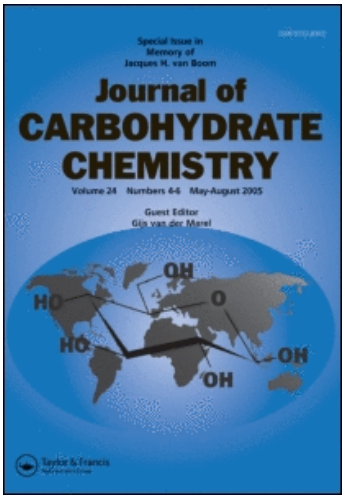


Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Syntheses of Model Oligosaccharides of Biological Significance. VI. Glycosylation at C-3 of Galactose: A Synthesis of Trideuterio-Methyl 3-O-(-Acetamido-2-Deoxy-β-D-Glucopyranosyl)-β-D-Galactopyranoside

Dennis M. Whitfield[ab]; Jeremy P. Carver[bc]; Jiri J. Krepinsky[abc]
[a] Ludwig Institute for Cancer Research, Toronto, Ont. [b] Departments of Medical Genetics, University of Toronto, Toronto, Ont., Canada [c] Medical Biophysics, University of Toronto, Toronto, Ont., Canada

To cite this Article Whitfield, Dennis M. , Carver, Jeremy P. and Krepinsky, Jiri J.(1985) 'Syntheses of Model Oligosaccharides of Biological Significance. VI. Glycosylation at C-3 of Galactose: A Synthesis of Trideuterio-Methyl 3-O-(-Acetamido-2-Deoxy-β-D-Glucopyranosyl)-β-D-Galactopyranoside', Journal of Carbohydrate Chemistry, 4: 3, 369 — 379
To link to this Article: DOI: 10.1080/07328308508070187
URL: http://dx.doi.org/10.1080/07328308508070187

J. CARBOHYDRATE CHEMISTRY, 4(3) 369-379 (1985)

SYNTHESES OF MODEL OLIGOSACCHARIDES OF BIOLOGICAL SIGNIFICANCE.VI. GLYCOSYLATION AT C-3 OF GALACTOSE: A SYNTHESIS OF TRIDEUTERIOMETHYL 3-O-(-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYL)-β-D-GALACTOPYRANOSIDE[1]

Dennis M. Whitfield,[1,2] Jeremy P. Carver[2,3] & Jiri J. Krepinsky[1,2,3]*

Ludwig Institute for Cancer Research, Toronto, Ont. M4Y 1M4
and
Departments of [2]Medical Genetics & [3]Medical Biophysics,
University of Toronto, Toronto, Ont. M5S 1A8, Canada

Received December 7, 1984 - Final Form July 7, 1985

ABSTRACT

The title disaccharide was prepared by glycosylation of either methyl trideuteriomethyl 2-O-benzoyl-4,6-benzylidene-β-D-galactopyranoside or trideuteriomethyl-4,6-O-benzylidene-β-D-galactopyranoside with 3,4,6-tri-O-acetyl 2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide using silver zeolite 13X or silver triflate as promoters.

INTRODUCTION

The title disaccharide 5C represents a fragment of the oligosaccharide moiety of glycoproteins of both mucin and N-linked type. In our NMR studies of N-linked oligosaccharides[2], we have found it advantageous to have available large amounts of model compounds representing fragments of the larger structure. Such models assist in the assignment of resonances and the interpretation of the data from NOE experiments.

0732-8303/85/0403-0369$3.50/0

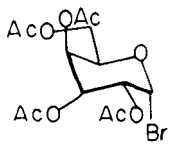
The selective glycosylation of one of the secondary hydroxyl groups of a carbohydrate is often a major challenge in carbohydrate synthesis and attempts to carry out such reactions frequently give unexpected results. The synthesis of 5C exemplifies this point. The synthesis of a very similar disaccharide 6, with the galactose unit as an α-methyl glycoside, has recently been described by Abbas and Matta[3]. We expected that following the procedure used to prepare 6[3] but employing trideuteriomethyl β-D-galactopyranoside (2B) as the starting substance, we should be able to prepare 5C smoothly. The derivative of choice for the appropriate coupling reaction was 2E with an unprotected OH-3[4]. The compound 2E was synthesized from 2A. The trideuteriomethyl group in the β-methyl galactoside used in the synthesis was chosen to avoid interference with the signals from the ring hydrogens in ^{1}H NMR spectroscopic analysis. The trideuteriomethyl group does not contribute to the relaxation pathways of the ring protons, thus simplifying the interpretation of NOE experimental results[5].

