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New Chain-Extended Analogues of Salacinol and Blintol and Their Glycosidase Inhibitory Activities. Mapping the Active-Site Requirements of Human Maltase Glucoamylase

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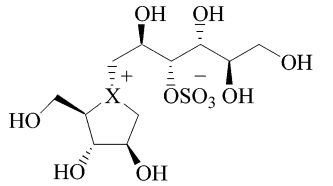
Received September 20, 2006



X= S, Se

The synthesis of new chain-extended sulfonium and selenonium salts of 1,4-anhydro-4-thio-(or 4-seleno)-D-arabinitol, analogues of the naturally occurring glycosidase inhibitor salacinol, is described. Nucleophilic attack at the least hindered carbon atom of 4,6-*O*-benzylidene-2,5-di-*O*-*p*-methoxybenzyl-D-mannitol-1,3-cyclic sulfate by 2,3,5-tri-*O*-*p*-methoxybenzyl-1,4-anhydro-4-thio-(or 4-seleno)-D-arabinitol gave the sulfonium and selenonium sulfates, respectively. Subsequent deprotection with trifluoroacetic acid yielded the target compounds. In these analogues, an extended polyhydroxylated aliphatic side chain has been incorporated while maintaining the stereochemistry of C-2′ and C-3′ of salacinol or blintol. These compounds were designed to probe the premise that they would bind with higher affinity to glucosidases than salacinol because the extra hydroxyl groups in the acyclic chain would make favorable polar contacts within the active site. Both target compounds inhibited recombinant human maltase glucoamylase, one of the key intestinal enzymes involved in the breakdown of glucose oligosaccharides in the small intestine, with K_i values in the low micromolar range. Comparison of these values to those of related compounds synthesized in previous studies has provided a better understanding of structure−activity relationships and the optimal stereochemistry at the different stereogenic centers required of an inhibitor of this enzyme. With respect to chain extension, the configurations at C-2′ and C-4′ are critical for activity, the configuration at C-3′, bearing the sulfate moiety, being unimportant. The desired configuration at C-5′ is also specified. However, comparison of the activities of the chain-extended analogues with those of salacinol and blintol indicates that there is no particular advantage of the chain-extension relative to salacinol or blintol. These results are similar to those reported earlier for kotalanol, a 7-carbon-extended derivative, versus salacinol against rat intestinal maltase, sucrase, and isomaltase.

Introduction

Glycosidases are responsible for the processing of complex carbohydrates, which are essential in numerous biological recognition processes.[1] Inhibition of these glycosidases can have profound effects on quality control, maturation, transport, and secretion of glycoproteins and can alter cell−cell or cell−virus recognition processes. This principle is the basis for the potential use of glycosidase inhibitors in the treatment of viral infections, cancer, and certain genetic disorders.[2,3] Enzyme-catalyzed glycosidic bond cleavage is usually mediated by two catalytic carboxylic acid residues and is believed to proceed via a positively charge oxacarbenium-ion transition state.[4] Many

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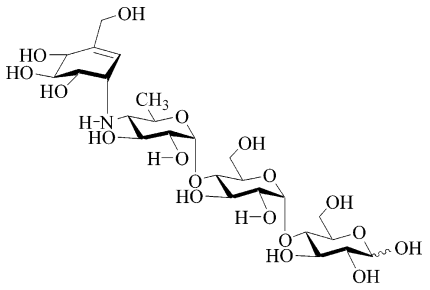
10.1021/jo061944v CCC: $37.00
Published on Web 12/06/2006

CHART 1



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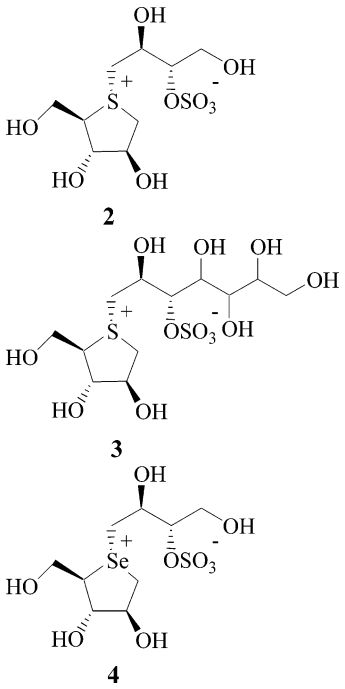
natural and synthetic glycosidase inhibitors mimic the charge and position in the active site of the enzyme,[5] enabling interaction with these carboxylate moieties. The naturally occurring glycosidase inhibitor acarbose (**1**), which contains a nitrogen atom in one of the linkages between sugar and pseudosugar units, has the highest known affinity for a carbohydrate-binding protein (Chart 1).[6] Acarbose is currently used for the oral treatment of diabetes, its mechanism of action being attributed to its ability to mimic the structure of the oxacarbenium ion formed during the enzyme-catalyzed hydrolysis process, and also to provide a basic site that forms favorable electrostatic contact with an active-site carboxylate residue.[7,8]

Several efforts have focused on a novel class of glycosidase inhibitor containing sulfonium ions with a permanent positive charge as putative mimics of the oxacarbenium ion transition states in hydrolysis reactions.[9] Isolation of the naturally occurring glycosidase inhibitors containing a zwitterionic sulfonium-sulfate structure, salacinol (**2**)[10] and kotalanol (**3**)[11] from *Salacia reticulata*, lent credence to this hypothesis (Chart 2). These compounds comprised a 1,4-anhydro-4-thio-D-arabinitol moiety

CHART 2



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3

4

and 1′-deoxy-L-erythrosyl-3′-sulfate or 1′-deoxyheptosyl-3-sulfate units, respectively. Interesting biological activities and the need for synthetic routes prompted us[12] and others[13] to synthesize salacinol and its stereoisomers. We have also reported the synthesis of the corresponding nitrogen and selenium congeners, ghavamiol[14] and blintol,[15] respectively. Seleno sugars have been reviewed in the literature.[16]

