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# THE SYNTHESIS OF SYMMETRICAL SPERMINE CONJUGATES USING SOLID-PHASE CHEMISTRY

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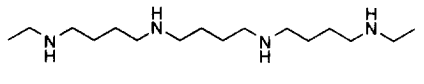
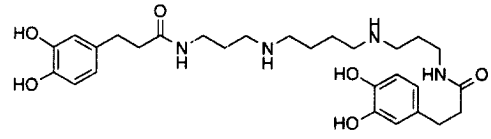
**Abstract:** The utility of spermine, selectively functionalised and immobilised on a solid support by means of the Wang "oxycarbonyl" linker is demonstrated by the solid-phase synthesis of a number of spermine conjugates including the natural product and potent antihypertensive agent kukoamine. The synthesis opens up the area of solid-phase spermine chemistry and library generation based on the symmetrical spermine scaffold. 

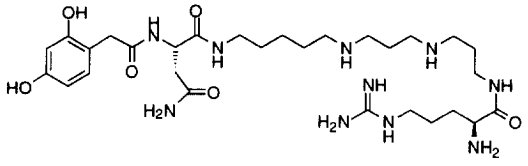
Naturally occurring and synthetic polyamine conjugates have been observed to play a myriad of important roles in a host of biological systems.[1] For example the polyamine conjugate $N^1,N^{16}$*bis*(ethyl) 1,6,11,16 tetraazahexadecane (DEHSPM) **(1)** is one member of a class of compounds found to be exceptionally active antidiarrhoeals and has potential in AIDS-related diarrhoea,[2] a major problem in the majority of AIDS patients. $N^1,N^{14}$*bis*(3,4-dihydroxyhydrocinnamoyl)-1,5,10,14-tetraazatetradecane or kukoamine A **(2)** is a powerful antihypertensive, the active ingredient of a ancient herbal extract isolated from *Lycium chinense*,[3] while compounds such as argiotoxin-636 **(3)** are under close scrutiny since they regulate the NMDA (*N*-methyl-D-aspartate) receptor,[4] while several polyamine analogs, such as the benzylated derivative **(4)**[5] interfere with polyamine synthesis and display antitumor[6] or antiparasitic[7] properties.



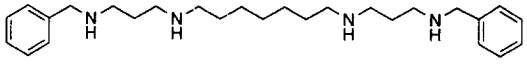
(1) $N^1$, $N^{16}$bis(ethyl)-1,6,11,16-tetraazahexadecane (DEHSPM)



(2) $N^1$, $N^{14}$bis(3,4-dihydroxyhydrocinnamoyl)-1,5,10,14-tetraaza tetradecane (kukoamine A)



(3) $N^1$ Arg , $N^{15}$Asn-1,4-dihydroxyphenylacetate-1,5,9,15-tetraaza pentadecane (argiotoxin-636)



(4) $N^1$, $N^{17}$dibenzyl-1,5,13,17-tetraazaheptadecane

**Figure 1.** Some biologically active polyamine conjugates.

There is thus a growing interest in polyamine conjugates. Solution-phase synthesis generally provides a significant challenge due to regioselective *N*-functionalization and problematic handling and purification issues although many impressive synthesis have recently been reported.[7] However, some of these problems can be solved by means of a solid-phase approach. For example, in 1995 we reported the immobilisation of a polyamine onto a 'tritylchloride resin' and selective acylation[8a] and in 1996 we reported the solid-phase synthesis of $N^1,N^8$-*bis*(glutathionyl)spermidine [trypanothione] and a library of some 10,000 analogues[8b] following the immobilisation of spermidine onto a solid support using an acid labile urethane linker in a manner that allowed

