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Note

Synthesis of thioswainsonine as a potential glycosidase inhibitor

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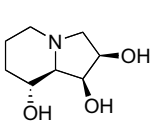
Abstract—The synthesis of a bicyclic sulfonium-ion analogue of a naturally occurring glycosidase inhibitor, swainsonine, in which the bridgehead nitrogen atom is replaced by a sulfonium ion, has been achieved by a multi-step synthesis starting from 1,4-anhydro-2,3-di-*O*-benzyl-4-thio-D-lyxitol. The synthetic strategy relies on the intramolecular displacement of a leaving group on a pendant acyclic chain by a cyclic thioether. This bicyclic sulfonium salt will serve as a candidate to test the hypothesis that a sulfonium salt carrying a permanent positive charge would be an effective glycosidase inhibitor.

Keywords: Glycosidase inhibitor; Swainsonine analogue; Bicyclic sulfonium salt; Indolizidine alkaloid analogue

Glycosidases play an important role in the metabolism of carbohydrates and in the processing of specific oligosaccharide structures on glycoproteins; the latter play important roles in intercellular recognition processes, and their modification has been implicated in disease states such as cancer.[1–7] Tumor cells display very complex carbohydrate structures that are usually restricted to embryonic tissues and it is believed that these structures provide signal stimuli for rapid proliferation and metastasis of tumor cells. Because tumor and normal cells have different rates of cell growth, a glycosidase inhibitor can be used to block the assembly of complex oligosaccharide structures, which might lead, in turn, to a therapeutic strategy for the treatment of cancer. For example, swainsonine (**1**), a plant-derived alkaloid, is an excellent inhibitor of Golgi α-mannosidase II (GMII) and has been shown to reduce tumor cell metastasis, enhance cellular immune responses, and slow tumor growth in mice.[8] Treatment with swainsonine (**1**) has led to a significant reduction of tumor mass in humans suffering from breast, liver, lung cancer, and other malignancies.[9,10] The most important class of reversible glycosidase inhibitors is composed of naturally occurring polyhydroxylated pyrrolidine, piperidine, pyrrolizidine, and indolizidine alkaloids[11–17] which are monocyclic and bicyclic amines such as 1-deoxynojirimycin (**2**), castanospermine (**3**), and swainsonine (**1**). It is postulated that these amines bind to glycosidase enzymes by mimicry of the shape and charge of the oxacarbenium ion transition state for the enzyme-catalyzed hydrolysis reaction.[18,19] The nitrogen atom is known to be protonated in the enzyme active site, thus providing the stabilizing electrostatic interactions between the inhibitor and the carboxylate residues in the enzyme active site. An alternative means of achieving the required charged state to provide such electrostatic interactions would be to synthesize compounds that incorporate a permanent positive charge at a suitable position.



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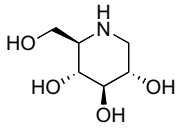

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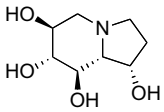

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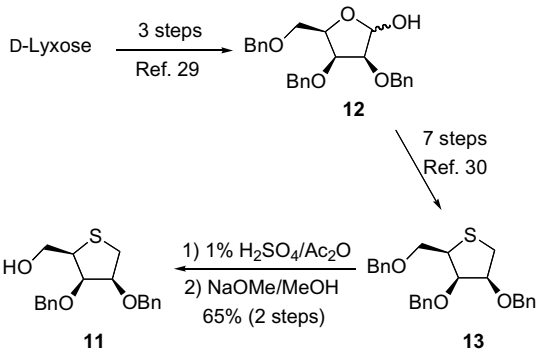
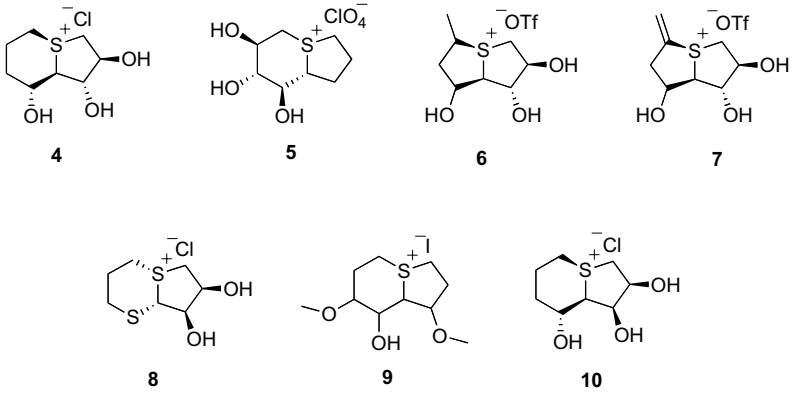
Recently, we described the synthesis of the sulfonium-ion analogue of 3-*epi*-swainsonine (**4**),[20] the synthesis, free and enzyme-bound conformations, and glycosidase inhibitory activity (albeit weak) of a sulfonium-ion analogue (**5**) of castanospermine (**3**),[21,22] and the synthesis of sulfonium ions (**6**, **7**) of the pyrrolizidine alkaloid, australine.[23] Siriwardena and co-workers have also reported the synthesis of bicyclic sulfonium salts to serve as glycosidase inhibitors.[24–26] Most recently they have reported the synthesis of compound **8**, which is not only a potent inhibitor of several mannosidases, but shows greater selectivity than swainsonine (**1**).[26] The syntheses of di-O-methylated sulfonium derivatives (**9**)[27] of swainsonine (**1**), as well as 5-*O*-methylthioswainsonine[28] have also been reported in the literature. We now report the first synthesis of the sulfonium-ion analogue (**10**) of swainsonine (**1**) as a potential glycosidase inhibitor.

