Synthesis of One-, Two- and Three-ring Macrocyclic, Bifunctional Compounds for Use in Radiolabelling Monoclonal Antibodies

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The synthesis of new linked bis- and tris-ring tetraazamacrocyclic (bifunctional) reagents for use in an alternative strategy for radiolabelling antibodies is described. For comparison with the above systems, a new single ring bifunctional system incorporating a dioxocyclam ring is also reported.

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Introduction.

Radiolabelled antibodies are of wide interest in cancer research and treatment [1-4] and the use of single ring macrocycles as the isotope-binding components for radioimmunoconjugates has been investigated for some time [5]. Macrocyclic systems offer potential advantages over corresponding open-chain systems [6] since both enhanced kinetic and thermodynamic stabilities are characteristic of such metal-bound macrocyclic species [7]. Individual macrocyclic polyamines, such as the tetraaza derivative, 1,4,8,11-tetraazacyclotetradecane (cyclam) [8], as well as a number of related macrocyclic polyaminocarboxylates [9], have been employed to radiolabel various monoclonal antibodies (mAbs) with selected metal isotopes and, recently, a hexaaza cage derivative has also been proposed for such a role [10].

The aim of the investigation now reported was the design and synthesis of new linked 1,4,8,11-tetrazacyclotetradecane (cyclam) derivatives that might yield new radioimmunoconjugates showing enhanced specific activity through binding two or three metal radionuclide ions in both a kinetically and thermodynamically stable manner. In this paper the synthetic procedures for obtaining the new linked macrocyclic products are reported; details of their conjugation to mAbs, their interaction with ^{64}Cu and subsequent animal studies employing the resulting radioimmunoconjugates will be reported in due course.

Results and Discussion.

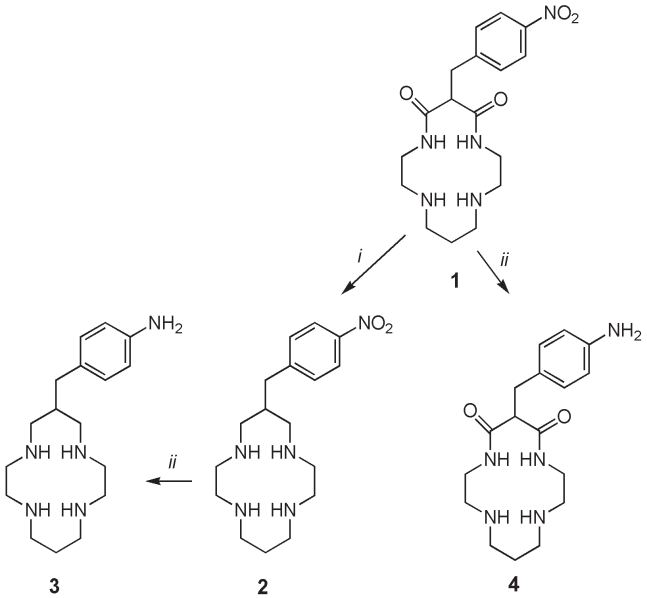
Mono-, bis- and tris-ring macrocyclic products, designed for use as bifunctional compounds for the radiolabelling of mAbs have been synthesised. In each of these an anilino group is incorporated in the structure to act as the site for subsequent linking to the mAbs.

Mono-macrocyclic Ring Derivatives.

The single-ring nitro-substituted macrocyclic species **1** and **2** were synthesised as described by Moreau *et al*. [11]. The anilino species **3** has been reported previously in the patent literature [12] and its synthesis from **2** (*via* H_2/PdC reduction) described in a recent paper [13]. The related anilino-substituted, dioxo derivative **4** was generated from **1** in the present study using a similar hydrogenation procedure.

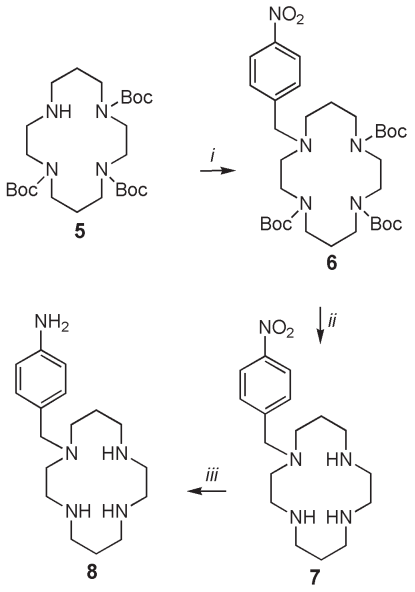
The *N*-substituted single-ring system **8** was prepared using the sequence shown in Scheme 2. Alkylation of the tri-*N*-boc protected derivative of cyclam **5** [14] with 4-nitrobenzyl bromide in the presence of caesium carbonate, followed by purification by column chromatography, yielded **6**. The boc groups were then cleaved with HCl/methanol to afford **7** in 50% overall yield. It needs to be noted that alternative syntheses for **7** using high dilution [15] and boron-protection [14] procedures have been reported previously. Finally, reduction of **7** to yield **8** has also been reported recently [11] involving H_2 (3 atm.) over Pd/C while in the present study sodium borohydride/10% Pd/C was employed.