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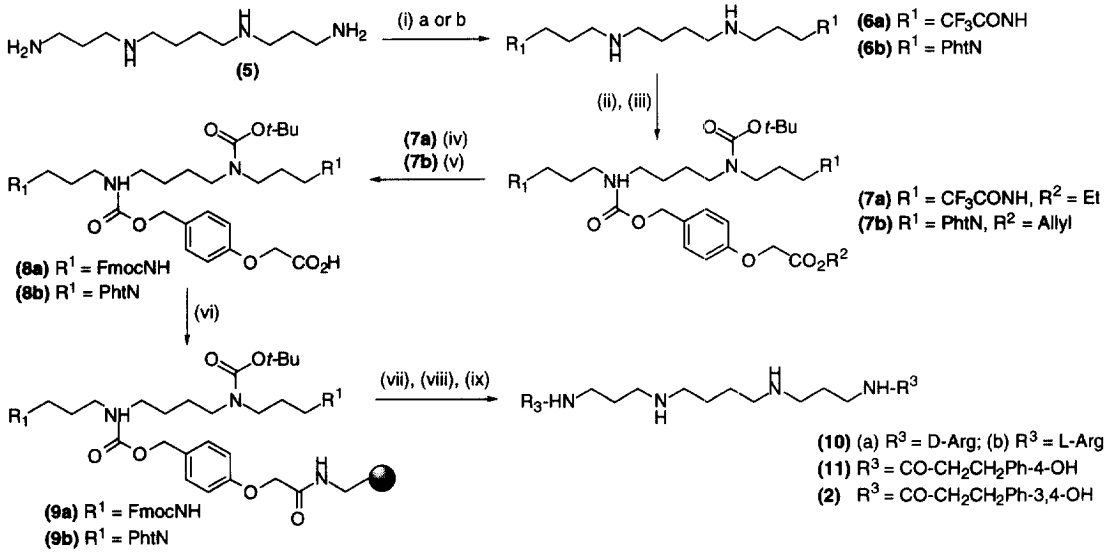
selection of the primary amino functionalities and library generation. Others have also reported the immobilisation of amines for example by attachment to a 'tritylchloride resin[9] and by the use of resin linked Wang based active carbonates.[10] We now wish to report an extension of our methodology which allows an efficient synthesis of symmetrical spermine conjugates using solid phase chemistry and the preparation of the natural product kukoamine (Scheme 1).




**Scheme 1.** (i) (a) $CF_3CO_2Et$, acetonitrile, water; (b) PhtN-$CO_2Et$, $CHCl_3$; (ii) 1 equiv. $Boc_2O$, $NEt_3$, DMAP, DMF; (iii) Allyl ester or $EtO_2CH_2OPhCH_2OCO_2Ph$-4-$NO_2$, $NEt_3$, DMF; (iv) (a) NaOH, dioxane; (b) pH 8.5, Fmoc-OSu, water, dioxane; (v) $Pd(PPh_3)_4$, dimedone, DCM/THF (1:1); (vi) Aminomethyl resin, DIC, HOBt, DCM; (vii) 20% piperidine, DMF or $NH_2NH_2.H_2O$, EtOH 80 °C; (viii) (a) $HO_2CCH_2CH_2Ph$-4-OH, DIC, HOBt, DCM or (b) $HO_2CCH_2CH_2Ph$-3,4-$(OH)_2$, DIC, HOBt, DCM or (c) Fmoc-Arg(Pmc)-OH, DIC, HOBt, DCM; (ix) TFA/TIS/Phenol/thioanisole/water.

Initially the primary amine groups of spermine (**5**) were selectively protected using 2 equiv. of ethyl trifluoroacetate to give $N^1,N^{14}$ *bis*(trifluoroacetyl)-1,5,10,14-tetraazatetradecane (**6a**) in a yield of 94%.[11] This was treated sequentially with 1 equiv. of $Boc_2O$ and 1.1 equiv. of 4-nitrophenyloxycarbonyl-4'-hydroxymethylphenoxy ethylacetate[8b] to give (**7a**) in an overall yield of 76% from spermine. (It should be noted that using another protecting group other than Boc at this stage would also allow access to one of the secondary amine sites for library generation). Following ester hydrolysis and trifluoroacetyl removal the primary amines were re-protected using Fmoc-OSu to give the linkage agent (**8a**), which was immobilised onto aminomethyl polystyrene resin to give (**9a**). The yield for the removal of the two trifluoroacetyl groups, ester hydrolysis and addition of the two Fmoc groups was as good as 64% but often very poor and irreproducible. For this reason we also utilised a phthaloyl protection group strategy for the primary amines, although this strategy required the use of the allyl rather than the ethyl ester to avoid phthaloyl ring opening under basic ester hydrolysis conditions. Thus, 1 equiv. of spermine (**5**) was treated with 2 equiv. of ethoxycarbonylphthalimide in chloroform at room temperature[12] to give *bis*-phthaloyl protected spermine (**6b**) in a yield of 70%. This was treated sequentially with

