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Expeditious synthesis of nicotianamine and 2′-deoxymugineic acid

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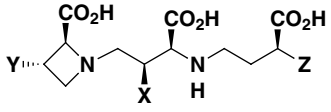
Abstract—A simple and efficient method of synthesizing nicotianamine **4** and 2′-deoxymugineic acid **3** was devised. We employed thioamide as an intermediate and the title compounds (**4** and **3**) were afforded via chemoselective reduction of the thioamides in 33% overall yield through 7 steps and 30% overall yield through 8 steps, respectively.

1. Introduction

Phytosiderophores **1**–**4** produced in higher plants as iron-chelating amino acids promote the uptake of iron from soil. The role of phytosiderophores is significant in plant physiology and has led several groups to synthesize them. 2′-Deoxymugineic acid **3** (Fig. 1) has been isolated from the washing of wheat (*Triticum aestivum* L.) under iron-deficient conditions.[1] It has been synthesized four times[2] and the syntheses have established its absolute configuration as depicted in Fig. 1. Nicotianamine **4** was first isolated from the leaves of *Nicotiana tabacum* L. and the first structure proposal was reported by Noma et al.[3a] Later, the structure was slightly corrected to **4** by Kristensen and Larsen.[3b] Nicotianamine **4** is now known as a key biosynthetic precursor of phytosiderophores. Various studies have proved that nicotianamine plays a significant role in plants as an iron transporter[4] and, therefore, nicotianamine also has become a synthetic target compound.[2c,d,4b,5] A comprehensive review of the previous syntheses of phytosiderophores has been published.[6]

The aim of the present work is to attempt a novel approach for an efficient synthesis of these compounds via peptide



1 Mugineic acid : X=Z=OH, Y=H
2 3-Epihydroxymugineic acid : X=Y=Z=OH
3 2'-Deoxymugineic acid : X=Y=H, Z=OH
4 Nicotianamine : X=Y=H, Z=NH_2

Figure 1.

intermediates to afford structurally diverse phytosiderophores in sufficient amount for biological studies. Our synthetic strategy toward **3** and **4** is illustrated in Scheme 1. In our previous work,[2d] we prepared tripeptide **D** by twice amide coupling, **C** with **B**, then with **A**. On the other hand, we have been interested in the biological activities of various analogues related to **3** and **4**. If these analogues show high iron-chelating and uptake-promoting ability, phytosiderophores would be applicable for practical use. One difficulty, which impedes their practical use, is the particular structure of L-azetidine-2-carboxylic acid (see **C**), so we intended to synthesize analogues without an azetidine ring. In order to afford the related analogues efficiently, it should be better to prepare the dipeptide from the righthand side. Thus, preparation of **A** and **B** and then coupling with **C** should give the target molecule. By changing the residue **C** with other amino acid derivatives, it would be possible to afford any type of phytosiderophore analogues very efficiently.

With this plan in mind, we first examined the synthesis of 2′-deoxymugineic acid **3** and nicotianamine **4**. Here we describe the synthesis of **3** and **4** in detail.

2. Results and discussion

The synthesis of nicotianamine **4** was carried out as shown in Scheme 2. Coupling of the known amine **5**[7] and carboxylic acid **6**[8] by use of DCC gave dipeptide **7** in 89% yield. The dipeptide **7** was hydrogenated, and the resulting carboxylic acid **8** was coupled with the amine **9**[9] by use of DCC to give tripeptide **10** in 75% yield. Thioamidation of **10** with Lawesson's reagent[10] gave thioamide **11** in 71% yield, which was converted to the fully protected nicotianamine **12** by reaction with excess amount of Raney-Ni[11] in 75% yield. Final deprotection with 4N HCl–EtOAc and Dowex purification afforded nicotianamine **4** in 94% yield. The spectral data were identical with those

