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Synthesis of novel amino acid carbohydrate hybrids via Mitsunobu glycosylation of nitrobenzenesulfonamides

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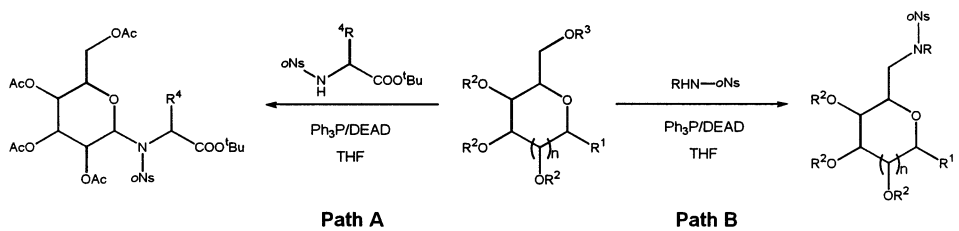
Abstract—Amino acid derived 2-nitrobenzenesulfonamides were successfully condensed under Mitsunobu conditions with the primary alcohol of various saccharide and nucleoside derivatives to furnish the fully protected hybrids in excellent yield. One such hybrid was incorporated into a short peptide sequence in good overall yield.

The most abundant post-translational modification of polypeptides is glycosylation, where carbohydrates are covalently attached to specific amino acid residues.[1] Such modifications impart structural diversity which can regulate activity and facilitate involvement in biological recognition events such as cell adhesion and infection.[2] Consequently, many glycosylated oligopeptides have been synthesised in the last couple of decades[2,3] with derivatives that deviate from the naturally occurring *N*- or *O*-glycosidic linkage becoming increasingly widespread as a result of efforts directed towards enhancing enzymatic stability.[4] Furthermore, the properties and/or conformation of biologically active peptide sequences have been modified by the synthesis and incorporation of glycosylated amino acids with unnatural linkages.[5,6] For instance, it was recently reported that the attachment of a carbohydrate core onto a cyclic RGD containing pentapeptide resulted in compounds with improved potency, selectivity and better pharmacokinetic properties.[6] The cyclic RGD glycopeptide derivatives synthesised either possessed a carbohydrate core attached to the side-chain of an amino acid or one affixed to the peptide backbone via an α-amino functionality. This latter modification is particularly interesting because (α)*N*-alkylated peptides have been shown to be more resistant to enzymatic degradation[7] however to date, only (α)*N*-glycosylated glycine residues have been successfully incorporated into peptide sequences.[5b,6] Moreover, (α)*N*-alkylated amino acids in general are often problematic with respect to their full incorporation (i.e. amino acid residues connected at both termini to backbone), frequently giving poor yields when extending from the N-terminus.[8]

Two years ago, we disclosed a novel route towards the synthesis of Amadori compounds by the Mitsunobu glycosylation of amino acid derived *ortho*-nitrobenzenesulfonamides[9] (path A, Scheme 1). It occurred to us that successful transference of this methodology from the anomeric to the primary alcohol of carbohydrate derivatives would provide glycosylated amino acids with enzymatically stable artificial linkages



Scheme 1.

