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A METHOD FOR REDUCTIVE CLEAVAGE OF *N*-GLYCOSYLAMIDE CARBOHYDRATE–PEPTIDE BOND*

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ABSTRACT

A new, mild method for the splitting of *N*-linked oligosaccharides from glycoproteins includes treatment of glycoproteins (ovomucoid, flavoprotein, ribonuclease B, hemagglutinin, or transferrin) with 2M $LiBH_4$ in 25mM LiOH–50mM Li citrate–70% aqueous *tert*-butyl alcohol (5 h, 45°), followed by hydrolysis of the resulting glycosylamine with aqueous acetic acid. The oligosaccharides formed were easily isolated by gel filtration and cation-exchange chromatography in 60–80% yields. The reaction was accompanied by the intense reductive cleavage of peptide bonds with formation of amino alcohols, but *N*-deacetylation of hexosamine was completely excluded. The optimal conditions of this reaction were chosen by use of a model glycopeptide, 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-1-*N*-(4-L-aspartoyl)-2-deoxy-β-D-glucopyranosylamine.

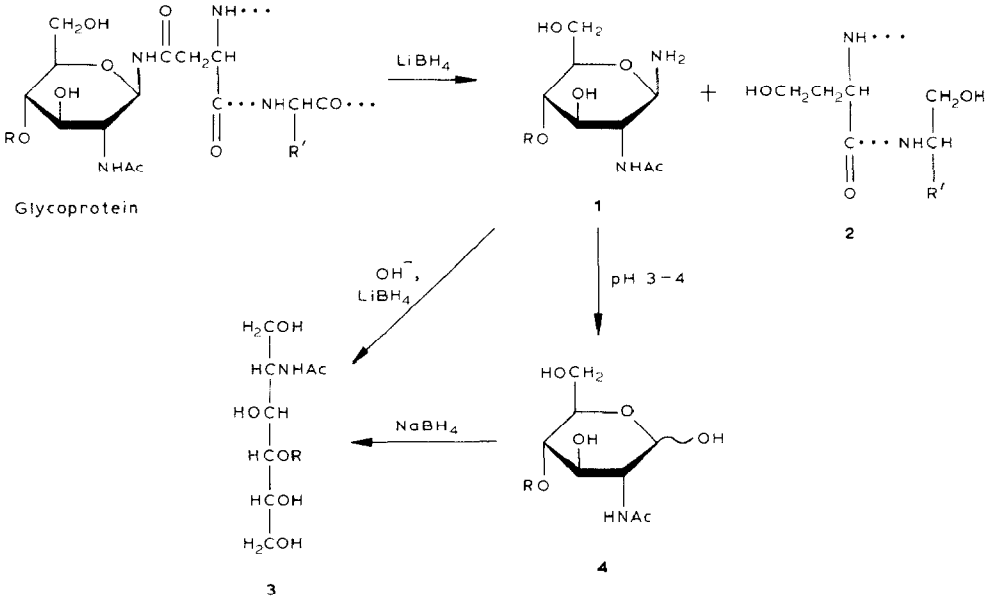
INTRODUCTION

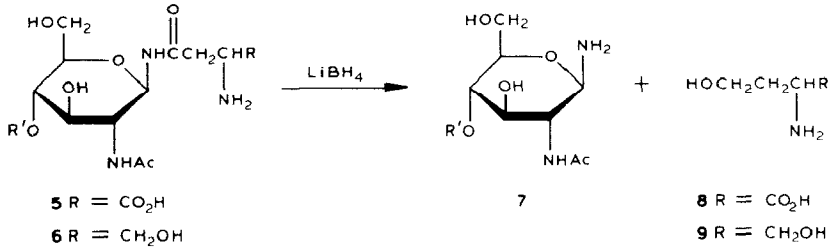
A few chemical and enzymic methods are known for the release of the *N*-asparagine-linked carbohydrate chains of glycoproteins[1–4]. The well known chemical method of cleavage by hydrazinolysis[1,5] results in good yields of oligosaccharide chains, but leads to *N*-deacetylation of the amino sugar residues, and several other side processes take place[6,7]. Other chemical methods for oligosaccharides liberation, such as drastic alkaline hydrolysis[3] and trifluoroacetolysis[2], are rarely used because of severe structural modifications of the original oligosaccharidic chains. In this paper, we report a new, mild method for the release of oligosaccharide units from glycoproteins, based on the reductive cleavage of the *N*-glycosyl bond.

