SYNTHESIS OF 5-*epi*-TREHAZOLIN (TREHALOSTATIN)

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Synthesis of 5-*epi*-trehazolin (trehalostatin) (**2**) was accomplished *via* the crucial intermediate, epoxide (**6α**), from D-glucose. The stereochemistry of epoxide (**6α**) and its isomer (**6β**) which were obtained from Sharpless epoxidation, was determined by comparison between the NMR relaxation times of relevant protons.

Trehazolin (**1**), a unique pseudodisaccharide possessing strong specific inhibitory activities towards various trehalases, was isolated from culture broths of both *Micromonospora* sp. strain SANK 62390 and *Amicoratopsis* sp. strain SANK 60791.[1)]

The absolute configuration of the trehazolin aminocyclitol moiety was determined as [1*R*-(1α,2β,-3α,4β,5β)] (aminocyclitol numbering) through the synthetic studies of trehazolin (**1**) and related compounds.[2~4)] Trehalostatin (**2**) was reported by the Suntory group. The C-5 stereochemistry of its aglycon moiety is different from that of trehazolin.[5)]

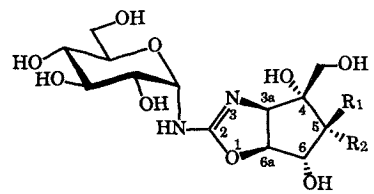
We attempted the synthesis of 5-*epi*-trehazolin[6)] (trehalostatin) (**2**) in order not only to reconfirm the difference in stereochemistry between trehazolin and trehalostatin, but also to investigate the structure activity relationship of trehazolin derivatives. Herein, we report the synthesis of 5-*epi*-trehazolin.

Synthesis of 5-*epi*-Trehazolin (Trehalostatin)

After benzylation of the allyl alcohol **3**, which was derived from D-glucose in 10 steps including [3+2] cycloaddition[7)] as a key step, cleavage of the silyl group and Sharpless epoxidation[8)] of the corresponding allyl alcohol **5** using diisopropyl L-tartrate (L-DIPT) furnished two stereoisomers of **6**. The major isomer was identical to the single isomer which was obtained by epoxidation using diisopropyl D-tartrate (D-DIPT) in lieu of L-DIPT (Table 1).

The coupling constants between 5-H and 4-H of both epoxides **6** are quite similar. In Sharpless epoxidation using L-DIPT, a NOE between 5-H and 2-H was observed in the minor isomer, while no NOE, specific for the stereochemistry of the epoxide, was observed in the major isomer. To compensate for the insufficient evidence for the stereochemistry of these compounds, the ^{1}H-^{1}H distance de-

Fig. 1. The structures of trehazolin (**1**) and 5-*epi*-trehazolin (trehalostatin) (**2**).



R_1=OH, R_2=H Trehazolin (**1**)
R_1=H, R_2=OH 5-*epi*-Trehazolin (Trehalostatin) (**2**)

Table 1. Stereoselectivities and yields of epoxidation of compound **5**.

