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Synthesis of novel ammonium and selenonium ions and their evaluation as inhibitors of UDP-galactopyranose mutase

Natacha Veerapen,[a] Yue Yuan,[a] David A. R. Sanders[b] and B. Mario Pinto[a,*]

[a]*Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6*
[b]*University of Saskatchewan, 110 Science Place, Saskatoon, Saskatchewan, Canada S7N 5C9*

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Abstract—The syntheses of two ammonium salts of 1,4-dideoxy-1,4-imino-D-galactitol containing erythritol sulfate side chains are described. The parent compound is a known inhibitor of the enzyme UDP-galactopyranose mutase (UDP-galactopyranose furanomutase, E.C. 5.4.99.9), which is responsible for the conversion of UDP-galactopyranose into UDP-galactofuranose and is presumably protonated in its active form. The side chain was chosen because it is present in a known sulfonium ion α-glucosidase inhibitor, salacinol. The syntheses of the selenonium analogues derived from 1,4-dideoxy-1,4-seleno-D-galactitol are also described. The synthetic strategy in the syntheses of all four salts involved the nucleophilic attack of a protected derivative of the alditol at the least hindered carbon of 2,4-*O*-benzylidene D- or L-erythritol-1,3-cyclic sulfate to give adducts that were subsequently deprotected. The importance of different protecting groups used in the various syntheses is also highlighted. Enzyme inhibition assays carried out on these compounds, and the corresponding sulfonium ions synthesized previously, show that they are poor inhibitors of UDP-galactopyranose mutase.

Keywords: Galactofuranose; Galactofuranosyltransferase; UDP-galactopyranose mutase; Inhibitors; Cyclic sulfate; Ammonium ions; Selenonium ions

1. Introduction

D-Galactofuranose (Gal*f*) residues are found in the cell walls of many mycobacteria[1] and in other microorganisms such as protozoa[2] and fungi.[3] Gal*f* structures are components of the oligosaccharide core of both the glycoinositolphospholipid (GIPL) and lipopeptidophosphoglycan (LPPG) of *Trypanosoma cruzi* as well as the lipophosphoglycan (LPG) of *Leishmania* species.[4–6] These protozoa are known to cause life-threatening diseases, like Chagas disease and leishmaniasis.[4] Mycobacteria cause pathogenic diseases like tuberculosis (*Mycobacterium tuberculosis*) and leprosy (*Mycobacterium leprae*).[1] Of particular interest is the fact that the cell walls of most of these mycobacteria consists largely of internal (1→5)-β-D-Gal*f* or (1→6)-β-D-Gal*f* residues.[1] Importantly, Gal*f* is absent in mammalian glycoconjugates,[4] and its presence in cell-wall structures appears to be essential for the survival and infectivity of microorganisms. Hence, the enzymes involved in both the formation and incorporation of Gal*f* in bacteria and parasites have become important drug targets.

UDP-galactofuranose (UDP-Gal*f*) is known to be the activated precursor for the construction of Gal*f*-containing oligosaccharides, and it is synthesized from UDP-galactopyranose (UDP-Gal*p*) in a reaction catalyzed by UDP-galactopyranose mutase.[7,8] Gal*f* is then transferred onto acceptors to form various glycoconjugates by galactofuranosyltransferase. UDP-galactopyranose mutase is essential for the viability of mycobacteria,[9] suggesting that the inhibition of either the interconversion of UDP-Gal*p* to UDP-Gal*f* or the transfer of the Gal*f* residue (galactosylation) is a promising strategy for the development of new therapeutic agents.

The mechanism of UDP-Gal*p* mutase, a flavoprotein in which the FAD coenzyme is noncovalently bound to the protein, is still the subject of active investigation.

* Corresponding author. Tel.: +1-604-291-4327; fax: +1-604-291-5424; e-mail: bpinto@sfu.ca

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Nonetheless, the direct participation of the flavin coenzyme[10,11] as well as the formation of the iminium ion intermediate **C**[11] have been established (Scheme 1). Whether the reduced flavin transfers a single electron to the oxacarbenium ion intermediate **A** (pathway **I**)[10,12] or acts as a nucleophile (pathway **II**), as suggested by Soltero-Higgin et al.,[11] to form the coenzyme–substrate adduct **B** remains uncertain at this time. However, both the radical mechanism, initially proposed by Fullerton et al.[12] and subsequently supported by Liu and co-workers[10] and that proposed by Soltero-Higgin et al.[11] seem to reconcile most of the findings obtained to date.

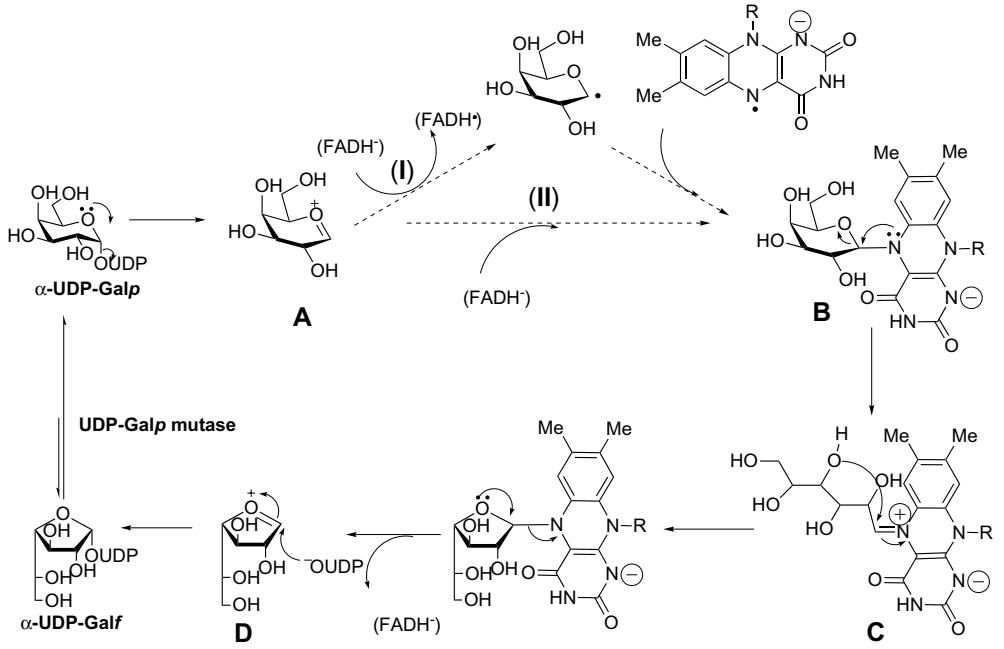
While the formation of a bicyclic intermediate proposed by Blanchard and co-workers[13,14] is now ruled out, the participation of the 4-OH in the ring formation is still invoked.[10,11] This is contradictory to the results obtained by Burton et al.[15] who found that UDP-[4-F] Gal*p*, which is expected to inhibit the enzyme mutase if the 4-OH group were crucial in the ring contraction, showed no inhibitory activity against the enzyme galactopyranose mutase in the direction pyranose → furanose, but it did inhibit the reverse reaction (furanose → pyranose).

Of interest to us was that the proposed mechanism involves the reversible cleavage of the anomeric C–O bond with the departure of the nucleotide to form a transient oxacarbenium ion-like transition state or intermediate, in accordance with preliminary studies carried out by Blanchard and co-workers.[13,14] The formation of such a species is further supported by a mechanistic investigation carried out on fluorinated analogues of UDP-Gal*f*, which revealed that UDP-[2-F]Gal*f* and UDP-[3-F]Gal*f* were potent inactivators of Gal*p* mutase.[16] The authors suggested that the inactivation could result from the formation of a covalent adduct between the enzyme and the inactivators. It was suggested further that compounds that lead to oxacarbenium ion-type intermediates or transition states resembling **A** and **D** could function as inhibitors of the enzyme by forming stable covalent adducts with the nucleophilic amino acid residues present in the enzyme active site.[16]

The second class of enzymes of interest, the galactofuranosyltransferases, transfers Gal*f* residues to glycosyl acceptors to give Gal*f*-containing glycoconjugates and oligosaccharides. Mechanistically, the reaction catalyzed by glycosyltransferases is believed to be similar to that of glycosidases,[17] also involving an oxacarbenium-ion-like transition state.[18,19]

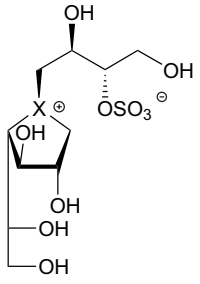
We report herein the syntheses of 4-imino Gal*f* ammonium salts **1** and **2** as potential inhibitors of UDP-Gal*p* mutase and Gal*f*-transferase. This reasoning is based on the fact that the parent iminogalactitol **7** is a known inhibitor of Gal*p* mutase[20] and is likely to be protonated in the active site.[21] The choice of the erythritol sulfate side chain derives from its presence in the known glycosidase inhibitors salacinol **8**[22–24] and ghavamiol **9**.[25] The sulfate counterion is also believed to contribute to the stability of these salts, thereby further supporting its presence in our designed compounds.

We report also the synthesis of the corresponding selenonium salts **3** and **4**; we have previously reported the synthesis of the analogous sulfonium ions **5** and **6**.[26] We propose to test the hypothesis that the permanent positive charge, as well as the shape of the salts

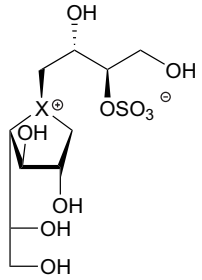


Scheme 1.

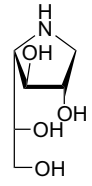
1–**6** could mimic the oxacarbenium-like transition states of the enzymatic reactions described above, and therefore function as inhibitors. We also report their evaluation as inhibitors of UDP-Gal*p* mutase.



