Rational Reduction of the Conformational Space of a Siderophore Analog through Nonbonded Interactions: The Role of Entropy in Enterobactin

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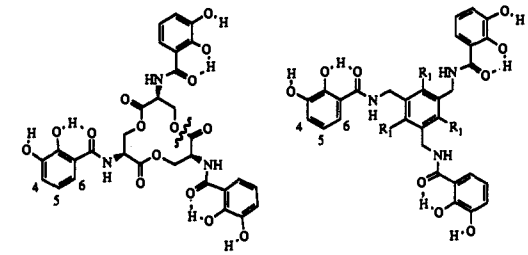
Predisposition[1] of ligands or receptors toward binding is a prevalent design feature in nature and significantly enhances selectivity and efficiency of complexation. Nature often achieves this organization through macrocyclic and nonbonded strategies whereby the conformational space of the ligand or receptor is severely constrained relative to a linear analog.[2] In this way, reorganizational costs of complexation are reduced. Biomimetic ligands and synthetic molecular receptors have generally relied on a macrocyclic strategy to achieve large selectivities.[3,4] Only recently have open-chain analogs extensively exploited nonbonded, conformational locking interactions[5] to organize and enhance the ligand binding efficiency.[6-8] Intramolecular hydrogen bonding has also been recently exploited to achieve similar enhancements of organization.[9]

Our interest in the predisposition of natural ligands stems from Enterobactin ($\mathbf{Ent_H}$) (Chart I), a siderophore[10] of *Escherichia coli*, which represents the ultimate in the arsenal of bacterial iron sequestering agents ($\log K_f(\mathbf{Ent_{Fe}}) \approx 49$).[11] To probe the design features of $\mathbf{Ent_H}$, many synthetic, tris-catecholamide analogs based on open-chain,[12] tripodal,[12-14] and macrocyclic[12,15-17] topologies have been synthesized, and all show lesser stability. One of the best analogs, $\mathbf{MecamH_6}$ ($\mathbf{2_H}$), has a K_f(Fe) ca. 10^6 smaller than that of $\mathbf{Ent_H}$[18,19] and equals that of linear Enterobactin ($\mathbf{l\text{-}Ent_H}$), the monohydrolyzed form.[20] Since the K_f(Fe) between $\mathbf{Ent_H}$ and $\mathbf{l\text{-}Ent_H}$ is greater than two-thirds entropically derived,[20] large enhancements in the stability of the synthetic complexes can be achieved by tailored predisposition of the free ligands. Reported here is the synthesis and the thermodynamic and structural characterization of a highly predisposed analog ($\mathbf{Et_3mecamH_6}$, $\mathbf{4_H}$) that solely exploits a network of nonbonded interactions for organization and displays the largest reported K_f(Fe) for a synthetic tris-catecholamide analog.[21]

Chart I



$\mathbf{Ent_H}$ Enterobactin $K_f(Fe)=10^{49}$

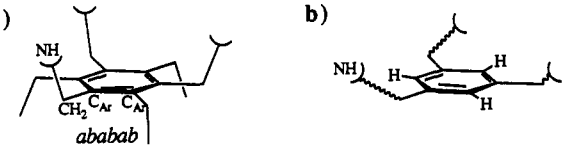
$\mathbf{l\text{-}Ent_H}$ linear Enterobactin $K_f(Fe)=10^{43}$ (hydrolyze at ξ)

$\mathbf{2_H}$ R_1 = H MecamH$_6$ $K_f(Fe)=10^{43}$

$\mathbf{3_H}$ R_1 = Me $(Me)_3$MecamH$_6$

$\mathbf{4_H}$ R_1 = Et $(Et)_3$MecamH$_6$ $K_f(Fe)=10^{47}$

Chart II



The concept behind the design of $\mathbf{4_H}$ derives from the conformation of hexaethylbenzene;[22,23] static gearing[24] of the methylene hydrogens assures that the β-groups alternate above (*a*) and below (*b*) the ring in a trigonally symmetric conformation (*ababab*).[25,26] This design orients the 1,3,5-catecholamide groups of $\mathbf{4_H}$ preferentially to one side of the central ring and maintains the $C_{Ar}C_{Ar}CH_2NH$ dihedral angles near 90° (Chart IIa).[22] This contrasts dramatically with the conformation of $\mathbf{MecamH_6}$ ($\mathbf{2_H}$) (Chart IIb).

