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FULL PAPER

Evaluation of a bispidine-based chelator for gallium-68 and of the porphyrin conjugate as PET/PDT theranostic agent

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Abstract: In this study a bispidine ligand has been applied to the complexation of gallium(III) and radiolabelled with gallium-68 for the first time. Despite its 5-coordinate nature, the resulting complex is stable in serum for over two hours, demonstrating a ligand system well matched to the imaging window of gallium-68 positron emission tomography (PET). To show the versatility of the bispidine ligand and its potential use in PET, the bifunctional chelator was conjugated to a porphyrin, producing a PET/PDT-theranostic, which showed the same level of stability to serum as the non-conjugated gallium-68 complex. The PET/PDT complex killed >90% of HT-29 cells upon light irradiation at 50 µM. This study shows bispidines have the versatility to be used as a ligand system for gallium-68 in PET.

Introduction

Positron emission tomography (PET) is a highly sensitive imaging technique with high tissue penetration.[1–3] This technique can allow for the *in vivo* imaging of diseased tissues by targeting biochemical processes; thus allowing for detection of disease before physical changes occur. Recently, gallium-68 (^{68}Ga) has found significant interest as a PET radionuclide due to its generator based production allowing for ease of access.[3–5] Incorporation of ^{68}Ga into a radiotracer is typically achieved through the use of a chelator that complexes the PET isotope.[3,6,7] When designing these chelators, special attention must be brought to the three following points: *(i)* they should form a unique radiolabeled complex, ideally in mild conditions; *(ii)* with high kinetic/thermodynamic stability; and *(iii)* strong resistance to hydrolysis and to transchelation reactions, occurring in particular with transferrin, and to transmetallation or competition with metals such as Cu(II) or Zn(II). Despite numerous attempts and studies only a few ligands fulfill all the criteria.[6–8] These ligands are depicted in Figure 1. DOTA (1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid) and its conjugates (with octreotide and derivatives) are clinically in use for imaging neuroendocrine tumours[9] although they require high temperatures and acidic conditions for radiolabeling.[10][11]The triazacyclononane derivative (NOTA), its analogue with phosphinic pendant arms (TRAP) and the acyclic chelator H_2dedpa also need acidic conditions but no heating.[6,12–14] All these chelators are however subject to competition with Cu(II) and Zn(II),[15,16] although TRAP displays an apparent improved selectivity for Ga(III).[13] 6-amino-1,4-diazepine triacetate (DATA) chelators[17] as well as siderophores (such as deferoxamine),[18] N,N'-Bis(2-hydroxybenzyl)ethylenediamine-N,N'-diacetic acid (HBED)[18]and tris(hydroxypyridinone) (THP)[18] have the advantage to be radiolabeled in a wide pH range, THP being the most promising as quantitative radiochemical yields can be obtained in mild conditions.[19,20] Several THP-bioconjugates have been studied *in vivo*, demonstrating either very promising tumor/body ratio[21,22] or disappointing results.[23,24] These observations demonstrate that both radiocomplex and biological vector have a synergic effect on the biodistribution of the radiopharmaceutical.

For all these considerations, it is of high interest to investigate the potential of other families of ligands such as bispidines. Bispidines (chelators based on a 3,7-diazabicyclo[3.3.1]nonane core) are widely used chelators with a highly preorganised coordinating site (Figure 1).[25,26] They have been used to complex a range of metals, including radiometals such as ^{64}Cu, complexes of which were found to be remarkably inert.[27,28] Their application to Ga(III) complexation, and particularly ^{68}Ga complexation, remains relatively unexplored.[29]

The field of theranostics aims at developing agents with combined therapeutic activity and diagnostic properties in a single agent.[30] Such theranostic agents allow for monitoring the uptake and real-time distribution of the therapeutic agent, the progression of the disease, as well as the therapeutic response. This enables an individualized treatment strategy which has been shown to be very efficient in selecting the optimal treatment, limiting adverse reactions, and implementing optimal dosing regimes.[31–33] In the case of cancer, "smart agents" with targeted drug delivery systems to the tumor are considered as a very promising alternative to conventional treatment, the effectiveness of which is limited by their absence of specificity. For these drugs, triggered by externally applied stimuli (e.g. radiation or light), monitoring their uptake and distribution is vital to optimize their application as

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it will allow for the appropriate timing of their trigger, hence maximizing their effectiveness.

