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Design and characterisation of novel hexadentate 3-hydroxypyridin-4-one ligands

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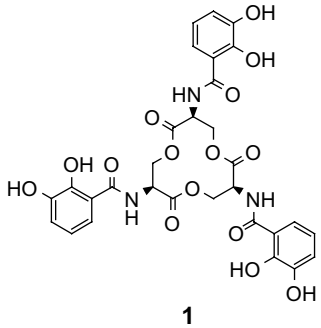
Abstract—Two novel hexadentate 3-hydroxypyridin-4-one ligands have been designed and synthesised. The physico-chemical properties of one of the hexadentate ligands have been determined and the results indicate that the hexadentate ligand possesses high affinity for iron(III).

Currently there is considerable interest in the design of therapeutically useful iron ligands,[1] and naturally occurring siderophores provide excellent models for such molecules. Enterobactin (**1**), a tricatecholate hexadentate ligand, possesses an extremely high stability constant ($\log K_1 = 49$)[2] and therefore very high affinity for iron(III) at physiological pH values.[3] However, the effectiveness of this molecule to scavenge iron at low pH values is limited by its weak acidity and the required loss of six protons on binding iron(III).[4] Furthermore, catechol-based siderophores are able to bind to siderophore receptors and thereby donate iron(III) to bacteria, resulting in undesirable side effects.[5] Many research groups have focused on siderophores and their analogues as promising candidates for pharmaceutical applications.[6] Several have focused on the synthesis of enterobactin analogues by using hydroxypyridinone subunits (**2** and **3**) in place of catechol.[7] Hydroxypyridinones are stronger acids than catechol and since they are monoprotic acids, hexadentate ligands formed from three of these units only need to lose three protons to form iron complexes.[4] Among the three classes of hydroxypyridinone, namely 1-hydroxypyridin-2-one, 3-hydroxypyridin-2-one and 3-hydroxypyridin-4-one, the 3-hydroxypyridin-4-one class possesses the highest affinity for iron(III). This can be attributed to the extensive delocalisation of the lone pair of electrons of the ring nitrogen. Although there are a number of reports on the synthesis of hexadentate ligands based on hydroxypyridinone chelating units, none have utilised 3-hydroxypyridin-4-one units.



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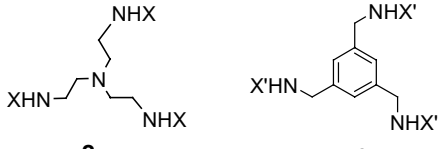
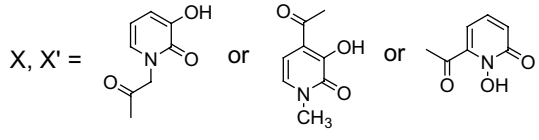


2 **3**



In order for the ligand to adopt the correct geometry for iron(III) binding, it is essential that the backbone be connected to the ring at the *ortho* position relative to

