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THE SYNTHESIS OF GLYCOPEPTIDE FRAGMENTS OF HUMAN PLASMA α_1-ACID GLYCOPROTEINS BY SEQUENTIAL ELONGATION AT THE TERMINAL-AMINO GROUP*

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ABSTRACT

Glycopeptides corresponding to sequences 27–28, 48–49, and 58–59 of human plasma α_1-acid glycoproteins have been synthesized by sequential elongation of the peptide chain at the terminal amino group. 2-Acetamido-3,4,6-tri-*O*-acetyl-1-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine was condensed with the *p*-nitrophenyl esters of protected amino acids to give the corresponding protected glycodipeptides having the sequences Gly-(GlcNAc-4-)Asn, Pro-(GlcNAc-4-)Asn, Val-(GlcNAc-4-)Asn, Leu-(GlcNAc-4-)Asn, Glu-(GlcNAc-4-)Asn, Tyr-(GlcNAc-4-)Asn, Ser-(GlcNAc-4-)Asn, and Cys-(GlcNAc-4-)Asn. Deprotection of the carbohydrate and of the peptide residues of these compounds was achieved, except for those having *N-tert*-butyloxycarbonyl protective groups, to give the corresponding free glycopeptides. The glycotripeptide 2-acetamido-1-*N*-{2-*N*-[*N*-(*tert*-butyloxycarbonyl)-L-glutam-1-oyl-L-tyrosyl]-L-aspart-4-oyl}-2-deoxy-β-D-glucopyranosylamine, having the amino acid sequence 10–12 of human plasma α_1-acid glycoprotein, was prepared by condensation of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-*N*-[2-*N*-(L-tyrosyl)-L-aspart-4-oyl]-β-D-glucopyranosylamine with 5-benzyl 1-*p*-nitrophenyl *N*-(*tert*-butyloxycarbonyl)-L-glutamate, followed by removal of the ester groups.

INTRODUCTION

Excretion glycoproteins, such as plasma glycoproteins[1], ribonuclease B[2], rat-liver microsomes[3], ovalbumin[4], *Aspergillus* α-amylase[4], pineapple bromelain[4],

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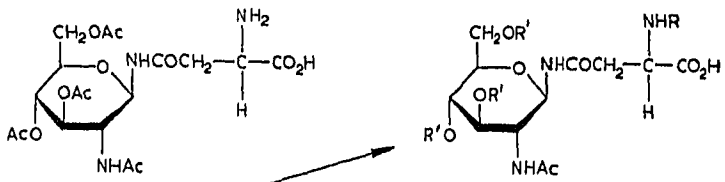
λ-G myeloma glycoprotein[5], silk fibroin[6], and thyroglobulin[7], are composed of carbohydrate chain(s) linked to the peptide chain through the 2-acetamido-1-*N*-(L-aspart-4-oyl)-2-deoxy-D-glucopyranosylamine residue. Mainly on the basis of degradative studies, the amino acid sequence of the peptide chains[8,9], as well as the sequence, position, and configuration of the interglycosidic linkages of the constituting monosaccharide units[10,11] of many of these glycoproteins have been elucidated.

Although the synthesis of part of the peptide[12] and carbohydrate[13] chains of various glycoproteins has been extensively studied, far less has been reported on the synthesis of glycopeptides. As a part of a program for the synthesis of oligosaccharide-L-asparagine compounds[14–19], as well as larger glycopeptides[20], we describe now the synthesis of some protected and free glycodipeptides and of one glycotripeptide containing the 2-acetamido-1-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glycopyranosylamine residue linked to one or two amino acid residues at the amino group of the aspartic acid residue. These glycopeptides include the sequences 10–12, 27–28, 48–49, and 58–59 of human plasma α_1-acid glycoprotein[9]. These compounds may be used as references for comparative characterization of fragments obtained from the degradation of glycopeptides and glycoproteins, as starting materials for the synthesis of larger glycopeptides, and as acceptors in the study of the biosynthesis of glycoproteins.

