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Synthesis of new analogues of salacinol containing a pendant hydroxymethyl group as potential glycosidase inhibitors

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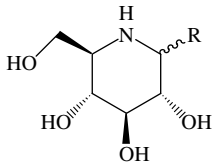
Abstract—The synthesis of new analogues of the naturally occurring glycosidase inhibitor, salacinol, and its ammonium analogue, ghavamiol is described. These analogues contain an additional hydroxymethyl group at C-1, which was intended to form additional polar contacts within the active site of glycosidase enzymes. The target zwitterionic compounds were synthesized by means of nucleophilic attack at the least hindered carbon atom of 2,4-*O*-benzylidene-L (or D)-erythritol 1,3-cyclic sulfate by 2,5-anhydro-1,3:4,6-di-*O*-benzylidene-2,5-dideoxy-5-thio (or 1,5-imino)-L-iditol.

Keywords: Glycosidase inhibitors; Analogues of salacinol and ghavamiol; Ammonium salt; Sulfonium salt; Cyclic sulfate

1. Introduction

Glycosidases are responsible for the hydrolysis of poly- and oligosaccharides into monomers or cleavage of bonds between sugars and a noncarbohydrate aglycon. These enzymes are involved in several metabolic pathways and an alteration of glycosidase activity by inhibitors in vivo has the potential for the control of certain cellular functions. Thus, glycosidase inhibitors have shown promising chemotherapeutic applications against diabetes,[1] cancer,[2] and viral infections including AIDS.[3]



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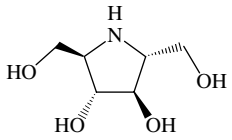
2. R = H



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The glycosidase-mediated reaction can occur with one of the two possible stereochemical outcomes—inversion or retention of configuration at the anomeric center. The currently accepted mechanisms of glycosidic bond cleavage involve general acid catalysis with protonation of the exocyclic oxygen atom at the anomeric center by a carboxylic acid group in the active site of the enzyme that results in the formation of an oxacarbenium ion transition state,[4] which then reacts with a molecule of water to form the products. An attractive approach to potent inhibitors is to create compounds that mimic the transition state of an enzyme-catalyzed reaction.[5] Many natural and synthetic polyhydroxylated pyrrolidines and polyhydroxylated pyrrolizidines alkaloids, such as nojirimycin **1**, 1-deoxynojirimycin (DNJ) **2**, and 2,5-dihydroxymethyl-3,4-dihydroxy-pyrrolidine (DMDP) **3** are carbohydrate mimics, commonly referred to as azasugars, act as glycosidase inhibitors. It has been postulated that their activity is due to their resemblance to the oxacarbenium-ion-like transition state that arises from the protonation of the ring nitrogen at physiological pH.[5a] DMDP (**3**), a common secondary metabolite, has been reported to be a good glucosidase inhibitor, with mild inhibition of some other glycosidases.

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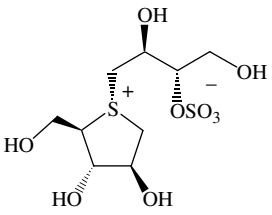

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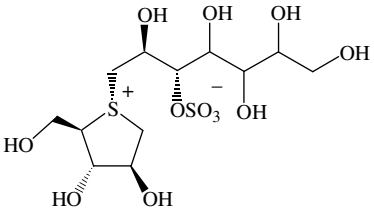

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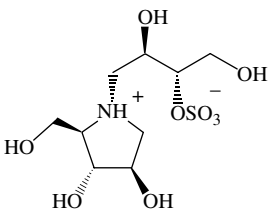

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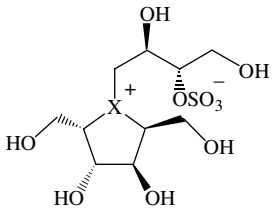
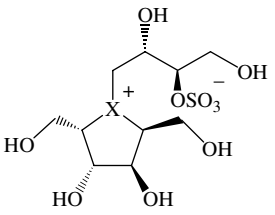
Recently, a new class of naturally occurring glycosidase inhibitor with an intriguing inner salt, sulfonium sulfate was isolated from *Salacia reticulata*[6] a plant from India and Sri Lanka known for its anti-diabetic properties. The bioassay-guided separation using intestinal α-glucosidase inhibitory activity resulted in the isolation of two potent α-glucosidase inhibitors, namely, salacinol (**4**) and kotalanol (**5**). The unique structure of salacinol is a sulfonium ion (1,4-anhydro-4-thio-D-pentitol cation) stabilized by an internal sulfate counter ion (1-deoxy-L-erythritol-3-sulfate anion). These glucosidase inhibitors are also presumably mimics of the oxacarbenium-ion transition state in glucosidase-mediated hydrolysis reactions, but could function simply by providing stabilizing electrostatic interactions with active site carboxylate residues.

