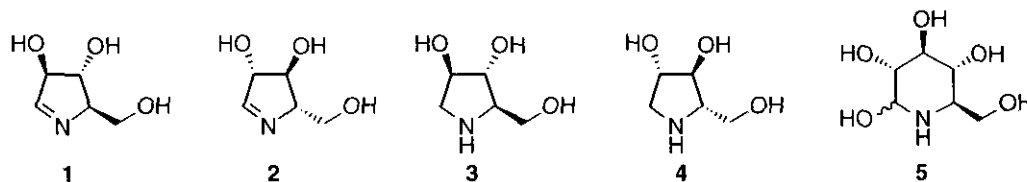


SYNTHESIS OF UNNATURAL ENANTIOMER OF NECTRISINE
AND ITS BIOLOGICAL ACTIVITYNaoto Kogoshi,^{a,b} Akira Takatsuki,^b Yong Jip Kim,^a and Takeshi Kitahara^{*a}^aDepartment of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan^bAnimal and Cellular Systems Laboratory, The Institute of Physical and Chemical Research (RIKEN), 2-1, Hirosawa, Wako-shi, Saitama 351-0198, Japan**Abstract** - *ent*-Nectrisine (**2**), the unnatural enantiomer of nectrisine (**1**), a potent α -glucosidase inhibitor, was synthesized from D-glucose derivative. Biological assay showed that **2** had no significant bioactivity.**Introduction**

Since nojirimycin (**5**)¹ was isolated as a potent glucosidase inhibitor, many glycosidase inhibitors, which have sugar-like skeleton and nitrogen function located in the ring, were isolated from natural origins.² Much efforts have also been paid to develop new and efficient glycosidase inhibitors synthetically.³

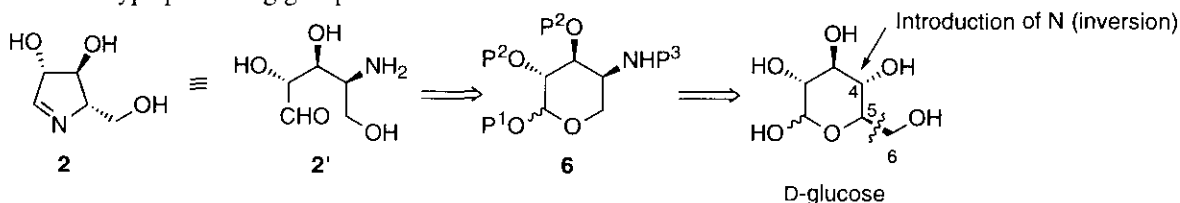
Nectrisine (**1**)^{4,5} was isolated as an immunomodulator from *Nectria lucida*, and was shown to be a potent inhibitor of α -glucosidase and α -mannosidase. Furthermore, **1** shows inhibitory activity against processing glucosidase, which is involved in glycoprotein synthesis,⁶ at cellular level as well as enzymatic level.⁷ While both DAB-1 (**3**),⁸ reduced form of nectrisine (**1**), isolated from *Arachniodes standishii* and *Angylocalyx boutiqueanus*, and its enantiomer LAB-1 (**4**), chemically synthesized, were reported to have inhibitory activity against glycosidases.⁹ Moreover, LAB-1 (**4**) inhibits HIV replication more effectively than DAB-1 (**3**), which implies that **4** is a more powerful inhibitor of processing glucosidase than **3**.¹⁰

**Figure 1**

Therefore we expected *ent*-nectrisine (**2**) to be also an efficient inhibitor of processing glucosidase. Although **2** has been already synthesized as a component of lipopolysaccharides,¹¹ bioactivity of **2** was not known. Thus we were interested in the synthesis and biological activity of **2**. We describe herein the synthesis of **2** from D-glucose derivative, and the results of bioassay on inhibitory activity of **2** against α -glucosidases and effects at cellular level.

