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Synthesis and application of an N^{δ}-acetyl-N^{δ}-hydroxyornithine analog: Identification of novel metal complexes of deferriferrichrysin

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ABSTRACT

Synthesis of Fmoc-protected N^{δ}-acetyl-N^{δ}-(*tert*-butoxy)-L-ornithine has revealed it to be a metal-chelating amino-acid precursor. This protected amino acid was compatible with the preparation of ferrichrome peptides by standard Fmoc-based solid-phase peptide synthesis. Evaluation of deferriferrichrysin for metal ion chelation revealed that zirconium(IV) and titanium(IV) formed complexes with deferriferrichrysin.

1. Introduction

Siderophores are iron(III)-chelating compounds produced by microorganisms such as bacteria and fungi that uptake iron ions under iron-deficient conditions.[1] Siderophores contain three bidentate functional groups (hydroxamate, catecholate, α-hydroxycarboxylate) which form an extremely stable hexadentate, octahedral complex with iron(III). The hydroxamate-type siderophore deferriferrichrysin (**1**) is a cyclic hexapeptide produced by *Aspergillus oryzae* (Fig. 1).[2] Deferriferrichrome (**2**) and deferriferricrocin (**3**) also belong to the hydroxamate family of siderophores, and are produced from *Ustilago sphaerogena*[3] and *Aspergillus fumigatus*,[4] respectively. All cyclic hexapeptides contain three consecutive N^{δ}-acetyl-N^{δ}-hydroxy-L-ornithines (Aho), which are produced from L-ornithine in a biosynthetic process by L-ornithine-N^{δ}-oxygenase [e.g., *Ustilago maydis sid1* protein (Sid1)[5] and *Aspergillus nidulans sidA* protein (SidA)[6]] and N^{δ}-transacetylase.[7]

Hydroxamic acid has metal-chelating functional groups that enable it to form complexes with various metals.[8] For example, deferriferrichrysin also forms chelate complexes with aluminum(III),[9] gallium(III)[10] and chromium(III).[11] A ^{89}Zr complex of the hydroxamate-type siderophore desferrioxamine B (**4**) has been applied to immuno-positron emission tomography (PET) in which the ^{89}Zr complex is conjugated to monoclonal antibodies (mAbs) for visualization of small tumors.[12] Deferriferricrocin (**3**) and desferritriacetylfusarinine C (**5**) are the predominant siderophores produced by *Aspergillus fumigatus*.[13] ^{68}Ga-labeled peptides of **3** and **5** have been investigated for PET-based diagnoses of invasive pulmonary aspergillosis (IPA).[14] These siderophore–metal chelate complexes can provide therapeutic and diagnostic agents, so identification of novel metal complexes of deferriferrichrysin derivatives would be of great interest.

There are two reports of the chemical synthesis of ferrichrome peptides containing three Aho residues. The first chemical synthesis of deferriferrichrome (**2**) was accomplished using L-δ-nitronorvaline by Keller-Schierlein and Maurer in 1969.[15,16] In that process, the linear hexapeptide *tert*-butoxycarbonylglycyl-tris(L-δ-nitronorvalyl)-glycyl-glycine methyl ester **7** was prepared using the mixed anhydride method. After cyclization of linear peptide **7**, reduction of the side-chain nitro group to a hydroxylamine group and subsequent acetylation produced deferriferrichrome (**2**) (Scheme 1). Kurita et al. also reported the total synthesis of ferrichrome using N^{δ}-tosyl-N^{δ}-benzyloxy-L-ornithine in 1974.[17] A linear hexapeptide, *tert*-butoxycarbonylglycyl-tris(N^{δ}-tosyl-N^{δ}-benzyloxy-L-ornithyl)-glycyl-glycine methyl ester **8**, was prepared using *N*,*N*′-dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccinimide (HOSu). After cyclization, removal of the tosyl group and acetylation of the protected cyclic hexapeptide **11** followed by hydrogenation afforded deferriferrichrome (**2**) (Scheme 1). However, these approaches are not applicable to the facile structure–activity relationship (SAR)

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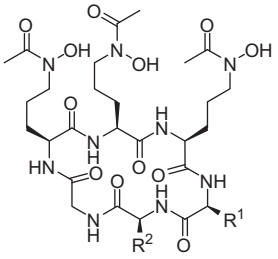
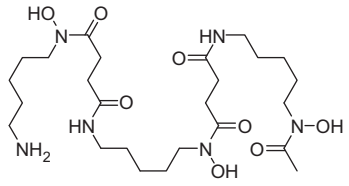
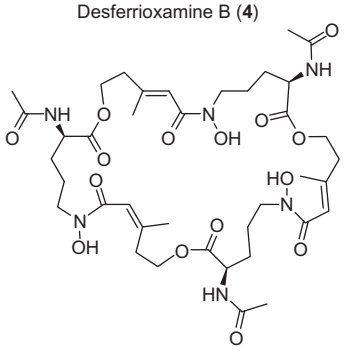