1 equiv. of $Boc_2O$ and 1.1 equiv. of 4-nitrophenyloxycarbonyl-4'-hydroxymethylphenoxy allylacetate[13] to give (**7b**) in an overall yield of 69%. (**7b**) was converted to the corresponding carboxylic acid (**8b**) in high yield (89%), using $Pd(PPh_3)_4$ and dimedone in dichloromethane/THF (1:1).[14] This was coupled, using standard conditions, to aminomethyl polystyrene resin to give resin linked spermine (**9b**). Fmoc removal from (**9a**) or Phthaloyl removal from (**9b**) allowed functionalization of the two primary amines. Functionalization with 4-hydroxyphenylpropanoic or 3,4-dihydroxyphenylpropanoic acid allowed access to compounds of the kukoamine type. Initially 2-ethoxy-1-ethoxycarbonyldihydroquinoline (EEDQ) was utilised as the coupling reagent due to its compatibility with unprotected hydroxyl functionalities during amide couplings,[15] however it was observed that diisopropylcarbodiimide coupled equally well and no ester by-products were observed. The desired products, kukoamine (**2**) and $N^1,N^{14}$-*bis*-(4-hydroxyphenyl propanoyl)-1,5,10,14 tetraazatetradecane (**11**) were cleaved from the solid support using trifluoroacetic acid/triisopropylsilane/phenol/thioanisole/water (92:2:2:2:2) and purified by reverse-phase HPLC. Kukoamine proved to be extremely sensitive to oxidation and required the broad scavenger cocktail to prevent serious alkylation of the aromatic functionalities. The HPLC's of crude and purified materials are shown in Figure 2.

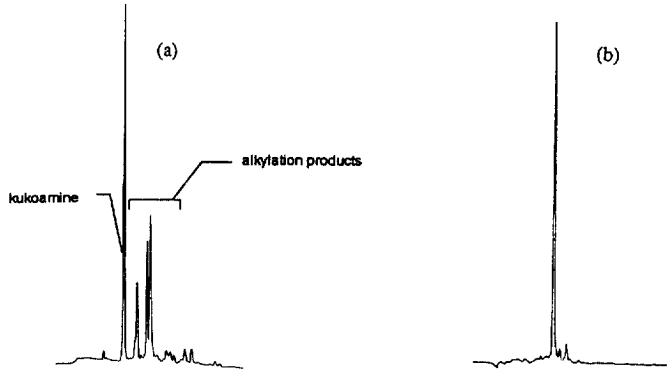



**Figure 2.** (a) Crude RP-HPLC (C-18) of kukoamine cleavage. (b) Purified material.

Using these linkers we have prepared many spermine conjugates both peptide and aromatic acid based. For example in view of the spectrum of biological activity of compounds such as argiotoxin-636 (**3**) and arginyl polyamine sFTX-3.3[7] and related polyamine conjugates and the difficulties often associated with removal of multiple arginine protecting groups we synthesised the diarginyl compounds (**10a,b**). The compounds were cleaved from the resin using TFA/TIS/phenol/thioanisole/water (92:2:2:2:2) to give the desired products in reasonable yields.

Overall we have devised an efficient and practical solid-phase method of synthesising a range of symmetrical spermine conjugates. The synthesis reported here, for the natural product kukoamine, is highly efficient compared to more conventional routes and amenable to library synthesis. The range of immobilised polyamines now available provides ideal scaffolds for library generation.

