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The tetrabenzylglucosyloxycarbonyl(BGloc)-group-A carbohydrate-derived enzyme-labile urethane protecting group

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Abstract

The development of the tetrabenzylglucosyloxycarbonyl (BGloc)-protecting group as an enzymatically removable urethane protecting function for peptide synthesis is described. BGloc-protected amino acids are readily synthesized by conversion of amino acid allyl esters into the respective isocyanates, subsequent treatment with 2,3,4,6-tetrabenzylglucose and *C*-terminal allyl ester cleavage. From BGloc masked dipeptide esters, which are accessible by standard methods of peptide chemistry, the *N*-terminal urethane is selectively cleaved off in high yield via removal of the benzyl ethers by hydrogenation followed by hydrolysis of the urethane by means of α- and β-glucosidase under very mild reaction conditions. © 1998 Elsevier Science Ltd.

Keywords: Protecting groups; Enzymatic deprotection; Peptides; Glucosyloxycarbonyl group

1. Introduction

The selective protection and deprotection of functional groups belong to the most important and regularly employed synthetic transformations in the construction of complex natural products. In particular, peptide and carbohydrate chemistry constitute the major areas in which protecting groups have to be applied extensively. However, although a variety of powerful protecting group techniques are available [1–4], in the construction of sensitive polyfunctional molecules, e.g., the glyco-, nucleo-, phospho- and lipopeptides [5–7] an increasing demand exists for further blocking functions removable by alternative methods and under exceptionally mild conditions.

Enzymatic protecting group techniques [4–8] offer advantageous alternatives to classical chemical methods since enzymes, in many cases, operate under the mildest reaction conditions (pH 6–8, room temperature) and may combine a high specificity for the structures they recognize and the reactions they catalyze with a broad substrate tolerance. Thus, we have for instance developed enzyme-labile blocking functions for carbohydrates [9,10], nucleosides, and nucleotides [11] as well as for the carboxy groups [12,13] and amino groups [9,14] of peptides.

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The development of enzyme-labile protecting groups for the *N*-terminus of peptides and peptide conjugates, like glycopeptides poses a major challenge. Firstly, these blocking functions have to be urethanes because, in the presence of a simple acyl group, the *N*-terminal amino acid racemizes upon activation. Unfortunately, the esterases, lipases and amidases available today do not attack urethanes [1] [15]. Secondly, the urethane must be cleaved chemoselectively by the biocatalysts, i.e. the peptide bonds and the C-terminal esters must remain intact. Thirdly, the protecting group should enhance the solubility of the compounds to be deprotected in aqueous solutions, i.e. the media in which enzymes operate best, in order to guarantee that the substrates are accessible to the biocatalyst. We now report that the 2,3,4,6-tetra-*O*-benzylglucosyloxycarbonyl (BGloc) group is an enzyme-labile blocking function which fulfills these requirements.

2. Results and discussion

In developing enzyme-labile urethanes we reasoned that, most probably, the established esterases, lipases and amidases are not able to attack the carbonyl group of urethanes for electronic reasons (diminished reactivity of the carbonyl group due to two + *M* substituents). An alternative strategy would be to employ a biocatalyst which attacks a different bond, e.g., an *O*-alkyl bond and to use an urethane designed accordingly.

Glycosidases hydrolyze glycosides by cleaving the bond between C-1 and O-1 (Fig. 1) and typically display a high specifity for the carbohydrate they splitt off, but not for the aglycon [16]. Therefore, they might also be able to cleave glycosyloxycarbonyl groups attached to peptides. This principle has recently also been employed for the development of a new strategy for antibody-directed enzyme prodrug therapy (ADEPT) [17], and glycosyl esters were cleaved from amino acids before [18]. Furthermore, glycosidases do not hydrolyze simple esters and amides, so that an attack on the peptide bonds and the *C*-terminal esters of the peptide has not to be feared. In addition, the presence of an *O*-deprotected carbohydrate in an otherwise fully masked peptide should

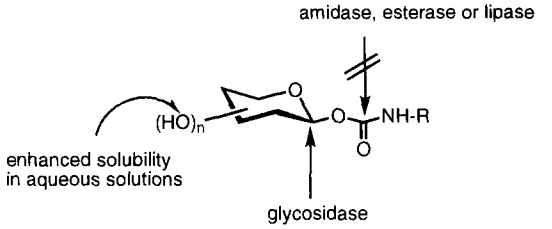


Fig. 1. Principle for the development of enzyme-labile carbohydrate derived urethane protecting groups.

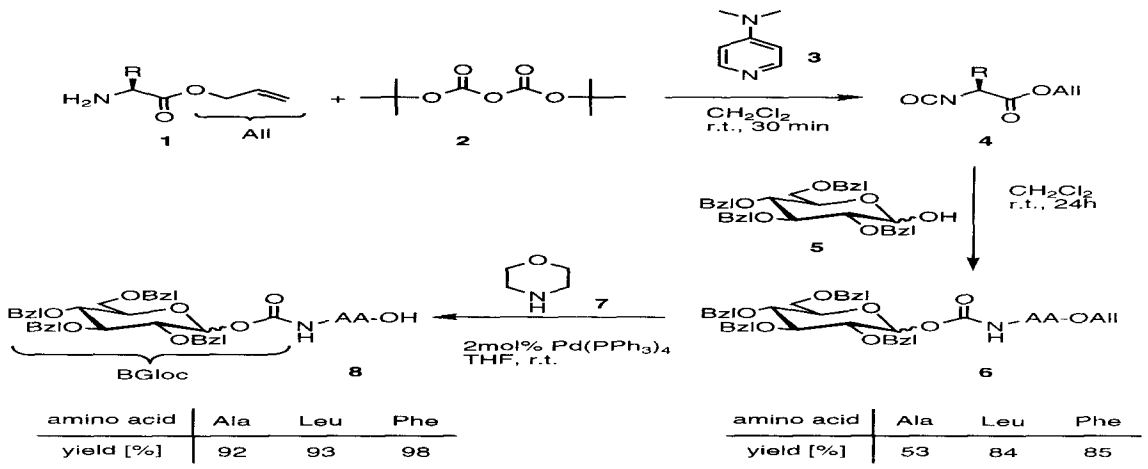
confer to the substrate a high solubility in aqueous solutions, i.e. the media in which the enzymatic deprotection has to be carried out.

Therefore, glycosyloxycarbonyl groups might constitute a general class of enzyme-labile urethane blocking functions. This hypothesis was investigated for the glucosyloxycarbonyl group and its removal by α- and β-glucosidases.

