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Synthesis of a biologically active isomer of kotalanol, a naturally occurring glucosidase inhibitor

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ABSTRACT

The syntheses of an isomer of kotalanol, a naturally occurring glucosidase inhibitor, and of kotalanol itself are described. The target compounds were synthesized by nucleophilic attack of PMB-protected 1,4-anhydro-4-thio-D-arabinitol at the least hindered carbon atom of two 1,3-cyclic sulfates, which were synthesized from D-mannose. Methoxymethyl ether and isopropylidene were chosen as protecting groups. The latter group was critical to ensure the facile deprotection of the coupled products in a one-step sequence to yield kotalanol and its isomer. The stereoisomer of kotalanol, with the opposite stereochemistry at the C-6′ stereogenic centre, inhibited the N-terminal catalytic domain of intestinal human maltase glucoamylase (ntMGAM) with a K_i value of 0.20 ± 0.02 μM; this compares to a K_i value for kotalanol of 0.19 ± 0.03 μM. The results indicate that the configuration at C-6′ is inconsequential for inhibitory activity against this enzyme.

1. Introduction

Glycosidases are enzymes that are involved in the catabolism of glycoproteins and glycoconjugates and the biosynthesis of oligosaccharides. Disruption in regulation of glycosidases can lead to diseases.[1,2] Over the years, glycosidase inhibitors have received considerable attention in the field of chemical and medicinal research[3] because of their effects on quality control, maturation, transport, secretion of glycoproteins, and cell–cell or cell–virus recognition processes. This principle has potential for many therapeutic applications, such as in the treatment of diabetes, cancer and viral infections.[1]

Bioactive components isolated from medicinal plants that are used in traditional medicine or folk medicine often provide the lead structures for modern drug-discovery programs. For example, the large woody climbing plant *Salacia reticulata*, known as Kothalahimbutu in Singhalese, is used in traditional medicine in Sri Lanka and Southern India for treatment of type 2 diabetes.[4,5] A person suffering from diabetes was advised to drink water stored overnight in a mug carved from Kothalahimbutu wood.[6] Several potent glucosidase inhibitors have been isolated from the water soluble fraction of this plant extract and also other plants that belong to the *Salacia* genus such as *Salacia chinensis*, *Salacia prinoides*, and *Salacia oblonga* which explain, at least in part, the antidiabetic property of the aqueous extract of this plant.[7–9] All these compounds share a common structural motif that comprises a 1,4-anhydro-4-thio-D-arabinitol and a polyhydroxylated side chain. So far, five components have been isolated, namely salaprinol (**1**),[9] salacinol (**2**),[7] ponkoranol (**3**),[9] kotalanol (**4**),[8] and de-O-sulfonated kotalanol (**5**)[10] (Fig. 1). The absolute stereostructure for these compounds, except salacinol, was not determined at the time of isolation, but synthetic work has led to their stereochemical structure elucidation.[11,12]

The synthesis of kotalanol **4** and its stereoisomer **6** (Fig. 2) are of interest here.

Our first attempt employed the reaction of the cyclic sulfates **8** in the coupling reaction (Scheme 1).[12] However, attempts to remove the methylene acetal in the coupled products required forcing conditions and resulted in de-O-sulfonation (Scheme 1).[12] We have also reported a successful synthesis of kotalanol using a cyclic sulfate derived from a naturally occurring heptitol, D-perseitol (Scheme 2).[12]

However, it was of interest to develop a synthesis of the isomer of kotalanol **6** in view of the fact that the C-6′ stereoisomer of de-O-sulfonated kotalanol was just as active an inhibitor as de-O-sulfo-

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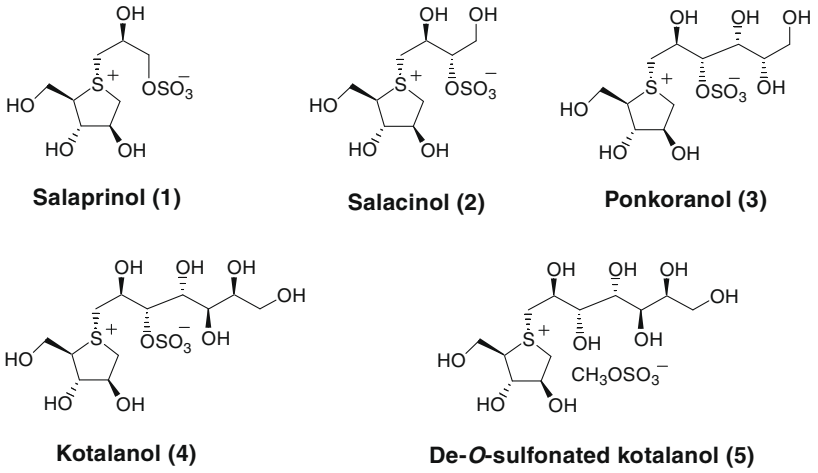


Figure 1. Components isolated from *Salacia* species.

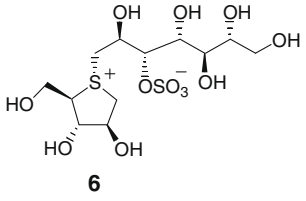


Figure 2. Kotalanol stereoisomer.

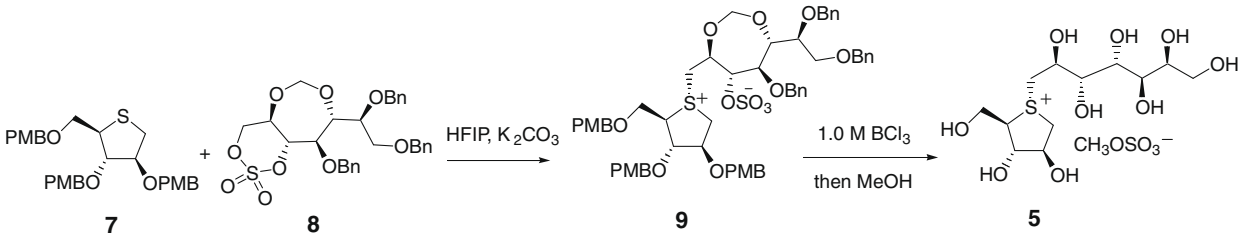
nated kotalanol **5** itself against a key intestinal enzyme, human maltase glucoamylase.[13] We report here a general synthetic route to this isomer **6** and also an alternative synthesis of kotalanol **4**.

