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SYNTHESIS AND EVALUATION OF GLUCOCEREBROSIDASE INHIBITORY ACTIVITY OF ANHYDRO DEOXYINOSITOLS FROM (+)-*EPI*- AND (–)-*VIBO*-QUERCITOLS

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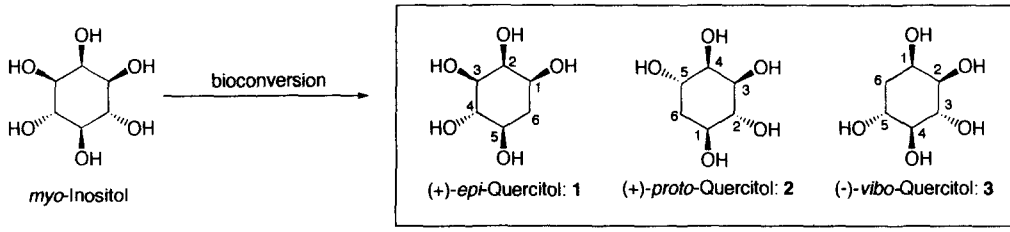
Abstract: Twelve 1,2- and 2,3-anhydro-1,2,3,4,5-cyclohexanepentols were synthesized from (+)-*epi*- and (–)-*vibo*-quercitols, readily available by bioconversion of *myo*-inositol, and assayed for inhibitory activity against glucocerebrosidase (mouse liver). Among them 1L-1,2-anhydro-1,2,4/3,5-cyclohexanepentol, the 3-deoxy derivative of the irreversible inhibitor conduritol B epoxide (CBE), has been demonstrated to be a highly potent and specific inhibitor, almost comparable to the parent compound.

Recently, three deoxyinositols, (+)-*epi*- (**1**), (+)-*proto*- (**2**), and (–)-*vibo*-quercitols (**3**), have been shown[1] to be produced by bioconversion of *myo*-inositol with bacteria isolated from soil, and each is obtainable in a crystalline form by chromatography. We have become interested in the application of these optically pure cyclitols as potential sources for development of biologically active compounds. The first paper of this series reports a synthesis[2] of twelve new optically active 1,2- and 2,3-anhydro derivatives of quercitols, including three pairs of optical antipodes, starting from readily available **1** and **3**, and their inhibitory activity against glucocerebrosidase (mouse liver) and β-glucosidase.

Cyclophellitol[3] (**4**) and conduritol B epoxide (CBE)[4,5] (**5**) have been known as potent and specific inhibitors of glucocerebrosidase. Their inhibitory activity has been explained[6] on the basis of their structural



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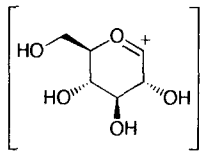
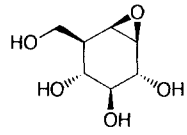
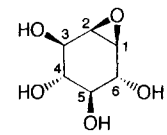
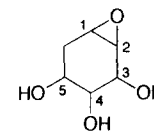
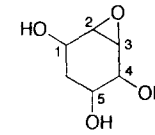

D-Glucopyranosyl cation

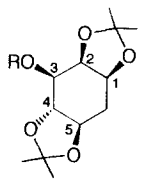

Cyclophellitol: **4**

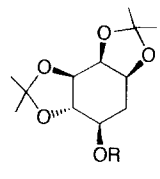

Conduritol B epoxide: **5**

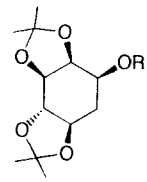

1,2-Anhydro-quercitols

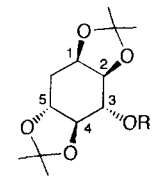

2,3-Anhydro-quercitols

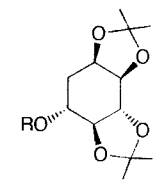
resemblance to D-glucopyranosyl cation probably formed during hydrolysis of the glucosides and covalent bonding to the active site of the enzyme through a nucleophilic cleavage of the epoxide ring by the carboxylate function of aspartate or glutamate residue. Therefore, determination of inhibitory activity of the corresponding dehydroxymethyl or 3-deoxy derivative is thought to be very important for further understanding of the structure activity relationship of the inhibitors of this kind.


