Synthesis of 1,4-anhydro-D-xylitol heteroanalogues of the naturally occurring glycosidase inhibitor salacinol and their evaluation as glycosidase inhibitors

Ahmad Ghavami, Blair D. Johnston, Matthew D. Maddess, Sarah M. Chinapoo, Morten T. Jensen, Birte Svensson, and B. Mario Pinto

Abstract: The syntheses of two 1,4-anhydro-D-xylitol heteroanalogues (**8** and **9**) of the naturally occurring sulfonium ion, salacinol (**3**), containing a sulfur or nitrogen atom in the ring are described. Salacinol (**3**) is one of the active principles in the aqueous extracts of *Salacia reticulata* that are traditionally used in Sri Lanka and India for the treatment of Type 2 diabetes. The synthetic strategy relies on the nucleophilic attack of sulfur or nitrogen analogues of 1,4-anhydro-D-xylitol at the least-hindered carbon of 2,4-*O*-benzylidene-L-erythritol-1,3-cyclic sulfate. The sulfonium ion **8** inhibited barley-α-amylase (AMY1) and porcine pancreatic-α-amylase (PPA), with K_i values of 109 ± 11 and 55 ± 5 μM, respectively. In contrast, the ammonium ion **9** showed no significant inhibition of either AMY1 or PPA. Compounds **8** and **9** also showed no significant inhibition of glucoamylase.

Key Words: glycosidase inhibitors, salacinol analogues, anhydro-D-xylitol heteroanalogues, enzyme inhibition.

Résumé : On décrit les synthèses de deux hétéroanalogues du 1,4-anhydro-D-xylitol (**8** et **9**) qui comportent un atome de soufre ou d'azote dans le cycle de l'ion sulfonium, salacinol (**3**), que l'on retrouve dans la nature. Le salacinol (**3**) est un des principes actifs que l'on retrouve dans la phase d'extraction aqueuse du *Salacia reticulata* utilisée au Sri-Lanka et aux Indes dans le traitement du diabète de type 2. La stratégie de la synthèse repose sur une attaque nucléophile d'analogues soufrés ou azotés du 1,4-anhydro-D-xylitol au niveau du carbone le moins encombré du sulfate 1,3-cyclique du 2,4-*O*-benzylidène-L-érythritol. L'ion sulfonium **8** inhibe l'α-amylase de l'orge (AMY1) et de l'α-amylase du pancréas de porc (PPA) avec des valeurs de K_i de 109 ± 11 et 55 ± 5 μM, respectivement. Par ailleurs, l'ion ammonium **9** ne provoque pas d'inhibition significative de l'AMY1 ou de la PPA. Les composés **8** et **9** n'inhibent pas de façon significative la glucoamylase.

Mots clés : inhibiteurs de glycosidase, analogues du salacinol, hétéroanalogues de l'anhydro-D-xylitol, inhibition enzymatique.

[Traduit par la Rédaction]

Introduction

A program of research to investigate the nature and origin of carbohydrate mimicry is in progress in our laboratory. Thus, we have recently reported the study of the peptide mimicry of carbohydrates recognized by antibodies directed against the Group A *Streptococcus* cell-wall polysaccharide (1) and have recently communicated our results with an antibody directed against the *Shigella flexneri* Y *O*-antigen (2). In the latter study, the crystal structures of the Ab-peptide mimetic and Ab-pentasaccharide complexes were compared. The results indicated that although both ligands engage some common groups on the Ab receptor in H-bonding and hydrophobic interactions, each ligand also displays unique interactions with groups on the protein, lending support to our previous hypothesis that "functional" and not "structural" mimicry might be the mode of mimicry with peptide mimetics (1).

We have also studied the mimicry of carbohydrates by glycomimetics as potential glycosidase inhibitors. Thus, for example, we have described the synthesis and conformational analysis of a sulfonium-ion analogue (**1**) of the glycosidase inhibitor castanospermine (**2**) (3). Our reasoning was inspired by the pioneering work of the late B. Belleau who synthesized sulfonium-ion analogues of the morphinans, levorphanol, and isolevorphanol, and showed that they were agonists or antagonists of morphine for the opiate receptor (*4a–d*). Recently, a new class of glycosidase inhibitor with an intriguing inner-salt sulfonium-sulfate structure was

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This work is dedicated, with respect and affection, to the memory of R.U. Lemieux.

