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Corynebactin and Enterobactin: Related Siderophores of Opposite Chirality

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The major role of siderophores in microbial iron transport and bacterial pathogenicity is now well established.[1] These low molecular weight chelators are expressed to overcome the insolubility of Fe^{3+} at pH 7 ($\sim 10^{-18}$ M).[2] The ferric complexes enter the bacterial cell via specific receptor proteins on the cell membrane. Once incorporated, iron is released via reduction, hydrolysis, or ligand-exchange mechanisms.[3] Enterobactin (**1**) is produced by both Gram-negative[4a,b] and Gram-positive[4c] bacteria, and has an extraordinarily high stability ($K_f = 10^{49}$)[5] with metal coordination at neutral pH accomplished through the six catecholate oxygens.[6] The chirality of the iron center in enterobactin is Δ,[6] and this chirality, while not essential for receptor recognition and outer membrane transport,[7] **is** essential for iron utilization; the mirror image enantio-enterobactin complex does not promote microbial growth.[8] Recently a closely related siderophore, corynebactin (**2**), was found to be produced by the Gram-positive *Corynebacterium glutamicum*.[9] Both siderophores are based on a trilactone backbone, consisting of L-serine units in enterobactin and L-threonine units in corynebactin. Each corynebactin side chain also contains one glycine spacer. Remarkably, the iron complexes of these two closely related siderophores have opposite chirality!

The synthetic analogue **7**, which contains the enterobactin triserine trilactone backbone and the corynebactin side chain, was synthesized to investigate the effect of this spacer and the methylation of the trilactone ring. This paper reports the relative stereochemistry of the ferric complexes of **1**, **2**, and **7** (Figure 1).

The preparation of the serine trilactone has been described previously in the high-yield synthesis of enterobactin.[10] The fluorination of BnGLYCAM (**3**) to yield 2,3-bis(benzyloxy)-glycinylfluoride benzoylamide (**4**) was based upon well-established peptide syntheses.[11,12] The trilactone **5** was then combined with **4** to give the hexabenzyl-protected ligand **6**.[13] Hydrogenation of **6** with a Pd/C catalyst under pressure afforded the serine corynebactin ligand **7** in quantitative yield[14] (Scheme 1).

The ferric complexes of **1**, **2**, and **7** were prepared from the solutions of the free ligands in water buffered at pH = 7 together with equivalent amounts of iron trichloride. All samples were purified with HPLC to remove contaminants.[15] ESI^- mass spectrometry displayed the molecular ion signals for these negatively charged ferric complexes.[16] Circular dichroism (CD) measurements were obtained for the iron(III) complexes of both corynebactin and serine-corynebactin.[17] In contrast to the Δ-iron(III)−enterobactin complex, the ferric corynebactin complex has a Λ conformation. The ferric serine analogue **7**, which can be considered a hybrid of enterobactin and corynebactin, appears to be a mixture of Δ- and Λ-isomers.

All ferric complexes of **1**, **2**, and **7** reveal intense CD bands at 270 nm corresponding to the carbonyl amide in the ligand. The bands of ferric corynebactin and serine-corynebactin (350 nm) and ferric enterobactin (330 nm) are due to the chiral trilactone scaffold

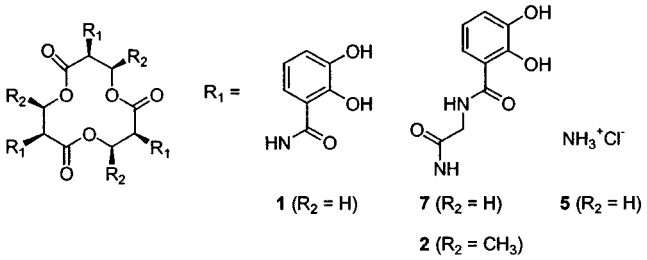
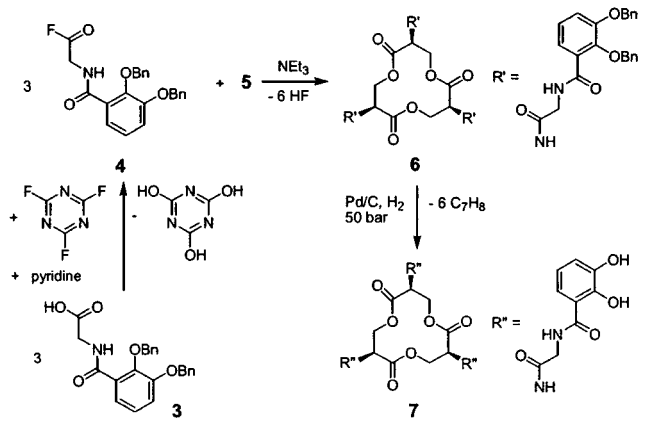


Figure 1. Siderophores enterobactin (**1**) and corynebactin (**2**). The synthetic analogue **7** is a hybrid, composed of the serine trilactone connected to the side chain of corynebactin. The triserine trilactone trihydrochloride (**5**) was used for the preparation of **7**.

