A FORMAL TOTAL SYNTHESIS OF (+)-GALACTOSTATIN *VIA* A TETRAHYDROXYNORLEUCINE DERIVATIVE

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Abstract—(2*S*,3*S*,4*S*,5*R*)-2,3:4,6-Bis(isopropylidenedioxy)-5-[[(*p*-methoxybenzyloxy)carbonyl]amino]hexan-1-ol (**1 4**), a synthetic intermediate for (+)-galactostatin (**1**), has been synthesized starting from (+)-diethyl tartarate using the aldol-type condensation of methyl isocyanoacetate (**2**) with 4-*O*-benzyl-2,3-*O*-isopropyliden-L-threose (**4**) *via* a 3,4,5,6-tetrahydroxynorleucine derivative (**8**) as a key reaction.

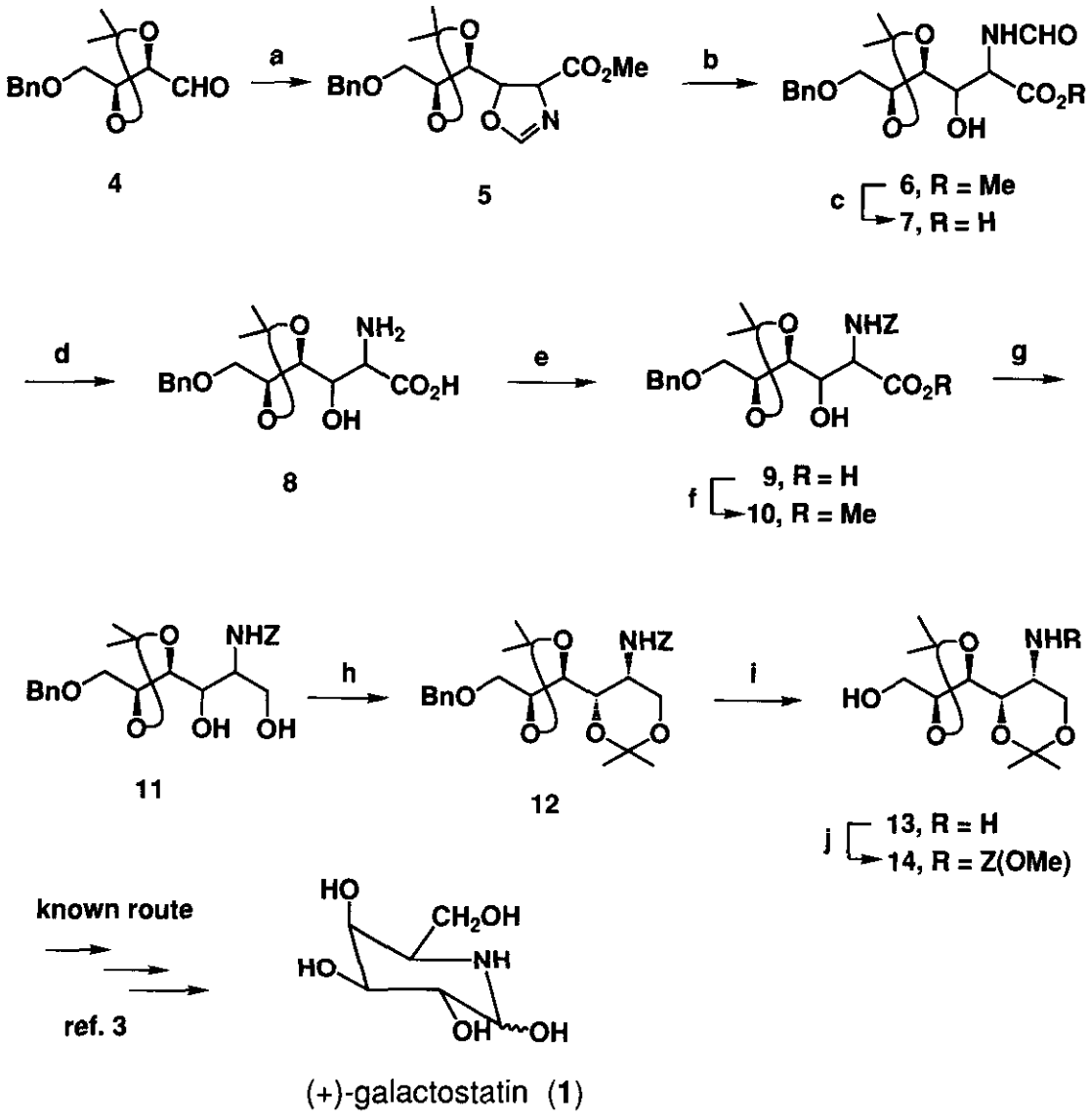
(+)-Galactostatin (**1**) is an azahexose which was isolated from the culture broth of *Streptomyces lydicus* PA-5725, and is a potent and specific inhibitor of several β-galactosidases,[1] therefore, much attention has been focused on synthetic studies of this compound. Control of the stereochemistry at the four contiguous chiral centers located at C(2)-C(5) is a difficult task in the synthesis of **1**. In an attempt to explore the potential azapyranoses, **1** was first synthesized from D-glucose by the chemical modification of existing functional groups.[2] Recently, Kibayashi *et al.*[3] have reported that the stereoselective synthesis of **1** using an asymmetric epoxidation of the allylic alchol which was prepared from nonsugar chiral pool.

