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The use of immobilized crown ethers as in-situ N-protecting groups in organic synthesis and their application under continuous flow

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ABSTRACT

In addition to their high affinity for inorganic cations, crown ethers have been shown to efficiently sequester ammonium ions, forming a stable adduct via hydrogen bonding. Using this principle, several authors have reported the use of crown ethers as protecting groups for amines however to date, their widespread use has been somewhat precluded by the difficulties associated with removal of the crown ether from the resulting reaction mixture. In order to address this problem, we report the preparation of an immobilized 18-crown-6 ether derivative and its incorporation into a flow reactor, demonstrating the ability to use and recycle the reagent for the chemoselective O-acylation and alkylation of bifunctional compounds such as 4-(2-aminoethyl)phenol and 4-nitrophenol.

1. Introduction

When conducting the synthesis of polyfunctionalized molecules it is often necessary to render one or more of the functionalities within the molecule temporarily inert in order to allow the selective reaction of another group. This process is achieved by appending a blocking group, which is stable to the reaction conditions under investigation whilst being readily removed; such a moiety is termed as a protecting or protective group.[1] While the use of protecting groups has enabled access to a vast library of complex molecules over the years, by removing functional group incompatibilities, their use can be disadvantageous as the introduction and removal of such groups generates additional synthetic steps. This can lead to increased costs and contribute to reductions in overall yield, along with the need to perform complex purifications in order to remove any protecting group residues from the final material. Furthermore, the deprotection strategy must be carefully selected in order to ensure that the product is obtained in the desired form i.e. as the free functionality or as a salt.

1.1. The use of crown ethers in organic synthesis

In addition to the propensity of crown ethers to form complexes with metal ions (Li^+, Na^+, K^+, Rb^+, Cs^+) and promote solubility in non-polar media, Pederson[2] also reported their ability to form complexes with ammonium ions ($R–NH_3^+$). Further work by Bushmann and Mutihac[3] compared the degree of ammonium ion complexation with various functionalized and un-functionalized crown ethers, concluding that 18-crown-6 ethers afforded superior complexation cf. to smaller crown ethers. As depicted in Figure 1, unlike metal ions, the ammonium ion is held above the cavity of the crown ether in a tetrahedral configuration via hydrogen bonding[4] with stabilization achieved due to distribution of the positive charge on the ammonium ion over the hydrogen atoms. The geometry of the complexed ammonium ion also leaves the remainder of the molecule unhindered and available to take part in subsequent reactions as demonstrated by Kunishima et al.[4b]

In the early 1990s, Mascagni and Hyde[5] exploited this phenomena for the synthesis of peptides and oligomers, proposing that the non-covalent nature of the interaction between the crown ether and the ammonium ion would provide a mild protection strategy for amines. Employing dibenzo-18-crown-6 ether (DB-18-c-6) and an array of alanine salts (HCl, TFA and *p*-TSA), the effect of solvent polarity on a coupling reaction was evaluated. During this investigation it was observed that tosylate salts formed the most stable complexes (*p*-TSA>TFA>HCl) and that complex stability increased with decreasing solvent polarity; an observation that was

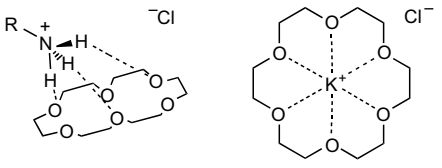

Figure 1. Schematic illustrating the modes of complexation observed for ammonium ions versus metal ions with 18-c-6 ether.

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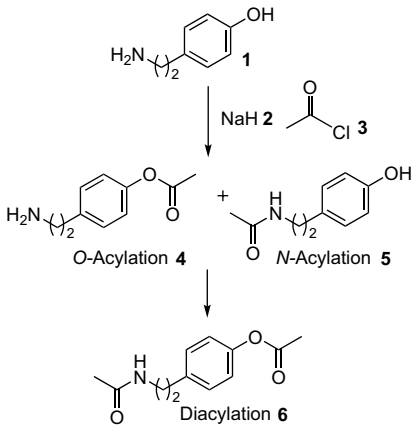
attributed to a reduction in competition between solvation of the cation and coordination by the crown ether.[5] Consequently, subsequent coupling reactions were performed in DCM and found to afford the desired complexed tripeptide in 80% yield, with the remainder comprising of mixed oligomers; the formation of which was attributed to partial decomplexation of the peptide during the reaction.[6] The authors subsequently reported the decomplexation of another tripeptide using aq KCl, exploiting the crown ethers affinity for metal ions to force out the ammonium ion and leaving the crown ether complexed with potassium, as depicted in Figure 1. Although this approach demonstrated the key components of a protecting group strategy, namely the ability to protect an amino acid, perform a coupling reaction and deprotect the resulting peptide, the use of stoichiometric quantities of crown ether proved disadvantageous due to difficulties associated with its removal from the reaction product. As such, isolation of the peptide, regeneration of the crown ether cavity and subsequent re-use of the crown ether were not demonstrated by the authors.

Based on the preliminary observations made by Mascagni et al.[5,6] it was proposed that the preparation of an immobilized 18-crown-6 ether derivative would facilitate the process of amine deprotection, along with product isolation and subsequent crown ether regeneration. With this in mind, our aim was to develop a non-covalent protecting group strategy using an immobilized 18-crown-6 ether derivative and combine it with continuous flow methodology, developed within our group, to demonstrate efficient O-acetylation/alkylation of substituted 1° amines.

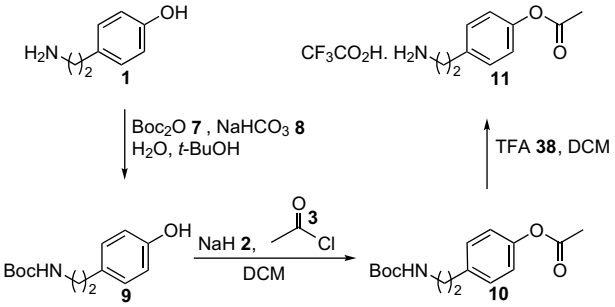
2. Results and discussion

Owing to the highly basic and nucleophilic nature of an amine group, protection is essential in reactions such as acylations and alkylations. This is illustrated for the bifunctional compound tyramine **1**, which contains both aromatic alcohol and aliphatic amine functionalities. As depicted in Scheme 1, failure to protect the amine group and direct the reaction to the phenolic group upon treatment of tyramine **1** with NaH **2** (1.0 equiv) and acetyl chloride **3** (1.2 equiv) results in a complex mixture containing the desired tyramine acetate (4-(2-aminoethyl)-phenyl acetate) **4** (23%), along with tyramine *N*-acetate (*N*-[2-(4-hydroxyphenyl)ethyl]-acetamide) (12%) **5**, tyramine diacetate (acetic acid 4-(2-acetylaminoethyl)-phenyl ester) (20%) **6** and residual starting material **1** (45%).



Scheme 1. Schematic illustrating the potential reaction products obtained when acetylating tyramine **1**.

In order to benchmark the developed non-covalent protecting group strategy against existing covalent approaches, the acetylation of tyramine **1** was subsequently performed using the acid labile *tert*-butoxycarbonyl (Boc) protecting group. As depicted in Scheme 2, the reaction sequence firstly involved the protection of tyramine **1**, using di-*tert*-butyl dicarbonate **7** in the presence of $NaHCO_3$ **8**, which afforded the intermediate Boc-tyramine **9** as a white solid (93% yield). Subsequent acylation, with acetyl chloride **3** (1.2 equiv) in the presence of NaH **2** (1.0 equiv) afforded the desired Boc-tyramine acetate **10** as a pale yellow gum (69% yield).



Scheme 2. Schematic of the covalent protecting group strategy employed to synthesize tyramine acetate TFA salt **11**.

