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The hydroxypyridinone (HOPO) journey into metal chelation

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Abstract

Hydroxypyridinones (HOPOs) form outstanding building blocks for the development of a variety of agents in the field of metal chelation. The pyridinone ring is easily synthesised and readily converted into tetradentate, hexadentate and octadentate chelators. There is considerable potential for the control of the stereochemistry of the resulting metal complex and hence the properties of these multidentate molecules. Their ability to rapidly bind hard metals in aqueous media has facilitated the development of efficient applications in both biological and medical contexts. In this review, an in-depth analysis of the synthetic methodologies for HOPO-based ligands is presented, as well as the many aspects to achieve optimal biological activity. Recent advances and current challenges for the future application of HOPO structures are outlined. The present flourishing development of drug candidates and diagnostic agents based on this chemical scaffold opens access to many new applications in analytical, environmental and clinical science.

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1. Introduction: the origins of hydroxypyridinone (HOPO) selection for iron(III) chelation

1.1. Design of orally active Iron chelators

Iron is essential for almost all microorganisms, plants and animals by virtue of its unique chemical properties; namely the ability to coordinate and activate oxygen, and the possession of an ideal redox chemistry ($\text{Fe}^{\text{II}} \rightleftharpoons \text{Fe}^{\text{III}} \rightleftharpoons \text{Fe}^{\text{IV}}$) for the facilitation of electron transport and metabolic processes.¹ However iron is toxic when present in excess. In the presence of molecular oxygen, 'loosely-bound' iron is able to redox cycle between Fe^{II} and Fe^{III} , thereby generating oxygen-derived free radicals, such as the hydroxyl radical.² A number of protective strategies are adopted by cells to prevent such damage, including iron storage, tightly controlled iron transport and controlled distribution of iron. However, there are situations when these protective mechanisms become saturated, either locally in ischemic tissue, or systemically, as with transfusion-induced iron overload. There is therefore a requirement for the selective removal of iron under such circumstances. Desferrioxamine-B (DFO, **1**), the most widely used therapeutic iron chelator for many years, has a major disadvantage of not being orally active,³ consequently there has been a search for orally active iron-selective chelators since the early 1970s.

Selection of appropriate ligands can be rationalised by adopting the concept of "hard" and "soft" acids and bases.⁴ High spin iron(III) is a tripositive cation of 0.65Å radius and as such is classified as a hard Lewis acid, by virtue of its high surface charge density. It forms stable bonds with charged oxygen atoms, such as phenolates. In contrast, the iron(II) cation has a lower charge density and favours nitrogen containing ligands. Such

ligands also bind other important divalent metals such as Cu(II) and Zn(II) with high affinity. In general, oxyanions are selective for tribasic metal cations over dibasic anions and as the majority of tribasic cations, for instance Al(III) and Ga(III), are not essential for living cells, iron(III) as opposed to iron(II), is the best target for 'selective iron chelator' design under biological conditions. A further advantage of high-affinity iron(III) chelators is that they chelate iron(II) and the resulting complex autoxidises under aerobic conditions to form iron(III).⁵ Thus high-affinity iron(III)-selective ligands scavenge both iron(III) and iron(II) under most physiological conditions.

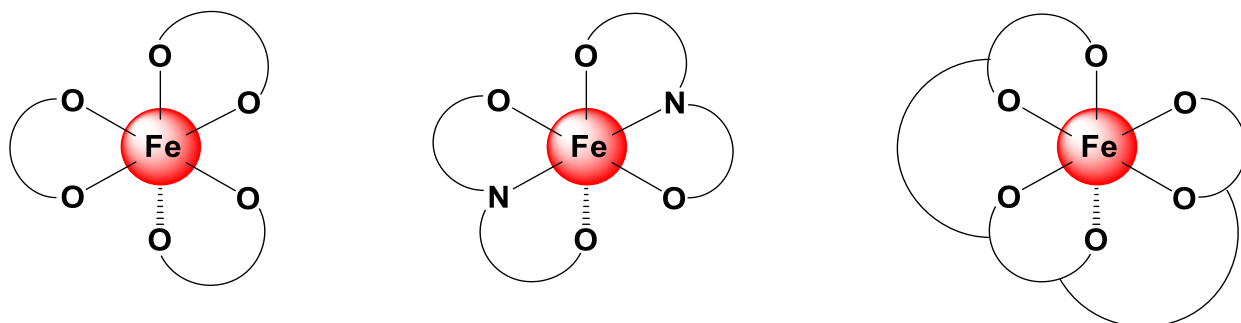


Figure 1. Octahedral iron complexes with six coordination sites. Bidentate ligands generate 3:1 complexes, tridentate ligands generate 2:1 complexes and hexadentate ligands generate 1:1 complexes.

High spin iron(III) favours an octahedral stereochemistry and this can be achieved by a single hexadentate ligand, two tridentate ligands or three bidentate ligands (Figure 1). The majority of iron scavenging compounds found in living systems are hexadentate ligands as demonstrated by the wide range of siderophore structures.⁶ However as

most of these compounds are hydrophilic and possess molecular weights in excess of 500, they possess low bioavailability *via* the oral route.⁷ Consequently, a search has been made for suitable bidentate and tridentate chelators, as these possess lower molecular weights and so are more likely to possess high oral bioavailability.⁸

1.2. Potential iron(III)-binding ligands

A range of established iron(III) bidentate ligands is presented in Figure 2.

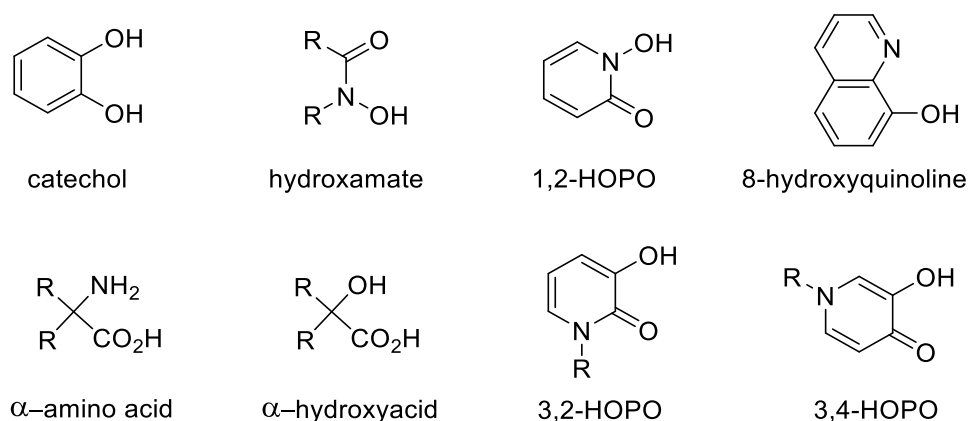


Figure 2. Bidentate iron(III) ligands.

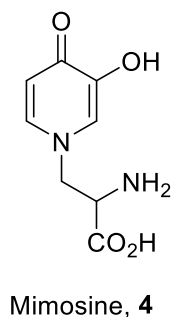
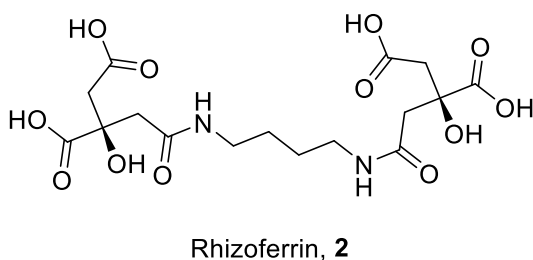
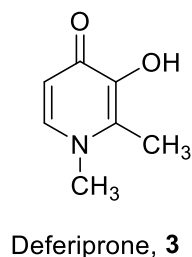
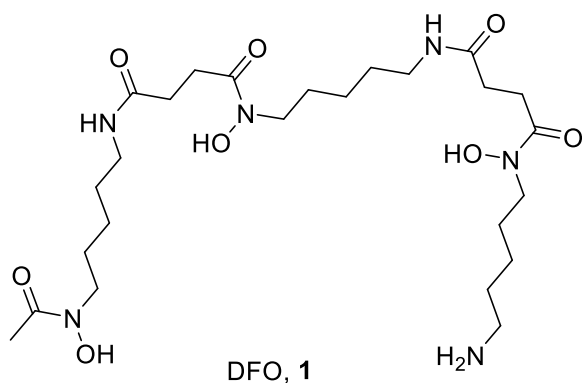
1.2.1. Catechols

Catechol residues possess a high affinity for tribasic metal ions resulting from the high electron density of both oxygen atoms. However, this pronounced charge density is also associated with a high affinity for protons (pK_a values, 12.1 and 8.4).⁹ Therefore, the binding of cations by catechols is highly pH sensitive.¹⁰ For iron(III) the $\log K_1$ value is 20 and the $\log \beta_3$ value is 40 (see Section 3.1). With bidentate catechols, the 2:1 complex is the dominant form in the pH range 5.5-7.5.¹¹ With such complexes, the iron atom is able

to interact with hydrogen peroxide or oxygen, possibly resulting in the generation of hydroxyl radicals. Moreover, catechol-based ligands are susceptible to oxidation.¹²

1.2.2. Hydroxamates

The hydroxamate moiety possesses a lower affinity for iron than catechol ($\log K_1 = 11.4$, $\log \beta_3 = 28$), but selectivity, as with catechols, favours tribasic cations over dibasic cations.¹³ Due to lower protonation constants, hydrogen ion interference at physiological pH values is less pronounced than for catechol, consequently the 3:1 complex dominates at pH 7.4. However, the affinity for iron is insufficient to solubilise iron(III) at pH 7.4 and thus bidentate hydroxamates are not suitable biological iron scavengers. In contrast hexadentate hydroxamates, for instance DFO (**1**), are powerful iron(III) chelators.



1.2.3. 8-Hydroxyquinolines

8-Hydroxyquinoline binds iron(III) tightly ($\log K_1 = 13.7$, $\log \beta_3 = 37$). This ligand also binds iron(II) tightly as indicated by the relatively high oxidation potential (-150mV). This is a result of the presence of 3 nitrogen atoms in the 3:1 complex. The value of the oxidation potential indicates that iron complexes are likely to redox cycle under most biological conditions¹³ and indeed many 8-hydroxyquinolines are toxic.

1.2.4. Aminocarboxylates

Bidentate aminocarboxylates do not form tight complexes with iron(III) at neutral pH values ($\log K_1 = 10$) and are unable to successfully compete with the hydroxide anion. In contrast multidentate aminocarboxylates, for instance EDTA and DTPA, bind iron tightly, but not with high selectivity;¹⁴ Ca(II) and Zn(II) also binding tightly to EDTA.

1.2.5. Hydroxycarboxylates

α -Hydroxycarboxylates bind iron(III), indeed the alcohol function frequently dissociates on binding iron(III),¹⁵ in contrast to most other transition metals. However bidentate α -hydroxycarboxylates, for instance lactic acid, possess a relatively weak affinity for iron(III) ($\log K_1 = 2.9$). In contrast, when incorporated in hexadentate siderophores, for instance rhizoferrin (**2**), they possess much higher affinities.¹⁶

1.2.6. Hydroxypyridinones

Hydroxypyridinones (HOPOs) combine the characteristics of both hydroxamate and catechol groups, forming 5-membered chelate rings in which the metal is coordinated by

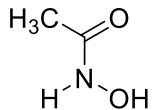
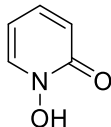
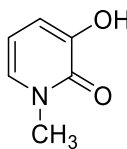
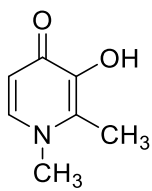
two vicinal oxygen atoms. The hydroxypyridinones are monoprotic acids at pH 7.4 and thus form neutral *tris*-iron(III) complexes. The affinity of such compounds for iron(III) reflects the pK_a values of the chelating oxygen atoms, the higher the affinity for iron(III), the higher the pK_a value (Table 1).^{17,18} There are three classes of metal chelating HOPO ligands, namely 1-hydroxypyridin-2-one (1,2-HOPO; $\log K_1 = 10.3$, $\log \beta_3 = 27$), 3-hydroxypyridin-2-one (3,2-HOPO; $\log K_1 = 11.7$, $\log \beta_3 = 32$) and 3-hydroxypyridin-4-one (3,4-HOPO; $\log K_1 = 14.2$, $\log \beta_3 = 37.2$). Of these, the pyridin-4-ones possess the highest affinity for iron(III) (Table 1) and are selective for tribasic metal cations over dibasic cations. The surprisingly high pK_a value of the carbonyl function of 3-hydroxypyridin-4-one results from extensive delocalisation of the lone pair electrons associated with the ring nitrogen atom. 3-Hydroxypyridin-4-ones form neutral 3:1 complexes with iron(III),¹⁹ which are stable over a wide range of pH values.¹⁸ The low redox potential of 3,4-HOPO iron complexes (-620 mV) indicates a strong bias towards iron(III) coordination.²⁰

1.3. Selection of an optimal bidentate iron-binding ligand class

A useful parameter for the comparison of the ability of chelating agents to bind iron(III) is the pFe^{3+} value (concentration of free Fe(III) in solution, i.e. $-\log[Fe^{3+}]$), which is typically defined by the following conditions: $[Fe^{3+}]_{total}$, 10^{-6} M; $[ligand]_{total}$, 10^{-5} M; pH 7.4.²¹ Hydroxamates, 1,2-HOPOs and 3,2-HOPOs are not suitable scavenging bidentate chelators for iron as they do not possess a sufficiently high pFe^{3+} value to function as an iron scavenger (Table 1). Catechols are not suitable due to their tendency to form 2:1 complexes at neutral pH values. α -Amino- and α -

hydroxycarboxylates do not compete efficiently with the hydroxide anion at neutral pH values. Iron complexes of 8-hydroxyquinolines are associated with the risk of redox cycling. This leaves 3-hydroxypyridin-4-one as the only bidentate ligand class presented in Figure 2 that is suitable for scavenging iron under biological conditions (see Section 5.2.1.1).

Table 1. pK_a values and iron(III) affinity constants of acetohydroxamic acid and three different HOPOs.

Ligand	Structure	pK_{a1}	pK_{a2}	$\log\beta_3$	$pFe^{3+ a}$
Acetohydroxamic acid		—	9.4	28.3	13
1-Hydroxypyridin-2-one		—	5.8	27	16
1-Methyl-3-hydroxypyridin-2-one		0.2	8.6	32	16
1,2-Dimethyl-3-hydroxypyridin-4-one (deferiprone, 3)		3.6	9.9	37.2	20.5

^a $pFe^{3+} = -\log[Fe^{3+}]$ when $[Fe^{3+}]_{total} = 10^{-6}$ M and $[ligand]_{total} = 10^{-5}$ M at pH = 7.4. An ideal value for efficient iron scavenging in biological matrices is $pFe^{3+} \geq 20$. Data sourced from references.^{17,18}

1.4. Selection of optimal multidentate iron-binding ligands

As indicated in the previous section, multidentate ligands are less likely to possess good oral activity, but they still have considerable potential as high affinity chelating agents for highly charged cations for instance Fe^{3+} , Ga^{3+} , Gd^{4+} , Pu^{4+} (see Section 4). Furthermore, their utility as chelating agents is not limited to 3,4-HOPOs; like hydroxamates, 1,2-HOPOs and 3,2-HOPOs when incorporated into multidentate ligands form powerful chelating agents. Thus, the design and synthesis of multidentate chelators is also a matter of some significance. Many naturally occurring hexadentate chelators (siderophores) which incorporate hydroxamate, catechol and α -hydroxycarboxylate functions are well characterized.⁶ The synthesis and properties of these molecules together with synthetic analogues have been reviewed.²²⁻²⁵ However analogues containing hydroxypyridinones have been less well covered and hence we overview such synthetic work in this review (Section 2). Hexadentate hydroxypyridinone chelating functions can adopt two broad structural designs, linear and tripodal (Figure 3). A range of tripodal bases are possible including cyclic esters, cyclic amides and sugars. Such molecules can also be capped (Figure 3). Synthetic approaches to these different structural designs are described in Section 2.

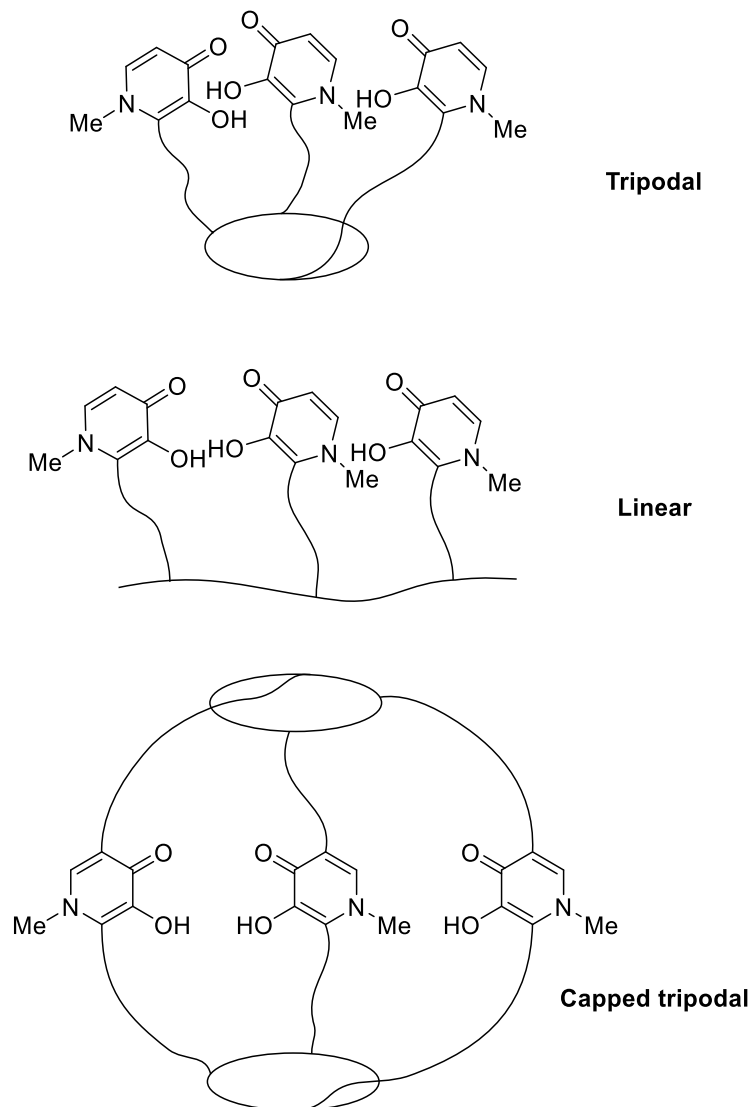


Figure 3. Structural design of hexadentate 3,4-HOPOs.

2. Synthesis of hydroxypyridinones

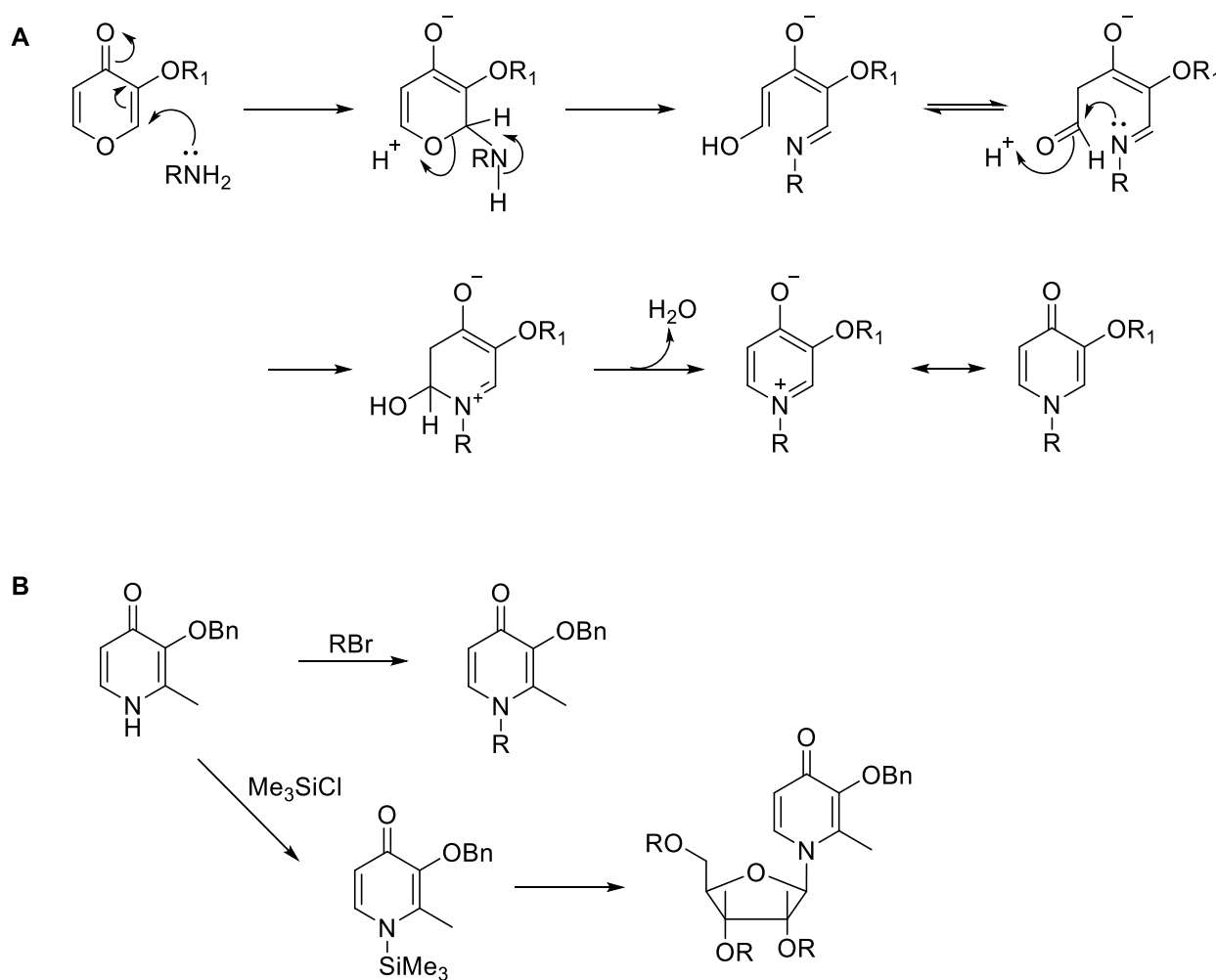
2.1. Bidentate

2.1.1. *N*-Substituted 3-hydroxypyridin-4-ones

The earliest record of the preparation of a 3-hydroxypyridin-4-one dates back to 1931²⁶ when Armit and Nolan converted pyromeconic acid to the protected 3-hydroxy-1-methylpyridin-4-one (Scheme 1A) *via* a double Michael addition reaction. This synthetic

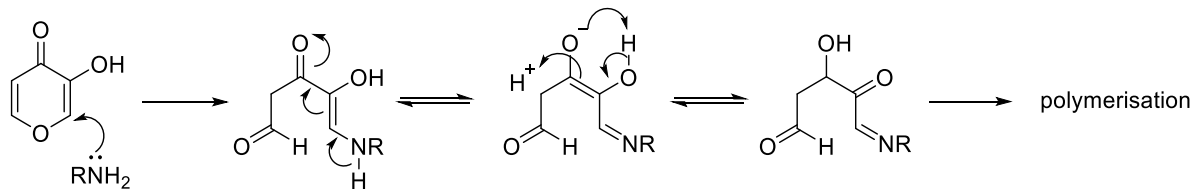
approach has been widely adopted since that time, as reported by Kleipool and Wibaut,²⁷ Heyns and Vogelsang,²⁸ Berson *et al.*,²⁹ and Adams *et al.*³⁰ It has been utilised for the synthesis of mimosine (**4**)^{31,32} and to prepare a range of 3-hydroxypyrid-4-ones with aliphatic *N*-substituents.³³

Scheme 1. A) Conversion of pyran-4-one to a 3,4-HOPO by reaction with an amine; B) substitution at position 1 of 3,4-HOPO by an alkyl function or protected sugar.



Although the double Michael addition occurs in acceptable yield without 3-hydroxy protection when non-bulky amines, for instance NH_3 and methyl amine, are used as the nucleophile, lower yields result when more bulky amines are reacted. The double Michael addition involves sequential ring cleavage and ring closure (Scheme 1A) and presumably the more rapid the ring closure, the higher the yield of the desired product and the lower the corresponding yield of side products, resulting from polymerisation of the acyclic intermediate. More bulky amines are likely to slow the ring closure step, so enhancing the life time of the unsaturated acyclic intermediate, thereby encouraging polymerisation. A factor which leads to enhanced yields of the pyridin-4-one is the protection of the 3-hydroxyl function. In two studies which lacked 3-hydroxyl protection, the range of yields were reported to be 3-18%³⁴ and 5-40%,³⁵ whereas when the 3-hydroxyl function was protected with, for instance a benzyl group, the range of yields was 53-84%.³⁶ Such protection avoids the generation of an α -hydroxy ketone intermediate (Scheme 2), accumulation of which will increase the rate of intermolecular reaction, leading to polymer formation. The 3-hydroxyl function can be protected by the formation of a methyl ether³⁷ or more commonly a benzyl ether.^{8,38,39} The methoxy group is typically cleaved by treatment with BBr_3 , whereas the benzyl group can be cleaved under more mild conditions, for instance with BCl_3 , catalytic hydrogenation or neat trifluoroacetic acid. This synthetic procedure has been reported in many patents.^{8,40-42}

Scheme 2. Formation of α -hydroxy ketone intermediate.

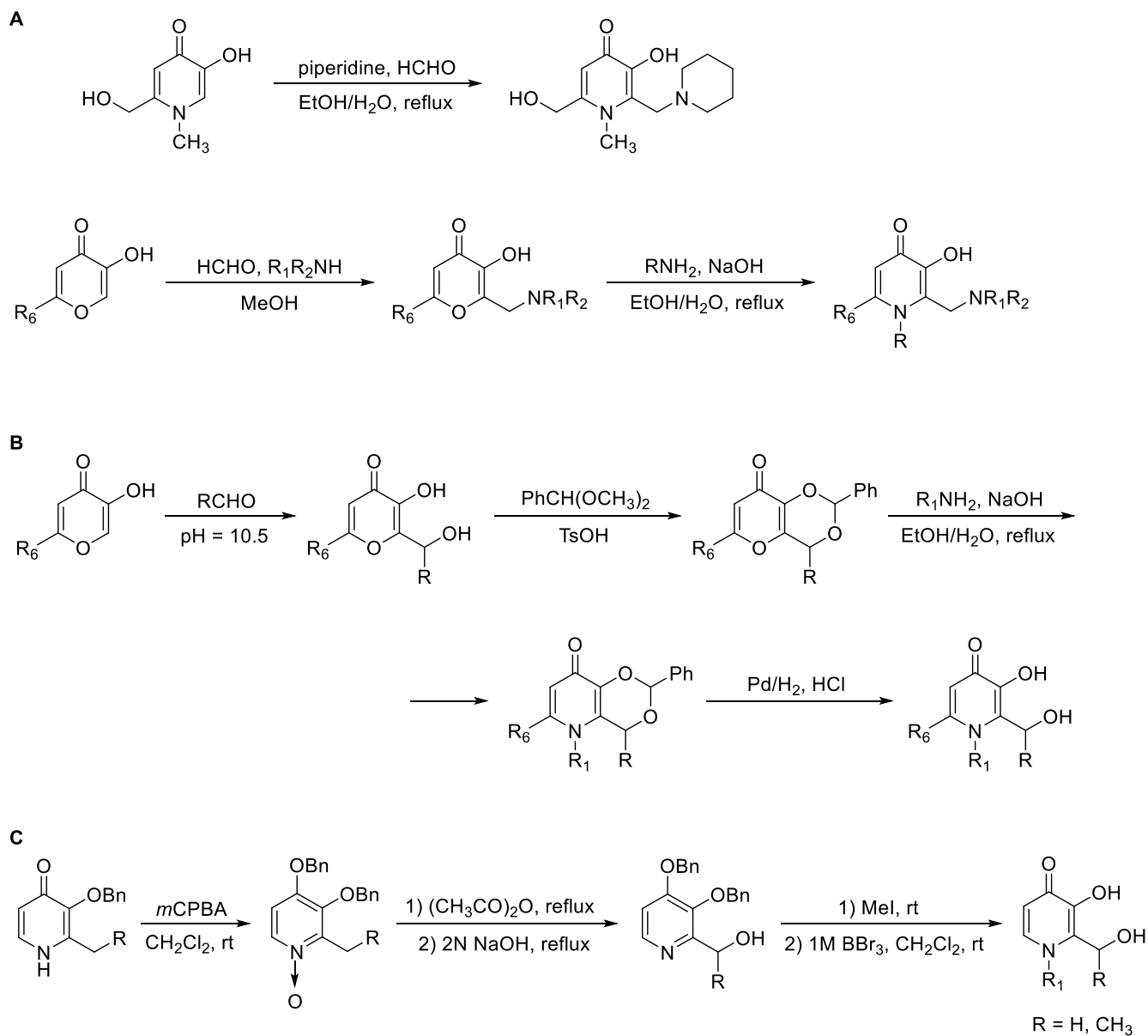


An alternative route to *N*-substituted 3-hydroxypyridin-4-ones is from the corresponding unsubstituted pyridinone (Scheme 1B).⁴³ This approach has been utilised for nucleoside synthesis, using trimethylsilyl intermediates (Scheme 1B).⁴⁴⁻⁴⁶

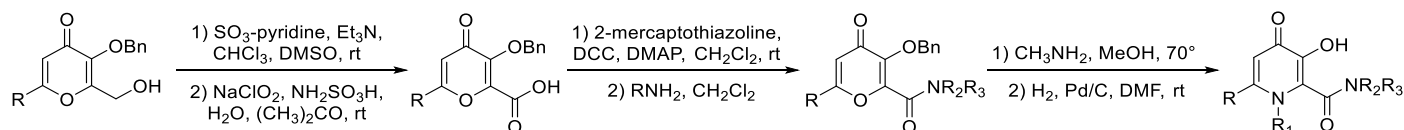
2.1.2. 2-Substituted 3-hydroxypyridin-4-ones

The Mannich reaction was first utilised by Taylor and coworkers for the preparation of 2-substituted pyridin-4-ones.⁴⁷ (Scheme 3A) This reaction also reaches high yields with the corresponding pyrones,^{47,48} which can then be subsequently converted to pyridin-4-ones (Scheme 3A). The aldol reaction has also been utilised to functionalise position 2 on pyromeconic acid and allomaltol (Scheme 3B) to provide a wide range of (1'-hydroxyalkyl)-3-hydroxypyridin-4-ones^{39,49} using benzaldehyde dimethyl acetal as a protecting group.⁴⁹ A convenient method for the production of 2-hydroxymethyl derivatives is *via* a pyridine N-oxide intermediate (Scheme 3C).⁵⁰ The 2-hydroxymethyl substituent has been further utilised to conjugate additional moieties, for instance pegylated side chains⁵¹ and by conversion to the corresponding 2-aminomethyl derivatives and conversion to carboxylic acids with subsequent amidation (Scheme 4).⁵²⁻⁵⁴

Scheme 3. Synthesis of 2-substituted 3,4-HOPOs. A) Mannich reactions with 3,4-HOPO and pyran-4-ones; B) aldol reaction with pyran-4-ones; C) preparation of 3,4-HOPOs *via* pyridine-*N*-oxide intermediate.



