Synthesis of pendant polyaminopolycarboxylic acid crown ethers: potential ligands for MRI contrasting agents

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The pendant polyamino crown ethers 8 and 11 and polyaminopolycarboxylic acid crown ethers 12 and 15, potentially useful as ligands for MRI contrasting agents as well as NMR shift reagents, have been synthesized from pentaerythritol.

Introduction

The synthesis of crown ethers is of importance since the compounds complex with alkali and alkaline-earth metal ions, which play an important role in biological systems. Further, modern medical techniques such as magnetic resonance imaging (MRI),[1] imaging with radioisotopes[2] and radiotherapy[3] require metal complexes with extreme kinetic and thermodynamic stability to metal ion release. Of the chelating compounds used either for chemical or medicinal purposes, polyaminopolycarboxylic acids are outstanding metal binding agents. Special attention has been paid to chelating agents based on aza crown ethers bearing acetic acid moieties such as diethylenetriaminepentaacetic acid (DTPA) **1**, 1,4,7,10-tetraazacyclodecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid (DOTA) **2** and 1,4,8,11-tetraazacyclotetradecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid (TETA) **3** (Fig. 1) because of their enhanced ligating abilities towards di- and tri-valent cations,[4] especially lanthanides,[5] and the kinetic and thermodynamic stability of their complexes. High stability is achieved with the Gd^{3+} complex of DTPA and $Gd(DTPA)^{2-}$ is the most commonly used MRI contrast agent today.[6] Work in recent years has focused on derivatives of macrocyclic amine carboxylic acids as suitable ligands for this purpose. Additionally, some of the polyaminopolycarboxylic acid ligands are also used as models for calcium binding proteins[7] and as antiviral agents against the viruses HIV-1 and 2.[8]

Compounds containing both crown ether and polyaminopolycarboxylic acid units are unknown and we felt such molecules would have interesting ligating properties. We report here the synthesis of crown ethers containing pendant polyaminopolycarboxylic acid chains, the first ionophores of this kind. These compounds can bind to alkali and alkaline metal ions *via* their crown ether cavity and complex to transition and lanthanide metal ions through their polyaminopolycarboxylic acid arms. The first set of metal ions are important in biological systems and the lanthanides, especially gadolinium, are widely used in MRI agents as well as NMR shift reagents for NMR-detectable alkali metal cations.[9] Our interest in their synthesis is therefore obvious.

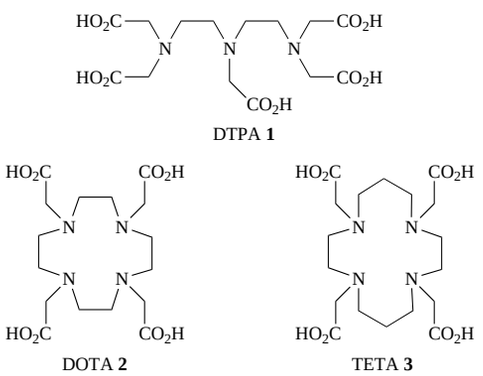
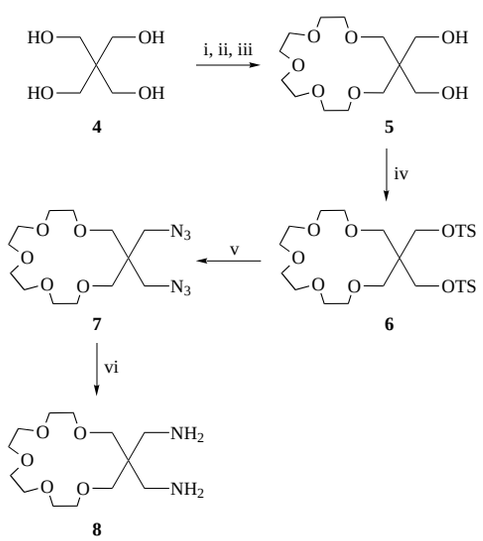


Fig. 1



Scheme 1 *Reagents and conditions:* i, PhCHO, HCl; ii, tetraethylene glycol ditosylate, NaH, THF, reflux, 12 h; iii, 0.05 M aq. H_2SO_4, heat, 12 h; iv, TsCl, NaOH, THF–H_2O; v, NaN_3, DMF; vi, LAH (6 equiv.), THF, reflux

