Carbohydrate Research, 39 (1975) 271–281

SYNTHESES OF 2-ACETAMIDO-2-DEOXY-4-*O*-β-D-GALACTOPYRANOSYL-D-MANNOSE AND -D-GLUCOSE AND OF 2-ACETAMIDO-2-DEOXY-4-*O*-β-D-MANNOPYRANOSYL-D-MANNOSE AND -D-GLUCOSE*

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(Received October 17th, 1974; accepted November 5th, 1974)

ABSTRACT

2-Acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-D-mannose (**6**) and -D-glucose (**7**) were prepared by addition of nitromethane to 3-*O*-β-D-galactopyranosyl-D-arabinose, followed by acetylation, ammonolysis, and application of the Nef reaction. Similarly, 2-acetamido-2-deoxy-4-*O*-β-D-mannopyranosyl-D-mannose (**14**) and -D-glucose (**15**) were prepared by the same scheme from 3-*O*-β-D-mannopyranosyl-D-arabinose. In the two series of experiments, **6** and **14** were the respective major products. Epimerization of the 2-acetamido-2-deoxy-D-mannose residue in **6** and **14** yielded **7** and **15**, respectively.

INTRODUCTION

Some 4-*O*-aldosyl derivatives of 2-acetamido-2-deoxy-D-glucose (*N*-acetyl-D-glucosamine) occupy important positions in complex carbohydrates. *N*-Acetyllactosamine [2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-D-glucose (**7**)] is not only found in milk oligosaccharides[1], but is an important structural unit in glycoproteins[2] and glycolipids[3]. Its 4-*O*-D-mannopyranosyl analog (**15**) has been shown to be a part of the core oligosaccharides of several glycoproteins[4,5]. However, only small quantities of **7** are obtainable from milk by laborious, chromatographic processes, and isolation of **15** from glycoproteins is even more difficult[4].

Our ongoing program of preparation of synthetic glycosides having suitable functional groups at the aglycon terminal[6] requires much larger quantities of these

*Contribution No. 815 from the McCollum-Pratt Institute, Johns Hopkins University. Supported by NIH Research Grant AM09970. Preliminary accounts of this work were presented at the 165th Meeting of the American Chemical Society, Dallas, Texas, April 9–11, 1973.

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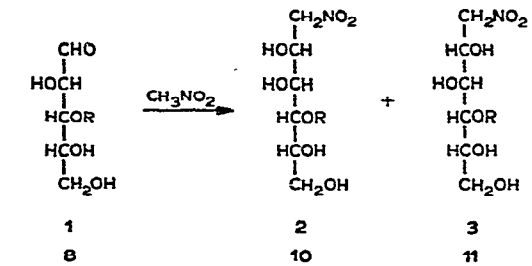
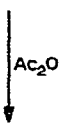
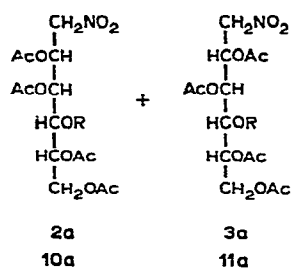
disaccharides than can conveniently be obtained from the natural sources. Although **7** has been synthesized from 3-*O*-*β*-D-galactopyranosyl-D-arabinose (**1**) by a Kiliani type of reaction[7], the use of dry hydrogen cyanide is objectionable from the standpoint of safety and convenience. Chemical synthesis of **15** has not hitherto been reported.



