Bioscience, Biotechnology, and Biochemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tbbb20

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Published online: 22 May 2014.

To cite this article: Jin Hwan LEE, Min Suk YANG, Kuy Young KANG, Yea Hwang MOON & Ki Hun PARK (2004) Synthesis of All Four Stereoisomers of 3-Amino-2-hydroxybutanoic Acids, Bioscience, Biotechnology, and Biochemistry, 68:3, 714-720, DOI: 10.1271/bbb.68.714

To link to this article: http://dx.doi.org/10.1271/bbb.68.714

Biosci. Biotechnol. Biochem., **68** (3), 714–720, 2004

Synthesis of All Four Stereoisomers of 3-Amino-2-hydroxybutanoic Acids

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Received October 27, 2003; Accepted December 3, 2003

All four stereoisomers of 3-amino-2-hydroxybutanoic acids were been obtained as single enantiomers *via* stereospecific reactions from D-gulonic acid *γ*-lactone and D-glucono-*δ*-lactone.

Key words: isothreonine; alloisothreonine; simultaneous dealkoxyhalogenation; D-gulonic acid *γ*-lactone; D-glucono-*δ*-lactone

α-Hydroxy-*β*-amino acid natural products exhibit a wide range of biological activity which includes antibiotic, antifungal and anti-tumor agents, as well as displaying protease inhibition.[1–5] The synthesis of homochiral *α*-hydroxy-*β*-amino acids has therefore attracted a considerable amount of interest in recent years, and accordingly, a number of synthetic methodologies including Lewis acid-promoted cyanation have been published.[6] These methodologies are ideally suited to the preparation of one stereoisomer of a target *α*-hydroxy-*β*-amino acid, but they still represent a limited stereodivergent approach. We have recently reported the synthesis of (2*S*,3*R*)- and (2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutanoic acid from a carbohydrate source.[7] This present report describes the transformation of sugars by a homochiral synthetic technique to obtain all 3-amino-2-hydroxybutanoic acid stereoisomers (**1**–**4**) with optical purity. (2*S*,3*R*)-3-Amino-2-hydroxybutanoic acid (L-isothreonine, **1**) is the key component of the glycopeptide, 1-*N*-(D-*threo*-3-amino-2-hydroxybutanoyl-2′,3′-dideoxykanamycin A,[8,9] which is known as to be a good antibacterial agent.[8,10] The derivatives of dideoxykanamycin A having (2*S*,3*S*)- and (2*R*,3*R*)-configuration (**2** and **3**) have shown potent antibacterial activity, similar to that of dideoxykanamycin A (Fig. 1).[8] Furthermore, L-leucyl peptides **1** and **3** have similar activity to that of bestatin-related compounds.[11]

Our approach to the synthesis of the target molecules (**1**–**4**) envisaged the use of epoxides and chiral aminoalcohols (**13**, **17**, **18** and **20**) which can be obtained from sugars (D-gulonic acid *γ*-lactone and D-glucono-*δ*-lactone, respectively) *via* a series of selective transformations (Scheme 1).

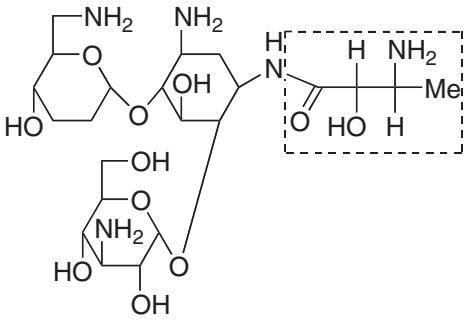
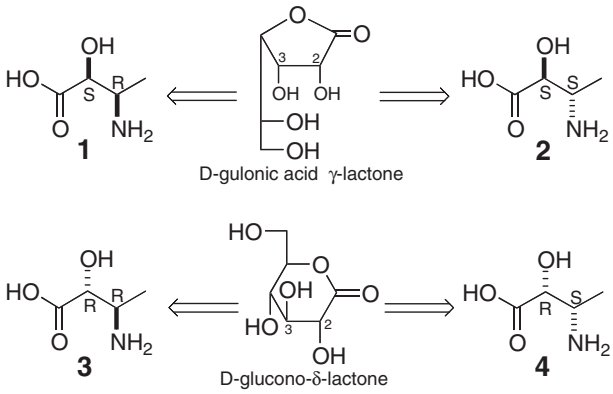


Fig. 1. Structure of Dideoxykanamycin A.



Scheme 1. Retrosynthesis of Target Molecules **1**–**4**.

Results and Discussion

The directly asymmetric conversion of *α*-hydroxy-*β*-amino acid is a highly desirable synthetic transformation, although this methodology suffers from the practical problem of a relatively low yield or limitated stereodivergence. Our objective is to obtain enantiomerically pure *α*-hydroxy-*β*-aminoacids from the well-defined absolute configuration of carbohydrates which have two stereocenters as required for the target

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Abbreviations: 10% Pd/C, palladium 10 wt.% on activated carbon; Pf-Br, 9-phenyl-9-fluorenyl bromide; Tf_2O, trifluoromethanesulfonic anhydride

molecules (**1**–**4**). Thus, the stereochemistry at C2 and C3 of D-gulonic acid γ-lactone was used as a basis for compounds **1** and **2**, whilst the stereochemistry at C2 and C3 of D-glucono-δ-lactone was used for compounds **3** and **4**.

