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

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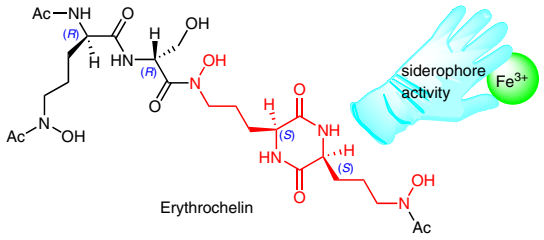

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Abstract Erythrochelin, a hydroxamate-type siderophore produced by *Saccharopolyspora erythraea*, is synthesized for the first time. A key building block of erythrochelin containing the 2,5-diketopiperazine ring is prepared by intramolecular cyclization 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.

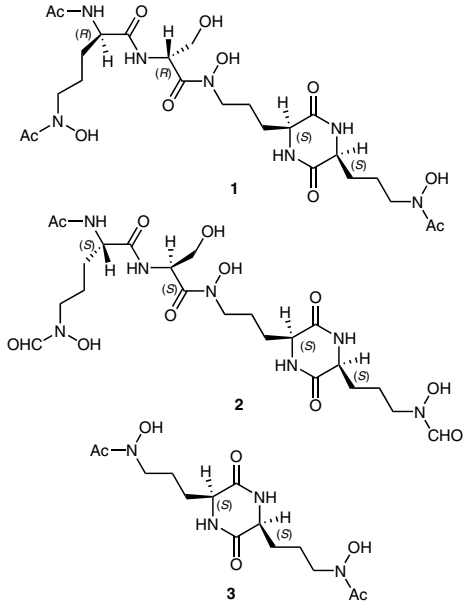

Figure 1 Chemical structures of erythrochelin (**1**), foroxymithine (**2**), and rhodotorulic acid (**3**)

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 bio-

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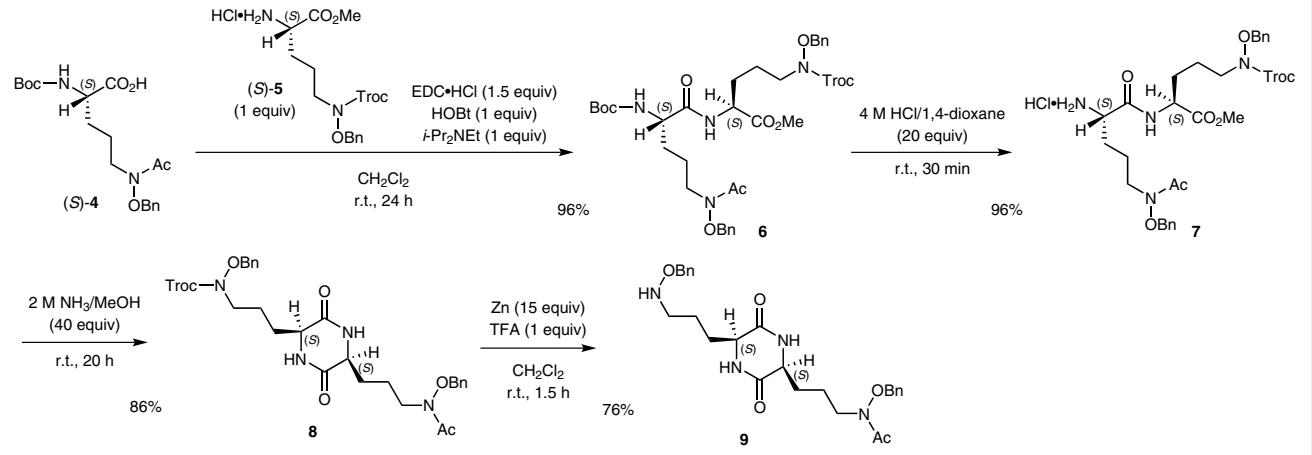
synthetic mechanism of **2** was predicted based on the NRPS domain organization.[9] In addition, rhodotorulic acid (**3**),[10] a structurally related hydroxamate-type siderophore isolated from *Rhodotorula pilimanae*, was synthesized by several groups, including ours.[11] A series of siderophores – triornicin,[12] isotriornicin,[13] dimerumic acid,[14] coprogen,[15] coprogen B,[14a] and α-*N*-methylcoprogen[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, the preparation of the key building block **9** containing the 2,5-DKP ring was investigated 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. A one-pot conversion of **6** into 2,5-DKP **8** was then tried 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, a stepwise construction of **8** was investigated. Deprotection of the Boc group of **6** with an excess amount of 4 M HCl in 1,4-dioxane afforded the dipeptide methyl ester hydrochloride **7** in 96% yield. Intramolecular cyclization of **7** on treatment with ammonia solution (2 M in MeOH) 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.