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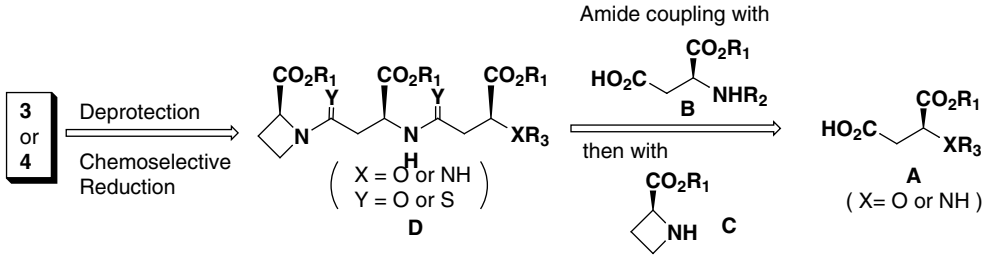
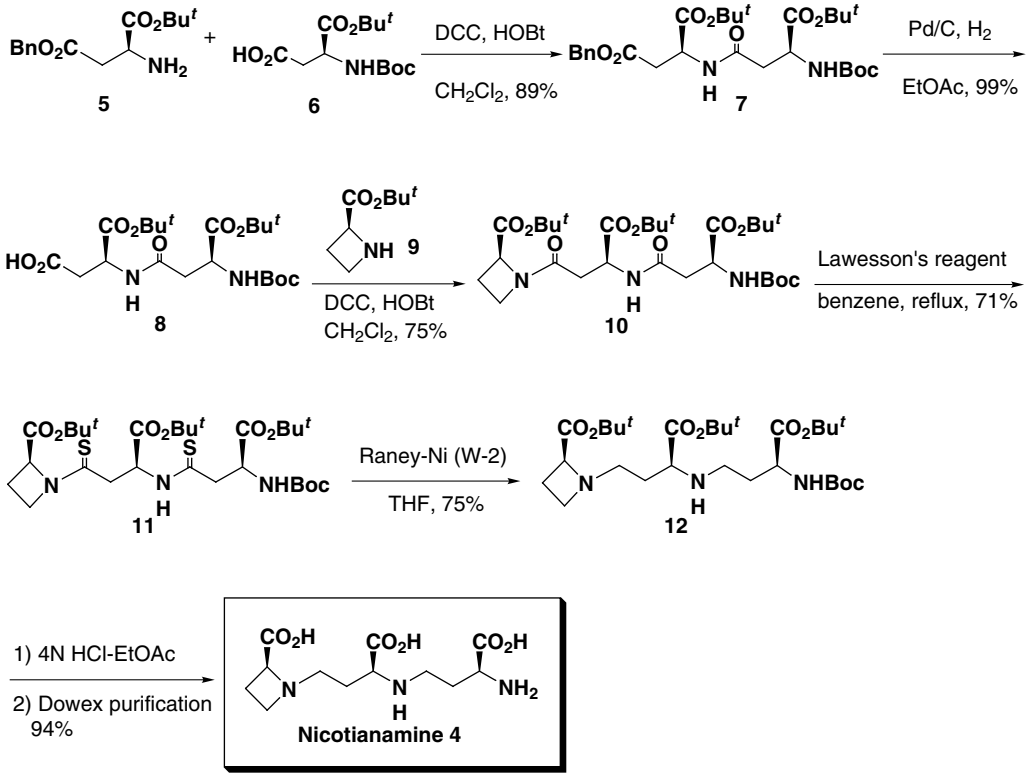


Scheme 1. Revised strategy.



Scheme 2.

reported[3a] (33.2% overall yield in 7 steps). Especially, the agreement of the value of optical rotation showed that no epimerization occurred through thioamidation and desulfurization steps.

