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Enantiospecific synthesis of 1-deoxythiomannojirimycin from a derivative of D-glucose

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ABSTRACT

1-Deoxythiomannojirimycin [**2**, (2*R*,3*S*,4*R*,5*S*)-3,4,5-trihydroxy-2-hydroxymethylthiane], a thio analogue of the glycosidase inhibitor 1-deoxymannojirimycin [**1**, (2*R*,3*S*,4*R*,5*S*)-3,4,5-trihydroxy-2-hydroxymethylpiperidine], has been synthesised from methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside. The key reactions involved inversion of configuration at C-2 with the introduction of a thio function and cyclisation to give methyl 2,6-anhydro-2-thio-α-D-mannopyranoside (**12**). Hydrolysis of the 3,4-di-*O*-benzyl derivative (**15**) of **12** and reduction of the product with borohydride gave (2*R*,3*S*,4*R*,5*S*)-3,4-dibenzyloxy-5-hydroxy-2-hydroxymethylthiane (**18**). Hydrogenolysis of **18** yielded **2**, which was a weak competitive inhibitor of yeast α-D-glucosidase but was inactive against almond β-D-glucosidase.

INTRODUCTION

1-Deoxymannojirimycin (**1**, 1,5-dideoxy-1,5-imino-D-mannitol), isolated[1] from the seeds of *Lonchocarpus sericeus* (Poir), is an inhibitor of α- and β-D-mannosidase[2], α- and β-D-glucosidase, and insect trehalase[3]. We now report the synthesis of the thio analogue (**2**) of **1** and some enzyme inhibitory properties.

Retrosynthesis indicated that **2** could be obtained from a suitable D-glucose derivative by introduction of a sulfide function between C-6 and C-2 with a single inversion of configuration at C-2 (**3** → **4**).

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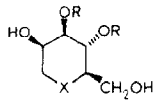
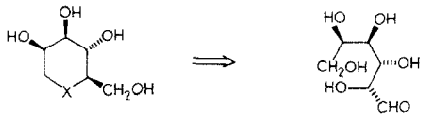


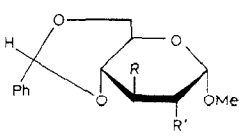
1 X = NH, R = H
2 X = S, R = H
18 X = S, R = Bn

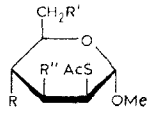


3 4

RESULTS AND DISCUSSION

The starting glucose derivative, methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside[4] (**5**) was converted into the 3-*O*-*tert*-butyldiphenylsilyl derivative **6**. *O*-Debenzoylation of **6** yielded **7**, which was converted into the 2-trifluoromethanesulfonate **8**. Reaction of **8** with potassium thioacetate in *N*,*N*-dimethylformamide effected inversion of configuration at C-2 to afford methyl 2-*S*-acetyl-4,6-*O*-benzylidene-3-*O*-*tert*-butyldiphenylsilyl-2-thio-α-D-mannopyranoside (**9**). The *manno* configuration of **9** was established on the basis of ^{1}H NMR data. Thus, the $J_{2,3}$ value of 1.2 Hz indicates H-2 to be equatorial (cf. $J_{2,3}$ 9 Hz for **8**).



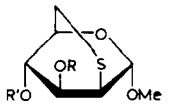


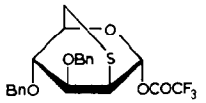
5 R = OH, R' = OBz
6 R = ^{t}BuPh$_2$SiO, R' = OBz
7 R = ^{t}BuPh$_2$SiO, R' = OH
8 R = ^{t}BuPh$_2$SiO, R' = OCF$_3$SO$_2$O

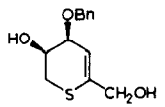
9 R,R' =  , R'' = ^{t}BuPh$_2$SiO
10 R = OBz, R' = Br, R'' = ^{t}BuPh$_2$SiO

Reaction of **9** with *N*-bromosuccinimide in carbon tetrachloride cleaved the 4,6-*O*-benzylidene ring to give the 6-bromo-6-deoxy derivative **10**, treatment of which with methanolic sodium methoxide afforded the 2,6-anhydro derivative **11**. A D-*altro* analogue of **11** has been reported[5]. The use of silica gel and ethyl acetate probably explains the formation of some of the 4-acetate of **11** during the purification process.

Desilylation of **11** gave methyl 2,6-anhydro-2-thio-α-D-mannopyranoside (**12**), attempted di-*O*-benzylation of which was unsuccessful. However, 4-*O*-benzylation of **11** gave **13**, desilylation of which afforded **14**. Benzylation of **14** then gave the desired 2,3-di-*O*-benzyl derivative **15**.







11 R = ^{t}BuPh$_2$Si, R' = H
12 R = R' = H
13 R = ^{t}BuPh$_2$Si, R' = Bn
14 R = H, R' = Bn
15 R = R' = Bn

16 **17**

Hydrolysis of **15** in aqueous trifluoroacetic acid gave a complex mixture of products from which two main fractions (**A** and **B**) were isolated, each of which had IR absorption for carbonyl. Fraction **A** did not react with sodium borohydride and was identified as 2,6-anhydro-3,4-di-*O*-benzyl-2-thio-1-*O*-trifluoroacetyl-α-D-mannopyranose (**16**). Treatment of fraction **B** with sodium borohydride gave (4*S*,5*S*)-4-benzyloxy-5-hydroxy-2-hydroxymethyl-2-thiene (**17**). The $J_{1,2}$ value of 2 Hz indicated **16** to be α as in the parent compound **15**.

Hydrolysis of **15** in aqueous acetic acid and hydrochloric acid gave a product of lower mobility (TLC) that was not isolated but treated with sodium borohydride to afford crystalline (2*R*,3*S*,4*R*,5*S*)-3,4-dibenzyloxy-5-hydroxy-2-hydroxymethylthiane (**18**). Hydrogenolysis (Pd–acetic acid) of **18** gave the target compound (2*R*,3*S*,4*R*,5*S*)-3,4,5-trihydroxy-2-hydroxymethylthiane (1-deoxythiomannojirimycin, **2**).

