Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

THE SYNTHESIS OF DISACCHARIDE–L-ASPARAGINE COMPOUNDS: DERIVATIVES OF *N*-(L-ASPART-4-OYL)-4-*O*-β-D-GALACTOPYRANOSYL-β-D-GLUCOPYRANOSYLAMINE (LACTOSYL-L-ASPARAGINE), 2-ACETAMIDO-*N*-(L-ASPART-4-OYL)-2-DEOXY-4-*O*-β-D-GALACTOPYRANOSYL-β-D-GLUCOPYRANOSYLAMINE (*N*-ACETYLLACTOSAMINYL-L-ASPARAGINE), AND 2-ACETAMIDO-*N*-(L-ASPART-4-OYL)-2-DEOXY-6-*O*-β-D-GALACTOPYRANOSYL-β-D-GLUCOPYRANOSYLAMINE*†

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(Received July 29th, 1970)

ABSTRACT

Three asparagine derivatives serving as intermediates in the synthesis of glycopeptides have been synthesized. 2,3,6-Tri-*O*-acetyl-*N*-[1-benzyl *N*-(benzyloxycarbonyl)-L-aspart-4-oyl]-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosylamine and the 2-acetamido-2-deoxy-β-D-glucopyranosylamine analog were respectively obtained from the fully acetylated lactose and lactosamine (2-amino-2-deoxy-4-*O*-β-D-galactopyranosyl-D-glucose) *via* the halides, azide, and amine, followed by condensation with 1-benzyl *N*-(benzyloxycarbonyl)-L-aspartate.

2-Acetamido-2-deoxy-β-D-glucopyranosyl azide was tritylated at O-6, the product was fully acetylated, the azide was reduced to the amine, and this was condensed with 1-benzyl *N*-(benzyloxycarbonyl)-L-aspartate; the resulting asparagine derivative was detritylated, and the product was condensed with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide, to give 2-acetamido-*N*-[1-benzyl *N*-(benzyloxycarbonyl)-L-aspart-4-oyl]-2-deoxy-3,4-di-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosylamine.

INTRODUCTION

For a long time, carbohydrate components have been known to be antigenic components at the surface of bacterial cells. In mammalian cells, however, their role

*Dedicated to Professor F. Micheel in celebration of his 70th birthday.

†Amino Sugars LXVI. This is publication No. 518 of the Robert W. Lovett Memorial Group for the Study of Crippling Diseases, Harvard Medical School at the Massachusetts General Hospital, Boston, Massachusetts. This work was supported by research grants from the National Institute of Arthritis and Metabolic Diseases (AM-03564-11) and from the National Cancer Institute (CA-08418-05) National Institutes of Health, U. S. Public Health Service; it was presented at a Symposium in honor of Professor F. Micheel in Münster (Germany) on July 3rd, 1970.

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as antigenic components at the surface of erythrocytes has been clearly demonstrated for the ABO and MN-antigen systems only, and has been suggested for the I, P, and T-antigen systems[1]. In view of (*a*) the interest in transplantation antigens[2] that might be of carbohydrate nature, and (*b*) the presence of carbohydrate components at the surface of the cancer cell[3], we have undertaken the synthesis of carbohydrate antigens. Carbohydrate moieties attached both to protein and lipid moieties are known to be antigenic; in the present work, however, attachment has been investigated of carbohydrate components to a peptide moiety only. In their pioneer investigations, Avery and Goebel[4] linked mono- and di-saccharides to a protein through an azo function; this structure is not found in natural substances, and it may elicit, by itself, a strong, antibody response. More recently, Arnon *et al.*[5] have linked a lactosylsphingosine group to a copolymer compound (containing residues of DL-alanine, L-glutamic acid, and L-tyrosine) *via* an amide linkage between the amino group of sphingosine and the γ-carboxyl group of L-glutamic acid. This type of linkage, also, has never been found in Nature. Rüde *et al.*[29] attached a nonantigenic monosaccharide, D-glucose, to nonantigenic synthetic polypeptides through a glycosidic linkage with L-serine; a linkage of D-xylose and 2-acetamido-2-deoxy-D-galactose with L-serine has been established in proteoglycans and in mucins.