Blintol (**4**) has been shown to be very effective in controlling blood glucose levels in rats after a carbohydrate meal, thus providing a lead candidate for the treatment of type-2 diabetes.[17]

Kotalanol (**3**), which has an extended polar side chain as compared to salacinol, was found to be a competitive inhibitor of sucrase and isomaltase.[11] As part of our program aimed at the synthesis of novel glycosidase inhibitors related to salacinol and kotalanol, we have synthesized chain-extended analogues of salacinol with different configurations at the stereogenic centers on the heterocyclic ring as well as on the poly hydroxylated, sulfate-containing aliphatic side chain to better understand structure−activity relationships.[18] The 6-carbon, chain-extended analogues **7** and **8** have shown competitive inhibitory activities against human maltase glucoamylase (MGA) in the same range as salacinol.[19] The analogue **9**, with the opposite stereochemistry at C-2′ from salacinol (**2**) and the opposite stereochemistry at C-5′ from **7** and **8**, is not active;

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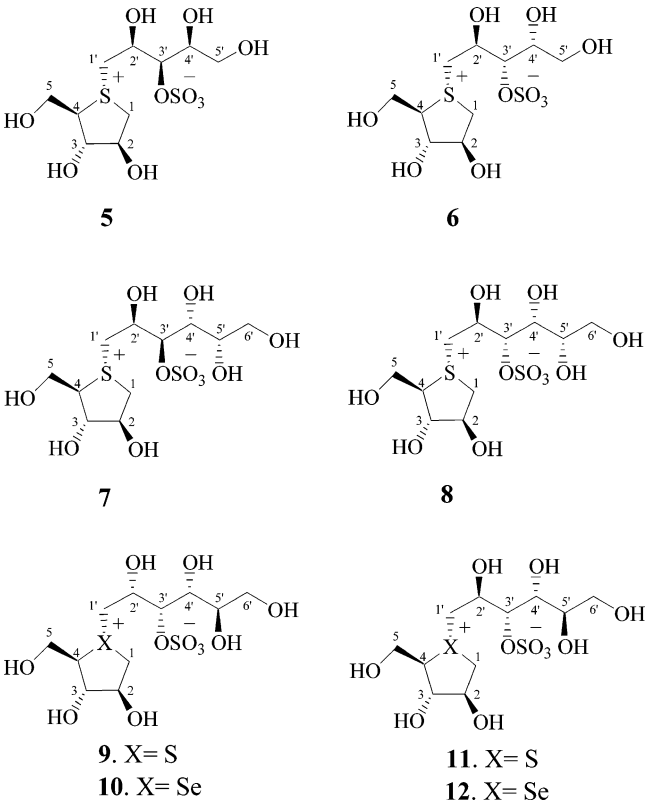
CHART 3



the selenium congener **10** is a weaker inhibitor than **7** or **8**.[18a] Comparison of data for the 5-carbon, chain-extended analogues **5** and **6** shows that the configuration at C-4′ is important because **5** is not active. To better understand these data and to further map the enzyme active site of MGA, we now report the synthesis of new chain-extended analogues **11** and **12**, with the same stereochemistry at C-2′ and C-3′ as salacinol (**2**) and with opposite stereochemistry at C-5′ from **7** and **8** (Chart 3). In particular, we intended to probe whether increased polar interactions within the active site of MGA would lead to more effective inhibition relative to salacinol **2** and blintol **4**.

Results and Discussion

Retrosynthetic analysis revealed that the target zwitterionic molecules could be synthesized by alkylation at the sulfur or selenium atom of suitably protected anhydro-alditols. The alkylating agent could be a cyclic sulfate whereby selective attack of the heteroatom at the least hindered primary center would afford the desired compounds.

Indeed, opening of 4,6-*O*-benzylidene-2,5-di-*p*-methoxybenzyl-D-mannitol-1,3-cyclic sulfate (**13**) by 2,3,5-tri-*O*-*p*-methoxybenzyl-1,4-anhydro-4-thio- (**14**) or 4-seleno-(**15**)-D-arabinitol proceeded smoothly to give the corresponding coupled products (Scheme 1). The reaction of the selenium compound, **15**, afforded *R*/*S* isomers at the stereogenic selenium center that were separated and characterized independently.

The thioarabinitol, **14**, and selenoarabinitol, **15**, were synthesized from L-xylose according to the reported procedure (Chart 4).[20] The desired cyclic sulfate **13** was synthesized from

SCHEME 1

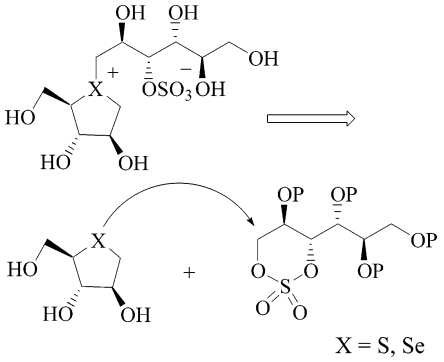
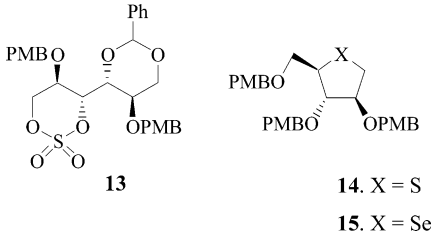
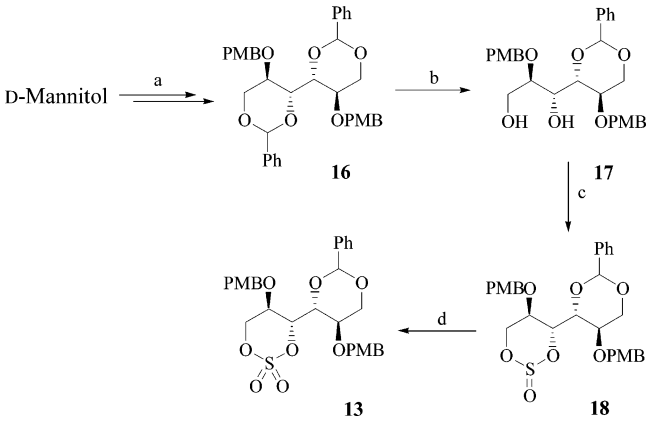