Kinetic studies have revealed that **2** does not inhibit almond β-D-glucosidase but is a weak competitive inhibitor of yeast α-D-glucosidase (K_i 10^{-3} M, IC_{50} 3.8×10^{-3} M).

EXPERIMENTAL

General methods.—Melting points were determined with an Electrothermal apparatus and are uncorrected. Solutions were dried over $MgSO_4$ before concentration under diminished pressure. The ^1H and ^{13}C NMR spectra were recorded with Bruker AM-300 and AM-360 spectrometers for solutions in $CDCl_3$ (internal Me_4Si). IR spectra were recorded with a Perkin–Elmer 782 instrument, and mass spectra with a Hewlett–Packard HP-5988-A spectrometer. Optical rotations were measured for solutions in $CHCl_3$ (1-dm tube) with a Perkin–Elmer 141 or a Jasco DIP-370 polarimeter. TLC was performed on Silica Gel G (Merck) with detection by charring with H_2SO_4. Column chromatography was performed on silica gel (Merck, 7734).

Methyl 2-O-benzoyl-4,6-O-benzylidene-3-O-tert-butyldiphenylsilyl-α-D-glucopyranoside (**6**).—To a stirred solution of methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside[4] (**5**; 19 g, 49 mmol) in dry DMF (30 mL) was added dropwise under N_2 a solution of imidazole (7.36 g, 108 mmol) and *tert*-butylchloro-

diphenylsilane (14.8 g, 53.8 mmol) in the same solvent (5 mL), and the mixture was heated for 2.5 h at 80°C. TLC (1:3 EtOAc–hexane) then showed the absence of **5** and the presence of a faster-running product. The solvent was evaporated under vacuum, and a solution of the residue in CH_2Cl_2 (50 mL) was washed with brine and water (2 × 50 mL), then concentrated. Column chromatography (1:5 EtOAc–hexane) of the residue gave **6**, isolated as a syrup (30.5 g, quantitative); $[\alpha]_D + 30°$ (*c* 1); ν_{max}^{film} 3074, 3051, 3016, and 3001 (C–H, aromatic), 2958, 2934, 2897, 2860 (C–H), 1729 (C=O, benzoate), 1429, 1138, 1115, 1087, 1060, 1044, 992 (C–O–C), 742 and 701 cm^{-1} (aromatic). NMR data: ^1H, δ 7.95–7.00 (m, 20 H, 4 Ph), 5.20 (dd, 1 H, $J_{1,2}$ 4, $J_{2,3}$ 9.3 Hz, H-2), 5.19 (s, 1 H, PhC*H*), 5.01 (d, 1 H, H-1), 4.40 (t, 1 H, $J_{3,4} = J_{4,5} = 9$ Hz, H-4), 4.21 (m, 1 H, H-5), 3.78–3.63 (m, 3 H, H-3,6a,6b), 3.25 (s, 3 H, OMe), and 0.83 (s, 9 H, Me_3C); ^{13}C, δ 166.22 (Ph*C*O), 101.62 (Ph*C*H), 97.76 (C-1), 81.90 (C-2), 74.93 (C-5), 70.67 (C-4), 68.83 (C-6), 61.89 (C-3), 55.27 (OMe), 26.55 (*Me*$_3$C), and 19.00 (Me$_3$*C*). Mass spectrum: *m/z* 568 (6.2%, $M^+ + 1 - Me_3C$), 567 (11.3, $M^+ - Me_3C$), 303 (6.2), 256 (8.1, $^tBuPh_2SiOH^+$), 199 (100, Ph_2SiOH^+), 106 (10.9), 105 (31.7), 83 (21.5), 78 (28.9), and 77 (29.3). *Anal.* Calcd for $C_{37}H_{40}O_7Si$: C, 71.12; H, 6.45. Found: C, 72.23; H, 7.12.

*Methyl 4,6-O-benzylidene-3-O-*tert*-butyldiphenylsilyl-α-*D*-glucopyranoside* (**7**).—A suspension of **6** (30.5 g, 49 mmol) in methanolic 0.5 M NaOMe (100 mL) was stirred at room temperature for 20 h. TLC (1:3 EtOAc–hexane) then revealed a new compound of lower mobility. The mixture was neutralised with acetic acid and concentrated, a solution of the residue in CH_2Cl_2 (100 mL) was washed with water and the solvent was evaporated. Column chromatography (1:10 → 1:2 EtOAc–hexane) of the syrupy residue gave, first, **6** (3.4 g) and then **7** (15.5 g, 69%); mp 135–137°C (from EtOAc–hexane); $[\alpha]_D + 87°$ (*c* 1); ν_{max}^{KBr} 3567 and 3500 (OH), 3064 and 3034 (C–H, aromatic), 2939 and 2854 (C–H), 1459 (benzyl), 1373, 1248, 1205, 1170, 1143, 1083, 1002, and 889 (C–O–C), 779 and 697 cm^{-1} (aromatic). NMR data: ^1H, δ 7.63–7.02 (m, 15 H, 3 Ph), 5.22 (s, 1 H, PhC*H*), 4.73 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 4.19 (dt, 1 H, $J_{4,5} = J_{5,6a} = 9$, $J_{5,6b}$ 5 Hz, H-5), 3.96 (t, 1 H, $J_{3,4}$ 9 Hz, H-4), 3.73 (dt, 1 H, $J_{2,3} = J_{2,OH} = 9$ Hz, H-2), 3.70–3.49 (2 m, 3 H, H-3,6a,6b), 3.33 (s, 3 H, OMe), 1.91 (d, 1 H, HO), and 1.01 (s, 9 H, Me_3C); ^{13}C, δ 136.97, 136.17, 135.77, 134.29, 133.29, 129.47, 129.28, 128.64, 127.80, 127.36, 127.20, and 126.21 (3 Ph), 101.45 (Ph*C*H), 100.01 (C-1), 81.43 (C-5), 73.79 and 73.76 (C-2,4), 68.84 (C-6), 62.34 (C-3), 55.28 (OMe), 26.95 (*Me*$_3$C), and 19.59 (Me$_3$*C*). *Anal.* Calcd for $C_{30}H_{36}O_6Si$: C, 69.20; H, 6.97. Found: C, 68.50; H, 7.20.