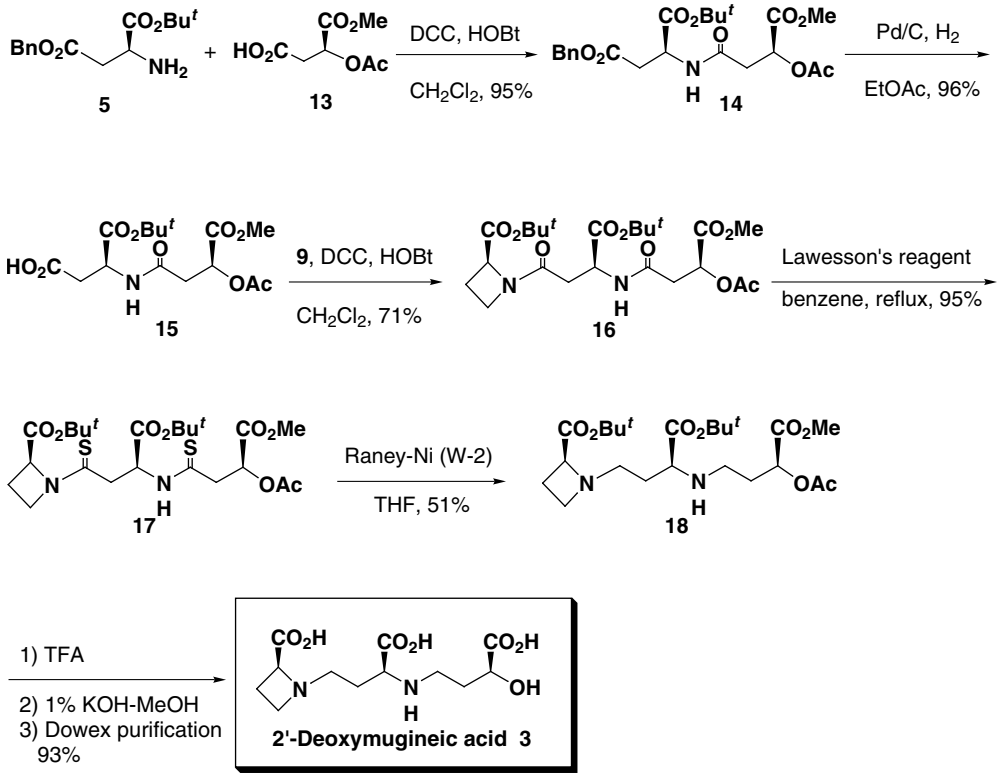
The synthesis of 2′-deoxymugineic acid **3** was realized according to Scheme 3. Amide coupling of the known amine **5** and carboxylic acid **13**[12] by use of DCC afforded dipeptide **14** in 95% yield. The dipeptide **14** was subjected to hydrogenation, and the resulting carboxylic acid **15** was coupled with amine **9** to give tripeptide **16** in 71% yield. The tripeptide **16** was converted to thioamide **17** with Lawesson's reagent in 95% yield, and the successive desulfurization was achieved with Raney-Ni to afford protected 2′-deoxymugineic acid **18** in 51% yield. Finally, treatment with TFA and 1% methanolic KOH solution followed by Dowex purification gave 2′-deoxymugineic acid **3** in 93% yield. The spectral data were in agreement with the literature values[1] (29.5% overall yield in 8 steps), and no epimerization occurred through thioamidation and desulfurization because of the same reason for the synthesis of nicotianamine.

In conclusion, we have achieved an efficient synthesis of nicotianamine **4** and 2′-deoxymugineic acid **3** by a simple and straightforward method. By this procedure, we are now able to afford **4** and **3** on several gram scale, which enables field study to be carried out. This method will be applied to synthesis of other phytosiderophores and analogues and, in fact, we already established the synthesis of avenic acid, also a natural congener of phytosiderophore, and other interesting analogues with significant biological activity as that of phytosiderophores. These results will be reported in the near future.

3. Experimental

3.1. General

Infrared spectra (IR) were measured on a Jasco FT/IR-230



Scheme 3.

spectrometer. Proton magnetic resonance spectra (^{1}H NMR) were recorded at 300 MHz on JEOL JNM-AL 300 spectrometer. Chemical shifts (δ) are reported in parts per million relative to internal chloroform (δ 7.24). Optical rotations were measured on a Jasco DIP-1000 polarimeter. Analytical thin-layer chromatography (TLC) was carried out using 0.25 mm Merck silica gel 60 F_{254} precoated glass-backed plates. Column chromatography was performed on Merck silica gel 60 and Cica silica gel 60N (neutral). All solvents used were of reagent grade. Tetrahydrofuran was distilled over benzophenone and sodium prior to use. Dichloromethane and benzene were dried and stored over 4 Å molecular sieves.

3.1.1. (2*S*,3′*S*)-2-(3-*t*-Butoxycarbonyl-3-*t*-butoxycarbonylaminopropanamido)-3-benzyloxycarbonylpropionic acid *t*-butyl ester (7). To a solution of HOBt (3.47 g, 25.7 mmol) in CH_2Cl_2 (31 ml) was added successively a solution of amine **5** (5.39 g, 19.3 mmol) in CH_2Cl_2 (10 ml), a solution of carboxylic acid **6** (5.59 g, 19.3 mmol) in CH_2Cl_2 (15 ml) and a solution of DCC (5.30 g, 25.7 mmol) in CH_2Cl_2 (10 ml) under argon at 0°C and the mixture was stirred for 15 h at rt. The reaction mixture was filtered through celite and the filtrate was concentrated under reduced pressure. The residue was diluted with EtOAc and filtered through celite again. The filtrate was washed with sat. $NaHCO_3$, 1N aq. HCl, sat. $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was diluted with EtOAc and filtered through celite. The filtrate was concentrated under reduced pressure and the residue was purified by chromatography on silica gel (hexane/EtOAc=4:1) to give the dipeptide **7** (9.44 g, 17.1 mmol, 88.6%) as a white foam; FT-IR (nujol cm^{-1}) 3314, 1747, 1729, 1692, 1655; $[\alpha]_D^{25}=-5.8$ (*c* 0.98, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.42 (27H, s, $-CO_2Bu^t$, Boc), 2.63 (1H, dd, *J*=4.5, 15.9 Hz, H-3), 2.81 (2H, m, H-2′), 2.98 (1H, dd, *J*=4.2, 15.9 Hz, H-3), 4.36 (1H, m, H-3′), 4.67 (1H, ddd, *J*=4.2, 4.5, 7.5 Hz, H-2), 5.09 (2H, m,– CH_2Ph), 5.64 (1H, d, *J*=8.4 Hz, *N*′-H), 6.45 (1H, d, *J*=7.5 Hz, *N*-H), 7.34 (5H, m, $-CH_2Ph$). Anal. calcd for $C_{28}H_{42}N_2O_9$: C, 61.07; H, 7.69; N, 5.09. Found: C, 61.02; H, 7.66; N, 5.11.

