THE UNIVERSITY OF HULL

The use of immobilised crown ethers as *in-situ* protecting groups for organic synthesis within flow reactors

Being a thesis submitted for the Degree of Doctor of Philosophy

The University of Hull

By

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(August 2008)

Abstract

Organic synthesis often requires one functional group of a bifunctionalised compound to be rendered temporarily inert to allow the selective reaction of another moiety. While protecting groups are used to remove the problem of the functional group incompatibility, they also raise other issues such as increasing the length of the synthetic pathway (by at least two steps – protection and deprotection), generally leading to an increase in cost and a decrease in yield. The protecting group is often selected based upon the deprotection conditions, leading to the requirement for orthogonal protecting groups. Crown ethers are commonly associated with the complexation of metal ions, but the 18-crown-6 species also readily bind ammonium ions with complexation occurring *via* hydrogen bonding. As discussed in Chapter 1, crown ethers have previously been employed for *N*-protection in this way, to successfully facilitate the reaction of bifunctional compounds, though they have exhibited very little selectivity and reaction control. Isolation of the desired product from the resulting reaction mixture has also proven to be problematic.

Over recent years there has been a large increase in the volume of organic protocols conducted in micro and continuous flow reactors. Utilising the high surface to volume ratio obtained under these reaction conditions, greater reaction control of many common and specialised organic syntheses has been reported. Building upon literature precedent, the work herein reports the immobilisation of an 18-crown-6 ether derivative onto a solid-support and its incorporation into a continuous flow reactor to enable sequestration of the primary amine salt of a bi-functionalised compound. This effectively affords a non-covalent *N*-protection strategy allowing the selective reaction of the remaining moiety. The desired product is subsequently recovered as the free amine by a simple process of decomplexation using an organic base.

Acknowledgements

The author would like to thank Professor Stephen J. Haswell for his supervision. Dr. Paul Watts, thank you for both your supervision and support through everything over five long years. Also to Dr. Charlotte Wiles, for your vast amounts of help, guidance, encouragement and support, not to mention your friendship, thank you does not even come close. Thanks are also given to all other members of the group, for their helpful, insightful discussions and encouragement, to the technical staff of Chemistry Department for their services and to the ESPRC for funding.

I must also thank my family for their support and general encouragement, especially Sarah and Annabel, my two ladies who make what I do worthwhile. They are the two people who will be most proud of this achievement, the two people who have suffered along with me and, most importantly, the two who have inspired me to never give up. A child, a marriage, a new house, a teaching qualification, not to mention your degree and new careers for us both; Sarah, we did it x.

Declaration

The work described in this thesis was carried out in the Department of Chemistry, The University of Hull under the supervision of Prof. S. J. Haswell and Dr. P. Watts between September 2003-September 2006. Except where indicated by references, this work is original and has not been submitted for any other degree.

Gareth Peter Wild

August 2008

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Abbreviations

AM-18-c-6	Aminomethyl-18-crown-6-ether
Ar	Aromatic
Boc	*t*-Butoxycarbonyl
br	Broad
Bu	Butyl
C_0	Quaternary carbon
CAB-18-c-6	Carboxybenzo-18-crown-6-ether
Cbz	Carbobenzyloxy
d	Doublet
D	Diffusion Coefficient
d	Diameter
DADB-18-c-6	Diaminodibenzo-18-crown-6-ether
DB-18-c-6	Dibenzo-18-crown-6 ether
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	*N,N*-Dicyclohexylcarbodiimide
DCM	Dichloromethane
dd	Double doublet
D_e	Effective diameter
DIEA	*N,N*-Diisopropylethylamine
Dmab	4-[*N*-(1-(4,4-Dimethyl-2,6-dioxocyclohexylidene)-3-methyl-butyl)amino]benzyl
DMAP	4-Dimethylaminopyridine
DMF	*N,N*-Dimethylformamide
DMSO	Dimethylsulfoxide
DNDB18-c-6	Dinitrodibenzo-18-crown-6-ether
DVB	Divinylbenzene
EDCI	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
EOF	Electroosmotic flow
EtOH	Ethanol
FEP	Fluorinated ethylenepropylene
Fmoc	9-Fluorenylmethoxycarbonyl
GC-MS	Gas Chromatography-Mass Spectrometry
η	Viscosity of the liquid
HCl	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
i.d.	Internal diameter
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry

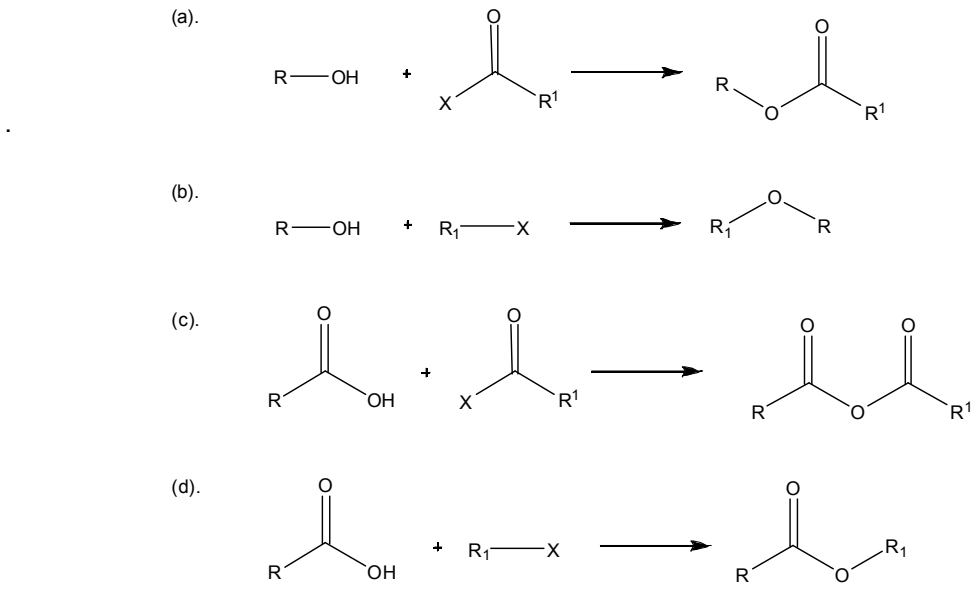
IR	Infra red
m	Multiplet
m/z	Mass to charge ratio
MALDI-MS	Matrix Assisted Laser Desorption Ionisation-Mass Spectrometry
MeCN	Acetonitrile
MeOH	Methanol
Merrifield peptide resin	MPR
MS	Mass Spectrometry
ν	Velocity
NMR	Nuclear Magnetic Resonance
o.d.	Outer diameter
ºC	Degrees Celcius
PASSflow	Polymer Assisted Solution Phase Synthesis
PCTFE	Polychlorotrifluoroethylene
PDF	Pressure driven flow
PEEK	Polyetheretherketone
PET	Positron Emission Tomography
PTFE	Polytetrafluoroethylene
p-TSA	*p*-Toluene sulphonic acid
ρ	Density of the liquid
R_e	Reynolds number
RSD	Relative standard deviation
R_T	Retention time
s	Singlet
SPPS	Solid phase peptide synthesis
SPS	Solid phase synthesis
t	Triplet
T	Temp
t	Time
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMEDA	*N,N,N′,N′*-Tetramethylethylenediamine
TMS	Tetramethylsilane
α	Thermal diffusivity
UV	Ultraviolet
15-c-6	15-crown-5-ether
18-c-6	18-crown-6-ether

Chapter 1: Introduction

1.0 Introduction

1.1 Chemistry of the -OH group

The -OH group can be found in two major classes of organic compound, alcohols and carboxylic acids. Alcohols are compounds whose molecules have a hydroxy group covalently bound to a saturated carbon atom, with compounds that possess a hydroxy group directly attached to an aromatic group known as phenols. Reactions of alcohols include acylations and alkylations which readily afford the respective ester or ether (Scheme 1 (a) and (b)), with the proton of a phenolic compound being far more labile, than an aliphatic alcohol, due to the delocalisation of electrons by the aromatic ring. In addition to the prevalence of the ester moiety in compounds ranging from polyesters and foodstuffs, it is also a common functionality present in pharmaceuticals, such as aspirin and cocaine. Conversely, the ether moiety can be found in compounds with properties ranging from solvents to anesthetics to naturally occurring essential oils.



Scheme 1. General scheme illustrating (a) the acylation and (b) alkylation of an alcohol, and (c) the acylation and (d) alkylation of a carboxylic acid.

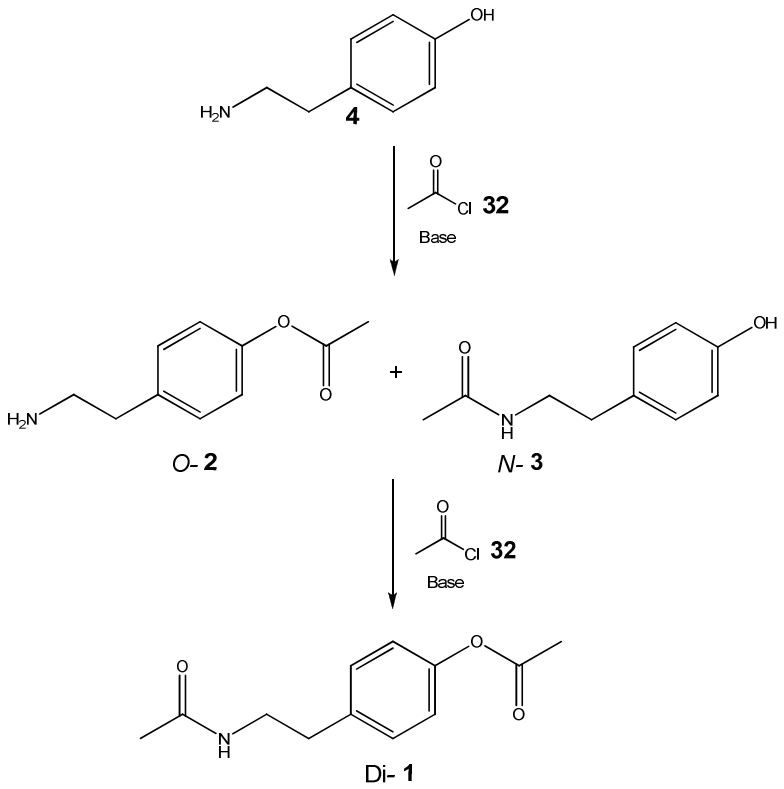
Analogous to the reaction of alcohols, carboxylic acids can also undergo acylation and alkylation reactions to afford anhydrides (Scheme 1 (c)) and esters (Scheme 1 (d)) respectively. Due to the versatility of the reaction products derived from the hydroxyl moiety, the selective *O*-acetylation and *O*-methylation of a series of phenolic derivatives forms the focus of the investigation reported herein, with the potential of the technique to be applied to amino acids discussed in Section 3.6.

1.2 Protecting group chemistry

When conducting the synthesis of polyfunctionalised molecules, it is often necessary to render one or more functionalities within the molecule temporarily unreactive in order to allow the reaction of another group and selectively obtain the desired product. This process is frequently achieved by appending a blocking group that is stable to the reaction conditions required for the transformation which can later be removed and as such is termed a protecting group.[1] Where more than one protecting group is required, orthogonal protecting groups must be selected to ensure removal can be conducted in the desired sequence. Careful selection of the protecting group is also paramount when designing the synthetic pathway, as the protecting group must be stable to the conditions employed for derivatisation of the remaining functionality, whilst requiring mild and selective conditions for its removal after conducting the desired reaction step.[1]

Owing to the fact that the amino functionality is both highly basic and nucleophilic,[2] protection of the group in bifunctional compounds is essential in even the most apparently trivial of reactions such as, alkyations and acylations.[3] This is illustrated in Scheme 2, where failure to protect the amine results in the undesirable formation of a complex reaction mixture containing three products, the diacetate **1**, the *O*-acetate **2** and the *N*-acetate **3**, along with residual starting material **4**. In addition, when performing

alkylation reactions the undesirable synthesis of an array of ammonium salts is also possible.



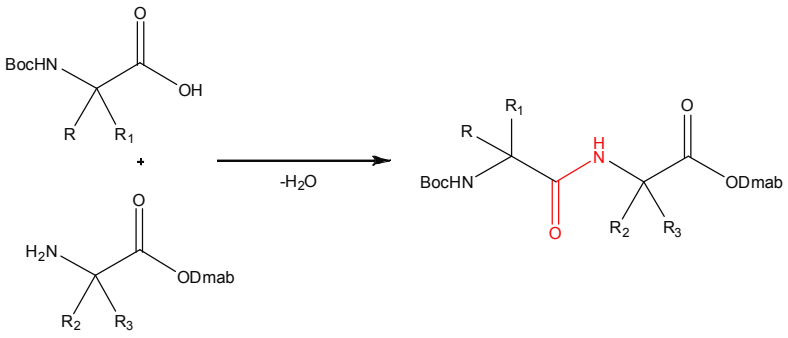
Scheme 2. Illustration of the complex reaction mixture obtained when acetylating unprotected tyramine **4**.

With this in mind, the history of protecting group chemistry, with respect to the amine group (-NH_2), is discussed along with a proposed alternative method to replace the current approach, which forms the basis of this investigation.

1.2.1 Common uses of *N*-protecting group chemistry

Amino acids are among the most common bifunctional compounds employed in synthetic organic chemistry. Owing to the diverse array of reactions available, amino acids have been employed in the preparation of peptides and proteins which have been employed as antibiotics, hormones, food additives, poisons, or painkillers.[4] As

illustrated in Scheme 3, whereby the peptide bond is highlighted in red, to ensure reaction control is obtained and the desired reaction product results, protecting groups are employed to block the respective amine and carboxylic acid moieties in order to prevent self condensation.[1,5]



Scheme 3. Illustration of a condensation reaction between two protected amino acid residues to afford the selective synthesis of a protected dipeptide.

The carboxylic acid is usually protected as an ester, with protecting groups including *tert*-butyl esters, benzyl esters and 4-(*N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]amino)-benzyl ester (Dmab). In contrast, the amino group may be protected by the acid labile *tert*-butoxycarbonyl (Boc) group or base labile 9-fluorenylmethoxycarbonyl (Fmoc) group, as illustrated in Figure 1. Where multiple protecting groups are employed within a molecule it is imperative that orthogonal groups are selected to enable control over the order of removal.[6-10]

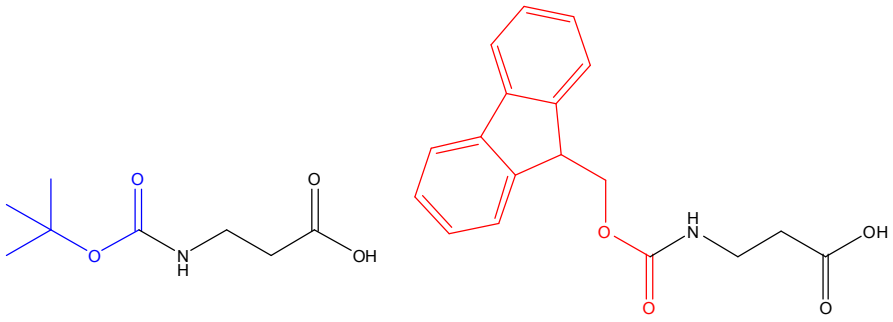
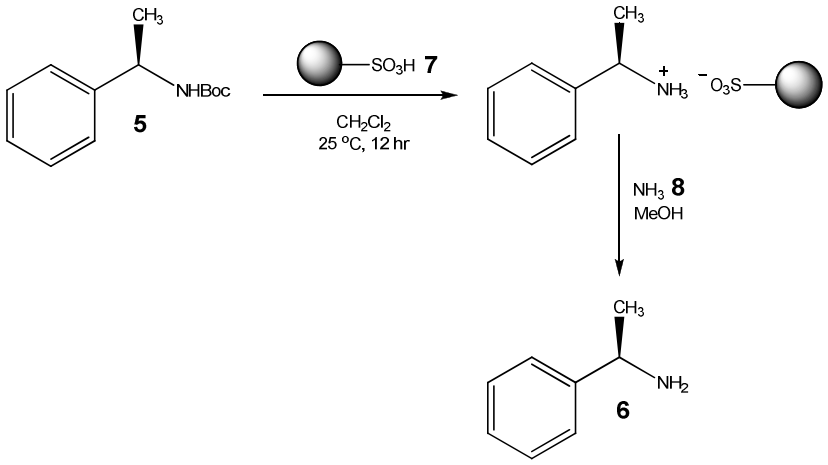


Figure 1. Schematic illustrating the protection of β-alanine with Boc and Fmoc respectively.

When considering the previously discussed acetylation of 4-(2-aminoethyl-phenol (tyramine) **4**, the introduction of a protecting group such as Boc, would enable the base promoted reaction to be conducted in order to react at the phenolic group whilst the acid labile protecting group remains unaffected. To afford the desired *O*-acetylated product, tyramine acetate **2**, however subsequent deprotection is required. This can be achieved using a variety of acids, to afford the respective acid salt of the amine compound (see Section 2.2).[10-13] However, it needs to be emphasised that in order to do one desired reaction, a long pathway of reactions is required.

An additional reaction step is then necessary if the desired product is required as the free amine. A novel solution to this problem was reported by Bergbreiter *et al.,*[14] who demonstrated the use of an ion-exchange resin for simultaneous deprotection and purification of Boc-protected compounds, simplifying the traditional approach of acidic deprotection greatly. Using principles first exploited by ion-exchange chromatography, the author reasoned that a strongly acidic ion-exchange resin should be able to sequester a Boc-protected amine **5** and be used to liberate the free amine **6** as the product (Scheme 4).



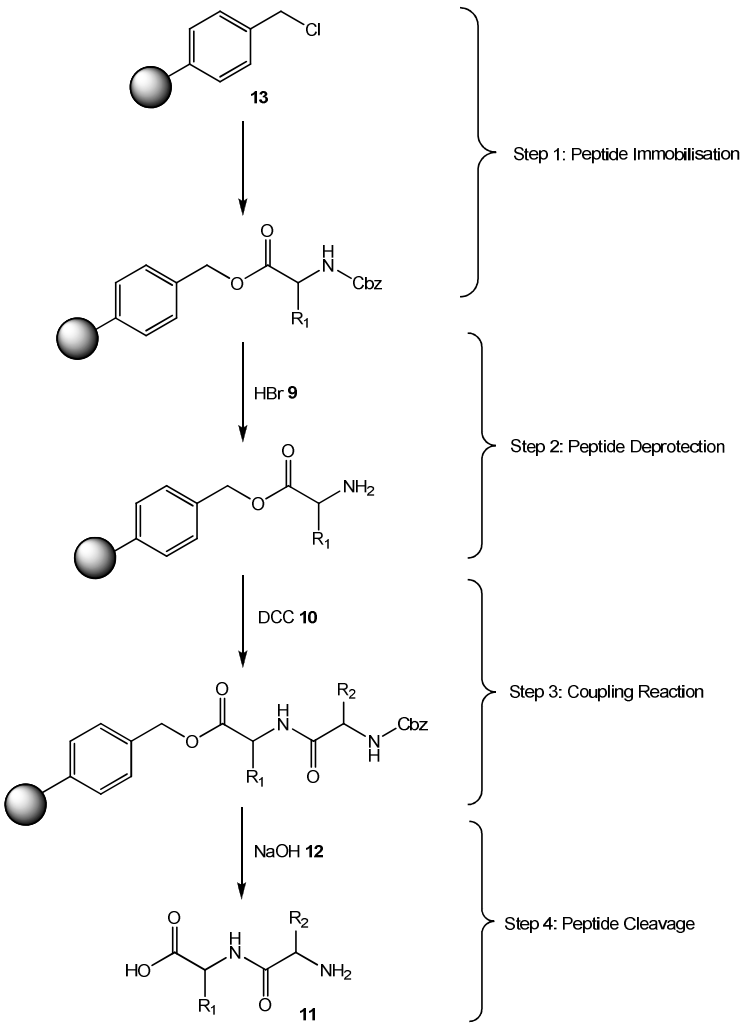
Scheme 4. The deprotection/purification of a Boc-protected amine using Amberlyst-15 **7**.

To facilitate deprotection, the compound **5** was stirred with Amberlyst-15 **7**, a macroreticular resin, for sufficient time to ensure that all of the material had been sequestered, typically 12 hr. The amine bound resin was then washed, to remove any by-products and subsequently treated with methanolic ammonia **8**, to afford the free amine **6** in yields > 80 %. Compared to the use of protic acids, this approach is advantageous as the free amine is recovered and not the respective acid salt.

It is, however, the time consuming nature of protection, reaction and subsequent deprotection that restricted the growth of solution phase peptide chemistry, largely due to the labour intensive nature of intermediate and product isolation. In order to address this, the field of solid phase peptide synthesis (SPPS) developed as a means of simplifying the process and enabling the preparation of peptide chains that would otherwise not be practical using solution phase chemistry.

1.2.2 Solid phase peptide synthesis

Dating back to the seminal work by Merrifield,[15] SPPS has become a popular tool for the synthesis of organic compounds due to the minimal amount of traditional post-reaction purification that is required. The principle of this technique is the stepwise addition of protected amino acids to a solid-supported amino acid, repeating these steps in order to selectively synthesise a growing peptide chain. Using this approach, Merrifield was able to synthesise a tetrapeptide and revolutionise this area of organic chemistry. His work also noted that traditional organic synthesis did not lend itself to the production of long chain polypeptides because of issues associated with purification and solubility, especially as the peptide chain increases in length. Having determined a co-polymer of chloromethylated styrene and divinylbenzene (200-400 mesh) to be the most effective solid support, due to the porous gel structure, readily allowing the penetration of reagents, the first step of Merrifield's process was to covalently attach a benzoyl ester protected amino acid to the resin, effectively protecting the NH_2 group. Deprotection of the Cbz group (HBr **9** in an organic acid) afforded the respective immobilised carboxylic acid which subsequently underwent a carbodiimide coupling reaction, in the presence of *N,N*-dicyclohexylcarbodiimide (DCC) **10**, to afford the supported dipeptide residue. At this stage, washing of the solid support removed any residual DCC **10** and *N,N*-dicylcohexylurea, prior to cleavage of the peptide from the support using sodium hydroxide **12**. This step was then followed by filtration of the solid support and concentration of the liquor afforded the desired peptide **12**. After this initial work, Merrifield evaluated the preparation of longer peptide fragments, by simply repeating Steps 2 and 3 (Scheme 5) until the desired chain length was obtained, followed by base promoted cleavage from the support. Using this approach, a tetrapeptide was synthesised and isolated in 80 % yield after cleavage from the support.



Scheme 5. A scheme showing the generic principle of SPPS as developed by Merrifield.

The use of Merrifeld peptide resin (MPR) **13** and the principle of SPPS is still widely applied today with peptide chains of up to 50 amino acids being reported.[16] The field of SPPS has also expanded to encapsulate many areas of chemistry not just peptide synthesis and, as such, the range of resins for solid phase synthesis (SPS) has expanded to facilitate the diversity required for this methodology.[17]

Although the technique undoubtedly facilitated the synthesis of compounds that would otherwise remain inaccessible with conventional solution phase chemistry, there are

several problems associated with the use of SPS for multi-step synthesis, most notably the loss of material during filtration and washing stages. The most prominent advantage however, remains the removal of by-products and the facile purification and isolation of the target molecule. While Merrifield reported an excellent yield, a lengthy purification process was still required in order to remove the random oligomers formed as a result of not all immobilised amino acids reacting at each stage of the peptide synthesis; an observation that has subsequently been overcome by the use of excess reactants at all stages.

With these factors in mind, the work herein endeavours to address these issues of incomplete reaction, by developing an immobilised non-covalent protecting group strategy that can be employed under continuous flow. The aim is to promote efficient reaction at each step, with minimal reagents, to afford the desired product in excellent purity, without the need for subsequent product purification (Section 1.5).

1.3 Crown ethers and their affinity for cations

First reported by Pederson[18,19] in 1967, cyclic polyethers or crown ethers as they are more commonly referred to due to their crown shape, have found many uses in both inorganic and organic chemistry. Pedersen initially synthesised thirty three crown ethers, the most common of which are illustrated in Figure 2, and trivially named the compounds based on (a) the number and kind of hydrocarbon rings, (b) the total number of atoms in the polyether ring, (c) the class name, crown, and (d) the number of oxygen atoms in the polyether ring, for example; dibenzo-18-crown-6 ether (DB-18-c-6) **14**.

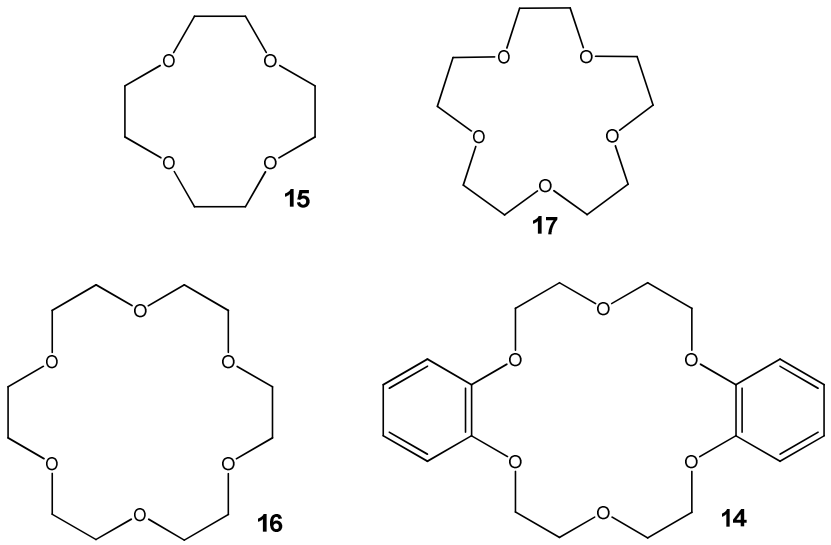


Figure 2. Schematic illustrating some of the common crown ethers synthesised by Pederson**,** 12-crown-4 ether **15**, 15-crown-5 ether **17**, 18-crown-6 **16** and DB-18-c-6 **14**.

During their evaluation, Pedersen noted that many of the polyethers he had synthesised possessed the propensity to form complexes with metal ions and/or their salts. Based on this observation, Pederson's[19] subsequent work was focussed on the complexation of various metal salts (Li^+, Na^+, K^+, Rb^+ and Cs^+) within the cavity of crown ethers. By comparing the binding constants obtained with the diameter of the metal under investigation and stating that a stable complex would not form if the ion was too large to lie in the cavity of the crown ether, Pedersen was able to define the potential uses of each crown ether. In accordance with this work, Table 1 illustrates the binding constants obtained for a series of metals within DB-18-c-6 **14**, which has a measured cavity size of 4.0 Å (Figure 2).[18] The high binding constant of K^+ corroborates the DB18-c-6 **14** cavity size as being large enough to accommodate the potassium cation (2.8 Å) (Figure 3).

Cation	Diameter of Cation (Å)	Binding constant (log K_s)
Li^+	1.5	1.00
Na^+	2.0	4.35
K^+	2.8	6.08
Rb^+	3.0	5.32
Cs^+	3.4	4.79

Table 1. The correlation of cation size and binding constant with respect to DB18-c-6 **14**.

As such, the affinity of crown ethers for metal ions and the stability of their complexes opened up a plethora of applications, with reported uses including a means of increasing the solubility of alkali metal salts, in non-polar solvents such as benzene[20] along with their use as an ion exchange site.[21] The early development and application of these crown ethers, and further macrocycles such as aza-crown ethers and 2,2,2-cryptate (Kryptofix),[22] is comprehensively reviewed by Gokel and Durst.[23]

1.3.1 The affinity of crown ethers for organic cations

In addition to the evaluation of metal complexes, Pederson[18] also included an investigation into the ammonium ion ($R\text{-}NH_3^+$), reporting that the HCl salt of glycine complexed with DB-18-c-6 **14**, stating "these compounds appear to be salt-polymer complexes formed by ion-dipole interaction between the cation and the negatively charged oxygen atoms symmetrically placed in the polyether ring". Further work by Bushman and Mutihac[24] compared the degree of ammonium ion complexation within various functionalised and unfunctionalised crown ethers. The authors found that 18-c-6 **16** afforded superior complexation compared to smaller crown ethers such as 15-crown-5-ether (15-c-5) **17** and as depicted in Figure 2. Compared to metal ions the ammonium ion is held above the cavity of the crown ether[25] **14** by hydrogen bonding that occurs

between the ammonium salt and the ether *cf.* within the cavity for metal cations (Figure 3). This phenomenon is further induced by the distribution of the positive charge on the ammonium ion over the hydrogen atoms,[26] with the length of the hydrogen bonds being approximately 2 Å.[27]

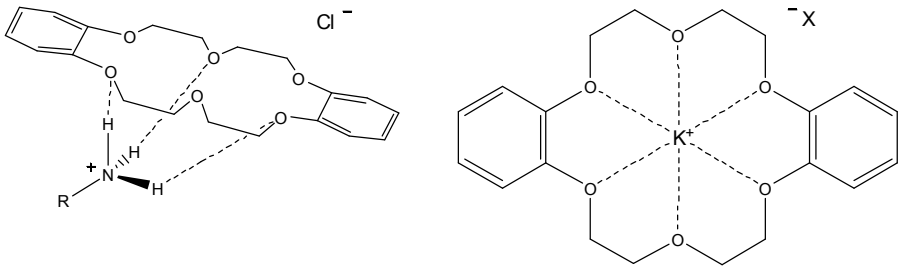


Figure 3. Schematic illustrating the different modes of complexation observed between an ammonium ion and an inorganic compound with DB-18-c-6 **14**.

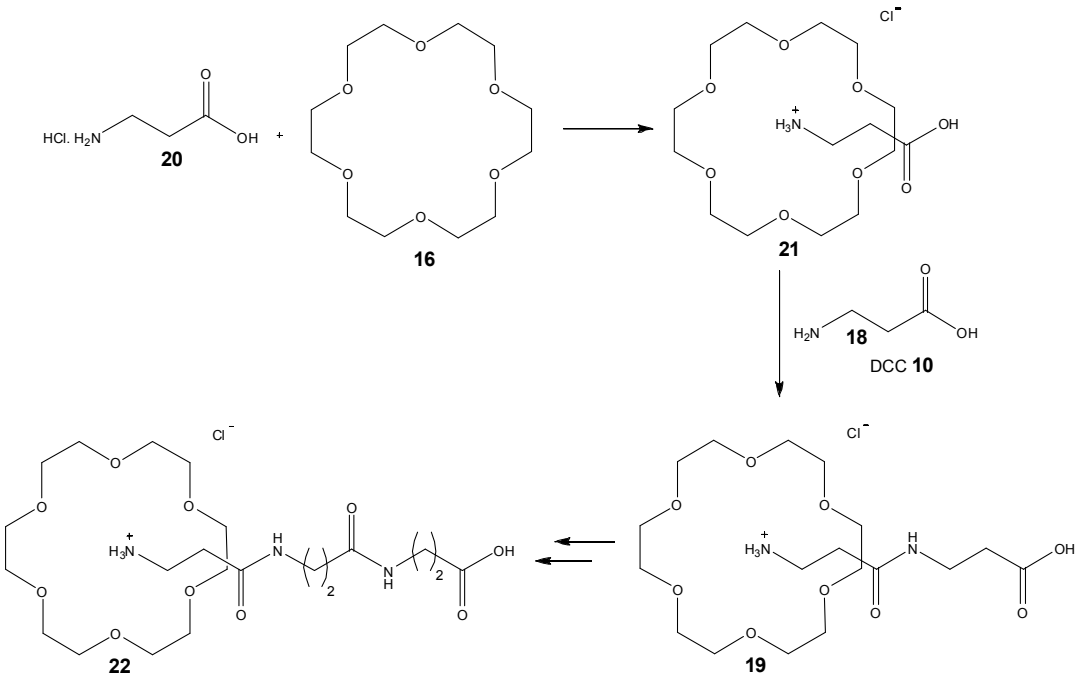
When studying the reactions of R-NH_3^+/18-crown-6-ether (18-c-6) in MeOH, Izzat *et al.*[28] determined a series of critical points about the complex formation, most notable being the stability of the ammonium salts followed the trend of $1^\circ > 2^\circ > 3^\circ$, an observation that was attributed to the primary salt having the greatest hydrogen bonding. They also determined the chain length emanating from the ammonium ion (R-NH_3^+) had no appreciable effect on the ability of the compound to complex. Steric hinderance was found to have an effect on the ability of the ammonium ion to complex, reasoning that this was due to an inability to completely form all possible hydrogen bonds. The authors also confirmed Pederson's[18] observation that an amine group would not complex unless present as the ammonium ion. Collectively, this evidence suggested that either 18-c-6 **16** or DB-18-c-6 **14** would be generally applicable to the extraction[29] or protection of amino acids and amine containing compounds.[30]

1.3.2 Application of crown ethers in organic chemistry

The ability of 18-c-6 ethers to complex with ammonium ions has facilitated the exploration of a wide range of chemistries,[23,31] including the analysis of amines by electrospray ionization mass spectrometry,[24] along with finding use in a range of biological applications to facilitate the transfer of ions across membranes. One of the most frequently reported uses however remains in the field of peptide synthesis.[32] One of the early reported uses of crown ethers in this was by Koga and co-workers[33] who employed functionalised 18-c-6 ethers to enhance the rate of thiolysis of amino ester salts to afford the corresponding thioester *via* the non-covalent complexation of the amino acid salt. However, the most interesting reports relating to the work described herein were a series of papers communicated by Mascagni and Hyde[34-37] who presented extensive work on the synthesis of peptidic oligomers using DB-18-c-6 **14** as a protecting group for the amine group of amino acids.

In the first instance, Mascagni and co-workers[34] reported the use of 18-c-6 **16** as a non covalent protecting group for the amine group of β-alanine and proposed the tetrahedral shape of the ammonium ion[26] when complexed would enable the selective reaction of the exposed carboxylic acid group. With this in mind, the authors' initial investigation centred on the ability to complex an ammonium salt of β-alanine **18**, with 18-c-6 **16**, to afford *N*-protection and subsequently investigated the condensation of the carboxylic acid with the amine of a second β-alanine **18** unit, affording the complexed dipeptide **19**, illustrated in Scheme 6. The selective masking of the amine group was achieved by stirring the β-alanine **18** (1.0 eq.) in water with HCl (1.1 eq.) to afford the HCl salt **20**. The hydrochloride salt **20** was then stirred in chloroform with 18-c-6 **16** (1.0 eq.) to afford the β-alanine crown ether complex **21** which was then treated with DCC **10** (1.0 eq.)

followed by β-alanine **18** (1.0 eq.). Using this approach, Mascagni and co-workers were able to isolate some of the complexed dipeptide **19** after 12 hr.



Scheme 6. Illustration of the non covalent strategy employed for the *N*-protection of β-alanine.

A further equivalent of DCC **10** and β-alanine **18** was then added and the reaction mixture analysed periodically over three weeks; after which evidence of the complexed tri-peptide **22** was determined by ^{1}H NMR spectroscopy. After isolation, the complexed peptide **22** was found in 80 % yield, with the remainder made up of mixed oligomers with the authors deducing that competition for the counterion by DCC **10** forced the ammonium ion complex to be in equilibrium. To reduce this effect, the procedure was also repeated using the *p*-toluene sulphonic acid (*p*-TSA) salt of β-alanine and was found to afford a far more stable complex than the HCl salt **20** due to conjugation of the counterion. Importantly, the authors noted that although this was critical to the complex stability, the counterion did not interfere with the reaction or have any effect on the

complex formation. In a further attempt to minimise oligomer formation and prevent partial *N*-deprotection, the authors subsequently complexed the dipeptide in an attempt to distance the site of the condensation reaction from the point of complex, thus removing the decomplexing effect of the coupling intermediate. This also allowed the use of more common solvents such DCM and DMF to be used *cf.* DMSO that was previously investigated as a means of circumventing this problem.[35] Further investigations with the complexed dipeptide focussed on evaluating the effect of the counterion, with respect to the stability of the complex and the proportion of oligomers formed, with the authors concluding that *p*-TSA > TFA > HCl. These observations were later corroborated by Bushman and Mutihac.[24]

It was at this point of their investigation that the authors considered isolating the decomplexed tri-peptide,[36] proposing for the first time that the non covalent nature of the interaction between crown ethers and the ammonium ion provided the basis for a rapid but mild protection and deprotection strategy. Removal of the oligomers from the crown ether protecting group was initially carried by washing with an aqueous solution of KCl **23**, exploiting the crown ethers affinity for metal ions (Table 1) to force out the ammonium ion, leaving the crown ether complexed with a metal ion (Figure 3) and unable to be re-used without regeneration.

In the fourth and final paper of the series, Mascagni *et al.*[37] synthesised and isolated a tri-peptide by combining their previous research with SPS. By coupling a resin *O*-protected amino acid with their crown ether *N*-protected dipeptide, the authors were able to afford the tri-peptide. This proved to be more selective that previous efforts, with a

further development being the implementation of an organic base, *N,N*-diisopropylethylamine (DIEA) **24** to promote deprotection.

