyrin (11c)**



To a stirred solution of 5-[4-aminophenyl]-10,15,20-tri(3-methoxyphenyl)porphyrin (57.6 mg, 0.079 mmol) in DCM:methanol (3:2, 10 ml) was added zinc acetate (50 mg, 0.27 mmol), and triethylamine (50 mg, 0.500 mmol). Imidazole-1-sulfonyl azide hydrogen sulfate (24 mg, 0.090 mmol) was added, and the mixture stirred at rt for 17 hours. The solvent was removed under reduced pressure and the crude purified by column chromatography (silica, DCM). The crude was precipitated from MeOH over DCM to yield the product as a purple solid (51.2 mg, 80.3%).

*UV-vis (DCM): λmax, nm  422, 548, 586. ε (422 nm)=778013$M^{-1}cm^{-1}$.$^1$H-NMR(CDCl$_3$): δ 3.80 (s, 9H, O-CH$_3$), 7.19 (m, 7H, 10,15,20-p-Ar, 5-m-Ar), 7.55 (m, 3H, 10,15,20-m-Ar), 7.66 (s, 3H, 5-2-Ar), 7.80 (m, 3H, 5-5-Ar), 8.88-9.03 (m, 8H, βH)). $^{13}$C-NMR (CDCl$_3$): δ 55.45 (O-CH$_3$), 113.32, 117.19, 119.64, 120.34, 120.80, 127.26 (β-C),127.60, 131.54, 131.95 (β-C),132.01, 135.54, 139.39, 139.83, 144.21, 150.00, 150.04, 157.76, 164.39. MS: (MALDI) m/z 807 (100[M ]+), HRMS: calcd. for C$_{47}$H$_{33}$N$_7$O$_3$Zn: 807.19309 found 807.19365.*

**Zinc 5-[4-azidophenyl]-10,15,20-tri-(4-chlorophenyl)porphyrin (11d)**



To a stirred solution of 5-[4-aminophenyl]-10,15,20- tri-(4-chlorophenyl)porphyrin (29 mg, 0.040 mmol) in DCM:methanol (3:2, 5 ml) was added zinc acetate (25 mg, 0.14 mmol), and triethylamine (50 mg, 0.500 mmol). Imidazole-1-sulfonyl azide hydrogen sulfate (12 mg, 0.045 mmol) was added, and the mixture stirred at rt overnight. The solvent was removed under reduced pressure and the crude purified by column chromatography (silica, DCM). The crude was precipitated from MeOH over DCM to yield the product as a purple solid (28.7 mg, 87.6%)

*UV-vis (DCM): λmax, nm 421, 549, 587. ε (421 nm)= 671335 $M^{-1}cm^{-1}$. $^{1}$H-NMR(CDCl$_3$): δ 7.44 (d, 2H, J= 8.36 Hz, 5-m-Ar), 7.75 (m, 6H, 10,15,20-m-Ar), 8.14 (m, 6H, 10,15,20-o-Ar),8.19 (d, 2H, J= 8.16, 5-m-Ar), 8.96 (m, 8H, β-H). $^{13}$C-NMR (CDCl$_3$): δ,117.37, 126.90, 126.89 (β-C), 132.01, 132.05, 132.13, 134.13, 135.36 (β-C), 139.28, 140.97, 150.07, 150.35. MS: (ESI) m/z 819 (100[M ]+), HRMS: calcd. for C$_{44}$H$_{24}$N$_7$Cl$_3$Zn: 819.04447 found 819.05085.*
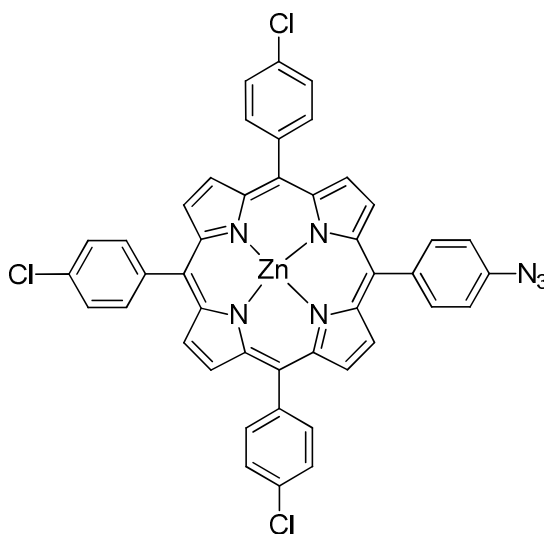
**Zinc 5-[4-azidophenyl]-10,15,20-tri-(4-bromophenyl)porphyrin (11e)**



To a stirred solution of 5-[4-aminophenyl]-10,15,20-tri-(4-bromophenyl)porphyrin (69.0 mg, 0.079 mmol) in DCM:methanol (3:2, 10 ml) was added zinc acetate (50 mg, 0.27 mmol), and triethylamine (50 mg, 0.500 mmol). Imidazole-1-sulfonyl azide hydrogen sulfate (24 mg, 0.090 mmol) was added, and the mixture stirred at rt for 17 hours. The solvent was removed under reduced pressure and the crude purified by column chromatography (silica, DCM). The crude was precipitated from MeOH over DCM to yield the product as a purple solid (63.0 mg, 83.9%).

*UV-vis (DCM): λmax, nm 422, 548, 589.  ε (422 nm)= 889816 $M^{-1}cm^{-1}$. $^1H$-NMR(CDCl$_3$): δ  7.41 (d, 2H, J= 7.96  Hz, 5-m-Ar), 7.94 (m, 6H, 10,15,20-m-Ar), 8.08 (m, 6H, 10,15,20-o-Ar), 8.18  (d, 2H, J= 7.92 Hz, 5-o-Ar), 8.87 (m, 8H, β-H). $^{13}C$-NMR (CDCl$_3$): δ,117.29, 119.53, 129.69 (β-C), 131.87, 132.05,135.63, 135.83 (β-C),  139.72, 141.97, 149.94, 150.27.  MS: (MALDI) m/z 950 (100[M ]+), HRMS: calcd. for C$_{44}$H$_{24}$Br$_3$N$_7$Zn: 950.89293 found 950.89327.*
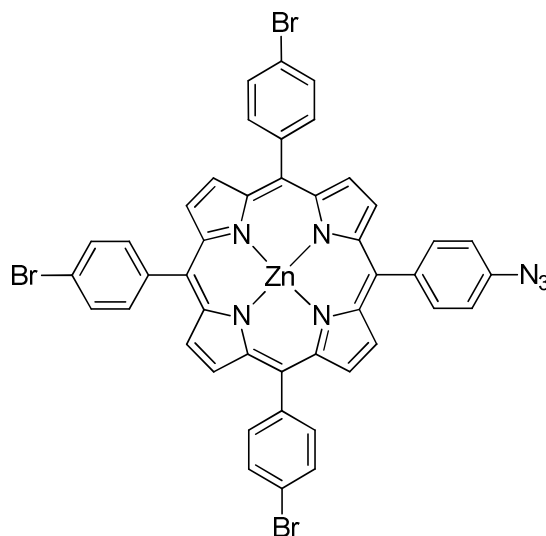
**Zinc 5-[4-azidophenyl]-10,15,20-tri-(4-iodophenyl)porphyrin (11f)**
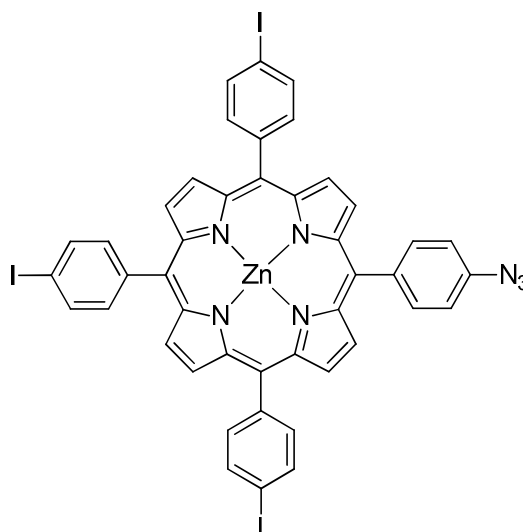


To a stirred solution of 5-[4-aminophenyl]-10,15,20-tri-(4-iodophenyl)porphyrin (80.6 mg, 0.079 mmol) in DCM:methanol (3:2, 10 ml) was added zinc acetate (50 mg, 0.270 mmol), and triethylamine (50 mg, 0.500 mmol). Imidazole-1-sulfonyl azide hydrogen sulfate (24 mg, 0.090 mmol) was added, and the mixture stirred at rt for 17 hours. The solvent was removed under reduced pressure and the crude purified by column chromatography (silica, DCM). The crude was precipitated from MeOH over DCM to yield the product as a purple solid (82.1 mg, 94.9%).

*UV-vis (DCM): λmax, nm 422, 550, 589. ε (422 nm)= 642287M$^{-1}$cm$^{-1}$. $^{1}$H-NMR(CDCl$_3$): δ 7.42 (d, 2H, J= 8.6 Hz, 5-m-Ar), 7.87 (m, 6H, 10,15,20-m-Ar), 8.07 (m, 6H, 10,15,20-o-Ar), 8.19 (d, 2H, J= 8.36 Hz, 5-m-Ar), 8.84 (m, 8H, β-H). $^{13}$C-NMR (CDCl$_3$): δ 93.74, 117.24, 119.53, 119.98, 131.81, 135.72 (β-C), 136.22 (β-C), 139.52, 139.91, 139.97, 142.70, 149.90, 150.18. MS: (ESI) m/z 1095 (100[M + H]+), HRMS: calcd. for C$_{44}$H$_{25}$I$_3$N$_7$Zn: 1095.8591 found 1095.8586.*

**Zinc 5-[4-azidophenyl]-10,15,20-tri-(2,3,4,5,6-pentafluorophenyl) porphyrin (11g)**
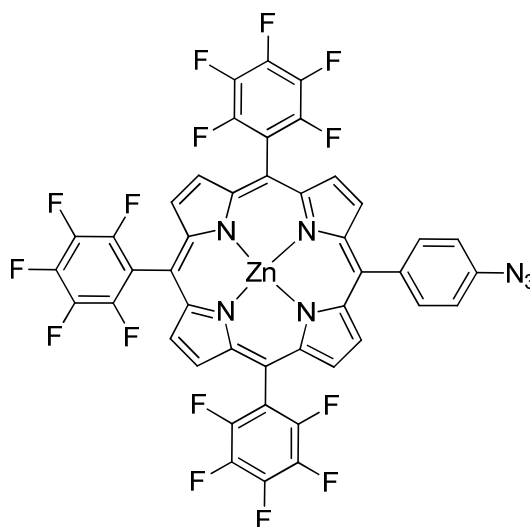


To a stirred solution of 5-[4-aminophenyl]-10,15,20-tri-(2,3,4,5,6-pentafluorophenyl) porphyrin (35.6 mg, 0.040 mmol) in DCM:methanol (3:2, 10 ml) was added zinc acetate (25 mg, 0.14 mmol), and triethylamine (25 mg, 0.250 mmol). Imidazole-1-sulfonyl azide hydrogen sulfate (12 mg, 0.045 mmol) was added, and the mixture stirred at rt for 17 hours. The solvent was removed under reduced pressure and the crude purified by column chromatography (silica, DCM). The crude was precipitated from MeOH over DCM to yield the product as a purple solid (34.0 mg, 88.4%).

*UV-vis (DCM): λmax, nm 416, 545, 578. ε (416 nm)= 669318 $M^{-1}cm^{-1}$. $^{1}H$-NMR (CDCl₃): δ 7.04 (d, 2H, J= 8.36 Hz, 5-m-Ar), 8.09 (d, 2H, J=8.36 Hz, 5-m-Ar),9.00 (m, 8H, βH). $^{13}C$-NMR (CDCl₃): δ 102.89, 103.78, 117.18 (β-C), 122.71, 130.64, 131.64, 131.95, 134.00, 135.48, 136.23 (β-C), 138.38, 138.80, 139.71, 140.74, 143.25, 145.32, 145.32, 147.80, 149.76, 149.93, 150.32, 150.93. MS: (MALDI) m/z 899 (100[M+H ]+), HRMS: calcd. for $C_{44}H_{13}N_7F_{15}Zn$: 988.02789 found 988.04271.*

**5-[4-Aminophenyl]-10,15,20-tri(4-sulfonatophenyl)porphyrin sodium salt (12)[184]**
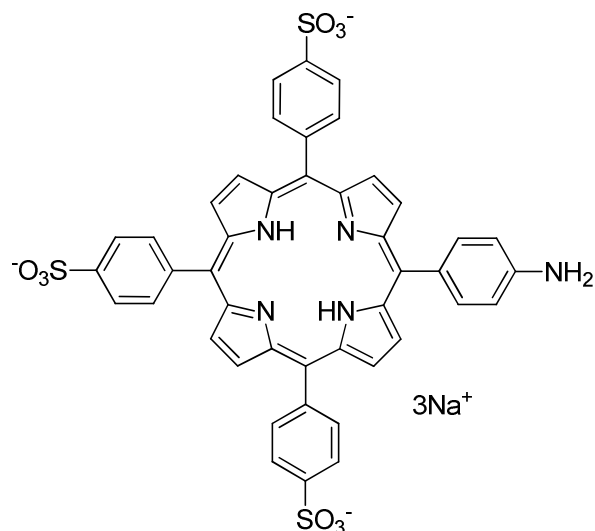


A solution of 5-[4-aminophenyl]-10,15,20-triphenylporphyrin (200 mg, 0.316 mmol) in sulfuric acid (5 ml) was heated to 110 °C for 48 hours. The mixture was cooled to room temperature, diluted with water and filtered. The solid product was neutralised with ammonium hydroxide and the solvent removed under reduced pressure. The residue was dissolved in water and triethylamine added. The mixture was stirred for 1 hour, and filtered. The solid was dissolved in acetone, and sodium iodide added. The mixture was filtered to yield the product as a purple solid (224 mg, 75.9%).

*UV-vis (H$_2$O): λmax, nm 416, 513, 553, 593, 647. $^1$H-NMR (D$_2$O): δ 7.16 (d, 2H, J=6.70 Hz, 5-m-Ar), 7.95 (d, 2H, J= 6.72 Hz, 5-o-Ar), 8.28 (m, 16H, 10,15,20-o,m-Ar). $^{13}$C-NMR (D$_2$O): δ 113.60, 118.69, 119.17, 124.27 (β-C), 134.18 (β-C), 135.63, 143.96, 144.85, 147.88. MS: (ESI) m/z 433 (100[M -2H]2-), HRMS: calcd. for C$_{44}$H$_{29}$N$_5$O$_9$S$_3$: 433.5569 found 433.5558.*

**Zinc 5-[4-azidophenyl]-10,15,20-tri(4-sulfonatophenyl)porphyrin sodium salt (13)**
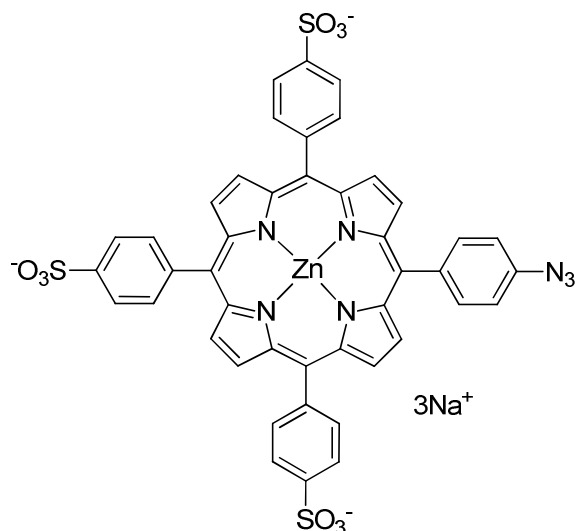


To a stirred solution of 5-[4-aminophenyl]-10,15,20-tri(4-sulfonatophenyl)porphyrin sodium salt (70 mg, 0.079 mmol) in methanol (20 ml) was added zinc acetate (50 mg, 0.270 mmol), and triethylamine (50 mg, 0.500 mmol). Imidazole-1-sulfonyl azide hydrogen sulfate (24 mg, 0.090 mmol) was added, and the mixture stirred at rt for 17 hours. The solvent was removed under reduced pressure and the crude redissolved in acetone. Sodium iodide was added and the precipitate removed by filtration. The crude was purified by column chromatography (C18 silica, 0-30% MeOH:water) and precipitated from acetone over methanol to yield the product as a purple solid (69.6 mg, 86.2%).

*UV-vis ($H_2O$): λmax, nm 424, 558, 597. ε (424 nm)= 650515 $M^{-1}cm^{-1}$.* *$^1H$ NMR (MeOH-$d_4$): δ 7.47 (d, 2H, J=8.40 Hz, 5-m-Ar), 8.26 (m, 18H, 5-o-Ar, 10,15,20-o, m-Ar), 8.85 (m, 8H β-H). $^{13}C$-NMR (MeOH-$d_4$): δ 118.18, 120.90, 120.95, 121.15, 125.13 (β-C), 132.51, 132.60, 135.40 (β-C),136.92, 140.98, 141.58, 145.48, 146.89, 151.18, 151.23, 151.26, 151.51. (ESI) m/z 318 (100[M -3Na]3-), HRMS: calcd. for $C_{44}H_{24}N_7O_9S_3Zn$: 318.0035 found 318.0030.*

## 5-[4-Acetamidophenyl]-10,15,20-tri-(4-pyridyl)porphyrin (14)[177]
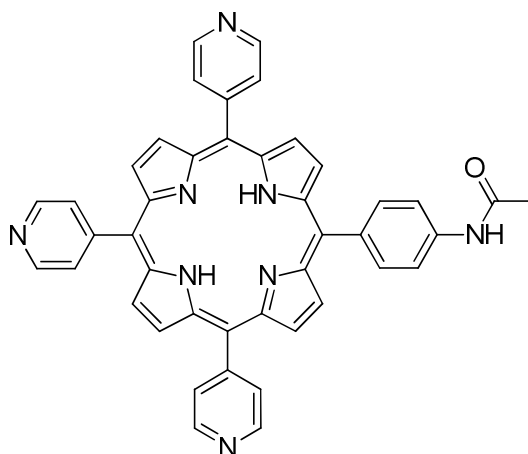


To a stirred solution of 4-acetamidobenzaldehyde (3.53 g, 21.5 mmol) in refluxing propionic acid (500 ml) was added 4-pyridinecarboxaldehyde (4.89 ml, 52 mmol). Pyrrole (5 ml, 72 mmol) was added dropwise and the mixture stirred under reflux for 1 hour, protected from light. The reaction was cooled to room temperature and the solvent removed under reduced pressure. The crude was purified by column chromatography (silica, 5-8% MeOH:DCM), and precipitated from MeOH over DCM to yield the product as a purple solid (800 mg, 5.52%).

$R_f$: (silica, 6% MeOH:DCM): 0.34. $^1$H-NMR(CDCl$_3$): δ -2.89 (s, 2H,- N$\underline{H}$),2.39 (s, 3H, -C$\underline{H_3}$), 7.94 (d, 2H, J=8.41 Hz, 5-m-Ph), 8.16 (m, 8H, βH), 8.85 (m, 6H, 10,15,20-o-Py), 9.04 (m, 2H, 5-o-Ph), 9.06 (m, 6H, 10,15,20-m-Py). $^{13}$C-NMR (CDCl$_3$): δ 24.14 (-$\underline{C}$H$_3$), 116.61, 117.06, 118.06, 118.15, 121.42, 129.46 (β-C), 134.97, 136.80, 138.42, 147.62, 147.75 (β-C), 150.38, 150.42, 169.76 (C=O).  MS: (ESI) m/z 675 (100[M +H]+), HRMS: calcd. for C$_{43}$H$_{31}$N$_8$O$_1$: 675.2615  found 675.2610.

## 5-[4-Aminophenyl]-10,15,20-tri-(4-pyridyl) porphyrin (15)[177]
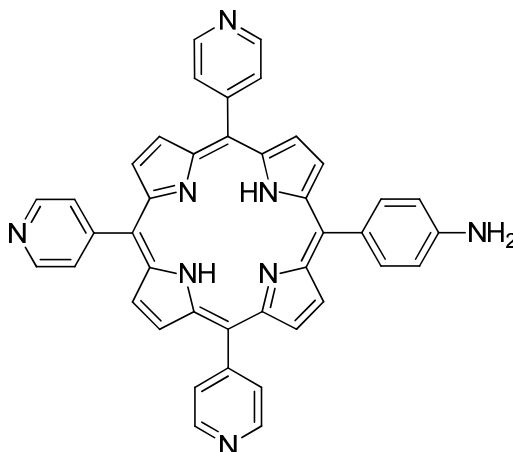


5-[4-acetamidophenyl]-10,15,20-tri-(4-pyridyl) porphyrin (100 mg, 0.148 mmol) was dissolved in $HCl_{(aq)}$ (5 M, 100 ml) and stirred under reflux for three hours. The reaction was cooled to room temperature and concentrated under reduced pressure. The residue was dissolved in DCM/TEA (9:1, 200 ml) and stirred for 10 minutes at room temperature. The solution was washed with water (3 x 200 ml) and the organic layer dried ($Na_2SO_4$). The solvent was removed under reduced pressure, and the residue precipitated from MeOH over DCM to yield the product as a purple solid (85 mg, 90%).

$R_f$: (silica, 6% MeOH:DCM): 0.41. $^1$H-NMR (CDCl$_3$): δ -2.84 (s, 2H,- N$\underline{H}$), 4.09 (m, 2H, -NH$_2$), 7.71 (d, 2H, J=8.11 Hz, 5-m-Ph), 7.99 (d, 2H, J=8.11 Hz, 5-o-Ph), 8.17 (m, 6H, 10,15,20-o-Py), 8.81 (m, 6H, 10,15,20-m-Py), 9.06 (m, 8H, βH). $^{13}$C-NMR (CDCl$_3$): δ 113.57 ($\underline{C}$-NH$_2$), 116.30, 116.98, 122.71, 129.49 (β-C), 131.40, 135.74, 146.40, 147.86 (β-C), 150.44, 150.53. MS: (ESI) m/z 633 (100[M +H]+), HRMS: calcd. for C$_{41}$H$_{29}$N$_8$: 633.2510 found 633.2505.

196

**5-[4-Azidophenyl]-10,15,20-tri-(4-pyridyl)porphyrin (16)**
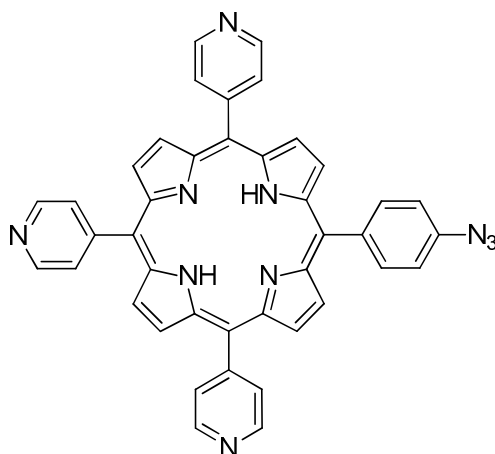


To a stirred solution of 5-[4-aminophenyl]-10,15,20-tri-(4-pyridyl) porphyrin (200 mg, 0.315 mmol) in TFA (2 ml) at 0 ˚C was added dropwise a solution of sodium nitrite (44 mg, 0.63 mmol) in water. The mixture was stirred for 15 minutes at 0 ˚C. A solution of sodium azide (83 mg, 1.26 mmol) in water was added dropwise and the reaction mixture stirred at 0 ˚C for 1 hour. The mixture was diluted with water and saturated sodium bicarbonate solution added until the colour changed from green to purple. The aqueous mixture was extracted with dichloromethane, and the organic layer dried ($Na_2SO_4$) and the solvent removed under reduced pressure. The crude was purified by column chromatography (3% MeOH:DCM) and precipitated from MeOH over DCM to yield the product as a purple solid (201 mg, 96.8%).

*$R_f$: (silica, 5% MeOH:DCM): 0.40. UV-vis (DCM): λmax, nm 418, 514, 547, 589, 647. ε (418 nm)= 375089 $M^{-1}cm^{-1}$. $^1$H-NMR (CDCl$_3$): δ -2.96 (s, 2H,- N<u>H</u>), 7.39 (d, 2H, J=8.44 Hz, 5-m-Ph), 8.14-8.24 (m, 5,10,15,20-o-Py), 8.79-8.88 (m, 6H, 10,15,20-m-Py), 8.88-9.14 (8H, βH )*. *$^{13}$C-NMR (CDCl$_3$): δ 117.54, 117.62, 121.35, 129.34 (β-C), 135.70, 138.17, 140.36, 148.38 (β-C), 149.94. MS: (ESI) m/z 660 (100[M + H]+), HRMS: calcd. for $C_{41}H_{27}N_{10}$: 659.2415 found 659.2412.*

197

**Zinc 5-[4-azidophenyl]-10,15,20-tri-(4-pyridyl)porphyrin (16Zn)**
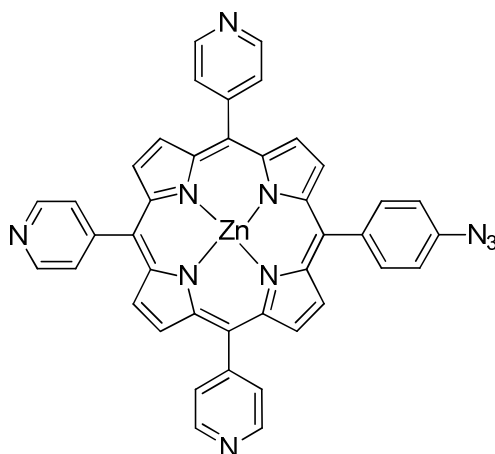


To a stirred solution of 5-[4-azidophenyl]-10,15,20-tri-(4-pyridyl) porphyrin (33 mg, 0.050 mmol) in dichloromethane (20 ml) was added zinc acetate (33 mg, 0.180 mmol) in methanol (0.5 ml). The mixture was stirred at room temperature for 1 hour, and the solvent removed under reduced pressure. The solid was washed with water and precipitated from MeOH over DCM to yield a deep purple solid. The desired product was not isolated.

*R_f: (silica, 10% MeOH:DCM): 0.61*

**5-[4-Azidophenyl]-10,15,20-tri-( N-methyl-4-pyridinium)porphyrin triiodide (17)**
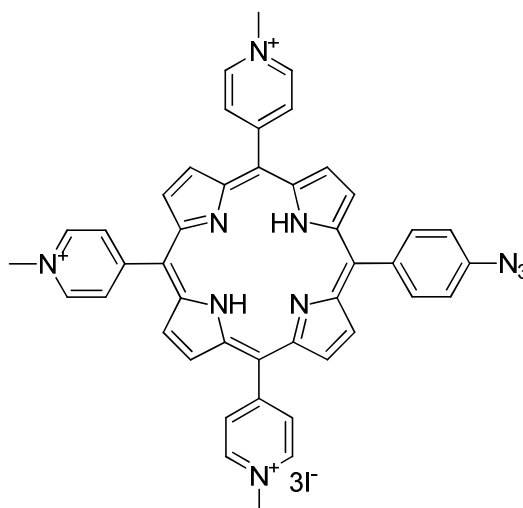


To a stirred solution of 5-[4-azidophenyl]-10,15,20-tri-(4-pyridyl) porphyrin (100 mg, 0.151 mmol) in DMF (10 ml) was added methyl iodide (2 ml, 32 mmol) *via* syringe. The reaction mixture was stirred at 40 °C overnight. The mixture was cooled to room temperature and cold diethyl ether (100 ml) was added. The mixture was filtered through cotton wool, and the residue redissolved in methanol. The solvent was removed under reduced pressure, and the porphyrin precipitated from diethyl ether over MeOH to yield the product as a dark brown solid (148 mg, 90.9%).

*$R_f$: 0.54 (silica, 1:1:8 sat. KNO$_3$ solution:water:MeCN), UV-vis (H$_2$O): λmax, nm 424, 520, 560, 590, 640. ε (424 nm)= 183361 M$^{-1}$cm$^{-1}$,$^1$H-NMR (DMSO-d$_6$): δ 4.72 (s, 9H, CH$_3$), 7.64 (d, 2H, J=8.36 Hz, 5-m-Ph), 8.26 (d, 2H, J=8.78 Hz, 5-o-Ph), 8.99 (m, 6H, 10,15,20-o-Py), 9.17 (m, 8H, βH), 9.49 (m, 6H, 10,15,20-m-Py).$^{13}$C-NMR (DMSO-d$_6$): δ 40.13 (CH$_3$), 114.60, 115.33, 118.11, 121.87, 132.08 (β-C), 135.71, 137.02, 139.98, 144.17 (β-C),156.43, 156.53. MS: (ESI) m/z 234 (100[M - 3Cl]3+), HRMS: calcd. for C$_{44}$H$_{33}$N$_{10}$ 234.4343 found 234.4339.*

**Zinc 5-[4-azidophenyl]-10,15,20-tri-(N-methyl-4-pyridinium)porphyrin trichloride (17Zn)**



To a stirred solution of 5-[4-azidophenyl]-10,15,20-tri-( *N*-methyl-4-pyridinium)porphyrin triiodide (100 mg, 0.092 mmol) in water (20 ml) was added a solution of zinc acetate (100 mg, 0.54 mmol) in water (10 ml). The mixture was stirred at room temperature for 30 minutes, and ammonium hexafluorophosphate added. The resulting solution was filtered and the precipitate redissolved in acetone. Tetrabutylammonium chloride was added, and the resulting solution filtered. The crude was precipitated from diethyl ether over MeOH to yield the product as a green solid (72 mg, 90%).

*HPLC: 5-60% B over 15 minutes. $R_f$ = 8.8 minutes. $R_f$: 0.54 (silica, 1:1:8 sat. $KNO_3$ solution:water:MeCN), UV-vis ($H_2O$): λmax, nm 427, 564, 608. ε (427 nm)= 202666 $M^{-1}cm^{-1}$. $^1H$-NMR (DMSO-$d_6$): δ 4.77 (s, 9H, $CH_3$), 8.27 (m, 4H, 5-o,m-Ph), 9.09 (m, 14H, 10,15,20-o-Py, βH), 9.60 (m, 6H, 10,15,20-m-Py). $^{13}C$-NMR (DMSO-$d_6$): δ 40.13 (CH3), 114.66, 115.42, 117.63, 122.87, 132.11, 132.25 (β-C), 135.53, 143.62 (β-C), 147.88, 148.15, 148.35, 150.19, 158.54. MS: (ESI) m/z 255 (100[M - 3Cl]3+), HRMS: calcd. for $C_{44}H_{33}N_{10}Zn$: 255.0722 found 255.0721.*
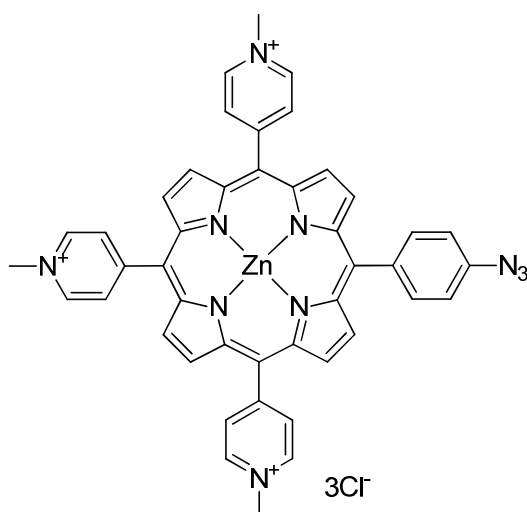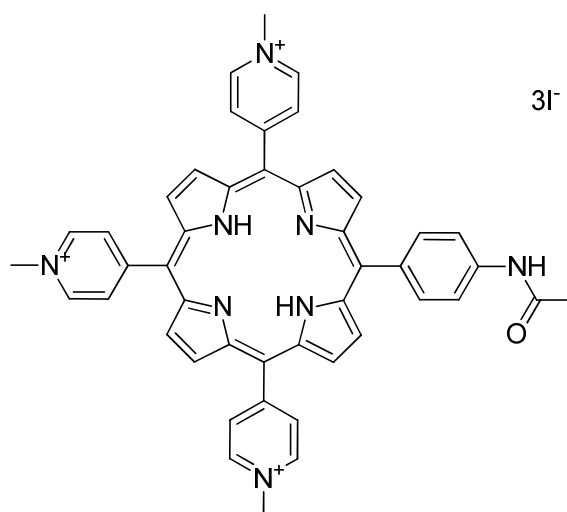
**5-[4-Acetamidophenyl]-10,15,20-tri-4-(N-methyl-4-pyridinium) porphyrin triiodide (18)**



To a stirred solution of 5-[4-acetamidophenyl]-10,15,20-tri-4-pyridylporphyrin (120 mg, 0.178 mmol) in DMF (10 ml) was added methyl iodide (2 ml, 32 mmol) and the mixture stirred at 40°C for 17 hours. Diethyl ether was added and the mixture filtered. The crude porphyrin was precipitated from diethyl ether over MeOH to yield the product as a brick red solid (180 mg, 91.9%).

*UV-vis (H$_2$O): λmax, nm 426, 517, 557, 594, 651. ε (426 nm)= 67470 M$^{-1}$cm$^{-1}$. $^1$H-NMR (DMSO-d$_6$): δ 2.25 (s, 3H, CH$_3$C=O),4.72 (s, 9H, N-CH$_3$),8.15 (m,4H,5-o,m-Ar), 9.00-9.20 (m, 14H, 10,15,20-m-Ar, βH), 9.51 (m, 6H, 10,15,20-o-Ar), 10.62 (s, 1H, NH). $^{13}$C-NMR (DMSO-d$_6$): δ 13.50 (CH$_3$-C=O), 48.13 (N-CH$_3$), 114.41, 115.29, 117.50, 122.88, 132.11 (β-C),134.82, 139.86, 144.18 (β-C), 156.60, 168.80 (C=O). MS: (ESI) m/z 238 (100[M -3I]3+), HRMS: calcd. for C$_{46}$H$_{39}$N$_8$O$_1$: 239.7743 found 239.7741.*

**5-[4-Aminophenyl]-10,15,20-(tri-N-methyl-4-pyridinium) porphyrin trichloride (19)**



5-[4-acetamidophenyl]-10,15,20-(tri-*N*-methyl-4-pyridinium) porphyrin triiodide (100 mg, 0.0909 mmol) was dissolved in $HCl_{(aq)}$ (18%, 50 ml) and stirred at reflux for 3 hours. The solvent was removed under reduced pressure and the residue redissolved in water (5 ml). The porphyrin was neutralised with 1M NaOH and ammonium hexafluorophosphate added. The mixture was filtered and the precipitate redissolved in acetone. Tetrabutyl ammonium chloride was added and the mixture filtered. The crude porphyrin was precipitated from diethyl ether over MeOH to yield the product as a brick red solid (67.0 mg, 94.2%).

*UV-vis (H$_2$O): λmax, nm 426, 518, 565, 594, 653. ε (426 nm)= 117904 M$^{-1}$cm$^{-1}$. $^1$H-NMR (DMSO-d$_6$): δ 4.73 (s, 9H, N-C$\underline{H}_3$), 5.78 (s, 2H, -N$\underline{H}_2$), 7.04-7.06 (m,2H,5-m-Ar),7.88-7.90 (m, 2H, 5-o-Ar), 8.99-9.20 (m, 14H, 10,15,20-m-Ar, βH), 9.50-9.52 (m, 6H, 10,15,20-o-Ar), 10.62 (s, 1H, NH). $^{13}$C-NMR (DMSO-d$_6$): δ 47.76 (N-C$\underline{H}_3$), 112.73, 113.83, 115.15, 124.98, 127.38, 132.07 (β-C), 135.97, 144.19, 144.25 (β-C), 156.51, 156.67. MS: (ESI) m/z 338 (100[M -3Cl]2+), HRMS: calcd. for C$_{44}$H$_{36}$N$_8$: 335.1526 found 335.1528.*
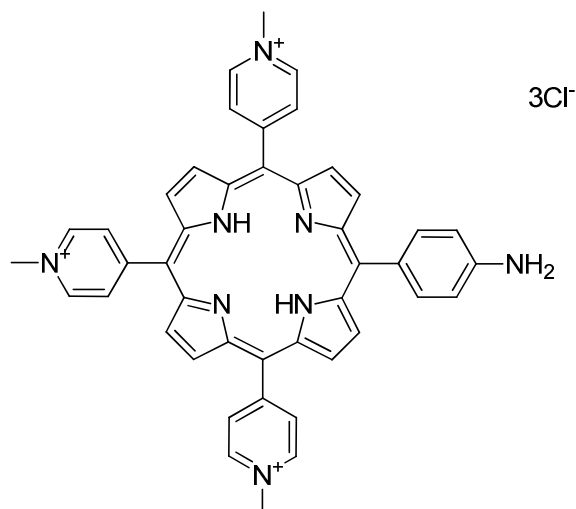
**Zinc 5-[4-azidophenyl]-10,15,20-tri-(N-methyl-4-pyridinium)porphyrin trichloride (17)**
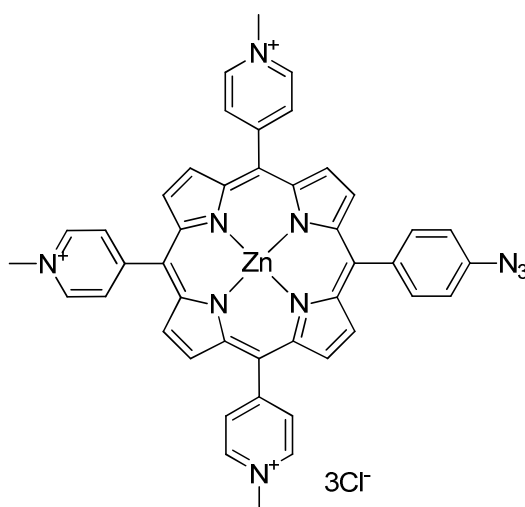


To a stirred solution of 5-[4-aminophenyl]-10,15,20-tri-(*N*-methyl-4-pyridinium) porphyrin trichloride (31.3 mg, 0.040 mmol) in methanol (3:2, 10 ml) was added zinc acetate (50 mg, 0.270 mmol), and triethylamine (25 mg, 0.250 mmol). Imidazole-1-sulfonyl azide hydrogen sulfate (24 mg, 0.090 mmol) was added and the mixture stirred at rt for 48 hours. The solvent was removed under reduced pressure and the product redissolved in water, and purified by column chromatography (C18 silica, 0-50% MeOH:water). Ammonium hexafluorophosphate was added to the aqueous solution and the mixture filtered. The precipitate was redissolved in acetone and tetrabutylammonium chloride added. The mixture was filtered and the product precipitated from diethyl ether over MeOH to yield the product as a green solid (23.2 mg, 66.8%).

*UV-vis (H$_2$O): λmax, nm 427, 564, 608. ε (427 nm)= 202666 M$^{-1}$cm$^{-1}$.$^{1}$H-NMR (DMSO-d$_6$): δ 4.77 (s, 9H, CH$_3$), 8.27 (m, 4H, 5-Ar), 9.09 (m, 14H, 10,15,20-o-Ar, βH), 9.60 (m, 6H, 10,15,20-m-Ar). $^{13}$C-NMR (DMSO-d$_6$): δ 40.13 (CH$_3$), 114.66, 115.42, 117.63, 122.87, 132.11, 132.25 (β-C), 135.53, 143.62 (β-C), 147.88, 148.15, 148.35, 150.19, 158.54. MS: (ESI) m/z 255 (100[M - 3Cl]3+), HRMS: calcd. for C$_{44}$H$_{33}$N$_{10}$Zn: 255.0722 found 255.0721.*

**5-(4-Methoxycarboxyphenyl)-10,15,20-tris-(4-pyridyl)porphyrin (20)[191]**
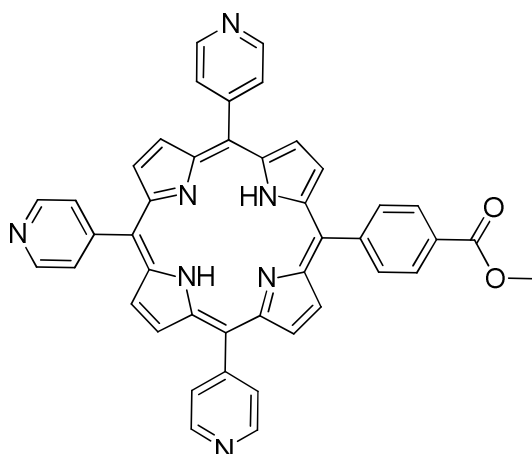


To a refluxing mixture of methyl-4-formylbenzoate (2.79 g, 17 mmol) and pyridine-4-carboxaldehyde (4.75 ml, 50 mmol) in propionic acid (500 ml) was added dropwise pyrrole (4.75 ml, 68 mmol). The mixture was refluxed for 1 hour, and allowed to cool to room temperature. The solvent was removed under reduced pressure, and any excess acid neutralised with sat. sodium bicarbonate solution. The product was extracted with dichloromethane, and the organic layer dried ($Na_2SO_4$). The crude was purified by column chromatography (silica, 2% MeOH:DCM) and precipitated from MeOH over DCM to yield the product as a purple solid (600 mg, 5.33%).

*$R_f$: (silica, 3% MeOH:DCM): 0.40. UV-vis (DCM): λmax, nm 417, 513, 548, 586, 645. ε (417 nm)= 428717 $M^{-1}cm^{-1}$. $^1$H-NMR (CDCl₃): δ -2.89 (s, 2H,- N$\underline{H}$), 4.12 (s, 3H, C=OOC$\underline{H}_3$), 8.16 (m, 6H, 10,15,20-o-Py), 8.31 (d, 2H, J=8.16 Hz, 5-m-Ph), 8.48 (d, 2H, J=8.36 Hz, 5-o-Ph), 8.86 (m, 8H, βH), 9.06 (m, 6H, 10,15,20-m-Py). $^{13}$C-NMR (CDCl₃): δ 52.51 ($\underline{C}H_3$), 117.66, 128.08, 129.33 (β-C), 134.51, 148.44, 149.86 (β-C), 167.07 (C=O). MS: (EI) m/z 675 (100[M]+), HRMS: calcd. for $C_{43}H_{29}N_7O_2$: 675.2377 found 675.2356.*

**5-[4-Carboxyphenyl]-10,15,20-tri-(4-pyridyl)porphyrin (21)**
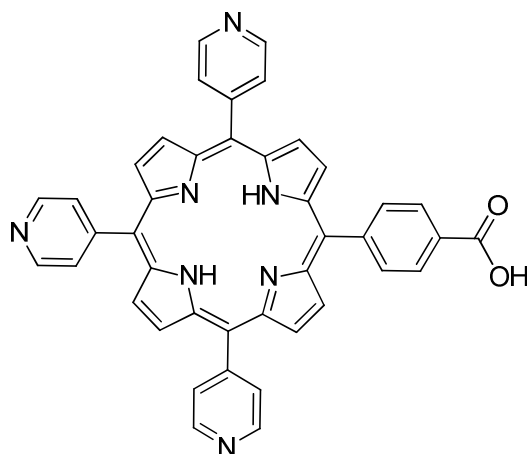


To a stirred solution of 5-(4-methoxycarboxyphenyl)-10,15,20-tris-(4-pyridyl)porphyrin (400 mg, 0.592 mmol) in DMF (40ml) was added a solution of potassium hydroxide (1.60 g, 28.6 mmol) in water (10 ml), and the mixture stirred at rt overnight. The solvent was removed under reduced pressure, and the residue neutralised with 1M HCl$_{(aq)}$. The mixture was filtered and the crude precipitated from MeOH over DCM to yield the product as a purple solid (300 mg, 76.7%).

$R_f$ = 0.29 (silica, 5% MeOH:DCM). UV-vis (MeOH), $\lambda_{max}$/nm: 413, 510, 543, 587, 642. $^1$H-NMR (CDCl$_3$:MeOH-d$_4$): δ 8.21 (m, 6H, 10,15,20-o-Py), 8.29 (d, 2H, J=8.36 Hz, 5-m-Ph), 8.51 (d, 2H, J=8.36 Hz, 5-o-Ph), 8.87 (br s, 8H, βH), 9.03 (m, 6H, 10,15,20-m-Py). $^{13}$C-NMR (CDCl$_3$:MeOH-d$_4$): δ 116.97, 117.24, 120.35, 128.22, 129.48 (β-C), 130.35, 134.36, 145.90, 147.73 (β-C), 150.40, 168.03 (C=O). MS: (NSI) m/z 660 (100[M -H]⁻), HRMS: calcd. for C$_{42}$H$_{26}$O$_2$N$_7$: 660.2153 found 660.2143.

**5-[4-Carboxyphenyl]-10,15,20-tri-(4-pyridyl)porphyrin (21)**
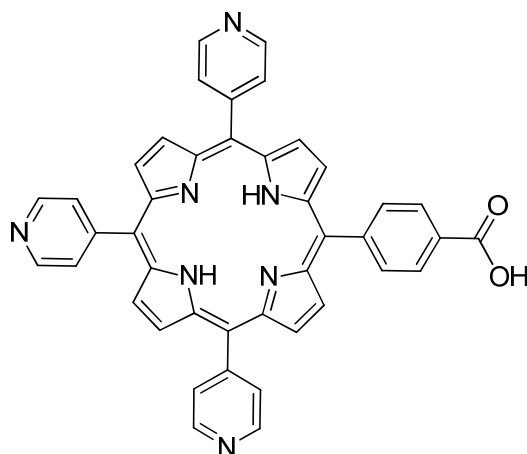


A mixture of 4-carboxybenzaldehyde (10.16 g, 68 mmol) and pyridine -4-carboxaldehyde (21.74 g, 19 ml, 203 mmol) in propanoic acid (500 ml) was heated to reflux with stirring. To this mixture, pyrrole (18.23 g, 19 ml, 272 mmol) was added dropwise, and the reaction mixture stirred for one hour under reflux. The crude was purified by column chromatography (silica, 8-15% MeOH:DCM) and precipitated from MeOH over DCM to yield the product as a purple solid (1.93 g, 4.29%).

$R_f$ = 0.29 (silica, 5% MeOH:DCM). UV-vis (MeOH), $\lambda_{max}$/nm: 413, 510, 543, 587, 642. $^1$H-NMR (CDCl$_3$:MeOH-d$_4$): δ 8.21 (m, 6H, 10,15,20-o-Py), 8.29 (d, 2H, J=8.36 Hz, 5-m-Ph), 8.51 (d, 2H, J=8.36 Hz, 5-o-Ph), 8.87 (br s, 8H, βH), 9.03 (m, 6H, 10,15,20-m-Py). $^{13}$C-NMR (CDCl$_3$:MeOH-d$_4$): δ 116.97, 117.24, 120.35, 128.22, 129.48 (β-C), 130.35, 134.36, 145.90, 147.73 (β-C), 150.40, 168.03 (C=O). MS: (NSI) m/z 660 (100[M -H]⁻), HRMS: calcd. for C$_{42}$H$_{26}$O$_2$N$_7$: 660.2153 found 660.2143.

**5-[4-(Succinimide-N-oxycarbonyl)phenyl]-10,15,20-tri-(4-pyridyl)porphyrin (22)**
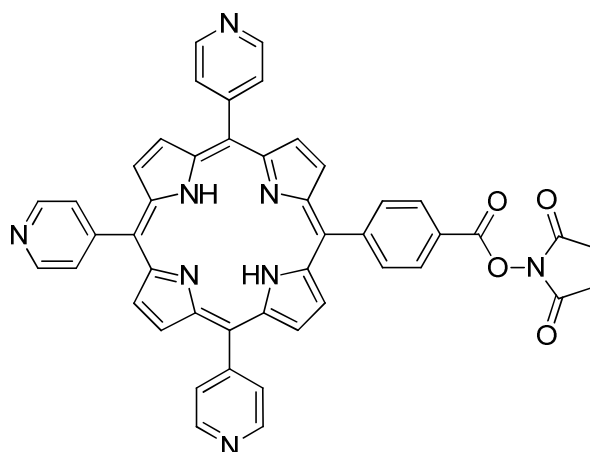


To a stirred solution of 5-(4-carboxyphenyl)-10,15,20-tri-(4-pyridyl)porphyrin (100 mg, 0.151 mmol) in dry pyridine (10 mL) was slowly added thionyl chloride (0.20 mL, 2.72 mmol). The reaction was then stirred at 50 °C, protected from light and atmospheric moisture, for 30 min. After this period, *N*-hydroxysuccinimide (400 mg, 3.47 mmol) was added and the mixture maintained under the previous conditions for 3 h. The solvent was removed under reduced pressure and the crude dissolved in DCM. The organic layer was washed with sat. sodium hydrogen carbonate solution and water, dried (MgSO$_4$), and the solvent removed under reduced pressure. The crude was precipitated from hexane over DCM to yield the product as a brick red solid (103 mg, 92%).

*$R_f$ = 0.82 (silica, 10% methanol:dichloromethane). UV-vis (DCM), $\lambda_{max}$, nm: 417, 512, 547, 588, 669. $\varepsilon$ (417 nm)= 323753 M$^{-1}$cm$^{-1}$. $^1$H-NMR (CDCl$_3$): $\delta$ 3.03 (br s, 4H, CH$_2$), 8.17 (m, 6H, 10,15,20-o-Py), 8.38 (d, 2H, J=8.13 Hz, 5-m-Ph), 8.59 (d, 2H, J=8.13 Hz, 5-o-Ph), 8.87 (m, 8H, $\beta$H), 9.06 (m, 6H, 10,15,20-m-Py). $^{13}$C-NMR (CDCl$_3$): $\delta$ 25.53, 117.66, 115.51, 120.69, 124.59, 129.12, 129.47 ($\beta$-C), 134.81, 148.07 ($\beta$-C), 150.26, 169.36 (C=O), 172.17 (C=O). MS: (ESI) m/z 759 (100[M +H]$^+$), HRMS: calcd. for C$_{46}$H$_{31}$N$_8$O$_4$: 759.2463 found 759.2445.*

## 2-(2-(2-Hydroxyethoxy)ethoxy)ethyl)isoindoline-1,3-dione (23)[192]
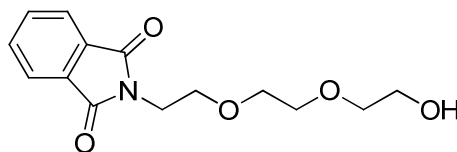


To a solution of potassium phthalimide (5.06 g, 27.3 mmol) in dry DMF (30ml) was added 2-(2-(2-chloroethoxy)ethoxy)ethanol (3.65 ml, 25.1 mmol). The mixture was heated to 100 ˚C with stirring under $N_2$ for 17 hours. The reaction mixture was cooled to room temperature and filtered to remove the white precipitate. The filtrate was evaporated under reduced pressure to yield a viscous yellow oil (6.94 g, 99.1%).