1 X= NH
3 X= Se
5 X= S



2 X= NH
4 X= Se
6 X= S



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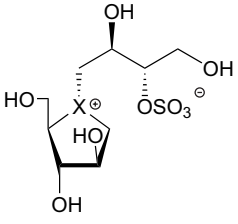
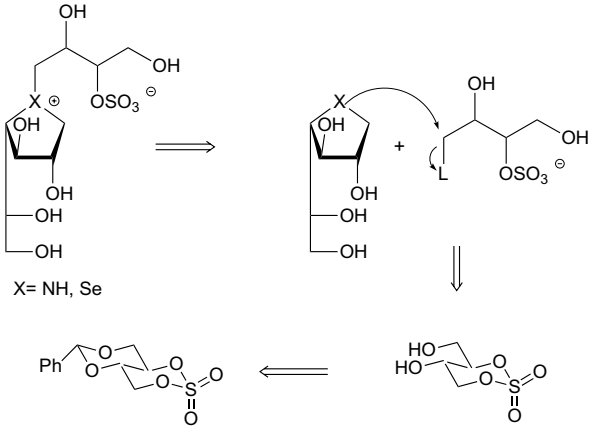


8 X= S
9 X= NH

2. Results and discussion

Retrosynthetic analysis showed that the target compounds could be obtained by the alkylation of the heteroatom of suitably protected cyclic alditols (Scheme 2). We chose 2,4-*O*-benzylidene-D-(**10**) and L-(**11**)erythritol-1,3-cyclic sulfates as alkylating agents, given the success of similar alkylation reactions in our laboratory.[24,25]

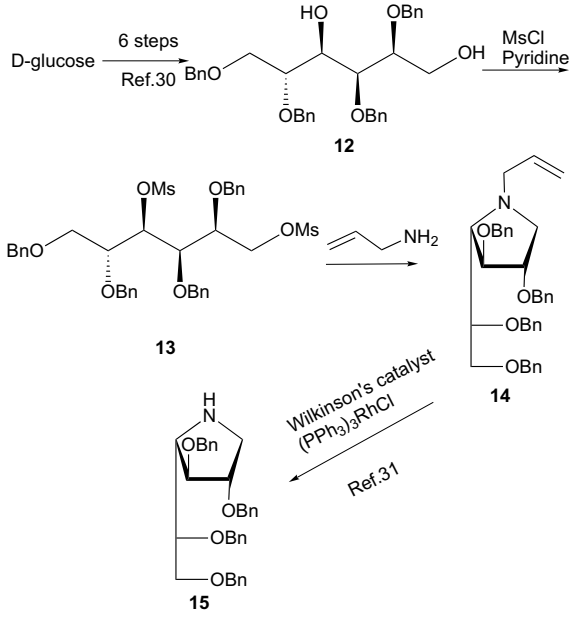


X= NH, Se

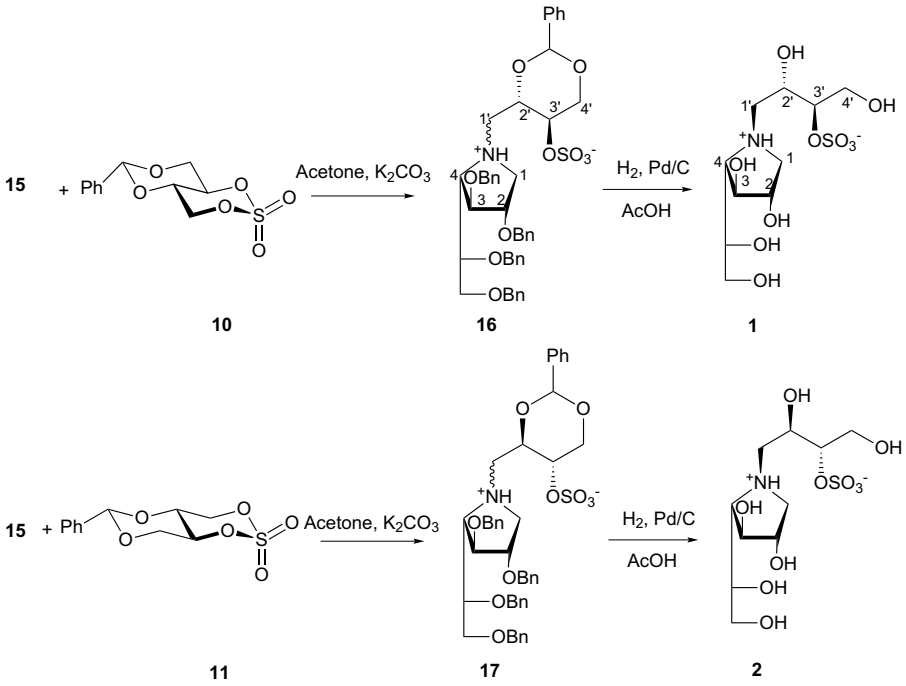
Scheme 2.

Various syntheses of 1,4-dideoxy-1,4-imino-D-galactitol have been previously reported by different research groups.[27–30] However, we used an alternative pathway to obtain 1,4-dideoxy-1,4-imino-D-galactitol en route to our target compounds. This method, similar to the procedure of Lee et al.,[27] involved the epimerization at C-4 of a benzyl-protected glucose derivative **13** as the key step (Scheme 3). The diol **12**, prepared from D-glucose,[31] was converted into the dimesylate **13**, which was then reacted with allylamine in an S_N2-type reaction to give the tertiary amine **14** in 83% yield. Unlike Lee et al.,[27] we chose allylamine rather than benzylamine because it permitted selective deprotection of the nitrogen atom, without affecting the benzyl protecting groups. Subsequent treatment of **14** with Wilkinson's catalyst[32] gave the iminogalactitol derivative **15** in 70% yield.

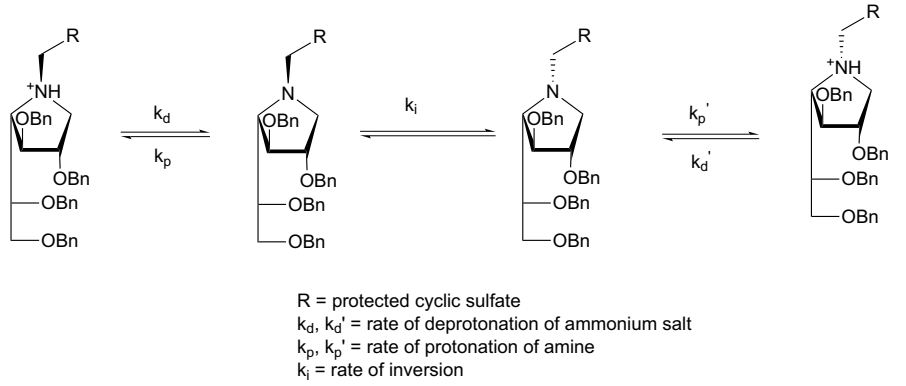
The coupling reaction of the benzylated derivative **15** with 2,4-*O*-benzylidene-D-erythritol-1,3-cyclic sulfate **10**[24] was then investigated. Thus, when a mixture of **15** and the cyclic sulfate **10** in dry acetone was heated at 70 °C in the presence of anhydrous potassium carbonate for 12 h, the desired coupled product **16** was obtained in 68% yield (Scheme 4). In an attempt to improve the yield of the coupling reaction and also to investigate the reactivity of the imino sugar, the deprotected galactitol **7**, obtained by hydrogenolysis of **15**, was reacted with the D-cyclic sulfate **10**. Owing to the insolubility of the deprotected compound **7** in acetone, the reaction was performed in dry methanol. When the mixture was heated at 60 °C for 12 h, TLC analysis showed the formation of two additional polar products as well as the presence of large amounts of both starting materials. Increasing



Scheme 3.



Scheme 4.



Scheme 5.

the temperature to 70 °C for longer reaction times only led to the decomposition of both the products and the cyclic sulfate.[33] It would appear that the protection of the hydroxyl groups as benzyl ethers makes the compound more nucleophilic. Diastereomer **17** was obtained similarly by the reaction of compound **15** with the L-cyclic sulfate **11**[24] in 74% yield (Scheme 4).

Compounds **16** and **17** were completely characterized by 2D NMR techniques. Initially, the NMR studies for both **16** and **17** were carried out in CD_2Cl_2 and CD_3OD, and it was observed that the peaks were broad and overlapping in both cases. However, when the NMR studies were performed on samples of compounds **16** and **17** in deuterated methanol, made basic with small amounts of sodium deuteroxide to give the corresponding amines, the peaks were more defined. We believe that the broadening of the peaks in the absence of base is due to chemical exchange between the ammonium salts and the corresponding tertiary amines, a process that is in the intermediate-exchange regime on the NMR time scale (Scheme 5). In the presence of base, only the rapidly inverting tertiary amine is present, and a conformationally averaged NMR spectrum in the fast-exchange regime is observed.

Deprotection of **16** and **17** was achieved by hydrogenolysis over Pd/C in 80% acetic acid to yield the diastereomers **1** and **2**, respectively. The ^1H and ^{13}C NMR spectra for both diastereomers obtained in CD_3OD were essentially identical, except for small changes in the chemical shifts. Interestingly, sharp peaks were obtained for the deprotected compounds **1** and **2** in the absence of base. Apparently, chemical exchange

between the ammonium salt and the corresponding tertiary amine in compounds **1** and **2** is slower than in compounds **16** and **17** (Scheme 5).