Results

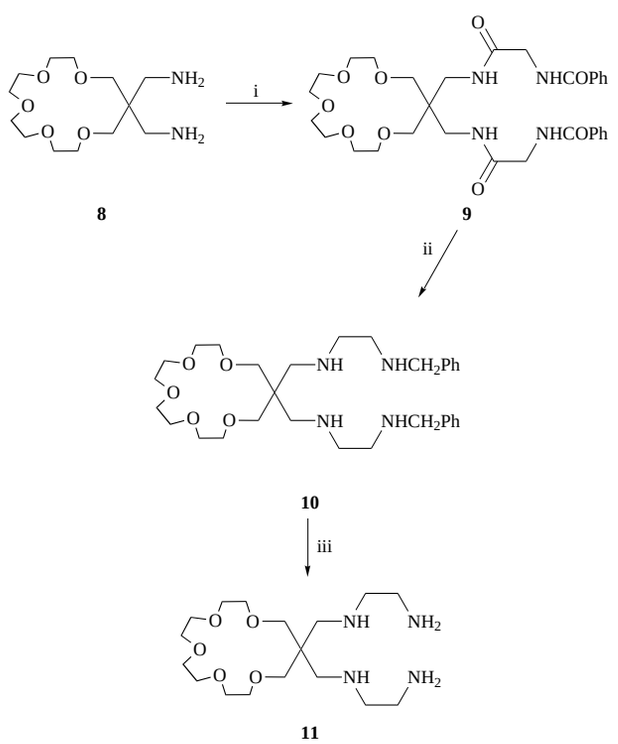
Synthesis of multi-loop spiro crowns with the crown diol moiety **5** was reported by Weber.[10] We realized the utility of **5** and related structures in the synthesis of our polyaminopolycarboxylic acid crown ethers since they provide a crown ether cavity as well as the hydroxy functionality which can be further transformed into the polyaminopolycarboxylic acid unit. Thus, the crown diol **5** was prepared starting from the easily available pentaerythritol **4** (Scheme 1). Treatment of pentaerythritol **4** with benzaldehyde gave the monobenzylidene acetal, which on further reaction with tetraethylene glycol ditosylate [bis-(toluene-*p*-sulfonate)] followed by hydrolysis afforded the required crown diol **5**, the physical and spectral properties of which were in agreement with those reported in the literature.[10]

An initial attempt at tosylation of the crown diol **5**, by treating it with toluene-*p*-sulfonyl chloride in pyridine gave a mixture and the required product could not be obtained in a pure form. Under the conditions employed by Ouchi,[11] we were able to tosylate the crown diol **5** in good yield (80%) and the desired product **6** was obtained as a white crystalline solid. Nucleophilic displacement of the crown ditosylate **6** with azide was performed by refluxing it with sodium azide (6 equiv.) in DMF for 3 days. Under these conditions, the crown diazide **7** was

obtained in almost quantitative yield. The IR spectrum of crown diazide **7** showed the azide absorption at 2100 cm^{-1}. Our initial attempts at reducing the diazide **7** to the diamine **8** with LAH (2.5 equiv.) at room temperature (RT) or at reflux were unsuccessful. Subsequently, other methods of reduction of the crown diazide **7** such as catalytic transfer hydrogenation using 10% Pd–C in cyclohexene[12] or triphenylphosphine–hydrobromic acid–acetic acid[13] were found to be of no avail. Surprisingly, when the reduction of **7** was carried out with a sixfold excess of LAH in THF under reflux, the required crown diamine **8** was obtained in quantitative yield. This compound served as the parent for all our multidentate ligand preparations. The diamine **8** was completely characterized from its spectral and analytical data.

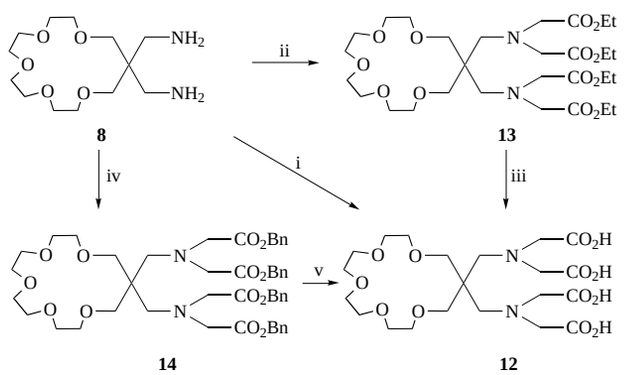
At this stage, our attention was focused on the synthesis of polyaminopolycarboxylic acid crown ethers starting from the crown diamine **8**. The crown diamine **8**, at the most, can only lead to a crown diaminetetraacetic acid **12** with 6 binding sites. In order to obtain compounds with more ligating sites (lanthanides are known to form complexes with higher coordination numbers, *e.g.* 8–12),[9,14] synthesis of the crown tetraamine **11** was planned, which in turn can be transformed to a crown tetraaminehexaacetic acid **15** having 10 binding sites.