Crown ethers have also been reported in the literature for applications other than peptide complexation, with Sato and Kawamura[38] developing immobilised crown ethers on magnetic nanoparticles as phase transfer catalysts. By coordinating Fe_3O_4 particles with either benzo-15-crown-5 or benzo-18-crown-6 ethers, the authors were able to improve the recyclability of the macrocycles by recovering them from the reaction mixture with a magnet.

With the previous work in mind, the investigation described in Chapter 2 aims to build upon the concept of 18-crown-6 ethers a non covalent protecting groups for amines coupled with the use of continuous flow reactors, as a means of offering a more selective and controlled method for the *O*-acylation and *O*-alkylation of bifunctionalised compounds.

1.4 Continuous flow reactors

The history of synthetic organic chemistry has been dominated by the use of round bottomed flasks, with reactions conventionally carried out in batches, on scales ranging from several milligrams to grams in research laboratories and kilograms to tonnes within pilot plant facilities; as such, solvent requirements range from millilitres to litres depending on the process under investigation. The desire to produce new and improved pharmaceutical products, coupled with huge developments in high throughput chemistry and the current competitive market have seen a shift from this batch-wise approach to micro scale organic techniques over the past decade.[39]

One of the major challenges that synthetic chemists frequently encounter, is the ability to successfully transfer synthetic protocols used for the preparation of a compound from a research and development environment, to a scale that is suitable for production of the target compound on a commercial scale. This observation has been largely attributed to changes in mixing and thermal properties as a result of modifications made to the size and dimensions of the reaction vessel. As Table 2 illustrates, as the size of the reaction vessel decreases, the surface to volume ratio increases, which in turn increases the rate at which changes occur to afford a homogeneous system.[40]

Parameter	Industrial Reactor (28, 000 l)	Lab Scale (250 ml)	Micro Scale (140 µl)	Nano Scale (125 nl)
Radius (m)	1.5	3.9×10^{-2}	3.0×10^{-2}	5×10^{-5}
Height (m)	4	N/A	5×10^{-3}	5×10^{-2}
Volume (m^3)	28	2.5×10^{-4}	1.4×10^{-7}	1.3×10^{-10}
Surface Area(m^2)	52	2×10^{-2}	1.5×10^{-4}	1.5×10^{-5}
S/V Ratio	2 :1	76 : 1	1080 : 1	8000 : 1

Table 2. The effect of reactor dimension on the surface to volume ratio.

1.4.1 The effect of reaction scale on mixing and temperature control

To ensure reaction selectivity is maintained, it is imperative that both reaction temperature and reactant concentrations are uniform over the reaction vessel. When conducting reactions in large batch vessels, mixing is achieved using mechanical stirrers and results in the formation of large eddies, which allow bulk diffusion to dominate. As such, the chance of a reaction occurring is based on collision theory and as the scale of the reaction increases, the efficiency of the stirring must also increase to prevent hindrance of the reaction, however this can be very difficult to achieve.

In comparison, at the micro-scale, high viscous forces dominate thus turbulence cannot be induced and mixing is limited to molecular diffusion. The importance of the inertial and viscous forces experienced by a moving liquid are determined by the Reynolds number (R_e) which is a dimensionless parameter (Equation 1) that can be used to determine the flow type experienced in a given reactor;

$$R_e = \frac{D_e \nu \rho}{\eta}$$

Equation 1. Determination of the Reynolds number (R_e).

Where ν is the velocity, ρ is the density of the liquid, η is the viscosity of the liquid and D_e is the effective diameter.

As Equation 1 illustrates, when considering the mixing of fluids, the size of the reaction vessel and the viscosity of the reaction mixture are the key factors to consider. This is exemplified for a large reaction vessel containing a non-viscous fluid ($R_e > 3000$) where turbulent flow is observed and a small reactor vessel containing the same fluid ($R_e < 1000$) where laminar flow dominates. In the case of micro reactors with dimensions in the order of micrometres, $R_e < 10$ and therefore diffusion only occurs at the interface of the two liquid streams. The rate at which diffusion occurs therefore is dependent upon the width of the reactor and as such, reaction vessels with high aspect ratios provide a simple route to increasing interfacial areas and hence a reduction in mixing time; an example exploiting this phenomenon can be found in Section 1.4.3, Table 3.

In addition to efficient mixing, the control of reaction temperature is also crucial when performing a reaction, not only from the point of view of product quality, but also from a safety perspective. In large-scale reaction vessels, fluctuations in reaction temperature occur and are difficult to correct due to the time taken for any amendments to have an

effect on the bulk. In comparison, when a change is made on the micro-scale, the effects are observed almost immediately, an effect which is in-line with Equation 2.

$$t \sim \frac{d^2}{\alpha}$$

Equation 2. Approximation of the time taken to obtain thermal mixing across a reactor.

Where *t* is the time scale, *d* is the diameter of the reactor and α is the thermal diffusivity of the fluid.

As a result, along with decreasing the time taken to change the reaction temperature employed, decreases in reactor volume also result in a high surface to volume ratio (Table 2) which reduce the risks associated with performing potentially explosive reactions as hot spot formation is removed, and the chance of thermal runaway when conducting highly exothermic reactions.[41] Consequently, owing to the unique reaction control attained in such continuous flow systems, the technology has opened up new horizons for both the synthetic organic chemist and the manufacturing industry.[42]

1.4.2 Fabrication and operation of continuous flow reactors

So called micro reactors or continuous flow reactors are commonly fabricated from glass,[43,44] but examples have also been reported where systems are manufactured from materials including plastic, polymers, ceramics and metals.[45] Irrespective of the material selected, the reactors all have in common the fact that they contain channels of the order of millimetres or below.[46] To perform reactions in such systems, solvents and reagents are manoeuvred through the channel(s) of the continuous flow reactor by one of two principle methods, pressure driven flow (PDF)[47] or electroosmotic flow (EOF).[48]

Pressure driven flow is by far the most common method reported within the literature, due to the ease with which it can be implemented through the use of commercially

available systems and connectors. The technique however suffers from disadvantages such as the size and expense of the syringe pumps *cf.* the reactors themselves, along with potential issues associated with back pressure generation within the reactor[49] and the generation of a parabolic flow profile.

An alternative method for pumping solvents and reactants around a reactor is electroosmotic flow, which employs a sequence of voltages applied to the solution *via* electrodes in order to induce pumping of the fluid. This methodology is preferred by some researchers due to the lack of mechanical parts, the small operating set-up *cf.* bulky syringe pumps and the flat flow profile obtained, compared to hydrodynamic pumping. A limitation of EOF however is the requirement of a polar solvent system to allow bulk flow of the reagents.[49,50]

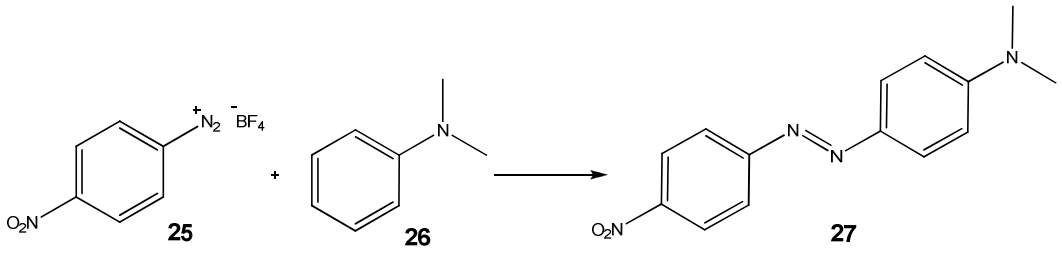
There are many benefits of using continuous flow reactors,[39,42,46,51,52] including excellent control of experimental conditions in terms of reaction time, temperature, stoichiometry and mixing. Highly exothermic reactions can be carried out without cooling as the small reaction volume, in combination with efficient heat dissipation, affords excellent levels of safety. Reduced reaction times can also be achieved through miniaturisation due to the increased mixing efficiencies obtained. The technique also enables a reaction to be fully optimised using only ng to mg quantities of material compared to conventional batchwise chemistry. The process of increasing throughput is addressed through the application of scale-out as supposed to scale-up. Historically bench top processes are difficult to scale up, especially for industrial processes (see Section 1.4.8), a feature that is overcome with the use of continuous flow reactors by increasing the number of

devices in operation, thus the total throughput is both increased and adjustable towards market demand.[53]

Integration of synthesis and analysis is readily achievable through the use of techniques such as on-line HPLC, MS and IR. With these factors in mind, the following section provides a brief insight into the types of chemistries conducted, to date, under continuous flow and aims to illustrate the advantages associated with the technology through the presentation of strategic examples.

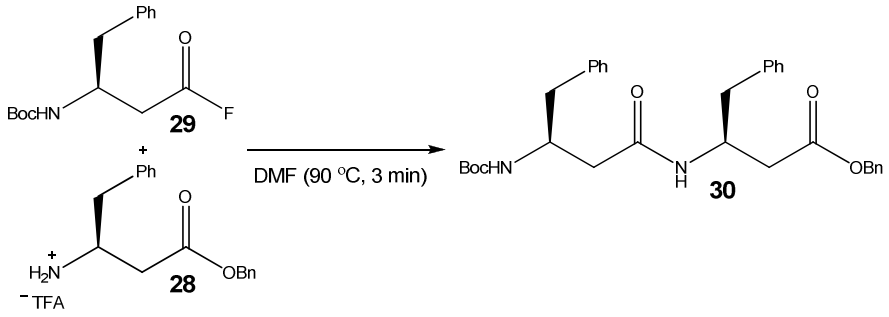
1.4.3 Single phase reactions within flow reactors

One of the earliest reported developments of synthetic chemistry performed in a micro reactor was the preparation of an azo dye by Harrison *et al.*[54] Using EOF to control the flow of polar solvents (MeOH and MeCN) *p*-nitrobenzenediazonium tetrafluoroborate **25** was reacted with *N*,*N*-dimethylaniline **26** in a fused silica reactor to produce the red azo dye **27** illustrated in Scheme 7. The diazotization of aromatic amines is an industrially important process that involves the synthesis of highly unstable and potentially explosive intermediates, therefore by utilizing the unique reaction conditions afforded by continuous flow technology there is the potential to minimise the safety issues frequently encountered in batch reactions.



Scheme 7. The single phase synthesis of a red azo dye **27** within an EOF-based continuous flow reactor.

More recently, Seeberger and co-workers[55] employed a silicon continuous flow reactor to investigate the solution phase synthesis of an array of β-peptides. Employing a reactor with an internal volume of 78.3 µl the authors reported the use of only small quantities of expensive reagents in order to optimise the β-peptide synthesis and subsequently generate a library of β-peptides. Scheme 8 illustrates, an example that the authors report involved the coupling of H-β^{3}hPheOBn **28** with an acyl fluoride **29**, in the presence of a base, to afford a 90 % yield of the desired dipeptide **30**.

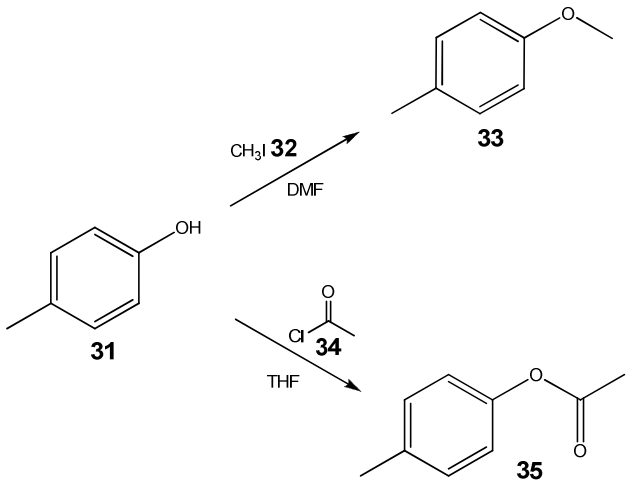


Scheme 8. The single phase synthesis of a *β*-dipeptide **30** within a continuous flow reactor.

Although this investigation illustrates the ability to synthesise *β*-peptides, the authors do not address some key requirements of peptide chemistry, namely the need to incorporate protection and deprotection steps, with the authors conducting these steps under batch conditions. In addition, the authors also performed the required aqueous work-ups in batch at each stage of the reaction.

In comparison, Watts and co-workers[9] reported the *in-situ* deprotection of various peptides demonstrating the removal of Dmab esters, using hydrazine, to afford the respective carboxylic acid which subsequently underwent an additional coupling in-line, to afford the desired tripeptide in an overall conversion of 30 %.

In a solution phase reaction, Hooper *et al.*[56] demonstrated the ability to perform various organic syntheses, including the alkylation and acetylation of phenols, and subsequently used the optimised protocol for the incorporation of stable isotope labels. Examples of the reactions evaluated are depicted in Scheme 9 and include the alkylation of 4-methylphenol **31** with methyl iodide **32** to afford 1-methoxy-4-methylbenzene **33** which was obtained in 96 % yield. The authors also performed the respective acylation, using acetyl chloride **34**, which afforded acetic acid-*p*-tolyl ester **35** in 98 % yield. It should, however be noted, these reactions were carried out using compounds with only one functional compounds and using the pre-formed lithium or sodium phenoxide intermediate, thus favouring the reaction.



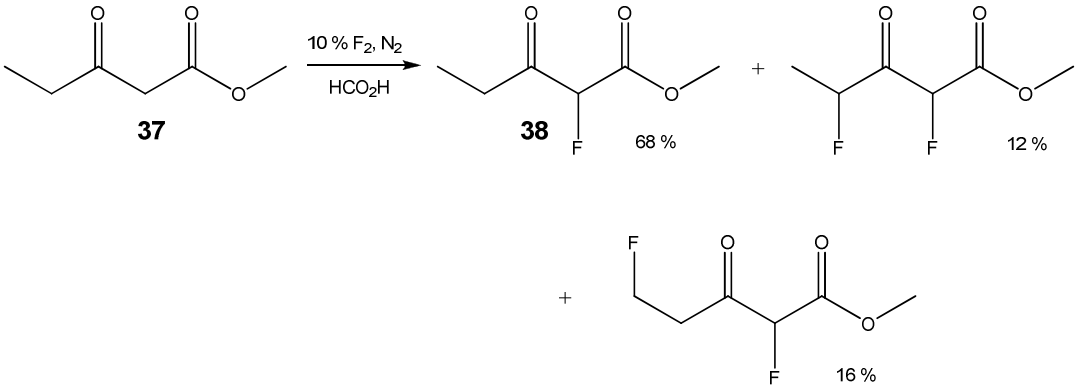
Scheme 9. The single phase synthesis of 1-methoxy-4-methylbenzene **33** and acetic acid *p*-tolyl ester **35** within a continuous flow reactor.

It should be noted that the authors found lithium hydride to be a preferable base for the preparation of the phenoxide anion *cf.* NaH **36**, when performing the acetylations, due to the higher solubility of the resulting metal salt in the reaction solvent (THF). Once optimised, the authors went on to report the incorporation of deuterium *via* the

substitution of methyl iodide **32** and acetyl chloride **34** with their tri-deuterated isotopologue to afford analogous yields of the target molecules.

1.4.4 Biphasic reactions within flow reactors

As the previous section illustrates, single phase continuous reactions have shown great promise however biphasic reactions open up a plethora of new possibilities. Leading the way in gas/liquid reactions were Chambers and Sandford[57] whose group have investigated the application of multi-channel gas-liquid reactors for both laboratory-scale and industrial applications. Using micro reactors with the aim of safely handling potentially hazardous reactants such as oxygen, chlorine and fluorine, the authors have reported the development of a nickel coated stainless steel, gas-tight flow reactor capable of employing a mixture of gaseous and liquid reagent streams.

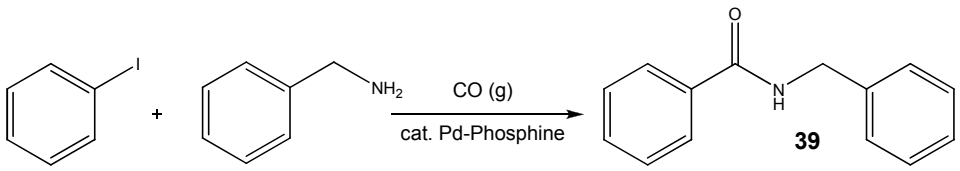


Scheme 10. The biphasic selective fluorination of a 1,3-dicarbonyl under continuous flow.

As the authors noted, while many fluorinating agents have been developed, elemental fluorine is one of the most economically viable reagents for the fluorination process. Using such a biphasic continuous flow reactor, Chambers and Sandford[58] were able to selectively fluorinate 1,3-ketoesters and 1,3-diketones by passing fluorine gas (10 % v/v in N_2) into the reactor along with a solution of the given reagent in formic acid. Products were generally isolated using fractional distillation techniques and as Scheme 10

illustrates, for the fluorination of β-ketoester **37**, a mixture of products were obtained however the mono-fluorinated compound **38** was isolated as the major product (68 %) in much higher selectivity than afforded by batch reactions.

de Mello *et al.*[59] subsequently employed pressure-driven methodology to manipulate biphasic reagents within a glass continuous flow reactor, to synthesise *N*-benzylbenzamides *via* a carbonylative cross coupling reaction (Scheme 11). The liquid and gaseous reagents were mixed on the chip and the reaction reported to occur at the interface between the thin film of liquid that was forced to the walls of the reactor and the gas that flowed through the centre. This approach generated a large surface to volume ratio and a gas flow of 5 μl min^{-1} led to an optimum conversion of 46 % to the desired amide **39**. This represented an increase in yield compared to batch, an observation that is attributed to an increase in interfacial gas-liquid contact area.

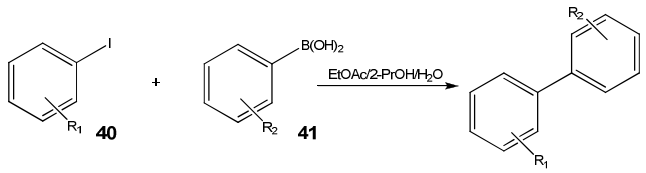


Scheme 11. The biphasic carbonylative coupling reaction conducted in a glass micro reactor.

In addition to gas/liquid reactions, biphasic continuous flow reactions have also exploited the use of the solid/liquid interface. Owing to our interest in the development of a packed-bed reactor, the solid/liquid continuous flow reactions have been categorised into three areas, those employing packed beds containing solid beads, monoliths and the use of membranes,[60] with examples of each discussed below. The introduction of a packed bed into a system often increases the size of the reactor channels (typical i.d. 1-

5 mm), but the small interparticle distance creates micro fluidic channels thus still affording all the benefits of micro reaction chemistry.

Membranes. Uozumi and co-workers[61] reported a novel approach by fabricating a catalytic membrane within a micro channel, enabling two reactant streams to flow through the divided channel while in contact with the surface of the catalytic membrane, demonstrating the Pd-catalysed Suzuki-Miyuara reaction (Table 3).



R_1	R_2	Yield (%)
H	4-MeO	99.0
H	3-Me	96.0
H	2-Me	99.0
3-$EtCO_2$	4-Me	99.0
3-Cl	4-MeO	88.0
4-CF_3	4-MeO	99.0

Table 3. Summary of the Suzuki-Miyaura coupling reactions conducted in a biphasic membrane reactor.

Utilising the stable solvent/solvent interface, which is a feature of laminar flow, the solid PA-TAP-Pd membrane (1.3 μm wide, 0.37 mmol g^{-1} Pd) was constructed within a glass micro channel of dimensions 100 μm (wide) x 40 μm (deep) x 1.4 cm (long). The coupling reaction was subsequently carried out using two solutions, the first containing the aryl iodide **40** (6.3 x 10^{-3} M) in EtOAc/2-PrOH (1:2.5) and the second the aryl boronic acid **41** (9.4 x 10^{-3} M) in an aqueous base (1.8 x 10^{-2} M). The reagents were passed

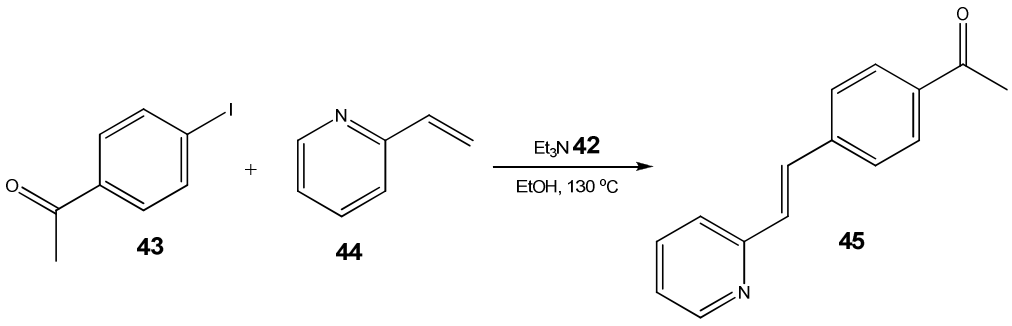
through the reactor, at 2.5 µl min^{-1}, which was heated to 50 °C; affording a residence time of 4 s, during which the biphasic reaction products were collected and the organic layer analysed off-line by GC and ^{1}H NMR spectroscopy. The authors reported that the system produced excellent yields ranging from 88 to 99 % depending on the substrate employed (see Table 3) and demonstrated the high catalytic activity of the PA-TAP-Pd membrane. Importantly, when the reaction was performed in the absence of the catalytic membrane, no reaction products were obtained.

Various membranes have been reported within micro reactors, especially with respect to the use of enzymes,[62] however a downside to their use is the relatively low loadings that are attainable using this approach; consequently many authors have investigated the use of monoliths which have a high surface area.

Monoliths. In comparison to membranes, monoliths are a single continuous porous material that can be fabricated *in-situ* within micro channels of any size and shape. Monoliths can be made using a variety of different materials, including polymers[63] and silica,[64] using polymerisation methods, such as thermal[65] and photoinitiation,[64] all of which allow control over pore size and support structure.[66] Compared to membranes, monoliths provide a high surface area, short diffusion paths and low back pressure operation making them suitable for a wide range of applications within continuous flow reactors.[63]

Recent work reported by Ley *et al.*[67] employed a monolith containing Pd-nanoparticles as a means of catalysing a series of C-C couplings, *via* the Heck reaction, in an automated flow through system. All polymerisations were carried out at 1 bar and the monoliths prepared *in-situ* within 6.6 mm (i.d.) x 70.0 mm (long) glass columns. Each

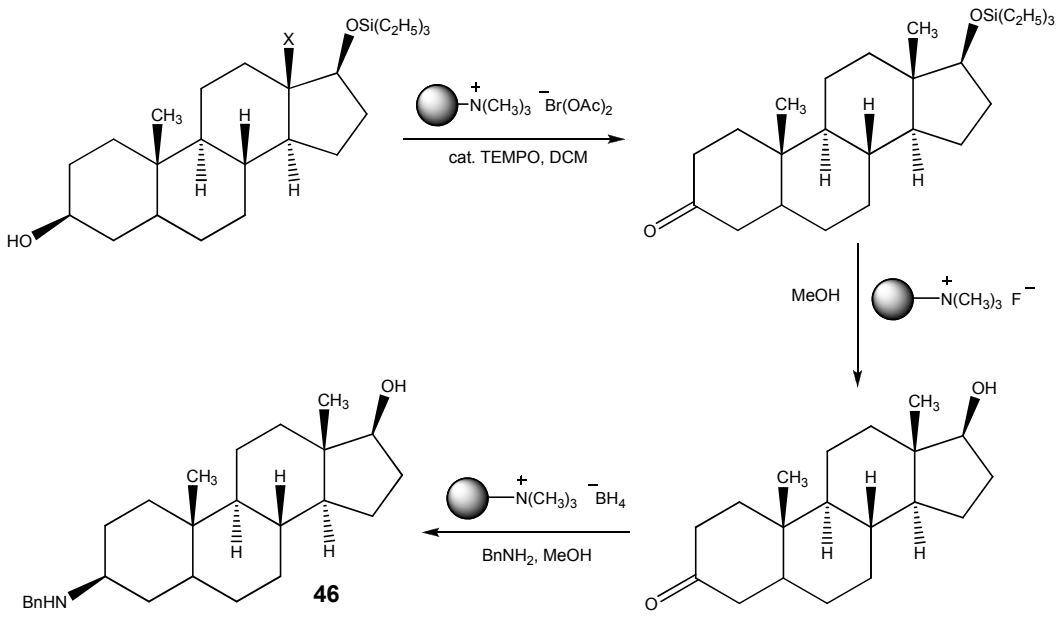
reactor was temporarily sealed at one end and placed in a multichannel convective heating device (80 ^{o}C) to induce polymerisation, in the presence of AIBN. Using an optimal polymerisation mixture (v/v) of vinyl benzyl chloride (35 %), divinyl benzene (20 %), 1-dodecanol (44 %) and AIBN (1 %), the authors reported the reproducible formation of a polymer monolith that was sufficiently porous to prevent the build-up of back pressure. The monolith was subsequently treated with aqueous sodium tetrachloropalladate, followed by aqueous sodium borohydride, to afford a black Pd dispersed monolith. The authors then reacted various aryl halides and alkenes, in the presence of Et_3N **42**, reporting the preparation of the desired products in both high yield (generally > 80 %) and purity (> 95 %). Scheme 12 illustrates an example of the couplings performed, demonstrating the reaction of 4-iodoacetophenone **43** and 2-vinylpyridine **44** to afford (*E*)-1-(4-(2-(pyridine-2-yl)vinyl)phenyl)ethanone **45** (86 % yield).



Scheme 12. The biphasic Heck coupling reaction conducted under flow employing a Pd-monolith.

Particles. In contrast, the most commonly used solid support reported for both batch and continuous flow chemistry is the use of polymeric or silica beads. These materials are widely employed due to their commercial availability (Section 1.2.2) and the ease with which they can be prepared and subsequently packed into tubes or columns makes them adaptable for flow processes. That said, the technique does present some

drawbacks when incorporated into a flow process, such as swelling of the polymer beads in various organic solvents, leading to the generation of high pressure and irreproducibilities with the quality of packing.[67] Despite these potential short comings, silica and polymer based solid supports remain a steadfast of biphasic flow reactions due to the robustness of the material and the vast array of chemistries that can be employed in order to prepare these immobilised catalysts and reagents. They also remove the issue associated with the loss of immobilised compounds through multiple filtrations, a common issue with SPS, due to the fact that the support is entrapped within the flow reactor; as such it can be washed and re-used without any loss.



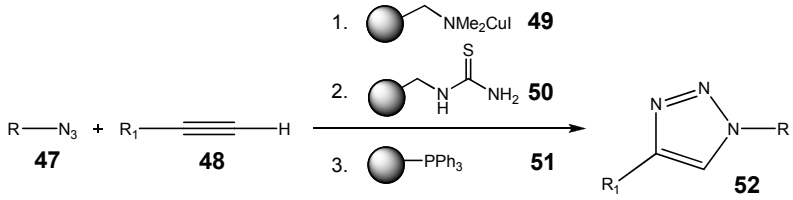
Scheme 13. Polymer-assisted derivatisation of a steroid **46** under continuous flow.

An example of this was reported by Kirschning and co-workers[68] who employed a PASSflow (Polymer Assisted Solution Phase Synthesis) reactor (5.0 mm (i.d.) x 10.0 cm (long)) packed with a series of immobilised reagents to carry out multiple synthetic

transformations. As Scheme 13 illustrates, the authors reported the synthesis of a steroid *via* an oxidation (99 %), silyl deprotection (90 %) and reductive amination (85 %), affording the derivatised steroid **46** in > 95 % purity.

In addition to the above example, organic compounds synthesised using immobilised reagents within continuous flow reactors include the chemoselective oxidation of primary alcohols to aldehydes or carboxylic acids (dependant on residence time employed)[69] the continuous flow synthesis of dimethyl acetals[70] and the chemoselective protection of carbonyl moieties as their 1,3-dithiane or 1,3-dithiolane.[71]

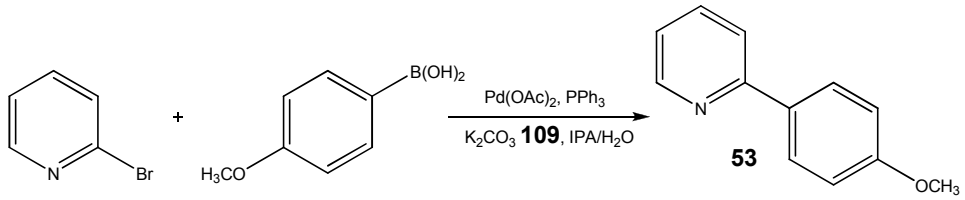
Although there are clearly many advantages associated with the use of flow reactors, the purification of reaction products prepared under continuous flow remains challenging, with many syntheses reporting purifications as being performed off-line and batch-wise. Ley *et al.*[72] has also reported the synthesis 1,4-disubstituted-1,2,3-triazoles using a series of immobilised reactants and scavengers in order to address this. As Scheme 14 illustrates, by pumping an azide **47** and an acetylene **48** (30 μl min^{-1}) over an immobilised copper iodide species **49**, followed by two sequential scavenger modules (QuadraPure TU **50** and phosphine resin **51**), the [3+2] cycloaddition of azides and terminal acetylenes afforded the desired 1,4-disubstituted-1,2,3-triazoles **52** in yields ranging from 70 to 93 %. Using this approach, the authors were able to produce 1.50 g of the desired product, over a 3 hr period, in 85 % yield and > 95 % purity.



Scheme 14. Illustration of the [3+2] cycloadditions and in-line purifications performed using a modular flow reactor.

This work by Ley[72] (Scheme 14), highlighted the successful approach of incorporating polymer-supported reagents and scavengers into packed-beds along with immobilised catalysts to enable the synthesis and purification of reaction products generated under continuous flow. Additional work by this author includes the recent investigation into the Curtius rearrangement under continuous flow.[73] Using this approach, Ley and co-workers reported the synthesis of thirty target compounds with yields in excess of 78 %. In a separate paper, Ley *et al.*[74] reported the synthesis of *N*-protected dipeptides employing a combination of immobilized reagents and scavengers, employing ion exchange resins to enable the development of a 'catch and release' protocol.

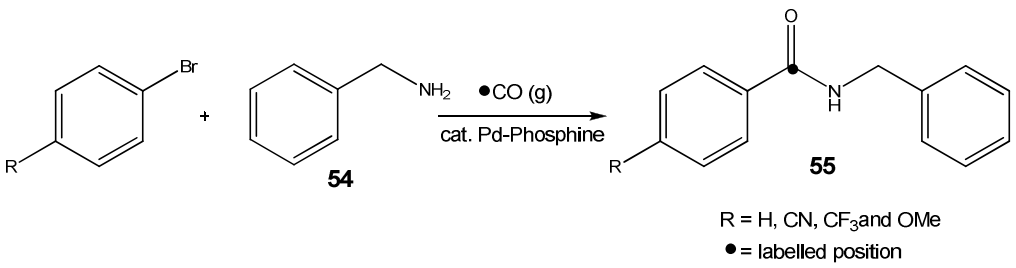
Given the increased use of continuous flow in the synthesis of pharmaceuticals and their intermediates, residual trace metal contamination is an area of significant importance. When investigating the Pd-catalysed Suzuki reaction illustrated in Scheme 15, Pitts and co-wokers[75] employed a plethora of scavengers, ranging from silica gel, carbon and QuadraPure (TU, IDA and AMPA) under continuous flow to scavenge the Pd content of the resulting product **53**. By employing a single pass of the reaction stream over thiourea derived QuadraPure the authors reported > 99.9 % Pd removal from the reaction mixture, to afford < 1 ppm residual Pd.



Scheme 15. Model reaction used to demonstrate the efficient removal of trace metals from continuous reaction streams.

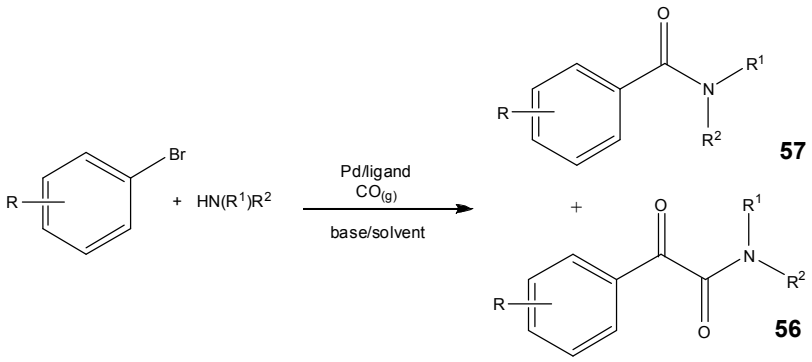
1.4.5 Tri-phasic reactions within flow reactors

In an addition to the earlier reported biphasic work (Scheme 11), de Mello and Long[76] developed a simple low cost and effective method for the gas-liquid-solid carbonylation of aryl halides employing a re-usable silica supported Pd catalyst (used 18 times with no loss of catalytic activity observed). Using this approach, the carbonylation of six aryl halides with benzylamine **54** was carried out affording the desired amides in yields ranging from 26 to 99 %. The authors subsequently reported the successful application of this methodology, using ^{11}CO to radiolabel the target molecule **55** (Scheme 16) for use in medical imaging using positron emission tomography (PET). Radiochemical yields of >33 % and purities of >70 % were obtained, which was highly favourable in comparison to batch syntheses.



Scheme 16. ^{11}CO carbonylative cross-coupling reactions conducted in a tri-phasic system, under continuous flow.

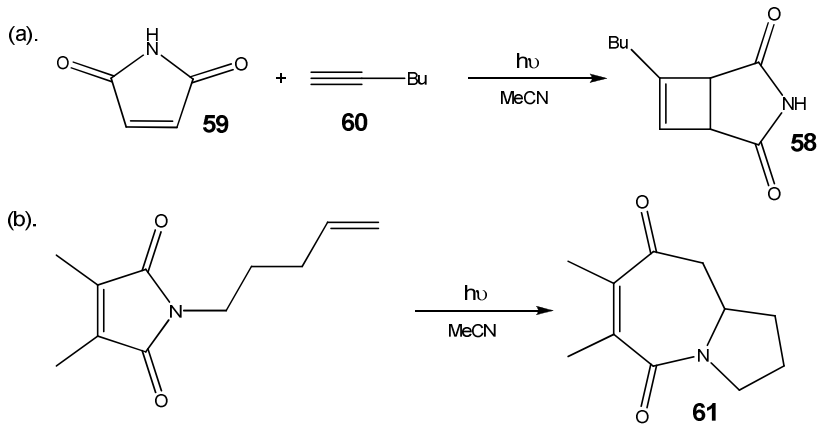
Employing a new method of sealing the fluidic inlets and outlet tubes of a continuous flow reactor, using a solder-based technique, Buckwald and Jensen[77] reported the ability to reach pressures exceeding 100 bar (approx 10 times the standard limit). Using this approach, the authors performed the aminocarbonylation of various aryl halides (Scheme 17) with conversions generally in excess of 99 %. Selectivity of aryl halides was temperature dependant, with higher temperatures favouring formation of the β-ketoester **56** over the amide **57**. The completely closed system not only facilitated the high pressure desired to drive the reaction, but also illustrated the improved safety aspects of the technology by encapsulating the toxic CO and air-sensitive Pd catalysts.



Scheme 17. Pd-catalysed aminocarbonylation conducted at high pressure under continuous flow.

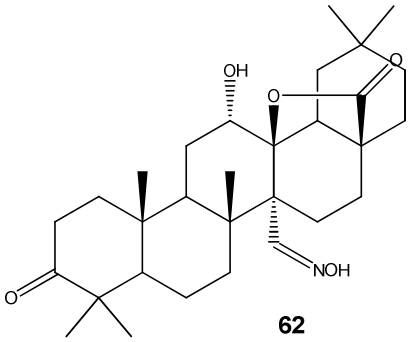
1.4.6 Photochemical and electrochemical reactions within flow reactors

While photochemistry can be an efficient method to synthesise complex molecules, the subsequent process of scaling up the light source to achieve industrial levels of production hamper the ability to employ such reactions in production. Consequently, its use in the pharmaceutical and fine chemistry is limited. However, the use of continuous flow reactors as a tool for organic synthesis has allowed the use of laboratory scale light sources in the synthesis of large volumes of organic compounds.



Scheme 18. (a) [2+2] Photocycloaddition of maleimide **59** and 1-hexyne **60** and (b) intramolecular [5+2] photocycloaddition to afford 7,8-dimethyl-1,2,3,9a-tetrahydropyrolo[1,2-a]azepine-6,9-dione **61**.

Booker-Milburn *et al.*[78] developed a continuous flow reactor using a commercially available mercury vapour discharge lamp and fluorinated ethylenepropylene (FEP) tubing (700 μm i.d.). Employing a HPLC pump to drive reactants through the tubing, which was coiled around the light source, afforded a high surface to volume ratio and efficient contact with the light source. The continuous flow reactor was used to perform a series of photocycloadditions including the synthesis of 6-butyl-3-azabicyclo[3.2.0]hept-6-ene-2,4-dione **58** (Scheme 18(a)), which was achieved by pumping a solution of maleimide **59** (0.10 M) and 1-hexyne **60** (0.15 M), in MeCN, through the reactor at 2.0 ml min^{-1}; affording 95 % conversion to the desired product **58** at a throughput of 2.1 g hr^{-1}. Further compounds synthesised by the group included 7,8-dimethyl-1,2,3,9a-tetrahydropyrrolo[1,2-a]azepine-6,9-dione **61** (Scheme 18(b)), which was obtained in 80.0 % yield, with a throughput of 7.4 g hr^{-1}.

