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# Synthesis of 1,2,3-tri-*O*-β-lactosyl-D-threitol and 1-*O*-benzyl-2,3,4-tri-*O*-β-lactosyl-D-threitol [1]

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**Abstract**

The coupling of 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-α-lactosyl bromide with 1,4-di-*O*-benzyl-D-threitol using mercury(II) cyanide as a promoter, with subsequent deprotection of one or both of the benzyl groups, further glycosylation, and deacetylation afforded the title compounds. This class of compound is useful in the assessment of binding properties of D-galactopyranose to human and rabbit hepatocytes. © 1998 Elsevier Science Ltd.

*Keywords:* Oligolactosides; Hepatocyte affinity

## 1. Introduction

Cell plasma membranes display arrays of receptors that recognize diverse ligands. The receptors provide portals for receptor-specific manipulations, including the targeting of xenobiotics to particular receptor-bearing cell types. The mammalian hepatocyte plasma membrane expresses the asialoglycoprotein receptor (ASGP-R) [1], a unique integral membrane receptor exhibiting specificity for terminal, nonreducing, β-D-galactopyranosyl or 2-acetamido-2-deoxy-β-D-galactopyranosyl residues. This specific binding to liver cells has been examined with a variety of oligogalactosides [2a,2b,2c,2d] and oligolactosides [3]. Compounds similar to the ones described have also found application as the polar moiety of non-ionic surfactants [4].

An ongoing project in this laboratory has been concerned with the synthesis of molecules bearing terminal D-galactopyranosyl residues. Previous studies have involved the synthesis of compounds having one, two, or three lactose units appended to a glycerol backbone [5a,5b]. Biological evaluation of the binding of the oligolactosides appended to a glycerol backbone demonstrated that, as the number of terminal D-galactose units increased, so did binding [2b,3]. The present work describes the synthesis of analogous compounds, using D-threitol as the backbone. The threitol backbone offers the advantage of optical purity; previous work [5b] with glycerol produced diastereomeric mixtures. Moreover, the extra hydroxyl group offers the possibility of adding a molecule that would be shuttled effectively to the liver.

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[1] Synthesis and binding of D-galactose-terminated ligands to human and rabbit asialoglycoprotein receptor, Part VIII.

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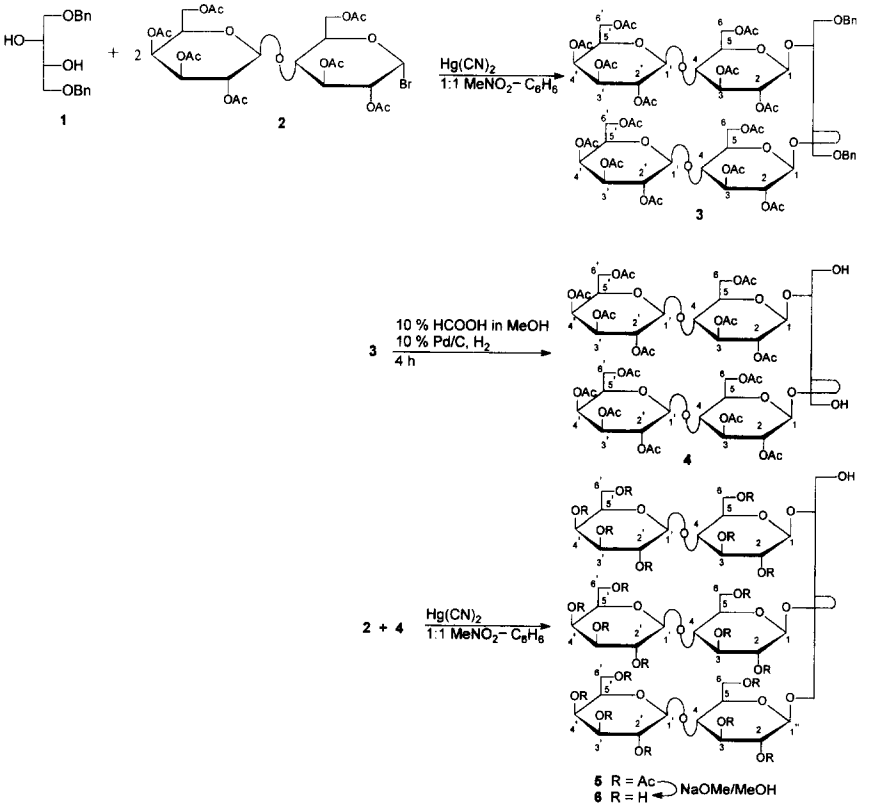
## 2. Results and discussion

