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The Synthesis of Antigenic Glycopeptides, 2-Acetamido-*N*-(β-L-aspartyl)-2-deoxy-4-*O*-β-(D-galactopyranosyl)-β-D-glucopyranosylamine. (*N*-Acetyllactosaminyl-L-asparagine)

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The title compound, serving as intermediate in the chemical and biochemical synthesis of glycopeptides and as a reference substance in the structure elucidation of glycoproteins, was synthesized. 2-Acetamido-4-*O*-allyl-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl azide was reduced to the corresponding β-D-glucosylamine and coupled with 1-benzyl *N*-benzyloxycarbonyl-L-aspartate to give 2-acetamido-4-*O*-allyl-3,6-di-*O*-benzyl-*N*-[(*S*)-3-benzyloxycarbonyl-3-benzyloxycarbonylamino)propionyl]-2-deoxy-β-D-glucopyranosylamine. Removal of the allyl group of the latter followed by condensation with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide gave the fully protected *N*-acetyllactosaminyl-L-asparagine. De-blocking of the *O*-acetyl, benzyl, and benzyloxycarbonyl groups gave the title compound.

Carbohydrate chains attached to protein and to lipid moieties are known to be antigenic both at the surface of bacterial and mammalian cells. The role of carbohydrate in antigenic components at the surface of erythrocytes has been well established for the ABO and MN-antigen systems and suggested for I, P, and T-antigen systems.[1] Carbohydrate chains are also components of the cancer cells[2] and transplantation antigens.[3]

In view of these facts and the interest in (a) studying the inhibition of lectins in relation to the structure of receptor sites[4–6] and (b) structural indentification by gas-liquid chromatography combined with mass spectrometry,[7] a research program has been launched for the synthesis of antigenic oligosaccharides,[8] glycopeptides,[9] and isoprenoid sugar phosphates.[10]

The synthesis of *N*-acetyllactosaminyl-L-asparagine has been undertaken as a part of this program and because 2-amino-2-deoxy-4-*O*-β-D-galactopyranosyl-D-glucose (lactosamine) is the determinant group in the antigenic, Type XIV pneumococcus polysaccharide.[11] The synthesized glycopeptide may also be used as a starting material for the synthesis[9,12] and biosynthesis of larger glycopeptides.

Results and Discussion

In synthesizing *N*-acetyl-D-glucosamine-containing glycopeptides[12–17] it has been shown that derivatives of 2-acetamido-2-deoxy-β-D-glucopyranosyl azide[18] were the most convenient starting materials since they are stable, crystalline, and can be transformed, under mild conditions, into β-D-glucopyranosylamine derivatives ready for coupling with amino acids. In addition, β-D-glycosyl azides showed no sign of changing their anomerization throughout reduction and coupling.[17,19,20]

Taking these facts into consideration while designing a synthetic pathway for the title compound, one of three possible approaches may be adopted (a) starting with *N*-acetyllactosamine and going through the α-glycosyl halide, β-glycosyl azide, β-glycosylamine, and finally *N*-acetyllactosaminyl-L-asparagine; (b) Koenigs-Knorr condensation of α-D-galactosyl halide with a suitably protected 2-acetamido-2-deoxy-β-D-glucopyranosyl azide, followed by reduction of the azido group and coupling with aspartic acid; and (c) Koenigs-Knorr condensation of a suitably blocked 2-acetamido-2-deoxy-β-D-glucopyranosylamine-L-aspartic acid derivative with α-D-galactopyranosyl halide. The first approach suffers the disadvantages of the inavailability of *N*-acetyllactosamine, and the possible splitting of the glycosidic linkage during the preparation of the glycosyl halide.[16,17] Executing either of the two other alternatives requires the synthesis of a 2-acetamido-2-deoxy-β-D-glucopyranosyl azide blocked at positions 3 and 6 and having the 4-hydroxyl group available for Koenigs-Knorr condensation.

Previous work[21–23] has shown that the 4-hydroxyl group in 2-acetamido-2-deoxy-D-glucopyranosyl residues is not reactive in the Koenigs-Knorr reaction, and that, when both 4- and 6-hydroxyl groups are free, this reaction occurs exclusively at *O*-6.[22,23] Recourse to open chain intermediates[24] was necessary to prepare (1→4) linked *N*-D-glucosamine-containing oligosaccharides.[8,24–26] Although 2-acetamido-3,6-di-*O*-acetyl-2-deoxy-D-glucopyranosyl derivatives showed no reactivity at the 4-hydroxyl group, benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α- and β-D-glucopyranosides[8,27] were successfully condensed to give (1→4) linked oligosaccharides.[8,28] We decided, therefore, to prepare 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl azide (**14**) as a suitable substrate for Koenigs-Knorr condensation with α-D-galactosyl halide to give β-*N*-acetyllactosaminyl azide.