**Experimental Section:**

***N*$^{1}$,*N*$^{14}$-bis(phthaloyl)-1,5,10,14-tetraazatetradecane (6b).**[12] $\delta_H$ (300 MHz, $CDCl_3$): 1.37 - 1.53 (4H, br m, $^{7,8}CH_2$), 1.80 (4H, tt, *J* = 7,7 Hz, $^{3,12}CH_2$), 2.45 - 2.63 (8H, m, $^{4,6\,9,11}CH_2NH$), 3.69 (4H, t, *J* = 7 Hz, $CH_2NPht$), 7.62 - 7.80 (8H, m, Ar); $\delta_C$ (75 MHz, $CDCl_3$): 27.92 ($^{7,8}CH_2$), 28.99 ($^{3,12}CH_2$), 36.03 ($^{6,9}CH_2NH$), 46.98 ($^{4,11}CH_2N$), 49.84 ($^{2,13}CH_2N$), 132.25 ($^{2,5}Ar$), 132.32 ($^{3,4}Ar$), 134.03 ($^{1,6}Ar$), 168.58 (4xC=O); ES-MS 463.3 $(M + H)^+$; HRMS, $C_{26}H_{30}O_4N_4$ calculated 463.2345; found 463.2333.

***N*$^{1}$,*N*$^{14}$-bis(phthaloyl)-N$^{5}$-$^{t}$butyloxycarbonyl-N$^{10}$-((4-hydroxymethylphenoxy)-allylacetate)oxy carbonyl-1,5,10,14-tetraazatetradecane (7b).** Compound **(6b)**, 3.41 g (7.37 mmol) was dissolved in DMF (100 mL) and triethylamine (3.1 mL (22.12 mmol)) was added. The solution was stirred and cooled in an ice bath at 0 °Cwhile di-t-butyl-dicarbonate (1.66 g (7.37 mmol)) was added over a 10 min period, followed by the addition of DMAP (50 mg). After a further 5 min at 0 °C the ice bath was removed and the mixture stirred for 5 h at room temperature. The reaction mixture was then cooled in an ice bath at 0 °C and the linker[13] (2.85 g, 7.37 mmol) was added dropwise over a 5 min period. The ice bath was removed and the mixture stirred for 17 h at room temperature. The resultant yellow solution was poured into ethyl acetate (200mL) which was washed with 2M $KHSO_4$ (2x50 mL), 5% $NaHCO_3$ (50 mL) and brine (50 mL), dried over $MgSO_4$ and concentrated *in vacuo* to give a yellow oil. The product was purified by column chromatography on silica gel (eluting EtOAc/Hexane, 2:1) to give 5.8 g of the title compound as a yellow oil, yield 98%. $\delta_H$ (300 MHz, $CDCl_3$): 1.26 - 1.52 (13H, s, $CH_3$, and br m, $C^{7,8}H_2$), 1.74 - 1.90 (4H, m, $C^{3,12}H_2$), 3.00 - 3.31 (8H, m $^{4,6,9,11}CH_2N$), 3.50 - 3.68 (4H, m, $CH_2NPht$), 4.57 (2H, s, $CH_2CO$), 4.63 (2H, d, *J* = 6 Hz, $C\underline{H}_2$-CH=), 4.93 (2H, s, $PhCH_2O$), 5.22 (2H, m, $C\underline{H}_2$=CH), 5.85 (1H, m, $C\underline{H}$=$CH_2$), 6.71 - 6.88 and 7.12 - 7.27 (4H, 2xm, Ar), 7.58 - 7.60 and 7.60 -7.80 (8H, 2xm, Pht); $\delta_C$ (75 MHz, $CDCl_3$): 26.03 ($^{7,8}CH_2$), 27.97 ($^{3,12}CH_2$), 28.51 ($CH_3$), 35.94 ($^{6,9}CH_2N$), 44.80 ($^{4,11}CH_2N$), 46.89 ($^{2,13}CH_2N$), 65.43 ($Ar\underline{C}H_2O$), 65.96 ($O\underline{C}H_2CO_2$), 66.74 ($O\underline{C}H_2CH$=), 79.51 ($O\underline{C}(CH_3)_3$), 114.72 (Ar), 119.24 ($CH_2$=, Allyl), 123.33 (Pht), 129.75 (Ar), 130.23 (Ar), 131.55 (CH=, Allyl), 132.20 (Pht), 134.07 (Pht), 155.50 and 156.09 (2xC=O, urethane), 157.62 (Ar), 168.37 (C=O, Pht), 168.64 (C=O, ester); ES-MS 811.2 $(M + H)^+$, 833.3 $(M + Na)^+$; HRMS, $C_{44}H_{50}O_{11}N_4$ calculated 811.3554; found 811.3551.

