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# Synthesis and Glycosidase-inhibitory Activity of Cyclophellitol Analogues

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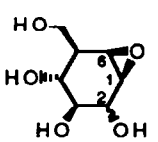
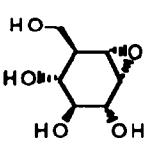
**Abstract**: The 6-deoxy-1,2-anhydro-analogues of cyclophellitol, **5** and **6**, have been synthesised from diol **11** *via* a regioselective ring opening of the cyclic sulfate **15**, an internal $S_N2$ reaction and hydrogenolysis. Compounds **5**, **6**, cyclophellitol **1** and its diastereoisomers **2-4** were assayed for inhibitory activity against six glycosidases. Oxirane **5** was shown to possess activity towards both β-mannosidase (*A. oryzae*) and β-glucosidase (almonds) but **6** showed no significant inhibitory activity.

## Introduction.

Cyclophellitol **1** was isolated from the culture filtrates of a mushroom, *Phellinus sp.*, by Umezawa *et al.* in 1989[1] and found to be a mechanism-based, active-site directed inhibitor of almond-derived β-D-glucosidase.[2] The potency ($IC_{50}$ = 0.8 μg/ml)[2] and the enzyme specificity of **1** have produced total syntheses[3] of cyclophellitol[1] as well as its diastereoisomers **2**,[4,3d] **3**,[3d] and **4**.[5,3d] Structurally, cyclophellitol **1** is a unique pseudo-pyranose with a β-epoxide moiety. The epoxide, the three hydroxy groups, and the hydroxymethyl group in **1** constitute the β-D-*gluco*-configuration. The unnatural diastereoisomers **2**, **3**, and **4** having the α-D-*gluco*-, β-D-*manno*- and α-D-*manno*-configuration should be expected to inhibit the corresponding glycosidases. Recently, syntheses of diastereoisomers **2** and **4** by the Umezawa group[4,5] have proved the above reasoning. The α-D-*gluco*-diastereoisomer **2** was found to be a specific baker's yeast α-D-glucosidase inhibitor with $IC_{50}$ of 10 μg/ml[4] and the α-D-*manno*-diastereoisomer **4** was a jack bean α-D-mannosidase inhibitor with $IC_{50}$ of 19 μg/ml.[5] As part of our ongoing programme to synthesise glycosidase inhibitors and to study their mode of action, we now disclose enantiospecific syntheses of 6-deoxy-1,2-epoxy analogues of **1**, i.e. **5** and **6**. Interestingly, these compounds lack the C-2 hydroxy group which defines them as being a glucosidase or a mannosidase inhibitor. We also report and compare the inhibitory effects of **1**, **2**, **3**, **4**, **5** and **6** on six glycosidases. Compounds **1**, **2**, **3** and **4** were prepared as described previously by us.[3d]



**1**



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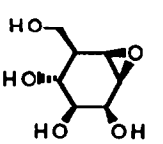

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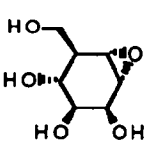
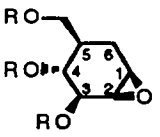
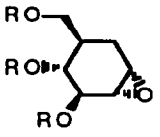


**4**



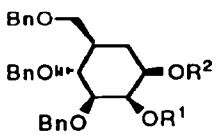
**5** : R = H
**7** : R = Bn
**9** : R = Ac



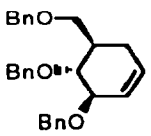
**6** : R = H
**8** : R = Bn
**10** : R = Ac

**Synthesis.**

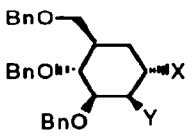
The readily available diol **11**, which was prepared from (-)-quinic acid in 8 steps with 27% overall yield,[3d] reacted with 1,1'-thiocarbonyldiimidazole in boiling toluene to give the thionocarbonate **12** which was then converted into the alkene **13** by boiling with trimethylphosphite.[6] The enantiopure alkene **13** could thus be obtained in 70% yield from the diol **11**; the alkenic protons in compound **13** resonated at δ 5.67 and 5.76 p.p.m. MCPBA epoxidation of the alkene **13** gave two diastereoisomeric epoxides **7** and **8** in 86% yield with a ratio of 1 : 4 respectively. The preparation of racemic **7** and **8** from (±)-**13** have been reported by Ogawa in the synthesis of (±)-2-amino-2-deoxy-validamine.[7]



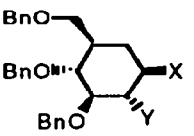
**11** : $R^1 = R^2 = H$
**12** : $R^1, R^2 = >C=S$
**15** : $R^1, R^2 = >SO_2$



