BIOACTIVE CARBOHYDRATE DERIVATIVES

I. ANALOGS OF THE GLYCOPEPTIDE JUNCTURE IN IMMUNOGLOBULINS

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ABSTRACT

A group of novel analogs of 2-acetamido-1-*N*-(β-L-aspartyl)-2-deoxy-β-glucospyranosylamine (**1**), the glycopeptide junction in immunoglobulins, were synthesized as potential regulators of the biosynthesis, secretion, and function of immunoglobulins. The 2-amino and carboxyl groups in the aspartyl moiety were incorporated into hydantoin, thiohydantoin, and dioxopiperazine systems, and converted into an N^2-toluenesulfonamide to mimic the neighboring peptide-linkages of this juncture. The amide linkage in **1** was replaced by glycosidic and by a sulfonamide linkage. The latter represents a new type of glycosylamine, the chemical stability of which was examined. The o.r.d. and c.d. spectra of these novel glycosyl derivatives are compared.

INTRODUCTION

Among numerous organic structures synthesized in recent years for biomedical applications, carbohydrate derivatives, with the exception of antibiotics and nucleoside analogs, have not been widely investigated[1]. From the biological point of view, the importance of carbohydrate components in various immunological processes that involve membrane receptor, surface antigen, mediators of delayed hypersensitivity, and glycoprotein biosynthesis have been well recognized[2]. The structural specificity of these carbohydrate moieties and the function of their biosynthetic enzymes, the glycosyl transferases, are actively being elucidated in many laboratories. In addition, physicochemical techniques such as magnetic resonance spectroscopy and optical rotatory measurements have also been applied to study the nature of protein–carbohydrate interactions. With the recent progress in synthetic carbohydrate chemistry, the exploration of novel carbohydrate derivatives as bioactive agents was undertaken in these laboratories. In this communication, a general consideration and the synthesis of a group of novel analogs of the glycopeptide juncture 2-acetamido-1-*N*-(β-L-aspartyl)-2-deoxy-β-glucopyranosylamine (**1**), which is present in certain glycoproteins such as immunoglobulin G (IgG), are described.

Our interest in these glycopeptide derivatives was initiated by their potential application for immunological disorders. For example, a heightened biosynthesis of

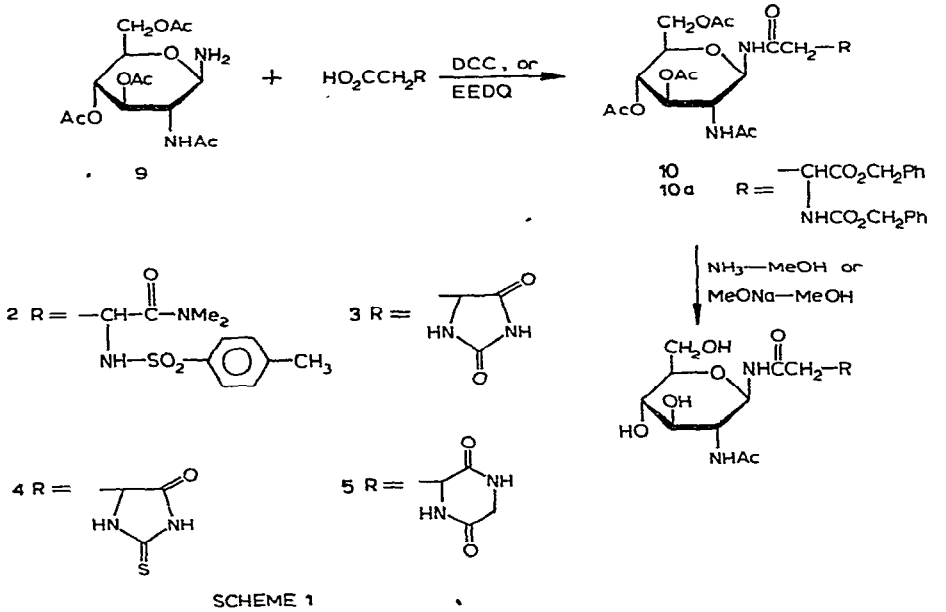
IgG by rheumatoid synovium *in vivo*, and its contribution to the pathogenesis of rheumatoid arthritis, are well documented[3]. A new approach to this disease is to interfere selectively with the biosynthesis or the cellular secretion of immunoglobins. Evidence accumulated to date depicts that the heavy and light chains of IgG are formed separately on membrane-bound polyribosomes[4,5]. It has been suggested that attachment to carbohydrate side-chains is a requisite for secretion of the protein from the cell[6,7]. The process of attachment is initiated while the polypeptides are still associated with the polyribosomes, and continues in a stepwise manner after their release. The point of attachment has been shown to be through the amide linkage of an asparagine residue in human[8,9,11], rabbit[10], and bovine[9] IgG, and in the mouse-immunoglobulin light chains[12]. In all cases, the first carbohydrate attached is GlcNAc.

Our working hypothesis is that analogs of the natural glycopeptide juncture (**1**) may act as inhibitors or acceptors of the glycosyl transferase enzyme, thus blocking the secretion of IgG. They may also interfere with other properties of immunoglobulins possibly involving the participation of the "hinge" region, such as catabolism and interaction with cellular-membrane binding-sites.

RESULTS AND DISCUSSION

The new derivatives and analogs of **1** in this work are conveniently classified into four groups according to the chemical modifications involved. Modifications were made only in the amino acid moiety.

Group I: conversion of the amino and the carboxylate groups of the asparagine moiety of **1** respectively into a sulfonamide and an amide (**2**).