3.1.2. (3*S*,3′*S*)-3-(3-*t*-Butoxycarbonyl-3-*t*-butoxycarbonylaminopropanamido)-3-*t*-butoxycarbonylpropionic acid (8). To a solution of dipeptide **7** (4.40 g, 7.99 mmol) in EtOAc (20 ml) was added 10% Pd/C (1.0 g) and the mixture was stirred for 3 h under hydrogen at rt. The reaction mixture was filtered through celite and the filtrate was concentrated under reduced pressure. The residue was purified by chromatography on silica gel (hexane/EtOAc=1:1) to give carboxylic acid **8** (3.63 g, 7.88 mmol, 98.6%) as a white solid; FT-IR (nujol cm^{-1}) 3313, 1731, 1698, 1654; $[\alpha]_D^{25}=+20.4$ (*c* 1.01, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.42 (27H, s, $-CO_2Bu^t$, Boc), 2.69 (1H, dd, *J*=3.6, 15.5 Hz, H-3), 2.76–2.92 (2H, m, H-2′), 2.99 (1H, dd, *J*=4.5, 15.5 Hz, H-3), 4.38 (1H, m, H-3′), 4.70 (1H, m, H-2), 5.72 (1H, d, *J*=8.4 Hz, *N*′-H), 6.62 (1H, d, *J*=8.4 Hz, *N*-H). Anal. calcd for $C_{21}H_{36}N_2O_9$: C, 54.77; H, 7.88; N, 6.08. Found: C, 54.77; H, 7.96; N, 5.68.

3.1.3. (2*S*,3′*S*,3″*S*)-*N*-[3-(3-*t*-Butoxycarbonyl-3-*t*-butoxycarbonylaminopropanamido)-3-*t*-butoxycarbonylpropanoyl]azetidine-2-carboxylic acid *t*-butyl ester (10). To a solution of HOBt (1.42 g, 10.5 mmol) in CH_2Cl_2 (7 ml) was

added successively a solution of amine **9** (1.27 g, 8.08 mmol) in CH_2Cl_2 (5 ml), a solution of carboxylic acid **8** (3.71 g, 8.06 mmol) in CH_2Cl_2 (10 ml) and a solution of DCC (2.16 g, 10.5 mmol) in CH_2Cl_2 (5 ml) under argon at 0°C and the mixture was stirred for 20 h at rt. The reaction mixture was filtered through celite and the filtrate was concentrated under reduced pressure. The residue was diluted with EtOAc and filtered through celite again. Then the filtrate was washed with sat. $NaHCO_3$, 1N aq. HCl, sat. $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was diluted with EtOAc, filtered through celite. The filtrate was concentrated under reduced pressure and the residue was purified by chromatography on silica gel (hexane/EtOAc=4:1–2:1) to give tripeptide **10** (3.64 g, 6.07 mmol, 75.3%) as a white foam; FT-IR (nujol cm^{-1}) 3340, 1742, 1660; $[\alpha]_D^{25}=-44.0$ (*c* 1.00, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.41 (9H, s, $-CO_2Bu^t$ or Boc), 1.43 (9H, s, $-CO_2Bu^t$ or Boc), 1.45 (9H, s, $-CO_2Bu^t$ or Boc), 1.48 (9H, s, $-CO_2Bu^t$ or Boc), 1.89 (1H, br, H-3), 2.10–2.23 (1H, m, H-3), 2.43–2.91 (4H, m, H-2′, H-2″), 3.85–4.20 (2H, m, H-4), 4.38 (1H, br, H-2 or H-3′ or H-3″), 4.51–4.72 (2H, m, H-2 or H-3′ or H-3″), 5.67 (1H, br, *N*″-H), 6.67 (0.5H, d, *J*=6.9 Hz, *N*′-H), 6.93 (0.5H, d, *J*=7.7 Hz, *N*′-H). Anal. calcd for $C_{29}H_{49}N_3O_{10}$: C, 58.08; H, 8.24; N, 7.01. Found: C, 57.64; H, 8.19; N, 6.95.