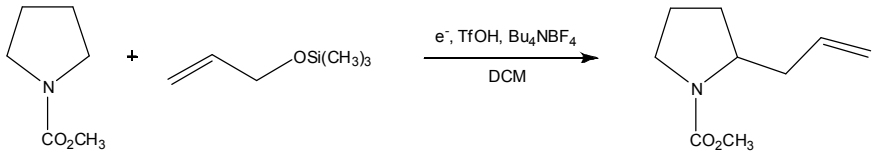


62

Figure 4. A key intermediate in the synthesis of an endothelin receptor antagonist **62** synthesised in a photochemical flow reactor.

In addition, Ryu *et al.*[79] performed the Barton reaction to afford a key intermediate in the synthesis of an endothelin receptor antagonist **62** (Figure 4) in a single step using a flow reactor subjected to black light, with a total residence time of 32 min afforded 71 % yield. Synthesis of this intermediate *via* batch is a three step process, thus again highlighting the advantages of chemistry carried out on a micro scale.

Much like their photochemical counterparts, electrochemical reactions are rarely employed for the large-scale production of chemicals, due to inhomogeneities that arise within the electric field when reactors are increased in size. Yoshida and co-workers[80] employed low temperature electrolysis to generate a continuous flow of carbocations, such as the *N*-acyliminium and alkoxycarbenium ions in a carrier stream. Using this 'cation flow' methodology, the reactant stream was subjected to cation generation and mixed with other reagents in a diaphragm flow cell affording a series of interesting products. Using this approach, the authors were able to readily screen their electrochemical process, in a serial manner, to afford an array of interesting compounds derived from the coupling of carbamates and allylsilanes (Scheme 19).

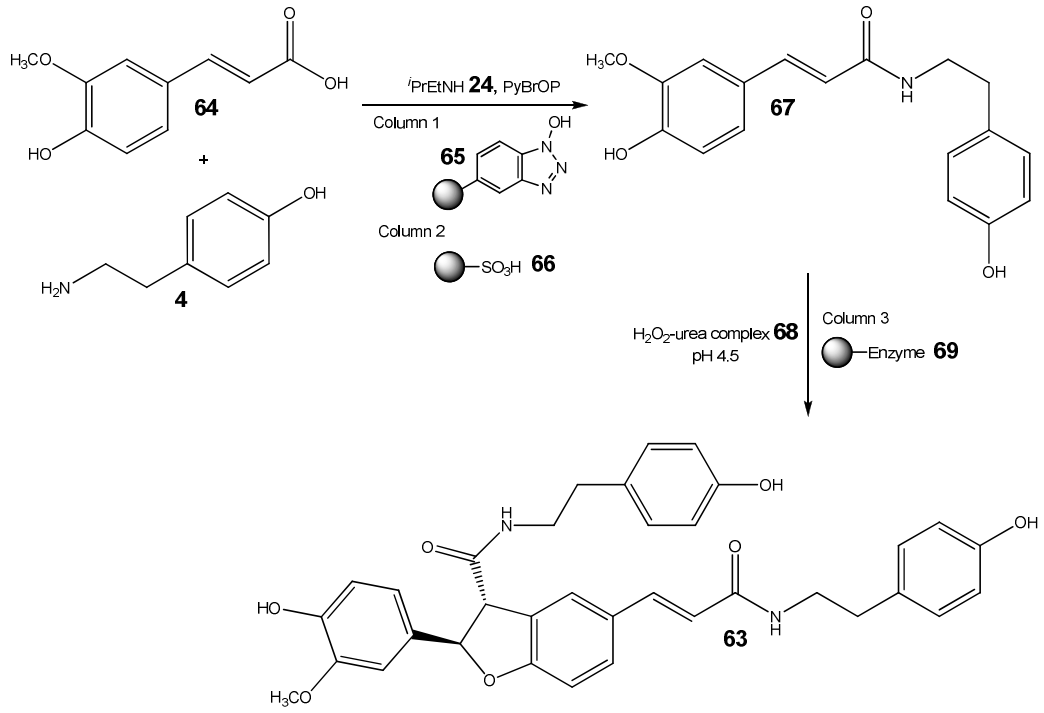


Scheme 19. An example of an electrochemical reaction conducted under 'cation flow' conditions.

1.4.7 Interesting examples of organic synthesis within flow reactors

The first enantioselective synthesis of 2-aryl-2,3-dihydro-3-benzofurancarboxyamide neolignan (grossamide) **63**, an interesting molecule belonging to the lignanamide family of compounds commonly used by plants in nature in response to pathogenic attacks such as fungal infections, using a fully automated and scalable continuous flow reactor was reported by Ley and co-workers.[81] With obvious pharmaceutical interest, the authors developed a flow reactor capable of synthesising gram quantities of the compound.

As illustrated in Scheme 20 the first step of the synthetic pathway involved the amide coupling of ferrulic acid **64** and tyramine **4**, using a column packed with polymer-supported HOBt **65**. Excess tyramine **4** was removed by flowing the reaction stream over a scavenger resin **66** and the reaction progress was monitored by in-line LC-MS. The purified amide **67** was then pre-mixed with a hydrogen peroxide:urea complex **68** and passed through a third column which contained silica-supported peroxidase **69**, which afforded the desired product, grossamide **63**, in excellent purity.

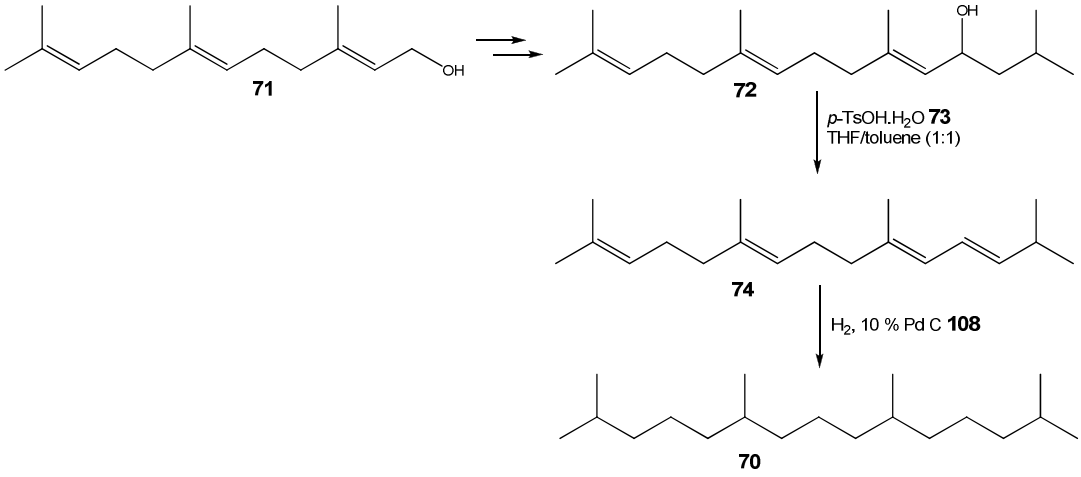


Scheme 20. Synthetic strategy employed for the continuous flow synthesis of the natural product grossamide **63**.

Pristane (2,6,10,14-tetramethylpentadecane) **70** is a naturally occurring immunoactivating agent found in basking sharks, *Cetorhinus maximus*. In 2002, basking sharks were listed as an endangered species, reducing the availability of pristane **70** from natural sources. With this in mind, Fukase and co-workers[82] evaluated a continuous flow approach to the large-scale synthesis of pristane **70** by employing the continuous flow dehydration of an alkanol as a key reaction step in the synthetic pathway (Scheme 21).

Farnesol **71** was initially treated with MnO_2 to afford the respective aldehyde, followed by treatment with *iso*butylmagnesium chloride to afford the allylic alcohol derivative **72**. The alcohol **72** (1.0 M in THF) was subsequently dehydrated within a micro mixer (Comet X-01) using *p*-toluene sulphonic acid **73** in THF/toluene (0.2 to 1.0 M), with a

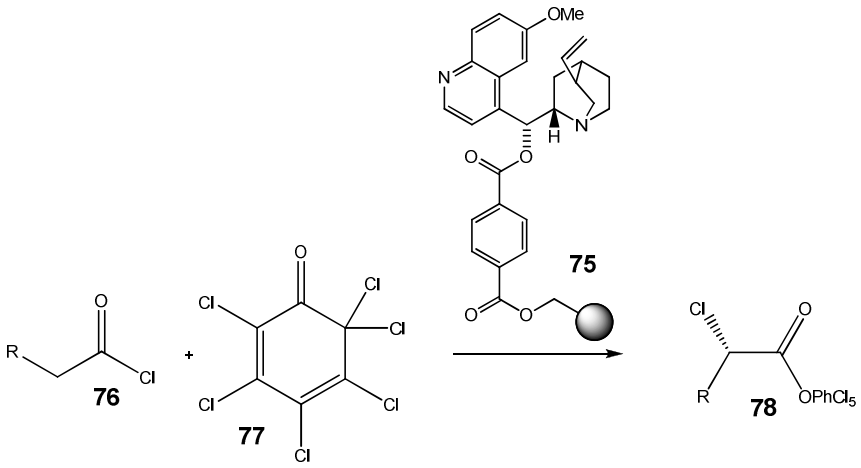
total flow rate of 600 µl min^{-1}, at 90 °C. The removal of the OH group allowed hydrogenation of the alkene **74** to afford the target molecule **70** in 80 % yield. Use of the continuous flow technique proved to be advantageous compared with batch syntheses due to the removal of complicated purification steps, enabling an efficient route to the multikilogram synthesis of pristane **70**, which is in-line with the current demand of ~5 kg $week^{-1}$.



Scheme 21. Synthesis of the natural product pristane **70** *via* continuous flow dehydration.

Letcka *et al.*[83] developed a flow system that featured a 'flush and flow' approach, where the reagents pumped over the solid support and subsequently immobilised. The second reactant is then added and the immobilised intermediate reacted to afford the desired product, which is subsequently released by treatment with DIEA **24**. As Scheme 22 illustrates, packing a column with a polymer bound quinine derivative **75** and sequentially flushing the acid chloride **76** and chlorinated reagent **77** through the column, the desired chloro ester **78** was collected in high yield and purity. Interestingly by using a 'flush' cycle of DIEA **24**, the authors were able to completely regenerate the

support, suggesting that theoretically an infinite number of flush/flow cycles could be carried out to continuously generate the product **78**.



Scheme 22. Column based flush/flow synthesis of α-chloro esters.

1.4.8 The role of continuous flow reactors in modern day chemistry

As previously discussed, a common problem associated with traditional batch-wise organic synthesis is the scaling-up of reactions from successful laboratory processes through the pilot plant stage and finally into large-scale industrial processes.

This is a flawed approach as after each stage, reactor modifications result in changes to surface to volume ratio, which in turn impact upon the thermal and mass transportation properties of the reaction (Table 2). The effect of these alterations to the chemical process mean that the process must be re-optimised each time the reaction is scaled, something which proves to be costly in both time and money. Continuous flow technology addresses this problem through the concept of scaling-out[84] rather than scaling-up, thus by increasing the number of devices running in parallel, the total throughput is both increased whilst being adjustable towards market demand (Figure 5).[85]

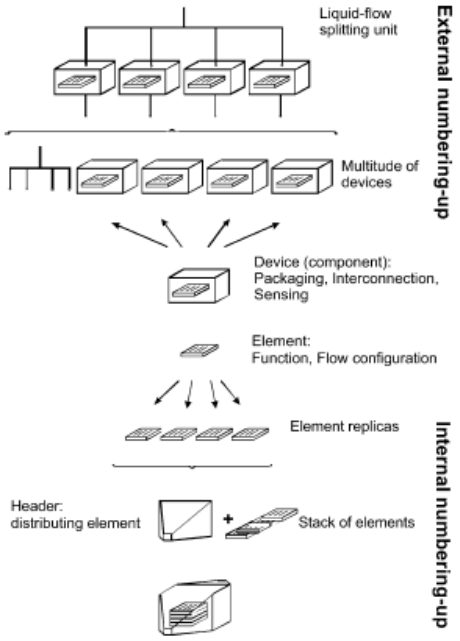


Figure 5. Schematic representation of the generic concepts of internal and external scaling-out.[24]

Further to their earlier reported work, which employed single channel reactors, Chambers and co-workers[86] (Scheme 10) recently reported the successful scale-out of their reactor whereby thirty reaction channels were operated in parallel. Using channels cut into a nickel block and lined with transparent polychlorotrifluoroethylene (PCTFE), the authors demonstrated the ability to directly fluorinate organic compounds in parallel.

As an alternative approach to the scale-out process detailed above, rather than running thirty single reactors the group combined the techniques and operated several internally numbered-up reactors in parallel. Initial efforts concentrated on running multi channel plates, though each channel required a separate reagent feed. Development of the process concluded with the use a micro reactor device fitted with a thirty channel plate, which still enabled the efficient heat exchange and gas/liquid mixing obtained in the single channel reactor, whilst providing an increased throughput without resorting to the

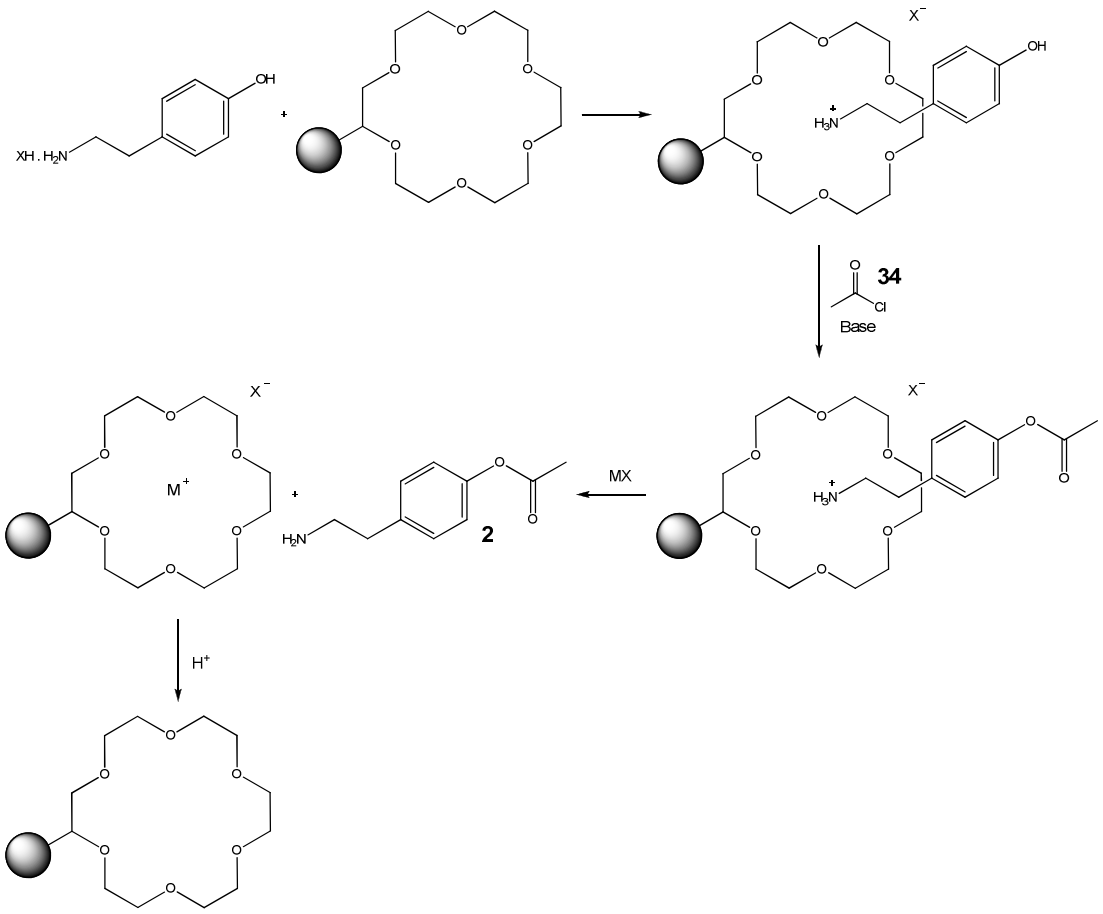
use of a hazardous batch-scale fluorinations. The technique and the scale of this methodology was demonstrated for the fluorination of ethylacetoacetate, whereby a throughput of 0.2 g channel^{-1} hr^{-1} was obtained which translates to 150.0 g day^{-1}.

1.5 Aim of the project

Whilst the use of protecting groups in both solution phase and solid phase chemistry has enabled access to a vast library of complex molecules over the years, by removing functional group incompatibilities, the employment of a protecting group can be disadvantageous as their introduction and subsequent removal generates the need for additional synthetic steps. This can contribute to reductions in overall yield and increased costs, along with the need to perform increasingly complex purifications in order to remove protecting group residues from the resulting reaction product. Furthermore, the deprotection strategy must be carefully selected in order to ensure that the product is obtained in the desired form, for example as the free amine or a salt and that decomposition of the reaction product is not observed. These factors therefore highlight the need for synthetic methodology that removes the aforementioned problems associated with the use of covalently bound protecting groups, whilst having the potential to be adapted for use in an automated system and as such, this provides focus for the investigation reported herein.

As eluded to in Section 1.1, the reaction of the hydroxy moiety is a common synthetic transformation, enabling the rapid synthesis of ethers and esters. However, when it comes to the derivatisation of bifunctional compounds, such as tyramine **4**, protection of the amine moiety is required in order to synthesise the desired product in high yield and selectivity. In this case, 4-(2-aminoethyl)phenyl acetate **2**, more commonly known as tyramine acetate **2**, represents the target molecule.

With this in mind, the work described herein aims to address the time consuming process of protection and deprotection by employing a non-covalent protecting group strategy, based on the use of crown ethers as a non covalent protecting group, which will enable the acetylation of the phenolic group whilst preventing competing reactions which could lead to by-products such as the *N*-acetate **3** and diacetate **1** (Scheme 2).



Scheme 23. Overview of the non-covalent *N*-protecting group strategy under investigation.

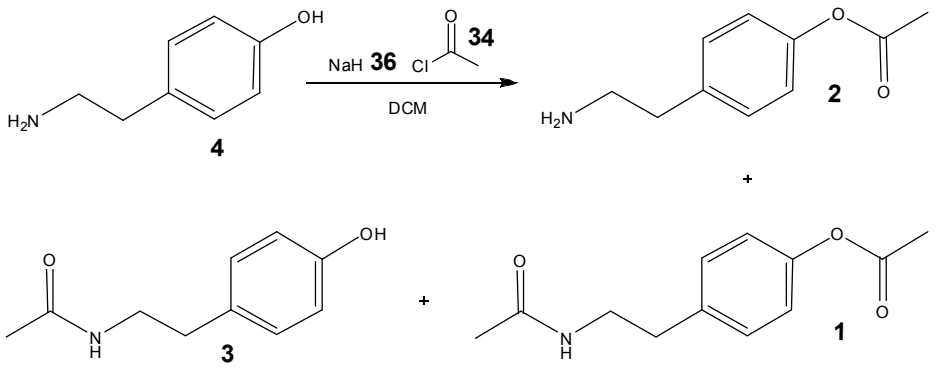
As illustrated in Scheme 23 it is proposed that by attaching an 18-crown-6 ether derivative to a solid support, a re-useable protecting group could be prepared that would be suitable for the *N*-protection of bifunctional compounds, present as the respective ammonium salt. Through the use of this approach, in a similar manner to the work reported by Letcka,[83] it was envisaged that the technique would allow for the facile isolation of the desired reaction product without the need for additional derivatisation steps, as required with covalent protection and deprotection strategies, the details of which form the basis of Chapter 2.

Chapter 2: Results and Discussion

2.0 Results and Discussion

2.1 Acetylation of tyramine in the absence of an *N*-protecting group

To highlight the problems associated with the acetylation of tyramine **4**, the model reaction was firstly performed in the absence of a protecting group (Scheme 24). Using NaH **36** (1.0 eq.) as the base and a slight excess of acetyl chloride **34** (1.2 eq.), acylation of tyramine **4** afforded a mixture of acetic acid 4-(2-acetylaminoethyl)phenyl acetate (tyramine diacetate) **1** (20 %), *N*-[2-(4-hydroxyphenyl)ethyl]acetamide (tyramine-*N*-acetate) **3** (12 %), the desired product 4-(2-aminoethyl)phenyl acetate **2** (23 %) and residual tyramine **4** (45 %), as determined by HPLC.



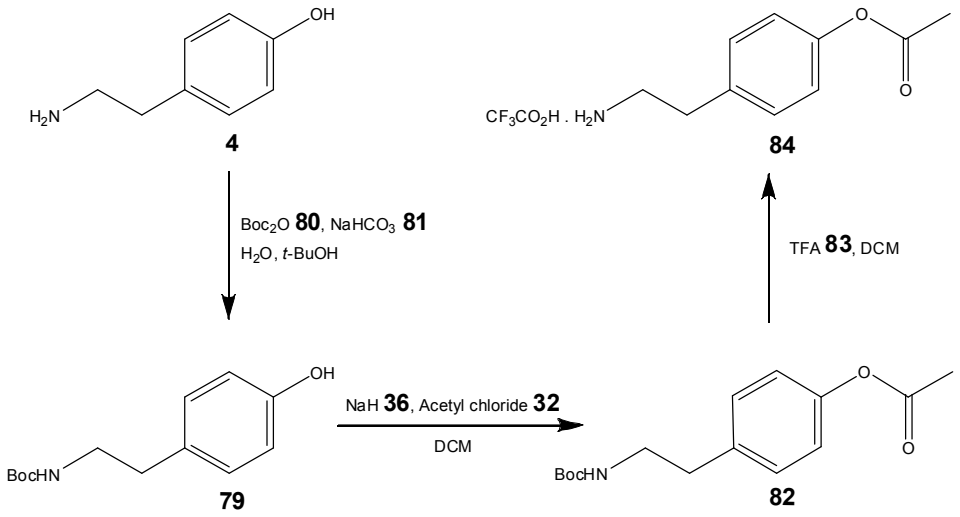
Scheme 24. Illustration of the complex reaction mixture obtained when acetylating tyramine **4**.

Owing to the complexity of the resulting reaction mixture, isolation of the desired product **2** was not practical due to the need to perform a lengthy separation procedure. It was therefore concluded, as first expected, that it would be necessary to employ a protecting group in future reactions.

2.2 The use of a protecting group strategy for the acetylation of tyramine

Having identified the need for a protecting group strategy, to enable the selective synthesis of tyramine acetate **2**, the reaction was repeated employing the precursor [2-(4-hydroxyphenyl)ethyl]carbamic acid *tert*-butyl ester (Boc-tyramine) **79**, as a means of circumventing the undesirable *N*-acetylation previously observed. As Scheme 25

illustrates, treatment of tyramine **4** with di-*tert*-butyl dicarbonate **80** in the presence of $NaHCO_3$ **81** afforded the intermediate Boc-tyramine **79**, as a white solid in 93 % isolated yield.

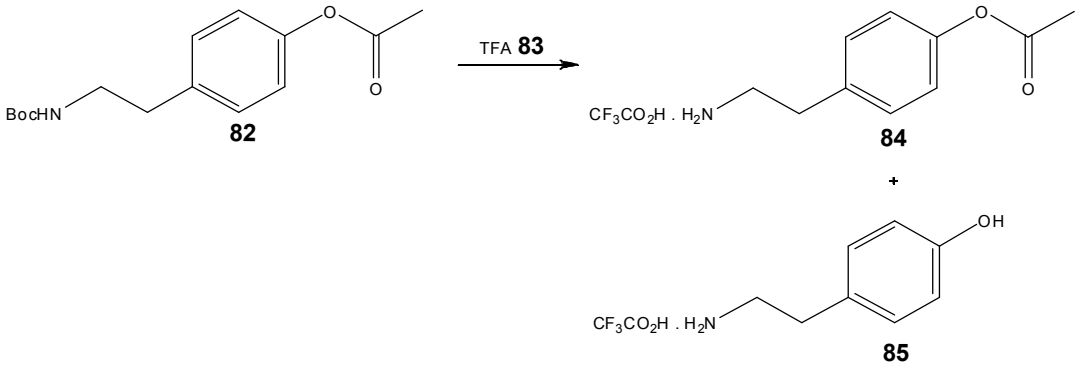


Scheme 25. Schematic of the conventional protecting group strategy employed to synthesise the TFA salt of tyramine acetate **84**.

Subsequent acetylation, with acetyl chloride **34** (1.2 eq.) in the presence of NaH **36** (1.0 eq.) followed and afforded the desired acetic acid 4-(2-*tert*-butoxycarbonylaminoethyl)phenyl ester (Boc-tyramine acetate) **82** as a pale yellow gum in 69 % isolated yield.

Although the use of a protecting group afforded a chemoselective route to the acylation of the phenolic moiety, in addition to increasing the number of steps employed in the synthetic pathway, removal of the protecting group often requires the use of harsh chemical conditions and the deprotection strategy must be selected carefully to ensure the desired product is obtained. In the case of Boc, deprotection has been reported using a plethora of reagents including trifluoroacetic acid (TFA) **83**, either neat or in a co-solvent such as DCM, 3 M HCl in EtOAc, 10 % H_2SO_4 in dioxane, $BF_3 \cdot OEt_2$ in acetic acid and even anhydrous HF.[1]

With respect to the deprotection of Boc-tyramine acetate **82**, it was found that dilute TFA **83** (0.1 M in DCM, 5.0 eq.) afforded minimal deprotection, 43 % by HPLC, to the desired tyramine acetate TFA salt **84** over a period of 1 hr. In order to increase the rate at which decomplexation occurred, neat TFA **83** was employed; however, analysis of the reaction mixture after 30 min revealed partial cleavage of the acetate moiety had also occurred affording a mixture of tyramine TFA salt **85** (16 %), tyramine acetate TFA salt **84** (69 %) and Boc-tyramine acetate (15 %) **82**, by HPLC (Scheme 26).



Scheme 26. Illustration of the reaction products obtained upon deprotection of Boc-tyramine acetate **82** with neat TFA **83**.

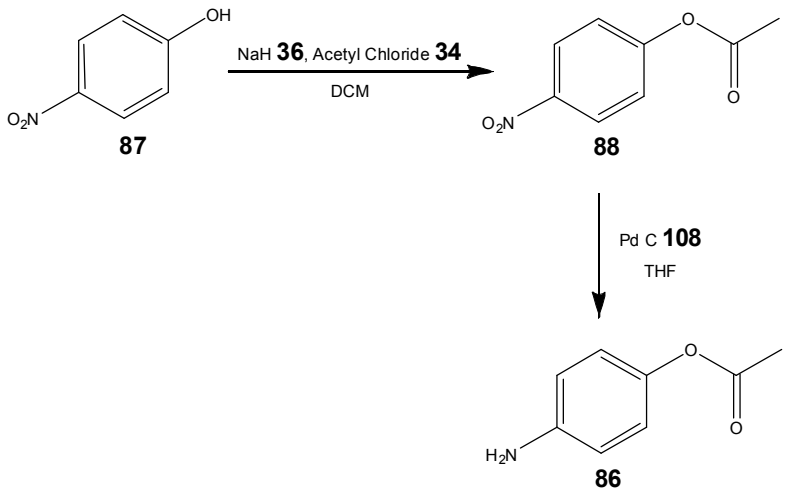
While for many organic reactions a 69 % yield would be deemed acceptable, the complexity of the resulting reaction mixture meant that in order to isolate the desired product **2**, purification by column chromatography would again be required.

In addition to the undesirable formation of by-products, it is frequently advantageous to convert TFA salts to the 'free' amine in order to prevent degradation of the compound; a phenomenon that is frequently observed upon storage of combinatorial libraries and is attributed to acid hydrolysis.[87] Numerous techniques have been reported to afford the free base (see Section 1.2.1), with the use of solid-phase extraction techniques, which employ solid-supported sulfonic acids as scavengers, becoming popular due to their

simplicity; again however, this results in additional reaction complexity to afford the desired product **84**.

2.3 The reduction of analogous nitro compounds

In order to reduce the number of reaction steps required to obtain the desired product **84**, an alternative synthetic strategy to the introduction of covalent protecting groups was sought. As Scheme 27 illustrates, one such approach is the use of nitro derivatives, which act as masked amine groups and thus enable acetylation of the phenolic moiety to be performed selectively. This is then followed by catalytic hydrogenation, as a means of incorporating the desired amine functionality into the reaction product. Although this technique reduces the overall process to a two step reaction, it is however limited to those compounds that have an analogous nitro form available and consequently, does not provide a sufficiently generic solution to the reaction of bifunctional compounds.



Scheme 27. The use of a masked amine to enable the selective acylation of a bifunctional compound.

As the tyramine analogue, 4-(2-nitroethyl)phenol, was not commercially available the principle was illustrated using the synthesis of 4-aminophenyl acetate **86** as a model reaction. Using this approach, 4-aminophenyl acetate **86** was obtained *via* the

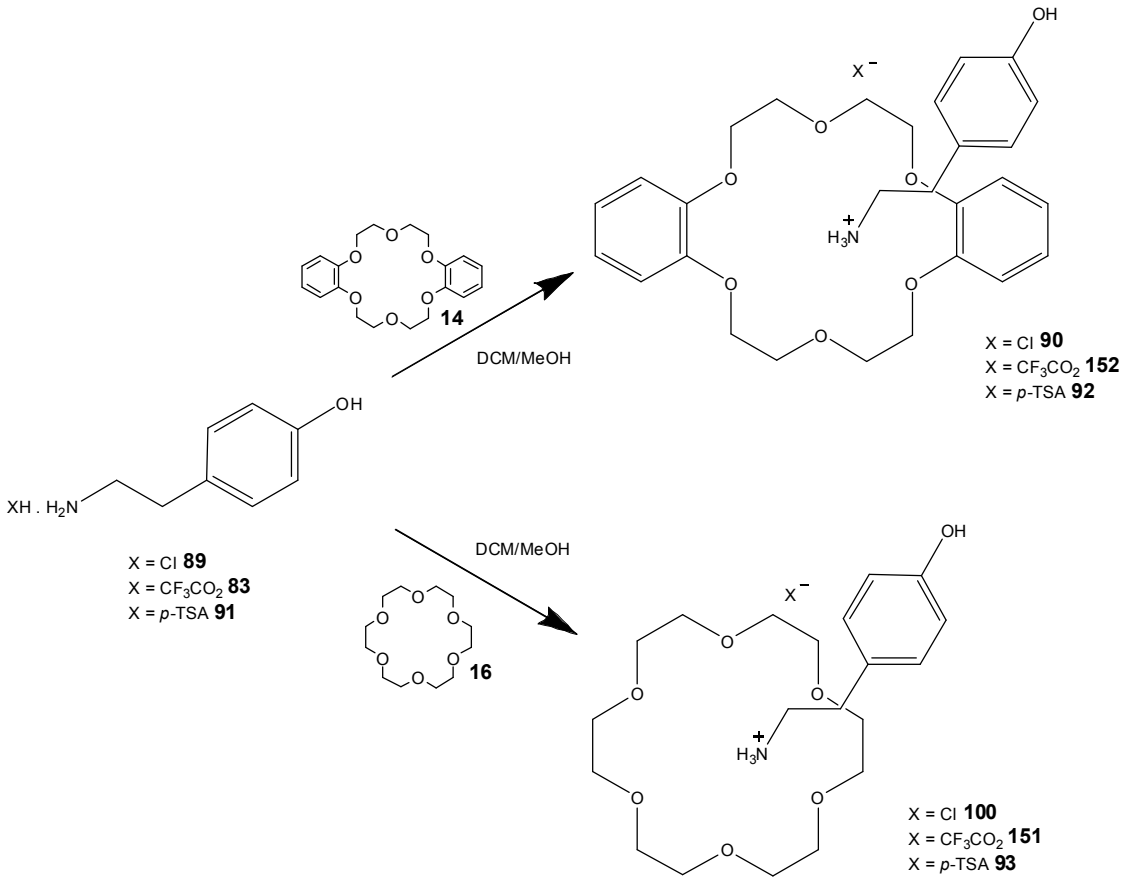
acetylation of 4-nitrophenol **87** (93 % yield) and subsequent reduction of 4-nitrophenyl acetate **88** afforded the desired 4-aminophenyl acetate **86** in 79 % yield, as illustrated in Scheme 27. In addition to the continued attempts into the synthesis of tyramine acetate **2** *via* a non-covalent protecting group strategy, the preparation of 4-aminophenyl acetate **86** under analogous conditions can be found in Section 2.11.

2.4 The use of crown ethers as 'protecting groups'

In pursuit of a non-covalent protecting group strategy, capable of affording a route to the chemoselective *O*-acylation of bifunctional compounds such as tyramine **4**, the potential of 18-crown-6 ether derivatives was firstly evaluated in solution prior to the development of an immobilised crown ether derivative, generalised in Scheme 23.

Based on previous reports by Mascagni and co-workers,[34-37] reviewed in section 1.3.2, the first step of the investigation was to demonstrate the ability to complex a salt of tyramine with DB-18-c-6 **14** (1.1 eq.) in DCM (5 ml) (Scheme 28). In the first instance, the HCl salt of tyramine **4** was evaluated owing to its commercial availability, however due to the relative insolubility of both the HCl salt **89** and crown ether **14**, it was found to be necessary to modify the published procedure. Addition of MeOH (approx. 0.1 ml) was found to aid the solubility of tyramine HCl **89** and hence facilitate the complex formation. Using this approach, the resulting reaction mixture was stirred for 1 hr prior to concentration *in vacuo* to afford a white solid. Subsequent evaluation of the material by MALDI-MS confirmed the formation of the desired complex **90** *via* detection of the protonated compound ($[M^+ +1] = 498$). In addition, the sodium ($[M^+ +1] = 383$) and potassium ($[M^+ +1] = 399$) adducts were also detected by MS due to sequestration of metal ions by unoccupied crown ether cavities, resulting from either an excess of crown ether **14** or uncomplexed sites, due to the presence of an equilibrium in solution.[36,37]

Having demonstrated the successful preparation of a complex **90**, the next step of the investigation was to evaluate the generality of the complexation strategy. To achieve this, tyramine TFA **83** and tyramine *p*-TSA **91** salts were synthesised, and their complexation with DB-18-c-6 **14** and the unfunctionalised 18-crown-6 ether (18-c-6) **16** (1.0 eq.) was also investigated, as illustrated in Scheme 28. In addition to analysis by MALDI-MS, the reaction mixtures were also monitored by HPLC in order to determine the proportion of tyramine salt complexed by the crown ether derivative under investigation.



Scheme 28. Schematic illustrating the generality of the DB-18-c-6 **14** and 18-c-6 **16** complex formation, with various salts of tyramine **89**, **83** and **91**.

At this stage it is worth highlighting the fact that upon analysis of the complexes by MALDI-MS, the molecular ion observed ([M^+ +1]) represented the crown ether and amine complex and not that of the counterion; an observation that is summarised in

Table 4. It must be noted that although Mascagni and co-workers[36] found it necessary to employ a 5:1 ratio of crown ether to amine salt, when adding a small proportion of MeOH to the reaction mixture, it was observed that 1.0 eq. of 18-c-6 **16** and 1.1 eq. of DB-18-c-6 **14** was sufficient to enable efficient complexation to occur. Compared to 18-c-6 **16**, the requirement for a slight excess of DB-18-c-6 **14** was attributed to the reduced flexibility of the cavity due to the presence of two aromatic groups.

			Complex data	
Tyramine Derivative	Crown ether	Complexation	HPLC R_T (min)	MS ([M^+ +1])
4	DB-18-c-6 **14**	No	N/A [a]	N/A [b]
HCl **89**	DB-18-c-6 **14**	Yes	4.6	498
TFA **85**	DB-18-c-6 **14**	Yes	5.2	498
p-TSA **91**	DB-18-c-6 **14**	Yes	5.7-6.5[c]	498
4	18-c-6 **16**	No	N/A [a]	N/A [b]
HCl **89**	18-c-6 **16**	Yes	3.6	402
TFA **85**	18-c-6 **16**	Yes	4.1	402
p-TSA **91**	18-c-6 **16**	Yes	4.5-5.2[c]	402

[a] Tyramine **4** only detected (R_T = 3.2 min), [b] Na^+ and K^+ adduct of relevant crown ether only, [c] due to co-elution of the complex with the *p*-TSA counterion, an exact R_T could not be obtained.

Table 4. Evaluation of the complexation ability of DB-18-c-6 **14** and 18-c-6 **16** for tyramine **4** and an array of tyramine salts **85**, **89** and **91** (employing DCM/MeOH as the solvent system).

