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Oxinobactin, a siderophore analogue to enterobactin involving 8-hydroxyquinoline subunits: Synthesis and iron binding ability

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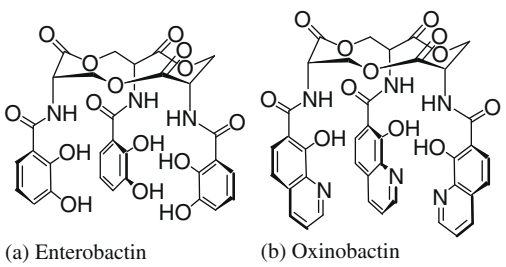
ABSTRACT

Oxinobactin, a siderophore analogue to enterobactin but possessing 8-hydroxyquinoline instead of catechol complexing subunits, has been synthesized starting from L-serine and 8-hydroxyquinoline. Comparative iron binding studies showed that oxinobactin is as effective as enterobactin for the complexation of Fe^{III} at physiological pH but with improved complexing ability at acidic pH.

Iron is vital for organisms, only *Lactobacillus* and *Borelia burgdoferie* bacteria are known to be iron independent. Due to its very low solubility ($pK_s = 38$) at neutral pH, Fe^{III} is poorly bio-available. So, in order to improve Fe^{III} uptake, microorganisms produce low-molecular weight compounds called siderophores. Siderophores and most of their synthetic models contain 3 catecholate or hydroxamate groups, giving stable Fe^{3+} octahedral complexes.[1] However efficient synthetic chelators have been developed involving other chelating subunits.[2] Siderophore-mediated iron transport in aerobic bacteria involves recognition of the ferri-siderophore by a receptor on the cell membrane. The recognition pattern is often sensitive to the chirality of the metal center, which could be induced by the chirality of the ligand[3] derived from naturally amino-acids: this is the case with enterobactin (Fig. 1a), the most efficient siderophore produced by *Escherichia coli* and *Salmonella typhimurium*. It derives from L-serine, forming a macrocyclic trilactone framework tailoring an exceptional preorganization favorable for the selective complexation of Fe^{III} by its three catechol subunits. Interest in synthetic siderophores includes their potential application as therapeutical iron removal agents (iron overload is one of the most common metal poisoning) and there is a renewed interest in this field since Fe^{3+} has been associated to Cu^{2+} and Zn^{2+} in the oxidative stress observed in amyloid plaques in Alzheimer disease.[4,5] Furthermore metal-chelating agents are able to dissolve Aβ-deposits from the amyloid aggregates by removing metal ions, showing that lipophilic metal chelator like clioquinol (an 8-hydroxyquinoline chelator) have some therapeutic potential in Alzheimer disease.[6] We have previously shown that chelators based on 8-hydroxyquinoline exhibit iron complexing abilities of the same order of magnitude as the catechol-based homologs in neutral medium, and higher complexing abilities in acidic medium.[7] It seemed to us interesting to design a chelator possessing the most efficient organizing framework, the trilactone derived from L-serine that also confers the 'chiral recognition area', but coupled to 8-hydroxyquinoline chelating subunits (Fig. 1b). The aim is to obtain a ligand as strong as enterobactin (which could eventually be recognized by its protein receptor), with improved efficiency in acidic medium for targeting other organs (e.g., brain is more acidic than other organs), and that can form neutral iron complex (a necessity for crossing the blood brain barrier). We present in this preliminary report the synthesis and the Fe^{III} complexing ability of oxinobactin, an analogue of enterobactin involving 8-hydroxyquinoline instead of the catechol subunits.



(a) Enterobactin (b) Oxinobactin

Figure 1. (a) Enterobactin (with catechol chelating subunits) and (b) oxinobactin (with 8-hydroxyquinoline subunits).

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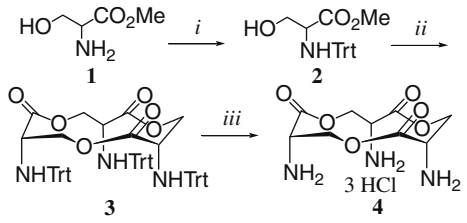


Scheme 1. Synthesis of the trilactone scaffold **4**. Reagents and conditions: (i) TrtCl, Et_3N, CH_2Cl_2, 79%; (ii) catalyst **b**, toluene, reflux, 75%; (iii) HCl, EtOH, rt, 95%.

