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Solid Phase Synthesis of Polyamine Conjugates for the Study of Trypanothione Reductase

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Abstract: A number of polyamine scaffolds were synthesised, enabling the facile preparation of a variety of polyamine conjugates using both Boc and Fmoc protecting group strategies. Products were released from the solid support by treatment with either triflic acid/trifluoroacetic acid or trifluoroacetic acid. The trypanosomal metabolite N^1, N^8-*bis*(glutathionyl)spermidine [trypanothione], and a range of related analogues were prepared for biological evaluation as previously communicated. © 1997 Elsevier Science Ltd.

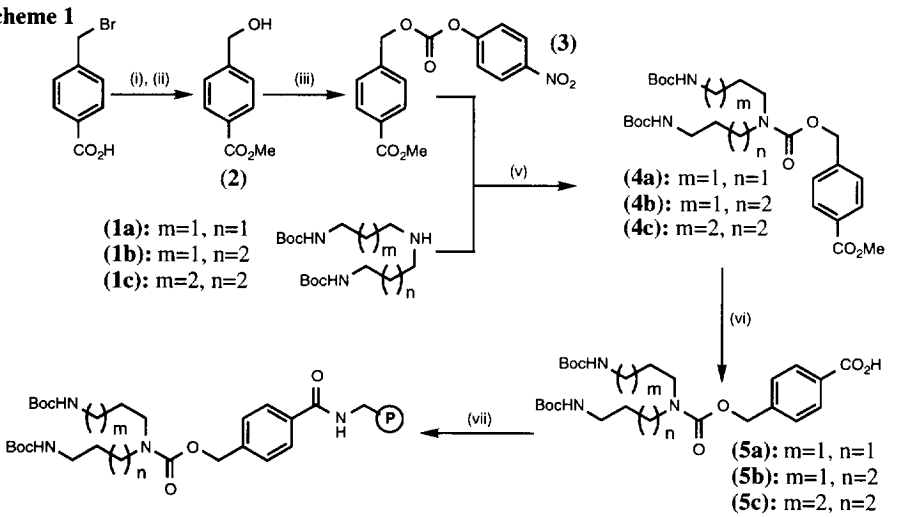
The inclusion of polyamines in a wide variety of biologically active compounds has led to a great deal of attention being focused on this class of compounds. For example many micro-organisms produce polyamine/peptide conjugates, for example, the edeines from *Bacillus brevis* exhibit broad spectrum anti-microbial activity.[1] Many of the glutamate receptor antagonists found in spider toxins[2] and wasp venom [3] contain polyamines conjugated to peptidic moieties and the ability to selectively block such neural pathways has implications in the treatment of neurological disorders such as Alzheimer's disease, Huntington's chorea and epilepsy. The funnel web spider toxins FTX 3.3 and sFTX 3.3 have potent paralytic properties arising from inhibition of voltage activated calcium channels at very low concentrations.[4] Polyamine conjugates are also produced by plants, and kukoamine A [N^1, N^{12}-*bis*(dihydrocaffeoyl)spermine] isolated from *Lycium chinense*[5] has been found to be a clinically effective anti-hypotensive agent, while they have been implicated in cell proliferation and have been studied with potential application in cancer chemotherapy.[6] The parasitic trypanosomes and leishmania utilise a novel cofactor, N^1, N^8-*bis*(glutathionyl)spermidine [trypanothione] in conjunction with the unique parasitic enzyme trypanothione reductase as their principal mechanism of oxidative stress management. This novel metabolism has been the subject of much research[7] and the ability to prepare a range of structural analogues of trypanothione was essential to our own efforts. We herein give full experimental details of our recent communications of a very reliable and attractive method to anchor a variety of protected polyamines to polystyrene based resins and their application in the synthesis of trypanothione and related polyamine conjugates to probe the active site of trypanothione reductase. It is unfortunate that a recent claim by Bycroft et al. with respect to the solid phase synthesis of trypanothione is substantially in error.[8]

SYNTHESIS OF LINKAGE AGENTS CONTAINING PROTECTED POLYAMINES

The first linkage agent employed to incorporate the polyamines was based around the benzyloxycarbonyl protecting group, such that the linkage would be stable to the cleavage conditions required for both Fmoc and Boc peptide synthesis, with the final release of the polyamine conjugates being achieved by treatment with triflic acid/TFA.[9] Linkage agents incorporating linear triamines were prepared, the link to the solid support being via the secondary amine, containing both *bis*-terminal and orthogonally protected polyamines.

Linkage agents (**5a,b,c**) containing three homologous *bis*-protected triamines were prepared as shown in Scheme 1. *Bis*-Boc protected norspermidine and spermidine (**1a,b**) were readily synthesised from the free triamine using the reagent Boc-ON,[10] to selectively protect the primary amino groups. The *bis*-protected homospermidine (**1c**) was prepared as shown in Scheme 2 from N^1-benzylputrescine.[11] The carbonate species (**3**) which was prepared in three steps from α-bromo-*p*-toluic acid[12] enabled both protection of the secondary amine and the introduction of the handle for attachment to a solid support.[13] Importantly, this reagent allows the purification of the linkage agents (**5**) prior to attachment and quantifiable incorporation of any suitably protected polyamine onto the solid phase. These scaffolds have proven themselves in our laboratories by the synthesis of many thousand polyamine conjugates.[13]

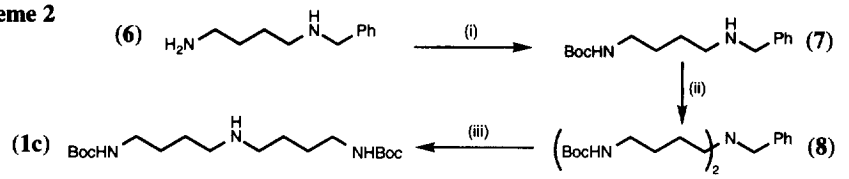
Scheme 1



Reagents: (i) H_2O, reflux, 96% (ii) MeOH, HCl, reflux, 99% (iii) *p*-nitrophenylchloroformate, pyridine, CH_2Cl_2, 80% (iv) Boc-ON, NaOH, dioxane, 81% (v) DMF, NEt_3, 40°C, 96% (vi) Aq. NaOH, dioxane (vii) DIC, HOBt, aminomethyl resin, DMF

After base hydrolysis of the methyl ester (**4a,b,c**), the resulting linkage agents (**5a,b,c**) were anchored onto an aminomethylated polystyrene resin using standard solid phase conditions.

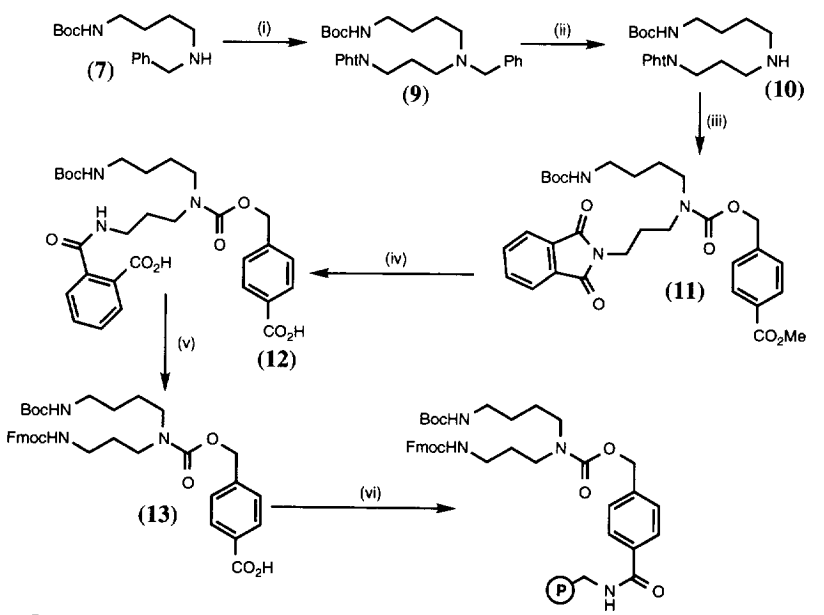
Scheme 2



Reagents: (i) Boc-ON, aq. NaOH, dioxane, 82% (ii) $BocHN(CH_2)_4OTs$, Na_2CO_3, KI, *n*BuOH, 86% (iii) Pd/C, H_2, EtOH, AcOH, 86%

In order to incorporate an orthogonally protected spermidine, the synthetic strategy was modified as shown in Scheme 3. The polyamine was constructed from N^1-Boc-N^4-benzylputrescine (**7**), by alkylation with 3-bromopropylphthalimide followed by hydrogenolysis to release the central secondary amine.[11] Reaction with the key carbonate (**3**) proceeded as before, although base hydrolysis of the methyl ester (**11**) also resulted in partial cleavage of the phthalimide protecting group. Cleavage of the resulting phthalamic acid followed by re-protection of the primary amine with Fmoc succinimide gave the linkage agent (**13**) incorporating an orthogonally protected spermidine which was attached to a solid support as before.

Scheme 3

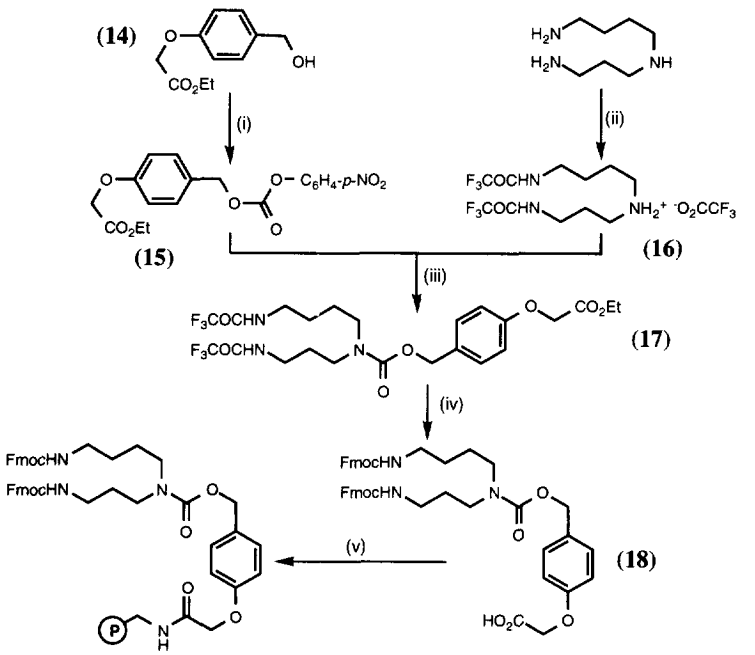


Reagents: (i) 3-bromopropylphthalimide, Na_2CO_3, KI, *n*BuOH, 62% (ii) H_2, Palladium/C, 83% (iii) 4-nitrophenyl-4'-hydroxymethylmethylbenzoate carbonate (**3**), NEt_3, DMF, 97% (iv) aq. NaOH, dioxane, 91% (v) N_2H_4. H_2O, EtOH; then Fmoc-succiminide, aq. $NaHCO_3$, dioxane, 75% (vi) aminomethyl resin, DIC, HOBt, DMAP, DMF

In addition to the linkage agents prepared above, another derivative was prepared for application to the synthesis of libraries of polyamine conjugates for screening in solution, enabling the release of products

under milder conditions (treatment with trifluoroacetic acid as opposed to triflic acid/trifluoroacteic acid) and being compatible with Fmoc amine protection.[9] The synthesis of this linkage agent **(18)** is shown in Scheme 4 and utilises a modified carbonate **(15)** to introduce the secondary amino protection and handle for resin attachment.[13] The handle differs only in the substitution of the carboxylic acid handle with an oxyacetic acid handle, thereby increasing the electron density of the aromatic system and susceptibility to cleavage under acidic conditions. The precursor to this carbonate, 4-hydroxymethylphenyloxyethyl acetate **(14)** was prepared from 4-hydroxybezaldehyde in two steps. N^1, N^8-*bis*(trifluoroacetyl)spermidine **(16)** was used to prepare the linkage agent, and was synthesised by the treatment of spermidine with ethyl trifluoroacetate.[14] Simultaneous hydrolysis of the ester and trifluoroacetyl protecting groups followed by re-protection of the amino groups with Fmoc succinimide gave the linkage agent **(18)** which was immobilised as before.

