Synthesis of Achiral α,α-Bis(aminomethyl)-β-alanines and Their Use in the Preparation of Branched β-Peptide Conjugates of *N*-2-Alkyl-1,2,3,4-tetrahydroisoquinolines on Solid Support

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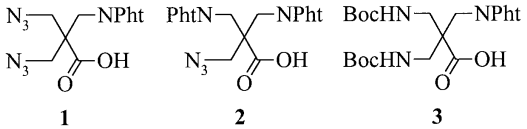
Three achiral branching units **1**–**3** derived from α,α-bis(aminomethyl)-β-alanine were synthesized and evaluated in the construction of branched and sterically compact β-peptide conjugates on a solid support. Several peptide conjugates bearing an *N*-alkylated 1,2,3,4-tetrahydroisoquinoline moiety were prepared to demonstrate the applicability of the branching units in a solid phase peptide synthesis. The most useful building block **3** incorporated Boc-protected aminomethyl side chains at the α-carbon of *N*-phthaloyl-β-alanine.

Introduction

Branched peptides and peptide-like oligomers have recently attracted increasing attention owing to their potential applications in medical sciences.[1] However, only a limited number of branching units are available. In fact most of the syntheses are simply based on the use of lysine for branching. We have previously reported on the synthesis of novel achiral α,α-disubstituted β-alanines, and their use in the preparation of linear β-peptide conjugates on a solid support.[2] In the present study, the methodology is extended to the preparation of branched β-peptide conjugates. Three novel achiral branching units (**1**−**3**) derived from α,α-bis(aminomethyl)-β-alanine were synthesized (Figure 1). The one bearing Boc-protected aminomethyl side chains at the α-carbon of *N*-phthaloyl-β-alanine (**3**) allowed convenient solid support preparation of branched β-peptides that contained either three identical peptide chains bound to the α-carbon of the branching unit, or two identical and one different chain. In both cases achirality is preserved. A set of such β-peptide conjugates containing an *N*-alkylated 1,2,3,4-tetrahydroisoquinoline core was prepared. The purpose of this core structure was to anchor the peptides close to the binding site of the α_2-adrenergic receptors to probe the receptor structure. The conjugate group does not, however, play any essential role in the assembly of the branched peptide chain, and **3** can be utilized equally well in solid phase syntheses of branched β-peptides bearing no conjugate group.



1 **2** **3**

Figure 1. Building blocks for the synthesis of branched β-peptides

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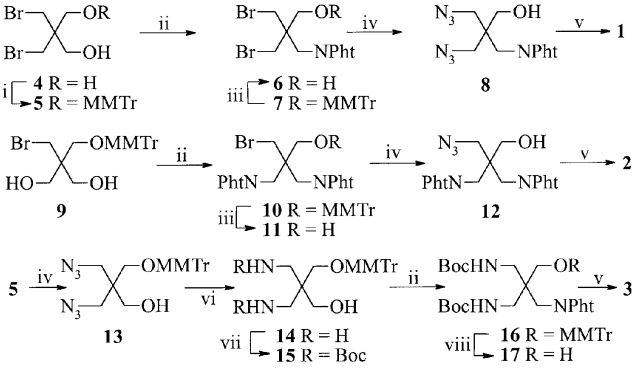
Results and Discussion

Synthesis of Protected Bis(aminomethyl)-β-alanine Building Blocks

Building block **1** was synthesized from commercially available 2,2-bis(bromomethyl)-1,3-propanediol (**4**), as depicted in Scheme 1. Two slightly different routes were employed: either one of the hydroxy functions of **4** was displaced with a phthalimido group under Mitsunobu conditions[3] without prior protection of the other hydroxy function (**6**), or one of the hydroxy functions was protected with a monomethoxytrityl group (**5**) before the Mitsunobu reaction (**7**) and subsequently deprotected (**6**). The latter route hence consists of two additional reaction steps, but **6** can still be obtained in a far better total yield. Displacement of the bromo substituents of **6** with azide ion, and Jones' oxidation of the hydroxy group to a carboxy group completed the synthesis of **1**.

The synthesis of building block **2** started from 2-bromomethyl-2-hydroxymethyl-1,3-propanediol.[4] One of the hydroxy functions was protected with a monomethoxytrityl group (→ **9**), and the remaining two were displaced with



Scheme 1. Synthesis of building blocks **1**−**3**: (i) MMTrCl, Py; (ii) phthalimide, PPh_3, DEAD, THF; (iii) DCA, CH_2Cl_2; (iv) NaN_3, LiCl, DMF; (v) CrO_3, H_2SO_4, acetone; (vi) $NaBH_4$, $HS(CH_2)_3SH$, *i*PrOH; (vii) Boc_2O, NaOH, MeCN, H_2O; (viii) I_2, MeOH, DCM

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phthalimide by Mitsunobu reaction (→ **10**). The monomethoxytrityl protection was removed (→ **11**) under acidic conditions, and an azido function was brought into the molecule by displacement of the bromo substituent with azide ion (→ **12**). Oxidation of the hydroxy function to a carboxy group gave the building block **2**.