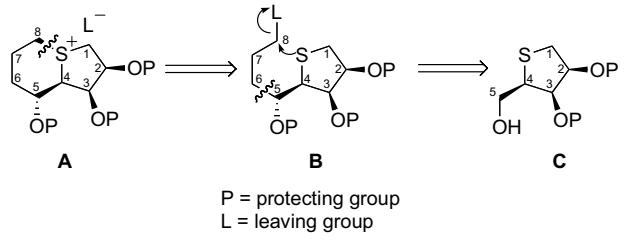


Scheme 2.



Retrosynthetic analysis indicates that the bicyclic sulfonium salt **A** could be synthesized by an intramolecular displacement of a suitable side-chain leaving group by a cyclic thioether (Scheme 1). The key intermediate **B** could, in turn, be synthesized from **C**.

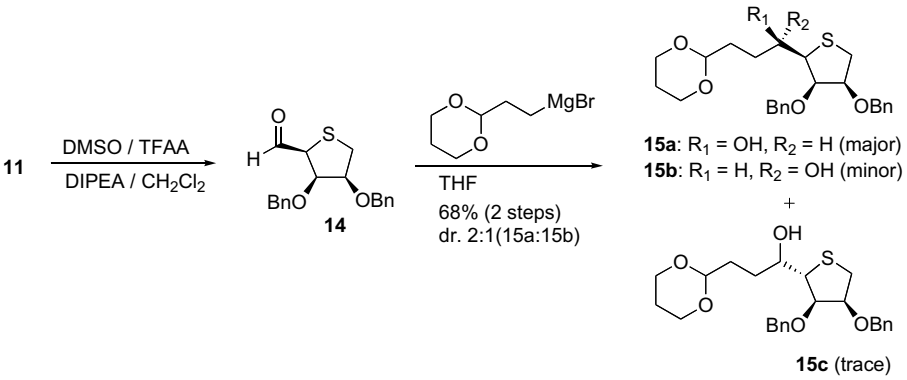
Compound **11**, corresponding to **C**, was synthesized from commercially available D-lyxose, as shown in Scheme 2. 2,3,5-Tri-*O*-benzyl-D-lyxofuranoside (**12**) was prepared in three steps starting from D-lyxose, as described by Postema et al.,[29] which was, in turn, transformed using a multi-step procedure into 1,4-anhydro-2,3,5-tri-*O*-benzyl-4-thio-D-lyxitol (**13**).[30] The primary benzyl ether was selectively removed using 1% sulfuric acid in acetic anhydride to give the corresponding acetate which was subsequently removed by methanolysis to give compound **11**.



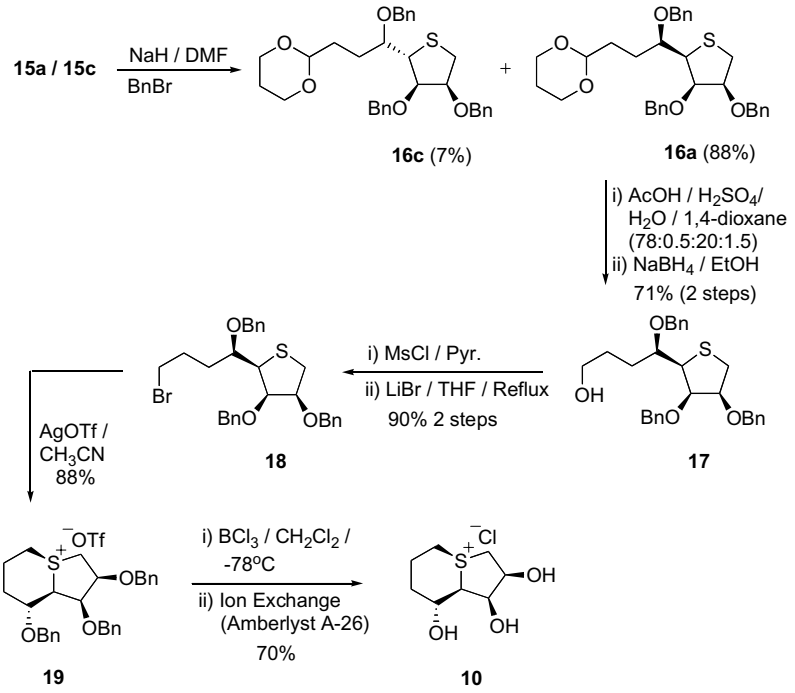
Scheme 1.

Swern oxidation of compound **11** using trifluoroacetic anhydride furnished aldehyde **14**, which was subsequently treated with a freshly prepared Grignard reagent from the reaction of 2-(2-bromoethyl)-1,3-dioxane and magnesium to give inseparable diastereomers **15a** and **15b** as a 2:1 mixture, along with a trace amount of diastereomer **15c** (Scheme 3). Compound **15c** was the result of epimerization at C-4, facilitated by the aldehyde group in **14**. After cyclization, it was found that the major compound **15a** had the required *R* configuration at C-5. Most of the minor isomer **15b** was recrystallized out from the mixture using ethyl acetate and hexane.