Of the carbohydrate–protein linkages found in glycoproteins and proteoglycans, the alkali-resistant L-aspartyl–glycosylamine bond is one of the most widely distributed in animal glycoproteins[6]. Glycoproteins containing a high proportion of this linkage, such as the α_1-acid glycoprotein of human plasma, are very poorly antigenic[7]. Consequently, it may be assumed that the L-aspartyl–glycosylamine linkage, widely distributed in all animals, is not antigenic, and it was decided to synthesize glycopeptides (and glycoproteins) containing this linkage, in order to study their antigenicity. These glycopeptides can be built by either attaching (*a*) the carbohydrate chain to a presynthesized peptide chain or (*b*) the carbohydrate chain to asparagine first and then synthesizing the peptide moiety. The second approach was selected. The resulting products may, in addition, be valuable tools for study of the influence of the peptide chain on the enzymic degradation or the biosynthesis of the carbohydrate chain, or, *vice versa*, of the influence of the carbohydrate chain on the enzymic degradation of the peptide chain.

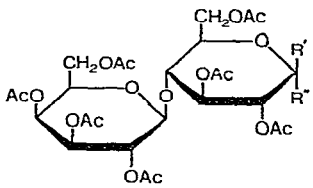
In the present article, the synthesis of compounds containing lactose (4-*O*-β-D-galactopyranosyl-D-glucose), lactosamine (2-amino-2-deoxy-4-*O*-β-D-galactopyranosyl-D-glucose), and 2-amino-2-deoxy-6-*O*-β-D-galactopyranosyl-D-glucose linked to L-asparagine is described. The first compound was selected because of its ready availability, and the second, because it is the determinant group in the antigenic, Type XIV pneumococcus polysaccharide[8]. The third compound was prepared as a model substance for the synthesis of derivatives having a tri- (or higher oligo-) saccharide chain. The attachment of peptide chains to the amino and carboxyl groups of these protected glycosylamine–aspartic acid derivatives will be described elsewhere.

DISCUSSION

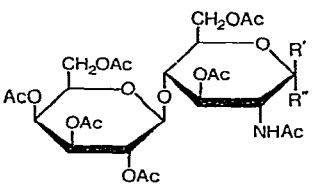
The synthesis of the fully protected lactose–L-asparagine (**4**), *N*-acetyllactosamine–L-asparagine (**9**), and 2-acetamido-2-deoxy-6-*O*-β-D-galactopyranosyl-D-glucose–L-asparagine (**16**) derivatives followed the route described earlier for the synthesis of *N*-(L-aspart-4-oyl)-β-D-glucopyranosylamine[9], 2-acetamido-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine[10–15], and 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine[16].

Condensation of hepta-*O*-acetyl-α-lactosyl bromide[17] (**1**) with silver azide, as originally described by Micheel and Wulff[18], gave the 1-azido derivative **2**, which could not be crystallized. It was directly hydrogenolyzed to the amine **3**, which was amorphous, but had an elementary analysis agreeing with that calculated. Condensation of the amine **3** with 1-benzyl *N*-(benzyloxycarbonyl)-L-aspartate in the presence of *N*,*N*′-dicyclohexylcarbodiimide gave (crystalline) L-asparagine derivative **4**.

In a parallel route, 2-acetamido-1,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-D-glucopyranose[19,20] (lactosamine octaacetate, **5**) was treated with acetyl chloride, and the impure, unstable chloride **6** resulting was immediately condensed with silver azide to give the impure azide **7**. Compound **7** could not be separated from unreacted **5**, and the mixture was reduced to a mixture of the amines **5** and **8** that was amorphous and unstable; crude amine **8** was characterized as the crystalline peracetate **9**. Condensation of amine **8** with 1-benzyl *N*-(benzyloxycarbonyl)-L-aspartate gave the crystalline derivative **10**.