For the synthesis of glucosyloxycarbonyl-protected peptide esters, the respective urethane masked amino acids had to be built up first. To this end, 2,3,4,6-tetra-*O*-benzylglucopyranose **5** was treated with the amino acid allyl ester isocyanates **4** to give the *N*-acylated amino acid esters **6** in satisfactory to high yields as mixtures of anomers (α:β ca. 2:1) (Scheme 1). The amino acid allyl ester isocyanates **4** were readily built up in high yields and without any racemization by treatment of the amino acid allyl esters **1** with phosgene [19] or, better, by means of the recently developed alternative method [20] which consists in the use of di-*tert*-butyldicarbonate **2** in the presence of 4-dimethylaminopyridine (DMAP) **3** (Scheme 1). From the *N*-acylated amino acid allyl esters **6**, the *C*-terminal ester blocking group was then removed selectively by means of Pd(0)-mediated allyl transfer to morpholine **7** as the accepting nucleophile (Scheme 1) [21]. Thereby, the 2,3,4,6-tetra-*O*-benzyl glucosyloxy carbonyl (BGloc)-protected amino acids **8** were obtained in high yields.

The acids **8** were then condensed with different amino acid *tert*-butyl or *n*-heptyl(Hep) esters [12] **9** in the presence of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) **10** [22] to deliver the BGloc-masked dipeptide esters **11** in high yields (Scheme 2). Careful inspection of the high-field ^{1}H and ^{13}C NMR spectra of the crude peptides **11** as well as of the selectively deprotected dipeptide esters **14** (vide infra) did not give any hint indicating that the *N*-terminal BGloc-protected amino acid might have racemized to a considerable extent. Although this is not a highly sensitive test, it shows that

[1] In one report, an enzyme-mediated removal of the *Z*- and the Boc-group from amino acids was described, where the biocatalyst did not attack peptide bonds [15].



amino acid	Ala	Leu	Phe
yield [%]	92	93	98

amino acid	Ala	Leu	Phe
yield [%]	53	84	85

Scheme 1. Synthesis of BGloc-protected amino acids **8**.

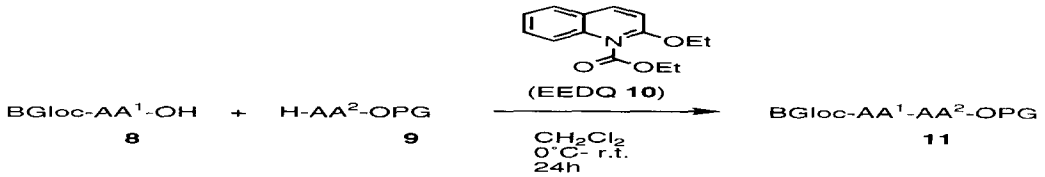
racemization of the *N*-terminal amino acid upon activation for peptide coupling is not a problem in the application of the BGloc group.

At the dipeptide stage, the anomers in many cases can be separated by means of flash chromatography if desired. However, since for both the α- and the β-anomer respective glycosidases are available (vide infra) the mixtures of the anomers can be subjected to the removal of the *N*-terminal urethane directly.

The benzyl ethers present in the carbohydrate moiety of **11** were removed without any problem and in quantitative yield by hydrogenolysis (Scheme 3) and the glucosyloxycarbonyl (Gloc) protected amino acid esters **12** obtained thereby were then subjected to the enzymatic deprotection reactions.

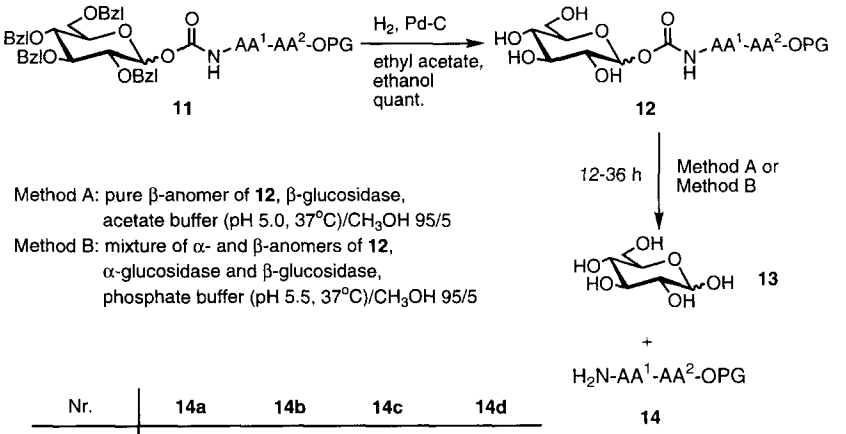
In initial exploring experiments, the pure β-anomers of the protected dipeptides **12** were treated with β-glucosidase from almonds in acetate buffer containing 5 vol% of methanol at pH 5 and 37 °C (Method A; Scheme 3). The substrates **12**, which are deprotected in the carbohydrate moiety are freely soluble in this medium. Furthermore, if the peptides do not embody two hydrophobic amino acids, the addition of methanol is not necessary at all. Thus, as expected, the hydrophilic carbohydrate moiety confers advantageous solubilizing properties to the substrates. It makes the use of solubility-enhancing cosolvents, which might denature the biocatalyst and/or slow down the biocatalyzed transformations, unnecessary or at least greatly reduces the need for them. Under these conditions the β-glycosidic bonds were smoothly cleaved by the enzyme and the desired *N*-terminally deprotected dipeptide esters **14b** and **14c** were obtained in excellent yields.

Since the Gloc protected dipeptide esters **12** are, however, obtained in the course of the synthesis described above as a mixture of α- and β-anomers with the α-configured compounds predominating, the simultaneous enzymatic cleavage of both diastereomers was investigated in further experiments. To



Nr.	11a	11b	11c	11d	11e
AA^1-AA^2	Phe-Ala	Phe-Val	Ala-Gly	Leu-Gly	Phe-Leu
PG	*t*Bu	Hep	*t*Bu	*t*Bu	*t*Bu
yield [%]	71	86	87	70	79

Scheme 2. Synthesis of BGloc-protected dipeptides **11**.



Nr.	14a	14b	14c	14d
AA^1-AA^2	Phe-Ala	Phe-Val	Ala-Gly	Leu-Gly
PG	*t*Bu	Hep	*t*Bu	*t*Bu
Method A yield [%]	-	98	91	-
Method B yield [%]	67	75	-	75

Scheme 3. Deprotection of the carbohydrate moiety and enzymatic removal of the Gloc urethane group.

this end, the mixtures of anomers of **12** were treated with both the β-glucosidase from almonds and an α-glucosidase. From several combinations of enzymes under different conditions, finally the use of α-glucosidase from bakers yeast together with the β-glucosidase at pH 5.5 and 37 °C gave the most advantageous results. Under these conditions, and in the presence of these two biocatalysts, the desired dipeptide esters **14** were formed in high yields (Scheme 3) indicating that both the α- and the β-glycosidase actively cleaved the α- and the β-anomers of the substrates **12**, respectively. In the course of the enzymatic transformations, absolutely no undesired side reactions were observed, i.e. the peptide bonds and the *C*-terminal esters were left intact. In particular, the glycosidases did not cleave the heptyl ester present in **12c** which is sensitive to the treatment with lipases [12].