Deferriferrichrysin (**1**) : R^1, $R^2 = CH_2OH$
Deferriferrichrome (**2**): R^1, $R^2 = H$
Deferriferricrocin (**3**) : $R^1 = H$, $R^2 = CH_2OH$

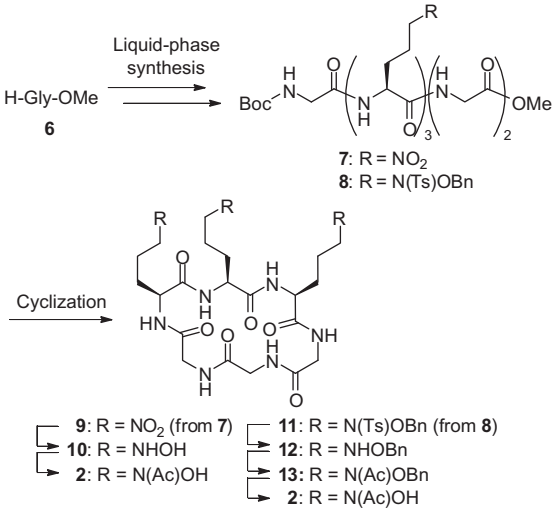


Desferrioxamine B (**4**)



Desferritriacetylfusarinine C (**5**)

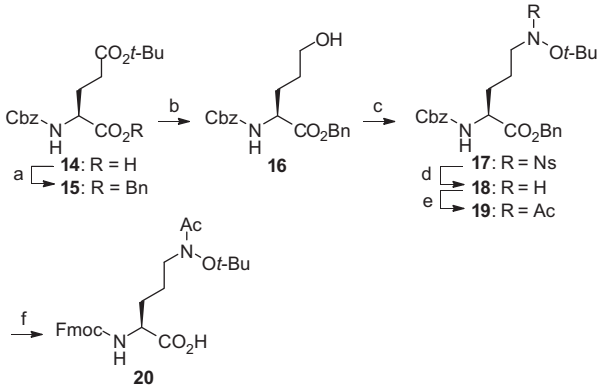
Figure 1. Structures of hydroxamate siderophores.



Scheme 1. Reported synthetic methods of deferriferrichrome.

studies of deferriferrichrysin derivatives because the liquid-phase syntheses were not particularly versatile.

In the present study, we investigated the synthesis of fully protected N^{δ}-acetyl-N^{δ}-hydroxy-L-ornithine, which can be utilized for the preparation of ferrichrome derivatives by Fmoc-based solid-phase peptide synthesis (SPPS). Additionally, formation of novel



Scheme 2. Synthesis of an Fmoc-protected hydroxamate building block for solid-phase peptide synthesis. Reagents and conditions: (a) BnOH, DCC, DMAP, CH_2Cl_2, rt, overnight (90%); (b) (i) TFA, CH_2Cl_2, rt, 2 h; (ii) EtOCOCl, Et_3N, THF, −10 °C, 1 h, then $NaBH_4$, H_2O, rt, 5 h (81%); (c) NsNH(O*t*-Bu), PPh_3, DEAD, THF, rt, 2 h; (d) thiophenol, K_2CO_3, DMF, rt, 5 h (84% from **16**); (e) Ac_2O, pyridine, DMAP, CH_2Cl_2, rt, overnight (95%); (f) (i) H_2, Pd/C, MeOH, rt, 2.5 h; (ii) FmocOSu, $(i\text{-Pr})_2NEt$, MeCN–H_2O, rt, overnight (quant.).

metal complexes of deferriferrichrysin was evaluated using the resulting cyclic peptides.