*Methyl 4,6-O-benzylidene-3-O-*tert*-butyldiphenylsilyl-2-O-trifluoromethanesulfonyl-α-*D*-glucopyranoside* (**8**).—To a stirred solution of **7** (11 g, 21.1 mmol) in dry CH_2Cl_2 (50 mL) and dry pyridine (50 mL) at −20°C was added trifluoromethanesulfonic anhydride (4.7 mL, 22.1 mmol) dropwise under Ar. The mixture was stirred at room temperature for 1 h. TLC (1:3 EtOAc–hexane) then showed the presence of a new, faster-running compound. The mixture was diluted with CH_2Cl_2 (50 mL), washed with aq 10% HCl, water, satd aq $NaHCO_3$, and water, then concentrated. Flash-column chromatography (1:7 EtOAc–hexane) of a part

(100 mg) of the amorphous orange residue (13.15 g) gave **8** as a colourless syrup that crystallised on standing; mp 109–111°C, $[\alpha]_D$ +50.5° (*c* 1); ν_{max}^{KBr} 3076, 3066, 3051, 3028, and 3001 (C–H, aromatic), 2989, 2978, 2960, 2929, 2909, and 2859 (C–H), 1454 (benzyl), 1373, 1170, 1149, 1141, 1117, 1102, 1088, 1073, 1051, 1040, 1029, 1015, 1006, and 992 (C–O–C), 744 and 700 cm^{-1} (aromatic). NMR data: ^1H, δ 7.60–7.55, 7.45–7.10, and 6.60–6.55 (3 m, 15 H, relative intensities 2:11:2, 3 Ph), 4.98 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 4.92 (dd, 1 H, $J_{2,3}$ 9 Hz, H-2), 4.66 (s, 1 H, PhC*H*), 4.34 (t, $J_{3,4}$ 9 Hz, H-3), 4.11 (dd, 1 H, $J_{4,5}$ 4.5 Hz, H-4), 3.62 (dt, 1 H, $J_{5,6a} = J_{5,6b} \approx 10$ Hz, H-5), 3.52 (t, 1 H, $J_{6a,6b}$ 10 Hz, H-6a), 3.46 (t, 1 H, H-6b), 3.38 (s, 3 H, OMe), and 0.94 (s, 9 H, Me_3C); ^{13}C, δ 136.19, 136.09, 135.10, 134.74, 131.49, 129.43, 129.14, 128.74, 127.62, 127.33, 127.15, and 126.36 (3 Ph), 101.71 (PhC*H*), 97.23 (C-1), 85.33 (C-2), 81.03 (C-5), 70.35 (C-4), 68.36 (C-6), 61.82 (C-3), 55.51 (OMe), 26.39 (*Me*$_3$C), and 19.51 (Me_3*C*). *Anal.* Calcd for $C_{31}H_{35}F_3O_8SSi$: C, 57.04; H, 5.41. Found: C, 57.90; H, 5.46.

*Methyl 2-S-acetyl-4,6-O-benzylidene-3-O-*tert*-butyldiphenylsilyl-2-thio-α-D-mannopyranoside* (**9**).—To a stirred solution of **8** (13.1 g, 20.1 mmol) in dry DMF (90 mL) was added potassium thioacetate (3.6 g, 31.5 mmol) portionwise, and the mixture was left at room temperature overnight. TLC (1:3 EtOAc–hexane) then revealed a new compound of slightly lower mobility. The solvent was evaporated, and a solution of the residue in CH_2Cl_2 was washed with brine and water, then concentrated. Column chromatography (1:6 EtOAc–hexane) of the residue gave **9** (11.52 g, quantitative), isolated as a thick yellow syrup. A portion (100 mg) was rechromatographed to yield **9** as a thick colourless syrup; $[\alpha]_D$ + 30° (*c* 0.62); ν_{max}^{film} 3067, 3046, and 3010 (C–H, aromatic), 2929 and 2854 (C–H), 1694 (C=O, thioacetate), 1587 (aromatic), 1494, 1379, 1353, 1255, 1211, 1195, 1056, 1016, 908, 845 (C–O–C and C–S–C), 757 and 697 cm^{-1} (aromatic). NMR data: ^1H, δ 7.65–7.55 and 7.40–7.10 (2 m, 15 H, relative intensities 4:11, 3 Ph), 5.32 (s, 1 H, PhC*H*), 4.55 (s, 1 H, H-1), 4.55 (dd, 1 H, $J_{3,4}$ 5.2, $J_{4,5}$ 9.5 Hz, H-4), 4.20–4.10 (m, 1 H, H-5), 3.96 (dd, 1 H, $J_{2,3}$ 1.2 Hz, H-3), 3.74–3.64 (m, 2 H, H-2,6a), 3.61–3.53 (m, 1 H, H-6b), 3.22 (s, 3 H, OMe), 2.37 (s, 3 H, SAc), and 0.98 (s, 9 H, Me_3C); ^{13}C, δ 194.10 (S*C*OMe), 137.12, 136.01, 135.84, 133.76, 132.96, 129.66, 129.31, 128.71, 127.88, 127.44, 127.17, and 126.23 (3 Ph), 102.32 (C-1), 101.80 (Ph*C*H), 81.24 (C-5), 68.68 (C-6), 67.62 (C-4), 63.41 (C-3), 55.04 (OMe), 51.14 (C-2), 30.64 (SCO*C*H$_3$), 26.69 (*Me*$_3$C), and 19.30 (Me_3*C*). Mass spectrum: *m/z* 522 (22.1%, $M^+ + 1 - CMe_3$), 521 (59.7, $M^+ - CMe_3$), 389 (8.8), 267 (15.3), 256 (12, $^tBuPh_2SiOH^+$), 199 (33.5, Ph_2SiOH^+), 149 (100), 121 (91.7), 91 (98.7, $C_7H_7^+$), and 43 (36.0, Ac^+).

