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Note

Synthesis of a tetrasaccharide of the extended core-region of the saccharide moiety of *N*-linked glycoproteins*

RAJAN N. SHAH[†], DALE A. CUMMING[†], ARTHUR A. GREY[†], JEREMY P. CARVER[†,‡], AND JIRI J. KREPINSKY[†,‡,**]

*Departments of [†]Medical Genetics and [‡]Medical Biophysics, University of Toronto, Toronto, Ontario M5S 1A8, and **Ludwig Institute for Cancer Research, Toronto Branch, Toronto, Ontario M4Y 1M4 (Canada)*

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Glycoproteins containing *N*-linked oligosaccharides are ubiquitous on cell surfaces. While all of these saccharides contain an invariant core-structure, the structures of their arms vary widely[2]. Even if the biological significance of these variations is unclear, it is certain that they are capable of influencing the overall shape of the oligosaccharide molecule[3]. As an aid in the study of the three-dimensional structures in solution of biologically derived, *N*-linked oligosaccharides by n.m.r. spectroscopy, we have synthesized several model compounds. These compounds aid in the interpretation of n.m.r. spectra of the larger, more complex glycopeptides in two ways. Firstly, ^{1}H-n.m.r. spectra of the model tri- and tetra-saccharides are simple enough to permit complete assignments, which then allows n.O.e. values in the spectra of the larger compounds to be assigned by analogy[4]. Secondly, the synthetic compounds can be prepared with ^{13}C (cf. lit.[5]) or ^{2}H (cf. lit.[6]) labels to aid in the conformational studies. We describe here the synthesis of a tetrasaccharide **2** that is a part of a carbohydrate structure **1**. The methoxyl group usually interferes with the observation of ring-proton signals and consequently perdeuterated methoxyl was used.

As a β-glycoside **5** was required, the bromide **4** had to have a nonparticipating group at C-2. The simplest precursor of **4** was the diacetate **3**, conveniently obtained by acetolysis of perbenzylated D-mannose[7]. The bromide **4** was prepared from **3** by treatment with HBr in dichloromethane at 0° and the desired trideuteriomethyl β-glycoside **5** was obtained from **4** in 95% yield by reaction with methanol-d_4 in dichloromethane with silver zeolite 3Å as a promoter at room

*Part V in the series Syntheses of Model Oligosaccharides of Biological Significance. For Part IV, see ref. 1.

**To whom all correspondence should be addressed.

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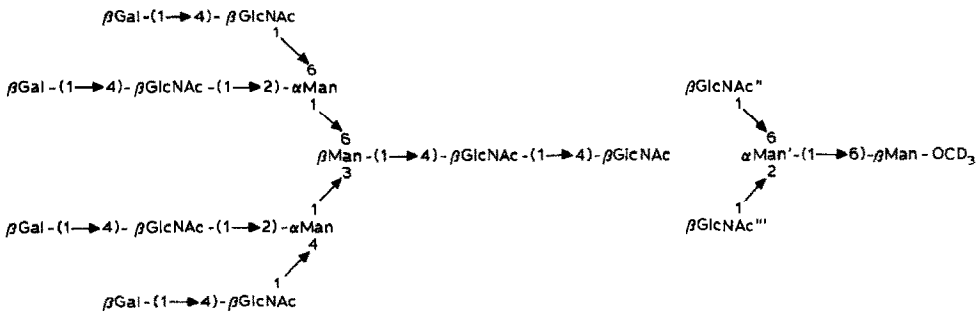


1 2

temperature[8a]. Zemplén deacetylation of **5** gave **6**, which subsequently served as the aglycon for glycosylation by the bromide **8**. The latter was prepared by treatment with HBr in dichloromethane of 1,2,6-tri-*O*-acetyl-3,4-di-*O*-benzyl-D-mannose (**7**), which in turn was readily made by acetolysis, as reported previously[7].

The glycosylation reaction was most efficiently promoted by thallium zeolite 3Å, as this promoter[8b] did not give any of the undesired β anomer and the yield of pure **9** was >30%, after 92 h at room temperature in dichloromethane. Three other promoters tried were all inferior (Table I). One of the advantages of thallium zeolite apparently is that the "soft" metal does not bring about any decomposition of the bromide, and the reaction may thus proceed for prolonged periods of time. The protected disaccharide **9** was deacetylated by Zemplén's procedure to give the diol **10**.

