Synthesis and Siderophore Activity of Vibrioferrin and One of Its Diastereomeric Isomers

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Total synthesis of vibrioferrin (1), a siderophore, was achieved *via* a chiral dibenzyl citrate obtained by optical resolution. The citrate moiety of vibrioferrin was determined to have the *R* configuration and one of the diastereomeric isomers (1b) of 1 did not exhibit siderophore activity.

Key words vibrioferrin; siderophore activity; synthesis; optical resolution; dibenzyl citrate

Vibrioferrin (**1**), which was isolated from a spent culture of *Vibrio parahaemolyticus* grown under iron-restricted conditions, is one of the high-affinity iron-chelating compounds called siderophore. We proposed the structure of **1** shown in Fig. 1 based on partial chemical degradation data and spectral analyses.[1a,b] We confirmed that **1** was a mixture of reversible epimeric isomers at the 2‴ position on the pyrrolidinone ring, and that the 2″ methine carbon bound to the pyrrolidinone ring was in the *S* form. However, the configuration of the center carbon at the 2 position in the citrate moiety could not be determined. We have presented a synthetic approach for the purpose of completely elucidating the structure of **1**.[2] In this paper, we describe the details of our synthetic method and the siderophore activity of **1a** and one of its diastereomeric isomers (**1b**).

Synthesis Dibenzyl citrate[3] (**2**) was prepared from citric acid (**3**) *via* 4,4-(5-oxo-1,3-dioxolane)diacetic acid[4] (**4**) and its anhydride[5] (**5**) (Chart 1). Attempts to synthesize **2** directly from **5** under a variety of conditions failed. However, the reaction of **5** with benzyl alcohol under reflux in dry chloroform for 1 d in the presence of dry pyridine gave benzyl 5-oxo-1,3-dioxolane-4,4-diacetate[6] (**6**) in 77% yield. Refluxing **6** with benzyl alcohol in chloroform for 1 week in the presence of dry triethylamine afforded **2** in 49% yield.

Both the optically active dibenzyl citrates ((*S*)-**2**, (*R*)-**2**) were obtained by optical resolution using several chiral alkaloids (Table 1). The optical resolution of **2** *via* recrystallization of the salt of **2** and (−)-cinchonidine afforded (*S*)-**2** in 25% yield with an enantiomeric excess of 83%. On the other hand, recrystallization of the salt of **2** and (−)-quinine gave (*R*)-**2** in 38% yield with an enantiomeric excess of 86%.

The two enantiomeric isomers, (*S*)-**2** ($[\alpha]_D$ −6.80°) and (*R*)-**2** ($[\alpha]_D$ +6.49°), were obtained with enantiomeric purity exceeding 99% ee (chiral HPLC) in 24% and 27% yield, respectively, by repeated recrystallization (Chart 2). The absolute configurations of the chiral citrates ((*S*)-**2** and (*R*)-**2**) were determined by transformation to a known chiral citrate (Chart 2). Replacement of the benzyl group in both (*S*)-**2** and (*R*)-**2** with a methyl group in methanol in the presence of sodium hydride gave (*S*)-**7** ($[\alpha]_D$ −3.39°) and (*R*)-**7** ($[\alpha]_D$ +3.87°) in 68% and 66% yields, respectively. The optical rotation of (*R*)-**7** was in good agreement with the reported value ($[\alpha]_D$ +4.0°).[7]

Compound **1a**, which has the *R* configuration at the 2 position, was successfully synthesized from (*S*)-**2** (Chart 3). Esterification of (*S*)-**2** with alcohol[8] (**10**), which was prepared in 58% yield from *N*-Boc-alanine (**8**) and ethanolamine (**9**) using 1-(3-*N*,*N*-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), afforded **11a** in 67% yield. Condensation of the amine afforded by debutoxycarbonylation of **11a** with 1-benzyl α-ketoglutarate, which was prepared from α-

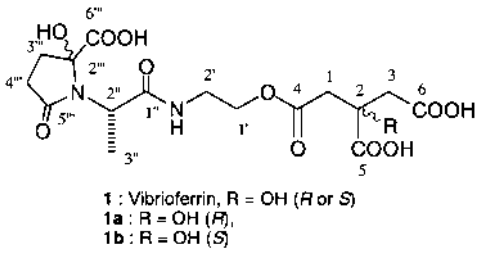


Fig. 1. Proposed Structure for Vibrioferrin (**1**)