*^{1}H-NMR (CDCl$_3$): δ 3.54 (m, 2H, CH$_2$ -OH), 3.60 (m, 2H), 3.65 (m, 4H), 3.76 (m, 2H), 3.92 (m, 2H, CH$_2$ -N), 7.75 (m, 2H), 7.85 (m, 2H). 13C-NMR (CDCl$_3$): δ 41.66 (CH$_2$N), 66.09 (CH$_2$OH), 72.34, 74.45, 74.77, 77.00, 127.71 (3,6), 136.50 (2a, 6a), 138.47 (4,5), 172.78 (C=O). MS: (NSI) m/z 280 (100[M +H]$^+$), HRMS: calcd. for $C_{14}H_{17}NO_5$H: 280.1179 found 280.1180. MS: (NSI) m/z 280 (100[M +H]$^+$), HRMS: calcd. for $C_{14}H_{17}NO_5$H: 280.1179 found 280.1180.*

## 2-(2-(2-(1,3-Dioxoisoindolin-2-yl)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (24)
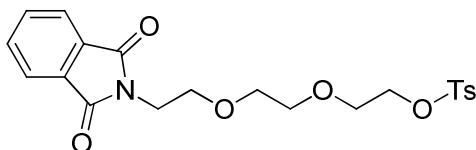


To a solution of 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethyl)isoindoline-1,3-dione (6.00 g, 21.4 mmol) in acetonitrile (50 ml) was added triethylamine (5.95 ml, 43.0 mmol) and trimethylamine hydrochloride (0.30 g, 3.20 mmol). The solution was cooled to 0 ˚C, and p-toluenesulfonyl chloride (6.16 g, 32.2 mmol) was added slowly. The mixture was stirred under these conditions for 20 minutes, and for 40 minutes at room temperature. The mixture was poured into water (100 ml) and extracted with ethyl acetate (3 x 100 ml). The organic fraction was dried (Na$_2$SO$_4$) and the solvent removed under reduced pressure. The crude was purified by column chromatography (silica, 5%-25% EtOAc:DCM) to yield the product as a yellow oil (5.64 g, 61.1%).

$^1$H-NMR (CDCl$_3$): δ2.34 (s, 2H, CH$_3$) 3.43 (m, 4H), 3.60 (m, 2H), 3.71 (m, 2H), 3.76 (m, 2H), 4.05 (m, 2H, CH$_2$ -N), 7.23, (d, 2H, J=8.13 Hz, o-toluene H), 7.62 (m, 2H, phthalimide),7.68 (d, 2H, J=8.44 Hz, m-toluene H) 7.73 (m, 2H, phthalimide). $^{13}$C-NMR (CDCl$_3$): δ21.34 (-CH$_3$), 36.99 (N-CH$_2$), 67.68, 68.40, 69.02, 69.78, 70.38, 122.94, 127.68, 129.57, 131.82, 132.81, 133.76, 144.53 (C-S), 167.94 (C=O). MS: (NSI) m/z 451 (100[M +NH4]$^+$), HRMS: calcd. for C$_{21}$H$_{23}$NO$_7$SNH$_4$: 451.1533 found 451.1534.

## 2-(2-(2-(2-Azidoethoxy)ethoxy)ethyl)isoindoline-1,3-dione (25)
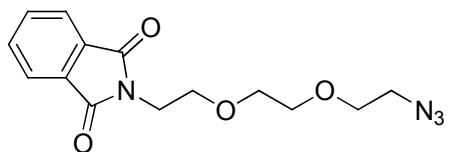


To a stirred solution of 2-(2-(2-(1,3-dioxoisoindolin-2-yl)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (4.33 g, 12.0 mmol) in acetonitrile (20 ml) was added sodium azide (1.98 g, 30.0 mmol). The mixture was refluxed under argon for 46 hours, then cooled to room temperature. The mixture was diluted with water (20 ml), and extracted with DCM (5 x 40 ml). The organic layer was dried ($Na_2SO_4$) and the solvent removed under reduced pressure to yield the product as a yellow oil (2.65 g, 72.8%).

$^1$H-NMR (CDCl$_3$): δ 3.24 (t, 2H, J=5.16 Hz, CH$_2$-N$_3$), 3.53 (m, 4H), 3.57 (m, 2H), 3.76 (m, 2H), 3.84 (t, 2H, J=5.94 Hz, CH$_2$ -N), 7.64 (m, 2H, phthalimide), 7.77 (m, 2H, phthalimide). $^{13}$C-NMR (CDCl$_3$): δ 37.20 (N-CH$_2$), 50.54, (CH$_2$-N$_3$), 67.91, 70.53, 76.69, 77.00, 123.12, 132.04, 133.84, 168.15 (C=O). MS: (NSI) m/z 322 (100[M +NH4]$^+$), HRMS: calcd. for C$_{14}$H$_{16}$N$_4$O$_4$NH$_4$: 322.1510 found 322.1512.

**2-(2-(2-Azidoethoxy)ethoxy)ethanamine (26)**
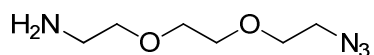


To a stirred solution of 2-(2-(2-(2-azidoethoxy)ethoxy)ethyl)isoindoline-1,3-dione (0.51 g, 1.64 mmol) in ethanol (30 ml) was added hydrazine monohydrate (1.60 ml, 32.8 mmol). The mixture was stirred for 20 hours at 25 ˚C. $HCl_{(aq)}$ (4M, 40 ml) was then added, and the reaction stirred for a further 30 minutes. The mixture was filtered, washed with DCM and basified to pH 14 with aqueous sodium hydroxide solution. The solvent was removed under reduced pressure, the residue taken up in dichloromethane and filtered. The organic layer was dried ($Na_2SO_4$) and the solvent removed under reduced pressure to yield the product as a yellow oil (156 mg, 55.0%).

$^1$H-NMR (CDCl$_3$): δ 2.87 (m, 2H, CH$_2$-N$_3$), 3.46 (m, 2H), 3.52 (m, 2H), 3.69 (m, 6H). $^{13}$C-NMR (CDCl$_3$): δ 45.08 (NH$_2$-_C_H$_2$), 54.21,(CH$_2$-N$_3$), 73.60, 73.86, 74.21, 77.00. MS: (ESI) m/z 175 (100[M + H]+), HRMS: calcd. for C$_6$H$_{15}$N$_4$O$_2$: 175.1190 found 175.1188.

**Tert-butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (27)**
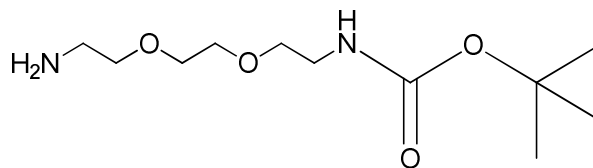


2,2'-(Ethane-1,2-diylbis(oxy))diethanamine (12.00g, 81 mmol) was dissolved in dry dichloromethane (20 ml) and cooled to 0 °C under nitrogen. Di-tert-butyl dicarbonate (2.62 g, 12.00 mmol) dissolved in dry dichloromethane (20 ml) was added dropwise, and the reaction mixture was allowed to return to room temperature, and stirred overnight under nitrogen. The solvent was removed under reduced pressure, and the residue redissolved in water. The aqueous layer was extracted with dichloromethane, the organic layer dried (Na$_2$SO$_4$) and the solvent removed under reduced pressure. The product was purified by column chromatography (silica, 5-25% MeOH:DCM) to yield the product as a pale yellow oil (0.87 g, 91.7% ).

*$^1$H-NMR (CDCl$_3$): δ 1.40 (s, 9H, C-(CH$_3$)$_3$), 2.02 (br s, 2H, NH), 2.80 (m, 2H, CH$_2$-NH$_2$), 3.29 (m, 2H, CH$_2$-NHBoc), 3.46 (m, 4H), 3.52 (m, 4H). 13C-NMR (CDCl$_3$): δ 26.30 (C-(CH$_3$)$_3$), 38.22 (CH$_2$-NHBoc), 39.55 (CH$_2$-NH$_2$), 68.10, 71.21, 75.40 (C-(CH$_3$)$_3$), 153.97 (C=O) MS: (ESI) m/z 249 (100[M +H]$^+$), HRMS: calcd. for C$_{11}$H$_{24}$N$_2$O$_4$H: 249.1809 found 249.1807.*

**Tert-butyl (2-(2-(2-azidoethoxy)ethoxy)ethyl)carbamate (28)**
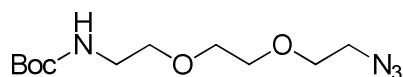


To a stirred solution of tert-butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (2.50 g, 10 mmol) in methanol (50 ml) was added copper (II) sulfate (1 mg), and anhydrous potassium carbonate (2.75 g, 20 mmol). Imidazole-1-sulfonyl azide hydrochloride (2.50 g, 12 mmol) was added, and the mixture stirred at rt for 24 hours. The solvent was removed under reduced pressure and the product purified by column chromatography (silica, 1% MeOH:DCM) to yield the product as a colourless oil (2.37 g, 86.5%).

$^{1}$H-NMR (CDCl$_3$): δ 1.44(s, 9H, C(C$\underline{H}_3$)$_3$), 3.34 (m, 2H, CH$_2$-N$_3$), 3.42 (m, 2H), 3.56 (m, 2H), 3.69 (m, 6H). $^{13}$C-NMR (CDCl$_3$): δ 28.35 (C(C$\underline{H}_3$)$_3$), 40.28, 50.60, 70.25, 70.50, 77.31 ($\underline{C}$(CH$_3$)$_3$), 155.97 ($\underline{C}$=O). MS: (ESI) m/z 275 (100[M + H]+), HRMS: calcd. for C$_{11}$H$_{23}$N$_4$O$_4$: 275.1714 found 275.1713.

**2-(2-(2-Azidoethoxy)ethoxy)ethanamine hydrochloride (29)**
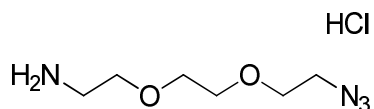


To a stirred solution of tert-butyl (2-(2-(2-azidoethoxy)ethoxy)ethyl)carbamate (2.20 g, 8.0 mmol) in ethyl acetate (20 ml) was added HCl in dioxane (10 ml), and the reaction mixture stirred at rt for 24 hours. The solvent was removed under reduced pressure, and the residue washed with diethyl ether to yield the product as a yellow oil (1.51 g, 89.9%).

$^{1}$*H-NMR (MeOH-d$_3$): δ 3.07 (m, 2H, CH$_2$-NH$_2$), 3.34 (m, 2H, CH$_2$-N$_3$), 3.60 (m, 6H), 3.66 (t, 2H). $^{13}$C-NMR (MeOH-d$_3$): δ 51.73, 67.78, 68.08, 70.95, 71.34. MS: (ESI) m/z 175 (100[M + H]+), HRMS: calcd. for C$_6$H$_{15}$N$_4$O$_2$: 175.1190 found 175.1188.*

**5-[4-2-(2-(2-Azidoethoxy)ethoxy)ethanaminocarbonyl]phenyl]-10,15,20-tris(4-pyridyl)porphyrin (30)**
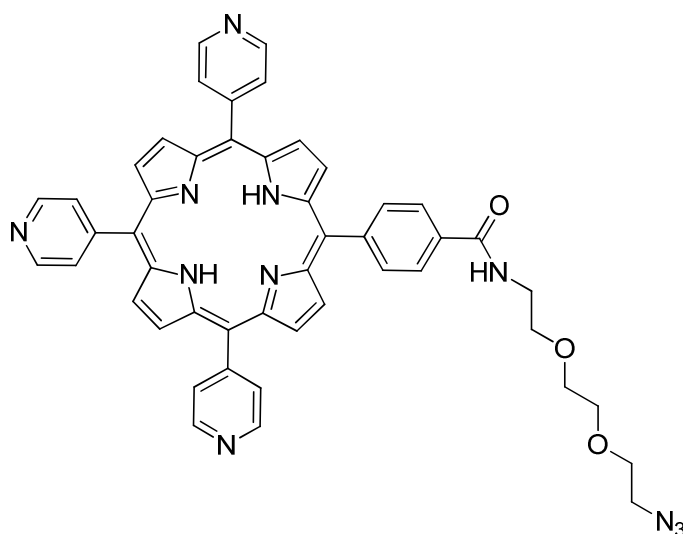


To a stirred solution of 5-[4-(Succinimide-*N*-oxycarbonyl)phenyl]-10,15,20-tri-(4-pyridyl) porphyrin (100 mg, 0.135 mmol) in dry DMSO (10 ml) was added  2-(2-(2-azidoethoxy)ethoxy)ethanamine  (65 mg, 0.33 mmol), and anhydrous potassium carbonate (58 mg, 0.416 mmol). The mixture was stirred for two days at 40 ˚C, protected from light and atmospheric moisture. Water (10 ml) was added, and the mixture centrifuged. The resulting solid was purified by column chromatography (silica, 6% MeOH:DCM) and precipitated from MeOH over DCM to yield the product as a purple solid (88 mg, 82%).

*$R_f$: (silica, 5% MeOH:DCM): 0.35. UV-vis (DCM): λmax, nm 417, 513, 547, 588, 642. ε (417 nm)= 376878 M$^{-1}$cm$^{-1}$. $^1$H-NMR (CDCl$_3$): δ -2.86 (s, 2H,- NH), 3.45 (m, 2H, CH$_2$-N$_3$), 3.76 (m, 6H), 3.87 (m, 4H), 7.57(t, 1H, NH), 8.18 (m, 6H, 10,15,20-o-Py), 8.19 (m, 2H, 5-m-Ph), 8.26 (m, 2H, 5-o-Ph), 8.86 (m, 8H, βH), 9.03 (m, 6H, 10,15,20-m-Py). $^{13}$C-NMR (CDCl$_3$): δ  39.82 (CH$_2$-N$_3$), 49.35 (CH$_2$-O), 69.91 (CH$_2$-O), 70.45 (CH), 76.67, 117.39, 120.24, 125.59, 129.22 (β-C), 134.25, 144.49, 148.05 (β-C),149.87, 167.55 (C=O).MS: (ESI) m/z 840 (100[M + Na]+), HRMS: calcd. for C$_{48}$H$_{39}$N$_{11}$O$_3$Na 840.3130 found 840.3125.*

**5-[4-2-(2-(2-Azidoethoxy)ethoxy)ethanaminocarbonyl]phenyl]-10,15,20-tris(N-methyl-4-pyridinium)porphyrin triiodide (31)**
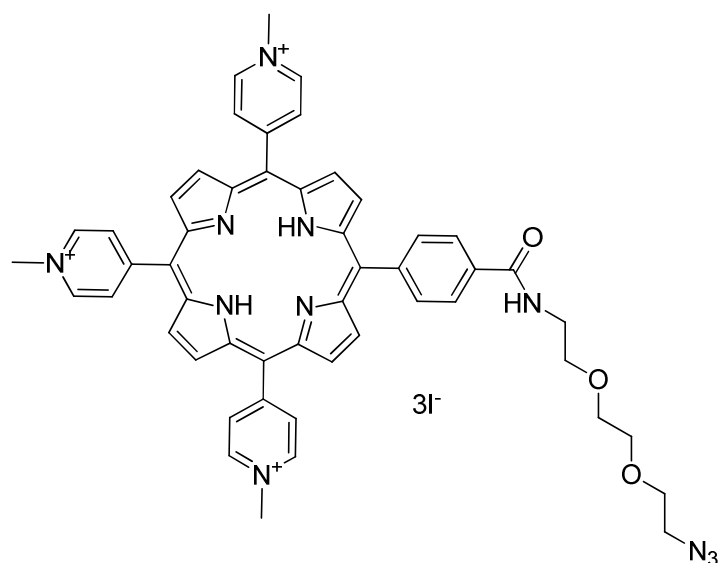


To a stirred solution of 5-[4-2-(2-(2-azidoethoxy)ethoxy)ethanaminocarbonyl]phenyl]-10,15,20-tris(4-pyridyl)porphyrin (87 mg, 0.106 mmol) in DMF (10ml) was added methyl iodide (2 ml, 32 mmol) *via* syringe. The reaction mixture was stirred at 40 °C overnight. The mixture was cooled to room temperature and cold diethyl ether (100 ml) was added. The mixture was filtered through cotton wool, and the residue redissolved in methanol. The solvent was removed under reduced pressure, and the porphyrin precipitated from diethyl ether over MeOH to yield the product as a purple solid (107 mg, 81.6%).

*$R_f$: 0.49 (silica, 1:1:8 sat. $KNO_3$ solution:water:MeCN). UV-vis ($H_2O$): λmax, nm 424, 521, 560, 580, 645. ε (424 nm)= 232892 $M^{-1}cm^{-1}$. $^1$H-NMR (DMSO-$d_6$): δ -2.98 (s, 2H,-NH), 3.45 (m, 2H, $CH_2$-$N_3$), 3.76 (m, 10H), 4.76 (m, 9H, N-C H$_3$), 8.39 (m, 4H, 5-m-Ph, 5-o-Ph), 8.96-9.25 (m, 14H, βH and 10,15,20-o-Py), 9.53 (m, 6H, 10,15,20-m-Py). $^{13}$C-NMR (DMSO-$d_6$): δ 47.94 ($CH_2$-NH), 50.02 (N-$CH_3$), 68.98, 69.25, 69.65, 114.70, 115.33, 121.79, 126.00, 132.05 (β-C), 134.27, 142.99, 144.15 (β-C), 156.47, 166.09 (C=O). MS: (MALDI-TOF) m/z 287 (100[M - 3Cl]3+), HRMS: calcd. for $C_{51}H_{46}N_{11}O_3$: 287.4642 found 287.4643*
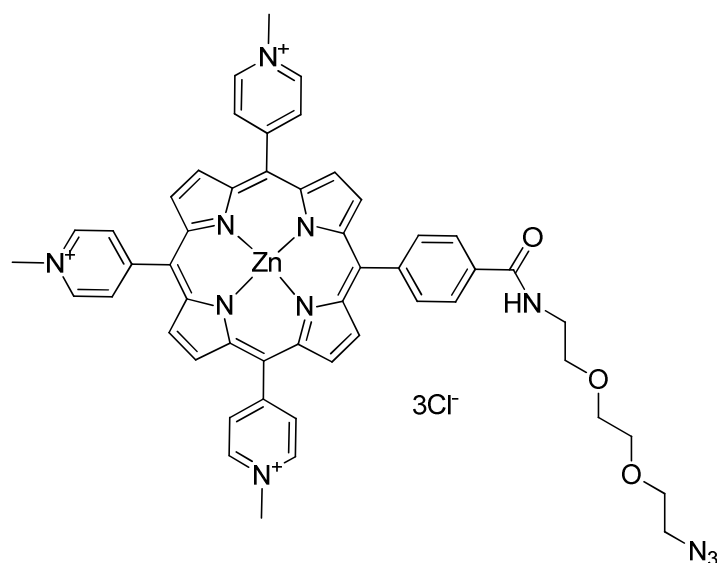
**Zinc 5-[4-2-(2-(2-azidoethoxy)ethoxy)ethanaminocarbonyl]phenyl]-10,15,20-tris(N-methyl-4-pyridinium)porphyrin trichloride (32)**



To a stirred solution of 5-[4-2-(2-(2-azidoethoxy)ethoxy)ethanaminocarbonyl]phenyl]-10,15,20-tris(*N*-methyl-4-pyridinium)porphyrin triiodide (107 mg, 0.086 mmol) in water (10 ml) was added a solution of zinc acetate (107 mg, 0.59 mmol) in water (10 ml). The mixture was stirred at room temperature for 3 hours, and ammonium hexafluorophosphate added. The resulting solution was filtered and the precipitate redissolved in acetone. Tetrabutylammonium chloride was added, and the resulting solution filtered. The product was precipitated from diethyl ether over MeOH to yield the product as a green solid (84 mg, 95%).

*HPLC: 5-60% B over 15 minutes. $R_f$ = 8.2 minutes. $R_f$: 0.49 (silica, 1:1:8 sat. KNO$_3$ solution:water:MeCN). UV-vis (H$_2$O): λmax, nm 434, 563, 607. ε (434 nm)= 181375 M$^{-1}$cm$^{-1}$. $^1$H-NMR (DMSO-d$_6$): δ 3.38 (m, 2H, CH$_2$-N$_3$), 3.67 (m, 10H), 4.72 (m, 9H, N-CH$_3$), 8.21 (m, 2H, 5-m-Ph), 8.35 (m, 2H, 5-o-Ph), 8.88-9.00 (m, 14H, βH and 10,15,20-o-Py), 9.44 (m, 6H, 10,15,20-m-Py). $^{13}$C-NMR (DMSO-d$_6$): δ 47.94 (CH$_2$-NH), 50.02 (N-CH$_3$), 68.98, 69.25, 69.65, 114.70, 115.33, 121.79, 126.00, 132.05 (β-C), 134.27, 142.99, 144.15 (β-C), 156.47, 166.09 (C=O). MS: (ESI) m/z 308 (100[M - 3Cl]3+), HRMS: calcd. for C$_{51}$H$_{46}$N$_{11}$O$_3$Zn: 308.1020 found 308.1025.*

**5-[4-Acetamidophenyl]-10,15,20-tri-(3,5-dimethoxyphenyl)porphyrin (33)[177]**
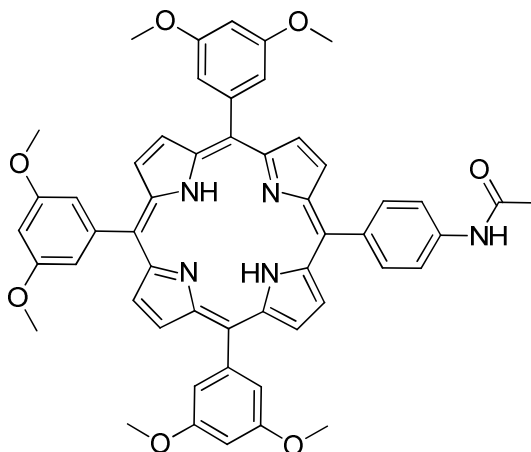


To a refluxing mixture of 4-acetamidobenzaldehyde (3.36 g, 0.02 mol) and 3,5-dimethylbenzaldehyde (10.0 g, 0.06 mol) in propionic acid (500 ml) was added pyrrole (5.5 ml, 0.08 mol) dropwise, and the mixture stirred vigorously under reflux for 1 hour, protected from light. The mixture was cooled to room temperature and the solvent removed under reduced pressure, and any excess acid neutralised with sat. sodium bicarbonate solution. The product was extracted with dichloromethane, and the organic layer dried (Na$_2$SO$_4$). The crude was purified by column chromatography (silica, 1-3% MeOH:DCM) to yield the product as a purple solid (1.42 g, 8.35%).

*$R_f$ = 0.20 (silica, 1% methanol:dichloromethane). UV-vis (DCM): λmax, nm 421, 459, 514, 550, 590. ε (421 nm)= 121458 M$^{-1}$cm$^{-1}$. $^1$H-NMR (CDCl$_3$): δ -2.83 (s, 2H,- N<u>H</u>), 2.35 (s, 3H, C=OC<u>H</u>$_3$), 3.96 (s, 18H, O-CH$_3$), 6.90 (m, 3H, 10,15,20-p-Ph),7.40 (m, 6H, 10,15,20-o-Ph), 7.89 (d, 2H, J=7.89 Hz, 5-m-Ph), 8.16 (d, 2H, J=8.14 Hz, 5-o-Ph), 8.93 (m, 8H, βH). MS: (MALDI-TOF) m/z 851.3 (100[M + H]+).*

## 5-[4-Aminophenyl]-10,15,20-tri-(3,5-dimethoxyphenyl)porphyrin (34)[177]
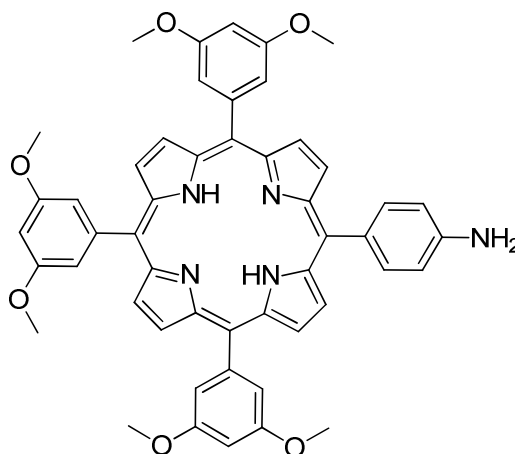


5-[4-acetamidophenyl]-10,15,20-tri-(3,5-dimethoxyphenyl)porphyrin (500 mg, 0.587 mmol) was dissolved in $HCl_{(aq)}$ (5 M, 100 ml) and stirred under reflux for two hours. The reaction was cooled to room temperature and concentrated under reduced pressure. The residue was dissolved in DCM/TEA (9:1, 200 ml) and stirred for 10 minutes at room temperature. The solution was washed with water (3 x 200 ml) and the organic layer dried ($Na_2SO_4$). The solvent was removed under reduced pressure, and the crude purified by column chromatography (silica, 1% MeOH:DCM) to yield the product as a purple solid (280 mg, 58.9%).

*$R_f$ = 0.80 (silica, 4% methanol:dichloromethane). UV-vis (DCM): λmax, nm 423, 464, 516, 553, 594. ε (423 nm)= 277370 $M^{-1}cm^{-1}$. $^1$H-NMR (CDCl$_3$): δ 3.96 (s, 18H, O-CH$_3$), 6.89 (m, 3H, 10,15,20-p-Ph), 7.05 (d, 2H, J=8.16 Hz, 5-m-Ph), 7.40 (m, 6H, 10,15,20-o-Ph), 7.99 (d, 2H, J=8.36 Hz, 5-o-Ph), 8.92 (m, 8H, βH). MS: (ESI) m/z 809.3 (100[M + H]+).*

# 5-[4-Aminophenyl]-10,15,20-tri-(3,5-dihydroxyphenyl)porphyrin (35)[177]
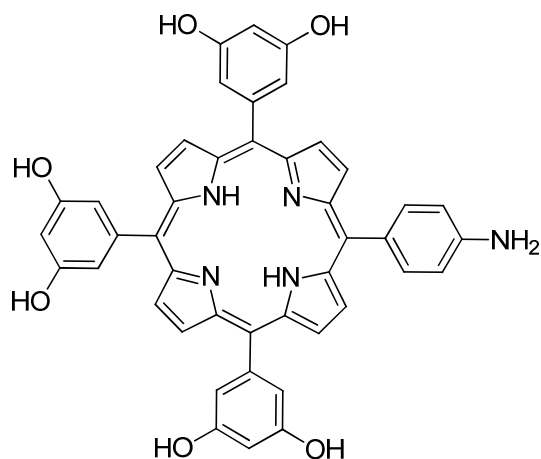


To a stirred solution of 5-[4-aminophenyl]-10,15,20-tri-(3,5-dihydroxyphenyl)porphyrin (250 mg, 0.31 mmol) in chloroform (20 ml) was added boron tribromide in hexanes (5.5 ml, 0.012 mol) dropwise, and the mixture stirred under $N_2$ for 24 hours. After this time, water (50 ml) was added, and the mixture maintained under previous conditions for 1 hour. The solvent was removed under reduced pressure, and the residue was dissolved in DCM/TEA (9:1, 200 ml) and stirred for 10 minutes at room temperature. The solution was washed with water (3 x 200 ml) and the organic layer dried ($Na_2SO_4$). The solvent was removed under reduced pressure, and the crude purified by column chromatography (silica, 10-12% MeOH:DCM) to yield the product as a purple solid (135 mg, 28.9%).

*$R_f$ = 0.80 (silica, 4% methanol:dichloromethane) UV-vis (MeOH): λmax, nm 419, 516, 551, 591, 649. ε (419 nm)= 292467 $M^{-1}cm^{-1}$. $^1$H-NMR (MeOH-$d_4$): δ 6.74 (m, 3H, 10,15,20-p-Ph), 7.10 (d, 2H, J=7.96 Hz, 5-m-Ph), 7.18 (m, 6H, 10,15,20-o-Ph), 7.93 (d, 2H, J=8.36 Hz, 5-o-Ph), 8.99 (m, 8H, βH). $^{13}$C-NMR (MeOH-$d_4$): δ 97.39, 109.05, 110.01 (β-C), 115.21, 115.58, 116.74, 126.81, 130.98, 139.27, 139.33, 143.21, 152.14 (β-C). MS: (ESI) m/z 726 (100[M +H]+), HRMS: calcd. for $C_{44}H_{32}N_5O_6$: 726.2347 found 726.2343.*

**5-[4-Azidophenyl]-10,15,20-tri-(3,5-dimethoxyphenyl)porphyrin (36)**



To a stirred solution of 5-[4-aminophenyl]-10,15,20-tri-(3,5-dimethoxyphenyl)porphyrin (600 mg, 0.74 mmol) in TFA (4 ml) at 0 ˚C was added dropwise a solution of sodium nitrite (220 mg, 3.15 mmol) in water. The mixture was stirred for 15 minutes at 0 ˚C. A solution of sodium azide (415 mg, 6.30 mmol) in water was added dropwise and the reaction mixture stirred at 0 ˚C for 1 hour. The mixture was diluted with water and saturated sodium bicarbonate solution added until the colour changed from green to purple. The aqueous mixture was extracted with dichloromethane, and the organic layer dried ($Na_2SO_4$) and the solvent removed under reduced pressure to yield a purple solid. Purification was attempted by column chromatography (silica, 1-10% MeOH-DCM). The desired product was not isolated.
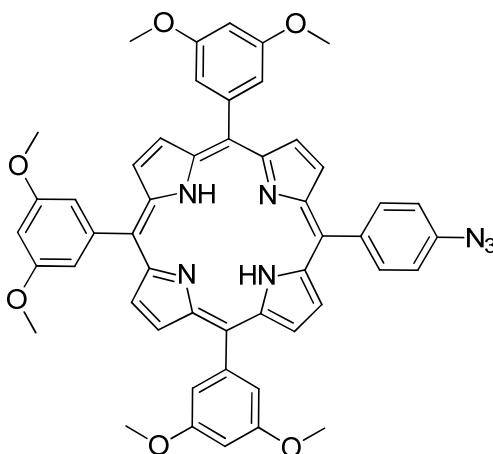
**5-[4-Azidophenyl]-10,15,20-tri-(3,5-dihydroxyphenyl)porphyrin (37)**
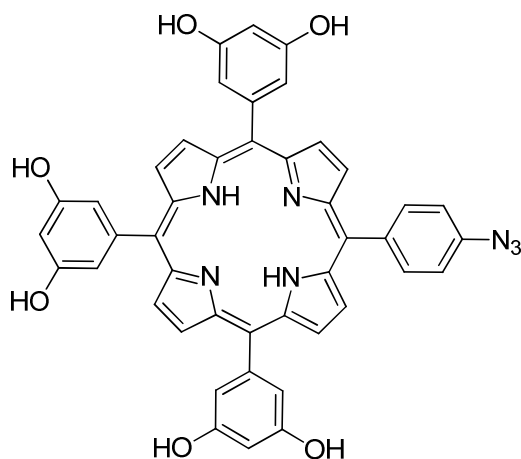


To a stirred solution of 5-[4-aminophenyl]-10,15,20-tri-(3,5-dihydroxyphenyl)porphyrin (65 mg, 0.089 mmol) in TFA (3 ml) at 0 ˚C was added dropwise a solution of sodium nitrite (44 mg, 0.63 mmol) in water. The mixture was stirred for 15 minutes at 0 ˚C. A solution of sodium azide (83 mg, 1.26 mmol) in water was added dropwise and the reaction mixture stirred at 0 ˚C for 1 hour. The mixture was diluted with water and saturated sodium bicarbonate solution added until the colour changed from green to purple. The desired product was not isolated.

**Zinc 5-[4-azidophenyl]-10,15,20-tri-(3,5-dihydroxyphenyl)porphyrin (37Zn)**



To a stirred solution of 5-[4-aminophenyl]-10,15,20-tri-(3,5-dimethoxyphenyl)porphyrin (57 mg, 0.079 mmol) in methanol (10 ml) was added zinc acetate (50 mg, 0.27 mmol), and anhydrous potassium carbonate (21 mg, 0.158 mmol). Imidazole-1-sulfonyl azide hydrochloride (16.5 mg, 0.095 mmol) was added, and the mixture stirred at rt for 24 hours. The solvent was removed under reduced pressure to yield the crude as a purple solid. The desired product was not isolated.

**Zinc 5-[4-azidophenyl]-10,15,20-tri-(3,5-dimethoxyphenyl)porphyrin (36Zn)**



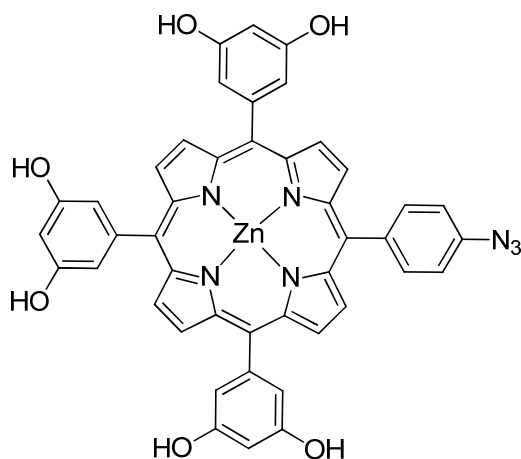To a stirred solution of 5-[4-aminophenyl]-10,15,20-tri-(3,5-dimethoxyphenyl)porphyrin (64 mg, 0.079 mmol) in DMSO:water (10:1, 5 ml) was added zinc acetate (64 mg, 0.34 mmol), and anhydrous potassium carbonate (21 mg, 0.158 mmol). Imidazole-1-sulfonyl azide hydrochloride (16.5 mg, 0.095 mmol) was added, and the mixture stirred at rt for 24 hours. The solvent was removed under reduced pressure to yield the crude as a purple solid, and the crude purified by column chromatography (silica, 0.5% MeOH:DCM) to yield the product as a purple solid (60.2 mg, 85.0%).

$^1$*H-NMR (CDCl$_3$:MeOH-d$_4$): δ 3.87 (s, 18H, O-CH$_3$), 6.78 (m, 3H, 10,15,20-p-Ph), 7.40 (m, 6H, 10,15,20-o-Ph), 8.29 (d, 2H, J=8.44 Hz, 5-m-Ph), 9.04 (d, 2H, J=8.44 5-o-Ph), 9.05 (m, 8H, βH). $^{13}$C-NMR (CDCl$_3$:MeOH-d$_4$): δ 55.54 (O-CH$_3$), 99.74, 113.82 (β-C), 117.09, 119.46, 120.44, 131.33, 135.53, 145.01, 149.71, 158.54 (β-C). MS: (ESI) m/z 898 (100[M + H]+), HRMS: calcd. for C$_{50}$H$_{40}$N$_7$O$_6$Zn$_1$ 898.2326 found 898.2325.*

**5-[4-Azidophenyl]-10,15,20-tri-(3,5-dihydroxyphenyl)porphyrin (37)**
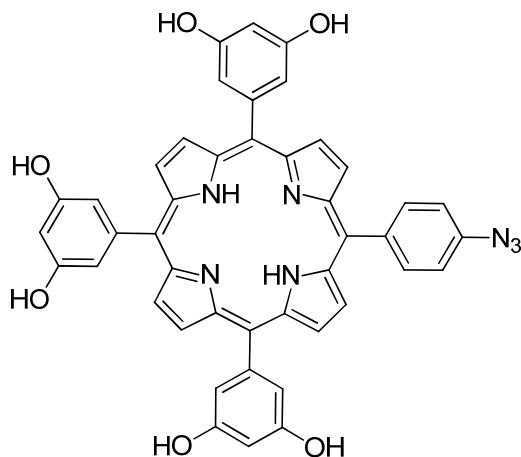


To a stirred solution of zinc 5-[4-azidophenyl]-10,15,20-tri-(3,5-dimethoxyphenyl)porphyrin (100 mg, 0.11 mmol) in chloroform (200 ml) was added boron tribromide (1.00 ml, 2.64 g, 10.5 mmol) dropwise, and the mixture stirred under $N_2$ for 24 hours. After this time, water (50 ml) was added, and the mixture maintained under previous conditions for 1 hour. The solvent was removed under reduced pressure, and the residue was dissolved in DCM/TEA (9:1, 200 ml) and stirred for 10 minutes at room temperature. The solution was washed with water (3 x 200 ml) and the organic layer dried ($Na_2SO_4$). The solvent was removed under reduced pressure, and the crude purified by column chromatography (silica, 10-12% MeOH:DCM) to yield a purple solid. The desired product was not isolated.

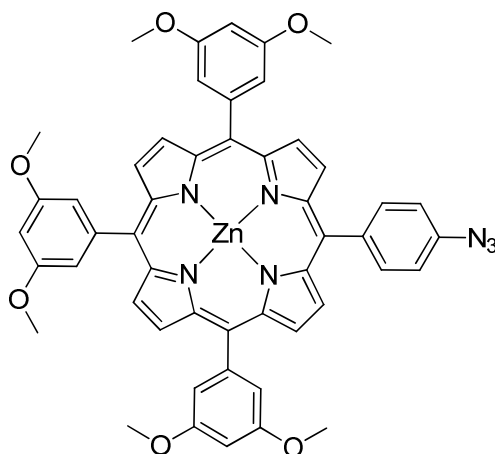**5-[4-2-(2-(2-Azidoethoxy)ethoxy)ethanthiourea]phenyl]-10,15,20-tri-(3,5-dihydroxyphenyl)porphyrin (38)**
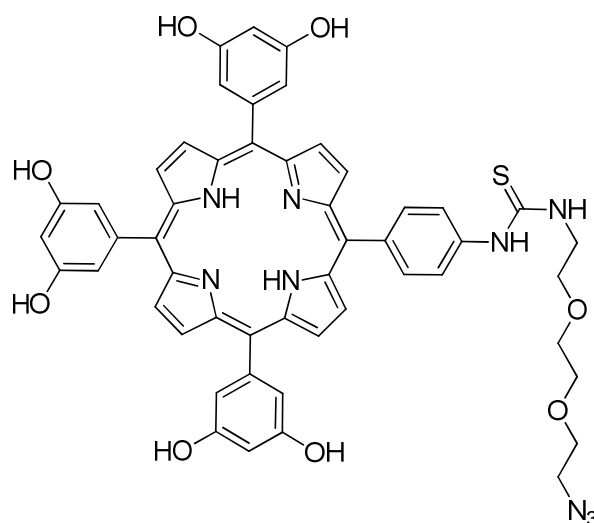


To a stirred solution of 5-[4-aminophenyl]-10,15,20-tri-(3,5-dihydroxyphenyl)porphyrin (100 mg, 0.137 mmol) in THF (25 ml) at 0 °C was added 1,1'-thiocarbonyldi-2-pyridone (64 mg, 0.276 mmol). The mixture was stirred under $N_2$ at 0 °C for 10 minutes, and hexane was added to precipitate out the porphyrin. The precipitate was filtered and the solid washed with DCM. The porphyrin was redissolved in methanol (25 ml) and triethylamine (60 mg) added. 2-(2-(2-azidoethoxy)ethoxy)ethanamine (64.5 mg, 0.37 mmol) was added and the mixture stirred at rt for one hour, under $N_2$. The solvent was removed under reduced pressure and the crude purified by column chromatography (silica, 10% MeOH:DCM) to yield the product as a purple solid (55 mg, 42.7%).

*$R_f$: 0.15 (silica, 10% MeOH:DCM), UV-vis (MeOH): λmax, nm 418, 514, 548, 588, 646. ε (418 nm)= 506968 $M^{-1}cm^{-1}$, $^1$H-NMR (CDCl$_3$:MeOH-d$_4$): δ 3.12 (m, 2H, C<u>H</u>$_2$-N$_3$) 3.30 (m, 6H), 3.32 (m, 2H), 3.63 (m, 2H, C<u>H</u>$_2$-NH), 6.83 (m, 3H, 10,15,20-p-Ph), 7.26 (m, 6H, 10,15,20-o-Ph), 7.64 (d, 2H, J=7.96 Hz, 5-m-Ph), 8.05 (d, 2H, J=7.72 Hz, 5-o-Ph), 9.11 (m, 8H, βH). $^{13}$C-NMR (CDCl$_3$:MeOH-d$_4$): δ 28.67, 48.58, 59.25, 71.08, 103.21, 108.53, 115.79 (β-C), 121.60, 144.99, 157.93(β-C). MS: (ESI) m/z 942 (100[M + H]+), HRMS: calcd. for C$_{51}$H$_{44}$N$_9$O$_8$S$_1$ 942.3027 found 942.3028.*
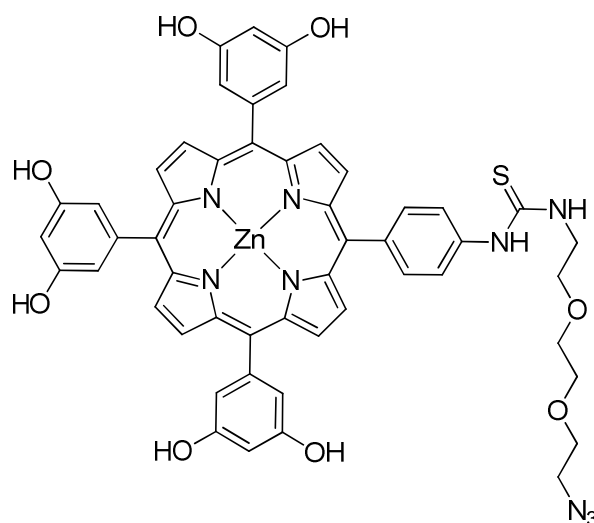
**Zinc 5-[4-2-(2-azidoethoxy)ethoxy)ethanthiourea]phenyl]-10,15,20-tri-(3,5-dihydroxyphenyl)porphyrin (39)**



To a stirred solution of 5-[4-2-(2-azidoethoxy)ethoxy)ethanthiourea]phenyl]-10,15,20-tri-(3,5-dihydroxyphenyl)porphyrin (55 mg, 0.058 mmol) in methanol (20ml) was added zinc acetate (50 mg, 0.270 mmol) and the mixture stirred at rt for 1 hour. The solvent was removed under reduced pressure and the crude purified by column chromatography (silica, 20% MeOH:DCM) to yield the product as a purple solid (56 mg, 96.4%).

$R_f$: 0.13 (silica, 10% MeOH:DCM). UV-vis (MeOH): $\lambda$max, nm 424, 556, 597. $\varepsilon$ (424 nm)= 440200 $M^{-1}cm^{-1}$. $^1$H-NMR (MeOH-$d_4$): $\delta$ 3.22 (m, 2H, C$\underline{H}_2$-N$_3$) 3.30 (m, 4H), 3.52 (m, 6H), 3.66 (m, 2H, C$\underline{H}_2$-NH), 3.94 (s, 1H, N$\underline{H}$), 6.73 (m, 3H, 10,15,20-p-Ph), 7.19 (m, 6H, 10,15,20-o-Ph), 7.73 (d, 2H, J=8.16 Hz, 5-m-Ph), 8.17 (d, 2H, J=8.36 Hz, 5-o-Ph), 8.89-9.01 (m, 8H, $\beta$H). $^{13}$C-NMR (MeOH-$d_4$): $\delta$ 21.01, 31.20, 48.57, 49.21, 102.62, 115.87 ($\beta$-C),121.82, 132.21, 142.40, 146. 69, 151.09, 157.40 ($\beta$-C). MS: (ESI) m/z 1004 (100[M + H]+), HRMS: calcd. for $C_{51}H_{41}N_9O_8S_1Zn_1H$ 1004.2168 found 1004.2163.

**2-(Prop-2-yn-1-yloxy)ethanamine hydrochloride (41)**
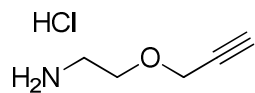


To a stirred solution of tert-butyl (2-(prop-2-yn-1-yloxy)ethyl)carbamate (0.41 g, 2.07 mmol) in ethyl acetate (5 ml) was added HCl in dioxane (4 M, 2 ml). The reaction was stirred at room temperature for one hour, and the solvent removed under reduced pressure. The residue was washed with diethyl ether to yield the hydrochloride salt as a pale pink solid (0.25 g, 89.8%).

*Mp 65-67 °C. $^1$H-NMR (MeOH-d$_4$): δ 1.38 (m, 1H, CH), 1.58 (m, 2H, CH$_2$-NH$_2$), 2.19 (m, 2H, O-CH$_2$-CH$_2$), 2.69 (m, 2H, CH$_2$-O). $^{13}$C-NMR (MeOH-d$_4$): δ 40.57 (CH$_2$-NH$_2$), 59.18 (CH$_2$-O), 66.46 (CH$_2$-O), 76.74 (CH), 79.91 (CH$_2$-C)*

**2-(Prop-2-yn-1-yloxy)ethanaminocarbonyl]phenyl]-10,15,20-tris(4-pyridyl)porphyrin (42)**
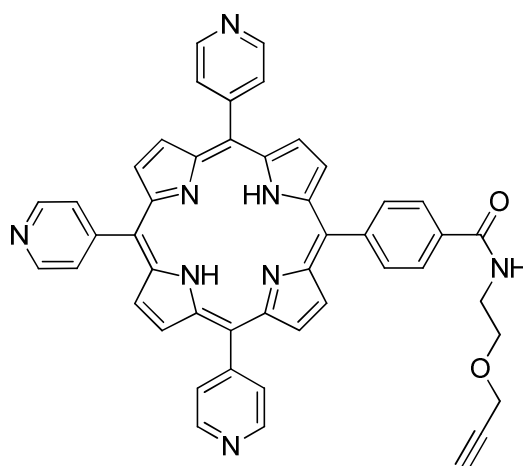


To a stirred solution of 5-[4-(succinimide-*N*-oxycarbonyl)phenyl]-10,15,20-tri-(4-pyridyl) porphyrin (100 mg, 0.14 mmol) in dry DMSO (10 ml) was added 2-(prop-2-yn-1-yloxy)ethanamine (36 mg, 0.33 mmol), and anhydrous potassium carbonate (58 mg, 0.42 mmol). The mixture was stirred for three days at 40 ˚C, protected from light and atmospheric moisture. Water (10 ml) was added, and the mixture centrifuged. The resulting solid was precipitated from MeOH over DCM to yield the product as a purple solid (61 mg, 60.8%).

*$^{1}$H-NMR (CDCl$_3$): δ -2.89 (s, 2H,- N$\underline{H}$), 2.54 (s, 1H, -C$\underline{H}$), 3.88 (m, 4H), 4.31 (m, 2H, C$\underline{H}_2$-NH), 8.15 (m, 6H, 10,15,20-o-Py), 8.22 (d, 2H, J= 8.44 Hz, 5-m-Ph), 8.30 (d, 2H, J=7.82 Hz, 5-o-Ph), 8.88 (m, 8H, βH), 9.06 (m, 6H, 10,15,20-m-Py), ).$^{13}$C-NMR (CDCl$_3$): δ 43.02 (C$\underline{H}_2$-NH$_2$), 60.54 (C$\underline{H}_2$-O), 70.88 (C$\underline{H}_2$-O), 77.00 (C$\underline{H}$), 79.34, 119.63, 127.59, 131.33 (β-C), 136.63, 150.42 (β-C),151.89, 169.47 (C=O). MS: (ESI) m/z 743 (100[M +H]+), HRMS: calcd. for C$_{47}$H$_{34}$N$_8$O$_2$H: 743.2877 found 743.2871.*

**Zinc 2-(prop-2-yn-1-yloxy)ethanaminocarbonyl]phenyl]-10,15,20-tris(N-methyl-4-pyridinium)porphyrin trichloride (43)**
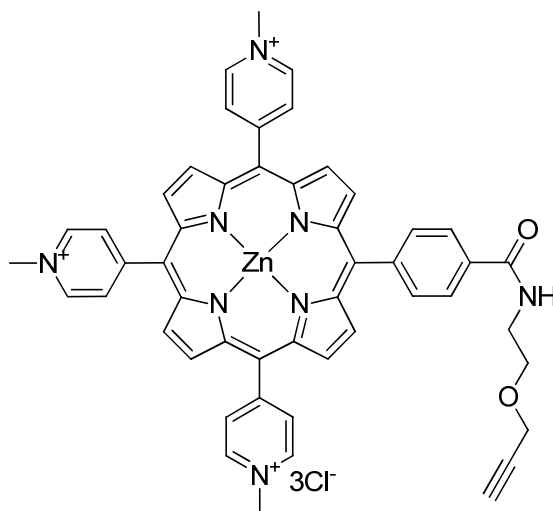


To a stirred solution 2-(prop-2-yn-1-yloxy)ethanaminocarbonyl]phenyl]-10,15,20-tris(4-pyridyl)porphyrin (50 mg, 0.067 mmol) in DMF (10 ml) was added methyl iodide (2 ml, 32 mmol) *via* syringe. The reaction mixture was stirred at 40 °C overnight. The mixture was cooled to room temperature and cold diethyl ether (100 ml) was added. The mixture was filtered through cotton wool, and the residue redissolved in methanol. Zinc acetate (50 mg, 0.27 mmol) was added and the mixture stirred at rt for 1 hour., The mixture was cooled to room temperature, and water (20 ml) added. Ammonium hexafluorophosphate was added and the resulting solution was filtered and the precipitate redissolved in acetone. Tetrabutylammonium chloride was added, and the resulting solution filtered to yield a green solid (47 mg, 73.2%).

*MS: (ESI) m/z 442 (100[M -2Cl-]2+).*

## Tert-butyl (2-hydroxyethyl)carbamate (45)
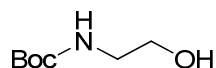


To a stirred solution of ethanolamine (1.04 ml, 17.2 mmol) in dichloromethane (40 ml) was added di-tert-butyl-dicarbonate (4.13 g, 18.9 mmol) in dichloromethane (10 ml), and the mixture was stirred at room temperature overnight. The mixture was washed with water and the organic layer dried (Na$_2$SO$_4$) and the solvent removed under reduced pressure. The crude was purified by column chromatography (1% methanol:DCM) to yield the product as a colourless oil (1.74 g, 63%).

^{1}H-NMR (CDCl$_3$): δ 1.39(s, 9H, C(CH$_3$)$_3$), 3.19 (m, 2H, CH$_2$-NH), 3.59 (quart, 2H, J=5.21 Hz, HO-CH$_2$), 3.84 (t, 1H, J=5.32 Hz, CH$_2$-NH), 5.34 (br s, 1H, OH). ^{13}C-NMR (CDCl$_3$): δ 25.6 (C(CH$_3$)$_3$), 40.52 (CH$_2$-NH), 59.41 (HO-CH$_2$), 77.00 (C(CH$_3$)$_3$), 154.32 (C=O)

**Tert-butyl (2-(but-3-yn-1-yloxy)ethyl)carbamate (46)**
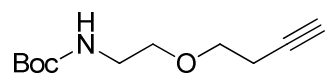


To a stirred solution of tert-butyl (2-hydroxyethyl)carbamate (1.48 g, 9.31 mmol) in dimethylformamide was added 4-bromo-1-butyne (1.35 g, 10.2 mol), tetrabutylammonium iodide (0.34 g, 0.93 mmol), and sodium iodide (0.19 g, 1.33 mmol). Potassium hydroxide was added in portions over 30 minutes, and the reaction stirred at rt under $N_2$ for 72 hours, and at 70 °C for a further 72 hours. The desired product was not isolated.