2. Results and discussion

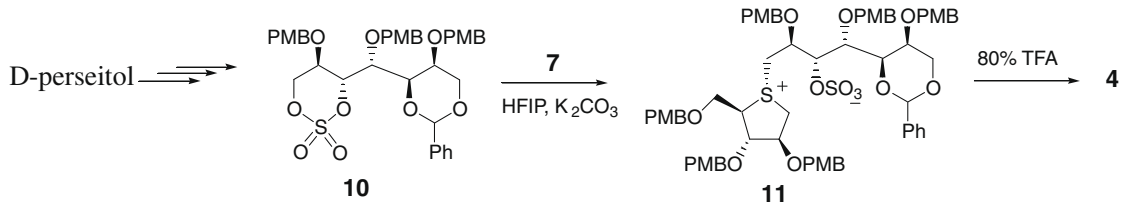
We chose to replace the problematic methylene acetal group of compounds **8** with an isopropylidene acetal (compound **12**) to ensure not only its facile removal after the coupling reaction but also to maintain some rigidity in the cyclic sulfate. We chose also to replace the benzyl ethers with methoxymethyl (MOM) ethers, because the latter can survive the hydrogenolysis conditions required for removal of the benzylidene acetal. The cyclic sulfate **12** could be synthesized from D-mannitol as shown in the retrosynthetic analysis (Scheme 3).

Thus, the D-mannitol-derived diol **15**,[14] was protected as the acetonide to give the C_2-symmetric compound **14** in 73% yield. Mild hydrolysis of this compound using catalytic PTSA in methanol effected the removal of one benzylidene group to give the corresponding diol in 70% yield based on recovered starting material. Selective protection of the primary hydroxyl group as its TBDMS ether followed by sequential protection of the secondary hydroxyl group as its MOM ether and removal of the TBDMS group with tetrabutylammonium fluoride (TBAF) gave **17** in 73% yield over three steps. Treatment of this alcohol with Dess–Martin periodinane provided the aldehyde which was reacted with methyltriphenylphosphonium bromide to yield the olefinic product **13** in 61% yield over two steps (Scheme 4).

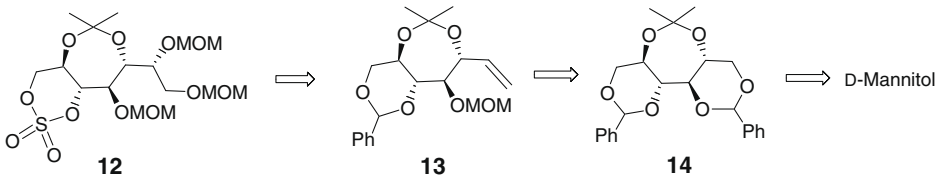
With compound **13** in hand, our next goal was to introduce the two hydroxyl groups. OsO_4-Catalyzed dihydroxylation of **13** afforded compound **18** (Scheme 4) as the major product with a diastereomeric ratio of **18**:**19** of 2.6:1. Kishi's rule predicts that the relative stereochemistry between the pre-existing hydroxyl group and the adjacent newly-introduced hydroxyl group in the major product should be erythro.[15] This result is also analogous to that



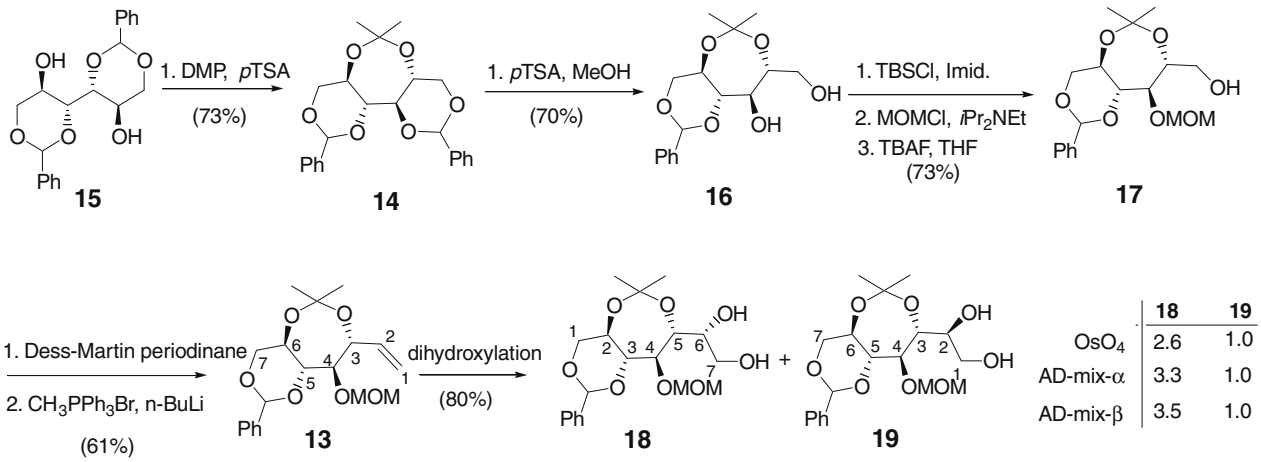
Scheme 1. First attempted synthesis of kotalanol.



Scheme 2. Synthesis of kotalanol **4**.



Scheme 3. Retrosynthetic analysis.



Scheme 4. Synthesis of the diols **18** and **19**.

