Synthesis and Glycosidase Inhibitory Activities of Nagstatin Triazole Analogs

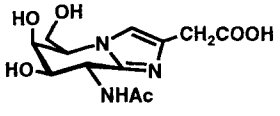
Sir:

Nagstatin (**1**) is a novel *N*-acetyl-β-D-glucosaminidase inhibitor isolated from culture filtrates of *Streptomyces amakusaensis*, and is structurally a nitrogenous *N*-acetylgalactosamine analog fused with an imidazole ring[1]. Recently, we have enatiospecifically synthesized nagstatin (**1**) and its analogs (for example, **2**) having different configurations and functionalities and clarified the structure-activity relationships[2~4].

Herein, we describe the synthesis of the triazole analogs (**3** and **4**) having a triazole ring in place of an imidazole ring, and evaluate their glycosidase inhibitory activities to better understand the mode of action. In the synthesis of the triazole analogs, since two regioisomers (for example, **11** and **11'**) would be possibly produced, it is intriguing heterocyclic chemistry which isomer is predominantly produced.

The galacto- and manno-analogs (**3** and **4**) were synthesized from L-ribofuranose and L-xylofuranose derivatives (**5** and **12**), respectively, as previously reported in the synthesis of the imidazole analogs[2~4]. The galacto-analog 3 has the same configurations as those of nagstatin (**1**).

Reaction of **5** with lithiated *N*-trityltriazole[5] **6** gave the L-altrose derivative **7**† [50%; mp 134~138°C (EtOAc), $[\alpha]_D$ −53° (*c* 0.75, $CHCl_3$)] and L-allose derivative **8** [41%; amorphous solid, $[\alpha]_D$ −135° (*c* 1.3, $CHCl_3$)]. De-*N*-tritylation and the S_N2-type cyclization of **7** were carried out in one-pot according to our procedure[2] by reaction with $BnSO_2Cl$ in pyridine at −15°C for 1 hour to give preferentially the 9-*O*-sulfonylated compound **9** followed by treatment with Ac_2O at 70°C for 12 hours to give the acetate **10**, which



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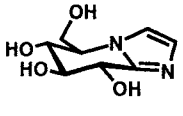

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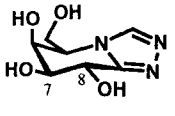

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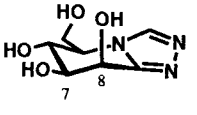

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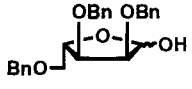

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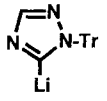

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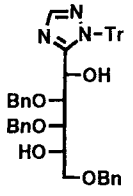

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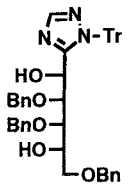

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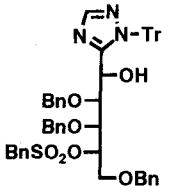

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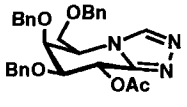

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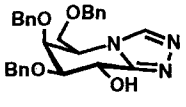

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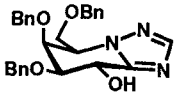

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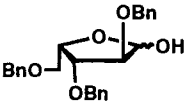

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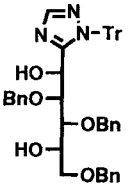

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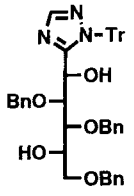

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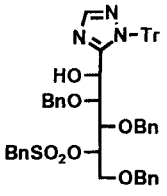

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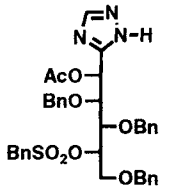

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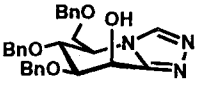

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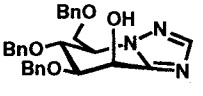

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† The nomenclature conveniently parallels that of carbohydrates.

Table 1. Physico-chemical properties of nagstatin analogs.

