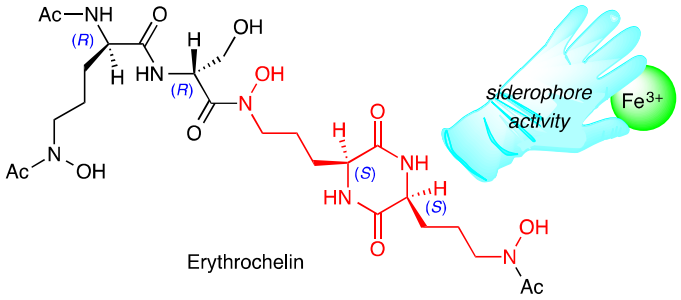
Synthesis of Erythrochelin: A Hydroxamate-type Siderophore from *Saccharopolyspora erythraea*

Michiyasu Nakao
Shunsuke Tsuji
Syuji Kitaike
Shigeki Sano*

Graduate School of Pharmaceutical Sciences, Tokushima University, Sho-machi, Tokushima 770-8505, Japan

ssano@tokushima-u.ac.jp

Click here to insert a dedication.



Received:
Accepted:
Published online:
DOI:

Abstract Erythrochelin, a hydroxamate-type siderophore produced by *Saccharopolyspora erythraea*, is synthesized for the first time. A key building block of erythrochelin containing the 2,5-diketopiperazine ring is prepared by intramolecular cyclization of the corresponding dipeptide precursor derived from two kinds of protected δ-*N*-hydroxy-L-ornithines. Consecutive condensation of the building block with protected D-serine and protected δ-*N*-hydroxy-D-ornithine, followed by deprotection, furnishes erythrochelin.

Key words erythrochelin, rhodotorulic acid, siderophore, 2,5-diketopiperazine, electrospray ionization mass spectrometry

Erythrochelin (**1**)[1], a hydroxamate-type tetrapeptide siderophore[2], was isolated as the first nonribosomal peptide synthetase (NRPS)-derived natural product of *Saccharopolyspora erythraea*.[3] In 2010, two groups independently reported the isolation and structural characterization of **1** (Figure 1).[4] Marahiel et al. identified the structure of **1** using a novel radio-LC-MS–guided genome mining methodology as well as NMR and MS analyses.[1a] On the other hand, Leadlay et al. isolated **1** as the metabolic product of the cryptic NRPS cluster and determined the structure based on NMR analysis of the Ga(III) complex of **1**.[1b] Both groups proposed that the chemical structure of **1** included a 2,5-diketopiperazine (2,5-DKP) ring derived from δ-*N*-acetyl-δ-*N*-hydroxy-L-ornithine and δ-*N*-hydroxy-L-ornithine. In addition, a dipeptide moiety comprised of D-serine and α-*N*-acetyl-δ-*N*-acetyl-δ-*N*-hydroxy-D-ornithine was presented. In the literature, a biosynthetic route was established *in vitro* for the generation of δ-*N*-acetyl-δ-*N*-hydroxy-L-ornithine starting from L-ornithine.[5] However, there has been no report on the chemical synthesis of **1**, and the specific rotation value of **1** has not been established.

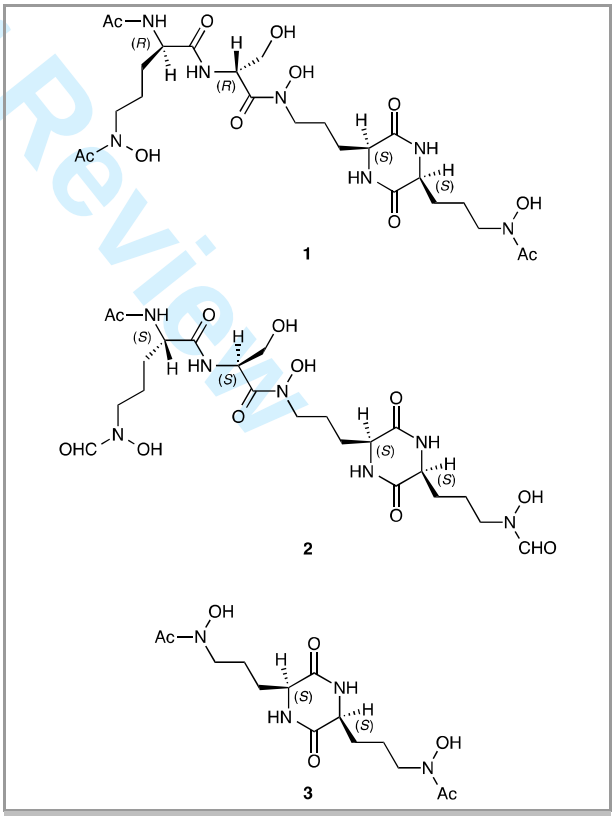
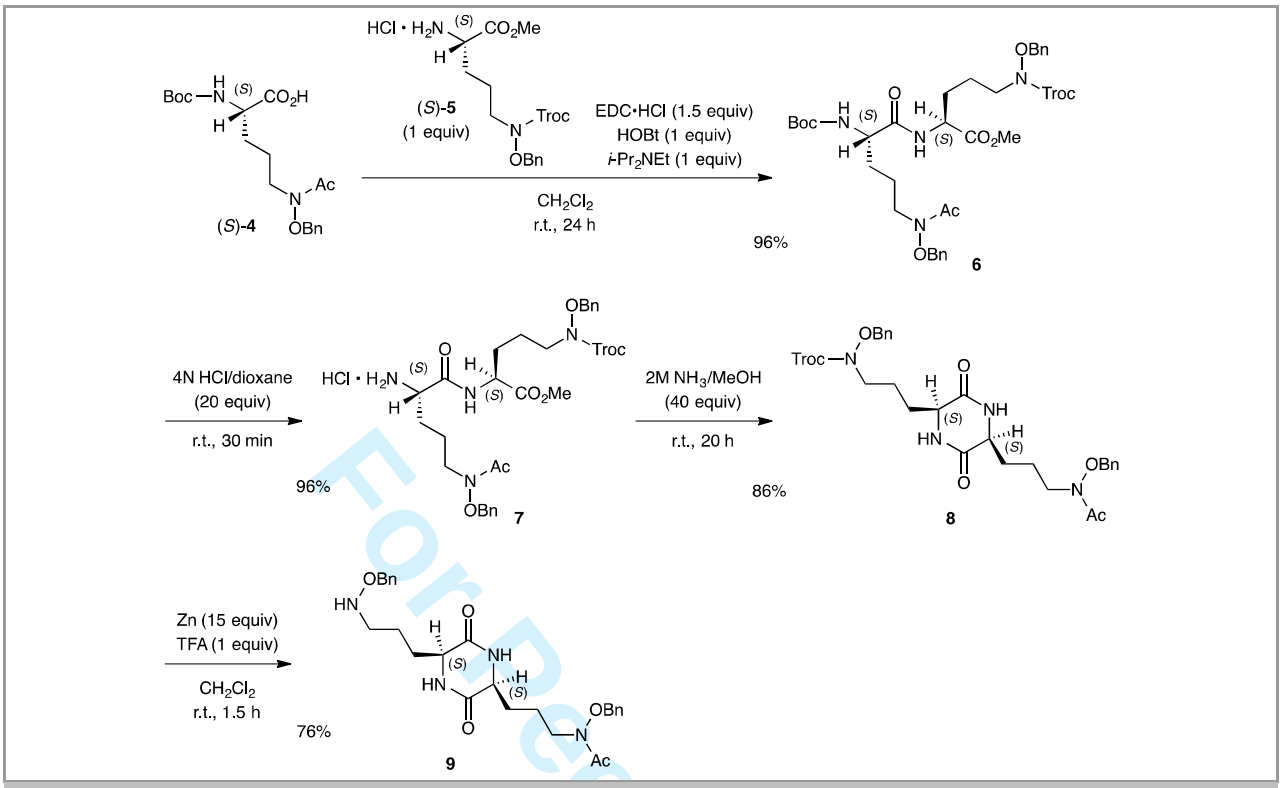


Figure 1 Chemical structures of erythrochelin (**1**), foroxymithine (**2**), and rhodotorulic acid (**3**).



Scheme 1 Synthesis of 2,5-DKP **9**.

In 1985, foroxymithine (**2**), which has a very similar chemical structure to **1**, was isolated from cultures of *Streptomyces nitrosporeus* as an angiotensin-converting enzyme inhibitor.[6] Interestingly, **2** was constructed from only L-α-amino acids, whereas corresponding L- and D-α-amino acids were contained in **1** as shown in Figure 1. Dolence and Miller achieved the total synthesis of **2**.[7] They established the chemical structure of **2** by comparing the spectroscopic data, including the specific rotation, with that of the natural product. The stereochemical structure of **2** isolated from *Streptomyces narbonensis* was also confirmed by Marfey's analysis of the corresponding Ga(III) complex of **2**.[8] The biosynthetic mechanism of **2** has been predicted based on the NRPS domain organization.[9] In addition, rhodotorulic acid (**3**)[10], a structurally related hydroxamate-type siderophore isolated from *Rhodotorula pilimanae*, has been synthesized by several groups, including ours.[11] A series of siderophores—triornicin,[12] isotriornicin,[13] dimerumic acid,[14] coprogen,[15] coprogen B,[14a] and α-*N*-methyl coprogen[14c,16]—are also known as hydroxamate-type siderophores. Each of these hydroxamate-type siderophores has a 2,5-DKP ring as a characteristic building block.[17] In this report, we present the first synthesis of **1** as a step toward the certain confirmation of its full stereochemistry.

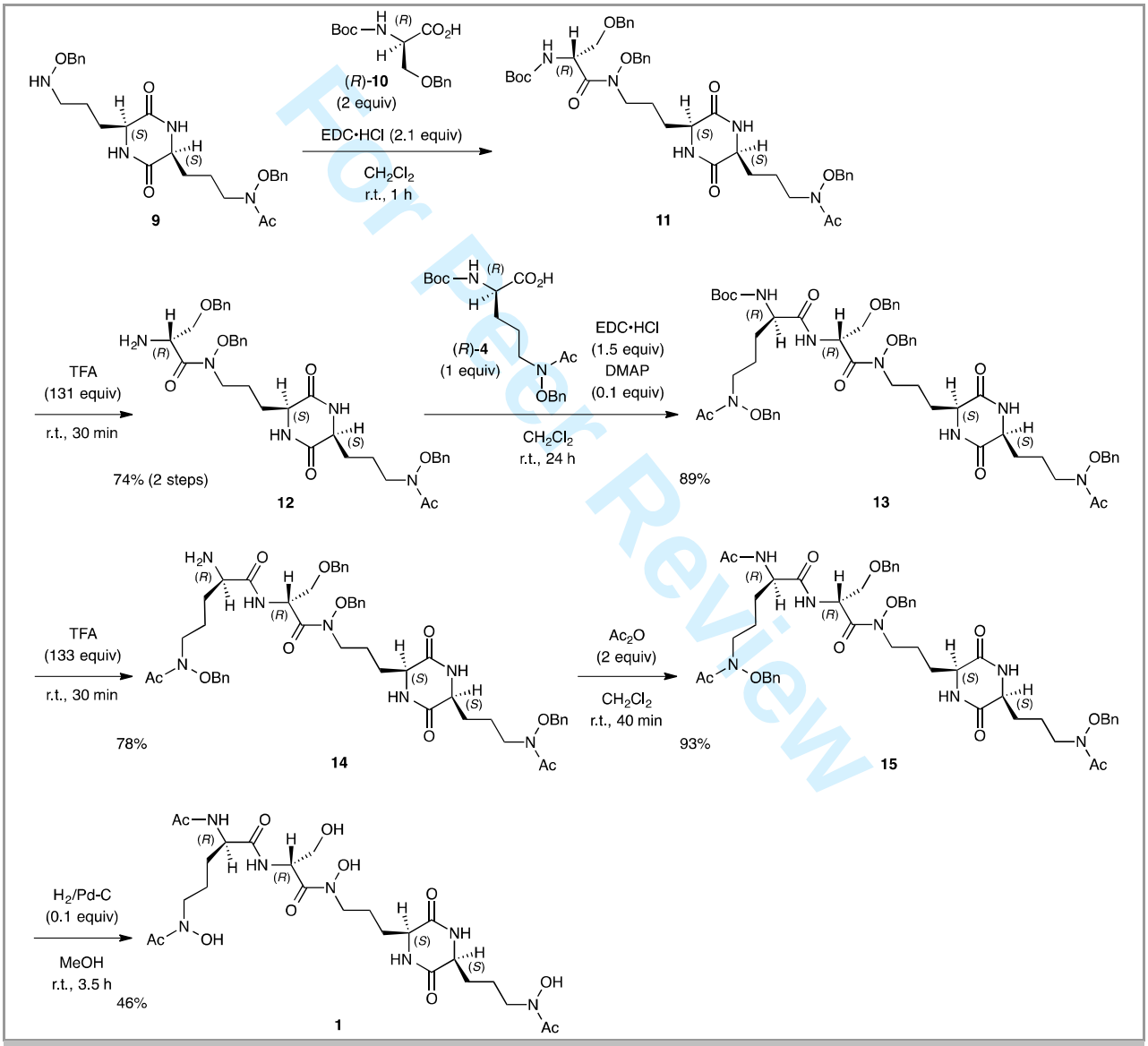
First, we investigated the preparation of the key building block **9** containing the 2,5-DKP ring as shown in Scheme 1. Protected amino acids as starting materials, α-*N*-Boc-δ-*N*-acetyl-δ-*N*-benzyloxy-L-ornithine [(*S*)-**4**][11b,d] and δ-*N*-benzyloxy-δ-*N*-(2,2,2-trichloroethoxy)carbonyl-L-ornithine methyl ester hydrochloride [(*S*)-**5**],[11d] were prepared from Boc-L-Glu(OBn)-OH. Condensation of (*S*)-**4** with (*S*)-**5** using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC□HCl) as a coupling reagent in the presence of 1-hydroxybenzotriazole (HOBt) and *N*,*N*-diisopropylethylamine (DIPEA) furnished the *N*-Boc-dipeptide methyl ester **6** in 96% yield. We then tried a one-pot conversion of **6** into 2,5-DKP **8** using microwave irradiation at 170 °C in a mixed solvent of water with methanol,[11e] but **8** was obtained in only moderate yield (58%). Therefore, we investigated a stepwise construction of **8**. Deprotection of the Boc group of **6** with an excess amount of 4N HCl in dioxane afforded the dipeptide methyl ester hydrochloride **7** in 96% yield. Intramolecular cyclization of **7** on treatment with ammonia solution (2M in methanol) afforded the 2,5-DKP **8** in 86% yield. Then, reductive cleavage of the 2,2,2-trichloroethoxycarbonyl (Troc) group of **8** with an excess amount of zinc powder in the presence of 1 equivalent of trifluoroacetic acid (TFA) gave the key building block **9** in 76% yield.