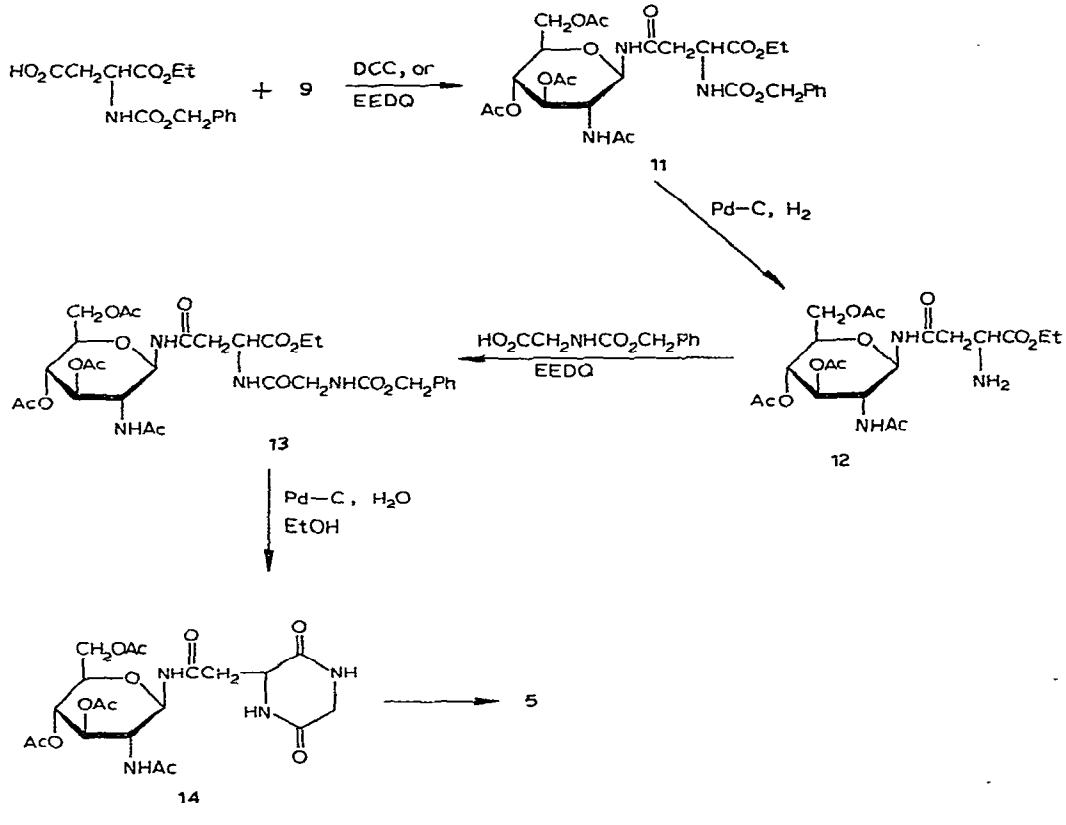
SCHEME 1

Group II: incorporation of the amino and the carboxylate groups of the asparagine moiety into a heterocycle through amide linkages (**3**, **4**, **5**).

Group III: replacement of the amide linkage of **1** by glycosidic linkage* (**6**).

Group IV: replacement of the amide linkage of **1** by a sulfonamide linkage (**7**, **8**). This represents a new type of glycosylamine.

For compounds of groups I and II, the corresponding carboxylic acids were condensed with 2-acetamido-2-deoxy-β-D-glucopyranosylamine (**9**), by using *N,N'*-dicyclohexylcarbodiimide (DCC) or 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) (Scheme 1). However, for the synthesis of **5**, the intermediate of **14** was also prepared by the stepwise synthesis of the sugar–dipeptide, followed by cyclization, as shown in Scheme 2.

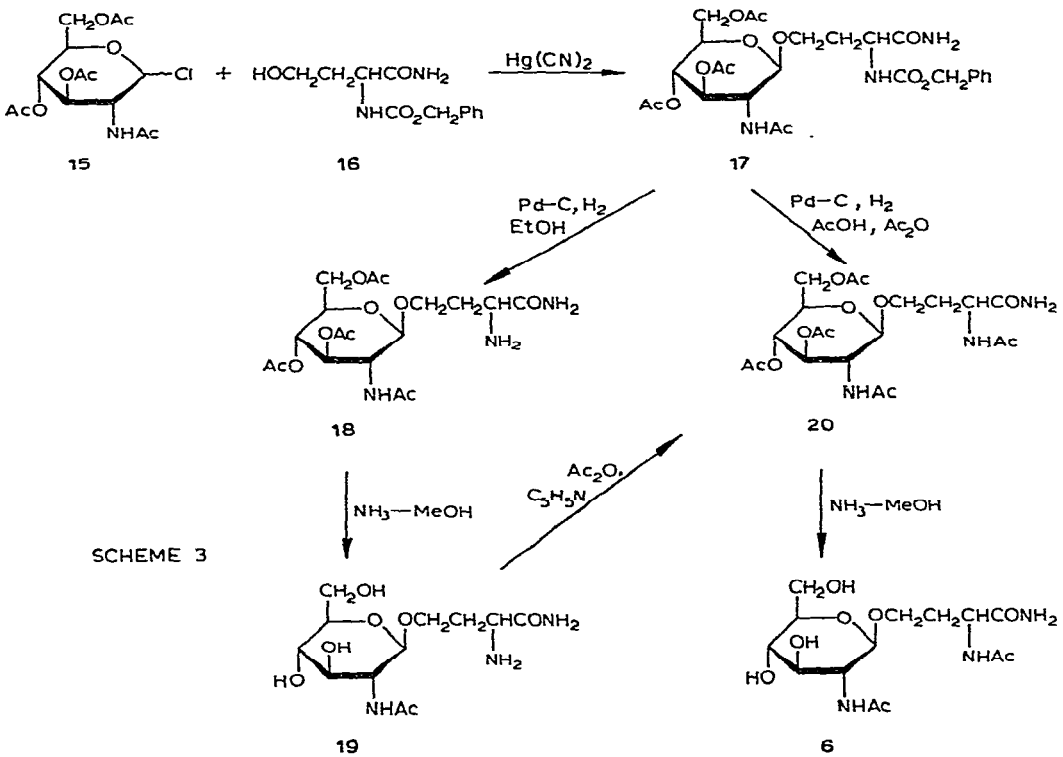


SCHEME 2

The glycoside **17** was prepared by a modified Helferich procedure and was converted into **19** via **18** by hydrogenolysis and ammonolysis. Compound **19** gave a fully acetylated derivative **20** that was also obtained directly from **17** by hydrogenolysis in the presence of acetic anhydride (Scheme 3).

*The glycosidic linkage is a natural linkage widely existing in glycoproteins. The amino acid residue for the carbohydrate attachment is usually serine, threonine, or hydroxylysine. See ref. 13.

Treatment of the amine **9** with the sulfonyl chloride **21** gave the sulfonamide **22**, which was debenzylated to the free amino acid **7**. When **7** was treated with a catalytic amount of ammonia in methanol at 5°, the desired product **23** could not be isolated or detected. The major product isolable was 3-aminosulfonylalanine (**24**) (Scheme 4); no 2-acetamido-2-deoxy-β-D-glucopyranosylamine was detected. The breakdown of **7** in the neutral, methanolic solution was monitored by t.l.c. Compound **7** was found to decompose completely in 48 h at room temperature. It appeared to be more stable in acidic solution (such as 90% acetic acid), but its decomposition was accelerated by bases (for example, ammonia and lithium methoxide). Likewise, the sulfonamide **22** was extensively fragmented by treatment with ammonia in methanol.