CHART 4



SCHEME 2[a]



[a] (a) Reference 20; (b) PTSA, MeOH (73%); (c) $SOCl_2$, Et_3N, CH_2Cl_2 (76%); (d) $NaIO_4$, $RuCl_3 \cdot 3H_2O$, CH_3CN:CCl_4 (1:1) (86%).

D-mannitol in five steps, as depicted in Scheme 2. Because of the difficulties experienced before in hydrogenolysis of the benzyl ethers in sulfonium and selenonium salts, we chose the acid labile protecting groups, *p*-methoxybenzyl and benzylidene groups, instead of benzyl ethers. 1,3:4,6-Di-*O*-benzylidene-2,5-di-*O*-*p*-methoxybenzyl-D-mannitol (**16**)[21] was subjected to mild acetolysis using catalytic *p*-toluenesulfonic acid (PTSA) in methanol to effect selective removal of one benzylidene group. The reaction proceeded smoothly to give the corresponding diol, **17**, in 73% yield. The target cyclic sulfate **13** was then obtained by treatment of the diol **17** with thionyl chloride in the presence of triethylamine to give a mixture of diastereomeric sufites **18**, followed by their oxidation with $NaIO_4/RuCl_3$.

The coupling reaction of 2,3,5-tri-*O*-*p*-methoxybenzyl-1,4-anhydro-4-thio- (**14**) and 4-seleno-D-arabinitol (**15**) with the

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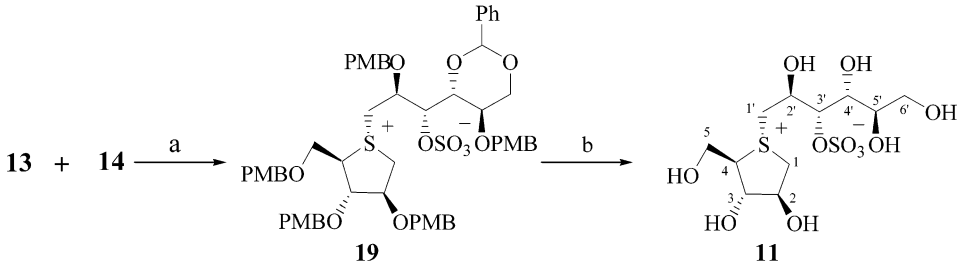
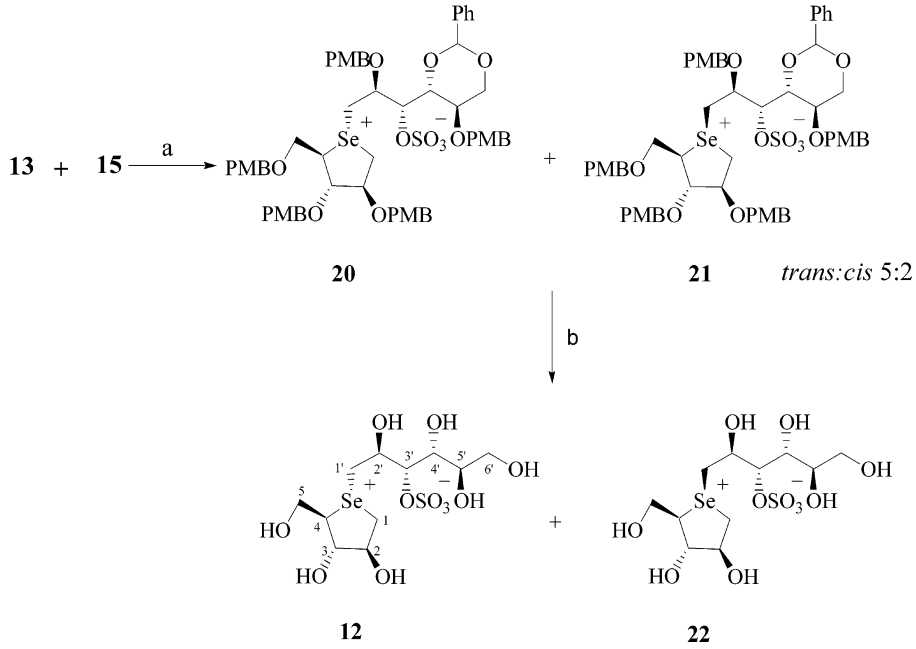
SCHEME 3[a]



[a] (a) K_2CO_3, HFIP, 65−70 °C (77%); (b) aq TFA.

SCHEME 4[a]



[a] (a) K_2CO_3, HFIP, 65−70 °C; (b) aq TFA.

cyclic sulfate **13** was then investigated. Thus, the mixture of the thioether (**14**) and the cyclic sulfate in 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) was heated at 65−70 °C in the presence of anhydrous potassium carbonate for 36 h to give the sulfonium salt, **19**, as the sole product in 77% yield. Deprotection of the coupled product using aqueous trifluoroacetic acid (TFA) gave the desired compound **11** in 82% yield (Scheme 3). The zwitterionic compound was assigned to be the isomer with an anti relationship between C-5 and C-1′ by analysis of transient one-dimensional nuclear Overhauser enhancement (NOE) experiments, which showed a correlation between H-1′ and H-4.

