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Design and synthesis of glycosidase inhibitor 5-amino-1,2,3,4-cyclohexanetetrol derivatives from (−)-*vibo*-quercitol☆

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Abstract—In continuation of development of bioactive inositol derivatives, a 1-*O*-methyl derivative of 5-amino-5-deoxy-L-*talo*-quercitol was designed and synthesized as an analogue of the strong α-fucosidase inhibitor, 5a-carba-α-L-fucopyranosylamine, the methyl branch being replaced with methoxyl, and demonstrated to be a moderate α-fucosidase inhibitor. The present approach provides a possible route to apply alkyl ethers of aminodeoxyinositols as hexopyranose mimics of biological interest.

1. Introduction

On chemical modification of a potent α-mannosidase inhibitor, mannostatin A[1,2] (**1**), the methyloxy analogue 1L-(1,2,3,5/4)-5-amino-4-*O*-methyl-1,2,3,4-cyclopentanetetrol[3] (**2**) was found to also possess very strong inhibitory activity, comparable with that of the parent compound. Furthermore, previous results[3,4] for chemical modification of 1D-(1,2,3,4/0)-4-amino-1,2,3-cyclopentanetriol, the core structure of **1**, suggested the presence of a methyloxy group, possibly an equivalent of hydroxymethyl, to render a certain hydrophobic region of space around C-5, effectively contributing to inhibitory activity. In the development of α-mannosidase inhibitors, design of novel candidates should essentially be based on mimicking structural features relating to the postulated transition-state mannopyranosyl cation.[5–7] The methylthio and methoxyl functions of **1** and **2** may match those of the 5-hydroxymethyl in the carbocation model.

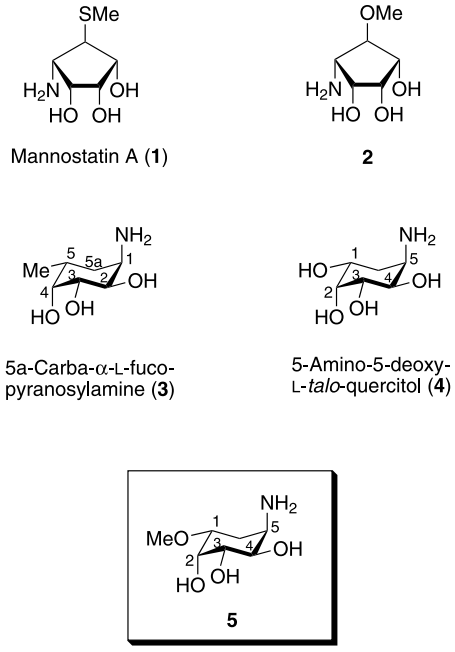

Figure 1. Methyloxy analogues **2** and **5** of respective mannostatin A (**1**) and 5a-carba-α-L-fucopyranosylamine (**3**).

☆In this paper, the nomenclature of aminocyclitols follows the IUPAC–IUB 1973 Recommendation for Cyclitols (*Pure Appl. Chem.* **1974**, *37*, 285–297).

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These considerations stimulated us to design, as glycosidase inhibitors, a series of methyl ethers of 5-amino-1,2,3,4-cyclohexanetetrols structurally related to 5a-carba-hexopyranosylamines (Fig. 1). Among all 5a-carba-glycosylamines, the ground-state mimicking glycosidase inhibitors, synthesized so far, 5a-carba-α-L-fucopyranosylamine[8,9] (**3**) was demonstrated to possess the strongest inhibitory activity against α-fucosidase. Therefore, it seemed desirable to choose it as a lead as well as mimetic compound, and new derivatives were generated by replacement of the methyl group with methyloxy elements: the methyloxy analogue, namely the 1-*O*-methyl derivative **5** of 5-amino-5-deoxy-L-*talo*-quercitol (**4**) was synthesized, and its enzyme-inhibitory activity was evaluated.

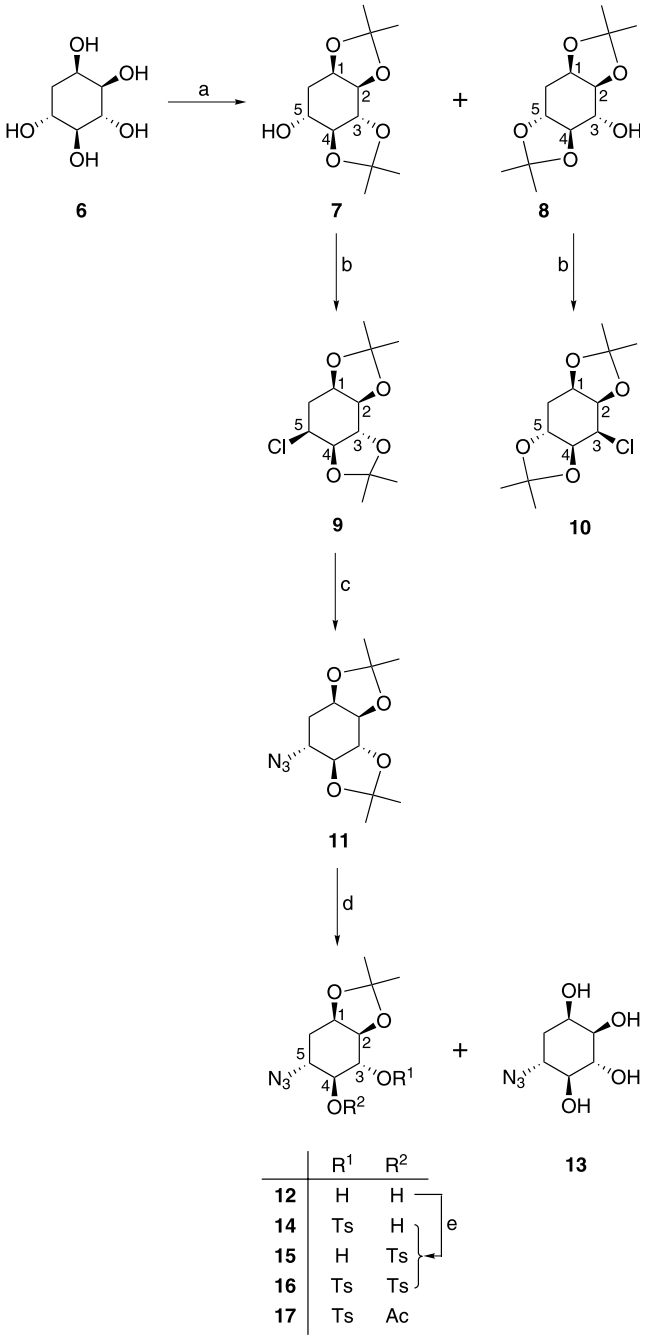
2. Results and discussion

Reaction of (−)-*vibo*-quercitol[10] (**6**) with large excess of 2,2-dimethoxypropane (10 M equiv) in DMF in the presence of *p*-toluenesulfonic acid for 3 h at room temperature gave an inseparable mixture of the 1,2:3,4- and 1,2:4,5-di-*O*-isopropylidene derivatives[11] (**7** and **8**) in 86% yield (Scheme 1). Treatment of the mixture with sulfuryl chloride (3 M equiv) in the presence of DMAP in pyridine at room temperature gave, after fractionation over a silica gel column, two chloro compounds **9** (40%) and **10** (58%), the structures of which were readily established on the basis of their ^{1}H NMR spectra, revealing a doublet of doublets of doublets ($\delta = 4.50$, $J = 2.3$, 3.1, and 5.2 Hz) and a doublet of doublets ($\delta = 4.62$, $J = 2.9$ and 5.6 Hz), respectively, due to the protons attached to the carbon atoms bonding to the chlorine atoms. These data indicated that halogen atoms were incorporated through direct S_N2 reactions with inversion of the configuration.

