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SYNTHESIS OF 2-ACETAMIDO-1-*N*-[*N*-(*tert*-BUTOXYCARBONYL)-L-ASPART-1-OYL-(L-PHENYLALANYL-L-SERINE METHYL ESTER)-4-OYL]-2-DEOXY-β-D-GLUCOPYRANOSYLAMINE AND ANALOGS*

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ABSTRACT

2-Acetamido-1-*N*-[*N*-(*tert*-butoxycarbonyl)-L-aspart-1-oyl-(L-phenylalanyl-L-serine methyl ester)-4-oyl]-2-deoxy-β-D-glucopyranosylamine and analogs containing D-glucopyranosyl, 4-*O*-β-D-glucopyranosyl-D-glucopyranosyl, L-Phe-L-Ala, and D-Phe-L-Ser were synthesized by condensation of glycosylamines having free hydroxyl groups with tripeptide esters activated with *N*-hydroxysuccinimide.

INTRODUCTION

N-Glycopeptides include a "core" region composed of two 2-acetamido-2-deoxy-β-D-glucopyranosyl and one β-D-mannopyranosyl residues, and a "sequon" tripeptide, Asn-X-Ser(Thr). The synthesis of *N*-glycopeptides including the "core", the "sequon", and their intermediates, has been studied by numerous investigators. Jeanloz and assoc. synthesized a 2-acetamido-2-deoxy-D-glucose–L-asparagine compound by the condensation of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosylamine with *N*-(benzyloxycarbonyl)-L-aspartic acid 1-benzyl ester[1], as well as several oligosaccharide–asparagine compounds by the condensation of glycosylamines of synthetic di- or tri-saccharide with *N*-(benzyloxycarbonyl)-L-aspartic acid 1-benzyl ester[2–7]. They also synthesized several "*N*-glyco-oligopeptides" in which the 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl residue is attached to the asparagine residue of a peptide chain[8–11]. Except for a few compounds[3–5,10], most *N*-glycopeptides synthesized had *O*-acetylated sugar residues. Removal of these acetyl groups without any cleavage of the peptide moiety is difficult because of instability of the aspartyl residue.

In order to synthesize *N*-glycopeptide having free hydroxyl groups, condensation of an unsubstituted glycosylamine with the free 4-carboxyl group of an aspartyl residue by use of an active ester was investigated. Thus, the *N*-hydroxysuccinimide ester of *N*-(*tert*-butoxycarbonyl)-L-aspartyl-L-phenylalanyl-L-serine methyl ester

*Studies on *N*-Glycopeptides I.

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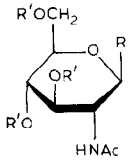
(**20**) was condensed with 2-acetamido-2-deoxy-β-D-glucopyranosylamine (**3**) to give 2-acetamido-1-*N*-[*N*-(*tert*-butoxycarbonyl)-L-aspart-1-oyl-(L-phenylalanyl-L-serine methyl ester)-4-oyl]-2-deoxy-β-D-glucopyranosylamine (**31**). Furthermore, several analogs were synthesized to ascertain the advantage of the active ester methods, *e.g.*, 2-acetamido-1-*N*-[*N*-(*tert*-butoxycarbonyl)-L-aspart-1-oyl-(L-phenylalanyl-L-alanine methyl ester)-4-oyl]-2-deoxy-β-D-glucopyranosylamine (**33**), 2-acetamido-1-*N*-[*N*-(*tert*-butoxycarbonyl)-L-aspart-1-oyl-(D-phenylalanyl-L-serine methyl ester)-4-oyl]-2-deoxy-β-D-glucopyranosylamine (**34**), and analogs containing a β-D-glucopyranosyl or 4-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl residue instead of the 2-acetamido-2-deoxy-β-D-glucopyranosyl residue (**35–40**).

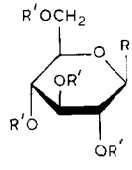
RESULTS AND DISCUSSION

2-Acetamido-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl azide[1] (**1**), 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide (**4**), and 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranosyl azide[12] (**7**), prepared in the usual manner, were *O*-deacetylated with saturated methanolic ammonia, and the resulting compounds (**2**, **5**, and **8**, respectively) were converted into the desired glycosylamines (**3**, **6**, and **9**, respectively) by catalytic hydrogenation.

In order to obtain the best conditions for the condensation of these glycosylamines with a peptide residue, the reaction of several active esters of *N*-(benzyloxycarbonyl)glycine, such as the pentachlorophenyl[13] (**10**), *p*-nitro-



1 R = N_3, R′ = Ac
2 R = N_3, R′ = H
3 R = NH_2, R′ = H



4 R = N_3, R′ = Ac
5 R = N_3, R′ = H
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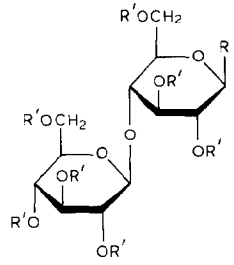
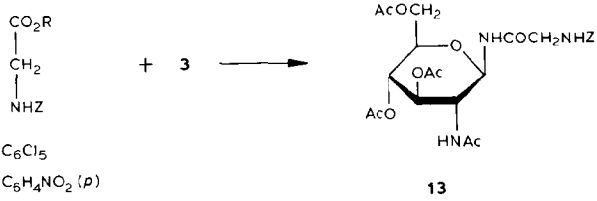


