

Bioorganic & Medicinal Chemistry Letters 8 (1998) 1751-1756

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

THE SYNTHESIS OF SYMMETRICAL SPERMINE CONJUGATES USING SOLID-PHASE CHEMISTRY

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Received 23 March 1998; accepted 3 June 1998

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. © 1998 Elsevier Science Ltd. All rights reserved.

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 $N^l, N^{l6}bis(\text{ethyl})$ 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. $N^l, N^{l6}bis(3,4\text{-dihydroxyhydrocinnamoyl})$ -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 antitumor⁶ or antiparasitic⁷ properties.



(1) N¹, N¹⁶bis(ethyl)-1,6,11,16-tetraazahexadecane (DEHSPM)





(2) N¹, N¹⁴ bis(3,4-dihydroxyhydrocinnamoyl)-1,5,10,14-tetraaza tetradecane (kukoamine A)



(4) / V, / V UDEnzy - 1,5, 13, 17 - Tetraaza

(3) N¹ Arg , N¹⁵ Asn-1,4-dihydroxyphenylacetate-1,5,9,15-tetraaza pentadecane (argiotoxin-636)

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

selection of the primary amino functionalities and library generation. Others have also reported the immobilisation of amines for example by attachment to a 'tritylchloride resin⁹ 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-CO₂Et, $CHCl_3$; (ii) 1 equiv. Boc₂O, NEt₃, DMAP, DMF; (iii) Allyl ester or $EtO_2CH_2OPhCH_2OCO_2Ph-4-NO_2$, NEt₃, DMF; (iv) (a) NaOH, dioxane; (b) pH 8.5, Fmoc-OSu, water, dioxane; (v) Pd(PPh₃)₄, dimedone, DCM/THF (1:1); (vi) Aminomethyl resin, DIC, HOBt, DCM; (vii) 20% piperidine, DMF or NH₂NH₂.H₂O, EtOH 80 °C; (viii) (a) HO₂CCH₂CH₂Ph-4-OH, DIC, HOBt, DCM or (b) HO₂CCH₂CH₂Ph-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 Boc₂O 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 temperature¹² 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 allylacetate¹³ to give (**7b**) in an overall yield of 69%. (**7b**) was converted to the corresponding carboxylic acid (**8b**) in high yield (89%), using Pd(PPh₃)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 4hydroxyphenylpropanoic 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 $\delta_{\rm H}$ (300 MHz, CDCl₃): 1.37 - 1.53 (4H, br m, ^{7.8}CH₂), 1.80 (4H, tt, J = 7,7 Hz, ^{3.12}CH₂), 2.45 - 2.63 (8H, m, ^{4.6.9.11}CH₂NH), 3.69 (4H, t, J = 7 Hz, CH₂NPht), 7.62 - 7.80 (8H, m, Ar); $\delta_{\rm C}$ (75 MHz, CDCl₃): 27.92 (^{7.8}CH₂), 28.99 (^{3.12}CH₂), 36.03 (^{6.9}CH₂NH), 46.98 (^{4.11}CH₂N), 49.84 (^{2.13}CH₂N), 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, C₂₆H₃₀O₄N₄ calculated 463.2345; found 463.2333.