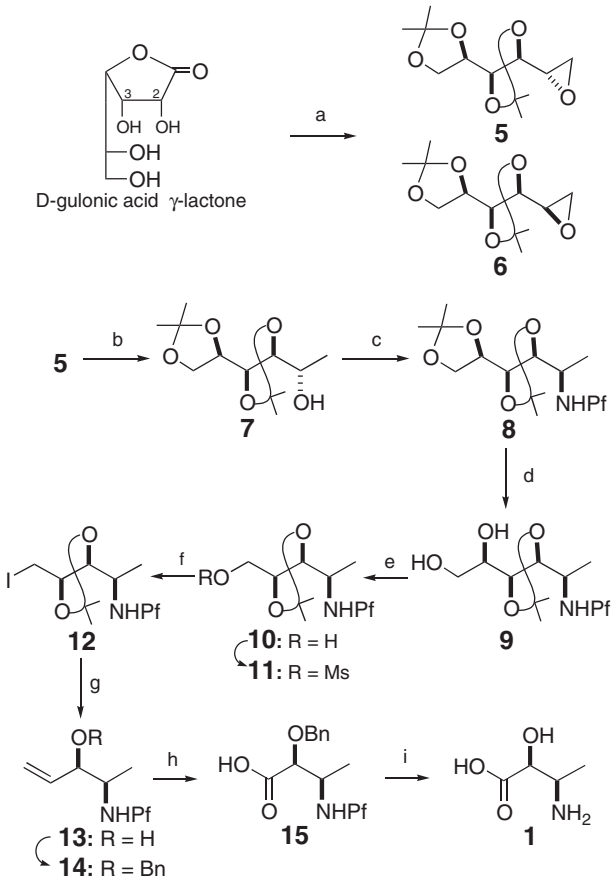
*(2S,3R)-3-Amino-2-hydroxybutanoic acid (L-isothreonine) (**1**)*

Diisopropylidene-D-glucitol (**5**) was synthesized in a high yield *via* a four-step process from D-gulonic acid γ-lactone.[7] Epoxide **5** was reacted with $LiAlH_4$ to give secondary alcohol **7** in an 81% yield, with no evidence of any product arising from an endoreductive cleavage pathway. Protected amine **8** was prepared in a 57% yield from the secondary alcohol according to our recently published methodology,[7,12] in which the addition of a pre-cooled solution of Tf_2O in CH_2Cl_2 at $-10°C$ suppressed epimerization in the triflation step. The 9-phenyl-9-fluorenyl (Pf) group was chosen to protect the amino moiety as it has been shown to be not only stable under basic conditions but also stable to organometallic reagents (Scheme 2).[13] The terminal isopropylidene group was selectively cleaved by treating diisopropylidene **8** with Dowex 50-X8 resin to give diol **9** in a 91% yield.[14]

Compound **9** was then treated with $NaIO_4$, and the resulting aldehyde was reduced with $NaBH_4$, leading to the formation of alcohol **10** in a 91% overall yield. Mesylation of alcohol **10** with MsCl in THF generated mesylate **11**, and subsequent treatment with LiI at 80°C gave iodide **12** in an 88% yield. Treatment of iodide **12** with *n*-BuLi at $-40°C$ generated (3*R*,4*R*) aminoalcohol **13** {$[\alpha]_D^{20}$ $-287.6°$ (*c* 4.00, $CHCl_3$)} in an 89% yield through simultaneous dealkoxyhalogenation.[12,15] The secondary hydroxyl group of **13** was easily protected with BnBr to give benzylate **14** in a 91% yield. Ozonolysis of benzylate **14** and subsequent H_2O_2 oxidation afforded protected (2*S*,3*R*)-3-amino-2-hydroxybutanoic acid **15**. To remove the Pf and Bn protecting groups, compound **15** was treated with H_2 and 10% Pd/C in MeOH. Subsequent purification by ion-exchange chromatography (Dowex 50W-X8) yielded the base form of (2*S*,3*R*)-3-amino-2-hydroxybutanoic acid (L-isothreonine) **1** as a solid. ^{1}H- and ^{13}C-NMR data and the optical rotation for **1** are consistent with those reported.[8,9,16]

*(2S,3S)-3-Amino-2-hydroxybutanoic acid (L-alloisothreonine) (**2**)*

Epoxide **6** was prepared from D-gulonic acid γ-lactone.[7] To prepare (2*S*,3*S*)-3-amino-2-hydroxybutanoic acid (**2**), called L-alloisothreonine, epoxide **6** was converted to compound **16** through triflation, azidation, hydrogenation, and an *N*-protection step. *N*-Protected **16** was subjected to the same reaction conditions as those already described above (**8**→**13**) to afford (3*R*,4*S*) aminoalcohol **17** {$[\alpha]_D^{20}$ $+290.7°$ (*c* 1.00, $CHCl_3$)} (Scheme 3). After benzylating aminoalcohol **17**, the resulting benzylate was subjected to ozonolysis and hydrogenolysis by a similar procedure to that for compound **1** to give (2*S*,3*S*)-3-amino-2-hydroxybutanoic acid **2**. The physical properties and ^{1}H- and ^{13}C-spectral data for compound **2** were identical to those previously reported.[10,17]

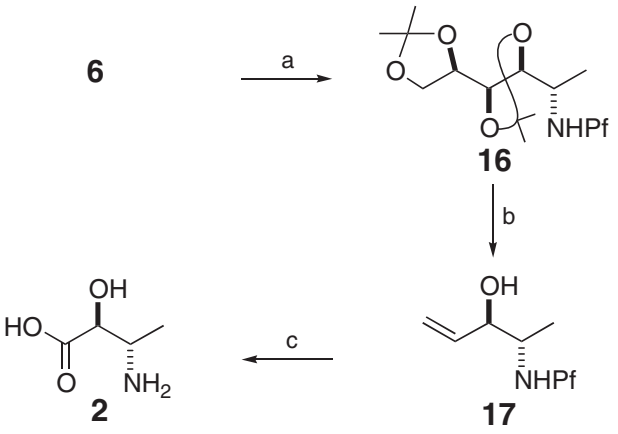


Scheme 2. Synthetic Route to the (2*S*,3*R*)-3-Amino-2-hydroxybutanoic Acid (**1**).

a: ref. 7; b: LAH, THF, 0°C (81% yield); c: i) Tf_2O, pyridine, CH_2Cl_2, $-10°C$ (90% yield); ii) NaN_3, DMF, rt (94% yield); iii) H_2, Pd/C, EtOAc, rt (85% yield); iv) Pf-Br, $Pb(NO_3)_2$, Et_3N, CH_2Cl_2, rt (79% yield); d: Dowex 50W-X8, MeOH, rt (91% yield); e: i) $NaIO_4$, EtOH-H_2O (2:1), rt, $NaBH_4$, 0°C (91% yield); ii) MsCl, Et_3N, THF, 0°C (96%, yield); f: LiI, DMF, 80°C (88% yield); g: i) *n*-BuLi, THF, $-40°C$ (89% yield); ii) BnBr, 60% NaH, Bu_4NI, THF, 0°C (91% yield); h: O_3, MeOH, $-78°C$, 30% H_2O_2, rt (90% yield); i: H_2, 10% Pd/C, MeOH, 70°C (83% yield).