2-Acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl azide[13] (**2**) was prepared by a modified method in which 2-acetamido-2-deoxy-β-D-glucopyranosyl azide[18] (**1**) was treated with benzaldehyde and anhydrous formic acid as a dessicant instead of

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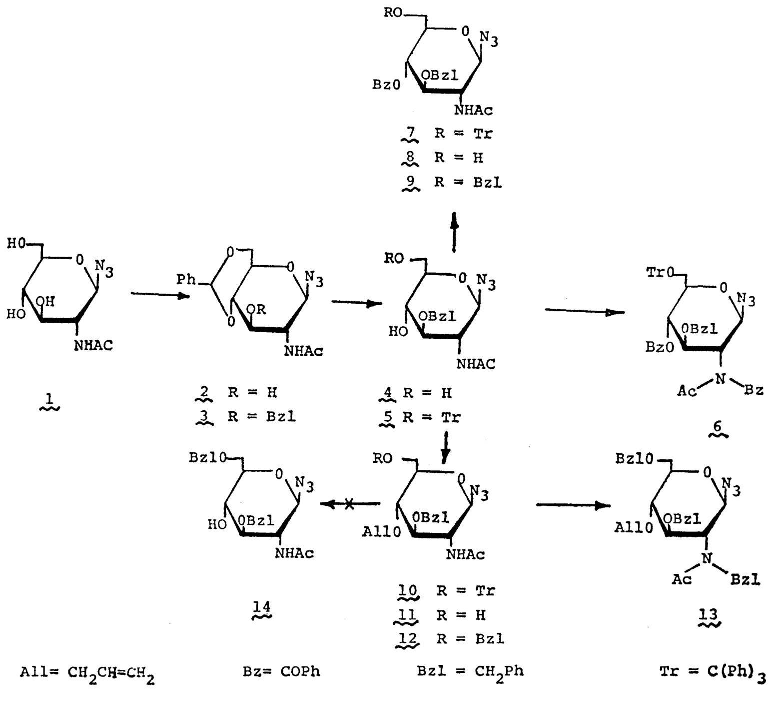
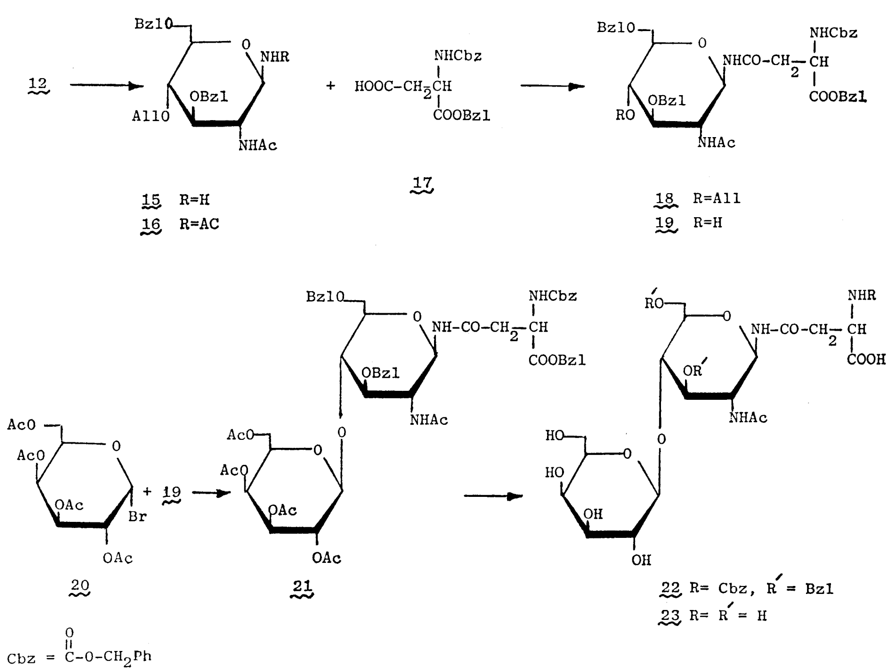
the usually used anhydrous zinc chloride. Benzylation of **2** with benzyl bromide in *N*,*N*-dimethylformamide and in the presence of a mixture of barium hydroxide and barium oxide, gave the 3-*O*-benzyl derivative **3**. Removal of the 4,6-*O*-benzylidene group was achieved by heating with 60% acetic acid to give **4**, which was tritylated to afford **5**. An attempt to prepare 2-acetamido-4-*O*-benzoyl-3-*O*-benzyl-2-deoxy-6-*O*-triphenylmethyl-*β*-D-glucopyranosyl azide (**7**) by benzoylating **5** with benzoyl chloride in the presence of pyridine at room temperature resulted in *N*- and *O*-benzoylation to give **6**. *O*-Benzylation, however, could be effected when the reaction was performed at −70 °C and in the presence of a limited amount of benzoyl chloride to give **7** in good yield (78%). Detritylation of **7** with hydrochloric acid in aqueous methanol for 4 h at room temperature gave **8**. Attempted conversion of 2-acetamido-4-*O*-benzoyl-3-*O*-benzyl-2-deoxy-*β*-D-glucopyranosyl azide (**8**) to 2-acetamido-4-*O*-benzoyl-3,6-di-*O*-benzyl-2-deoxy-*β*-D-glucopyranosyl azide (**9**) by benzylation with benzyl bromide and silver oxide, according to the method described by Sinaÿ[27] for a similar derivative, gave a mixture difficult to separate either by chromatography or fractional crystallization. This result, together with a previous similar one,[8] may be attributed to the partial migration of the 4-*O*-benzoyl group under the slightly alkaline conditions of the reaction.

