Solid-Phase Assisted N-1 Functionalization of Azamacrocycles

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Abstract: A simple solid-phase assisted strategy for the N-1 functionalization of azamacrocycles is described. Compounds such as cyclen, cyclam and piperazine can be selectively modified by temporary attachment to solid-phase resins providing an efficient and clean method to prepare biomedically interesting moieteies.

Key words: solid-phase synthesis, azo-macrocycles, protecting groups, contrast agents

Our interest in the synthesis and development of contrast agents for biomedical imaging has resulted in the development of a simple solid phase-based methodology for the selective functionalization of azacrowns and their polyacetate derivatives, such as, cyclen (1,4,7,10-tetraazacyclododecane, **1**) and DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid, **2**; see Figure 1).

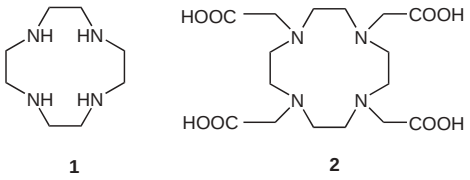

Figure 1 Cyclen (**1**) and DOTA (**2**).

Azacrowns, and their polyacetic acid derivatives are a family of unnatural compounds that display excellent metal chelating properties, especially for heavy metal cations and lanthanides. This makes this class of compounds useful in a host of biological and biomedical applications, as part of MRI contrast agents,[1] luminescent probes,[2] DNA/RNA cleavers,[3] and in radioimmunotherapy medicines[4] for treatment of diseases such as cancer and even anti-HIV therapies. For instance, AMD-3100 (**3**), was recently in a Phase II clinical trials for treatment of HIV[5] and is also being investigated for stem cell immobilization (Figure 2).[6]

Selective N-functionalization (alkylation) is an important step in preparing interesting variants of both azacrowns and their polyacetate derivatives. These approaches are

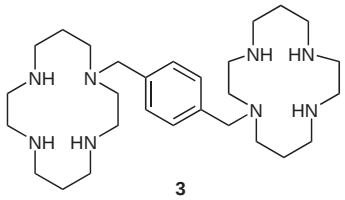

Figure 2 AMD-3100 (**3**).

typically characterized by: (i) introducing the alkyl group prior to the cyclization step,[7] a tedious method in which the choice of alkyl group is limited, (ii) use of large excesses of the expensive polyamines in the presence of alkylating agents[8] or (iii) employing metals to afford temporary protection of the appropriate amine groups.[9] These methods tend to suffer from variable yields, significant amounts of di-and tri-finctionalized by-products and thus difficult purification steps. More recently improved boron[10] and phosphorous[11] protection approaches have been developed, for the functionalization of cyclen (**1**) and cyclam (1,4,8,11-tetraazacyclotetradecane). However, these methods still suffer from production of by-products and are limited to the aforementioned tetraazacrowns

It is known that a range of functional groups (carboxylic acids, alcohols, amines) can be immobilized onto 2-chlorotrityl resin (**4**), affording a temporary protection of that functional group and thus permitting modification of the remainder of the molecule.[12] The bulky resin provides excellent protection against highly nucleophilic and basic conditions. This concept has been used successfully for the functionalization of linear polyamines where the terminal primary amine functional group is immobilized on the resin. For instance, in the synthesis of Philanthotoxin-343[13] and DTPA (diethylenetriaminepentaacetic acid) derivatives.[14] We have extended this methodology for the functionalization of cyclic polyamines, immobilizing via a secondary amine functionalization, to allow production of two classes of compounds namely selectively protected azacrowns (see Scheme 1 and Table 1) and their polyacetate derivatives (see Scheme 2 and Table 2). Our simple methodology allows production of these compounds in good yields (70–98%) and excellent purity (≥96%). These synthons provide invaluable precursors for a range of compounds[1–5] and could also be also useful in labelling of proteins[15] and peptides[16] for imaging purposes in addition to other uses mentioned above.