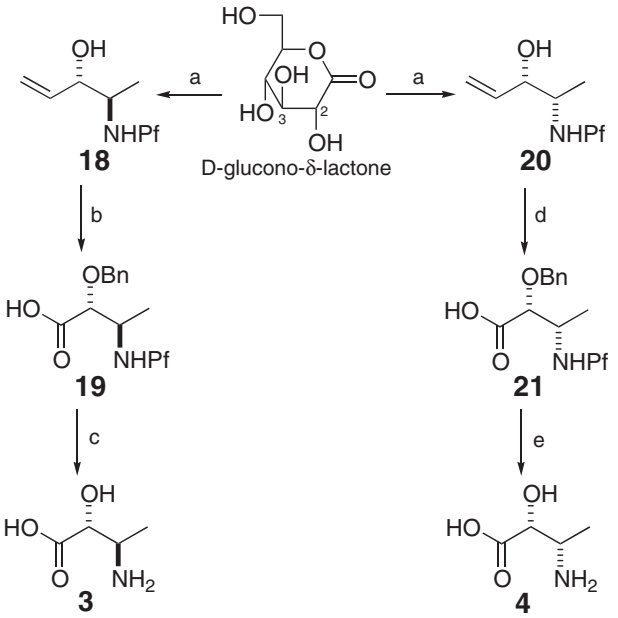
*(2R,3R)- and (2R,3S)-3-Amino-2-hydroxybutanoic acid (D-alloisothreonine and D-isothreonine; **3** and **4**)*

To further demonstrate the versatility of this synthetic strategy, we prepared the (2*R*,3*R*)- and (2*R*,3*S*)-3-amino-2-hydroxybutanoic acids (**3** and **4**). The aminoalcohols (**18** and **20**) were obtained as single stereoisomers from D-glucono-δ-lactone by the same procedure as that already described (**5**→**13**). After benzylating the chiral aminoalcohols (**18** and **20**), the resulting chiral benzylates were sequentially subjected to ozonolysis and hydrogenolisis to afford either (2*R*,3*R*)- or (2*R*,3*S*)-3-amino-2-hydroxybutanoic acid (**3** or **4**) as a single



Scheme 3. Synthetic Route to the (2*S*,3*S*)-3-Amino-2-hydroxybutanoic Acid (**2**).

a: i) LAH, THF, 0°C; ii) Tf_2O, pyridine, CH_2Cl_2, −10°C; iii) NaN_3, DMF, rt; iv) H_2, Pd/C, EtOAc, rt; v) Pf-Br, $Pb(NO_3)_2$, Et_3N, CH_2Cl_2, rt (58% yield, 5 steps); b: i) Dowex 50W-X8, MeOH, rt; ii) $NaIO_4$, EtOH-H_2O (2:1), rt, $NaBH_4$, 0°C; iii) MsCl, Et_3N, THF, 0°C; iv) LiI, DMF, 80°C; v) *n*-BuLi, THF, −40°C (63% yield, 5 steps); c: i) BnBr, 60% NaH, Bu_4NI, THF, 0°C; ii) O_3, MeOH, −78°C, 30% H_2O_2, rt; iii) H_2, 10% Pd/C, MeOH, 70°C (65% yield, 3 steps).



Scheme 4. Synthetic Route to the (2*R*,3*R*)- and (2*R*,3*S*)-3-Amino-2-hydroxybutanoic Acids (**3** and **4**).

a, b, c, d, e: i) ref. 7; ii) (2*R*,3*R*)- and (2*R*,3*S*)-3-amino-2-hydroxybutanoic acids (**3** and **4**) were obtained by the same procedure as that used for the conversion of **5** to **1**.

diastereomer in each case. The spectroscopic data for **3** and **4** are consistent with those reported (Scheme 4).[9,10,17]

In conclusion, we report here the stereospecific synthesis of chiral aminoalcohols **13**, **17**, **18**, and **20** *via* simultaneous dealkoxyhalogenation from sugars (D-gulonic acid γ-lactone and D-glucono-δ-lactone, respectively). These compounds are important precursors for the asymmetric synthesis of bioactive α-hydroxy-β-amino acids. We additionally report that all four stereoisomers of 3-amino-2-hydroxybutanoic acids (**1**–**4**) could be obtained in enantiomerically pure form from the respective chiral aminoalcohol.

Experimental

All non-aq. reactions were carried out in an inert nitrogen atmosphere. THF was distilled from Na/benzophenone, and 2,2-dimethoxypropane, DMF, and methylene chloride were distilled from CaH_2. Column chromatography was carried out with 230–400 mesh silica gel. The final solution before evaporation was washed with brine and dried over anhydrous Na_2SO_4. All melting point(mp) data were measured by Thomas Scientific capillary melting point apparatus and are uncorrected. IR spectra were recorded by a Bruker IFS66 infrared Fourier transform spectrophotometer, and ^{1}H-NMR and ^{13}C-NMR experiments were conducted with a Brucker AW-500 spectrometer. Optical rotation values were measured with a Jasco DIP-1000 polarimeter, and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}.

*1,2-Anhydro-3,4;5,6-di-O-isopropylidene-D-gulitol (**5**).* This compound was prepared from D-gulonic acid γ-lactone as previously described.[7]

*1-Deoxy-3,4;5,6-di-O-isopropylidene-D-gulitol (**7**).* To an ice-cooled solution of $LiAlH_4$ (0.31 g, 8.19 mmol) in THF (14 ml) was added a solution of epoxide compound **5** (1.00 g, 4.09 mmol) in THF (6 ml). The reaction mixture was warmed to room temperature, stirred for 25 min, and then quenched by the sequential addition of water (1.0 ml), 15% aq. NaOH (1.0 ml), and water (3.0 ml). The mixture was filtered and evaporated. The resulting residue was chromatographed on silica gel (EtOAc-hexane, 1:3) to give compound **7** (0.82 g, 81%) as an oil; $[\alpha]_D^{20} = +7.8°$ (*c* 4.00, $CHCl_3$); NMR δ_H ($CDCl_3$): 1.27 (d, $J = 6.0$ Hz, 3H), 1.38 (s, 3H), 1.40 (s, 3H), 1.43 (s, 3H), 1.44 (s, 3H), 2.24 (d, $J = 3.1$ Hz, 1H), 3.87 (m, 2H), 3.94 (t, $J = 7.5$ Hz, 1H), 4.06 (m, 2H), and 4.23 (m, 1H); NMR δ_C ($CDCl_3$): 19.4, 25.5, 26.1, 27.0, 27.3, 65.9, 68.3, 75.6, 77.7, 80.8, 109.4, and 109.7; IR ν_{max} (KBr) cm^{-1}: 3477, 2998, 2940, 2904. *Anal*. Found: C, 58.51; H, 9.01%. Calcd. for $C_{12}H_{22}O_5$: C, 58.52; H, 9.00%.