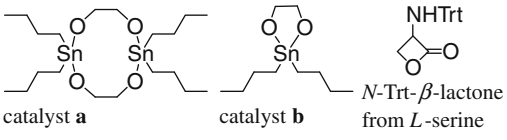


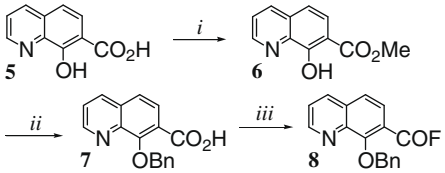
Figure 2. Formulae of the organotin catalysts a, b and α-lactone of L-serine.

The key compound of the synthesis of enterobactin and oxinobactin is a trilactone ring derived from L-serine (product **4**, Scheme 1). Since the pioneering work of Corey et al.,[8] who obtained enterobactin in low yield (about 1%), improvement of the synthesis focused on the macrolactonisation step. The use of an organotin template by Shanzer et al.[9] (Fig. 2, catalyst **a**) allowed the formation of the triserine lactone **4** starting from *N*-trityl-β-lactone of L-serine (Fig. 2) in 26%.

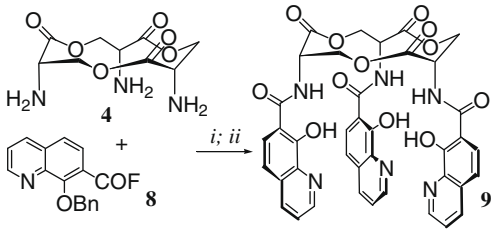
Then in 1997, Gutierrez et al.[10] and Raymond et al.[11] raised the yield (85% and 56%, respectively) following the Shanzer approach but starting from methyl-*N*-trityl-serinate **2** in place of *N*-trityl-β-lactone, which is more tedious to obtain. In our hands, following these conditions (Scheme 1), reproductive yields of 75% could be reached only if 0.2 M concentration of 2,2-dibutyl-1,3,2-dioxastannolane[12] (Fig. 2, catalyst **b**) was used as the organotin catalyst in refluxing toluene.

Removal of the trityl group under acidic conditions gave the triamine lactone **4** as a Tris–HCl salt in quantitative yield. The phenol group of 8-hydroxyquinoline carboxylic acid **5** (Scheme 2), the chelating subunit, was protected in two steps with a benzyl group. Finally, the carboxylic acid group of compound **7** was activated and then combined with the trilactone **4**.

For this step, we found that better yields and better purity were obtained with the fluoro acid derivative **8** in place of the more common chloro derivative. When DAST was used for the fluorination step, some decomposition occurred. Cyanuryl trifluoride proved to be more effective for this transformation. This result is in accordance with the well-established procedures of peptide syntheses, and with similar results obtained in the synthesis of Corynebactin.[13] Then, removing the benzyl group with Pd/C catalyst under slightly positive hydrogen pressure afforded oxinobactin **9** in 16% yield (Scheme 3) as a pale yellow hygroscopic compound. In order to ovoid iron contamination of oxinobactin during the deprotective step, the glassware was treated as previously described by Marinez et al.[14] Characterization of the product was carried out by ^{1}H NMR, mass spectrometry and by elemental analysis.[15]



Scheme 2. Protection and activation of 8-hydroxyquinolinecarboxylic acid. Reagents and conditions: (i) BF_3, MeOH, reflux, 97%; (ii) BnCl, K_2CO_3, KI, EtOH, reflux, 95%; then NaOH, MeOH, reflux, then pH 7, 82%; (iii) $C_3F_3N_3$, DIPEA, CH_2Cl_2, 0 °C, 75%.



Scheme 3. Coupling step. Reagents and conditions: (i) DIPEA, CH_2Cl_2, 0 °C, 66%; (ii) H_2, Pd/C, EtOH, 16%.

The low yield obtained for the final step is explained by the competitive hydrogenation of the quinoline nucleus as shown by MS analysis of the crude mixture. Indeed, a similar reaction has been reported by Shanzer et al. with Hopobactin, a hydroxamate analogue of enterobactin.[16] In order to circumvent this problem they reported that quantitative debenzylation may be accomplished with Fe^{III}, however the ferric complex is formed during the process. We did not apply this methodology since we needed the free ligand for competitive study. We also prepared enterobactin following the procedure described in Scheme 3 but starting from 1,2-dihydroxybenzoic acid.