We attempted the condensation of 2,5-DKP **9** with two protected D-α-amino acids toward the synthesis of **1** (Scheme 2). Condensation of **9** with Boc-D-Ser(OBn)-OH [(*R*)-**10**] using EDC□HCl as a coupling reagent afforded **11**. Deprotection of the Boc group of **11** with an excess amount of TFA provided **12** in 74% yield (two steps). Amine **12** was coupled with α-*N*-Boc-δ-*N*-acetyl-δ-*N*-benzyloxy-D-ornithine [(*R*)-**4**], which was prepared from Boc-D-Glu(OBn)-OH, to furnish **13** in 89% yield. Amine **14** was obtained by acidic deprotection of the Boc group of **13** in 78% yield. Acetylation of **14** with 2 equivalent of acetic anhydride gave **15** in 93% yield. Finally, catalytic hydrogenolysis of **15** under hydrogen with palladium on carbon (10 wt% loading) provided erythrochelin (**1**) in 46% yield by recrystallization from chloroform–methanol. The chemical structure of **1** was fully characterized by

spectroscopic methods and agreed well with the reported ^{1}H and ^{13}C NMR data.[1] In addition, the negative specific rotation value {$[\alpha]_D^{24}$ –10.3 (*c* 1.00, MeOH)} was observed.

To investigate the coordination pattern of **1** with Fe(III), electrospray ionization mass spectrometry (ESI-MS) was used for the detection of metal-chelate complexes.[18] As a result, **1** was suggested to form a 1:1 complex with Fe(III) from *m*/*z* of 679.1879 [(M–3H)+Fe(III)+Na]$^+$ (calcd: *m*/*z* of 679.1876) in the presence of 1 equiv of iron(III) chloride.[19] Furthermore, a similar 1:1 complex with Mg(II) was indicated from *m*/*z* of 648.2449 [(M–2H)+Mg(II)+Na]$^+$ (calcd: *m*/*z* of 648.2456) in the ESI-MS analysis with 1 equiv of magnesium(II) chloride.[19]

In conclusion, we have achieved the synthesis of erythrochelin (**1**) and determined its specific rotation. In addition, **1** was found to form a 1:1 complex with not only Fe(III) but also Mg(II) based on ESI-MS analysis. The present work will be valuable for the confirmation of the full stereochemistry of **1** isolated from *Saccharopolyspora erythraea* and for the synthesis of stereoisomers of **1** and their structurally related derivatives with various metal chelating abilities.



Scheme 2 Synthesis of erythrochelin (**1**).

The experimental section has no title; please leave this line here.

All melting points were determined on a Yanagimoto micro melting point apparatus and 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, respectively. Chemical shifts are given in δ values (parts per million) using tetramethylsilane (TMS) as an internal standard. ESI-MS were recorded on a Waters LCT Premier spectrometer. Elemental combustion analyses were performed using a J-SCIENCE LAB JM10. Microwave-assisted reaction was performed utilizing an automated single-mode microwave synthesizer (Initiator™ 60; Biotage AB). 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)]. Anhydrous CH_2Cl_2 was used as purchased from Kanto Chemical. DIPEA was distilled prior to use. All other reagents were used as purchased.

Methyl (*S*)-5-{(Benzyloxy)[(2,2,2-trichloroethoxy)carbonyl]amino}-2-{(*S*)-5-[*N*-(benzyloxy)acetamido]-2-[(*tert*-butoxycarbonyl)amino]pentanamido}pentanoate (6)

To a solution of (*S*)-**4** (498 mg, 1.31 mmol) in anhydrous CH_2Cl_2 (6.5 mL) were added HOBt (177 mg, 1.31 mmol), DIPEA (226 µL, 1.31 mmol), EDC□HCl (376 mg, 1.96 mmol), and (*S*)-**5** (608 mg, 1.31 mmol) at 0 °C under argon. The reaction mixture was allowed to warm to r.t. and stirred for 24 h. AcOEt (40 mL) was added to the reaction mixture, and then washed with 5% citric acid (10 mL), 1N HCl (10 mL), H_2O (10 mL) and brine (10 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The oily residue was purified by column chromatography [Silica Gel 60N: $CHCl_3$–MeOH (50:1)] to afford **6** (987 mg, 96%) as a colorless oil.

$[\alpha]_D^{20}$ +5.1 (*c* 1.00, $CHCl_3$).

IR (neat): 3305, 2953, 1683, 1506, 1456, 1367 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 7.46–7.32 (m, 10H), 7.12–7.00 (m, 1H), 5.24 (brd, 1H), 4.93 (s, 2H), 4.87–4.76 (m, 4H), 4.56–4.48 (m, 1H), 4.42–4.31 (m, 1H), 4.27–4.12 (m, 1H), 3.65 (s, 3H), 3.54 (t, *J* = 6.5 Hz, 2H), 3.49–3.45 (m, 1H), 2.10 (s, 3H), 1.89–1.63 (m, 7H), 1.55–1.46 (m, 1H), 1.43 (s, 9H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 173.3, 172.4, 172.3, 155.8, 155.1, 134.8, 134.2, 129.6, 129.2, 129.0, 128.82, 128.76, 128.5, 95.3, 79.6, 76.3, 75.1, 52.2, 52.1, 51.7, 49.1, 43.4, 30.7, 29.2, 28.3, 23.2, 23.0, 20.4.

HRMS (ESI): *m/z* $[M + Na]^+$ calcd for $C_{35}H_{47}Cl_3N_4NaO_{10}$: 811.2255; found: 811.2256.

Methyl (*S*)-2-{(*S*)-2-Amino-5-[*N*-(benzyloxy)acetamido]pentanamido}-5-{(benzyloxy)[(2,2,2-trichloroethoxy)carbonyl]amino}pentanoate Hydrochloride (7)

A mixture of **6** (1 g, 1.26 mmol) and 4N HCl in dioxane (6.3 mL, 25.3 mmol) was stirred at r.t. for 30 min. The reaction mixture was concentrated *in vacuo*. The residue was washed with *n*-hexane and $CHCl_3$ to afford **7** (881 mg, 96%) as a white solid.

$[\alpha]_D^{21}$ +4.7 (*c* 1.00, $CHCl_3$).

IR (KBr): 3552, 3477, 3419, 3033, 2946, 2870, 1742, 1684 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 8.49 (brs, 3H), 8.26 (brd, 1H), 7.44–7.40 (m, 2H), 7.38–7.30 (m, 8H), 4.91 (s, 2H), 4.86–4.75 (m, 4H), 4.54–4.46 (m, 1H), 4.37 (brs, 1H), 4.02-3.87 (m, 1H), 3.69–3.47 (m, 3H), 3.59 (s, 3H), 2.06 (s, 3H), 2.10–1.72 (m, 8H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 173.4, 171.9, 169.1, 155.1, 134.8, 134.2, 129.6, 129.3, 129.0, 128.8, 128.7, 128.5, 95.3, 77.1, 76.4, 75.2, 52.3, 52.2, 52.0, 48.8, 43.8, 28.53, 28.47, 23.3, 22.6, 20.4.

HRMS (ESI): *m/z* $[M - HCl + Na]^+$ calcd for $C_{30}H_{39}Cl_3N_4NaO_8$: 711.1731; found: 711.1729.

2,2,2-Trichloroethyl Benzyloxy{3-{(2*S*,5*S*)-5-{3-[*N*-(benzyloxy)acetamido]propyl}-3,6-dioxopiperazin-2-yl}propyl}carbamate (8)

A mixture of **7** (53.0 mg, 0.073 mmol) and 2M NH_3 in MeOH (1.46 mL, 2.92 mmol) was stirred at r.t. for 20 h. The reaction mixture was concentrated *in vacuo*. The residue was filtered with $CHCl_3$ and concentrated *in vacuo*. It was then purified by column chromatography [Silica Gel 60N: $CHCl_3$–MeOH (15:1)] to afford **8** (41.5 mg, 86%) as a white solid.

Mp 146–147 °C (white powder, MeOH) ; $[\alpha]_D^{21}$ –29.8 (*c* 1.00, $CHCl_3$).

IR (KBr): 3190, 3058, 2951, 2899, 1708, 1678 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 7.44–7.32 (m, 10H), 6.88 (d, *J* = 1.0 Hz, 1H), 6.67 (d, *J* = 1.2 Hz, 1H), 4.92 (s, 2H), 4.82 (s, 2H), 4.80 (s, 2H), 4.00–3.91 (m, 2H), 3.73–3.58 (m, 2H), 3.57–3.45 (m, 2H), 2.07 (s, 3H), 1.95–1.68 (m, 8H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 172.7, 168.1, 167.8, 155.2, 134.8, 134.3, 129.6, 129.2, 129.1, 128.9, 128.8, 128.6, 95.2, 76.5, 75.2, 54.5, 54.3, 49.1, 44.6, 31.0, 30.9, 22.71, 22.67, 20.5.

HRMS (ESI): *m/z* $[M + Na]^+$ calcd for $C_{29}H_{35}Cl_3N_4NaO_7$: 679.1469; found: 679.1462.

Anal. Calcd for $C_{29}H_{35}Cl_3N_4O_7$: C, 52.94; H, 5.36; N, 8.52. Found: C, 52.87; H, 5.32; N, 8.55.

***N*-(Benzyloxy)-*N*-{3-{(2*S*,5*S*)-5-{3-[(benzyloxy)amino]propyl}-3,6-dioxopiperazin-2-yl}propyl}acetamide (9)**

To a solution of **8** (1.16 g, 1.76 mmol) and zinc (1.73 g, 26.4 mmol) in anhydrous CH_2Cl_2 (17.6 mL) was added TFA (135 µL, 1.76 mmol) at 0 °C under argon. The reaction mixture was allowed to warm to r.t. and stirred for 1.5 h. The reaction mixture was filtered with $CHCl_3$ and 5% $NaHCO_3$ (20 mL) was added to the filtrate, and then extracted with $CHCl_3$ (30 mL x 3). The extract was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography [Silica Gel 60N: $CHCl_3$–MeOH (20:1)] to afford **9** (650 mg, 76%) as a white solid.

Mp 94–95 °C ; $[\alpha]_D^{20}$ –52.5 (*c* 1.04, $CHCl_3$).

IR (KBr): 3033, 2926, 2890, 1665, 1456 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 7.43–7.28 (m, 10H), 6.70 (brs, 1H), 6.44 (brs, 1H), 5.57 (brs, 1H), 4.84–4.78 (m, 2H), 4.70 (s, 2H), 4.02–3.97 (m, 1H), 3.91–3.85 (m, 1H), 3.74–3.58 (m, 2H), 3.00–2.89 (m, 2H), 2.09 (s, 3H), 2.06–1.98 (m, 1H), 1.94–1.86 (m, 1H), 1.83–1.56 (m, 6H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 172.5, 168.6, 168.4, 137.8, 134.3, 129.2, 129.0, 128.7, 128.5, 128.4, 127.9, 76.4, 76.1, 54.8, 54.4, 51.3, 44.7, 32.0, 31.1, 23.0, 22.7, 20.5.

HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{26}H_{35}N_4O_5$: 483.2607; found: 483.2601.

***tert*-Butyl {(*R*)-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 (11)**

To a solution of **9** (530 mg, 1.10 mmol) and (*R*)-**10** (649 mg, 2.20 mmol) in anhydrous CH_2Cl_2 (11 mL) was added EDC□HCl (442 mg, 2.31 mmol) at 0 °C under argon. The reaction mixture was allowed to warm to r.t. and stirred for 1 h. AcOEt (60 mL) was added to the reaction mixture, and then washed with 1N HCl (20 mL), H_2O (10 mL), 5% $NaHCO_3$ (20 mL), and brine (20 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The oily residue was purified by column chromatography [Silica Gel 60N: $CHCl_3$–MeOH (40:1 to 10:1)] to afford **11** (763 mg) as a colorless oil containing small amounts of impurities. In the next step, **11** was used without further purification.