Scheme 4



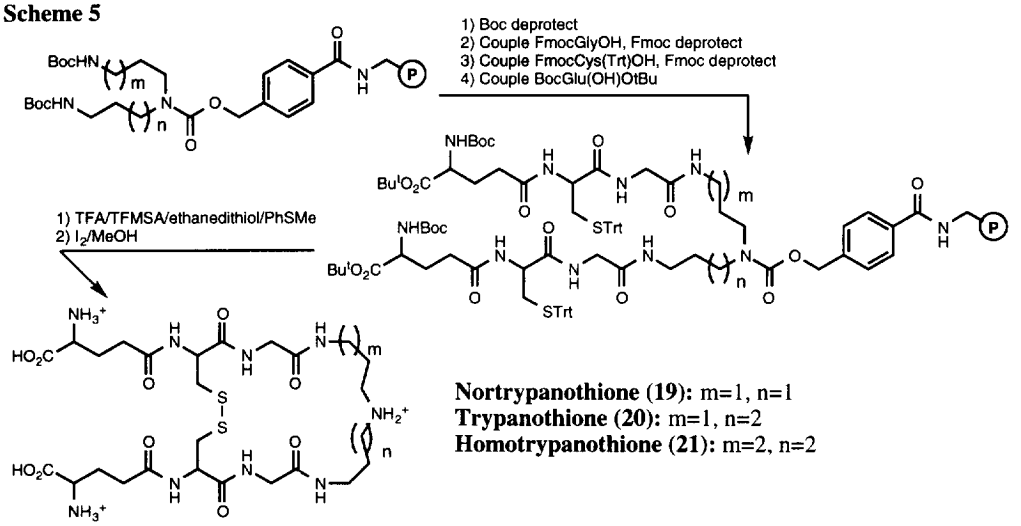
Reagents: (i) *p*-nitrophenylchloroformate, CH_2Cl_2, pryidine, 84% (ii) CF_3CO_2Et, dioxane, H_2O, Δ, 71% (iii) DMF, NEt_3, 45°C, 75% (iv) aq. NaOH, dioxane, followed by $KHSO_4$, then Fmoc-OSu, dioxane, aq. $NaHCO_3$, 85% (v) DIC, HOBt, DMAP, CH_2Cl_2, aminomethyl resin.

SYNTHESIS OF TRYPANOTHIONE AND ANALOGOUS POLYAMINE CONJUGATES

The linkage agents **(5a,b,c)** were used to prepare the three polyamine/glutathione conjugates nortrypanothione **(19)**, trypanothione **(20)** and homotrypanothione **(21)** respectively (Scheme 5). After attachment to the solid support and removal of the Boc protecting groups, the peptidic moiety was constructed by sequential coupling and Fmoc deprotection of FmocGlyOH, FmocCys(Trt)OH followed by

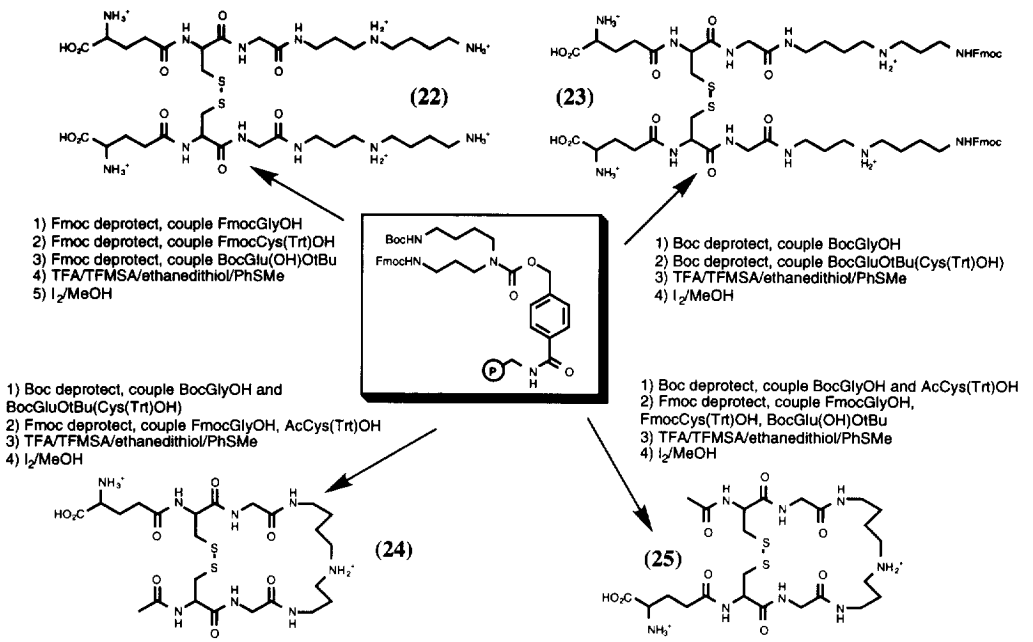
final coupling of BocGlu(OH)OtBu, all couplings being monitored by ninhydrin tests.[15] The products were cleaved from the solid support using a 'low-high' procedure where the side chain protecting groups were removed by initial treatment with trifluoroacetic acid/ethanedithiol/thioanisole, and the product released subsequently by treatment with a combination of TFA/triflic acid/thioanisole for 2 hours at 40°C. After ether precipitation, disulphide bond formation was achieved using methanolic iodine[16] and the products purified using RP HPLC. Synthetic trypanothione (**20**) was assayed with trypanothione reductase and was found to exhibit kinetic properties consistent with the accepted literature values.[17,18,19] Both nortrypanothione (**19**) and homotrypanothione (**21**) were found to be equally good substrates for trypanothione reductase.[19]

Scheme 5



The linkage agent (**13**) containing orthogonally protected spermidine allowed a combination of both Boc and Fmoc peptide synthesis to be carried out, thus enabling the selective manipulation of either primary amino group of spermidine, an essential factor in the preparation of analogues for probing the active site of trypanothione reductase (Scheme 6).[19] In the first instance, Fmoc chemistry alone was used to prepare N^1-glutathionylspermidine (**22**) which was isolated after cleavage and solution phase disulphide bond formation. Conversely, Boc chemistry was used to prepare N^1-Fmoc-N^8-glutathionylspermidine (**23**), with the dipeptide BocGlu(Cys(Trt)OH)OtBu being coupled to enable the use of S-trityl protection. A combination of both Boc and Fmoc chemistries were used to prepare the two isomeric compounds N^1-acetylcysteinylglycinyl-N^8-glutathionylspermidine (**24**) and N^1-glutathionyl-N^8-acetylcysteinylglycinyl-spermidine (**25**). These two substrates were used to investigate the preference, if any, of trypanothione reductase for the binding orientation of the polyamine in its natural substrate, but little difference was observed between them.[19] The more acid labile linkage agent (**18**) (Scheme 4) has been used in the preparation of a small polyamine library which was screened for inhibition of trypanothione reductase.[13]

Scheme 6



In summary, we have developed a method of anchoring any suitably protected polyamine to a solid support and successfully prepared a range of polyamine conjugates for biological examination. The application to combinatorial synthesis has been investigated and studies are ongoing in this area.

Acknowledgements. The authors would like to thank the EPSRC for a quota award (I. R. M.) and the Royal Society (M. B.).

EXPERIMENTAL

4-Hydroxymethylbenzoic acid[12]

α-Bromo-*p*-toluic acid (13.52g, 63.0mmol) was dissolved in water with heating and stirring. On dissolution the mixture was heated under reflux for 2 hours. The reaction mixture was allowed to cool, and left to stand overnight at 4°C whereupon the product crystallised to give colourless needles. The crystals were collected by filtration and the filtrate concentrated to furnish a second crop. Yield 9.17g (96%), Tlc : ethyl acetate/petroleum ether/acetic acid (19:19:2) R_f 0.52, mp: 176-178°C, lit. 181-183°C, δ_H (d_6-DMSO, 300MHz, *J*/Hz): 4.57 (s, 2H, CH_2), 5.35 (s, 1H, OH), 7.42 (d, *J* 8, 2H, Aryl $C^{3,5}H$), 7.90 (d, *J* 8, 2H, Aryl $C^{2,6}H$), 12.85 (s, 1H, CO_2H), δ_C (d_6-DMSO, 75MHz): 62.54 (CH_2), 126.25 (Aryl $C^{3,5}H$), 129.17 (Aryl C^1), 129.30 (Aryl $C^{2,6}H$), 147.83 (Aryl C^4), 167.41 (CO_2H), IR (Nujol),υ (cm^{-1}): 3289 (OH), 1701 (acid C=O).

4-Hydroxymethylbenzoic acid methyl ester (2)

4-Hydroxymethylbenzoic acid (9.17g, 60.3mmol) was dissolved in methanol (250ml) and hydrochloric acid (25ml) and heated under reflux for 4 hours. The acid was neutralised with triethylamine and the solvents removed under reduced pressure. The residue was dissolved in dichloromethane (150ml) and washed with 5% aq. $NaHCO_3$ (2x50ml). The organic phase was separated, dried ($MgSO_4$), and the solvent removed *in vacuo* to give the product as an off-white solid. Yield 10.0g (99%), Tlc : ethyl acetate/petroleum ether/acetic acid (19:19:2) R_f 0.60, mp: 45-46°C, δ_H ($CDCl_3$, 300MHz, *J*/Hz): 2.52 (s, 1H, OH), 3.90 (s, 3H, COOMe), 4.73 (s, 2H, CH_2), 7.39 (d, *J* 8, 2H, Aryl $C^{3,5}H$), 7.99 (d, *J* 8, 2H, Aryl $C^{2,6}H$), δ_C ($CDCl_3$, 75MHz): 52.30 (CO_2*Me*), 64.71 (CH_2), 126.60 (Aryl $C^{3,5}H$), 129.31 (Aryl C^1), 129.95 (Aryl $C^{2,6}H$), 146.28 (Aryl C^4), 167.25 (*C*O_2Me), IR ($CHCl_3$),υ (cm^{-1}): 3464 (OH), 1716 (ester).

4-Nitrophenyl-4'-hydroxymethylmethylbenzoate carbonate (3)[13]

4-Hydroxymethylbenzoic acid methyl ester (**2**) (10.0g, 60.2mmol) was dissolved in dichloromethane (150ml) and pyridine (7.3ml, 90.3mmol) and cooled in an ice-salt bath. A solution of *p*-nitrophenylchloroformate (13.39g, 66.3mmol) in dichloromethane (50ml) was added dropwise over 30 minutes and stirring continued for a further 2 hours. The reaction mixture was poured into water (200ml) and the organic phase separated. The aqueous phase was extracted with dichloromethane (2x30ml), the organic extracts combined, dried ($MgSO_4$), and the solvent removed under reduced pressure. The crude product was recrystalised from ether/dichloromethane as pale yellow crystals. Yield 15.90g (80%), Tlc : ethyl acetate/petroleum ether (1:1) R_f 0.64, mp: 98-100°C, δ_H ($CDCl_3$, 300MHz, *J*/Hz): 3.93 (s, 3H, CO_2Me), 5.34 (s, 2H, CH_2), 7.38 (d, *J* 9, 2H, Aryl $C^{3,5}H$), 7.50 (d, *J* 9, 2H, Aryl $C^{3',6'}H$), 8.07 (d, *J* 9, 2H, Aryl $C^{2,6}H$), 8.26 (d, *J* 9, 2H, Aryl $C^{2',6'}H$), δ_C ($CDCl_3$, 75MHz): 52.43 (CO_2*Me*), 70.18 (CH_2), 121.91 (Aryl $C^{3',5'}H$), 125.50 (Aryl $C^{2',6'}H$), 128.25 (Aryl $C^{3,5}H$), 130.21 (Aryl $C^{2,6}H$), 130.81 (Aryl C^1), 139.21 (carbonate), 145.62 (Aryl C^4), 152.53 (Aryl $C^{1'}$), 155.54 (Aryl $C^{4'}$), 166.69 (*C*O_2Me), IR ($CHCl_3$),υ (cm^{-1}): 1767 (carbonate), 1721 (ester), 1528, 1349 (C-NO_2), M/Z (FAB, NBA matrix): 332 $(M+H)^+$, 300 $(M+H\text{-}OMe)^+$.