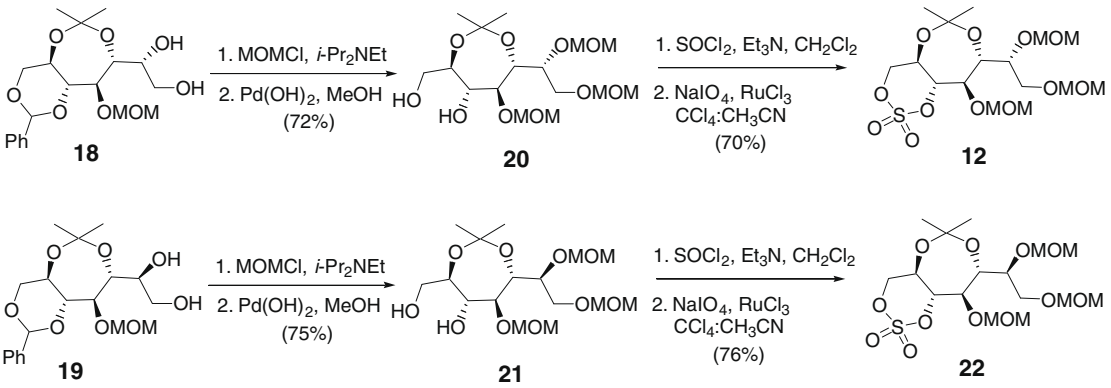
obtained for dihydroxylation of a corresponding methylene acetal.[12]

Interestingly, AD-mix-α and AD-mix-β also afforded compound **18** as a major product, with a diastereomeric ratio of 3.3:1 and 3.5:1 (determined by 600 MHz ^{1}H NMR), respectively. The unsatisfactory selectivity can be explained by the steric hindrance imposed by the bicyclic structure, observed previously with a similar structure.[16] The two isomers were separated by column chromatography and each was converted into its cyclic sulfate **12** or **22** as follows. The hydroxyls in **18** were protected with MOM groups and the product was subjected to hydrogenolysis to effect removal of the benzylidene group and to yield the corresponding diol **20** in 72% yield over two steps. The cyclic sulfate **12** was then obtained by treatment of **20** with thionyl chloride in the presence of triethylamine to give the mixture of diastereomeric sulfites, followed by their oxidation with sodium periodate and ruthenium(III) chloride as a catalyst. A similar sequence of reactions with the diol **19** yielded the cyclic sulfate **22** (Scheme 5).

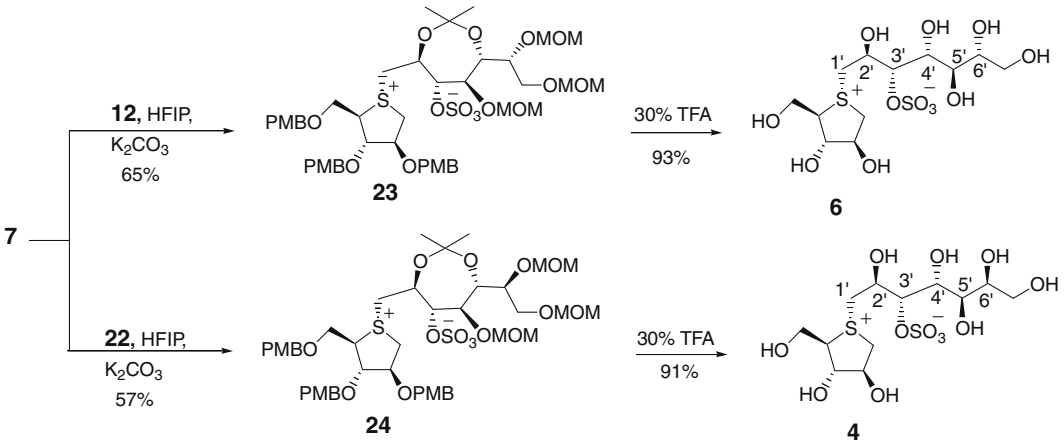
The target compounds were prepared by opening of the cyclic sulfates **12** and **22** by nucleophilic attack of the sulfur atom in 2,3,5-tri-*O*-*p*-methoxybenzyl-1,4-anhydro-4-thio-D-arabinitol **7**.[11] Reactions were carried out at 72 °C in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) containing K_2CO_3[17] for six days to give the sulfonium salts **23** and **24** in 65% and 57% yield, respectively. Finally, deprotection of the coupled products **24** and **23** using aqueous 30% trifluoroacetic acid (TFA) at 50 °C gave the desired compounds **4** and **6** in 91% and 93% yields, respectively (Scheme 6).

Comparison of the ^{1}H and ^{13}C NMR spectra of kotalanol **4** with those reported[13] revealed identical data and served, therefore, to confirm the stereochemistry at C-6′, and, by inference, the stereochemistry at C-2 in each of **18** and **19**.

Finally, the inhibitory activities of compounds **4** and **6** were examined against the N-terminal catalytic domain of recombinant human maltase glucoamylase (ntMGAM), a critical intestinal glucosidase for processing starch-derived oligosaccharides into glucose. The stereoisomer of kotalanol **4** inhibited ntMGAM with a



Scheme 5. Synthesis of the cyclic sulfates **12** and **22**.



Scheme 6. Coupling reactions.

K_i value of 0.20 ± 0.02 μM; this compares to a K_i value for kotalanol of 0.19 ± 0.03 μM,[18] and K_i values of 0.10 ± 0.02 μM and 0.13 ± 0.02 μM for other stereoisomers of **4** with opposite configurations at C-5′ or both C-5′ and C-6′, respectively.[16] It is clear, therefore, that the configurations at C-5′ and C-6′ are not critical for dictating enzyme inhibitory activity against ntMGA.

3. Experimental section

3.1. General

Optical rotations were measured at 23 °C. ^{1}H and ^{13}C NMR spectra were recorded at 600 and 150 MHz, respectively. All assignments were confirmed with the aid of two-dimensional ^{1}H, ^{1}H (COSYDFTP) or ^{1}H, ^{13}C (INVBTP) experiments using standard pulse programs. Column chromatography was performed with Silica 60 (230–400 mesh). High resolution mass spectra were obtained by the electrospray ionization method, using an Agilent 6210 TOF LC/MS high resolution magnetic sector mass spectrometer.