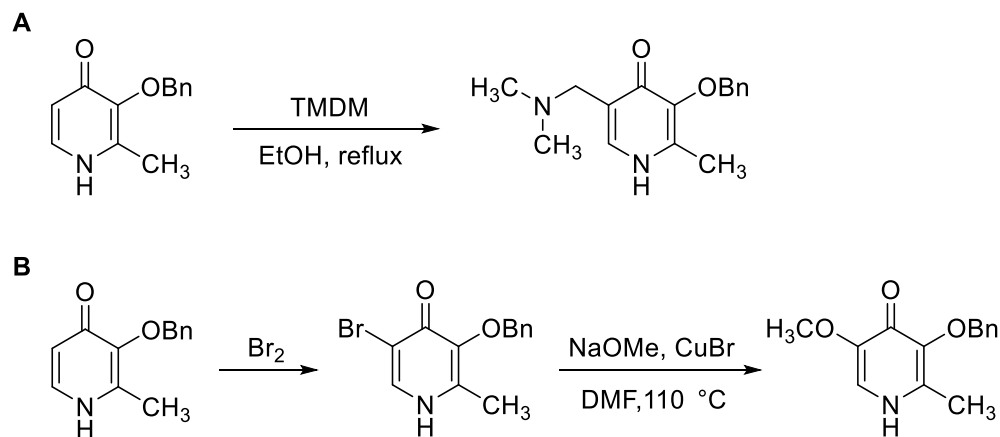
Scheme 4. Synthesis of 2-carboxyamido-3,4-HOPOs.



2.1.3. 5-Substituted 3-hydroxypyridin-4-ones

Aminomethylation of 2-methyl-3-hydroxypyridin-4-ones leads to functionalisation of position 5,⁴⁷ this approach has been utilised to produce an extensive range of substituted pyridin-4-ones (Scheme 5A).⁵⁵ Position 5 can also be selectively brominated and then subsequently substituted by the methoxy group (Scheme 5B).⁵⁶

Scheme 5. Synthesis of 5-substituted 3,4-HOPOs.

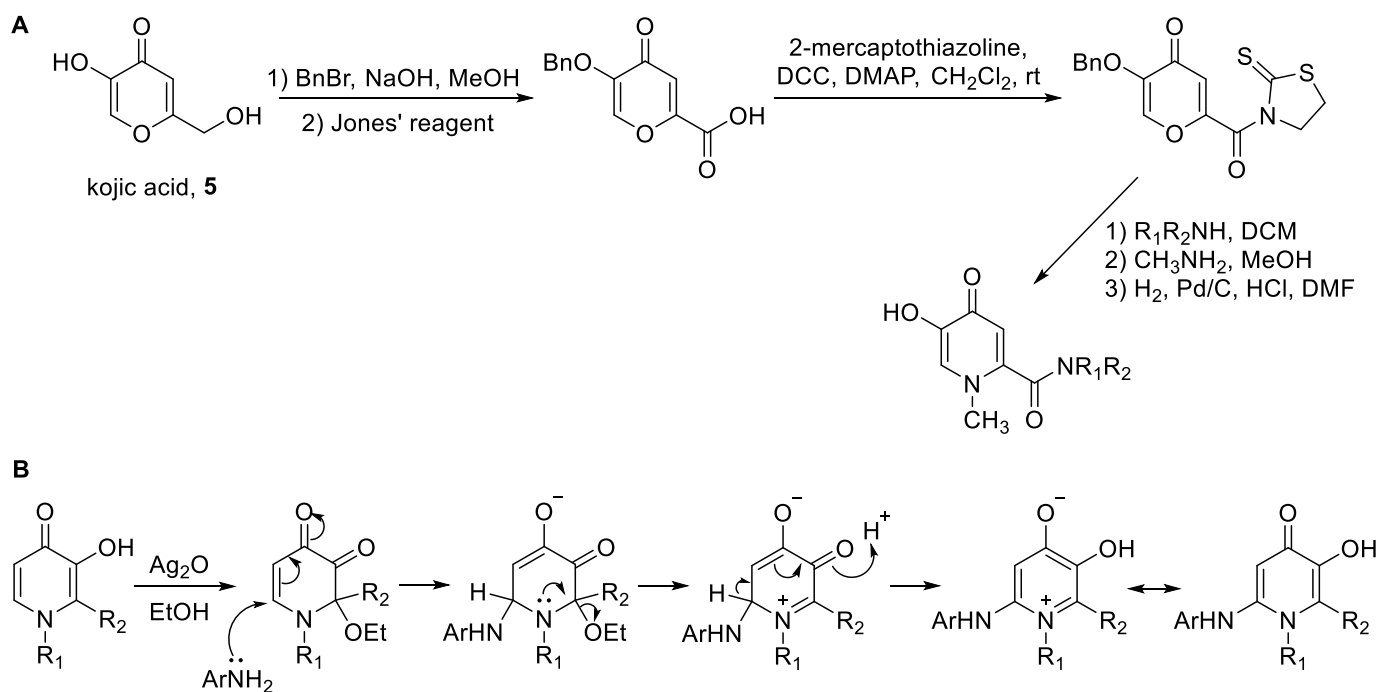


2.1.4. 6-Substituted 3-hydroxypyridin-4-ones

Kojic acid (**5**) is a convenient starting point for the synthesis of a range of 6-substituted pyridin-4-ones, for instance pegylated derivatives.⁵¹ In addition, oxidation and subsequent amide formation has led to the synthesis of a wide range of analogues

(Scheme 6A).⁵⁷ 6-Substituted-2-alkyl-3-hydroxypyridin-4-ones can also be prepared in a one-pot procedure from the parent pyridinone in good yield (Scheme 6B).⁵⁸

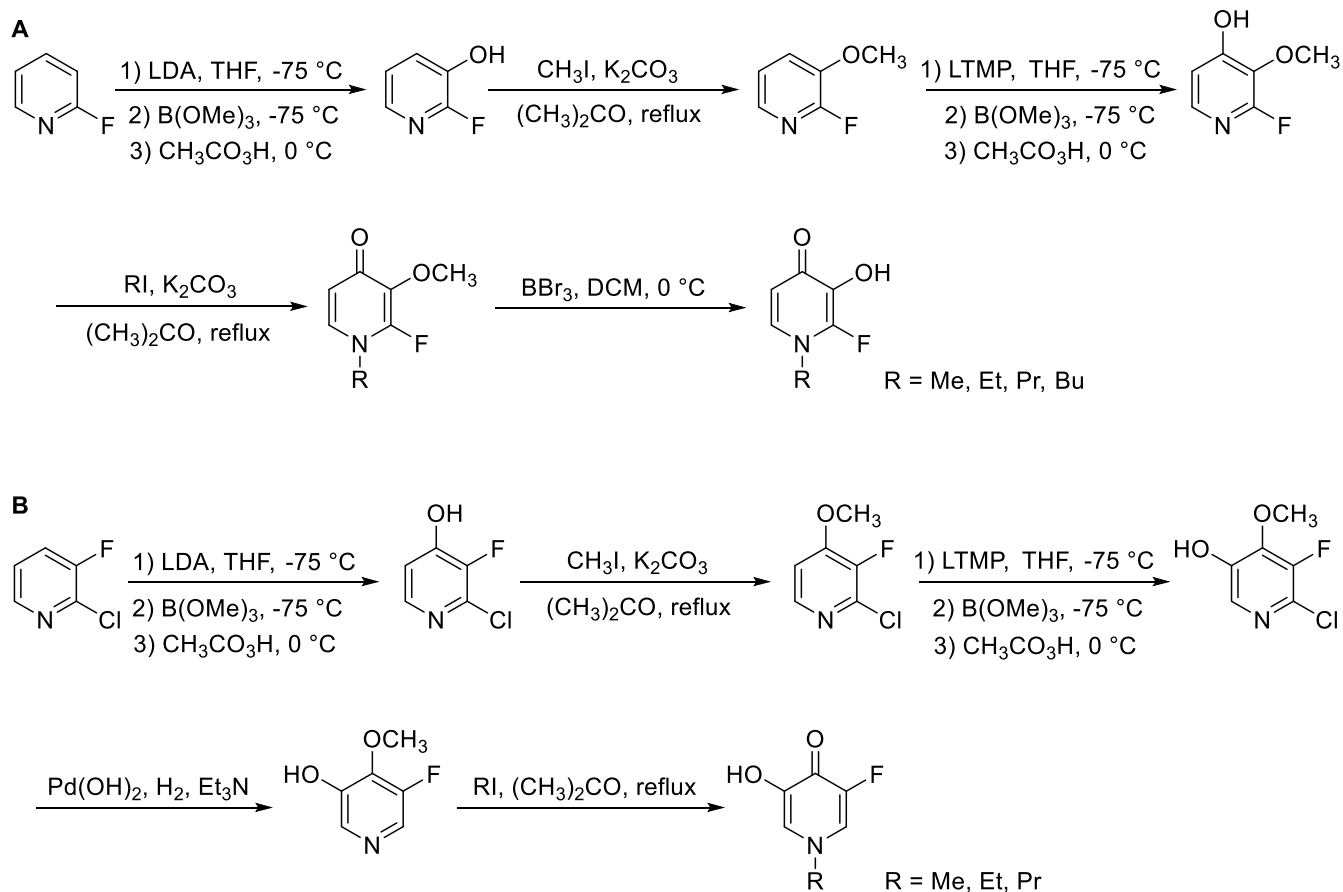
Scheme 6. Synthesis of 6-substituted 3,4-HOPOs. A) Conversion of kojic acid (**5**) to 6-carboxyamido-3,4-HOPOs; B) conversion of 1,2-dialkyl-3,4-HOPOs to 6-arylamino-1,2-dialkyl-3,4-HOPOs.



2.1.5. Fluorine substituted 3-hydroxypyridin-4-ones

A series of both 2-fluoro- and 5-fluoro-derivatives have been prepared from fluorinated pyridines.^{59,60} The general synthetic strategy for the synthesis of 2-fluoro derivatives is based on Li salt-catalysed aromatic hydroxylation and is presented in Scheme 7A. The strategy for synthesis of 5-fluoro derivatives is presented in Scheme 7B. Trifluoromethyl derivatives can be prepared in an analogous fashion.⁵⁹

Scheme 7. Synthesis of fluorine-substituted 3,4-HOPOs. A) Synthesis of 2-fluoro-3,4-HOPOs; B) synthesis of 3-fluoro-3,4-HOPOs.



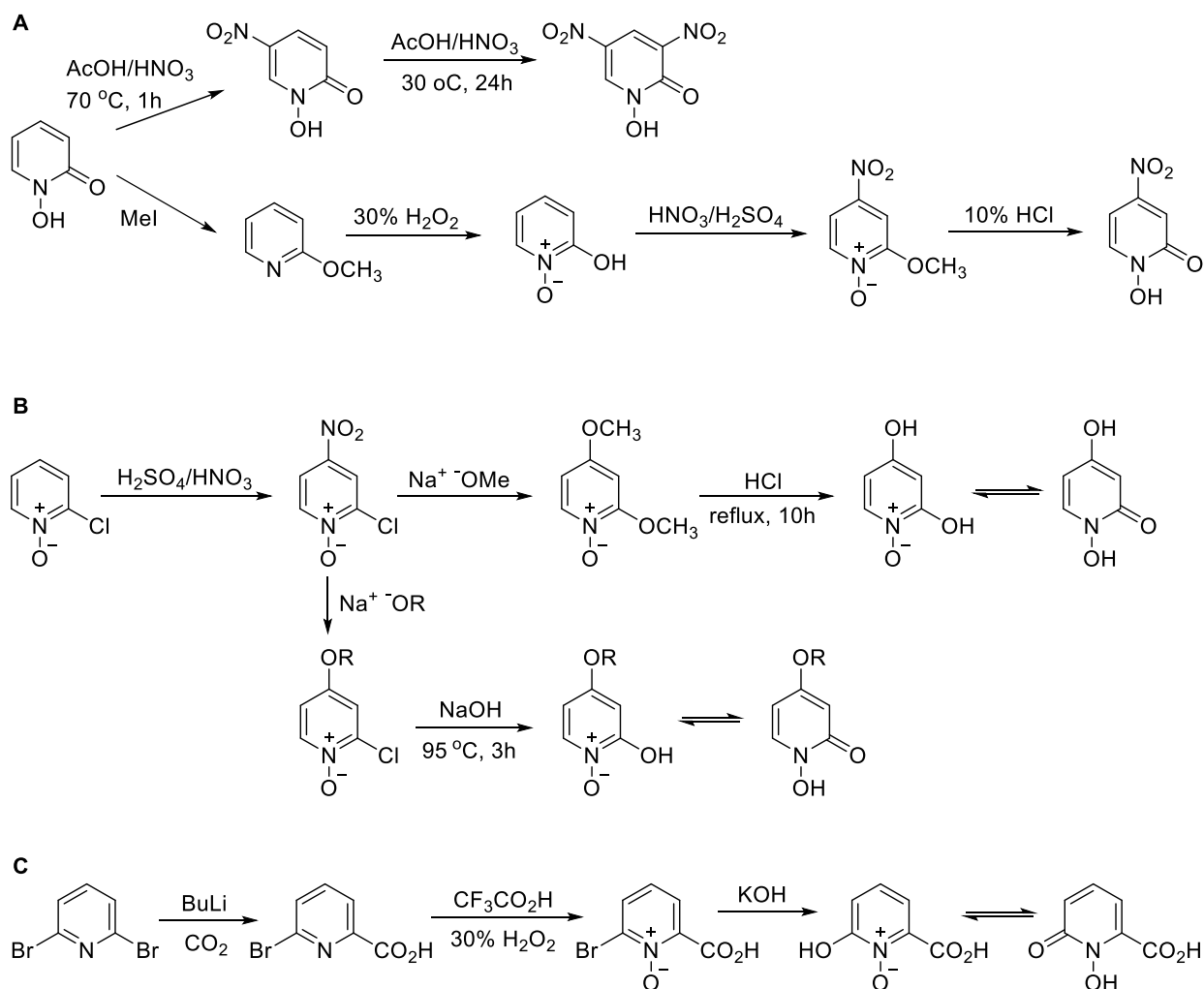
2.1.6. Methyl substituted 3-hydroxypyridin-4-ones

The complete range of 1, 2, 5 and 6 monomethyl-3-hydroxypyridin-4-ones, 1,2,-; 1,5,-; 1,6,- and 2,5,- dimethyl-3-hydroxypyridin-4-ones and 1,2,5-trimethyl-3-hydroxypyridin-4-one have been synthesised⁶¹ in order to compare their properties with those of deferiprone (1,2-dimethyl-3-hydroxypyridin-4-one) (**3**), an iron chelator with wide clinical application (see Section 5.2.1.1).^{13,62}

2.1.7. Synthesis of bidentate 1-hydroxy- and 3-hydroxypyridin-2-ones

1-Hydroxypyridin-2-one can be directly nitrated to yield three different nitro derivatives (Scheme 8A)⁶³⁻⁶⁵ and 2-chloropyridine-*N*-oxide is a precursor of 4-alkoxy and 4-hydroxy derivatives (Scheme 8B).^{66,67}

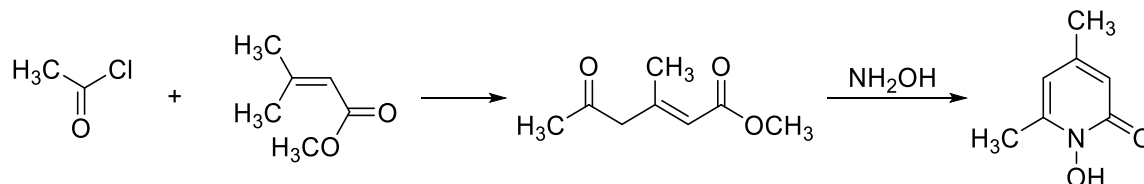
Scheme 8. Synthesis of bidentate 1,2-HOPOs.



6-Carboxy-1-hydroxypyridin-2-one, which is useful for the synthesis of oligopyridinone chelators (see Section 2.4.1.1), can be prepared from 2,6-dibromopyridine (Scheme

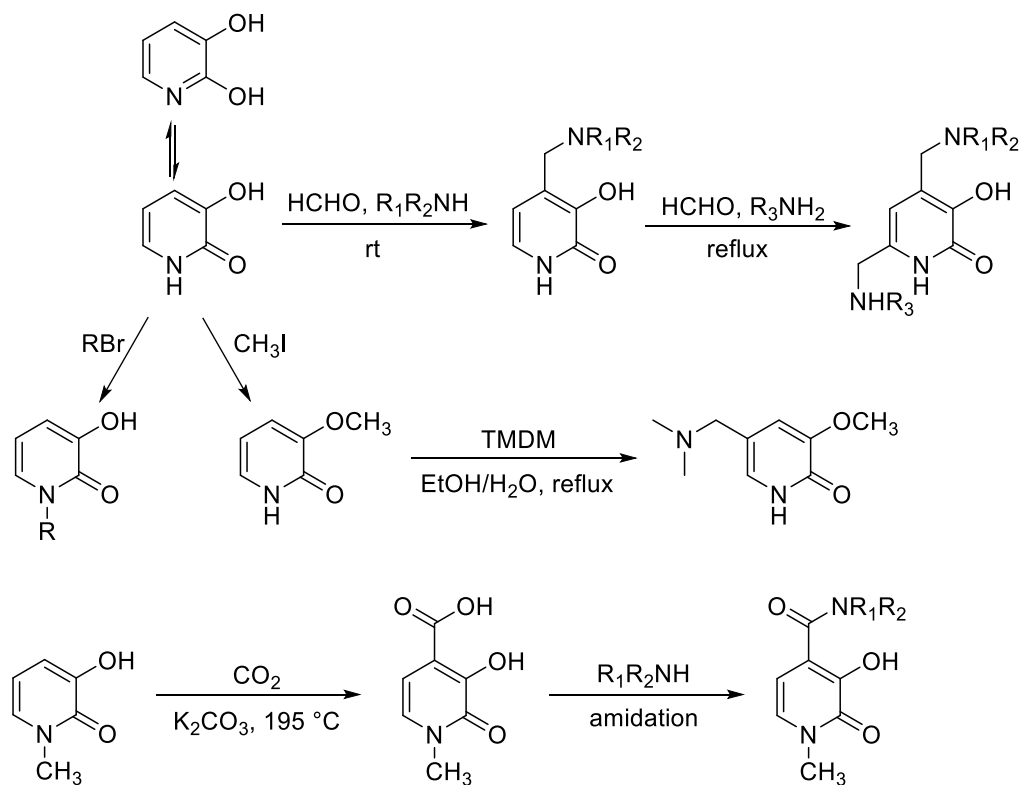
8C).^{68,69} 1-Hydroxy-pyridin-2-ones can also be formed from acyclic precursors (Scheme 9).⁷⁰

Scheme 9. Formation of a 1,2-HOPO from an acyclic precursor.



2,3-Dihydropyridine is a useful starting point for the synthesis of a wide range of 3-hydroxypyridin-2-ones as initially reported by Mohrle and Weber.⁷¹ A series of *N*-substituted derivatives have been prepared⁷² and derivatisation of the ring at the 4, 5 and 6 positions is possible by use of the Mannich reaction (Scheme 10).⁴⁷ A carboxyl function can be readily introduced at position 4 and this in turn can be amidated.⁷³

Scheme 10. Synthesis of bidentate 3,2-HOPOs.



2.2. Potential tridentate hydroxypyridinones

In principle tridentate ligands are likely to be more kinetically stable than the corresponding bidentate analogues. Putative tridentate hydroxypyridin-4-one analogues were synthesised by the addition of either a phenol or carboxylate function at ring position 2 (Figure 4). 2-Aminomethyl-1,6-dimethyl-3-hydroxypyridin-4-one was condensed with a range of carboxylic acids *via* *N*-hydroxysuccinimide intermediates.⁵² None of the compounds were found to bind iron(III) in tridentate mode, each favouring the formation of 3:1 complexes with the hydroxypyridin-4-one units binding iron in bidentate mode.

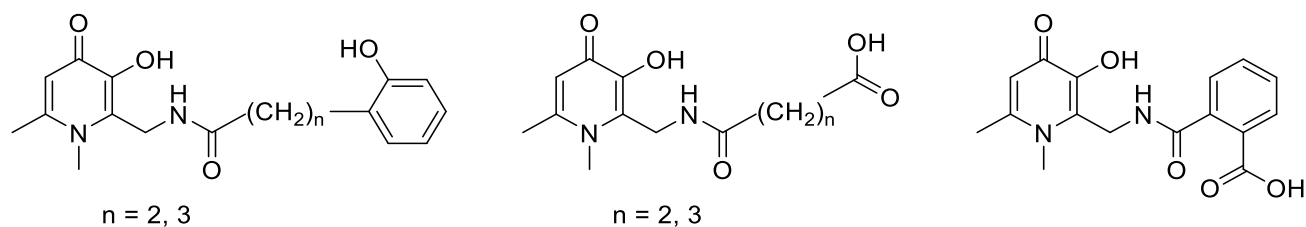


Figure 4. Potential tridentate 3,4-HOPOs.

2.3. Tetradentate hydroxypyridinones

There are two possible structures for the iron complexes of tetradentate hydroxypyridinones, **6** and **7**, both with a metal:ligand ratio of 2:3 (Figure 5). Structure **6** is apparently the most favoured form for both hydroxamate siderophores⁷⁴ and 1-hydroxypyridin-2-ones.⁷⁵ With larger cations, for example europium(III), complexes with the metal to ligand ratio of 1:2 are formed (**8**).⁷⁶ In contrast, with oxycation species such as UO_2^{2+} and MoO_2^{2+} stable 1:1 complexes result (**9**) (Figure 5).

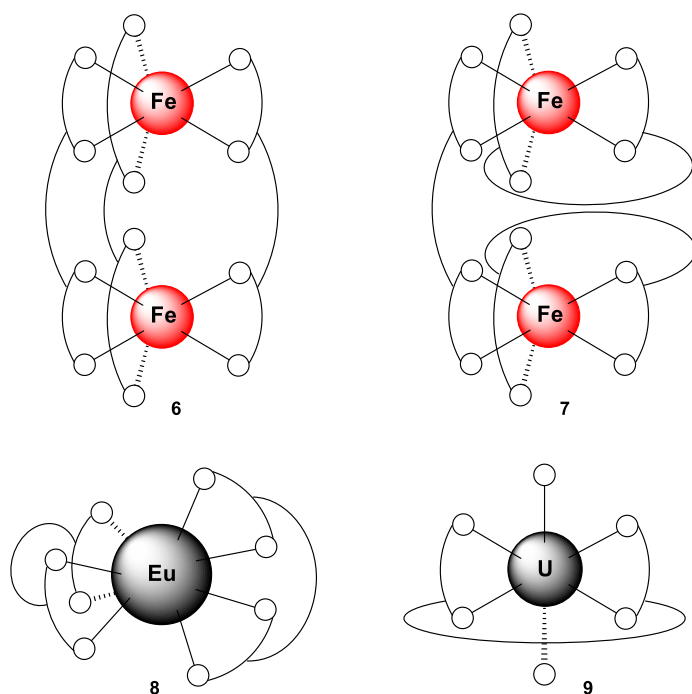
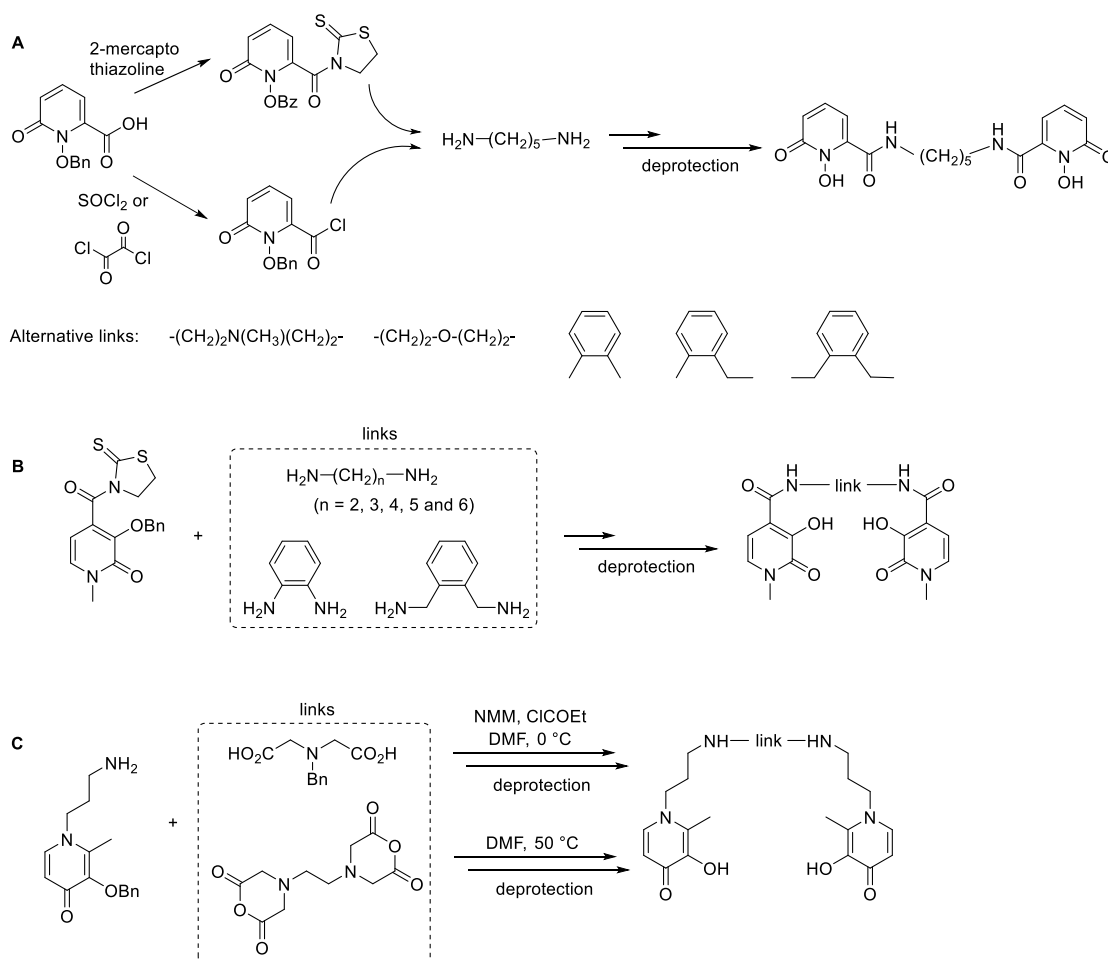


Figure 5. Possible structures for metal complexes of tetradentate hydroxypyridinones.

