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Synthesis and spectroscopic characterization of a new tripodal hexadentate iron chelator incorporating catechol units

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ABSTRACT

We report the synthesis and physicochemical properties of a new tripodal hexadentate chelator (**catTHC**) synthesized by reaction of a flexible tripodal backbone with three bidentate catechol units. To improve the efficiency of the amide coupling reaction, classical conditions using two pairs of coupling reactants were tested, and a significant reduction in reaction time was achieved by using microwave irradiation with the reactants DCC/HOBt. Subsequent removal of the benzyl protecting groups using BCl_3 in dichloromethane provided the final chelator in good yield. The acid–base properties of **catTHC** in aqueous solution and the affinity of the ligand towards iron(III) were investigated at variable pH and in the presence of iron(III) using spectroscopic methods. The hexadentate ligand forms a 1:1 complex with iron(III) whose stability constant was determined by competition with EDTA. The values obtained for the stability constant and pFe^{3+} are $\log\beta_{110} = 36.70$ and $pFe^{3+} = 26.7$.

1. Introduction

The design of new biomimetic siderophores is a field of extensive and continuing research that aims to find ideal candidates for: (a) iron removal agents to be used in the treatment of iron overloaded patients [1–3]; (b) iron complexes as iron delivery agents in agriculture [4,5], and (c) iron removal in environmental applications [6,7]. Biomimetic siderophores are also applied in the study of iron metabolism and iron uptake in living systems [8] antibiotic drug-delivery strategies [9–13] and detection of iron(III) [14].

Siderophores are iron-specific chelators produced by microorganisms, fungi and plants which are used to scavenge iron from the environment and make this essential element available to the organisms. Siderophores can be divided in four broad groups based on the chemical nature of the chelating units: catechol, hydroxamate, hydroxypyridones and aminocarboxylic acids [15]. The characterization of catecholate siderophores was initiated in 1958 after the identification of a glycine conjugate of 2,3-dihydroxybenzoic acid when growing *Bacillus subtilis* under low-iron conditions [4]. Among natural siderophores containing catecholate ligands, enterobactin (Fig. 1) isolated from *Salmonella typhimurium* in 1970 is on the top of the affinity scale for iron(III), with $\log\beta_{110}$ (Fe^{3+}) = 49.0 and pFe^{3+}=35.5 [16].

Our group has been investigating the potential anti-microbial activity of a set of iron tripodal 3-hydroxy-4-pyridinone hexadentate chelators and the results revealed that the fluorescent ligands, in particular a rhodamine derived chelator, (CP777 = 4 = MRB7), are effective in inhibiting the intramacrophagic growth of *Mycobacterium avium* [17–19]. All chelators are based on the same chelating unit and compound **CP256** (Fig. 1) was isolated to assess the chelating properties of the new compounds. The acidity and iron(III) stability constants were determined for **CP256**, which showed an affinity for iron ($\log\beta_{110}$ (Fe^{3+}) = 34.4 and pFe^{3+} = 29.8) higher than those previously reported for mycobacterial siderophores ($\log\beta_{110}$ (Fe^{3+}) = 31 and pFe^{3+} = 29.0) [18].

Since we aim to design antimicrobial agents based on the concept of their ability to restrict the iron sources in bacteria and taking into consideration the extremely high affinity of catecholate ligands for iron, we decided to synthesize a hexadentate catecholate ligand, **catTHC** (Fig. 1), based on the same tripodal unit used for **CP256** and three catecholate bidentate units.

Since in **CP256** the tripodal backbone has terminal carboxylate functions, then an amide bond can be formed by reaction with a catecholamine. This will lead to structural differences between **CP256** and enterobactin; while in enterobactin the amide connec-

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tor is directly linked to the catechol through –CONH– function, in **catTHC** the connector is inverted (–NHCO–) and there is an additional CH_2 spacer between the amide function and the catechol unit. Therefore, the electronic density of the catecholate ring is more affected by amide bond in enterobactin than in **catTHC**. Considering this structural differences, also differences in binding modes to iron are expecting to be achieved. While for the ferric enterobactin two binding modes are possible with different conformation stabilities and previous studies showed that the protonated salicylate binding mode is energetically favored over the protonated catecholate mode, [20] for **catTHC**, we are expecting a lower stability constant with iron(III) than the one reported for enterobactin [16], although the catechol chelating unit should provide higher stability constants compared with 3,4-HPO ligands.

