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SYNTHESIS OF THREE STEREOISOMERS OF ERYTHROCHELIN, A HYDROXAMATE-TYPE TETRAPEPTIDE SIDEROPHORE FROM *SACCHAROPOLYSPORA ERYTHRAEA*

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Abstract – Details of the synthesis of three stereoisomers of erythrochelin, a hydroxamate-type tetrapeptide siderophore produced by *Saccharopolyspora erythraea*, were described. Both enantiomers of protected δ-*N*-hydroxyornithine were used as key intermediates in the synthesis of stereoisomers of erythrochelin containing a (3*S*,6*S*)-3,6-disubstituted-2,5-diketopiperazine ring. From comparisons of ^{1}H and ^{13}C NMR spectra, neither of stereoisomers provided a match for the erythrochelin spectral data, and the absolute configuration of erythrochelin was unambiguously reconfirmed to be (*R*,*R*,*S*,*S*).

Relatively low molecular weight ion(III)-chelators produced by some microorganisms are called siderophores, which mediate the biological uptake of iron(III) under iron-deficient conditions.[1-7] Erythrochelin (**1**), a hydroxamate-type tetrapeptide siderophore isolated as the first nonribosomal peptide synthetase (NRPS)-derived natural product of *Saccharopolyspora erythraea*, has four stereogenic centers.[8-10] The chemical structure of (*R*,*R*,*S*,*S*)-**1**, including absolute configurations, was proposed by Marahiel and co-workers from a dedicated radio LC-MS guided genome mining methodology.[11-13] Erythrochelin [(*R*,*R*,*S*,*S*)-**1**] has a (3*S*,6*S*)-2,5-diketopiperazine (2,5-DKP) ring[14,15] derived from δ-*N*-acetyl-δ-*N*-hydroxy-L-ornithine and δ-*N*-hydroxy-L-ornithine, and a dipeptide moiety comprised of D-serine and α-*N*-acetyl-δ-*N*-acetyl-δ-*N*-hydroxy-D-ornithine as shown in Figure 1.[9,16] The same structure for (*R*,*R*,*S*,*S*)-**1** was independently proposed by Leadlay and co-workers based on NMR analysis of the Ga(III) complex of natural **1**.[17] Recently, we reported the first chemical synthesis of (*R*,*R*,*S*,*S*)-**1** based on the proposed absolute configurations at four stereogenic centers.[18] The obtained ^{1}H and ^{13}C NMR spectra of synthetic (*R*,*R*,*S*,*S*)-**1** agreed well with the corresponding data reported for natural **1**.[11,17] In addition, the specific rotation value of synthetic (*R*,*R*,*S*,*S*)-**1** was negative {$[\alpha]_D^{24}$ –10.3 (*c* 1.00, MeOH)}, whereas that of natural **1** has not been reported. In this report, we synthesized three stereoisomers,

(*S*,*S*,*S*,*S*)-**1**, (*S*,*R*,*S*,*S*)-**1**, and (*R*,*S*,*S*,*S*)-**1**, of erythrochelin [(*R*,*R*,*S*,*S*)-**1**] associated with the linear dipeptide moiety for the certain reconfirmation of the absolute configuration of natural **1** (Figure 1).