To verify the hypothesis of chemical exchange, 1D-transient NOE experiments were performed on **16** and **1** in deuterated methanol at 285 K. If chemical exchange were to occur, exchange peaks would display the same phase as that of the originally saturated resonance.[34] On the other hand, in the absence of any chemical exchange, only positive NOE enhancements would be observed.[34] For the protected compound **16**, when the multiplets containing H-1′a and H-1′b were selectively irradiated in separate experiments, enhancement of the H-1′b and H-1′a resonances, respectively, were observed. The irradiated and observed peaks were of the same phase, thereby confirming that chemical exchange, together with NOE transfer, were occurring on the NMR time scale (Fig. 1b and c). It should be noted that the tentative assignments of the broad NMR spectrum of **16** (Fig. 1a) are based on the assignments made for the spectrum in basic CD_3OD. With deprotected compound **1**, when the multiplets containing H-1b and H-1′a, were selectively irradiated, in separate experiments, only positive NOE enhancements of the relevant resonances were observed (Fig. 1e and f). Irradiation of the multiplet containing H-1b and H-4 gave rise to enhancements in the resonances corresponding to H-1′b and H-1′a while irradiation of the multiplet containing H-1′a gave rise to an enhancement of the resonance corresponding to both protons H-1b and H-4. This study suggests that the stereochemistry at the nitrogen center is fixed or that chemical exchange is slow on the NMR time scale in the deprotected compounds. The 1D-transient NOE results obtained at 285 K did not allow us to determine which pair of protons is on the same face of the ring. However, a NOESY experiment carried out at 298 K showed correlations between H-4 and H-1′b and H-1b and H-1′a, respectively, indicating that the stereochemistry at the stereogenic nitrogen center in **1** is such that the erythritol side chain is preponderantly trans to the chain at C-5 of the anhydrogalactitol moiety.

The stereochemistry at the stereogenic nitrogen center in **2** was also established by means of a NOESY experiment. The correlation between H-1′ and H-4 confirmed the trans relationship between the erythritol side chain and the chain at C-5 of the anhydrogalactitol moiety, and also confirmed that the compound maintained its stereochemical integrity.

The selenonium analogues **3** and **4** were synthesized using a similar approach as for their ammonium congeners. However, previous experience with selenonium salts in our laboratory had shown that the hydrogenolysis of the benzyl ethers in these compounds is difficult

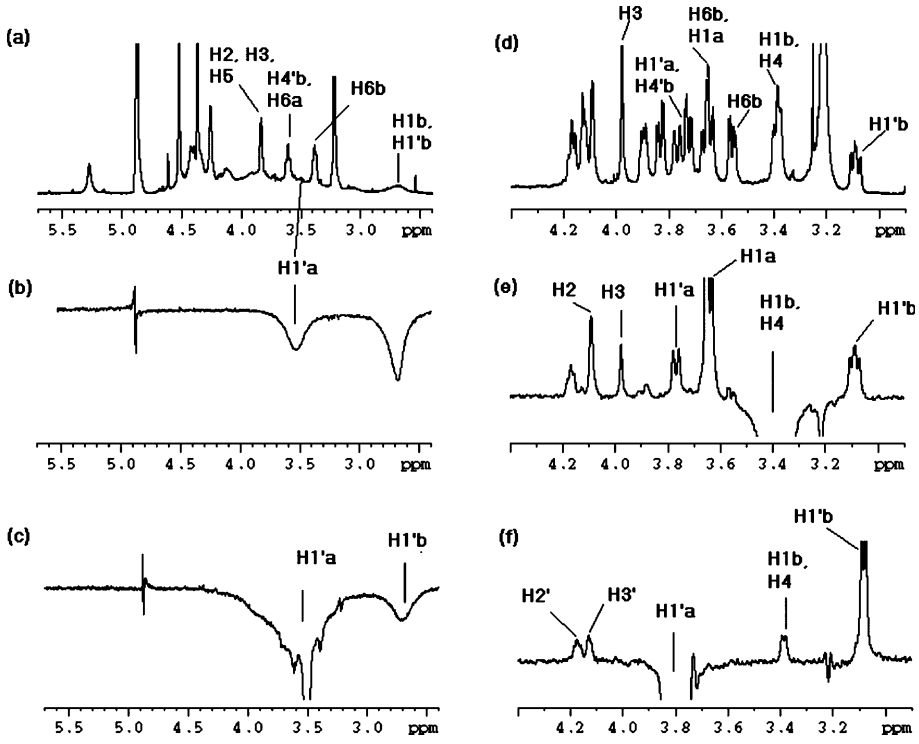


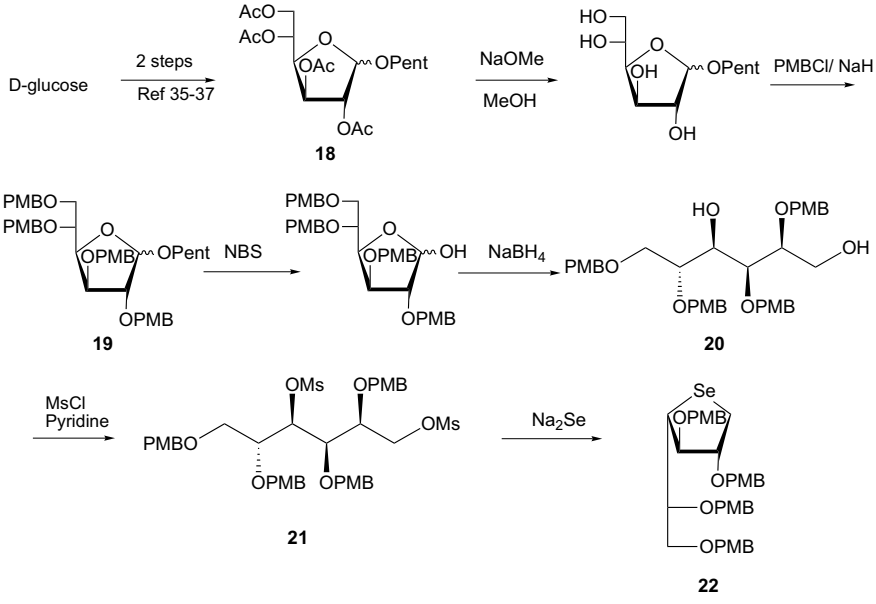
Figure 1. (a) 1D ^{1}H NMR spectrum of **16** in CD_3OD, (b) 1D-transient NOE spectrum of **16** in CD_3OD upon irradiation of the multiplet containing H-1′b, (c) 1D-transient NOE spectrum of **16** in CD_3OD upon irradiation of the multiplet containing H-1′a, (d) 1D ^{1}H NMR spectrum of **1** in CD_3OD, (e) 1D-transient NOE spectrum of **1** in CD_3OD upon irradiation of the multiplet containing H-1b and H-4 and (f) 1D-transient NOE spectrum of **1** in CD_3OD upon irradiation of the multiplet containing H-1′a.

and low yielding, explaining our choice of *p*-methoxybenzyl (PMB) ether groups instead. *p*-Methoxybenzyl (PMB) ethers, while having similar chemical properties as benzyl ethers, do not require hydrogenolysis. However, they are acid labile and therefore noncompliant with the synthetic route used to make the protected iminogalactitol derivative **15** (Scheme 3). A modified synthetic pathway in which the anomeric OH could be selectively deprotected in the presence of PMB ethers was therefore desired. Both allyl and pentenyl glycosides are suitable options for this modified synthetic route. However, in our hands, allyl deprotection was low yielding and expensive due to the requirement of $PdCl_2$ in virtually stoichiometric amounts.

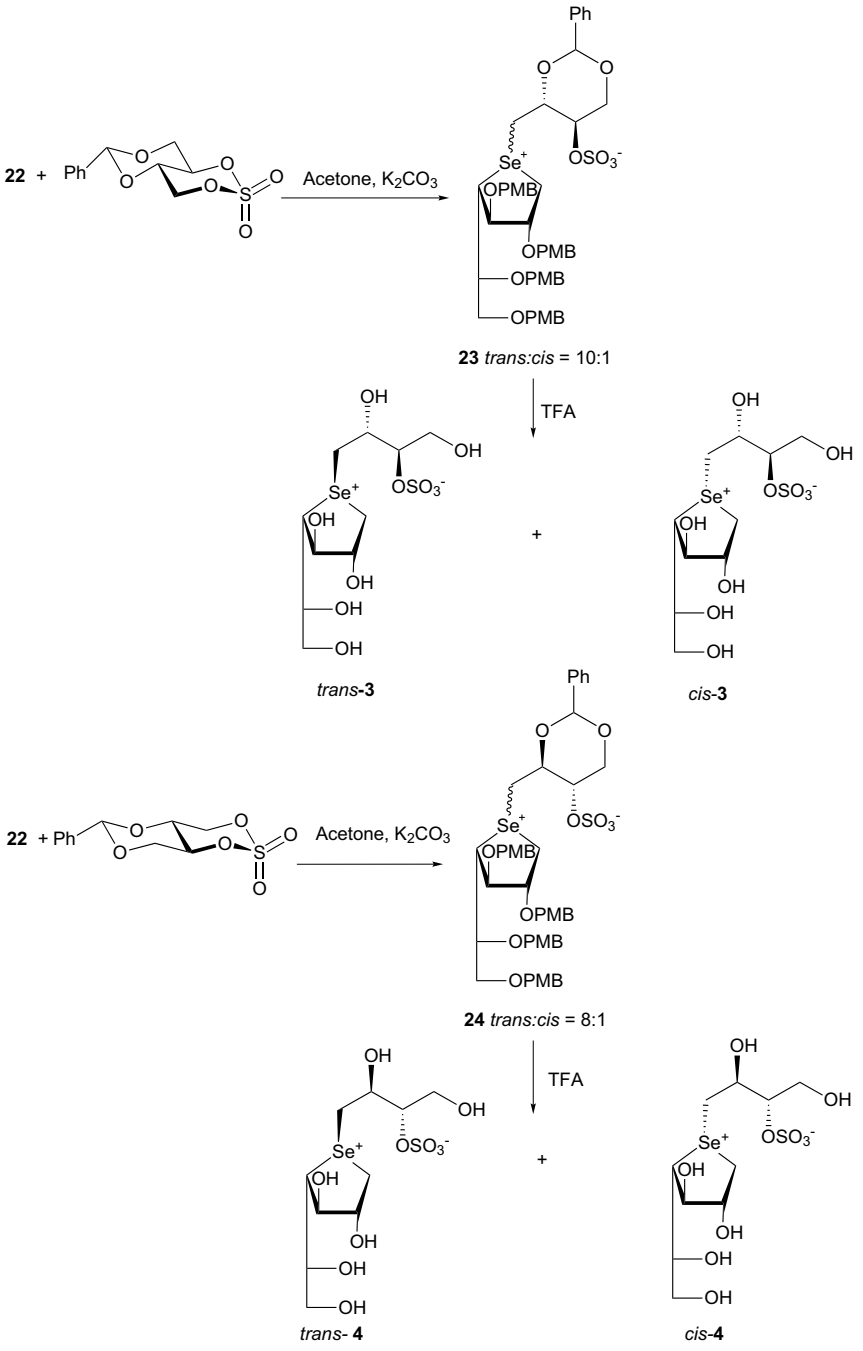
The pentenyl glycoside **18** (Scheme 6), obtained in 75% yield from D-glucose[35–37] in a 1:1 α,β ratio, on the other hand, gave better results. Cleavage of the pentenyl glycoside to the hemiacetal was achieved in high yields using *N*-bromosuccinimide (NBS).[38] The reaction was performed in the dark to ensure that no radical reaction leading to the bromination of the methylene moiety of the PMB group would occur. The hemiacetal was then reduced to the diol, which was subsequently converted to the desired dimesylated compound **21**. Na_2Se prepared freshly in situ was used to displace the primary mesylate. Cyclization then ensued to give the protected 1,4-dideoxy-1,4-seleno-D-galactitol (**22**) in 98% yield.