*1,2-Dideoxy-3,4;5,6-di-O-isopropylidene-2-[(9-phenyl-9-fluorenyl)-amino]-D-iditol (**8**).* To a solution of gulitol **7** (0.70 g, 2.84 mmol) in CH_2Cl_2 (10 ml) at −10°C was added pyridine (0.70 ml, 8.53 mmol), before an ice-cooled solution of Tf_2O (0.72 ml, 4.26 mmol) in CH_2Cl_2 (6 ml) was dropped for 5 min at the same temperature. The reaction mixture was stirred for 10 min at −10°C, and then quenched with saturated aq.

$NaHCO_3$ (16 ml). The organic layer was washed with saturated aq. $CuSO_4$ (16 ml) and then evaporated to give a triflate (0.97 g, 90%) which was used without further purification. A mixture of this triflate (0.97 g, 2.57 mmol) and NaN_3 (0.50 g, 7.70 mmol) in DMF (10 ml) was stirred for 2 h at room temperature. The reaction mixture was quenched with H_2O (50 ml) and extracted with EtOAc (90 ml). After evaporating the organic layer, the remaining residue was subjected to flash column chromatography to give an azide compound (0.65 g, 94%). This compound was directly hydrogenated with 10% Pd/C (0.07 g) in EtOAc (10 ml) to the corresponding free amine (0.50 g, 85%). To a solution of this free amine (0.50 g, 2.04 mmol) in CH_2Cl_2 (10 ml) were added Pf-Br (0.98 g, 3.06 mmol), $Pb(NO_3)_2$ (1.01 g, 3.06 mmol), and Et_3N (0.60 ml, 4.08 mmol). After stirring for 24 h at room temperature, the mixture was filtered, poured into an excess of H_2O, and extracted with CH_2Cl_2 (60 ml). After concentrating the combined extracts, the resulting residue was chromatographed on silica gel (EtOAc-hexane, 1:6) to give *N*-protected compound **8** (0.78 g, 79%) as a solid, mp 40–42°C; $[\alpha]_D^{20} = -103.7^\circ$ (*c* 1.00, $CHCl_3$); NMR δ_H ($CDCl_3$): 0.62 (d, $J = 6.6$ Hz, 3H), 1.35 (s, 3H), 1.36 (s, 3H), 1.40 (s, 3H), 1.48 (s, 3H), 2.19 (br, 1H), 2.31 (m, 1H), 3.69 (m, 2H), 3.90 (m, 2H), 4.18 (dd, $J = 3.5$, 7.9 Hz, 1H), and 7.18–7.68 (m, 13H); NMR δ_C ($CDCl_3$): 20.9, 26.2, 26.7, 27.4, 28.0, 48.5, 66.2, 72.9, 76.1, 82.5, 109.4, 109.8, 120.3, 120.4, 125.8, 126.1, 126.5, 127.5, 128.0, 128.2, 128.6, 128.7, 128.7, 140.7, 140.9, 146.0, 149.5, and 152.0; IR ν_{max} (KBr) cm^{-1}: 3319, 3070, 2991, 2940, 2904, 1728. *Anal*. Found: C, 76.68; H, 7.26; N, 2.87%. Calcd. for $C_{31}H_{35}NO_4$: C, 76.67; H, 7.26; N, 2.88%.

*1,2-Dideoxy-3,4-O-isopropylidene-2-[(9-phenyl-9-fluorenyl)-amino]-D-iditol (**9**).* To a solution of *N*-protected compound **8** (3.00 g, 6.18 mmol) in 90% MeOH (30 ml) was added Dowex 50W-X8 resin (0.30 g) at room temperature. After stirring for 24 h, the reaction mixture was filtered, and then the filtrate was evaporated. The resulting residue was chromatographed on silica gel (EtOAc-hexane, 1:2) to give diol **9** (2.50 g, 91%) as a solid, mp 66–68°C; $[\alpha]_D^{20} = -120.5^\circ$ (*c* 1.00, $CHCl_3$); NMR δ_H ($CDCl_3$): 0.66 (d, $J = 6.6$ Hz, 3H), 1.33 (s, 3H), 1.42 (s, 3H), 2.37 (m, 1H), 2.56 (br, 1H), 3.50 (m, 1H), 3.69 (m, 2H), 3.75 (dd, $J = 3.6$, 8.2 Hz, 1H), 4.16 (dd, $J = 2.8$, 8.2 Hz, 1H), and 7.19–7.69 (m, 13H); NMR δ_C ($CDCl_3$): 19.8, 27.4, 27.8, 48.6, 65.5, 70.0, 73.1, 78.7, 81.6, 109.3, 120.4, 120.5, 125.7, 125.9, 126.4, 127.6, 128.1, 128.2, 128.7, 128.8, 128.9, 140.8, 140.9, 145.5, 149.3, and 151.4; IR ν_{max} (KBr) cm^{-1}: 3429, 3341, 3070, 2990, 2931, 1600. *Anal*. Found: C, 75.50; H, 7.00; N, 3.14%. Calcd. for $C_{28}H_{31}NO_4$: C, 75.48; H, 7.01; N, 3.14%.