Photodynamic therapy (PDT) is an example of such a therapeutic technique in which a drug is administered, and then an external trigger (in this case irradiation with a high intensity light source, such as a laser) causes the therapeutic effect. PDT agents are typically photosensitizers that are activated by absorption of visible light which first populate their excited singlet state and then, after energy transfer, their long-lived excited triplet state. This triplet state can undergo photochemical reactions in the presence of oxygen to form reactive oxygen species (ROS), including singlet oxygen.[34] The localized production of these highly reactive species in the diseased tissues further causes the destruction of the neoplasm. Thus, PDT can allow for highly targeted toxicity with minimal off-target toxicity by only irradiating the target tissue.[34] Tetrapyrrole structures such as porphyrins, chlorins, bacteriochlorins and phthalocyanines derivatives have been widely investigated in PDT.[35] Porphyrins have been selected in this study due to their low toxicity, high phototoxicity, ease of synthesis, and innate tumour targeting properties.[34,36–40]

In view of the multimodal biomedical applications of metalloporphyrin, and in particular for PET/PDT purposes, ^{68}Ga has also been used previously with porphyrins.[41–47] Work in this area started with the incorporation of ^{68}Ga into the porphyrin core directly; however this involved vigorous heating using a microwave for efficient radiolabeling.[41–44] Furthermore, this prevents the incorporation of other metals into the porphyrin cavity, reducing the options available for optimizing the system.[48] More recent work has therefore involved conjugating a chelator to the porphyrin and then radiolabeling the chelator.[45,46] First experiments were performed using DOTA and NOTA. However, although they are commonly used for ^{68}Ga complexation, they were only poorly labelled when conjugated to the porphyrin.[45] The acyclic chelator H_3Dpaa was able to be readily radiolabeled with ^{68}Ga when conjugated to a porphyrin;[46] however, the resulting complex was insufficiently stable.[46,49] As for bioconjugates, there is a need of finding a good chelator/porphyrin match in order to optimize both radiolabeling and PET imaging properties and PDT efficiency.

In this study we investigated the ability of a bispidine chelator to complex Ga(III) and to be radiolabeled with ^{68}Ga. Further, we conjugated this chelator to a water soluble porphyrin and assessed the resulting conjugate's potential as a PET/PDT theranostic agent.

Results and Discussion

Complex Synthesis: The bispidine ligand, **L_1**, was prepared as previously described.[28] This pentadentate ligand is expected to coordinate to Ga(III) via the two ternary amines of the diazabicyclononane, the two nitrogen of the pyridyl groups and the acetate arm, in a similar fashion to that observed with Zn(II) for an analogue of L1 bearing a glycine substituent instead of the (L)-lysine.[27] Complexation of Ga(III) by **L_1** was performed at pH 4.5 at reflux (Scheme 1). Upon complexation of Ga(III), the pyridyl protons are significantly deshielded (Figure 2), with a downfield shift of 0.3-0.8, indicating the donation of electrons from the ring due to complexation of the cationic metal. The methyl group attached to the amine of the bispidine ring is also significantly deshielded (CH_3, $\Delta\delta_H$ = 0.9 ppm); this suggests that the amine of the ring is involved in complexation. The proton alpha to the carboxylate of the lysine unit (H_{10}) is also greatly shielded; with an upfield shift of 1.2 ppm (Figure S1).

The backbone of the cyclic structure is also locked in place, as by the coordination of the carboxylate giving an asymmetric nature evidenced by many of the resonances corresponding to protons in these environments; the resonances of H_2 and H_4 are distinct; these are also significantly deshielded compared to the analogous resonances in the free ligand with an upfield shift of 1.1 ppm. The proton at the apex of the ligand ring structure (H_9) is also deshielded by 0.7 ppm. Protons in the 6 and 8 positions show geminal coupling of 13.5 Hz and are also deshielded.

Radiochemistry: Radiolabeling of **L_1** with ^{68}Ga was followed by radio-TLC. Achieving a high radiochemical yield required heating due to the rigid nature of the chelator. Furthermore, an acidic pH was required for effective radiolabeling; pH 4 was found to be optimal (Figure 3a), likely due to the formation of kinetically inert Ga(III) hydroxides at higher pHs.[4,50] A relatively high ligand concentration was also required; a radiochemical yield of 89% was achieved at a ligand concentration of 100 µM whereas at 200 µM a radiochemical yield of 94% was achieved (Figure 3b). These results are comparable to those previously reported for macrocyclic chelators such as DOTA, which shows a 95% RCY under similar conditions (upon heating at 85 °C at pH 4.0 for 30 minutes with a 100 µM ligand concentration)[3,51,52] In terms of complexation kinetics and radiolabeling efficiency, ligand **L_1** is not as efficient as NOTA (95% RCY are obtained with no heating at pH 3.5 for 10 minutes with a 10 µM ligand concentration) and the phosphinic analogue TRAP,[13] which can be radiolabeled at much lower concentrations (c < 3µM, 5 min, pH 3.2) at 95°C or even at room temperature when using a large excess of ligand. A similar trend is observed, when comparing to acyclic ligands such as THP (5 min, pH 6.5 at 25°C), H_2dedpa (5-10 min, pH 4.5 at 25°C) and H_3dpaa (99% RCY, pH 4.5, 25°C at 110 µM ligand concentration). These differences are not surprising when looking at the chemical structure of ligand **L_1**, which is a pentadentate ligand and therefore not optimized for Ga(III) complexation in terms of kinetic, selectivity and thermodynamic stability.