3.1.4. (2*S*,3′*S*,3″*S*)-*N*-[3-(3-*t*-Butoxycarbonyl-3-*t*-butoxycarbonylaminopropanethioamido)-3-*t*-butoxycarbonylpropanethioyl]azetidine-2-carboxylic acid *t*-butyl ester (11). To a solution of tripeptide **10** (4.92 g, 8.20 mmol) in benzene (82 ml) was added Lawesson's reagent (3.65 g, 9.02 mmol) and the mixture was stirred for 2 h under argon at 80°C. The reaction mixture was filtered through celite and the filtrate was concentrated under reduced pressure. The residue was purified twice by chromatography on silica gel (CH_2Cl_2/EtOAc=1:0–10:1, hexane/EtOAc=20:1–10:1) to give thioamide **11** (3.68 g, 5.82 mmol, 71.0%) as a white foam; FT-IR (nujol cm^{-1}) 3304, 1729; $[\alpha]_D^{29}=+7.3$ (*c* 1.17, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.42 (9H, s, $-CO_2Bu^t$ or Boc), 1.44 (9H, s, $-CO_2Bu^t$ or Boc), 1.46 (9H, s, $-CO_2Bu^t$ or Boc), 1.52 (9H, s, $-CO_2Bu^t$ or Boc), 2.15–2.27 (1H, m, H-3), 2.45–2.70 (1H, m, H-3), 3.03–3.23 (4H, m, H-2′, H-2″), 4.09–4.37 (2H, m, H-4), 4.48–4.58 (1H, m, H-3′ or H-3″), 4.75–4.85 (1H, m, H-3′ or H-3″), 5.24 (0.5H, dt, *J*=4.2, 7.9 Hz, H-2), 5.39 (0.5H, dt, *J*=4.2, 8.1 Hz, H-2), 5.75 (1H, d, *J*=8.4 Hz, *N*″-H), 8.73 (0.5H, d, *J*=7.5 Hz, *N*′-H), 8.89 (0.5H, d, *J*=7.5 Hz, *N*′-H); HRFAB: $[M]^+$ calcd for $C_{29}H_{49}N_3O_8S_2$ 631.2961, found 631.2933.

3.1.5. (2*S*,3′*S*,3″*S*)-*N*-[3-(3-*t*-Butoxycarbonyl-3-*t*-butoxycarbonylaminopropylamino)-3-*t*-butoxycarbonylpropyl]-azetidine-2-carboxylic acid *t*-butyl ester (12). To a solution of thioamide **11** (1.05 g, 1.66 mmol) in THF (10 ml) was added 10 ml of THF suspension of Raney-Ni (W-2) (0.5 g/ml) and the mixture was stirred for 4 h under argon at rt. The reaction mixture was filtered through celite by washing with hot THF and the filtrate was concentrated under reduced pressure. The residue was purified by chromatography on silica gel (hexane/EtOAc=3:2–0:1) to give amine **12** (712 mg, 1.25 mmol, 75.3%) as a yellow oil; FT-IR (neat cm^{-1}) 3355, 2977, 2932, 1730, 1708; $[\alpha]_D^{21}=-42.2$ (*c* 1.60, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.30 (9H, s, $-CO_2Bu^t$ or Boc), 1.33 (9H, s, $-CO_2Bu^t$ or Boc), 1.35 (9H, s, $-CO_2Bu^t$ or Boc), 1.40 (9H, s, $-CO_2Bu^t$ or Boc), 1.56–1.84 (4H, m, H-1′, H-1″), 2.00–2.23 (2H, m, H-3), 2.34–2.42 (2H, m, H-2′ or H-2″), 2.55–2.70 (2H, m, H-2′ or H-2″), 3.00 (1H, dd, *J*=7.6, 5.6 Hz, H-3″), 3.26 (1H, td, *J*=7.4, 2.4 Hz, H-3′), 3.38 (1H, t, *J*=8.3 Hz, H-2), 4.08 (2H, br, H-4), 5.61 (1H, d, *J*=7.5 Hz, N″-H); HRFAB: $[M+H]^+$ calcd for $C_{29}H_{54}N_3O_8$ 572.3911, found 572.3925.

