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Preliminary communication

Synthesis of the smallest tris-(catecholamide) siderophore analogue

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Abstract

The tris-(catecholamide) (H_6L) (**8**) was synthesised in six steps and characterized. This is the smallest analogue of naturally occurring siderophores structurally related to an approved enterobactin and protochelin model, known up to now.

Keywords: Siderophore; Catecholate; Enterobactin; Protochelin

1. Introduction

In order to capture iron, many bacteria and microorganisms produce and excrete high-affinity chelators, so called siderophores [1–4]. These are compounds of low molecular-weight which solubilise, bind and assimilate extra cellular iron for transport into the cell. Among these natural siderophores, enterobactin and protochelin are largely studied. The first is composed of 2,3-dihydroxybenzoyl units appended to a cyclic L-serine triester isolated from *Escherichia coli* [5] and *Salmonella typhirium* [6]. The second has been identified in the culture medium of *Azotobacter vinelandii* [7]. The hydroxyl functions of each ligand bind ferric ions with a very high formation constant ($K = 49$) [8] and ($K = 44.6$) [9], respectively, to give a charged octahedral tris-catecholate. However, it is well established that chirality and shape of siderophore iron complexes are important factors involved in the recognition process. The complex interacts with a specific receptor in the outer cell membrane. Iron is then taken and released from siderophores to the agents which are directly involved in cell metabolisms. Another factor, presumably, is the importance of reducing the conformational space of the ligand to assure maximal binding efficiency. This can be achieved equally well through macrocyclic or non-bonded conformation as has been shown by Stack et al. [10]. In this article, we describe the synthesis of a low molecular-weight enterobactin and protochelin analogue. To our knowledge and by comparison with compounds MECAM [11] and TRENCAM [12] two types of synthetic siderophore models, the H_6L (**8**) is the smallest iron chelator model known until now.

The synthesis of this complex was accomplished in six steps. The triamine or 1,1,1-tris-(aminomethyl)-ethane (**4**), was prepared by converting 1,1,1-tris-(hydroxymethyl)-ethane (**1**), to 1,1,1-tris-(benzenesulfonyloxymethyl)-ethane (**2**) [13] and then into the 1,1,1-tris-(phtalimidomethyl)-ethane (**3**) by condensation with potassium phtalimide in freshly distilled dry dimethylformamide at 170°C over 15 h.

The hydrolysis of (**3**) was carried out with aqueous KOH in a high-pressure autoclave (20 h at 180°C) to