1 R′ = H; R″ = Br
2 R′ = N_3; R″ = H
3 R′ = NH_2; R″ = H
4 R′ = $NHCOCH_2CHCO_2CH_2Ph$; R″ = H
$NHCO_2CH_2Ph$

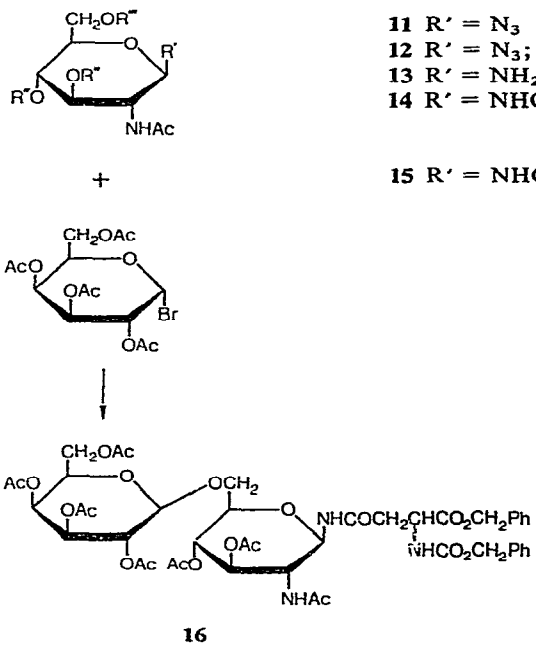


5 R′ = H; R″ = OAc
6 R′ = H; R″ = Cl
7 R′ = N_3; R″ = H
8 R′ = NH_2; R″ = H
9 R′ = NHAc; R″ = H
10 R′ = $NHCOCH_2CHCO_2CH_2Ph$; R″ = H
$NHCO_2CH_2Ph$

In order to synthesize *N*-(L-aspart-4-oyl)glycosylamine derivatives having a tri- (or higher oligo-) saccharide chain, the potential starting material **15** was prepared. All of the groups of this compound are masked, except the 6-hydroxyl group of the 2-acetamido-2-deoxy-D-glucose residue. 2-Acetamido-2-deoxy-β-D-glucopyranosyl azide[18] (**11**) was tritylated at O-6 to give crystalline **12**, which was reduced to the crystalline amine **13**. Condensation of **13** with 1-benzyl *N*-(benzyloxycarbonyl)-L-aspartate in the presence of *N*,*N*′-dicyclohexylcarbodiimide gave the crystalline

L-asparagine derivative **14**, from which the trityl group was removed. The reactivity of the crystalline, asparagine derivative **15** resulting was determined by condensation with 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl bromide to give, in 16% yield, the L-asparagine–disaccharide derivative **16**.

Use of n.m.r. spectrometry for the determination of the configuration of the linkage of the carbohydrate moiety to L-asparagine was precluded by the complexity of the spectra. Although the Hudson rules of isorotation can be used only as a first approximation[21], they gave sufficient information for the determination of the anomeric configuration, because of the relatively large differences of optical rotations between anomers. This procedure can be used only with pure compounds, and, fortunately, compounds **4**, **10**, **15**, and **16** were obtained in crystalline form and showed only one spot in t.l.c. By use of the isorotation rules, Bertho[22–24] had suggested that the β anomer is produced for numerous azides of mono- and di-saccharides prepared from α-D-halides and for the amines derived therefrom by catalytic hydrogenation. Bolton *et al.*[14] firmly established the configuration of C-1 of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glycopyranosyl azide from an examination of its n.m.r. spectrum. As further transformations of this compound did not involve the configuration at C-1 of the sugar residue, the configuration at C-1 of



11 R′ = N_3 R″ = R‴ = H
12 R′ = N_3; R″ = Ac; R‴ = Tr
13 R′ = NH_2; R″ = Ac; R‴ = Tr
14 R′ = $NHCOCH_2CHCO_2CH_2Ph$ ($NHCO_2CH_2Ph$); R″ = Ac; R‴ = Tr
15 R′ = $NHCOCH_2CHCO_2CH_2Ph$ ($NHCO_2CH_2Ph$); R″ = Ac; R‴ = H

