Enterobactin Analogues Prepared by Cross-Linking Catechol Derivative with *cis,cis*-1,3,5-Tris(aminomethyl)cyclohexane

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Enterobactin (Ent) is a representative tricatecholate siderophore produced from *Escherichia coli* and related bacteria under iron-deficient conditions.[1] In the treatment of iron-overload diseases, there is a critical need for effective chelating.[2] Thus, considerable attention has been paid to the design and synthesis of Ent analogues. To probe the design features of Ent, many synthetic tris-catecholamide analogues based on tripodal[3] and macrocyclic[4] skeletons have been synthesized. One of the best analogues, MECAM, was extensively studied by Raymond.[5] Structurally similar $(Et)_3$MECAM[6] showed a 10^4 increase relative to MECAM. This enhanced stability compared to MECAM is attributed predominantly to entropy. However, $(Et)_3$MECAM-Fe was one hundred times less stable than Ent-Fe. In the structure of the X-ray crystal structure of its vanadium(VI) complex,[7] benzylic carbons deviate (~13°) from the benzene plane. Since such deviations increase ring strain markedly, due to the rigidity of the benzene ring, we report here the synthesis of **1a** and **1b**, in which a cyclohexane ring replaces the benzene ring in MECAM (Figure 1). We hoped that the flexibility introduced by this replacement would reduce ring strain in the complex.

The synthetic route of **1a** and **1b** was outlined in Scheme 1. Synthesis of triamine **3a** from commercially available *cis,cis*-1,3,5-cyclohexanetricarboxylic acid **2a** was accomplished in 68% overall yield in 4 steps. Activated ester **5** was prepared from 1,2-dihydroxy benzoic acid according to the known procedure.[3] **3a** and **5** was coupled and debenzylated by catalytic hydrogenolysis to obtain **1a**[8] in 60% overall yield from **2a**. **1b**[9] was prepared from cyclohexanetricarboxylic acid **2b** according to the same procedure as in the preparation of **1a**.

Using previously described procedure,[10] the K_f values of **1a** and **1b** were estimated. With the K_f being accurately

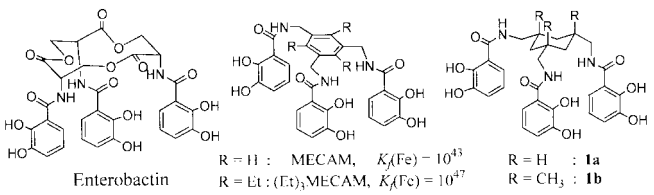

Figure 1. Structure of Enterobactin and its analogues.

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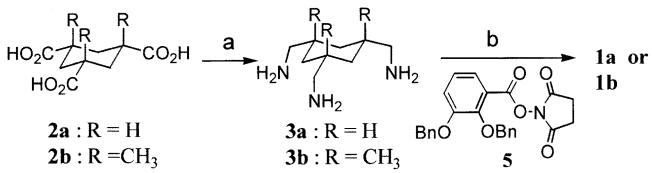

Scheme 1. Reagents and Conditions: (a) i. BH_3-THF, reflux, 16h; ii. TsCl/pyridine, rt, 3h; iii. NaN_3/DMF, 70 °C,16h; iv. H_2 (1 atm), Pd/C (10%)/MeOH, rt, 5h; (b) i. CH_2Cl_2, rt, 6h; ii. Pd/C (10%)/MeOH, rt, 5h.

determined, MECAM was used as a reference to determine the K_f values of **1a** and **1b**. Analogues **1a**, **1b** and MECAM were subjected to the competition experiments against EDTA (K_f~10^{25})[11] for Fe(III). The absorbance with no EDTA added was the reference and the decrease in absorbance, where a particular concentration of EDTA was added, was presented in percentage, as shown in Table 1. The proton dependent formation constant, K^*, can be calculated from eq 1: $([Fe\text{-}L^{3-}][H^+]^6[EDTA^{4-}])/([Fe\text{-}EDTA^-][H_6L] = K^*/K_{Fe\text{-}EDTA}$, where $K_{Fe\text{-}EDTA} = 10^{25}$, and $K^* = ([Fe\text{-}L^{3-}][H^-]^6)/([Fe^{3+}][H_6L])$. Assuming the overall pK_a values for **1a**-Fe, **1b**-Fe, and MECAM-Fe are the same,[5,10] the difference in order of magnitudes of K^* is, therefore, also the difference in order of magnitude of K_f, where, the proton-independent formation contant, $K_f = [Fe\text{-}L^{3-}]/([Fe^{3+}][L^{6-}]) = (K^*[H_6L]/([H^-]^6[L^{6-}])$. Knowing that the K_f for MECAM-Fe is 10^{43}, the K_f values for **1a**-Fe and **1b**-Fe are estimated on the differences, in order of magnitude (Table 1).

Both ligands **1a** and **1b** had higher affinities ($K_f = 10^{44.0}$ and $K_f = 10^{43.3}$, respectively) for Fe(III) than MECAM ($K_f = 10^{43.0}$). To compare the strain energy of ferric complexes,

Table 1. Decrease (%)[a] of Absorbance[b] upon Addition of [EDTA] to L-Fe^{3+} Complex[d]

Concentration of EDTA (mM)	**1a**-Fe	**1b**-Fe	MECAM-Fe
0.25	8.2	15.2	19.5
0.5	9.4	20.1	22.5
2.5	13.8	27.2	35.2
3.75	14.8	29.5	44.9
Estimated $\log_{10} K_f$	**44.0**	**43.3**	**43.0**[e]

[a]Relative to the absorbance where no EDTA was added. [b]At max = 495 nm, at pH7. [c][Ligand]$_{total}$ = [Fe^{3+}]$_{total}$ = 0.1 mM. [d]In 0.01 M phosphate buffer, μ = 0.1 M KNO_3, 25 °C. [e]Reference 5.