RESULTS AND DISCUSSION

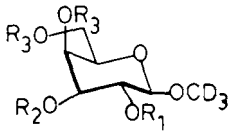
2,3,4,6-Tetra-O-acetyl-α-D-galactosyl bromide (1)[6] was glycosylated with CD_3OD in dichloromethane using silver zeolite[7] to give expected trideuteriomethyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (2A). Zemplen deacetylation of 2A gave 2B which on treatment with benzaldehyde and zinc chloride according to Sorkin and Reichstein[8] yielded the 4,6-benzylidene derivative 2C.

The next step in the synthesis involved differentiating the reactivity of the two remaining hydroxyls of 2C, OH-2 and OH-3. It was shown in the case of methyl 4,6-O-benzylidene-α-D-galactopyranoside[3,9] that a monobenzoylation of either OH-2 or OH-3 can be achieved by variation of phase-transfer conditions; addition of a very polar aprotic solvent (HMPA) to the reaction medium led to the exclusive benzoylation of OH-3, while the reaction without HMPA gave exclusively the OH-2 benzoylated product[9]. However, we used β-anomer 2C and, under either set of reaction conditions, the

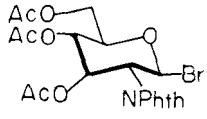
3-benzoate 2D was formed predominantly and as the kinetic product. It has been previously reported[10] that while benzyl 4-6-O-benzylidene-β-D-galactopyranoside also forms the 3-O-benzoyl derivative, the latter compound can be isomerized to the 2-O-benzoyl derivative by treatment with mild alkali. The reaction is presumably driven to completion by the precipitation of the 2-O-benzoyl derivative from the acetone solution. This isomerization also worked in the case of our trideuteriomethyl 3-O-benzoyl-4,6-O-benzylidene-β-D-galactopyranoside (2D). In practice, the crude reaction mixture from the phase-transfer benzoylation (containing 2D, some 2-O-benzoyl derivative 2E, and some 2,3-di-O-



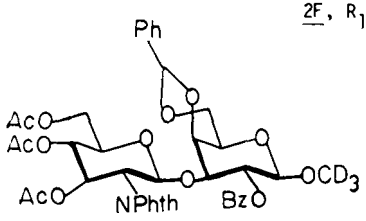
1



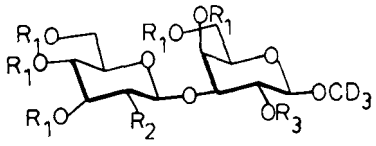
2A, R_1=R_2=R_3=Ac
2B, R_1=R_2=R_3=H
2C, R_1=R_2=H, R_3=PhCH
2D, R_1=H, R_2=Bz, R_3=PhCH
2E, R_1=Bz, R_2=H, R_3=PhCH
2F, R_1=R_2=Bz, R_3=PhCH



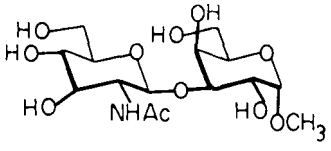
3



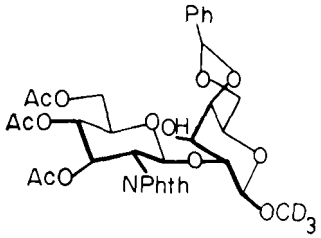
4



5A, R_1=Ac, R_2=NPhth, R_3=Bz
5B, R_1=Ac, R_2=NHAc
5C, R_1=H, R_2=NHAc



6



7

benzoyl derivative 2F) was subjected to the conditions cited, and the resulting crude 2E was purified by flash chromatography on silica gel. The overall yield of 2E from pentaacetyl galactose was 32%.