To obtain building block **3**, the two bromo substituents of **5** were displaced with the azide ion (→ **13**), and the azido functions were reduced with sodium borohydride/propanedithiol (→ **14**).[5] The amino groups were protected with Boc anhydride (→ **15**), and the phthaloyl protected third amino function was introduced into the molecule by Mitsunobu reaction (→ **16**). The monomethoxytrityl protection was removed by treatment with iodine in methanol/dichloromethane,[6] and oxidation to acid **3** completed the synthesis.

Deprotection of the Amino Functions and Coupling of the Amino Acid Building Blocks 1−3 on a Solid Support

The amino acid building blocks **1**−**3** could be coupled through their carboxy function almost quantitatively to hydroxymethyl polystyrene-supported free amine functions. On using HATU activation, the peptide coupling of **1** or **2** to the support-bound primary amine was completed in an hour, and that of **3** in 5 hours. In spite of its slower coupling, **3** turned out to be superior to **1** and **2** as a building block for the assembly of branched β-peptides on a solid support. The main reason for this was that reduction of the azido functions of **1** and **2** to amino groups, allowing the next peptide coupling, turned out to be unexpectedly difficult. Most of the methods described in the literature for the reduction of an azido to an amino function on a solid support[7] were tested. The best, but still not satisfactory, result was achieved with a tin(II) chloride/thiophenol/triethylamine system. Different concentration ratios and reaction times were tried without significant improvement of the yield. In the case of **1**, the reduction yielded 34% of the desired product in 10 minutes. If shorter reaction times were used, the deprotection was incomplete. Longer reaction times, in turn, gave rise to several side products. Tentative explanations for the low yields obtained with **1** include the interference of one azido group in the reduction of the other, and the instability of the phthaloyl protection under the applied reaction conditions. Removal of the phthaloyl protection from **2** was also problematic. The hydrazinolysis of the phthaloyl moiety proceeded slowly in the presence of an azido group and led to the formation of side products. The Boc protections of **3** could be removed cleanly with 1 M HCl in a 1:2 mixture of MeOH and dichloromethane (v/v) or with aluminium chloride.[8] The standard TFA treatment, in turn, resulted in a marked increase in the number of side products. The removal of the phthaloyl protection also proceeded slowly in this case, but a quantitative deprotection was achieved in 6 hours using 2 M hydrazine hydrate in a 1:2 mixture of DMF and dioxane (v/v) at 80 °C.

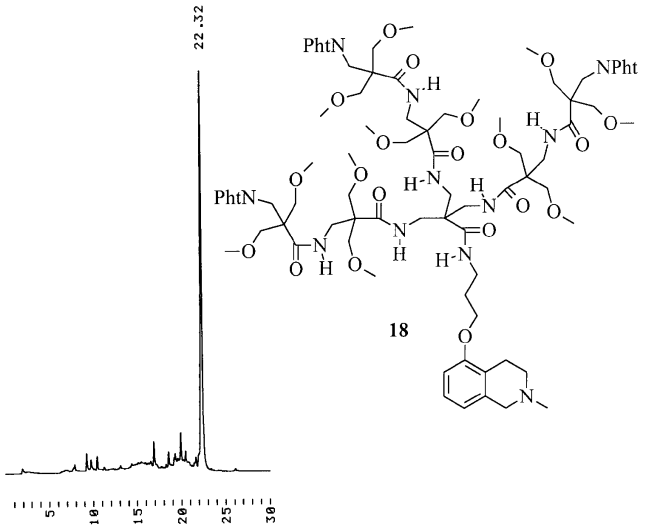

Figure 2. The model compound **18** analysed by HPLC (Hypersil Hypurity C18, gradient from aqueous 0.1% TFA to acetonitrile in 30 min, flow 1.0 mL/min, detection at 276 nm); MS (ESI): **18** (peak at 22.32 min) found 1695.9 $[M + H]^+$ and 848.5 $[M + 2\ H]^+$, $C_{84}H_{115}N_{11}O_{26}$ + H requires 1694.8

The Solid Phase Synthesis of Branched β-Peptide Conjugates

β-Peptide conjugate **18** was prepared to verify the compatibility of building block **3** with the solid phase peptide synthesis (Figure 2). The synthesis was carried out on hydroxymethyl polystyrene, to which the desired conjugate group, Boc protected 5-(3-aminopropoxy)-1,2,3,4-tetrahydroisoquinoline, was tethered through a diethyl sulfone linker.[2] The support-bound amino group was deprotected and subjected to HATU activated acylation with building block **3**. The phthaloyl protection of the introduced branching unit was removed by hydrazinolysis, and the β-peptide chain was elongated with an achiral *N*-phthaloyl-β-alanine building block. Subsequently, the Boc protection of the branching unit was removed and the exposed amino groups were acylated with a β-alanine unit. Finally, all phthaloyl protections were removed and the chain assembly was accomplished by introducing, in a single step, a β-alanine block onto each of the three amino groups. The conjugate **18** was released from the solid support by quaternization of *N*-2 of the tetrahydroisoquinoline moiety and subsequent treatment with triethyl amine.