7 R = N_3, R′ = Ac
8 R = N_3, R′ = H
9 R = NH_2, R′ = H



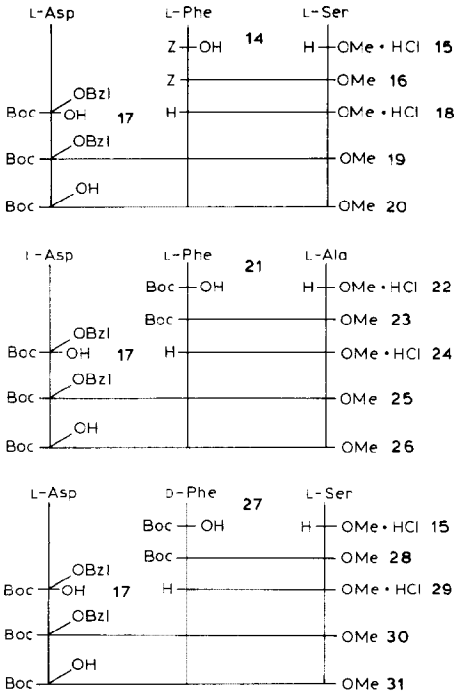
10 R = C_6Cl_5
11 R = $C_6H_4NO_2$ (*p*)
12 R = $N(COCH_2)_2$
Z = Benzyloxycarbonyl

13

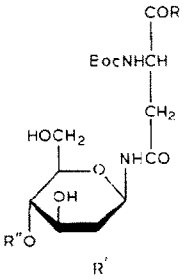
phenyl[14–16] (**11**), and *N*-hydroxysuccinimide[17,18] (**12**) esters, with 2-acetamido-2-deoxy-β-D-glucopyranosylamine was investigated. It showed that **12** was the most effective intermediate to give **13**.

N-(Benzyloxycarbonyl)-L-phenylalanine (**14**) was condensed with L-serine methyl ester (**15**) in the presence of dicyclohexylcarbodiimide[19] to give **16** which was hydrogenolyzed in the presence of palladium black[20] and hydrogen chloride. The resulting dipeptide ester hydrochloride **18** was condensed with *N*-(*tert*-butoxycarbonyl)-L-aspartic acid 4-benzyl ester (**17**) by the carbodiimide method to afford **19**, and the benzyl group was hydrogenolyzed to give **20**. *N*-(*tert*-Butoxycarbonyl)-L-phenylalanine (**21**) was coupled with L-alanine methyl ester (**22**) by the mixed anhydride method[21,22], and the resulting compound **23** treated with hydrogen chloride in 1,4-dioxane to remove the *tert*-butoxycarbonyl residue. The resulting dipeptide ester hydrochloride **24** was condensed with **17** as just described, and hydrogenolysis gave **26**. *N*-(*tert*-Butoxycarbonyl)-L-phenylalanine (**27**) and **15** were condensed, as described for the preparation of **16**, and the resulting **28** was treated with hydrogen chloride in 1,4-dioxane to give **29**, which was converted into **31** as just described.

Condensation of an unsubstituted glycosylamine with a peptide residue was then investigated. The *N*-hydroxysuccinimide group was introduced into the acyltripeptide esters **20**, **26**, and **31** by the carbodiimide method. After removal of the



Boc = *tert*-Butoxycarbonyl



32 R = L-Phe-L-Ser-OMe, R′ = NHAc, R″ = H
33 R = L-Phe-L-Ala-OMe, R′ = NHAc, R″ = H
34 R = D-Phe-L-Ser-OMe, R′ = NHAc, R″ = H
35 R = L-Phe-L-Ser-OMe, R′ = OH, R″ = H
36 R = L-Phe-L-Ala-OMe, R′ = OH, R″ = H
37 R = D-Phe-L-Ser-OMe, R′ = OH, R″ = H
38 R = L-Phe-L-Ser-OMe, R′ = OH, R″ = β-D-Glc
39 R = L-Phe-L-Ala-OMe, R′ = OH, R″ = β-D-Glc
40 R = D-Phe-L-Ser-OMe, R′ = OH, R″ = β-D-Glc

N,N-dicyclohexylurea, the active ester intermediates were condensed with the glycosylamine derivatives **3, 6,** and **9**, respectively, to give the glycopeptides **21–40**. In the condensation of *N*-(*tert*-butoxycarbonyl)-L-aspartyl-L-phenylalanyl-L-alanine methyl ester (**26**) with the glycosylamine derivatives **3, 6,** and **9**, only the crystalline *N*-glycopeptides **33, 36,** and **39**, respectively, were obtained. However, the condensation of the peptides that included a serine residue gave two products; the major one was the desired *N*-glycopeptide and the minor one a by-product involving the hydroxyl group of the serine residue. The desired products were obtained after chromatography on silica gel. The free hydroxyl groups of the carbohydrate residue were not involved. When **3** and **20** were condensed in the presence of 2-ethoxy-1-*N*-ethoxycarbonyl-1,2-dihydroquinoline, many compounds (including one giving a ninhydrin-positive reaction), formed by the reaction of the 4-carboxyl group with hydroxyl groups of the sugar and serine residues, were observed on t.l.c. In contrast, condensation *via* the hydroxysuccinimide intermediates did not give esters, and all compounds found were ninhydrin negative.

