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Potent Glycosidase Inhibitors, *N*-Phenyl Cyclic Isourea Derivatives of 5-Amino- and 5-Amino-1-*C*-(hydroxymethyl)-cyclopentane-1,2,3,4-tetraols

Chikara Uchida, Hiroshi Kimura, and Seiichiro Ogawa*

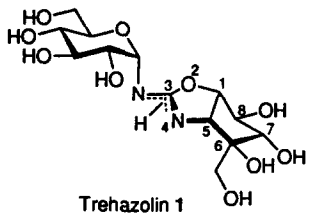
Department of Applied Chemistry, Faculty of Science and Technology, Keio University, Hiyoshi, Kohoku-ku, Yokohama, 223 Japan

ABSTRACT: Nine *N*-phenyl cyclic isourea derivatives of 5-aminocyclopentane-polyols were prepared conventionally, and assayed for enzyme-inhibitory activity against five sugar hydrolases. Two derivatives derived from 1L-(1,2,4,5/3)-5-amino-1-*C*-(hydroxymethyl)cyclopentane-1,2,3,4-tetraol, and two from 1D-(1,2,3,5/4)- and DL-(1,4,5/2,3)-5-aminocyclopentane-1,2,3,4-tetraols have been shown to possess strong potency against α-glucosidase and β-galactosidase, respectively.

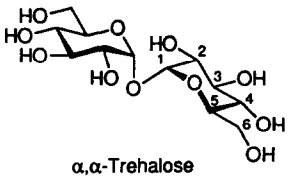
In connection with the synthetic studies[1] on sugar hydrolase inhibitors, interests in the unique structure of a potent and specific trehalase inhibitor trehazolin **1**[2] has prompted us to synthesize several aminocyclopentanetetraol derivatives containing cyclic isourea functions and to subject them to bioassay on inhibitory activities against several enzymes.

Trehazolin **1** possesses a pseudo-disaccharide structure composed of α-D-glucopyranosylamine and trehazolamine **D-2**, 1D-(1,3/2,4,5)-5-amino-1-*C*-(hydroxymethyl)cyclopentane-1,2,3,4-tetraol,[3] linked by way of a cyclic isourea group. On the basis of the structural feature of **1**, the aminocyclitol moiety having cyclic isourea may be assumed to correspond with one of the two α-D-glucopyranose residues as the mimic of symmetric α,α-trehalose (Scheme 1). In fact, topology of the hydroxyl groups of the cyclitol part has been shown[4,5] to play an important role for exerting the activity. On the other hand, the cyclic isourea moiety would constitute the charge distribution part for binding the active site of the enzymes.[6] Therefore, by analogy, it may be possible to design a new sugar hydrolase inhibitors composed of modified aminocyclitols, having the 3-amino-2-oxa-4-azabicyclo[3.3.0]oct-3-ene structure.

Scheme 1



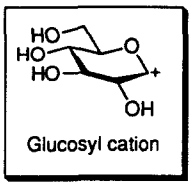
Trehazolin 1

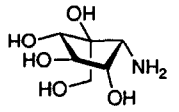


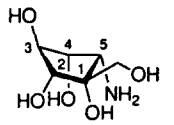
α,α-Trehalose

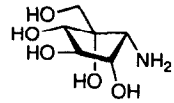
Since compound **D-2** had been described[7] as a mild inhibitor against β-glucosidase, we reinvestigated the inhibitory activity of **D-2**, as well as its 1-epimers,[4] 1D, 1L-(1,2,4,5/3)-5-amino-1-*C*-(hydroxymethyl)-cyclopentane-1,2,3,4-tetraols **D,L-3** against α- and β-glucosidases. When the structural features[8] of **D-2** and **D,L-3** were compared, **L-3** was thought to have more similarity to the flattened half-chair conformation of the glucosyl cation probably formed during hydrolysis[6] (Scheme 2). As had been expected, it was demonstrated that **L-3** possessed a strong potency against Baker's yeast α-glucosidase and a mild activity against almonds β-glucosidase, while **D-3** had a weak potency against α-glucosidase (Table 1). On the other hand, **D-2** was shown to be a weak α-glucosidase inhibitor (IC_{50} 2.62 x 10^{-4} M) and a mild β-glucosidase inhibitor (IC_{50} 2.12 x 10^{-5} M). These results would seemingly support the feasibility to design new glycosidase-inhibitors on the structural basis.