The compatibility of commercial amino acid building blocks with **3** was checked by the synthesis of peptide conjugate **19** (Figure 3). After attachment of building block **3** to the support-bound conjugate group, the phthaloyl protection was removed and an achiral β-alanine building block was coupled to the free amino group. The Boc protections were then removed and a dipeptide side chain was assembled onto each of the exposed amino groups by using standard Boc chemistry. Conjugate **20** was prepared in order to illustrate that more than one branching unit can be inserted in a single peptide (Figure 3). Compounds such as **20** can obviously be used as a scaffold in the preparation of

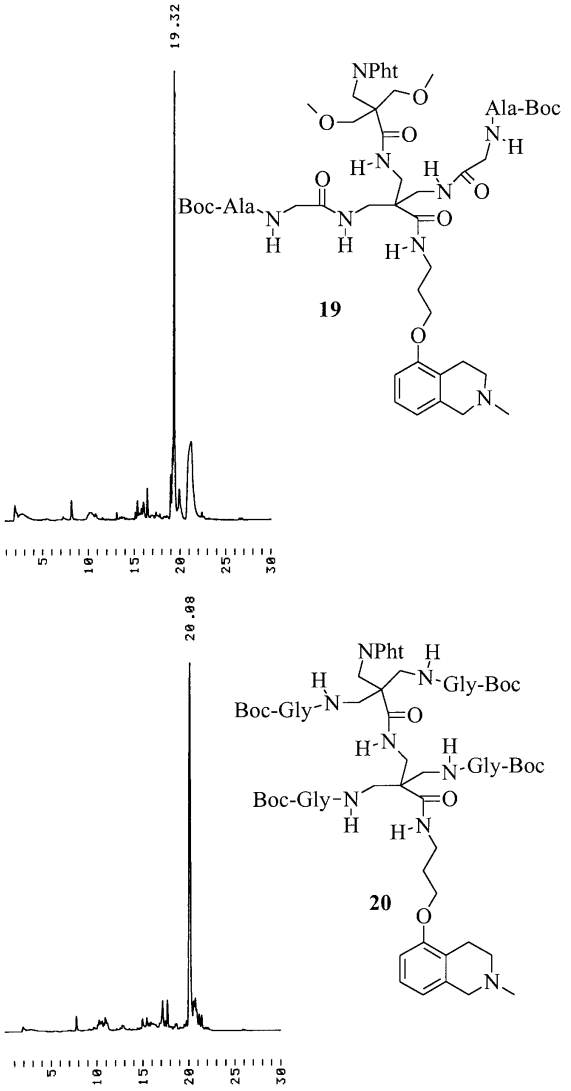

Figure 3. The model compounds **19** and **20** analysed by HPLC (Hypersil Hypurity C18, gradient from aqueous 0.1% TFA to acetonitrile in 30 min, flow 1.0 mL/min, detection at 276 nm); MS (ESI): **19** (peak at 19.32 min) found 1096.1 $[M + H]^+$, $C_{53}H_{78}N_{10}O_{15}$ + H requires 1096.3; **20** (peak at 20.08) 1522.4 $[M + H]^+$, $C_{71}H_{108}N_{16}O_{21}$ + H requires 1522.8

branched peptides by automated solid phase peptide synthesis.

Experimental Section

Spectroscopy: The NMR spectra were recorded on JEOL JNM-GX 400 or Bruker 200 NMR spectrometers. The chemical shifts are given in ppm from internal TMS. − The mass spectra of small molecular compounds were recorded on a 7070E VG mass spectrometer. − RP HPLC analyses were performed using Hypersil 150 × 4.6 mm, 5 µm HyPurity Elite C18 column, gradient from aqueous 0.1% TFA to acetonitrile in 30 min, flow rate 1.0 mL/min, detection at 276 nm. − LC/ESI-MS analyses were performed on a Perkin−Elmer Sciex API 365 LC/MS/MS triple quadrupole mass spectrometer.

Removal of *N*-Phthaloyl Protection on a Solid Support: 3 mL of 2 M hydrazine hydrate in a 1:2 mixture of DMF and dioxane (v/v) was added to a reaction vessel containing 5 to 50 mg of the solid support. The stoppered vessel was held at 55 °C for 3 h and the mixture was shaken occasionally. The solid support was filtered off, washed with dichloromethane and methanol, and dried under reduced pressure.

Peptide Coupling on a Solid Support: 20 mg of the solid support was suspended in 0.5 mL of DMF. To this suspension was added the appropriate amino acid (5 mol equiv. in 0.5 mL of DMF), *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) (5 mol equiv. in 0.5 mL of DMF), and diisopropyl ethyl amine (10 mol equiv. in 0.1 mL of DMF). The mixture was shaken for 6 h and the solid support was filtered off, washed with dichloromethane and methanol, and dried under reduced pressure.

Cleavage from the Solid Support: The solid support was shaken with 1.5 mmol of methyl iodide in DMF for 1 h, filtered off, washed with dichloromethane and methanol, and dried under reduced pressure. The solid support was suspended in a mixture of triethylamine and dichloromethane (1:1, v/v). After 1 h shaking, the solution was collected by filtration and evaporated under reduced pressure.