The yield of the condensation of the hydroxyl-free glycosylamines was slightly inferior to that of the corresponding protected glycosylamines. The 3,4,6-tri-*O*-acetyl derivative of **3** was condensed with **26** to give the *O*-acetyl derivative of **33** in more than 50% yield. However, **33** could not be obtained by alkaline hydrolysis with sodium hydroxide owing to degradation of the peptide chain. Thus, the condensation of hydroxyl-free glycosylamines with a peptide residue by the active ester method is more convenient than the condensation with hydroxyl-protected glycosylamines, because deblocking is not required in the final step.

EXPERIMENTAL

General methods. — Melting points are uncorrected. Optical rotations were determined for solutions in a 0.1-dm tube with a Union model PM-101 polarimeter. I.r. spectra were recorded, for KBr discs, with a JASCO spectrophotometer, Model IRA-1. Amino acid analysis was performed with an h.l.c. amino acid analyzer, KLA-5 type. T.l.c. was carried out on Kieselgel G (60) with the following solvent systems (all v/v): (*A*) 4:1:1:2 1-butanol–acetic acid–pyridine–water, and (*B*) 5:1 chloroform–methanol. Spots of materials having a free amino group were detected on the t.l.c. plate with ninhydrin, and those having a blocked amino group, by spraying with 25% HBr–acetic acid and then with ninhydrin. Sugar derivatives giving a negative ninhydrin reaction were detected by spraying with M H_2SO_4 and then heating. Column chromatography on silica gel was performed on Kieselgel 60 (70–230 mesh, Merck), the ratio of the column to its length being 4:125 and the flow rate 1–2 mL/min. Prior to analysis, the compounds were dried in the presence of phosphorus pentaoxide at 66° and 0.26 MPa for 2 h. Amino acid derivatives were prepared in the usual manner with benzyloxycarbonyl chloride[23], 2-*tert*-butoxycarbonylimino-2,2-phenylacetonitrile[24], thionyl chloride–methanol[25], and benzyl alcohol–hydrochloric acid[26].

*2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl azide* (**1**). — This was prepared by the method of Bolton and Jeanloz[1] in 50% yield, m.p.165–167° (dec.), $[\alpha]_D^{24}$ −46° (*c* 1, chloroform); lit.[27] m.p. 166–168°, $[\alpha]_D^{24}$ −60° (*c* 2, chloroform); lit.[28] m.p. 160–161° (dec.), $[\alpha]_D^{30}$ −40° (*c* 1, chloroform); ν_{max}^{KBr} 3325 (NH), 2130 (N_3), 1750 (OAc), and 1660 cm^{-1} (CONH).

Anal. Calc. for $C_{14}H_{20}N_4O_8$: C, 45.09; H, 5.41; N, 15.05. Found: C, 45.09; H, 5.48; N, 14.89.

*2-Acetamido-2-deoxy-β-*D*-glucopyranosyl azide* (**2**). — A solution of **1** (1.79 g) in methanol (20 mL), which had been previously saturated with dry ammonia at 0°, was stored in a glass-stoppered bottle at room temperature for 24 h. The mixture was evaporated *in vacuo*, and additioning ethyl acetate to the residual oil gave **2** (1.13 g, 92%), m.p. 120–121°, $[\alpha]_D^{24}$ −39° (*c* 1, methanol); ν_{max}^{KBr} 3240 (br. OH and NH), 2100 (N_3), and 1650 cm^{-1} (CONH).

Anal. Calc. for $C_8H_{14}N_4O_5$: C, 39.02; H, 5.73; N, 22.76. Found: C, 38.81; H, 5.78; N, 22.61.

*2-Acetamido-2-deoxy-β-*D*-glucopyranosylamine* (**3**). — A solution of **2** (369 mg) in methanol (15 mL) was hydrogenated at atmospheric pressure in the presence of PtO_2 (Adam's catalyst) (400 mg) for 3 h. The catalyst was filtered off, and the solution (which gave a positive ninhydrin reaction) was evaporated *in vacuo*. Crystallization from ethyl acetate gave crystals (291 mg, 88%), m.p. 115°, $[\alpha]_D^{24}$ −4° (*c* 1, water); ν_{max}^{KBr} 3250 (br. OH and NH) and 1670 cm^{-1} (CONH), and no N_3 band.

Anal. Calc. for $C_8H_{16}N_2O_5$: C, 43.63; H, 7.32; N, 12.72. Found: C, 43.50; H, 7.38; N, 12.75.

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl azide (**4**). — This compound was obtained, in 81% yield, by azidolysis of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide, under conditions similar to those described by Bolton and Jeanloz[1], m.p. 129–130°, $[\alpha]_D^{24}$ −29° (*c* 1, chloroform); ν_{max}^{KBr} 2110 (N_3) and 1760 cm^{-1} (OAc).

Anal. Calc. for $C_{14}H_{19}N_3O_9$: C, 45.04; H, 5.13; N, 11.26. Found: C, 45.02; H, 5.13; N, 11.21.