N^1, N^7-*bis*(Boc)Norspermidine (1a) and N^1, N^8-*bis*(Boc)Spermidine (1b)

The polyamine (11.5mmol) was dissolved in dioxane (25ml) and sodium hydroxide (2M, 11.5ml) and cooled on ice. A solution of Boc-ON (5.66g, 23.0mmol) in dioxane (15ml) was added dropwise with stirring and the mixture stirred for 90 minutes. The solvents were removed under reduced pressure and the residue partitioned between dichloromethane (30ml) and sodium hydroxide (0.5M, 40ml). The organic layer was separated and the aqueous phase extracted with dichloromethane (3x20ml). The organic extracts were combined, dried ($MgSO_4$), and the solvent removed *in vacuo*. The product was purified by column chromatography on silica gel eluting with chloroform/methanol (9:1) and obtained as a white solid.

N^1, N^7-*bis*(Boc)Norspermidine (1a) Yield 3.07g (81%), Tlc : chloroform/methanol (9:1) R_f 0.24, mp 70-72°C, δ_H ($CDCl_3$, 300MHz, *J*/Hz): 1.41 (s, 18H, 2xCMe3), 1.62 (quin, *J* 7, 4H, C^2H_2 + C^6H_2), 2.62 (t, *J* 7, 4H, C^3H_2 + C^5H_2), 3.17 (dt, *J* 6, 7, 4H, C^1H_2 + C^7H_2), 5.25 (broad s, 2H, NH), δ_C ($CDCl_3$, 75MHz): 28.55 (*CMe*3), 29.89 (C^2H_2 + C^6H_2), 39.04 (C^3H_2 + C^5H_2), 47.53 (C^1H_2 + C^7H_2), 79.06 (*C*Me3), 156.25 (urethane), IR ($CHCl_3$),υ (cm^{-1}): 1705 (urethane C=O), 1509 (urethane), M/Z (FAB, NBA matrix): 332 $(M+H)^+$, 132 $(M+H\text{-}2Boc)^+$. HRMS $[M+H]^+$ $C_{16}H_{34}O_4N_3$ Calcd. 332.2573, Found 332.2549

N^1, N^8-*bis*(Boc)Spermidine (1b) Yield 2.50g (70%), Tlc : chloroform/methanol (9:1) R_f 0.17, mp 77-78°C, lit. 86-87°C, δ_H ($CDCl_3$, 300MHz, *J*/Hz): 1.41 (s, 18H, 2xCMe3), 1.49 (m, 4H, C^6H_2 + C^7H_2), 1.62 (quin, *J* 7, 2H, C^2H_2), 2.57 (t, *J* 7, 2H, C^5H_2), 2.63 (t, *J* 6, 2H, C^3H_2), 3.09 (dt, *J* 6, 7, 2H, C^8H_2), 3.17 (dt, *J* 6,7, 2H, C^1H_2), 4.91 (broad s, 1H, N^8H), 5.23 (broad s, 1H, N^1H), δ_C ($CDCl_3$, 75MHz): 27.48, 27.94 (C^6H_2 + C^7H_2), 28.55 (*CMe*3), 29.96 (C^2H_2), 39.32 (C^1H_2), 40.54 (C^8H_2), 47.84 (C^3H_2), 49.55 (C^5H_2), 79.04 (*C*Me3), 156.16, 156.25 (urethanes), IR ($CHCl_3$),

υ (cm^{-1}): 1705, 1509 (urethane), M/Z (FAB, NBA matrix): 346 (100%, $[M+H]^+$), 290 (12%, $[M+H-{}^tBu]^+$), 234 (23 %, $[M+H-2{}^tBu]^+$). HRMS $[M+H]^+$ $C_{17}H_{36}O_4N_3$ Calcd. 346.2706, Found 346.2706.

N^1-Benzylputrescine (6)

Putrescine (96g, 109.5ml, 1.09mol) was carefully dissolved in formic acid (360ml) whilst cooling in an ice bath. The solution was cooled to 0-5°C and benzaldehyde (28.9g, 27.8ml, 0.27mol) added dropwise over 30 minutes. The mixture was stirred and slowly warmed to reflux over a period of 2 hours. Heating was continued overnight resulting in a reddish-brown solution which was allowed to cool and poured into 6M HCl (800ml). The resulting orange/red solution was heated under reflux for a further 20 hours before evaporating to dryness. The semi-solid residue was dissolved in water (500ml) and made basic (pH 10-11) by the addition of solid sodium hydroxide. The cloudy solution was extracted with chloroform (5x175ml). The extracts were combined, dried (Na_2SO_4) and the solvent removed *in vacuo*. The crude product was purified by distillation under reduced pressure, collecting the fraction which distilled at 108-110°C (0.6mmHg). Yield 33.7g (70%), δ_H ($CDCl_3$, 300MHz, *J*/Hz): 1.22 (s, 3H, NH + NH_2), 1.39-1.56 (m, 4H, C^2H_2 + C^3H_2), 2.59-2-68 (2xt, *J* 7, 4H, C^1H_2 + C^4H_2), 3.75 (s, 2H, PhCH_2), 7.18-7.25 (m, 5H, Ph), δ_C ($CDCl_3$, 75MHz): 27.51 (C^2H_2), 31.64 (C^3H_2), 42.20 (C^4H_2), 49.33 (C^1H_2), 54.09 (PhCH_2), 126.91, 128.12, 128.41, 140.54 (Ph), IR ($CHCl_3$),υ (cm^{-1}): 3367, 3277 (NH), 2928, 2854 (CH), 1588, 1492 (Ph), 1452 (CH), M/Z (ES): 179.2 (100%, $[M+H]^+$), 162.2 (37%, $[M-NH_3+H]^+$).

N^1-Boc-N^4-benzylputrescine (7)

N^1-Benzylputrescine **(6)** (3.0g, 16.9mmol) was dissolved in dioxane (30ml) and sodium hydroxide (2M, 8.4ml, 16.9mmol) with stirring and cooling on ice. A solution of Boc-ON (4.15g, 16.9mmol) in dioxane (20ml) was added dropwise over 30 minutes and the reaction mixture stirred for 3 hours. The solvents were removed under reduced pressure and the residue dissolved in chloroform (50ml) and sodium hydroxide (0.5M, 40ml). The organic layer was separated and the aqueous phase extracted with chloroform (2x30ml). The organic extracts were combined, dried (Na_2SO_4), and the solvent removed *in vacuo*. The product was purified by column chromatography on silica gel eluting with chloroform/methanol (19:1) and obtained as a colourless oil. Yield 2.85g (82%), Tlc : chloroform/methanol (9:1) R_f 0.33, δ_H ($CDCl_3$, 300MHz, *J*/Hz): 1.44 (s, 9H, CMe3), 1.53 (quin, *J* 3, 4H, C^2H_2 + C^3H_2), 2.64 (t, *J* 5, 2H, C^4H_2), 3.11 (broad dt, *J* 5, 2H, C^1H_2), 3.77 (s, 2H, PhCH_2), 4.93 (broad s, 1H, C^1H_2NH), 7.21-7.33 (m, 5H, Ph), δ_C ($CDCl_3$, 75MHz): 27.59, 28.04 (C^2H_2 + C^3H_2), 28.61 (C*Me*3), 40.63 (C^1H_2), 49.15 (C^4H_2), 54.21 (PhCH_2), 79.10 (*C*Me3), 127.10, 128.29, 128.55, 140.52 (Ph), 156.20 (urethane), IR ($CHCl_3$),υ (cm^{-1}): 1705, 1509 (urethane), M/Z (ES): 279.2 (100%, $[M+H]^+$), 223.2 (79%, $[M-{}^tBu+H]^+$).

N^1, N^9-*bis*(Boc)-N^5-Benzylhomospermidine (8)

A stirred suspension of N^1-Boc-N^4-benzylputrescine **(7)** (3.50g, 12.6mmol), potassium iodide (0.5g) and sodium carbonate (1.38g, 13.0mmol) in *n*-butanol (40ml) was warmed to 50°C. A solution of N-Boc-O-(*p*-tolylsulphonyl)-4-amino-1-butanol (5.19g, 15.1mmol) in *n*-butanol (30ml) was added dropwise over 90 minutes and stirring continued overnight. After 24 hours more sodium carbonate (1.38g, 13.0mmol) and a few crystals of DMAP were added. After 48 hours N-Boc-O-(*p*-tolylsulphonyl)-4-amino-1-butanol (2.00g, 5.8mmol) was added and after 72 hours 0.45g (1.3mmol) portions were added periodically until 9.89g (28.8mmol) of N-Boc-O-(*p*-tolylsulphonyl)-4-amino-1-butanol had been added and only a trace of starting material remained. The reaction mixture was filtered and the residue washed with ethanol (3x10ml). The solvents were removed from the filtrate *in vacuo*, the residue was dissolved in chloroform (60ml) and washed with sodium hydroxide solution (0.5M, 80ml). The aqueous phase was then extracted with chloroform (2x20ml). The organic extracts were combined, dried (Na_2SO_4) and the solvent removed *in vacuo*. The crude product was purified by column chromatography on silica gel eluting with chloroform/methanol (99:1) and then chloroform/methanol (95:5) to yield the product as an oil. Yield 4.86g

(86%), Tlc : chloroform/methanol (9:1) R_f 0.29, δ_H ($CDCl_3$, 300MHz, *J*/Hz): 1.43 (s, 9H, CMe_3), 1.41-1.44 (m, 8H, $C^2H_2 + C^3H_2 + C^7H_2 + C^8H_2$), 2.38 (t, *J* 7, 4H, $C^4H_2 + C^6H_2$), 3.09 (broad dt, *J* 5,7, 4H, $C^1H_2 + C^9H_2$), 3.51 (s, 2H, Ph*CH*$_2$), 4.84 (broad s, 2H, NH), 7.18-7.30 (m, 5H, Ph), δ_C ($CDCl_3$, 75MHz): 24.53, 27.07 ($C^2H_2 + C^3H_2 + C^7H_2 + C^8H_2$), 28.61 (C*Me*$_3$), 40.65 ($C^1H_2 + C^9H_2$), 53.55 ($C^4H_2 + C^6H_2$), 58.76 (Ph*CH*$_2$), 79.05 (*C*Me$_3$), 126.95, 128.29, 129.02, 139.87 (Ph), 156.18 (urethane), IR ($CHCl_3$),υ (cm^{-1}): 3453 (NH), 1705, 1509 (urethane), M/Z (FAB, NBA matrix): 450 (60%, $[M+H]^+$), 291 (72%, $[M\text{-Boc-}^tBu]^+$), 91 (100 %, $[C_7H_7]^+$). HRMS $[M+H]^+$ $C_{25}H_{44}O_4N_3$ Calcd. 450.3332, Found 450.3325.