The readily accessible galactose derivative 2E can be used as an acceptor in glycosylation reactions with a variety of glycosyl donors. The conditions described below illustrate the utilization of solid state promoters,[11] in this case silver zeolite,[7] with glycosyl donors such as 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-D-glucopyranosyl bromide (3). Silver triflate gave in this particular reaction much inferior yields since it brought about rapid decomposition of 2E. Thus silver zeolite was the promoter of choice in this glycosylation yielding 4, despite the long reaction times required. The protected disaccharide 4 can also be prepared from the diol 2C, using silver zeolite or silver triflate as promoters, but the main product of such a glycosylation is the 1→2 linked oligosaccharide 7.

Removal of the benzylidene group from 4 was accomplished by hydrogenolysis in acetic acid over 10% Pd/C. The hydrolytic condition for removal of a benzylidene group (80% aqueous acetic acid at 100 °C for 30 min) led to considerable cleavage of the glycosidic bond and could not be used. Acetylation of the crude product in pyridine/acetic anhydride gave the pentaacetate 5A. The phthaloyl group was cleaved next by treatment with hydrazine hydrate at 70 °C for 20 min; all ester groups were also removed under these conditions. The heptaacetate 5B was prepared by acetylation of 5A with acetic anhydride/pyridine, and after chromatographic purification the acetyl groups were finally removed by Zemplen deacetylation. The resulting disaccharide 5C was isolated as a microcrystalline solid and its ^{1}H NMR data are summarized in Table 1, together with ^{1}H NMR data of the intermediates (cf also Fig 1).

Methyl 3-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranoside was used in a study of a receptor for pneumococci adhering to human pharyngeal epithelial cells.[13] While the method

TABLE ^{1}H NMR SPECTRAL DATA FOR COMPOUND 5C AND ITS INTERMEDIATES