3.2. Enzyme inhibition assays

Compounds **4** and **6** were tested for inhibition of ntMGAM, as previously described.[16]

3.3. 1,3,4,6-Di-*O*-benzylidene-2,5-*O*-isopropylidene-D-mannitol (14)

Compound **15** (9.30 g, 26.00 mmol) was dissolved in 2,2-dimethoxypropane (150 mL), PTSA (1.50 g, 0.3 equiv) was added, and the mixture was rotated on a rotary evaporator at room temperature under reduced pressure for 1 h. The reaction mixture was quenched by addition of Et_3N to pH >9. The reaction mixture was concentrated under vacuum to give a white solid which was dissolved in $CHCl_3$ (200 mL) and washed with water (3 × 50 mL). The separated organic layer was dried over Na_2SO_4, concentrated, and the residue was purified by column chromatography with EtOAc/hexanes (1:4) as eluent to afford **14** as a white solid (7.55 g, 73%). Mp 160–162 °C; $[\alpha]_D^{23} = -83$ (*c* 1.1, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 7.54–7.37 (10H, m, Ar), 5.54 (2H, s, 2C*H*–Ph), 4.24 (2H, dd, $J_{1a,1b}$ = 10.8, $J_{2,1}$ = 5.3 Hz, H-1), 3.95–3.91 (2H, m, H-6a, H-5), 3.84–3.80 (2H, m, H-3, H-4), 3.74 (2H, t, $J_{1,2} = J_{2,3} = J_{5,6b} = J_{6a,6b}$ = 10.5, H-2, H-6b), 1.42 (6H, s, 2Me). ^{13}C NMR ($CDCl_3$) δ 137.5 (*C*Me_2), 129.9–126.2 (m, Ar), 100.7 (*C*H–Ph), 82.2 (C-3, C-4), 69.4 (C-1, C-6), 61.7 (C-2, C-5), 24.4 (2Me). HRMS Calcd for $C_{23}H_{27}O_6$ (M+H): 399.1802. Found: 399.1809.

3.4. 1,3-*O*-Benzylidene-2,5-*O*-isopropylidene-D-mannitol (16)

To a solution of compound **14** (7.50 g, 18.84 mmol) in MeOH (300 mL), was added PTSA (300 mg), and the reaction was stirred at room temperature for 30 min. The reaction mixture was then quenched by addition of Et_3N to pH >9, and the solvent were removed under vacuum to give a solid. The solid was dissolved in CH_2Cl_2 (100 mL) and washed with water (50 mL). The organic solution was dried (Na_2SO_4), concentrated, and the crude product was purified through a silica column with EtOAc/hexanes (1:1) as eluent to yield **16** as a foam (4.1 g, 70%). $[\alpha]_D^{23} = -15$ (*c* 1, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 7.42–7.30 (5H, m, Ar), 5.40 (1H, s, C*H*–Ph), 4.12 (1H, dd, $J_{1a,1b}$ = 10.8, $J_{1a,2}$ = 5.5 Hz, H-1a), 3.81 (1H, dd, $J_{6a,6b}$ = 10.9, $J_{6a,5}$ = 4.3 Hz, H-6a), 3.76–3.72 (2H, m, H-3, H-5), 3.66 (1H, m, H-6b), 3.60–3.53 (2H, m, H-4, H-1b), 3.43 (1H, t, $J_{1,2}$ = 8.9 Hz, H-2), 2.23 (2H, br, 2OH), 1.30 (6H, s, 2Me). ^{13}C NMR ($CDCl_3$) δ 137.3 (*C*Me_2), 129.3- 101.7 (m, Ar), 101.1 (*C*H–Ph), 85.2 (C-2), 73.9 (C-4), 70.3 (C-5), 69.3 (C-1), 63.6 (C-6), 61.2 (C-3), 24.8, 24.6 (2Me). HRMS Calcd for $C_{16}H_{23}O_6$ (M+H): 311.1489. Found: 311.1487.

3.5. 1,3-*O*-Benzylidene-2,5-*O*-isopropylidene-4-*O*-methoxymethyl-D-mannitol (17)

To a solution of **16** (6.80 g, 21.93 mmol) in DMF (125 mL) was added imidazole (4.47 g, 65.81 mmol). The reaction was cooled in an ice bath, TBDMSCl (3.79 g, 24.13 mmol) was added portionwise, and the mixture was stirred at 0 °C under N_2 for 2 h. The reaction was quenched by the addition of ice-water, and the reaction mixture was extracted with Et_2O (3 × 75 mL). The combined organic solvents were dried (Na_2SO_4) and concentrated to give the crude product which was used directly in the next step without further purification. The crude product was dissolved in DMF (60 mL), and *i*-Pr_2NEt (26 mL, 150.75 mmol) and MOMCl (5.7 mL, 75.38 mmol) were added. The reaction mixture was heated at 60 °C overnight, then quenched with ice, and extracted with ether (3 × 50 mL). The organic solution was dried (Na_2SO_4) and concentrated to give a crude product. The crude residue was dissolved in THF (100 mL), TBAF (1.0 M solution in THF, 13.8 mL, 24.12 mmol) was added, and the reaction mixture was stirred at room temperature. After 4 h it was concentrated and the residue was purified by flash chromatography (EtOAc/hexanes (1:3)) to yield **17** as a white

solid (5.67 g, 73%). Mp 65–67 °C; $[\alpha]_D^{23}$ = +22 (*c* 1, MeOH). ^{1}H NMR ($CDCl_3$) δ 7.49–6.37 (5H, m, Ar), 5.50 (1H, s, C*H*–Ph), 4.93, 4.73 (2H, 2d, $J_{A,B}$ = 6.4 Hz, CH_2OMe), 4.20 (1H, dd, $J_{1a,1b}$ = 10.9, $J_{1a,2}$ = 5.5 Hz, H-1a), 3.89–3.79 (4H, m, H-2, H-5, H-6a,b), 3.74 (1H, t, $J_{3,4}$ = $J_{5,4}$ = 8.1 Hz, H-4), 3.69–3.65 (2H, m, H-1b, H-3), 3.40 (3H, s, OMe), 2.69 (1H, t, $J_{6,OH}$ = 8.5 Hz, OH), 1.41, 1.38 (6H, 2s, 2Me). ^{13}C NMR ($CDCl_3$) δ 137.5 (*C*Me_2), 128.9–101.5 (m, Ar), 100.9 (*C*H–Ph), 98.6 (*C*H_2–OMe) 85.3 (C-3), 78.2 (C-4), 70.4 (C-5), 69.5 (C-1), 63.1 (C-6), 61.3 (C-2), 56.4 (OMe), 24.7, 24.4 (2Me). HRMS Calcd for $C_{18}H_{27}O_7$ (M+H): 355.1751. Found: 355.1741.