DISCUSSION

In a previous publication from this laboratory[20], the synthesis of protected glycotetra- and glycopenta-peptides containing the 2-acetamido-1-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosyl amino residue and the amino acid sequences 34–37 and 34–38, respectively, of beef ribonuclease B, was described. The method selected for the synthesis of these glycopeptides was the attachment of a preformed oligopeptide to the free 1-carboxylic group of the glycosyl-L-asparagine derivative, and the advantages of this method were discussed. In the present study, we have investigated the conditions for sequential elongation of a glycopeptide in the direction of the amino-terminal group. Condensation of this group with a protected *p*-nitrophenyl ester of an amino acid is known to give good yields and to proceed without racemization[21]. Isolation, from the unreacted starting material, of the glycopeptide thus formed was facilitated by use of an excess of the protected amino acid *p*-nitrophenyl ester. By monitoring the reaction with t.l.c., we have found that the aminolysis reaction proceeds smoothly and that the only by-product present, in addition to the unreacted, protected amino acid starting material, is *p*-nitrophenol. These by-products were readily removed by washing the reaction product with ether. Thus, 2-acetamido-3,4,6-tri-*O*-acetyl-1-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine[22] (**1**) was condensed with the *p*-nitrophenyl esters of *N*-(benzyloxycarbonyl)-glycine (**2**), *N*-(benzyloxycarbonyl)-L-proline (**3**), *N*-(benzyloxycarbonyl)-L-valine (**4**), *N*-(benzyloxycarbonyl)-L-leucine (**5**), 5-benzyl *N*-(*tert*-butyloxycarbonyl)-L-glutamate



2 R = *N*-Zglycyl
3 R = *N*-Z-L-prolyl
4 R = *N*-Z-L-valyl
5 R = *N*-Z-L-leucyl
6 R = 5-benzyl *N*-Boc-L-glutam-l-oyl
7 R = *N*-Z-L-tyrosyl

8 R = *O*-Bzl-*N*-Boc-L-seryl

9 R = *S*-Bzl-*N*-Z-L-cysteinyl

10 R = *N*-Zglycyl, R′ = Ac
11 R = *N*-Z-L-prolyl, R′ = Ac
12 R = *N*-Z-L-valyl, R′ = Ac
13 R = *N*-Z-L-leucyl, R′ = Ac
14 R = 5-benzyl *N*-Boc-L-glutam-l-oyl, R′ = Ac
15 R = *N*-Z-L-tyrosyl, R′ = Ac

16 R = *O*-Bzl-*N*-Boc-L-seryl, R′ = Ac
17 R = *S*-Bzl-*N*-Z-L-cysteinyl, R′ = Ac

18 R = *N*-Zglycyl, R′ = H
19 R = *N*-Z-L-prolyl, R′ = H
20 R = *N*-Z-L-valyl, R′ = H
21 R = *N*-Z-L-leucyl, R′ = H
22 R = *N*-Boc-L-glutam-l-oyl, R′ = H
23 R = *N*-Z-L-tyrosyl, R′ = H

24 R = *O*-Bzl-*N*-Boc-L-seryl, R′ = H
25 R = *S*-Bzl-*N*-Z-L-cysteinyl, R′ = H

26 R = glycyl, R′ = H
27 R = L-prolyl, R′ = H
28 R = L-valyl, R′ = H
29 R = L-tyrosyl, R′ = H
30 R = L-tyrosyl, R′ = Ac

31 R = 5-benzyl *N*-Boc-L-glutam-l-oyl-L-tyrosyl, R′ = H
32 R = *N*-Boc-L-glutam-l-oyl-L-tyrosyl, R′ = H

Z = $PhCH_2OC(=O)-$
Boc = $Me_3COC(=O)-$
Bzl = $PhCH_2-$

(**6**), *N*-(benzyloxycarbonyl)-L-tyrosine (**7**), *O*-benzyl-*N*-(*tert*-butyloxycarbonyl)-L-serine (**8**), and *S*-benzyl-*N*-(benzyloxycarbonyl)-L-cysteine (**9**) to give the corresponding protected glycopeptides **10**–**17**, respectively.

O-Deacetylation of the carbohydrate residues of **10**–**13** and **15**–**17** was performed with a catalytic amount of sodium methoxide to give **18**–**21** and **23**–**25**, respectively. In order to avoid transesterification of the glutamoyl residue, previously observed during de-esterification of protected glycosyl-L-asparaginyl compounds with sodium methoxide[18], simultaneous removal of the *O*-acetyl groups of the sugar residues and of the benzyl group of the glutamic acid of derivative **14** to give **22** was achieved by aqueous lithium hydroxide.

Removal of the *N*-benzyloxycarbonyl groups from **18**–**20** and **23** to give **26**–**28** and **29**, respectively, was achieved by hydrogenolysis in the presence of 10% palladium-on-charcoal at room temperature and under normal pressure. Attempted removal of the *N*-*tert*-butyloxycarbonyl group of **22** and **24** with hydrogen bromide in acetic acid failed; it gave degraded products, probably because the glycosyl–asparagine linkage was sensitive to the drastic acid conditions.

In order to prepare the protected glycotripeptide **32**, the *N*-benzyloxycarbonyl group of **15** was hydrogenolyzed without previous *O*-deacetylation, to give 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-*N*-[2-*N*-(L-tyrosyl)-L-aspart-4-oyl]-β-D-glucopyranosylamine (**30**). The free, terminal amino group was then condensed with 5-benzyl 1-*p*-nitrophenyl *N*-(*tert*-butyloxycarbonyl)-L-glutamate to give the protected glycotripeptide **31**, which was further hydrolyzed to give **32**. Retention of the *O*-acetyl groups of **30** was a prerequisite in order to maintain a reasonable solubility in the solvent used for the condensation.