COMPOUND RESIDUE	H_1 (J_{12})	H_2 (J_{23})	H_3 (J_{34})	H_4 (J_{45})	H_5 (J_{56})	H_6 ($J_{66'}$)	$H_{6'}$ ($J_{56'}$)	OTHERS
2A Gal	4.40 d (7.9)	5.21 dd (10.5)	5.02 dd (3.4)	5.39 dd (0.9)	3.91 td (6.9)	4.13 dd (-11.2)	4.21 dd (6.6)	2.15 s, 2.07 s, 2.06 s, 1.99 s[b]
2B Gal	4.31 d (7.9)	3.50 dd (9.9	3.64 dd (3.4)	3.92 dd (0.6)	3.69 ddd (4.4)	3.75 dd (-11.6)	3.80 dd (7.5)	-
2C Gal	4.22 d (7.2)	3.75 m[d]	3.75 m[d]	4.22 m[d]	3.51 m (1.9)	4.13 dd (-12.5)	4.36 brd (1.4)	7.50 m(2), 7.36 m(3)[e] 5.56 s[f] 2.50 m[g]
2E Gal	4.55 d (7.9)	5.37 dd (9.9)	3.91 ddd (3.9)	4.28 dd (0.6)	3.59 brd (1.8)	4.13 dd (-12.4)	4.41 dd (1.4)	8.07 m(2), 7.56 m(3), 7.39 m(5)[e] 5.59 s[f] 2.59 d (J=11.1)[g]
7 Gal	4.23 d (7.7)	3.60 dd (9.3)	3.45 ddd (3.9)	3.98 m[d]	3.38 brs	3.98 m[d] (-12.0)	4.27 dd (1.2)	7.81 m(2), 7.69 m(2), 7.43 m(5)[e,h] 2.12 d (J=9.2)[g]
Glc	5.55 d (8.5)	4.38 dd (10.6)	5.88 dd (9.1)	5.20 dd (10.1)	3.90 m (4.7)	4.19 dd (-12.2)	4.33 dd (2.3)	2.12 s, 2.04 s, 1.88 s[b]
4 Gal	4.45 d[d] (7.9)	5.40 dd (10.1)	3.96 dd (3.5)	4.15 m[d]	3.49 brs	4.4 m[d]	4.4 m[d]	7.65 m(2), 7.55 m(3) 7.47 m(3), 7.37 m(6)[e,h] 5.58 s[f]
Glc	5.61 d[d] (8.5)	4.45 m[d] (10.7)	5.65 dd (9.2)	5.16 dd (10.1)	3.85 m	4.17 m[d]	4.4 m[d]	2.07 s, 2.01 s, 1.75 s[b]
5A Gal	4.37 d[d] (7.9)	5.23 dd (9.9)	3.96 dd (3.6)	5.49 brs[d] (0.6)	3.87 brt (6)	4.15 m[d]	4.15 m[d] (6)	7.7 m(2), 7.5 m(4), 7.3 m(3)[e,h]
Glc	5.47 d[d] (8.4)	4.15 m[d] (10.7)	5.66 dd (9.2)	5.17 dd (9.9)	3.83 dt	4.2 m[d] (-12.4)	4.37 ddd (2.5)	2.17 s, 2.16 s, 2.09 s, 2.00 s, 1.78 s[b,h]
5B Gal	4.42 d[d] (8.1)	5.11 dd (9.9)	3.83 dd (3.4)	5.37 dd[d]	3.82 m[d]	4.15 m[d]	4.15 m[d]	2.16 s(1), 2.12 s(1), 2.11 s(1), 2.08 s(1), 2.03 s(2) 1.91 s(1)[b,h]
Glc	5.51 d[d]	3.30 ddd (9.5)	5.53 dd[d]	5.03 dd (9.6)	3.68 dt (3.8)	4.09 dd	4.30 dd[d] (2.6)	5.07 d
5C Gal	4.30 d (8.0)	3.54 dd[d] (8.0)	3.69 m[d] (3.2)	4.14 brd (<0.5)	3.69 m[d] (4.1)	3.8 m[d]	3.8 m[d]	-
Glc	4.69 d (8.4)	3.73 dd[d] (8.0)	3.52 dd[d] (8.0)	3.47 m[d] (9.7)	3.45 m[d]	3.8 m[d] (-11.8)	3.90 dd (1.7)	2.03 s[b]