16

azide **12** and amine **13** is well established. The impure azides **2** and **17**, and the impure amines **3** and **8** respectively derived from them, were synthesized in a way similar to that used for **12** and **13**, and they may be assumed to consist preponderantly of the β anomer. Because condensation with the L-asparagine derivative in the presence of *N*,*N*′-dicyclohexylcarbodiimide was performed in anhydrous dichloromethane, no

change in configuration at C-1 presumably occurred. The results of application of the isorotation rules to the fully protected mono- and di-saccharide–L-asparagine derivatives (see Table I) show good correlation for the monosaccharides and the lactose derivative **4** with an A value of $\sim -7{,}000$ to $-8{,}000$. The discrepancies observed for the disaccharides may be the result of uncertainties in the B values calculated and of incorrect assumptions: for example, the same B value was used for **16** as for **10**. Comparison of a series of peracetylated disaccharides shows that, in general, the β-D-(1→6)-linked disaccharides have a rotation of +5 to +10° higher than those of the corresponding β-D-(1→4)-linked disaccharides. Introduction of this difference in the calculation of the A value of **16** would give a value of −3,000 to −8,000, instead of the value of +2,300 reported in Table I. As compound **16** was obtained from **15** *via* a Koenigs–Knorr reaction, which generally gives the β-D anomer, it is safe to assume that **16**, like compounds **4** and **10**, possesses the β-D configuration at both of its anomeric carbon atoms.

TABLE I

MOLAR OPTICAL ROTATIONS AND A VALUES OF VARIOUS *N*-[1-BENZYL *N*-(BENZYLOXYCARBONYL)-L-ASPART-4-OYL]-β-D-GLYCOPYRANOSYLAMINES[3]

Derivative of the β-D-glycopyranosylamine related to	$[M]_D \times 10^{-2}$, *degrees*	$A \times 10^{-2}$
D-Glucose, 2,3,4,6-tetraacetate[9]	+131[a]	−76[b]
2-Acetamido-2-deoxy-D-glucose, 3,4,6-triacetate[25]	+117[a]; +82[a]	−98[c]; −53[c]
3,4-diacetate (**15**)	+121[d]	−74[e]
Chitobiose, heptaacetate[16]	+185[a]	−140[f]
Lactose, heptaacetate (**4**)	+93[a]	−73[g]
Lactosamine, heptaacetate (**10**)	+136[a]	−116[k]
2-Acetamido-2-deoxy-6-*O*-β-D-galactopyranosyl-D-glucose, heptaacetate (**16**)	+263[a]	+23[l]

[a]Rotation determined in chloroform. [b]Based on a B value of +20,700 (ref. 26). [c]Based on a B value of +18,000, calculated from the optical rotation of the pentaacetates (ref. 27). [d]Rotation determined in methyl sulfoxide. [e]Based on a B value of +19,500, calculated from an A value of +17,600 for the acetate [based on the anomers of D-glucosamine pentaacetate (ref. 27)] and $[M]_D$ +1,900 of 2-acetamido-1,3,4-tri-*O*-acetyl-β-D-glucopyranose (ref. 27). [f]Based on a B value of −4,500, calculated from the optical rotations of the azide and glycosylamine derivatives[16] and from the values obtained by Bertho[29]. [g]Based on a B value of +16,800 (ref. 28). [k]Based on a B value of +24,600, calculated from A = +17,600 for the acetate (see footnote *e*) and $[M]_D$ +41,600 (ref. 20). [l]Based on the B value (+24,000) for **10**.