EXPERIMENTAL

General methods. — Melting points were determined with a Mettler FP-2 apparatus, and correspond to "corrected melting points". Optical rotations were determined for solutions in 1-dm semimicro tubes with a Perkin–Elmer model 141 polarimeter. I.r. spectra were recorded with a Perkin–Elmer Model 237 spectrophotometer. The homogeneity of compounds was verified by ascending t.l.c. on precoated Silica Gel G plates (Silplate 22; E. Merck, Darmstadt, Germany; layer thickness 0.25 mm). Unless otherwise stated, the protected amino acid *p*-nitrophenyl esters were supplied by Cyclo Chemical Corp., Los Angeles, California 90001, U.S.A. The spray reagent used was anisaldehyde–sulfuric acid–ethanol (1:1:18, v/v) and the plates were developed by heating for a few min on a hot plate. Evaporations were conducted *in vacuo*, with a bath temperature below 45°. Solutions (<5 ml) in volatile solvents were evaporated under a stream of nitrogen. Microanalyses were performed by Dr. W. Manser, Zurich, Switzerland.

*2-Acetamido-3,4,6-tri-*O*-acetyl-1-*N*-{2-*N*-[*N*-(benzyloxycarbonyl)glycyl]-L-aspart-4-oyl}-2-deoxy-β-D-glucopyranosylamine* (**10**). — A solution of 2-acetamido-3,4,6-tri-*O*-acetyl-1-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine[22] (**1**,

100 mg) in a mixture of *N,N*-dimethylformamide (1 ml) and chloroform (10 ml) was treated with triethylamine (10 drops) and *N*-(benzyloxycarbonyl)glycine *p*-nitrophenyl ester (**2**, 100 mg; prepared according to Benoiton[23]) and stirred for 24 h at room temperature. The reaction was monitored by t.l.c. in 1:1 (v/v) chloroform–methanol; the starting material (R_F 0.19) was gradually transformed into **10** (R_F 0.46). The mixture was stirred with Dowex-50 (H^+) cation-exchange resin (2 ml) for 5 min and filtered through a sintered-glass funnel. The exchange resin was washed with chloroform (5 ml), and the filtrate and washings were evaporated. The residue, which contained *p*-nitrophenol as a by-product, was washed repeatedly with warm ether until the ether washings gave no yellow color with sodium methoxide. Crystallization of the residue from water–methanol gave 105 mg (79%) of needles, m.p. 238–240°, $[\alpha]_D^{20}$ +22° (*c* 0.6, 50% methanol); ν_{max}^{KBr} 3480 (shoulder, OH of CO_2H), 3300 (NH), 1745 (OAc and CO_2H), 1655 (Amide I), 1535 (Amide II), 735, and 695 cm^{-1} (Ph).

Anal. Calc. for $C_{28}H_{36}N_4O_{14}$: C, 51.53; H, 5.56; N, 8.59; O, 34.33. Found: C, 51.50; H, 5.37; N, 8.68; O, 34.68.

*2-Acetamido-1-*N-{*2-*N-[N-(*benzyloxycarbonyl*)*glycyl-*L-*aspart-4-oyl*}*-2-deoxy-β-*D-*glucopyranosylamine* (**18**). — A suspension of **10** (65 mg) in methanol (10 ml) was treated with a solution (2 ml) of 0.1M sodium methoxide in methanol. The mixture was stirred at room temperature until complete dissolution of the starting material occurred and then kept for a further 2 h. The solution was deionized by stirring it with Dowex-50 (H^+) cation-exchange resin (1 ml). After filtration and evaporation of the filtrate, the residue crystallized from methanol–ether to give 43 mg (82%), m.p. 181–182° (dec.), $[\alpha]_D^{22}$ −39° (*c* 0.6, methanol); ν_{max}^{KBr} 3280 (broad, OH and NH), 1720 (CO_2H), 1655 (Amide I), 1540 (Amide II), 725, and 680 cm^{-1} (Ph).

Anal. Calc. for $C_{22}H_{30}N_4O_{11}$: C, 50.20; H, 5.74; N, 10.64; O, 33.43. Found: C, 50.11; H, 5.78; N, 10.69; O, 33.52.