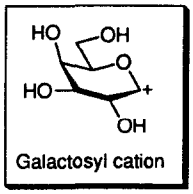
Scheme 2

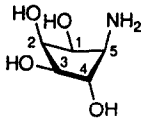


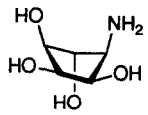

Trehazolamine **D-2**

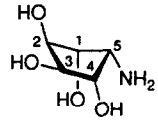

D-3


L-3




D-4

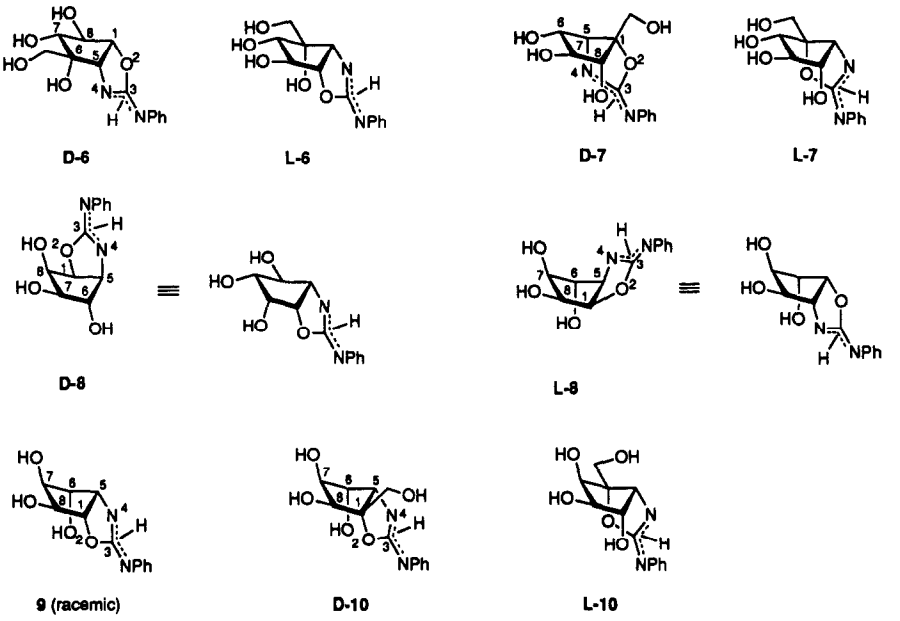

L-4


5 (meso)

Next, readily available two 5-aminocyclopentane-1,2,3,4-tetraols **4** and **5** , which mimic the half-chair conformation of galactosyl cation, were chosen to test the inhibitory activity against galactosidases (Scheme 2). The penta-*N*,*O*-acetyl derivative[9] of **DL-4** and **5**[10] have been known so far. Then the enantiomeric **D,L-4** were newly synthesized[11] by four-step sequence starting from the 2,3-*O*-cyclohexylidene derivative **10**[12] of (1,4/2,3,5)-5-acetamidocyclopentan-1,2,3,4-tetraol. Among them, as had been expected on the basis of the structural model, only **D-4** was shown to be a mild inhibitor against *E. coli* β-galactosidase (Table 1). Interestingly, **D-4** and **5** also possessed a weak potency against β-glucosidase.

The above observation has stimulated us to study in detail the biological activity of the derivatives readily accessible by modification of the aminocyclitols of this kind. Therefore, in order to increase the inhibitory activities by changing somewhat charge distribution and their conformation, *N*-phenyl cyclic isourea groups having hydrophobic functions were introduced into them. Thus, nine *N*-phenyl isoureas **D,L-6**, **D,L-7**, **D,L-8**, **9**, and **D,L-10** were synthesized conventionally[4] from the corresponding aminocyclitols by coupling with phenylisothiocyanate, followed by treatment with yellow mercury(II) oxide[13] (Scheme 3).