Although the use of a protecting group afforded a chemoselective route to the O-acetylation of tyramine **4**, in addition to increasing the number of reaction steps employed in the synthetic pathway, removal of the protecting group proved problematic, affording a mixture of tyramine acetate TFA salt **11** (69%), tyramine TFA salt **12** (16%) and Boc-tyramine acetate **10** (15%) as determined by HPLC. In an analogous manner to the direct acetylation of tyramine **1** (Scheme 1), the use of a protecting group afforded a complex reaction mixture, largely due to problems associated with the efficient removal of the Boc-protecting group. Furthermore, in order to isolate the desired tyramine acetate **4** it was necessary to free-base the TFA salt **11** thus incurring additional reaction steps.

2.1. Evaluation of immobilized crown ethers as non-covalent protecting groups

In order to evaluate the use of an immobilized crown ether as a protecting group, preliminary investigations were conducted using the commercially available di-*tert*-butylcyclohexano-18-crown-6 ether on an inert chromatographic support (0.37 mmol g^{-1}) (Eichrom Technologies, France). To assess the potential of di-*tert*-butylcyclohexano 18-c-6 as a non-covalent protecting group, the ability of the material to complex tyramine HCl **13** and subsequently decomplex tyramine **1** was firstly investigated. This was achieved by stirring the crown ether with tyramine HCl **13** in MeOH, prior to filtration, under suction, to remove any uncomplexed tyramine HCl **13**. To confirm sequestration had occurred, the immobilized complex was treated with methanolic KCl **14**, to induce decomplexation, and the resulting filtrate analyzed by HPLC. Initial results were pleasing and detection of tyramine **1** confirmed the ability of the material to complex tyramine HCl **13** and release tyramine **1** from the crown ether cavity. To demonstrate re-use of the supported crown ether, the cavity was regenerated and the above procedure repeated; unfortunately, upon analysis of the filtrate no tyramine **1** was detected. Employing a fresh portion of the solid-supported crown ether again confirmed sequestration of tyramine HCl **13** and release of the amine **1**. It was therefore postulated that during the regeneration step the crown ether, which was only adsorbed onto the solid support, leached from the support and therefore could not be efficiently recycled or employed in a continuous system.

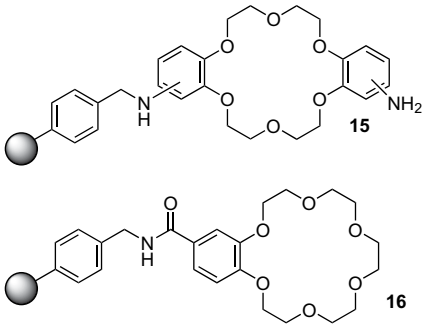


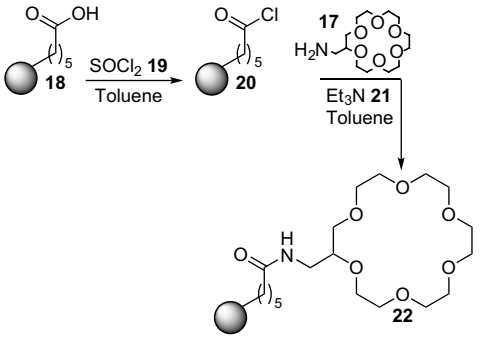
Figure 2. Illustration of two immobilized crown ethers that was found to complex metal ions but not ammonium ions.

2.1.1. Preparation of an immobilized crown ether

To address if desorption of the crown ether was the problem, we evaluated the covalent immobilization of three 18-crown-6 ether derivatives and subsequently evaluated the materials towards the sequestration of ammonium salts under continuous flow. With this in mind, several immobilization techniques were employed, with starting materials including diamino-dibenzo-18-crown-6 ether and carboxybenzo-18-crown-6 ether to afford crown ethers **15** and **16**, respectively, as depicted in Figure 2.

Unfortunately, due to the increased ring strain observed as a result of immobilization, neither material was able to complex ammonium ions, a process, which is reliant on hydrogen bonding (Fig. 1), however, both materials successfully sequestered potassium permanganate (0.12 mmol g^{-1} and 0.98 mmol g^{-1}, respectively) confirming the presence of active crown ether moieties.

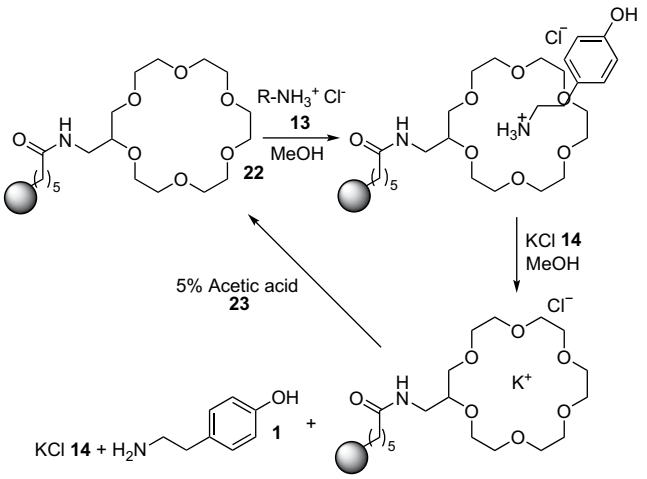
Owing to the reliance of ammonium ion complexation on hydrogen bonding (Fig. 1), it is imperative for the immobilized crown ether to be free of strain in order to form a stable complex. With this in mind, a further attempt was made to prepare a covalently bound 18-crown-6 ether with no ring strain, this was achieved by employing aminomethyl-18-crown-6 ether (AM-18-c-6) **17** as a precursor. As Scheme 3 illustrates, treatment of carbxoypolystyrene **18** with thionyl chloride **19** afforded the immobilized acid chloride **20**, to which was added Et_3N **21** followed by AM-18-c-6 **17** to afford immobilized AM-18-c-6 **22**.



Scheme 3. Synthetic protocol employed for the covalent immobilization of AM-18-c-6 **17** to carboxypolystyrene **18**.

To evaluate the materials ability to sequester ammonium ions, the crown ether **22** was stirred at room temperature in a methanolic solution of tyramine HCl **13**, filtered under suction and washed with MeOH prior to treatment with methanolic KCl **14** to release any complexed tyramine HCl **13** as the free amine **1**. Analysis of the filtrate by HPLC confirmed the presence of tyramine **1** and elemental analysis of the crown ether **22**, coupled with sequestration of $KMnO_4$ followed by ICP-MS analysis, confirmed the material to have a loading of 0.37 mmol g^{-1}.

Having identified the presence of active crown ether cavities on the solid-support **22**, the ability to recycle the material was subsequently investigated, with removal of the potassium cation achieved using methanolic acetic acid **23**. Once successfully regenerated, the cycle was repeated a further four times and unlike the commercially available di-*tert*-butylcyclohexano-18-c-6, all subsequent cycles yielded tyramine **1**, with an 11.4% RSD (n=5) (Scheme 4).



Scheme 4. Reaction scheme illustrating the complexation of tyramine HCl **13**, using immobilized AM-18-c-6 **22**, and subsequent decomplexation and cavity regeneration.

Although the material was able to be recycled, illustrating the robustness required for continuous processing, the irreproducible quantities of tyramine **1** recovered were still undesirable. In order to identify the origin of any irreproducibility, an aliquot of immobilized crown ether was taken at each stage of the process and subjected to elemental analysis. As Table 1 illustrates, the decomplexation step was found to be 100% efficient and as such, the gradual decrease in the proportion of tyramine **1** recovered was attributed to loss of immobilized crown ether **22** upon filtration and washing.