EXPERIMENTAL

General. — Melting points were determined with a Mettler FP-2 apparatus and correspond to "corrected melting point". Rotations were determined for solutions in 1-dm, semimicro tubes with a Perkin–Elmer No. 141 polarimeter; the chloroform used was analytical-reagent grade and contained approximately 0.75% of ethanol. Infrared spectra were recorded 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 (0.05–0.2 mm); the proportion (w/w) of substance to silica gel was 1:50–60. The ratio of diameter to length of the column was 1:8–10. The proportion of substance (in g) to fraction of eluate (in ml) was 1:100. The homogeneity of nonpolar substances was verified by ascending t.l.c. on precoated plates of Silica Gel (Merck); the zones were revealed by spraying with 1:1:18 (v/v) anisaldehyde–conc. sulfuric acid–ethanol. The microanalyses were performed by Dr. M. Manser, Zürich, Switzerland.

2,3,6-Tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl azide (**2**). — A suspension of silver azide in dry chloroform (100 ml) was prepared by mixing sodium azide (0.4 g) and silver nitrate (1.0 g) in the minimum volume of water for dissolution, and then washing the precipitate extensively by successive decantation* with water, ethanol, ether, and chloroform. 2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranosyl bromide[17] (hepta-*O*-acetyl-α-lactosyl bromide, **1**, 2.0 g) was added to the suspension, and the mixture was boiled for 2 h under reflux, cooled, and filtered. The filtrate was evaporated to dryness, and the residue, dissolved in 19:1 chloroform–ethanol, was chromatographed on silica gel. The fractions showing R_F 0.8 on t.l.c. in the same solvent system, and an i.r. absorption band at 2120 cm^{-1}, were evaporated, to give an amorphous product (1.0 g, 53%) which was used without further purification.

2,3,6-Tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosylamine (**3**). — To a solution of **2** (1.5 g) in abs. ethanol (100 ml) was added platinum oxide (200 mg), and the suspension was shaken with hydrogen for 1 h at atmospheric pressure and room temperature. The suspension was filtered, the filtrate was evaporated to dryness, and the residue was dissolved in 19:1 chloroform–ethanol and chromatographed on silica gel. The fractions showing R_F 0.3 on t.l.c. (same solvent mixture) were evaporated, to give an amorphous product (750 mg, 52%), $[\alpha]_D^{22}$ +8.6° (*c* 1.0, ethanol), $[\alpha]_D^{20}$ +4.8° (*c* 0.62, chloroform).

Anal. Calc. for $C_{26}H_{37}NO_{17}$: C, 49.13; H, 5.86; N, 2.20; O, 42.79. Found: C, 48.99; H, 5.84; N, 2.09; O, 42.76.

2,3,6-Tri-O-acetyl-N-[1-benzyl N-(benzyloxycarbonyl)-L-aspart-4-oyl]-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosylamine (**4**). — To a solution of **3** (400 mg, 0.63 mmole) in dry dichloromethane (15 ml) was added 1-benzyl *N*-(benzyloxycarbonyl)-L-aspartate[16] (225 mg, 0.63 mmole) and *N,N'*-dicyclohexylcarbodiimide (200 mg). The mixture was stirred for 4 h at room temperature, and filtered; after evaporation of the filtrate, the residue was dissolved in 19:1 chloroform–ethanol and chromatographed on silica gel. The fractions showing R_F 0.6 on t.l.c. in the same solvent mixture were evaporated. Recrystallization from ethanol–ether gave 300 mg (49%) of **4**, m.p. 91–92°, $[\alpha]_D^{22}$ +9.5° (*c* 1.0, chloroform).

Anal. Calc. for $C_{45}H_{54}N_2O_{22}$: C, 55.43; H, 5.58; N, 2.87; O, 36.10. Found: C, 55.34; H, 5.60; N, 2.83; O, 36.00.

*Silver azide should be washed by decantation only, and should always be kept covered with solvent, as drying may result in a severe explosion.