2,2-Bis(bromomethyl)-3-(4-methoxytrityloxy)propanol (5): 2,2-bis-(bromomethyl)-1,3-propanediol (5.27 g, 20.1 mmol) was dried by repeated evaporation with dry pyridine and then dissolved in dry pyridine (50 mL). 4-Methoxytrityl chloride (6.21 g, 20.1 mmol) was added to this solution and the reaction mixture was stirred for 20 h. Methanol (10 mL) was added and most of the pyridine was removed by evaporation. The remaining mixture was dissolved in dichloromethane and the solution was washed with aq $NaHCO_3$ (3 × 20 mL). The organic layer was dried with Na_2SO_4, evaporated to dryness, and the oily residue was purified by silica gel chromatography (dichloromethane) to yield 8.0 g (74%) of a foamy product. R_f (dichloromethane) = 0.48. − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.63 (t, J = 5.7 Hz, 1 H, OH), 3.23 (s, 2 H, CH_2OMMTr), 3.51 (s, 4 H, CH_2Br), 3.61 (d, J = 5.3 Hz, 2 H, CH_2OH), 3.78 (s, 3 H, MMTr OCH_3), 6.81 (m, 2 H, MMTr CH), 7.1−7.5 (m, 12 H, 12 × MMTr CH). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 35.1 (CH_2Br), 44.8 (C_q, pentaerythritol), 55.2 (MMTr OCH_3), 62.4, 63.5 (CH_2OH, CH_2OMMTr), 86.7 (C_q, MMTr), 113.3, 127.1, 128.0, 128.3, 130.3, 134.8, 143.8, 158.7 (MMTr C). − MS (EI^+): 534 $[M^+]$ (5), 457 $[M^+ - Br]$ (4), 273 (100). − HRMS (EI): M^+ found 472.1078. $C_{25}H_{27}O_4{}^{81}Br$ requires 472.1072.

2,2-Bis(bromomethyl)-3-(4-methoxytrityloxy)-*N*-phthaloylpropylamine (7): Diethyl azodicarboxylate (1.93 mL, 12.3 mmol) was added dropwise to a solution of **5** (5.26 g, 9.9 mmol), phthalimide (1.80 g, 12.3 mmol) and triphenylphosphane (3.22 g, 12.3 mmol) in THF. The reaction mixture was stirred for 22 h and concentrated by evaporation. The product was separated by silica gel chromatography (5:95 hexane/dichloromethane) to yield 6.0 g (92%) of a pale yellow foam. R_f (dichloromethane) = 0.79. − ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.31 (s, 2 H, CH_2OMMTr), 3.56 (d, J = 10.9 Hz, 2 H, 2 × CH*H*Br), 3.65 (d, J = 10.9 Hz, 2 H, 2 × C*H*HBr), 3.77 (s, 3 H, MMTr OCH_3), 3.93 (s, 2 H, CH_2Npht), 6.8 (m, 2 H, 2 × MMTr CH), 7.2−7.4 (m, 12 H, 12 × MMTr CH), 7.72 (dd, J = 5.7 and 2.9 Hz, 2 H, 2 × Npht CH), 7.82 (dd, J = 5.7 and 2.9 Hz, 2 H, 2 × Npht CH). − MS (EI^+): 663 (M^+, 2), 374 (4), 289 (2), 273 (100), 160 (15). − HRMS (FAB): MH^+ found 663.0430. $C_{33}H_{29}NO_4Br^{81}Br$ requires 663.0443.

2,2-Bis(bromomethyl)-3-phthalimidopropanol (6): Compound **7** (6.0 g, 9.1 mmol) was dissolved in dichloromethane (120 mL) and methanol (15 mL) and dichloroacetic acid (15 mL) were added. After 24 h stirring, the reaction mixture was washed with aqueous $NaHCO_3$

until neutral, dried with Na_2SO_4 and evaporated to dryness. The product was purified by silica gel chromatography (5:95 hexane/dichloromethane) to yield 2.9 g (85%) of a colourless oil. R_f (dichloromethane) = 0.31. − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.50 (d, J = 5.3 Hz, 2 H, CH_2OH), 3.58 (d, J = 4.2 Hz, 2 H, 2 × CH*H*Br), 3.65 (s, 2 H, CH_2Npht), 3.74 (d, J = 4.2 Hz, 2 H, 2 × C*H*HBr), 7.81 (dd, J = 5.3 and 2.6 Hz, 2 H, 2 × Npht CH), 7.90 (dd, J = 5.3 and J = 2.6 Hz, 2 H, 2 × Npht CH). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 36.1 (CH_2Br), 39.9 (CH_2Npht), 44.9 (C_q, pentaerythritol), 63.3 (CH_2OH), 123.8, 131.4, 134.7 (Npht C), 169.4 (C=O, Npht). − MS (EI$^+$): 391 [M$^+$] (1), 310 (2), 292 (1), 280 (3), 200 (21), 160 (100). − HRMS (FAB): MH$^+$ found 391.9333. $C_{13}H_{14}NO_3Br^{81}Br$ requires 391.9320.

2,2-Bis(azidomethyl)-3-phthalimidopropanol (8): Compound **6** (0.75 g, 1.9 mmol), NaN_3 (0.62 g, 9.6 mmol), and a catalytic amount of LiCl were dissolved in DMF (35 mL). The mixture was refluxed for 6 h and evaporated to dryness. The residue was dissolved in dichloromethane and washed with water. After drying with Na_2SO_4, and evaporation of the solvent, the oily residue was purified by silica gel chromatography (3:97 MeOH/dichloromethane) to yield 0.38 g (63%) of **8** as a colourless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.61 (s, 1 H, OH), 3.41 (s, 2 H, CH_2OH), 3.45 (q_{AB}, J = 14.1 Hz, 4 H, 2 × CH_2N_3), 3.72 (s, 2 H, CH_2Npht), 7.72−7.95 (m, 4 H, 4 × Npht CH). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 38.7 (CH_2Npht), 45.6 (C_q, pentaerythritol), 52.3 (CH_2N_3), 61.9 (CH_2OH), 123.8, 131.6, 134.6 (Npht C), 169.5 (C=O, Npht).