*2-Acetamido-2-deoxy-1-*N-[*2-*N-(*glycyl*)-L-*aspart-4-oyl*]-*β-*D-*glucopyranosylamine* (**26**). — A solution of **18** (52 mg), in 1:1 (v/v) methanol–water (50 ml) was hydrogenated for 2 h with 10% palladium-on-charcoal (50 mg) under atmospheric pressure and at room temperature. The catalyst was filtered off through a bed of Celite and washed with water (5 ml). The combined filtrate and washings were evaporated to dryness, and the residue was crystallized from water–methanol to give needles (32 mg), m.p. 242–243°, $[\alpha]_D^{20}$ +29° (*c* 0.6, water); ν_{max}^{KBr} 3550 (OH), 3280 (NH), 1680, 1650 (Amide I), 1620 (CO_2^-), and 1545 cm^{-1} (Amide II).

Anal. Calc. for $C_{14}H_{24}N_4O_9 \cdot H_2O$: C, 40.98; H, 6.39; N, 13.65; O, 38.99. Found: C, 40.96; H, 6.47; N, 13.69; O, 38.87.

*2-Acetamido-3,4,6-tri-*O*-acetyl-1-*N-{*2-*N-[N-(*benzyloxycarbonyl*)-L-*prolyl*]-L-*aspart-4-oyl*}*-2-deoxy-β-*D-*glucopyranosylamine* (**11**). — A solution of **1** (100 mg) in 10:1 (v/v) chloroform–*N,N*-dimethylformamide (11 ml) was treated with triethylamine (10 drops), and then with *N*-(benzyloxycarbonyl)-L-proline *p*-nitrophenyl ester (**3**, 100 mg, prepared according to Bodanszky and du Vigneaud[24]. The reaction mixture was stirred for 24 h at room temperature, and then processed as described for the preparation of **10**. The product (135 mg, 82%) crystallized from methanol in

needles, m.p. 219–221° (dec.), $[\alpha]_D^{20}$ −7.2° (*c* 0.6, methanol); ν_{max}^{KBr} 3500 (shoulder, OH of CO_2H), 3285 (NH), 1740 (OAc and CO_2H), 1690, 1660 (Amide I), 1540 (Amide II), 765, and 690 cm^{-1} (Ph).

Anal. Calc. for $C_{31}H_{40}N_4O_{14}$: C, 53.76; H, 5.82; N, 8.09; O, 32.33. Found: C, 53.63; H, 5.78; N, 8.06; O, 32.19.

*2-Acetamido-1-*N-{*2-*N-[N-(*benzyloxycarbonyl*)-L-*prolyl*]-L-*aspart-4-oyl*}-*2-deoxy-β-*D-*glucopyranosylamine* (**19**). — A solution of **11** (70 mg) in methanol (10 ml) was treated with 0.1M sodium methoxide in methanol (2 ml), and the mixture was processed as described for **18**. The product (49 mg, 88%) crystallized from methanol; m.p. 198–200° (dec.), $[\alpha]_D^{20}$ +6.0° (*c* 0.4, methanol); ν_{max}^{KBr} 3450 (OH), 3285 (NH), 1725 (CO_2H), 1670, 1660, 1635 (Amide I), 1540 (Amide II), 745, and 690 cm^{-1} (Ph).

Anal. Calc. for $C_{25}H_{34}N_4O_{11}$: C, 52.99; H, 6.05; N, 9.89; O, 31.06. Found: C, 52.90; H, 6.02; N, 9.81; O, 31.20.

*2-Acetamido-2-deoxy-1-*N-[*2-*N-(L-*prolyl*)-L-*aspart-4-oyl*]-*β-*D-*glucopyranosylamine* (**27**). — A solution of **19** (57 mg) in 50% methanol (50 ml) was hydrogenolyzed for 2 h at room temperature and under normal pressure in the presence of 10% palladium-on-charcoal (50 mg). The residue (39 mg, 93%), obtained after removal of the catalyst by filtration and evaporation of the solvent, was crystallized from water–methanol to give **27**, m.p. 214–215° (dec.), $[\alpha]_D^{20}$ +13° (*c* 0.4, 50% methanol); ν_{max}^{KBr} 3300 (broad, OH and NH), 1675, 1640 (Amide I), 1580 (CO_2^-), and 1540 cm^{-1} (Amide II).

Anal. Calc. for $C_{17}H_{28}N_4O_9$: C, 47.22; H, 6.53; N, 12.96; O, 33.30. Found: C, 47.29; H, 6.50; N, 12.96; O, 33.45.

*2-Acetamido-3,4,6-tri-*O-*acetyl-1-*N-{*2-*N-[N-(*benzyloxycarbonyl*)-L-*valyl*]-L-*aspart-4-oyl*}-*2-deoxy-β-*D-*glucopyranosylamine* (**12**). — To a solution of **1** (460 mg) in 10:1 (v/v) chloroform–*N,N*-dimethylformamide (55 ml) was added triethylamine (2.5 ml) and *N*-(benzyloxycarbonyl)-L-valine *p*-nitrophenyl ester[25] (**4**, 400 mg, "Cyclo"). The mixture was stirred for 24 h at room temperature and processed as described for **10** to give 500 mg (72%), of **12** after crystallization from methanol; m.p. 231–233° (dec.), $[\alpha]_D^{20}$ −4.3° (*c* 0.3, methanol); ν_{max}^{KBr} 3500 (shoulder, OH of CO_2H), 3300 (NH), 1740 (OAc and CO_2H), 1680, 1650 (Amide I), 1530 (Amide II), 745, and 685 cm^{-1} (Ph).