### Tert-butyl (3-hydroxypropyl)carbamate (47)

$$\text{Boc} \overset{\underset{\displaystyle H}{|}}{N} \diagup\diagdown\diagup\diagdown \text{OH}$$
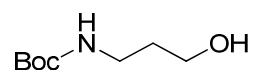
To a stirred solution of 3-aminopropan-1-ol (2.04 g, 0.027 mol) in dichloromethane (130 ml) at 0 °C was added a solution of di-tert-butyl-dicarbonate (6.58 g, 0.0298 mol) slowly. The mixture was stirred at rt overnight. The solvent was removed under reduced pressure to yield the product as a colourless oil (4.70 g, 99%).

$^{1}$H-NMR (CDCl$_3$): $\delta$ 1.44(s, 9H, C(C$\underline{H}_3$)$_3$), 1.67 (m, 2H, CH$_2$-C$\underline{H}_2$-CH$_2$), 3.27 (m, 2H, C$\underline{H}_2$-NH), 3.66 (m, 2H, HO-C$\underline{H}_2$), 5.31 (br s, 1H, O$\underline{H}$). $^{13}$C-NMR (CDCl$_3$): $\delta$ 28.25 (C($\underline{C}$H$_3$)$_3$), 32.61 (CH$_2$-$\underline{C}$H$_2$-CH$_2$), 36.94 ($\underline{C}$H$_2$-NH), 59.17 (HO-$\underline{C}$H$_2$), 79.39 ($\underline{C}$(CH$_3$)$_3$), 157.03 ($\underline{C}$=O)

**Tert-butyl (3-(prop-2-yn-1-yloxy)propyl)carbamate (48)**
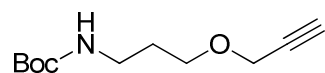


To a stirred solution of tert-butyl (3-hydroxypropyl)carbamate (4.70 g, 0.0268 mol) in THF (130 ml) was added propargyl bromide (5.5 ml, 80% in toluene, 7.34 g, 0.050 mol), tetrabutylammonium iodide (0.98 g, 2.68 mmol), and sodium iodide (0.98 g, 6.6 mmol). Potassium hydroxide (1.84 g) was added in portions over 30 minutes, and the reaction stirred at rt under $N_2$ for 24 hours. The solvent was removed under reduced pressure and the residue purified by column chromatography (silica, 2% MeOH:DCM) to yield the product as a yellow-orange oil (3.08 g, 54%).

*$^1$H-NMR (CDCl$_3$): δ 1.47(s, 9H, C(CH$_3$)$_3$), 1.79 (m, 2H, CH$_2$-CH$_2$-CH$_2$),2.44 (m, 1H, C≡CH), 3.24 (m, 2H, CH$_2$-NH), 3.60 (m, 2H, O-CH$_2$), 4.14 (d, 2H, J=2.50 Hz, O-CH$_2$-C). $^{13}$C-NMR (CDCl$_3$): δ 25.68 (C(CH$_3$)$_3$),26.97 (CH$_2$-CH$_2$-CH$_2$), 35.54 (CH$_2$-NH), 55.44 (O-CH$_2$-C), 65.39 (CH$_2$-O), 71.63 (C≡CH), 76.32 (C≡CH), 77.00 (C(CH$_3$)$_3$), 153.25 (C=O)*

### 3-(Prop-2-yn-1-yloxy)propan-1-amine hydrochloride (49)
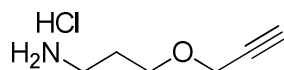


To a stirred solution of tert-butyl (3-(prop-2-yn-1-yloxy)propyl)carbamate (1.00 g, 4.69 mmol) in ethyl acetate (10 ml) was added HCl in dioxane (4 ml) and the mixture stirred at rt overnight. The solvent was removed under reduced pressure and the residual oil washed with diethyl ether to yield the product as a brown solid (0.50 g, 94.3%).

*Mp 69-71 °C.. $^1$H-NMR (CDCl$_3$): δ 2.10 (m, 2H, CH$_2$-CH$_2$-CH$_2$), 2.48 (m, 1H, C≡CH), 3.19 (m, 2H, CH$_2$-NH), 3.70 (m, 2H, O-CH$_2$), 4.22 (d, 2H, J=2.50 Hz, O-CH$_2$-C), 8.19 (br s, 2H, NH$_2$). $^{13}$C-NMR (CDCl$_3$): δ 24.60 (CH$_2$-CH$_2$-CH$_2$), 35.93 (CH$_2$-NH), 55.88 (O-CH$_2$-C), 64.63 (CH$_2$-O), 64.98 (C≡CH), 72.53 (C≡CH).*

**3-(Prop-2-yn-1-yloxy)propan-1-aminocarbonyl]phenyl]-10,15,20-tris(4-pyridyl)porphyrin (50)**
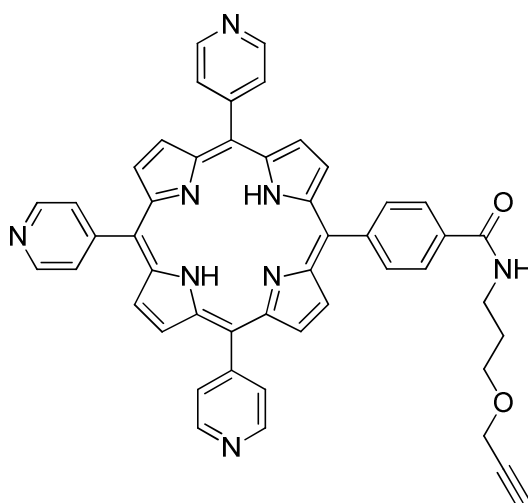


To a stirred solution of 5-[4-(Succinimide-*N*-oxycarbonyl)phenyl]-10,15,20-tri-(4-pyridyl) porphyrin (90 mg, 0.100 mmol) in dry DMSO (10 ml) was added  3-(prop-2-yn-1-yloxy)propan-1-amine (38 mg, 0.26 mmol), and anhydrous potassium carbonate (58 mg, 0.416 mmol). The mixture was stirred for three days at 40 ˚C, protected from light and atmospheric moisture. Water (10 ml) was added, and the mixture centrifuged. The crude was precipitated from MeOH over DCM to yield the product as a purple solid (45 mg, 59.5%)

*$R_f$ = 0.79 (silica, 10% methanol:dichloromethane). $^1$H-NMR (CDCl$_3$): δ -2.91 (s, 2H,-NH), 2.10 (m, 2H, CH$_2$-CH$_2$-CH$_2$), 2.53 (m, 1H, C≡CH), 3.77 (m, 2H, CH$_2$-NH), 3.86 (m, 2H, O-CH$_2$), 4.28 (m, 2H, O-CH$_2$-C), 8.18 (m, 8H, 10,15,20-o-Py, 5-m-Ph), 8.27 (m, 2H, 5-o-Ph), 8.88 (m, 8H, βH), 9.03 (m, 6H, 10,15,20-m-Py). MS: (NSI) m/z 757 (100[M + H]+), HRMS: calcd. for C$_{48}$H$_{37}$N$_8$O$_2$: 757.3034 found 757.3029.*

**Zinc- 3-(prop-2-yn-1-yloxy)propan-1-aminocarbonyl]phenyl]-10,15,20-tris(N-methyl-4-pyridinium)porphyrin trichloride (51)**
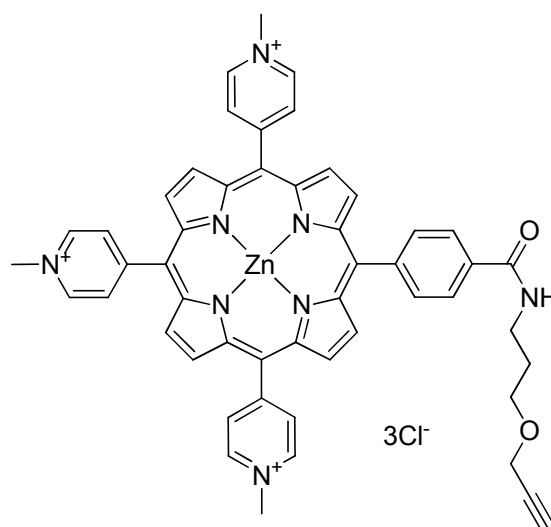


To a stirred solution of 3-(prop-2-yn-1-yloxy)propan-1-aminocarbonyl]phenyl]-10,15,20-tris(4-pyridyl)porphyrin (80 mg, 0.105 mmol) in DMF (10 ml) was added methyl iodide (2 ml, 32 mmol) *via* syringe. The reaction mixture was stirred at 40 °C overnight. The mixture was cooled to room temperature and cold diethyl ether (100 ml) was added. The mixture was filtered through cotton wool, and the residue redissolved in methanol. Zinc acetate (150 mg, 0.76 mmol) in water (10 ml) was added and the mixture was stirred at room temperature for 3 hours. Ammonium hexafluorophosphate was added, and resulting solution was filtered and the precipitate redissolved in acetone. Tetrabutylammonium chloride was added, and the resulting solution filtered to yield the product as a green solid (96.8 mg, 81.2%).

*$R_f$: 0.51 (silica, 1:1:8 sat. KNO$_3$ solution:water:MeCN). UV-vis (H$_2$O): λmax, nm 435, 563, 610. ε (435 nm)= 127552M$^{-1}$cm$^{-1}$. $^1$H-NMR (MeOH-d$_4$): δ 2.88 (s, 1H, C≡CH), 3.62 (m, 4H), 4.22 (m, 2H, CH$_2$-NH), 4.79 (m, 4H, O-CH$_2$), 4.80 (s, 9H, N-CH$_3$), 8.22 (m, 4H, 5-m-Ph, 5-o-Ph), 8.90 (m, 6H, 10,15,20-o-Py), 9.12 (m, 8H, βH), 9.33 (m, 6H, 10,15,20-m-Py). UV-vis (H$_2$O): λmax, nm 435, 563, 610. ε (435 nm)= 127552 M$^{-1}$cm$^{-1}$. MS: (ESI) m/z 255 (100[M - 3Cl]3+), HRMS: calcd. for C$_{44}$H$_{33}$N$_{10}$Zn: 255.0722 found 255.0721.*

**Methyl 14-(3-methoxy-3-oxopropyl)-2,2-dimethyl-4-oxo-3,8,11-trioxa-5,14-diazaheptadecan-17-oate (52)**
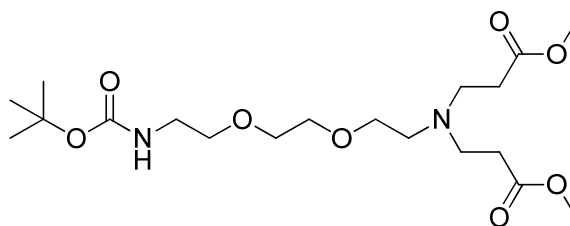


Tert-butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (1.12 g, 4.5 mmol) was dissolved in methanol (50 ml) and cooled to 0 °C under dry nitrogen. Methyl acrylate (1.45 g, 1.53 ml, 16.9 mmol) was added dropwise, and the reaction allowed to return to room temperature. The reaction was stirred for 96 hours at room temperature, and the solvent removed under vacuum.  The crude was purified on a plug (silica, 3% methanol:DCM) to yield the product as a pale yellow oil (1.60 g, 85%)

$^{1}$H -NMR (CDCl$_3$): δ 1.47 (s, 9H, C-(CH$_3$)$_3$), 2.48 (4H, t, J=7.04 Hz, CO-CH$_2$), 2.69 (t, 2H, J=6.10 Hz, O-CH$_2$-CH$_2$-N), 2.87 (t, 4H, J=7.19 Hz, CH$_2$-N), 3.31 (m, 2H,CH$_2$-NH-CO), 3.53 (m, 4H), 3.58 (m, 4H), 3.66 (s, 6H, O-CH$_3$). $^{13}$C-NMR (CDCl$_3$): δ 28.28 (C-(CH$_3$)$_3$), 32.46 (CH$_2$C=O),40.23 (CH-NH), 49.49 (O-CH$_3$), 51.40 (CH$_2$-CH$_2$-CO), 53.11 (CH$_2$-N), 53.35, 69.58, 70.12, 70.26, 77.00 (C-(CH$_3$)$_3$) 155.89 (NH-C=O), 172.86 (CH$_2$C=O). MS: (ESI) m/z 421 (100[M +H]$^{+}$), HRMS: calcd. for C$_{19}$H$_{37}$O$_8$N$_2$: 421.2544 found 421.2537.

## G$_0$ PAMAM Dendron (53)
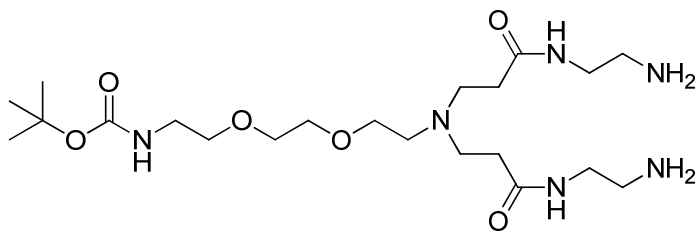


Methyl 14-(3-methoxy-3-oxopropyl)-2,2-dimethyl-4-oxo-3,8,11-trioxa-5,14-diazaheptadecan-17-oate (0.52 g, 1.25 mmol) was dissolved in methanol (40 ml) and cooled to -30 ˚C in dry ice. Ethylene diamine (9.00 g, 150 mmol) was cooled to -30 ˚C and slowly added to the mixture. The reaction was allowed to return to room temperature and stirred for five days. After this time, the solvent was removed under reduced pressure. The desired product was not isolated.

## G$_0$ PAMAM Dendron PEG alkyne (54)
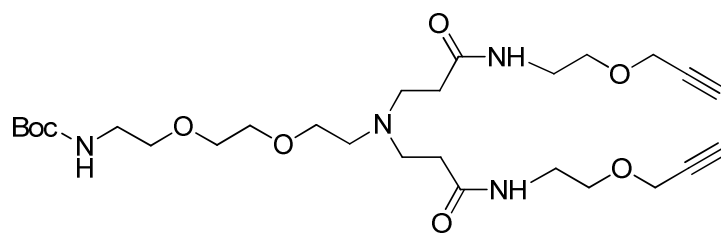


To a stirred solution of methyl 14-(3-methoxy-3-oxopropyl)-2,2-dimethyl-4-oxo-3,8,11-trioxa-5,14-diazaheptadecan-17-oate (0.2 g, 0.475 mmol) in methanol (10 ml) was added 3-(prop-2-yn-1-yloxy)propan-1-amine hydrochloride (0.18 g, 1.9 mmol) and the mixture stirred at rt for 5 days, followed by 24 hours at 50 ˚C. The desired product was not isolated.

**G$_0$ PAMAM Dendron PEG azide (55)**
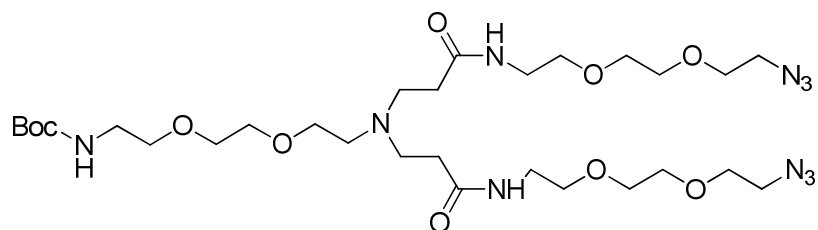


To a stirred solution of methyl 14-(3-methoxy-3-oxopropyl)-2,2-dimethyl-4-oxo-3,8,11-trioxa-5,14-diazaheptadecan-17-oate (150 mg, 0.35 mmol) in methanol (10 ml) was added 2-(2-(2-azidoethoxy)ethoxy)ethanamine (156 mg, 0.89 mmol) and the mixture stirred at rt for 5 days, followed by 3 days at 50 ˚C. The desired product was not isolated.

## G_0 PAMAM dendron alkyne (56)
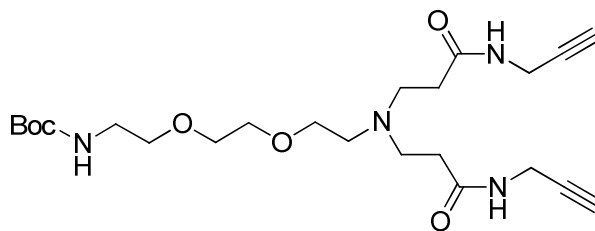


To a stirred solution of methyl 14-(3-methoxy-3-oxopropyl)-2,2-dimethyl-4-oxo-3,8,11-trioxa-5,14-diazaheptadecan-17-oate (420 mg, 1.0 mmol) in DMF (20 ml) was added propargyl amine  (1.14 g, 20.0 mmol) and the mixture stirred at 100 ˚C for 5 days. The desired product was not isolated.

## Propargyl acrylate (57)[209]
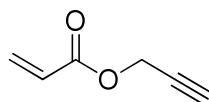


Propargyl alcohol (0.75 ml, 13 mmol) and triethylamine (2.5 ml) were dissolved in dichloromethane (70 ml) and cooled to 0 °C with stirring. Acryloyl chloride (1.2 ml, 14 mmol) was added dropwise, and the mixture allowed to return to room temperature. The reaction was quenched with saturated sodium hydrogencarbonate solution, and the organic layer was washed with 10% $HCl_{(aq)}$ , saturated sodium hydrogencarbonate solution and water. The organic layer was dried ($Na_2SO_4$) and filtered through neutral alumina. The volatiles were removed under reduced pressure to yield the product as a pale yellow oil (1.12 g, 78.3%).

$^1$H-NMR (CDCl$_3$): δ 2.50 (t, 1H, J=2.40 Hz, C≡C$\underline{H}$), 4.77 (d, 2H, J=2.56 Hz, C$\underline{H}_2$-C ), 5.91 (dd, 1H, J=4.20 Hz, 9.16 Hz, C$\underline{H}_2$=CH,), 6.17 (dd, 1H, J=10.48Hz, 15.04 Hz,CH$_2$=C$\underline{H}$), 6.46 (dd, 1H, J=1.48, 17.40, C$\underline{H}_2$=CH). $^{13}$C-NMR (CDCl$_3$): δ 54.08 ($\underline{C}$H$_2$-C), 77.00, 129.60, 133.97, 167.31 (C=O). MS: (GC-CIP) m/z 128 (100[M + H$_2$O]+).

## Alkyne PAMAM dendron (58)
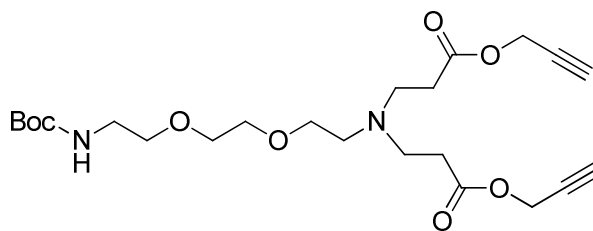


A stirred solution of tert-butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (0.55 g, 2.2 mmol) in methanol (50 ml) was cooled to 0 °C, and propargyl acrylate (0.60 g, 5.5 mmol) added over a period of 5 minutes. The solution was allowed to return to rt, and stirred for 48 hours. The solvent was removed under reduced pressure, and the crude product purified by column chromatography (1-5% MeOH:DCM) to yield a colourless oil. The desired product was not isolated.

$^1H$ -NMR (MeOH-$d_4$): δ 1.42 (s, 9H, C-(C$\underline{H}_3$)$_3$), 2.51 (m, 4H, CO-C$\underline{H}_2$), 2.67 (t, 2H, J=6.10 Hz, O-CH$_2$-C$\underline{H}_2$-N), 2.85 (t, 4H, J=7.19 Hz, C$\underline{H}_2$-N), 3.21 (m, 2H, C$\underline{H}_2$-NH-CO), 3.39 (m, 1H, N$\underline{H}$), 3.50 (m, 2H), 3.57, (m, 2H), 3.59 (m, 4H), 3.66 (s, 6H, O-C$\underline{H}_3$).

**Alkyne PAMAM dendron (58)**
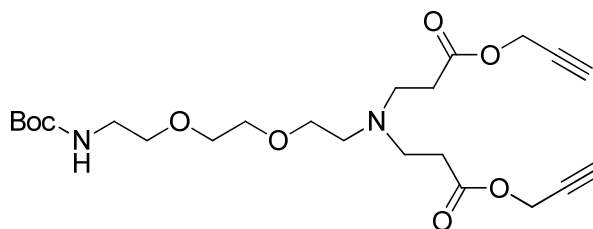


A stirred solution of tert-butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (0.55 g, 2.2 mmol) in THF (50 ml) was cooled to 0 °C, and propargyl acrylate (0.60 g, 5.5 mmol) added over a period of 5 minutes. The solution was allowed to return to rt, and stirred for 96 hours. The solvent was removed under reduced pressure, and the crude product purified by column chromatography (silica, 1-5% MeOH:DCM) to yield the product as a colourless oil (0.81 g, 78.6%).

*$^1$H -NMR (CDCl$_3$): δ 1.44 (s, 9H, C-(CH$_3$)$_3$), 2.47 (m, 2H, C≡CH), 2.51 (m, 4H, CH$_2$-CH$_2$-C=O), 2.68 (m, 2H, N-CH$_2$-CH$_2$-O), 2.85 (m, 4H, CH$_2$-CH$_2$-C=O), 3.34 (m, 2H, NH-CH$_2$),3.53 (m, 4H), 3.58 (m, 4H), 4.68 (m, 4H,CH$_2$-C). $^{13}$C-NMR (CDCl$_3$): δ 28.40 (C(CH$_3$)$_3$), 32.64, 40.33, 49.83, 51.87, 53.23, 70.23 (C≡CH), 74.85 (C≡CH), 76.69, 77.32 (C(CH$_3$)$_3$), 155.78 (C=O Boc), 171.63 (C=O dendrimer). MS: (ASP) m/z 469 (100[M + H]+), HRMS: calcd. for C$_{23}$H$_{37}$N$_2$O$_8$: 469.2544 found 469.2533.*

**Deprotected alkyne PAMAM dendron (58a)**
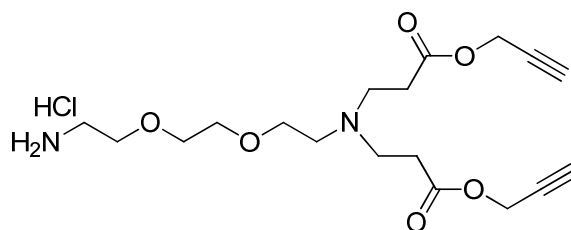


To a stirred solution of Dendron 2 (0.99 g, 2.11 mmol) in ethyl acetate (15 ml) was added HCl in dioxane (4 M, 5 ml) and the mixture stirred at rt overnight. The solvent was removed under reduced pressure and the residue washed with diethyl ether to yield the product as a viscous yellow oil (0.57 g, 66.9%).

$^1$H -NMR (MeOH-d$_3$): δ 3.05 (m, 6H, C≡C*H*, C*H$_2$*-CH$_2$-C=O), 3.17 (m, 2H, N-C*H$_2$*-CH$_2$-O), 3.53 (m, 4H, CH$_2$-C*H$_2$*-C=O), 3.82 (m, 6H), 3.92 (m, 2H, NH-C*H$_2$*), 4.79 (m, 4H,C*H$_2$*-C).

## 2-(2-(2-Azidoethoxy)ethoxy)ethanol (59)
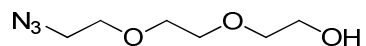


To a stirred solution of 2-(2-(2-chloroethoxy)ethoxy)ethanol (4.99 g, 30 mmol) in DMF (100 ml) was added sodium azide (3.03 g, 45 mmol), and the mixture heated to 100 °C under $N_2$ for 48 hours. The mixture was cooled to rt, and the white solid removed by filtration. The solvent was removed under reduced pressure to yield the product as a yellow oil (5.24 g, 99%).

$^1$H-NMR (CDCl$_3$): δ 2.63, (br s, 1H, O*H*), 3.34 (t, 2H, J=5.00 Hz, CH$_2$-N$_3$), 3.55 (m, 2H), 3.66 (m, 8H). $^{13}$C-NMR (CDCl$_3$): δ 55.09 (*C*H$_2$-N$_3$), 66.14 (*C*H$_2$-OH), 77.00, 81.28, 81.61, 81.92. MS: (NSI) m/z 193 (100[M + NH$_4$]+), HRMS: calcd. for C$_6$H$_{17}$N$_4$O$_3$: 193.1295 found 193.1296.

**2-(2-(2-Azidoethoxy)ethoxy)ethyl acrylate (60)**
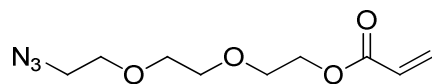


2-(2-(2-azidoethoxy)ethoxy)ethanol (2.28 g, 13 mmol) and triethylamine (2.5 ml) were dissolved in dichloromethane (70 ml) and cooled to 0 °C with stirring. Acryloyl chloride (1.2 ml, 14 mmol) was added dropwise, and the mixture stirred overnight at room temperature. The reaction was quenched with saturated sodium hydrogencarbonate solution, and the organic layer was washed with 10% $HCl_{(aq)}$ , saturated sodium hydrogencarbonate solution and water. The organic layer was dried ($Na_2SO_4$) and filtered through neutral alumina. The volatiles were removed under reduced pressure to yield the product as a pale yellow oil (1.97 g, 66.3%).

$^1$*H-NMR (CDCl$_3$): δ 3.40 (t, 2H, J=5.16 Hz, CH$_2$-N$_3$), 3.68 (m, 6H), 3.77 (t, 2H), 4.41 (t, 2H,CH$_2$-OC=O), 5.86 (dd, 1H, J=0.92, 9.4Hz), 6.19, (dd, 1H, J=10.64, 17.52Hz), 6.46 (dd, 1H, J=0.60, 16.6Hz). $^{13}$C-NMR (CDCl$_3$): δ 50.40 (CH$_2$-N$_3$), 63.38 (CH$_2$-O-C=O), 68.90, 69.82, 70.38, 76.67, 128.04 (O=C-CH=CH), 130.68 (O=C-CH=CH), 165.81 (C=O)*

**Azide PAMAM dendron (61)**
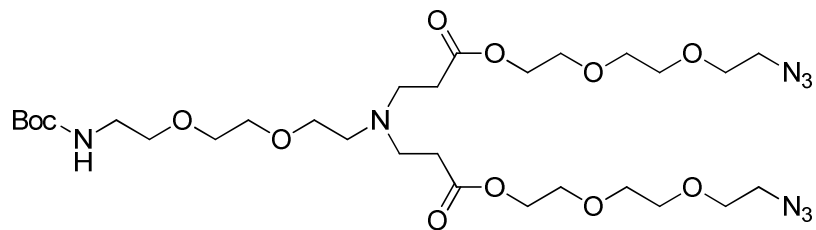


A stirred solution of tert-butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (0.55 g, 2.2 mmol) in THF (50 ml) was cooled to 0 °C, and 2-(2-(2-azidoethoxy)ethoxy)ethyl acrylate (1.26 g, 5.5 mmol) added over a period of 5 minutes. The solution was allowed to return to rt, and stirred for 10 days. The solvent was removed under reduced pressure, and the crude product purified by column chromatography (silica, 1-5% methanol:DCM) to yield a colourless oil. The desired product was not isolated.

**Propargyl acrylamide (62)[210]**
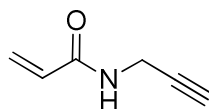


Propargylamine (1.28 ml, 20.0 mmol) was dissolved in dry DCM (40 ml) and DIPEA (4 ml, 22.48 mmol) was added. The mixture was cooled to 0 °C under $N_2$ and acryloyl chloride (1.82 ml, 22.48 mmol) was added dropwise over 10 minutes. The mixture was stirred at rt under $N_2$ for 24 hours. The reaction mixture was washed with saturated $NaHCO_3$ solution and brine, and the organic layer dried ($MgSO_4$). The solvent was removed under reduced pressure to yield the product as a yellow oil (1.45 g, 66.4%).

*$^1$H-NMR (CDCl$_3$): δ 2.11 (m, 1H, C≡CH), 3.91 (s, 2H, CH$_2$-NH), 5.48 (m, 1H, CH=CH$_2$), 6.32 (m, 2H, CH=CH$_2$).*

**Tert-butyl (12-oxo-9-(3-oxo-3-(prop-2-yn-1-ylamino)propyl)-3,6-dioxa-9,13-diazahexadec-15-yn-1-yl)carbamate (63)**
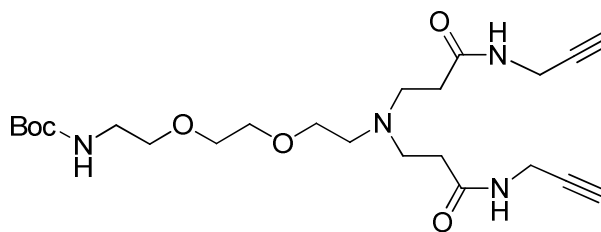


To a stirred solution of tert-Butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (0.50 g, 2.2 mmol) in THF (40 ml) was added propargyl acrylamide (1.0 g, 9.1 mmol) and the mixture stirred at rt for 72 hours under $N_2$. No sign of reaction was observed by TLC. The desired product was not isolated.

**Tert-butyl (12-oxo-9-(3-oxo-3-(prop-2-yn-1-ylamino)propyl)-3,6-dioxa-9,13-diazahexadec-15-yn-1-yl)carbamate (63)**
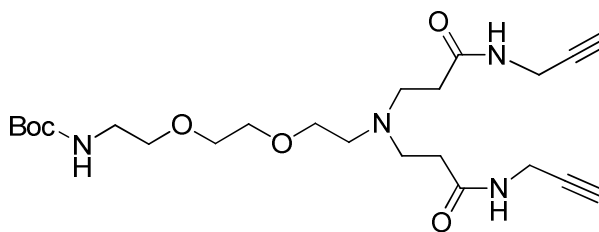


To a stirred solution of tert-Butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (0.50 g, 2.2 mmol) in 10% aq. methanol (40 ml) was added propargyl acrylamide (1.0 g, 9.1 mmol) and the mixture stirred at 50 °C for 96 hours under $N_2$. No sign of reaction was observed by TLC. The desired product was not isolated.

## 2-Bromo-N-(prop-2-yn-1-yl)acetamide (64)[212]
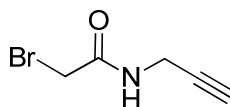


To stirred solution of bromoacetyl bromide (0.50 ml, 5.61 mmol) in dichloromethane (25ml) at 0 °C was added propargylamine (0.36 ml, 6.61 mmol) and triethylamine (0.78 ml, 5.61 mmol). The mixture was maintained at 0 °C for 1 hour, and then concentrated under reduced pressure. The residue was resuspended in ethyl acetate and filtered through a pad of silica gel. The solvent was removed under reduced pressure to yield the product as a yellow-orange oil (0.97 g, 98.9%).

*$^1$H-NMR (CDCl$_3$): δ 2.26 (t, 1H, J=2.48 Hz, C≡CH), 3.90 (s, 2H, CH$_2$-Br), 4.10 (dd, 2H, J=2.68, 5.28 Hz, CH$_2$-NH), 6.70 (br s, 1H, NH). $^{13}$C-NMR (CDCl$_3$): δ 28.64 (CH$_2$-Br), 29.93 (CH$_2$-NH), 72.23 (C≡CH), 77.31 (C≡CH), 165.16 (C=O)*

**Tert-butyl (11-oxo-9-(2-oxo-2-(prop-2-yn-1-ylamino)ethyl)-3,6-dioxa-9,12-diazapentadec-14-yn-1-yl)carbamate (65)**
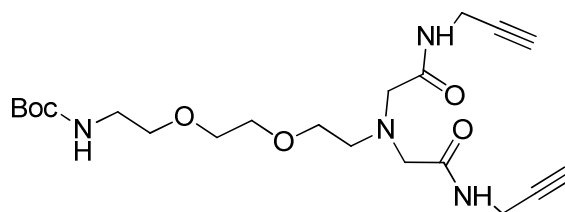


To a stirred solution of tert-Butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (124 mg, 0.5 mmol) in dry acetonitrile (6 ml) was added DBU (0.2 ml, 1.3 mmol) and 2-bromo-*N*-(prop-2-yn-1-yl)acetamide (270 mg, 1.5 mmol). The mixture was stirred for 17 hours at rt under $N_2$. The solvent was removed under reduced pressure and the crude purified by a silica plug (6% MeOH:DCM). The desired product was not isolated.

## G₀ Alcohol-functionalised PAMAM dendron (66)
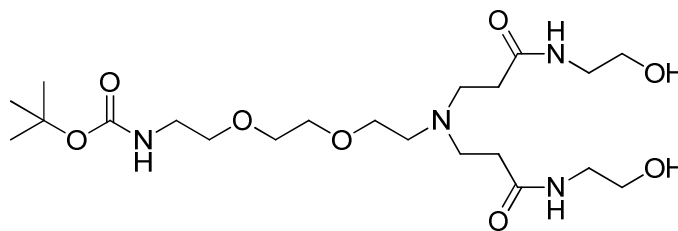


Methyl 14-(3-methoxy-3-oxopropyl)-2,2-dimethyl-4-oxo-3,8,11-trioxa-5,14-diazaheptadecan-17-oate (0.35 g, 0.83 mmol) was dissolved in methanol (40 ml) and cooled to 0 ˚C in ice. Ethanolamine (4.50 g, 73.7 mmol) was slowly added to the mixture, and the reaction was stirred for five days. After this time, the solvent was removed under reduced pressure, and the excess ethanolamine removed as an azeotrope with chlorobenzene to yield the product as a viscous yellow oil (366 mg, 92.4%)

*IR (DCM) cm$^{-1}$: 1654 (HN-C=O), 3443 (O-H). $^{1}$H -NMR (MeOH-d₄): δ 1.34 (s, 9H, C-(CH₃)₃), 2.30 (m, 4H, (C=O)-CH₂), 2.61 (m, 2H, O-CH₂-CH₂-N), 2.73 (t, 4H, CH₂-N), 3.13 (m, 2H, CH₂-NH-Boc), 3.21 (m, 4H), 3.40 (m, 2H), 3.51 (m, 10H). $^{13}$C-NMR (MeOH-d₄): δ 23.74 (C-(CH₃)₃), 29.42 (CH₂C=O),37.82 (CH-NH), 46.36 (O-CH₃), 48.93 (CH₂-CH₂-CO), 53.20 (CH₂-N),56.48, 65.12, 65.90, 66.16, 66.24, , 74.92 (C-(CH₃)₃), 153.25 (NH-C=O), 170.16 (CH₂C=O). MS: (ESI) m/z 479 (100[M + H]+), HRMS: calcd. for C₂₁H₄₃N₄O₈: 479.3075 found 479.3070.*

**Tert-butyl (12-oxo-9-(3-oxo-3-((2-(prop-2-yn-1-yloxy)ethyl)amino)propyl)-3,6,16-trioxa-9,13-diazanonadec-18-yn-1-yl)carbamate (67)**
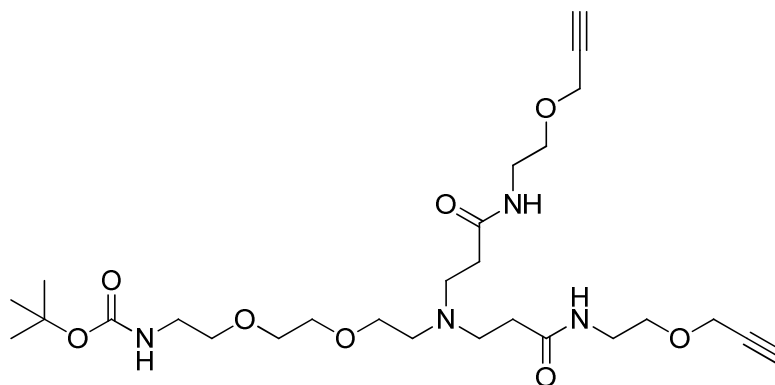


To a stirred solution of $G_0$ alcohol-functionalised PAMAM dendron (0.40 g, 0.9 mmol) in THF (30 ml) was added tetrabutylammonium iodide (0.08 g, cat.), sodium iodide (0.08g, cat.) and propargyl bromide (80% in toluene, 0.60 ml, 5.3 mmol). Potassium hydroxide (0.20 g, 3.6 mmol) was added and the mixture stirred for 17 hours at rt. The solvent was removed under reduced pressure and the crude purified by column chromatography (silica, 5-10% MeOH:DCM) to yield the product as a colourless oil (0.40 g, 80.2%).

$^{1}$H -NMR (MeOH-$d_4$): δ 1.38 (s, 9H, C-(C$\underline{H}_3$)$_3$), 2.33 (m, 4H, (C=O)-C$\underline{H}_2$), 2.44 (m, 2H, C≡C$\underline{H}$), 2.66 (m, 2H,O-CH$_2$-C$\underline{H}_2$-N), 2.77 (m, 4H, C$\underline{H}_2$-N), 3.25 (m, 2H, C$\underline{H}_2$-NH-Boc), 3.40 (m, 8H), 3.55 (m, 12H), 4.11 (m, 4H, C$\underline{H}_2$-C≡CH). $^{13}$C-NMR (MeOH-$d_4$): δ 28.50 (C-(C$\underline{H}_3$)$_3$), 33.82 (C$\underline{H}_2$C=O), 39.07 (C$\underline{H}$-NH), 40.34, 50.91, 53.32 (C$\underline{H}_2$-N), 58.28, 68.79, 69.15, 70.30, 74.98(C≡CH), 79.28 (C-(CH$_3$)$_3$),79.54 (C≡CH), 156.12 (NH-C=O), 172.71(CH$_2$C=O). MS: (ESI) m/z 555 (100[M +H]+), HRMS: calcd. for $C_{27}H_{47}N_4O_8$: 555.3388  found 555.3383.

**Tert-butyl (1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl)carbamate (68)[85]**
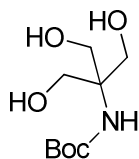


To a stirred solution of tris(hydroxymethyl)aminoethane (1.00 g, 8.20 mmol) in a mixture of water and methanol (1:1, 50 ml) was added di-tert-butyl-dicarbonate (2.35 g, 10.7 mmol). The mixture was stirred at room temperature for 24 hours, and the solvent removed under reduced pressure. The crude was recrystallised from ethyl acetate to yield the product as a white crystalline solid (1.75 g, 96%).

*Mp 146–148 °C. $^1$H-NMR (MeOH-d$_4$): δ 1.50 (s, 9H, (CH$_3$)$_3$), 3.67 (s, 6H, CH$_2$-OH). $^{13}$C-NMR (MeOH-d$_4$): δ 28.67 (C(CH$_3$)$_3$), 61.46 (C), 62.75 (C-CH$_2$), 158.05 (C=O). MS: (ESI) m/z 220 (100[M -H]-), HRMS: calcd. for C$_9$H$_{18}$N$_1$O$_5$: 220.1190 found 220.1186.*

**Tert-butyl (1,3-bis(prop-2-yn-1-yloxy)-2-((prop-2-yn-1-yloxy)methyl)propan-2-yl)carbamate (69)[85]**
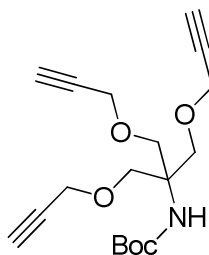


To a stirred solution of tert-butyl (1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl)carbamate (1.00 g, 4.52 mmol) in DMF (12ml) was added propargyl bromide (80% in toluene, 3ml, 27.2mmol). Potassium hydroxide (1.9 g, 27.2 mmol) was added portionwise over a period of 15 minutes. The mixture was heated to 35 ˚C and stirred under $N_2$ for 24 hours. The solvent was removed under reduced pressure, and the product purified by column chromatography (silica, DCM) to yield the product as a colourless oil (600 mg, 39.6%).

*$^1$H-NMR (CDCl$_3$): δ 1.41 (s, 9H, (CH$_3$)$_3$), 2.39 (m, 3H, C≡CH), 3.70 (s, 6H, CH$_2$-O), 4.08 (m, 6H,O-CH$_2$-C), 4.86 (br s, 1H, NH). $^{13}$C-NMR (CDCl$_3$): δ 28.30 (C(CH$_3$)$_3$), 58.00 (C), 58.61 (C-(CH$_2$)$_3$), 74.52 (O-CH$_2$), 76.68 (C≡CH), 79.57 (C≡CH), 154.71 (C=O). MS: (ESI) m/z 336 (100[M + H]+), HRMS: calcd. for C$_{18}$H$_{26}$N$_1$O$_5$: 336.1805 found 336.1810.*

**Methyl 3,5-bis(prop-2-yn-1-yloxy)benzoate (70)**[213]
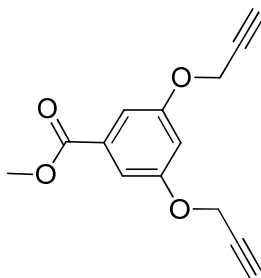


Methyl-3,5-dihydroxybenzoate (1.82 g, 10.8 mmol) was dissolved in acetone (100 ml) and propargyl bromide (80% in toluene, 4.2 ml, 38.0 mmol) was added. The mixture was stirred at rt for 10 minutes then potassium carbonate (10.0 g, 72.4 mmol) added, and the mixture heated to reflux for 17 hours. The reaction was cooled to rt and the solvent removed under reduced pressure. The residue was redissolved in dichloromethane and filtered, and the filtrate collected. The solvent was removed under reduced pressure to yield the product as a white solid (2.60 g, 98.7%).

*Mp 108–109 °C. $^1$H-NMR (CDCl$_3$): δ 2.56 (m, 2H, C≡CH̲), 3.91 (s, 3H, CH̲$_3$), 4.75 (m, 4H, CH̲$_2$-C), 6.80 (m, 1H, 4-Ar-H), 7.29 (m, 2H, 2,6-Ar-H). $^{13}$C-NMR (CDCl$_3$): δ 52.29 (C̲H$_3$), 56.02 (C̲H$_2$-C), 75.95 (C≡CH), 77.89 (C̲≡CH), 107.40 (4-Ar), 108.77 (2,6-Ar), 132.06 (1-Ar), 158.41 (3,5-Ar), 166.37 (C=O). MS: (ESI) m/z 245 (100[M +H]+), HRMS: calcd. for C$_{14}$H$_{13}$O$_4$: 245.0808 found 245.0812.*

## 3,5-Bis(prop-2-yn-1-yloxy)benzoic acid (71)[213]



To a stirred solution of methyl 3,5-bis(prop-2-yn-1-yloxy)benzoate (2.0 g, 8.2mmol) in dioxane:methanol (14:5, 114 ml) was added sodium hydroxide solution (4 M, 15 ml) in one portion, and the mixture stirred at rt for 5 hours. The mixture was neutralised with 1 M $HCl_{(aq)}$ and the solvent removed under reduced pressure. The product was redissolved in ethyl acetate, washed with saturated sodium bicarbonate solution and brine and the organic layer dried ($MgSO_4$). The solvent was removed under reduced pressure to yield the product as a white solid (1.54 g, 81.7%).

*Mp 177–179 °C. $^1$H-NMR (MeOH-d$_4$): δ 2.97 (m, 2H,C≡C<u>H</u>), 4.76 (m, 4H, C<u>H</u>$_2$-C), 6.84 (m, 1H, 4-Ar-H), 7.27 (m, 2H, 2,6-Ar-H). $^{13}$C-NMR (MeOH-d$_4$): δ 49.43 (<u>C</u>H$_2$-C), 77.18 (C≡CH), 79.35 (<u>C</u>≡CH), 108.09 (4-Ar), 110.06 (2,6-Ar), 134.00 (1-Ar), 160.11 (3,5-Ar), 169.19 (C=O). MS: (ESI) m/z 229 (100[M -H]-), HRMS: calcd. for C$_{13}$H$_9$O$_4$: 229.0506  found 229.0501.*

## Tert-butyl (2-(2-(2-(3,5-bis(prop-2-yn-1-yloxy)benzamido)ethoxy)ethoxy)ethyl)carbamate (72)



To a stirred solution of 3,5-bis(prop-2-yn-1-yloxy)benzoic acid (0.253 g, 1.1 mmol) in dichloromethane (100 ml) was added tert-Butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (0.272 g, 1.1 mmol) in dichloromethane (50 ml). DIPEA (0.35 ml, 2.5 mmol) was added, followed by addition of PyBOP (0.572 g, 1.1 mmol), and the mixture was stirred at rt overnight. The solvent was removed under reduced pressure and the crude purified by column chromatography (silica, 5% MeOH:DCM) to yield the product as a colourless oil (0.42 g, 83.0%)

$^{1}$H-NMR (CDCl$_3$): δ 1.42 (s, 9H, C(C$\underline{H}_3$)$_3$), 2.57 (m, 2H, C≡C$\underline{H}$), 3.30 (m, 2H, C$\underline{H}_2$-NHBoc), 3.56 (m, 2H, C$\underline{H}_2$-NH-C=O), 3.82 (m, 8H), 4.71 (m, 4H, C$\underline{H}_2$-C), 6.74 (m, 1H, 4-Ar-H), 7.04 (m, 2H, 2,6-Ar-H). $^{13}$C-NMR (CDCl$_3$): δ 28.27 (C(C$\underline{H}_3$)$_3$), 39.70 (C$\underline{H}_2$-NH), 40.14 (C$\underline{H}_2$-NH), 55.97 (C$\underline{H}_2$-C),69.52, 69.99, 70.10, 75.97 (C≡CH), 77.00 ($\underline{C}$(CH$_3$)$_3$), 77.32 ($\underline{C}$≡CH), 105.04 (4-Ar), 106.66 (2,6-Ar), 136.78 (1-Ar), 155.71 (C=O Boc), 158.53 (3,5-Ar), 166.65 (C=O dendron). MS: (ESI) m/z 461 (100[M+H]+), HRMS: calcd. for C$_{24}$H$_{33}$N$_2$O$_7$: 461.2282  found 461.2279.
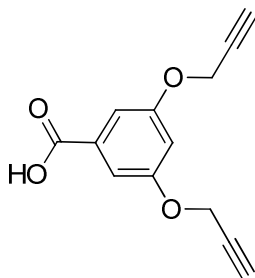
**Methyl 3,4,5-tris(prop-2-yn-1-yloxy)benzoate (73)**
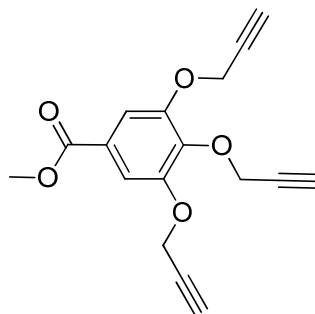


Methyl-3,4,5-trihydroxybenzoate (2.0 g, 10.8 mmol) was dissolved in acetone (100 ml) and propargyl bromide (80% in toluene, 4.2 ml, 38.0 mmol) was added. The mixture was stirred at rt for 10 minutes then potassium carbonate (10.0 g, 72.4 mmol) added, and the mixture heated to reflux for 17 hours. The reaction was cooled to rt and the solvent removed under reduced pressure. The residue was redissolved in dichloromethane and filtered, and the filtrate collected. The solvent was removed under reduced pressure to yield the product as a pale yellow solid (3.09 g, 95.5%).

*Mp 90–91 °C. $^1$H-NMR (CDCl$_3$): δ 2.45 (m, 1H, C≡CH), 2.53 (m, 2H, C≡CH), 3.89 (s, 3H, CH$_3$), 4.78 (m, 6H, CH$_2$-C), 7.44 (m, 2H, Ar-H). $^{13}$C-NMR (CDCl$_3$): δ 52.57 (CH$_3$), 57.12 (CH$_2$-C), 60.42 (CH$_2$-C), 75.70 (C≡CH),76.33 (C≡CH), 78.01 (C≡CH), 78.75 (C≡CH), 109.79 (2,6-Ar), 125.76 (4-Ar), 141.14 (1-Ar), 151.33 (3,5-Ar), 166.31 (C=O). MS: (ESI) m/z 299 (100[M +H]+).*
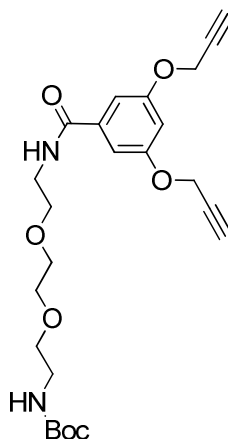
**3,4,5-Tris(prop-2-yn-1-yloxy)benzoic acid (74)**
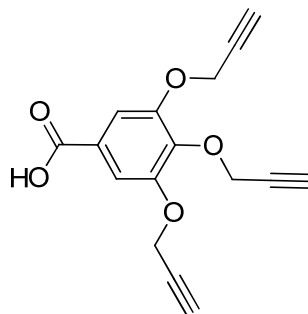


To a stirred solution of methyl 3,4,5-tris(prop-2-yn-1-yloxy)benzoate (1.0 g, 3.35 mmol) in dioxane:methanol (14:5, 114 ml) was added sodium hydroxide solution (4 M, 15 ml) in one portion, and the mixture stirred at rt for 5 hours. The mixture was neutralised with 1 M HCl$_{(aq)}$ and the solvent removed under reduced pressure. The product was redissolved in ethyl acetate, washed with saturated sodium bicarbonate solution and brine and the organic layer dried (MgSO$_4$). The solvent was removed under reduced pressure to yield the product as a white solid (0.66 g, 69.4%).

*Mp 150–151 °C. $^1$H-NMR (MeOH-d$_4$): δ 2.86 (m, 1H, C≡C$\underline{H}$), 2.99 (m, 2H, C≡C$\underline{H}$), 4.77 (m, 2H, C$\underline{H}_2$-C), 4.83 (m, 4H, C$\underline{H}_2$-C), 7.49 (s, 2H, Ar-H). $^{13}$C-NMR (MeOH-d$_4$): δ 56.57 ($\underline{C}H_2$-C), 59.67 ($\underline{C}H_2$-C), 75.62 (C≡CH), 76.16 (C≡CH), 77.99 ($\underline{C}$≡CH), 78.39 ($\underline{C}$≡CH), 109.75 (2,6-Ar), 140.71 (1-Ar), 151.37 (3,4,5-Ar), 168.13 (C=O). MS: (ESI) m/z 283 (100[M -H]-), HRMS: calcd. for C$_{16}$H$_{11}$O$_5$: 283.0612 found 283.0608.*

**Tert-butyl (2-(2-(2-(3,4,5-tris(prop-2-yn-1-yloxy)benzamido)ethoxy)ethoxy)ethyl)carbamate (75)**
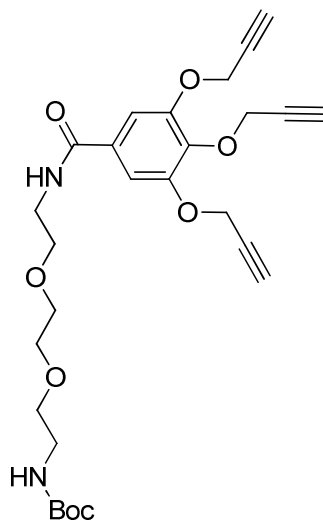


To a stirred solution of 3,4,5-tris(prop-2-yn-1-yloxy)benzoic acid (0.312 g, 1.1 mmol) in dichloromethane (100 ml) was added tert-Butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (0.272 g, 1.1 mmol) in dichloromethane (50 ml). DIPEA (0.35 ml, 2.5 mmol) was added, followed by addition of PyBOP (0.572 g, 1.1 mmol), and the mixture was stirred at rt overnight. The solvent was removed under reduced pressure and the crude purified by column chromatography (silica, 5% MeOH:DCM) to yield the product as a colourless oil (0.487 g, 86.1%).