In conclusion, the results demonstrate that the strategy delineated above for the development of enzyme-labile urethane protecting groups in general, and by means of the class of glycosyloxycarbonyl groups and glycosidases in particular, is valid. In the case studied, and described in this paper, the glucosidases did successfully remove the α- and the β-glucosyloxycarbonyl group from the peptides without undesired side reaction. The blocking group has advantageous solubilizing properties which obviate or reduce the need for undesired organic cosolvents, and it suppresses racemization in the course of peptide bond formation. The conditions under which it can be removed are so mild that this blocking function may advantageously be applied in the construction of acid- and base-labile complex peptide conjugates, i.e. glyco-, nucleo-, phospho- and lipopeptides.

Finally, it should be pointed out that, by switching from the couple glucose–glucosidase to different carbohydrates and the respective glycosidases, an entire set of enzyme-labile carbohydrate-based blocking functions may be generated. This could, for instance, open up the possibility to have two or more carbohydrate-derived protecting groups present in one compound all of which can be cleaved off selectively by choosing the appropriate glycosidase. In such a scenario, the principles for the protection and for the removal of the different blocking functions would be the same and the selectivity would be introduced exclusively by means of the chosen biocatalyst.

3. Experimental

General methods.—^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC250, AM400 and DRX-500. Mass spectra were measured on a Finnigan MAT MS 70 spectrometer. FAB-spectra were obtained using 3-nitrobenzyl alcohol (3-NBA) or glycerol as matrix. Specific rotations were measured with a Perkin-Elmer polarimeter 241. Analytical chromatography was performed on E. Merck Silica Gel 60 F_{254} plates. Flash

chromatography was performed on Baker silica gel (40–64 μm). Tetrahydrofuran was distilled from potassium metal and CH_2Cl_2 from P_2O_5. α-Glucosidase from bakers yeast and β-glucosidase from almonds were obtained from Sigma. One unit of enzymatic activity is equivalent to the amount of enzyme converting 1.0 μmol of maltose to 2.0 μmol of D-glucose per min at pH 6.0 at 25 °C (α-glucosidase); one unit of β-glucosidase will liberate 1.0 μmol of D-glucose from salicin per min at pH 5.0 at 37 °C.

Synthesis of BGloc-protected amino acid esters; N-*(2,3,4,6-tetra*-O-*benzyl-α / β*-D-*glucopyranosyloxycarbonyl)*-L-*amino acid allyl esters* **6a**, **b**, **c**; *General procedure.*—To a soln of di-*tert*-butyldicarbonate **2** (306 mg, 1.4 mmol) in 10 mL of CH_2Cl_2 was added successively a soln of 4-dimethylaminopyridine **3** (122 mg, 1.0 mmol) in 5 mL of CH_2Cl_2 and a soln of the amino acid allyl ester **1** (1.0 mmol) in 5 mL of CH_2Cl_2. After stirring for 30 min at room temperature, a solution of 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose **5** (541 mg, 1.4 mmol) in 10 mL of CH_2Cl_2 was added. The reaction mixture was stirred overnight at room temperature. Evaporation of the solvent followed by flash chromatography (EtOAc–hexane) of the residue on silica gel provided the BGloc-protected amino acid allyl esters **6**. Characteristic signals: ^{1}H NMR ($CDCl_3$): δ 7.3–7.1 (m, 20 H, arom. CH), 6.3 (d, 2/3 H, $J_{1,2}$ 3.4 Hz, αH-1), 5.6 (d, 1/3 H, $J_{1,2}$ 8.1 Hz, βH-1), 5.4 (d, 1 H, *J* 7.6 Hz, NH), 4.9–4.3 (m, 8 H, 4 CH_2Ph), 3.8–3.5 (m, 6 H, H-2, H-3, H-4, H-5, H-6, H-6′); ^{13}C NMR ($CDCl_3$): δ 153.7 (OCONH), 138.3, 138.0, 137.9, 137.8 (ipso C), 128.4–127.7 (arom. CH), 95.3 (β C-1), 91.1 (α C-1), 84.7, 80.8, 77.1, 75.1 (CH β-anomer, C-2, C-3, C-4, C-5), 81.7, 78.9, 76.8, 72.4 (CH α-anomer, C-2, C-3, C-4, C-5), 75.7–73.1 (4 CH_2Ph), 68.0 (CH_2, C-6). Furthermore, the spectra displayed the characteristic signals of the respective amino acid and of the allyl ester group [21].

N-*(2,3,4,6-Tetra*-O-*benzyl-α / β*-D-*glucopyranosyloxycarbonyl)*-L-*alanine allyl ester* **6a** (369 mg, 53%): mp 79 °C; $[\alpha]_D^{20}$ +9.2° (*c* 1.0, $CHCl_3$); FABMS (3-NBA): *m/z* 718.2, $[M + Na]^+$; Anal. Calcd for $C_{41}H_{45}NO_9$: C, 70.77; H, 6.51; N, 2.01. Found: C, 70.96; H, 6.51; N, 1.95.

N-*(2,3,4,6-Tetra*-O-*benzyl-α / β*-D-*glucopyranosyloxycarbonyl)*-L-*leucine allyl ester* **6b** (620 mg, 84%): colourless amorphous solid; $[\alpha]_D^{20}$ +22.2° (*c* 1.0, $CHCl_3$); HRMS (FAB, 3-NBA) *m/z*: Calcd for $C_{44}H_{51}NaNO_9$: 760.346, $[M + Na]^+$. Found: 760.362.

N-*(2,3,4,6-Tetra*-O-*benzyl-α / β*-D-*glucopyranosyloxycarbonyl)*-L-*phenylalanine allyl ester* **6c** (656 mg, 85%): mp 84 °C; $[\alpha]_D^{20}$ +23.6° (*c* 1.0, $CHCl_3$); FABMS (3-NBA): *m/z* 794.3, $[M + Na]^+$; Anal. Calcd for $C_{47}H_{49}NO_9$: C, 73.13; H, 6.39; N, 1.81. Found: C, 72.72; H, 6.34; N, 1.77.