2. Results and discussion

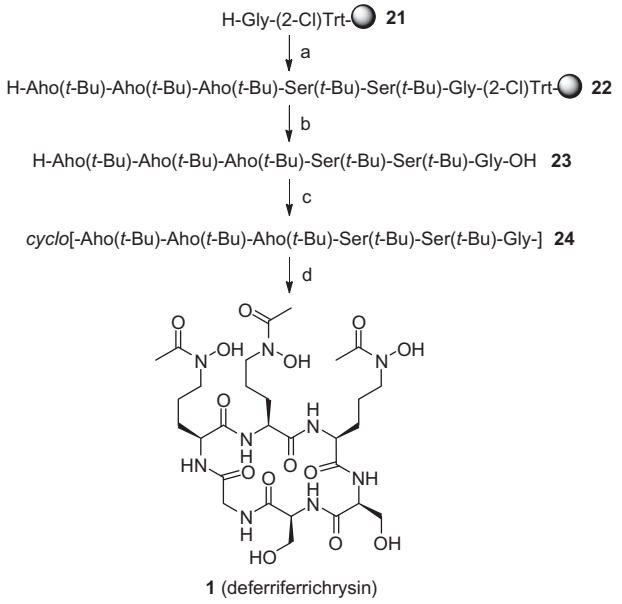
2.1. Synthesis of fully protected N^{δ}-acetyl-N^{δ}-hydroxy-L-ornithine

N^{α}-Fmoc-protected N^{δ}-acetyl-N^{δ}-hydroxy-L-ornithine derivative **20** was designed as a 'building block' for Fmoc-based SPPS (Scheme 2). We expected that a side-chain C3 unit with a terminal functional group of ornithine could be constructed by manipulations of derivatives of glutamic acid. Initially, the carboxylic acid in the commercially available Cbz-Glu(*t*-Bu)-OH **14** was protected with a benzyl group to afford an ester **15**. After removal of the side-chain *t*-Bu group of **15**, the resulting carboxylic acid was converted to an alcohol **16** by in situ preparation of a mixed anhydride followed by $NaBH_4$-mediated reduction. Treatment of **16** with NsNH(O*t*-Bu)[18,19] under the Mitsunobu condition gave a protected hydroxylamine **17**. The hydroxylamine **17** was converted to a protected hydroxamate **19** by removal of the Ns group and acetylation. Hydrogenation of **19** followed by N^{α}-Fmoc protection provided the desired product **20**. This synthetic scheme was applied to the large-scale synthesis of **20**, in which 12 g of the key building block **20** was obtained from 30 g of the starting material **14** (29% in eight steps).

2.2. Preparation of ferrichrome peptides by Fmoc-based SPPS

Using the resulting amino acid **20**, peptide resin **22** was prepared on a 2-chlorotrityl [(2-Cl)Trt] resin **21** using standard Fmoc-based SPPS (Scheme 3). The consecutive couplings of three protected Aho **20** using *N*,*N*′-diisopropylcarbodiimide (DIC)/HOBt proceeded smoothly as the standard amino acids. After treatment of the resin with 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), a protected linear peptide **23** was cyclized using diphenylphosphoryl azide (DPPA)/$NaHCO_3$[20,21] to give a cyclic peptide **24**. Removal of the side chain-protecting groups in **24** provided deferriferrichrysin (**1**). The overall yield from resin loading was 8%.

To demonstrate the potential utility of the established protocol, deferriferrichrome (**2**) and deferriferricrocin (**3**) were also synthesized. These two compounds were obtained efficiently by merely altering the coupling components in SPPS. Spectral data showed good agreement with the reported spectra.[4,22,23]



Scheme 3. Synthesis of deferriferrichrysin. Reagents and conditions: (a) Fmoc-based SPPS; (b) HFIP, CH_2Cl_2, rt, 2 h; (c) DPPA, $NaHCO_3$, DMF, −40 °C to rt, 61.5 h; (d) TFA, H_2O, rt, 9 h (8% from **21**); Aho = N^{δ}-acetyl-N^{δ}-hydroxy-L-ornithine.

Table 1
HPLC and ESI-MS analyses of deferriferrichrysin–metal complexes

Entry	Metal[a]	t_R[b] (min)	m/z (ESI)
1	None	18.7	748 [MH^+]
2	$Sr(NO_3)_2$	18.6	748 [MH^+]
3	$Y(NO_3)_3$[c]	18.9	748 [MH^+]
4	$InCl_3$	18.6	748 [MH^+]
5	$Gd(NO_3)_3$	18.8	748 [MH^+]
6	$Re(CO)_5Cl$[d]	18.8	748 [MH^+]
7	$ReCl_5$[e]	15.9	700 [$(M-3O)H^+$]
8	$ZrCl_4$	15.5	834 [$(M+Zr-3H)^+$]
9	$Ti(SO_4)_2$[f]	18.0	792 [$(M+Ti-3H)^+$]
		18.3	792 [$(M+Ti-3H)^+$]
10	$Fe(NO_3)_3$[g]	20.1	801 [$(M+Fe-3H)H^+$]

[a] Metal salt was dissolved in H_2O.
[b] HPLC conditions: a linear gradient of solvent B in solvent A (0–25% over 25 min); COSMOSIL 5C18-ARII column (4.6 × 250 mm); flow rate, 1 mL/min; solvent A: 0.1% TFA–H_2O; solvent B: 0.1% TFA–MeCN; detection at 220 nm.
[c] Yttrium standard solution [$Y(NO_3)_3$ in 1 mol/L HNO_3] was used.
[d] $Re(CO)_5Cl$ was dissolved in DMF.
[e] $ReCl_5$ was dissolved in MeOH.
[f] 30% $Ti(SO_4)_2$ solution was used.
[g] Iron standard solution [$Fe(NO_3)_3$ in 0.1 mol/L HNO_3] was used.