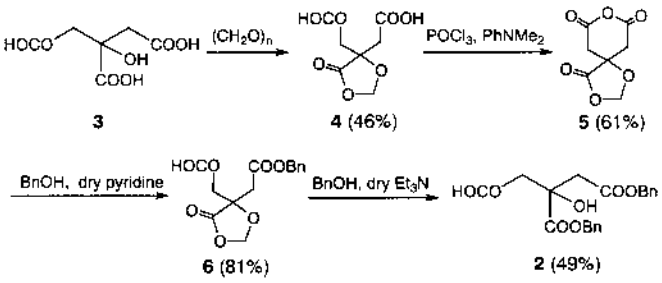
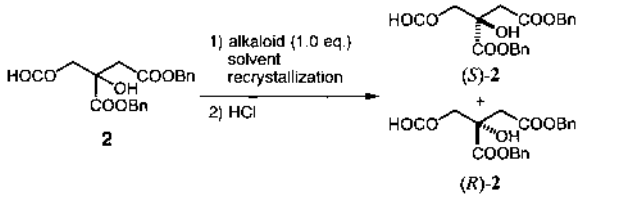


Chart 1

Table 1. Optical Resolution of Dibenzyl Citrate (**2**)



Alkaloid	Solvent	Yield (%)	ee (%)[a]
(−)-Cinchonidine	AcOEt	(*S*)-**2** 25	83.4
(+)-Cinchonidine	$CHCl_3$–AcOEt	(*S*)-**2** 12	16.4
(−)-Quinine	AcOEt	(*R*)-**2** 38	86.1
(+)-Quinidine	AcOEt	No crystallization	

a) Determind by HPLC with a chiral column.

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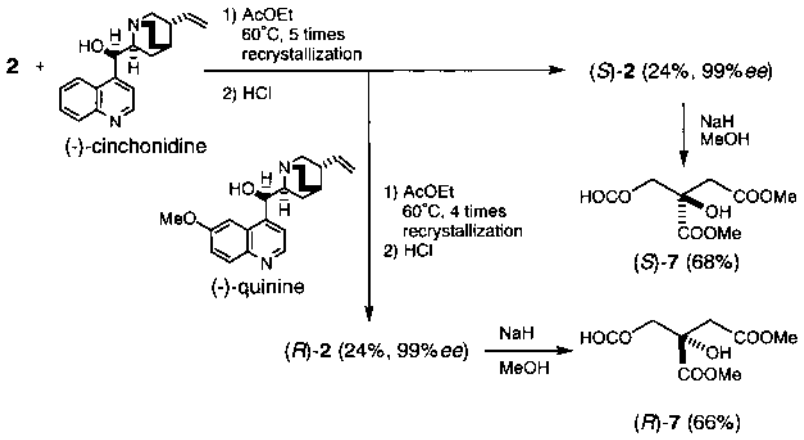

Chart 2

Table 2. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) Data for **1**, **1a**, and **1b**

Position	^{1}H-NMR chemical shift (ppm (δ))			^{13}C-NMR chemical shift (ppm (δ))		
	1	**1a**	**1b**	**1**	**1a**	**1b**
1	2.84 (d, J=15.0 Hz)	2.85 (d, J=15.0 Hz)	2.83 (d, J=15.0 Hz)	43.4	43.5, 43.6	43.5, 43.7
	2.96 (d, J=15.0 Hz)	2.97 (d, J=15.0 Hz)	2.96 (d, J=15.0 Hz)			
2				73.6	73.8	73.8
3	2.81 (d, J=16.0 Hz)	2.83 (d, J=16.0 Hz)	2.83 (d, J=16.0 Hz)	44.2, 44.3	44.4, 44.5	44.5, 44.7
	2.95 (d, J=16.0 Hz)	2.98 (d, J=16.0 Hz)	2.98 (d, J=16.0 Hz)			
4				170.0	169.9	169.7, 169.8
5				175.4, 175.5	175.48, 175.52	175.65, 175.74
6				171.6, 171.7	171.5, 171.6	171.5, 171.6
1′	4.04—4.16 (m)	4.07—4.17 (m)	4.00—4.18 (m)	63.9, 64.0	63.9, 64.0	63.9, 64.3
2′	3.32—3.52 (m)	3.32—3.51 (m)	3.36—3.49 (m)	39.0	39.2, 39.3	39.1, 39.3
1″				171.9, 172.6	172.1, 172.7	171.9, 172.8
2″				52.5, 52.7	52.71, 52.74	52.6, 52.8
	4.00 (q, J=7.0 Hz)	4.01 (q, J=7.0 Hz)	4.00 (q, J=7.0 Hz)			
3″	4.33 (q, J=7.5 Hz)	4.32 (q, J=7.5 Hz)	4.34 (q, J=7.5 Hz)	14.3, 14.7	14.4, 14.7	14.4, 14.8
	1.45 (d, J=7.5 Hz)	1.47 (d, J=7.0 Hz)	1.47 (d, J=7.0 Hz)			
2‴	1.48 (d, J=7.0 Hz)	1.50 (d, J=7.5 Hz)	1.50 (d, J=7.5 Hz)	90.7, 90.9	90.8, 90.9	90.9, 91.0
3‴				33.0, 34.3	33.2, 34.5	33.0, 34.5
	2.17—2.26 (m)	2.17—2.27 (m)	2.12—2.33 (m)			
4‴	2.36—2.58 (m)	2.40—2.58 (m)	2.39—2.58 (m)	—	30.6	30.6
5‴	2.36—2.58 (m)	2.40—2.58 (m)	2.39—2.58 (m)	175.6, 175.9	175.5, 175.7	175.7, 175.9
6‴				173.0, 174.2	173.1, 174.4	173.1, 174.4

