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Note

The synthesis of the 1-*N*-(L-aspart-4-oyl)glucosylamine linkage. New synthesis of 1-*N*-(L-aspart-1- and 4-oyl)-4-*O*-β-D-galactopyranosyl-β-D-glucopyranosylamines*

HARI G. GARG AND ROGER W. JEANLOZ†

Laboratory for Carbohydrate Research, Departments of Biological Chemistry and Medicine, Harvard Medical School and Massachusetts General Hospital, Boston, Massachusetts 02114 (U. S. A.)

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Synthetic oligosaccharide–asparagine compounds are of interest for determining the specificity of lectins for which the asparagine residue may be a part of the immunodeterminant[1], and for determining the chemical structure of antigenic sites containing a 1-*N*-(L-aspart-4-oyl)glycosylamine component[2].

The methods of synthesis of 2-acetamido-1-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine, the glycosylamino acid residue found as the carbohydrate–protein linkage of many glycoproteins of excretory origin[3], include both the reaction of the glycosylamine with *N*-(benzyloxycarbonyl)-L-aspartic anhydride[4] (**1**) and with a partially protected L-aspartoyl derivative, 1-benzyl *N*-(benzyloxycarbonyl)-L-aspartate[5] (**3**). In the first reaction, both the L-aspart-1- and 4-oyl derivatives are obtained and, consequently, yields lower than 50% are expected for each derivative. The possibility exists, however, that the reaction shows some specificity, which may vary with the type of reagent used[6]. One advantage of the first method over the second is that it avoids the preparation of the derivative protected at C-1, which may be both lengthy and give poor yields when applied to long-chain peptides. In order to test the stereoselectivity and the yield of the first method with an oligosaccharide, *N*-(benzyloxycarbonyl)-L-aspartic anhydride[7] (**1**) was condensed with 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosylamine[8] (**2**) to give the L-aspart-1- and 4-oyl compounds (**5** and **8**, respectively), which were separated by column chromatography.

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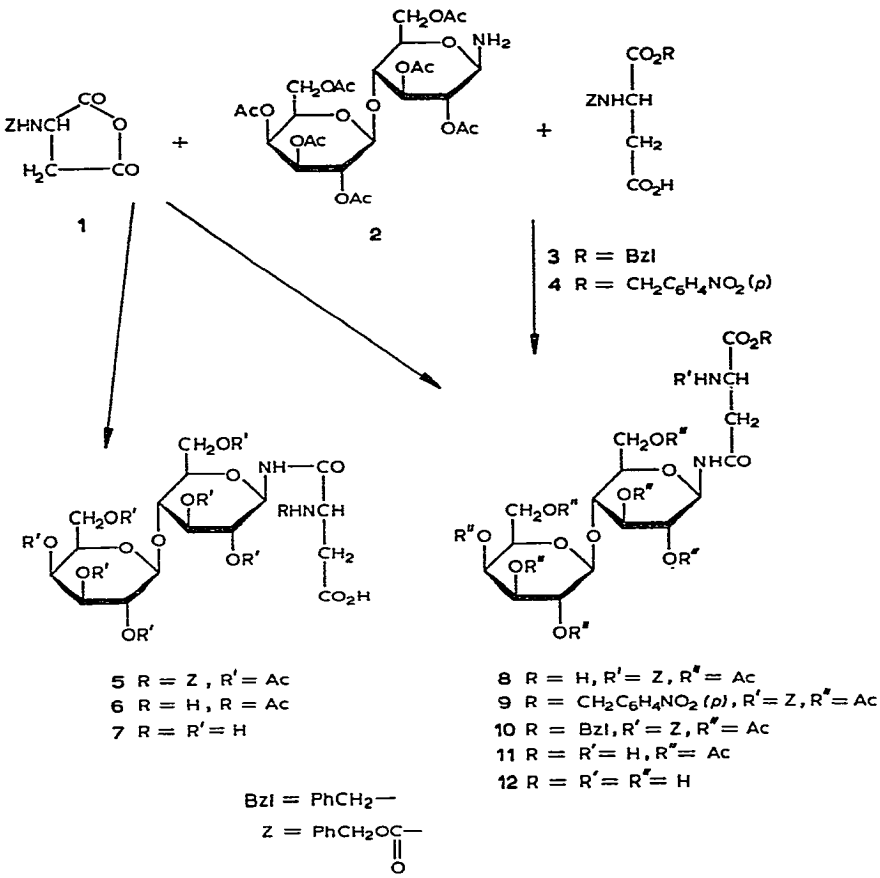
†To whom correspondence should be addressed.