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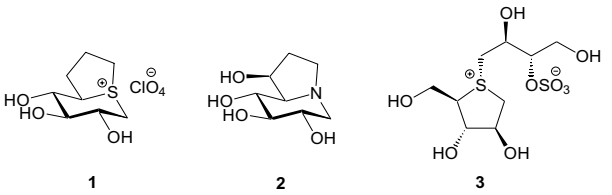
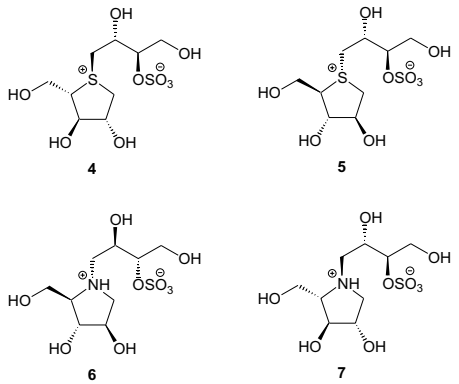
isolated from the roots and stems of the plant *Salacia reticulata*. Extracts of this plant have been traditionally used in the Ayurdevic method of Indian medicine as a treatment



1 **2** **3**

for diabetes. One of the active ingredients of these extracts is the sulfonium salt salacinol (**3**) (5).

We (6) and others (7) have recently reported the synthesis of salacinol (**3**) and its stereoisomers (**4**, **5**), and provided conclusive proof of structure of the natural product. We have also reported the syntheses of the hitherto unknown nitrogen congeners (**6**, **7**) as potential glycosidase inhibitors (8). Enzyme inhibition assays indicated that salacinol (**3**) is a weak (K_i = 1.7 mM) inhibitor of glucoamylase, whereas compounds **6** and **7** inhibit glucoamylase with K_i values in the range about 10-fold higher. The nitrogen analogues **6** and **7** showed no significant inhibitory effect of either barley α-amylase (AMY1) or porcine pancreatic α-amylase (PPA) at concentrations of 5 mM. In contrast, salacinol (**3**) inhib-



4 **5** **6** **7**

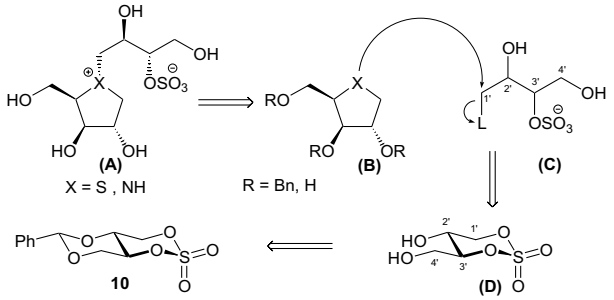
ited AMY1 and PPA in the micromolar range, with K_i values of 15 ± 1 μM and 10 ± 2 μM, respectively (8).

Yuasa and et al. (9) have also investigated the glucosidase inhibitory activities of compounds (**3**) and (**5**) and showed that although salacinol (**3**) is a better inhibitor (IC_{50} = 1.1 μM) of rice α-mannosidase than its diastereomer **5** (IC_{50} = 0.38 mM), the inhibitory activities are comparable for almond α-glucosidase (IC_{50} = 2.1 mM for **3**; 3.6 mM for **5**). In the case of almond β-glucosidase, (**5**) is a better inhibitor than (**3**) ((**3**) showed no activity; IC_{50} = 3.4 mM for (**5**)).

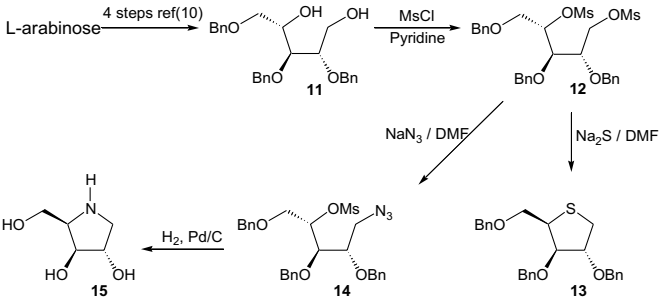
In a recent study, Muraoka et al. (10) showed that salacinol (**3**) inhibits intestinal α-glucosidases: maltase, sucrase, and isomaltase with IC_{50} values of 9.6 μM, 2.5 μM, and 1.8 μM, respectively, whereas compound (**6**) inhibits these enzymes with IC_{50} values of 306 μM, 44 μM, and 136 μM, respectively.