On the other hand, we have reported that the stereoselective synthesis of 2-amino-2-deoxy-D-arabinose using the *erythro*-selective aldol-type condensation of metalated methyl isocyanoacetate (**2**) with 2,3-*O*-isopropylidene D-gryceraldehyde. These aspects prompted us to explore an efficient synthesis of (+)-galactostatin by appling these results.

The present paper deals with a facile synthesis of (2*S*,3*S*,4*S*,5*R*)-2,3:4,6-bis(isopropylidene-dioxy)-5-[[(*p*-methoxybenzyloxy)carbonyl]amino]hexan-1-ol (**14**), the Kibayashi's intermediate for the synthesis of (+)-galactostatin by the efficient route shown in Scheme.

The protected L-*threose* (**4**), *was prepared by Swern oxidation from* 4-*O*-*benzyl*-2,3-isopropylidene-L-threitol which was prepared from commercially available (+)-diethyl tartarate according to the literature method.[5] The reaction of **4** with **2** in the presence of sodium cyanide in methanol gave a diastereoisomeric mixture of unstable oxazoline (**5**) *which was then treated with 10% acetic acid to give unseparable N-formylamino acid* methyl ester (**6**) in a 75% overall yield from **4**. The ^{1}H nmr spectra of **6** exhibited that this compound was a mixture of two isomers in a ratio of *ca.* 1: 5.5 (based on the proton integration of the two singlets appearing at δ 3.78 and 3.84 which correspond to the methyl esters). This compound was used for the following reaction without further separation of isomers. Hydrolysis of **6** with sodium hydroxide followed by deformylation with hydrazine provided the tetrahydroxynorleucine derivative (**8**) in a 79% yield from **6**.

With the amino acid **8** in hand, we next turned to elaborate this to the target compund **14** *in a six-step sequence as outlined in Scheme. Thus, protectiont of* **8** *with Z-Cl followed by* esterification with diazomethane gave the methyl ester (**10**) in a 66% overall yield from **8**. Reduction of **10** with lithium aluminum hydride in ether provided the diol (**11**), which was then treated with dimethoxypropane in the presence of PPTS to afford the corresponding bisacetonide as a diastereoisomeric mixture in a 52% overall yield. In this stage, the desired bisacetonide (**12**) was easily separated by flash chromatography on silica gel in a 44% yield (based on **10**). Catalytic hydrogenolysis of **12** in ethanol over 10% Pd-carbon at 50 atm pressure gave the amine (**13**), which was then re-protected with *p*-(methoxybenzyl-oxy)carbony { Z(OMe)} group to furnish **14** in an 80% yield from **12**. The spectral data (ir, nmr and ms) of **14** were identical with those recorded in the literature.[3] The specific rotation ($[\alpha]_D^{25}$ -35.7°, lit.,[3] -32.7°) was in good accord with that of the literature. The alcohol (**14**) has already been transformed into (+)-galactostatin by Kibayashi *et al.*, in three steps.[3]



Scheme 1. Reagent and Conditions:

(a) :$CNCH_2CO_2Me$ (**2**), NaCN, MeOH, 81%; (b) 10% AcOH, 15-20 °C, 3 h, 93%; (c) 1N NaOH, 20 °C, 3 h, 88%; (d) H_2NNH_2, 90% MeOH, reflux, 4 h, 85%; (e) Z-Cl, $NaHCO_3$, 0 °C, 12 h, 74%; (f) CH_2N_2, ether, 91%; (g) LAH, ether, 58%; (h) dimethoxypropane, PPTS, acetone, silica gel column, 76%; (i) 10% Pd-C, EtOH, 50 °C, 50 atm, 6 h, 97%; (j) *p*-methoxybenzyl *S*-(4,6-dimethylpyrimidin-2-yl)thiol-carbonate, Et_3N, dioxane, 82%.