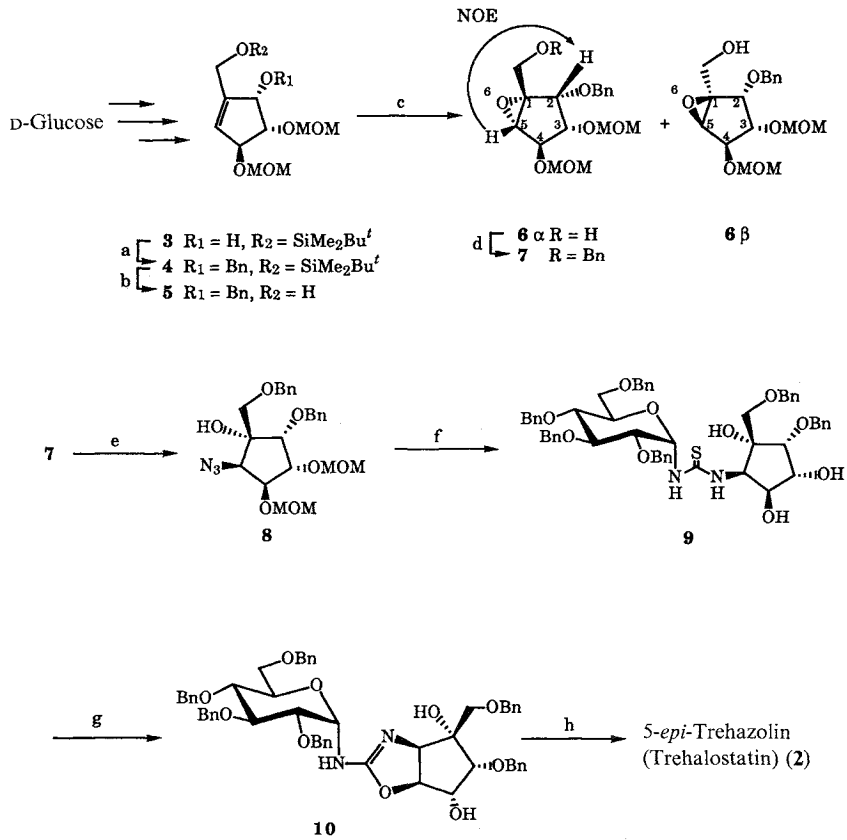
Entry	Condition	Yield (%)	Ratio (**6α** : **6β**)
1	1.5 equiv of L-DIPT, 1.4 equiv of $Ti(O^iPr)_4$ 2.0 equiv of *tert*-BuOOH, CH_2Cl_2, −25°C→ room temperature, 5 hours	71%	1 : 2
2	1.5 equiv of D-DIPT, 1.5 equiv of $Ti(O^iPr)_4$ 2.0 equiv of *tert*-BuOOH, CH_2Cl_2, −25°C→ room temperature, 14 hours	62%	**6β** only

Table 2. The observed T_1 (sec) values of respective protons for compounds **6α** and **6β**.

	6α	**6β**
2-H	2.8	3.2
3-H	[a]	2.6
4-H	3.5	2.6
5-H	6.2	3.9
6-H	1.9	1.6

[a] The peak of 3-H overlapped with that of 6-H at 4.01 ppm, therefore T_1 of this peak could not be observed.

Scheme 1.



(a) 1.5 equiv of BnBr, 1.5 equiv of NaH, DMF, 0°C, 2 hours, 94%. (b) 1.5 equiv of n-Bu_4NF, THF, 0°C, 1 hour, 91%. (c) Table 1. (d) 1.9 equiv of BnBr, 1.7 equiv of NaH, DMF, 0°C, 2 hours, 86%. (e) 13 equiv of NaN_3, 14 equiv of NH_4Cl, DMF-ethylene glycol (5 : 1), 125°C, 48 hours, 62%. (f) 5.5 equiv of $LiAlH_4$, Et_2O, 0°C, 3 hours, 5% HCl-MeOH, 60°C, 7 hours, and then 1.2 equiv of 2,3,4,6-tetra-*O*-benzyl-1-deoxy-α-D-glucopyranosyl isothiocyanate, 1.2 equiv of Et_3N, THF, room temperature, 21 hours, 54% (from **8**). (g) 2.6 equiv of 2-chloro-3-ethylbenzoxazolium tetrafluoroborate, MeCN, 0°C, 1 hour, then quenched with 5.2 equiv of Et_3N, 0°C, 3 hours, 71%. (h) H_2, $Pd(OH)_2$-C, MeOH, 60°C, 30 minutes, 20%.

pendencies of longitudinal relaxation times (T_1) were utilized.[9~11]

The T_1s were measured by the conventional inversion recovery method and shown in Table 2. The T_1 value of 5-H in the minor isomer of **6** was almost twice as long as that in the major one. Since the T_1 value of a specific proton is virtually determined by the dipole-dipole interaction with its proximately located protons, the T_1 value of 5-H is determined by the interaction between 4-H and 5-H. As a result, the distance between 4-H and 5-H was evaluated to be longer in the minor isomer than in the major one. Thus, the stereochemistry between 4-H and 5-H of the former and the latter could be *trans* and *cis*, respectively. Hence, it was concluded that the minor isomer of **6** is the α-epoxide (**6α**) and the major is the β-epoxide (**6β**). This result constitutes a rare case that is opposite to proposed Sharpless rule. The reason why **6β** was produced as the major isomer by Sharpless epoxidation using L-DIPT is that the C-5 benzyl group of **5** sterically impeded this epoxidation.