*Methyl-2-S-acetyl-4-O-benzoyl-6-bromo-6-deoxy-3-O-*tert*-butyldiphenylsilyl-2-thio-α-D-mannopyranoside* (**10**).–To a stirred solution of **9** (1.9 g, 3.28 mmol) in dry CCl_4 (30 mL) were added $BaCO_3$ (2.5 g) and NBS (600 mg, 3.3 mmol), and the mixture was boiled under reflux for 1 h. TLC (1:6 EtOAc–hexane) then revealed a new compound of slightly higher mobility. The mixture was cooled, filtered through a Hyflo pad, washed with aq 10% sodium thiosulfate and water, then concentrated. Column chromatography (1:6 EtOAc–hexane) of the residue gave

10 (1.84 g, 85%); mp 134–135°C (from EtOAc–hexane); $[\alpha]_D$ + 37° (*c* 1); ν_{max}^{KBr} 3069 and 3045 (C–H, aromatic), 2995, 2951, 2929, and 2856 (C–H), 1723 (C=O, benzoate), 1689 (C=O, thioacetate), 1600 and 1587 (aromatic), 1263, 1114, 1056, and 998 (C–O–C and C–S–C), 703 and 618 cm^{-1} (aromatic). NMR data: ^1H, δ 7.86–7.83, 7.60–7.25, and 7.18–7.14 (3 m, 15 H, relative intensities 2:11:2, 3 Ph), 5.15 (t, 1 H, $J_{3,4} = J_{4,5}$ = 10 Hz, H-4), 4.71 (dd, 1 H, $J_{2,3}$ 5.1 Hz, H-3), 4.60 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1), 3.88 (ddd, 1 H, $J_{5,6a}$ 3.5, $J_{5,6b}$ 6.5 Hz, H-5), 3.79 (dd, 1 H, H-2), 3.39–3.37 (m, 2 H, H-6a,6b), 3.31 (s, 3 H, OMe), 2.33 (s, 3 H, SCOMe), 0.90 (s, 9 H, Me_3C); ^{13}C, δ 194.02 (S*C*OMe), 165.37 (Ph*C*O), 135.87, 135.81, 133.26, 132.93, 132.52, 129.95, 129.91, 129.58, 129.20, 128.20, 127.58, and 127.38 (3 Ph), 101.00 (C-1), 73.12 (C-5), 70.12 (C-4), 68.23 (C-3), 55.41 (OMe), 50.82 (C-2), 31.91 (C-6), 30.65 (SCO*Me*), 26.55 (*Me*$_3$C), and 19.08 (Me$_3$*C*). *Anal.* Calcd for $C_{32}H_{37}BrO_6SSi$: C, 58.44; H, 5.67, Br, 12.15; S, 4.87. Found: C, 58.30, H, 5.46; Br, 12.02; S, 4.74.

Scale-up of the procedure lowered the yield.

*Methyl 2,6-anhydro-3-*O*-tert-butyldiphenylsilyl-2-thio-α-*D*-mannopyranoside* (**11**). —To a stirred suspension of **10** (1.82 g, 2.8 mmol) in dry MeOH (20 mL) was added methanolic M NaOMe (4 mL), and the mixture was left at room temperature overnight. TLC (1:2 EtOAc–hexane) then revealed a new compound of lower mobility. The solvent was evaporated, and a solution of the residue in EtOAc was washed with water, then concentrated. Column chromatography (1:4 EtOAc–hexane) of the residue gave **11**, isolated as a syrup (1 g, 83%); $[\alpha]_D$ – 45° (*c* 1); ν_{max}^{film} 3514 (OH), 3067 and 3047 (C–H, aromatic), 2995, 2931, and 2854 (C–H), 1587 (aromatic), 1425, 1360, 1239, 1106, 1008, 846, and 822 (C–O–C and C–S–C), 740 and 702 cm^{-1} (aromatic). NMR data: ^1H, δ 7.73–7.69 and 7.46–7.34 (2 m, 10 H, relative intensities 4:6, 2 Ph), 4.90 (d, 1 H, $J_{1,2}$ 2.8 Hz, H-1), 4.36 (d, 1 H, $J_{2,3}$ 4.7 Hz, H-3), 4.23 (dm, 1 H, H-5), 3.52 (dm, 1 H, H-4), 3.25 (s, 3 H, OMe), 2.97 (dd, 1 H, $J_{5,6a}$ 5.6, $J_{6a,6b}$ 11.3 Hz, H-6a), 2.74 (dd, 1 H, $J_{5,6b}$ 1.1 Hz, H-6b), 2.65 (d, 1 H, $J_{4,OH}$ 11.2 Hz, HO-4), 2.56 (m, 1 H, H-2), 1.11 (s, 9 H, Me_3C); ^{13}C, δ 135.78, 135.75, 133.77, 133.36, 129.88, 129.80, 127.74, and 127.70 (2 Ph), 102.55 (C-1), 76.57 (C-4), 72.92 and 71.76 (C-3,5), 55.23 (OMe), 40.62 (C-2), 27.10 (C-6), 26.91 (*Me*$_3$C), and 19.27 (Me$_3$*C*). Mass spectrum: *m/z* 375 (11.9%, $M^+ + 2 - CMe_3$), 374 (26.9, $M^+ + 1 - CMe_3$), 373 (100, $M^+ - CMe_3$), and 199 (96.9, Ph_2SiOH^+).