Table 1
Elemental analyses illustrating the efficient complexation and decomplexation achieved using immobilized crown ether **22**

Polymeric material	N (%)	N (mmol g^{-1})
Blank **18**	0.00	0.00
Immobilized AM-18-c-6 **22**	0.52	0.37
Complexed	0.97	0.69
Decomplexed	0.52	0.37

2.1.2. Continuous flow evaluation of crown ether

In order to address the irreproducibility associated with the batch process and develop an efficient, re-useable system for the non-covalent protection of amines, the incorporation of the immobilized crown ether into a continuous flow reactor was

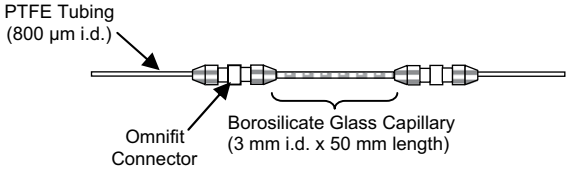


Figure 3. Schematic of the continuous flow reactor used herein for the evaluation of an immobilized crown ether **22**.

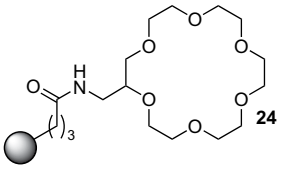


Figure 4. SILICA AM-18-c-6 **24** prepared via the acid chloride.

investigated. It was proposed that such an approach would enable material **22** to be recycled with ease as solutions of the various reactants would be pumped through crown ether **22** in order to conduct the desired reaction step; thus removing the main source of error observed thus far, loss of material upon filtration.

As Figure 3 illustrates, the flow reactor comprised of a borosilicate glass capillary (50 mm (length)×3 mm (i.d.)) packed with ~0.15 g of immobilized crown ether **22**. Reactant solutions were passed through the reactor using a syringe pump and reaction products collected in a sample vial prior to off-line analysis by HPLC.[7] To increase the reproducibility of the technique further, reactants were introduced into the system via a Rheodyne valve (200 μL sample loop).

Employing a solvent stream of MeOH, tyramine HCl **13** (0.3 M, 6.0×10^{-2} mmol) was introduced into the flow reactor, containing immobilized AM-18-c-6 **22** (0.15 g, 5.6×10^{-2} mmol) at a flow rate of 100 μL min^{-1}. Decomplexation was again achieved using KCl **14** in MeOH (0.3 M, 6.0×10^{-2} mmol) and the column purged with 5% acetic acid **23** in MeOH to regenerate the crown ether **22**. Using this approach, 0.35 mmol g^{-1} of tyramine **1** was released with an RSD of 4.3% (n=10), demonstrating a dramatic increase in reproducibility cf. the 11.4% obtained in batch.

While the use of MeOH was found to be ideal for complex formation, decomplexation and cavity regeneration, the solvent is not suitable for the reactions under investigation herein. As such, a series of alternative solvents were investigated, these included DMF, THF, DCM and MeCN. Unfortunately, when this series of common organic solvents were employed, the immobilized AM-18-c-6 **22** was observed to swell, leading to blockages within the reactor and inconsistencies in the volume of solution passing through the packed bed at any one time. This observation was attributed to the low degree of crosslinking within the carboxypolystyrene **18** (1% DVB) employed as a solid support and was confirmed by packing the reactor with polystyrene crosslinked with 2% DVB whereby no swelling was observed. Regrettably, carboxypolystyrene **18** was not available with a higher degree of crosslinking and as such, 3-carboxypropyl functionalized silica gel was evaluated as an alternative, non-swelling support material.

2.1.3. Preparation and evaluation of SILICA AM-18-c-6

Having successfully immobilized AM-18-c-6 **17** onto carboxypolystyrene **18** via the acid chloride, an analogous approach was employed for the derivatization of 3-carboxypropyl functionalized silica gel, as depicted in Figure 4, affording a loading of 0.16 mmol g^{-1}. Prior to performing a reaction, material's **24** stability to DMF, THF, DCM and MeCN was evaluated and unlike immobilized crown ether **22**, all solvents were able to be pumped through the reactor at 100 μL min^{-1} with no sign of swelling or restricted flow over a period of 8 h. Having demonstrated the materials stability, its ability to complex tyramine HCl **13** and release tyramine **1** was evaluated. Employing a solvent stream of MeOH, tyramine HCl **13** (0.12 M, 2.4×10^{-2} mmol) was introduced into the flow reactor, containing SILICA AM-18-c-6 **24** (0.15 g, 2.4×10^{-2} mmol) at a flow rate of 100 μL min^{-1}. Decomplexation was again achieved using KCl **14** in MeOH (0.12 M, 2.4×10^{-2} mmol) and the column washed with 5% acetic acid **23** in MeOH to regenerate the crown ether **24**. Using this approach, a loading of 0.16 mmol g^{-1} was obtained, which was in agreement with ICP-MS analysis performed on the respective potassium complex.

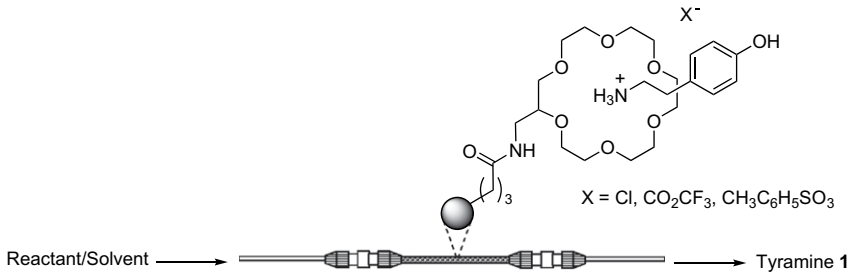
2.1.4. Evaluation of complex stability

Once complexed, it was important to determine how stable the ammonium salt was to various solvents and reactants that may be employed in the derivatization of the complexed material. To ensure the investigation provided general conclusions for this non-covalent protecting group strategy, three ammonium salts were investigated (tyramine HCl **13**, then tyramine TFA **12** and finally tyramine *p*-TSA **25**). As before, complexation was achieved by injecting a 200 μL plug of the tyramine salt under investigation (0.12 M, 2.4×10^{-2} mmol) into a continuous MeOH stream (100 μL min^{-1}) (Scheme 5), which ensured complete washing of the resin prior to evaluating the stability of the complex under the selected reaction condition, followed by decomplexation with KCl **14** in MeOH (0.12 M, 2.4×10^{-2} mmol) and analysis by HPLC (Tables 2–5).

2.1.4.1. Solvent stability. To determine the stability of the ammonium salts of tyramines **12**, **13** and **25**, each solvent illustrated in Table 2 was pumped through the reactor at 100 μL min^{-1} for 5 min prior to analysis of the reactor effluent by HPLC; all solvents were evaluated five times and the average resulted is presented. With the

Table 2
Evaluation of complex stability to a series of common organic solvents (n=5)

Solvent	Stability of complex (%)		
	Tyramine HCl **13**	Tyramine TFA **12**	Tyramine *p*-TSA **25**
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



Scheme 5. Set-up used to evaluate the stability of tyramine HCl **13**, TFA **12** and *p*-TSA **25** salts to an array of reactants and solvents.