However, based on previous observations with ^{64}Cu-analogues,[53] promising results in terms of kinetic inertness were expected when using a bispidine scaffold. This was indeed the case when assessed for radiochemical stability against foetal bovine serum (FBS) no decomplexation was observed over 2 hours incubation at 37ºC (Figure S5). This is the ideal imaging window time for gallium-68's half-life, thus showing that the bispidine chelator is suitable for translation to *in vivo* PET applications with ^{68}Ga. In addition, it is foreseen that radiochemical yields and labelling conditions may be further improved by utilizing other bispidine derivatives, in particular with hexadentate coordination mode.

Conjugation to porphyrin: To show the bispidine ligand can be utilized for applications in PET, not only as a chelator for gallium-68 but as a functional tool, we conjugated the ligand to a water-soluble porphyrin to produce a PET/PDT theranostic agent. **L_1**

was coupled to a water soluble porphyrin through the terminal lysine residue. The NHS-ester of the water soluble porphyrin, **L_2**, was prepared as previously described[46,54,55] and the amide bond formation was undertaken in DMF. Following semi-preparative HPLC purification, the desired bispidine-porphyrin conjugate, **L_3**, was obtained in a 48% yield (Scheme 2). Conjugate formation was confirmed by mass spectrometry (*m/z* = 405.3 $[M]^{3+}$, Figure S11).

It is evident from the ^{1}H NMR (Figure S9) that the product contains both porphyrin and bispidine moieties; the aromatic porphyrin ^{1}H resonances, corresponding to 24 protons, are evident at δ_H = 9.46, 9.04 and 8.29 for the three pyridyl units and the beta hydrogens of the porphyrin ring. The bispidine is evident through the additional aromatic resonances, corresponding to 8 protons, at 7.48 and 8.70 due to the pyridyl arms.

Conjugate complexation reaction: Complexation of Ga(III) was undertaken under the same conditions as for **L_1**. Evidence for complexation was obtained from the ^{1}H NMR of the complex due to the increased shielding of the bispidine pyridyl protons (downfield shift of 0.3 ppm). The retention of the protons within the porphyrin ring (δ_H = -3.08) confirms that complexation did not take place within the porphyrin ring (Figure S12). While complexation of Ga(III) by porphyrins has been previously reported,[42] this required more forcing conditions such as microwave heating and as such complexation within the porphyrin ring was not expected.

Radiolabelling of conjugate: Radiolabelling of the conjugate, **L_3**, was achieved under the optimized conditions determined for the ligand **L_1**. Complete complexation of the ^{68}Ga was achieved by 200 µM L_3 at pH 4.5 within 15 minutes when heated to 95 °C. This radiolabelled conjugate was assessed for its stability in FBS – all of the activity was retained within the complex over 2 hours (Figure S15). As a control, the porphyrin **L_2** was radiolabeled under the same conditions; radiochemical yields <30% were achieved, showing that the conjugate **L_3** has a selectivity for ^{68}Ga in the bispidine chelator, with a stability to FBS within the PET imaging window.

Phototoxicity: To assess the viability of this system as a potential theranostic agent, the photo- and cytotoxicities of both the conjugate, **L_3**, and the Ga(III) complex, **[Ga(L_3)]**, were assessed in human adenocarcinoma (HT-29) cells (Figure S16). Cells were incubated with either **L_3** or **[Ga(L_3)]** at varying concentrations and irradiation was carried out using a constant dose of visible light (20 J cm^{-2}; 400-700 nm). The results were compared to a non-irradiated control. Although in a clinical setting red light is more commonly used for PDT, clinical lasers used for PDT are significantly more powerful than the quartz tungsten halogen light source used in this study. To compensate for the lower power, white light was used covering the whole porphyrin absorbance band including the strong Soret band at 422 nm.