Also isolated, as a minor product, was the 4-acetate of **11**; $[\alpha]_D$ – 32° (*c* 1.7); ν_{max}^{film} 3075, 3071, and 3000 (C–H, aromatic), 2961, 2934, 2898, and 2861 (C–H), 1745 (C=O, acetate), 1247, 1233, 1114, 1106, 1062, and 1038 (C–O–C and C–S–C), 737 and 703 cm^{-1} (aromatic). NMR data: ^1H, δ 7.71–7.60 and 7.46–7.31 (2 m, 10 H, relative intensities 4:6, 2 Ph), 4.94 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.78 (dd, 1 H, $J_{3,4}$ 3.3, $J_{4,5}$ 0.7 Hz, H-4), 4.64 (t, 1 H, $J_{2,3}$ 3.3 Hz, H-3), 4.20 (m, 1 H, H-5), 3.17 (s, 3 H, OMe), 3.00 (dd, 1 H, $J_{5,6a}$ 3.9, $J_{6a,6b}$ 11.5 Hz, H-6a), 2.87 (dd, 1 H, $J_{5,6b}$ 2.3 Hz, H-6b), 2.56 (dd, 1 H, H-2), 1.91 (s, 3 H, Ac), and 1.08 (s, 9 H, Me_3C); ^{13}C, δ 170.75 (Me*C*O), 135.87, 133.62, 133.59, 129.91, 129.83, 127.80, and 127.66 (2 Ph), 103.52 (C-1), 80.05 (C-4), 70.51 and 70.39 (C-3,5), 55.17 (OMe), 41.67 (C-2), 29.01 (C-6), 26.92 (*Me*$_3$C), 21.06 (*C*H$_3$CO), and 19.30 (Me$_3$*C*). Mass spectrum: *m/z* 417 (2.2%,

$M^+ + 2 - CMe_3$), 416 (4.8 $M^+ + 1 - CMe_3$), 415 (16.7, $M^+ - CMe_3$), 242 (14.1), 241 (71.2), 199 (91.8, Ph_2SiOH^+), 157 (44), 129 (50.1), and 43 (100, Ac^+).

*Methyl 2,6-anhydro-2-thio-α-*D*-mannopyranoside* (**12**).—To a stirred solution of **11** (3.06 g, 7.1 mmol) in dry THF (20 mL) was added M tetrabutylammonium fluoride trihydrate in THF (8 mL), and the mixture was left at room temperature for 24 h under Ar, then concentrated. TLC (3:1 EtOAc–hexane) then revealed a new compound of lower mobility. Column chromatography (1:1 → 3:1 EtOAc–hexane) of the residue gave **12** (1.15 g, 84.5%) that crystallised on standing; mp 42–44°C; $[\alpha]_D$ + 3.3° (*c* 1); ν_{max}^{film} 3417 (OH), 2937 and 2838 (C–H), 1244, 1114, 1060, 1047, and 920 cm^{-1} (C–O–C and C–S–C). NMR data: ^1H, δ 5.10 (d, 1 H, $J_{1,2}$ 2.7 Hz, H-1), 4.24 (dm, 1 H, H-5), 4.19 (d, 1 H, $J_{2,3}$ 4.6 Hz, H-3), 3.46 (s, 3 H, OMe), 3.39 (t, 1 H, $J_{2,4} = J_{4,5}$ = 1.2 Hz, H-4), 3.04 (bm, 3 H, H-2, HO-3,4), 2.95 (dd, 1 H, $J_{5,6a}$ 5.3, $J_{6a,6b}$ 11.6 Hz, H-6a), 2.61 (dd, 1 H, $J_{5,6b}$ 1.3 Hz, H-6b); ^{13}C, δ 101.93 (C-1), 76.22 (C-4), 71.36 and 70.32 (C-3,5), 55.70 (OMe), 41.42 (C-2), and 26.86 (C-6). Mass spectrum: *m/z* 192 (1.2%, M^+), 161 (4.5, $M^+ - OMe$), 149 (2.4), 132 (14.0), 87 (23.3), 76 (100), and 57 (69.8).

*Methyl 2,6-anhydro-4-*O*-benzyl-3-*O*-tert-butyldiphenylsilyl-2-thio-α-*D*-manno-pyranoside* (**13**).—To stirred solution of **11** (970 mg, 2.25 mmol) in dry THF (10 mL) was added potassium *tert*-butoxide (400 mg, 3.56 mmol) under Ar. The mixture was heated at 60°C for 10 min, the resulting orange solution was cooled, a solution of benzyl bromide (0.4 mL, 3.36 mmol) in THF (3 mL) was added dropwise, and the mixture was heated at 60°C for 15 min. TLC (1:3 ether–hexane) then showed the absence of **11** and the presence of a new compound of higher mobility. The mixture was diluted with ether, washed with water, and concentrated. Column chromatography (1:4 ether–hexane) of the residue yielded **13** (750 mg, 64%), isolated as a syrup; $[\alpha]_D$ + 1.6° (*c* 1); ν_{max}^{film} 3074, 3050, and 3035 (C–H, aromatic), 2960, 2934, 2895, and 2860 (C–H), 1113 and 1054 (C–O–C and C–S–C), 735 and 702 cm^{-1} (aromatic). NMR data: ^1H, δ 7.75–7.65 and 7.44–7.22 (2 m, 15 H, relative intensities 4:11, 2 Ph and $PhCH_2$), 4.92 (d, 1 H, $J_{1,2}$ 2 Hz, H-1), 4.70 (t, 1 H, $J_{2,3} = J_{3,4}$ = 3.2 Hz, H-3), 4.51 and 4.43 (2 d, 2 H, *J* 12 Hz, $PhCH_2$), 4.30 (m, 1 H, H-5), 3.56 (d, 1 H, H-4), 3.18 (s, 3 H, OMe), 3.00 (dd, 1 H, $J_{5,6a}$ 4.2, $J_{6a,6b}$ 11.3 Hz, H-6a), 2.67 (dd, 1 H, $J_{5,6b}$ 2.2 Hz, H-6b), 2.51 (dd, 1 H, H-2), and 1.10 (s, 9 H, Me_3C); ^{13}C, δ 138.27, 136.06, 136.00, 135.54, 134.19, 129.81, 128.34, 127.78, 127.70, and 127.58 (3 Ph), 103.26 (C-1), 85.19 (C-4), 72.64 (C-3), 71.01 ($PhCH_2$), 69.51 (C-5), 55.14 (OMe), 41.78 (C-2), 29.64 (C-6), 27.08 (Me_3C), and 19.41 (Me_3C). Mass spectrum: *m/z* 463 (2.7%, $M^+ - CMe_3$), 199 (80, Ph_2SiOH^+), and 91 (100, $C_7H_7^+$).