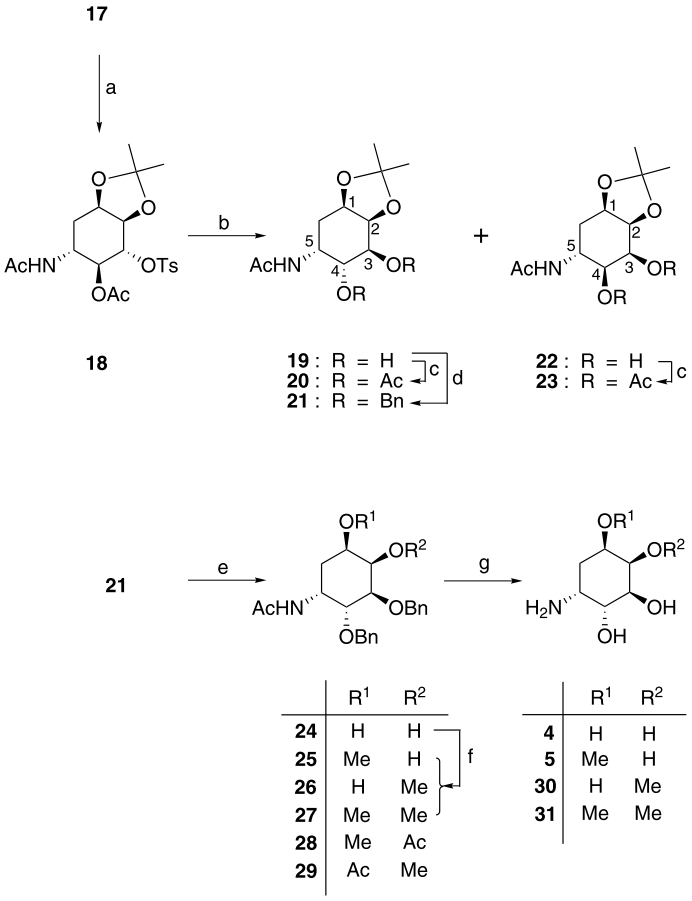
Azidolysis of the desired chloride **9** with an excess of sodium azide in DMF at 100 °C gave selectively the azide **11** (50%), accompanied by some elimination products.[12]

Attempted selective *O*-deisopropylidenation of **11** was conducted under the influence of a trace of *p*-toluenesulfonic acid in methanol at 0 °C, formation of mono-*O*-isopropylidene derivative **12** being monitored by TLC. The mixture of products was successfully separated by a silica gel column to give **12** (71%), along with **11** (∼10%) and the tetrol **13** (∼7%). Selective tosylation of **12** was carried out by treatment with excess *p*-toluenesulfonyl chloride (5 M equiv) in pyridine at −18 °C. After 4 days, two mono-tosylates **14** (43%) and **15** (29%), and the ditosylate **16** (15%) were afforded.[13] Compounds **14** and **15** isolated by silica gel chromatography could be readily differentiated, their structures being firmly established from their NMR spectra as 3- and 4-tosylates, respectively, combined with data obtained for the acetyl derivative **17** of **14**.

The azido tosylate **17** was first transformed into the *N*-acetyl derivative **18**, in the expectation that, under acetolysis conditions, the amide may participate as a neighboring group to give rise preferentially to the desired aminodeoxyquercitol with a *talo*-configuration (Scheme 2). Thus, **17** was hydrogenolyzed in ethanol containing acetic anhydride in the presence of Raney nickel to give crystalline amide tosylate **18**, quantitatively. Treatment of **18** with sodium acetate (5 M equiv) in 90% aqueous 2-methoxyethanol for three days at 120 °C produced an approximately 10:1 mixture of the two diols **19** and **22** with *talo*- and *allo*-configurations. Alternatively, a similar reaction was carried out using aqueous DMF to give an about 1:10 mixture of **19** and **22**. The mixture was conventionally acetylated and



Scheme 1. Synthesis of tosyl derivative **17** of 5-azido-5-deoxy-L-*vibo*-quercitol. Reagents and conditions: (a) $Me_2C(OMe)_2$ (∼6 M equiv), DMF, *p*-TsOH·H_2O, rt; (b) $SOCl_2$ (3 M equiv), DMAP, pyridine, 0 °C; (c) NaN_3 (10 M equiv), 15-crown-5 ether, DMF, 100 °C; (d) MeOH, *p*-TsOH·H_2O, ∼pH 4; (e) TsCl (5 M equiv), pyridine, −18 °C.



Scheme 2. Synthesis of methyl ethers **5**, **30**, and **31** of 5-amino-5-deoxy-L-*talo*-quercitol (**4**). Reagents and conditions: (a) H_2, Raney Ni, EtOH, Ac_2O; (b) anhydrous NaOAc (5 M equiv), 90% aqueous $MeOCH_2CH_2OH$, 110 °C, 2 days; (c) Ac_2O, pyridine; (d) benzyl bromide (3 M equiv), NaH, (4 M equiv), DMF, 0 °C; (e) 80% aqueous AcOH, 50 °C; (f) CH_3I, Ag_2O, CH_3CN, reflux temp, silica gel chromatography; (g) H_2, 10% Pd/C, EtOH, rt, aqueous 1 M $Ba(OH)_2$, 80 °C, 2 h, column of Dowex-50 W × 2 (H^+) resin, 1% aqueous NH_3.

the di-*O*-acetyl derivatives **20** and **23** were isolated pure, respectively. Mechanistically, two compounds were predicted to be produced mainly by the acetolysis of **18** (Fig. 2). Thus, in an aprotic solvent DMF, direct S_N2 reaction with an acetate ion would undergo preferentially to afford the product with *allo*-configuration, and, on the other hand, in a protic solvent aqueous 2-methoxyethanol, the 4-acetoxyl would participate at C-3 to form an intermediate acetoxonium ion between C-3 and 4, which was cleaved to give rise to two products with *allo*- and *talo*-configurations. The latter was obtained as major product through subsequent neighboring participation of the amide. Thus, the two compounds **20** and **23** were likely to be provided by direct nucleophilic substitution and by preferential opening of the acetoxonium ion, respectively.

In the ^1H NMR spectra of **20** and **23**, adopting the preferred conformations (Fig. 3), the signals due to axially oriented H-4 appeared as doublets of doublets ($J = 5.1$ and 7.4 Hz) and ($J = 3.8$ and 9.3 Hz) at δ 5.30 and 4.95, respectively (Fig. 3). In the former, the signal was deshielded by close proximity to the ketal oxygen atoms. Signals due to H-5 attached to the carbon atoms bonding to the amido groups resonated at δ 4.69 and 4.53. The latter axial hydrogen seemed to be appreciably deshielded by the axial 3-acetoxyl and the ketal oxygens. The ^1H NMR data thus provided clear support for the assigned *talo*- and *allo*-configurations of **20** and **23**.