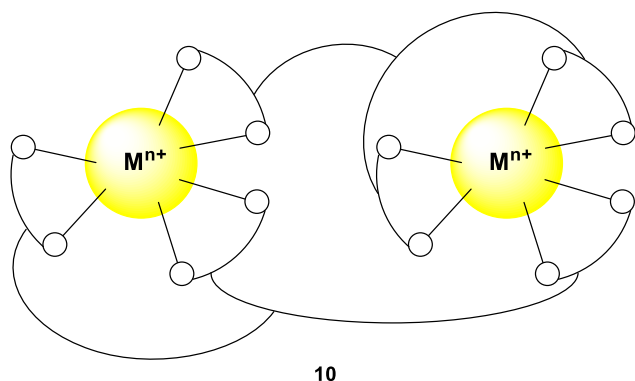
The majority of tetradentate 1,2-HOPOs are synthesised from 1-benzyloxy-6-carboxypyridin-2-one (Scheme 11A), with the carboxylic acid function being activated for amide formation.⁷⁶⁻⁷⁸ Tetradentate 3,2-HOPOs have been designed for uranyl chelation⁷⁹ and are largely based on the coupling of 4-carboxy-3-hydroxy-1-methylpyridin-2-one to various diamines using identical coupling methods (Scheme 11B).⁸⁰ Tetradentate 3,4-HOPOs have also been developed by Santos *et al* for the chelation of molybdenum(VI)⁸¹ (Scheme 11C).

Scheme 11. Synthesis of tetradentate HOPOs.



2.4. Hexadentate hydroxypyridinones

As indicated in the introduction (Figure 3), there are three common basic structural designs for metal complexes formed from hexadentate hydroxypyridinones, tripodal, linear and capped tripodal. However, if the ligand structure is not optimal for 1:1 hexadentate coordination, a 2:2 metal:ligand complex (**10**) may also form and even dominate.⁸²



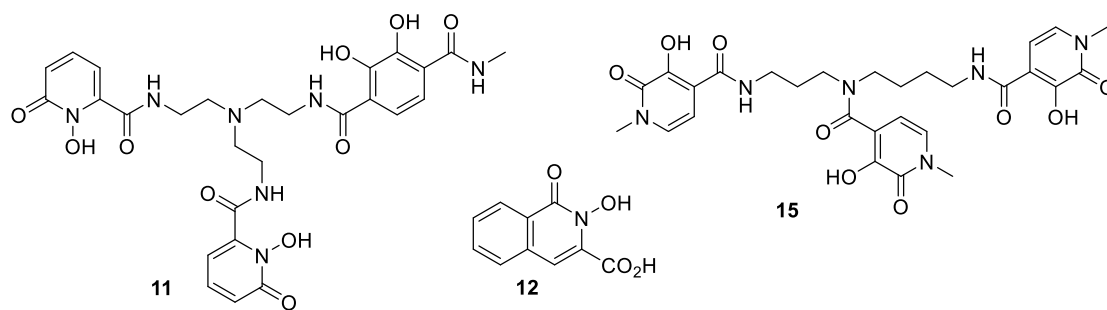
2.4.1. Synthesis of tripodal hexadentate ligands

2.4.1.1. 1-Hydroxypyridin-2-ones (1,2-HOPOs)

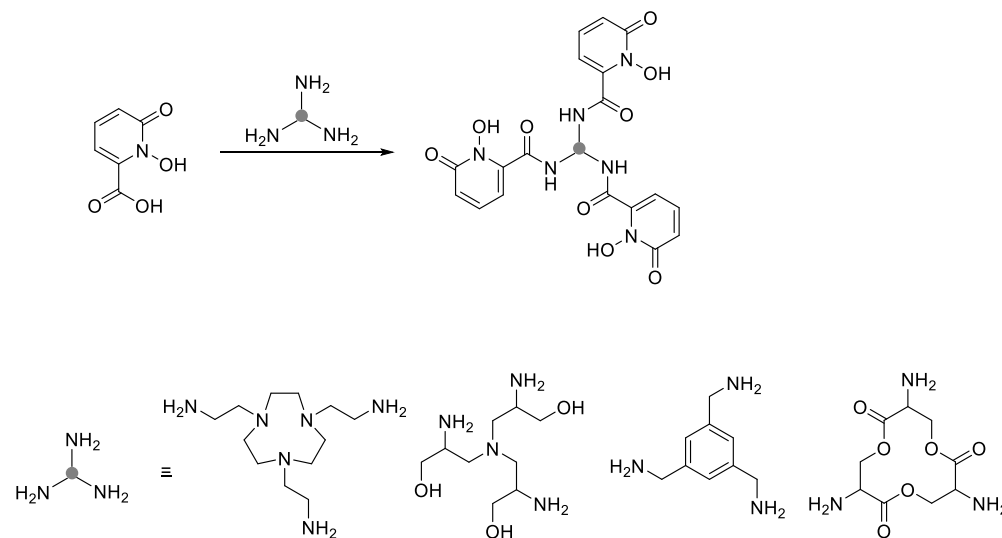
6-Carboxy-1-hydroxypyridin-2-one is the main bidentate ligand adopted for tripod 1,2-HOPO synthesis. It has been attached to a range of structures, including cyclic amines,⁸³ tertiary amines,⁸⁴ cyclic esters⁸⁵ and aromatic amines⁷³ (Scheme 12).

Typically the 6-carboxy-1-hydroxypyridin-2-one is protected by the benzyl group and the carboxylic acid function is activated with 2-mercaptothiazoline for subsequent reaction with the tri-amines.⁸⁴ Deprotection is generally achieved by treatment with HBr/AcOH.

Activation with pentachlorophenol has also been successfully utilised for such conjugations.⁸⁵ Hybrid molecules containing both catechol and 1-hydroxypyridin-2-one ligands, for instance **11**, have been prepared by stepwise addition of the activated ligands under high dilution conditions.⁸⁴ 3-Carboxy-2-hydroxyisoquinolin-1-one (**12**) has also been used to synthesise tripodal ligands,⁸⁶ with the reported advantage that it is possible to readily form sulfonated derivatives which facilitate the water solubility of such ligands.



Scheme 12. Synthesis of hexadentate 1,2-HOPOs. A range of methods have been used to facilitate amide bond formation.

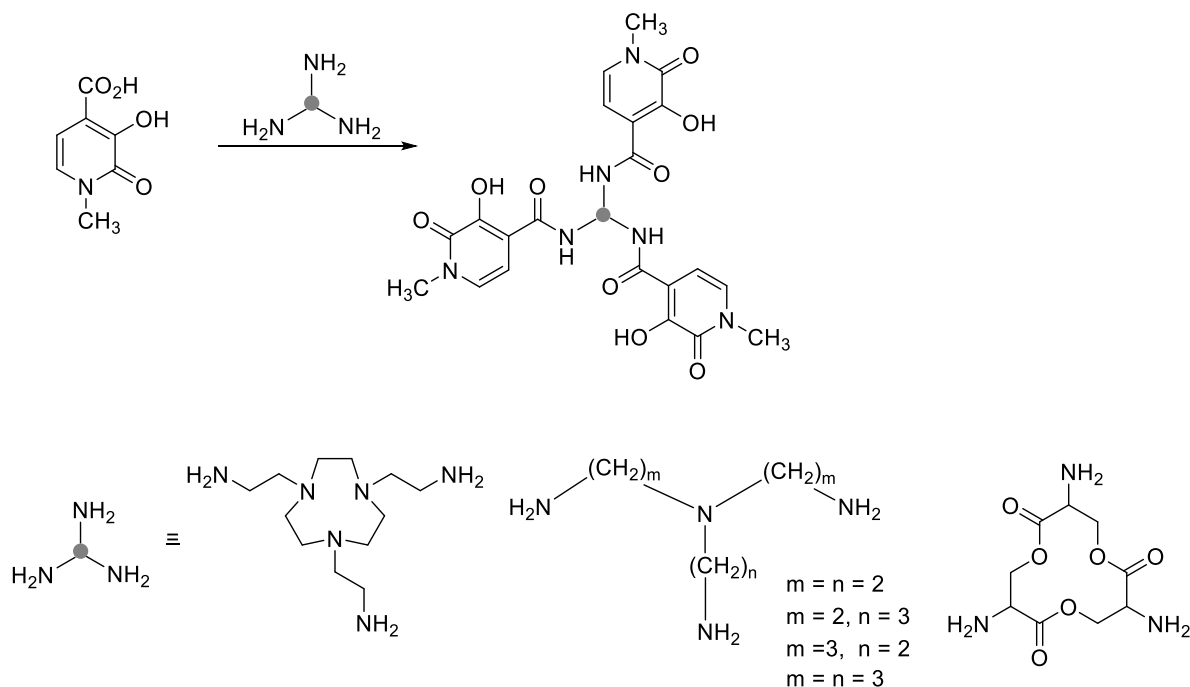


2.4.1.2. 3-Hydroxypyridin-2-ones (3,2-HOPOs)

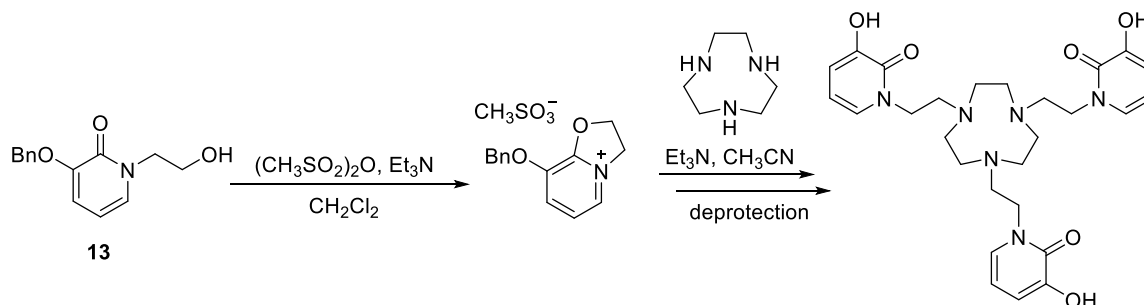
4-Carboxy-3-hydroxy-1-methylpyridin-2-one has been used extensively to prepare tripodal chelators, using tertiary amines,⁸⁷ cyclic amines⁸⁸ and cyclic esters⁸⁹ (Scheme 13). 4-Carboxy-3-hydroxyl-1-methylpyridin-2-one is protected by the benzyl group and conjugated with 2-mercaptothiazoline before condensation with various tri-amines. Deprotection is achieved in HCl/AcOH at RT over two days.⁸⁷ The corresponding acid chloride can also be utilised for the conjugation.⁸⁹ Various derivatives of 3-hydroxypyridin-2-one can also be utilised to form hexadentate ligands; for instance the 1-(2'-hydroxyethyl) derivative (**13**) can be converted to an imidate salt which is capable of direct reaction with triamines (Scheme 14)⁹⁰ and 1-carboxymethyl-3-hydroxypyridin-2-one can be coupled to various triamines subsequent to activation with *N*-hydroxysuccinimide⁷² or TBTU-facilitated condensation⁹¹ (Scheme 15).

A capped tripodal chelator based on 3-hydroxypyridin-2-one as the ligand has been prepared from 6-methyl-2,3-hydroxypyridinone dithiazolide (**14**) (Scheme 16)⁹² and a linear hexadentate ligand (**15**) has been synthesised as a result of condensation of 4-carboxy-3-hydroxyl-1-methylpyridin-2-one and spermidine. As with hexadentate 1-hydroxypyridin-2-ones a range of hybrid molecules have been prepared with terephthalamide units.^{83,93}

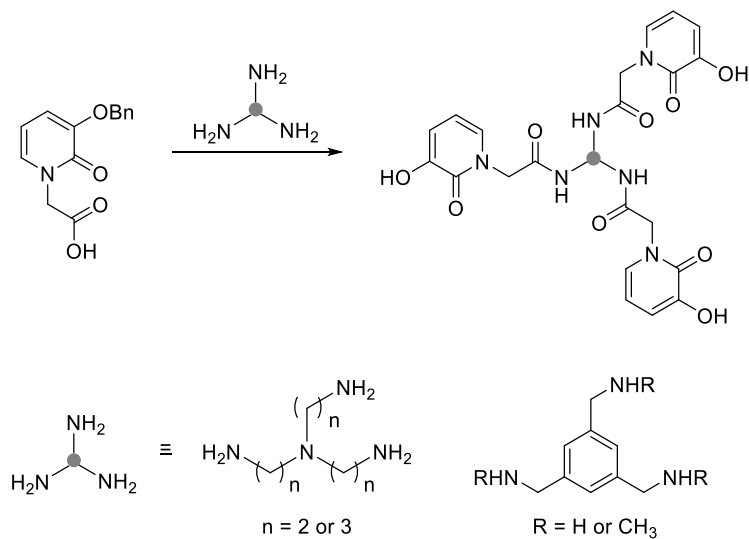
Scheme 13. Synthesis of hexadentate 3,2-HOPOs using 4-carboxy-3-hydroxy-1-methylpyridin-2-one as the chelating function. A range of methods have been used to facilitate amide bond formation.



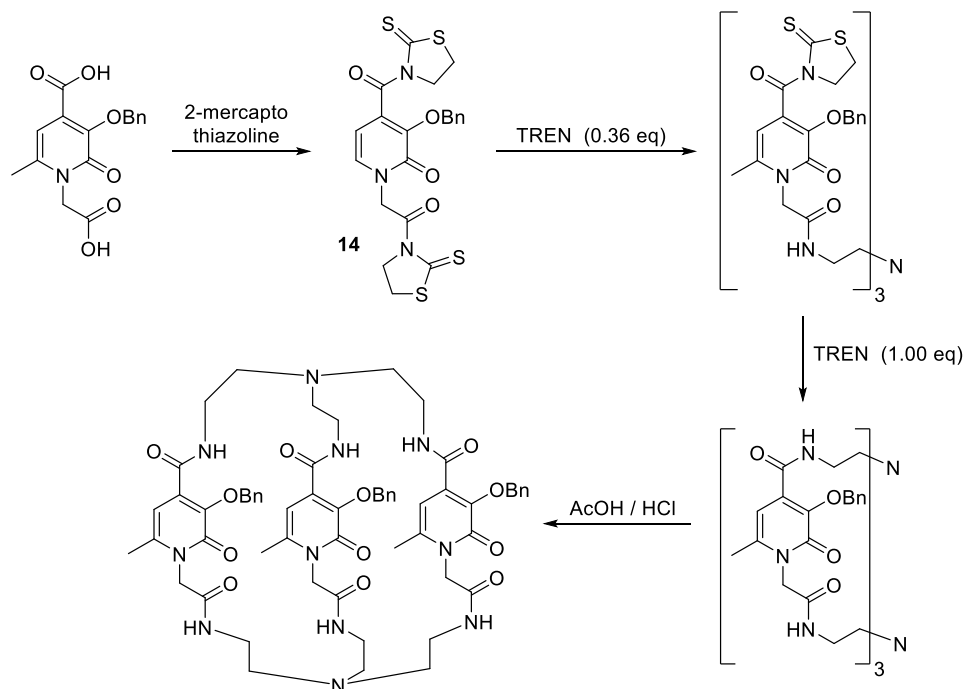
Scheme 14. Synthesis of hexadentate 3,2-HOPO using 1-ethylene-3-hydroxypyridin-2-one as the chelating function.



Scheme 15. Synthesis of hexadentate 3,2-HOPOs using 1-carboxymethyl-3-hydroxypyridin-2-one as the chelating function. A range of methods have been used to facilitate amide bond formation.



Scheme 16. Synthesis of capped hexadentate 3,2-HOPOs.

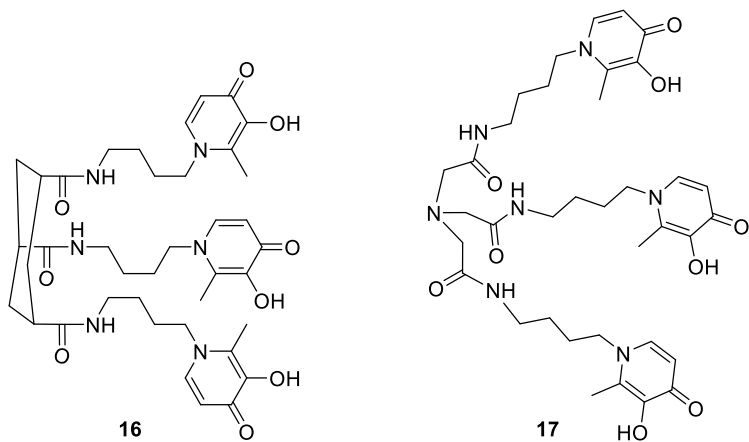


2.4.1.3. 3-Hydroxypyridin-4-ones (3,4-HOPOs)

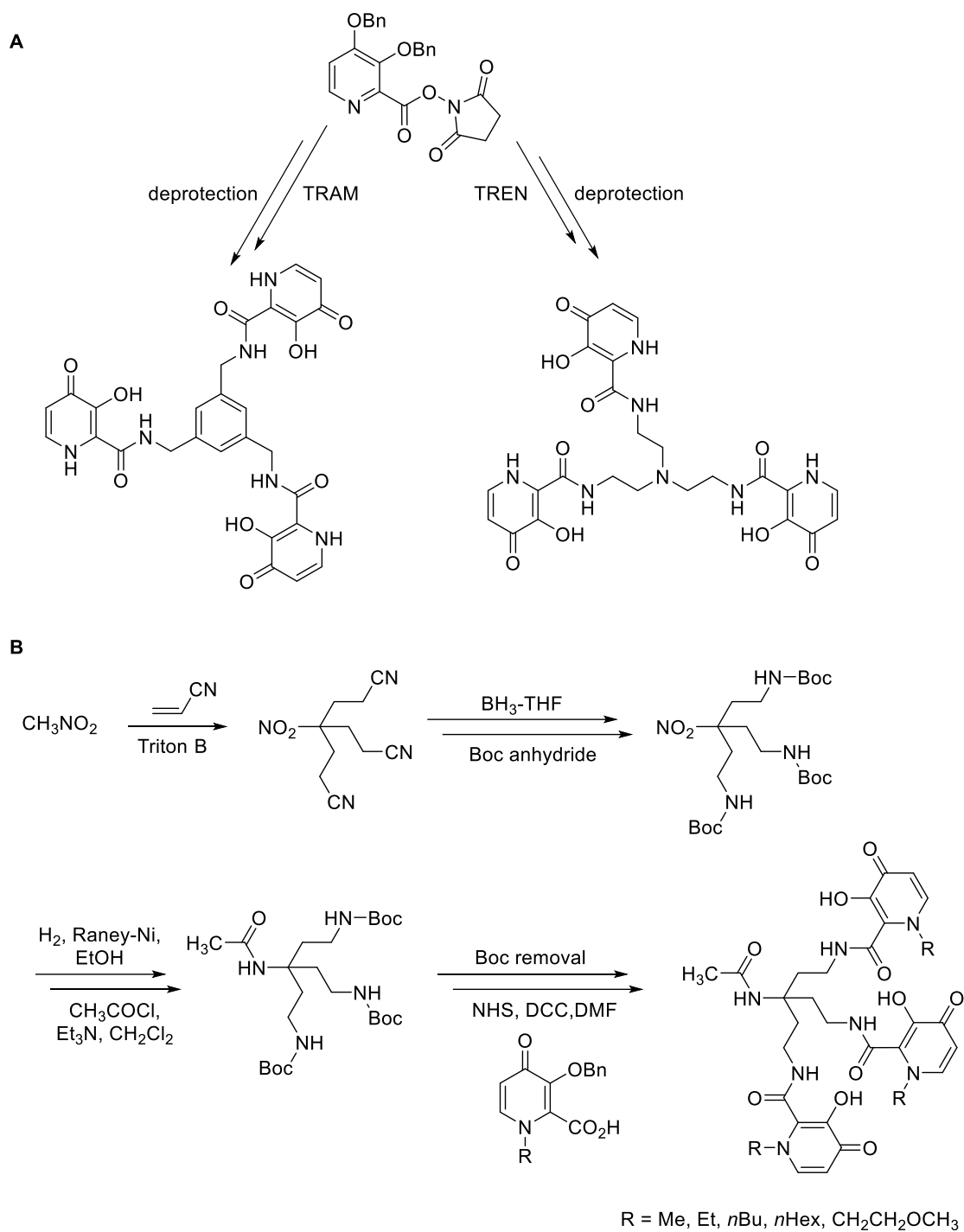
Hexadentate 3-hydroxypyridin-4-ones, based on simple tripodal structures have been prepared *via* two major routes, one by direct conjugation of the protected pyridinone with tertiary nitrogen-based tripodal triamines (Scheme 17A)^{94,95} and a second utilising carbon-based tripodal triamines (Scheme 17B);⁹⁶ the latter having the advantage of ready coupling to other biological active moieties for instance enzyme inhibitors or “address” systems (see Section 5).

There are some reports of hexadentate 3-hydroxypyridin-4-ones being prepared directly from double Michael reactions.⁹⁷⁻⁹⁹ However, this leads to the tripodal base being attached to the 1-position of the 3-hydroxypyridin-4-one, which frequently generates a non-ideal stereochemistry for metal chelation. With wide tripodal structures such as that

provided by saturated carbocyclic rings, chelation in the hexadentate mode is possible, for instance with KEMP derivatives (e.g. **16**). However with simple tripodal structures (**17**) a suitable orientation of the three coordinating units is more difficult to achieve, under which circumstances 2:2 complexes (**10**) may result.⁸²

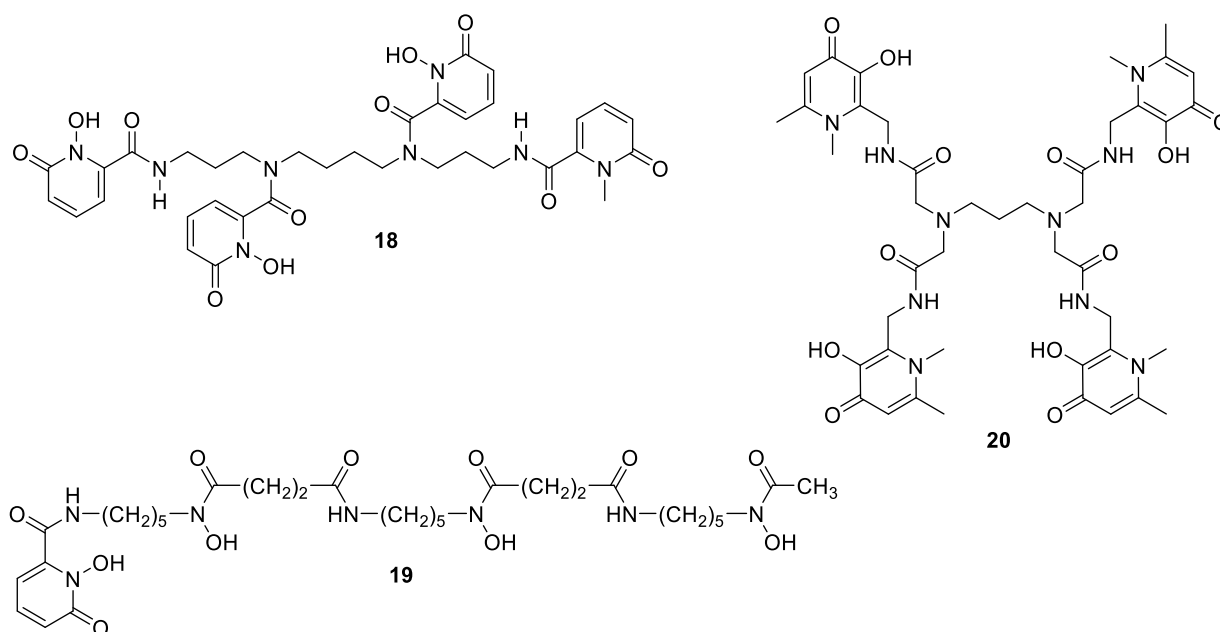


Scheme 17. Synthesis of tripodal hexadentate 3,4-HOPOs based on A) TRAM or TREN, and B) on ANPD.

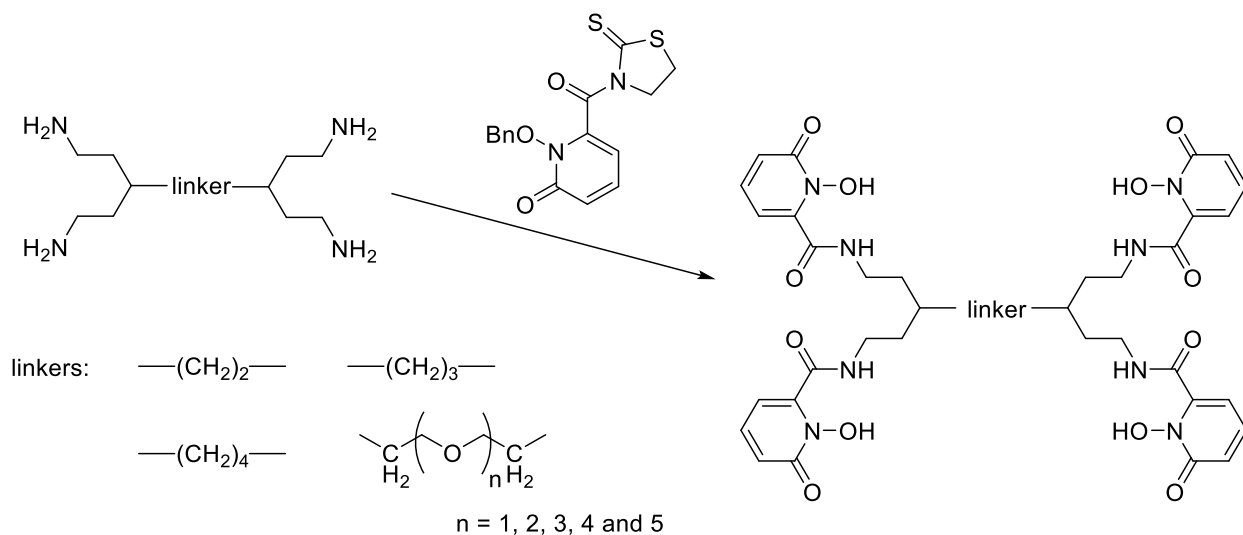


2.5. Octadentate hydroxypyridinones

Tetrapodal ligands have been synthesised by coupling protected 1-hydroxypyridin-2-ones to a range of tetrapodal amines (Scheme 18)¹⁰⁰ and analogous linear octadentate chelators (**18**) have been prepared in a similar fashion.^{101,102} Hybrid linear chelators (**19**) have also been prepared.^{103,104} The tetrapodal 3-hydroxypyridin-4-one (**20**) has been synthesised by amide bond formation by performing the coupling in a microwave reactor. This procedure decreased the reaction time and increased the yield.¹⁰⁵



Scheme 18. Synthesis of octadentate 1,2-HOPOs.