***N*$^{1}$,*N*$^{14}$-bis(phthaloyl)-*N*$^{5}$-t-butyloxycarbonyl-*N*$^{10}$-((4-hydroxymethylphenoxy)-aceticacid)-oxy carbonyl-1,5,10,14-tetraazatetradecane (8b).** $\delta_H$ (300 MHz, $CDCl_3$): 1.23 - 1.51 (13H, m, $CH_3$ and $^{7,8}CH_2$), 1.51 - 1.91 (4H, br m, $^{3,12}CH_2$), 2.94 - 3.37 (8H, m, $^{4,6,9,11}CH_2N$), 3.48 - 3.68 (4H, m, $CH_2NPht$), 4.62 (2H, $CH_2C(O)$), 4.99 (2H, s, $PhCH_2O$), 6.60 - 6.84 and 7.04 - 7.22 (4H, m, Ar), 7.56 - 7.68 and 7.68 - 7.79 (8H, 2xm, Pht), 11.0 (1H, br s, $CO_2H$); $\delta_C$ (75 MHz, $CDCl_3$): 25.67 ($^{7,8}CH_2$), 27.97 ($^{3,12}CH_2$), 28.52 ($CH_3$), 35.94 ($^{6,9}CH_2N$), 44.81 ($^{4,11}CH_2N$), 46.90 ($^{2,13}CH_2N$), 65.12 ($Ar\underline{C}H_2O$), 65.91 ($O\underline{C}H_2CO_2$), 79.96 ($O\underline{C}(CH_3)_3$), 122.74 (Ar), 123.42 (Pht), 129.93 (Ar), 129.94 (Ar), 132.17 (Pht), 134.18 (Pht), 155.7 and 156.42 (2xC=O, urethane), 157.61 (C=, Ar), 168.51 (C=O, Pht), 171.80 (C=O, acid); ES-MS, 771.3 $(M + H)^+$, 793.3 $(M + Na)^+$; HRMS $C_{41}H_{46}O_{11}N_4$ calculated 771.3241; found 771.3223.

**Solid-phase synthesis procedure of kukoamine A (2).**

***N*$^{1}$,*N*$^{14}$-bis(phthaloyl)-*N*$^{5}$-t-butyloxycarbonyl-*N*$^{10}$-((4-hydroxymethylphenoxy)-acetamido methyl resin)-oxycarbonyl-1,5,10,14-tetraazatetradecane (9b).** To 230 mg (1.1 $mmolg^{-1}$, 253 μmol) of aminomethyl PS-resin (1% cross-linked) (pre-swolled in DCM for 20min and filtered) was added a solution of **(8b)** (310 mg, 400 μmol) dissolved in DCM (3 mL) and a few drops of DMF, DIC (60 μl, 400 μmol) and HOBt (53 mg, 400 μmol). The suspension was shaken at room temperature for 5 h. The resin was filtered and washed successively with DMF (10 mL), DCM (10 mL), MeOH (10 mL) and ether (10mL). The resin was dried under vacuum and gave a negative ninhydrin test.[16]

***N*$^{5}$-*t*-butyloxycarbonyl-*N*$^{10}$-((4-hydroxymethylphenoxy)-acetamido methyl resin)-oxycarbonyl-1,5,10,14-tetraazatetradecane (9c).** To a shaken suspension of the previous resin **(9b)** (425 mg, pre-swolled in DCM and filtered) was added absolute EtOH (10 mL), hydrazine monohydrate (250 μl, 5 mmol) and the solution was degassed by a gentle flow of nitrogen and then heated at 80 °C overnight. The resin was then

filtered and washed successively with hot water (20 mL), EtOH (20 mL), MeOH (10 mL), DCM (10 mL), MeOH (10 mL) and ether (10 mL). The resin was dried under vacuum and a quantitative ninhydrin test recorded, giving a loading of 1.3mmolg$^{-1}$ (87% expected).