ketoglutaric acid and benzyl bromide in the presence of dicyclohexylamine[9] in 67% yield, gave **13a** in 40% yield and subsequent debenzylation gave **1a** in 98% yield. By a similar method, **1b**, a diastereomer of **1a**, was synthesized from (*R*)-**2**.

We could not determine whether **1a** or **1b** is vibrioferrin (**1**) from only the ^{1}H-NMR or ^{13}C-NMR data, because no distinguishing differences were observed between the compounds (Table 2). The optical rotation ($[\alpha]_D$ +13.3°)[1a] suggested that vibrioferrin was **1a** ($[\alpha]_D$ +8.67°) and not **1b** ($[\alpha]_D$ −12.4°), although no satisfactory agreement was observed. We can not satisfactorily explain the reason for the disagreement of the optical rotations. However, we think that our synthetic compound is almost optically pure, because it was obseved that the ratio (1 : 1) of a mixture of epimers due to the 2‴ position of the synthetic compounds (**1a** or **1b**) is the same as the natural one (**1**).

Biological Activity The addition of compound **1a** alleviated the growth inhibition imposed by ethylenediamine-di(*o*-hydroxyphenyl)acetic acid (EDDA), indicating that it can sequester Fe^{3+} from a complex of Fe^{3+} and EDDA and deliver iron to MY-1 cells. Furthermore, the growth promotion activity of **1a** at concentrations of 5 and 10 μM was almost the same as that of **1**. On the other hand, **1b** was not observed to promote growth. The remarkable difference between the siderophore activity of **1a** and **1b** strongly supports the hypothesis that **1** and **1a** are the same compounds, and that the configuration of the citrate moiety of **1** is *R*.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were recorded on a JASCO A-102 spectrometer. Mass spectra (MS) were recorded on a VG-70SE spectrometer. ^{1}H- and ^{13}C-NMR spectra were run on a Hitachi R-1500 or a Varian VXR-500 spectrometer. Optical rotations were measured on a JASCO DIP-4 spectrometer. Analytical HPLC was performed on Chemcosorb 5Si-U (Chemco) or Chiralcel OD (Daicel). Merck silica gel 60 (230—400 mesh) was employed for column chromatography. Extracts were dried over anhydrous $MgSO_4$.

5-Oxo-1,3-dioxolane-4,4-diacetic Acid (4) A mixture of citric acid (**3**, 60.0 g, 312 mmol) and paraformaldehyde (18.0 g) was stirred and heated at 160 °C for 0.5 h and then cooled. The precipitate was collected by filtration