Compound **20** was *O*-deacetylated with methanolic sodium methoxide (→**19**) and the diol obtained was again protected with a benzyl group (→**21**). Then, removal of the isopropylidene group generated the 1,2-unprotected derivative **24**. Removal of the benzyl groups of **24** by hydrogenolysis in ethanol in the presence of Pd/C and subsequent hydrolysis with aqueous barium hydroxide gave, after purification over a column of Dowex-50 W × 2 (H^+) resin with aqueous 1% ammonia, 5-amino-5-deoxy-L-*talo*-quercitol **4** quantitatively. Then, attempted selective methylation of **24** was carried out with 2 M equiv of iodomethane in acetonitrile in the presence of silver oxide at reflux temperature. The mixture of products was fractionated over a silica gel column to give an inseparable mixture (52%) of two monomethyl derivatives **25** and **26**, and one dimethyl form **27** (33%). The former mixture was conventionally acetylated to give the acetyl derivatives, which were found to be separable on a silica gel column to give **28** (34%) and **29** (49%). Compounds **25**, **26**, and **27** were deprotected followed by base hydrolysis, affording the corresponding free bases **5**, **30**, and **31**.

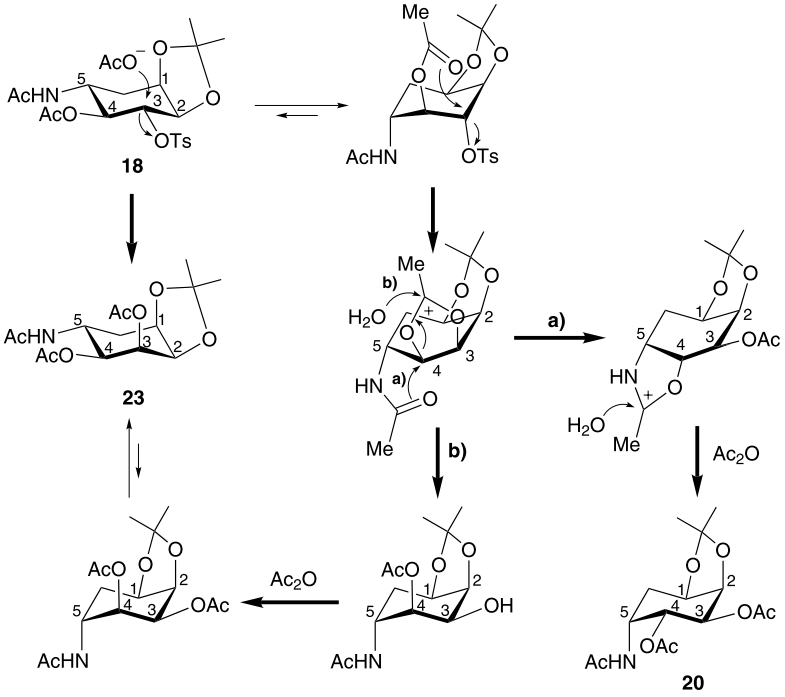


Figure 2. Postulated reaction mechanism for formation of compounds **20** and **23** by treatment of **18** with sodium acetate in appropriate solvents, followed by acetylation.

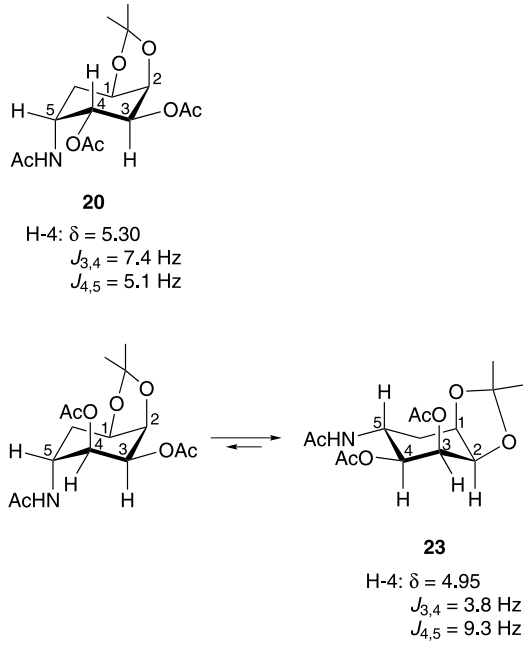


Figure 3. Preferred conformations of compounds **20** and **23**.

3. Biological assay

Data for inhibitory activity[14] of compounds **4**, **5**, **30**, and **31** toward three glycosidases are summarized in Table 1. Compounds **5** and **30** possessed moderate inhibitory activity toward α-fucosidase (bovine liver), as expected, while, the parent free base **4** and the dimethyl ether **31** did not show any potential. Interestingly, compound **30** showed cross-inhibitory action toward β-galactosidase (Jack beans). Thus, the present study has demonstrated that methyloxy groups are likely to function as equivalents of the hydrophobic 5-methyl branching in **1**, allowing us to design deoxyinosamine-type glycosidase inhibitors other than α-fucosidase. From the present results we therefore propose incorporation of methyl ether groups as one effective route for application of chemically modified inositols as pseudo-sugar (carba-sugar) derivatives of biological interest.

Table 1. Data for inhibitory activity of 5-amino-5-deoxy-L-*talo*-quercitol (**4**) and its methyl ethers **5**, **30**, and **31** against three glycosidase, compared with 5a-carba-L-fucopyranosylamine (**3**)

Compound[a]	IC_{50} (M)		
	α-Fucosidase (bovine kidney)	α-Galactosidase (green coffee beans)	β-Glucosaminidase (bovine kidney)
3[b]	9.3×10^{-6}	NI	NI
4	NI	4.9×10^{-4}	NI
5	2.3×10^{-5}	NI	NI
30	1.0×10^{-4}	2.0×10^{-5}	4.0×10^{-4}
31	NI	NI	NI

NI: no inhibition < 10^{-3} M.

[a] None of the compounds showed any notable inhibitory activity against β-galactosidase (bovine liver), α-glucosidase (sucrase from rat), β-glucosidase (almond), and α-mannosidase (Jack beans).

[b] Compound **3** has been observed[9] to exhibit strong activity ($K_i = 1.2 \times 10^{-8}$ M) against α-fucosidase (bovine kidney).

4. Experimental

4.1. General methods

Optical rotations were measured with a JASCO DIP-370 polarimeter, and $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. ^{1}H NMR spectra were recorded for

solutions in deuteriochloroform and deuteriomethanol with internal tetramethylsilane (TMS) as a reference with a JEOL JNM Lambda-300 (300 MHz) instrument. Mass spectra were determined with Hitachi M-8000 ion trap mass spectrometer. TLC was performed on silica gel 60 F-254 (E. Merck, Darmstadt). The silica gel used for a column chromatography was Wakogel C-300 (Wako Junyaku Kogyo Co., Osaka, 200–300 mesh) or silica gel 60 KO (Katayama Kagaku Kogyo Co., Osaka, 70–230 mesh). Organic solutions were dried over anhydrous Na_2SO_4 and concentrated at >45 °C under diminished pressure.