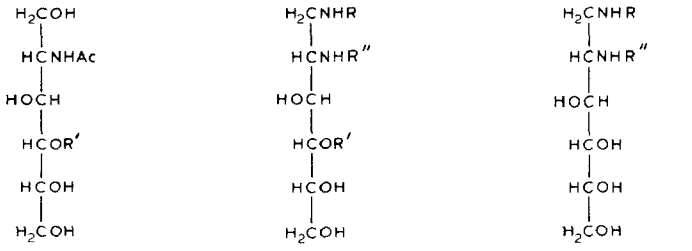
RESULTS AND DISCUSSION

In a preliminary communication[8] it was reported that treatment of some glycoproteins with alkaline lithium borohydride in 70% aquous *tert*-butyl alcohol resulted in the reductive cleavage of *N*-glycosylamide carbohydrate–peptide bonds,

*Dedicated to Professor Walter T. J. Morgan.


LiBH4
Glycoprotein
1
2
OH⁻, LiBH4
pH 3–4
NaBH4
3
4
R = carbohydrate chain
R′ = peptide chain


LiBH4
5 R = CO2H
6 R = CH2OH
7
8 R = CO2H
9 R = CH2OH


10
11 R = H, R″ = Ac
12 R = Ac, R″ = H
13 R = R″ = H
14 R = H, R″ = Ac
R′ = β-D-GlcpNAc

to give rise to oligosaccharide glycosylamines (**1**), oligosaccharide alditols (**3**) and amino alcohols (**2**). Subsequent hydrolysis of **1** with acetic acid and reduction of **4** with sodium borohydride gave **3** in a yield of 35–60%.

In a study using a model glycopeptide, 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-1-*N*-(4-L-aspartoyl)-2-deoxy-β-D-glucopyranosylamine[9] (**5**), the optimal conditions of this reaction were chosen by varying the solvent, concentrations of lithium borohydride (1–2.5M), lithium hydroxide (0–0.1M) and trilithium citrate (0–0.1M), time, and temperature. It should be noted that the reaction did not proceed in anhydrous media, and that 70% aqueous *tert*-butyl alcohol was the best solvent; with other solvents examined (aqueous butanol, 2-propanol, 2-methoxyethanol, oxolane, dimethyl sulfoxide, and *N,N,N',N'*-tetramethylurea), the reductive cleavage of the *N*-glycosylamide bond was substantially lower.

The optimal conditions found were as follows: 2M lithium borohydride in 25mM lithium hydroxide–50mM trilithium citrate–70% aqueous *tert*-butyl alcohol for 5 h at 45°. The mixture obtained after treatment of **5** with lithium borohydride contained 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranosylamine[9,10] (**7**, 72%), 2-amino-4-hydroxybutyric acid (**8**, 80%), the starting glycopeptide **5** (15%), and the reduced disaccharide **10** (5%); identification of **8** as the main product of the reaction confirmed the reductive cleavage of the *N*-glycosylamide bond. Alkaline hydrolysis of this bond with formation of aspartic acid proceeded insignificantly (~0.5%). The low content of the reduced disaccharide **10** indicated that alkaline hydrolysis of glycosylamine **7** and subsequent reduction to **10** occurred only to a small extent. The by-products of the reaction 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-[1-amino-1,2-dideoxy-D-glucitol (**11**) and the corresponding $N^2 \rightarrow N^1$ acetyl-migration product, 1-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-amino-1,2-dideoxy-D-glucitol (**12**)] were also identified in a total yield of 8% by use of an authentic sample of **11**; the details of the $N^2 \rightarrow N^1$ acetyl migration will be published elsewhere.