6a,b

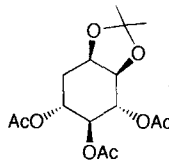

7a,b

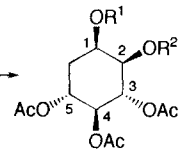

8a,b


9a,b


10a,b

a: R = H
b: R = Ts

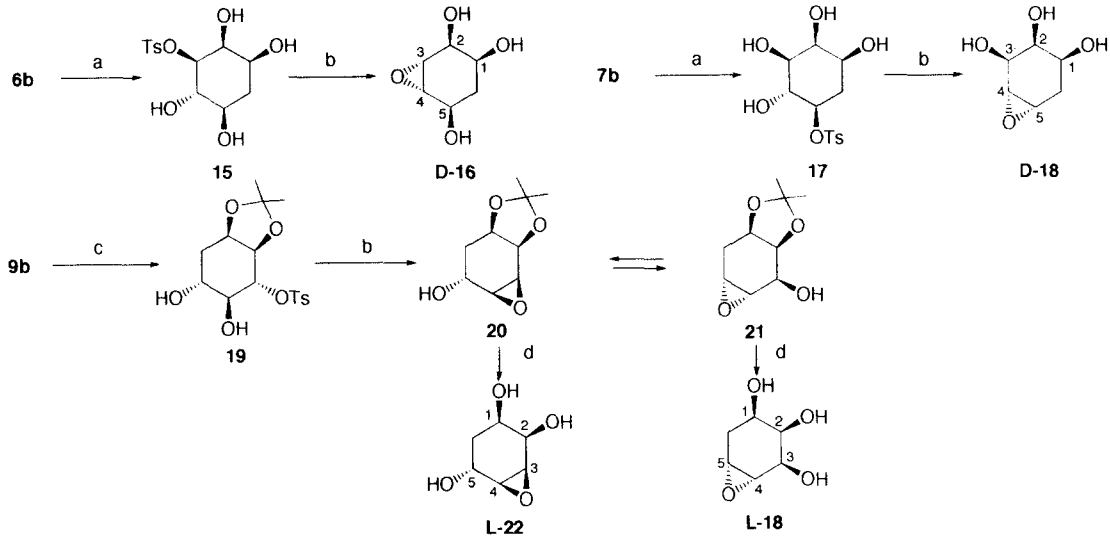

11



	R^1	R^2
12	H	H
13	H	Ts
14	Ts	Ts

O-Isopropylidenation of (+)-*epi*-quercitol[7] (**1**), 1D-1,2,3,5/4-cyclohexanepentol[8,9] was conducted with an excess of 2,2-dimethoxypropane (5 molar equiv) in DMF in the presence of TsOH (0.1 molar equiv) at room temperature, a progress of the reaction being monitored by TLC. When the reaction reached at the equilibrium after ~5 h, the mixture was neutralized with sodium carbonate and the products were isolated by a silica gel column to give an inseparable mixture (9%) of two mono-*O*-isopropylidene derivatives, and three di-*O*-isopropylidene derivatives **6a** (26%), **7a** (24%), and **8a** (31%). Compounds **6a**–**8a** were treated with *p*-toluenesulfonyl chloride in pyridine to give the corresponding tosylates **6b**–**8b**. Similar treatment of (–)-*vibo*-quercitol (**3**), 1L-1,2,4/3,5-cyclohexanepentol, with 2,2-dimethoxypropane gave an inseparable mixture (86%) of two di-*O*-isopropylidene derivatives **9a** and **10a**. When the mixture was tosylated, the resulting tosylates were easily separable by a silica gel column to give the tosylates **9b** (56%) and **10b** (43%). The mixture of **9a** and **10a** was partially de-*O*-isopropylidenated by treatment with a trace of *p*-toluenesulfonic acid in methanol, and then acetylated with acetic anhydride in pyridine, giving the 1,2-*O*-isopropylidene triacetate (**11**) (80%).

