

Design of iron chelators: syntheses and iron (III) complexing abilities of tripodal tris-bidentate ligands

Amaury du Moulinet d'Hardemare, Stéphane Torelli, Guy Serratrice & Jean-Louis Pierre*

Laboratoire de Chimie Biomimétique (ICMG, LEDSS, UMR CNRS 5616), Université J. Fourier BP 53, 38041, Grenoble Cedex 9, France; *Author for correspondence: (E-mail: Jean-Louis.Pierre@ujf-grenoble.fr)

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Abstract

The interest in synthetic siderophore mimics includes therapeutic applications (iron chelation therapy), the design of more effective agents to deliver Fe to plants and the development of new chemical tools in order to study iron metabolism and iron assimilation processes in living systems. The design of ligands needs a rational approach for the understanding of the metal ion complexing abilities. The octahedral arrangement of donor atoms is the most favourable geometry, allowing the maximum possible distance between their formal or partial negative charges. Hexadentate chelators, usually of the tris-bidentate type, can accommodate the metal coordination sphere and are well-suited to obtain high pFe values. The first part of this review is dedicated to selected synthetic routes, taking into account (i) the nature of the chelating subunits, connecting groups and spacers, (ii) the water-solubility and hydrophilic/lipophilic balance, (iii) the chirality and (iv) the possibility of grafting probes or vectors. In the second part, we discuss the role of the molecular design on complexing abilities (thermodynamics and kinetics). The bidentate 8-hydroxyquinoline moiety offers an alternative to the usual coordinating hydroxamic acids, catechols and/or α -hydroxycarboxylic acids groups encountered in natural siderophores. The promising results obtained with the tris-hydroxyquinoline-based ligand *O*-TRENSOX are summarized. *O*-TRENSOX exhibits a high and selective affinity for Fe(III) complexation. Its efficiency in delivering Fe to plants, iron mobilization, cell protection, and antiproliferative effects has been evidenced. Other chelators derived from *O*-TRENSOX (mixed catechol/8-hydroxyquinoline ligands, lipophilic ligands) are also described. Some results question the relevance of partition coefficients to foresee the activity of iron chelators. The development of probes (fluorescent, radioactive, spin labelled) based on the *O*-TRENSOX backbone is in progress in order to get insights in the complicated iron metabolism processes.

Introduction

All organisms need iron. Siderophores are low-molecular weight compounds produced by micro-organisms to facilitate the uptake of ferric iron (Telford & Raymond 1996). Since iron overload is lethal for humans, one can easily imagine the necessity of developing synthetic analogues of siderophores for clinical purposes (Hider et al. 1994). Water-soluble iron complexes can also be

used to alleviate iron deficiency in plants, preventing and even reversing iron chlorosis.

The design of ligands needs a rational approach to understand the metal ion complexing abilities. The $pFe = -\log[Fe^{3+}]_{aq}$ values (Telford & Raymond 1996) calculated for $[Fe^{3+}]_{tot} = 1$ mM, $[L]_{tot} = 10$ mM at pH = 7.4) are directly correlated to the affinity of the ligands for ferric iron. Hexadentate ligands (usually tris-bidentate) offer an optimum preorganization to accommodate an

octahedral geometry around the iron and to get high pFe values. Hider (Tilbrook & Hider 1998) already discussed the point of denticity, which is out of the scope of this review. The ferric iron being a hard acid, it is not surprising that natural or synthetic siderophores mostly contain three hard catecholates or hydroxamates coordinating units. From these types of chelating groups result stable octahedral Fe(III) complexes stabilised by the ligand field (Telford & Raymond 1996). On the other hand, the water solubility and the hydrophilic/lipophilic balance are important aspects related to the 'assimilation' of the chelator or its ferric complex. The crucial point of bio-availability and biological properties are also out of the scope of this review, which is limited to chemical synthesis and physicochemical properties. Furthermore, additional features may be needed for a specific purpose (*i.e.* probes for studying iron transport, diagnostic tools, vectorisation agent, etc...). In order not to interfere with the chelating abilities, these properties are usually added *via* modification of the second coordination sphere. Several reviews have already described one or the other of these fields (Bergeron & Mc Manis 1991; Telford & Raymond 1996; Albrecht & Crumbliss 1998; Shanzer & Libman 1998; Tilbrook & Hider 1998; Rosenberg *et al.* 2000; Liu & Hider 2002).

Organic synthesis

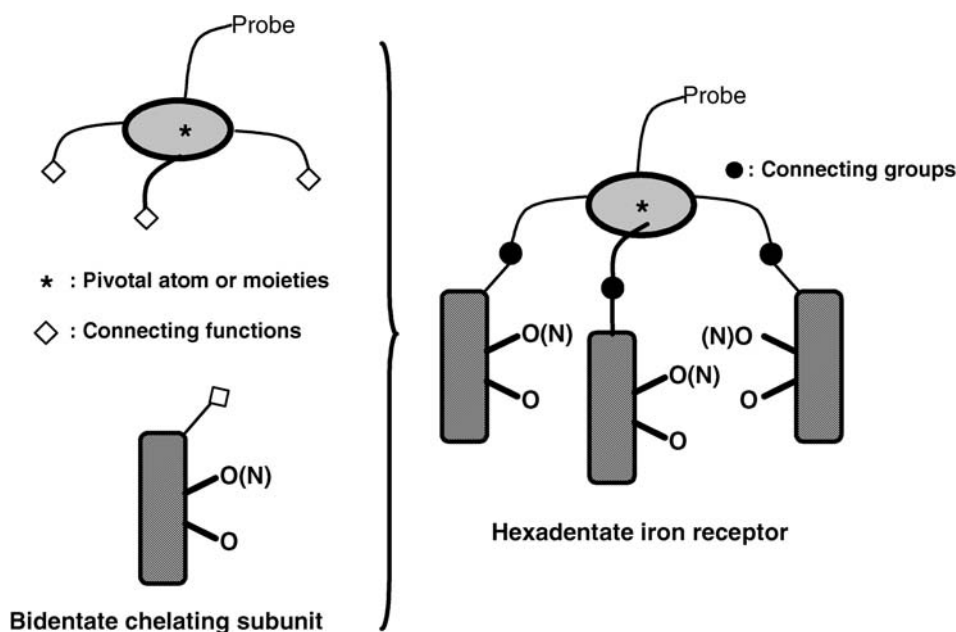
Three distinct moieties are at least involved in tris-bidentate iron chelators:

- three chelating subunits, which can be or not be identical,
- the spacer organized around a pivotal atom or a pivotal moiety,
- the connecting groups resulting from the reaction of complementary functional groups (Scheme 1).

Depending on the purpose, an additional link is needed to connect the group supposed to give additional properties (*i.e.* design of molecular probes or vectors).

The nature of the chelating subunits

In order to well accommodate the hard ferric ion, bidentate subunits have to possess hard donor atoms. It is so why Nature has used oxygen donor sets with catecholate, hydroxamate or hydroxyacid groups. The abiotic iron sequestering agents encountered in literature are thus often biomimetic in nature (Bergeron & Mc Manis 1991; Shanzer & Libman 1991 and 1998; Rosenberg *et al.* 2000). The poorly selective EDTA or analogues (ED-DHA) have also been described (Yunta *et al.*



Scheme 1. General synthesis of tripodal iron chelators

2003) as well as pyridoxal isonicotinoylhydrazone and its derivatives (Richardson & Ponka 1998) depicted in Figure 1.

More recently, other chelating bidentate units such as 8-hydroxyquinolines (Baret *et al.* 1995; Pierre *et al.* 2003) or hydroxypyridinones (Raymond & Xu 1994) have been used. Except for isonicotinoylhydrazones (which exhibit a higher denticity), the bidentate subunits mentioned above involve a five-membered chelating ring size, whose

role will be discussed in the physicochemical part of this paper. Pyridinophenol subunits allow a six-membered coordination ring with the same donor atoms as in the 8-hydroxyquinoline backbone (Baret *et al.* 2000) whereas a seven-membered ring chelate can be achieved with *o, o'*-dihydroxybiphenyl subunits (Baret *et al.* 1994, 1998). The more usual bidentate groups encountered in tripodal ligands are depicted in Figure 2. Corresponding bidentate chelators (monopodal derivatives) are

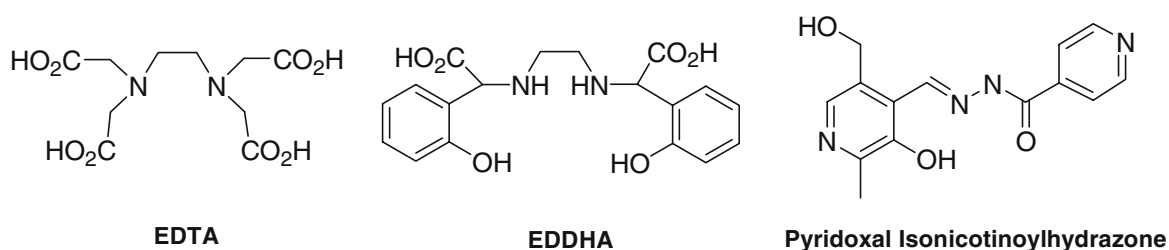


Figure 1. Structures of EDTA, EDDHA and pyridoxal isonicotinoylhydrazone.

