Journal of Peptide Science
J. Peptide Sci. **8**: 663–670 (2002)
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/psc.427

Synthesis of N^{α}-Boc-N^{ε}-Tetrabenzyl-DTPA-L-Lysine and N^{α}-Fmoc-N^{ε}-Tetra-t-butyl-DTPA-L-Lysine, Building Blocks for Solid Phase Synthesis of DTPA-containing Peptides

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Received 28 June 2002
Accepted 4 July 2002

Abstract: Two building blocks, Boc-Lys(Bn_4-DTPA)-OH and Fmoc-Lys(Bu^t_4-DTPA)-OH have been synthesized via the acylation of the ε-amino group of N^{α}-protected lysine, using suitably protected tetra-esters of diethylene triamine pentaacetic acid (DTPA), a ligand with wide application as a chelating agent for complexing metal ions to peptides. Copyright © 2002 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: DTPA; diethylene triamine pentaacetic acid derivatives; lysine building blocks

INTRODUCTION

Diethylene triamine pentaacetic acid (DTPA) has been used extensively as a metal chelator in radiopharmaceuticals and contrast agents. Its uses in magnetic resonance imaging (MRI) as a Gd(III) chelate [1], in radiotherapy as e.g. ^{117m}Sn-DTPA in the treatment of bone metastasis [2] and in radiodiagnostics [3] have been recently reviewed. There is increasing interest in attaching this chelating group to biologically active peptides, which can deliver the labelling metal ion to the site of biological action. Our interest in the field was derived from the need to use cyclic RGD peptides as contrasting agents for visualizing blood-clot formation [4]. In a similar approach by Dupont researchers [5], a cyclic RGD analogue was proven to give good imaging results in dogs. Recently, two cyclic RGD peptides have been synthesized and characterized [6], bearing ^{111}In — DTPA conjugates, and have been compared with ^{90}Y-DTPA complexes on the same cyclic peptide scaffold [7]. The somatostatin analogue octreotide has also been chelated with ^{111}In via a DTPA-complex giving the commercially used OctreoScan — 111 {[^{111}In-DTPA-D-Phe^1]-octreotide} [8,9].

The traditional approaches for attaching the DTPA ligand to peptides in a 'global approach' have used the readily available cyclic diethylene triamine pentaacetic dianhydride **1**, or a *p*-isothiocyanatobenzyl derivative **2** [10], but these have several disadvantages. The former, with its two anhydride groups, can undergo inter-molecular cross-linking, and one of the potential chelator carboxyls is used in the peptide–DTPA link. Acylation of the phenolic group of tyrosine has also been reported using this reagent [11]. The isothiocyanato group has sometimes shown instability and incompatible solubility properties. To overcome such problems, other derivatives of DTPA have been synthesized as exemplified by the recent novel additions to the range such as **3** [12], **4** [13], **5** [14] and **6** [9].

Having experienced some problems with the global approach in our work on RGD-chelated analogues [4], we were attracted to the building block approach by its success in glycopeptide synthesis [15], where quite large suitably protected conjugates are attached to the protected amino

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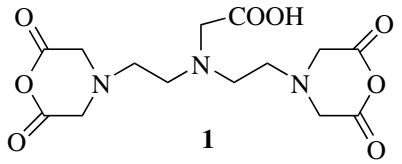
Contract/grant sponsor: Altajir Trust.



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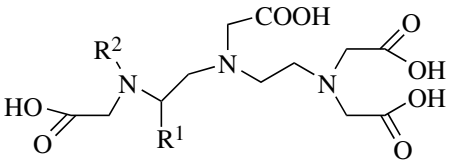
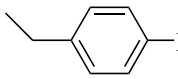


2 R^1 =  $R^2 = CH_2COOH$

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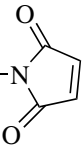
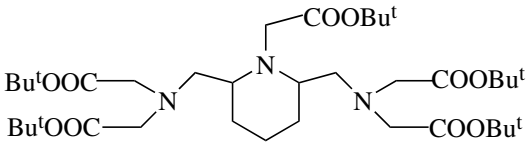
4 $R^1 = H$ $R^2 = CH_2CONHCH_2CH_2$ 



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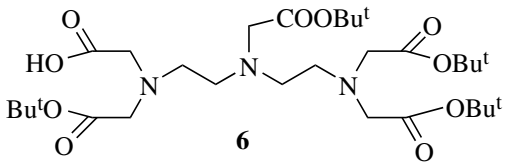

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acids in a form compatible with solid phase peptide synthesis protocols. We were therefore challenged to synthesize protected derivatives of lysine in which the ε-amino group was linked to solid phase-compatible tetra esters of DTPA. Building blocks of this kind, but with different conjugate groups, have been previously announced in the literature, e.g. N^{α}-Boc-N^{ε}-tribenzyl-EDTA-L-lysine [16] and N^{α}-Fmoc-N^{ε}-tetrabutyl-EDTA-L-lysine [17], the latter based on earlier work [18] on t-butyl-protected EDTA derivatives. Bifunctional DTPA-like ligands have also been attached to lysine [19], but are less useful in the building block approach as they are linked to the α-amino group.

