1,2-Hopobactin: a hydroxamate analog of enterobactin

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Hopobactin is a 1-hydroxy-2-oxopyridine-6-carboximide analog of enterobactin that forms isostructural complexes.

Enterobactin is a catechol-based siderophore that is one of the strongest ferric iron binders.[1] The remarkable binding properties of enterobactin are attributed to a unique binding cavity that stabilizes the metal complex.[2] This hexacoordinate cavity is composed of a trislactone ring where three 2,3-dihydroxybenzamide units are attached to the L-serine α-amino groups (Fig 1). Upon Fe^{3+} binding, the lactone ring allows for coplanarity of the amide bond and the coordinated catecholate moiety. The conjugation with the amide increases the acidity of the phenol groups and results in an inter-chain hydrogen bond between the amide NH and the 2-catecholate oxygen. As a consequence of the trislactone chirality and the hydrogen bonds, the natural enterobactin iron complex preferentially adopts a Δ configuration. Synthetic analogs of enterobactin have demonstrated that deviations from the enterobactin structure result in less profound properties.[3] Here we present the synthesis of a hydroxamic acid analog of enterobactin, 1,2-hopobactin (HOPO = 1-hydroxy-2-oxopyridine-6-carboximide, Fig. 1). The HOPO ligand and its anion have resonance forms that are isoelectronic with pyrocatechol and have a similar structure. Attaching the HOPO moiety through an amide linkage to the trislactone results in a perfect structural analog of enterobactin that forms neutral complexes.

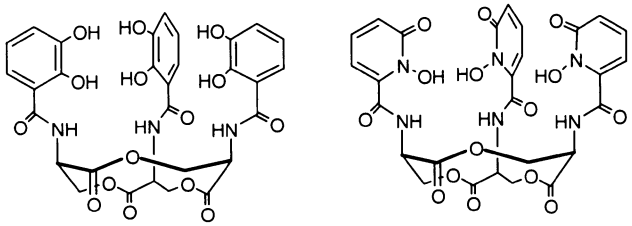
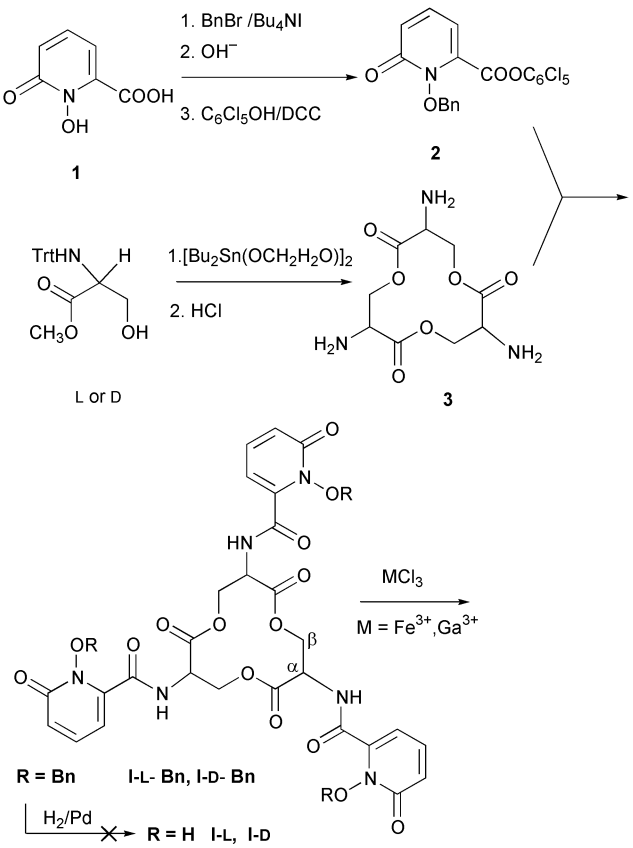


Fig. 1 Enterobactin (left) and its hydroxamic acid analog (right).