4.2. 5-Chloro-5-deoxy-1,2:3,4-di-*O*-isopropylidene-L-*muco*-quercitol (9) and 3-chloro-3-deoxy-1,2:4,5-di-*O*-isopropylidene-L-*allo*-quercitol (10)

To a solution of (−)-*vibo*-quercitol[10] (7.97 g, 49 mmol) in DMF (120 mL) were added 2,2-dimethoxypropane (80 mL, 0.77 mol) and *p*-toluenesulfonic acid monohydrate (0.93 g, 4.9 mmol), and the mixture was stirred for 90 min at room temperature. TLC showed a formation of two components with similar R_f values (R_f = ~0.5, 2-butanone–toluene 1:2). After neutralization with triethylamine, the mixture was evaporated and the residue was chromatographed on a column of silica gel (300 g, acetone–hexane 1:2) to give an inseparable mixture (10.2 g, 86.3%) of 1,2:3,4- and 1,2:4,5-di-*O*-isopropylidene-L-*vibo*-quercitol (**7** and **8**) as a syrup.

To a 113 mg (0.46 mmol) portion of the mixture in pyridine (2.3 mL) was added DMAP (5.6 mg, 0.05 mmol) and sulfuryl chloride (112 μL, 1.4 mmol) at 0 °C, and the mixture was stirred for 21 h at room temperature. The reaction mixture was roughly co-evaporated with toluene to dryness, and the residue was diluted with chloroform (24 mL). The solution was washed with saturated aqueous sodium thiosulfate, water, and saline, dried, and evaporated. The residue was chromatographed on a column of silica gel (14 g, acetone–hexane 1:60 → 1:30) to give the chlorides **9** (49 mg, 40%) and **10** (70 mg, 58%) as crystalline solid.

For **9**: R_f = 0.37 (acetone–hexane 1:5); $[\alpha]_D^{21}$ −8.5 (*c* 0.57, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.50 (ddd, 1H, $J_{5,6eq}$ = 2.3 Hz, $J_{4,5}$ = 3.1 Hz, $J_{5,6ax}$ = 5.2 Hz, H-5), 4.38 (ddd, 1H, $J_{1,6eq}$ = 1.8 Hz, $J_{1,6ax}$ = 4.9 Hz, $J_{1,2}$ = 5.1 Hz, H-1), 4.22 (m, 2H, H-2, H-3), 3.48 (dd, 1H, $J_{4,5}$ = 3.1 Hz, $J_{3,4}$ = 9.2 Hz, H-4), 2.69 (ddd, 1H, $J_{1,6eq}$ = 1.8 Hz, $J_{5,6eq}$ = 2.3 Hz, J_{6gem} = 16.6 Hz, H-6eq), 2.30 (ddd, 1H, $J_{1,6ax}$ = 4.9 Hz, $J_{5,6ax}$ = 5.2 Hz, J_{6gem} = 16.6 Hz, H-6ax), 1.56, 1.49, 1.47, and 1.36 (4 s, each 3H, 2 × CMe_2).

For **10**: R_f = 0.40 (acetone–hexane 1:5); $[\alpha]_D^{19}$ +74 (*c* 0.61, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.62 (dd, 1H, $J_{3,4}$ = 2.9 Hz, $J_{2,3}$ = 5.6 Hz, H-3), 4.49 (m, 1H, $J_{1,6ax}$ = 5.5 Hz, H-1), 4.35 (m, 2H, H-2, H-5), 3.46 (dd, 1H, $J_{3,4}$ = 2.9 Hz, $J_{4,5}$ = 9.2 Hz, H-4), 2.65 (m, 1H, $J_{5,6eq}$ = 5.0 Hz, J_{6gem} = 14.2 Hz, H-6eq), 1.87 (ddd, 1H, $J_{1,6ax}$ = 5.5 Hz, $J_{5,6ax}$ = 11.8 Hz, J_{6gem} = 14.2 Hz, H-6ax), 1.64, 1.47, 1.45, and 1.36 (4s, each 3H, 2 × CMe_2).

4.3. 5-Azido-5-deoxy-1,2:3,4-di-*O*-isopropylidene-L-*vibo*-quercitol (11)

A mixture of **9** (605 mg, 2.3 mmol), sodium azide (1.5 g, 23 mmol), 15-crown-5 ether (4.6 mL, 23 mmol), and DMF (12 mL) was stirred for 15 h at 100 °C, and then co-evaporated with butanol and toluene. The residue was suspended with ethyl acetate (120 mL) and the mixture was washed with water and saline, dried, and evaporated. The residue was chromatographed on a column of silica gel (60 g, acetone–hexane 1:80) to give the azide **11** (310 mg, 50%) as a crystalline solid, R_f = 0.38 (acetone–hexane 1:5); $[\alpha]_D^{20}$ −43 (*c* 5.2, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.40 (ddd, 1H, $J_{1,6eq}$ = 2.4 Hz, $J_{1,6ax}$ = 5.0 Hz, $J_{1,2}$ = 5.1 Hz, H-1), 4.19 (dd, 1H, $J_{1,2}$ = 5.1 Hz, $J_{2,3}$ = 8.8 Hz, H-2), 3.84 (ddd, 1H, $J_{5,6eq}$ = 5.6 Hz, $J_{4,5}$ = 10.0 Hz, $J_{5,6ax}$ = 10.2 Hz, H-5), 3.58 (dd, 1H, $J_{2,3}$ = 8.8 Hz, $J_{3,4}$ = 9.7 Hz, H-3), 3.26 (dd, 1H, $J_{3,4}$ = 9.7 Hz, $J_{4,5}$ = 10.0 Hz, H-4), 2.69 (ddd, 1H, $J_{1,6eq}$ = 2.4 Hz, $J_{5,6eq}$ = 5.6 Hz, J_{6gem} = 15.4 Hz, H-6eq), 1.71 (ddd, 1H, $J_{1,6ax}$ = 5.0 Hz, $J_{5,6ax}$ = 10.2 Hz, J_{6gem} = 15.4 Hz, H-6ax), 1.51, 1.45, 1.44, and 1.35 (4s, each 3H, 2 × CMe_2).

4.4. 5-Azido-5-deoxy-1,2-*O*-isopropylidene-L-*vibo*-quercitol (12) and 5-azido-5-deoxy-L-*vibo*-quercitol (13)

To a stirred solution of the azide **11** (5.35 g, 19.9 mmol) in methanol (100 mL) was added gradually $TsOH \cdot H_2O$ until pH of the mixture being adjusted to ~4. After neutralization with triethylamine, the mixture was evaporated, and the residue was chromatographed on a column of silica gel (500 g, acetone–hexane 1:3 → 1:1, methanol) to give **12** (3.22 g, 71%), as a crystalline solid, and **13** (0.71 g, 7%), together with **11** (0.55 g, 10%) recovered.

For **12**: R_f = 0.23 (acetone–hexane 1:2); $[\alpha]_D$ −74 (*c* 0.91, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.39 (m, 1H, H-1), 3.96 (dd, 1H, $J_{1,2}$ = 5.1 Hz, $J_{2,3}$ = 7.7 Hz, H-2), 3.61 (m, 1H, H-5), 3.59 (dd, 1H, $J_{2,3}$ = 7.7 Hz, $J_{3,4}$ = 9.8 Hz, H-3), 3.30 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, H-4), 2.40 (ddd, 1H, $J_{1,6eq}$ = 2.1 Hz, $J_{5,6eq}$ = 4.8 Hz, J_{6gem} = 15.0 Hz, H-6eq), 1.76 (ddd, 1H, $J_{1,6ax}$ = 3.7 Hz, $J_{5,6ax}$ = 11.7 Hz, J_{6gem} = 15.0 Hz, H-6ax), 1.58 and 1.42 (2s, each 3H, CMe_2); ITMS-APCI (negative mode): *m*/*z* 228 $[M-H]^-$.