**13**



**14** : X = I , Y = OH
**16** : X = OAc, Y = OH
**18** : X = OAc, Y = OMs



**17** : X =OH , Y = OAc

In order to synthesise the protected epoxides **7** and **8** in a stereoselective fashion, differentiation of the reactivity of the two hydroxy groups in the diol **11** is necessary. Fabrication of the β-epoxide **7** *via* an internal $S_N2$ reaction requires a β-oxygen nucleophile and an α-leaving group; the reversal of reactivity is applicable for the synthesis of the α-epoxide **8**. The iodo alcohol **14**, which was obtained previously from nucleophilic opening of the cyclic sulfate **15**,[3d] was treated with NaOMe/MeOH to give **7** stereospecifically in 95% yield. Stereospecific synthesis of **8** could also be realized by ring opening of the cyclic sulfate **15** by $^nBu_4NOAc$ to give the *trans*-diaxial hydroxy-acetate **16** (80%) together with its C-2 regioisomer **17** (12%). The regiochemistry is supported by their $^1H$ NMR spectra, (**16**, H-1, δ 5.12, m; **17**, H-2, δ 5.06, *J* 7.3 and 9.2 Hz). The free hydroxy group in **16** was esterified with methanesulfonyl chloride to give the mesylate **18** in 93% yield (H-2, δ 5.00, t, *J* 3.3 Hz). Deacetylation of the acetyl group in **18** using NaOMe/MeOH proceeded with concomitant epoxide formation to give the α-epoxide **8** in 3 steps from the cyclic sulfate **15** (69% overall yield). Thus, we have provided an enantiospecific route to the blocked epoxides **7** and **8** from diol **11**. This reaction sequence also confirmed the structures of products from epoxidation of the alkene **13**. Finally, hydrogenolysis of epoxides **7** and **8** separately with palladium-on-charcoal as the catalyst gave the epoxy analogues of cyclophellitol, **5** and **6**, in 80% and 92% respectively. Epoxides **5** and **6** were further characterised as their triacetates **9** and **10**.

**Effects on glycosidases.**

The inhibiting activities of the six oxiranes **1**, **2**, **3**, **4**, **5** and **6** against six glycosidases were determined and the data are listed in Table 1. These epoxides are expected to have a half-chair or sofa conformation since the X-ray crystallographic analysis of cyclophellitol **1** has already indicated that **1** has a half-chair conformation.[1] Our assay results suggest that transition-state analogue inhibitors for glycosidases should possess a conformation close to that of a flattened chair of the glycopyranosyl cation together with the correct configuration at C-2, -3, -4, and -5. The $IC_{50}$s of cyclophellitol **1** and its diastereoisomers **2** and **4** were the same as those reported by the Umezawa group within experimental error.[1,4,5] The (2*S*)-diastereoisomer **3** was determined to be a specific inhibitor of β-D-mannosidase (*A. oryzae*) and epoxide **5** was found to be an inhibitor for both β-D-glucosidase (almonds) and β-D-mannosidase (*A. oryzae*). The lack of a C-2 hydroxy group in **5** results in non-specificity towards glucosidase and mannosidase. Concerning the stereochemistry of the oxirane moiety, epoxides **1**, **3**, and **5** which possess β-epoxides, are β-glycosidase inhibitors whereas epoxides **2** and **4**, which possess α-epoxides, are α-glycosidase inhibitors. Thus, the synthesised inhibitors are glycosidase-specific with respect to the epoxide stereochemistry. Since it was found that the oxirane ring of conduritol epoxides were opened regiospecifically at C-1,[8-10] we envisaged that, by analogy, the epoxide in cyclophellitol **1** would also be opened by β-D-glucosidase regiospecifically at C-1. As illustrated in Figure 1, an acidic group AH transfers a proton to the oxirane of the inhibitor and then a carboxylate group forms an ester bond with the activated oxirane, giving a covalent-inhibitor complex. The high potency of cyclophellitol **1** among the six inhibitors may be attributed to the facile *trans*-diaxial opening of the epoxide ring which follows the Fürst and Plattner[11] rule. Compounds **3** and **5** are also expected to undergo *trans*-diaxial oxirane opening. Comparatively, the ring opening at C-1 for epoxides **2**, **4**, and **6**, is energetically unfavourable because of the diequatorial opening. This was further demonstrated by **6** which was inactive towards all six glycosidases, particularly the α-D-glucosidase and α-D-mannosidase.

Structurally, **5** and **6** have the oxirane attached between C-1,2 instead of C-1,6 as in cyclophellitol **1**. The relatively weak activity of **5** towards β-D-glucosidase and β-D-mannosidase suggests that the position of the epoxide ring is very important; this may be attributed to the oxygen atom of the epoxide ring is not at close proximity to the amino acid of the glycosidase. It is noteworthy that the oxygen atom of the oxirane in cylcophellitol has more or less the same orientation as the glucopyranosyl ring oxygen (O-5), thus allowing facile protonation (activation) of the epoxide by the active site acidic group. Another plausible reason for the relatively weak activity of **5** is the significance of the C-2 hydroxy group in the recognition of the specific enzyme. It is not surprising that **5** is an inhibitor for both β-D-glucosidase and β-D-mannosidase as it contains the minimal structural features necessary for the inhibition of both enzymes. The specificty of this group of mechanism-based inactivator, as described by Legler,[8] lies on non-covalent interactions of the glycon binding site with the hydroxy groups of the inhibitor and the catalytic features of the enzyme active site. The above factors combined with the energetically unfavorable diequatorial opening may account for the loss of inhibition effect for epoxide **6** towards the α-glycosidases.