*Methyl 2,6-anhydro-4-*O*-benzyl-2-thio-α-*D*-mannopyranoside* (**14**).—To a stirred solution of **13** (750 mg, 1.44 mmol) in dry THF (6 mL) was added a solution of tetrabutylammonium fluoride trihydrate (530 mg, 1.68 mmol) in the same solvent (3 mL) under Ar. The mixture was stirred at room temperature for 24 h. TLC (2:1 ether–hexane) then revealed a new compound of lower mobility. The solvent was evaporated, and a solution of the residue in ether (15 mL) was washed with brine

and water, then concentrated. Column chromatography (1:1 ether–hexane) of the residue yielded **14** (330 mg, 81.3%), isolated as a colourless syrup; $[\alpha]_D + 59°$ (*c* 2.1); ν_{max}^{film} 3446 (OH), 3090, 3066, and 3033 (C–H, aromatic), 2936, 2880, and 2837 (C–H), 1455 (benzyl), 1369, 1244, 1055, 1030, and 983 (C–O–C and C–S–C), 739 and 700 cm^{-1} (aromatic). NMR data: ^1H, δ 7.40–7.23 (m, 5 H, CH_2Ph), 5.15 (d, 1 H, $J_{1,2}$ 2 Hz, H-1), 4.76 and 4.64 (2 d, 2 H, *J* 12.4 Hz, PhCH_2), 4.45 (dd, 1 H, $J_{2,3}$ 3.5, $J_{3,4}$ 2.6 Hz, H-3), 4.30 (m, 1 H, H-5), 3.44 (s, 3 H, OMe), 3.25 (t, 1 H, H-4), 2.98 (dd, 1 H, $J_{5,6a}$ 4.1, $J_{6a,6b}$ 11.5 Hz, H-6a), 2.91 (dd, 1 H, H-2), 2.70 (bs, 1 H, HO-3), 2.53 (dd, 1 H, $J_{5,6b}$ 2 Hz, H-6b); ^{13}C, δ 137.94, 128.43, 127.92, and 127.74 (Ph), 102.15 (C-1), 83.66 (C-4), 70.20 (PhCH_2), 69.70 and 69.55 (C-3,5), 55.37 (OMe), 42.32 (C-2), and 28.94 (C-6). Mass spectrum: *m/z* 251 (0.6%, $M^+ + 1 -$ MeOH), 250 (0.8, $M^+ -$ MeOH), 147 (5.8), 91 (100, $C_7H_7^+$), and 65 (22).

*Methyl 2,6-anhydro-3,4-di-O-benzyl-2-thio-α-*D*-mannopyranoside* (**15**).—To a stirred solution of **14** (950 mg, 3.37 mmol) in dry THF (15 mL) was added potassium *tert*-butoxide (570 mg, 5 mmol) under Ar. The mixture was heated at 60°C for 10 min, the resulting orange solution was cooled, and a solution of benzyl bromide (0.6 mL, 5 mmol) in dry THF (5 mL) was added dropwise. The mixture was heated at 60°C for 15 min. TLC (1:3 EtOAc–hexane) then revealed the absence of **14** and the presence of a faster-running compound. Water was added, the mixture was extracted with ether (3 × 15 mL), and the combined extracts were washed with brine and water, then concentrated. Column chromatography (1:6 EtOAc–hexane) of the residue gave **15** (1.19 g, 95%), isolated as a syrup; $[\alpha]_D - 2.3°$, $[\alpha]_{435} - 12°$ (*c* 1.5); ν_{max}^{film} 3091, 3066, and 3033 (C–H, aromatic), 2997, 2932, 2879, and 2836 (C–H), 1455 (benzyl), 1243, 1100, 1057, and 1029 (C–O–C and C–S–C), 737 and 697 cm^{-1} (aromatic). NMR data: ^1H, δ 7.40–7.24 (m, 10 H, 2 Ph), 5.15 (d, 1 H, $J_{1,2}$ 2 Hz, H-1), 4.63 and 4.58 (2 d, 2 H, *J* 12.3 Hz, PhCH_2O-3), 4.60 and 4.50 (2 d, 2 H, *J* 11.7 Hz, PhCH_2O-4), 4.36 (t, 1 H, $J_{2,3} = J_{3,4} = 3.3$ Hz, H-3), 4.37–4.33 (m, 1 H, H-5), 3.51 (d, 1 H, H-4), 3.45 (s, 3 H, OMe), 3.03 (dd, 1 H, $J_{5,6a}$ 4, $J_{6a,6b}$ 11.3 Hz, H-6a), 3.00 (dd, 1 H, H-2), and 2.65 (dd, 1 H, $J_{5,6b}$ 2 Hz, H-6b); ^{13}C, δ 138.00, 137.93, 128.45, 128.40, 127.97, 127.80, and 127.74 (2 *Ph*CH_2), 102.97 (C-1), 81.95 (C-4), 78.07 (C-3), 70.97 and 70.59 (2 PhCH_2), 69.75 (C-5), 55.44 (OMe), 38.28 (C-2), and 29.44 (C-6). Mass spectrum: *m/z* 372 (0.04%, M^+), 341 (0.04, $M^+ -$ OMe), 312 (0.27), 281 (0.07, $M^+ - C_7H_7$), 181 (0.45), 147 (1.01), 91 (100, $C_7H_7^+$), and 65 (13.74).