*1,2-Dideoxy-3,4-O-isopropylidene-2-[(9-phenyl-9-fluorenyl)-amino]-L-xylitol (**10**).* To a solution of diol **9** (2.00 g, 4.49 mmol) in EtOH-H_2O (30 ml:15 ml) was added $NaIO_4$ (1.44 g, 6.73 mmol) at room temperature. After stirring for 3 h, the mixture was cooled to 0°C, $NaBH_4$ (0.25 g, 6.73 mmol) was added, and the resulting mixture was stirred for 10 min. After evaporating EtOH, the mixture was poured into an excess of H_2O and extracted with EtOAc (120 ml). After concentrating the combined extracts, the residue was chromatographed on silica gel (EtOAc-hexane, 1:3) to give alcoholic compound **10** (1.70 g, 91%) as a solid, mp 49–51°C; $[\alpha]_D^{20} = -79.5^\circ$ (*c* 2.00, $CHCl_3$); NMR δ_H ($CDCl_3$): 0.81 (d, $J = 6.8$ Hz, 3H), 1.20 (s, 3H), 1.32 (s, 3H), 2.46 (m, 1H), 3.38 (dd, $J = 4.1$, 8.3 Hz, 3H), 3.62 (m, 1H), 3.74 (dd, $J = 4.4$, 11.0 Hz, 1H), 4.05 (m, 1H), and 7.02–7.72 (m, 13H); NMR δ_C ($CDCl_3$): 17.9, 27.2, 27.2, 27.4, 48.8, 63.7, 73.5, 76.7, 82.9, 108.6, 120.6, 120.7, 125.1, 126.1, 126.3, 126.3, 127.8, 128.3, 128.4, 128.8, 128.9, 129.0, 129.0, 129.1, 140.7, 141.4, 144.6, 149.1, and 150.2; IR ν_{max} (KBr) cm^{-1}: 3436, 3319, 3070, 3019, 2990, 2931, 2873, 1739, 1607. *Anal*. Found: C, 78.04; H, 7.02; N, 3.36. Calcd. for $C_{27}H_{29}NO_3$: C, 78.04; H, 7.03; N, 3.37%.

*1,2-Dideoxy-3,4-O-isopropylidene-5-O-methanesulfonyl-2-[(9-phenyl-9-fluorenyl)-amino]-L-xylitol (**11**).* To a solution of alcohol **10** (1.50 g, 3.60 mmol) in THF (16 ml) were added triethylamine (1.00 ml, 7.21 mmol) and methanesulfonyl chloride (0.56 ml, 7.21 mmol) at 0°C. The reaction mixture was stirred for 10 min at the same temperature, before being quenched with saturated aq. $NaHCO_3$ (35 ml). The organic phase was separated, and the aq. phase was extracted with EtOAc (105 ml). After concentrating the combined extracts, the resulting residue was chromatographed on silica gel (EtOAc-hexane, 1:5) to give mesylate **11** (1.70 g, 96%), mp 48–50°C; $[\alpha]_D^{20} = -98.6^\circ$ (*c* 1.00, $CHCl_3$); NMR δ_H ($CDCl_3$): 0.66 (d, $J = 6.6$ Hz, 3H), 1.33 (s, 3H), 1.42 (s, 3H), 2.13 (br, 1H), 2.36 (dd, $J = 3.9$, 6.6 Hz 1H), 3.05 (s, 3H), 3.52 (dd, $J = 3.9$, 8.2 Hz, 1H), 4.12 (dd, $J = 6.1$, 11.2 Hz, 1H), 4.37 (m, 1H), 4.46 (dd, $J = 2.6$, 11.2 Hz, 1H), and 7.18–7.71 (m, 13H); NMR δ_C ($CDCl_3$): 19.1, 26.8, 27.0, 27.3, 37.8, 48.2, 69.8, 72.7, 75.1, 81.0, 109.5, 120.0, 120.1, 120.2, 125.2, 125.4, 126.1, 127.2, 127.8, 127.9, 128.4, 128.4, 128.5, 140.3, 140.6, 145.2, 149.0, and 151.0; IR ν_{max} (KBr) cm^{-1}: 3346, 2983, 2933, 1735, 1662. *Anal*. Found: C, 68.14; H, 6.33; N, 2.85%. Calcd. for $C_{28}H_{31}NO_5S$: C, 68.13; H, 6.33; N, 2.84%.

*1,2,5-Trideoxy-3,4-O-isopropylidene-5-iodo-2-[(9-phenyl-9-fluorenyl)-amino]-L-xylitol (**12**).* To a solution of mesylate **11** (1.50 g, 3.04 mmol) in DMF (15 ml) was added LiI (1.22 g, 9.12 mmol) over 12 h at 80°C. To the reaction mixture was added saturated aq. $NaHCO_3$ (40 ml), before extracting with EtOAc (90 ml). The extract was evaporated and chromatographed on silica gel (EtOAc-hexane, 1:12) to give iodonate **12** (1.40 g, 88%) as a solid, mp 51–54°C; $[\alpha]_D^{20} = -103.2^\circ$ (*c* 2.00, $CHCl_3$); NMR δ_H ($CDCl_3$): 0.62 (d, $J = 6.6$ Hz, 3H),

1.40 (s, 3H), 1.46 (s, 3H), 2.15 (br, 1H), 2.34 (m, 1H), 3.09 (dd, $J = 6.0$, 10.6 Hz, 1H), 3.27 (dd, $J = 4.0$, 10.6 Hz, 1H), 3.41 (dd, $J = 3.4$, 7.5 Hz, 1H), 4.11 (m, 1H), and 7.17–7.69 (m, 13H); NMR δ_C ($CDCl_3$): 7.9, 20.4, 27.9, 28.0, 48.7, 73.0, 76.6, 86.1, 109.3, 120.4, 120.5, 125.8, 126.0, 126.5, 127.6, 128.2, 128.3, 128.7, 128.7, 128.8, 140.7, 140.9, 145.8, 149.5, and 151.7; IR ν_{max} (KBr) cm^{-1}: 3341, 3078, 2990, 2939, 1607. *Anal*. Found: C, 61.72; H, 5.36; N, 2.67%. Calcd. for $C_{27}H_{28}INO_2$: C, 61.72; H, 5.37; N, 2.67%.