The results described above suggest that the stereochemistry at the different stereogenic centres and the nature of the ring heteroatom in the candidate inhibitors play a significant role in discriminating between different glycosidase

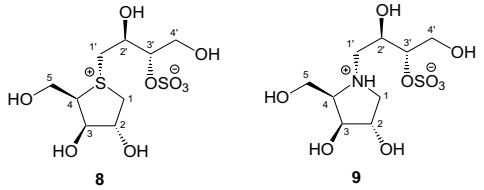
Scheme 1.



Scheme 2.



enzymes. Therefore, to probe these structure–function studies further, we now report the syntheses of the thio and iminoxylitol analogues (**8**, **9**) of salacinol (**3**) and their evaluation as glycosidase inhibitors of AMY1, PPA, or glucoamylase.



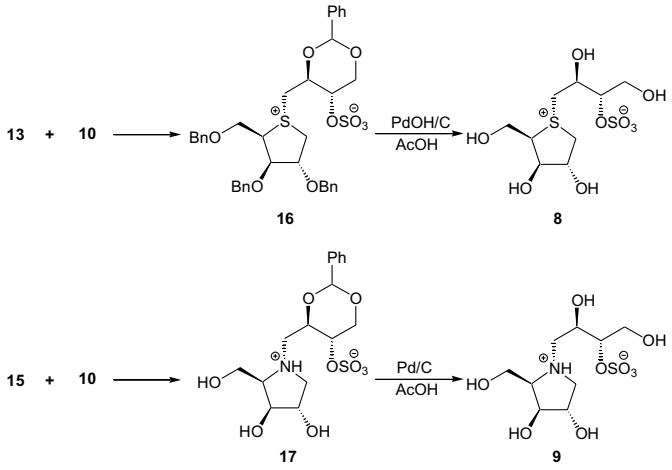
8 **9**

Results and discussion

Retrosynthetic analysis indicated that salacinol (**3**) or its analogues (**A**) could be obtained by alkylation of anhydroalditol derivatives at the ring heteroatom (Scheme 1). As in our previous work (6, 8), the benzylidene acetal **10** of **D** was chosen as the alkylating agent. We envisaged that selective attack of the heteroatom at the least-hindered primary centre would afford the desired sulfonium or ammonium ions.

The cyclic sulfate (**10**) was synthesized in five steps starting from L-glucose (6). The thio- and iminoxylitols (**13**, **15**) were synthesized from L-arabinose. Thus, the diol (**11**) was synthesized from L-arabinose in four steps according to the procedure used by van der Klein et al. (11) to synthesize its enantiomer (Scheme 2). Treatment of the diol (**11**) with methanesulfonyl chloride in pyridine then afforded the dimesylated compound (**12**) (88% yield). Compound (**12**) was used as a key intermediate to synthesize both the thio- and iminoxylitols. Treatment of (**12**) with sodium sulfide in DMF produced compound (**13**) in 95% yield, whereas treatment with sodium azide in DMF followed by

Scheme 3.



hydrogenolysis afforded the iminoxylitol (**15**) in 55% yield for the two steps (12).

To synthesize the target sulfonium ion **8**, compound **16** was first synthesized by alkylation of 1,4-anhydro-2,3,5-tri-*O*-benzyl-4-thio-D-xylitol (**13**) with the cyclic sulfate **10** (1.2 equiv) in acetone containing K_2CO_3 at 60–70°C in 72% yield. Compound **16** was obtained as the sole coupled product (Scheme 3). The stereochemistry at the stereogenic sulfonium centre in **16** was established by means of a NOESY experiment. Thus, a correlation between H-1′ and H-4, confirmed the trans relationship between the erythritol side chain and C-4 substituent on the anhydroxylitol moiety, which is similar to the stereochemistry at the stereogenic sulfur atom in salacinol (**3**). Deprotection of **16** by hydrogenolysis over a palladium hydroxide catalyst on carbon was problematic because of poisoning of the catalyst but afforded compound **8** in 50% yield.

The corresponding nitrogen congener **17** was synthesized in an analogous manner although, in this case, the increased nucleophilicity of the nitrogen atom did not necessitate benzylation of the hydroxyl groups. Thus, alkylation of 1,4-dideoxy-1,4-imino-D-xylitol (**15**) with the cyclic sulfate **10** (1.2 equiv) in methanol containing K_2CO_3 at 60–70°C afforded compound **17** in 63% yield. The stereochemistry at the stereogenic nitrogen centre in **17** was established by means of a NOESY experiment, as above. In this case, a correlation between H-1′ and H-3, confirmed the trans relationship between the erythritol side chain and the C-3 substituent on the iminoxylitol moiety. Deprotection of **17** by hydrogenolysis over a Pd/C catalyst afforded compound **9** in 83% yield.