C-Terminal deprotection of the BGloc-protected amino acid esters; N-*(2,3,4,6-tetra*-O-*benzyl-α / β*-D-*glucopyranosyloxycarbonyl)*-L-*amino acids* **8a**, **b**, **c**; *General procedure.*—To a soln of the *N*-(2,3,4,6-tetra-*O*-benzyl-α/β-D-glucopyranosyloxycarbonyl)-L-amino acid allyl ester **6** (1 mmol) and $(Ph_3P)_4Pd$ (23 mg, 2 mol%) in 20 mL of THF under an argon atmosphere was added a soln of morpholine **7** (105 mg, 1.2 mmol) in 5 mL of THF. The mixture was stirred for 1 h, the solvent was removed under reduced pressure and the residue was purified by flash chromatography (EtOAc–hexane + 1% acetic acid). Characteristic signals: ^{1}H NMR ($CDCl_3$): δ 10.4 (br, 1 H, COOH), 7.3–7.1 (m, 20 H, arom. CH), 6.3 (d, 2/3 H, $J_{1,2}$ 3.4 Hz, αH-1), 5.6 (d, 1/3 H, $J_{1,2}$ 8.1 Hz, βH-1), 5.4 (d, 1 H, *J* 7.6 Hz, NH), 4.9–4.3 (m, 8 H, 4 CH_2Ph), 3.8–3.5 (m, 6 H, H-2, H-3, H-4, H-5, H-6, H-6′); ^{13}C NMR ($CDCl_3$): δ 177.0 (COOH), 153.7 (OCONH), 138.3, 138.0, 137.9, 137.8 (ipso C), 128.4–127.7 (arom. CH), 95.3 (βC-1), 91.1 (αC-1), 84.7, 80.8, 77.1, 75.1 (CH β-anomer, C-2, C-3, C-4, C-5), 81.6, 78.7, 76.7, 72.3 (CH α-anomer, C-2, C-3, C-4, C-5), 75.7–73.4 (4 CH_2Ph), 68.0 (CH_2, C-6). Furthermore, the spectra display the characteristic signals of the respective amino acid.

N-*(2,3,4,6-Tetra*-O-*benzyl-α / β*-D-*glucopyranosyloxycarbonyl)*-L-*alanine* **8a** (603 mg, 92%): mp 139 °C; $[\alpha]_D^{20}$ +8.7° (*c* 1.0, $CHCl_3$); FABMS (3-NBA): *m/z* 678.3, $[M + Na]^+$; Anal. Calcd for $C_{38}H_{41}NO_9 \cdot 0.5H_2O$: C, 68.71; H, 6.30; N, 2.10. Found: C, 68.69; H, 6.31; N, 1.70.

N-*(2,3,4,6-Tetra*-O-*benzyl-α / β*-D-*glucopyranosyloxycarbonyl)*-L-*leucine* **8b** (649 mg, 93%): colourless amorphous solid; $[\alpha]_D^{20}$ +38.6° (*c* 1.0, $CHCl_3$); FABMS (3-NBA): *m/z* 720.2, $[M + Na]^+$; Anal. Calcd for $C_{41}H_{47}NO_9 \cdot H_2O$: C, 68.79; H, 6.89; N, 1.95. Found: C, 68.56; H, 6.57; N, 1.43.

N-*(2,3,4,6-Tetra*-O-*benzyl-α / β*-D-*glucopyranosyloxycarbonyl)*-L-*phenylalanine* **8c** (717 mg, 98%): mp 139 °C; $[\alpha]_D^{20}$ +69.6 (*c* 1.0, $CHCl_3$); FABMS (3-NBA): *m/z* 754.2, $[M + Na]^+$; Anal. Calcd for $C_{44}H_{45}NO_9$: C, 72.21; H, 6.19; N, 1.91. Found: C, 71.81; H, 6.06; N, 1.60.

Peptide couplings to give BGloc-protected dipeptides **11**; *General procedure.*—To a soln of BGloc-protected amino acid **8** (1 mmol), and the relevant

amino acid ester hydrochloride **9** (1 mmol) in 50 mL of dry CH_2Cl_2, was added at 0 °C NEt_3 (0.17 mL, 1.2 mmol) and a soln of EEDQ **10** (495 mg, 2 mmol) in 10 mL of dry CH_2Cl_2. After 2 h at 0 °C, the mixture was stirred overnight at room temperature. The soln was extracted three times each with aq HCl (0.5 N, 100 mL), satd $NaHCO_3$-soln and water. The organic layer was dried on $MgSO_4$, filtered, and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (hexane–EtOAc).

N-*(2,3,4,6-Tetra*-O*-benzyl-α / β-*D*-glucopyranosyloxycarbonyl)*-L*-phenylalanyl*-L*-alanine* tert*-butyl ester* **11a** (610 mg, 71%): colourless amorphous solid; $[\alpha]_D^{20}$ +50.3° (*c* 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.34–7.12 (m, 25 H, arom. CH), 6.27 (d, 2/3 H, $J_{1,2}$ 3.4 Hz, αH-1), 6.19 (d, *J* 6.9 Hz, CONH), 5.53 (d, 1/3 H, $J_{1,2}$ 8.1 Hz, βH-1), 5.41 (d, 1 H, *J* 8.3 Hz, OCONH), 4.97–4.29 (m, 10 H, 4 CH_2Ph, α-CH Phe, α-CH Ala), 3.92–3.53 (m, 6 H, H-2, H-3, H-4, H-5, H-6, H-6′), 3.19–3.14 (m, 1 H, β-CH_{2a} Phe), 3.03–2.93 (m, 1 H, β-CH_{2b} Phe), 1.43 (s, 9 H, $C(CH_3)_3$), 1.29 (d, *J* 7.0 Hz, β-CH_3 Ala); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 171.4, 169.5 (COO, CONH), 154.0 (OCONH), 138.6–136.0 (ipso C), 129.4–127.1 (arom. CH), 95.5 (αC-1), 91.4 (βC-1), 82.1 ($C(CH_3)_3$), 84.8, 80.7, 77.3, 75.3 (CH β-anomer, C-2, C-3, C-4, C-5), 81.7, 78.8, 75.7, 72.6 (CH α-anomer, C-2, C-3, C-4, C-5), 75.7–73.0 (4 CH_2Ph), 68.0 (CH_2, C-6), 55.9 (α-CH Phe), 48.8 (α-CH Ala), 38.6 (β-CH_2 Phe), 27.9 ($C(CH_3)_3$), 18.5 (β-CH_3 Ala); HRMS (FAB, 3-NBA) *m/z* 881.390; Calcd for $C_{51}H_{58}NaN_2O_{10}$: 881.398, $[M + Na]^+$.