With the selenoether in hand, the coupling reaction with the 2,4-*O*-benzylidene-D- and L-erythritol-1,3-cyclic sulfates **6** and **7** was first carried out in hexafluoro-2-propanol (HFIP) at 65 °C to afford the coupled products as a mixture of diastereomers in both cases. The coupling reactions with the D- and L-cyclic sulfates were not very selective as diastereomers were obtained in a 3:2 ratio in both cases, as judged by the ratio of the benzylidene proton resonances in the ^1H NMR spectra. The NMR spectra of the coupled products proved to be complicated and difficult to analyze due to a high degree of overlap of the peaks. In an attempt to improve the stereoselectivity of the reaction, we carried out the coupling in dry acetone at 60 °C (Scheme 7). Despite the reaction being slower and lower yielding, we were pleased to observe a net enhancement in stereoselectivity. Hence, when a mixture of the selenoether **22** and the D-cyclic sulfate **10** was heated at 60 °C for 48 h, the diastereomers were obtained in a ratio of 10:1. Similar results were observed in the coupling reaction with the L-cyclic sulfate **11** that afforded **24** as a mixture of diastereomers in a 8:1 ratio. The major isomer in both cases was determined, by means of NOESY experiments, to be that in which the erythritol side chain is trans to the chain at C-5. These diastereomers were then treated with trifluoroacetic acid to give the deprotected compounds in quantitative yields. Compounds **3** and **4** were fully characterized by 2D NMR spectroscopy, and the stereochemistry at the stereogenic selenium center in the major diastereomers was established by means of NOESY experiments.

The iminogalactitol and selenogalactitol derivatives **1**–**4**, as well as their thio congeners **5** and **6**, were tested for their inhibition of UDP-Gal*p* mutase. The HPLC assay for detecting UDP-Gal*f* and UDP-Gal*p* was initially performed on enzyme and substrate alone to determine the optimal time for examining the inhibitors. We found the addition of ice-cold HCl, followed by immediate freezing in liquid nitrogen, greatly increased the reproducibility of our results, presumably by preventing further enzyme activity while the samples were re-



Scheme 6.



Scheme 7.

thawed prior to HPLC analysis. At high UDP-Gal*p* concentrations, there was a slight evidence of a breakdown product of UDP-Gal*p* (less than 1%), and this was included in the calculation for UDP-Gal*p* when necessary. A time point of 5 min was chosen for the inhibitor studies, as this gave a turnover of $56.8 \pm 4.0\%$, allowing us to follow any small changes in the rate of turn-over.

Compounds **1**–**6** proved to be very poor inhibitors of UDP-galactopyranose mutase. Concentrations of 10 mM were required to begin to see inhibition (Table 1). Previous site-directed mutagenesis studies showed that the presence of a tryptophan (Trp) residue located in the putative active-site cleft away from the isoalloxazine ring of the FAD was required for enzyme activity.[39] We believe that this Trp residue forms a base-stacking interaction with the UDP moiety of the sugar substrate as has been seen with a number of other nucleotide–sugar interactions with proteins.[19,39–42] UDP has been demonstrated to be an inhibitor of mutase

Table 1. Inhibition of UDP-galactopyranose mutase by inhibitors

Inhibitor (10 mM final conc.)	% Turn-over[a]	% Inhibition[b]
1	33.7 ± 4.8	40.7
2	47.3 ± 1.2	16.7
3	57.7 ± 1.6	None
4	46.3 ± 4.0	18.5
5	32.0 ± 2.0	43.7
6	56.8 ± 0.6	None
No inhibitor	56.8 ± 4.0	N/A

[a] The % turn-over was calculated by integration of the two peaks.
[b] The % inhibition was calculated from the turn-over of the inhibited reaction compared to the reactions with no inhibitor. Each result was measured in triplicate.

(IC_{50} = 200 μM),[42] while we have been unable to show any inhibitory activity of galactose (to 50 mM concentrations, data not shown).

As all of the molecules **1**–**6** lack the nucleotide moiety, a promising strategy to enhance their inhibitory activities might be to elaborate analogous structures incorporating nucleotide moieties.

3. Experimental section

3.1. General

Optical rotations were measured with a Rudolph Research Autopol II automatic polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX 400 NMR spectrometer at 400.13 and 100.6 MHz, for ^{1}H and ^{13}C, respectively. Chemical shifts are given in ppm downfield from Me_4Si for those spectra measured in $CDCl_3$ or CD_3OD and from 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) for those spectra measured in D_2O. Chemical shifts and coupling constants were obtained from a first-order analysis of the spectra. All assignments were confirmed with the aid of two-dimensional ^{1}H/^{1}H COSY, ^{1}H/^{13}C HMQC, and ^{1}H NOESY experiments, using standard Bruker pulse programs. Processing of the spectra was performed with standard UXNMR (Bruker) and WINNMR software. Zero filling of the acquired data (512 t_1 values and 2K data points in t_2) led to a final data matrix of 1K × 1K ($F_1 \times F_2$) data points. 1D Transient NOE experiments were recorded at 285 K on a Bruker AMX 600 NMR spectrometer. For each 1D transient NOE spectrum, 1024 scans, preceded by 16 dummy scans were acquired. 1D transient NOE experiments were performed with 80-ms Gaussian-shaped pulses (2K data points, truncation level 1%) for selective inversion as described previously.[43,44] A corrected mixing time of 100 and 500 ms was used in 1D transient NOE experiments for compound **16** and compound **1**, respectively. Analytical thin-layer chromatography (TLC) was performed on aluminum plates precoated with Merck silica gel $60F_{254}$ as the adsorbent. The developed plates were air-dried, exposed to UV light and/or sprayed with a solution containing 1% $Ce(SO_4)_2$ and 1.5% molybdic acid in 10% aqueous H_2SO_4, and heated. Compounds were purified by flash chromatography on Kieselgel 60 (230–400 mesh). Solvents were distilled before use and were dried as necessary by literature procedures. Solvents were evaporated under reduced pressure and below 50 °C. High-resolution mass spectra were liquid secondary ionization fast-atom bombardment (LSIMS(FAB)), using Cs^+ ions, run on a Kratos Concept H double-focusing mass spectrometer at 10000 RP, using *meta*-NO_2-benzyl alcohol as matrix or, in the case of compounds **1** and **2**, with glycerine as matrix and PEG-sulfate as the mass reference.

3.2. Protein over-expression and purification

UDP-galactopyranose mutase (UDP-galactopyranose furanomutase, E.C. 5.4.99.9) from *Klebsiella pneumonia* was purified as described previously.[39] The protein was flash-frozen in liquid nitrogen (20 μL aliquots) and stored at 193 K. The enzyme does not require glycerol for storage, provided the aliquots are small and very rapidly frozen. Protein concentrations were determined by the method of Bradford.[45]

3.3. 1,2,3,5,6-Penta-*O*-benzyl-4-di-*O*-methanesulfonyl-D-glucitol (13)

Methanesulfonyl chloride (15 mL, 0.19 mol) was dissolved in pyridine (15 mL) and this mixture was added dropwise to a stirred solution of the diol **12** (2.2 g, 41 mmol) in dry CH_2Cl_2 (30 mL). TLC analysis after 3 h showed the absence of starting material and formation of a less polar compound. The reaction mixture was quenched with solid $NaHCO_3$, and the mixture was stirred for 30 min. The mixture was then partitioned between water (100 mL) and CH_2Cl_2 (50 mL). The CH_2Cl_2 extract was washed with 5% HCl (2 × 10 mL), water (2 × 10 mL), satd aq $NaHCO_3$ solution (2 × 10 mL) and finally with satd aq NaCl solution (2 × 10 mL). The organic extract was then dried (Na_2SO_4) and evaporated. The residue was then purified by flash chromatography using 3:2 hexanes–EtOAc to give the dimesylated product **13** (2.53 g, 89%). $[\alpha]_D$ +18 (*c* 1.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.52–7.22 (20H, m, Ar), 5.21 (1H, dd, $J_{4,5}$ 6.8, $J_{4,3}$ 2.9 Hz, H-4), 4.79, 4.62 (2H, 2d, $J_{A,B}$ 11.3 Hz, CH_2Ph), 4.58–4.46 (6H, m, 3 × CH_2Ph), 4.31 (1H, dd, $J_{1a,1b}$ 10.9, $J_{1a,2}$ 4.2 Hz, H-1a), 4.25 (1H, dd, $J_{1b,2}$ 4.3, H-1b), 3.96–3.85 (3H, m, H-5, H-4, H-2), 3.76 (1H, dd, $J_{6a,6b}$ 10.3, $J_{6a,5}$ 5.5 Hz, H-6a), 3.62 (1H, dd, $J_{6b,5}$ 5.6 Hz, H-6b), 2.96 (3H, s, CH_3), 2.81 (3H, s, CH_3). ^{13}C NMR ($CDCl_3$): δ 137.72, 137.50, 137.32, 137.22 (4 × C_{ipso}), 128.62–127.80 (20C_{Ar}), 81.85 (C-4), 76.60 (2C C-5, C-2), 76.05 (C-3), 74.95, 73.48, 73.03, 72.40 (4 × *C*H_2Ph), 68.72 (C-1),

68.62 (C-6), 39.98 (O*Ms*), 37.08 (O*Ms*). Anal. Calcd for $C_{36}H_{42}O_{10}S_2$: C, 61.87; H, 6.06. Found: C, 61.97; H, 6.01.

