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New syntheses of 1D- and 1L-1,2-anhydro-*myo*-inositol and assessment of their glycosidase inhibitory activities

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Abstract

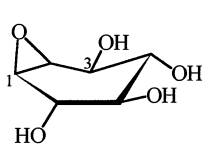
The 1D and 1L enantiomers of 1,2-anhydro-*myo*-inositol (conduritol B epoxide) were synthesised from 1D-pinitol and 1L-quebrachitol, respectively, and their activities were compared in selected glycosidase inhibition assays. The 1D enantiomer was found to be the active isomer, functioning as an irreversible inhibitor of sweet almond β-D-glucosidase. Neither isomer was active against the α-D-glucosidase from *Bacillus stearothermophilus* or the β-D-galactosidase from *Aspergillus oryzae*.

Keywords: Conduritol B epoxide; 1,2-Anhydro-*myo*-inositol; Pinitol; Quebrachitol; *chiro*-Inositol; Glycosidase inhibition

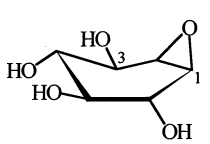
1. Introduction

Inhibitors of glycosidase enzymes have potential for the treatment of various disorders and diseases such as diabetes, cancer, and AIDS [1]; for example acarbose, a pseudotetrasaccharide, is an inhibitor of α-D-glucosidase and is in clinical use for the treatment of diabetes. Glycosidase inhibitors have also proved useful in the investigation of metabolic disorders such as Gaucher's disease [2].

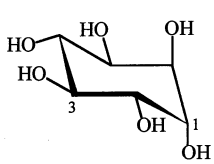
1,2-Anhydro-*myo*-inositol (conduritol B epoxide) is of considerable interest because of its ability to act as an irreversible inhibitor of various β-glucosidases [3], but it has yet to be conclusively determined by direct comparison whether the 1L (**1**) or the 1D (**2**) enantiomer is responsible for this observed inhibition.



1L-1,2-anhydro-*myo*-inositol **1**



1D-1,2-anhydro-*myo*-inositol **2**



1D-*chiro*-inositol **3**

The mechanism of glycosidase inhibition by vicinal anhydro-inositols depends upon binding in the active site of the enzyme in such a manner that the oxygen atom of the epoxide ring may be protonated by a proximate amino acid and the ring opened by an amino acid acting as a nucleophile. In this way an active epoxide is ring opened and becomes covalently bound to the enzyme which is thereby irreversibly inhibited. Because the bound product of the reaction of racemic 1,2-anhydro-*myo*-inositol with the β-glucosidase of *As-*

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pergillus wentii was 1D-*chiro*-inositol (**3**) it has been concluded that the inhibitor was the β-D-glucopyranoside-like D enantiomer (**2**) [4], which is consistent with several observations that, while racemic 1,2-anhydro-*myo*-inositol is a β-glucosidase inhibitor, the L enantiomer is inactive [4–7].

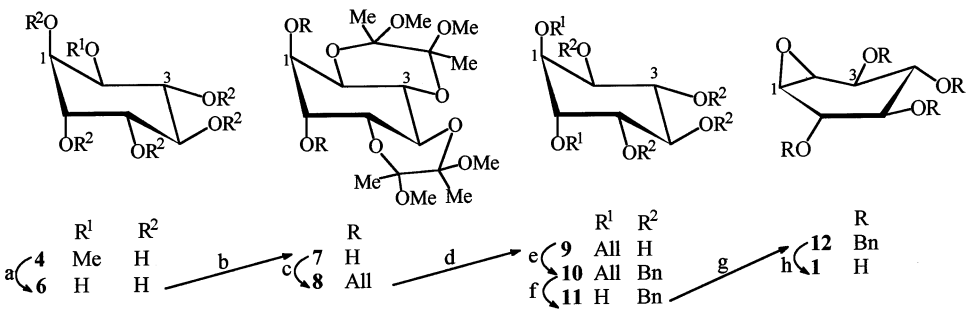
The literature contains reports of the syntheses of racemic 1,2-anhydro-*myo*-inositol from *myo*-inositol via racemic 6-bromo-6-deoxy-*chiro*-inositol [8], and racemic conduritol B tetraacetate [9,10], and the anhydride has also been obtained from benzene via *trans*-5,6-dihydroxycyclohexa-1,3-diene and hence the racemic tetrahydroxycyclohexene conduritol B [11]. In addition, 1L-1,2-anhydro-*myo*-inositol (**1**) has been produced by treatment with sodium methoxide of 1L-1-*O*-tosyl-*chiro*-inositol, derived in three steps from 1L-quebrachitol (**4**) [5,12]. Alternatively, the 1D enantiomer (**2**) has been synthesised ultimately from D-glucose [13,14] by epoxidation of 1,2,3-tri-*O*-benzyl-conduritol B derived from 2*S*,3*R*,4*S*-2,3,4-tribenzyloxylcyclohex-5-enone followed by debenzylation to yield the product [13].