The selenium congener **12** was synthesized in a manner analogous to that described for the sulfonium analogue **11** (Scheme 4). The coupling reaction of the selenoether, **15**, with the cyclic sulfate **13** in HFIP at 70 °C afforded a mixture of diastereomeric selenonium salts **20** and **21** in a 5:2 ratio, as judged by the ratio of the benzylidene proton resonances in the ^{1}H NMR spectrum of the crude product, and NOE experiments (as above). This mixture results from the electrophilic attack on both the α and the β faces of the selenoarabinitol moiety to afford **20** and **21**, respectively. This result is attributed to the longer C−Se bond as compared to the C−S bond, which leads to less steric hindrance in the formation of the C−Se bond. The two isomers, **20** and **21**, were isolated successfully in pure form after two rounds of column chromatography and were characterized by NMR spectroscopy. Deprotection of the coupled products **20** and **21** using aqueous trifluoroacetic acid (TFA) gave the desired zwitterionic compounds **12** and **22**, respectively, in quantitative yields. The major isomer **12** was assigned to be that with an anti relationship between C-5 and C-1′ by means of a 1D-transient NOE experiment.

It is of interest to comment on the inhibitory activities and structure−activity relationships of the compounds synthesized in this study and previous studies against recombinant human maltase glucoamylase (MGA), a critical intestinal glucosidase involved in the processing of oligosaccharides of glucose into glucose itself. Comparison of the data in Table 1 indicates that compounds **11** and **12**, with the same configuration at the stereogenic heteroatom center (and the same configuration at C-2′ and C-3′ as salacinol or blintol), have K_i values of 0.65 ± 0.10 and 0.14 ± 0.03 μM, respectively. The latter selenonium ion is the most active compound to date within this class of compounds. Interestingly, however, compound **7**, with opposite configurations at C-2′ and C-3′ from **9** (and opposite configuration at only C-3′ in **11** and **12**), is also active, with a K_i value of 0.25 ± 0.02 μM. The *S*-configuration at C-5′ also appears to be important because **8** and **11** (with opposite configurations at only C-5′) show significantly different inhibitory activities. Our previous data with the 5-carbon-extended analogues **5** and **6** also permit us to specify the required configuration at C-4′ as

TABLE 1. Experimentally Determined K_i Values[a]

inhibitor	K_i (μM)
5	NA[b,c]
6	0.26 ± 0.03[c]
7	0.25 ± 0.02[c]
8	0.17 ± 0.02[c]
9	NA[b,d]
10	41.0 ± 7.00[d]
11	0.65 ± 0.10
12	0.14 ± 0.03[e]
22	10.1 ± 0.2[e]
salacinol (**2**)	0.19 ± 0.02[c]
blintol (**4**)	0.49 ± 0.05[c]

[a] Analysis of MGA inhibition was performed using maltose as the substrate, and measuring the release of glucose. Absorbance measurements were averaged to give a final result. [b] NA: not active. [c] Reference 19. [d] Reference 18a. [e] Analysis of MGA inhibition was performed using *p*-nitrophenyl α-D-glucopyranoside as the substrate.

4′*R* because **6** is active and **5** is not. It would appear then that the common motif for activity is the *S*-configuration at C-2′ and an *R*-configuration at C-4′, the configuration of the stereogenic center C-3′ bearing the sulfate group being unimportant. The fact that compound **10**, with the *R*-configuration at C-2′, binds more weakly to MGA (K_i value of 41 ± 7 μM), while the sulfur analogue **9** lacks inhibitory activity, corroborates this conclusion, although these two compounds also possess the unwanted stereochemistry at C-5′. The selenonium ion **22**, the enantiomer of **12** at the stereogenic selenium ion, is less active (Table 1).

Finally, we comment on the effect of chain extension for inhibition of this enzyme. Comparison of K_i values for the active chain-extended analogues with those of salacinol **2** and blintol **4** (Table 1) indicates that increased polar interactions with the extra hydroxyl groups do not confer any large advantage (e.g., order of magnitude) for inhibition of this particular intestinal enzyme. The differences in inhibitory activities between the chain-extended analogues **7**, **8**, and **11** seem to suggest that, in the case of **11**, steric clashes of the hydroxyl groups with active site groups because of chain-extension might even be detrimental. These results are similar to those reported for kotalanol, with a 7-carbon-extended chain, in that no major differences were observed from the inhibitory activities of salacinol.[11] For comparison, the IC_{50} values of kotalanol were 0.58 μg/mL against sucrase, 2.8 μg/mL against maltase, and 1.9 μg/mL against isomaltase. The corresponding values for salacinol were 0.84, 3.2, and 0.59 μg/mL, respectively.

Experimental Section

Enzyme Activity Assay. Analysis of MGA inhibition was performed using maltose as the substrate, and measuring the release of glucose. Reactions were carried out in 100 mM MES buffer pH 6.5 at 37 °C for 15 min. The reaction was stopped by boiling for 3 min, and 20 μL aliquots were taken and added to 100 μL of glucose oxidase assay reagent (Sigma) in a 96-well plate. Reactions were allowed to proceed for 1 h, and the absorbance was measured at 450 nm to determine the amount of glucose produced by MGA activity in the reaction mixture. One unit of activity is defined as the hydrolysis of 1 mol of maltose per minute. All reactions were performed in triplicate, and absorbance measurements were averaged to give a final result.

Enzyme Kinetics. Kinetic parameters of recombinant MGA for compound **11** were determined as before[17a,21] using the glucose oxidase assay to follow the production of glucose upon addition of enzyme (15 nM) at increasing maltose concentrations (from 1 to 3.5 mM) with a reaction time of 15 min. The program GraFit 4.0.14 was used to fit the data to the Michaelis−Menten equation and estimate the kinetic parameters, K_m and V_{max}, of the enzyme. K_i values for each inhibitor were determined by measuring the rate of maltose hydrolysis by MGA at varying inhibitor concentrations. Data were plotted in Lineweaver−Burk plots (1/rate vs 1/[substrate]), and K_i values were determined by the equation $K_i = K_m$-$[I]/(V_{max})m - K_m$, where "*m*" is the slope of the line. The K_i reported for each inhibitor was estimated by averaging the K_i values obtained from each of the different inhibitor concentrations.