 N^{I} , N^{I4} -bis(phthaloyl)- N^{5} -'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 linker¹³ (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 KHSO₄ (2x50 mL), 5% NaHCO₃ (50 mL) and brine (50 mL), dried over MgSO₄ 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%. δ_{μ} (300 MHz, CDCl₃): 1.26 -1.52 (13H, s, CH₃, and br m, C^{7.8}H₂), 1.74 - 1.90 (4H, m, C^{3,12}H₂), 3.00 - 3.31 (8H, m^{-4.6.9,11}CH₂N), 3.50 -3.68 (4H, m, CH₂NPht), 4.57 (2H, s, CH₂CO), 4.63 (2H, d, J = 6 Hz, CH₂-CH=), 4.93 (2H, s, PhCH₂O), 5.22 (2H, m, CH₂=CH), 5.85 (1H, m, CH=CH₂), 6.71 - 6.88 and 7.12 - 7.27 (4H, 2xm, Ar), 7.58 - 7.60 and 7.60 -7.80 (8H, 2xm, Pht); δ_{C} (75 MHz, CDCl₃): 26.03 (^{7.8}CH₂), 27.97 (^{3,12}CH₂), 28.51 (CH₃), 35.94 (^{6,9}CH₂N), 44.80 (^{4.11}CH₂N), 46.89 (^{2.13}CH₂N), 65.43 (ArCH₂O), 65.96 (OCH₂CO₂), 66.74 (OCH₂CH₂), 79.51 (OC(CH₃)₃), 114.72 (Ar), 119.24 (CH₂=, 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^{I} , N^{I4} -bis(phthaloyl)- N^{5} -t-butyloxycarbonyl- N^{I0} -((4-hydroxymethylphenoxy)-aceticacid)-oxy carbonyl-1,5,10,14-tetraazatetradecane (8b). $\delta_{\rm H}$ (300 MHz, CDCl₃): 1.23 - 1.51 (13H, m, CH₃ and ^{7.8}CH₂), 1.51 - 1.91 (4H, br m, ^{3.12}CH₂), 2.94 - 3.37 (8H, m, ^{4.6.9.11}CH₂N), 3.48 - 3.68 (4H, m, CH₂NPht), 4.62 (2H, CH₂C(O)), 4.99 (2H, s, PhCH₂O), 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, CO₂H); $\delta_{\rm C}$ (75 MHz, CDCl₃): 25.67 (^{7.8}CH₂), 27.97 (^{3.12}CH₂), 28.52 (CH₃), 35.94 (^{6.9}CH₂N), 44.81 (^{4.11}CH₂N), 46.90 (^{2.13}CH₂N), 65.12 (ArCH₂O), 65.91 (OCH₂CO₂), 79.96 (OC(CH₃)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 C₄₁H₄₆O₁₁N₄ 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 μ mol) of aminomethyl PS-resin (1% cross-linked) (pre-swolled in DCM for 20min and filtered) was added a solution of (8b) (310 mg, 400 μ mol) dissolved in DCM (3 mL) and a few drops of DMF, DIC (60 μ l, 400 μ mol) and HOBt (53 mg, 400 μ mol). 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, preswolled 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 was isolated 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. $\delta_{\rm H}$ (300 MHz, D₂O): 1.65 - 1.77 (8H, m, ^{37,8,12}CH₂), 2.56 and 2.62 (8H, 2xt, *J* = 8 Hz, PhCH₂CH₂), 2.81 (4H, *J* = 7 Hz, NHC^{6,9}H₂), 2.88 - 2.92 (4H, m, NHC^{4,11}H₂), 3.20 (4H, t, *J* = 7 Hz, CONHCH₂), 6.69 (2H, dd, *J* = 8 Hz, Ar), 6.76 (2H, t, *J* = 2Hz, Ar), 6.83 (2H, dd, *J* = 8Hz Ar); $\delta_{\rm c}$ (75Mhz, D₂O): 28.16 (^{7,8}CH₂), 31.04 (^{3,12}CH₂), 35.18 (CH₂Ar), 35.88 (^{6,9}CH₂N), 40.85 (^{4,11}CH₂N), 42.49 (CH₂CO), 50.04 (^{2,13}CH₂NC(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 C₂₈H₄₃O₆N₄ calculated 531.3183; found 531.3168. *N*¹,*N*¹⁴-bis(*b*-arginyl)-1,5,10,14-tetraazatetradecane (10). (71% yield, 39% pure by HPLC integration

A *j*, *V* -Dis(b-arginy)-1,3,10,14-tetrazzatetradectate (10). (71% yield, 39% pute by III EC integration at 220 nm). $\delta_{\rm H}$ (300 MHz, CD₃OD): 1.66 (4H, quin J = 7 Hz, $^{7.8}C\underline{\rm H}_2$), 1.76 - 2.00 (12H,m, $^{3.12}CH_2$, COCH(NH₂)C<u>H₂CH₂</u>), 3.09 (8H, br t, J = 7 Hz, $^{4.6.9,11}C\underline{\rm H}_2$ NH), 3.22 (4H, t, J = 7 Hz, $^{2.13}C\underline{\rm H}_2$ NH), 3.41 (4H, t, J = 7 Hz, C<u>H</u>₂NHC(NH)NH₂), 3.96 (2H, t, J = 7 Hz, H₂NC<u>H</u>CONH); $\delta_{\rm C}$ (75 MHz, CD₃OD): 23.9 (COCH(NH₂)C<u>H</u>₂), 25.0 (CH₂CH₂C(NH)NH₂), 26.7 (C^{7.8}H₂), 29.3 ($^{3.12}CH_2$ N), 37.5 (C^{6.9}H₂N), 41.5 (CH₂NHC(NH)NH₂), 46.3 (C^{4.11}H₂N), under solvent resonances (C²H₂, C¹³H₂), 54.0 (H₂NCHCONH), 158.1 (H₂NC(NH)NH), 170.8 (CONH); ES-MS 258.5 (M + 2H)²⁺; HRMS C₂₂H₅₁O₂N₁₂ calculated 515.4258; found 515.4286.