Table 3. Inhibitory activities of 5-*epi*-trehazolin (trehalostatin) (**2**) and trehazolin (**1**) towards silkworm and porcine trehalases (IC_{50}).

Enzyme	5-*epi*-Trehazolin (Trehalostatin) (**2**)	Trehazolin (**1**)
Silkworm trehalase	>100 μg/ml	0.011 μg/ml
Porcine trehalase	20 μg/ml	0.006 μg/ml

Benzylation of the α-epoxide (**6α**) and subsequent azidation[12] of **7** afforded the corresponding azido alcohol **8**. After reduction of the azido group and cleavage of the two methoxymethyl (MOM) groups of the latter compound, coupling of the corresponding amino alcohol hydrochloride with 2,3,4,6-tetra-*O*-benzyl-1-deoxy-α-D-glucopyranosyl isothiocyanate[13] in the presence of triethylamine furnished a thiourea derivative **9**. Cyclization of **9** using 2-chloro-3-ethylbenzoxazolium tetrafluoroborate[14~16] gave an aminooxazoline **10**. Finally, hydrogenolysis of compound **10** and purification on Amberlite CG-50 (NH_4^+ type/H^+ type, 3/2) afforded 5-*epi*-trehazolin (**2**).

Inhibition of Trehalases

Although all spectral data of **2** were quite similar to those of trehazolin, it possesses much weaker inhibitory activities towards two trehalases than trehazolin (Table 3). It is obvious that the stereochemistry at C-5 position of trehazolin aglycon has a significant influence towards the inhibitory activities of trehalases.

Experimental

General

Melting points are uncorrected. 270 MHz ^{1}H NMR spectra were recorded on a JEOL JNM-EX-270 or JNM-GX-270 spectrometer, 400 MHz ^{1}H NMR spectrum on a JNM-GX-400, using tetramethylsilane as an internal reference. Infrared spectra were recorded using a JASCO FT/IR-8900 or A-102 spectrometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Low and high resolution mass spectra were obtained from a JEOL JMS-AX-505H spectrometer. FAB and FAB high resolution mass spectra were recorded from a JEOL HX-100 or SX-102A spectrometer. Elemental analyses were performed by the Institute of Science and Technology, Inc. Analytical chromatography was performed on Merck Art 5715 silica gel $60F_{245}$ plates. Flash chromatography was performed on Merck Art 9385 silica gel 60 (230~400 mesh). Tetrahydrofuran (THF) was distilled from $LiAlH_4$ and used immediately thereafter. Diethyl ether (Et_2O) was dried by passing through ICN Alumina N-Super I. Dichloromethane (CH_2Cl_2) was dried by passing through ICN Alumina B-Super I. Dimethylformamide (DMF) and pyridine were dried by storing over 4A-molecular sieves. Acetonitrile (MeCN) was dried by storing over 3A-molecular sieves. All other commercially available reagents were used directly as received.