N-*(2,3,4,6-Tetra*-O*-benzyl-α / β-*D*-glucopyranosyloxycarbonyl)*-L*-phenylalanyl*-L*-valine heptyl ester* **11b** (799 mg, 86%): wax; $[\alpha]_D^{20}$ +23.5° (*c* 0.7, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.33–7.12 (m, 25 H, arom. CH), 6.27 (d, 2/3 H, $J_{1,2}$ 3.2 Hz, αH-1), 6.12 (d, 1 H, *J* 8.3 Hz, CONH), 5.53 (d, 1/3 H, $J_{1,2}$ 8.1 Hz, βH-1), 5.46 (d, 1 H, *J* 8.4 Hz, OCONH), 4.97–4.40 (m, 10 H, 4 CH_2Ph, α-CH Phe, α-CH Val), 4.07 (t, *J* 6.7 Hz, 2 H, OCH_2 Hep), 3.92–3.53 (m, 6 H, H-2, H-3, H-4, H-5, H-6, H-6′), 3.15–2.97 (m, 2 H, β-CH_2 Phe), 2.10–2.06 (m, 1 H, β-CH Val), 1.61–1.59 (m, 2 H, CH_2 Hep), 1.31–1.28 (m, 8 H, 4 CH_2 Hep), 0.90–0.80 (m, 9 H, 2 γ-CH_3 Val, CH_3 Hep); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 171.2, 170.2 (COO, CONH), 153.9 (OCONH), 138.4–136.1 (ipso C), 129.4–127.1 (arom. CH), 95.5 (βC-1), 91.4 (αC-1), 84.9, 80.7, 77.3, 75.2 (CH β-anomer, C-2, C-3, C-4, C-5), 81.7, 78.8, 76.8, 72.6 (CH α-anomer, C-2, C-3, C-4, C-5), 75.7–73.0 (4 CH_2Ph), 68.0 (CH_2, C-6), 65.5 (OCH_2 Hep), 57.4, 56.3 (α-CH Phe, α-CH Val), 38.5 (β-CH_2 Phe), 31.7 (CH_2 Hep), 31.3 (β-CH Val), 28.9, 28.5, 22.8, 22.6 (4 CH_2 Hep), 18.8, 17.8 (2 CH_3 Val), 14.1 (CH_3 Hep). HRMS (FAB, 3-NBA) *m/z* 928.466, 951.5 $[M + Na]^+$; Calcd for $C_{56}H_{68}N_2O_{10}$: 928.487, $[M]^+$. Anal. Calcd for $C_{56}H_{68}N_2O_{10} \cdot 0.5H_2O$: C, 71.69; H, 7.41; N, 2.98. Found: C, 71.86; H, 7.38; N, 2.60.

N-*(2,3,4,6-Tetra*-O*-benzyl-α / β-*D*-glucopyranosyloxycarbonyl)*-L*-alanyl-glycine* tert*-butyl ester* **11c** (669 mg, 87%): colourless amorphous solid; $[\alpha]_D^{20}$ +16.7° (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31–7.10 (m, 20 H, arom. CH), 6.54 (t, 1 H, *J* 5.1 Hz, CONH), 6.25 (d, 1/3 H, $J_{1,2}$ 3.4 Hz, αH-1), 5.56 (d, 1 H, *J* 8.0 Hz, OCONH), 5.48 (d, 2/3 H, *J* 7.9 Hz, βH-1), 4.96–4.41 (m, 8 H, 4 CH_2Ph), 4.29 (qd, 1 H, $J_{NH,CH} = J_{CH,CH_3} = 7.2$ Hz, α-CH Ala), 3.95–3.83 (m, 2 H, α-CH_2 Gly), 3.78–3.54 (m, 6 H, H-2, H-3, H-4, H-5, H-6, H-6′), 1.45 (s, 9 H, $C(CH_3)_3$), 1.39 (d, *J* 7.0 Hz, β-CH_3 Ala); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 171.8, 168.6 (COO, CONH), 153.9 (OCONH), 138.3, 138.0, 137.9, 137.8 (ipso C), 128.4–127.7 (arom. CH), 95.4 (βC-1), 91.3 (αC-1), 82.4 ($C(CH_3)_3$), 84.7, 80.9, 77.2, 75.2 (CH β-anomer, C-2, C-3, C-4, C-5), 81.6, 78.7, 76.7, 72.5 (CH α-anomer, C-2, C-3, C-4, C-5), 75.7–73.1 (CH_2Ph), 68.0 (CH_2, C-6), 50.4 (α-CH Ala), 42.0 (α-CH_2 Gly), 28.0 ($C(CH_3)_3$), 18.6 (β-CH_3 Ala); HRMS (FAB, 3-NBA) *m/z* 791.361; Calcd for $C_{44}H_{52}NaN_2O_{10}$: 791.352, $[M + Na]^+$.

N-*(2,3,4,6-Tetra*-O*-benzyl-α / β-*D*-glucopyranosyloxycarbonyl)*-L*-leucyl-glycine* tert*-butyl ester* **11d** (568 mg, 70%): colourless amorphous solid; $[\alpha]_D^{20}$ +34.6° (*c* 1.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.31–7.12 (m, 20 H, arom. CH), 6.45 (t, *J* 5.0 Hz, CONH), 6.26 (d, 2/3 H, $J_{1,2}$ 3.4 Hz, αH-1), 5.53 (d, 1/3 H, $J_{1,2}$ 8.1 Hz, βH-1), 5.29 (d, 1 H, *J* 8.4 Hz, OCONH), 4.95–4.42 (m, 8 H, 4 CH_2Ph), 4.28–4.21 (m, 1 H, α-CH Leu), 3.96–3.54 (m, 8 H, α-CH_2 Gly, H-2, H-3, H-4, H-5, H-6, H-6′), 1.83–1.65 (m, 2 H, γ-CH, β-CH_{2a} Leu), 1.56–1.50 (m, 1 H, β-CH_{2b} Leu), 1.46 (s, 9 H, $C(CH_3)_3$), 0.96–0.93 (m, δ-CH_3 Leu); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 171.7, 168.6 (COO, CONH), 154.5 (OCONH), 138.6–137.7 (ipso C), 128.4–127.7 (arom. CH), 95.5 (βC-1), 91.3 (αC-1), 82.4 ($C(CH_3)_3$), 84.8, 80.9, 77.3, 75.3 (CH β-anomer, C-2, C-3, C-4, C-5), 81.7, 78.9, 76.9, 72.5 (CH α-anomer, C-2, C-3, C-4, C-5), 75.6–73.0 (4 CH_2Ph), 68.0 (CH_2, C-6), 53.3 (α-CH Leu), 42.0 (α-CH Gly), 41.7 (β-CH_2 Leu), 28.0

($C(CH_3)_3$), 24.6 (γ-CH Leu), 22.9, 21.9 (δ-CH_3 Leu); HRMS (FAB, 3-NBA) *m/z* 811.401; Calcd for $C_{47}H_{58}NaN_2O_{10}$: 811.417, $[M + Na]^+$. Anal. Calcd for $C_{47}H_{58}N_2O_{10}$: C, 69.61; H, 7.20; N, 3.45. Found: C, 69.37; H, 7.21; N, 3.09.

