

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

Seiichiro Ogawa^{a*}, Satoko Uetsuki^a, Yoji Tezuka^a, Takayuki Morikawa^a,
Atsushi Takahashi^b, and Kiyoshi Sato^b

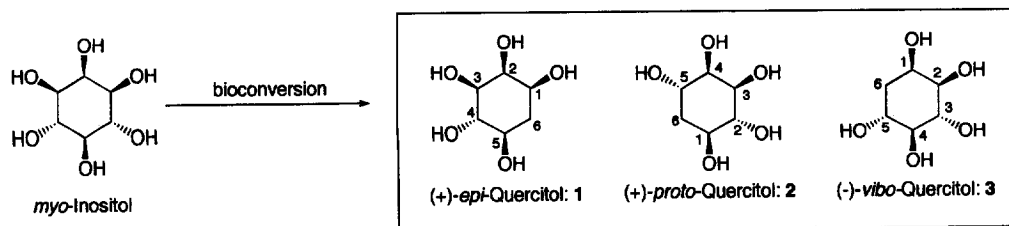
^aDepartment of Applied Chemistry, Faculty of Science and Technology, Keio University,
Hiyoshi, Kohoku-ku, Yokohama, 223-8522 Japan; ^bCentral Research Laboratories,
Hokko Chemical Industry Co. Ltd., Toda, Atsugi, 243-0023 Japan

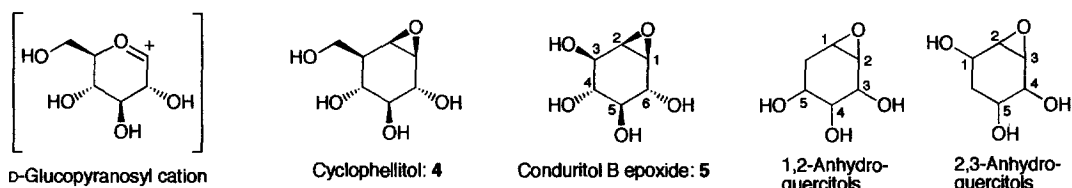
Received 25 January 1999; accepted 9 April 1999

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. © 1999 Elsevier Science Ltd. All rights reserved.

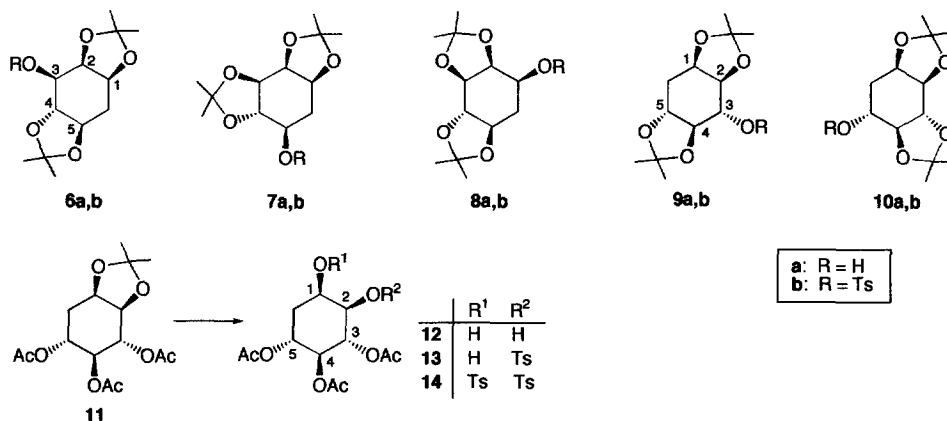
Recently, three deoxyinositols, (+)-*epi*- (1), (+)-*proto*- (2), and (-)-*vibo*-quercitols (3), have been shown^[1] to be produced by bioconversion of *myo*-inositol with bacteria isolated from soil, and each is obtainable in a crystalline form by chromatography. We have become interested in the application 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