Keywords: Mitsunobu glycosylation; nitrobenzenesulfonamides; amino acids; carbohydrates; nucleosides.
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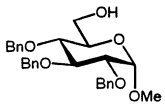
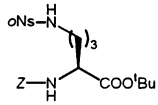
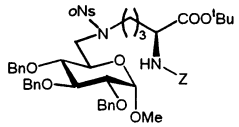
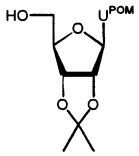
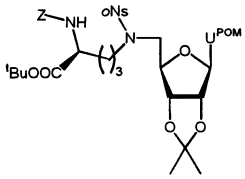
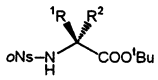
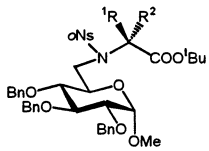
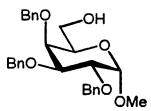
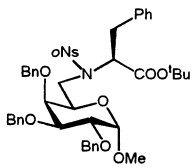
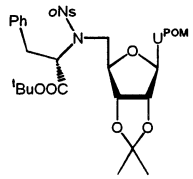
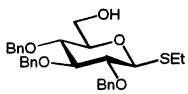
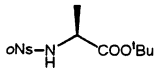
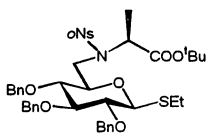
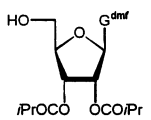
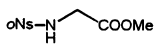
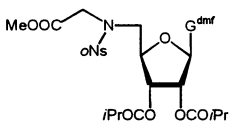
(path B). Due to the fact that the side-chain glycosylation of an amino acid does not significantly affect its incorporation into peptide sequences,[2] modifications of this type were attempted first. To this end, 2,3,4-tri-*O*-benzyl-α-D-methyl-glucopyranose **1**[10] was condensed with N^{α}-benzyloxycarbonyl-N^{δ}-(2-nitrobenzenesulfonyl)-L-ornithine *tert*-butyl ester **2**[11] furnishing the desired side-chain glycosylated amino acid **3** in excellent yield (entry 1, Table 1).[12] A more complex example, that of ornithine/uridine conjugate **5** was also obtained in a similar yield by reacting uridine derivative **4** with **2** (entry 2). In this case, the pivaloyloxymethyl (POM) protecting group on N^3 proved to be essential for the prevention of known intramolecular cyclisation.[13] Hav-

Table 1. Mitsunobu condensation of carbohydrates and nucleosides with amino acids

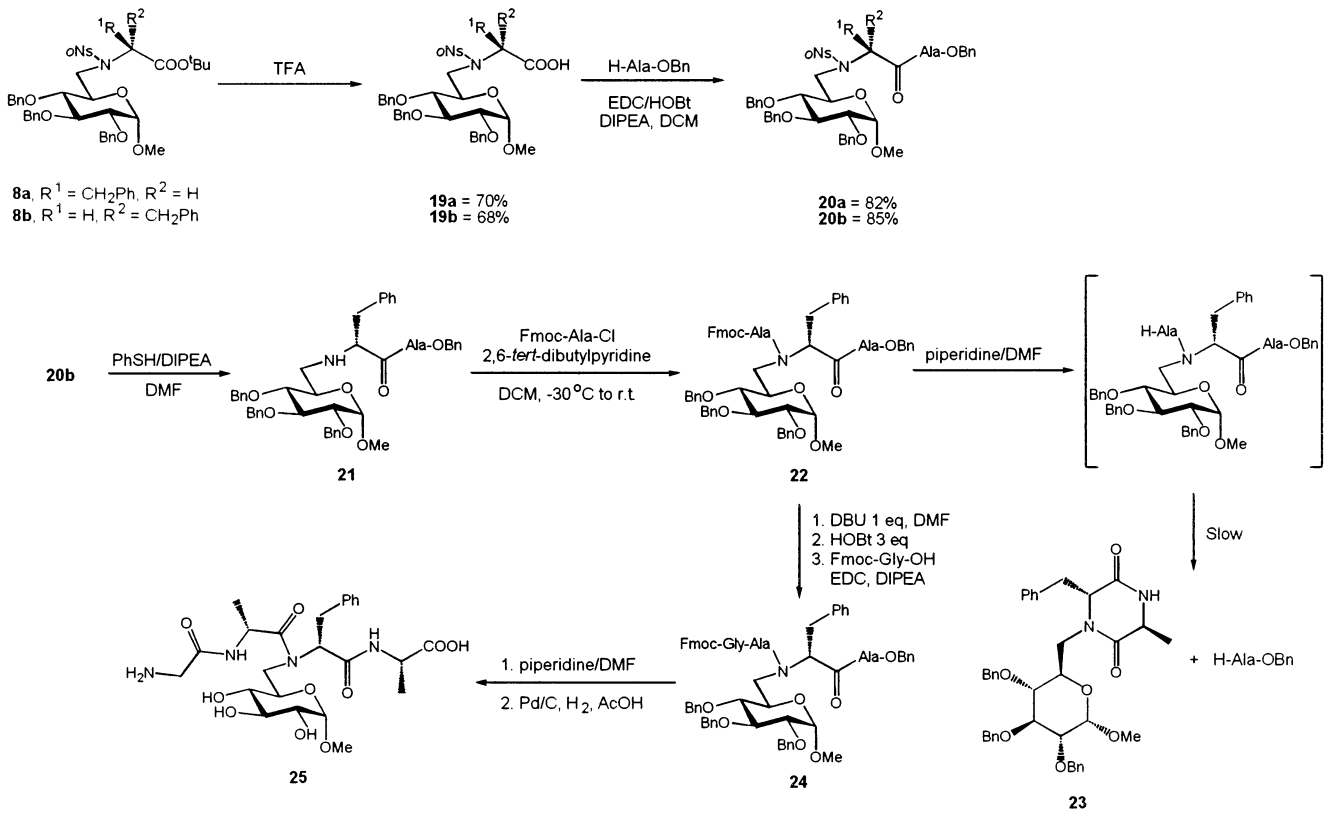
Entry[a]	Primary alcohol	R-NH*o*Ns	Product	Yield (%)
1	 **1**	 **2**	 **3**	95
2	 **4**	**2**	 **5**	96
3	**1**			**8a** = 93 **8b** = 92 **9** = 99
		6a, $R^1 = CH_2Ph$, $R^2 = H$ **6b**, $R^1 = H$, $R^2 = CH_2Ph$ **7**, $R^1 = CH_2CH_2COO^tBu$, $R^2 = H$	**8a**, $R^1 = CH_2Ph$, $R^2 = H$ **8b**, $R^1 = H$, $R^2 = CH_2Ph$ **9**, $R^1 = CH_2CH_2COO^tBu$, $R^2 = H$	
4[b]	 **10**	**6a**	 **11**	95
5	**4**	**6a**	 **12**	93
6	 **13**	 **14**	 **15**	92
7[c]	 **16**	 **17**	 **18**	94