3.4. *N*-Allyl-2,3,5,6-tetra-*O*-benzyl-1,4-dideoxy-1,4-imino-D-galactitol (14)

The dimesylate **13** (2.53 g, 3.6 mmol) was dissolved in allylamine (60 mL) and the solution heated to reflux for 12 h. The solvent was then removed under reduced pressure, and the residue was dissolved in water (50 mL) and extracted with CH_2Cl_2 (3×50 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated. Purification of the resulting oil by column chromatography (2:1 hexanes–EtOAc) gave the desired tertiary amine **14** as a colorless oil (1.7 g, 83%). $[\alpha]_D$ +27 (*c* 2.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.41–7.22 (20H, m, Ar), 5.96–5.85 (1H, m, $NCH_2CH{=}CH_2$), 5.18 (1H, d, J_{trans} = 17.0 Hz, $NCH_2CH{=}CH_2$), 5.10 (1H, d, J_{cis} = 10.1 Hz, $NCH_2CH{=}CH_2$), 4.83 (1H, d, $J_{A,B}$ 11.9 Hz, CH_2Ph), 4.76 (1H, d, $J_{A,B}$ 11.9 Hz, CH_2Ph), 4.45–4.40 (6H, m, $3 \times CH_2Ph$), 4.00 (1H, bs, H-3), 3.92–3.88 (3H, m, H-2, H-5, H-6a), 3.62 (1H, dd, $J_{6b,6a}$ 10.8, J_{vic} 7.6 Hz, H-6b), 3.54 (1H, dd, J_{gem} 13.6, J_{vic} 4.2 Hz, $NCH_2CH{=}CH_2$), 3.21 (1H, d, $J_{1a,1b}$ 10.7 Hz, H-1a), 3.05 (1H, dd, $NCH_2CH{=}CH_2$), 2.91 (1H, m, H-4), 2.62 (1H, dd, J_{1b-2} 4.4 Hz, H-1b). ^{13}C NMR ($CDCl_3$): δ 139.01, 138.72, 138.31, 138.28 ($4 \times C_{ipso}$), 135.9 (1C, $NCH_2CH{=}CH_2$), 128.42–127.40 ($20C_{Ar}$), 116.96 ($NCH_2CH{=}CH_2$), 84.18 (C-3), 81.22 (C-5), 79.13 (C-2), 73.18 (CH_2Ph), 72.78 (CH_2Ph), 72.01 (C-6), 71.15 (C-4), 70.70 (2C, $2 \times CH_2Ph$), 58.78 ($NCH_2CH{=}CH_2$), 57.05 (C-1). Anal. Calcd for $C_{37}H_{41}NO_4$: C, 78.83; H, 7.33; N, 2.48. Found: C, 78.59; H, 7.45; N, 2.50.

3.5. 2,3,5,6-Tetra-*O*-benzyl-1,4-dideoxy-1,4-imino-D-galactitol (15)

The tertiary amine **14** (1.7 g, 3.3 mmol) was dissolved in a mixture of CH_3CN–H_2O, 8:1 (100 mL). N_2 gas was bubbled through the solution for 10 min, after which Wilkinson's catalyst (80 mg) was added. The reaction mixture was heated to reflux for 15 h. The solvents were then removed, and the oil was purified by flash chromatography (1:1 EtOAc–hexanes) as eluent. The deprotected amine **15** was obtained as a yellow oil (1.1 g, 70%). $[\alpha]_D$ −20 (*c* 4.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.51–7.25 (20H, m, Ar), 4.78, 4.36 (2H, 2d, $J_{A,B}$ 11.6 Hz, CH_2Ph), 4.54–4.44 (6H, m, 6 C*H*Ph), 3.97 (1H, m, H-2), 3.88–3.80 (2H, m, H-3, H-5), 3.76–3.68 (2H, 2 dd, $J_{6a,6b}$ 10.4, $J_{6a,5}$ 6.1, $J_{6b,5}$ 4.6 Hz, H-6a, H-6b), 3.13 (1H, d, $J_{1a,1b}$ 12.5 Hz, H-1a), 3.07 (1H, dd, $J_{4,5}$ 5.9, $J_{4,3}$ 3.7 Hz, H-4), 2.97 (1H, dd, $J_{1b,2}$ 5.0 Hz, H-1b). ^{13}C NMR ($CDCl_3$): δ 138.58, 138.27, 138.20, 138.05 ($4 \times C_{ipso}$), 128.41–127.52 ($20C_{Ar}$), 85.97 (C-3), 84.51 (C-2), 77.12 (C-5), 79.42, 73.29 (2C, $2 \times CH_2Ph$), 72.61 (CH_2Ph), 72.06 (C-6), 71.2 (C-4), 70.85 (CH_2Ph), 66.46 (C-3), 51.25 (C-1). Anal. Calcd for $C_{37}H_{41}NO_4$: C, 77.98; H, 7.12; N, 2.67. Found: C, 78.11; H, 7.12; N, 2.59.

3.6. 1′-((2,3,5,6-Tetra-*O*-benzyl-1,4-dideoxy-1,4-imino-D-galactitol)-4-*N*-ammonium)-2′,4′-*O*-benzylidene-1′-deoxy-D-erythritol-3′-sulfate (16)

A mixture of the imino sugar **15** (1.1 g, 2.1 mmol) and the D-cyclic sulfate **10** (0.7 g, 2.6 mmol, 1.2 equiv) was dissolved in dry acetone (0.5 mL) containing anhyd K_2CO_3 (20 mg). The mixture was stirred in a sealed tube in an oil bath (70 °C) overnight. The solvent was removed under reduced pressure and the product was purified by column chromatography (2:1 EtOAc–MeOH). The coupled product was obtained as a white solid (1.1 g, 64%). $[\alpha]_D$ +1.2 (*c* 0.85, $CHCl_3$); ^{1}H NMR (CD_3OD made basic with NaOD): δ 7.40–7.10 (25H, m, Ar), 5.35 (1H, s, C*H*Ph), 4.54–4.50 (3H, m, $2CH_2$ Ph, H-4′a), 4.36–4.24 (6H, m, 6 CH_2Ph), 4.18–4.11 (1H, ddd, $J_{3',4'a}$ $J_{3',2'}$ 9.9, $J_{3',4'b}$ Hz, H-3′), 3.87 (1H, t, $J_{2',1'a}$ $J_{2',1'b}$ 8.7 Hz, H-2′), 3.85–3.78 (3H, m, H-2, H-3, H-5), 3.75–3.67 (2H, m, H-4′b, H6a), 3.42–3.39 (1H, m, H-6b), 3.37 (1H, brd, H-1′a), 3.24 (1H, d, $J_{1a,1b}$ 10.9 Hz, H-1a), 3.13 (1H, dd, $J_{4,5}$ 5.2, $J_{4,3}$ 2.6 Hz, H-4), 2.82–2.73 (2H, m, H-1b, H-1′b). ^{13}C NMR (CD_3OD): δ 142.46, 142.09, 141.88, 141.78, 141.43 ($5 \times C_{ipso}$), 135.61–129.89 ($25C_{Ar}$), 104.67 (*C*HPh), 86.85 (C-5), 85.29 (C-3), 83.10 (C-2′), 82.05 (C-2), 76.48 (CH_2Ph), 76.48, 76.01 (2C, CH_2Ph), 74.79 (C-6), 74.53 (C-4), 74.39 (C-4′), 72.92 (C-3′), 72.28 (CH_2Ph), 61.59 (C-1), 58.52 (C-1′). Anal. Calcd for $C_{45}H_{49}O_{10}S$: C, 67.91; H, 6.21; N, 1.76. Found: C, 68.04; H, 6.07; N, 2.01.