The secondary hydroxyl group at C-5 in each of compounds **15a** and **15c** was protected as a benzyl ether to give compounds **16a** and **16c**, respectively (Scheme 4), which were separated by flash chromatography. The 1,3-dioxane protecting group in compound **16a** was hydrolyzed to give the corresponding aldehyde, which was subsequently reduced using sodium borohydride to furnish compound **17**. Treatment of **17** with methanesulfonyl chloride furnished the corresponding sulfonate ester, which was subsequently treated with LiBr to give bromide **18**. Compound **18** was treated with sil-



Scheme 3.



Scheme 4.

ver triflate in acetonitrile to promote cyclization, giving the desired sulfonium salt **19** as a stable, colorless oil. The ring junction of this bicyclic compound **19** was cis as expected.[20,28,31] The configuration at C-5 of compound **19** was assigned by means of a 1D-NOESY experiment, which showed a correlation between H-3 and H-5; the stereochemistry of compound **15a** was thus assigned by inference. The benzyl protecting groups were removed with boron trichloride at −78 °C and the product was subsequently treated with Amberlyst A-26 (chloride form) to completely exchange the triflate counterion with chloride ion to give compound **10**, because our previous work had shown that some of the triflate counterion was exchanged to chloride ion during the course of deprotection.[20]

1. Experimental

1.1. General methods

Optical rotations were measured at 23 °C and reported in deg dm^{-1} g^{-1} cm^3. ^1H and ^{13}C NMR spectra were recorded with frequencies of 500 and 125 MHz, respectively. All assignments were confirmed with the aid of two-dimensional $^1H,^1H$ (gCOSY) and $^1H,^{13}C$ (gHMQC) experiments using standard Varian pulse programs. Processing of the spectra was performed with MestRec software. 1D-NOESY experiments were recorded at 295 K on a 500 MHz spectrometer. For each 1D-NOESY spectrum, 512 scans were acquired with a Q3 Gaussian Cascade pulse. A mixing time of 500 ms was used in the

1D-NOESY experiments. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectra were obtained using 2,5-dihydroxybenzoic acid as a matrix. Analytical thin-layer chromatography (TLC) was performed on aluminum plates precoated with 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. Column chromatography was performed with silica gel 60 (230–400 mesh).

1.2. 1,4-Anhydro-2,3-di-*O*-benzyl-4-thio-D-lyxitol (11)

A solution of 1,4-anhydro-2,3,5-tri-*O*-benzyl-4-thio-D-lyxitol (**13**) (5.00 g, 11.9 mmol) in 1.0% H_2SO_4/Ac_2O (50.0 mL) was stirred at ambient temperature for 14 h and then partitioned between EtOAc (200 mL) and H_2O (100 mL). The organic layer was washed with H_2O (100 mL), satd aq $NaHCO_3$ (2 × 100 mL), followed by brine (100 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was dissolved in CH_3OH (200 mL) and 1 M $NaOCH_3/CH_3OH$ solution was added until the solution was basic. The reaction mixture was stirred at room temperature for 1 h and then neutralized with HOAc. The mixture was concentrated and then partitioned between EtOAc (300 mL) and H_2O (100 mL). The organic phase was washed with H_2O (100 mL) and brine (100 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product was purified by flash chromatography (3:1, hexanes/EtOAc) to give compound **11** as a colorless oil (2.55 g, 65%): $[\alpha]_D$ −12.9 (*c* 1.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 7.38–7.28 (m, 10H, Ar), 4.79 and 4.64 (2d, each 1H, $J_{a,b} = 11.9$ Hz, CH_2Ph), 4.69 and 4.61 (2d, each 1H, $J_{a,b} = 12.1$ Hz, CH_2Ph), 4.17–4.15 (m, 2H, H-2, H-3), 3.92 (dd, 1H, $J_{4,5a} = 7.1$ Hz, $J_{5a,5b} = 11.5$ Hz, H-5a), 3.77 (dd, 1H, $J_{4,5b} = 4.9$ Hz, H-5b), 3.55 (ddd, 1H, $J_{3,4} = 5.4$ Hz, H-4), 3.04 (dd, 1H, $J_{1a,2} = 4.2$ Hz, $J_{1a,1b} = 10.5$ Hz, H-1a), 2.92 (dd, 1H, $J_{1b,2} = 4.6$ Hz, H-1b); ^{13}C NMR ($CDCl_3$): δ 138.1, 138.0 (2C$_{ipso}$), 128.8–127.8 (10C$_{Ar}$), 81.7 (C-2), 81.2 (C-3), 73.4, 72.4 (2CH_2Ph), 63.1 (C-5), 47.5 (C-4), 31.2 (C-1); MALDI-TOFMS: *m*/*z* 331.21 $[M+H]^+$, 352.84 $[M+Na]^+$, 368.97 $[M+K]^+$. Anal. Calcd for $C_{19}H_{22}O_3S$: C, 69.06; H, 6.71. Found: C, 69.20; H, 6.85.