2-Acetamido-3,6-di-O-acetyl-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galacto-pyranosyl)-β-D-glucopyranosyl azide (**7**). — The hepta-*O*-acetyl derivative **5** of 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-D-glucose[19] was prepared by the method of Zilliken *et al.*[20]. A solution of **5** (2.0 g) in acetyl chloride (20 ml) pre-saturated with hydrogen chloride at −20° was kept for 30 h at room temperature and then evaporated. The resulting crude 2-acetamido-3,6-di-*O*-acetyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranosyl chloride (**6**) was treated with silver azide, as described for the preparation of **2**. After chromatography, the fractions having R_F 0.3 on t.l.c. in the same solvent mixture were combined and evaporated, to give a crystalline product (1.2 g) consisting of a mixture of the azide **7** and of starting material **5** that could not be separated. It showed an i.r. absorption band typical for azide at 2120 cm^{-1}, and was used without further purification.

2-Acetamido-3,6-di-O-acetyl-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galacto-pyranosyl)-β-D-glucopyranosylamine (**8**). — Crude compound **7** (1.2 g) was hydrogenated as described for the preparation of **3**. Chromatography on silica gel with 9:1 chloroform–ethanol gave an amorphous product (600 mg; 31% based on **5**) showing R_F 0.4 on t.l.c. in the same solvent system. The product was very unstable, and could not be obtained in crystalline form. It was characterized by acetylation with acetic anhydride in the presence of pyridine, to give the *N*-acetyl derivative **9**, which was recrystallized from acetone–ether, m.p. 195–196°, $[\alpha]_D^{20}$ +5.4° (*c* 1.0, chloroform).

Anal. Calc. for $C_{28}H_{40}N_2O_{17}$: C, 49.70; H, 5.95; N, 4.13; O, 40.19. Found: C, 49.60; N, 5.92; H, 4.14; O, 40.23.

2-Acetamido-3,6-di-O-acetyl-N-[1-benzyl N-(benzyloxycarbonyl)-L-aspart-4-oyl]-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl-amine (**10**). — Compound **8** (300 mg, 474 μmoles) in dry dichloromethane (12 ml) was treated with 1-benzyl *N*-(benzyloxycarbonyl)-L-aspartate[16] (169.2 mg, 474 μmoles) and *N,N'*-dicyclohexylcarbodiimide, as described for the preparation of **4**, to give 200 mg (43%) of **10** after recrystallization from ethanol–ether; m.p. 167–169°, $[\alpha]_D^{20}$ +14° (*c* 1.0, chloroform); R_F 0.5 by t.l.c. in 19:1 chloroform–ethanol.

Anal. Calc. for $C_{45}H_{55}N_3O_{21}$: C, 55.49; H, 5.69; N, 4.31. Found: C, 55.38; H, 5.66; N. 4.28.

2-Acetamido-3,4-di-O-acetyl-2-deoxy-6-O-trityl-β-D-glucopyranosyl azide (**12**). — A solution of 2-acetamido-2-deoxy-β-D-glucopyranosyl azide[18] (**11**, 1.15 g, 4.73 mmoles) and chlorotriphenylmethane (1.8 g, 6.6 mmoles) in dry pyridine (20 ml) was kept for 3 days at room temperature. Acetic anhydride (13 ml) was added, and the solution was kept overnight at room temperature, and then poured into ice–water. The resulting crystalline product was filtered off and recrystallized from ethanol to give 1.5 g (56%) of **12**, m.p. 182–183°, $[\alpha]_D^{20}$ +0.3° (*c* 1.0, chloroform).

Anal. Calc. for $C_{31}H_{32}N_4O_7$: C, 65.02; H, 5.63; N, 9.78, O. 19.55. Found: C, 65.02; H, 5.67; N, 9.75; O. 19.76.

2-Acetamido-3,4-di-O-acetyl-2-deoxy-6-O-trityl-β-D-glucopyranosylamine (**13**). — Compound **12** (1.0 g) in 1:1 (v/v) ethyl acetate–ethanol (100 ml) was reduced with hydrogen at atmospheric pressure in the presence of platinum oxide (200 mg) for

15 min at room temperature. The suspension was filtered, the filtrate was evaporated to dryness, the residue was dissolved in 5:1 (v/v) chloroform–ethanol, and the solution was chromatographed on silica gel. The crystalline fractions were recrystallized from ether-pentane, to give 600 mg (63%) of **13**, m.p. 125–126°; $[\alpha]_D^{20}$ +20° (*c* 1.0, chloroform); R_F 0.5 by t.l.c. in 5:1 (v/v) chloroform–ethanol.