3.7. 1′-((2,3,5,6-Tetra-*O*-benzyl-1,4-dideoxy-1,4-imino-D-galactitol)-4-*N*-ammonium)-2′,4′-*O*-benzylidene-1′-deoxy-L-erythritol-3′-sulfate (17)

A mixture of the imino sugar **15** (0.25 g, 0.48 mmol) and the L-cyclic sulfate **11** (0.15 g, 0.58 mmol, 1.2 equiv) was dissolved in dry acetone (0.5 mL) containing anhyd K_2CO_3 (10 mg) added. The mixture was stirred in a sealed tube in an oil bath (70 °C) overnight. The solvent was removed under reduced pressure, and the product was purified by column chromatography (2:1 EtOAc–MeOH). The coupled product was obtained as a white solid (0.28 g, 74%). $[\alpha]_D$ +31 (*c* 1, $CHCl_3$); ^{1}H NMR (CD_3OD made basic with NaOD): δ 7.29–7.12 (25H, m, Ar), 5.44 (1H, s, C*H*Ph), 4.52 (1H, dd, $J_{4'a,4'b}$ 11.1, $J_{4'a,3'}$ 5.3 Hz, H-4′a), 4.49 (2H, d, $2CH_2Ph$), 4.13 (1H, ddd, $J_{3',4'b}$ $J_{3',2'}$ 9.92 Hz, H-3′), 3.91 (1H, t, $J_{2',1'a}$ $J_{2',1'b}$ 8.7 Hz, H-2′), 3.88–3.85 (2H, m, H-2, H-4), 3.75–3.67 (3H, m, H-4′b, H-5, H-6a), 3.42 (1H, dd, $J_{6a,6b}$ 10.8, $J_{6a,5}$ 6.9 Hz, H-6a), 3.28 (1H, brd, H-1a), 3.16 (1H, d, H-1′a), 3.01 (1H, dd, $J_{4,5}$ 5.3, $J_{4,3}$ 2.4 Hz,

H-4), 2.93 (1H, dd, $J_{1'b,1'a}$ 14.0, $J_{1'b,2'}$ 8.5 Hz, H-1′b), 2.76 (1H, dd, $J_{1b,1a}$ 11.1 Hz, H-1b). ^{13}C NMR (CD_3OD): δ 140.89, 140.53, 140.27, 140.20, 139.96 (5′C_{ipso}), 130.03–128.50 (25C_{Ar}), 103.35 (*C*HPh), 85.85 (C-3), 83.55 (C-2′), 81.03 (C-5), 80.89 (C-2), 74.99 (*C*H_2Ph), 74.69 (*C*H_2Ph), 73.29 (*C*H_2Ph), 73.17 (*C*H_2Ph), 72.99 (C-6), 72.89 (C-4), 71.33 (C-4′), 70.98 (C-3′), 60.17 (C-1), 59.13 (C-1′). Anal. Calcd for $C_{45}H_{49}NO_{10}S$: C, 67.91; H, 6.21; N, 1.76. Found: C, 67.67; H, 6.14; N, 1.82.

3.8. 1′-(1,4-Dideoxy-D-1,4-imino-galactitol)-4-*N*-ammonium)-1′-deoxy-D-erythritol-3′-sulfate (1)

Compound **16** (900 mg, 1.1 mmol) was dissolved in AcOH–H_2O (5 mL) and stirred with Pd–C (100 mg) under H_2 (100 psi). After 48 h, the reaction mixture was filtered through a pad of Celite, which was subsequently washed with MeOH. The combined filtrates were concentrated and the residue was purified by column chromatography (1:1 EtOAc–MeOH) to give the product as an off-white solid (240 mg, 62%). $[\alpha]_D$ +5.9 (*c* 0.15, MeOH); ^{1}H NMR (CD_3OD): δ 4.29 (1H, ddd, $J_{3',2'}$ 5.9, $J_{3',4'a}$ $J_{3',4'b}$ 4.1 Hz, H-3′), 4.16 (1H, ddd, $J_{2',1'a}$ 8.1 Hz, $J_{2',1'b}$ 7.9, H-2′), 4.10 (1H, dd, H-2), 4.01 (1H, bs, H-3), 3.91 (2H, dd, ddd, H-4′a, H-5), 3.81 (1H, dd, $J_{4'b,4'a}$ 12.1 Hz, H-4′b), 3.74 (1H, dd, $J_{6a,6b}$ 11.60, $J_{6a,5}$ 4.3 Hz, H-6a), 3.64 (1H, dd, $J_{6b,5}$ 4.3 Hz, H-6b), 3.61 (1H, dd, H-1′a), 3.54 (1H, d, $J_{1a,1b}$ 11.8 Hz, H-1a), 3.25 (1H, dd, $J_{1b,2}$ 3.9 Hz, H-1b), 3.19 (1H, d, $J_{4,5}$ 7.0 Hz, H-4), 2.98 (1H, dd, $J_{1'a,1'b}$ 13.1 Hz, H-1′b). ^{13}C NMR (CD_3OD): δ 84.08 (C-3′), 81.65 (C-3), 80.30 (C-4), 79.55 (C-2), 74.99 (C-5), 72.06 (C-2′), 67.38 (C-6), 64.78 (C-1′), 64.61 (C-1), 64.41 (C-4′). HRMS Calcd for $C_{10}H_{22}NO_{10}S$ [M+1]: *m*/*z* 348.0964. Found: *m*/*z* 348.0961.

3.9. 1′-(1,4-Dideoxy-D-1,4-imino-galactitol)-4-*N*-ammonium)-1′-deoxy-L-erythritol-3′-sulfate (2)

Compound **17** (190 mg, 0.24 mmol) was dissolved in 4:1 AcOH–H_2O (5 mL) and stirred with Pd–C (40 mg) under H_2 (100 psi). After 48 h, the reaction mixture was filtered through a pad of Celite, which was subsequently washed with MeOH. The combined filtrates were concentrated and the residue was purified by column chromatography (1:1 EtOAc–MeOH) to give the product as an off-white solid (72 mg, 86%). $[\alpha]_D$ +24.9 (*c* 0.75, MeOH); ^{1}H NMR (CD_3OD): δ 4.26 (1H, ddd, $J_{3',2'}$ 6.3, $J_{3',4'b}$ $J_{3',4'a}$ 4.3 Hz, H-3′), 4.11–4.06 (2H, m, H-2′, H-2), 4.01 (1H, bs, H-3), 3.91 (1H, ddd, H-5), 3.88 (1H, dd, H-4′a)3.81 (1H, dd, $J_{4'b,4'a}$ 12.1 Hz, H-4′b), 3.73 (1H, dd, $J_{6a,6b}$ 11.5, $J_{6a,5}$ 4.7 Hz, H-6a), 3.63 (1H, dd, $J_{6b,5}$ 5.5 Hz, H-6b), 3.40 (1H, d, $J_{1a,1b}$ 11.0 Hz, H-1a), 3.26 (1H, d, $J_{1'a,1'b}$ 10.1 Hz, H-1′a), 3.16 (1H, dd, H-1′b), 3.14–3.09 (2H, m, H-1b, H-4). ^{13}C NMR (CD_3OD): δ 84.08 (C-3′), 82.24 (C-3), 79.39 (C-4), 79.34 (C-2), 75.12 (C-5), 71.14 (C-2′), 67.43 (C-6), 64.49 (C-4′), 64.18 (C-1′), 62.84 (C-1). HRMS Calcd for $C_{10}H_{22}NO_{10}S$ [M+1]: *m*/*z* 348.0964. Found: *m*/*z* 348.0959.

3.10. Pent-4-enyl 2,3,5,6-tetra-*O*-*p*-methoxybenzyl-α,β-D-glucofuranoside (19)

To a solution of pent-4-enyl 2,3,5,6-tetra-*O*-acetyl-α,β-D-glucofuranoside **18** (3.9 g, 9.4 mmol) in MeOH (50 mL) was added 1 M solution of sodium methoxide in MeOH (0.02 mol, 20 mL). The mixture was stirred overnight at room temperature and then neutralized with Rexyn 101 [H^+]. The Rexyn was filtered off and the filtrate was concentrated to a straw-colored syrup. To a stirring solution of this syrup in dry DMF (50 mL) at 0 °C, NaH (3 g, 47 mmol, 5 equiv) was added in small portions. *p*-Methoxybenzylchloride (7.3 g, 47 mmol, 5 equiv) was then added and the mixture stirred at room temperature for 4 h. The reaction mixture was quenched with MeOH and the solution diluted with water. This mixture was extracted with CH_2Cl_2 (3 × 30 mL). The organic extracts were dried (Na_2SO_4) and evaporated to an oil that was purified by column chromatography (2:1 hexanes–EtOAc) to give **19** as a mixture of anomers in a 1:1 ratio. (5.7 g, 84%). $[\alpha]_D$ −18.5 (*c* 2.0, $CHCl_3$); ^{1}H NMR of one anomer ($CDCl_3$): δ 7.33–6.72 (16H, m, Ar), 5.88–5.74 (1H, m, $OCH_2CH_2CH_2CH{=}CH_2$), 5.05–4.89 (m, 3H, H-1, $OCH_2CH_2CH_2CH{=}CH_2$), 4.65 (1H, d, $J_{A,B}$ 10.9 Hz, C*H*Ph), 4.54–4.36 (8H, m, 7 C*H*Ph, H-3), 4.25 (1H, dd, $J_{4,5}$ 8.9, $J_{4,3}$ 4.6 Hz, H-4), 4.02 (1H, m, H-5), 3.93 (1H, bd, H-2), 3.80 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 3.77 (3H, s, OCH_3), 3.70–3.58 (m, 3H, H-6a, H-6b, $OCHCH_2CH_2CH{=}CH_2$), 3.42–3.34 (1H, m, $OCHCH_2CH_2CH{=}CH_2$), 2.14–2.02 (2H, $OCH_2CH_2CH_2CH{=}CH_2$), 1.74–1.61 (2H, m, $OCH_2CH_2CH_2CH{=}CH_2$). ^{13}C NMR ($CDCl_3$): δ 159.55, 159.32, 159.21, 159.15 (4 × C_{para}), 138.53 (OCH-$CH_2CH_2CH{=}CH_2$), 131.39, 131.06, 130.42, 129.92 (4 × C_{ipso}), 129.54, 129.51, 129.46, 129.40 (4 × C_{ortho}), 114.95 ($OCHCH_2CH_2CH{=}CH_2$), 114.06–113.78 (4 × C_{meta}), 107.80 (C-1), 85.52 (C-2), 80.63 (C-3), 80.11 (C-4), 76.41, 73.19, 72.29, 71.66 (4 × CH_2PH), 71.51 (C-5), 70.86 (C-6), 68.05 ($OCHCH_2CH_2CH{=}CH_2$), 55.57–55.37 (4 × OCH_3), 30.50 ($OCHCH_2CH_2CH{=}CH_2$), 29.05 ($OCHCH_2CH_2CH{=}CH_2$). Anal. Calcd for $C_{38}H_{46}O_{10}$: C, 68.86; H, 7.00. Found: C, 68.95; H, 7.14.