1.3. 1,4-Anhydro-6,7-dideoxy-2,3-di-*O*-benzyl-4-thio-7-(1′,3′-dioxan-2′-yl)-D-*manno*-heptitol (15a); 1,4-anhydro-6,7-dideoxy-2,3-di-*O*-benzyl-4-thio-7-(1′,3′-dioxan-2′-yl)-L-*gluco*-heptitol (15b) and 1,4-anhydro-6,7-dideoxy-2,3-di-*O*-benzyl-4-thio-7-(1′,3′-dioxan-2′-yl)-(D-*talo*/L-*allo*)-heptitol (15c)

To a stirred solution of DMSO (1.5 mL, 21.5 mmol) in CH_2Cl_2 (15 mL) at −78 °C under N_2 atmosphere was added a solution of trifluoroacetic anhydride (0.87 mL, 11.6 mmol) in CH_2Cl_2 (5 mL) dropwise, and the mixture was stirred at −78 °C for 30 min. A solution of compound **11** (1.01 g, 3.06 mmol) in CH_2Cl_2 (15 mL) was added dropwise while maintaining the temperature below −78 °C and the stirring was continued for 1.5 h. A solution of diisopropylethylamine (2.3 mL, 12.6 mmol) in CH_2Cl_2 (15 mL) was added dropwise and the stirring was continued at −78 °C for an additional 2 h. The reaction was quenched by the addition of aqueous HCl (0.5 M, 5 mL) and the mixture was partitioned between Et_2O (200 mL) and H_2O (50 mL). The Et_2O layer was washed with H_2O (50 mL) and brine (50 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product was purified by flash chromatography (6:1, hexanes/EtOAc) to give the aldehyde **14**. Compound **14** was diluted with THF (15 mL) and cooled to 0 °C under N_2. A Grignard reagent, freshly prepared from 2-(2-bromoethyl)-1,3-dioxane (0.75 mL, 5.49 mmol) and Mg (272 mg, 10.90 mmol) in THF (15 mL), was added dropwise. The reaction mixture was stirred at ambient temperature for 14 h and then the reaction was quenched by the addition of 0.5 M HCl (5 mL), and concentrated. The residue was diluted with Et_2O (200 mL) and washed with H_2O (2 × 50 mL) and brine (50 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product was purified by flash chromatography (3:1, hexanes/EtOAc) to give a 2:1 mixture of compounds **15a** and **15b**, respectively, along with a trace amount of compound **15c** as a white solid (0.92 g, 68%).

Data for the major diastereomer (**15a**): ^{1}H NMR ($CDCl_3$): δ 7.40–7.31 (m, 10H, Ar), 4.98 and 4.74 (2d, each 1H, $J_{a,b} = 11.6$ Hz, CH_2Ph), 4.63 and 4.58 (2d, each 1H, $J_{a,b} = 12.1$ Hz, CH_2Ph), 4.54 (dd, 1H, $J_{2',7a} = J_{2',7b} =$ 4.8 Hz, H-2′), 4.27 (dd, 1H, $J_{2,3} = 3.0$ Hz, $J_{3,4} = 3.9$ Hz, H-3), 4.15– 4.08 (m, 2H, H-4′eq, H-6′eq), 4.02 (ddd, 1H, $J_{1b,2} = 6.2$ Hz, $J_{1a,2} = 9.0$ Hz, H-2), 3.91 (ddd, 1H, $J_{5,6b} = 2.0$ Hz, $J_{4,5} = J_{5,6a} = 9.2$ Hz, H-5), 3.72 (ddd, 1H, $J_{4'ax,5'eq} = 2.4$ Hz, $J_{4'ax,4'eq} = J_{4'ax5'ax} = 12.1$ Hz, H-4′ax), 3.72 (ddd, 1H, $J_{5'eq,6'ax} = 2.4$ Hz, $J_{6'ax,6'eq} = J_{5'ax,6'ax} = 12.1$ Hz, H-6′ax), 3.19 (dd, 1H, H-4), 3.08 (dd, 1H, $J_{1a,1b} = 9.3$ Hz, H-1a), 2.90 (dd, 1H, H-1b), 2.07 (ddddd, 1H, $J_{4'eq,5'ax} = J_{5'ax,6'eq} = 4.9$ Hz, $J_{5'ax,5'eq} = 12.7$ Hz, H-5′ax), 1.79–1.64 (m, 3H, H-6a, H-7a, H-7b), 1.43–1.29 (m, 2H, H-5′eq, H-6b); ^{13}C NMR ($CDCl_3$): δ 138.7, 138.2 (2C$_{ipso}$), 128.7–127.7 (10C$_{Ar}$), 102.4 (C-2′), 83.5 (C-2), 79.6 (C-3), 73.9, 72.4 (2CH_2Ph), 71.5 (C-5), 67.13 (C-4′), 67.11 (C-6′), 51.6 (C-4), 31.4 (C-7), 31.0 (C-1), 29.7 (C-6), 25.9 (C-5′).

For the mixture of **15a**,**b** and **15c**: MALDI-TOF MS: *m*/*z* 445.30 $[M+H]^+$, 467.32 $[M+Na]^+$, 483.30 $[M+K]^+$. Anal. Calcd for $C_{25}H_{32}O_5S$: C, 67.54; H, 7.27. Found: C, 67.20; H, 7.45.

