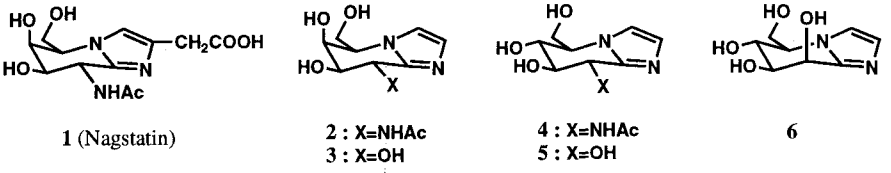
## Syntheses and Glycosidase Inhibiting Activities of Nagstatin Analogs

Sir:

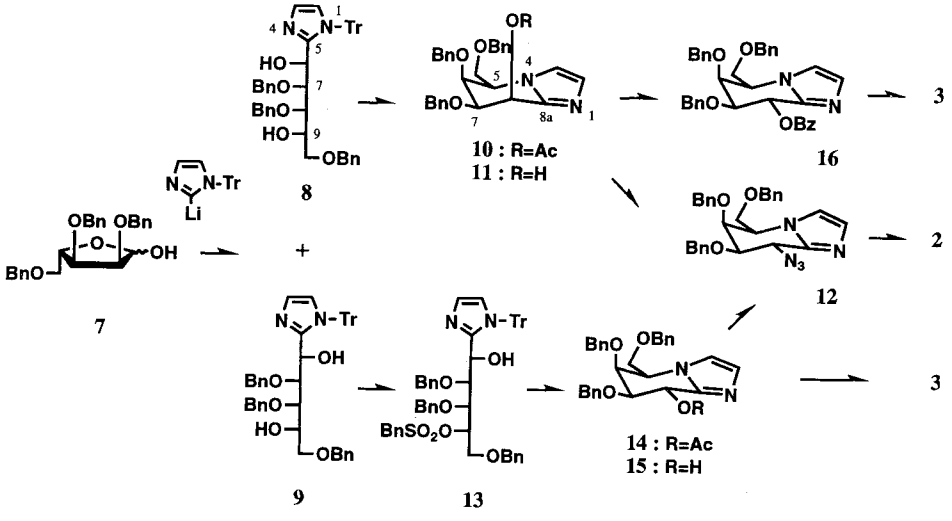
Nagstatin (**1**) is a novel *N*-acetyl-β-D-glucosaminidase inhibitor isolated from culture filtrates of *Streptomyces amakusaensis*, and is structurally a nitrogenous *N*-acetyl-galactosamine analog fused with an imidazole ring.[1] Recently, we have synthesized de-branched nagstatin analogs having different configurations and functionalities, and then determined the absolute structure of nagstatin (**1**).[2]

Herein, we describe the syntheses and evaluation of the glycosidase inhibiting activities of analogs **2**~**6** as

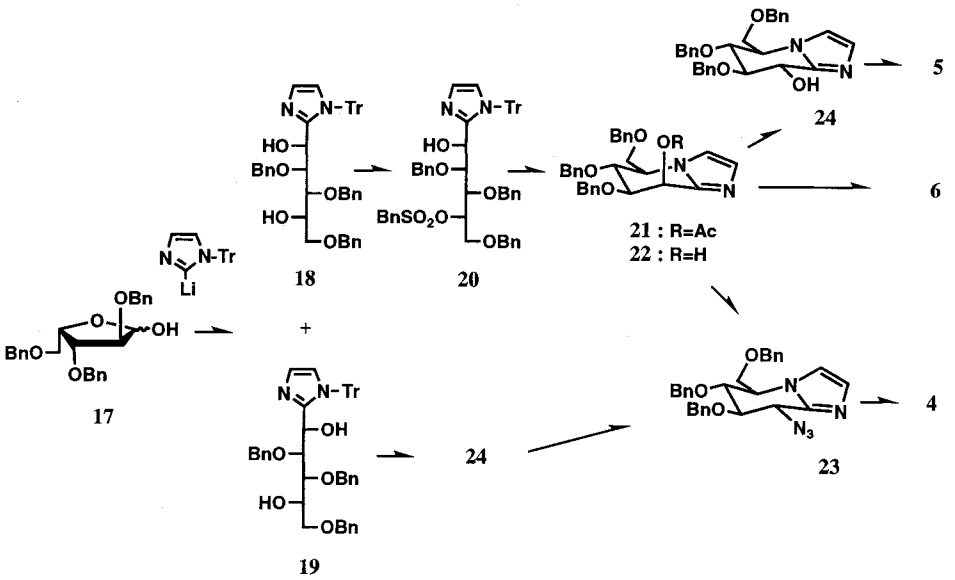
Scheme 1.




Scheme 2.




Scheme 3.