Enzyme Kinetics for Compounds 12 and 22. Kinetic parameters of MGA with compounds **12** and **22** were determined using the pNP-glucose assay to follow the production of *p*-nitrophenol upon addition of enzyme (500 nM). The K_i obtained for salacinol **2** in this assay was identical to that obtained using the glucose oxidase assay above. The assays were carried out in 96-well microtiter plates containing 100 mM MES buffer pH 6.5, inhibitor (at three different concentrations), and *p*-nitrophenyl-D-glucopyranoside (pNP-glucose, Sigma) as substrate (2.5, 3.5, 5, 7.5, 15, and 30 mM) with a final volume of 50 μL. Reactions were incubated at 37 °C for 35 min and terminated by addition of 50 μL of 0.5 M sodium carbonate. The absorbance of the reaction product was measured at 405 nm in a microtiter plate reader. All reactions were performed in triplicate, and absorbance measurements were averaged to give a final result. Reactions were linear within this time frame. The program GraFit 4.0.14 was used to fit the data to the Michaelis−Menten equation and estimate the kinetic parameters, K_m, K_{mobs} (K_m in the presence of inhibitor), and V_{max}, of the enzyme. K_i values for each inhibitor were determined by the equation $K_i = [I]/((K_{mobs}/K_m) - 1)$. The K_i reported for each inhibitor was determined by averaging the K_i values obtained from three different inhibitor concentrations.

4,6-*O*-Benzylidene-2,5-di-*O*-*p*-methoxybenzyl-D-mannitol (17). To a solution of 1,3:4,6-di-*O*-benzylidene-2,5-di-*O*-*p*-methoxybenzyl-D-mannitol (**16**)[22] (5.8 g, 9.6 mmol) in MeOH (150 mL) was added *p*-toluenesulfonic acid (160 mg), and the reaction mixture was stirred for 4 h at rt. The reaction was then quenched by addition of Et_3N, and the solvents were removed under vacuum to give a pale yellow syrup that was purified by flash column chromatography to give **15** as a white solid (3.62 g, 73%). mp 71−72 °C; $[\alpha]^{23}_D$ = −53.1° (c = 1.5, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 7.27−6.80 (13H, Ar−H), 5.31 (1H, s, Ph−CH−), 4.60 (2H, dd, MeO−Ph−CH_2−), 4.50 (2H, dd, MeO−Ph−CH_2−), 4.25 (1H, dd, $J_{6a,6b}$ = 10.6, $J_{6a,5}$ = 4.9 Hz, H-6a), 4.01 (1H, dd, $J_{3,2}$ = 9.6, $J_{3,3-OH}$ = 10.2 Hz, H-3), 3.93−3.78 (4H, m, H_2-1, H-4, H-5), 3.80, 3.72 (2×-OMe), 3.58 (2H, m, H-2, H-6b), 2.17 (1H, dd, OH-1), 2.09 (1H, d, OH-3). ^{13}C NMR ($CDCl_3$): δ 159.7−114.1 (18C, Ar), 100.6 (Ph−CH−), 78.5 (C-4), 76.6 (C-2), 72.8, 71.8 (2×-MeO−Ph−CH_2−), 69.8 (C-6), 68.9 (C-3), 67.6 (C-5), 61.8 (C-1), 55.5, 55.4 (2×-OMe). MALDI: *m*/*e* 532.48 (M^+ + Na). Anal. Calcd for $C_{29}H_{34}O_8$: C, 68.22; H, 6.71. Found: C, 68.02; H, 6.82.

4,6-*O*-Benzylidene-2,5-di-*O*-*p*-methoxybenzyl-D-mannitol 1,3-Cyclic Sulfite (18). A mixture of **17** (3.31 g, 6.5 mmol) and Et_3N (3.64 mL, 26.0 mmol) in CH_2Cl_2 (100 mL) was stirred in an ice bath. Thionyl chloride (0.7 mL, 9.7 mmol) in CH_2Cl_2 (10 mL) was then added dropwise over 20 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 (2 × 100 mL). The combined organic layers were washed with brine solution, dried over Na_2SO_4, and concentrated. Column chromatography (8:1, 5:1, 3:1 hexanes: EtOAc) gave the diastereomeric mixture of cyclic sulfites, **18** (2.74 g, 76%). Data for major isomer, ^{1}H NMR ($CDCl_3$): δ 7.48−6.84 (Ar−H), 6.02 (1H, Ph−CH−), 4.83 (1H, d, H-1a), 4.80 (1H, br t, H-3), 4.72−4.56 (4H, 2×-MeO−Ph−CH_2−), 4.22 (1H, dd, $J_{6a,6b}$ = 12.8, $J_{6a,5}$ = 3.7 Hz, H-6a), 4.16 (1H, m, H-5), 4.03 (1H, d, H-6b), 4.02 (1H, m, H-2), 3.96 (1H, br t, H-4), 3.81, 3.79 (6H,

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2×-OMe), 3.61 (1H, dd, $J_{1a,1b}$ = 12.9, $J_{1b,2}$ = 3.2 Hz, H-1b). ^{13}C NMR ($CDCl_3$): δ 159.6−114.0 (18C, Ar), 103.9 (Ph−CH−), 80.1 (C-5), 79.3 (C-3), 77.3 (C-4), 76.7 (C-2), 73.1, 72.0 (2×-Ph−CH_2−), 61.4 (C-6), 58.2 (C-1), 55.5 (2C, 2×-OMe). MALDI: *m*/*e* 579.6 (M^+ + Na). Anal. Calcd for $C_{29}H_{32}O_9S$: C, 62.58; H, 5.79. Found: C, 62.80; H, 6.08.