The glycosylamine **7** was hydrolyzed with dilute acetic acid and reduced with sodium borohydride to give **10** in 62% yield. The structure of **10** was confirmed by hydrolysis with 4M hydrochloric acid and the quantitative determination (amino acid analyzer) of 2-amino-2-deoxyglucose and 2-amino-2-deoxyglucitol (1:1). It is necessary to emphasize that the rate of glycosylamine hydrolysis with acetic acid is greatly dependent on the concentration of salts present. Hydrolysis of **7** proceeded rather rapidly in salt-free solution (5–6 h at 20° in 0.1M acetic acid).

This new reaction of *N*-glycosylamide carbohydrate–peptide bond cleavage was applied to the release of intact carbohydrate chains from some glycoproteins (ovomucoid, flavoprotein, ribonuclease B, transferrin, and hemagglutinin). As was reported previously[8], *N*-deacetylation of hexosamines and rupture of glycosidic linkages were not observed upon reductive cleavage of glycoproteins. Lithium borohydride treatment of 2-acetamido-2-deoxy-D-glucose and a hexasaccharide[11],

α-D-Glc*p*NAc-(1→4)-β-D-Gal*p*-(1→3)-[α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→6)]-D-GalNAcol, gave almost quantitatively 2-acetamido-2-deoxy-D-glucitol from the former whereas the latter was not changed.

The general procedure for isolation of oligosaccharides includes the treatment of a glycoprotein with lithium borohydride, acidification, and chromatography. After gel-filtration on Sephadex G-15, two fractions were obtained. Fraction I was eluted in the void volume and contained all carbohydrates, and some amino acids and amino alcohols residues. Low-molecular-weight Fraction II contained only amino acids and amino alcohols residues, and salts. Fraction I was further separated on AG 50W-X2 (H^+) cation-exchange resin into oligosaccharide (elution with water) and glycopeptide (elution with ammonium hydroxide) fractions. The former fraction contained oligosaccharide alditols **3** and oligosaccharides **4** with a ratio of ~1:7. This was confirmed by an eight-fold increase in 2-acetamido-2-deoxy-D-glucitol content after sodium borohydride reduction of the oligosaccharide fraction.

The yield of oligosaccharides was calculated from quantitation of 2-acetamido-2-deoxy-D-glucose and -D-glucitol in oligosaccharide and glycopeptide fractions (Table I). As can be seen, the yields of oligosaccharides are satisfactory (60–80%). The repeated treatment of a glycopeptide fraction with lithium borohydride gave an additional amount of oligosaccharides. For example, such a treatment of the fraction from ovomucoid and flavoprotein increased the total yield of oligosaccharides up to 70–75%.

The side reaction of glycosylamine **1** derived from glycoproteins, namely, its reduction into amino alditols of the type **11** and **12**, did not exceed 5%, as followed from determination of 1,2-diamino-1,2-dideoxy-D-glucitol (**13**) after hydrolysis of glycopeptide fractions with 4M hydrochloric acid. It should be especially noted that sialic acid residues in transferrin also survived the reductive cleavage.

In order to render the analysis of oligosaccharides more convenient, the oligosaccharide fraction was reduced with sodium borohydride, and the mixtures of reduced oligosaccharides could be separated into individual compounds by liquid chromatography[13].

As had been shown previously[8], lithium borohydride treatment caused an extensive, reductive cleavage of peptide bonds as well. For example, treatment of ovomucoid with lithium borohydride in 0.05M lithium hydroxide–70% aqueous *tert*-butyl alcohol (16 h, 50°) resulted in the survival of 80% of proline, valine, isoleucine, and ~30–45% of aspartic acid, threonine, serine, glutamic acid, glycine, alanine, tyrosine, and phenylalanine residues. Similtaneously, the formation of amino alcohols was observed, such as 2-amino-1,4-butanediol, 2-amino-1,3-butanediol, 2-amino-1,3-propanediol, 2-amino-1,5-pentanediol, 2-aminoethanol, 2-aminopropanol, and prolinol. Analogous treatment of polyglycine and polyserine resulted in a decrease in glycine and serine content by 45 and 57% with formation of 2-aminoethanol (44.5%) and 2-aminopropanol (54%), respectively.