2.3. Metal-chelating properties of deferriferrichrysin

Formation of the complex of deferriferrichrysin with various metals was investigated. We chose strontium(II) [Sr(II)], yttrium(III) [Y(III)], indium(III) [In(III)], gadolinium(III) [Gd(III)], rhenium(I) [Re(I)], rhenium(V) [Re(V)] and zirconium(IV) [Zr(IV)]. These have been used for clinical diagnoses and treatments. Mixtures of a metal salt and deferriferrichrysin were analyzed by high-performance liquid chromatography (HPLC) and electrospray ionization-mass spectrometry (ESI-MS) (Table 1).[24]

Formation of a metal–deferriferrichrysin complex in the mixture was not observed using Sr(II), Y(III), In(III), Gd(III) or Re(I) (entries 2–6). When using Re(V), a deferriferrichrysin peak disappeared and a new peak was observed (t_R = 15.9 min; entry 7). ESI-MS analyses revealed that the product in the Re(V) mixture (m/z 700) was not the expected Re(V)–deferriferrichrysin complex (m/z 931). However, the reduced deferriferrichrysin analog was obtained, in which three hydroxamate groups were converted to amide groups (entry 7). Alcohols work as reducing agents for transition metals including rhenium,[25] palladium[26] and ruthenium,[27] so Re(V) might be reduced by methanol to a reactive low-valent ion, which reduced deferriferrichrysin. When DMF was used as the solvent, fully reduced deferriferrichrysin was not observed.[28] This observation supported our proposal. In the mixture of Zr(IV) solution, formation of the desired Zr(IV)–deferriferrichrysin complex was observed (t_R = 15.5 min; m/z 834; entry 8), which was characterized by ultraviolet–visible (UV–vis) spectroscopy, circular dichroism (CD) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, and fast atom bombardment-high resolution mass spectrometry (FAB-HRMS). The physiochemical properties of this complex are being investigated.[29]

Furthermore, we examined the formation of the titanium(IV) [Ti(IV)]–deferriferrichrysin complex because Ti and Zr are group-4 elements. When a mixture of Ti(IV) and deferriferrichrysin was analyzed by HPLC, two new peaks with an identical molecular weight of the Ti(IV)–deferriferrichrysin complex (m/z 792) were obtained (t_R = 18.0, 18.3 min; entry 9). Each product was interconverted to the other product. This finding suggested that the Ti(IV) complex may have existed as a mixture of two equilibrated structures.[30]

3. Conclusions

We established a facile synthetic protocol for a fully protected Aho derivative **20**. Using this key component **20** for Fmoc-based SPPS, deferriferrichrysin and derivatives were synthesized. This facile synthetic protocol may be useful for the preparation of Aho-containing peptides to conduct SAR studies and structure–function relationship studies. We also identified two new metal-chelating complexes of deferriferrichrysin with Zr(IV) and Ti(IV). Some Zr(IV) and Ti(IV) complexes have been used as catalyst materials in chemical reactions such as olefin polymerization,[31–33] epoxidation,[34,35] and aldol reaction.[36,37] Hence, these new peptide–metal complexes could be novel reagents with unique reactivity. Further studies on the application of these deferriferrichrysin–metal complexes are in progress.

4. Experimental

4.1. Synthesis

4.1.1. General methods

All moisture-sensitive reactions were performed using syringe-septum cap techniques under an argon atmosphere and all glassware was dried in an oven at 80 °C for 2 h prior to use. Melting points were measured by a hot stage melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO P-1020 polarimeter. For flash chromatography, Wakosil C-300E was employed. For analytical HPLC, a COSMOSIL 5C18-ARII column (4.6 × 250 mm, Nacalai Tesque Inc., Kyoto, Japan) was employed with a linear gradient of MeCN containing 0.1% (v/v) TFA at a flow rate of 1 mL/min on Shimadzu LC-20ADvp (Shimadzu corporation, Ltd, Kyoto, Japan). Preparative HPLC was performed using a COSMOSIL 5C18-ARII column (20 × 250 mm, Nacalai Tesque Inc.) with a linear gradient of MeCN containing 0.1% (v/v) TFA at a flow rate of 8 mL/min on Shimadzu LC-6AD (Shimadzu corporation, Ltd). ^1H NMR spectra were recorded using a JEOL ECA-500 spectrometer, and chemical shifts are reported in δ (ppm) relative to TMS (in $CDCl_3$), DMSO (in DMSO-d_6) or H_2O (in D_2O) as an internal standard. ^{13}C NMR spectra were recorded using a JEOL ECA-500 spectrometer and referenced to the residual $CHCl_3$ (in $CDCl_3$), DMSO (in DMSO-d_6), or MeOH or dioxane (in D_2O) signal. Chemical shifts were reported in parts per million with the residual solvent peak used as