Hydrolysis of **15**.—(*a*) To a solution of **15** (1.15 g, 3.10 mmol) in THF (13 mL) was added aq 30% trifluoroacetic acid (3.5 mL). The mixture was heated at 50°C for 3.5 h, then left at room temperature overnight. TLC (2:3 EtOAc–hexane) then revealed a complex mixture. The solvent was evaporated and dry toluene was distilled twice from the residue. Column chromatography (1:3 EtOAc–hexane) then gave **15** (237 mg), fraction *A* (456 mg), and fraction *B* (208 mg). Fractions *A* and *B* each showed IR absorption for C=O (1738 and 1692 cm^{-1}, respectively).

Fraction *A* was treated, at room temperature, with $NaBH_4$ (80 mg) in dry MeOH (10 mL) for 4 h. Conventional work-up of the mixture followed by column

chromatography (1:3 EtOAc–hexane) yielded 2,6-anhydro-3,4-di-*O*-benzyl-2-thio-1-*O*-trifluoroacetyl-α-D-mannopyranose (**16**, 130 mg), isolated as a colourless syrup; $[\alpha]_D - 1°$, $[\alpha]_{435} - 10°$ (*c* 1.2); ν_{max}^{film} 3090, 3066 and 3034 (C–H, aromatic), 1738 (C=O, trifluoroacetatc), 1455 (benzyl), 1242, 1100, 1042, 1029, and 1008 (C–O–C and C–S–C), 737 and 698 cm^{-1} (aromatic). NMR data: ^1H, δ 7.40–7.23 (m, 10 H, 2 *Ph*CH_2), 5.62 (d, 1 H, $J_{1,2}$ 2 Hz, H-1), 4.58 and 4.53 (2 d, 2 H, *J* 12 Hz, PhCH_2O-3), 4.48 and 4.32 (2 d, 2 H, *J* 11.7 Hz, PhCH_2O-4), 4.40–4.36 (m, 2 H, H-3,5), 3.50 (d, 1 H, $J_{3,4}$ 2.7 Hz, H-4), 3.17 (t, 1 H, $J_{2,3}$ 2 Hz, H-2), 3.03 (dd, 1 H, $J_{5,6a}$ 4.2, $J_{6a,6b}$ 11.4 Hz, H-6a), 2.64 (dd, 1 H, $J_{5,6b}$ 2 Hz, H-6b); ^{13}C, δ 137.92, 137.81, 128.44, 128.37, 128.07, 127.98, 127.91, 127.81, 127.72 (2 *Ph*CH_2), 99.28 (C-1), 81.88 (C-4), 77.73 (C-3), 70.85 and 70.58 (2 PhCH_2), 69.91 (C-5), 38.08 (C-2), and 29.12 (C-6).

Fraction *B* (208 mg) was treated with $NaBH_4$ (50 mg), as described above, to afford, after work-up and column chromatography (1:3 EtOAc–hexane) unstable (4*S*,5*S*)-4-benzyloxy-5-hydroxy-2-hydroxymethyl-2-thiene (**17**, 50 mg); $[\alpha]_D + 235°$ (*c* 1); ν_{max}^{film} 3407 (OH), 3090, 3065, and 3033 (C–H, aromatic), 2928 and 2871 (C–H), 1633 (C=C), 1455 (benzyl), 1109, 1086, 1066, and 1029 (C–O–C and C–S–C), 739 and 699 cm^{-1} (aromatic). NMR data: ^1H, δ 7.41–7.26 (m, 5 H, *Ph*CH_2), 5.89 (dt, 1 H, $J_{3,4}$ 4.5, J_{3,CH_2OH} 1 Hz, H-3), 4.72 and 4.59 (2 d, 2 H, *J* 11.6 Hz, PhCH_2), 4.11 (d, 2 H, CH_2OH), 4.04 (dt, 1 H, $J_{4,5} = J_{5,6eq} = 3.3$, $J_{5,6ax}$ 10 Hz, H-5), 3.98 (m, 1 H, H-4), 3.05 (dd, 1 H, $J_{6ax,6eq}$ 12.3 Hz, H-6*ax*), 2.81 (ddd, 1 H, $J_{4eq,6eq}$ 1 Hz, H-6*eq*); ^{13}C, δ 139.17 (C-2), 137.90, 128.80, 128.05, 127.94 (*Ph*CH_2), 115.57 (C-3), 71.78 (C-4), 71.02 (PhCH_2), 66.46 (C-5), 65.57 (CH_2OH), and 28.54 (C-6).