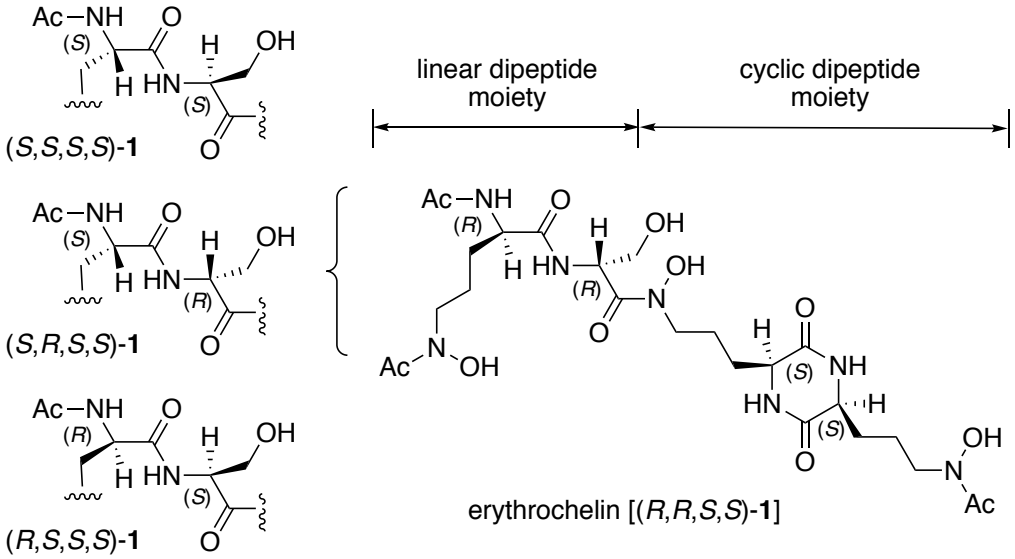
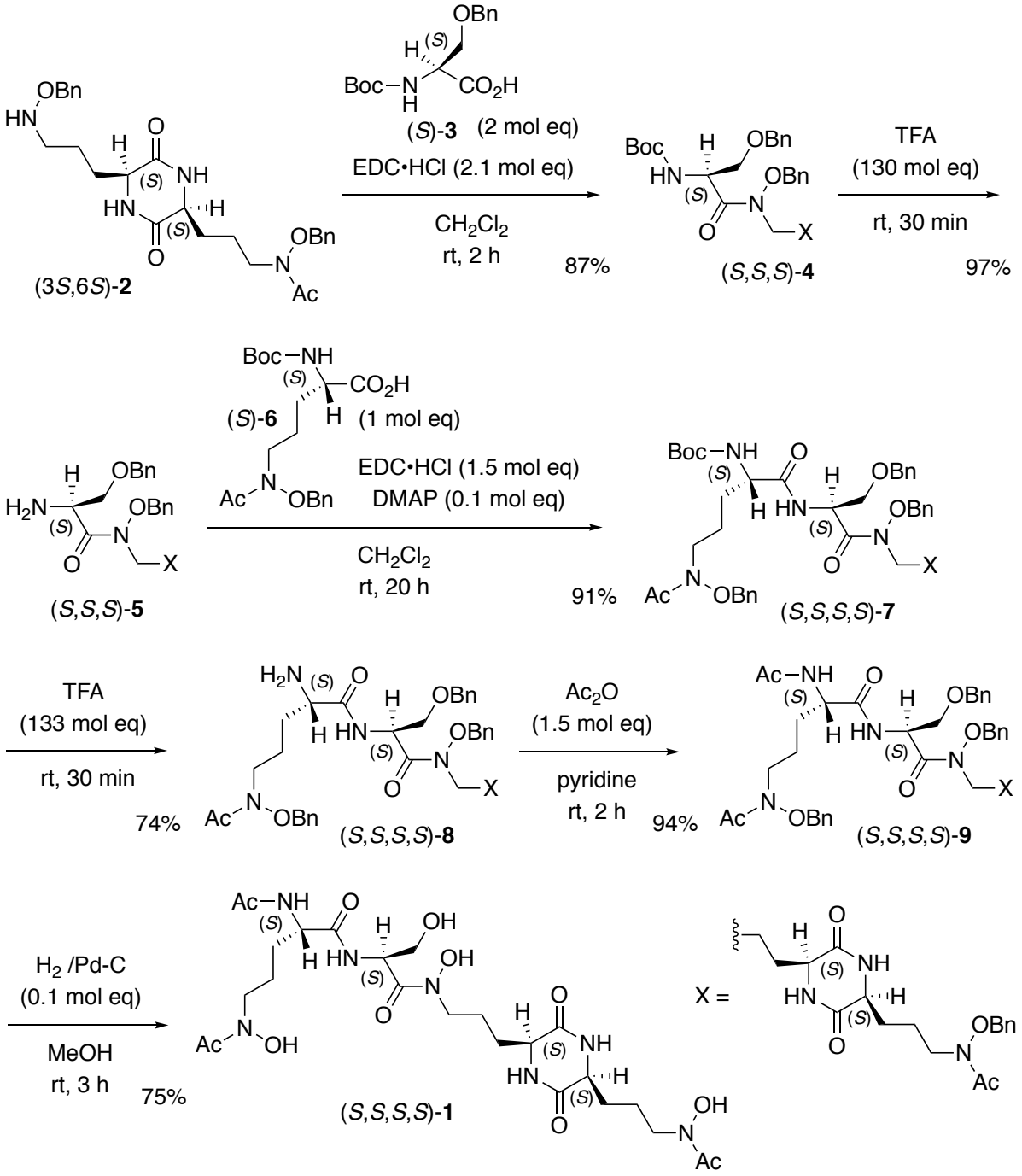


Figure 1. Chemical structures of erythrochelin [(*R*,*R*,*S*,*S*)-**1**] and its stereoisomers (*S*,*S*,*S*,*S*)-**1**, (*S*,*R*,*S*,*S*)-**1**, and (*R*,*S*,*S*,*S*)-**1**

The synthetic route of (*S*,*S*,*S*,*S*)-**1**, one of the stereoisomers of (*R*,*R*,*S*,*S*)-**1**, is shown in Scheme 1. The common key precursor used for the synthesis of all three stereoisomers is (3*S*,6*S*)-**2**, which was obtained in four steps starting from α-*N*-Boc-δ-*N*-acetyl-δ-*N*-benzyloxy-L-ornithine and δ-*N*-benzyloxy-δ-*N*-(2,2,2-trichloroethoxy)carbonyl-L-ornithine methyl ester hydrochloride according to our reported procedure.[18,19] Condensation of (3*S*,6*S*)-**2** with Boc-L-Ser(OBn)-OH [(*S*)-**3**] using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC•HCl) as a coupling reagent afforded (*S*,*S*,*S*)-**4**. The *N*-Boc group of (*S*,*S*,*S*)-**4** was removed rapidly using neat trifluoroacetic acid (TFA). The resultant amine (*S*,*S*,*S*)-**5** was coupled with α-*N*-Boc-δ-*N*-acetyl-δ-*N*-benzyloxy-L-ornithine [(*S*)-**6**], which was prepared from Boc-L-Glu(OBn)-OH in six steps,[18,19] by EDC•HCl to furnish (*S*,*S*,*S*,*S*)-**7**. Primary amine (*S*,*S*,*S*,*S*)-**8** was obtained by *N*-Boc deprotection of (*S*,*S*,*S*,*S*)-**7** by using neat TFA. Subsequent treatment of (*S*,*S*,*S*,*S*)-**8** with acetic anhydride afforded (*S*,*S*,*S*,*S*)-**9**. Finally, catalytic hydrogenolysis of (*S*,*S*,*S*,*S*)-**9** catalyzed by palladium on carbon (Pd-C) under a hydrogen atmosphere provided the stereoisomer (*S*,*S*,*S*,*S*)-**1** in 40% yield over six steps from the common key precursor (3*S*,6*S*)-**2**. In a similar way, stereoisomers (*S*,*R*,*S*,*S*)-**1** and (*R*,*S*,*S*,*S*)-**1** were also prepared from the common key precursor (3*S*,6*S*)-**2** using (*R*)-**3** and (*R*)-**6** in 40% and 45% yields over six steps, respectively.