*(3R,4R)-3-Hydroxy-4-[(9-phenyl-9-fluorenyl)-amino]-1-pentene (**13**).* To a solution of iodinate **12** (1.30 g, 2.47 mmol) in THF (15 ml) was dropped 2.5 M *n*-BuLi (1.98 ml, 4.95 mmol, 200 mol%) over 10 min *via* a syringe at −40°C. The reaction mixture was stirred for an additional 15 min at the same temperature and then quenched with saturated aq. NH_4Cl (20 ml). The mixture was extracted with EtOAc (60 ml) and concentrated. The resulting residue was chromatographed on silica gel (EtOAc-hexane, 1:4) to give allylic alcohol **13** (0.75 g, 89%) as a solid, mp 51–52°C; $[\alpha]_D^{20} = -287.6°$ (*c* 4.00, $CHCl_3$); NMR δ_H ($CDCl_3$): 0.55 (d, $J = 6.4$ Hz, 3H), 2.12 (dt, $J = 6.5$, 12.9 Hz, 1H), 2.90 (br, 1H), 3.58 (t, $J = 6.7$ Hz, 1H), 5.04–5.07 (m, 1H), 5.17–5.21 (m, 1H), 5.51–5.58 (m, 1H), and 7.19–7.71 (m, 13H); NMR δ_C ($CDCl_3$): 19.1, 53.0, 72.5, 77.3, 117.0, 120.0, 120.0, 125.1, 125.6, 125.9, 127.2, 127.7, 128.1, 128.3, 138.4, 140.1, 140.6, 145.1, 148.7, and 151.0; IR ν_{max} (KBr) cm^{-1}: 3404, 3317, 3078, 3027, 2933, 1815, 1735. *Anal*. Found: C, 84.41; H, 6.79; N, 4.11%. Calcd. for $C_{24}H_{23}NO$: C, 84.42; H, 6.79; N, 4.10%.

*(3R,4R)-3-O-Benzyl-4-[(9-phenyl-9-fluorenyl)-amino]-1-pentene (**14**).* Allylic alcohol **13** (1.00 g, 2.93 mmol) was dissolved in THF (15 ml) and treated with 60% NaH (0.23 g, 5.86 mmol) and Bu_4NI (0.32 g, 0.88 mmol) at 0°C. After stirring for 10 min at the same temperature, BnBr (0.70 ml, 5.86 mmol) was added. The reaction mixture was stirred for 24 h at room temperature and then quenched with saturated aq. NH_4Cl (30 ml), before the mixture was extracted with EtOAc (90 ml). After concentrating the combined extracts, the residue was purified by silica gel column chromatography (EtOAc-hexane, 1:8) to give benzylate **14** (1.15 g, 91%) as an oil, $[\alpha]_D^{20} = -1.2°$ (*c* 4.00, $CHCl_3$); NMR δ_H ($CDCl_3$): 0.56 (d, $J = 6.5$ Hz, 3H), 2.43 (dt, $J = 6.4$, 12.7 Hz, 1H), 3.48 (dd, $J = 6.3$, 7.0 Hz, 1H), 4.14 (d, $J = 12.2$ Hz, 1H), 4.36 (d, $J = 12.2$ Hz, 1H), 5.23–5.29 (m, 2H), 5.74–5.81 (m, 1H), and 7.16–7.68 (m, 13H); NMR δ_C ($CDCl_3$): 18.5, 51.7, 70.5, 73.4, 84.9, 119.0, 120.2, 120.3, 125.8, 126.1, 126.5, 127.4, 127.7, 127.9, 128.0, 128.1, 128.4, 128.5, 128.6, 128.7, 136.1, 136.3, 139.2, 140.7, 140.9, 146.4, 150.2, and 151.6; IR ν_{max} (neat) cm^{-1}: 3334, 3070, 3026, 2975, 2939, 2873, 1951. *Anal*. Found: C, 87.56; H, 6.57; N, 2.76%. Calcd. for $C_{31}H_{29}NO$: C, 86.27; H, 6.77; N, 3.25%.

*(2S,3R)-2-O-Benzyl-3-[(9-phenyl-9-fluorenyl)-amino]-butanoic acid (**15**).* A solution of benzylate **14** (0.80 g, 1.85 mmol) in CH_3OH (16 ml) was ozonized at −78°C until the solution turned blue, the residual ozone then being removed by purging with N_2 gas. The reaction mixture was left to reach room temperature, 30% H_2O_2 (16 ml) was added, and the mixture was stirred overnight. After concentrating the combined extracts, the residue was chromatographed on silica gel (EtOAc-hexane, 1:1) to give compound **15** (0.75 g, 90%) as a solid, mp 76–78°C; $[\alpha]_D^{20} = -59.4°$ (*c* 2.00, $CHCl_3$); NMR δ_H ($CDCl_3$): 0.89 (d, $J = 6.7$ Hz, 3H), 2.57–2.62 (m, 1H), 3.26 (d, $J = 3.7$ Hz, 1H), 4.13 (d, $J = 12.2$ Hz, 1H), 4.56 (d, $J = 12.2$ Hz, 1H), and 7.04–7.74 (m, 19H); NMR δ_C ($CDCl_3$): 17.6, 50.0, 72.6, 72.8, 78.7, 120.5, 120.5, 124.2, 125.4, 125.7, 127.7, 127.7, 127.9, 128.3, 128.4, 128.5, 128.9, 129.2, 129.3, 137.4, 139.9, 141.2, 142.9, 146.9, 148.3, and 171.8; IR ν_{max} (KBr) cm^{-1}: 3331, 3062, 3010, 2920, 1708, 1603. *Anal*. Found: C, 80.15; H, 6.04; N, 3.12%. Calcd. for $C_{30}H_{27}NO_3$: C, 80.15; H, 6.05; N, 3.12%.

*(2S,3R)-3-Amino-2-hydroxybutanoic acid [(**1**), L-isothreonine].* Protected butanoic acid **15** (0.50 g, 1.11 mmol) and 10% Pd/C (0.05 g) were stirred in CH_3OH (12 ml) under an atmosphere of hydrogen at 70°C for 12 h. After filtering the catalyst off with Celite, to the filtrate was added Dowex 50W-X8. The mixture was filtered, and then washed with MeOH. The remaining residue was eluted with 3 N NH_4OH. The ammoniacal solution was evaporated, and co-evaporation with toluene gave L-isothreonine **1** (0.11g, 83%) as a solid, mp 213–216°C; $[\alpha]_D^{20} = -13.2°$ (*c* 0.70, H_2O) {lit.[16] $[\alpha]_D^{25} = -12.4°$ (*c* 0.75, H_2O), mp > 225°C (decomp.)}; NMR δ_H (D_2O): 1.27 (d, $J = 11.3$ Hz, 3H), 3.59–3.63 (m, 1H), and 4.24 (d, $J = 7.9$ Hz, 1H); NMR δ_C (D_2O): 14.5, 49.2, 70.8, and 174.1; IR ν_{max} (KBr) cm^{-1}: 3416, 3063, 2930, 1740. *Anal*. Found: C, 40.31; H, 7.62; N, 11.77%. Calcd. for $C_4H_9NO_3$: C, 40.33; H, 7.62; N, 11.76%.