[3*S*-(3α,4β,5β)]-5-Benzyloxy-1-*tert*-butyldimethylsilyloxymethyl-3,4-di(methoxymethoxy)-1-cyclopentene (**4**)

To a solution of **3** (425 mg, 1.22 mmol) in DMF (8.5 ml) were added sodium hydride (70 mg, 2.06 mmol, 55% oil dispersion) and benzyl bromide (0.2 ml, 1.68 mmol) with stirring at 0°C. After completion of the reaction (2 hours), to the reaction mixture was added MeOH (2.0 ml) with stirring at 0°C. After 30 minutes, this reaction mixture was diluted with EtOAc and washed with brine. The aqueous layer was extracted twice with EtOAc. The combined organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a crude product, which was chromatographed on a silica gel column. Elution with benzene - EtOAc (5 : 1) gave 534 mg (94%) of **4** as a colorless oil. $[\alpha]_D^{24}$ +77.6° (*c* 1.34, $CHCl_3$); IR (film) ν_{max} 2953, 2930, 2889, 2857 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.40~7.20 (5H, m), 5.95~5.89 (1H, m), 4.84~4.70 (5H, m including dd×4 (4.81, 4.80, 4.76 and 4.72, 1H, d, *J*=6.6 Hz)), 4.62 (1H, d, *J*=11.2 Hz), 4.54 (1H, d, *J*=5.9 Hz), 4.51 (1H, d, *J*=11.2 Hz), 4.24 (1H, dt, *J*=15.0, 1.9 Hz), 4.15 (1H, dt, *J*=15.0, 1.9 Hz), 4.13 (1H, dd, *J*=5.9, 4.6 Hz), 3.44 (3H, s), 3.39 (3H, s), 0.90 (9H, s), 0.05 (6H, s); MS *m/z* 438 (M^+), 407 ($M^+ - OMe$); Rf 0.57 (benzene - EtOAc, 5 : 1).

Anal Calcd for $C_{23}H_{38}O_6Si$: C 62.98, H 8.73.
Found: C 62.92, H 9.00.

[3*S*-(3α,4β,5β)]-5-Benzyloxy-1-hydroxymethyl-3,4-di(methoxymethoxy)-1-cyclopentene (**5**)

To a solution of **4** (504 mg, 1.15 mmol) in THF (10 ml) was added tetra-*n*-butylammonium fluoride (1M - THF solution, 1.7 ml) with stirring at 0°C. After completion of the reaction (1 hour), to the reaction mixture was added water with stirring at 0°C. After 30 minutes, this reaction mixture was diluted with EtOAc and washed with brine. The aqueous layer was extracted twice with EtOAc. The combined organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a crude product, which was chromatographed on a silica gel column. Elution with hexane - EtOAc (1 : 1) gave 339 mg (91%) of **5** as white crystals: $[\alpha]_D^{24}$ +81.1° (*c* 1.20, $CHCl_3$); mp 30~31°C (recrystallized from hexane); IR (KBr) ν_{max} 3324, 3237, 2951, 2896 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.40~7.20 (5H, m), 5.95~5.90 (1H, m), 4.83 (1H, d, *J*=6.6 Hz), 4.79 (1H, d, *J*=4.6 Hz), 4.78 (1H, d, *J*=6.6 Hz), 4.77 (1H, d, *J*=6.6 Hz), 4.71 (1H, d, *J*=6.6 Hz), 4.69 (1H, d, *J*=11.2 Hz), 4.60 (1H, br d, *J*=5.3 Hz), 4.53 (1H, d, *J*=11.2 Hz), 4.24 (1H, br d, *J*=15.2 Hz), 4.15 (1H, br d, *J*=15.2 Hz), 3.44 (3H, s), 3.39 (3H, s), 1.96 (1H, br s); MS *m/z* 307 ($M^+ + 1 - H_2O$); Rf 0.23 (hexane - EtOAc, 1 : 1).

Anal Calcd for $C_{17}H_{24}O_6$: C 62.95, H 7.46.
Found: C 62.88, H 7.40.