Scheme 1. Synthesis of (*S*,*S*,*S*,*S*)-**1**

We compared the ^{1}H NMR spectra of natural and synthetic **1**[17,18] to those of stereoisomers (*S*,*S*,*S*,*S*)-**1**, (*S*,*R*,*S*,*S*)-**1**, and (*R*,*S*,*S*,*S*)-**1**. Selected chemical shifts are shown in Figure 2. As a result, an accidentally equivalent singlet signal at δ 1.97 ppm originating from the two acetyl groups were observed in the ^{1}H NMR spectra of natural and synthetic **1**. But the stereoisomers (*S*,*R*,*S*,*S*)-**1** and (*R*,*S*,*S*,*S*)-**1** exhibited independent singlet signals corresponding to the two kinds of acetyl group at δ 1.966 and 1.972 ppm for (*S*,*R*,*S*,*S*)-**1**, and δ 1.966 and 1.971 ppm for (*R*,*S*,*S*,*S*)-**1**. In addition, the ^{1}H NMR spectra of the two methine groups of the linear dipeptide moiety of (*S*,*R*,*S*,*S*)-**1** (δ 4.31–4.39 and 4.91–4.97 ppm) and

(*R*,*S*,*S*,*S*)-**1** (δ 4.31–4.39 and 4.91-4.98 ppm) appeared at lower magnetic fields than those of natural and synthetic **1** (natural: δ 4.30–4.35 and 4.82–4.86 ppm, synthetic: δ 4.29–4.36 and 4.85–4.92 ppm). However, stereoisomer (*S*,*S*,*S*,*S*)-**1** and natural and synthetic **1** have ^{1}H NMR spectra that are so similar as to be indistinguishable. Therefore, we analyzed the ^{13}C NMR spectra of natural and synthetic **1** and of the three stereoisomers (*S*,*S*,*S*,*S*)-**1**, (*S*,*R*,*S*,*S*)-**1**, and (*R*,*S*,*S*,*S*)-**1** as shown in Figure 3. As a result, α-carbon of ornithine of a linear dipeptide moiety of (*S*,*R*,*S*,*S*)-**1** (δ 51.7 ppm) and that of (*R*,*S*,*S*,*S*)-**1** (δ 51.6 ppm) were observed at higher magnetic fields than those of natural and synthetic **1** (natural: δ 52.1 ppm; synthetic: δ 52.03 ppm), but the stereoisomer (*S*,*S*,*S*,*S*)-**1** (δ 52.04 ppm) showed about the same chemical shift value to natural **1** (δ 52.1 ppm) and an almost equal value to synthetic **1** (δ 52.03 ppm). Finally, δ-carbon of ornithine linking L-serine and the 2,5-DKP ring of stereoisomers (*S*,*S*,*S*,*S*)-**1** (δ 47.4 ppm) was definitely observed at a lower magnetic field than those of natural and synthetic **1** (δ 47.0 ppm for both). The absolute configuration of the serine moiety in the natural **1** was determined to be *R* using amino acid analysis based on 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA, Marfey's reagent) derivatization.[11,20-22] Therefore, the stereochemistry of natural **1** was evidently established to be (*R*,*R*,*S*,*S*) as shown in Figure 1.