The key step to the synthesis of $\mathbf{4_H}$ is the reduction of the 1,3,5-tricyano-2,4,6-triethylbenzene[27] to the triamine,[28,29] which is converted to the final ligand according to previously described methods.[30] A crystal structure of $\mathbf{4_H}$ confirms the expected *ababab* conformation in which a cavity is formed by three upwardly positioned catecholamide groups (Figure 1).[31] This cavity is filled by two acetone solvate molecules (not shown) which are hydrogen-bonded to two of the inwardly projected amide protons. The ortho-hydroxyl protons strongly hydrogen-bond to the amide carbonyl oxygen, resulting in the outward projection of the catecholate binding groups,[32] consistent with other related structures.[33-36] Significantly, this highly predisposed configu-

(1) The term "preorganized" will be reserved for those cases where the movement of the ligating atoms is on the order of the bond lengths in question, *i.e.*, <3 Å. Other ligands will be described as highly "predisposed".
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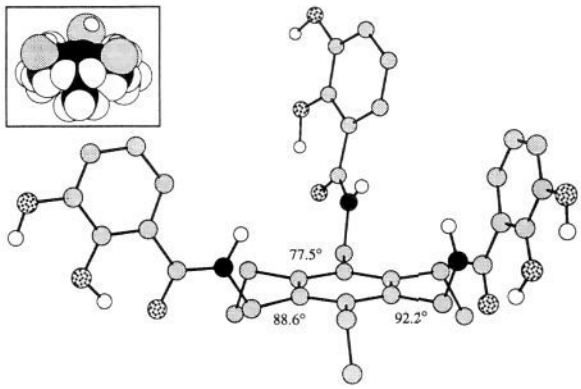

Figure 1. Structure of $Et_3mecamH_6$ ($\mathbf{4_H}$). Atoms shown include the hydroxyl and amide protons. The inset diagram is a space-filling model of the essential portions of the structure to convey the gearing of the methylene groups around the central ring.

Table I. ^1H NMR Shift (δ) Values of Ligands[a]

	ligand					
compd	NH	4-catechol	5-catechol	6-catechol	3-OH	2-OH
$\mathbf{Ent_H}$	9.24 (8.40)	6.95 (7.02)	6.71 (6.74)	7.34 (7.22)	(8.65)	(11.9)
$\mathbf{2_H}$	9.39 (8.70)	6.91 (6.95)	6.65 (6.60)	7.29 (7.25)	9.2	12.3
$\mathbf{3_H}$	8.65 (7.96)	6.89 (6.93)	6.64 (6.65)	7.34 (7.23)	9.2 (7.7)	12.2 (12.9)
$\mathbf{4_H}$	8.72 (7.92)	6.89 (6.94)	6.65 (6.64)	7.39 (7.23)	9.3 (7.7)	12.1 (12.9)
$\mathbf{Eba_H}$[b]	8.79 (8.12)	6.89 (6.95)	6.66 (6.70)	7.26 (7.23)	9.1 (7.6)	12.9 (13.0)

[a] Chemical shifts are referenced to TMS at 300 K in DMSO-d_6 (acetone-d_6). [b] 2,3-Dihydroxyethylbenzamide.