In this paper we wish to record our results for the synthesis of Fmoc-Lys(Bu^t_4-DTPA)-OH (**12**) and Boc-Lys(Bn_4-DTPA)-OH (**17**), using the principles used for the EDTA derivatives [17], but adapting them to a synthetic sequence based on work in the unrelated field of cobalt complexes [20]. The stages in the syntheses are outlined in Scheme 1.

MATERIALS AND METHODS

Analytical Methods

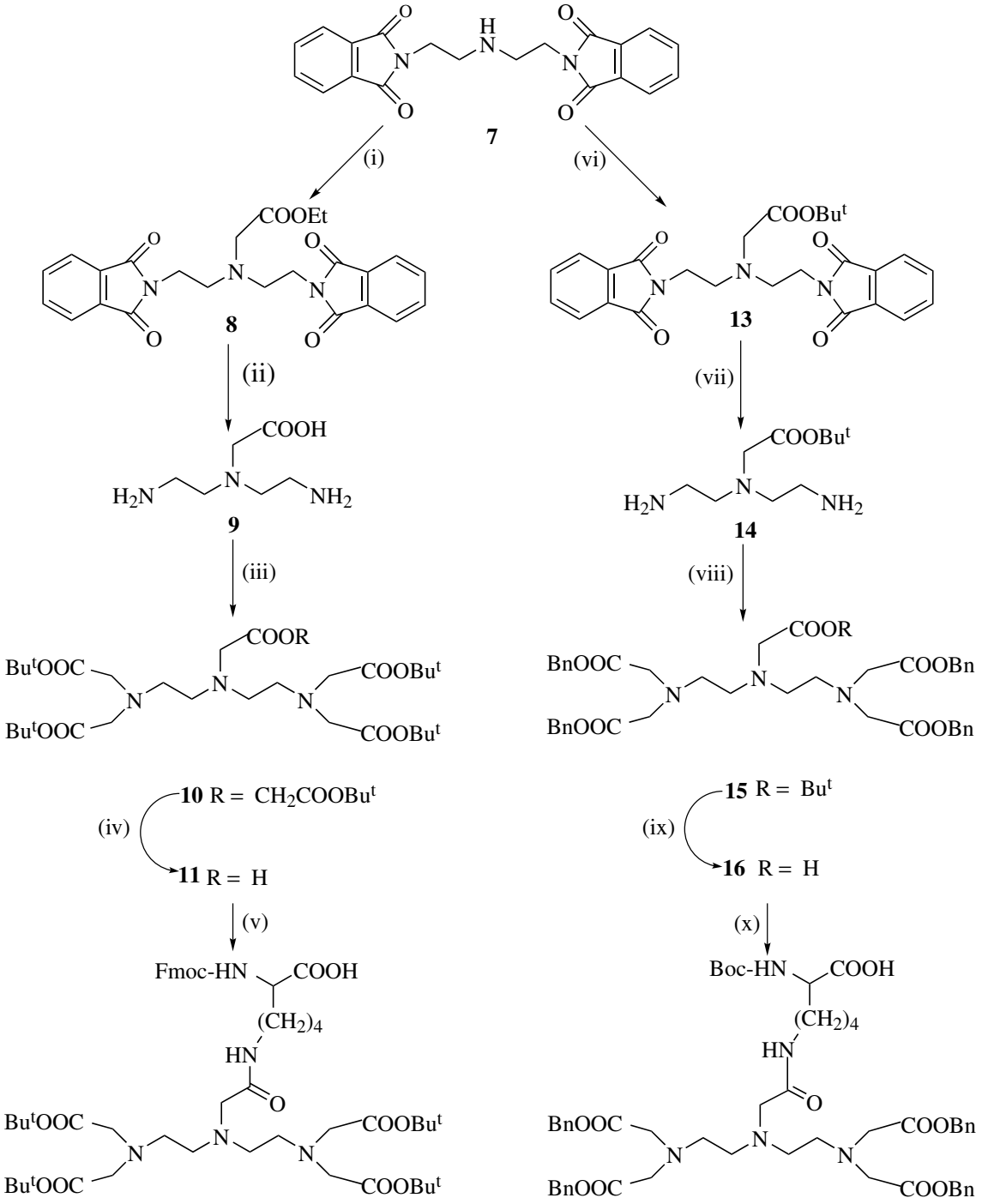
NMR spectra were recorded on a Bruker AC-400 FT spectrometer at 400 MHz for ^1H and 100 MHz for ^{13}C, or where noted in the experimental on a Bruker WM 250 spectrometer at 250 MHz for ^1H and 63 MHz for ^{13}C. Low resolution EI/CI and electrospray (ES) mass spectra were obtained using a Quattro II triple quadruple instrument, or a MAT900 instrument. Samples were loop injected into a stream of methanol/water (1 : 1). Low resolution FAB-MS analyses were run on an AutoSpec instrument. Cs^+ bombardment was used at 25 kV onto the sample held in a matrix liquid (3-nitrobenzyl alcohol). Accurate mass measurements were obtained on the MAT 900 instrument by manual peak matching. All MS analyses were courtesy of the EPSRC National MS Centre at Swansea.