Consequently, a new route for the synthesis of 2-acetamido-3,6-di-*O*-benzyl-*β*-D-glucopyranosyl azide (**14**) was contrived with intermediates containing the *O*-allyl protective group[29,30] instead of the benzoyl group. Allylation of **5** with allyl bromide and sodium hydroxide in benzene for 72 h gave 2-acetamido-4-*O*-allyl-3-*O*-benzyl-2-deoxy-6-*O*-triphenylmethyl-*β*-D-glucopyranosyl azide (**10**) in 77% yield. However, the reaction could be expedited (5 h) and the yield improved (89%) by carrying out the allylation in *N*,*N*-dimethylformamide. Detritylation of **10** with 60% acetic acid gave 2-acetamido-4-*O*-allyl-3-*O*-benzyl-2-deoxy-*β*-D-glucopyranosyl azide (**11**). Benzylation of the latter with benzyl bromide in *N*,*N*-dimethylformamide and in the presence of sodium hydroxide gave the expected *O*-benzylated product **12** in addition to the *O*- and *N*-benzylated product **13** in a 1: 1.7 ratio. Attempted removal of the 4-*O*-allyl group of **12** by isomerization with tris(triphenylphosphine)rhodium chloride in the presence of 1,4-diazabicyclo[2.2.2]octane[8,31,32] followed by splitting of the resulting *O*-1-propenyl group with mercury(II) chloride,[8,33] failed giving a complex mixture of highly polar compounds devoid of the azido group. This may be attributed to the reaction of the azido group with the rhodium triphenylphosphine complex to give glycosylphosphinimines.[34,35] It was necessary, therefore, to reduce the azido group of **12**, and coupling the resulting glycosylamine with L-aspartic acid prior to the removal of the 4-*O*-allyl group.

The commonly used method[12–17] of reducing glycosyl azides by hydrogenation in the presence of Adam's catalyst, would be unsuitable for reducing compound **12** as it would lead to indiscriminate reduction of the azido and allyl groups. Saturation to *O*-propyl groups deprive the *O*-allyl groups their desirable selective removal in the presence of *O*-benzyl groups. Sodium borohydride was attempted as a selective reductant of the azido group without affecting the *O*-allyl function. In methanol, no such reduction took place and compound **12** was recovered unchanged. In *N*,*N*-dimethylformamide, however, the reduction took place smoothly at room temperatures to give the unstable glycosylamine **15** which showed a positive ninhydrin reaction.[36] The amine **15** was characterized by acetylation into the stable and crystalline 2-acetamido-*N*-acetyl-4-*O*-allyl-3,6-di-*O*-benzyl-2-deoxy-*β*-D-glucopyranosylamine (**16**).





In order to prevent the formation of bis(glycosylamines),[17,19,37] the unstable amine **15** was used without further purification in the next step. Condensation of **15** with 1-benzyl-*N*-benzyloxycarbonyl-L-aspartate[38,39] (**17**) in the presence of dicyclohexylcarbodiimide gave crystalline 2-acetamido-4-*O*-allyl-3,6-di-*O*-benzyl-*N*-[(*S*)-3-benzyloxycarbonyl-3-(benzyloxycarbonylamino)propionyl]-2-deoxy-B-D-glucopyranosylamine (**18**) in 53% yield. Removal of the 4-*O*-allyl group of **18** using tris(triphenylphosphine)rhodium chloride and mercury(II)chloride gave crystalline **19**.

Koenigs-Knorr condensation of **19** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide[40] (**20**) in the presence of mercury(II) cyanide gave the fully protected *N*-acetyllactosaminyl-L-asparagine **21**. Assigning the β-D-configuration to the D-galactopyranosyl residue was based on the result of a previous synthesis.[16] Treatment of **21** with lithium hydroxide removed the protective *O*-acetyl groups of the D-galactopyranosyl moiety and the benzyl ester of L-aspartic acid giving **22**. Hydrogenolysis of the *N*-benzyloxycarbonyl and *O*-benzyl groups of **22** gave the title compound **23**.

Experimental

General. Melting points were determined with a Mettler FP2 hot stage equipped with a microscope, and correspond to "corrected" melting points. Optical rotations were determined for solutions in 1-dm, semimicro tubes with a Perkin-Elmer Model 141 polarimeter. Infrared spectra were recorded, for potassium bromide discs or for thin film, with a Perkin-Elmer Model 237 spectrophotometer. Chloroform used was analytical grade and contained about 0.75% ethanol. All solvent mixtures used were v/v. Solvents were dried over molecular sieve (type 5A, Grade 522, 8—12 mesh, Fisher Scientific Co., Fairlawn, N. J.). The cation-exchange resin used was in at least two-fold over the quantity necessary to effect complete ion exchange. Evaporations were performed in a rotary evaporator under diminished pressure, with an outside bath-temperature kept below 40 °C. Solutions ($<$5 ml) were evaporated under a stream of nitrogen. The microanalyses were performed by Dr. M. Manser, Zurich, Switzerland.