β-D-Glucopyranosylamine (**5**). — Compound **4** (7.46 g) was *O*-deacetylated as described for the preparation of **2**, and the resulting oil was hydrogenated as described for the preparation of **3** to give a powder (2.83 g), m.p. 119–120° (dec.), $[\alpha]_D^{24}$ +18° (*c* 1, water); ν_{max}^{KBr} 3250 cm^{-1} (broad OH and NH); and no N_3 band.

Anal. Calc. for $C_6H_{13}NO_5$: C, 40.22; H, 7.31; N, 7.91. Found: C, 40.44; H, 7.30; N, 8.04.

2,3,6-Tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-β-D-glucopyranosyl azide (**7**). — This compound was prepared, in 72% yield, by the method of Dunstan and Hough[12], m.p. 180–181°, $[\alpha]_D^{24}$ −31° (*c* 1, chloroform); lit.[12] m.p. 182–183°, $[\alpha]_D^{24}$ −31.2° (*c* 1, chloroform); ν_{max}^{KBr} 2110 (N_3) and 1760 cm^{-1} (OAc).

Anal. Calc. for $C_{26}H_{35}N_3O_{17}$: C, 47.20; H, 5.33; N, 6.35. Found: C, 47.30; H, 5.38; N, 6.17.

4-O-β-D-Glucopyranosyl-β-D-glucopyranosylamine (**8**). — Compound **7** (3.85 g) was *O*-deacetylated as described for the preparation of **2**. The resulting oil was hydrogenated as described for the preparation of **3** to give **8** (1.40 g), m.p. 159° (dec.), $[\alpha]_D^{24}$ +20° (*c* 1, water); ν_{max}^{KBr} 3240 cm^{-1} (br. OH and NH), and no N_3 band.

Anal. Calc. for $C_{12}H_{23}NO_{10}$: C, 42.23; H, 6.79; N, 4.10. Found: C, 42.16; H, 6.38; N, 3.98.

*Condensation of N-(benzyloxycarbonyl)glycine active esters (***10, 11,** *and* **12***) with 2-acetamido-2-deoxy-β-D-glucopyranosylamine* (**3**). — To a solution of **3** (264 mg, 1.2 mmol) in *N,N*-dimethylformamide (1 mL) was added a solution of *N*-(benzyloxycarbonyl)glycine active ester (**10, 11,** or **12**) (1 mmol) in oxolane (2 mL). The mixture was stirred for 6 h and evaporated *in vacuo*. The oily residue was dissolved in water (10 mL) and the solution was extracted with ethyl acetate (total 500 mL). The extract was dried (Na_2SO_4) and evaporated. 2-Acetamido-1-*N*-[*N*-(benzyloxycarbonyl)glycyl]-2-deoxy-β-D-glycopyranosylamine (**13**) was obtained, by crystallization from ether, in yields 58% from **10**, 51% from **11**, and 62% from **12**; m.p. 188–190° (dec.), $[\alpha]_D^{24}$ +39° (*c* 1, methanol); ν_{max}^{KBr} 3250 (br. OH and NH), 1680 (benzyloxycarbonyl CO), and 1630 cm^{-1} (br. amide I).

Anal. Calc. for $C_{18}H_{25}N_3O_8$: C, 52.55; H, 6.13; N, 10.21. Found: C, 52.43; H, 6.21; N, 10.18.

N-(Benzyloxycarbonyl)-L-phenylalanyl-L-serine methyl ester (**16**). — To a solution of *N*-(benzyloxycarbonyl)-L-phenylalanine **14** (4.50 g), L-serine methyl ester hydrochloride (**15**) (2.33 g), and *N*-methylmorpholine (1.65 mL) in chloroform (20 mL) and *N,N*-dimethylformamide (10 mL) was added dicyclohexylcarbodiimide (3.05 g) with stirring for 1 h at 0°. The mixture was stirred

overnight at room temperature. *N,N*-Dicyclohexylurea was filtered off and the filtrate evaporated *in vacuo*. The oily residue was dissolved in ethyl acetate, the solution washed with 4% $NaHCO_3$, 2% HCl, and water, and dried (Na_2SO_4). The solvent was evaporated *in vacuo* and the residual oil crystallized from ether and petroleum ether (yield 78%), m.p. 123–124°, $[\alpha]_D^{24}$ −9° (*c* 1, methanol); R_F (*A*) 0.95, R_F (*B*) 0.67; ν_{max}^{KBr} 3250 (OH and NH), 1730 (COOMe), 1690 (benzyloxycarbonyl CO), and 1650 cm^{-1} (peptide amide I).

Anal. Calc. for $C_{21}H_{24}N_2O_6$: C, 62.99; H, 6.04; N, 7.00. Found: C, 62.84; H, 5.99; N, 6.84.

L-*Phenylalanyl*-L-*serine methyl ester hydrochloride* (**18**). — Compound **16** (12.01 g), dissolved in methanol (30 mL) and 3.5M HCl in 1,4-dioxane (30 mL), was hydrogenated in the presence of Pd black as a catalyst for 5 h. After removal of the catalyst, the filtrate was evaporated, and **18** (8.09 g, 89%) was obtained by crystallization from ether, m.p. 184–185°, $[\alpha]_D^{24}$ +89° (*c* 1, methanol); R_F (*A*) 0.82, R_F (*B*) 0.59; ν_{max}^{KBr} 2950 (NH_3^+), 1740 (CO_2Me), and 1650 cm^{-1} (peptide amide I).