Table 4 illustrates that using the aforementioned methodology, quantitative complexation was achieved for all tyramine salts (**85**, **89** and **91**) and as expected, no complex formation was observed for the free amine, tyramine **4**. In the case of tyramine *p*-TSA **91**, analysis by HPLC proved problematic due to co-elution of the *p*-TSA counterion with the complex; in these cases formation of the complexes **92** and **93** was corroborated by disappearance of the tyramine *p*-TSA **91** peak *via* HPLC and identification of the complex by MS analysis (Table 4). As expected, it was established

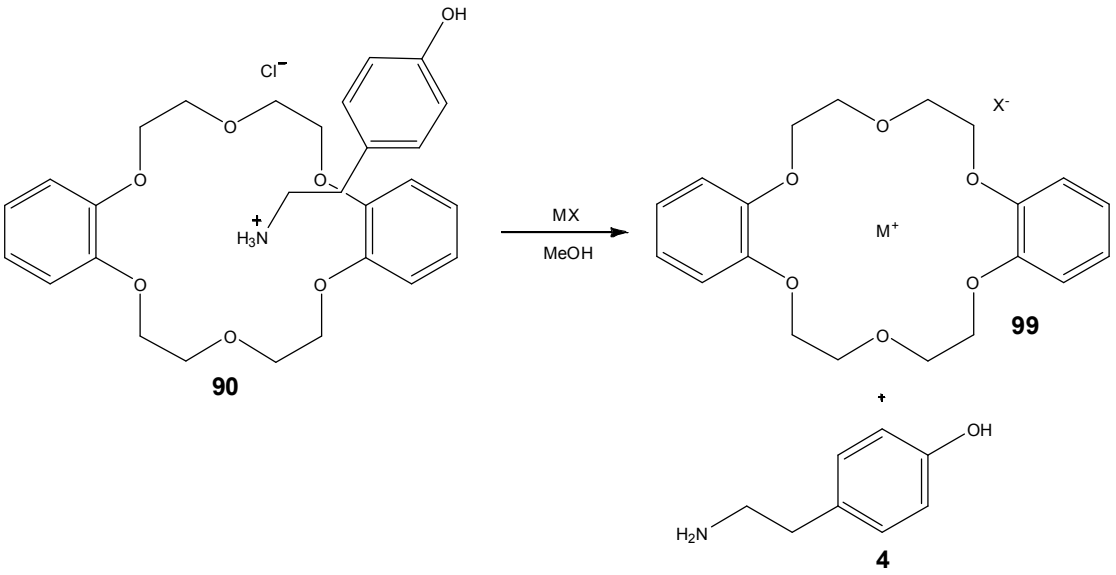
that all ammonium salts would readily complex, while the free amine did not complex to any degree.

Further studies therefore employed tyramine HCl **89**, due to commercial availability and the ease of analysing the resulting complex, owing to the lack of a UV active chromophoric counterion. In addition, based on reports within the literature, of the three counterions investigated, chloride was observed to form the least stable complex[35] thus working with the worst case scenario allowed the development of a robust technique with future scope for improvement *via* the selection of an alternative counterion (*p*-TSA > TFA > HCl).

2.4.1 Investigation into solution phase decomplexation

Having confirmed the ability to prepare a series of crown ether complexes, along with a suitable analytical technique for their quantification in hand, the next step of the investigation was to determine a method by which decomplexation could be performed in order to liberate the free amine **4**. Literature precedent shows that the decomplexation of an amine salt from a crown ether can be achieved using a reagent containing an inorganic metal with a cation diameter similar to the size of the crown ether cavity (Table 1). In the case of 18-c-6 ethers, where the cavity size has been determined as *ca.* 4 Å, Pederson *et al.*[18] demonstrated the dissociation of an amine salt, to afford the free amine, using methanolic NaOH **11** (Na^+ *ca.* 2 Å).

In order to evaluate the efficiency of NaOH **11** and an array of metal salts for the decomplexation step described herein, the complex of DB-18-c-6 **14** and tyramine HCl **89** (Scheme 29) was firstly prepared using the protocol previously described. Off-line analysis by HPLC (R_T = 4.6 min) and MS, confirmed complex formation had indeed occurred prior to the addition of the decomplexing agent under investigation.



Scheme 29. Schematic illustrating the decomplexation of tyramine **4** from a DB-18-c-6 **90** complex using a series of metal salts.

Decomplexation of tyramine **4** was subsequently studied by dissolution of the complex **90** (0.30 g) in MeOH (15 ml) followed by addition of the inorganic salt (1.0 eq.) under investigation. The resulting reaction mixture was stirred for 30 min prior to analysis by HPLC; whereby decomplexation was confirmed *via* the detection of tyramine **4** (R_T = 3.2 min) and loss of the peak attributed to the complex at R_T = 4.6 min. With respect to the model reaction under evaluation herein, the use of NaOH **11** as a decomplexing agent was thought to be undesirable as it could potentially lead to cleavage of the acetate group, resulting in the decomposition of the target molecule **2**, again increasing the complexity of the reaction mixture. However, in order to benchmark alternative inorganic compounds and to potentially circumvent this problem, a series of metal alkoxides **11**, **94** to **95**, were evaluated alongside various metal salts **96** to **98**.

As Table 5 illustrates, in addition to the previously investigated NaOH **11**, the metal chlorides **96** to **98** were also found to provide a suitable method of decomplexation for DB-18-c-6 **14** complexes of tyramine HCl **89**, TFA **85** and *p*-TSA **91** salts, whilst presenting no risk of product degradation. In addition, the ability to decomplex tyramine

salts from a series of 18-c-6 **16** complexes was also evaluated using the aforementioned methodology, again affording the free amine **4** in all cases.

Inorganic salt	Crown ether	Decomplexation[a]
NaOH **11**	DB-18-c-6 **14**	Yes
LiOH **94**	DB-18-c-6 **14**	Yes
KOH **95**	DB-18-c-6 **14**	Yes
NaCl **96**	DB-18-c-6 **14**	Yes
LiCl **97**	DB-18-c-6 **14**	Yes
KCl **98**	DB-18-c-6 **14**	Yes
NaOH **11**	18-c-6 **16**	Yes
LiOH **94**	18-c-6 **16**	Yes
KOH **95**	18-c-6 **16**	Yes
NaCl **96**	18-c-6 **16**	Yes
LiCl **97**	18-c-6 **16**	Yes
KCl **98**	18-c-6 **16**	Yes

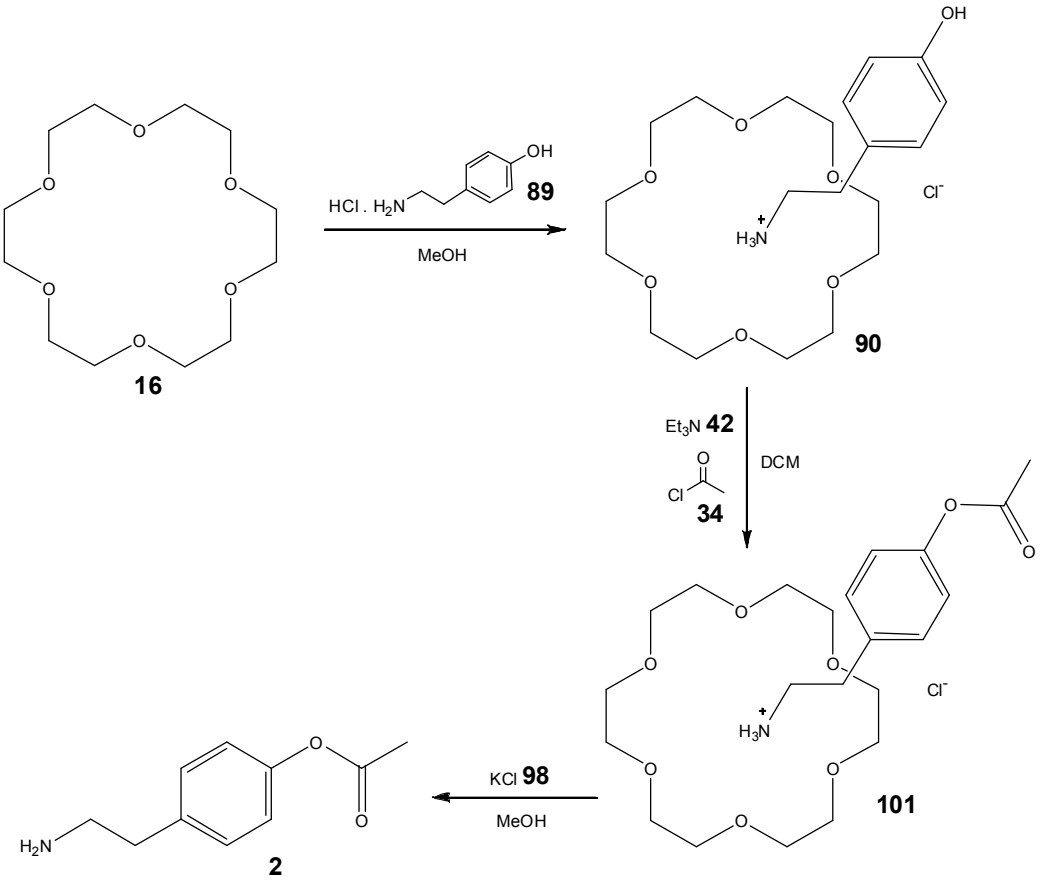
[a] Based on the detection of tyramine **4** by HPLC (R_T = 3.2 min).

Table 5. Evaluation of a range of inorganic salts for the decomplexation of tyramine HCl **89** from DB-18-c-6 **14** and 18-c-6 **16** complexes.

As Scheme 29 illustrates, once the amine **4** has been successfully released from the complex **90**, prior to re-use of the crown ether **14** the metal salt must be removed in order to regenerate the crown ether cavity **99** and enable subsequent sequestration of ammonium ions.

2.4.2 Solution phase reaction of a crown ether complex

Upon establishing a suitable protocol for the protection of tyramine HCl **89** and subsequent deprotection of tyramine **4**, reaction of the phenolic moiety was subsequently investigated. As Scheme 30 illustrates, 18-c-6 **16** was selected as the complexing agent due to the ease of complex formation **100** and intermediate analysis by HPLC, again due to the lack of a chromophore in the crown ether itself.



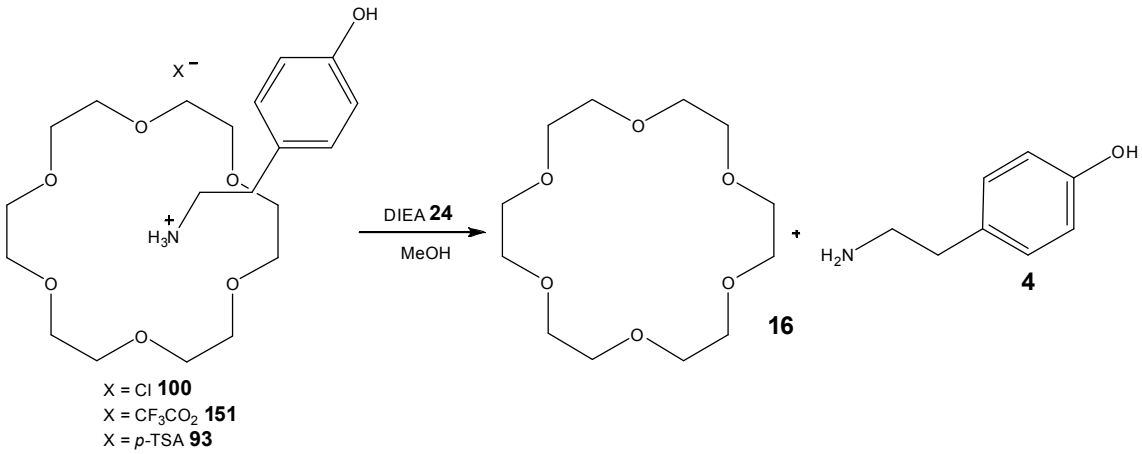
Scheme 30. Illustration of the solution phase acetylation of tyramine HCl **89**, in the presence of 18-crown-6 ether **16**, employing Et_3N **42** as the base.

Owing to the fact that inorganic bases had been shown to readily destabilise ammonium complexes (Table 5), the organic base triethylamine (Et_3N) **42** was initially selected as a base to deprotonate the phenolic group, due to the reported stability of such complexes to this base by Hyde and co-workers.[35] The complex **90** was initially formed in DCM/MeOH, as previously described, then concentrated *in vacuo* to remove residual

MeOH, prior to dissolution in DCM. As Scheme 30 illustrates, deprotonation of the phenolic group using Et_3N **42** (1.2 eq.) was followed by acetylation with acetyl chloride **34** (1.5 eq.) in DCM and the resulting reaction mixture stirred at room temperature for 1 hr after which, residual reagents were removed *in vacuo* to afford the complexed tyramine acetate **2** as a pale yellow oil. Analysis of the intermediate **101** by HPLC (R_T= 6.3 min) and MS ([M^+ +1] = 444) confirmed that acetylation had indeed occurred to afford the desired intermediate **101**.

As the decomplexation step had previously been performed in MeOH and due to the poor solubility of the metal salts in DCM, the complexed acetate **101** (0.35 g) was dissolved in MeOH (10 ml) and subsequently treated with KCl **98** (0.1 M in MeOH), whereby decomplexation afforded a mixture of the desired product tyramine acetate **2** (64 %), starting material **4** (24 %) and the diacetate **1** (12 %); as determined by HPLC analysis. Although initially surprised by the observation that diacetylation had occurred, it can be attributed to partial decomplexation of tyramine HCl **89** during the reaction or due to the presence of an equilibrium.[35-37]

As eluded to previously, the affinity of the crown ether cavity to inorganic cations necessitates washing of the cavity in order to regenerate it once inorganic ions are sequestered. Although this can be achieved by treatment of the crown ether with acetic acid (5% v/v in MeOH),[88] it does however add an additional step to the overall process and as such another approach was sought. As an alternative to the use of an inorganic salt for destabilisation of the ammonium complex **101**, Botti *et al.*[34] reported the use of an organic base, DIEA **24**, to facilitate decomplexation of an aminated compound complexed within a crown ether cavity. The advantage of this technique being that dissociation is achieved without a cation residing within the crown ether cavity, thus removing a reaction step (Scheme 31).



Scheme 31. Schematic illustrating the decomplexation of tyramine **4** from a series of 18-c-6 **16** complexes using the organic base DIEA **24**.

Unfortunately however, even if the need to regenerate the crown ether cavity can be avoided, separation of the product from the crown ether **16**, ammonium salt and residual organic base **24** is still necessary in order to isolate the desired product and enable subsequent use of the crown ether **16**. While the above method showed promise as a protecting group strategy, separation from the complex reaction mixture was not performed; consequently, tyramine **4** was not isolated *via* this route.

2.5 Immobilisation of crown ethers

As discussed in Section 1.2.2, the immobilisation of crown ethers has the potential to address many of the issues that have prevented their adoption as an alternative to covalent protecting groups, namely their separation from the reaction products in order to facilitate efficient recycle. As such, their immobilisation would allow increased reaction control, compared to solution phase analogues, as after each reaction step the immobilised complex could be washed, in order to remove excess reagents and any uncomplexed material, thus affording the resulting reaction products in higher purity.

2.5.1 Evaluation of immobilised di-*t*-butylcyclohexano 18-crown-6 ether

In order to evaluate the use of an immobilised crown ether as a protecting group, preliminary investigations were conducted using the commercially available di-*t*-butylcyclohexano 18-crown-6 ether on an inert chromatographic support **102** (Figure 6), obtained from Eichrom Technologies. Designed for use as a stationary phase within HPLC columns and as a Sr sequestering resin employed in gravity fed columns,[89] the crown ether is adsorbed onto a chromatographic support, affording a loading of 0.37 mmol g^{-1}, as reported by the supplier.

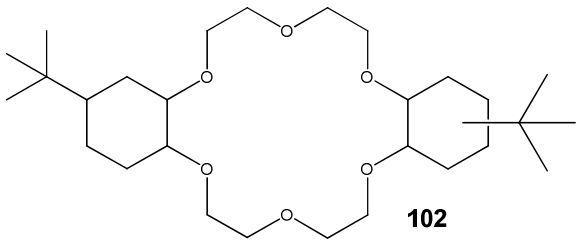


Figure 6. Schematic of the crown ether moiety, di-*t*-butylcyclohexano 18-crown-6 ether **102**, supplied by Eichrom Technologies.

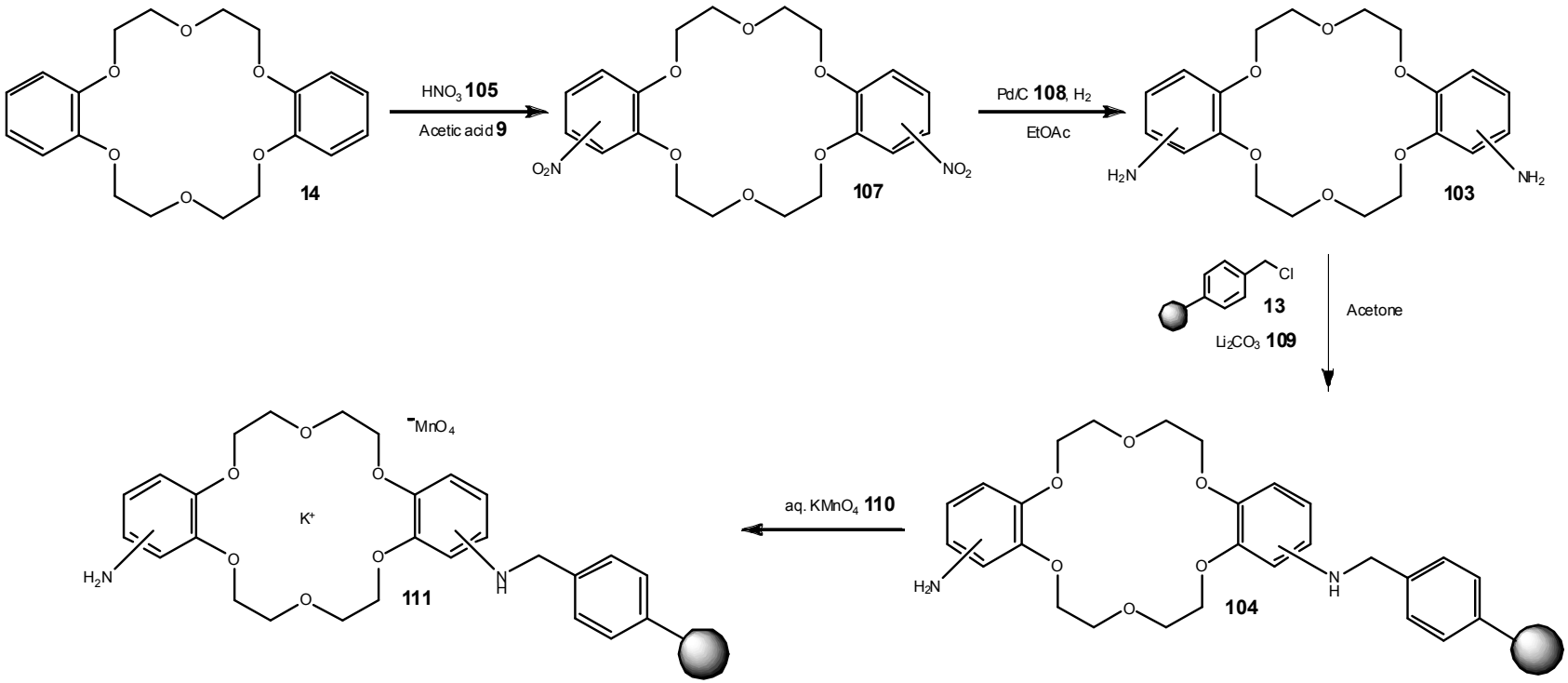
To assess the use of the di-*t*-butylcyclohexano 18-c-6 **102** as a non covalent protecting group, the ability of the material to complex and decomplex an amine salt was firstly investigated. This was achieved by stirring the crown ether **102** (2.0 mmol) with tyramine HCl **89** (2.0 mmol) in MeOH for 1 hr, prior to filtration and washing with MeOH, followed by treatment with methanolic KCl **98** (15 ml, 0.1 M) to afford decomplexation. Analysis of the filtrate by HPLC confirmed complexation and decomplexation was achieved *via* detection of tyramine **4** (R_T = 3.2 min). The recyclability of the immobilised crown ether **102** was subsequently evaluated, by firstly treating the material with methanolic acetic acid (10 ml, 5 % v/v) to regenerate the cavity, followed by complexation of tyramine HCl **89** (1.0 eq.) in MeOH (10 ml). Upon decomplexation and analysis of the resulting reaction mixture, it was surprising that no tyramine **4** was detected.

When the aforementioned process was repeated with a new portion of the resin, successful complexation and decomplexation of tyramine **4** was once again observed. Following further investigation, it was proposed that under the reaction conditions evaluated the di-*t*-butylcyclohexano 18-c-6 **102** was washed off of the support owing to the fact that the crown ether is adsorbed onto the polymer support rather than covalently bound, and therefore could not be reused.

With this in mind, it was proposed that covalent immobilisation of the crown ether was required in order to obtain sufficient stability and to enable recycling of the supported crown ether and its potential use in a continuous flow system. Unfortunately however, no such materials were commercially available and thus their preparation was investigated; the results of which are reported herein.

2.5.2 Preparation of a covalently immobilised 18-crown-6 ether derivative

To enable the covalent immobilisation of an 18-crown-6 ether derivative onto a polymeric support, it was firstly necessary to functionalise the parent crown ether in order to append a suitable functional group through which the immobilisation could take place. As 18-c-6 **16** has no obvious positions to functionalise, the investigation initially concentrated on the immobilisation of DB-18-c-6 **14**; especially as the previously observed issues with solubility and complications with HPLC analysis would be removed once immobilised. In order to immobilise DB-18-c-6 **14**, it was proposed that aromatic nitration followed by reduction of the nitro group to the amine **103**, would allow facile attachment of the functionalised crown ether to MPR **13** in the presence of a base, as illustrated in Scheme 32. In addition to being a rapid route to the immobilised crown ether **104**, it was proposed that the introduction of nitrogen from the crown ether moiety **103**, would afford facile determination of the crown ether loading by elemental analysis, based on an increase in nitrogen content of the solid-supported crown ether **104** *cf.* the blank resin **13**.



Scheme 32. Derivatisation of DB-18-c-6 ether **14** and immobilisation of DADB-18-c-6 ether **103** onto Merrifield peptide resin **13** and subsequent sequestration of $KMnO_4$ **110** to confirm the presence of an active cavity.

Under standard nitration conditions, comprising of nitric acid **105** (1.0 eq.) and sulfuric acid **106** (1.1 eq.),[2] the synthesis of dinitro-dibenzo-18-crown-6 ether (DNDB-18-c-6) **107** was attempted. Unfortunately, not only did the electrophilic substitution fail, but the reaction conditions also led to decomposition of the parent crown ether **16**, an observation that was confirmed by MS analysis. Subsequent investigations, including a method reported by Lai *et al.*[90] that solely employed nitric acid **105**, in the belief that the nitronium ion could be generated in the absence of sulfuric acid **106** also proved fruitless. In a final attempt, the nitration was conducted using a protocol reported by Feigenbaum *et al.*[91] whereby DB-18-c-6 **14** and nitric acid **105** (1.0 eq.) were heated under reflux in acetic acid (1.1 eq.) for 8 hr. Analysis of the resulting yellow solid by ^{1}H NMR spectroscopy confirmed formation of DNDB-18-c-6 **103** as the sole product, in 76 % yield. It should be emphasised that the exact positions of the two nitro groups was unknown, however the compound was observed to be symmetrical (Figure 7) from the ^{1}H-NMR data.

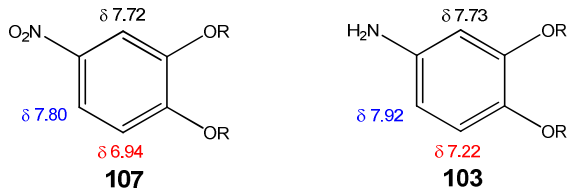


Figure 7. Schematic representation of the chemical shifts observed in ^{1}H NMR spectra that confirmed the reduction of DNDB-18-c-6 **107** to afford DADB-18-c-6 **103**.

To enable attachment of the DNDB-18-c-6 **107** to a polymeric support, the compound **103** was reduced to afford the respective amine moieties required. To achieve this, various methods of reduction were investigated, including catalytic hydrogenation using 5 % Pd on Carbon **108** and hydrazine monohydrate, but the most efficient technique investigated employed 5 % Pd on Carbon **108** and H_2, in EtOAc, which afforded the

respective diaminodibenzo-18-crown-6 ether (DADB-18-c-6) **103** as a cream solid in 78 % yield. The reduction was confirmed by disappearance of the NO_2 band in the IR spectrum (1347 cm^{-1}) and by the shift of the aromatic doublet (*J* 9.0) from δ 6.94 to δ 7.22 (red) and the double doublet (*J* 9.0 and 2.6) from δ 7.80 to δ 7.92 (blue), in the ^1H NMR spectra, as illustrated in Figure 7.

Immobilisation of the DADB-18-c-6 **103** (Scheme 32) was subsequently undertaken using a method reported by Simpson *et al.,*[92] whereby MPR **13** (1.5 mmol Cl g^{-1}, 2 % crosslinked with DVB) was heated to reflux with the amine under investigation (1.0 eq.) in the presence of an inorganic base in acetone (6 hr). Due to the increased affinity of the DADB-18-c-6 **103** cavity for potassium ions (see Section 1.3), the protocol was modified and potassium carbonate **109** was substituted with lithium carbonate **110** (1.0 eq.) as the base (Scheme 32). The immobilised crown ether **104** was subsequently washed with water (50 ml), followed by acetone (50 ml), prior to drying in an oven at 60 °C to afford a pale yellow free flowing solid. The immobilised DADB-18-c-6 **104** was then evaluated by elemental analysis, whereby an increase in nitrogen content was used to determine the proportion of DADB-18-c-6 **103** covalently bound to the MPR **13**. Using this approach, an increase of 0.33 % N c*f.* unfunctionalised MPR **13** was obtained, confirming a loading of 0.12 mmol g^{-1} of crown ether (Equation 3, Section 4.4.4). Based on the loading of the MPR **13** this equates to 7.9 % functionalisation with respect to available benzyl chloride groups (1.50 mmol g^{-1}).

Having confirmed the successful immobilisation of DADB-18-c-6 **103**, to afford **104**, by elemental analysis, the next step was to evaluate the viability of the immobilised crown ether cavities. To achieve this, a sample of the material **104** (0.30 g) was stirred in an

aqueous solution of potassium permanganate **110** (0.1 M) for 1 hr (Scheme 32), prior to filtration and washing. The resulting beige solid **111** was digested and the liquor analysed by ICP-MS, affording a measure of both potassium and manganese. Comparison of the results obtained for the material **111** with unfunctionalised MPR **13**, quantified the loading of accessible crown ether sites to be 0.12 mmol g^{-1}, which was in agreement with the elemental analyses previously performed. As the potassium and manganese results corroborated one another, for simplicity only the potassium results are subsequently reported.

In an attempt to further increase the loading of the material **104**, using the residual benzyl chloride moieties, attachment of DADB-18-c-6 **14** was repeated using the aforementioned protocol depicted in Scheme 32; however this afforded no further increase in loading and added credence to the theory that the bulky DADB-18-c-6 **103** prevented access to all of the benzyl chloride moieties on the polymeric support **13**. An observation that is corroborated by Kim *et al.*[93] who attained a significantly higher loading of the less hindered 4-amino-18-crown-6 ether (AM-18-c-6) **112** onto MPR **13** by refluxing in DMF for 72 hr (2.25 mmol g^{-1}). Having confirmed that the crown ether had been successfully immobilised, and that the cavity was able to complex potassium ions, the materials ability to complex ammonium salts was subsequently evaluated.

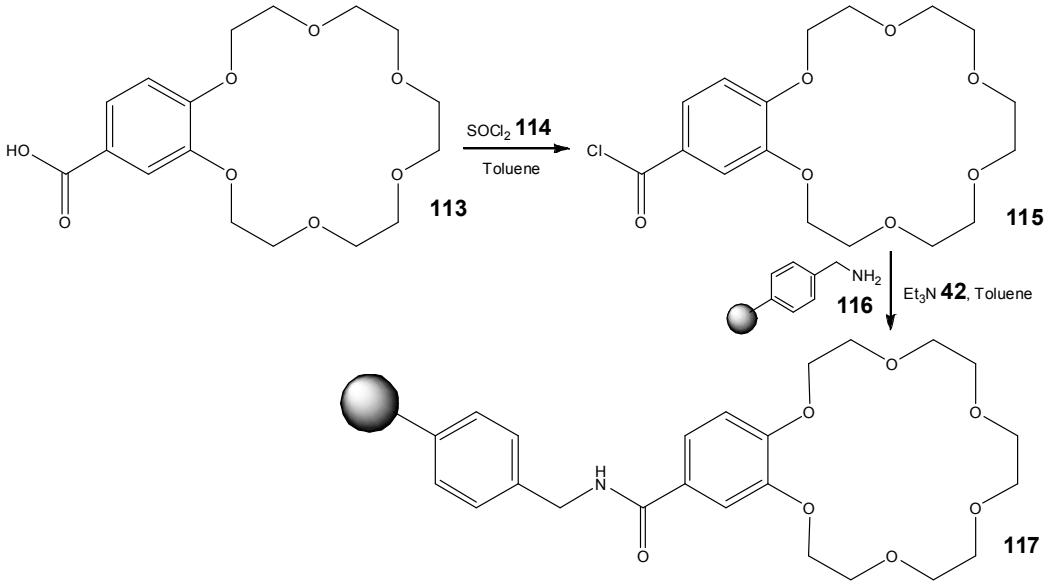
Employing an excess of tyramine HCl **89** (1.5 eq.), in the first instance, the immobilised DADB-18-c-6 **104** (0.10 g, 1.20 x 10^{-2} mmol) was stirred at room temperature in MeOH for 1 hr prior to filtration and washing with MeOH (100 ml), to remove any uncomplexed and potentially adsorbed tyramine HCl **89** from the solid-support. The decomplexation step was subsequently performed using methanolic KCl **98** (1 ml, 0.1 M) and the filtrate

concentrated *in vacuo* prior to off-line analysis by HPLC. Based on previous observations for the immobilised crown ether **104**, it was surprising at this point that no tyramine **4** was detected. To ensure that the problem was not due to the decomplexation strategy employed, the process was repeated with a second aliquot of material **104** and prior to decomplexation, the polymer was oven dried (at 60 °C) and subjected to elemental analysis. Comparison of the results obtained for the immobilised DADB-18-c-6 **104** (0.33 % N) prior to complexation and those obtained after complexation showed no increase in nitrogen content (0.33 % N), indicating that the problematic step was in fact ammonium ion complexation. Treatment of the material with $KMnO_4$ and analysis by ICP-MS confirmed that the crown ether cavity was still accessible to inorganic cations and it was postulated that the presence of lithium ions, from the immobilisation step, may still be present and would be readily displaced by potassium ions but not by the ammonium salt **89** under investigation. With this in mind, the immobilised DADB-18-c-6 **104** was treated with methanolic acetic acid (5 % v/v) prior to any attempts to complex tyramine HCl **89**, with the aim of removing any inorganic cations from the crown ether cavity. Unfortunately, this approach also failed to enable complexation of tyramine HCl **89** and it was concluded that the immobilised material **104** was unable to perform the desired complexation of ammonium salts. This is an observation that was found to be in agreement with Bradshaw and co-workers,[94] who reported that immobilisation of DB-18-c-6 **14** led to a lesser propensity for ammonium salts, whilst maintaining the materials ability to sequester inorganic cations. The rationale for this is believed to be due to the fact that the ammonium ion is less reliant on cavity size, than inorganic cations, and more so on hydrogen bonding. Figure 3 (Section 1.3.1) depicts how the ammonium ion sits above the crown ether cavity and is held in place by hydrogen bonding, between the ammonium and ether groups. It is

therefore suggested that immobilisation of the DB-18-c-6 **14** leads to further straining of the crown ether structure, which in turn affects its ability to accommodate the ammonium group, whilst retaining the ability to host metal ions within its cavity. This is further corroborated by reports of Shchori and co-workers[95] who observed a marked decrease in the rate of ammonium ion complexation as a result of substitution with nitro groups.

2.5.3 Immobilisation of carboxybenzo-18-crown-6 ether

To investigate this theory further, the immobilisation of a second crown ether was performed, selecting carboxybenzo-18-crown-6 ether (CAB-18-c-6) **113** due to the presence of only one aromatic ring, enabling the reduced cavity strain to be evaluated. As Scheme 33 illustrates, the immobilisation was achieved *via* treatment of the commercially available CAB-18-c-6 **113** (1.0 eq.) with thionyl chloride **114** (1.2 eq.), to afford the respective acid chloride **115**. The acid chloride **115** was subsequently reacted, without isolation, with amino methyl polystyrene **116** (1.50 mmol N g^{-1}, crosslinked with 2 % DVB, 200-400 mesh), in the presence of Et_3N **42** (1.2 eq.).



Scheme 33. Synthetic methodology employed for the immobilisation of CAB-18-c-6 **113** onto aminomethyl functionalised polystyrene beads **116**.

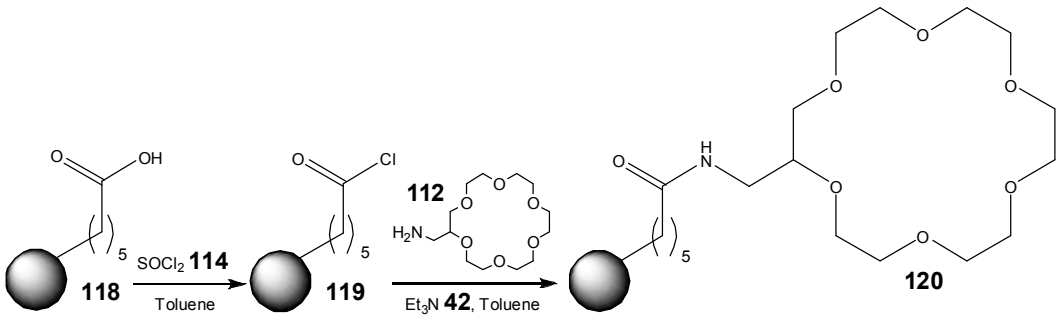
The reaction mixture was stirred overnight, filtered under suction and sequentially washed with DI water (50 ml) and acetone (100 ml), prior to oven drying at 60 °C, to afford a pale yellow free flowing solid. Due to the presence of nitrogen within the polymer matrix, elemental analysis afforded no reliable means of determining the crown ether loading; consequently, the complexation of $KMnO_4$ **110** was used, as previously discussed, to quantify the available crown ether groups. Using this approach, a loading of 0.98 mmol g^{-1} was determined, which is equivalent to 89.0 % functionalisation of the solid support **117**; representing a considerable increase in loading compared to the previous immobilisation strategy (Section 2.5.2) whereby an optimal loading of 0.12 mmol g^{-1} was obtained which equated to 7.9 % functionalisation.

Having confirmed the presence of active crown ether cavities, by $KMnO_4$ **110** sequestration, the next step was to evaluate the materials ability to complex ammonium ions; this was achieved using the aforementioned approach of tyramine HCl **89** complexation. Again, it was surprising to see that the filtrate contained no tyramine **4**, an observation that suggested deformation of the crown structure was still a problem and prevented ammonium ion complexation.

2.5.4 Immobilisation of aminomethyl-18-crown-6 ether

In a final attempt to remove all ether strain from the system, a third covalent strategy involving the immobilisation of aminomethyl-18-crown-6 ether (AM-18-c-6) **112** onto carboxypolystyrene **118** (0.4-0.6 mmol g^{-1}, 1 % cross linked with DVB, 200-400 mesh size) was studied. As Scheme 34 illustrates, the immobilisation was achieved by treating the carboxypolystyrene **118** with thionyl chloride **114** (1.2 eq.), to afford the immobilised acid chloride **119**, prior to the addition of AM-18-c-6 **112** (1.0 eq.) in the presence of Et_3N **42** (1.2 eq.). The resulting reaction mixture was stirred at room

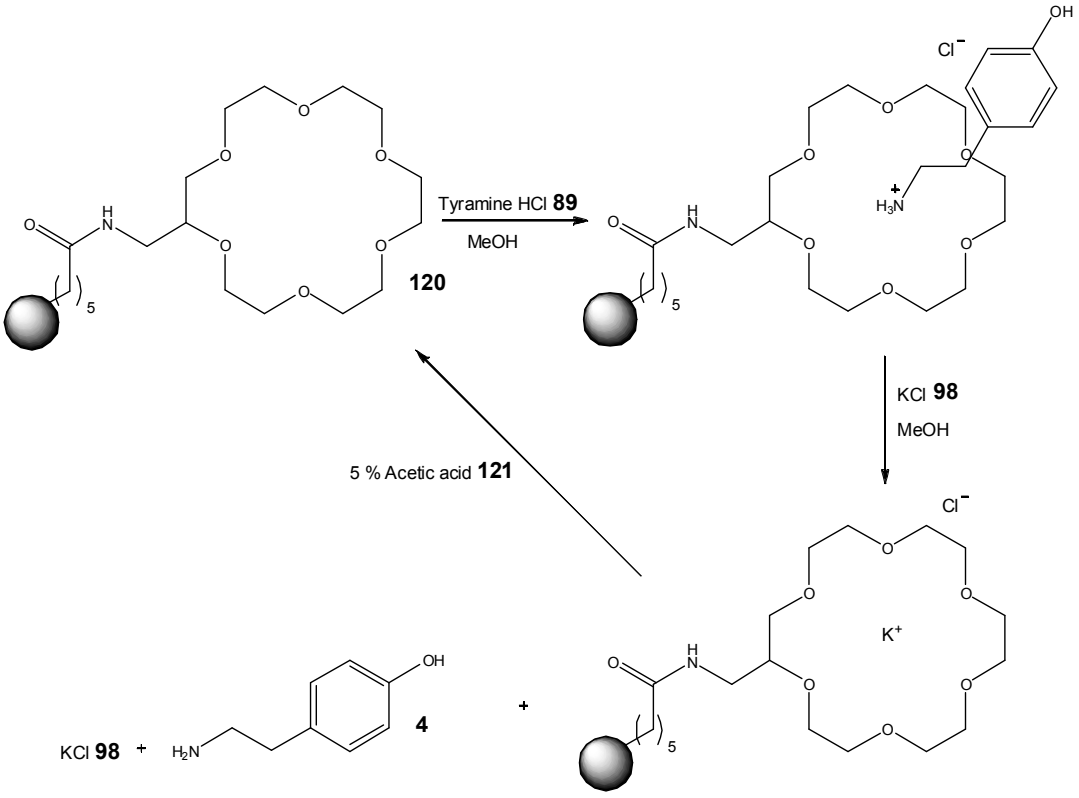
temperature overnight; the polymer was then filtered, washed with DI water (100 ml) and acetone (100 ml) and oven dried at 60 °C to afford a white, free flowing solid **120**.