$^1$H-NMR (CDCl$_3$): $\delta$ 1.25 (s, 9H, C(C$\underline{H}_3$)$_3$), 2.38 (m, 1H, C≡C$\underline{H}$), 2.48 (m, 2H, C≡C$\underline{H}$), 3.11 (m, 2H, C$\underline{H}_2$-NHBoc), 3.45 (m, 2H, C$\underline{H}_2$-NH-C=O), 3.47 (m, 8H), 4.62 (m, 6H, C$\underline{H}_2$-C), 7.01 (m, 2H, 2,6-Ar-H). $^{13}$C-NMR (CDCl$_3$): $\delta$ 27.99 (C(C$\underline{H}_3$)$_3$), 39.54 (C$\underline{H}_2$-NH), 39.83 (C$\underline{H}_2$-NH), 56.73 (C$\underline{H}_2$-C), 59.83 (C$\underline{H}_2$-C), 69.31, 69.70, 69.77, 75.46 (C≡CH), 76.10 (C(CH$_3$)$_3$), 77.79 (C≡CH), 107.40 (2,6-Ar), 129.98 (1-Ar), 139.25 (4-Ar), 150.95 (3,5-Ar), 155.65 (C=O Boc), 166.34 (C=O dendron). MS: (ESI) m/z 461 (100[M+H]+), HRMS: calcd. for C$_{24}$H$_{33}$N$_2$O$_7$: 461.2282 found 461.2279.

**(3,5-Bis(prop-2-yn-1-yloxy)phenyl)methanol (76)**[213]
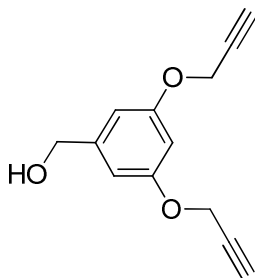


A stirred suspension of lithium aluminium hydride (2.99 g, 90.0 mmol) in dry THF (60 ml) was cooled to 0 °C under $N_2$ and a solution of methyl 3,5-bis(prop-2-yn-1-yloxy)benzoate (2.20 g, 9.0 mmol) in THF (20 ml) was added dropwise. The mixture was stirred for 1 hour at 0 °C and a further 5 hours at rt. Water (1 ml) and 4 M sodium hydroxide solution (2 ml) was added and the solid residue removed by filtration. The filter cake was washed thoroughly with DCM and the washings combined and dried ($MgSO_4$). The solvent was removed under reduced pressure to yield the product as a white waxy solid (1.85 g, 95.2%).

*Mp 67–70 °C. $^1$H-NMR (CDCl$_3$): δ 2.54 (m, 2H, C≡CH), 2.67 (br s, 1H, -OH), 4.59 (s, 2H, CH$_2$-OH), 4.65 (m, 4H, CH$_2$-C), 6.51 (m, 1H, 4-Ar-H), 6.60 (m, 2H, 2,6-Ar-H). $^{13}$C-NMR (CDCl$_3$): δ 55.77 (CH$_2$-C), 64.85 (CH$_2$-OH), 75.63 (C≡CH), 78.31 (C≡CH), 101.25 (4-Ar), 106.03 (2,6-Ar), 143.62 (1-Ar), 158.65 (3,5-Ar). MS: (ESI) m/z 217 (100[M +H]+), HRMS: calcd. for C$_{13}$H$_{13}$O$_3$: 217.0859  found 217.0861.*

## 1-(Bromomethyl)-3,5-bis(prop-2-yn-1-yloxy)benzene (77)[213]
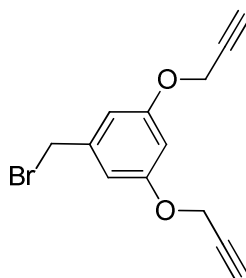


To a stirred solution of (3,5-bis(prop-2-yn-1-yloxy)phenyl)methanol (2.06 g, 9.5 mmol) in dry dichloromethane (150 ml) was added dropwise phosphorus tribromide (0.89 ml, 9.5 mmol) and the mixture stirred for 17 hours at rt. The product was partitioned between dichloromethane and water, the organic layer was dried (MgSO$_4$) and the solvent removed under reduced pressure to yield the product as an off-white waxy solid (2.37 g, 89.8%).

*Mp 60–61 °C. $^1$H-NMR (CDCl$_3$): δ 2.48 (m, 2H, C≡CH), 4.34 (s, 2H, CH$_2$-Br), 4.60 (m, 4H, CH$_2$-C), 6.47 (m, 1H, 4-Ar-H), 6.57 (m, 2H, 2,6-Ar-H). $^{13}$C-NMR (CDCl$_3$): δ 33.37 (CH$_2$-Br), 55.97 (CH$_2$-C), 76.10 (C≡CH), 78.31 (C≡CH), 102.47 (4-Ar), 108.83 (2,6-Ar), 140.00 (1-Ar), 158.83 (3,5-Ar). MS: (ESI) m/z 279 (100[M +H]+), HRMS: calcd. for C$_{13}$H$_{12}$O$_2$Br: 279.0015  found 279.0018.*

**Methyl 3,5-bis((3,5-bis(prop-2-yn-1-yloxy)benzyl)oxy)benzoate (78)**[213]
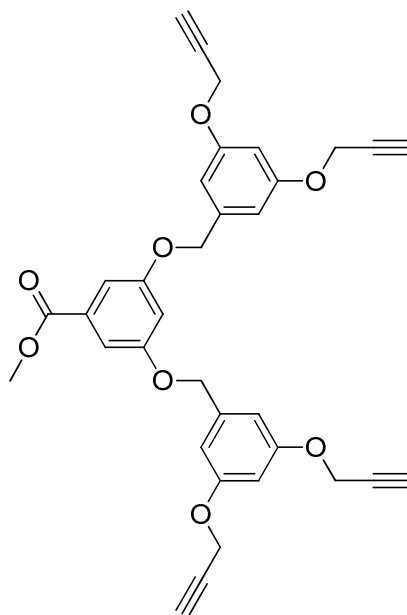


To a stirred solution of 1-(bromomethyl)-3,5-bis(prop-2-yn-1-yloxy)benzene (0.75 g, 2.8 mmol) and methyl-3,5-dihydroxybenzoate (0.21 g, 1.40 mmol) in acetone (80 ml) was added potassium carbonate (0.19 g, 1.38 mmol) and 18-crown-6 (2.5 mg, 0.001 mmol) and the mixture heated to reflux for 17 hours. The mixture was cooled to rt and the solvent removed under reduced pressure. The residue was redissolved in dichloromethane, magnesium sulfate added and the mixture filtered. The filtrate was concentrated under reduced pressure to yield the crude, which was purified by column chromatography (silica, DCM) to yield the product as a pale yellow solid (0.62 g, 78.5%).

*Mp 114–116 °C. $^1$H-NMR (CDCl$_3$): δ 2.55 (m, 4H, C≡C$\underline{H}$), 3.86 (s, 3H, C$\underline{H}_3$), 4.63 (m, 8H, C$\underline{H}_2$-C≡CH), 4.95 (m, 4H, O-C$\underline{H}_2$), 6.53 (m, 2H, Ar-H), 6.64 (m, 4H, Ar-H), 6.73 (m, 1H, Ar-H), 7.22 (m, 2H, Ar-H). $^{13}$C-NMR (CDCl$_3$): δ 52.27 ($\underline{C}$H$_2$-C), 53.66 ($\underline{C}$H$_2$-C), 56.06 ($\underline{C}$H$_3$), 76.23 (C≡CH), 78.43 ($\underline{C}$≡CH), 101.60, 106.99, 108.59, 139.34, 158.92, 159.72, 166.91 (C=O). MS: (ESI) m/z 582 (100[M +NH$_4$]+), HRMS: calcd. for C$_{34}$H$_{32}$O$_8$N: 582.2122  found 582.2119.*

**3,5-Bis((3,5-bis(prop-2-yn-1-yloxy)benzyl)oxy)benzoic acid(78a)**[213]
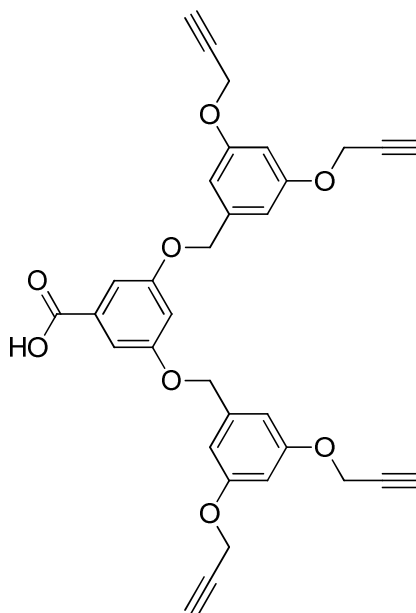


To a stirred solution of methyl 3,5-bis((3,5-bis(prop-2-yn-1-yloxy)benzyl)oxy)benzoate (0.5 g, 0.88 mmol) in dioxane:methanol (14:5, 57 ml) was added sodium hydroxide solution (4 M, 10 ml) in one portion, and the mixture stirred at rt for 5 hours. The mixture was neutralised with 1 M $HCl_{(aq)}$ and the solvent removed under reduced pressure. The product was redissolved in ethyl acetate, washed with saturated sodium bicarbonate solution and brine and the organic layer dried ($MgSO_4$). The solvent was removed under reduced pressure to yield the product as a white solid (0.43 g, 88.8%).

*Mp 177–179 °C. $^1$H-NMR (CDCl$_3$): δ 2.51 (m, 4H, C≡CH), 4.64 (m, 8H, CH$_2$-C≡CH), 4.99 (m, 4H, O-CH$_2$), 6.53 (m, 2H, Ar-H), 6.64 (m, 4H, Ar-H), 6.73 (m, 1H, Ar-H), 7.26 (m, 2H, Ar-H). $^{13}$C-NMR (CDCl$_3$): δ 55.98, (CH$_2$-C), 69.95 (CH$_2$-C), 75.86 (C≡CH), 78.23 (C≡CH), 102.02, 106.91, 107.47, 108.80, 132.42, 139.11, 158.96, 159.57 (C=O). MS: (ESI) m/z 549 (100[M -H]-), HRMS: calcd. for C$_{33}$H$_{25}$O$_8$: 549.1555 found 549.1542.*

**Tert-butyl (2-(2-(2-(3,5-bis((3,5-bis(prop-2-yn-1-yloxy)benzyl)oxy)benzamido)ethoxy)ethoxy)ethyl)carbamate (79)**
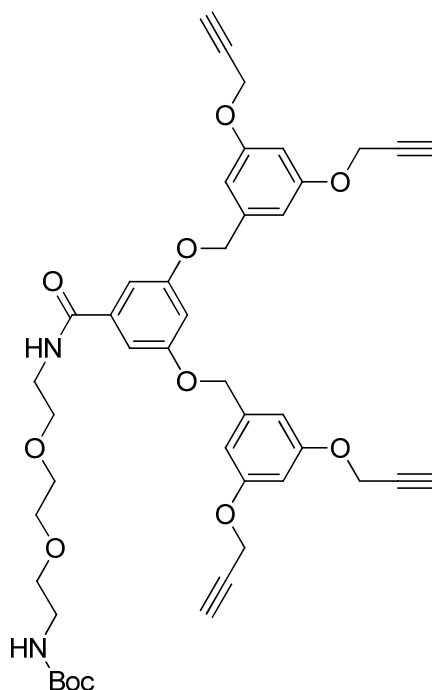


To a stirred solution of 3,5-bis((3,5-bis(prop-2-yn-1-yloxy)benzyl)oxy)benzoic acid (0.440 g, 0.80 mmol) in dichloromethane (100 ml) was added tert-Butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (0.198 g, 0.80 mmol) in dichloromethane (20 ml). DIPEA (0.25 ml, 1.81 mmol) was added, followed by addition of PyBOP (0.416 g, 0.80 mmol), and the mixture was stirred at rt overnight. The solvent was removed under reduced pressure and the crude purified by column chromatography (silica, 4% MeOH:DCM) to yield the product as a colourless oil (0.61 g, 97.8%).

$^1$H-NMR (THF-$d_8$): δ 1.36 (s, 9H, C(C$\underline{H}_3$)$_3$, 2.53 (m, 4H, C≡C$\underline{H}$), 3.22 (m, 2H), 3.31 (s, 1H, O=C-N$\underline{H}$), 3.48 (m, 2H), 3.59 (m, 8H), 4.62 (m, 8H, C$\underline{H}_2$-C≡CH), 4.94 (m, 4H, O-C$\underline{H}_2$),  6.50 (m, 2H, Ar-H), 6.61 (m, 4H, Ar-H), 6.82 (m, 1H, Ar-H), 6.98 (m, 2H, Ar-H). $^{13}$C-NMR (THF-$d_8$): δ 26.91 (C(C$\underline{H}_3$)$_3$), 38.95 (C$\underline{H}_2$-NH), 39.46 (C$\underline{H}_2$-NH), 54.66, (C$\underline{H}_2$-C),68.99, 69.30, 69.34, 69.39, 75.02 (C≡C$\underline{H}$), 77.78 ($\underline{C}$≡CH), 100.42, 103.40, 105.63, 136.39, 138.70, 154.81 (C-O), 158.98 ($\underline{C}$-O), 159.16 ($\underline{C}$=O Boc), 165.15 ($\underline{C}$=O dendron). MS: (ESI) m/z 781 (100[M+H]+), HRMS: calcd. for C$_{44}$H$_{49}$N$_2$O$_{11}$: 781.3331 found 781.3334.

**Conjugate (80)**
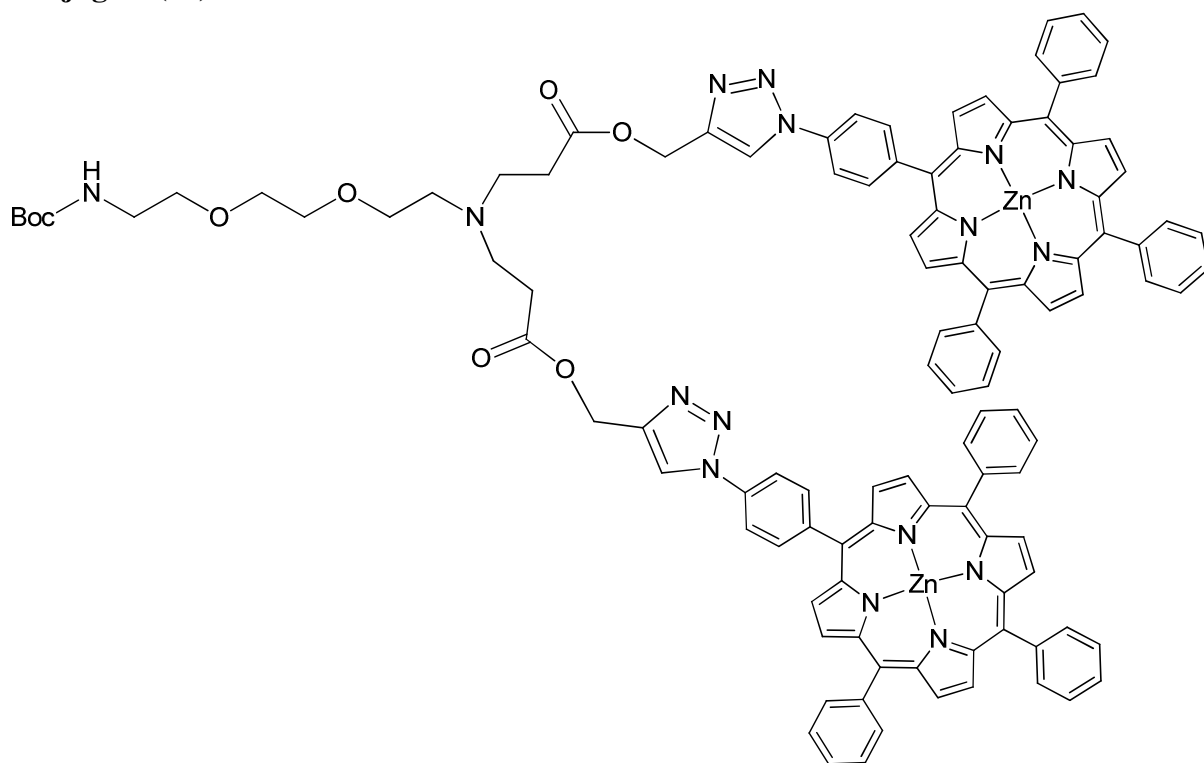


To a stirred solution of zinc 5-[4-azidophenyl]-10,15,20-triphenylporphyrin (50 mg, 0.076 mmol) and alkyne PAMAM dendron (17 mg, 0.038 mmol) in toluene (10 ml) was added copper (II) sulfate pentahydrate (5 mg) and sodium ascorbate (10 mg) in water (5 ml). The resulting mixture was stirred at 80 °C for 48 hours and 100 °C for 48 hours. The solvent was removed under reduced pressure to yield the crude, which was purified by column chromatography (silica, 1-3% MeOH:DCM) to yield the product as a purple solid (22 mg, 32.8%).

*$R_f$: 0.50 (silica, 3% MeOH:DCM). UV-vis (DCM): λmax, nm 420, 548, 588. ε (420 nm)= 786947 $M^{-1}cm^{-1}$. $^1$H-NMR (CDCl$_3$):  δ 1.37 (s, 9H, C-(C$\underline{H}_3$)$_3$), 2.38 (m, 4H, C$\underline{H}_2$-CH$_2$-C=O), 2.54 (m, 2H, N-C$\underline{H}_2$-CH$_2$-O), 2.72  (m, 4H, CH$_2$-C$\underline{H}_2$-C=O), 3.04 (m, 2H, NH-CH$_2$), 3.12 (m, 6H), 3.36 (m, 4H), 3.42 (m, 4H,C$\underline{H}_2$-C), 7.18 (s, 2H, triazole $\underline{H}$), 7.59 (m, 18H, 10,15,20-m,p-Ph), 7.88 (m, 4H, 5-m-Ph), 8.07 (m, 12H, 10,15,20-o-Ph), 8.10 (m, 4H, 5-o-Ph), 8.81 (m, 16H, βH). MS: (MALDI) m/z 1907 (100[M +H]+).*

**Conjugate (80)**



To a stirred solution of zinc 5-[4-azidophenyl]-10,15,20-triphenylporphyrin (50 mg, 0.076 mmol) and alkyne PAMAM dendron (17 mg, 0.038 mmol) in toluene (5 ml) was added copper (II) sulfate pentahydrate (5 mg) and sodium ascorbate (10 mg) in water (2 ml). The resulting mixture was heated to 80 ˚C for 1 hour by MW (75 W, max pressure 50 bar, max stirring). The solvent was removed under reduced pressure to yield the crude, which was purified by column chromatography (silica, 1-3% MeOH:DCM) to yield the product as a purple solid (31 mg, 46.2%).

*$R_f$: 0.50 (silica, 3% MeOH:DCM). UV-vis (DCM): λmax, nm 420, 548, 588. ε (420 nm)= 786947 $M^{-1}cm^{-1}$. $^1$H-NMR (CDCl$_3$): δ 1.37 (s, 9H, C-(C$\underline{H}_3$)$_3$), 2.38 (m, 4H, C$\underline{H}_2$-CH$_2$-C=O), 2.54 (m, 2H, N-C$\underline{H}_2$-CH$_2$-O), 2.72 (m, 4H, CH$_2$-C$\underline{H}_2$-C=O), 3.04 (m, 2H, NH-CH$_2$), 3.12 (m, 6H), 3.36 (m, 4H), 3.42 (m, 4H,C$\underline{H}_2$-C), 7.18 (s, 2H, triazole $\underline{H}$), 7.59 (m, 18H, 10,15,20-m,p-Ph), 7.88 (m, 4H, 5-m-Ph), 8.07 (m, 12H, 10,15,20-o-Ph),8.10 (m, 4H, 5-o-Ph), 8.81 (m, 16H, βH). MS: (MALDI) m/z 1907 (100[M +H]+).*

**Conjugate (81)**



Conjugate 2 (20 mg, 0.0105 mmol) was dissolved in TFA (1 ml) and stirred at RT for 4 hours. The mixture was neutralised with sodium hydrogencarbonate and extracted with DCM. The organic layer was dried (MgSO$_4$) and the solvent removed under reduced pressure to yield the product as a purple solid (16 mg, 90.9%)

*$R_f$: 0.16 (silica, 5% MeOH:DCM). UV-vis (DCM): λmax, nm 419, 448, 513, 549, 592. ε (419 nm)= 819693M$^{-1}$cm$^{-1}$,$^1$H-NMR (CDCl$_3$): δ -2.87 (s, 4H, inner NHs), 2.52 (m, 4H, C$\underline{H}_2$-CH$_2$-C=O), 2.54 (m, 2H, N-C$\underline{H}_2$-CH$_2$-O), 2.72 (m, 4H, CH$_2$-C$\underline{H}_2$-C=O), 3.00 (m, 6H), 3.02 (m, 2H), 3.56 (m, 6H), 3.59 (m, 2H), 7.60 (m, 18H, 10,15,20-m,p-Ph), 8.07 (m, 20H, 5-m-Ph, 5,10,15,20-o-Ph), 8.10, (s, 2H, triazole $\underline{H}$), 9.05 (m, 16H, βH).*
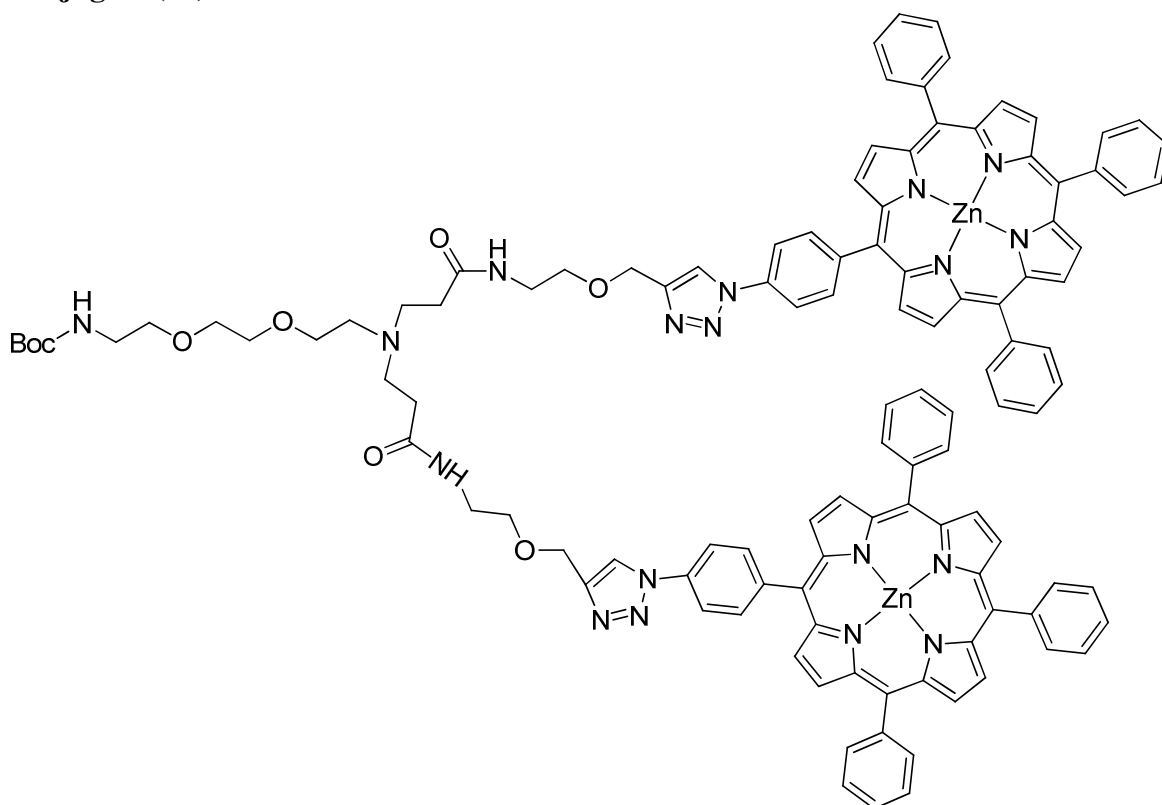
# Conjugate (82)



To a 10ml microwave tube was added zinc 5-[4-azidophenyl]-10,15,20-triphenylporphyrin (50 mg, 0.070 mmol) and tert-butyl (12-oxo-9-(3-oxo-3-((2-(prop-2-yn-1-yloxy)ethyl)amino)propyl)-3,6,16-trioxa-9,13-diazanonadec-18-yn-1-yl)carbamate (19 mg, 0.035 mmol) in THF:water (7:1, 8 ml). Copper (II) sulfate pentahydrate (2 mg) and sodium ascorbate (2 mg) in THF:water (1:1, 1 ml) was added, and the mixture heated to 80°C by MW (75W, max pressure 100 bar, max stirring) for 2 hours. The solvent was removed under reduced pressure and the product purified by column chromatography (silica, 3-20% MeOH:DCM) to yield the product as a purple solid (45 mg, 64.7%).

*UV-vis (DCM): λmax, nm 420, 549, 590. ε (420 nm)= 929385 $M^{-1}cm^{-1}$. $^1$H-NMR (CDCl$_3$): δ 1.37 (s, 9H, C-(C$\underline{H}_3$)$_3$), 3.16 (m, 4H,(C=O)-C$\underline{H}_2$), 3.31 (m, 2H, O-CH$_2$-C$\underline{H}_2$-N), 3.41 (t, 4H, C$\underline{H}_2$-N), 3.47 (m, 2H, C$\underline{H}_2$-NH-Boc), 3.59 (m, 10H), 3.73 (m, 2H), 4.50 (m, 4H), 7.68-7.72 (m, 18H, 10,15,20-m,p-Ph), 7.99 (m, 4H, 5-m-Ph), 8.19 (m, 12H, 10,15,20-o-Ph), 8.30 (m, 6H, 5-o-Ph, triazole H), 8.82-8.89 (m, 16H, βH). $^{13}$C-NMR (CDCl$_3$): δ 28.15 (C-(C$\underline{H}_3$)$_3$), 29.58 (C$\underline{H}_2$C=O),39.13 (C$\underline{H}$-NH), 48.80 (O-C$\underline{H}_3$), 49.23 (C$\underline{H}_2$-CH$_2$-CO), 50.09 (C$\underline{H}_2$-N),52.97, 53.36, 58.60, 63.66, 68.81, 69.90, 70.15 (C-(CH$_3$)$_3$), 118.19, 118.25, 120.80, 121.03, 121.32, 126.29 (β-C), 127.19, 130.99, 131.71, 131.94, 134.41 (β-C), 135.41, 135.83, 143.06, 144.27, 149.44, 149.94, 150.06, 150.12 (NH-C$\underline{}$=O), 170.16 (CH$_2$C$\underline{}$=O). MS: (MALDI-TOF) m/z 1993.6 (100[M + H]+).*
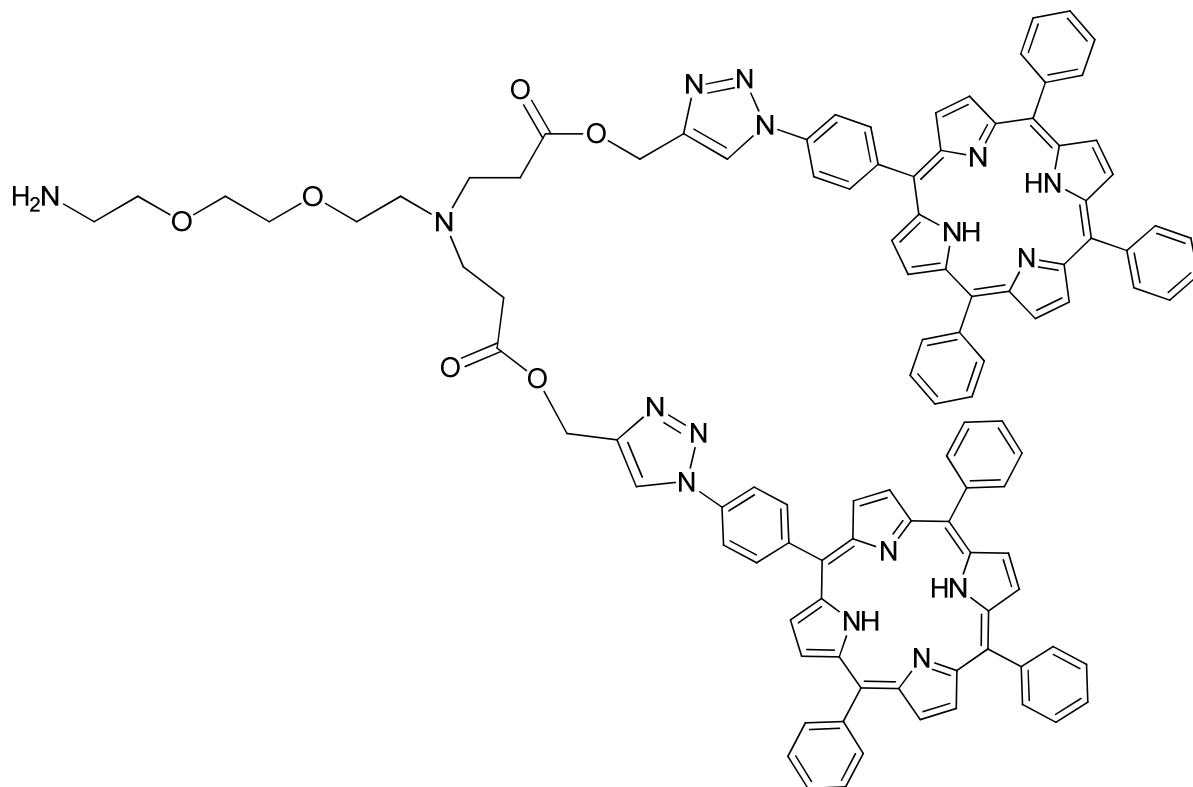
**Conjugate (83)**
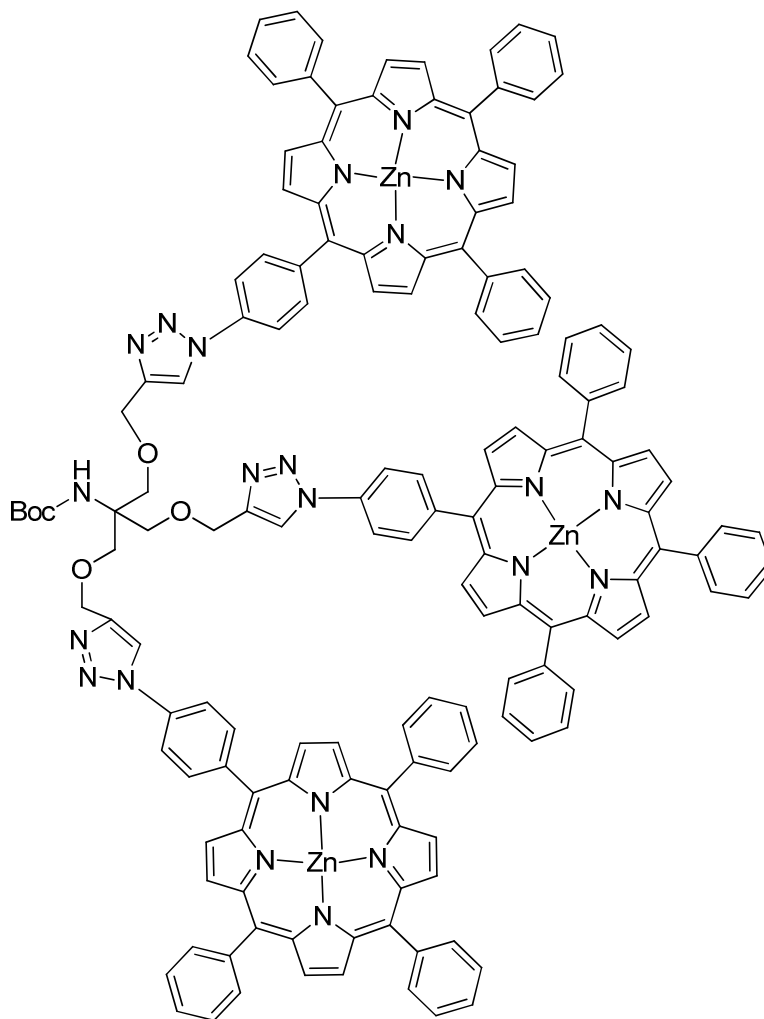


To a stirred solution of zinc 5-[4-azidophenyl]-10,15,20-triphenylporphyrin (50 mg, 0.076 mmol) and tert-butyl (1,3-bis(prop-2-yn-1-yloxy)-2-((prop-2-yn-1-yloxy)methyl)propan-2-yl)carbamate (8.5 mg, 0.025 mmol) in toluene (5 ml) was added copper (II) sulfate pentahydrate (5 mg) and sodium ascorbate (10 mg) in water (2 ml). The resulting mixture was heated to 80 ˚C for 1 hour by MW (75 W, max pressure 50 bar, max stirring), followed by heating to 90 ˚C for 3 hours. The solvent was removed under reduced pressure to yield the crude, which was purified by column chromatography (silica, 4% MeOH:DCM) to yield the product as a purple solid (33 mg, 56.4%).

*$R_f$: 0.18 (silica, 4% MeOH:DCM). UV-vis (DCM): λmax, nm 420, 446, 548, 581. ε (420 nm)= 1228314 $M^{-1}cm^{-1}$.$^1$H-NMR (CDCl$_3$): δ 1.46 (s, 9H, (CH$_3$)$_3$), 3.86 (s, 6H, CH$_2$-O), 4.55 (m, 6H,O-CH$_2$-C), 7.57 (m, 18H, 10,15,20-m -Ph), 7.72 (m, 9H, 10,15,20-p-Ph), 8.07 (m, 6H, 5-m-Ph), 8.13 (m, 18H, 10,15,20-o-Ph), 8.28 (s, 3H, triazole H), 8.34 (m, 6H, 5-o-Ph), 8.87 (m, 24H, βH), $^{13}$C-NMR (CDCl$_3$): δ 14.01, 22.56, 28.37, 31.50, 64.51, 69.38, 118.36, 120.80, 126.23 (β-C), 126.299, 131.69, 134.37, 134.44 (β-C) , 149.54, 149.97, 150.04, 150.10, MS: (MALDI-TOF) m/z 2492 (100[M+]),*

**Conjugate (84)**
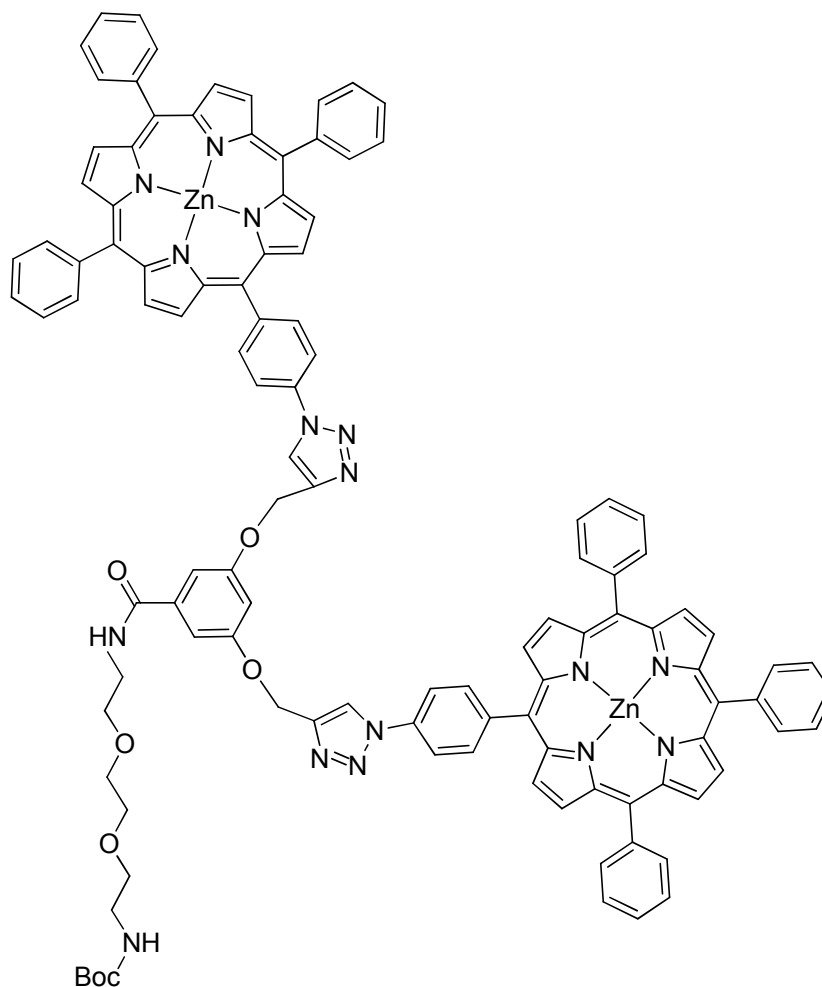


To a 10ml microwave tube was added zinc 5-[4-azidophenyl]-10,15,20-triphenylporphyrin (50 mg, 0.070 mmol) and tert-butyl (2-(2-(2-(3,5-bis(prop-2-yn-1-yloxy)benzamido)ethoxy)ethoxy)ethyl)carbamate (16 mg, 0.035 mmol) in THF:water (7:1, 8 ml). Copper (II) sulfate pentahydrate (2 mg) and sodium ascorbate (2 mg) in THF:water (1:1, 1 ml) was added, and the mixture heated to 80°C by MW (75W, max pressure 100 bar, max stirring) for 60 minutes. The solvent was removed under reduced pressure and the product purified by column chromatography (silica, 2-5% MeOH:DCM) to yield the product as a purple solid (60 mg, 90.5%).

*UV-vis (DCM): λmax, nm 420, 548, 588. ε (420 nm)= 809403 $M^{-1}cm^{-1}$. $^{1}$H-NMR (CDCl$_3$): δ 1.30 (s, 9H, C(C$\underline{H}_3$)$_3$), 3.04 (m, 4H, C$\underline{H}_2$-NH), 3.35 (m, 8H), 7.68 (m, 20H, 10,15,20-m,p-Ph, triazole H), 8.17 (m, 20H, 10,15,20-o-Ph, 5-o,m-Ph), 8.93 (m, 16H, βH). $^{13}$C-NMR (CDCl$_3$): δ 14.24, 21.32, 28.39 (C($\underline{C}$H$_3$)$_3$), 29.76, 40.09, 53.44, 60.60, 62.46, 65.63, 70.17, 79.47 ($\underline{C}$(CH$_3$)$_3$), 107.35 (2,6-Ar), 117.24, 117.58, 120.11,121.13, 121.47, 125.64, 126.52, 127.40, 131.21, 131.62, 134.71, 135.74, 143.29, 149.65, 150.15, 150.25, 150.31, 150.50, 158.61 (C=O Boc), 165.90 (C=O dendron). MS: (ESI) m/z 1898.7 (100[M]+).*

**Conjugate (85)**



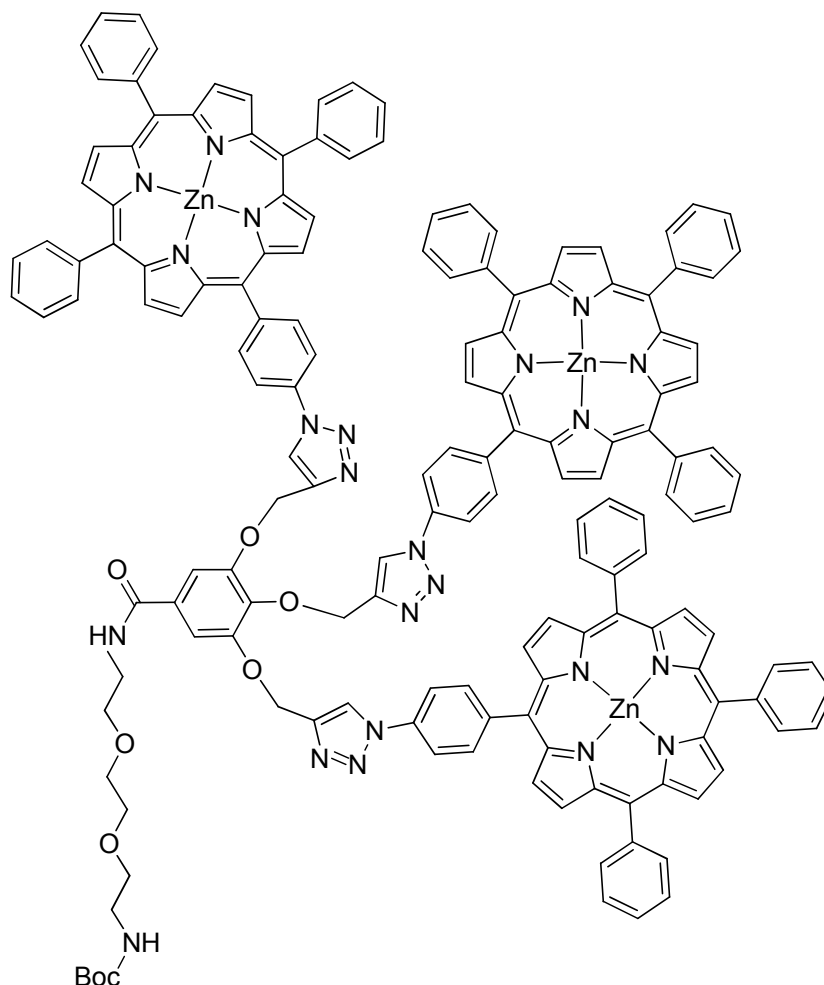To a 10ml microwave tube was added zinc 5-[4-azidophenyl]-10,15,20-triphenylporphyrin (50 mg, 0.070 mmol) and tert-butyl (2-(2-(2-(3,4,5-tris(prop-2-yn-1-yloxy)benzamido)ethoxy)ethoxy)ethyl)carbamate (12 mg, 0.023 mmol) in THF:water (7:1, 8 ml). Copper (II) sulfate pentahydrate (2 mg) and sodium ascorbate (2 mg) in THF:water (1:1, 1 ml) was added, and the mixture heated to 80°C by MW (75W, max pressure 100 bar, max stirring) for 100 minutes. The solvent was removed under reduced pressure and the product purified by column chromatography (silica, 2-5% MeOH:DCM) to yield the product as a purple solid (30 mg, 48.9%).

*UV-vis (DCM): λmax, nm 420, 548, 586. ε (420 nm)= 1316562 $M^{-1}cm^{-1}$. $^1$H-NMR (CDCl$_3$): δ 1.31 (s, 9H, C(CH$_3$)$_3$), 3.45 (br s, 4H), 3.58 (br s, 6H), 4.79 (m, 2H, CH$_2$-C),4.95 (m, 4H, CH$_2$-C), 7.13 (br s, 2H, 2,6-Ar-H), 7.49 (m, 18H, 10,15,20-m-Ph), 7.67 (m, 9H, 10,15,20-p-Ph), 7.87-8.38 (m, 33H), 8.80 (m, 24H, βH). $^{13}$C-NMR (CDCl$_3$): δ 14.20, 21.08, 28.38 (C(CH$_3$)$_3$), 29.83, 30.44, 40.02, 40.12, 69.50, 69.89, 70.08, 79.46 (C(CH$_3$)$_3$), 104.04 (2,6-Ar),118.31, 118.44, 118.48, 120.89, 120.96, 121.08, 121.16, 121.51, 121.81, 122.11, 126.36, 126.47, 127.25, 127.38, 131.27, 131.88, 132.04, 132.11, 134.48, 134.60, 135.65, 135.94, 140.11, 143.10, 143.21, 144.01, 144.08, 144.18, 144.34,*

*144.64, 149.69, 150.03, 150.09, 150.19, 150.24, 151.85, 156.21 (C=O Boc), 166.84 (C=O dendron). MS: (MALDI) m/z 2671.7 (100[M]+).*

**Conjugate (86)**
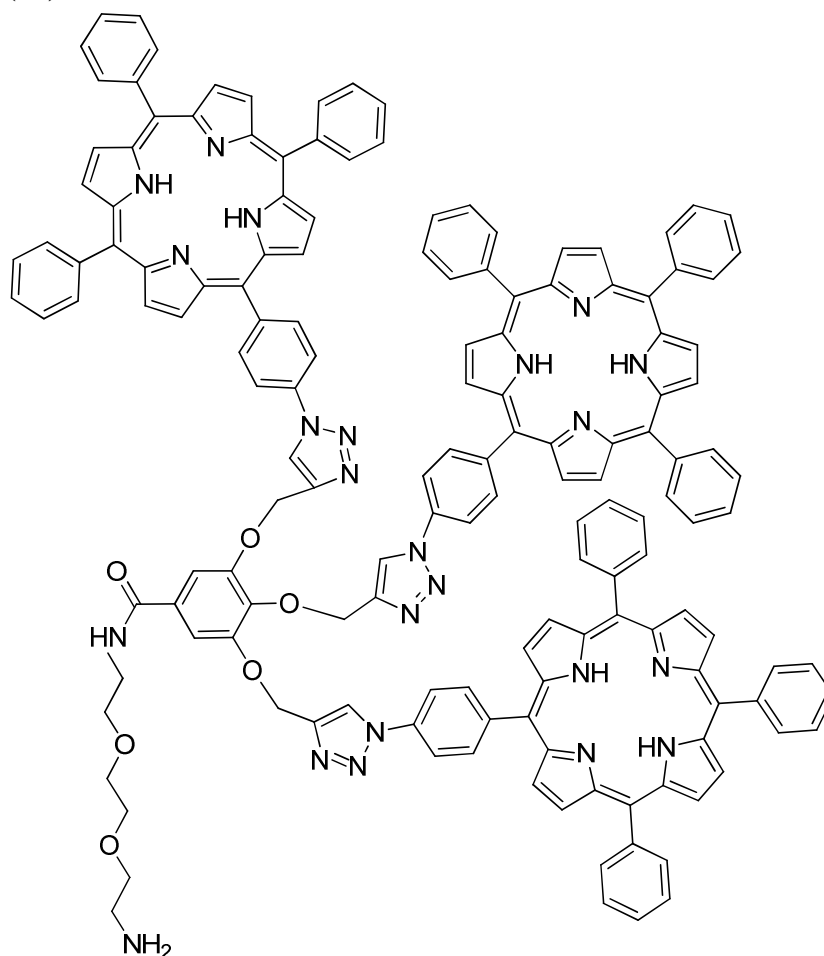


Dendron (30 mg, 0.011 mmol) was dissolved in TFA (2 ml) and stirred overnight at rt. The acid was neutralised with saturated sodium bicarbonate solution and the aqueous layer extracted with dichloromethane. The organic layer was dried ($MgSO_4$) and the solvent removed under reduced pressure to yield the product as a purple solid (26.6 mg, 99.3%).

*UV-vis (DCM): λmax, nm 419, 448, 515, 548, 601, 665 . ε (420 nm)= 1483288 $M^{-1}cm^{-1}$.*
*$^1$H-NMR (CDCl$_3$): δ 3.40 (br s, 2H, N$\underline{H}_2$), 3.81 (m, 12H), 5.63 (m, 6H, C$\underline{H}_2$-C), 7.49 (m, 18H, 10,15,20-m-Ph), 7.67 (m, 12H), 7.81-8.35 (m, 33H), 8.48 (br s, 2H, 2,6-Ar-H), 8.77 (m, 24H, βH). $^{13}$C-NMR (CDCl$_3$): δ 14.28, 22.86, 28.39, 29.92, 32.03, 40.47, 50.87, 53.64, 62.88, 66.08, 67.54, 107.47 (2,6-Ar), 118.04, 118.10, 118.79, 118.85, 120.36, 120.44, 120.53, 120.62, 122.37, 122.91, 126.54, 126.62, 126.74, 127.58, 127.69, 127.82, 130.52, 134.40, 134.45, 134.57, 135.66, 136.49, 136.66, 139.87, 141.80, 141.86, 141.90, 142.04, 142.09, 142.91, 143.08, 144.80, 145.21, 152.11, 167.35 (C=O dendron). MS: (ESI) m/z 2380.9 (100[M]+).*

**Conjugate (87)**
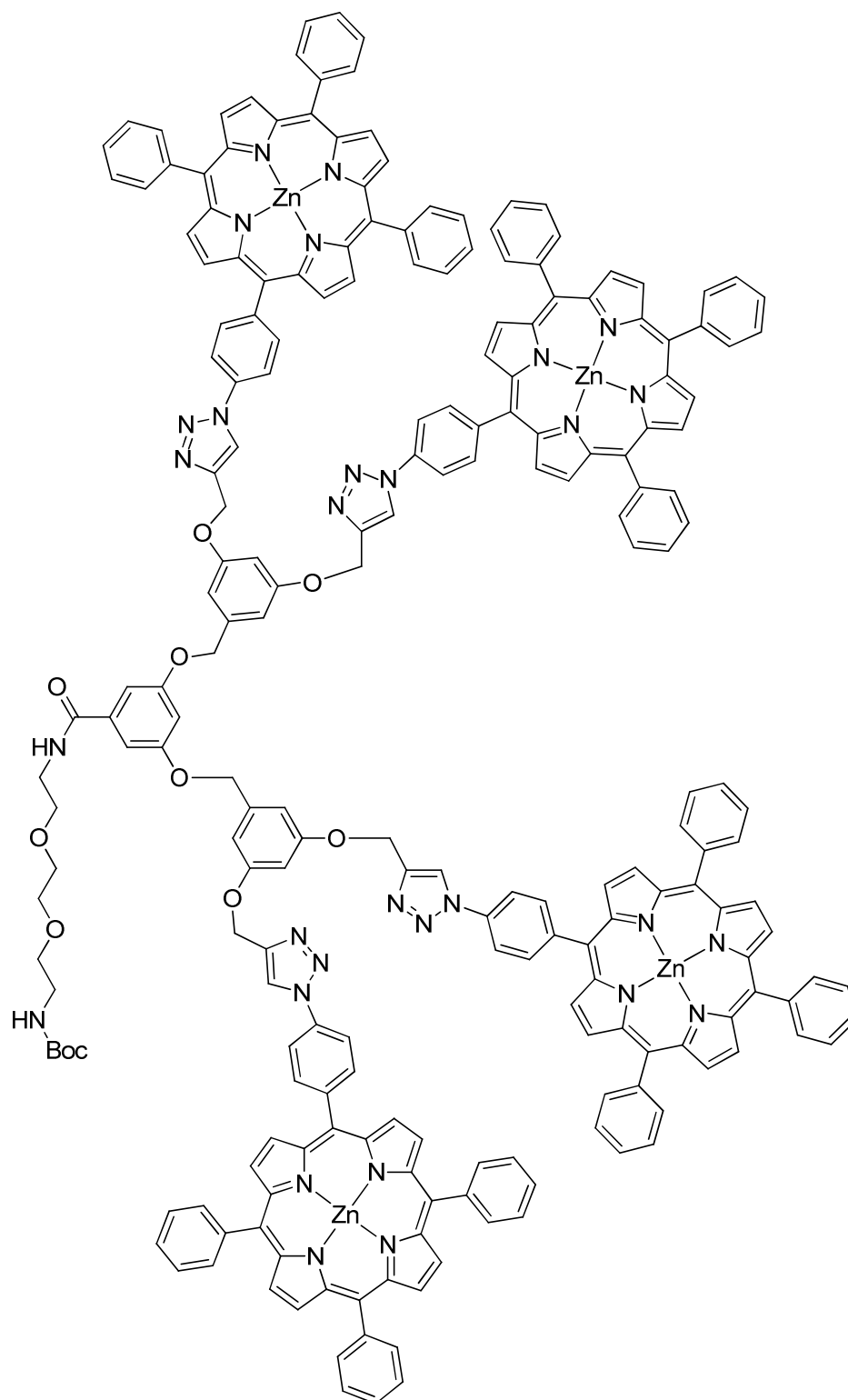


To a 10ml microwave tube was added zinc 5-[4-azidophenyl]-10,15,20-triphenylporphyrin (50 mg, 0.070 mmol) and tert-butyl (2-(2-(2-(3,5-bis((3,5-bis(prop-2-yn-1-yloxy)benzyl)oxy)benzamido)ethoxy)ethoxy)ethyl)carbamate (14 mg, 0.018 mmol) in THF:water (7:1, 8 ml). Copper (II) sulfate pentahydrate (2 mg) and sodium ascorbate (2 mg) in THF:water (1:1, 1 ml) was added, and the mixture heated to 80°C by MW (75W, max pressure 100 bar, max stirring) for 100 minutes. The solvent was

removed under reduced pressure and the product purified by column chromatography (silica, 1-4% MeOH:DCM) to yield the product as a purple solid (33 mg, 51.7%).