In the search for new glycosidase inhibitors, we have reported the synthesis and glucosidase inhibitory properties of the different stereoisomers and heteroatom congeners of salacinol[7] in which the ring sulfur atom has been substituted by the cognate atoms nitrogen and selenium. Enzyme inhibition assays with a panel of glycosidase enzymes have indicated that the stereochemistry at the different stereogenic centers, the nature of the heteroatom, the size of the heterocyclic ring, and the length and nature of the side chain play a significant role in discrimination between different glycosidase enzymes.[8] Our recent investigation by X-ray crystallography[9] of the interaction of salacinol and its analogues with Golgi α-mannosidase II (GMII) indicated that, as speculated, in all of these derivatives, the positively charged sulfonium center was in close proximity to an aspartate residue in the enzyme active site. In addition, a comparison of the interactions with those of the naturally occurring inhibitor, swainsonine demonstrated that the coordination of salacinol **4** with the Zn atom in the enzyme active site is pentacoordinate (T5) whereas that of swainsonine is hexacoordinate (T6). We speculated that octahedral coordination was important to generate a good inhibitor. We report herein the synthesis of new analogues **7**–**10** of salacinol (**4**) and the corresponding ammonium analogue, ghavamiol **6** that incorporate an additional hydroxymethyl group at C-1 that might facilitate T6 coordination in the active site of GMII but might also provide favorable polar contacts in the active site of other glycosidase enzymes.



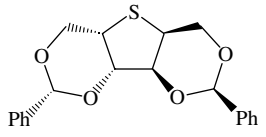
7. X = S
9. X = NH



8. X = S
10. X = NH

2. Results and discussion

The general synthetic strategy involved alkylation of the anhydroalditol at the heteroatom by a cyclic sulfate derivative, whereby selective attack of the heteroatom at the least hindered primary center would afford the desired target molecules.



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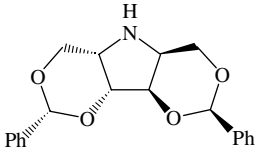

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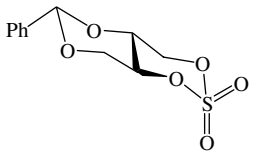

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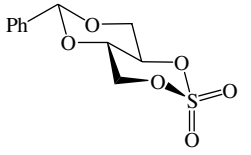

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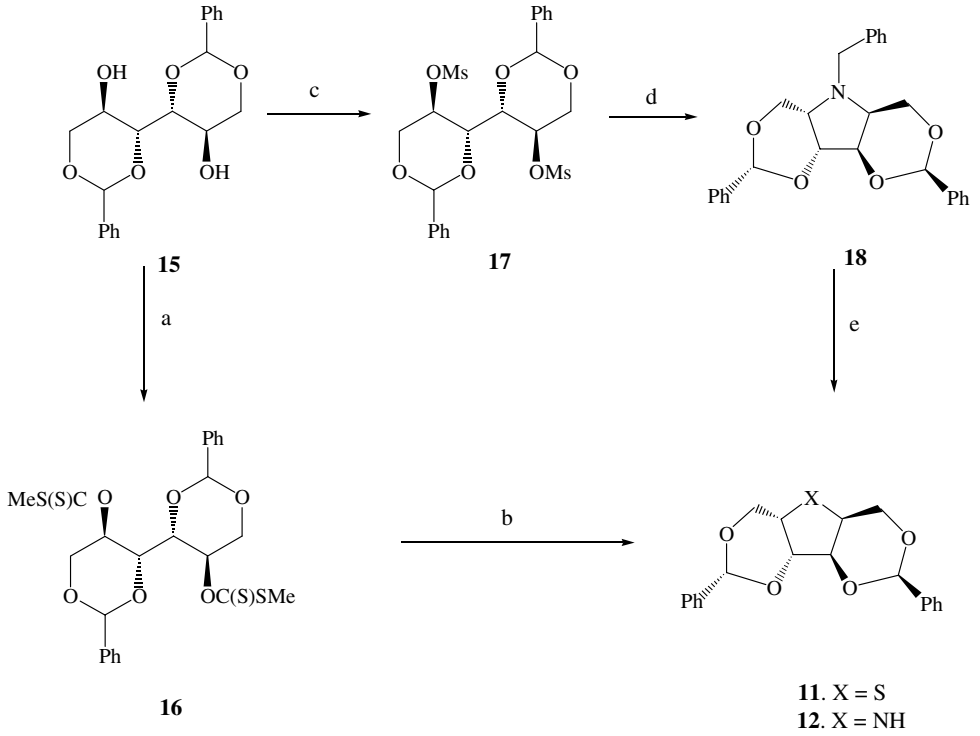
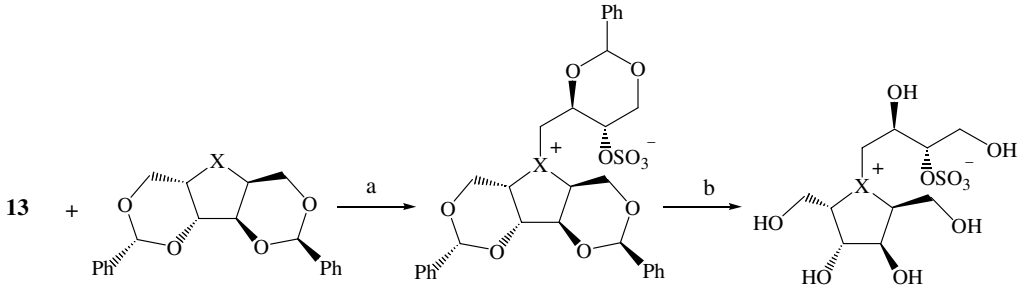
Thioether (**11**) was synthesized according to a reported method[10] by the radical-mediated cyclization of the corresponding 1,5-bis-dithiocarbonate derivative using tributyltin hydride with α,α-diazoisobutyronitrile (AIBN) as a radical initiator (Scheme 1). The corresponding dithiocarbonate was synthesized, in turn, from 1,3:4,6-di-*O*-benzylidene-D-mannitol by the sequential addition of sodium hydride, carbon disulfide, and methyl iodide in THF (Scheme 1).