Under these conditions, >90% cell death was seen at a concentration of 50 µM for **[Ga(L_3)]** when irradiated (Figure 4). Minimal dark toxicity was observed at all concentrations tested. This shows phototoxicity at a similar concentration to Photofrin®, a clinically relevant porphyrin PDT agent, in HT-29 cells.[56]

Conclusions

We describe the application of a bispidine ligand, **L_1**, to the complexation of Ga(III). Furthermore, we demonstrate that this ligand can be successfully radiolabeled with ^{68}Ga producing a serum stable complex for the first time. Radiolabeling required high temperature (95°C) and concentrations (200 µM) to achieve near-quantitative yields (94%). Although a higher ligand concentration than traditional chelators for gallium-68, further optimization of the denticity of the ligand and of the functional groups attached to the bispidine core may improve upon this in the future.[25]

The bifunctional bispidine, **L_1**, was conjugated to a water-soluble porphyrin; the resulting conjugate, L_3, was also applied to gallium(III) complexation and radiolabeling was achieved under the same conditions as for **L_1**. These conditions are milder than those previously reported for insertion of ^{68}Ga into the porphyrin core as microwave heating was not required. Furthermore, ^{1}H NMR analysis of the Ga(III) complex confirms the presence of the protons within the porphyrin ring; this confirms that the radiolabeling is taking place at the chelator site and not at the porphyrin site. This will allow for future developments of this system to potentially incorporate alternate metals into the porphyrin ring.

L_3 and **[Ga(L_3)]** were shown to have low toxicity in the absence of light. Upon irradiation these systems were significantly more toxic with over 90% of HT-29 cells being killed by 50 µM of **[Ga(L_3)]**, and 79% by L_3, upon irradiation.

This work demonstrates the viability of the bispidine framework for Ga(III) complexation and radiolabeling with ^{68}Ga for applications in PET imaging. The combination of bispidine and porphyrin produces a PDT agent that can be effectively radiolabeled with ^{68}Ga to produce a serum stable theranostic probe for PET/PDT.

Experimental Section

NMR spectra were recorded on a JEOL ECP 400 MHz/JEOL Lambda 400 MHz spectrometer using the residual protic solvent signal as an internal reference. Chemical shifts are given in ppm (δ) and coupling constants (J) are given in Hertz (Hz). Mass spectrometry data were obtained from the EPSRC National Mass Spectrometry Facility at Swansea University. UV-vis spectroscopy was carried out on a Varian Cary 50 Bio UV-vis spectrophotometer. pH measurements were carried out using a Jenway model 3520 pH/mV/temperature meter with a three point calibration. All commercially available starting material used in synthesis were obtained from Sigma Aldrich, Fluorochem, and Alfa Aesar and were used without further purification. Deionised water was obtained from a Millipore Milli-Q reagent water system. All solvents were obtained from Fisher Scientific and VWR.

HPLC analysis were performed on Agilent HPLC system. The separations were performed on a Gemini® 5µm C18 110 Å LC column 150×4.6 mm (Phenomenex, UK) at a flow rate of 1 mL min^{-1}, with a mobile phase consisting of 0.1% TFA in water (solvent A) and 0.1% TFA in acetonitrile (solvent B). Gradient [time/min](solvent A:solvent B): [0-2](95:5). [2-3](95:5 – 78:22). [3-23](78:22 – 77:23). [23-24](77:23 – 70:30). [24-25](70:30 – 5:95). [25-26](5:95). [26-27](5:95 – 95:5). [27-30](95:5).

Radiochemistry: The IGG100 generator was eluted with 0.6 M aq. HCl (3 mL). This eluate (300–200 MBq) was diluted with H_2O (15 mL) and passed through a Strata-X-C 33 µM Cation Mixed-mode polymeric support. The activity was liberated from the column using 98:2 acetone:0.1 M aq. HCl (1 mL). Aliquots (~30 MBq) of this solution were dried under a stream of inert gas at 90 °C and allowed to cool before use. 100 µL of ligand solution was added to the dried ^{68}Ga and shaken at the appropriate temperature. 5 µL aliquots were taken for analysis by TLC. TLC analysis was performed on Kieselgel 60 F_{254} plates (Merck) with an eluate of 0.1 M citric acid in water. 100 µL of radiolabelling solution was added to 1.5 mL of foetal bovine serum and incubated at 37 °C. Aliquots were taken every 30 minutes for TLC analysis.