Analytical HPLC analyses were carried out using LDC/Milton Roy equipment. A Spherisorb ODS C_{18} analytical column (25× 0.43 cm) at a flow rate of 1 cm^3 min^{-1} was used to check sample purity, with detector wavelength at 220 nm. The mobile phase was an isocratic ratio of CH_3CN : H_2O (40 : 60) containing 0.1% TFA. Solvents for HPLC were filtered before hand through 0.45 μm filters.

Melting points (m.p.) were recorded on an electrothermal digital melting point apparatus. Flash column chromatography on silica gel was carried out using Merck Matrex silica 60 (35–70 μm).

Synthesis of 1,7-Diphthaloyl-4-diethylenetriamine (7) {Nomenclature for the series can also be based on Bis-(2-(1,3-oxo-isoindol-2-yl)-ethyl)-amine}

2,2′-Dichlorodiethylamine hydrochloride [20] (113 mmol, 20.06 g) and potassium phthalimide (407 mmol, 75.4 g) in DMF (250 ml) was heated overnight in the range 90°–110 °C. The hot solution was then poured slowly into water (250 ml) containing ice (250 g) and potassium carbonate (25 g). After 1 h, the precipitate was filtered, washed with ice water, and dried *in vacuo* over P_2O_5 to



Reagents / Conditions: (i) $BrCH_2COOEt$ / Na_2CO_3 / EtOH / reflux; (ii) 6M HCl ; (iii) $BrCH_2COOBu^t$ / DIEA / reflux; (iv) NaSPh / Et_3N / MeCN: DMF 80:20 / 100°C 24 hr, r.t 48 hr; (v) Fmoc-Lys-OH / DCC / HOBt; (vi) $BrCH_2COOBu^t$ / DIEA / reflux; (vii) N_2H_4 / r.t 40 hr; (viii) $BrCH_2COOBn$ / DIEA / r.t.12 hr then reflux; (ix) trifluoroacetic acid / r.t 1.5 hr; (x) Boc-Lys-OH / DCC / HOBt.

Scheme 1

give white crystals (87% yield) of *1,7-diphthaloyl-4-diethylenetriamine* (**7**) m.p. 177°–179 °C, Lit. [20] 178°–180 °C HPLC $t_R = 3.07$ min. δ_H d_6 DMSO 2.73–2.76 (4H, t, *J* 6.2, 2 × *CH*$_2$), 3.57–3.60 (4H, t, *J* 6.2, 2 × *CH*$_2$NPht), 7.64–7.67 (2H, s, N*H*$_2^+$), 7.73–7.85 (8H, m, 2 × *Pht*); δ_c 37.2, 46.1 (*C*H$_2$), 121.9, 122.8 (aromatic *C*H), 131.7 (aromatic *C*), 133.0, 134.2 (aromatic *C*H), 168.0 (*C*O), FAB–m/z $[M + H]^+ = 364$, $[M + Na]^+ = 386$.