In the present report we describe the syntheses of the 1L (**1**) and 1D (**2**) enantiomers of 1,2-anhydro-*myo*-inositol from 1L- and 1D-*chiro*-inositol which were derived from commercially available 1L-quebrachitol (**4**) and 1D-pinitol (**5**), respectively. Additionally, we describe the direct comparison of selected glycosidase inhibitory activities of **1** and **2**.

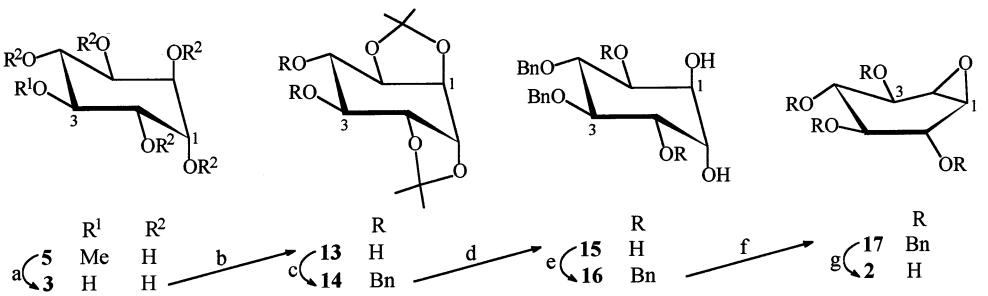
2. Results and discussion

Synthesis.—Given the ready availability of 1L-*chiro*-inositol (**6**) by the demethylation of the rubber tree natural product 1L-quebrachitol (**4**), and the enantiomer 1D-*chiro*-inositol (**3**) by similar treatment of the naturally-occurring 1D-pinitol (**5**), these compounds have been used as the respective starting materials for the preparation of the 1,2-anhydro-*myo*-inositol enantiomers **1** and **2**.

In Scheme 1, the eight-step synthesis of 1L-1,2-anhydro-*myo*-inositol (**1**) from 1L-quebrachitol (**4**) is illustrated. Protection of the vicinal diequatorial diols of 1L-*chiro*-inositol (**6**) as the bis-diacetal derivative **7** [15] left the axial 1,6-hydroxyl groups free. Although the fully protected *myo*-epoxide was obtainable from **7** in 74% yield, several attempts to remove the diketal groups from the former using acid resulted in opening of the epoxide ring and regeneration of the starting material **6**. Consequently, the more robust tetrabenzyl epoxide analogue **12** was made from the diketal **7** by standard functional group interconversions (**7** → **8** → **9** → **10** → **11** → **12**). Hydrogenation of **12**, employing the conditions described by Jaramillo et al. [13], gave the desired 1L-1,2-anhydro-*myo*-inositol product **1** in 9% overall yield. Use of too little solvent or too much catalyst in the final step led to undesired side products, possibly due to hydrogenolysis of the epoxide.



Scheme 1. Reagents: (a) 57% HI (b) cat. CSA, 2,3-butanedione, $CH(OMe)_3$, MeOH; (c) AllBr, NaH, DMF; (d) TFA, water, MeOH, DCM; (e) BnBr, NaH, DMF; (f) cat. *p*-TSA, Pd–C, water, MeOH; (g) Ph_3P, DEAD, toluene; (h) 10% Pd–C, H_2, 1:5 EtOAc:MeOH.



Scheme 2. Reagents: (a) 57% HI (b) cat. TsOH, acetone, 2,2-dimethoxypropane, DMF; (c) BnBr, Bu_4NI, NaH, DMF; (d) TFA, water; (e) $(Bu_3Sn)_2O$, BnBr, Bu_4NBr, toluene; (f) Ph_3P, DIAD, toluene; (h) 10% Pd–C, H_2, 1:5 EtOAc:MeOH.