† The nomenclature conveniently parallels that of carbohydrates.

Table 1. Physico-chemical properties of nagstatin analogs (**2**~**6**).

| No. | Mp (°C) | $[\alpha]_D$ | $^1$H NMR (ppm) |
|---|---|---|---|
| **2** | 210~212 | +109° (*c* 1.3, $H_2O$) | 270 MHz ($CD_3OD$): $\delta$ 2.07 (3H, s), 4.10 (2H, d, $J$=6 Hz), 4.15 (1H, dd, $J$=9, 2 Hz), 4.34 (1H, m), 4.37 (1H, dd, $J$=2, 2 Hz), 5.08 (1H, d, $J$=9 Hz), 7.39 (1H, d, $J$=2 Hz), 7.78 (1H, d, $J$=2 Hz) |
| **3** | ~80 | +29.2° (*c* 1.6, $CH_3OH$) | 270 MHz ($CD_3OD$): $\delta$ 3.87 (1H, dd, $J$=7, 2 Hz), 4.03 (2H, d, $J$=5 Hz), 4.23 (1H, dt, $J$=5, 4 Hz), 4.38 (1H, dd, $J$=4, 2 Hz), 4.76 (1H, d, $J$=7 Hz), 7.05 (1H, d, $J$=1 Hz), 7.36 (1H, d, $J$=1 Hz) |
| **4** | 249~451 | +52.6° (*c* 0.90, $H_2O$) | 500 MHz, (pyridine-$d_5$): $\delta$ 2.11 (3H, s), 4.34 (1H, ddd, $J$=9, 5, 2 Hz), 4.40 (1H, dd, $J$=11, 5 Hz), 4.44 (1H, dd, $J$=9, 9 Hz), 4.57 (1H, dd, $J$=9, 9 Hz), 4.66 (1H, dd, $J$=11, 2.5 Hz), 5.85 (1H, dd, $J$=9, 9 Hz), 7.35 (1H, d, $J$=1.5 Hz), 7.18 (1H, d, $J$=1.5 Hz), 9.17 (1H, d, $J$=9 Hz) |
| **5** | 169~174 | −8.0° (*c* 0.97, $CH_3OH$) | 270 MHz ($D_2O$): $\delta$ 3.85 (1H, dd, $J$=10, 9 Hz), 3.98 (1H, d, $J$=10, 9 Hz), 4.11 (1H, dd, $J$=13, 3 Hz), 4.12 (1H, m), 4.27 (1H, dd, $J$=13, 3 Hz), 4.72 (1H, d, $J$=9 Hz), 7.30 (1H, d, $J$=1.5 Hz), 7.43 (1H, d, $J$=1.5 Hz) |
| **6** | 111~114 | −36.2° (*c* 1.01, $CH_3OH$) | 270 MHz (pyridine-$d_5$): $\delta$ 4.39 (1H, dd, $J$=13, 4 Hz), 4.40 (1H, dd, $J$=7.5, 4 Hz), 4.41 (1H, dd, $J$=13, 4 Hz), 4.76 (1H, ddd, $J$=9, 4, 4 Hz), 5.04 (1H, dd, $J$=9, 7.5 Hz), 5.66 (1H, d, $J$=4 Hz), 7.39 (1H, d, $J$=1.5 Hz), 7.78 (1H, d, $J$=1.5 Hz) |

part of an ongoing program[3] to clarify the mode of action of glycosidase inhibitors.

The syntheses of the *N*-acetyl-D-galactosamine† and D-galactose analogs (**2** and **3**) originated from L-ribofuranose and the *N*-acetyl-D-glucosamine, D-glucose and D-mannose analogs (**4**, **5** and **6**) from L-xylofuranose respectively, through the intermolecular and intramolecular nucleophilic reactions with the imidazole moieties(Scheme 2 and 3).

Reaction of the protected ribofuranose[4] **7** with lithiated *N*-tritylimidazole[5] gave the L-allose derivative **8**† [47%; mp 62~67°C (amorphous solid), $[\alpha]_D$ −111° (*c* 1.0, $CHCl_3$)] and L-altrose derivative **9** [40%; mp 132.5~133.5°C (EtOAc), $[\alpha]_D$ −31° (*c* 1.0, $CHCl_3$)]. Both compounds **8** and **9** were effectively converted into **2** and **3** as follows. De-*N*-tritylation and the $S_N2$-type intramolecular cyclization of **8** were realized in one-pot by reaction with $BnSO_2Cl$ in pyridine at −15°C for 1.5 hours to give preferentially the 9-*O*-sulfonylated compound followed by treatment with $Ac_2O$ at 65°C for 1.5 hours to give the desired acetate **10**, which was de-*O*-acetylated with MeONa to the nitrogenous D-talose analog **11** [84%; mp 77~78°C (hexane-EtOAc), $[\alpha]_D$ −7.8° (*c* 1.0, $CHCl_3$)]. The effective de-*N*-tritylation seemed to be affected by the producing pyridinium acetate and was supported by the stepwise conversions of **13** and **20** into **14** and **21** as shown later. The inversion of the hydroxyl group in **11** was carried out by using $HN_3$, *n*-$Bu_3P$ and DEAD in THF and PhMe at room temperature for 30 minutes[6] to afford the azido derivative **12** [68%; oil, $[\alpha]_D$ +98° (*c* 1.2, $CHCl_3$)], which was subjected to hydrogenolysis on Pd-C in AcOH and *N*-acetylation with $Ac_2O$ in MeOH to give the *N*-acetyl-D-galactosamine analog **2** in 65% yield (Table 1), which was corresponding to de-branched nagstatin.