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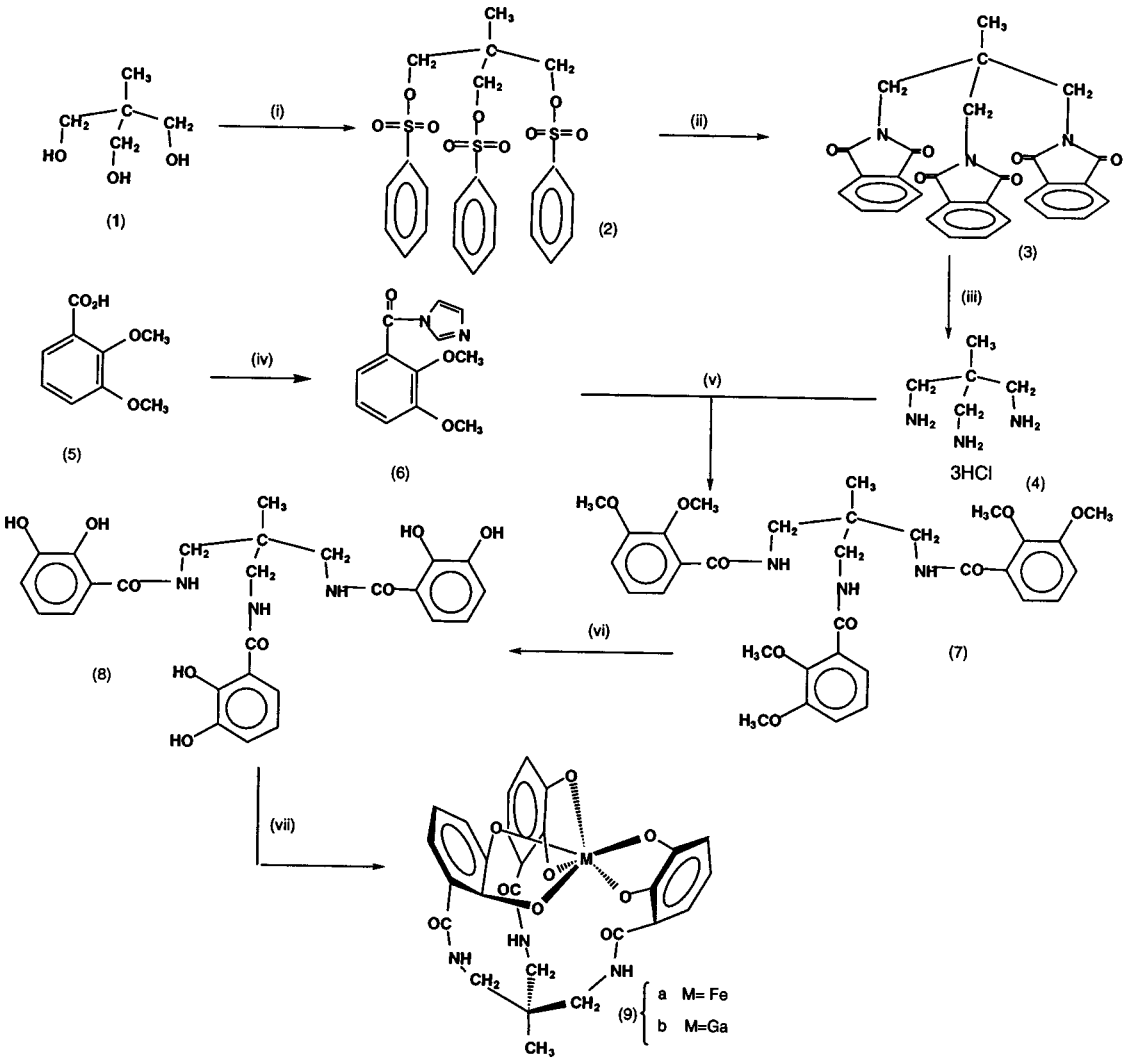
give 70% of 1,1,1-tris-(aminomethyl)-ethane (**4**). The solution was diluted, acidified to pH 3 and sorbed directly onto a column containing strong-acid cation-exchange resin (H^+ form) [14]; a white crystalline structure was obtained after evaporation of water.

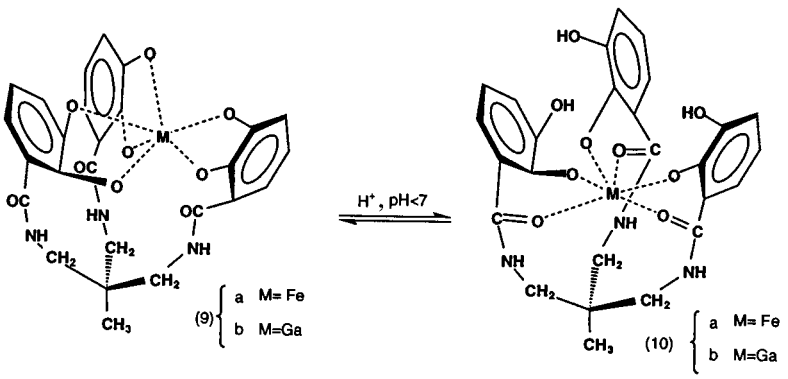
The second step involved, the preparation of the activated form (**6**) of 2,3-dimethoxybenzoic acid (**5**) using carbonyldiimidazole (CDI) [15]. No attempt was made to isolate this intermediate which was directly treated with a mixture of three equivalents of triethylamine and one equivalent of triamine (**4**) in dichloromethane. Removal of the methyl groups (**7**) using BBr_3 gave the tris-catecholate (**8**) obtained from the reaction mixture by evaporation of solvent under reduced pressure. The crystalline compound obtained (**8**) was then treated with $Fe(acac)_3$ in methanol to give ferric complex (**9a**) as an analogue of enterobactin and protochelin (Scheme 1).