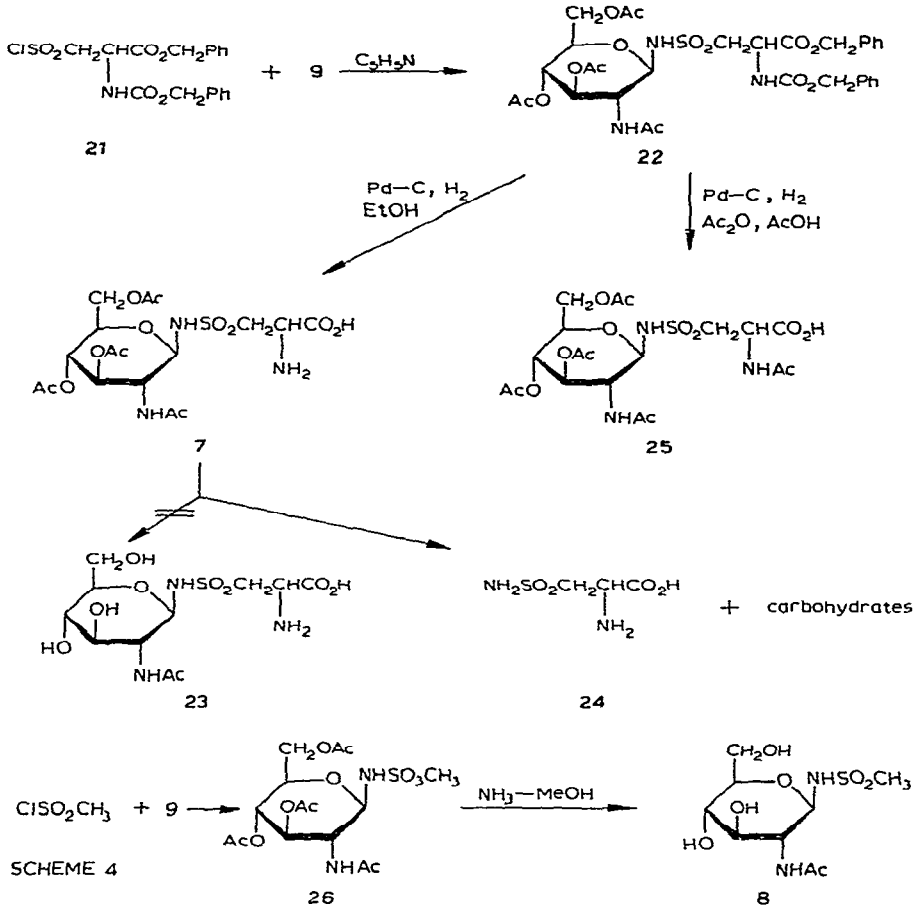
SCHEME 3

Hydrogenolysis of **22** in the presence of acetic anhydride gave the *N*-acetylated product **25**, which appeared to be more stable than **7**, but still decomposed upon prolonged storage in solution.

In order to investigate the stability of *N*-alkylsulfonylglucosylamines, the simple methanesulfonamido derivative **26** was prepared and subjected to conditions similar to those that caused the cleavage of **7**. However, the *O*-deacetylated product **8** was obtained, and it proved to be stable. The foregoing results suggest that the instability of **7** is due to some effect of the amino acid moiety.

Compound **11** gave a molecular ion at *m/e* 623. Cleavage between C-1 and C-2

of the aspartate ($-CO_2CH_2CH_3$) led to the same ion at *m/e* 550 from **10a**, by a similar splitting out of CO_2CH_2Ph. Other major ions produced directly from the molecular ion were *m/e* 577 ($-CH_3CH_2OH$) and 564 ($-CH_3CONH_2$), in agreement with the fragmentation patterns reported in the literature[14,15]. The o.r.d. and c.d.



SCHEME 4

spectra of some of these compounds have been measured. The o.r.d. spectrum of **1** was described by Austen and Marshall[16], who reported that both **1** and 2-acetamido-1-*N*-acetyl-2-deoxy-β-D-glucopyranosylamine exhibited two positive Cotton effects having extrema at 227 and 204 nm. However, compound **1** prepared in this work exhibited only one peak at 229 nm (Fig. 1). A similar, positive o.r.d. curve showing one single peak at 230 nm is given by the dioxopiperazine **5** (Fig. 1). On the other hand, the hydantoin **3** shows one broad peak at 228 nm and a peak of approximately equal magnitude at 209 nm. The curves of **2** and **4** are more complicated, as shown by Fig. 2. Both **6** and **8** exhibit a peak at *ca.* 200 nm. In addition to this, **6** also shows a trough at 225 nm (Fig. 3).

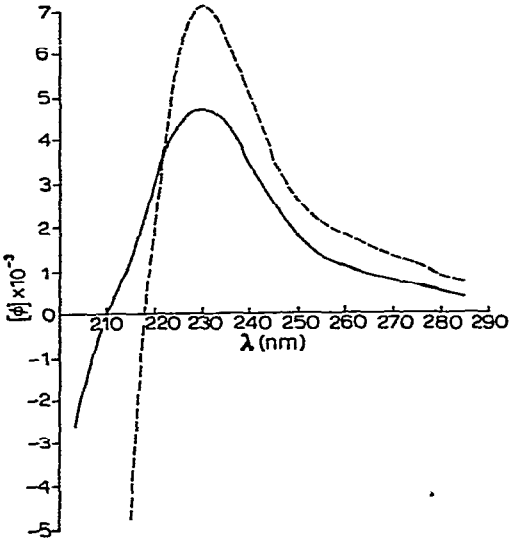


Fig. 1. The o.r.d. spectra of **1** (—) and **5** (– – –) in water at 22°.

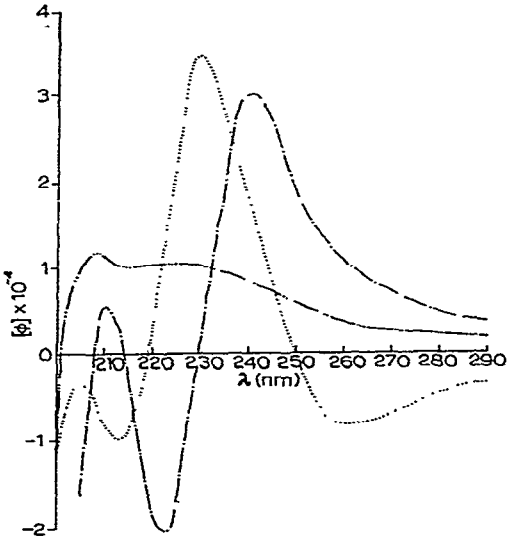


Fig. 2. The o.r.d. spectra of **2** (—·—·—), **3** (—···—···—), and **4** (·····) in water at 22°.

In Figs. 3 and 4 the c.d. measurements are summarized. Compounds **1**, the hydantoin **3**, and the dioxopiperazine **5** show a positive maximum at 197 nm and a shoulder at *ca.* 220 nm, whereas the thiohydantoin **4** gives two positive maxima of about equal magnitude at the same wavelengths. Both **6** and **8** show a negative maximum at 212 nm.