an internal standard. ^{1}H NMR spectra are tabulated as follows: chemical shift, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), number of protons, and coupling constant(s). Exact mass (HRMS) spectra were recorded on a JMS-HX/HX 110A mass spectrometer. Infrared (IR) spectra were obtained on a JASCO FT/IR-4100 FT-IR spectrometer with JASCO ATR PRO410-S. UV–vis and CD spectra were recorded on Shimadzu UV-2450 UV–vis spectrophotometer and JASCO J-720 circular dichroism spectrometer. The preparation and characterization of compounds **15**,[38] and **16**[39] were reported previously.

4.1.2. Benzyl (*S*)-2-[*N*-(benzyloxycarbonyl)amino]-5-[*N*-(*tert*-butoxy)-*N*-(2-nitrophenylsulfonyl)amino]pentanoate (17)

To a solution of the alcohol **16**[39] (50.3 mg, 0.14 mmol), PPh_3 (75.4 mg, 0.29 mmol) and NsNH(O*t*-Bu) (78.0 mg, 0.28 mmol) in THF (1.4 mL) was added DEAD in toluene (2.2 M, 0.13 mL, 0.28 mmol) at 0 °C under argon, and the mixture was stirred at room temperature for 2 h. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane–EtOAc (2:1) gave the crude title compound **17** (91.2 mg), which was used in the next step without further purification.

Compound **17** (purified for characterization): $[\alpha]_D^{26}$ +6.17 (*c* 1.02, $CHCl_3$); IR (neat): 1725 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.27 (s, 9H), 1.56–1.66 (m, 1H), 1.66–1.79 (m, 2H), 1.83–1.93 (m, 1H), 3.02–3.16 (m, 1H), 3.31–3.43 (m, 1H), 4.30–4.44 (m, 1H), 5.05–5.20 (m, 4H), 5.27 (d, *J* = 8.0 Hz, 1H), 7.28–7.39 (m, 10H), 7.46 (d, *J* = 5.7 Hz, 1H), 7.60–7.75 (m, 2H), 8.00–8.06 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.9, 27.7 (3C), 29.8, 53.6, 55.2 67.0, 67.3, 84.0, 123.4, 127.1, 128.1 (2C), 128.2, 128.3 (2C), 128.49, 128.54 (2C), 128.6 (2C), 130.8, 132.9, 134.5, 135.1, 136.2, 149.4, 155.8, 171.8; HRMS (FAB), *m/z* calcd for $C_{30}H_{36}N_3O_9S$ (MH^+) 614.2167, found: 614.2166.

4.1.3. Benzyl (*S*)-2-[*N*-(benzyloxycarbonyl)amino]-5-[*N*-(*tert*-butoxy)amino]pentanoate (18)

To a stirred solution of crude ester **17** (91.2 mg) in DMF (1.5 mL) were added thiophenol (28.8 μL, 0.28 mmol) and K_2CO_3 (60.2 mg, 0.42 mmol) at room temperature, and the mixture was stirred at the same temperature for 5 h. After concentration under reduced pressure, the residue was extracted with EtOAc, and washed with saturated NH_4Cl, brine, saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane–EtOAc (3:1) gave the title compound **18** (50.8 mg, 0.12 mmol, 84% from **16**) as a colorless oil: $[\alpha]_D^{27}$ −1.14 (*c* 1.13, $CHCl_3$); IR (neat): 1725 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.13 (s, 9H), 1.41–1.53 (m, 2H), 1.68–1.78 (m, 1H), 1.85–1.95 (m, 1H), 2.81 (t, *J* = 6.9 Hz, 2H), 4.40–4.47 (m, 1H), 5.10 (s, 2H), 5.11–5.22 (m, 2H), 5.47 (d, *J* = 8.0 Hz, 1H), 7.27–7.39 (m, 10H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 23.1, 26.7 (3C), 30.5, 52.1, 53.8, 66.9, 67.1, 76.7, 128.07 (2C), 128.12, 128.2 (2C), 128.4, 128.5 (2C), 128.6 (2C), 135.3, 136.2, 155.9, 172.2; HRMS (FAB), *m/z* calcd for $C_{24}H_{33}N_2O_5$ (MH^+) 429.2384, found: 429.2384.