*UV-vis (DCM): λmax, nm 420, 522, 590  ε (420 nm)= 1483288 M$^{-1}$cm$^{-1}$. $^1$H-NMR (CDCl$_3$): δ 0.52 (s, 9H, C(CH$_3$)$_3$), 2.33 (m, 2H, CH$_2$-NH), 2.73 (m, 10H), 3.87 (s, 8H, CH$_2$-C), 4.27 (m, 4H, CH$_2$-C), 5.69 (s, 2H), 5.87 (s, 4H), 5.97(s, 1H), 6.36 (s, 2H), 6.83 (m, 44H, 5,10,15,20-m-Ph, 10,15,20-p-Ph),7.01 (s, 4H, triazole H), 7.33 (m, 32H, 5,10,15,20-o-Ph), 7.88-8.12 (m, 32H, βH). $^{13}$C-NMR (CDCl$_3$): δ 28.14 (C(CH$_3$)$_3$), 29.66, 39.74, 39.90, 61.74, 69.72, 69.88, 69.99, 70.04, 79.44 (C(CH$_3$)$_3$), 101.72, 105.12, 106.38, 106.54, 106.60, 118.06, 118.14, 120.85, 121.08, 126.30, 127.19, 130.99, 131.74, 131.80, 132.00, 134.47, 135.43, 136.60, 139.44, 143.19, 143.23, 144.02, 144.37, 149.50, 150.03, 150.15, 150.21, 156.38, 156.42, 159.39, 159.74, 159.74, 156.21,167.94, 168.03 (C=O dendron).MS: (MALDI-TOF) m/z 3656.9 (100[M + H]+)*

**Conjugate (88)**
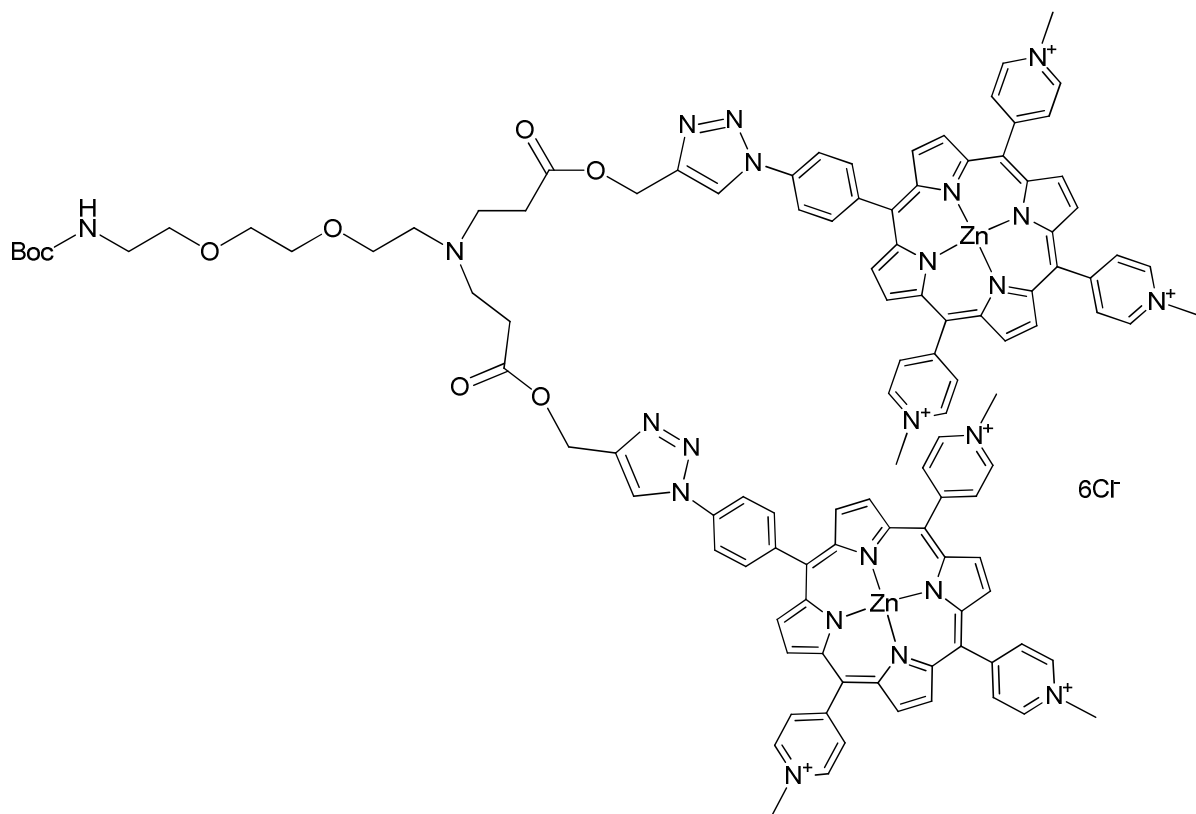


To a 10ml microwave tube was added alkyne PAMAM dendron (8.6 mg, 0.019 mmol) and zinc 5-[4-azidophenyl]-10,15,20-tri-(*N*-methyl-4-pyridinium)porphyrin trichloride (33 mg, 0.038 mmol) dissolved in t-butanol:water (2:5, 7 ml). Copper (II) sulfate pentahydrate (5 mg), sodium ascorbate (5 mg) and TBTA (1 mg) were added, and the mixture heated to 40 °C for 20 min in the microwave (75 W, max stirring). The reaction was shown as complete on TLC, and ammonium hexafluorophosphate added to the mixture. . The resulting solution was filtered and the precipitate redissolved in acetone. Tetrabutylammonium chloride was added, and the resulting solution filtered. The product was precipitated from diethyl ether over MeOH to yield the product as a green solid (30 mg, 79.9%).

*$R_f$: 0.06 (silica, 1:1:8 sat. $KNO_3$ solution:water:MeCN). $^1$H-NMR (MeOH-$d_4$): δ 1.40 (s, 9H, C-(C$\underline{H}_3$)$_3$), 3.19 (m, 2H), 3.30 (m, 8H), 3.34 (m, 6H), 3.50 (m, 8H), 4.79 (s, 18H,C$\underline{H}_3$), 8.06 (m, 4H, 5-m-Ph), 8.21 (m, 4H, 5-o-Ph), 8.82 (m, 18H, βH, triazole Hs), 9.09 (m, 12H, 10,15,20-m-Py), 9.30 (m, 12H, 10,15,20-o-Py).*

**Conjugate (89)**



Conjugate 3 (20 mg, 0.010 mmol) was dissolved in TFA (1 ml) and stirred at RT for 4 hours. The mixture was neutralised with sodium hydrogencarbonate and ammonium hexafluorophosphate added to the mixture. . The resulting solution was filtered and the precipitate redissolved in acetone. Tetrabutylammonium chloride was added, and the resulting solution filtered to yield the product as a green solid. The desired product was not isolated.
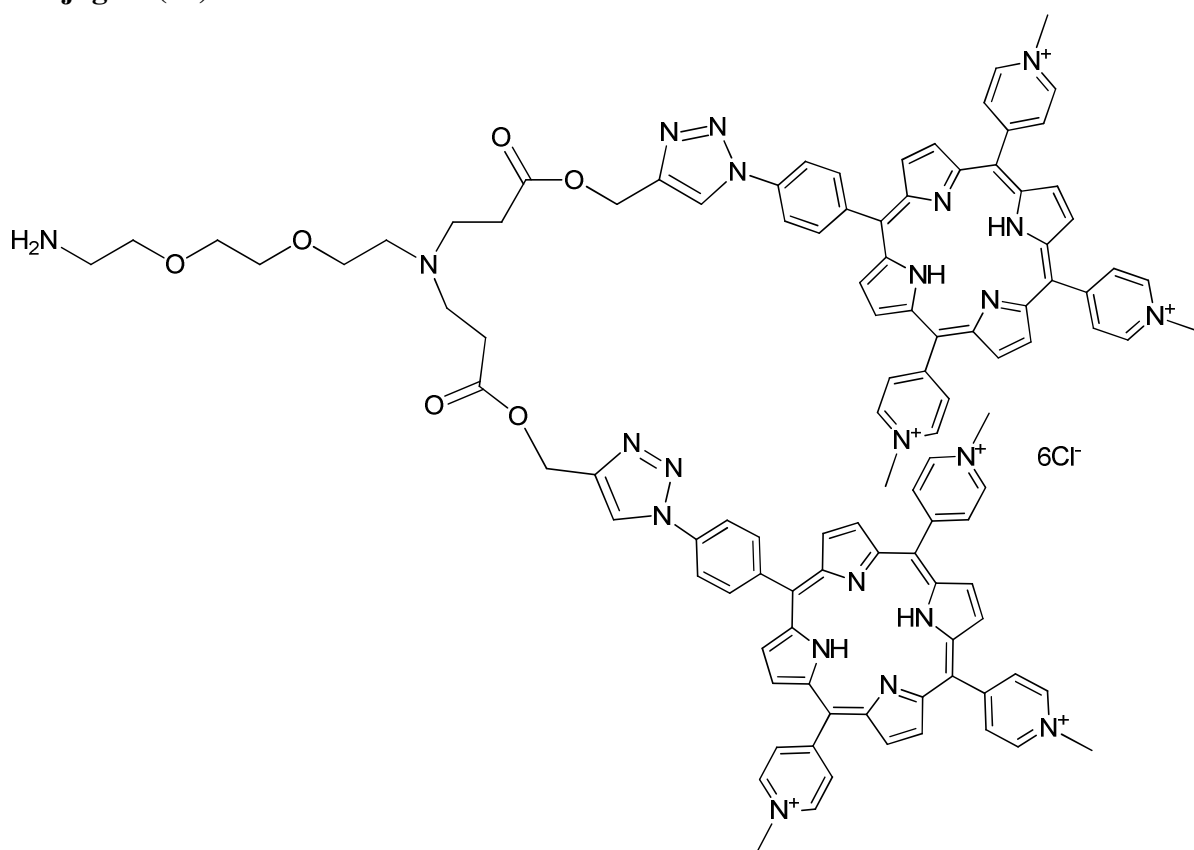
**Conjugate (90)**
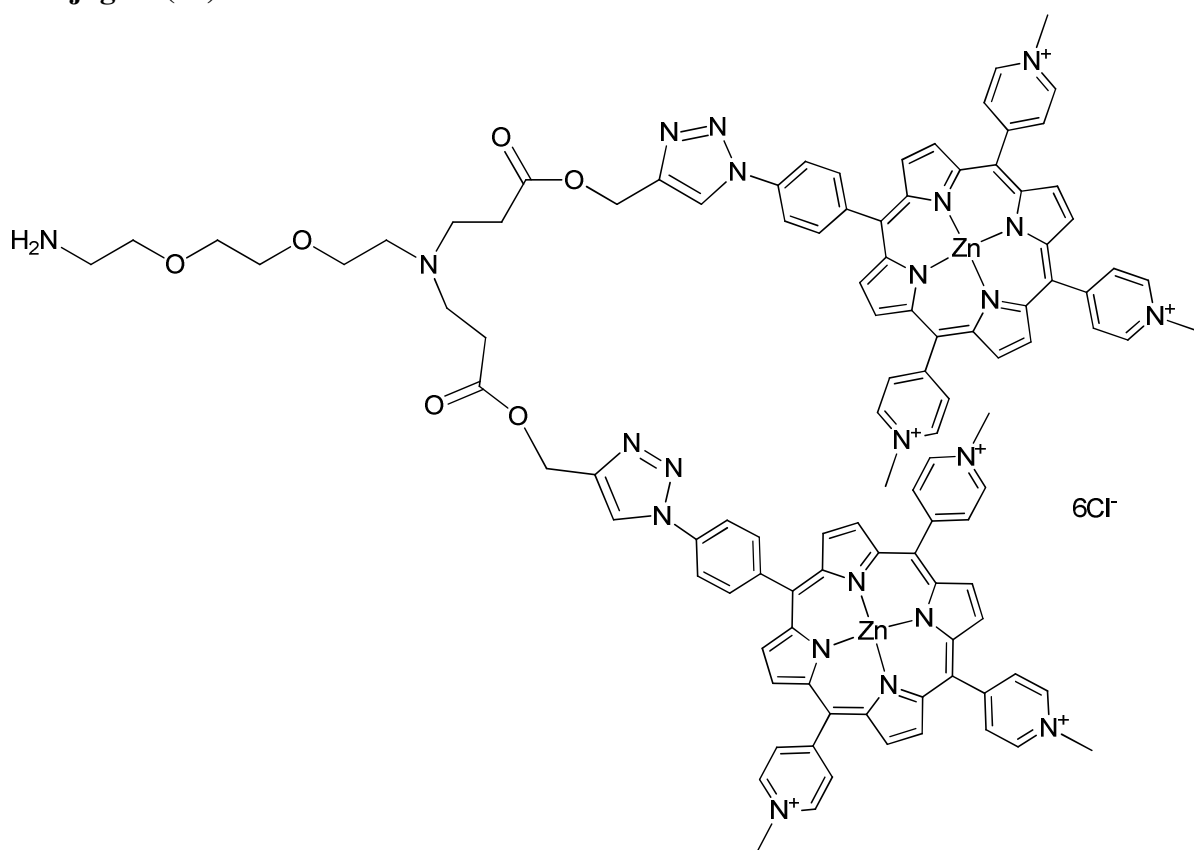


To a 10 ml microwave tube was added deprotected alkyne PAMAM dendron (8.5 mg, 0.0210 mmol) and zinc 5-[4-azidophenyl]-10,15,20-tri-(*N*-methyl-4-pyridinium)porphyrin trichloride (30 mg, 0.0344 mmol) dissolved in t-butanol:water (2:5, 7 ml). Copper (II) sulfate pentahydrate (5 mg), sodium ascorbate (5 mg) and TBTA (1 mg) were added, and the mixture heated to 40 °C for 20 min in the microwave (75 W, max stirring). The reaction was shown as complete on TLC, and ammonium hexafluorophosphate added to the mixture. The resulting solution was filtered and the precipitate redissolved in acetone. Tetrabutylammonium chloride was added, and the resulting solution filtered. The product was precipitated from diethyl ether over MeOH to yield a green solid. The desired product was not isolated.

*$^{1}$H-NMR (MeOH-d$_4$): δ 4.57, (s, 2H, C$\underline{H}_2$-OH),  4.83 (s, 9H, CH$_3$), 8.38 (m, 4H, 5-Ph),8.79 (m, 1H, triazole H), 8.91 (m, 6H, 10,15,20-o-Py) 9.02-9.14 (m, 8H, βH), 9.32 (m, 6H, 10,15,20-m-Py).*

**Conjugate (91)**
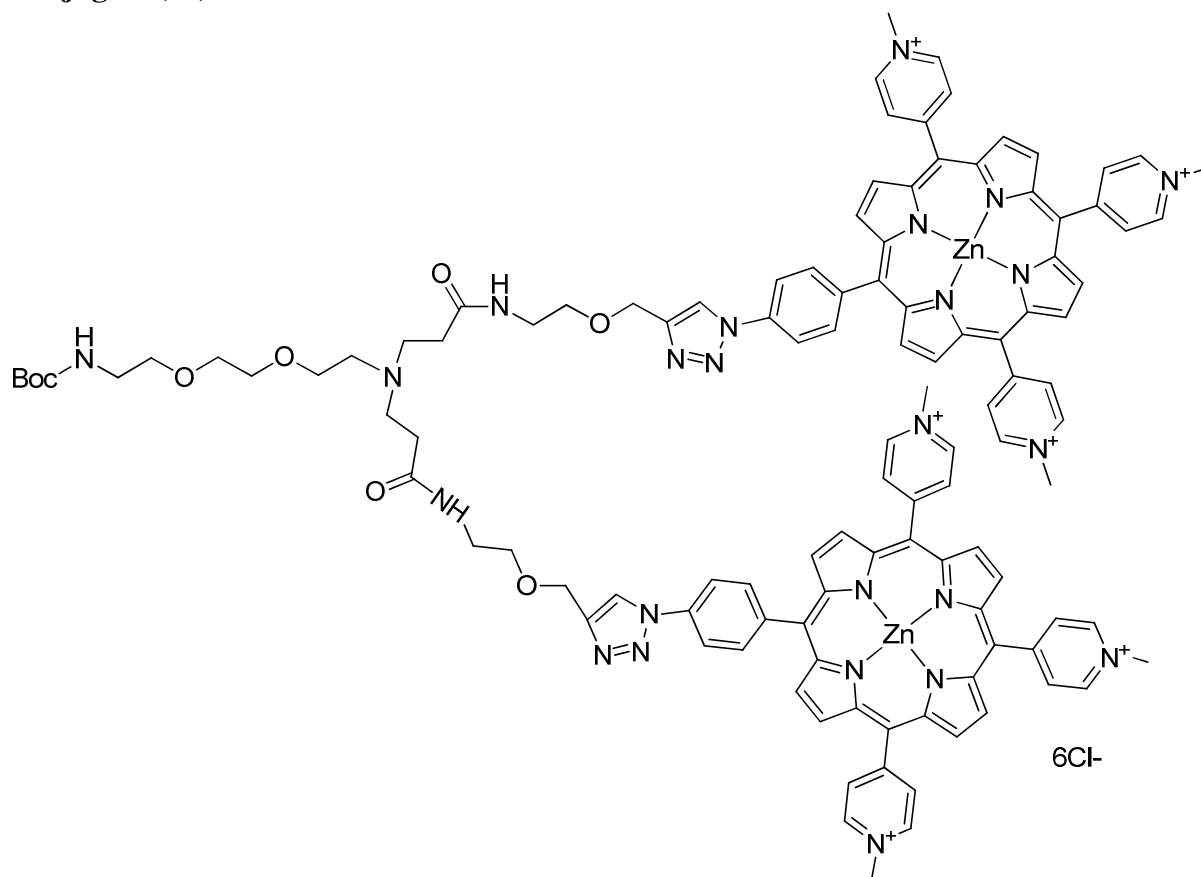


To a 10ml microwave tube was added zinc 5-[4-azidophenyl]-10,15,20-tri-(*N*-methyl-4-pyridinium porphyrin trichloride (45 mg, 0.048 mmol) and tert-butyl (12-oxo-9-(3-oxo-3-((2-(prop-2-yn-1-yloxy)ethyl)amino)propyl)-3,6,16-trioxa-9,13-diazanonadec-18-yn-1-yl)carbamate (12.0 mg, 0.026 mmol) in tBuOH:water (1:1, 8 ml). Copper (II) sulfate pentahydrate (5 mg), sodium ascorbate (5 mg) and TBTA (1 mg) was added, and the mixture heated to 40°C by MW (75W, max pressure 100 bar, max stirring) for 90 minutes. The mixture was concentrated under reduced pressure, and ammonium hexafluorophosphate added to the mixture. The resulting solution was filtered and the precipitate redissolved in acetone. Tetrabutylammonium chloride was added, and the resulting solution filtered. The product was precipitated from diethyl ether over MeOH to yield the product as a green solid (49.0 mg, 90.0%).

*HPLC: 5-60% B over 15 minutes. $R_f$ = 9.1 minutes. UV-vis (H$_2$O): λmax, nm 435, 565, 612. ε (435 nm)= 382647 M$^{-1}$cm$^{-1}$. $^1$H-NMR (DMSO-d$_6$): δ 1.34 (s, 9H, C-(CH$_3$)$_3$), 2.74 (m, 4H, (C=O)-CH$_2$), 3.07 (m, 2H, O-CH$_2$-CH$_2$-N), 3.51 (m, 10H), 3.62 (m, 4H), 4.71 (s, 18H, N-CH$_3$), 4.77 (m, 4H), 8.19 (s, 2H, NH), 8.34-8.44 (m, 8H, 5-m,o-Ar), 8.82-9.08 (m, 28H, 10,15,20-o-Ar, βH), 9.21 (s, 2H, 5-triazole-H), 9.35-9.50 (m, 12H, 10,15,20-m-Ar). $^{13}$C-NMR (DMSO-d$_6$): δ 28.67 (C(CH$_3$)$_3$), 38.88 (N-CH$_3$), 48.10, 63.84, 69.01, 69.65, 69.99, 70.17, 78.08 (C(CH$_3$)$_3$), 115.30, 115.99, 118.79, 122.13, 123.03, 132.15, 132.61 (β-C), 132.79, 133.58, 135.73, 136.81, 142.73, 144.11 (β-C), 145.89, 148.42, 148.69, 148.84, 150.50, 156.07 (C=O), 158.92 (C=O Boc).*

**Conjugate (92)**



To a 10ml microwave tube was added zinc 5-[4-2-(2-(2-azidoethoxy)ethoxy)
ethanaminocarbonyl]phenyl]-10,15,20-tris(*N*-methyl-4-pyridinium)porphyrin trichloride
(26.8 mg, 0.026 mmol) and tert-butyl (12-oxo-9-(3-oxo-3-((2-(prop-2-yn-1-
yloxy)ethyl)amino)propyl)-3,6,16-trioxa-9,13-diazanonadec-18-yn-1-yl)carbamate (7.3
mg, 0.013 mmol) in tBuOH:water (1:1, 8 ml). Copper (II) sulfate pentahydrate (5 mg),
sodium ascorbate (5 mg) and TBTA (1 mg) was added, and the mixture heated to 40°C
by MW (75W, max pressure 100 bar, max stirring) for 90 minutes. The mixture was
concentrated under reduced pressure, and ammonium hexafluorophosphate added to the
mixture. The resulting solution was filtered and the precipitate redissolved in acetone.
Tetrabutylammonium chloride was added, and the resulting solution filtered. The
product was precipitated from diethyl ether over MeOH to yield the product as a green
solid (28.7 mg, 84.5%).

*HPLC: 5-60% B over 15 minutes. $R_f$ = 9.1 minutes. UV-vis ($H_2O$): λmax, nm 438, 565,
609. ε (438 nm)= 342320 $M^{-1}cm^{-1}$. $^1$H-NMR (DMSO-$d_6$): δ 1.35 (s, 9H, C-(C$\underline{H}_3$)$_3$), 2.08
(s, 1H, N$\underline{H}$), 3.03 (m, 4H, (C=O)-C$\underline{H}_2$), 3.24 (m, 6H), 3.41 (m, 8H), 3.62 (m, 16H), 3.88
(s, 4H), 4.72 (s, 18H, N-C$\underline{H}_3$), 8.14 (s, 2H, triazole),8.26 (m, 4H, 5-m-Ar), 8.34 (m, 4H,
5-o-Ar), 8.82-9.05 (m, 28H, 10,15,20-o-Ar, βH), 9.39-9.51 (m, 12H, 10,15,20-m-Ar).
$^{13}$C-NMR (DMSO-$d_6$): δ 28.81 (C($\underline{C}H_3$)$_3$), 48.26 (N-C$\underline{H}_3$), 49.91, 63.92, 68.80, 69.33,
69.54, 69.73, 69.89, 70.07, 70.14, 78.18 ($\underline{C}$(CH$_3$)$_3$), 115.34, 116.03, 122.79,
124.96,126.25, 132.15, 132.70 (β-C), 132.87, 133.62, 134.21, 134.56, 144.18 (β-C),
144.28, 145.44, 148.47, 148.73, 148.91, 150.44,156.17, 159.06 (C=O), 166.83 (C=O
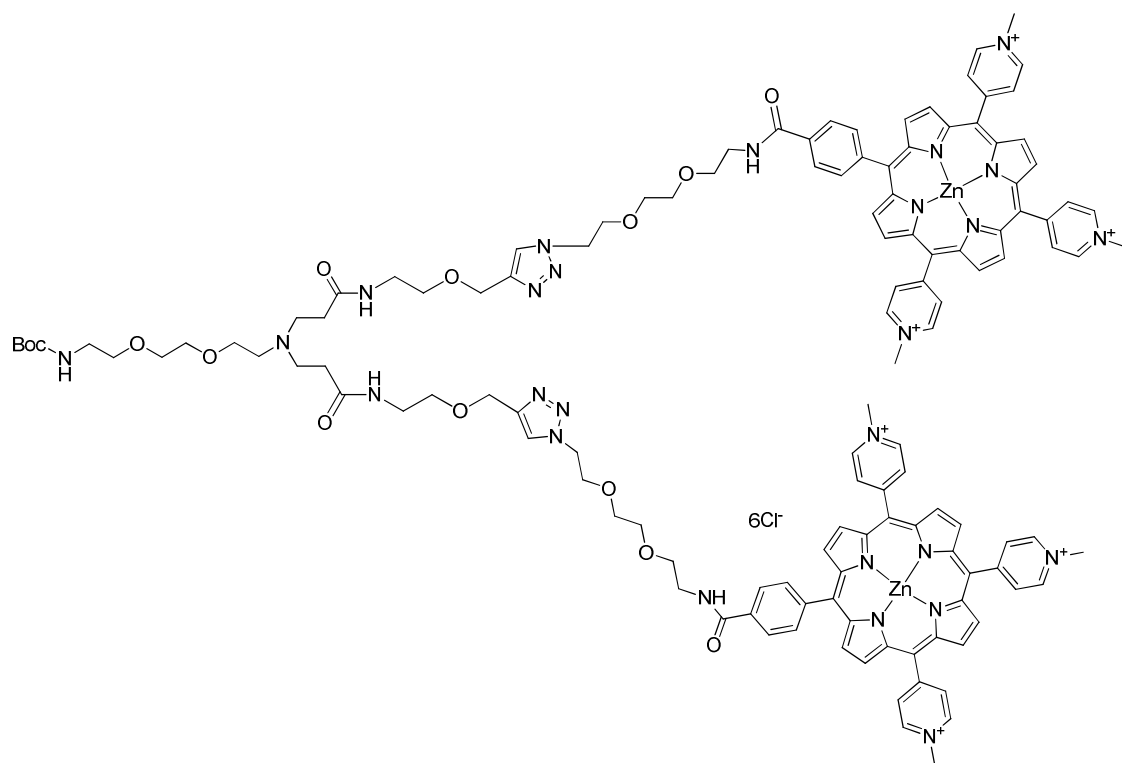Boc).*

**Conjugate (93)**
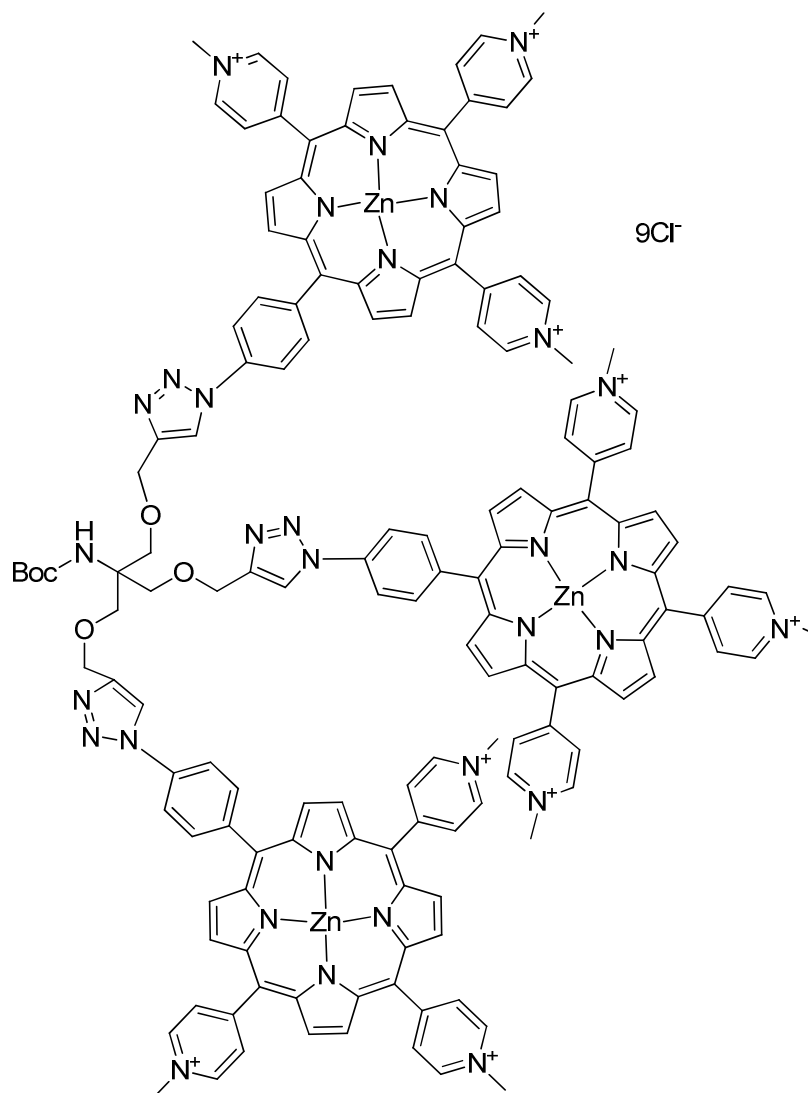


To a 10 ml microwave tube was added zinc 5-[4-azidophenyl]-10,15,20-tri-(*N*-methyl-4-pyridinium)porphyrin trichloride (33 mg, 0.038 mmol) and tert-butyl (1,3-bis(prop-2-yn-1-yloxy)-2-((prop-2-yn-1-yloxy)methyl)propan-2-yl)carbamate (4.3 mg, 0.013 mmol) in t-butanol:water (2:5, 7 ml). Copper (II) sulfate pentahydrate (5 mg), sodium ascorbate (5 mg) and TBTA (1 mg) were added, and the mixture heated to 45 °C for 5 hours in the microwave (75 W, max stirring). The reaction was shown as complete on TLC, and ammonium hexafluorophosphate added to the mixture. The resulting solution was filtered and the precipitate redissolved in acetone. Tetrabutylammonium chloride was added, and the resulting solution filtered. The desired product was not isolated.

**Conjugate (94)**



To a 10ml microwave tube was added zinc 5-[4-azidophenyl]-10,15,20-tri-(*N*-methyl-4-pyridinium)porphyrin trichloride (45 mg, 0.048 mmol) and tert-butyl (2-(2-(2-(3,5-bis(prop-2-yn-1-yloxy)benzamido)ethoxy)ethoxy)ethyl)carbamate (12 mg, 0.027 mmol) in tBuOH:water (1:1, 8 ml). Copper (II) sulfate pentahydrate (5 mg), sodium ascorbate (5 mg), and TBTA (1 mg) was added, and the mixture heated to 40°C by MW (75W, max pressure 100 bar, max stirring) for 30 minutes. The mixture was concentrated under reduced pressure, and ammonium hexafluorophosphate added to the mixture. The resulting solution was filtered and the precipitate redissolved in acetone. Tetrabutylammonium chloride was added, and the resulting solution filtered. The product was precipitated from diethyl ether over MeOH to yield the product as a green solid (50.1 mg, 94.7%).

*HPLC: 5-60% B over 15 minutes. $R_f$ = 9.0 minutes. UV-vis ($H_2O$): λmax, nm 435, 567, 615. ε (435 nm)= 258265 $M^{-1}cm^{-1}$. $^1$H-NMR (DMSO-d$_6$): δ 1.38 (s, 9H, C-(C$\underline{H}_3$)$_3$), 3.21 (m, 2H), 3.51 (m, 2H), 3.63-3.71 (m, 10H), 4.78 (s, 12H, 10,20- N-C$\underline{H}_3$), 4.80 (s, 6H, 15-N-C$\underline{H}_3$), 5.25 (s, 4H, C$\underline{H}_2$), 6.89 (s, 1Hs, 4-Ar-H), 7.16 (s, 2H, 2,5-Ar-H), 8.31 (m, 4H, 5-m-Ph), 8.41 (m, 4H, 5-o-Ph), 8.88-8.91 (m, 14H, 10,15,20-o-Ph, triazole H), 9.01-9.11 (m, 16H, βH), 9.33 (m, 12H, 10,15,20-m-Ph). $^{13}$C-NMR (DMSO-d$_6$): δ 28.30 (C(C$\underline{H}_3$)$_3$), 47.60 (N-C$\underline{H}_3$), 48.50, 61.47, 68.79, 68.82, 69.09, 69.40,69.49, 77.49 ($\underline{C}$(CH$_3$)$_3$), 114.81 (2,6-Ar), 115.51, 118.35, 121.55, 123.13, 131.67, 132.02 (β-C),*

*132.29, 133.07, 135.17, 136.19, 136.65, 143.55 (β-C), 143.93, 147.86, 148.12, 148.27, 149.92, 158.26 (C=O), 159.12 (C=O Boc).*
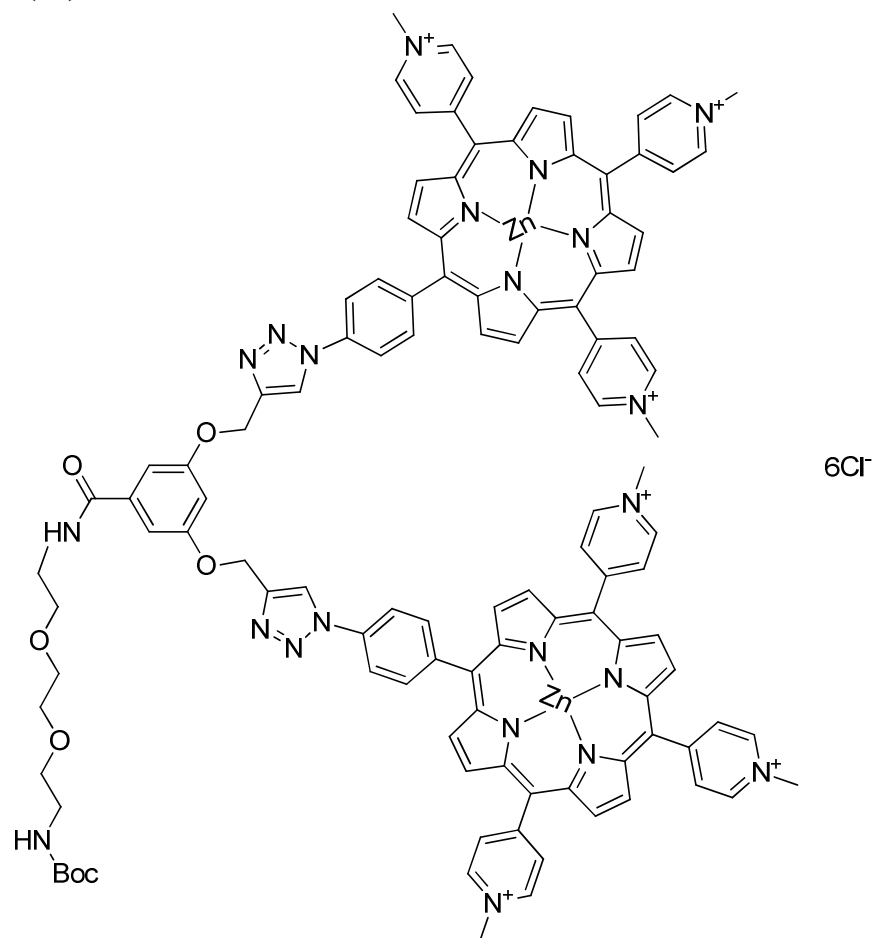
**Conjugate (95)**



To a 10ml microwave tube was added zinc 5-[4-2-(2-(2-azidoethoxy)ethoxy)ethanaminocarbonyl]phenyl]-10,15,20-tris(*N*-methyl-4-pyridinium)porphyrin trichloride (35.0 mg, 0.034 mmol) and tert-butyl (2-(2-(2-(3,5-bis(prop-2-yn-1-yloxy)benzamido)ethoxy)ethoxy)ethyl)carbamate (7.4 mg, 0.017 mmol) in tBuOH:water (1:1, 8 ml). Copper (II) sulfate pentahydrate (5 mg), sodium ascorbate (5 mg), and TBTA (1 mg) was added, and the mixture heated to 40°C by MW (75W, max pressure 100 bar, max stirring) for 30 minutes. The mixture was concentrated under reduced pressure, and ammonium hexafluorophosphate added to the mixture. The resulting solution was filtered and the precipitate redissolved in acetone. Tetrabutylammonium chloride was added, and the resulting solution filtered. The product was precipitated from diethyl ether over MeOH to yield the product as a green solid (39.8 mg, 93.0%).

*HPLC: 5-60% B over 15 minutes. $R_f$ = 8.8 minutes. UV-vis ($H_2O$): λmax, nm 438, 566, 610. ε (438 nm)= 301692 $M^{-1}cm^{-1}$. $^1$H-NMR (DMSO-$d_6$): δ 1.33 (s, 9H, C-(C$\underline{H}_3$)₃), 3.01 (s, 4H), 3.60 (s, 20H), 3.88 (s, 4H), 4.58 (s, 6H), 4.71 (s, 20H, 10,20- N-C$\underline{H}_3$, PEG C$\underline{H}_2$), 5.20 (s, 4H, C$\underline{H}_2$O), 6.75 (s, 1H, N$\underline{H}$), 6.93 (s, 1H, 4-Ar-H), 7.18 (s, 2H, 2,5-Ar-H), 8.09-8.44 (m, 8H, 5-o,m-Ph), 8.72-9.14 (m, 30H, 10,15,20-o-Ph, triazole H, βH), 9.44 (s, 12H, 10,15,20-m-Ph). $^{13}$C-NMR (DMSO-$d_6$): δ 28.74 (C(C$\underline{H}_3$)₃), 48.19 (N-C$\underline{H}_3$), 50.04, 55.50, 61.99, 69.31, 69.38, 69.55, 69.69, 69.98, 70.06, 70.14, 78.14 ($\underline{C}$(CH₃)₃), 79.24, 79.57, 79.90, 106.92 (4-Ar), 115.36 (2,6-Ar), 116.06, 122.83, 125.58, 126.62, 132.17, 132.68 (β-C), 132.86, 133.64, 134.23, 134.56, 137.03, 142.89, 144.20 (β-C), 145.40, 148.48, 148.75, 148.90, 150.45, 156.14, 159.66, 166.23 (C=O Boc), 166.85 (C=O dendron).*
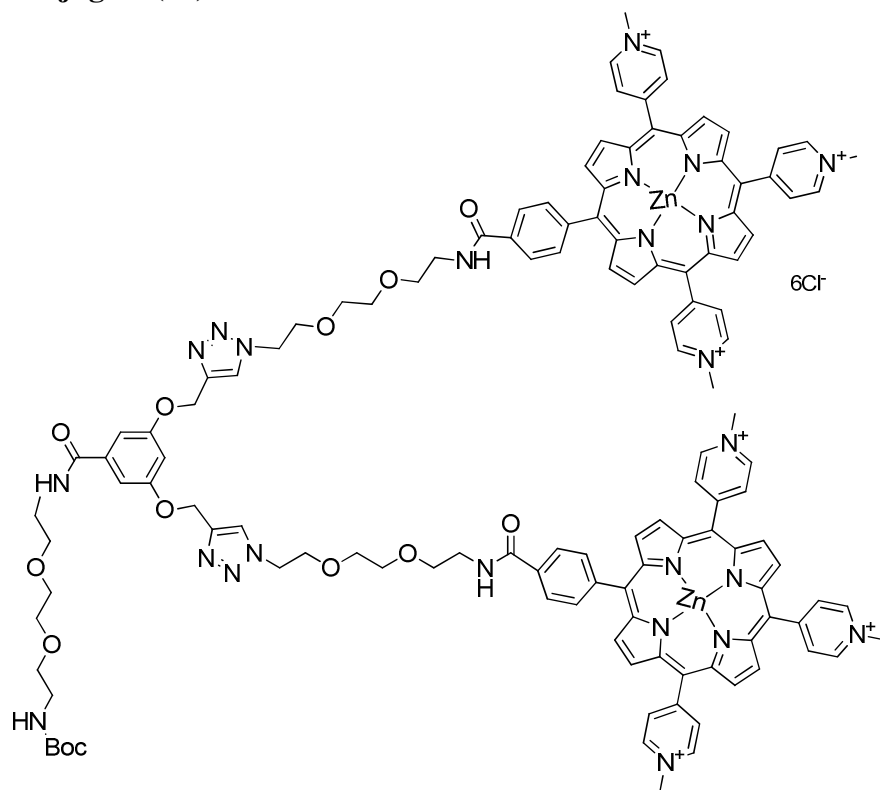
**Conjugate (96)**



To a 10ml microwave tube was added zinc 5-[4-azidophenyl]-10,15,20-tri-(*N*-methyl-4-pyridinium)porphyrin trichloride (30 mg, 0.035 mmol) and tert-butyl (12-oxo-9-(3-oxo-3-((2-(prop-2-yn-1-yloxy)ethyl)amino)propyl)-3,6,16-trioxa-9,13-diazanonadec-18-yn-1-yl)carbamate (6.0 mg, 0.012 mmol) in tBuOH:water (1:1, 8 ml). Copper (II) sulfate pentahydrate (5 mg), sodium ascorbate (5 mg) and TBTA (1 mg) was added, and the mixture heated to 40°C by MW (75W, max pressure 100 bar, max stirring) for 90 minutes. The mixture was concentrated under reduced pressure, and ammonium hexafluorophosphate added to the mixture. The resulting solution was filtered and the precipitate redissolved in acetone. Tetrabutylammonium chloride was added, and the resulting solution filtered. The desired product was not isolated.
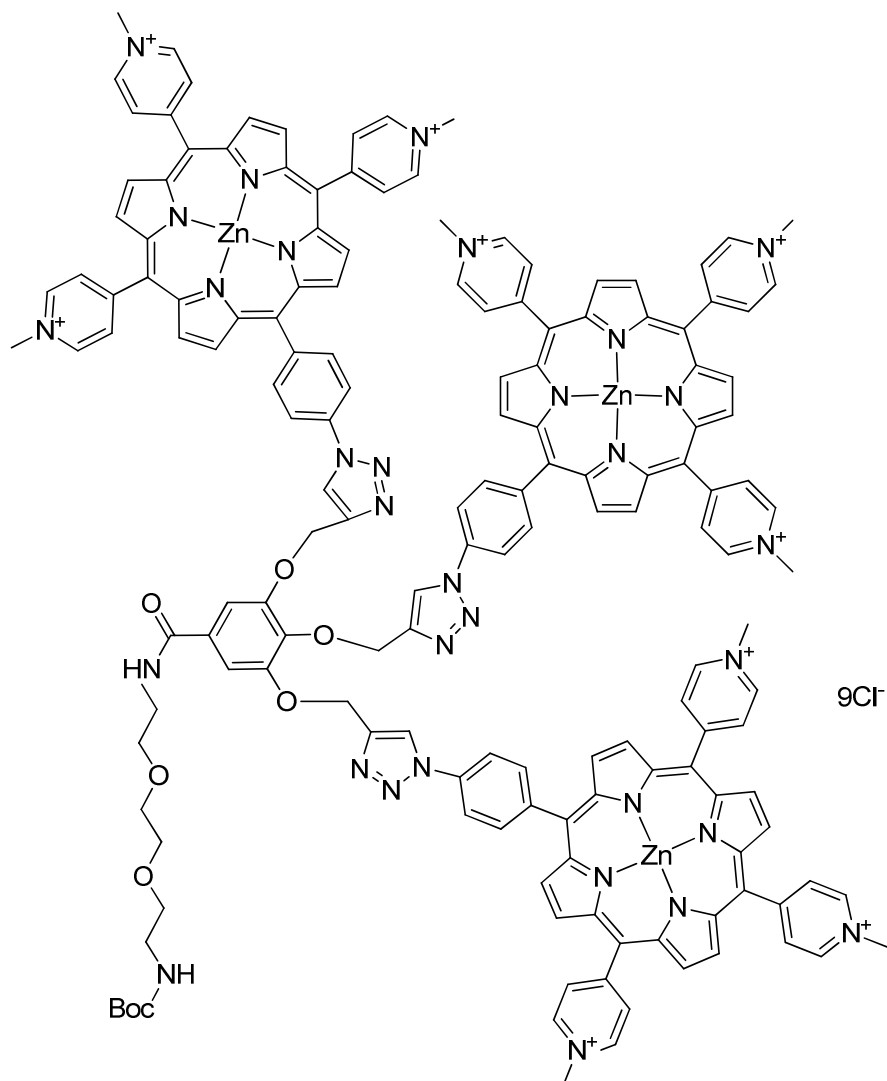
**Conjugate (97)**



To a 10ml microwave tube was added zinc 5-[4-2-(2-(2-azidoethoxy)ethoxy)ethanaminocarbonyl]phenyl]-10,15,20-tris(*N*-methyl-4-pyridinium)porphyrin trichloride (36.0 mg, 0.035 mmol) and tert-butyl (12-oxo-9-(3-oxo-3-((2-(prop-2-yn-1-yloxy)ethyl)amino)propyl)-3,6,16-trioxa-9,13-diazanonadec-18-yn-1-yl)carbamate (6.0 mg, 0.012 mmol) in tBuOH:water (1:1, 8 ml). Copper (II) sulfate pentahydrate (5 mg), sodium ascorbate (5 mg) and TBTA (1 mg) was added, and the mixture heated to 40°C by MW (75W, max pressure 100 bar, max stirring) for 60 minutes. The mixture was concentrated under reduced pressure, and ammonium hexafluorophosphate added to the mixture. The resulting solution was filtered and the precipitate redissolved in acetone. Tetrabutylammonium chloride was added, and the resulting solution filtered. The product was precipitated from diethyl ether over MeOH to yield the product as a green solid (33.2 mg, 86.6%).
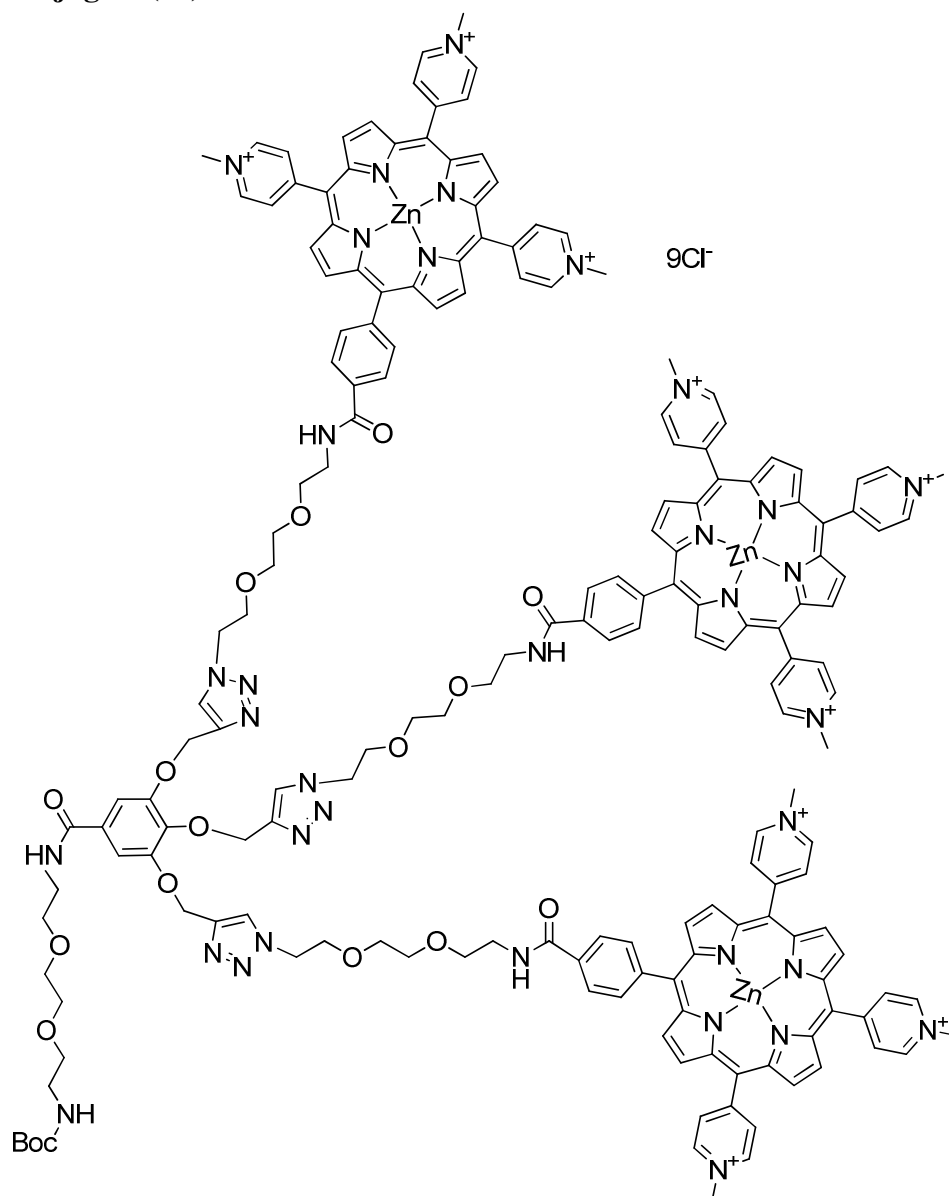
*HPLC: 5-60% B over 15 minutes. $R_f$ = 9.0 minutes. UV-vis (H$_2$O): λmax, nm 438, 567, 612. ε (438 nm)= 406281 M$^{-1}$cm$^{-1}$. $^1$H-NMR (DMSO-d$_6$): δ 1.33 (s, 9H, C(C$\underline{H}_3$)$_3$), 3.01 (br s, 4H), 3.46-3.69 (m, 30H), 3.88 (s, 6H),4.56 (m, 8H),4.70 (s, 27H, N-C$\underline{H}_3$), 5.10 (s,*

2H, O-C*H*$_2$-C), 5.24 (s, 2H, O-C*H*$_2$-C), 6.75 (s, 1H, N*H*), 7.48 (br s, 2H, 2,6-Ar-H), 8.07 (m, 1H, N*H*),  8.19-8.38 (m, 15H, 5-o,m-Ar, 5-triazole-H), 8.80-9.07 (m, 42H, 10,15,20-o-Ph, βH), 9.26-9.59 (m,18H, 10,15,20-m-Ph). $^{13}$C-NMR (DMSO-d$_6$): δ 28.73 (C(*C*H$_3$)$_3$), 48.26 (N-C*H*$_3$), 50.07, 62.96, 65.51, 66.13, 69.32, 69.53, 69.68, 69.96, 70.04, 70.09, 70.14, 78.16  (*C*(CH$_3$)$_3$), 107.58 (4-Ar), 115.33 (2,6-Ar), 116.04, 122.82, 125.48, 126.21, 132.13, 132.68 (β-C), 132.84, 133.64, 134.18, 134.57, 143.12, 144.20 (β-C), 145.44, 148.47, 148.73, 148.92, 150.45, 156.12, 159.03, 166.83 (C=O).