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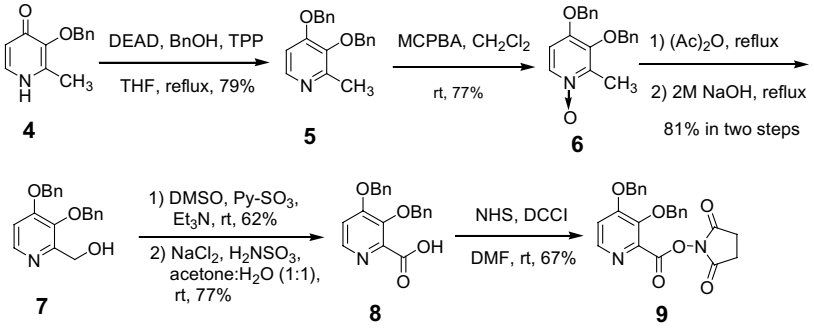
the oxygen anion.[8] Therefore an amide linkage was introduced at the 2-position of the ring. The advantage of having an amide group adjacent to the 3-hydroxyl group is that it can form an intramolecular hydrogen bond, which results in the stability of the iron complex at neutral pH values.[8] In this letter we present a novel synthetic route to build the 3-hydroxypyridin-4-one with a carboxylic acid function at the 2-position of the ring (Scheme 1). The pyridone oxygen of **4** was protected[9] before it was allowed to further oxidise to form **6**.[10] The *N*-oxide group of **6** was subjected to acetylation where intramolecular rearrangement led to the formation of an acetylated alcohol on the 2-methyl group.[11] Subsequent saponification with base gave **7**, which upon oxidation yielded the carboxylic acid **8**. Activation of **8** to an active ester **9** was achieved in the presence of *N*-hydroxysuccinimide (NHS) and DCCI. Ligands **10**[12] and **11**[13] were synthesised by the coupling of the active ester **9** with primary amines including tris(2-aminoethyl)amine (TREN) and 1,3,5-tris(aminomethyl)benzene (TRAM) backbones followed by the removal of the benzyl protecting groups (Scheme 2). Studies have demonstrated that when there is no *N*-alkyl substitution in the ring, the amide *NH* and the 3-hydroxyl group can form a coplanar intramolecular hydrogen bond (Fig. 1a). However, such a bond is not so well favoured in the presence of *N*-alkyl substitution, as appreciable steric repulsion exists between the 1-alkyl group and the amide oxygen atom (Fig. 1b).

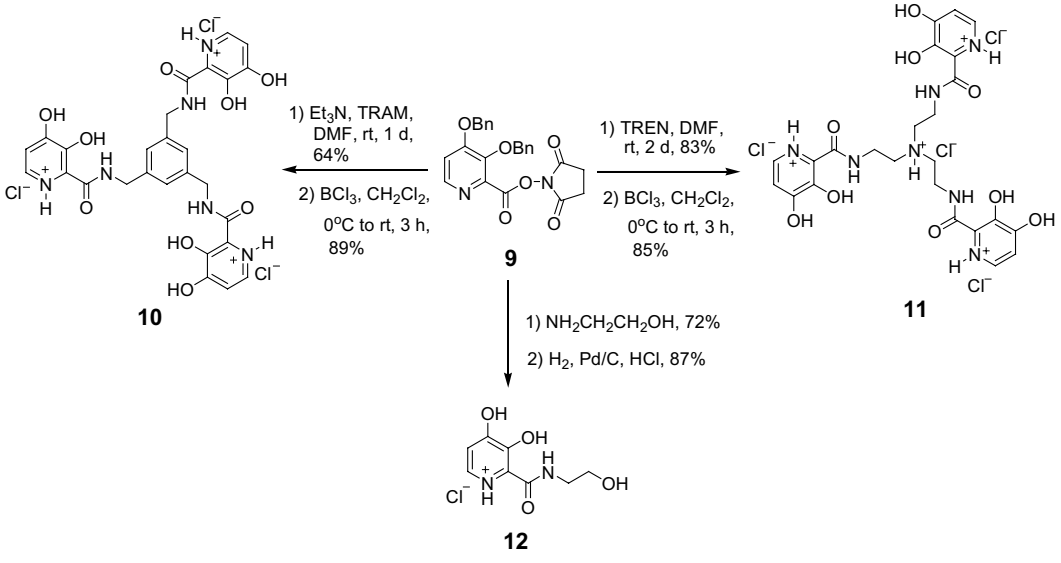
The pK_a values of ligand **11** were investigated by spectrophotometric titration.[14] Ligand **11** can be considered as a trimer of the bidentate ligand **12** and therefore possesses two sets of intrinsic site pK_a values, namely 0.66, 1.88, 3.60 and 6.58, 7.65, 8.10, which are similar to the corresponding two pK_a values of **12**, namely 1.99, 6.32. The tertiary amine function has a pK_a value of 4.52.