**$N^1$,$N^{14}$-bis(3,4-dihydroxyhydrocinnamoyl)-$N^5$-t-butyloxycarbonyl-$N^{10}$-((4-hydroxymethyl phenoxy)-acetamido methyl resin)-oxycarbonyl-1,5,10,14-tetraazatetradecane (9d).** To a shaken suspension resin **(9c)** (352 mg, pre-swollen in DCM and filtered) was added a solution of 3,4-dihydroxyhydrocinnamic acid (460 mg, 2.53 mmol) dissolved in DCM (3 mL) and a few drops of DMF, DIC (396 µl, 2.53 mmol) and HOBt (350 mg, 2.53 mmol). This suspension was shaken at room temperature for 3 h. The resin was filtered and washed successively with DMF (10 mL), DCM (10 mL), MeOH (10 mL) and ether (10 mL). The resin was dried under vacuum and gave a negative ninhydrin result.

**$N^1$,$N^{14}$-bis(3,4-dihydroxyhydrocinnamoyl)-1,5,10,14-tetraazatetradecane or kukoamine A (2).** Resin **(9d)** (422 mg) was pre-swollen in DCM for 30 min and the resin filtered. To this resin was added 92% TFA/2% water/2% thioanisole/2% TIS/2% Phenol (3 mL) and the suspension was shaken at room temperature for 2 h. The resin was filtered, the filtrate concentrated (ca. 0.5 mL) and 'butylmethylether (15 mL) was added. After 30 min centrifugation (5000rpm/25 °C), a white-yellow solid which was redissolved in water with a few drops of acetonitrile and freeze-dried (160 mg). Yield calculated from the loading of aminomethyl resin, 85%. HPLC analysis at 220 nm: 45% pure. $\delta_H$ (300 MHz, $D_2O$): 1.65 - 1.77 (8H, m, $^{3,7,8,12}C\underline{H}_2$), 2.56 and 2.62 (8H, 2xt, $J$ = 8 Hz, PhC$\underline{H}_2$C$\underline{H}_2$), 2.81 (4H, $J$ = 7 Hz, NHC$^{6,9}\underline{H}_2$), 2.88 - 2.92 (4H, m, NHC$^{4,11}\underline{H}_2$), 3.20 (4H, t, $J$ = 7 Hz, CONHC$\underline{H}_2$), 6.69 (2H, dd, $J$ = 8 Hz, Ar), 6.76 (2H, t, $J$ = 2Hz, Ar), 6.83 (2H, dd, $J$ = 8Hz Ar); $\delta_C$ (75Mhz, $D_2O$): 28.16 ($^{7,8}CH_2$), 31.04 ($^{3,12}CH_2$), 35.18 ($CH_2Ar$), 35.88 ($^{6,9}CH_2N$), 40.85 ($^{4,11}CH_2N$), 42.49 ($CH_2CO$), 50.04 ($^{2,13}\underline{C}H_2NC(O)$), 121.24 (Ar), 125.68 (Ar), 128.28 (Ar), 138.1 (Ar), 147.97 (Ar), 149.51 (Ar), 181.5 (C=O); ES-MS 531.4 $(M + H)^+$; HRMS $C_{28}H_{43}O_6N_4$ calculated 531.3183; found 531.3168.