The complexing abilities of the new ligand were studied by spectrophotometry competition between enterobactin and oxinobactin. The 1:1 iron(III) complex of oxinobactin was prepared in methanol owing to the poor water solubility of the ligand with adding diisopropylethylamine (DIPEA) as a base to adjust pH. The complexation was still achieved in acidic medium (pH ~ 3) and the addition of base has only a slight effect on the UV–visible spectrum that exhibits two bands at λ_{max} = 450 nm (ε = 6600 M^{-1} cm^{-1}) and λ_{max} = 590 nm (ε = 5000 M^{-1} cm^{-1}) (Fig. 3).

These features are very close to those of the Tris-(hydroxyquinolinate) coordination sphere of Fe^{III} by comparison to the UV–visible spectrum of Fe-O-TRENSOX complex.[17,18] The spectrum of the ferric complex of enterobactin in methanol at neutral pH is depicted in Figure 4 (λ_{max} = 510 nm, ε = 5100 M^{-1} cm^{-1}).

A comparative study of the Fe^{III} complexing abilities of oxinobactin and enterobactin was monitored by UV–visible spectrophotometric competition at pH 3.8, 7.1, and 8.9. The equilibrium was approached from both directions with enterobactin and Fe^{III}oxinobactin or Fe^{III}enterobactin and oxinobactin as starting reagents.[19] The same spectra were obtained. The spectrum recorded at

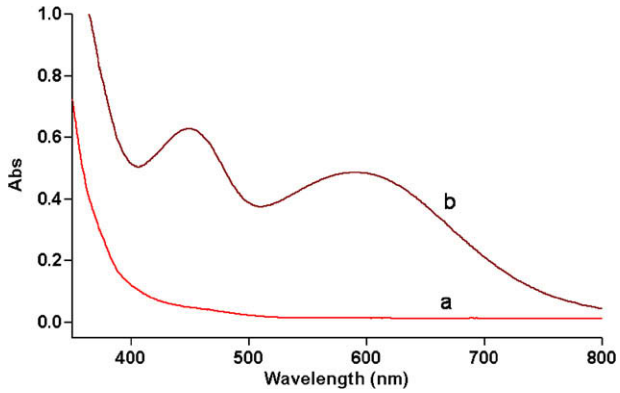


Figure 3. UV–visible absorption spectra of (a) oxinobactine and (b) ferric oxinobactin in methanol; [Oxinobactin] = 0.95 × 10^{-4} M. [Fe^{III}-Oxinobactin] = 0.95 × 10^{-4} M, pH = 7.1.

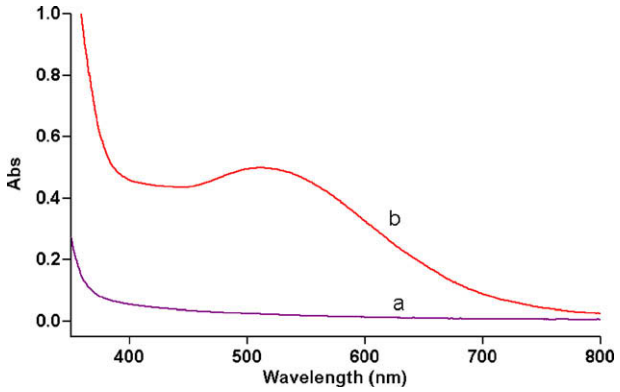


Figure 4. UV–visible absorption spectra of (a) enterobactin and (b) ferric enterobactin in methanol; [Enterobactin] = 0.98×10^{-4} M. [Fe^{III}-Enterobactin] = 0.98×10^{-4} M, pH = 7.1.

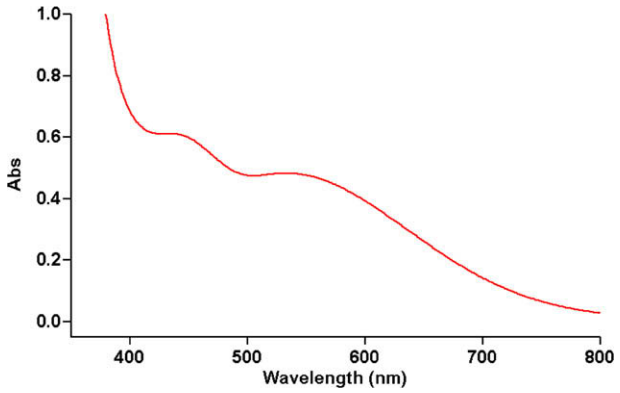


Figure 5. UV–visible absorption spectrum of a mixture of ferric enterobactine and oxinobactine in methanol; [Fe^{III}-Enterobactin] = 1.05×10^{-4} M, [Oxinobactin] = 0.95×10^{-4} M, pH = 7.1.

