Bis-(hydroxyamino)triazines: highly stable hydroxylamine-based ligands for iron(III) cations†

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Bis-(hydroxyamino)triazines (BHTs) constitute a new, general and highly versatile group of tridentate iron(III) chelating agents exhibiting higher affinity to iron(III) than other tridentate iron(III) chelators and superior iron(III) over iron(II) selectivity compared to desferrioxamine-B (DFO), EDTA as well as other tridentate ligands.

Currently catechols, hydroxamates, hydroxypyridinones, and carboxylates[1,2a] are the main general classes of iron(III) selective chelators. In this manuscript we show that bis-(hydroxyamino)-triazines constitute a fifth potent class. Like hydroxamates, and unlike most hydroxylamines, their interaction with iron(III) leads to complex formation rather than to a charge transfer step yielding iron(II) and oxygenated nitrogen products.

Iron chelators have attracted considerable attention[1–3] due to their importance in environmental,[4] forensic,[5] and bioanalytical chemistry;[6] due to their catalytic[7] and electrocatalytic[8] activity, and above all due to their ability to regulate the bioavailability of iron.[1] A central thrust of research effort is devoted to the design of new siderophores—for treatment of β-thalassemia,[2] cardiac disorders,[9] malaria,[10] renal failure,[11] tumor cell growth,[12] and Parkinson's[13] and Alzheimer's[14] diseases. From the known classes of chelators only hydroxamates provide a complete exclusion of iron(III) ions from redox transformations thus inhibiting formation of free radicals through the Fenton process. However, hydroxamates, including DFO, possess a number of disadvantages such as low bioavailability and metabolic stability. The disadvantages of hydroxamate ligands call for an alternative approach toward iron(III) ligands. Desirable ligands should possess high affinity toward iron(III) cations, metabolic stability, selectivity for iron(III), and low redox potential, preferably coupled with the capability to adjust their physical properties for the control of pharmacokinetics. Many of these requirements are not compatible with the amide bonding that is intrinsic to hydroxamate ligands.

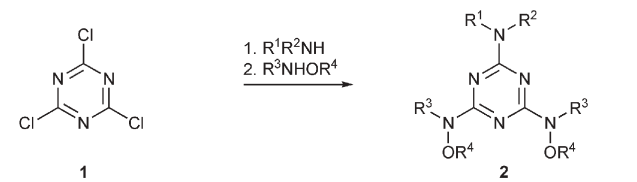
Hydroxylamine based ligands have been shown to form stable complexes with main group and titanium cations.[15] Their use for iron(III) cations is complicated due to their easy oxidation and disproportionation. The attachment of hydroxyamino groups to the electron-poor 1,3,5-triazine system provides a number of advantages that are critical for strong ligation: (a) a dramatic increase in the stability of hydroxyamino groups to oxidation; (b) easy *O*-deprotonation of hydroxyamino group; and (c) the participation of the nitrogen atoms of the 1,3,5-triazine cycle in the binding with metal cations thus providing tridentate ligands.

All BHT ligands (Scheme 1) were synthesized[16] through a monosubstitution in trichloro-1,3,5-triazine with secondary amines R^1R^2NH followed by the replacement of two remaining chlorine atoms with R^3NHOR^4 functions.[17] A variety of ligands of type **2** possessing different substituents R^1, R^2, R^3, and R^4 can be synthesized using this convergent methodology. More complex derivatives can be easily prepared through the attachment of bis(hydroxyamino)-1,3,5-triazine ligands possessing carboxylate-terminated alkyl chains at positions R^1, R^2 to other organic molecules and biopolymers.

For the current studies we selected a range of compounds, **2a–f** with R^1, R^2 representing different peripheral functional groups without interfering with the bis[hydroxy(methyl)amino]1,3,5-triazine backbone. As a control we incorporated compound **2g** possessing both OH and NH coordination sites and compound **2h** for which the OH coordination sites are blocked by benzyl groups. In the following LH_2 is used to denote the bis[hydroxy(methyl)-amino]1,3,5-triazine ligands.

^{1}H NMR spectra of compounds **2a–d**, **g**, **h** show single sets of protons and identical chemical shifts for both methyl groups R^3. In compounds **2e**, **f**, the chemical shifts of the two methyl groups R^3 are different. This difference can be attributed to a low rotational barrier for hydroxy(methyl)amino groups while preserving a high rotational barrier for the NR^1R^2 group[18] thus providing a different environment for methyl groups R^3 in **2e**, **f**.