a) 1.5 eq PPh_3, 1.5 eq DEAD, 1.3 eq alcohol w.r.t. R-NH*o*Ns in THF, -50 °C-r.t. overnight; b) 2 h, reflux; c) 0 °C-r.t. overnight.

ing substrates with side-chain modifications in hand, attention was turned to the synthesis of (α)*N*-alkylated amino acids. Thus, **1** was reacted with *ortho*-nitrobenzenesulfonamide derived phenylalanine *tert*-butyl esters **6a** and **6b** and the corresponding L-glutamic di-*tert*-butyl ester **7** providing **8a**, **8b** and **9**, respectively, in excellent yields (entry 3). The more sterically hindered 2,3,4-tri-*O*-benzyl-α-D-methyl-galactopyranose **10** was more sluggish in its reaction with **6a**. However, phenyalanine/galactose conjugate **11** was obtained in near quantitative yield when the mixture was heated under reflux (entry 4). Uridine derivative **4** again provided no problems, smoothly reacting with **6a** to furnish the desired product **12** (entry 5). To examine further the scope of this methodology, ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranose **13** was successfully condensed with alanine derivative **14** to give the thioethyl donor **15** in 92% yield (entry 6). The thioethyl functionality of **15** should facilitate a variety of transformations at the anomeric centre. Finally, the ability to form (α)*N*-guanosylated amino acids is also demonstrated (entry 7). Synthetic transformations on guanosine are notoriously difficult, however the use of the *N*,*N*-dimethylformamidine (dmf) protecting group for N^2 of the nucleobase has previously been shown to prevent intramolecular condensation under Mitsunobu conditions.[14] Investigations revealed that the secondary hydroxyl protecting groups employed also play a vital role in the success of the reaction. For instance, the use of conformationally constrained isopropylidene diol protection only yielded intramolecular condensation,[15] whereas isobutyryl ester masking of the secondary hydroxyls allowed intermolecular condensation, furnishing glycine/guanosine conjugate **18** in excellent yield.

As previously mentioned, the side-chain attachment of carbohydrate moieties is unlikely to affect the full incorporation of an amino acid into a peptide sequence but (α)*N*-alkylation often does. Therefore, it was considered necessary to investigate the assimilation of an example of the latter. To keep the synthetic route as concise as possible, the feasibility of performing coupling reactions using the *ortho*-nitrobenzenesulfonyl group as a replacement for a urethane protecting group was investigated.