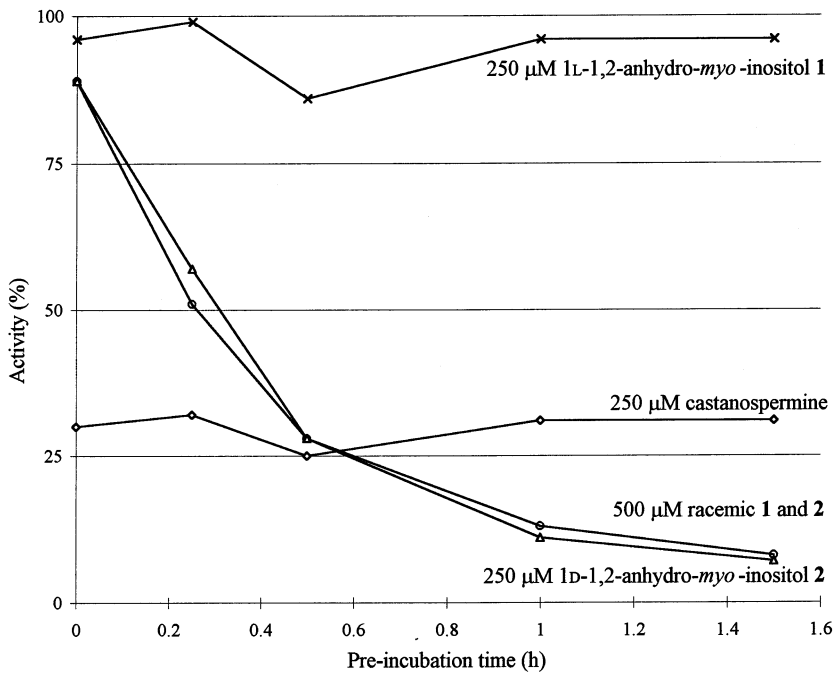


Fig. 1. Time-scale of sweet almond β-glucosidase inhibition. Assay details are described in Section 3.

The synthesis of the D enantiomer of 1,2-anhydro-*myo*-inositol (**2**) from 1D-pinitol (**5**) is illustrated in Scheme 2. The tetrabenzyl ether **16**, which is the enantiomer of compound **11** (Scheme 1), was made via the diacetonide **13**, the acetal groups now protecting the vicinal *cis*-1,2 and 5,6-diols rather than the vicinal 2,3- and 4,5-diequatorial *trans*-diols of the starting materials. The 3,4-hydroxyl groups were benzylated to give compound **14**. After removal of the acetals, the resulting tetraol **15** was subjected to regioselective stannylene-mediated benzylation of the 2,5-equatorial hydroxyls [16] to give the expected tetrabenzyl intermediate **16** which was treated with diisopropyl azodicarboxylate and triphenylphosphine [17] to form the epoxide **17**. Hydrogenolysis of the tetrabenzyl epoxide **17** gave the target compound, 1D-1,2-anhydro-*myo*-inositol **2** in 29% (unoptimised) overall yield in seven steps from 1D-pinitol (**5**).

A notable feature of the procedures described above is their applicability to the synthesis of either enantiomer of the required products from the corresponding enantiomer of *chiro*-inositol.

Glycosidase inhibition activity.—Initially, the inhibitory effects of **1** and **2** were compared with those of castanospermine, a well known and potent inhibitor of many glycosi-

dases [18]. To observe significant inhibitory activity it was necessary to pre-incubate 1,2-anhydro-*myo*-inositol with the selected enzyme, the β-glucosidase from sweet almonds. In Fig. 1 the dramatic, irreversible inhibition by 1D-1,2-anhydro-*myo*-inositol (**2**) with increasing pre-incubation time is demonstrated. In contrast, the corresponding 1L enantiomer **1** shows no significant inhibiting action. Correspondingly, a racemic mixture of the 1D- and 1L-1,2-anhydro-*myo*-inositols exhibits the inhibitory effect expected of its 1D-component (**2**). On this time-scale, the positive control castanospermine behaved in a reversible manner, producing a constant level of inhibition with increasing pre-incubation time.

Under the conditions described in Section 3, IC_{50} values were determined using a 1 h pre-incubation time and these data are illustrated in Fig. 2. Under these conditions, the IC_{50} value for 1D-1,2-anhydro-*myo*-inositol (**2**) is 67.5 μM (or 10.8 μg/mL) while that for castanospermine is 109.5 μM (or 20.1 μg/mL).

The inhibitory activity of the D enantiomer **2** appears to be a reflection of its ability to mimic the structure of β-D-glucopyranosides. Consistent with this are the observations that **2** did not inhibit either the β-D-galactosidase from *Aspergillus oryzae* or the α-D-glucosidase from *Bacillus stearothermophilus*. The latter observation can be rationalised by the fact that the trans-diequatorial opening required of the epoxide **2** by α-D-glucosidases functioning in a concerted process is energetically unfavourable, in contrast with the energetically easier trans-diaxial opening of **2** which is effected by β-D-glucosidases [3].

1L-1,2-Anhydro-*myo*-inositol (**1**) has previously been reported to be a weak competitive inhibitor of yeast α-D-glucosidase [5]; however, we observed no detectable inhibition of the α-D-glucosidase from *B. stearothermophilus* by **1**. The present result is consistent with the more general observation that α-D-glucosidases from most other sources show no or minimal inhibition of activity in the presence of **1** [3]. This result, together with the inactivity apparent against sweet almond β-D-glucosidase, can be ascribed to the inability of **1** to mimic the structure of either α- or β-D-glucopyranosides.