Chromatographic Methods. Column chromatography on silica gel was performed on Silica gel Merck (10—325 mesh, E. Merck A. G., Darmstadt, Germany) used without pretreatment. The ratio of the diameter of the column to its length was 1:8 to 1:12. The volume of the fractions eluted was 2 to 3 ml/g of substance to be chromatographed. The proportion of weight of substance to weight of silica gel was 1:60 to 1:100. Thin-layer chromatography (TLC) was performed on plates precoated with Silica gel G (layer thickness 0.25 mm. Merck), the plates supplied were cut to a length of 6 cm before use, but otherwise they were used without pretreatment. The distance of solvent-travel was 5 cm and the zones were detected by spraying the chromatograms with 1:1:18 *p*-anisaldehyde-sulfuric acid–ethanol[40] following by heating on a hot plate for a few minutes.

*2-Acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl Azide (2).* A suspension of 2-acetamido-2-deoxy-β-D-glucopyranosyl azide[18] (**1**) (1.88 g) in freshly distilled benzaldehyde (15 ml) was stirred for 5 min at room temperature and then treated with 99% formic acid (20 ml). After stirring for a further 30 min, the reaction mixture was cooled in an ice-bath and gradually neutralized with a saturated aqueous solution of potassium carbonate. The heterogeneous mixture was distilled under reduced pressure and the excess benzaldehyde removed by repeated co-distillation with water. The residue, which remained, was triturated with water, filtered, washed with water, and dried. Crystallization from methanol gave 1.95 g (76%) of **2** as needles, mp and mixed mp[13] 214—216 °C (dec).

*2-Acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl Azide (3).* A solution of **2** (1.4 g) in dry *N,N*-dimethylformamide (30 ml) was treated with benzyl bromide (1.3 ml), barium oxide (5 g), and barium hydroxide octahydrate (1.3 g) and the mixture was stirred for 2 h at room temperature. Chloroform (50 ml) was added and the mixture was heated under reflux for 2 h while stirring and then filtered while hot. The inorganic residue was washed with hot chloroform (3×50 ml) and the filtrate and washings were evaporated to dryness. Crystallization of the residue from chloroform–methanol gave 1.45 g (82%) of **3** as needles, mp 224—225 °C (dec); $[\alpha]_D^{20}$ −30° (*c* 2.1, pyridine); ν_{max}^{KBr} 3290 (NH), 2130 (N_3), 1655 (Amide I), 1555 (Amide II), 750, 725, and 690 cm^{-1} (Ph); TLC in 19:1 chloroform–ethanol R_f 0.32. Found: C, 62.18; H, 5.71; N, 13.16; O, 18.70%. Calcd for $C_{22}H_{24}N_4O_5$: C, 62.25; H, 5.70; N, 13.20; O, 18.85%.

2-Acetamido-3-O-benzyl-2-deoxy-β-D-glucopyranosyl Azide (4). A suspension of **3** (1.06 g) in 60% acetic acid (50 ml) was heated for 2 h at 90—95 °C. The clear solution was evaporated under diminished pressure and the residue was dried by several co-distillations with toluene. Chromatography of the residue on a column of silica gel with 19:1 chloroform–ethanol gave 655 mg (78%) of **4**, which crystallized from methanol–chloroform–ether; mp 173—174 °C (dec); $[\alpha]_D^{20}$ −29° (*c* 2.24, methanol); ν_{max}^{KBr} 3350 (OH), 3290 (NH), 2130 (N_3), 1645 (Amide I), 1550 (Amide II), 730, and 690 cm^{-1} (Ph); TLC in 4:1 chloroform–ethanol R_f 0.52. Found: C, 53.47; H, 5.99; N, 16.71; O, 23.58%. Calcd for $C_{15}H_{20}N_4O_5$: C, 53.57; H, 5.99; N, 16.66; O, 23.78%.

2-Acetamido-3-O-benzyl-2-deoxy-6-O-triphenylmethyl-β-D-glucopyranosyl Azide (5). A solution of **4** (700 mg) in dry pyridine (30 ml) was treated with chlorotriphenylmethane (1.4 g) for 30 h at room temperature. The mixture was poured onto a mixture of ice (200 g) and potassium carbonate (5 g), and then extracted with chloroform (4×50 ml). The extracts were washed with water (3×50 ml), dried (K_2CO_3) and evaporated. The last traces of pyridine was removed by several additions and evaporations of toluene. The residue was chromatographed on a column of silica gel with 19:1 chloroform–ethanol containing 0.1% of triethylamine, to give 1.16 g (84%) of **5**, which crystallized from dichloromethane–carbon tetrachloride as needles, mp 116—118 °C; $[\alpha]_D^{20}$ −22° (*c* 2.3, chloroform); ν_{max}^{KBr} 3500 (OH), 3280 (NH), 2120 (N_3), 1650 (Amide I), 1550 (Amide II), 740, and 690 cm^{-1} (ph); TLC in 19:1 chloroform–ethanol, R_f 0.32. Found: C, 63.79; H, 5.27; N, 8.62; O, 12.13%. Calcd for $C_{34}H_{34}N_4O_5 \cdot CH_2Cl_2$: C, 63.99; H, 5.37; N, 8.29; O, 11.84%.