3.1.6. Nicotianamine (4). To the amine **12** (519 mg, 0.908 mmol) was added 15 ml of 4N HCl in EtOAc under argon at 0°C and the mixture was stirred for 10 h at rt. The reaction mixture was concentrated under reduced pressure and purified by ion exchange resin (Dowex 50W-X8, H_2O then 2N aq. NH_3) to give nicotiamanine **4** (260 mg, 0.857 mmol, 94.4%) as a white solid; FT-IR (nujol cm^{-1}) 3410, 1617; $[\alpha]_D^{34}=-56.4$ (*c* 0.81, H_2O), [lit. $[\alpha]_D^{23}=-60.5$ (*c* 2.7, H_2O)]; ^1H NMR (300 MHz, D_2O) (ppm) 1.91–2.21 (4H, m, H-2′, H-2″), 2.32–2.46 (1H, m, H-3), 2.59 (1H, qd, *J*=9.3, 4.3 Hz, H-3), 3.12 (2H, t, *J*=6.9 Hz, H-1″), 3.15–3.35 (2H, m, H-1′), 3.66 (1H, dd, *J*=4.4, 8.3 Hz, H-3′), 3.74 (1H, t, *J*=5.7 Hz, H-3″), 3.83 (1H, t, *J*=9.6 Hz, H-4), 3.95 (1H, td, *J*=9.8, 6.6 Hz, H-4), 4.75 (1H, t, *J*=9.6 Hz, H-2); HRFAB: $[M+H]^+$ calcd for $C_{12}H_{22}N_3O_6$ 304.1509, found 304.1512.

3.1.7. (2*S*,3′*S*)-2-(3-Acetoxy-3-methoxycarbonylpropanamido)-3-benzyloxycarbonylpropionic acid *t*-butyl ester (14). To a solution of HOBt (7.01 g, 51.9 mmol) in CH_2Cl_2 (155 ml) was added successively a solution of amine **5** (11.5 g, 41.2 mmol) in CH_2Cl_2 (10 ml), a solution of carboxylic acid **13** (7.83 g, 41.2 mmol) in CH_2Cl_2 (15 ml) and a solution of DCC (10.7 g, 51.9 mmol) in CH_2Cl_2 (10 ml) under argon at 0°C and the mixture was stirred for 6 h at rt. The reaction mixture was filtered through celite and the filtrate was concentrated under reduced pressure. The residue was diluted with EtOAc and filtered through celite again. Then the filtrate was washed with sat. $NaHCO_3$, 1N aq. HCl, sat. $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was diluted with EtOAc and filtered through celite. The filtrate was concentrated under reduced pressure and the residue was purified by chromatography on silica gel (hexane/EtOAc=1:1) to give dipeptide **14** (17.7 g, 39.3 mmol, 95.3%) as a white foam; FT-IR (nujol cm^{-1}) 3320, 1747, 1650; $[\alpha]_D^{26}=+32.4$ (*c* 1.07, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.38 (9H, s, $-CO_2Bu^t$), 2.08 (3H, s, –OAc), 2.72 (1H, d, *J*=7.5 Hz, H-2′), 2.74 (1H, d, *J*=4.8 Hz, H-2′), 2.84 (1H, dd, *J*=4.2, 17.0 Hz, H-3), 2.99 (1H, dd, *J*=4.2, 17.0 Hz, H-3), 3.74 (3H, s, $-CO_2Me$), 4.70 (1H, dd, *J*=4.2, 7.8 Hz, H-2), 5.10 (2H, q, *J*=12.0 Hz, $-CH_2Ph$), 5.40 (1H, dd, *J*=4.8, 7.5 Hz, H-3′), 6.55 (1H, d, *J*=7.8 Hz, *N*-H), 7.33 (5H, m, $-CH_2Ph$). Anal. calcd for $C_{22}H_{29}NO_9$: C, 58.53; H, 6.47; N, 3.10. Found: C, 58.59; H, 6.47; N, 3.21.

3.1.8. (3*S*,3′*S*)-3-(3-Acetoxy-3-methoxycarbonylpropanamido)-3-*t*-butoxycarbonylpropionic acid (15). To a solution of dipeptide **14** (7.22 g, 16.0 mmol) in EtOAc (20 ml) was added 10% Pd/C (2.0 g) and the mixture was stirred for 2.5 h under hydrogen at rt. The reaction mixture was filtered through celite and the filtrate was concentrated

to give carboxylic acid **15** (5.55 g, 15.4 mmol, 96.0%) as a white solid; FT-IR (nujol cm^{-1}) 3354, 1754, 1668; $[\alpha]_D^{26}=+21.1$ (*c* 1.01, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.42 (9H, s, $-CO_2Bu^t$), 2.10 (3H, s, –OAc), 2.79 (1H, d, *J*=7.2 Hz, H-2′), 2.80 (1H, d, *J*=5.1 Hz, H-2′), 2.83 (1H, dd, *J*=4.5, 17.0 Hz, H-2), 2.98 (1H, dd, *J*=4.5, 17.0 Hz, H-2), 3.69 (3H, s, $-CO_2Me$), 4.70 (1H, dt, *J*=4.5, 7.5 Hz, H-3), 5.40 (1H, dd, *J*=5.1, 7.2 Hz, H-3′), 6.88 (1H, d, *J*=7.5 Hz, *N*-H), 9.86 (1H, br, $-CO_2H$). Anal. calcd for $C_{15}H_{23}NO_9$: C, 49.86; H, 6.42; N, 3.88. Found: C, 49.93; H, 6.43; N, 3.92.