The purity of each compound was readily established from relative integration of key signals in the ^{1}H-NMR and ^{13}C-NMR spectra.

Owing to the similarities of the coordination chemistry of Fe(III) and Ga(III), the same ^{1}H-NMR assignment of LGa(III) (**9b**) (diamagnetic) can be made for the ferric complex LFe(III) (**9a**) (paramagnetic). Upon complexation of Ga^{3+} by H_6L (**8**), the aromatic protons are shifted from (δ_H: 6.75, 6.93, 7.29) to (δ_H: 6.38, 6.60, 7.76) and amide protons are shifted from (δ_H: 8.86) observed in the free ligand to (δ_H: 10.17) thus indicating intramolecular hydrogen bonding of the amide protons and ortho catecholate oxygen atom [16]. However, when the pH is below 7, the catecholates FeL^{3-} (**9a**) or GaL^{3-} (**9b**) are converted to salicylate modes H_3FeL (**10a**) or H_3GaL (**10b**) and the neutral complexes precipitate from solutions (Scheme 2). This behaviour is in agreement with the observations obtained for enterobactin and MECAM compounds [17]. Indeed, the



Scheme 1. Reagents and conditions: (i) $PhSO_2Cl$, pyridine; (ii) Potassium phtalimide, DMF; (iii) Autoclave, 170°C, KOH; (iv) CDI, CH_2Cl_2; (v) Et_3N, CH_2Cl_2; (vi) BBr_3, CH_2Cl_2; (vii) $Fe(acac)_3$, CH_2Cl_2, or $Ga(NO_3)_3$, CH_2Cl_2.



Scheme 2.

ESI-MS spectra (negative mode) and ^{1}H-NMR further confirmed the integrity of the two forms of the complexes. In the case of compounds (**10a**) and (**10b**) the ESI-MS spectra showed peaks at 577 $(M-H)^-$ and 590 $(M-H)^-$, respectively, corresponding to salicylate forms. In contrast, for the catecholate forms (**9a**) and (**9b**), peaks at 575 $(M-3K)^-$ and 588 $(M-3K)^-$, respectively, were obtained.

The synthesis of tris-catecholate ligands, based on the reduction of conformational space, furnishes a new type of siderophore models. With its low molecular-weight, this type of molecule could provide a useful biological probe; to elucidate and to reproduce specific functions involved in microbial growth promotion and hence in cell metabolism. Quantitative determination of the formation constant and biological studies for ferric ligand are in progress.

2. Materials and methods

All reagents were of the finest quality available commercially. All solvents were distilled prior to use. Ethylether was distilled and stored over sodium. DMF was distilled and freshly used. The triethylamine was stored over potassium hydroxide, dichloromethane was distilled on $CaCl_2$ and freshly used.

^{1}H-NMR spectra were run on Bruker AM 250 and AC 200 instruments at 250 and 200 MHz, ^{13}C-NMR were run on Bruker AC 200 instruments at 50 MHz. Chemical shifts are reported in parts per million (δ) downfield from internal Me_4Si. The abbreviations used are: s, singlet; d, doublet; t, triplet; q, quadriplet; m, multiplet. All compounds showed consistent NMR and mass spectral data.CI-MS spectra were determined on Nermag spectrometer using direct insertion probe, a source pressure 10^{-1} torr and ammonia as the reactant gas. Therefore $(M^+ + 1)$ and $(M^+ + 18)$ values are reported. ESI-MS were recorded on a Finnigan spectrometer MAT 95.

^{1}H-NMR spectra are only recorded for the non-paramagnetic complexes (**9b**) and (**10b**).

Selected data for (**2**): δ_H ($CDCl_3$) 0.90 (s, 3H), 3.90 (s, 6H), 7.56 (t, 6H), 7.68 (t, 3H), 7.81 (d, 6H); δ_C ($CDCl_3$) 15.94 (s, CH_3), 39.34 (s, C-quat), 69.69 (s, $(CH_2)_2$), 127.72 (s, C-aroma); 129.37 (s, C-aroma); 134.15 (s, C-aroma); 134.72 (s, C-aroma); IC-MS (NH_4^+) m/z 558 $((M+18)^+, 100)$.