Anal. Calc. for $C_{31}H_{34}N_2O_7$: C, 68.11; H, 6.26; N, 5.12; O, 20.48. Found: C, 68.10; H, 6.31; N, 5.02; O, 20.55.

*2-Acetamido-3,4-di-*O*-acetyl-*N*-[1-benzyl* N*-(benzyloxycarbonyl)-*L*-aspart-4-oyl]-2-deoxy-6-*O*-trityl-β-*D*-glucopyranosylamine* (**14**). — A solution of **13** (1.5 g, 2.75 mmoles), 1-benzyl *N*-(benzyloxycarbonyl)-L-aspartate[16] (0.98 g, 2.75 mmoles), and *N,N'*-dicyclohexylcarbodiimide (0.7 g) in dichloromethane (35 ml) was treated as described for the preparation of **4**. Crystallization from ethanol gave 1.3 g (53%) of **14**, m.p. 196–197°, $[\alpha]_D^{20}$ +34° (*c* 1.0, chloroform); R_F 0.4 by t.l.c. in 19:1 (v/v) chloroform–ethanol.

Anal. Calc. for $C_{50}H_{51}N_3O_{12}$: C, 67.78; H, 5.80; N, 4.74; O, 21.67. Found: C, 67.62; H, 5.61; N, 4.60; O, 21.75.

*2-Acetamido-3,4-di-*O*-acetyl-*N*-[1-benzyl* N*-(benzyloxycarbonyl)-*L*-aspart-4-oyl]-2-deoxy-β-*D*-glucopyranosylamine* (**15**). — A solution of **14** (900 mg) in acetic acid (3 ml) was treated for 2 min at room temperature in a tightly closed flask with acetic acid (0.40 ml) presaturated at 0° with hydrogen bromide. The suspension was poured into a mixture of ice–water and solid potassium hydrogen carbonate, and extracted thrice with chloroform. The extracts were combined, dried (sodium sulfate), and evaporated, and the residue was chromatographed on silica gel with 19:1 (v/v) chloroform–ethanol. Crystallization from ethanol gave 250 mg (38%) of **15**, m.p. 212–214°, $[\alpha]_D^{20}$ +19° (*c*, 1.0, methyl sulfoxide); R_F 0.2 by t.l.c. in 19:1 (v/v) chloroform–ethanol.

Anal. Calc. for $C_{31}H_{37}N_3O_{12}$: C, 57.85; H, 5.79; N, 6.52; O, 29.82. Found: C, 57.94; H, 5.78; N, 6.42; O, 29.99.

*2-Acetamido-3,4-di-*O*-acetyl-*N*-[1-benzyl* N*-(benzyloxycarboxyl)-*L*-aspart-4-oyl]-2-deoxy-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranosylamine* (**16**). — A mixture of **15** (400 mg, 623 μmoles), 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (256 mg, 623 μmoles), silver oxide (500 mg), Drierite (1.2 g), and iodine (70 mg) in dry dichloromethane (10 ml) was stirred for 24 h at room temperature. The suspension was filtered, the filtrate was evaporated, and the residue was chromatographed twice on silica gel, and eluted with 3:1 (v/v) chloroform–acetone from the first column and 19:1 (v/v) chloroform–ethanol from the second column. Crystallization from methanol–ether gave 100 mg (16%) of **16**, m.p. 135–137°, $[\alpha]_D^{20}$ +27° (*c* 1.0, chloroform); R_F 0.3 by t.l.c. in 3:1 (v/v) chloroform–acetone.

Anal. Calc. for $C_{45}H_{55}N_3O_{21}$: C, 55.49; H, 5.69; N, 4.31; O, 34.49. Found: C, 55.55; H, 5.66; N, 4.35; O, 34.62.

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