Scheme 34. Synthetic strategy employed for the immobilisation of AM-18-c-6 **112** onto carboxypolystyrene **118**.

Elemental analysis was used to quantify the crown ether loading, again based on an increase in nitrogen content compared to the blank carboxypolystyrene **118**, which afforded a loading of 0.37 mmol N g^{-1} (Entries 1 and 2, Table 6). ICP-MS was subsequently employed in order to corroborate the loading, whereby complexation of $KMnO_4$ afforded a loading of 0.37 mmol g^{-1}.

Having confirmed the presence of active crown ether cavities within the immobilised material **120**, the complexation of tyramine HCl **89** was investigated. To achieve this, the immobilised AM-18-c-6 **120** was stirred in a methanolic solution of tyramine HCl **89**, filtered under suction and washed with MeOH to remove any residual tyramine HCl **89** (Scheme 35).



Scheme 35. Reaction scheme illustrating the complexation of tyramine HCl **89**, using immobilised AM-18-c-6 **120**, and decomplexation to afford tyramine **4** (performed in batch).

At this stage, an aliquot of the resin underwent elemental analysis where it was found that the % N had increased from 0.52 % to 0.97 %, confirming an increase equivalent to the complexation of 0.32 mmol g^{-1} of tyramine HCl **89** (Table 6). The remaining material was treated with methanolic KCl **98** (0.1 ml, 0.1 M) to achieve decomplexation and the filtrate concentrated *in vacuo* prior to analysis by HPLC, which confirmed the release of tyramine **4** from the immobilised crown ether **120**. The recovery of the free amine, not the salt, was confirmed by comparison of the retention time of the reaction product (R_T of 3.2 min) with a series of synthetic standards; tyramine **4** (R_T = 3.2 min) and tyramine HCl **89** (R_T = 3.6 min). In addition, a portion of the material was again submitted for

elemental analysis where the % N was found to have returned to the original 0.52 %, illustrating quantitative decomplexation of tyramine **4**.

Polymeric Material	N (%)	N (mmol g^{-1})
Blank **118**	0.00	0.00
Immobilised AM-18-c-6 **120**	0.52	0.37
Complexed	0.97	0.69
Decomplexed	0.52	0.37

Table 6. Summary of the elemental analyses performed, which served to illustrate successful complexation of tyramine HCl **89** using immobilised AM-18-c-6 **120**.

Having successfully demonstrated, for the first time, the ability to complex the ammonium salt **89** using an immobilised crown ether **120**, the material was subsequently treated with methanolic acetic acid (5 % v/v) to regenerate the cavity and the process was repeated a further 5 times. This served to illustrate the ability to not only sequester ammonium ions and release the free amine, but also the ability to readily recycle the immobilised crown ether **120** (Figure 8), unlike the commercially available di-*t*-butylcyclohexano 18-crown-6 ether **102** previously evaluated (Section 2.5.1), which was adsorbed onto the support material.

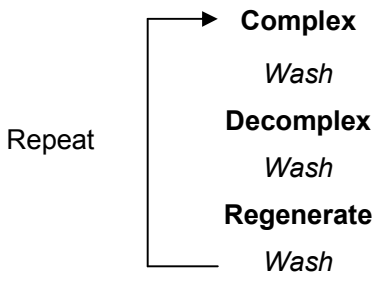


Figure 8. Schematic illustrating the processes of complexation, decomplexation and cavity regeneration used to evaluate the immobilised crown ethers ability to be recycled.

Repetitive complexation, decomplexation and regeneration confirmed that this methodology could be used to capture tyramine HCl **89** and release tyramine **4** on multiple occasions. Compared to the use of solution phase crown ethers, this approach is advantageous as it affords the product with ease; removing the lengthy process of product isolation, whilst allowing recovery and regeneration of the crown ether. The one drawback of this technique when used within a batch reaction is the need to filter and wash the resin **120** under suction at each stage, in order to remove excess reagents, leading to a somewhat laborious, time consuming process. In addition, the methodology is solvent intensive and can result in significant loss of immobilised material **120** with repeated use; this is illustrated by a reduction in recovered tyramine **4** afforded by the decomplexation step in consecutive runs leading to an 11.4 % RSD (n =5).

2.6 The incorporation of immobilised crown ethers in flow reactors

In order to address the problems associated with the batch process and develop a reusable system that enables the non covalent protection of amine groups, the incorporation of the immobilised crown ether **120** into a flow reactor was evaluated. It was proposed that such an approach would enable the material to be recycled with ease, as the immobilised crown ether **120** would be held in a cartridge and solutions of the various reactants would simply be pumped over the material in order to affect the desired reaction step; thus removing the main source of error which was material loss upon repetitive filtration.

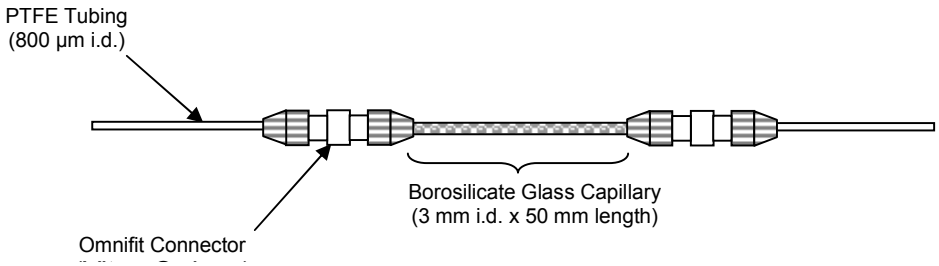
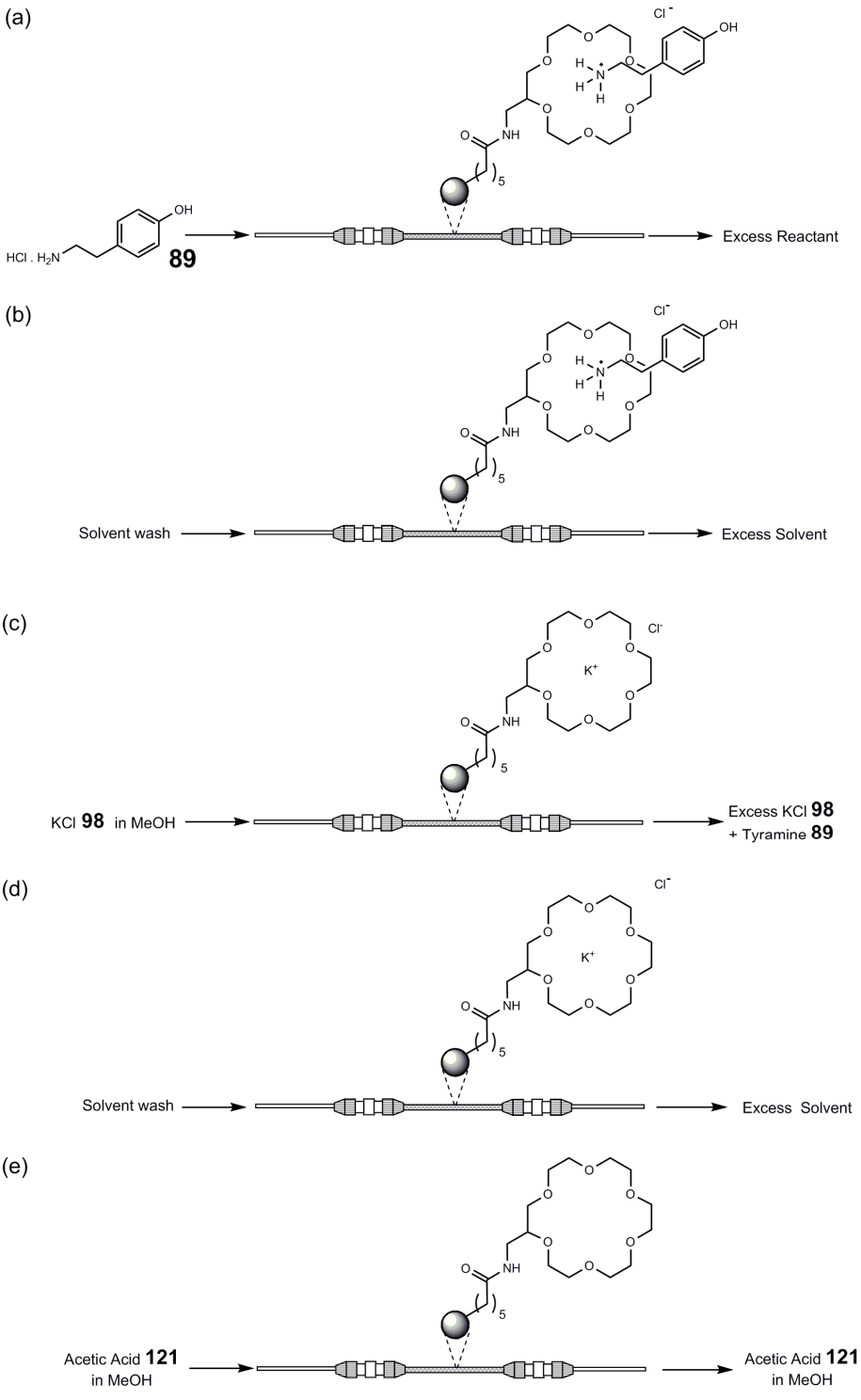


Figure 9. Schematic illustrating the basic flow reactor set-up employed herein for the continuous flow evaluation of immobilised crown ethers.

As Figure 9 illustrates, the flow reactor evaluated herein comprised of a borosilicate glass capillary (50 mm (length) x 3 mm (i.d.)) packed with approximately 0.15 g of immobilised crown ether **120**. The reactor (volume experimentally determined to be 35 µl) was connected to a syringe driver *via* a length of PTFE tubing (0.8 mm i.d.) using commercially available Omnifit connectors. Solvent and reactant solutions were passed through the system under pressure-driven flow, using a Harvard syringe pump, and collected in a 1.5 ml sample vials prior to off-line analysis, by HPLC.

2.6.1 Evaluation of immobilised AM-18-c-6 in a flow reactor

Up to now, it had been shown that MeOH enables the complexation of ammonium salts, with a solution of methanolic KCl **98** affording efficient decomplexation of the respective amines and methanolic acetic acid efficiently regenerating the cavity. However up to this point, nothing was known about the optimum volume of reagents required to conduct each step of the aforementioned process, with an excess employed during the development stages. Consequently, before attempting to perform a reaction of the phenolic moiety, a feasibility study was performed in order to evaluate the effect of various reaction parameters such as flow rate and analyte stoichiometry on the efficiency of the overall process of sequestration, decomplexation and cavity regeneration.



Scheme 36. Illustration of the principle used to evaluate the efficiency of each reaction step utilising the immobilised crown ether **120** under continuous flow.

Using the flow reactor set-up depicted in Scheme 36, the ability to perform continuous flow complexations and decomplexations was firstly investigated. To achieve this, a solution of tyramine HCl **89** (500 μl, 0.3 M, 0.15 mmol, 2.5 eq.) in MeOH was pumped (10 μl min^{-1}) through the flow reactor, containing immobilised AM-18-c-6 **120** (0.15 g, 0.37 mmol g^{-1}, 0.06 mmol). After 50 min, the column was washed with MeOH (500 μl, 100 μl min^{-1}), in order to remove any residual tyramine HCl **89**, before treating the complex with methanolic KCl **98** (500 μl, 0.2 M, 0.10 mmol) at a flow rate of 10 μl min^{-1}. The reaction products were collected in a sample vial and an aliquot of the reaction mixture was then analysed by HPLC. Using biphenyl as the internal standard, the proportion of tyramine **4** that had been decomplexed, and hence initially sequestered by the immobilised crown ether **120**, was quantified to be 0.32 mmol g^{-1} (86 % complexation). In addition, the reaction product was also analysed by HPLC and MS, which confirmed the presence of tyramine **4** (R_T = 3.2 min, $[M^+ + 1]$ = 138).

Having demonstrated the ability to perform the complexation and decomplexation steps under continuous flow, the regeneration and re-use of the material **120** was subsequently investigated. In order to demonstrate the ease of re-using the supported crown ether **120**, the reactor was purged with methanolic acetic acid (10 % v/v) at 200 μl min^{-1}, thus removing the potassium ion from within the crown ether cavity (Scheme 36(e)) and enabling re-use after a short solvent wash (500 μl). The process depicted in Scheme 36 was then repeated a further nine times in order to confirm that the process was reproducible. Using this approach, a % RSD of 4.3 (n = 10) was obtained *cf.* 11.4 previously obtained in batch (n = 5). In addition, as Table 7 illustrates, the proportion of tyramine **4** decomplexed was found to be in-line with the loading of the immobilised AM-

18-c-6 **16**; previously determined by both elemental analysis (Table 6) and ICP-MS, indicating excellent efficiency.

Run No.	Tyramine **4** (mmol) [a]	Calculated loading of AM-18-c-6 **120** (mmol g^{-1}) [b]
1	0.051	0.340
2	0.054	0.360
3	0.052	0.347
4	0.056	0.373
5	0.056	0.373
6	0.052	0.347
7	0.049	0.327
8	0.055	0.367
9	0.055	0.367
10	0.054	0.360
Mean	0.053	0.356
% RSD	4.3	(n = 10)

[a] Monitored by HPLC, based on the detection of tyramine **4** (R_T = 3.2 min)

[b] Calculated based on 0.15 g of resin in the flow reactor (actual = 0.37 mmol g^{-1})

Table 7. Summary of the results obtained for the repeated complexation and decomplexation of immobilised AM-18-c-6 **120** performed in a flow reactor.

Before investigating a reaction within the flow system, it was important to consider the regeneration step in order to ensure all active crown ether sites were cleared of inorganic ions before the next complexation step and minimal quantities of reagents were employed. Table 8 shows that increasing the percentage of acetic acid **121** in MeOH beyond 5 % (v/v), did not improve the recovery of decomplexed tyramine **4**, though a decrease to 2 % (v/v) significantly affected reproducibility and tyramine **4** recovery. This was confirmed with subsequent complexation/decomplexation steps yielding a decreased proportion of tyramine **4**. Treatment of the packed bed with 5 % (v/v) acetic acid **121** in MeOH afforded reproducible recovery on subsequent cycles

(Table 8) and as such, this concentration was used from this point forward for cavity regeneration.

Acetic acid **121** in MeOH (% v/v)	Recovery of tyramine **4** (mmol g^{-1})	
	Run 1	Run 2
15 %	0.306	0.333
10 %	0.319	0.338
5 %	0.350	0.351
2 %	0.151	0.150

Table 8 Investigation into the effect of acetic acid **121** concentration on crown ether cavity regeneration and subsequent efficiency with respect to the complexation of tyramine HCl **89**.

2.6.2 Improved reagent introduction technique

While the aforementioned technique demonstrated the ability to recycle the immobilised crown ether **120** with greater ease and reproducibility under continuous flow *cf.* batch, the time taken to complex and decomplex aminated compounds at these reagent volumes and flow rates was undesirable (approx. 2 hr run^{-1}). It was therefore proposed that by exposing the immobilised crown ether **120** to a stoichiometric quantity of ammonium salt **89**, in a smaller aliquot of solvent, that the time taken to perform the steps could be significantly reduced. This was achieved by introducing a plug of tyramine HCl **89** (200 µl, 0.3 M, 0.06 mmol) into a continuous MeOH solvent stream (10 µl min^{-1}), using a Rheodyne injection valve as depicted in Figure 10.

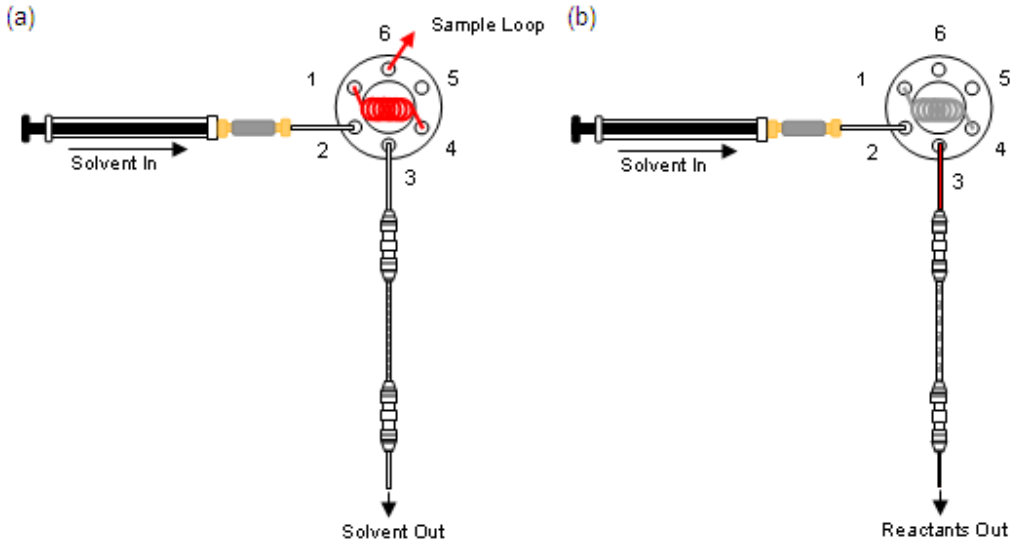


Figure 10. The use of a Rheodyne injection valve to reduce reaction time within the flow system (a) sample loading and (b) sample introduction in continuous a solvent stream.

At this stage, the amine decomplexation was still performed using an excess of methanolic KCl **98** (500 μl, 0.3 M, 0.06 mmol), direct from a syringe at a flow rate of 10 μl min^{-1}. This technique was kept constant from previous studies, in which 5.3 x 10^{-2} mmol of tyramine **4** was obtained, to firstly confirm that the complexation efficiency was not compromised by a reduction in plug volume. It was pleasing to see that increasing the flow rate of the decomplexation step from 10 μl min^{-1} to 100 μl min^{-1} also afforded quantitative decomplexation (0.05 mmol of **4**) (Table 9). Further increases in flow rate however led to irreproducible recoveries of tyramine **4**, a factor that was attributed to the insufficient pressure tolerance of the reactor.

In a final attempt to reduce the overall processing time, optimisation of the decomplexation step was performed using the Rheodyne injector valve in a similar manner to the previously described complexation step. Injecting a 200 μl plug of methanolic KCl **98** (0.3 M, 0.06 mmol) into a continuous stream of MeOH (10 μl min^{-1}) it

was observed that comparable tyramine **4** recoveries could be achieved (0.05 mmol of **4**) using less reagent again; increasing the flow rate further reduced reaction time, with 100 μl min^{-1} proving to be optimal (Table 9). No further reductions were feasible due to the solubility of tyramine HCl **89** in MeOH being limited to 0.3 M at 25 °C.

With the decomplexation step optimised, the flow rate for complexation was investigated and it was found that increasing the flow rate to 100 μl min^{-1} had no erroneous effect on the recovery of tyramine **4**. Although as observed for the decomplexation step, an increase beyond this did result in a decrease in the proportion of amine **4** recovered, an observation which was again attributed to insufficient pressure tolerance of the reactor.

Process	Introduction Technique	Flow Rate (μl min^{-1})	Tyramine **4** (mmol) [a]	Calculated loading **120** (mmol g^{-1}) [b]
Complexation	Constant stream[c]	100	0.052	0.351
Complexation	Fixed volume[d]	10	0.053	0.338
Complexation	Fixed volume[d]	100	0.051	0.329
Decomplexation	Constant stream[c]	10	0.049	0.353
Decomplexation	Constant stream[c]	100	0.052	0.354
Decomplexation	Fixed volume[d]	10	0.053	0.345
Decomplexation	Fixed volume[d]	100	0.052	0.349

[a] Determined *via* HPLC analysis [b] calculated based on 0.15 g of resin in the flow reactor (actual loading 0.37 mmol g^{-1}) [c] 200 μl *via* Rheodyne valve and [d] 500 μl direct from syringe of reactant employed.

Table 9. Summary of the effect of flow rate and sample introduction technique on complexation/decomplexation efficiency using immobilised AM-18-c-6 **120**.

2.6.3 Reaction of immobilised tyramine

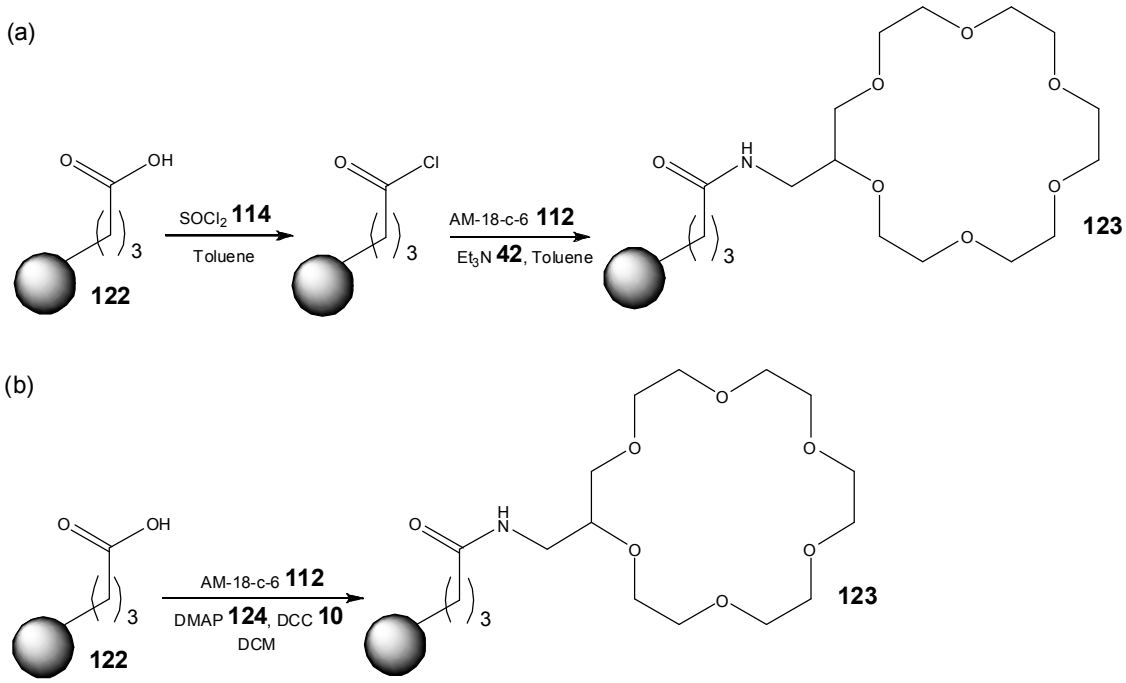
Having optimised both the complexation and decomplexation steps under continuous flow, the next phase of the investigation was to perform a reaction on the complexed tyramine **4**. While the use of MeOH was found to be ideal for both the complexation and

decomplexation steps of the process, the fact that the solvent is incompatible with the reagents used for the reaction, namely acetyl chloride **34** in the acylation reaction, alternative solvent systems were investigated; these included DMF, THF, DCM and MeCN. Unfortunately, when this series of common organic solvents were employed, the immobilised AM-18-c-6 crown ether **120** was observed to swell, leading to blockages within the reactor and inconsistencies in the amount of solution passing through the packed bed at any one time. This observation was attributed to the low degree of crosslinking employed in the solid-support **117** (1 % crosslinked with DVB) and was confirmed by the preparation of a packed bed containing polystyrene beads (2 % crosslinked with DVB); whereby no swelling was observed under the aforementioned conditions. Regrettably the carboxy polystyrene **117** was not commercially available with a higher degree of crosslinking and as such, 3-carboxypropyl functionalised silica gel **122** was investigated as an alternative, non-swelling support material.

2.7 Preparation of silica-supported aminomethyl-18-crown-6 ether

Based on the success of the technique employed for the immobilisation of AM-18-c-6 **112** onto carboxy polystyrene **118**, an analogous approach was employed for the functionalisation of 3-carboxypropyl functionalised silica gel **122** (1.60 mmol g^{-1}, 200-400 mesh) (Scheme 37(a)). Owing to the use of a silica support, elemental analysis of the immobilised crown ether **123** could not be performed and hence evaluation of the loading was conducted solely *via* ICP-MS, which afforded a loading of 0.16 mmol g^{-1}. In an attempt to increase the loading of AM-18-c-6 **112**, preparation of an immobilised AM-18-c-6 **123** *via* a carbodiimide coupling reaction was also investigated. As Scheme 37(b) illustrates, the reaction involved treatment of AM-18-c-6 **112** (1.0 eq.) and 3-carboxypropyl functionalised silica gel **118** (1.0 eq.) with DCC **10** (3.0 eq.) and a

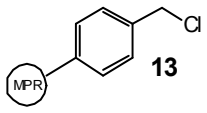
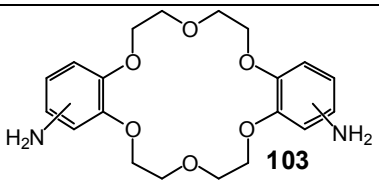
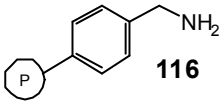
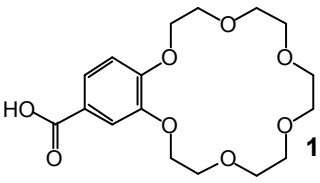
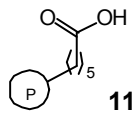
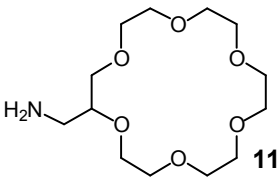
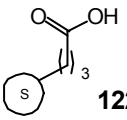
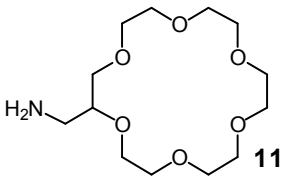
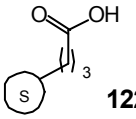
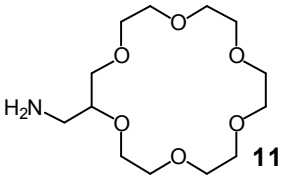
catalytic quantity of DMAP **124** (0.5 eq.) in DCM. The reaction mixture was stirred at room temperature for 24 hr, filtered under vacuum, washed with DI water (100 ml) then acetone (100 ml) and oven dried (60 °C) to afford a free flowing white powder. Complexation of the immobilised crown ether **123** with $KMnO_4$ **110** followed by ICP-MS analysis, afforded an improved loading of 0.21 mmol g^{-1}.



Scheme 37. Illustration of the two immobilisation techniques employed for the preparation of SILICA AM-18-c-6 **123** *via* (a) acid chloride and (b) carbodiimide coupling methods.

Employing an excess of tyramine HCl **89** (1.5 eq.) both materials were evaluated in batch by stirring in a solution of amine **89** in MeOH at room temperature for 1 hr prior to filtration and washing with MeOH, in order to remove any uncomplexed tyramine HCl **89**. Decomplexation was subsequently performed using methanolic KCl **98** (0.2 M) and the filtrate concentrated *in vacuo* prior to analysis by HPLC, upon which tyramine **4** was

detected (R_T = 3.2 min), correlating to the loadings indicated by ICP-MS analysis of 0.16 mmol g^{-1} and 0.21 mmol g^{-1} respectively (Table 10).

Solid support	Crown ether	Loading (mmol g^{-1})	Loading (%) [a]	Capture **89** [b]
 **13**	 **103**	0.12	8.4	No
 **116**	 **113**	0.98	65	No
 **118**	 **112**	0.37	62-93[c]	Yes*
 **122**	 **112**	0.16 [d]	10	Yes
 **122**	 **112**	0.21[e]	12	Yes

MPR = Merrifield peptide resin, P = Polymer, S = Silica. * Resin swelled in organic solvents.
[a] Determined with respect to the loading of the solid support, [b] monitored by HPLC, based on the detection of tyramine **4** (R_T = 3.2 min), [c] manufacturers loading reported as a range and materials prepared *via* [d] acid chloride and [e] carbodiimide coupling method.

Table 10. Summary of the immobilised crown ethers prepared herein and their ability to complex the hydrochloride salt of tyramine **89**.

Table 10 compares the immobilised crown ethers and support materials evaluated, summarising the crown ether loading, along with the percentage loading attained with respect to the functionality available on the solid support. Of the methods evaluated, it is clear that the silica gel supported AM-18-c-6 **123** prepared *via* carbodiimide coupling, affords an immobilised material with the greatest complexation capacity and potential solvent stability, and as such was selected for further evaluation under continuous flow.

2.7.1 Evaluation of AM-18-c-6 functionalised silica gel under continuous flow

Using the reaction set-up illustrated in Figure 10, the SILICA AM-18-c-6 **123** (0.15 g, 2.4 x 10^{-2} mmol) was subsequently evaluated under continuous flow, firstly employing the material prepared *via* the acid chloride route (Scheme 37(a)). Prior to performing any investigations into the materials ability to complex ammonium salts under continuous flow, the stability of the packed bed to common organic solvents, such as THF, DMF, DCM and MeCN, was initially investigated and unlike the polymer supported AM-18-c-6 **120**, it was found that all solvents could be pumped through the reactor at a flow rate of 100 μl min^{-1} with no sign of swelling or restricted flow over an extended period of operation (8 hr). Having overcome the limiting factor associated with the polymer supported crown ether **123** prepared herein, the next step was to evaluate the continuous flow complexation of tyramine HCl **89** using SILICA AM-18-c-6 **123**.

Employing a solvent stream of MeOH, tyramine HCl **89** (200 μl, 0.12 M, 2.4 x 10^{-2} mmol) was introduced into the system *via* a Rheodyne injection valve (200 μl) and pumped through the reactor containing 0.15 g of immobilised crown ether **123** (2.4 x 10^{-2} mmol). Again, decomplexation was achieved using KCl **98** in MeOH (200 μl, 0.12 M, 2.4 x 10^{-2} mmol) and the column was purged with 5 % acetic acid (v/v) in MeOH between steps, in order to regenerate the crown ether cavity. As Table 11 illustrates, operating the reactor

at a flow rate of 100 μl min^{-1} afforded reproducible complexation of tyramine HCl **89** and subsequent decomplexation of tyramine **4** (RSD = 7.3 %), whereby the loading of 0.15 mmol g^{-1} corresponded with that obtained *via* ICP-MS analysis.

Run No.	Moles of tyramine **4** mmol	Calculated loading of **123** (mmol g^{-1}) [a]
1	0.024	0.158
2	0.021	0.139
3	0.024	0.157
4	0.023	0.150
5	0.019	0.128
6	0.023	0.152
7	0.022	0.145
8	0.021	0.139
9	0.022	0.148
10	0.025	0.165
mean	0.022	0.148
% RSD	7.3	n = 10

[a] Calculated based on 0.15 g of resin in the flow reactor (actual loading 0.16 mmol g^{-1}).

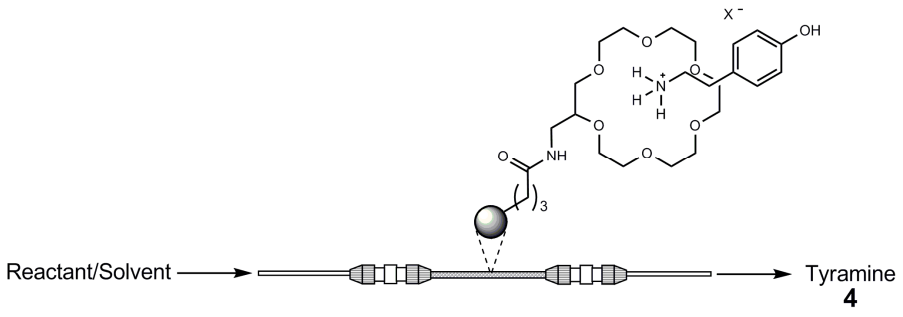
Table 11. Summary of the results obtained for the evaluation of SILICA AM-18-c-6 **123** recyclability under continuous flow.

Having demonstrated the ability to perform the complexation of tyramine HCl **89**, the decomplexation of tyramine **4** and confirmed the supported reagents stability to a range of common organic solvents, the flow reactor set-up was improved to enable the use of two packed-beds, in parallel, thus increasing the throughput of the system and allowing all subsequent evaluations to be conducted in duplicate. Aside from the syringe pump, the reactors were independent of one another, with reactants introduced from separate Rheodyne injection valves and solvent streams from separate gas-tight syringes. Again,

the flow reactors consisted of a borosilicate glass capillary (50 mm (length) x 3 mm (i.d.)) packed with 0.15 g of the immobilised crown ether material under investigation (Figure 9).

2.8 Evaluation of the stability of complexed tyramine HCl

Once complexed, it was important to determine how stable the complexed ammonium salt **89** was, to various solvents and reactants that may be employed to derivatise the complexed material, prior to evaluating a reaction. To ensure the investigation provided general conclusions for this non covalent protecting group strategy, the experiments were conducted using three ammonium salts, firstly tyramine HCl **89**, then tyramine TFA **85** and finally tyramine *p*-TSA **91**. Complexation was achieved by injecting a 200 µl plug of the tyramine salt under investigation (0.12 M, 2.4 x 10^{-2} mmol) into a continuous MeOH solvent stream (100 µl min^{-1}) (Scheme 38), using a Rheodyne injection valve, as per the previous procedure. The use of the continuous solvent stream ensured complete washing of the resin prior to evaluating the stability of the complex under the selected reaction condition (Tables 12-14).



Scheme 38. Set-up used to evaluate the stability of tyramine HCl **89**, TFA **85** and *p*-TSA **91** to a variety of common organic solvents and reactants in a flow system.

2.8.1 Solvent stability

To determine the stability of the ammonium salts of tyramine **85, 89** and **91**, each solvent was pumped through the reactor at 100 μl min^{-1} for 5 min, prior to analysis of the resulting reaction stream by HPLC; all parameters were repeated five times and the average result presented. As Table 12 illustrates, the complexes were found to be stable to all solvents except DMF, whereby the proportion of displaced tyramine **4** ranged from 66 to 82 % depending on the ammonium salt under investigation. An observation that is in-line with reports made by Mascagni and co-workers[37] who found the use of DMF increased the likelihood of random oligomer formation and decreased reaction control (see Section 1.3.2). The above study suggests that the basic nature of DMF induces decomplexation, thus removing the protecting capacity of the crown ether and allowing undesirable reaction of the free amine functionality. Mascagni *et al.*[35] also reported a trend in the complex stability, *p*-TSA > TFA > HCl, and attributed this to an increase in conjugation of the counterions; owing to the fact that the dissociation of electrons over the anion increases, the stability of the counterion is enhanced and so too is the stability of the complex.

This observation is verified in Table 12 with respect to the complex stability in the presence of DMF, whereby 82 % of the HCl salt **89** was lost upon washing with DMF, which decreases to 66 % for the TFA salt **85** and 56 % for the *p*-TSA **91** salt over a 5 min period; showing a direct link between complex stability and counterion conjugation.

	Stability of complex (%)		
Solvent	Tyramine HCl **89**	Tyramine TFA **85**	Tyramine *p*-TSA **91**
Acetone	100	100	100
Methanol	100	100	100
Ethanol	100	100	100
Dichloromethane	100	100	100
Diethyl ether	100	100	100
Hexane	100	100	100
Toluene	100	100	100
Water	100	100	100
Acetonitrile	100	100	100
Tetrahydrofuran	100	100	100
DMF	18	34	44

Table 12. Summary of the stability of tyramine HCl **89**, TFA **85** and *p*-TSA **91** complexes to a variety of common organic solvent systems (n = 5).