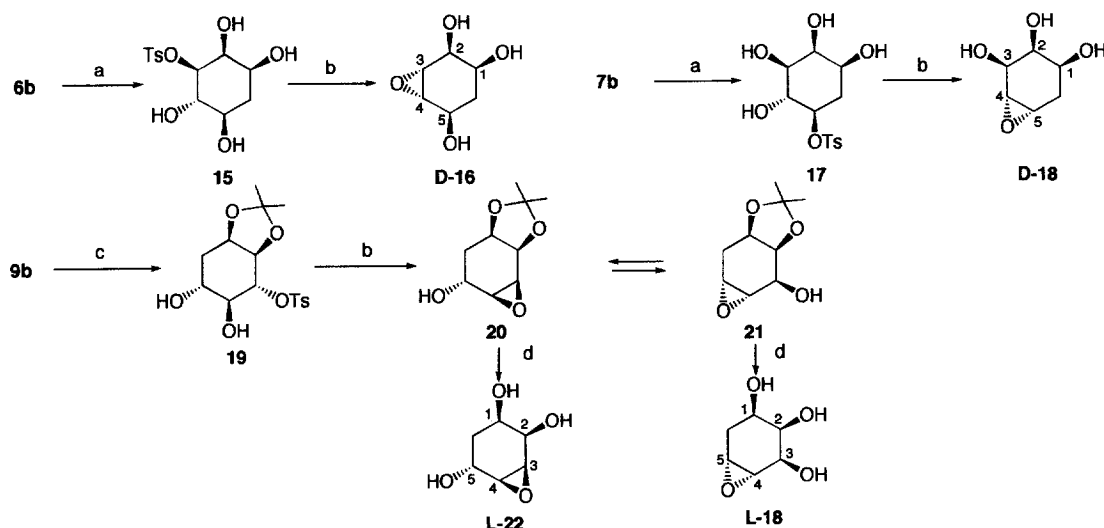


resemblance to D-glucopyranosyl cation probably formed during hydrolysis of the glucosides and covalent bonding to the active site of the enzyme through a nucleophilic cleavage of the epoxide ring by the carboxylate function of aspartate or glutamate residue. Therefore, determination of inhibitory activity 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.



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 ¹H NMR spectra.

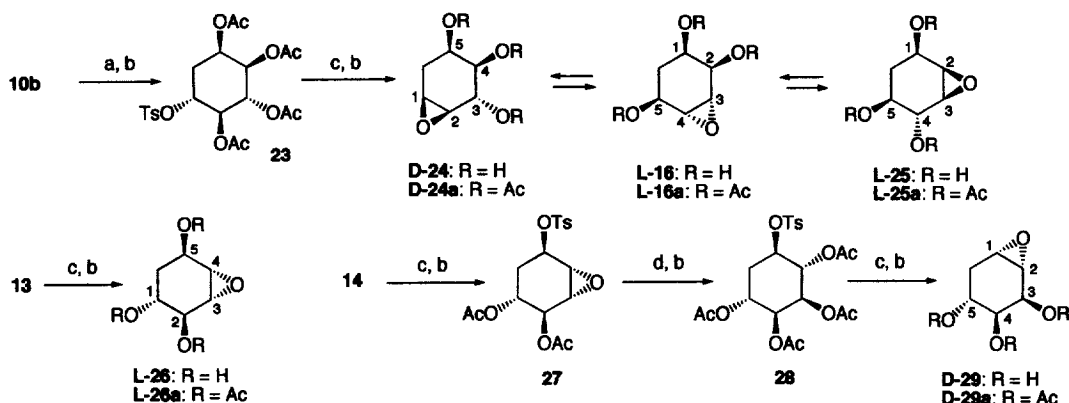


Scheme 1. *Reagents and conditions* (a) 80% aq AcOH, 1.5 h, 50 °C; (b) NaOMe (1.5 molar equiv)/MeOH, rt, 3 h; after neutralization with 1 M HCl, elution from a silica gel column with chloroform–methanol (5–10:1, v/v) as an eluent; (c) a trace of TsOH, MeOH, 0 °C; (d) 50% aq AcOH, 50 °C.

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 ¹H 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).

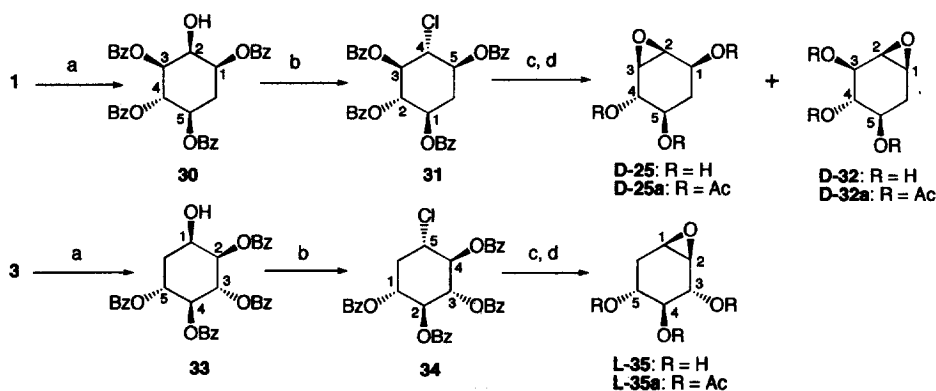
The 5-*O*-tosylate **23** obtained from **10b** was similarly treated with methanolic sodium methoxide, and the products were acetylated to give the triacetyl derivative **D-24a** (34%) and ca. 3:1 mixture (28%) of the



Scheme 2. *Reagents and conditions:* (a) 80% aq AcOH, 80 °C; (b) Ac₂O, pyridine; (c) NaOMe (1.5 molar equiv)/MeOH, rt, overnight; (d) 10% H₂SO₄/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-cyclohexanepentol 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) SO₂Cl₂ (1.1 molar equiv), pyridine, 0 °C → rt, 2 h; (c) NaOMe (1.5 molar equiv)/MeOH, rt; (d) Ac₂O, 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 SO₂Cl₂ 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 ¹H NMR data (270 or 300 MHz, CD₃OD) of 1,2- and 2,3-anhydro-1,2,3,4,5-cyclohexanepentols