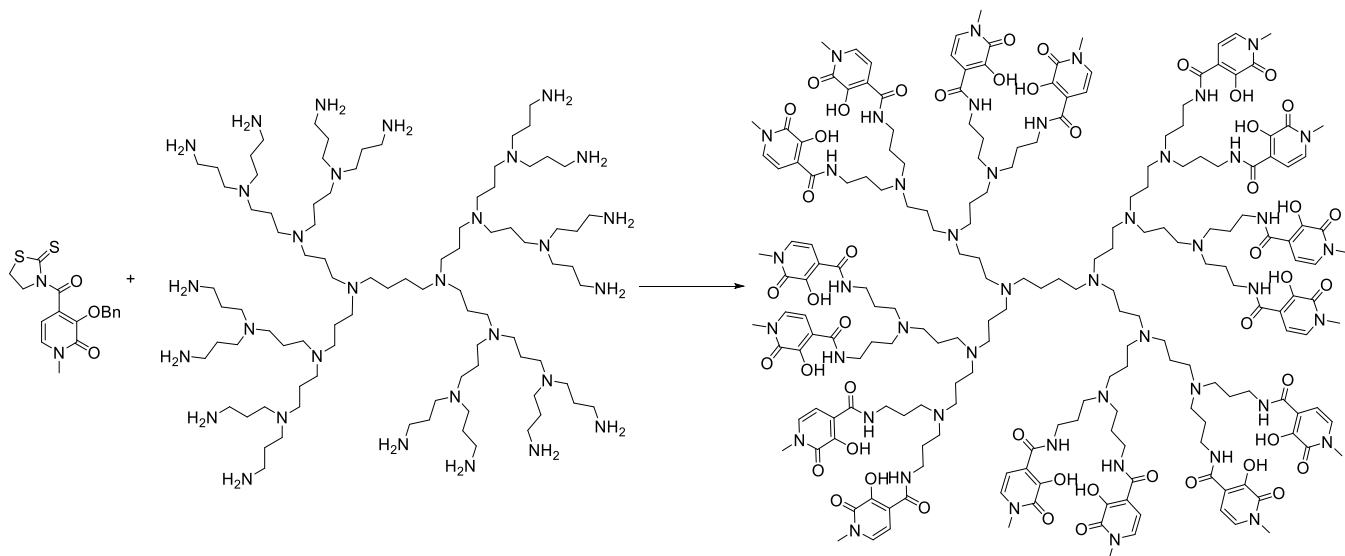


2.6. Dendrimers

Dendritic chelators are capable of binding large numbers of metal ions and may find application as metal sequestering agents for waste remediation and metal separation technologies. Raymond and co-workers reported the synthesis of salicylate-, catecholate-, and 3-hydroxypyridin-2-one functionalized dendrimers by attaching bidentate moieties to either poly(propyleneimine) or poly(amidoamine) dendrimers.¹⁰⁶

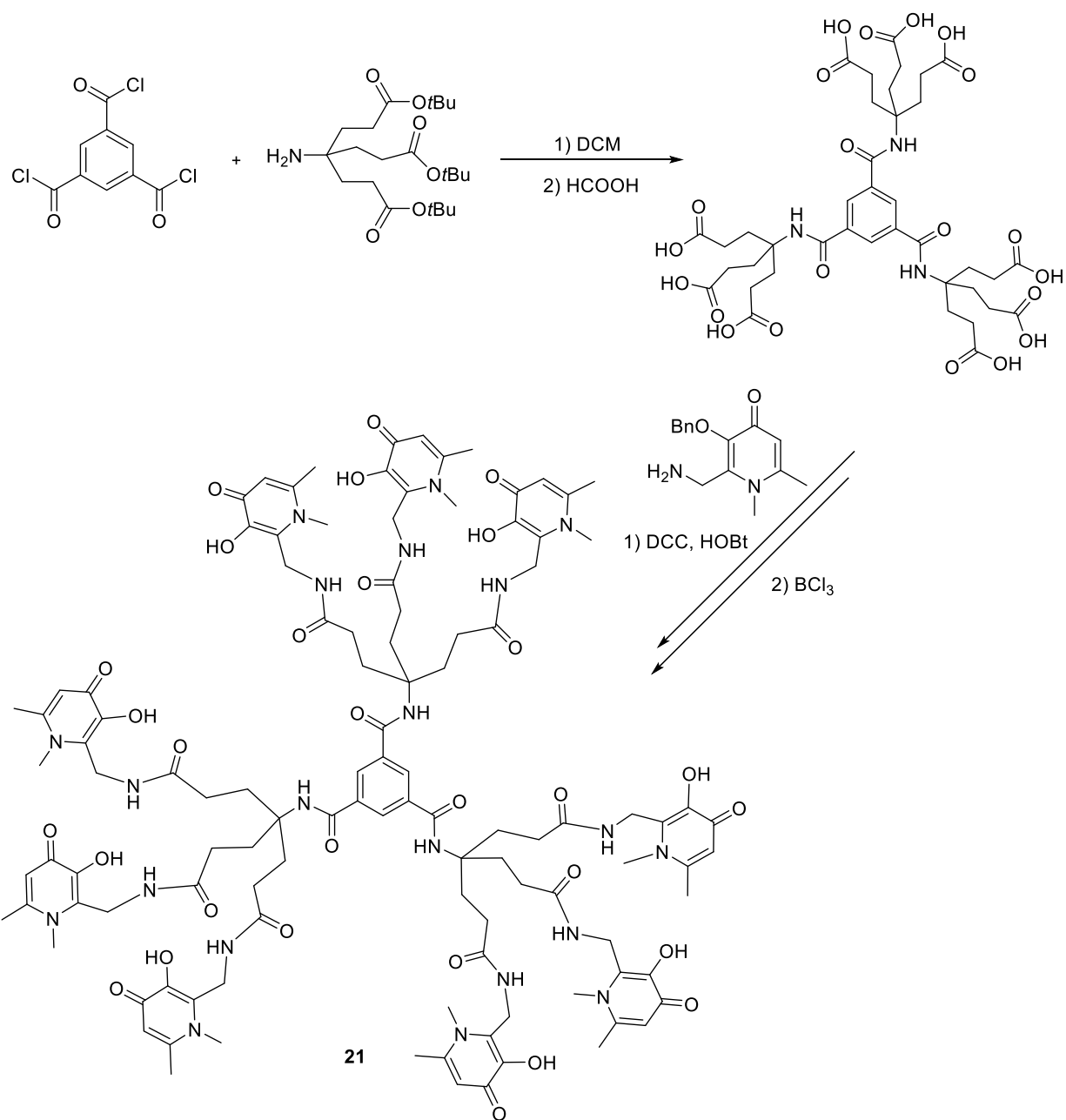
The synthesis of an example of a 3-hydroxypyridin-2-one-based dendrimeric chelator is presented in Scheme 19.

Scheme 19. Synthesis of PAMAM dendrimers conjugated with bidentate 3,2-HOPOs.

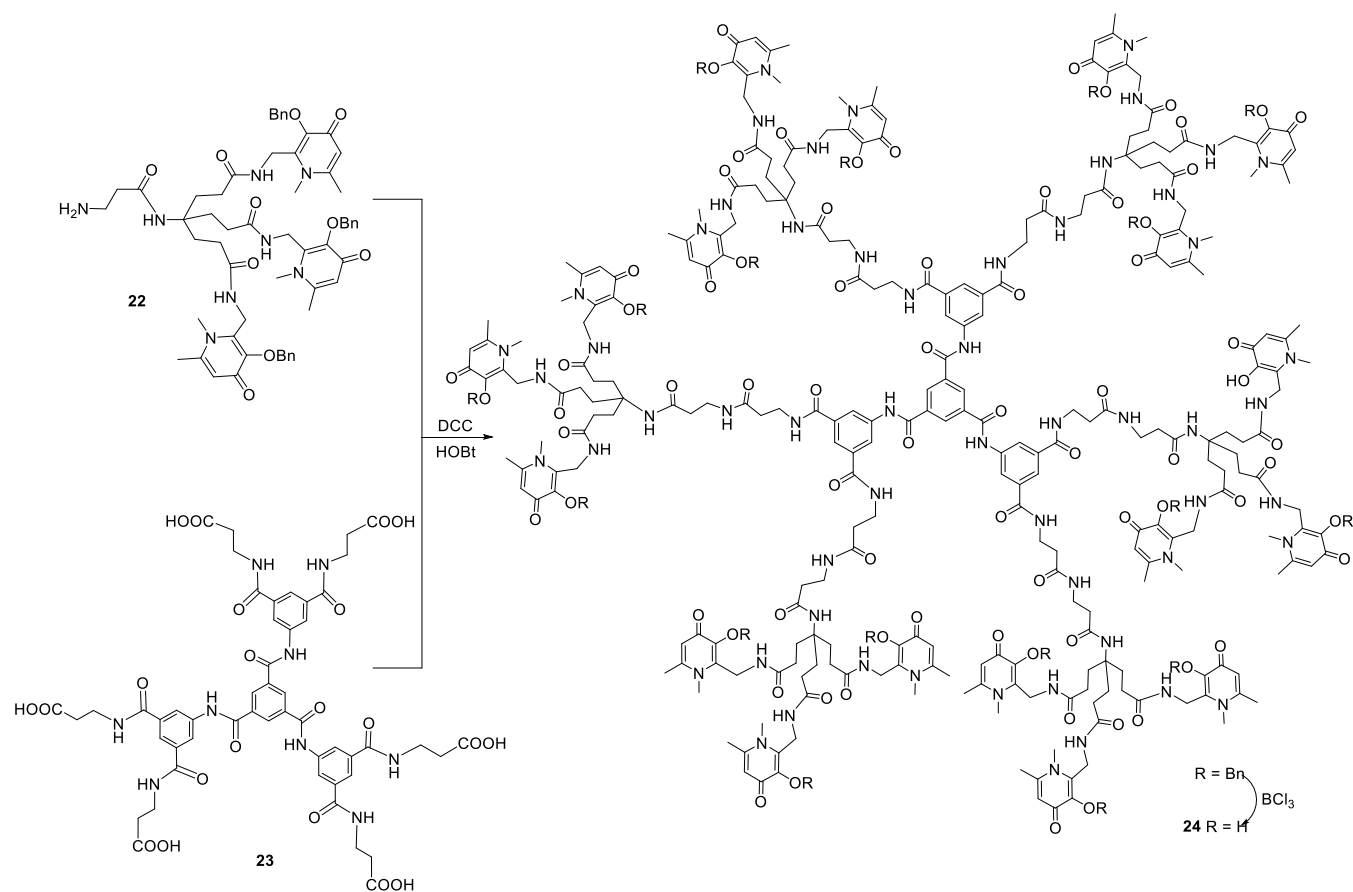


The synthesis of a range of 3-hydroxypyridin-4-one hexadentate-containing dendrimers, together with the evaluation of their iron binding properties has also been reported. A first generation dendrimer (**21**) was prepared using a divergent synthetic strategy (Scheme 20).¹⁰⁷ A convergent strategy was employed for the synthesis of a second generation dendrimeric chelator. In this case, a dendron containing three HOPO moieties (**22**) and multi-acid core (**23**) were combined to form dendrimer (**24**) (Scheme 21).¹⁰⁷ These dendrimeric chelators possess a high selectivity and affinity for iron(III).¹⁰⁷ The first generation dendrimer (**25**) which contains three hydroxypyridinone hexadentate moieties has been synthesised using a similar strategy (Scheme 22).¹⁰⁸ Dendrimer **25** includes amide functions adjacent to the coordinating phenolates, which contribute to the stability of the iron-complex via a hydrogen bond effect.¹⁰⁹

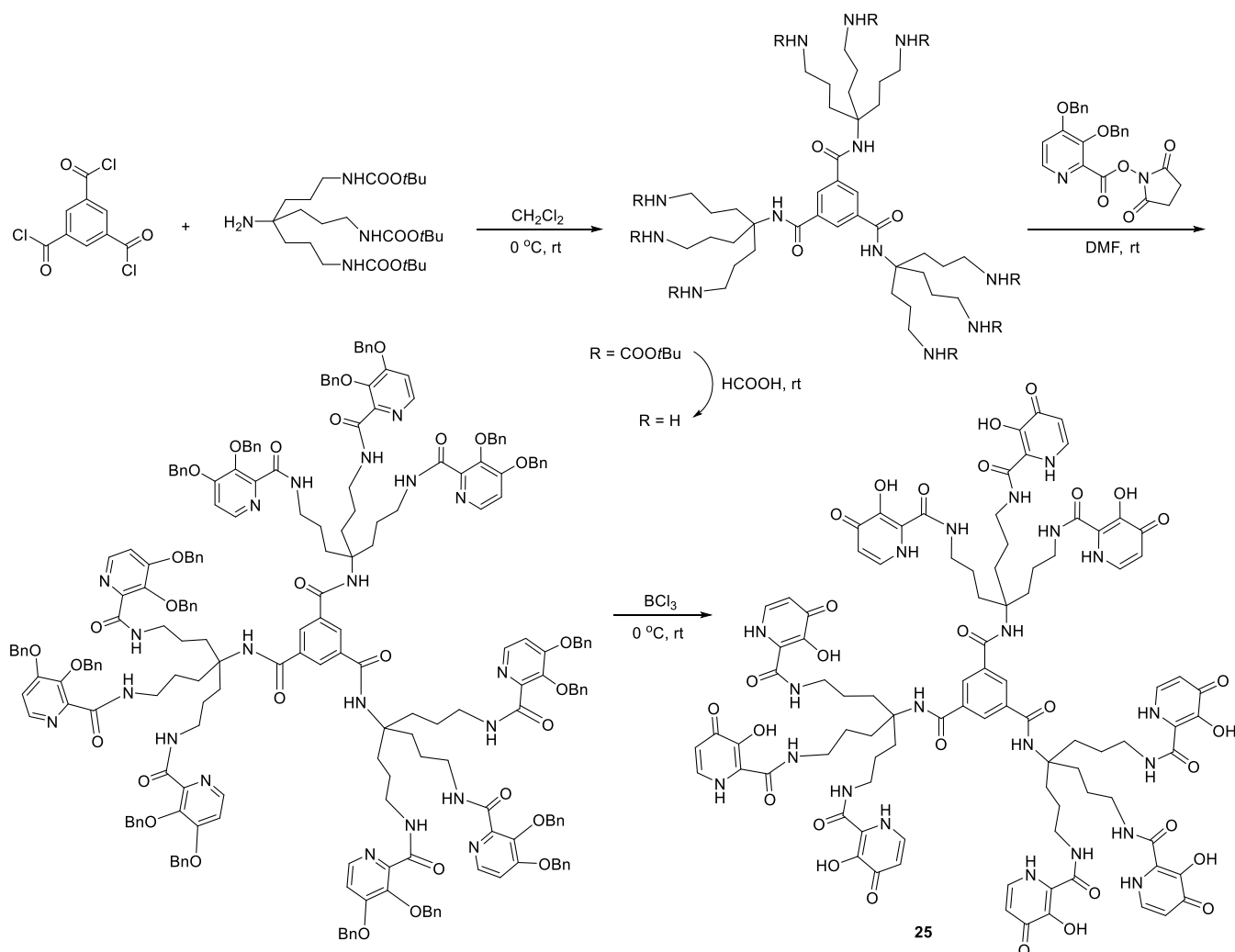
Scheme 20. Synthesis of first generation dendrimeric chelator (**21**) with 3,4-HOPO chelating groups.



Scheme 21. Synthesis of second generation dendrimeric chelator (**24**) with 3,4-HOPO chelating groups.



Scheme 22. Synthesis of first generation dendrimeric chelator (**25**) with 3,4-HOPO chelating groups.

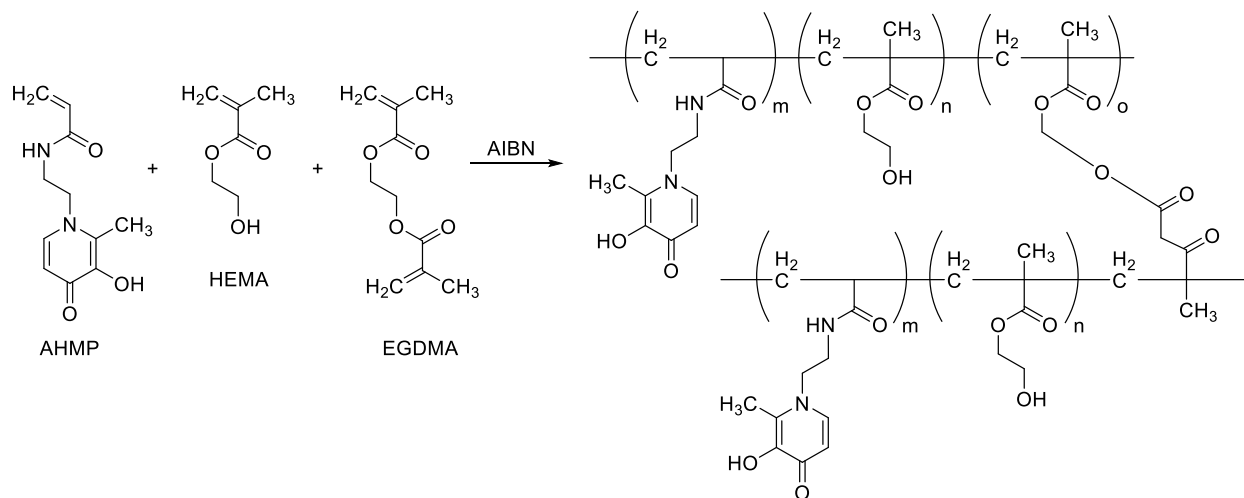


2.7. Polymers

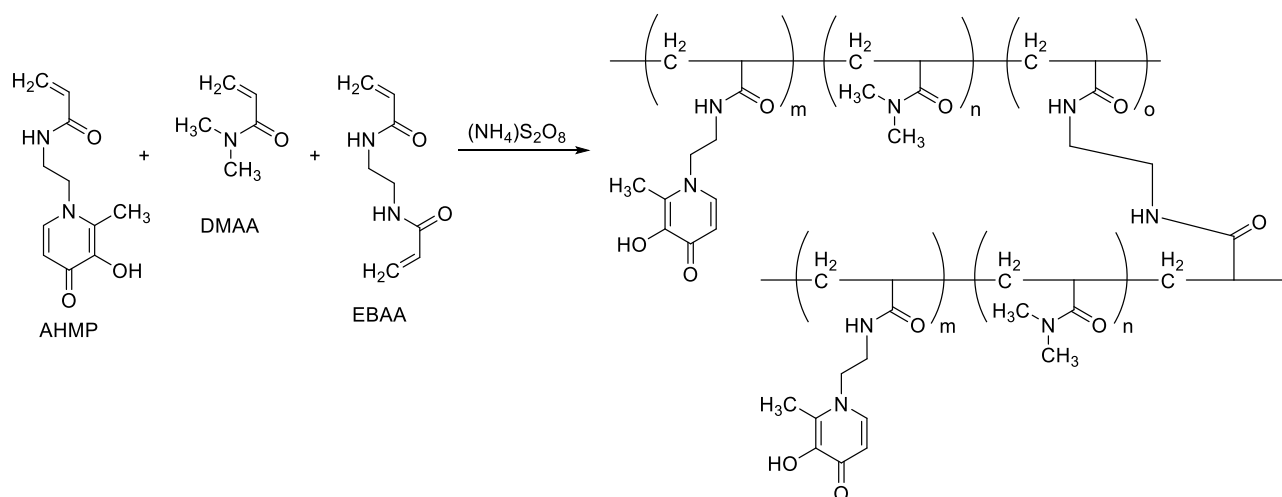
As with dendritic chelators, polymeric chelators have potential for waste remediation and metal separation technologies. Hydroxypyridinone-functionalized Sepharoses have been synthesised in order to scavenge 'hard' metal ions. Both epoxy-activated- and CNBr-activated Sepharoses have been utilised to form gels with iron(III) chelating capacities between 200 and 500 $\mu\text{mol/g}$ dry weight.¹¹⁰ A similar procedure utilising 1-ethyl-3-hydroxy-2-methyl pyridin-4-one and mimosine (**4**) has also been used to prepare functionalized Sepharose.¹¹¹ These gels possess a high affinity for hard metal cations, as typified by iron(III).¹¹²

A series of hydroxypyridinone-based iron binding resins have been synthesised; for instance, by copolymerisation of 1-(β -acrylamidoethyl)-3-hydroxy-2-methyl-4-(1*H*)-pyridinone (AHMP) with 2-hydroxyethyl methacrylate and ethyleneglycol dimethacrylate as the crosslinking agent to form iron(III)-chelating beads (Scheme 23).¹¹³ Iron(III) chelating resins have also been synthesised by copolymerisation of AHMP with *N,N*-dimethylacrylamide, and *N,N'*-ethylene-*bis*-acrylamide as a crosslinking agent (Scheme 24).¹¹²

Scheme 23. Synthesis of crosslinked 3,4-HOPO-containing polymers from AHMP and HEMA.



Scheme 24. Synthesis of crosslinked 3,4-HOPO-containing polymers from AHMP and DMAA.

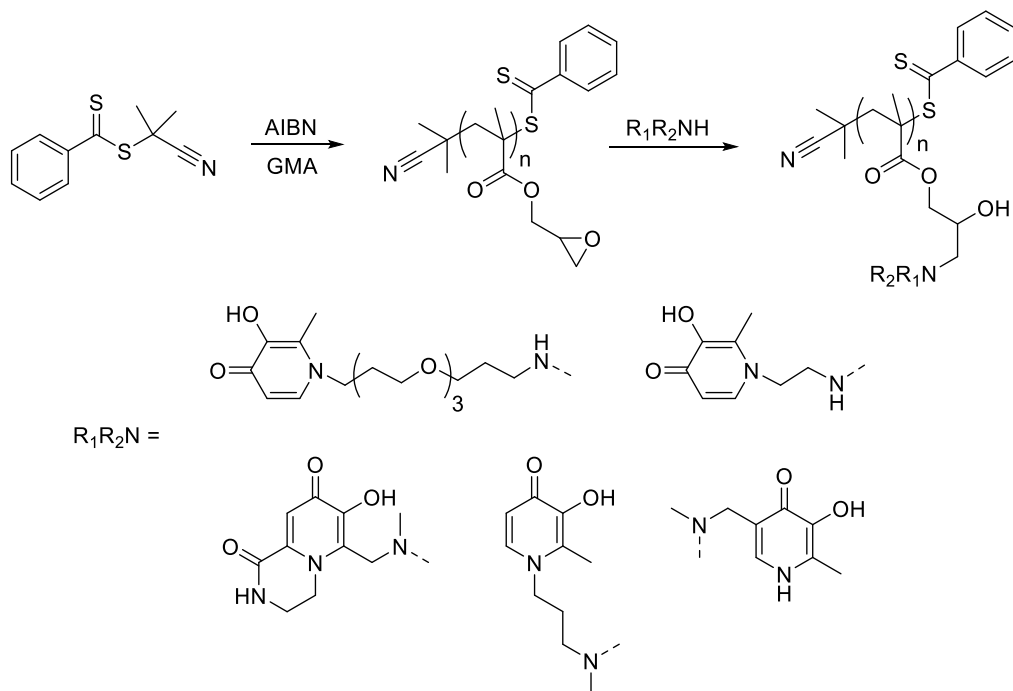


A series of linear poly(glycidyl methacrylate) (PGMA) polymers with well-defined properties and low polydispersities ($PDI < 1.1$) have been synthesized *via* RAFT polymerisation of GMA in the presence of CPBD as the chain-transfer agent. PGMA was conjugated with 3-hydroxypyridin-4-ones (3,4-HOPOs) containing free amino groups by a ring-opening reaction to generate a panel of HOPO-containing materials with controlled structures and specific iron-binding functions (Scheme 25).¹¹⁴ The synthetic method is simple with high yield and low cost, and thus the approach can be readily applied to the synthesis of related polymers. A hybrid 3,4-ethylenedioxythiophene/thiophene precursor functionalized with an hydroxypyridinone group has been subjected to electropolymerisation.¹¹⁵

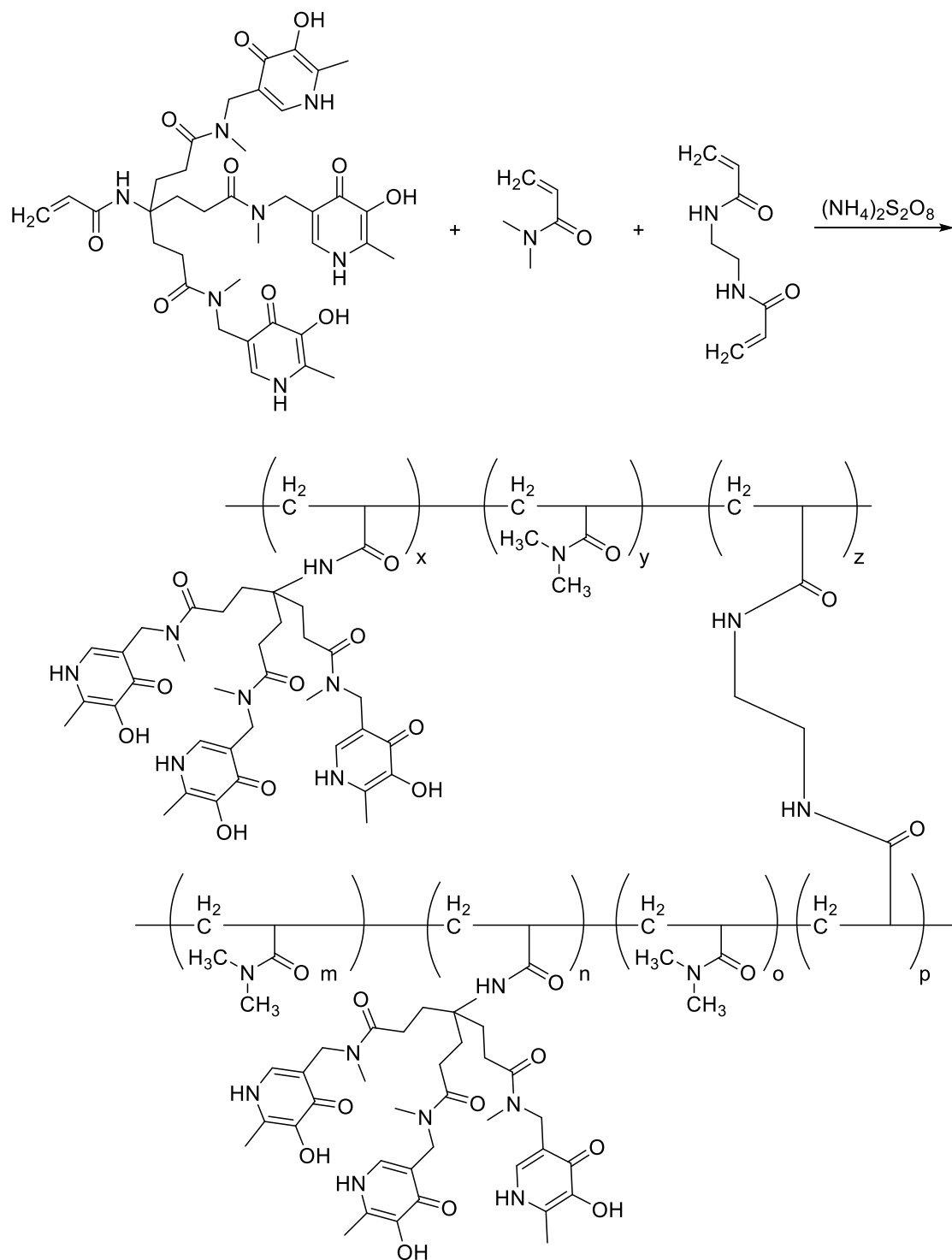
Apart from the polymers on which some natural hexadentate ligands (such as DFO) are immobilised,^{116,117} other hexadentate chelator-containing polymers have rarely been reported. However the synthesis of 3-hydroxypyridin-4-one hexadentate ligand-containing copolymers by copolymerisation of a 3-hydroxypyridin-4-one hexadentate ligand with *N,N*-dimethylacrylamide (DMAA), and *N,N*-ethylene-*bis*-acrylamide (EBAA) (Scheme 26), leads to copolymers which possess a high selectivity and affinity for

iron(III).¹¹⁸ A novel soluble 3-hydroxypyridin-4-one hexadentate based polymer has also been synthesised by copolymerisation of the hexadentate ligand with 2-hydroxyethyl acrylate (HEA) (Scheme 27).¹¹⁹ Hexadentate chelator-conjugated polymers have a distinct advantage over bidentate chelator conjugates in that they provide uniform metal chelating sites (Figure 6). Bidentate chelator-conjugated polymers lack a uniform high affinity for iron, because it is impossible for each bidentate moiety to form part of an ideal octahedral iron(III) coordination site. Thus the complexation of three bidentate ligands with iron will not be consistently strong and partial chelation of iron is likely.¹¹⁸

Scheme 25. RAFT-based synthesis of PGMA-conjugated 3,4-HOPO-containing polymers.



Scheme 26. Synthesis of hexadentate 3,4-HOPO-based chelating resin.



Scheme 27. Synthesis of hexadentate 3,4-HOPO-based chelating copolymer.