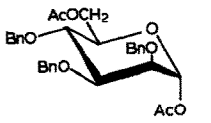
The glycosylation agent **11**, previously used with success[1,9], was utilized for the introduction of two molecules of the precursor of 2-acetamido-2-deoxyglucose into **10**. The reaction was promoted by silver triflate and required acetonitrile as the solvent; the temperature was allowed to rise slowly from −25° to room temperature during 24 h. Interestingly, when the same reaction partners were allowed to react in dichloromethane (temperature range −40° to room temperature), the trisaccharide **13** (71%) was formed, which, after chromatography (silica gel, 1:1 hexane–ethyl acetate), had $[\alpha]_D$ +3.01° (*c* 5.5, $CHCl_3$); ^{1}H-n.m.r.: δ 7.0–8.0 [m, 29 H, $N(CO)_2C_6H_4$ and $C_6H_5CH_2$], 5.38 (d, 1 H, *J* 8.28 Hz, H-1"), 4.83 (d, 1 H, *J* 1.44 Hz, H-1'), 4.23 (d, 1 H, *J* <1 Hz, H-1), and 1.85–2.07 (3 s, 9 H, $OCOCH_3$).

TABLE I

COMPARISON OF PROMOTERS IN THE REACTION OF **8** WITH **6** TO GIVE **10** (α/β)

Promoter	$\alpha:\beta$	*Yield*[a] *(%)*	*Solvent*
Ag zeolite (3A)	99:1	<8	CH_2Cl_2
Tl zeolite (3A)	99:1	30	CH_2Cl_2
Ag triflate	50:50	47	CH_2Cl_2
$HgBr_2$–$Hg(CN)_2$	63:37	45	CH_3CN

[a]After chromatographic separation of anomers.



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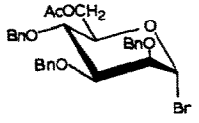

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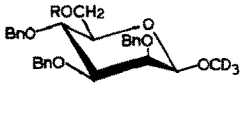
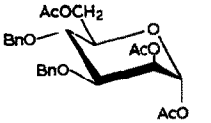


5 , R = Ac
6 , R = H



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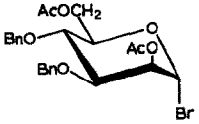
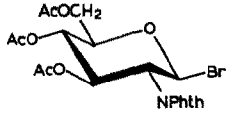
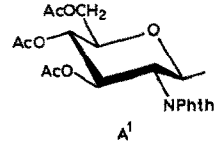


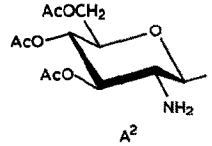
8



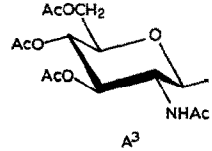
11



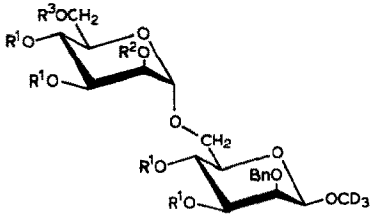
A^1



A^2



A^3



9 , R^1 = Bn , R^2 = R^3 = Ac
10 , R^1 = Bn , R^2 = R^3 = H
12 , R^1 = Bn , R^2 = R^3 = A^1
13 , R^1 = Bn , R^2 = H , R^3 = A^1
14 , R^1 = Bn , R^2 = R^3 = A^2
15 , R^1 = Bn , R^2 = R^3 = A^3
16 , R^1 = Ac , R^2 = R^3 = A^3

Deprotection of **12** was done first by hydrazinolysis to give **14** followed by acetylation. The acetylated product **15**, after debenzylation by hydrogenolysis on Pd/C followed by acetylation, gave **16**. The final tetrasaccharide **2** was obtained by Zemplén deacetylation overnight and desalting of the mixture with a mixed-bed resin. The deblocking sequence had to be performed in the way described, and each step had to be followed by acetylation and chromatography. When the acetylation step was omitted, the intermediates were very difficult to purify. In particular, after hydrogenolysis, the product contained cations (including Pd, Fe, and Ca) that caused considerable broadening of n.m.r. signals. These cations could not be removed by treatment with Chelex.

Complete analysis of the ^{1}H-n.m.r. spectrum of **2** will be published separately in connection with our conformational study of this oligosaccharide.

EXPERIMENTAL

General methods. — Optical rotations were measured with a Perkin–Elmer polarimeter (model 140) at 26 ±1°. Microanalyses were performed by the Microanalytical Laboratory Ltd., Markham, Ontario. ^{1}H-N.m.r. spectra were recorded at 360 MHz with a Nicolet spectrometer at the Toronto Biomedical NMR Centre, University of Toronto. They were obtained at 23 ±2° either in $CDCl_3$ containing 1% Me_4Si as the internal standard or in D_2O (99.996%, Merck, Sharpe, and Dohme) with acetone [0.1%, 2.225 p.p.m. relative to sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS)] as the internal standard. F.a.b. mass spectrometry was performed with a VG Analytical ZAB HF reverse-geometry instrument (for general conditions see ref. 10 and references therein) at the Institute of Physiological Chemistry, University of Bonn.