All the examined BHT ligands possessing unprotected hydroxy groups were found to be capable of forming highly stable, intensively colored 2 : 1 ligand–iron(III) complexes at neutral pH. The diligand structure of the iron(III)–**2a** complex was confirmed by titration of 1 mM methanol solution of ligand **2a** by



1 2

a	R^1, R^2 = -$CH_2CH_2OCH_2CH_2$-	R^3=Me	R^4=H
b	$R^1 = R^2 = c\text{-}C_6H_{11}$	R^3=Me	R^4=H
c	$R^1 = R^2 = CH_2CO_2Et$	R^3=Me	R^4=H
d	$R^1 = R^2 = CH_2CO_2H$	R^3=Me	R^4=H
e	R^1 = Me R^2=CH_2CO_2Et	R^3=Me	R^4=H
f	R^1 = Me R^2=CH_2CO_2H	R^3=Me	R^4=H
g	$R^1 = R^2 = c\text{-}C_6H_{11}$	R^3=H	R^4=H
h	$R^1 = R^2 = c\text{-}C_6H_{11}$	R^3=H	R^4=CH_2Ph

Scheme 1

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concentrated solution of $FeCl_3$ in methanol taking advantage of the light absorption of the iron complex of **2a** at $\lambda = 535$nm. The visible spectra and the peak wavelengths did not change by the introduction of as much as a ten fold larger amount of the ligands showing that diligand complex is the predominant form and other stoichiometries are not abundant even if they exist at all. Additionally, ESI-MS studies showed a base peak at 566 amu at positive mode or 564 amu at negative mode corresponding to $(FeL_2H_2)^+$ and $(FeL_2)^-$. As can be expected, the deprotonated hydroxy groups are essential for the complex formation and *O*-benzyl-protected ligand **2h** did not form the iron(III) complex.

The molecular structure of ligand **2a**–iron(III) complex was determined by X-ray diffraction (Fig. 1).[19] In the asymmetric unit the complex appears as a dimer with two units connected by a hydrogen bond through two molecules of methanol. Each unit possesses a highly distorted octahedral geometry with deprotonation of three OH groups from the original four. The iron–OH bond is substantially (2.45 *vs.* 2.0 Å) longer than the remaining iron–oxygen bonds. The participation of nitrogen atoms in the coordination is evident from both bond distances (2.0 Å) and from the highly distorted bond angles of the 1,3,5-triazine cycle.

The acid dissociation constants of the different LH_3^+ ligands were determined by base titration. Stability constants of the set of Fe^{III} chelating agents were determined by competition tests against EDTA at pH 7. Since the $pK_{a,3}$ of the ligands (LH_3^+) was too high to resolve in the current study we provide the cumulative formation constant from the LH_2 form, the more stable form in neutral conditions. Table 1 shows that the $^*\beta$-values of all the examined BHTs are very similar.

A comparison of the formation constants of differently coordinated complexes is not straightforward, and therefore it is customary[1] to compare the pFe^{3+} values. pFe^{3+} is defined as

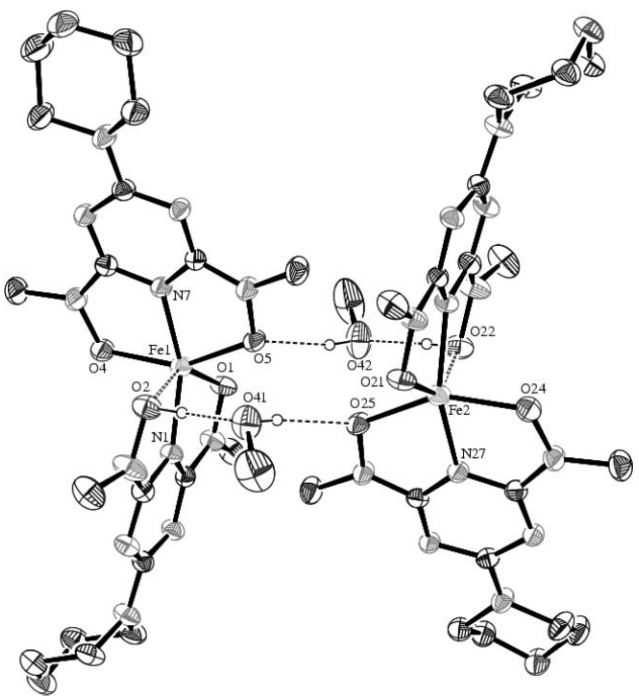

Fig. 1 Molecular structure of 2 : 1 dimeric **2a**–iron(III) complex with two molecules of MeOH. Selected bond length [Å]: Fe1–O4 1.993(18); Fe1–O5 2.025(17); Fe1–O1 1.947(17);Fe1–O2 2.454(19); Fe1–N7 1.976(2).