Scheme 3



Inhibitory-activities of the *N*-phenyl cyclic isourea derivatives[14] were listed in Table 1. Surprisingly enough, significant improvement in rather specific inhibitory activity against α-glucosidase were observed for the derivatives **L-6** and **L-7** (diastereomeric pairs).[15] Also, the derivatives **D,L-8** were shown to act strongly on both β-glucosidase and β-galactosidase. The configurations of the hydroxyl groups, including the oxygen atoms of the isoureas, seemed to be essentially important for obtaining specificity on action. However, the topology of hydroxyl groups on the positions (C-8 of **D-8**, and C-7 of **9** and **D-10**) possibly corresponding to the C-4 of glycosyl cations were not so much important for its binding to the enzymes. In fact, *N*-phenyl isourea derivative **9** (racemic) of **5** was a potent β-galactosidase inhibitor as well as a mild β-glucosidase inhibitor. Furthermore, although the 8-epimer **D-10** of **D-7** possessed a significant potency against both β-glucosidase and β-galactosidase, its enantiomer **L-10**, which in appearance more resembled the galactosyl cation, was a weak inhibitor.[16] It is interesting of note that the respective enantiomers **D-6**, **D-7** and **L-8** in all cases still possess the activity toward β-galactosidase to a considerable extent. Therefore, these new inhibitors would belong both to the mannostatin[17] type inhibitor that has an exocyclic nitrogen and to the deoxynojirimycin[18] type inhibitor that has a nitrogen in the ring.

In summary, the present results have demonstrated that the *N*-substituted 3-amino-2-oxa-4-azabicyclo-[3.3.0]oct-3-ene-6,7,8-triols and the 6-(hydroxymethyl) derivatives thereof would be leading compounds suitable for designing new potential sugar-hydrolase inhibitors.

Table 1 Inhibitory activity of compounds **D,L-3**, **D,L-4**, **5**, **D,L-6—8**, **9**, and **D,L-10** against five sugar hydrolases

Compound	Inhibitory activity (IC_{50})/M				
	α-Glucosidase[19] (Baker's yeast)[a]	β-Glucosidase[20] (Almonds)[b]	α-Galactosidase[21] (*E. coli*)[c]	β-Galactosidase[22] (*E. coli*)[d]	β-Galactosidase[23] (Bovine liver)[d]
D-3	1.62×10^{-4}	—[e]	—	—	—
L-3	4.02×10^{-7}	2.93×10^{-5}	—	—	3.63×10^{-4}
D-4	—	8.38×10^{-5}	—	7.78×10^{-6}	4.69×10^{-5}
L-4	—		—	1.29×10^{-4}	—
5	—	1.17×10^{-5}	—	—	2.41×10^{-4}
D-6	2.32×10^{-6}	—	—	—	3.00×10^{-6}
L-6	2.93×10^{-8}	—	—	—	1.53×10^{-4}
D-7	9.99×10^{-7}	—	—	—	1.89×10^{-6}
L-7	1.03×10^{-8}	—	—	—	5.17×10^{-5}
D-8	—	2.16×10^{-6}	—	2.00×10^{-7}	—
L-8	—	6.39×10^{-6}	—	9.39×10^{-7}	—
9 (racemic)	—	2.40×10^{-5}	—	—	2.40×10^{-7}
D-10	—	4.89×10^{-6}	—	—	5.71×10^{-7}
L-10	—	2.11×10^{-4}	1.18×10^{-4}	—	2.39×10^{-5}
Deoxynojirimycin[18]	9.19×10^{-5}	1.47×10^{-4}	----[f]	----	—

[a] *p*-Nitrophenyl α-D-glucopyranoside (0.66 mM), phosphate buffer (100 mM), pH 6.8. [b] *p*-Nitrophenyl β-D-glucopyranoside (0.33 mM), Acetate buffer (100 mM), pH 5.0, [c] *p*-Nitrophenyl α-D-galactopyranoside (2.0 mM), phosphate buffer (100 mM), pH 6.5. [d] *o*-Nitrophenyl β-D-galactopyranoside (2.5 mM), phosphate buffer (50 mM), pH 7.3, $MgCl_2$ (1.3 mM), 2-mercaptoethanol (100 mM). [e] Activity less than IC_{50} 3.0×10^{-4} M. [f] Not measured.