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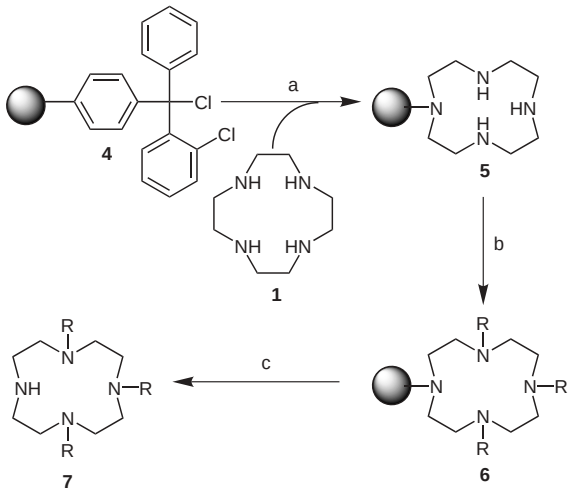


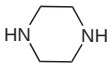
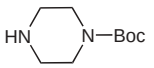
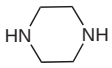
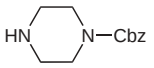
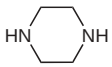
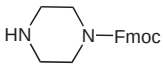
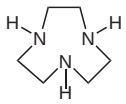
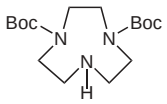
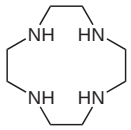
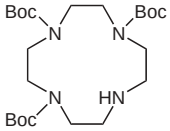
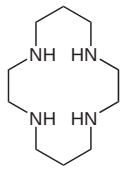
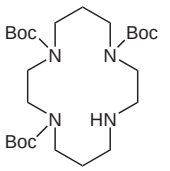
Scheme 1 a) Cyclic polyamines **1** (10 equiv), *i*-Pr_2Et_3N (20 equiv), CH_2Cl_2, r.t., 12 h, MeOH (capping reagent), 10 min; b) protecting group (Boc_2O, CbzCl, FmocCl*) (5 equiv per free amine), Et_3N (10 equiv), CH_2Cl_2, r.t., 4 h; c) 1% TFA in CH_2Cl_2, 3 × 90 s. Pyridine (20 equiv) was used as a base for addition of FmocCl. The scheme shown depicts functionalization of **1**, however **1** can easily be replaced with **8**, **9** and **10**.

Selectively protected cyclic polyamines were synthesized as follows (see Scheme 1):

First resin [2-chlorotrityl resin (**4**), 0.8 $mmolg^{-1}$] was loaded by treatment with excess of a given cyclic polyamine in the presence of base. The sterics of the resin ensured immobilization via one secondary amine functional group only. Excess cyclic polyamine was isolated by filtration and recycled. Free secondary amine functional groups of the resin bound cyclic polyamine were then functionalized with a range of possible protecting groups (Boc, Cbz and Fmoc). In each case the chloranil test[17] was used to check for incomplete reaction products. Final products were cleaved easily from the resin under very mild acidic conditions, affording selectively protected cyclic polyamines in good yields and high purity (>97%), as determined by HPLC and ^{1}H NMR and ^{13}C NMR.[18] This methodology is compatible with a range of cyclic amines (see Table 1), including the frequently used cyclen (**1**) and 1,4,7-triazacylonane (**9**).