2-(N-Acetylbenzamido)-4-O-benzoyl-3-O-benzyl-2-deoxy-6-O-triphenylmethyl-β-D-glucopyranosyl Azide (6). A solution of **5** (800 mg) in dry pyridine (30 ml) was treated with benzyl chloride (2 ml) for 2 h at room temperature. The mixture was poured into an ice-cold solution of sodium hydrogencarbonate and then extracted with chloroform (4×50 ml). The chloroform extracts were washed with water (3×50 ml), and dried (K_2CO_3). Evaporation of the solvent and removal of the residual pyridine by several co-distillations with toluene gave a residue which was chromatographed on a column of silica gel with chloroform. Fractions containing **6** were evaporated and the residue crystallized from dichloromethane–ether–pentane as needles (863 mg; 91%), mp 154—157 °C; $[\alpha]_D^{22}$ −33.4° (*c* 2.3, chloroform); ν_{max}^{KBr} 2120 (N_3), 1725 (O. COPh), 1700 (N. COPh), 1670 (N. $COCH_3$), 745, and 690 cm^{-1} (Ph); TLC in chloroform R_f 0.54. Found: C, 73.23; H, 5.42; N, 7.15; O, 14.20%. Calcd for $C_{48}H_{42}N_4O_7$: C, 73.27; H, 5.38; N, 7.12; O, 14.23%.

2-Acetamido-4-O-benzoyl-3-O-benzyl-2-deoxy-6-O-triphenylmethyl-β-D-glucopyranosyl Azide (7). A solution of **5** (664 mg) in dry pyridine (10 ml) was cooled to −70 °C and treated with a 14% (w/v) solution (1 ml) of benzoyl chloride in dry benzene. The mixture was kept at −20 °C for 16 h and then treated with a few drops of methanol, evaporated, and the residual pyridine removed by repeated addition and distillation of toluene. The syrupy residue which obtained could not be crystallized, and it was, therefore, chromatographed on a column of silica gel with 19:1 chloroform–ethanol to give 532 mg (78%) of **7**. It crystallized from dichloromethane–ether as needles, mp 169—170 °C; $[\alpha]_D^{20}$ +0.5° (*c* 2.8, chloroform); ν_{max}^{KBr} 3280 (NH), 2130 (N_3), 1730 (O. COPh), 1655 (Amide I), 1550 (Amide II), 740, and 680 cm^{-1} (Ph); TLC in 19:1 chloroform–ethanol R_f 0.38. Found: C, 72.10; H, 5.54; N, 8.12; O, 14.14%. Calcd for $C_{41}H_{38}N_4O_6$: C, 72.12; H, 5.61; N, 8.20; O, 14.06%.

2-Acetamido-4-O-benzoyl-3-O-benzyl-2-deoxy-β-D-glucopyranosyl Azide (8). A solution of **7** (1.9 g) in methanol (75 ml) was treated with 2 M hydrochloric acid until the mixture becomes just turbid (about 10 ml) and kept for 4 h at room temperature. The mixture was diluted with cold water (200 ml), extracted with chloroform (5×75 ml), and the extracts were washed with water (2×50 ml), saturated sodium hydrogencarbonate solution (2×50 ml), and water (3×50 ml), dried (Na_2SO_4), and evaporated. The residue was chromatographed on a column of silica gel with 9:1 chloroform–ethanol, to give 1.03 g (84%) of **8**, which crystallized from dichloromethane–ether; mp 178—190 °C (dec); $[\alpha]_D^{20}$ −28° (*c* 2.0, chloroform); ν_{max}^{KBr} 3480 (OH), 3280 (NH), 2130 (N_3), 1720 (O. COPh), 1655 (Amide I), 1550 (Amide II), 745, and 700 cm^{-1} (Ph); TLC in 19:1 chloroform–ethanol, R_f 0.20, and in 9:1 chloroform–ethanol, R_f 0.40. Found: C, 60.06; H, 5.49; N, 12.76; O, 21.98%. Calcd for $C_{22}H_{24}N_4O_6$: C, 59.99; H, 5.49; N, 12.72; O, 21.79%.

Attempted Synthesis of 2-Acetamido-4-O-benzoyl-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl Azide (9). Compound **8** (500 mg) in anhydrous benzene (30 ml) was treated with benzyl bromide (1.5 ml) and silver oxide (700 mg) and the mixture stirred for 24 h at 60—70 °C in the dark. After cooling to room temperature, the mixture was diluted with dichloromethane (30 ml) and filtered through a celite layer. To the filtrate pyridine (3 ml) was added and the mixture was evaporated and dried by several additions and distillations of toluene. The syrup obtained showed, on TLC, to contain more than four products. Attempted separation of these products by fractional crystallization or by repetitive column chromatography on silica gel using different solvent systems was unsuccessful.