**$N^1$,$N^{14}$-bis(D-arginyl)-1,5,10,14-tetraazatetradecane (10).** (71% yield, 39% pure by HPLC integration at 220 nm). $\delta_H$ (300 MHz, $CD_3OD$): 1.66 (4H, quin $J$ = 7 Hz, $^{7,8}C\underline{H}_2$), 1.76 - 2.00 (12H,m, $^{3,12}CH_2$, COCH($NH_2$)C$\underline{H}_2$C$\underline{H}_2$), 3.09 (8H, br t, $J$ = 7 Hz, $^{4,6,9,11}C\underline{H}_2NH$), 3.22 (4H, t, $J$ = 7 Hz, $^{2,13}C\underline{H}_2NH$), 3.41 (4H, t, $J$ = 7 Hz, C$\underline{H}_2$NHC(NH)$NH_2$), 3.96 (2H, t, $J$ = 7 Hz, $H_2$NC$\underline{H}$CONH); $\delta_C$ (75 MHz, $CD_3OD$): 23.9 (COCH($NH_2$)$\underline{C}H_2$), 25.0 ($\underline{C}H_2CH_2C(NH)NH_2$), 26.7 ($C^{7,8}H_2$), 29.3 ($^{3,12}CH_2N$), 37.5 ($C^{6,9}H_2N$), 41.5 ($\underline{C}H_2NHC(NH)NH_2$), 46.3 ($C^{4,11}H_2N$), under solvent resonances ($C^2H_2$, $C^{13}H_2$), 54.0 ($H_2N\underline{C}HCONH$), 158.1 ($H_2N\underline{C}(NH)NH$), 170.8 ($\underline{C}ONH$); ES-MS 258.5 $(M + 2H)^{2+}$; HRMS $C_{22}H_{51}O_2N_{12}$ calculated 515.4258; found 515.4286.

**$N^1$,$N^{14}$-bis(4-hydroxyhydrocinnamoyl)-1,5,10,14-tetraazatetradecane (11).** (86% yield, 45% pure by HPLC integration at 220 nm). $\delta_H$ (300 MHz, $D_2O$): 1.45 - 1.62 and 1.86 - 1.92 (8H, m, $NH^{3,7,8,12}C\underline{H}_2$), 2.40 (4H, t, $J$ = 7 Hz, PhC$\underline{H}_2$), 2.73 (4H, t, $J$ = 7 Hz, $CH_2CONH$), 2.88 - 3.09 (12H, m, $NH^{2,4,6,9,11,13}C\underline{H}_2$), 6.68 (4H, d, $J$ = 8 Hz, Ar), 6.97 (4H, d, $J$ = 8 Hz, Ar); ES-MS 250.4 $(M + 2H)^{2+}$, 499.3 $(M + H)^+$, 521.3 $(M + Na)^+$.

**Synthesis of bis-Fmoc protected spermine derivative (8a).** The title compound **(8a)** was prepared in an analogous manner to **(8b)**. Thus spermine was protected with trifluoracetyl groups according to the literature procedure[11] to give compound **(6a)**. This was treated with $Boc_2O$ and 4-nitrophenyloxycarbonyl-4'-hydroxymethylphenoxy ethylacetate[8] to give **(7a)**. After treatment with NaOH in dioxane for 4h, the solution was neutralised with 2 M $KHSO_4$ (pH 8.5), 2.1 equiv. of Fmoc-OSu added and the reaction mixture stirred for 2h. The solution was acidified with $KHSO_4$ to pH 5 and extracted with DCM to give the linkage agent **(8a)** in 64% yield after purification by column chromatography on silica gel (eluting with EtOAc/petroleum ether (1:1)).