Both compounds were obtained in approximately the same yield and no stereoselectivity was observed. The total yield, based on the carbohydrate starting material, was low (33%), but the peptide starting material may be recovered, which is advantageous for peptides requiring a lengthy synthesis. Thus, the method may be used for the condensation of glycosylamines of readily obtainable disaccharides with peptides bearing an L-aspartoyl residue as C-terminal.

On hydrogenolysis in the presence of 10% palladium-on-charcoal, 2,3,6-tri-*O*-acetyl-1-*N*-[*N*-(benzyloxycarbonyl)-L-aspart-4-oyl]-4-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-*β*-D-glucopyranosylamine (**8**) gave **11**, which on treatment with methanolic ammonia afforded 1-*N*-(L-aspart-4-oyl)-4-*O*-(*β*-D-galactopyranosyl)-*β*-D-glucopyranosylamine (**12**), prepared earlier from the corresponding benzyl ester derivative[8,9] (**10**). Similarly, 2,3,6-tri-*O*-acetyl-1-*N*-[*N*-(benzyloxycarbonyl)-L-aspart-1-oyl]-4-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-*β*-D-glucopyranosylamine (**5**) gave, on hydrogenation, 2,3,6-tri-*O*-acetyl-1-*N*-[L-aspart-1-oyl]-4-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-*β*-D-glucopyranosylamine (**6**), which was treated with

methanolic ammonia to afford 1-*N*-(L-aspart-1-oyl)-4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosylamine (**7**).

In the second type of synthesis of the 1-*N*-(L-aspart-4-oyl)glycosylamine linkage[5], the 1-benzyl ester, which is used as a protecting group, has the disadvantage of being labile under the acidic conditions[10,11] required for removal of the *N*-(benzyloxycarbonyl) protecting group, thus limiting the usefulness of this derivative for the elongation of the chain toward the N-terminal. In contrast, the *p*-nitrobenzyl ester exhibits a marked stability to acid cleavage[12], which permits selective removal of the *N*-(benzyloxycarbonyl) group. Therefore, the condensation of *N*-(benzyloxycarbonyl)-L-aspartic *p*-nitrobenzyl ester[13] with **2** in the presence of 2-ethoxy-*N*-ethoxycarbonyl-1,2-dihydroquinoline[14] (E.E.D.Q.) at room temperature gave pure 2,3,6-tri-*O*-acetyl-1-*N*-[1-*p*-nitrobenzyl *N*-(benzyloxycarbonyl)-L-aspart-4-oyl]-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosylamine (**9**) in almost quantitative yield, without requiring purification by chromatography. Hydrogenolysis of **9** in the presence of palladium-on-charcoal gave the partially protected derivative[8] **11**.

EXPERIMENTAL

General methods. — Melting points were determined with a Mettler FP-2 apparatus and correspond to "corrected melting points". Rotations were determined for solutions in 1-dm, semimicro tubes with a Perkin–Elmer No. 141 polarimeter. The *N*,*N*-dimethylformamide used was spectro-reagent grade. I.r. spectra were recorded for potassium bromide discs, with a Perkin–Elmer spectrophotometer Model 237. Evaporations were performed *in vacuo*, the bath temperature being kept below 45°. Column chromatography was performed on Silica Gel Merck (70–325 mesh, E. Merck, Darmstadt, Germany), used without pretreatment; the ratio of the weight of the substance to the weight of silica gel was 1:50; the volume of the fractions collected was 4 ml per g of the substance; and the ratio of diameter of the column to its length was 1:16. The homogeneity of compounds was verified by ascending t.l.c. on precoated plates of Silica Gel (Merck); the spots were detected by spraying with 20% sulfuric acid and heating for a few min at 200°. Spots of free amino glycopeptides were detected by spraying with a ninhydrin solution in acetone and heating the plates for a few seconds. Microanalyses were performed by Dr. W. Manser, Zurich, Switzerland.