Carrying the reaction at lower temperature (40—50 °C) and for a shorter duration (16 h) led to the same result.

2-Acetamido-4-O-allyl-3-O-benzyl-2-deoxy-6-O-triphenylmethyl-β-D-glucopyranosyl Azide (10). *Method A*: To a solution of **5** (500 mg) in dry benzene (20 ml) allyl bromide (110 mg) and powdered sodium hydroxide (1.25 g) were added and the mixture stirred for 72 h at room temperature. The mixture was filtered on a celite layer and the inorganic residue was washed with benzene (2×50 ml). The filtrate and washings were washed with water (3×50 ml), dried (Na_2SO_4), and evaporated. The residue which obtained was chromatographed on a column of silica gel with 19:1 chloroform–ethanol to give 360 mg (77%) of **10**, which crystallized from dichloromethanol–pentane as needles, mp 190—191 °C; $[\alpha]_D^{20}$ +1.4° (*c* 1.04, chloroform); ν_{max}^{KBr} 3300 (NH), 2130 (N_3), 1650 (Amide I), 1550 (Amide II), 740, and 680 cm^{-1} (Ph); TLC in 19:1 chloroform–ethanol R_f 0.44. Found: C, 71.72; H, 6.20; N, 9.05; O, 13.04%. Calcd for $C_{37}H_{38}N_4O_5$: C, 71.82; H, 6.19; N, 9.06; O, 12.93%.

Method B: A solution of **5** (500 mg) in *N,N*-dimethylformamide (20 ml) was treated with the same amounts of allyl bromide and sodium hydroxide as described in Method A. After completion of the reaction (5 h), the mixture was diluted with chloroform (200 ml), washed with water (4×50 ml), and dried (Na_2SO_4). Evaporation of the solvents and chromotography of the residue gave 416 mg (89%) of **10** having the same properties as those just described.

2-Acetamido-4-O-allyl-3-O-benzyl-2-deoxy-β-D-glucopyranosyl Azide (11). A suspension of **10** (619 mg) in 60% acetic acid (50 ml) was heated for 2 h at 90 °C. The clear solution was evaporated and the residue, which obtained, was dried by several co-distillations with toluene and then chromatographed on a column of silica gel with 19:1 chloroform–ethanol to give 285 mg (79%) of **11**. It crystallized from dichloromethane–ether–pentane as needles, mp 188 °C; $[\alpha]^{20}_{D}$ −11° (*c* 2, chloroform); ν^{KBr}_{max} 3250 (OH), 3480 (NH), 2140 (N_3), 1650 (Amide I), 1550 (Amide II), 745, and 680 cm^{-1} (Ph); TLC in 19:1 chloroform–ethanol R_f 0.21 and in 9:1 chloroform–ethanol R_f 0.43. Found: C, 57.41; H, 6.45; N, 14.89; O, 21.89%. Calcd for $C_{18}H_{24}N_4O_5$: C, 57.44; H, 6.43; N, 14.88; O, 21.25%.

2-Acetamido-4-O-allyl-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl azide (12) and 2-(N-Acetylbenzylamino)-4-O-allyl-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl Azide (13). Benzyl bromide (1.2 ml) and powdered potassium hydroxide (3 g) were added to a solution of compound **11** (600 mg) in a mixture of anhydrous benzene (30 ml) and *N,N*-dimethylformamide (5 ml) and stirred for 3 h at room temperature. The mixture was cooled in an ice-bath, diluted with chloroform (200 ml), washed with ice–water (3×50 ml), 5% citric acid solution (2×50 ml), saturated sodium hydrogencarbonate solution (3×30 ml), and water (3×50 ml), and dried (Na_2SO_4). Evaporation of the solvents gave a syrup which on chromatography on a column of silica gel with 99:1 chloroform–ethanol gave 191 mg (33%) of **13** in the fast-moving fractions as a syrup which could not be crystallized, $[\alpha]^{20}_{D}$ +2.15° (*c* 1.6, chloroform), ν^{KBr}_{max} 2125 (N_3), 1650 (Amide I), 725, and 680 cm^{-1} (Ph); TLC in 29:1 chloroform–ethanol R_f 0.62. Found: C, 69.06; H, 6.46; N, 10.04; O, 14.60%. Calcd for $C_{32}H_{36}N_4O_5$: C, 69.05; H, 6.52; N, 10.06; O, 14.37%.

From the slow-moving fractions, 365 mg (56%) of **12** were obtained, which crystallized from methanol as needles, mp 159—160 °C; $[\alpha]^{20}_{D}$ +7.5° (*c* 2.7, chloroform), ν^{KBr}_{max} 3250 (NH), 2125 (N_3), 1645 (Amide I), 1550 (Amide II), 735, and 680 cm^{-1} (Ph); TLC in 29:1 chloroform–ethanol R_f 0.34. Found: C, 64.45; H, 6.52; N, 12.04; O, 17.25%. Calcd for $C_{25}H_{30}N_4O_5$: C, 64.36; H, 6.48; N, 12.01; O, 17.15%.