Ethyl 1,7-diphthaloyl-4-diethylenetriamine acetate (8)

A mixture of triamine derivative (**7**) (50 mmol, 18.17 g), ethyl bromoacetate (80 mmol, 8.87 ml) and sodium carbonate (50 mmol, 5.3 g) in ethanol (350 ml) was refluxed for 24 h. The solvent was then evaporated and the residual oily product re-dissolved in chloroform (100 ml), and washed with 10% citric acid (50 ml), sodium hydrogen carbonate (1 M, 50 ml) and distilled water (50 ml). The organic layer was dried over $MgSO_4$, filtered and evaporated to dryness. The resulting oily product was triturated with ethanol and the precipitate filtered and washed with ice-cold ethanol, when *ethyl 1,7-diphthaloyl-4-diethylenetriamine acetate* (**8**), a white solid was obtained in 78% yield m.p. 100°–102 °C (Lit. [20] 100°–102 °C). HPLC t_R = 4.40 min. δ_H d_6 DMSO 1.18–1.22 (3H, t, *J* 7.1 C*H*$_3$), 3.00–3.02 (4H, t, *J* 6.4, 2 × *CH*$_2$), 3.55 (2H, s, C*H*$_2$), 3.70–3.73 (4H, t, *J* 6.4, 2 × *CH*$_2$NPht), 4.06–4.11 (2H, q, *J* 7.1, C*H*$_2$) 7.64–7.71 (8H, m, 2 × *Pht*); δ_c 14.2 (*C*H$_3$) 35.9, 51.6, 53.9, 60.2 (*C*H$_2$), 123.0 (aromatic *C*H), 132.1 (aromatic *C*), 133.7 (aromatic *C*H), 168.2, 170.1 (*C*O), FAB–m/z $[M + H]^+ = 450$, $[M + Na]^+ = 472$.

t-Butyl 1,7-dinaphthoyl-4-diethylenetriamine acetate (13)

A mixture of triamine derivative (**7**) (13.3 mmol, 4.8 g), t-butyl 2-bromoacetate (21.8 mmol, 3.5 ml) and DIPEA (13.3 mmol, 2.3 ml) in dichloromethane (100 ml) was refluxed for 36 h, under nitrogen. It was then washed with 10% citric acid (50 ml), sodium hydrogen carbonate (1 M, 50 ml) and distilled water (50 ml). The organic layer was dried over $MgSO_4$, filtered and evaporated to dryness. The resulting oily product was recrystallized from ethanol, filtered and washed with ice-cold ethanol to give *t-butyl 1,7-dinaphthoyl-4-diethylenetriamine acetate* (**13**) as a white solid (51% yield), m.p. 119°–121 °C HPLC $t_R = 5.87$ min. δ_H $CDCl_3$ 1.42 (9H, s, C(C*H*$_3$)$_3$), 3.01–3.04 (4H, t, *J* 6.5, 2 × *CH*$_2$), 3.45 (2H, s, C*H*$_2$), 3.70–3.74 (4H, t, *J* 6.5, 2 × *CH*$_2$NPht), 7.64–7.72 (8H, m, 2 × *Pht*); δ_C $CDCl_3$ 21.2 (Bu^t *C*H$_3$), 36.0, 51.6 (*C*H$_2$), 81.0 (Bu^t *C*), 123.0 (aromatic *C*H), 132.1 (aromatic *C*), 133.7 (aromatic *C*H), 168.2, 170.5 (*C*O); FAB–m/z $[M + H]^+ = 478$, $[M + Na]^+ = 500$; $C_{26}H_{27}N_3O_6$ requires 478.1978, measured $[M + H]^+ = 478.1983$.

Diethylenetriamine acetic acid salt (9) (or Bis-(2-amino-ethyl)-amino-acetic acid)

A suspension of the triamine ester derivative (**8**) (35 mmol, 16.0 g) in hydrochloric acid (6M, 70 ml) was refluxed for 6 h. After cooling, filtering, and washing with hydrochloric acid (6 M, 4 × 5 ml), the combined filtrates were evaporated leaving an oily product, which was further dried over P_2O_5 *in vacuo*. *Diethylenetriamine acetic acid salt* (**9**) was obtained as a brown oil (98% yield); δ_H D_2O 3.05 (4H, br s, 2 × *CH*$_2$), 3.17 (4H, br s, 2 × *CH*$_2$NH$_2$), 3.77 (2H, br s, C*H*$_2$), 8.29 (6H, br s, 2 × *N*H$_3^+$); δ_C D_2O 36.4, 52.8 (C*H*$_2$), 172.3 (CO). EI-m/z $[M\text{-}H_2O]^+ = 143$.