Table 3
Summary of the stability of tyramine HCl **13**, TFA **12** and *p*-TSA **25** to a variety of common amines in MeOH (n=5)

	Amine	pK_b	Stability of complex (%)		
			13	**12**	**25**
3°	Et_3N **21**	11.06	100	100	100
3°	DIEA	10.50	48	54	69
3°	*N*-Methylpiperidine	10.08	51	53	68
3°	TMEDA **26**	6.10	0	0	0
3°	Lutidine	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	4.63	27	58	78
1°	Benzylamine	9.33	50	64	73
1°	2-Phenylethylamine	9.58	39	61	53
1°	3-Phenylpropylamine	9.68	49	57	63

Table 4
Stability of tyramine HCl **13** complexed SILICA AM-18-c-6 **24** when exposed to a plethora of common reactants

Substrate type	Compound	Stability of complex (%)
Cation/anion	KOH	0
Cation/anion	NaOH	0
Cation/anion	LiOH	0
Cation/anion	KCl	0
Cation/anion	NaCl	0
Cation/anion	LiCl	0
Cation/anion	K_2CO_3	46
Cation/anion	Na_2CO_3	0
Cation/anion	Li_2CO_3	50
Reactant	Acetyl chloride **3**	100
Reactant	Acetic anhydride **26**	100
Reactant	DMAP	47
Reactant	DCC	45
Reactant	EDCI	50
Reactant	Methyl iodide **27**	0
Acid	TFA **38**	100
Acid	HCl	100
Acid	Acetic acid **23**	100
Acid	Sulfuric acid	100

Table 5
Summary of the ammonium salts evaluated under continuous flow and their ability to form complexes with SILICA AM-18-c-6 **24**

Effect	Amine	Complexation (%)
Cation	Tyramine *p*-TSA **25**	100
Cation	Tyramine TFA **12**	100
Cation	Tyramine HCl **13**	100
No cation	Tyramine **1**	0
Free amine	Aniline	0
Free amine	Benzylamine	0
Free amine	2-Phenylethylamine	0
Free amine	3-Phenylpropylamine	0
Chain length	Aniline HCl	100
Chain length	Benzylamine HCl	100
Chain length	Phenylethylamine HCl	100
Chain length	Phenylpropylamine HCl	100
Chain length	4-Aminophenol HCl **35**	100
Steric	L-β-Alanine benzyl ester HCl **29**	0
Steric	(*S*)-(−)-2-Amino-3-phenyl-1-propanol HCl **30**	0
Functionality	Benzamide HCl **31**	0

exception of DMF, the complexes were found to be stable to all solvents investigated and interestingly, the proportion of tyramine **1** displaced by DMF ranged from 66 to 82% depending on the ammonium salt under investigation. The results from this study herein suggest therefore that the basic nature of DMF induces decomplexation, thus removing the protecting capacity of the crown ether and potentially resulting in undesirable reaction of the free amine functionality. Mascagni and Hyde[5a] also observed this trend, *p*-TSA>TFA>HCl, suggesting that stability increased as a function of cation conjugation.

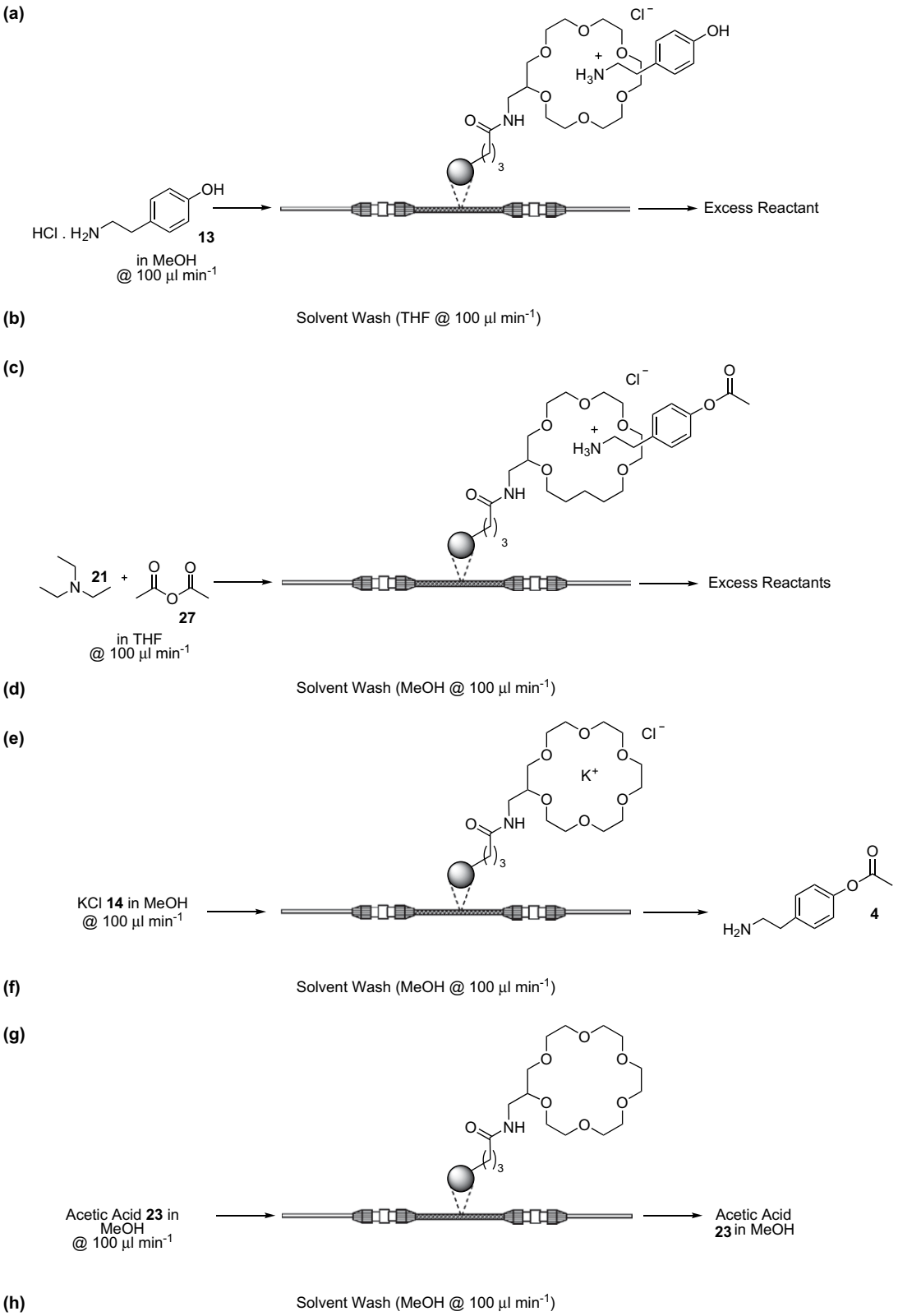
2.1.4.2. Complex stability in the presence of amines. In addition to the use of organic solvents, many reactions that require the protection of amine functionalities involve the use of compounds containing other amine moieties; as such it was important to identify, which of those reagents were compatible with the protecting group strategy under investigation. Using the aforementioned complexation strategy, the effect of an array of amines (0.12 M, 2.4×10^{-2} mmol) on the complex stability was investigated, with the data presented in Table 3 highlighting an obvious trend of increased complex stability towards 3° amines; with 1° amines causing the greater degree of destabilization. This general trend of complex stability, 1°<2°<3°, has some obvious exceptions such as lutidine and Et_3N **21**, which have no destabilizing effect, an observation that is attributed to their steric bulk. As a result, however, these bases may be useful as reagents in future reactions, such as in the deprotonation of the phenolic moiety in the model reaction.

Interestingly, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine **26** (TMEDA) was found to afford quantitative decomplexation for all three salts **12**, **13** and **25**; an observation that compares favourably with reports by Hyde et al.[5b] who found diisopropylethylamine (DIEA) induced decomplexation of an ammonium salt. In comparison to TMEDA **26**, however, DIEA afforded only 31–52% decomplexation, depending upon the salt employed. As such, the advantages of TMEDA **26** as a decomplexation agent are discussed in Section 2.1.5.

2.1.4.3. Stability to common reactants and by-products. Further to investigating the complex stability to possible bases and solvents, it was also important to consider other reagents common to synthetic reactions that require the protection of amines. Table 4 illustrates the stability of the tyramine HCl **13** complexed with SILICA AM-18-c-6 **24** when exposed to a plethora of possible reactants (0.12 M, 2.4×10^{-2} mmol) such as those used for peptide couplings, acetylations and methylations.

Common reagents for acylation (acetyl chloride **3**, acetic anhydride **27**) and alkylation (methyl iodide **28**) were observed to have no destabilizing effect, however, reagents employed in coupling reactions such as DMAP, DCC and EDCI were all found to cause varying degrees of decomplexation. This is an observation that would go some way towards explaining the poor reaction control reported by Mascagni et al.,[5] as upon deprotection, the amino acid could take part in random solution phase couplings to afford oligomers.