(*b*) To a solution of **15** (182 mg, 0.49 mmol) in dry THF (5 mL) were added 30% acetic acid (1 mL) and concd HCl (6 drops). The mixture was left at room temperature for 30 h and then heated at 60°C for 1 h. TLC (2:1 ether–hexane) then revealed the absence of **15** and the presence of a new compound of lower mobility. The mixture was neutralised (aq $NaHCO_3$) and $NaBH_4$ (150 mg) was added. After 1 h, TLC (4:1 ether–hexane) revealed the absence of **15** and a new compound of lower mobility. The THF was evaporated, the residue was extracted with $CHCl_3$ (10 mL), and the extract was washed with brine and water, then concentrated. Column chromatography (4:1 ether–hexane) of the residue gave (2*R*,3*S*,4*R*,5*S*)-3,4-dibenzyloxy-5-hydroxy-2-hydroxymethylthiane (**18**; 76 mg, 43%); mp 118–120°C; $[\alpha]_D + 16°$ (*c* 0.6); ν_{max}^{KBr} 3377 (OH), 3089, 3067, and 3033 (C–H, aromatic), 2961, 2929, 2895, and 2854 (C–H), 1453 (benzyl), 1133, 1102, 1067, 1056, and 1011 (C–O–C and C–S–C), 745, 726, 705, and 691 cm^{-1} (aromatic). NMR data: ^1H, δ 7.42–7.26 (m, 10 H, 2 *Ph*CH_2), 4.82 and 4.67 (2 d, 2 H, *J* 11 Hz, PhCH_2), 4.69 and 4.63 (2 d, 2 H, *J* 11.4 Hz, PhCH_2), 4.25 (ddd, 1 H, H-5), 3.93 (t, 1 H, $J_{2,3} = J_{3,4} = 8$ Hz, H-3), 3.89–3.73 (m 2 H, CH_2OH), 3.45 (dd, 1 H, $J_{4,5}$ 3 Hz, H-4), 2.87 (dt, 1 H, J_{2,CH_2OH} 5 Hz, H-2), 2.80 (dd, 1 H, $J_{5,6a}$ 6, $J_{6a,6b}$ 14 Hz, H-6a), 2.69 (dd, 1 H, $J_{5,6b}$ 2.6 Hz, H-6b), 2.68 (d, 1 H, $J_{5,OH}$ 6.7 Hz, HO-5), 2.15 (bt, 1 H, CH_2OH); ^{13}C, δ 137.96, 137.70, 128.65, 128.62, 128.18, 128.14, 128.05 (2 *Ph*CH_2S),

82.83 (C-3), 77.52 (C-4), 74.89 and 73.10 (2 PhCH$_2$), 67.37 (C-5), 62.33 (CH$_2$OH), 47.56 (C-2), and 31.09 (C-6). Mass spectrum: *m/z* 269 (0.4%, $M^+ - C_7H_7$), 251 (0.8, $M^+ - C_7H_7 - H_2O$), 238 (0.3, $M^+ - C_7H_7 - CH_2OH$), 163 (19.0), 91 (100, $C_7H_7^+$), and 65 (8.4).

1-Deoxythiomannojirimycin (**2**).—A solution of **18** (63 mg, 0.175 mmol) in acetic acid (1.5 mL) was hydrogenated over palladium oxide (100 mg) at 4 atm for 5 days. TLC (10:1 ether–MeOH) then revealed a new compound of lower mobility. The mixture was filtered and concentrated. Column chromatography (5:1 ether–MeOH) of the residue afforded **2** (20 mg, 63.5%); mp 119–121°C (from ether–MeOH); $[\alpha]_D$ −43° (*c* 0.7, MeOH). NMR data (CD_3OD): ^1H, δ 4.13 (m, 1 H, H-5), 3.95 (dd, 1 H, $J_{7a,7b}$ 11.4, $J_{2,7a}$ 4.3 Hz, H-7a), 3.80 (t, 1 H, $J_{2,3} = J_{3,4} = 8.7$ Hz, H-3), 3.72 (dd, 1 H, $J_{2,7b}$ 6.9 Hz, H-7b), 3.36 (dd, 1 H, $J_{4,5}$ 3 Hz, H-4), 2.84 (dd, 1 H, $J_{5,6a}$ 2.2, $J_{6a,6b}$ 14 Hz, H-6a), 2.80 (ddd, 1 H, H-2), 2.68 (dd, 1 H, $J_{5,6b}$ 5.2 Hz, H-6b); ^{13}C, δ 76.74 (C-3), 72.16 (C-4), 70.22 (C-5), 63.34 (C-7), 50.18 (C-2), and 32.99 (C-6). Mass spectrum: *m/z* 164 (27.3%, $M^+ + 1 - OH$), 163 (41.9, $M^+ - OH$), 162 (20.9, $M^+ - H_2O$), 149 (36.4, $M^+ - CH_2OH$), 145 (15.5, $M^+ + 1 - 2H_2O$), 144 (23.4, $M^+ - 2H_2O$), 133 (51.2, $M^+ + 1 - OH - CH_2OH$), 132 (85.8, $M^+ - OH - CH_2OH$), 131 (55.0, $M^+ - H_2O - CH_2OH$), 115 (29.7, $M^+ + 1 - OH - H_2O - CH_2OH$), 114 (45.0, $M^+ - OH - H_2O - CH_2OH$), 113 (62.0, $M^+ - 2H_2O - CH_2OH$), 89 (86), 85 (98), and 45 (100).

Determination of enzyme activities and inhibition constants.—The enzymes, obtained from Sigma, were used without further purification. The activity of β-D-glucosidase (from almonds) was measured as follows: a mixture of 50 mM trisodium citrate (200 μL, pH 4.8), 2 mM *p*-nitrophenyl β-D-glucopyranoside (200 μL), and enzyme (200 μL, 5 μg/mL) was incubated for 15 min at 25°C, 0.1 M NaOH (400 μL) was added, and the absorbance at 400 nm was determined.

The activity of α-D-glucosidase (from yeast) was measured as follows: a mixture of 50 mM trisodium citrate (200 μL, pH 6.8), 2 mM *p*-nitrophenyl α-D-glucopyranoside (200 μL), and enzyme (200 μL, 5 μg/mL) was incubated for 15 min at 25°C, 0.1 M NaOH (400 μL) was added, and the absorbance at 400 nm was determined.

For the inhibition studies, 1-deoxythiomannojirimycin (**2**) was incorporated variously into each assay buffer to give a final concentration in the range 10^{-4}–10^{-2} M.

Dissociation constants for competitive inhibition by the inhibitor were calculated from the slopes of plots $1/v$ against $1/[S]$ from the rates of substrate hydrolysis in the absence and presence of inhibitor (Lineweaver–Burk plots).

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