1 **2** **3**

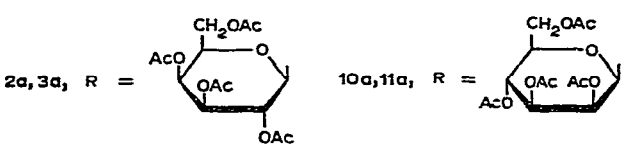
8 **10** **11**

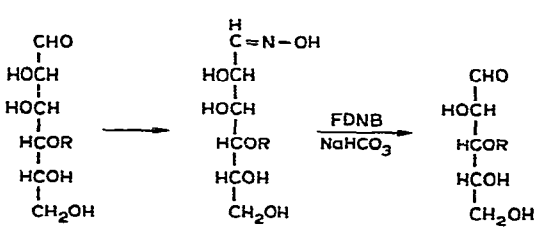




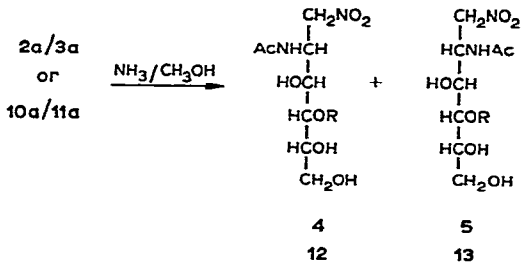
2a **3a**

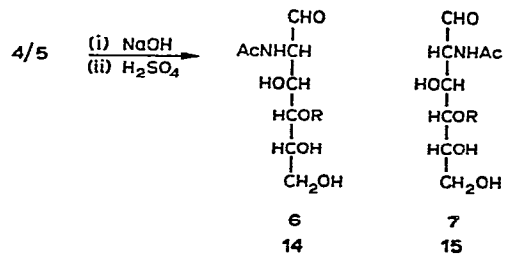
10a **11a**

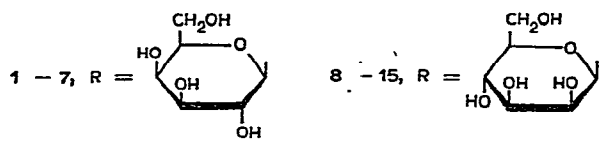




9 **8**







We now describe practical procedures for the synthesis of **7** and **15** by the addition of nitromethane to **1** and **8**, followed by a series of reactions, namely, acetylation, ammonolysis, and the Nef reaction. Although the products from such a reaction series favor the D-*manno* configuration (**6** and **14**) at the reducing terminus, their 2-epimers (**7** and **15**) can readily be obtained by epimerization of **6** and **14** with ammonia. Such reaction schemes have been successfully applied to the synthesis of amino sugars (monosaccharides)[8].

DISCUSSION

The results presented here demonstrate the extended utility of the nitromethane synthesis for the preparation of reducing disaccharides containing an amino sugar residue at the reducing end. In both cases examined, the stereochemistry of nitromethane addition followed the Maltby rule[16], and the product having the D-*manno* configuration preponderated; this predominance of the D-*manno* configuration also persists at the stage of amino sugar formation, as has been observed for similar reactions for monosaccharides[8], and so the overall reaction still gives mainly the disaccharide having a 2-acetamido-2-deoxy-D-mannose residue at the reducing end. This circumstance is not a great disadvantage, even when disaccharides containing

2-acetamido-2-deoxy-D-glucose are the end products desired, because epimerization with ammonia readily converts the 2-acetamido-2-deoxy-D-mannose residue into a 2-acetamido-2-deoxy-D-glucose residue. When the procedure was conducted without isolation of intermediates, enrichment in **7** and **15** (over **6** and **14**) was accomplished by treatment of the mixture with aqueous ammonia prior to column chromatography on an anion-exchange resin.

To the best of our knowledge, this is the first report of the synthesis of **14** and **15**. In view of the importance of compound **15** in glycoprotein chemistry[2,4], the synthesis of **15** described here is expected to contribute significantly to the field.

EXPERIMENTAL

General. — Unless otherwise mentioned, the general techniques used in this work were essentially identical to those previously described[6]. Precoated plates of silica gel (0.25 mm thickness; Q-5 Quantum Industries, Fairfield, N.J.) were used for thin-layer chromatography (t.l.c.). The solvents used for t.l.c. were: (*A*) 9:4:2 (v/v) ethyl acetate–2-propanol–water, (*B*) 9:1 (v/v) toluene–ethanol, (*C*) 12:5:4 (v/v) ethyl acetate–pyridine–water, (*D*) 3:2:1 (v/v) ethyl acetate–acetic acid–water, and (*E*) 10:3:3 (v/v) butanol–pyridine–water. P.m.r. spectra were recorded with a JEOL NMH-100 spectrometer. Analyses of mono- and di-saccharides were conducted by use of established, automatic systems[9]. For column chromatography, microcrystalline cellulose (Avicel, American Viscose Division, FMC Corporation, Newark, Delaware), and silica gel (70–235 mesh, Merck) were used; the columns were packed dry, and solutions of the samples in suitable solvents were applied to the dry columns and eluted. For gel filtration, a column (5 × 150 cm) of Bio Gel P-2 (200–400 mesh) in 0.1M acetic acid was used exclusively.