3. Experimental

General methods.—NMR spectra were acquired on a Bruker Avance NMR spectrometer operating at 300 MHz for ^1H and 75 MHz

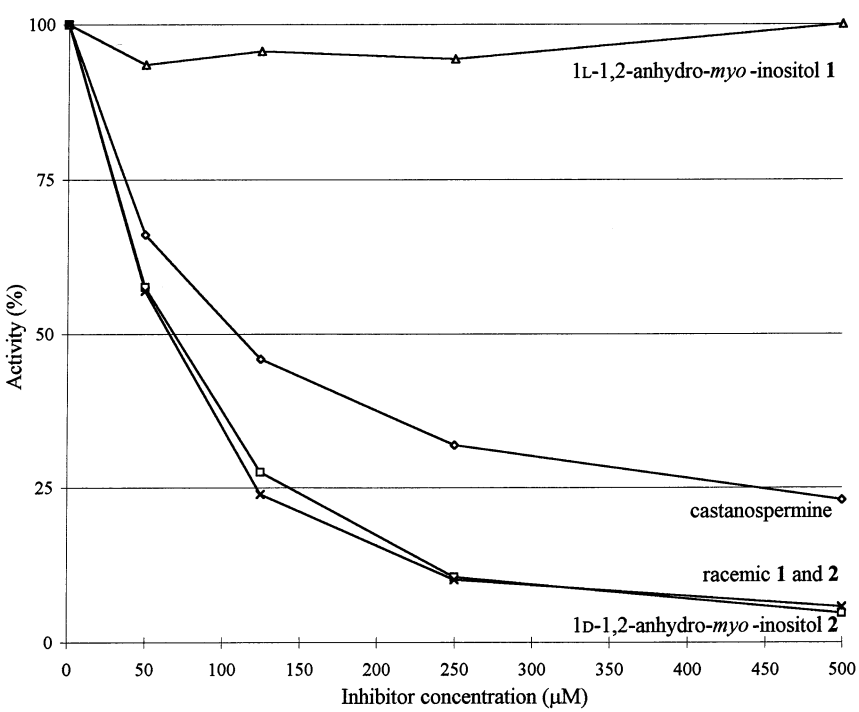


Fig. 2. Sweet almond β-glucosidase inhibition. Assay details are described in Section 3.

for ^{13}C nuclei and chemical shifts are listed in ppm. NMR experiments performed in $CDCl_3$ are referenced to Me_4Si (0 ppm), those in D_2O to external acetone (δ 218.1 ppm (C=O) and δ 33.2 ppm (Me) for ^{13}C and δ 2.22 ppm for ^1H) and those in CD_3OD to external MeOH (δ 49.0 ppm for ^{13}C and δ 3.30 ppm (Me) for ^1H). Chemical shift assignments were made on the basis of ^{13}C-DEPT, HH-COSY and HC-COSY spectral analysis. Melting points were determined using a Reichert hot stage microscope apparatus. All solvents used were AR grade or were distilled unless otherwise stated. Flash chromatography (FC) was performed using Scharlau silica gel 60 (0.04–0.06 mm, 230–400 mesh ASTM). Optical rotations were acquired with a Perkin–Elmer 241 polarimeter, and mass spectra were obtained on a VG70-250S double focusing magnetic sector mass spectrometer. Pinitol was supplied by New Zealand Pharmaceuticals Ltd. and the Rubber Research Institute of Malaysia supplied quebrachitol.

Sweet almond β-glucosidase was obtained from Boehringer-Mannheim and *p*-nitrophenyl β-D-glucopyranoside from Sigma; *A. oryzae* β-galactosidase from Sigma and *p*-nitrophenyl β-D-galactopyranoside from Appli-Chem; *B. stearothermophilus* α-glucosidase and *p*-nitrophenyl α-D-glucopyranoside were from Sigma.

L-*Anhydro-3,4,5,6-tetra*-O-*benzyl*-myo-*inositol* (**12**).—1L-*chiro*-Inositol (**6**) was prepared from 1L-quebrachitol (**3**) by the method of Angyal and Hoskinson [19] as white crystals in 93% yield; ^1H NMR (D_2O): δ 4.06 (br s, 2 H, H-1 and H-6), 3.79 (m, 2 H, H-2 and H-5), 3.63 (m, 2 H, H-3 and H-4); ^{13}C NMR (D_2O): δ 75.6 (C-3 and C-4), 74.5 (C-1 and C-6), 73.3 (C-2 and C-5), lit. [20].

Crude 1L-*chiro*-inositol (**6**, 10 g, 55.6 mmol) and anhydrous camphorsulfonic acid (2.8 g, 12.1 mmol) were dissolved in dry MeOH (140 mL) and trimethylorthoformate (37 mL, 338.2 mmol) and 2,3-butanedione (10 mL, 114.0 mmol) were added. The resulting soln was heated under reflux for 72 h under argon and NEt_3 (10 mL) was added and the solvents were removed in vacuo. The residue was purified by FC (1:1 hexanes–EtOAc) to give 1L-2,3:4,5-bis-[*O*-(2,3-dimethoxybutane-2,3-diyl)]-*chiro*-inositol (**7**) as a pale brown foam (15.9 g, 70%); $[\alpha]_D^{19}$ +184° (*c* 1.5, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 3.93–3.82 (m, 6 H, H-1–H-6), 3.19 (s, 6 H, OMe × 2), 3.17 (s, 6 H, OMe × 2), 3.00 (br s, 2 H, OH × 2), 1.23 (s, 6 H, Me × 2), 1.20 (s, 6 H, Me × 2); ^{13}C NMR ($CDCl_3$): δ 100.1 (O–*C*–O), 98.8 (O–*C*–O), 70.3, 68.5, 66.0 (C-1–C-6), 47.9, 47.8 (OMe × 2), 17.6, 17.5 (Me × 2); FAB$^+$-MS (CH_2Cl_2–NBA): Calcd for $C_{18}H_{31}O_{10}$ [M − H$^+$]: *m*/*z* 407.1917. Found: 407.1886.