*2,3,6-Tri-*O*-acetyl-1-*N*-[N-(benzyloxycarbonyl)-L-aspart-1-oyl]-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosylamine* (**5**) *and 2,3,6-tri-*O*-acetyl-1-*N*-[N-(benzyloxycarbonyl)-L-aspart-4-oyl]-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosylamine* (**8**). — A solution of *N*-(benzyloxycarbonyl)-L-aspartic anhydride[7] (**1**, 0.45 g, calc. on the basis of a 50% yield of **2**) in ethyl acetate (5 ml) was added to a solution of 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosylamine [**2**, obtained[8] from 2.0 g of 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl azide] in ethyl acetate (10 ml). The solution was kept for 24 h at room temperature. The ethyl acetate layer was washed with M hydrochloric acid and water,

and dried (sodium sulfate). Removal of the solvent gave 1.75 g of product (93% yield, based on **1**) showing on t.l.c. in 19:1 (v/v) chloroform–methanol two spots, for **5** (R_F 0.4) and **8** (R_F 0.15), in addition to three spots having R_F 0.7, 0.9, and 0.95, corresponding to sugar compounds devoid of aspartoyl residue. A solution of the mixture (1.0 g) in chloroform was applied to a column of silica gel. Successive elutions with a linear gradient (1:0 to 10:1 v/v) of chloroform–methanol, gave at first 0.45 g containing only sugar residues, and then **5** (0.18 g, 18%), which was crystallized from methanol; m.p. 234.5–235.5°, $[\alpha]_D^{23}$ +20° (*c* 1.2, chloroform); ν_{max}^{KBr} 3350 (NH), 1750 (OAc), 1650 (shoulder, benzyloxycarbonyl CO), and 1550–1650 cm^{-1} (peptide Amide I); t.l.c. 9:1 (v/v) chloroform–methanol: R_F 0.37.

Anal. Calc. for $C_{38}H_{48}N_2O_{22}$: C, 51.58; H, 5.47; N, 3.17· O, 39.77. Found: C, 51.50· H, 5.40; N, 3.11; O, 39.61.

Compound **8** (0.15 g, 15%) was eluted after complete elution of **5**, and was crystallized from abs. ethanol; m.p. 177–178° (shrinks at 175°), $[\alpha]_D^{23}$ −6.7° (*c* 1.2, chloroform) ν_{max}^{KBr} 3400 (NH), 1760 (OAc), 1650 (shoulder, benzyloxycarbonyl CO), and 1525–1600 cm^{-1} (peptide Amide I); t.l.c. (9:1, v/v, chloroform–methanol): R_F 0.21.

Anal. Calc. for $C_{38}H_{48}N_2O_{22}$: C, 51.58 H, 5.47 N, 3.17 O, 39.77. Found: C, 51.65; H, 5.49; N, 3.15; O, 39.23.

2,3,6-Tri-O-*acetyl-1*-N-[*1*-p-*nitrobenzyl* N-(*benzyloxycarbonyl*)-L-*aspart-4-oyl*]-*4*-O-(*2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl*)-*β*-D-*glucopyranosylamine* (**9**). — To a solution of **2** (1 g) in benzene–ethanol (1:1, v/v, 20 ml) was added 1-*p*-nitrobenzyl *N*-(benzyloxycarbonyl)-L-aspartate[13] (**4**, 0.63 g) and 2-ethoxy-*N*-ethoxycarbonyl-1,2-dihydroquinoline[14] (0.4 g). The mixture was stirred overnight at room temperature, the solvents were evaporated, and the residue was dissolved in chloroform. The organic layer was washed successively with M hydrochloric acid, water, 1% sodium hydrogencarbonate, and water, dried (sodium sulfate), and evaporated. The residue crystallized from 1:1 (v/v) benzene–alcohol as needles (1.0 g, 98%), m.p. 113–114°, $[\alpha]_D^{22}$ +13° (*c* 1.3, chloroform); ν_{max}^{KBr} 3350 (NH) 1750 (OAc), 1610 (benzyloxycarbonyl CO), and 1525 cm^{-1} (peptide Amide I).

Anal. Calc. for $C_{45}H_{53}N_3O_{24}$: C, 52.98; H, 5.25; N, 4.13; O, 37.73. Found: C, 52.64; H, 5.20; N, 4.13; O, 38.12.

2,3,6-Tri-O-*acetyl-1*-N-(L-*aspart-4-oyl*)-*4*-O-(*2,3,4,6-tetra*-O-*acetyl-β*-D-*gluco-pyranosyl*)-*β*-D-*glucopyranosylamine* (**11**). — *A*. A solution of **9** (0.5 g) in 10:1 (v/v) methanol–water (110 ml) was hydrogenated in the presence of 10% palladium-on-charcoal (0.15 g) for 3 h at room temperature and at a pressure of 2.4 atm. The catalyst was filtered off, and the filtrate evaporated to dryness to give **11** (0.35 g, 93%), m.p. 196–197°, $[\alpha]_D^{22}$ −6.8° (*c* 0.91, chloroform); ν_{max}^{KBr} 1750 (OAc) cm^{-1}; t.l.c. (4:1:5, v/v, butanol–acetic acid–water): R_F 0.32 (brown color with ninhydrin reagent).