Scheme 1



Reagents and Conditions: (*i*) BH_3-THF, reflux 20 h. (*ii*) H_2 at 3 atm, 5% Pd/C.

Scheme 2

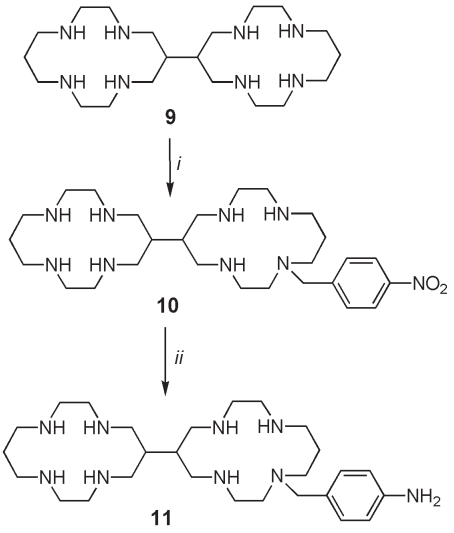


Boc = *tert*-butoxycarbonyl. Reagents and Conditions (*i*) Dry acetonitrile, caesium carbonate, $BrCH_2C_6H_4NO_2$; silica gel-CH_2Cl_2-CH_3OH-25% NH_3OH, 150 :1:0.1. (*ii*) 3 *M* HCl-MeOH, NaOH/$CHCl_3$. (*iii*) $NaBH_4$-10% Pd/C, HCl, 10% NaOH-$CHCl_3$.

Bis-macrocyclic Ring Derivative.

The synthesis of the double-ring macrocyclic ligand system **11**, starting from the previously reported [17,18] bis-ring precursor **9**, was carried out using the sequence shown in Scheme 3. Alkylation of **9** with 4-nitrobenzyl bromide yielded **10**. As initially isolated, crude **10** was obtained as its hydrobromide salt together with the hydrobromide salts of corresponding bis- and tris-alkylated species as bi-products. Following neutralisation and chloroform extraction of the crude product, attempts at purification using column chromatography on silica gel were unsuccessful due to strong adsorption of the polyamine mixture on the column. In view of this the mixture was reacted with di-*tert*-butyl dicarbonate to convert the secondary amines to their boc-derivatives and hence reduce their polarity. The hepta-'boc' derivative of **10** was successfully separated employing silica gel and the boc groups then removed with methanolic HCl. After workup, pure **10** was obtained as a glassy solid. Hydrogenation ($NaBH_4$-10% Pd/C) of the product yielded the pendant anilino derivative 11.

Scheme 3



Reagents and Conditions: (*i*) $BrCH_2C_6H_4NO_2$-anhydrous $CHCl_3$, 10% NaOH-$CHCl_3$, CH_2Cl_2-di-*tert*-butyl dicarbonate, silica gel-MeOH-CH_2Cl-25% NH_3OH, 200:1:0.1, 10% NaOH-$CHCl_3$. (*ii*) $NaBH_4$-10% Pd/C, HCl, 10% NaOH-$CHCl_3$.

Tris-macrocyclic Ring Derivative.

The three-ring macrocyclic system **15** was synthesised as shown in Scheme 4. The initial step involved the alkylation of the two available amines (4- and 8-positions) of **1** with 4-(chloromethyl)benzoyl chloride to yield **12** which was then reacted with a 2.1-fold molar equivalent of **5** in dry acetonitrile in the presence caesium carbonate to afford **13** in 47% yield after column chromatography on silica gel. The boc groups were then removed (HCl/methanol) to give **14** in 95% yield. Reduction of the nitro substituent of **14** to yield the corresponding anilino derivative **15** was once again achieved by reaction with sodium borohydride in the presence of 10% Pd/C. Workup involved chloroform extraction of the product from 10% sodium hydroxide solution to yield **15** as an oil.

Concluding Remarks.

While a focus of the present study was the development of new linked reagents for the radiolabelling of antibodies for use in cancer therapy, it needs to be noted that linked macrocyclic ligand systems and their metal complexes continue to receive attention in other areas [19]. In particular, very considerable interest in such systems has also been generated by the observation that linked, two-ring, tetraaza macrocycles, and their (selected) metal complexes, inhibit particular HIV strains with low toxicity [20-24]. The present ligand systems are closely structurally related to the bis-ring systems employed in the latter studies and hence may also prove of interest in this context.