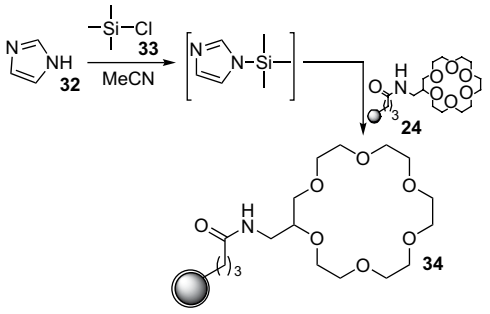
Entries 1–9 (Table 4) were investigated for the purpose of gaining further understanding into the decomplexation process as well as identifying possible reagents for use in future reactions; all solutions evaluated were saturated in MeOH. Of the three metal salts investigated, lithium was found to have the least affinity for the 18-c-6 ether due to its small cation size, followed by sodium. In addition to decomplexation, potassium hydroxide was found to be unsuitable for use with the solid-supported crown ether **24** as it was found to cleave AM-18-c-6 **17** from the support (confirmed by ICP-MS analysis). In comparison to the metal alkoxides, carbonates are weaker inorganic bases, which exhibit relatively poor dissociation in solution, resulting in a lower concentration of available



Scheme 6. Schematic illustrating the reaction steps used to evaluate the synthesis of tyramine acetate **4** under continuous flow.

metal ions, which leads to the observed increase in complex stability.

In addition, acid catalyzed reactions are also commonly employed and thus a range of acids, which are known to remove inorganic ions from crown ether cavities, were tested for their effect on ammonium complex stability, this time focusing on the tyramine HCl **13** complex as it had been shown to be the least stable of the three salts evaluated thus far. As Table 4 illustrates, it was pleasing to see that the acids evaluated were found to have no effect on the complex stability and thus could be



Scheme 7. Schematic illustrating the end-capping protocol employed to prepare TMS SILICA 18-c-6 **34**.

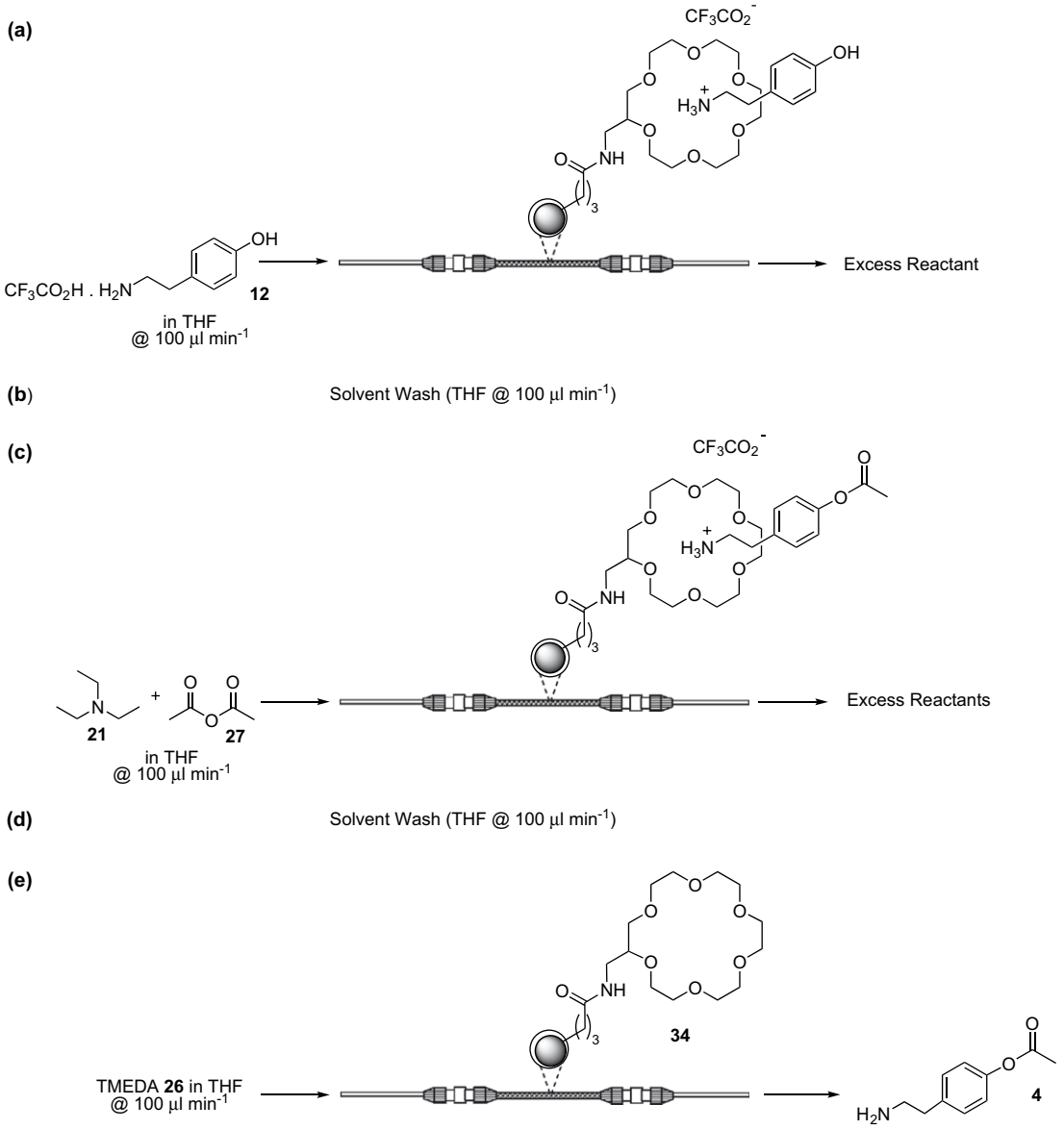
employed as reactants alongside this non-covalent protecting group strategy.

2.1.4.4. Generality of complexation. Recognizing the need for the technique to *N*-protect compounds other than tyramine **1**, a general study was undertaken in order to evaluate factors such as chain length, steric hinderance, as well as the effect of counterions. Again MeOH was used as the reaction solvent (100 μL min^{-1}), reactant concentrations of 0.12 M (2.4×10^{-2} mmol) were employed, methanolic KCl **14** (0.12 M, 2.4×10^{-2} mmol) afforded decomplexation and the reaction products were analyzed off-line by HPLC.

Table 5 summarizes the results obtained and confirms initial observations whereby no complexation is observed for free amines. In keeping with previous findings the HCl salts of all amines investigated, independent of chain length or aromaticity, were found to complex efficiently. The issue of steric hinderance on complexation was also evaluated, employing L-β-alanine benzyl ester HCl **29** and (*S*)-(−)-2-amino-3-phenyl-1-propanol HCl **30**, in both cases no complexation was observed. Benzamide HCl **31** also failed to complex due to resonance effects experienced by the carbonyl group, which reduces hydrogen bonding between the crown ether and results in the formation of an unstable complex.

2.1.5. The selective acetylation of tyramine

Having successfully developed a supported crown ether **24** capable of sequestering ammonium ions and subsequently evaluated



Scheme 8. Summary of the protocol employed for the continuous flow acetylation of tyramine **1**, using TMS SILICA AM-18-c-6 **34**.

the stability of the complex to a range of common reaction conditions, the final step of the investigation was to perform a reaction on the exposed phenolic moiety. When conducting the acetylation of tyramine in batch, NaH **2** was employed as the base (Scheme 1), however, when employing a crown ether as the protecting group, the presence of sodium is undesirable due to its ability to deprotect the amine (Table 4), consequently Et_3N **21** and acetic anhydride **27** were selected suitable as reactants for the transformation.