Anal. Calc. for $C_{30}H_{42}N_2O_{20}$: C, 48.00; H, 5.64; N, 3.73; O, 42.62. Found: C, 48.08; H, 5.66; N, 3.80; O, 42.50.

B. A solution of **8** (0.08 g) in 6:1 (v/v) ethanol–acetic acid (35 ml) was hydrogenated in the presence of 10% palladium-on-charcoal (0.05 g) for 3 h at room

temperature. The catalyst was filtered off and the filtrate was evaporated. The residue crystallized from hot abs. ethanol to give 40 mg (58%), m.p. 197–198; i.r. spectra and t.l.c. mobility were identical with those of the compound obtained from method *A*.

1-N-(L-*Aspart-4-oyl*)-*4*-O-*β*-D-*galactopyranosyl*-*β*-D-*glucopyranosylamine* (**12**). — A solution of crude **11** (obtained from 0.5 g of **9**) in dry methanol (15 ml) was saturated with ammonia at 0° and kept overnight. The solvent was evaporated *in vacuo* at room temperature. The residue was washed with acetone to remove acetamide and crystallized from water–methanol to give **12** (0.11 g, 52%), m.p. 235–235° (dec.), $[\alpha]_D^{22}$ +1.8° (*c* 1.1, water); ν_{max}^{KBr} 3350 (broad, OH) and 1550–1675 cm^{-1} (peptide Amide I); t.l.c. (4:1, v/v pyridine–water): R_F 0.12 (brown color with ninhydrin); lit.[9]: m.p. 235° (dec.), $[\alpha]_D^{22}$ +1.0° (water).

Anal. Calc. for $C_{16}H_{28}N_2O_{13}\cdot H_2O$: C, 40.60; H, 6.37; N, 5.90; O, 47.21. Found: C, 40.38; H, 6.44; N, 5.90; O, 47.40.

2,3,6-Tri-O-*acetyl*-*1*-N-(L-*aspart*-*1*-*oyl*)-*4*-O-(*3,2,4,6-tetra*-O-*acetyl*-*β*-D-*gluco*-*pyranosyl*)-*β*-D-*glucopyranosylamine* (**6**). — A solution of **5** (0.35 g) in 10:1 (v/v) ethanol–acetic acid was hydrogenated in the presence of 10% palladium-on-charcoal for 3 h at room temperature and at a pressure of 2.0 atm. The catalyst was filtered off, the solution evaporated, and the residue crystallized from hot abs. ethanol to give **6** (0.27 g, 95%), m.p. 154–155° (dec.), $[\alpha]_D^{22}$ +3.8° (*c* 1.2, chloroform); ν_{max}^{KBr} 1750 (OAc) and 1575 cm^{-1} (peptide Amide I); t.l.c. (4:1:5, v/v, butanol–acetic acid–water): R_F 0.30 (purple color with ninhydrin reagent).

Anal. Calc. for $C_{30}H_{42}N_2O_{20}$: C, 48.00; H, 5.64; N, 3.73; O, 42.62. Found: C, 47.94; H, 5.68; N, 3.81; O, 42.69.

1-N-(L-*Aspart*-*1*-*oyl*)-*4*-O-*β*-D-*galactopyranosyl*-*β*-D-*glucopyranosylamine* (**7**). — A solution of crude **6** (obtained from 0.25 g of **5**) in dry methanol (20 ml) was saturated with ammonia at 0°, and kept overnight at 0°. The solution was concentrated *in vacuo* at room temperature. The resultant crystalline material was filtered off, washed with acetone (75 mg), and recrystallized from water–methanol, giving **7** (50 mg, 48%), m.p. 218–220° (dec.) (browning at 196°), $[\alpha]_D^{22}$ +14° (*c* 0.79, water); ν_{max}^{KBr} 3350 (broad, OH) and 1550–1680 cm^{-1} (peptide Amide I); t.l.c. (4:1, v/v pyridine–water): R_F 0.16 (purple color with ninhydrin).

Anal. Calc. for $C_{16}H_{28}N_2O_{13}\cdot H_2O$: C, 40.60; H, 6.37; N, 5.90; O, 47.21 Found: C, 40.39; H, 6.44; N, 5.90; O, 47.40.

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