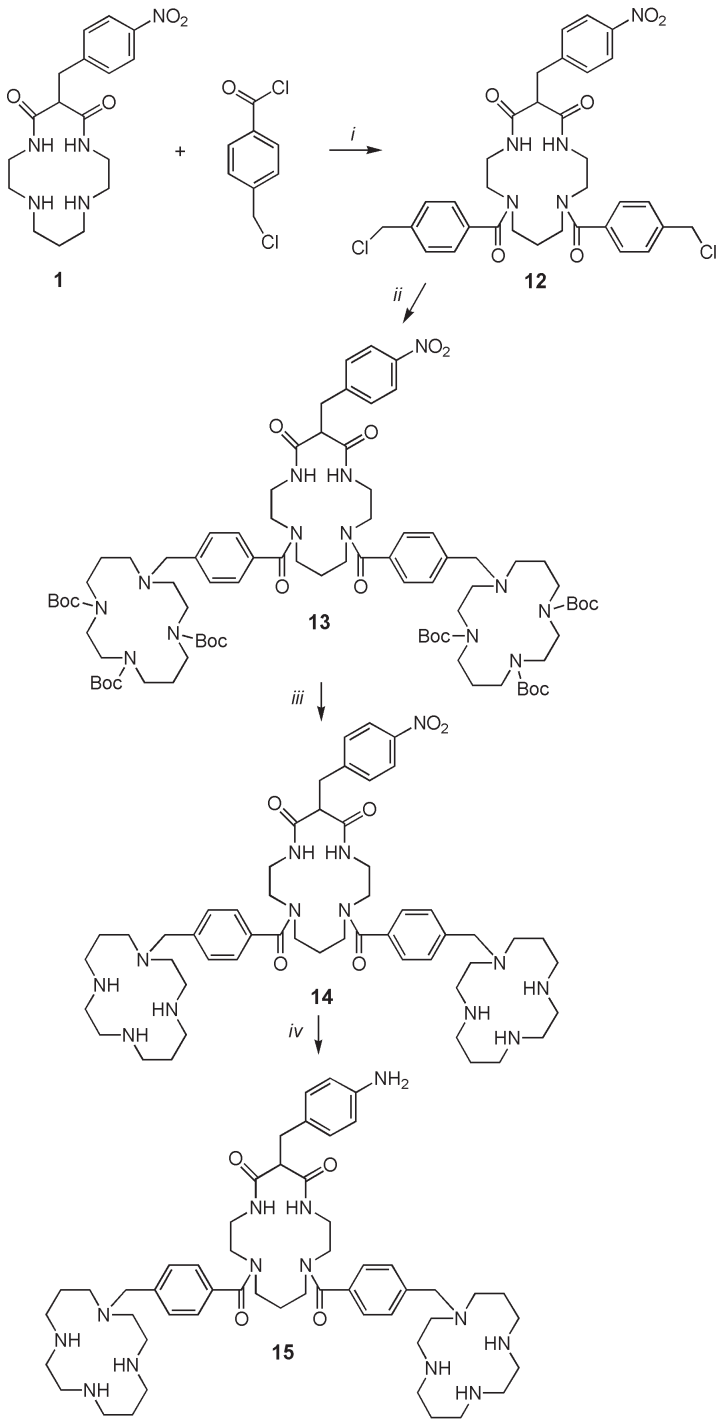
EXPERIMENTAL

Materials and Apparatus.

Reagents were of the highest grade available commercially. Dry acetonitrile was prepared by shaking acetonitrile with Linde 4A molecular sieves, stirring with calcium hydride until no further hydrogen was evolved, and then fractionally distill-

ing from fresh calcium hydride. Dichloromethane was shaken with portions of sulfuric acid until the acid layer remained colourless; it was then washed with water, aqueous 5% sodium carbonate, and finally again with water. It was then pre-dried with calcium chloride before being distilled from calcium hydride. Dry tetrahydrofuran was prepared by heating tetrahy-

Scheme 4



Boc = *tert*-butoxycarbonyl. Reagents and Conditions: (*i*) Dry CH_2Cl_2, triethylamine, 4-(chloromethyl)benzoyl chloride, silica gel, CH_2Cl_2/CH_3OH (50:1). (*ii*) **5**, dry acetonitrile, potassium iodide, caesium carbonate, silica gel-CH_2Cl_2-CH_3OH; 50 : 1; (*iii*) 3 M HCl-MeOH, NaOH/$CHCl_3$. (*iv*) $NaBH_4$-10% Pd/C, HCl, 10% NaOH-$CHCl_3$.

drofuran at reflux over phosphorus pentoxide before distillation. The process was then repeated over sodium wire followed finally by fractional distillation. All water used for experimental purposes was of Milli-Q™ grade.

NMR spectra were obtained on Bruker Advance DPX 200, 300 or 400 spectrometers; δ_H values are relative to TMS; δ_C values are relative to $CDCl_3$ (at 77.1 ppm), and *J* values are given in Hertz (Hz). HRMS spectra were obtained on a Crates M25RFA spectrometer and low resolution positive ion (ES) spectra on a Finnegan LCQ-8 spectrometer.

Syntheses.

1,4,8,11-Tetraazaundecane used for the synthesis of **1** was prepared and characterised in a similar manner to that described by Fabbrizzi *et al*. [17]. Cyclam and its precursor 1,5,8,12-tetrazadodecane were synthesised as described by Barefield *et al*. [25]. 6,6'-Bis(1,4,8,11-tetraazacyclotetradecane-5,7-dione), a precursor for **9**, was also prepared as described previously [17].