^1H NMR (500 MHz, $CDCl_3$): δ = 7.41–7.34 (m, 10H), 7.31–7.20 (m, 5H), 6.50 (brs, 1H), 6.41 (brs, 1H), 5.49 (d, *J* = 8.4 Hz, 1H), 5.00 (brs, 1H), 4.94–4.76 (m, 4H), 4.52–4.44 (m, 2H), 4.00–3.82 (m, 2H), 3.76–3.58 (m, 5H), 3.54–3.45 (m, 1H), 2.08 (s, 3H), 1.91–1.83 (m, 1H), 1.79–1.67 (m, 7H), 1.45 (s, 9H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 172.6, 171.6, 168.0, 167.7, 155.5, 137.6, 134.3, 133.9, 129.3, 129.2, 129.1, 129.0, 128.80, 128.76, 128.4, 127.79, 127.77, 79.8, 76.9, 76.5, 73.1, 70.0, 54.3, 54.1, 50.8, 44.8, 44.7, 30.7, 29.9, 28.4, 22.7, 22.1, 20.5.

(*R*)-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 (12)

A solution of **11** (763 mg) in TFA (10 mL, 131 mmol) was stirred at r.t. for 30 min. The reaction mixture was concentrated *in vacuo*. The residue was dissolved in 5% $NaHCO_3$ (40 mL) and then extracted with $CHCl_3$ (25 mL x 3). The extract was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. It was then purified by column chromatography [Silica Gel 60N: $CHCl_3$–MeOH (15:1)] to afford **12** (534 mg, 74%, 2 steps) as a colorless oil.

$[\alpha]_D^{20}$ –29.9 (*c* 1.00, $CHCl_3$).

IR (neat): 3452, 3244, 2936, 2871, 2516, 1664, 1454 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 7.41–7.23 (m, 15H), 6.94 (brs, 1H), 6.90 (brs, 1H), 4.87–4.76 (m, 4H), 4.48 (s, 2H), 4.06 (brs, 1H), 3.93 (brs, 1H), 3.82 (brs, 1H), 3.76–3.57 (m, 5H), 3.55–3.49 (m, 1H), 2.06 (s, 3H), 1.91–1.65 (m, 10H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 175.0, 172.6, 168.1, 168.0, 137.9, 134.3, 134.1, 129.3, 129.2, 129.1, 129.0, 128.80, 128.75, 128.4, 127.74, 127.70, 76.6, 76.5, 73.3, 72.7, 54.3, 54.2, 51.1, 44.7, 30.8, 30.4, 22.7, 22.5, 20.5.

HRMS (ESI): *m/z* [M + Na]$^+$ calcd for $C_{36}H_{45}N_5NaO_7$: 682.3217; found: 682.3210.

tert-Butyl {(5*R*,8*R*)-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-triazatetradecan-8-yl}carbamate (13)

To a solution of **12** (44.1 mg, 0.0668 mmol) and (*R*)-**4** (25.4 mg, 0.0668 mmol) in anhydrous CH_2Cl_2 (0.7 mL) were added EDC□HCl (19.2 mg, 0.100 mmol) and 4-dimethylaminopyridine (DMAP) (0.82 mg, 0.00668 mmol) at 0 °C under argon. The reaction mixture was allowed to warm to r.t. and stirred for 24 h. AcOEt (20 mL) was added to the reaction mixture, and then washed with 1N HCl (5 mL), H_2O (5 mL), 5% $NaHCO_3$ (5 mL), and brine (5 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by by column chromatography [Silica Gel 60N: $CHCl_3$–MeOH (20:1)] to afford **13** (60.6 mg, 89%) as a white solid.

Mp 52–55 °C ; $[\alpha]_D^{23}$ –31.9 (*c* 1.00, $CHCl_3$).

IR (KBr): 3064, 3034, 2931, 2869, 1708, 1675, 1497, 1454 cm^{-1}.

^1H NMR (500 MHz, DMSO-d_6): δ = 8.17–8.09 (m, 3H), 7.48–7.34 (m, 15H), 7.31–7.19 (m, 5H), 6.89 (brd, 1H), 5.26–5.15 (m, 1H), 5.01–4.94 (m, 1H), 4.89–4.82 (m, 5H), 4.44–4.35 (m, 2H), 4.08–3.99 (m, 1H), 3.84–3.75 (m, 3H), 3.64–3.40 (m, 7H), 1.99 (s, 3H), 1.98 (s, 3H), 1.72–1.44 (m, 12H), 1.36 (s, 9H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 173.2, 173.1, 172.7, 171.1, 168.3, 167.8, 155.8, 137.5, 134.4, 134.3, 134.1, 129.3, 129.25, 129.19, 129.06, 129.02, 129.0, 128.8, 128.7, 128.4, 128.0, 127.8, 79.5 76.5, 76.3, 73.3, 69.2, 54.3, 53.6, 52.6, 49.9, 44.8, 44.3, 43.9, 30.4, 29.9, 28.4, 23.0, 22.7, 22.4, 20.5.

HRMS (ESI): *m/z* [M + Na]$^+$ calcd for $C_{55}H_{71}N_7NaO_{12}$: 1044.5058; found: 1044.5054.

(*R*)-2-Amino-*N*-{(*R*)-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 (14)

A solution of **13** (60.6 mg, 0.0593 mmol) in TFA (0.6 mL, 7.86 mmol) was stirred at r.t. for 30 min. The reaction mixture was concentrated *in vacuo*. The residue was dissolved in 5% $NaHCO_3$ (7 mL) and then extracted with $CHCl_3$ (7 mL x 3). The extract was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography [Silica Gel 60N: $CHCl_3$–MeOH (15:1)] to afford **14** (42.6 mg, 78%) as a colorless oil.

$[\alpha]_D^{23}$ –25.8 (*c* 1.00, $CHCl_3$).

IR (neat): 3231, 2934, 2872, 1652, 1506, 1456 cm^{-1}.

^1H NMR (500 MHz, DMSO-d_6): 8.22 (brd, 1H), 8.14 (brd, 2H), 7.48–7.34 (m, 15H), 7.32–7.18 (m, 5H), 5.25–5.16 (m, 1H), 5.02–4.94 (m, 1H), 4.90–4.80 (m, 5H), 4.44–4.35 (m, 2H), 3.84–3.74 (m, 3H), 3.67–3.44 (m, 7H), 3.23–3.17 (m, 1H), 1.983 (s, 3H), 1.980 (s, 3H), 1.82 (brs, 2H), 1.72–1.50 (m, 11H), 1.39–1.28 (m, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 175.6, 173.7, 172.6, 171.2, 168.2, 167.7, 137.6, 134.4, 134.3, 134.0, 129.4, 129.24, 129.19, 129.0, 128.9, 128.78, 128.76, 128.71, 128.4, 127.9, 127.8, 76.5, 76.3, 73.1, 69.5, 54.4, 54.3, 53.8, 49.3, 44.7, 44.4, 32.1, 30.7, 30.0, 23.2, 22.7, 22.2, 20.51, 20.48.

HRMS (ESI): *m/z* [M + Na]$^+$ calcd for $C_{50}H_{63}N_7NaO_{10}$: 944.4534; found: 944.4532.

(*R*)-2-Acetamido-*N*-{(*R*)-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 (15)

To a solution of **14** (29.5 mg, 0.0320 mmol) in anhydrous CH_2Cl_2 (0.64 mL) was added Ac_2O (6 μL, 0.0640 mmol) at r.t. under argon. The reaction mixture was stirred for 40 min. AcOEt (20 mL) was added to the reaction mixture, and then washed with 5% $NaHCO_3$ (5 mL x 2), H_2O (5 mL), and brine (5 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography [Silica Gel 60N: $CHCl_3$–MeOH (20:1)] to afford **15** (28.7 mg, 93%) as a white solid.

Mp 65–70 °C (white powder, $CHCl_3$–*n*-hexane) ; $[\alpha]_D^{22}$ –34.3 (*c* 1.00, $CHCl_3$).

IR (KBr): 3267, 3213, 3065, 3034, 2934, 2870, 1670, 1535, 1498, 1455 cm^{-1}.

^1H NMR (500 MHz, DMSO-d_6): δ = 8.30 (brd, 1H), 8.139 (d, *J* = 9.9 Hz, 1H), 8.136 (d, *J* = 9.8 Hz, 1H), 7.99 (d, *J* = 8.4 Hz, 1H), 7.47–7.35 (m, 15H), 7.31–7.20 (m, 5H), 5.23–5.14 (m, 1H), 5.02–4.95 (m, 1H), 4.89–4.82 (m, 5H), 4.44–4.36 (m, 3H), 3.83–3.75 (m, 3H), 3.64–3.39 (m, 7H), 1.985 (s, 3H), 1.978 (s, 3H), 1.83 (s, 3H), 1.72–1.42 (m, 12H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 173.7, 173.4, 172.7, 171.4, 170.7, 168.9, 168.4, 137.4, 134.3, 134.2, 129.21, 129.17, 129.1, 129.0, 128.9, 128.8, 128.7, 128.4, 128.0, 127.9, 76.51, 76.46, 76.3, 73.4, 68.7, 54.2, 53.2, 50.4, 44.8, 44.0, 43.4, 30.3, 29.8, 29.6, 23.2, 23.0, 22.9, 22.6, 20.5.

HRMS (ESI): *m/z* [M + Na]$^+$ calcd for $C_{52}H_{65}N_7NaO_{11}$: 986.4640; found: 986.4631.

(*R*)-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 [Erythrochelin (1)]

A mixture of **15** (155 mg, 0.161 mmol) and 10% Pd-C (17.1 mg, 0.0161 mmol) in MeOH (3.2 mL) was stirred at r.t. for 3.5 h under hydrogen. The reaction mixture was filtered and concentrated *in vacuo*. The residue was recrystallized from $CHCl_3$–MeOH to afford erythrochelin (**1**) (44.7 mg, 46%) as a hygroscopic white powder.

$[\alpha]_D^{24}$ –10.3 (*c* 1.00, MeOH).

IR (KBr): 3109, 2930, 2872, 1671, 1536, 1457 cm^{-1}.

^1H NMR (500 MHz, DMSO-d_6): δ = 9.82 (brs, 1H), 9.67 (brs, 1H), 9.66 (brs, 1H), 8.13 (brs, 1H), 8.08 (brs, 1H), 7.96 (d, *J* = 8.3 Hz, 1H), 7.74 (brd, 1H), 4.92–4.85 (m, 1H), 4.78 (brt, 1H), 4.36–4.29 (m, 1H), 3.81 (brs, 2H), 3.67–3.35 (m, 8H), 1.97 (s, 6H), 1.85 (s, 3H), 1.71–1.39 (m, 12H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 171.5, 170.17, 170.15, 169.2, 169.1, 167.9, 167.8, 60.8, 53.7, 53.5, 52.05, 52.03, 47.0, 46.7, 46.6, 30.2, 30.0, 29.4, 23.0, 22.4, 22.0, 21.7, 20.2.

HRMS (ESI): *m/z* [M + Na]$^+$ calcd for $C_{24}H_{41}N_7NaO_{11}$: 626.2762; found: 626.2762.

ESI-MS Analysis of Erythrochelin (1)–Fe(III) Complex

A solution of Fe(III)Cl_3 (0.21 mg) was prepared at 0.7 μM in MeOH and added to an equimolar amount of **1** (0.79 mg). The mixture was further

diluted (1:1000 v/v) in MeOH before it was injected in the ESI source for MS analysis.

ESI-MS Analysis of Erythrochelin (1)–Mg(II) Complex

A solution of Mg(II)Cl_2 (0.09 mg) was prepared at 0.5 μM in MeOH and added to an equimolar amount of **1** (0.56 mg). The mixture was further diluted (1:1000 v/v) in MeOH before it was injected in the ESI source for MS analysis.

Acknowledgment

This work was supported in part by a JSPS KAKENHI Grant (Number 15K18829).

Supporting Information

Is there **Supporting Information** to be published? Click here to indicate YES or NO (text and links will be updated prior to publication).

Primary Data

Is there **Primary Data** to be associated with this manuscript? Click here to indicate YES or NO (text and links will be updated prior to publication).

References

(1) (a) Robbel, L.; Knappe, T. A.; Linne, U.; Xie, X.; Marahiel, M. A. *FEBS J.* **2010**, *277*, 663. (b) Lazos, O.; Tosin, M.; Slusarczyk, A. L.; Boakes, S.; Cortés, J.; Sidebottom, P. J.; Leadlay, P. F. *Chem. Biol.* **2010**, *17*, 160.

(2) (a) Howard, D. H. *Clin. Microbiol. Rev.* **1999**, *12*, 394. (b) Renshaw, J. C.; Robson, G. D.; Trinci, A. P. J.; Wiebe, M. G.; Livens, F. R.; Collison, D.; Taylor, R. J. *Mycol. Res.* **2002**, *106*, 1123. (c) Saha, R.; Saha, N.; Donofrio, R. S.; Bestervelt, L. L. *J. Basic Microbiol.* **2013**, *53*, 303. (d) Raymond, K. N.; Allred, B. E.; Sia, A. K. *Acc. Chem. Res.* **2015**, *48*, 2496.

