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Amine-linked diquercitols as new α-glucosidase inhibitors

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ABSTRACT

Two new diastereomeric amine-linked diquercitols **7** and **8** were synthesized by reductive amination of ketoquercitol **4** and epimeric aminoquercitols **3** and **6**. The ketone and amines were successfully prepared, without the formation of byproducts, from naturally available (+)-*proto*-quercitol (**1**). The amine-linked diquercitols showed inhibitory effect against α-glucosidases with more pronounced potency than their original aminoquercitol monomers.

Carbasugars are glycomimics in which the oxygen atom of the pyranose ring is replaced by carbon. They involve several biological events by inhibiting glycosidases, leading to potential applications in the therapy of viral infections,[1] cancer[2] and diabetes.[3] Prominent examples of carbasugars include aminocyclitols or pseudoaminosugars, whose structures comprise one or more amino groups in place of hydroxy moieties. Aminocyclitols are exclusively produced by Actinomycetes bacteria. The presence of amino group as *N*-linked glycosidic bond in various aminocyclitols has been noted for pronounced glycosidase inhibition, which was later elaborated by the unusually tight binding between amino linkage of inhibitor and acidic group of the enzyme.[4] The most recognized aminocyclitols (Fig. 1) that mimic polysaccharide include acarbose, whose structure contains one aminocyclitol connected with trisaccharide. Its potent inhibition against human intestinal glucosidase and wide use for diabetes therapy have triggered the synthesis of aminocyclitols connected with sugar or cyclitol moieties such as oligobiosaminide.[5]

In our previous studies, we have synthesized diastereomeric aminocyclitols from naturally available (+)-*proto*-quercitol.[6] Subsequent investigation by installing a variety of aryl and alkyl moieties onto amino group led to the discovery of more pronounced α-glucosidase inhibitors; some of which are 2–8 times more potent than antidiabetic drug acarbose.[6] To date, over 40 aminoquercitols[6,7] and related analogues[8,9] have been generated from (+)-*proto*-quercitol.

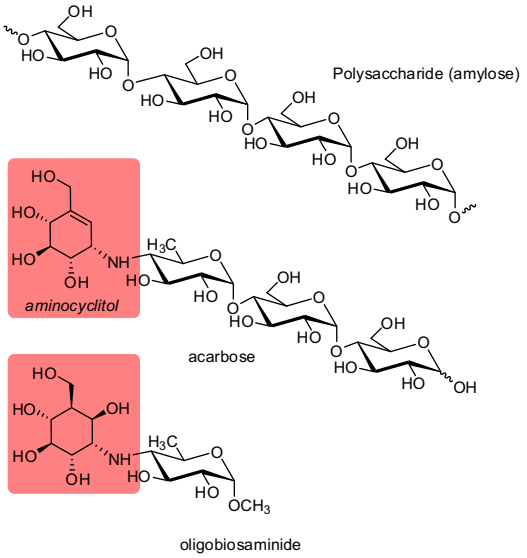

Figure 1. Polysaccharide-mimic aminocyclitols.

The success of preparing diverse aminocyclitol derivatives is due mainly to exclusive formation of single bisacetonide in the first step; in contrast, up to 50% of material is lost if other stereoisomers of quercitol are employed.[10] With the success of our approach in hand, we herein plan to synthesize new aminocyclitols containing two (+)-*proto*-quercitol residues connected through *N*-linked glycosidic bond and evaluate their α-glucosidase inhibition. In fact,