In summary, the stereoselective aldol-type condensation of methyl isocyanoacetate with chiral aldehyde has examined and the formal synthesis of the key intermediate for galactostatin (**1**) was achieved.

EXPERIMENTAL

Melting points are uncorrected. Ir spectra were measured with a Perkin Elmer FT-IR spectrometer. Nmr spectra were taken on a JEOL-MH GSX 270 spectrometer. Unless otherwise stated, tetramethylsilane was used as the internal standard. Mass spectra were obtained with a JMX-AX 500 mass spectrometer. Optical rotation was measured with a JASCO DIP-360 polarimeter. Distillation was performed with a Kugelrohr apparatus.

***(2RS,3RS,4S,5S)*-Methyl 6-benzyloxy-2-formylamino-3-hydroxy-4,5-(isopropylidenedioxy)heptanoate (6)** To a stirred solution of oxalyl chloride (1.4 g, 11 mmol) in CH_2Cl_2 (5 ml) was added dropwise a solution of dimethyl sulfoxide (1.88 g, 24 mmol) in CH_2Cl_2 (5 ml) over a period of 5 min at -78 °C. After 10 min to this mixture was added dropwise a solution of 4-*O*-benzyl-2,3-isopropylidene-L-threitol [5] (2.44 g, 10 mmol) in CH_2Cl_2 (10 ml) over 5 min, and stirring was continued for 15 min. Triethylamine (5.06 g, 50 mmol) was added to the reaction mixture, and the reaction was allowed to warm to ambient temperature. After addition of water (30 ml) the layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 50 ml). The combined extracts were washed with water, dried (Na_2SO_4) and evaporated. Distillation (150-155 °C / 1 mmHg) of the resulting oil gave **4** (2.05 g, 82%) as a colorless oil; ir ν_{max} (neat) cm^{-1} 1734, 1215, 1090, 849; ^1H nmr ($CDCl_3$) δ 1.43 (6H, s), 3.50-3.80 (2H, m), 4.10-4.30 (2H, m), 4.60 (2H, s), 7.33 (5H, m), 9.77 (1H, s). This material was used immediately for the next reaction without further purification.

To an ice-cooled mixture of sodium cyanide (59 mg, 1.2 mmol) in dry MeOH (90 ml) was added dropwise a solution of **4** (1.19 g, 1.2 mmol) and **2** (3.0 g, 12 mmol) in dry MeOH (20 ml) over 30 min under nitrogen. The mixture was stirred at 0 °C for 1 h and at ambient temperature 1.5 h. After removal of the solvent *in vacuo*, the resulting oil was extracted with CH_2Cl_2 (3 x 30 ml). The combined extracts were worked up by the usual manner to give an

oil, which was distilled to provide practically pure **5** (3.41 g, 81%) as a colorless oil; bp 200-205°C / 1 mmHg); ir ν_{max} (neat) cm^{-1} 2988, 1746, 1696, 1631, 1373, 1211, 1069; ^1H nmr ($CDCl_3$) δ 1.42 (6H, s), 3.56-3.76 (2H, m), 3.78 (3H, s) 3.98-4.29 (2H, m), 4.59 (2H, s), 4.71-4.88 (2H, m), 6.88-6.93 (1H, m), 7.27-7.34 (5H, m). This material deteriorated upon storage and was used directly in the next reactions.

A mixture of **5** (2.50 g, 15.7 mmol) and 10% AcOH (9 ml) in EtOAc (16 ml) was stirred at 15-20 °C for 3 h. The organic layer was separated, washed with aq. 10% $NaHCO_3$, dried (Na_2SO_4) and evaporated *in vacuo*. Flash chromatography on silica gel with EtOAc-hexane (6:4) gave **6** (2.44 g, 93%) as a colorless oil; ir ν_{max} (neat) cm^{-1} 3357, 2988, 1741, 1659, 1382, 1248, 1091, 912; ^1H nmr ($CDCl_3$) δ 1.34 (3H, s), 1.39 (3H, s), 3.41-3.42 (2H, m), 3.74 and 3.84 (3H, two s), 3.94-4.27 (3H, m) 4.57 (2H, s) 4.79 (1H, m), 6.25-6.47 (1H, m, NH), 7.28-7.41 (5H, m), 8.03-8.34 (1H, m); ms m/z (relative intensity): 368 (M^++ 1, 1), 260 (1), 188 (3), 160 (2) 107 (5) 91 (100). FABms: m/z 368 (M^++ 1). Anal. Calcd for $C_{18}H_{25}NO_7$: C, 58.85; H, 6.86; N, 3.81. Found: C, 58.98; H, 6.97 ; N, 3.89.