13-(4-Nitrobenzyl)-1,4,8,11-tetraazacyclotetradecane-12,14-dione (**1**).

This product was prepared as reported previously [11]. Yield 43%. Mass spectrum: *m/z* 364.3 [M+H$^+$]. ^{1}H-NMR ($CDCl_3/D_2O$): δ 1.62 (2 H, quintet, $NCH_2CH_2CH_2N$), 2.52-2.79 (8 H, m, CH_2NHCH_2), 3.18-3.54 (7 H, m, NO_2ArCH_2, COCHCO, $CONHCH_2$), 7.46 (2 H, *J* 4.3, d, Ar*H*), 8.13 (2 H, *J* 4.3, d, NO_2ArH).

Anal. Calcd. for $C_{17}H_{25}N_5O_4$: C, 56.19; H, 6.93; N, 19.27. Found: C, 55.96; H, 6.80; N, 19.08.

13-(4-Nitrobenzyl)-1,4,8,11-tetraazacyclotetradecane ·0.5CH_3OH·0.5H_2O (**2**).

This product was prepared as reported previously [11]. Yield 80%. Mass spectrum: *m/z* 336.5 [M+H$^+$]. ^{1}H-NMR (DMSO): δ 1.64 (2 H, quintet, $NCH_2CH_2CH_2N$), 2.42-2.76 (19 H, m, $NCH_2CH_2CH_2N$, NCH_2CH_2N, NO_2ArCH_2), 7.49, (2 H, *J* 4.3, d, Ar*H*), 8.15 (2 H, *J* 4.3, d, NO_2ArH). ^{13}C-NMR (DMSO): δ 27.7, 30.1, 38.3, 39.6, 48.4, 48.6, 50.3, 55.0, 62.4, 123.6, 129.7, 146.6, 147.8.

Anal. Calcd. for $C_{17}H_{29}N_5O_4$.0.5CH_3OH.0.5H_2O: C, 58.31; H, 8.95; N, 19.43. Found: C, 58.58; H, 8.37; N, 19.98.

13-(4-Aminobenzyl)-1,4,8,11-tetraazacyclotetradecane ·0.5CH_3OH (**3**).

This compound was synthesised in a related manner to that reported previously [15]. Yield 92%. Mass spectrum: *m/z* 306.5 [M+H$^+$]. ^{1}H-NMR ($CDCl_3$): δ 1.85 (2 H, quintet, $NCH_2CH_2CH_2N$), 2.44-3.98 (19 H, m, $NCH_2CH_2CH_2N$, NCH_2CH_2N, NH_2ArCH_2), 6.61 (2 H, *J* 4.1, d, NH_2ArH), 6.93 (2 H, *J* 4.1, d, Ar*H*). ^{13}C-NMR ($CDCl_3$): δ 27.6, 37.6, 39.4, 48.1, 50.3, 55.3, 115.2, 129.7, 144.5.

Anal. Calcd. for $C_{17}H_{31}N_5$·0.5CH_3OH: C, 65.38; H, 10.35; N, 21.78. Found: C, 65.26; H, 10.40; N, 21.34.

13-(4-Aminobenzyl)-1,4,8,11-tetraazacyclotetradecane-12,14-dione·CH_3OH·H_2O (**4**).

Hydrogenation of **1** to yield **4** as a light yellow solid was carried out as in similar manner to that described for the hydrogenation of **2**. Yield: 89%. Mass spectrum: *m/z* 334.3 [M+H$^+$]. ^{1}H-NMR (CD_3OD): δ 1.68 (2H, quintet, $NCH_2CH_2CH_2N$), 2.67-2.80 (8 H, m, CH_2NCH_2), 2.99-3.07 (4 H, m, $CONHCH_2$), 3.34-3.58 (3 H, m, NH_2ArCH_2, COCHCO), 6.76 (2 H, *J* 4.2, d, NH_2ArH), 7.04 (2 H, *J* 4.2, d, Ar*H*). ^{13}C-NMR (DMSO): δ 27.4, 32.6, 37.0, 38.2, 47.9, 49.3, 55.2, 113.7, 126.6, 129.0, 146.5, 168.7.

Anal. Calcd. for $C_{17}H_{27}N_5O_2$·CH_3OH·H_2O: C, 56.38; H, 8.67; N, 18.26. Found: C, 56.72; H, 8.23; N, 18.29.

1,4,8-Tris-(*tert*-butoxycarbonyl)-1,4,8,11-tetraazacyclotetradecane (**5**).

This compound was prepared in a similar manner to that described by Guilard *et al*. [14]. Yield: 75%. Mass Spectrum: *m/z* 501.0 [M+H$^+$]. The ^{1}H-NMR spectrum was identical to that reported previously [14].

11-(4-Nitrobenzyl)-1,4,8-tris-(*tert*-butoxycarbonyl)-1,4,8,11-tetraazacyclotetradecane (**6**).