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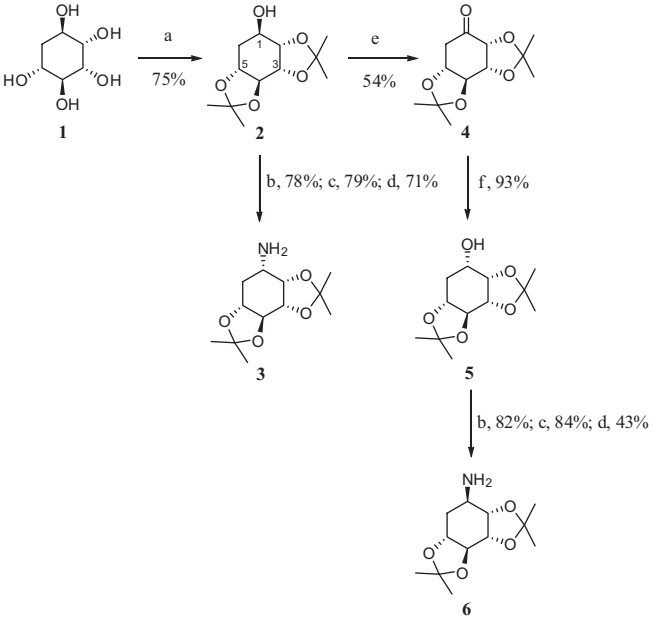
the idea of *N*-linked dicyclitols was introduced by Hudlicky and coworkers since 2002;[11] however, all synthesized targets (Fig. 2) showed weak inhibition (IC_{50} 370–2,000 μM). In the recent study, we hope that the replacement of the above cyclitols by (+)-*proto*-quercitol would promote inhibitory effect more potent than other *N*-linked dicyclitols previously reported.

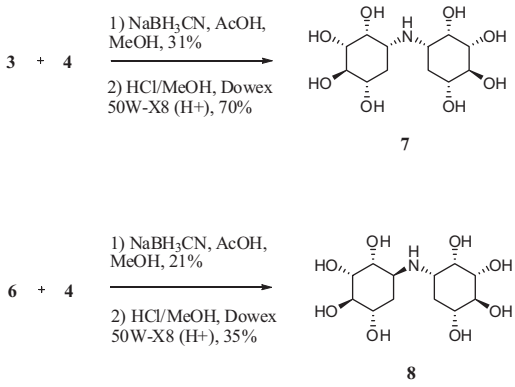
The starting material (+)-*proto*-quercitol (**1**) utilized in this investigation was obtained from the stems of *Arfeuillea arborescens* using the procedure described elsewhere.[6a] Crucial to the successful synthesis of amine-linked diquercitols (**7** or **8**) was the use of reductive amination of aminoquercitols **3** or **6** and ketoquercitol **4** (Scheme 1), in which high diastereoselectivity was observed in each step.

All key coupling intermediates **3**, **4** and **6** were synthesized from (+)-*proto*-quercitol (**1**) (Scheme 2), using our previous methodology.[6a] Initially, hydroxyl groups in **1** were protected by reaction with $Me_2C(COMe)_2$ in the presence of *p*-TsOH as a catalyst, yielding exclusively bisacetonide **2** in 75% yield. This observation could be rationalized by initially favored formation of *cis*-acetonide at C-2 and C-3 followed by inevitable *trans*-acetonide formation at C-4 and C-5, resulting in the free hydroxyl group at C-1 for further functionalization. The remaining hydroxyl group in **2** was converted to a better leaving group (a mesyl moiety), which was further substituted by azide group (NaN_3). The desired aminoquercitol **3** was eventually obtained by hydride reduction using $LiAlH_4$. Noticeably, transformation of **2**–**3** proceeded stereospecifically with inversion of configuration.

Having aminoquercitol **3** in hand, we turned our plan to generate another coupling motif, ketoquercitol **4**. Starting from **2**, the target ketone **4** was synthesized in moderate yield (54%) by Albright-Goldman oxidation[12] using Ac_2O-DMSO. At this step, we also have an idea to produce another aminoquercitol that is epimeric to **3**, which would be coupled to ketone **4** to produce diastereomeric *N*-linked diquercitol. Ketone **4** was first reduced by $LiAlH_4$, affording the hydroxybisacetonide **5** with excellent yield (93%) as sole product. The desired aminoquercitol **6** was thus generated using the similar conditions applied for **3**.