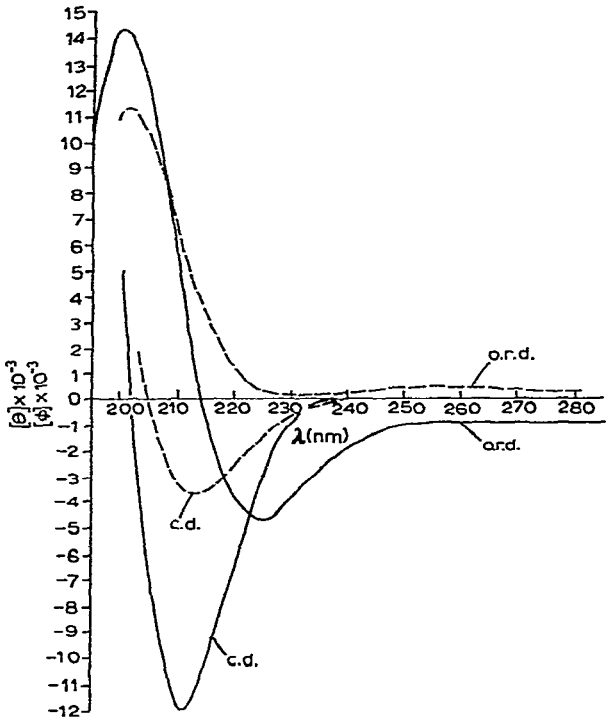


Fig. 3. The o.r.d. and c.d. spectra of **6** (—) and **8** (– – –) in water at 22°.

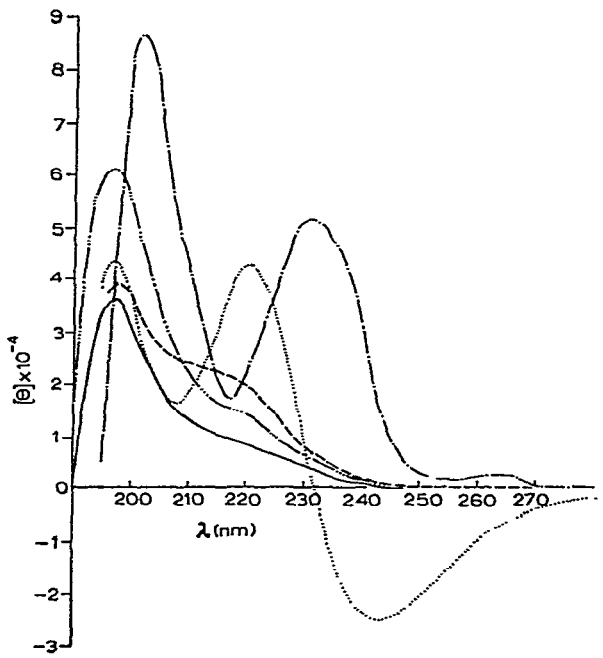


Fig. 4. C.d. spectra of **1** (—), **2** (—·—·—), **3** (—···—···—), **4** (·····), and **5** (– – –) in water at 22°.

EXPERIMENTAL

General. — O.r.d. and c.d. measurements were effected with a Cary 60 Spectropolarimeter and a Cary Model 6001 circular-dichroism accessory at 22° in water as solvent. Unless otherwise stated, t.l.c. was performed on silica Gel G, products were detected by charring with sulfuric acid, and single spots were observed. Evaporations were performed *in vacuo*, and products were dried over phosphorus pentaoxide.

*2-Acetamido-1-*N-(*β*-L-*aspartyl*)*-2-deoxy-β-*D-*glucopyranosylamine* (**1**). — This compound has been synthesized by several groups[17–24]. It was prepared from **10** according to Bolton's procedure[19]; o.r.d. data: $[\phi]_{229}$ +4696 peak, $[\phi]_{210}$ 0.0 cross; c.d. data: $[\theta]_{225}$ 0.0, $[\theta]_{215}$ +10938 shoulder, $[\theta]_{197}$ +35901 max, $[\theta]_{190}$ 0.0 cross.

*Methyl 3-(benzyloxycarbonyl)amino-*N,N-*dimethylsuccinamate* (**27**). — To an ice-cooled solution of methyl *N*-(benzyloxycarbonyl)-4-aspartate (5.6 g) in *p*-dioxane (40 ml) and tetrahydrofuran (40 ml) was added triethylamine (2.2 g) dropwise. After 5 min, ethyl chloroformate (2.4 g) was added, and the resulting mixture was stirred for 15 min. Aqueous dimethylamine (40%, 2.5 g) was then added. The reaction mixture was warmed to room temperature, stirred for 3 h, and concentrated. Benzene–diethyl ether (1:1) was added to the residue and the resulting mixture was washed with dilute hydrochloric acid, water, saturated sodium hydrogen carbonate solution, and water. The organic extract was dried (Na_2SO_4) and concentrated to give the product as an oil (3.6 g). The material was used in the subsequent reaction without further purification.

*Methyl 3-amino-*N,N-*dimethylsuccinamate hydrobromide* (**28**). — The crude compound **27** (3.6 g) was stirred with a mixture of 30% hydrobromic acid (60 ml) and glacial acetic acid (30 ml) for 1 h. The reaction mixture was concentrated to give an oil (2.5 g), which was used in the subsequent reaction without further purification.

Methyl N,N-*dimethyl-3-*(p-*toluenesulfonamido*)*succinamate* (**29**). — The crude product **28** (2.5 g) was dissolved in chloroform (200 ml) and cooled in an ice–water bath. Immediately following the addition of triethylamine (4 g), *p*-toluenesulfonyl chloride (2.3 g) was added. The mixture was stirred at room temperature for 90 min and then concentrated. A 1:1 mixture of benzene–ether (200 ml) was added and the resulting mixture filtered to remove the precipitate, which showed no C=O absorption in the infrared. The filtrate was concentrated to a thick oil that gradually solidified under hexane. The solid (1.75 g) was collected and recrystallized from isopropyl alcohol to give the pure product (1.1 g), m.p. 132–135°.

Anal. Calc. for $C_{14}H_{20}N_2O_5S$: C, 51.21; H, 6.14; N, 8.53. Found: C, 51.06; H, 6.03; N, 8.37.

N,N-*Dimethyl-3-*(p-*toluenesulfonamido*)*succinamic acid* (**30**). — To a suspension of compound **29** (3.3 g) in water (20 ml) was added M sodium hydroxide (21 ml). The reaction mixture was stirred for 2 h at room temperature, acidified with M hydrochloric acid (25 ml), filtered, and the filtrate concentrated to an oil. Addition of water (10 ml) followed by cooling gave the crystalline product (1.9 g), m.p. 147–149°.

Anal. Calc. for $C_{13}H_{18}N_2O_5S$: C, 49.66; H, 5.77; N, 8.91. Found: C, 49.80; H, 5.84; N, 9.10.

N'-(*2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-glucopyranosyl*)-N,N-*dimethyl-2-*(p-*toluenesulfonamido*)*succinamide* (**31**). — *Procedure A.* Compounds **9** (0.68 g) and **30** (0.62 g) were dissolved in ethyl acetate (75 ml) and *N,N'*-dicyclohexylcarbodiimide (0.42 g) was added. The reaction mixture was stirred for 5 h at room temperature and filtered to give a solid (0.99 g), which appeared to consist of the product and *N,N'*-dicyclohexylurea. The solid was combined with a sample (0.83 g) obtained from a similar experiment in which tetrahydrofuran had been used as the solvent, and was purified by chromatography on silica gel. The crude material (1.82 g) was adsorbed onto silica gel (15 g) and chromatographed on a 100-g silica gel column prepared with 2% isopropyl alcohol in dichloromethane. Elution with 6 l of 2% isopropyl alcohol in chloroform–dichloromethane (1:3) gave dicyclohexylurea (0.485 g). Subquent elution with a mixture of isopropyl alcohol–chloroform–dichloromethane (200:1000:1000 ml) gave the product (1.26 g), m.p. 275–276° (dec.).