References and Notes

1 Ogawa, S.; Aso, D. *Carbohydr. Res.*, **1993**, *250*, 177–184.

2 Ando, O.; Satake, H.; Itoi, K.; Sato, A.; Nakajima, M.; Takahashi, S.; Haruyama, H.; Okuma, Y.; Kinoshita, Y.; Enokita, R. *J. Antibiot.*, **1991**, *44*, 1165–1168.

3 In this paper, nomenclature of cyclitols follows IUPAC-IUB 1973 Recommendations for Cyclitol (*Pure Appl. Chem.*, **1974**, *37*, 285–297). The stereochemical feature of cyclitol is shown by a fractional notation whereby numerals in the numerator denote hydroxy or other groups above the plane of the ring while numerals in the denominator denote hydroxy or other groups below the plane.

4 Uchida, C.; Yamagishi, T.; Ogawa, S. *J. Chem. Soc., Perkin Trans. 1*, 1994, 589–602.

5 Uchida, C.; Kitahashi, H.; Yamagishi, T.; Iwaisaki, Y.; Ogawa, S. *J. Chem. Soc., Perkin Trans. 1.*, 1994 in press.

6 a) Legler, G. *Advan. Carbohydr. Chem. Biochem.*, **1990**, *48*, 319–384; b) Look, G. C.; Fotsch, C. H.; Wong, C-H. *Acc. Chem. Res.*, **1993**, *26*, 182–190.

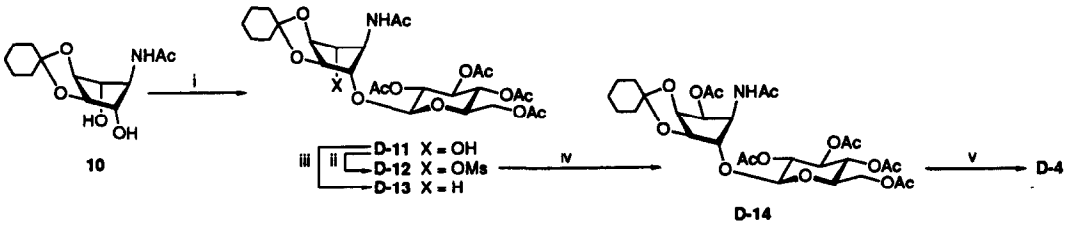
7 Ando, O.; Nakajima, M.; Hamano, K.; Itoi, K.; Takahashi, S.; Takamatsu, Y.; Sato, A.; Enokita, R.; Okazaki, T.; Haruyama, H.; Kinoshita, T. *J. Antibiot.*, **1993**, *46*, 1116–1125.

8 For convenience, all conformation formulae of the amino cyclitols and derivatives thereof only depict with reference to those of glycosyl cations postulated during hydrolysis, being not based on the ^1H NMR data.

9 Suami, T.; Tadano, K.; Nishiyama, S.; Lichtenthaler, F. W. *J. Org. Chem.*, **1973**, *38*, 3691–3696.

10 Suami, T.; Nishiyama, S.; Tadano, K.; Lichtenthaler, F. W. *Bull. Chem. Soc. Jpn.*, **1973**, *46*, 2562–2564.

Scheme 4



Scheme 4 *Reagents and conditions*: i, 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide, AgOTf, tetramethylurea, CH_2Cl_2, 0°C; ii, $MeSO_2Cl$, pyridine, room temp.; iii, a) 1,1'-thiocarbonyldiimidazole, 1,2-dichloroethane, 100°C; b) *n*-Bu_3SnH, AIBN, $PhCH_3$, reflux; iv, a) AcONa, aq. 80% *N,N*-dimethylformamide, 110°C; b) Ac_2O, pyridine, room temp.; v, 2 N HCl, 80°C.