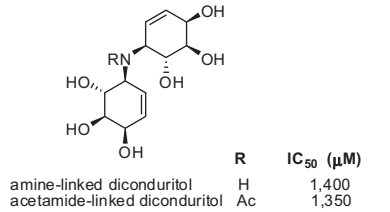
Scheme 2. Reagents and conditions: (a) $Me_2C(OMe)_2$, DMF, *p*-TsOH; (b) $MeSO_2Cl$, Et_3N, DMAP; (c) NaN_3, DMF, 15-crown-5-ether, 100 °C; (d) $LiAlH_4$; (e) Ac_2O, DMSO; (f) $LiAlH_4$.



Scheme 3.

Having aminoquercitols **3** and **6** together with ketone **4** in hand, the required *N*-linked diquercitols were synthesized by reductive amination[13] using $NaBH_3CN$[14] (Scheme 3). Coupling of **3** and **4** followed by deprotection under acid condition afforded amine-linked diquercitol **7** while compound **8** was generated from **6** and **4**. Although amine-linked diquercitols **7** and **8** were obtained as single product in each synthetic route, the configuration of newly generated chiral center (C-1) remained unclear.

The severely overlapped ^1H NMR signals, particularly in diagnostic region (δ 3.0–4.0 ppm), made impossible determination of configuration. We therefore turned our attempt to inspect ^1H NMR data of the bisacetonides (**7a** and **8a**) of **7** and **8** because they displayed well-separated spectra. We have demonstrated, in our previous report,[6a] that ^1H NMR pattern and coupling constants of methylene protons in **3** and **6** are distinct enough to apply for predicting configuration of amino-connected chiral carbon as α- or β-oriented. Apparently, bisacetonide **7a** revealed a single set of methylene protons (H_2-6/and H_2-6′) identical to those of **3** whereas bisacetonide **8a** contained two different patterns of **3** and **6** (Fig. 3).



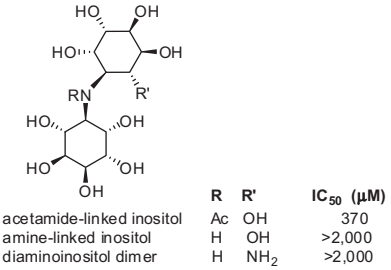
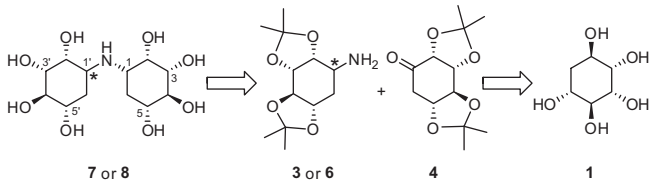


Figure 2.



Scheme 1.