Cytotoxicity assays: A stock solution was made by dissolving **L_3** or **[Ga(L_3)]** in medium (2 mL). The stock was sterilized by filtration through 0.22 µm PES syringe filter unit (Millex-GP). The concentration of the stock was calculated by UV-vis spectroscopy using the extinction coefficient of the conjugate. The stock was diluted further with medium to give the desired concentration range. 800 µl of the appropriate cells (HT-29 colon adenocarcinoma, adjusted to a concentration of $1x10^6$ cells /ml in medium with L-glutamine, was added to 200 µL conjugate solution in a 12×75 mm polystyrene FACS tube (Falcon). The cells were allowed to incubate in the dark for 1 hour at 37 °C and 5% CO_2, after which they were centrifuged with 3× excess of medium to remove unbound **L_3** or **[Ga(L_3)]**. The pellet of cells was resuspended in 1 ml medium and 4 x100 µl of each concentration was put in two 96 wells plates. One plate was irradiated with white light to a dose of 20 J cm^{-2} while the other serves as a dark control. After irradiation, 5 µl of foetal bovine serum (FBS) was added to each well and the plates are returned to the incubator overnight. After 18 to 24 hours, the cell viability was determined using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. 10 µL of 12 mM MTT solution was added to each well and incubated between 1 and 4 hours at 37 °C to allow MTT metabolisation. The crystals formed were dissolved by adding 150 µL of acid-alcohol mixture (0.04M HCl in absolute 2-propanol). The absorbance at 570 nm was measured on a Biotek ELX800 Universal Microplate Reader. The results were expressed with respect to control values

Synthesis of [Ga(L_1)]: L_1 (21 mg, 33.6 µmol) was dissolved in water (5 mL). $GaCl_3$ (11.8 mg, 67.2 µmol) was added and the pH adjusted to 4.0 with NaOH and HCl. The solution was heated to reflux for 20 hours before being concentrated. The white solid was washed with acetonitrile before being dissolved in 1:1 Acetonitrile:water, filtered, and dried to give a white solid (18 mg, 90%). ^{1}H NMR (400 MHz, D_2O, 298 K), *δ*: 8.75 (br s, 2 H, H_a), 8.37 (t, 1 H, H_c, $^3J_{HH}$ = 7.8 Hz), 8.31 (t, 1 H, H_c, $^3J_{HH}$ = 7.6 Hz), 8.02 (d, 1 H, H_b/H_d, $^3J_{HH}$ = 7.8 Hz), 7.81 – 7.92 (m, 3 H, H_b, H_d), 5.52 (s, 1 H, H_2/H_4), 5.48 (s, 1 H, H_2/H_4), 4.48 (s, 1 H, H_9), 3.41 (d, 1 H, H_{10}, $^3J_{HH}$ = 8.9 Hz), 3.36 (d, 1 H, H_6/H_8, $^2J_{HH}$ = 13.7 Hz), 3.14 (d, 1 H, H_6/H_8, $^2J_{HH}$ = 13.3 Hz), 2.88 (br s, 2 H, H_{14}), 2.67 (d, 1 H, H_6/H_8, $^2J_{HH}$ = 13.5 Hz), 2.59 (d, 1 H, H_6/H_8, $^2J_{HH}$ = 13.5 Hz), 2.45 (s, 3 H, -CH_3), 1.70 (br s, 1 H, - CH_2-), 1.59 (br s, 4 H, - CH_2-), 1.29 (br s, 1 H, -CH_2-). ^{13}C NMR (100 MHz, D_2O, 298 K), *δ*: 175.14, 171.35, 171.14, 151.31, 151.04, 147.09, 144.50, 128.21, 128.03, 70.58, 68.69, 64.29, 63.79, 57.81, 51.55, 51.26, 50.79, 46.16, 38.99, 26.68, 24.52, 22.83. MS (ESI) *m/z*: 297.58 $[M]^{2+}$

Synthesis of L_3: L_2 (50 mg, 42 µmol) and **L_1** (50 mg, 80 µmol) were taken up in dry DMF (5 mL) and triethylamine (50 µL, 360 µmol) was added. The reaction was allowed to proceed at room temperature overnight protected from light. Solvent was removed under reduced pressure and the reaction was purified using semi-preparative HPLC. The solvent was concentrated under reduced pressure, washed with diethyl ether, and dried under vacuum. The residue was precipitated by addition of diethyl ether over a methanol solution to yield a purple solid (27 mg, 20 µmol, 48%). ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 9.46 (s, 6H, porphyrin-m-Py), 9.16-8.92 (m, 14H, βH, porphyrin-o-Py), 8.81-8.52 (m, 2H, bispidine-Py), 8.29 (d, *J* = 16.4 Hz, 4H, porphyrin-o,m-Ph), 7.00-7.08 (m, 6H, bispidine-Py), 5.02 (br s, 2H), 4.69 (s, 9H, N-CH_3), 3.95 (2, 1H), 3.60-3.43 (m, 4H), 3.20-2.94 (m, 4H), 2.28-1.51 (m, 8H), -3.06 (s, 2H, NH). ^{13}C NMR (100 MHz, DMSO-D_6) *δ*: 204.09, 198.95, 198.73, 172.05, 166.35, 158.37, 158.06, 156.99, 149.46, 148.08, 144.76 (βC), 137.45, 135.04, 132.66 (βC), 127.03, 126.58, 126.22, 123.33, 123.19, 122.42, 119.43, 118.86, 116.43, 115.26, 75.01, 74.27, 67.65, 62.26, 61.38, 58.60, 50.57, 48.29, 44.07, 43.32, 42.06, 29.27, 19.70. MS (ESI) *m/z*: 405.3 $[M]^{3+}$, HRMS (ESI) *m/z*: 405.1727 (calculated for $C_{71}H_{67}N_{12}O_8$ 405.1729). UV-Vis (H_2O), *nm*: 422, 519, 558, 582, 639. *ε*(422 nm) = 258300 M^{-1} cm^{-1}.

