Synthesis and evaluation of sulfonium analogues of isofucofagomine as glycosidase inhibitors

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A series of L-fucopyranose analogues having a sulfur substituent at the anomeric position were synthesised. A sulfide, a methylsulfonium ion, a sulfoxide and a sulfone analogue were made. The synthesised compounds were tested for inhibition of two α-fucosidases. The methylsulfonium ion was found to be a moderately strong competitive inhibitor, and 700 times more potent than the corresponding sulfide.

Introduction

Glycosidases and related enzymes are crucial in many biological processes. Potent and selective inhibitors of these enzymes are important, because they can be used to interfere with such processes. Thus glycosidase inhibitors may be potential agents against diabetes,[1] cancer,[2] AIDS,[3] hepatitis,[4] Gaucher's disease[4] and influenza.[5] The search for new potent glycosidase inhibitors has therefore been intense. An interesting recent finding is the observation that the natural product sulfonium ions salacinol (**1**) and kotalanol (**2**), are potent α-glucosidase inhibitors.[6,7] The related synthetic glycosidase inhibitors, **3** and **4**, have also been reported (Fig. 1).[8,9] Sulfonium ion analogues of castanospermine have also been made.[10] It is reasonable that the inhibitors **1**–**4** may owe their biological activity to a resemblance with the oxocarbenium ion like transition state **A** (Fig. 2). Since strong glycosidase inhibitors have also been found among compounds with the ability to mimic charge at anomeric carbon atoms (*i.e.* isofagomine),[11] the idea was conceived that introducing the sulfonium ion moiety at the C-1 position of a monosaccharide might create potent glycosidase inhibitors by analogy with transition state **B** (Fig. 2). A charged sulfur atom has some interesting differences compared to nitrogen; for instance, longer C–S bonds and the larger S atom. In the present paper we have investigated this idea. The piperidines **5** and **6** are potent α-fucosidase inhibitors and are possibly binding to the enzyme in a protonated form.[12,13] We report herein the synthesis of two sulfonium ion analogues of **5** as well as the synthesis of other sulfur analogues of **5**, and report that these compounds are indeed α-fucosidase inhibitors.

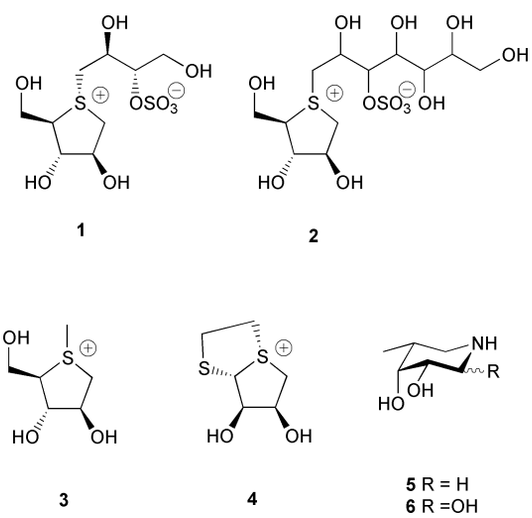


Fig. 1

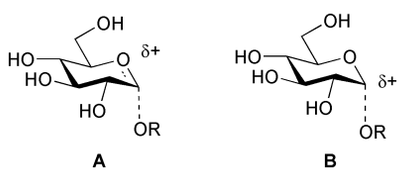
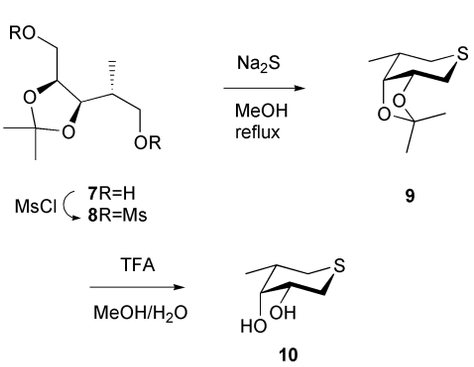


Fig. 2

Results and discussion

In order to synthesise various types of charged sulfur compounds, a cyclic sulfide analogue of **5** appeared to be a good synthetic intermediate. 6-Membered cyclic sulfides have previously been obtained from 1,5-dihalides or 1,5-disulfonates using Na_2S as a reagent even on carbohydrate type substrates.[14,15] Therefore, the dimesylate (bis(methanesulfonate)) **8** was chosen as a starting material (Scheme 1). Mesylation of the known diol **7**[16] with mesyl chloride at 5 °C in pyridine containing DMAP as catalyst afforded **8** in 98% yield. This compound was pure enough to be used in the next step without further purification. Reaction of **8** with anhydrous Na_2S in refluxing



Scheme 1

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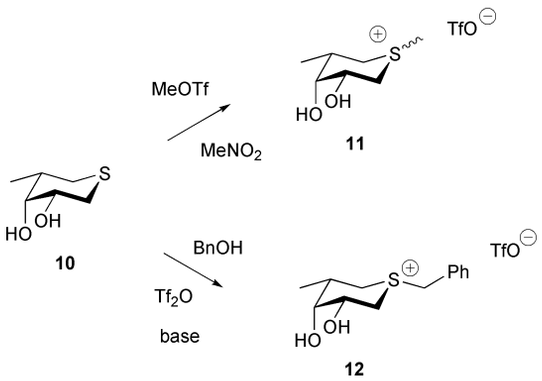
MeOH gave a 57% yield of the cyclic sulfide **9** after 12 h. The relatively low yield of this reaction is explained by the formation of a less polar aromatic by-product whose exact identity has not been determined. Deprotection of **9** was carried out with TFA in MeOH–H_2O which led to **10** in 85% yield after chromatography.