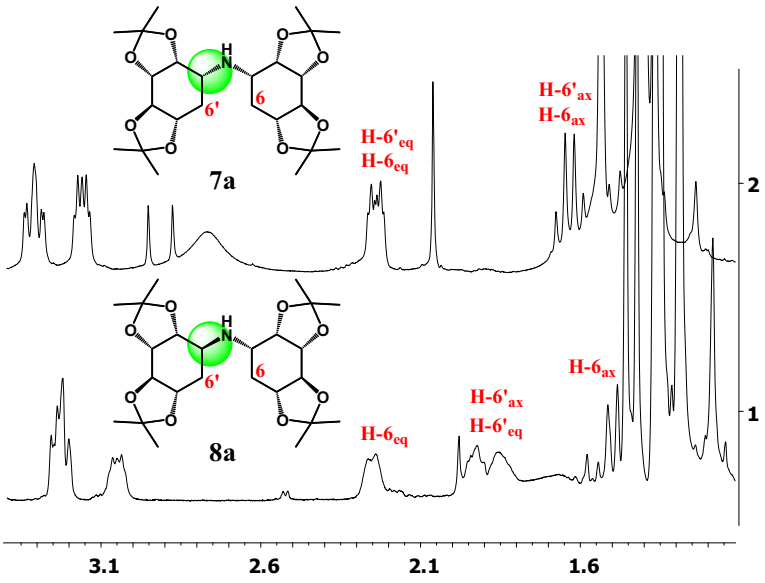
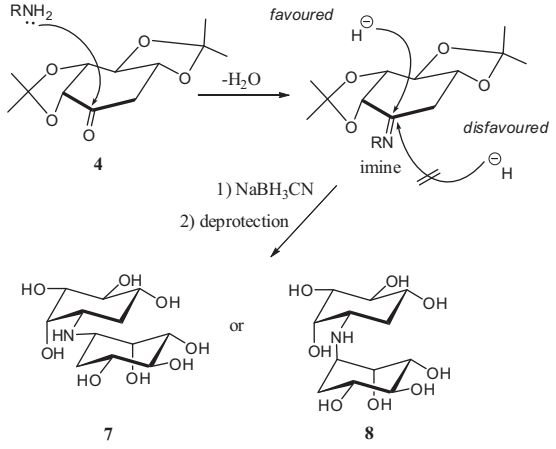


Figure 3. Partial ^{1}H NMR spectra of **7a** and **8a** ($CDCl_3$) focusing on signals of methylene protons (H_2-6 and H_2-6′).



Scheme 4. Proposed mechanistic formations of **7** and **8**.

Table 1
α-Glucosidase inhibitory effect of synthesized compounds

Compound	IC_{50} (μM)		
	Baker's yeast	Maltase	Sucrase
3a	2,890.0	5.8	7.3
6a	12.5	4.4	6.8
7	32.3	3.1	3.7
8	40.1	3.6	4.0
Acarbose	403.9	1.5	2.4

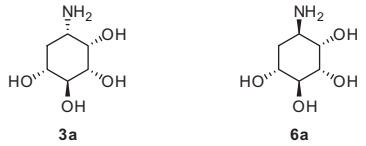


Figure 4.

Therefore, the newly generated chiral C-6s in **7a** and **8a** would be α-oriented as shown. Noticeably, the occurrence of α-oriented C-6 and C-6′ in **7a** resulted in its meso compound character, which was consistent with only six carbon signal observed in ^{13}C NMR spectrum.

Based on aforementioned data, the proposed mechanism on the formation of amine-linked diquercitols **7** and **8** would be rationalized in Scheme 4. Noticeably, a more steric hindrance on the bottom face of imine, contributed by sterical control of acetonides, allowed α-attack of hydride (Na BH_3CN).

Amine-linked diquercitols **7** and **8** were evaluated for inhibitory effect toward α-glucosidases (Table 1) from two different sources; baker's yeast (type I) and rat intestine (maltase & sucrase, type II).[15] They showed moderate inhibition (32.3–40.1 μM) against yeast α-glucosidase while 10-time more potent inhibition (3.1–4.0 μM) toward rat intestine was observed. Although **7** and **8** are diastereomers different in configuration of C-1′, they revealed comparable inhibition against all α-glucosidases tested. Compared to the original aminoquercitols **3a** and **6a** (Fig. 4) obtained from acetonide deprotection of **3** and **6**, the amine-linked diquercitols **7** and **8** showed slightly improved inhibitory effect against maltase and sucrase. In addition, amine-linked diquercitols **7** and **8** revealed much more potent inhibition (ca. 93–500 times) than other related *N*-linked dicyclitols.[11] It is likely that structural motif of cyclitol would also critically participate in exerting the inhibitory effect, in addition to the presence of *N*-linked glycosidic bond.