Anal. Calc. for $C_{27}H_{38}N_4O_{12}S$: C, 50.46; H, 5.96; N, 8.72. Found: C, 50.42; H, 6.02; N, 8.96.

Procedure B. Compounds **9** (0.53 g) and **30** (0.58 g) were dissolved in anhydrous tetrahydrofuran (40 ml). 2-Ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ, 0.444 g) was added. The mixture was stirred overnight at room temperature, and concentrated to give the crude product (1.4 g), which was purified by recrystallizing from ethanol–chloroform. The pure product was identical by t.l.c. with a sample obtained by procedure *A*.

N'-(*2-Acetamido-2-deoxy-β-*D-*glucopyranosyl*)-N,N-*dimethyl-2-*(p-*toluenesulfonamido*)*succinamide* (**2**). — Compound **31** (0.43 g) was dissolved by warming in anhydrous methanol (200 ml), mixed with 0.2M methanolic sodium methoxide solution (0.2 ml), and stirred for 3 h at room temperature. After evaporating the solution, the residue was taken up in water (2 ml), neutralized with acetic acid (3 drops), and evaporated to dryness. Recrystallizing the residue from methanol afforded the pure product (0.26 g), m.p. 251–252° (dec.). Concentration of the mother liquor followed by addition of diethyl ether gave a second crop (0.07 g), m.p. 233–234° (dec); total yield: 0.33 g (89%). T.l.c. (chloroform–methanol 8:2) showed both materials to be homogeneous; o.r.d. data: $[\phi]_{241}$ +30071 peak, $[\phi]_{230}$ 0.0 cross, $[\phi]_{223}$ −20214 trough, $[\phi]_{214}$ 0.0 cross, $[\phi]_{210}$ +5680 peak, $[\phi]_{208}$ 0.0 cross; c.d. data: $[\theta]_{270}$ 0.0, $[\theta]_{231}$ +51493 max, $[\theta]_{217}$ +16981 min, $[\theta]_{202}$ +86552 max.

Anal. Calc. for $C_{21}H_{32}N_4O_9S \cdot 0.5\ H_2O$: C, 47.99; H, 6.33; N, 10.65; S, 6.10. Found: C, 47.88, H, 6.12; N, 10.41; S, 6.38.

Anal. Calc. for $C_{21}H_{32}N_4O_9S \cdot H_2O$: C, 47.18; H, 6.41; N, 10.48; S, 6.00. Found: C, 47.27; H, 6.25; N, 10.39; S, 5.84.

5-Hydantoinacetic acid (**32**). — To a slurry of aspartic acid (46 g) in water (170 ml) at 80° was added, with warming and stirring, 50% sodium hydroxide solution (18 ml) to bring the pH to 5.5. Potassium cyanate (68 g) was added, whereupon the pH increased to 7.2. The mixture was stirred and maintained for 1 h at 85° while

concentrated hydrochloric acid was added dropwise to maintain the pH at 7.0 (16 ml was used). Dissolution resulted during this course of treatment. After the pH had been adjusted to 3.5, additional concentrated hydrochloric acid (43 ml) was added. The mixture was maintained for 2 h at 85°, allowed to cool to room temperature and to stand overnight. The product was collected, washed with cold water, and dried at 50° in a high vacuum. Yield: 20 g, m.p. 214–217°; ν_{max}^{Nujol} 3330, 3175, 1760, 1690 cm^{-1}.

Anal. Calc. for $C_5H_6N_2O_4$: C, 37.98; H, 3.83; N, 17.72. Found: C, 37.98; H, 3.72; N, 17.70.

*2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-*N*-(5-hydantoinacetyl)-β-*D*-glucopyranosylamine* (**33**). — *Method A.* *N,N'*-Dicyclohexylcarbodiimide (2.2 g) was added to a solution of **32** (1.58 g) in *N,N*-dimethylformamide (10 ml) and acetonitrile (60 ml) and the mixture was stirred. Compound **9** (3.46 g) was then added and the mixture was stirred overnight. The precipitated *N,N'*-dicyclohexylurea was filtered off and the filtrate concentrated. Triturating the oily residue with diethyl ether gave the product (4.7 g), which was purified by recrystallization from methanol. The compound was obtained in three forms: (**1**) anhydrous form, m.p. 249–250°; (**2**) hemihydrate, m.p. 248–250°; (**3**) monohydrate, m.p. 245–246°. All three gave a single spot having R_F 0.58 on a thin-layer plate with chloroform–methanol (8:2). I.r. data: ν_{max}^{Nujol} 3310, 3275, 1730, 1700 cm^{-1}.

Anal. Calc. for $C_{19}H_{26}N_4O_{11}$: C, 46.91; H, 5.39; N, 11.52. Found: C, 47.18; H, 5.75; N, 11.38.

Anal. Calc. for $C_{19}H_{26}N_4O_{11} \cdot 0.5\ H_2O$: C, 46.06; H, 5.49; N, 11.31. Found: C, 45.93; H, 5.27; N, 11.24.

Anal. Calc. for $C_{19}H_{26}N_4O_{11} \cdot H_2O$: C, 45.24; H, 5.59; N, 11.11. Found: C, 45.63; H, 5.41; N, 11.28.

*2-Acetamido-2-deoxy-*N*-(5-hydantoinacetyl)-β-*D*-glucopyranosylamine* (**3**). — A suspension of **33** (0.5 g) in anhydrous methanol (100 ml) was mixed with 0.2M methanolic sodium methoxide solution (0.3 ml). The mixture was stirred overnight at room temperature. The resulting solution was evaporated. The white crystalline residue was dissolved in water (4 ml) and acidified with acetic acid (3 drops). Evaporating the solution afforded a syrupy residue, which was recrystallized twice from methanol–diehtyl ether to give the pure product (0.2 g). The material was hygroscopic and was dried *in vacuo*; it melted at 160–180° and decomposed at 245–250° (slow heating). T.l.c. developed in two solvent systems [ethyl acetate–acetone–methanol (2:2:1) and diethyl ether–methanol (7:3)] showed one single spot. Elemental analyses indicated this sample was the monohydrate. A second crop of material (0.12 g) was obtained from the mother liquor. An analytical sample was prepared by recrystallization from methanol–ethyl acetate and dried at 110°, m.p. 187–188° (foamed), o.r.d. data: $[\phi]_{228}$ +10581 shoulder, $[\phi]_{209}$ +11749 peak, $[\phi]_{201}$ 0.0 cross; c.d. data: $[\theta]_{245}$ 0.0, $[\theta]_{215}$ +16410 shoulder, $[\theta]_{197}$ +61127 max; ν_{max}^{Nujol} 3350, 3250, 1750, 1720 cm^{-1}.