Anal. Calc. for $C_{31}H_{42}N_4O_{14}$: C, 53.60; H, 6.09; N, 8.07; O, 32.24. Found: C, 53.47; H, 6.00; N, 7.97; O, 32.10.

*2-Acetamido-1-*N-{*2-*N-[N-(*benzyloxycarbonyl*)-L-*valyl*]-L-*aspart-4-oyl*}-*2-deoxy-β-*D-*glucopyranosylamine* (**20**). — A solution of **12** (189 mg) in methanol (30 ml) was treated with 0.1M sodium methoxide in methanol (6 ml) as described for **19**. The product (62 mg, 86%) was crystallized from water–methanol; m.p. 263° (dec.), $[\alpha]_D^{22}$ +14.5° (*c* 0.3, *N,N*-dimethylformamide); ν_{max}^{KBr} 3546, 3460 (OH), 3300, 3260 (NH), 1730 (CO_2H), 1680, 1660 (Amide I), 1570–1530 (Amide II), 750, and 690 cm^{-1} (Ph).

Anal. Calc. for $C_{25}H_{36}N_4O_{11}$: C, 52.81; H, 6.38; N, 9.85; O, 30.95. Found: C, 52.78; H, 6.36; N, 9.75; O, 31.03.

*2-Acetamido-2-deoxy-1-*N-[*2-*N-(L-*valyl*)-L-*aspart-4-oyl*]-*β-*D-*glucopyranosylamine* (**28**). — A solution of **20** (225 mg) in 1:1 (v/v) methanol–water (50 ml) was

hydrogenolyzed for 2 h at room temperature and under normal pressure in the presence of 10% palladium-on-charcoal (50 mg). Removal of the catalyst and evaporation of the solvent gave 212 mg (94%) of **20**, which crystallized from water–methanol as a monohydrate, m.p. 213–215° (dec.), $[\alpha]_D^{20}$ +39° (*c* 0.4, methanol); ν_{max}^{KBr} 3290 (broad, OH and NH), 1675–1645 (Amide I), 1620 (CO_2^-), and 1545–1530 cm^{-1} (Amide II).

Anal. Calc. for $C_{17}H_{30}N_4O_9 \cdot H_2O$: C, 45.13; H, 7.13; N, 12.36; O, 35.36. Found: C, 45.08; H, 6.90; N, 12.16; O, 35.64.

*2-Acetamido-3,4,6-tri-*O*-acetyl-1-*N*-{2-*N*-[*N*-(benzyloxycarbonyl)-*L*-leucyl]-*L*-aspart-4-oyl}-2-deoxy-β-*D*-glucopyranosylamine* (**13**). — To a solution of **1** (230 mg) in 25:3 (v/v) chloroform–*N*,*N*-dimethylformamide (28 ml) was added triethylamine (1.5 ml) and *N*-(benzyloxycarbonyl)-L-leucine *p*-nitrophenyl ester[24] (**5**, 200 mg, "Cyclo"). The mixture was stirred at room temperature for 24 h, and then processed as described for **10** to give a product (297 mg, 84%) that crystallized from methanol–ether–pentane; m.p. 234–236° (dec.), $[\alpha]_D^{20}$ +13° (*c* 0.3, methanol); ν_{max}^{KBr} 3500 (shoulder OH of CO_2H), 3305 (NH), 1740 (OAc and CO_2H), 1680, 1660 (Amide I), 1530 (Amide II), 740, and 690 cm^{-1} (Ph).

Anal. Calc. for $C_{32}H_{44}N_4O_{14}$: C, 54.25; H, 6.26; N, 7.91; O, 31.61. Found: C, 54.15; H, 6.24; N, 7.86; O, 31.70.

*2-Acetamido-1-*N*-{2-*N*-[*N*-(benzyloxycarbonyl)-*L*-leucyl]-*L*-aspart-4-oyl}-2-deoxy-β-*D*-glucopyranosylamine* (**21**). — A solution of **13** (100 mg) in methanol (25 ml) was treated with 0.1M sodium methoxide in methanol (4 ml) and the mixture was processed as described for **18**. The product (71 mg, 85%) crystallized from methanol–ether; m.p. 206–207° (dec.), $[\alpha]_D^{20}$ +24° (*c* 0.7, methanol); ν_{max}^{KBr} 3450 (shoulder, OH), 3290 (NH), 1715 (CO_2H), 1680, 1650 (Amide I), 1530 (Amide II), 730, and 680 cm^{-1} (Ph).

