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SYNTHESES OF MODEL OLIGOSACCHARIDES OF BIOLOGICAL SIGNIFICANCE. 8. A SYNTHESIS OF A SPECIFICALLY DEUTERATED 2-PROPYL 3,6-DI-O-[α-D-MANNOPYRANOSYL]-β-D-MANNOPYRANOSIDE.†

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SUMMARY

The title trisaccharide has been synthesized containing a 3-deuterated central mannopyranosyl unit and 2,3,4,6,6'-pentadeuterio mannopyranosyl units in both arms. 2-Propyl 2-O-benzyl-4,6-O-benzylidene-3-deuterio--β-D- mannopyranoside was obtained by stereoselective deuteride reduction of the corresponding 3-oxo derivative and was reacted with 2,3,4,6,6'-penta- deuterio-α-D-mannopyranosyl bromide. The disaccharide obtained was glycosylated with another equivalent of the same bromide after hydrolysis of the benzylidene group. This sequence led to the title compound after deblocking of all the hydroxyl groups. The 2,3,4,6,6'-pentadeuterio mannopyranosyl bromide was obtained by a

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reaction sequence starting with a controlled deuterium exchange in deuterium oxide over Raney nickel catalyst of methyl α-D-manno-pyranoside, followed by acetolysis and purification via the corresponding orthoester. The latter compound gave the bromide on reaction with trimethylsilyl bromide.

Key words: 2-propyl 3,6-di-O-(α-D-mannopyranosyl)-β-D-mannopyranoside, perdeuterated trimannoside, 2,3,4,6,6'-pentadeuteriomannoside, 3-deuteriomannoside.

INTRODUCTION

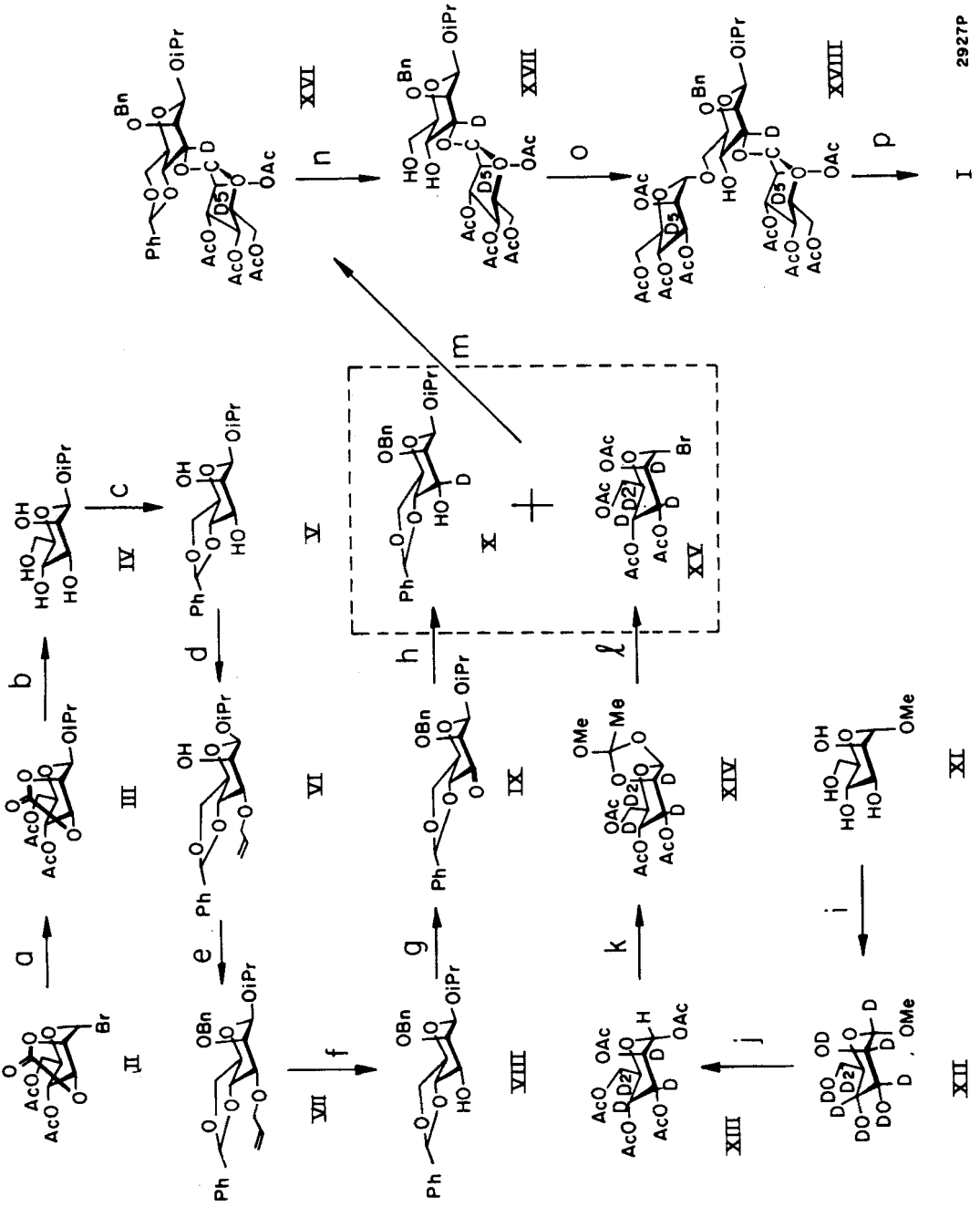
The oligosaccharide moieties of glycoproteins on cell surfaces have several important biological functions that are understandably dependent on their three-dimensional structures. The solution conformation of oligosaccharides is most conveniently studied by nuclear magnetic resonance (NMR) spectroscopy, in particular by using the nuclear Overhauser effect (NOE).[1] When measured under appropriate experimental conditions, NOE's enable direct measurement of the distances between uncoupled nuclei close in space. After irradiation of one nucleus the observed signal of another is enhanced, the magnitude of this enhancement is a function of the internuclear distance . As this enhancement is also dependent on the longitudinal relaxation times of the hydrogens experiencing the enhancement, and since the primary relaxation pathways are via neighbouring hydrogens, the magnitude of the observed NOE's can be increased by replacing neighbouring hydrogens with deuteria. Such