3-*O*-β-D-Galactopyranosyl-D-arabinose (**1**) and ivory-nut meal were purchased from Pfanstiehl Laboratories (Waukegan, Illinois). An authentic sample of **7** was kindly provided by Dr. Saul Roseman. Nitromethane (J. T. Baker) was of spectrographic grade. Dimethyl sulfoxide was redistilled from calcium hydride, and stored over calcium hydride. Rexyn 201 (a strong, anion-exchange resin) was purchased from Fisher Scientific Co.

*1-Deoxy-4-*O*-β-D-galactopyranosyl-1-nitro-*D*-mannitol* (**2**) *and* *-D-glucitol* (**3**). — To a solution of **1** (4 g, 12.8 mmoles) in a mixture of dimethyl sulfoxide (50 ml) and nitromethane (35 ml) was added 4M sodium methoxide (4 ml), and the mixture was stirred in the dark for 6.5 h at room temperature. The mixture was diluted with diethyl ether (400 ml), and stored in the cold for 4 h to precipitate the products. The precipitate was harvested by filtration, washed with diethyl ether, immediately mixed into a suspension of Dowex-50 X-8 (H^+) (100–200 mesh) in cold water (150 ml), and the suspension stirred in the cold for 15 min. The resin was filtered off and washed with water, and the filtrate and washings were combined and evaporated to dryness. The mixture of products was purified on a column (5 × 60 cm) of Avicel, with solvent *C* as the eluant. The effluent was collected in a fraction collector, and monitored by

carbohydrate determination (phenol–sulfuric acid method[10]) and t.l.c. (solvent *A*). The combined yield of **2** (R_F 0.38) and **3** (R_F 0.33) was 83%. From the elution profile of the cellulose chromatography, the ratio of **2** to **3** was estimated to be 6:1. Compound **2** was obtained crystalline from ethanol; m.p. 151–152°, $[\alpha]_D$ +10° (in water).

Anal. Calc. for $C_{12}H_{23}NO_{12}$ (373.31 daltons): C, 38.61; H, 6.21; N, 3.75. Found: C, 38.43; H, 6.29; N, 3.70.

Optical rotatory dispersion (o.r.d.) curves of samples of **2** and **3** taken at the peak fraction from the cellulose chromatography are shown in Fig. 1.

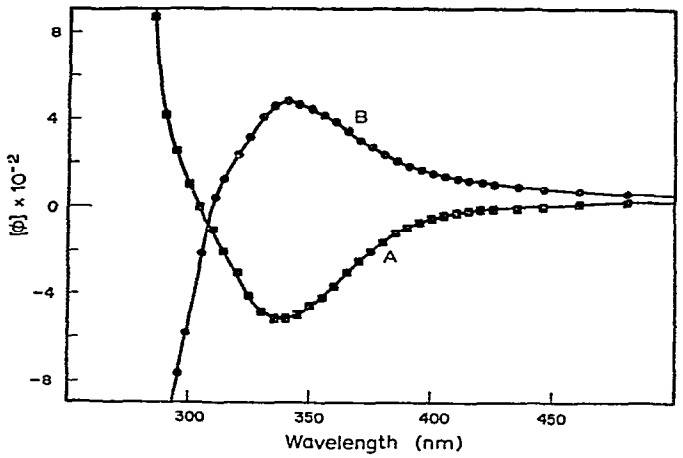


Fig. 1. Optical rotatory dispersion curves of compounds **2** and **3**. (Curve A: o.r.d. curve of **2** ; curve B: o.r.d. curve of **3**. $[\phi]$ = molecular weight × $[\alpha]_{sp}/100$.)