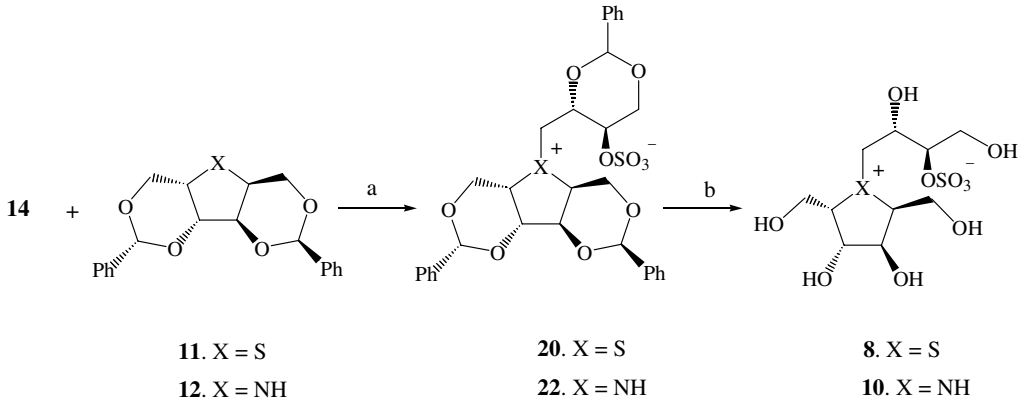
Scheme 1. Reagents and conditions: (a) NaH, CS_2, THF and then MeI (82%); (b) Bu_3SnH, AIBN, toluene, 80 °C (77%); (c) MsCl/Py, CH_2Cl_2 (89%); (d) $BnNH_2$, 130 °C (84%); (e) $Pd(OH_2)/C$, H_2, EtOAc–MeOH (90%).

The synthesis of the C_2-symmetric iminoalditol (**12**) was patterned after that of Masaki et al.[11] The C-2 and C-5 hydroxyl groups in **15** were activated by mesylation, and pyrrolidine ring formation was effected by heating the dimesylate in benzylamine for 12 h at 130 °C. Cyclization proceeded with complete inversion at both centers leading to 2,5-dideoxy-2,5-*N*-benzylimino-1,3:4,6-di-*O*-benzylidene-L-iditol in 87% yield. Unlike Masaki et al., we chose to use $Pd(OH)_2/C$ and hydrogen for the removal of the *N*-benzyl group. The origin of this selectivity is unknown at present. The ^{13}C NMR spectrum of compound **12** exhibited only three carbon resonances, thus confirming that the molecule possessed a C_2 axis of symmetry.