*1,2-Anhydro-3,4;5,6-di-O-isopropylidene-D-iditol (**6**).* This compound was prepared from D-gulonic acid γ-lactone as previously described.[7]

*1,2-Dideoxy-3,4;5,6-di-O-isopropylidene-2-[(9-phenyl-9-fluorenyl)-amino]-D-mannonate (**16**).* *N*-protected **16** was obtained by the same procedure with the conversion of **5** to **8**; the overall yield for this conversion was 58%; mp 48–52°C; $[\alpha]_D^{20} = +0.4°$ (*c* 2.00, $CHCl_3$); NMR δ_H ($CDCl_3$): 0.77 (d, $J = 6.5$ Hz, 3H), 1.30 (s, 3H), 1.32 (s, 3H), 1.33 (s, 3H), 1.34 (s, 3H), 2.23 (m, 2H), 3.28 (t, $J = 8.0$ Hz, 1H), 3.45 (dd, $J = 6.5$, 8.2 Hz, 1H), 3.55 (dd, $J = 5.4$, 7.8 Hz, 1H), 3.62 (dd, $J = 3.7$, 7.8 Hz, 1H), 3.80–3.84 (m, 1H), and 7.17–7.71 (m, 13H); NMR δ_C ($CDCl_3$): 17.6, 25.7, 26.3, 27.0, 27.1, 49.6, 65.2, 73.0, 76.8, 78.7, 81.4, 109.3, 109.4, 120.0, 120.0, 125.3, 125.5, 126.1, 127.1, 127.9, 127.9,

128.2, 128.3, 128.4, 140.3, 140.4, 145.3, 150.1, and 150.5; IR ν_{max} (KBr) cm^{-1}: 3303, 3063, 2991, 2896, 1598. *Anal*. Found: C, 76.67; H, 7.26; N, 2.86%. Calcd. for $C_{31}H_{35}NO_4$: C, 76.67; H, 7.26; N, 2.88%.

*(3R,4S)-3-Hydroxy-4-[(9-phenyl-9-fluorenyl)-amino]-1-pentene (**17**).* Allylic alcohol **17** was obtained by the same procedure with the conversion of **8** to **13**; the overall yield for this conversion was 63%; mp 48–52°C; $[\alpha]_D^{20} = +290.7°$ (*c* 1.00, $CHCl_3$); NMR δ_H ($CDCl_3$): 0.66 (d, $J = 6.7$ Hz, 3H), 2.29 (m, 1H), 3.12 (br, 1H), 3.52 (m, 1H), 5.01–5.10 (m, 2H), 5.59 (m, 1H), and 7.20–7.73 (m, 13H); NMR δ_C ($CDCl_3$): 17.6, 52.8, 73.0, 74.5, 115.7, 120.4, 120.5, 125.1, 125.7, 126.3, 127.7, 128.3, 128.5, 128.8, 128.9, 137.7, 140.3, 141.4, 145.5, 149.6, and 150.8; IR ν_{max} (KBr) cm^{-1}: 3462, 3027, 2933, 1735, 1644. *Anal*. Found: C, 84.40; H, 6.79; N, 4.12%. Calcd. for $C_{24}H_{23}NO$: C, 84.42; H, 6.79; N, 4.10%.

*(2S,3S)-3-Amino-2-hydroxybutanoic acid [(**2**), L-alloisothreonine].* Hydroxybutanoic acid **2** was obtained by same procedure with the conversion of **13** to **1**; the overall yield for this conversion was 65%; mp 243–245°C; $[\alpha]_D^{20} = -24.8°$ (*c* 1.00, H_2O) {lit.[17] $[\alpha]_D^{23} = -25.7°$ (*c* 1.10, H_2O), mp 240–241°C}; NMR δ_H (D_2O): 1.23 (d, $J = 6.8$ Hz, 3H), 3.81–3.83 (m, 1H), and 4.51 (d, $J = 3.4$ Hz, 1H); NMR δ_C (D_2O): 12.3, 49.5, 70.4, and 174.4; IR ν_{max} (KBr) cm^{-1}: 3406, 3075, 2940, 1740, 1620. *Anal*. Found: C, 40.33; H, 7.61; N, 11.77%. Calcd. for $C_4H_9NO_3$: C, 40.33; H, 7.62; N, 11.76%.

*(3S,4R)-3-Hydroxy-4-[(9-phenyl-9-fluorenyl)-amino]-1-pentene (**18**).* Compound **18** was obtained by the same procedure with the conversion of D-gulonic acid γ-lactone to **13**; mp 51–52°C; $[\alpha]_D^{20} = -293.9°$ (*c* 1.00, $CHCl_3$); NMR δ_H ($CDCl_3$): 0.66 (d, $J = 6.7$ Hz, 3H), 2.27–2.44 (m, 1H), 3.51–3.54 (m, 1H), 5.02–5.10 (m, 2H), 5.51–5.62 (m, 1H), and 7.20–7.89 (m, 13H); NMR δ_C ($CDCl_3$): 17.2, 52.4, 72.6, 74.1, 77.3, 115.3, 120.0, 120.1, 124.7, 125.3, 125.9, 127.3, 127.9, 128.1, 128.4, 128.5, 137.3, 139.8, 141.0, 145.1, 149.2, and 150.4; IR ν_{max} (KBr) cm^{-1}: 3448, 3317, 3070, 2976, 1648. *Anal*. Found: C, 84.42; H, 6.78; N, 4.10%. Calcd. for $C_{24}H_{23}NO$: C, 84.42; H, 6.79; N, 4.10%.