With this in mind, Scheme 6 summarizes the proposed reaction sequence for the continuous flow acetylation of tyramine **1**, comprising of N-protection (step (a)), followed by O-acetylation (step (c)), deprotection (step (e)) and crown ether regeneration (step (g)) all punctuated with solvent wash (steps (b), (d), (f) and (h)). Upon evaluation of the reaction under continuous flow, it was disappointing to observe that analysis of the reaction products obtained from step (c) (Scheme 6), afforded only tyramine **1** (2.4×10^{-2} mmol). Therefore, in order to promote the acetylation (step (c)), a range of flow rates (10–200 μL min^{-1}), hence reaction times, were subsequently investigated; unexpectedly all flow rates failed to prepare the desired product **4**. Based on this observation it was postulated that the THF flush performed between reaction steps was not removing MeOH from the reactor prior to the introduction of the acetylating reagents and hence the volume of THF was increased (from 1 mL to 2 mL), however, all subsequent reactions failed, yielding only un-reacted tyramine **1** (2.4×10^{-2} mmol).

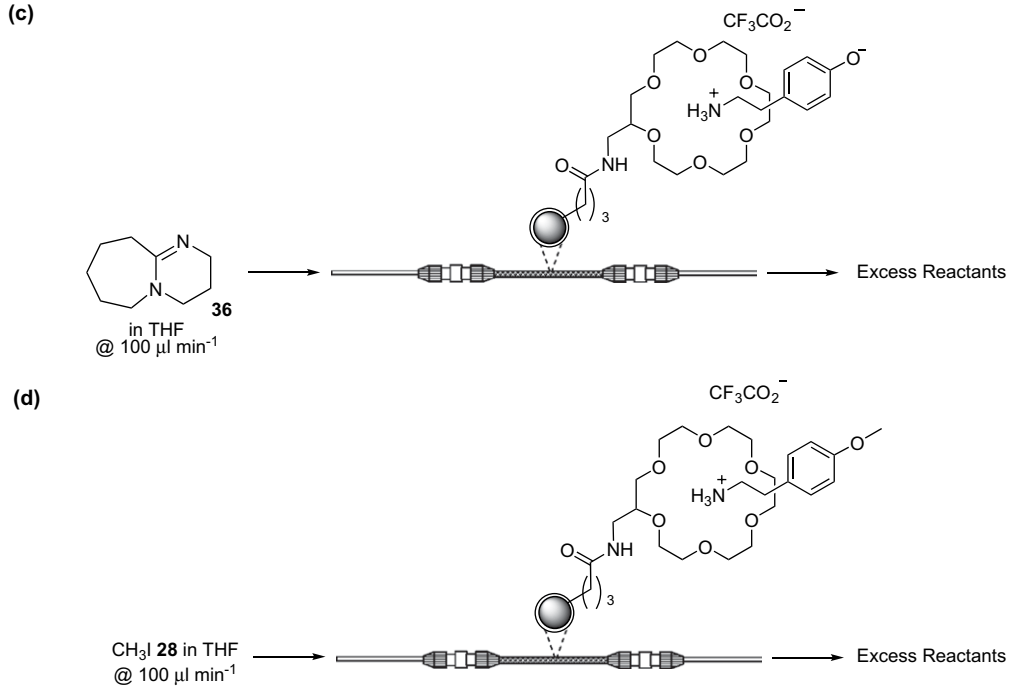
As Mascagni[5] and the work conduct within our laboratory had shown analogous reactions to be possible in the solution phase, the only remaining explanation for the reaction failing was an interaction between the reagents and the solid-support itself, resulting in either quenching or adsorption of the reactants. Consequently, the SILICA AM-18-c-6 **24** was treated with imidazole **32** and chlorotrimethylsilane **33** in MeCN to afford trimethylsilane (TMS) end-capped SILICA AM-18-c-6 **34** (Scheme 7), rendering the support hydrophobic (represented as a white sphere around the solid support).[8]

The TMS modified material **34** was subsequently filtered, washed with acetone and oven dried prior to packing into the flow reactor (0.15 g, 2.4×10^{-2} mmol). At this stage, the reaction sequence illustrated in Scheme 6 was repeated, affording 42% conversion to tyramine acetate **4** and 58% un-reacted tyramine **1**; quantified via internal standardization with biphenyl. Although a proportion of the *N*-protected tyramine had been successfully acetylated and no *N*- or di-acetylation was observed, the detection of un-reacted tyramine **1** was again attributed to the presence of residual MeOH, owing to its use as a reaction solvent for the complexation and decomplexation steps ((a) and (e)). Consequently, all efforts to remove MeOH from the process were, therefore, investigated.

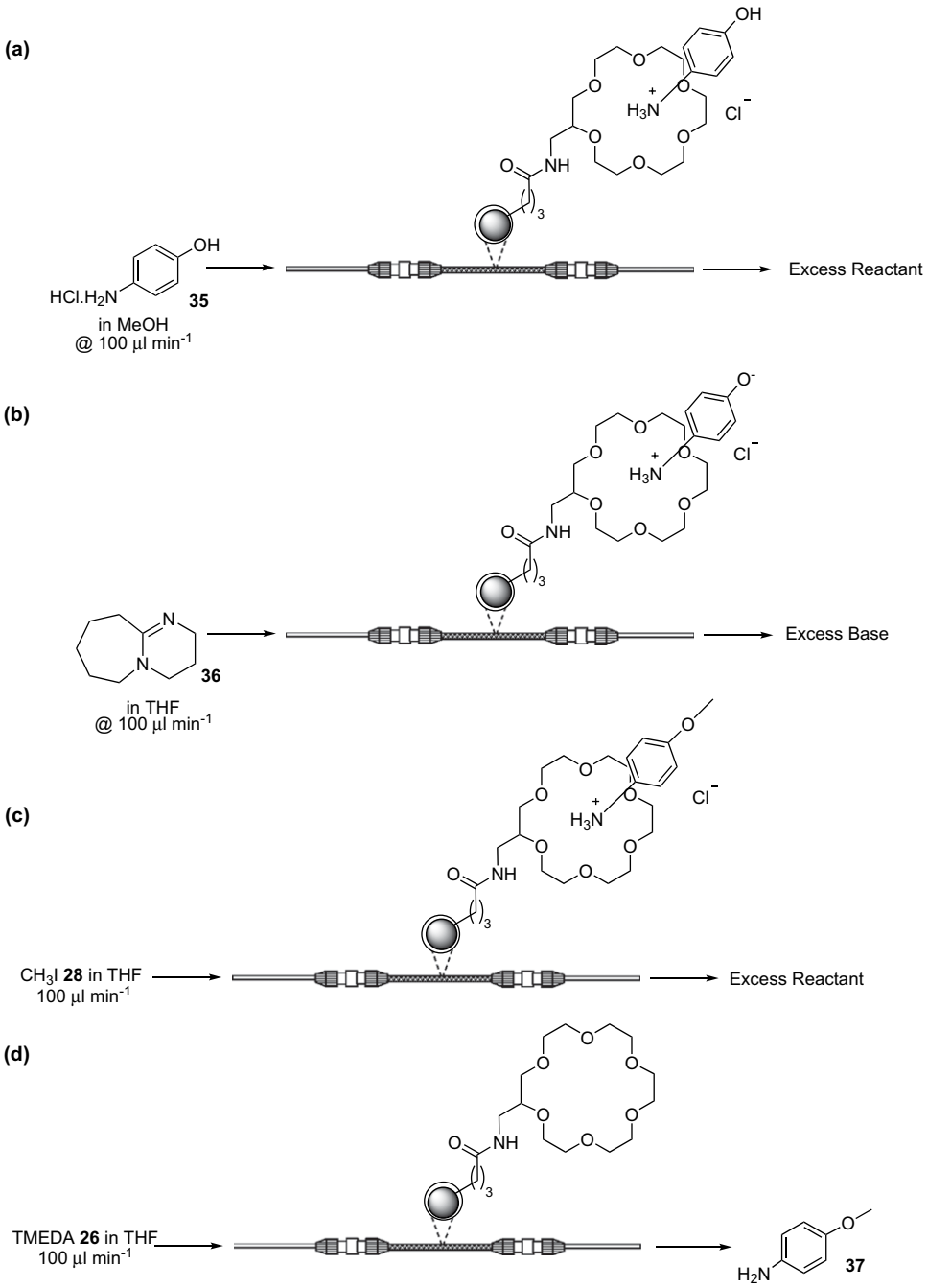
To achieve this, two approaches were investigated, the first involved exploring an alternative decomplexation strategy as KCl **14** was found to be insoluble in THF. As Table 3 illustrates, TMEDA **26** (0.12 M, 2.4×10^{-2} mmol) was shown to quantitatively decomplex all three salts evaluated and owing to its increase solubility in THF cf. KCl **14** it provided an alternative means of decomplexation (% RSD=4.4 (n=5)). Furthermore, by employing an organic base, the regeneration steps (f), (g) and (h) illustrated in Scheme 6 became unnecessary as TMEDA **26** destabilized the complexed amine without residing within the cavity, thus reducing the reaction cycle to complexation, reaction and decomplexation. Using the modified decomplexation strategy, the reaction was repeated and found to afford 63% conversion to tyramine acetate **4** and 27% residual tyramine **1**. At this stage, the complexation step (a) was the only methanolic step remaining, which proved necessary due to the insolubility of tyramine HCl **13** in non-polar solvents. To circumvent this, tyramine TFA **12** was employed, which was found to be extremely soluble in THF, alongside TMEDA **26** as illustrated in Scheme 8. Using the refined protocol, complete removal of MeOH from the reaction enabled 100% conversion of tyramine TFA **12** to tyramine acetate **4**, affording 2.4×10^{-2} mmol $reaction^{-1}$ (4.3 mg) (Scheme 8).