3.6. 5,7-*O*-Benzylidene-1,2-dideoxy-3,6-*O*-isopropylidene-4-*O*-methoxymethyl-D-manno-hep-1-enitol (13)

Compound **17** (2.60 g, 7.34 mmol) was dissolved in CH_2Cl_2 (50 mL) and $NaHCO_3$ (2.77 g, 33.03 mmol) and Dess–Martin periodinane (3.73 g, 8.81 mmol) were added. The reaction mixture was stirred for 2 h at room temprature, diluted with ether (100 mL), and poured into saturated aqueous $NaHCO_3$ (100 mL) containing a seven fold excess of $Na_2S_2O_3$. The mixture was stirred to dissolve the solid, and the ether layer was separated and dried over Na_2SO_4. The ether was removed to give the aldehyde that was further dried under high vacuum for 1 h. Methyltriphenylphosphonium bromide (2.99 g, 8.80 mmol) in dry THF (15 mL), was cooled to −78 °C and *n*-BuLi (*n*-hexane solution, 14.67 mmol) was added dropwise under N_2. The reaction mixture was stirred at the same temperature for 1 h, and a solution of the previously made aldehyde in THF (10 mL) was added. The resulting mixture was allowed to warm to rt and was stirred overnight. The reaction was quenched by the addition of acetone (1.5 mL), and the mixture was extracted with ether (3 × 100 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated in vacuo. Chromatographic purification of the crude product (EtOAc/hexanes (1:10)) gave **13** as a foam (1.56 g, 61%). $[\alpha]_D^{23}$ = +4 (*c* 0.5, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 7.50–7.36 (5H, m, Ar), 6.05 (1H, ddd, $J_{5,6}$ = 6.1, $J_{6,7b}$ = 10.5, $J_{6,7a}$ = 16.6 Hz, H-6), 5.51 (1H, s, C*H*–Ph), 5.39 (1H, ddd, $J_{7b,7a}$ = 17.1, $J_{6,7a}$ = 3.3, $J_{5,7a}$ = 1.5 Hz, H-7a), 5.36 (1H, ddd, $J_{7a,7b}$ = 10.7, $J_{6,7b}$ = 3.1, $J_{5,7b}$ = 1.5 Hz, H-7b), 5.27, 5.26 (2H, 2d, $J_{A,B}$ = 6.25 Hz, CH_2OMe), 4.25 (1H, m, H-5), 4.20 (1H, dd, $J_{1a,1b}$ = 10.8, $J_{1a,2}$ = 5.4 Hz, H-1a), 3.90 (1H, dt, $J_{2,3}$ = 5.4, $J_{2,1}$ = 9.9 Hz, H-2), 3.68 (2H, m, H-3, H-1b), 3.56 (1H, dd, $J_{3,4}$ = 8.1, $J_{4,5}$ = 9.7 Hz, H-4), 3.33 (3H, s, OMe), 1.40, 1.37 (6H, 2s, 2Me). ^{13}C NMR ($CDCl_3$) δ 137.6 (*C*Me_2), 136.2 (C-6), 128.9–101.3 (m, Ar), 116.8 (C-7), 100.7 (*C*H–Ph), 97.9 (*C*H_2OMe), 85.5 (C-3), 80.2 (C-4), 71.1 (C-5), 69.6 (C-1), 61.4 (C-2), 56.4 (OMe), 24.8,24.1 (2Me). HRMS Calcd for $C_{19}H_{26}NaO_6$ (M+Na): 373.1622. Found: 373.1606.

3.7. 1,3-*O*-Benzylidene-2,5-*O*-isopropylidene-4-*O*-methoxymethyl-D-glycero-D-manno-heptitol (18)

To a solution of **13** (2.00 g, 5.71 mmol) in acetone/water (9:1, 6 mL) at rt were added NMO (*N*-methylmorpholine-*N*-oxide) (735 mg, 6.28 mmol) and OsO_4 (40 mg, 2.5 wt % solution in 2-methyl-2-propanol). The reaction mixture was stirred at rt for 48 h before it was quenched with a saturated solution of $NaHSO_3$. After being stirred for an additional 15 min the reaction mixture was extracted with ethyl acetate and the organic layer was washed with water and brine, dried (Na_2SO_4), and concentrated in vacuo. The crude material was purified by column chromatography on silica gel (MeOH/CH_2Cl_2 (1:100)) to give **18** (1.27 g, 58%) and **19** (0.48 g, 22%) as foams. $[\alpha]_D^{23}$ = +5.8 (*c* 4.6, MeOH). ^{1}H NMR (MeOD) δ 7.49–7.36 (5H, m, Ar), 5.54 (1H, s, C*H*–Ph), 4.82 (1H, s, CH_2OMe), 4.13 (1H, dd, br, H-1a), 4.00 (1H, br, q, H-6), 3.87–3.77 (3H, m, H-4, H-5, H-2), 3.68–3.55 (4H, H-1b, H-3, H-7a, H-7b), 3.32 (3H, s, OMe), 1.39, 1.34 (6H, 2s, 2Me). ^{13}C NMR (MeOD) δ 138.0 (*C*Me_2), 128.4–101.1 (m, Ar), 100.8 (*C*H–Ph), 97.7 (*C*H_2OMe), 85.3 (C-4), 77.1 (C-2), 69.2 (C-6), 69.1 (C-5), 69.0 (C-1), 62.3 (C-7), 61.1 (C-3), 55.3 (OMe), 23.5, 23.4 (2Me). HRMS Calcd for $C_{19}H_{29}O_8$ (M+H): 385.1857. Found: 385.1875.