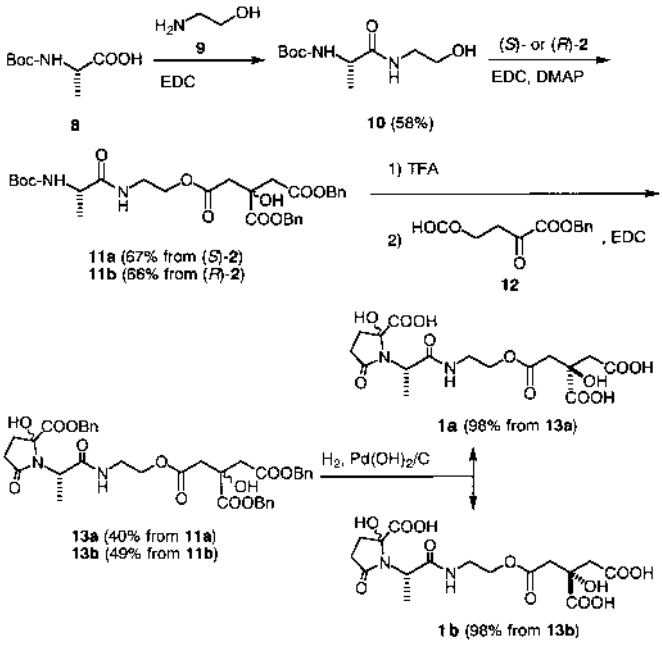

Chart 3

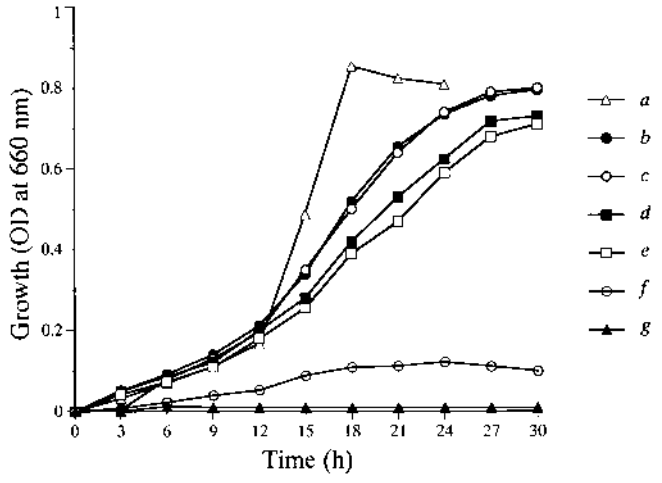

Fig. 2. Growth of Vibrioferrin-Deficient Mutant MY-1 of *V. Parahaemolyticus* AQ3354

The following ingredients were added to the iron-deficient Tris-buffered minimal medium.[1c] *a*) 5.0 μM Fe^{3+} (positive control). *b*) 10.0 μM **1a**, 5.0 μm Fe^{3+}, and 17.5 μM ethylenediamine-di(*o*-hydroxyphenylacetic acid) (EDDA). *c*) 10.0 μM Vibrioferrin (**1**), 5.0 μM Fe^{3+}, and 17.5 μM EDDA. *d*) 5.0 μM **1a**, 5.0 μM Fe^{3+}, and 17.5 μM EDDA. *e*) 5.0 μM vibrioferrin (**1**), 5.0 μM Fe^{3+}, and 17.5 μM EDDA. *f*) No addition (negative control). *g*) 5.0 μM Fe^{3+} and 17.5 μM EDDA; 5.0 μM **1b**, 5.0 μM Fe^{3+}, and 17.5 μM EDDA; 10.0 μM **1b**, 5.0 μM Fe^{3+}, 17.5 μM EDDA. Note that both EDDA and ferric ion were added to the medium one day before to accomplish complete complexation. After inoculation with MY-1 cells at OD660 of 0.05, the cultures were incubated at 37 °C with a reciprocal shaker (120 rpm), and cell density at OD660 was measured at various intervals.

and recrystallized from H_2O to give **4** (29.3 g, 46%), as colorless prisms, mp 205—212 °C (lit.[4] mp 208 °C). IR (Nujol) cm^{-1}: 3040, 1800, 1720, 1715, 1260, 1060. ^{1}H-NMR (60 MHz, DMSO-d_6) δ: 2.86 (4H, s), 5.45 (2H, s), 12.64 (2H, br s).

5-Oxo-1,3-dioxolane-4,4-diacetic Anhydride (5) Phosphorus oxychloride (100 ml, 1.07 mol) was added dropwise to a stirred solution of **4** (200 g, 0.98 mol) and *N*,*N*-dimethylaniline (248 ml, 1.96 mol) in $CHCl_3$ (500 ml) at room temperature. The mixture was stirred at room temperature and cooled to 0 °C. The precipitate was collected by filtration and washed with $CHCl_3$ and recrystallized from AcOEt to give **5** (111 g, 61%) as colorless needles, mp 154—156 °C (lit.[5] mp 153 °C). IR (Nujol) cm^{-1}: 1810, 1780, 1220, 1060. ^{1}H-NMR (60 MHz, DMSO-d_6) δ: 3.38 (4H, s), 5.64 (2H, s).

Benzyl 5-Oxo-1,3-dioxolane-4,4-diacetate (6) A mixture of **5** (16.0 g, 86 mmol), pyridine (14.0 ml, 173 mmol), and benzyl alcohol (24.0 ml, 232 mmol) in $CHCl_3$ (288 ml) was refluxed for 1 d and the solvent was removed. The residue was poured into 10% HCl solution, extracted with AcOEt, and then extracted with saturated $NaHCO_3$ solution. The aqueous layer was made acidic with 10% HCl solution and extracted with AcOEt. The AcOEt layer was washed with brine, dried, and then solvent was removed. Recrystallization of the residue from AcOEt gave **6** (20.4 g, 81%) as colorless needles, mp 147—149 °C (lit.[6] mp 143—145 °C). IR (Nujol) cm^{-1}: 3000, 1790, 1730, 1700, 1260, 1050. ^{1}H-NMR (60 MHz, acetone-d_6) δ: 2.98 (2H, s), 3.03 (2H, s), 5.16 (2H, s), 5.45, 5.50 (each 1H, each s), 7.38 (5H, s), 10.5—12.0 (1H, br).