3.11. 2,3,5,6-Tetra-*O*-(*p*-methoxybenzyl)-D-glucitol (20)

To a cold solution of **19** (5.7 g, 7.8 mmol) in 40% aq CH_3CN (80 mL) was added NBS (3.48 g, 19.5 mmol, 2.5 equiv). The reaction mixture was stirred at 0 °C for 1 h in the dark and then quenched with a 10% $Na_2S_2O_3$ solution. The mixture was concentrated to 20 mL and

the residue extracted with EtOAc (3 × 30 mL). The organic extracts were combined, dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (1:1 hexanes–EtOAc) to give the hemiacetal as a colorless syrup (4.13 g, 80%). $NaBH_4$ (0.75 g, 25 mmol, 4 equiv) was added in small amounts to a cold solution of the hemiacetal in MeOH (50 mL). The reaction mixture was then stirred at room temperature for 2 h, after which the solvent was evaporated. The residue was dissolved in water and extracted with EtOAc (3 × 30 mL). The organic extracts were dried (Na_2SO_4) and concentrated. The crude diol was purified by column chromatography (1:1 hexanes–EtOAc) to afford **20** as a colorless syrup (3.2 g, 78%). $[\alpha]_D$ −20.5 (*c* 8.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.28–7.01 (16H, m, Ar), 4.64 (1H, d, $J_{A,B}$ 11.3 Hz, C*H*Ph), 4.55–4.44 (5H, m, 5 C*H*Ph), 4.30–4.24 (2H, m, C*H*Ph), 3.90–3.85 (2H, m, H-3, H-4), 3.83 (1H, dd, $J_{1a,1b}$ 10.2, $J_{1a,2}$ 2.9 Hz, H-1a), 3.78 (3H, s, OCH_3), 3.77 (3H, s, OCH_3), 3.75 (6H, s, 2 × OCH_3), 3.78–3.70 (m, 2H, H-6a, H-6b), 3.67 (1H, dd, $J_{1b,2}$ 4.9 Hz, H-1b), 3.63–3.57 (2H, m, H-5, H-2), 3.20–2.81 (2H, bs, 2 × O*H*). ^{13}C NMR ($CDCl_3$): δ 159.55, 159.49, 159.40, 159.36 (4 × C_{para}), 130.83, 130.53, 130.41, 130.35 (4 × C_{ipso}), 129.95, 129.79, 129.65, 129.58 (4 × C_{ortho}), 114.04–113.93 (4 × C_{meta}), 77.98, 77.86 (C-5, C-2), 75.36 (C-3), 73.37, 73.29, 71.94, 71.63 (4 × *C*H_2Ph), 70.30 (C-1), 69.44 (C-4), 60.88 (C-6), 55.46 (4 × O*C*H_3). Anal. Calcd for $C_{38}H_{46}O_{10}$: C, 68.86; H, 7.00. Found: C, 68.95; H, 7.14.

3.12. 1,4-Di-*O*-methanesulfonyl-2,3,5,6-tetra-*O*-(*p*-methoxybenzyl)-D-glucitol (21)

Methanesulfonyl chloride (15 mL, 0.19 mol) was dissolved in pyridine (15 mL), and this mixture was added dropwise to a stirred solution of the diol **20** (2.2 g, 3.3 mmol) in dry CH_2Cl_2 (30 mL). TLC analysis after 3 h showed the absence of starting material and formation of a less polar compound. The reaction mixture was quenched with solid $NaHCO_3$, and the mixture was stirred for 30 min. The mixture was then partitioned between water (100 mL) and CH_2Cl_2 (50 mL). The CH_2Cl_2 extract was washed with water (2 × 10 mL), satd aq $NaHCO_3$ solution (2 × 10 mL) and finally with satd aq NaCl solution (2 × 10 mL). The organic extract was then dried (Na_2SO_4) and evaporated. The residue was then purified by flash chromatography using 3:2 hexanes–EtOAc to give the dimesylated product **21** (2.2 g, 82%). $[\alpha]_D$ +17.5 (*c* 4.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.28–6.78 (16H, m, Ar), 5.14 (1H, dd, $J_{4,5}$ 7.2, $J_{4,3}$ 2.8 Hz, H-4), 4.71, 4.38 (2H, 2d, $J_{A,B}$ 11.1 Hz, CH_2Ph), 4.54–4.40 (6H, m, 3 × CH_2Ph), 4.24 (1H, dd, $J_{1a,1b}$ 10.8, $J_{1a,2}$ 5.9 Hz, H-1a), 4.15 (1H, dd, $J_{1b,2}$ 4.1, H-1b), 3.85–3.81 (2H, m, H-5, H-3), 3.80 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 3.73 (4H, m, OCH_3, H-2), 3.68 (1H, dd, $J_{6a,6b}$ 10.1, $J_{6a,5}$ 5.9 Hz, H-6a), 3.56 (1H, dd, $J_{6b,5}$ 5.6 Hz, H-6b), 2.97 (3H, s, CH_3), 2.83 (3H, s, CH_3). ^{13}C NMR ($CDCl_3$): δ 159.69, 159.66, 159.56, 159.52 (4 × C_{para}), 130.60–129.51 (4 × C_{ipso}, 4 × C_{ortho}), 114.2–114.0 (4 × C_{meta}), 82.56 (C-4), 76.87 (C-5), 76.47 (C-2), 75.64 (C-3), 74.66, 73.32, 72.79, 72.24 (4 × CH_2Ph), 69.13 (C-1), 68.67 (C-6), 55.49 (4 × O*C*H_3), 39.23 (O*Ms*), 37.27 (O*Ms*). Anal. Calcd for $C_{40}H_{50}O_{14}S_2$: C, 58.66; H, 6.15. Found: C, 58.68; H, 6.04.

3.13. 1,4-Anhydro-2,3,5,6-tetra-*O*-(*p*-methoxybenzyl)-4-seleno-D-galactitol (22)

Selenium metal (0.32 g, 4.1 mmol) was added to liquid NH_3 (60 mL) in a −50 °C bath, and small pieces of sodium (0.20 g) were added until a blue color appeared. NH_3 was removed by warming on a water bath, and DMF was added, and removed under high vacuum to remove the rest of NH_3. A solution of the mesylated compound **21** (2.2 g, 2.7 mmol) in DMF (20 mL) was added and the mixture was stirred under Ar in a 50 °C bath for 3 h. The reaction mixture was taken up in water (50 mL) and extracted with CH_2Cl_2 (3 × 25 mL). The combined extracts were washed with water (50 mL) and brine (50 mL), then dried ($MgSO_4$) and concentrated. The crude product was purified by flash chromatography (3:1 hexanes–EtOAc) to give a light-yellow oil (1.5 g, 80%). $[\alpha]_D$ −37.7 (*c* 6.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.26–6.81 (16H, m, Ar), 4.61, 4.26 (2H, 2d, $J_{A,B}$ 11.1 Hz, CH_2Ph), 4.54, 4.47 (2H, 2d, $J_{A,B}$ 11.4 Hz, CH_2Ph), 4.43–4.39 (4H, m, 4 × C*H*Ph), 4.09 (1H, ddd, H-2), 3.94 (1H, dd, $J_{2,3}$ $J_{3,4}$ 6.7 Hz, H-3), 3.82–3.80 (1H, m, H-5), 3.80 (3H s, OCH_3), 3.79 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 3.75 (3H,s, OCH_3), 3.63 (1H, dd, $J_{4,5}$ 6.3 Hz, H-4), 3.51–3.44 (2H, m, H-6a, H-6b), 2.80 (1H, dd, $J_{1a,1b}$ 9.8, $J_{1a,2}$ 5.6 Hz, H-1a), 2.96 (1H, dd, $J_{1b,2}$ 7.6 Hz H-1b). ^{13}C NMR ($CDCl_3$): δ 159.20, 159.15, 155.28, 159.20 (4 × C_{para}), 132.74–127.99 (4 × C_{ipso}, 4 × C_{ortho}), 113.74–111.06 (4 × C_{meta}), 85.69 (C-2), 84.15 (C-3), 78.24 (C-5), 72.99–71.38 (4 × CH_2Ph), 70.52 (C-6), 56.23, 55.24 (4 × O*C*H_3), 44.25 (C-4), 21.77 (C-1). Anal. Calcd for $C_{38}H_{44}O_8Se$: C, 64.49; H, 6.27. Found: C, 64.49; H, 6.19.

3.14. 1′-((2,3,5,6-Tetra-*O*-(*p*-methoxybenzyl)-1,4-dideoxy-1,4-seleno-D-galactitol)-4-*Se*-selenonium)-2′,4′-*O*-benzylidene-1′-deoxy-D-erythritol-3′-sulfate (23)

The seleno sugar **22** (110 mg, 0.15 mmol) and the D-cyclic sulfate **10** (48 mg, 0.17 mmol, 1.2 equiv) was dissolved in dry acetone (1 mL) containing anhydrous K_2CO_3 (10 mg). The mixture was stirred in a sealed tube in an oil bath (60 °C) for 48 h. The solvent was removed under reduced pressure, and the product was purified by column chromatography (2:1 EtOAc–MeOH). The coupled product was obtained as a white solid (100 mg, 68%). $[\alpha]_D$ +1.9 (*c* 0.53, $CHCl_3$); ^{1}H NMR

(CD_2Cl_2) data for the major diastereomer trans-**23**: δ 7.25–6.80 (21H, m, Ar), 5.49 (1H, s, C*H*Ph), 4.50–4.41 (3H, m, H-3′, H-2, H-4), 4.40–4.31 (8H, m, 7 C*H*Ph, H-3), 4.25 (1H, d, $J_{A,B}$ 11.5 Hz, C*H*Ph), 4.22 (1H, dd, $J_{1'a,1'b}$ 12.4, $J_{1'a,2'}$ 3.1 Hz, H-1′a), 4.15 (1H, dd, $J_{4'a,4'b}$ 9.4, $J_{4'a,3'}$ 1.3 Hz, H-4′a), 4.07 (1H, ddd, $J_{2',3'}$ 9.3, $J_{2',1'a}$ $J_{2',1'b}$ 3.2 Hz, H-2′), 3.88 (1H, dd, H-1′b), 3.88–3.76 (13H, m, H-1a, 4 × OCH_3), 3.75–3.70 (2H, m, H-5, H-4′b), 3.49 (1H, dd, H-1b), 3.45 (1H, dd, $J_{6a,6b}$ 10.8, $J_{6a,5}$ 3.5 Hz, H-6a), 3.41 (1H, dd, $J_{6b,5}$ 4.4 Hz, H-6b). ^{13}C NMR (CD_2Cl_2): δ 160.0, 159.98, 159.94, 159.75 (4 × C_{para}), 137.09 (1 × C_{ipso}), 130.39–126.77 (21 C_{Ar}), 114.16 (2C), 114.13, 114.04 (4 × C_{meta}), 101.95 (*C*HPh), 83.44 (C-3), 83.07 (C-2), 76.95 (C-2′), 73.76 (C-5), 73.49, 72.16, 72.04, 71.82 (4 × *C*H_2Ph), 70.27 (C-4), 69.48 (C-6), 69.42 (C-4′), 67.62 (C-3′), 45.13 (C-1′), 44.55 (C-1). Anal. Calcd for $C_{45}H_{56}O_{14}SSe$: C, 60.05; H, 5.76. Found: C, 60.03; H, 5.61.