3.8. 5,7-*O*-Benzylidene-3,6-*O*-isopropylidene-4-*O*-methoxymethyl-D-glycero-D-galacto-heptitol (19)

$[\alpha]_D^{23}$ = −20 (*c* 0.1, MeOH). ^{1}H NMR (MeOD) δ 7.48–7.34 (5H, m, Ar), 5.51(1H, s, C*H*–Ph), 4.49, 4.47 (2H, 2d, $J_{A,B}$ = 6.2 Hz, CH_2OMe), 4.13 (1H, dd, $J_{7a,7b}$ = 10.7, $J_{6,7b}$ = 5.4 Hz, H-7a), 4.08 (1H, m, H-2), 3.95 (1H, dd, $J_{3,4}$ = 9.7, $J_{5,4}$ = 2.8 Hz, H-4), 3.85 (1H, dd, $J_{1a,1b}$ = 11.4, $J_{2,1a}$ = 3.6 Hz, H-1a), 3.78 (1H, dt, $J_{6,7}$ = 9.9, $J_{5,6}$ = 5.4 Hz, H-6), 3.67–3.60 (4H, m, H-5, H-7b, H-1b, H-3), 3.35 (3H, s, OMe), 1.37, 1.36 (6H, 2s, 2Me). ^{13}C NMR (MeOD) δ 137.9 (*C*Me_2), 128.5 -101.3 (m, Ar), 100.6 (*C*H–Ph), 97.7 (*C*H_2OMe), 86.0 (C-5), 78.2 (C-3), 72.1 (C-4), 71.3 (C-2), 69.1 (C-7), 61.3 (C-1), 61.0 (C-6), 55.6 (OMe), 23.6, 23.4 (2Me). HRMS Calcd for $C_{19}H_{29}O_8$ (M+H): 385.1857. Found: 385.1865.

3.9. 2,5-*O*-Isopropylidene-4,6,7-tri-*O*-methoxymethyl-D-glycero-D-manno-heptitol (20)

Compound **18** (580 mg, 1.51 mmol), was dissolved in DMF (20 mL) and *i*-Pr_2NEt (4.21 mL, 24.16 mmol) and MOMCl (0.9 mL, 12.08 mmol) were added. The reaction mixture was heated at 60 °C for 2 h, then quenched with ice, and extracted with ether (3 × 30 mL). The organic solution was dried (Na_2SO_4) and concentrated to give a crude product that was further dried under high vacuum for 1 h. The crude product was dissolved in MeOH (50 mL) and the solution was stirred with $Pd(OH)_2$ 20 wt % on carbon (520 mg) under 100 Psi of H_2 for 1 h. The catalyst was removed by filtration through a bed of Celite, then washed with methanol. The solvents were removed under reduced pressure and the residue was purified by flash column chromatography (EtOAc/hexanes (1.5:1)) to give **20** as a colorless syrup (420 mg, 72%). $[\alpha]_D^{23}$ = +48.0 (*c* 0.1, MeOH). ^{1}H NMR (MeOD) δ 4.90–4.63 (6H, m, 3CH_2OMe), 4.20 (1H, dd, br, H-6), 3.95 (1H, d, br, $J_{4,5}$ = 8.6 Hz, H-5), 3.86–380 (2H, m, H-1a, H-7a), 3.68–3.58 (3H, m, H-2, H-7b, H-1b), 3.45, 3.42, 3.36 (9H, 3s, 3OMe), 3.34 (2H, m, H-4, H-3), 1.35 (6H, s, 2Me). ^{13}C NMR ($CDCl_3$) δ 100.7 (*C*Me_2), 98.4, 96.3, 95.5 (3CH_2OMe), 83.9 (C-4), 75.0 (C-6), 74.9 (C-3), 71.2 (C-2), 70.5 (C-5), 66.2 (C-7), 62.5 (C-1), 55.3, 54.5, 54.1 (3OMe), 22.6, 22.4 (2Me). HRMS Calcd for $C_{16}H_{33}O_{10}$ (M+H): 385.2068. Found: 385.2083.

3.10. 3,6-*O*-Isopropylidene-1,2,4-tri-*O*-methoxymethyl-D-glycero-D-galacto-heptitol (21)

Compound **21** was obtained as a colorless syrup (285 mg, 75%) from **19** (380 mg, 1 mmol) using the same procedure that was used to obtain **20**. $[\alpha]_D^{23}$ = −30 (*c* 0.4, MeOH). ^{1}H NMR (MeOD) δ 4.84–4.61 (6H, m, 3CH_2OMe), 4.08 (1H, ddd, $J_{3,2}$ = 1.3, $J_{2,1a}$ = 5.6, $J_{2,1b}$ = 7.2 Hz, H-2), 3.86–3.84 (2H, m, H-7a, H-3), 3.74 (1H, dd, $J_{1a,1b}$ = 9.5, $J_{1a,2}$ = 5.6 Hz, H-1a), 3.69 (1H, ddd, $J_{6,5}$ = 2.9, $J_{6,7b}$ = 6.8, $J_{6,7a}$ = 9.8 Hz, H-6), 3.60–3.55 (2H, m, H-1b, H-7b), 3.45(1H, m, H-5), 3.44, 3.38, 3.35 (9H, 3s, 3OMe), 3.34 (1H, m, H-4), 1.36, 1.32 (6H, 2s, 2Me). ^{13}C NMR ($CDCl_3$) δ 100.1 (*C*Me_2), 97.8, 96.8, 95.9 (3CH_2OMe), 83.3 (C-5), 75.1 (C-2), 73.8 (C-4), 70.3 (C-6), 67.8 (C-3), 65.9 (C-1), 61.8 (C-7), 54.3, 54.1, 53.7 (3OMe), 22.9, 22.8 (2Me). HRMS Calcd for $C_{16}H_{33}O_{10}$ (M+H): 385.2068. Found: 385.2067.