The proof that the component of R_F 0.33 was compound **3** came from the following data. (*a*) It contained the expected amount of D-galactose. (*b*) It could be converted into lactose (4-*O*-β-D-galactopyranosyl-D-glucose) by the Nef reaction. (*c*) Its o.r.d. curve (Fig. 1) had a positive maximum at 340 nm, whereas that of **2** has[11] a negative maximum at 335–340 nm.

2,3,5,6-Tetra-O-*acetyl-1-deoxy-1-nitro-4*-O-(*2,3,4,6-tetra*-O-*acetyl*-β-D-*galactopyranosyl*)-D-*mannitol* (**2a**) *and* -D-*glucitol* (**3a**). — A portion (4.0 g, 10.7 mmoles) of the 6:1 mixture of **2** and **3** in acetic anhydride (75 ml) containing concentrated sulfuric acid (0.75 ml) was heated for 3.5 h at 100°. The colored, acetylation mixture was poured into ice water (1.5 liters) and stirred overnight in the cold. The dark gum thus precipitated was extracted with chloroform, and the solution was successively washed with cold, saturated sodium hydrogen carbonate and cold water, and evaporated to dryness. The residue was dissolved in ethanol, and the solution was decolorized with charcoal, and evaporated to an amorphous solid (6.1 g, 85%) which was mostly a mixture of **2a** and **3a** (presumably in the same ratio as **2** to **3** in the starting mixture).

2-Acetamido-1,2-dideoxy-4-O-β-D-*galactopyranosyl-1-nitro*-D-*mannitol* (**4**) *and* -D-*glucitol* (**5**). — The mixture (8.5 mmoles total) of **2a** and **3a** obtained from the

acetylation reaction was suspended in dry methanol (60 ml), chilled in an ice bath, and saturated with ammonia. The mixture was then kept for 12 h at room temperature and evaporated to a syrup, which was purified on a column (5 × 60 cm) of cellulose powder by eluting with solvent *C*. The column effluent was monitored by t.l.c. (solvent *A*), and by analysis for carbohydrate. The fractions containing components of R_F 0.26 corresponded to a mixture of **4** and **5**, and were pooled and evaporated. The combined yield of **4** and **5** was 2.86 g (81%).

*2-Acetamido-2-deoxy-4-*O*-β-*D*-galactopyranosyl-*D*-mannose* (**6**) *and* -D-*glucose* (**7**). — The syrupy mixture of **4** and **5** (1.86 g, 4.49 mmoles) obtained as just described was dissolved in water (10 ml), and the solution mixed with cold, 2M sodium hydroxide (10 ml). The alkaline solution was immediately added dropwise to cold, 9M sulfuric acid (20 ml); the mixture was kept for 20 min at room temperature, diluted with cold water (200 ml), and the sulfate ions were precipitated by addition of 1M barium acetate

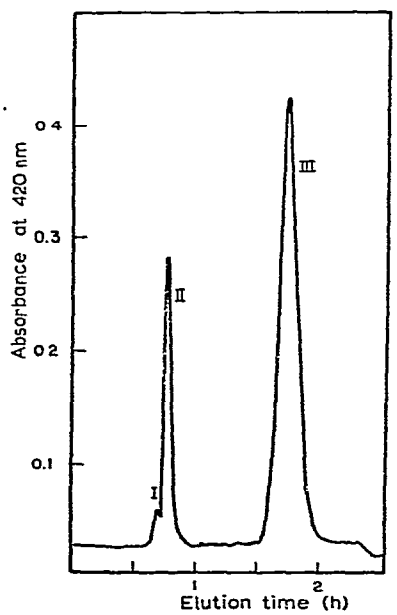


Fig. 2. Elution pattern of Nef reaction products from **4** and **5** on a column (0.3 × 70 cm) of Rexyn 201 ion-exchange eluted with 0.27M sodium borate buffer (pH 7.8) at 55°. (Peaks II and III contained **7** and **6**, respectively. Sugars were determined by the orcinol color reaction.)