For (**3**): δ_H ($CDCl_3$) 0.99 (s, 3H), 2.90 (d, 1/3 (2 CH_3)), 3.82 (s, 6H),7.74 (m, 6H), 7.86 (m, 3H); δ_C ($CDCl_3$) 19.23 (s, $\underline{C}H_3$), 29 (s, C-quat), 44.81 (s, $(\underline{C}H_2)_2$), 123.40 (s, $\underline{C}$-aroma), 131.85 (s, $\underline{C}$-aroma), 134.12 (s, $\underline{C}$-aroma), 168.91 (s, $\underline{C}$=O); IC-MS (NH_4^+) m/z 508 $((M^+ + 1)^+, 100)$; 525 $((M+18)^+, 35)$.

For (**4**): δ_H (D_2O) 1.09 (s, 3H), 3.04 (s, 6H); δ_C (D_2O) ($CDCl_3$ as reference) 17.20 (s, $\underline{C}H_3$); 35.61 (s, -$\underline{C}$-quat); 43.00 (s, -$\underline{C}H_2$); ESI-MS (positive): m/z 118 $(M+1)^+$.

For (**7**): δ_H ($CDCl_3$) 0.98 (s, 3H, CH_3), 3.02 (d, 6H, $2\times$ -(CH_2)-), 3.82 (s, 9H, $3\times OCH_3$), 3.97 (s, 9H, $3\times$ OCH_3), 7.21 (m, 6H), 7.55 (dd, 3H), 8.68 (t, 3H, $3\times$ NH); δ_C ($CDCl_3$) 18.71 (s, $\underline{C}H_3$), 42.06 (s, $\underline{C}$-quat), 43.05 (s, -$(\underline{C}H_2)$-), 55.90 (s, $O\underline{C}H_3$), 61.41 (s, $O\underline{C}H_3$), 115.07 (s, 1C), 122.28 (s, 1C-aroma), 123.90 (s, 1C-aroma), 127.08 (s, 1C-aroma), 147.5 (s, 1C-aroma), 152.57 (s, 1C-aroma), 166.41 (s, 1C-aroma), IC-MS (NH_4^+) m/z 610 $((M+1)^+, 74)$.

For (**8**): δ_H (DMSO-d_6) 0.87 (s, 3H, CH_3), 3.27 (d, 6H, CH_2), 6.72–6.77 (t, 3H, H-aroma), 6.92–6.95 (d, 3H, H-aroma), 7.28–7.31 (d, 3H, H-aroma), 8.86 (t, 3H, $3\times$ NH), 9.29 (s, 3H, $3\times$ OH), 12.03 (s, 3H, $3\times$ OH); δ_C (DMSO-d_6) 19.09 (s, CH_3), 42.07 (s, CH_2), 42.98 (s, C-quat), 115.78 (s, C-aroma), 117.30 (s, C-aroma), 146.27 (s, C-aroma), 148.77 (s, C-aroma), 169.75 (s, C=O); ESI-MS (positive): m/z 526 $((M+1)^+$, 32), 548 $((M+23)^+, 100)$.

For (**9a**): K_3(FeL) ESI-MS (negative): m/z 575 $(M-3K)^-$.

For (**9b**): K_3(GaL) δ_H (DMSO-d_6) 0.92 (s, 3H, CH_3), 3.15 (d, 6H, CH_2), 6.35–6.42 (t, 3H, H-aroma), 6.59–6.61 (d, 3H, H-aroma), 6.75–6.78 (d, 3H, H-aroma), 10.17 (t, 3H, $3\times$ NH); ESI-MS (negative) m/z 588 $(M-3K)^-$.

For (**10a**): H_3(FeL) ESI-MS (negative) m/z 577 $(M-H)^-$.

For (**10b**): ^{1}H-NMR (200 MHz, DMSO-d_6) $\delta = 0.92$ (s, 3H, CH_3); 3.15 (d, 6H, CH_2); 6.61–6.70 (t, 3H, H-aroma); 6.92–7.01 (d, 3H, H-aroma); 7.27–6.34 (d, 3H, H-aroma); 9.02 (t, 3H, $3 \times$ NH); 12.03 (s, 3H, $3 \times$ OH). H_3(GaL) ESI-MS (negative) m/z 590 $(M-H)^-$.

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