Enzyme inhibition assays

Compounds **8** and **9** were tested for their inhibition of three glycosidase enzymes, namely glucoamylase G2, (13, 14) porcine pancreatic α-amylase, and barley α-amylase (15). The effects were compared to those of salacinol (**3**). Glucoamylase G2 was weakly inhibited by salacinol (**3**) (K_i = 1.7 mM) whereas compounds **8** and **9** showed no significant inhibition of glucoamylase. The sulfonium ion **8** inhibited barley α-amylase (AMY1) and porcine pancreatic α-amylase (PPA), with K_i values of 109 ± 11 and 55 ± 5 μM, respectively, as compared to salacinol (**3**), with K_i values of 15 ± 1 and 10 ± 2 μM, respectively. In contrast, the ammonium ion **9** showed no significant inhibition of either AMY1 or PPA (Table 1). It would appear then that analogues **8** and **9** and salacinol (**3**) show discrimination or selectivity for certain glycosidase enzymes, and further testing against a wider panel of enzymes that includes human small intestinal maltase–glucoamylase (16) and human pancreatic α-amylase (17) is planned to map the enzyme selectivity profiles of these compounds.

Table 1. K_i (mM) values of compounds **1** and **3**–**9** against barley α-amylase (AMY1), porcine pancreatic α-amylase (PPA), and glucoamylase.

Compound	AMY1	PPA	Glucoamylase
1	>5	>5	1.32
3	0.015	0.01	1.71
4	>5	>5	2.17
5	>5	>5	1.06
6	>5	>5	>2.5
7	>5	>5	>8
8	0.109	0.052	>5
9	>5	>5	>30

Experimental section

General

Optical rotations were measured at 23°C. ^1H and ^{13}C NMR spectra were recorded at 400.13 and 100.6 MHz. All assignments were confirmed with the aid of two-dimensional ^1H, ^1H (COSYDFTP), or ^1H, ^{13}C (INVBTP) experiments using standard Bruker pulse programs. Column chromatography was performed with Merck Silica gel 60 (230–400 mesh). High resolution mass spectra were measured with liquid secondary ionization fast atom bombardment (LSI-MS (FAB)), run on a Kratos Concept H double focussing mass spectrometer at 10 000 RP, using *meta*-NO_2-benzyl alcohol as the matrix.

Enzyme inhibition assays

The glucoamylase G2 form from *Aspergillus niger* was purified from a commercial enzyme (Novo Nordisk, Bagsvaerd, Denmark) as described (13, 14). The initial rates of glucoamylase G2-catalyzed hydrolysis of maltose was tested with 1 mM maltose as substrate in 0.1 M sodium acetate pH 4.5 at 45°C using an enzyme concentration of 7.0×10^{-8} M and five inhibitor concentrations in the range 1 μm – 5 mM. The effect of the inhibition on rates of substrate hydrolysis were compared for the different compounds. The glucose released was analyzed in aliquots removed at appropriate time intervals using a glucose oxidase assay adapted to microtiter plate reading and using a total reaction volume for the enzyme reaction mixtures of 150 or 300 μL (18). The K_i values were calculated assuming competitive inhibition from $1/v = (1/V_{max}) + [(K_m)/(V_{max}[S]K_i)] \times [I]$, where v is the rate measured in the presence or absence of inhibitor, $[I]$ and $[S]$ the concentrations of inhibitor and substrate, K_m 1.6 mM, and k_{cat} 11.3 s^{-1}, using ENZFITTER (19).