**Figure 1** Proposed reaction of **1** and **2** with β- and α-D-glucosidases respectively.

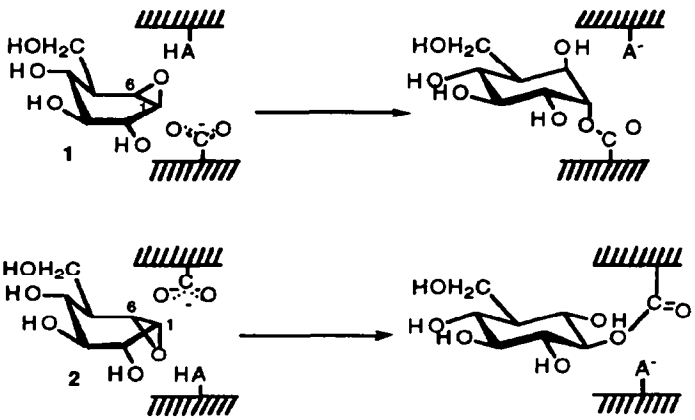


**Table 1** Inhibitory activities of compounds **1** - **6** against six enzymes.

| Enzyme | pH, buffer | % Inhibition | | | | | |
|---|---|---|---|---|---|---|---|
| | | **1** | **2** | **3** | **4** | **5** | **6** |
| α-D-galactosidase (*E. coli*) | 6.5, sodium phosphate | 2[b] | 18[c] | 6[d] | 17[e] | 12[f] | 2[f] |
| β-D-galactosidase (*A. oryzae*) | 4.5, sodium acetate | 3 | 19 | 15 | 11 | 7[f] | 0[f] |
| α-D-glucosidase (brewers yeast) | 6.0, sodium phosphate | 0 | 47 (23) | 0 | 13 | 0[e] | 0[e] |
| β-D-glucosidase (almonds) | 5.0, sodium acetate | 99 (0.3) | 15 | 3 | 7 | 83 (50) | 3 |
| α-D-mannosidase (jack beans) | 4.5, sodium acetate | 0 | 2 | 12 | 100 (9) | 0 | 0 |
| β-D-mannosidase[a] (*A. oryzae*) | 4.0, sodium acetate | 3[e] | 2[e] | 30[e] (28) | 3[e] | 12[e] (53) | 2[e] |

[a] Purification up to ammonium sulfate precipitation stage for preliminary screening. [b] Inhibition at the final concentraion of 40 μg/ml, numbers in parentheses denote the $IC_{50}$ values. [c] Inhibition at the final concentration of 80 μg/ml. [d] Inhibition at the final concentration of 200 μg/ml. [e] Inhibition at the final concentration of 100 μg/ml. [f] Inhibition at the final concentration of 160 μg/ml.

**Experimental.**

For experimental generalisation, see ref. 3d. J values are given in Hz. Optical rotations were measured in chloroform unless stated otherwise.

(1R,2R,3S,4R,5R)-3,4-*Di*-O-*benzyl*-5-*benzyloxymethyl*-1,2-O-*thiocarbonylcyclohexan*-1,2,3,4-*tetraol* **12**.—To a solution of the diol **11**[3d] (109.7 mg, 0.245 mmol) in toluene (5 ml) was added 1,1'-thiocarbonyldiimidazole (131 mg, 0.735 mmol) at room temperature. The mixture was heated under reflux for 24 h and poured into $H_2O$ (2 ml). The aqueous layer was extracted with diethyl ether (3 × 2 ml). The combined organic extracts were washed with brine (2 × 2 ml), dried ($MgSO_4$) and filtered. Concentration of the filtrate followed by flash chromatography [hexane–diethyl ether (5:4 v/v)] afforded the *title compound* **12** (103.3 mg, 86%) as a colourless oil; $R_f$ 0.31 [hexane–diethyl ether (1:1 v/v)] (Found: C, 71.0; H, 6.2. $C_{29}H_{30}O_5S$ requires C, 71.0; H, 6.2%); $[\alpha]_D^{21}$ – 27.3 (*c* 1.1); $\nu_{max}$(film)/cm$^{-1}$ 1350 (C=S); $\delta_H$ ($CDCl_3$, 250 MHz) 1.56–1.70 (1H, m), 1.90–2.16 (2H, m), 3.26 (1H, dd, *J* 5.9 and 9.1, H-7), 3.28 (1H, dd, *J* 5.1 and 9.1, H-7'), 3.52 (1H, dd, *J* 6.7 and 3.7, H-3), 3.75 (1H, t, *J* 3.5, H-4), 4.20 and 4.35 (2H, ABq, *J* 11.6, C*H*$_2$Ph), 4.33 (2H, s, C*H*$_2$Ph), 4.45 and 4.63 (2H, ABq, *J* 12.0, C*H*$_2$Ph), 4.88–4.96 (2H, m, H-1,H-2), 7.03–7.29 (15H, m, 3Ph); *m/z* (EI) 399 ($M^+ - C_7H_7$, 4%), 91 (100).