substitutions with deuterium also eliminates another complicating factor - the "three-spin effect".[2] Still another reason for the introduction of deuteria into an oligosaccharide molecule is the resulting simplification of the overall pattern of the spectrum by an elimination of overlapping signals.

The study of the conformation of the 1,3-glycosidic bond[3] required the availability of the title compound having a deuterium instead of the proton on C-3 of the central mannopyranoside, and as few protons as possible in mannopyranoses in both arms (except the anomeric protons). The regiospecific deuteration, and synthesis of the title compound, has been achieved by the separate deuteration of the mannose units and the addition of the two substituents of the central mannopyranoside in two consecutive steps.[4]

RESULTS AND DISCUSSION

Scheme 1 outlines the synthesis of an appropriately protected 2-propyl 3-deuterio-ß-D-manno- pyranoside unit by the sequence a-h. While the scheme is self-explanatory, it should be noted that IX is unstable under even mildly alkaline conditions as the corresponding gluco configuration is apparently more stable. The use of pyridinium chlorochromate[5] led to the best results. The stereoselectivity of reduction (step h) is excellent and no formation of the altro configuration was observed. The sequence of steps d-f was necessary since OH-2 was always much less reactive than OH-3.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The synthesis of the 2,3,4,6,6'-pentadeuterio acetobromomannose is described by the sequence i-l. The deuteration of methyl α-D-manno-pyranoside (XI) by deuterium oxide in the presence of Raney nickel at elevated temperatures[6] led to significant epimerization at all carbons involved (presumably C-2, C-3, C-4). However the triacetyl mannose orthoester (XIV) crystallizes well, and consequently all the unwanted by-products could be easily removed after step k.

The glycosylation and subsequent deprotection of the hydroxyl groups was optimized in the preparation of the unlabelled trimannosides, which have been synthesized as α- and β-glycosides[4,7] at the central mannopyranoside unit. Compound X and the bromide XV gave the disaccharide XVI under the promotion of mercuric salts (cyanide and bromide)[8] in an excellent yield. After hydrolysis of the benzylidene ring by acetic acid, the resulting XVII reacted regiospecifically at C-6 with the bromide XVI under Helferich conditions as in the step n. Protected trisaccharide XVIII was the sole product of the step o, and the deprotection step p consisted of its debenzylation by hydrogenation on Pd/C catalyst, followed by Zemplén deacetylation. The product I was extensively characterized by NMR spectroscopy (cf. experimental section and Ref.3).

The conformation of the compound I has been determined by using NMR methods. The results of this study are reported and discussed in a separate communication[3].