N-*(2,3,4,6-Tetra*-O-*benzyl-α / β-*D-*glucopyranosyloxycarbonyl)-*L-*phenylalanyl-*L-*leucine* tert-*butyl ester* **11e** (712 mg, 79%): colourless amorphous solid; $[\alpha]_{20}^{D}$ +47.4° (*c* 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.35–7.12 (m, 25 H, arom. CH), 6.29 (d, 2/3 H, $J_{1,2}$ 3.4 Hz, αH-1), 6.10 (d, *J* 8.2 Hz, CONH), 5.53 (d, 1/3 H, *J* 8.1 Hz, βH-1), 5.45 (d, 1 H, *J* 8.1 Hz, OCONH), 4.98–4.39 (m, 10 H, 4 CH_2Ph, α-CH Leu, α-CH Phe), 3.92–3.53 (m, 6 H, H-2, H-3, H-4, H-5, H-6, H-6′), 3.17–2.96 (m, 2 H, β-CH_2 Phe), 1.56–1.51 (m, 2 H, γ-CH, β-CH_{2a} Leu), 1.47–1.40 (m, 10 H, β-CH_{2b} Leu, $C(CH_3)_3$), 0.90–0.88 (m, 6 H, δ-CH_3 Leu); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 171.5, 169.7 (COO, CONH), 154.0 (OCONH), 138.6–136.0 (ipso C), 129.5–127.0 (arom. CH), 95.5 (βC-1), 91.3 (αC-1), 81.9 ($C(CH_3)_3$), 84.8, 80.6, 77.2, 75.2 (CH β-anomer, C-2, C-3, C-4, C-5), 81.7, 78.9, 75.7, 72.6 (CH α-anomer, C-2, C-3, C-4, C-5), 77.2–73.0 (4 CH_2Ph), 68.0 (CH_2, C-6), 55.9 (α-CH Phe), 51.4 (α-CH Leu), 41.9 (β-CH_2 Leu), 38.4 (β-CH_2 Phe), 28.0 ($C(CH_3)_3$), 24.7 (γ-CH Leu), 22.6, 22.1 (δ-CH_3 Leu); HRMS (FAB, 3-NBA) *m/z* 923.428; Calcd for $C_{54}H_{64}NaN_2O_{10}$: 923.445, $[M + Na]^+$.

Removal of the O-*benzyl protecting groups from the BGloc-protected peptide esters* **11a**, **b**, **c**, **d**, **e**; General procedure.—To a soln of the BGloc-protected dipeptide esters **11** (1 mmol) in a mixture of EtOAc–EtOH (3:1, 40 mL) was added Pd–C (10%, 30 mg). After stirring for 12–24 h under a hydrogene atmosphere, the reaction mixture was filtered through Celite, and the filtrate was concentrated under reduced pressure to yield the hygroscopic, debenzylated glucose derivatives **12**.

N-*(α / β-*D-*glucopyranosyloxycarbonyl)-*L-*phenylalanyl-*L-*alanine* tert-*butyl ester* **12a** (499 mg, quant.): wax; $[\alpha]_D^{20}$ +24.4° (*c* 1, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD): δ 7.28–7.17 (m, 5 H, arom. CH), 5.87 (d, 2/3 H, $J_{1,2}$ 3.7 Hz, αH-1), 5.27 (d, 1/3 H, $J_{1,2}$ 8.0 Hz, βH-1), 4.43–4.38 (m, 1 H, α-CH Phe), 4.27 (q, 1 H, *J* 7.2 Hz, α-CH Ala), 3.80–3.27 (m, 6 H, H-2, H-3, H-4, H-5, H-6, H-6′), 3.17–3.13 (m, 1 H, β-CH_{2a} Phe), 2.94–2.83 (m, 1 H, β-CH_{2b} Phe), 1.46 (s, 9 H, $C(CH_3)_3$), 1.35 (d, *J* 7.2 Hz, β-CH_3 Ala); ^{13}C NMR (100.6 MHz, CD_3OD): δ 173.4, 173.1 (COO, CONH), 156.8 (OCONH), 138.3 (ipso C), 130.3–127.6 (arom. CH), 96.8 (βC-1), 94.6 (αC-1), 82.6 ($C(CH_3)_3$), 78.4, 77.7, 73.8, 70.8 (CH β-anomer, C-2, C-3, C-4, C-5), 75.3, 74.5, 72.1, 70.8 (CH α-anomer, C-2, C-3, C-4, C-5), 62.0 (CH_2, C-6), 57.3 (α-CH Phe), 50.1 (α-CH Ala), 38.8 (β-CH_2 Phe), 28.1 ($C(CH_3)_3$), 17.5 (β-CH_3 Ala); HRMS (FAB, glycerol) *m/z* 499.217; 521.197, $[M + Na]^+$. Calcd for $C_{23}H_{35}N_2O_{10}$: 499.229, $[M + H]^+$.

N-*(α / β-*D-*glucopyranosyloxycarbonyl)-*L-*phenylalanyl-*L-*valine heptyl ester* **12b** (563 mg, 99%): wax; $[\alpha]_D^{20}$ +25.8° (*c* 1, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD): δ 7.27–7.18 (m, 5 H, arom. CH), 5.89 (d, 2/3 H, $J_{1,2}$ 3.7 Hz, αH-1), 5.29 (d, 1/3 H, $J_{1,2}$ 8.1 Hz, βH-1), 4.51–4.30 (m, 1 H, α-CH Phe), 4.13–4.06 (m, 1 H, α-CH Val), 4.09 (t, 2 H, *J* 6.4 Hz, OCH_2 Hep), 3.81–3.30 (m, 6 H, H-2, H-3, H-4, H-5, H-6, H-6′), 3.13–3.08 (m, 1 H, β-CH_{2a} Phe), 2.94–2.85 (m, 1 H, β-CH_{2b} Phe), 2.16–2.09 (m, 1 H, β-CH Val), 1.65–1.60 (m, 2 H, CH_2 Hep), 1.36–1.22 (m, 8 H, 4 CH_2 Hep), 0.95–0.93 (m, 6 H, 2 γ-CH_3 Val), 0.90–0.87 (m, 3 H, CH_3 Hep); ^{13}C NMR (125.7 MHz, CD_3OD): δ 173.9, 172.8 (COO, CONH), 156.8 (OCONH), 138.2 (ipso C), 130.3–127.7 (arom. CH), 96.9 (βC-1), 94.7 (αC-1), 78.4, 77.7, 73.9, 70.9 (CH β-anomer, C-2, C-3, C-4, C-5), 75.3, 74.6, 72.2, 70.8 (CH α-anomer, C-2, C-3, C-4, C-5), 66.2 (OCH_2 Hep), 62.0 (CH_2, C-6), 59.2, 57.4 (α-CH Phe, α-CH Val), 38.9 (β-CH_2 Phe), 32.8 (CH_2 Hep), 31.9 (β-CH Val), 29.9, 29.6, 26.9, 23.6 (4 CH_2 Hep), 19.5, 18.5 (2 γ-CH_3 Val), 14.4 (CH_3 Hep); HRMS (FAB, glycerol) *m/z* 591.301. Calcd for $C_{28}H_{44}NaN_2O_{10}$: 591.289, $[M + Na]^+$. Anal. Calcd for $C_{28}H_{44}N_2O_{10} \cdot H_2O$: C, 57.32; H, 7.90; N, 4.77. Found: C, 57.66; H, 7.83; N, 4.54.