(3R,4R,5R)-3,4-*Di*-O-*benzyl*-5-*benzyloxymethyl*-1-*cyclohexen*-3,4-*diol* **13**.—To a solution of the diol **11**[3d] (1.0 g, 2.23 mmol) in toluene (50 ml) was added 1,1'-thiocarbonyldiimidazole (1.2 g, 6.70 mmol). The mixture was heated for 6 h at 100 °C and poured into water (25 ml). The aqueous layer was extracted with diethyl ether (4 × 25 ml). The combined extracts were washed with brine (2 × 15 ml), dried ($MgSO_4$) and filtered. Concentration of the filtrate provided a yellow oil **12** which was dissolved in trimethylphosphite (50 ml) and the solution was heated for 3 d at 120 °C. Removal of the solvent under reduced pressure followed by flash chromatography [hexane–diethyl ether (7:1 v/v)] provided the *title compound* **13** (0.65 g, 70%) as a colourless oil; $R_f$ 0.50 [hexane–diethyl ether (5:1 v/v)] (Found: C, 81.15; H, 7.4. $C_{28}H_{30}O_3$ requires C, 81.1; H, 7.3%); $[\alpha]_D^{21}$ + 2.4 (*c* 1.2); $\nu_{max}$(film)/cm$^{-1}$ 1650 (C=C); $\delta_H$ ($CDCl_3$, 250 MHz) 2.01–2.20 (3H, m), 3.58 (1H, dd, *J* 3.1 and 9.0, H-7), 3.64–3.74 (2H, m, H-4,H-7'), 4.18 (1H, dd, *J* 2.1 and 5.0, H-3), 4.18 (2H, br s, C*H*$_2$Ph), 4.65 and 4.70 (2H, ABq, *J* 11.8, C*H*$_2$Ph), 4.88 and 5.62 (2H, ABq, *J* 11.0, C*H*$_2$Ph), 5.67 (1H, dd, *J* 1.2 and 10.2) and 5.76 (1H, br d, *J* 10.2), H-1 and H-2, 7.22–7.37 (15H, m, 3Ph); *m/z* (EI) 323 ($M^+ - C_7H_7$, 1.4%), 217 ($M^+ - C_7H_7 - C_7H_6O$, 5), 91 (100).

(1S,2R,3S,4R,5R)-1-O-*Acetyl*-3,4-*di*-O-*benzyl*-5-*benzyloxymethylcyclohexan*-1,2,3,4-*tetraol* **16** and (1R,2S,3S,4R,5R)-2-O-*Acetyl*-3,4-*di*-O-*benzyl*-5-*benzyloxymethylcyclohexan*-1,2,3,4-*tetraol* **17**.—A solution of the cyclic sulfate **15**[3d] (279.4 mg, 0.548 mmol), $^nBu_4NOAc$ (198 mg, 0.658 mmol) in THF (15 ml) was heated under reflux for 1 h under nitrogen. Conc. $H_2SO_4$ (1 drop) and $H_2O$ (1 drop) were added and the solution stirred for 30 min at 60 °C. The mixture was filtered through a pad of silica gel topped with Celite and washed with $CH_2Cl_2$. Concentration of the filtrate followed by flash chromatography [hexane–diethyl ether (4:3 v/v)] afforded the *title compound* **16** (215 mg, 80%) first and then **17** (32 mg, 12%), both as colourless oils. Compound **16**: $R_f$ 0.35 [hexane–diethyl ether (1:1 v/v)] (Found: C, 73.3; H, 7.1. $C_{30}H_{34}O_6$ requires C, 73.45; H, 7.0%); $[\alpha]_D^{19}$ + 33.0 (*c* 1.9); $\nu_{max}$(film)/cm$^{-1}$ 1737 (C=O), 3450 (OH); $\delta_H$ ($CDCl_3$, 270 MHz) 1.66-2.08 (3H, m), 2.03(3H, s, *Ac*), 2.57 (1H, d, *J* 2.3, O*H*), 3.50 (1H, dd, *J* 2.4 and 8.9, H-7), 3.65 (1H,

dd, *J* 4.6 and 8.9, H-7'), 3.74 (1H, dd, *J* 3.0 and 8.6, H-3), 3.80 (1H, dd, *J* 8.6 and 8.9, H-4), 4.03 (1H, m, H-2), 4.48 (2H, s, C*H*$_2$Ph), 4.55 and 4.85 (2H, ABq, *J* 10.9, C*H*$_2$Ph), 4.66 and 4.73 (2H, ABq, *J* 11.6, C*H*$_2$Ph), 5.12 (1H, m, H-1), 7.26-7.36 (15H, m, 3Ph); *m/z* (CI-CH$_4$) 491 ($M^+$ + 1, 2%), 399 ($M^+$ − $C_7H_7$, 3), 91 (100); Compound **17**: $R_f$ 0.12 [hexane–diethyl ether (1:1 v/v)] (Found: C, 73.2; H, 7.2. $C_{30}H_{34}O_6$ requires C, 73.45; H, 7.0%); $[\alpha]_D^{19}$ + 28.0 (*c* 0.8); $\nu_{max}$(film)/cm$^{-1}$ 1739 (OH), 3450 (OH); $\delta_H$ ($C_6D_6$, 270 MHz) 1.30-1.56 (2H, m), 1.70 (3H, s, O*Ac*), 1.83 (1H, br s, O*H*), 1.91-1.95 (1H, m), 3.27 (1H, dd, *J* 2.6 and 8.9, H-7), 3.33-3.43 (4H, m, H-1,H-3,H-4,H-7'), 4.20 (2H, s, C*H*$_2$Ph), 4.44 and 4.81 (2H, ABq, *J* 11.6, C*H*$_2$Ph), 4.63 and 4.77 (2H, ABq, *J* 11.9, C*H*$_2$Ph), 5.06 (1H, dd, *J* 7.3 and 9.2, H-2), 7.04-7.31 (15H, m, 3Ph); *m/z* (CI-CH$_4$) 491 ($M^+$ + 1, 13%), 399 ($M^+$ − $C_7H_7$, 75), 91 (100).