Sulfide **10** now served as a source for several compounds (Scheme 2). A way to prepare positively charged potential



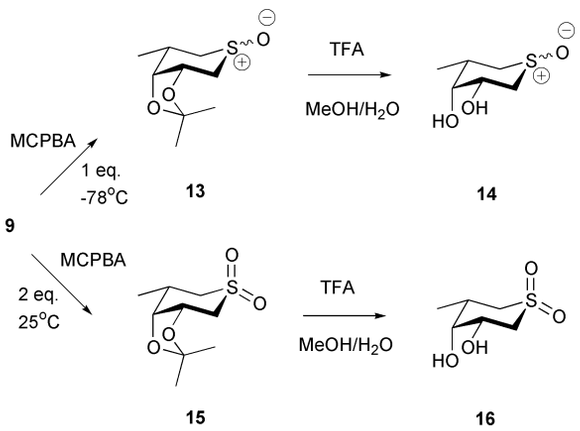
Scheme 2

glycosidase inhibitors derived from thiosugars having sulfur in the ring is the introduction of a positive charge on the sulfur atom by alkylation, that is, the preparation of sulfonium salts. It is noteworthy that the alkylating agent should be carefully selected because it is known that nucleophilic counterions (*e.g.* halides) slowly decompose the sulfonium cation *via* nucleophilic substitution reactions.[10,17] For this reason alkyl perchlorates[18] and triflates †[19–22] have been used as alkylating reagents. Due to the inherent risk of explosion in the handling of organic perchlorates we decided to use triflates in these reactions. In fact methyl triflate is commercially available and benzyl triflate can be easily prepared by esterification of benzyl alcohol with triflic anhydride in the presence of a base.[20–22]

Alkylation of **10** using methyl triflate in nitromethane at 0 °C gave **11** in 97% yield (Scheme 2), obtained as a chromatographically irresolvable 4 : 1 mixture of stereoisomers as determined by digital integration of the ^{1}H NMR spectrum. Analytical TLC of the crude product of reaction showed the absence of compounds less polar than **10**, confirming that no *O*-alkylation side reactions had occurred. The ^{13}C NMR spectrum of the sulfonium salt was used for assigning the configuration of the stereoisomers. As is known, the steric interaction between an axial methyl group and the *syn* axial protons in a cyclohexane ring results in the shielding of the carbons attached to these protons (γ effect).[23] In this sense the observation that the C-3 (66.53 ppm) and C-5 (32.34 ppm) carbons of the minor isomer are shielded with respect to the major isomer (69.90 and 35.46 ppm respectively) led us to assign the major isomer as being the *S* equatorial isomer. The observed chemical shift of the *S*-methyl group in the major isomer (28.01 ppm) compared with the minor isomer (20.99 ppm) also supports our assignment. This result indicates that, as expected, the alkylation process takes place preferentially from the less hindered face of the thiane ring.

In order to test how an increase in the lipophilicity of the sulfonium salt would affect its inhibitory activity, we decided to prepare the *S*-benzyl sulfonium salt by benzylation of **10**. In this case benzyl triflate was made *in situ* in CH_2Cl_2 from benzyl alcohol, Tf_2O and 2,6-di-*tert*-butylpyridine, and then reacted with sulfide **10** in dichloromethane. This gave the *S*-benzyl derivative **12** in 90% yield after chromatography. In this case the ^{1}H NMR spectrum indicated that the compound was composed almost exclusively of only one stereoisomer. The ^{13}C chemical shift of the methylene of the *S*-benzyl group (48.14 ppm) is close to that observed for *trans*-1-benzyl-4-*tert*-butylthianium tetrafluoroborate[24] (47.59 ppm). This fact and the similarity observed between the chemical shifts of carbons C-3 and C-5 (68.51 and 33.94 ppm respectively) and those of **11*S*** (69.60 and 35.46 ppm) led us to identify **12** as the equatorial *R* (or β) stereoisomer (as depicted in Scheme 2).

The polar nature of the S–O bond in sulfoxides and sulfones agrees with the existence of a high positive charge density on the sulfur atom in these types of compounds. Therefore, they also resemble transition state **B** (Fig. 2). In this sense, we synthesised the epimeric mixture of sulfoxides **14** and the sulfone **16** by oxidation of **9** with either an equimolecular or an excess amount of *m*-chloroperbenzoic acid and subsequent deacetonation (Scheme 3). Treatment of **9** with 1 equivalent of



Scheme 3

MCPBA at −78 °C in EtOAc gave the sulfoxide **13** as mixture of isomers in 81% yield. The solvent was selected due to its ability to dissolve both the peroxy acid and the sulfide at low temperatures.[25] The product was a chromatographically irresolvable 4 : 1 mixture of epimeric stereoisomers as determined by digital integration of the ^{1}H NMR spectrum.