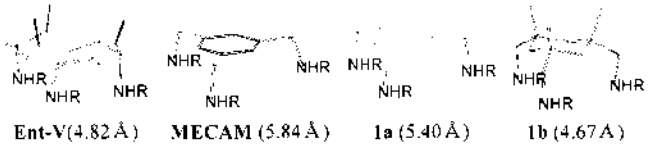

Figure 2. Average *N-N* distance in spacers of Ent and its analogues. The structure of Ent is in its vanadium(IV) complex state and the structures of analogues are in metal free state.

N-N distances of spacers were evaluated. Energy minimized structures[12] of MECAM. **1a** and **1b,** in which three catechol moieties were oriented suitably for formation of ferric complexes. were estimated to have *N-N* distances of 5.84 Å. 5.40 Å and 4.67 Å respectively (Figure 2). In order to achieve optimum metal-ligand interaction, the three binding arms of MECAM have to bend inward. This bending caused large deviations of the benzylic carbons from the plane of the benzene ring. Due to the rigidity of benzene, distortions can increase ring strain energy seriously in MECAM-Fe as shown in $(Et)_3$MECAM-V. In **1a**-Fe complex a lower degree of deviation is expected compared to MECAM-Fe because the *N-N* distance of **1a** (5.40 Å) is much closer to that of Ent-V (4.82 Å) than that of MECAM (5.84 Å). These positive enthalpic contributions. reduced *N-N* distance and reduced ring strain. overcome the negative entropic contribution from the conformational flexibility of the cyclohexane ring. in formation of the **1a**-Fe complex.

The introduction of methyl groups at 1,3.5-positions of the cyclohexane ring in **1b** makes the *N-N* distance (4.67 Å) almost equivalent to that of Ent-Fe (4.82 Å). In addition to this positive enthalpic contribution, considering predisposition effect of methyl group. the higher stability of **1b**-Fe. compared to **1a**-Fe, was expected. as in the stability of $(Et)_3$MECAM[6] *vs.* MECAM. However, competition experiments gave the reverse result: **1a**-Fe was slightly more stable than **1b**-Fe. The lower stability of **1b**-Fe can be explained by 1,3-diaxial steric strain caused by the methyl groups. In the ring flip of the cyclohexane ring of **1a**, **1a-eq**. in which conformation of catechol moieties is equatorial, is much more stable than **1a-ax** (Figure 3). In the case of **1b** (R = CH_3). 1.3-diaxial steric strain of methyl groups destabilize **1b-eq**. The increase of **1b-ax** is unfavorable for the formation of the ferric complex owing to its high strain energy. Thus. the predisposition effects of methyl groups in positioning the catechols and the enthalpic contribution based on *N-N* distance are overpowered by 1,3-diaxial steric strain of methyl groups.

In conclusion, our prediction of a high stability **1a**-Fe complex, based on the flexibility of the cyclohexane ring compared to the rigid benzene ring, was proved correct. However. the expected positive contribution of 1.3,5-substituents on the cyclohexane of **1b** was not observed. *cis,cis*-1.3,5-Tris(aminomethyl)cyclohexane (**3a**) is a good spacer for Ent analogues, considering the high stability of the **1a**-Fe complex and easy preparation of this compound. In addition. **3a** can be used to design Pu-sequestering agents because of the similarity in the coordination properties of Fe(III) and Pu (IV).[14] Furthermore. **3a** can be used as a spacer with other donor units for the complexation of noble metals Pd, Pt, Rh. etc..[15] in modifying other benzene-based tripodal ligands for molecular recognition.[16] Modification of cyclohexane ring spacer is currently under study.

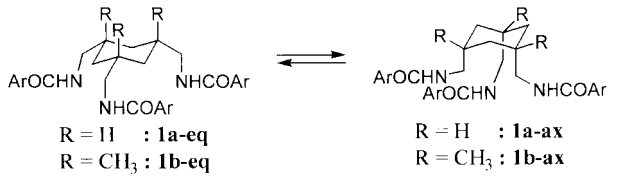

Figure 3. Effect of methyl groups on the conformation of **1b**: **1a-ax** and **1b-ax** are unfavorable for formation of ferric complex due to its high ring strain.

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8. **1a**: ^{1}H NMR (DMSO-d_6) δ 0.68 (dd, J = 12.3 Hz and 24.0 Hz, 3H), 1.73 (br, 3H), 1.91 (d, J = 15.2 Hz, 3H), 3.24 (dd, J = 6.0 Hz and 6.0 Hz, 6H), 6.64 (dd, J = 8.0 Hz and 8.2 Hz, 9H), 6.96 (d, J = 7.8 Hz , 3H), 7.20 (d, J = 8.1 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 34.8, 36.6, 45.2, 115.0, 117.8, 118.7, 146.2, 149.6, 169.8. HRMS calcd for $C_{30}H_{33}N_3O_9$ 579.2217, found 579.2210.
9. **1b**: ^{1}H NMR (DMSO-d_6) δ 1.08 (s, 9H), 1.09 (m, 3H), 1.28 (m, 3H), 3.15 (d, J = 4.6 Hz, 6H), 6.69 (dd, J = 8.0 Hz, 9H), 6.91 (d, J = 7.7 Hz, 3H), 7.36 (d, J = 7.4 Hz, 3H), 8.58 (br, 3H). ^{13}C NMR ($CDCl_3$) δ 15.2, 26.4, 35.4, 39.5, 115.9, 117.9, 118.0, 118.6, 146.1, 148.8, 169.5. HRMS calcd for $C_{30}H_{33}N_3O_9$ 621.2686, found 621.2687.
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