(1S,2R,3S,4R,5R)-1-O-*Acetyl*-3,4-*di*-O-*benzyl*-5-*benzyloxymethyl*-2-O-*methanesulfonylcyclohexan*-1,2,3,4-*tetraol* **18**.— To a solution of the alcohol **16** (136.5 mg, 0.279 mmol) in pyridine (5 ml) was added methanesulfonyl chloride (26 μl, 0.335 mmol) at room temperature. The solution was stirred at room temperature for 3 h and then diluted with $CHCl_3$ (15 ml) and saturated aqueous $NH_4Cl$ (5 ml). The aqueous phase was extracted with $CHCl_3$ (3 × 5 ml) and the combined extracts were washed with brine (2 × 8 ml), dried ($MgSO_4$), and filtered. Concentration of the filtrate followed by flash chromatography [hexane–diethyl ether (4:3 v/v)] afforded the *title compound* **18** (148 mg, 93%) as a pale yellow syrup; $R_f$ 0.28 [hexane–diethyl ether (1:1 v/v)] (Found: C, 65.3; H, 6.4. $C_{31}H_{36}SO_8$ requires C, 65.5; H, 6.4%); $[\alpha]_D^{19}$ +18.0 (*c* 1.8); $\nu_{max}$(film)/cm$^{-1}$ 1744 (C=O); $\delta_H$ ($CDCl_3$, 270 MHz) 1.88-2.05 (3H, m), 2.01 (3H, s, *Ac*), 3.02 (3H, s, *Ms*), 3.47 (1H, dd, *J* 2.6 and 8.9, H-7), 3.63 (1H, dd, *J* 4.6 and 8.9, H-7'), 3.73 (1H, dd, *J* 8.6 and 8.9, H-4), 3.82 (1H, dd, *J* 2.6 and 8.6, H-3), 4.46 (2H, s, C*H*$_2$Ph), 4.52 and 4.82 (2H, ABq, *J* 10.9, C*H*$_2$Ph), 4.68 and 4.74 (2H, ABq, *J* 10.8, C*H*$_2$Ph), 5.00 (1H, t, *J* 3.3, H-2), 5.18 (1H, m, H-1), 7.22-7.34 (15H, m, 3Ph); *m/z* (CI-CH$_4$) 477 ($M^+$ − $C_7H_7$, 1), 371 ($M^+$ − $C_7H_7$ − $C_7H_7O$, 1),91 (100).

(1R,2R,3S,4R,5R)- and (1S,2S,3S,4R,5R)-1,2-*Anhydro*-3,4-*di*-O-*benzyl*-5-*benzyloxymethylcyclohexan*-1,2,3,4-*tetraol* **7** and **8**.—To a solution of the alkene **13** (117 mg, 0.282 mmol) in $CH_2Cl_2$ (10 ml) was added MCPBA (102 mg, 0.56 mmol). The mixture was stirred at room temperature for 24 h and poured into aqueous NaOH (1 M, 10 ml). The aqueous phase was extracted with $CH_2Cl_2$ (2 × 10 ml). The combined extracts were washed with aqueous $NH_4Cl$ (2 × 4 ml), brine (2 × 4 ml), dried ($MgSO_4$) and filtered. Concentration of the filtrate followed by flash chromatography [hexane–diethyl ether (4:1 v/v)] gave **8** (82.2 mg, 68%) and then **7** (20.5 mg, 17%), both as white solids. Compound **7**: m.p. 60—62 °C; $R_f$ 0.56 [hexane–ethyl acetate (3:1 v/v)]; $[\alpha]_D^{21}$ + 29.3 (*c* 1.2); $\nu_{max}$(film)/cm$^{-1}$ 3000–3100 (aromatic C–H); $\delta_H$ ($CDCl_3$, 250 MHz) 1.81 (1H, m, H-5), 2.04-2.16 (2H, m, H-6,H-6'), 3.25 (1H, t, *J* 4.0, H-1), 3.33 (1H, dd, *J* 1.9 and 4.0, H-2), 3.48 (2H, m, H-7,H-7'), 3.65 (1H, dd, *J* 8.1 and 10.8, H-4), 3.83 (1H, dd, *J* 1.9 and 8.1, H-3), 4.45 (2H, s, C*H*$_2$Ph), 4.52 and 4.84 (2H, ABq, *J* 10.8, C*H*$_2$Ph), 4.82 (2H, s, C*H*$_2$Ph), 7.22–7.43 (15H, m, 3Ph); *m/z* (EI) 339 ($M^+$ − $C_7H_7$, 6%), 91 (100). Compound **8**: m.p. 32—34 °C; $R_f$ 0.36 [hexane–diethyl ether (3:1 v/v)] (Found: C, 77.8; H, 7.1. $C_{28}H_{30}O_4$ requires C, 78.1; H, 7.0%); $[\alpha]_D^{21}$ + 18.2 (*c* 1.1); $\nu_{max}$(film)/cm$^{-1}$ 3000–3100 (aromatic C–H); $\delta_H$ ($CDCl_3$, 250 MHz) 1.80 (1H, m), 2.07 (1H, dt, *J* 11.8 and 1.5), 2.23 (1H, ddd, *J* 15, 4.4 and 1.8), 3.15 (1H, d, *J* 3.7, H-2), 3.23 (1H, m, H-1), 3.40 (1H, dd, *J* 2.6 and 9.0, H-7), 3.44 (1H, dd, *J* 8.0 and 11.3, H-4), 3.72 (1H, dd, *J* 4.5 and 9.0, H-7'), 3.78 (1H, d, *J* 8.0, H-3), 4.45 (2H, br s, C*H*$_2$Ph), 4.69 and 4.80 (2H, ABq, *J* 11.4, C*H*$_2$Ph), 4.56 and 4.84 (2H, ABq, *J* 11.0, C*H*$_2$Ph), 7.20–7.36 (15H, m, 3Ph); *m/z* (EI) 339 ($M^+$ − $C_7H_7$, 31%), 91 (100).