(200 ml). The barium sulfate was removed by centrifugation and washed with water, the supernatant liquor was treated with Dowex 50-W X-8 (H^+) (100–200 mesh) and the suspension filtered, and the filtrate was evaporated to afford a crystalline mass. Examination of this material on a column (0.3 × 70 cm) of Rexyn 201 (20–40 μm) that had been equilibrated and was eluted with[9] a borate buffer (0.27M in borate, pH 7.8) at 55° gave the profile shown in Fig. 2. Preparative separation of **6** from **7** was effected on a column (5 × 55 cm) of Rexyn 201 (200–400 mesh) operated under similar con-

ditions. The effluent was monitored by the phenol–sulfuric acid method*, and the fractions containing materials corresponding to peaks II and III were decationized with Dowex-50 X-8 (H^+) (100–200 mesh); boric acid was then removed by the usual method of addition of methanol and evaporation as methyl borate. The yields from peaks II (**7**) and III (**6**) were 13 and 72%, respectively.

Compound **7** was obtained crystalline from methanol; m.p. 168–169°, $[\alpha]_D^{25}$ +24.7° (*c* 1.11, water); lit.[7] m.p. 168–170°, $[\alpha]_D$ +28.5° (in water). This material was homogeneous in t.l.c. (solvent *D*), and, on acid hydrolysis, yielded one molar proportion each of galactose and 2-amino-2-deoxyglucose. P.m.r. data (D_2O): δ 2.52 (s, 3, Ac), 4.86 (d, *J* 7 Hz, H-1β), and 5.74 (d, *J* 3 Hz, H-1α). It was indistinguishable from an authentic sample of **7** by paper chromatography (Whatman No. 1) in solvents *D* and *E*.

Anal. Calc. for $C_{14}H_{25}NO_{11}\cdot CH_4O$ (monomethanolate, 415.39 daltons): C, 43.37; H, 7.04; N, 3.37. Found: C, 43.27; H, 7.13; N, 3.30.

The material from peak III, which contained exactly one molar proportion each of galactose and 2-amino-2-deoxymannose, crystallized from aqueous ethanol; m.p. 232–235°, $[\alpha]_D^{25}$ +28.2° (*c* 1.31, water); lit.[12] m.p. 237°, $[\alpha]_D$ +32.5° (in water). It was homogeneous in t.l.c. (solvent *D*). P.m.r. data (D_2O): δ 2.51 and 2.56 (s, 3, Ac), 4.83 (d), 4.95 (d), and 5.52 (d), anomeric protons.

Anal. Calc. for $C_{14}H_{25}NO_{11}$ (383.35 daltons): C, 43.86; H, 6.57; N, 3.65. Found: C, 43.79; H, 6.63; N, 3.60.

Conversion of **6** *into* **7**. — Treatment of a mixture of **6** and **7** (1 g, see preceding) with 7.4M ammonium hydroxide (25 ml) for 25 h at room temperature, followed by the same procedure of column chromatography (borate) yielded **7** and **6** in .52 and 14% yield, respectively.

Preparation of **6** *and* **7** *from* **1** *without isolation of intermediates.* — Compound **1** (4 g, 12.8 mmoles) was treated with nitromethane as described for the preparation of **2** and **3**. After de-ionization with Dowex-50 X-8 (H^+), the resin was filtered off, and the filtrate evaporated to dryness. The residue was then treated with a mixture of acetic anhydride (75 ml) and concentrated sulfuric acid (0.3 ml), and processed as described for the preparation of **2a** and **3a**. After evaporation of the chloroform, the residue was dissolved in dry methanol, and the solution treated with ammonia as described for the preparation of **4** and **5**. The reaction mixture was evaporated to a syrup, dissolved in water (10 ml), and purified by passage through a column (5 × 150 cm) of Bio Gel P-2. Gel filtration of **4** plus **5** obtained from the Bio Gel column did not separate **4** from **5**, but separated **4** plus **5** from some impurities of both larger and smaller molecular sizes. Upon t.l.c., the major peak was found to contain mainly one carbohydrate zone corresponding to **4** plus **5**. At this point, the overall yield of **4** plus **5** was ~33% (estimated by the phenol–sulfuric acid method). The fractions containing **4** plus **5** were combined, and evaporated, and twice evaporated after addition of