{Bis-(2-(bis-t-butoxycarbonylmethyl-amino)-ethyl)-amino}-acetic acid t-butoxycarbonylmethyl ester (10)

To a solution of acid salt (**9**) (0.025 mol, 4.03 g) in acetonitrile (100 ml) was added 5 mol equivalents of t-butylbromoacetate (0.125 mol, 18.5 ml) and DIPEA (0.125 mol, 21.8 ml). The mixture was stirred at room temperature overnight, then refluxed for 24 h. The solvent was then evaporated to dryness and the residual oily product dissolved in dichloromethane (100 ml), which was then washed with 10% citric acid (50 ml), sodium hydrogen carbonate (1M, 50 ml) and distilled water (50 ml). After drying the organic layer over $MgSO_4$, filtration and evaporation gave an oily product which was chromatographed on a silica column using an eluant of 30% ethyl acetate in hexane and 1% triethylamine.

{Bis-[2-(bis-t-butoxycarbonylmethyl-amino)-ethyl]-amino}-acetic acid t-butoxycarbonylmethyl ester (**10**) was obtained as a yellow oil (61% yield); δ_H 250 MHz, $CDCl_3$ 1.33 (45H, s, 5 × *C*(*CH*$_3$)$_3$), 2.70 (8H, br s, 2 × *CH*$_2$*CH*$_2$), 3.27 [8H, s, 2 × *N*(*CH*$_2$CO$_2$)$_2$], 3.44 (2H, s, NHC*H*$_2$CO$_2$CH$_2$), 4.38 (2H, s, O$_2$CC*H*$_2$OCO); δ_C $CDCl_3$, 27.9, 28.0 (Bu^t *C*H$_3$), 52.0, 52.5, 54.5, 55.9, 60.8 (*C*H$_2$), 80.7, 82.1 (Bu^t *C*), 170.5 (*C*O); FAB — m/z $[M + H]^+ = 732$, $[M + Na]^+ = 754$; $C_{36}H_{65}N_3O_{12}$ requires 732.4647, measured $[M + H]^+ = 732.4643$.

{Bis-(2-(bis-t-butoxycarbonylmethyl-amino)-ethyl)-amino}-acetic acid (11)

The t-butoxycarbonylmethyl ester (**10**) (6.83 mmol, 5 g), sodium thiophenoxide (6.83 mmol, 0.90 g) and triethylamine (8.61 mmol, 1.2 ml) in MeCN : DMF 80 : 20 (100 ml) was heated at 100 °C for 24 h, then stirred at room temperature for 48 h. Solvent was evaporated and the residual oily product re-dissolved in dichloromethane (100 ml) and washed with sodium hydrogen carbonate (1M, 2 × 50 ml) and distilled water. Work-up of the organic layer as described previously gave an oily product which was chromatographed on silica gel, using a linear gradient of 0–40% methanol in diethyl ether. *{Bis-[2-(bis-t-butoxycarbonylmethyl-amino)-ethyl]-amino}-acetic acid* (**11**) was obtained as a brown oil (22% yield): δ_H 250 MHz, $CDCl_3$ 1.40 (36H, s, 4× C(CH_3)$_3$), 2.82 (8H, br s, 2 × CH_2CH_2), 3.42 [8H, s, 2 × N(CH_2CO_2)$_2$], 3.43 (2H, s, NHCH_2CO_2H); δ_C $CDCl_3$, 27.8 (Bu^t CH_3), 50.0, 54.2, 56.2, 61.0 (CH_2), 80.3, (Bu^t *C*), 170.1, 170.3 (*C*O); FAB–m/z $[M + H]^+ = 618$, $[M + Na]^+ = 640$; $C_{30}H_{55}N_3O_{10}$ requires 618.3965, measured $[M + H]^+$ by ES-m/z = 618.3963.

N^{α}-9-Fluorenylmethoxycarbonyl – N^{ε} — {Bis-(2-(bis-t-butoxycarbonylmethyl-amino)-ethyl)-amino}-acetyl lysine (Fmoc-Lys(But$_4$-DTPA)-OH) (12)