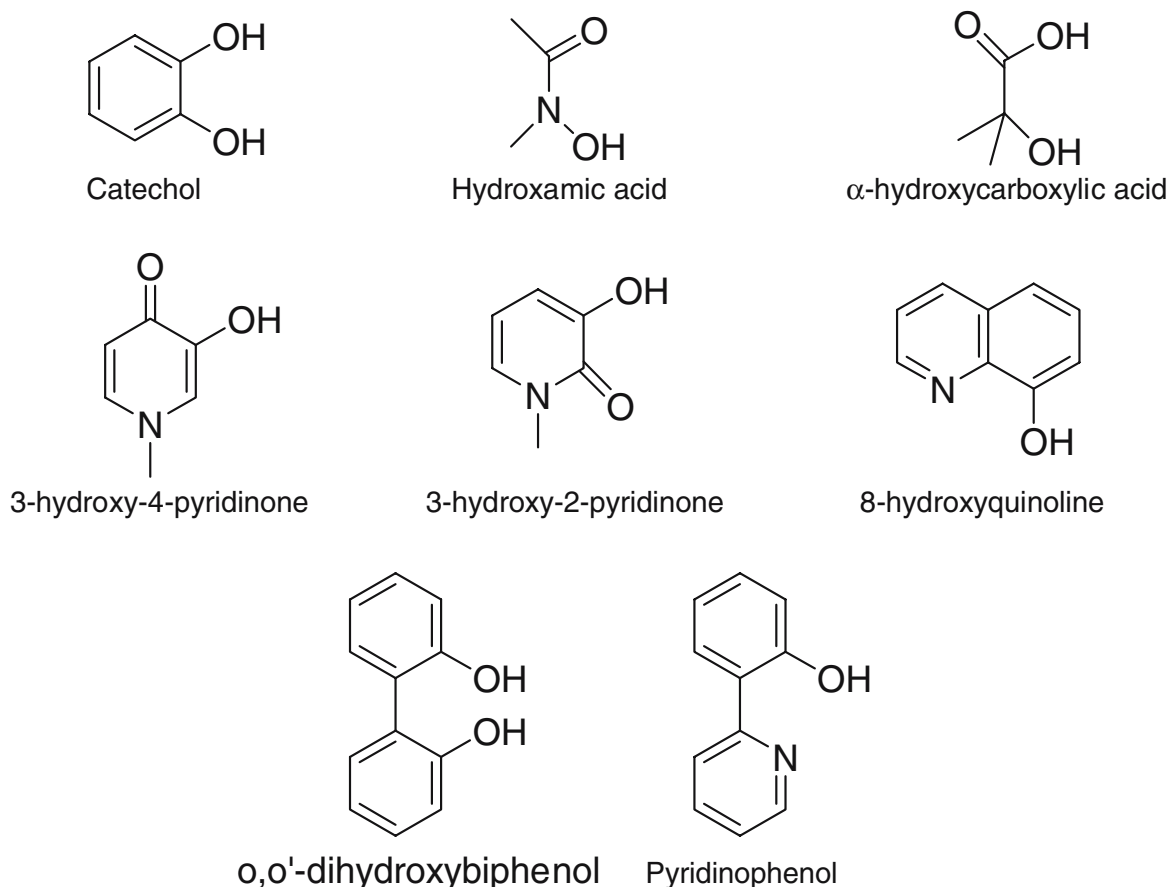


Figure 2. Usual bidentate subunits.

often used for comparisons with the more efficient corresponding tripodal compound.

Connecting groups and synthons

The amide function is widely encountered and the mostly biomimetic relevant connecting unit. Experimentally, amides are obtained by the condensation of primary amines with activated carboxylic acids located in the α -position of the bidentate subunits (Figure 3). The activation is usually promoted by the corresponding acid chloride or a synthetic equivalent such as 3-carbonyl-1,3-thiazolidine-2-thione or *N*, *N'*-carbonyldiimidazole (CDI) derivatives. Siderophores in which the amide connector --CO--NH-- is reversed (--NH--CO-- , Shanzer & Libman 1998) have also been prepared, but they cannot give salicylate coordination in acidic medium (see below). More rarely, amine, ether, or imine connectors have been considered. The latest result from the condensation of an aldehyde synthon with the primary amine function of the spacer but they are less stable (prone to hydrolysis) compared to the amine (directly obtained by one pot condensation of the aldehyde synthon with the amine group of the spacer in presence of sodium borohydride or its cyano-derivative).

The spacer

The spacer (Figure 4) plays a paramount role in the complexing abilities. It has to allow the unstressed octahedral coordination sphere involving the three arms of the tripodal ligand. Tren (tris(2-aminoethyl)amine), is the most widely used spacer.

However, the *N* pivotal atom offers a severe limitation since its alkylation (or protonation) results in the formation of quaternary ammonium salts that alter the optimum electronic and geometric properties of the overall molecule. Consequently, the needs of grafting additional chemical potentialities lead to introduce carbon pivotal spacers. However, the tetrahedral nature of the sp^3 carbon atom involves 109.5° angles (120° for the pivotal *N* atom) and requires longer arms compared to Tren in order to allow an unconstrained octahedral coordination.

A wide range of tripodal spacers are commercially available, while the trisamino-*C*-pivotal ones are commonly obtained by the reduction of the corresponding triscyano derivatives. As a typical example, the synthetic pathway for the ligand *O*-TRENSEX is depicted in Figure 5 (Baret *et al.* 1995). The preparation starts from 8-hydroxyquinoline, a very cheap precursor, which is transformed into the 7-carboxylated derivative by a Kolbe procedure. Activation of the carboxylic group, conveniently achieved using *N*, *N'*-carbonyldiimidazole (CDI), allows the conjugation with the tripodal Tren. The last step consists of the regiospecific *para*-sulphonation in the fifth position in order to increase both phenol acidity and aqueous solubility.

Water solubility

Water solubility is required in order to take advantage of the synthetic iron chelators for most of the clinical purposes. Sulphonation of the ligand with concentrated sulphuric acid is usually performed to introduce hydrophilicity, but the purification on the

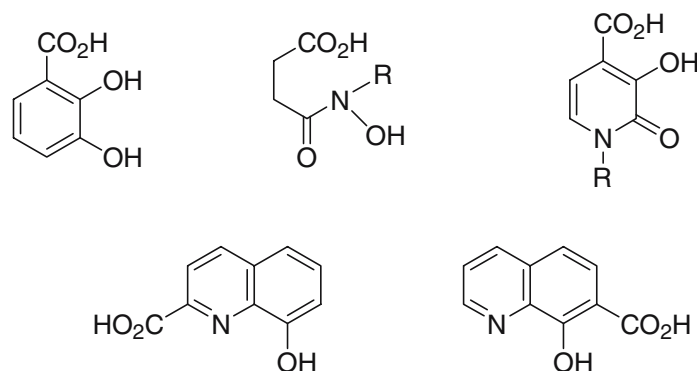


Figure 3. Typical synthons (the hydroxyl groups are often protected).