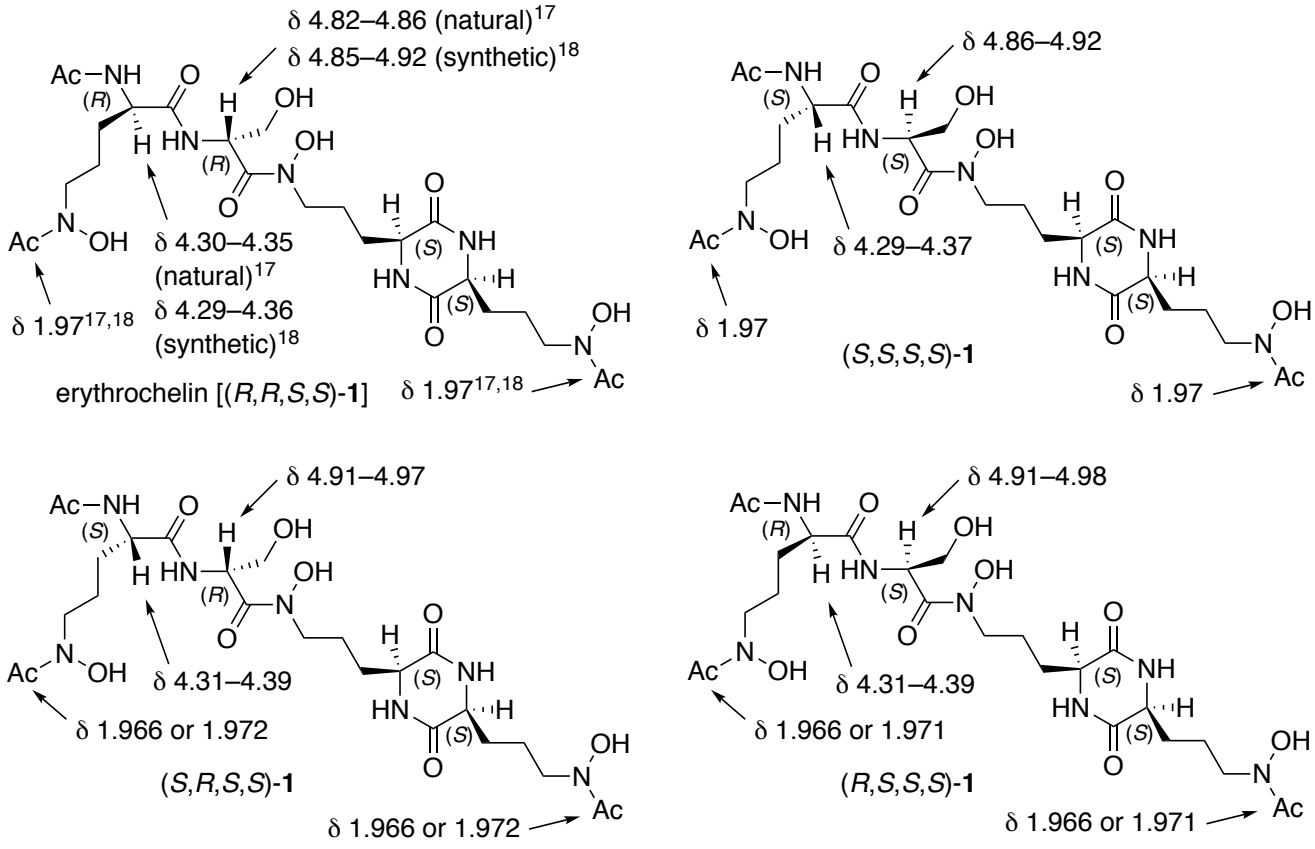


Figure 2. Selected ^{1}H NMR (500 MHz, DMSO-d_6) chemical shifts of erythrochelin [(*R*,*R*,*S*,*S*)-**1**] and its stereoisomers (*S*,*S*,*S*,*S*)-**1**, (*S*,*R*,*S*,*S*)-**1**, and (*R*,*S*,*S*,*S*)-**1**