Anal. Calc. for $C_{13}H_{19}ClN_2O_2$: C, 51.57; H, 6.33; N, 9.25. Found: C, 51.70; H, 6.28; N, 9.36.

[N-(tert-*Butoxycarbonyl*)-L-*aspartyl-4-benzyl ester*]-L-*phenylalanyl*-L-*serine methyl ester* (**19**). — To a solution of **18** (6.06 g), *N*-(*tert*-butoxycarbonyl)-L-aspartic acid 4-benzyl ester (**17**) (6.47 g), and *N*-methylmorpholine (2.2 mL) in chloroform (20 mL) and *N,N*-dimethylformamide (20 mL) was added dicyclohexylcarbodiimide (4.13 g) at 0°. The mixture was processed, as described for the preparation of **16**, with 4% citric acid instead of 2% HCl. The crystalline residue (8.91 g, 78%) was obtained by evaporation, m.p. 111–115° (dec.), $[\alpha]_D^{24}$ −30° (*c* 1, methanol); R_F (*A*) 0.98, R_F (*B*) 0.70; ν_{max}^{KBr} 3300 (OH and NH), 1735 and 1720 (ester), 1680 (*tert*-butoxycarbonyl CO), and 1640–1520 cm^{-1} (peptide amide I).

Anal. Calc. for $C_{29}H_{37}N_3O_9$: C, 60.93; H, 6.52; N, 7.35. Found: C, 61.18; H, 6.61; N, 7.43.

N-(tert-*Butoxycarbonyl*)-L-*aspartyl*-L-*phenylalanyl*-L-*serine methyl ester* (**20**). — Compound **19** (5.70 g), dissolved in methanol (10 mL) and acetone (10 mL), was hydrogenated as described for the preparation of **18**, to give crystals (4.16 g, 83%), m.p. 176–179° (dec.), $[\alpha]_D^{24}$ −33° (*c* 1, *N,N*-dimethylformamide: R_F (*A*) 0.88, R_F (*B*) 0.61; ν_{max}^{KBr} 3300 (OH and NH), 1750 and 1730 (CO_2H) and CO_2Me), 1670 (*tert*-butoxycarbonyl CO), and 1690–1520 cm^{-1} (peptide amide I).

Anal. Calc. for $C_{22}H_{31}N_3O_9$: C, 54.88; H, 6.49; N, 8.73. Found: C, 54.72; H, 6.61; N, 8.59.

N-(tert-*Butoxycarbonyl*)-L-*phenylalanyl*-L-*alanine methyl ester* (**23**). — To a solution of *N*-(*tert*-butoxycarbonyl)-L-phenylalanine (**21**) (5.30 g) and *N*-methylmorpholine (2.2 mL) in oxolane (20 mL) was added ethyl chloroformate (2 mL) at −5°. After 10 min, a mixture of L-alanine methyl ester hydrochloride (**22**) (2.80 g) and *N*-methylmorpholine (2.2 mL) in *N,N*-dimethylformamide (10 mL) and chloroform (10 mL) was added. The mixture was kept overnight at room temperature, evaporated *in vacuo*, and the oily residue dissolved in ethyl acetate. The solu-

tion was washed with 4% $NaHCO_3$, 4% citric acid, and water, and dried (Na_2SO_4). The solvent was evaporated *in vacuo* and the residual oil crystallized by addition of ether and petroleum ether (yield 87%), m.p. 111°, $[\alpha]_D^{24}$ −18° (*c* 1, methanol); R_F (*A*) 0.98, R_F (*B*) 0.77; ν_{max}^{KBr} 3300 (NH), 1750 (CO_2Me), 1690 (*tert*-butoxycarbonyl CO), and 1650 cm^{-1} (peptide amide I).

Anal. Calc. for $C_{18}H_{26}N_2O_5$: C, 61.70; H, 7.48; N, 8.00. Found: C, 61.77; H, 7.60; N, 7.95.

[N-(tert-*Butoxycarbonyl*)-L-*aspartyl-4-benzyl ester*]-L-*phenylalanyl*-L-*alanine methyl ester* (**25**). — A solution of **23** (5.50 g) in 4.3M HCl in 1,4-dioxane (30 mL) was kept for 3 h at room temperature and then evaporated, to give hygroscopic crystals (**24**) (4.45 g) by addition of ether. Compound **24** (4.30 g), **17** (4.85 g), and *N*-methylmorpholine (1.65 mL) were dissolved in *N,N*-dimethylformamide (15 mL) and chloroform (15 mL), and then dicyclohexylcarbodiimide (3.09 g) was added at 0° with stirring for 1 h. The reaction was carried out, as described for the preparation of **16**, by use of 4% citric acid instead of 2% HCl to give crystals (6.24 g) after evaporation, m.p. 133–134° (dec.), $[\alpha]_D^{24}$ −38° (*c* 1, methanol); R_F (*A*) 0.98, R_F (*B*) 0.80; ν_{max}^{KBr} 3300 (NH), 1730 (ester), 1690 (*tert*-butoxycarbonyl CO), and 1640–1530 cm^{-1} (peptide amide I).