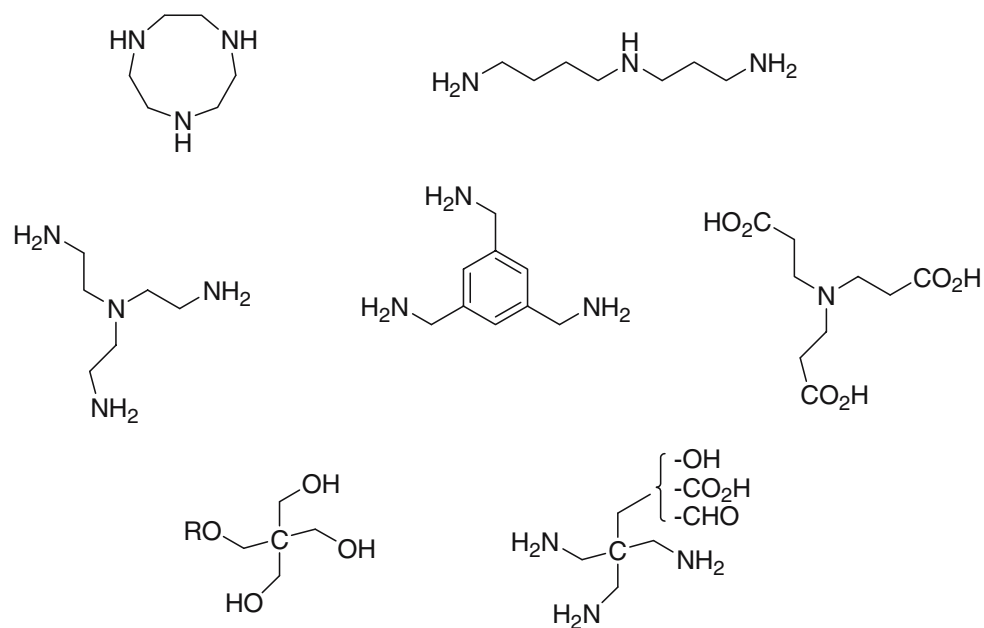
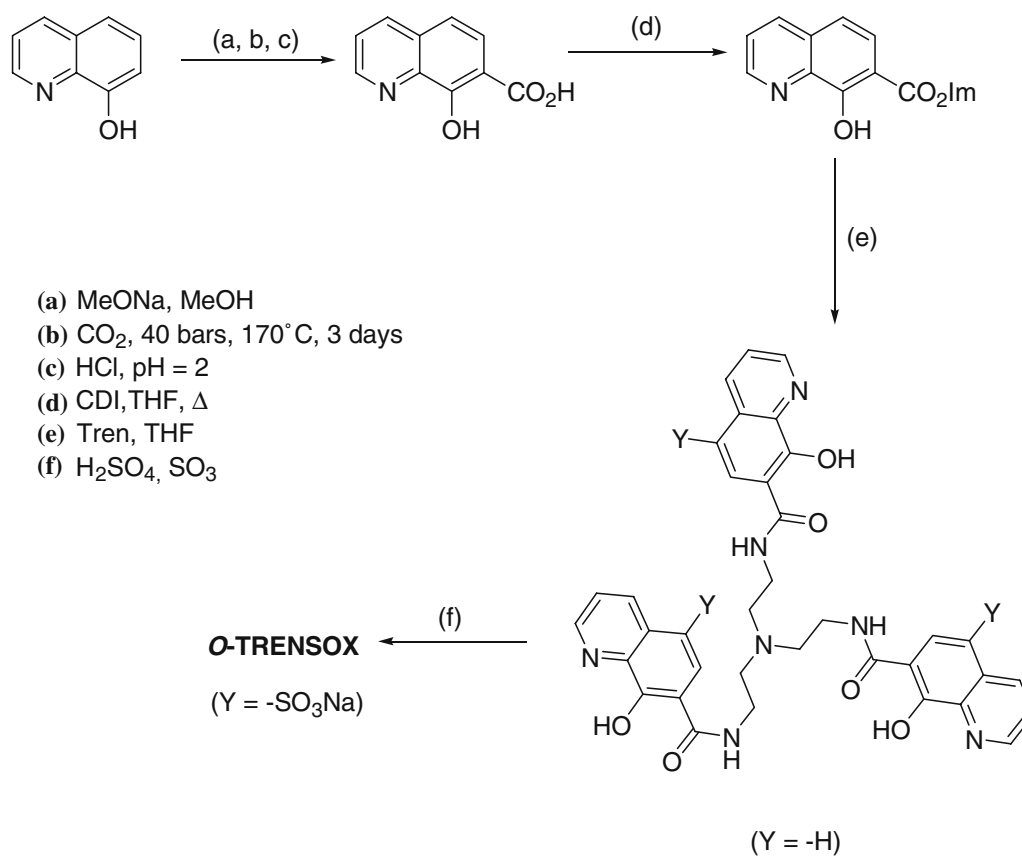


Figure 4. Usual spacers.

Figure 5. Synthesis of *O*-TRENSEX.

resulting compounds (sulphonic acid or sodium salt) turns out to be rather difficult. The sulphonated compounds are highly water-soluble and the ionised derivatives insoluble in lipophilic medium. A more discerning approach, based on the grafting of polyoxyethylene chains (Imbert *et al.* 2002), can allow both water and lipophilic solubility without introducing ionisable (*i.e.* $-\text{SO}_3\text{H}$) or charged groups (*i.e.* quaternary ammonium groups).

Chirality

Siderophore-mediated iron transport by aerobic bacteria involves recognition of the iron-siderophore complex based on chirality of the octahedral metal centre. The Δ or Λ configuration is enantioselectively induced by the chirality of the ligand upon complexation (diastereoisomeric complexes may be obtained). Optically pure chiral synthetic siderophores have been reported (Tor *et al.* 1987; Meyer *et al.* 1997; Hara & Akiyama 2001; Bluhm *et al.* 2002), including chiral biomimetic compounds using aminoacids precursors. However, the enantioselective creation of the chiral metal centre has rarely been investigated. An interesting potential application of the chirality has been performed by Bergeron (Bergeron *et al.* 2001): owing to the fact that the chiral configuration of natural ferrisiderophores is recognized by the producing bacteria, the authors prepared the non natural corresponding enantiosiderophores and showed that deferration agents obtained are still effective for clearing iron from animals, but do not promote microbial growth.

Mixed ligands

As the synthesis of such compounds needs protection and deprotection steps, only few mixed tripodal tris-bidentate ligands (*i.e.* ligands involving different complexing units) have been reported (Cohen *et al.* 2000; Albrecht-Gary *et al.* 2003). The first paper described the synthesis and iron complexing abilities of ligands based on two hydroxypyridinonates and one catecholate or one 2-hydroxyisophthalamide units (Figure 6).

Other mixed ligands involving either two 8-hydroxyquinolinates and one catecholate (TRENSEX2CAMS, L^1) or one hydroxyquinoline and two catecholates moieties (TREN-

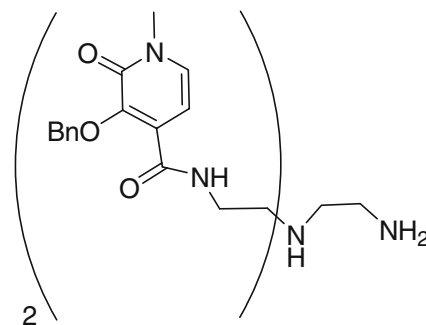


Figure 6. Synthon for mixed hydroxypyridinonate-based ligands.

SOXCAMS2, L^2) have also been reported. These chelators derived from the homoleptic *O*-TRENSEX and TRENCAMS ligands, and allowed detailed solution studies *i.e.* pH dependence, cooperative effects and exchange processes in iron chelation (Albrecht-Gary *et al.* 2002). L^2 has the highest affinity towards Fe^{3+} ever reported (to the best of our knowledge) for an abiotic synthetic siderophore with a pFe value of 32.2.

The synthetic route for the hetero-tripodal hexadentate ligands is presented in Figure 7. The two catecholate subunits of L^2 were connected to the tripodal scaffold by direct condensation of two equivalents of 2, 3-dimethoxybenzoic chloride with one equivalent of Tren (Figure 7, path a). The oxinate subunit was then grafted onto the free primary amine group by coupling with CDI-activated 7-carboxy-8-hydroxyquinoline. The synthesis of L^1 required the selective protection of one arm of Tren with a trityl group (Figure 7, path e), while phenol groups were respectively benzylated (oxine) and methylated (catechol). Upon deprotection with BBr_3 , regiospecific sulphonation in the fifth position of both oxinate and catecholate subunits afforded the hydrosoluble ligands.

Lipophilic and amphiphilic siderophores

In order to be used for the treatment of iron overload or deficiency, the (ferri)siderophore has to be soluble in physiological conditions. It is indeed generally claimed that access to the cell through biological membranes depends on the subtle balance between hydrophilicity and lipophilicity of the chelator or its iron complex.

