SYNTHESIS OF UNNATURAL ENANTIOMER OF NECTRISINE AND ITS BIOLOGICAL ACTIVITY

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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]

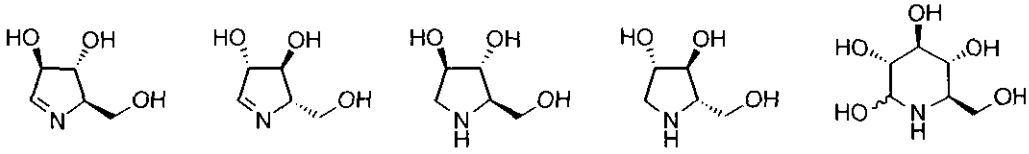
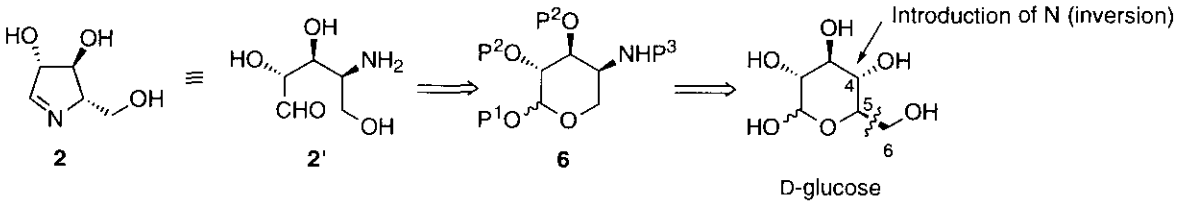


Figure 1

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.

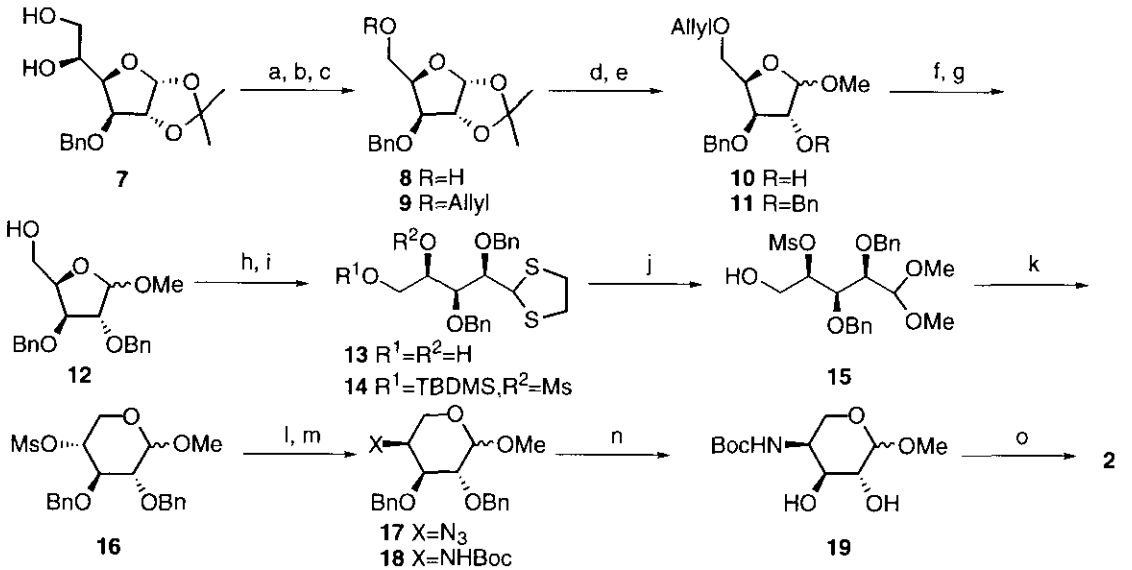
Results and Discussions

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 $NaIO_4$, and the resulting aldehyde was reduced with $NaBH_4$ 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) $NaIO_4$, Et_2O-H_2O; b) $NaBH_4$, MeOH; 2 steps, 95%; c) NaH, allyl bromide, THF; quant.; d) Amberlyst -15(H^+), MeOH; 88%; e) NaH, BnBr, *n*-Bu_4NI, THF; 86%; f) *t*-BuOK, DMSO, 100°C, 20 min; g) formic acid, Et_2O; 2 steps, 66%; h) 1,2-ethanedithiol, conc. HCl, $CHCl_3$; 71%; i) TBDMSCl, DMAP, Et_3N, CH_2Cl_2, 0°C, overnight; then MsCl, 0°C, 5 h; 91%; j) $Hg(ClO_4)_2$, MeOH, 1 h; k) 10% HCl in MeOH; 2 steps; 78%; l) NaN_3, DMF, 120°C, 10 h; 91%; m) H_2, 5% Pd-Al_2O_3, Boc_2O, EtOAc; 93%; n) H_2, 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 method[13] (*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 azide[14]) in DMF at elevated temperature (90-120°C) did not give the desired product, and attempted reaction to obtain more reactive trifluoromethansulfonate 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 $Hg(ClO_4)_2$ 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 (Boc_2O)[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° (c=0.49, H_2O), which had almost the same absolute value and opposite sign compared to that of natural nectrisine (**1**)($[\alpha]_D^{23}$+21.8° (c=0.6, H_2O)).[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, IC_{50} 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. Tyms, 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. Tyms, 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}H-NMR (300 MHz, D_2O) δ 3.08-4.15 (5H, m), 7.70 (1H, s); ^{13}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.

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