*(2R,3R)-2-O-Benzyl-3-[(9-phenyl-9-fluorenyl)-amino]-butanoic acid (**19**).* The procedure to obtain alcohol compound **19** was the same as that described for the conversion of **13** to **15**; mp 70–73°C; $[\alpha]_D^{20} = +48.6°$ (*c* 1.00, $CHCl_3$); NMR δ_H ($CDCl_3$): 0.65 (d, $J = 6.7$ Hz, 3H), 2.83–2.85 (m, 1H), 3.58 (d, $J = 4.1$ Hz, 1H), 4.31 (dd, $J = 4.9$, 11.5 Hz, 1H), 4.66 (t, $J = 11.3$ Hz, 1H), and 7.14–7.70 (m, 19H); NMR δ_C ($CDCl_3$): 17.1, 51.6, 72.5, 72.5, 80.9, 120.3, 120.3, 125.5, 125.7, 125.7, 127.6, 127.9, 128.1, 128.1, 128.2, 128.2, 128.4, 128.6, 128.9, 129.0, 129.0, 137.4, 140.2, 140.6, 143.4, 147.0, 148.4, and 173.4; IR ν_{max} (KBr) cm^{-1}: 3280, 3060, 3017, 2931, 2870, 1729, 1624. *Anal*. Found: C, 80.14; H, 6.05; N, 3.12%. Calcd. for $C_{30}H_{27}NO_3$: C, 80.15; H, 6.05; N, 3.12%.

*(2R,3R)-3-Amino-2-hydroxybutanoic acid [(**3**), D-alloisothreonine].* (2*R*,3*R*)-Hydroxybutanoic acid **3** was obtained by same procedure as that for the conversion of **15** to **1**; the overall yield for this conversion was 62%; mp 239–240°C; $[\alpha]_D^{20} = +23.4°$ (*c* 0.90, H_2O) {lit.[9] $[\alpha]_D^{25} = +23.0°$ (*c* 0.50, H_2O), mp 238–239°C}; NMR δ_H (D_2O): 1.24 (d, $J = 6.8$ Hz, 3H), 3.80–3.85 (m, 1H), and 4.52 (d, $J = 3.4$ Hz, 1H); NMR δ_C (D_2O): 12.3, 49.5, 70.4, and 174.4; IR ν_{max} (KBr) cm^{-1}: 3429, 2930, 1634. *Anal*. Found; C, 40.35; H, 7.63; N, 11.76%. Calcd. for $C_4H_9NO_3$: C, 40.33; H, 7.62; N, 11.76%.

*(3S,4S)-3-Hydroxy-4-[(9-phenyl-9-fluorenyl)-amino]-1-pentene (**20**).* Aminoalcohol **20** was obtained from D-glucono-δ-lactone by the same procedure as that described for the preparation of **13**, mp 48–52°C; $[\alpha]_D^{20} = +267.4°$ (*c* 1.00, $CHCl_3$); NMR δ_H ($CDCl_3$): 0.55 (d, $J = 6.4$ Hz, 3H), 2.12 (m, 1H), 3.58 (dd, $J = 6.8$, 7.5 Hz, 1H), 5.05–5.08 (m, 1H), 5.17–5.21 (m, 1H), 5.51–5.58 (m, 1H), and 7.20–7.71 (m, 13H); NMR δ_C ($CDCl_3$): 19.1, 53.1, 72.5, 77.3, 117.1, 120.0, 120.1, 125.2, 125.6, 125.9, 127.2, 127.8, 128.1, 128.4, 138.5, 140.1, 140.7, 145.1, 148.7, and 151.1; IR ν_{max} (KBr) cm^{-1}: 3426, 3310, 3078, 2933. *Anal*. Found: C, 84.42; H, 6.78; N, 4.11%. Calcd. for $C_{24}H_{23}NO$: C, 84.42; H, 6.79; N, 4.10%.

*(2R,3S)-2-O-Benzyl-3-[(9-phenyl-9-fluorenyl)-amino]-butanoic acid (**21**).* The procedure to obtain alcohol compound **21** was the same as that described for the conversion of **13** to **15**; mp 75–77°C; $[\alpha]_D^{20} = +50.3°$ (*c* 1.50, $CHCl_3$); NMR δ_H ($CDCl_3$): 0.88 (d, $J = 6.3$ Hz, 3H), 2.58 (t, $J = 5.2$ Hz, 1H), 3.28 (d, $J = 3.4$ Hz, 1H), 4.08 (m, 1H), 4.50 (br, 1H), and 7.03–7.73 (m, 19H); NMR δ_C ($CDCl_3$): 17.5, 49.9, 72.6, 72.7, 78.3, 120.5, 120.5, 124.2, 125.3, 125.6, 127.6, 127.7, 128.0, 128.3, 128.4, 128.5, 128.9, 129.3, 129.4, 137.3, 139.7, 141.1, 142.6, 146.6, 147.9, and 172.1; IR ν_{max} (KBr) cm^{-1}: 3280, 3060, 3017, 2931, 2870, 1729, 1624. *Anal*. Found: C, 80.16; H, 6.03; N, 3.11%. Calcd. for $C_{30}H_{27}NO_3$: C, 80.15; H, 6.05; N, 3.12%.

*(2R,3S)-3-Amino-2-hydroxybutanoic acid [(**4**), D-isothreonine].* Compound **4** was obtained by same procedure as that for the conversion of **15** to **1**; the overall yield for this conversion was 67%; mp 218–219°C; $[\alpha]_D^{20} = +14.1°$ (*c* 0.70, H_2O) {lit.[17] $[\alpha]_D^{20} = +21.6°$ (*c* 1.10, H_2O), mp 223–225°C (decomp.)}; NMR δ_H (D_2O): 1.12 (d, $J = 6.8$ Hz, 3H), 3.44–3.47 (m, 1H), and 4.08 (d, $J = 4.8$ Hz, 1H); NMR δ_C (D_2O): 15.0, 49.8, 71.3, and 174.6; IR ν_{max} (KBr) cm^{-1}: 3410, 3070, 2932, 1612. *Anal*. Found: C, 40.33; H, 7.60; N, 11.75%. Calcd. for $C_4H_9NO_3$: C, 40.33; H, 7.62; N, 11.76%.

Acknowledgments

This research was supported by the Korea Science and Engineering Foundation (KOSEF) through the Regional Animal Industry Research Center at Jinju National University, Jinju, Korea. We are also grateful for the financial support of Brain Korea 21 program.

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