11 The glucoside **D-11**, obtained by diastereospecific glycosylation of **10**[12] with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (31% yield), was converted into the 1-mesylate **D-12** (94%). Treatment of **D-11** with sodium acetate in aq. *N,N*-dimethylformamide resulted in inversion of the configuration at C-1 through neighboring assistance to give, after acetylation, **D-14** quantitatively. Finally, the protecting groups and glucose residue were removed by hydrolysis with 2 N hydrochloric acid to give **D-4**, $[\alpha]_D^{20}$ +8.2 (*c* 0.50, H_2O), quantitatively. Likewise, the enantiomer **L-4**, $[\alpha]_D^{20}$ –7.3 (*c* 0.55, H_2O) was obtained from the diastereoisomer. For establishment of the absolute configuration, **D-11** was conventionally transformed[4] into the deoxy derivative **D-13** , which was then converted into the known optically active (2*R*)-2-acetamido-1,4-di-*O*-acetylbutane-1,4-diol by periodate oxidation and subsequent reduction.[4]

12 Ahluwalia, R; Angyal, S. J.; Luttrell, B. M. *Aust. J. Chem.*, **1970**, *23*, 1819–1829.

13 In general, the amine was allowed to react with phenylisothiocyanate (1.5 molar equiv.) in aq. 60% ethanol for 3 h at room temp. The thiourea obtained was then treated with yellow mercury(II) oxide (3 molar equiv.) in acetone–ethanol (1:1, v/v) for 3–5 h at room temperature to give the *N*-phenyl cyclic isourea in >90% overall yield.

14 The D,L-notation of the compound-numbers (**6—8, 10**) refers to that of the absolute configuration of the cyclitol moiety. All new compounds were fully characterized by IR, ^{1}H NMR and elemental analyses. The pair of enantiomers used in this paper were obtained by chromatographic separation of their diastereoisomers derivatized. The biological data suggested that they are pure enough for the present study. We thank Drs. T. Ouchi and Y. Fukuda (Meiji Seika Kaisha, Yokohama) for helpful discussion on carrying out bioassay.[19–23]

15 The *N*-phenyl cyclic isourea derivative of **D-2** also showed an improved potency against α-glucosidase (IC_{50} 1.34 x 10^{-6} M). In addition to enhancement of the charge distoribution, introduction of the isourea rings probably tends to fix the conformations of the inhibitors, as well as to share hydrophobic parts to the molecules, being favorable for binding to the active sites of the enzymes.

16 Although the 2-epimers of **D,L-3**, the parent aminocyclitols of **D,L-10**, had first been expected to possess a high potency against α- and/or β-galactosidases based on molecular modeling study, they did not show any observable potency at the concentration less than 3.0 x 10^{-4} M. Therefore, derivatization into the *N*-phenyl cyclic isourea compounds dramatically changed their biological property toward the enzymes.

17 Aoyagi, T.; Yamamoto, T.; Kojiri, K.; Morishima, H.; Nagai, M.; Hamada, M.; Takeuchi, T.; Umezawa, H. *J. Antibiot.*, **1989**, *42*, 883–889.

18 Chida, N.; Furuno, Y.; Ikemoto, H.; Ogawa, S. *Carbohydr. Res.*, **1992**, *237*, 185–194.

19 Halvorson, H.; Ellias, L. *Biochim. Biophys. Acta.*, **1958**, *30*, 28–40.

20 Kobayashi, A. *Agr. Biol. Chem.*, **1962**, *26*, 203–207.

21 Suzuki, H.; Li, S-C.; Li, Y-T. *J. Biol. Chem.*, **1970**, *245*, 781–786.

22 Craven, G. R.; Steers, Jr., E.; Anfinsen, C. B. *J. Biol. Chem.*, **1965**, *240*, 2468–2477.

23 Aoyagi, T.; Hazato, T.; Kumagai, M.; Hamada, M.; Takeuchi, T.; Umezawa, H. *J. Antibiot.*, **1975**, *28*, 1006–1008.

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