De-*O*-isopropylidenation of **11** with 80% aq AcOH gave the diol **12**, selective tosylation (1.5 molar equiv TsCl/pyr at room temperature) of which gave the 2-tosylate **13** (93%), along with the 1,2-ditosylate **14** (7%). The structures of all new compounds[10] were fully assigned on the basis of their ^{1}H NMR spectra.

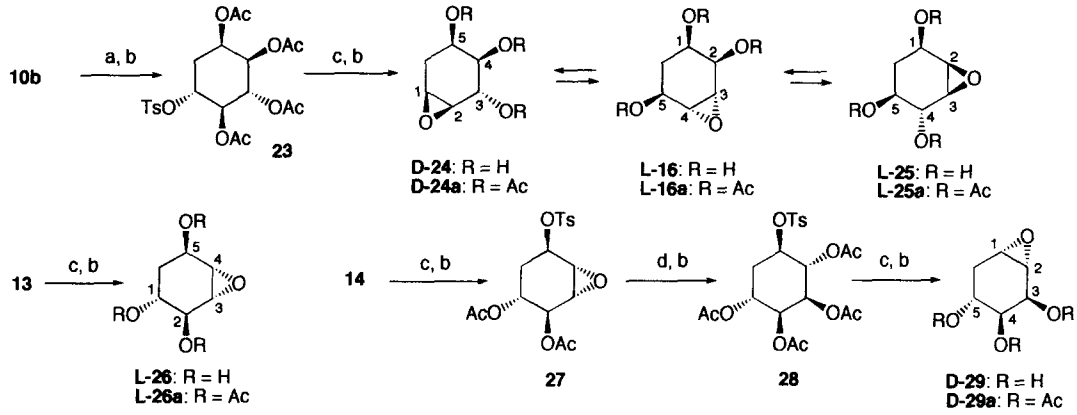


Scheme 1. *Reagents and conditions* (a) 80% aq AcOH, 1.5 h, 50 °C; (b) NaOMe (1.5 molar equiv)/MeOH, rt, 3 h; after neutralization with 1 M HCl, elution from a silica gel column with chloroform–methanol (5~10:1, v/v) as an eluent; (c) a trace of TsOH, MeOH, 0 °C; (d) 50% aq AcOH, 50 °C.

Removal of two isopropylidene groups of **6b** with 80% aq AcOH gave the tosylate **15**, which without isolation was treated with methanolic sodium methoxide (1.5 molar equiv) in methanol at room temperature. The resulting anhydride **D-16** was isolated by a silica gel column in 59% yield. Similarly **17** derived from **7b** was converted into **D-18** (65%). The structures of **D-16** and **D-18** were assigned 1D-3,4-anhydro-1,2,5/3,4- and 1D-4,5-anhydro-1,2,3/4,5-cyclohexanetriols, respectively, on the basis of the reaction sequence and their ^{1}H NMR spectra.

The trans 4,5-*O*-isopropylidene group of **9b** was selectively removed to give, after acetylation, **19** (77%), a similar treatment of which with methanolic sodium methoxide overnight at room temperature gave **20** (69%) and **21** (11%). They were shown to be interconvertible each other through epoxide group migration under the basic conditions. The initially formed 3,4-anhydride **20** is likely to be attacked by *trans*-situated 5-hydroxyl group to give the 4,5-anhydride **21**. The product-ratio of these anhydrides at the equilibrium would reflect their relative stability, i.e. thermodynamical features under the basic conditions. Treatment of **20** and **21** with 50% aq AcOH gave **L-22** (82%) and **L-18** (68%), respectively (Scheme 1).