2.8.2 Complex stability in the presence of amines

In addition to the use of an organic solvent, many reactions requiring the protection of amine functionalities often involve the presence or use of compounds containing other amine moieties. As previously discussed it was desirable to investigate the use of organic bases for decomplexation; in addition, it was advantageous to identify those organic bases that could potentially be used as deprotonating agents. As a result, it was important to evaluate the stability of the complexes of salts **85**, **89** and **91** to a range of 1°, 2° and 3° amines. Using the aforementioned complexation strategy, the effect of amines (200 µl, 0.12 M, 2.4 x 10^{-2} mmol) on the system was evaluated, followed by decomplexation and analysis of the reactor effluent off-line by HPLC.

Using the data illustrated in Table 13 it is clear that there is an obvious trend of increased stability towards 3° amines with the 1° amines causing the greater degree of decomplexation. This is to be expected as primary amines are far more likely to drive

the equilibrium between the complexed and free amine *cf.* secondary amines, which in turn will be more powerful decomplexing agents than tertiary amines. This general trend of complex stability, 1° < 2° < 3°, does have some obvious exceptions, such as lutidine **125** and Et_3N **42** whereby their steric hinderance forbids the close proximity required to facilitate decomplexation.

	Amine	p*K*b[96]	Stability of complex (%) Tyramine HCl **89**	Tyramine TFA **85**	Tyramine *p*-TSA **91**
3°	Et_3N **42**	11.06	100	100	100
3°	DIEA **24**	10.50	48	54	69
3°	*N*-Methylpiperidine	10.08	51	53	68
3°	TMEDA **126**	6.10	0	0	0
3°	Lutidine **125**	6.75	100	100	100
2°	Diethylamine	11.09	27	48	89
2°	Piperazine	9.82	37	47	57
2°	Piperidine	11.22	40	54	81
2°	Diisopropylamine	11.05	57	61	73
2°	Dimethylamine	10.73	50	58	69
1°	Aniline **136**	4.63	27	58	78
1°	Benzylamine **54**	9.33	50	64	73
1°	2-Phenylethylamine **137**	9.58	39	61	53
1°	3-Phenylpropylamine **138**	9.68	49	57	63

Table 13. Summary of the stability of tyramine HCl **89**, TFA **85** and *p*-TSA **91** to a variety of common amines (n = 5) in MeOH.

As a result, these bases may be useful as reagents for future reactions such as the deprotonation of the phenolic moiety in the model system described herein. Interestingly, *N,N,N′,N′*-tetramethylethylenediamine (TMEDA) **126** was found to afford 100 % decomplexation for all three salts **85**, **89** and **91**; an observation that can be

attributed to the presence of two amine groups, which potentially doubles any interaction with the complex. This observation compares favourably with reports by Hyde *et al.*[37] who found DIEA **24** to initiate decomplexation. In comparison to TMEDA **126**, DIEA **24** afforded only 31-52 % decomplexation depending on the salt employed (Entry 2, Table 13); consequently, the advantages of TMEDA **126** as a reagent for decomplexation are discussed in Section 2.9.

Table 13 also shows the previously reported variation in the stability of the salts is still evident, demonstrated by the increase in decomplexation exhibited for the HCl salt **89** by many amines *cf*. the *p*-TSA salt **91**. Given the evidence that the HCl salt **89** is the least stable of the three salts investigated, all subsequent investigations were carried out using tyramine HCl **89**. Consequently, if stability proved to be an issue with any particular reaction condition evaluated, the more stable TFA or *p*-TSA complexes could be investigated, enabling a more generic methodology to be developed.

2.8.3 Stability to common reactants and by-products

Further to investigating the stability of the complex to possible bases (Section 2.8.2) and solvents (Section 2.8.1) it is important to consider other reagents common to synthetic reactions requiring the protection of amine groups. Table 14 shows the stability of the tyramine HCl **89** complexed with SILICA AM-18-c-6 **123** when exposed to a plethora of possible reagents such as those used for peptide couplings and the derivatisation of the phenolic moiety reported herein. From herein, reactants were introduced to the reactor as a 200 μl plug (0.12 M, 2.4 x 10^{-2} mmol) at a flow rate of 100 μl min^{-1}. Results are determined from the recovery of tyramine after decomplexation with methanolic KCl (200 μl, 0.2 M, 0.06 mmol). Common reagents for acylation (acetyl chloride **34**, acetic anhydride **127**) and alkylation (methyl iodide **32**) had no effect on the stability of the

complex with no decomplexation of tyramine observed, however reagents employed in coupling reactions such as DMAP **124**, DCC **10** and EDCI **128**, were all found to cause varying degrees of decomplexation. This is an observation which would go some way towards explaining the poor reaction control reported by Mascagni[36,37] (Section 1.3.2) if the reagents were causing decomplexation, thus exposing the amine functionality to undesired reactions and is attributed to their basic nature (see Table 13).

Entries 1-9 (Table 14) were investigated for the purpose of gaining further understanding into the decomplexation process as well as identifying possible reagents for use in future reactions; all solutions were saturated in MeOH. Of the three metal salts investigated, lithium has the least affinity for the 18-c-6 ether due to the small size of its cation, followed by sodium (see Section 1.3). In comparison to the metal alkoxides, carbonates are the weaker inorganic bases, Li_2CO_3 **109**, Na_2CO_3 **129**, K_2CO_3 **130**, due to the relatively poor dissociation exhibited, as a result they provide a lower concentration of metal ions available to decomplex the ammonium ion, hence an increase in complex stability is observed. This was not the case with any of the sodium analogues, which can be accounted for by the increased affinity of the 18-c-6 ether for the sodium ion.

Sodium and lithium hydroxide both proved to be suitable decomplexing agents, causing complete instability, however as previously discussed inorganic hydroxides are unsuitable with respect to the model reaction reported herein, due to their tendency to hydrolyse the acetate moiety, affording recovery of the starting material **4**. It is this propensity that caused the strong inorganic base potassium hydroxide to cleave the AM-18-c-6 **112** from the silica support at the amide linkage, proving to be the only incompatible reagent evaluated thus far.

	Compound	Stability of Complex (%) Reactor 1	Reactor 2
Cation/Anion	KOH **95** [b]	0 [a]	0 [a]
Cation/Anion	NaOH **11** [b]	0	0
Cation/Anion	LiOH **94** [b]	0	0
Cation/Anion	KCl **98** [b]	0	0
Cation/Anion	NaCl **96** [b]	0	0
Cation/Anion	LiCl **97** [b]	0	0
Cation/Anion	K_2CO_3 **130** [b]	48	44
Cation/Anion	Na_2CO_3 **129** [b]	0	0
Cation/Anion	Li_2CO_3 **109** [b]	50	49
Reactant	Acetyl chloride **34** [c]	100	100
Reactant	Acetic anhydride **127**	100	100
Reactant	DMAP **124**	47	48
Reactant	DCC **10**	46	44
Reactant	EDCI **128**	51	49
Reactant	Methyl iodide **32** [c]	0	0
Acid	TFA **83**	100	100
Acid	HCl **131**	100	100
Acid	Acetic acid **121**	100	100
Acid	Sulfuric acid **106**	100	100

[a] Cleaved the crown ether moiety from the support, [b] saturated solution in MeOH, and [c] in THF

Table 14. Stability of tyramine HCl **89** complexed by SILICA AM-18-c-6 **123** when exposed to a plethora of common reagents.

This observation was confirmed by ICP-MS analysis of an aliquot of SILICA AM-18-c-6 **123** treated with KOH **95** and a portion that had not been subjected to the inorganic base, both treated with $KMnO_4$ **110**. As expected, the KOH treated support returned a negative result for the presence of potassium, confirming that the crown ether was no longer present on the solid support.

In addition, acid catalysed reactions are also common in organic synthesis and thus a range of acids which are known to remove inorganic ions from crown ether cavities were tested for their effects on the stability of the ammonium ion complexation. Trifluroacetic acid (TFA) **83**, hydrochloric acid (HCl) **131**, acetic acid **121** and sulfuric acid (H_2SO_4) **106** were found to have no effect on the complex stability and therefore would be suitable for use in reactions within the flow reactor.

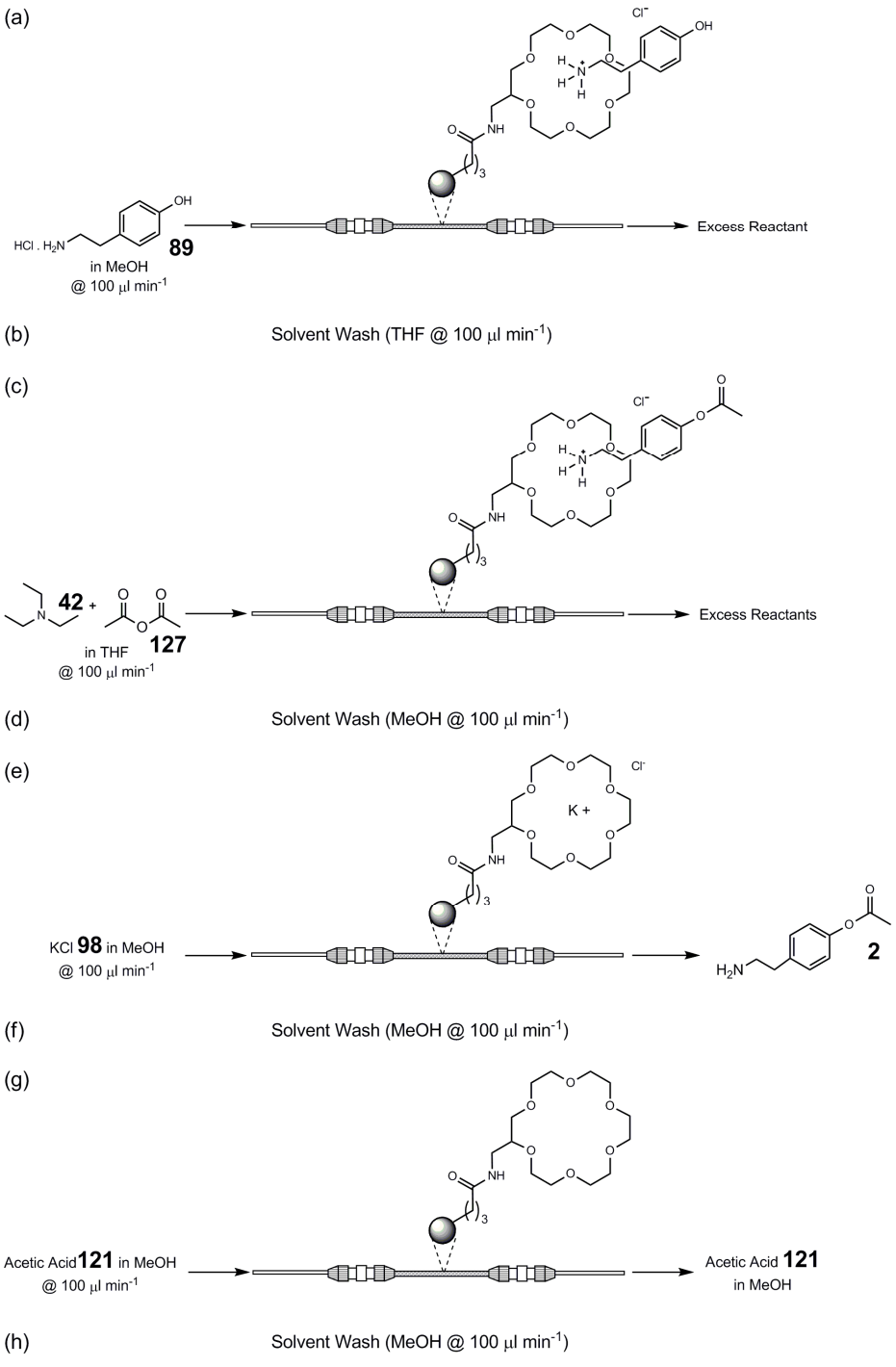
2.9 The acetylation of tyramine

Based on the successful development of a supported crown ether **123** capable of sequestering the ammonium cation under continuous flow, along with those observations made during the base, solvent and reagent studies detailed above, the next step of the investigation was to evaluate reaction of the phenolic moiety (Scheme 39). As it is not possible to perform acylation reactions in a methanolic solvent stream, due to the tendency of the solvent to react with the acetylating agent to afford the respective methyl ester, THF was chosen as the solvent for the reaction step based on the previously observed complex stability (Table 12). The solubility of other reagents in the reaction sequence however necessitated the use of MeOH for stages such as complexation of tyramine HCl **89** (Scheme 39(a)) and decomplexation with KCl **98** (Scheme 39(e)).

Previously reported batch acetylations were carried out using either Et_3N **42** and acetyl chloride **34** or NaH **36** and acetyl chloride **34**[56] with the latter method being preferable in batch reactions as the only by-products formed are NaCl and H_2. Within the flow reactor, the use of acetic anhydride **127** was found to be the preferred method of acetylation due to the reaction process being carried out in one step (using a pre mixed

solution of acetic anhydride **127** and Et_3N **42**), rather than the two steps that would be required for an approach such as the NaH **36** and acetyl chloride **34** method. Batch studies showed this to be a feasible synthetic approach and under flow conditions the removal of acetic acid **121**, which was an issue in batch synthesis, from the reaction product is addressed readily, as it is simply washed away to waste (Scheme 39(d)) prior to decomplexation of the desired product **2**. It was also desired to move away from employing inorganic reagents due to their tendency to initiate decomplexation.

Once the tyramine HCl **89** solution (200 μl, 0.12 M, 2.4 x 10^{-2} mmol) had passed though the flow reactor at 100 μl min^{-1}, as illustrated in Scheme 39(b), the system was purged with THF (1000 μl direct from the syringe at 100 μl min^{-1}) in an attempt to remove all MeOH prior to performing the acetylation reaction. The reaction step depicted in Scheme 39(c) employed a 200 μl plug (0.12 M, (200 μl, 0.12 M, 2.4 x 10^{-2} mmol in THF) of both acetic anhydride **127** and Et_3N **42** which passed through the flow reactor at 100 μl min^{-1}, facilitating the deprotonation of the phenolic moiety and acetylation in one step. Et_3N **42** was employed for deprotonation as solution studies proved it a suitable base for acetylation reactions and as previously reported it does not affect complex stability (Table 13).

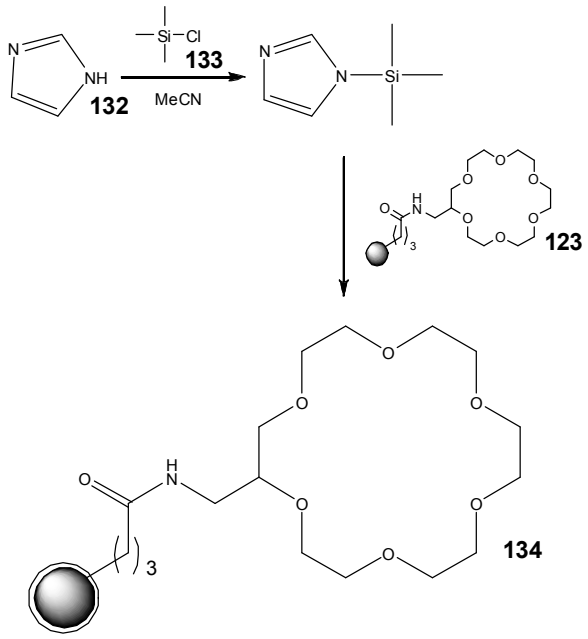


Scheme 39. The complete reaction sequence employed for the acetylation of tyramine in a flow reactor.

Once all reagents had passed through the flow system the solvent stream was purged with MeOH (Scheme 39(d)) before being decomplexed with methanolic KCl **98** (200 μl, 0.12 M, 2.4 x 10^{-2} mmol) at 100 μl min^{-1} (Scheme 39(e)). As previously reported, the low solubility of KCl **98** in other solvents, such as THF, necessitated the use of MeOH as the decomplexation solvent in order to prevent precipitation of KCl **98** within the flow reactor and the build-up of back-pressure.

Unfortunately, upon analysis of the decomplexation products only tyramine **4** (2.4 x 10^{-2} mmol) was detected by HPLC analysis with no trace of the desired product tyramine acetate **2**. A range of flow rates (10-200 μl min^{-1}) were investigated for the reaction step in order to vary the reaction time employed, the only step of the process that had not been optimised to date; unfortunately, all flow rates failed to produce any of the desired product **2**. From these observations, it was postulated that the THF purge was not efficient enough, so various combinations of both flow rate and solvent volume were investigated to ensure all MeOH was removed from the packed reactor bed, thus ensuring the acetylation reagents were not quenched prior to contacting the complexed tyramine salt **89**. This approach only produced further negative results, as did attempts to promote the reaction through the employment of a stopped flow technique, whereby quantitative recovery of tyramine **4** was again obtained. The only remaining possible explanation for reaction failure was reagent interaction with the support material, as both Mascagni[35,36] and the work discussed in Section 2.4.1, illustrated analogous reactions to be possible in the solution phase.

Given the large surface to volume ratio obtained in flow reactors combined with the interaction of unreacted silanol groups on the support with the low quantities of reagents being used, it was proposed that the support was quenching or adsorbing the reagents. Consequently, the SILICA AM-18-c-6 **123** was treated with imidazole **132** and chlorotrimethylsilane **133** in MeCN to afford trimethysilane (TMS) coated SILICA AM-18-c-6 **134** (Scheme 40), rendering the support hydrophobic.[97]

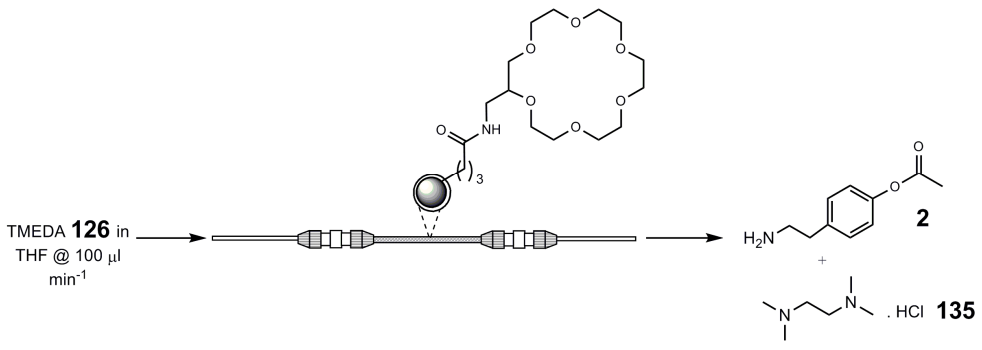


Scheme 40. Schematic illustrating the protocol employed to afford TMS SILICA AM-18-c-6 **134**.

The TMS modified material **134**, was subsequently filtered, washed with acetone (50 ml) and dried in the oven (60 °C) before being packed into the flow reactor (0.15 g, 2.4 x 10^{-2} mmol). The complexation/reaction/decomplexation process detailed in Scheme 39 (steps (a) to (h)), was repeated and analysis of the reaction mixture *via* HPLC indicated 42 % conversion to tyramine acetate **2**, quantified *via* internal standardisation with biphenyl.

Despite an excess of reactants being subsequently passed through the system and successful acetylation of a portion of the available phenolic moieties, it was apparent that the reaction was still not going to completion. Given previous successes with solution phase reactions reported by Mascagni and co-workers,[36,37] coupled with the increased control and surface to volume ratio afforded in the continuous flow reactor, a logical conclusion was that some MeOH was being retained by the silica and as such all efforts to remove the solvent from the process were investigated.

As illustrated in Section 2.8.3, in addition to quantitative decomplexation afforded by the organic base TMEDA **126**, its increased solubility in THF *cf.* the inorganic counterparts evaluated in Table 14, meant it provided an alternative means of decomplexation that was free of MeOH. Furthermore, by exchanging the decomplexation illustrated in Scheme 39(e) with that illustrated in Scheme 41, also made the steps (f), (g) and (h) redundant as washing with TMEDA **126** not only relinquished the desired tyramine acetate **2**, but also left the crown ether cavity free and available for subsequent complexation steps.

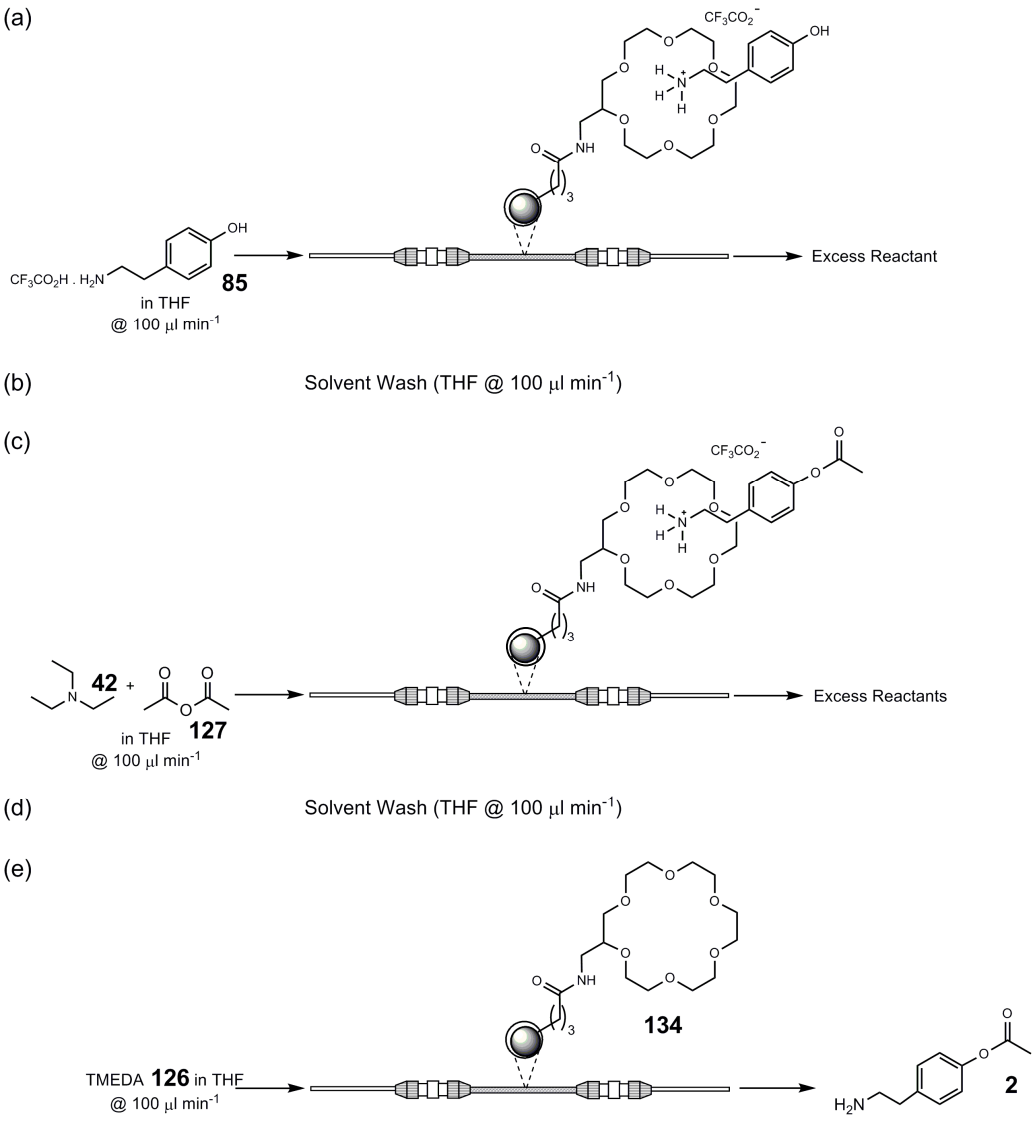


Scheme 41. Schematic illustrating the use of TMEDA **126** as a decomplexing agent with in a flow reactor

In comparison to the previous methodology which required acid washing to regenerate the cavity. As with the KCl **98** decomplexation (Scheme 39(e)), the free amine is also released and the resulting *N,N,N',N'*-tetramethylethylenediamine hydrochloride **135** salt is readily removed *via* an off-line aqueous extraction, to afford the desired product **2** (Scheme 42).

Reproducibility of the new decomplexation step was investigated, prior to performing the reaction, using the optimised complexation of tyramine HCl **89**, THF purge followed by HPLC analysis of the decomplexation solution; the flow system was then purged with MeOH before re-complexation and decomplexation. This was repeated five times with a % RSD = 4.4. Using the modified methodology reported above, conversion of tyramine HCl **89** to the desired acetate **2** increased to 63 %, with 37 % unreacted tyramine **4** recovered.

At this stage, the only place MeOH was introduced into the reaction system was during the complexation step, which was a necessity due to the insolubility of tyramine HCl **89** in other organic solvents. However, tyramine TFA **91** was found to be extremely soluble in THF, so step (a) in Scheme 39 was replaced with complexation step shown in Scheme 42(a).



Scheme 42. Synthetic pathway for the acetylation of tyramine TFA **85** to tyramine acetate **2** within a flow reactor packed with TMS SILICA AM-18-c-6 **134**.

Complexation reproducibility was investigated using the same principles already reported for studies of this nature, again using HPLC analysis, confirming a % RSD of 3.7. Using the refined reaction protocol detailed in Scheme 42, complete removal of MeOH from the flow system enabled 100 % conversion of the complexed amine **91** to the tyramine acetate **2**, quantified by internal standardisation, affording a throughput of

2.4 x 10^{-2} mmol (4.3 mg) per reaction. This observation confirmed that the incomplete reaction observed previously was due to the retention of MeOH within the packed, which resulted in quenching of the reactant stream, preventing the desired acetylation from taking place.

2.10 Generality of complexation

Recognising the requirement of the methodology to protect more complex compounds, other than salts of tyramine **85**, **89** and **91**, a general study was undertaken in order to investigate the effect of factors such as chain length and steric hinderance, as well as looking at the effect of counterions. While issues with MeOH as a solvent were experienced for the acetylation of tyramine **4**, it was employed as the solvent for this study due to the varying degrees of solubility exhibited by the compounds under evaluation in other organic solvents and as such, decomplexation was again performed using methanolic KCl **98**. It was not envisaged that issues with solubility will affect future reactions, it is just necessary to select a solvent system that is compatible with all reagents, as with traditional batch-wise chemistry. As previously reported in Table 12, the only solvent that seems to be incompatible for this non-covalent protection methodology is DMF.

Complexation was again achieved by injecting a plug of the amine salt (200 μl, 0.12 M, 2.4 x 10^{-2} mmol) into a continuous MeOH solvent stream (100 μl min^{-1}), using a Rheodyne injection valve, as per the previous procedure. The use of the continuous solvent stream ensured the complete washing of the resin before a plug of methanolic KCl **98** (200 μl, 0.12 M, 2.4 x 10^{-2} mmol) was introduced into the solvent stream (100 μl min^{-1}). The resulting reaction products were collected and analysed off-line by HPLC in order to quantify the proportion of amine released, using an internal standard.

Effect	Amine	Complexation (%)[a] Reactor 1	Reactor 2
Cation	Tyramine *p*-TSA **91**	100	100
Cation	Tyramine TFA **85**	100	100
Cation	Tyramine HCl **89**	100	100
No Cation	Tyramine **4**	0	0
Free amine	Aniline **136**	0	0
Free amine	Benzylamine **54**	0	0
Free amine	2-Phenylethylamine **137**	0	0
Free amine	3-Phenylpropylamine **138**	0	0
Chain Length	Aniline HCl **139**	100	100
Chain Length	Benzylamine HCl **140**	100	100
Chain Length	Phenylethylamine HCl **141**	100	100
Chain Length	Phenylpropylamine HCl **142**	100	100
Chain Length	4-Aminophenol HCl **143**	100	100
Steric Hinderance	L-β-Alanine benzyl ester HCl **144**	0	0
Steric Hinderance	(S)-(-)-2-Amino-3-phenyl-1 -propanol HCl **145**	0	0
Functionality	Benzamide HCl **146**	0	0

[a] Based on the recovery of amine after decomplexation *via* HPLC analysis.

Table 15. Summary of the complexation ability of a variety of amine and their salts.

Initial results summarised in Table 15, confirmed the observations made when studying tyramine salts were general, for example free amines (tyramine **4**, aniline **136**, benzylamine **54**, 2-phenylethylamine **137**, and 3-phenylpropylamine **138**) did not complex. In keeping with previous findings the HCl **89**, TFA **85** and *p*-TSA **91** salts of tyramine complexed well, as did the respective HCl salts **139**-**143** of the other amines studied of varying chain lengths. Given the propensity for all three types of salt to complex and due to their commercial availability, other amines were investigated as their HCl salts. The issue of steric effects on complexation was also addressed with the

exploration of L-β-alanine benzyl ester hydrochloride **144** and (*S*)-(-)-2-amino-3-phenyl-1-propanol hydrochloride **145**, in both cases no complexation was observed.

Benzamide HCl **146** also failed to complex when passed through the flow system due to the resonance effects experienced by the carbonyl group, thus resulting in an unstable complex with the crown ether. In contrast, investigations employing 4-aminophenol HCl **143** and aniline HCl **139** showed that the amine moiety does not need to be an alkyl derivative, with aromatic amines also complexing well.

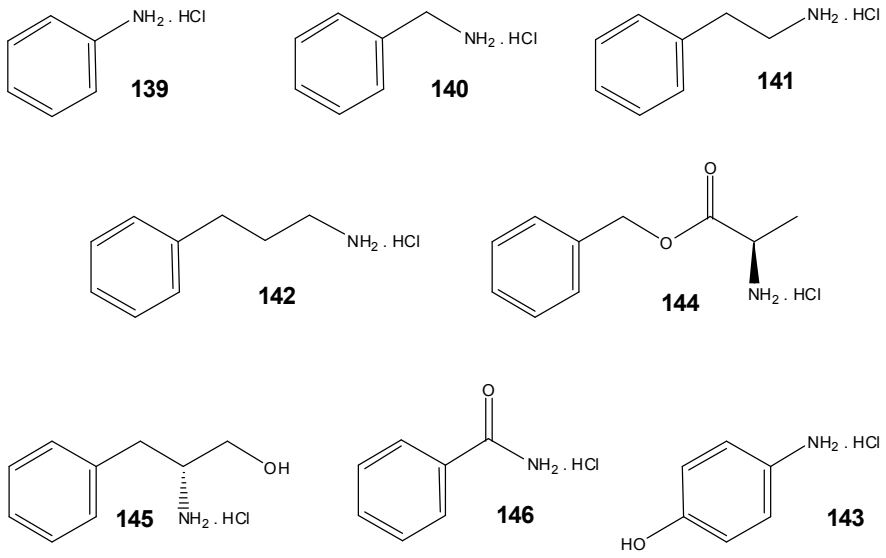
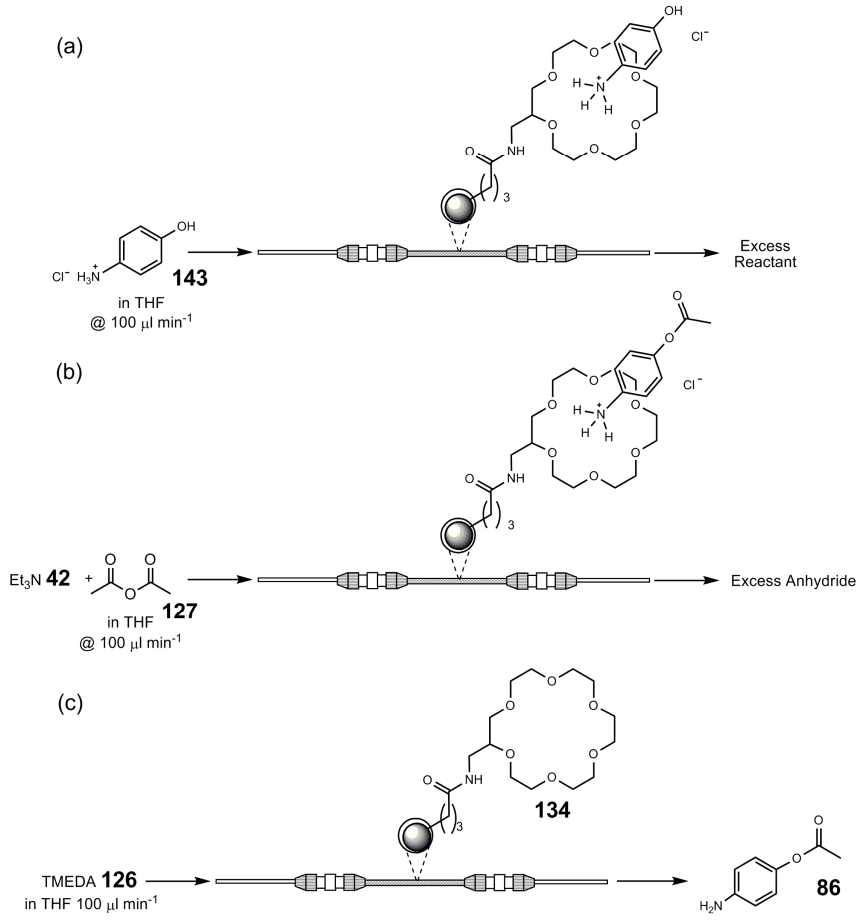


Figure 11. A selection of the amine salts evaluated using SILICA AM-18-c-6 **134**.

2.11 Further reactions

Having demonstrated the ablilty to complex bi-functional compounds other than tyramine **4** (Table 15), the observed complexation of 4-aminophenol HCl **143** with TMS SILICA AM-18-c-6 **134** prompted an investigation into the acetylation of 4-aminophenol **147** under continuous flow. The previously discussed issues regarding MeOH as a solvent system were again found to be problematic because when a methanolic solution of 4-

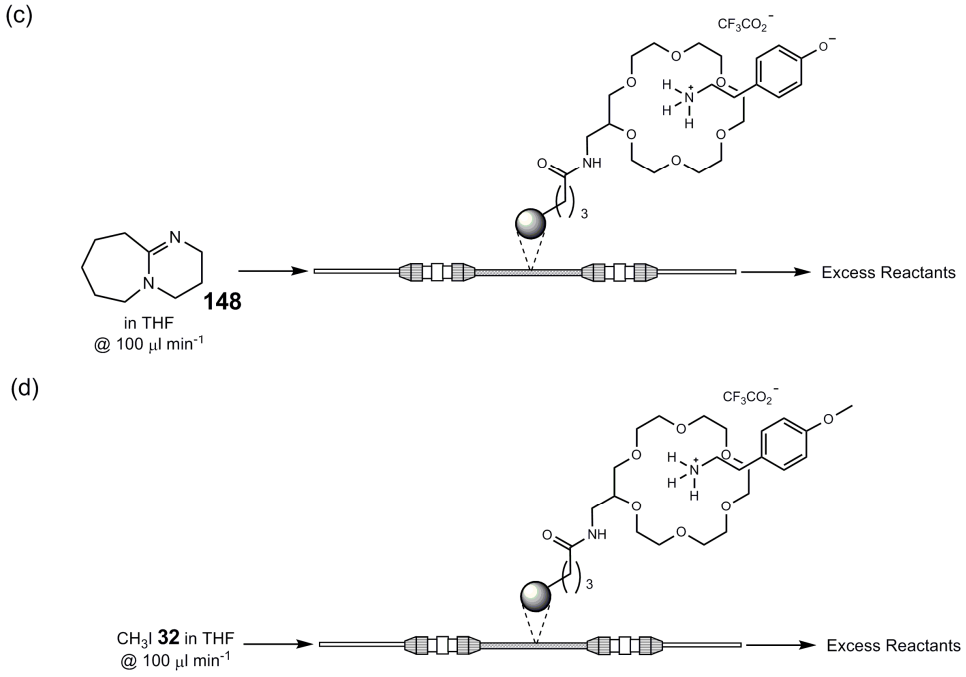
aminophenol HCl **143** (200 μl, 0.12 M, 2.4 x 10^{-2} mmol) was employed, followed by purging of the system with THF, subsequent acetylation (Et_3N **42**/Ac_2O **127**) afforded only 84 % conversion to 4-aminophenyl acetate **86**, with 16 % 4-aminophenol **147** recovered.



Scheme 43. Schematic of the synthetic pathway used for the continuous synthesis of 4-aminophenyl acetate **86**.