The formation of either of the threitol-based oligolactosides **6** or **9** required the protection of the two primary hydroxyls of threitol. It has been demonstrated, on the analogous glycerol backbone, that reaction at the sterically favored primary hydroxyls occurs first, impeding reaction at the secondary hydroxyls [5b]. Thus, 1,4-di-*O*-benzyl-D-threitol (**1**) was synthesized in five steps from readily available D-tartaric acid using well-established reactions [6]. Coupling of 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-α-lactosyl bromide (**2**), prepared in a two-step, one-pot reaction by the method of Kartha and Jennings [7], was achieved using the Helferich modification [8] of the Königs–Knorr reaction.

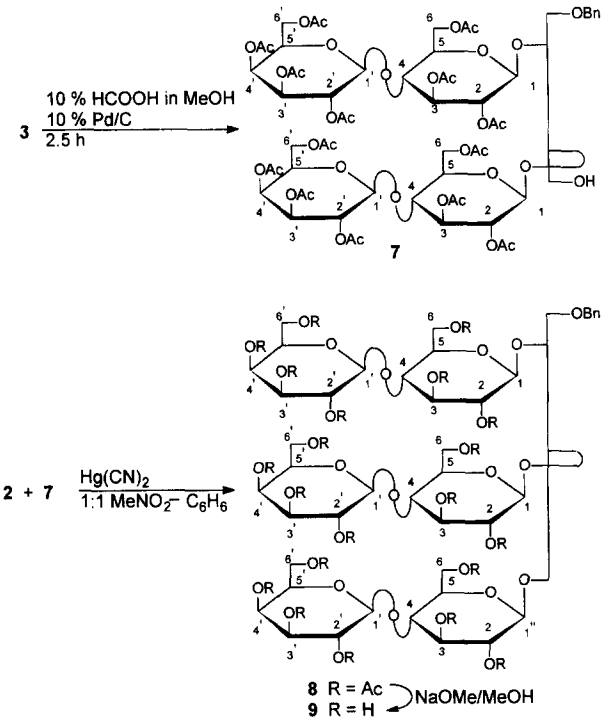
The coupling of **1** (Scheme 1) with **2** proceeded using $Hg(CN)_2$ as a promoter in a 1:1 (v/v) mixture of nitromethane and benzene to afford 1,4-di-*O*-benzyl-2,3-bis-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-D-lactosyl)-D-threitol (**3**), in a 58% yield after purification. Confirmation of the structure of this molecule was achieved with the aid of two-dimensional Fourier-transform, proton chemical-shift spectroscopy (COSY) [9]. The ready assignment of the signals of the anomeric protons revealed that the glycosidic linkage between the lactose units and the threitol unit had the β-D-configuration. However, the detection limit of the NMR experiment would have precluded the identification of trace amounts of material having α-D-linkages.

Catalytic hydrogenolysis of **3** in formic acid–methanol using 10% palladium-on-charcoal, afforded the diol **4** in an 83% yield. An effort to convert **4** into 1,2,3,4-tetra-*O*-β-lactosyl-D-threitol by attempting coupling of the lactosyl bromide **2** using the same conditions previously reported [5b] for the analogous glycerol compounds was unsuccessful. Instead, the trilactoside **5** was isolated. Even after extensive chromatography none of the tetralactoside was isolated. Electrospray-ionization mass spectrometry (ESIMS) unequivocably demonstrated that **5** had indeed been produced. It is possible that the third lactose unit significantly alters the conformation of the substrate in solution, thereby preventing reaction at the remaining hydroxyl group.