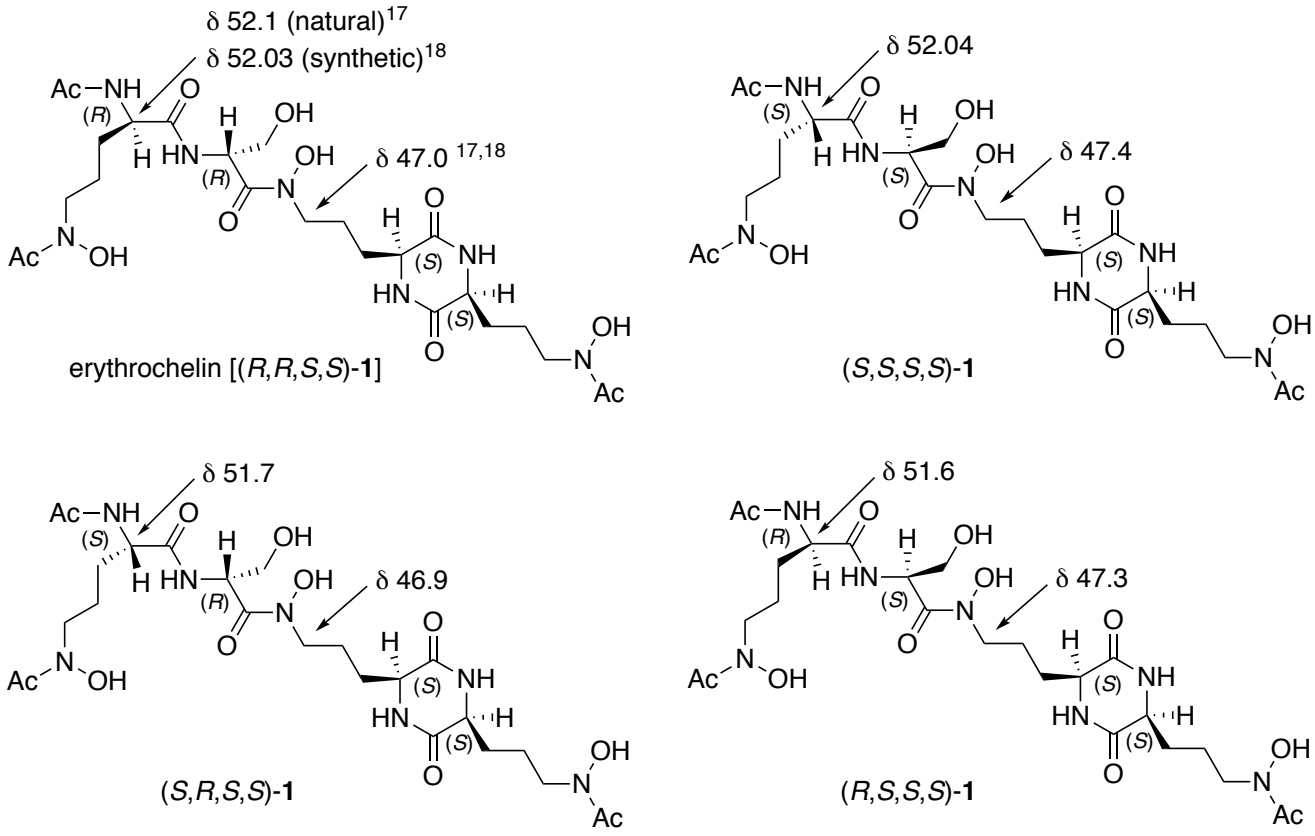


Figure 3. Selected ^{13}C NMR (125 MHz, DMSO-d_6) chemical shifts of erythrochelin [(*R*,*R*,*S*,*S*)-**1**] and its stereoisomers (*S*,*S*,*S*,*S*)-**1**, (*S*,*R*,*S*,*S*)-**1**, and (*R*,*S*,*S*,*S*)-**1**

In conclusion, we have synthesized three stereoisomers, (*S*,*S*,*S*,*S*)-**1**, (*S*,*R*,*S*,*S*)-**1**, and (*R*,*S*,*S*,*S*)-**1**, associated with the linear dipeptide moiety of natural erythrochelin (**1**). The absolute configuration of natural erythrochelin (**1**) was unambiguously reconfirmed to be (*R*,*R*,*S*,*S*) by the comparison of ^{1}H and ^{13}C NMR spectra of natural and synthetic **1** with those of these three stereoisomers.

EXPERIMENTAL

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were obtained using a JASCO FT/IR-6200 IR Fourier transform spectrometer. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Bruker AV500 spectrometer. Chemical shifts are given in δ values (parts per million) using tetramethylsilane (TMS) as an internal standard. Electron spray ionization mass spectra (ESI-MS) were recorded on a Waters LCT Premier spectrometer. Optical rotations were recorded on JASCO digital polarimeter P-2200. All reactions were monitored by TLC employing 0.25-mm silica gel plates (Merck 5715; 60 F_{254}). Column chromatography was carried out on silica gel [Kanto Chemical 60N (spherical, neutral); 63-210 μm]. Anhydrous CH_2Cl_2 and pyridine were used as purchased from Kanto Chemical. All other reagents were used as purchased.

***tert*-Butyl {(*S*)-3-(Benzyloxy)-1-{(benzyloxy){3-{(2*S*,5*S*)-5-{3-[*N*-(benzyloxy)acetamido]propyl}-3,6-dioxopiperazin-2-yl}propyl}amino}-1-oxopropan-2-yl}carbamate [(*S*,*S*,*S*)-4]**

To a solution of (3*S*,6*S*)-**2**[18] (78.0 mg, 0.162 mmol) and (*S*)-**3** (95.5 mg, 0.323 mmol) in anhydrous CH_2Cl_2 (2 mL) was added EDC•HCl (65.1 mg, 0.339 mmol) at 0 °C under an argon atmosphere. After being stirred at rt for 2 h, AcOEt (30 mL) was added and the mixture was washed with 1N HCl (10 mL), H_2O (10 mL), aq 5% $NaHCO_3$ (10 mL), and brine (10 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by chromatography on a silica gel column [Silica Gel 60N: $CHCl_3$–MeOH (40:1 to 10:1)] to afford (*S*,*S*,*S*)-**4** (106.5 mg, 87%).

White amorphous solid; mp 43–46 °C; $[\alpha]_D^{20}$ -23.9 (*c* 1.07, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.45 (s, 9H), 1.60–1.81 (m, 7H), 1.82–1.90 (m, 1H), 2.08 (s, 3H), 3.36–3.44 (m, 1H), 3.59–3.70 (m, 3H), 3.75–3.81 (m, 1H), 3.82–3.87 (m, 1H), 3.91–3.96 (m, 1H), 3.99–4.09 (m, 1H), 4.50 (s, 2H), 4.76–4.82 (m, 2H), 4.85–4.91 (m, 2H), 4.91–4.98 (m, 1H), 5.49 (brd, 1H), 6.25 (brs, 1H), 6.41 (brs, 1H), 7.22–7.31 (m, 5H), 7.32–7.41 (m, 10H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.5, 22.3, 22.7, 28.4, 30.5, 30.9, 44.0, 44.7, 51.1, 53.7, 54.3, 69.7, 73.1, 76.4, 79.7, 127.7, 127.8, 128.3, 128.7, 128.8, 129.0, 129.1, 129.2, 129.3, 133.9, 134.3, 137.5, 155.4, 167.9, 168.4, 171.3, 172.5; IR (KBr) 3213, 3064, 3033, 2932, 2872, 1677, 1454, 1168, 1110 cm^{-1}; HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{41}H_{53}N_5O_9Na$: 782.3741; found: 782.3737.