The stability constant ($\log K_1$) of **11** was determined spectrophotometrically by competition with the well characterised hexadentate ligand *N*,*N*′-di(2-hydroxybenzyl)ethylenediamine-*N*,*N*′-diacetic acid (HBED), at pH 4.27. The UV/visible spectra of **11**, in the presence of iron(III) and HBED are shown in Figure 2 where the λ_{max} of the spectra were found to shift appreciably when **11** was added to the system. The absolute stability constant of **11** was determined to be 30.7 ± 0.6.

The speciation plot of compound **11**–iron(III) complexes (Fig. 3) demonstrated that the 1:1 ligand–iron(III) complex is the dominant species over the



Scheme 1. Synthetic route leading to the construction of 3-hydroxypyridin-4-one ligands.



Scheme 2. Coupling of the active ester with various primary amines resulted in both bi- and hexadentate ligands.

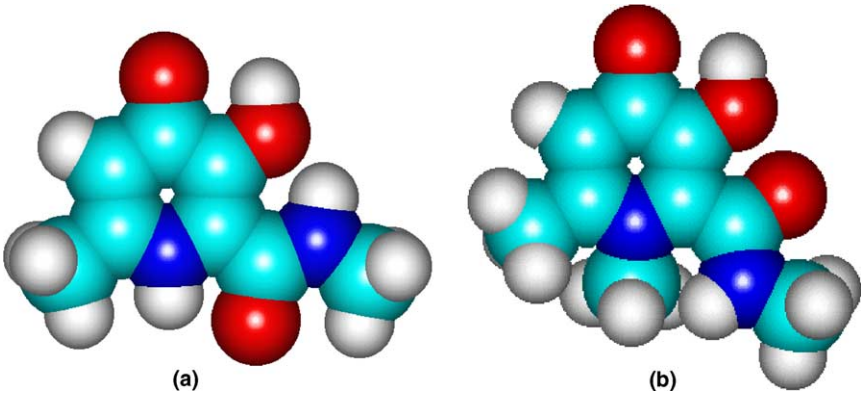


Figure 1. Energy minimised conformer of the 2-amido-3-hydroxypyridin-4-ones: (a) without *N*-alkyl substitution, (b) with *N*-methyl substitution.

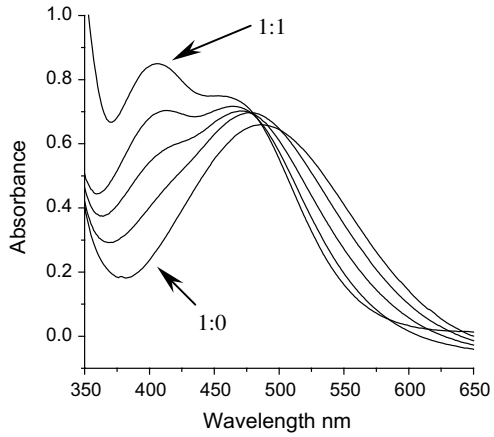


Figure 2. Visible spectra of an **11**–iron(III) complex solution in competition with HBED at pH 4.27. $[Fe^{3+}]_{total}$ = 0.2 mM, $[HBED]_{total}$ = 0.4 mM, the concentration ratio of Fe^{3+} to **11** was varied from 1:0 to 1:1.

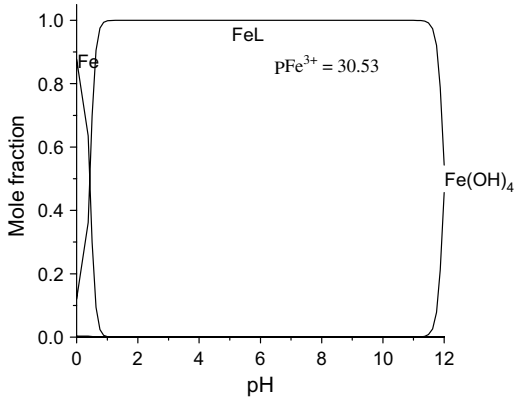


Figure 3. Speciation plot of **11** in the presence of iron(III). pFe^{3+} values were determined by calculating the equilibrium concentration of free hexaaquoiron(III) in a solution of pH 7.45 containing 10^{-6} M iron(III) and 10^{-5} M ligand.