Attempted Preparation of 2-Acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl Azide (14). A solution of **12** (500 mg) and 1,4-diazabicyclo[2.2.2]octane (75 mg; Eastman Kodak Co., Rochester, N. Y. 14650) in 90% methanol (25 ml) was heated to boiling. Tris(triphenylphosphine)rhodium chloride (200 mg; Alpha Products, Ventron Corporation, Danvers, MA 01923) was added, and the mixture was boiled under reflux for 4 h, cooled to room temperature, filtered, and the filtrate, evaporated without being dried. A solution of the residue in chloroform (100 ml) was successively washed with 5% citric acid solution (2×25 ml) and water (2×25 ml), and evaporated without being dried. The residue was dissolved in 90% acetone (25 ml), and the solution was stirred with mercury(II) chloride (500 mg) and yellow mercury(II) oxide (500 mg) for 30 min at room temperature. The mixture was filtered on a celite layer, the inorganic residue was washed with acetone, and the filtrate and washings were evaporated to a syrupy residue (600 mg). TLC of this residue revealed the presence of a complex mixture of highly polar compounds and infrared showed the total loss of the characteristic azido group absorption.

2-Acetamido-N-acetyl-4-O-allyl-3,4-O-benzyl-2-deoxy-β-D-glucopyranosylamine (16). Attempted reduction of a solution of **12** (117 mg) in methanol (29 ml) with sodium borohydride (100 mg) for 16 h at room temperature or for 6 h at 80 °C was unsuccessful and gave the unchanged azido compound. Therefore, a solution of **12** (117 mg) in *N,N*-dimethylformamide (10 ml) was cooled to 0 °C and treated with sodium borohydride (100 mg) and then allowed to attain room temperature for 6 h. The mixture was treated with a few pieces of Dry Ice, diluted with chloroform (100 ml) and washed with water (5×25 ml), and dried (Na_2SO_4). Evaporation of the solvents gave a residue of 2-acetamido-4-*O*-allyl-3,6-di-*O*-benzyl-2-deoxy-*β*-D-glucopyranosylamine (**15**) which gave positive test with ninhydrin. The residue was dissolved in methanol (10 ml), treated with acetic anhydride (0.5 ml) for 2 h at room temperature, and evaporated. After several co-distillations with toluene, the substance was chromatographed on a column of silica gel with 19:1 chloroform–ethanol to give 95 mg (79%) of **16**, which crystallized from methanol, mp 214—216 °C; $[\alpha]^{20}_{D}$ +33° (*c* 1.12, methanol); ν^{KBr}_{max} 3280 (NH), 1650 (Amide I), 1550 (Amide II), 730, 680 cm^{-1} (Ph); TLC in 9:1 chloroform–ethanol R_f 0.53. Found: C, 67.11; H, 7.06; N, 5.69%. Calcd for $C_{27}H_{34}N_2O_6$: C, 67.20; H, 7.10; N, 5.80%.

2-Acetamido-4-O-allyl-3,6-di-O-benzyl-N[(S)-3-benzyloxycarbonyl-3-(benzyloxycarbonylamino)propionyl]-2-deoxy-β-D-glucopyranosylamine(18). A solution of compound **12** (1.1 g) in *N,N*-dimethylformamide (10 ml) was reduced with sodium borohydride (1 g), as described for the preparation of **15**. Carbon dioxide was bubbled into the solution while maintaining the temperature at 0 °C. The mixture was diluted with chloroform (300 ml), washed with water (6×50 ml), and the solvents were evaporated under reduced pressure with the bath temperature being kept below 30 °C. The residue, without further purification, was dissolved in dichloromethane (30 ml) and treated with 1-benzyl *N*-(benzyloxycarbonyl)-L-asparate[39] (**17**, 1 g) and dicyclohexylcarbondiimide (700 mg), and the mixture was stirred for 16 h at room temperature. The *N,N'*-dicyclohexylurea that had precipitated was filtered off, and the filtrate was evaporated. The residue was chromatographed on a column of silica gel which was first eluted with chloroform to remove the non-carbohydrate compounds, and then with 29:1 chloroform–ethanol to give 975 mg (53%) of **18**. It crystallized from dichloromethane–methanol as needles, mp 216—218 °C; $[\alpha]^{20}_{D}$ +43° (*c* 1.83, chloroform); ν^{KBr}_{max} 3300 (NH), 1740 (C=O ester), 1650 (Amide I), 1550 (Amide II), 725, and 680 cm^{-1} (Ph); TLC in 19:1 chloroform–ethanol R_f 0.33. Found: C, 67.73; H, 6.31; N, 5.34%. Calcd for $C_{44}H_{49}N_3O_{10}$: C, 67.76; H, 6.33; N, 5.39%.