1,2-Dibenzyl Hydrogen Citrate (2)[3] A mixture of **6** (10.0 g, 34.0 mmol), triethylamine (31.0 ml, 222 mmol), and benzyl alcohol (36.0 ml, 348 mmol) in $CHCl_3$ (200 ml) was refluxed for 1 week and the solvent was removed. The residue was poured into 10% HCl solution, extracted with Et_2O, and then the solvent was removed. The residue was dissolved in saturated $NaHCO_3$ solution and washed with Et_2O. The aqueous layer was made acidic with 10% HCl solution and extracted with Et_2O. The Et_2O layer was washed with brine, dried, and then the solvent was removed. The hot hexane extract of the residue was recrystallized from Et_2O gave **2** (6.16 g, 49%) as colorless needles, mp 80—83 °C. IR ($CHCl_3$) cm^{-1}: 3500, 3020, 1720. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 2.88 (4H, s), 5.06 (2H, s), 5.14 (2H, s), 5.88—6.11 (1H, br s), 7.31 (10H, s).

(*S*)- and (*R*)-1,2-Dibenzyl Hydrogen Citrate ((*S*)-2, (*R*)-2) A mixture of **2** (28.6 g, 76.8 mmol) and (−)-cinchonidine (22.6 g, 76.8 mmol) was dissolved in AcOEt (150 ml) at 60 °C and stirred at room temperature overnight. The precipitate was collected by filtration. The precipitate for purification of (*S*) citrate and the filtrate for purification of (*R*) citrate were separated by repeating this recrystallization five times. The precipitate was poured into 10% HCl solution, and then extracted with Et_2O. The Et_2O layer was washed with brine, dried, and then solvent was removed. The resulting oil was cooled for 2 d in the freezer to give (*S*)-**2** (6.89 g, 24%, 99% ee) as a colorless crystalline solid. The solvent of above filtrate was removed. A mixture of the residue (20.2 g, 54.2 mmol) and (−)-quinine (17.6 g, 54.2 mmol) was dissolved in AcOEt (300 ml) at 60 °C and stirred at room temperature overnight. This recrystallization was repeated four times. The precipitate was poured into 10% HCl solution and extracted with Et_2O. The Et_2O layer was washed with brine, dried, and then solvent was removed. The resulting oil was cooled for 2 d in the freezer to give (*R*)-**2** (6.88 g, 24%, 99% ee) as a colorless crystalline solid. The enantiomeric excess was determined by HPLC (chiral column, Daicel Chiralcel OD; column temperature, room temperature; eluent, hexane : isopropyl alcohol : trifluoroacetic acid = 80 : 10 : 1; flow rate = 2.0 ml/min; wavelength, 254 nm; (*S*)-**2**, t_R = 11.2 min; (*R*)-**2**, t_R = 8.4 min).

(*S*)-**2**: mp 45—46 °C, $[\alpha]_D^{24}$ −6.80° (*c* = 1.03, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 3500, 3020, 1740, 1720. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.85, 2.87, 2.93, 2.96 (each 1H, each d, *J* = 16.0 Hz), 4.20 (2H, br s), 5.08 (2H, s), 5.16 (2H, s), 7.28—7.36 (10H, m). FAB-MS (positive ion mode) *m*/*z*: 373 $(M+1)^+$. *Anal*. Calcd for $C_{20}H_{20}O_7$: C, 64.51; H, 5.41. Found: C, 64.41; H, 5.56.

(*R*)-**2**: mp 43—47 °C, $[\alpha]_D^{24}$ +6.79° (*c* = 1.00, $CHCl_3$). *Anal*. Calcd for $C_{20}H_{20}O_7$: C, 64.51; H, 5.41. Found: C, 64.30; H, 5.59.