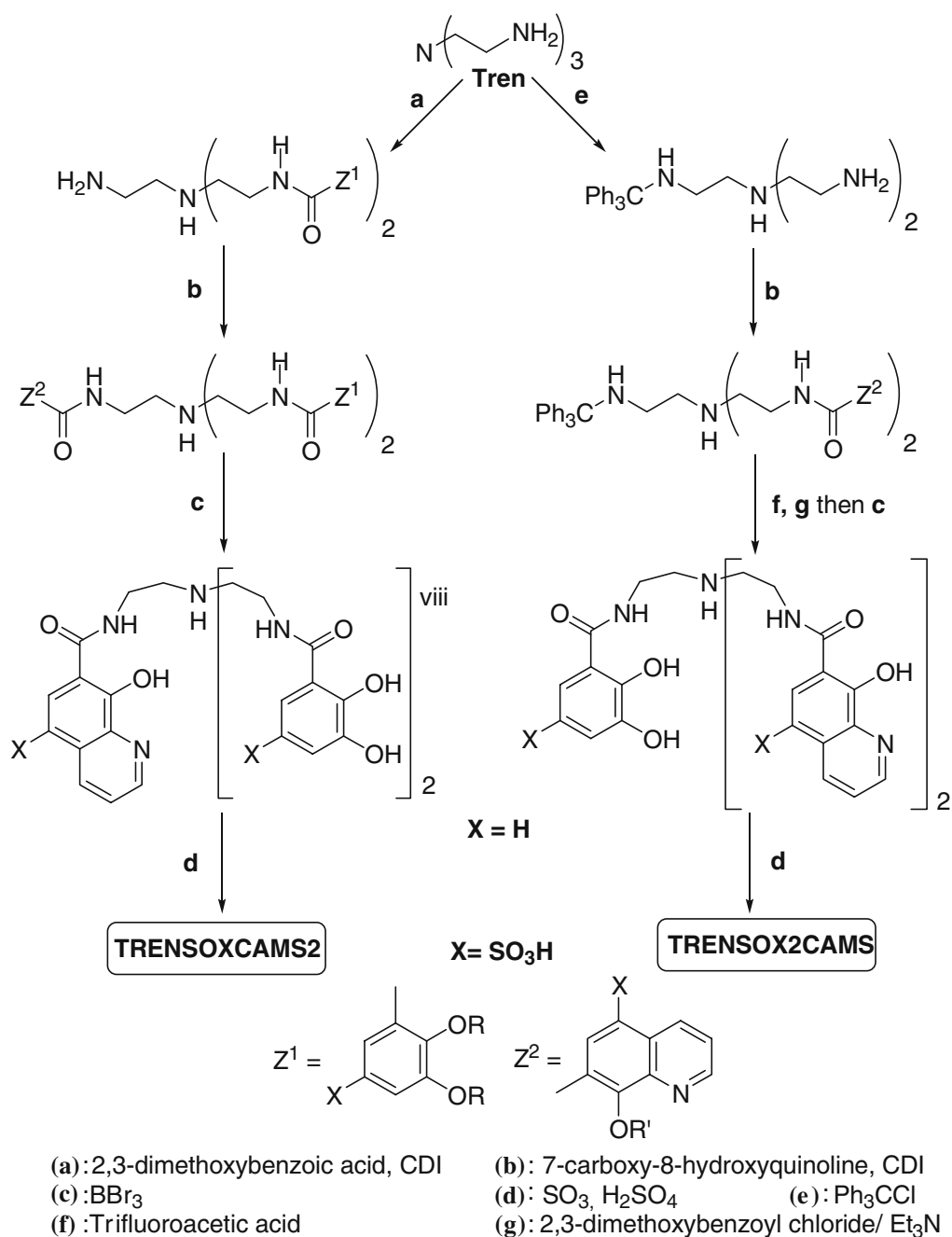


Figure 7. Synthetic pathways for mixed ligands.

Lipophilic enterobactin analogues (tris-catecholates) have been described (Weil & Raymond 1981) using a spermidine spacer in which the *N* terminal nitrogen atom is substituted by long alkyl chains. Lipophilic chelators derived from EDTA (Galey *et al.* 2000; Serratrice *et al.* 2001) or tris-

aminophenol (Bollinger *et al.* 1995) have also been reported. An elegant strategy has been developed by Shanzer and collaborators in which the lipophilic ferrichrome analogues with masked hydrophilic moieties (Figure 8a) turn hydrophilic upon esterase mediated hydrolysis of the lipophilic

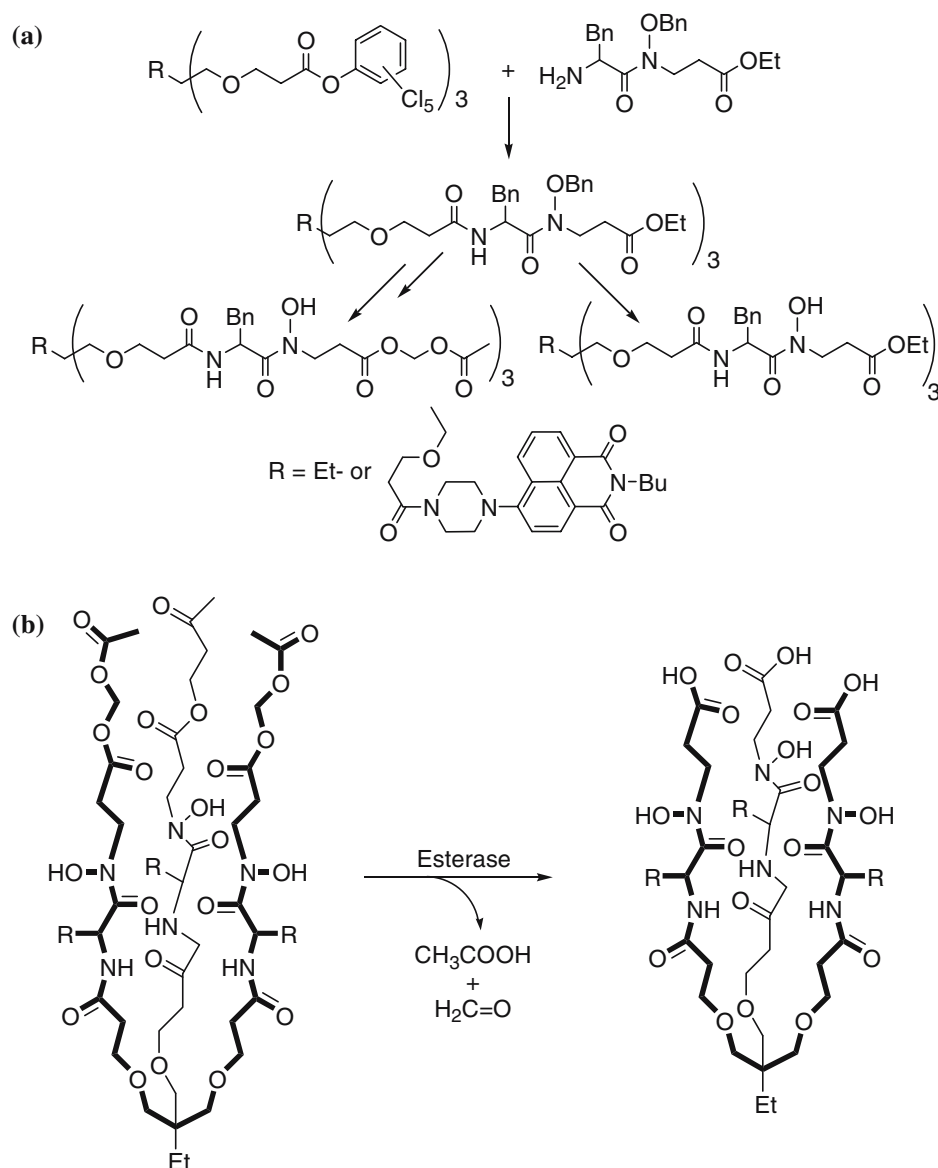


Figure 8. Synthesis (a) and hydrolysis (b) of lipophilic ferrichrome analogues.

methylenoxyacetate groups (Figure 8b, Meijler *et al.* 2002).

A series of three tris-hydroxyquinolate derivatives of *O*-TRENSOX containing a *C*-pivotal atom as spacer and grafted with various polyoxyethylene chains (POE) has been prepared in our group (Figure 9a, Imbert *et al.* 2002), leading to the observation that the hydrophilic-lipophilic balance (quantified by the partition coefficients) of the overall neutral ferric complexes is tuned by the length of the polyoxyethylene chain. This

aspect was particularly well illustrated by Hider and gave conflicting results with the ligands of the 8-hydroxyquinoline family (Thomas *et al.* 1999a, b; Henry *et al.* 2001; Imbert *et al.* 2002). Contrary to the results of Hider, which are beyond doubt, the biological results obtained with the hydroxyquinoline chelators do not depend on partition coefficient: *O*-TRENSOX and its iron complex are water soluble and insoluble in lipophilic medium, but exhibit the same biological activity as the amphiphilic COX 750 (Figure 9a).