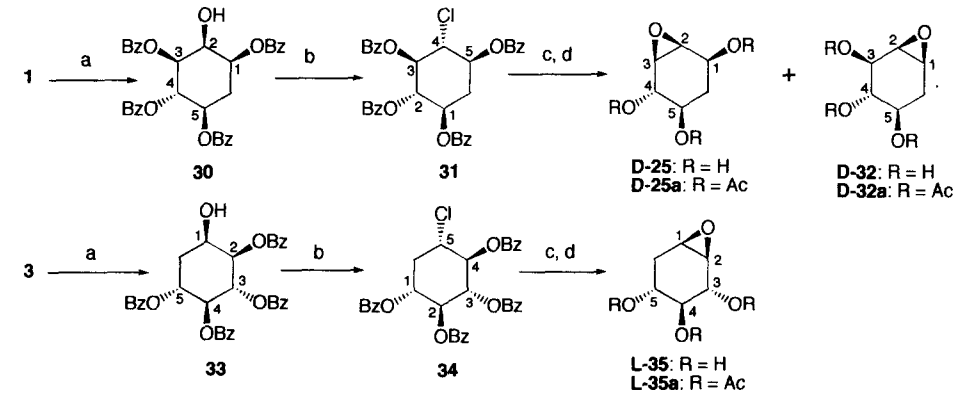
The 5-O-tosylate **23** obtained from **10b** was similarly treated with methanolic sodium methoxide, and the products were acetylated to give the triacetyl derivative **D-24a** (34%) and ca. 3:1 mixture (28%) of the



Scheme 2. *Reagents and conditions:* (a) 80% aq AcOH, 80 °C; (b) Ac_2O, pyridine; (c) NaOMe (1.5 molar equiv)/MeOH, rt, overnight; (d) 10% H_2SO_4/aq acetone, rt. The free epoxides were first isolated and characterized as the triacetates, which were then converted into the free epoxides by de-*O*-acetylation with NaOMe (a catalytic amount)/MeOH, rt, and, after neutralization with 1 M HCl, subsequent elution from a silica gel column with chloroform–methanol (5~10:1, v/v) as an eluent.

triacetates **L-16a** and **L-25a**. The Zemplén de-*O*-acetylation of **D-24a** gave **D-24** (74%). De-*O*-acetylation of the mixture afforded, after chromatography, **D-16** (14%) and 1L-2,3-anhydro-1,2,3,5/4-cyclohexanepentols (**L-25**, 7%) (Scheme 2). The structures of three anhydrides formed from **23** were deduced by considering the epoxide group-migration, and assigned on the basis of the ^1H NMR spectra.

Similar epoxidation of **13** followed by acetylation gave 1L-3,4-anhydro-1,3,4/2,5-cyclohexanepentol triacetate **L-26a** (85%), which afforded **L-26** (~100%). Treatment of **14** with methanolic sodium methoxide under kinetic control gave, after acetylation, **27**, which was hydrolyzed with 10% sulfuric acid in aqueous acetone followed by acetylation, giving a sole tetraacetate **28**. Similar epoxidation of **28** gave 1D-1,2-anhydro-1,2,5/3,4-cyclohexanepentrol triacetate **D-29a** (72%), which was converted into **D-29** (65%) (Scheme 2).