Porcine pancreatic α-amylase (PPA) and bovine serum albumin (BSA) were purchased from Sigma. Amylose EX-1 (DP17; average degree of polymerization 17) was purchased from Hayashibara Chemical Laboratories (Okayama, Japan). Recombinant barley α–amylase isozyme 1 (AMY1) was produced and purified as described (15). An aliquot of the porcine pancreatic α-amylase (PPA) crystalline suspension (in ammonium sulfate) was dialyzed extensively against the assay buffer without BSA. The enzyme concentration was determined by aid of amino acid analysis as determined using an LKB model Alpha Plus amino acid analyzer. The inhibition of AMY1 (3×10^{-9} M) and PPA (9×10^{-9} M) activity towards DP17 amylose was measured at 37°C in 20 mM sodium acetate (pH 5.5, 5 mM $CaCl_2$, 0.005% BSA (for AMY1)) and 20 mM sodium phosphate (pH 6.9, 10 mM NaCl, 0.1 mM $CaCl_2$, 0.005% BSA (for PPA)). Six different final inhibitor concentrations were used in the range 1 μM – 5 mM. The inhibitor was preincubated with enzyme for 5 min at 37°C before addition of substrate. Initial rates were determined by measuring reducing sugar by the copper-bicinchoninate method as described (15, 20). The K_i values were calculated assuming competitive inhibition, as described above for the case of glucoamylase, and a K_m of 0.57 mg mL^{-1} and k_{cat} of 165 s^{-1} for AMY1 and 1 mg mL^{-1} and 1200 s^{-1} for PPA, as determined in the substrate concentration range 0.03–10 mg mL^{-1} using ENZFITTER (19). For the K_i determinations, [S] = 0.7 mg mL^{-1} amylose DP 17 for the AMY1 binding and [S] = 2.5 mg mL^{-1} amylose DP 17 for the PPA binding.

2,3,5-Tri-O-benzyl-L-arabinitol (11)

The diol **11** was synthesized from L-arabinose according to the procedure used by van der Klein et al. (11) to synthesize its enantiomer. $[\alpha]_D^{22}$ –4.6° (*c* 1.0, CH_2Cl_2) (lit. (10) value +6.8° (*c* 1, $CHCl_3$)). ^{1}H NMR (CD_2Cl_2) δ: 7.38–7.25 (15H, m, Ar), 4.65 and 4.61 (2H, 2d, $J_{A,B}$ = 11.4 Hz, C*H*$_2$Ph), 4.60 and 4.57 (2H, 2d, $J_{A,B}$ = 11.2 Hz, C*H*$_2$Ph), 4.55 and 4.51 (2H, 2d, $J_{A,B}$ = 11.9 Hz, C*H*$_2$Ph), 4.00 (1H, dddd, H-4), 3.81–3.74 (3H, m, H-2, H-1a, H-1b), 3.70 (1H, dd, $J_{3,4}$ = 7.0, $J_{2,3}$ = 3.6 Hz, H-3), 3.67 (1H, dd, $J_{5a,5b}$ = 12.2, $J_{4,5a}$ = 3.9 Hz, H-5a), 3.63 (1H, dd, $J_{4,5b}$ = 5.2 Hz, H-5b), 2.90 (1H, d, $J_{OH,4}$ = 5.4 Hz, 4-OH), 2.20 (1H, dd, $J_{OH,1a}$ = 6.1, $J_{OH,1b}$ = 4.6 Hz, 1-OH). ^{13}C NMR (CD_2Cl_2) δ: 138.68, 138.58 (3C_{ipso}), 128.76–128.07 (15C_{Ar}), 80.08 (C-2), 79.16 (C-3), 74.14, 73.72, 73.15 (3*C*H$_2$Ph), 71.63 (C-5), 70.84 (C-4), 61.82 (C-1).

2,3,5-Tri-O-benzyl-1,4-di-O-methanesulfonyl-L-arabinitol (12)