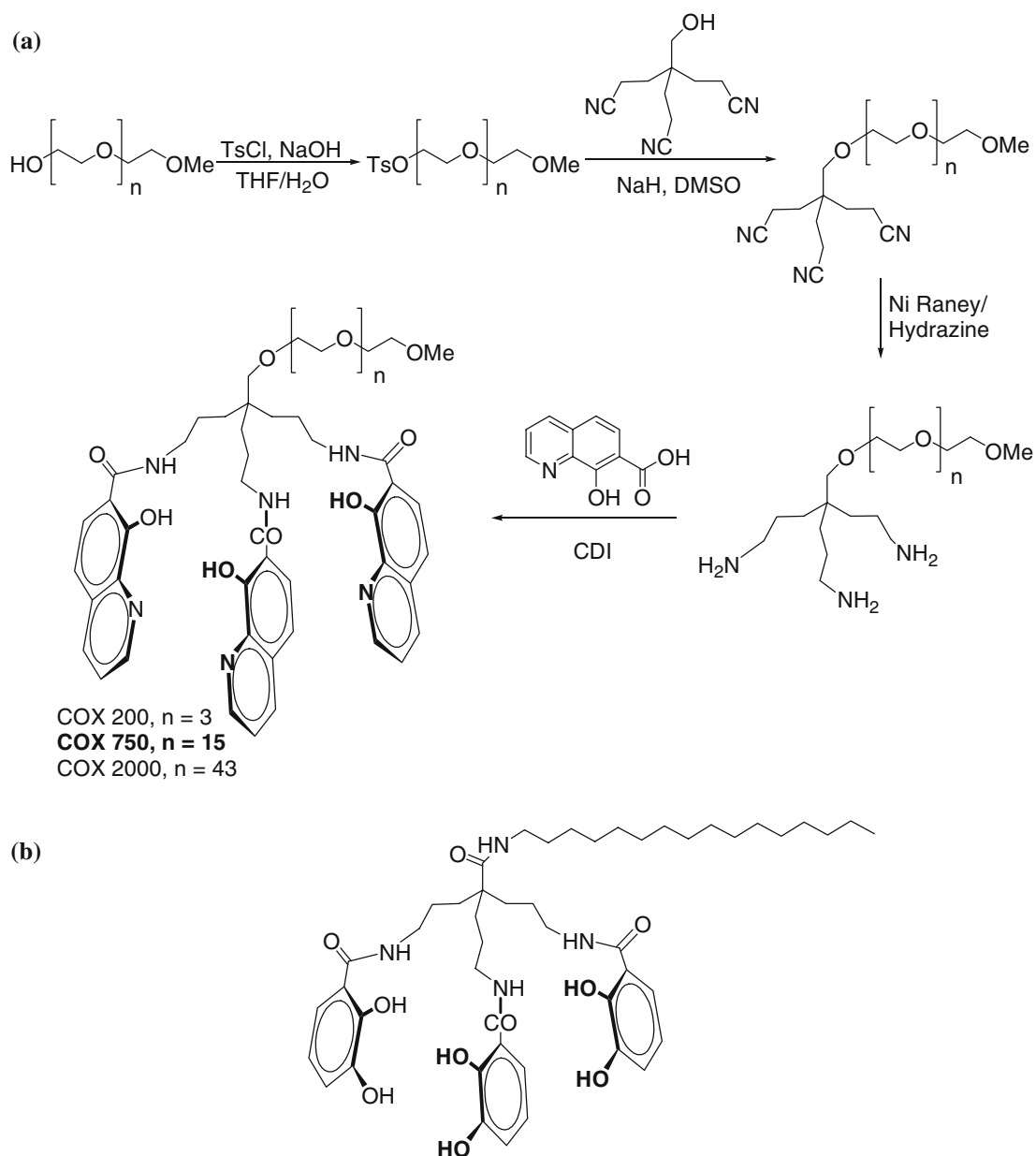


Figure 9. (a) Syntheses of POE-grafted tris-hydroxyquinolinates COX 200, COX 750 and COX 2000; (b) hexadecyl-grafted tris-catecholate.

Another recent approach involves chelators possessing an anionic hydrophilic head (the ferric chelate) and a neutral lipophilic tail capable of self-organization, leading to vesicles that mimic the behaviour of marine siderophores (Apostol *et al.* 2005). In this case, the tripodal tris-catecholate ligand was obtained by reaction of the 2, 2, 2-tris[3-(2, 3-dimethoxy-benzamido)propyl]-acetic acid precursor (Imbert *et al.* 2000)

with the acyl chloride derivative and hexadecylamine, and then deprotection of the catechol groups (Figure 9b).

Vectorisation, probes

As previously described for the POE or the long alkyl chains derivatives, the grafting of different probes (fluorescent, paramagnetic, etc...) can be

considered in order to get insights in the incorporation and localization of the chelator (or its iron complex) into cells or membranes. The incorporation of a fluorophore is an elegant way to track the active species at the cellular scale (Figure 8, Meijler *et al.* 2002). One can also imagine therapeutic investigations by association of either an antibacterial or a vector agent with the functionalized siderophore. By example, the ‘Trojan horse’ approach developed by Miller using siderophores as selective drug delivery agents in siderophore-drug conjugates (Figure 10) has shown great ability to target pathogenic microbes (Miller & Malouin 1993; Roosenberg *et al.* 2000).

The nature of the linker will determine whether the drug remains attached to the siderophore or is enzymatically released into the cell. The synthesis of conjugates can proceed by derivatization of natural siderophores or by total synthesis. Miller has illustrated both strategies, with an example depicted in Figure 11.

Complexing abilities

The knowledge of the main physicochemical features is an essential prerequisite for the understanding, at the molecular level, of the biological properties of a given iron chelator. The-

modynamic, kinetic, structural, spectroscopic, and surface properties have been studied in numerous cases. An excellent review on the coordination chemistry of iron chelators has been recently published (Albrecht-Gary & Crumbliss 1998). However, such synthetic analogues are relatively complicated molecules, and the properties of the corresponding iron complexes can be influenced by:

- the spacers
- the connecting modes and the nature of chelating moieties
- the pH.

The role of the spacer and the connecting mode of the chelating moieties

If different spacers allow the same coordination geometry such as an undistorted octahedral coordination sphere, the pFe of the corresponding ligands would be the same for siderophores containing identical connecting mode and complexing units. *Vice versa*, changes in pFe should reflect modifications in the metal coordination sphere. Thus, the same pFe values are observed for *O*-TRENSEX and for the POE-grafted chelators (Imbert *et al.* 2002).

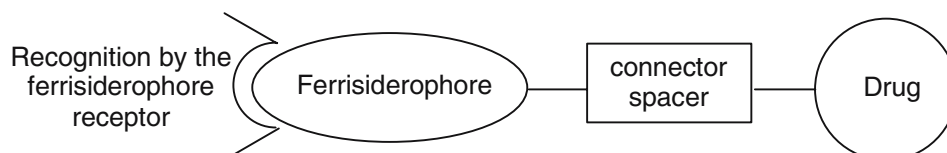


Figure 10. Siderophore drug-conjugate or the ‘Trojan horse’.

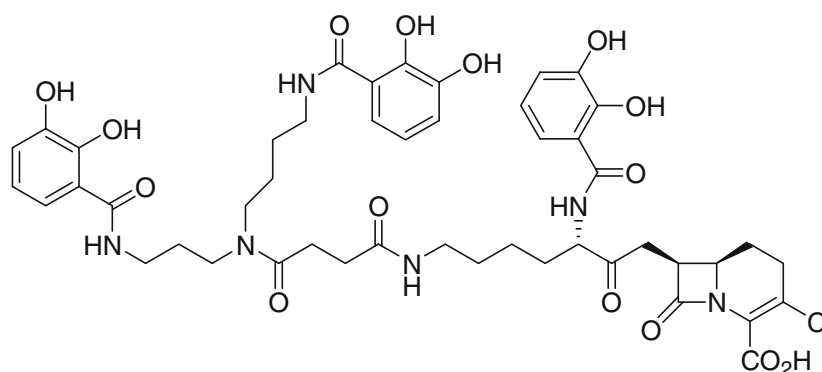


Figure 11. Tris-catechol β -lactam conjugate.

The role of the spacer can be exemplified in the series of tris(3-hydroxy-2-pyridinone) based chelators (Figure 12): the affinity of the tris-anchored propoxymethyl based ligand TRISPYR (Sun *et al.* 1998) for iron is higher compared to CP130 and its tris(aminopropyl) derivative (Streater *et al.* 1990). In this case, the enhanced complexing ability of TRISPYR is assigned to the greater flexibility of the spacer.

The influence of the spacer can also be shown with TRENCAMS (pFe=29.6), MECAMS (pFe=29.4), CYCAMS (pFe=24.9) (Harris *et al.* 1981) which respectively involve Tren, tris(1, 3, 5-aminomethyl)benzene and 1, 5, 9-triazacyclodecane spacers. For MECAMS, one can highlight the drastic effect of the inversion of the connecting units with a pFe value of 25.1 for TRIMCAMS, the latest possessing reversed connecting amide units (Harris *et al.* 1981).