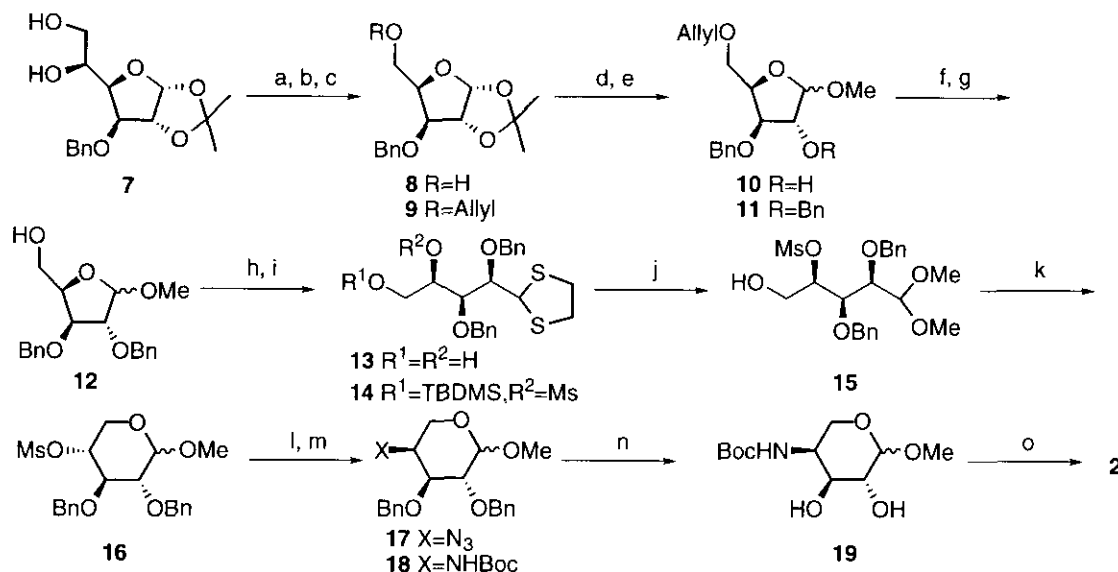
Results and Discussions

We planned to synthesize **2** from D-glucose, cleaving C5-C6 bond and introducing nitrogen function at C4 by SN2 reaction. For this purpose, multi-hydroxyl functions of glucose had to be protected with different type protecting groups.



Scheme 1

We started the synthesis from known 1,2-*O*-isopropylidene-3-*O*-benzyl- α -D-glucofuranose (**7**),¹² which was readily prepared from commercially available diacetone-D-glucose. 1,2-Diol part of **7** was cleaved with NaIO₄, and the resulting aldehyde was reduced with NaBH₄ to give 1,2-*O*-isopropylidene-3-*O*-benzyl- α -D-xylofuranose (**8**) (2 steps, 95%). Hydroxyl group at C5 of **8** was protected as allyl ether (quant.), which is resistant against both acidic and basic conditions. Methanolysis of **9** with acidic ion-exchange resin in methanol gave methyl xylofuranoside (**10**) (88%) and 2-hydroxyl group of **10** was protected as benzyl ether (86%), similarly as 3-hydroxyl group.



a) NaIO₄, Et₂O-H₂O; b) NaBH₄, MeOH; 2 steps, 95%; c) NaH, allyl bromide, THF; quant.; d) Amberlyst -15(H⁺), MeOH; 88%; e) NaH, BnBr, *n*-Bu₄NI, THF; 86%; f) *t*-BuOK, DMSO, 100°C, 20 min; g) formic acid, Et₂O; 2 steps, 66%; h) 1,2-ethanedithiol, conc. HCl, CHCl₃; 71%; i) TBDMSCl, DMAP, Et₃N, CH₂Cl₂, 0°C, overnight; then MsCl, 0°C, 5 h; 91%; j) Hg(ClO₄)₂, MeOH, 1 h; k) 10% HCl in MeOH; 2 steps; 78%; l) NaN₃, DMF, 120°C, 10 h; 91%; m) H₂, 5% Pd-Al₂O₃, Boc₂O, EtOAc; 93%; n) H₂, 20% Pd(OH)₂-C, MeOH, overnight; 79%; o) 6N HCl, 80°C, 40 min; then Dowex 1X2 (OH⁻); 51%