a $CDCl_3$ as solvent, b $\underline{CH_3}CO$, c D_2O as solvent, d resonances overlapped,
e Ar$\underline{H}$, f Ph$\underline{CH}$, g $\underline{OH}$, h Glc and Gal combined, i $\underline{NH}$.

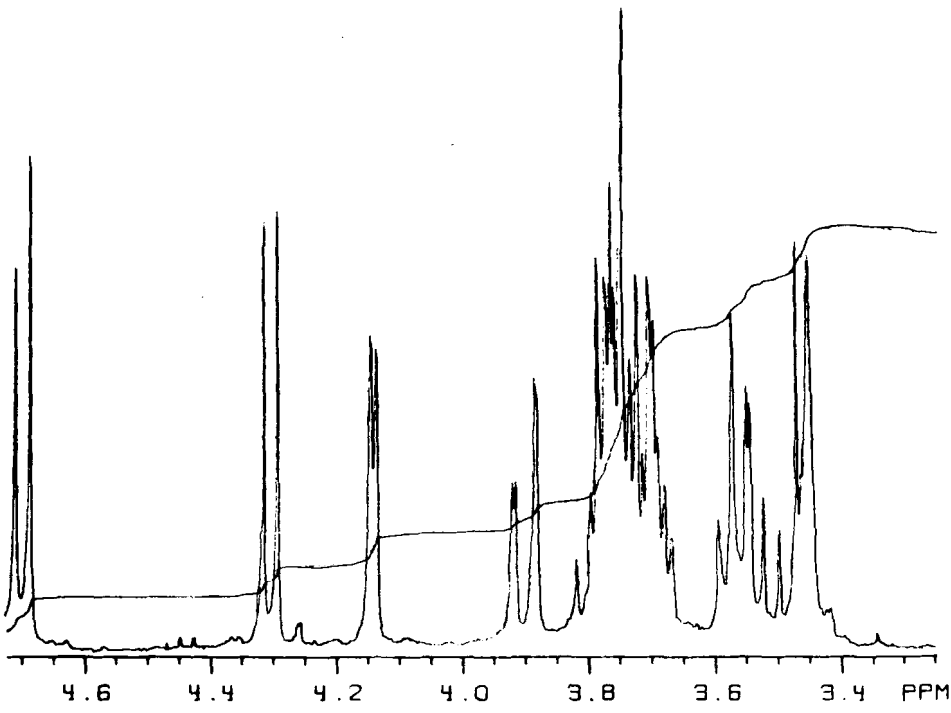


FIG 1 ^{1}H NMR spectrum showing the ring protons resonances of 5C.

of the synthesis for the compound was not described in the paper,[13] some physico-chemical data were reported which closely correspond with the data reported in this communication.

EXPERIMENTAL

General Methods. Melting points were determined on a Reichert Thermovar melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer polarimeter (Model 140) at 26 ° ± 1 °C. Microanalyses were performed by the Micro analytical Laboratory Ltd., Markham, Ontario. ^{1}H NMR spectra were recorded at 360 MHz with a Nicolet spectrometer at the Toronto Biomedical NMR Center, University of Toronto. They were obtained

at 19 ± 2 °C either in $CDCl_3$ containing 1% TMS as the internal standard or in D_2O(99.996%, Merck, Sharpe and Dohme) with acetone (0.1%, 2.225 ppm relative to internal DSS) as the internal standard. IR spectra were recorded on a Perkin-Elmer 1430 spectrometer using thin films on NaCl plates. Thin-layer chromatography (TLC) was performed on silica gel $60F_{254}$ (Merck) plastic plates and visualized by quenching of ultraviolet fluorescence and/or spraying with 50% aq. sulfuric acid and heating at 200 °C. Silica gel 60 (230-400 mesh; Merck) was used for flash chromatography. All glycosylation reactions were performed under an argon atmosphere. All starting materials were dried overnight under vacuum (10^{-3}mm Hg) prior to use and the solvents were distilled from appropriate drying agents. Solutions were concentrated at water aspirator pressure.

Trideuteriomethyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (2A). The bromide 1 (15 g, 0.0365 mol) prepared according to Ref[6] was added to a stirred suspension of silver zeolite (21 g; prepared from $AgNO_3$ and molecular sieve Linde 13X) in dry CH_2Cl_2 (50 mL) containing CD_3OD (3 mL) under argon, and the stirring continued for 16 h at room temperature. The solids were removed by filtration, washed twice with methylene chloride, and the combined washings and filtrates were evaporated to dryness. The amorphous residue was purified by flash chromatography on silica gel (hexane:ethyl acetate 6:4) yielding pure 2A (10.9 g, 82%), mp 97-8 °C $[\alpha]_D$ - 10.7(c=1.49, chloroform); IR (neat): 1750 cm^{-1}(CO). Anal. Calcd for $C_{15}H_{14}D_3O_{10}$ (365.31): C,49.32; H,5.24. Found: C,49.01; H,5.43.

Trideuteriomethyl 2-O-benzoyl-4,6-O-benzylidene-β-D-galactopyranoside (2E). To the solution of 2C (1.85 g; 6.5 mmol) obtained from 2A by Zemplen deacetylation followed by benzylidenation according to Ref[8], in methylene chloride (40 mL) were added sequentially[9] tetrabutylammonium hydrogen sulphate (0.109 g, 0.33 mmol), 40% aq. NaOH (6 mL), and benzoyl chloride (0.85 mL, 7.3 mmol). The reaction mixture was stirred for 10 min, diluted with methylene chloride (50 mL) and water (100 mL), and the