On the another hand, in **Lo** and **Lm** (**o** and **m** standing for *ortho* and *meta* substitution, Figure 13), the role of the position of the connecting group is clearly shown: the salicylate moieties in **Lo** allows a better pre-organisation of the three arms, *via* a hydrogen bonding network (Garrett *et al.* 1992). Similar conclusions can be drawn if we compare *O*-TRENSOX (pFe=29.5) and *N*-TRENSOX (pFe=21.6) that differ only by the position of the link between the amide connectors and the 8-hydroxyquinoline moieties (7th position for *N*-TRENSOX and 2nd position for *O*-TRENSOX (Baret *et al.* 1995)).

Role of the chelating subunit and of the chelate ring sizes

The relative complexing abilities of catecholates, 8-hydroxyquinolines, hydroxamates and

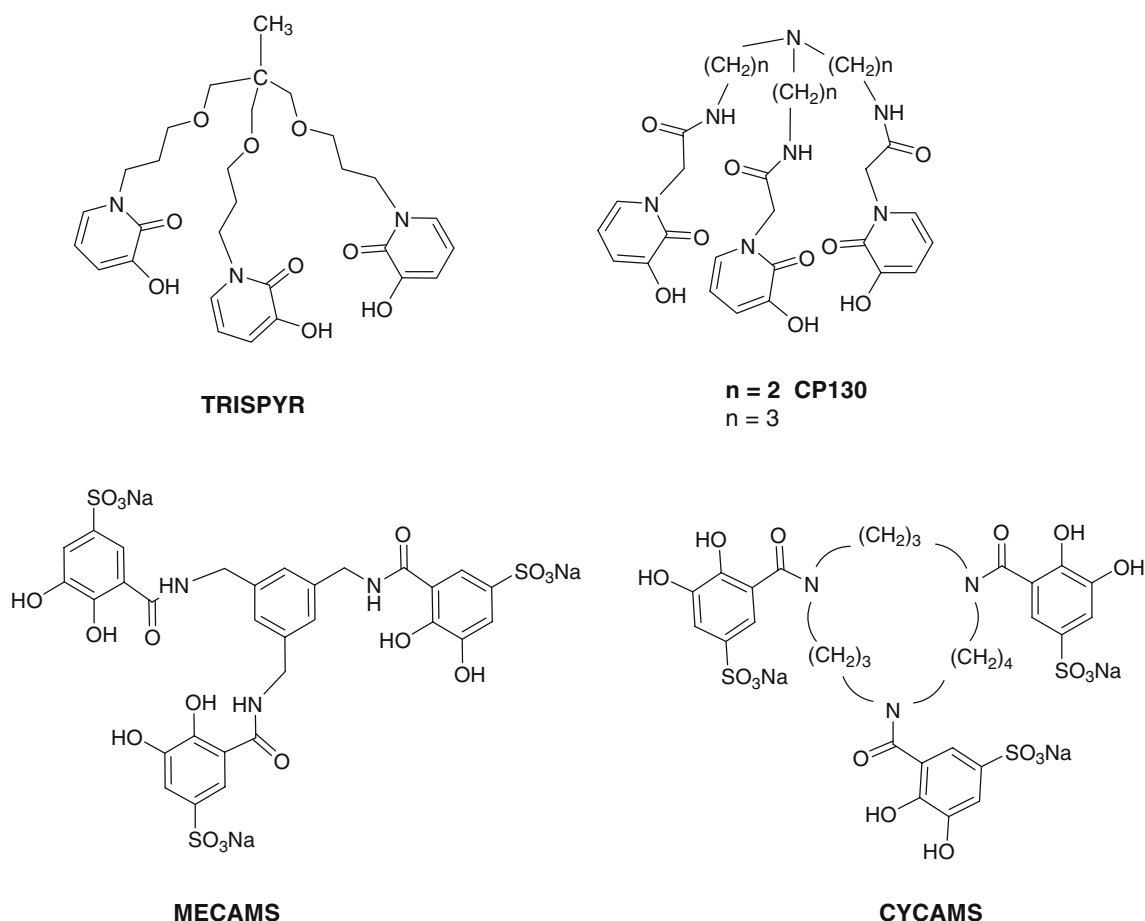


Figure 12. Chemical structures of TRISPYR, CP130, MECAMS and CYCAMS.

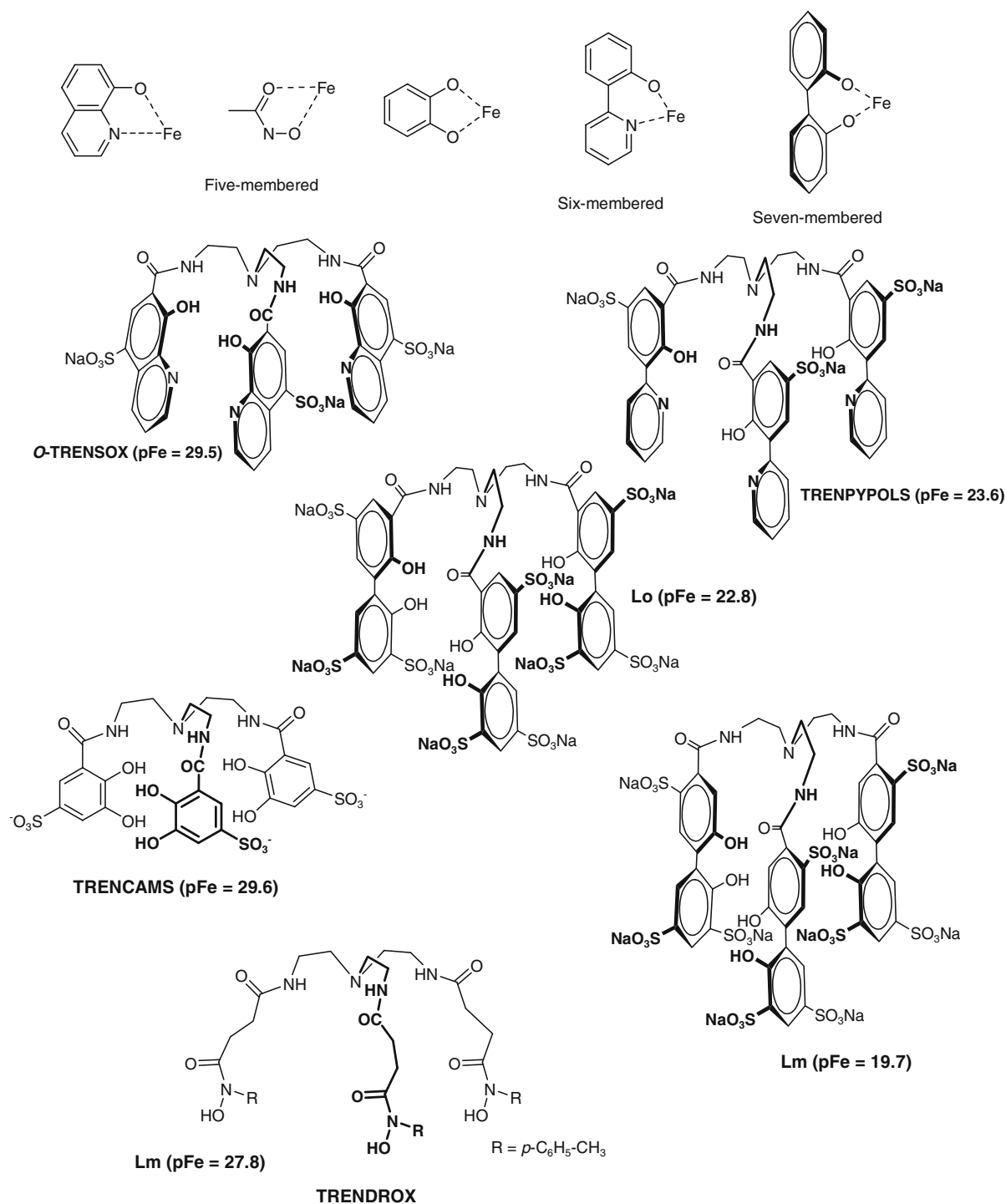


Figure 13. Chelating ring sizes and tripodal derivatives.