2-Acetamido-3,6-di-O-benzyl-N-[(S)-3-benzyloxycarbonyl-3-(benzyloxycarbonylamino)propionyl]-2-deoxy-β-D-glucopyranosylamine (19). A boiling solution of **18** (780 mg) and 1,4-diazabicyclo[2.2.2]octane (80 mg) in 95% methanol (60 ml) was treated with tris(triphenylphosphine)rhodium chloride (250 mg) and the mixture was boiled under reflux for 5 h, and then processed as described for the attempted synthesis of **14**. The residue obtained was chromatographed on a column of silica gel with 19:1 chloroform–ethanol to give 503 mg (68%) of **19**, which crystallized from dichloromethane–ether; mp 163—165 °C, $[\alpha]^{20}_{D}$ +26° (*c* 2.4, chloroform), ν^{KBr}_{max} 3500 (OH), 3300 (NH), 1740 (C=O ester), 1650 (Amide I), 1545 (Amide II), 750, and 690 cm^{-1} (Ph); TLC in 19:1 chloroform–ethanol R_f 0.24. Found: C, 66.28; H, 6.27; N, 5.51%. Calcd for $C_{41}H_{45}N_3O_{10}$: C, 66.56; H, 6.13; N, 5.68%.

*2-Acetamido-3,6-di-*O*-benzyl-*N*-[(*S*)-3-benzyloxycarbonyl-3-(benzyloxycarbonylamino)propionyl]-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosylamine (**21**).* A solution of **19** (740 mg) and mercury(II) cyanide (1.2 g) in 1:1 benzene-1,2-dichloroethane (250 ml) was distilled at atmospheric pressure until 100 ml of the solvents were distilled. The mixture was cooled to room temperature and treated with a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide[40] **20** (1.5 g) in dry 1,2-dichloroethane (50 ml) during 3 h, while being stirred. The mixture was stirred for a further 48 h, treated with additional amounts of mercury(II) cyanide (1 g) and **20** (1 g), and stirring was continued for a further 24 h. The mixture was diluted with chloroform (300 ml), filtered through a celite layer, and the filtrate was successively washed with water (3×50 ml), a saturated solution of sodium hydrogencarbonate (2×50 ml), a saturated solution of potassium iodide (3×25 ml), and water (3×50 ml), and dried (Na_2SO_4). Evaporation of the solvents gave a residue that was chromatographed on a column of silica gel with 19:1 chloroform–ethanol to give 480 mg (42%) of pure **21**. This could not be crystallized and was obtained as an amorphous powder by precipitation from an ethereal solution by addition of pentane; $[\alpha]_D^{20}$ $+29°$ (*c* 1.1, methanol), ν_{max}^{KBr} 3300 (NH), 1740 (C=O ester), 1665 (Amide I), 1555 (Amide II), 750, and 690 cm^{-1} (Ph); TLC in 19:1 chloroform–ethanol R_f 0.29. Found: C, 57.84; H, 6.26; N, 3.63%. Calcd for $C_{55}H_{61}N_3O_{19}\cdot 4H_2O$: C, 57.79; H, 6.35; N, 3.68%.

*2-Acetamido-3,6-di-*O*-benzyl-*N*-[*N*-(benzyloxycarbonyl)-β-L-aspartyl]-2-deoxy-4-*O*-β-D-galactopyranosyl-β-D-glucopyranosylamine (**22**).* Compound **21** (570 mg) was stirred with a solution of lithium hydroxide (80 mg) in water (30 ml) for 2 h at room temperature. The solution was deionized by passage through Amberlite IRC-50 (H^+) ion-exchange resin, and then evaporated. The residue could not be induced to crystallize and **22** was obtained as an amorphous powder 351 mg (82%) from methanol–ether–pentane; $[\alpha]_D^{20}$ $+13°$ (*c* 2.1, methanol), ν_{max}^{KBr} 3350 (broad, NH and OH), 1720, (COOH), 1650 (Amide I), 1550 (Amide II), 750, and 690 cm^{-1} (Ph); TLC in 8:2 chloroform–ethanol R_f 0.40. Found: C, 55.74; H, 5.80; N, 4.77%. Calcd for $C_{40}H_{49}N_3O_{15}\cdot 2.5\cdot H_2O$: C, 56.07; H, 6.35; N, 4.90%.

*2-Acetamido-*N*-(β-L-aspartyl)-2-deoxy-4-*O*-β-D-galactopyranosyl-β-D-glucopyranosylamine (**23**).* A solution of **22** (215 mg) in 50% methanol (100 ml) was hydrogenolyzed with hydrogen in the presence of 10% palladium-on-charcoal (100 mg) for 24 h at room temperature and 2 atm. The mixture was filtered on a celite layer and the filtrate was hydrogenolyzed for a second time as just described. Evaporation of the solvents gave a residue (108 mg, 81%) that showed a positive ninhydrin reaction.[36] This residue could not be crystallized and **23** was obtained as a hygroscopic amorphous powder from methanol–acetone–pentane; $[\alpha]_D^{20}$ $+6°$ (*c* 1.1, methanol), ν_{max}^{KBr} 3400 (broad, NH_2 and OH), 1725 (COOH), 1650 (Amid I), and 1550 cm^{-1} (Amide II). Found: C, 40.51; H, 6.59; N, 7.72%. Calcd for $C_{18}H_{31}N_3O_{13}\cdot 2H_2O$: C, 40.53; H, 6.61; N, 7.88%.

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