(3) (a) Xu, W.; Li, L.; Du, L.; Tan, N. *Acta Biochim. Biophys. Sin.* **2011**, *43*, 757. (b) Licona-Cassani, C.; Marcellin, E.; Quek, L.-E.; Jacob, S.; Nielsen, L. K. *Anton. Leeuw.* **2012**, *102*, 493.

(4) Oves-Costales, D.; Challis, G. L. In *Drug Discovery from Natural Products, RSC Drug Discovery Series No. 25*; Genilloud, O.; Vicente, F. Ed.; The Royal Society of Chemistry: Cambridge, **2012**, 145.

(5) Robbel, L.; Helmetag, V.; Knappe, T. A.; Marahiel, M. A. *Biochemistry* **2011**, *50*, 6073.

(6) (a) Umezawa, H.; Aoyagi, T.; Ogawa, K.; Obata, T.; Iinuma, H.; Naganawa, H.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1985**, *38*, 1813. (b) Aoyagi, T.; Wada, T.; Iinuma, H.; Ogawa, K.; Kojima, F.; Nagai, M.; Kuroda, H.; Obayashi, A.; Umezawa, H. *J. Appl. Biochem.* **1985**, *7*, 388.

(7) Dolence, E. K.; Miller, M. J. *J. Org. Chem.* **1991**, *56*, 492.

(8) (a) Ahmad, M. Ph.D. thesis, University of Warwick, Coventry, UK (2011). (b) Kodani, S.; Komaki, H.; Suzuki, M.; Kobayakawa, F.; Hemmi, H. *Biometals* **2015**, *28*, 791.

(9) Chen, Y.; Ntai, I.; Ju, K.-S.; Unger, M.; Zamdborg, L.; Robinson, S. J.; Doroghazi, J. R.; Labeda, D. P.; Metcalf, W. W.; Kelleher, N. L. *J. Proteome Res.* **2012**, *11*, 85.

(10) Atkin, C. L.; Neilands, J. B. *Biochemistry* **1968**, *7*, 3734.

(11) (a) Isowa, Y.; Takashima, T.; Ohmori, M.; Kurita, H.; Sato, M.; Mori, K. *Bull. Chem. Soc. Jpn.* **1972**, *45*, 1467. (b) Fujii, T.; Hatanaka, Y. *Tetrahedron* **1973**, *29*, 3825. (c) Widmer, J.; Keller-Schierlein, W. *Helv. Chim. Acta* **1974**, *57*, 1904. (d) Lee, B. H.; Gerfen, G. J.; Miller, M. J. *J. Org. Chem.* **1984**, *49*, 2418. (e) Nakao, M.; Fukayama, S.; Kitaike, S.; Sano, S. *Heterocycles* **2015**, *90*, 1309.

(12) Frederick, C. B.; Szaniszlo, P. J.; Vickrey, P. E.; Bentley, M. D.; Shive, W. *Biochemistry* **1981**, *20*, 2432.

(13) Frederick, C. B.; Bentley, M. D.; Shive, W. *Biochem. Biophys. Res. Commun.* **1982**, *105*, 133.

(14) (a) Burt, W. R. *Infect. Immun.* **1982**, *35*, 990. (b) Aniya, Y.; Ohtani, I. I.; Higa, T.; Miyagi, C.; Gibo, H.; Shimabukuro, M.; Nakanishi, H.; Taira, J. *Free Radical Biol. Med.* **2000**, *28*, 999. (c) Bertrand, S.; Larcher, G.; Landreau, A.; Richomme, P.; Duval, O.; Bouchara, J.-P. *Biometals* **2009**, *22*, 1019. (d) Tseng, W.-T.; Hsu, Y.-W.; Pan, T.-M. *Pharm. Biol.* **2016**, in press.

(15) (a) Hantke, K. *Mol. Gen. Gent.* **1983**, *191*, 301. (b) Simionato, A. V. C.; de Souza, G. D.; Rodrigues-Filho, E.; Glick, J.; Vouros, P.; Carrilho, E. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 193.

(16) Bertrand, S.; Bouchara, J.-P.; Venier, M.-C.; Richomme, P.; Duval, O.; Larcher, G. *Med. Mycol.* **2010**, *48*, S98.

(17) (a) Borthwick, A. D. *Chem. Rev.* **2012**, *112*, 3641. (b) Giessen, T. W.; Marahiel, M. A. *Front. Microbiol.* **2015**, *6*, 785. (c) Sano, S.; Nakao, M. *Heterocycles*, **2015**, *91*, 1349.

(18) (a) Spasojević, I.; Boukhalfa, H.; Stevens, R. D.; Crumbliss, A. L. *Inorg. Chem.* **2001**, *40*, 49. (b) Nguyen-van-Duong, M. K.; Guillot, V.; Nicolas, L.; Gaudemer, A.; Lowry, L.; Spasojević, I.; Crumbliss, A. L. *Inorg. Chem.* **2001**, *40*, 5948. (c) Dell'mour, M.; Koellensperger, G.; Quirino, J. P.; Haddad, P. R.; Stanetty, C.; Oburger, E.; Puschenreiter, M.; Hann, S. *Electrophoresis* **2010**, *31*, 1201. (d) Dimkpa, C. *Endocytobiosis Cell Res.* **2016**, *27*, 7. (e) Pluháček, T.; Lemr, K.; Ghosh, D.; Milde, D.; Novák, J.; Havlíček, V. *Mass Spectrom. Rev.* **2016**, *35*, 35.

(19) The ESI-MS spectrum is shown in Supporting Information.

Supporting Information

for

Synthesis of Erythrochelin: A Hydroxamate-type Siderophore from *Saccharopolyspora erythraea*

Michiyasu Nakao, Shunsuke Tsuji, Syuji Kitaike, and Shigeki Sano*

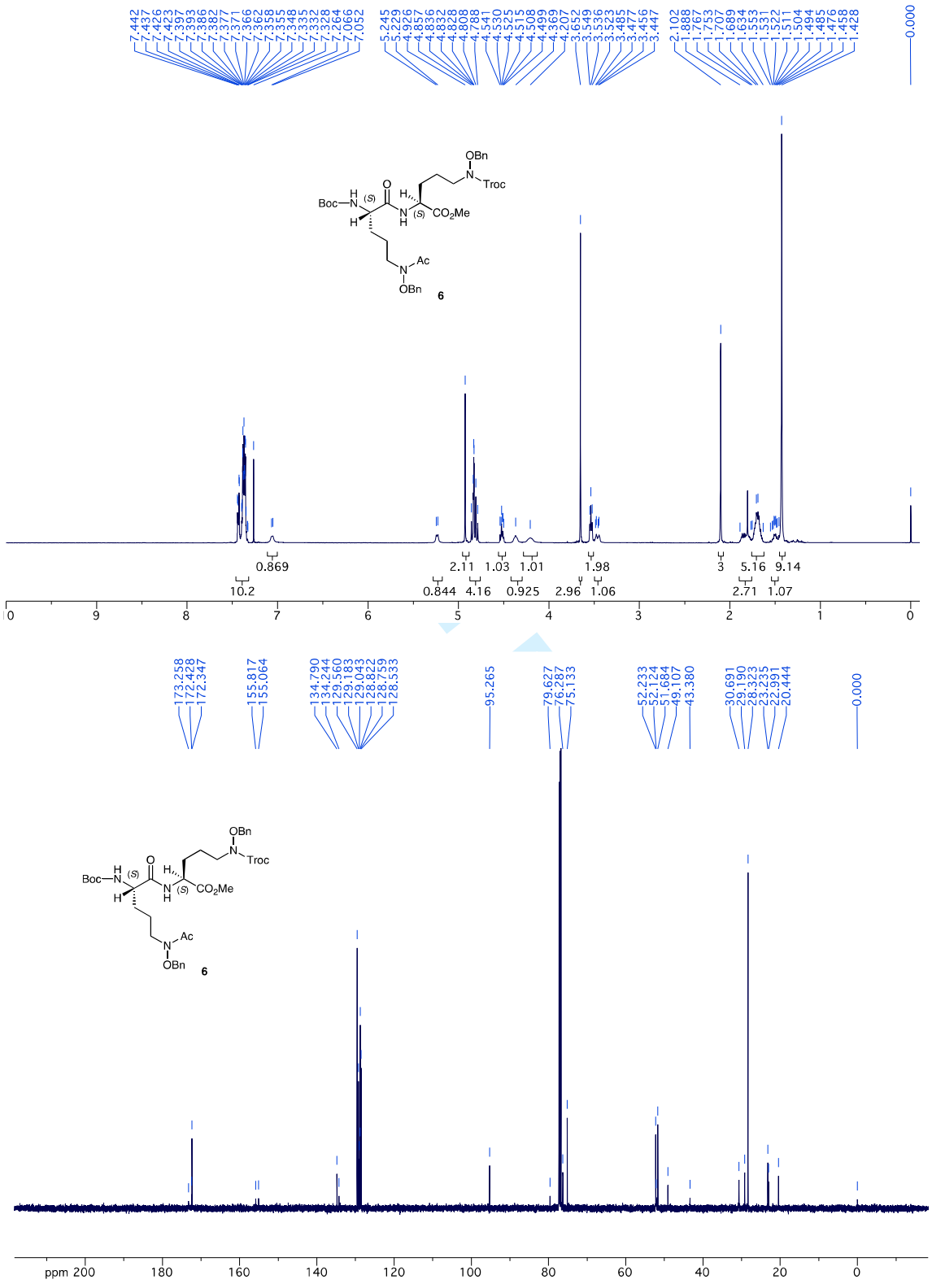
Graduate School of Pharmaceutical Sciences, Tokushima University