To this end, both D and L phenylalanine/glucose conjugates **8a** and **8b** were treated with neat TFA under dry conditions followed by coupling of L-alanine benzyl ester under the agency of EDC/HOBt/DIPEA to give the coupled products **20a** and **20b** in good yield as depicted in Scheme 2. Comparison of both products using HPLC revealed that the coupling did not lead to racemisation.[16] In order to fully incorporate the D-phenylalanine/glucose conjugate into a peptide sequence, dipeptide **20b** was subjected to *ortho*-nitrobenzenesulfonyl cleavage conditions[9] followed by coupling of Fmoc protected L-alanine acid chloride in the presence of 2,6-*tert*-dibutylpyridine[17] to give fully protected tripeptide **22** in a gratifying 92% yield over the 2 steps. It should be noted that attempted coupling of Fmoc-Ala-OH employing established peptide coupling reagents was wholly unsuccessful with no product formation observed.[18]



Scheme 2.

Having established that the conjugate could be coupled at both the amino and the acid functionalities we wished to confirm that standard coupling protocols could be used again. Therefore, fully protected tripeptide **22** was subjected to Fmoc cleavage followed by coupling of Fmoc protected glycine in a one-pot procedure furnishing the fully protected tetrapeptide **24** in 83% yield. The necessity for the one-pot procedure arises from the observation that the product resulting from cleavage of the Fmoc group, slowly forms diketopiperazine **23** upon standing. Finally, removal of the protecting groups of **24** gave the requisite free tetrapeptide **25** in 75% isolated yield.

In summary, we have presented an efficient and facile method for the synthesis of novel amino acid/carbohydrate and amino acid/nucleoside conjugates and fully incorporated an (α)*N*-glycated amino acid into a short peptide sequence in good overall yield. In addition, it has been shown that the *ortho*-nitrobenzenesulfonyl group can be considered as a potential replacement for the urethane group in peptide synthesis. Finally, the coupling of hindered secondary amines was also shown to proceed in excellent yield utilising an Fmoc amino acid chloride in conjunction with a weak non-nucleophilic base.

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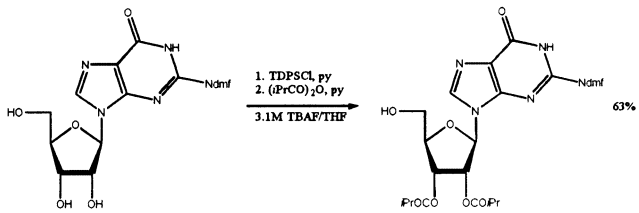
10. Compound **1** was synthesised according to Sahai, P.; Chawla, M.; Vishwakarma, R. A. *J. Chem. Soc. Perkin Trans. 1* **2000**, 1283–1290. Compound **10** was synthesised in the same manner. Compound **13** was synthesised according to Ray, A. K.; Roy, N. *Carbohydr. Res* **1990**, *196*, 95–100. Compound **4** was synthesised from $O^{2'},O^{3'}$-isopropylidene-uridine[10b] utilising standard POM functionalisation conditions according to Brown, J. M.; Cristodoulou, C.; Jones, S. S.; Modak, A. S.; Reese, C. B.; Sibanda, S.; Ubusawa, A. *J. Chem. Soc. Perkin Trans. 1* **1989**, 1735–1753. Guanosine derivative **16** was prepared in 3 steps from dmf protected guanosine[10c] according to Scheme 3; (b) Yamane, A.; Inoue, H.; Ueda, T. *Chem. Pharm. Bull* **1980**, *28*, 157–162; (c) Grøtli, M.; Douglas, M.; Beijer, B.; García, R. G.; Eritja, R; Sproat, B. *J. Chem. Soc. Perkin Trans. 1* **1997**, 2779–2788.



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Scheme 3.