Thus, according to Scheme 2, the crown diamine **8** was condensed with hippuric acid in the presence of DCC to afford the diamide **9** (75%). The crown diamide **9** on reduction with LAH (6 equiv.) in THF at reflux afforded the crown *N*,*N*′-dibenzyltetraamine **10** in quantitative yield as a brown syrup. An excess of LAH was needed for the complete reduction of **9** to **10**. Difficulty in purifying **10** due to its highly polar nature precluded its elemental analysis. Hence its hydrochloride salt was prepared by passing dry HCl gas into a dichloromethane solution of **10**. The salt, however, was highly sensitive to moisture. Finally, **10** was characterized as its (tetrakis)hexafluorophosphate salt, a white solid prepared by treating the hydrochloride salt of **10** with ammonium hexafluorophosphate (4 equiv.).



Scheme 2 *Reagents and conditions:* i, hippuric acid, DCC, dichloromethane, 5 °C; ii, LAH, THF, reflux, 12 h; iii, H_2, 20% $Pd(OH)_2$–C, EtOH, 65 psi, 5 h

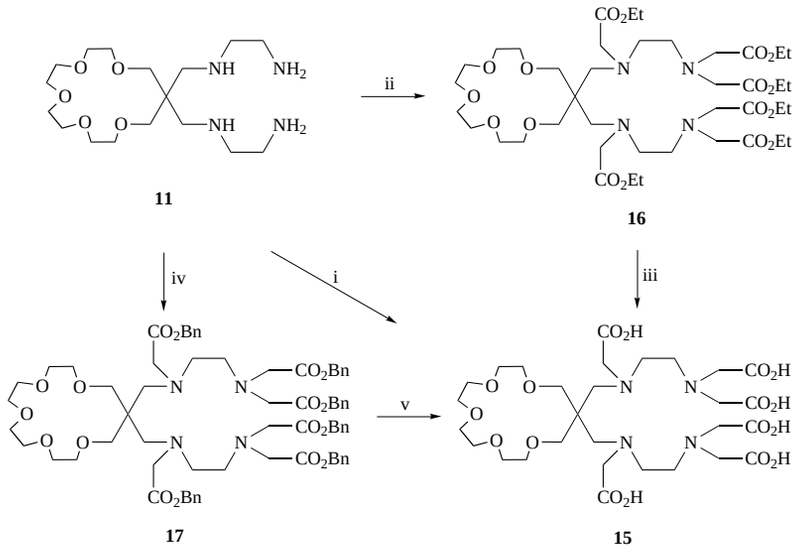
Removal of the *N*-benzyl groups of **10** was attempted next. No cleavage occurred when **10** was treated with: (1) formic acid in methanol and 10% Pd–C,[15] (2) sodium in liquid ammonia,[16] (3) ammonium formate and 10% Pd–C,[17] (4) hydrogenation with 10% Pd–C in the presence of acetic acid[18] and (5) hydrogenation with 10% Pd–C in ethanolic HCl.[19] Finally, the *N*-benzyl group was cleaved using 20% $Pd(OH)_2$–C (Pearlman catalyst).[20] Thus, when the *N*,*N*′-dibenzyltetraamine **10** was hydrogenated in ethanol using 20% $Pd(OH)_2$–C (by weight; 2 equiv.) at 65 psi for 4 h, the desired crown tetraamine **11** was obtained in good yield as a hygroscopic foamy material. During the course of this reaction, it was observed that the amount of 20% $Pd(OH)_2$–C used was a decisive factor. When a catalytic amount (or 1 equiv.) of 20% $Pd(OH)_2$–C was used, cleavage of the *N*-benzyl group was incomplete. This is attributed to the complexation of the crown tetraamine **11** with palladium, thus poisoning the catalyst for further reaction. The hygroscopic nature of **11** precluded direct elemental analysis. For the complete characterization of **11**, preparation of its hexafluorophosphate salt was attempted, but without success. However, the bis(tetraphenylborate) salt of **11** could be prepared by treating **11** in 0.1 M aqueous HCl solution with aqueous sodium tetraphenylborate.



Scheme 3 *Reagents and conditions:* i, $BrCH_2CO_2H$, KOH, H_2O; ii, $BrCH_2CO_2Et$, K_2CO_3, DMF, 100 °C, 48 h; iii, KOH, H_2O, reflux, 12 h; iv, $BrCH_2CO_2Bn$, K_2CO_3, DMF, 120 °C, 72 h; v, H_2, 20% $Pd(OH)_2$–C, EtOH, 65 psi, 3 h

Having the crown diamine **8** and the crown tetraamine **11** in hand, we endeavoured to prepare the corresponding tetra- and hexa-acetic acids as depicted in Schemes 3 and 4. Initially, direct *N*-alkylation using bromoacetic acid[21] was attempted. Thus, when the crown diamine **8** and the crown tetraamine **11** were subjected to *N*-alkylation with bromoacetic acid in the presence of potassium hydroxide, the crude crown diaminetetraacetic acid **12** and the crown tetraaminehexaacetic acid **15** were obtained. Purification of **12** and **15** by either chromatography or ion-exchange techniques was, however, unsuccessful.