For **13**: R_f = 0.01 (acetone–hexane 1:2); $[\alpha]_D^{20}$ −13 (*c* 0.44, MeOH); ^{1}H NMR (300 MHz, CD_3OD): δ 4.05 (m, 1H, H-1), 3.62 (m, 1H, H-5), 3.58 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 3.43 (dd, 1H, $J_{1,2}$ = 2.5 Hz, $J_{2,3}$ = 9.7 Hz, H-2), 3.32 (dd, 1H, $J_{3,4}$ = 9.5 Hz, $J_{2,3}$ = 9.7 Hz, H-3), 2.09 (ddd, 1H, $J_{1,6eq}$ = 3.8 Hz, $J_{5,6eq}$ = 4.1 Hz, J_{6gem} = 14.2 Hz, H-6eq), 1.53 (ddd, 1H, $J_{5,6ax}$ = ~1.5 Hz, $J_{1,6ax}$ = J_{6gem} = 14.2 Hz, H-6ax); ITMS-APCI (negative mode): *m*/*z* 188 $[M-H]^-$.

4.5. 5-Azido-5-deoxy-1,2-*O*-isopropylidene-3-*O*-tosyl- (14), 4-*O*-tosyl- (15), and 3,4-di-*O*-tosyl-L-*vibo*-quercitols (16)

A mixture of **12** (423 mg, 1.84 mmol) in pyridine (8.5 mL) was added tosyl chloride (1.76 g, 9.22 mmol)

at −18 °C, and the mixture was stirred for 4 days at the similar temperature. The reaction was quenched by addition of methanol (1 mL) and then the mixture was co-evaporated with toluene. The residue was diluted with ethyl acetate (90 mL), and the solution was washed with water and saline, dried, and evaporated. The residue was chromatographed on a silica gel column (70 g, EtOAc–toluene 1:30 → 1:10) to give **14** (305 mg, 43%), **15** (208 mg, 29%), and **16** (150 mg, 15%) as crystalline solid.

For **14**: R_f = 0.50 (acetone–hexane 1:6); $[\alpha]_D^{20}$ −76.5 (*c* 1.71, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.88–7.33 (m, 4H, 2 × Ph), 4.58 (dd, 1H, $J_{2,3}$ = 7.4 Hz, $J_{3,4}$ = 9.8 Hz, H-3), 4.27 (m, 1H, H-1), 4.00 (dd, 1H, $J_{1,2}$ = 5.2 Hz, $J_{2,3}$ = 7.4 Hz, H-2), 3.74 (ddd, 1H, $J_{5,6eq}$ = 4.6 Hz, $J_{4,5}$ = 9.5 Hz, $J_{5,6ax}$ = 11.8 Hz, H-5), 3.52 (dd, 1H, $J_{4,5}$ = 9.5 Hz, $J_{3,4}$ = 9.8 Hz, H-4), 2.45 (s, 3H, PhCH_3), 2.29 (ddd, 1H, $J_{1,6eq}$ = 2.2 Hz, $J_{5,6eq}$ = 4.6 Hz, J_{6gem} = 15.5 Hz, H-6eq), 1.62 (ddd, 1H, $J_{1,6ax}$ = 3.8 Hz, $J_{5,6ax}$ = 11.8 Hz, J_{6gem} = 15.5 Hz, H-6ax), 1.39 and 1.29 (2s, each 3H, CMe_2); ITMS-ESI (positive mode): *m*/*z* 406 $[M+Na]^+$.

For **15**: R_f = 0.34 (acetone–hexane 1:6); $[\alpha]_D^{20}$ +5.5 (*c* 6.52, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.89–7.36 (m, 4H, Ph), 4.35 (dd, 1H, $J_{3,4}$ = 9.5 Hz, $J_{4,5}$ = 10.0 Hz, H-4), 4.31 (m, 1H, H-1), 4.04 (dd, 1H, $J_{1,2}$ = $J_{2,3}$ = 6.3 Hz, H-2), 3.81 (dd, 1H, $J_{2,3}$ = 6.3 Hz, $J_{3,4}$ = 9.5 Hz, H-3), 3.74 (m, 1H, H-5), 2.46 (s, 3H, PhCH_3), 2.45 (m, 1H, H-6eq), 1.74 (ddd, 1H, $J_{1,6ax}$ = 3.7 Hz, $J_{5,6ax}$ = 12.2 Hz, J_{6gem} = 15.6 Hz, H-6ax), 1.49 and 1.35 (2s, each 3H, CMe_2); ITMS-ESI (positive mode): *m*/*z* 406 $[M+Na]^+$.

For **16**: R_f = 0.59 (acetone–hexane 1:6); $[\alpha]_D^{20}$ +0.9 (*c* 1.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.84–7.32 (m, 8H, 2 × Ph), 4.76 (dd, 1H, $J_{2,3}$ = 6.2 Hz, $J_{3,4}$ = 7.4 Hz, H-3), 4.44 (dd, 1H, $J_{3,4}$ = 7.5 Hz, $J_{4,5}$ = 8.5 Hz, H-4), 4.32 (m, 1H, H-1), 4.17 (dd, 1H, $J_{1,2}$ = 6.1 Hz, $J_{2,3}$ = 6.2 Hz, H-2), 3.78 (ddd, 1H, $J_{5,6eq}$ = 4.4 Hz, $J_{4,5}$ = 8.5 Hz, $J_{5,6ax}$ = 12.3 Hz, H-5), 2.44 (s, 6H, 2 × PhCH_3), 2.29 (ddd, 1H, $J_{1,6eq}$ = 2.4 Hz, $J_{5,6eq}$ = 4.4 Hz, J_{6gem} = 14.9 Hz, H-6eq), 1.77 (ddd, 1H, $J_{1,6ax}$ = 3.9 Hz, $J_{5,6ax}$ = 12.3 Hz, J_{6gem} = 14.9 Hz, H-6ax), 1.42 and 1.24 (2s, each 3H, CMe_2); ITMS-ESI (positive mode): *m*/*z* 560 $[M+Na]^+$.