*It should be noted that 2-acetamido-2-deoxy-D-mannose gives a color reaction on application of the phenol–sulfuric acid test[13].

absolute ethanol and toluene (to remove residual acetic acid). The final products, **6** and **7**, were obtained by the Nef reaction, as already described. Analysis of the mixture showed the ratio of **6** to **7** to be ~2:1. Compound **6** crystallized out from methanol or ethanol in a yield of 30% of the mixture of **4** plus **5**. Examination by means of a column of Rexyn 201 showed that the purity of the crystals of **6** thus obtained exceeded 97%. Recrystallization from ethanol removed remaining contaminants, to give pure **6**. Compound **7** could not be crystallized from the mother liquor, but was obtained crystalline after chromatography on a column of Rexyn 201 as described in an earlier section.

3-O-β-D-Mannopyranosyl-D-arabinose (**8**). — 4-*O*-β-D-Mannopyranosyl-D-mannose (**9**), prepared by acetolysis of ivory-nut mannan[14], was converted into **8** by the method of Weygand *et al.*[15]. A mixture of compound **9** (1.46 g, 4.27 mmoles) in 50% pyridine (13 ml) with 0.5M hydroxylamine in absolute ethanol (21 ml)* was heated for 10 h at 60°, cooled, poured into toluene (500 ml), and the solution evaporated to a syrup which was placed over concentrated sulfuric acid in a vacuum desiccator (to remove remaining pyridine). A solution of the residue in 0.25M sodium hydrogen carbonate (75 ml) was heated at 60° while carbon dioxide gas was bubbled continuously through the mixture and into a trap containing silver nitrate solution. After 0.5 h, a solution of 1-fluoro-2,4-dinitrobenzene (2.0 g, 10.7 mmoles) in 2-propanol (30 ml) was added, and heating with bubbling of carbon dioxide was continued for an additional 2 h; at this time, further formation of a precipitate of silver cyanide in the trap appeared to have ceased. The clear solution was cooled, separated from a gummy residue by decantation, acidified with Dowex-50 X-8 (H^+) to pH 3.5, and the suspension filtered. The filtrate was concentrated by evaporation, and the concentrate applied to a column (2.5 × 40 cm) of Rexyn 201 ion-exchange resin which had been equilibrated with 0.19M sodium borate buffer of pH 7.3. Elution with the same buffer produced three well-separated peaks (see Fig. 3). Fractions in the second peak contained **9**; these were recycled to produce more **8**. Fractions of the third peak were combined; sodium borate was removed by acidification with Dowex 50 and removal of boric acid as methyl borate. The dry residue contained **8**, yield 1.33 g (60%), and, on acid hydrolysis, afforded mannose and arabinose in an equimolar ratio. Compound **8** was crystallized (m.p. 184–188°) from aqueous ethanol.

1-Deoxy-4-O-β-D-mannopyranosyl-1-nitro-D-mannitol (**10**) *and -D-glucitol* (**11**). — To a solution of compound **8** (1.9 g, 6 mmoles) in dry dimethyl sulfoxide (25 ml) were added dry nitromethane (17 ml) and 3.42M sodium methoxide in methanol (2.3 ml), and the mixture was stirred overnight at room temperature. T.l.c. examination (solvent *A*) then indicated that the reaction was virtually complete. The mixture was poured into cold ether (500 ml), and the precipitate was filtered off and successively washed in the cold with ether, methanol, and ether. The precipitate was then

*Hydroxylamine was prepared from its hydrochloride by titrating in absolute ethanol with 4M sodium methoxide in methanol to the phenolphthalein end-point. The mixture was diluted with absolute ethanol to make it 0.5M in hydroxylamine. The salt deposited after cooling was removed by filtration.