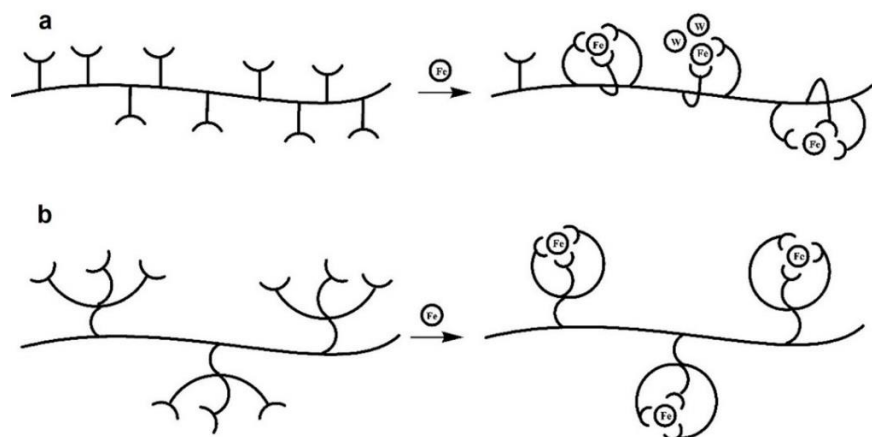
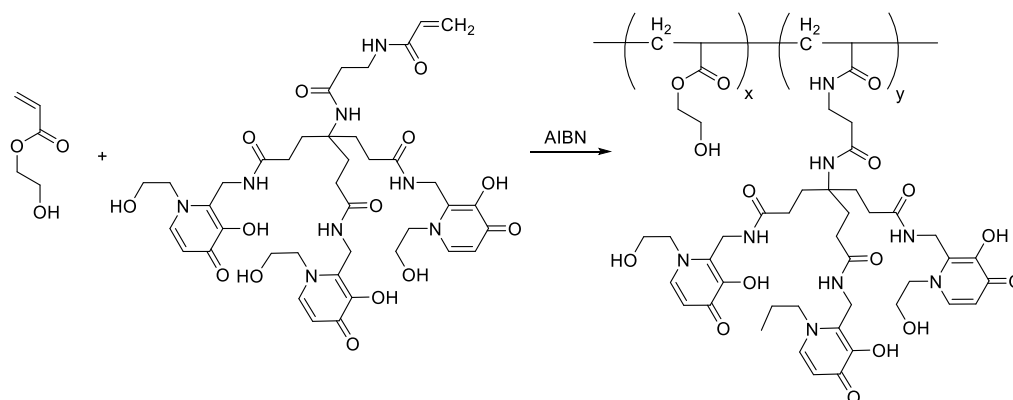


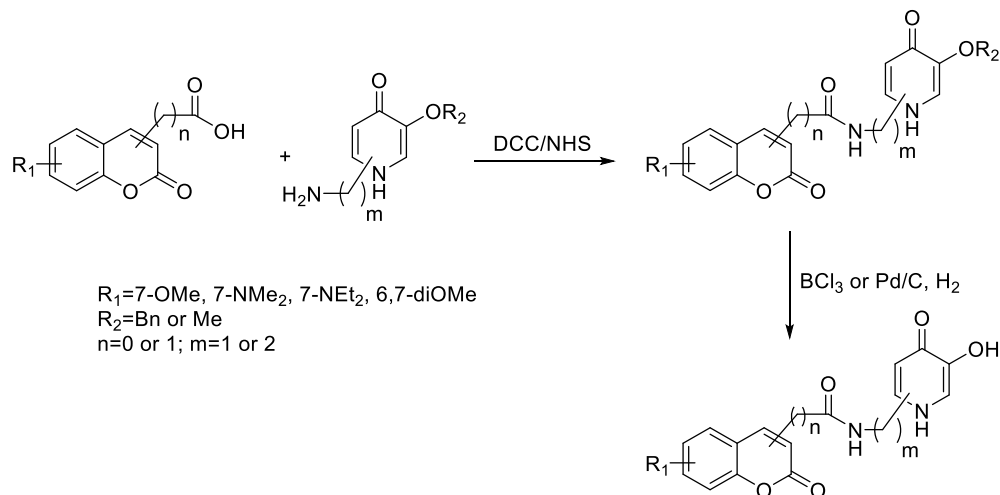
Figure 6. (a) Iron chelation by a bidentate ligand-containing polymeric chelator: three bidentate moieties bind one iron with ideal stereochemistry (left), only two bidentate moieties bind one iron and two coordination sites are occupied by water molecules (middle), three bidentate moieties bind one iron in a non-ideal geometry (right); (b) Iron chelation by a hexadentate ligand-containing polymeric chelator: all the hexadentate moieties bind iron with an ideal stereochemistry. Reproduced with permission from Ref.¹¹⁸. Copyright 2008 American Chemical Society.

2.8. Hydroxypyridinone-based fluorescent probes

2.8.1. Coumarin-, benzothiazole- and fluorescein-linked bidentate hydroxypyridinones

The most common fluorescent moieties used as metal sensors are coumarin, fluorescein and rhodamine.^{120,121} The coumarin molecule possesses strong fluorescence due to the presence of electron-donating groups at the 6- and 7-positions.¹²² The synthetic route of such coumarin-linked bidentate hydroxypyridinones is summarised in Scheme 28.¹²³ The coumarin acid is activated with dicyclohexylcarbodiimide (DCC) / 2-mercaptothiazoline, catalyzed by 4-(dimethylamino)pyridine or DCC / *N*-hydroxysuccinimide (NHS) before coupling with an aminopyridinone. The aminopyridinones are typically synthesised from commercially available maltol or kojic acid *via* multistep reactions (Schemes 3 and 4).¹²³ The amino group can be located in the 1-, 2-, 5- or 6-position of the pyridinone ring. After the methyl or benzyl protecting group of the resulting conjugate is removed, a range of fluorescent chelators is produced. The absorption and emission wavelengths of such probes fall in the ranges 325-432 nm and 380-474 nm, respectively. These probes are bidentate and chelate iron in a 3:1 ratio. The resulting fluorescence quenches on forming an iron complex.

Scheme 28. Synthesis of coumarin-linked bidentate 3,4-HOPOs.

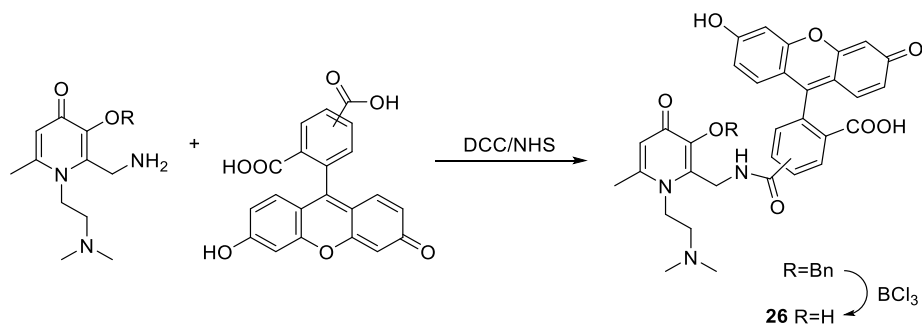


More specifically, a lysosomal targeting probe¹²⁴ and a mitochondria-targeted probe¹²⁵ have also been synthesised. The syntheses of these compounds are presented in Schemes 29 and 30, respectively. Probe **26** is produced by coupling carboxylated fluorescein acid with an amine-based pyridinone in the presence of DCC/NHS (Scheme 29); for the preparation of probes **27** and **28**, the hydroxypyridinone component was coupled to the resin in position 2 or 4, respectively, using PyOxP/DIPEA chemistry. Prior to hydrazine treatment, the Fmoc-group on the N-terminus was removed and the resin treated with $\text{BOC}_2\text{O}/\text{DIPEA}$ in DMF. Following cleavage from the resin, probes **27** and **28** were obtained following BCl_3 treatment (Scheme 30).

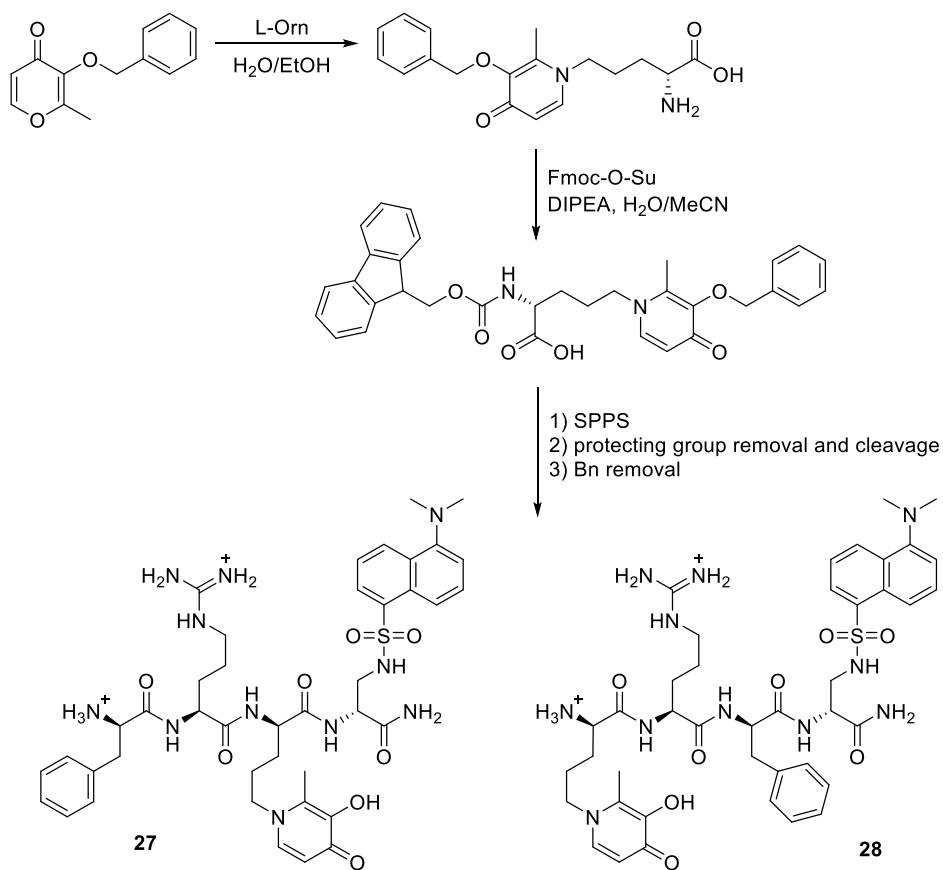
Orvig *et al* reported the synthesis of 1-(4-benzo[d]oxazol-2-yl)phenyl-3-hydroxy-2-methylpyridin-4(1*H*)-one (**29a**) and 1-(4-benzo[d]thiazol-2-yl)phenyl-3-hydroxy-2-methylpyridin-4(1*H*)-one (**29b**) to determine interaction with amyloid protein implicated in Alzheimer's disease.¹²⁶ The synthesis of these two compounds was initiated from maltol which coupled with 4-aminobenzoic acid, followed by cyclisation with 2-

aminophenol or 2-aminobenzenethiol afforded the fluorescent chelators (**29**) (Scheme 31).

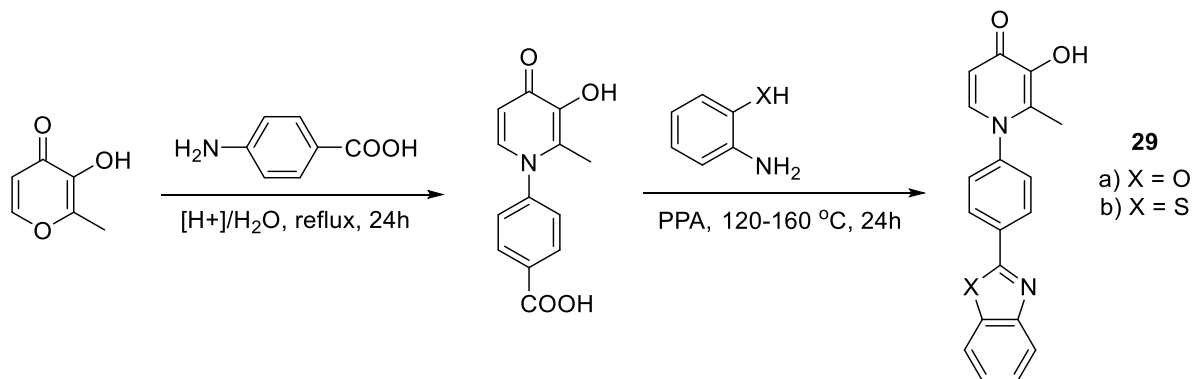
Scheme 29. Synthesis of a fluorescein-linked bidentate 3,4-HOPO.



Scheme 30. Synthesis of peptide-based dansyl-linked bidentate 3,4-HOPOs.



Scheme 31. Synthesis of benzothiazole and benzoxazole functionalized 3,4-HOPOs.

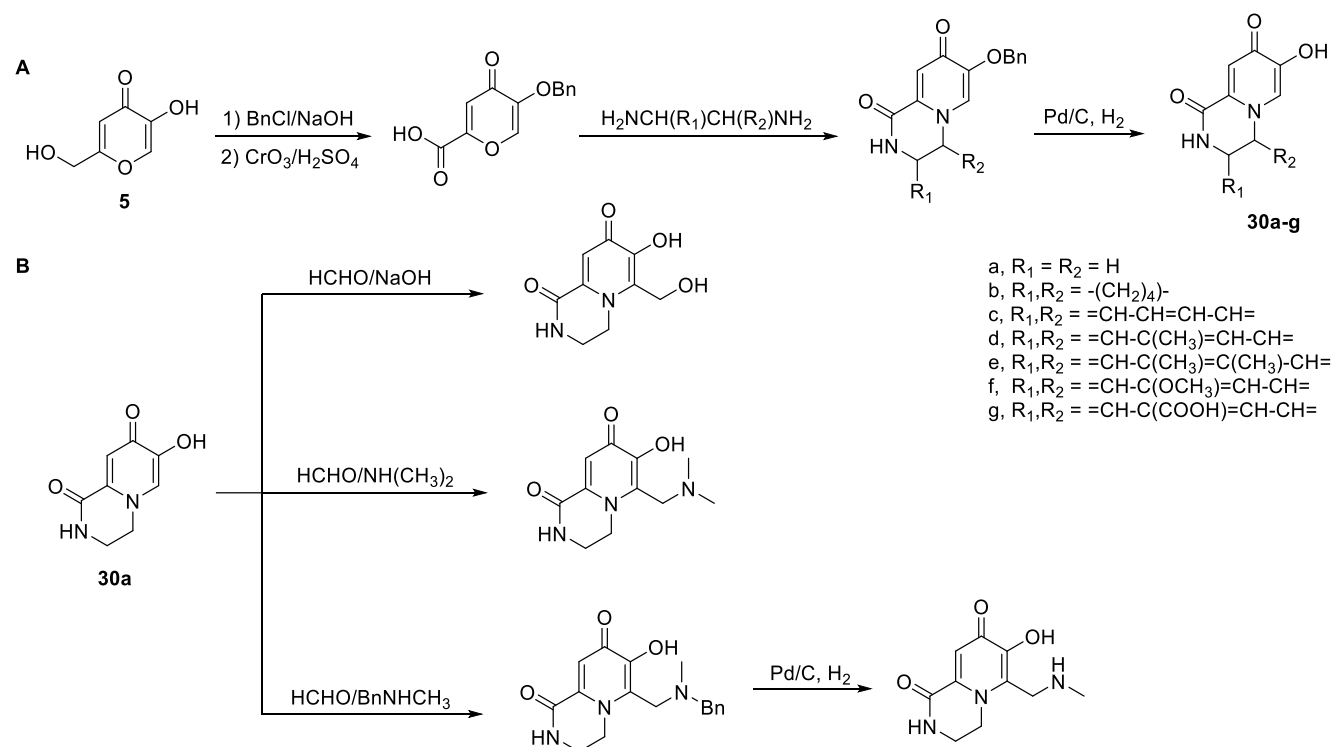


2.8.2. Bicyclic fluorescent hydroxypyridinones

The introduction of fluorescent moieties appreciably influences the overall size of the probe molecule which is generally undesirable in biological studies. To minimise this effect, fluorescent probes have been synthesised where the optical moiety is merged with the chelating entity of the molecule.^{127,128} The synthetic procedure for the target fluorescent chelators is outlined in Scheme 32A. 3-Benzoxycomenic acid was prepared from kojic acid (**5**) by oxidation. The conversion of the pyranone to the corresponding bicyclic lactams was carried out by reaction with a range of diamino derivatives. The resulting bicyclic compounds were then hydrogenated in the presence of catalytic amounts of palladium to afford high yields of the desired fluorescent chelators (**30**). Chelator **30a** can be further reacted using the procedures outlined in Scheme 32B. The position *ortho* to the enolic hydroxyl group can be functionalised in an analogous fashion to the aldol condensation, under alkaline aqueous conditions. Similarly, the 2-position to the pyridinone ring is readily susceptible to aminomethylation by the Mannich reaction to form the corresponding aminomethyl derivatives. The emission wavelengths

of this type of probe are in the range of 454-470 nm and the average quantum yields are higher than those of coumarin-based probes.¹²⁸

Scheme 32. Synthesis of bicyclic fluorescent 3,4-HOPO probes.

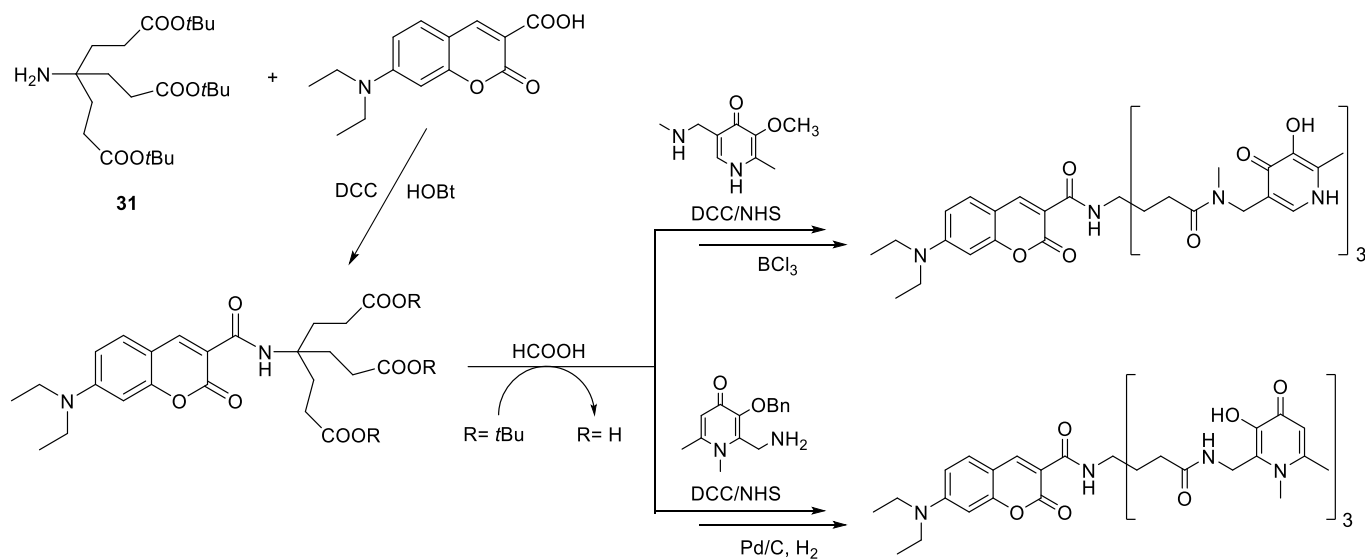


2.8.3. Hexadentate fluorescent hydroxypyridinones

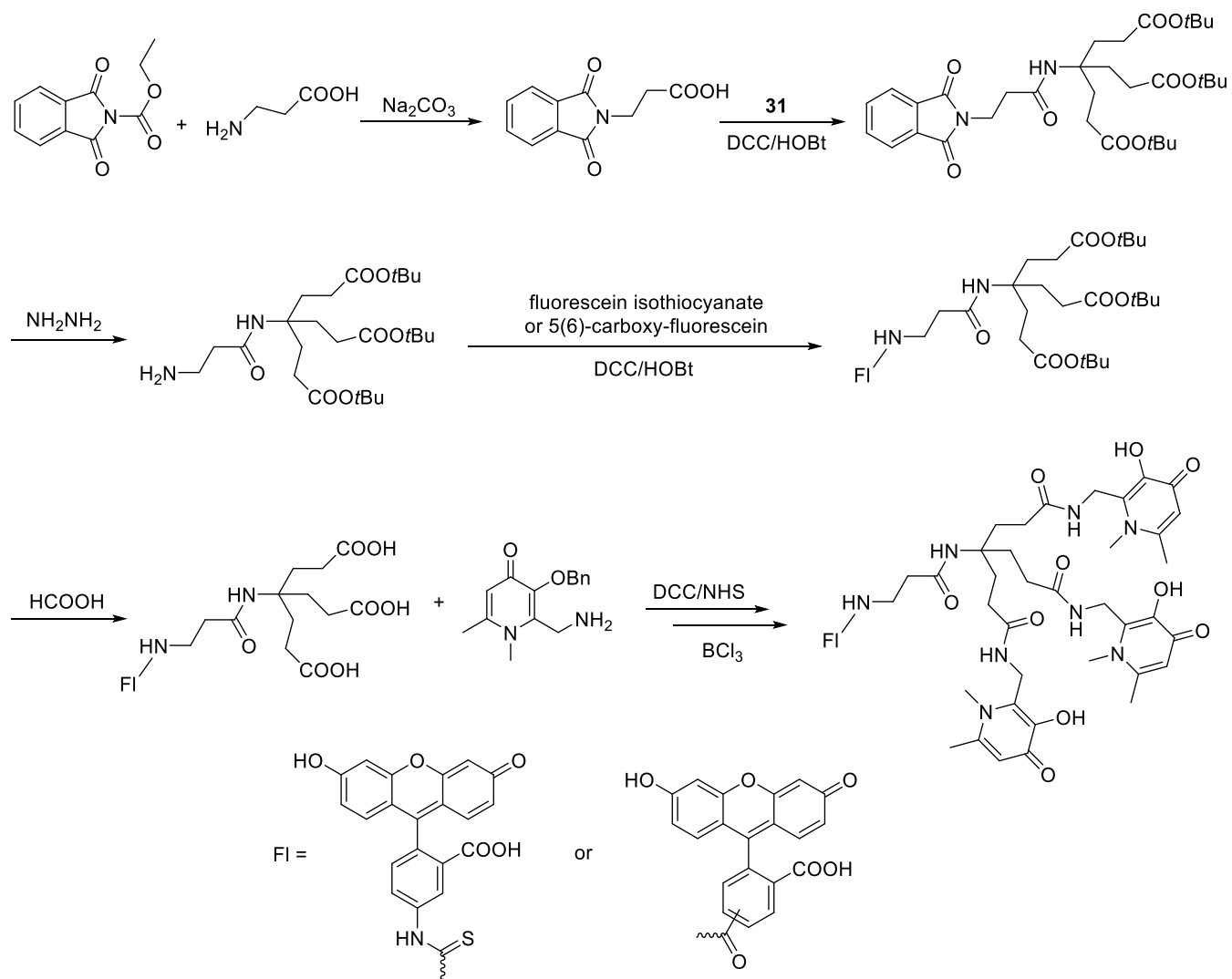
In addition to bidentate optical probes, a few hexadentate fluorescent agents have been reported.^{129,130} The syntheses of the coumarin-, fluorescein- and rhodamine-based hexadentate probes are presented in Schemes 33-35. Tri-*tert*-butoxycarbonylethyl methylamine (**31**) was treated with coumarin-3-carboxylic acid in DMF in the presence of DCC/HOBt, to form the coumarin-link. Hydrolysis of the resulting ester was achieved by treatment with formic acid, followed by activation of the triacid with DCC/NHS and coupled with aminopyridinones to furnish the corresponding protected coumarin-linked hexadentate molecules (Scheme 33). For fluorescein probes, tri-*tert*-

butoxycarbonyl ethyl methylamine was initially coupled to β -alanine in the presence of DCC/HOBt, followed by deprotection to release the amino group. This amine group can couple with fluorescein isothiocyanate or activated fluorescein 5(6)-carboxylic acid. Following deprotection, the resulting triacid was coupled to an aminopyridinone and deprotected to yield the corresponding hexadentate probes^{129,130} (Scheme 34). In a similar manner, β -alanine was protected and coupled with tri-*tert*-butoxycarbonyl ethyl methylamine, followed by hydrolysis. The resulting triacid was coupled to an aminopyridinone, followed by the removal of the phthalimide protecting group to afford a free amine. This amine was coupled with rhodamine isothiocyanate and after deprotection, yielded the rhodamine-linked fluorescent probe (Scheme 35).

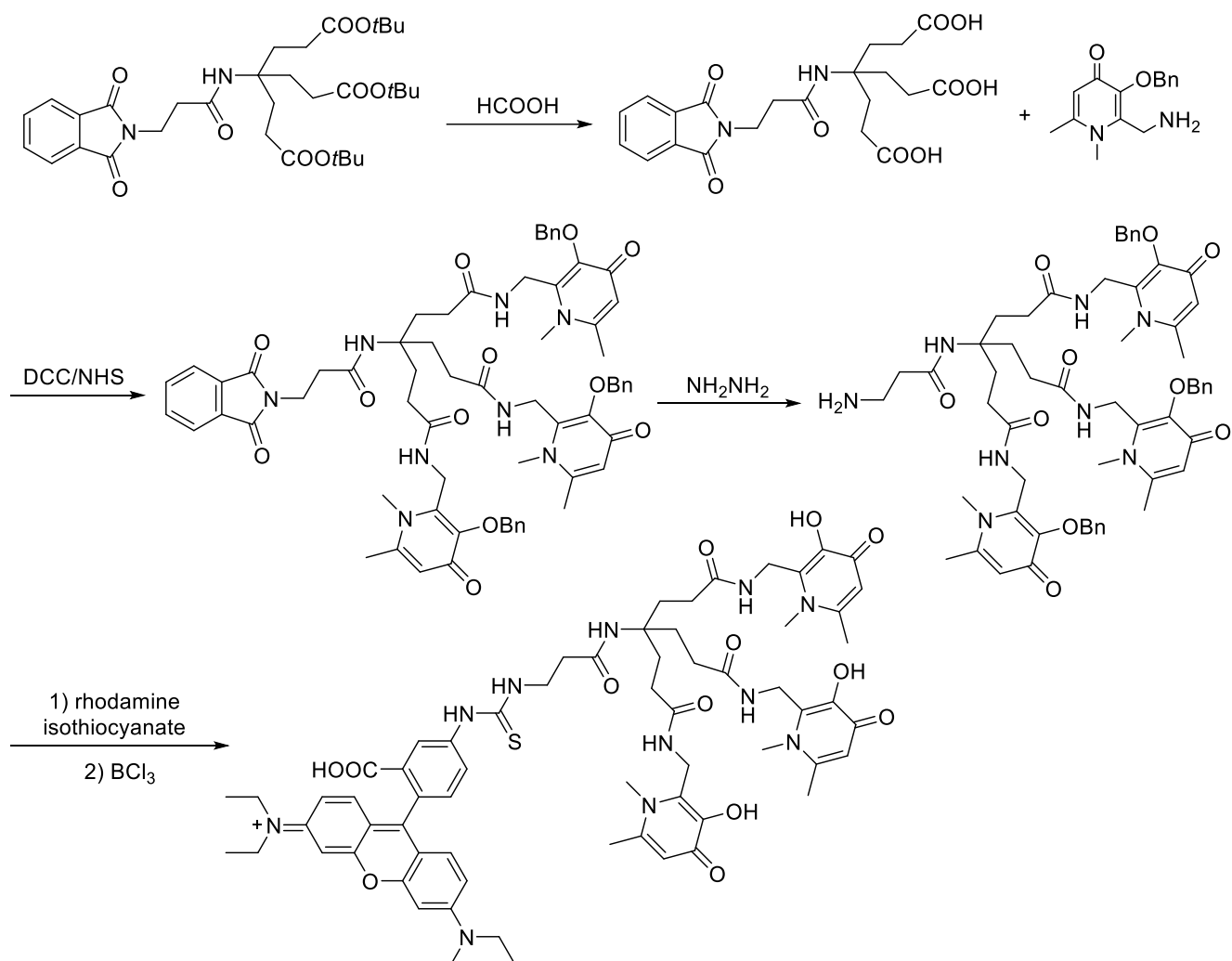
Scheme 33. Synthesis of coumarin-based hexadentate 3,4-HOPO probes.



Scheme 34. Synthesis of fluorescein-based hexadentate 3,4-HOPO probes.



Scheme 35. Synthesis of rhodamine-based hexadentate 3,4-HOPO probes.