The minor isomer (**15b**) was obtained by crystallization from hexanes/EtOAc as a white solid: mp 116–118 °C;

$[\alpha]_D$ +5.2 (*c* 0.6, CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 7.36–7.29 (m, 10H, Ar), 4.86 and 4.63 (2d, each 1H, $J_{a,b} = 11.7$ Hz, CH_2Ph), 4.70 and 4.59 (2d, each 1H, $J_{a,b} = 12.1$ Hz, CH_2Ph), 4.50 (dd, 1H, $J_{2',7a} = J_{2',7b} =$ 5.0 Hz, H-2′), 4.16–4.03 (m, 4H, H-2, H-3, H-4′eq, H-6′eq), 4.01 (ddd, 1H, $J_{4,5} = J_{5,6a} = 4.2$ Hz, $J_{5,6b} = 8.4$ Hz, H-5), 3.74 (ddd, 1H, $J_{4'ax,5'eq} = 2.4$ Hz, $J_{4'ax,4'eq} =$ $J_{4'ax,5'ax} = 12.0$ Hz, H-4′ax), 3.71 (ddd, 1H, $J_{5'eq,6'ax} = 2.5$ Hz, $J_{6'ax,6'eq} = J_{5'ax,6'ax} = 11.8$ Hz, H-6′ax), 3.42 (dd, 1H, $J_{3,4} = 4.1$ Hz, H-4), 3.05 (dd, 1H, $J_{1a,2} = 7.0$ Hz, $J_{1a,1b} = 10.5$ Hz, H-1a), 2.94 (dd, 1H, $J_{1b,2} = 5.5$ Hz, H-1b), 2.04 (ddddd, 1H, $J_{4'eq,5'ax} = J_{5'ax,6'eq} = 5.0$ Hz, $J_{5'ax,5'eq} = 12.6$ Hz, H-5′ax), 1.77 (dddd, 1H, $J_{6b,7a} = 5.2$ Hz, $J_{6a,7a} = 9.3$ Hz, $J_{7a,7b} = 14.0$ Hz, H-7a), 1.65 (dddd, 1H, $J_{6a,7b} = 6.7$ Hz, $J_{6b,7b} = 8.6$ Hz, H-7b), 1.53 (dddd, 1H, $J_{6a,6b} = 14.1$ Hz, H-6b) 1.46 (m, 1H, H-6a), 1.31 (ddddd, 1H, $J_{4'eq,5'eq} = J_{5'eq,6'eq} = 1.2$ Hz, $J_{5'ax,5'eq} = 13.4$ Hz, H-5′eq); ^{13}C NMR ($CDCl_3$): δ 138.1, 137.7 (2C$_{ipso}$), 128.8–127.9 (10C$_{Ar}$), 102.3 (C-2′), 82.1 (C-2), 81.6 (C-3), 73.5, 72.5 (2*C*H$_2$Ph), 69.1 (C-5), 67.10 (C-4′), 67.08 (C-6′), 52.4 (C-4), 31.7 (C-7), 30.9 (C-1), 30.4 (C-6), 26.0 (C-5′); MALDI-TOFMS: *m*/*z* 445.26 $[M+H]^+$, 467.30 $[M+Na]^+$, 483.27 $[M+K]^+$. Anal. Calcd for $C_{25}H_{32}O_5S$: C, 67.54; H, 7.27. Found: C, 67.80; H, 7.49.

1.4. 1,4-Anhydro-6,7-dideoxy-2,3,5-tri-*O*-benzyl-4-thio-7-(1′,3′-dioxan-2′-yl)-D-*manno*-heptitol (16a) and 1,4-anhydro-6,7-dideoxy-2,3,5-tri-*O*-benzyl-4-thio-7-(1′,3′-dioxan-2′-yl)-(D-*talo*/L-*allo*)-heptitol (16c)

A mixture of compounds **15a**/**15c** (145 mg, 0.33 mmol) and 60% NaH (20 mg, 1.5 equiv) in DMF (8 mL) was stirred in an ice bath for 15 min. Benzyl bromide (66 μL, 1.3 equiv) was added and the solution was stirred at room temperature for 2 h. The reaction was quenched by the addition of ice H_2O (1 mL) and the mixture was diluted with Et_2O (50 mL). The organic layer was washed with H_2O (20 mL) and brine (20 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product was purified by flash chromatography (5:1, hexanes/EtOAc) to give **16a** (153 mg, 88%) and **16c** (12 mg, 7%).

Data for the major diastereomer (**16a**): $[\alpha]_D$ −5.3 (*c* 1.1, CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 7.36–7.24 (m, 15H, Ar), 5.07 and 4.59 (2d, each 1H, $J_{a,b} = 11.4$ Hz, CH_2Ph), 4.56 (s, 2H, CH_2Ph), 4.56 and 4.32 (2d, each 1H, $J_{a,b} = 11.0$ Hz, CH_2Ph), 4.50 (dd, 1H, $J_{7a,2'} = J_{7b,2'} =$ 5.1 Hz, H-2′), 4.34 (dd, 1H, $J_{2,3} = J_{3,4} = 2.8$ Hz, H-3), 4.09–4.05 (m, 2H, H-4′eq, H-6′eq), 4.02 (ddd, 1H, $J_{1b,2} = 6.5$ Hz, $J_{1a,2} = 10.5$ Hz, H-2), 3.98 (ddd, 1H, $J_{5,6a} = J_{5,6b} = 4.3$ Hz, $J_{4,5} = 9.9$ Hz H-5), 3.72 (ddd, 1H, $J_{4'ax,5'eq} = 2.4$ Hz, $J_{4'ax,4'eq} = J_{4'ax,5'ax} = 12.0$ Hz, H-4′ax), 3.71 (ddd, 1H, $J_{5'eq,6'ax} = 2.4$ Hz, $J_{6'ax,6'eq} = J_{5'ax,6'ax} = 11.9$ Hz, H-6′ax), 3.44 (dd, 1H, H-4), 3.13 (dd, 1H, $J_{1a,1b} = 9.6$ Hz, H-1a), 2.95 (dd, 1H, H-1b), 2.06 (ddddd, 1H, $J_{4'eq,5'ax} = J_{5'ax,6'eq} = 5.0$ Hz, $J_{5'ax,5'eq} =$ 12.6 Hz, H-5′ax), 1.93 (dddd, 1H, $J_{6a,7a} = 4.7$ Hz, $J_{6a,7b} = 11.2$ Hz, $J_{6a,6b} = 14.8$ Hz, H-6a), 1.78–1.63 (m, 2H, H-7a, H-7b), 1.51 (dddd, 1H, $J_{6b,7b} = 5.0$ Hz, $J_{6b,7a} = 10.7$ Hz, H-6b), 1.31 (m, 1H, H-5′eq); ^{13}C NMR ($CDCl_3$): δ 139.3, 138.9, 138.2 (3C$_{ipso}$), 128.7–127.5 (15C$_{Ar}$), 102.5 (C-2′), 84.7 (C-2), 78.6 (C-3), 77.6 (C-5), 73.8, 72.4, 71.2 (3*C*H$_2$Ph), 67.1 (2C, C-4′, C-6′), 49.4 (C-4), 30.7 (C-1), 29.4 (C-7), 26.0 (C-5′), 25.1 (C-6); MALDI-TOFMS: *m*/*z* 535.08 $[M+H]^+$, 556.96 $[M+Na]^+$, 573.32 $[M+K]^+$. Anal. Calcd for $C_{32}H_{38}O_5S$: C, 71.88; H, 7.16. Found: C, 71.59; H, 7.35.