pH range 1–11. In contrast, the iron complexes of catechol hexadentates, for instance MECAM,[15] begin to dissociate at pH values below 5.0, despite a higher stability constant ($\log K_1$ = 43.6). The pFe^{3+} value, defined as the negative logarithm of concentration of the free iron(III) in solution, is a more suitable factor for comparison than the stability constant, since it takes into account the effect of ligand basicity, denticity, degree of protonation and difference in metal–ligand complexes.[16] The application of pFe^{3+} values permits the comparison of different ligands under specified conditions. Since the formation constant of a hexadentate ligand has only first order dependence on the free ligand concentration, whilst that of bidentate ligands has third order dependence, the pFe^{3+} values of hexadentate ligands are proportionally increased when compared to those of bidentate ligands. The pFe^{3+} value of **11**, obtained by using measured stability constant and pK_a values, is 30.5 at pH 7.45. Thus, even though the stability constant of **11** is lower than its bidentate counterpart **12** ($\log \beta_3$ = 31.4), when comparing the pFe^{3+} value the situation is reversed to 8 log units higher than that of **12** (pFe^{3+} value at pH 7.45 = 21.97). In fact, **11** gives the highest ever reported pFe^{3+} value of a hydroxypyridinone hexadentate ligand. The physico-chemical properties of ligand **10** was not investigated because of its poor solubility in water.

In summary, two novel hexadentate 3-hydroxypyridin-4-one ligands have been synthesised by conjugating three protected bidentate carboxyl units on a backbone amine. The hexadentate ligand **11** possesses a high affinity for iron(III). The potential of **11** as a therapeutic iron chelator is currently under investigation.

Acknowledgements

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12. Data for hexadentate ligand **10**: white solid (89%): mp 240 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 4.56 (d, J = 6.0 Hz, 6H; CH_2), 7.24 (d, J = 6.1 Hz, 3H; pyridine C–5H), 7.26–7.48 (m, 3H; ArH), 7.95 (d, J = 6.1 Hz, 3H; pyridine C–6H), 9.49 (t, J = 6.0 Hz, 3H; CONH); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 42.53 (CONH*C*H$_2$), 112.33 (*C*-5 in pyridine ring), 125.10 (benzene *C*H), 126.86 (*C*-2 in pyridine ring), 136.02 (*C*-6 in pyridine ring), 138.91 (benzene *C*), 147.38 (*C*-3 in pyridine ring), 161.81 (*C*-4 in pyridine ring), 162.06 (C=O). MS (FAB): *m/z* 577 [(M−H$_2$Cl$_3$)$^+$]; HR-MS (FAB) calcd for $C_{27}H_{25}N_6O_9$: 577.1683; found: 577.1664.
13. Data for hexadentate ligand **11**: white solid (85%): mp 200 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.51 (t, J = 6.0 Hz, 6H; CH_2), 3.85 (q, J = 6.0 Hz, 6H; CH_2), 7.19 (d, J = 6.0 Hz, 3H; pyridine C–5H), 7.94 (d, J = 6.0 Hz, 3H; pyridine C–6H), 9.30 (t, J = 6.0 Hz, 3H; CONH); ^{13}C NMR (100 MHz, DMSO-d_6) δ = 34.51 (CONH*C*H$_2$), 51.44 (*C*H$_2$NH), 113.03 (*C*-5 in pyridine ring), 127.54 (*C*-2 in pyridine ring), 136.59 (*C*-6 in pyridine ring), 146.56 (*C*-3 in pyridine ring), 161.38 (*C*-4 in pyridine ring), 162.65 (C=O); MS (FAB): *m/z* 558 [(M−H$_3$Cl$_4$)$^+$]; HR-MS (FAB) calcd for $C_{24}H_{28}N_7O_9$: 558.1949; found: 558.1941.
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