The condensation of 2,5-DKP **9** was attempted 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 equivalents 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, a 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 – 3 H) + Fe(III) + Na]$^+$ (calcd: *m*/*z* of 679.1876) in the presence of 1 equivalent of iron(III) chloride.[19] Furthermore, a similar 1:1 complex with Mg(II) was indicated from *m*/*z* of 648.2449 [(M – 2 H) + Mg(II) + Na]$^+$ (calcd: *m*/*z* of 648.2456) in the ESI-MS analysis with 1 equivalent 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 with 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



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

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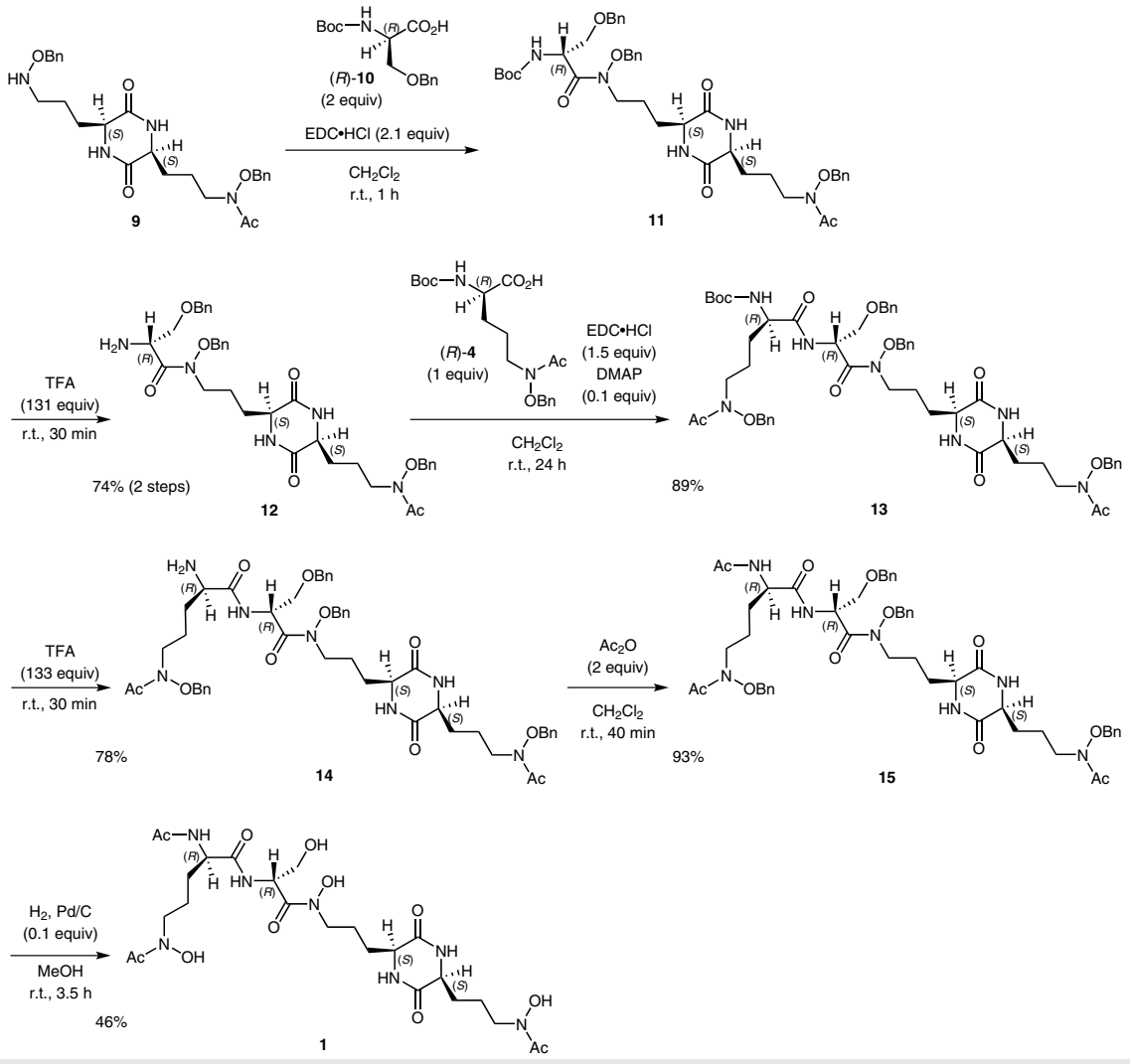

Scheme 2 Synthesis of erythrochelin (**1**)

their structurally related derivatives with various metal chelating abilities.

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 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. Optical rotations were recorded on JASCO digital polarimeter P-2200. 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)]. Anhyd 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 anhyd CH_2Cl_2 (6.5 mL) were added HOBt (177 mg, 1.31 mmol), *i*-Pr_2NEt (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. EtOAc (40 mL) was added to the reaction mixture, and the organic layer was washed with aq 5% citric acid solution (10 mL), aq 1 M HCl (10 mL), H_2O (10 mL), and brine (10 mL). The organic layer was dried (anhyd $MgSO_4$), filtered, and concentrated in vacuo. The oily residue was purified by column chromatography [silica gel 60 N: $CHCl_3$–MeOH (50:1)] to afford **6** (987 mg, 96%) as a colorless oil; $[\alpha]_D^{20}$ +5.1 (*c* 1.00, $CHCl_3$).