2.1.6. Further reactions of N-protected compounds

Having demonstrated the ability to complex a bifunctional compound and selectively *O*-acetylate it under continuous flow (Scheme 8), the next step of the investigation was to evaluate the



Scheme 9. Schematic illustrating the consecutive reaction steps required for the synthesis of 2-(4-methoxyphenyl)ethylamine.



Scheme 10. Summary of the reaction protocol employed for the continuous flow synthesis of 4-methoxyphenylamine **37**.

generality of the protecting group strategy. With the ability to complex other bifunctional compounds previously illustrated (Table 5), this prompted us to investigate the acetylation of 4-aminophenol. Once again, the use of MeOH as a complexation solvent for 4-aminophenol HCl **35** (0.12 M, 2.4×10^{-2} mmol) was found to be problematic, affording only 84% conversion to 4-aminophenyl acetate. Replacing MeOH with THF and employing the protocol depicted in Scheme 8, quantitative conversion to 4-aminophenyl acetate was obtained, again affording a throughput of 2.4×10^{-2} mmol reaction^{-1}.

2.1.6.1. Alkylations. Having acetylated two bifunctional compounds, the scope of the technique was extended to the O-alkylation of tyramine TFA **12** and 4-aminophenol HCl **35**, to afford the respective methyl esters. However, unlike the acylations, where a pre-mixed solution was employed to achieve deprotonation and acetylation, the reagents selected for the alkylation, namely methyl iodide **28** and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) **36**,[9,10] could potentially react together. As such, the steps were performed separately, as illustrated in Scheme 9, employing DBU **36** (0.12 M, 2.4×10^{-2} mmol) in THF at 100 µL min^{-1}, followed by methyl iodide **28** (0.12 M, 2.4×10^{-2} mmol) in THF at 100 µL min^{-1}. Decomplexation was again achieved using TMEDA **26** and analysis of the reaction products by HPLC confirmed quantitative conversion of tyramine TFA **12** to 2-(4-methoxyphenyl)ethylamine.

Using analogous reaction conditions to those reported for the alkylation of tyramine **1**, the methylation of 4-aminophenol HCl **35** (0.12 M, 2.4×10^{-2} mmol) was investigated and afforded 100%

conversion of the starting material to 4-methoxyphenylamine **37** (2.4×10^{-2} mmol) (Scheme 10), with no *N*-methylation or dimethylation observed.

In addition to the generality observed for both the complex formation and subsequent reaction of the protected compounds, it must be noted that a full 12 months after the initial investigation was performed, the alkylation of 4-aminophenol HCl **35** was repeated using the same aliquot of TMS SILICA AM-18-c-6 **34** and found to afford analogous results, demonstrating long term stability of the immobilized crown ether **34**.

3. Conclusions

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 removing the selectivity issues associated with the reaction of bifunctional amines. 18-Crown-6 ethers have been shown to efficiently complex ammonium salts and examples of reactions employing these complexes have been reported within the literature. Application of the technique to the protection of amines was, however, limited due to problems largely associated with the removal of the crown ether from the resulting reaction product. It was, therefore, proposed that through the immobilization of an 18-crown-6 ether derivative, that many of the issues that have prevented adoption of this technique could be overcome.

Having investigated a range of immobilization strategies and crown ether derivatives, it was found that the covalent immobilization of AM-18-c-6 **17** onto 3-carboxypropyl functionalized silica gel (0.16 mmol g^{-1}) combined the properties of efficient complexation and suitability for use in a continuous flow reactor. Using the aforementioned material, a plethora of ammonium salts were complexed (Table 5) and their stability to a wide range of solvents (Table 3) and reactants (Table 4) was evaluated.

Once the scope and limitations of the operating conditions had been evaluated, reaction of the complexed tyramine salt was performed and found to afford the selective O-acetylation, providing an efficient route to the synthesis of tyramine acetate **4** cf. the laborious route required when employing covalent protecting groups (Scheme 2). Having demonstrated the quantitative conversion of tyramine TFA salt **12** to the free tyramine acetate **4**, the investigation was extended to *O*-alkylation, demonstrating again the selective synthesis of 2-(4-methoxyphenyl)ethylamine in quantitative conversion, with no sign of competing di-alkylation or *N*-alkylation products. The generality of the technique was subsequently explored using 4-aminophenol HCl **35**, which enabled the facile synthesis of 4-aminophenyl acetate and 4-methoxyphenylamine **37** in quantitative yield, respectively. In all cases, the free amine was afforded and products were obtained in higher purity than those prepared using conventional *N*-protecting group strategies.

In summary, the work described herein presents a broad investigation into the viability of immobilized crown ethers as a replacement for traditional covalent *N*-protecting group chemistry and combines their use with continuous flow technology to afford a technique that has potential for future automation.

4. Experimental section

4.1. Reagents and materials

Unless otherwise stated, the chemicals employed herein were used as received and purchased from Sigma Aldrich, Acros and Avocado. Where available, reactions were performed using puriss grade solvents, which were stirred over molecular sieves (<0.005% H_2O) (Fluka, UK), with the exception of DCM and *tert*-butanol, which were of laboratory grade (Fisher Scientific, UK). All chromatography employed HPLC grade solvents (Fisher Scientific) and purified water (5 MΩ cm^{-1}) was prepared by reverse osmosis and ion exchange using a water purifier (Elgast, UK) fitted with an Option 4 cartridge. PTFE tubing (1/16″ o.d.×800 μm i.d.), femal 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 Supelco (UK) and Kinesis (UK). Borosilicate glass capillary (3 mm i.d.) (Duran®, UK) was cut into the desired 50 cm lengths and flame polished.

4.2. 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 TMS as the 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 Ionization (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 (UK) SMP10 apparatus and are reported 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 degasser, two PU-1580 pumps, an HG-1580-32 mixer, a UV-1575 detector and an AS-1555 autosampler. Analytical measurements were made using a Jupiter 10 μm C18, 300 A (250×4.60 mm) column (Phenomenx, UK). Reagents and solutions were delivered to the continuous flow reactor using a Harvard syringe pump (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 introduces into the continuous flow reactor using a Rheodyne injector valve, model 7125 (Supelco, UK).