^{1}H NMR studies on thiane oxides have revealed three effects which allow the sulfoxide configuration in these compounds to be assigned.[26,27] The first is the *syn*-axial effect that implies a deshielding of the axial β-hydrogens in axial sulfoxides. Secondly, geminal coupling constants in α-methylene groups are smaller for equatorial sulfoxides, and finally equatorial α-hydrogens are shielded (*ca.* 0.3 ppm) in axial sulfoxides. A comparison between the chemical shifts of H-3 and H-5 and the $^2J_{2ax,2eq}$ coupling constant of the major isomer (4.63 ppm, 2.82 ppm and 14.1 Hz, respectively) and those observed for the minor isomer (4.31 ppm, 2.05 ppm and 12.0 Hz) clearly indicates that the major isomer is the axial *R* sulfoxide. This conclusion is also supported by the observation that the H-2eq and H-6eq protons of this stereoisomer appear shielded (*ca.* 0.37 ppm) with respect to the minor sulfoxide. Interestingly, we noticed that the geminal coupling constant values ($^2J_{2ax,2eq}$ and $^2J_{6ax,6eq}$) of the α-methylenes of the sulfonium salt **11** are appreciably higher in the axial isomer (14.3 and 14.8 Hz) than in the equatorial isomer (11.1 and 11.8 Hz), as is also observed in the isomers of the sulfoxide **14**. Deacetonation of **13** with trifluoroacetic acid in MeOH–H_2O at room temperature afforded **14** in 88% yield. Despite reports describing the acid lability of the sulfoxide grouping,[28] in our hands the hydrolysis of the isopropylidene group did not alter the axial : equatorial ratio.

Oxidation of **9** using two equivalents of MCPBA at 25 °C gave the sulfone **15** in 86% yield. TFA treatment gave the unprotected sulfone **16** in 80% yield.

The vicinal coupling constants data for compounds **9**–**16** agrees with preference for the $^1C_4$ conformation in solution. The

† The IUPAC name for triflate is trifluoromethanesulfonate.

values for $^3J_{5,6ax}$ are in the range 11.6–14.8 Hz indicating a *trans* diaxial relationship between these protons. The coupling constant $^3J_{3,2ax}$ is more sensitive to conformational distortions due to the presence of the 1,3-dioxolane ring in **9**, **13** and **15**, but nevertheless, the values for the *O*-unprotected derivatives fall in the range 11.2–12.4 Hz supporting the $^1C_4$ conformation. This conformation is also confirmed by the observance of long range $^4J_{H,H}$ coupling constants between H-2eq, H-4 and H-6eq. Of the three possible "w" paths the path involving the sulfur atom of the thiane ring (that is, the coupling between H-6eq and H-2eq) results in $^4J_{H,H}$ values in the interval 1.5–3.9 Hz. The *cis* fused 1,3-dioxolane ring introduces a torsional tension in the thiane ring forcing it to adopt a distorted chair conformation. This results in a decrease in the dihedral angles between H-3 and the vicinal protons H-2eq, H-2ax and H-4. While $^3J_{3,2ax}$ experiences a decrease, $^3J_{3,2eq}$ and $^3J_{3,4}$ increase due to the fact that the corresponding dihedral angles are closer to 0°.

The new fucose analogues **10**, **11**, **12**, **14** and **16** were tested for inhibition of two α-fucosidases. All were either competitive inhibitors or they did not inhibit. The sulfide **10** was an extremely poor inhibitor of both enzymes. This is not surprising since neither in terms of charge or geometry does it mimic the transition state. However as soon as the sulfur atom becomes charged, as in the methyl sulfonium analogue **11**, the inhibition is increased 700 times. This very clearly demonstrates the advantage of having a positive charge in this position. The increased binding may be a result of a salt bridge between the catalytic nucleophile in the enzyme and the charged sulfur. Compound **11** is a 25–50 times weaker fucosidase inhibitor than **5**. This may be explained to some part by the poor fit of the methyl group in the enzyme active site. *N*-Methylated analogues of isofagomines are generally much weaker inhibitors than the secondary amines themselves presumably because of unfavourable interactions of the methyl group in that area.[29] The *S*-benzyl sulfonium salt **12** has an inhibitory activity much like **11**. This suggests that the size of the *S*-alkyl group is of minor importance. Interestingly the sulfoxide **14** is a considerably weaker inhibitor than **11** although still a 100 times stronger inhibitor than **10**. This suggests that **14** can benefit from some electrostatic interaction between the enzyme nucleophile and the charged sulfur atom. However, the negative oxygen atom appears to be unfavourable in the electronegative surroundings around the anomeric carbon. This situation is intensified in the sulfone **16**, which is a weaker inhibitor. This may however also be due to limited space in the active site to accommodate the sulfone.