An alternative method, also reported in the literature, involves the use of ethyl bromoacetate as the alkylating reagent, followed by hydrolysis[22] to afford the free acid. Accordingly, the crown ethyl esters **13** and **16** were obtained by *N*-alkylation of the corresponding amines **8** and **11** with ethyl bromoacetate in the presence of potassium carbonate. Basic hydrolysis of **13** and **16** yielded the crude crown diaminetetraacetic acid **12** and the crown tetraaminehexaacetic acid **15**, respectively. In this case also purification of **12** and **15** was unsuccessful either by chromatography or ion-exchange techniques. This difficulty perhaps arises due to the use of strongly basic and acidic conditions in the course of hydrolysis and work-up. In order to avoid acidic and basic conditions in the preparation and purification of **12** and **15**, either in reaction or in the work-up procedure, we adopted a modification of the method reported recently by Tweedle.[23] This method comprises the preparation of benzyl esters of **14** and **17**, followed by hydrogenolysis. Accordingly, **8** and **11** were treated with benzyl bromoacetate in the presence



Scheme 4 *Reagents and conditions:* i, $BrCH_2CO_2H$, KOH, H_2O; ii, $BrCH_2CO_2Et$, K_2CO_3, DMF, 100 °C, 72 h; iii, KOH, H_2O, reflux, 12 h; iv, $BrCH_2CO_2Bn$, K_2CO_3, DMF, 120 °C, 72 h; v, H_2, 20% $Pd(OH)_2$–C, EtOH, 65 psi, 5 h

of potassium carbonate to obtain the corresponding benzyl esters **14** and **17** in 54 and 12–15% yields, respectively. Attempts to improve the yield of **17** by treating **11** with benzyl iodoacetate–potassium carbonate or caesium carbonate in DMF as well as in acetonitrile under reflux conditions were of no avail.

Finally, the desired crown diaminetetraacetic acid **12** and the crown tetraaminehexaacetic acid **15** were synthesized by hydrogenolysis of **14** and **17**, respectively, using 20% $Pd(OH)_2$–C (by weight; 2 equiv.) in ethanol.

In conclusion, we have synthesized in a novel and facile way, the crown ethers with pendant amino groups **8** and **11** and polyaminopolycarboxylic acid arms **12** and **15**. These multidentate compounds are useful ligands because of their multiligating sites. Metal-ion complexes of these ligands may find application as MRI agents as well as NMR shift reagents for NMR active alkali metal ions. Their charge and side chains are added points in their favour. Currently, metal ion-binding studies of these multidentate ligands are in progress.

Experimental

Melting points were determined on a Superfit melting-point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AF 200 NMR spectrometer operating at a magnetic field strength of 4.7 T in [^{2}H]chloroform solutions with tetramethylsilane (TMS) as internal standard unless otherwise stated. *J* Values are given in Hz. Elemental analyses were obtained using Perkin-Elmer model 240C-CHN analyser. FAB spectra were recorded on a JEOL SX 102/DA-6000 spectrometer (*m*-nitrobenzyl alcohol as matrix) and were obtained from CDRI Lucknow.

Materials

Unless specified otherwise, reagent grade reactants and solvents were used as received from chemical suppliers. Solvents were dried by appropriate methods wherever needed. All organic extracts were dried over anhydrous magnesium sulfate. Acme silica gel 100–200 mesh and Acme alumina were used for column chromatography.

Crown ditosylate 6

A mixture of sodium hydroxide (1.20 g, 30.0 mmol) in water (6 cm^3) and the crown diol **5** (3.0 g, 10.2 mmol) in THF (12 cm^3) was cooled in an ice-bath with magnetic stirring. After being cooled for 0.5 h the mixture was treated with toluene-*p*-sulfonyl chloride (4.08 g, 21.4 mmol), added in small portions and then stirred for 1 h at 5 °C. The reaction mixture was then poured into ice–water to give a white solid which was filtered off and dried. The product was recrystallized by dissolution in a minimum of ethyl acetate to which a few drops of hexane were added until the solution became turbid. Storage of the solution at RT gave the crown ditosylate **6** (4.90 g, 80%) as a white crystalline solid; mp 124–126 °C (from ethyl acetate–hexane) (Found: C, 53.71; H, 6.35. Calc. for $C_{27}H_{38}O_{11}S_2$: C, 53.80; H, 6.35%); ν_{max}(KBr)/cm^{-1} 3050, 2900, 1600, 1353, 1187 and 1119; δ_H 7.14–7.55 (AA′BB′ system, *J* 8, 8 H), 3.78 (s, 4 H), 3.23–3.35 (m, 20 H) and 2.24 (s, 6 H); δ_C 144.0, 132.0, 129.0, 128.0, 70.80, 70.50, 70.20, 68.50, 68.20, 44.0 and 21.0.