3.15. 1′-((2,3,5,6-Tetra-*O*-(*p*-methoxybenzyl)-1,4-dideoxy-1,4-seleno-D-galactitol)-4-*Se*-selenonium)-2′,4′-*O*-benzylidene-1′-deoxy-L-erythritol-3′-sulfate (24)

The seleno sugar **22** (150 mg, 0.21 mmol) and the L-cyclic sulfate **11** (70 mg, 0.25 mmol, 1.2 equiv) was dissolved in dry acetone (1 mL) containing anhydrous K_2CO_3 (10 mg). The mixture was stirred in a sealed tube in an oil bath (60 °C) for 48 h. The solvent was removed under reduced pressure, and the product was purified by column chromatography (2:1 EtOAc–MeOH). The coupled product was obtained as a white solid (128 mg, 62%). $[\alpha]_D$ +2.9 (*c* 0.35, $CHCl_3$); ^{1}H NMR (CD_2Cl_2) data for the major diastereomer trans-**24**: δ 7.42–6.80 (21H, m, Ar), 5.35 (1H, s, C*H*Ph), 4.56 (1H, dd, $J_{4'a,4'b}$ 11.0, $J_{4'a,3'}$ 5.4 Hz, H-4′a), 4.47, 4.28 (2H, d, $J_{A,B}$ 11.3 Hz, CH_2Ph), 4.41–4.38 (1H, m, H-2), 4.37–4.29 (9H, m, 6C*H*Ph, H-3, H-4, H-3′), 4.10 (1H, ddd, $J_{2',3'}$ 9.8, $J_{2',1'a}$ 5.5, $J_{2',1'b}$ 4.6 Hz, H-2′), 3.99 (1H, dd, $J_{1'a,1'b}$ 12.2, H-1′a), 3.87 (1H, dd, H-1′b), 3.79, 3.78, 3.77, 3.76 (12H, 4s, 4 × OCH_3), 3.72–3.66 (2H, m, H-5, H-4′b), 3.62 (1H, dd, $J_{1'a,1'b}$ 12.2, $J_{1'a,2'}$ 2.8 Hz, H-1a), 3.47–3.40 (3H, m, H-1b, H-6a, H-6b). ^{13}C NMR (CD_2Cl_2): δ 160.0, 159.98, 159.94, 159.75 (4 × C_{para}), 137.09 (1 × C_{ipso}), 130.13–126.39 (21 C_{Ar}), 114.16 (2C), 114.13, 114.04 (4 × C_{meta}), 101.56 (*C*HPh), 83.56 (C-2), 82.65 (C-3), 76.76 (C-2′), 73.68 (C-5), 73.44, 72.19, 71.94, 71.86 (4 × *C*H_2Ph), 70.28 (C-4), 69.70 (C-4′), 69.45(C-6), 68.37 (C-3′), 47.94(C-1′), 44.66 (C-1). Anal. Calcd for $C_{45}H_{56}O_{14}SSe$: C, 60.05; H, 5.76. Found: C, 60.00; H, 5.49.

3.16. 1′-(1,4-Dideoxy-D-1,4-seleno-galactitol)-4-*Se*-selenonium)-1′-deoxy-D-erythritol-3′-sulfate (3)

Compound **23** (100 mg, 0.1 mmol) was dissolved in TFA (5 mL) and stirred for 1 h at room temperature. The solvent was then evaporated, and the residue was purified by column chromatography (1:1 EtOAc–MeOH) to give the product as a white solid (37 mg, 90%). $[\alpha]_D$ +11.9 (*c* 0.25, MeOH); ^{1}H NMR (CD_3OD) for major *trans* isomer: δ 4.60 (1H, ddd, $J_{2,3}$ 3.9 Hz, H-2), 4.41 (1H, dd, $J_{3,4}$ 3.9 Hz, H-3), 4.32–4.24 (2H, m, H-2′, H-3′), 4.11–4.04 (2H, m, H-4, H-5), 3.98 (1H, dd, $J_{1'a,1'b}$ 12.1, $J_{1'a,2'}$ 3.5 Hz, H-1′a), 3.92 (1H, dd, $J_{4'a,4'b}$ 12.2, $J_{4'a,3'}$ 3.8 Hz, H-4′a), 3.86 (1H, dd, $J_{1'b,2'}$ 6.7 Hz, H-1′b), 3.81 (1H, dd, $J_{4'b,3'}$ 4.1 Hz, H-4′b), 3.76 (1H, d, $J_{6a,6b}$ 11.7, $J_{6a,5}$ 3.5 Hz, H-6a), 3.72–3.67 (2H, m, H-1a, H-6b), 3.57 (1H, dd, $J_{1b,2}$ 4.2 Hz, H-1b). ^{13}C NMR (CD_3OD): δ 80.25 (C-3′), 77.60 (C-3), 70.88 (C-2), 68.96 (C-4), 66.58 (C-5), 60.57 (C-2′), 64.77 (C-6), 60.59 (C-4′), 48.66 (C-1′), 44.92 (C-1). HRMS: Calcd for $C_{10}H_{21}O_{10}SSe$ [M+1]: *m*/*z* 413.0021. Found: *m*/*z* 413.0022.

3.17. 1′-(1,4-Dideoxy-D-1,4-seleno-galactitol)-4-*Se*-selenonium)-1′-deoxy-L-erythritol-3′-sulfate (4)

Compound **24** (150 mg, 0.15 mmol) was dissolved in TFA (5 mL) and stirred for 1 h at room temperature. The solvent was then evaporated, and the residue was purified by column chromatography (1:1 EtOAc–MeOH) to give the product as a white solid (58 mg, 92%). $[\alpha]_D$ +5.7 (*c* 0.15, MeOH); ^{1}H NMR (CD_3OD) data for major *trans* isomer: δ 4.64 (1H, ddd, $J_{2,3}$ 4.1 Hz, H-2), 4.44 (1H, dd, $J_{3,4}$ 3.8 Hz, H-3), 4.30 (1H, ddd, $J_{2',1'a}$ $J_{2',3'}$ 6.1, $J_{2',1'b}$ 2.4 Hz, H-2′), 4.26 (1H, ddd, H-3′), 4.18 (1H, dd, $J_{4,5}$ 5.2 Hz, H-4), 4.01 (1H, ddd, $J_{5,6a}$ $J_{5,6a}$ 12.1 Hz, H-5), 3.94 (1H, dd, $J_{4'a,4'b}$ 12.1, $J_{4'a,3'}$ 3.8 Hz, H-4′a), 3.91–3.88 (2H, m, H-1′a, H-1′b), 3.83 (1H, dd, $J_{4'b,3'}$ 3.8 Hz, H-4′b), 3.79–3.67 (3H, m, H-1a, H-6a, H-6b), 3.57 (1H, dd, $J_{1b,1a}$ 12.2, $J_{1b,2}$ 4.2 Hz, H-1b). ^{13}C NMR (CD_3OD): δ 81.92 (C-3′), 81.67 (C-3), 79.43 (C-2), 72.62 (C-4), 70.38 (C-5), 67.64 (C-2′), 65.98 (C-6), 61.83 (C-4′), 48.63 (C-1′), 45.89 (C-1). Anal. Calcd for $C_{10}H_{20}O_{10}SSe$: C, 29.20; H, 4.90. Found: C, 29.00; H, 4.63.

3.18. Enzyme assays

Enzyme assays were carried out following a procedure similar to that described by Zhang and Liu.[46] Mutase (20 μg/mL) in 100 mM MOPS, pH 8.0 was pre-incubated with 20 mM freshly prepared Na_2S_2O on ice for 1 min. Inhibitors were then added and incubated for an additional minute. UDP-Gal*f* (63 μM) was then added and the reaction was allowed to proceed at room temperature. The reactions were stopped at different times by addition of ice-cold HCl and immediate freezing in liquid nitrogen. Samples were analyzed by HPLC (Agilent 1100 series) using a 5-μm Luna $C_{18}(2)$ column (4.6 × 250 mm, Phenomenex). The column was run isocratically with 1.5% acetonitrile in 50 mM Et_3NHOAc

buffer, pH 6.8 at a flow rate of 1 mL/min. The two sugars separated with base line resolution, and the extent of conversion was determined by integration of the peaks and Eq. 1:

$$(\text{area of UDP} - \text{Gal}p\ \text{peak})/[(\text{area of UDP} - \text{Gal}p\ \text{peak}) + (\text{area of UDP} - \text{Gal}f\ \text{peak})] \quad (1)$$

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