4.1.4. Benzyl (*S*)-5-[*N*-acetyl-*N*-(*tert*-butoxy)amino]-2-[*N*-(benzyloxycarbonyl)amino]pentanoate (19)

To a solution of ester **18** (97.2 mg, 0.23 mmol) in CH_2Cl_2 (4.5 mL) were added pyridine (36.5 μL, 0.45 mmol), 4-(dimethylamino)pyridine (DMAP, 14.1 mg, 0.12 mmol) and Ac_2O (32.1 μL, 0.34 mmol) at room temperature. After stirring for 1 h, pyridine (36.5 μL, 0.45 mmol) and Ac_2O (32.1 μL, 0.34 mmol) were added to the mixture, and the resulting mixture was stirred overnight. After dilution with CH_2Cl_2, the mixture was washed with saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane–AcOEt (1:1) gave the title compound **19** (101 mg, 0.22 mmol, 95%) as a colorless oil: $[\alpha]_D^{22}$ +1.01 (*c* 1.03, $CHCl_3$); IR (neat): 1721 (C=O), 1654 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6, 100 °C) δ 1.24 (s, 9H), 1.60–1.75 (m, 4H), 1.99 (m, 3H), 3.52–3.59 (m, 2H), 4.11–4.17 (m, 1H), 5.05 (s, 2H), 5.09–5.16 (m, 2H), 7.27–7.39 (m, 11H); ^{13}C NMR (125 MHz, DMSO-d_6, 100 °C) δ 20.5, 22.2, 26.9 (3C), 27.9, 48.7, 53.5, 65.1, 65.4, 81.6, 127.05 (2C), 127.12 (2C), 127.36, 127.42, 128.8 (4C), 135.5, 136.5, 155.4, 171.3, 173.6; HRMS (FAB), *m/z* calcd for $C_{26}H_{35}N_2O_6$ (MH^+) 471.2490, found: 471.2489.

4.1.5. (*S*)-5-[*N*-Acetyl-*N*-(*tert*-butoxy)amino]-2-[*N*-(9-fluorenylmethoxycarbonyl)amino]pentanoic acid (20)

The ester **19** (101.7 mg, 0.22 mmol) was treated with 10% Pd/C (43.3 mg) in MeOH (1.2 mL) under H_2 atmosphere at room temperature for 3.5 h. After filtration and concentration under reduced pressure, the residue was dissolved in MeCN (1.4 mL) and water (1.4 mL). (*i*-Pr)$_2$NEt (75.3 μL, 0.43 mmol) and Fmoc-OSu (148 mg, 0.43 mmol) were added at room temperature, and the mixture was stirred overnight at the same temperature. The reaction was quenched by addition of 1 M HCl. After concentration under reduced pressure, the residue was extracted with EtOAc. The extract was washed with 1 N HCl and brine, and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with EtOAc containing 0.5% AcOH gave the title compound **20** (104 mg, 0.22 mmol, quant.) as white amorphous solid: $[\alpha]_D^{22}$ −5.4 (*c* 1.09, DMSO); IR (neat): 3373 (NH), 1718 (C=O), 1650 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6, 100 °C) δ 1.26 (s, 9H), 1.56–1.74 (m, 4H), 2.02 (s, 3H), 3.53–3.61 (m, 2H), 3.96–4.02 (m, 1H), 4.20–4.24 (m, 1H), 4.28–4.32 (m, 2H), 7.12–7.20 (m, 1H), 7.29–7.34 (m, 2H), 7.38–7.43 (m, 2H), 7.67–7.72 (m, 2H), 7.85 (d, *J* = 6.9 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6, 100 °C) δ 20.6, 22.4, 26.8 (3C), 28.1, 46.4, 48.9, 53.2, 65.4, 81.6, 119.4 (2C), 124.6 (2C), 126.4 (2C), 126.9 (2C), 140.3 (2C), 143.5 (2C), 155.4, 172.7, 173.6; HRMS (FAB), *m/z* calcd for $C_{26}H_{33}N_2O_6$ (MH^+) 469.2333, found: 469.2335.