Anal. Calc. for $C_{29}H_{37}N_3O_8$: C, 62.69; H, 6.71; N, 7.56. Found: C, 57.02; H, 6.92; N, 9.02.

N-(tert-*Butoxycarbonyl*)-L-*aspartyl*-L-*phenylalanyl*-L-*alanine methyl ester* (**26**). — Compound **30** (5.56 g), dissolved in methanol (10 mL) and acetone (10 mL), was hydrogenated as described for the preparation of **18** to give a crystalline residue (4.46 g, 96%), m.p. 160–162°, $[\alpha]_D^{24}$ −50° (*c* 1, *N,N*-dimethylformamide); R_F (*A*) 0.85, R_F (*B*) 0.67; ν_{max}^{KBr} 3300 (NH), 1730 and 1710 (CO_2H and CO_2Me), 1660 (*tert*-butoxycarbonyl CO), and 1640–1520 cm^{-1} (peptide amide I).

Anal. Calc. for $C_{22}H_{31}N_3O_8$: C, 56.76; H, 6.71; N, 9.03. Found: C, 57.02; H, 6.92; N, 9.02.

N-(tert-*Butoxycarbonyl*)-D-*phenylalanyl*-L-*serine methyl ester* (**28**). — To a solution of *N*-(*tert*-butoxycarbonyl)-D-phenylalanine (**27**) (7.95 g), **15** (4.67 g), and *N*-methylmorpholine (3.3 mL) in *N,N*- dimethylformamide (15 mL) and chloroform (15 mL) was added dicyclohexylcarbodiimide (6.19 g) at 0° with stirring for 1 h. The mixture was processed as described for the preparation of **19**, to give crystals (8.08 g, 74%) from ether–petroleum ether, m.p. 96–98°, $[\alpha]_D^{24}$ +9° (*c* 1, methanol); R_F (*A*) 0.96, R_F (*B*) 0.73; ν_{max}^{KBr} 3250 (OH and NH), 1740 (CO_2Me), 1690 (*tert*-butoxycarbonyl CO), and 3240 cm^{-1} (peptide amide I).

Anal. Calc. for $C_{18}H_{26}N_2O_6$: C, 59.00; H, 7.15; N, 7.65. Found: C, 59.15; H, 7.24; N, 7.72.

[N-(tert-*Butoxycarbonyl*)-L-*aspartyl-4-benzyl*]-D-*phenylalanyl*-L-*serine methyl ester* (**30**). — Compound **28** (7.33 g) was treated with 4.2M HCl in 1,4-dioxane (60 mL). The resulting dipeptide ester hydrochloride **29** (5.87 g), **17** (6.47 g), and *N*-methylmorpholine (2.2 mL) were dissolved in *N,N*-dimethylformamide (10 mL) and chloroform (10 mL), dicyclohexylcarbodiimide (4.13 g) was added, and the

reaction was carried out in the same manner as described for **19** to give a crystalline residue (8.23 g), m.p. 119–121°, $[\alpha]_D^{24}$ +8° (*c* 1, methanol); R_F (*A*) 0.98, R_F (*B*) 0.69; ν_{max}^{KBr} 3250 (OH and NH), 1750 and 1720 (ester), 1670 (*tert*-butoxycarbonyl CO), and 1640–1520 cm^{-1} (peptide amide I).

Anal. Calc. for $C_{29}H_{37}N_3O_9$: C, 60.93; H, 6.52; N, 7.35. Found: C, 60.69; H, 6.67; N, 7.31.

N-(tert-*Butoxycarbonyl*)-L-*aspartyl*-D-*phenylalanyl*-L-*serine methyl ester* (**31**). — Compound **30** (6.86 g) was hydrogenated as described for the preparation of **18** to give a powder (5.33 g, 92%), m.p. 117–118° (dec.), $[\alpha]_D^{24}$ −14° (*c* 1, *N,N*-dimethylformamide); R_F (*A*) 0.88, R_F (*B*) 0.61; ν_{max}^{KBr} 3250 (OH and NH), 1750 and 1725 (CO_2H and CO_2Me), 1650 (*tert*-butoxycarbonyl CO), and 1680–1530 cm^{-1} (peptide amide I).

Anal. Calc. for $C_{22}H_{31}N_3O_9$: C, 54.88; H, 6.49; N, 8.73. Found: C, 55.11; H, 6.49; N, 8.73.

2-Acetamido-1-N-[N-(tert-*butoxycarbonyl*)-L-*aspart-1-oyl*)-L-*phenylalanyl*-L-*serine methyl ester*)-*4-oyl*]-*2-deoxy*-β-D-*glucopyranosylamine* (**32**). — To a solution of **20** (481 mg) and *N*-hydroxysuccinimide (138 mg) in *N,N*-dimethylformamide (1 mL) and oxolane (2 mL) was added dicyclohexylcarbodiimide (227 mg) at 0° with stirring for 1 h. The mixture was stirred for a further 24 h at 0°. *N,N*-Dicyclohexylurea was filtered off, and a solution of **3** (291 mg) in *N,N*-dimethylformamide (2 mL) was added to the filtrate. The mixture was stirred for 6 h at room temperature, and then ethyl acetate was added. The solution was washed with 4% $NaHCO_3$, 4% citric acid, and water, and dried (Na_2SO). After evaporation *in vacuo*, the residual oil was dissolved in a minimal volume of 3:1 benzene–acetone and purified by silica gel column chromatography. Fractions containing **32** were collected and evaporated to give a crystalline residue (82 mg, 21%), m.p. 170–172° (dec.) (ether–petroleum ether), $[\alpha]_D^{24}$ −36° (*c* 1, methanol); R_F (*A*) 0.85, R_F (*B*) 0.62; ν_{max}^{KBr} 3300 (br. OH and NH), 1740 (CO_2Me), 1670 (*tert*-butoxycarbonyl CO), and 1700–1520 cm^{-1} (peptide amide I); amino acid analysis: Asp:Phe:Ser 1.00:1.05:1.09.