4.6. 4-*O*-Acetyl-5-azido-5-deoxy-1,2-*O*-isopropylidene-3-*O*-tosyl-L-*vibo*-quercitol (17)

Compound **14** (1.33 g, 3.47 mmol) was treated with acetic anhydride (6.7 mL) in pyridine (13 mL) for 14 h at room temperature, and then co-evaporated with toluene. The residue was dissolved in ethyl acetate (300 mL) and the solution was washed with water and saline, dried, and evaporated. The product was chromatographed on a column of silica gel (150 g, acetone–hexane 1:15 → 1:5) to give **17** (1.44 g, 97%) as a syrup, R_f = 0.61 (acetone–toluene 1:4); $[\alpha]_D^{20}$ −63 (*c* 1.7, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.88–7.33 (m, 4H, Ph), 4.96 (t, 1H, $J_{4,5}$ = 9.8 Hz, $J_{3,4}$ = 10.5 Hz, H-4), 4.84 (dd, 1H, $J_{2,3}$ = 7.3 Hz, $J_{3,4}$ = 10.5 Hz, H-3), 4.31 (m, 1H, H-1), 4.05 (dd, 1H, $J_{1,2}$ = 5.1 Hz, $J_{2,3}$ = 7.3 Hz, H-2), 3.88 (ddd, 1H, $J_{5,6eq}$ = 4.6 Hz, $J_{4,5}$ = 9.8 Hz, $J_{5,6ax}$ = 12.0 Hz, H-5), 2.48 (ddd, 1H, $J_{1,6eq}$ = 2.1 Hz, $J_{5,6eq}$ = 4.6 Hz, J_{6gem} = 15.4 Hz, H-6eq), 2.43 (s, 3H, PhCH_3), 2.14 (s, 3H, Ac), 1.72 (ddd, 1H, $J_{1,6ax}$ = 3.7 Hz, $J_{5,6ax}$ = 12.0 Hz, J_{6gem} = 15.4 Hz, H-6ax), 1.57 and 1.33 (2s, each 3H, CMe_2); ITMS-ESI (positive mode): *m*/*z* 448 $[M+Na]^+$.

4.7. 5-Acetamido-4-*O*-acetyl-5-deoxy-1,2-*O*-isopropylidene-3-*O*-tosyl-L-*vibo*-quercitol (18)

A solution of **17** (1.47 g, 3.46 mmol) in ethanol (25 mL) was hydrogenated in the presence of Raney Ni (two spoonful) and acetic anhydride (0.66 mL) under atmospheric pressure of hydrogen for 4.5 h at room temperature. A catalyst was removed by filtration and the filtrate was evaporated to dryness. The residue was chromatographed on a column of silica gel (160 g, acetone–hexane 2:3) to give **18** (1.57 g, 99%) as a crystalline solid, R_f = 0.14 (acetone–toluene 1:4); $[\alpha]_D^{19.5}$ −45.5 (*c* 1.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.58 (d, 1H, $J_{5,NH}$ = 8.5 Hz, NH), 4.92 (dd, 1H, $J_{2,3}$ = 7.3 Hz, $J_{3,4}$ = 10.3 Hz, H-3), 4.79 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 10.3 Hz, H-4), 4.42 (m, 1H, H-5), 4.27 (m, 1H, H-1), 4.02 (dd, 1H, $J_{1,2}$ = 5.1 Hz, $J_{2,3}$ = 7.3 Hz, H-2), 2.55 (ddd, 1H, $J_{1,6eq}$ = 2.0 Hz, $J_{5,6eq}$ = 4.6 Hz, J_{6gem} = 15.1 Hz, H-6eq), 1.63 (m, 1H, H-6ax), 2.45 (s, 3H, PhCH_3), 2.05 and 1.98 (2s, each 3H, 2 × Ac), 1.51 and 1.40 (2s, each 3H, CMe_2); ITMS-ESI (positive mode): *m*/*z* 464 $[M+Na]^+$.

4.8. 5-Acetamido-5-deoxy-1,2-*O*-isopropylidene-L-*talo*-quercitol (19) and 5-acetamido-5-deoxy-1,2-*O*-isopropylidene-L-*allo*-quercitol (22)

(a) A mixture of **18** (1.25 g, 2.74 mmol), anhydrous NaOAc (1.12 g, 13.7 mmol), and 90% aqueous 2-methoxyethanol (25 mL) was stirred for 2 days at 110 °C, and then co-evaporated with ethanol and toluene. The residue was triturated with acetone (50 mL) and an insoluble material was removed by filtration. The filtrate was evaporated and the residue was chromatographed on a column of silica gel (60 g, MeOH–$CHCl_3$ 1:10) to give ca. 10:1 mixture (0.56 g, 83%) of the diols **19** and **22** as a syrup.

(b) A mixture of **18** (49 mg, 0.11 mmol), anhydrous NaOAc (44 mg, 0.53 mmol), and 90% aqueous DMF (1.0 mL) was stirred for 3 days at 115 °C. The reaction mixture was processed similarly and the products were chromatographed on a column of silica gel to give to give ca. 1:10 mixture of **19** and **22** (12 mg, 35%), together with **18** (10 mg, 20%) recovered.

Compounds **19** and **22** were further characterized by converting into the corresponding di-*O*-acetyl derivatives **20** and **23** in the conventional manner.

For **20**: R_f = 0.52 (MeOH–$CHCl_3$ 1:5); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.66 (d, 1H, $J_{5,NH}$ = 8.3 Hz, NH), 5.30 (dd, 1H, $J_{4,5}$ = 5.1 Hz, $J_{3,4}$ = 7.4 Hz, H-4), 5.19 (dd, 1H, $J_{2,3}$ = 3.5 Hz, $J_{3,4}$ = 7.4 Hz, H-3), 4.69 (m, 1H, H-5), 4.42 (m, 2H, H-1, H-2), 2.06 (m, 1H, H-

6eq), 1.95 (m, 1H, H-6ax), 2.16, 2.06 and 1.95 (3 s, each 3H, 3 × Ac), 1.57 and 1.30 (2s, each 3H, CMe_2); ITMS-ESI (positive mode): *m/z* 352 $[M+Na]^+$.

For **23**: $R_f = 0.52$ (MeOH–$CHCl_3$ 1:5); ^1H NMR (300 MHz, $CDCl_3$): δ 5.59 (d, 1H, $J_{5,NH} = 8.3$ Hz, NH), 5.36 (dd, 1H, $J_{3,4} = 3.8$ Hz, $J_{2,3} = 4.3$ Hz, H-3), 4.95 (dd, 1H, $J_{3,4} = 3.8$ Hz, $J_{4,5} = 9.3$ Hz, H-4), 4.53 (m, 1H, H-5), 4.41 (m, 1H, H-1), 4.31 (dd, 1H, $J_{2,3} = 4.3$ Hz, $J_{1,2} = 6.2$ Hz, H-2), 2.42 (ddd, 1H, $J_{1,6eq} = 2.4$ Hz, $J_{5,6eq} = 4.9$ Hz, $J_{6gem} = 14.9$ Hz, H-6eq), 2.16, 2.06 and 1.95 (3s, each 3H, 3 × Ac), 1.71 (ddd, 1H, $J_{1,6ax} = 4.6$ Hz, $J_{5,6ax} = 11.2$ Hz, $J_{6gem} = 14.9$ Hz, H-6ax), 1.54 and 1.34 (2s, each 3H, CMe_2); ITMS-ESI (positive mode): *m/z* 352 $[M+Na]^+$.