Anal. Calc. for $C_{26}H_{38}N_4O_{11}$: C, 53.62; H, 6.57; N, 9.62; O, 30.21. Found: C, 53.50; H, 6.48; N, 9.65; O, 30.52.

*2-Acetamido-3,4,6-tri-*O*-acetyl-1-*N*-{2-*N*-[5-benzyl* N-(tert-*butyloxycarbonyl*)-*L*-glutam-1-oyl]-*L*-aspart-4-oyl}-2-deoxy-β-*D*-glucopyranosylamine* (**14**). — To a solution of **1** (460 mg) in 17:3 (v/v) chloroform–*N*,*N*-dimethylformamide (40 ml) was added 5-benzyl 1-*p*-nitrophenyl *N*-(*tert*-butyloxycarbonyl)-L-glutamate[26] (**6**, 500 mg, "Cyclo") and triethylamine (3 ml). The mixture was stirred for 24 h at room temperature, and **14** (508 mg, 75%), obtained as described for **10**, crystallized from chloroform–ether; m.p. 182–184°, $[\alpha]_D^{20}$ +37° (*c* 0.62, chloroform); ν_{max}^{KBr} 3550 (shoulder, OH of CO_2H), 3325 (NH), 1740 (ester C=O and CO_2H), 1680, 1660 (Amide I), 1530 (Amide II), 740, and 690 cm^{-1} (Ph).

Anal. Calc. for $C_{35}H_{48}N_4O_{16}$: C, 53.84; H, 6.18; N, 7.18; O, 32.78. Found: C, 53.90; H, 6.25; N, 7.08; O, 32.81.

*2-Acetamido-1-*N*-{2-*N*-[*N*-(tert-*butyloxycarbonyl*)-*L*-glutam-1-oyl]-*L*-aspart-4-oyl}-2-deoxy-β-*D*-glucopyranosylamine* (**22**). — A suspension of **14** (390 mg) in 0.1M lithium hydroxide (15 ml) was stirred for 2 h at room temperature. The solution was deionized with Dowex-50 (H^+) cation-exchange resin, and evaporated *in vacuo*. The

residue (231 mg, 82%) was crystallized from methanol to give **22**, m.p. 189–191°, $[\alpha]_D^{20}$ +26° (*c* 0.3, methanol); ν_{max}^{KBr} 3300 (broad, OH and NH), 1730 (CO_2H), 1675, 1650 (Amide I), and 1530 cm^{-1} (Amide II).

Anal. Calc. for $C_{22}H_{36}N_4O_{13} \cdot 0.5H_2O$: C, 46.06; H, 6.51; N, 9.77. Found: C, 46.11; H, 6.54; N, 9.47.

*2-Acetamido-3,4,6-tri-*O*-acetyl-1-*N*-{2-*N*-[*N*-(benzyloxycarbonyl)-*L*-tyrosyl]-*L*-aspart-4-oyl}-2-deoxy-β-*D*-glucopyranosylamine* (**15**). — Condensation of **1** (460 mg) with *N*-(benzyloxycarbonyl)-L-tyrosine *p*-nitrophenyl ester[27] (**7**, 460 mg, "Cyclo") in 5:1 (v/v) chloroform–*N*,*N*-dimethylformamide (30 ml) in the presence of triethylamine (2 ml), as described for **10** gave 560 mg (72%) of **15**, which was not purified but was used directly to prepare the unprotected derivatives **23**, **29**, and **30**.

*2-Acetamido-1-*N*-{2-*N*-[*N*-(benzyloxycarbonyl)-*L*-tyrosyl]-*L*-aspart-4-oyl}-2-deoxy-β-*D*-glucopyranosylamine* (**23**). — A solution of **15** (190 mg) in methanol (20 ml) was *O*-deacetylated with 0.1M sodium methoxide in methanol (2 ml) for 2 h at room temperature. The solution was deionized by filtration through Dowex-50 (H^+) cation-exchange resin and evaporated. The residue crystallized from methanol to give 129 mg (82%) of **23**, m.p. 217° (dec.), $[\alpha]_D^{20}$ +14° (*c* 0.9, methanol); ν_{max}^{KBr} 3450 (OH), 3300 (NH), 1690 (CO_2H), 1650 (Amide I), 1535 (Amide II), 740, and 690 cm^{-1} (Ph).

Anal. Calc. for $C_{29}H_{36}N_4O_{12}$: C, 55.07; H, 5.74; N, 8.86; O, 30.35. Found: C, 54.90; H, 5.84; N, 8.95; O, 30.35.