Data for the minor diastereomer (**16c**): $[\alpha]_D$ −17.1 (*c* 0.4, CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 7.37–7.22 (m, 15H, Ar), 4.62 and 4.58 (2d, each 1H, $J_{a,b} = 12.4$ Hz, CH_2Ph), 4.55 and 4.46 (2d, each 1H, $J_{a,b} = 11.4$ Hz, CH_2Ph), 4.46 and 4.42 (2d, each 1H, $J_{a,b} = 11.9$ Hz, CH_2Ph), 4.43 (dd, 1H, $J_{7a,2,} = J_{7b,2'} = 3.6$ Hz, H-2′), 4.13–4.04 (m, 2H, H-4′eq, H-6′eq), 4.00 (dd, 1H, $J_{2,3} = J_{3,4} = 3.3$ Hz, H-3), 3.96 (ddd, 1H, $J_{1b,2} = 5.7$ Hz, $J_{1a,2} = 8.7$ Hz, H-2), 3.69 (ddd, 1H, $J_{4'ax,5'eq} = 2.5$ Hz, $J_{4'ax,4'eq} = J_{4'ax,5'ax} =$ 12.1 Hz, H-4′ax), 3.68 (ddd, 1H, $J_{5'eq,6'ax} = 2.5$ Hz, $J_{6'ax,6'eq} = J_{5'ax,6'ax} = 12.0$ Hz, H-6′ax), 3.60 (dd, 1H, $J_{4,5} = 7.0$ Hz H-4), 3.35 (m, 1H, H-5), 3.03 (dd, 1H, $J_{1a,1b} = 10.2$ Hz, H-1a), 2.85 (dd, 1H, H-1b), 2.04 (ddddd, 1H, $J_{4'eq,5'ax} = J_{5'ax,6'eq} = 4.9$ Hz, $J_{5'ax,5'eq} =$ 12.6 Hz, H-5′ax), 1.77–1.59 (m, 4H, H-6a, H-6b, H-7a, H-7b), 1.31 (m, 1H, H-5′eq); ^{13}C NMR ($CDCl_3$): δ 138.32, 138.27, 138.11 (3C$_{ipso}$), 128.8–127.6 (15C$_{Ar}$), 102.1 (C-2′), 80.7 (C-2), 79.8 (C-3), 79.7 (C-5), 71.99, 71.96, 71.36 (3*C*H$_2$Ph), 66.9 (2C, C-4′, C-6′), 51.3 (C-4), 30.9 (C-1), 30.4 (C-7), 25.8 (C-5′), 25.5 (C-6); MALDI-TOFMS: *m*/*z* 535.15 $[M+H]^+$, 557.12 $[M+Na]^+$, 572.95 $[M+K]^+$. Anal. Calcd for $C_{32}H_{38}O_5S$: C, 71.88; H, 7.16. Found: C, 72.05; H, 7.27.

1.5. 1,4-Anhydro-6,7-dideoxy-2,3,5-tri-*O*-benzyl-4-thio-D-*manno*-octitol (17)

A solution of **16a** (135 mg, 0.25 mmol) in 5 mL AcOH–H_2O–H_2SO_4–1,4-dioxane (78:20:0.5:1.5) was stirred at room temperature for 20 h. The mixture was diluted with Et_2O (50 mL) and washed with H_2O (20 mL), satd aq $NaHCO_3$ (2 × 20 mL), and brine (20 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was diluted with 95% EtOH (30 mL) and the solution was cooled to 0 °C. $NaBH_4$ (10 mg, 1 equiv) was added and the mixture was stirred in an ice bath for 1 h. The reaction was quenched by the addition of AcOH (0.2 mL) and the mixture was concentrated. The residue was diluted with Et_2O (50 mL) and washed with H_2O (20 mL) and brine (20 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product was purified by flash chromatography (3:1, hexanes/EtOAc) to give compound **17** as a white solid (86 mg, 71%): mp 98–100 °C; $[\alpha]_D$ −6.0 (*c* 0.5, CH_2Cl_2); ^1H NMR ($CDCl_3$):