N-*(α / β-*D-*glucopyranosyloxycarbonyl)-*L-*alanylglycine* tert-*butyl ester* **12c** (409 mg, quant.): wax; $[\alpha]_D^{20}$ −12.0° (*c* 0.5, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD): δ 5.95 (d, 2/3 H, $J_{1,2}$ 3.7 Hz, αH-1), 5.34 (d, 1/3 H, *J* 8.0 Hz, βH-1), 4.22–4.17 (m, 1 H, α-CH Ala), 3.87–3.29 (m, 8 H, α-CH_2 Gly, H-2, H-3, H-4, H-5, H-6, H-6′), 1.45 (s, 9 H, $C(CH_3)_3$), 1.37 (d, *J* 7.2 Hz, β-CH_3 Ala); ^{13}C NMR (125.7 MHz, CD_3OD): δ 175.6, 170.3 (COO, CONH), 156.6 (OCONH), 96.8 (βC-1), 94.6 (αC-1), 82.8 ($C(CH_3)_3$), 78.5, 77.8, 73.9, 71.0 (CH β-anomer, C-2, C-3, C-4, C-5), 75.5, 74.7, 72.3, 71.0 (CH α-anomer, C-2, C-3, C-4, C-5), 62.3 (CH_2, C-6), 51.8 (α-CH Ala), 42.8 (α-CH_2 Gly), 28.2 ($C(CH_3)_3$), 18.3 (β-CH_3 Ala); HRMS (FAB, glycerol) *m/z* 431.154; Calcd for $C_{16}H_{28}NaN_2O_{10}$: 431.164, $[M + Na]^+$.

N-*(α / β-*D-*glucopyranosyloxycarbonyl)-*L-*leucylglycine* tert-*butyl ester* **12d** (451 mg, quant.): wax;

$[\alpha]_D^{20}$ +27.3° (*c* 0.7, CH_3OH); ^1H NMR (500 MHz, CD_3OD): δ 5.96 (d, 2/3 H, $J_{1,2}$ 3.8 Hz, αH-1), 5.35 (d, 1/3 H, *J* 7.9 Hz, βH-1), 4.22–4.17 (m, 1 H, α-CH Leu), 3.89–3.30 (m, 8 H, α-CH_2 Gly, H-2, H-3, H-4, H-5, H-6, H-6′), 1.79–1.69 (m, 1 H, γ-CH Leu), 1.66–1.55 (m, 2 H, β-CH_2 Leu), 1.46 (s, 9 H, $C(CH_3)_3$), 0.97–0.94 (m, 6 H, δ-CH_3 Leu); ^{13}C NMR (100.6 MHz, CD_3OD): δ 175.5, 170.3 (COO, CONH), 157.1 (OCONH), 96.8 (βC-1), 94.7 (αC-1), 82.8 ($C(CH_3)_3$), 78.5, 77.8, 74.0, 70.9 (CH β-anomer, C-2, C-3, C-4, C-5), 75.4, 74.7, 72.3, 71.0 (CH α-anomer, C-2, C-3, C-4, C-5), 62.2 (CH_2, C-6), 54.8 (α-CH Leu), 42.8, 42.2 (2 CH_2, α-CH_2 Gly, β-CH_2 Leu), 28.3 ($C(CH_3)_3$), 25.7 (γ-CH Leu), 23.5, 21.9 (δ-CH_3 Leu); HRMS (FAB, glycerol) *m/z* 423.204; Calcd for $C_{19}H_{34}NaN_2O_{10}$: 473.211, $[M + Na]^+$.

N-(α / β-D-*glucopyranosyloxycarbonyl*)-L-*phenylalanyl*-L-*leucine* tert-*butyl ester* **12e** (541 mg, quant.): wax; $[\alpha]_D^{20}$ +16.3° (*c* 1, CH_3OH); ^1H NMR (500 MHz, CD_3OD): δ 7.29–7.18 (m, 5 H, arom. CH), 5.88 (d, 2/3 H, $J_{1,2}$ 3.7 Hz, αH-1), 5.28 (d, 1/3 H, $J_{1,2}$ 8.1 Hz, βH-1), 4.46–4.41 (m, 1 H, α-CH Phe), 4.35–4.32 (m, 1 H, α-CH Leu), 3.81–3.29 (m, 6 H, H-2, H-3, H-4, H-5, H-6, H-6′), 3.18–3.14 (m, 1 H, β-CH_{2a} Phe), 2.95–2.86 (m, 1 H, β-CH_{2b} Phe), 1.72–1.66 (m, 1 H, γ-CH Leu), 1.61–1.57 (m, 2 H, β-CH_2 Leu), 1.46 (s, 9 H, $C(CH_3)_3$), 0.97–0.91 (m, 6 H, δ-CH_3 Leu); ^{13}C NMR (125.7 MHz, CD_3OD): δ 173.6, 173.2 (COO, CONH), 156.8 (OCONH), 138.4 (ipso C), 130.4–127.7 (arom. CH), 96.9 (βC-1), 94.7 (αC-1), 82.7 ($C(CH_3)_3$), 78.5, 77.8, 73.9, 71.0 (CH β-anomer, C-2, C-3, C-4, C-5), 75.3, 74.6, 72.3, 70.9 (CH α-anomer, C-2, C-3, C-4, C-5), 62.1 (CH_2, C-6), 57.3 (α-CH Phe), 52.9 (α-CH Leu), 41.6 (β-CH_2 Leu), 38.9 (β-CH_2 Phe), 28.2 ($C(CH_3)_3$), 25.9 (γ-CH Leu), 23.2, 22.1 (δ-CH_3 Leu); HRMS (FAB, glycerol) *m/z*; 541.266; 563.247, $[M + Na]^+$. Calcd for $C_{26}H_{41}N_2O_{10}$: 541.276, $[M + H]^+$.