Taking into account that reductive cleavage of the polypeptide backbone

could interfere with oligosaccharide liberation, we investigated the behavior of a glycopeptide, 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-1-*N*-(3-amino-4-hydroxybutyryl)-2-deoxy-β-D-glucopyranosylamine (**6**). It was found that structural differences (carboxylic or oxymethylenic groups) of glycopeptides **5** and **6** did not affect *N*-glycosylamide bonds cleavage. Features of the glycoprotein polypeptide chain also appeared to be of insignificant influence on reductive cleavage. Alkaline lithium borohydride treatment of ovomucoid and flavoprotein glycopeptides containing mostly a single asparaginic residue (after Pronase digestion), led to the liberation of oligosaccharides in a yield close to that from the intact glycoproteins. The reason for decreased yields (60–65%) of oligosaccharides enriched with D-mannose or 2-acetamido-2-deoxy-D-glucose residues (ribonuclease B, ovomucoid, and flavoprotein) and high yields (80%) of complex oligosaccharides (transferrin and hemagglutinin) is so far unclear.

The results presented herein suggest that this new, mild method of cleavage of the *N*-glycosylamide carbohydrate–peptide linkage will be a very convenient tool for the structural analysis of many natural glycoproteins. In this laboratory, it was successfully used for the splitting off of intact carbohydrate chains of hemagglutinin[13,14] and flavoprotein.

EXPERIMENTAL

General methods. — Optical rotations were measured with a Perkin–Elmer 141 polarimeter. Solutions were evaporated *in vacuo* at 30–35°. *Tert*-butyl alcohol was crystallized up to minimal u.v. absorbance; $LiBH_4$ (95%) was from Fluka, trilithium citrate tetrahydrate p.a. from Chemapol, ribonuclease B type III from Sigma, and transferrin (human) p.a. from Serva. Other glycoproteins examined were ovomucoid[15] and egg white flavoprotein[16], as well as respective glycopeptides obtained after Pronase digestion[12] and influenza virus hemagglutinin[17].

Analytical methods. — Quantitative analysis of 2-amino-2-deoxy-D-glucose and -D-glucitol after hydrolysis with 4M HCl (4 h, 100°), amino acids and amino alcohols after hydrolysis with 4M HCl (16 h, 100°), and amino alditols used a post-column ninhydrin reaction after separation with the amino acid analyzer Biotronik LC 4010, equipped with an Aminex A-6 column (0.9 × 23 cm). Sodium citrate–HCl buffers used were 0.2M Na^+, pH 3.25 at 63° (*A*), and 0.35M Na^+, pH 5.28 at 63° (*B*), at a flow rate of 80 mL/h. Quantitative analysis of 1,2-diamino-1,2-dideoxy-D-glucitol (**13**, retention time 20 min) after hydrolysis with 4M HCl (6 h, 100°) was performed on an amino acid analyzer, equipped with an Aminex A-6 column (0.9 × 8 cm), using an elution with buffer *B* containing 5 g/L of boric acid titrated to pH 7.8–7.9 (buffer *C*). For post-column derivatization, a freshly prepared 0.1% solution of 2,4,6-trinitrobenzenesulfonic acid in 0.25M $K_2B_4O_7$, titrated with KOH to pH 10.2, was used; the reagent and the buffer were mixed in proportion 1:2 and the reaction was carried out for 10 min at 60°. Sialic acids were determined by the thiobarbituric method of Warren[18].