[1*R*-(1α,2β,3β,4α,5α)]-2-Benzyloxy-1-hydroxymethyl-3,4-di(methoxymethoxy)-6-oxabicyclo[3.1.0]hexane (**6α**) and [1*S*-(1α,2α,3α,4β,5α)]-2-Benzyloxy-1-hydroxymethyl-3,4-di(methoxymethoxy)-6-oxabicyclo[3.1.0]hexane (**6β**)

A solution of titanium tetraisopropoxide (0.22 ml, 0.9 mmol) and L-DIPT (0.21 ml, 0.9 mmol) in CH_2Cl_2 (12 ml) was stirred at −25°C under a nitrogen atmosphere for 20minutes. To this mixture was added a solution of **5** (188 mg, 0.58 mmol) in CH_2Cl_2 (4 ml). After 20 minutes, to this reaction mixture was added *tert*-BuOOH (3M-toluene solution, 0.4 ml) maintaining −25° and the nitrogen atmosphere and the temperature was elevated to room temperature. After completion of the reaction (22 hours), the reaction mixture was diluted with Et_2O, and 10% NaOH - brine was added to the mixture with stirring at 25°C. After 15 minutes, the reaction mixture was extracted twice with Et_2O. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a crude product, which was chromatographed on a silica gel column. Elution with hexane - EtOAc (1 : 2) gave 46 mg (24%) of **6α** as white crystals and 94 mg (49%) of **6β** as a colorless oil: **6α**: $[\alpha]_D^{24}$ −64.9° (*c* 0.53, $CHCl_3$); mp 62~63°C (recrystallized from Et_2O - hexane), IR (KBr) ν_{max} 3360, 3267, 2938, 2890 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.40~7.25 (5H, m), 4.77 (1H, d, *J*=11.9 Hz), 4.75 (1H, d, *J*=6.6 Hz), 4.71 (1H, d, *J*=6.6 Hz), 4.64 (2H, d, *J*=6.6 Hz), 4.51 (1H, d, *J*=11.9 Hz), 4.23 (1H, d, *J*=5.8 Hz), 4.21 (1H, s), 4.03 (1H, dd, *J*=12.5, 3.3 Hz), 3.99 (1H, d, *J*=5.8 Hz), 3.85 (1H, dd, *J*=12.5, 9.1 Hz), 3.52 (1H, s), 3.43 (3H, s), 3.37 (3H, s), 1.72 (1H, dd, *J*=9.1, 3.3 Hz); MS *m/z* 295 ($M^+ - CH_2OCH_3$), 263, 259, 233, 217, 189; Rf 0.19 (hexane - EtOAc, 1 : 2).

Anal Calcd for $C_{17}H_{24}O_7$: C 59.99, H 7.11.
Found: C 59.90, H 6.85.

6β: $[\alpha]_D^{25}$ +17.7° (*c* 1.30, $CHCl_3$); IR (film) ν_{max} 3465, 3090, 3064, 3032, 2947, 2894, 2826, 2788 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.42~7.25 (5H, m), 4.87 (1H, d, J=11.2 Hz), 4.79 (2H, s), 4.72 (1H, d, J=6.8 Hz), 4.64 (1H, d, J=11.2 Hz), 4.63 (1H, d, J=6.8 Hz), 4.23 (1H, dd, J=7.3, 1.0 Hz), 4.16 (1H, d, J=5.2 Hz), 4.05 (1H, d, J=12.5 Hz), 3.97 (1H, dd, J=7.3, 5.2 Hz), 3.71 (1H, d, J=12.5 Hz), 3.64 (1H, br s), 3.43 (3H, s), 3.39 (3H, s), 1.80 (1H, broad s); MS *m/z* 308 (M^++1−CH_3−H_2O), 295 (M^+−CH_2OCH_3); Rf 0.52 (hexane - EtOAc, 1 : 2).

Anal Calcd for $C_{17}H_{24}O_7 \cdot 1/4H_2O$: C 59.20, H 7.16.
Found: C 59.20, H 6.91.

[1*S*-(1α,2α,3α,4β,5α)]-2-Benzyloxy-1-hydroxymethyl-3,4-di(methoxymethoxy)-6-oxabicyclo[3.1.0]hexane (**6β**)

Using the above procedure except for addition of D-tartrate in lieu of L-tartrate, **5** was converted to **6β** in 62% yield. However, after addition of *tert*-BuOOH, the temperature was elevated to room temperature and maintained for 14 hours. The physical data of **6β** is the same as that of **6β** derived by the procedure using L-tartrate.