4.9. 5-Acetamido-3,4-di-*O*-benzyl-5-deoxy-1,2-*O*-isopropylidene-L-*talo*-quercitol (21)

To a 390 mg (1.60 mmol) portion of the ca. 10:1 mixture of **19** and **22** dissolved in DMF (1.0 mL) was added NaH (60% in oil, 0.26 g, 6.4 mmol), and the mixture was stirred for 30 min at 0 °C. Benzyl bromide (570 μL, 4.8 mmol) was added to the mixture and it was stirred for 3 h at room temperature. After being quenched by addition of MeOH, the reaction mixture was evaporated and further co-evaporated with *n*-BuOH and toluene. The residue was diluted with ethyl acetate and the solution was washed with water and saline, dried, and evaporated. The residual product was chromatographed on a column of silica gel (70 g, acetone–hexane 1:4 → 1:3) to give mainly the dibenzyl ether **21** (494 mg, 73%) as a syrup, $R_f = 0.53$ (acetone–toluene 1:2); $[\alpha]_D^{22}$ +41 (*c* 0.63, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.41–7.23 (m, 10H, Ph), 5.65 (d, 1H, $J_{5,NH} = 7.8$ Hz, NH), 4.79 and 4.66 (ABq, $J_{gem} = 12.2$ Hz), and 4.66 and 4.44 (ABq, $J_{gem} = 11.5$ Hz) (2 × CH_2Ph), 4.58 (m, 1H, H-5), 4.41 (dd, 1H, $J_{2,3} = 3.8$ Hz, $J_{1,2} = 6.3$ Hz, H-2), 4.32 (m, 1H, H-1), 3.85 (dd, 1H, $J_{4,5} = 5.3$ Hz, $J_{3,4} = 5.8$ Hz, H-4), 3.67 (dd, 1H, $J_{2,3} = 3.8$ Hz, $J_{3,4} = 5.8$ Hz, H-3), 1.88 (m, 1H, H-6eq), 1.85 (s, 3H, Ac), 1.70 (m, 1H, H-6ax), 1.56 and 1.34 (2s, each 3H, CMe_2); ITMS-ESI (positive mode): *m/z* 448 $[M+Na]^+$.

4.10. 5-Acetamido-3,4-di-*O*-benzyl-5-deoxy-L-*talo*-quercitol (24)

A mixture of **21** (13 mg, 0.03 mmol) and 80% aqueous acetic acid (0.4 mL) was stirred for 1.5 h at 50 °C, and then co-evaporated with ethanol and toluene. The residue was chromatographed on a column of silica gel (1 g, MeOH–$CHCl_3$ 1:20) to give the diol **24** (12 mg, ~100%) as a syrup, $R_f = 0.65$ (MeOH–$CHCl_3$ 1:20); $[\alpha]_D^{22}$ +69 (*c* 0.51, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.42–7.20 (m, 10H, 2 × Ph), 5.45 (d, 1H, $J_{5,NH} = 8.5$ Hz, NH), 4.71 and 4.60 (ABq, $J_{gem} = 11.5$ Hz), and 4.56 and 4.31 (ABq, $J_{gem} = 11.8$ Hz) (2 × CH_2Ph), 4.48 (m, 1H, H-5), 3.93–3.77 (m, 4H, H-1, H-2, H-3, H-4), 3.08 and 2.82 (2br s, each 1H, 2 × OH), 2.00 (ddd, 1H, $J_{5,6eq} = 3.3$ Hz, $J_{1,6eq} = 3.8$ Hz, $J_{6gem} = 13.5$ Hz, H-6eq), 1.83 (s, 3H, Ac), 1.79 (m, 1H, H-6ax); ITMS-ESI (positive mode): *m/z* 408 $[M+Na]^+$.

4.11. 5-Amino-5-deoxy-L-*talo*-quercitol (4)

A solution of **24** (30 mg, 0.08 mmol) in ethanol (1 mL) containing a drop of 1 M hydrochloric acid was hydrogenated in the presence of 10% Pd/C (a spoonful) in atmospheric pressure for 14 h at room temperature. A catalyst was removed by filtration and the filtrate was evaporated. The residual product was treated with 6 M hydrochloric acid for 15 h at 80 °C, and then evaporated. The product was purified by a column of Dowex-50 W × 2 (1.2 g, 1% aqueous ammonia) to give the free bose **4** (13.4 mg, ~100%) as a syrup, $R_f = 0.53$ (H_2O–AcOH–*n*-BuOH 1:1:2); $[\alpha]_D^{20}$ −35 (*c* 0.53, H_2O); ^1H NMR (300 MHz, D_2O): δ 3.86 (m, 2H, H-1, H-2), 3.70 (dd, 1H, $J_{4,5} = 3.5$ Hz, $J_{3,4} = 8.3$ Hz, H-4), 3.64 (dd, 1H, $J_{2,3} = \sim 3$ Hz, $J_{3,4} = 8.3$ Hz, H-3), 3.32 (m, 1H, H-5), 1.77 (ddd, 1H, $J_{5,6ax} = 4.0$ Hz, $J_{1,6ax} = 9.9$ Hz, $J_{6gem} = 13.8$ Hz, H-6ax), 1.64 (ddd, 1H, $J_{5,6eq} = 4.6$ Hz, $J_{1,6eq} = 5.1$ Hz, $J_{6gem} = 13.8$ Hz, H-6eq); ITMS-ESI (positive mode): *m/z* 164 $[M+H]^+$.

4.12. 5-Acetamido-3,4-di-*O*-benzyl-5-deoxy-1-*O*-methyl- (25), 2-*O*-methyl- (26), and 1,2-di-*O*-methyl-L-*talo*-quercitols (27)

To a mixture of **24** (81 mg, 0.21 mmol) and acetonitrile (1.6 mL), were added iodomethane (2.6 mL, 42 mmol) and silver oxide (146 mg, 0.63 mmol), and the mixture was refluxed for 4 h, and then evaporated to dryness. The residue was chromatographed on a column on silica gel (11 g, acetone–toluene 1:6) to give the dimethyl ether **27** (28.4 mL, 33%) as a crystalline solid, and an inseparable mixture (44 mg, 52%) of the methyl ethers **25** and **26**. The latter mixture was treated with acetic anhydride (0.5 mL) in pyridine (1 mL) for 13 h at room temperature. The reaction mixture was processed in the usual manner and the products were chromatographed on a column of silica gel (5 g, acetone–toluene 1:8) to give the respective *O*-acetyl methyl ethers **28** (17 mg, 34%) and **29** (24 mg, 49%) as a syrup.

For **27**: $R_f = 0.56$ (acetone–toluene 1:1); $[\alpha]_D^{21}$ +4.7 (*c* 0.37, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.21 (m, 10H, 2 × Ph), 5.51 (br s, 1H, NH), 4.79 and 4.72 (ABq, $J_{gem} = 12.2$ Hz), and 4.55 and 4.44 (ABq, $J_{gem} = 11.3$ Hz) (2 × CH_2Ph), 4.39 (m, 1H, H-5), 3.81 (m, 1H, H-4), 3.68 (br s, 1H, H-2), 3.59 (m, 1H, H-3), 3.52 (s, 3H, OMe), 3.43 (m, 1H, H-1), 3.37 (s, 3H, OMe), 2.05 (m, 2H, 2 × H-6), 1.90 (s, 3H, Ac); ITMS-ESI (positive mode): *m/z* 414 $[M+H]^+$.