**Conjugate (98)**



To a 10ml microwave tube was added zinc 5-[4-azidophenyl]-10,15,20-tri-(*N*-methyl-4-pyridinium)porphyrin trichloride (30 mg, 0.035 mmol) and tert-butyl (2-(2-(2-(3,5-bis((3,5-bis(prop-2-yn-1-yloxy)benzyl)oxy)benzamido)ethoxy)ethoxy)ethyl)carbamate (6.8 mg, 0.875 mmol) in THF:water (1:1, 8 ml). Copper (II) sulfate pentahydrate (5 mg), sodium ascorbate (5 mg) and TBTA (1 mg) was added, and the mixture heated to 40°C by MW (75W, max pressure 100 bar, max stirring) for 90 minutes. The mixture was concentrated under reduced pressure, and ammonium hexafluorophosphate added to the mixture. The resulting solution was filtered and the precipitate redissolved in acetone. Tetrabutylammonium chloride was added, and the resulting solution filtered. The desired product was not isolated.
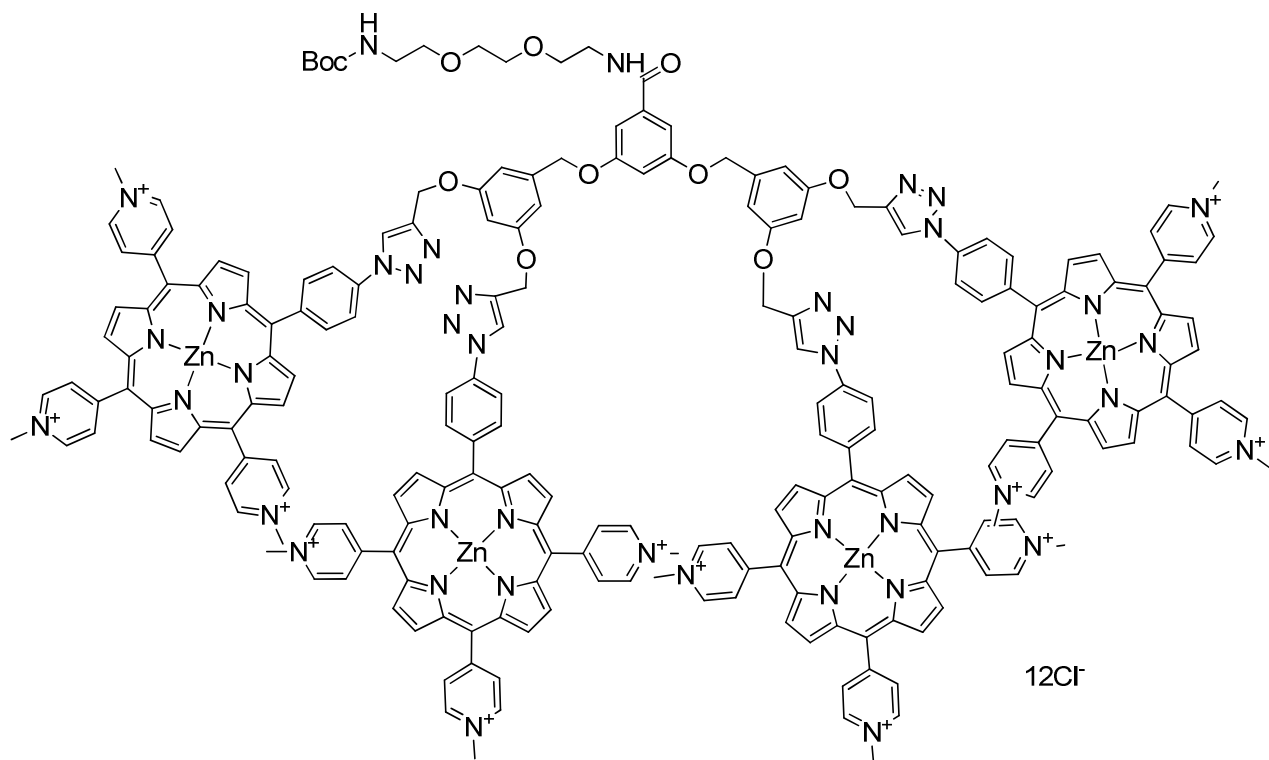
**Conjugate (99)**



To a 10ml microwave tube was added zinc 5-[4-2-(2-(2-azidoethoxy)ethoxy)ethanaminocarbonyl]phenyl]-10,15,20-tris(*N*-methyl-4-pyridinium)porphyrin trichloride (36.0 mg, 0.035 mmol) and tert-butyl (2-(2-(2-(3,5-bis((3,5-bis(prop-2-yn-1-yloxy)benzyl)oxy)benzamido)ethoxy)ethoxy)ethyl)carbamate (6.8 mg, 0.875 mmol) in THF:water (1:1, 8 ml). Copper (II) sulfate pentahydrate (5 mg), sodium ascorbate (5 mg) and TBTA (1 mg) was added, and the mixture heated to 40°C by MW (75W, max pressure 100 bar, max stirring) for 90 minutes. The mixture was concentrated under reduced pressure, and ammonium hexafluorophosphate added to the mixture. The resulting solution was filtered and the precipitate redissolved in acetone. Tetrabutylammonium chloride was added, and the resulting solution filtered. The product was precipitated from diethyl ether over MeOH to yield the product as a green solid (26.0 mg, 60.7%).

*HPLC: 5-60% B over 15 minutes. $R_f$ = 9.4 minutes. UV-vis ($H_2O$): λmax, nm 440, 567, 612. ε (440 nm)= 653651 $M^{-1}cm^{-1}$. $^1$H-NMR (DMSO-d$_6$): δ 1.32 (s, 9H, C(C$\underline{H}_3$)$_3$), 3.00 (br s, 4H), 3.51-3.69 (m, 40H), 3.86 (s, 8H),4.56 (m, 10H),4.70 (s, 36H, N-C$\underline{H}_3$), 5.04 (s, 4H, O-C$\underline{H}_2$-C), 5.16 (s, 8H, O-C$\underline{H}_2$-C), 6.74 (m, 6H), 6.84 (m, 1H), 7.15 (m, 2H), 8.07 (m, 1H, N$\underline{H}$), 8.20-8.35 (m, 20H, 5-o,m-Ar, 5-triazole-H), 8.81-9.06 (m, 56H, 10,15,20-o-Ph, βH), 9.36-9.49 (m 24H, 10,15,20-m-Ph). $^{13}$C-NMR (DMSO-d$_6$): δ 28.19 (C(C$\underline{H}_3$)$_3$), 47.74 (N-C$\underline{H}_3$), 48.61, 49.34, 61.21, 64.93, 68.75, 68.91, 69.00, 69.14, 69.43, 69.49, 69.56 ($\underline{C}$(CH$_3$)$_3$), 106.48 (4-Ar), 114.84 (2,6-Ar), 115.52, 122.29, 125.07, 125.67, 131.63, 132.14 (β-C), 132.36, 133.10, 133.65, 134.05, 142.43, 143.65 (β-C), 144.85, 147.95, 148.19, 148.36, 149.91, 158.45, 159.36, 165.70 (C=O Boc), 166.31 (C=O dendron).*

**Dibromomaleic anhydride (100)**
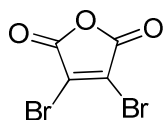


A mixture of maleic anhydride (1.50 g, 15.3 mmol), bromine (1.57 ml, 30.6 mmol) and aluminium chloride (28 mg, cat.) was heated for 16 hours at 160 °C in a sealed tube. The tube was cooled to rt and the solid suspended in ethyl acetate. The mixture was filtered and the filter cake washed with ethyl acetate. The washings were combined and the solvent removed under reduced pressure to yield the product as a tan solid (3.68 g, 94.8%).

*Mp 114–115 °C. $^{13}$C-NMR (CDCl$_3$): δ 131.35 (C-Br), 158.43 (C=O). MS: (ESI) m/z 288(100[M + MeOH]+), HRMS: calcd. for C$_5$H$_4$Br$_2$O$_4$: 286.8549 found 286.8546.*

**5-[4-(3,4-Dibromomaleimide)phenyl]-10,15,20-triphenylporphyrin (101)**
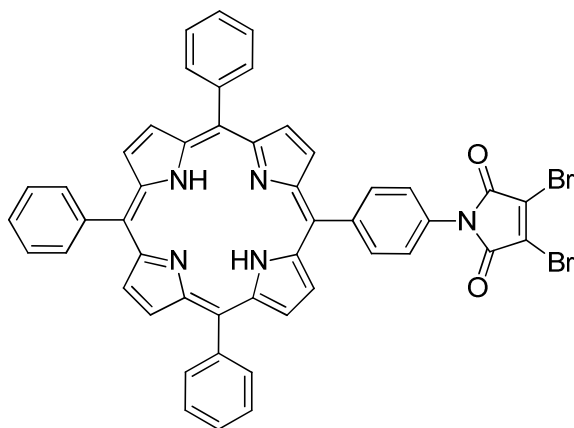


To a stirred solution of 5-[4-aminophenyl]-10,15,20-triphenylporphyrin (30 mg, 0.048 mmol) in acetic acid (50 ml) was added dibromomaleic anhydride (28 mg, 0.111 mmol) and the mixture heated to 120 °C for 5 hours. The solvent was removed under reduced pressure and the crude redissolved in DCM. The organic layer was washed with sat. NaHCO$_3$ solution and water, and dried (MgSO$_4$). The solvent was removed under reduced pressure and the crude precipitated from MeOH over DCM to yield the product a purple solid (16 mg, 38.6%).

*UV-vis (DCM): λmax, nm 418, 514, 550, 590, 646. ε (418 nm)= 434530 M$^{-1}$cm$^{-1}$. $^1$H-NMR (CDCl$_3$): δ 7.69-7.95 (m, 11H, 5,10,15,20-m,p-Ph), 8.21-8.45 (m, 8H, 5,10,15,20-o-Ph), 8.82-8.93 (m, 8H, βH). $^{13}$C-NMR (CDCl$_3$): δ 113.27, 118.48, 120.53, 120.67, 124.09, 126.89 (β-C), 127.93, 130.20 (C-Br), 130.78, 134.73 (β-C), 135.31, 142.19, 142.58, 163.18 (C=O). MS: (MALDI) m/z 867(100[M + H]+).*

**5-[4-(3,4-Dibromomaleimide)phenyl]-10,15,20-tri-(4-pyridyl)porphyrin (102)**



To a stirred solution of 5-[4-aminophenyl]-10,15,20-triphenylporphyrin (50 mg, 0.080 mmol) in acetic acid (50 ml) was added dibromomaleic anhydride (28 mg, 0.111 mmol) and the mixture heated to 120 °C for 5 hours. The solvent was removed under reduced pressure and the crude redissolved in DCM. The organic layer was washed with sat. NaHCO$_3$ solution and water, and dried (MgSO$_4$). The solvent was removed under reduced pressure and the product precipitated from MeOH over DCM. The desired product was not isolated.

**5-[4-(3,4-Dibromomaleimide)phenyl]-10,15,20-tri-(4-pyridyl)porphyrin (102)**
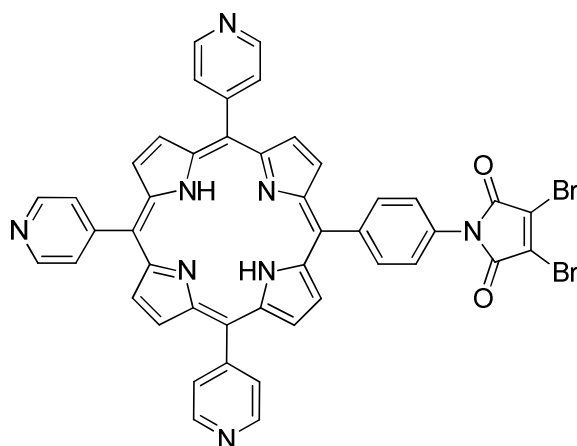


To a stirred solution of 5-[4-aminophenyl]-10,15,20-triphenylporphyrin (50 mg, 0.080 mmol) in nitrobenzene (7.5 ml) was added dibromomaleic anhydride (48.4 mg, 0.191 mmol) and the mixture stirred at 100 °C under $N_2$ for 22 hours. The mixture was cooled to rt and hexane added, and the mixture filtered. The precipitate was redissolved in acetic anhydride (10 ml) and sodium acetate (7.5 mg, 0.191 mmol) added. The mixture was heated to 100 °C for 3.5 hours under $N_2$. The desired product was not isolated.

## Methyl 3,4-dibromomaleimide-1-carboxylate (103)[230]
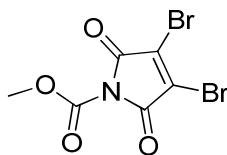


To a stirred solution of dibromomaleimide (1.00 g, 3.9 mmol) and 4-methylmorpholine (0.433 ml, 3.9 mol) in THF (40 ml) was added methyl chloroformate (0.304 ml, 3.9 mmol) and the mixture stirred at rt for 20 minutes. The mixture was diluted with dichloromethane (40 ml) and washed with water. The organic layer was dried and the solvent removed under reduced pressure to yield the product as a pale pink solid (1.14 g, 94.1%).

*Mp 129-131 °C. $^1$H-NMR (CDCl$_3$): δ 3.97 (s, 3H, CH$_3$) $^{13}$C-NMR (CDCl$_3$): δ 54.91 (CH$_3$), 131.57 (C-Br), 147.03 (C=OCH$_3$), 159.48 (C=O). MS: (ESI) m/z 335(100[M + H]+), HRMS: calcd. for C$_6$H$_3$Br$_2$NO$_4$Na: 333.8321 found 333.8325.*
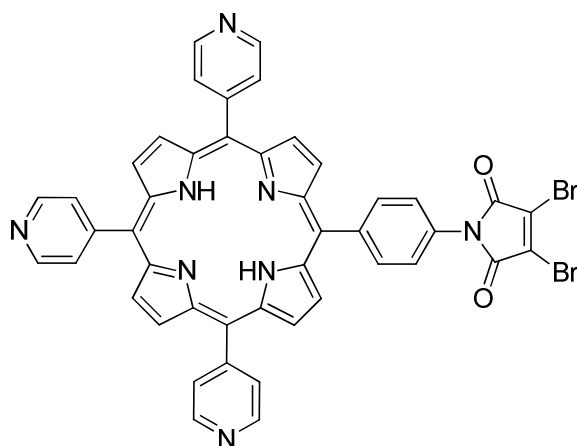
**5-[4-(3,4-Dibromomaleimide)phenyl]-10,15,20-triphenylporphyrin (104)**
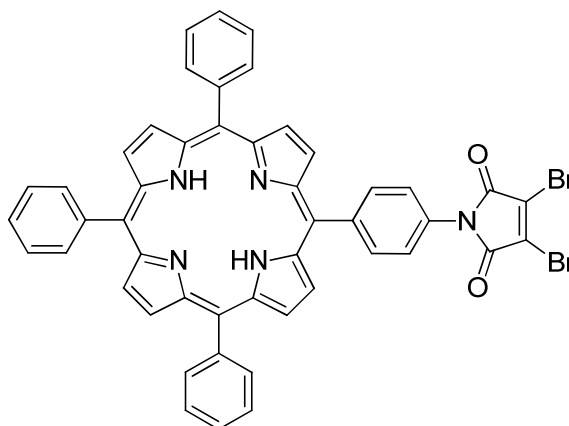


To a stirred solution of 5-[4-aminophenyl]-10,15,20-triphenylporphyrin (30 mg, 0.048 mmol) in DCM (5 ml) was added methyl 3,4-dibromomaleimide-1-carboxylate (14.8 mg, 0.064 mmol) and the mixture stirred at rt for 17 hours. The solvent was removed under reduced pressure and the crude purified by column chromatography (silica, 2% MeOH:DCM). The crude was precipitated from MeOH over DCM to yield the product as a purple solid (38 mg, 91.5%)

*UV-vis (DCM): λmax, nm 418, 514, 550, 590, 646. ε (418 nm)= 434530 $M^{-1}cm^{-1}$. $^1$H-NMR (CDCl$_3$): δ 7.69-7.95 (m, 11H, 5,10,15,20-m,p-Ph), 8.21-8.45 (m, 8H, 5,10,15,20-o-Ph), 8.82-8.93 (m, 8H, βH). $^{13}$C-NMR (CDCl$_3$): δ 113.27, 118.48, 120.53, 120.67, 124.09, 126.89 (β-C), 127.93, 130.20 (C-Br), 130.78, 134.73 (β-C), 135.31, 142.19, 142.58, 163.18 (C=O). MS: (MALDI) m/z 867(100[M + H]+).*

**5-[4-(3,4-Dibromomaleimide)phenyl]-10,15,20-tri-(4-pyridyl)porphyrin (105)**
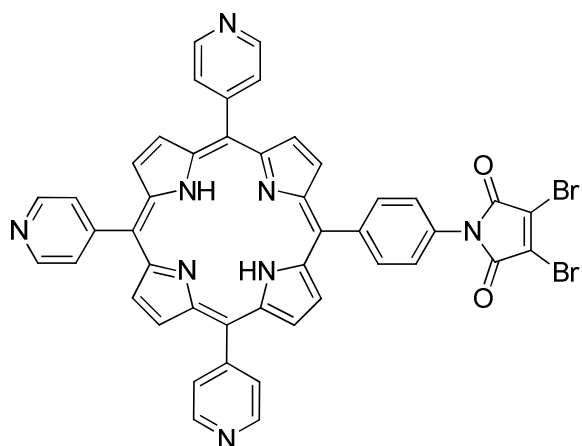


To a stirred solution of 5-[4-aminophenyl]-10,15,20-tri-4-pyridylporphyrin (61 mg, 0.096 mmol) in DMF (5 ml) was added methyl 3,4-dibromomaleimide-1-carboxylate (30 mg, 0.096 mmol) and the mixture stirred at 40°C for 17 hours. Diethyl ether was added and the mixture filtered. The crude porphyrin was purified by column chromatography (silica, 5% MeOH:DCM). The desired product was not isolated.

**5-[4-(3,4-Dibromomaleimide)phenyl]-10,15,20-(tris –N-methyl-4-pyridinium)porphyrin triiodide (106)**
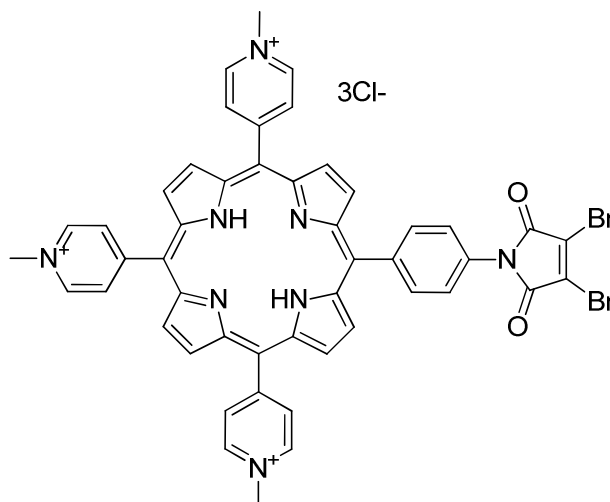


To a stirred solution of 5-[4-aminophenyl]-10,15,20-tri-4-pyridinium porphyrin trichloride (20.0 mg, 0.026 mmol) in DMF (5 ml) was added methyl 3,4-dibromomaleimide-1-carboxylate (20.0 mg, 0.048 mmol) and the mixture stirred overnight at rt. Diethyl ether was added and the mixture filtered. The desired product was not isolated.

**5-[4-(3,4-Dibromomaleimide)phenyl]-10,15,20-tris-(3,5-dihydroxyphenyl)porphyrin (107)**
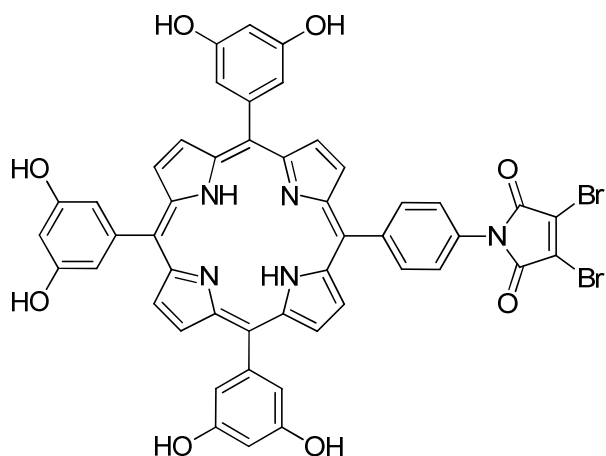


To a stirred solution of 5-[4-aminophenyl]-10,15,20-tris(3,5-dihydroxyphenyl)porphyrin (70 mg, 0.096 mmol) in DMF (5 ml) was added methyl 3,4-dibromomaleimide-1-carboxylate (30 mg, 0.096 mmol) and the mixture stirred at rt for 17 hours. Diethyl ether was added and the mixture filtered. The desired product was not isolated.

## 3,4-Dibromo-1-(prop-2-yn-1-yl)-maleimide (108)
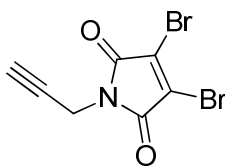


To a stirred solution of 2,3-dibromomaleic anhydride (0.50 g, 2.0 mmol) in acetic acid (10 ml) was added propargylamine (0.21 g, 4.0 mmol) and the mixture heated to 120 °C for 17 hours under $N_2$. The solvent was removed under reduced pressure and the crude purified by column chromatography (75% DCM:hexane) to yield the product as a white solid (0.214 g, 36.8%).

*Mp 120–121 °C. $^1$H-NMR (CDCl$_3$): δ 2.33 (m, 1H, C≡CH), 4.42 (m, 2H, CH$_2$, $^{13}$C-NMR (CDCl$_3$): δ 28.50 (CH$_2$), 72.52 (C≡CH), 76.00 (C≡CH), 129.69 (C-Br), 162.55 (C=O). MS: (ESI) m/z 293(100[M + H]+), HRMS: calcd. for $C_7H_4Br_2N_1O_2$: 291.8603 found 291.8605.*

**Propargyltetraethylene glycol (109)**
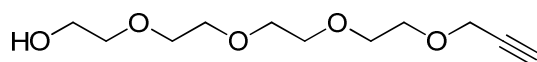


To a stirred solution of sodium hydride (60% in mineral oil, 1.16 g, 30 mmol) in dry THF (30 ml) at -20 °C was added slowly tetraethylene glycol (4.00 g, 20.6 mmol) and the mixture stirred until no more hydrogen was released. Propargyl bromide (80% in toluene, 3.00 ml, 29.3 mmol) was added dropwise to the solution and the mixture stirred overnight at rt under nitrogen. Methanol (5 ml) was added to the mixture and the solution filtered. The solvent was removed under reduced pressure and the crude purified by column chromatography (silica, 4.5% MeOH:DCM) to yield the product as a colourless oil (1.82 g, 38.1%).

$^1$*H-NMR (CDCl$_3$): δ 2.38 (s, 1H, C≡C$\underline{H}$), 3.45-3.66 (m, 16H), 4.05-4.13 (m, 2H, C$\underline{H}_2$-C).* $^{13}$*C-NMR (CDCl$_3$): δ 58.33 (C$\underline{H}_2$-C), 61.58 (C$\underline{H}_2$-OH), 69.06, 70.29, 70.35, 70.49, 70.53, 70.58, 72.59, 74.75 (C≡CH), 79.65 ($\underline{C}$≡CH). MS: (ESI) m/z 233 (100[M + H]+), HRMS: calcd. for C$_{11}$H$_{21}$O$_5$: 233.1384 found 233.1380.*

## 3,6,9,12-Tetraoxapentadec-14-yn-1-yl 4-methylbenzenesulfonate (110)
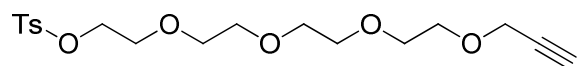


To a stirred solution of propargyl tetraethylene glycol (1.00 g, 4.31 mmol) in DCM was added triethylamine (1.2 ml, 8.6 mmol) and p-toluenesulfonyl chloride (0.99 g, 5.2 mmol) and the mixture stirred overnight at rt under nitrogen. The solvent was removed under reduced pressure and the crude purified by column chromatography (silica, 4% MeOH:DCM)to yield the product as a pale yellow oil (1.53 g, 91.9%).

$^1$H-NMR (CDCl$_3$): $\delta$ 2.40 (s, 4H, C≡C$\underline{H}$, C$\underline{H}_3$), 3.48-3.82 (m, 14H), 4.07-4.30 (m, 4H, C$\underline{H}_2$-C, CH$_2$), 7.30 (d, 2H, J=7.9 Hz), 7.55 (d, 2H, J=7.1 Hz). $^{13}$C-NMR (CDCl$_3$): $\delta$ 21.74 (C$\underline{H}_3$), 58.55 (C$\underline{H}_2$-C), 68.74, 69.14, 69.37, 70.42, 70.56, 70.63, 70.75, 74.68 (C≡CH), 79.74 (C≡CH), 128.01, 129.93, 133.02 (4-Ar), 144.89 (1-Ar). MS: (ESI) m/z 387 (100[M + H]+), HRMS: calcd. for C$_{18}$H$_{27}$O$_7$S$_1$: 387.1472 found 387.1464.

**Tert-butyl (2-(2-hydroxyethoxy)ethyl)carbamate (111)**

$$\text{Boc} - \overset{\text{H}}{\underset{}{\text{N}}} - \text{CH}_2\text{CH}_2 - \text{O} - \text{CH}_2\text{CH}_2 - \text{OH}$$
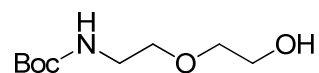
To a stirred solution of 2-(2-aminothoxy)ethanol (2.00 g, 19.0 mmol) in dichloromethane (100 ml) was added di-tert-butyl-dicarbonate (4.56 g, 21.0 mmol) and the mixture stirred at rt overnight. The solvent was removed under reduced pressure and the crude purified by column chromatography (silica, 2.5% MeOH:DCM) to yield the product as a colourless oil (3.42 g, 88.3%)

$^{1}$*H-NMR (CDCl$_3$): δ 1.39 (s, 9H, C(CH$_3$)$_3$), 3.02 (s, 1H, CH$_2$-OH), 3.24-3.31 (m, 2H, CH$_2$-NH), 3.47-3.56 (m, 4H), 3.68 (s, 2H, CH$_2$-OH). $^{13}$C-NMR (CDCl$_3$): δ 28.44 (C(CH$_3$)$_3$), 40.39 (CH$_2$-NH$_2$), 61.63 (CH$_2$-OH), 70.36 (C(CH$_3$)$_3$), 156.26 (C=O). MS: (ESI) m/z 206 (100[M + H]+), HRMS: calcd. for C$_9$H$_{20}$N$_1$O$_4$: 206.1387 found 206.1387.*

**Tert-butyl 3,6,9,12,15,18-hexaoxahenicos-20-yn-1-ylcarbamate (112)**
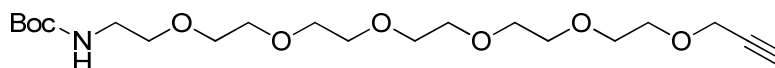


A stirred suspension of sodium hydride (60% dispersion in mineral oil, 168 mg, 4.20 mmol) in THF (40 ml) was cooled to 0 °C and tert-butyl (2-(2-hydroxyethoxy)ethyl)carbamate (0.57 g, 2.80 mmol) was added slowly. The mixture was stirred until gas evolution had ceased, and 3,6,9,12-tetraoxapentadec-14-yn-1-yl 4-methylbenzenesulfonate (0.12 g, 3.06 mmol)was added dropwise. The mixture was stirred overnight under $N_2$. Methanol (5 ml) was added, and the solid removed from the mixture by filtration. The crude was concentrated under reduced pressure and purified by column chromatography (silica, 5% MeOH:DCM) to yield the product as a colourless oil (804 mg, 68.5%).

$^1$H-NMR (CDCl$_3$): δ 1.38 (s, 9H, C(C$\underline{H}_3$)$_3$), 2.40 (s, 1H, C≡C$\underline{H}$), 3.45-3.52 (m, 4H), 3.55-3.70 (m, 20H), 4.12-4.16 (m, 2H, C$\underline{H}_2$-C≡CH). $^{13}$C-NMR (CDCl$_3$): δ 28.47 (C(C$\underline{H}_3$)$_3$), 40.41 (C$\underline{H}_2$-NH$_2$), 53.56 (C$\underline{H}_2$-C≡CH), 58.50, 59.08, 61.71, 69.15, 70.26 (C$\underline{C}$(CH$_3$)$_3$), 70.32, 70.43, 70.55, 70.60, 71.96, 72.28, 74.66 (C≡CH), 79.72 (C$\underline{C}$≡CH), 156.19 (C$\underline{C}$=O). MS: (ESI) m/z 320 (100[M + H]+), HRMS: calcd. for C$_{20}$H$_{38}$N$_1$O$_8$: 420.2592 found 420.2589.

**3,6,9,12,15,18-Hexaoxahenicos-20-yn-1-amine (113)**
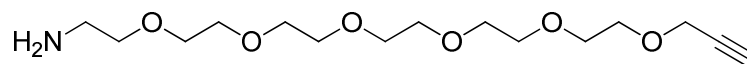


To a stirred solution of tert-butyl 3,6,9,12,15,18-hexaoxahenicos-20-yn-1-ylcarbamate (800 mg, 1.91 mmol) in ethyl acetate (5 ml) was added HCl in dioxane (4 M, 5 ml) and the mixture stirred overnight at rt. The solvent was removed under reduced pressure, and the product washed with diethyl ether. The crude was neutralised with triethylamine and purified by flash chromatography (silica, DCM-20:80:1 MeOH:DCM:TEA) to yield the product as a colourless oil (558 mg, 91.6%).

*MS: (ESI) m/z 320 (100[M + H]+), HRMS: calcd. for $C_{15}H_{30}N_1O_6$: 320.2068 found 320.2070.*

**3,4-Dibromo-1-(3,6,9,12,15,18-hexaoxahenicos-20-yn-1-yl)-1H-pyrrole-2,5-dione (114)**
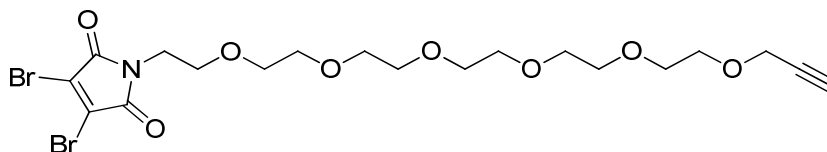


To a stirred solution of methyl 3,4-dibromomaleimide-1-carboxylate (50 mg, 0.16 mmol) in THF (20 ml) was added 3,6,9,12,15,18-hexaoxahenicos-20-yn-1-amine (51 mg, 0.16 mmol) and the mixture stirred overnight at rt. After this time, no reaction had occurred.

**2-(2-(2-Azidoethoxy)ethoxy)ethanol (115)**
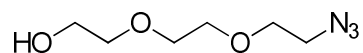


To a stirred solution of 2-(2-(2-chloroethoxy)ethoxy)ethanol (2.50 g, 14.8 mmol) in dry DMF (25 ml) was added sodium azide (1.45 g, 27.2 mmol) and the mixture heated to 100 °C for 17 hours. The suspension was cooled to rt and the solvent removed under reduced pressure. Ethyl acetate (100 ml) was added and the organic layer washed with water. The organic layer was dried (MgSO$_4$) and the solvent removed under reduced pressure to yield the product as a colourless oil (2.05 g, 79.1%).

*$^1$H-NMR (CDCl$_3$): δ 3.30-3.39 (m, 2H, CH$_2$-N$_3$), 3.52-3.58 (m, 2H), 3.59-3.62 (m, 6H), 3.65-3.70 (m, 2H). $^{13}$C-NMR (CDCl$_3$): δ 50.66 (CH$_2$-N$_3$), 61.66 (CH$_2$-OH), 70.02, 70.37, 70.64, 72.59. MS: (ESI) m/z 176(100[M + H]+), HRMS: calcd. for C$_6$H$_{14}$N$_3$O$_3$: 176.1030 found 176.1025.*

**1-Azido-3,6,9,12,15,18,21-heptaoxatetracos-23-yne (116)**
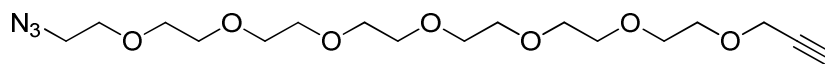


To a stirred solution of sodium hydride (60% in mineral oil, 168 mg, 2.8 mmol) in dry THF (30 ml) at -20 °C was added slowly 2-(2-(2-azidoethoxy)ethoxy)ethanol (0.50 g, 2.8 mmol) and the mixture stirred until no more hydrogen was released. A solution of 3,6,9,12-Tetraoxapentadec-14-yn-1-yl 4-methylbenzenesulfonate (1.00 g, 2.8 mmol) in dry THF (10 ml) was added slowly and the mixture allowed to return to rt. The solution was stirred at rt overnight under nitrogen. Methanol (5 ml) was added to the mixture and the solution filtered. The solvent was removed under reduced pressure and the crude purified by column chromatography (silica, 5% MeOH:DCM)to yield the product as a pale yellow oil (0.89 g, 81.7%).

$^{1}$*H-NMR (CDCl$_3$): δ 2.40-2.43 (m, 1H, C≡CH̲), 3.34-3.39(m, 2H, CH̲$_2$-N$_3$), 3.60-3.69 (m, 26H), 4.16-4.19 (m, 2H, CH̲$_2$-C). $^{13}$C-NMR (CDCl$_3$): δ  50.75 (C̲H$_2$-N$_3$), 58.47 (C̲H$_2$-C), 69.17, 70.10, 70.47, 70.65, 70.69, 70.73, 70.76, 74.64 (C≡C̲H), 79.74 (C̲≡CH). MS: (ESI) m/z 390 (100[M + H]+), HRMS: calcd. for C$_{17}$H$_{32}$N$_3$O$_7$: 390.2235 found 390.2229.*

**3,6,9,12,15,18,21-Heptaoxatetracos-23-yn-1-amine (117)**
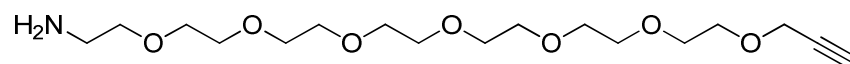


To a stirred solution of 1-azido-3,6,9,12,15,18,21-heptaoxatetracos-23-yne (545 mg, 1.4 mmol) in THF (20 ml) was added tributylphosphine (0.518 ml, 2.10 mmol) and the mixture stirred at rt for 1 hour under $N_2$. Water was added and the mixture stirred for 1 hour. The solvent was removed under reduced pressure and the crude purified by flash chromatography (silica, DCM-20:80:1 MeOH:DCM:TEA) to yield a colourless oil. The desired product was not isolated from O=PBu$_3$.

*MS: (ESI) m/z 364 (100[M + H]+), HRMS: calcd. for $C_{17}H_{34}NO_7$: 364.2348 found 364.2335.*

**3,4-Dibromo-1-(3,6,9,12,15,18,21-heptaoxatetracos-23-yn-1-yl)-1H-pyrrole-2,5-dione (118)**
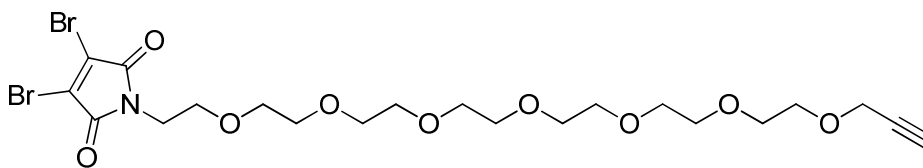


To a stirred solution of added methyl 3,4-dibromomaleimide-1-carboxylate (100 mg, 0.322 mmol) in THF (30 ml) was added 3,6,9,12,15,18,21-heptaoxatetracos-23-yn-1-amine (0.121 g, 0.322 mmol) and the mixture stirred overnight at rt. After this time, no reaction had occurred.

## 3,4-Dibromo-1-(2-(2-hydroxyethoxy)ethyl)-1H-pyrrole-2,5-dione (119)
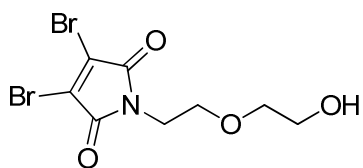


To a stirred solution of methyl 3,4-dibromomaleimide-1-carboxylate  (100 mg, 0.32 mmol) in THF (20 ml) was added 2-(2-aminothoxy)ethanol (33.6 mg, 0.32 mmol) and the mixture stirred overnight at rt. The solvent was removed under reduced pressure and the product purified by column chromatography (silica, 1-2% MeOH:DCM) to yield the product as a colourless oil (96 mg, 88.0%).

$^1$H-NMR (CDCl$_3$): δ 3.50-3.56(m, 2H, C<u>H</u>$_2$-OH), 3.60-3.67 (m, 4H), 3.76-3.82 (m, 2H, C<u>H</u>$_2$-N). $^{13}$C-NMR (CDCl$_3$): δ  39.25 (<u>C</u>-N), 61.66, 67.89, 72.20, 129.61 (C-Br), 164.07 (C=O).

**3,4-Dibromo-1-(3,6,9,12,15,18-hexaoxahenicos-20-yn-1-yl)-1H-pyrrole-2,5-dione (120)**
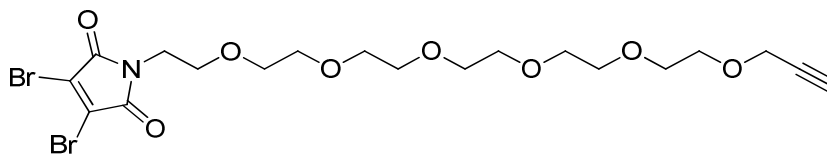


A stirred suspension of sodium hydride (60% dispersion in mineral oil, 17 mg, 0.420 mmol) in THF (10 ml) was cooled to 0 °C and 3,4-dibromo-1-(2-(2-hydroxyethoxy)ethyl)-1H-pyrrole-2,5-dione (95 mg, 0.279 mmol) was added slowly. The mixture was stirred until gas evolution had ceased, and 3,6,9,12-tetraoxapentadec-14-yn-1-yl 4-methylbenzenesulfonate (118 mg, 0.306 mmol) was added dropwise. The mixture was stirred overnight under $N_2$. Methanol (5 ml) was added, and the solid removed from the mixture by filtration. The desired product was not isolated.

**3,4-Dibromo-1-(3,6,9,12-tetraoxapentadec-14-yn-1-yl)-1H-pyrrole-2,5-dione (121)**
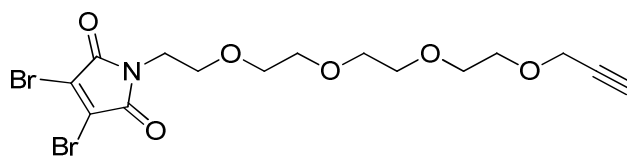


A solution of triphenylphosphine (0.43 g, 1.65 mmol) in anhydrous THF was cooled to -78 °C. Successive portions of diisopropyl azodicarboxylate (0.26 ml, 1.65 mmol), propargyltetraethylene glycol (0.383 g, 1.65 mmol) and dibromomaleimide (0.41 g, 1.65 mmol) were added, and the mixture was stirred at -78 °C for 5 minutes, then at rt overnight. The solvent was removed under reduced pressure and purification attempted by column chromatography (0-10% EtOAc:DCM). The desired product was not isolated.

## 3,4-Dibromo-1-(3,6,9,12-tetraoxapentadec-14-yn-1-yl)-1H-pyrrole-2,5-dione (121)
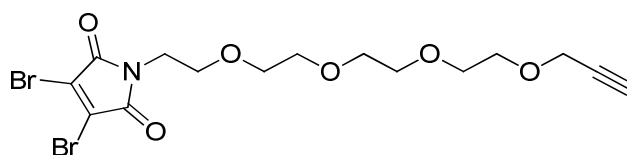


A solution of triphenylphosphine (0.43 g, 1.65 mmol) in anhydrous THF was cooled to -78 °C. Successive portions of di-p-chlorobenzyl azodicarboxylate (0.41 g, 1.65 mmol), propargyltetraethylene glycol (0.383 g, 1.65 mmol) and dibromomaleimide (0.41 g, 1.65 mmol) were added, and the mixture was stirred at -78 °C for 5 minutes, then at rt overnight. The solvent was removed under reduced pressure , and the crude redissolved in DCM, then filtered. The solvent was removed under reduced pressure, and the crude purified by column chromatography (silica, 0-10% EtOAc:DCM) to yield the product as a colourless oil (200 mg, 26.0%)

$^{1}$H-NMR (CDCl$_3$): δ 2.40-2.43 (m, 1H, C≡C<u>H</u>), 3.55-3.80 (m, 14H), 3.77-3.83 (m, 2H), 4.17-4.20 (m, 2H, <u>CH</u>$_2$-C). $^{13}$C-NMR (CDCl$_3$): δ 38.97 (<u>CH</u>$_2$-N), 58.53 (<u>CH</u>$_2$-C), 67.65 (<u>CH</u>$_2$-OH), 69.20, 70.14, 70.48, 70.65, 70.69, 74.63 (C≡<u>C</u>H), 79.76 (<u>C</u>≡CH), 129.52 (<u>C</u>-Br),163.90 (C=O). MS: (ESI) m/z 469 (100[M + H]+), HRMS: calcd. for C$_{15}$H$_{20}$Br$_2$N$_1$O$_6$: 467.9652  found 467.9651.

## 3,4-Dibromo-1-(3,6,9,12-tetraoxapentadec-14-yn-1-yl)-1H-pyrrole-2,5-dione (121)



A solution of triphenylphosphine (0.43 g, 1.65 mmol) in anhydrous THF was cooled to -78 °C. Successive portions of di-2-methoxyethyl azodicarboxylate (0.386 g, 1.65 mmol), propargyltetraethylene glycol (0.383 g, 1.65 mmol) and dibromomaleimide (0.41 g, 1.65 mmol) were added, and the mixture was stirred at -78 °C for 5 minutes, then at rt overnight. The mixture was diluted with DCM, washed with water and dried (MgSO$_4$). The solvent was removed under reduced pressure and the crude purified by column chromatography (silica, 0-10% EtOAc:DCM) to yield the product as a colourless oil (0.48 g, 62.3%).

$^1$H-NMR(CDCl$_3$): δ 2.40-2.43 (m, 1H, C≡C<u>H</u>), 3.55-3.80 (m, 14H), 3.77-3.83 (m, 2H), 4.17-4.20 (m, 2H, <u>CH</u>$_2$-C). $^{13}$C-NMR (CDCl$_3$): δ 38.97 (<u>CH</u>$_2$-N), 58.53 (<u>CH</u>$_2$-C), 67.65 (<u>CH</u>$_2$-OH), 69.20, 70.14, 70.48, 70.65, 70.69, 74.63 (C≡<u>C</u>H), 79.76 (<u>C</u>≡CH), 129.52 (<u>C</u>-Br),163.90 (C=O). MS: (ESI) m/z 469 (100[M + H]+), HRMS: calcd. for C$_{15}$H$_{20}$Br$_2$N$_1$O$_6$: 467.9652 found 467.9651.

**Propargylhexaethylene glycol (122)**
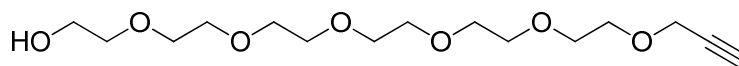


To a stirred solution of sodium hydride (60% in mineral oil, 0.78 g, 19.5 mmol) in dry THF (40 ml) at -20 °C was added slowly hexaethylene glycol (5.00 g, 17.6 mmol) and the mixture stirred until no more hydrogen was released. Propargyl bromide (80% in toluene, 2.20 ml, 19.5 mmol) was added dropwise to the solution and the mixture stirred overnight at rt under nitrogen. Methanol (5 ml) was added to the mixture and the solution filtered. The solvent was removed under reduced pressure and the crude purified by column chromatography (silica, 4% MeOH:DCM)to yield the product as a colourless oil (2.63 g, 42.1%).

$^1$*H-NMR(CDCl$_3$): δ 2.39-2.41 (m, 1H, C≡C$\underline{H}$), 3.52-3.57 (m, 2H, C$\underline{H}_2$-OH), 3.57-3.70 (m, 22H), 4.12-4.17 (m, 2H, C$\underline{H}_2$-C). $^{13}$C-NMR (CDCl$_3$): δ 58.44 (*$\underline{C}$H$_2$-C), 61.72 (*$\underline{C}$H$_2$-OH), 69.14, 70.38, 70.44, 70.62, 72.59, 74.66 (C≡$\underline{C}$H), 79.71 ($\underline{C}$≡CH). MS: (ESI) m/z 321 (100[M + H]+), HRMS: calcd. for C$_{15}$H$_{29}$O$_7$: 321.1908 found 321.1910.*
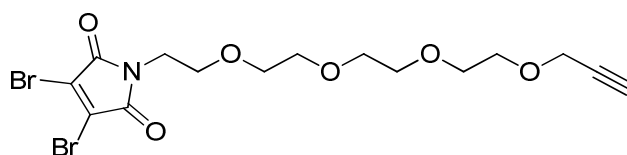
**3,4-Dibromo-1-(3,6,9,12,15,18-hexaoxahenicos-20-yn-1-yl)-1H-pyrrole-2,5-dione (123)**
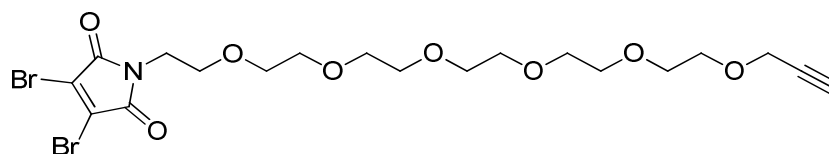


A solution of triphenylphosphine (0.43 g, 1.65 mmol) in anhydrous THF was cooled to -78 ˚C. Successive portions of di-2-methoxyethyl azodicarboxylate (0.386 g, 1.65 mmol), propargylhexaethylene glycol (0.528 g, 1.65 mmol) and dibromomaleimide (0.41 g, 1.65 mmol) were added, and the mixture was stirred at -78 ˚C for 5 minutes, then at rt overnight. The solvent was removed under reduced pressure and purification attempted by column chromatography (silica, 0-10% EtOAc:DCM). The desired product was not isolated.

**Zinc 5-(4-(3,4-dibromomaleimide-N-methyl(1-H-1,2,3-triazol-4-yl)phenyl)-10,15,20-tris-phenylporphyrin (124)**
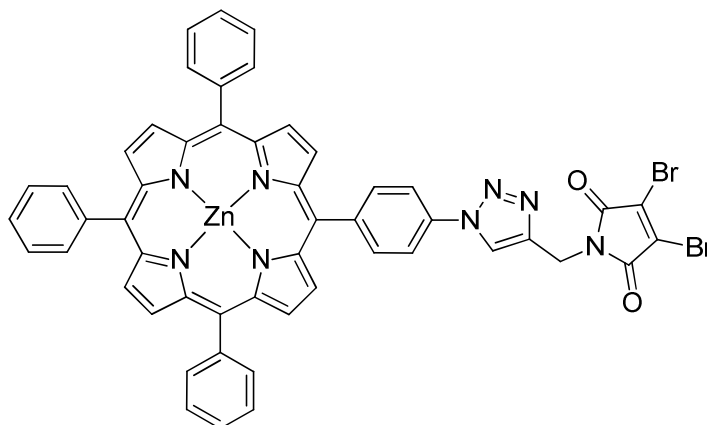


To a 10ml microwave tube was added zinc 5-[4-azidophenyl]-10,15,20-triphenylporphyrin (25 mg, 0.035 mmol) and 3,4-dibromo-1-(prop-2-yn-1-yl)-1H-pyrrole-2,5-dione (20 mg, 0.070 mmol) in THF:water (7:1, 8 ml). Copper (II) sulfate pentahydrate (2 mg) and sodium ascorbate (2 mg) in THF:water (1:1, 1 ml) was added, and the mixture heated to 80°C by MW (75W, max pressure 100 bar, max stirring) for 120 minutes. The solvent was removed under reduced pressure and the product purified by column chromatography (silica, 0-3% MeOH:DCM) to yield the product as a purple solid (14 mg, 39.7%).