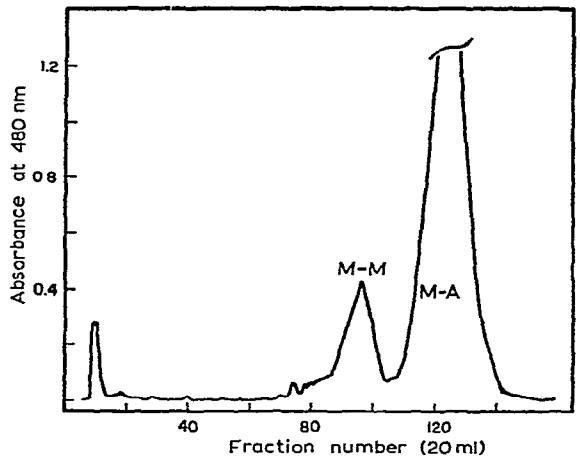


Fig. 3. Separation of **8** from **9** by chromatography on a column (2.5 × 40 cm) of Rexyn 201 ion-exchange resin. [Elution was performed with 0.19M sodium borate buffer, pH 7.3. The second peak (M-M) contained **9**, and the third (M-A) contained **8**. Sugars were determined by the phenol–sulfuric acid method.]

treated with a suspension of Rexyn 101 (H^+) (20–50 mesh; 20 ml) in water (120 ml). The resin was filtered off, the filtrate was evaporated to a syrup, and residual water was removed by co-evaporation with absolute ethanol and toluene. As judged by t.l.c., the material contained 80% of **10**, the rest being **8** plus **11**. Without further purification, this material was used in the next step.

2,3,5,6-Tetra-O-acetyl-1-deoxy-1-nitro-4-O-(2,3,4,6-tetra-O-acetyl-β-D-manno-pyranosyl)-D-mannitol (**10a**) *and -D-glucitol* (**11a**). — The mixture obtained in the preceding experiment was heated with acetic anhydride (32 ml) and concentrated sulfuric acid (0.1 ml) on a boiling-water bath. As acetylation progressed, the suspension became a clear solution, and the reaction was essentially complete in 2.5 h, as shown by t.l.c. in solvent *B*. The mixture was cooled and poured into a mixture of cold water (750 ml) and chloroform (~10 ml), and the resulting mixture was stirred overnight in the cold. The chloroform layer was separated, and the aqueous solution was extracted with chloroform. The chloroform solutions were combined and successively washed with cold, saturated sodium hydrogen carbonate (twice) and 1M sodium chloride (twice), dried (anhydrous sodium sulfate), and evaporated, to give, presumably, a mixture of **10a** and **11a**. T.l.c. (solvent *B*) revealed the presence of a single spot.

2-Acetamido-1,2-dideoxy-4-O-β-D-mannopyranosyl-1-nitro-D-mannitol (**12**) *and -D-glucitol* (**13**). — A solution of the mixture of **10a** and **11a** (described in the preceding experiment) in dry methanol (20 ml) was cooled in ice and saturated with ammonia by bubbling in dry ammonia gas. The mixture was kept overnight at room temperature, and then evaporated to dryness. T.l.c. (solvent *A*) revealed one major spot with some minor spots. Purification of this material on a column (5 × 150 cm) of Bio Gel

P-2 eluted with 0.1M acetic acid removed the contaminants represented by the minor spots. Fractions containing mainly **12** and **13** (t.l.c. with solvent *A*) were combined, evaporated, and re-evaporated after addition of absolute ethanol and toluene. The combined, overall yield of **12** plus **13** was estimated by the phenol–sulfuric acid method to be 60%.