{Bis-[2-(bis-t-butoxycarbonylmethyl-amino)-ethyl]-amino}-acetic acid (**11**) (1.28 mmol, 0.539 g), HOBt (1.28 mmol 0.173 g) and DCCI (1.28 mmol, 0.264 g), dissolved in dichloromethane (7 ml) and DMF (3 ml) were stirred together at 0 °C for 1 h and at room temperature for 2 h, followed by filtration of the precipitated dicyclohexylurea. The filtrate, containing the activated ester, was evaporated to dryness and the residue re-dissolved in DMF (10 ml) and dichloromethane (4 ml), when N^{α}-Fmoc — L-lysine (1.28 mmol, 0.472 g) partially dissolved in ethylene glycol monoethyl ether (50 ml) was added, followed by DIEA (1.4 mmol, 0.24 ml). After stirring the reaction overnight, the solvent was evaporated, and the residue re-dissolved in dichloromethane (100 ml), the excess N^{α}-Fmoc–Lys-OH being removed by extraction with 10% citric acid (3 × 50 ml). After work-up of the organic layer as previously described, it was evaporated to dryness to yield a solid product which was chromatographed on silica gel using a linear gradient 0–40% methanol in diethyl ether. *N^{α}-9-Fluorenylmethoxycarbonyl — N^{ε}-{bis-[2-(bis-t-butoxycarbonylmethyl-amino)-ethyl]-amino}-acetyl lysine [Fmoc-Lys(But$_4$-DTPA)-OH]* (**12**) was obtained as a yellow gum (42% yield); δ_H 250 MHz, $CDCl_3$ 1.32 (36H, br s, 4 × $C(CH_3)_3$), 1.23–1.40 (4H, overlapping peaks, γ CH_2, δ CH_2), 1.82 (2H, br s, β CH_2), 2.72–2.74 (2H, br m, ω CH_2), 2.85–3.00 (8H, br s, 2 × CH_2CH_2), 3.23–3.40 [10H, br s, 5 × NCH_2CO], 3.50 (2H, br s, Fmoc CH_2) 4.09–4.11 (1H, d, Fmoc-C*H*), 4.21 (1H, br m, α C*H*), 6.86 (1H, m, ε N*H*) 7.20–7.29 (4H, m, Fmoc *Ar*), 7.47–7.55 (3H, br s, *Fmoc* and α N*H*) 7.61–7.64 (3H, d, *J* 7.3, *Fmoc*); δ_C $CDCl_3$, 22.9 (CH_2), 28.0 (t-Bu CH_3), 29.6, 30.2, 39.1 (CH_2), 47.1, 53.8 (*C*H), 55.5, 56.1, 63.6, 66.8 (CH_2), 81.8 (Bu^t *C*), 119.8, 124.9, 125.1, 125.9, 127.0, 127.6 (aromatic *C*H), 141.1, 142.6 (aromatic *C*) 167.0, 170.6 (*C*O); FAB–m/z $[M + H]^+ = 968$, $[M + Na]^+ = 990$; $C_{51}H_{77}N_5O_{13}$ Na requires 990.5416, measured $[M + Na]^+$ by ES-m/z = 990.5425.

t-Butyl–4-diethylenetriamine acetate (14) {Bis-(2-aminoethyl)-amino-acetic acid t-butyl ester}

To a solution of t-butyl 1,7-dinaphthoyl-4-diethylenetriamine acetate (**13**) (4.19 mmol) in 95% acetonitrile/water (40 ml), 4 mmol equivalent of hydrazine hydrate was added and the reaction mixture stirred at room temperature, until HPLC analysis showed no starting material to be present (40 h). The resulting white precipitate was filtered, washed with acetonitrile, and the combined filtrates were evaporated using a rotary evaporator at 25 °C under high vacuum to give *t-butyl–4-diethylenetriamine acetate* (**14**) as a colourless solid (87% yield); δ_H 250 MHz, d_6DMSO, 1.10 (9H, s, C (CH_3)$_3$), 2.59–2,71 (4H, m, 2 × CH_2), 2.91 (2H, s, NCH_2CO_2), 3.26–3.47 (4H, m, 2 × CH_2NH_2), 4.20–4.70 (4H, br s, 2 × NH_2); δ_C d_6 DMSO 27.8 (Bu^t CH_3), 37.3, 48.7 (CH_2), 80.4 (Bu^t *C*), 167.8 (*C*O); CI-m/z $[M + H]^+$ = 218.

If esters (e.g. benzyl or allyl) other than the t-butyl ester (**13**) were used, the cyclized product, 4-(2-aminoethyl)-piperazine-2-one(**18**) was the main product of phthaloyl group removal and was present as a brown oil δ_H d_6DMSO, 2.30 (2H, s, NH_2), 2.39–2.42 (2H, t, *J* 6.5 NCH_2), 2.58–2.60 (2H, t, *J* 5.5, CH_2N), 2.66–2.69 (2H, t, *J* 6.5, CH_2NH_2), 2.96 (2H, s, COCH_2), 3.18–3.21(2H, m, CH_2NH), 7.79 (1H, s, N*H*); δ_C d_6DMSO 38.0, 40.4, 48.9, 57.0, 63.0 (CH_2), 142.5 (*C*O); EI-m/z $[M + H]^+ = 144$; $C_6H_{13}N_3O$ requires 144.1137, measured $[M + H]^+$ by ES-m/z = 144.1136.