Formation of epoxide **7** from the iodo alcohol **14**.—To a solution of the iodo alcohol **14**[3d] (116 mg, 0.208 mmol) in anhydrous methanol (5 ml) was added sodium methoxide (12.4 mg, 0.229 mmol) at room temperature and the suspension was stirred for 5 min. The mixture was then filtered through a pad of silica gel topped with Celite and washed with $CH_2Cl_2$ (10 ml). Concentration of the filtrate followed by flash chromatography [hexane–diethyl ether (3:1 v/v)] afforded the oxirane **7** ( 85.1 mg, 95%) as a white solid.

Formation of epoxide from **8** the acetoxy-mesylate **18**.—To a solution of the mesylate **18** (60.9 mg, 0.107 mmol) in anhydrous methanol (5 ml) was added sodium methoxide (14 mg, 0.268 mmol) at room temperature and the suspension was stirred for 5 h. The mixture was then filtered through a pad of silica gel and washed with EtOAc (10 ml). Concentration of the filtrate followed by flash chromatography [hexane-diethyl ether (4:1 v/v)] gave the oxirane **8** (37.2 mg, 81%) as a white solid.

(1R,2R,3S,4R,5R)-1,2-*Anhydro*-5-*hydroxymethylcyclohexan*-1,2,3,4-*tetraol* **5**.—To a suspension of palladium-on-charcoal (25 mg, 5% w/w) in absolute EtOH (1.5 ml) under $H_2$ at atmospheric pressure was added a solution of the compound **7** (208 mg, 0.484 mmol) in absolute EtOH (10 ml). The suspension was stirred for 6 h at room temperature and filtered through a pad of Celite. The residue was washed with methanol (15 ml). Concentration of the filtrate followed by flash chromatography [chloroform–methanol (5:1 v/v)] gave the *title compound* **5** (62.0 mg, 80%) as colourless needles, m.p. 125.5—127 °C (MeOH); $R_f$ 0.45 [chloroform–methanol (4:1 v/v)] (Found: C, 52.2; H, 7.6. $C_7H_{12}O_4$ requires C, 52.5; H, 7.55%); $[\alpha]_D^{26}$ – 2 (*c* 0.5, $H_2O$); $\nu_{max}$(KBr)/cm$^{-1}$ 3400 (OH); $\delta_H$ ($D_2O$, DOH=4.80, 250 MHz) 1.70–1.82 (2H, m), 2.12–2.22 (1H, m), 3.40–3.51 (3H, m, H-1,H-2,H-4), 3.56 (1H, dd, *J* 11.3 and 5.5, H-7), 3.64 (1H, dd, *J*, 11.3 and 3.4, H-7'), 3.98 (1H, dd, *J* 1.4 and 8.6, H-3); $\delta_C$ ($D_2O$-dioxane as internal reference at 67.4 ppm, 62.9 MHz) 26.6, 41.2, 55.6, 58.9, 62.9, 71.5, 74.1; *m/z* (CI) 161 ($MH^+$, 30%).