We chose to alkylate compounds **11** and **12** with the protected D- and L-erythritol cyclic sulfates (**13** and **14**) as the source of the sulfated alkyl side chain, both of which were synthesized from D-glucose.[5,12] Alkylation of thioether **11** with the cyclic sulfate **13** in 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) proceeded smoothly in 24 h to give the sulfonium salt **19** as the sole product in 81% yield (Scheme 2). Subsequent removal of all the three benzylidene groups by treatment with trifluoroacetic acid afforded the target compound **7**. The ^1H



Scheme 2. Reagents and conditions: (a) HFIP, K_2CO_3, 70 °C for **19** (81%) and acetone, K_2CO_3, 60 °C for **21** (88%); (b) TFA, for **7** (82%) and Pd/C, aq acetic acid, H_2 for **9** (86%).



Scheme 3. Reagents and conditions: (a) HFIP, K_2CO_3, 70 °C for **20** (78%) and acetone, K_2CO_3, 60 °C for **22** (80%); (b) TFA, for **8** (79%) and Pd/C, aq acetic acid, H_2 for **10** (81%).

NMR and ^{13}C NMR spectra of **7** were completely assigned with the help of ^{1}H–^{1}H COSY, HMQC, and HMBC experiments. In a similar manner, the reaction of **11** with the enantiomeric cyclic sulfate **14** yielded the corresponding sulfonium sulfate **20** in 78% yield, and deprotection with TFA produced the desired product **8** (Scheme 3). The ^{1}H NMR and ^{13}C NMR spectra for compound **8** were similar to those of **7**. Each of the sulfonium salts (**7** and **8**) were obtained as a single isomer at the sulfur atom, correlated with the C_2 axis of the starting thioether (**11**).

Amine **12** reacted with L-cyclic sulfate **13** in dry acetone containing K_2CO_3 to give the ammonium salt **21** in 88% yield. Deprotection was accomplished with Pd/C/H_2 in aqueous acetic acid to effect the hydrogenolytic cleavage of the benzylidene groups, to give the target compound **9**. The ^{1}H NMR resonances for compounds were extremely broad in D_2O, but sharpened when the sample was made basic with K_2CO_3. Analogously, the reaction of amine **12** with D-cyclic sulfate (**14**) produced the corresponding ammonium salt **22** in 80% yield; subsequent deprotection by hydrogenolysis, as before, gave the desired product, **10**.

3. Experimental

3.1. General

Optical rotations were measured at 23 °C. ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively. All assignments were confirmed with the aid of two dimensional experiments (^{1}H–^{1}H COSY, HMQC, and HMBC) using standard Varian pulse programs. Processing of the spectra was performed using Mestrac software. Column chromatography was performed with Merck silica gel 60 (230–400 mesh), and thin layer chromatography (TLC) was performed on aluminum plates precoated with E. Merck Silica Gel 60F-254 as the absorbent. MALDI-TOF mass spectra were recorded on a perSeptive Biosystems Voyager-DE spectrometer, using 2,5-dihydroxybenzoic acid as a matrix.

3.2. 2,5-Dideoxy-2,5-*N*-benzylimino-1,3:4,6-di-*O*-benzylidene-L-iditol (18)

Compound **17** (3.2 g, 5.89 mmol) in benzylamine (10 mL) was stirred at 130 °C for 12 h. The excess benzylamine was removed under high vacuum, and the residue was purified by flash column chromatography to yield compound **18** (2.14 g, 84%) as a white solid; mp 118–120 °C (lit. 120 °C); $[\alpha]_D$ +90 (*c* 1.6, $CHCl_3$); lit.[10] $[\alpha]_D$ +90.6 (*c* 0.3, $CHCl_3$). The spectral data were in agreement with those reported.

3.3. 2,5-Dideoxy-2,5-imino-1,3:4,6-di-*O*-benzylidene-L-iditol (12)

2,5-Dideoxy-2,5-*N*-benzylimino-1,3:4,6-di-*O*-benzylidene-L-iditol (**18**, 1.98 g, 4.6 mmol) was dissolved in 100 mL EtOAc–MeOH (1:1), and 20% $Pd(OH)_2$/C (200 mg) was added. The solution was stirred under an atmosphere of H_2 for 3 h. The catalyst was removed by filtration, the solvents were removed under vacuum, and the residue was purified by flash chromatography (20:1, EtOAc–MeOH) to afford **12** (1.42 g, 90%) as a white solid; mp 130–131 °C (lit. 130 °C); $[\alpha]_D$ +15.2 (*c* 1.0, $CHCl_3$) (lit.[10] $[\alpha]_D$ +7.7 (*c* 0.5, $CHCl_3$). The spectral data were in agreement with those reported.