***(2RS,3RS,4S,5S)*-2-Amino-6-benzyloxy-3-hydroxy-4,5-isopropylidenedioxy-hexanoic acid (8).** A solution of **6** (3.10 g, 8.4 mmol) in 1 N NaOH (10.2 ml) was stirred at 20 °C. After 3 h the mixture was acidified with 2 N HCl and evaported. The residue was chromatographed on silica gel using CH_2Cl_2-MeOH (9:1) as eluent to give practically pure **7** (2.64 g, 88%) as a pale yellow oil; ir ν_{max} (neat) cm^{-1} 3362, 2989, 1731, 1670, 1217; ^1H nmr (CD_3OD) δ 1.34 (3H, s), 1.39 (3H, s), 3.65-3.96 (3H, m), 4.05-4.58 (3H, m), 4.68 (2H, s), 4.92-4.94 (1H, m), 7.35-7.46 (5H, m), 8.12-8.20 (1H, m). This material was provided directly in the next reaction.

A stirred mixture of **7** (2.5 g, 7.1 mmol) and 100% hydrazine hydrate (2.12 g, 42.4 mmol) in 90% MeOH (15 ml) was refluxed for 4 h. The mixture was evaporated to dryness, and the residue was chromatographed on silica gel with CH_2Cl_2-MeOH (8:2) to afford **8** as a colorless solid. Recrystallization from water gave a pure **8** (1.96 g, 85%); mp 170-172 °C (decomp.); ir ν_{max} (KBr disk) cm^{-1} 3450, 2990, 1631, 1522, 1385; ^1H nmr (D_2O) δ 1.44

(3H, s), 1.51 (3H, s), 3.76-4.03 (4H, m), 4.17-4.45 (2H, m), 4.69 (2H, s), 7.48 (5H, m); FABms m/z: 326 (M^++ 1); Anal. Calcd for $C_{16}H_{23}NO_6$: C, 59.07; H, 7.12; N, 4.30. Found: C, 59.52; H, 7.49; N, 4.59.

***(2RS,3RS,4S,5S)*-Methyl 6-benzyloxy-2-[(benzyloxy)carbonyl]amino-3-hydroxy-4,5-isopropylidenedioxyhexanoate (10).** To a solution of **8** (1 g, 3.1 mmol) and $NaHCO_3$ (0.80 g, 3.7 mmol) in water (10 ml) was added a solution of carbobenzyloxy chloride (0.79 g, 4.6 mmol) in ether (7 ml) over 2 h at 0 °C. After 3 h $NaHCO_3$ (0.4 g, 4.8 mmol) and carbobenzyloxy chloride (0.16 g, 0.9 mmol) were added to the mixture, and the mixture was stirred for 12 h at 0 °C. After being neutralized with 1N HCl, the mixture was extracted with EtOAc (3 x 20 ml). The combined extracts were washed with brine and dried over Na_2SO_4. The solvent was evaporated to give an oil, which was purified by column chromatography on silica gel with EtOAc-hexane (4:6) to give **9** (1 g, 74%) as a pale yellow oil. To an ice-cooled solution of **9** (1.2 g, 2.6 mmol) in ether (10 ml) was added ethereal solution of diazomethane in small portions with swiring until the yellow color of diazomethane persisted. After being concentrated, the resulting oil was chromatographed on silica gel with EtOA-hexane (1:3) to afford **1 0** (1.1 g, 91%) as a colorless oil; ir ν_{max} (neat) cm^{-1} *3386, 2987, 1730, 1517, 1215*; ^1H *nmr* ($CDCl_3$) δ *1.33* (3H, *s*), *1.38* (3H, *s*), 3.54-3.81 (2H, m), 3.77 (3H, s), 3.97-4.18 (3H, m), 4.57 (2H, s), 4.67 (1H, d, *J*=9.8 Hz), 5.16 (2H, s), 5.56 (1H,, d, *J*=9.8 Hz), 7.26-7.38 (10H, m); HRms: found, m/z 473.5279 (M^+); calcd for $C_{25}H_{31}NO_8$, 473.5268. Anal. Calcd for $C_{25}H_{31}NO_8$: C, 63.41; H, 6.60; N, 2.96. Found: C, 63.31; H, 6.65; N, 3.08.