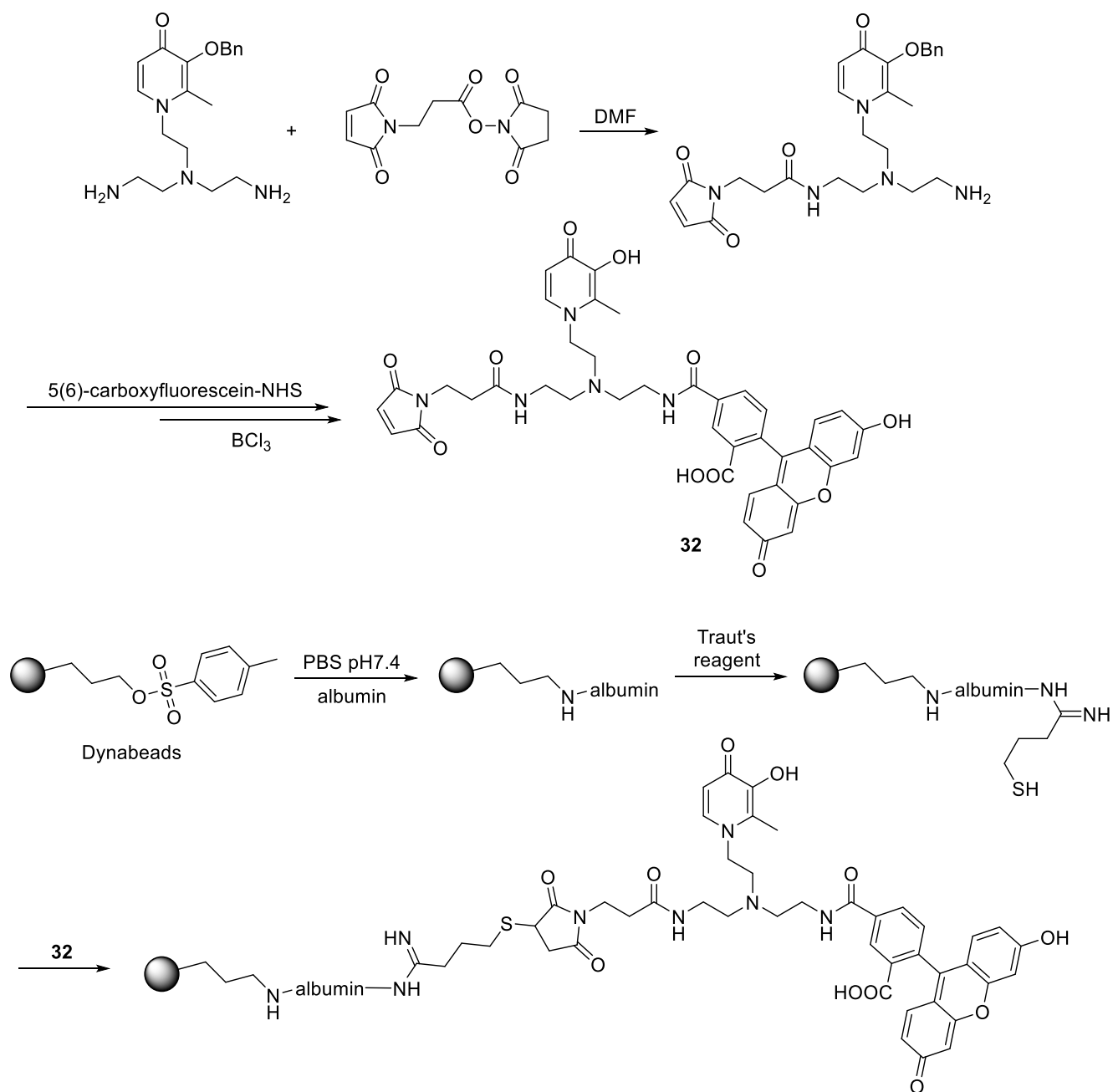


2.8.4. Fluorescent hydroxypyridinone-containing beads

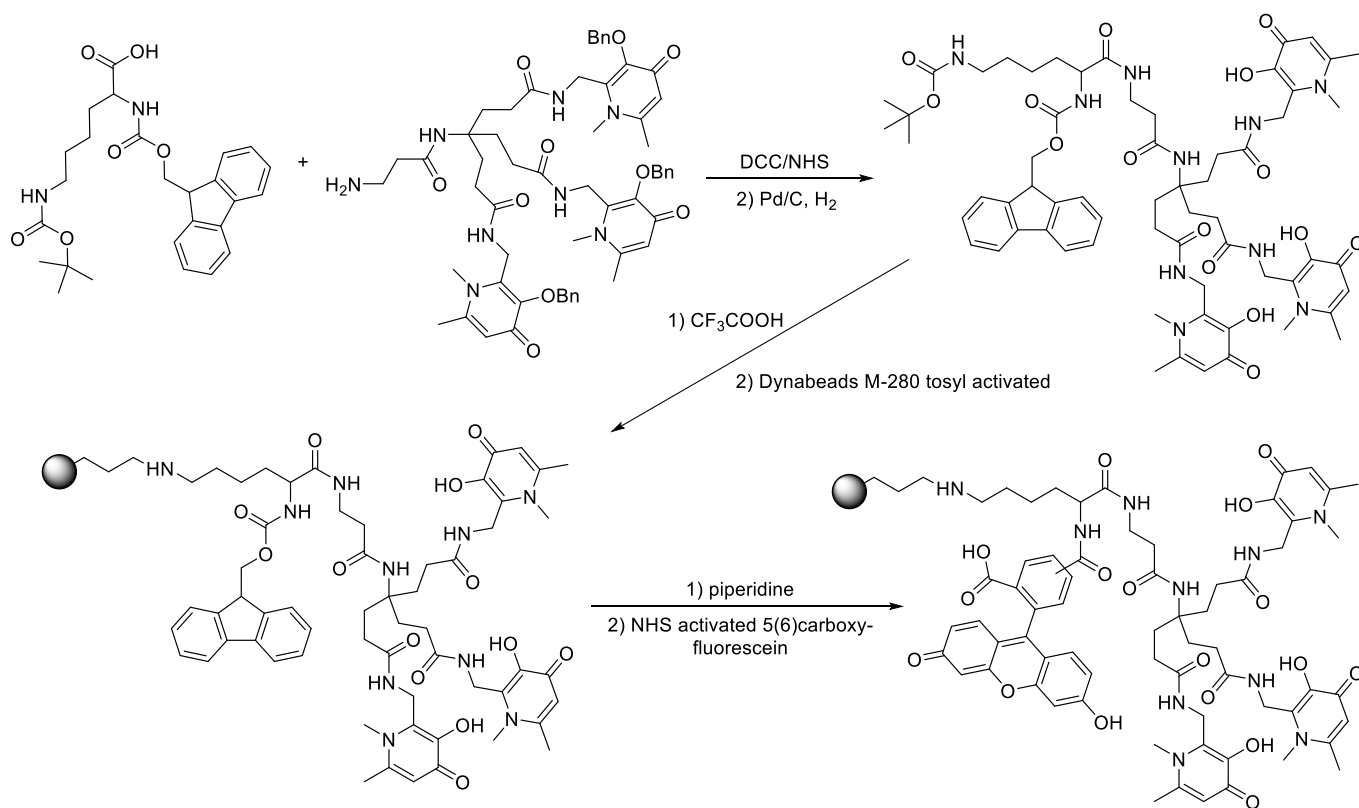
A simple and fast method for the quantification of iron in serum has been developed using chelating fluorescent beads (CFBs). The synthetic route of the first generation CFB is shown in Scheme 36. The substituted pyridinone was treated with an equivalent of NHS-activated 3-maleimidopropionic acid to provide single substituted intermediate, where the free amino group reacts with NHS-activated 5(6)-carboxyfluorescein to form the benzyl protected compound. The benzyl group was deprotected using BCl_3 . This chelating probe (**32**) was covalently bound to Dynabeads. Incubation of the beads with

bovine serum albumin led to covalent binding of albumin to the beads *via* amino and thiol groups. The remaining albumin amino groups were reacted with Traut's Reagent, and subsequently the thiol group from Traut's reagent covalently bound to the iron sensor to afford the final probe-labelled beads (Scheme 36). These beads can be processed *via* a FACS instrument in order to provide information of the iron-content of solutions in which the beads have been incubated (see Section 5.1.1.3).¹³¹ Improved iron-sensitive beads were prepared using a hexadentate ligand (Scheme 37).^{107,123} Lysine was selected as the linker between the beads, chelator and probe. The coupling of Fmoc-lys(Boc)-OH with the protected chelator *via* activation of the acid, followed by deprotection by hydrogenation leads to loss of the benzyl group, without influencing the Fmoc and Boc protected amines. The Boc group was then removed by trifluoroacetic acid, followed by coupling to Dynabeads. The Fmoc group was subsequently released by treatment with piperidine and the free amino group coupled with NHS activated 5(6)-carboxyfluorescein to obtain the final chelatable fluorescent beads (Scheme 37).

Scheme 36. Synthesis of bidentate 3,4-HOPO-containing fluorescent beads (CFBs).



Scheme 37. Synthesis of hexadentate 3,4-HOPO-containing fluorescent beads.



2.9. Considerations specific to HOPO synthetic chemistry

In this section, we give a general overview of specific tips worthy of consideration when synthesising HOPO-based ligands. We highlight a number of practical aspects that will facilitate the efficient production of this class of chelator. We present a list of considerations for the optimisation of both synthetic and purification methods which are generally necessary to achieve the quality and amounts of chelator required for successful biological investigation.

Chemical suppliers. Many of the reagents (e.g. starting materials) used in synthetic chemistry laboratories contain different traces of contaminants, depending on the brand and batch. Some of these contaminants are frequently metals or metal ions that can be complexed by the ligand under preparation. Attention should be paid to the

information relating to purity, procedure of purification and expected contaminants, as indicated by the specific supplier.

Glassware. Particular care should be taken when cleaning the glassware. Metal spatulas and vials using caps with a metallic surface should be avoided as this is a potential source of metal contamination. The use of plastic spatulas to weigh both protected- and free-chelators is highly recommended. Moreover, each piece of glassware used in a chelator synthesis should firstly be acid treated with concentrated HCl (37%) and washed with methanol and dichloromethane, before drying.

Analytical characterisation and purification procedures. Several analytical/purification methods have been adopted in the field of HOPO chelators, including precipitation and/or crystallisation from various solvents. Flash column chromatography can be used for protected bidentate ligands (pre-treatment of the stationary phase with EDTA is desirable); analytical/preparative TLC (i.e. plastic support sheet) and HPLC. HPLC presents numerous advantages as it can permit the rapid purification of free ligand. Also, in the case of tri- and tetra-podal HOPOs (i.e. hexadentate and octadentate ligands), preparative HPLC often represents the only option to obtain pure material. Crucial requirements for ideal HPLC procedures (both analytical and preparative) with regards to HOPO-based free ligands are:

- (i) perform a “wash run” of the system with a solution of 1mM EDTA, in order to minimise trace metal contamination during the following preparative/analytical run;
- (ii) use plastic tubes and plastic vial inserts to inject and/or collect the samples;
- (iii) use HPLC grade solvents (e.g. extra-pure MilliQ water, MeCN or MeOH).

In some cases, pre-treatment of the mobile phases with Chelex[®] is also required in order to avoid unwanted chelation of trace metals. Despite these measures, small amounts of metal complexes are nearly always detected during HPLC-based analyses and purifications. This contamination results from the metal parts associated with the equipment (e.g. column pumps, needles for injection and collection tubing). An HPLC unit with a titanium pump can be an advantage.

Deprotection step. Removal of the protecting groups to unmask the chelating units is typically the final step in the synthesis of HOPO chelators. This step requires the above mentioned HCl pre-treatment of glassware to avoid the chelation of metals by the newly formed ligands. Typical procedures to remove specific protecting groups include: 1) H₂, Pd/C (benzyl removal); 2) BCl₃ (benzyl removal; aluminum contamination has been detected when using this reagent, possibly due to traces present in the purchased batch); 3) BBr₃ (methyl removal, benzyl removal that cannot be accomplished with BCl₃); 4) neat trifluoroacetic acid (benzyl removal); 5) NH₃OH 25% v/v (acetyl removal).

Amide bond formation, coupling agents and solid-phase peptide synthesis (SPPS). Amino (see Scheme 3A) and carboxylate (see Scheme 4) functionalised HOPOs are the most common building blocks to generate oligomeric-HOPO chelators. These scaffolds are amenable to standard SPPS procedures. Commonly, unprotected chelators can be loaded on to the growing polypeptide chain immediately before the final cleavage from the resin. However, protected HOPOs can also be loaded on to the resin-attached peptide at any time during SPPS procedures and final deprotection (to unmask the chelating units) is usually performed in solution as the last step (see Scheme 30). Tripodal HOPO-based chelators (e.g. **22**) have also been used in solid-

phase synthesis, mainly in the unprotected form due to difficulties in fully accomplishing the final deprotection. Typical coupling reagents used include DIC, EDC, HATU, TBTU, PyOxP. Ethyl cyanoglyoxylate-2-oxime and HOBt are the usual auxiliary agents (i.e. anti-epimerisation) employed when standard carbodiimide are used, whereas DIPEA and Et₃N are usually used as bases to favour the nucleophilic substitution. Amide coupling performance can be greatly facilitated by using microwave irradiation, both in terms of reaction rate and isolated yield of the desired product. For instance, the final step presented in Scheme 17 (i.e. reaction of a tri-COOH linker with a NH₂-functionalised HOPO) proceeds to completion within 30 minutes, with the minimal occurrence of side reactions and producing a final yield of 70% for the desired product when subjected to microwave conditions. In the absence of microwave irradiation, the reaction took over two days to reach completion and was associated with a much reduced yield.

3. Physico-chemical properties of hydroxypyridinones

3.1. Thermodynamic stability constants

Thermodynamic stability constants are the main indicators for chelator metal affinities. In aqueous solution, apart from metal ions, protons, can compete for chelators. For many trivalent metal ions, including iron(III), the optimal coordination number is six, which requires three bidentate or one hexadentate chelator to form a stable metal complex (Figure 1). The dominant chemical equations and corresponding equilibrium/stability constants of metal-chelation reactions for 3-hydroxypyridin-4-ones, e.g. deferiprone (**3**), are listed in Scheme 38. Deferiprone (**3**) possesses two pK_a values,

3.7 and 9.8, over the pH 2-12 range and forms a stable iron complex at pH 7.4 with the molar ratio of ligand:ferric iron being 3:1, $\log\beta_3(\text{Fe}^{3+}) = 37.4^{18}$ (Table 2 and Figure 7). In general, the higher proton affinity a chelator possesses, the higher its metal affinity. This observation is demonstrated by the existence of a linear relationship between $\log K_1(\text{Fe}^{3+})$ and sum of two $\text{p}K_a$ values for 3-hydroxypyridin-4-ones (Figure 8).

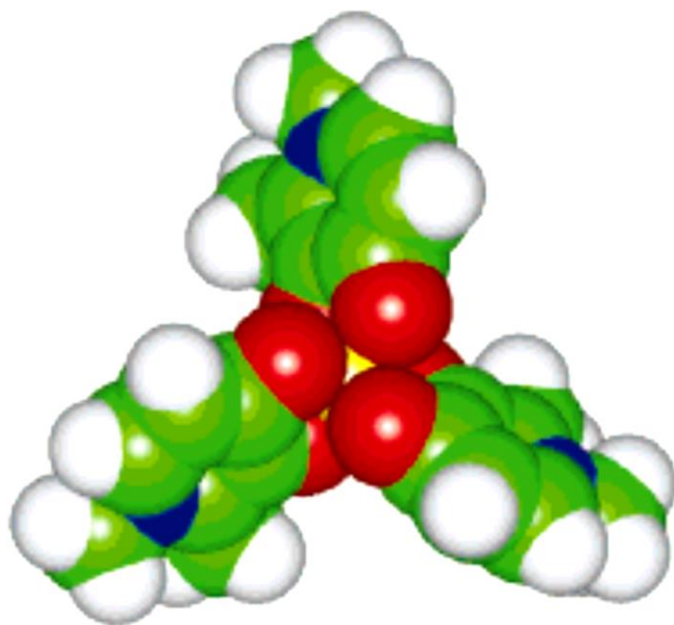
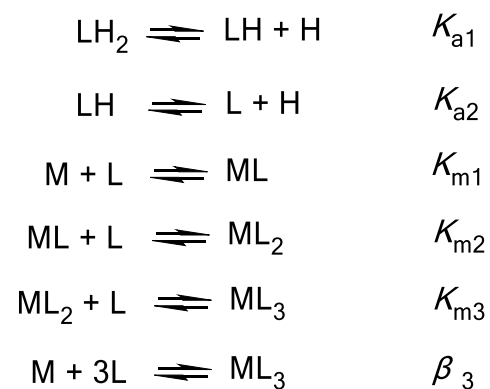


Figure 7. Optimised Fe^{3+}L_3 structure of deferiprone, left-hand propeller (λ), $\text{Fac}(e)$ stereoisomer. Reproduced with permission from Ref.¹⁷. Copyright 2002 John Wiley & Sons, Inc.

Scheme 38. Typical stability constants of a bidentate 3-hydroxypyridin-4-one. 2 pK_a s values and 3 metal $\log K$ equilibrium equations describe the metal-HOPO interaction; L: ligand, M: metal, β_3 is a cumulative constant; charges of species are neglected for simplicity.



$$K_{a1} = \frac{[\text{LH}][\text{H}]}{[\text{LH}_2]}$$

$$K_{a2} = \frac{[\text{L}][\text{H}]}{[\text{LH}]}$$

$$K_{m1} = \frac{[\text{ML}]}{[\text{M}][\text{L}]}$$

$$K_{m2} = \frac{[\text{ML}_2]}{[\text{ML}][\text{L}]}$$

$$K_{m3} = \frac{[\text{ML}_3]}{[\text{ML}_2][\text{L}]}$$

$$\beta_3 = \frac{[\text{ML}_3]}{[\text{M}][\text{L}]^3} = K_{m1} \times K_{m2} \times K_{m3}$$

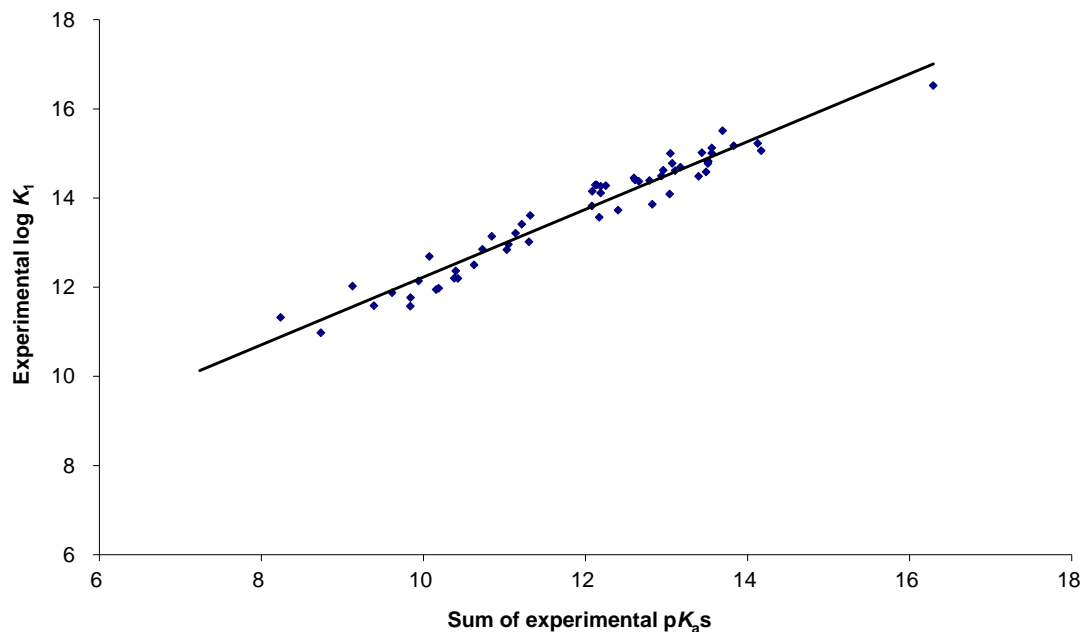


Figure 8. $\log K_1(\text{Fe}^{3+})$ values *versus* sum of pK_a values for 57 3,4-HOPOs. Reproduced from Ref.¹³² with permission from the Royal Society of Chemistry.

Another example for this relationship is the trend between $\log \beta_3(\text{Fe}^{3+})$ and pK_a values of hydroxypyridinone analogues (Table 1). The superior affinity of deferiprone for metals results from the extensive delocalisation of electrons in its resonance structures (Figure 9). Because of the competition effect at different pH values in aqueous solution Since the HOPO chelators differ in the denticity of their metal coordination and also in their relative acidities, metal stability constants are not directly comparable for all the ligands. The pM value (normally defined as $-\log[\text{free hydrated metal}]$, when $[\text{Ligand}]_{\text{total}} = 10^{-5} \text{ M}$ and $[\text{Metal}]_{\text{total}} = 10^{-6} \text{ M}$, at pH 7.4) is introduced as a comparative indicator.²¹ This term is calculated from the associated stability constants and multiple equilibria amongst the various chemical species, under defined conditions. The pM value changes as a function of $[\text{Metal}]$, $[\text{Ligand}]$ and pH. This is clearly shown in Ringbom coefficients,¹³³

derived from the mass balance equation of associated chemical reactions, as part of pM calculation (the ligand Ringbom coefficient is exemplified in Scheme 39). The influence of pH on pM values is illustrated by the pFe³⁺ value of deferiprone (**3**) over the pH range 2-12 (Figure 10).

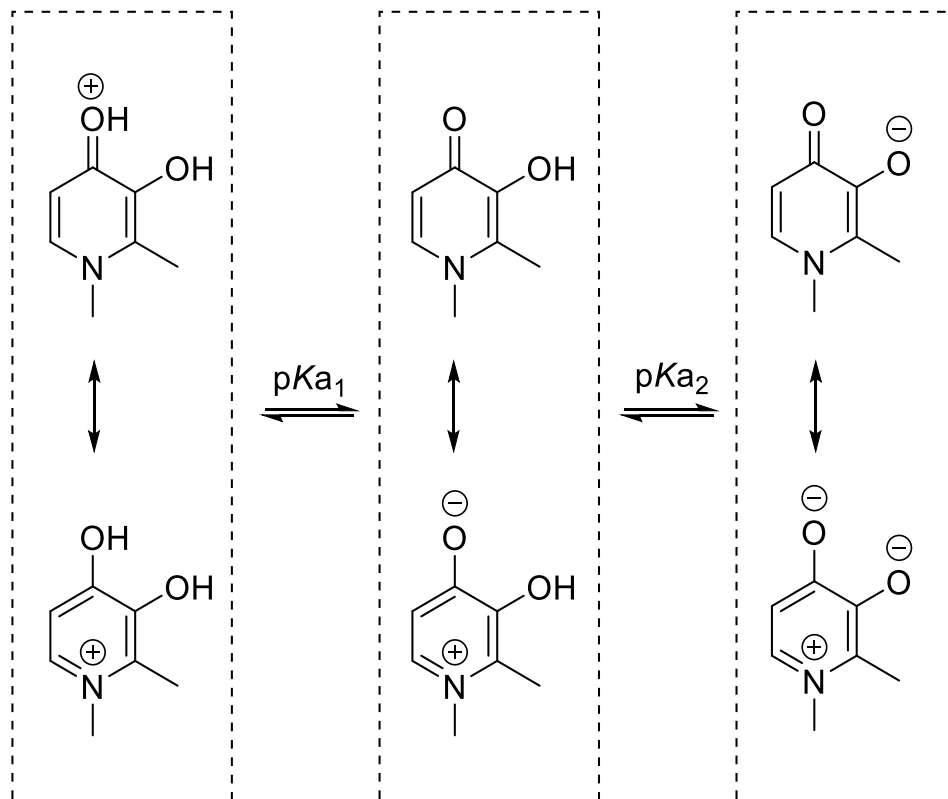


Figure 9. Proton equilibria and resonance structures of deferiprone (**3**), a typical *N*-alkyl-3,4-HOPO with 2 pK_a values.

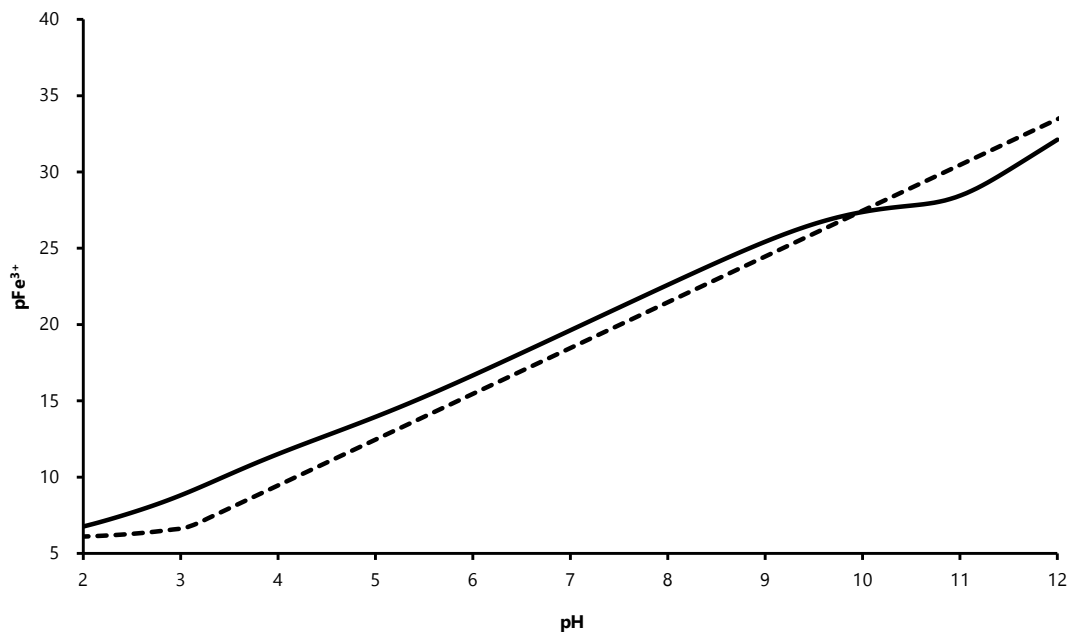


Figure 10. pH dependence of the $p\text{Fe}^{3+}$ value of deferiprone (**3**) under the conditions of $[\text{deferiprone}]_{\text{total}} = 10^{-5} \text{ M}$ and $[\text{Fe}^{3+}]_{\text{total}} = 10^{-6} \text{ M}$ (solid line), compared to $p\text{Fe}^{3+}$ values of iron hydroxide species (dashed line). The formation constants of hydroxide species are as follows: $\log K \text{FeOH} = 11.2$, $\log K \text{Fe}(\text{OH})_2 = 22.3$, $\log K \text{Fe}(\text{OH})_3 (\text{s}) = -38.8$, $\log K \text{Fe}(\text{OH})_4 = 34.4$, $\log K \text{Fe}_2(\text{OH})_2 = 24.7$, $\log K \text{Fe}_3(\text{OH})_4 = 49.7$.¹³⁴

Scheme 39. Ligand Ringbom coefficient (α_L) from the mass balance equation of ligand, K_f : formation constant, the inverse form of conventional acid equilibrium constant.

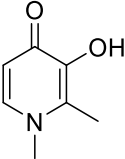
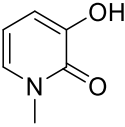
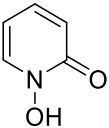
$$L_{\text{total}} = [\text{L}] + [\text{LH}] + [\text{LH}_2] + \dots$$

$$L_{\text{total}} = [\text{L}] + K_{f1}[\text{L}][\text{H}] + \beta_{f2}[\text{L}][\text{H}]^2 + \dots$$

$$L_{\text{total}} = [\text{L}](1 + K_{f1}[\text{H}] + \beta_{f2}[\text{H}]^2 + \dots)$$

$$\alpha_L = 1 + K_{f1}[\text{H}] + \beta_{f2}[\text{H}]^2 + \dots$$

Table 2. Stability constant of selected HOPOs. Affinity constants data taken from references^{18,135-137}.

Ligand	Structure	pK_{a1}	pK_{a2}	Log cumulative constants				
				Fe^{3+}	Al^{3+}	Ga^{3+}	Cu^{2+}	Zn^{2+}
3-hydroxy-1,2-dimethylpyridin-4-one (deferiprone, 3)		3.7	9.8	37.4	32.6	35.8	19.1	13.5
3-hydroxy-1-methylpyridin-2-one		-	8.9	30.0	25.1	29.7	N/A	N/A
1-hydroxypyridin-2-one		-	5.9	27.2	21.6	N/A	13.1	12.0

Under defined conditions of $[Metal]_{total}$ and $[Ligand]_{total}$, the (relative) concentrations of associated chemical species over a pH range can be calculated to obtain a speciation plot. This is exemplified by the ferric iron in the presence and absence of deferiprone over the pH range 2-12 (Figure 11). In general, the higher the pM value a chelator possesses, the stronger its metal affinity. Bidentate chelators possessing chelating atoms with pK_a values above 9, dominantly form metal complexes with molar ratios of ligand:metal of 2:1 at pH 7.4, the remaining metal coordination sites being occupied by water. In contrast, deferiprone (**3**), with pK_a values of 3.7 and 9.8, forms 3:1 iron complexes at pH 7.4 (Figure 7).