Anal. Calc. for $C_{30}H_{45}N_5O_{13}$: C, 52.70; H, 6.63; N, 10.24. Found: C, 52.88; H, 6.53; N, 10.24.

2-Acetamido-1-N-[N-(tert-*butoxycarbonyl*)-L-*aspart-1-oyl*)-L-*phenylalanyl*-L-*alanine methyl ester*)-*4-oyl*]-*2-deoxy*-β-D-*glucopyranosylamine* (**33**). — Compound **16** (465 mg), *N*-hydroxysuccinimide (138 mg), dicyclohexylcarbodiimide (227 mg), and **3** (291 mg) were treated as described for the preparation of **32**. The crystalline residue (234 mg, 35%), obtained without silica gel column chromatography, had m.p. 133–135° (dec.), $[\alpha]_D^{24}$ −79° (*c* 1, methanol); R_F (*A*) 0.85, R_F (*B*) 0.70; ν_{max}^{KBr} 3300 (br. OH and NH), 1730 (CO_2Me), 1680 (*tert*-butoxycarbonyl CO), and 1660–1530 cm^{-1} (peptide amide I); amino acid analysis: Asp:Phe:Ala 1.00:0.92:1.10.

Anal. Calc. for $C_{30}H_{45}N_5O_{12}$: C, 53.96; H, 6.79; N, 10.49. Found: C, 54.08; H, 6.75; N, 10.54.

*2-Acetamido-1-*N-[N-*(*tert-*butoxycarbonyl)-*L-*aspart-1-oyl)-*D-*phenylalanyl-*L-*serine methyl ester)-4-oyl]-2-deoxy-β-*D-*glucopyranosylamine* (**34**). — Compound **31** (481 mg), *N*-hydroxysuccinimide (138 mg), dicyclohexylcarbodiimide (227 mg), and **3** (291 mg) were treated as described for the preparation of **32**. The crystalline residue (102 mg, 15%) was obtained after purification by silica gel column chromatography, m.p. 96–97° (dec.), $[\alpha]_D^{24}$ +28° (*c* 1, methanol); R_F (*A*) 0.86, R_F (*B*) 0.69; ν_{max}^{KBr} 3300 (br. OH and NH), 1740 (CO_2Me), 1660 (*tert*-butoxycarbonyl CO), and 1690–1520 cm^{-1} (peptide amide I); amino acid analysis: Asp:Phe:Ser 1.00:1.11:0.95.

Anal. Calc. for $C_{30}H_{45}N_5O_{13}$: C, 52.70; H, 6.63; N, 10.24. Found: C, 52.93; H, 6.50; N, 10.29.

*1-*N-[N-*(*tert-*Butoxycarbonyl)-*L-*aspart-1-oyl-(*L-*phenylalanyl-*L-*serine methyl ester)-4-oyl]-β-*D-*glucopyranosylamine* (**35**). — Compound **20** (962 mg), *N*-hydroxysuccinimide (276 mg), dicyclohexylcarbodiimide (454 mg), and **6** (448 mg) were treated as described for the preparation of **32**. The residual oil was purified by silica gel column chromatography to give a crystalline residue (555 mg, 43%), m.p. 158–162° (dec.), $[\alpha]_D^{24}$ −23° (*c* 1, *N,N*-dimethylformamide: R_F (*A*) 0.85, R_F (*B*) 0.67; ν_{max}^{KBr} 3300 (br. OH and NH), 1740 (CO_2Me), 1675 (*tert*-butoxycarbonyl CO), and 1690–1520 cm^{-1} (peptide amide I); amino acid analysis: Asp:Phe:Ser 1.00:1.12:0.92.

Anal. Calc for $C_{28}H_{42}N_4O_{13}$: C, 52.33; H, 6.59; N, 8.72. Found: C, 52.15; H, 6.65; N, 8.58.

*1-*N-[N-*(*tert-*Butoxycarbonyl)-*L-*aspart-1-oyl-(*L-*phenylalanyl-*L-*alanine methyl ester)-4-oyl]-β-*D-*glucopyranosylamine* (**36**). — Compound **26** (465 mg), *N*-hydroxysuccinimide (138 mg), dicyclohexylcarbodiimide (227 mg), and **6** (224 mg) were treated as described for the preparation of **32** to give a crystalline residue (254 mg, 41%) by evaporation, m.p. 142–146° (dec.), $[\alpha]_D^{24}$ −62° (*c* 1, methanol); R_F (*A*) 0.86, R_F (*B*) 0.73; ν_{max}^{KBr} 3300 (br. OH and NH), 1740 (CO_2Me), 1670 (*tert*-butoxycarbonyl CO), and 1660–1520 cm^{-1} (peptide amide I); amino acid analysis: Asp:Phe:Ala 1.00:1.06:1.10.