TABLE I

YIELDS OF OLIGOSACCHARIDES AFTER LITHIUM BOROHYDRIDE TREATMENT OF GLYCOPROTEINS

Glycoprotein	*Yield of oligosaccharides (%)*	*Ratio of GlcNAc to GlcNAcol*[a]	*Preponderant type of oligosaccharide chains*
Ribonuclease B	64	1.3:1	Oligomannoside
Ovomucoid and its glycopeptides	60–61.5(73)[b]	4.5–4.7:1	GlcNAc-rich
Flavoprotein and its glycopeptides	61–63(74)[b]	4.5–4.8:1	GlcNAc-rich
Transferrin	80	3.3:1	Complex
Hemagglutinin	81	4.0–4.2:1	Complex

[a]After reduction of oligosaccharides with $NaBH_4$. [b]Yields in parentheses refer to repeated $LiBH_4$ treatment.

Liberation of oligosaccharides from glycoproteins (standard procedures using ovomucoid as example). — Ovomucoid (8 mg containing 3.7 μmol of 2-amino-2-deoxy-D-glucose) and trilithium citrate tetrahydrate (28 mg) were dissolved in water (0.5 mL), and 0.5M LiOH (0.1 mL) and *tert*-butyl alcohol (1.4 mL) added. The mixture was cooled in ice and $LiBH_4$ (90 mg) was added portionwise. After an incubation for 5 h at 45°, the mixture was cooled to 10°, diluted with cold water (10 mL), and then cold acetone (2 mL, portionwise) and acetic acid (1 mL) were added. The solution was concentrated to ~5 mL, kept overnight, and repeatedly evaporated with methanol (~5 mL) and several drops of acetic acid up to ~2 mL. The residue was fractionated on a Sephadex G-15 column (1.84 × 85 cm) in 0.1M acetic acid. Orcinol–H_2SO_4-positive fractions were combined (Fraction I) and kept overnight in order to achieve complete hydrolysis of the glycosylamine linkage. Fraction II eluted later contained the main portion of amino acids and amino alcohols. Fraction I was evaporated to ~2 mL, acetic acid added (0.4 mL), and the solution applied to a column (0.5 × 5 cm) of Bio-Rad AG 50W-X2 (H^+) cation exchanger. The oligosaccharide fraction was eluted with water (10 mL), and the glycopeptide fraction with 0.7M NH_4OH (10 mL). Both fractions were evaporated to dryness and analyzed for amino acids, diamino alditol **13**, 2-amino-2-deoxy-D-glucose, and -D-glucitol. The oligosaccharide fraction contained 2.06 μmol (60%) of 2-amino-2-deoxy-D-glucose and 54 nmol (1.5%) of 2-amino-2-deoxy-D-glucitol, and the glycopeptide fraction 1.33 μmol (38.5%) of 2-amino-2-deoxy-D-glucose. The yields of oligosaccharides from glycoproteins are presented in Table I.

Reduction to oligosaccharide alditols. — The oligosaccharide fraction was repeatedly evaporated with toluene, dissolved in 0.1M NaOH (0.4 mL), and $NaBH_4$ (15 mg) added. The mixture was incubated for 4 h at 40°, diluted with water to ~5 mL, acetic acid (0.5 mL) was added dropwise, and boric acid was removed by evaporation with methanol (5 × 10 mL). The residue was assayed for 2-amino-2-deoxy-D-glucose and -D-glucitol (Table I).

Lithium borohydride treatment of 2-acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-1-N-(4-L-aspartoyl)-2-deoxy-β-D-glucopyranosylamine (**5**) *and*

2-acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-1-N-(3-amino-4-hydroxybutyryl)-2-deoxy-β-D-glucopyranosylamine hydrochloride (**6**). — To a solution of **5** (1.08 mg) or **6** (1.1 mg) in water (0.5 mL) were added trilithium citrate tetrahydrate (28 mg), 0.5M LiOH (0.1 mL) and *tert*-butyl alcohol (1.4 mL). The mixture was cooled to ~4° and $LiBH_4$ (90 mg) added. After an incubation for 5 h at 45°, the mixture was cooled to ~10°, and then were added cold water (10 mL), cold acetone (2 mL portionwise in 2–3 min intervals), and finally acetic acid (1 mL). The mixture was evaporated to ~2 mL, diluted with water to 10 mL, and aliquots were analyzed with the amino acid analyzer.