No.	mp (°C)	$[\alpha]_D$	^{1}H NMR (ppm)
3	223~230	+29° (*c* 1.3, H_2O)	270 MHz (D_2O): δ 4.05 (1H, dd, J=9, 2 Hz), 4.12 (2H, d, J=6 Hz), 4.46 (1H, dt, J=3, 6 Hz), 4.49 (1H, dd, J=3, 2 Hz), 4.98 (1H, d, J=9 Hz), 8.75 (1H, s)
4	198~201	−53° (*c* 0.5, H_2O)	400 MHz (D_2O): δ 4.04 (1H, dd, J=13, 5 Hz), 4.06 (2H, dd, J=9, 4 Hz), 4.14 (1H, ddd, J=8, 5, 3 Hz), 4.24 (1H, dd, J=9, 8 Hz), 4.27 (1H, dd, J=13, 3 Hz), 5.17 (1H, d, J=4 Hz), 8.69 (1H, s)
11		+43° (*c* 1.2, $CHCl_3$)	500 MHz ($CDCl_3$): δ 3.81 (1H, ddd, J=10, 9 Hz), 3.87 (1H, dd, J=10, 3 Hz), 4.10 (1H, dd, J=6, 2 Hz), 4.37 (1H, dd, J=6, 2 Hz), 4.44 (1H, ddd, J=9, 6, 3 Hz), 5.31 (1H, d, J=6 Hz), 8.30 (1H, s)
17	104~106	−2.2° (*c* 2.4, $CHCl_3$)	400 MHz ($CDCl_3$): δ 3.62 (1H, dd, J=10, 8 Hz), 3.66 (1H, dd, J=10, 4 Hz), 4.00 (1H, dd, J=7, 4 Hz), 4.03 (1H, dd, J=7, 3 Hz), 4.28 (1H, ddd, J=8, 4, 4 Hz), 5.24 (1H, dd, J=6, 3 Hz), 8.37 (1H, s)
18		−46° (*c* 1.0, $CHCl_3$)	500 MHz ($CDCl_3$): δ 3.98 (2H, d, J=6 Hz), 4.04 (1H, dd, J=7, 4 Hz), 4.40 (1H, dt, J=4, 6 Hz), 4.53 (1H, dd, J=7, 4 Hz), 5.13 (1H, dd, J=6, 4 Hz), 7.83 (1H, s)

was de-*O*-acetylated with MeONa to the triazole analog **11** [78% in total; syrup, $[\alpha]_D$ +43° (*c* 1.2, $CHCl_3$)]. The triazole structure was determined by the ^{1}H NMR NOE studies: on irradiation at H-5 (δ 4.44), the NOE enhancement (3.9%) of H-3 signal was clearly detected to support the structure **11**, but not the other regioisomer **11'**. Catalytic hydrogenolysis (H_2/Pd-C) of **11** in AcOH afforded the galacto-analog **3** [70%, mp 223~230°C (MeOH), $[\alpha]_D$ +29° (*c* 1.3, H_2O)]. Similar treatment of the other product **8** gave complex mixtures and, therefore, was discontinued.

On the other hand, reaction of the xylofuranose derivative **12** with lithiated *N*-trityltriazole **6** gave the L-gulose analog **13** [52%; amorphous solid, $[\alpha]_D$ −53° (*c* 2.0, $CHCl_3$)] and L-idose analog **14** [19%; amorphous solid, $[\alpha]_D$ +13° (*c* 1.0, $CHCl_3$)]. Since the aforesaid one-pot cyclization reaction of **13** and **14** gave complex mixtures, conversion of **13** into **4** was carried out stepwise as follows. Benzylsulfonylation of **13** gave exclusively the sulfonate **15** [90%; amorphous solid, $[\alpha]_D$ −50° (*c* 1.28, $CHCl_3$)]. This was acetylated with Ac_2O in pyridine to give the *N*,*O*-diacetate, followed by de-*N*-acetylation with 80% HCO_2H at room temperature for 1 hour, to give the triazole **16** [79% in total; amorphous solid, $[\alpha]_D$ −22° (*c* 1.5, $CHCl_3$)]. On heating in pyridine at 80°C for 2 days, **16** gave two cyclized products, which were deacetylated with MeONa to give the regioisomers **17** [90% in 2 steps; mp 104~106°C (EtOAc), $[\alpha]_D$ −2.2° (*c* 2.4, $CHCl_3$)] and **18** [4% in 2 steps; syrup, $[\alpha]_D$ −46° (*c* 1.0, $CHCl_3$)]. Their structures were determined by the ^{1}H NMR NOE studies. On irradiationg at H-5 (δ 4.28) in **17**, the NOE enhancement (8.7%) of H-3 signal was observed, while, in **18**, no NOE enhancement was detected between H-2 and H-5, supporting the structures **17** and **18**. Hydrogenolysis of **17** gave the manno-analog **4** [72%; mp 198~201°C (MeOH), $[\alpha]_D$ −53° (*c* 0.5, H_2O)].

The glycosidase inhibiting activities were generally assayed according to the method reported by SAUL *et al.* as summarized in Table 2[2~4]. The galacto-analog **3** and manno-analog **4** showed very specific inhibiting activities against β-D-galactosidase and β-D-mannosidase, respectively, as expected from our previous results[2~4]. The strong β-D-glycosidase inhibiting activities indicated that these analogs **3** and **4** serve essentially as the antagonists of the corresponding β-D-galacto- and mannopyranosides, respectively.

Table 2. Inhibitory activity of nagstatin analogs (**3** and **4**) against glycosidases.

Glycosidases	IC_{50} (μg/ml)	
	3	**4**
α-D-Glucosidase[a]	>100	>100
β-D-Glucosidase[b]	2.5	57
α-D-Mannosidase[c]	>100	13
β-D-Mannosidase[d]	>100	0.078
α-D-Galactosidase[e]	>100	>100
β-D-Galactosidase[f]	0.081	>100

[a] Bakers yeast; [b] Almonds; [c] Jack beans; [d] Snail; [e] *Escherichia coli*; [f] *Escherichia coli*.

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