Dry DMF (30 mL) was added under argon to dissolve the diketal **7** (3.34 g, 8.2 mmol) and the soln was cooled to 0 °C and NaH (0.72 g of 60% w/w NaH in oil, 18 mmol) was added followed by allyl bromide (2.97 g, 24.5 mmol) under argon. The ice bath was removed and the mixture was stirred at rt under argon for 72 h. EtOH (100 mL) was added to quench the reaction and the soln was poured into water (150 mL). A combined EtOAc extract (3 × 75 mL) of the resulting mixture was washed with water (50 mL), then dried ($MgSO_4$), filtered and the solvent was removed to yield 1L-1,6-di-*O*-allyl-2,3:4,5-bis-[*O*-(2,3-dimethoxybutane-2,3-diyl)]-*chiro*-inositol (**8**) as a yellow oil (3.05 g, 76%); ^1H NMR ($CDCl_3$): δ 5.90 (m, 2 H, CH_2=C*H*), 5.23 (dd, 2 H, *J* 17.3, 1.6 Hz, CH_2=CH), 5.11 (dd, 2 H, *J* 10.4, 1.4 Hz, CH_2=CH), 4.32 (m, 2 H, H-3, H-4 or H-2, H-5), 4.15–3.89 (m, 6 H, CH_2–O, H-3, H-4 or H-2, H-5), 3.70 (d, 2 H, *J* 1.6 Hz, H-1 and H-6), 3.26 (s, 6 H, OMe × 2), 3.23 (s, 6 H, OMe × 2), 1.27 (s, 6 H, Me × 2), 1.26 (s, 6 H, Me × 2); ^{13}C NMR ($CDCl_3$): δ 135.6 (CH_2=*C*H), 116.2 (*C*H_2=CH–CH_2–O), 99.8, (O–*C*–O), 98.6 (O–*C*–O), 76.3 (C-1 and C-6), 72.2 (*C*H_2–O), 68.8, 66.9 (C-2, C-5 and C-3, C-4), 48.0 (OMe), 47.6 (OMe), 17.8 (Me).

Compound **8** (2.98 g) was dissolved in CH_2Cl_2 (20 mL) and water (20 mL), trifluoroacetic acid (5 mL) and MeOH (2 mL) were added and the mixture was stirred at rt for 5 days. The solvents were removed in vacuo and the resulting material subjected to FC (5:3:2 CH_2Cl_2–EtOAc–MeOH) to yield 1L-1,6-di-*O*-allyl-*chiro*-inositol (**9**) (1.24 g, 78%) as a white solid; ^1H NMR (CD_3OD): δ 5.95 (m, 2 H, CH_2=C*H*), 5.30 (dd, 2 H, *J* 17.2, 1.6 Hz, CH_2=CH), 5.19 (dd, 2 H, *J* 10.4, 1.2 Hz, CH_2=CH), 4.18 (m, 4 H, CH_2–O), 3.78 (d, 2 H, *J* 2.4 Hz, H-1 and H-6), 3.69 (m, 2 H,

H-2, H-5 or H-3, H-4), 3.53 (m, 2 H, H-3, H-4 or H-2, H-5); ^{13}C NMR (CD_3OD): δ 136.9 (CH_2=*C*H), 117.7 (*C*H_2=CH), 79.1 (C-1 and C-6), 75.3 (C-2, C-5 or C-3, C-4), 73.9 (*C*H_2–O), 73.0 (C-3, C-4 or C-2, C-5); FAB$^+$-MS (MeOH–glycerol): Calcd for $C_{12}H_{21}O_6$ [M + H$^+$]: *m*/*z* 261.1338. Found: 261.1335.