2,2-Bis(azidomethyl)-3-phthalimidopropanoic Acid (1): A solution of CrO_3 (110 mg, 1.1 mmol) and H_2SO_4 (0.11 mL) in the minimum amount of water was added to a solution of **8** (350 mg, 1.1 mmol) in acetone at 0 °C. After 1 h stirring at 0 °C, and an additional 2 h stirring at room temperature, the mixture was filtered. Methanol and water were added and the solution was concentrated by evaporation. The crude product was extracted with chloroform, dried with Na_2SO_4, and evaporated to dryness. Purification by silica gel chromatography (5:95 MeOH/dichloromethane) yielded 170 mg (47%) of the product as a white foam. ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 3.63 (q_{AB}, J = 12.6 Hz, 4 H, 2 × CH_2N_3), 3.76 (s, 2 H, CH_2Npht), 7.8 (m, 4 H, 4 × Npht CH). − ^{13}C NMR (50 MHz, [D$_6$]DMSO): δ = 50.9 (CH_2Npht), 52.3 (CH_2N_3), 123.2, 131.6, 134.6 (Npht C), 168.1 (C=O, Npht), 172.1 (COOH). − MS (FAB$^+$): 330 (MH$^+$, 100). − IR (KBr): $\tilde{\nu}$ = 3430 (br), 3030, 2939, 2114 (s), 1722 (s), 1396, 1298, 717 cm^{-1}. − $C_{13}H_{11}N_7O_4$ (329.3) calcd. C 47.4, H 3.37, N 29.8; found C 47.5, H 3.52, N 29.0.

2-Bromomethyl-2-(4-methoxytrityloxy)methyl-1,3-propanediol (9): 2-Bromomethyl-2-hydroxymethyl-1,3-propanediol[4] (4.00 g, 20.1 mmol) was co-evaporated twice with dry pyridine and then dissolved in the same solvent (50 mL). 4-Methoxytrityl chloride (6.21 g, 20.1 mmol) was added to this solution and the reaction mixture was stirred for 20 h. Methanol (10 mL) was added and most of the pyridine was removed by evaporation. The remaining mixture was dissolved in dichloromethane and the solution was washed with aq. $NaHCO_3$ (3 × 20 mL). The organic phase was dried with Na_2SO_4, evaporated to dryness, and the oily residue was purified by silica gel chromatography (1:99 MeOH/dichloromethane) to yield 5.27 g (58%) of white foam. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.05 (br. s, 2 H, OH), 3.21 (s, 2 H, CH_2OMMTr), 3.61 (s, 2 H, CH_2Br), 3.67 (s, 4 H, 2 × CH_2OH), 3.79 (s, 3 H, MMTr OCH_3), 6.82 (m, 2 H, 2 × MMTr CH), 7.1−7.5 (m, 12 H, 12 × MMTr CH). − MS (EI$^+$): 472 [M$^+$] (3), 470 [M$^+$] (3), 395 (3), 393 (3), 273 (100). − IR (KBr): $\tilde{\nu}$ = 3410 (br), 3057, 2930, 1607, 1512, 1251, 1070 cm^{-1}. − HRMS (EI): M$^+$ found 470.1093. $C_{25}H_{27}O_4Br$ requires 470.1093.

2-Bromomethyl-2-(4-methoxytrityloxy)methyl-*N*,*N*′-diphthaloylpropane-1,3-diamine (10): Diethyl azodicarboxylate (4.6 mL, 29 mmol) was added dropwise to a solution of **9** (5.2 g, 11.4 mmol), phthalimide (4.2 g, 29 mmol) and triphenylphosphane (7.6 g, 29 mmol) in THF. The reaction mixture was stirred for 22 h and concentrated by evaporation. The product was separated by silica gel chromatography (1:99 MeOH/dichloromethane) to yield 5.7 g (68%) of white foam. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.42 (s, 2 H, CH_2OH), 3.75 (s, 3 H, MMTr OCH_3), 3.77 (s, 2 H, CH_2Br), 3.80 (d, J = 14.5 Hz, 2 H, 2 × CH*H*Npht), 3.95 (d, J = 14.5 Hz, 2 H, 2 × C*H*HNpht), 6.75 (m, 2 H, 2 × MMTr CH), 7.1−7.5 (m, 12 H, 12 × MMTr CH), 7.6−7.9 (m, 4 H, 4 × Npht CH). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 37.4 (CH_2Br), 42.5 (CH_2Npht), 45.5 (C_q, pentaerythritol), 55.1 (MMTr OCH_3), 65.7 (CH_2OMMTr), 87.1 (C_q, MMTr), 112.9, 123.4, 126.8, 127.6, 128.7, 130.7, 132.0, 134.0, 134.9, 143.8, 158.5 (MMTr C and Npht C), 168.7 (C=O, Npht). − IR (KBr): $\tilde{\nu}$ = 1774, 1716 (s), 1633, 1394, 1251, 718 cm^{-1}. − HRMS (FAB): MH$^+$ found 731.1602. $C_{41}H_{34}N_2O_6{}^{81}Br$ requires 731.1580.