pH = 3.8 closely resemble that of Fe^{III}oxinobactin spectrum. On raising the pH up to 7.1 (Fig. 5) a shift of the band from 590 nm to 535 nm was observed; this characteristic showed that both the species Fe^{III}oxinobactin and Fe^{III}enterobactin were still in solution at this pH. More precisely, analysis of the curve gave at equilibrium a composition of 60% Fe^{III}enterobactin and 40% Fe^{III}oxinobactin. The spectrum recorded at pH = 8.9 was similar to that of Fe^{III}enterobactin. These data did not allow to calculate the formation constant of Fe^{III}oxinobactin since such a calculation requires the formation constant of Fe^{III}enterobactin and the pK_a's of the two ligands in methanol, that are not known in this solvent.

Nevertheless, these experiments demonstrated that the two ligands exhibit similar complexing abilities in neutral medium, and that oxinobactin is a stronger iron-chelating agent in acidic medium (i.e., over the pH range 3–7) and a lower one in basic medium. In a previous study, considering the relevant parameter pFe = $-\log[Fe^{3+}]$ calculated for $[Fe^{3+}]_{tot} = 10^{-6}$ M and $[ligand]_{tot} = 10^{-5}$ M, we have compared the efficiency of two iron tripodal chelators, O-TRENSOX based on 8-hydroxyquinoline and TRENCAMS based on catechol

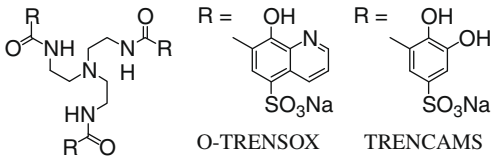


Figure 6. Formulae of O-TRENSOX and TRENCAMS.

groups (Fig. 6).[20] A higher pFe indicates a stronger iron complex. The pFe values at pH 4, 7.4, and 9 in aqueous solution are 18.8, 29.5, and 31.7, respectively, for O-TRENSOX and 12.8, 29.6, and 35.2, respectively, for TRENCAMS. The results of the spectrophotometric competition of oxinobactin and enterobactin towards Fe^{III} are consistent with these values.

In summary these results clearly demonstrate that oxinobactin, an iron chelator possessing the trilactone framework of enterobactin and 8-hydroxyquinoline groups instead of catechol groups, is as strong as enterobactin in neutral pH but is more effective at lower pH's. Various applications of oxinobactin may be expected. The lipophilicity and neutrality of oxinobactin and its ferric complex are useful properties that may be of interest in chelation therapy especially in neurodegenerative diseases (lipophilicity and neutrality are fundamental characteristics to cross the blood brain barrier). Moreover, the iron center in ferrioxinobactin is necessarily of the same stereochemistry as in ferrienterobactin as it is imposed by the chirality of the trilactone ligand. We can hypothesize that ferrioxinobactin would be recognized by the receptor of *E. coli* or other pathogenic microorganisms, but its transport properties would be modified since the ferrioxinobactin is a neutral complex (ferrienterobactin is trisanionic). Therefore ferrioxinobactin may act as an antimetabolite. Biological studies are now in progress.