3.11. 2,5-*O*-Isopropylidene-4,6,7-tri-*O*-methoxymethyl-D-glycero-D-manno-heptitol-1,3-cyclic sulfate (12)

A mixture of **20** (400 mg, 1.04 mmol) and Et_3N (0.57 mL, 4.16 mmol) in CH_2Cl_2 (10 mL) was stirred in an ice bath. Thionyl chloride (0.12 mL, 1.56 mmol) in CH_2Cl_2 (2 mL) was then added

dropwise over 15 min, and the mixture was stirred for an additional 30 min. The mixture was poured into ice-cold water and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was dried under high vacuum for 1 h. The diasteromeric mixture of cyclic sulfites was dissolved in a mixture of CH_3CN/CCl_4 (1:1, 25 mL) and sodium periodate (333 mg, 1.56 mmol) and $RuCl_3$ (10 mg) were added, followed by water (2 mL). The reaction mixture was stirred for 2 h at rt, then filtered through a bed of Celite, and washed with ethyl acetate. The volatile solvents were removed, and the aqueous solution was extracted with EtOAc (2 × 30 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, concentrated under reduced pressure, and the residue purified by flash column chromatography (EtOAc/hexanes (1:2)) to give **12** as a colorless syrup (325 mg, 70%). $[\alpha]_D^{23}$ = +1.2 (*c* 0.85, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 4.77–4.65 (6H, m, 3CH_2OMe), 4.62 (1H, t, $J_{2,3} = J_{4,3}$ = 9.1 Hz, H-3), 4.54 (1H, t, $J_{1a,1b} = J_{2,1a}$ = 11.1 Hz, H-1a), 4.37 (1H, dd, $J_{2,1a}$ = 5.4, $J_{1a,1b}$ = 11.1 Hz, H-1b), 4.16 (2H, m, H-2, H-6), 3.98 (1H, d, $J_{4,5}$ = 9.8 Hz, H-5), 3.80 (1H, dd, $J_{6,7a}$ = 4.8, $J_{7a,7b}$ = 10.8 Hz, H-7a), 3.75 (1H, t, $J_{3,4} = J_{4,5}$ = 8.6 Hz, H-4), 3.64 (1H, t, $J_{7a,7b} = J_{6,7b}$ = 8.9 Hz, H-7b), 3.44, 3.41, 3.39 (9H, 3s, 3OMe), 1.38, 1.36 (6H, 2s, 2Me). ^{13}C NMR ($CDCl_3$) δ 102.3 (*C*Me_2), 97.9, 96.7, 96.1 (3CH_2OMe), 89.2 (C-3), 76.9 (C-4), 74.6 (C-6), 72.2 (C-1), 71.0 (C-5), 66.8 (C-5), 66.8 (C-7), 56.5 (C-2), 56.6, 55.7, 55.3 (3OMe), 24.4, 23.9 (2Me). HRMS Calcd for $C_{16}H_{31}O_{12}S$ (M+H): 447.1531. Found: 447.1516.

3.12. 3,6-*O*-Isopropylidene-1,2,4-tri-*O*-methoxymethyl-D-glycero-D-galacto-heptitol-5,7-cyclic sulfate (22)

Compound **22** was obtained as a colorless syrup (220 mg, 76%) from **21** (250 mg, 0.65 mmol) using the same procedure that was used to obtain **12**. $[\alpha]_D^{23} = -32$ (*c* 0.46, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 4.83–4.63 (6H, m, 3CH_2OMe), 4.70 (1H, m, H-5), 4.55 (1H, t, $J_{7a,7b}$ = -$J_{6,7a}$ = 11.1 Hz, H-7a), 4.39 (1H, dd, $J_{6,7a}$ = 4.9, $J_{7a,7b}$ = 10.7 Hz, H-7b), 4.24 (1H, td, $J_{5,6}$ = 5.7, $J_{6,7}$ = 10.5 Hz, H-6), 4.09 (1H, ddd, $J_{1a,2}$ = 6.9, $J_{1b,2}$ = 5.3, $J_{3,2}$ = 1.4 Hz, H-2), 3.97 (1H, dd, $J_{4,3}$ = 10.0, $J_{3,2}$ = 1.5 Hz, H-3), 3.89 (1H, dd, $J_{3,4}$ = 10.0, $J_{5,4}$ = 7.7 Hz, H-4), 3.80 (1H, dd, $J_{2,1a}$ = 5.4, $J_{1a,1b}$ = 9.8 Hz, H-1a), 3.58 (1H, t, $J_{1a,1b} = J_{2,1b}$ = 9.2 Hz, H-1b), 3.45, 3.41, 3.39 (9H, 3s, 3OMe), 1.43, 1.37 (6H, 2s, 2Me). ^{13}C NMR ($CDCl_3$) δ 102.32 (*C*Me_2), 98.1, 98.0, 97.9 (3CH_2OMe), 89.6 (C-5), 76.6 (C-4), 75.1(C-2), 72.0 (C-7), 68.9 (C-3), 66.2 (C-1), 59.5 (C-6), 56.4, 56.0, 55.7 (3OMe), 24.8, 23.8 (2Me). HRMS Calcd for $C_{16}H_{30}NaO_{12}S$ (M+Na): 470.1383. Found: 470.1399.