3-hydroxy-4-pyridinonates can be compared in the series of the tripodal TRENCAMS, O-TRENSEX, TRENDROX (Ng *et al.* 1989, Figure 13) and CP

130 (Streater *et al.* 1990, Figure 12) ligands which possess the same spacer (Tren), the same connecting amide groups, and three identical chelating

units mentioned above (Streater *et al.* 1990; Rai *et al.* 1999). The respective pFe values are 29.6 for catecholates, 29.5 for 8-hydroxyquinolinates, 27.8 for hydroxamates and 25.8 for 3-hydroxy-4-pyridinonates. Even if the pFe of natural hydroxamates-containing siderophores is the highest, this trend clearly indicates that catecholates and 8-hydroxyquinolinates can also act, in a thermodynamic sense, as efficient iron(III) chelating units. Even if almost all the natural and abiotic chelators involve five membered ring sizes (see above), it seems interesting to compare the complexing abilities (revealed by the pFe values) of ligands leading to other chelating ring sizes. The respective pFe of TRENYPOLs (pFe=23.6) which involves a six-membered chelate ring and of *O*-TRENSEX (pFe=29.5) corresponding to a five-membered chelate ring may allow a good comparison: the two water soluble chelators have the same atom donor sets, the same spacers and the same connecting groups (Figure 13; Baret *et al.* 2000). In the same way, the tris-catecholate TRENEX (pFe=29.6, Thomas *et al.* 1999a, b) and the two tris-2, 2'-dihydroxybiphenyl derivatives **Lo** (pFe=22.8) and **Lm** (pFe=19.7, Baret *et al.* 1998) allow comparisons between five-membered and seven-membered chelate rings (Figure 13). As revealed by the pFe values, the five-membered chelate-rings lead to drastically higher pFe values that can be explain, for an octahedral geometry, by a better minimization of the free energy of the coordination sphere.

Role of the sulphonate group

The pFe value is also tuned by the presence of one or several sulphonate groups on the siderophore. Nevertheless, its influence is often difficult to quantify since the unsulphonated counterparts are very poorly soluble in water, leading the determination of the pFe value very difficult. The comparison has however been possible in the case of TRENEX (pFe=29.6) and its unsulphonated counterpart TRENEX (pFe=27.8, Thomas *et al.* 1999). Sulphonated bidentate units exhibit slightly better complexing abilities ($\Delta pFe=1.8$ at pH=7.4) due to the electron withdrawing effect of the sulphonate group which renders the phenol group more acidic, since it is less prone to competitive reprotonation.

pH-dependence of the complexing abilities

Acidic media are encountered in the gastrointestinal tract, and acidification may be implicated in the release of iron from its complexes. So, the knowledge of the iron complexing properties of chelators at pH values lower than *usual* biological pH (pFe are given at pH=7.4) is needed for a better understanding of their biological metabolism. Nevertheless, only few studies deal with changes in pFe according to pH. It is well known that amide connectors allow salicylate complexation at low pH, in catechol (Cass *et al.* 1989) or hydroxyquinoline-based chelators (Serratrice *et al.* 1999, Figure 14).

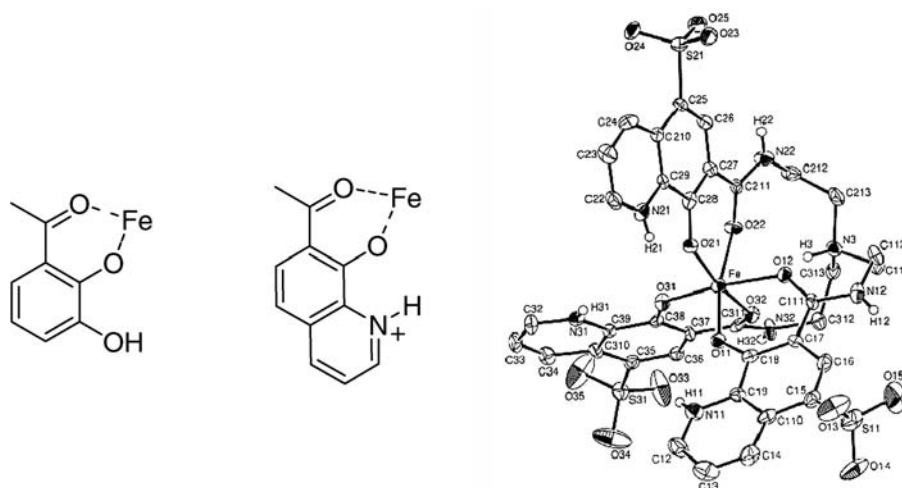


Figure 14. Salicylate complexation for catechol and 8-hydroxyquinoline-based chelators in acidic medium illustrated by the X-ray crystal structure of tris-salicylate Fe(III) complex with *O*-TRENSEX.

The salicylate complexation is often evidenced in protonation experiments (see below: kinetic studies). A systematic study of $pFe = f(pH)$ has been performed for some usual chelators (Serratrice *et al.* 1997; Albrecht-Gary *et al.* 2003, Figure 15).

Enterobactin (Figure 16), a natural siderophore, appears to be the better iron complexing

agent in a broad pH range. Among artificial siderophores, *O*-TRENTOX is a better chelator compared to Desferal® (Figure 16) which is the widely clinically used chelator in the overall pH range. If TRENCAMS is more efficient than *O*-TRENTOX at $pH > 7.4$, this latter is the more interesting chelator in acidic conditions. The

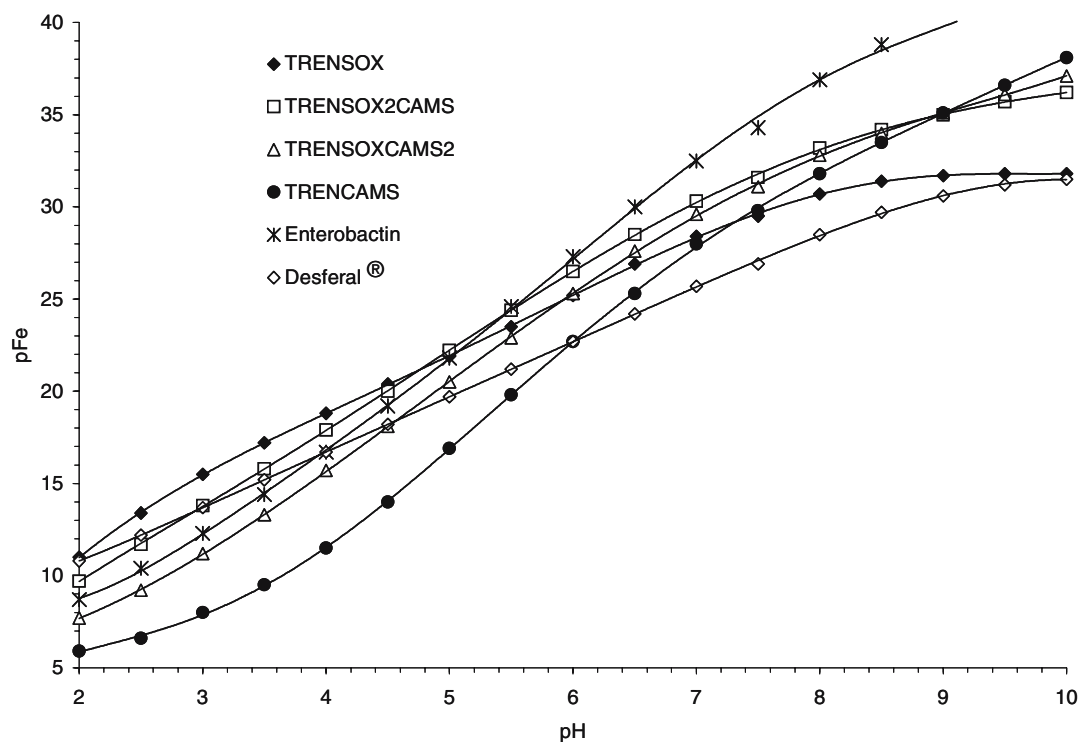
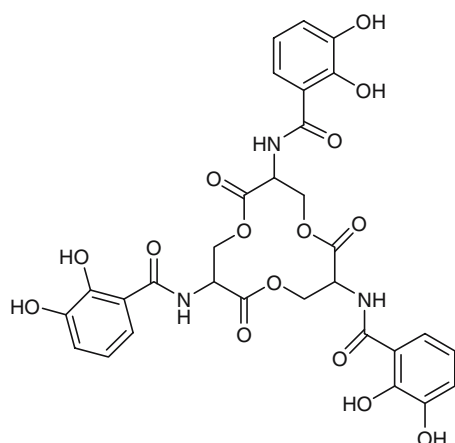
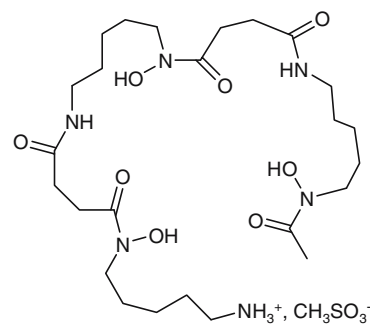


Figure 15. $pFe = f(pH)$ for usual chelators.



ENTEROBACTIN



DESFERAL®

Figure 16. Enterobactin and Desferal.

cooperative effects exhibited by the mixed ligands of the Figure 7 allow higher complexing abilities compared to the homoleptic parents in the overall pH range (Figure 15, Albrecht *et al.* 2003): TRENSOXCAMS2 is, to date and to the best of our knowledge, the more efficient abiotic iron chelating agent in the pH range of 5 to 9.