4.1.6. Peptide synthesis

The protected linear peptide **23** was constructed by Fmoc-based solid-phase synthesis on H-Gly-(2-Cl)Trt resin (0.80 mmol/g, 125 mg, 0.10 mmol). *t*-Bu group for Ser was employed for side-chain protection. Fmoc-Ser(*t*-Bu)-OH (0.50 mmol) and Fmoc-protected ornithine derivative **20** (0.30 mmol) were coupled by using DIC (46.4 μL, 0.3 mmol/77.4 μL, 0.5 mmol) and HOBt·H_2O (45.9 mg, 0.3 mmol/76.6 mg, 0.5 mmol) in DMF. Completion of each coupling reaction was ascertained using the Kaiser ninhydrin test. The Fmoc-protecting group was removed by treating the resin with 20% piperidine in DMF. The resulting protected peptide resin **22** was subjected to HFIP–CH_2Cl_2 (2:8, 25 mL) treatment at room temperature for 2 h. After filtration of the residual resin, the filtrate was concentrated under reduced pressure to give a crude linear peptide **23**.

By use of a procedure identical with that described for the preparation of **23**, the protected linear peptides for deferriferrichrome (**2**) and deferriferricrocin (**3**) were obtained.

4.1.7. Deferriferrichrysin (1)

To a mixture of the resulting linear peptide **23** (55.6 mg) and $NaHCO_3$ (42.0 mg, 0.500 mmol) in DMF (40 mL) was added DPPA (53.9 μL, 0.250 mmol) at −40 °C. The mixture was stirred for 48 h with warming to room temperature and then filtered. The filtrate was concentrated under reduced pressure, followed by flash chromatography over silica gel with $CHCl_3$–MeOH (9:1) to give the crude protected cyclic peptide **24**. The crude peptide **24** was treated with 95% TFA (2 mL) at room temperature for 6 h. The mixture was poured into ice-cold dry Et_2O (10 mL). The resulting powder was collected by centrifugation and the washed three times with ice-cold dry Et_2O. The crude product was purified by preparative HPLC to afford the deferriferrichrysin **1** (6.1 mg, 0.0082 mmol, 8.2% yield based on H-Gly-(2-Cl)Trt resin) as yellow freeze-dried

powder: $[\alpha]_D^{31}$ −16.8 (*c* 1.03, H_2O); ^1H NMR (500 MHz, D_2O) δ 1.63–2.03 (m, 12H), 2.12–2.19 (m, 9H), 3.60–3.75 (m, 6H), 3.85–3.98 (m, 5H), 4.08 (d, *J* = 16.6 Hz, 1H), 4.29–4.35 (m, 1H), 4.36–4.48 (m, 4H); ^{13}C NMR (125 MHz, D_2O, internal standard; dioxane) δ −47.3 (3C), −44.2, −44.1, −43.9, −38.8, −38.63, −38.61, −23.5, −19.4 (2C), −19.3, −13.0, −12.4 (2C), −10.6, −10.5, −6.1, −6.0, 104.7, 105.1, 105.4, 106.9, 107.1, 107.3 (4C); HRMS (FAB), *m/z* calcd for $C_{29}H_{50}N_9O_{14}$ (MH^+) 748.3472, found: 748.3479. The NMR data was in good agreement with the literature data.[4,22]

4.1.8. Deferriferrichrome (2)

By use of a procedure identical with that described for the preparation of the deferriferrichrysin **1**, the deferriferrichrome **2** (4.9 mg, 0.0071 mmol, 7.1% based on H-Gly-(2-Cl)Trt resin) was obtained as yellow freeze-dried powder: $[\alpha]_D^{27}$ −2.6 (*c* 0.351, H_2O); ^1H NMR (500 MHz, D_2O) δ 1.63–2.02 (m, 12H), 2.12–2.19 (m, 9H), 3.61–3.78 (m, 6H), 3.91 (d, *J* = 16.6 Hz, 1H), 3.95–4.11 (m, 4H), 4.15 (d, *J* = 17.2 Hz, 1H), 4.30–4.39 (m, 1H), 4.39–4.49 (m, 2H); ^{13}C NMR (125 MHz, D_2O, internal standard; dioxane) δ −47.30, −47.27 (2C), −44.5, −44.2, −44.0, −39.2, −38.4, −37.9, −24.0, −23.9, −23.6, −19.4 (2C), −19.3, −12.8, −12.7, −12.6, 105.0, 105.4, 105.6, 107.2, 107.3 (2C), 107.4, 107.48, 107.55; HRMS (FAB), *m/z* calcd for $C_{27}H_{46}N_9O_{12}$ (MH^+) 688.3260, found: 688.3256. The NMR data was in good agreement with the literature data.[4,22]