{Bis-(2-(bis-benzyloxycarbonylmethyl-amino)-ethyl)-amino}-acetic acid t-butyl ester (15)

t-Butyl–4-diethylenetriamine acetate (**14**) (2.3 mmol, 0.5 g) was dissolved in dichloromethane (100 ml) and benzyl 2-bromoacetate (14.9 mmol, 2.4 ml) was added. After the addition of di-isopropylethylamine (14.9 mmol, 2.6 ml), the reaction mixture was stirred overnight at room temperature under argon, and then heated under reflux for 24 h. Following washing of the organic layer in turn with 10% citric acid, sodium hydrogen carbonate and water, and the usual drying procedures, the residual oily product obtained was chromatographed on silica gel using an eluant of 30% ethyl acetate in hexane containing 1% triethylamine. {*Bis-[2-(bis-benzyloxycarbonylmethyl-amino)-ethyl]-amino*}*-acetic acid t-butyl ester* (**15**) was obtained from collected fractions as a yellow oil (59% yield); δ_H 250 MHz $CDCl_3$, 1.35 (9H, s, C (CH_3)$_3$), 2.71–2.77 (8H, m, 2 × CH_2CH_2), 3.56 (10 H, s, 5 × NCH_2CO_2), 5.03 (8H, s, 4 × $PhCH_2O_2C$), 7.25 (20H, s, 4 × C_6H_5); δ_C $CDCl_3$ 28.1 (Bu^t CH_3), 51.8, 52.4, 55.1, 55.8, 66.1 (CH_2), 80.8 (Bu^t *C*), 128.3, 128.4, 128.5 (aromatic *C*H), 135.6 (aromatic *C*), 170.6, 171.0 (*C*O); ES-m/z $[M + H]^+ = 810$, $[M + Na]^+ = 832$; $C_{46}H_{55}N_3O_{10}$ requires 810.3965, measured $[M + H]^+$ by ES-m/z = 810.3969.

{Bis-(2-(bis-benzyloxycarbonylmethyl-amino)-ethyl)-amino}-acetic acid (16)

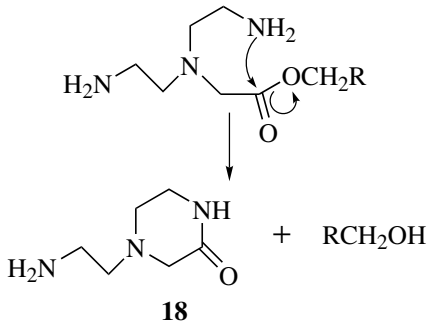
t-Butyl ester (**15**) (0.4 mmol, 0.324 g) was stirred in 70% trifluoroacetic acid in dichloromethane (5 ml) at room temperature for 1.5 h. Evaporation of the solvent, followed by neutralization with triethylamine (5 ml), followed by further evaporation, gave an oily product which was dissolved in dichloromethane (50 ml), washed three times with water, before the organic layer was dried over $MgSO_4$, filtered and evaporated to yield an oily product which was purified on a silica gel column, using a linear gradient of 0–40% methanol in diethyl ether (or 0–10% methanol in dichloromethane). {*Bis-[2-(bis-benzyloxy-carbonylmethyl-amino)-ethyl]-amino*}*-acetic acid* (**16**) was obtained as yellow oil (78% yield); δ_H 250 MHz $CDCl_3$, 2.89–2.96 (8H, m, 2 × CH_2CH_2), 3.31 (10H, s, 5 × NCH_2CO_2), 4.94 (8H, s, 4 × $PhCH_2O_2C$), 7.16 (20H, s, 4 × C_6H_5); δ_C $CDCl_3$ 50.2, 52.8, 54.9, 56.8, 66.5 (CH_2), 128.2, 128.3, 128.5 (aromatic *C*H), 135.4 (aromatic *C*), 171.2, 171.8 (*C*O); FAB-m/z $[M + H]^+ = 754$, $[M + Na]^+ = 776$; $C_{42}H_{47}N_3O_{10}$ requires 754.3340, measured $[M + H]^+$ by ES-m/z = 754.3340.