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

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

^{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 $C_{35}H_{47}Cl_3N_4O_{10}Na$: 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 aq 4 M HCl in 1,4-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 hygroscopic 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 (br s, 3 H), 8.26 (br d, 1 H), 7.44–7.40 (m, 2 H), 7.38–7.30 (m, 8 H), 4.91 (s, 2 H), 4.86–4.75 (m, 4 H), 4.54–4.46 (m, 1 H), 4.37 (br s, 1 H), 4.02–3.87 (m, 1 H), 3.69–3.47 (m, 3 H), 3.59 (s, 3 H), 2.06 (s, 3 H), 2.10–1.72 (m, 8 H).

^{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 $C_{30}H_{39}Cl_3N_4O_8Na$: 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 2 M 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, 10 H), 6.88 (d, *J* = 1.0 Hz, 1 H), 6.67 (d, *J* = 1.2 Hz, 1 H), 4.92 (s, 2 H), 4.82 (s, 2 H), 4.80 (s, 2 H), 4.00–3.91 (m, 2 H), 3.73–3.58 (m, 2 H), 3.57–3.45 (m, 2 H), 2.07 (s, 3 H), 1.95–1.68 (m, 8 H).

^{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 $C_{29}H_{35}Cl_3N_4O_7Na$: 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 Zn (1.73 g, 26.4 mmol) in anhyd 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 mixture was filtered with $CHCl_3$ and aq 5% $NaHCO_3$ (20 mL) was added to the filtrate, and then extracted with $CHCl_3$ (3 × 30 mL). The extract was dried (anhyd $MgSO_4$), 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, 10 H), 6.70 (br s, 1 H), 6.44 (br s, 1 H), 5.57 (br s, 1 H), 4.84–4.78 (m, 2 H), 4.70 (s, 2 H), 4.02–3.97 (m, 1 H), 3.91–3.85 (m, 1 H), 3.74–3.58 (m, 2 H), 3.00–2.89 (m, 2 H), 2.09 (s, 3 H), 2.06–1.98 (m, 1 H), 1.94–1.86 (m, 1 H), 1.83–1.56 (m, 6 H).

^{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 anhyd 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. EtOAc (60 mL) was added to the mixture, and the organic layer was washed with aq 1 M HCl (20 mL), H_2O (10 mL), aq 5% $NaHCO_3$ (20 mL), and brine (20 mL). The organic layer was dried (anhyd $MgSO_4$), 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, 10 H), 7.31–7.20 (m, 5 H), 6.50 (br s, 1 H), 6.41 (br s, 1 H), 5.49 (d, *J* = 8.4 Hz, 1 H), 5.00 (br s, 1 H), 4.94–4.76 (m, 4 H), 4.52–4.44 (m, 2 H), 4.00–3.82 (m, 2 H), 3.76–3.58 (m, 5 H), 3.54–3.45 (m, 1 H), 2.08 (s, 3 H), 1.91–1.83 (m, 1 H), 1.79–1.67 (m, 7 H), 1.45 (s, 9 H).

^{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 aq 5% $NaHCO_3$ (40 mL) and then extracted with $CHCl_3$ (3 × 25 mL). The extract was dried (anhyd $MgSO_4$), 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}.

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

^{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 $C_{36}H_{45}N_5O_7Na$: 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 anhyd 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. EtOAc (20 mL) was added to the mixture, and the organic layer was washed with aq 1 M HCl (5 mL), H_2O (5 mL), aq 5% $NaHCO_3$ (5 mL), and brine (5 mL). The organic layer was dried (anhyd $MgSO_4$), 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}.

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

^{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 $C_{55}H_{71}N_7O_{12}Na$: 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 aq 5% $NaHCO_3$ (7 mL) and then extracted with $CHCl_3$ (3 × 7 mL). The combined organic extracts were dried (anhyd $MgSO_4$), 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}.

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

^{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 $C_{50}H_{63}N_7O_{10}Na$: 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 anhyd 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. EtOAc (20 mL) was added to the reaction mixture, and then washed with aq 5% $NaHCO_3$ (2 × 5 mL), H_2O (5 mL), and brine (5 mL). The organic layer was dried (anhyd $MgSO_4$), 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}.

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

^{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 $C_{52}H_{65}N_7O_{11}Na$: 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 H_2 atmosphere. 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}.

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

^{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 $C_{24}H_{41}N_7O_{11}Na$: 626.2762; found: 626.2762.

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

A solution of $FeCl_3$ (0.21 mg) was prepared at a concentration of 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 $MgCl_2$ (0.09 mg) was prepared at a concentration of 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.

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Supporting Information

Supporting information for this article is available online at http://dx.doi.org/10.1055/s-0035-1560564.

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(19) For the ESI-MS spectra of the complexes, see the Supporting Information.

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