Synthesis of [Ga(L_3)]: L_3 (1 mg, 0.76 µmol) was dissolved in water (0.4 mL). To this solution was added acetate buffer (pH 4.5, 1 M, 50 µL) and a solution of $GaCl_3$ (70 µL, 57 mM, 4.0 µmol). The reaction was allowed to proceed overnight at 100 ºC. Concentration of the reaction solution, followed by purification by semi-preparative HPLC, yielded the product as a purple solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.49 (s, 6H, porphyrin-m-Py), 9.23-8.67 (m, 16H, bispidine-Py, βH, porphyrin-o-Py), 8.62 (s, 1H, bispidine-Py), 8.42-8.11 (m, 6H, bispidine-Py, porphyrin-o,m-Ph), 7.94-7.64 (m, 3H, bispidine-Py), 5.48-4.99 (m, 2H), 4.69 (s, 9H, N-CH_3), 4.04 (s, 1H), 3.10-2.52 (m, 4H), 2.36-0.70 (m, 12H), -3.08 (s, 2H, NH). MS (ESI), *m/z*: 433.8 $[M-3Cl+H_2O]^{3+}$, HRMS (ESI), *m/z*: 433.8077 (calculated. for $C_{71}H_{66}GaN_{12}O_9$ 433.8107). UV-Vis (H_2O) *nm*: 422, 519, 556, 585, 640. *ε*(422 nm) = 240000 M^{-1} cm^{-1}.