4,6-*O*-Benzylidene-2,5-di-*O*-*p*-methoxybenzyl-D-mannitol 1,3-Cyclic Sulfate (13). To a solution of compound **18** (2.58 g, 4.63 mmol) in a mixture of CH_3CN:CCl_4 (100 mL) were added sodium periodate (1.48 g, 6.95 mmol) and $RuCl_3$ (100 mg), followed by H_2O (20 mL). The mixture was then stirred for 2 h at rt. The reaction mixture was filtered through a silica bed and washed repeatedly with EtOAc. The volatile solvents were removed, and the aqueous solution was extracted with EtOAc (2 × 100 mL). The combined organic layer was washed with saturated NaCl, dried over Na_2SO_4, and evaporated under diminished pressure. The residue was purified by flash column chromatography to give **13** as a white solid (2.30 g, 86%). mp 98 °C; $[\alpha]^{23}_D$ = −44.6° (*c* = 0.1, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 7.39−6.77 (13H, Ar−H), 5.32 (1H, s, Ph−CH−), 5.08 (1H, d, $J_{3,2}$ = 9.8 Hz, H-3), 4.50 (4H, 2×-MeO−Ph−CH_2−), 4.46 (1H, dd, $J_{1a,2}$ = 4.8 Hz, H-1a), 4.32 (1H, dd, $J_{1b,1a}$ = 10.1, $J_{1b,2}$ = 9.6 Hz, H-1b), 4.29 (1H, dd, $J_{6a,5}$ = 5.6 Hz, H-6a), 4.21 (1H, ddd, H-2), 3.98 (1H, d, $J_{4,5}$ = 9.6 Hz, H-4), 3.81, 3.72 (2×-OMe), 3.80 (1H, m, H-5), 3.60 (1H, dd, $J_{6a,6b}$ = 10.4, $J_{6b,5}$ = 10.2 Hz, H-6b). ^{13}C NMR ($CDCl_3$): δ 160.0−114.0 (18C, Ar), 101.5 (Ph−CH−), 82.2 (C-3), 76.3 (C-4), 73.3, 72.8 (2×-MeO−Ph−CH_2−), 71.9 (C-1), 69.6 (C-6), 66.0 (C-5), 64.8 (C-2), 55.5, 55.4 (2×-OMe). MALDI: *m*/*e* 595.6 (M^+ + Na). Anal. Calcd for $C_{29}H_{32}O_{10}S$: C, 60.83; H, 5.63. Found: C, 60.81; H, 5.66.

2,3,5-Tri-*O*-*p*-methoxybenzyl-1,4-dideoxy-1,4-[[(2*S*,3*S*,4*R*,5*R*)-4,6-*O*-benzylidene 2,5-Di-*O*-*p*-methoxybenzyl-3-(sulfooxy)hexyl]-(*R*)-epi-sulfoniumylidene]-D-arabinitol Inner Salt (19). The thio-arabinitol **14** (212 mg, 0.42 mmol) and the cyclic sulfate **13** (296 mg, 0.52 mmol) were added to 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) (3 mL) containing anhydrous K_2CO_3 (40 mg). The mixture was stirred in a sealed tube at 65−70 °C for 42 h. The solvent was removed under reduced pressure, and the residue was purified by flash column chromatography (3:1 hexanes/EtOAc and then 20:1, 15:1 EtOAc/MeOH). The coupled product, **19**, was obtained as a white amorphous solid (350 mg, 77%). $[\alpha]^{23}_D$ = −53.6° (*c* = 0.1, CH_2Cl_2). ^{1}H NMR (*d*$_6$-acetone): δ 5.37 (1H, s, Ph−CH−), 4.92 (1H, d, $J_{3',2'}$ = 9.1 Hz, H-3′), 4.83−4.54 (10H, 5×-MeO−Ph−CH_2−), 4.50 (1H, m, H-3), 4.32 (3H, m, H-1a′, H-2′, H-6a′), 4.23 (1H, dd, $J_{1b',1a'}$ = 13.6, $J_{1b',2'}$ = 4.5 Hz, H-1b′), 4.16 (1H, m, H-5′), 4.06 (1H, m, H-1a), 3.97−3.81 (3H, m, H-4, H-4′, H-1b), 3.80−3.66 (15H, 5×-OMe), 3.70 (2H, m, H_2-5), 3.51 (1H, m, H-6b′). ^{13}C NMR (*d*$_6$-acetone): δ 159.9−113.5 (36C, Ar), 100.6 (Ph−CH−), 83.6 (C-3), 81.9 (C-2), 77.9 (C-4′), 72.9 (C-2′), 72.4 (C-3′), 72.7, 72.6, 71.7, 71.4, 71.3 (5×-MeO−Ph−CH_2−), 69.9 (C-6′), 67.2 (C-5′), 66.7 (C-5), 65.2 (C-4), 54.8, 55.7 (5×-OMe), 50.9 (C-1′), 48.0 (C-1). MALDI: *m*/*e* 1083.64 (M^+ + H), 1003.29 (M^+ + H − SO_3). Anal. Calcd for $C_{58}H_{66}O_{16}S_2$: C, 64.31; H, 6.14. Found: C, 64.61; H, 5.82.