3.13. 2,3,5-Tri-*O*-*p*-methoxybenzyl-1,4-dideoxy-1,4-[[2*S*,3*S*,4*R*,5*R*,6*R*]-2,5-isopropylidene-4,6,7-tri-*O*-methoxymethyl-3-(sulfooxy)heptyl]-(*R*)-*epi*-sulfoniumylidine-D-arabinitol inner salt (23)

The cyclic sulfate **12** (260 mg, 0.58 mmol) and the thiosugar **7** (360 mg, 0.70 mmol) were dissolved in HFIP (1.5 mL), containing anhydrous K_2CO_3 (10 mg). The mixture was stirred in a sealed reaction vessel in an oil bath at 72 °C for six days. The progress of the reaction was followed by TLC analysis (developing solvent EtOAc/MeOH, 10:1). The mixture was cooled, then diluted with EtOAc and evaporated to give a syrupy residue. Purification by column chromatography (EtOAc/MeOH 99:1) gave the sulfonium salt **23** as a syrup (360 mg, 65%). $[\alpha]_D^{23}$ = +62 (*c* 0.85, CH_2Cl_2). ^{1}H NMR (acetone-d_6) δ 7.32–6.91(12H, m, Ar), 5.12–4.52 (12H, m, 3CH_2OMe, 3CH_2–Ph), 4.69 (1H, m, H-2), 4.55 (1H, m, H-3), 4.39–4.30 (4H, m, H-1′a, H-2′, H-3′, H-6′), 4.08 (1-H, t, $J_{3,4} = J_{5,4}$ = 7.4 Hz, H-4), 4.02–3.90 (4H, m, H-1a, H-1′b, H-5′), 3.85–3.78 (3H, m, H-5a, H-7′a, H-1b), 3.82 (9H, s, 3Ph–OMe), 3.60 (1H, t, $J_{7'a,7'b} = J_{6',7'b}$ = 9.1 Hz, H-7′b), 3.42 (1H, m, H-4′), 3.39, 3.36, 3.33 (9H, 3s, 3CH_2OMe) 1.37, 1.32 (6H, 2s, 2Me). ^{13}C NMR (acetone-d_6) δ 159.8–129 (m, Ar), 101.6 (*C*Me_2), 98.7, 96.5, 95.2 (3CH_2OMe), 83.5 (C-3), 81.2 (C-2), 79.7 (C-2′), 78.6 (C-4′), 74.0 (C-6′), 72.7, 71.6, 71.4 (3CH_2Ph), 71.3 (C-5′), 66.9 (C-7′), 66.6 (C-3′), 66.5 (C-5), 65.1 (C-4), 55.9–54.2 (6OMe), 51.5 (C-1′), 47.4 (C-1), 24.4, 23.5 (2Me). HRMS Calcd for $C_{45}H_{65}O_{18}S_2$ (M+H): 957.3607. Found: 957.3604.

3.14. 1,4-Dideoxy-1,4-[[2*S*,3*S*,4*R*,5*R*,6*R*-2,4,5,6,7-pentahydroxy-3-(sulfooxy)heptyl]-(*R*)-*epi*-sulfoniumylidine]-D-arabinitol inner salt (6)

The protected sulfonium salt **23** (150 mg, 0.16 mmol) was dissolved in 30% aqueous solution of TFA (25 mL) and the mixture was stirred at 50 °C for 5 h. The solvent was removed under reduced pressure and the residue was dissolved in water (5 mL) and washed with CH_2Cl_2 (3 × 5 mL). The water layer was evaporated to give the crude product that was purified on silica gel with EtOAc/MeOH/H_2O 6:3:1 (v/v) as eluent to give compound **6** as a colorless solid (61 mg, 93%). Mp 82–84 °C $[\alpha]_D^{23}$ = +5.5 (*c* 0.55, CH_2Cl_2). ^{1}H NMR (D_2O) δ 4.67 (1H, dd, $J_{1a,2}$ = 3.7, $J_{1b,2}$ = 7.4 Hz, H-2), 4.56 (1H, d, $J_{2',3'}$ = 8.2 Hz, H-3′), 4.39 (1H, t, $J_{2,3} = J_{3,4}$ = 3.1 Hz, H-3), 4.35 (1H, dt, $J_{2',3'}$ = 3.3, $J_{2',1'}$ = 7.8 Hz, H-2′), 4.02 (3H, m, H-5a, H-1′a, H-4), 3.91–3.83 (5H, m, H-6′, H-5′, H-5b, H-4′, H-1′b), 3.81 (2H, d, $J_{1,2}$ = 3.9 Hz, H-1a,b), 3.71 (1H, dd, $J_{7'a,7'b}$ = 3.2, $J_{7'b,6'}$ = 11.9 Hz, H-7′b), 3.62 (1H, dd, $J_{7'b,7'a}$ = 7.8, $J_{7'a,6'}$ = 11.6 Hz, H-7′a). ^{13}C NMR (D_2O) δ 78.3 (C-3′), 77.7 (C-3), 76.7 (C-2), 72.9 (C-6′), 70.7 (C-5′), 70.0 (C-4), 69.1 (C-4′), 66.0 (C-2′), 61.6 (C-7′), 59.2 (C-5), 50.7 (C-1′), 47.7 (C-1). HRMS Calcd for $C_{12}H_{25}O_{12}S_2$ (M+H): 425.0782. Found: 425.0778.

3.15. 1,4-Dideoxy-1,4-[[2*S*,3*S*,4*R*,5*R*,6*S*-2,4,5,6,7-pentahydroxy-3-(sulfooxy)heptyl]-(R-)*epi*-sulfoniumylidine]-D-arabinitol inner salt (4)

A mixture of the thiosugar **7** (100 mg, 0.224 mmol) and the cyclic sulfate **22** (137 mg, 0.269 mmol) in HFIP (1 mL) containing K_2CO_3 (5 mg) was placed in a sealed reaction vessel and heated at 72 °C with stirring for six days. The progress of the reaction was followed by TLC analysis (developing solvent EtOAc/MeOH, 10:1). The mixture was cooled, then diluted with EtOAc and evaporated to give a syrupy residue. Purification by column chromatography (EtOAc/MeOH 95:5) gave the protected sulfonium salt as a foam (120 mg, 57%). The protected sulfonium salt **24** (100 mg, 0.11 mmol) was dissolved in 30% aqueous TFA (10 mL) and stirred at 50 °C for 5 h. The solvents were removed under reduced pressure and the residue was dissolved in water (5 mL) and washed with CH_2Cl_2 (3 × 5 mL). The water layer was evaporated to give the crude product that was purified on silica gel column with EtOAc/MeOH/H_2O 6:3:1 (v/v) as eluent to give compound **4** as a colorless solid (40 mg, 91%).[12]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.03.027.

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