4.1.9. Deferriferricrocin (3)

By use of a procedure identical with that described for the preparation of the deferriferrichrysin **1**, the deferriferricrocin **3** (16.0 mg, 0.0223 mmol, 22% based on H-Gly-(2-Cl)Trt resin) was obtained as yellow freeze-dried powder: $[\alpha]_D^{30}$ −16.0 (*c* 1.14, H_2O); ^1H NMR (500 MHz, D_2O) δ 1.63–1.96 (m, 12H), 2.16 (s, 9H), 3.60–3.75 (m, 6H), 3.85 (d, *J* = 16.6 Hz, 1H), 3.90 (dd, *J* = 11.5, 5.2 Hz, 1H), 3.95 (dd, *J* = 11.5, 5.7 Hz, 1H), 3.98–4.09 (m, 2H), 4.16 (d, *J* = 16.6 Hz, 1H), 4.27–4.33 (m, 1H), 4.35–4.45 (m, 2H), 4.50–4.55 (m, 1H); ^{13}C NMR (125 MHz, D_2O, internal standard; dioxane) δ −47.3 (3C), −44.4, −44.2 (2C), −39.2, −38.3, −38.0, −23.9, −23.7, −19.4 (2C), −19.3, −12.8, −12.7, −12.6, −11.4, −6.2, 104.7, 105.0, 105.4, 106.8, 107.2, 107.27 (3C), 107.34; HRMS (FAB), *m/z* calcd for $C_{28}H_{48}N_9O_{13}$ (MH^+) 718.3366, found: 718.3371. The NMR data was in good agreement with the literature data.[22,23]

4.1.10. Zirconium(IV)–deferriferrichrysin complex (S1)

To a solution of deferriferrichrysin (14.4 mg, 0.0193 mmol) in H_2O (1 mL) was added a solution of $ZrCl_4$ (17.4 mg, 0.0747 mmol) in H_2O (1 mL) at room temperature. The mixture was purified by preparative HPLC to afford the titled compound **S1** (8.9 mg, 0.0106 mmol, 55% yield) as colorless freeze-dried powder: $[\alpha]_D^{29}$ +89.4 (*c* 0.926, DMSO); ^1H NMR (500 MHz, D_2O) δ 1.34–1.46 (m, 1H), 1.50–1.59 (m, 1H), 1.65–1.80 (m, 4H), 1.82–1.89 (m, 1H), 1.92–2.11 (m, 13H), 2.22–2.36 (m, 1H), 3.36–3.51 (m, 2H), 3.51–3.63 (m, 1H), 3.67–3.77 (m, 2H), 3.77–3.93 (m, 5H), 4.02–4.12 (m, 1H), 4.12–4.19 (m, 2H), 4.22–4.27 (m, 1H), 4.36 (t, *J* = 3.4 Hz, 1H), 4.48 (dd, *J* = 13.2, 2.9 Hz, 1H), 4.83–4.89 (m, 1H), 6.86–6.93 (m, 1H), 8.51–8.58 (m, 1H), 9.00–9.05 (m, 1H); ^{13}C NMR (125 MHz, D_2O, internal standard; MeOH) δ 17.3, 17.5, 17.9, 22.9, 26.0, 26.4, 27.0, 27.2, 29.5, 44.1, 50.5, 50.8, 51.8, 54.1, 55.9, 56.0, 59.2, 59.9, 61.45, 61.49, 165.5, 166.1, 166.2, 171.7, 171.9, 173.2, 173.7, 174.9, 177.8; HRMS (FAB), *m/z* calcd for $C_{29}H_{46}N_9O_{14}Zr$ (M^+) 834.2206, found: 834.2202.

4.2. Metal-chelating experiment

To a 100 μL solution of deferriferrichrysin in solvent (1.0 mM) was added a 100 μL solution of metal salts in solvent (5.0 mM) at room temperature. $Sr(NO_3)_2$, Y standard solution [$Y(NO_3)_3$ in 1 mol/L HNO_3], $InCl_3$, $Gd(NO_3)_3$, $ZrCl_4$, 30% $Ti(SO_4)_2$ solution and Fe standard solution [$Fe(NO_3)_3$ in 0.1 mol/L HNO_3] were dissolved in H_2O; $Re(CO)_5Cl$ and $ReCl_5$ were dissolved in DMF and methanol, respectively. After 24 h, the mixture was analyzed by HPLC and assessment of ESI-MS spectra. *HPLC conditions*: a linear gradient of solvent B in solvent A (0–25% over 25 min); COSMOSIL 5C18-ARII column (4.6 × 250 mm); flow rate, 1 mL/min; solvent A: 0.1% TFA–H_2O; solvent B: 0.1% TFA–MeCN; detection at 220 nm.

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Supplementary data

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