3-Bromo-2,2-bis(phthalimidomethyl)propanol (11): Compound **10** (5.5 g, 7.5 mmol) was dissolved in dichloromethane (100 mL). Ethanol (20 mL) and trifluoroacetic acid (10 mL) were then added. After the reaction was complete according to TLC (1:99 MeOH/dichloromethane), the solution was evaporated to dryness. The product was separated by silica gel chromatography (gradient of MeOH in dichloromethane) to yield 3.5 g (100%) of a colourless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.52 (s, 2 H, CH_2Br), 3.60 (d, J = 7.7 Hz, 2 H, CH_2OH), 3.87 (t, J = 8.2 Hz, OH), 3.95 (q_{AB}, J = 14.6 Hz, 4 H, 2 × CH_2Npht), 7.7−7.9 (m, 8 H, 8 × Npht CH). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 36.1 (CH_2Br), 40.8 (CH_2Npht), 45.6 (C_q, pentaerythritol), 123.7, 131.7, 134.4 (Npht C), 169.3 (C=O, Npht). − MS (EI$^+$): 458 [M$^+$] (5), 456 [M$^+$] (5), 359 (8), 347 (45), 200 (21), 160 (100).

3-Azido-2,2-bis(phthalimidomethyl)propanol (12): Compound **11** (3.5 g, 7.5 mmol), NaN_3 (3.5 g, 54 mmol) and a catalytic amount of LiCl were dissolved in DMF. The mixture was refluxed for 12 h and evaporated to dryness. The product was separated by silica gel chromatography (ethyl acetate) to yield 1.0 g (32%) of white foam. − ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.23 (s, 1 H, OH), 3.45* (s, 2 H, CH_2OH), 3.47* (s, 2 H, CH_2N_3), 3.83 (q_{AB}, J = 14.6 Hz, 4 H, 2 × CH_2Npht), 7.7−7.9 (m, 8 H, 8 × Npht CH); assignments denoted by * may be interchanged. − ^{13}C NMR (100 MHz, $CDCl_3$): 40.0 (CH_2Npht), 46.3 (C_q, pentaerythritol), 53.6 (CH_2N_3), 62.4 (CH_2OH), 123.7, 131.7, 134.4 (Npht C), 169.3 (C=O, Npht). − MS (EI$^+$): 419 [M$^+$] (1), 346 (38), 328 (24), 199 (40), 186 (19), 160 (100). − IR (KBr): $\tilde{\nu}$ = 3356 (br), 2930, 2100 (s), 1730, 1670 (s), 1412 cm^{-1}.

3-Azido-2,2-bis(phthalimidomethyl)propanoic Acid (2): A solution of CrO_3 (56 mg, 0.56 mmol) and H_2SO_4 (0.06 mL, 1.1 mmol) in the minimum amount of water was added to a solution of **12** (235 mg, 0.56 mmol) in acetone. After 2 h stirring at room temperature, another portion of oxidizing solution (1/2 of the initial volume used) was added and the reaction mixture was stirred for an additional 2 h. The mixture was filtered, methanol and water were added, and the solution was concentrated by evaporation. The crude product was extracted into chloroform and the solution was dried with Na_2SO_4 and evaporated to dryness. The yield of the crude product was 190 mg (79%). Purification by silica gel chromatography (1:9 MeOH/dichloromethane) gave 39% recovery as a white foam. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.65 (s, 2 H, CH_2N_3), 4.03 (d, J = 14.4 Hz, 2 H, 2 × CH*H*Npht), 4.25 (d, J = 14.4 Hz, 2 × C*H*HNpht), 7.70−7.95 (m, 8 H, 8 × Npht CH). − ^{13}C NMR

(50 MHz, [D$_6$]DMSO): δ = 40.6 (C_q, pentaerythritol), 51.4 (CH_2Npht), 54.3 (CH_2N_3), 123.0, 131.7, 134.3 (Npht C), 168.3 (C=O, Npht), 171.9 (COOH). − MS (EI$^+$): 433 (M^+, 1), 299 (41), 227 (100), 160 (24). − IR (KBr): ṽ = 3575 (br), 3057, 2932, 2110 (s), 1776, 1720, 1593, 1390, 1265, 1045, 736 cm^{-1}. − HRMS (FAB): MH^+ found 434.1086. $C_{21}H_{16}N_5O_6$ requires 434.1101.

2,2-Bis(azidomethyl)-3-(4-methoxytrityloxy)propanol (13): Compound **5** (8.0 g, 15 mmol), NaN_3 (5.0 g, 77 mmol), and a catalytic amount of LiCl were dissolved in DMF. The reaction mixture was refluxed for 12 h and most of DMF was removed by evaporation under vacuum. The residue was dissolved in dichloromethane and washed with aq $NaHCO_3$. The organic fraction was dried with Na_2SO_4, evaporated to dryness, and the residue was purified by silica gel chromatography (dichloromethane) to yield 3.4 g (49%) of an off-white foamy product. − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.78 (t, *J* = 5.9 Hz, 1 H, OH), 3.10 (s, 2 H, CH_2OMMTr), 3.44 (s, 4 H, CH_2N_3), 3.55 (d, *J* = 6.0 Hz, 2 H, CH_2OH), 3.82 (s, 3 H, MMTr OCH_3), 6.87 (m, 2 H, 2 × MMTr CH), 7.25−7.5 (m, 12 H, 12 × MMTr CH). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 44.9 (C_q, pentaerythritol), 51.8 (CH_2N_3), 55.2 (MMTr OCH_3), 62.4* (CH_2OMMTr), 63.5* (CH_2OH), 86.8 (C_q, MMTr), 113.2, 127.1, 128.0, 128.2, 130.3, 134.8, 143.9, 158.7 (MMTr C); assignments denoted by * may be interchanged. − IR (KBr): ṽ = 3470 (br), 2933, 2104 (s), 1608, 1510 cm^{-1}. − MS (EI$^+$): 458 [M^+] (8), 381 (3), 273 (100). − HRMS (EI): M^+ found 458.2072. $C_{25}H_{26}N_6O_3$ requires 458.2066.