References and notes

1. Telford, J. R.; Raymond, K. N. Siderophores. In *Comprehensive Supramolecular Chemistry*; Lehn, J. M., Gokel, G. W., Eds.; Pergamon Press: London, 1996; Vol. 1, pp 45–266.
2. Albrecht-Gary, A. M.; Crumbliss, A. L. In *Metal ions in biological systems*; Sigel, A., Sigel, H., Eds.; Iron Transport and Storage in Microorganisms, Plants and Animals; M. Dekker: New York, 1998; Vol. 35, p 239.
3. (a) Raymond, K. N.; Dertz, E. A.; Kim, S. S. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 3584; (b) Winkelmann, G. In *Bioinorganic Chemistry: Transition Metals in Biology and their Coordination Chemistry*; Trautwein, A., Ed.; Wiley, John & Sons, 1997; p 108.
4. Deraeve, C.; Pitié, M.; Mazarguil, H.; Meunier, B. *New J. Chem.* **2007**, *31*, 193.
5. Deraeve, C.; Boldron, C.; Maraval, A.; Mazarguil, H.; Gornitzka, H.; Vendier, L.; Pitié, M.; Meunier, B. *Chem. Eur. J.* **2008**, *14*, 682.
6. Ritchie, C.; Bush, A.; Mackinnon, A.; Macfarlane, S.; Mastwyk, M.; McGregor, L.; Kiers, L.; Cherny, R.; Li, Q.; Tammer, A.; Carrington, D.; Mavros, C.; Volitakis, I.; Xilinas, M.; Ames, D.; Davis, S.; Beyreuther, K.; Tanzi, R.; Masters, C. *Arch. Neurol.* **2003**, *60*, 1685.
7. Pierre, J.-L.; Baret, P.; Serratrice, G. *Curr. Med. Chem.* **2003**, *10*, 1077.
8. Corey, E. J.; Bhattacharyya, S. *Tetrahedron Lett.* **1977**, *45*, 3919.
9. Shanzer, A.; Libman, J. *J. Chem. Soc., Chem. Commun.* **1983**, *15*, 846.
10. Ramirez, R. J. A.; Karamanukyan, L.; Ortiz, S.; Guterriez, C. G. *Tetrahedron Lett.* **1997**, *38*, 749.
11. Meyer, M.; Telford, J. R.; Cohen, S. M.; White, D. J.; Xu, J.; Raymond, K. N. *J. Am. Chem. Soc.* **1997**, *119*, 10093.
12. Deleuze, H.; Maillard, B. *J. Organometallic Chem.* **1995**, *490*, C14.
13. Bluhm, M. E.; Kim, S. S.; Dertz, E.; Raymond, K. N. *J. Am. Chem. Soc.* **2002**, *124*, 2436.
14. Marinez, E. R.; Salmassian, E. K.; Lau, T. T.; Gutierrez, C. G. *J. Org. Chem.* **1996**, *61*, 3548.
15. All the glassware was treated according Marinez[14] in order to avoid iron contamination. A solution of compound **8** (0.48 mmol) in 60 mL of freshly distilled ethanol was subjected to hydrogenation over Pd/C (10%, 100 mg) under a slightly positive pressure of H_2 and debenzylation was monitored by ^{1}H NMR spectroscopy. After completion of the reaction, the catalyst was removed by filtration and the solvent removed by distillation under vacuum. The residue then obtained was carefully washed with several portion of freshly distilled acetonitrile. The remaining solid was then dried under vacuum to afforded 9 as a pale yellow hygroscopic product (0.046 mmol, 16% yield). Analytical data of oxinobactin **9**. ^{1}H NMR (DMSO, 300 MHz) δ: 4.64 (m, 9H, CH–, CH_2); 7.40 (m, 3H, H Ar); 7.53 (m, 3H, H Ar); 7.64 (m, 3H, H Ar); 7.92 (m, 3H, H Ar); 8.32 (m, 3H, H Ar). MS (ESI) *m/z*: 773 $[M-H]^-$ 100%. Elemental analysis (calculated for the trihydrate) $C_{39}H_{36}N_6O_{15}$: calculated C = 56.52; H = 4.38; N = 10.14; found C = 56.50; H = 4.41; N = 9.44.
16. Weizman, H.; Shanzer, A. *Chem. Commun.* **2000**, 2013.
17. Baret, P.; Béguin, C.; Boukhalfa, H.; Caris, C.; Laulhère, J.-P.; Pierre, J.-L.; Serratrice, G. *J. Am. Chem. Soc.* **1995**, *117*, 9760.
18. Serratrice, G.; Boukhalfa, H.; Béguin, C.; Baret, P.; Caris, C.; Pierre, J.-L. *Inorg. Chem.* **1997**, *36*, 3898.
19. Spectra were recorded 48 h after mixing to ensure equilibrium condition. The complexes are stable during this time.
20. Albrecht-Gary, A. M.; Blanc, S.; Biaso, F.; Thomas, F.; Baret, P.; Gellon, G.; Pierre, J. L.; Serratrice, G. *Eur. J. Inorg. Chem.* **2003**, *14*, 2596.