The removal of one of two identical benzyl groups can be achieved, albeit in less than spectacular yield, by simply limiting the amount of time that the molecule is subjected to the deprotection conditions. To obtain the optimum yield from such a reaction, it is desirable to be able to monitor the course of the




Scheme 1.




Scheme 2.

reaction easily by TLC, precluding the use of standard hydrogenolysis using hydrogen gas. Cleavage of benzyl ethers by hydrogenolysis can be effected using a variety of sources of hydrogen other than the gas [10]. In the present work, **3** was treated with 10% formic acid in methanol in the presence of 10% palladium-on-charcoal for 2.5 h, at which time TLC indicated that the optimum amount of **7** (Scheme 2) with respect to **4** had been produced. In many attempts, with varying amounts of both formic acid and catalyst, the best yield obtained was 45%. On subjecting **7** to the glycosylation conditions employed for the conversion of **4** into **5**, the desired 1-*O*-benzyl-2,3,4-tris-*O*-(hepta-*O*-acetyl-β-D-lactosyl)-D-threitol (**8**) was obtained, a result which suggests that it was the size of the third lactose unit that prevented incorporation of an additional lactose unit in the attempted synthesis of 1,2,3,4-tetra-*O*-β-D-lactosyl-D-threitol.

Deacetylation of **5** and **8** was achieved under standard conditions using sodium methoxide in methanol [11] to afford the target compounds **6** and **9**, respectively. Biological evaluation of the binding to the ASGP-R of these compounds and some conjugates will be reported separately.

## 3. Experimental

*General methods.*—Melting points were determined using a Mel-Temp II apparatus, and are uncorrected. $^1$H NMR spectra were obtained on a Bruker AM 400 (400 MHz) spectrometer. Proton chemical shifts are given relative to $Me_4Si$ ($\delta$ 0). Chemical-shift and coupling assignments were made with the aid of spin–spin decoupling experiments (COSY, HETCOR). The multiplicities given are those observed within the resolving power of the spectrometer, and are not necessarily true multiplicities. Optical rotations were recorded at room temperature with a Perkin–Elmer model 241 automatic polarimeter. Elemental analyses were performed by M–H–W Laboratories, Pheonix, Arizona. Thin-layer chromatography (TLC) was performed using glass-backed E. Merck Silica Gel 60 F-254 plates, and column chromatography used E. Merck Silica Gel 60. The developed plates were sprayed with 1% ceric sulfate and 1.5% molybdic acid in 10% aqueous sulfuric acid and heated at about 150 °C. Benzene was dried by distilling a solution in the presence of blue sodium benzyl ketyl. Methanol was dried by distilling a solution in the presence of $Mg(OMe)_2$. Nitromethane was dried by stirring in the presence of $CaCl_2$, followed by filtration and fractional distillation. Routine drying during processing of all compounds was accomplished over $MgSO_4$.

*1,4-Di-O-benzyl-2,3-bis-O-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-lactosyl)-*D*-threitol* (**3**).—To a 1:1 (v/v) mixture of nitromethane and benzene (40 mL) were added **1** (1.3 g, 4.30 mmol), **2** (6.99 g, 10.19 mmol), and $Hg(CN)_2$ (3.05 g, 12.1 mmol). The mixture was stirred under argon at 40 °C for 40 h. The mixture was diluted with toluene (60 mL) and washed sequentially with a satd aq solution of $NaHCO_3$ (2 × 35 mL) and $H_2O$ (35 mL). The organic layer was dried, filtered, and the solvent was removed under vacuum. Column chromatography [3:2 (v/v) PhMe–EtOAc] afforded **3** as a white solid (3.56 g, 58%): mp 90–92°C; $[\alpha]_D$ −0.7° (*c* 22, $CHCl_3$); $^1$H-NMR ($CDCl_3$): $\delta$ 1.87 (s, 6 H, 2 OAc), 1.90 (s, 6 H, 2 OAc), 1.95 (s, 18 H, 6 OAc), 2.02 (s, 6 H, 2 OAc), 2.14 (s, 6 H, 2 OAc), 3.45 (m, 2 H, 2 threityl H), 3.55 (m, 2 H, 2 threityl H), 3.61 (m, 2 H, 2 threityl H), 3.79 (t, 2 H, $J_{5,6}$ 8.6 Hz, H-5,5), 3.82 (br t, 2 H, H-5′,5′), 3.95–4.15 [m, 8 H, H-6(4), 6′(4)], 4.50 (d, 4 H, *J* 7.9 Hz, 2 C$H_2$Ph), 4.52 (d, 2 H, $J_{1',2'}$ 8.1 Hz, H-1′,1′), 4.61 (br d, 2 H, H-4,4), 4.81 (d, 2 H, $J_{1,2}$ 8.0 Hz, H-1,1), 4.83 (t, 2 H, $J_{2,3}$ 8.2 Hz, H-2,2), 4.95 (dd, 2 H, $J_{3',2'}$ 8.9,