layers were separated. The organic phase was washed with water (3x100 mL), dried over $MgSO_4$, and evaporated to dryness. The amorphous residue containing three substances (TLC on silica gel, chloroform:acetone 9:1; 2F, 15%, R_F.64; 2D, 80%, R_F.22; 2E, 5%, R_F.43) was dissolved in acetone (50 mL), cooled in an ice-water bath, and an ice-cold aqueous NaOH (0.05M, 100 mL) was added to the solution bringing about an immediate precipitation[10]. The mixture was allowed to stand for another 10 min, then cold water (100 mL) was added, the precipitate was filtered off, washed with cold water (500 mL), and dried overnight in vacuo. Flash chromatography of the precipitate (silica gel, chloroform:acetone 9:1) gave pure 2E (2.0 g, 78%), mp.237-9 °C; IR(neat) 3540 cm^{-1}(OH), 1705 cm^{-1} (CO, $[\alpha]_D$ + 30.6°(c=1.10, chloroform). Anal. Calcd for $C_{21}H_{19}D_3O_7$ (389.38): C,64.78; H, 4.92. Found: C,64.51; H,5.21.

Trideuteriomethyl 2-O-benzoyl-4,6-O-benzylidene-3-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-gluco-pyranosyl)-β-D-galactopyranoside (4). A solution of the alcohol 2E (95 mg, 0.24 mmol) in dry dichloromethane (3 mL) was added to silver zeolite 13X (1.0 g) under argon and stirred for 30 min. Solid bromide 3[12] (325 mg, .65 mmol) was added to the above suspension and the stirring was continued for 70 h. The solids were removed by filtration, rinsed with dichloromethane (25 mL), and the combined filtrates were evaporated to dryness. Flash chromatography of the residue (silica gel; chloroform followed by chloroform:acetone 20:1) yielded pure 4 (68 mg, 40%), mp 288-94 °C; $[\alpha]_D$+53.7°(c=53.7°, chloroform); IR(neat): 1780, 1760, 1725, 1715 cm^{-1}(CO). Anal. Calcd for $C_{41}H_{38}D_3NO_{16}$ (806.75): C,61.04; H,4.75; N,1.74. Found: C,60.93; H,5.03; N,1.81.

Trideuteriomethyl 2-O-benzoyl-4,6-tri-O-acetyl-3-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-β-D-galacto-pyranoside (5A). Compound 4 (200 mg, 0.25 mmol) was dissolved in glacial acetic acid (30 mL), Pd/C (10%, 275 mg) was added, and the mixture was stirred under hydrogen at normal pressure for 20 h. The solids were filtered off using Celite bed, washed with methylene chloride (25 mL) and toluene (50 mL) and the combined

filtrates were evaporated to dryness. Toluene (50 mL) was added, and evaporated azeotropically with residual acetic acid. The syrupy residue was dissolved in dry pyridine (10 mL) and acetic anhydride (5 mL) and stirred under argon for 18 h. After evaporation of the reaction mixture to dryness, followed by twofold addition of toluene and again evaporation to dryness, the resulting syrup was purified by flash chromatography (silica gel, chloroform:acetone 19.1) to give 5A (169 mg, 92%), mp 218-22 °C; $[\alpha]_D$+ 32.6°(c=0.45, chloroform); IR(neat): 1780, 1750, 1720 cm^{-1}(CO).

Trideuteriomethyl 4,6-O-benzylidene-2-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-β-D-galactopyranoside (7). Compound 7 was obtained following the method used for the preparation of 4 from the bromide 3 and diol 2C. The product was purified by flash chromatography (silica gel, ethyl acetate:hexane 7:3) to yield first the C-3 isomer which was characterized as its 2-O-benzoyl derivative 4 (8%), and 7 (17%), mp 219-23 °C, $[\alpha]_D$+65.6° (c=0.92, chloroform); IR(neat): 3500 cm^{-1}(br, OH), 1780, 1750, 1715 cm^{-1}(CO). Anal. Calcd for $C_{34}H_{34}D_3NO_{15}$ (702.64): C,58.12; H,4.89; N,1.99. Found: C,58.31; H,5.12; N,2.02.