*UV-vis (DCM): λmax, nm 420, 547, 586. ε (420 nm)= 455775 $M^{-1}cm^{-1}$. $^1H$-NMR(CDCl$_3$): δ 4.08 (s, 2H, CH$_2$-C), 7.69 (s, 1H, triazole H), 7.71-7.84 (m, 11H, 5,10,15,20-m,p-Ph), 8.21-8.36 (m, 8H, 5,10,15,20-o-Ph), 8.82-9.07 (m, 8H, βH). $^{13}C$-NMR (CDCl$_3$): δ 33.65 (CH$_2$), 118.46, 118.67(5-triazole), 120.87, 121.37, 121.55 (4-triazole), 126.66 (β-C), 127.59, 129.55 (C-Br), 131.38, 132.19, 132.26, 132.42, 134.66 (β-C), 135.51, 135.38, 141.89, 143.07, 144.29, 149.77, 150.31, 150.42, 150.50, 162.92 (C=O). MS: (MALDI) m/z 948(100[M + 2H - Zn]+).*

**Zinc 5-(4-(3,4-dibromomaleimide1-(1-2,5,8,11-tetraoxatridecan-13-yl))(1-H-1,2,3-triazol-4-yl)phenyl-10,15,20-tris-phenylporphyrin (125)**
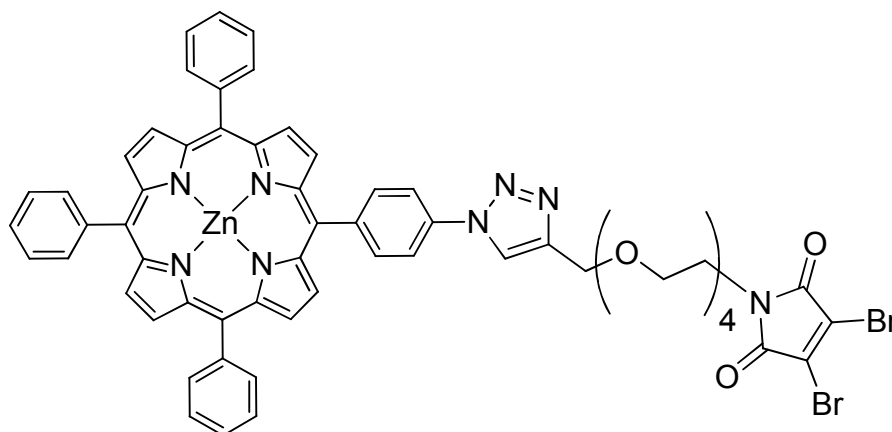


To a 10ml microwave tube was added zinc 5-[4-azidophenyl]-10,15,20-triphenylporphyrin (25 mg, 0.035 mmol) and 3,4-dibromo-1-(3,6,9,12-tetraoxapentadec-14-yn-1-yl)-1H-pyrrole-2,5-dione (33 mg, 0.070 mmol) in THF:water (7:1, 8 ml). Copper (II) sulfate pentahydrate (2 mg) and sodium ascorbate (2 mg) in THF:water (1:1, 1 ml) was added, and the mixture heated to 90°C by MW (75W, max pressure 100 bar, max stirring) for 40 minutes. The solvent was removed under reduced pressure and the product purified by column chromatography (silica, 1.5% MeOH:DCM) to yield the product as a purple solid (36.2 mg, 87.2%).

*UV-vis (DCM): λmax, nm 420, 548, 586. ε (420 nm)= 407736 $M^{-1}cm^{-1}$. $^1$H-NMR(CDCl₃): δ 3.37-4.07 (s, 12H), 4.64-5.19 (m, 4H), 6.50 (s, 3H, triazole H, 5-m-Ph), 7.04-7.39 (m, 2H, 5 -o-Ph), 7.71 (s, 9H, 10,15,20-m,p-H), 8.01-8.32 (m, 6H, 10,15,20-o-Ph), 8.54-9.07 (m, 8H, βH). $^{13}$C-NMR (CDCl₃): δ 33.44, 46.09, 64.71, 68.29, 70.09, 70.16, 70.74, 118.62 (5-triazole), 120.91, 121.12 (4-triazole), 126.28, 126.45 (β-C), 127.35, 128.66 (C-Br), 131.21, 131.91, 132.13, 134.67 (β-C), 135.74, 135.83, 136.88, 143.31, 149.66, 150.15, 150.24, 156.49, 167.18 (C=O).*

**Zinc 5-(4-(3,4-dibromomaleimide-N-methyl(1-H-1,2,3-triazol-4-yl)phenyl)-10,15,20-tris-N-methyl-4-pyridinium porphyrin trichloride (126)**
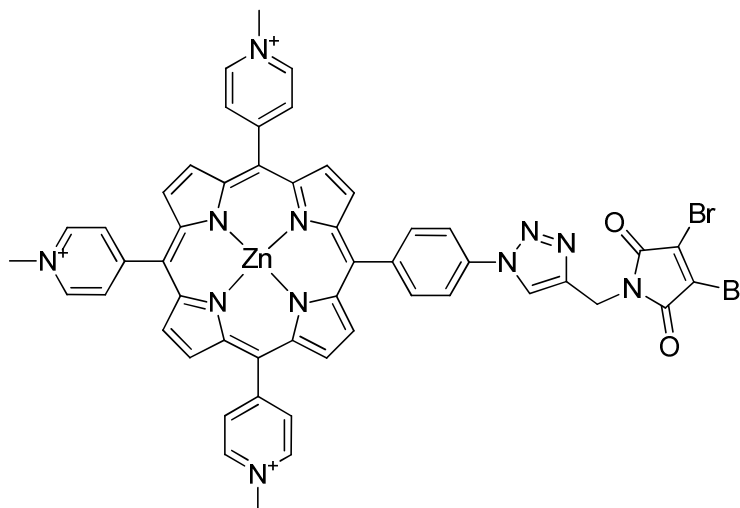


To a 10ml microwave tube was added zinc 5-[4-azidophenyl]-10,15,20-tri-(*N*-methyl-pyridinium4-yl)porphyrin trichloride (10 mg, 0.012 mmol) and 3,4-dibromo-1-(prop-2-yn-1-yl)-1H-pyrrole-2,5-dione (6 mg, 0.023 mmol) in THF:water (1:1, 8 ml). Copper (II) sulfate pentahydrate (2 mg) and sodium ascorbate (2 mg) in THF:water (1:1, 1 ml) was added, and the mixture heated to 45°C by MW (75W, max pressure 100 bar, max stirring) for 75 minutes. The mixture was concentrated under reduced pressure, and ammonium hexafluorophosphate added to the mixture. The resulting solution was filtered and the precipitate redissolved in acetone. Tetrabutylammonium chloride was added, and the resulting solution filtered. The crude was precipitated from diethyl ether over MeOH. The desired product was not isolated.

**Zinc 5-(4-(3,4-dibromomaleimide1-(1-2,5,8,11-tetraoxatridecan-13-yl) )(1-H-1,2,3-triazol-4-yl)phenyl-10,15,20-tris- N-methyl-4-pyridinium porphyrin trichloride (127)**
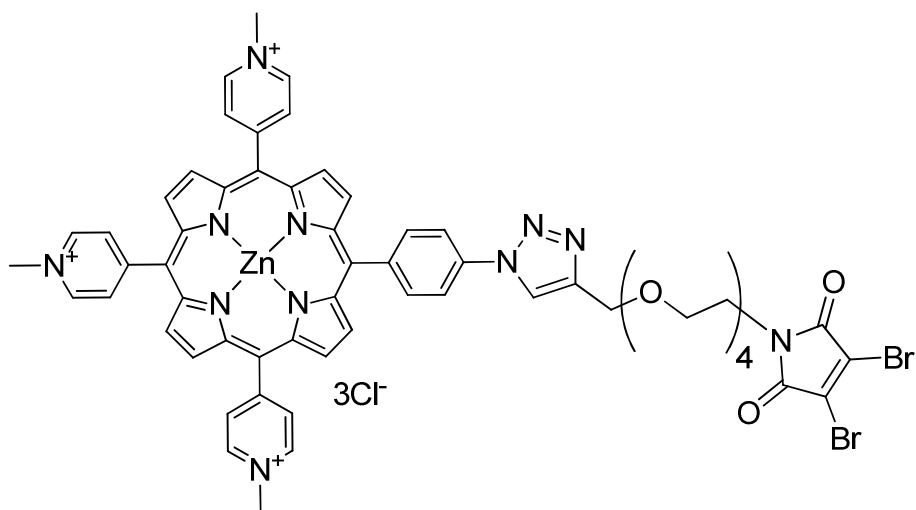


To a 10ml microwave tube was added zinc 5-[4-azidophenyl]-10,15,20-tri-(*N*-methyl-pyridinium4-yl)porphyrin trichloride (10 mg, 0.012 mmol) and 3,4-dibromo-1-(prop-2-yn-1-yl)-1H-pyrrole-2,5-dione (11 mg, 0.023 mmol) in THF:water (1:1, 8 ml). Copper (II) sulfate pentahydrate (2 mg) and sodium ascorbate (2 mg) in THF:water (1:1, 1 ml) was added, and the mixture heated to 45°C by MW (75W, max pressure 100 bar, max stirring) for 40 minutes. The mixture was concentrated under reduced pressure, and ammonium hexafluorophosphate added to the mixture. The resulting solution was filtered and the precipitate redissolved in acetone. Tetrabutylammonium chloride was added, and the resulting solution filtered. The crude was precipitated from diethyl ether over MeOH. The desired product was not isolated.

**Conjugate (128)**



Conjugate **94** (30 mg, 0.014 mmol) was stirred overnight with Quadrapure BZA (5 mg) in water:methanol (1:1) to remove residual copper. HCl$_{(aq)}$ (4 M, 0.5 ml) was added and the mixture stirred for 4 hours at rt. The mixture was neutralised with saturated sodium bicarbonate and ammonium hexafluorophosphate added to the mixture. The resulting solution was filtered and the precipitate redissolved in acetone. Tetrabutylammonium chloride was added, and the resulting solution filtered. The product was precipitated from diethyl ether over MeOH to yield the product as a green solid (20 mg, 83.1%).

*UV-vis (H$_2$O): λmax, nm 428, 521, 566. ε (428 nm)= 370084 M$^{-1}$cm$^{-1}$. $^1$H-NMR (DMSO-d$_6$): δ 4.71 (s, 18H, N-CH$_3$), 5.50 (s, 4H, CH$_2$), 8.37-8.53 (m, 8H, 5-o,m-Ph), 8.86-9.05 (m, 30H, 10,15,20-o-Ph, triazole H, βH), 9.40-9.57 (m, 12H, 10,15,20-m-Ph).*

**N-(2-(2-(2-Aminoethoxy)ethoxy)ethyl)-3,5-bis(prop-2-yn-1-yloxy)benzamide (129)**
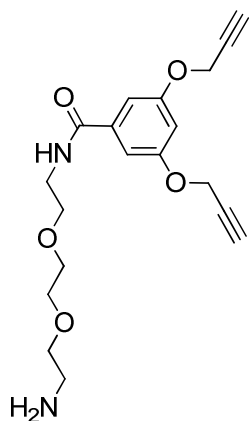


To a stirred solution of tert-butyl (2-(2-(2-(3,5-bis(prop-2-yn-1-yloxy)benzamido)ethoxy)ethoxy)ethyl)carbamate (0.60g, 1.30 mmol) in ethyl acetate (5 ml) was added HCl in dioxane (4 M, 2 ml) and the mixture stirred at rt overnight. The solvent was removed under reduced pressure and the residue washed with diethyl ether to yield the product as a yellow oil (0.41 g, 87.6%).

$^1$*H-NMR(CDCl$_3$): δ 2.60 (s, 2H, C≡CH), 3.07-3.60 (m, 6H), 3.49-3.60 (m, 8H), 4.67 (s, 4H, CH$_2$-C), 6.63 (s, 1H, 4-Ar-H), 7.09-7.15 (m, 2H, 2,6-Ar-H).* $^{13}$*C-NMR (CDCl$_3$): δ 39.73 (CH$_2$-NH), 40.05 (CH$_2$-NH$_2$),45.37, 47.27, 66.64, 69.72, 70.07, 70.18, 76.32 (C≡CH), 78.38 (C≡CH), 105.71 (4-Ar), 107.06 (2,6-Ar), 136.26 (1-Ar), 158.53 (3,5-Ar), 167.56 (C=O dendron). MS: (ESI) m/z 361 (100[M + H]+), HRMS: calcd. for C$_{19}$H$_{25}$N$_2$O$_5$: 361.1758 found 361.1759.*
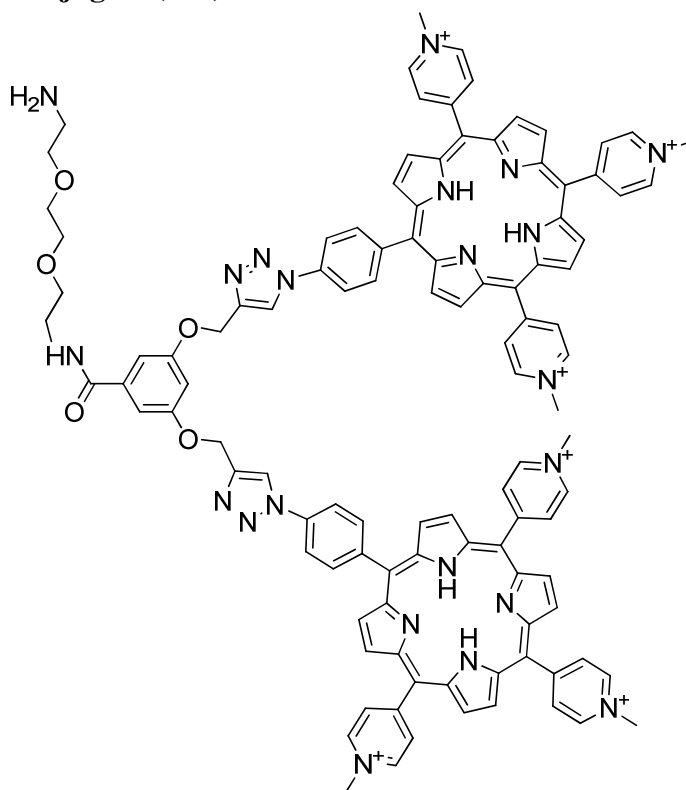
**Conjugate (130)**
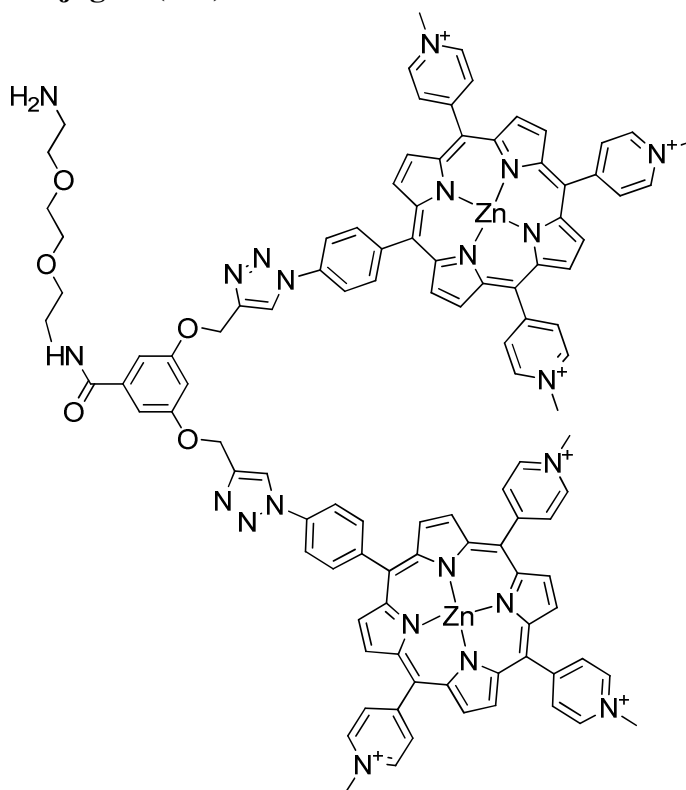


To a 10ml microwave tube was added zinc 5-[4-azidophenyl]-10,15,20-tri-(*N*-methyl-4-pyridinium)porphyrin trichloride (38 mg, 0.044 mmol) and 2-(2-(2-(2-aminoethoxy)ethoxy)ethyl)-3,5-bis(prop-2-yn-1-yloxy)benzamide (8 mg, 0.022 mmol) in tBuOH:water (1:1, 8 ml). Copper (II) sulfate pentahydrate (5 mg), sodium ascorbate (5 mg), and TBTA (1 mg) was added, and the mixture heated to 40°C by MW (75W, max pressure 100 bar, max stirring) for 30 minutes. The mixture was concentrated under reduced pressure, and ammonium hexafluorophosphate added to the mixture. The resulting solution was filtered and the precipitate redissolved in acetone. Tetrabutylammonium chloride was added, and the resulting solution filtered. The product was precipitated from diethyl ether over MeOH to yield the product as a green solid (30 mg, 71.4%).

*HPLC: 5-60% B over 15 minutes. $R_f$ = 11.5 minutes. UV-vis ($H_2O$): λmax, nm 435, 567, 617. ε (435 nm)= 281847 $M^{-1}cm^{-1}$. $^1$H-NMR(DMSO-$d_6$): δ 2.97 (m, 4H, N<u>H</u>), 3.62 (s, 10H), 4.65 (s, 2H), 4.71 (s, 18H, N-C<u>H</u>$_3$), 5.49 (s, 4H, C<u>H</u>$_2$), 7.16 (s, 2H, 2,5-Ar-H), 8.34-8.47 (m, 8H, 5-o,m-Ph), 8.85-9.05 (m, 30H, 10,15,20-o-Ph, triazole H, βH), 9.38-9.49 (m, 12H, 10,15,20-m-Ph). $^{13}$C-NMR (DMSO-$d_6$): δ 48.26, 65.49, 67.26, 70.09, 70.13, 99.99, 107.31, 116.08 (2,6-Ar), 122.15, 122.61, 132.25, 132.70 (β-C), 132.89, 133.70, 133.87, 143.02, 144.21 (β-C), 144.44, 144.59, 148.52, 148.80, 148.94, 150.56, 159.04 (C=O).*

**Conjugate (131)**
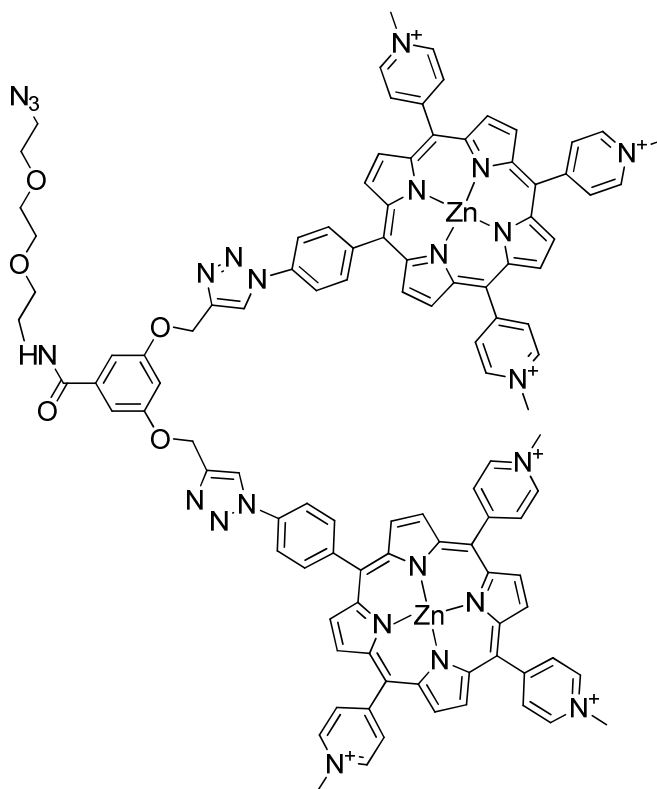


To a stirred solution of conjugate **130** (5 mg, 0.0026 mmol) in methanol (2 ml) was added potassium carbonate (1 mg, 0.0072 mmol) and imidazole-1-sulfonyl azide hydrogen sulfate (1 mg, 0.036 mmol), followed by copper (II) sulfate pentahydrate (10 µg, cat.), and the mixture was stirred overnight at rt. The desired product was not isolated.

**Conjugate (132)**
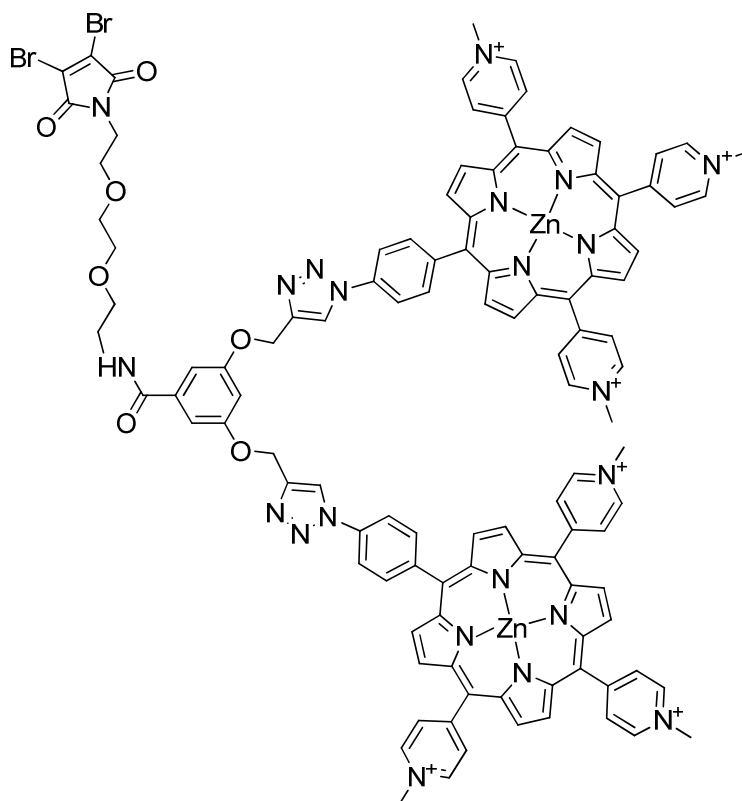


To a stirred solution of conjugate **130** (5 mg, 0.0026 mmol) in NMP:H₂O(1:1, 2 ml) was added methyl 3,4-dibromomaleimide-1-carboxylate (1 mg, 0.0032 mmol) and the mixture was stirred overnight at rt. The desired product was not isolated.

**Conjugate (133)**
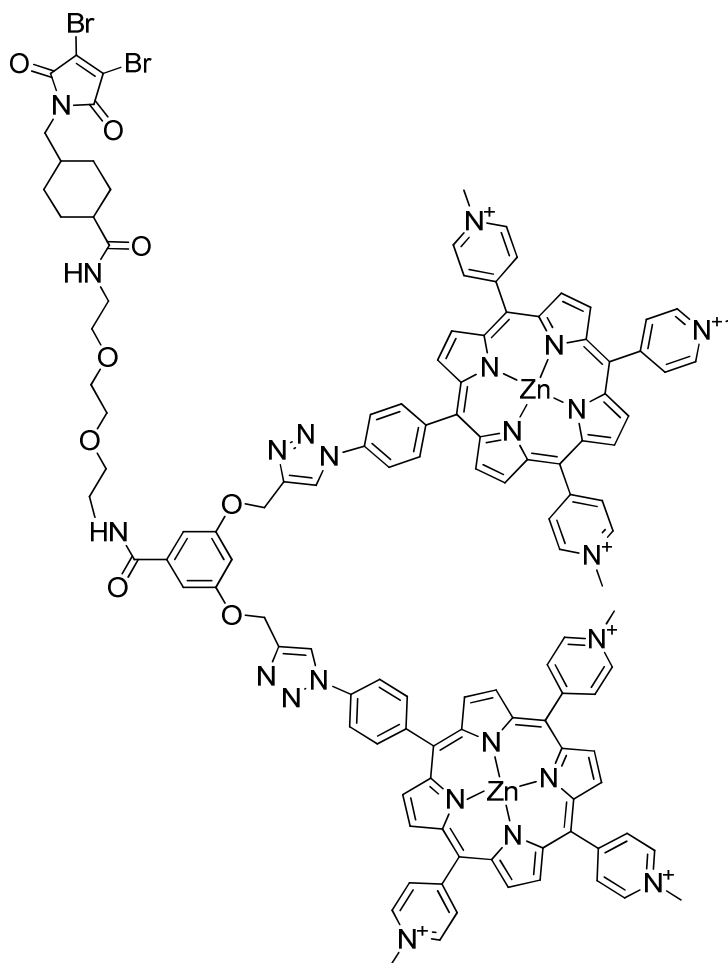


To a stirred solution of conjugate **130** (5 mg, 0.0026 mmol) in THF:H$_2$O(1:1, 2 ml) was added 2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl 4-((3,4-dibromo-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)methyl)cyclohexanecarboxylate (5 mg, 0.010 mmol) and potassium carbonate (1 mg, 0.0072 mmol) and the mixture was stirred overnight at rt. The desired product was not isolated.

**2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl 4-((3,4-dibromo-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)methyl)cyclohexanecarboxylate (134)[249]**
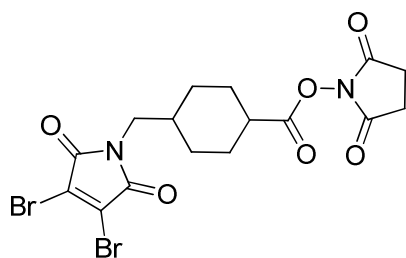


To a stirred solution of trans-4-(aminomethyl)cyclohexanecarboxylic acid (50 mg, 0.32 mmol) in DMF (10 ml) was added methyl 3,4-dibromomaleimide-1-carboxylate (100 mg, 0.32 mmol) and the mixture stirred overnight at rt. The solvent was removed under reduced pressure and the crude redissolved in THF (10 ml). N,N'-Dicyclohexylcarbodiimide (66.0 mg, 0.32 mmol) and N-hydroxysuccinimide (73.9 mg, 0.64 mmol) were added, and the mixture stirred overnight at rt. The mixture was filtered, and the solvent removed under reduced pressure. The crude was purified by column chromatography (silica, 5% EtOAc:DCM) to yield the product as a white solid (71 mg, 45.5%).

*Mp 243–244 °C. $^1$H-NMR (THF-$d_8$): δ 0.04-0.19 (m, 2H),0.46-0.60 (m, 2H), 0.82-0.89 (m, 2H), 1.09-1.17 (m, 2H), 1.58-1.69 (m, 1H), 1.77 (s, 4H, succinimide C<u>H</u>$_2$), 2.47 (d, 2H, J=7.1 Hz, C<u>H</u>$_2$-N). $^{13}$C-NMR (THF-$d_8$): δ 24.34 (succinimide <u>C</u>H$_2$), 27.18, 28.09, 35.45, 39.33, 43.85, 128.09 (C-Br), 162.98 (C=O dibromomaleimide), 168.02 (C=O). MS: (ESI) m/z 490 (100[M + H]+), HRMS: calcd. for $C_{16}H_{17}Br_2N_2O_6$: 490.9448 found 490.9442.*

**Tert-butyl (1-(3,5-bis(prop-2-yn-1-yloxy)phenyl)-1-oxo-5,8,11,14,17,20,23,26,29,32,35-undecaoxa-2-azaheptatriacontan-37-yl)carbamate (135)**
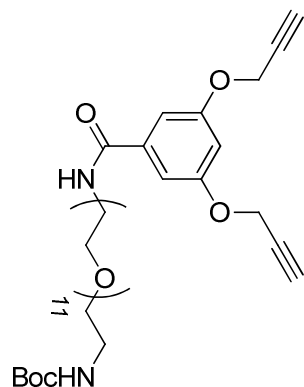


To a stirred solution of 3,5-bis(prop-2-yn-1-yloxy)benzoic acid (0.126 g, 0.55 mmol) in dichloromethane (50 ml) was added *O*-(2-Aminoethyl)-*O'*-[2-(Boc-amino)ethyl]decaethylene glycol (0.355 g, 0.55 mmol). DIPEA (0.18 ml, 1.2 mmol) was added, followed by addition of PyBOP (0.285 g, 0.55 mmol), and the mixture was stirred at rt overnight. The solvent was removed under reduced pressure and the crude purified by column chromatography (silica, 4% MeOH:DCM) to yield the product as a colourless oil (0.380 g, 80.7%)

*1H-NMR (CDCl3): δ 1.31 (s, 9H, C(CH3)3), 2.51 (s, 2H, C≡CH), 3.13-3.20 (m, 2H, CH2-NHBoc), 3.38-3.43 (m, 2H, CH2-NH-C=O), 3.46-3.58 (m, 44H), 4.52-4.67 (m, 4H, CH2-C), 6.60 (s, 1H, 4-Ar-H), 6.95-6.98 (m, 2H, 2,6-Ar-H). 13C-NMR (CDCl3): δ 28.50 (C(CH3)3), 39.68 (CH2-NH), 42.68 (CH2-NH), 54.54 (CH2-C), 56.04, 69.76, 69.81, 69.86, 69.98, 70.04,70.07, 70.10, 70.18, 70.21, 70.25, 70.38, 76.14 (C≡CH), 78.18 (C≡CH), 79.08 (C(CH3)3), 105.52 (4-Ar), 106.78 (2,6-Ar), 136.83 (1-Ar), 156.34 (C=O Boc), 158.71 (3,5-Ar), 167.13 (C=O dendron). MS: (ESI) m/z 874 (100[M + NH4]+), HRMS: calcd. for C42H72N3O16: 874.4907 found 874.4902.*

**N-(35-amino-3,6,9,12,15,18,21,24,27,30,33-undecaoxapentatriacontyl)-3,5-bis(prop-2-yn-1-yloxy)benzamide (136)**
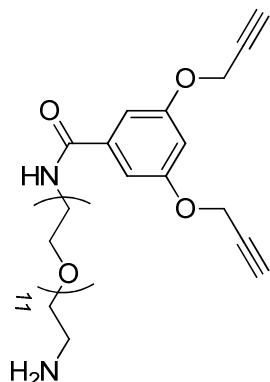


To a stirred solution of tert-butyl (2-(2-(2-(3,5-bis(prop-2-yn-1-yloxy)benzamido)ethoxy)ethoxy)ethyl)carbamate (0.38 g, 0.44 mmol) in ethyl acetate (5 ml) was added HCl in dioxane (4 M, 2 ml) and the mixture stirred at rt overnight. The solvent was removed under reduced pressure and the residue washed with diethyl ether to yield the product as a yellow oil (0.32 g, 95.5%).

$^1$H-NMR (CDCl$_3$): δ 2.48-2.52 (m, 2H, C≡C*H*), 3.66-3.75 (m, 2H), 3.46-3.62 (m, 46H), 4.59-4.65 (m, 4H, C*H$_2$*-C), 6.59 (s, 1H, 4-Ar-H), 7.01-7.04 (m, 2H, 2,6-Ar-H). $^{13}$C-NMR (CDCl$_3$): δ 39.77 (*C*H$_2$-NH), 40.09, 42.34 (*C*H$_2$-NH), 54.00 (*C*H$_2$-C), 56.19, 66.78, 69.56, 69.79, 69.86, 70.02, 70.05, 70.12, 70.16, 70.22, 70.27, 70.31, 70.36, 70.41, 70.47, 76.12 (C≡CH), 78.24 (*C*≡CH), 105.51 (4-Ar), 106.84 (2,6-Ar), 136.79 (1-Ar), 158.57 (3,5-Ar), 166.95 (C=O dendron). MS: (ESI) m/z 757 (100[M + H]+), HRMS: calcd. for C$_{37}$H$_{61}$N$_2$O$_{14}$: 757.4117 found 757.4107.

**Conjugate (137)**
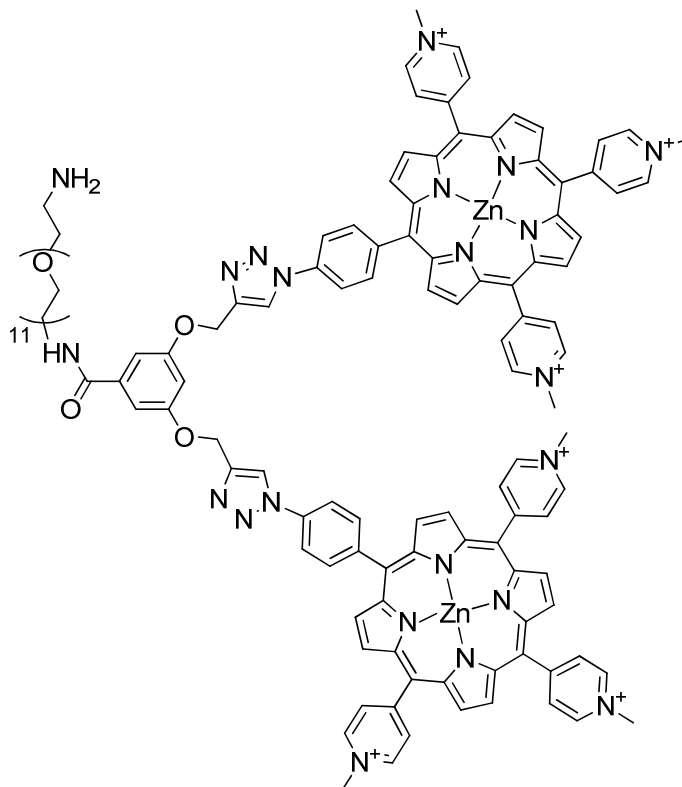


To a 10ml microwave tube was added zinc 5-[4-azidophenyl]-10,15,20-tri-(*N*-methyl-4-pyridinium)porphyrin trichloride (38 mg, 0.044 mmol) and *N*-(35-amino-3,6,9,12,15,18,21,24,27,30,33-undecaoxapentatriacontyl)-3,5-bis(prop-2-yn-1-yloxy)benzamide (19 mg, 0.022 mmol) in tBuOH:water (1:1, 8 ml). Copper (II) sulfate pentahydrate (5 mg), sodium ascorbate (5 mg), and TBTA (1 mg) was added, and the mixture heated to 40°C by MW (75W, max pressure 100 bar, max stirring) for 1 hour. The mixture was concentrated under reduced pressure, and ammonium hexafluorophosphate added to the mixture. The resulting solution was filtered and the precipitate redissolved in acetone. Tetrabutylammonium chloride was added, and the resulting solution filtered. The product was precipitated from diethyl ether over MeOH to yield the product as a green solid (47 mg, 82.5%).

*HPLC: 5-60% B over 25 minutes. $R_f$ = 14.9 minutes. UV-vis (H$_2$O): λmax, nm 435, 567, 615. ε (435 nm)= 369545 M$^{-1}$cm$^{-1}$. $^1$H-NMR(DMSO-d$_6$): δ 3.49 (s, 48H), 4.71 (s, 18H, N-C$\underline{H_3}$), 5.48 (s, 4H, C$\underline{H_2}$), 7.15 (s, 1H, 4-Ar),7.37 (s, 2H, 2,5-Ar-H), 8.43 (s, 8H, 5-o,m-Ph), 8.82-9.07 (m, 30H, 10,15,20-o-Ph, triazole H, βH), 9.34-9.47 (m, 12H, 10,15,20-m-Ph). $^{13}$C-NMR (DMSO-d$_6$): δ 48.28, 62.12, 65.49, 69.39, 70.16, 70.30, 115.42 (2,6-Ar), 116.13, 119.05, 122.18, 122.84, 123.84, 132.28, 132.72 (β-C), 132.91, 133.69, 133.87, 135.87, 136.83 (1-Ar), 143.00, 144.19 (β-C), 144.61, 148.52, 148.78, 148.94, 150.59, 159.77(3,5-Ar), 166.29 (C=O dendron).*

**Conjugate (138)**
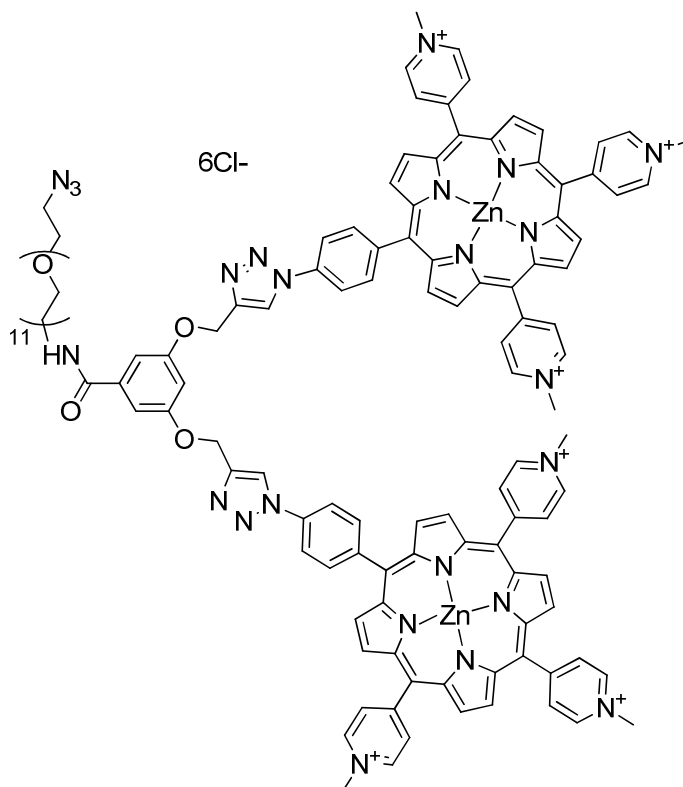


To a stirred solution of conjugate **137** (15 mg, 0.0066 mmol) in methanol:water (1:1, 2 ml) was added potassium carbonate (5 mg, 0.036 mmol) and imidazole-1-sulfonyl azide hydrogen sulfate (5 mg, 0.036 mmol), followed by copper (II) sulfate pentahydrate (10 µg, cat.), and the mixture was stirred overnight at rt. The mixture was concentrated under reduced pressure, and ammonium hexafluorophosphate added to the mixture. The resulting solution was filtered and the precipitate redissolved in acetone and the solid removed by filtration. Tetrabutylammonium chloride was added, and the resulting solution filtered. The product was precipitated from diethyl ether over MeOH to yield the product as a green solid (12 mg, 80.0%)

*HPLC: 5-60% B over 25 minutes. $R_f$ = 16.1 minutes. UV-vis ($H_2O$): λmax, nm 436, 567, 616. ε (436 nm)= 405034 $M^{-1}cm^{-1}$. $^1$H-NMR(DMSO-$d_6$): δ 3.48 (s, 48H), 4.71 (s, 18H, N-C$\underline{H}_3$), 5.49 (s, 4H, C$\underline{H}_2$), 7.16 (s, 1H, 4-Ar),7.37 (s, 2H, 2,5-Ar-H), 8.38-8.48 (s, 8H, 5-o,m-Ph), 8.76 (s, 2H, triazole), 8.85-9.05 (m, 28H, 10,15,20-o-Ph, βH), 9.38-9.50 (m, 12H, 10,15,20-m-Ph). $^{13}$C-NMR (DMSO-$d_6$): δ 48.23, 50.52, 62.20, 69.77, 70.31, 107.28, 116.11 (2,6-Ar), 116.48, 118.78, 119.00, 122.12, 132.20, 132.68 (β-C), 132.87, 133.66, 135.83, 136.84 (1-Ar), 144.23 (β-C), 144.67, 148.52, 148.78, 148.94, 150.59, 159.05, 159.82 (3,5-Ar), 166.24 (C=O dendron).*

**Conjugate (139)**
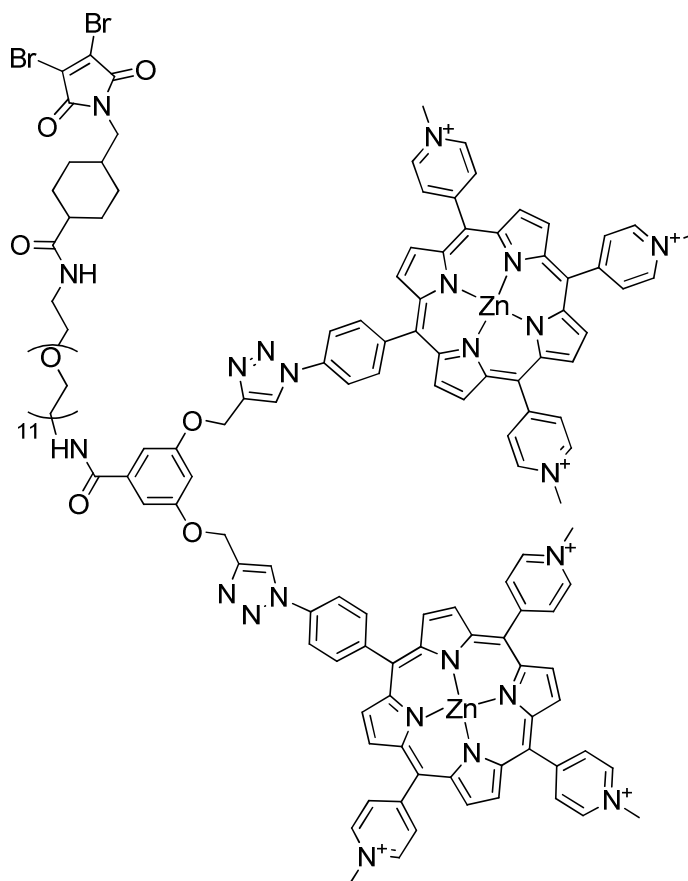


To a stirred solution of conjugate 137 (15 mg, 0.0066 mmol) in DMSO (2 ml) was added potassium carbonate (5 mg, 0.036 mmol) and 2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl 4-((3,4-dibromo-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)methyl)cyclohexanecarboxylate (18 mg, 0.036 mmol), and the mixture was stirred overnight at rt. The mixture was concentrated under reduced pressure, and ammonium hexafluorophosphate added to the mixture. The resulting solution was filtered and the precipitate redissolved in acetone and the solid removed by filtration. Tetrabutylammonium chloride was added, and the resulting solution filtered. The desired product was not isolated.

**Conjugate (140)**
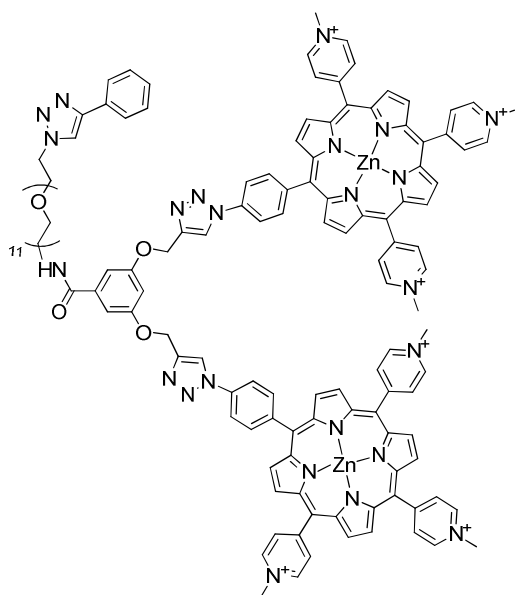


To a 10ml microwave tube was added conjugate **138** (10 mg, 3.9 x 10$^{-4}$ mmol) and phenyl acetylene (30 mg, 0.29 mmol) in THF:water (1:3, 8 ml). Copper (II) sulfate pentahydrate (5 mg), sodium ascorbate (5 mg), and TBTA (1 mg) was added, and the mixture heated to 45°C by MW (75W, max pressure 100 bar, max stirring) for 3 hours. The desired product was not isolated.

**Conjugate (141)**
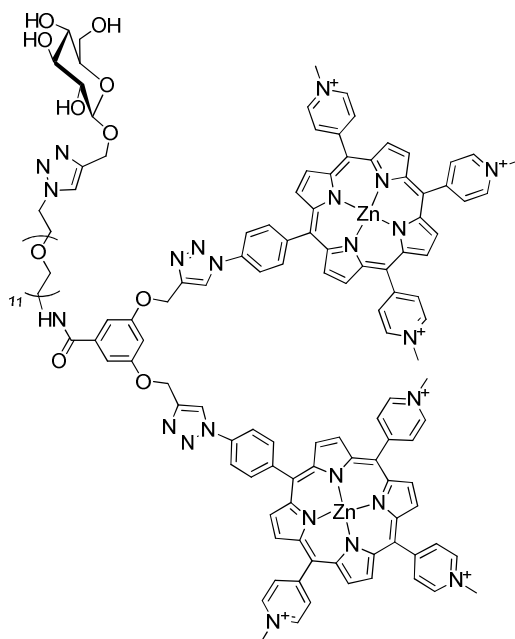


To a stirred solution of conjugate **138** (20 mg, 7.8 x 10$^{-4}$ mmol) and propargylglucose (5 mg, 0.0024 mol) in water (10 ml). was added copper (II) sulfate pentahydrate (5 mg), sodium ascorbate (5 mg), and TBTA (1 mg) was added, and the mixture stirred overnight at rt. The mixture was concentrated under reduced pressure, and ammonium hexafluorophosphate added to the mixture. The resulting solution was filtered and the precipitate redissolved in acetone. Tetrabutylammonium chloride was added, and the resulting solution filtered. The product was precipitated from diethyl ether over MeOH.

*HPLC: 5-60% B over 25 minutes. $R_f$ = 15.2 minutes.*

## 6.2.Bioconjugation of porphyrins and Fab fragments

Fab synthesis, reduction and functionalisation, all linker chain and porphyrin conjugation to Fab fragments, and analysis of these conjugates was carried out by Antoine Maruani, University College London.

**Preparation of trastuzumab Fab fragment (11) using sequential digests with pepsin and papain**

Immobilized pepsin (0.15 ml) was washed with digestion buffer (20 mM sodium acetate trihydrate, pH 3.1) four times and trastuzumab (0.5 ml, 6.41 mg/ml in digestion buffer) was added. The mixture was incubated for 5 h at 37 °C whilst shaking (1100 rpm). The resin was separated from the digest using a filter column, and washed with digest buffer (50 mM phosphate, 1 mM EDTA, 150 mM NaCl, pH 6.8) three times. The digest was combined with the washes and the volume adjusted to 0.5 ml. The sample was analysed by LCMS and revealed formation of trastuzumab-F(ab')$_2$, LCMS observed mass: 97303.
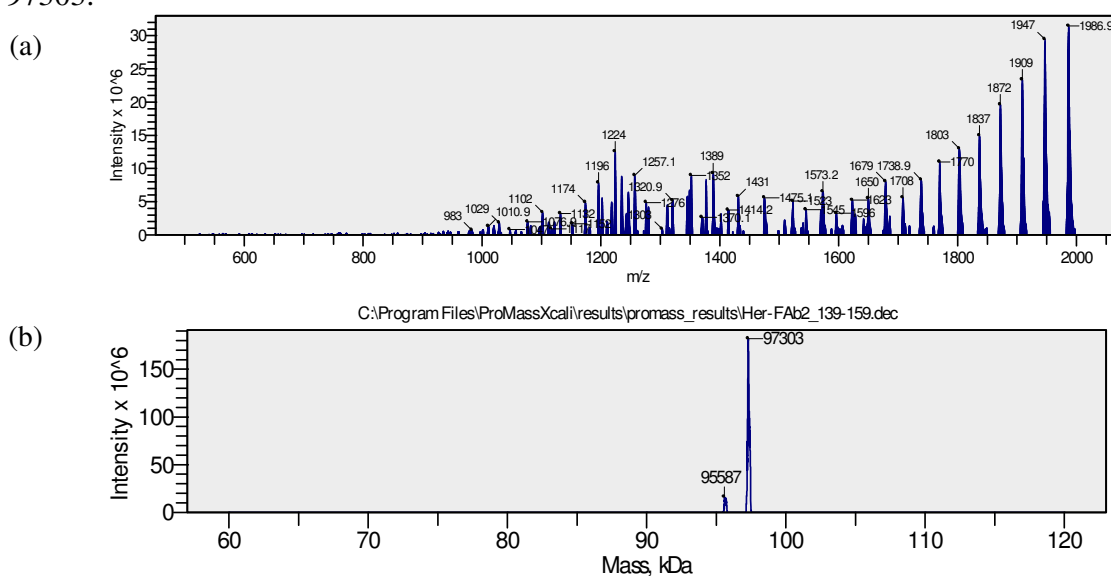


**Figure S1.** (a) non-deconvoluted and (b) deconvoluted MS data for digestion of trastuzumab with pepsin to afford trastuzumab-F(ab')$_2$.

After this, papain (1 ml, 0.25 mg/ml) was activated with 10 mM DTT (in digest buffer: 50 mM phosphate, 1 mM EDTA, 150 mM NaCl, pH 6.8) under an argon atmosphere whilst shaking (1100 rpm) for 1 h at 25 °C in the dark. The resin was washed with digest buffer (without DTT) four times and the 0.5 mL of Herceptin-F(ab')$_2$ added. The mixture was incubated for 16 h at 37 °C whilst shaking (1100 rpm) in the dark. Then the resin was separated from the digest using a filter column, and washed with PBS (pH 7.0) three times. The digest was combined with the washes and the buffer was exchanged completely for PBS (pH 7.4) using diafiltration columns (10 KDa MWCO) and the volume adjusted to 0.4 ml. The digest was analysed by SDS-PAGE and LCMS to reveal formation of a single Fab fragment: observed mass 47644. The concentration of Fab fragment was determined by UV/Vis using a molecular extinction coefficient of $\varepsilon_{280} = 68590$ M$^{-1}$.cm$^{-1}$. [Fab] 3.33 mg/ml (0.4 ml), 64%.

(a)

(b)

C:\Program Files\ProMassXcali\results\promass_results\Fab-Her_138-148.dec

341
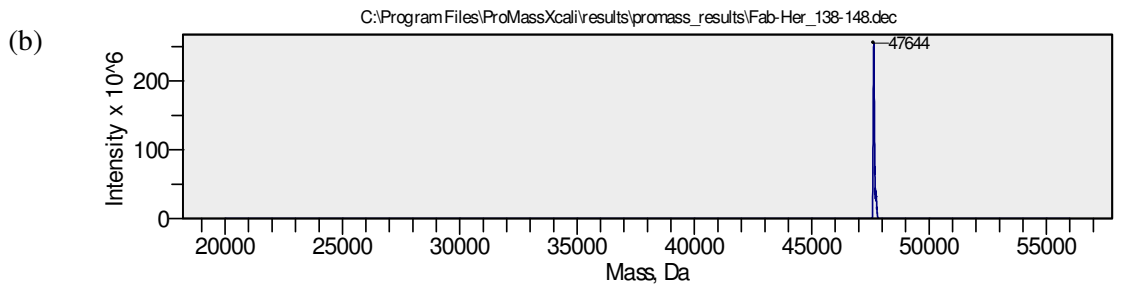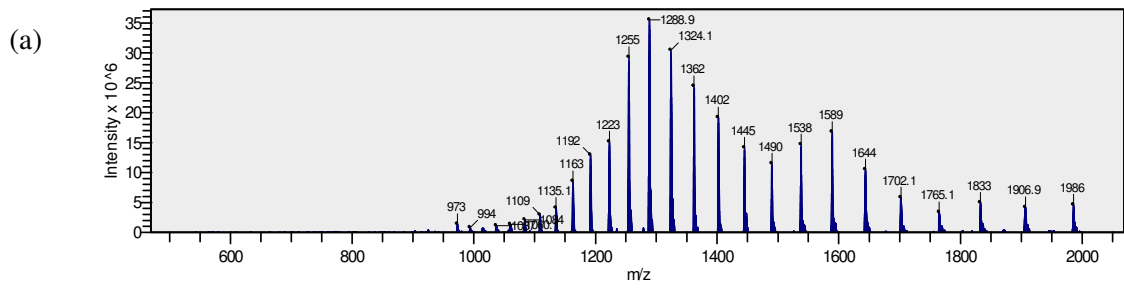
**Reduced trastuzumab Fab**

To a solution of Fab fragment (50 μl, 1.72 mg/ml, 25 mM sodium borate, 25 mM NaCl, 1 mM EDTA, pH 8.0) was added TCEP (15μl, 0.36 mM) to affect reduction of the interchain disulfide. After 1.5 h at 37 °C, the reaction mixture was analysed by LCMS to reveal the heavy and light chains only (*i.e.* the reduced fragment).