N^1, N^9-*bis*(Boc)-Homospermidine (1c)

N^1, N^9-*bis*(Boc)-N^5-Benzylhomospermidine (**8**) (4.34g, 9.67mmol) was dissolved in ethanol (80ml) and acetic acid (1ml). The solution was twice degassed and the reaction vessel filled with nitrogen. Palladium on activated charcoal (10%, 0.5g) was added and the solution degassed. The reaction vessel was filled with hydrogen and the solution degassed again. This procedure was repeated three times. The reaction mixture was stirred at room temperature for 90 minutes. The reaction mixture was filtered through celite and the residue washed with ethanol (2x20ml). The solvent was removed from the filtrate under reduced pressure and the crude product dissolved in dichloromethane (70ml). This solution was washed with sodium hydroxide (0.5M, 2x50ml), dried (Na_2SO_4) and the solvent removed *in vacuo* to give a colourless oil. Yield 3.0g (86%), Tlc : chloroform/methanol (9:1) R_f 0.21, δ_H ($CDCl_3$, 300MHz, *J*/Hz): 1.42 (s, 9H, CMe_3), 1.50 (t, *J* 3, 8H, $C^2H_2 + C^3H_2 + C^7H_2 + C^8H_2$), 2.59 (t, *J* 7, 4H, $C^4H_2 + C^6H_2$), 3.09 (broad dt, *J* 6,7, 4H, $C^1H_2 + C^9H_2$), 4.91 (broad s, 2H, NH), δ_C ($CDCl_3$, 75MHz): 27.55, 28.03 ($C^2H_2 + C^3H_2 + C^7H_2 + C^8H_2$), 28.58 (C*Me*$_3$), 40.60 ($C^1H_2 + C^9H_2$), 49.64 ($C^4H_2 + C^6H_2$), 79.09 (*C*Me$_3$), 156.19 (urethane), IR ($CHCl_3$),υ (cm^{-1}): 3454 (NH), 1705, 1509 (urethane), M/Z (FAB, NBA matrix): 360 (100%, $[M+H]^+$), 304 (8%, $[M+H\text{-}^tBu]^+$), 248 (12%, $[M+H\text{-}2^tBu]^+$). HRMS $[M+H]^+$ $C_{18}H_{38}O_4N_3$ Calcd. 360.2862, Found 360.2857.

N^1, N^7-*bis*(Boc)-N^4-(4-Benzyloxycarbonyl-1-methylbenzoate)norspermidine (4a)

N^1, N^7-*bis*(Boc)Norspermidine (**1a**) (0.26g, 0.80mmol) was dissolved in DMF (8ml) and triethylamine (0.2ml) and stirred at room temperature. A solution of 4-nitrophenyl-4'-hydroxymethylmethylbenzoate carbonate (**3**) (0.25g, 0.76mmol) in DMF (4ml) was added dropwise and the mixture stirred at room temperature for 30 minutes and then at 50°C for 2.5 hours. The yellow reaction mixture was poured into water (30ml), acidified (2M $KHSO_4$) and the mixture extracted with dichloromethane (3x20ml). The extracts were combined, washed with 10% $NaHCO_3$ (3x25ml), then brine, dried ($MgSO_4$) and the solvents removed *in vacuo*. The crude product was purified by column chromatography on silica gel eluting with petroleum ether/ethyl acetate (3:1) to remove *p*-nitrophenol and then petroleum ether/ethyl acetate (1:1) to elute the title compound which was obtained as a viscous oil. Yield 0.39g (96%), Tlc : petroleum ether/ethyl acetate (1:1) R_f 0.29, δ_H ($CDCl_3$, 300MHz, *J*/Hz): 1.39 (s, 18H, 2xCMe_3), 1.65-1.69 (m, 4H, $C^2H_2 + C^5H_2$), 3.06 (dt, *J* 5,6, 4H, $C^1H_2 + C^7H_2$), 3.28 (t, *J* 6, 4H, $C^3H_2 + C^5H_2$), 3.87 (s, 3H, CO_2Me), 4.66 (broad s, 1H, NH), 5.15 (s, 2H, Ar*CH*$_2$), 5.20 (broad s, 1H, NH), 7.38 (d, *J* 8, 2H, Aryl $C^{3,5}H$), 8.00 (d, *J* 8, 2H, Aryl $C^{2,6}H$), δ_C ($CDCl_3$, 75MHz): 28.27, 29.24 ($C^2H_2 + C^6H_2$), 28.55 (C*Me*$_3$), 37.33, 37.97 ($C^1H_2 + C^7H_2$), 44.49 ($C^3H_2 + C^5H_2$), 52.21 (CO_2*Me*), 66.60 (Ar*CH*$_2$), 79.01, 79.34 (*C*Me$_3$), 127.48 (Aryl $C^{3,5}H$), 129.89 (Aryl C^1), 130.02 (Aryl $C^{2,6}H$), 141.74 (Aryl C^4), 156.08, 156.11, 156.34 (urethanes), 166.81 (*CO*$_2$Me), IR ($CHCl_3$),υ (cm^{-1}): 1707 (urethane + ester), 1507 (urethane), M/Z (FAB, NBA matrix): 524 $(M+H)^+$, 424 $(M+H\text{-Boc})^+$, 324 $(M+H\text{-2Boc})^+$, HRMS $[M+H]^+$ $C_{26}H_{42}O_8N_3$ Calcd. 524.2990, Found 524.2972.

N^1, N^8-*bis*(Boc)-N^4-(4-Benzyloxycarbonyl-1-methylbenzoate)spermidine (4b)

The procedure used to form N^1, N^7-*bis*(Boc)-N^4-(4-benzyloxycarbonyl-1-methylbenzoate)norspermidine (**4a**) was followed using N^1, N^8-*bis*(Boc)spermidine (**1b**) (2.20g, 6.38mmol) and 4-nitrophenyl-4'-

hydroxymethylmethylbenzoate carbonate **(3)** (2.05g, 6.20mmol). Yield 3.22g (97%), Tlc : petroleum ether/ethyl acetate (1:1) R_f 0.37, δ_H (d_6-DMSO, 300MHz, *J*/Hz): 1.36 (s + m, 20H, 2xCMe3 + C^7H_2), 1.40-1.45 (m, 2H, C^6H_2), 1.62-1.56 (m, 2H, C^2H_2), 2.92 (dt, *J* 5,6, 4H, C^1H_2 + C^8H_2), 3.19 (m, 4H, C^3H_2 + C^5H_2), 3.84 (s, 3H, CO_2Me), 5.14 (s, 2H, Ar*CH*$_2$), 6.80 (t, *J* 6, 2H, NH), 7.47 (d, *J* 8, 2H, Aryl $C^{3,5}H$), 7.96 (d, *J* 8, 2H, Aryl $C^{2,6}H$), δ_C (d_6-DMSO, 75MHz): 25.03, 25.69, 26.95 (C^6H_2 + C^7H_2), 28.28 (C*Me*3), 29.00 (C^2H_2), 37.61, 39,59 (C^1H_2 + C^8H_2), 44.37, 44.81, 46.33, 46.80 (C^3H_2 + C^5H_2), 52.15 (CO_2*Me*), 65.39 (Ar*CH*$_2$), 77.41, 77.54 (*C*Me3), 127.17 (Aryl $C^{3,5}H$), 128.89 (Aryl C^1), 129.38 (Aryl $C^{2,6}H$), 142.74 (Aryl C^4), 155.12, 155.67 (urethanes), 166.05 (*CO*$_2$Me), IR ($CHCl_3$),υ (cm^{-1}): 1698 (urethane + ester), 1509 (urethane), M/Z (FAB, NBA matrix): 538 (4%, $[M+H]^+$), 438 (19%, $[M+H\text{-Boc}]^+$), 382 (95%, $[M+H\text{-Boc-}^tBu]^+$), 338 (33%, $[M+H\text{-2Boc}]^+$). HRMS $[M+H]^+$ $C_{27}H_{44}O_8N_3$ Calcd. 538.3128, Found 538.3182.

N^1, N^9-*bis*(Boc)-N^5-(4-Benzyloxycarbonyl-1-methylbenzoate)-homospermidine (4c)

The procedure used to form N^1, N^7-*bis*(Boc)-N^4-(4-benzyloxycarbonyl-1-methylbenzoate)norspermidine **(4a)** was followed using N^1, N^9-*bis*(Boc)-homospermidine **(1c)** (2.58g, 7.20mmol) and 4-nitrophenyl-4'-hydroxymethylmethylbenzoate carbonate **(3)** (2.30g, 6.90mmol). Yield 3.26g (86%), Tlc : petroleum ether/ethyl acetate (1:1) R_f 0.30, mp: 65-66°C, δ_H ($CDCl_3$, 300MHz, *J*/Hz): 1.40 (s + m, 22H, 2xCMe3 + C^2H_2 + C^8H_2), 1.52 (m, 4H, C^3H_2 + C^7H_2), 3.05 (m, 4H, C^1H_2 + C^9H_2), 3.22 (t, *J* 7, 4H, C^4H_2 + C^6H_2), 3.89 (s, 3H, CO_2Me), 4.61 (s, 1H, NH), 4.70 (s, 1H, NH), 5.11 (s, 2H, Ar*CH*$_2$), 7.35 (d, *J* 8, 2H, Aryl $C^{3,5}H$), 7.98 (d, *J* 8, 2H, Aryl $C^{2,6}H$), δ_C ($CDCl_3$, 75MHz): 25.47, 26.04, 27.52 (C^2H_2 + C^3H_2 + C^7H_2 + C^8H_2), 28.55 (C*Me*3), 40.26 (C^1H_2 + C^9H_2), 46.77, 47.35 (C^4H_2 + C^6H_2), 52.27 (CO_2*Me*), 66.35 (Ar*CH*$_2$), 79.23 (*C*Me3), 127.51 (Aryl $C^{3,5}H$), 129.79 (Aryl C^1), 129.97 (Aryl $C^{2,6}H$), 142.19 (Aryl C^4), 156.01, 156.15 (urethanes), 166.92 (*CO*$_2$Me), IR ($CHCl_3$),υ (cm^{-1}): 1700 (urethane + ester), 1508 (urethane), M/Z (FAB, NBA matrix): 552 (3%, $[M+H]^+$), 452 (21%, $[M+H\text{-Boc}]^+$), 396 (57%, $[M+H\text{-Boc-}^tBu]^+$), 352 (56%, $[M+H\text{-2Boc}]^+$). HRMS $[M+H]^+$ $C_{28}H_{46}O_8N_3$ Calcd. 552.3285, Found 552.3309.

N^1, N^7-*bis*(Boc)-N^4-(4-Benzyloxycarbonyl-1-benzoic acid)norspermidine (5a)

N^1, N^7-*bis*(Boc)-N^4-(4-Benzyloxycarbonyl-1-methylbenzoate)norspermidine **(4a)** (0.28g, 0.54mmol) was dissolved in dioxane (9ml) and sodium hydroxide (0.5M, 4ml) and stirred at room temperature for 2 hours and then at 50°C for 2.5 hours. The solvents were removed under reduced pressure. The oily residue was dissolved in ether (20ml) and extracted with sodium hydroxide (0.5M, 3x15ml). The extracts were combined, acidified (2M $KHSO_4$) and re-extracted with dichloromethane (3x15ml). The organic extracts were combined, dried ($MgSO_4$) and the solvent removed *in vacuo* to give the title compound as a colourless glassy solid. Yield 0.22g (80%), Tlc : petroleum ether/ethyl acetate/acetic acid (19:19:1) R_f 0.43, δ_H ($CDCl_3$, 300MHz, *J*/Hz): 1.42 (s, 18H, 2xCMe3), 1.70 (m, 4H, C^2H_2 + C^5H_2), 3.09 (dt, *J* 6,6, 4H, C^1H_2 + C^7H_2), 3.31 (m, 4H, C^3H_2 + C^5H_2), 4.67 (broad s, 1H, NH), 5.18 (s, 2H, Ar*CH*$_2$), 5.21 (broad s, 1H, NH), 7.40 (d, *J* 8, 2H, Aryl $C^{3,5}H$), 8.06 (d, *J* 8, 2H, Aryl $C^{2,6}H$), δ_C ($CDCl_3$, 75MHz): 28.06, 29.15 (C^2H_2 + C^6H_2), 28.46 (C*Me*3), 37.36, 37.95 (C^1H_2 + C^7H_2), 44.54 (C^3H_2 + C^5H_2), 66.62 (Ar*CH*$_2$), 79.15, 79.35 (*C*Me3), 127.36 (Aryl $C^{3,5}H$), 130.11 (Aryl C^1), 130.33 (Aryl $C^{2,6}H$), 141.78 (Aryl C^4), 156.17, 156.37 (urethanes), 166.81 (CO_2H), IR ($CHCl_3$),υ (cm^{-1}): 3450-3300 (CO_2H), 1699 (urethane + acid), 1509 (urethane), M/Z (FAB, NBA matrix): 510 (9%, $[M+H]^+$), 410 (23%, $[M+H\text{-Boc}]^+$), 354 (100%, $[M+H\text{-Boc-}^tBu]^+$), 310 (32%, $[M+H\text{-2Boc}]^+$). HRMS $[M+H]^+$ $C_{25}H_{40}O_8N_3$ Calcd. 510.2815, Found 510.2803.