(1S,2S,3S,4R,5R)-1,2-*Anhydro*-5-*hydroxymethylcyclohexan*-1,2,3,4-*tetraol* **6**.—Epoxide **8** (313.8 mg, 0.730 mmol) was debenzylated as described in the preparation of **5**. Purification by flash chromatography [chloroform–methanol (5.5:1 v/v)] gave the *title compound* **6** (107.5 mg, 92%) as a colourless oil which solidified at – 20 °C; $R_f$ 0.34 [chloroform–methanol (5:1 v/v)] (Found: C, 52.3; H, 7.5. $C_7H_{12}O_4$ requires C, 52.5; H, 7.55%); $[\alpha]_D^{26}$ + 37.1 (*c* 0.6, $H_2O$); $\nu_{max}$(KBr)/cm$^{-1}$ 3400 (OH); $\delta_H$ ($D_2O$, DOH=4.80, 250 MHz) 1.53 (1H, m), 1.81 (1H, ddd *J* 13.7, 12.2 and 1.6), 2.26 (1H, dt, *J* 15 and 2.1), 3.21 (1H, d, *J* 3.93, H-2), 3.25 (1H, dd, *J* 11.3 and 8.55, H-7), 3.44 (1H, br d, *J* 1.8, H-1), 3.65–3.71 (3H, m, H-3,H-4,H-7'); $\delta_C$ ($D_2O$-dioxane as internal reference at 67.4 ppm, 62.9 MHz)) 27.3, 35.3, 54.9, 57.6, 62.8, 72.8, 74.1; *m/z* (CI) 161 ($MH^+$, 92%).

(1R,2R,3S,4R,5R)-5-*Acetoxymethyl*-3,4-*di*-O-*acetyl*-1,2-*anhydro*-cyclohexan-1,2,3,4-*tetraol* **9**.—A solution of the epoxide **5** (14.1 mg, 0.088 mmol), acetic anhydride (0.3 ml), and a crystal of DMAP in pyridine (2 ml) was stirred at room temperature for 15 h. The solution was diluted with $CH_2Cl_2$ (5 ml) and an aqueous saturated solution of $NH_4Cl$ (2 ml) was added. The aqueous phase was extracted with $CH_2Cl_2$ (4 × 5 ml) and the combined organic extracts were washed with brine (2 × 5 ml), dried ($MgSO_4$), and filtered. Concentration of the filtrate follwed by flash chromatography [hexane–diethyl ether (3:4 v/v)] gave the *triacetate* **9** (19.0 mg, 75%) as a colourless oil; $R_f$ 0.25 [hexane–diethyl ether (2:3 v/v)] (Found C, 54.3; H, 6.4. $C_{13}H_{18}O_7$ requires

C, 54.5; H, 6.3%); $[\alpha]_D^{25}$ – 9.6 (*c* 0.7); $\nu_{max}$(film)/cm$^{-1}$ 1743 (C=O); $\delta_H$ ($CDCl_3$, 250 MHz) 1.89–2.16 (3H, m), 1.95 (3H, s, *Ac*), 1.97 (3H, s, *Ac*), 2.04 (3H, s, *Ac*), 3.27 (1H, dd, *J* 4.0 and 4.3, H-1) and 3.35 (1H, dd, *J*, 1.8 and 4.0, H-2), 3.79 (1H, dd, *J* 3.1 and 11.3, H-7), 3.99 (1H, dd, *J* 4.8 and 11.3, H-7'), 5.07 (1H, dd, *J* 8.8 and 10.2, H-4), 5.17 (1H, dd, *J* 1.8 and 8.8, H-3); $\delta_C$ ($CDCl_3$) 20.6 (× 2), 20.7, 26.3, 37.5, 52.6, 54.5, 63.2, 69.6, 73.4, 169.7, 170.6 (× 2); *m/z* (EI) 244 ($M^+ - C_2H_2O$, 23%), 43 (100).

(1S,2S,3S,4R,5R)-5-*Acetoxymethyl*-3,4-*di*-O-*acetyl*-1,2-*anhydro*-cyclohexan-1,2,3,4-*tetraol* **10**.—Epoxide **6** (12.5 mg, 0.078 mmol) was acetylated as described in the preparation of **9**. Purification by flash chromatography [hexane–diethyl ether (2:3 v/v)] gave the *triacetate* **10** (18.2 mg, 81%) as a white solid, m.p. 59—61 °C; $R_f$ 0.35 [hexane–diethyl ether (2:3 v/v)] (Found C, 54.4; H, 6.4. $C_{13}H_{18}O_7$ requires C, 54.5; H, 6.3%); $[\alpha]_D^{26}$ + 41.7 (*c* 0.5); $\nu_{max}$(film)/cm$^{-1}$ 1742 (C=O); $\delta_H$ ($CDCl_3$, 250 MHz) 1.98–2.11 (2H, m), 2.03 (3H, s, *Ac*), 2.04 (3H, s, *Ac*), 2.08 (3H, s, *Ac*), 2.32 (1H, m), 3.08 (1H, dd, *J* 0.5 and 3.6, H-2), 3.32 (1H, m, H-1), 3.84 (1H, dd, *J* 2.5 and 11.3, H-7), 4.20 (1H, dd, *J* 4.0 and 11.3, H-7'), 4.91–5.03 (2H, m, H-3,H-4); $\delta_C$ ($CDCl_3$) 20.45, 20.5, 20.6, 27.2, 31.1, 52.2, 54.0, 62.8, 71.0, 71.9, 169.8, 170.0, 170.4; *m/z* (EI) 243 ($M^+ - C_2H_3O$, 55%), 124 (100).