Kinetic studies and exchange reactions

Kinetics studies involving the acid hydrolysis of the Fe(III) complexes and the Fe(III) exchange between the tripodal ligands or with EDTA were investigated in order to suggest a molecular description of Fe(III) removal from the ligand. Proposed structures for the transition states and rates of coordination bonds cleavage in the complexes should be helpful for designing new ligands for appropriate Fe(III) exchange in biological systems (Albrecht & Crumbliss 1998). Analysis of kinetic data for the acid dissociation kinetics of the Fe(III) complexes with a series of tripodal ligands (Serratrice *et al.* 2004) shown that there is a common mechanism in all the series when the ligand structure varies. The acid hydrolysis reaction was found to proceed *via* a stepwise mechanism. The first step is very fast and was attributed to the dissociation of one arm of the tripodal ligand. This indicates that the complex has one labile arm (thus favouring the substitution by an arm of a competing ligand). The second and the third steps involve protonation of the complex that induce coordination changes, giving the corresponding tetra-coordinated bis-salicylate complex (coordination with carbonyl and *o*-hydroxyl oxygens). The final dissociation of the ligand proceeds in the fourth step for which an important structural effect is observed: from 1.2×10^{-5} to $2.2 \times 10^{-2} \text{ s}^{-1}$ for the rate constant of the proton-independent step and from 1.2×10^{-6} to $0.18 \text{ M}^{-1} \text{ s}^{-1}$ for the rate constant of the proton-dependent step. Differences in rate and mechanism between the different tripodal ligands are interpreted in terms of global electrostatic charge of the protonated ligand and donor ability of the chelating subunit (catechol, pyridinophenol *vs.* 8-hydroxyquinoline). The C–N bond rotation of the amide moiety and the importance, depending on the charge, of the hydrogen bonding network in the partially de-coordinated complexes are also emphasized.

Catechol and pyridinophenol subunits thus exhibit larger dissociation rates than 8-hydroxyquinolines.

The iron exchange between two tripodal ligands or between EDTA and a tripodal ligand occurs in several steps involving the formation of a precursor species (a ternary complex with the competitive ligand) and a successive substitution by the arms of the competing ligand. The kinetic data show that the substitution by a catechol group is faster compared to a 8-hydroxyquinoline chelating agent, presumably due to a greater electron donor ability and a lower bulkiness of the catechol group.

Redox potentials

The knowledge of the electrochemical data ($\text{Fe}^{3+}/\text{Fe}^{2+}$ redox potentials of the complexes) is required for a better understanding of the iron delivering system. Even if quite all the processes implicated in iron metabolism are based on the use of ferric iron, Fe^{2+} represents an interesting target due to its toxicity (see above), and the design of complexing agents remains of interest. This is not the case for natural siderophores, which exhibit very low redox potentials and have higher affinity for ferric iron (Albrecht & Crumbliss 1998 have reviewed a great number of redox potential for natural siderophores): compared to them, *O*-TRENSOX possesses a relatively high redox potential ($E_{1/2} = +87 \text{ mV vs. NHE}$) with a pFe^{2+} value of 17.9. It can be claimed that O_6 coordination spheres induce very low redox potentials, while N_3O_3 coordination spheres lead to slightly positive redox potentials. By a subtle choice of the chelating subunits, the chemist can tune the redox potential and prepare chelators well-suited for specific biological applications: a low redox potential is more interesting for anti iron overload therapy, while a higher redox potential is better for iron nutrition.

Polytopic tripodal ligands

A recent paper (Hara & Akiyama 2001) tackles the promising area of 'iron reservoir', *i.e.* ligands capable to accommodate more than one iron ion. A tripodal ligand (Figure 17) with a Tren spacer and amide connector based on bis-hydroxamate

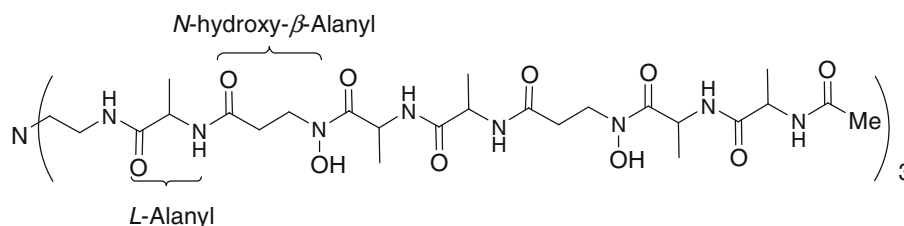


Figure 17. Ditopic tripodal ligand based on *L*-alanyl and *N*-hydroxy- β -alanyl sequence.

arms with a *L*-alanyl-*L*-alanyl-*N*-hydroxy- β -alanyl sequence allows the complexation of two ferric ions.

The two stability constants of the iron chelate are almost the same ($\log K_{\text{FeL}} = 28.5$ and 29.0) and close to that of ferrichrome. The design of such 'iron reservoir' constitutes a promising alternative to design new efficient iron chelators for removing iron from overloaded patients.

Design of iron chelators from molecular mechanic calculations

Owing to the fact that a large number of possible architectures exist for a given set of donor groups and that only a limited number of them can be examined experimentally, molecular mechanic calculations are helpful to predict how the structure of the ligand will influence metal ion complexation. The first general study, by the EFF (Empirical Force Field) method, of catecholate and hydroxamate siderophores and abiotic models has been reported by Shanzer (Shanzer *et al.* 1992). The lowest calculated conformation energies of synthetic models, as well as the parent natural siderophore, have underlined the paramount role of the hydrogen bonding network in enhancing binding efficiency. The results have confirmed the conclusions drawn from experiments. More recently tris-catecholamide siderophores have been studied by Raymond, using an extended MM3 model (Hay *et al.* 2001). Force field parameters were fitted to geometries and energies from electronic structure calculations, and to crystal structure data. The authors have shown that $\log \beta_{110}$ was roughly correlated with the calculated strain in the metal complex, *i.e.* with an energetic quantity that measures Fe^{3+} complementarity. An evaluation of the degree of

the binding site organization in tris-catecholamides revealed that none of the backbones examined in their study provides architectures with optimum complementarity for Fe^{3+} . Nevertheless, this study has highlighted the tools needed to screen potential backbone structures to obtain more effective tris-catecholate siderophores.

Selectivity towards other biologically relevant metal ions

The toxicity of an iron chelator may be an indication of the chelation of other essential elements. To be efficient, a chelator has thus to be selective (or even specific) of a given metal ion if the targeted therapy deals, for example, with metal overload disease. In this context, we investigated the ability of some chelators towards several biological relevant cations (Table 1, Biaso *et al.* 2002). In this case, we do observe a pronounced selectivity for iron(III).

The selectivity towards ferric iron remains true in biological relevant pH range of 4 to 9 (Biaso *et al.* 2002). Furthermore, the ferrous complex of *O*-TRENSEX does not promote the toxic Fenton chemistry in presence of hydrogen peroxide (Baret *et al.* 1995). This is a crucial result for therapeutic purposes because many reducers, as ascorbates and reductases, are present in living organisms.

Table 1. pM values for some selected iron chelators.

Ligand	pFe ³⁺	pFe ²⁺	pCu ²⁺	pZn ²⁺	pAl ³⁺	pCa ²⁺
<i>O</i> -TRENSEX	29.5	17.4	22.9	21.7	20.0	13.6
TRENCAMS	29.5	6.0	18.9	11.7	26.2	6.0
Desferal	26.6	6.0	11.7	6.4	19.4	6.0
EDTA	23.4	–	16.9	14.6	16.0	8.8

Conclusion

To date, synthetic procedures and essential physicochemical features are available for a great number of tripodal iron(III) chelators. Shanzer (Shanzer *et al.* 1992) has underlined the advantage of synthetic carriers for biological applications, which resides in their versatility. Contrary to natural siderophores, biomimetic analogues may be tuned to either act as growth promoters or inhibitors and may even be modified in order to have opposite activity towards different organisms. Shanzer has also claimed the interest of adding probes at exogenous positions that do not interfere with receptor recognition. The future age of the research on abiotic siderophores, suited for iron chelation therapy and/or iron nutrition, involves a better knowledge of the penetration of the free ligand or its iron complex through biological membranes. The permeability and the biological activity of siderophores are dictated by their three-dimensional structure and chirality. How does their shapes fit specific membrane receptors (whose structures are often unknown) remains the main question and chemists have to turn their attention to this point. The study of the interaction between iron chelators with well-suited peptide-based models may be a first approach at the molecular level. The chemistry of the iron assimilation processes thus remains a fascinating and attracting field of innovating research.

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