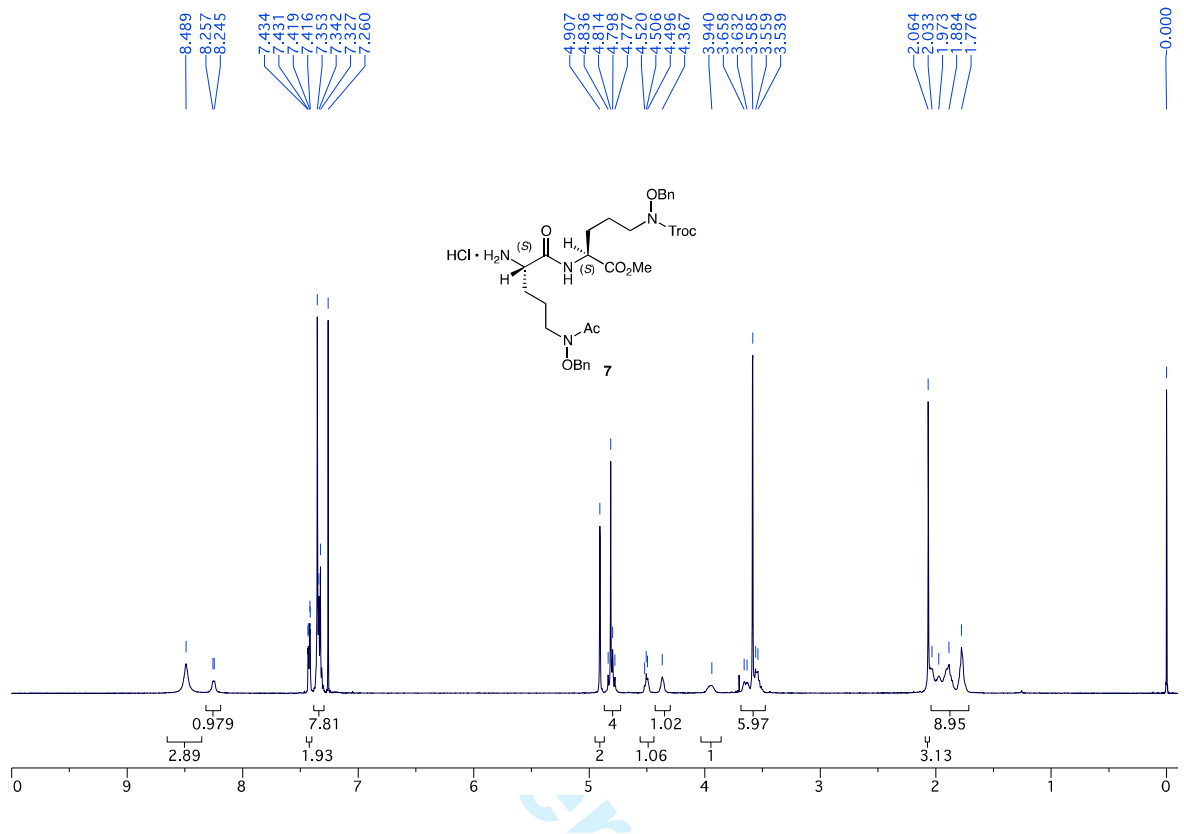
Sho-machi, Tokushima 770-8505, Japan

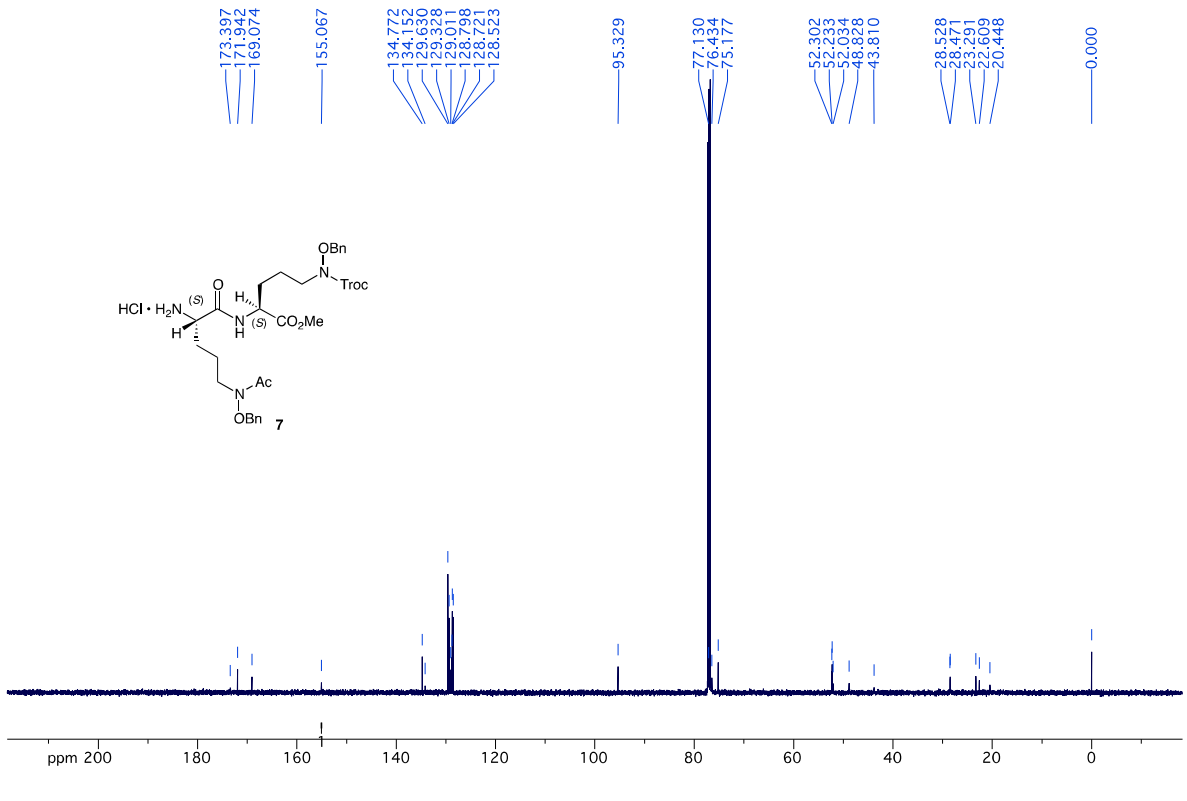
1. ^{1}H and ^{13}C NMR spectra

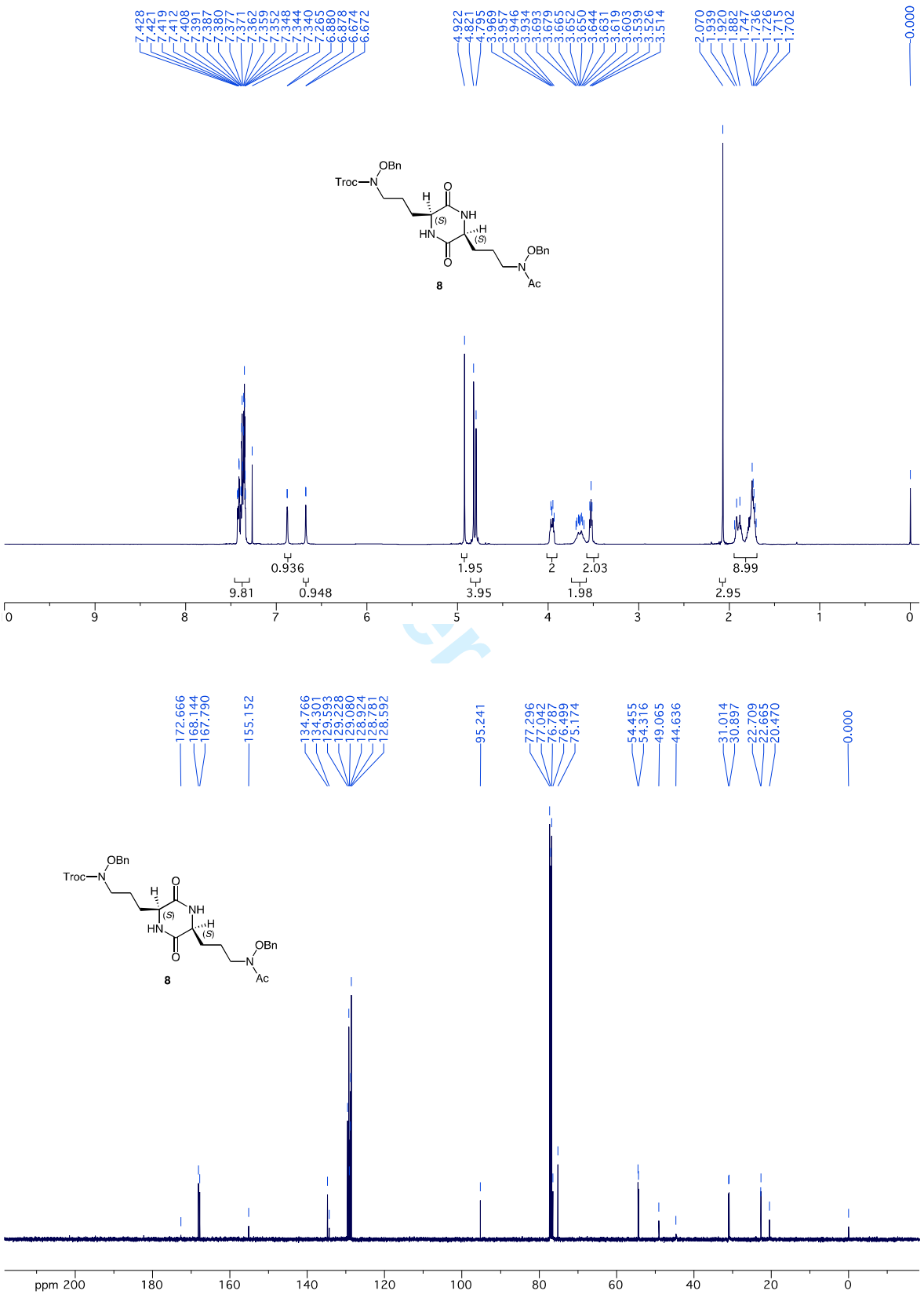
2. Electrospray ionization mass spectra

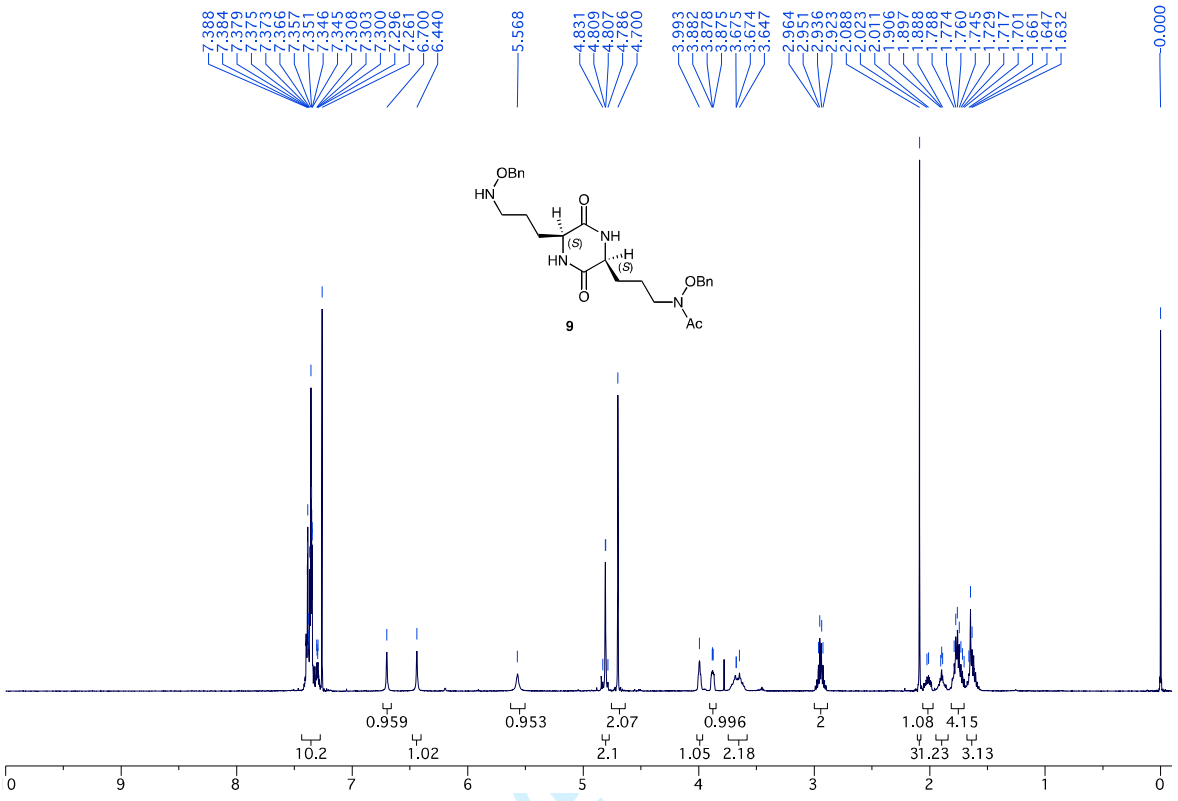
1. ^{1}H and ^{13}C NMR spectra

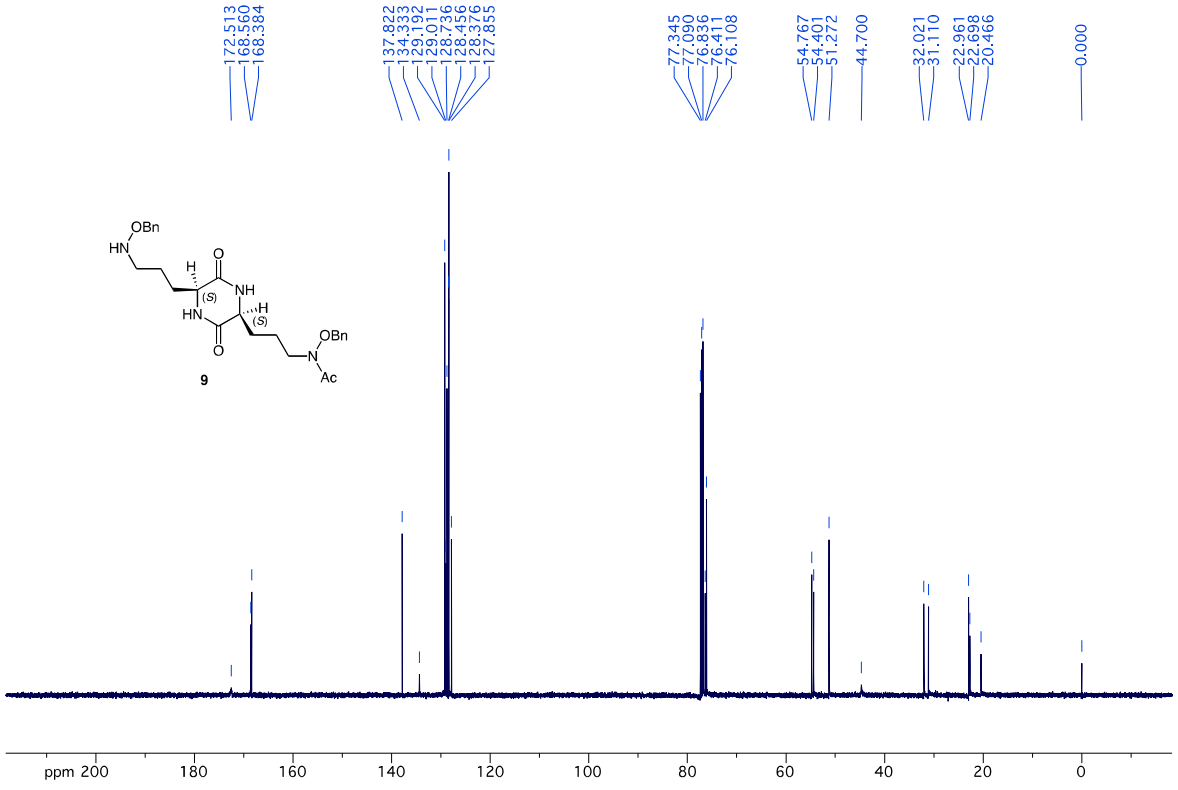


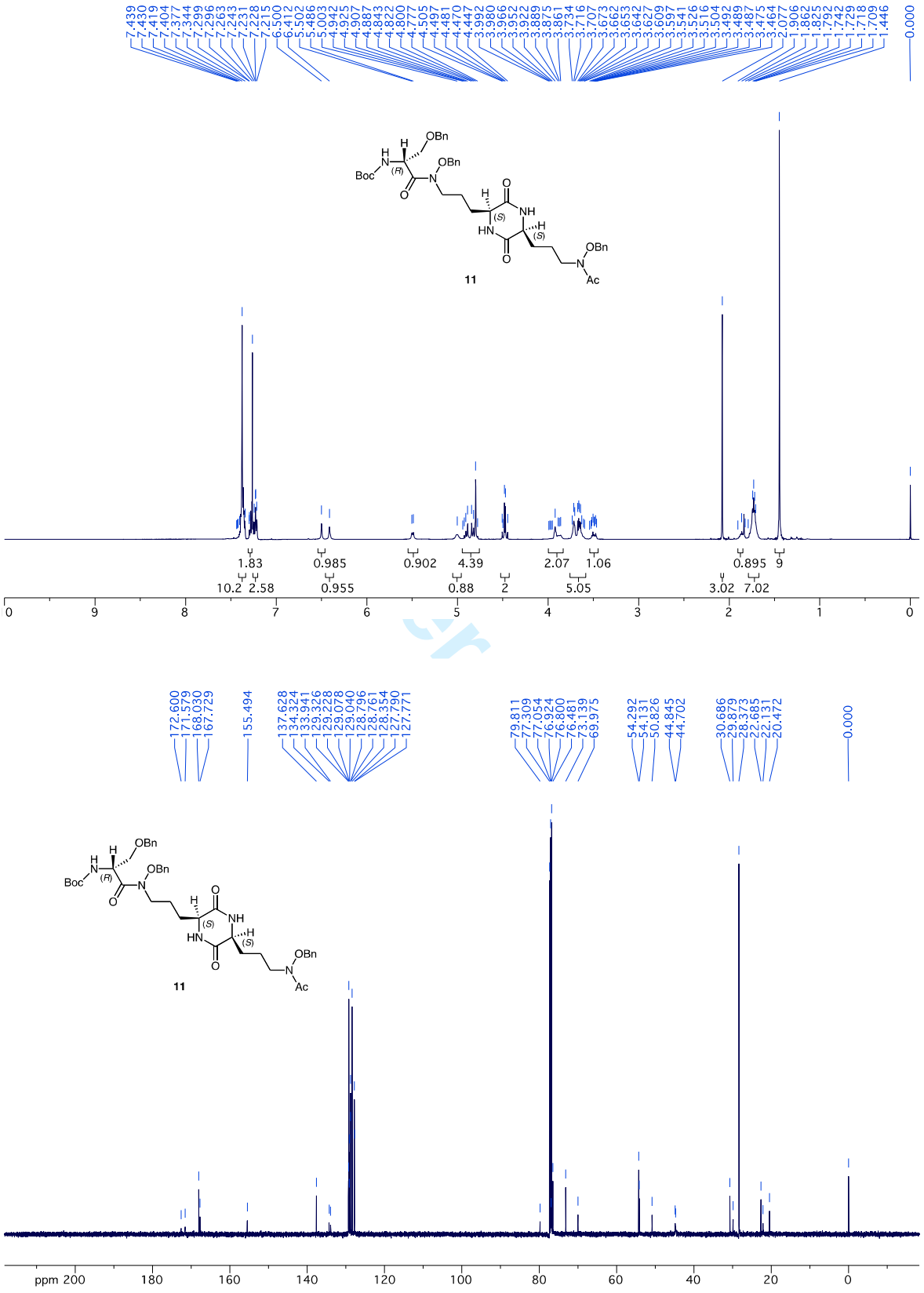

8.489
8.257
8.245
7.434
7.431
7.419
7.416
7.353
7.342
7.327
7.260
4.907
4.836
4.814
4.798
4.777
4.520
4.506
4.496
4.367
3.940
3.658
3.632
3.585
3.559
3.539
2.064
2.033
1.973
1.884
1.776
0.000
HCl • H2N
OBn
N
Troc
CO2Me
Ac
7
0.979
7.81
4
1.02
5.97
8.95
2.89
1.93
2
1.06
1
3.13

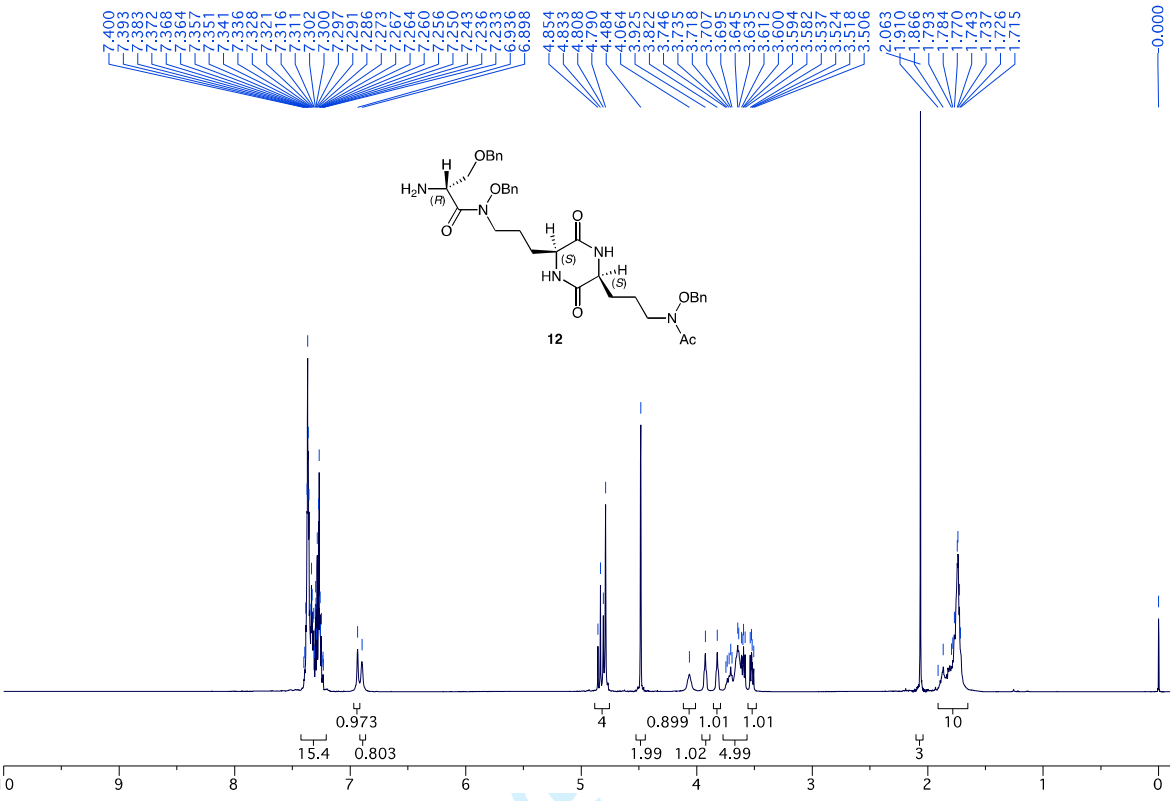

173.397
171.942
169.074
155.067
134.772
134.152
129.630
129.328
129.011
128.798
128.721
128.523
95.329
77.130
76.434
75.177
52.302
52.233
52.034
48.828
43.810
28.528
28.471
23.291
22.609
20.448
0.000
HCl • H2N
OBn
N
Troc
CO2Me
Ac
7
ppm 200

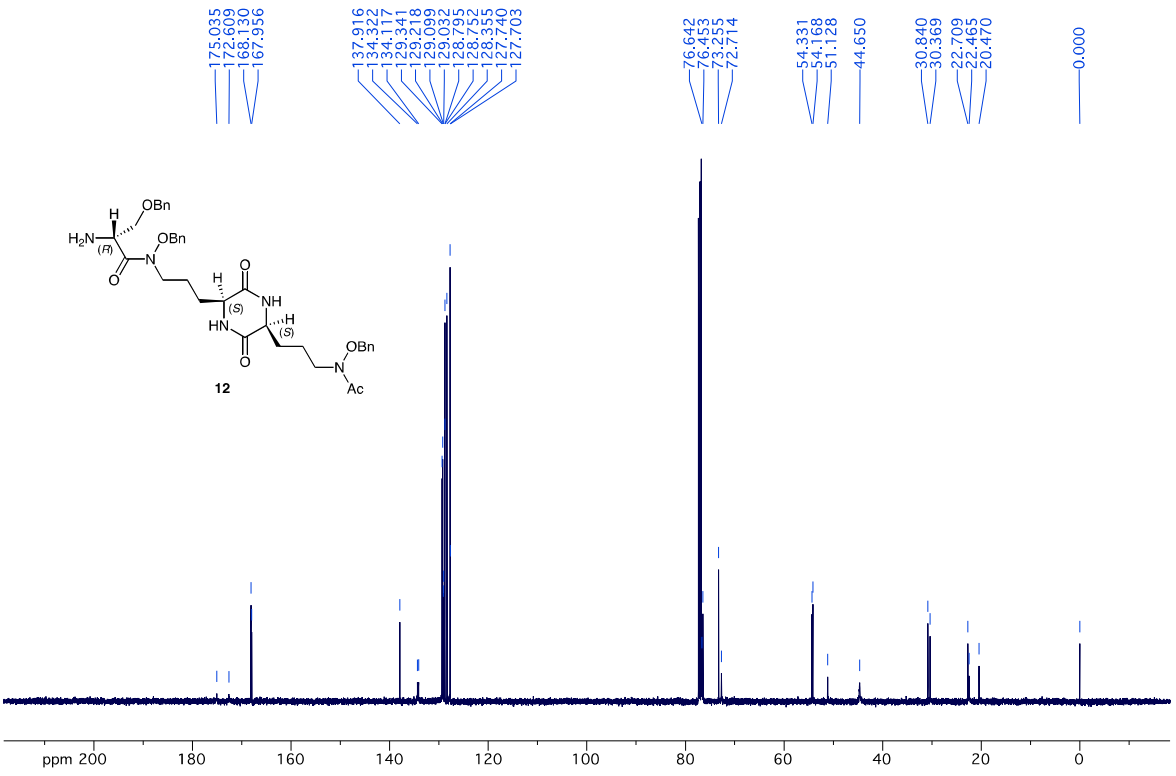

7.428
7.421
7.419
7.412
7.408
7.391
7.387
7.380
7.377
7.371
7.362
7.359
7.352
7.348
7.344
7.340