4-Nitrobenzyl bromide (1.8 g, 8.20 mmol), caesium carbonate (4.8 g, 15.0 mmol) and **5** (3.7 g, 7.40 mmol), were heated in dry acetonitrile (50 mL) at reflux for 4 h under a N_2 stream. The solid was removed by filtration and was washed with warm chloroform. The filtrate was evaporated under reduced pressure and the residue was then partitioned between a mixture of dichloromethane (50 mL) and water (20 mL). The phases were separated and the aqueous solution was shaken with further dichloromethane (3 x 50 mL). The combined dichloromethane extracts were dried (anhydrous Na_2SO_4) and evaporated under reduced pressure to give a light brown oil, which was purified by column chromatography on silica gel (eluting with dichloromethane-methanol-25% ammonia, 150:1:0.1) to give **6** as a light yellow glassy solid. Yield: 3.4 g (74 %). Mass spectrum: *m/z* 636.4 [M+H$^+$]. ^{1}H-NMR ($CDCl_3$): δ 1.39 (9 H, s, Bu^t), 1.43 (9 H, s, Bu^t), 1.47 (9 H s, Bu^t), 1.69 (2 H, quintet, $NO_2ArCH_2NCH_2CH_2CH_2NBoc$), 1.72 (2 H, quintet, $BocNCH_2CH_2CH_2NBoc$), 2.40 (2 H, m, NO_2Ar-NCH_2CH_2-CH_2NBoc), 2.63 (2 H, t, $NO_2ArCH_2NCH_2CH_2N$), 3.25– 3.37 (12 H, m, $NO_2ArCH_2NCH_2CH_2CH_2$, CH_2NBoc), 3.63 (2 H, s, NO_2ArCH_2), 7.45 (2 H, *J* 4.2, d, Ar*H*), 8.16 (2 H, *J* 4.2, d, NO_2ArH). ^{13}C-NMR ($CDCl_3$): δ 28.3, 28.4, 46.3, 47.2, 47.9, 52.0, 53.3, 59.2, 123.4, 129.5, 132.4, 147.1, 155.5.

Anal. Calcd. for $C_{32}H_{53}N_5O_8$: C, 60.45; H, 8.40; N, 11.02. Found: C, 60.54; H, 8.55; N, 11.08.

Anal. Calcd. for $C_{32}H_{53}N_5O_8$, [M+Na$^+$]: 658.3787. Found (FAB): [M+Na$^+$], 658.3795.

11-(4-Nitrobenzyl)-1,4,8,11 tetraazacyclotetradecane·0.25H_2O (**7**).

A mixture of methanol (18 mL) and concentrated hydrochloric acid (6 mL) was added to 6 (2.0 g, 0.31 mmol). The reaction mixture was allowed to stir overnight at room temperature after which the white solid that formed was collected by filtration. The solid obtained was dispersed in a mixture of 10% aqueous sodium hydroxide (25 mL) and chloroform (50 mL) and the mixture shaken. The phases were separated and the aqueous phase was further shaken with chloroform (6 x 50 mL). The combined chloroform extracts were dried (anhydrous Na_2SO_4) and the solvent removed under reduced pressure. Further purification involved formation of the hydrobromide salt. The title compound was dissolved in absolute ethanol (2 mL) (any undissolved material was removed by filtration). To this solution was added 45% hydrobromic acid in acetic acid (0.5 mL). The mixture was stirred for two h after which the white solid that had formed was collected by filtration. This solid was washed spar-

ingly with cold absolute ethanol and it was then partitioned between a mixture of 10% aqueous sodium hydroxide (10 mL) and chloroform (25 mL). The phases were separated and the aqueous phase shaken with further chloroform (6 x 25 mL). The combined chloroform extracts were dried (anhydrous Na_2SO_4) and evaporated under reduced pressure to give **7** as a light yellow powder. Yield 0.95 g (90%); Mass spectrum: *m/z* 336.7 [M+H$^+$]. ^{1}H-NMR ($CDCl_3$): δ 1.74 (2 H, quintet, $PhCH_2NCH_2CH_2CH_2N$), 1.87 (2 H, quintet, $NCH_2CH_2CH_2N$), 2.51- 2.89 (16 H, m, NCH_2), 3.66 (2 H, s, NO_2PhCH_2), 7.57 (2 H, *J* 4.4, d Ar*H*), 8.15 (2 H, *J* 4.3, d, NO_2ArH). ^{13}C-NMR ($CDCl_3$): δ 26.1, 28.3, 47.3, 47.9, 48.6, 49.0, 49.3, 50.9, 53.4, 54.9, 57.7, 123.4, 129.5, 147.1.

Anal. Calcd. for $C_{17}H_{29}N_5O_2 \cdot 0.25H_2O$: C, 60.06; H, 8.75; N, 20.60. Found: C, 60.05; H, 8.66; N, 20.36.

11-(4-Aminobenzyl)-1,4,8,11-tetraazacyclotetradecane (**8**).