$J_{3',4'}$ 3.4 Hz, H-3',3'), 5.11 (t, 2 H, $J_{2',1'}$ 8.0 Hz, H-2',2'), 5.20 (t, 2 H, $J_{3,2}$ 8.0 Hz, H-3,3), 5.34 (d, 2 H, $J_{4',3'}$ 3.4 Hz, H-4',4'), 7.29 (m, 10 H, 2 Ph). Anal. Calcd for $C_{70}H_{90}O_{38}$: C, 54.61; H, 5.89. Found: C, 54.86; H, 5.97.

*2,3-Bis-*O*-(2,3,6,2',3',4',6'-hepta-*O*-acetyl-β-lactosyl)-*D*-threitol* (**4**).—To a solution of **3** (3.0 g, 1.95 mmol) in MeOH (6 mL) containing 10% formic acid was added 10% palladium-on-charcoal (200 mg) (Caution: Extreme fire hazard!). The mixture was subjected to a hydrogen atmosphere (50 psig) for 4 h, the catalyst was removed by filtration through Celite, and the Celite was washed with MeOH. The solvent was removed and the residue was chromatographed [1:1 (v/v) EtOAc–hexane] to afford **4** as a white solid (2.2 g, 83%): mp 127–130 °C; $[\alpha]_D$ −1.3° (*c* 17, $CHCl_3$); $^1$H NMR ($CDCl_3$): δ 1.96 (s, 6 H, 2 OAc), 2.03–2.07 (4 s, 24 H, 8 OAc), 2.15 (s, 6 H, 2 OAc), 2.16 (s, 6 H, 2 OAc), 2.70 (br t, 2 H, *J* 6.1 Hz, 2 OH), 3.49 3.75 (m, 8 H, 6 threityl H, H-6',6'), 3.88 (m, 4 H, H-5',5',6',6'), 3.98 (m, 2 H, H-5,5), 4.09 [m, 4 H, H-6(4)], 4.51 (d, 2 H, $J_{1'',2'}$ 7.9 Hz, H-1''), 4.59 (d, 2 H, $J_{1,2}$ 8.1 Hz, H-1,1), 4.65 (br d, 2 H, H-4,4), 4.85 (t, 2 H, $J_{2,1}$ 8.1 Hz, H-2,2), 4.98 (dd, 2 H, $J_{3',2'}$ 10.4, $J_{3',4'}$ 3.4 Hz, H-3',3'), 5.10 (dd, 2 H, $J_{2',3'}$ 8.0, $J_{2',1'}$ 7.8 Hz, H-2',2'), 5.18 (t, 2 H, $J_{3,2}$ 8.9 Hz, H-3,3), 5.34 (d, 2 H, $J_{4',3'}$ 3.4 Hz, H-4',4'). Anal. Calcd for $C_{56}H_{78}O_{38}$: C, 49.50; H, 5.79. Found: C, 49.90; H, 5.43.