Compd	[α] _D ²¹ (°) (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		<i>J</i> _{3,4} 3.34, <i>J</i> _{4,5} ~0
L-16	+33						
D-18	+26				3.01	3.26	<i>J</i> _{3,4} ~0, <i>J</i> _{4,5} 3.67, <i>J</i> _{5,6e} 1.47
L-18	−26						
L-22	+89			3.31	3.17		<i>J</i> _{2,3} 4.40, <i>J</i> _{3,4} 3.66, <i>J</i> _{4,5} ~0
D-24	−49	3.25	3.11				<i>J</i> _{1,2} 3.66, <i>J</i> _{2,3} ~0
D-25	−53		3.22	3.05			<i>J</i> _{2,3} 3.66, <i>J</i> _{3,4} ~0
L-25	+49						
L-26	+30			3.05–3.10			<i>J</i> _{2,3} ~0, <i>J</i> _{3,4} 4.03, <i>J</i> _{4,5} ~0
D-29	−125	3.13–3.19					<i>J</i> _{1,2} 3.66, <i>J</i> _{1,6e} 4.40, <i>J</i> _{2,3} 2.57
D-32	+18	3.16	3.24				<i>J</i> _{1,2} 3.67, <i>J</i> _{2,3} 1.83
L-35	−11	3.25	2.98				<i>J</i> _{1,2} 3.66, <i>J</i> _{1,6a} 1.47, <i>J</i> _{1,6e} 2.20, <i>J</i> _{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 ¹H 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 (IC₅₀ 9.6 × 10^{−7} M) for glucocerebrosidase, which compares favorably with the IC₅₀ 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-gluc-

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

1. Takahashi, A.; Kanbe, K.; Tamamura, T.; Sato, K. *The First International Symposium on Disease Prevention by IP₆ & Other Rice Components*, Kyoto, 1998, June 8–9, Abstract PS-64.
2. Presented part at the 74th Annual Meeting of Japan Chemical Society, Tanabe, Kyoto, 1998, March 27–30, 3 E6 05, Abstract II, p 1103..
3. Atsumi, S.; Umezawa, K.; Iinuma, H.; Nakamura, H.; Iitaka, Y.; Takeuchi, T. *J. Antibiot.*, **1990**, *43*, 49.
4. Lee, K. J.; Boyd, S. A.; Radin, N. S. *Carbohydr. Res.* **1985**, *144*, 148, references cited therein.
5. Legler, G.; Bieberich, E.; *Arch. Biochem. Biophys.* **1988**, *260*, 437. For biochemical application, see van Weekly, S.; van den Berg, M.; Barranger, J. A.; Sa Miranda, M. C.; Tager, J. M.; Aerts, J. M. F. G. *J. Clin. Invest.* **1993**, *91*, 1167, references cited therein.
6. (a) Legler, G. *Advan. Carbohydr. Chem. Biochem.*, **1990**, *48*, 319. (b) Look, G.; Fotsch, C. H.; Wong, C.-H. *Acc. Chem. Res.* **1993**, *26*, 182. (c) Legler, G. *Carbohydrate Mimics*; Chapleur, Y. Ed.; Wiley-VCH: Weinheim, 1998; pp. 463–490.
7. (a) McCasland, G. E. *Advan. Carbohydr. Chem.* **1965**, *20*, 12. (b) Anderson, L. *The Carbohydrates IA*, Pigman, W.; Horton, D. Ed.; Academic Press, New York and London, 1972, pp 519–579.
8. In this paper, nomenclature of cyclitols follows IUPAC-IUB 1973 Recommendations for Cyclitol (*Pure Appl. Chem.*, **1974**, *37*, 285).
9. Recently, (+)-*epi*-quercitol has been synthesized from D-galactose: Dubreuil, D.; Cleophax, J.; de Almeida, M. V.; Verre-Sebrié, C.; Liaigre, J.; Vass, G.; Gero, S. D. *Tetrahedron*, **1997**, *53*, 16747.
10. All new compounds were characterized on the basis of ¹H NMR spectra and elementary analyses.
11. The pentols **1** and **3** were treated with 7 molar equiv of benzoyl chloride in pyridine at room temperature for 2–3 days, giving the tetrabenzoates (~60% isolated yield), together with the pentabenzoates (~10%).
12. (a) Inokuchi, J.-i.; Jimbo, M.; Momosaki, K.; Shimeno, H.; Nagamatsu, A.; Radin, N. S. *Cancer Res.* **1990**, *50*, 6731. (b) Radin, N. S.; Shayman, J. A.; Inokuchi, J.-i. *Advan. Lipid Res.* **1993**, 183.
13. Raghavan, S.; Krusell, A. *Biochim. Biophys. Acta.* **1986**, *877*, 1.
14. Conduritol B epoxide **5** has been assayed to possess strong inhibitory activity (IC_{50} 1.1×10^{-6} M) against glucocerebrosidase (mouse liver): Ogawa, S.; Ashiura, M.; Uchida, C.; Watanabe, S.; Yamazaki, C.; Yamagishi, K.; Inokuchi, J.-i. *Bioorg. Med. Chem. Lett.*, **1996**, *6*, 929.