[1*R*-(1α,2β,3β,4α,5α)]-2-Benzyloxy-1-benzyloxymethyl-3,4-di(methoxymethoxy)-6-oxabicyclo[3.1.0]hexane (**7**)

To a solution of **6α** (45 mg, 0.13 mmol) in DMF (1.4 ml) were added 55% sodium hydride (5.3 mg, 0.22 mmol, 55% oil dispersion) and benzyl bromide (0.03 ml, 0.25 mmol) with stirring at 0°C. After 5 minutes, the mixture was warmed to room temperature and stirred for 2 hours. After completion of the reaction, to the reaction mixture was added EtOH (1 ml) with stirring at 0°C. After 30 minutes, the reaction mixture was diluted with EtOAc and washed with brine. The aqueous layer was extracted twice with EtOAc. The combined organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a crude product, which was chromatographed on a silica gel column. Elution with hexane - EtOAc (1 : 1) gave 49 mg (86%) of **7** as a colorless oil: $[\alpha]_D^{25}$ −32.2° (*c* 0.55, $CHCl_3$); IR (film) ν_{max} 2935, 2891, 2825 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.45~7.20 (10H, m), 4.74 (1H, d, J=9.6 Hz), 4.73 (1H, d, J=9.6 Hz), 4.725 (1H, d, J=6.6 Hz), 4.69 (1H, d, J=9.6 Hz), 4.625 (1H, d, J=6.6 Hz), 4.62 (1H, d, J=9.6 Hz), 4.57 (1H, d, J=11.2 Hz), 4.51 (1H, d, J=11.2 Hz), 4.37 (1H, d, J=7.2 Hz), 4.19 (1H, s), 3.963 (1H, d, J=11.2 Hz), 3.956 (1H, d, J=7.2 Hz), 3.60 (1H, d, J=11.2 Hz), 3.47 (1H, s), 3.41 (3H, s), 3.37 (3H, s); MS *m/z* 365 (M^+−CH_2OCH_3); Rf 0.73 (hexane - EtOAc, 1 : 1).

Anal Calcd for $C_{24}H_{30}O_7$: C 66.95, H 7.02.
Found: C 66.56, H 7.18.

[1*R*-(1α,2α,3α,4β,5β)]-5-Azido-2-benzyloxy-1-benzyloxymethyl-3,4-di(methoxymethoxy)-1-cyclopentanol (**8**)

To a solution of **7** (136 mg, 0.32 mmol) in DMF (6.4 ml) and ethylene glycol (1.4 ml) were added sodium azide (266 mg, 4.1 mmol) and ammonium chloride (240 mg, 4.1 mmol) with stirring at room temperature and this mixture was warmed to 125°C and stirred for 48 hours. After completion of the reaction, the reaction mixture was diluted with EtOAc and washed with brine. The aqueous layer was extracted twice with EtOAc. The combined organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a crude product, which was chromatographed on a silica gel column. Elution with benzene - EtOAc (3 : 1) gave 92 mg (62%) of **8** as a pale yellow oil: $[\alpha]_D^{24}$ +4.6° (*c* 0.69, $CHCl_3$); IR (film) ν_{max} 3494, 2934, 2894, 2109 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.45~7.25 (10H, m), 4.85~4.55 (8H, m), 4.33 (1H, t, J=5.3 Hz), 4.10 (1H, dd, J=5.3, 6.5 Hz), 4.06 (1H, d, J=5.3 Hz), 3.57 (1H, d, J=9.9 Hz), 3.49 (1H, d, J=9.9 Hz), 3.40 (1H, s), 3.39 (3H, s), 3.37 (3H, s); MS *m/z* 428 (M^+−CH_2OCH_3), 400, 354, 322, 292; Rf 0.26 (benzene - EtOAc, 5 : 1).

Anal Calcd for $C_{24}H_{31}N_3O_7$: C 60.88, H 6.60, N 8.87.
Found: C 60.52, H 6.96, N 8.81.