In conclusion the results show that monosaccharide derivatives having positively charged sulfur atoms at anomeric positions are indeed glycosidase inhibitors. They are however in this case much weaker than the corresponding amines.

Experimental

General

Solvents were distilled under an inert atmosphere. Nitromethane was dried over anhydrous $CaCl_2$ and then distilled over 4 Å molecular sieves. Similarly, dichloromethane was dried over anhydrous $CaCl_2$ and then distilled over CaH_2. All reagents were used as purchased without further purification. Columns were packed with silica gel 60 (230–400 mesh) as the stationary phase. TLC-plates (Merck, $60F_{254}$) were visualised using CEMOL (1% ceric sulfate and 1.5% ammonium molybdate in 10% aqueous H_2SO_4) or 10% ethanolic H_2SO_4 and heating until coloured spots appeared. ^{13}C and ^1H NMR spectra were recorded on Varian Gemini 200, Bruker AMX300 and Bruker AMX500 instruments. Mass spectra were obtained on Micromass LCT-QTOF and Micromass AutoSpec instruments. Specific optical rotations were measured on a Perkin-Elmer 241 Polarimeter and are given in 10^{-1} deg cm^2 g^{-1}. Products were concentrated on a rotary evaporator below 40 °C. All enzymes and substrates for the enzyme assays were purchased from Sigma.

2-Deoxy-3,4-*O*-isopropylidene-2-*C*-methyl-1,5-bis(*O*-methylsulfonyl)-L-ribitol (8)

To a solution of 2-deoxy-3,5-*O*-isopropylidene-2-*C*-methyl-L-ribitol **7** (1.0 g, 5.68 mmol) and DMAP (5 mg) in dry pyridine (10 mL) was added mesyl chloride (1.3 g, 11.36 mmol) at 0 °C. The obtained mixture was left overnight at 5 °C, then concentrated *in vacuo* and taken up in 1 : 1 H_2O–CH_2Cl_2 (100 mL). After separation of both phases the aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phases were washed with 1 M HCl (5 × 50 mL), saturated aq. $NaHCO_3$ (50 mL) and H_2O (50 mL), dried (Na_2SO_4) and concentrated to dryness to give **8** (1.8 g, 98%) which was pure enough to be used without further purification in the next step. ^1H NMR ($CDCl_3$, 200 MHz) δ 4.34–3.90 (m, 6H, H-1, H-1′, H-3, H-4, H-5, H-5′), 3.02, 2.96 (2s, 3H each, $MeSO_3$), 2.02 (m, 1H, H-2), 1.42, 1.28 (2s, 3H each, Me_2C), 1.10 (d, 3H, $J_{2,2'}$ 7.0 Hz, H-2′); *m*/*z* (ES) 332.0598 (M + Na^+). Calc. for $C_{10}H_{20}O_8S_2$ + Na^+: *m*/*z* 332.0600.

(3*R*,4*R*,5*R*)-3,4-Isopropylidenedioxy-5-methylthiane (9)

To a solution of the dimesylate **8** (808 mg, 2.33 mmol) in MeOH (75 mL) was added anhydrous Na_2S (364 mg, 4.66 mmol). The mixture was stirred under reflux for 12 h. The resulting solution was then concentrated to give a yellowish oil which was dissolved in CH_2Cl_2 (20 mL) and extracted with H_2O (2 × 20 mL). The organic phase was dried (Na_2SO_4) and evaporated to dryness. The resulting oil was purified by column chromatography on silica gel using pentane–Et_2O (20 : 1) as eluent (R_f 0.25). The yield was 251 mg (57%) of **9** as a white solid. $[a]_D^{22}$ +24.8 (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 4.11 (ddd, 1H, $J_{3,4}$ 4.8 Hz, $J_{3,2ax}$ 9.4 Hz, $J_{3,2eq}$ 6.4 Hz, H-3), 4.05 (br t, 1H, $J_{4,5}$ 3.5 Hz, H-4), 2.59 (dd, 1H, $J_{2ax,2eq}$ 13.4 Hz, H-2ax), 2.55 (dddd, 1H, $J_{2eq,6eq}$ 1.8 Hz, $J_{4,2eq}$ 0.4 Hz, H-2eq), 2.49 (dd, 1H, $J_{5,6ax}$ 12.0 Hz, $J_{6ax,6eq}$ 13.0 Hz, H-6ax), 2.20 (dddd, 1H, $J_{5,6eq}$ 4.0 Hz, $J_{4,6eq}$ 0.6 Hz, H-6eq), 2.14 (m, 1H, H-5), 1.47, 1.31 (2s, 3H each, Me_2C), 1.10 (d, 3H, $J_{5,5'}$ 7.0 Hz, H-5′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 107.86 (Me_2C), 76.59 (C-4), 73.36 (C-3), 35.95 (C-5), 29.26 (C-6), 29.17 (C-2), 28.56, 26.29 (Me_2C), 18.64 (C-5′); *m*/*z* (ES) 211.0771 (M + Na^+). Calc. for $C_9H_{16}O_2S$ + Na^+: *m*/*z* 211.0769.