1,4-Dideoxy-1,4-[[(2*S*,3*S*,4*R*,5*R*)-2,4,5,6-tetrahydroxy-3-(sulfooxy)hexyl]-(*R*)-epi-sulfoniumylidene]-D-arabinitol Inner Salt (11). To a solution of compound **19** (240 mg, 0.22 mmol) in CH_2Cl_2 (1 mL) were added trifluoroacetic acid (10 mL), followed by H_2O (4 mL). The mixture was stirred at room temperature for 2 h. The solvents were then evaporated under diminished pressure, and the residue was purified by flash column chromatography to give **11** as a white amorphous solid (72 mg, 82%). $[\alpha]^{23}_D$ = −3.6° (*c* = 0.1, MeOH). ^{1}H NMR (D_2O): δ 4.77 (1H, ddd, $J_{2,1}$ = 4.0, $J_{2,3}$ = 3.7 Hz, H-2), 4.67 (1H, d, $J_{3',2'}$ = 8.1 Hz, H-3′), 4.48 (1H, dd, $J_{3,4}$ = 3.2 Hz, H-3), 4.44 (1H, ddd, $J_{2',1a'}$ = 3.3, $J_{2',1b'}$ = 7.7 Hz, H-2′), 4.14 (1H, dd, $J_{5a,4}$ = 5.0 Hz, $J_{5a,5b}$ = 10.8, H-5a), 4.10 (2H, m, H-1a′, H-4), 3.98 (1H, dd, $J_{5b',4}$ = 7.1 Hz, H-5b), 3.93 (1H, dd, $J_{1b',1a'}$ = 13.6 Hz, H-1a′), 3.91−3.87 (5H, m, H_2-1, H-4′, H-5′, H-6a′), 3.54 (1H, m, H-6b′). ^{13}C NMR (D_2O): δ 78.3 (C-3′), 77.8 (C-3), 76.8 (C-2), 70.1 (C-4′, C-4), 68.8 (C-5′), 66.1 (C-2′), 62.9 (C-6′), 59.2 (C-5), 50.8 (C-1′), 47.9 (C-1). MALDI: *m*/*e* 395.36 (M^+ + H), 417.58 (M^+ + Na). HRMS calcd for $C_{11}H_{22}O_{11}S_2$ (M − H): 393.05119. Found: 393.05215.

2,3,5-Tri-*O*-*p*-methoxybenzyl-1,4-dideoxy-1,4-[[(2*S*,3*S*,4*R*,5*R*)-4,6-*O*-benzylidene-2,5-di-*O*-*p*-methoxybenzyl-3-(sulfooxy)hexyl]-(*R*/*S*)-epi-seleniumylidene]-D-arabinitol Inner Salt (20 and 21). To HFIP (3 mL) were added 1,4-dideoxy-2,3,5-tri-*O*-*p*-methoxybenzyl-1,4-anhydro-4-seleno-D-arabinitol **15** (254 mg, 0.45 mmol), 4,6-*O*-benzylidene-2,5-*O*-di-*p*-methoxybenzyl-D-mannitol-1,3-cyclic sulfate (**13**) (318 mg, 0.55 mmol), and anhydrous K_2CO_3 (40 mg). The mixture was stirred in a sealed tube at 65−70 °C for 36 h. The solvent was removed under reduced pressure, and the residue was purified by flash column chromatography (3:1 hexanes:EtOAc and then 15:1 EtOAc:MeOH) to give **20** (286 mg, 56%) and **21** (122 mg, 23%) in a 5:2 ratio as white amorphous solids. Data for trans isomer (**20**): $[\alpha]^{23}_D$ = −19.5° (*c* = 0.4, CH_2Cl_2). ^{1}H NMR (*d*$_6$-acetone): δ 7.45−6.79 (25H, Ar−H), 5.21 (1H, s, Ph−CH−), 4.83 (1H, br d, $J_{3',2'}$ = 8.9 Hz, H-3′), 4.81−4.39 (10H, 5×-MeO−Ph−CH_2−), 4.73 (1H, br dd, H-2), 4.55 (1H, br dd, H-3), 4.30−4.19 (4H, m, H_2-1′, H-2′, H-6a′), 4.13 (1H, m, H-5′), 4.09 (1H, br d, $J_{1a,1b}$ = 13.1, H-1a), 4.04 (1H, m, H-4), 3.84−3.63 (15H, 5×-OMe), 3.81 (1H, m, H-4′), 3.79 (1H, m, H-5a), 3.72 (1H, m, H-1b), 3.71 (1H, dd, H-5b), 3.46 (1H, dd, H-6b′). ^{13}C NMR (*d*$_6$-acetone): δ 159.6−113.2 (36C, Ar), 100.2 (Ph−CH−), 84.4 (C-3), 82.4 (C-2), 77.9 (C-4′), 73.0 (C-3′), 72.4 (C-2′), 72.8, 72.6, 71.5, 71.4, 71.3 (5×-MeO−Ph−CH_2−), 69.8 (C-6′), 67.4 (C-5′), 67.3 (C-5), 64.8 (C-4). 54.7 (5×-OMe), 50.0 (C-1′), 45.8 (C-1). MALDI: *m*/*e* 1131.51 (M^+ + H), 1051.74 (M^+ + H − SO_3). Anal. Calcd for $C_{58}H_{66}O_{16}SSe$: C, 61.64; H, 5.89. Found: C, 61.31; H, 5.66. Data for cis isomer (**21**): $[\alpha]^{23}_D$ = −15.7° (*c* = 0.1, CH_2Cl_2). ^{1}H NMR (*d*$_6$-acetone): δ 7.31−6.81 (25H, Ar−H), 5.30 (1H, s, Ph−CH−), 4.86 (1H, br d, $J_{3',2'}$ = 9.0 Hz, H-3′), 4.82−4.45 (10H, 5×-MeO−Ph−CH_2−), 4.74 (1H, br s, H-2), 4.68 (1H, br s, H-3), 4.41 (1H, m, H-4), 4.29 (2H, m, H-2′, H-6a′), 4.21 (1H, dd, $J_{5a,5b}$ = 9.9, $J_{5a,4}$ = 5.4 Hz, H-5a), 4.18 (1H, m, H-5′), 4.06 (2H, m, H_2-1), 4.00 (1H, dd, $J_{5b,4}$ = 9.4 Hz, H-5b), 3.83 (1H, m, H-4′), 3.80−3.65 (15H, 5×-OMe), 3.63 (1H, m, H-1a), 3.56 (1H, br d, $J_{1b,1a}$ = 12.9, H-1b), 3.48 (1H, dd, $J_{6b',6a'}$ = 10.4, $J_{6b',5'}$ = 10.2 Hz, H-6b′). ^{13}C NMR (*d*$_6$-acetone): δ 159.8−114.0 (36C, Ar), 100.6 (Ph−CH−), 83.2 (C-2), 82.6 (C-3), 77.8 (C-4′), 73.1 (C-3′), 72.8 (C-2′), 72.7, 72.1, 71.6, 71.2, 71.2 (5×-MeO−Ph−CH_2−), 69.8 (C-6′), 67.2 (C-5′), 65.6 (C-5), 58.4 (C-4), 54.8, 54.7 (5×-OMe), 42.3 (C-1), 41.0 (C-1′). MALDI: *m*/*e* 1131.31 (M^+ + H), 1051.62 (M^+ + H − SO_3). Anal. Calcd for $C_{58}H_{66}O_{16}SSe$: C, 61.64; H, 5.89. Found: C, 61.30; H, 5.97.