2. Material and methods

Reagents and solvents were purchased as reagent-grade and used without further purification unless otherwise stated. NMR spectra were recorded on a Bruker Avance III 400 spectrometer, operating at 400.15 MHz for protons and 100.62 MHz for carbons, equipped with pulse gradient units, capable of producing magnetic field pulsed gradients in the *z*-direction of 50.0 G/cm. Two-dimensional $^{1}H/^{1}H$ correlation spectra (COSY), gradient selected $^{1}H/^{13}C$ heteronuclear single quantum coherence (HSQC) and $^{1}H/^{13}C$ heteronuclear multiple bond coherence (HMBC) spectra were acquired using the standard Bruker software. Mass spectra were obtained from Unidade de Espectrometria de Masas of Santiago de Compostela and microanalyses were obtained from Unidad de Análisis Elemental of Santiago de Compostela. Flash chromatography was carried out using silica gel purchased from Merck (230–400 mesh). Electronic absorption spectra were recorded on a Shimadzu–UV 3600 UV–Vis-NIR equipped with a Shimadzu TCC-Controller. A Crison pH meter Basic 20+, equipped with a combined glass electrode (model 50 29), and standardized at 25 °C, was used for the spectrophotometric titrations.

2.1. Synthesis

2.1.1. 4-Acetamido-4-(2-carboxyethyl)heptanedioic acid (*1*)

Compound **1** [18] was synthesized through the reaction of nitromethane and *tert*-butyl acrylate, followed by reduction of the nitro to amino group with Raney nickel using the methodology described by Newkome et al. [21]. Further acylation with acetyl chloride and removal of the protecting *tert*-butyl groups using formic acid afforded anchor **1** [18].

2.1.2. 2,3-Dibenzyloxybenzylamine (*2*)

Compound **2** was prepared from benzylation of 2,3-dihydroxybenzaldehyde with benzyl bromide followed by condensation with *tert*-butylcarbamate in the presence of triethylsilane and subsequent hydrolysis in TFA using a reductive amination protocol [22].

2.1.3. Protected hexadentate ligand (*3*)

Compound **3** was synthesized using two different strategies involving: (a) the use of 1-[3-dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDAC)/*N*-hydroxysuccinimide (NHS) at room temperature and (b) the use of *N*,*N*′-dicyclohexylcarbodiimide (DCC)/*N*-hydroxybenzotriazole (HOBt) under microwave irradiation.

(a). Using EDAC/NHS at room temperature: to a solution of 4-acetamido-4-(2-carboxy-ethyl)heptanedioic acid **1** (0.20 g, 0.70 mmol) in anhydrous DMF (4.0 mL) EDAC (0.48 g, 2.56 mmol) and NHS (0.30 g, 2.56 mmol) were added, under argon atmosphere and the reaction mixture was protected from light. After stirring for 4.5 h, EDAC (0.080 g, 0.40 mmol) was added and the reaction proceeded for two more hours, then 2,3-dibenzyloxybenzylamine **2** (0.84 g, 2.56 mmol) was added. The stirring was maintained for 64 h. Then the reaction mixture was purified by flash chromatography using a mixture of chloroform–methanol (9:1) as eluent giving compound **3** (0.70 g, 84%);

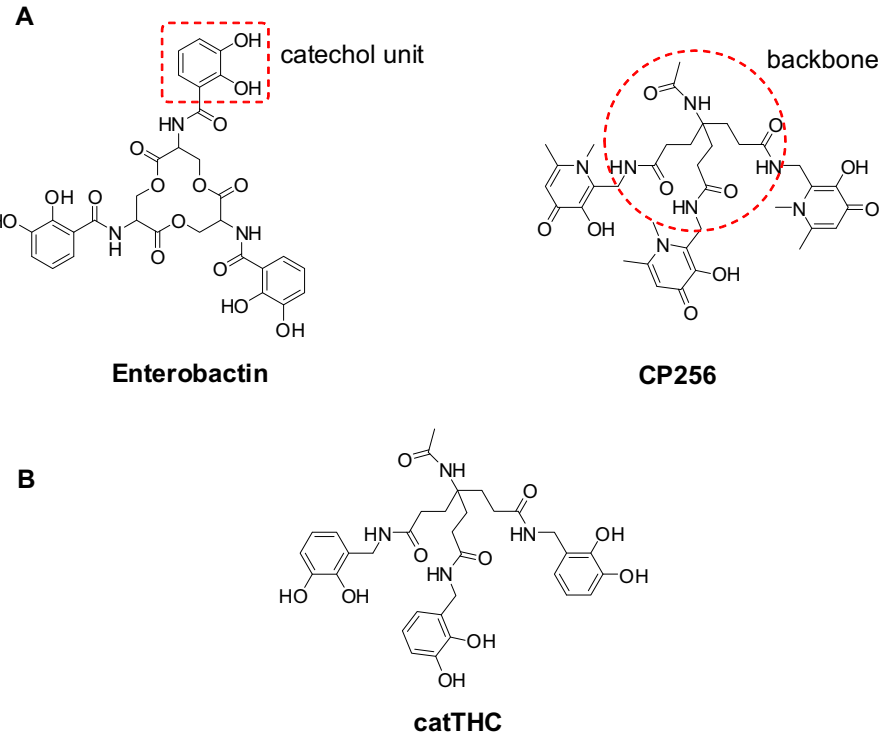

Fig. 1. (A) Structures of enterobactin and **CP256**. (B) Structure of **catTHC**.