EXPERIMENTAL

The ^1H NMR spectra were recorded at 360 MHz at 23±2°C either in $CDCl_3$ containing 1% TMS as an internal standard or in D_2O with acetone (0.1%, 2.225 ppm relative to internal DSS) as the internal standard. Dichloromethane was dried by distillation under dry argon or nitrogen in the presence of P_2O_5. Acetonitrile was dried by 3h-reflux over CaH_2 and subsequent distillation under nitrogen or argon onto 4A molecular sieves. Methanol was dried by a 4h-reflux over Mg and a trace of iodine and subsequent distillation under dry nitrogen or argon onto 3A molecular sieves. Mercuric bromide was dissolved in hot toluene, dried by azeotropic distillation of some toluene, and crystallized upon cooling. Solutions were dried over sodium sulfate. Molecular sieves were heated at 300 °C at 0.01 mm Hg for 1 hour. Deuterated Raney nickel and deuterium oxide were purchased from Merck, Sharp, and Dohme, Montreal, P.Q., Canada. The purity of the reaction products was monitored either by TLC on silica gel or HPLC on silica gel or reversed phase (C_{18}).

2-Propyl 2,4-di-O-acetyl-2,3-O-carbonyl-β-D-manno-pyranoside (III). The bromide II[9] (31.2 g; 0.068mol) was added to a solution of 2-propanol (31.2 mL) in methylene chloride (150 mL) containing 4A molecular sieve (7.5 g) and silver silicate on alumina[10] (27 g) previously stirred at room temp. for 30 min. The reaction mixture was stirred overnight, then filtered through a celite bed, washed with a

sat. aq. $NaHCO_3$ (250 mL), dried, and evaporated to dryness in vacuo yielding III as a colorless thick oil (28.3 g; 0.085 mol; 97%).

2-Propyl β-D-mannopyranoside (IV). A solution of III (32.6 g; 0.098 mol) in methanol (50 mL) was added to sodium methoxide in methanol (prepared from 0.4g Na and 100 mL MeOH) and the reaction mixture was strirred at room temp. for 40 mins. After neutralization with AG 50W-X2 (H^+ form), the resin was filtered off, the solvent evaporated to dryness giving IV as a colorless syrup (21.4 g; 0.097 mol; 98%).

2-Propyl 4,6-O-benzylidene-β-D-mannopyranoside (V). A mixture of IV (5.55 g; 0.025 mol), 95-97% formic acid (35 mL) and benzaldehyde (35 mL) was allowed to react at room temp. for 6 min. Then it was poured into a mixture of heptane (450 mL) and water (450 mL) containing potassium carbonate (148 g), allowed to stand for 30 min. at room temp., and in a refrigerator for 16 hours. The precipitated amorphous solid was filtered off and after drying gave V (2.78g; 8.9 mmol; 36%).

2-Propyl 3-O-allyl-4,6-O-benzylidene-β-D-mannopyranoside (VI). To a solution of V (0.9 g; 2.9 mmol) in dry tetrahydrofurane (30 mL) cooled to -75 °C was added dropwise a solution of nBuLi in hexane (1.78M in hexane, 3.3 mL). The mixture was allowed to warm up to room temperature and stirred for an additional 10 min. Then allyl bromide (0.77g; 6.4 mol) was added, and the resulting reaction mixture was

refluxed for 16 hours. Then it was poured into water (150 mL), extracted with chloroform (50 mL, four times), and the chloroform was evaporated after drying, yielding a thick syrup of VI (0.99 g; 2.8 mmol; 98%). Crystallization from hexane-ethyl acetate 95:5 gave pure VI, mp 123-5 °C.

2-Propyl 3-O-allyl-2-O-benzyl-4,6-O-benzylidene-β-D-mannopyranoside (VII). To DMF (15 mL) containing NaH prepared from 50% dispersion in oil (0.64g; 1.34mmol) by washing with dry benzene was added a solution of VI (0.98 g) in DMF (5 mL), and the resulting mixture was stirred at room temperature for 10 min. After addition of benzyl bromide (2.44 g; 1.42 mmol) the reaction mixture was stirred at room temperature for 16 hours, poured in water (150 mL) and extracted with methylene chloride (3x75 mL). The combined extracts were washed with water, dried, and evaporated to dryness. The residue was subjected to flash chromatography on silica gel and the fractions eluted with hexane-ethyl acetate 9:1 gave VII as a pale yellow syrup (1.23 g; 2.8 mmol; 98%).