N-(2,3,4,6-Tetra-*O*-benzyl-1-deoxy-α-D-glucopyranosyl)-*N'*-[[1*R*-(1α,2α,3α,4β,5β)]-2-benzyloxy-1-benzyloxymethyl-1,3,4-trihydroxycyclopentan-5-yl]thiourea (**9**)

A solution of **8** (80 mg, 0.17 mmol) in Et_2O (4.0 ml) was added dropwise to a suspension of $LiAlH_4$

(35 mg, 0.93 mmol) in Et_2O (4.1 ml) with stirring at 0°C under a nitrogen atmosphere. After completion of the reaction (3 hours), the reaction mixture was diluted with Et_2O, and saturated aqueous Na_2SO_4 was added to the mixture at 0°C. After stirring at room temperature for 1 hour, the mixture was extracted twice with Et_2O. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a crude product, which was chromatographed on a silica gel column. Elution with CH_2Cl_2 - MeOH (18 : 1) gave 55 mg (72%) of the corresponding amine as a colorless oil. To a solution of this amine (45 mg) in MeOH (2.3 ml) was added 10% hydrogen chloride in methanol (2.3 ml) with stirring at 0°C, and after 5 minutes, the mixture was warmed to 60°C and stirred for 7 hours. After completion of the reaction, the reaction mixture was concentrated *in vacuo* to give a residue, which was dried under reduced pressure for 3 hours. Then, to a solution of this residue in THF (2.3 ml) were added a solution of 2,3,4,6-tetra-*O*-benzyl-1-deoxy-α-D-glucopyranosyl isothiocyanate (71 mg, 0.12 mmol) in THF (2.1 ml) and Et_3N (0.0168 ml, 0.12 mmol) with stirring at 0°C. After being stirred for 5 minutes at 0°C, this mixture was warmed to room temperature and stirred for 21 hours. After completion of the reaction, this reaction mixture was diluted with EtOAc and washed with brine. The aqueous layer was extracted twice with EtOAc. The combined organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a crude product, which was chromatographed on a silica gel column. Elution with benzene - MeCN (4 : 1) gave 71 mg (2 steps, 74%) of **9** as a colorless foamy glass: $[\alpha]_D^{25}$ +113.6° (*c* 1.01, $CHCl_3$); IR (KBr) ν_{max} 3320, 3088, 3063, 3031, 2913, 2869, 1534 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.57 (1H, d, J=4.4 Hz), 7.45~7.05 (30H, m), 6.61 (1H, br s), 5.21 (1H, br s), 4.98 (1H, br s), 4.95~4.40 (9H, m), 4.35~4.15 (4H, m), 4.00 (3H, br d, J=11.9 Hz), 3.70 (4H, br s), 3.54 (2H, br d, J=9.9 Hz), 3.29 (2H, br d, J=8.6 Hz), 2.94 (1H, br s), 2.86 (1H, br d, J=9.9 Hz); FAB-MS, positive m/z 941 $(M+H)^+$; negative m/z 939 $(M-H)^-$; Rf 0.44 (benzene - EtOAc, 2 : 1); High resolution mass: Calcd. for $C_{55}H_{61}N_2O_{10}S$: 941.4047; Found m/z 941.4033 $(M+H)^+$.

[[3a*R*-(3aα,4α,5α,6α,6aα)]-*N*-(5-Benzyloxy-4-benzyloxymethyl-4,6-dihydroxy-3a,5,6,6a-tetrahydro-4*H*-cyclopentoxazol-2-yl)]-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosylamine (**10**)