Scheme 2

Then allyl ether of **11** was deprotected by conventional method¹³ (*t*-BuOK, DMSO, 100°C, 20 min; H⁺; 2 steps, 66%). Acetal linkage was opened by dithioacetal formation (78%). The primary hydroxyl group of the resulting diol (**13**) was protected as TBDMS ether and secondary hydroxyl group is subsequently mesylated in one pot to give **14** (71%).

Substitution reaction of **14** with azide salts (sodium azide, or tetra-*n*-butylammonium azide¹⁴) in DMF at elevated temperature (90-120°C) did not give the desired product, and attempted reaction to obtain more reactive trifluoromethanesulfonate corresponding to **14** only gave decomposed products, presumably because of intramolecular reaction between sulfonate and nucleophilic sulfur atom. Therefore we removed dithioacetal before substitution reaction to form methyl pyranoside (**16**). Treatment of **14** with Hg(ClO₄)₂ in methanol cleaved also TBDMS ether as expected by acid generated during deprotection of dithioacetal, to give dimethyl acetal (**15**), but not **16**. The dimethyl acetal (**15**) was further treated with HCl in methanol to give methyl pyranoside (**16**) (2 steps, 78%). Then, mesyl group was substituted with sodium azide in DMF at 120°C to give the azide (**17**) (91%). Catalytic hydrogenation of azide in the presence of di-*t*-butyl dicarbonate (Boc₂O)¹⁵ gave the *t*-butyl carbamate (**18**) (93%), followed by deprotection of benzyl ethers by catalytic hydrogenation to give **19** (79%).

Finally, removal of Boc protecting group and methyl acetal under acidic condition, followed by treatment with basic ion-exchange resin, gave *ent*-nectrisine (**2**)¹⁶ (51%). Specific rotation of synthesized **2** was $[\alpha]_D^{22} -19.4^\circ$ (*c*=0.49, H₂O), which had almost the same absolute value and opposite sign compared to that of natural nectrisine (**1**)($[\alpha]_D^{23} +21.8^\circ$ (*c*=0.6, H₂O)).⁴

Synthetic *ent*-nectrisine (**2**) was assayed for its inhibitory activity against α -glucosidase (yeast), and processing glucosidase (rat liver microsome). But contrary to our expectation, IC₅₀ values of **2** against these glucosidases were larger than 1mM. At cellular level, **2** inhibits syncythium formation of Newcastle disease virus-infected BHK cells only above 2.0 mM.¹⁷

In conclusion, we completed the stereospecific synthesis of *ent*-nectrisine (**2**) in 8.2% of total yield through 15 steps from D-glucose derivative (**7**). Bioassay of the synthetic *ent*-nectrisine (**2**) revealed that it shows extremely low inhibitory activity against glucosidases at enzymatic and cellular level. These results indicate that the relationship of natural nectrisine (**1**) and *ent*-nectrisine (**2**) is not parallel to that of DAB-1 (**3**) and LAB-1 (**4**). Further study of structure-activity relationship of nectrisine-type analogs should be required and are in progress in this group.¹⁸ Detailed results will be reported in a full account.

REFERENCES AND NOTES

1. N. Ishida, K. Kumagai, T. Niida, T. Tsuruoka, and H. Yumoto, *J. Antibiot. Ser. A*, 1967, **20**, 66; S. Inouye, T. Tsuruoka, T. Ito, and T. Niida, *Tetrahedron*, 1968, **24**, 2125.
2. L. E. Fellows, *Chem. Brit.*, 1987, **23**, 842; B. Winchester, and G. W. J. Fleet, *Glycobiology*, 1992, **2**, 199; and references cited therein.
3. A. Karpas, G. W. J. Fleet, R. A. Dwek, S. Petursson, S. K. Namgoong, N. G. Ramsden, G. S. Jacob, and T. W. Rademacher, *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 9229; P. S. Sunkara, D. L. Taylor, M.S.