A solution of sodium borohydride (150 mg) in water (3 mL) was added to a suspension of 10% palladium on charcoal (70 mg) in water (3 mL) under a N_2 stream. The mixture was stirred for five min before a solution of **7** (220 mg, 0.65 mmol) in 2% sodium hydroxide in methanol (6 mL) was added. The reaction was allowed to proceed at room temperature under a N_2 stream for 30 min. The palladium on charcoal was removed by filtration (using a 0.45 μm filter) after which concentrated hydrochloric acid was added dropwise to the filtrate until gas evolution ceased (~ pH 7). This solution was then evaporated under reduced pressure to give a white solid, which was then dispersed in a mixture of 10% sodium hydroxide (20 mL) and chloroform (30 mL). The phases were separated and the aqueous phase was further shaken with chloroform (5 x 30 mL). The combined chloroform extracts were dried (anhydrous Na_2SO_4) and evaporated under reduced pressure to give 8 as a light yellow oil. Yield 180 mg (90%); Mass spectrum: *m/z* 306.5 (MH$^+$). ^{1}H-NMR ($CDCl_3$): δ 1.82-1.90 (4 H, m, $ArCH_2NCH_2CH_2CH_2N$, $NCH_2CH_2CH_2N$), 2.56-2.93 (16 H, m, NCH_2), 3.49 (2 H, s, NO_2ArCH_2), 6.65, (2 H, *J* 4.1, d, NH_2ArH), 7.12 (2H, *J* 4.1, d, Ar*H*). ^{13}C-NMR (DMSO): δ 25.9, 28.1, 47.0, 47.8, 48.0, 48.2, 48.6, 49.5, 51.7, 53.6, 56,6, 113,5, 125.3, 129.7, 147.2.

Anal. Calcd. for $C_{17}H_{31}N_5$; [M+H$^+$]: 306.2652. Found (FAB): [M+H$^+$], 306.2656.

6,6'-Bis-(1,4,8,11-tetraazacyclotetradecane)·0.25H_2O (**9**).

6,6'-Bis(1,4,8,11-tetraazacyclotetradecane-5,7-dione) was initially prepared in similar manner to that described by Fabbrizzi *et al*. [18]. Yield 24%. Mass spectrum: *m/z* 455.3 [M+H$^+$]. ^{1}H-NMR ($CDCl_3$): δ 1.64 (4 H, quintet, $NCH_2CH_2CH_2N$), 2.62 – 3.67 (24 H, m, NC*H*), 4.02 (2 H, d, C*H*CO).

Anal. Calcd. for $C_{20}H_{38}N_8O_4$: C, 52.84; H, 8.43; N, 24.65. Found: C, 52.98; H, 8.11; N, 24.76.

This compound was then converted to **9** using the published procedure [18]. Yield 35.8%; *m/z* 399.5 [M+H$^+$]. ^{1}H-NMR (D_20): δ 1.66-1.70 (6 H, m, $NCH_2CH_2CH_2N$, C*HCH*), 2.55- 2.74 (32 H, m, NCH_2CH_2N, $NCH_2CH_2CH_2N$).

Anal. Calcd. for $C_{20}H_{46}N_8.0.25H_2O$: C, 59.59; H, 11.63; N, 27.80. Found: C, 59.34; H, 11.12; N, 27.56.

Compound **9** was also isolated as its octa-hydrochloride salt.

Anal. Calcd. for $C_{20}H_{46}N_8 \cdot 8HCl \cdot 2H_2O$: C, 33.07; H, 8.05; N, 15.43. Found: C, 33.08; H, 8.37; N, 15.16.

1-(4-Nitrobenzyl)-6,6-bis-(1,4,8,11-tetraazacyclotetradecane) (**10**).

6,6'-Bis-(1,4,8,11-tetraazacyclotetradecane), **9**, (1.0 g, 2.50 mmol) was dissolved in dry chloroform (50 mL). To this solution was added 4-nitrobenzyl bromide (0.54 g, 2.50 mmol) in anhydrous chloroform (50 mL) using motorised syringes employing a 0.62 mL/h flow rate. When the addition was complete, the solution was brought to dryness under reduced pressure after which it was dispersed between a mixture of 10% sodium hydroxide (20 mL) and chloroform (50 mL). The organic phase was separated and the aqueous phase was shaken further with chloroform (6 x 50 mL). The combined chloroform extracts were dried (anhydrous Na_2SO_4) and evaporated to give a light yellow residue, which was then dissolved in dichloromethane (200 mL). To this solution was added di-*tert*-butyl dicarbonate (3.5 g, 17.5 mmol) in dichloromethane. The mixture was stirred for 2 h and again brought to dryness. The intermediate [1-(4-nitrobenzyl)-4,8,11,1',4',8',11'-hepta(*tert*-butoxycarbonate)]-6,6'-bis(tetraazacyclotetradecane) was purified by column chromatography on silica gel (eluting with dichloromethane-methanol-25% ammonium hydroxide, 200:1:0.1). The solvent was removed under reduced pressure and the residue was dissolved in a mixture of methanol (18 mL) and concentrated hydrochloric acid (6 mL). The mixture was stirred overnight and the white solid that formed was collected by filtration and carefully washed with cold methanol. This product was dispersed between a mixture of 10% sodium hydroxide (10 mL) and chloroform (30 mL). The phases were separated and the aqueous phase was shaken with further chloroform (5 x 30 mL). The combined chloroform extracts were dried (anhydrous Na_2SO_4) and the solvent was removed under reduced pressure to give **10** as a light brown glassy solid. Yield: 0.20 g (15%); *m/z* 535 [M+H$^+$]. ^{1}H-NMR (D_2O): δ 1.72-1.90 (6 H, m, $NCH_2CH_2CH_2N$, C*HCH*), 2.70- 3.60 (34 H, m, NCH_2CH_2N, $NCH_2CH_2CH_2N$, NCH_2Ar), 7.54 (2 H, *J* 4.3, d, Ar*H*), 8.31, (2 H, *J* 4.2, d, NO_2ArH). ^{13}C-NMR (D_2O): δ 17.4, 25.5, 26.9, 41.1, 46.7, 47.5, 47.6, 47.9, 48.8, 49.4, 52.8, 53.3, 53.5, 54,5, 54,7, 55,5, 58.1, 124.2, 130,7, 147,3, 148.8.