*1,2,3-Tris-*O*-(2,3,6,2',3',4',6'-hepta-*O*-acetyl-β-*D*-lactosyl)-*D*-threitol* (**5**).—A mixture of **2** (503 mg, 0.72 mmol), **4** (250 mg, 0.18 mmol), and $Hg(CN)_2$ (182 mg, 0.72 mmol) in a 1:1 (v/v) mixture of benzene and nitromethane (20 mL) was maintained at 40 °C under argon for 36 h and processed in the usual way. Purification was achieved by column chromatography using 3:1 (v/v) EtOAc–PhMe as eluent to give the product as a white solid (100 mg, 28%): mp 88–90 °C; $[\alpha]_D$ +3.02° (*c* 1.5, $CH_2Cl_2$); $^1$H NMR ($CDCl_3$): δ 1.8–2.2 (multiple s's, 63 H, 21 OAc), 2.65 (br t, 1 H, OH), 3.50 (m, 6 H, 6 threityl H), 3.58–3.91 [m, 8 H, H-5(3), 5'(3), 6'(2)], 3.95–4.15 [m, 10 H, H-6(6), 6'(4)], 4.37 (d, 1 H, $J_{1'',2}$ 7.9 Hz, H-1''), 4.46 (d, 2 H, $J_{1,2}$ 8.15 Hz, H-1,1), 4.52 (t, 3 H, $J_{1',2'}$ 8.43 Hz, H-1',1',1'), 4.62 (m, 3 H, H-4,4,4), 4.75 (m, 3 H, H-2,2,2), 4.93 (m, 3 H, H-3',3',3'), 5.07 (m, 3 H, H-2',2',2'), 5.17 (m, 3 H, H-3,3,3), 5.31 (br s, 3 H, H-4',4',4'); ESIMS (positive-ion): Expected for $C_{82}H_{112}O_{55}$ $[M + Na]^+$: 2000.2. Found: 1999.7. Anal. Calcd for $C_{82}H_{112}O_{55}$: C, 49.81; H, 5.71. Found: C, 49.74; H, 5.60.

*1,2,3-Tris-*O*-β-lactosyl-*D*-threitol* (**6**).—To a solution of **5** (80 mg, 40 μmol) in anhydrous MeOH was added a trace of sodium metal. The mixture was stirred under argon for 2 h. Amberlite 120-IR cation-exchange resin ($H^+$) was added, and stirring was continued a further 30 min. The resin was removed, and the product was purified on Sephadex LH-20 using 1:1 (v/v) $CHCl_3$–MeOH as eluent to afford **6** as a white solid (35 mg, 80%): mp 190–192 °C; $[\alpha]_D$ +12.31° (*c* 1.3, $H_2O$); $^1$H-NMR ($D_2O$): δ 3.20 (m, 3 H, CHO and/or C$H_2$OH), 3.41–4.52 (m, 45 H, CHO and/or C$H_2$OH); ESIMS (positive-ion): Expected for $C_{40}H_{70}O_{34}$ $[M + Na]^+$: 1117.3. Found: 1116.8. Anal. Calcd for $C_{40}H_{70}O_{34}$: C, 43.89; H, 6.45. Found: C, 43.65; H, 6.70.

*1-*O*-Benzyl-2,3-bis-*O*-(2,3,6,2',3',4',6'-hepta-*O*-acetyl-β-lactosyl)-*D*-threitol* (**7**).—To a solution of **3** (7.1 g, 4.6 mmol) in 10% formic acid in MeOH (76 mL) was added 10% palladium-on-charcoal (1.5 g). The mixture was stirred vigorously for 2.5 h and then filtered through a bed of Celite; the Celite was washed with MeOH, and the solvent was removed under vacuum. Column chromatography using 2:3 (v/v) PhMe–EtOAc as eluent afforded **7** as a white solid (3.0 g, 45%): mp 115–118 °C; $[\alpha]_D$ −5.47° (*c* 12, $CHCl_3$); $^1$H NMR ($CDCl_3$): δ 1.96 (s, 6 H, 2 OAc), 2.03–2.07 (4 s, 24 H, 8 OAc), 2.15 (s, 6 H, 2 OAc), 2.16 (s, 6 H, 2 OAc), 2.70 (br t, 1 H, *J* 6.0 Hz, OH), 3.52 (m, 6 H, 6 threityl H), 3.72 (m, 2 H, H-6,6), 3.74 (m, 2 H, H-5',5'), 3.9–4.18 [m, 8 H, H-5(2), 6(2), 6'(4)], 4.51 (s, 2 H, C$H_2$Ph), 4.54 (d, 2 H, $J_{1',2'}$ 8.1 Hz, H-1', 1'), 4.57 (d, 1 H, $J_{4,3}$ 7.7 Hz, H-4), 4.68 (d, 1 H, $J_{4,3}$ 7.1 Hz, H-4), 4.82 (m, 2 H, H-2,2), 4.98 (m, 2 H, H-3',3'), 5.13 (br t, 2 H, $J_{2',3'}$ 8.9 Hz, H-2',2'), 5.18 (m, 2 H, H-3,3), 5.31 (br s, 2 H, H-4',4'), 7.30 (m, 5 H, Ph). Anal. Calcd for $C_{63}H_{84}O_{38} \cdot H_2O$: C, 51.58; H, 5.91. Found: C, 51.55; H, 6.35.