δ 7.36–7.21 (m, 15H, Ar), 5.12 and 4.58 (2d, each 1H, $J_{a,b} = 11.3$ Hz, CH_2Ph), 4.59 and 4.56 (2d, each 1H, $J_{a,b} = 12.6$ Hz, CH_2Ph), 4.53 and 4.37 (2d, each 1H, $J_{a,b} = 11.1$ Hz, CH_2Ph), 4.34 (dd, 1H, $J_{2,3} = J_{3,4} =$ 2.9 Hz, H-3), 4.05 (ddd, 1H, $J_{1b,2} = 6.5$ Hz, $J_{1a,2} = 10.4$ Hz, H-2), 4.00 (ddd, 1H, $J_{4,5} = J_{5,6b} =$ 3.9 Hz, $J_{5,6a} = 9.9$ Hz, H-5), 3.62 (ddd, 1H, $J_{7a,8a} = J_{7b,8a} = 5.7$ Hz, $J_{8a,8b} = 10.9$ Hz, H-8a), 3.56 (ddd, 1H, $J_{7b,8b} = 5.7$ Hz, $J_{7a,8a} = 7.1$ Hz, H-8b), 3.50 (br s, 1H, H-4), 3.13 (dd, 1H, $J_{1a,1b} = 10.4$ Hz, H-1a), 2.95 (dd, 1H, H-1b), 1.85–1.50 (m, 4H, H-6a, H-6b, H-7a, H-7b); ^{13}C NMR ($CDCl_3$): δ 139.2, 138.5, 138.1 (3C$_{ipso}$), 128.7–127.6 (15C$_{Ar}$), 84.9 (C-2), 78.6 (C-3), 78.0 (C-5), 73.8, 72.5, 71.8 (3CH$_2$Ph), 63.3 (C-8), 49.2 (C-4), 30.7 (C-1), 27.8 (C-6), 27.3 (C-7); MALDI-TOF MS: m/z 478.97 $[M+H]^+$, 501.07 $[M+Na]^+$, 516.91 $[M+K]^+$. Anal. Calcd for $C_{29}H_{34}O_4S$: C, 72.77; H, 7.16. Found: C, 73.01; H, 7.27.

1.6. 1,4-Anhydro-8-bromo-6,7,8-trideoxy-2,3,5-tri-*O*-benzyl-4-thio-D-*manno*-octitol (18)

To a stirred solution of **17** (60 mg, 0.13 mmol) in pyridine (4 mL) at 0 °C under N_2 was added methanesulfonyl chloride (12 μL, 1.2 equiv). The reaction mixture was stirred at 0 °C for 2 h and then quenched by the addition of ice (0.5 mL). The mixture was concentrated under high vacuum and then diluted with Et_2O (50 mL). The organic phase was washed with H_2O (20 mL), 1 M HCl (20 mL), satd aq $NaHCO_3$ (20 mL), and brine (20 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue in THF (4 mL) together with LiBr (55 mg, 5 equiv) was heated at reflux under N_2 for 2 h and then concentrated. The residue was diluted with Et_2O (50 mL) and washed with H_2O (2 × 20 mL) and brine (20 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product was purified by flash chromatography (5:1, hexanes/EtOAc) to give compound **18** as a colorless oil (61 mg, 90%): $[\alpha]_D$ −6.2 (c 0.8, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 7.38–7.23 (m, 15H, Ar), 5.11 and 4.61 (2d, each 1H, $J_{a,b} = 11.3$ Hz, CH_2Ph), 4.61 and 4.58 (2d, each 1H, $J_{a,b} = 12.2$ Hz, CH_2Ph), 4.51 and 4.37 (2d, each 1H, $J_{a,b} = 11.4$ Hz, CH_2Ph), 4.35 (dd, 1H, $J_{2,3} = J_{3,4} =$ 3.0 Hz, H-3), 4.06 (ddd, 1H, $J_{1b,2} = 6.6$ Hz, $J_{1a,2} =$ 10.5 Hz, H-2), 3.98 (ddd, 1H, $J_{5,6b} = 3.3$ Hz, $J_{5,6b} = 4.2$ Hz, $J_{4,5} = 10.0$ Hz, H-5), 3.43 (dd, 1H, H-4), 3.41–3.36 (m, 2H, H-8a, H-8b), 3.15 (dd, 1H, $J_{1a,1b} = 9.4$ Hz, H-1a), 2.97 (dd, 1H, H-1b), 2.01–1.89 (m, 3H, H-6a, H-7a, H-7b), 1.56 (m, 1H, H-6b); ^{13}C NMR ($CDCl_3$): δ 139.2, 138.6, 138.1 (3C$_{ipso}$), 128.7–127.6 (15C$_{Ar}$), 84.9 (C-2), 78.6 (C-3), 77.5 (C-5), 73.8, 72.5, 71.6 (3CH$_2$Ph), 49.4 (C-4), 34.3 (C-8), 30.7 (C-1), 29.6 (C-6), 27.3 (C-7); MALDI-TOFMS: m/z 461.29 $[M-Br]^+$. Anal. Calcd for $C_{29}H_{33}BrO_3S$: C, 64.32; H, 6.14. Found: C, 64.08; H, 6.32.