In conclusion, we reported the first synthesis of diastereomeric amine-linked diquercitols **7** and **8** through reductive amination of ketone **4** and epimeric amines **3** and **6**. The coupling intermediates were successfully prepared, without the formation of byproducts, from naturally available (+)-*proto*-quercitol (**1**). The synthesized amine-linked diquercitols **7** and **8** showed comparable inhibition against α-glucosidases, while the inhibitory effect toward maltase and sucrase were more enhanced than the original aminoquercitol. Interestingly, they inhibited glucosidase function much more potent than other related *N*-linked dicyclitols, therefore suggesting pivotal role of quercitol residue in addition to the presence of nitrogen-linkage.

Acknowledgments

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Supplementary data

Supplementary data (experimental procedure and NMR spectra) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.09.064.

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13. *General procedure for reductive amination*: To a solution of aminocyclitols **3** or **6** (1 equiv) in methanol (1.0 mL/0.1 mmol of aminoquercitol) under an atmosphere of N_2 were treated with sodium cyanoborohydride (2 equiv), acetic acid (4 μL/0.05 mmol of aminoquercitol) and ketone **4** (1–2 equiv). After stirring at room temperature for 24 h, the reaction mixture was evaporated to dryness, quenched with water and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford crude product, which was purified by silica gel or Sephadex LH-20 column chromatography to afford bisacetonides **7a** or **8a**. To a solution of the bisacetonide (**7a** or **8a**) in 1.25 M methanolic HCl (1 mL) were stirred at room temperature for 4 h. The reaction mixture was evaporated to dryness, redissolved in H_2O, loaded onto Dowex 50W-X8 (H^+) column and eluted with H_2O followed by 50% NH_3-H_2O. Fractions eluted with 50% NH_3–H_2O were evaporated to give **7** or **8**. (1*R*,2*S*,3*S*,4*S*,5*S*)-5-((1′*R*,2′*R*,3′*R*,4′*R*,5′*S*)-2,3,4,5-tetrahydroxycyclohexylamino)-cyclohexane-1′,2′,3′,4′-tetraol (**7**) or amine-linked diquercitol **7**: white solid, ^{1}H NMR (D_2O, 400 MHz) δ 3.95 (br s, 1H), 3.34–3.37 (m, 2H), 3.26 (m, 1H), 3.15 (br d, J = 9.6 Hz, 1H), 1.88 (br d, J = 12.0 Hz, 1H), 1.58 (q, J = 11.2 Hz, 1H); ^{13}C NMR (D_2O, 100 MHz) δ 73.7, 71.9, 69.2, 67.8, 50.8, 30.2; HRMS m/z 310.1499 $[M+H]^+$ (calcd for $C_{12}H_{24}NO_8$, 310.1502). (1*R*,2*S*,3*S*,4*S*,5*S*)-5-((1′*S*,2′*R*,3′*R*,4′*R*,5′*S*)-2,3,4,5-tetrahydroxycyclohexylamino)cyclohexane-1′,2′,3′,4′-tetraol (**8**) or amine-linked diquercitol **8**: pale yellow oil, ^{1}H NMR (D_2O, 400 MHz) δ 3.88 (br s, 1H), 3.63–3.66 (m, 3H), 3.51 (m, 1H), 3.25–3.34 (m, 3H), 2.99 (br s, 1H), 2.76 (br d, J = 8.0 Hz, 1H), 1.67–1.80 (m, 3H), 1.39 (m, 1H); ^{13}C NMR (D_2O, 100 MHz) δ 74.4, 73.7, 72.8, 71.8, 71.5, 70.2, 69.5, 69.2, 52.3, 51.3, 32.6, 30.8; HRMS m/z 332.1321 $[M+Na]^+$ (calcd for $C_{12}H_{23}NNaO_8$, 332.1321).
14. Of several hydride reagents examined, reductive amination using $NaBH_3CN$ afforded highest yield of the desired products. Similar approach was also applied for the synthesis of validoxylamine G. See Fukase, H.; Horii, S. *J. Org. Chem.* **1992**, *57*, 3651.
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