Scheme 1. Synthesis of serine corynebactin (**7**)



(Figure 2). Two characteristic ferric catechol transitions are observed in the visible region at 435 nm and between 520 and 540 nm. These bands arise from ligand-to-metal charge transfer (LMCT) transitions and are therefore sensitive to the chirality at the metal center[18] (Table 1).

Molecular modeling was used to probe the effect of both the addition of the glycine spacer and also the methylation of the trilactone backbone.[19] The lowest-energy conformation for both the ferric corynebactin and ferric serine-corynebactin complexes is the Λ-isomer, while the lowest-energy conformation of the ferric enterobactin complex is the Δ-isomer. The effect of the glycine spacer is highlighted by a comparison of serine-corynebactin to enterobactin. The longer side chains of serine-corynebactin favor the Λ conformation, while the shorter side chains of enterobactin favor the Δ-isomer. A comparison of corynebactin to serine-corynebactin shows that methylation of the trilactone backbone does not change the metal center chirality of the lowest-energy conformer. However, methylation of the trilactone does influence the chirality of higher-energy conformers. Therefore, both effects are important in determining the overall stability of the ferric complex.

In summary, the chirality of the ferric complexes of corynebactin

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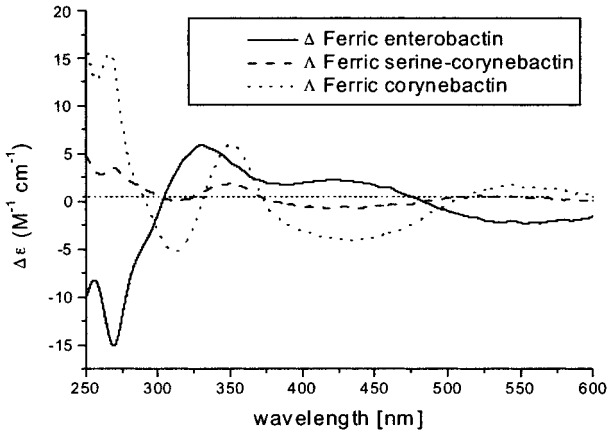

Figure 2. Circular dichroism spectra of ferric enterobactin, ferric corynebactin, and ferric serine-corynebactin in water. $T = 22$ °C.

Table 1. Circular Dichroism Results of Ferric Complexes

ferric complex	diastereomer	λ_{max} [nm]	$\Delta\epsilon$ [M^{-1} cm^{-1}]
enterobactin (**1**)	Δ	553	−2.2
corynebactin (**2**)	Λ	545	+1.7
serine-corynebactin (**7**)	Λ (slight)	520	+0.6

and a new serine trilactone analogue (**7**) have been determined. While the chirality of ferric enterobactin is Δ, ferric corynebactin (**2**) is Λ. The hybrid analogue **7** is a mixture of Δ- and Λ-isomers. It will be interesting to see how the microbial transport properties respond to these different chiralities.

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Supporting Information Available: Detailed synthetic procedures for **1**, **2**, and **7** and intermediates (PDF). This material is available at http://pubs.acs.org.

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(12) **3**: ^{1}H NMR ($CDCl_3$) δ 7.72−7.75 (m, 1H, N*H*), 7.14−7.47 (m, 13H, H_{ar}), 5.18 (s, 2H, CH_2), 5.14 (s, 2H, CH_2), 4.06 (d, 2H, glycine CH_2). EI$^+$-MS *m*/*z* 391 (M^+, 20). Anal. Calcd (Found) for **3** C, 70.58 (70.90); H, 5.41 (5.37); N 3.58 (3.22). **4**: ^{1}H NMR ($CDCl_3$) δ 4.17 (dd, 2H, CH_2), 5.17 (d, 4H, CH_2), 7.13−7.71 (m, 13H, H_{ar}), 8.52 (t, 1H, N*H*). ^{19}F NMR ($CDCl_3$) δ −16 (s). EI$^+$-MS *m*/*z* 393 (M^+, 80).