*2-Acetamido-2-deoxy-1-*N*-[2-*N*-(*L*-tyrosyl)-*L*-aspart-4-oyl]-β-*D*-glucopyranosylamine* (**29**). — A solution of **23** (63 mg) in 1:1 methanol–water (50 ml) was hydrogenolyzed in the presence of 10% palladium-on-charcoal (50 mg) for 2 h at room temperature and under atmospheric pressure. The catalyst was filtered off on Celite, washed, and the filtrate evaporated. The residue was crystallized from water–methanol to give 47 mg (93%) of **29**, m.p. 212–214° (dec.), $[\alpha]_D^{20}$ +46° (*c* 0.4, 50% methanol); ν_{max}^{KBr} 3300 (broad, OH and NH), 1660–1650 (Amide I), 1610 (CO_2^-), 1550 cm^{-1} (Amide II).

Anal. Calc. for $C_{21}H_{30}N_4O_{10} \cdot H_2O$: C, 48.83; H, 6.25; N, 10.85; O, 34.08. Found: C, 48.56; H, 6.22; N, 10.87; O, 34.30.

*2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-*N*-[2-*N*-(*L*-tyrosyl)-*L*-aspart-4-oyl]-β-*D*-glucopyranosylamine* (**30**). — A solution of **15** (300 mg) in 90% acetic acid (100 ml) was hydrogenolyzed at room temperature and under atmospheric pressure for 4 h in the presence of 10% palladium-on-charcoal (200 mg). The catalyst was filtered off on Celite, and washed with water. The filtrate and washings were evaporated and the residue dried by repeated addition and distillation of toluene. It was crystallized from water–methanol to give 235 mg (94%) of **30**, m.p. 207–208°, $[\alpha]_D^{20}$ +16.0° (*c* 0.35, 50% methanol); ν_{max}^{KBr} 3625, 3490 (OH), 3310, 3250 (NH), 1740, 1725 (OAc), 1675 (Amide I), 1620 (CO_2^-), 1575, 1550, 1530 (Amide II), 740, and 690 cm^{-1} (Ph).

Anal. Calc. for $C_{27}H_{36}N_4O_{13}$: C, 51.92; H, 5.81; N, 8.97; O, 33.31. Found: C, 51.73; H, 5.89; N, 8.82; O, 33.38.

*2-Acetamido-3,4,6-tri-*O*-acetyl-1-*N*-{2-*N*-[O-benzyl-*N*-(tert-butyloxycarbonyl)-*L*-seryl]-*L*-aspart-4-oyl}-2-deoxy-β-*D*-glucopyranosylamine* (**16**). — A solution of **1**

(230 mg) in 4:1 (v/v) of chloroform–*N*,*N*-dimethylformamide (25 ml) was treated with *O*-benzyl-*N*-(*tert*-butyloxycarbonyl)-L-serine *p*-nitrophenyl ester (**8**, 300 mg, "Cyclo") and triethylamine (1 ml). After being stirred for 24 h at room temperature, the mixture was processed as described for **10**, and **16** (248 mg, 67%) was crystallized from methanol–ether; m.p. 175–176°, $[\alpha]_D^{20}$ +22° (*c* 0.35, methanol); ν_{max}^{KBr} 3550 (shoulder, OH of CO_2H), 3300 (NH), 1740 (OAc and CO_2H), 1680, 1660 (Amide I), 1545, 1535 (Amide II), 740, and 695 cm^{-1} (Ph).

Anal. Calc. for $C_{33}H_{46}N_4O_{15}$: C, 53.64; H, 6.28; N, 7.58; O, 32.48. Found: C, 53.52; H, 6.31; N, 7.58; O, 32.29.

2-Acetamido-1-N-{*2*-N-[O-*benzyl*-N-(tert-*butyloxycarbonyl*)-L-*seryl*]-L-*aspart-4-oyl*}-*2-deoxy-β*-D-*glucopyranosylamine* (**24**). — A solution of **16** (185 mg) in methanol (20 ml) was treated with 0.1M sodium methoxide in methanol (2 ml) for 2 h at room temperature. The solution was deionized with Dowex-50 (H^+) cation-exchange resin and evaporated under diminished pressure. The residue was crystallized from methanol to give 129 mg (84%) of **24**, m.p. 171–173°, $[\alpha]_D^{20}$ +43° (*c* 0.5, methanol); ν_{max}^{KBr} 3300 (broad, OH and NH), 1715 (CO_2H), 1650 (Amide I), 1535 (Amide II), 730, and 695 cm^{-1} (Ph).

Anal. Calc. for $C_{27}H_{40}N_4O_{12}$: C, 52.93; H, 6.58; N, 9.15; O, 31.34. Found: C, 52.66; H, 6.52; N, 9.03; O, 31.59.