Thin-layer chromatography (t.l.c.) was performed on silica gel $60F_{254}$ (Merck) plastic plates and spots made visible by quenching of u.v. fluorescence and/or spraying with 50% aq. sulfuric acid and heating at 100°. Silica gel 60 (230–400 mesh; Merck) was used for flash chromatography. All glycosylation reactions were performed in flame-dried glassware under argon. All starting materials were dried overnight under vacuum (10^{-3} mm Hg) prior to use and solvents were distilled onto 4Å molecular sieves from appropriate drying agents (CH_2Cl_2 from P_2O_5, CH_3CN from CaH_2, and CH_3OH from Mg turnings) all under dry argon. Solvents were removed at water-aspirator pressure unless indicated *in vacuo* (10^{-3} mm Hg).

Trideuteriomethyl 6-O-acetyl-2,3,4-tri-O-benzyl-β-D-mannopyranoside (**5**). — To a cooled solution (−8°) of 1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (**3**, 4.0 g; 7.49 mmol)[7] in CH_2Cl_2 (30 mL) was added a saturated solution of HBr in CH_2Cl_2 (60 mL) under stirring. The mixture was kept for 2 h at 0°, the solvent was evaporated, benzene (15 mL) was twice azeotropically distilled from the residue. A solution of the residue in CH_2Cl_2 (25 mL) was added dropwise under vigorous stirring to a suspension of Ag zeolite (6.85 g) in CH_2Cl_2 (30 mL) containing methanol-d_3 (1.2 mL) which had previously been stirred under argon for 10 min. The stirring was continued for 2 h, the solids were filtered off, and the syrupy residue after removal of the solvent *in vacuo* was chromatographed on a column of silica gel prepared in 3:1 hexane–ethyl acetate. Hexane–ethyl acetate (2:1) eluted **5** (2.3 g, 60.5%) as a colorless syrup, $[\alpha]_D$ −21° (*c* 2.9, $CHCl_3$); ^{1}H-n.m.r.: δ 7.2–7.6 (m, 15 H, $C_6H_5CH_2$), 4.3 (d, 1 H, J <1 Hz, H-1), and 2.13 (s, 3 H, $OCOCH_3$).

Anal. Calc. for $C_{30}H_{31}D_3O_7$ (509.60): C, 70.70; H, 7.32. Found: C, 70.92; H, 7.12.

Trideuteriomethyl 2,3,4-tri-O-benzyl-β-D-mannopyranoside **6**. — Compound **5** (2.2 g, 4.32 mmol) was stirred with sodium methoxide in methanol (prepared from 44 mg of Na and 75 mL of dry methanol) for 2 h at room temperature. To this solution was added Dowex 50W-8X, H^+ (5 mL) prewashed with methanol, and the mixture was slowly stirred for 30 min. The resin was filtered off, washed with

methanol (15 mL), and the combined washings and filtrate were evaporated to dryness *in vacuo* yielding syrupy **6** (1.51 g, 75%); $[\alpha]_D$ −55.4° (*c* 7.6, $CHCl_3$).

Anal. Calc. for $C_{28}H_{29}D_3O_6$ (467.56): C, 71.92; H, 7.54. Found: C, 71.73; H, 7.33.

*Trideuteriomethyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,6-di-*O*-acetyl-3,4-di-*O*-benzyl-α-*D*-mannopyranosyl)-β-*D*-mannopyranoside* (**9**). — To a solution of 1,2,6-tri-*O*-acetyl-3,4-di-*O*-benzyl-D-mannopyranose (**7**; 2.0 g, 4.1 mmol)[7] in CH_2Cl_2 (25 mL) was added a saturated solution of HBr in CH_2Cl_2 (130 mL) at −10° and the mixture was kept for 3 h at 0° (monitored by t.l.c. 1:1 hexane–ethyl acetate). The solvent was removed *in vacuo* and the residual, syrupy **8** was twice azeotropically dried by distilling dry benzene (25 mL) from it. The residue was dried under high vacuum and dissolved before use in dry CH_2Cl_2 (30 mL).