3.4. 1,3:4,6-Di-*O*-benzylidine-2,5-dideoxy-1,5-[[(2*S*,3*S*)-2,4-*O*-benzylidene-2,4-dihydroxy-3-(sulfooxy)butyl]sulfoniumylidene]-L-iditol inner salt (19)

To 1,1,1,3,3,3-hexafluoro propanol (2 mL) were added 1,3:4,6-di-*O*-benzylidene-2,5-dideoxy-1,5-thio-L-iditol (**11**) (282 mg, 0.79 mmol), 2,4-*O*-benzylidine-L-erythritol-

1,3-cyclic-sulfate (**13**) (260 mg, 0.94 mmol), and K_2CO_3 (45 mg) in a sealed tube and the reaction mixture was stirred for 48 h at 75 °C. The solvent was removed and the crude product was purified by column chromatography (15:1, EtOAc–MeOH) to afford the coupled product **19** as a colorless foam (405 mg, 81%); ^{1}H NMR ($CDCl_3$): δ 5.54, 5.52, 5.42 (3H, 3 × Ph-CH–), 4.94 (2H, d, $J_{1a,1b} = 14.0$, $J_{1a',1b'} = 14.0$ Hz, H-1a, H-1a′), 4.80 (1H, br s, H-3), 4.79 (1H, br s, H-4), 4.66 (1H, d, $J_{6a,6b'} = 14.0$ Hz, H-6a), 4.56 (1H, dd, $J_{4a,'4b'} = 10.3$, $J_{3',4a'} = 5.6$ Hz, H-4a′), 4.48 (1H, ddd, $J_{3',4a'} = 5.6$, $J_{3',4b'} = 10.7$, $J_{3',2'} = 9.7$ Hz, H-3′), 4.37 (1H, ddd, $J_{2',1a'} = 3.0$, $J_{2',1b} = 6.1$ Hz, H-2′), 4.32 (1H, br s, H-5), 4.30 (1H, dd, $J_{1b,2} = 3.2$ Hz, H-1b), 4.21 (1H, br s, H-2), 4.13 (1H, dd, H-1b′), 3.97 (1H, dd, $J_{6b,5} = 1.8$ Hz, H-6b), 3.75 (1H, dd, H-4b′); ^{13}C NMR (CD_2Cl_2): δ 136.2–126.3 (18C), 101.5, 100.8, 100.4 (3H, Ph–CH–), 83.0 (C-3), 80.6 (C-4), 76.8 (C-2′), 69.0 (C-4′), 65.6 (C-3′), 64.0 (C-5), 63.9 (C-6), 63.4 (C-1), 54.3 (C-2), 47.4 (C-1′); MALDI-MS: *m*/*z* 651.20 $[M+Na]^+$, 629.48 $[M+H]^+$, 549.37 $[M+H-SO_3]^+$. HRMS Calcd for $C_{31}H_{32}O_{10}S_2Na$ $[M+Na]^+$: 651.1329. Found: 651.13263.

3.5. 2,5-Dideoxy-1,5-[[(2*S*,3*S*)-2,4-dihydroxy-3-(sulfooxy)butyl]sulfoniumylidene]-L-iditol inner salt (7)

Compound **19** (220 mg, 0.35 mmol) was dissolved in CH_2Cl_2 (1 mL), and 50% aqueous TFA (15 mL) was added and the mixture was stirred at room temperature for 2 h. The solvents were removed by a high vacuum to give a brown gummy product. Purification by column chromatography (10:1, EtOAc–MeOH) gave **7** as an amorphous solid (109 mg, 85%); ^{1}H NMR (D_2O): δ 4.58 (1H, dd, $J_{3,2} = 2.4$, $J_{3,4} = 2.2$ Hz, H-3), 4.50 (1H, dd, $J_{4,5} = 2.3$ Hz, H-4), 4.44 (1H, ddd, $J_{5,6a} = 5.0$, $J_{5,6b} = 9.0$ Hz, H-5), 4.41 (1H, dd, $J_{2,1a} = 6.2$, $J_{2,1b} = 9.6$ Hz, H-2), 4.27 (1H, ddd, $J_{2',1b'} = 7.6$, $J_{2',1a'} = 10.5$, $J_{2',3'} = 2.2$ Hz, H-2′), 4.16 (1H, dd, $J_{6a,6b} = 12.4$ Hz, H-6a), 4.14 (1H, ddd, $J_{3',4a'} = 3.2$, $J_{3',4b'} = 3.2$ Hz, H-3′), 4.07 (1H, dd, $J_{1a',1b'} = 14.0$ Hz, H-1b′), 4.04 (1H, dd, $J_{1a,1b} = 12.4$ Hz, H-1a), 4.02 (1H, dd, H-1b), 4.00 (1H, dd, H-6b), 3.82 (1H, dd, $J_{4a',4b'} = 12.8$ Hz, H-4a′), 3.72 (1H, dd, H-4b′), 3.39 (1H, dd, H-1b′); ^{13}C NMR (D_2O): δ 80.9 (C-3′), 78.4 (C-4), 76.9 (C-3), 70.0 (C-2), 66.6 (C-2′), 63.1 (C-5), 59.7 (C-4′), 57.7 (C-1), 55.5 (C-6), 44.8 (C-1′); MALDI-MS: *m*/*z* 387.15 $[M+Na]^+$, 365.02 $[M+H]^+$, 285.26 $[M+H-SO_3]^+$; HRMS Calcd for $C_{10}H_{20}O_{10}S_2Na$ $[M+Na]^+$: 387.03901. Found: 387.03906.