(a) 2-Amino-4-hydroxybutyric acid (**8**) *and free aspartic acid.* To an aliquot (1.5 mL) (from **5**) was added water (1.5 mL) and the solution acidified with conc. HCl to pH ~1.5; **8** and aspartic acid were determined (buffer *A*) in 80% and 0.5% yields, respectively.

(b) 2-Amino-1,4-butanediol (**9**). An aliquot (3 mL) (from **6**) was acidified with conc. HCl to pH ~1.5; **9** and the starting **6** were determined in 77% (buffer *B*) and 20% (buffer *A*) yields, respectively.

(c) Bound aspartic acid. To an aliquot (0.5 mL) (from **5**) was added 8M HCl (0.5 mL) and aspartic acid determined (buffer *A*) after hydrolysis (16 h, 100°) in 15% yield.

(d) 2-Amino-2-deoxy-D-glucose and -D-glucitol. To an aliquot (2 mL) (from **5** or **6**) was added acetic acid (0.5 mL), the solution was diluted with water to 10 mL and kept for 2 days at room temperature. The solution was concentrated to ~5 mL, and Bio-Rad AG 50W-X2 (H^+) cation exchanger (3 mL) added and stirred for 2 h at room temperature. The resin was filtered off and washed with water. The filtrate was concentrated to ~3 mL and then evaporated with toluene (5 × 10 mL). The residue was reduced with $NaBH_4$ as described above. Following hydrolysis, 2-amino-2-deoxy-D-glucose (62.5 and 59%) and 2-amino-2-deoxy-D-glucitol (61 and 57%) were determined (buffer *B*) for **5** and **6**, respectively.

(e) 2-Acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-1-amino-1,2-dideoxy-D-glucitol (**11**) *and 1-acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-amino-1,2-dideoxy-D-glucitol* (**12**). To an aliquot (1.5 mL) (from **5** or **6**) was added conc. HCl to pH ~1.5. The yield of **11** was found to be 4.5% and that of **12** was 3.5% (buffer *B*). Retention times were 26 min for **11**, 29 min for **12**, and 49 min for 2-amino-2-deoxy-D-glucose.

Determination of 2-acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranosylamine (**7**). — This determination was carried out after reduction with $NaBH_3CN$ to amino alditol **11**. The glycopeptide **5** (1.08 mg) was treated with $LiBH_4$ as described with the exception that the reaction mixture was diluted with water (4 mL), concentrated to ~1.5 mL, and $NaBH_3CN$ (50 mg) and formic acid (1 mL) were added. The mixture was kept for 2 h at room temperature and diluted with water to 15 mL. The yield of **11** was 80% (buffer *B*).

Hydrolysis of 2-acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranosylamine (**7**). — (*a*). The solution of **7** (1.9 mg) in 0.1M

acetic acid (0.5 mL) was kept at room temperature. Aliquots (0.05 mL) were taken after 1, 4, and 6 h, formic acid (0.02 mL) and $NaBH_3CN$ (2.5 mg) were added, and the mixtures were kept for 2 h at room temperature. Water (5 mL) was added to each reaction mixture, which was analyzed with buffer *A*. The yields of **11** were 25% (1 h), 1% (4 h) and 0% (6 h).

(*b*). A solution of 25mM LiOH, 50mM trilithium citrate, and 2M $LiBH_4$ in 70% aqueous *tert*-butyl alcohol (1 mL) was treated with cold water (6 mL), cold acetone (1 mL, portionwise), and acetic acid (0.25 mL). The solution was concentrated to ~3 mL, and glycosylamine **7** (0.92 mg) and water (to 5 mL) were added. The mixture was kept overnight at room temperature and two aliquots (2 mL) were taken. The first one was concentrated to ~0.3 mL, formic acid (0.2 mL) and $NaBH_3CN$ (12 mg) were added, and the solution was kept for 2 h at room temperature. The second aliquot was diluted with water up to 6 mL, kept for 2 days at room temperature, concentrated to ~0.3 mL, and reduced with $NaBH_3CN$ as just described. Both reaction mixtures were diluted with water up to 10 mL and analyzed for amino alditol **11** (buffer *A*, retention time 35 min). The yields were 69 and 0%, respectively.