N^1, N^8-*bis*(Boc)-N^4-(4-Benzyloxycarbonyl-1-benzoic acid)spermidine (5b)

The above procedure was repeated for 4 hours with N^1, N^8-*bis*(Boc)-N^4-(4-Benzyloxycarbonyl-1-methylbenzoate)spermidine **(4b)** (3.03g, 5.60mmol). Yield 2.28g (82%), Tlc : petroleum ether/ethyl acetate (1:1) R_f 0.23, δ_H ($CDCl_3$, 300MHz, *J*/Hz): 1.37 (s + m, 20H, 2xCMe3 + C^7H_2), 1.50 (broad,

2H, C^6H_2), 1.63 (m, 2H, C^2H_2), 3.03 (dt, *J* 6,6, 4H, C^1H_2 + C^8H_2), 3.14-3.30 (m, 4H, C^3H_2 + C^5H_2), 4.62 (broad s, 1H, NH), 4.69 (broad s, 1H, NH), 5.11 (s, 2H, Ar*CH*$_2$), 7.32 (d, *J* 8, 2H, Aryl $C^{3,5}H$), 8.00 (d, *J* 8, 2H, Aryl $C^{2,6}H$), δ_C ($CDCl_3$, 75MHz): 25.37, 25.93, 27.42, 28.35 (C^6H_2 + C^7H_2), 28.55 (C*Me*$_3$), 29.34 (C^2H_2), 37.43, 38.06, 40.21 (C^1H_2 + C^8H_2), 44.62, 46.74, 47.41 (C^3H_2 + C^5H_2), 66.58 (Ar*CH*$_2$), 79.28 (*C*Me$_3$), 127.43 (Aryl $C^{3,5}H$), 129.69 (Aryl C^1), 130.50 (Aryl $C^{2,6}H$), 142.40 (Aryl C^4), 156.27 (urethanes), 170.35 (CO_2H), IR ($CHCl_3$),υ (cm^{-1}): 3450-3300 (CO_2H), 1697 (urethane + acid), 1510 (urethane), M/Z (FAB, NBA matrix): 524 (4%, $[M+H]^+$), 424 (18%, $[M+H\text{-Boc}]^+$), 368 (100%, $[M+H\text{-Boc-}^t\text{Bu}]^+$), 324 (33%, $[M+H\text{-2Boc}]^+$). HRMS $[M+H]^+$ $C_{26}H_{42}O_8N_3$ Calcd. 524.2972, Found 524.3012.

N^1, N^9-*bis*(Boc)-N^5-(4-Benzyloxycarbonyl-1-benzoic acid)homospermidine (5c)

The above procedure was repeated for 4 hours with N^1, N^9-*bis*(Boc)-N^5-(4-Benzyloxycarbonyl-1-methylbenzoate) homospermidine (**4c**) (2.95g, 5.40mmol). Yield 2.56g (88%), Tlc : petroleum ether/ethyl acetate (1:1) R_f 0.25, δ_H ($CDCl_3$, 300MHz, *J*/Hz): 1.43 (s + m, 22H, 2xCMe$_3$ + C^2H_2 + C^8H_2), 1.55 (m, 4H, C^3H_2 + C^7H_2), 3.08 (m, 4H, C^1H_2 + C^9H_2), 3.23 (t, *J* 6, 4H, C^4H_2 + C^6H_2), 4.69 (broad s, 1H, NH), 4.76 (broad s, 1H, NH), 5.16 (s, 2H, Ar*CH*$_2$), 7.38 (d, *J* 8, 2H, Aryl $C^{3,5}H$), 8.04 (d, *J* 8, 2H, Aryl $C^{2,6}H$), δ_C ($CDCl_3$, 75MHz): 25.45, 26.03, 27.44 (C^2H_2 + C^3H_2 + C^7H_2 + C^8H_2), 28.56 (C*Me*$_3$), 40.28 (C^1H_2 + C^9H_2), 46.79, 47.37 (C^4H_2 + C^6H_2), 66.45 (Ar*CH*$_2$), 79.41 (*C*Me$_3$), 127.50 (Aryl $C^{3,5}H$), 129.64 (Aryl C^1), 130.46 (Aryl $C^{2,6}H$), 142.58 (Aryl C^4), 156.12, 156.25 (urethanes), 170.36 (CO_2H), IR ($CHCl_3$),υ (cm^{-1}): 3450-3300 (COOH), 1697 (urethane + acid), 1510 (urethane), M/Z (FAB, NBA matrix): 538 (4%, $[M+H]^+$), 438 (15%, $[M+H\text{-Boc}]^+$), 382 (45%, $[M+H\text{-Boc-}^t\text{Bu}]^+$), 338 (15%, $[M+H\text{-2Boc}]^+$). HRMS $[M+H]^+$ $C_{27}H_{44}O_8N_3$ Calcd. 538.3128, Found 538.3171.

N^1-Phthalimido-N^4-benzyl-N^8-Boc-spermidine (9)

A warm solution of 3-brompropylphthalimide at 40°C (40.28g, 0.15mol) in *n*-butanol (100ml) was added dropwise to a suspension of N^1-Boc-N^4-benzylputrescine (**7**) (17.00g, 61.1mmol), sodium carbonate (6.81g, 64.2mmol) and potassium iodide (2.5g, 15.0mmol) in *n*-butanol (150ml) at 50°C. Stirring and warming were maintained for 51 hours. The mixture was allowed to cool, resulting in formation of a white precipitate which was removed by filtration and washed with *n*-butanol (3x30ml). The filtrate and washes were combined and the solvent removed *in vacuo*. The crude product was purified by column chromatography on silica gel, eluting with ethyl acetate/petroleum ether (3:1) then ethyl acetate/petroleum ether (1:1) to elute the product. The crude product was dry-loaded by prior mixing of a solution in ethyl acetate with silica (≈40g) and evaporation of the solvent to leave the crude mixture absorbed on the silica) to give a gummy solid. Yield 17.72g (62%), Tlc : ethyl acetate/petroleum ether (3:2) R_f 0.44, δ_H ($CDCl_3$, 300MHz, *J*/Hz): 1.43 (s, 9H, CMe$_3$), 1.48 (m, 4H, C^6H_2 + C^7H_2), 1.82 (quin, *J* 7, 2H, C^2H_2), 2.37-2.50 (2xt, *J* 7, 4H, C^3H_2 + C^5H_2), 3.07 (m, 2H, C^8H_2), 3.52 (s, 2H, $PhCH_2$), 3.68 (t, *J* 7, 2H, C^1H_2), 4.82 (broad s, 1H, NH), 7.17-7.30 (m, 5H, Ph), 7.68-7.72 (m, 2H, Pht C^3H), 7.79-7.83 (m, 2H, Pht C^2H), δ_C ($CDCl_3$, 75MHz): 24.55, 27.90 (C^6H_2 + C^7H_2), 26.16 (C^2H_2), 28.55 (C*Me*$_3$), 36.51 (C^1H_2), 40.58 (C^8H_2), 51.21 (C^3H_2), 53.42 (C^5H_2), 58.67 ($PhCH_2$), 123.24 (Pht C^2H), 126.92, 128.24, 128.96 (Ph), 132.27 (Ph), 133.95 (Pht C^3H), 139.62 (Pht C^1), 156.11 (urethane), 168.47 (Pht amide).

N^1-Phthalimido-N^8-Boc-spermidine acetate (10)

A solution of N^1-phthalimido-N^4-benzyl-N^8-Boc-spermidine (**9**) (17.72g, 3.81mmol) in ethanol (350ml) and acetic acid (4.4ml, 76.2mmol) was degassed and the reaction vessel filled with nitrogen. This procedure was repeated twice before adding palladium on activated charcoal (1.5g). The reaction mixture and vessel were degassed and filled with hydrogen three times before stirring at room temperature under hydrogen overnight. The reaction mixture was filtered through celite before repeating the degassing procedure above. Fresh palladium on activated charcoal (1.5g) was added and degasssing and filling with

hydrogen repeated as before. Stirring under hydrogen was continued for a further 48 hours. The reaction was again filtered through celite and the solvent removed *in vacuo*. The product was contaminated with residual starting material, but nmr analysis showed that the extent of reaction was ≈83%. This crude product used directly in the next reaction step., Tlc : chloroform/methanol (9:1) R_f 0.22, δ_H ($CDCl_3$, 300MHz, *J*/Hz): 1.40-1.60 (s + m, 13H, CMe_3 + C^6H_2 + C^7H_2), 1.72 (m, 2H, C^2H_2), 1.94 (s, 3H, CH_3CO_2H), 2.87 (m, 4H, C^3H_2 + C^5H_2), 3.10 (m, 2H, C^8H_2), 3.76 (m, 2H, C^1H_2), 5.18 (broad s, 1H, NH), 7.69-7.72 (m, 2H, Pht), 7.79-7.82 (m, 2H, Pht), 9.96 (s, 2H, NH_2^+), δ_C ($CDCl_3$, 75MHz): 22.69 (CH_3CO_2H), 23.72, 27.02 (C^6H_2 + C^7H_2), 25.94 (C^2H_2), 28.52 (CMe_3), 35.11 (C^1H_2), 39.72 (C^8H_2), 45.36, 47.55 (C^3H_2 + C^5H_2), 79.15 (CMe_3), 123.51, 132.02, 134.26 (Pht), 156.32 (urethane), 168.55 (Pht amide), IR ($CHCl_3$),υ (cm^{-1}): 3453 (NH), 1771 (Pht), 1710 (urethane + Pht), 1512 (urethane + Ar), 1399, 1367 (CMe_3), M/Z (ES): 376.2 (100%, $[M+H]^+$), 466.2 (38%, $[M+CH_2Ph+H]^+$).

N^1-Phthaloyl-N^4-(4-benzyloxycarbonyl-1-methylbenzoate)-N^8-Boc-spermidine (11)

N^1-Phthalimido-N^8-Boc-spermidine acetate (**10**) (7.96g, 18.30mmol) was dissolved in DMF (50ml) and triethylamine (7.6ml, 55.0mmol) and stirred at room temperature. A solution of 4-nitrophenyl-4'-hydroxymethylmethylbenzoate carbonate (**3**) (4.00g, 12.10mmol) in DMF (15ml) was added dropwise while the mixture was warmed to 55°C. Stirring and warming were maintained for 2.5 hours. The yellow reaction mixture was poured into water (200ml), acidified (2M $KHSO_4$) and the mixture extracted with ether (5x50ml). The extracts were combined, dried ($MgSO_4$) and the solvents removed *in vacuo*. The crude product was purified by column chromatography on silica gel eluting with petroleum ether/ethyl acetate (3:1) to remove *p*-nitrophenol and then petroleum ether/ethyl acetate (1:1) to elute the title compound which was obtained as a straw coloured oil. Yield 6.66g (97%), Tlc : ethyl acetate/petroleum ether (1:1) R_f 0.33, δ_H ($CDCl_3$, 300MHz, *J*/Hz): 1.41 (s + m, 11H, CMe_3 + C^7H_2), 1.56 (quin, *J* 7, 2H, C^6H_2), 1.93 (quin, *J* 7, 2H, C^2H_2), 3.10 (broad s, 2H, C^8H_2), 3.24-3.34 (m, 4H, C^3H_2 + C^5H_2), 3.67 (m, 2H, C^1H_2), 3.89 (s, 3H, CO_2Me), 4.66 (2xbroad s, 1H, NH), 5.12 (d, *J* 8, 2H, ArCH_2), 7.34 (2xd, *J* 7, 2H, Aryl $C^{3,5}H$), 7.67-7.72 (m, 2H, Pht C^3H), 7.76-7.83 (m, 2H, Pht C^2H), 7.97 (2xd, *J* 7, 2H, Aryl $C^{2,6}H$), δ_C ($CDCl_3$, 75MHz): 25.53, 26.06 (C^6H_2), 27.46 (C^7H_2), 28.04 (C^2H_2), 28.52 (CMe_3), 35.74 (C^1H_2), 40.24 (C^8H_2), 44.97, 45.46 (C^3H_2), 46.86, 47.50 (C^5H_2), 52.22 (CO_2Me), 66.43 (ArCH_2), 79.21 (CMe_3), 123.37 (Pht C^2H), 127.39 (Aryl C^3H), 129.69, 132.05 (Aryl C^1 + Pht C^1), 129.93 (Aryl C^2H), 133.98 (Pht C^3H), 141.90 (Aryl C^4), 156.08, 155.90 (urethanes), 166.85 (CO_2Me), 168.35 (Pht amide), IR ($CHCl_3$),υ (cm^{-1}): 3454 (urethane NH), 1771 (Pht), 1712 (urethanes + ester + Pht), M/Z (FAB, NBA matrix): 568 (8%, $[M+H]^+$), 468 (100%, $[M+H\text{-Boc}]^+$). HRMS $[M+H]^+$ $C_{30}H_{38}O_8N_3$ Calcd. 568.2659, Found 568.2635.