Anal. Calc. for $C_{13}H_{20}N_4O_8$: C, 43.34; H, 5.59; N, 15.55. Found: C, 43.06; H, 5.45; N, 15.44.

2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-N-(2-thio-5-hydantoinacetyl)-β-D-glucopyranosylamine (**35**). — *Method A.* To a solution of the thiohydantoinacetic acid[25] (**34**) (1.74 g) in *N,N*-dimethylformamide (10 ml) and acetonitrile (50 ml) were added DCC (2.2 g) and **9** (3.46 g). The mixture was stirred overnight and filtered. The filtrate was evaporated to dryness in a high vacuum, and the residue taken up in hot methanol (40 ml), treated with charcoal, filtered, mixed with diethyl ether (100 ml) and petroleum ether (15 ml), and kept overnight in the cold, to give the crude product (2.2 g), which was purified by chromatographing on a 150-g silica gel column. Elution with 3% methanol in dichloromethane gave **9**. Elution with 5–7% methanol in dichloromethane gave the product **35**, which was recrystallized from methanol; yield: 120 mg, m.p. 241–242° (dec.).

Anal. Calc. for $C_{19}H_{26}N_4O_{10}S$: C, 45.41; H, 5.22; N, 11.15; S, 6.38. Found: C, 44.75; H, 5.25; N, 11.02; S, 6.18.

Method B. A mixture of **34** (0.487 g), **9** (1.0 g), and EEDQ (0.72 g) in *N,N*-dimethylformamide (10 ml) and acetonitrile (50 ml) was stirred overnight, and then concentrated in high vacuum. Addition of diethyl ether (120 ml) to the concentrated solution caused the separation of a heavy oil, which crystallized upon trituration. The solid was collected and washed with diethyl ether until free of quinoline odor; yield: 1.2 g. The material was recrystallized from methanol.

2-Acetamido-2-deoxy-N-(2-thio-5-hydantoinacetyl)-β-D-glucopyranosylamine (**4**). — A mixture of **35** (0.35 g) and 0.2M methanolic sodium methoxide solution (0.2 ml) in anhydrous methanol (100 ml) was stirred for 3 days at room temperature, evaporated *in vacuo* to a solid, which was taken up in water (2 ml), acidified with acetic acid (2 drops), and evaporated to dryness. Recrystallization of the residue from water–methanol afforded the product (0.146 g), m.p. 270–271° (dec.); o.r.d. data: $[\phi]_{263}$ −7576 trough, $[\phi]_{250}$ 0.0 cross, $[\phi]_{231}$ +34422 peak, $[\phi]_{219}$ 0.0 cross, $[\phi]_{213}$ −9882 trough, $[\phi]_{205}$ −3458 peak; c.d. data: $[\phi]_{300}$ 0.0, $[\theta]_{244}$ −24729 max, $[\theta]_{232}$ 0.0 cross, $[\theta]_{221}$ +45391 max, $[\theta]_{208}$ +16346 min, $[\theta]_{197}$ +43423 max.

Anal. Calc. for $C_{13}H_{20}N_4O_7S \cdot 0.5\ H_2O$: C, 40.51; H, 5.49; N, 14.54; S, 8.32. Found: C, 40.84; H, 5.47; N, 14.80; S, 8.19.

2,5-Dioxopiperazine-3-acetic acid (**36**). — A solution of methyl 2,5-dioxopiperazine-3-acetate[26] (1.86 g) was mixed with 2.5M sodium hydroxide solution (4.0 ml), stirred for 1 h, and then treated with Dowex 50W-X4 (50–100 mesh, H^+ form, 1.3 meq/ml) for 5 min. The resin was filtered off and the filtrate was evaporated at 45°. The white, crystalline product was washed with methanol and diethyl ether; yield: 1.55 g, m.p. 214–216°. Recrystallization from water gave pure product, m.p. 222–224°.

Anal. Calc. for $C_6H_8N_2O_4$: C, 41.86; H, 4.68; N, 16.28. Found: C, 41.94; H, 4.70; N, 16.35.

2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-N-(2,5-dioxopiperazine-3-acetyl)-β-D-glucopyranosylamine (**14**). — *Method A.* A solution of **36** (1.72 g) and triethylamine (1.01 g) in *N,N*-dimethylformamide (25 ml) was cooled to −5°. Isobutyl chloroformate (1.50 g, 10% excess) was added and the mixture was stirred for 5 min. Compound **9** (3.46 g) was added, and the resulting mixture was warmed to room temperature and

stirred overnight. The precipitate was removed and the filtrate treated with water (30 ml). The crystalline product thus obtained was recrystallized from acetone, m.p. 264–265°.

Anal. Calc. for $C_{20}H_{28}N_4O_{11}\cdot H_2O$: C, 46.33; H, 5.83; N, 10.81. Found: C, 46.02; H, 5.45; N, 10.81.

Method B. From **13**. Compound **13** (0.68 g) was dissolved in ethanol (100 ml) and then hydrogenated in the presence of 10% palladium-on-charcoal (0.3 g) at *ca.* 40 lb.in.$^{-2}$ for 3 h. Removal of catalyst and solvent afforded a white solid (0.54 g), m.p. 149–150°. T.l.c. of this substance developed in chloroform–methanol (8:2) gave two spots, at R_F 0.32 and 0.48, the former corresponding to **14** obtained by method *A*.

The foregoing material was adsorbed on silicic acid (2 g) and chromatographed on a column of silicic acid (28 g) having an inside diameter of 1.5 cm. The column was eluted with 5% methanol in chloroform. The fast-moving material was eluted in the first 300 ml of eluant. The next 280 ml of eluent gave the product, which was purified by recrystallization.

Ethyl N-(*2-acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl*)-*2*-L-(*benzyloxycarbonyl*)*aminosuccinamate* (**11**). — *Method A*. A solution of **9** (0.70 g) and ethyl *N*-benzyloxycarbonyl-L-1-aspartate[27] **37** (0.60 g) in tetrahydrofuran (25 ml) was mixed with EEDQ (0.55 g), stirred overnight, and evaporated to dryness *in vacuo*. Recrystallization of the residue from dichloromethane–ethanol gave the product (0.88 g, 74%), m.p. 228–229°. One more recrystallization from the same solvent mixture afforded the pure substance as a white crystalline powder, m.p. 231–232°; R_F 0.63; t.l.c. (9:1 chloroform–methanol); *m/e* (principal ions) 623, 577, 564, 550, 534, 517, 504, 458, 431, 428, 398, 385, 368, 356, 343, 330, 329, 270, 241, 228, 227, 210, 168, 150.

Anal. Calc. for $C_{28}H_{37}N_3O_{13}$: C, 53.79; H, 5.98; N, 6.51. Found: C, 53.93; H, 5.98; N, 6.74.