(*S*)-1,2-Dimethyl Hydrogen Citrate ((*S*)-7) Methanol (50 ml) was added dropwise at 0 °C to a suspension of NaH (2.53 g, 62.5% despersion in mineral oil, 65.9 mmol) in Et_2O (10 ml) and a solution of (*S*)-**2** (2.50 g, 6.71 mmol) in MeOH (50 ml) was added dropwise at 0 °C to the mixture. The mixture was stirred at 0 °C for 1 h, poured into 10% HCl solution, and then extracted with AcOEt. The AcOEt layer was washed with brine, dried, and concentrated *in vacuo*. The residue was purified by flash column chromatography (SiO_2, AcOEt : hexane = 1 : 4) to give (*S*)-**7** (1.01 g, 68%) as colorless needles, mp 54—57 °C, $[\alpha]_D^{24}$ −3.39° (*c* = 1.00, MeOH). IR ($CHCl_3$) cm^{-1}: 3500, 3000, 1710. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.82, 2.85, 2.91, 2.94 (each 1H, each d, *J* = 15.5 Hz), 3.70 (3H, s), 3.83 (3H, s). FAB-MS (positive ion mode) *m*/*z*: 221 $(M+1)^+$. *Anal*. Calcd for $C_8H_{12}O_7$: C, 43.64; H, 5.49. Found: C, 43.59; H, 5.43.

(*R*)-1,2-Dimethyl Hydrogen Citrate ((*R*)-7) From (*R*)-**5** (2.50 g, 6.71 mmol), (*R*)-**7** (0.78 g, 66%, colorless needles) was prepared by the same procedure mentioned above, mp 53—54 °C, $[\alpha]_D^{24}$ +3.78° (*c* = 1.03, MeOH) (lit.[7] $[\alpha]_D$ +4.0° (MeOH)). High-resolution positive ion FAB-MS: Calcd for $C_8H_{13}O_7$ $(M+1)^+$: 221.0661. Found: 221.0695.

(*S*)-2-(*N*-*tert*-Butoxycarbonylamino)-*N*′-(2-hydroxyethyl)propanamide (10) A mixture of Boc-L-alanine (**8**, 2.00 g, 10.6 mmol) and 1-(3-*N*,*N*-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 5.00 g, 26.1 mmol) was dissolved at 0 °C with stirring in CH_2Cl_2 (50 ml). Ethanolamine (**9**, 2.0 ml, 33 mmol) was added to the solution and the mixture was

stirred at room temperature for 12 h. After removal of solvent, the residue was purified by column chromatography (SiO_2, AcOEt) to give **10** (1.43 g, 58%) as a colorless oil, $[\alpha]_D^{24}$ −16.8° (c=1.00, MeOH) (lit.[7]) $[\alpha]_D$ −17° (c=1, MeOH)). IR ($CHCl_3$) cm^{-1}: 3430, 3320, 1650. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.37 (3H, d, J=7.0 Hz), 1.44 (9H, s), 3.10 (1H, t, J=5.0 Hz), 3.36—3.48 (2H, m), 3.71 (2H, dt, J=5.0 Hz), 4.15 (1H, m), 5.17 (1H, m), 6.77 (1H, m). High-resolution positive ion FAB-MS: Calcd for $C_{10}H_{21}N_2O_4$ $(M+1)^+$: 233.1501. Found: 233.1529.

(*R*)-2-[(*S*)-2-(*N*-*tert*-Butoxycarbonylamino)propanamido]ethyl 2,3-Dibenzyl Citrate (11a) A mixture of (*S*)-**2** (3.00 g, 8.06 mmol), **10** (4.85 g, 20.9 mmol), and 4-dimethylaminopyridine (DMAP, 1.54 g, 12.6 mmol) in CH_2Cl_2 (100 ml) was stirred at 0 °C. EDC (2.00 g, 10.4 mmol) was added to the solution and the mixture was stirred at room temperature for 3 h. After removal of the solvent, the residue was poured into 10% HCl solution and extracted with AcOEt. The AcOEt layer was washed with brine, dried, and concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2, AcOEt : hexane=1 : 2) to give **11a** (3.18 g, 67%) as a light yellow oil, $[\alpha]_D^{24}$ +16.0° (c=1.14, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 3430, 1730, 1670. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.34 (3H, d, J=7.0 Hz), 1.43 (9H, s), 2.79, 2.93 (each 1H, each d, J=15.0 Hz), 2.86, 2.98 (each 1H, each d, J=16.0 Hz), 3.14—3.59 (2H, m), 3.92—4.24 (3H, m), 4.54 (1H, br s), 5.09 (1H, br s), 5.09 (2H, s), 5.14, 5.20 (each 1H, each d, J=12.0 Hz), 6.66 (1H, br s), 7.31—7.35 (10H, m). High-resolution positive ion FAB-MS: Calcd for $C_{30}H_{39}N_2O_{10}$: $(M+1)^+$: 587.2605. Found: 587.2556.