**Studies of Glycosidase Inhibition.**

*Instrumentation, inhibitors, Substrates.* Spectrophotometric measurements were made on a Spectronic 3000 or 601 spectrophotometer. Cyclophellitol **1** and its diastereoisomers **2-4** were prepared as reported by us.[3d] *p*-Nitrophenyl-α-D-galactopyranoside (PNP-αGal), *p*-nitrophenyl-β-D-galactopyranoside (PNP-βGal), *p*-nitrophenyl-α-D-glucopyranoside (PNP-αGlu), *p*-nitrophenyl-β-D-glucopyranoside (PNP-βGlu), *p*-nitrophenyl-α-D-mannopyranoside (PNP-αMan), *p*-nitrophenyl-β-D-mannopyranoside (PNP-βMan) were purchased from Sigma Chemical Co., St. Louis, U. S. A. All reagents were of the highest grade available and were used as purchased. pH values were determined at 25 °C.

*Enzymes.* α-D-Galactopyranosidase (EC 3.2.1.22) from *E. coli*, β-D-galactopyranosidase (EC 3.2.1.23) from *A. oryzae*, α-D-glucosidase (EC 3.2.1.20) from brewers yeast, β-D-glucosidase (EC 3.2.1.21) from almonds and α-D-mannosidase (EC 3.2.1.24) from jack beans were obtained from Sigma Chemical Co. and used without further purification.

*Partial purification of β-D-mannosidase from A. oryzae*. The spore suspension of *A. oryzae* (0.5 ml) in each culture flask were inoculated with 125 ml potato dextrose broth (24 g/L) supplemented with glucose (5 g/L). Cultivation was carried out for 3.5 d at 28 °C under shaking. 49 g of wet mycelia were obtained from 2 L of medium solution. The mycelia were homogenized with sand in 37 ml cold sodium acetate buffer at pH 4.0. The mixture was centrifuged at 27,500*g* for 20 min at 4 °C to obtain the supernatant as the crude preparation. 49 ml of supernanant was fractionated by ammonium sulfate precipitation (70-95% saturation). The protein pellet was obtained after centrifugation at 20,200*g* and redissolved in 2.0 ml 100 mM sodium acetate buffer, the dialysed extract were chromatographed on 43.4 ml CM-Sepharose CL-6B (Pharmacia) with UV/VIS detector (280 nm, ISCO UA-6). It was equilibrated first with 10 mM sodium acetate buffer (pH 4.0) at 2.0 ml/min. After loading the sample, 80 ml of 10 mM sodium acetate buffer was used to elute the unbound proteins. The bound proteins were then eluted with stepwise gradient of 0.1 to 0.5 M sodium chloride in the same buffer. The fractions containing β-D-mannosidase activity were collected mainly at 0.2 M sodium chloride solution. All the

active fractions were dialysed and β-D-mannosidase was collected after lyophilization with 55 fold increase in purity (Table 2).

**Table 2** Isolation of β-D-mannosidase from *A. oryzae*

| Fractions | Protein content (mg) | Total activity (μmole/min) | Specific activity (μmol/mg/min) | Purification (fold) |
|---|---|---|---|---|
| Crude enzyme | 120.6 | 0.7987 | $6.62 \times 10^{-3}$ | 1 |
| Ammonium sulfate precipitation | 2.456 | 0.3012 | $1.23 \times 10^{-1}$ | 19 |
| CM-Sepharose chromatography | 0.4896 | 0.1786 | $3.65 \times 10^{-1}$ | 55 |

*Enzyme Assays.* The enzyme activities for the commercial available enzymes were determined according to the method described by Saul[12] with slight modification. The reaction mixture contained 20 mM of the appropriate buffer, 5 mM *p*-nitrophenyl glycoside, the inhibitor and glycosidase in a final volume of 0.5 ml. Enough enzyme was used to give an absorbance of 1.0 within 10 min. Control was included by replacing the inhibitor in the reaction mixture with water. After incubation at 30 °C for a definite period of time, 2.5 ml glycine-NaOH buffer (0.4 M, pH 10.) was added to quench the reaction and the liberated *p*-nitrophenol was measured at 410 nm ($\varepsilon_{410}$ 17,000 L $mol^{-1}$ $cm^{-1}$).

Enzyme assay for **3** against partially purified β-D-mannosidase (*A. oryzae*) was described as follows: the inhibitor was preincubated with β-D-mannosidase in sodium acetate buffer (pH 4.0) for 15 min at 30 °C. The reaction was started by the addtition of 0.2 ml PNP-βMan. The reaction mixture contained a final concentration of 20 mM sodium acetate buffer, 5 mM substrate, β-D-mannosidase (15.28 mU/ml, specific activity 0.172 U/mg), and epoxide **3** in 0.5 ml volume. After incubation for 20 min at 30 °C, 2.5 ml glycine-NaOH buffer (0.4 M, pH 10) was added and the liberated p-nitrophenol was measured at 410 nm ($\varepsilon_{410}$ 17,700 L $mol^{-1}$ $cm^{-1}$).

Enzyme assay for **5** against partially purified β-D-mannosidase (*A. oryzae*) (8.703 mU/ml, specific activity 0.172 U/mg) was the same as for **3**. The preincubation time was 15 min and the reaction time was 30 min.

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