ration observed in the solid state is maintained in polar solutions, as indicated by the ^1H NMR spectra. Variable-temperature ^1H NMR studies[37] of $\mathbf{4_H}$ provide spectra consistent only with trigonal symmetry.[38] The intramolecular hydrogen-bonding motif is maintained in polar aprotic solvents, as evidenced by the strong NOE between the amide proton and the 6-H of the catecholamide rings in addition to the large downfield shift of the ortho-catechol hydrogen (Table I). The preferred inward projection of the amide proton over the central ring can be inferred from the upfield shift of ca. 0.7 ppm relative to $\mathbf{2_H}$ while the signals for the remaining protons are nearly identical.[39] Molecular mechanics calculations support this inward projection of the amide protons from primarily steric considerations.[40] Thus the combination of the geared hexaalkylaryl backbone of $\mathbf{4_H}$ and the steric constraints of the amide group preorganizes the amide functionality for metal complexation. In contrast, the catecholate rings can, at best, be described as only predisposed toward binding; a significant reorganization of the catecholate groups is required for complexation (180-deg rotations). Similar reorganizational requirements apply to $\mathbf{Ent_H}$.[41]

Thermodynamic characterization of $\mathbf{4_{Fe}}$ by spectrophotometric competition and potentiometric experiments[11] gives $K_f(\mathbf{4_{Fe}}) \approx 10^{47}$,[42] a 10^4 increase (ca. 5.5 kcal/mol at 300 K) relative to $K_f(\mathbf{2_{Fe}})$. Given the structural similarity and restricted conformational space of $\mathbf{4_H}$ relative to $\mathbf{2_H}$, this increase is attributed predominantly to entropy. Estimates of the entropic contribution may be obtained by assessing the reduced rotational degrees of freedom of $\mathbf{4_H}$ relative to $\mathbf{2_H}$. The buttressing ethyl groups in $\mathbf{4_H}$ serve not only to severely restrict the three C_{Ar}–CH_2 rotations[38] but also to restrict, to a lesser degree, the three N_{amide}–CH_2 rotations. An upper estimate of the entropic contribution of locking three rotational degrees of freedom (C_{Ar}–CH_2) at 300 K is ~4.5 kcal/mol.[43] The remaining 1.0 kcal/mol difference between the observed stability and this upper estimate results from either further reductions in rotational degrees of freedom (N_{amide}–CH_2) or enthalpic contributions. Further circumstantial evidence in support of the major role of entropy results from the matching of the enhanced stability of $\mathbf{4_{Fe}}$ to $\mathbf{2_{Fe}}$ to the entropic energetic difference between $\mathbf{Ent_{Fe}}$ and $\mathbf{l\text{-}Ent_{Fe}}$.[20] The designed, nonbonded interactions of $\mathbf{4_{Fe}}$ have nearly equaled the entropic contribution afforded by macrocyclic closure in $\mathbf{Ent_{Fe}}$. The remaining difference in stability (~3 kcal/mol) of $\mathbf{4_{Fe}}$ relative to $\mathbf{Ent_{Fe}}$ can be attributed to the greater enthalpic strain in the former caused by the suboptimal orientation of the CH_2–N_{amide} bond vectors.[44-46]

In summary, a comparison of **4** and **2** has shown first the importance of reducing the conformational space of a ligand to assure maximal binding efficiency and second that this reduction can be achieved equally well through macrocyclization or nonbonded conformational locking.[5] These results support the notion that the catechol moieties in $\mathbf{Ent_H}$ exists in an all-axial conformation,[46] yet conflict with the idea that $\mathbf{Ent_H}$, under physiological conditions, is preorganized[1] as one might anticipate for nature's strongest siderophore. In light of the role of siderophores as both prospecting and sequestering agents, we suggest that $\mathbf{Ent_H}$ adopts a free ligand conformation that is optimally tuned to perform both tasks. Rapid initial binding of an iron atom by an outwardly projected catecholate group triggers a conformational change in the catecholamide group (180-deg aryl rotation) to maximize favorable hydrogen-bonding interactions. This deprotonation-induced conformational change allows facile movement of the partially complexed metal into a position appropriate for full complexation. Such a mechanism would suggest that $\mathbf{Ent_H}$ avoids a preorganized geometry with associated slow kinetics to optimize overall biological performance.

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Supplementary Material Available: Synthetic scheme, thermodynamic parameters, and tables of atoms coordinates and thermal parameters for $Et_3mecamH_6$, $\mathbf{4_H}$ (6 pages). Ordering information is given on any current masthead page.

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