1,4-Dideoxy-1,4-[[(2*S*,3*S*,4*R*,5*R*)-2,4,5,6-tetrahydroxy-3-(sulfooxy)hexyl]-(*R*/*S*)-epi-seleniumylidene]-D-arabinitol Inner Salts (12 and 22). The selenonium salts **20** (240 mg, 0.212 mmol) and **21** (102 mg, 0.09 mmol) were deprotected separately using aq TFA following the same procedure that was used for compound **11**, to give compounds **12** (80 mg, 85%) and **22** (32 mg, 81%), respectively. Data for the trans isomer, **12**: $[\alpha]^{23}_D$ = +16.1° (*c* = 0.1, MeOH). ^{1}H NMR (D_2O): δ 4.82 (1H, dt, $J_{2,1}$ = 3.8, $J_{2,3}$ = 3.7 Hz, H-2), 4.63 (1H, d, $J_{3',2'}$ = 7.7 Hz, H-3′), 4.52 (1H, dd, $J_{3,4}$ = 3.2 Hz, H-3), 4.44 (1H, ddd, $J_{2',1a'}$ = 4.0, $J_{2',1b'}$ = 7.2 Hz, H-2′), 4.19 (1H, ddd, $J_{4,5a}$ = 5.1 Hz, $J_{4,5b}$ = 8.9, H-4), 4.13 (1H, dd, $J_{1a',1b'}$ = 12.3 Hz H-1a′), 4.10 (1H, dd, $J_{5a,5b}$ = 12.6 Hz, H-5a), 3.98 (1H, dd, H-5b), 3.95 (1H, dd, H-1b′), 3.86 (3H, m, H-4′, H-5′, H-6a′), 3.82 (2H, d, H_2-1), 3.69 (1H, dd, $J_{6b',6a'}$ = 12.3, $J_{6b',5'}$ = 5.7 Hz, H-6b′). ^{13}C NMR (D_2O): δ 78.6 (C-3′), 77.6 (C-3), 77.6 (C-2), 70.1 (C-5′), 70.0 (C-4), 68.9 (C-4′), 66.1 (C-2′), 62.9 (C-6′), 59.4 (C-5), 49.1 (C-1′), 44.9 (C-1). MALDI: *m*/*e* 465.37 (M^+ + Na), 363.29 (M^+ + H − SO_3). HRMS calcd for $C_{11}H_{22}O_{11}SSe$ (M − H): 440.9964. Found: 440.9963. Data for the cis isomer, **22**: $[\alpha]^{23}_D$ = +41.6° (*c* = 0.1, MeOH). ^{1}H NMR (D_2O): δ 4.75 (1H, ddd, $J_{2,1b}$ = $J_{2,1b}$ = 3.4, $J_{2,3}$ = 3.8 Hz, H-2), 4.64 (1H, d, $J_{3',2'}$ = 8.0 Hz, H-3′), 4.56 (1H, t, $J_{3,4}$ = 3.8 Hz, H-3), 4.41 (1H, ddd, $J_{2',1a'}$ = 3.8, $J_{2',1b'}$ = 7.8 Hz, H-2′), 4.26 (1H, ddd, $J_{4,5a}$= 5.5 Hz, $J_{4,5b}$ = 9.0,

H-4), 4.21 (1H, dd, $J_{5a,5b}$ = 12.2 Hz H-5a), 4.10 (2H, m, H-5b, H-1a′), 3.91 (1H, dd, $J_{1b',1a'}$ = 12.5 Hz, H-b′), 3.84 (4H, m, H-1a, H-4′, H-5′, H-6a′), 3.67 (1H, dd, $J_{6b',6a'}$ = 12.3, $J_{6b',5'}$ = 4.9 Hz, H-6b′), 3.62 (1H, dd, $J_{1b,1a}$ = 12.7 Hz, H-1b). ^{13}C NMR (D_2O): δ 79.0 (C-3′), 78.6 (C-2), 78.2 (C-3), 70.1 (C-5′), 69.0 (C-4′), 66.0 (C-2′), 64.1 (C-4), 62.9 (C-6′), 58.2 (C-5), 42.6 (C-1), 41.5 (C-1′). MALDI: *m/e* 465.29 ($M^+ + Na$), 363.23 ($M^+ + H - SO_3$). HRMS calcd for $C_{11}H_{22}O_{11}SSe$ (M − H): 440.9964. Found: 440.9961.

Acknowledgment. We are grateful to the Canadian Institutes of Health Research for financial support and to B. D. Johnston for helpful discussions.

Supporting Information Available: General experimental and copies of ^{1}H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

JO061944V