A typical hexadentate 3-hydroxypyridin-4-one possesses six pK_a values and one $\log K(\text{Fe}^{3+})$ value. In terms of Fe^{3+} -complex formation and provided with suitable stereochemistry, a hexadentate ligand can fully coordinate a single ferric cation in a 1:1 molar ratio.

The determination of thermodynamic equilibrium/stability constants in complexes with a slow kinetics is dependent on sufficient time being allowed for the association to reach equilibrium after each addition, during titration studies. Metal ions possess dramatically different kinetic properties (Figure 12); for instance the rate constant for the substitution of water on iron(III) is approximately 5 log units faster than for that of aluminum(III).¹³⁸

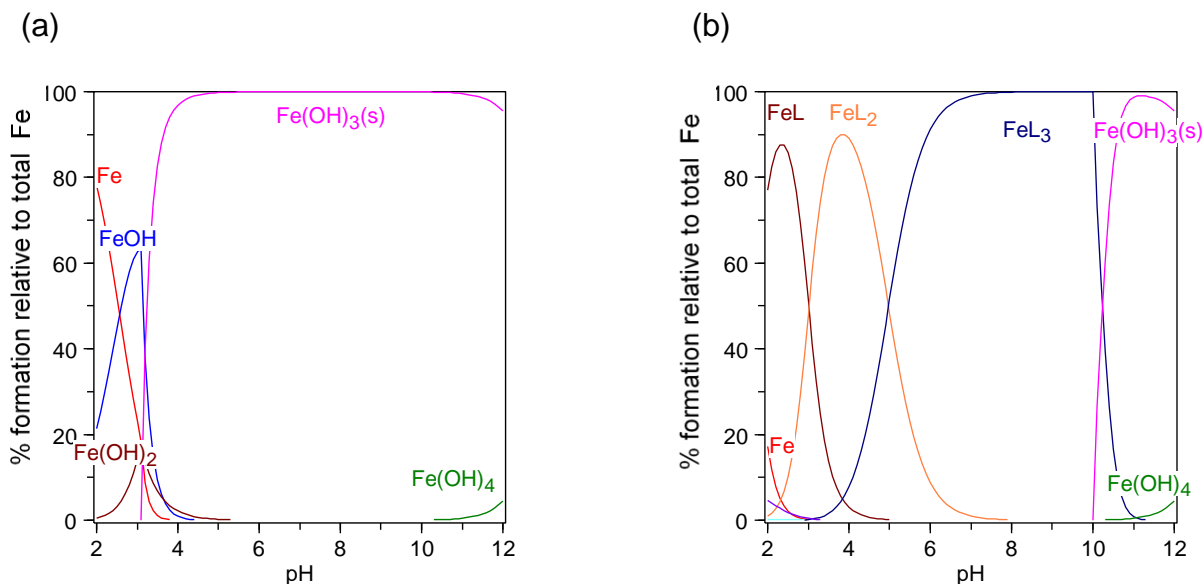


Figure 11. Speciation plots of ferric iron in the presence and absence of deferiprone (**3**) over the pH range 2-12. (a) Ferric iron in water. (b) Ferric iron in the presence of deferiprone (**3**) under the conditions $[\text{deferiprone}]_{\text{total}} = 10^{-5} \text{ M}$ and $[\text{Fe}^{3+}]_{\text{total}} = 10^{-6} \text{ M}$. Charges of species are neglected for simplicity. (s): solid.

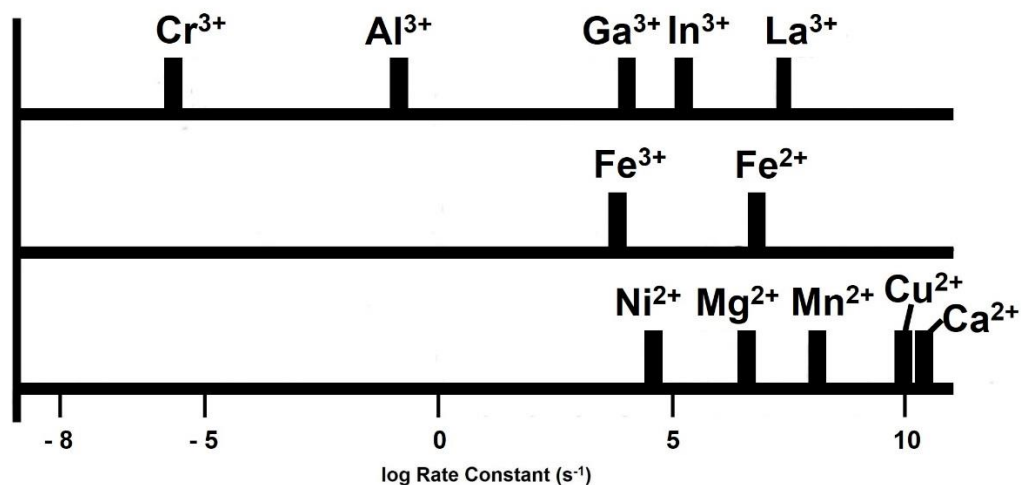


Figure 12. Logarithms of characteristic rate constants (s^{-1}) for substitution of inner-sphere water molecules on various metal ions.¹³⁹

3.2. Metal Selectivity

Selectivity between metal ions and ligands is important for the therapeutic design of chelating agents. The traditional “*Hard and Soft Acid and Base Principle*”¹⁴⁰ states that metal ions classified as soft prefer less electronegative chelating atoms, e.g. iodide, while metal ions classified as hard prefer more electronegative chelating atoms, e.g. fluoride. Ferric iron, which possesses a high charge density, is usually classified as a hard metal ion and prefers high charge density chelating atoms, e.g. charged oxygen species. In contrast, ferrous iron prefers relative low charge density chelating atoms, e.g. aromatic nitrogen or sulphur. Ligand Field Theory is also associated with the selectivity. This is highlighted here such that a transition metal ion in the presence of ligands with an octahedral coordination geometry will experience the splitting of the d -shell into e_g (higher energy) and t_{2g} (lower energy) levels.¹³⁹ This is especially

pronounced for partially-occupied d-shell metal ions, with different electron configurations and spin states of metal-complexes, thus leading to different stability constants.

The linear free energy relationship between a single metal ion and different ligands is presented in Figure 8. A linear free energy relationship also exists between a single ligand and Z^2/r of different metal ions (Figure 13), a factor which can be adopted for ligand design. Deferiprone (**3**) possesses high selectivity for ferric ion due to its two chelating oxygen atoms with appropriate pK_a values, compared to the analogues, 3-hydroxy-1-methylpyridin-2-one and 1-hydroxypyridin-2-one (Table 2).

The design of hexadentate HOPOs is controlled by a number of steric effects and involves the choice of linker (length and type) and linking positions on the hydroxypyridinone ring. The combination of choices results in various pre-organised ligands and sterically-strained metal-complex structures, which in turn lead to different binding affinities.⁸² Hexadentate and octadentate HOPO chelators possess a high affinity for hard cations, for instance gallium(III), gadolinium(III) and iron(III).

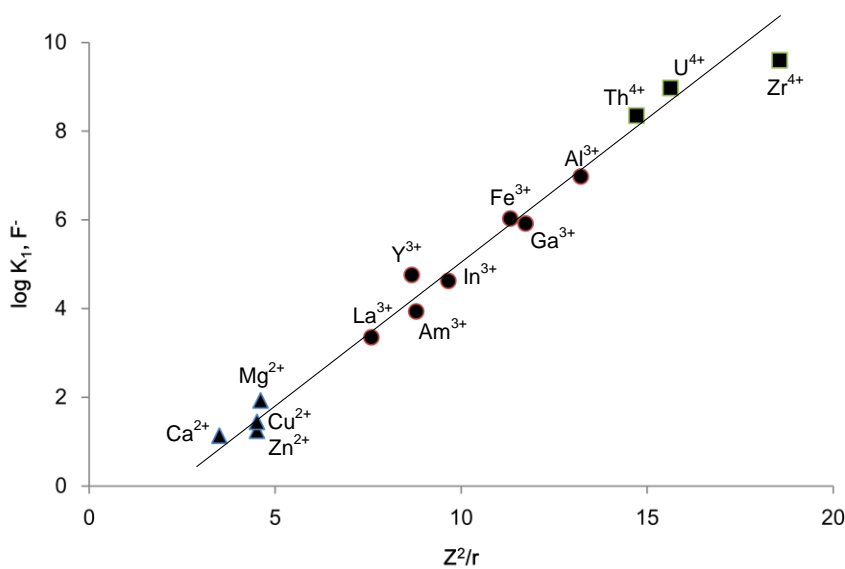


Figure 13. Relationship between $\log K_1$ for fluoride complexes of metal ions *versus* Z^2/r , where Z is the cationic charge on the metal ion, and r the ionic radius.¹³⁹ ■ tetra-positive iron; ● tri-positive iron; ▲ di-positive iron.

4. Hydroxypyridinones as chelators

4.1. Iron(III)

Bidentate 3,4-HOPOs have been used to chelate and sense iron in biological matrices for both therapeutic and analytical purposes. Deferiprone (**3**)¹⁴¹ and more recently 1-(*N*-acetyl-6-aminohexyl-3-hydroxypyridin-4-one (**33**)¹⁴² have been developed for the selective removal of iron from biological tissues (Figure 14). Hexadentate 3,4-HOPOs have also been developed for iron scavenging, for instance tripodal ligands based on a *tris*-carboxylic acid linker (**34**)¹⁴³ or *tris*-(2-aminoethylamine) (TREN) scaffold⁹⁴ (**35**) (Figure 14) and compounds using KEMP as a tripodal base (**16**).⁹⁷ The lipophilicity of these compounds can be readily modified by *N*-substitution on the pyridinone ring.⁹⁶

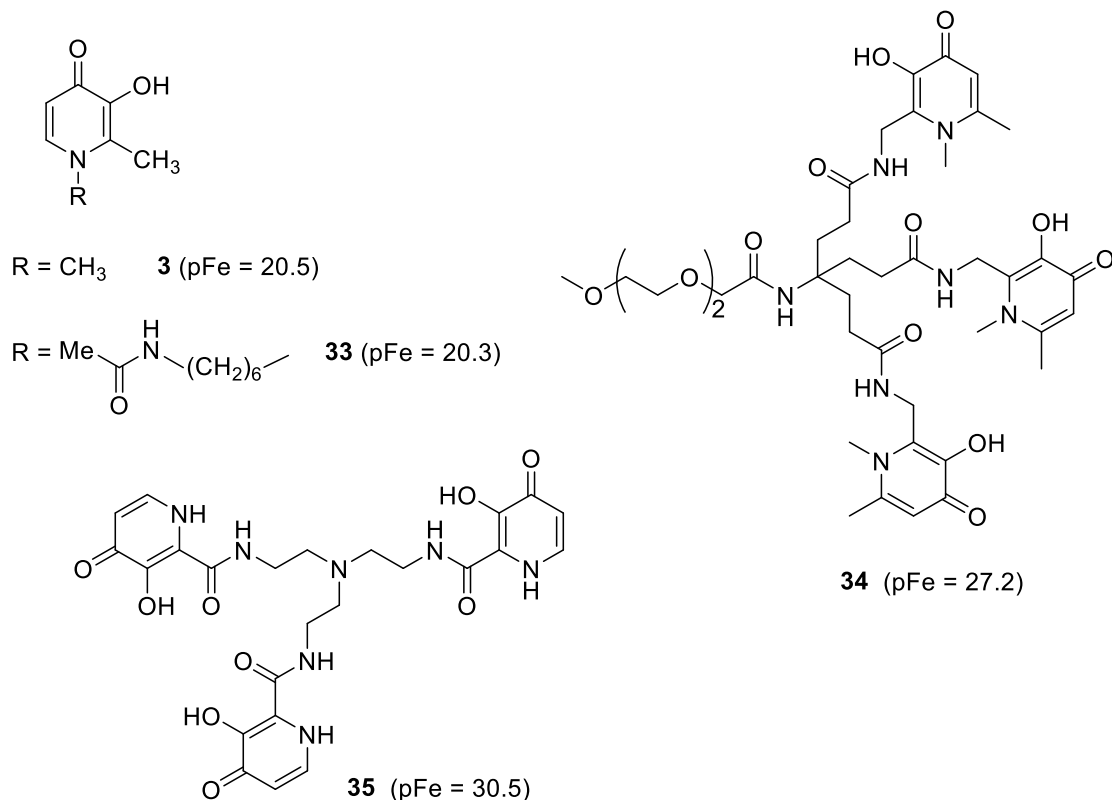


Figure 14. Compounds adopted for iron(III) chelation.

4.2. Gallium(III)

Gallium(III) possesses a similar ionic radius and surface charge density to that of iron(III) (Table 3) and consequently ligands that bind iron(III) also bind gallium(III) with similar affinity. Thus bidentate 3-hydroxypyridin-4-ones, for instance deferiprone (**3**), bind gallium(III) tightly,¹⁴⁴ with a pGa of 20.5. A series of bidentate *N*-carboxyalkyl-3-hydroxypyridin-4-ones (**36**) have been developed for gallium chelation in biological matrices (Figure 15).¹⁴⁵ Hexadentate, tripodal 3,4-HOPOs have been designed specifically for gallium chelation, for instance the THP-derivative (**37**) (Figure 15).¹⁴⁶ This and related chelators find application in diagnostic therapeutics (see Section 5.1.3).

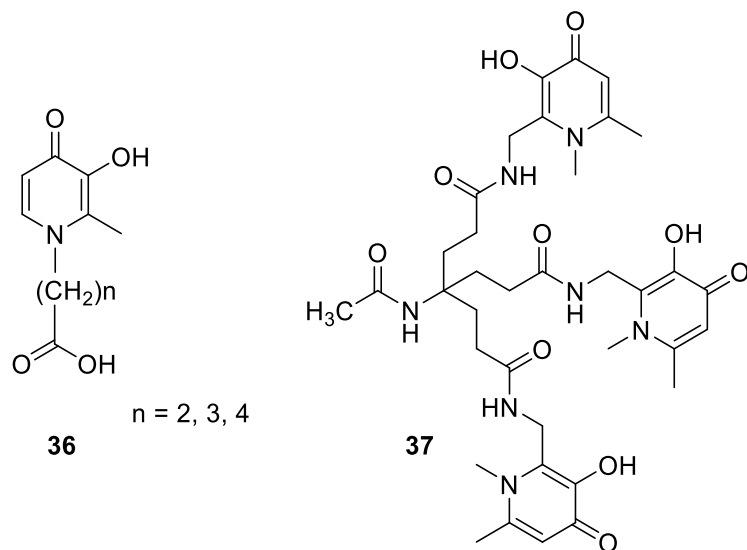


Figure 15. Compounds adopted for gallium(III) chelation.

Table 3. Ionic properties of cations chelated by HOPOs.

	Charge on ion	Ionic Radius (Å)	Surface Charge density $e\text{Å}^{-2}$
Aluminum	3 ⁺	0.54	0.82
Europium	3 ⁺	1.17	0.17
Gallium	3 ⁺	0.62	0.62
Gadolinium	3 ⁺	0.94	0.27
Iron	3 ⁺	0.65	0.57
Plutonium	4 ⁺	0.87	0.42
Zirconium	4 ⁺	0.72	0.62

4.3. Aluminum(III)

Aluminum(III) possesses a smaller radius than iron(III) (Table 3), which renders it one of the 'hardest' cations known, never-the-less aluminum binds to most iron(III)-chelating molecules, albeit with a lower affinity. Bidentate, tetradentate and hexadentate 3-hydroxypyridin-4-ones have been used for the selective removal of aluminum from biological tissue.^{99,147-149} As expected there is an increase of pAI with denticity: bidentate, 16.0; tetradentate, 19.0; and hexadentate 22.0.^{99,147} Examples of 3,4-HOPO ligands developed for aluminum scavenging are presented in Figure 16.

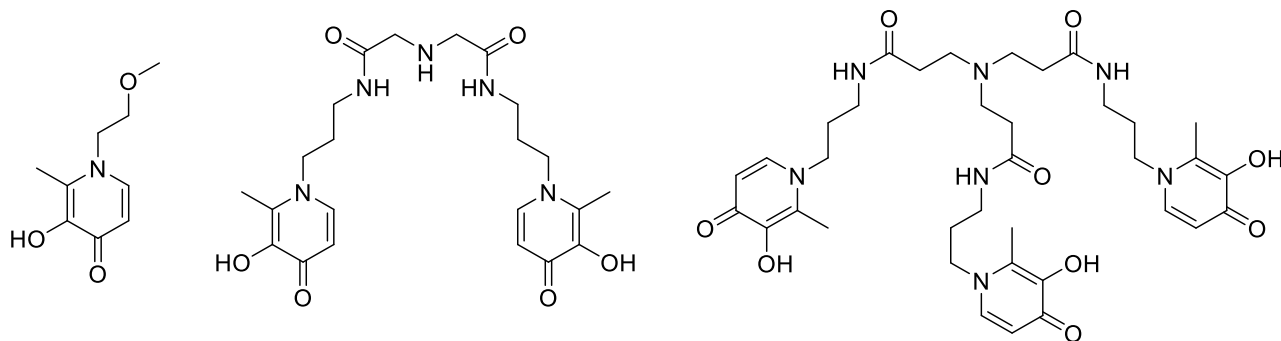


Figure 16. Compounds adopted for aluminum chelation.

4.4. Zirconium(IV)

By virtue of the tetrapositive nature of Zr(IV), the cation possesses a high surface charge density (Table 3) and so binds to hydroxypyridinones tightly. Zirconium-89 is a β^+ emitter with desirable nuclear imaging properties, including a relatively long half life. Because of the larger radius and tetrapositive nature of the cation, octadentate ligands

have found application. The formation of a desferrioxamine / hydroxypyridinone conjugate (**19**) has proved useful in this regard.¹⁰⁴ Guerard *et al* have reported a comparative study of the aqueous chemistry of Zr(IV) HOPO complexes,¹⁵⁰ not surprisingly the 3,4-HOPO compounds were found to possess the highest affinity for Zr(IV). Octadentate 3,2-HOPOs (**38**)¹⁵¹ and 1,2-HOPOs (**39**)¹⁰¹ have been investigated and demonstrated to be capable of binding Zr(IV), but with insufficient affinity to provide robust complex stability *in vivo*. However, Buchwalder *et al*¹⁰⁵ have reported that the tetrapodal 3,4-HOPO chelator (**40**) has largely overcome these problems and possesses a high *in vivo* stability (Figure 17). Hexadentate ligands have also been investigated for Zr(IV) binding, but for instance the tripodal 3,4-HOPO (**37**) demonstrated poor *in vivo* stability.¹⁵² A detailed comparison of zirconium-89 chelation chemistry has been reviewed by Wadas and co-workers.^{153,154}

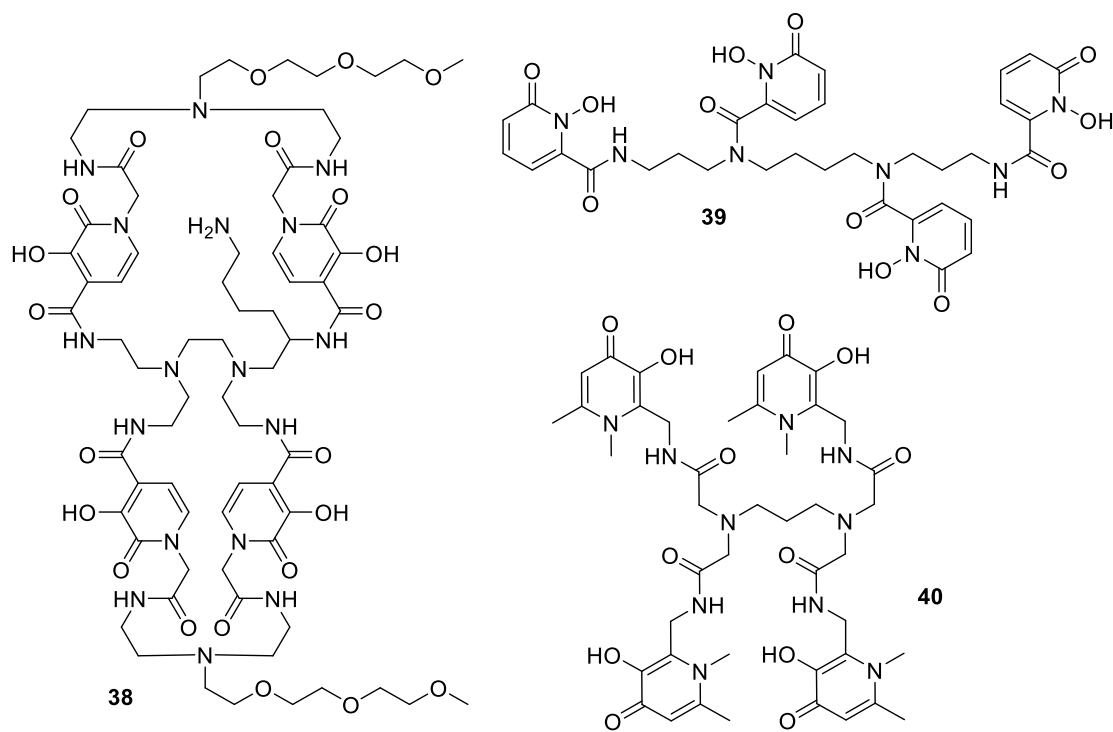


Figure 17. Compounds adopted for zirconium chelation.

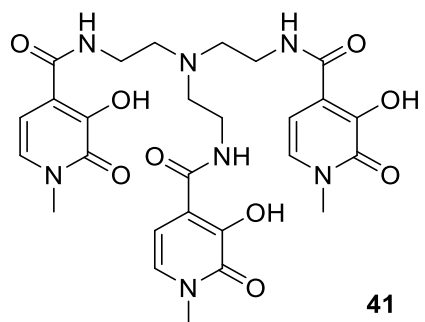
4.5. Gadolinium(III)

Among the various paramagnetic ions (Mn^{2+} , Gd^{3+} , Fe^{3+} , VO^{2+}) used as contrast agents, the highly paramagnetic lanthanide Gd(III) , with its seven unpaired electrons and long electronic relaxation time, has the most favourable electronic properties as a relaxation agent for *in vivo* magnetic resonance imaging (MRI).¹⁵⁵ MRI images are improved by administration of paramagnetic agents, which increase the relaxation rates of adjacent water protons, thereby enhancing the MRI signal. Although Gd^{3+} provides excellent MRI contrast, the high *in vivo* toxicity of $[\text{Gd}(\text{H}_2\text{O})_8]^{3+}$ necessitates that the metal is coordinated by high affinity chelators before it can be used *in vivo*.¹⁵⁶

The image enhancing capacity of Gd^{3+} -based contrast agents is proportional to the number of water molecules which can be exchanged in the inner coordination sphere of the Gd^{3+} ion.¹⁵⁵ The complexation of Gd^{3+} by organic chelators will reduce the number of inner sphere water molecules and therefore reduce this sensitivity. Current research in the field of Gd -based contrast agents focuses on obtaining micro- to nanomolar sensitivities, thereby reducing the toxicity due to Gd^{3+} accumulation.

Hydroxypyridinones have been developed for this purpose by exploiting the relatively low pK_a values of the chelating functions. The first ligand developed was (41), which forms an eight-coordinate Gd(III) centre, complexed by 6 hydroxypyridinone oxygen atoms and two water molecules.¹⁵⁷ Such a complex is associated with a rapid rate of water-exchange, moreover the stability of the complex is higher than many of the oxygen/nitrogen donor complexes previously utilised for this purpose.¹⁵⁸ In an analogous fashion, the tripodal 3,4-HOPO ligand presented in Figure 16 also

demonstrates a high thermodynamic stability for Gd(III) associated to an improved relaxivity (higher hydration number) of the complex.¹⁵⁹



Raymond and co-workers have systematically studied many variants on this structural theme.⁸³ Hybrid ligands, for instance with terephthalic acid (**42**) and salicylamide (**43**), are effective relaxation agents. Hybrid ligands containing different hydroxypyridinones (**44**) also show potential.⁸⁴ A further variation is to replace the TREN backbone with triazacyclononane (**45**) which provides a broader base to the Gd(III) complex (Figure 18).⁸⁸

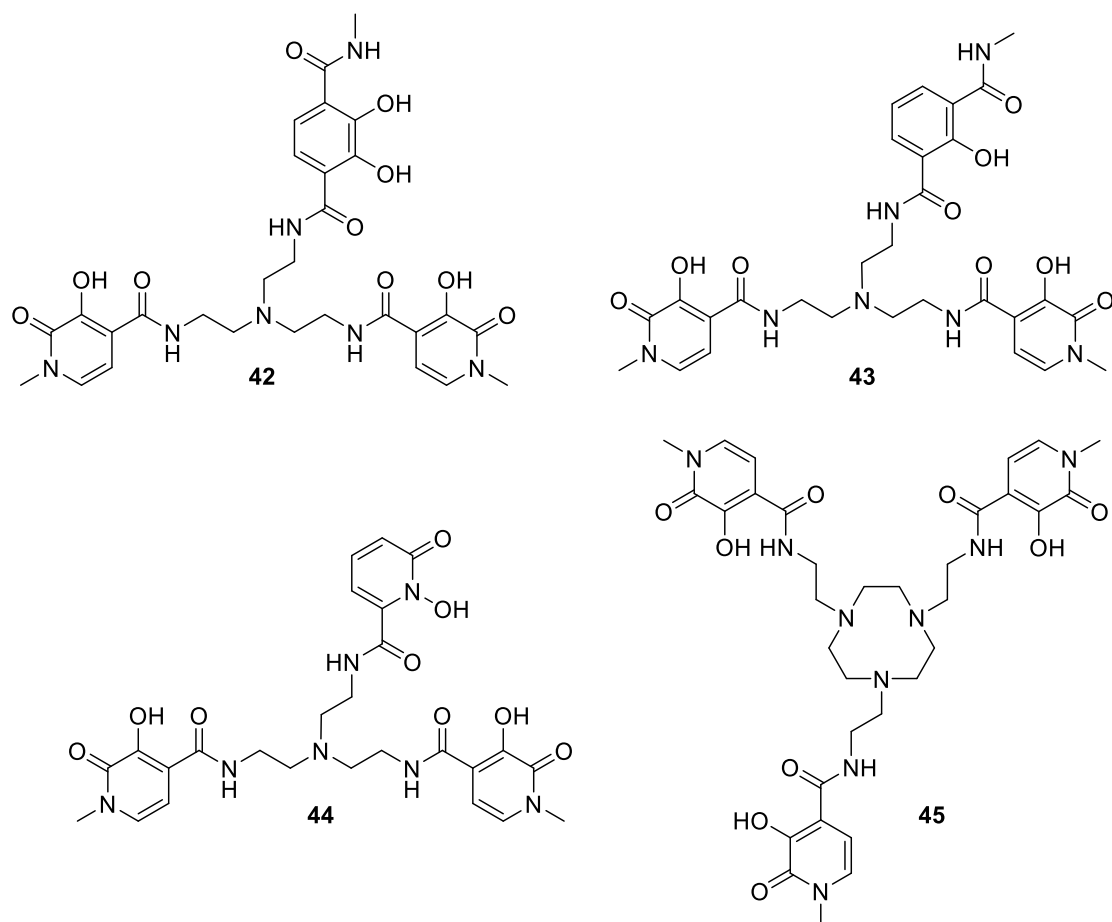


Figure 18. Compounds adopted for gadolinium chelation.