(*S*)-2-Amino-*N*,3-bis(benzyloxy)-*N*-{3-{(2*S*,5*S*)-5-{3-[*N*-(benzyloxy)acetamido]propyl}-3,6-dioxopiperazin-2-yl}propyl}propanamide [(*S*,*S*,*S*)-5]

A solution of (*S*,*S*,*S*)-**4** (260 mg, 0.342 mmol) in TFA (3.4 mL) was stirred at rt for 30 min. The reaction mixture was concentrated *in vacuo*. The residue was dissolved in aq 5% $NaHCO_3$ (20 mL) and then extracted with $CHCl_3$ (2 x 20 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by chromatography on a silica gel column [Silica Gel 60N: $CHCl_3$–MeOH (10:1)] to afford (*S*,*S*,*S*)-**5** (220 mg, 97%).

White hygroscopic solid; $[\alpha]_D^{18}$ -29.7 (*c* 1.07, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.60–1.90 (m, 10H), 2.08 (s, 3H), 3.52–3.72 (m, 5H), 3.84–3.92 (m, 2H), 3.93–3.97 (m, 1H), 3.99–4.06 (m, 1H), 4.50 (s, 2H), 4.77–4.83 (m, 3H), 4.86 (d, *J* = 10.6 Hz, 1H), 6.43 (brs, 1H), 6.49 (brs, 1H), 7.24-7.42 (m, 15H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.5, 22.3, 22.7, 30.6, 31.0, 44.1, 44.6, 51.2, 53.9, 54.3, 72.5, 73.2, 76.36, 76.44, 127.7, 127.8, 128.3, 128.7, 128.8, 129.0, 129.1, 129.2, 129.3, 134.1, 134.3, 137.8, 168.1, 168.4, 172.5, 174.9; IR (KBr) 3196, 3062, 3033, 2933, 2870, 1959, 1880, 1814, 1677, 1454, 1334, 1211, 1099 cm^{-1}; HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{36}H_{45}N_5O_7Na$: 682.3217; found: 682.3196.

***tert*-Butyl {(5*S*,8*S*)-12-Acetyl-3-{3-{(2*S*,5*S*)-5-{3-[*N*-(benzyloxy)acetamido]propyl}-3,6-dioxopiperazin-2-yl}propyl}-5-[(benzyloxy)methyl]-4,7-dioxo-1,14-diphenyl-2,13-dioxa-3,6,12-triaza-**

tetradecan-8-yl}carbamate [(*S*,*S*,*S*,*S*)-7]

To a solution of (*S*,*S*,*S*)-**5** (220 mg, 0.333 mmol) and (*S*)-**6** (127 mg, 0.333 mmol) in anhydrous CH_2Cl_2 (3.3 mL) were added EDC•HCl (95.9 mg, 0.500 mmol) and 4-dimethylaminopyridine (DMAP) (4.07 mg, 0.0333 mmol) at 0 °C under an argon atmosphere. After the reaction mixture was stirred at rt for 20 h, AcOEt (40 mL) was added and the mixture was washed with 1N HCl (20 mL), H_2O (20 mL), aq 5% $NaHCO_3$ (20 mL), and brine (20 ml). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by chromatography on a silica gel column [Silica Gel 60N: $CHCl_3$–MeOH (10:1)] to afford (*S*,*S*,*S*,*S*)-**7** (312 mg, 91%).

White amorphous solid; mp 50–52.5 °C; $[\alpha]_D^{21}$ -18.6 (*c* 1.01, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.41 (s, 9H), 1.47–1.56 (m, 2H), 1.58–1.92 (m, 9H), 2.02–2.14 (m, 1H), 2.06 (s, 3H), 2.11 (s, 3H), 3.35–3.45 (m, 2H), 3.59–3.66 (m, 3H), 3.69–3.75 (m, 1H), 3.76–3.84 (m, 1H), 3.94–3.98 (m, 1H), 3.99–4.03 (m, 1H), 4.15–4.26 (m, 1H), 4.36 (brt, 1H), 4.50–4.56 (m, 2H), 4.75–4.86 (m, 6H), 5.15 (q, *J* = 6.1 Hz, 1H), 6.15–6.25 (m, 2H), 7.24–7.41 (m, 21H), 7.52 (brs, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.41, 20.44, 21.5, 22.4, 22.9, 28.4, 29.4, 29.6, 30.0, 43.3, 44.8, 45.5, 49.7, 52.2, 53.7, 54.2, 69.0, 73.3, 76.2, 76.4, 79.2, 127.8, 127.9, 128.4, 128.69, 128.71, 128.88, 128.94, 129.0, 129.1, 129.2, 129.3, 134.2, 134.27, 134.33, 137.4, 156.1, 168.1, 168.6, 171.9, 172.5, 173.2, 173.8; IR (KBr) 3269, 3063, 3033, 2934, 2872, 1675, 1454, 1366, 1167 cm^{-1}; HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{55}H_{71}N_7O_{12}Na$: 1044.5058; found: 1044.5060.