2-Propyl 2-O-benzyl-4,6-O-benzylidene-β-D-mannopyranoside (VIII). To a solution of potassium t.butoxide (0.8 g; 7.15 mmol) in DMSO (15 mL) was added VII (1.43 g; 3.25 mmol) and the amber mixture was stirred at 100 °C for 30 min. Then it was poured into brine (200 mL), extracted with chloroform (3x50 mL), the combined extracts were washed with water, dried, and evaporated to dryness. The resulting syrupy vinyl ether was taken up in acetone (200 mL) and treated with 0.1N aq. HCl

(1 mL) at room temperature overnight. The reaction mixture then was poured into brine (500 mL), extracted with chloroform (4x50 mL), the combined extracts were washed with sat. aq. $NaHCO_3$, dried, evaporated to dryness, and purified by a filtration through silica gel using hexane-ethyl acetate. The yield of syrupy VIII was 0.97 g (2.43 mmol; 75%).

2-Propyl 2-O-benzyl-4,6-O-benzylidene-3-oxo-β-D-manno-pyranoside (IX). A solution of VIII (0.685 g; 1.71 mmol) in benzene (70 mL) containing pyridimium chlorochromate (PCC, 1.0 g; 4.65 mmol) was refluxed and the progress of the reaction was monitored by TLC on silica gel (hexane: ethyl acetate 9:1). After 2 hours another portion (1.0 g) of PCC was added and the refluxing continued for another 75 min. The final addition of PCC (1.0 g) followed by 80 min reflux led to the completion of the oxidation, the reaction mixture was cooled to room temperature, diethyl ether (300 mL) was added, the colored solution filtered through a Florisil bed, and evaporated to dryness. The light yellow-green syrupy IX (0.34 g; 0.96 mmol; 56%) was used in the following reduction without purification.

2-Propyl 2-O-benzyl-4,6-O-benzylidene-3-deuterio-β-D-manno-pyranoside (X). A solution of IX (0.31 g; 0.78 mmol) in methanol-methylene chloride mixture (1:1, 10 mL) to which solid $NaBD_4$ (66 mg; 1.6 mmol) was added was stirred at room temperature for 90 min. Then it was poured in water (50 mL), extracted with chloroform (3x50 mL), the

combined chloroform extracts were washed with water (50 mL), dried, evaporated, and subjected to chromatography on silica gel. The elution with hexane–ethyl acetate 3:1 gave X (0.15 g; 0.37 mmol; 48%).

Methyl 1,2,3,4,6,6'-hexadeuterio-α-D-mannopyranoside (XII). A solution of methyl α-D-mannopyranoside (XI; 17 g) in D_2O (100 mL) containing Raney nickel (50 mL suspension in D_2O) was stirred overnight under mild reflux (bath temperature 123 °C), and the course of deuterium exchange was monitored by ^1H NMR. As both H-6's were exchanged to less than 50%, the reflux was continued for an additional 24 hours. The exchange still was not complete, therefore the residue after filtration and evaporation of heavy water was redissolved in fresh D_2O (100 mL) and stirred under reflux with another portion of Raney nickel (50 mL) for 6 hours. Approx. 15% of H-6 remained unexchanged; epimerization to gluco was about 33%. After filtration and evaporation of heavy water, the residue was crystallized from a small volume of methanol, to give slightly impure XII.

1,2,3,4,6-Pentaacetyl-2,3,4,6,6'-pentadeuterio-α-D-mannopyranoside (XIII). To a solution of sulfuric acid (96%; 0.9 mL) in acetic anhydride (35 mL) was added XII (4.3 g) and the reaction mixture was stirred at room temperature for 18 hours. An ice-cold sat. aq. $NaHCO_3$ soln. was added until pH 6 under external ice-cooling, and the liquids evaporated

to dryness. The residue was taken up with chloroform (4x50 mL), combined chloroform washings were washed with water, cold sat. aq. $NaHCO_3$ (with external cooling), water, then dried, and evaporated to dryness to give semisolid XIII (3.9 g), ^1H NMR ($CDCl_3$) ∂ 2.0-2.2 (15 H, $OOCCH_3$), 4.05 (s, 1 H, H-5, 6.13 (s, 1 H, H-1), and a trace of H-6 (5.205, s). The compound was used without further purification.