N^1-Phthalamic acid-N^4-(4-benzyloxycarbonyl-1-benzoic acid)-N^8-Boc-spermidine (12)

A solution of N^1-phthaloyl-N^4-(4-benzyloxycarbonyl-1-methylbenzoate)-N^8-Boc-spermidine (**11**) (6.41g, 11.30mmol) in dioxane (60ml) and sodium hydroxide (0.5M, 60ml) was stirred at room temperature for 1.5 hours. The mixture was concentrated under reduced pressure, acidified (2M $KHSO_4$) and extracted with dichloromethane (4x50ml). The extracts were combined, dried ($MgSO_4$) and the solvent removed *in vacuo*. Yield 5.86g (91%), Tlc : ethyl acetate/petroleum ether/acetic acid (19:19:2) R_f 0.10, mp: 78-80°C, δ_H (d_6-DMSO, 300MHz, *J*/Hz): 1.38 (s + m, 11H, CMe_3 + C^7H_2), 1.48 (m, 2H, C^6H_2), 1.73-1.77 (m, 2H, C^2H_2), 2.90-2.93 (m, 2H, C^8H_2), 3.17-3.29 (m, 6H, C^1H_2 + C^3H_2 + C^5H_2), 5.15 (s, 2H, ArCH_2), 6.80 (t, *J* 6, 1H, C^8H_2NH), 7.33-7.57 (m + d, *J* 8, 5H, Pht + Aryl $C^{3,5}H$), 7.77 (dd, *J* 1,7, 1H, Pht), 7.94 (t, *J* 8, 2H, Aryl $C^{2,6}H$), 8.27 (broad s, 1H, C^1H_2NH), 12.94 (broad s, 2H, CO_2H), δ_C (d_6-DMSO, 75MHz): 24.97, 25.61 (C^6H_2), 26.85 (C^7H_2), 27.52, 28.34 (C^2H_2), 28.18 (CMe_3), 36.70 (C^1H_2), 39.50 (C^8H_2), 44.41, 44.89 (C^5H_2), 46.29, 46.75 (C^3H_2), 65.40 (ArCH_2), 77.33 (CMe_3), 126.97 (Aryl $C^{3,5}H$), 127.51, 128.97, 129.17, 131.16 (Pht CH), 129.42 (Aryl $C^{2,6}H$),

130.46 (Aryl C^1), 131.16, 138.73 (Pht Cq), 142.13 (Aryl C^4), 155.09, 155.57 (urethanes), 167.04, 167.82, 168.50 (amide + CO_2H), IR ($CHCl_3$),υ (cm^{-1}): 3400-3200 (CO_2H), 1697 (urethanes + amide + acid), 1520 (urethane + amide), M/Z (ES): 994.8 (30%, $[2M+Na]^+$), 594.0 (5%, $[M+Na]^+$), 572.0 (2%, $[M+H]^+$), 424.2 (100%, $[M\text{-Pht}+H]^+$). HRMS $[M+H]^+$ $C_{29}H_{38}O_9N_3$ Calcd. 572.2608, Found 572.2573.

N^1-Fmoc-N^4-(4-Benzyloxycarbonyl-1-benzoic acid)-N^8-Boc-spermidine (13)

A solution of N^1-phthalamic acid-N^4-(4-benzyloxycarbonyl-1-benzoic acid)-N^8-Boc-spermidine **(12)** (5.30g, 9.30mmol) in ethanol (90ml) and hydrazine monohydrate (10ml) was stirred at 80°C for 21 hours resulting in precipitation of phthalhydrazide. The mixture was allowed to cool and filtered. The precipitate was washed with ethanol (4x20ml) and the washes combined with the filtrate. The solvent was removed under reduced pressure to give a sticky product oil The product was triturated by washing with ethanol (4x20ml). The washes were combined and the solvent removed *in vacuo*. This product was dissolved in dioxane (50ml) followed by addition of sodium hydrogencarbonate solution (10% aq., 15ml) and cooling in an ice bath. Fmoc-succinimide (5.1g, 15mmol) was added and stirring continued overnight whilst warming to room temperature. The mixture was poured into water (200ml), acidified to pH 3 (2M $KHSO_4$) and extracted with dichloromethane (80ml then 3x40ml). The extracts were combined, dried ($MgSO_4$) and the solvent removed *in vacuo*. The crude product was purified by column chromatography on silica gel, eluting with ethyl acetate/petroleum ether/acetic acid (19:57:4) then ethyl acetate/petroleum ether/acetic acid (19:19:2) and obtained as a sticky oil. Yield 2.23g (75%), Tlc : ethyl acetate/petroleum ether/acetic acid (19:19:2) R_f 0.44, δ_H (d_6-DMSO, 300MHz, *J*/Hz): 1.30-1.40 (s + m, 13H, CMe_3 + C^6H_2 + C^7H_2), 1.66 (m, 2H, C^2H_2), 2.92 (dt, *J* 5,7, 2H, C^8H_2), 3.02 (dt, *J* 6,7, 2H, C^1H_2), 3.21 (broad m, 4H, C^3H_2 + C^5H_2), 4.21 (m, 1H, Fmoc CH), 4.31 (d, *J* 7, 2H, Fmoc CH_2), 5.14 (s, 2H, Ar*CH*$_2$OCONR$_2$), 6.81 (t, *J* 5, 1H, C^8H_2*NH*), 7.10-7.40 (m, 5H, Fmoc CH + C^1H_2*NH*), 7.45 (d, *J* 8, 2H, Aryl $C^{3,5}H$), 7.65 (m, 2H, Fmoc CH), 7.87 (d, *J* 7, 2H, Fmoc CH), 7.96 (d, *J* 8, 2H, Aryl $C^{2,6}H$), δ_C (d_6-DMSO, 75MHz): 24.94, 25.60 (C^6H_2), 26.85 (C^7H_2), 28.18 (*CMe*$_3$), 28.79 (C^2H_2), 37.96 (C^1H_2), 39.48 (C^8H_2), 44.13, 44.71, 46.24 (C^3H_2 + C^5H_2), 46.72 (Fmoc CH), 65.17 (Fmoc CH_2), 65.40 (Ar*CH*$_2$OCONR$_2$), 77.30 (*C*Me3), 120.03, 125.10, 127.50, 128.13, 128.84 (Fmoc CH), 126.96 (Aryl $C^{3,5}H$), 129.42 (Aryl $C^{2,6}H$), 130.19 (Aryl C^1), 140.70, 143.86 (Fmoc Cq), 142.03 (Aryl C^4), 155.05, 155.57, 156.05 (urethanes), 167.09 (CO_2H), IR ($CHCl_3$),υ (cm^{-1}): 3500-3300 (CO_2H), 3447 (NH), 1699, 1514 (urethanes), M/Z (ES): 1312.7 (25%, $[2M+Na]^+$), 683.8 (15%, $[M+K]^+$), 667.9 (100%, $[M+Na]^+$), 645.9 (21%, $[M+H]^+$), 468.2 (41%, [N^1-Fmoc-N^8-Boc-spermidine + H]$^+$). HRMS $[M+H]^+$ $C_{36}H_{44}O_8N_3$ Calcd. 646.3128, Found 646.3182.

4-Nitrophenyl-4'-hydroxymethylphenyl-1'-oxyethylacetate carbonate (15)[13]

4-Hydroxymethylphenyloxyethyl acetate **(14)** (2.20g, 11.0mmol) was dissolved in dichloromethane (25ml) and pyridine (1.24ml, 15.0mmol) and cooled in an ice-salt bath. A solution of *p*-nitrophenylchloroformate (2.06g, 10.0mmol) in dichloromethane (25ml) was added dropwise over 40 minutes with stirring and cooling being maintained for a further 2 hours. The reaction mixture was poured into water (50ml) and the organic phase separated. The aqueous phase was extracted with dichloromethane (2x30ml), the organic extracts combined, dried ($MgSO_4$), and the solvent removed under reduced pressure. The crude product was crystallised from ether/dichloromethane as pale yellow crystals. Yield 3.12g (84%), Tlc : ethyl acetate/petroleum ether (1:1) R_f 0.59, mp: 48-50°C, δ_H ($CDCl_3$, 300MHz, *J*/Hz): 1.30 (t, *J* 7, 3H, CH_2CH_3), 4.27 (q, *J* 7, 2H, CH_2CH_3), 4.64 (s, 2H, ArO*CH*$_2$CO$_2$Et), 5.23 (s, 2H, Ar*CH*$_2$OCOOAr'), 6.93 (d, *J* 8, 2H, Aryl $C^{2,6}H$), 7.35-7.40 (2xd, *J* 9,8, 4H, Aryl $C^{3,5}H$ + Aryl $C^{3',5'}H$), 8.26 (d, *J* 9, 2H, Aryl $C^{2'}H$), δ_C ($CDCl_3$, 75MHz): 14.32 (CH_2CH_3), 61.65 (CH_2CH_3), 65.48 (ArO*CH*$_2$CO$_2$Et), 70.85 (Ar*CH*$_2$OCOOAr'), 115.02 (Aryl C^2H), 121.96 (Aryl $C^{2',6'}H$), 125.44 (Aryl $C^{3',5'}H$), 127.57 (Aryl C^4), 130.87 (Aryl $C^{3,5}H$), 145.52 (carbonate), 152.60 (Aryl $C^{4'}$), 155.70

(Aryl $C^{1'}$), 158.61 (Aryl C^1), 168.83 (CO_2Et), IR ($CHCl_3$),υ (cm^{-1}): 1760 (carbonate + ester), 1613 (aryl ring), 1523 (aryl ring + NO_2), 1348 (NO_2), M/Z (ES): 772.7 (12%, $[2M+Na]^+$), 414.0 (5%, $[M+K]^+$), 398.1 (5%, $[M+Na]^+$), 272.2 (35%, $[M-OCH_2CO_2Et]^+$). HRMS $[M+H]^+$ $C_{18}H_{18}O_8N$ Calcd. 376.1032, Found 376.1022.