Tetraol **9** (1.14 g, 4.4 mmol) was dissolved in dry DMF (30 mL) and treated with NaH (0.42 g, 60% w/w in oil, 10.5 mmol) at 0 °C, followed by BnBr (1.8 g, 10.5 mmol) under argon. The mixture was stirred at rt overnight and a second portion of NaH (0.42 g, 60% w/w in oil, 10.5 mmol) was added followed by another portion of BnBr (1.8 g, 10.5 mmol) at 0 °C. After 24 h at rt with stirring, the reaction was quenched with EtOH (100 mL) and the mixture was poured into water (150 mL). The resulting soln was extracted with EtOAc (3 × 75 mL) and the combined EtOAc extract was washed with water (50 mL), dried ($MgSO_4$), filtered and the solvent removed in vacuo to yield crude **10** as a yellow oil contaminated with parafin oil (3.26 g). To 3.0 g of this oil, *p*-toluenesulfonic acid monohydrate (1.0 g, 5.3 mmol) and Pd–C (1.5 g of 10% w/w) were added, followed by water (10 mL) and MeOH (45 mL) and the mixture was heated under reflux for 2.25 h [21]. The mixture was cooled and the catalyst removed by filtration through Celite pre-washed with MeOH. The filtrate was concd and partitioned between $CHCl_3$ (100 mL) and water (75 mL). The organic phase was washed with water (75 mL), dried ($MgSO_4$), filtered and the filtrate was taken to dryness. FC of the residue (3:2 hexanes–EtOAc) gave 1L-2,3,4,5-tetra-*O*-benzyl-*chiro*-inositol (**11**) (0.95 g, 40% from **9**) as a clear, colourless oil; ^{1}H NMR ($CDCl_3$): δ 7.55–7.22 (m, 20 H, Ar), 4.84 (s, 4 H, CH_2), 4.81–4.56 (m, 4 H, H-2, H-5 and H-3, H-4), 4.11 (s, 2 H, H-1 and H-6), 3.83 (m, 4 H, CH_2), 2.68 (br s, 2 H, OH × 2); ^{13}C NMR ($CDCl_3$): δ 139.0, 138.2, 128.6, 128.4, 128.0, 127.7, 127.6, 127.0, Ar), 81.6 (C-3 and C-4), 80.3 (C-2 and C-5), 75.9, 73.3 (CH_2), 69.1 (C-1 and C-6); FAB$^+$-MS (MeOH–NBA): Calcd for $C_{34}H_{35}O_6$ [M − H$^+$]: *m*/*z* 539.2434. Found: 539.2441.

The tetraether **11** (590 mg, 1.1 mmol) in dry toluene (3 mL) was added under argon dropwise, with stirring, to Ph_3P (858.7 mg, 3.3 mmol) and diethyl azodicarboxylate (515.5 μL, 3.3 mmol) in dry toluene (3 mL). After 7 days with stirring at rt the reaction mixture was fractionated directly by FC (1:5 EtOAc–hexanes) to give the anhydride **12** (353 mg, 62%) as a white solid. Recrystallisation (1:1 MeOH–water) gave white needles; mp 106–107 °C; $[\alpha]_D^{20}$ −34° (*c* 0.72, $CHCl_3$); ^{1}H NMR ($CDCl_3$): 7.50–7.20 (m, 20 H, Ar), 4.80–4.70 (m, 8 H, CH_2), 3.90 (dd, 1 H, *J* 8.5, <2 Hz, H-3 or H-6), 3.89 (d, 1 H, *J* 8.7 Hz, <2 Hz, H-6 or H-3), 3.62 (dd, 1 H, *J* 10.3, 8.7 Hz, H-4 or H-5), 3.47 (dd, 1 H, *J* 10.3, 8.7 Hz, H-5 or H-4), 3.32 (br d, 1 H, *J* 3.6, <2 Hz, H-1 or H-2), 3.19 (d, 1 H, *J* 3.8 Hz, H-2 or H-1); ^{13}C NMR ($CDCl_3$): 83.9 (C-4 or C-5), 79.8 and 79.7 (C-6 and C-3), 79.5 (C-5 or C-4), 76.4, 75.9, 73.7, 73.5 (CH_2), 55.6 and 55.4 (C-1 and C-2); FAB$^+$-MS: Calcd for $C_{34}H_{33}O_5$ [M − H$^+$]: *m*/*z* 521.2328. Found: 521.2324; Anal. Calcd for $C_{34}H_{34}O_5$: C, 78.14; H, 6.56. Found: C, 77.90; H, 6.61.

*1*L*-1,2-Anhydro*-myo-*inositol [1*L*-*conduritol B epoxide*] (**1**).—Palladium-on-carbon catalyst (90 mg of 10% w/w) was added to compound **12** (250 mg, 0.48 mmol) in 5:1 MeOH–EtOAc (55 mL) and shaken under hydrogen at atmospheric pressure overnight at rt. The catalyst was removed by filtration through Celite and FC (1:3 MeOH–$CHCl_3$) of the concentrate yielded anhydride **1** (71.3 mg, 94%) as a white solid. Recrystallisation (EtOH) gave white cubic crystals; mp 158–159 °C, lit. 160 °C [5]; $[\alpha]_D^{20}$ −65° (*c* 0.99, water), lit. −70° [5]; ^{1}H NMR (D_2O): 3.94 (m, 1 H, H-3 or H-6), 3.80 (m, 1 H, H-6 or H-3), 3.49 (dd, 1 H, *J* 1.9, 1.6 Hz, H-1 or H-2), 3.33–3.28 (m, 3 H, H-2 or H-1 and H-4 and H-5); ^{13}C NMR (D_2O): 75.2 (C-4 or C-5), 71.8 (C-3 or C-6), 71.0 (C-6 or C-3), 70.8 (C-5 or C-4), 57.7 (C-1 or C-2), 57.0 (C-2 or C-1); Anal. Calcd for $C_6H_{10}O_5$: C, 44.45; H, 6.22. Found: C, 44.47; H 6.33.