Keywords: Gallium-68• Bispidine • PET • PDT • theranostic

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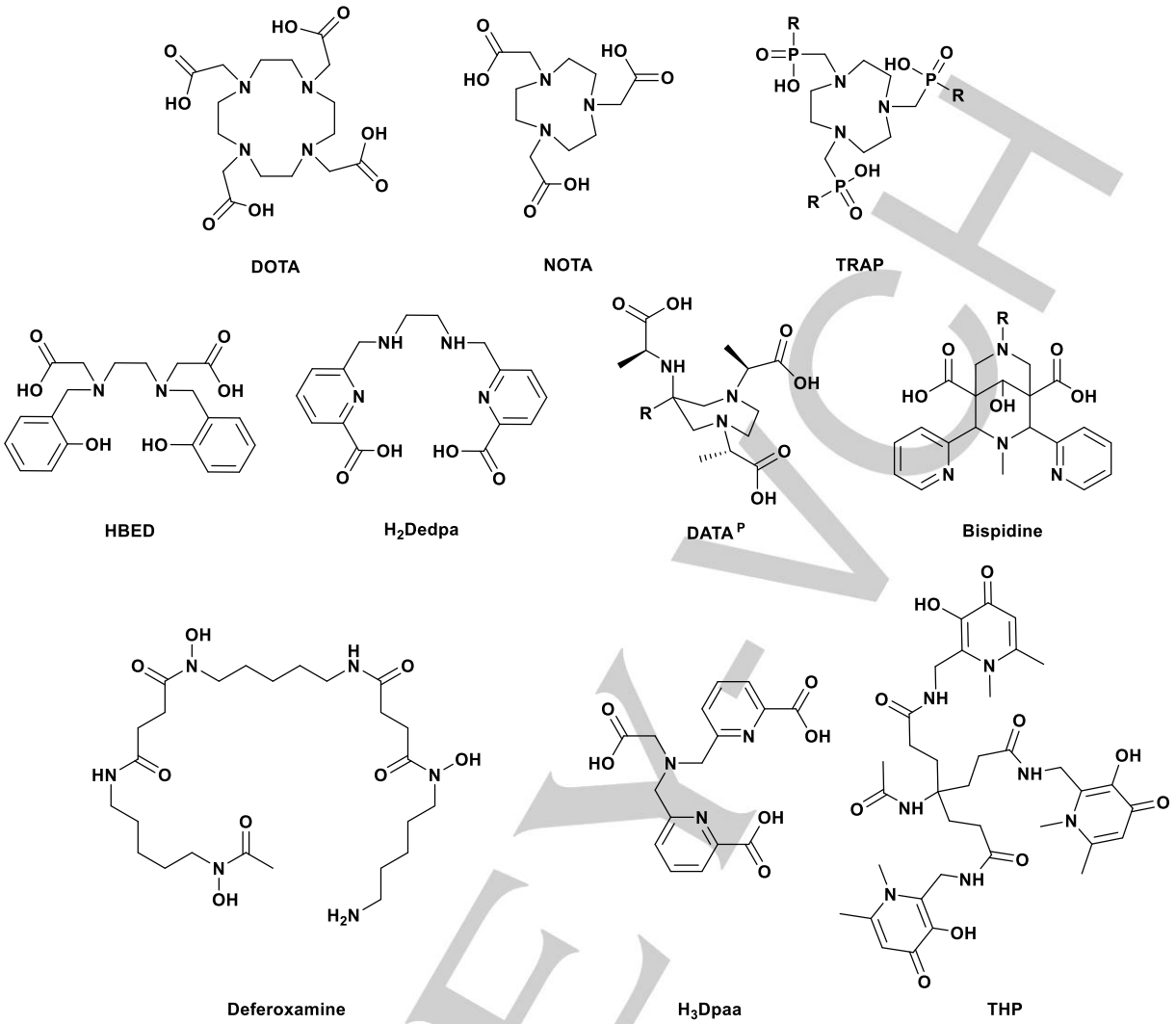
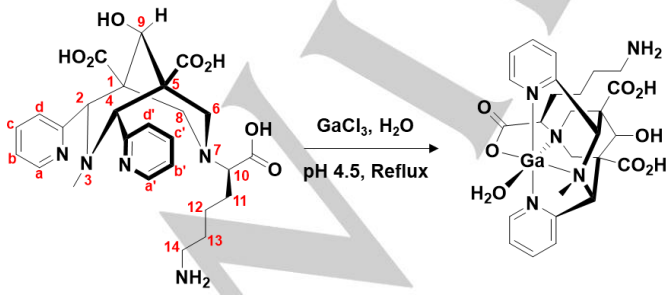


Figure 1. Ligands discussed



Scheme 1. Complexation of Ga(III) by bispidine L_1.

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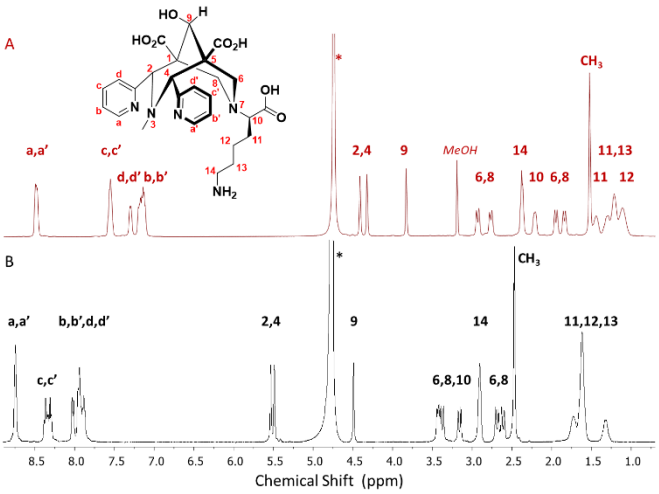

Figure 2. ^{1}H NMR (400 MHz, 298 K) of A) **L$_1$** (d_4-MeOH) and B) [Ga(**L$_1$**)] (D_2O, p*D* = 5.4).Figure Caption.

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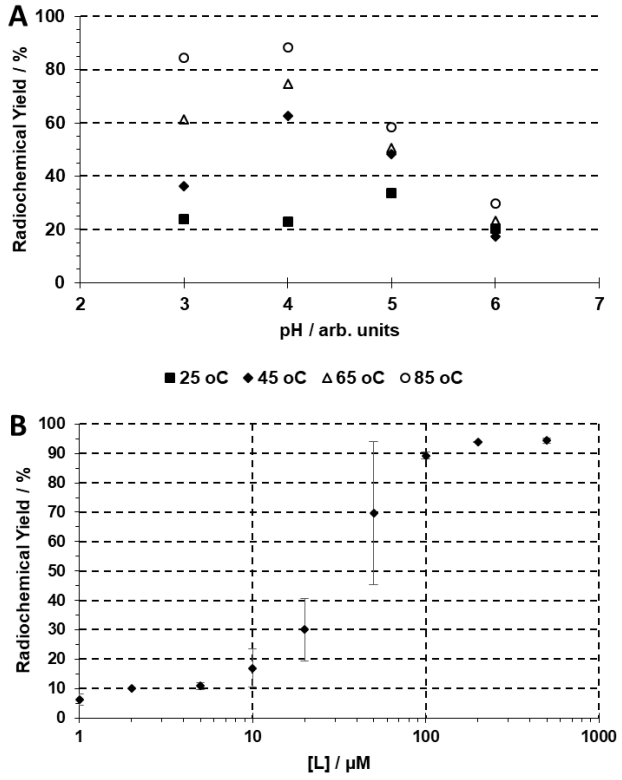