Two enantiomeric trilactones were prepared from L and D-serine (Scheme 1).[4a] 1,2-dihydro-1-hydroxy-2-oxopyridine-6-carboxylic acid [4b] was protected with benzyl groups, activated and coupled with the trislactone. Hydrogenolysis of the protected product gave a mixture of products and the desired compound could not be isolated.[5] Yet, by addition of ferric or gallium acetylacetonates to the mixture, the corresponding complexes could be purified by preparative TLC in very low yields. The complexes were characterized by NMR (for Ga), IR and MS.[6] Removal of the benzyl protecting groups was accomplished quantitatively using Lewis acids like $FeCl_3$ or $GaCl_3$. After the reaction was complete, the pH was adjusted to 8 and the complexes were purified by chromatography. The spectroscopic data for the ferric and gallium complexes were identical to those of the complexes isolated by addition of ferric and gallium acetylacetonate as described above.

Both gallium complexes I-D-Ga and I-L-Ga were characterized by NMR (Table 1). Since Ga^{3+} has the same charge as Fe^{3+} and a similar radius, it is a good diamagnetic equivalent of iron.[7] I-L-Ga displays one set of signals in the NMR time scale. Due to the homochirality of the trislactone, the formation of the diasteromeric complexes (L,L,L-Δ and L,L,L-Λ) would be expected to form two sets of NMR signals. The observance of only one set indicates the formation of only one diastereoisomer (within the limits of detection of NMR). The possibility of fast exchange between the two isomers can be disregarded, as isomerization reactions of hydroxamates are slow enough to allow detection of two geometrical isomers.



Scheme 1 Synthetic scheme for the preparation of 1,2-hopobactin (I-L) and enantioenterobactin (I-D).

I-D-Ga gave an NMR spectrum identical to that of I-L-Ga. Because the two compounds are based on enantiomeric trislactones, the identical spectra reveal that the complexes are

Table 1 ^1H NMR Spectra of enterobactin and analogs

Compound	H^{α}	$H^{\beta}{}_1$	$H^{\beta}{}_2$	H^{N}
Enterobactin[a]	4.94 (m)	4.66 (dd)	4.41 (dd)	9.06
	J = 8.06, 4.19	*J* = 8.06, 10.8	*J* = 4.19, 10.8	*J* = 6.52
Enterobactin-Ga[a]	5.12	5.22	3.80	11.72
		J = 10.68	*J* = 10.68	*J* = 9.83
I-protected[b]	4.80 (d)	4.76 (d)	3.68 (d)	8.12
	J = 10.9	*J* = 10.9	*J* = 10.3	
I-D-Ga[b]	5.37 (t of d)	5.56 (dd)	3.96 (dd)	10.70 (d)
I-L-Ga[b]	*J* = 1.3, 6.4	*J* = 1.1, 7.2	*J* = 1.7, 7.2	*J* = 6.3
TBA[c]	5.05 (m)	4.91 (dd)	4.65 (dd)	7.25
	J = 7.8	*J* = 3.1, 11.5	*J* = 3.3, 11.5	

[a] Data for enterobactin in DMSO-d_6.[8] [b] $CDCl_3$. [c] Trisbenzylamide enterobactin $CDCl_3$.[2a]

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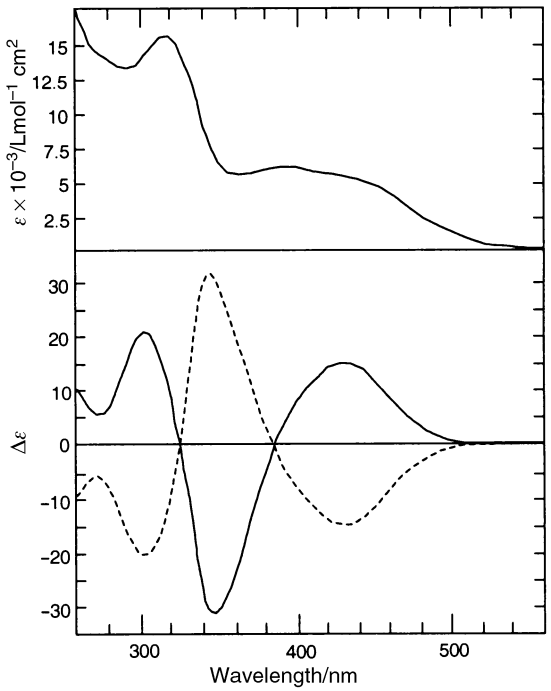