(3*R*,4*R*,5*S*)-3,4-Dihydroxy-5-methylthiane (10)

A solution of **9** (200 mg, 1.06 mmol) and TFA (0.5 mL) in 5 : 1 MeOH–H_2O (6 mL) was left at room temperature for 1 h. The solution was concentrated and the residue was purified by column chromatography on silica gel using pentane–EtOAc (1 : 1) as eluent (R_f 0.38). The yield was 134 mg (85%) of **10** as a white solid. $[a]_D^{22}$ +16.9 (*c* 0.9, $CHCl_3$); ^1H NMR (CD_3OD, 500 MHz) δ 3.67 (br t, 1H, $J_{3,4}$ 2.4 Hz, $J_{4,2eq}$ 1.5 Hz, H-4), 3.63 (ddd, 1H, $J_{3,2ax}$ 11.2 Hz, $J_{3,2eq}$ 4.2 Hz, H-3), 2.82 (dd, $J_{2ax,2eq}$ 12.6 Hz, H-2ax), 2.55 (dd, 1H, $J_{5,6ax}$ 11.9 Hz, $J_{6ax,6eq}$ 13.2 Hz, H-6ax), 2.18 (ddt, 1H, $J_{2eq,6eq}$ 1.5 Hz, H-2eq), 1.93 (ddd, 1H, $J_{5,6eq}$ 3.6 Hz, H-6eq), 1.86 (m, 1H, H-5), 1.00 (d, 3H, $J_{5,5'}$ 6.9 Hz, H-5′); ^{13}C NMR (CD_3OD, 75 MHz) δ 74.16 (C-4), 73.56 (C-3), 40.75 (C-5), 27.88 (C-6), 27.72 (C-2), 19.26 (C-5′); *m*/*z* (CI) 148.0557 (M^+). Calc. for $C_6H_{12}O_2S^+$: *m*/*z* 148.0558.

(3*R*,4*R*,5*S*)-3,4-Dihydroxy-1,5-dimethylthianium triflate (11)

To a solution of **10** (79 mg, 0.53 mmol) in $MeNO_2$ (1 mL) cooled at 0 °C was slowly added methyl triflate (60 μL, 0.53 mmol) under N_2. After 2 h the solution was concentrated to give pure **11** (161 mg, 97%) as mixture of stereoisomers. $[a]_D^{22}$ +2.0 (*c* 0.9, H_2O); *S* stereoisomer (equatorial): ^1H NMR (D_2O, 500 MHz) δ 4.07 (ddd, 1H, $J_{3,4}$ 2.4 Hz, $J_{3,2ax}$ 12.0 Hz, $J_{3,2eq}$ 3.7

Hz, H-3), 3.91 (br m, 1H, H-4), 3.47 (ddd, 1H, $J_{2ax,2eq}$ 11.1 Hz, $J_{2eq,6eq}$ 2.5 Hz, H-2eq), 3.28 (ddd, 1H, $J_{5,6eq}$ 3.1 Hz, $J_{6ax,6eq}$ 11.8 Hz, H-6eq), 3.22 (t, 1H, H-2ax), 3.02 (dd, 1H, $J_{5,6ax}$ 13.1 Hz, H-6ax), 2.98 (s, 3H, SMe), 2.21 (m, 1H, H-5), 1.17 (d, 3H, $J_{5,5'}$ 7.0 Hz, H-5′). ^{13}C NMR (D_2O, 125 MHz) δ 122.24 (q, 1C, $J_{C,F}$ 315 Hz, TfO^-), 72.50 (C-4), 69.60 (C-3), 39.83 (C-6), 38.67 (C-2), 35.45 (C-5), 28.01 (SMe), 19.70 (C-5′). *R* stereoisomer (axial): ^{1}H NMR (D_2O, 500 MHz) δ 4.25 (ddd, 1H, $J_{3,4}$ 2.2 Hz, $J_{3,2ax}$ 11.3 Hz, $J_{3,2eq}$ 4.4 Hz, H-3), 3.91 (br m, 1H, H-4), 3.31 (dd, 1H, $J_{2ax,2eq}$ 14.3 Hz, H-2ax), 3.20 (ddd, 1H, $J_{2eq,6eq}$ 2.4 Hz, H-2eq), 3.17 (dd, 1H, $J_{5,6ax}$ 12.3 Hz, $J_{6ax,6eq}$ 14.8 Hz, H-6ax), 3.01 (ddd, 1H, $J_{5,6eq}$ 3.9 Hz, H-6eq), 2.93 (s, 3H, SMe), 2.49 (m, 1H, H-5), 1.15 (d, 3H, $J_{5,5'}$ 7.1 Hz, H-5′). ^{13}C NMR (D_2O, 125 MHz) δ 122.24 (q, 1C, $J_{C,F}$ 315 Hz, TfO^-), 72.50 (C-4), 66.53 (C-3), 34.87 (C-6), 32.90 (C-2), 32.34 (C-5), 20.99 (SMe), 19.40 (C-5′); *m/z* (ES) 475.1103 [2 $(C_7H_{15}O_2S)^+ + TfO^-$]. Calc. for $C_{15}H_{30}F_3O_7S_3^+$: *m/z* 475.1106.