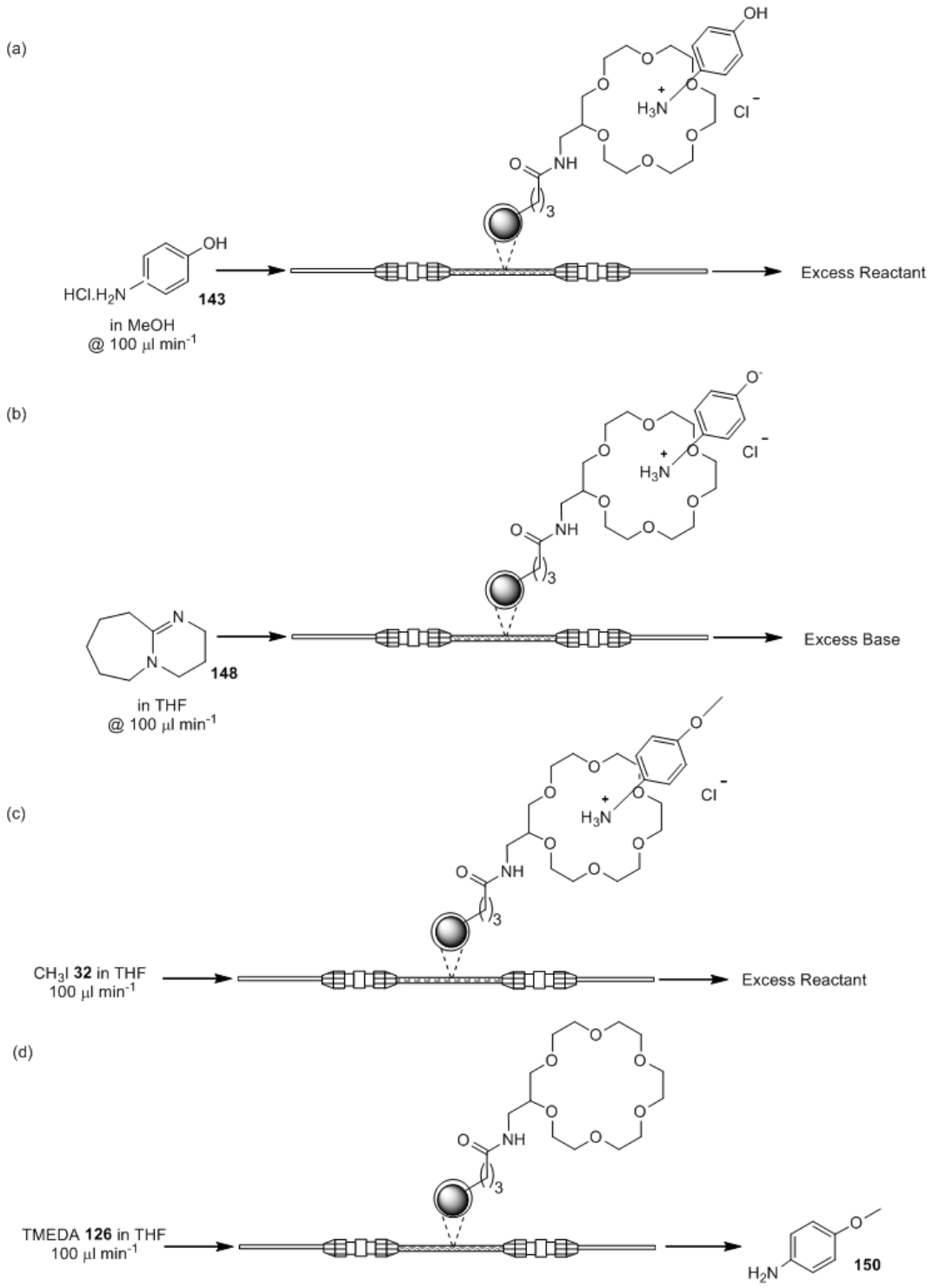
As detailed in Scheme 43, this was improved by replacing MeOH/THF with THF as the sole solvent and initiating decomplexation with TMEDA **126**, as in Section 2.9. It was not necessary in this case to alter the counterion employed, due to solubility of 4-aminophenol HCl **143** in THF. Using this approach, quantitative conversion to 4-aminophenyl acetate **86** was obtained and could be readily isolated after an off-line aqueous extraction, affording a throughput of 2.4 x 10^{-2} mmol reaction^{-1} (Scheme 43).

Having acetylated several compounds, investigation into extending the scope of the methodology began with the methylation of tyramine TFA **91** (Scheme 44). A 200 µl plug (0.12 M, 2.4 x 10^{-2} mmol) of the TFA salt **91** in THF was introduced into a continuous THF stream (100 µl min^{-1}) *via* a Rheodyne valve. To ensure the amine salt had passed through the system, before introducing further reagents, THF was pumped through the system for 2 min. The first of the reaction steps employed a 200 µl plug of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) **148** (0.12 M, 2.4 x 10^{-2} mmol) through the system, followed by methyl iodide **32** (200 µl, 0.12 M, 2.4 x 10^{-2} mmol). As these two reagents have the potential to react together, the reaction step could no longer be carried out in as a single step using a pre-mixed solution, thus the reaction step (c) (Scheme 39) was replaced by reaction steps (c) and (d) in Scheme 44 facilitating the deprotonation of the phenolic moiety and methylation in consecutive steps. Decomplexation was again initiated *via* a 200 µl plug of TMEDA **126** (0.12 M, 2.4 x 10^{-2} mmol) at 100 µl min^{-1}, affording 2-(4-methoxyphenyl)ethylamine **149** in quantitative conversion at a throughput of 2.4 x 10^{-2} mmol reaction^{-1}.



Scheme 44. Schematic illustrating the consecutive reaction steps required for the synthesis of 2-(4-methoxyphenyl)ethylamine **149**.

The generality of the alkylation methodology was subsequently evaluated to conduct the methylation of 4-aminophenol HCl **143** to selectively afford 4-methoxyphenylamine (*p*-anisidine) **150** (Scheme 45). Using analogous conditions to those reported for the alkylation of tyramine TFA **91**, the methylation[98] of 4-aminophenol HCl **143** was investigated under continuous flow, affording 100 % conversion of 4-aminophenol HCl **143** to *p*-anisidine **150**, with no *N*-alkylation or dialkylation observed.



Scheme 45. Schematic of the synthetic pathway used for the continuous synthesis of 4-methoxyphenylamine **150**.

In addition to the observed generality of the protecting group strategy, the stability of the TMS SILICA AM 18-c-6 **134** was evaluated by performing the methylation of 4-aminophenol HCl **143** under continuous flow, a full 12 months after the initial evaluation was conducted. It was pleasing to observe that with no special treatment of the material, simply washing the SILICA AM 18-c-6 **134** with solvent prior to storing it in the borosilicate glass flow reactor that analogous results could be obtained with ease.

2.12 Summary

Having investigated a range of immobilisation strategies and functionalised crown ethers, it was found that aminomethyl-18-c-6 **112** covalently bound to 3-carboxypropyl functionalised silica gel **122**, to afford immobilised crown ether **134** (0.16 to 0.21 mmol g^{-1}), offered the best combination of properties with respect to ammonium ion complexation and compatibility with a flow system. Using the aforementioned material **134**, the complexation of a plethora of ammonium salts were investigated (Table 15) in order to determine the methodologies scope. With this in mind, the only limiting factor was found to be sterically hindered molecules, such as L-β-alanine benzyl ester HCl **144** and alcohol **145**, whereby no complexation was observed. In addition, the stability of the ammonium complexes was evaluated in the presence of various common organic solvents (Table 12), along with a series of organic and inorganic reagents (Tables 13 and 14). The overall trend obtained illustrated that the complexes, in general, were not stable to amines, with DMF causing destabilisation of all complexes regardless of the counterion employed; however the system demonstrated excellent stability to a wide range of common reactants. As an extension, the acylation and alkylation of a series of bifunctional compounds, tyramine **4** and 4-aminophenol **143**, was investigated and found to afford the selective synthesis of tyramine acetate **2**, 4-aminophenyl acetate **86**,

2-(4-methoxyphenyl)ethylamine **149** and 4-methoxyphenylamine **150** in quantitative conversion. In summary, the work described herein presents a broad investigation into the viability of immobilised crown ethers as a replacement for traditional covalent protecting group chemistry and combines their use with continuous flow technology to afford a technique with potential for future development into an automated system.

Chapter 3: Conclusions and Future Work

3.0 Conclusions and Future work

3.1 Immobilisation of crown ethers

As outlined in Chapter 1, the use of protecting groups has been widely adopted by the modern day synthetic chemist as a means of enabling selective reactions to be performed. As such, for each functional group present within the molecule that is not the desired reaction site, a protecting group is required thus increasing the number of reaction steps to include the introduction, and subsequent removal, of each protecting group. In addition further complications include the potential need to remove the groups in a specific sequence and hence orthogonal groups are required along with the need to select protecting groups that are tolerant to the reaction conditions and subsequent work-up procedures employed.

Owing to the complex nature of conventional covalent protecting group chemistry, it was the aim of this work to devise a non-covalent protecting group strategy which would enable the facile *N*-protection of bifunctional compounds, thus reducing the time taken to *N*-protect and deprotect the amine moiety of the precursor. Consequently, the work discussed herein focused on the *N*-protection of compounds containing amine and phenolic functionalities, *via* the complexation of the ammonium salt of the amine with an immobilised 18-crown-6-ether. In addition, it was the aim of this work to develop a strategy that would be able to be transferred into a continuous flow system in order to provide further time and cost savings to the methodology. Also the higher surface to volume ratio should enable the process to be more efficient.

Initial investigations were performed using the commercially available immobilised di-*t*-butylcyclohexano 18-crown-6 ether **102** and although the material was shown to

complex tyramine HCl **89** and decomplex to afford the free amine, tyramine **4**, the supported crown ether **102** was not recyclable, due to leaching of the crown ether from the support and consequently no further investigations were performed using this material. Having identified a need to covalently immobilise the crown ether functionality, a variety of immobilisation techniques were investigated, employing several derivatised 18-crown-6-ethers as precursors (Table 10).

As reported in Section 2.4.2, the first immobilisation strategy evaluated involved the nitration of DB-18-c-6 **14** and subsequent reduction to afford DADB-18-c-6 **163**, which enabled covalent immobilisation to commercially available Merrifield peptide resin (MPR) **13**, as illustrated in Figure 32. Analysis of the resulting material **102** by elemental analysis confirmed the crown ether loading of the material to be 0.12 mmol g^{-1} and the materials ability to sequester ammonium ions was subsequently evaluated. Unfortunately, the material was unable to complex the ammonium salt **89**; an observation that was attributed to increased crown ether strain as a result of immobilisation *via* the aromatic ring.

In order to reduce the proposed distortion of the crown ether and promote hydrogen bonding between the complex and crown ether, the next strategy involved the immobilisation of CAB-18-c-6 **113** onto aminomethyl polystyrene **116**, as illustrated in Figure 33. This strategy afforded a loading of 0.98 mmol g^{-1}, as determined by ICP MS analysis, however, subsequent attempts to complex tyramine HCl **89** again proved unsuccessful. In a final attempt to reduce cavity strain, attachment of AM-18-c-6 **112** to carboxypolystyrene **118** (Scheme 34) provided a supported crown ether with a loading of 0.37 mmol g^{-1} and most importantly, a reagent with the ability to complex tyramine

HCl **89**. Unfortunately, however upon repeated complexation of the supported AM-18-c-6 **120** with tyramine HCl **89**, inconsistencies in the proportion of tyramine **4** recovered (11.4 % RSD) were observed and the losses attributed to inefficient recovery of the material **120** during the washing and filtration stages of this batch procedure.

3.2 Incorporation of immobilised crown ethers into a flow reactor

In order to remove the need for filtration steps, and hence reduce the loss of immobilised AM-18-c-6, incorporation of the immobilised crown ether **120** into a flow reactor was investigated. As Section 2.4.1 illustrates, using this approach the reproducibility of the complexation and decomplexation strategy was improved, leading to a reduction in the RSD from 11.4 % (in batch) to 4.3 % (in flow). Having demonstrated the preparation of an immobilised crown ether **120** that was capable of sequestering ammonium ions, under both stirred and flow conditions, the next step of the investigation was to evaluate the stability of the material to the reaction the phenolic moiety. As initial investigations had been performed in MeOH, a change of reaction solvent was necessary in order to investigate the acetylation of complexed tyramine under continuous flow.

Unfortunately, the solid support was observed to swell in the presence of alternative organic solvents in which the reaction could be conducted, leading to the build-up of pressure within the system, meaning that the reaction could not be evaluated under continuous flow. This observation was attributed to the low cross-linking of the polymer support and with no higher cross-linked carboxypolystyrene commercially available, the attachment of AM-18-c-6 **112** onto 3-carboxypropyl functionalised silica gel **122** was investigated in order to remove the issue of solvent incompatibility.

As Scheme 37 illustrates, two immobilisation techniques were evaluated, the first employed an *in-situ* generated acid chloride and the second approach was the previously demonstrated carbodiimide coupling reaction. While both methods produced immobilised crown ethers **123** that were found to be effective at sequestering the ammonium salts, the carbodiimide approach afforded the higher loading (0.21 mmol g^{-1}) compared to acid chloride approach (0.16 mmol g^{-1}). Compared to immobilised AM-18-c-6 **120**, SILICA AM-18-c-6 **123** was found to be stable to all common organic solvents, exhibiting no swelling and enabling continued operation without the build-up of pressure.

3.3 Stability studies

Having developed a material **123** suitable for incorporation into a flow reactor, the stability of the various tyramine salt **89**, **85** and **91** complexes were evaluated in a range of common organic solvents. As Table 12 illustrates, with the exception of DMF the complex was found to be stable in common organic solvents, with the instability attributed to the basic nature of DMF. This induction of decomplexation, thus removes the protecting capacity of the crown ether and allows potential undesirable reaction of the free amine functionality, as such the solvent was not used in further investigations. The varying stability of the three tyramine salts investigated was found to be *p*-TSA > TFA > HCl based on the instability when treated with DMF whereby 82 % of the HCl salt **89** was lost upon washing with DMF, which decreased to 66 % for the TFA salt **85** and 56 % for the *p*-TSA **91** salt. In addition to the solvent study, the complex stability was also evaluated in the presence of a plethora of amines and as Table 13 illustrates, an obvious trend was observed with 1^{o} amines causing the greater degree of decomplexation. This general trend of complex stability, $1^{o} < 2^{o} < 3^{o}$, does have some obvious exceptions, such as lutidene **125** and triethylamine **42** whereby steric

hinderance forbids the close proximity required to facilitate decomplexation. Again, these results highlighted the variation in the stability of the salts discussed above showing an increase in decomplexation for the HCl salt **82** *cf*. the *p*-TSA salt **91**. In addition, the effect of a series of common reagents was evaluated, with the complexes proving to be stable to acetyl chloride **34**, acetic anhydride **127** and methyl iodide **32**, however reagents employed in coupling reactions such as DMAP **124**, DCC **10** and EDCI **128**, were all found to cause varying degrees of decomplexation, which is again attributed to their basic nature. Metal alkoxides and carbonates were investigated for the purpose of understanding the decomplexation process and to identify possible decomplexation reagents. In comparison to the metal alkoxides, carbonates are the weaker inorganic bases due to the relatively poor dissociation exhibited, as a result they provide a lower concentration of metal ions available to decomplex the ammonium ion, hence an increase in complex stability is observed. This was not observed to be the case with any of the sodium analogues, which can be accounted for by the increased affinity of the 18-c-6 ether for the sodium ion.

3.4 Complexation generality

Recognising the need to consider starting materials other than tyramine **4**, a variety of amines salts were subsequently investigated for their ability to complex. Factors such as chain length and steric hinderance, as well as the effect of counterions were considered. Results summarised in Table 15 confirmed the observations made when studying tyramine salts were general, for example free amines (tyramine **4**, aniline **136**, benzylamine **54**, 2-phenylethylamine **137**, and 3-phenylpropylamine **138**) did not complex. In keeping with previous findings the HCl **89**, TFA **85** and *p*-TSA **91** salts of tyramine complexed well and due to their commercial availability, additional amines

were investigated as their HCl salts. With this in mind, 4-aminophenol HCl **143** and aniline HCl **139** showed that the amine moiety does not need to be an alkyl derivative, with aromatic amines also complexing well. The issue of steric effects on complexation was also addressed with the exploration of L-β-alanine benzyl ester hydrochloride **144** and (*S*)-(-)-2-amino-3-phenyl-1-propanol hydrochloride **143**, in both cases no complexation was detected.

3.5 Reactions evaluated

Having determined the scope and limitations of the non-covalent protecting group strategy, the final step was to evaluate the reaction of the phenolic moiety. The first reaction under investigation was the synthesis of tyramine acetate **2** and employed a pre-mixed solution of acetic anhydride **127** and Et_3N **42**, enabling the deprotonation and subsequent reaction to be incorporated into a single step (Scheme 39).

Unfortunately, upon analysis of the decomplexation products only tyramine **4** was detected by HPLC analysis with no trace of the desired product tyramine acetate **2**, a situation that did not improve by a thorough evaluation of reaction times (10-200 μl min^{-1}) and solvent systems. The only remaining explanation for the lack of reaction was reagent interaction with the support material, as the reaction has been shown to be possible in the solution phase. To address this, the SILICA AM-18-c-6 **123** was silylated to afford a hydrophobic barrier as a means of preventing any undesirable interactions observed between the reactants and the silica surface.

Employing the TMS SILICA AM-18-c-6 **134** under the aforementioned conditions, it was pleasing to see 42 % conversion to tyramine acetate **2**, with 58 % unreacted tyramine **4** recovered. Although the reaction remained incomplete, the process demonstrated the

successful *N*-protection, further optimisation was of the reaction step was therefore required.

Having previously removed any hydrophobic interactions, it was postulated that retention of MeOH, by the reagent **134**, may be retarding the reaction. In an effort to remove MeOH from the system, TMEDA **126** was investigated as an alternative decomplexing agent due to its solubility in THF *cf.* KCl. In addition, the use of TMEDA **126** was also advantageous as decomplexation with an organic base left the crown ether cavity free for subsequent complexations, rendering the acid wash (Scheme 39, steps (f)-(h)) unnecessary. At this stage, the conversion to tyramine acetate **2** increased to 63 % however, MeOH was still employed for the complexation step and accounted for the incomplete reaction observed. Due to the insolubility of tyramine HCl **89** in a solvent other than MeOH, an alternative tyramine salt was investigated.

By excluding MeOH from the reaction and employing tyramine TFA **85** in THF (Scheme 42), the target compound **2** was obtained in 100 % conversion (% RSD = 3.7), with no competing *N*-acylation **3** or diacylation **1** (Scheme **24**). Based on the use of 0.15 g of TMS SILICA AM-18-c-6 **134**, this afforded a throughput of 2.4 x 10^{-2} mmol reaction^{-1} which is equivalent to 4.3 mg, with a reaction time of typically 10 min. Under analogous conditions the generality of the technique was evaluated for the acetylation of 4-aminophenol HCl **143** (Scheme 43) where the desired product, 4-aminophenyl acetate **86**, was obtained in quantitative conversion and a throughput of 2.4 x 10^{-2} mmol reaction^{-1}.

Having demonstrated the ability to acylate numerous compounds, investigation into extending the scope of the methodology began with the methylation of tyramine TFA **85**,

using DBU **148** and methyl iodide **32**. As these two reagents have the potential to react together, the deprotonation and reaction step could no longer be carried out as a single step therefore sequential base **148** deprotonation followed by methylation was employed (Scheme 44). Using this approach, the methyl ester of tyramine **149** was obtained in 100 % conversion, with no competing *N*-alkylation observed. The generality of the method was proven by the subsequent methylation of 4-aminophenol HCl **143** to selectively afford 4-methoxyphenylamine (*p*-anisidine) **150**.

In addition to exploring the generality of the complexation and the resulting stability, solvent/reactant compatibility of the complex, it was pleasing to find that a full 12 months after initial evaluation of the immobilised crown ether **134**, the same aliquot of the reagent demonstrated no signs of degradation, enabling the facile quantitative methylation of 4-aminophenol HCl **143** to 4-*p*-anisidine **150**, further demonstrating the synthetic potential of crown ethers as recyclable, non-covalent protecting groups.

In summary, it has been shown that AM-18-c-6 crown ether **112** can be immobilised onto a solid silica support **122** before being treated to achieve a TMS coated silica support **134**. This can then be readily incorporated into a continuous flow reactor to sequest a primary amine salt of a bi-functionalised compound such as tyramine **4**. This effectively affords a non-covalent *N*-protection strategy allowing the desired *O*-reaction to be carried out utilising all of the commonly reported benefits of flow reactor synthesis.

3.6 Future work

Having discussed the initial evaluation of immobilised crown ethers as non-covalent protecting groups, the potential applications for this technique are vast and although the work reported herein limits the examples to those containing an aromatic group, due to the analytical methodology, the technique is suitable for the complexation and reaction of aliphatic ammonium salts such as amino acids and peptides.

Ley *et al*.[99] reported the development of a multi-purpose flow reactor as an automated platform for the rapid synthesis of oxazoles, capable of producing gram quantities of material. It is proposed that by connecting the flow reactor described herein to an automated injector, the efficiency of the process could be increased *cf*. the use of a manual Rheodyne valve as described herein. In addition, interfacing the flow reactor with a HPLC would allow the on-line analysis of material produced, with the further incorporation of a switching valve enabling the collection of the desired reaction product. This process could easily be controlled using current HPLC software, with no further development necessary and would greatly increase the number of reactions that could be evaluated.

In order to ensure uptake of this methodology by the scientific community, it is proposed that further investigations are conducted into increasing the loading of the immobilised crown ether to increase the throughput of the system. This could be achieved by employing an excess of crown ether during immobilisation, in order to approach maximum loading for the given solid support. Alternatively, larger packed-beds could be prepared and the fabrication of monoliths investigated as a means of reducing the back-pressure within such a system.

In addition to increasing the throughput of the system, the technique could also be applied to the preparation of immobilised chiral crown ethers, in an extension to work by Karakaplan and co-workers[100] who reported the ability selectively interact with *L*- and *D*-amino acid derivatives. Using the immobilisation strategy described herein, it is proposed that by incorporating a chiral sub-unit into the crown ether, chiral recognition of the ammonium salt would enable enantioselective reactions to be performed under continuous flow.

Chapter 4: Experimental Details

4.0 Experimental details

4.1 Chemicals and materials

Reagents. Unless otherwise stated, the chemicals listed were used as received. Trifluoroacetic acid **83** (spectrophotometric grade), di-*tert*-butyl dicarbonate **80** (97 %), hydrochloric acid (1.0 M in diethyl ether), acetyl chloride **34** (98 %), tyramine **4** (99 %), tyramine hydrochloride **89** (99 %), sodium hydroxide **11** (98 %), chlorotrimethylsilane **133** (>98 %), biphenyl (>99 %), (*S*)-(-)-2-amino-3-phenyl-1-propanol hydrochloride **145** (98 %), potassium hydroxide **95** (90 %), 4-aminophenol **147** (>98 %), 4-nitrophenol **87** (>99 %), 18-crown-6 ether **16** (99.5%), sodium chloride **96** (>99 %), sodium carbonate **129** (> 99.5 %), dibenzo-18-crown-6 ether **14** (98 %), lithium chloride **97** (99 %), lithium hydroxide **94** (98 %), lithium carbonate **109** (98 %), potassium chloride **98** (>99%), ammonium chloride (99.5 %), potassium carbonate **130** (99 %), potassium permanganate **110** (99 %), aminomethyl polystyrene **116** (1.5 mmol N g^{-1}, 2 % DVB, 200-400 mesh), aminomethyl-18-crown-6-ether **112** (95 %), thionyl chloride **114** (+99%), triethylamine **42** (99.5 %), *p*-toluene sulfonic acid monohydrate **73** (+99 %), imidazole **132** (99 %), diethylamine (99.5 %), Merrifield peptide resin **13** (1.15 mmol Cl^- g^{-1}, 2 % DVB, 200-400 mesh), diisopropylamine (99 %),*N,N*-dicyclohexylcarbodiimide **10** (99 %), 4-dimethylaminopyridine **124** (99 %), *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide **128** (98 %), *N,N*-diisopropylethylamine **24** (99.5 %), polystyrene beads (2 % DVB, 200-400 mesh), 1,8-diazabicyclo[5.4.0]undec-7-ene **148** (98 %), 2-phenylethylamine **137** (99 %), 3-phenylpropylamine **138** (99 %), piperazine (99 %), piperidine (99.5 %), carboxypolystyrene **118** (0.4-0.6 mmol g^{-1}, 1 % DVB, 200-400 mesh), *N*-methylpiperidine (99 %), L-β-alanine benzyl ester hydrochloride **144** (99 %), 2,4-lutidene **125** (99 %), *N,N,N',N'*-tetramethylethylenediamine **126** (99.5 %), benzylamine **54** (99 %), acetic anhydride **127** (99.7 %), 3-carboxypropyl functionalised silica gel **122** (1.60

mmol g^{-1}, 200-400 mesh), *p*-anisidine (99 %) and aniline **136** (99.5 %) were purchased from Sigma-Aldrich (Gillingham, UK). Sulfuric acid **106** (98 %) and acetic acid **121** (Analytical Grade) were obtained from Fisher Scientific (Leicestershire, UK). Dimethylamine (40 % wt. in H_2O), aminomethyl polystyrene **116** (1.50 mmol N g^{-1}, 2 % DVB, 200-400 mesh) and silica gel 60 were purchased from Fluka (Gillingham, UK). Supported di-*t*-butylcyclohexano-18-crown-6-ether **102** was kindly donated by Eichrom Technologies (France). Palladium on Carbon **108** (5 %, 64.28 % H_2O), methyl iodide **32** (99 %) and carboxybenzo-18-crown-6 ether **117** (99 %) were obtained from Precious Metals Corporation (USA), Avocado (Lancashire, UK) and Acros (Leicestershire, UK) respectively. Sodium hydride **36** (60 % wt/wt suspension in mineral oil) was obtained from Sigma-Aldrich and was washed free of mineral oil, in accordance with general procedure 6, prior to use.

Solvents. Reactions were performed using, where available, puriss grade solvents stored over molecular sieves (< 0.005 % H_2O) (Fluka, UK), with the exception of dichloromethane and *tert*-butanol which were of laboratory reagent grade (Fisher Scientific, UK). All chromatography, including HPLC, employed HPLC grade solvents (Fisher Scientific, UK). Purified water (5 $M\Omega\ cm^{-1}$) was prepared by reverse osmosis and ion exchange using a water purifier (Elgast, UK) fitted with an Option 4 cartridge.

Materials. PTFE tubing (1/16" o.d. x 800 μm i.d.), female luers 10-32 (Tefzel), 1/16" unions (Tefzel), o-rings (Viton®), gas-tight syringes (5 ml and 10 ml, Hamilton, UK) and Omnifit connectors employed for the flow reactor system were sourced from Supleco (UK) and Kinesis (UK). Borosilicate glass capillary (3 mm i.d.) (Duran®, UK) was cut into the desired 50 mm lengths and flame polished. Thin layer chromatography plates were sourced from Merck (Kieselgel 60, HF_{254} aluminium backed).

4.2 Chromatographic techniques

Column chromatography was performed using silica gel 60 as the solid support and compounds were eluted using mixtures of either ethyl acetate/hexane or MeOH/DCM of varying polarity. Thin layer chromatography (TLC) was carried out using Kieselgel 60, HF_{254} aluminium backed TLC plates, with the appropriate mobile phase as eluent. Visualisation was achieved upon exposure to short wave ultra violet light (λ 254 nm).

4.3 Instrumentation

Nuclear Magnetic Resonance (NMR) spectra were recorded at room temperature as solutions in either deuterated chloroform ($CDCl_3$) or deuterated MeOH (CD_3OD), using tetramethylsilane (TMS) as an internal standard. All spectra were recorded on a Jeol GX400 spectrometer and the chemical shifts given in parts per million (ppm) with coupling constants in Hertz (Hz). Elemental analyses were performed using a Fisons (UK) Carlo Erba EA1108 analyser, with measurements repeated until concurrent data was obtained, typically n = 2. Matrix-Assisted Laser Desorption Ionisation (MALDI)-Mass Spectrometry was performed using a Bruker Reflex 4 instrument operated in reflector mode. Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) measurements were made at 257.61 nm and 766.49 nm using a Perkin Elmer (UK) Optima 5300DV instrument. Melting points were obtained using a Stuart Scientific SMP10 apparatus and are uncorrected.

High Performance Liquid Chromatography (HPLC) data was obtained using a Jasco (UK) modular system comprising of a LV-1580-03 solvent selector, a DG-1580-53 de-gasser, two PU-1580 pumps, a HG-1580-32 mixer, a UV-1575 detector and an AS-1555 auto sampler. Analytical measurements were made using a Jupiter 10 μm C18 300 Å (250 x 4.60 mm) column purchased from Phenomenex (UK). Gas Chromatography-

Mass Spectrometry (GC-MS) was performed using a Varian (UK) gas chromatograph (CP-3800) coupled to a Varian (UK) mass spectrometer (2000) with a CP-Sil 8 (30 m) column (Phenomenex, UK) and ultra high purity helium (99.999 %) (Energas, UK) carrier gas. Reagents and solutions were delivered to the continuous flow reactors using syringe pumps (Harvard, UK) capable of delivering liquids at flow rates ranging from 0.1 to 1000.0 μl min^{-1} based on a 5 ml gas-tight syringe. Where necessary, aliquots of reactants were introduced into the continuous flow reactor using a Rheodyne injector valve, model 7125 (Supleco, UK).

Hydrogenations were carried out using PARR (UK) hydrogenation apparatus (696EF).

4.4 Instrumental methodology

4.4.1 GC-MS

The following procedure was employed for all samples analysed by GC-MS; injector temperature 200 °C, helium flow rate 1 ml min^{-1}, oven temp 50 °C for 4 min then ramped to 250 °C at 30 ° C min^{-1} and held for 30 min with a 3.5 min filament delay.

4.4.2 HPLC

Using gradient elution at a flow rate of 1.5 ml min^{-1}, the aqueous portion of the mobile phase was decreased from 60 % to 40 % over a period of 7 min and then maintained at 40 % for the remaining 13 min of the method. Both the organic phase (MeOH) and aqueous phases contained 0.1 % TFA in order to improve peak shape. An injection volume of 20 μl was employed and biphenyl was used as an internal standard.

4.4.3 ICP-MS

The dry powdered sample was weighed into a 120 ml PTFE digestion vessel and 5 ml nitric acid **105** added. The vessels were then heated in the CEM MDS 2100 microwave

digestion system to 80 psi for 10 min. When cool the digests were eluted with water (Elga UHQ grade) to 25 ml then transferred into plastic sample vials. The limits of detection, defined as three times the standard deviation of the mean of two blank check solutions, were found to be 0.75 ppb for manganese at 257.61 nm and 11.4 ppb for potassium at 766.49 nm. The digests, analysed at 250 ml final volume, were corrected for dilution and the original sample weight and metal concentrations in the original solid were reported as % w/w.

4.4.4 Interpretation of elemental analyses (on solid-supported materials)

In order to determine the loading (mmol g^{-1}) of available crown ether moieties within the solid-supported materials prepared herein, the raw data obtained from CHN and ICP-MS was treated in accordance with Equation 3.

$$\text{mmol g}^{-1} = \frac{\%\text{ of A}}{100} \times \frac{1}{\text{B} \times \text{Mr}_\text{A}} \times 1000$$

Equation 3. The equation used to calculate the loading of available crown ether moieties within the solid-supported materials.

Where A = the element in question, B = the number of A within the molecule and Mr_A = the molecular weight of the element under investigation.

4.5 General experimental procedures

4.5.1 General procedure 1: Preparation of the HCl salts of 1° amines

To a stirred solution of the 1° amine (10.00 mmol) in DCM (10 ml), HCl in ether (10 ml, 1.0 M, 10.00 mmol) was added dropwise over a period of 10 min. After 1 hr at room temperature, the organic solvents were removed *in vacuo*, to afford the respective hydrochloride salt as a white crystalline solid. The hydrochloride salts were subsequently used without additional purification.

4.5.2 General procedure 2: Preparation of the TFA salts of 1° amines

To a stirred solution of the 1° amine (10.00 mmol) in DCM (10 ml) was added trifluoroacetic acid **83** (0.76 ml, 10.00 mmol) over a period of 10 min. After 1 hr at room temperature, the organic solvent was removed *in vacuo* to afford the respective trifluoroacetate salt as a white crystalline solid. The resulting materials were used without further purification.

4.5.3 General procedure 3: Preparation of the *p*-TSA salts of amines

p-Toluene sulfonic acid monohydrate **73** (1.89 g, 10.00 mmol) was carefully added to a stirred solution of the 1° amine (10 mmol) in DCM (10 ml). After 1 hr at room temperature, the organic solvent was removed *in vacuo*, leaving the respective *p*-toluenesulfonic acid salt as a pale yellow solid. The materials were subsequently used without further purification.

4.5.4 General procedure 4: Ammonium/DB-18-c-6 complex formation in solution

The amine salt, HCl, TFA or *p*-TSA (1.00 mmol), was dissolved in a mixture of DCM and MeOH (15:0.1 ml) prior to the addition of DB-18-c-6 **14** (0.40 g, 1.10 mmol, 1.1 eq.). The resulting reaction mixture was then stirred at room temperature for 1 hr prior to removal of the organic solvents, *in vacuo,* to afford the desired complex as a white solid.

4.5.5 General procedure 5: Ammonium/18-c-6 complex formation in solution

The amine salt, HCl, TFA or *p*-TSA (1.00 mmol), was dissolved in a mixture of DCM and MeOH (15:0.1 ml) prior to the addition of 18-c-6 **16** (0.26 g, 1.00 mmol, 1.0 eq.). The reaction mixture was stirred at room temperature for 1 hr, prior to removal of the organic solvents *in vacuo*, to afford the desired complex as a white solid.

4.5.6 General procedure 6: Purification of sodium hydride

Commercially available sodium hydride **36** (60 % dispersion in mineral oil) was stirred in hexane (2.5 ml $mmol^{-1}$) for 1 hr prior to decantation. A second aliquot of hexane (2.5 ml $mmol^{-1}$) was then employed and the washing repeated, prior to drying to afford purified NaH **36** as a pale grey solid. The resulting material was used immediately due to the instability of the material when stored.

4.5.7 General procedure 7: Acylation of phenols using acetyl chloride and NaH

Dried NaH **36** (0.16 g, 6.68 mmol, 1.0 eq.) was added, with care, to a stirred solution of the phenolic material (6.68 mmol) under investigation in DCM (10 ml). Upon complete addition, the mixture was stirred for 15 min, under N_2, prior to the dropwise addition of acetyl chloride **34** (0.57 ml, 8.02 mmol, 1.2 eq.). After an initial evolution of heat, the mixture was allowed to stir at room temperature for a further 2 hr. Volatiles were removed *in vacuo* and the crude product dissolved in DCM (100 ml), washed with water (3 x 50 ml) and the organic portion dried over $MgSO_4$. Filtration and evaporation of the DCM *in vacuo* afforded the corresponding acetic acid phenyl ester.

4.5.8 General procedure 8: Acylation of phenols using acetyl chloride and Et_3N

Triethylamine **42** (0.51 ml, 3.70 mmol, 1.2 eq.) was added to a stirred solution of phenolic material (3.08 mmol) in THF (5 ml). Upon complete addition, the mixture was stirred for 15 min under N_2, followed by the dropwise addition of acetyl chloride **34** (0.33 ml, 4.59 mmol, 1.5 eq.). The resulting reaction mixture was stirred at room temperature for a further 2 hr, prior to the removal of volatiles *in vacuo.* The crude product was subsequently dissolved in DCM (100 ml), the organic layer washed with aqueous ammonium chloride (1 x 50 ml), water (2 x 50 ml) and subsequently dried over $MgSO_4$.

Filtration and evaporation of the DCM *in vacuo* afforded the corresponding acetic acid phenyl ester.

4.5.9 General procedure 9: Acetylation of phenols using acetic anhydride and Et_3N

Acetic anhydride **127** (0.48 ml, 5.04 mmol, 1.2 eq.) was dissolved, along with Et_3N **42** (0.70 ml, 5.04 mmol, 1.2 eq.), in THF (5 ml) and the phenolic derivative (4.20 mmol) added with care. Upon complete addition of the phenolic derivative under investigation, the reaction mixture was stirred for 2 hr prior to the removal of volatiles *in vacuo*. The crude product was dissolved in DCM (100 ml), washed with sat. $NaHCO_3$ (1 x 50 ml), water (2 x 50 ml) and the organic portion dried over $MgSO_4$. Filtration and evaporation of the organic solvent *in vacuo* afforded the corresponding acetic acid phenyl ester.

4.5.10 General procedure 10: Flow reactor set-up

As illustrated in Figure 10 the syringe driver was interfaced to inlet 2 of the Rheodyne valve (7125) using a series of commercially available connectors and a length of PTFE tubing (0.8 mm i.d.). Interconnects were made between the PTFE tubing and the luer lock gas-tight syringes using a female to male 10-32 (Tefzel) luer, a 1/16" union (Tefzel) and a 1/16" HPLC connector (PEEK). The flow reactor comprised of a borosilicate glass capillary (50 mm (length) x 3 mm (i.d.)), packed with ~ 0.15 g of the immobilised crown ether under investigation, the inlet of which was connected to the Rheodyne valve *via* outlet 3 (see Figure 10, Chapter 2). The stainless steel sample loop (200 µl) was positioned across outlets 1 and 4, with excess reactants diverted to waste *via* outlet 6 of the valve and reaction products collected in a sample vial (1.5 ml) at the reactor outlet.