Fig. 2 UV/CD spectra of I-L-Fe (…..) and I-D-Fe (__) in $CHCl_3$.

enantiomers and that the D,D,D trislactone is directing the formation of a complex with opposite helicity. Therefore, the NMR indicates that the enterobactin skeleton serves as a stereospecific template for the formation of exclusively one stereoisomer depending on the chirality of the serine units.

For I-Ga, the vicinal proton–proton coupling constants for the $C_\alpha H$-$C_{\beta 1}H$ and $C_\alpha H$-$C_{\beta 2}H$ were both small (J = 1.1 and 1.7 Hz). These values indicate equatorial–equatorial and axial–equatorial relationships between the vicinal protons and, therefore, an axial arrangement of the HOPO ring. The pronounced shift of the amide proton to a lower field upon Ga^{3+} complexation compared to the protected I-D is compatible with hydrogen-bonding. A similar shift was previously observed in enterobactin and several triscatecholamides as well. In enterobactin the shift was attributed to a hydrogen bond between the amide NH and the 2-catecholate oxygen in the complex. This suggestion was supported by theoretical calculations, and it was shown experimentally that replacement of the NH with *N*-Me results in a lower binding affinity. It is suggested that a similar stabilization occurs in I-Ga between the amide proton and the HOPO N-oxygen.

The isolated iron complexes exhibit two absorption maxima at 321 nm (ε = 16430 M^{-1} cm^{-1}) and 398 nm (ε = 6375 M^{-1} cm^{-1}) in chloroform (Fig. 2). The LMCT band has an additional shoulder at 450 nm (ε = 5575 M^{-1} cm^{-1}). The CD spectra of I-L-Fe and I-D-Fe showed opposite Cotton effects. The fact that the spectra are mirror images confirms that the enantiomeric trislactone skeleton induced opposite helicity around the iron center.

The determination of metal center chirality of the metal complex in solution is possible through comparison of the solution CD spectra. However, the correlation of the rotary power with left or right-handed helical stereochemistry requires an absolute assignment based on crystal structure data. As this data is not available, the ferric enterobactin CD spectrum was used as a reference.[9a] This comparison is based on the high structural and electronic similarity between the HOPO and enterobactin complexes together with the resemblance in their CD spectral shape.[9b] This correlation suggests that I-L induces the formation of the Δ iron complex while the I-D ligand induces the formation of the Λ isomer. This chiral induction is compatible with the one observed for enterobactin.

These results indicate that the unique structure of the enterobactin complex is preserved in the HOPO analog. This work demonstrates that careful design of ligands allows for the mimicking of the unique features of enterobactin while changing only desired properties. While the enterobactin complex is negatively charged and, therefore, water-soluble, hopobactin forms neutral M^{3+} complexes that are soluble in non polar organic solvents. Therefore, hopobactin is a potential ligand for liquid-membrane separations. The evaluation of these complexes as siderophore mimics is underway.

Notes and references

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5 The hydrogenolysis of HOPO resulted in hydrogenation of the ring or reduction to the lactam.
6 **I-Fe**: IR ($CDCl_3$)ν cm^{-1}: 3258 (NH), 1768 (COOC), 1676 (CONO, CONH) 1610 (CONO-Fe). FAB-MS *m/z* 725.20 [L-Fe]$^+$. **I-Ga**: IR ($CDCl_3$)ν cm^{-1}: 3286 (NH), 1766 (COOC), 1676 (CONO, CONH) 1610 (CONO-Ga). FAB-MS *m/z* 738.04 [L-^{69}Ga]$^+$.
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