For **28**: $R_f = 0.37$ (acetone–toluene 1:1); $[\alpha]_D^{21}$ −9.4 (*c* 0.24, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.32 (m, 10H, Ph), 5.58 (br s, 1H, H-2), 5.51 (d, 1H, $J_{5,NH} = 5.9$ Hz, NH), 4.77 and 4.58 (ABq, $J_{gem} = 12.0$ Hz), and 4.60 and 4.45 (ABq, $J_{gem} = 11.2$ Hz) (2 × CH_2Ph), 4.43 (m, 1H, H-5), 3.77 (dd, 1H, $J_{4,5} = 4.4$ Hz, $J_{3,4} = 7.8$ Hz, H-4), 3.65 (dd, 1H, $J_{2,3} = 2.9$ Hz, $J_{3,4} = 7.8$ Hz, H-3), 3.44 (m, 1H, H-1), 3.36 (s, 3H, OMe), 2.20 (m, 1H, H-6eq), 2.13 and 1.92 (2s, each 3H, 2 × Ac), 1.86 (m, 1H, H-6ax); ITMS-ESI (positive mode): *m/z* 464 $[M+Na]^+$.

For **29**: $[\alpha]_D^{21}$ +23.5 (*c* 0.19, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.33 (m, 10H, 2 × Ph), 5.49 (d, 1H, $J_{5,NH}$ = 6.1 Hz, NH), 5.17 (m, 1H, H-1), 4.79 and 4.68 (ABq, J_{gem} = 12.2 Hz), and 4.60 and 4.45 (ABq, J_{gem} = 11.7 Hz) (2 × CH_2Ph), 3.82 (dd, 1H, $J_{4,5}$ = 3.7 Hz, $J_{3,4}$ = 6.4 Hz, H-4), 3.77 (m, 1H, H-2), 3.66 (br s, 1H, H-3), 3.45 (s, 3H, OMe), 1.96 (m, 2H, 2 × H-6), 2.06 and 1.87 (2s, each 3H, 2 × Ac); ITMS-ESI (positive mode): *m*/*z* 464 $[M+Na]^+$.

4.13. 5-Amino-5-deoxy-1-*O*-methyl-L-*talo*-quercitol (5)

Compound **25** (17 mg, 0.04 mmol) was hydrogenated as in the preparation of **4**, and the product was treated with 1 M aqueous $Ba(OH)_2$ (1 mL) for 20 h at 80 °C. After neutralization with CO_2, an insoluble material was removed by filtration and the filtrate was evaporated. The residue was similarly purified by a column of Dowex 50 W × 2 (H^+) resin (1 g, 1% aqueous ammonia) to give **5** (7 mg, ~100%) as a syrup, R_f = 0.26 (H_2O–AcOH–*n*-BuOH 1:1:4); $[\alpha]_D^{20}$ −10 (*c* 0.24, H_2O); ^1H NMR (300 MHz, D_2O): δ 4.00 (br s, 1H, H-2), 3.75 (dd, 1H, $J_{2,3}$ = 3.7 Hz, $J_{3,4}$ = 7.8 Hz, H-3), 3.62 (dd, 1H, $J_{4,5}$ = ~3 Hz, $J_{3,4}$ = 7.8 Hz, H-4), 3.52 (m, 1H, H-1), 3.29 (m, 1H, H-5), 3.22 (s, 3H, OMe), 1.85 and 1.71 (2m, each 1H, 2 × H-6); ITMS-ESI (positive mode): *m*/*z* 178 $[M+H]^+$.

4.14. 5-Amino-5-deoxy-2-*O*-methyl-L-*talo*-quercitol (30)

A solution of **26** (17 mg, 0.04 mmol) in ethanol (1 mL) containing a drop of 1 M hydrochloric acid was hydrogenated in the presence of 10% Pd/C (a spoonful) under an atmospheric pressure of hydrogen for 16 h at room temperature. The product was purified by a column of Dowex-50 W × 2 (1 g, 1% aqueous ammonia) to give the free base **30** (7 mg, ~100%) as a syrup: R_f = 0.26 (H_2O–AcOH–*n*-BuOH 1:1:4); $[\alpha]_D^{20}$ −38.5 (*c* 0.12, H_2O); ^1H NMR (300 MHz, D_2O): δ 4.01 (br s, 1H, H-5), 3.79 (br s, 1H, H-3), 3.74 (br s, 1H, H-2), 3.44 (br s, 1H, H-4), 3.35 (s, 3H, OMe), 3.29 (m, 1H, H-1), 1.72 (m, 2H, 2 × H-6); ITMS-ESI (positive mode): *m*/*z* 178 $[M+H]^+$.

4.15. 5-Amino-5-deoxy-1,2-di-*O*-methyl-L-*talo*-quercitol (31)

The dibenzyl ether **27** (20 mg, 0.05 mmol) was hydrogenolyzed and subsequently hydrolyzed as in the preparation of **4**. The product was also purified by a column of Dowex-50 W × 2 resin to give the dimethyl ether **31** (4.3 mg, 46%) as a syrup, R_f = 0.36 (H_2O–AcOH–*n*-BuOH 1:1:4); $[\alpha]_D^{20}$ −85 (*c* 0.04, H_2O); ^1H NMR (300 MHz, D_2O): δ 3.67 (m, 4H, H-2, H-3, H-4, H-5), 3.24 and 3.19 (2s, each 3H, 2 × OMe), ~3.2 (m, 1H, H-1), 1.79 and 1.64 (m, each 1H, 2 × H-6); ITMS-ESI (positive mode): *m*/*z* 192 $[M+H]^+$.

5. Biological assay

Determination of IC_{50}: The IC_{50} values were determined for all inhibitors and correspond to the inhibition concentration required for 50% inhibition of the enzyme in our experimental conditions. α-Fucosidase (bovine kidney) was assayed using *p*-nitrophenyl α-L-fucopyranoside (4 mM) as a substrate at pH 5.5 (30 mM CH_3COONa) at 37 °C. α-Galactosidase (green coffee beans) was assayed using *p*-nitrophenyl α-D-galactopyranoside as a substrate at pH 6.8 (20 mM, phosphate buffer) at 27 °C. β-Galactosidase (bovine liver) assayed similarly using *p*-nitrophenyl β-D-galactopyranoside at 37 °C. α-Glucosidase (Baker's yeast) was assayed similarly using *p*-nitrophenyl α-D-glucopyranoside (4 mM) as a substrate at pH 5.5 (30 mM CH_3COONa) at 27 °C. β-Glucosidase (almond) was assayed similarly using *p*-nitrophenyl β-D-glucopyranoside (4 mM) as a substrate at pH 5.5 (30 mM CH_3COONa) at 27 °C. β-Glucosaminidase (bovine kidney) was assayed using *p*-nitrophenyl *N*-acetyl-β-D-glucosaminide (4 mM) as a substrate at pH 5.5 (30 mM CH_3COONa) at 37 °C. α-Mannosidase was assayed using *p*-nitrophenyl α-D-mannopyranoside (4 mM) as a substrate at pH 5.5 (30 mM CH_3COONa) at 27 °C.

Test compound was added to each reaction mixture described above and it was incubated for 1 h at 27 or 37 °C. After 1 h, the reaction was quenched by addition of three volumes of aqueous 0.2 M sodium carbonate, and the absorbance of the liberated *p*-nitrophenol was measured at 410 nm. The percentage inhibition was calculated by the formula $BA \times 100$, where *A* is the *p*-nitrophenol liberated by the enzyme without an inhibitor and *B* is that with an inhibitor.

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12. The reaction conditions have not been optimized yet in order to reduce the elimination products.
13. The reaction conditions have not been optimized yet.
14. All biological assays were carried out in a standard manner by Dr. A. Tomoda.