 N^{I} , N^{I4} -bis(4-hydroxyhydrocinnamoyl)-1,5,10,14-tetraazatetradecane (11). (86% yield, 45% pure by HPLC integration at 220 nm). $\delta_{\rm H}$ (300 MHz, D₂O): 1.45 - 1.62 and 1.86 - 1.92 (8H, m, NH^{3.7,8.12}CH₂), 2.40 (4H, t, J = 7 Hz, PhCH₂), 2.73 (4H, t, J = 7 Hz, CH₂CONH), 2.88 - 3.09 (12H, m, NH^{2.4,6.9,11,13}CH₂), 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 procedure¹¹ to give compound (6a). This was treated with Boc_2O and 4-nitrophenyloxycarbonyl-4'-hydroxymethylphenoxy ethylacetate⁸ to give (7a). After treatment with NaOH in dioxane for 4h, the solution was neutralised with 2 M KHSO₄ (pH 8.5), 2.1 equiv. of Fmoc-OSu added and the reaction mixture stirred for 2h. The solution was acidified with KHSO₄ 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', N'^4 -bis(trifluoroacetyl)- N^5 -t-butyloxycarbonyl- N'^0 -((4-hydroxymethylphenoxy)-ethyl

acetate)-oxycarbonyl-1,5,10,14-tetraazatetradecane (7a). $\delta_{\rm H}$ (300 MHz, CD₃SOCD₃): 1.24 (3H, t, J = 7 Hz, CO₂CH₂CH₃), 1.31 - 1.54 (9H, br s, CH₃), 1.57 - 1.76 (8H, broad m, C^{3.7.8.12}H₂), 2.98 - 3.28 (12H, m, C^{2.4.6.9.11.13}H₂N), 4.16 (2H, q, J = 7 Hz, CO₂CH₂CH₃), 4.78 (2H, s, CH₂CO), 4.98 (2H, s, PhCH₃O), 6.89

 $(2H, d, J = 8 Hz, Ar), 7.25 (2H, d, J = 8 Hz, Ar), 9.4 (2H, s, NH); \delta_{C} (75 MHz, CD_{3}OD): 14.4 (CH_{2}CH_{3}), 14.4 (CH_{2}CH_{3}$ 26.8 (^{7.8}CH₂), 28.58 (^{3,12}CH₂), 28.6 (CH₃), 38.2 (^{6.9}CH₂N), 45.80 (^{4,11}CH₂N), 46.6 (^{2,13}CH₂N), 61.4 (ArCH₂O), 62.63 (CH₁CH₂), 66.0 (OCH₂CO₂), 81.0 (OC(CH₁)₂), 115.6 (Ar), 119.3 (q. CF₃), 129.7, 130.8 (Ar), 155.40 and 156.1 (2xC=O, urethane), 157.8 (Ar), 159.3 (q, CF, C=O), 170.7 (C=O, ester); ES-MS 731.5 $(M + H)^+$, 753.5 $(M + Na)^+$.

 N^{i} , N^{I4} -bis(fluorenylmethoxycarbonyl)- N^{5} -t-butyloxycarbonyl- N^{I0} -((4-hydroxymethylphenoxy) -acetic acid)-oxycarbonyl-1,5,10,14-tetraazatetradecane (8a). δ_μ (300 MHz, CD₃SOCD₃): 1.28 - 1.45 $(13H, s + br m CH_3 and {}^{7.8}CH_2), 1.50 - 1.68 (4H, m, {}^{3.12}CH_2), 2.85 - 3.21 (12H, 3xm, {}^{2.4.6.9,11,13}CH_2N)), 4.16 - 3.21 (12H, 3xm, {}^{2.4.6.9,11,13}CH_2N)), 4.16 - 3.21 (12H, 3xm, {}^{2.4.6.9,11,13}CH_2N))$ 4.33 (8H, 2x m, Fmoc-CH₂, Fmoc-C⁹H, OCH₂CO), 4.94 (2H, s, CH₂Ph), 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); $\delta_{\rm C}$ (75 MHz, CD₃SOCD₃): 25.6 (C^{7,8}H₂), 26.2 (C^{3,12}H₂), 28.6 (CH₃), 37.8 (C^{6,9}H₂N), 43.7 (C^{4.11}H₂N), 46.5 and 46.9 (C^{2.13}H₂), 47.4 (FmocC⁹H), 61.1 (ArCH₂O), 66.8 (OCH₂CO₂), 73.1 (FmocCH₂), 80.0 (C(CH₃)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 (CO₂H); ES-MS 855.6 (M - Boc + H)⁺, 911.6 (M - Bu¹ + H)⁺, 955.6 $(M + H)^+$, 977.3 $(M + Na)^+$.

Acknowledgements: We thank the Wellcome Trust for a Project Grant 043503/PMG/AH and the Royal Society for a University Research Fellowship (MB).

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