A solution **6** (1.2 g, 2.57 mmol) in CH_2Cl_2 (25 mL) was added under argon to a suspension of Tl-zeolite (12 g)[8b] and molecular sieves 4Å (16 g) in dry CH_2Cl_2 (50 mL), which was stirred slowly under argon for 15 min before the addition of the solution of **8** prepared as already described. The mixture was stirred for 92 h at room temperature under argon. After conventional processing, the products were subjected to chromatography on a column of silica gel prepared in 3:1 hexane–ethyl acetate. Elution with 1:1 hexane–ethyl acetate gave the desired pure, syrupy α anomer **9** (0.824 g, 35.8%); $[\alpha]_D$ −13.1° (*c* 2.6, $CHCl_3$); ^{1}H-n.m.r.: δ 7.2–7.5 (m, 25 H, $C_6H_5CH_2$), 4.88 (d, 1 H, *J* 1.8 Hz, H-1′), 4.27 (d, 1 H, *J* <1 Hz, H-1), 2.13 and 1.99 (2 s, 6 H, $OCOCH_3$).

Anal. Calc. for $C_{52}H_{55}D_3O_{13}$ (894.01): C, 69.86; H, 6.88. Found: C, 70.11; H, 7.01.

*Trideuteriomethyl 2,3,4-tri-*O*-benzyl-6-*O*-(3,4-di-*O*-benzyl-α-*D*-mannopyranosyl)-β-*D*-mannopyranoside* (**10**). — To compound **9** (0.82 g, 0.92 mmol) dissolved in dry methanol (20 mL) was added a solution of methoxide in methanol (prepared from 66 mg of Na and 200 mL of dry methanol), and the mixture was stirred for 1 h at room temperature. To this solution was added Dowex 50W-8X, H^+ (3 mL) prewashed with methanol, and the mixture was stirred slowly for 30 min. The resin was filtered off, washed with methanol (15 mL), and the combined washings and filtrate were evaporated to dryness *in vacuo* yielding syrupy **10** (0.7 g, 94%); $[\alpha]_D$ −90° (*c* 2.0, $CHCl_3$); ^{1}H-n.m.r.: δ 7.1–7.6 (m, 25 H, $C_6H_5CH_2$), 5.02 (d, 1 H, *J* 1.58 Hz, H-1′), and 4.27 (d, 1 H, *J* <1 Hz, H-1).

Anal. Calc. for $C_{48}H_{51}D_3O_{11}$ (809.93): C, 71.18; H, 7.09. Found: C, 71.23; H, 7.02.

*Trideuteriomethyl 2,3,4-tri-*O*-benzyl-6-*O*-[3,4-di-*O*-benzyl-2,6-di-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-α-*D*-mannopyranosyl]-β-*D*-mannopyranoside* (**12**). — To a suspension of granular molecular sieves (4Å, 8.4 g) and powdered molecular sieve (4Å, 9.9 g) in dry acetonitrile (20 mL) was added a solution of **10** (0.384 g, 0.47 mmol) in dry acetonitrile (20 mL) under slow stirring. The mixture was cooled to −25–−30°, silver triflate (0.846 g, 3.29 mmol) was added, and the mixture was stirred for 5 min at −30° before dropwise addition

of a solution of the bromide **11** (1.29 g, 2.59 mmol) in dry acetonitrile (60 mL)[1,9]. The mixture was protected from light, the temperature was allowed to rise slowly to ambient, and stirring was continued overnight. Then it was filtered, the solids were washed twice with CH_2Cl_2 (10 mL), and the filtrate and washings were combined, evaporated, and subjected to flash chromatography on a column of silica gel with 6:1 chloroform–acetone, followed by preparative l.c. on μPorasil using 2:3 hexane–ethyl acetate. The yield of pure, syrupy, protected tetrasaccharide **12**, was 0.20 g (26%); $[\alpha]_D$ −10.3° (*c* 1.5, $CHCl_3$); ^{1}H-n.m.r.: δ 7.00–8.00 (m, 33 H, $N(CO)_2C_6H_4$ and $C_6H_5CH_2$), 5.51 (d, 1 H, *J* 8.29 Hz, H-1″), 4.86 (d, 1 H, *J* 9.2 Hz, H-1‴), 4.56 (d, 1 H, *J* <1 Hz, H-1′), 4.25 (d, 1 H, *J* <1 Hz, H-1), and 1.95–2.05 (6 s, 18 H, $OCOCH_3$). Molecular ion observed $[MNa]^+$ 1666.

Anal. Calc. for $C_{88}H_{89}D_3N_2O_{29}$ (1644.66): C, 64.21; H, 5.82; N, 1.70. Found: C, 64.40; H, 5.93; N, 1.83.