7.265
6.880
6.878
6.674
6.672
4.922
4.821
4.755
3.969
3.957
3.946
3.934
3.693
3.679
3.665
3.652
3.650
3.644
3.631
3.619
3.603
3.539
3.526
3.514
2.070
1.939
1.920
1.882
1.747
1.736
1.726
1.715
1.702
0.000
Troc
OBn
N
H
O
NH
HN
(S)
(S)
OBn
N
Ac
8
0.936
1.95
2
2.03
8.99
9.81
0.948
3.95
1.98
2.95
0
9
8
7
6
5
4
3
2
1
0
172.666
168.144
167.790
155.152
134.766
134.301
134.093
129.293
129.268
129.080
128.924
128.781
128.592
95.241
77.296
77.042
76.787
76.499
75.174
54.455
54.316
49.065
44.636
31.014
30.897
22.709
22.665
20.470
0.000
Troc
OBn
N
H
O
NH
HN
(S)
(S)
OBn
N
Ac
8
ppm 200
180
160
140
120
100
80
60
40
20
0

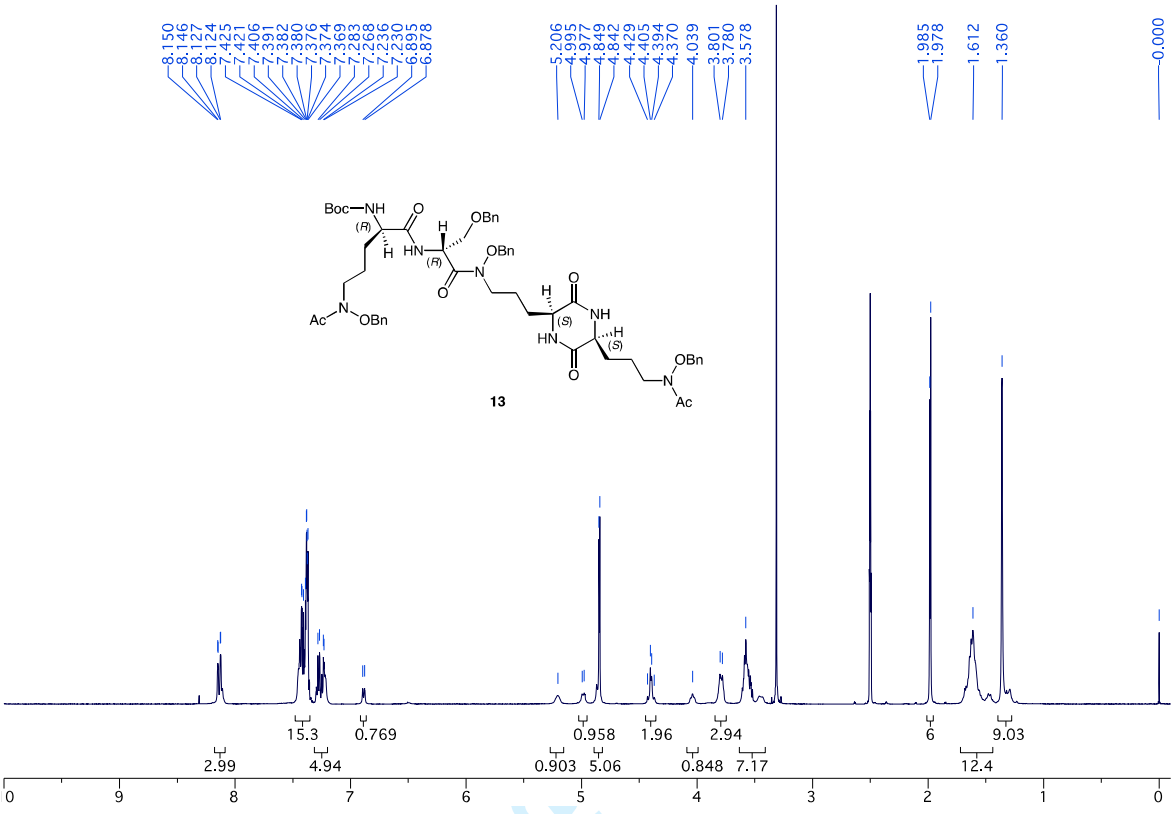

7.388
7.384
7.379
7.375
7.373
7.366
7.357
7.351
7.346
7.345
7.308
7.303
7.300
7.296
7.261
6.700
6.440
5.568
4.831
4.809
4.807
4.786
4.700
3.993
3.882
3.878
3.875
3.675
3.674
3.647
2.964
2.951
2.936
2.923
2.088
2.023
2.011
1.906
1.897
1.888
1.788
1.774
1.760
1.745
1.729
1.717
1.701
1.661
1.647
1.632
0.000
OBn
HN
H
(S)
O
NH
HN
H
(S)
O
OBn
N
Ac
9
0.959
0.953
2.07
0.996
2
1.08
4.15
10.2
1.02
2.1
1.05
2.18
31.23
3.13
0
9
8
7
6
5
4
3
2
1
0