- Kang, T. L. Bowlin, P. S. Liu, A. S. Tymes, and A. Sjoerdsma, *Lancet*, 1989, **i**, 1206; T. M. Jespersen, W. Dong, M. R. Sierks, T. Skrydstrup, I. Lundt, and M. Bols, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 1778; C. Uchida, H. Kimura, S. Ogawa, *Bioorg. Med. Chem. Lett.*, 1994, **4**, 2643.
4. T. Shibata, O. Nakayama, Y. Tsurumi, M. Okuhara, H. Terano, and M. Kohsaka, *J. Antibiot.*, 1998, **41**, 296.
 5. For chemical syntheses of **1**, see; H. Kayakiri, K. Nakamura, S. Takase, H. Setoi, I. Uchida, H. Terano, M. Hashimoto, T. Tada, and S. Koda, *Chem. Pharm. Bull.* 1991, **39**, 2807; S.-H. Chen and S. J. Danishefsky, *Tetrahedron Lett.*, 1990, **31**, 2229; Y. J. Kim and T. Kitahara, *ibid.*, 1997, **38**, 3423.
 6. R. Kornfeld, and S. Kornfeld, *Ann. Rev. Biochem.* 1985, **54**, 631; U. Fuhrmann, E. Bause, and H. Ploegh, *Biochim. Biophys. Acta*, 1985, **825**, 95; A. D. Elbein, *FASEB J.*, 1991, **5**, 3056.
 7. E. Tsujii, M. Muroi, N. Shiragami, and A. Takatsuki, *Biochem. Biophys. Res. Commun.*, 1996, **220**, 459.
 8. J. Furukawa, S. Okuda, K. Saito, and S. Hatanaka, *Phytochemistry*, 1985, **24**, 593; R. J. Nash, J. M. Williams, and E. A. Bell, *Phytochemistry*, 1985, **24**, 1620.
 9. G. W. J. Fleet, S. J. Nicholas, P. W. Smith, S. V. Evans, L. E. Fellows, and R. J. Nash, *Tetrahedron Lett.*, 1985, **26**, 3127.
 10. G. W. J. Fleet, A. Karpas, R. A. Dwek, L. E. Fellows, A. S. Tymes, S. Petursson, S. K. Namgoong, N. G. Ramsden, P. W. Smith, J. C. Son, F. Wilson, D. R. Witty, G. S. Jacob, and T. W. Rademacher, *FEBS Lett.*, 1988, **237**, 128; and references cited therein.
 11. J. J. Naleway, C. R. H. Raetz, and L. Anderson, *Carbohydr. Res.*, 1988, **179**, 199.
 12. R. E. Gramera, R. M. Bruce, S. Hirase, and R. L. Whistler, *J. Org. Chem.*, 1963, **28**, 1401.
 13. J. Gigg, and R. Gigg, *J. Chem. Soc. C*, 1966, 82.
 14. A. Brändström, B. Lamm, and I. Palmertz, *Acta Chem. Scand. B*, 1974, **28**, 699.
 15. S. Saito, H. Nakajima, M. Inaba, T. Moriwake, *Tetrahedron Lett.*, 1989, **30**, 837.
 16. Spectroscopic data of synthetic **2**: $^1\text{H-NMR}$ (300 MHz, D_2O) δ 3.08-4.15 (5H, m), 7.70 (1H, s); $^{13}\text{C-NMR}$ (75 MHz, D_2O) δ 61.7, 77.3, 78.7, 83.8, 170.9; IR (KBr) 3270, 2920, 1640, 1560, 1400, 1050, 870 cm^{-1} .
 17. Methods of the bioassay were the same as described in reference 7
 18. Y. J. Kim, A. Takatsuki, N. Kogoshi, and T. Kitahara, *Tetrahedron*, in press.

Received, 30th April, 1998