To a solution of the diol **11** (4.0 g, 9.5 mmol) in pyridine (20 mL) at 0°C was added a solution of methanesulfonyl chloride (1.8 mL, 2.5 equiv) in pyridine (3.0 mL). Stirring was continued at 0°C, and under an N_2 atmosphere until TLC (hexanes–EtOAc, 3:2) showed complete disappearance of the starting material. The solvent was removed under high vacuum, and the residue was dissolved in CH_2Cl_2 (100 mL) and washed with 1 M aq HCl (2 × 30 mL), H_2O (30 mL), and sat. aq $NaHCO_3$ (30 mL), and dried (Na_2SO_4). The solution was concentrated on a rotary evaporator and the product was purified by flash chromatography (hexanes–EtOAc, 3:2) to give **12** as a colourless oil (4.8 g, 88%). $[\alpha]_D^{22}$ +4.1° (*c* 1.5, CH_2Cl_2). ^{1}H NMR (CD_2Cl_2) δ: 7.42–7.20 (15H, m, Ar), 5.01 (1H, ddd, $J_{4,5b}$ = 6.8, $J_{4,5a}$ = $J_{3,4}$ = 3.2 Hz, H-4), 4.72 and 4.59 (2H, 2d, $J_{A,B}$ = 11.1 Hz, C*H*$_2$Ph), 4.65 and 4.61 (2H, 2d, $J_{A,B}$ = 11.0 Hz, C*H*$_2$Ph), 4.53 (2H, s, C*H*$_2$Ph), 4.34–4.29 (2H, m, H-1a, H-1b), 3.96–3.91 (2H, m, H-2, H-3), 3.87 (1H, dd, $J_{5a,5b}$ = 11.3 Hz, H-5a), 3.81 (1H, dd, H-5b), 3.00 (3H, s, OSO_2CH_3), 2.93 (3H, s, OSO_2CH_3). ^{13}C NMR (CD_2Cl_2) δ: 137.95, 137.87, 137.72 (3C_{ipso}), 128.84–128.29 (15C_{Ar}), 81.52 (C-4), 77.98 (C-2), 77.32 (C-3), 74.75, 73.96, 73.77 (3*C*H$_2$Ph), 6916 (C-5), 68.64 (C-1), 39.04 (OSO_2CH_3), 37.67(OSO_2CH_3). Anal. calcd. for $C_{28}H_{34}O_9S_2$: C 58.12, H 5.92; found: C 58.21, H 6.02.

1,4-Anhydro-2,3,5-tri-O-benzyl-4-thio-D-xylitol (13)

Compound **12** (1.6 g, 2.8 mmol) was dissolved in DMF (10 mL) and $Na_2S \cdot H_2O$ (1.1 g, 1.5 equiv) was added. The mixture was stirred at 100°C until TLC (hexanes–EtOAc, 4:1) showed complete disappearance of the starting material. The solvent was removed under high vacuum, and the residue was dissolved in EtOAc (100 mL) and washed with H_2O (30 mL). The organic phase was dried (Na_2SO_4) and concentrated on a rotary evaporator. The product was purified by flash chromatography (hexanes–EtOAc, 4:1) to give a colourless syrup (1.1 g, 95%). $[\alpha]_D^{22}$ +67° (*c* 1.3, CH_2Cl_2). ^{1}H NMR (CD_2Cl_2) δ: 7.37–7.25 (15H, m, Ar), 4.57–4.47 (6H, m, 3C*H*$_2$Ph), 4.21 (1H, ddd, H-2), 4.13 (1H, dd, $J_{2,3}$ = 3.8, $J_{3,4}$ = 3.5 Hz, H-3), 3.85 (1H, dd, $J_{4,5a}$ = 7.5 Hz, H-5a), 3.80 (1H, ddd, H-4), 3.60 (1H, dd, $J_{5a,5b}$ = 8.2, $J_{4,5b}$ = 5.5 Hz, H-5b), 3.10 (1H, dd, $J_{1a,1b}$ = 11.4, $J_{1a,2}$ = 4.4 Hz, H-1a), 2.85 (1H, dd, $J_{1b,2}$ = 2.1 Hz, H-1b). ^{13}C NMR (CD_2Cl_2) δ: 138.87, 138.56 (3C_{ipso}), 128.70–127.86 (15C_{Ar}), 83.52 (C-3), 83.47 (C-2), 73.52, 72.95, 71.60 (3*C*H$_2$Ph), 69.85 (C-5), 48.55 (C-4), 33.46 (C-1). Anal. calcd. for $C_{26}H_{28}O_3S$: C 74.25, H 6.71; found: C 74.05, H 6.63.

2,3,5-Tri-O-benzyl-1,4-dideoxy-1,4-[[(2′S,3′S)-2′,4′-O-benzylidene-3′-(sulfooxy)butyl]-episulfoniumylidene]-D-xylitol inner salt (16)