Anal. Calcd. for $C_{27}H_{51}N_9O_2$, [M+H$^+$]: 534.4238. Found (FAB): [M+H$^+$], 534.4247.

1-(4-Aminobenzyl)-6,6-bis-(1,4,8,11-tetraazacyclotetradecane) (**11**).

Hydrogenation of **9** to yield **10** was performed in similar manner to that described for the preparation of 8. (Yield 95%). ^{1}H-NMR (D_2O): δ 1.72-1.90 (6 H, m, $NCH_2CH_2CH_2N$, C*HCH*) 2.70- 3.60 (34 H, m, NCH_2CH_2N, $NCH_2CH_2CH_2N$, NCH_2Ar), 6.81 (2 H, *J* 5.5, d, Ar*H*), 7.17 (2 H, *J* 5.5, d, Ar*H*). ^{13}C-NMR (D_2O): δ 17.4, 21.7, 27.1, 28.8, 40.3, 42.9, 42.7, 48.7, 49.5, 49.7, 50.0, 50.9, 51.5, 55.0, 57.0, 57.5, 59.5, 121.3, 132.4, 133.4, 148.8.

Anal. Calcd. for $C_{27}H_{53}N_9$, [M+H$^+$]: 504.4497. Found (FAB): [M+H$^+$], 504.4508.

[13-(4-Nitrobenzyl)-4,8-bis(chloromethyl)benzoyl)]-1,4,8,11-tetraazacyclotetradecane-12,14-dione·2H_2O (**12**).

Triethylamine (2.1 g, 20.07 mmol) and a solution of 4-(chloromethyl)benzoyl chloride (3.4 g, 18.25 mmol) in dry dichloromethane were added (over five min) to a suspension of **12** (3.0 g, 8.25 mmol) in dry dichloromethane (200 mL). The mixture was stirred overnight at room temperature to give a clear yellow solution, which was then washed with water (50 mL). The organic phase was further washed with water (50 mL). A white solid formed during the washing procedure, which was collected

by filtration and then dried over phosphorus pentoxide. This crude product was then purified by column chromatography on silica gel (eluting with dichloromethane-methanol, 50:1). The eluant was left to stand and evaporate at room temperature to yield **12** as a white shiny solid, which was again collected by filtration. Yield 3.43 g (64%); Mass spectrum: *m/z* 668.3 [M+H$^+$]. ^{1}H-NMR (CD_3CN): δ 1.75 (2 H, q, $NCH_2CH_2CH_2N$), 2.59–3.69 (15 H, m, NCH_2, $COCHCO$, CH_2ArNO_2), 4.70 (4H, s, $ArCH_2Cl$), 7.36-7.60 (10 H, d, overlapping, Ar*H*), 8.12 (2H, *J* 4.3, d, NO_2ArH). ^{13}C-NMR (CD_3CN): δ 37.9, 38,2, 47.0, 49,5, 50.1, 57.8, 124.8, 128.2, 130.0, 131.4, 138.8, 140.5, 148.1, 148,3, 170.4, 172.6.

Anal. Calcd. for $C_{35}H_{35}N_5O_6{\cdot}2H_2O$: C, 56.25; H, 5.58; N, 9.94. Found: C, 56.46; H, 5.61; N, 9.76.

Anal. Calcd. for $C_{35}H_{35}N_5O_6$; [M+H$^+$]: 668.2038. Found (FAB): [M+H$^+$], 668.2048.

Compound **13**.