11. (a) Amino acid derived *ortho*-nitrobenzenesulfonamides **6**, **7** and **14** were synthesised as reported previously.[9] Compound **17** was obtained by reacting glycine methyl ester hydrochloride with *ortho*-nitrobenzenesulfonyl chloride under Schotten–Baumann conditions. Compound **2** was synthesised by *tert*-butyl protection[11b] of the corresponding free carboxylic acid.[11c] (b) Callahan, F. M.; Anderson, G. W.; Paul, R.; Zimmerman, J. E. *J. Am. Chem. Soc.* **1963**, *85*, 201–207; (c) Buijsman, R.; PhD Thesis, Leiden **1998**.
12. All new compounds were fully characterised by ^1H and ^{13}C NMR spectroscopy as well as mass spectrometry. Data for selected examples is as follows. **5**: ^1H NMR (300 MHz, $CDCl_3$): δ 8.00 (m, 1H, *o*Ns), 7.67–7.43 (m, 3H, *o*Ns), 7.40–7.29 (m, $5H_{arom.}$), 7.18 (d, 1H, H_5 $J_{5,6}=8.1$ Hz), 5.90 (AB, 2H, CH_2 Z, $J=9.5$ Hz), 5.74 (d, 1H, H_6), 5.52 (app. s, 1H, $H_{1'}$), 5.35 (d, 1H, NH, $J_{NH,\alpha}=7.9$ Hz), 5.09 (s, 2H, POM), 5.00 (app. d, 1H, $H_{2'}$), 4.76–4.73 (m, 1H, $H_{3'}$), 4.33–4.30 (m, 1H, $H_{4'}$), 4.28–4.08 (m, 1H, $H_{\alpha'}$), 3.59 (m, 2H, $H_{5'}$), 3.44–3.25 (m, 2H, H_δ), 1.86–1.55 (m, 4H, $2\times H_\beta$ and $2\times H_\gamma$), 1.52 and 1.32 (2s, $2\times 3H$, CH_3 isopropyl), 1.43 and 1.18 (2s, $2\times 9H$, CH_3 ^{t}Bu); ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.4 (C=O POM), 171.0 (C=O ester Orn), 161.4 (C_4), 155.8 (NH-C=O, Z), 150.2 (C_2), 147.2 (C-NO_2), 141.7 (C_5), 136.2 ($C_{q\ arom.}$, Z), 133.5 ($CH_{arom.}$