Figure 3. Radiolabelling of $\mathbf{L_1}$ with ^{68}Ga. A) Effect of pH and temperature on radiolabelling. *[L_1]* = 100 µM, *t* = 15 minutes, *I* = 0.1 M NH_4OAc. B) Effect of concentration on radiolabelling. *pH* = 4, *T* = 95 °C, *t* = 15 minutes, *I* = 0.1 M NH_4OAc.

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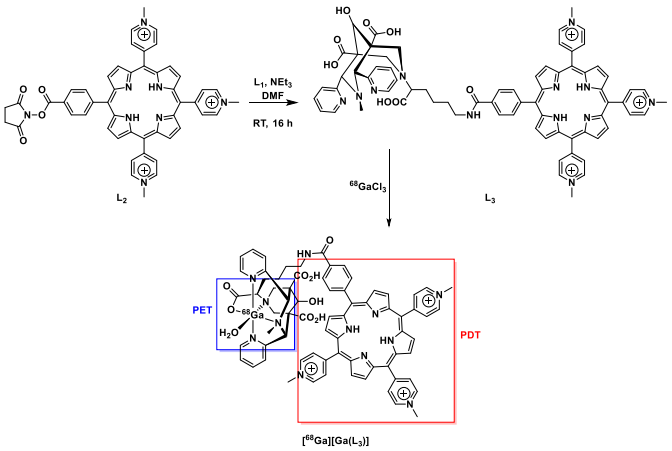

Scheme 2. Synthesis and radiolabelling of **L_3** to produce a PET/PDT theranostic agent.

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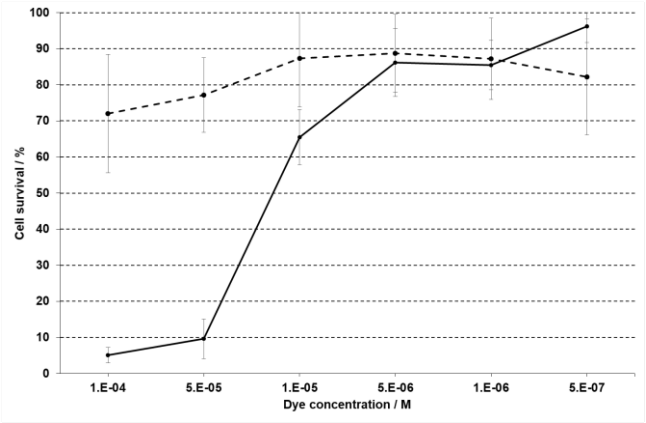

Figure 4. Toxicity of **[Ga(L_3)]** in HT-29 cells as measured by MTT assay. Solid line indicates irradiated toxicity, dashed line indicates non-irradiated toxicity.

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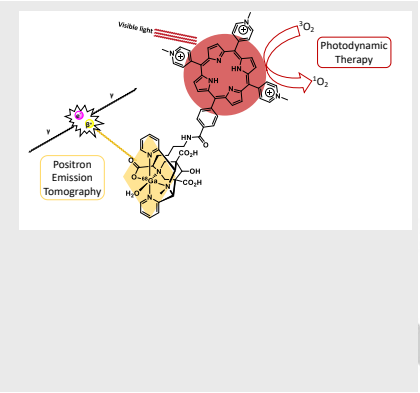
In this study a bispidine ligand has been applied to the complexation of gallium(III) and radiolabelled with gallium-68 for the first time. The resulting complex is stable in serum for over two hours, showing a ligand system perfectly matched to the imaging window of PET. To show the versatility of ligand the bifunctional chelator was conjugated to porphyrin, producing a PET/PDT-theranostic. This killed >90% of HT-29 cells upon light irradiation at 100 µM.



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Title: Evaluation of a bispidine-based chelator for gallium-68 and of the porphyrin conjugate as PET/PDT theranostic agent