Trideuteriomethyl 3-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-O-acetyl-β-D- galactopyranoside (5B). Compound 5A (155 mg,0.21 mmol) was dissolved in ethanol (6 mL) and hydrazine hydrate (85%,2 mL) and heated at 70 °C for 25 min. After concentration of the reaction mixture in vacuo, the obtained residue was dissolved in ethanol (2x15 mL) and the solvent evaporated. The resulting residue was stirred in acetic anhydride (8 mL) and dry pyridine (4 mL) for 16 h under argon. Crude peracetylated disaccharide 5B was obtained after distilling off the reagents at reduced pressure, last traces being removed by co-distillation with toluene (2x15 mL). The residue yielded after flash chromatography (silica gel; dichloromethane: methanol 25:1) pure 5B (135 mg, 98%); mp 190-95°C,$[\alpha]_D$+20.4°(c=0.84, chloroform); IR(neat): 3280, 3100 cm^{-1}(NH), 1750 cm^{-1}(COO), 1650, 1430 cm^{-1}(CON). Anal. Calcd.

for $C_{27}H_{36}D_3NO_{17}$ (652.58): C,49.70; H,5.66; N,2.15. Found: C,49.61; H,5.84; N,2.22.

Trideuteriomethyl 3-O-(-2-acetamido-2-deoxy-β-D-gluco-pyranosyl)-β-D-galactopyranoside (5C). Compound 5B (109 mg, 0.16 mmol) was dissolved in dry methanol (100 mL), 1 M NaOMe (1.0 mL) was added, and the resulting reaction mixture was stirred under argon for 16 h. After neutralization with Dowex 80W-8X H^+, the resin was filtered off, washed with methanol (150 mL) and the combined filtrates evaporated to dryness to give 5B (62 mg, 96%); mp 85-92 °C, $[\alpha]_D$-4.1° (c=5.4, methanol); IR(neat): 3300 cm^{-1}(br,OH), 1650, 1430 cm^{-1}(CON). Anal. Calcd. for $C_{15}H_{24}D_3NO_{11}$ (400.35): C,45.00; H,6.04; N,3.50. Found: C,45.84, H,6.22; N,3.03.

ACKNOWLEDGEMENTS

This work was in part supported by MRC (Canada) through grants MT-3732 and MA-6499 (to J.P.C.).

REFERENCES AND FOOTNOTES

1. Communication V in this series: Carbohydr. Res., in press.

2. J. P. Carver and J. -R. Brisson in;"Biology of Carbohydrates", Vol.2; V. Ginsburg and P.W. Robbins, Eds; Wiley, New York, N.Y. 1984, p. 289.

3. S. A. Abbas and K. L. Matta, Carbohydr.Res., 123, 53 (1983).

4. A.F. Bochkov and G.E. Zaikov, "Chemistry of the O-Glycosidic Bond", Pergamon Press, Oxford, England, 1979.

5. D. A. Cumming, R. N. Shah, A. A. Grey, J. J. Krepinsky and J. P. Carver, J. Biol. Chem., in press.

6. R. W. Jeanloz and P. J. Stoffyn in; "Methods in Carbohydrate Chemistry", Vol. 1; R. L. Whistler and M. L. Wolfrom, Eds; Academic Press, New York, N.Y. 1962, p. 224.

7. P. J. Garegg and P. Ossowski, Acta Chem. Scand., B37, 249 (1983).

8. E. Sorkin and T. Reichstein, Helv.Chim.Acta, 28, 1 (1945).

9. W. Szeja, Synthesis, 821 (1979).

10. G. J. F. Chittenden and J. G. Buchanan, Carbohydr.Res., 11, 379 (1969).

11. D. M. Whitfield, R. N. Shah, J. P. Carver, and J. J. Krepinsky, Synth.Commun., in press.

12. a. R. U. Lemieux, T. Takeda, and B. Y. Chung, Am.Chem.Soc.Symp.Ser., 39, 90 (1976); b, R. U.Lemieux, S. Z. Abbas, and B. Y. Chung, Can.J.Chem., 60, 109 (1982); c, O. Hindsgaul, T. Norberg, J. LePendu, and R. U. Lemieux, Carbohydr.Res., 109, 109 (1982); purchased from Toronto Research Chemicals, 100 College Street, Toronto, Ont. Canada.

13. B. Andersson, J. Dahmen, T. Frejd, H. Leffler, G. Magnusson, G. Noori, and C. S. Eden, J.Exp.Med., 158, 559 (1983).