Alternatively, **12** was prepared from the other isomer **9**. Treatment of **9** with $BnSO_2Cl$ in pyridine followed by acetylation gave the cyclized compound **14** [90%; oil, $[\alpha]_D$ +75° (*c* 1.4, $CHCl_3$)] with de-*N*-tritylation as described above, which was de-*O*-acetylated to **15** [95%; mp 112~113°C (hexane-EtOAc), $[\alpha]_D$ +38° (*c* 1.0, $CHCl_3$)]. The reaction of **15** with $HN_3$ by the aforesaid conditions gave **12** in 68% yield with retention of the C-8 configuration as expected from the nucleophilic reaction of the C-2 equatorial group in carbohydrates.[7] This retention was confirmed by the fact that **15** was treated with benzoic acid, *n*-$Bu_3P$ and DEAD to give the benzoate **16** [75%; mp 97~98°C (EtOAc), $[\alpha]_D$ +98° (*c* 1.1, $CHCl_3$)], which was deacylated to the starting **15**. Catalytic hydrogenolysis of **15** afforded the nitrogenous D-galactose analog **3** in 90% yield (Table 1).

Similarly, the enantiomeric *N*-acetyl-L-galactosamine analog **2'** and L-galactose analog **3'** were prepared from D-ribofuranose by the same procedures as mentioned above: **2'**: mp 210~212°C (decomp.), $[\alpha]_D$ −104° (*c* 0.65, MeOH); **3'**: mp ~80°C (amorphous solid), $[\alpha]_D$ −32° (*c* 0.95, MeOH).

Nitrogenous *N*-acetyl-D-glucosamine, D-glucose and D-mannose analogs (**4**, **5** and **6**) were also efficiently prepared from the protected L-xylofuranose[4] by the similar fashion as described above. Reaction with lithiated *N*-tritylimidazole gave the L-gulose analog **18** [64%; mp 40~44°C (amorphous solid), $[\alpha]_D$ −55° (*c* 0.87, $CHCl_3$)] and L-idose analog **19** [16%; mp 43~46°C (amorphous solid), $[\alpha]_D$ +18° (*c* 1.2, $CHCl_3$)]. Benzylsulfonylation of **18** followed by acetylation with concomitant cyclization gave the nitrogenous D-mannose analog **21**, which was deacetylated to **22** [95%; mp 116.5~117.5°C (hexane-EtOAc), $[\alpha]_D$ −4.0° (*c* 0.95, $CHCl_3$)]. Hydrogenolysis of **22** gave the D-mannose analog **6** in 91% yield (Table 1). Mitsunobu inversion of the hydroxyl group of **22** with $HN_3$ by the aforesaid conditions gave **23** [71%; oil, $[\alpha]_D$ +59° (*c* 1.0, $CHCl_3$)], which was converted into the nitrogenous *N*-acetyl-D-glucosamine analog **4** in 65% yield (Table 1) by hydrogenolysis and *N*-acetylation as described above.

Table 2. Inhibitory activity of nagstatin (**1**) and its analogs (**2**~**6**) against glycosidases.

| Glycosidases | $IC_{50}$ (μg/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **2′** | **3′** |
| α-D-Glucosidase[a] | >100 | >100 | >100 | >100 | 21 | >100 | >100 | >100 |
| β-D-Glucosidase[b] | >100 | 77 | 0.10 | 32 | 0.14 | 3.5 | >100 | >100 |
| α-D-Mannosidase[c] | >100 | >100 | >100 | >100 | 2.5 | 0.12 | >100 | >100 |
| β-D-Mannosidase[d] | >100 | >100 | 74 | >100 | 60 | 0.023 | >100 | >100 |
| α-D-Galactosidase[e] | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| β-D-Galactosidase[f] | >100 | 2.6 | 0.0016 | >100 | >100 | >100 | 71 | >100 |
| *N*-acetyl-β-D-Glucosaminidase[g] | 0.004 | 0.0015 | 12 | 0.0017 | >100 | >100 | 31 | >100 |
| *N*-acetyl-α-D-Galactosaminidase[h] | 19 | 2.4 | 25 | >100 | >100 | >100 | >100 | >100 |

[a] Bakers yeast; [b] Almonds; [c] Jack beans; [d] Snail; [e] Escherichia coli; [f] Escherichia coli; [g] Bovine kidney; [h] Chicken liver.