*o*Ns), 133.2 (C-SO_2), 131.5, 131.1 (2×$CH_{arom.}$ *o*Ns), 128.5–128.1 ($CH_{arom.}$ Z), 124.2 ($CH_{arom.}$ *o*Ns), 114.5 (C_q isopropyl), 102.2 (C_6), 96.2 ($C_{1'}$), 85.8 ($C_{4'}$), 84.2 ($C_{2'}$), 82.4 ($C_{3'}$), 81.6 (C_q ^{t}Bu ester Orn), 66.9 (CH_2 POM), 64.6 (CH_2 Z), 53.7 (C_α), 49.6 ($C_{5'}$), 47.9 (C_δ), 38.8 (C_q ^{t}Bu POM), 29.7 (C_β), 27.9, 26.9 (2×CH_3 ^{t}Bu), 26.9, 25.1 (2×CH_3 isopropyl), 23.7 (C_γ); MS (ESI): *m*/*z* 910.3 [M+Na]$^+$. **11**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.83 (m, 1H, *o*Ns), 7.61–7.53 (m, 2H, *o*Ns), 7.47–7.16 (m, 21H: 15$H_{arom.}$ Bn, 5$H_{arom.}$ Phe, 1H *o*Ns), 4.87 (AB, 2H, CH_2 Bn), 4.81 (AB, 2H, CH_2 Bn), 4.74 (AB, 2H, CH_2 Bn), 4.66 (m, 1H, H_α Phe), 4.49 (d, 1H, H_1, $J_{1,2}$=3.4 Hz), 4.02–3.96 (m, 3H, H_2, H_4, H_5), 3.89 (dd, 1H, H_3, $J_{2,3}$= 10.1 Hz, $J_{3,4}$=2.1 Hz), 3.66 (ABX, 2H, H_6), 3.25 (s, 3H, OMe), 3.15 (ABX, 2H, H_β Phe), 1.23 (s, 9H, ^{t}Bu); ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.9 (C=O), 148.1 (C-NO_2), 138.6, 138.6, 138.4 (3×C_q Bn), 136.7 ($C_{q\ arom.}$, Phe), 133.5 (C-SO_2), 133.3, 131.5, 131.4 (3×$CH_{arom.}$ *o*Ns), 129.1–126.8 ($CH_{arom.}$ Bn), 123.8 (1×$CH_{arom.}$ *o*Ns), 98.9 (C_1), 82.2 (C_q ^{t}Bu), 79.2 (C_3), 75.9, 75.3 (C_2/C_4), 74.4, 73.5, 73.2 (3×CH_2 Bn), 69.7 (C_5), 62.9 (C_α Phe), 55.7 (OMe), 47.9 (C_6), 36.9 (C_β Phe), 27.6 (CH_3 ^{t}Bu); MS (ESI): *m*/*z* 875.5 [M+Na]$^+$. **18**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.76 (bs, 1H, H_1), 8.90 (s, 1H, N=C-H dmf), 7.92 (m, 1H, 1×*o*Ns), 7.78–7.51 (m, 4H, 3×*o*Ns, H_8), 6.16 (dd, 1H, $H_{3'}$, $J_{2',3'}$=5.9Hz, $J_{3',4'}$=9.2Hz), 6.03 (dd, 1H, $H_{2'}$, $J_{1',2'}$=1.3 Hz), 5.73 (d, 1H, $H_{1'}$), 4.36 (dt, 1H, $H_{4'}$, $J_{4',5'}$=3.3 Hz), 4.15 (AB, 2H, CH_2 Gly), 3.87 (d, 2H, $H_{5'}$), 3.48 (s, 3H, OMe), 3.22 and 3.12 (2s, 2×3H, 2×CH_3 dmf), 2.68–2.57 (m, 2H, 2×CH isobutyryl), 1.28–1.06 (m, 12H, 4×CH_3 isobutyryl). ^{13}C NMR (75 MHz, $CDCl_3$): δ 176.0, 175.4 (2×C=O isobutyryl ester), 168.8 (C=O methyl ester), 159.2 (N=CH dmf), 158.0 (C_6), 157.2 (C_2), 149.5 (C_4), 147.8 (C-NO_2), 138.3 (C_8), 133.7 ($CH_{arom.}$ *o*Ns), 132.5 (C-SO_2), 131.5, 130.5, 123.9 (3×$CH_{arom.}$ *o*Ns), 120.9 (C_5), 88.7 ($C_{1'}$), 79.6 ($C_{4'}$), 73.0 ($C_{2'}$), 69.4 ($C_{3'}$), 51.9 (OMe), 48.8 (CH_2 Gly), 46.8 ($C_{5'}$), 41.1, 35.3 (2×CH_3 dmf), 33.6, 33.4 (2×CH isobutyryl), 18.7, 18.5 (2×CH_3 isobutyryl); MS (ESI): *m*/*z* 735.4 [M+H]$^+$. **22**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.77–7.75 (m, 2H, 2×$H_{arom.}$ Fmoc), 7.58–7.55 (m, 2H, 2×$H_{arom.}$ Fmoc), 7.41–7.37 (m, 2H, 2×$H_{arom.}$ Fmoc), 7.33–7.10 (m, 28H: 2×$H_{arom.}$ Fmoc, 20×$H_{arom.}$ Bn, 5×$H_{arom.