Anal. Calc for $C_{28}H_{42}N_4O_{12}$: C, 53.66; H, 6.76; N, 8.94. Found: C, 53.52; H, 6.84; N, 8.84.

*1-*N-[N-*(*tert-*Butoxycarbonyl)-*L-*aspart-1-oyl-(*D-*phenylalanyl-*L-*serine methyl ester)-4-oyl]-β-*D-*glucopyranosylamine* (**37**). — Compound **31** (481 mg), *N*-hydroxysuccinimide (138 mg), dicyclohexylcarbodiimide (227 mg), and **6** (224 mg) were treated as described for the preparation of **32**. The residual oil was purified by silica gel column chromatography to give hygroscopic crystals (149 mg, 23%), $[\alpha]_D^{24}$ +30° (*c* 1, methanol); R_F (*A*) 0.85, R_F (*B*) 0.70; ν_{max}^{KBr} 3300 (br. OH and NH), 1740 (CO_2Me), 1670 (*tert*-butoxycarbonyl CO), and 1700–1530 cm^{-1} (peptide amide I); amino acid analysis: Asp:Phe:Ser 1.00:1.03:1.08.

Anal. Calc. for $C_{28}H_{42}N_4O_{13}$: C, 52.33; H, 6.59; N, 8.72. Found: C, 52.41; H, 6.50; N, 8.66.

*1-*N-[N-*(*tert-*Butoxycarbonyl)-*L-*aspart-1-oyl-(*L-*phenylalanyl-*L-*serine methyl

ester)-oyl]-4-O-(β-D-glucopyranosyl)-β-D-glucopyranosylamine (**38**). — Compound **20** (481 mg), *N*-hydroxysuccinimide (138 mg), dicyclohexylcarbodiimide (227 mg), and **9** (341 mg) were treated as described for the preparation of **32**. The residual oil was purified by silica gel column chromatography with 3:1 benzene–acetone as eluent to give a crystalline residue (144 mg, 18%), m.p. 168–169° (dec.), $[\alpha]_D^{24}$ −38° (*c* 1, methanol); R_F (*A*) 0.85, R_F (*B*) 0.63; ν_{max}^{KBr} 3300 (br. OH and NH), 1740 (CO_2Me), 1680 (*tert*-butoxycarbonyl CO), and 1700–1530 cm^{-1} (peptide amide I); amino acid analysis: Asp:Phe:Ser 1.00:0.96:1.04.

Anal. Calc. for $C_{34}H_{52}N_4O_{18}$: C, 50.74; H, 6.51; N, 6.96. Found: C, 50.92; H, 6.46; N, 7.00.

1-N-[N-(tert-*Butoxycarbonyl*)-L-*aspart-1-oyl*-(L-*phenylalanyl*-L-*alanine methyl ester)-oyl*]-*4*-O-(*β*-D-*glucopyranosyl*)-*β*-D-*glucopyranosylamine* (**39**). — Compound **26** (481 mg), *N*-hydroxysuccinimide (138 mg), dicyclohexylcarbodiimide (227 mg), and **9** (341 mg) were treated as described for the preparation of **32** to give a crystalline residue (209 mg, 26%), m.p. 140–145° (dec.), $[\alpha]_D^{24}$ −65° (*c* 1, methanol); R_F (*A*) 0.87, R_F (*B*) 0.70; ν_{max}^{KBr} 3300 (br. OH and NH), 1740 (CO_2Me), 1670 (*tert*-butoxycarbonyl CO), and 1690–1520 cm^{-1} (peptide amide I); amino acid analysis: Asp:Phe:Ala 1.00:0.96:1.01.

Anal. Calc. for $C_{34}H_{52}N_4O_{17}$: C, 51.77; H, 6.65; N, 7.10. Found: C, 51.95; H, 6.54; N, 6.99.

1-N-[N-(tert-*Butoxycarbonyl*)-L-*aspart-1-oyl*-(D-*phenylalanyl*-L-*serine methyl ester)-4-oyl*]-*4*-O-(*β*-D-*glucopyranosyl*)-*β*-D-*glucopyranosylamine* (**40**). — Compound **31** (481 mg), *N*-hydroxysuccinimide (138 mg), dicyclohexylcarbodiimide (227 mg) and **9** (341 mg) were treated as described for the preparation of **32**. The residual oil was purified by silica gel column chromatography to give a crystalline residue (144 mg, 18%), m.p. 124–127° (dec.), $[\alpha]_D^{24}$ +22° (*c* 1, methanol); R_F (*A*) 0.86, R_F (*B*) 0.68; ν_{max}^{KBr} 3300 (br. OH and NH), 1740 (CO_2Me), 1670 (*tert*-butoxycarbonyl CO), and 1700–1530 cm^{-1} (peptide amide I); amino acid analysis: Asp:Phe:Ser 1.00:1.08:0.97.

Anal. Calc. for $C_{34}H_{52}N_4O_{18}$: C, 50.74; H, 6.51; N, 6.96. Found: C, 50.63; H, 6.41; N, 7.01.

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