*1-*O*-Benzyl-2,3,4-tris-*O*-(2,3,6,2',3',4',6'-hepta-*O*-acetyl-β-lactosyl)-*D*-threitol* (**8**).—A mixture of **2** (0.99 g, 1.4 mmol), **7** (1.37 g, 0.94 mmol), and $Hg(CN)_2$ (0.389 g, 1.52 mmol) in a 1:1 (v/v) mixture of benzene and nitromethane (40 mL) was maintained at 40 °C under argon for 36 h and processed in the usual way. Purification was achieved by column chromatography using 2:1 (v/v) EtOAc–PhMe as eluent to give the product as a glassy solid (842 mg, 43%): mp 119–120 °C; $[\alpha]_D$ +6.74° (*c* 0.46, $CH_2Cl_2$); $^1$H NMR ($CDCl_3$): δ 1.9–2.2 (multiple s's, 63 H, 21 OAc), 3.45–3.60 (m, 6 H, 6 threityl H), 3.77 (br t, 3 H, $J_{5,6}$ 9.4 Hz, H-5,5,5), 3.84 (br t, 3 H,

$J_{5',6'}$ 6.8 Hz, H-5',5',5'), 4.0–4.15 [m, 12 H, H-6(6), 6'(6)], 4.34 (d, 1 H, $J_{1'',2}$ 8.2 Hz, H-1''), 4.40–4.54 [m, 7 H, H-1(2), H-1'(3), C$H_2$Ph], 4.68–4.81 [m, 6 H, H-2(3), 4(3)], 4.95–4.99 (m, 3 H, H-3',3',3'), 5.08 (br t, 3 H, H-2',2',2'), 5.16 (m, 3 H, H-3,3,3), 5.34 (br s, 3 H, H-4',4',4'), 7.31 (m, 5 H, Ph); ESIMS (positive-ion): Expected for $C_{89}H_{118}O_{55}$ $[M + Na]^+$: 2089.6. Found: 2089.5. Anal. Calcd for $C_{89}H_{118}O_{55}$: C, 51.68; H, 5.75. Found: C, 51.89; H, 6.26.

*1-O-Benzyl-2,3,4-tris-O-β-lactosyl-D-threitol* (**9**).—To a solution of **8** (100 mg, 0.05 mmol) in anhydrous MeOH was added a trace of sodium metal. The mixture was stirred under argon for 2 h. Amberlite IR-120($H^+$) cation exchange resin was added, and stirring was continued a further 30 min. The resin was removed, and the product was purified on Sephadex LH-20 using 1:1 (v/v) $CHCl_3$–MeOH as eluent to afford **9** as a white solid (46 mg, 78%): mp 176–177 °C; $[\alpha]_D$ +2.29° (*c* 1.75, $H_2O$); $^1H$ NMR ($D_2O$): δ 3.1–4.5 (m, 48 H, CHO and/or C$H_2$OH), 7.35 (m, 5 H, Ph); ESIMS (positive-ion): Expected for $C_{47}H_{76}O_{34}$ $[M + Na]^+$: 1207.4. Found: 1206.7. Anal. Calcd for $C_{47}H_{76}O_{34} \cdot 6H_2O$: C, 43.67; H, 6.86. Found: C, 43.94; H, 6.83.

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