3.6. 1,3:4,6-Di-*O*-benzylidene-2,5-dideoxy-1,5-[[(2*R*,3*R*)-2,4-*O*-benzylidene-2,4-dihydroxy-3-(sulfooxy)butyl]sulfoniumylidene]-L-iditol inner salt (20)

Thio-L-iditol (**11**) (240 mg, 0.67 mmol) was coupled to 2,4-*O*-benzylidene-D-erythritol-1,3-cyclic-sulfate (**14**) (214 mg, 0.78 mmol) in HFIP (2 mL), following the same procedure that was used for the synthesis of **19**. Column chromatography (15:1, EtOAc–MeOH) of the crude product gave **20** as an amorphous solid (338 mg, 79%); ^{1}H NMR (CD_2Cl_2): δ 5.74 (1H, Ph–CH–), 5.62, 5.46 (2H, Ph–CH–), 5.20 (1H, d, $J_{1a,1b} = 14.8$ Hz, H-1a), 4.98 (1H, br s, H-3), 4.87 (1H, br s, H-4), 4.78 (1H, d, $J_{1a',1b'} = 13.9$ Hz, H-1a′), 4.72 (1H, ddd, $J_{3',2'} = 9.5$, $J_{3',4a'} = 10.5$ Hz, H-3′), 4.62 (1H, dd, $J_{1b,2} = 3.1$ Hz, H-1b), 4.58 (1H, dd, $J_{4a'4b'} = 10.5$ Hz, H-4a′), 4.56 (1H, br s, H-2), 4.45 (1H, dd, $J_{2',1b'} = 3.0$ Hz, H-2′), 4.30 (1H, d, $J_{6a,6b} = 14.0$ Hz, H-6a), 4.06 (1H, br s, H-5), 3.89 (1H, dd, H-1b′), 3.83 (1H, d, H-4b′), 3.79 (1H, dd, H-6b); ^{13}C NMR (CD_2Cl_2): δ 136.2–126.3 (18C), 101.5, 100.8, 100.4 (3H, Ph–CH–), 83.0 (C-3), 80.6 (C-4), 76.8 (C-2′), 69.0 (C-4′), 65.6 (C-3′), 64.0 (C-5), 63.9 (C-6), 63.4 (C-1), 54.3 (C-2), 47.4 (C-1′); MALDI-MS: *m*/*z* 651.20 $[M+Na]^+$, 629.48 $[M+1]^+$, 549.37 $[M-SO_3]^+$; HRMS Calcd for $C_{31}H_{32}O_{10}S_2Na$ $[M+Na]^+$: 651.13291. Found: 651.13289.

3.7. 2,5-Dideoxy-1,5-[[(2*R*,3*R*)-2,4-dihydroxy-3-(sulfooxy)butyl]sulfoniumylidene]-L-iditol inner salt (8)