*1*D*-1,2-Anhydro-3,4,5,6-tetra-*O*-benzyl-*myo-*inositol* (**17**).—Demethylation of 1D-pinitol (**5**) was performed as previously reported [22] to give 1D-*chiro*-inositol **3** (77%) as off-white crystals; ^{1}H and ^{13}C NMR data (D_2O) were identical to those of compound **6**.

1D-*chiro*-Inositol (**3**) (8.0 g, 44 mmol) was dissolved in dry DMF (80 mL) and acetone

(80 mL), and *p*-toluenesulfonic acid monohydrate (2.5 g, 133 mmol) and 2,2-dimethoxypropane (16 mL) were added with stirring. After 15 h, NEt_3 (1 mL) was added and the solvents were removed in vacuo. The residue was redissolved in MeOH (160 mL) and camphorsulfonic acid (250 mg) was added to favour conversion of any triisopropylidene derivative formed to the desired 1D-1,2:5,6-di-*O*-isopropylidene-*chiro*-inositol (**13**). After a further 50 min NEt_3 (1 mL) was added and the solvents were again removed. FC (EtOAc) of the residue dissolved in CH_2Cl_2 gave **13** (7.9 g, 68%) as an amorphous white solid; ^{1}H NMR ($CDCl_3$): δ 4.33 (m, 2 H, H-2 and H-5), 4.20 (br s, 2 H, H-1 and H-6), 3.94 (2 H, OH), 3.57 (m, 2 H, H-3 and H-4), 1.52 (s, 6 H, Me), 1.37 (s, 6 H, Me); ^{13}C NMR ($CDCl_3$): δ 109.5 (O–*C*–O), 78.6 (C-1, C-6 or C-2, C-5), 76.2 (C-6, C-1 or C-5, C-2), 72.2 (C-3 and C-4), 27.5 (Me), 25.0 (Me).

To a soln of compound **13** (7.9 g, 30 mmol) in THF (80 mL) and dry DMF (40 mL), BzBr (14.4 mL, 120 mmol) was added followed by tetrabutylammonium iodide (5.6 g, 15 mmol). The soln was cooled to 0 °C, NaH was added (2.7 g, 113 mmol) and the mixture was stirred overnight at rt. Toluene (100 mL) was added and extracted with water (2 × 100 mL), dried ($MgSO_4$), filtered and then the solvents were removed. FC (1:8 → 1:5 EtOAc–hexanes) gave 1D-1,2:5,6-di-*O*-isopropylidene-3,4-di-*O*-benzyl-*chiro*-inositol **14** (11.1 g, 83%) as a pale yellow syrup; ^{1}H NMR ($CDCl_3$): δ 7.39–7.23 (m, 10 H, Ar), 4.82 (m, 4 H, CH_2), 4.29 (m, 2 H, H-2, H-5 or H-3, H-4), 4.25 (m, 2 H, H-5, H-2 or H-4, H-3), 3.57 (m, 2 H, H-1 and H-6), 1.47 (s, 6 H, Me), 1.35 (s, 6 H, Me); ^{13}C NMR ($CDCl_3$): δ 138.6, 128.2, 127.9, 127.5 (Ar), 109.5 (O–*C*–O), 79.9, 79.3 (C-1 and C-6, C-2 and C-5), 76.5 (C-3 and C-4), 73.8 (CH_2), 27.8 (Me), 25.4 (Me).

Acetal **14** (11.1 g, 25 mmol) in aq trifluoroacetic acid (100 mL of 50% v/v) was kept at rt for 1 h. The solvent was evaporated to give a white solid which was dissolved in hot EtOAc and hexanes were added to cause precipitation of 1D-3,4-di-*O*-benzyl-*chiro*-inositol (**15**) (8.0 g, 88%) as a white solid. Recrystallisation (EtOAc) gave **15** as white needles; mp 139–140 °C; $[\alpha]_D^{20}$ −4° (*c* 1.02, MeOH); ^{1}H NMR (CD_3OD): δ 7.38–7.22 (m, 10 H, Ar), 4.91–4.78 (m, 4 H, CH_2), 3.93 (s, 2 H, H-1 and H-6), 3.90 (m, 2 H, H-2 and H-5), 3.61 (m, 2 H, H-3 and H-4); ^{13}C NMR (CD_3OD): δ 141.0, 129.6, 129.4, 128.8 (Ar), 83.8 (C-3 and C-4), 76.6 (CH_2), 74.3 (C-1 and C-6), 73.1 (C-2 and C-5); FAB$^+$-MS (MeOH–glycerol): Calcd for $C_{20}H_{25}O_6$ [M + H$^+$]: *m*/*z* 361.1651. Found: 361.1650.