4.3. HPLC method

Using a 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 30 min of the method. Both the organic phase (MeOH) and aqueous phases contained 0.1% TFA. An injection volume of 20 μL was employed and biphenyl used as the internal standard.

4.4. Preparation of silica gel immobilized aminomethyl-18-crown-6 ether 24

Thionyl chloride **19** (0.37 mL, 5.11 mmol) was added to a stirred solution of oven dried 3-carboxypropyl functionalized silica gel (1.06 g, 1.6 mmol g^{-1}, 200–400 mesh) in toluene (20 mL) and the reaction mixture heated to reflux for 3 h. 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 **17** (0.50 g, 1.70 mmol), followed by triethylamine **21** (0.26 mL, 1.86 mmol). The reaction mixture was stirred overnight, under N_2, prior to filtration under vacuum. The supported crown ether **24** 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 0.162 mmol g^{-1}.

4.5. Silanisation of SILICA AM-18-c-6 24 to afford TMS SILICA AM-18-c-6 34

Imidazole **32** (1.36 g, 20.00 mmol) and chlorotrimethylsilane **33** (2.51 mL, 20.00 mmol) were added to a stirred solution of SILICA AM-18-c-6 **24** (0.50 g, 0.16 mmol g^{-1}) in MeCN (10 mL) under N_2. After stirring at room temperature for 1 h, the reaction mixture was filtered under suction and washed with MeCN (20 mL), acetone (20 mL) and DCM (20 mL) prior to oven drying at 60 °C to afford a free flowing white powder **34**.

4.6. Flow reactor set-up

As illustrated in Figure 3, 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 (800 μm 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)×3 mm (i.d.)), packed with ~0.15 g of the immobilized crown ether under investigation, the inlet of which was connected to the Rheodyne valve via outlet 3. 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.

4.7. General flow reaction protocol

Prior to performing a flow reaction, the solvent under investigation (THF, MeOH or DCM) was driven through the packed-bed reactor at 100 μL min^{-1} to waste.

4.7.1. Crown ether complexation

To prepare the immobilized crown ether complex, the sample loop was filled with a solution of the ammonium salt under investigation (200 μL) 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 100 μL min^{-1} and the reactant stream diverted to waste.

4.7.1.1. Acetylation of the ammonium complex. To acetylate the immobilized crown ether complex, a pre-mixed solution of acetic anhydride **27** and Et_3N **21** (200 μL) in THF was pumped through the reactor at 100 μL min^{-1}, which facilitated both deprotonation of the phenolic moiety and the subsequent acetylation in a single step. THF was then pumped through the system for 2 min, prior to initiating decomplexation.

4.7.1.2. Alkylation of the ammonium complex. To alkylate the immobilized crown ether complex, a solution of DBU **36** (200 μL) in THF was pumped through the reactor at a flow rate of 100 μL min^{-1}, followed by THF for 2 min prior to the introduction of the alkylating agent (200 μL) in THF at 100 μL min^{-1}. This process was found to enable deprotonation of the phenolic moiety and subsequent alkylation; throughout this step, the reaction products were diverted to waste.

4.7.2. Decomplexation of the immobilized product

The decomplexing agent, KCl **14** in MeOH (200 μL) or TMEDA **26** in THF (200 μL), was pumped through the packed bed at a flow rate of 100 μL min^{-1} and at this point, the reactant stream was diverted from waste to sample collection; the reaction products were analyzed off-line by HPLC.

4.7.3. Regeneration of the immobilized crown ether

In the cases where KCl **14** in MeOH was employed for the decomplexation step, the immobilized crown ether cavity was regenerated using methanolic acetic acid **23** (200 μL, 5% v/v). 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 steps.

4.8. Flow synthesis of 4-(2-aminoethyl)phenyl acetate 4

Using the general flow procedure detailed above, tyramine acetate **4** was synthesized under continuous flow. To achieve this, tyramine TFA **12** (200 μL, 0.12 M, 2.4×10^{-2} mmol), in THF, was introduced into a continuous stream of THF (100 μL min^{-1}) via a Rheodyne valve. To ensure the tyramine salt **12** had passed through the system containing TMS SILICA AM-18-c-6 **34** (0.15 g, 2.4×10^{-2} mmol) before introducing additional reactants, THF 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 **27** and Et_3N **21** (200 μL, 0.12 M, 2.4×10^{-2} 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 **26** (200 μL, 0.12 M, 2.4×10^{-2} 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[10] (HPLC, t_R=6.3 min) confirmed quantitative conversion of tyramine TFA **12** to 4-(2-aminoethyl)phenyl acetate **4** with a throughput of 4.3×10^{-2} g $reaction^{-1}$ (2.3×10^{-2} mmol).

4.9. Flow synthesis of 4-aminophenyl acetate

Using the general flow protocol detailed above, 4-aminophenyl acetate was synthesized under continuous flow. To achieve this, 4-aminophenol HCl **35** (200 μL, 0.12 M, 2.4×10^{-2} mmol), in THF, was introduced into a continuous stream of THF (100 μL min^{-1}) via a Rheodyne valve. To ensure salt **35** had passed through the system before introducing additional reagents, THF 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 **27** and Et_3N **21** (200 μL, 0.12 M, 2.4×10^{-2} mmol) at a flow rate of 100 μL min^{-1}. The system was purged with THF (2 min) prior to initiating decomplexation using TMEDA **26** (200 μL, 0.12 M, 2.4×10^{-2} 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. Comparison with a synthetic standard (HPLC, t_R=3.2 min) confirmed quantitative conversion of 4-aminophenol HCl **35** to 4-aminophenyl acetate, with a throughput of 2.3×10^{-2} mmol $reaction^{-1}$.

4.10. Flow synthesis of 2-(4-methoxyphenyl)ethylamine

Using the general flow procedure detailed above, tyramine TFA **12** (200 μL, 0.12 M, 2.4×10^{-2} mmol) in THF was introduced into a continuous THF stream (100 μL min^{-1}) via a Rheodyne valve. To ensure tyramine salt **12** had passed through the system containing TMS SILICA AM-18-c-6 **34** (0.15 g, 2.4×10^{-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 **36** (0.12 M, 2.4×10^{-2} mmol), followed by methyl iodide **28** (200 μL, 0.12 M, 2.4×10^{-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 2 min

was allowed before initiating decomplexation via a 200 μL plug of TMEDA **26** (0.12 M, 2.4×10^{-2} mmol) at 100 μL min^{-1}. At this point, the solvent stream was diverted from waste to sample collection and the reaction products analyzed off-line by HPLC (t_R=6.1 min) whereby 100% conversion to 3-(4-methoxyphenyl)ethylamine was obtained (2.3×10^{-2} mmol $reaction^{-1}$).

4.11. Flow synthesis of 4-methoxyphenylamine 37

4-Aminophenol HCl **35** (200 μL, 0.12 M, 2.4×10^{-2} mmol) in THF was introduced into a continuous THF stream (100 μL min^{-1}) via the Rheodyne valve. To ensure 4-aminophenol salt **35** has passed through the system containing TMS SILICA AM-18-c-6 **34** (0.15 g, 2.4×10^{-2} mmol), before introducing further reagents, THF (100 μL min^{-1}) was pumped through the reactor for 2 min. The reaction steps firstly incorporated a 200 μL plug of DBU **36** (0.12 M, 2.4×10^{-2} mmol), followed by methyl iodide **28** (200 μL, 0.12 M, 2.4×10^{-2} mmol); both of which were pumped at a flow rate of 100 μL min^{-1}, facilitating the deprotonation and methylation of the complexed phenolic derivative. Again, 2 min was allowed before initiating decomplexation via a 200 μL plug of TMEDA **26** (0.12 M, 2.4×10^{-2} mmol). At this point the solvent stream was diverted from waste to sample collection and the reaction products analyzed off-line by HPLC, affording 100% conversion with a throughput of 2.4×10^{-2} mmol $reaction^{-1}$; HPLC, t_R=5.7 min.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.12.052.

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