Scheme 3. *Reagents and conditions:* (a) BzCl (7 molar equiv), pyridine, rt; (b) SO_2Cl_2 (1.1 molar equiv), pyridine, 0 °C→rt, 2 h; (c) NaOMe (1.5 molar equiv)/MeOH, rt; (d) Ac_2O, pyridine, rt; (e) NaOMe (a catalytic amount)/MeOH

Alternatively, selective benzoylation[11] of **1** and **3** afforded directly **30** (68%) and **33** (60%), respectively. The axially-oriented hydroxyl groups were hardly esterified. They were treated with a slight excess of SO_2Cl_2 in pyridine at room temperature to give the respective chlorides **31** (93%) and **34** (95%) with inversion of the configurations. Similar treatment of **31** with methanolic sodium methoxide followed by acetylation afforded two epoxides, **D-32a** (43%) and **D-25a** (17%). On the other hand, **34** afforded a sole anhydride **L-35a** in 45% yield under kinetic control. The Zemplén de-*O*-acetylation of the triacetates gave the free anhydrides **D-25**, **D-32**, and **L-35** in quantitative yields (Scheme 3).

Table 1. Specific rotations and partial ^{1}H NMR data (270 or 300 MHz, CD_3OD) of 1,2- and 2,3-anhydro-1,2,3,4,5-cyclohexanepentols

Compd	$[\alpha]^{21}_D$ (°) (in MeOH)	^{1}H NMR signals (δ) of epoxide protons					Coupling constant (Hz)
		1-H	2-H	3-H	4-H	5-H	
D-16	–22[a]			3.11	3.20		$J_{3,4}$ 3.34, $J_{4,5}$ ~0
L-16	+33						
D-18	+26				3.01	3.26	$J_{3,4}$ ~0, $J_{4,5}$ 3.67, $J_{5,6e}$ 1.47
L-18	–26						
L-22	+89			3.31	3.17		$J_{2,3}$ 4.40, $J_{3,4}$ 3.66, $J_{4,5}$ ~0
D-24	–49	3.25	3.11				$J_{1,2}$ 3.66, $J_{2,3}$ ~0
D-25	–53		3.22	3.05			$J_{2,3}$ 3.66, $J_{3,4}$ ~0
L-25	+49						
L-26	+30			3.05–3.10			$J_{2,3}$ ~0, $J_{3,4}$ 4.03, $J_{4,5}$ ~0
D-29	–125	3.13–3.19					$J_{1,2}$ 3.66, $J_{1,6e}$ 4.40, $J_{2,3}$ 2.57
D-32	+18	3.16	3.24				$J_{1,2}$ 3.67, $J_{2,3}$ 1.83
L-35	–11	3.25	2.98				$J_{1,2}$ 3.66, $J_{1,6a}$ 1.47, $J_{1,6e}$ 2.20, $J_{2,3}$ ~0

[a] Compound **L-16** seemed to contain a trace of an interconvertible **L-24** during the processing under basic conditions.

The specific rotations and partial ^{1}H NMR spectral data were listed in Table 1. All epoxides were tested for the inhibitory activity against glucocerebrosidase[12] (mouse liver) and galactocerebrosidase[13] (mouse liver). Among twelve stereoisomers synthesized, **L-35** exhibited a highly potent and specific inhibitor (IC_{50} 9.6 × 10^{-7}M) for glucocerebrosidase, which compares favorably with the IC_{50} value of 8.9 × 10^{-6} M for **5**[14]. Other epoxides did not show inhibitory activity at <10^{-4} M. Interestingly, **L-35** did not show any inhibitory activity against galactocerebrosidase. In the three deoxy derivatives **L-25**, **D-32**, and **L-35** of **5**, the latter 3-deoxy derivative only possesses inhibitory activity comparable to **5**. Therefore, its contiguous three hydroxyl functions at C-3, -4, and -5 appear to be very important in correlation to those at C-2, -3, and -4 of D-gluco-

pyranosyl cation, respectively. Furthermore, the 4-, 5-, and 6-hydroxyl groups of **5** seems to be indispensable for the epoxide group to suffer a nucleophilic attack[5] of the carboxylate function of the enzyme. Interestingly, **L-26** having no structural similarity to either glucopyranosyl cation or known inhibitors of this kind has been shown to be a moderate inhibitor (IC_{50} 9.8×10^{-5} M) of glucocerebrosidase.

References and Notes

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