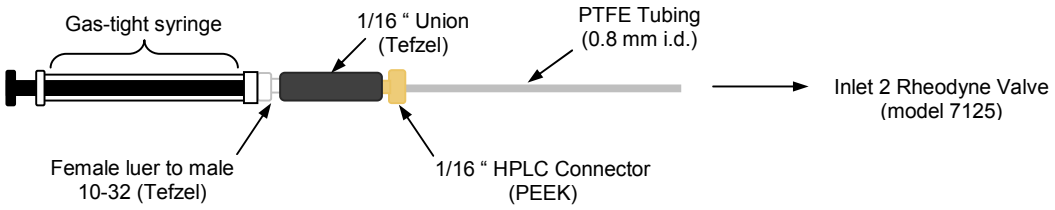


Figure 11. Schematic detailing the interconnection strategy used to enable the continuous introduction of solvent through the immobilised crown ether reactor.

4.5.11 General procedure 11: Flow reaction protocol

Prior to performing a flow reaction, the Rheodyne valve was positioned to load and the solvent under investigation (THF, MeOH or DCM) was driven through inlet 2 of the valve using a Harvard syringe pump at the desired flow rate (10 to 100 µl min^{-1}). The solvent then passed through the valve, out of inlet 3 and through the packed bed reactor to waste.

Crown ether complexation. To prepare the immobilised crown ether complex, the sample loop was filled with a solution of the ammonium salt under investigation (200 µl, 0.12 M, 0.06 mmol) with the valve in the load position. Once filled, the valve was turned to the inject position and the solvent stream diverted through the sample loop and the ammonium salt pumped through the packed bed at the desired flow rate (10 to 100 µl min^{-1}). A period of 2 min was then allowed for the plug to pass through the flow reactor and any uncomplexed ammonium salt was diverted to waste. This step also served to ensure that the sample loop was purged of any residual ammonium salt solution prior to the next step of the reaction.

Reaction of the ammonium complex. To perform a reaction on the immobilised crown ether complex, a solution of the desired reactant(s) (200 µl, 0.12 M, 0.06 mmol) was

introduced into the reactor *via* the Rheodyne valve at a flow rate of 100 µl min^{-1}. The reactor effluent was again diverted to waste in order to remove any unused reagents.

Decomplexation of the immobilised product. After a period of 2 min, the decomplexing agent (200 µl, 0.12 M, 0.06 mmol) was introduced into the flow system *via* the Rheodyne valve (10 to 100 µl min^{-1}) and at this point, the solvent stream was diverted from waste to sample collection. The reaction products were then analysed off-line, by HPLC.

Regeneration of the immobilised crown ether. In the cases where an inorganic compound was employed in the decomplexation step, the immobilised crown ether was regenerated using methanolic acetic acid **121** (200 µl, 5 % v/v) at 100 µl min^{-1}. In this case, the reactor effluent was again diverted to waste and the system purged with the reaction solvent for 2 min prior to repeating the aforementioned reaction steps.

4.5.12 General procedure 12: Acetylation under continuous flow

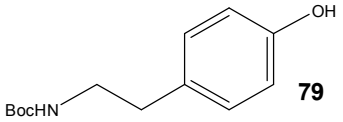
Acetylation reactions were carried using the following optimised protocol; a 200 µl plug of the desired amine salt in THF was introduced into a continuous THF stream (100 µl min^{-1}) *via* the Rheodyne injection valve. To ensure the amine salt had passed through the system before introducing further reagents, THF was pumped through the packed bed for 2 min. Reaction of the phenolic moiety was achieved *via* introduction of a pre-mixed solution of acetic anhydride **127** and Et_3N **42** (200 µl) at a flow rate of 100 µl min^{-1}, which facilitated both deprotonation of the phenolic moiety and the subsequent acetylation in a single step. Again, THF was pumped through the system for 2 min, prior to initiating decomplexation using TMEDA **126** (200 µl), in THF, at 100 µl min^{-1}. At this point the solvent stream was diverted from waste to sample collection and the reaction products collected prior to off-line analysis by HPLC.

4.5.13 General procedure 13: Methylation under continuous flow

Continuous flow methylation reactions were carried using the following optimised protocol; a 200 μl plug of the desired amine salt, in THF, was introduced into a continuous stream of THF (100 μl min^{-1}) *via* a Rheodyne valve. To ensure the amine salt had passed through the system, prior to the introduction of further reagents, THF was pumped through the packed bed at a flow rate of 100 μl min^{-1} for 2 min. The first reaction step involved the introduction of DBU **148** (200 μl) as a solution in THF at a flow rate of 100 μl min^{-1}. This was followed by THF, for 2 min, prior to the introduction of methyl iodide **32** (200 μl) in THF at 100 μl min^{-1} which enabled deprotonation of the phenolic moiety to be followed by methylation in consecutive steps. Again, purging with THF for 2 min was performed prior to initiating decomplexation with TMEDA **126** (200 μl) at 100 μl min^{-1}. At this point the solvent stream was diverted from waste to sample collection and the resulting reaction products analysed off-line by HPLC.

4.6 Synthetic Protocols

[2-(4-Hydroxyphenyl)ethyl]carbamic acid *tert*-butyl ester (Boc-protected tyramine)[101]

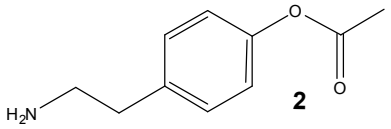


79

Tyramine **4** (2.13 g, 15.53 mmol) was stirred with di-*tert*-butyl dicarbonate **80** (3.39 g, 15.54 mmol) and $NaHCO_3$ (3.92 g, 46.64 mmol) in aq. THF (25 % H_2O) (20 ml) overnight at room temperature, prior to the removal of THF *in vacuo*. The resulting crude material was dissolved in DCM (100 ml), washed with water (3 x 50 ml) and the organic portion dried over $MgSO_4$ and filtered. Evaporation of the organic portion *in vacuo*, followed by recrystallisation (DCM/hexane) of the resulting pale yellow gum

afforded [2-(4-hydroxyphenyl)ethyl]carbamic acid *tert*-butyl ester **79** (3.43 g, 93 % yield) as a white solid; δ_H (400 MHz, $CDCl_3$/TMS) 1.44 (9H, s, 3 x CH_3), 2.70 (2H, brt, J 7.4, $NHCH_2\mathit{CH}_2$), 3.32 (2H, m, $NH\mathit{CH}_2CH_2$), 4.60 (1H, brs, OH), 6.77 (2H, AB_x, J_{AB}, J 8.4, 2 x $ArHC_0CH_2$), 7.01 (2H, AB_x, J_{BA}, J 8.4, 2 x $ArHC_0OH$) and NH not observed; δ_C (100 MHz, $CDCl_3$) 28.5 (3 x CH_3), 35.7 (CH_2), 41.8 (CH_2), 79.4 (C_0) 121.7 (2 x CH), 129.8 (2 x CH), 136.7 (C_0), 149.3 (C_0OH) and 169.7 (CO); *m/z* (E.I.) 238 (M^+ +1, 5%), 237 (3), 181 (72), 180 (100), 136 (25), 121 (15), 120 (25), 109 (24) and 76 (10); HPLC R_T = 8.4 min.

Batch preparation of 4-(2-aminoethyl)phenyl acetate (tyramine acetate)

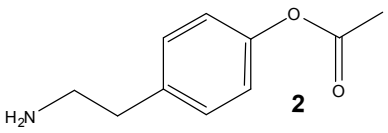


2

In accordance with general procedure 8, [2-(4-hydroxyphenyl)ethyl]carbamic acid *tert*-butyl ester **79** (4.11 g, 17.34 mmol) was acetylated in the presence of acetyl chloride **34** (2.84 ml, 20.80 mmol) and Et_3N **42** (1.48 ml, 20.80 mmol) in THF (30 ml). After 2 hr, the volatiles were removed *in vacuo* and the crude product dissolved in DCM (100 ml), the organic layer washed with sat. NH_4Cl (1 x 50 ml) and water (2 x 50 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting acetic acid 4-(2-*tert*-butoxycarbonylaminoethyl)phenyl ester **82** (1.56 g, 5.61 mmol) was used without further purification and subsequent deprotection achieved *via* dissolution in DCM (50 ml), followed by the addition of TFA **85** (2.08 ml, 28.05 mmol). The resulting reaction mixture was stirred at room temperature for 30 min, the volatiles removed *in vacuo* and the resulting pale yellow gum dissolved in DCM (100 ml), washed with water (3 x 50 ml) and the organic portion dried over $MgSO_4$. The respective TFA salt **84** was subsequently stirred with Amberlyst 15 **7** (13.36 g, 4.20 mmol g^{-1}) in DCM (100 ml) for 1

hr. After filtration, the filtrate was discarded; the Amberlyst 15 **7** was retained, washed with MeOH/NH_3 **9** (20 ml) and the filtrate concentrated *in vacuo*. The resulting yellow gum was purified by column chromatography and eluted using 10 % MeOH in DCM to afford the title compound **2** as a cream crystalline solid (0.92 g, 69 %); (Found C, 67.29; H, 7.52; N, 7.66 %, $C_{10}H_{13}NO_2$ requires C, 67.02; H, 7.31: N; 7.82 %); mpt. 134-136 °C, υ_{max}/cm^{-1} 792, 1088, 1172, 1469, 1703, 2847, 2924 and 3412; δ_H (400 MHz, d-MeOH) 1.88 (3H, s, CH_3), 2.68 (2H, t, J 7.4, $NH_2CH_2CH_2$), 3.33 (2H, brt, J 7.4, $NH_2CH_2CH_2$), 4.89 (2H, brs, NH_2), 6.70 (2H, AB_x, J_{AB}, J 8.4, 2 x $ArHC_0CH_2$) and 7.02 (2H, AB_x, J_{BA}, J 8.4, 2 x $ArHC_0O$); δ_C (100 MHz, d-MeOH) 22.5 (CH_3), 36.6 (CH_2), 42.4 (CH_2), 116.2 (2 x CH), 130.7 (2 x CH), 131.2 (C_0), 156.9 (C_0O) and 173.2 (CO); *m/z* (E.I.) 180 (M^+ +1, 5%), 179 (4), 121 (30), 120 (100), 108 (25), 107 (20), 91 (5) and 77 (20); HPLC R_T = 6.3 min and GC-MS R_T = 8.7.

Flow synthesis of 4-(2-aminoethyl)phenyl acetate (tyramine acetate)

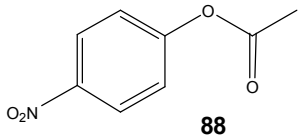


2

Using general procedure 12, 4-(2-aminoethyl)phenyl acetate **2** was synthesised under continuous flow. To achieve this, tyramine TFA **85** (200 μl, 0.12 M, 0.06 mmol), in THF, was introduced into a continuous THF stream (100 μl min^{-1}) *via* a Rheodyne valve. To ensure the tyramine salt **85** had passed through the system containing TMS SILICA AM-18-c-6 **134** (0.15 g, 2.4 x 10^{-2} mmol) before introducing additional reagents, THF (100 μl min^{-1}) was pumped through the system for 2 min. Reaction of the phenolic moiety was achieved *via* introduction of a pre-mixed solution of acetic anhydride **127** and Et_3N **42** (200 μl, 0.12 M, 0.06 mmol) at a flow rate of 100 μl min^{-1}. The system was again purged

with THF (2 min), prior to initiating decomplexation using TMEDA **126** (200 μl, 0.12 M, 0.06 mmol), in THF, at 100 μl min^{-1}. At this point the solvent stream was diverted from waste to sample collection and the reaction products collected prior to off-line analysis by HPLC whereby comparison with a synthetic standard (HPLC R_T = 6.3 min) confirmed quantitative conversion of tyramine TFA **85** to 4-(2-aminoethyl)phenyl acetate **2** with a throughput of 4.3 x 10^{-2} g $reaction^{-1}$ (2.4 x 10^{-2} mmol $reaction^{-1}$); data as previously reported.

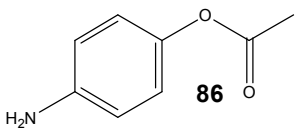
Preparation of 4-nitrophenyl acetate [102]



88

4-Nitrophenol **87** (0.93 g, 6.68 mmol) was acetylated in accordance with general procedure 7 employing dried NaH **36** (0.16 g, 6.68 mmol) and acetyl chloride **34** (0.57 ml, 8.02 mmol) in DCM (10 ml). The reaction mixture was stirred at room temperature for a further 2 hr, prior to removal of volatiles *in vacuo*. The crude product was then dissolved in DCM (100 ml), washed with water (3 x 50 ml) and the organic portion dried over $MgSO_4$. Filtration and evaporation of the organic solvent *in vacuo* afforded 4-nitrophenyl acetate **88** and recrystallised in DCM/Hexane to afford a yellow crystalline solid (1.12 g, 93 %); δ_H (400 MHz, $CDCl_3$/TMS) 2.13 (3H, s, CH_3), 6.63 (2H, AB_x, J_{AB}, J 8.6, 2 x ArH C_0NO_2) and 6.79 (2H, AB_x, J_{BA}, J 8.6, 2 x $ArHC_0O$); δ_c (100 MHz, $CDCl_3$/TMS), 18.3 (CH_3), 110.2 (2 x CH), 119.2 (2 x CH), 138.3 (C_0N), 160.1 (C_0O) and 170.2 (CO); *m/z* (E.I.) 182 (M^+ +1, 55 %), 139 (45), 123 (75) and 107 (18); HPLC R_T = 3.9 min and GC-MS R_T = 9.9 min.

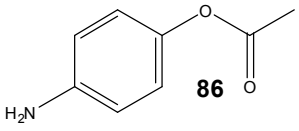
Batch preparation of 4-aminophenyl acetate[103]



86

4-Nitrophenyl acetate **88** (1.64 g, 9.04 mmol) was dissolved in THF (500 ml) prior to the addition of Pd on Charcoal **108** (0.55 g, 5 % w/w) and hydrogenated overnight. The resulting reaction mixture was filtered under suction, using Celite as a filter aid and the filtrate concentrated *in vacuo* to afford a pale yellow gum. The crude material was purified by column chromatography and eluted using 20 % MeOH in DCM to afford the title compound **86** as a cream crystalline solid (1.08 g, 79 %); δ_H (400 MHz, $CDCl_3$/TMS) 2.29 (3H, s, CH_3), 1.65 (2H, brs, NH_2), 6.66 (2H, AB_x, J_{AB}, J 8.7, 2 x $ArHC_0NH_2$) and 6.81 (2H, AB_x, J_{BA}, J 8.7, 2 x $ArHC_0O$); δ_c (100 MHz, $CDCl_3$/TMS), 21.1 (CH_3), 115.6 (2 x CH), 122.2 (2 x CH), 142.9 (C_0N), 144.2 (C_0O), 179.3 (CO); *m/z* (E.I.) 152 (M^+ +1, 100 %), 108 (72) and 78 (35); HPLC R_T = 3.2 min and GC-MS R_T = 9.1 min.

Flow synthesis of 4-aminophenyl acetate



86

Using general procedure 12, 4-aminophenylacetate **86** was synthesised under continuous flow. To achieve this, 4-aminophenol HCl **143** (200 μl, 0.3 M, 0.06 mmol), in THF, was introduced into a continuous THF stream (100 μl min^{-1}) *via* a Rheodyne valve. To ensure the salt **143** had passed through the system before introducing additional reagents, THF (100 μl min^{-1}) was pumped through the system for 2 min. Reaction of the phenolic moiety was achieved *via* introduction of a pre-mixed solution of acetic

anhydride **127** and Et_3N **42** (200 μl, 0.3 M, 0.06 mmol) at a flow rate of 100 μl min^{-1}. The system was again purged with THF (2 min), prior to initiating decomplexation using TMEDA **126** (200 μl, 0.3 M, 0.06 mmol), in THF, at 100 μl min^{-1}. At this point the solvent stream was diverted from waste to sample collection and the reaction products collected prior to off-line analysis by HPLC whereby comparison with a synthetic standard (HPLC R_T = 3.2 min) confirmed quantitative conversion of 4-aminophenol HCl **143** to 4-aminophenyl acetate **86**, with a throughput of 2.3 x 10^{-2} mmol $reaction^{-1}$; data as previously reported.

Preparation of the crown ether complexes of tyramine in solution

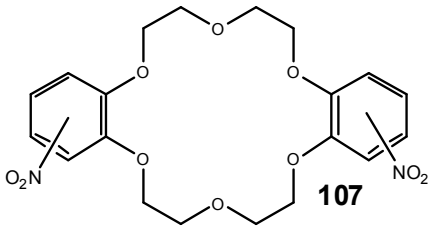
Using general procedures 1 to 3 the HCl, TFA and *p*-TSA salts of tyramine **85**, **89** and **91** were prepared. The resulting materials were subsequently complexed with DB-18-c-6 **14** or 18-c-6 **16** in accordance with general procedures 4 and 5 respectively. Analysis by HPLC and MALDI-MS confirmed complex formation, the results of which are depicted in Table 16.

Complex	Crown ether	Tyramine salt	MS ($[M^+ +1]$)	HPLC R_T (min)
100	18-c-6 **16**	HCl **89**	402	4.6
151	18-c-6 **16**	CF_3CO_2 **85**	402	4.1
93	18-c-6 **16**	*p*-TSA **91**	402	4.5-5.2[a]
90	DB-18-c-6 **14**	HCl **89**	498	4.6
152	DB-18-c-6 **14**	CF_3CO_2 **85**	498	5.2
92	DB-18-c-6 **14**	*p*-TSA **91**	498	5.7-6.5[a]

[a] Due to co-elution of the complex formed and the *p*-TSA counterion, an exact retention time could not be determined.

Table 16. Summary of the data obtained upon analysis of various crown ether/amine salt complexes.

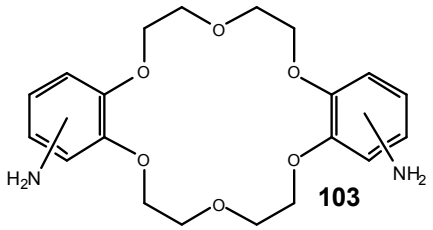
Preparation of dinitro-dibenzo-18-crown-6 ether[91]



107

To a stirred solution of dibenzo-18-crown-6-ether **14** (0.51 g, 1.42 mmol) in DCM (10 ml) and acetic acid **121** (7 ml), a solution of nitric acid **105** (0.07 ml, 1.56 mmol) in acetic acid **121** (1 ml) was added dropwise. The reaction mixture was stirred for 1 hr at room temperature and then heated to reflux for an additional 8 hr, during which time a precipitate formed. Upon cooling the precipitate was filtered and the filtrate retained. The precipitate was washed with hexane, to remove any residual acetic acid and the filtrate extracted with water (3 x 50 ml) dried over $MgSO_4$ and concentrated *in vacuo* to afford a yellow powder. The two aliquots of material products were analysed separately and ^{1}H NMR confirmed them both to be the title compound **107** (total yield 0.44 g, 79 %); δ_H (400 MHz, $CDCl_3$/TMS) 4.01-4.12 (8H, m, 2 x CH_2CH_2), 4.18-4.25 (8H, m, 2 x CH_2CH_2), 6.94 (2H, d, J 9.0, 2 x ArH), 7.72 (2H, d, J 2.6, 2 x ArH) and 7.80 (2H, dd, J 9.0 and 2.6, 2 x ArH); δ_c (100 MHz, $CDCl_3$/TMS) 67.8 (4 x CH_2), 68.6 (4 x CH_2), 106.6 (2 x CH), 110.2 (2 x CH), 117.2 (2 x CH), 140.6 (2 x C_0NO_2), 147.5 (2 x C_0), 153.4 (2 x C_0).

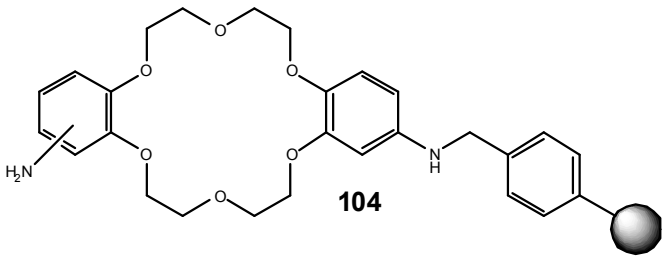
Preparation of diamino-dibenzo-18-crown-6 ether[91]



103

To a stirred solution of dinitro-dibenzo-18-crown-6-ether **107** (0.15 g, 0.33 mmol) in EtOAc (50 ml) was added Palladium on Carbon **108** (0.02 g, 5 % w/w) and the reaction mixture hydrogentated overnight. The resulting solution was then filtered under suction, employing Celite as a filter aid, prior to removal of the reaction solvent *in vacuo* to afford diamino-dibenzo-18-crown-6-ether **103** as a cream crystalline solid (0.30 g, 78 %); δ_H (400 MHz, $CDCl_3$/TMS) 3.91-4.00 (8H, m, 2 x CH_2CH_2), 4.23-4.32 (8H, m, 2 x CH_2CH_2), 7.22 (2H, d, J 8.9, 2 x ArH), 7.73 (2H, d, J 2.6, 2 x ArH), 7.92 (2H, dd, J 8.9 and 2.6, 2 x ArH) and 2 x NH_2 not observed; δ_c (100 MHz, $CDCl_3$/TMS), 68.0 (4 x CH_2), 68.4 (4 x CH_2), 106.6 (2 x CH), 111.2 (2 x CH), 117.6 (2 x CH), 140.5 (2 x C_0), 147.6 (2 x C_0), 153.8 (2 x C_0).

Preparation of MPR immobilised diamino-dibenzo-18-crown-6 ether

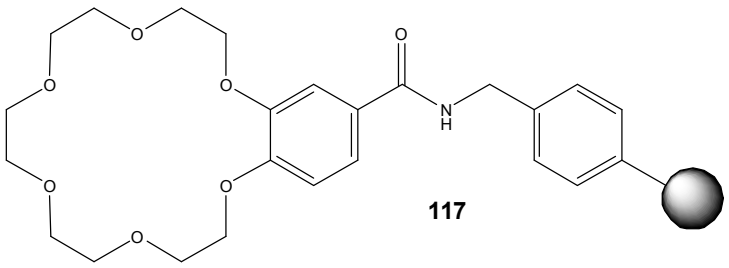


104

Merrifield peptide resin **13** (0.54 g, 1.5 mmol Cl g^{-1}, 200-400 mesh) was added to a stirred solution of diamino-dibenzo-18-crown-6-ether **103** (0.40 g, 3.75 mmol) and lithium carbonate **109** (0.28 g, 3.75 mmol) in acetone (10 ml) and heated to reflux for 6

hr. Upon cooling, the reaction mixture was filtered under suction and the polymeric material washed with DI water (100 ml), DCM (100 ml) and acetone (100 ml). The material **104** was subsequently oven dried, at 60 °C, to afford a pale yellow free flowing solid (0.51 g); Found C 83.99; H 7.82; N 0.33 %, ICP-MS 0.66 % Mn and 0.47 % K. If 100 % of the active sites on the Merrifield peptide resin **13** had been functionalised, this would be equivalent to 4.20 % N, 8.40 % Mn and 5.98 % K. Consequently, a 7.8 % loading of diamino-dibenzo-18-crown-6-ether **103** was achieved which is a loading of 0.12 mmol g^{-1}.

Preparation of immobilised carboxybenzo-18-crown-6 ether

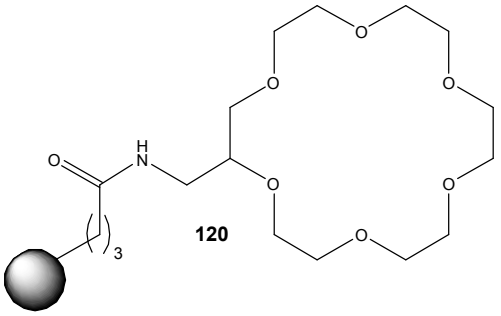


117

Thionyl chloride **114** (0.38 ml, 5.12 mmol) was added to a stirred solution of carboxybenzo 18-crown-6-ether **113** (1.52 g, 4.30 mmol) in toluene (20 ml) and the reaction mixture heated to reflux for 3 hr. The resulting acid chloride **117** was concentrated *in vacuo* to afford a free flowing white solid, which was subsequently redispersed in toluene (20 ml), prior to the addition of amino methyl polystyrene (1.5 mmol N g^{-1}, crosslinked with 2 % DVB, 200-400 mesh) (2.82 g, 4.30 mmol), followed by triethylamine **42** (0.71 ml, 5.12 mmol). The reaction mixture was stirred overnight, under N_2, prior to filtration under vacuum. The supported crown ether was then washed (H_2O, acetone and DCM) and oven dried, at 90 °C, to afford a free flowing white powder (3.78

g, 87 %) with a calculated loading of 0.98 mmol g^{-1}; ICP-MS results 0.98 mmol g^{-1}, 3.88 %.

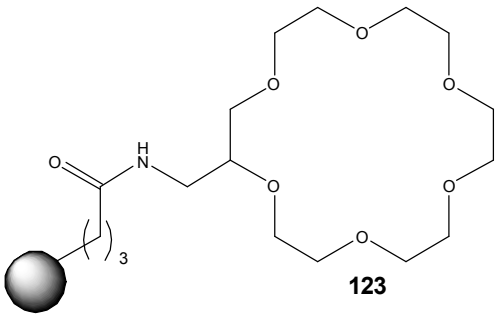
Preparation of carboxy polystyrene immobilised aminomethyl-18-crown-6-ether



120

Thionyl chloride **114** (0.05 ml, 0.72 mmol) was added to a stirred solution of oven dried carboxypolystyrene **118** (1.00 g, 0.40-0.60 mmol g-1, 1 % cross linked, 200-400 mesh) in toluene (20 ml) and the reaction mixture heated to reflux for 3 hr. The resulting supported acid chloride was concentrated *in vacuo* to afford a free flowing white solid, which was subsequently redispersed in toluene (20 ml), under N_2, prior to the addition of 2-aminomethyl-18-crown-6 ether **112** (0.18 g, 0.60 mmol), followed by triethylamine **42** (0.10 ml, 0.72 mmol). The reaction mixture was stirred overnight, under N_2, prior to filtration under vacuum. The supported crown ether **120** was then washed (H_2O, acetone and DCM) and oven dried, at 90 °C, to afford a free flowing white powder (0.99 g, 83 %); (Found C 83.50; H 8.83; N 0.52 %, looking for 0.84 % N). This is the equivalent to a loading of 0.37 mmol g^{-1} of 2-aminomethyl-18-crown-6-ether **112** onto the resin which is a 61.7 % loading of crown ether with respect to active sites on the resin (based on maximum 0.6 mmol g^{-1} loading). ICP-MS confirmed a loading of 0.37 mmol g^{-1} with 2.05 % K.

Preparation of silica gel immobilised aminomethyl-18-crown-6-ether



123

Method 1: *via* the acid chloride

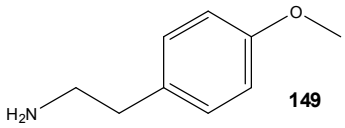
Thionyl chloride **114** (0.37 ml, 5.11 mmol) was added to a stirred solution of oven dried 3-carboxypropyl functionalised silica gel **122** (1.06 g, 1.60 mmol g^{-1}, 200-400 mesh) in toluene (20 ml) and the reaction mixture heated to reflux for 3 hr. The resulting silica supported acid chloride was concentrated *in vacuo* to afford a free flowing white solid, which was subsequently redispersed in toluene (20 ml), prior to the addition of 2-aminomethyl-18-crown-6 ether **112** (0.50 g, 1.70 mmol), followed by triethylamine **42** (0.26 ml, 1.86 mmol). The reaction mixture was stirred overnight, under N_2, prior to filtration under vacuum. The supported crown ether **123** was then washed (H_2O, acetone and DCM) and oven dried, at 90 °C, to afford a free flowing white powder (1.08 g, 72.2 %); ICP-MS results 0.162 mmol g^{-1}, 10.06 %.

Method 2: *via* a carbodiimide coupling reaction

N,N-Dicyclohexylcarbodiimide **10** (0.50 g, 2.415 mmol) was added to a stirred solution of 3-carboxypropyl functionalised silica gel **122** (0.50 g, 1.60 mmol g^{-1}, 200-400 mesh) and 2-aminomethyl-18-crown-6 ether **112** (0.24 g, 0.81 mmol) in DCM (50 ml). The resulting reaction mixture was heated to reflux for 12 hr, prior to filtering under suction and washing with H_2O, acetone and DCM. The resulting functionalised silica gel **123** was

oven dried, at 90 °C, to afford a free flowing white powder (0.51 g, 78.4 %); ICP-MS results 0.21 mmol g^{-1}, 12.76 %.

Batch production of 2-(4-methoxyphenyl)ethylamine[104]

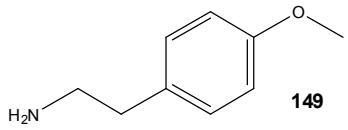


149

[2-(4-Hydroxyphenyl)ethyl]carbamic acid *tert*-butyl ester **79** (1.69 g, 8.03 mmol) was dissolved in DCM (10 ml) and treated with DBU **148** (1.19 ml, 8.03 mmol). The reaction mixture was stirred at room temperature for 10 min prior to the addition of methyl iodide **32** (0.50 ml, 8.03 mmol). After 30 min, the reaction mixture was diluted with DCM (100 ml) and washed with sat. NH_4Cl (1 x 100 ml) and water (2 x 100 ml). The organic portion was dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford a pale yellow gum. A portion of the resulting material (0.69 g, 5.61 mmol) was dissolved in DCM (50 ml), to which was added TFA **83** (2.08 ml, 28.05 mmol) and the reaction mixture stirred at room temperature for 30 min. The solvents were removed *in vacuo* and the resulting pale yellow solid was dissolved in DCM (100 ml), washed with water (3 x 50 ml) and the organic portion dried over $MgSO_4$. Filtration followed by evaporation of the DCM *in-vacuo* afforded the respective TFA salt which was subsequently stirred with Amberlyst 15 **7** (13.36 g, 4.20 mmol g^{-1}) in DCM (10 ml) for 1 hr. Upon filtration, the filtrate was discarded and the Amberlyst 15 **7** washed with MeOH/NH_3 **8** (10 ml) and the filtrate concentrated *in vacuo*. The material was purified by column chromatography and eluted using 10 % MeOH in DCM to afford a cream crystalline solid **149** (1.04 g, 78 %); δ_H (400 MHz, $CDCl_3$/TMS) 2.40 (2H, m CH_2), 2.78 (2H, m, C*H*$_2NH_2$), 3.60 (3H, s, CH_3), 4.89 (2H, brs, NH_2), 6.65 (2H, AB_x, J_{AB}, J 8.6, 2 x $ArHC_0CH_2$) and 6.97 (AB_x, J_{BA}, J 8.6, 2 x

$ArHC_0OCH_3$); δ_C (100 MHz, $CDCl_3$/TMS) 32.8 (CH_2), 41.4 (CH_2NH_2), 55.6 (CH_3), 114.1 (2 x CH), 130.7 (2 x CH), 128.7 (C_0O) and 129.9 (C_0); HPLC R_T = 6.1 min.

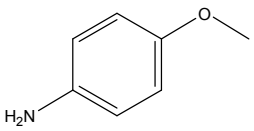
Flow synthesis of 2-(4-methoxyphenyl)ethylamine



149

Tyramine TFA **85** (200 μl, 0.12 M, 0.06 mmol) in THF was introduced into a continuous THF stream (100 μl min^{-1}) *via* a Rheodyne valve. To ensure the tyramine salt **85** had passed through the system containing TMS SILICA AM-18-c-6 **134** (0.15 g, 2.4 x 10^{-2} mmol), before introducing further reagents, THF (100 μl min^{-1}) was pumped through for 2 min. The first of the reaction steps incorporated a 200 μl plug of DBU **148** (0.12 M, 0.06 mmol), followed by methyl iodide **32** (0.12 M, 2.4 x 10^{-2} mmol); both of which were passed through the flow reactor at 100 μl min^{-1}, facilitating the deprotonation of the phenolic moiety and methylation in consecutive steps. Again, two minutes was allowed before initiating decomplexation *via* a 200 μl plug of TMEDA **126** (0.12 M, 2.4 x 10^{-2} mmol) at 100 μl min^{-1}. At this point, the solvent stream was diverted from waste to sample collection and the reaction products analysed off-line by HPLC (R_T = 6.1 min) whereby 100 % conversion to 3-(4-methoxyphenyl)ethylamine **149** was obtained, corresponding to 2.3 x 10^{-2} mmol $reaction^{-1}$; data as previously reported

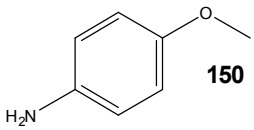
Batch production of 4-methoxyphenylamine (*p*-anisidine)[105]



4-Aminophenol **143** (1.20 g, 11.04 mmol) was stirred with di-*tert*-butyl dicarbonate **80** (2.40 g, 11.04 mmol) and $NaHCO_3$ (2.72 g, 33.12 mmol) in aq. THF (25 % H_2O) (20 ml) at room temperature overnight prior to removal of THF *in vacuo*. The resulting crude product was dissolved in DCM (100 ml), washed with water (3 x 50 ml) and the organic portion dried over $MgSO_4$. Evaporation of the DCM *in vacuo*, followed by recrystallisation (DCM/hexane) of the resulting solid afforded the Boc-protected amine (1.37 g, 6.52 mmol) which was subsequently treated with DBU **148** (0.97 ml, 6.52 mmol, 1.0 eq.) and methyl iodide **32** (0.41 ml, 8.03 mmol, 1.0 eq.) in DCM (10 ml) and allowed to stir for 30 min. A portion of the resulting solid (0.63 g, 5.52 mmol) was dissolved in DCM (50 ml), to which was added TFA **83** (1.97 ml, 27.60 mmol) and stirred for 30 min at room temperature. The solvents were removed *in vacuo* and the resulting pale yellow solid was dissolved in DCM (100 ml), washed with water (3 x 50 ml) and the organic portion dried over $MgSO_4$. Filtration followed by evaporation of the DCM *in vacuo* afforded the respective TFA salt which was subsequently stirred with Amberlyst 15 **7** (13.14 g, 4.20 mmol g^{-1}) in DCM (10 ml) for 1 hr. After filtration, the filtrate was discarded and the Amberlyst 15 **7** washed with MeOH/NH_3 **9** (20 ml) and the filtrate concentrated *in vacuo*. The material was purified by column chromatography and eluted using 10 % MeOH in DCM to afford 4-methoxyphenylamine **150** as a grey crystalline solid δ_H (400 MHz, $CDCl_3$/TMS) 3.38 (2H, brs, NH_2), 3.78 (3H, s, CH_3), 6.66 (2H. AB_x, J_{AB}, J 8.6, 2 x $ArHC_0NH_2$) and 6.76 (2H, AB_x, J_{BA}, J 8.6, 2 x $ArHC_0OCH_3$), δ_c (100 MHz),

55.6 (CH_3), 114.7 (2 x CH), 116.3 (2 x CH), 139.8 (C_0N), 152.7 (C_0O); HPLC R_T = 5.7 min (compared to a commercially available standard).

Flow synthesis of 4-methoxyphenylamine



150

4-Aminophenol HCl **143** (200 μl, 0.12 M, 0.06 mmol) in THF was introduced into a continuous THF stream (100 μl min^{-1}) *via* the Rheodyne valve. To ensure the 4-aminophenol salt **143** had passed through the system containing TMS SILICA AM-18-c-6 **134** (0.15 g, 2.4 x 10^{-2} mmol), before introducing further reagents, THF (100 μl min^{-1}) was pumped through for 2 min. The reaction steps firstly incorporated a 200 μl plug of DBU (0.12 M, 2.4 x 10^{-2} mmol) **148** being passed through the system followed by methyl iodide **32** (200 μl, 0.12 M, 2.4 x 10^{-2} mmol) both of which were passed through the flow reactor at 100 μl min^{-1}, facilitating the deprotonation of the phenolic moiety and methylation in consecutive steps. Again, two minutes was allowed before initiating decomplexation *via* a 200 μl plug of TMEDA **126** (0.12 M, 2.4 x 10^{-2} mmol) at 100 μl min^{-1}. At this point the solvent stream was diverted from waste to sample collection and the reaction products analysed off-line by HPLC, affording 100 % conversion with a throughput of 2.4 x 10^{-2} mmol $reaction^{-1}$; HPLC R_T = 5.7 min.

Chapter 5: References

5.0 References

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Chapter 6: Publications and Proceedings

6.0 Publications and Proceedings

6.1 Publications

'Flow Reactors for Organic Chemistry', G. P. Wild, C. Wiles and P. Watts, *Letters in Organic Chemistry*, 2006, **3**, 419.

'The use of immobilised crown ethers as *in-situ* protecting groups for organic synthesis within flow reactors', G. P. Wild, C. Wiles, P. Watts and S. J. Haswell, in preparation.

6.2 Conference Proceedings

Immobilised crown ethers as *in-situ* protecting groups within flow reactors', G. P. Wild, C. Wiles, P. Watts and S. J. Haswell, in proc. '*9th International conference on microreaction technology*', 2006, 326.

'Immobilised crown ethers as *in-situ* protecting groups within flow reactors', G. P. Wild, C. Wiles, P. Watts and S. J. Haswell, *Abstr. Pap. Am. Chem.,* 2008, ORGN 235.