3,4,6-Tri-O-acetyl-2,3,3,6,6'-pentadeuterio-β-D-mannopyranose-1,2-methylorthoacetate (XIV). The orthoester XIV was prepared in two steps from XIII (4.0 g) following the procedure described by Mazurek and Perlin[11]; yield: 35% after twofold crystallization (mp. 110-111 °C) from abs. methanol. ^1H NMR ($CDCl_3$) ∂ 1.76 (s, 3H, CH_3), 2.06 (s, 3H, $OOCCH_3$), 2.08 (s, 3H, $OOCCH_3$), 2.13 (s, 3H, $OOCCH_3$), 3.28 (s, 3H, OCH_3), 3.70 (s, 1H, H-5), 5.51 (s, 1H, H-1).

2,3,4,6-Tetra-O-acetyl-2,3,4,6,6'-pentadeuterio-αD-mannopyranosyl bromide (XV). The bromide XV was prepared according to Winnik et al.[12] from XIV in 95% yield and was used without further purification.

2-Propyl 2-O-benzyl-3-deuterio-3-O-(2,3,4,6,6'-pentadeuterio-2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-4,6-O-benzylidene-β-D-mannopyranoside (XVI). The reaction was performed under

argon. A solution of X (140 mg; 0.35 mmol) in acetonitrile (7 mL) was added into a flask containing molecular sieves 4A (0.5 g), $Hg(CN)_2$ (88 mg) and $HgBr_2$ (125 mg), the mixture was stirred for 30 min at room temperature and then a solution of XV (prepared from 162 mg of XIV) in acetonitrile (7 mL). After stirring for 18 hours at room temperature, the usual work-up and chromatography on silica gel, the fractions eluted with hexane- ethyl acetate yielded slightly yellow syrupy XVI (174 mg; 0.23 mmol; 67%).

2-Propyl 2-O-benzyl-3-deuterio-3-O-(2,3,4,6,6'-pentadeuterio-2,3,4,6-tetra-O-acetyl-α-D-manno pyranosyl)-β-D-manno-pyranoside (XVII). A solution of XVI (174 mg; 0.233 mmol) in acetic acid (60%, 10 mL) was heated at 80 °C for 30 min. After pouring into cold water (100 mL) and extraction with chloroform (4x25 mL), the chloroform extracts were washed with sat. aq. $NaHCO_3$, water, dried, and the solvent evaporated to give XVII (142 mg; 0.215 mmol; 93%).

2-Propyl 2-O-benzyl-3-deuterio-3,6-di-O-(2,3,4,6,6'-pentadeuterio-2,3,4,6-tetra-O-acetyl-α-D- mannopyranosyl)-β-D-mannopyranoside (XVIII). The coupling was performed as described for the preparation of XVI, using XVII (160 mg; 0.243 mmol), XV (prepared from 186 mg of XIV), $HgBr_2$(96 mg, 0.267 mmol), and $Hg(CN)_2$ (67 mg, 0.26 mmol). Chromatography on silica gel using a gradient of hexane-ethyl acetate 60:40 -33:66 yielded syrupy XVIII (214 mg; 0.21 mmol; 89%).

2-Propyl 3-deuterio-3,6-di-O-(2,3,4,6,6'-pentadeuterio-α-D-mannopyranosyl)-β-D-mannopyranoside (I). Compound XVIII (214 mg) was deprotected by hydrogenation in methanol (25 mL) over Pd/C (10%) at normal pressure. After filtration and evaporation, the residue (164 mg, without purification) was redissolved in methanol (10 mL) and treated with sodium methoxide (prepared from 100 mg Na in 10 mL methanol) for 1 hour. The neutralized solution (with Ag 50W-X2, H^+ form), was filtered, and evaporated to dryness to yield amorphous I (74 mg; 0.131 mmol; 72 %). For values of chemical shifts see Table I.

TABLE I

*Values of Chemical Shifts for Deuterated Trimannoside I**

	H 1	H 2	H 3	H 4	H 5	H 6	H 6'
Mannose M 6	4.902	-	-	-	3.682	-	-
Central Mannose	4.775	4.080	-	3.784	3.557	3.771	3.948
Mannose M 3	5.100	-	-	-	3.790	-	-

Values of Coupling Constants (Hz)

	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$
Mannose M 6	-	-	-	-	-	-	-
Central Mannose	<1.0	-	-	9.9	1.8	5.2	-11.2
Mannose M 3	-	-	-	-	-	-	-

*We are grateful for the table to Dr D. A. Cumming, cf. Ref. 3.

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FOOTNOTES AND REFERENCES

+For Part 7, see Ref [13].

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