2-Acetamido-3,4,6-tri-O-*acetyl-1*-N-{*2*-N-[S-*benzyl*-N-(*benzyloxycarbonyl*)-L-*cysteinyl*]-L-*aspart-4-oyl*}-*2-deoxy-β*-D-*glucopyranosylamine* (**17**). — A solution of **1** (115 mg) in 1:5 (v/v) of chloroform–*N*,*N*-dimethylformamide (18 ml) was stirred for 24 h with *S*-benzyl-*N*-(benzyloxycarbonyl)-L-cysteine *p*-nitrophenyl ester[24] (**9**, 180 mg, "Cyclo") in the presence of triethylamine (1 ml). After the mixture had been processed as described for **10**, the product (**17**, 164 mg, 83%) was crystallized from chloroform–methanol; m.p. 211–212° (dec.), $[\alpha]_D^{20}$ +10° (*c* 0.22, chloroform); ν_{max}^{KBr} 3500 (shoulder, OH of CO_2H), 3300 (NH), 1740 (OAc and CO_2H), 1655 (Amide I), 1530 (Amide II), 750, and 690 cm^{-1} (Ph).

Anal. Calc. for $C_{36}H_{44}N_4O_{14}S$: C, 54.83; H, 5.62; N, 7.10; S, 4.06. Found: C, 54.74; H, 5.62; N, 7.05; S, 4.23.

2-Acetamido-1-N-{*2*-N-[S-*benzyl*-N-(*benzyloxycarbonyl*)-L-*cysteinyl*]-L-*aspart-4-oyl*}-*2-deoxy-β*-D-*glucopyranosylamine* (**25**). — A solution of **17** (113 mg) in methanol (10 ml) was treated with 0.1M sodium methoxide in methanol (1 ml) for 2 h at room temperature. The mixture was passed through Dowex-50 (H^+) cation-exchange resin and evaporated. The residue crystallized from water–methanol to give 87 mg (93%) of **25**, m.p. 229–230° (dec.), $[\alpha]_D^{20}$ +9° (*c* 0.3, methanol–water 1:1); ν_{max}^{KBr} 3430 (OH), 3290 (NH), 1685 (CO_2H), 1645 (Amide I), 1540 (Amide II), 750, and 690 cm^{-1} (Ph).

Anal. Calc. for $C_{30}H_{38}N_4O_{11}S$: C, 54.37; H, 5.78; N, 8.45; S, 4.84. Found: C, 54.38; H, 5.86; N, 8.32; S, 4.91.

2-Acetamido-1-N-{*2*-N-[N-(tert-*butyloxycarbonyl*)-L-*glutam-1-oyl*-L-*tyrosyl*]-L-*aspart-4-oyl*}-*2-deoxy-β*-D-*glucopyranosylamine* (**32**). — A solution of **30** (312 mg) in 2:1 (v/v) chloroform–*N*,*N*-dimethylformamide (20 ml) was treated with 5-benzyl

1-*p*-nitrophenyl *N*-(*tert*-butyloxycarbonyl)-L-glutamate[26] (**6**, 500 mg, "Cyclo") and triethylamine (2 ml) and stirred for 2 h at room temperature. The mixture was stirred for 5 min with Dowex-50 (H^+) cation-exchange resin (3 ml) and filtered. The filtrate was evaporated to give a residue of 2-acetamido-3,4,6-tri-*O*-acetyl-1-*N*-{2-*N*-[5-benzyl *N*-(*tert*-butyloxycarbonyl)-L-glutam-1-oyl-L-tyrosyl]-L-aspart-4-oyl}-2-deoxy-β-D-glucopyranosylamine (**31**); ν_{max}^{KBr} 3500 (shoulder, OH of CO_2H), 3300 (NH), 1730–1720 (ester C=O and CO_2H), 1685–1655 (Amide I), 1540 (Amide II), 750, and 695 cm^{-1} (Ph). The residue was dissolved in methanol (20 ml) and treated with 0.1M sodium methoxide in methanol (3 ml) for 2 h at room temperature. The solution was passed through Dowex 50 (H^+) cation-exchange resin, and evaporated. Crystallization of the residue from methanol–ethyl acetate gave 225 mg (61%) of **32**, m.p. 177–179° (dec.), $[\alpha]^{20}$ +15° (*c* 0.3, methanol); ν_{max}^{KBr} 3290 (broad, OH and NH), 1710 (CO_2H) 1685–1645 (Amide I), and 1530 cm^{-1} (Amide II).

Anal. Calc. for $C_{31}H_{45}N_5O_{15}\cdot 0.5H_2O$: C, 50.55; H, 6.30; N, 9.48; O, 33.67. Found: C, 50.93; H, 6.30; N, 9.04; O, 33.31.

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