2,2-Bis(aminomethyl)-3-(4-methoxytrityloxy)propanol (14): Compound **13** (9.3 g, 20.3 mmol), 1,3-propanedithiol (0.2 mL, 2 mmol), and triethylamine (6 mL, 40 mmol) were dissolved in 2-propanol (200 mL). $NaBH_4$ (3.8 g, 20 mmol) was added under vigorous stirring in several portions and the mixture was stirred overnight at room temperature. The solvent was evaporated and the residue was dissolved in dichloromethane. The solution was washed with 10% NaOH in saturated aqueous NaCl, saturated aqueous NaCl, and then dried with $NaSO_4$. The solvent was evaporated and the resulting oil was separated on a silica gel column (85:5:10 dichloromethane/TEA/MeOH). Evaporation of the appropriate fractions yielded 4.2 g (51%) of the product as an almost colourless oil. − ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 2.54 (s, 4 H, 2 × NH_2), 2.86 (s, 2 H, CH_2OMMTr), 3.40 (s, 2 H, CH_2OH), 3.73 (s, 3 H, MMTr OCH_3), 6.87 (m, 2 H, 2 × MMTr CH), 7.15−7.41 (m, 12 H, 12 × MMTr CH). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 42.8 (CH_2NH_2), 43.8 (C_q, pentaerythrithol), 55.0 (MMTr OCH_3), 62.6* (CH_2OH), 63.1* (CH_2OMMTr), 85.0 (C_q, MMTr), 126.7, 127.8, 127.9, 130.0, 135.2, 144.5, 158.0 (MMTr C); assignments denoted by * may be interchanged. − IR (KBr): ṽ = 3369, 3306, 3057, 2932, 1607, 1510, 1447, 1252, 1034, 634, 710 cm^{-1}. − HRMS (FAB): MH^+ found 407.2325. $C_{25}H_{31}N_2O_3$ requires 407.2335.

2,2-Bis(*tert*-butoxycarbonylaminomethyl)-3-(4-methoxytrityloxy)-propanol (15): Compound **14** (4.18 g, 10.3 mmol) was dissolved in aqueous MeCN. NaOH (aq. 2 M, 11 mL, 22 mmol) and Boc_2O (6.6 g, 30 mmol) were added and the reaction mixture was stirred overnight. Dichloromethane was added, and the organic fraction was washed with water and brine, dried with Na_2SO_4, and evaporated to dryness. The resulting oil was purified by silica gel chromatography (3:97 MeOH/dichloromethane) to yield 5.34 g (86%) of the product as a white foam. − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.41 [s, 18 H, 2 × $C(CH_3)_3$], 2.65 (dd, *J* = 14.3 and 5.1 Hz, 2 H, 2 × CH*H*NHBoc), 2.99 (s, 2 H, CH_2OMMTr), 3.13 (dd, *J* = 14.3 and 8.4 Hz, 2 H, 2 × C*H*HNHBoc), 3.38 (d, *J* = 7.6 Hz, CH_2OH), 3.79 (s, 3 H, MMTr OCH_3), 4.22 (t, *J* = 7.6 Hz, 1 H, OH), 4.89 (m, 2 H, 2 × NH), 6.8−6.9 (m, 2 H, 2 × MMTr CH), 7.2−7.5 (m, 12 H, 12 × MMTr CH). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 28.3 (Boc CH_3), 39.6 (CH_2NHBoc), 46.0 (C_q, pentaerythritol), 55.2 (MMTr OCH_3), 61.4* (CH_2OMMTr), 61.7* (CH_2OH), 79.5 (C_q, Boc), 86.2 (C_q, MMTr), 113.2, 127.0, 128.0, 128.3, 130.2, 135.3, 144.2 (MMTr C), 157.5 (C=O, Boc), 158.6 (MMTr C); assignments denoted by * may be interchanged. − IR (KBr): ṽ = 3423 (br), 1698, 1510, 1252, 1173, 1036 cm^{-1}. − HRMS (EI): M^+ found 606.3303. $C_{35}H_{46}N_2O_7$ requires 606.3305.