(*S*)-2-[(*S*)-2-(*N*-*tert*-Butoxycarbonylamino)propanamido]ethyl 2,3-Dibenzyl Citrate (11b) From (*R*)-**2** (4.00 g, 10.7 mmol), **11b** (4.18 g, 66%, colorless oil) was prepared by the same procedure mentioned above. $[\alpha]_D^{24}$ −9.11° (c=1.09, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 3390, 1730, 1670. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.36 (3H, d, J=7.5 Hz), 1.43 (9H, s), 2.79, 2.94 (each 1H, each d, J=15.0 Hz), 2.84, 2.97 (each 1H, each d, J=16.0 Hz), 3.40 (2H, m), 4.03—4.20 (3H, m), 4.34 (1H, br s), 5.09 (2H, s), 5.13, 5.20 (each 1H, each d, J=12.0 Hz), 5.31 (1H, br s), 6.59 (1H, br s), 7.30—7.36 (10H, m). High-resolution positive ion FAB-MS: Calcd for $C_{30}H_{39}N_2O_{10}$ $(M+1)^+$: 587.2605. Found: 587.2516.

1-Benzyl Hydrogen 2-Oxoglutarate (12) A mixture of 2-oxoglutaric acid (2.00 g, 13.7 mmol) and dicyclohexylamine (1.36 ml, 6.83 mmol) was dissolved at 50 °C with stirring in dimethylformamide (DMF, 80 ml). Benzyl bromide (0.82 ml, 6.9 mmol) was added to the solution and the mixture was heated at 50 °C with stirring for 1 h. The mixture was poured into water and extracted with Et_2O. The Et_2O layer was washed with brine, dried, and concentrated *in vacuo*. The residue was washed with petr. ether and Et_2O and recrystallized from Et_2O to give **12** (1.09 g, 67%) as colorless needles, mp 68—70 °C. IR ($CHCl_3$) cm^{-1}: 3000, 1780, 1720, 1265. ^{1}H-NMR (60 MHz, CDCl3) δ: 2.50—3.40 (m, 4H), 5.28 (s, 2H), 7.38 (s, 5H). FAB-MS (positive ion mode) m/z: 237 $(M+1)^+$. *Anal*. Calcd for $C_{12}H_{12}O_5$: C, 61.02; H, 5.12. Found: C, 61.15; H, 5.25.

(*R*)-2-[(*S*)-2-(2-Benzyloxycarbonyl-2-hydroxy-5-oxo-1-pyrrolidinyl)-propanamido]ethyl 2,3-Dibenzyl Citrate (13a) A mixture of **11a** (1.01 g, 1.72 mmol) and trifluoroacetic acid (3 ml, 38.9 mmol) was stirred at 0 °C for 3 h. After removal of the solvent, the residue was poured into a saturated $NaHCO_3$ solution and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with brine, dried, and concentrated *in vacuo*. A mixture of the residue and **12** (500 mg, 2.12 mmol) in CH_2Cl_2 (40 ml) was stirred at 0 °C. EDC (470 mg, 2.45 mmol) was added to the solution and the mixture was stirred at room temperature for 24 h. After removal of the solvent, the residue was poured into 10% HCl solution and extracted with AcOEt. The AcOEt layer was washed with brine, dried, and concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2, AcOEt : hexane=1 : 2) to give **13a** (487 mg, 40%) as a yellow oil, $[\alpha]_D^{24}$ +15.9° (c=1.01, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 3400, 1730. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.35, 1.40 (each 1.5H, each d, J=7.5, 7.0 Hz), 2.13—2.24 (1H, m), 2.34—2.58 (3H, m), 2.79, 2.80 (each 0.5H, each d, J=15.0, 15.5 Hz), 2.83, 2.84 (each 0.5H, each d, J=16.0 Hz), 2.92, 2.94 (each 0.5H, each d, J=15.0, 15.5 Hz), 2.95, 2.96 (each 0.5H, each d, J=16.0 Hz), 2.98—3.53 (2H, m), 3.83, 4.57 (each 0.5H, each q, J=7.5, 7.0 Hz), 3.89—4.18 (2H, m), 4.58, 4.82, 5.42, 5.81 (each 0.5H, each br s), 5.075, 5.081 (each 1H, each s), 5.11, 5.13, 5.16, 5.18 (each 0.5H, each d, J=12.0 Hz), 5.18, 5.23, 5.26, 5.30 (each 0.5H, each d, J=12.0 Hz), 6.90, 7.03 (each 0.5H, each m), 7.31—7.37 (15H, m). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 14.1, 14.4, 29.2, 29.3, 32.1, 33.8, 38.4, 38.5, 43.6, 43.8, 43.8, 44.1, 52.2, 52.4, 63.5, 63.6, 66.8, 68.05, 68.12, 68.4, 68.6, 73.26, 73.27, 89.5, 90.2, 128.3—128.9, 134.2, 134.3, 134.9, 135.2, 168.7, 168.9, 169.3, 171.0, 171.2, 172.2, 172.5, 173.4, 173.7, 175.0, 175.6. High-resolution positive ion FAB-MS: Calcd for $C_{37}H_{41}N_2O_{12}$ $(M+1)^+$: 705.2660. Found: 705.2585.