3.1.9. (2*S*,3′*S*,3″*S*)-*N*-[3-(3-Acetoxy-3-methoxycarbonylpropanamido)-3-*t*-butoxycarbonylpropanoyl]azetidine-2-carboxylic acid *t*-butyl ester (16). To a solution of HOBt (10.4 g, 76.6 mmol) in CH_2Cl_2 (230 ml) was added successively a solution of amine **9** (9.56 g, 60.8 mmol) in CH_2Cl_2 (10 ml), a solution of carboxylic acid **15** (22.0 g, 60.8 mmol) in CH_2Cl_2 (10 ml) and a solution of DCC (15.8 g, 76.6 mmol) in CH_2Cl_2 (10ml) under argon at 0°C and the mixture was stirred for 6 h at rt. The reaction mixture was filtered through celite and the filtrate was concentrated under reduced pressure. The residue was diluted with EtOAc and filtered through celite again. Then the filtrate was washed with sat. $NaHCO_3$, 1N aq. HCl, sat. $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was diluted with EtOAc and filtered through celite. The filtrate was concentrated under reduced pressure and the residue was purified by chromatography on silica gel (hexane/EtOAc=1:3–0:1) to give tripeptide **16** (21.6 g, 43.2 mmol, 71.2%) as a white foam; FT-IR (nujol cm^{-1}) 3315, 1770, 1739, 1687, 1639; $[\alpha]_D^{25}=-53.9$ (*c* 0.99, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.45 (18H, s, $-CO_2Bu^t$), 2.10 (3H, s, –OAc), 2.06–2.14 (1H, m, H-3), 2.42–2.60 (2H, m, H-3, H-2′), 2.69 (0.5H, dd, *J*=4.2, 16.5 Hz, H-2′), 2.74 (0.5H, d, *J*=6.0 Hz, H-2″), 2.75 (0.5H, d, *J*=5.1 Hz, H-2″), 2.76 (0.5H, d, *J*=6.6 Hz, H-2″), 2.77 (0.5H, d, *J*=8.4 Hz, H-2″), 2.80 (0.5H, dd, *J*=4.2, 16.5 Hz, H-2′), 3.69(3H, s, $-CO_2Me$), 3.91–4.18 (2H, m, H-4), 4.55 (0.5H, dd, *J*=4.2, 8.7 Hz, H-2), 4.58 (0.5H, dd, *J*=4.8, 8.1 Hz, H-2), 4.63 (0.5H, dt, *J*=5.1, 6.9 Hz, H-3′), 4.75 (0.5H, dt, *J*=4.2, 8.4 Hz, H-3′), 5.41 (0.5H, dd, *J*=6.0, 8.4 Hz, H-3″), 5.44 (0.5H, dd, *J*=5.1, 6.6 Hz, H-3″), 6.77 (0.5H, d, *J*=6.9 Hz, *N*′-H), 7.07 (1H, d, *J*=8.4 Hz, *N*′-H). Anal. calcd for $C_{23}H_{36}N_2O_{10}$: C, 55.19; H, 7.25; N, 5.60. Found: C, 55.19; H, 7.23; N, 5.69.