*2-Acetamido-2-deoxy-4-*O*-β-*D*-mannopyranosyl-*D*-mannose* (**14**) *and* -D-*glucose* (**15**). — A solution of the residue obtained (in the preceding experiment) in water (7.5 ml) was cooled in ice, and mixed with cold, 2M sodium hydroxide (7.5 ml). This alkaline solution was then slowly added to cold, 9M sulfuric acid (15 ml) with constant, vigorous shaking. After 0.5 h at room temperature, water (150 ml) and 1M barium acetate (150 ml) were added, and the resulting precipitate was removed by centrifugation at 10,000 r.p.m. for 0.5 h. The supernatant liquor was treated with Rexyn 101 (H^+) (20–50 mesh, 75 ml) suspended in water, the suspension was filtered, and the filtrate was evaporated. A trace of water was removed from the residue by evaporating twice with absolute ethanol and toluene. The white, solid residue thus obtained contained **14** and **15** in the ratio of ~2:1. Attempted crystallization from hot 95% ethanol or methanol produced crystals containing **14** and **15** in the ratio of ~1.5:1. Complete separation of **14** from **15** was achieved by preparative chromatography on a column (2.5 × 40 cm) of Rexyn 201 as described for the preparation of **6** and **7**. The elution produced two peaks; the first peak contained **15**, and the second, **14**. The fractions in each peak were combined, acidified with glacial acetic acid, and evaporated. Boric acid was removed as methyl borate by evaporation with methanol. Each residue was then applied to a column (5 × 150 cm) of Bio Gel P-2, and eluted with 0.1M acetic acid to remove salt. Both **14** and **15** each produced a single, sharp peak in this elution. Peak fractions were combined, evaporated, and co-evaporated with absolute ethanol and toluene to remove residual acetic acid.

Compound **14** was obtained as hygroscopic crystals from 80% ethanol by addition of ether to turbidity. The crystals were washed with a small volume of hot isopropyl alcohol and then, by repeated recrystallization from 80% ethanol plus ether, nonhygroscopic crystals were obtained. The overall yield of **14** was 22% (0.52 g, 1.35 mmoles); m.p. 153–155°, $[\alpha]_D^{25}$ +2.4° (*c* 1.19, water). The material was homogeneous in t.l.c. (solvent *D*), and yielded one molar proportion each of mannose and 2-amino-2-deoxymannose on acid hydrolysis. P.m.r. spectra in D_2O showed double peaks[17] for *N*-acetyl group (δ 2.48 and 2.52), and anomeric proton signals at δ 4.95 (H-1′β), 5.48 (H-1β), and 5.58 (H-1α), respectively. Owing to retention of moisture in the compound after several recrystallizations, elemental analyses were not performed.

Compound **15** was crystallized from 80% ethanol by addition of ether to turbidity; overall yield 8% (0.18 g, 0.46 mmole). It was recrystallized twice from the same solvent system; m.p. 167–169°; $[\alpha]_D^{25}$ +0.4° (*c* 5.4, water). On acid hydrolysis, it yielded one molar proportion each of mannose and 2-amino-2-deoxyglucose. P.m.r. in D_2O showed a single peak for *N*-acetyl group, δ 2.48, and signals for anomeric protons at δ 5.25 (H-1β) and 5.64 (H-1α).

Anal. Calc. for $C_{14}H_{25}NO_{11} \cdot H_2O$ (401.37 daltons); C, 41.89; H, 6.78; N, 3.49. Found: C, 41.71; H, 6.77; N, 3.54.

Conversion of **14** *into* **15**. — In order to obtain a larger quantity of compound **14**, the reaction series (from **8** to a mixture of **14** and **15**) was conducted as already described but with an additional treatment with ammonia just before the fractionation of **14** and **15** on a column of Rexyn 201. The residue, a mixture of **14** and **15** in the ratio of ~2:1, was dissolved in 3.4M NH_4OH, and the solution was kept at room temperature. After 24 h, the ratio of **14** to **15** had changed to 1:1.7, but further storage under these conditions did not change the ratio significantly. After evaporation, the material was fractionated with a column (2.4 × 40 cm) of Rexyn 201, desalted with a column (5 × 150 cm) of Bio Gel P-2, and the products obtained crystalline as already described.

ACKNOWLEDGMENT

We are indebted to Dr. Agnes T. Wu for her assistance in recording and measuring the p.m.r. spectra.

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