1.7. 2(*S*),3(*R*),4(*R*),5(*R*)-2,3,5-Tribenzyloxy-*cis*-1-thioniabicyclo[4.3.0]-nonane triflate (19)

A solution of compound **18** (55 mg, 0.10 mmol) in CH_3CN (3 mL) was treated with AgOTf (26 mg, 1 equiv) for 14 h at ambient temperature. Another equivalent of AgOTf (26 mg) was added and the reaction mixture was stirred for an additional 10 h. The solvent was removed and the crude product was purified by flash chromatography (20:1, CH_2Cl_2–CH_3OH) to give compound **19** as a colorless syrup (55 mg, 88%): $[\alpha]_D$ −38.2 (c 0.5, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 7.37–7.20 (m, 15H, Ar), 4.98 and 4.59 (2d, each 1H, $J_{a,b} = 11.0$ Hz, CH_2Ph), 4.85 (ddd, 1H, $J_{2,3} = 2.8$ Hz, $J_{1b,2} = 7.2$ Hz, $J_{1a,2} = 10.0$ Hz, H-2), 4.69 and 4.65 (2d, each 1H, $J_{a,b} = 11.5$ Hz, CH_2Ph), 4.66 and 4.46 (2d, each 1H, $J_{a,b} = 11.9$ Hz, CH_2Ph), 4.54 (dd, 1H, $J_{3,4} = 3.0$ Hz, H-3), 4.39 (br s, 1H, H-4), 4.17 (dd, 1H, $J_{1a,1b} = 12.2$ Hz, H-1b), 3.93 (br s, 1H, H-5), 3.65 (m, 1H, H-8eq), 3.34 (ddd, 1H, $J_{7eq,8ax} = 2.8$ Hz, $J_{7ax,8ax} =$ 10.9 Hz, $J_{8ax,8eq} = 13.3$ Hz, H-8ax), 3.19 (dd, 1H, H-1a), 2.05–1.76 (m, 4H, H-6ax, H-6eq, H-7ax, H-7eq); ^{13}C NMR ($CDCl_3$): δ 137.1, 137.1, 136.8 (3C$_{ipso}$), 129.0–128.2 (15C$_{Ar}$), 120.9 (q, 1C, $J_{C,F} = 318.5$ Hz, OTf), 82.8 (C-2), 79.4 (C-3), 75.1, 74.1, 71.1 (3CH$_2$Ph), 69.7 (C-5), 52.8 (C-4), 43.5 (C-1), 40.4 (C-8), 25.8 (C-6), 16.2 (C-7); MALDI-TOFMS: m/z 461.25 $[M-OTf]^+$. Anal. Calcd for $C_{30}H_{33}F_3O_6S_2$: C, 59.00; H, 5.45. Found: C, 59.24; H, 5.52.

1.8. 2(*S*),3(*R*),4(*R*),5(*R*)-2,3,5-Trihydroxy-*cis*-1-thioniabicyclo[4.3.0]-nonane chloride (10)

BCl_3 gas was bubbled vigorously through a solution of **19** (50 mg, 82 μmol) in CH_2Cl_2 (5 mL) at −78 °C under N_2 for 10 min. The mixture was stirred at −78 °C for 2 h and a stream of dry air was blown vigorously over the solution to remove excess BCl_3. The reaction was quenched by the addition of CH_3OH (2 mL) and the solvent was removed. The residue was co-evaporated with CH_3OH (2 × 5 mL) and then washed with CH_2Cl_2 (2 × 2 mL) to give a white solid. The solid was dissolved in CH_3OH (5 mL) and a freshly washed ion exchange resin (Amberlyst A-26 (chloride form), 60 mg) was added. The mixture was stirred at room temperature for 30 min and filtered. The filtrate was concentrated and recrystallized from CH_3OH–CH_2Cl_2 to give compound **10** as white crystals (13 mg, 70%): mp > 219 °C (decomp.); $[\alpha]_D$ −6.8 (c 0.3, CH_3OH); ^{1}H NMR (CD_3OD): δ 4.52–4.42 (m, 3H, H-2, H-3, H-5), 3.80 (dd, 1H, $J_{1b,2} = 7.7$ Hz, $J_{1a,1b} = 12.2$ Hz, H-1b), 3.79 (br s, 1H, H-4), 3.68 (m, 1H, H-8eq), 3.58, (ddd, 1H, $J_{7eq,8ax} = 3.1$ Hz, $J_{7ax,8ax} = 11.1$ Hz, $J_{8ax,8eq} = 14.0$ Hz, H-8ax), 3.15 (dd, 1H, $J_{1a,2} = 9.9$ Hz, H-1a), 2.36 (dddd, 1H, $J_{5,6ax} = 2.6$ Hz, $J_{6ax,7eq} = 3.7$ Hz, $J_{6ax,6eq} = J_{6ax,7ax} =$ 14.0 Hz, H-6ax), 2.14 (ddddd, 1H, $J_{7ax,8eq} = J_{6eq,7ax} =$

3.2 Hz, $J_{7ax,7eq} = 15.1$ Hz, H-7ax), 1.97 (ddddd, 1H, $J_{7eq,8eq} = J_{6eq,7eq} = 3.6$ Hz, H-7eq), 1.86 (dddd, 1H, $J_{5,6eq} = 3.4$ Hz, H-6eq); ^{13}C NMR (CD_3OD): δ 74.5 (C-3), 74.3 (C-2), 63.3 (C-5), 57.5 (C-4), 43.8 (C-1), 40.6 (C-8), 27.5 (C-6), 15.3 (C-7); MALDI-TOF MS: m/z 191.27 $[M-Cl]^+$. Anal. Calcd for $C_8H_{15}ClO_3S$: C, 42.38; H, 6.67. Found: C, 42.29; H, 6.73.

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