}$ Phe, NH Ala^2), 5.48 (d, 1H, NH Ala^1, $J_{NH,\alpha}$=6.9 Hz), 5.14 (m, 1H, H_α Phe), 5.08 (AB, 2H, CH_2 Bn), 4.86 (AB, 2H, CH_2 Bn), 4.70 (AB, 2H, CH_2 Bn), 4.70 (m, 1H, H_α Ala^1), 4.65 (AB, 2H, CH_2 Bn), 4.54–4.52 (m, 1H, H_α Ala^2), 4.43 (d, 1H, H_1, $J_{1,2}$=3.4 Hz), 4.34–4.27 (m, 2H, CH_2 Fmoc), 4.20–4.18 (m, 1H, CH Fmoc), 4.11–4.09 (m, 1H, 1×H_6), 3.92 (app. t, 1H, H_3), 3.68–3.65 (m, 1H, H_5), 3.46–3.44 (m, 2H, H_2, 1×H_β Phe), 3.16–3.14 (m, 1H, 1×H_β Phe), 3.08 (dd, 1H, H_4, J=9.8 Hz, J=8.9 Hz), 2.95 (s, 3H, OMe), 2.95–2.92 (m, 1H, 1×H_6), 1.32 (d, 3H, H_β Ala^2, $J_{\beta,\alpha}$=7.2 Hz), 1.14 (d, 3H, H_β Ala^1, $J_{\beta,\alpha}$= 6.7 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 175.6, 172.5, 169.6 (3×C=O), 155.7 (NH-C=O, Fmoc), 143.7, 143.6, 141.2, 141.2 (4×C_q Fmoc), 138.6, 138.0, 137.9, 137.8 (4×C_q Bn), 135.4 ($C_{q\ arom.}$, Phe), 128.7–125.0 ($CH_{arom.}$ Fmoc, Bn, Phe), 97.6 (C_1), 81.6 (C_3), 79.8 (C_2), 79.5 (C_4), 75.5, 75.0, 73.2 (3×CH_2 Bn), 69.9 (C_5), 67.0 (1×CH_2 Bn), 66.8 (CH_2 Fmoc), 60.7 (C_α Phe), 55.3 (OMe), 48.5 (C_6), 48.4 (C_α Ala^1), 47.0 (C_α Ala^2), 46.9 (CH Fmoc), 34.1 (C_β Phe), 18.3 (C_β Ala^2), 17.7 (C_β Ala^1). MS (ESI): *m*/*z* 1088.8 [M+Na]$^+$. **25**: ^{1}H NMR (600 MHz, D_2O): δ 7.35–7.24 (m, 5H, $H_{arom.}$ Phe), 5.08 (q, 1H, H_α Ala^1, $J_{\alpha,\beta}$=6.9 Hz), 4.93 (app. t, 1H, H_α Phe), 4.63 (d, 1H, H_1, $J_{1,2}$=3.7 Hz), 4.17 (q, 1H, H_αAla^2, $J_{\alpha,\beta}$=7.1 Hz), 3.91 (app. d, 1H, 1×H_6), 3.78 (s, 2H, H_β Gly), 3.66 (app. t, 1H, H_5), 3.56 (app. t, 1H, H_3), 3.45 (dd, 1H, H_2, $J_{2,3}$=9.8 Hz), 3.29–3.16 (m, 7H, OMe, 2H_β Phe, H_4 and 1×H_6), 1.27 (d, 3H, H_β Ala^2), 1.04 (d, 3H, H_β Ala^1); ^{13}C NMR (150 MHz, D_2O): δ 177.8, 176.0, 170.5, 165.6 (4×C=O), 136.7 ($C_{q\ arom.}$, Phe), 129.1, 128.8, 127.0 ($CH_{arom.}$ Phe), 99.1 (C_1), 72.8 (C_3), 71.5 (C_4), 71.0 (C_2), 70.2 (C_5), 61.4 (C_α Phe), 55.3 (OMe), 50.0 (C_α Ala^2), 47.5 (C_6), 46.4 (C_α Ala^1), 40.2 (C_β Gly), 34.0 (C_β Phe), 17.4 (C_β Ala^2), 17.0 (C_β Ala^1); MS (ESI): *m*/*z* 541.3 [M+H]$^+$.

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14. Bhat, B.; Swayze, E. E.; Wheeler, P.; Dimock, S.; Perbost, M.; Sanghvi, Y. S. *J. Org. Chem.* **1996**, *61*, 8186–8199.
15. As confirmed by mass spectrometry.
16. HPLC detection limit >95%.
17. (a) The use of DIPEA led to rapid decomposition of Fmoc-Ala-Cl. The amino acid chloride was synthesised as described by Carpino, L. A.; Cohen, B. J.; Stephens, K. E., Jr.; Sadat-Aalaee, S.-Y.; Tien, J.-H.; Langridge, D. C. *J. Org. Chem.* **1986**, *51*, 3732–3734; (b) partial racemisation (5%) of the amino acid chloride was prevented by starting the reaction at −30°C, instead of at room temperature, and allowing the mixture to warm up after a 30 min period.
18. For example: BOP/HOBt, EDC/HOBt, HATU.