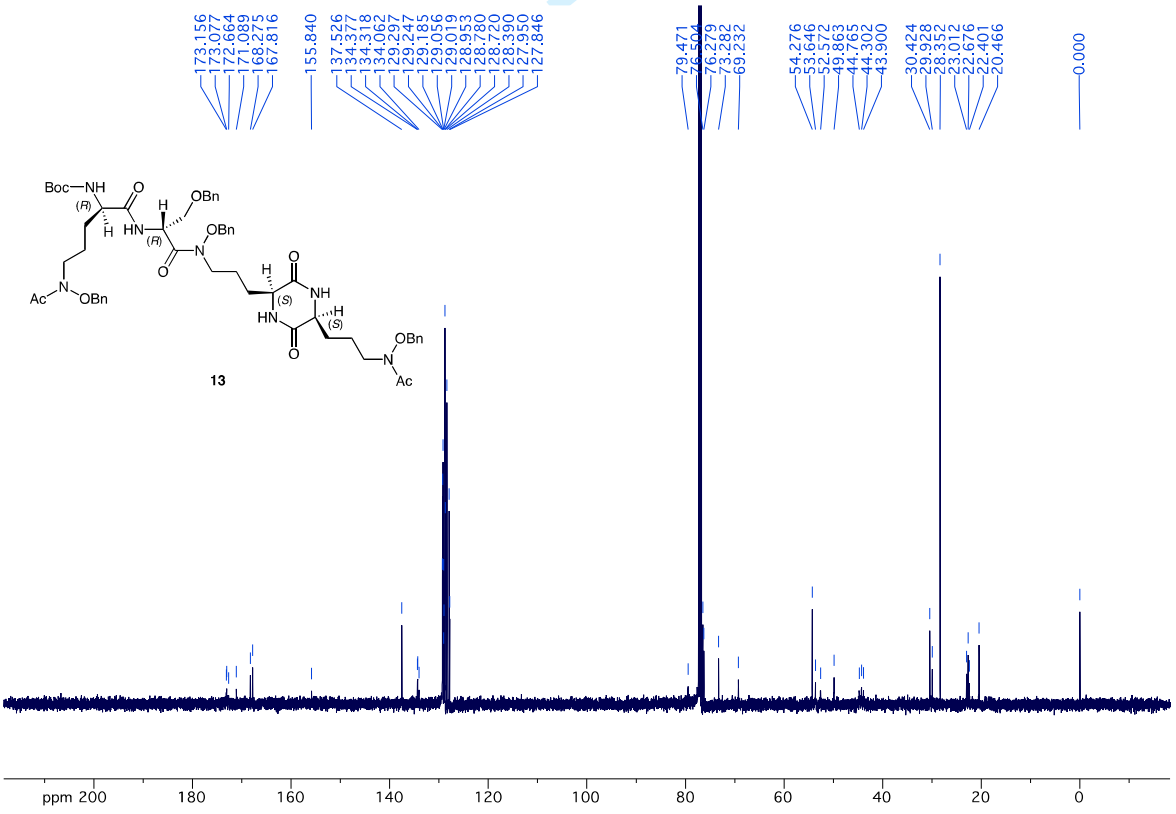

172.513
168.560
168.384
137.822
134.333
134.132
129.131
129.071
128.736
128.456
128.376
127.855
77.345
77.090
76.836
76.411
76.108
54.767
54.401
51.272
44.700
32.021
31.110
22.961
22.698
20.466
0.000
OBn
HN
H
(S)
O
NH
HN
H
(S)
O
OBn
N
Ac
9
ppm 200
180
160
140
120
100
80
60
40
20
0

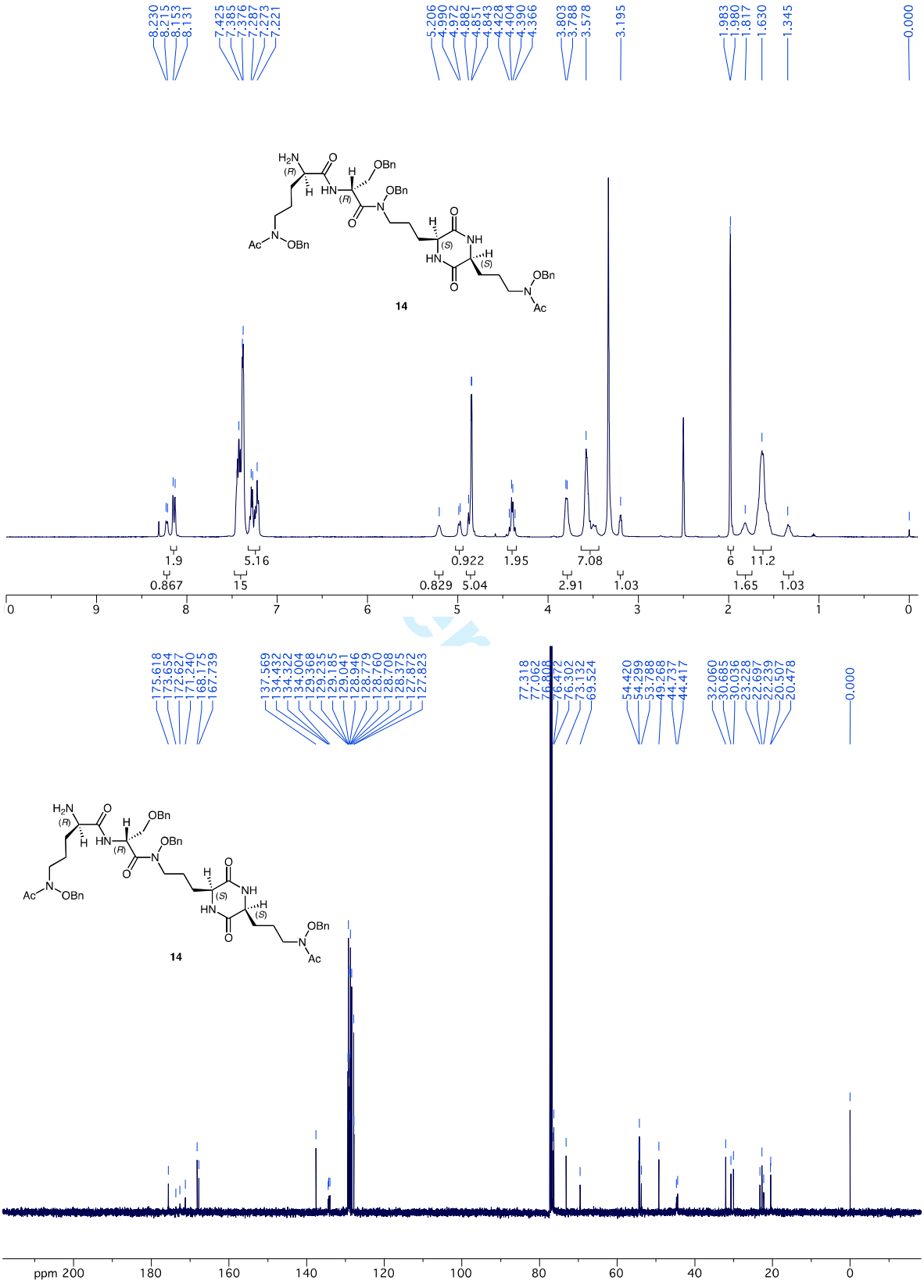

7.439
7.430
7.419
7.404
7.377
7.344
7.299
7.296
7.263
7.243
7.231
7.228
7.215
6.500
6.412
5.502
5.486
5.003
4.942
4.925
4.907
4.887
4.843
4.822
4.800
4.777
4.505
4.497
4.481
4.470
4.447
3.992
3.980
3.966
3.952
3.922
3.889
3.875
3.861
3.734
3.716
3.707
3.673
3.662
3.653
3.642
3.627
3.609
3.597
3.541
3.526
3.516
3.504
3.492
3.489
3.487
3.475
3.464
2.077
1.906
1.862
1.825
1.792
1.742
1.729
1.718
1.709
1.446
0.000
Boc
OBn
OBn
11
1.83
0.985
0.902
4.39
2.07
1.06
0.895
9
10.2
2.58
0.955
0.88
2
5.05
3.02
7.02
172.600
171.379
168.030
167.729
155.494
137.628
134.324
133.941
133.326
129.628
129.040
128.796
128.761
128.554
127.790
127.771
79.811
77.309
77.034
76.924
76.800
76.481
73.139
69.975
54.292
54.131
50.826
44.845
44.702
30.686
29.879
28.373
22.685
22.131
20.472
0.000
ppm