(a)
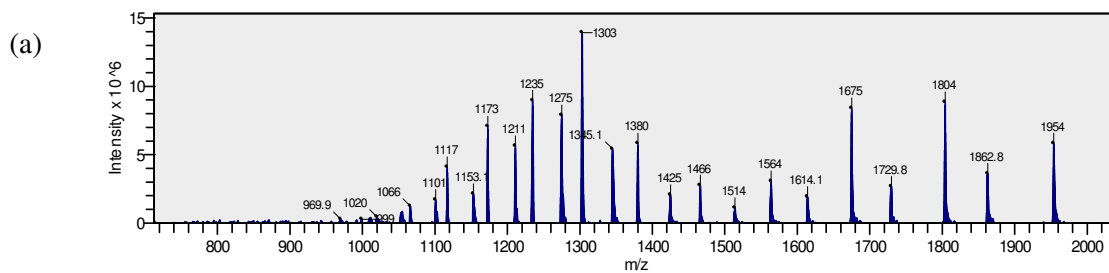
**General procedure for the preparation of the Her-Fab-Maleimide conjugate (Her-Fab-Mal)**

To a solution of Her-Fab (50 μl, 30 μM, 1.4 mg/ml, 1 eq) in borate buffer (25 mM sodium borate, 25 mM NaCl, 1 mM EDTA, pH 8.0) was added TCEP (final concentration 90 μM, 3 eq) and the reaction mixture incubated at 37 °C for 90 min. After this time, was added a solution of maleimide in DMF (final concentration 1.5 mM, 5 eq) and the reaction mixture incubated at 37 °C for 1 h. Following this, analysis by LCMS revealed >99% conversion to the conjugate. The excess reagents were then removed by repeated diafiltration into fresh buffer using VivaSpin sample concentrators (GE Healthcare, 10,000 MWCO).

Fab Her + MalAlk

C:\Program Files\ProMassXcali\results\promass_results\Fab_Her_MalAlk_136-152.dec
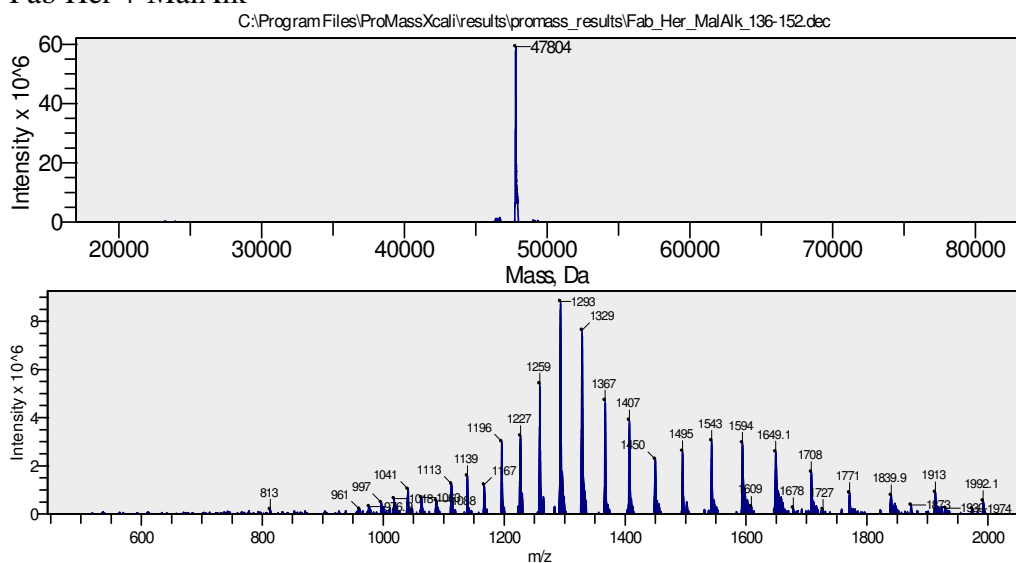
**General procedure for Azide-Alkyne Huisgen Cycloaddition (CuAAC)**

To a solution of 'clickable'-Her-Fab-Mal (50 μL, 21 μM, 1 mg/ml) in PBS (pH 7.4) containing tris(3-hydroxypropyltriazolylmethyl)amine[251] (THPTA) (500 μM), CuSO$_4$ (100 μM), aminoguanidine (5 mM) was added a cargo molecule (azide) (final concentration 150 μM, 3 eq) and sodium ascorbate (final concentration 5 mM) and the reaction mixture incubated at 25 °C for 1 h. Following this, analysis by LCMS revealed >99% conversion to the conjugate. The excess reagents were then removed by repeated diafiltration into fresh buffer using VivaSpin sample concentrators (GE Healthcare, 10,000 MWCO).

Fab Her-**17**
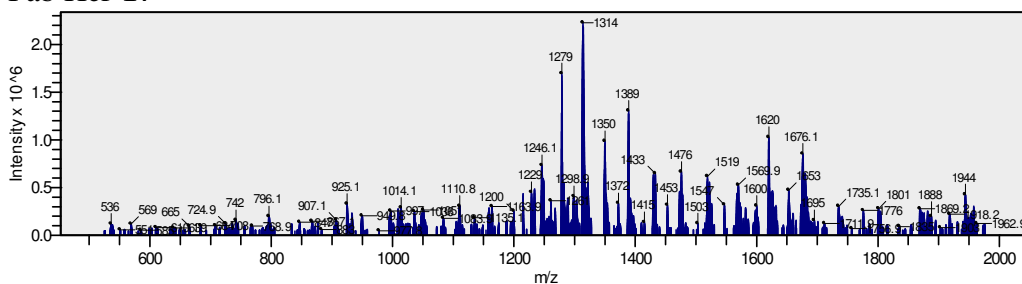


Fab Her-**32**
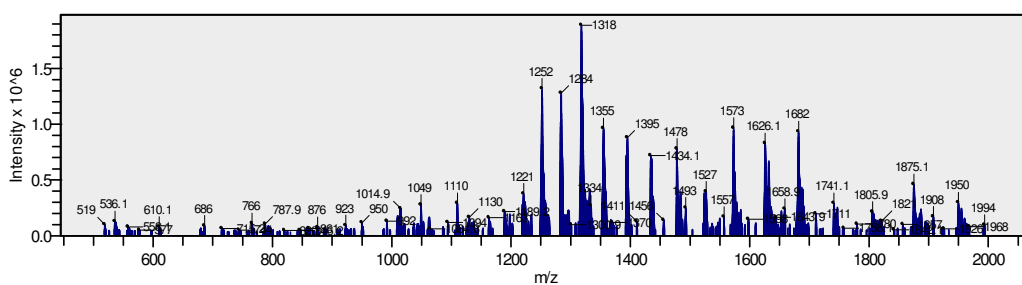
### 6.3.Biological evaluation

All cytotoxicity assays were carried out and analysed by Huguette Savoie.

Materials :

Medium:      DMEM (Gibco 10938) and

Nutrient Mixture F-12 Ham (Sigma N4888)

L-Glutamine (Gibco 25030)

Fetal Bovine Serum (BioSera S1810)


MTT (Thiazolyl blue; Sigma M5655)

Light Source: Oriel 1000W Quartz Tungsten Halogen Lamp


#### 6.3.1.1. *Protocol for preliminary cytotoxicity experiment*

The 2 porphyrin-antibodies conjugates were made up to a volume of 320µl with a 1:1 mixture of DMEM and Ham F12 media supplemented with 2mM L-glutamine. 160µl of each diluted conjugate was added to 640µl of either BT-474 (HER2 negative) or MDA-MB-468 (HER2 positive) cells adjusted to a concentration of $1 \times 10^6$ cells /ml. The cells were incubated in the dark for an hour at 37 $^o$C and 5% $CO_2$, after which they were washed in a 3x excess of medium to eliminate any unbound porphyrin-antibody conjugate. The pellets of cells were re-suspended in 800 µl of the above mentioned medium and 4 x 100 µl of each concentration was put in two 96 well plates. One plate was irradiated with white light to a dose of 20 J/cm$^2$ (2 x 14 minutes with a 'rest" period of 10 minutes in the incubator in between) while the other served as dark control. After irradiation was completed, 5 µl of Fetal Bovine Serum was added to each well and the plates were returned to the incubator overnight. After 18 to 24 hours, an MTT cell viability assay was performed (see below) and the results expressed as% of cell viability versus the porphyrin-antibody conjugate concentration.

#### 6.3.1.2. *MTT assay*

The cell viability is determined using MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyltetrazolium bromide) colorimetric assay. Briefly: 10µL of 12mM MTT solution is added to each well and incubated between 1 and 4 hours at 37°C to allow MTT metabolisation. The crystals formed are dissolved by adding 150µL of acid-alcohol mixture (0.04M HCL in absolute 2-propanol). The absorbance at 570nm is measured on a Biotek ELX800 Universal Microplate Reader. The results are expressed with respect to control values.

## 6.4. Singlet oxygen measurement

All singlet oxygen data was collected by Geraldine Rosser (Durham University)

Singlet oxygen was detected by monitoring the intensity of its phosphorescence at 1270 nm. The samples were held in a four-sided quartz cuvette and excited at 355 nm using Nd:YAG laser and the emission collected at 90° to the excitation source, passed through a 1270 nm interference filter (Infra-Red Engineering) and detected using a cooled germanium photodiode (North coast EO-817P). The emission signal was digitised and averaged using an oscillosocpe and transferred to a PC for analysis. The singlet oxygen quantum yield was calculated with reference to Rose Bengal, (phi-delta = 0.76 [252]),[253]. All measurements were run in $D_2O$ and samples were prepared with an absorbance between 0.10 and 0.15 at 355 nm.

# 7. References

1.  E. S. Nyman and P. H. Hynninen, *J Photochem Photobiol B*, 2004, **73**, 1-28.
2.  S. B. Brown, E. A. Brown and I. Walker, *Lancet Oncol*, 2004, **5**, 497-508.
3.  A. P. Castano, Demidova, T.N., Hamblin, M.R., *Photodiag Photodyn*, 2004, **1**, 279-293.
4.  K. Plaetzer, B. Krammer, J. Berlanda, F. Berr and T. Kiesslich, *Laser Med Sci*, 2009, **24**, 259-268.
5.  L. B. Josefsen and R. W. Boyle, *Met Based Drugs*, 2008, **2008**, 276109.
6.  F. Wilkinson, W. P. Helman and A. B. Ross, *J Phys Chem Ref Data*, 1993, **22**, 113-262.
7.  S. Hatz, J. D. C. Lambert and P. R. Ogilby, *Photochem Photobiol Sci*, 2007, **6**, 1106-1116.
8.  S. Hatz, L. Poulsen and P. R. Ogilby, *Photochem Photobiol*, 2008, **84**, 1284-1290.
9.  R. Bonnett, *Chem Soc Rev*, 1995, **24**, 19-33.
10. R. Bonnett, *Rev Contemp Pharmaco*, 1999, **10**, 1-17.
11. K. Plaetzer, T. Kiesslich, T. Verwanger and B. Krammer, *Med Laser Appl*, 2003, **18**, 7-19.
12. A. P. Castano, Demidova, T.N., Hamblin, M.R., *Photodiag Photodyn*, 2005, **2**, 1-23.
13. C. Fabris, G. Valduga, G. Miotto, L. Borsetto, G. Jori, S. Garbisa and E. Reddi, *Cancer Res*, 2001, **61**, 7495-7500.
14. S. Nagata, A. Obana, Y. Gohto and S. Nakajima, *Laser Surg Med*, 2003, **33**, 64-70.
15. C. R. Goodlett and K. H. Horn, *Alcohol Res Health*, 2001, **25**, 175-184.
16. C. Abels, *Photochem Photobiol Sci*, 2004, **3**, 765-771.
17. D. Preise, O. Mazor, N. Koudinova, M. Liscovitch, A. Scherz and Y. Salomon, *Neoplasia*, 2003, **5**, 475-480.
18. L. B. Josefsen and R. W. Boyle, *Brit J Pharmacol*, 2008, **154**, 1-3.
19. P. Morliere, J. C. Maziere, R. Santus, C. D. Smith, M. R. Prinsep, C. C. Stobbe, M. C. Fenning, J. L. Golberg and J. D. Chapman, *Cancer Res*, 1998, **58**, 3571-3578.
20. M. R. Detty, S. L. Gibson and S. J. Wagner, *J Med Chem*, 2004, **47**, 3897-3915.
21. A. E. O'Connor, W. M. Gallagher and A. T. Byrne, *Photochem Photobiol*, 2009, **85**, 1053-1074.
22. D. Wohrle, A. Hirth, T. Bogdahn-Rai, G. Schnurpfeil and M. Shopova, *Russ Chem B+*, 1998, **47**, 807-816.
23. H. L. L. M. Vanleengoed, V. Cuomo, A. A. C. Versteeg, N. Vanderveen, G. Jori and W. M. Star, *Brit J Cancer*, 1994, **69**, 840-845.
24. E. Reddi, *J Photochem Photobiol B*, 1997, **37**, 189-195.
25. C. Ell, L. Gossner, A. May, H. T. Schneider, E. G. Hahn, M. Stolte and R. Sroka, *Gut*, 1998, **43**, 345-349.
26. R. Sinha, G. J. Kim, S. Nie and D. M. Shin, *Mol Cancer Ther*, 2006, **5**, 1909-1917.
27. G. Zheng, J. Chen, H. Li and J. D. Glickson, *P Natl Acad Sci USA*, 2005, **102**, 17757-17762.
28. L. Polo, G. Valduga, G. Jori and E. Reddi, *Int J Biochem Cell B*, 2002, **34**, 10-23.
29. S. Sugio, A. Kashima, S. Mochizuki, M. Noda and K. Kobayashi, *Protein Eng*, 1999, **12**, 439-446.

30. W. M. Sharman, J. E. van Lier and C. M. Allen, *Adv Drug Deliver Rev*, 2004, **56**, 53-76.
31. R. Schneider, L. Tirand, C. Frochot, R. Vanderesse, N. Thomas, J. Gravier, F. Guillemin and M. Barberi-Heyob, *Anticancer Agents Med Chem*, 2006, **6**, 469-488.
32. C. Alonso, Boyle, R.W., in *Handbook of Porphyrin Science: With Applications to Chemistry, Physics, Materials Science, Engineering, Biology and Medicine* ed. K. M. Kandish, Smith, K.M., Guilard, R., World Scientific Publishing, Editon edn., 2010, vol. 1, pp. 121-190.
33. M. Sibrian-Vazquez, T. J. Jensen, F. R. Fronczek, R. P. Hammer and M. G. H. Vicente, *Bioconjugate Chem*, 2005, **16**, 852-863.
34. M. Sibrian-Vazquez, T. J. Jensen, R. P. Hammer and M. G. H. Vicente, *J Med Chem*, 2006, **49**, 1364-1372.
35. X. Zheng and R. K. Pandey, *Anticancer Agents Med Chem*, 2008, **8**, 241-268.
36. J. G. Moser, Montforts, F. P., Kusch, D., Vervoorts, A., Kirsch, D., Berghahn, M., Akgün, N., Rueck, A., Andrees, S., Wagner, B., *Proc SPIE Int Soc Opt Eng*, 1996, **2924**, 22.
37. I. Laville, T. Figueiredo, B. Loock, S. Pigaglio, P. Maillard, D. S. Grierson, D. Carrez, A. Croisy and J. Blais, *Bioorgan Med Chem*, 2003, **11**, 1643-1652.
38. G. A. M. S. van Dongen, G. W. Visser and M. B. Vrouenraets, *Adv Drug Deliver Rev*, 2004, **56**, 31-52.
39. R. Hudson, M. Carcenac, K. Smith, L. Madden, O. J. Clarke, A. Pelegrin, J. Greenman and R. W. Boyle, *Brit J Cancer*, 2005, **92**, 1442-1449.
40. M. B. Vrouenraets, G. W. M. Visser, C. Loup, B. Meunier, M. Stigter, H. Oppelaar, F. A. Stewart, G. B. Snow and G. A. M. S. van Dongen, *Int J Cancer*, 2000, **88**, 108-114.
41. G. Jori, L. Schindl, A. Schindl and L. Polo, *J Photochem Photobiol A*, 1996, **102**, 101-107.
42. S. M. Deyev and E. N. Lebedenko, *BioEssays*, 2008, **30**, 904-918.
43. A. Worn and A. Pluckthun, *J Mol Biol*, 2001, **305**, 989-1010.
44. N. S. Soukos, M. R. Hamblin, S. Keel, R. L. Fabian, T. F. Deutsch and T. Hasan, *Cancer Res*, 2001, **61**, 4490-4496.
45. C. Staneloudi, K. A. Smith, R. Hudson, N. Malatesti, H. Savoie, R. W. Boyle and J. Greenman, *Immunology*, 2007, **120**, 512-517.
46. M. Birchler, F. Viti, L. Zardi, B. Spiess and D. Neri, *Nat Biotechnol*, 1999, **17**, 984-988.
47. M. Fabbrini, E. Trachsel, P. Soldani, S. Bindi, P. Alessi, L. Bracci, H. Kosmehl, L. Zardi, D. Neri and P. Neri, *Int J Cancer*, 2006, **118**, 1805-1813.
48. A. Palumbo, F. Hauler, P. Dziunycz, K. Schwager, A. Soltermann, F. Pretto, C. Alonso, G. F. Hofbauer, R. W. Boyle and D. Neri, *Br J Cancer*, 2011, **104**, 1106-1115.
49. M. Bhatti, G. Yahioglu, L. R. Milgrom, M. Garcia-Maya, K. A. Chester and M. P. Deonarain, *Int J Cancer*, 2008, **122**, 1155-1163.
50. M. K. Kuimova, M. Bhatti, M. Deonarain, G. Yahioglu, J. A. Levitt, I. Stamati, K. Suhling and D. Phillips, *Photochem Photobiol Sci*, 2007, **6**, 933-939.
51. A. F. Hussain, F. Kampmeier, V. von Felbert, H.-F. Merk, M. K. Tur and S. Barth, *Bioconjugate Chem*, 2011, **22**, 2487-2495.
52. L. R. Duska, M. R. Hamblin, M. P. Bamberg and T. Hasan, *Brit J Cancer*, 1997, **75**, 837-844.
53. M. R. Hamblin, J. L. Miller and T. Hasan, *Cancer Res*, 1996, **56**, 5205-5210.
54. K. L. Molpus, M. R. Hamblin, I. Rizvi and T. Hasan, *Gynecol Oncol*, 2000, **76**, 397-404.

55.    L. R. Duska, M. R. Hamblin, J. L. Miller and T. Hasan, *J Natl Cancer I*, 1999, **91**, 1557-1563.

56.    Z. R. Lu, J. G. Shiah, P. Kopečková and J. Kopeček, *STP Pharma Sci*, 2003, **13**, 69-75.

57.    C. M. A. Alonso, A. Palumbo, A. J. Bullous, F. Pretto, D. Neri and R. W. Boyle, *Bioconjugate Chem*, 2010, **21**, 302-313.

58.    E. Buhleier, W. Wehner and F. Vögtle, *Synthesis*, 1978, **1978**, 155-158.

59.    D. A. Tomalia, H. Baker, J. Dewald, M. Hall, G. Kallos, S. Martin, J. Roeck, J. Ryder and P. Smith, *Macromolecules*, 1986, **19**, 2466-2468.

60.    B. Klajnert and M. Bryszewska, *Acta Biochim Pol*, 2001, **48**, 199-208.

61.    A. K. Patri, I. J. Majoros and J. R. Baker, *Curr Opin Chem Biol*, 2002, **6**, 466-471.

62.    R. Esfand and D. A. Tomalia, *Drug Discov Today*, 2001, **6**, 427-436.

63.    A. Kumar, V. K. Yellepeddi, G. E. Davies, K. B. Strychar and S. Palakurthi, *Int J Pharm*, 2010, **392**, 294-303.

64.    M. Liu and J. M. J. Fréchet, *Pharm Sci Tech To*, 1999, **2**, 393-401.

65.    J. Peterson, Ebber, A., Allikmaa, V., and Lopp, M. , *Proc Estonian Acad Sci Chem*, 2001, **50**, 156-166.

66.    J. W. Lee, J. H. Kim, B.-K. Kim, J. H. Kim, W. S. Shin and S.-H. Jin, *Tetrahedron*, 2006, **62**, 9193-9200.

67.    J. W. Lee, J. H. Kim and B.-K. Kim, *Tetrahedron Lett*, 2006, **47**, 2683-2686.

68.    J. W. Lee, H. J. Kim, S. C. Han, J. H. Kim and S.-H. Jin, *J Polym Sci Pol Chem*, 2008, **46**, 1083-1097.

69.    J. W. Lee, J. H. Kim, H. J. Kim, S. C. Han, J. H. Kim, W. S. Shin and S.-H. Jin, *Bioconjugate Chem*, 2007, **18**, 579-584.

70.    I. K. Martin and L. J. Twyman, *Tetrahedron Lett*, 2001, **42**, 1119-1121.

71.    L. Tao, J. Geng, G. Chen, Y. Xu, V. Ladmiral, G. Mantovani and D. M. Haddleton, *Chem Commun*, 2007, 3441-3443.

72.    N. Pollock, G. Fowler, L. J. Twyman and S. L. McArthur, *Chem Commun*, 2007, 2482-2484.

73.    J. Bu, R. Li, C. W. Quah and K. J. Carpenter, *Macromolecules*, 2004, **37**, 6687-6694.

74.    C.-C. Chu, N. Ueno and T. Imae, *Chem Mater*, 2008, **20**, 2669-2676.

75.    H.-C. Tsai, T. Imae, G. Calderó and C. Solans, *J Biomed Mater Res A*, 2012, **100A**, 746-756.

76.    H. Yu, Y. Nie, C. Dohmen, Y. Li and E. Wagner, *Biomacromolecules*, 2011, **12**, 2039-2047.

77.    N. Georgiev and V. Bojinov, *J Fluoresc*, 2011, **21**, 51-63.

78.    N. I. Georgiev, V. B. Bojinov and N. Marinova, *Sensor Actuat B-Chem*, 2010, **150**, 655-666.

79.    T. Mizugaki, M. Murata, S. Fukubayashi, T. Mitsudome, K. Jitsukawa and K. Kaneda, *Chem Commun*, 2008, 241-243.

80.    G. R. Newkome, Z. Yao, G. R. Baker and V. K. Gupta, *J Org Chem*, 1985, **50**, 2003-2004.

81.    Y. M. Chabre, D. Giguère, B. Blanchard, J. Rodrigue, S. Rocheleau, M. Neault, S. Rauthu, A. Papadopoulos, A. A. Arnold, A. Imberty and R. Roy, *Chem-Eur J*, 2011, **17**, 6545-6562.

82.    J. J. Lundquist and E. J. Toone, *Chem Rev*, 2002, **102**, 555-578.

83.    G. J. Miller and J. M. Gardiner, *Org Lett*, 2010, **12**, 5262-5265.

84.    K. H. Schlick, J. R. Morgan, J. J. Weiel, M. S. Kelsey and M. J. Cloninger, *Bioorg Med Chem Lett*, 2011, **21**, 5078-5083.

85.    Y. M. Chabre, C. Contino-Pepin, V. Placide, T. C. Shiao and R. Roy, *J Org*

*Chem*, 2008, **73**, 5602-5605.

86. S. Lebreton, S.-E. How, M. Buchholz, B.-E. Yingyongnarongkul and M. Bradley, *Tetrahedron*, 2003, **59**, 3945-3953.

87. R. Kaplánek, T. Bříza, M. Havlík, P. Martásek and V. Král, *J Fluorine Chem*, 2007, **128**, 179-183.

88. S. J. Atkinson, V.-J. Ellis, S. E. Boyd and C. L. Brown, *New J Chem*, 2007, **31**, 155-162.

89. C. Hawker and J. M. J. Frechet, *Chem Commun*, 1990, **0**, 1010-1013.

90. T. Fukuda, E. Matsumoto, S. Onogi and Y. Miura, *Bioconjugate Chem*, 2010, **21**, 1079-1086.

91. P. Rajakumar, V. Kalpana, S. Ganesan and P. Maruthamuthu, *Tetrahedron Lett*, 2011, **52**, 5812-5816.

92. B. P. Dash, R. Satapathy, B. P. Bode, C. T. Reidl, J. W. Sawicki, A. J. Mason, J. A. Maguire and N. S. Hosmane, *Organometallics*, 2012, **31**, 2931-2935.

93. I. Deguise, D. Lagnoux and R. Roy, *New J Chem*, 2007, **31**, 1321-1331.

94. S. L. Elmer and S. C. Zimmerman, *J Org Chem*, 2004, **69**, 7363-7366.

95. Y.-L. Zhao, A. Trabolsi and J. F. Stoddart, *Chem Commun*, 2009, 4844-4846.

96. P. Rajamalli and E. Prasad, *Langmuir*, 2013, **29**, 1609-1617.

97. P. Rajamalli and E. Prasad, *Soft Matter*, 2012, **8**, 8896-8903.

98. N. Baek, Y. Kim, S.-G. Roh, D. Lee, K. Seo and H. Kim, *Macromol Res*, 2009, **17**, 672-681.

99. C. Agarwal and E. Prasad, *New J Chem*, 2012, **36**, 1859-1865.

100. Z.-X. Liu, Y. Feng, Z.-C. Yan, Y.-M. He, C.-Y. Liu and Q.-H. Fan, *Chem Mater*, 2012, **24**, 3751-3757.

101. M. Dilek and I. Erol, *J Macromol Sci A*, 2010, **47**, 26-32.

102. A. Sánchez-Méndez, E. de Jesús, J. C. Flores and P. Gómez-Sal, *Eur J Inorg Chem*, 2010, 141-151.

103. W.-S. Li and T. Aida, *Chem Rev*, 2009, **109**, 6047-6076.

104. D. L. Jiang and T. Aida, *Prog Polym Sci*, 2005, **30**, 403-422.

105. A. Y. Lebedev, A. V. Cheprakov, S. Sakadzic, D. A. Boas, D. F. Wilson and S. A. Vinogradov, *ACS Appl Mater Interfaces*, 2009, **1**, 1292.

106. I. Dunphy, S. A. Vinogradov and D. F. Wilson, *Anal Biochem*, 2002, **310**, 191.

107. S. A. Vinogradov, L. W. Lo and D. F. Wilson, *Chem-Eur J*, 1999, **5**, 1338.

108. S. Thyagarajan, T. Leiding, S. P. Arskold, A. V. Cheprakov and S. A. Vinogradov, *Inorg Chem*, 2010, **49**, 9909-9920.

109. T. H. Xu, R. Lu, X. P. Qiu, X. L. Liu, P. C. Xue, C. H. Tan, C. Y. Bao and Y. Y. Zhao, *Eur J Org Chem*, 2006, 4014-4020.

110. A. Merhi, S. Drouet, N. Kerisit and C. O. Paul-Roth, *Tetrahedron*, 2012, **68**, 7901-7910.

111. H. Imahori, *J Phys Chem B*, 2004, **108**, 6130-6143.

112. G. J. Capitosti, S. J. Cramer, C. S. Rajesh and D. A. Modarelli, *Org Lett*, 2001, **3**, 1645-1648.

113. M. S. Choi, T. Yamazaki, I. Yamazaki and T. Aida, *Angew Chem Int Ed*, 2004, **43**, 150-158.

114. P. Bhyrappa, J. K. Young, J. S. Moore and K. S. Suslick, *J Am Chem Soc*, 1996, **118**, 5708-5711.

115. H. Ozawa, M. Kawao, H. Tanaka and T. Ogawa, *Langmuir*, 2007, **23**, 6365-6371.

116. J. B. Oh, Y. H. Kim, M. K. Nah and H. K. Kim, *J Lumin*, 2005, **111**, 255-264.

117. S.-i. Kimata and T. Aida, *Tetrahedron Lett*, 2001, **42**, 4187-4190.

118. S. Sengupta, S. Uemura, S. Patwardhan, V. Huber, F. C. Grozema, L. D. A. Siebbeles, U. Baumeister and F. Würthner, *Chem-Eur J*, 2011, **17**, 5300-5310.

119. T. Palacin, H. L. Khanh, B. Jousselme, P. Jegou, A. Filoramo, C. Ehli, D. M. Guldi and S. p. Campidelli, *J Am Chem Soc*, 2009, **131**, 15394-15402.
120. K. H. Le Ho, L. Rivier, B. Jousselme, P. Jegou, A. Filoramo and S. Campidelli, *Chem Commun*, 2010, **46**, 8731-8733.
121. F. Brégier, S. M. Aly, C. P. Gros, J.-M. Barbe, Y. Rousselin and P. D. Harvey, *Chem-Eur J*, 2011, **17**, 14643-14662.
122. P. D. Harvey, F. Brégier, S. M. Aly, J. Szmytkowski, M. F. Paige and R. P. Steer, *Chem-Eur J*, 2013, **19**, 4352-4368.
123. S. Battah, S. O'Neill, C. Edwards, S. Balaratnam, P. Dobbin and A. J. MacRobert, *Int J Biochem Cell B*, 2006, **38**, 1382-1392.
124. A. Casas, S. Battah, G. Di Venosa, P. Dobbin, L. Rodriguez, H. Fukuda, A. Batlle and A. J. MacRobert, *J Control Release*, 2009, **135**, 136-143.
125. S. H. Battah, C.-E. Chee, H. Nakanishi, S. Gerscher, A. J. MacRobert and C. Edwards, *Bioconjugate Chem*, 2001, **12**, 980-988.
126. D. Ma, Z.-H. Liu, Q.-Q. Zheng, X.-Y. Zhou, Y. Zhang, Y.-F. Shi, J.-T. Lin and W. Xue, *Macromol Rapid Comm*, 2013, **34**, 548-552.
127. S. Ballut, D. Naud-Martin, B. Loock and P. Maillard, *J Org Chem*, 2011, **76**, 2010-2028.
128. S. Ballut, A. Makky, B. Loock, J. P. Michel, P. Maillard and V. Rosilio, *Chem Commun*, 2009, 224-226.
129. R. Ballardini, B. Colonna, M. T. Gandolfi, S. A. Kalovidouris, L. Orzel, F. M. Raymo and J. F. Stoddart, *Eur J Org Chem*, 2003, 288-294.
130. V. Morosini, C. Frochot, M. Barberi-Heyob and R. Schneider, *Tetrahedron Lett*, 2006, **47**, 8745-8749.
131. H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew Chem, Int Ed*, 2001, **40**, 2004-2021.
132. J. E. Moses and A. D. Moorhouse, *Chem Soc Rev*, 2007, **36**, 1249-1262.
133. V. D. Bock, H. Hiemstra and J. H. van Maarseveen, *Eur J Org Chem*, 2006, **2006**, 51-68.
134. V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew Chem, Int Ed*, 2002, **41**, 2596-2599.
135. C. W. Tornoe, C. Christensen and M. Meldal, *J Org Chem*, 2002, **67**, 3057-3064.
136. F. Himo, T. Lovell, R. Hilgraf, V. V. Rostovtsev, L. Noodleman, K. B. Sharpless and V. V. Fokin, *J Am Chem Soc*, 2004, **127**, 210-216.
137. J. E. Hein and V. V. Fokin, *Chem Soc Rev*, 2010, **39**, 1302-1315.
138. L. Liang and D. Astruc, *Coordin Chem Rev*, 2011, **255**, 2933-2945.
139. H. C. Kolb and K. B. Sharpless, *Drug Discov Today*, 2003, **8**, 1128-1137.
140. G. C. Tron, T. Pirali, R. A. Billington, P. L. Canonico, G. Sorba and A. A. Genazzani, *Med Res Rev*, 2008, **28**, 278-308.
141. W. H. Binder and R. Sachsenhofer, *Macromol Rapid Comm*, 2007, **28**, 15-54.
142. C. O. Kappe, *Angew Chem, Int Ed*, 2004, **43**, 6250-6284.
143. P. Lidstrom, J. Tierney, B. Wathey and J. Westman, *Tetrahedron*, 2001, **57**, 9225-9283.
144. C. O. Kappe, D. Dallinger and S. S. Murphree, *Practical Microwave Synthesis for Organic Chemists: Strategies, Instruments, and Protocols*, Wiley VCH, 2009.
145. D. Stuerga, in *Microwaves in Organic Synthesis*, ed. L. Loupy, Wiley-VCH, Weinheim, Editon edn., 2006, vol. 1, pp. 1-61.
146. D. Obermayer, B. Gutmann and C. O. Kappe, *Angew Chem, Int Ed*, 2009, **48**, 8321-8324.
147. C. O. Kappe and E. Van der Eycken, *Chem Soc Rev*, 2010, **39**, 1280-1290.
148. A. Barge, S. Tagliapietra, A. Binello and G. Cravotto, *Curr Org Chem*, 2011, **15**,

189-203.

149.  P. Appukkuttan, W. Dehaen, V. V. Fokin and E. Van der Eycken, *Org Lett*, 2004, **6**, 4223-4225.

150.  G. Garcia, D. Naud-Martin, D. Carrez, A. Croisy and P. Maillard, *Tetrahedron*, 2011, **67**, 4924-4932.

151.  E. M. Scanlan, O. B. Locos, C. C. Heindl, A. Corral and M. O. Senge, *Eur J Org Chem*, 2010, 1026-1028.

152.  M. E. Bakleh, V. Sol, K. Estieu-Gionnet, R. Granet, G. Déléris and P. Krausz, *Tetrahedron*, 2009, **65**, 7385-7392.

153.  P. Rothemund, *J Am Chem Soc*, 1935, **57**, 2010-2011.

154.  R. H. Ball, G. D. Dorough and M. Calvin, *J Am Chem Soc*, 1946, **68**, 2278-2281.

155.  A. D. Adler, F. R. Longo, J. D. Finarelli, J. Goldmacher, J. Assour and L. Korsakoff, *J Org Chem*, 1967, **32**, 476-476.

156.  J. S. Lindsey, I. C. Schreiman, H. C. Hsu, P. C. Kearney and A. M. Marguerettaz, *J Org Chem*, 1987, **52**, 827-836.

157.  J. S. Lindsey, in *The Porphyrin Handbook: Synthesis and organic chemistry*, eds. K. M. Kadish, K. M. Smith and R. Guilard, Elsevier, London, Editon edn., 2000, vol. 2, pp. 45-119.

158.  J. S. Lindsey, in *The Porphyrin Handbook: Synthesis and organic chemistry*, ed. K. M. S. Karl M. Kadish, Roger Guilard, Elsevier, Editon edn., 2000, vol. 1, pp. 46-112.

159.  N. K. Devaraj, R. A. Decreau, W. Ebina, J. P. Collman and C. E. D. Chidsey, *J Phys Chem B*, 2006, **110**, 15955-15962.

160.  F. Dumoulin and V. Ahsen, *J Porphyr Phthalocyanines*, 2011, **15**, 481-504.

161.  J. E. A. Webb, F. Maharaj, I. M. Blake and M. J. Crossley, *Synlett*, 2008, **14**, 2147-2149.

162.  M. Fathalla, S.-C. Li, U. Diebold, A. Alb and J. Jayawickramarajah, *Chem Commun*, 2009, **0**, 4209-4211.

163.  Y. Liu, C.-F. Ke, H.-Y. Zhang, J. Cui and F. Ding, *J Am Chem Soc*, 2007, **130**, 600-605.

164.  E. Hao, T. J. Jensen and M. G. H. Vicente, *J Porphyr Phthalocya*, 2009, **13**, 51-59.

165.  F. d. C. Santos, A. C. Cunha, M. C. B. V. de Souza, A. C. Tomé, M. G. P. M. S. Neves, V. F. Ferreira and J. A. S. Cavaleiro, *Tetrahedron Lett*, 2008, **49**, 7268-7270.

166.  J.-S. Marois, K. Cantin, A. Desmarais and J.-F. Morin, *Org Lett*, 2007, **10**, 33-36.

167.  C. Maeda, S. Yamaguchi, C. Ikeda, H. Shinokubo and A. Osuka, *Org Lett*, 2008, **10**, 549-552.

168.  W.-H. Wen, M. Lin, C.-Y. Su, S.-Y. Wang, Y.-S. E. Cheng, J.-M. Fang and C.-H. Wong, *J Med Chem*, 2009, **52**, 4903-4910.

169.  M. Séverac, L. L. Pleux, A. Scarpaci, E. Blart and F. Odobel, *Tetrahedron Lett*, 2007, **48**, 6518-6522.

170.  V. S. Shetti and M. Ravikanth, *Eur J Org Chem*, 2010, **2010**, 494-508.

171.  N. W. Smith and S. V. Dzyuba, *Arkivoc*, 2010, 10-18.

172.  L. Sun, H. Chen, Z. Zhang, Q. Yang, H. Tong, A. Xu and C. Wang, *J Inorg Biochem*, 2012, **108**, 47-52.

173.  E. D. Goddard-Borger and R. V. Stick, *Org Lett*, 2007, **9**, 3797-3800.

174.  N. Fischer, E. D. Goddard-Borger, R. Greiner, T. M. Klapötke, B. W. Skelton and J. Stierstorfer, *J Org Chem*, 2012, **77**, 1760-1764.

175.  M. G. Walter, C. C. Wamser, J. Ruwitch, Y. Zhao, D. Braden, M. Stevens, A. Denman, R. Pi, A. Rudine and P. J. Pessiki, *J Porphyr Phthalocya*, 2007, **11**,

601-612.

176. P. Battioni, O. Brigaud, H. Desvaux, D. Mansuy and T. G. Traylor, *Tetrahedron Lett*, 1991, **32**, 2893-2896.

177. *US Pat.*, US2003/203888 2003.

178. J.-P. Tremblay-Morin, H. Ali and J. E. van Lier, *Tetrahedron Lett*, 2006, **47**, 3043-3046.

179. I. Hatay, B. Su, M. A. Méndez, C. Corminboeuf, T. Khoury, C. P. Gros, M. Bourdillon, M. Meyer, J.-M. Barbe, M. Ersoz, S. Záliš, Z. Samec and H. H. Girault, *J Am Chem Soc*, 2010, **132**, 13733-13741.

180. M. d. Carvalho, A. E. P. M. Sorrilha and J. A. R. Rodrigues, *J Brazil Chem Soc*, 1999, **10**, 415-420.

181. G. G. Meng, B. R. James, K. A. Skov and M. Korbelik, *Can J Chem*, 1994, **72**, 2447-2457.

182. C. E. Stilts, M. I. Nelen, D. G. Hilmey, S. R. Davies, S. O. Gollnick, A. R. Oseroff, S. L. Gibson, R. Hilf and M. R. Detty, *J Med Chem*, 2000, **43**, 2403-2410.

183. Z. Dong and P. J. Scammells, *J Org Chem*, 2007, **72**, 9881-9885.

184. X.-a. Zhang, K. S. Lovejoy, A. Jasanoff and S. J. Lippard, *P Natl Acad Sci USA*, 2007, **104**, 10780-10785.

185. J. W. Winkelman and G. H. Collins, *Photochem Photobiol*, 1987, **46**, 801-807.

186. D. M. Collins and J. L. Hoard, *J Am Chem Soc*, 1970, **92**, 3761-3771.

187. E. B. Fleischer and A. M. Shachter, *Inorg Chem*, 1991, **30**, 3763-3769.

188. F. Giuntini, F. Dumoulin, R. Daly, V. Ahsen, E. M. Scanlan, A. S. P. Lavado, J. W. Aylott, G. A. Rosser, A. Beeby and R. W. Boyle, *Nanoscale*, 2012, **4**, 2034-2045.

189. S. Asayama, T. Moris, S. Nagaoka and H. Kawakami, *J Biomat Sci- Polym E*, 2003, **14**, 1169.

190. R. Schneider, F. Schmitt, C. Frochot, Y. Fort, N. Lourette, F. Guillemin, J. F. Muller and M. Barberi-Heyob, *Bioorg Med Chem*, 2005, **13**, 2799-2808.

191. Y. Ishikawa, A. Yamashita and T. Uno, *Chem Pharm Bull*, 2001, **49**, 287-293.

192. G. L. Lu, S. Lam and K. Burgess, *Chem Commun*, 2006, 1652-1654.

193. *Int. Pat.*, WO2009/60158 A1, 2009.

194. T. Suzuki, S. Hisakawa, Y. Itoh, N. Suzuki, K. Takahashi, M. Kawahata, K. Yamaguchi, H. Nakagawa and N. Miyata, *Bioorg Med Chem Lett*, 2007, **17**, 4208-4212.

195. J. Rao and A. K. Saxena, *Indian J Chem B*, 1989, **28**, 620-625.

196. H. Marom, K. Miller, Y. Bechor-Bar, G. Tsarfaty, R. Satchi-Fainaro and M. Gozin, *J Med Chem*, 2010, **53**, 6316-6325.

197. S. Punna, Finn, M.G., *Synlett*, 2004, **1**, 99-100.

198. S. Roth, W. C. Drewe and N. R. Thomas, *Nat Protoc*, 2010, **5**, 1967-1973.

199. C. Hein, X.-M. Liu and D. Wang, *Pharmaceut Res*, 2008, **25**, 2216-2230.

200. *Int. Pat.*, 2009/113828, 2009.

201. P. Wu, A. K. Feldman, A. K. Nugent, C. J. Hawker, A. Scheel, B. Voit, J. Pyun, J. M. J. Fréchet, K. B. Sharpless and V. V. Fokin, *Angew Chem, Int Ed*, 2004, **43**, 3928-3932.

202. M. J. Joralemon, R. K. O'Reilly, J. B. Matson, A. K. Nugent, C. J. Hawker and K. L. Wooley, *Macromolecules*, 2005, **38**, 5436-5443.

203. M. Malkoch, K. Schleicher, E. Drockenmuller, C. J. Hawker, T. P. Russell, P. Wu and V. V. Fokin, *Macromolecules*, 2005, **38**, 3663-3678.

204. J. Camponovo, J. Ruiz, E. Cloutet and D. Astruc, *Chem-Eur J*, 2009, **15**, 2990-3002.

205. K. Yoon, P. Goyal and M. Weck, *Org Lett*, 2007, **9**, 2051-2054.

206. D. T. S. Rijkers, G. W. van Esse, R. Merkx, A. J. Brouwer, H. J. F. Jacobs, R. J. Pieters and R. M. J. Liskamp, *Chem Commun*, 2005, 4581-4583.
207. I. Dijkgraaf, A. Y. Rijnders, A. Soede, A. C. Dechesne, G. W. van Esse, A. J. Brouwer, F. H. M. Corstens, O. C. Boerman, D. T. S. Rijkers and R. M. J. Liskamp, *Org Biomol Chem*, 2007, **5**, 935-944.
208. D. D. Díaz, S. Punna, P. Holzer, A. K. McPherson, K. B. Sharpless, V. V. Fokin and M. G. Finn, *J Polym Sci Pol Chem*, 2004, **42**, 4392-4403.
209. D. F. Harvey, K. P. Lund and D. A. Neil, *J Am Chem Soc*, 1992, **114**, 8424-8434.
210. M. Malkoch, R. J. Thibault, E. Drockenmuller, M. Messerschmidt, B. Voit, T. P. Russell and C. J. Hawker, *J Am Chem Soc*, 2005, **127**, 14942-14949.
211. L. V. Christensen, C.-W. Chang, W. J. Kim, S. W. Kim, Z. Zhong, C. Lin, J. F. J. Engbersen and J. Feijen, *Bioconjugate Chem*, 2006, **17**, 1233-1240.
212. C.-L. Do-Thanh, M. M. Rowland and M. D. Best, *Tetrahedron*, 2011, **67**, 3803-3808.
213. H. M. Branderhorst, R. Ruijtenbeek, R. M. J. Liskamp and R. J. Pieters, *Chembiochem*, 2008, **9**, 1836-1844.
214. M. V. Trivedi, J. S. Laurence and T. J. Siahaan, *Curr Protein Pept Sci*, 2009, **10**, 614-625.
215. V. A. S. F.F. Schumacher, B. Tolner, Z.V.F. Wright, C.P. Ryan, M.E.B. Smith, J.M. Ward, S. Caddick, C.W.M. Kay, G. Aeppli, K.A. Chester & J.R. Baker, *Sci Rep*.
216. M. E. B. Smith, F. F. Schumacher, C. P. Ryan, L. M. Tedaldi, D. Papaioannou, G. Waksman, S. Caddick and J. R. Baker, *J Am Chem Soc*, 2010, **132**, 1960-1965.
217. S. Lang, D. E. Spratt, J. G. Guillemette and M. Palmer, *Anal Biochem*, 2005, **342**, 271-279.
218. K. E. Borbas, H. L. Kee, D. Holten and J. S. Lindsey, *Org Biomol Chem*, 2008, **6**, 187-194.
219. Z. Yang and A. B. Attygalle, *Journal of Mass Spectrometry*, 2007, **42**, 233-243.
220. H. Liu, C. Chumsae, G. Gaza-Bulseco, K. Hurkmans and C. H. Radziejewski, *Anal Chem*, 2010, **82**, 5219-5226.
221. T. E. Michaelsen, O. H. Brekke, A. Aase, R. H. Sandin, B. Bremnes and I. Sandlie, *P Natl Acad Sci USA*, 1994, **91**, 9243-9247.
222. P. Moody, M. E. B. Smith, C. P. Ryan, V. Chudasama, J. R. Baker, J. Molloy and S. Caddick, *Chembiochem*, 2012, **13**, 39-41.
223. M. W. Jones, R. A. Strickland, F. F. Schumacher, S. Caddick, J. R. Baker, M. I. Gibson and D. M. Haddleton, *J Am Chem Soc*, 2011, **134**, 1847-1852.
224. R. P. Joyce, J. A. Gainor and S. M. Weinreb, *J Org Chem*, 1987, **52**, 1177-1185.
225. C. Antczak, B. Bauvois, C. Monneret and J.-C. Florent, *Bioorgan Med Chem*, 2001, **9**, 2843-2848.
226. D. Royer, Y.-S. Wong, S. Plé, A. Chiaroni, K. Diker and J. Lévy, *Tetrahedron*, 2008, **64**, 9607-9618.
227. A. Martinez, M. Alonso, A. Castro, I. Dorronsoro, J. L. Gelpí, F. J. Luque, C. Pérez and F. J. Moreno, *J Med Chem*, 2005, **48**, 7103-7112.
228. *Int. Pat.*, WO2013085925, 2013.
229. U. Muus, C. Hose, W. Yao, T. Kosakowska-Cholody, D. Farnsworth, M. Dyba, G. T. Lountos, D. S. Waugh, A. Monks, T. R. Burke Jr and C. J. Michejda, *Bioorgan Med Chem*, 2010, **18**, 4535-4541.
230. L. Castañeda, Z. V. F. Wright, C. Marculescu, T. M. Tran, V. Chudasama, A. Maruani, E. A. Hull, J. P. M. Nunes, R. J. Fitzmaurice, M. E. B. Smith, L. H. Jones, S. Caddick and J. R. Baker, *Tetrahedron Lett*, 2013, **54**, 3493-3495.
231. J. Carlsson, H. Nordgren, J. Sjostrom, K. Wester, K. Villman, N. O. Bengtsson, B. Ostenstad, H. Lundqvist and C. Blomqvist, *Br J Cancer*, 2004, **90**, 2344-

2348.

232. M. A. Cobleigh, C. L. Vogel, D. Tripathy, N. J. Robert, S. Scholl, L. Fehrenbacher, J. M. Wolter, V. Paton, S. Shak, G. Lieberman and D. J. Slamon, *J Clin Oncol*, 1999, **17**, 2639.

233. D. J. Slamon, B. Leyland-Jones, S. Shak, H. Fuchs, V. Paton, A. Bajamonde, T. Fleming, W. Eiermann, J. Wolter, M. Pegram, J. Baselga and L. Norton, *New Engl J Med*, 2001, **344**, 783-792.

234. Y. Lu, X. Zi, Y. Zhao, D. Mascarenhas and M. Pollak, *J Natl Cancer I*, 2001, **93**, 1852-1857.

235. A. Thor *J Natl Cancer I*, 2001, **93**, 1120-1121.

236. M. Moreau, O. Raguin, J.-M. Vrigneaud, B. Collin, C. Bernhard, X. Tizon, F. Boschetti, O. Duchamp, F. Brunotte and F. Denat, *Bioconjugate Chem*, 2012, **23**, 1181-1188.

237. D. Gianolio, C. Rouleau, W. Bauta, D. Lovett, W. Cantrell, Jr., A. Recio, III, P. Wolstenholme-Hogg, M. Busch, P. Pan, J. Stefano, H. Kramer, J. Goebel, R. Krumbholz, S. Roth, S. Schmid and B. Teicher, *Cancer Chemother Pharmacol*, 2012, **70**, 439-449.

238. O. Germershaus, T. Merdan, U. Bakowsky, M. Behe and T. Kissel, *Bioconjugate Chem*, 2006, **17**, 1190-1199.

239. L. T. Rosenblum, M. Mitsunaga, J. W. Kakareka, N. Y. Morgan, T. J. Pohida, P. L. Choyke and H. Kobayashi, in *Reporters, Markers, Dyes, Nanoparticles, and Molecular Probes for Biomedical Applications Iii*, eds. S. Achilefu and R. Raghavachari, Spie-Int Soc Optical Engineering, Bellingham, Editon edn., 2011, vol. 7910.

240. Y. Wang, Q. Lu, S.-L. Wu, B. L. Karger and W. S. Hancock, *Anal Chem*, 2011, **83**, 3133-3140.

241. M. Dubernet, V. Caubert, J. Guillard and M.-C. Viaud-Massuard, *Tetrahedron*, 2005, **61**, 4585-4593.

242. K.-L. Dao, R. R. Sawant, J. A. Hendricks, V. Ronga, V. P. Torchilin and R. N. Hanson, *Bioconjugate Chem*, 2012, **23**, 785-795.

243. K. P. Antonsen and A. S. Hoffman, in *Poly(Ethylene Glycol) Chemistry: Biotechnical and Biomedical Applications*, ed. J. M. Harris, Springer, Editon edn., 1992, pp. 15-28.

244. J. C. Pelletier and S. Kincaid, *Tetrahedron Lett*, 2000, **41**, 797-800.

245. B. H. Lipshutz, D. W. Chung, B. Rich and R. Corral, *Org Lett*, 2006, **8**, 5069-5072.

246. K. Hagiya, N. Muramoto, T. Misaki and T. Sugimura, *Tetrahedron*, 2009, **65**, 6109-6114.

247. T. Y. S. But and P. H. Toy, *J Am Chem Soc*, 2006, **128**, 9636-9637.

248. C. Ringot, V. Sol, M. Barrière, N. m. Saad, P. Bressollier, R. Granet, P. Couleaud, C. l. Frochot and P. Krausz, *Biomacromolecules*, 2011, **12**, 1716-1723.

249. S. Kimura, S.-i. Masunaga, T. Harada, Y. Kawamura, S. Ueda, K. Okuda and H. Nagasawa, *Bioorgan Med Chem*, 2011, **19**, 1721-1728.

250. D. B. G. Williams and M. Lawton, *J Org Chem*, 2010, **75**, 8351-8354.

251. V. Hong, S. I. Presolski, C. Ma and M. G. Finn, *Angew Chem Int Edit*, 2009, **48**, 9879-9883.

252. M. C. DeRosa and R. J. Crutchley, *Coordin Chem Rev*, 2002, **233–234**, 351-371.

253. S. Nonell and S. E. Braslavsky, in *Methods in Enzymology*, ed. H. S. Lester Packer, Academic Press, Editon edn., 2000, vol. Volume 319, pp. 37-49.