(1*R*,3*R*,4*R*,5*S*)-1-Benzyl-3,4-dihydroxy-5-methylthianium triflate (12)

To a stirred solution of triflic anhydride (36 μL, 0.22 mmol) in CH_2Cl_2 (2 mL) cooled at −78 °C was slowly added under N_2 a solution of benzyl alcohol (22 μL, 0.22 mmol) and 2,6-di-*tert*-butylpyridine (48 μL, 0.22 mmol) in CH_2Cl_2 (1 mL). After 10 min a solution of thiane **10** (32 mg, 0.22 mmol) in CH_2Cl_2 (1 mL) was added and the resulting solution was left at −20 °C overnight. The crude product was evaporated to give an oily residue which was purified by column chromatography on silica gel using CH_2Cl_2–MeOH (10 : 1) as eluent (R_f 0.12). The yield was 76 mg (90%) of **12** as a colourless oil. $[a]_D^{22}$ +12.0 (*c* 1.2, H_2O); ^{1}H NMR (D_2O, 500 MHz) δ 7.52–7.44 (m, 5H, Ph), 4.70 (d, 1H, *J* 13.1 Hz, SCH_2Ph), 4.66 (d, 1H, SCH_2Ph), 4.03 (ddd, 1H, $J_{3,4}$ 2.4 Hz, $J_{3,2ax}$ 12.0 Hz, $J_{3,2eq}$ 3.8 Hz, H-3), 3.83 (br t, 1H, H-4), 3.29 (ddd, 1H, $J_{2ax,2eq}$ 10.9 Hz, $J_{2eq,6eq}$ 3.0 Hz, H-2eq), 3.14 (dd, 1H, H-2ax), 3.04 (dt, 1H, $J_{5,6eq}$ 3.0 Hz, $J_{6ax,6eq}$ 11.6 Hz, H-6eq), 2.90 (dd, 1H, $J_{5,6ax}$ 13.0 Hz, H-6ax), 2.14 (m, 1H, H-5), 1.10 (d, 3H, $J_{5,5'}$ 6.9 Hz, H-5′); ^{13}C NMR (D_2O, 125 MHz) δ 130.28, 129.97, 129.24, 125.71 (Ph), 120.90 (q, 1C, $J_{C,F}$ 316 Hz, TfO^-), 71.21 (C-4), 68.51 (C-3), 48.14 (S*C*H_2Ph), 35.54 (C-6), 34.53 (C-2), 33.94 (C-5), 18.45 (C-5′); *m/z* (ES) 627.1734 [2 $(C_{13}H_{19}O_2S)^+ + TfO^-$]. Calc. for $C_{27}H_{38}F_3O_7S_3^+$: *m/z* 627.1732.

(3*R*,4*R*,5*R*)-3,4-Isopropylidenedioxy-5-methylthiane *S*-oxide (13)

To a stirred solution of **9** (106 mg, 0.56 mmol) in EtOAc (3 mL) cooled at −78 °C under N_2 was slowly added a solution of 77% *m*-chloroperbenzoic acid (126 mg, 0.56 mmol) in EtOAc (3 mL). After 10 min the reaction was stopped by adding saturated aq. $Na_2S_2O_3$ (20 mL). The organic layer was then washed with saturated aq. $NaHCO_3$ (1 × 20 mL) and water (1 × 20 mL), dried (Na_2SO_4) and concentrated to dryness. The residue was purified by column chromatography on silica gel using CH_2Cl_2–MeOH (30 : 1) as eluent to give **13** (93 mg, 81%, R_f 0.25) as a mixture of stereoisomers. A small quantity of sulfone **15** could also be isolated (5 mg, 4%, R_f 0.63). $[a]_D^{21}$ +54.4 (*c* 1.2, $CHCl_3$); *R* sulfoxide (axial): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.63 (ddd, 1H, $J_{3,4}$ 5.6 Hz, $J_{3,2ax}$ 7.9 Hz, $J_{3,2eq}$ 4.7 Hz, H-3), 4.17 (dd, 1H, $J_{4,5}$ 2.8 Hz, H-4), 3.01 (ddd, 1H, $J_{2eq,6eq}$ 1.9 Hz, $J_{2ax,2eq}$ 14.1 Hz, H-2eq), 2.82 [m, 1H, ½($J_{5,6ax}$ + $J_{5,6eq}$) 8.7 Hz, H-5), 2.77 (dd, 1H, H-2ax), 2.66 (d, 2H, H-6eq, H-6ax), 1.43, 1.34 (2s, 3H each, Me_2C), 1.20 (d, 3H, $J_{5,5'}$ 7.0 Hz, H-5′). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 108.54 (Me_2C), 75.22 (C-4), 70.12 (C-3), 46.28 (C-6), 45.91 (C-2), 27.45, 25.41 (*Me*$_2$C), 23.61 (C-5), 17.39 (C-5′). *S* sulfoxide (equatorial): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.31 (ddd, 1H, $J_{3,4}$ 4.8 Hz, $J_{3,2ax}$ 10.4 Hz, $J_{3,2eq}$ 5.6 Hz, H-3), 3.97 (dd, 1H, $J_{4,5}$ 2.9 Hz, H-4), 3.38 (ddd, 1H, $J_{2eq,6eq}$ 2.9 Hz, $J_{2ax,2eq}$ 12.0 Hz, H-2eq), 3.03 (dt, $J_{5,6eq}$ 2.9 Hz, $J_{6ax,6eq}$ 11.9 Hz, H-6eq), 2.83 (dd, $J_{5,6ax}$ 11.5 Hz, H-6ax), 2.75 (dd, 1H, H-2ax), 2.05 (m, 1H, H-5), 1.53, 1.34 (2s, 3H each, Me_2C), 1.24 (d, 3H, $J_{5,5'}$ 7.0 Hz, H-5′). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 109.44 (Me_2C), 74.92 (C-4), 71.85 (C-3), 52.53 (C-2), 51.36 (C-6), 28.19, 25.41 (*Me*$_2$C), 27.12 (C-5), 17.79 (C-5′); *m/z* (ES) 227.0722 (M + Na^+). Calc. for $C_9H_{16}O_3S + Na^+$: *m/z* 227.0718.