***N,N′*-Bis(*tert*-butoxycarbonyl)-2-(4-methoxytrityloxy)methyl-2-phthalimidomethylpropane-1,3-diamine (16):** Diethyl azodicarboxylate (2.1 mL, 13.3 mmol) was added dropwise to a solution of **15** (5.34 g, 9.2 mmol), phthalimide (1.95 g, 13.3 mmol) and triphenylphosphane (3.7 g, 13.3 mmol) in THF. The reaction mixture was stirred overnight and then evaporated to dryness. Purification by silica gel chromatography (3:7 ethyl acetate/petroleum ether) yielded 4.36 g (66%) of the product as a yellow foam. − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.39 [s, 18 H, 2 × $C(CH_3)_3$], 3.05 (dd, *J* = 14.5 and 6.5 Hz, 2 H, 2 × CH*H*NHBoc), 3.20 (s, 2 H, CH_2OMMTr), 3.25 (dd, *J* = 14.6 and 6.5 Hz, 2 × C*H*HNHBoc), 3.66 (s, 2 H, CH_2Npht), 3.75 (s, 3 H, MMTr OCH_3), 5.53 (t, *J* = 6.5 Hz, 2 H, NH), 6.76 (m, 2 H, 2 × MMTr CH), 7.1−7.4 (m, 12 H, 12 × MMTr CH), 7.65−7.85 (m, 4 H, 4 × Npht CH). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 28.2 (Boc CH_3), 40.2 (CH_2Npht), 41.7 (CH_2Npht), 45.7 (C_q, pentaerythritol), 55.0 (MMTr OCH_3), 64.7 (CH_2OMMTr), 78.8 (C_q, Boc), 86.6 (C_q, MMTr), 112.9, 123.2, 126.7, 127.6, 128.3, 130.2, 131.8, 132.8, 133.9, 134.0, 134.8, 143.7 (MMTr C, Npht C), 156.4 (C=O, Boc), 158.3 (MMTr C), 168.9 (C=O, Npht).

2,2-Bis(*tert*-butoxycarbonylaminomethyl)-3-phthalimidopropanol (17): Compound **16** (3.50 g, 4.76 mmol) was dissolved in dichloromethane. A solution of iodine (1%, m/v; 50 mL) was added and the reaction mixture was stirred for 2 h at room temperature. The reaction solution was washed with aqueous Na_2SO_3 and brine, dried with Na_2SO_4, and evaporated to dryness. Purification by silica gel chromatography (gradient of MeOH in dichloromethane) yielded 1.9 g (87%) of the product as a yellow foam. − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.46 [s, 18 H, 2 × $C(CH_3)_3$], 2.98 (dd, *J* = 14.7 and 6.4 Hz, 2 H, 2 × CH*H*NHBoc), 3.11 (dd, *J* = 14.6 and 7.0 Hz, 2 H, 2 × C*H*HNHBoc), 3.32 (d, *J* = 7.2 Hz, 2 H, CH_2OH), 3.63 (s, 2 H, CH_2Npht), 4.48 (t, *J* = 7.2 Hz, 1 H, OH), 6.25 (t, *J* = 6.4 Hz, 2 H, NH), 7.7−7.9 (m, 4 H, 4 × Npht CH). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 27.6 (Boc CH_3), 38.4* (CH_2Npht), 40.0* (CH_2NHBoc), 45.4 (C_q, pentaerythritol), 78.5 (C_q, Boc), 122.2, 130.9, 133.1 (Npht C), 156.4 (C=O, Boc), 168.7 (C=O, Npht); assignments denoted by * may be interchanged. − IR (KBr): ṽ = 3416, 3208, 3063, 2980, 1744 (s), 1516, 1388, 1308, 1053, 717 cm^{-1}. − HRMS (EI): M^+ found 463.2311. $C_{23}H_{33}N_3O_7$ requires 463.2319.

2,2-Bis(*tert*-butoxycarbonylaminomethyl)-3-phthalimidopropanoic Acid (3): A solution of CrO_3 (192 mg, 1.92 mmol) and H_2SO_4 (0.19 mL) in the minimum amount of water was added to a solution of **17** (890 mg, 1.92 mmol) in acetone at 0 °C. After 1 h stirring at 0 °C, and an additional 2 h stirring at room temperature, the mixture was filtered, methanol and water were added, and the solution was concentrated by evaporation. The crude product was extracted with chloroform and the solution was dried with Na_2SO_4 and evaporated to dryness. Purification by silica gel chromatography (6:94 MeOH/dichloromethane) yielded 0.6 g (65%) of the product as a pale yellow solid foam. − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.42 [s, 18 H, 2 × $C(CH_3)_3$], 3.38 (br d, *J* = 33.7 Hz, 4 H, 2 × CH_2NHBoc), 3.93 (s, 2 H, CH_2Npht), 5.95 (br s, 3 H, 2 × NH and OH), 7.6−7.8 (m, 4 H, 4 × Npht CH). − ^{13}C NMR

(50 MHz, $CDCl_3$): δ = 28.4 (Boc CH_3), 39.6 (CH_2NHBoc), 41.4 (C_q, pentaerythritol), 52.4 (CH_2Npht), 79.7 (C_q, Boc), 123.5, 131.8, 134.2 (Npht C), 156.8 (C=O, Boc), 168.9 (C=O, Npht), 184.9 (COOH). − IR (KBr): $\tilde{\nu}$ = 3416, 3055, 2984, 1776, 1714 (s), 1514, 1394, 1265, 1167, 738 cm^{-1}. − $C_{23}H_{31}N_3O_8$ (477.5) Calcd. C 57.9, H 6.54, N 8.80; found C 57.5, H 6.31, N 8.81.

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