N^1, N^8-*bis*(Trifluoroacetyl)spermidine trifluoroacetate (16)[14]

Water (0.3ml, 17.0mmol) and ethyl trifluoroacetate (6.85g, 5.73ml, 48.0mmol) were added to a solution of spermidine (2.00g, 14.0mmol) in acetonitrile (45ml) and the mixture heated under reflux for 21 hours. The solvents were removed under reduced pressure to give a pale brown solid which was washed with dichloromethane (3x10ml) and recrystallised from ethyl acetate. Yield 4.49g (71%), Tlc: chloroform/methanol (8:2) R_f 0.43, mp: 146-148°C, lit. 146-147°C [50], δ_H (d_6-DMSO, 300MHz, *J*/Hz): 1.55 (m, 4H, C^6H_2 + C^7H_2), 1.82 (quin, *J* 7, 2H, C^2H_2), 2.91 (m, 4H, C^3H_2 + C^5H_2), 3.17-3.29 (2xdt, *J* 5,7, 4H, C^1H_2 + C^8H_2), 8.68 (s, 2H, NH_2^+), 9.53 (broad t, *J* 5, 1H, $C^8H_2NHCOCF_3$), 9.61 (broad t, *J* 5, 1H, $C^1H_2NHCOCF_3$), δ_C (d_6-DMSO, 75MHz, *J*/Hz): 23.16, 25.61 (C^6H_2 + C^7H_2), 25.33 (C^2H_2), 36.81 (C^1H_2), 38.82 (C^8H_2), 44.74, 46.63 (C^3H_2 + C^5H_2), 110.41-121.86, 110.48-121.94 (q, *J* 288, CF_3CONH), 111.38-123.27 (q, *J* 298, $CF_3CO_2^-$), 155.86-157.28, 156.01-157.49 (q, *J* 36, CF_3CONH), 158.35-159.61 (q, *J* 32, $CF_3CO_2^-$), IR (Nujol),υ (cm^{-1}): 3317, 3197, 3087 (NH), 1711 (CO_2H), 1671, 1560 (amide), M/Z (ES): 338.4 (100%, $[M+H]^+$).

N^1,N^8-*bis*(Trifluoroacetyl)-N^4-(4-Benzyloxycarbonyl-1-oxyethylacetate)-spermidine (17)

N^1, N^8-*bis*(Trifluoroacetyl)spermidine trifluoroacetate **(16)** (2.50g, 5.54mmol) was dissolved in DMF (10ml) and triethylamine (2.5ml) added. A solution of 4-nitrophenyl-4'-hydroxymethylphenyl-1'-oxyethylacetate carbonate **(15)** (1.96g, 5.25mmol) in DMF (8ml) was added dropwise over 45 minutes whilst stirring and warming the reaction mixture to 50°C. Stirring was maintained at 50°C for a further $2\frac{1}{4}$ hours, whereupon the mixture was poured into water (100ml), acidified to pH6 with 2M $KHSO_4$ and extracted with ether (3x30ml). The extracts were combined, dried ($MgSO_4$) and the solvent removed under reduced pressure. The product was purified by column chromatography on silica gel, eluting with ethyl acetate/petroleum ether (2:5) to elute *p*-nitrophenol then ethyl acetate/petroleum ether (2:1) to elute the product and obtained as a white foam. Yield 2.26g (75%), Tlc: ethyl acetate/petroleum ether (1:1) R_f 0.28, mp: 62-64°C, δ_H (d_6-DMSO, 300MHz, *J*/Hz): 1.21 (t, *J* 7, 3H, CH_2CH_3), 1.44 (m, 4H, C^6H_2 + C^7H_2), 1.70 (quin, *J* 7, 2H, C^2H_2), 3.18 (m, 8H, C^1H_2 + C^3H_2 + C^5H_2 + C^8H_2), 4.16 (q, *J* 7, 2H, CH_2CH_3), 4.76 (s, 2H, $ArOCH_2CO_2Et$), 4.98 ($ArCH_2OCONR_2$), 6.89 (d, *J* 9, 2H, Aryl C^2H), 7.27 (d, *J* 9, 2H, Aryl C^3H), 9.39 (broad s, 2H, NH), δ_C (d_6-DMSO, 75MHz, *J*/Hz): 14.19 (CH_2CH_3), 25.10, 25.67 (C^6H_2), 25.78 (C^7H_2), 27.88, 27.28 (C^2H_2), 37.21, 39.15 (C^1H_2 + C^8H_2), 44.22, 44.68 (C^3H_2 + C^5H_2), 60.85 (CH_2CH_3), 64.86 ($ArOCH_2CO_2Et$), 66.07 ($ArCH_2OCONR_2$), 110.45-121.91, 110.50-121.95 (q, *J* 288, CF_3CONH), 114.58 (Aryl C^2H), 129.50 (Aryl C^3H), 129.92 (Aryl C^4), 155.56, 155.59 (urethanes), 155.74-157.18 (q, *J* 36, CF_3CONH), 157.55 (Aryl C^1), 168.91 (CH_2CO_2Et), IR ($CHCl_3$),υ (cm^{-1}): 3315 (NH), 1716 (urethane + ester + amides), 1175 (C-F), M/Z (ES): 596.3 (100%, $[M+Na]^+$), 612.3 (10%, $[M+K]^+$), 1169.4 (12%, $[2M+Na]^+$). HRMS $[M+H]^+$ $C_{23}H_{30}O_7N_3F_6$ Calcd. 574.1988, Found 574.1987.

N^1, N^8-*bis*(Fmoc)-N^4-(4-Benzyloxycarbonyl-1-oxyacetic acid)spermidine (18)

N^1, N^8-*bis*(Trifluoroacetyl)-N^4-(4-benzyloxycarbonyl-1-oxyethyl acetate)spermidine **(17)** (2.05g, 3.60mmol) was dissolved in dioxane (25ml). Sodium hydroxide (1M, 20ml) was added and the mixture stirred for 1.5hours at room temperature resulting in a yellow solution. The pH of the solution was adjusted to 4 by addition of 2M $KHSO_4$ prior to additon of sodium hydrogencarbonate (10% aq., 15ml) to bring the pH to 8. Fmoc-succinimide (2.67g, 7.90mmol) was added and the mixture stirred at room temperature for 1.5hours. The mixture was poured into water (150ml), acidified to pH 3 with 2M $KHSO_4$ and extracted with dichloromethane (50ml, then 2x30ml). The extracts were combined, dried ($MgSO_4$) and the solvent

removed *in vacuo*. The crude product was purified by column chromatography on silica gel, eluting with ethyl acetate/petroleum ether (1:1) to elute excess Fmoc-succinimide then ethyl acetate/petroleum ether/methanol (9:9:2) to elute the product as a white foam. Yield 2.45g (85%), Tlc: ethyl acetate/petroleum ether/acetic acid (19:19:2) R_f 0.21, mp: 81-83°C, δ_H (d_6-DMSO, 300MHz, *J*/Hz): 1.35 (broad m, 2H, C^7H_2), 1.43 (broad m, 2H, C^6H_2), 1.62 (t, *J* 7, 2H, C^2H_2), 2.98 (m, 4H, C^1H_2 + C^8H_2), 3.17 (m, 4H, C^3H_2 + C^5H_2), 4.20 (t, *J* 7, 2H, Fmoc CH), 4.31 (d, *J* 7, 4H, Fmoc CH_2), 4.65 (s, 2H, ArOCH_2CO_2H), 4.97 (ArCH_2OCONR_2), 6.89 (d, *J* 9, 2H, Aryl C^2H), 7.25-7.42 (m, 12H, Fmoc CH + Aryl C^3H + NH), 7.68 (d, *J* 7, 4H, Fmoc CH), 7.87 (d, *J* 7, 4H, Fmoc CH), δ_C (d_6-DMSO, 75MHz): 25.17, 25.77 (C^6H_2), 26.92 (C^7H_2), 28.33, 28.87 (C^2H_2), 38.23, 39.96 (C^1H_2 + C^8H_2), 44.31, 44.74, 46.29 (C^3H_2 + C^5H_2), 46.99 (Fmoc CH), 64.64 (ArOCH_2CO_2H), 65.39 (Fmoc CH_2), 65.97 (ArCH_2OCONR_2), 114.49 (Aryl C^2H), 129.44 (Aryl C^3H), 120.31, 125.32, 127.24, 127.79 (Fmoc CH), 129.74 (Aryl C^4), 140.96, 144.13 (Fmoc Cq), 155.52, 156.13 (urethanes), 157.60 (Aryl C^1), 170.36 (CO_2H), IR ($CHCl_3$),υ (cm^{-1}): 3447 (NH), 1713, 1515 (urethanes), M/Z (ES): 836.5 (27%, $[M+K]^+$), 820.3 (11%, $[M+Na]^+$), 590.4 (53%, [N^1, N^8-*bis*(Fmoc)spermidine + $H]^+$). HRMS $[M+H]^+$ $C_{47}H_{48}O_9N_3$ Calcd. 798.3391, Found 798.5017.

Anchoring of linkage agents to solid support

All polyamine containing linkage agents were anchored to aminomethyl polyatyrene resin (0.7mmol/g) using standard peptide coupling procedures. Aminomethyl resin (1 equiv) was added to a solution containing the linkage agent (1-1.1 equiv), HOBt (1.2 equiv), DIC (1.2 equiv) and a few crystals of DMAP in DMF. The mixture was shaken for several hours whereupon almost complete coupling was observed (ninhydrin test[15]). Any residual amines were capped by treatment with acetic anhydride. The resin was finally washed twice with DMF, four times with methanol and twice with CH_2Cl_2 before drying *in vacuo*.

Solid Phase Synthesis of Trypanothione (20), Nortrypanothione (19) and Homotrypanothione (21)[13,17,18,19]

A sample of functionalised resin (ca. 0.75g) was treated with a solution of TFA in CH_2Cl_2 (25ml), filtered and washed with CH_2Cl_2 (3x30ml) followed by neutralisation of the free amines by treatment with a solution of DIPEA (10% v/v in CH_2Cl_2, 3x30ml) and final washing with CH_2Cl_2 (5x30ml). The deprotected resin was derivatised using a BioSearch 9500 peptide synthesiser equipped with programs for Fmoc peptide synthesis which were modified for the purpose of this procedure. Sequential coupling of the first two residues was performed using DIC/HOBt activation with FmocGlyOH and FmocCys(Trt)OH, followed by Fmoc deprotection with a 20% solution of piperidine in DMF. BocGlu(OH)OtBu was then coupled in similar fashion. All couplings were show to be complete using a ninhydrin test. The resin was suspended in a solution of TFA (15ml), thioanisole (1.25ml) and ethanedithiol (1.25ml) under an atmosphere of nitrogen with stirring. Triflic acid (0.45ml, 5mmol) was added and stirring continued for 3 hours at room temperature. After this time a quantitative ninhydrin test indicated that cleavage was incomplete with free amines still left attached to the solid support at a level of 0.19mmol/g. Another aliquot of triflic acid (0.45ml) was added and the mixture stirred for a further 4 hours whereupon a quantitative ninhydrin test confirmed complete release of the product from the solid support. (Nortrypanothone and homotrypanothione were cleaved from the solid support (0.5g resin) using a modified procedure: The resin was first treated with a combination of TFA (10ml), thioanisole (0.28ml) and ethanedithiol (0.20ml) for 30 minutes, filtered and treated again with a solution of TFA (8ml) containing thioanisole (0.28ml) and triflic acid (0.2ml) for 4 hours at 40°C).

The residual resin was filtered and the product isolated by precipitation of the filtrate into cold ether (120ml). The residual resin was washed with methanol (5x3ml) and the washes were combined, concentrated to a volume of about 3ml, and added to cold ether (40ml) to precipitate any remaining product. The precipitate was collected by centrifugation and washed with ether before dissolving in methanol. A

methanolic solution of iodine (0.2M, 1.2ml) was added dropwise to the solution of the crude product until a yellow colour persisted. The solution was concentrated under reduced pressure to a volume of approximately 1ml and the oxidised product was again isolated by precipitation into ether followed by centrifugation.

Analysis of the crude products by analytical reverse-phase HPLC (C18 column, 4.5mm x 25cm), using a linear gradient from H_2O + 0.1% TFA (solvent A) to acetonitrile + 0.1% TFA (solvent B) with a flow rate of 1ml/min, monitoring at 220nm, combined with MALDI-TOF MS showed that the desired polyamine-peptide conjugate was the predominant product. The gradient conditions used were t=0, 100% A; t=10, 100% A; t=60, 100% B. The crude products were purified by semi-prep reverse-phase HPLC (C18 column, 9mm x 25cm) using a linear gradient from H_2O + 0.1% TFA (solvent A) to acetonitrile + 0.1% TFA (solvent B) with a flow rate of 2ml/min. The gradient conditions used were t=0, 100% A; t=10, 100% A; t=60, 100% B.