Table 1 Cumulative formation constants and the formal potentials of BHT–iron complexes

	$\log {}^*\beta_{Fe(III)}$	$E^{0'}$/V	$\log {}^*\beta_{Fe(II)}$	$pK_{a,1}$	$pK_{a,2}$	pFe^{3+}
2a	−2.73	−0.80	−26.0	5.2	8.8	22.6
2c	−2.10	—0.795	−25.3	5.0	8.6[b]	23.3
2d	−3.07	−0.97	−29.2	6.4	9.2	22.2
2e	−2.16	—0.785	−25.4	5.4[b]	8.7[b]	23.2
2f	−2.97	−0.88	−27.6	5.8	8.8	22.4

[a] The formal potentials are reported *vs.* sat'd Ag/AgCl ref. $K_{a,1}$, $K_{a,2}$ are the first and second acid dissociation constants of LH_3^+; [b] Value obtained in 4 : 6 (*v*/*v*) methanol : water solution. $^*\beta$ is the formation constant based on the reaction: $2LH_2 + Fe^{n+} = [FeL_2]^{(n-4)+} + 4H^+$. Error margins: pK_a values are within ± 0.1 units; $\log {}^*\beta_{Fe(III)}$ are within ± 0.3 and E^0 are within ± 20 mV.

$-\log[Fe^{3+}]$ in a pH 7.4 aqueous solution containing 10^{-6} M of total ferric ions and 10^{-5} M of the ligand. This definition is somewhat biased towards high-dentate chelating agents. The most commonly explored hexadentate ligands DFO and EDTA have pFe^{3+} values of 26 and 21.3, (corresponding to log $^*\beta$ values of −1.21 and −3.48) and even higher pFe^{3+} values were reported for some hexadentate aminocarboxylates and their polymers.[1,20] In contrast, all the tridentate ligands cited in Hider's comprehensive review on iron(III) chelators[1] have pFe^{3+} values lying in the range 15–22.5. Novartis' lead tridentate drug, Deferasirox, 4-[3,5-bis(2-hydroxyphenyl)-1,2,4-triazol-1-yl]benzoic acid has a pFe^{3+} of value of 22.5.[21] Bidentate ligands have even lower pFe^{3+} and the promising deferiprone chelator (1,2-dimethyl-3-hydroxy-pyridine-4-one) has a pFe^{3+} value of 19.[1] Table 1 shows that, based on pFe^{3+} values all the BHT ligands are superior to the state-of-the-art bidentate and tridentate ligands.

The iron(III) over iron(II) selectivity was determined by electrochemical studies using a hanging-mercury-drop electrode in 1 M KCl adjusted to the set pH with concentrated HCl or NaOH solutions. The **2a–e** complexes exhibited well defined anodic and cathodic peaks in cyclic voltammetry studies (Fig. 2) which allowed determination of their formal potential over a large pH range.

In all cases, the formal potential was constant at least in the pH range 5–10, showing that the complex composition was unaltered by the electroreduction, which allowed direct derivation of $^*\beta$ of FeL_2^- from the shift of the formal potential, $E^{0'}$ relative to the $E^{0'}$ of the free iron pair.[22] The complex should exhibit low redox potential ($\leqslant$440 mV at physiological pH) in order to guarantee

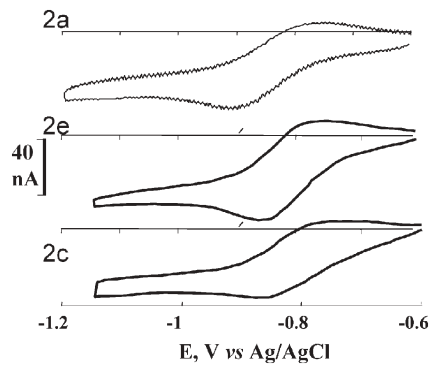

Fig. 2 CV of **2a**, **2e**, and **2c** BHT–Fe(III) complexes using hanging-mercury-drop electrode. (Sat'd Ag/AgCl ref; pH 5.5; 50 mV s^{-1} scan rate).

that it will not be able to participate in aqueous Fenton reactions. Table 1 shows that in all cases the formal potential ranged between −0.78 and −0.97 V *vs.* Ag/AgCl, much lower than the DFO complex (−0.67 V).[2] Indeed, immediately upon addition of iron(II)–BHT complexes to aqueous solutions the solutions turned purple indicating the oxidation of the complex.

In summary, BHTs provide a new general group of siderophores. Their high iron(III) affinity, low redox potential, tridentate character, small size and above all the versatility of their synthesis which allows tuning of their physico-chemical properties open the door for widely different potential applications in medicine, plant nutrition, analysis and bioanalysis.

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