Crown diazide 7

A mixture of the crown ditosylate **6** (3.0 g, 4.97 mmol) and sodium azide (1.95 g, 29.9 mmol) in dry dimethylformamide (15 cm^3) was heated to 120 °C for 3 days and then cooled to RT, diluted with water and extracted with dichloromethane. The organic layer was dried and evaporated and the residue was purified by column chromatography using 50% ethyl acetate–hexane as eluent to give the product **7** (1.68 g, 98%) as a colourless oil (Found: C, 45.70; H, 7.24; N, 22.35. Calc. for $C_{13}H_{24}N_6O_5$: C, 45.34; H, 7.02; N, 24.40%); ν_{max}(neat)/cm^{-1} 2870, 2102, 1454, 1356, 1249 and 1118; δ_H 3.40 (s, 4 H), 3.46 (s, 4 H) and 3.64 (m, 16 H); δ_C 70.24, 51.71 and 45.0.

Crown diamine 8

The crown diazide **7** (1.7 g, 4.91 mmol) in dry THF (10 cm^3) was added dropwise at RT to a stirred suspension of LAH (1.12 g, 29.5 mmol) in dry THF (10 cm^3). After the addition was complete, the reaction mixture was refluxed overnight. The reaction mixture was cooled to RT and quenched by the dropwise addition of saturated aqueous sodium sulfate. The resulting granular precipitate was filtered off and washed with acetone. The filtrate was evaporated and the residue was purified by chromatography on a neutral alumina column using 50% ethyl acetate–acetone as eluent to give the crown diamine **8** (1.40 g, 98%) as a light yellow syrup (Found: C, 53.56; H, 9.64; N, 9.65. Calc. for $C_{13}H_{28}N_2O_5$: C, 53.40; H, 9.65; N, 9.58%); ν_{max}(neat)/cm^{-1} 3315, 2866, 1662, 1450, 1359, 1120, 939 and 879; δ_H 3.60–3.65 (m, 16 H), 3.46 (s, 4 H) and 2.89 (s, 4 H); δ_C 72.24, 70.77, 64.59, 44.59 and 35.59.

Crown diamide 9

DCC (445 mg, 2.15 mmol) was added to a stirred and ice-

cooled solution of hippuric acid (250 mg, 0.86 mmol) in dry dichloromethane (8 cm^3) and then stirred for 30 min at 5 °C. The crown diamine **8** (385 mg, 2.15 mmol) in dry dichloromethane (8 cm^3) was added to the mixture which was then stirred for 3 h with ice cooling and then at RT overnight. After dilution with water, the mixture was further stirred to afford a white solid which was filtered off. The dichloromethane and aqueous layers were separated and the former was dried and evaporated to give a residue. This was chromatographed using ethyl acetate as eluent to obtain the product **9** (400 mg, 76%) as a white, hygroscopic foam (Found: C, 60.38; H, 6.95; N, 9.28. Calc. for $C_{31}H_{42}N_4O_9$: C, 60.56; H, 6.88; N, 9.11%); ν_{max}(neat)/cm^{-1} 3300, 3050, 2850, 1650, 1530, 1300 and 1110; δ_H 7.91–7.94 (d, 4 H), 7.40–7.44 (d, 6 H), 4.12–4.15 (d, 4 H) and 3.35–3.53 (m, 24 H); δ_C 170.19, 167.50, 133.58, 131.69, 128.47, 127.28, 127.10, 74.24, 71.42, 70.50, 70.32, 69.72 and 43.94.

Crown *N,N′*-dibenzyldiamine 10

The crown diamide **9** (400 mg, 0.65 mmol) in dry THF was added dropwise to a stirred suspension of LAH (148 mg, 3.90 mmol) in dry THF (8 cm^3) and the mixture was refluxed overnight under a nitrogen atmosphere. The reaction mixture was then cooled to RT and quenched by the addition to it of saturated aqueous sodium sulfate. The resulting precipitate was filtered off and the cake was washed thoroughly with acetone. The combined organic layer filtrate and washings were dried and concentrated and the residue was chromatographed to yield the pure product **10** (360 mg, 96%) as a brown syrup; ν_{max}(neat)/cm^{-1} 3317, 3028, 2868, 1454, 1358, 1120, 738 and 700; δ_H 7.29 (m, 10 H), 3.76 (s, 4 H), 3.59–3.63 (m, 20 H) and 2.55–2.68 (m, 12 H); δ_C 140.40, 128.20, 128.0, 126.0, 70.60, 70.20, 53.50, 51.80, 49.0, 47.0 and 42.80; *m/z* 559 $(M + H)^+$.