Compound **20** (190 mg, 0.30 mmol) was dissolved in CH_2Cl_2 (1 mL) and 50% aqueous TFA (15 mL) was added and stirred at room temperature for 2.5 h. The solvents were removed by a high vacuum to give a brown residue which was purified by column chromatography (10:1, EtOAc–MeOH) to give **8** as an amorphous solid (83 mg, 76%); ^{1}H NMR (D_2O): δ 4.60 (1H, dd, $J_{3,2} = 2.4$, $J_{3,4} = 3.4$ Hz, H-3), 4.50 (1H, $J_{4,5} = 2.1$ Hz, H-4), 4.47 (1H, dd, $J_{5,6a} = 5.3$, $J_{5,6b} = 9.8$ Hz, H-5), 4.34 (1H, ddd, $J_{2,1a} = 4.8$,$J_{2,1b} = 9.6$ Hz, H-2), 4.30 (1H, ddd, $J_{2',1b'} = 10.5$, $J_{2',1a'} = 2.0$, $J_{2',3'} = 3.6$ Hz, H-2′), 4.18 (1H, ddd, $J_{3'4a'} = 3.4$, $J_{3',4b'} = 3.3$ Hz, H-3′), 4.11 (1H, dd, $J_{1a',1b'} = 12.6$ Hz, H-1a′), 4.04 (1H, d, $J_{1a,1b} = 12.5$ Hz, 12.5 Hz, H-1a), 3.98 (1H, dd, H-6b), 3.88 (1H, dd, H-1b), 3.83 (1H, dd, $J_{1a'1b'} = 13.0$ Hz, H-1a′), 3.82 (1H, dd, $J_{4a',4b'} = 12.8$ Hz, H-4a′), 3.73 (1H, dd, H-4b′), 3.38 (1H, dd, H-1b′); ^{13}C NMR (D_2O): δ 80.6 (C-3′), 78.1 (C-4), 77.1 (C-3), 70.2 (C-2), 64.5 (C-2′), 62.5 (C-5), 59.7 (C-4′), 57.8 (C-6), 55.5 (C-1), 44.0 (C-1′); MALDI-MS: *m*/*z* 651.20 $[M+Na]^+$, 629.48 $[M+H]^+$, 549.37 $[M+H-SO_3]^+$; HRMS Calcd for $C_{31}H_{32}O_{10}S_2Na$ $[M+Na]^+$: 387.03901. Found: 387.03904.

3.8. 1,3:4,6-Di-*O*-benzylidene-2,5-dideoxy-1,5-[[(2*S*,3*S*)-2,4-*O*-benzylidene-2,4-dihydroxy-3-(sulfooxy)butyl]iminonium]-L-iditol inner salt (21)

Imino alditol **12** (260 mg, 0.76 mmol) and cyclic sulfate **13** (228 mg, 0.83 mmol) were added to anhydrous acetone (2 mL) containing K_2CO_3 (50 mg), and the mixture

was stirred in a sealed tube for 12 h at 65 °C. The solvent was removed and the mixture was purified by column chromatography. The coupled product **21** was obtained as a colorless foam (398 mg, 85%); ^{1}H NMR (acetone-d_6, pH > 8): δ 7.40–7.20 (15H), 5.57 (2H, 2 × Ph–CH–), 5.48 (1H, Ph–CH–), 4.69 (2H, d, $J_{1a,1b(6a,6b)}$ = 12.8 Hz, H-1a, H-6a), 4.60 (1H, m, H-4a′), 4.35 (2H, br s, H-3, H-4), 4.20 (1H, m, H-3′), 4.02 (2H, d, H-1b, H-6b), 3.98 (1H, d, $J_{1a',1b'}$ = 12.5 Hz, H-1a′), 3.91 (1H, m, H-2′), 3.68 (1H, m, H-4b′), 3.44 (2H, br s, H-2, H-5), 2.98 (1H, m, H-1b′); MALDI-MS: *m*/*z* 634.08 $[M+Na]^+$, 612.16 $[M+H]^+$, 532.17 $[M+H-SO_3]^+$; HRMS Calcd for $C_{31}H_{32}NO_{10}S$ $[M-H]^-$: 610.1752. Found: 610.1755.

3.9. 2,5-Dideoxy-1,5-[[(2*S*,3*S*)-2,4-dihydroxy-3-(sulfooxy)butyl]iminonium]-L-iditol inner salt (9)

Compound **21** (230 mg, 0.37 mmol) was dissolved in a 4:1 mixture of CH_3COOH–H_2O (20 mL) and the solution was stirred with 10% Pd/C (180 mg) under 100 psi of H_2 for 30 h. The catalyst was removed by filtration through a silica bed, and washed with water (25 mL). The filtrate was evaporated, and the mixture was purified by column chromatography (10:3:1, EtOAc–MeOH–H_2O) to give an amorphous solid (**9**) (112 mg, 86%); ^{1}H NMR (D_2O, pH > 8): δ 4.20 (3H, m, H-3, H-4, H-3′), 4.00 (1H, ddd, $J_{2',3'}$ = 2.2, $J_{2',1a'}$ = 5.6, $J_{2',1b'}$ = 10.2 Hz, H-2′), 3.81 (1H, dd, $J_{4a',3'}$ = 3.5, $J_{4a',4b'}$ = 12.6 Hz, H-4a′), 3.75 (1H, dd, $J_{4b',3'}$ = 4.7 Hz, H-4b′), 3.70 (2H, dd, $J_{1a,1b(6a,6b)}$ = 12.1, $J_{1a,2\ (6a,5)}$ = 4.3 Hz, H-1a, H-6a), 3.59 (2H, dd, $J_{1b,2(6b,5)}$ = 3.0 Hz, H-1b, H-6b), 3.16 (3H, m, H-2, H-5, H-1a′), 2.59 (1H, dd, $J_{1b',1a'}$ = 13.9 Hz, H-1b′); ^{13}C NMR: δ 81.6 (C-3′), 76.3 (C-3, C-4), 67.3 (C-2′), 62.4 (C-2, C-5), 60.0 (C-4′), 58.1 (C-1, C-6), 50.9 (C-1′); MALDI-MS: *m*/*z* 370.19 $[M+Na]^+$, 347.99 $[M+H]^+$, 268.32 $[M+H-SO_3]^-$. Anal. Calcd for $C_{10}H_{21}NO_{10}S$: C, 34.58; H, 6.09; N, 4.03. Found: C, 34.82; H, 5.89; N, 3.94.