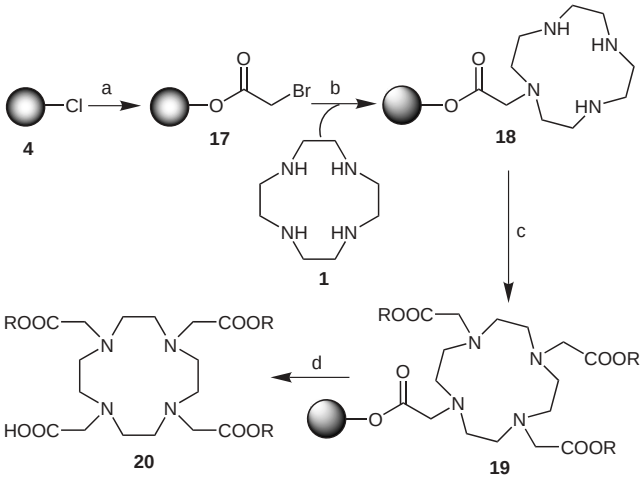
Polyacetate derivatives for selective mono-functionalization were constructed using a similar solid phase methodology by means of a bromoacetate 2-chlorotrityl resin (**17**; see Scheme 2). This resin is commercially available,[12] but was easily made by treatment of 2-chlorotrityl resin (0.8 $mmolg^{-1}$) with excess bromoacetic acid in the presence of base. Treatment of the solid supported bromide with a given cyclic polyamine generated monosubstituted cyclic polyamine (see Scheme 2). Excess unreacted cyclic polyamine was easily recycled as described previously. The remaining free secondary amine functional groups of the resin bound cyclic polyamine were then functionalized with appropriately protected bromoacetate analogues (*t*-butylbromoacetate, 2-benzylbromoacetate). Attempts to obtain polyacetate analogues of 1,4,7-triazacyclonane

Table 1 Selective Protection of Cyclic Polyamines and Azacrowns

Cyclic polyamine	Protecting group (*R*)	Product	Yield (purity, %)[a]
	Boc		98 (≥99)
	Cbz		96 (≥98)
	Fmoc		96 (≥98)
	Boc		88 (≥98)
	Boc		80 (≥97)[b]
	Boc		77 (≥98)[b,c]

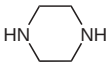
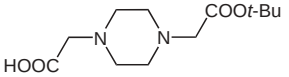
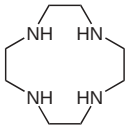
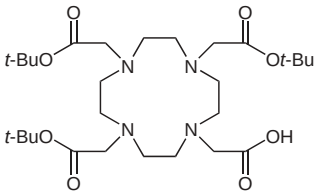
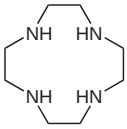
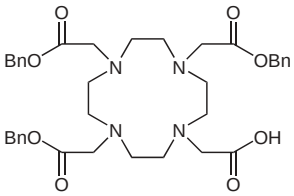
[a] Purity determined by HPLC and ^{1}H NMR.
[b] Yields based on amount of recovered azacrown.
[c] A small silica gel chromatographic was performed to improve purity.

(**9**) and cyclam **10** failed to yield sufficiently pure products under a number of conditions. We attribute this to a combination of poor solubility of starting material and steric factors. Final products were cleaved from the solid support using mild acidic conditions [CH_2Cl_2 (2):trifluoroethanol (1)], rendering the desired products, in good yields and excellent purity.[19]



Scheme 2 a) Bromoacetic acid (10 equiv), *i*-Pr_2Et_3N (20 equiv), CH_2Cl_2, r.t. 12 h, MeOH (capping reagent), 10 min; b) azacrown **1** (10 equiv), *i*-Pr_2Et_3N (20 equiv), CH_2Cl_2, 4 h, r.t; c) *t*-butylbromoacetate/2-benzylbromoacetate (5 equiv per free amine), Et_3N, DMF, 12 h, r.t; d) CH_2Cl_2 (2):trifluoroethanol (1), 2 h, r.t. The scheme shown depicts functionalization of **1**, however **1** can easily be replaced with **8**.

Table 2 Selective Protection of Polyacetate Analogues

Azacrown	R	Product	Yield (purity, %)[a]
	*t*-Bu		82 (≥98)
	*t*-Bu		66 (≥98)
	Bn		69 (≥95)

[a] Purity determined by HPLC and ^{1}H NMR.

In conclusion, we have demonstrated a novel solid phase methodology for selective functionalization of cyclic polyamines, azacrowns and their polyacetate derivatives. This methodology is widely applicable to a broad range of cyclic polyamines, yielding the products in excellent yield and purity in the absence of any purification steps.