*Trideuteriomethyl 2,3,4-tri-*O*-benzyl-6-*O*-[3,4-di-*O*-benzyl-2,6-di-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-α-*D*-mannopyranosyl]-β-*D*-mannopyranoside* (**15**). — A solution of **12** (10 mg, 6 μmol) in ethanol (95%, 25 mL) containing hydrazine hydrate (85%, 2 mL) was boiled under reflux for 5 h. Evaporation *in vacuo* gave a residue that was dried by azeotropic distillation of dry benzene (20 mL). The resulting syrup was stirred overnight with 1:1 pyridine–acetic anhydride (10 mL) at room temperature, dried *in vacuo,* and subjected to chromatography on column of silica gel prepared in 2:1 hexane–ethyl acetate. Ethyl acetate eluted a syrup that was further purified by l.c. on a column of μPorasil with 2:3 hexane–ethyl acetate to give pure **15**; $[\alpha]_D$ +5.14° (*c* 0.74, $CHCl_3$); ^{1}H-n.m.r.: δ 7.00–7.25 (m, 25 H, $C_6H_5CH_2$), 5.21 (d, 1 H, *J* 8.28 Hz, H-1″), 5.38 (d, 1 H, *J* 9.00 Hz, H-1‴), 4.58 (d, 1 H, *J* 1 Hz, H-1′), 4.31 (d, 1 H, *J* <1 Hz, H-1), and 2.06–1.93 (8 s, 24 H, $OCOCH_3$ and $NHCOCH_3$).

Anal. Calc. for $C_{76}H_{89}D_3N_2O_{27}$ (1468.54): C, 62.15; H, 6.52; N, 1.91. Found: C, 62.21; H, 6.42; N, 1.99.

*Trideuteriomethyl 2,3,4-tri-*O*-acetyl-6-*O*-[3,4-di-*O*-acetyl-2,6-di-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-α-*D*-mannopyranosyl]-β-*D*-mannopyranoside* (**16**). — Compound **15** (0.065 g, 0.044 mmol) was stirred with 10% palladium-on-charcoal (0.058 g) in 5:1 ethanol–ethyl acetate (24 mL) under hydrogen (1 atm) for 18 h. Following filtration and washing the catalyst with ethanol (20 mL), the combined washings and filtrate were concentrated *in vacuo,* dried by azeotropic evaporation of benzene (15 mL), and the colorless syrup was directly acetylated with 1:1 pyridine–acetic anhydride (15 mL) overnight at room temperature. The volatiles were distilled off *in vacuo,* and the residue was further purified by l.c. on a column of μPorasil with 1:6 hexane–ethyl acetate to give pure, syrupy **16** (0.041 g); $[\alpha]_D$ −2.94° (*c* 0.71, $CHCl_3$); ^{1}H-n.m.r.: δ 5.38 (d, 1 H, *J* 8.28 Hz, H-1″), 4.29 (d, 1 H, *J* 9.72 Hz, H-1‴), 4.88 (d, 1 H, *J* <1 Hz, H-1′), 4.57 (d, 1 H, *J* <1 Hz, H-1), and 2.06–1.89 (13 s, 39 H, $OCOCH_3$ and $NHCOCH_3$).

Anal. Calc. for $C_{51}H_{69}D_3N_2O_{32}$ (1228.13): C, 49.87; H, 6.15; N, 2.28. Found: C, 50.11; H, 6.31; N, 2.33.

Trideuteriomethyl 6-O-[2,6-di-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranosyl]-β-D-mannopyranoside (**2**). — To compound **16** (0.041 g) dissolved in dry methanol (10 mL) a solution of sodium methoxide in methanol (prepared from 33 mg of Na and 15 mL dry methanol) was added and the mixture was stirred for 3 h at room temperature. To this solution was added mixed-bed resin (BioRad AG501-X8, 1 mL) prewashed with methanol, and the mixture was stirred slowly for 30 min. The resin was filtered off, washed with methanol (5 mL), and the combined washings and filtrate were evaporated to dryness *in vacuo* yielding amorphous **2** (0.014 g, 50%); $[\alpha]_D$ −30.74° (*c* 0.5, CH_3OH); ^{1}H-n.m.r.: δ 4.55 (d, 1 H, *J* 8.28 Hz, H-1″), 4.60 (d, 1 H, *J* 6.8 Hz, H-1‴), 4.88 (d, 1 H, *J* <1 Hz, H-1′), 4.58 (d, 1 H, *J* <1 Hz, H-1), and 2.04–2.06 (2 s, 6 H, $NHCOCH_3$).

ACKNOWLEDGMENTS

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