A mixture of the thioxylitol **13** (100 mg, 0.24 mmol) and 2,4-*O*-benzylidene-L-erythritol-1,3-cyclic sulfate (**10**) (80 mg, 1.2 equiv) was dissolved in dry acetone (0.5 mL) and anhyd K_2CO_3 (15 mg) was added. The mixture was stirred in a sealed tube in an oil bath (60–70°C) overnight. The solvent was removed under reduced pressure and column chromatography ($CHCl_3$–MeOH, 10:1 + 0.1% Et_3N) of the crude product gave an amorphous solid (120 mg, 72%). $[\alpha]_D^{22}$ +28° (*c* 0.3, CH_2Cl_2). ^{1}H NMR (CD_2Cl_2) δ: 7.54–7.07 (20H, m, Ar), 5.52 (1H, s, C*H*Ph), 4.60 and 4.49 (2H, 2d, $J_{A,B}$ = 11.7 Hz, C*H*$_2$Ph), 4.57–4.47 (2H, m, H-3′, H-4′ eq), 4.47 and 4.44 (2H, 2d, $J_{A,B}$ = 11.3 Hz, C*H*$_2$Ph), 4.41 (1H, dd, H-1′a), 4.41–4.36 (2H, m, H-2, H-3), 4.27 (1H, ddd, $J_{2',3'}$ = 9.1, $J_{1'a,2'}$ = $J_{1'b,2'}$ = 3.3 Hz, H-2′), 4.09 (1H, ddd, $J_{4,5a}$ = 9.4, $J_{4,5b}$ = 6.0, $J_{3,4}$ = 3.2 Hz, H-4), 4.02 and 3.96 (2H, 2d, $J_{A,B}$ = 11.4 Hz, C*H*$_2$Ph), 3.92 (1H, dd, $J_{1'b,1'a}$ = 13.6 Hz, H-1′b), 3.87 (1H, dd, $J_{1a,1b}$ = 14.4, $J_{1a,2}$ = 3.1 Hz, H-1a), 3.82 (1H, dd, $J_{5a,5b}$ = 9.4 Hz, H-5a), 3.76 (1H, dd, $J_{4'ax,4'eq}$ = $J_{3',4'ax}$ = 12.2 Hz, H-4′ ax), 3.71 (1H, brd, H-1b), 3.67 (1H, dd, H-5b). ^{13}C NMR (CD_2Cl_2) δ: 137.42, 137.17, 136.46 (4C_{ipso}), 129.82–126.65 (20C_{Ar}), 101.76 (*C*HPh), 82.64 (C-3), 81.74 (C-2), 76.63 (C-2′), 73.92, 73.84, 72.64 (3*C*H$_2$Ph), 69.54 (C-4′), 66.56 (C-4), 66.40 (C-3′), 64.00 (C-5), 51.51 (C-1′),

47.42 (C-1). Anal. calcd. for $C_{37}H_{40}O_9S_2$: C 64.14, H 5.82; found: C 64.45, H 5.85.

1,4-Dideoxy-1,4-[[(2′S,3′S)-2′,4′-dihydroxy-3′-(sulfooxy)butyl]-episulfoniumylidene]-D-xylitol inner salt (8)

The protected compound **16** (150 mg, 0.22 mmol) was dissolved in AcOH–H_2O (4:1, 3 mL) and stirred with palladium hydroxide catalyst on carbon (100 mg) under H_2 (52 psi). After 72 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 ($CHCl_3$–MeOH–H_2O, 7:3:1) to give an amorphous solid (36 mg, 50%). $[\alpha]_D^{22}$ +20° (*c* 1.2, MeOH). HR-MS calcd. for $C_9H_{18}O_9S_2$ (M + H): 335.0471; found: 335.0471. ^{1}H NMR (CD_3OD) δ: 4.64–4.59 (1H, m, H-2), 4.55 (1H, dd, $J_{2,3} = J_{3,4}$ = 2.9 Hz, H-3), 4.37 (1H, ddd, H-4), 4.35 (1H, ddd, $J_{1'a,2'}$ = 3.5 Hz, H-2′), 4.25 (1H, ddd, $J_{2',3'}$ = 7.2, $J_{3',4'a} = J_{3',4'b}$ = 3.4 Hz, H-3′), 4.16 (1H, dd, $J_{5a,5b}$ = 11.7, $J_{4,5a}$ = 6.0 Hz, H-5a), 4.07 (1H, dd, $J_{4,5b}$ = 9.0 Hz, H-5b), 3.95 (1H, dd, $J_{1'a,1'b}$ = 13.4 Hz, H-1′ a) 3.93 (1H, dd, H-4′ a), 3.91 (1H, dd, H-1a) 3.88 (1H, dd, $J_{1'b,2'}$ = 8.0 Hz, H-1′ b), 3.81 (1H, dd, $J_{4'b,4'a}$ = 12.1 Hz, H-4′ b), 3.58 (1H, brd, $J_{1b,1a}$ = 13.7 Hz, H-1b). ^{13}C NMR (CD_3OD) δ: 81.05 (C-3′), 79.55 (C-2), 78.56 (C-3), 71.69 (C-4), 67.71 (C-2′), 61.66 (C-4′), 58.92 (C-5), 52.60 (C-1′), 49.26 (C-1).