To a solution of 2-chloro-3-ethylbenzoxazolium tetrafluoroborate (48 mg, 0.18 mmol) in MeCN (3.3 ml) was added a solution of **9** (66 mg, 0.07 mmol) in MeCN (3.0 ml) with stirring at 0°C under nitrogen. After being stirred for 1 hour, to this mixture was added Et_3N (0.05 ml, 0.36 mmol) maintaining 0°C. After completion of the reaction (1 hour), the reaction mixture was diluted with EtOAc and washed with saturated aqueous $NaHCO_3$. The aqueous layer was extracted twice with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a crude product, which was chromatographed on a silica gel column. Elution with benzene - MeCN (5 : 2) gave 45 mg (71%) of **10** as a white foamy glass: $[\alpha]_D^{24}$ +12.0° (*c* 1.13, $CHCl_3$); IR (KBr) ν_{max} 3420, 3331, 3088, 3063, 3031, 2923, 2867, 1672 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.45~7.10 (30H, m), 5.37 (1H, d, J=5.3 Hz), 4.95 (1H, d, J=7.9 Hz), 4.88 (1H, d, J=10.6 Hz), 4.79 (1H, d, J=10.6 Hz), 4.76 (1H, d, J=10.6 Hz), 4.67 (1H, d, J=11.9 Hz), 4.65 (1H, d, J=11.9 Hz), 4.59 (1H, d, J=11.9 Hz), 4.58 (1H, d, J=11.9 Hz), 4.50 (2H, s), 4.49 (1H, d, J=11.9 Hz), 4.47 (1H, d, J=11.9 Hz), 4.42 (1H, d, J=7.9 Hz), 4.41 (1H, d, J=11.9 Hz), 4.17 (1H, br s), 3.85~3.55 (8H, m), 3.49 (1H, d, J=5.3 Hz), 3.35 (1H, br s), 2.60~1.40 (2H, br s); FAB-MS, positive m/z 907 $(M+H)^+$, negative m/z 905 $(M-H)^-$; Rf 0.23 (benzene - MeCN, 3 : 1); High resolution mass: Calcd for $C_{55}H_{59}N_2O_{10}$: 907.4173; Found m/z 907.4163 $(M+H)^+$.

[[3a*R*-(3aα,4α,5α,6α,6aα)]-*N*-(3α,5,6,6α-tetrahydro-4,5,6-trihydroxy-4-hydroxymethyl-4*H*-cyclopentoxazol-2-yl)]-α-D-glucopyranosylamine (5-*epi*-Trehazolin, Trehalostatin) (**2**)

To a solution of **10** (43 mg, 0.05 mmol) in MeOH (8.6 ml) was added $Pd(OH)_2$ on carbon (1.3 g) at room temperature and the mixture was hydrogenolyzed at 60°C for 30 minutes. After completion of the reaction, this reaction mixture was filtered, and concentrated *in vacuo* to give a crude product, which was chromatographed on Amberlite CG-50 (NH_4^+ type/H^+ type, 3 : 2, 5 ml). Elution with 0.5 M aqueous NH_3 gave 3.4 mg (20%) of **2** as a white powder: $[\alpha]_D^{24}$ +110.3° (*c* 0.34, H_2O). IR (KBr) ν_{max} 3368, 2932, 1667 cm^{-1}; ^1H NMR (400 MHz, D_2O/external TMS) δ 5.13 (1H, br d, J=2.8 Hz), 4.78 (1H, dd, J=8.3, 1.0 Hz), 4.19 (1H, d, J=8.3 Hz), 4.00 (1H, dd, J=5.4, 1.0 Hz), 3.75 (1H, d, J=5.4 Hz), 3.62 (1H, dd, J=12.2, 2.4 Hz), 3.58~3.48 (3H, m including doublet at 3.35 ppm, J=11.7 Hz), 3.454 (1H, t, J=9.3 Hz), 3.45 (1H, d, J=11.7 Hz), 3.37 (1H, ddd, J=9.3, 5.4, 2.4 Hz), 3.21 (1H, t, J=9.3 Hz); FAB-MS, positive

m/z 367 $(M+H)^+$, negative *m/z* 365 $(M-H)^-$; Rf 0.32 ($MeCN:H_2O$-AcOH, 13:5:2); High resolution mass: Calcd for $C_{13}H_{23}N_2O_{10}$: 367.1355. Found *m/z* 367.1354 $(M+H)^+$.

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