Hydrochloride salt of the crown dibenzyldiamine 10

HCl gas was passed through a solution of the crown *N,N′*-dibenzyldiamine **10** in dichloromethane until it became turbid. The resulting mixture was evaporated to yield the salt as a white hygroscopic solid. Because of its high sensitivity to moisture, the compound could not be characterized and hence its hexafluorophosphate salt was prepared; $\delta_H(D_2O)$ 7.31–7.34 (m, 10 H), 4.14 (s, 4 H) and 3.15–3.53 (br, 32 H).

Tetrakis(hexafluorophosphate) salt of the crown dibenzyldiamine 10

A solution of ammonium hexafluorophosphate (4 equiv.) in methanol was added to a solution of the hydrochloride salt of the crown dibenzyldiamine **10** in methanol to which a few drops of acetone were added until the solution became turbid; the mixture was then allowed to stand at RT. The white solid thus formed was filtered off, washed with methanol–acetone and dried; decomposes at 200 °C (Found: C, 32.45; H, 4.72; N, 4.85. Calc. for $C_{31}H_{54}N_4O_5P_4F_{24}$: C, 32.58; H, 4.76; N, 4.90%); ν_{max}(KBr)/cm^{-1} 3337, 3038, 2430, 1624, 1431, 1271, 1186, 1132 and 721; $\delta_H(D_2O)$ 7.36 (s, 10 H), 4.19 (s, 4 H) and 3.18–3.56 (m, 32 H).

Crown tetraamine 11

The crown *N,N′*-dibenzyldiamine **10** (90 mg, 0.16 mmol) in ethanol (5 cm^3) was hydrogenated using 20% $Pd(OH)_2$–C (200 mg) in a Parr apparatus at 65 psi for 4 h. The catalyst was filtered off and the cake was washed with methanol. Evaporation of the combined filtrate and washings gave the crown tetraamine **11** as a hygroscopic foam (58 mg, 95%); ν_{max}(KBr)/cm^{-1} 3290, 2866, 1574, 1454, 1116 and 943; δ_H 5.50 (br) and 3.35–3.51 (m); δ_C 73.69, 70.63, 70.45, 62.50, 59.23 and 39.73.

Bis(tetraphenylborate) salt of the crown tetraamine 11

An aq. solution of sodium tetraphenylborate (180 mg, 0.52 mmol) was added to a solution of the crown tetraamine **11** (30 mg, 0.07 mmol) in 0.1 M aq. HCl. After complete addition, the white solid formed was filtered off and dried. The product was recrystallized by dissolution in a minimum of ethyl acetate to which solution diethyl ether was added until it became turbid. Refrigeration gave a white solid (60 mg, 74%) which, on drying, became granular; mp 78–80 °C (Found: C, 76.19; H, 7.64; N, 5.18. Calc. for $C_{65}H_{80}N_4O_5B_2$: C, 76.61; H, 7.91; N, 5.49%); ν_{max}(KBr)/cm^{-1} 3425, 3055, 1579, 1479, 1427, 1109, 736 and 707; δ_H 7.80–7.06 (m) and 3.61 (br s).

Crown diaminetetraacetic acid 12: by direct alkylation with bromoacetic acid

A solution of bromoacetic acid (238 mg, 1.71 mmol) in water (2.8 cm^3) was brought to pH 10 with 7 M aqueous KOH, the temperature being maintained <5 °C. To this, a solution of the crown diamine **8** (100 mg, 0.34 mmol) in ethanol (5 cm^3) was added and the reaction mixture was heated to 70 °C for 4 h. The mixture was maintained at pH 10 with 7 M aqueous KOH. After cooling to RT, the reaction mixture was acidified to pH 2 with 47% aqueous HBr and then evaporated. The resulting residue, dissolved in water (1 cm^3), was loaded on a column of Amberlite 120 cation-exchange resin (washed with dilute aqueous HCl). The column was eluted with water (500 cm^3) followed by 2% aqueous NH_3 (500 cm^3). The combined water and aqueous NH_3 fractions were concentrated to afford the product **12** (115 mg, 64%, crude) as a hygroscopic white foam. Further purification either by column chromatography or by preparative TLC was unsuccessful and hence a thorough characterization was not possible; ν_{max}(KBr)/cm^{-1} 3408, 2922, 1743, 1439, 1249, 1109 and 945; $\delta_H(D_2O)$ 3.68–4.08 (m); δ_C 172.59, 74.0, 73.87, 72.47, 62.65, 62.35, 62.0, 60.35, 60.24, 55.94 and 45.88.