Acknowledgment

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(17) Chloranil test: To 1–5 mg of resin add one drop of acetaldehyde in DMF followed by one drop of 2% *p*-chloranil in DMF. Allow to stand at r.t. for 5 min blue beads indicate the prescence of secondary amines.

(18) Sample data for production of protected cyclic amines. Product: Tri-Boc cyclen (**15**). FT-IR (film): ν_{max} = 3417 (amine), 2960 (alkyl), 1712 (carbonyl), 1681 (amide), 1470 (alkyl) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.45 [$C(CH_3)_3 \times 2$], 1.46 [$C(CH_3)_3$], 3.25–3.34 (4 H, m, $CH_2 \times 2$), 3.39–3.46 (8 H, m, $CH_2 \times 4$), 3.54–3.59 (4 H, m, $CH_2 \times 2$). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.1 [$C(CH_3)_3$], 28.2 [$C(CH_3)_3 \times 2$], 46.8 ($CH_2 \times 2$), 47.6 ($CH_2 \times 2$), 50.7 ($CH_2 \times 2$), 52.7 ($CH_2 \times 2$), 82.0 [$C(CH_3)_3$], 82.2 [$C(CH_3)_3 \times 2$], 156.8 (COO*t*-Bu × 2), 157.8 (COO*t*-Bu). MS (ESI+ve): *m/z* = 473 (M + H). FAB-MS: *m/e* calcd for $C_{23}H_{45}N_4O_6$ (M + H): 473.3316; found: 473.3339. HPLC analysis: t_R = 24.0 min, column Vydac C-4 peptide, mobile phases MeCN (0.1% TFA) and H_2O (0.1% TFA), gradient H_2O/MeCN, 0–20 min [100/0] to [0/100], 20–25 min [0/100], 25.1 min [100/0], 40.0 min [100/0], flow rate 1 mL/min.

(19) Sample data for production of selectively protected polyacetic acid derivatives. Product: Tri-*t*-Bu-DOTA (**21**). FT-IR (film): ν_{max} = 3540 (amine), 2957 (alkyl), 2933(alkyl), 2850 (alkyl), 1744(carbonyl), 1632 (amide), 1454(alkyl) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.42 [27 H, s, $C(CH_3)_3 \times 3$], 2.71–2.82 (4 H, m, $CH_2 \times 2$), 2.97–3.12 (8 H, m, $CH_2 \times 4$), 3.26–3.31 (4 H, m, $CH_2 \times 2$), 3.37 (2 H, s, CH_2COO*t*-Bu), 3.36 (2 H, s, br, CH_2COOH), 3.70 (4 H, s, CH_2COO*t*-Bu × 2). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.0 [$C(CH_3)_3 \times 3$], 48.3 ($CH_2 \times 2$), 50.1 ($CH_2 \times 2$), 53.2 ($CH_2 \times 2$), 53.3 (CH_2), 53.4 (CH_2), 55.6 (CH_2COO*t*-Bu), 55.9 (CH_2COO*t*-Bu × 2), 56.7 (CH_2COOH), 81.6 [$C(CH_3)_3$], 81.7 [$C(CH_3)_3 \times 2$], 167.4 (COO*t*-Bu), 169.8 (COO*t*-Bu × 2), 170.5 (COOH). MS (FAB+ve): *m/z* = 573 (M + H). FAB-MS: *m/e* calcd for $C_{28}H_{53}N_4O_8$ (M + H): 573.3863; found: 573.3885. HPLC analysis t_R = 22.0 min, column Vydac C-4 peptide, mobile phases MeCN (0.1% TFA) and H_2O (0.1% TFA), gradient H_2O/MeCN, 0–20 min [100/0] to [0/100], 20–25 min [0/100], 25.1 min [100/0], 40.0 min [100/0], flow rate 1 mL/min.