(*S*)-2-Amino-*N*-{(*S*)-3-(benzyloxy)-1-{(benzyloxy){3-{(2*S*,5*S*)-5-{3-[*N*-(benzyloxy)acetamido]propyl}-3,6-dioxopiperazin-2-yl}propyl}amino}-1-oxopropan-2-yl}-5-[*N*-(benzyloxy)acetamido]pentanamide [(*S*,*S*,*S*,*S*)-8]

A solution of (*S*,*S*,*S*,*S*)-**7** (312 mg, 0.305 mmol) in TFA (3.1 mL) was stirred at rt for 30 min. The reaction mixture was concentrated *in vacuo*. The residue was dissolved in aq 5% $NaHCO_3$ (10 mL) and then extracted with $CHCl_3$ (2 x 20 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by chromatography on a silica gel column [Silica Gel 60N: $CHCl_3$–MeOH (10:1)] to afford (*S*,*S*,*S*,*S*)-**8** (208 mg, 74%).

White hygroscopic solid; $[\alpha]_D^{23}$ -22.4 (*c* 1.09, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.44–1.54 (m, 1H), 1.62–1.95 (m, 11H), 1.97 (brs, 2H), 2.07 (s, 3H), 2.08 (s, 3H), 3.44–3.50 (m, 1H), 3.54–3.82 (m, 8H), 3.89–3.95 (m, 2H), 4.48 (s, 2H), 4.75–4.83 (m, 4H), 4.85 (d, *J* = 10.2 Hz, 1H), 4.88 (d, *J* = 10.2 Hz, 1H), 5.16–5.25 (m, 1H), 6.50 (brs, 1H), 6.82 (brs, 1H), 7.22–7.31 (m, 5H), 7.33–7.40 (m, 15H), 7.86 (d, *J* = 7.6 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.5, 21.9, 22.6, 23.2, 30.1, 30.6, 31.9, 44.6, 49.4, 53.7, 54.26, 54.31, 69.3, 73.2, 76.3, 76.5, 127.8, 127.9, 128.4, 128.71, 128.75, 128.77, 128.9, 129.0, 129.17, 129.21, 129.4, 134.0, 134.3, 134.4, 137.5, 167.8, 168.4, 171.3, 172.6, 175.6; IR (KBr) 3209, 3063, 3033,

2930, 2871, 1674, 1454, 1369, 1333, 1212, 1109 cm^{-1}; HRMS (ESI): *m*/*z* [M + Na]$^+$ calcd for $C_{50}H_{63}N_7O_{10}Na$: 944.4534; found: 944.4506.

(*S*)-2-Acetamido-*N*-{(*S*)-3-(benzyloxy)-1-{(benzyloxy){3-{(2*S*,5*S*)-5-{3-[*N*-(benzyloxy)acetamido]-propyl}-3,6-dioxopiperazin-2-yl}propyl}amino}-1-oxopropan-2-yl}-5-[*N*-(benzyloxy)acetamido]-pentanamide [(*S*,*S*,*S*,*S*)-9]

To a solution of (*S*,*S*,*S*,*S*)-**8** (208 mg, 0.226 mmol) in anhydrous pyridine (4.5 mL) was added Ac_2O (32.0 μL, 0.338 mmol) at rt under an argon atmosphere. The reaction mixture was stirred at rt for 2 h. 1N HCl (20 mL) was added and then extracted with $CHCl_3$ (2 x 40 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by chromatography on a silica gel column [Silica Gel 60N: $CHCl_3$–MeOH (15:1)] to afford (*S*,*S*,*S*,*S*)-**9** (205 mg, 94%).

White amorphous solid; mp 42–46.5 °C; $[\alpha]_D^{25}$ -26.3 (*c* 1.01, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.38–1.58 (m, 3H), 1.60–1.87 (m, 7H), 1.94–2.04 (m, 1H), 1.97 (s, 3H), 2.06 (s, 3H), 2.11 (s, 3H), 2.18–2.29 (m, 1H), 3.13 (brt, 1H), 3.28–3.36 (m, 1H), 3.55–3.72 (m, 4H), 3.97–4.04 (m, 2H), 4.09 (brs, 1H), 4.29 (brt, 1H), 4.53 (d, *J* = 11.8 Hz, 1H), 4.56 (d, *J* = 11.8 Hz, 1H), 4.71–4.86 (m, 7H), 5.10 (q, *J* = 6.8 Hz, 1H), 6.28 (brs, 1H), 7.22–7.41 (m, 20H), 7.44–7.50 (m, 1H), 7.94 (d, *J* = 8.7 Hz, 1H), 8.34 (brs, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.4, 21.0, 22.3, 22.8, 22.9, 28.4, 29.2, 29.4, 42.9, 44.8, 46.6, 49.9, 50.0, 53.7, 54.1, 68.7, 73.5, 76.2, 76.4, 76.9, 127.9, 128.0, 128.4, 128.66, 128.73, 128.75, 128.80, 129.00, 129.03, 129.1, 129.2, 129.3, 134.2, 134.3, 134.4, 137.3, 168.7, 168.9, 171.0, 172.6, 172.7, 173.5, 174.4; IR (KBr) 3267, 3215, 3064, 3033, 2933, 2872, 1667, 1453, 1411, 1372, 1212, 1110 cm^{-1}; HRMS (ESI): *m*/*z* [M + Na]$^+$ calcd for $C_{52}H_{65}N_7O_{11}Na$: 986.4640; found: 986.4661.