Method B. A solution of **9** (0.70 g) and **37** (0.60 g) in dichloromethane (20 ml) was mixed with DCC (0.45 g) and stirred for 3 days. Acetic acid (1 drop) was added and stirring was continued for 1 h. The mixture was filtered to remove the *N,N'*-dicyclohexylurea, and the filtrate was evaporated to dryness to give the product as a white solid (1.0 g). After one recrystallization from dichloromethane–ethyl acetate, the substance melted at 222–223° and was identical by t.l.c. with an authentic sample.

Ethyl N-(*2-acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl*)-*2*-L-*aminosuccinamate* (**12**). — A solution of **11** (0.252 g) in ethanol (20 ml) was hydrogenated in the presence of 10% palladium-on-charcoal at room temperature and atmospheric pressure for 3 h. Removing the catalyst and the solvent afforded the product (0.19 g), which was recrystallized from ethanol–diethyl ether, m.p. 161–163°; R_F 0.35; t.l.c. (in 9:1 chloroform-methanol).

Ethyl N-(*2-acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl*)-*2*-L-[N-(*benzyloxycarbonyl*)*glycyl*]*aminosuccinamide* (**13**). — A solution of **12** (0.06 g) and *N*-(benzyloxycarbonyl)glycine (0.0255 g) in dichloromethane (10 ml) was mixed with EEDQ (0.0331 g), stirred for 20 h, and evaporated to dryness to give the crude pro-

duct as a white solid (0.1 g). Recrystallization from ethanol–diethyl ether gave the pure product as a white crystalline powder, m.p. 221–222°; R_F 0.69 t.l.c. (*in* 8:2 chloroform–methanol).

Anal. Calc. for $C_{30}H_{40}N_4O_{14}$: C, 52.94; H, 5.92; N, 8.23. Found: C, 52.79; H, 6.04; N, 8.00.

*2-Acetamido-*N*-(2,5-dioxopiperazine-3-acetyl)-2-deoxy-β-*D*-glucopyranosylamine* (**5**). — Compound **14** (0.60 g) was dissolved in anhydrous methanol (160 ml), mixed with 0.2M methanolic sodium methoxide solution (1 ml), and stirred for 18 h. The mixture became turbid after 45 min, and some white crystals separated at the end of the reaction. Acetic acid (6 drops) was added, and the mixture evaporated to dryness, giving a white, crystalline residue (0.50 g). Two recrystallizations of the residue from water–methanol afforded the pure product as white, fine needles, m.p. 261° (dec.); t.l.c. R_F 0.176 (in 3:2 ethyl acetate–methanol); o.r.d. data: $[\phi]_{230}$ +7135 peak, $[\phi]_{218}$ 0.0 cross; c.d. data: $[\theta]_{250}$ 0.0, $[\theta]_{215}$ +22469 shoulder, $[\theta]_{198} \sim +39000$ max; ν_{max}^{Nujol} 1670, 1640 cm^{-1}.

Anal. Calc. for $C_{14}H_{22}N_4O_8$: C, 44.92; H, 5.92; N, 14.97. Found: C, 44.53; H, 5.96; N, 14.87.

*4-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-*D*-glucopyranosyl)-*N*-(benzyloxycarbonyl)homoserine amide* (**17**). — Racemic 2-benzyloxycarbonylamino-4-hydroxybutyramide[28] (**16**) (1.26 g) was suspended in anhydrous benzene (110 ml), and the mechanically stirred mixture was heated in a 250-ml three-necked flask in an oil bath at 95° to distil off some benzene (26 ml). Mercuric cyanide (1.77 g) was then added, and more benzene (25 ml) was removed. Compound[29] **15** (2.0 g) was then added. The mixture was stirred and refluxed for 2 h, and anhydrous benzene (50 ml) was added to thin the mixture. Stirring and refluxing were continued overnight. After cooling, the solid was collected, washed with ethyl acetate, boiled with chloroform (100 ml), and filtered rapidly. The filtrate was concentrated to a volume of 10 ml. Diethyl ether (20 ml) was added slowly with stirring to give the product (1.42 g); t.l.c. (in 9:1 chloroform–methanol), two adjacent spots (two diastereoisomers). The material was recrystallized from absolute ethanol as a white, crystalline powder, m.p. 230–235° (shrank at 220°); ν_{max}^{Nujol} 3420, 3310, 1745, 1720, 1687, 1670 cm^{-1}.

Anal. Calc. for $C_{26}H_{35}N_3O_{12}$: C, 53.70; H, 6.07; N, 7.22. Found: C, 53.93; H, 6.09; N, 7.22.

*4-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-*D*-glucopyranosyl)homoserine amide* (**18**). — A mixture of **17** (0.50 g), 10% palladium-on-charcoal (0.30 g) and absolute ethanol (10 ml) was hydrogenated at room temperature and atmospheric pressure. Removal of the catalyst and solvent afforded the product (0.42 g) as a syrupy liquid. It had a different i.r. spectrum and a much lower mobility on t.l.c. than compound **17**.

*4-O-(2-Acetamido-2-deoxy-β-*D*-glucopyranosyl)homoserine amide* (**19**). — Compound **18** (0.355 g) was dissolved in anhydrous methanol (5 ml), mixed with a saturated solution of ammonia in methanol (1 ml), kept for 7 h at room temperature, evaporated, and dried overnight. The residue was triturated with diethyl ether and

collected. The product thus obtained was an amorphous solid, R_F 0.365 by t.l.c. with 3:3:3:1 butyl alcohol–acetone–water–pyridine.

Anal. Calc. for $C_{12}H_{23}N_3O_7 \cdot 0.5\ H_2O$: C, 43.63; H, 7.32; N, 12.72. Found: C, 43.65; H, 7.48; N, 12.22.

4-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-N-acetylhomoserine amide (**20**). — *Procedure A.* Compound **19** (0.17 g) was dissolved in pyridine (2 ml), mixed with acetic anhydride (2 ml), and kept overnight. Evaporating afforded a pale solid (0.26 g) that had a greater mobility (R_F 0.80 in the same t.l.c. system used to detect **19**) than **19**. The material was recrystallized from absolute ethanol as white needles, m.p. 237–239°; ν_{max}^{Nujol} 3420, 3324, 3290, 3226, 3110, 1749, 1725, 1675, 1658, 1640, 1625 cm^{-1}; t.l.c. (in 8:2 chloroform–methanol), one major spot at R_F 0.42 and a minor spot at R_F 0.38.

Anal. Calc. for $C_{20}H_{31}N_3O_{11}$: C, 49.07; H, 6.38; N, 8.58. Found: C, 48.79; H, 6.31; N, 8.54.

Processing of the mother liquor afforded a second crop of product, m.p. 222–225°, which gave two spots of equal intensity on t.l.c.

Procedure B. A mixture of **17** (0.87 g), 10% palladium-on-charcoal (0.30 g), acetic anhydride (5 ml), and 90% acetic acid (30 ml) was hydrogenated at 44 lb.$in.^{-2}$ for 3 h at 20°. The catalyst and solvents were removed and the residue was repeatedly evaporated with water until free of acetic acid (odor), and was finally dried to give a gel-like solid (0.67 g). It was triturated with diethyl ether, collected, and recrystallized from absolute ethanol as a crystalline powder (0.575 g), m.p. 223–224°. The i.r. spectrum and t.l.c. behavior were identical to those of a sample obtained by procedure *A*, except that the two spots on t.l.c. were of equal intensity.