Synthesis of model compounds. — (a) Preparation of amino alditols **11** *and* **14**. A solution of glycosylamine **7** or 2-acetamido-2-deoxy-β-D-glucopyranosylamine[9,10] (1 mmol) in 10% aqueous formic acid (10 mL) containing $NaBH_3CN$ (250 mg, 4 mmol) was kept for 2 h at room temperature and then diluted with water (10 mL), Cationite CU-2 or Dowex 50W-X8 (H^+) (10 mL) cation-exchange resin was added and stirred for 30 min. The resin was filtered off, washed with water (100 mL), and then with cold 0.7M NH_4OH (100 mL) for 45 min. The basic fraction was evaporated to ~2 mL, acidified with conc. HCl to pH ~2.5, and purified by gel-chromatography on a Sephadex G-10 column (1.64 × 158 cm) in 0.1M acetic acid. The effluent was monitored at 230 nm and by the ninhydrin reaction (amino acid analyzer). The peak fractions containing amino alditols were pooled, evaporated to ~5 mL, acidified with conc. HCl to pH ~2.5, and evaporated with toluene to dryness to give amino alditols **11** and **14** in 80% yields.

2-Acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-1-amino-1,2-dideoxy-D-glucitol (**11**) *hydrochloride*. M.p. 140–144° (abs. ethanol–2-propanol), $[\alpha]_D^{23}$ −14.4° (*c* 1.2, water).

Anal. Calc. for $C_{16}H_{32}ClN_3O_{10}$: C, 41.61; H, 6.98; N, 9.09. Found: C, 41.84; H, 7.22; N, 8.86.

2-Acetamido-1,2-dideoxy-1-amino-D-glucitol (**14**) *hydrochloride*. M.p. 151–152° (from aqueous ethanol), $[\alpha]_D^{22}$ −24.5° (*c* 0.82, water).

Anal. Calc. for $C_8H_{19}ClN_2O_5$: C, 37.14; H, 7.40; N, 10.82. Found: C, 37.01; H 7.47; N, 10.71.

(b) 1,2-Diamino-1,2-dideoxy-D-glucitol (**13**) *dihydrobromide*. A solution of **14** (150 mg) in 2M HBr (5 mL) was heated for 2 h at 100° and evaporated to dryness. The residue was dissolved in methanol, abs. ethanol added, and the solution concentrated. The product was filtered off, washed with abs. ethanol, and dried *in*

vacuo to give **13** in 64% yield as a hygroscopic amorphous solid, $[\alpha]_D^{23}$ +3.6° (*c* 0.9, water); lit.[19] m.p. 146–147.5°, $[\alpha]_D^{22}$ +4.0° (*c* 5.06, water).

*(c) 2-Acetamido-4-*O*-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-1-*N*-(3-amino-4-hydroxybutyryl)-2-deoxy-β-*D*-glucopyranosylamine hydrochloride* (**6**). This compound was synthesized from 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-1-*N*-(1-benzyl *N*-benzyloxycarbonyl-4-L-aspartoyl)-2-deoxy-β-D-glucopyranosylamine[9] by $LiBH_4$ treatment (4 h, 0°), followed by hydrogenolysis on Pd–C as reported previously[9]. The glycopeptide **6** was obtained in 63% yield as an amorphous solid, $[\alpha]_D^{20}$ +2.6° (*c* 2.1, water).

Anal. Calc. for $C_{20}H_{37}ClN_4O_{12}$: C, 42.82; H, 6.65; N, 9.98. Found: C, 42.49; H, 6.84; N, 9.84.

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