1′-((1,4-Dideoxy-1,4-imino-D-xylitol)-4-N-ammonium)-2′,4′-O-benzylidene-1′-deoxy-L-erythritol-3′-sulfate (17)

A mixture of 1,4-dideoxy-1,4-imino-D-arabinitol (**15**) (100 mg, 0.74 mmol) and 2,4-*O*-benzylidene-L-erythritol-1,3-cyclic sulfate (**10**) (240 mg, 1.2 equiv) was dissolved in dry MeOH (0.5 mL) and anhyd K_2CO_3 (15 mg) was added. The mixture was stirred in a sealed tube in an oil bath (60–70°C) overnight. The solvent was removed under reduced pressure, and column chromatography (CH_2Cl_2–MeOH, 5:1) of the crude product gave a white solid (191 mg, 63%) that was recrystallized from methanol; mp 202–204°C, $[\alpha]_D^{22}$ +30° (*c* 0.5, H_2O). ^{1}H NMR (D_2O) δ: 7.73–7.58 (5H, m, Ar), 5.93 (1H, s, C*H*Ph), 4.68 (1H, dd, $J_{4'eq,4'ax}$ = 11.0, $J_{3',4'eq}$ = 5.5 Hz, H-4′eq), 4.54 (1H, brt, $J_{2',3'} = J_{1'b,2'}$ = 9.9 Hz, H-2′), 4.50–4.41 (3H, m, H-3, H-3′, H-2), 4.20 (1H, dd, $J_{5a,5b}$ = 12.6, $J_{4,5a}$ = 5.5 Hz, H-5a), 4.18–4.12 (2H, m, H-1′a, H-5b), 4.10 (1H, dd, $J_{3',4'ax}$ = 11.0 Hz, H-4′ax), 4.11–4.01 (2H, m, H-1a, H-4), 3.66 (1H, br dd, $J_{1'b,1'a}$ = 11.6 Hz, H-1′b), 3.56–3.50 (1H, brd, H-1b). ^{13}C NMR (D_2O) δ: 138.50 (C_{ipso}), 132.45 (C_{para}), 131.26 (2C) and 128.62 (2C) (C_{ortho} and C_{meta}), 103.38 (*C*HPh), 78.08 (C-2′), 77.38 (C-3), 76.70 (C-2), 73.31 (C-4), 70.77 (C-4′), 70.41 (C-3′), 63.18 (C-1), 60.01 (C-1′), 59.55 (C-5). Anal. calcd. for $C_{16}H_{22}O_9SN$: C 47.51, H 5.49, N, 3.47; found: C 47.29, H 5.80, N 3.22.

1′-((1,4-Dideoxy-1,4-imino-D-xylitol)-4-N-ammonium)-1′-deoxy-L-erythritol-3′-sulfate (9)

The protected compound **17** (75 mg, 0.18 mmol) was dissolved in AcOH–H_2O (4:1, 5 mL) and stirred with Pd/C (30 mg) under H_2. After 16 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 ($CHCl_3$–MeOH–H_2O, 7:3:1) to give an amorphous solid (49 mg, 83%). $[\alpha]_D^{22}$ –5.3° (c 0.8, H_2O). HR-FAB-MS calcd. for $C_9H_{18}O_9SN$ (M + H): 318.0859; found: 318.0859. ^{1}H NMR (D_2O) δ: 4.59–4.40 (4H, m, H-3, H-2, H-2′, H-3′), 4.27–4.17 (2H, m, H-5a, H-5b), 4.18–4.06 (2H, m, H-1a, H-4), 4.11 (1H, dd, $J_{4'a,4'b}$ = 12.6, $J_{3',4'a}$ = 2.0 Hz, H-4′a), 4.02 (1H, dd, $J_{3',4'b}$ = 3.3 Hz, H-4′b), 4.11–3.95 (1H, m, H-1′a), 3.68–3.57 (1H, m, H-1b), 3.57–3.45 (1H, m, H-1′b). ^{13}C NMR (D_2O) δ: 82.52 (C-3′), 77.46 (C-3), 76.56 (C-2′), 73.20 (C-4), 68.42 (C-2), 63.09 (C-1), 62.38 (C-1′), 61.93 (C-4′), 59.38 (C-5).

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