A suspension of the dibenzyl ether **15** (0.25 g, 0.69 mmol) in toluene (10 mL) was heated with bis(tributyltin) oxide (0.83 g, 1.4 mmol) under reflux in a Dean–Stark apparatus for 2 h. Tetrabutylammonium bromide (0.11 g, 0.35 mmol) and BzBr (0.41 mL, 3.5 mmol) were added to the mixture which was heated under reflux overnight. After cooling, it was fractionated directly by FC (1:2 EtOAc–hexanes) to give 1D-2,3,4,5-tetra-*O*-benzyl-*chiro*-inositol (**16**) (317 mg, 84%) isolated as a syrup; $[\alpha]_D^{20}$ +15.6° (*c* 0.8, $CHCl_3$); ^{1}H and ^{13}C NMR data ($CDCl_3$) were identical to those obtained for compound **11**; FAB$^+$-MS (DCM–NBA): Calcd for $C_{34}H_{35}O_6$ [M − H$^+$]: *m*/*z* 539.2434. Found: 539.2448.

A soln of the tetrabenzyl ether **16** (270 mg, 0.5 mmol) in toluene (5 mL) was added to a pre-formed mixture of diisopropyl azodicarboxylate (202 mg, 1 mmol) and Ph_3P (262 mg, 1 mmol) in toluene (5 mL) and the mixture was stirred at rt for 2 days, and fractionated directly by FC (1:5 EtOAc–hexanes) to give 245 mg (93%) of the title compound **17** as a white solid. Recrystallisation (1:1 MeOH–water) gave tiny white needles; mp 103–105 °C; $[\alpha]_D^{20}$ +33° (*c* 1.2, $CHCl_3$); ^{1}H and ^{13}C NMR data ($CDCl_3$) were identical to those obtained for compound **12**; FAB$^+$-MS (DCM–NBA): Calcd for $C_{34}H_{33}O_5$ [M − H$^+$]: *m*/*z* 521.2328. Found: 521.2324; Anal. Calcd for $C_{34}H_{34}O_5$: C, 78.14; H, 6.56. Found: C, 77.78; H, 6.72.

1D-1,2-Anhydro-myo-*inositol [1D-conduritol B epoxide]* (**2**).—The tetrabenzyl anhydride **17** (280 mg, 0.54 mmol) in MeOH (50 mL) and EtOAc (10 mL) was hydrogenated at atmospheric pressure and rt overnight in the presence Pd–C catalyst (100 mg, 10% w/w). The catalyst was removed by filtration through Celite (pre-washed with 5:1 MeOH–EtOAc) and the solvents were concd. FC (1:3 MeOH–$CHCl_3$) gave **2** (84 mg, 97%) as a white solid.

Recrystallisation (EtOH) gave white cubic crystals; mp 160–161 °C, lit. 158–160 °C [13]; $[\alpha]_D^{19}$ + 67° (*c* 0.93, water), lit. + 66° [13]; ^1H and ^{13}C NMR data (D_2O) were identical with those obtained for compound **1**, lit. [13]; Anal. Calcd for $C_6H_{10}O_5$: C, 44.45; H, 6.22. Found: C, 44.37; H, 6.45.

Glycosidase inhibition studies.—All buffers and solns were prepared using Millipore-filtered water. Assays were performed in duplicate at 37 °C using 96 well microtitre plates with a final assay volume of 300 μL. All assays used 200 μL of 250 μM *p*-nitrophenyl glycoside (substrate) soln, 50 μL test inhibitor soln and 50 μL enzyme soln (0.2 U/mL), buffered to pH 5.0 in 0.12 M phosphate (Pi) buffer. The liberated *p*-nitrophenol (PNP) was measured at 405 nm after quenching the reactions with 100 μL borate buffer (pH 9.8, 0.2 M); standards were included in the assay to generate a standard curve.

For the time-scale inhibition studies, at each pre-incubation time-step enzyme (50 μL of 0.2 U/mL) was added to the test inhibitor (50 μL of 500 μM [or 1000 μM for the racemate, **1**,**2**]) or Pi buffer for the negative control (50 μL). At the end of the pre-incubation time, the corresponding *p*-nitrophenyl glycopyranoside (200 μL of 250 μM) was added to each well and incubated for a further 5 min at 37 °C. For the α-glycosidase inhibition assay, a reaction time of 20 min was used with varying inhibitor concns up to 10 mM (corresponding to a maximum assay concn of 1.7 mM). Results are expressed as percentage activities relative to the corresponding enzyme controls.

To determine the inhibitory concns for the various inhibitors, enzyme (50 μL of 0.2 U/mL) was added to each well containing 50 μL of various concns of inhibitor (0, 100, 250, 500 or 1000 μM; to give final assay concns of 0, 16.7, 41.7, 83.3 or 166.7 μM, respectively) and pre-incubated for 1 h. After this time, the corresponding *p*-nitrophenyl glycopyranoside was added (200 μL of 250 μM) and incubated for a further 5 min. The activities are expressed as a percentage relative to the control wells (with no inhibitor present).

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