***(2S,3S,4S,5R)*-1-Benzyloxy-5-[(benzyloxycarbonyl)amino]-2,3;4,6-bis(isopropylidenedioxy)hexane (13).** To an ice-cooled suspension of lithium aluminum hydride (0.36 g, 9.5 mmol) in dry ether (20 ml) was added dropwise a solution of **1 0** (0.95 g, 2.0 mmol) in dry ether (8 ml) over 30 min under nitrogen, and stirring was continued for 20 h at room temperature. Water (5 ml) was added to the mixture at 5 °C, and the mixture was filtered through a pad of Celite. The filtrate was extracted with EtOAc (3 x 30 ml). The combined extracts were washed with brine, dried (Na_2SO_4) and evaporated to

give an oil, which was chromatographed on silica gel with CH_2Cl_2-hexane (4:6) to afford **11** (0.52 g, 58%) as a colorless oil; ir ν_{max} (neat) cm^{-1} 3420, 2986, 1706, 1513, 1246, 1075. ^1H nmr ($CDCl_3$ + D_2O) δ 1.34 (3H, s), 1.36 (3H, s), 3.48-3.83 (5H, m), 3.98-4.18 (3H, m), 4.56 (2H, s), 5.16 (2H, s), 5.49 (1H, d, *J*=8.9 Hz, NH), 7.28-7.65 (10H, m). A mixture of **11** (0.61 g, 1.4 mmol), dimethoxypropane (1.43 g, 13.7 mmol) and pyridinum *p*-toluenesulfonate (34 mg, 0.14 mmol) in dry acetone (12 ml) was stirred at room temperature for 20 h, and refluxed for 2 h. Removal of the solvent left a thick oil, which was extracted with CH_2Cl_2 (2 x 20 ml). The combined organic layers were washed with aq. 10% $NaHCO_3$ and dried ($MgSO_4$). Evaporation of the solvent gave an oil, which was chromatographed on silica gel with EtOAc-hexane (1:9 to 2:8) to afford **12** (0.50 g, 76%) and minor product (90 mg). **12**; $[\alpha]_D^{24}$ -22.4° (*c* 1.09, $CHCl_3$); ir ν_{max} (neat) cm^{-1} 3032, 2989, 1721, 1502, 1079, 915; ^1H nmr ($CDCl_3$) δ 1.21 (3H, s) 1.39 (9H, s), 3.43 (1H, dd, *J*=6.7, 10.4 Hz), 3.60 (1H, dd, *J*=2.4, 10.4 Hz), 3.68 (1H, t, *J*=7.9 Hz), 3.78-3.92 (3H, m), 4.00-4.05 (2H, m), 4.53 (1H, d, *J*=12.5 Hz), 4.63 (1H, d, *J*=12.5 Hz), 5.08 (1H, d, *J*=12.2), 5.15 (1H, d, *J*=11.9 Hz), 5.47 (1H, d, *J*=9.8 Hz), 7.28-7.38 (10H, m); FABms m/z: 486 (M^++ 1); HRms: found, m/z 485.2450 (M^+); calcd for $C_{27}H_{35}NO_7$, 485.2455; Anal. Calcd for $C_{27}H_{35}NO_7$: C, 66.79; H, 7.26; N, 2.88. Found: C, 66.85; H, 7.32; N, 2.96.

***(2S,3S,4S,5R)*-2,3:4,6-Bis(isopropylidenedioxy)-5-[[(*p*-methoxybenzyloxy)-carbonyl]amino]hexan-1-ol (14).** To a mixture of **12** (0.21 g, 0.43 mmol) and 10% Pd-carbon (80 mg) in EtOH (7 ml) was stirred at 50°C in hydrogen at 50 atm pressure for 6 h. After filtration the solvent was removed *in vacuo* and the residue was chromatographed on silica gel with EtOAc-MeOH (9:1) to give **13** as an oil (0.11 g, 97%); $[\alpha]_D^{20}$ +7.4° (*c* 1.36, $CHCl_3$) {lit.,[3] $[\alpha]_D^{25}$ +7.6° (*c* 0.7, $CHCl_3$)}. To a mixtire of **13** (0.120 g, 0.46 mmol) and triethylamine (51.2 mg, 0.50 mmol) in dioxane (1.5 ml) was added a solution of *p*-methoxybenzyl (*S*)-(4,6-dimethylpyrimidin-2-yl)thiolcarbonate (0.15 g, 0.5 mmol) in dioxane (0.8 ml) at room temperature for 4 h.[3] To this mixture was added CH_2Cl_2 (20 ml) and water (5 ml). The separated organic layer was washed with water, dried ($MgSO_4$) and evaporated. The

residue was purified by a flash column on silica gel with EtOAc-hexane (2:8 to 4:6) to afford pure **1 4** (0.16 g, 82%) as a colorless oil; $[\alpha]_D^{22}$ -35.7° (*c* 0.97, $CHCl_3$) {lit.,[3] $[\alpha]_D^{25}$ -32.7° (*c* 1.3, $CHCl_3$)}.

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