(*S*)-2-Acetamido-*N*-{(*S*)-3-hydroxy-1-{hydroxy{3-{(2*S*,5*S*)-5-[3-(*N*-hydroxyacetamido)propyl]-3,6-dioxopiperazin-2-yl}propyl}amino}-1-oxopropan-2-yl}-5-(*N*-hydroxyacetamido)pentanamide [(*S*,*S*,*S*,*S*)-1]

A mixture of (*S*,*S*,*S*,*S*)-**9** (155 mg, 0.161 mmol) and 10% Pd-C (17.1 mg, 0.0161 mmol) in MeOH (3.2 mL) was stirred at rt for 3 h under a hydrogen atmosphere. The reaction mixture was filtered and concentrated *in vacuo*. The residue was purified by chromatography on a silica gel column [Silica Gel 60N: $CHCl_3$–MeOH–H_2O (40:10:1)] to afford (*S*,*S*,*S*,*S*)-**1** (72.4 mg, 75%).

Pale yellow hygroscopic solid; $[\alpha]_D^{26}$ -36.0 (*c* 1.09, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.35–1.75 (m, 12H), 1.85 (s, 3H), 1.97 (s, 6H), 3.33–3.68 (m, 8H), 3.79-3.86 (m, 2H), 4.29–4.37 (m, 1H), 4.81 (brs, 1H), 4.86–4.92 (m, 1H), 7.76 (brd, 1H), 7.98 (d, *J* = 8.2 Hz, 1H), 8.09 (brs, 1H), 8.13 (brs, 1H), 9.69 (brs, 2H), 9.84 (brs, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 20.2, 21.8, 22.0, 22.4, 23.0, 29.4, 30.0, 30.1, 46.6, 46.7, 47.4, 51.98, 52.04, 53.6, 53.7, 60.8, 167.76, 167.80, 169.1, 169.2, 170.2, 171.5; IR (KBr) 3173,

2932, 2871, 1665, 1631, 1539, 1458, 1376, 1335 cm^{-1}; HRMS (ESI): *m*/*z* [M + Na]$^+$ calcd for $C_{24}H_{41}N_7O_{11}Na$: 626.2762; found: 626.2734.

(*S*)-2-Acetamido-*N*-{(*R*)-3-hydroxy-1-{hydroxy{3-{(2*S*,5*S*)-5-[3-(*N*-hydroxyacetamido)propyl]-3,6-dioxopiperazin-2-yl}propyl}amino}-1-oxopropan-2-yl}-5-(*N*-hydroxyacetamido)pentanamide [(*S*,*R*,*S*,*S*)-1]

White hygroscopic solid; $[\alpha]_D^{26}$ -33.1 (*c* 1.03, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.40–1.72 (m, 12H), 1.85 (s, 3H), 1.966 (s, 3H), 1.972 (s, 3H), 3.38–3.65 (m, 8H), 3.78-3.85 (m, 2H), 4.31–4.39 (m, 1H), 4.81 (brt, 1H), 4.91–4.97 (m, 1H), 7.79 (brd, 1H), 8.01 (d, *J* = 8.4 Hz, 1H), 8.11 (brs, 1H), 8.15 (brs, 1H), 9.68 (brs, 1H), 9.70 (brs, 1H), 9.85 (brs, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 20.2, 21.7, 22.0, 22.4, 23.0, 29.4, 30.0, 30.2, 46.6, 46.7, 46.9, 51.7, 52.0, 53.5, 53.7, 60.9, 167.75, 167.84, 169.1, 169.2, 170.1, 171.4; IR (KBr) 3199, 2933, 2873, 1665, 1541, 1458, 1376, 1335 cm^{-1}; HRMS (ESI): *m*/*z* [M + Na]$^+$ calcd for $C_{24}H_{41}N_7O_{11}Na$: 626.2762; found: 626.2725.

(*R*)-2-Acetamido-*N*-{(*S*)-3-hydroxy-1-{hydroxy{3-{(2*S*,5*S*)-5-[3-(*N*-hydroxyacetamido)propyl]-3,6-dioxopiperazin-2-yl}propyl}amino}-1-oxopropan-2-yl}-5-(*N*-hydroxyacetamido)pentanamide [(*R*,*S*,*S*,*S*)-1]

Pale yellow hygroscopic solid; $[\alpha]_D^{27}$ -12.8 (*c* 1.01, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.39–1.73 (m, 12H), 1.85 (s, 3H), 1.966 (s, 3H), 1.971 (s, 3H), 3.37–3.65 (m, 8H), 3.79-3.87 (m, 2H), 4.31–4.39 (m, 1H), 4.82 (brt, 1H), 4.91–4.98 (m, 1H), 7.79 (brd, 1H), 8.01 (d, *J* = 8.4 Hz, 1H), 8.10 (brs, 1H), 8.14 (brs, 1H), 9.687 (brs, 1H), 9.697 (brs, 1H), 9.86 (brs, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 20.2, 21.8, 22.0, 22.4, 23.0, 29.5, 30.0, 30.1, 46.6, 46.7, 47.3, 51.6, 52.0, 53.6, 53.7, 60.9, 167.77, 167.79, 169.1, 169.2, 170.1, 171.4; IR (KBr) 3172, 2931, 2870, 1667, 1542, 1459, 1376, 1334 cm^{-1}; HRMS (ESI): *m*/*z* [M + Na]$^+$ calcd for $C_{24}H_{41}N_7O_{11}Na$: 626.2762; found: 626.2755.

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