Crown *N,N′*-tetraethyl ester 13

A mixture of the crown diamine **8** (100 mg, 0.34 mmol), ethyl bromoacetate (1.20 g, 7.18 mmol) and potassium carbonate was heated at 100 °C for 48 h after which it was cooled to RT and evaporated under reduced pressure. The residue was extracted with dichloromethane and this extract was washed with aqueous sodium hydrogen carbonate, dried and concentrated; the residue was purified by column chromatography with ethyl acetate as the eluent to give the product **13** (140 mg, 64%) as a light-yellow syrup; ν_{max}(neat)/cm^{-1} 2872, 1734, 1662, 1464, 1371, 1186, 1116 and 1032; δ_H 4.09–4.19 (q, 8 H), 3.48–3.64 (m, 28 H), 2.80 (s, 4 H) and 1.22–1.25 (t, 12 H); δ_C 172.08, 70.60, 70.30, 60.30, 57.10, 56.80 and 47.40; *m/z* 637 $(M + H)^+$.

Hydrolysis of the crown tetraethyl ester 13

A mixture of the crown tetraethyl ester **13** (200 mg, 0.31 mmol) and KOH (350 mg, 6.24 mmol) in aqueous methanol (8 cm^3) was heated at 100 °C overnight after which it was neutralized with conc. HCl to pH 7. The solvent mixture was then evaporated under reduced pressure and the residue thus obtained was dissolved in methanol and filtered. Concentration of the solvent gave a hygroscopic foam (145 mg, crude) which was not amenable to further purification; ν_{max}(KBr)/cm^{-1} 3410, 2191, 1734, 1635, 1402, 1248, 1107 and 945; $\delta_H(D_2O)$ 3.50–3.80 (br).

Crown tetrabenzyl ester 14

A mixture of the crown diamine **8** (100 mg, 0.34 mmol), benzyl bromoacetate (470 mg, 2.05 mmol) and anhydrous potassium carbonate (285 mg, 2.05 mmol) in dry dimethylformamide (5 cm^3) was heated to 100 °C for 3 days. The mixture was concentrated under reduced pressure and the residue thus obtained was extracted with dichloromethane. The extract was dried and evaporated and the residue was chromatographed with ethyl acetate as eluent to give the product **14** (165 mg, 54%) as a brown syrup with a fruity smell (Found: C, 66.62; H, 6.88; N, 3.25. Calc. for $C_{49}H_{60}N_2O_{13}$: C, 66.49; H, 6.83; N, 3.16%); ν_{max}(neat)/cm^{-1} 3034, 2876, 1743, 1170, 1114, 736 and 698; δ_H 7.32 (s, 20 H), 5.10 (s, 8 H), 3.43–3.60 (m, 28 H) and 2.81 (s, 4

H); δ_C 171.76, 135.92, 128.56, 128.23, 70.65, 70.40, 70.26, 70.12, 66.10, 57.27, 56.89 and 47.43.

A similar reaction with benzyl iodoacetate in dimethylformamide under reflux conditions gave the same product (64%).

Hydrogenolysis of the crown ether tetrabenzyl ester 14

The crown tetrabenzyl ester **14** (60 mg, 0.07 mmol) was hydrogenated with 20% $Pd(OH)_2$–C (100 mg) in dry ethanol (8 cm^3) at 65 psi in a Parr apparatus for 3 h. After this, the catalyst was filtered off and washed with methanol. Evaporation of the combined filtrate and washings gave the pure crown diaminetetraacetic acid **12** (35 mg, 95%) as a hygroscopic white solid (Found: C, 48.15; H, 6.88; N, 5.31. Calc. for $C_{21}H_{36}N_2O_{13}$: C, 48.08; H, 6.91; N, 5.34%); ν_{max}(KBr)/cm^{-1} 3422, 2926, 1739, 1249 and 1113; $\delta_H(D_2O)$ 3.20–3.80 (br, m); δ_C 170.45, 69.13, 71.22, 59.72, 57.90 and 43.06.