(3*R*,4*R*,5*S*)-3,4-Dihydroxy-5-methylthiane *S*-oxide (14)

To a solution of **13** (30 mg, 0.15 mmol) in 4 : 1 MeOH–H_2O (1.25 mL) was added TFA (0.5 mL) at room temperature. After 30 min the solution was evaporated and the crude product was purified by column chromatography on silica gel using CH_2Cl_2–MeOH (10 : 1) as eluent to give **14** (21 mg, 88%, R_f 0.19) as a mixture of stereoisomers. $[a]_D^{21}$ −24.9 (*c* 2.1, H_2O); *R* sulfoxide (axial): ^{1}H NMR (D_2O, 500 MHz) δ 4.27 (ddd, 1H, $J_{3,4}$ 2.4 Hz, $J_{3,2ax}$ 12.0 Hz, $J_{3,2eq}$ 3.8 Hz, H-3), 3.92 (br s, 1H, H-4), 3.08 (dddd, 1H, $J_{2eq,6eq}$ 2.9 Hz, $J_{2ax,2eq}$ 14.1 Hz, $J_{4,2eq}$ 1.0 Hz, H-2eq), 2.81 (ddd, 1H, $J_{5,6eq}$ 2.6 Hz, $J_{6ax,6eq}$ 14.1 Hz, H-6eq), 2.77 (dd, 1H, H-2ax), 2.60 (dd, 1H, $J_{5,6ax}$ 12.9 Hz, H-6ax), 2.53 (m, 1H, H-5), 1.11 (d, 3H, $J_{5,5'}$ 6.8 Hz, H-5′). ^{13}C NMR (D_2O, 125 MHz) δ 69.86 (C-4), 62.03 (C-3), 41.92, (C-6), 40.87 (C-2), 25.40 (C-5), 15.35 (C-5′). *S* sulfoxide (equatorial): ^{1}H NMR (D_2O, 500 MHz) δ 3.85 (ddd, 1H, $J_{3,4}$ 2.6 Hz, $J_{3,2ax}$ 12.4 Hz, $J_{3,2eq}$ 3.3 Hz, H-3), 3.76 (br t, 1H, H-4), 3.45 (dt, 1H, $J_{2ax,2eq}$ 10.9 Hz, $J_{2eq,6eq}$ 3.1 Hz, H-2eq), 3.16 (ddd, 1H, $J_{6ax,6eq}$ 11.8 Hz, $J_{5,6eq}$ 2.5 Hz, H-6eq), 2.91 (dd, 1H, H-2ax), 2.69 (dd, 1H, $J_{5,6ax}$ 13.1 Hz, H-6ax), 1.89 (m, 1H, H-5), 1.12 (d, 3H, $J_{5,5'}$ 6.8 Hz, H-5′). ^{13}C NMR (D_2O, 125 MHz) δ 68.59 (C-4), 64.75 (C-3), 47.00 (C-2), 46.94 (C-6), 27.26 (C-5), 15.14 (C-5′); *m/z* (ES) 187.0404 (M + Na^+). Calc. for $C_6H_{12}O_3S + Na^+$: *m/z* 187.0405.