**$N^1$,$N^{14}$-bis(trifluoroacetyl)-$N^5$-t-butyloxycarbonyl-$N^{10}$-((4-hydroxymethylphenoxy)-ethyl acetate)-oxycarbonyl-1,5,10,14-tetraazatetradecane (7a).** $\delta_H$ (300 MHz, $CD_3SOCD_3$): 1.24 (3H, t, $J$ = 7 Hz, $CO_2CH_2C\underline{H}_3$), 1.31 - 1.54 (9H, br s, $CH_3$), 1.57 - 1.76 (8H, broad m, $C^{3,7,8,12}H_2$), 2.98 - 3.28 (12H, m, $C^{2,4,6,9,11,13}H_2N$), 4.16 (2H, q, $J$ = 7 Hz, $CO_2C\underline{H}_2CH_3$), 4.78 (2H, s, $CH_2CO$), 4.98 (2H, s, $PhCH_2O$), 6.89

(2H, d, $J$ = 8 Hz, Ar), 7.25 (2H, d, $J$ = 8 Hz, Ar), 9.4 (2H, s, NH); $\delta_C$ (75 MHz, $CD_3OD$): 14.4 ($CH_2\underline{C}H_3$), 26.8 ($^{7,8}CH_2$), 28.58 ($^{3,12}CH_2$), 28.6 ($CH_3$), 38.2 ($^{6,9}CH_2N$), 45.80 ($^{4,11}CH_2N$), 46.6 ($^{2,13}CH_2N$), 61.4 ($Ar\underline{C}H_2O$), 62.63 ($CH_3\underline{C}H_2$), 66.0 ($O\underline{C}H_2CO_2$), 81.0 ($O\underline{C}(CH_3)_3$), 115.6 (Ar), 119.3 (q, $CF_3$), 129.7, 130.8 (Ar), 155.40 and 156.1 (2xC=O, urethane), 157.8 (Ar), 159.3 (q, $CF_3\underline{C}$=O), 170.7 (C=O, ester); ES-MS 731.5 $(M + H)^+$, 753.5 $(M + Na)^+$.

***N*$^1$,*N*$^{14}$-bis(fluorenylmethoxycarbonyl)-*N*$^5$-*t*-butyloxycarbonyl-*N*$^{10}$-((4-hydroxymethylphenoxy)-acetic acid)-oxycarbonyl-1,5,10,14-tetraazatetradecane (8a).** $\delta_H$ (300 MHz, $CD_3SOCD_3$): 1.28 - 1.45 (13H, s + br m $C\underline{H}_3$ and $^{7,8}C\underline{H}_2$), 1.50 - 1.68 (4H, m, $^{3,12}C\underline{H}_2$), 2.85 - 3.21 (12H, 3xm, $^{2,4,6,9,11,13}C\underline{H}_2N$)), 4.16 - 4.33 (8H, 2x m, Fmoc-$C\underline{H}_2$, Fmoc-$C^9\underline{H}$, $OC\underline{H}_2CO$), 4.94 (2H, s, $C\underline{H}_2Ph$), 6.85 and 7.20 (4H, 2xd, $J$ = 8 Hz, Ar $C^{2,3,5,6}H$), 7.31 and 7.40 (8H, 2xt, $J$ = 7Hz, Fmoc Ar-$C^{2,3,6,7}H$), 7.67 and 7.86 (8H, 2xd, $J$ = 8Hz, Fmoc Ar-$C^{1,4,5,8}H$); $\delta_C$ (75 MHz, $CD_3SOCD_3$): 25.6 ($C^{7,8}H_2$), 26.2 ($C^{3,12}H_2$), 28.6 ($CH_3$), 37.8 ($C^{6,9}H_2N$), 43.7 ($C^{4,11}H_2N$), 46.5 and 46.9 ($C^{2,13}H_2$), 47.4 (Fmoc$\underline{C}^9H$), 61.1 ($Ar\underline{C}H_2O$), 66.8 ($O\underline{C}H_2CO_2$), 73.1 (Fmoc$CH_2$), 80.0 ($\underline{C}(CH_3)_3$), 120.1, 120.3, 125.4, 127.2, 127.80, 128.4, 129.8, 141.5, 142.6, 144.2 (Ar), 155.2, 155.6 and 156.8 (3xOCONH), 157.8 (Ar), 168.7 ($CO_2H$); ES-MS 855.6 $(M - Boc + H)^+$, 911.6 $(M - Bu^t + H)^+$, 955.6 $(M + H)^+$, 977.3 $(M + Na)^+$.

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## References and Notes

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