3.10. 1,3:4,6-Di-*O*-benzylidene-2,5-dideoxy-1,5-[[(2 *R*,3*R*)-2,4-*O*-benzylidene-2,4-dihydroxy-3-(sulfooxy)butyl]iminonium]-L-iditol inner salt (22)

Imino alditol (**12**) (210 mg, 0.61 mmol) was reacted with 2,4-*O*-benzylidene-L-erythritol-1,3-cyclicsulfate (192 mg, 0.70 mmol) in acetone (2 mL) following the same procedure that was used for the synthesis of **21**. Column chromatography (15:1, EtOAc–MeOH) of the crude product gave **22** as an amorphous solid (338 mg, 89%); ^{1}H NMR (acetone-d_6, pH > 8): δ 7.38–7.20 (15H), 5.60, 5.55 (3H, 3 × Ph–CH–), 4.68 (2H, d, $J_{1a,1b(6a,6b)}$ = 12.7 Hz, H-1a, H-6a), 4.57 (1H, dd, $J_{4a',4b'}$ = 10.7, $J_{4a',3'}$ = 5.4 Hz, H-4a′), 4.34 (2H, br s, H-3, H-4), 4.24 (1H, m, H-3′), 4.00 (2H, d, H-1b, H-6b), 3.94 (2H, m, H-1a′, H-2′), 3.66 (1H, dd, $J_{4b',3'}$ = 10.4 Hz, H-4b′), 3.52 (2H, br s, H-2, H-5), 3.46 (1H, br s, H-1b′); MALDI-MS: *m*/*z* 634.04 $[M+Na]^+$, 612.15 $[M+H]^+$, 532.19 $[M+H-SO_3]^+$.

3.11. 2,5-Dideoxy-1,5-[[(2*R*,3*R*)-2,4-dihydroxy-3-(sulfooxy)butyl]iminonium]-L-iditol inner salt (10)

Compound **22** (216 mg, 0.35 mmol) was dissolved in a 4:1 mixture of CH_3COOH–H_2O (20 mL) and the solution was stirred with 10% Pd/C (160 mg) under 100 psi of H_2 for 44 h. The catalyst was removed by filtration through a silica bed, and washed with water (30 mL). The filtrate was evaporated, and the mixture was purified by column chromatography (10:3:1, EtOAc–MeOH–H_2O) to give an amorphous solid (**10**) (103 mg, 83%); ^{1}H NMR (D_2O, pH > 8): δ 4.29 (1H, ddd, $J_{3',2'}$ = 2.1, $J_{3',4a'}$ = 3.4, $J_{3',4b'}$ = 5.1 Hz, H-3′), 4.19 (2H, m, H-3, H-4),4.03 (1H, ddd, $J_{2',1a'}$ = 7.7, $J_{2',1b'}$ = 5.6 Hz, H-2′), 3.82 (1H, dd, $J_{4a',4b'}$ = 12.7 Hz, H-4a′), 3.74 (1H, dd, H-4b′), 3.73 (2H, dd, $J_{1a,1b(6a,6b)}$ = 12.0, $J_{1a,2(6a,5)}$ = 6.2 Hz, H-1a, H-6a), 3.61 (2H, dd, H-1b, H-6b), 3.17 (2H, m, H-2, H-5), 2.94 (1H, dd, $J_{1a',1b'}$ = 3.9 Hz, H-1a′), 2.85 (1H, dd, H-1b′); ^{13}C NMR: δ 81.5 (C-3′), 76.2 (C-3, C-4), 69.9 (C-2′), 63.8 (C-2, C-5), 59.8 (C-4′), 58.4 (C-1, C-6), 51.0 (C-1′); MALDI-MS: *m*/*z* 370.03 $[M+Na]^+$, 348.05 $[M+H]^+$. Anal. Calcd for $C_{10}H_{21}NO_{10}S$: C, 34.58; H, 6.09; N, 4.03. Found: C, 34.27; H, 6.27; N, 3.81.

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