Enzymatic removal of the Gloc-group from the peptides **12a**, **b**, **c**, **d**; *Method A*.—A soln of the *N*-(β-D-glucopyranosyloxycarbonyl) dipeptide ester **12** (0.2 mmol) in MeOH (10 mL) was added to a soln of β-glucosidase (40 units) in 0.25 M acetate buffer (pH 5.0, 190 mL). The reaction mixture was shaken for 12–36 h at 37 °C, the pH was adjusted to 10–11 and the soln was extracted with EtOH (100 mL × 4). The combined organic phases were dried on $MgSO_4$, filtered and evaporated to dryness to give the N-terminal deprotected dipeptide esters **14** as colourless oils.

Method B.—A soln of the *N*-(α/β-D-glucopyranosyloxycarbonyl) dipeptide ester **12** (0.2 mmol) in MeOH (10 mL) was added to a soln of α-glucosidase (16 units) and β-glucosidase (40 units) in 0.07 M phosphate buffer (pH 5.5, 190 mL). The reaction mixture was shaken for 12–36 h at 37 °C and then lyophilized. The residue was dissolved in satd $NaHCO_3$ soln (20 mL) and the soln was extracted with CH_2Cl_2 (40 mL × 3). The combined organic phases were dried on $MgSO_4$, filtered and evaporated to dryness to give the N-terminal deprotected dipeptide esters **14** as colourless oils.

L-*Phenylalanyl*-L-*alanine* tert-*butyl ester* **14a** (Method B: 39 mg, 67%): colourless oil; $[\alpha]_D^{20}$ −43.2° (*c* 0.9, CH_3OH); ^1H NMR (400 MHz, $CDCl_3$): δ 7.79 (d, 1 H, *J* 7.2 Hz, NH), 7.32–7.21 (m, 5 H, arom. CH Phe), 4.45 (qd, 1 H, *J* 7.2 Hz, α-CH Ala), 3.68–3.66 (m, 1 H, α-CH Phe), 3.25–3.21 (m, 1 H, β-CH_{2a} Phe), 2.77–2.72 (m, 1 H, β-CH_{2b} Phe), 2.00 (bs, 2 H, NH_2), 1.46 (s, 9 H, $C(CH_3)_3$), 1.35 (d, 3 H, *J* 7.1 Hz, CH_3 Ala); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 173.44, 172.19 (COO, CONH), 137.54 (ipso C), 129.35, 128.66, 126.83 (arom. CH), 81.79 (C, $C(CH_3)_3$), 56.15 (CH, α-CH Phe), 48.28 (CH, α-CH Ala), 40.70 (β-CH_2 Phe), 27.93 (CH_3, $C(CH_3)_3$), 18.53 (CH_3 Ala); HRMS (EI) *m/z*; 292.179. Calcd for $C_{16}H_{24}N_2O_3$: 292.178, $[M]^+$.

L-*Phenylalanyl*-L-*valine heptyl ester* **14b** (Method A: 71 mg, 98%; Method B: 54 mg, 75%): characterized as the hydrochloride, colourless oil; $[\alpha]_D^{20}$ +9.4° (*c* 1.0, CH_3OH); ^1H NMR (500 MHz, $CDCl_3$): δ 8.46 (bs, 3 H, NH_3^+), 7.37–7.19 (m, 6 H, arom. CH Phe, NH), 4.72–4.65 (m, 1 H, α-CH), 4.30–4.28 (m, 1 H, α-CH), 4.03 (t, *J* 6.7 Hz, O–CH_2 Hep), 3.45–3.41 (m, 1 H, β-CH_{2a} Phe), 3.29–3.24 (m, 1 H, β-CH_{2b} Phe), 2.10–2.06 (m, 1 H, β-CH Val), 1.59–1.55 (m, 2 H, CH_2 Hep), 1.29–1.26 (m, 8 H, 4 CH_2 Hep), 0.88–0.85 (m, 9 H, 2 δ-CH_3 Val, CH_3 Hep); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 170.9, 168.3 (COO, CONH), 134.4 (ipso C), 129.9, 128.8, 127.5 (arom. CH), 65.4 (OCH_2 Hep), 58.4, 54.7 (α-CH Phe, α-CH Val), 37.2 (β-CH_2 Phe), 31.7 (CH_2 Hep), 30.8 (β-CH Val), 28.9, 28.5, 25.8, 22.5 (4 CH_2 Hep), 18.9, 18.2 (2 γ-CH_3 Val), 14.0 (CH_3 Hep); HRMS (EI) *m/z*; 362.255. Calcd for $C_{21}H_{34}N_2O_3$: 362.256, $[M]^+$.

L-*Alanyl-glycine* tert-*butyl ester* **14c** (Method A: 37 mg, 91%): colourless oil; $[\alpha]_D^{20}$ +0.7° (*c* 0.95, CH_3OH); ^1H NMR (500 MHz, $CDCl_3$): δ 7.72 (bs, 1 H, NH), 3.95–3.92 (m, 2 H, CH_2 Gly), 3.61–3.52 (m, 1 H, α-CH Ala), 1.67 (bs, 2 H, NH_2), 1.47 (s, 9

H, $C(CH_3)_3$), 1.36 (d, 3 H, J 6.9 Hz, CH_3 Ala); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 175.89, 169.20 (COO, CONH), 82.10 (C, $C(CH_3)_3$), 50.63 (CH, α-CH Ala), 41.65 (α-CH_2 Gly), 28.05 (CH_3, $C(CH_3)_3$), 21.67 (CH_3 Ala); HRMS (EI) m/z; 202.130. Calcd for $C_9H_{18}N_2O_3$: 202.131, $[M]^+$.

L-*Leucyl-glycine* tert-*butyl ester* **14d** (Method B: 37 mg, 75%): colourless oil; $[\alpha]_D^{20}$ −48.1° (c 0.96, CH_3OH); ^1H NMR (500 MHz, $CDCl_3$): δ 7.74 (bs, 1 H, NH), 3.98–3.89 (m, 2 H, CH_2 Gly), 3.46–3.44 (m, 1 H, α-CH Leu), 1.78–1.68 (m, 4 H, NH_2, γ-CH, β-CH_{2a} Leu), 1.47 (s, 9 H, $C(CH_3)_3$), 1.44–1.35 (m, 1 H, β-CH_{2b} Leu), 0.97–0.93 (m, 6 H, δ-CH_3 Leu); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 175.7, 169.3 (COO, CONH), 82.1 ($C(CH_3)_3$), 53.4 (α-CH Leu), 43.9, 41.7 (2 CH_2, α-CH_2 Gly, β-CH_2 Leu), 28.0 ($C(CH_3)_3$), 24.8 (γ-CH Leu), 23.4, 21.3 (δ-CH_3 Leu); HRMS (EI) m/z; 244.178. Calcd for $C_{12}H_{24}N_2O_3$: 244.178, $[M]^+$.

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