Caesium carbonate (3.2 g, 10 mmol), potassium iodide (0.15 g, 0.90 mmol) and **12** (3.0 g, 4.49 mmol) in dry acetonitrile (10 mL) were heated at reflux under a N_2 stream for 30 min before adding **5** (4.71 g, 9.43 mmol) in a dry acetonitrile (38 mL). The mixture was heated at reflux for 24 h after which the solid was collected by filtration using filter-aid Celite. The solid which collected was washed with warm chloroform. The filtrate was evaporated under reduced pressure and the residue was then partitioned between a mixture of dichloromethane (50 mL) and water (20 mL). The phases were then separated and the aqueous solution was shaken with further dichloromethane (3 x 50 mL). The combined dichloromethane extracts were dried (anhydrous Na_2SO_4) and evaporated under reduced pressure to give a light brown oil which was purified *via* column chromatography on silica gel (eluting with dichloromethane-methanol, 50:1 and 50:2). Yield 3.39 g (47%); Mass spectrum: *m/z* 1596.9 [M+H$^+$]. ^{1}H-NMR ($CDCl_3$): δ 1.39 (27 H, s, Bu^t), 1.43 (27 H, s, Bu^t), 1.64 (4H, m, $ArCH_2NCH_2CH_2CH_2N$), 1.85 (6 H, m, $BocNCH_2CH_2CH_2NBoc$, $CONCH_2CH_2CH_2NCO$), 2.33 (4 H, m, $ArCH_2NCH_2CH_2CH_2N$), 2.59 (4 H, m, $ArCH_2NCH_2CH_2NBoc$), 2.90–3.52 (43 H, m, $BocNCH_2$, $CONCH_2$, $CONHCH_2$,$COCHCO$, $CHCH_2ArNO_2$, $ArCH_2N$), 7.28-7.43 (10 H, m, overlapping, Ar*H*), 8.09 (2H, *J* 3.9, d, NO_2ArH). ^{13}C-NMR ($CDCl_3$): δ 26.1, 26.7, 28.1, 28.3, 28.4, 28.6, 36.3, 38.4, 45.9, 46.9, 47.5, 48.5, 51.4, 52.7, 57.1, 59.2, 76.6, 79.5, 79.6, 123.7, 126.5, 129.1, 129.6, 134.7, 141,1, 145.6, 146.8, 155.3, 155.5, 169.2, 173.0.

Anal. Calcd. for $C_{83}H_{129}N_{13}O_{18}$: C, 62.42; H, 8.14; N, 11.40. Found: C, 62.35; H, 7.93; N, 11.06.

Anal. Calcd. for $C_{83}H_{129}N_{13}O_{18}$, [M+H$^+$]: 1596.9650. Found (FAB): [M+H$^+$], 1596.9662.

Compound **14**•$CHCl_3$.

Conversion of **13** to **14** was performed in an analogous manner to that described for the preparation of **7**. Yield 1.18 g (95 %); Mass spectrum: *m/z* 996.7 [M+H$^+$]. ^{1}H-NMR ($CDCl_3$): δ 1.63-1.78 (10 H, m, overlapping, $NCH_2CH_2CH_2N$), 2.39-2.76 (32 H, m, $ArCH_2NCH_2$, CH_2NHCH_2), 3.21–3.51 (19 H, m, overlapping, $CHCH_2ArNO_2$, $CONCH_2CH_2CH_2NCO$, $CONHCH_2CH_2NCO$, $COCHCO$, $ArCH_2N$), 7.25-7.48 (10 H, d, overlapping, Ar*H*), 8.03 (2 H, *J* 4.0, d, NO_2ArH). ^{13}C-NMR ($CDCl_3$): δ 25.9, 27.7, 29.5, 30.0, 36.0, 37.6, 47.1, 47.6, 48.3, 49.1, 49.3, 51.0, 53.4, 54.2, 55.5, 57.7, 76.6, 123.5, 126.9, 128.9, 129.8, 135.0, 141.1, 146.2, 146.6, 169.2, 172.6.

Anal. Calcd. for $C_{53}H_{81}N_{13}O_6{\cdot}CHCl_3$: C, 58.13; H, 7.41; N, 16.32. Found: C, 57.86; H, 7.52; N, 16.69.

Anal. Calcd. for $C_{53}H_{81}N_{13}O_6$, [M+H$^+$]: 996.6506. Found (FAB): [M+H$^+$), 996.6494.

Compound **15**.

Hydrogenation of **14** to obtain **15** was achieved in a similar manner to that described for the formation of 8. (Yield 91%). Mass spectrum: *m/z* 966.7 [M+H$^+$]. ^{1}H-NMR (D_2O): δ 1.85-2.11 (10 H, m, overlapping, $NCH_2CH_2CH_2N$,), 2.75-3.35 (32 H, m, $ArCH_2NCH_2$, CH_2NCH_2), 3.52–3.84 (19 H, m, overlapping, $CHCH_2ArNO_2$, $CONCH_2CH_2CH_2NCO$, $CONHCH_2CH_2NCO$, $COCHCO$, $ArCH_2N$), 6.79 (2 H, *J* 9.0, d, NH_2ArH), 7.09 (2 H, d, *J* 9.5, Ar*H*), 7.39-7.56 (8 H, d, overlapping, Ar*H*). ^{13}C-NMR (DMSO): δ 25.9, 28.0, 30.0, 46.9, 47.5, 48.1, 49.6, 52.4, 54.1, 57.3, 113.7125.9, 126.2, 128.6, 128.9, 135.5, 140.4, 146.7, 169.1, 170.9.

Anal. Calcd. for $C_{53}H_{83}N_{13}O_4$, [M+H$^+$]: 966.6763. Found (FAB): [M+H$^+$], 966.6773.

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