3.1.10. (2*S*,3′*S*,3″*S*)-*N*-[3-(3-Acetoxy-3-methoxycarbonylpropanethioamido)-3-*t*-butoxycarbonylpropanethioyl]-azetidine-2-carboxylic acid *t*-butyl ester (17). To a solution of tripeptide **16** (8.01 g, 16.0 mmol) in benzene (70 ml) was added Lawesson's reagent (7.12 g, 17.6 mmol) and the mixture was stirred for 3 h under argon at 80°C. The reaction mixture was filtered through celite and the filtrate was concentrated under reduced pressure. The residue was purified by chromatography on silica gel (CH_2Cl_2/EtOAc=1:0–10:1) to give thioamide **17** (8.06 g, 15.1 mmol, 94.6%) as a white foam; FT-IR (nujol cm^{-1}) 3285, 1759, 1737, 1711; $[\alpha]_D^{27}=-25.8$ (*c* 1.06, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.49 (18H, s, $-CO_2Bu^t$), 2.11 (3H, s, –OAc), 2.09–2.20 (1H, m, H-3), 2.50–2.60 (1H, m, H-3), 3.01–3.26 (4H, m, H-2′, H-2″), 3.68 (3H, s, $-CO_2Me$), 4.04–4.32 (2H, m, H-4), 4.79 (1H, m, H-2), 5.16 (0.5H, dt, *J*=4.2, 6.6 Hz, H-3′), 5.40 (0.5H, dt, *J*=4.8, 7.8 Hz, H-3′), 5.48 (0.5H, dd, *J*=3.9, 8.4 Hz, H-3″), 5.57 (0.5H, dd, *J*=4.2, 8.4 Hz, H-3″), 8.78 (0.5H, d, *J*=6.6 Hz, *N*′-H), 9.12 (0.5H, d, *J*=7.8 Hz, *N*′-H); HRFAB: $[M+H]^+$ calcd for $C_{23}H_{37}N_2O_8S_2$ 533.1991, found 533.1971.

3.1.11. (2*S*,3′*S*,3″*S*)-*N*-[3-(3-Acetoxy-3-methoxycarbonylpropylamino)-3-*t*-butoxycarbonylpropyl]azetidine-2-carboxylic acid *t*-butyl ester (18). To a solution of thioamide **17** (5.00 g, 9.39 mmol) in THF (15 ml) was added 100 ml of THF suspension of Raney-Ni (W2) (0.5 g/ml) and the mixture was stirred for 4 h under argon at rt. The reaction mixture was filtered through celite by washing with hot THF and the filtrate was concentrated under reduced pressure. The residue was purified by chromatography on silica gel (hexane/EtOAc=1:3–0:1) to give amine **18** (2.28 g, 4.82 mmol, 51.4%) as a yellow oil; FT-IR (neat cm^{-1}) 3454, 1731; $[\alpha]_D^{21}=-76.6$ (*c* 1.13, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.43 (18H, s, $-CO_2Bu^t$), 1.37–1.54 (3H, m, H-2′, *N*′-H), 1.96 (2H, q, *J*=6.3 Hz, H-2″), 2.01–2.11 (1H, m, H-3), 2.10 (3H, s, –OAc), 2.23 (1H, dt, *J*=8.4, 9.0 Hz, H-3), 2.41–2.56 (2H, m, H-1′ or H-1″), 2.61–2.78 (3H, m, H-4, H-1′ or H-1″), 3.08 (1H, m, H-4), 3.32 (1H, t, *J*=6.6 Hz, H-3′), 3.46 (1H, t, *J*=8.4 Hz, H-2), 3.70 (3H, s, $-CO_2Me$), 5.09 (1H, t, *J*=6.3 Hz, H-3″); HRFAB: $[M+H]^+$ calcd for $C_{23}H_{41}N_2O_8$ 473.2863, found 473.2848.

3.1.12. 2′-Deoxymugineic acid (3). To the amine **18** (670 mg, 1.42 mmol) was added 10 ml of TFA under argon at 0°C and the reaction mixture was stirred for 3 h at rt. To this was added 1% KOH–MeOH and the mixture was stirred overnight at rt. The mixture was concentrated and purified by ion exchange resin (Dowex 50W-X8, H_2O then 1N aq. NH_3) to give 2′-deoxymugineic acid **3** (400 mg, 1.31 mmol, 93.0%) as a white solid; FT-IR (nujol cm^{-1}) 3441, 1703, 1605; $[\alpha]_D^{29}=-74.5$ (*c* 0.74, H_2O), [lit. $[\alpha]_D^{34}=-70.5$ (*c* 0.31, H_2O)]; ^1H NMR (300 MHz, D_2O) δ (ppm) 1.83 (2H, m, H-2′ or H-2″), 2.09 (1H, m, H-2′ or H-2″), 2.38 (2H, H-3, H-2′ or H-2″), 2.58 (1H, m, H-3), 3.01 (1H, m, H-1″), 3.25 (3H, m, H-1′, H-1″), 3.75 (1H, q, *J*=9.0 Hz, H-3′), 3.92 (1H, m, H-4), 4.21 (1H, dd, *J*=5.7, 9.0 Hz, H-4), 4.33 (1H, t, *J*=9.0 Hz, H-3″), 4.58 (1H, t, *J*=9.3 Hz, H-2); HRFAB: $[M+H-H_2O]^+$ calcd for $C_{12}H_{19}N_2O_6$ 287.1243, found 287.1266.

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