(*S*)-2-[(*S*)-2-(2-Benzyloxycarbonyl-2-hydroxy-5-oxo-1-pyrrolidinyl)-propanamido]ethyl 2,3-Dibenzyl Citrate (13b) From **11b** (2.97 g, 5.06 mmol), **13b** (1.73 g, 49%, light yellow oil) was prepared by the same procedure mentioned above, $[\alpha]_D^{24}$ −17.6° (c=1.01, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 3400, 1730. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.33, 1.41 (each 1.5H, each d, J=7.5, 7.0 Hz), 2.14—2.29 (1H, m), 2.35—2.61 (3H, m), 2.78, 2.80 (each 0.5H, each d, J=15.0 Hz), 2.85, 2.86 (each 0.5H, each d, J=16.0 Hz), 2.93 (1H, d, J=15.0 Hz), 2.959, 2.964 (each 0.5H, each d, J=16.0 Hz), 2.99—3.63 (2H, m), 3.85, 4.53 (each 0.5H, each q, J=7.5, 7.0 Hz), 3.92—4.17 (2H, m), 4.60, 4.66, 5.42, 5.50 (each 0.5H, each s), 5.07, 5.08 (each 1H, each s), 5.13, 5.15, 5.17, 5.19 (each 0.5H, each d, J=12.0 Hz), 5.18, 5.23, 5.27, 5.30 (each 0.5H, each 0.5H, J=12.0 Hz), 6.98 (1H, m), 7.29—7.39 (15H, m). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 13.9, 14.3, 29.3, 29.4, 31.7, 33.9, 38.3, 43.7, 43.8, 44.0, 52.10, 52.14, 63.6, 64.0, 66.76, 66.80, 68.1, 68.2, 68.5, 68.6, 73.27, 73.30, 89.4, 90.1, 128.2—128.9, 134.3, 134.8, 134.9, 135.2, 168.77, 168.85, 169.4, 171.0, 171.3, 172.3, 173.6, 173.8, 174.8, 175.5. High-resolution positive ion FAB-MS: Calcd for $C_{37}H_{41}N_2O_{12}$ $(M+1)^+$: 705.2660. Found: 705.2759.

(*R*)-2-[(*S*)-2-(2-Carboxy-2-hydroxy-5-oxo-1-pyrrolidinyl)propanamido]-ethyl Dihydrogen Citrate (1a) A mixture of **13a** (487 mg, 0.691 mmol) and 20% Pd/C (70 mg, 0.13 mmol) in AcOEt (30 ml) was stirred at room temperature for 4 h under H_2 atmosphere. The mixture was filtered off and the solvent was removed to give **1a** (295 mg, 98%) as a colorless amorphous solid, $[\alpha]_D^{24}$ +8.67° (c=1.00, MeOH) (lit.[1a]) $[\alpha]_D^{20}$ +13.3° (c=1.4, MeOH)). IR (Nujol) cm^{-1}: 3310, 1720, 1650, 1540. The data for ^{1}H-NMR and ^{13}C-NMR is described in Table 2. High-resolution positive ion FAB-MS: Calcd for $C_{16}H_{23}N_2O_{12}$ $(M+1)^+$: 435.1251. Found: 435.1264.

(*R*)-2-[(*S*)-2-(2-Carboxy-2-hydroxy-5-oxo-1-pyrrolidinyl)propanamido]-ethyl Dihydrogen Citrate (1b) From **13b** (411 mg, 0.583 mmol), **1b** (248 mg, 98%, colorless amorphous) was prepared by the same procedure mentioned above, $[\alpha]_D^{24}$ −12.4° (c=1.19, MeOH). IR (Nujol) cm^{-1}: 3300, 1700. The data for ^{1}H-NMR and ^{13}C-NMR is described in Table 2. FAB-MS: Calcd for $C_{16}H_{23}N_2O_{12}$ $(M+1)^+$: 435.1251. Found: 435.1302.

Siderophore Activity Assays and evaluation of siderophore activities were carried out according to the methods described previously.[1c]

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