The azide **23** was also obtained from **24** with retention of the C-8 configuration as described in the preparation of **12** from **15**. The intermediate **24** was prepared from **19** by benzylsulfonylation and acetylation with cyclization followed by de-*O*-acetylation as described in the preparation of **15** from **9**, and also derived from **22** in 85% overall yield by Mitsunobu inversion with BzOH followed by de-*O*-benzoylation. Then, the D-glucose analog **5** (Table 1) was prepared from **24** by hydrogenolysis.

The glycosidase inhibiting activities were generally assayed according to the method reported by SAUL *et al.* as summarized in Table 2. The D-galactose, D-glucose and D-mannose analogs (**3**, **5** and **6**) showed very much stronger inhibiting activities against β-D-galactosidase, β-D-glucosidase and β-D-mannosidase, respectively, than activities against the corresponding α-D-glycosidases. *N*-Acetyl-D-glucosamine analog **4** inhibited strongly *N*-acetyl-β-D-glucosaminidase activity and weakly β-D-glucosidase activity. *N*-Acetyl-D-galactosamine analog **2** exhibited the strong activity even against *N*-acetyl-β-D-glucosaminidase and, consequently, was expected to inhibit *N*-acetyl-β-D-galactosaminidase, although this glycosidase was not available now. Remarkably, the L-galactose analogs **2′** and **3′** showed no significant glycosidase inhibitory activities.

Structurally, all analogs possess a quasi-equatorially oriented C-8a~N-1 bond, which corresponds to an equatorial C-1~O bond of β-glycopyranosides, due to the fused imidazole ring. The configurations from C-8a to C-5 of the analogs parallel the alignment from C-1 to C-5 of the corresponding glycopyranosides. The strong β-D-glycosidase inhibiting activities of the analogs **2**~**6** indicated that the β-D-glycosidases including *N*-acetyl-β-D-glucosaminidase recognized especially their C-8a portions as the C-1 position of β-D-glycopyranosides. Furthermore, their substrate-specific activities emphasized that the analogs serve essentially as the antagonists of the corresponding stereochemically oriented β-D-glycopyranosides.

Acknowledgments

We are grateful to Shikoku Chemicals Co., Yamanouchi Pharmaceutical Co., Ltd., Meiji Seika Kaisha, Ltd. and the Institute of Microbial Chemistry for the generous support of our program.

KUNIAKI TATSUTA*
SHOZO MIURA
SHIGERU OHTA
HIROKI GUNJI

Graduate School of Science and Engineering,
Waseda University,
3-4-1 Ohkubo, Shinjuku-ku, Tokyo 169, Japan

(Received November 21, 1994)

## References

1) AOYAMA, T.; H. NAGANAWA, H. SUDA, K. UOTANI, T. AOYAGI & T. TAKEUCHI: The structure of nagstatin, a new inhibitor of *N*-acetyl-β-D-glucosaminidase. J. Antibiotics 45: 1557~1558, 1992
2) TATSUTA, K.; S. MIURA, S. OHTA & H. GUNJI: Total syntheses of de-branched nagstatin and its analogs having glycosidase inhibiting activities. Tetrahedron Lett., in press.
3) TATSUTA, K.; Y. NIWATA, K. UMEZAWA, K. TOSHIMA & M. NAKATA: Syntheses and enzyme inhibiting activities of cyclophellitol analogs. J. Antibiotics 44: 912~914, 1991
4) KAWANA, M.; H. KUZUHARA & S. EMOTO: The use of Grignard reagents in the synthesis of carbohydrates. III. The one-way anomerization of methyl glycofuranosides and the opening of their furanose ring. Bull. Chem. Soc. Jpn. 54: 1492~1504, 1981
5) KIRK, K. L.: Facile synthesis of 2-substituted imidazoles. J. Org. Chem. 43: 4381~4383, 1978
6) LOIBNER, H. & E. ZBIRAL: Sturukuturelle Abwandlungen an Nucleosiden mit Hilfe von Triphenylphosphan/Azodicarbonsäureester. Justus Liebigs Ann. Chem. 1978: 78~86, 1978
7) HOUGH, L. & A. C. RICHARDSON: Stereo-electronic factors in displacement and esterification reactions. "Rodd's Chemistry of Carbon Compounds $I_F$" ed by S. Coffey, Elsevier, Amsterdam (1967), p. 118