(13) **6**: UV−vis λ [nm] = 215, 268, 444 (charge-transfer bands); IR $\nu = 1641$ (amide CO), 1750 (ester CO). ^{1}H NMR ($CDCl_3$) δ 3.69−3.90 (ddd, J = 5, 15, 23 Hz, 6H, CH_2), 4.09 (dd, J = 3, 11 Hz, 3H, gly-CH_2), 4.82 (d, J = 8 Hz, 3H, C*H*), 4.90 (dd, J = 3, 11 Hz, 3H, gly-CH_2), 5.00−5.17 (m, 12H, benzyl-CH_2), 7.09−7.72 (m, 39H, H_{ar}), 8.30 (d, J = 8 Hz, 3H, N*H*), 8.64 (t, J = 5 Hz, 3H, N*H*). ^{13}C NMR ($CDCl_3$) δ 43.4, 64.9, 71.2, 117.5, 123.0, 124.5, 126.5, 127.7, 128.3, 128.5, 128.6, 128.7, 129.1, 136.3, 147.3, 152.0, 165.7 (CO), 168.8 (CO), 169.7 (CO). FAB$^+$-LR-MS: *m*/*z* 1381 (M^+, 80), 1290 (M^+ − benzyl, 5). FAB$^+$-HR-MS *m*/*z* 1381.495410 (calcd), found 1381.498135 for $C_{78}H_{73}N_6O_{18}$, δ = 2.7 mDa. Anal. Calcd (Found) for **6**: C 67.82 (67.87), H 5.25 (5.23), N 6.08 (5.70); mp = 78 °C.

(14) **7**: ^{1}H NMR (d_6-acetone) δ 4.11 (s, 6H, gly-CH_2), 4.32 (d, J = 11 Hz, 3H, CH_2), 4.61 (d, J = 11 Hz, 3H, CH_2), 4.78 (s, 3H, C*H*), 6.68 (t, J = 8 Hz, 3H, H_{ar}), 6.93 (d, J = 8 Hz, 3H, H_{ar}), 7.27 (d, J = 8 Hz, 3H, H_{ar}), 7.95 (s, br, N*H*), 8.45 (s, br, N*H*). ^{13}C NMR (d_6-acetone) δ 42.9, 52.9, 65.1, 114.9, 117.6, 118.8, 119.1, 146.6, 149.9, 169.1 (CO), 169.4 (CO), 171.0 (CO). FAB$^+$-LR-MS *m*/*z* 841 (M^+, 85). FAB$^+$-HR-MS *m*/*z* 840.210375 (calcd), found: 840.208609 for $C_{36}H_{36}N_6O_{18}$, δ = 1.8 mDa; mp = 130 °C.

(15) Ligands (**1**, **2**, and **7**) were dissolved in a mixture of 2 mL water and 3 mL of methanol. The initial concentration of the complex was 0.5 mM. Ferric ion dissolved in 10 mM HCl was added to the solution to make a 1:1 complex. A color change was observed, and the solution was centrifuged for 10 min (14000 rpm Eppendorf) to remove solid precipitate. Impurities were removed by preparative HPLC eluting with H_2O:MeOH (35:65), with a pressure of approximately 1000 psi and a flow rate of 10 mL/min. The intensity of the eluent was measured at 254 nm. The colored fraction of each ligand was collected.

(16) Ferric enterobactin: ESI$^-$-HR-MS *m*/*z* 721.047857 (calcd), found 721.048500 for $C_{30}H_{23}N_3O_{15}Fe$ [M − H]$^-$, δ = 0.6 mDa, ferric corynebactin: ESI$^-$-HR-MS *m*/*z* 934.159198 (calcd), found 934.160100 for $C_{39}H_{38}N_6O_{18}Fe$ [M − H]$^-$, δ = 0.9 mDa, ferric serine corynebactin: ESI$^-$-HR-MS *m*/*z* 892.112248 (calcd), found 892.112100 for $C_{36}H_{32}N_6O_{18}Fe$ [M − H]$^-$, δ = 0.1 mDa.

(17) The pure fraction collected from HPLC was measured by UV−vis spectrophotometry (Cary 300 Scan). The concentrations of the ferric compounds were approximately 0.066 mM (ϵ = 15000 M^{-1} cm^{-1} at 330 nm). The CD spectra of the complexes were measured using a Jasco J-810 spectrometer.

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