N^{α}-t-Butyloxycarbonyl – N^{ε} — {bis-(2-(bis-benzyloxycarbonylmethyl-amino)-ethyl)-amino}-acetyl lysine (Boc-Lys (Bn_4-DTPA)-OH) (17)

N^{α}-Boc — L-lysine and bis-[2-(bis-benzyloxycarbonylmethyl-amino)-ethyl]-amino-acetic acid (**16**) were coupled in the same mole ratio and using the same coupling agents and conditions as described under the preparation of Fmoc-Lys(Bu^t_4-DTPA)-OH (**12**) above. Purification by column chromatography, yielded N^{α}*-t-Butyloxycarbonyl* — N^{ε}-{*bis-[2-(bis-benzyloxycarbonylmethyl-amino)-ethyl]-amino*}*-acetyl lysine [Boc-Lys (*Bn_4*-DTPA)-OH]* (**17**) as a colourless gum (48% yield); δ_H 250 MHz $CDCl_3$, 1.31 (9H, s, C(CH_3)$_3$), 1.1–1.36 (4H, overlapping peaks, γ CH_2, δ CH_2), 1.60 (2H, br s, β CH_2), 2.50–2.84 (8H, br m, 2 × CH_2CH_2), 3.05–3.10 (2H, br m, ω CH_2), 3.43 (10H, s, 5 × NCH_2CO_2), 4.30 (1H, br s, α-C*H*), 5.03 (8H, s, 4 × $PhCH_2O_2C$), 5.30 (1H, br s, ε-N*H*), 6.80 (1H, s, α-N*H*), 7.18–7.30 (20H, s, 4 × C_6H_5); δ_C $CDCl_3$ 21.8 (CH_2), 28.3 (C(CH_3)$_3$), 28.9, 30.1, 40.0, 51.6, 52.7 (CH_2), 54.5 (*C*H), 54.8, 55.1, 66.3 (CH_2), 80.0 (*C*(CH_3)$_3$), 127.3, 128.3, 128.5 (aromatic *C*H), 131.8 (aromatic *C*), 168.0, 171.1 (*C*O); FAB-m/z $[M + H]^+ = 982$, $[M + Na]^+ = 1004$.

RESULTS AND DISCUSSION

The strategy adopted was to synthesize symmetrically protected tetra esters (**11**) and (**16**), having free carboxyl groups attached to the middle acetate branch, which could be used for coupling to the ε-amino group of a suitably protected lysine derivative. To us, the most accessible starting material was the diphthaloyl derivative (**7**), and if orthogonal ester protecting groups could be used to insert the central acetate group, we would achieve a short and streamlined route to the required synthons. However, the removal of the phthaloyl protecting groups under the standard hydrazine deprotection conditions, proved to be incompatible with the presence of ethyl, allyl or benzyl ester protection on the central acetate. Only in the case of the t-butyl analogue (**13**) did this strategy work. The explanation to these failures is summarized in Scheme 2. Once the two phthaloyl groups were removed, there was an immediate cyclization to form the piperidone (**18**), in all cases except (**13**), where its deprotected product



18

Scheme 2

(**14**) showed less tendency to cyclize, presumably due to the steric hindrance of the t-butyl group.

The synthetic route (**7–11**) was therefore achieved under acid cleavage conditions in the early stages, and very critical to the success of the sequence was the previous observation of Rana and co-workers [17] that a t-butoxycarbonylmethyl ester can be removed by sodium thiophenoxide, in the presence of other t-butyl esters. Thus the stage (**10–11**) was carried out using sodium thiophenoxide, but the conditions had to be modified from those previously published [17], and even then was not a high-yielding reaction. The final stage (**11–12**) proceeded without problems, but the yield was not optimized, and probably would benefit from a previous observation [17] that such couplings would be higher yielding, if *N*- and *C*-protected lysine derivatives were used.

As pointed out earlier, it was found that the synthetic sequence (**7–17**) was only possible due to the compatibility of the t-butyl, and phthaloyl protecting group, during the latter's deprotection by hydrazine. However, we recommend that the deprotection should be carried out at room temperature and with only a mole equivalent of hydrazine hydrate to avoid any chance of the cyclization reaction taking place to form (**18**). The latter was sometimes detected as a by-product if excess hydrazine was used. Removal of solvent at as low a temperature as possible (25 °C) under high vacuum also prevented cyclization. Care should also be taken during the subsequent step (**14–15**) to control the temperature to ambient for the first 12 h, adding the DIEA only after the benzyl bromoacetate had been added. The deprotection of the t-butyl group in the presence of the benzyl esters occurred smoothly, and in good yield using the conventional trifluoroacetic acid method. The recently reported [21] zinc bromide-catalysed removal of the t-butyl group gave much poorer results for this stage.

Acknowledgements

One of us, L. Al-Jamri, gratefully acknowledges a grant from the Altajir Trust, which enabled him to pursue this research. We are indebted to the Chemistry Department for all services relating to the implementation of the work.

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