Crown tetraaminehexaacetic acid 15: by direct alkylation of the crown tetraamine 11 with bromoacetic acid

A solution of bromoacetic acid (850 mg, 6.12 mmol) in water (3 cm^3) was brought to pH 10 with 7 M aqueous KOH at 5 °C and then treated with a solution of the tetraamine **11** (115 mg, 0.30 mmol) in ethanol (3 cm^3). The reaction mixture was stirred at 70 °C for 12 h, with addition of 7 M aqueous KOH to maintain it at pH 10. After this the reaction mixture was cooled to RT, acidified to pH 2 with 47% aqueous HBr and then extracted with diethyl ether to remove the organic impurities. The resulting aqueous mixture was concentrated, loaded on a column of cation exchange resin (IR 120, H^+ form) and eluted first with water (100 cm^3) and then by 2% aqueous ammonia (100 cm^3). The two fractions were combined and concentrated to yield the crude product **15** (140 mg, 64%) which, once again, was not amenable to further purification; ν_{max}(KBr)/cm^{-1} 3408, 2926, 1745, 1651, 1454, 1248, 1091 and 945; $\delta_H(D_2O)$ 3.20–3.70 (br).

Crown tetraaminehexaacetic acid hexaethyl ester 16

A mixture of the crown tetraamine **11** (75 mg, 0.20 mmol), ethyl bromoacetate (1.50 g, 9.00 mmol) and potassium carbonate (620 mg, 4.50 mmol) in dry dimethylformamide was heated at 100 °C for 3 days after which dimethylformamide was removed under reduced pressure and the residue was extracted with dichloromethane. The dichloromethane extract was filtered, dried and concentrated and the residue was chromatographed using ethyl acetate as eluent to give the hexaester **16** (100 mg, 56.4%) as a dark brown syrup; ν_{max}(neat)/cm^{-1} 2982, 1732, 1192, 1116, 1032 and 733; δ_H 4.20–4.13 (q, 12 H), 3.63–3.53 (m, 32 H), 2.80–2.75 (m, 12 H) and 1.29–1.22 (t, 18 H); δ_C 172.10, 171.20, 70.40, 70.0, 60.45, 60.16, 56.63, 55.23, 54.61, 52.94, 46.90 and 14.31; *m/z* 895 $(M + H)^+$.

Saponification of the hexaethyl ester 16

A mixture of the hexaethyl ester **16** (150 mg, 0.17 mmol) and KOH (500 mg) in aqueous methanol (8 cm^3) was heated at 100 °C overnight and then concentrated. The residue, loaded on a column of ion exchange resin (IR 120, H^+ form) was eluted with water and then with 2% aqueous ammonia. These two fractions were combined and concentrated under reduced pressure to afford the crude crown tetraaminehexaacetic acid **15** (90 mg, 73% crude) as a yellow hygroscopic solid. Its further purification was not successful for adequate characterization; ν_{max}(KBr)/cm^{-1} 3423, 2935, 1728, 1614, 1462, 1390, 1116 and 1030; $\delta_H(D_2O)$ 3.00–3.90 (br).

Crown tetraaminehexaacetic acid hexabenzyl ester 17

A mixture of the crown tetraamine **11** (100 mg, 0.26 mmol), benzyl bromoacetate (545 mg, 4.75 mmol) and anhydrous K_2CO_3 (330 mg, 2.38 mmol) in dry dimethylformamide (5 cm^3) was heated to 120 °C for 3 days and then evaporated *in vacuo*. The resulting residue was extracted with dichloromethane. The organic layer was dried, filtered and concentrated and the resulting residue was purified by column chromatography using ethyl acetate as the eluent to give the pure product **17** as a brown syrup (40 mg, 12%) (Found: C, 66.98; H, 6.62; N, 4.01. Calc. for $C_{71}H_{86}N_4O_{17}$: C, 67.27; H, 6.83; N, 4.42%); ν_{max}(neat)/cm^{-1} 3034, 2922, 1743, 1498, 1172, 736 and 698; δ_H 7.28–7.36 (m, 30 H), 5.09 (s, 12 H), 3.45–3.55 (m, 32 H) and 2.78 (m, 12 H); δ_C 171.40, 170.60, 135.36, 128.90, 128.30, 128.0, 126.20, 70.50, 68.80, 66.30, 66.0, 55.0 and 46.20.

Hydrogenolysis of the ester 17

The crown hexaester **17** (70 mg, 0.05 mmol) was hydrogenated with 20% $Pd(OH)_2$–C (150 mg) in ethanol (5 cm^3) at 65 psi for 5 h in a Parr apparatus. The catalyst was filtered off and washed with methanol. The combined filtrate and washings were evaporated to dryness to give the crown tetraaminehexaacetic acid **15** (30 mg, 75%) as an hygroscopic foam; because of its hygroscopicity, its melting point could not be determined (Found: C, 48.15; H, 6.98; N, 7.78. Calc. for $C_{29}H_{50}N_4O_{17}$: C, 47.92; H, 6.93; N, 7.71%); ν_{max}(KBr)/cm^{-1} 3429, 2926, 1736, 1651, 1385, 1249 and 1109; $\delta_H(D_2O)$ 3.12–3.69 (br) and 2.45–2.76 (br).

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