4-O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-N-acetylhomoserine amide (**6**). — Compound **20** (0.27 g) was dissolved in methanol (10 ml), mixed with a saturated solution of ammonia in methanol (5 ml), and kept for 20 h in the cold. Evaporation followed by drying afforded a glassy solid (0.24 g) that was triturated with diethyl ether, collected, and rinsed with acetone. The product thus obtained was hygroscopic, and was dried to a powder; t.l.c. R_F 0.11 (in 2:1:1 acetone–chloroform–methanol). This sample gave satisfactory analyses for carbon, hydrogen, and nitrogen.

One sample of the amorphous product was recrystallized from methanol–diethyl ether as white granules, m.p. 225° (dec.), homogeneous on t.l.c.; ν_{max}^{Nujol} 3640–3040 (broad, with several bands), 1675 (shoulder), 1653 cm^{-1}; o.r.d. data: $[\phi]_{225}$ −4776 trough, $[\phi]_{214}$ 0.0. cross, $[\phi]_{200}$ +14409 peak; c.d. data: $[\theta]_{235}$ 0.00, $[\theta]_{212}$ −11962 max, $[\theta]_{202}$ 0.0 cross.

Anal. Calc. for $C_{14}H_{25}N_3O_8 \cdot 0.5\ H_2O$: C, 45.15; H, 7.04; N, 11.28. Found: C, 45.14; H, 7.20; N, 11.02.

2-Acetamido-3,4,6-tri-O-acetyl-N-[2-(benzyloxycarbonyl)-2-(benzyloxycarbonyl)-aminoethanesulfonyl]-2-deoxy-β-D-glucopyranosylamine (**22**). — A solution of **9** (0.692 g) in pyridine (3 ml) was stirred and cooled in an ice bath, while 2-(benzyloxycarbonyl)-2-(benzyloxycarbonyl)aminoethanesulfonyl chloride[30] (**21**) (0.824 g) was added. The mixture was then stirred for 1 h at room temperature and concentrated

to a gum. The gummy residue was taken up in ethyl acetate, washed with water, dried, and evaporated to dryness. Recrystallizing the residue from dilute ethanol gave the pure product as white needles (0.271 g), m.p. 187–189° (dec.).

Anal. Calc. for $C_{32}H_{39}N_3O_{14}S$: C, 53.26; H, 5.45; N, 5.82. Found: C, 53.16; H, 5.26; N, 5.91.

*2-Acetamido-3,4,6-tri-*O*-acetyl-*N*-(2-amino-2-carboxyethanesulfonyl)-2-deoxy-β-*D*-glucopyranosylamine* (7). — Compound **22** was dissolved in ethanol (200 ml) by warming, and was then hydrogenated in the presence of 10% palladium-on-charcoal (0.25 g) at 40 lb.in.$^{-2}$ for 90 min. Removal of the catalyst and solvent gave a solid residue, which was dissolved in methanol. Diethyl ether was added to precipitate the product. The precipitation process was repeated. The product thus obtained was a slightly yellow, non-crystalline solid (0.26 g) that was homogeneous, R_F 0.65, by t.l.c. on a cellulose plate developed in butyl alcohol–pyridine–acetic acid–water (15:10:3:12 vol.); spot detected by ninhydrin.

Anal. Calc. for $C_{17}H_{27}N_3O_{12}S$: C, 41.04; H, 5.47; N, 8.45. Found: C, 40.74; H, 5.89; N, 7.61.

Attempted deacetylation of **7** in diluted ammonia in methanol gave the decomposition product 3-aminosulfonylalanine[30] (**24**).

*2-Acetamido-*N*-(2-acetamido-2-carboxyethanesulfonyl)-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosylamine* (**25**). — A mixture of **22** (0.72 g), 90% acetic acid (40 ml), acetic anhydride (2 ml) and 10% palladium-on-charcoal was hydrogenated for 2 h at room temperature and at atmospheric pressure. The catalyst and solvents were removed, and the residue evaporated several times with a small amount of water until free of acetic acid odor, and dried. The residue thus obtained was a white, glassy solid (0.53 g), m.p. 115° (dec.). Recrystallization from ethanol–ether gave white, hygroscopic crystals m.p. 121° (dec.).

Anal. Calc. for $C_{19}H_{29}N_3O_{13}S$: C, 42.30; H, 5.42; N, 7.79; S, 5.94. Found: C, 40.89; H, 5.45; N, 7.68; S, 6.23.

*2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-*N*-methylsulfonyl-β-*D*-glucopyranosylamine* (**26**). — A solution of **9** (0.697 g) in pyridine (10 ml) was stirred and cooled to −10°. Methanesulfonyl chloride (0.25 g) was added dropwise. After stirring overnight, the mixture was evaporated to dryness at <30°. The residue was taken up in water and extracted with ethyl acetate. Evaporating the extract afforded the product, which was recrystallized from chloroform–diethyl ether as a white crystalline powder, m.p. 216–218° (dec.); ν_{max}^{Nujol} 3340, 3275, 1750, 1747, 1660, 1540, 1320 cm^{-1}, t.l.c., R_F 0.53 (in 9:1 chloroform–methanol).

Anal. Calc. for $C_{15}H_{24}N_2O_{10}S$: C, 42.45; H, 5.70; N, 6.60; S, 7.55. Found: C, 42.23; H, 5.67; N, 6.34; S, 7.84.

*2-Acetamido-2-deoxy-1-*N*-methanesulfonyl-β-*D*-glucopyranosylamine* (**8**). — A solution of **26** (1.27 g) in dry methanol (10 ml) was mixed with a saturated solution of ammonia in methanol (3 ml), kept overnight in the cold, and evaporated to dryness to give an oily residue that crystallized upon stirring. The crude product was recrystallized from dilute methanol and dried at 100°, m.p. 196–197°; ν_{max}^{Nujol} 3650–3050,

1645, 1550, 1320 cm^{-1}; p.m.r. data (in methyl sulfoxide-d_6): τ 2.05–2.45 (2-proton pyramid), 5.06 (2-proton doublet, J 4 Hz), 5.35–5.85 (2-proton pyramid), 6.05–7.3 (7 protons), 7.03 (3-proton singlet), 8.16 (3-proton singlet); o.r.d. data: $[\phi]_{202}$ +11344 peak; c.d. data: $[\theta]_{245}$ 0.0, $[\theta]_{212}$ −3646 max, $[\theta]_{205}$ 0.0 cross.

Anal. Calc. for $C_9H_{18}N_2O_7S \cdot 0.5\ H_2O$: C, 35.17; H, 6.23; N, 9.13; S, 10.43. Found: C, 35.23; H, 6.37; N, 8.89; S, 10.26.

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