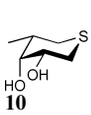
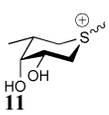
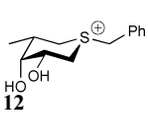
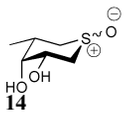
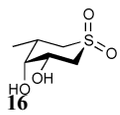
(3*R*,4*R*,5*R*)-3,4-Isopropylidenedioxy-5-methylthiane *S*,*S*-dioxide (15)

To a stirred solution of **9** (60 mg, 0.32 mmol) in EtOAc (2 mL) was slowly added at room temperature a solution of 77% *m*-chloroperbenzoic acid (143 mg, 0.64 mmol) in EtOAc (2 mL). After 10 min the reaction was stopped by adding saturated aq. $Na_2S_2O_3$ (20 mL). The organic layer was then washed with saturated aq. $NaHCO_3$ (1 × 20 mL) and water (1 × 20 mL), dried (Na_2SO_4) and concentrated to dryness. The residue was purified by column chromatography on silica gel using pentane-EtOAc (1 : 1) as eluent to give **15** (60 mg, 86%, R_f 0.56) as a white solid. $[a]_D^{21}$ +3.6 (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.51 (ddd, 1H, $J_{3,4}$ 4.7, $J_{3,2ax}$ 8.9 Hz, $J_{3,2eq}$ 5.9 Hz, H-3), 4.13 (dd, 1H, $J_{4,5}$ 3.0 Hz, H-4), 3.25 (ddd, 1H, $J_{2eq,6eq}$ 3.5 Hz, $J_{2ax,2eq}$ 14.3 Hz, H-2eq), 3.01 (dd, 1H, $J_{5,6ax}$ 12.7 Hz, $J_{6ax,6eq}$ 14.0 Hz, H-6ax), 3.00 (dd, 1H, H-2ax), 2.77 (dt, 1H, $J_{5,6eq}$ 3.6 Hz, H-6eq), 2.58 (m, 1H, H-5), 1.50, 1.37 (2s, 3H each, Me_2C), 1.25 (d, 3H, $J_{5,5'}$ 7.0 Hz, H-5′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 109.09 (Me_2C), 74.72 (C-4), 72.39 (C-3), 51.03 (C-2), 50.83 (C-6), 30.04 (C-5), 27.91, 25.63 (*Me*$_2$C), 17.22 (C-5′); *m/z* (ES) 243.0663 (M + Na^+). Calc. for $C_9H_{16}O_4S + Na^+$: *m/z* 243.0667.

(3*R*,4*R*,5*S*)-3,4-Dihydroxy-5-methylthiane *S*,*S*-dioxide (16)

To a solution of **15** (42 mg, 0.38 mmol) in 4 : 1 MeOH–H_2O (1.25 mL) was added TFA (0.5 mL) at room temperature. After 30 min the solution was evaporated and the crude was purified by column chromatography on silica gel using EtOAc as eluent to give **16** (27 mg, 80%, R_f 0.38) as a white solid. $[a]_D^{21}$ −6.6 (*c* 1.8, H_2O); ^{1}H NMR (D_2O, 500 MHz) δ 4.15 (ddd, 1H, $J_{3,4}$ 2.4 Hz, $J_{3,2ax}$ 11.7 Hz, $J_{3,2eq}$ 4.8 Hz, H-3), 3.91 (m, 1H, H-4), 3.35 (dd, $J_{2ax,2eq}$ 13.6 Hz, H-2ax), 3.28 (ddd, $J_{2eq,6eq}$ 3.9 Hz, H-2eq), 3.09 (dd, 1H, $J_{5,6ax}$ 13.0 Hz, $J_{6ax,6eq}$ 14.3 Hz, H-6ax), 2.99 (dt, 1H, $J_{5,6eq}$ 3.5 Hz, H-6eq), 2.27 (m, 1H, H-5), 1.13 (d, 3H, $J_{5,5'}$ 6.9 Hz, H-5′); ^{13}C NMR (D_2O, 125 MHz) δ 71.91 (C-4), 69.50 (C-3), 51.40 (C-2), 51.16 (C-6), 32.55 (C-5), 17.46 (C-5′); *m/z* (ES) 187.0404 (M + Na^+). Calc. for $C_6H_{12}O_3S + Na^+$: *m/z* 187.0405.

Table 1 Inhibition constants (K_i) in μM

Enzyme	 **10**	 **11**	 **12**	 **14**	 **16**
α-L-Fucosidase (bovine kidney)	73000	102	161	740	2000
α-L-Fucosidase (human placenta)	>100000	303	309	>1000	>100000

Enzyme kinetics

The enzyme assays were carried out as described previously.[30] All assays were performed at pH 6.8 and 25 °C. Steady state kinetics was performed and reaction rates were measured after possible slow-onset inhibition was essentially complete. The inhibition constants (K_i) were obtained from the formula $K_i = [I]/(K_{M'}/K_M - 1)$, where $K_{M'}$ and K_M are Michaelis–Menten constants with and without inhibitor present (Table 1). $K_{M'}$ and K_M were obtained from a Hanes plot, which was also used to ensure that inhibition was competitive. The following K_M values (without inhibitor) were obtained using 4-nitrophenyl glycosides as substrates and the above conditions: α-fucosidase (human placenta), 0.23 mM; α-fucosidase (bovine kidney), 0.24 mM.

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