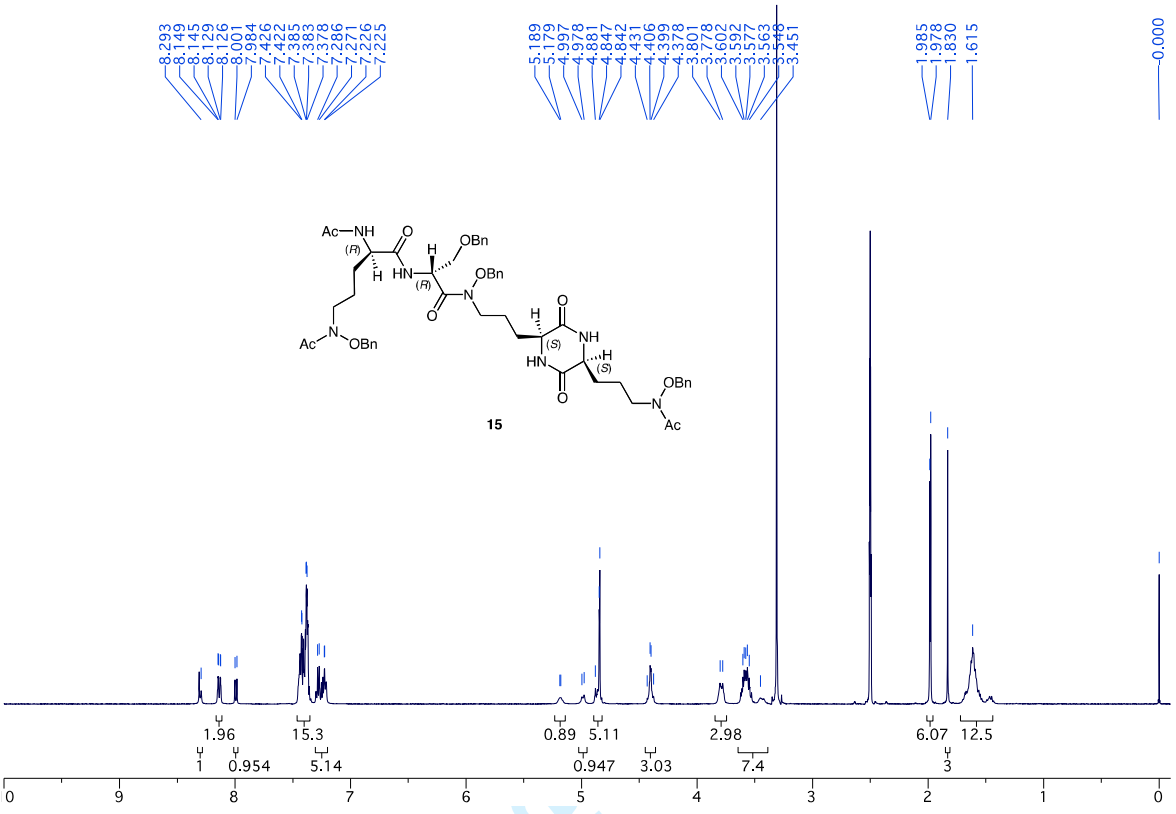

12
0.973
4
0.899
1.01
1.01
10
15.4
0.803
1.99
1.02
4.99
3

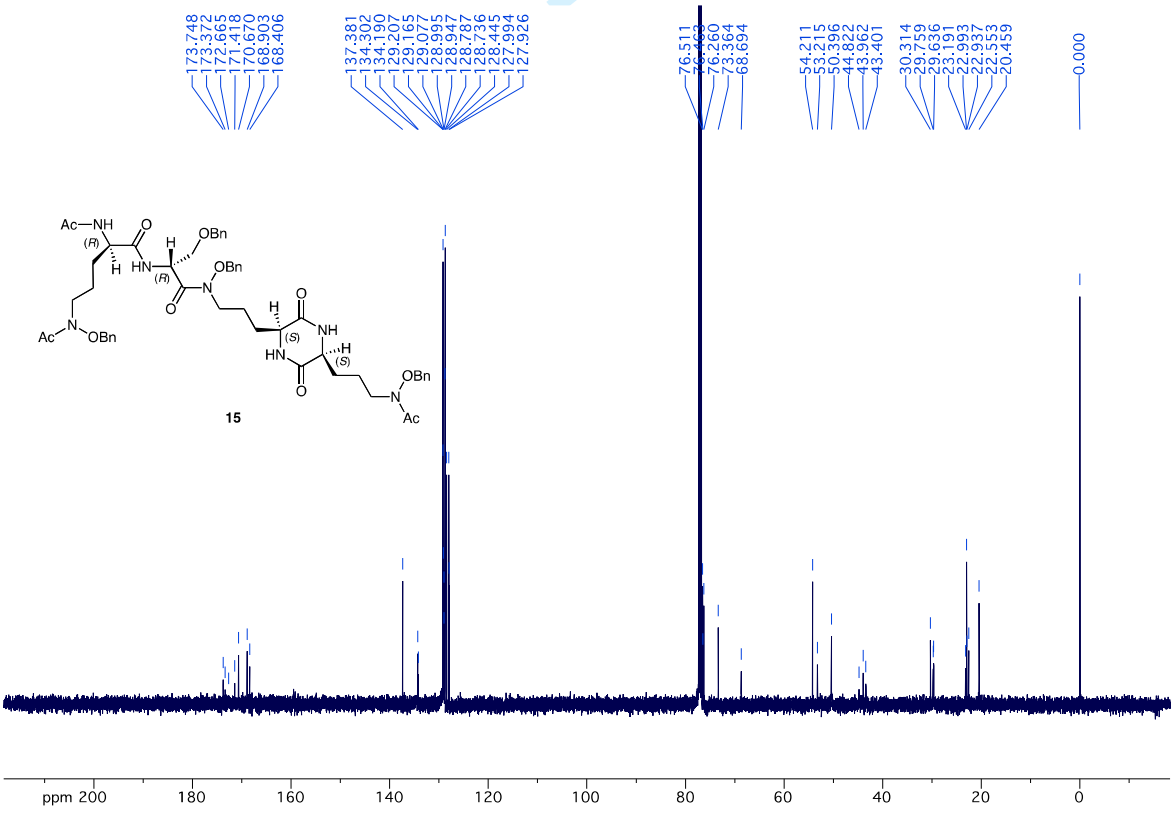

12
ppm 200
180
160
140
120
100
80
60
40
20
0

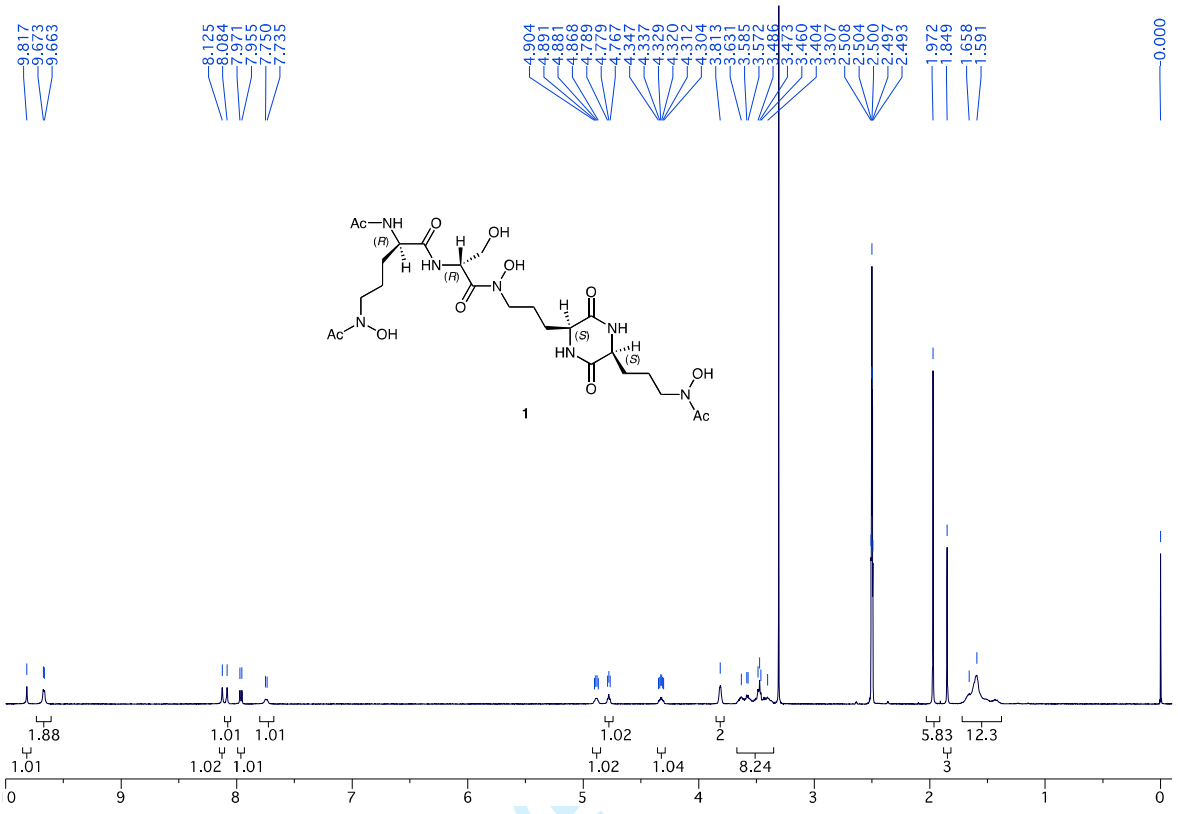

8.150
8.146
8.127
8.124
7.425
7.421
7.406
7.391
7.382
7.380
7.376
7.374
7.369
7.283
7.268
7.236
7.230
6.895
6.878
5.206
4.995
4.977
4.849
4.842
4.429
4.405
4.394
4.370
4.039
3.801
3.780
3.578
1.985
1.978
1.612
1.360
0.000
13
15.3
0.769
0.958
1.96
2.94
6
9.03
2.99
4.94
0.903
5.06
0.848
7.17
12.4

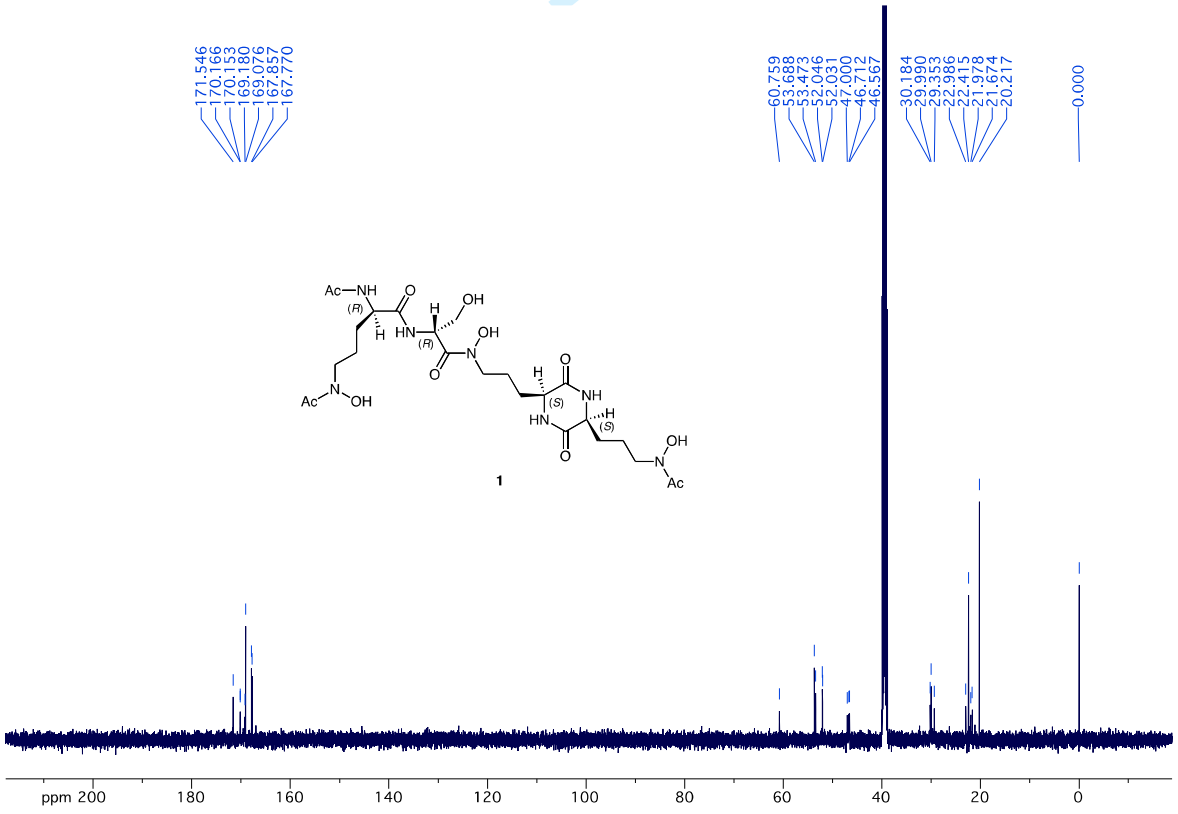

173.156
173.077
172.664
171.089
168.275
167.816
155.840
137.526
134.377
134.318
134.062
129.297
129.247
129.185
129.056
129.019
128.953
128.780
128.720
128.390
127.950
127.846
79.471
76.504
76.279
73.282
69.232
54.276
53.646
52.572
49.863
44.765
44.302
43.900
30.424
29.928
28.352
23.012
22.676
22.401
20.466
0.000
13
ppm 200
180
160
140
120
100
80
60
40
20
0


8.230
8.215
8.153
8.131
7.425
7.385
7.376
7.287
7.273
7.221
5.206
4.990
4.972
4.882
4.851
4.843
4.428
4.404
4.390
4.366
3.803
3.788
3.578
3.195
1.983
1.980
1.817
1.630
1.345
0.000
14
1.9
5.16
0.922
1.95
7.08
6
11.2
0.867
15
0.829
5.04
2.91
1.03
1.65
1.03
175.618
173.654
172.627
171.240
168.175
167.739
137.569
134.432
134.322
134.004
129.368
129.235
129.185
129.041
128.946
128.779
128.760
128.708
128.375
127.872
127.823
77.318
77.063
76.808
76.472
76.302
73.132
69.524
54.420
54.299
53.788
49.268
44.737
44.417
32.060
30.685
30.036
23.228
22.697
22.239
20.507
20.478
0.000
14
ppm 200
180
160
140
120
100
80
60
40
20
0


8.293
8.149
8.145
8.129
8.126
8.001
7.984
7.426
7.422
7.385
7.383
7.378
7.286
7.271
7.226
7.225
5.189
5.179
4.997
4.978
4.881
4.847
4.842
4.431
4.406
4.399
4.378
3.801
3.778
3.602
3.592
3.577
3.563
3.548
3.451
1.985
1.978
1.830
1.615
0.000
15
1.96
15.3
0.89
5.11
2.98
6.07
12.5
1
0.954
5.14
0.947
3.03
7.4
3
0
9
8
7
6
5
4
3
2
1
0


173.748
173.372
172.665
171.418
170.670
168.903
168.406
137.381
134.302
134.190
129.207
129.165
129.077
128.995
128.947
128.787
128.736
128.445
127.994
127.926
76.511
76.468
76.260
73.364
68.694
54.211
53.215
50.396
44.822
43.962
43.401
30.314
29.759
29.636
23.191
22.993
22.937
22.553
20.459
0.000
15
ppm 200
180
160
140
120
100
80
60
40
20
0


9.817
9.673
9.663
8.125
8.094
7.971
7.955
7.750
7.735
4.904
4.881
4.881
4.888
4.789
4.779
4.767
4.347
4.337
4.329
4.320
4.312
4.304
3.813
3.631
3.585
3.572
3.486
3.473
3.460
3.404
3.307
2.508
2.504
2.500
2.497
2.493
1.972
1.849
1.658
1.591
0.000
1
1.88
1.01
1.01
1.02
1.01
1.01
1.02
1.04
2
8.24
5.83
3
12.3
0
9
8
7
6
5
4
3
2
1
0


171.546
170.166
170.153
169.180
169.076
167.857
167.770
60.759
53.688
53.473
52.046
52.031
47.000
46.712
46.567
30.184
29.990
29.353
22.386
22.475
21.978
21.674
20.217
0.000
1
ppm 200
180
160
140
120
100
80
60
40
20
0

2. Electrospray ionization mass spectra