Trypanothione (20). Yield 36mg. δ_H (D_2O, 360MHz, *J*/Hz)[18,19]: 1.61 (m, 2H, C^7H_2), 1.68 (m, 2H, C^6H_2), 1.90 (quin, *J* 7, 2H, C^2H_2), 2.22 (m, 4H, Gluβ), 2.58 (m, 4H, Gluγ), 2.97-3.08 (m, 6H, Cysβ + C^3H_2 + C^5H_2), 3.24 (dd, *J* 5,10, 2H, Cysβ), 3.27 (t, *J* 5, 2H, C^8H_2), 3.35 (t, *J* 5, 2H, C^1H_2), 3.82 (d, *J* 17, 1H, Glyα), 3.84 (d, *J* 17, 1H, Glyα), 3.93 (d, *J* 17, 2H, Glyα), 4.04 (t, *J* 7, 2H, Gluα), 4.73 (m, 2H, Cysα), δ_C (D_2O, 90MHz): 25.17 (C^6H_2), 28.09 (C^7H_2), 28.19 (C^2H_2), 33.47 (Gluβ), 38.27 (Gluγ), 40.38 (C^8H_2), 40.80 (C^1H_2), 45.58 (Cysβ), 45.67 (Glyα), 47.19 (C^3H_2), 49.86 (C^5H_2), 55.04 (Cysα), 55.16 (Gluα), 173.61, 174.44, 174.51, 175.40, 174.43 (amides), 177.29 (CO_2H), M/Z: MALDI-TOF, 722.3 $(M+H)^+$, 743.3 $(M+Na)^+$, 761.1 $(M+K)^+$: ES, 722.2 (45%, $[M+H]^+$), 362.1 (100%, $[M+2H]^{2+}$). HRMS $[M+H]^+$ $C_{27}H_{48}O_{10}N_9S_2$ Calcd. 722.2966, Found 722.2957.

Nortrypanothione (19).[19] Yield 28 mg. δ_H (CD_3OD, 360MHz, *J*/Hz): 1.85-1.95 (m, 4H, C^2H_2 + C^6H_2), 2.21 (m, 4H, Gluβ), 2.63 (m, 4H, Gluγ), 3.01-3.08 (m, 6H, Cysβ + C^3H_2 + C^5H_2), 3.27 (dd, *J* 5,14, 2H, Cysβ), 3.30-3.41 (m, 4H, C^1H_2 + C^7H_2), 3.81 (d, *J* 17, 1H, Glyα), 3.85 (d, *J* 17, 1H, Glyα), 3.98 (t, *J* 7, 2H, Gluα), 4.67 (dd, *J* 5,9, 2H, Cysα), δ_C (CD_3OD, 75MHz): 27.12 (C^2H_2 + C^6H_2), 27.37 (Gluβ), 32.08 (Gluγ), 36.96 (C^1H_2 + C^7H_2), 41.32 (Cysβ), 44.03 (Glyα), 46.47 (C^3H_2 + C^5H_2), 53.81 (Cysα), 54.71 (Gluα), 172.10, 172.43, 173.28 (amides), 174.87 (CO_2H), M/Z: MALDI-TOF, 708.3 $(M+H)^+$, 730.3 $(M+Na)^+$: ES, 708.2 (20%, $[M+H]^+$), 355.0 (100%, $[M+2H]^{2+}$). HRMS $[M+H]^+$ $C_{26}H_{46}O_{10}N_9S_2$ Calcd. 708.2809, Found 708.2855.

Homotrypanothione (21).[19] Yield 35 mg. δ_H (CD_3OD, 360MHz, *J*/Hz): 1.61 (m, 4H, C^2H_2 + C^8H_2), 1.70 (m, 4H, C^3H_2 + C^7H_2), 2.21 (m, 4H, Gluβ), 2.61 (m, 4H, Gluγ), 2.99 (dd, *J* 10,14, 2H, Cysβ), 3.02 (t, *J* 7, 4H, C^4H_2 + C^6H_2), 3.26-3.32 (m, 6H, Cysβ + C^1H_2 + C^9H_2), 3.78 (s, 4H, Glyα), 3.98 (t, *J* 7, 2H, Gluα), 4.62 (dd, *J* 4,10, 2H, Cysα), δ_C (CD_3OD, 75MHz): 24.20 (C^3H_2 + C^7H_2), 27.10 (C^2H_2 + C^8H_2), 27.28 (Gluβ), 32.17 (Gluγ), 39.06 (C^1H_2 + C^9H_2), 40.31 (Cysβ), 44.19 (Glyα), 48.51 (C^4H_2 + C^6H_2), 53.70 (Cysα), 54.57 (Gluα), 171.74, 172.01, 173.52 (amides), 175.06 (CO_2H), M/Z: MALDI-TOF, 736.4 $(M+H)^+$: ES, 736.2 (88%, $[M+H]^+$), 369.0 (37%, $[M+2H]^{2+}$). HRMS $[M+H]^+$ $C_{28}H_{50}O_{10}N_9S_2$ Calcd. 736.3122, Found 736.3083.

N^1-Glutathionylspermidine disulphide (22).[19]

A sample of resin functionalised with linker **(13)** (0.50g, 0.5mmol substitution of linkage agent) was derivatised as above using a BioSearch 9500 peptide synthesiser with semi-automatic Fmoc peptide synthesis protocols. Removal of the N^1-Fmoc protecting group by treatment with 20% piperidine in DMF was followed by sequential coupling and deprotection of Fmoc-Gly-OH, Fmoc-Cys(Trt)-OH, and final coupling of Boc-Glu(OH)-O^{t}Bu. The couplings were monitored using a ninhydrin test. An identical cleavage and disulphide formation procedure to that employed for nortrypanothione was used. The crude product was again analysed and purified using RP HPLC using the same conditions as above. Yield 5mg. δ_H (D_2O, 360MHz, *J*/Hz): 1.75 (m, 8H, C^6H_2 + C^7H_2), 1.88 (quin, *J* 7, 4H, C^2H_2), 2.17 (m, 4H,

Gluβ), 2.53 (m, 4H, Gluγ), 2.97 (dd, *J* 5,14, 2H, Cysβ), 3.05 (m, 12H, C^3H_2 + C^5H_2 + C^8H_2), 3.28 (dd, *J* 5,14, 2H, Cysβ), 3.31 (t, *J* 5, 4H, C^1H_2), 3.42 (t, *J* 6, 2H, Gluα), 3.91 (s, 4H, Glyα), 4.70 (m, 2H, Cysα), M/Z: MALDI-TOF, 866.7 $(M+H)^+$, 889.4 $(M+Na)^+$

N^1-Fmoc-N^8-glutathionylspermidine disulphide (23).[19]

A sample of resin functionalised with linker **(13)** (0.50g, 0.5mmol substitution of linkage agent) was derivatised using a BioSearch 9500 peptide synthesiser with semi-automatic Boc peptide synthesis protocols. Removal of the N^8-Boc protecting group by treatment with 50% TFA in CH_2Cl_2 was followed by two hour coupling of Boc-Gly-OH (0.25g, 1.43mmol) with and DIC activation. The deprotection and coupling cycles were repeated for the dipeptide Boc-Glu-α-O^{t}BuCys(Trt)OH (0.93g, 1.43mmol). Couplings were again monitored using a ninhydrin test. An identical cleavage and disulphide formation procedure to that employed for nortrypanothione was used. The crude product was again analysed and purified using RP HPLC using the same conditions as above. Yield 11mg. δ_H (D_2O, 360MHz, *J*/Hz): 1.55 (broad m, 8H, C^6H_2 + C^7H_2), 1.70 (broad m, 4H, C^2H_2), 2.19 (m, 4H, Gluβ), 2.53 (m, 4H, Gluγ), 2.80-2.95 (broad m, 10H, C^3H_2 + C^3H_2 + Cysβ), 3.10-3.30 (broad m, 10H, C^1H_2 + C^8H_2 + Cysβ), 3.73 (s, 4H, Glyα), 3.90 (t, *J* 6, 2H, Gluα), 7.37-7.45 (m, 8H, Fmoc CH), 7.60 (d, *J* 7, 4H, Fmoc CH), 7.81 (d, *J* 7, 4H, Fmoc CH), M/Z: MALDI-TOF, 1312.9 $(M+H)^+$

N^1-Acetylcysteinylglycinyl-N^8-glutathionyl-spermidine (24).[19]

A sample of resin functionalised with linker **(13)** (0.40g, 0.4mmol substitution of linkage agent) was derivatised using a BioSearch 9500 peptide synthesiser using Boc and Fmoc peptide synthesis protocols. Removal of the N^8-Boc protecting group was followed by coupling/deprotection of Boc-Gly-OH (0.25g, 1.43mmol) and then Boc-Glu-α-O^{t}BuCys(Trt)OH (0.93g, 1.43mmol) with HOBt/DIC activation. Removal of the N^1-Fmoc protecting group by treatment with 20% piperidine in DMF was followed by coupling/deprotection of Fmoc-Gly-OH (0.27g, 0.9mmol) and Ac-Cys(Trt)-OH (0.36g, 0.9mmol) using HOBt/DIC activation. Couplings were monitored by a ninhydrin test. An identical cleavage and disulphide formation procedure to that employed for nortrypanothione was used. The crude product was again analysed and purified using RP HPLC using the same conditions as above. Yield 5mg. δ_H (D_2O, 360MHz, *J*/Hz): 1.62-1.71 (m, 4H, C^6H_2 + C^7H_2), 1.90 (m, 2H, C^2H_2), 2.05, 2.09 (2xs, 3H, CH_3), 2.18 (m, 2H, Gluβ), 2.58 (m, 2H, Gluγ), 3.02 (m, 6H, Cysβ + C^3H_2 + C^5H_2), 3.25-3.40 (m, 6H, C^1H_2 + C^8H_2 + Cysβ), 3.75-3.90 (m, 5H, Glyα + Gluα), 4.61 (m, 2H, Cysα), M/Z (ES): 635.2 (51%, $[M+H]^+$), 318.4 (100%, $[M+2H]^{2+}$).

N^1-Glutathionyl-N^8-acetylcysteinylglycinylspermidine (25).[19]

A sample of resin functionalised with linker **(13)** (0.40g, 0.4mmol substitution of linkage agent) was derivatised using a BioSearch 9500 peptide synthesiser using Boc and Fmoc peptide synthesis protocols. Removal of the N^8-Boc protecting group was followed by coupling/deprotection of Boc-Gly-OH (0.25g, 1.43mmol) and then Ac-Cys(Trt)-OH (0.58g, 1.43mmol) with HOBt/DIC activation. Removal of the N^1-Fmoc protecting group by treatment with 20% piperidine in DMF was followed by coupling/deprotection of Fmoc-Gly-OH (0.27g, 0.9mmol), Fmoc-Cys(Trt)-OH and Boc-Glu(OH)-O^{t}Bu using HOBt/DIC activation. Couplings were monitored by a ninhydrin test. An identical cleavage and disulphide formation procedure to that employed for nortrypanothione was used. The crude product was again analysed and purified using RP HPLC using the same conditions as above. Yield 12mg. δ_H (D_2O, 360MHz, *J*/Hz): 1.61-1.73 (m, 4H, C^6H_2 + C^7H_2), 1.90 (t, *J* 6, 2H, C^2H_2), 2.06 (2xs, 3H, CH_3), 2.19 (m, 2H, Gluβ), 2.58 (m, 2H, Gluγ), 3.03 (m, 6H, Cysβ + C^3H_2 + C^5H_2), 3.25-3.35 (m, 6H, C^1H_2 + C^8H_2 + Cysβ), 3.75-3.90 (m, 5H, Glyα + Gluα), 4.61 (m, 2H, Cysα), M/Z (ES): 635.2 (42%, $[M+H]^+$), 318.5 (100%, $[M+2H]^{2+}$). HRMS $[M+H]^+$ $C_{24}H_{43}O_8N_8S_2$ Calcd. 635.2645, Found 635.2689.

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