4.6. Plutonium(IV) and other actinides

Plutonium is an actinide metal used extensively in the nuclear industry. It has high toxicity as an alpha emitter and is readily accumulated in a range of tissues, including lung and bone. Pu(IV) is the most common oxidation state found in biological tissue. It has a high surface charge (Table 3) and so binds to iron(III) chelators, such as deferoxamine and other siderophores. Tetradentate 3,2-HOPO's, and in particular **46**, have shown excellent potential for mobilisation of plutonium, forming 2:1 complexes,¹⁶⁰ as has the hexadentate 3,2-HOPO (**47**).¹⁶⁰ However, a range of octadentate

hydroxypyridinones has been found to possess the best potential for the selective removal of plutonium.^{73,160} The two spermine backbone-derived oligo-hydroxypyridinones (**48**) and (**49**) have been demonstrated to be particularly efficient Pu(III) scavengers in biological tissue (Figure 19).^{161,162} HOPOs can chelate a range of actinides including thorium, americium and neptunium.¹⁶⁰

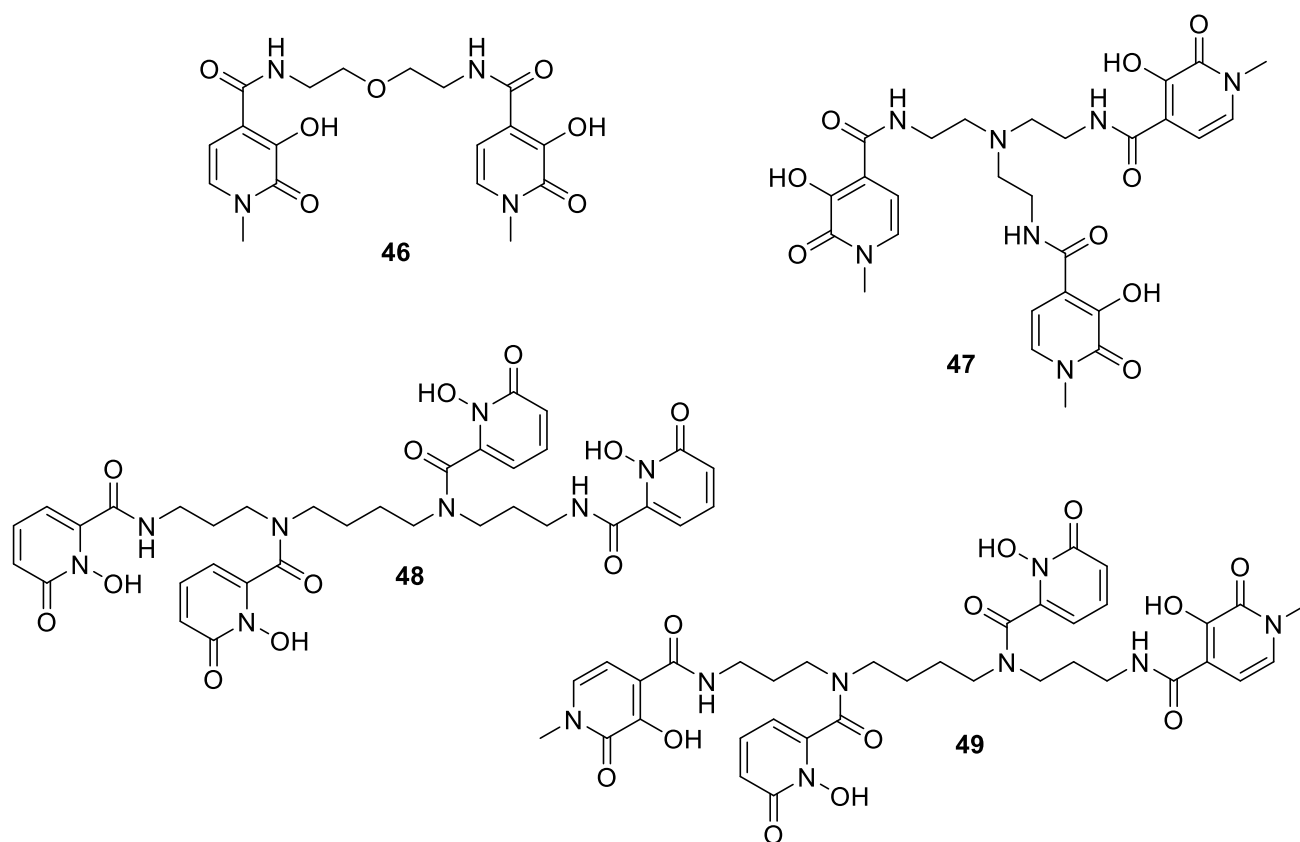


Figure 19. Compounds adopted for plutonium chelation.

4.7. Europium(III)

Organic complexes of luminescent lanthanides such as europium(III) have become increasingly useful for biological assays and high-throughput screening applications

where their long-lived luminescence leads to improvements in the signal-to-noise ratio. The larger radii (Table 3) renders octadentate ligands ideal for tight chelation, indeed the tetrapodal 1,2-HOPO (**50**) has proved to be an efficient chelator for europium(III).⁷⁶ The efficiency of Eu(III) luminescence by energy transfer is strongly dependent on the ion coordination geometry and this has been systematically modified in an attempt to increase the luminescence efficiency.^{77,100} Ligand **51** was found to be associated with an excellent luminescence quantum yield (Figure 20).¹⁰⁰

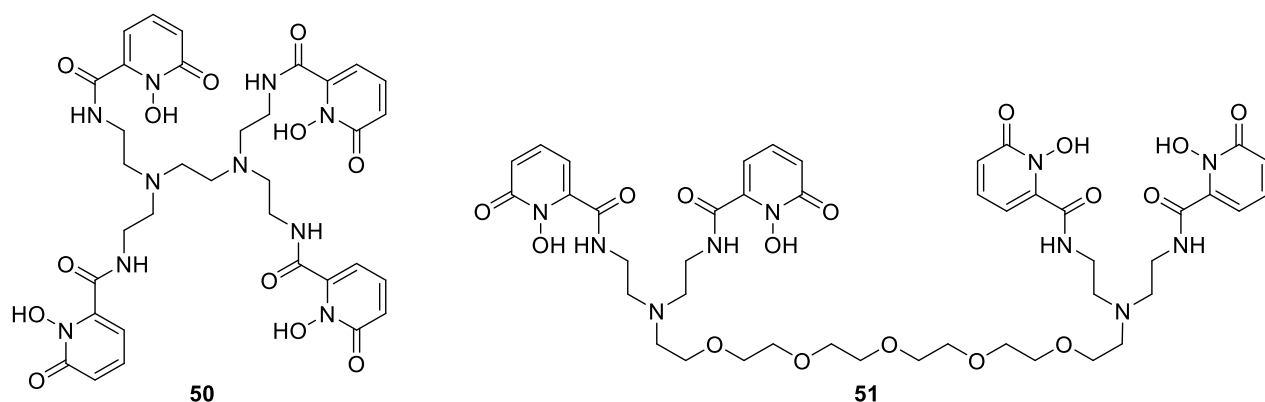


Figure 20. Compounds adopted for europium chelation.

5. Applications centred on HOPO chelators

5.1. Analytical applications

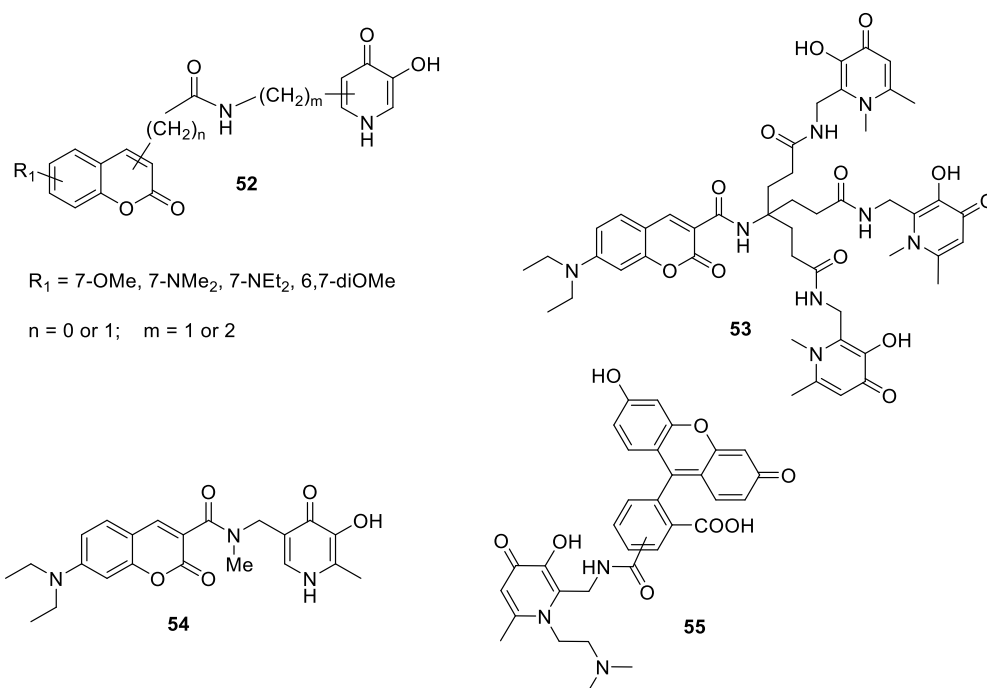
5.1.1. Fluorescence sensing

Over the last two decades, fluorescent probes have been widely used for the selective quantification of metal ions because they can provide a simple, sensitive, precise and economical method for online monitoring of low concentrations of target metal ions without destruction of the biological matrix, together with the advantages of spatial and

temporal resolution. Several reviews regarding fluorescent probes for measuring metal ions (especially Zn^{2+} , Cu^{2+} , Fe^{2+} and Fe^{3+}) in biological systems have been published.^{120,121,163} With the design of fluorescent probes, the chelating moiety should have the potential to selectively complex the target metal ion. The fluorophore moiety, which is covalently attached to the chelating moiety, should produce a distinct fluorescence signal upon chelation. This fluorescence change can be monitored for both qualitative and quantitative determination of the target metal ion. The fluorescence signals can be observed in three ways: decrease, increase, or shift in the fluorescence maxima due to either electron transfer, charge transfer or energy transfer processes.^{164,165} Fluorescence quenching, enhancement and shift is related to turn-OFF, turn-ON and ratiometric probes chelating with the target metal ion. This process is generally reversible. *d*-Block ions such as Fe^{3+} often open excited state de-excitation pathways *via* electronic energy transfer and/or photoinduced electron transfer involving the metal center. Therefore, it is not surprising that there are many more turn-OFF probes that have been reported as compared to turn-ON probes. All hydroxypyridinone-based fluorescent probes reported to date are turn-OFF probes.

The fluorescence of hydroxypyridinone probes (**52**) is sensitive towards the presence of ferric ions,¹²³ quenching ratios falling in the range 46-96%. The fluorescence quenching mechanism has been assessed and found to generate a non-linear relationship in the Stern-Volmer plot, indicating a static rather than dynamic mechanism of quenching. Further investigation of these probes led to the finding that a linear relationship exists between the fluorescence intensity and Fe^{3+} concentration when the metal-to-ligand ratio is less than 1:3. No additional quenching of fluorescence was observed upon

further increasing the metal-to-ligand ratio to greater than 1:3. Probes of the type **52** possess a high Fe³⁺ and Fe²⁺ sensitivity (>90%) and are much less sensitive (<15%) towards the presence of other metal ions such as Zn²⁺, Ni²⁺, Cu⁺, Co²⁺, Ca²⁺, Mn²⁺, Mg²⁺, Na⁺ and K⁺, with the exception of Cu²⁺, which has a sensitivity of 40-50%.^{166,167} The sensitivity towards Fe²⁺ is due to rapid autooxidation of iron(II) subsequent to coordination to the probe. The sensitivity towards Cu²⁺ is not a serious problem for most living cells, as the level of copper in the cytosol, lysosome and mitochondria is maintained at an extremely low level (< 10⁻²⁰ M) by selective copper(II) pumps and chaperone proteins.¹⁶⁸



5.1.1.1. Measurement of pFe³⁺ values

The fluorescence intensity of the probe in the presence of iron and a competing ligand is associated with the pFe³⁺ value of the competing ligand. A linear correlation was

found to exist between the fluorescence of such a mixture and the pFe^{3+} values of competing ligands. As a result of this finding, a fluorescence method was set up in order to determine the pFe^{3+} values of ligands which are difficult to measure using conventional spectrophotometric or potentiometric methods.¹⁶⁹ The method can operate with sub-milligram quantities of competing ligand, a real advantage with natural products which are difficult to isolate. By using one of these fluorescent probes, ligands which possess pFe^{3+} values in the range of 17-23 can be measured. Several hydroxypyridinone-based hexadentate fluorescent probes were also found to be selective for Fe over other metals such as Cu, Zn, Ni, Mn and Co. By using one of these hexadentate probes (**53**), the pFe^{3+} values of a range of hexadentate chelators, dendrimers and polymers (which are difficult to measure by conventional spectrophotometric and potentiometric assays) were determined.^{107,170}

5.1.1.2. Measurement of the concentration of intracellular iron pools

Compound **54** is a typical iron-sensitive fluorescent probe. Due to its relatively small molecular size (MW < 500), possession of less than 5 H-bond donors, possession of less than 10 H-bond acceptors and a $clogP$ value less than 5, this probe is predicted to efficiently permeate biological membranes by passive diffusion.¹⁷¹ The permeability of representative probes across human erythrocyte ghost membranes was investigated and the rate of permeability was found to be related to the corresponding $clogP$ values.¹²³ Thus these probes have the potential for monitoring intracellular labile iron pools. The moderately lipophilic fluorescent probe (**54**) was found to be the most sensitive for monitoring labile iron. The concentration of the intracellular chelatable iron

pool in rat hepatocytes was determined, by this probe, to be $5.4 \pm 1.3 \mu\text{M}$.¹⁶⁷

Furthermore, this probe was used to determine the labile iron pool of human lymphocytes and a value of $0.57 \pm 0.27 \mu\text{M}$ was determined for healthy human lymphocytes.¹⁷²

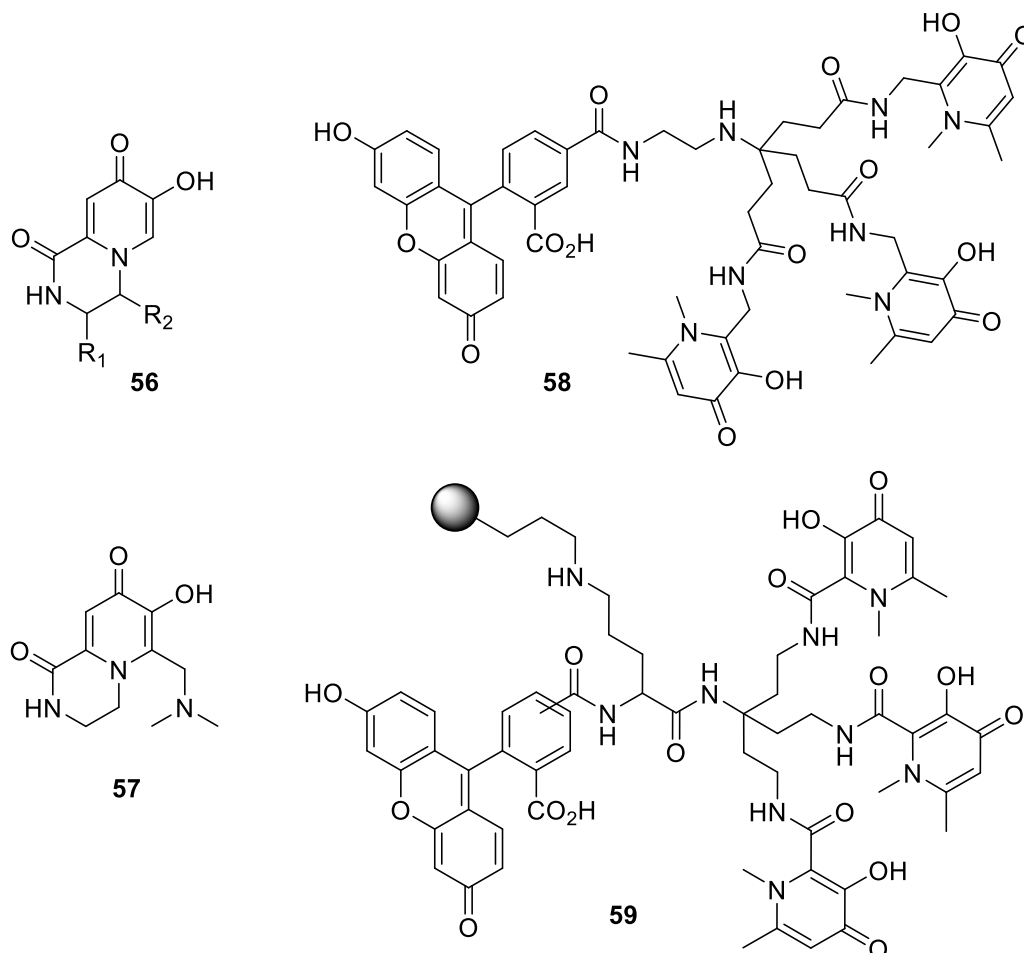
The lysosomal compartment of cells typically contains a relatively high level of labile iron but the measurement of this pool is difficult because of its small size; approximately 1% of the cell volume. Compound **55** is a probe specifically designed to target the lysosome.¹²⁴ The probe distribution is limited to the lysosomal compartment as demonstrated by colocalisation studies in macrophages. The fluorescence of the probe is highly responsive toward alterations of vesicular labile iron concentrations in the lysosome. This has permitted the assessment of cellular iron status with high sensitivity in response to the clinically applied medications desferrioxamine, deferiprone and deferasirox.¹²⁴ Mitochondria are major utilisers of iron, as well as being an important source of the superoxide anion. The mitochondrial labile iron pool plays a crucial role in oxidative injuries and pathologies. Delocalised lipophilic cations, are able to cross the inner mitochondrial membrane and hence be accumulated with a distribution associated with the Nernst potential, typically in excess of 1000 fold.¹⁷³ This useful property of lipophilic cations has been applied to basic hydrophobic peptides which are capable of targeting fluorescent iron probes to the mitochondrion. Two such hydroxypyridinone-based fluorescent peptides (**27** and **28**, Scheme 30) have been designed to be selectively accumulated by mitochondria as confirmed by confocal microscopy.¹²⁵ The iron-sensing ability of these two peptides was confirmed by fluorescent quenching and dequenching studies both in solution and in FEK4 cells.¹⁷⁴ The probes represent the

first example of highly sensitive mitochondria-directed fluorescent iron chelators, with potential to monitor mitochondrial labile iron pool. This probe range represents a powerful class of sensors which are suitable for quantitative iron detection and clinical real-time monitoring of subcellular labile iron pools in iron-overloaded patients.^{124,175}

Orvig's group have designed a range of hydroxypyridinone derivatives in order to investigate their interaction with amyloid protein.¹²⁶ Using the fluorescent signal as the readout, **29b** was found to be capable of interacting strongly with amyloid-beta protein fibrils.¹²⁶

Bicyclic lactam probes **56** and **57** have been designed to replace the established fluorescent probe chelator conjugates (**52**) by a single chemical moiety and thus a smaller molecular size. The quantum yields of this type of probe are higher than those of probes typified by **52**. The relatively low pK_a values of these probes result from an inductive effect of the amido group at the 6-position of the pyridinone ring. As a result, the pFe^{3+} values are correspondingly higher and therefore these probes bind iron tightly.¹²⁸ The probe fluorescence is almost completely quenched in the presence of an equivalent amount of Fe^{3+} or Fe^{2+} . In addition, these probes are also very sensitive to the presence of Cu^{2+} but much less sensitive towards other metals such as Ni^{2+} , Co^{2+} and Mn^{2+} . Furthermore, the combination of these probes with Zn^{2+} , Mg^{2+} and Ca^{2+} results in a blue shift of the maximum emission wavelength and an increase of the fluorescence intensity. Although the probes can become completely quenched by the addition of equimolar Cu^{2+} , the influence of this cation under *in vivo* conditions can be neglected as intracellular Cu^{2+} is very tightly bound to proteins and not accessible to

quench the probe fluorescence.¹⁷⁶ **57** was found to penetrate the cell membranes quickly and therefore has potential as an intracellular iron probe.¹²⁷



5.1.1.3. Measurement of the extracellular labile iron pool ('non-transferrin bound iron')

The ability to scavenge iron from oligomeric Fe³⁺ citrate complexes demonstrated that the probes **53** and **58** scavenge iron faster than both deferiprone (**3**) and desferrioxamine (**1**). The limit of detection for iron by the fluorescein-based probe **58** is

10^{-8} M. However, when this direct method using either **53** or **58**, was applied to various serum samples, the measured non-transferrin-bound iron (NTBI) concentrations were found to be variable, the main reason being that autofluorescence varies between serum samples. As the fluorescence method is so sensitive, any small change of fluorescence leads to a relatively large change in fluorescence emission.¹²⁹ In order to avoid the influence of variable autofluorescence from serum samples on NTBI determination, it is necessary to separate the iron probe from serum during the fluorescence measurement. Consequently, chelating fluorescent beads (CFBs) have been designed which consist of various hydroxypyridinones linked to fluorescein and are coupled to magnetic Dynabeads[®]. Upon incubation with serum samples, NTBI is captured by the CFBs and the complex is separated from the serum proteins by flow cytometry by virtue of its different size.¹⁷⁷ The autofluorescence interference is therefore avoided. The hexadentate pyridinone-based CFB (**59**) was designed as a result of a number of optimization steps. This probe has proved to be reliable in quantifying iron levels within the 0.1-10 μ M range, which is the expected NTBI concentration range in the iron-overloaded patients.¹⁷⁷ NTBI quantification from the sera of patients was found to be consistent with the known iron status of the patients. This new CFB assay has several advantages over other NTBI assays by virtue of its simplicity and avoidance of filtration steps.¹³¹

5.1.2. Time-resolved luminescence sensing

Time-resolved luminescence resonance energy transfer (TR-LRET) assays are under continuous development for high-throughput screening of molecular interactions¹⁷⁸ and

in particular the interaction between proteins and inhibitors.¹⁷⁹ Spectral and time resolved discrimination of the luminescent signal from background autofluorescence results in a marked improvement in sensitivity. In many assays undertaken in biological matrices, it is necessary to quantify a specific analyte at relatively low concentrations (typically 10^{-8} M). Thus chelators are required, which possess sufficient water solubility and a high affinity for europium(III), such that no complex dissociation occurs even in the presence of the commonly used chelators EDTA and DTPA. Octadentate hydroxypyridinones offer this possibility, providing eight chelating oxygen atoms,¹⁸⁰ for instance the tetra 1,2-HOPO (**50**). Optimisation of this octadentate structure in order to increase brightness has been achieved by removing the inner sphere water molecules and adjusting the geometry around the europium(III) cation by increasing the spacing between the two tetradentate components, using a pentoethylene oxide chain (**51**). This latter compound has potential for *in vitro* and cell biological measurements.

5.1.3. Positron emission tomography (PET) imaging

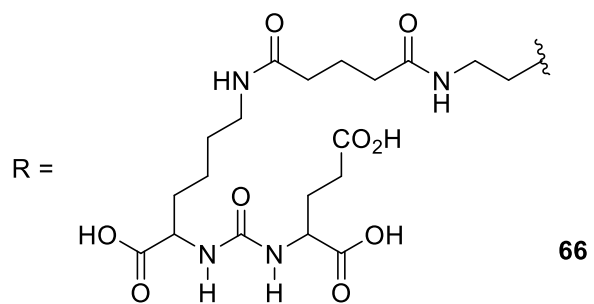
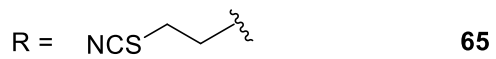
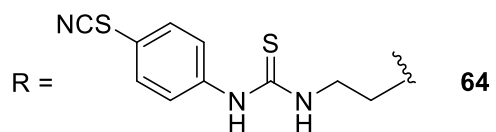
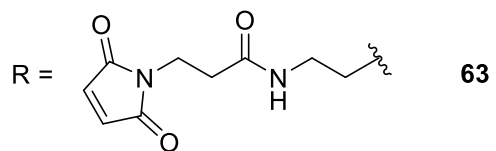
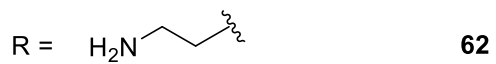
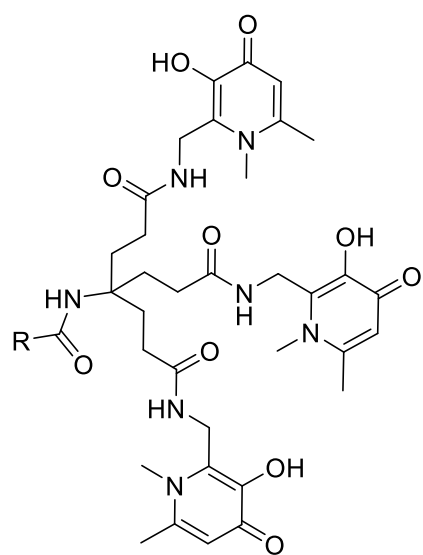
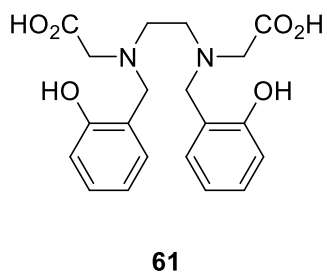
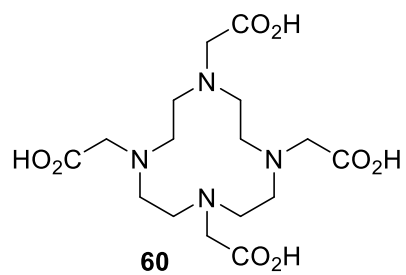
Positron emission tomography is a whole body diagnostic, three – dimensional molecular imaging modality, used in nuclear medicine that detects radiation arising from the decay of unstable positron emitting radioisotopes. The increasing availability of gallium-68 has had a significant clinical impact on the use of PET for molecular imaging.¹⁸¹ Clinical use of ⁶⁸Ga receptor-targeting radiopharmaceuticals has found wide application in the detection of neuroendocrine cancers¹⁸² and prostate cancers.¹⁸³ ⁶⁸Ga has a half-life of 68 mins. A second radioisotope, also with potential for PET imaging is zirconium-89, which possesses the longer half-life of 78h.

Both these radioisotopes bind to oxygen-rich chelators (see Sections 4.2 and 4.4) and two widely adopted ligands are DOTA (**60**) and HBED (**61**). Clinical radiosyntheses of ^{68}Ga -DOTA and ^{68}Ga -HBED involve heating at 80 – 100°C, for 5 – 20 min at pH 3 – 5¹⁸³ followed by post-synthetic purification. In the case of DOTA, heating is required to speed up the complexation of $^{68}\text{Ga}^{3+}$; for HBED, three geometric isomers result on gallium chelation and heating favours the formation of the thermodynamically preferred species. For ^{68}Ga to be adopted for routine clinical practice, chelators that provide efficient and reproducible kit-based radiolabelling methods are required. Chelators based on 3,4-HOPOs, in contrast to those based on DOTA and HBED, allow a rapid one-step quantitative $^{68}\text{Ga}^{3+}$ radiolabelling at neutral pH and ambient temperature. Thus, the hexadentate ligand **37** binds $^{68}\text{Ga}^{3+}$ extremely tightly, such that there is no exchange with transferrin under *in vivo* conditions. Furthermore the ^{68}Ga complex of **37** is rapidly excreted *via* the kidneys, ensuring a low background for imaging studies.¹⁸⁴

5.1.3.1. *Tris*-(3,4-HOPO) bioconjugates

The β -alanine-derived compound **62** can be functionalised for bioconjugation via maleimide^{146,152} or isothiocyanate links^{185,186} (**63**, **64** and **65**). These bifunctional chelators can be readily coupled to peptides and proteins, for instance protein C2A,¹⁴⁶ C(RGDFK),¹⁸⁵ TATE¹⁸⁶ and Trastuzumab.¹⁸⁴ A particularly successful application involves **66** which binds to prostate-specific membrane antigen (PSMA), which is over expressed in prostate cancer.¹⁸⁷ The ^{68}Ga -labelled THP-PSMA bioconjugate has been preclinically evaluated as a radiotracer for PSMA imaging. Interestingly, radiolabel procedures with ^{68}Ga carried out by adding unprocessed generator eluate directly to a

vial containing the ligand THP-PSMA (**66**) with no further manipulation, led to efficient radiolabelling (i.e. 95% radiochemical yield) within 5 min, at room temperature. The product was a single radioactive species that demonstrated specific binding to PSMA protein in PSMA-expressing DU145 cells and was stable in human serum for more than 6 h.¹⁸⁷ Clearly, ⁶⁸Ga-THP-PSMA (Galliprost[®]) has potential to achieve 1-step kit-based labelling.¹⁸⁸ *Ex vivo* and *in vivo* PET imaging and biodistribution studies have been conducted in mice bearing xenografts of the same cell lines, in comparison to commonly used ⁶⁸Ga-HBEDCC-PSMA,¹⁸⁹ demonstrating that ⁶⁸Ga-THP-PSMA selectively accumulates in PSMA-expressing tumours, with a good signal-to-background ratio delineation of PSMA-positive tumour lesions similar to the reference imaging agent. This compound is currently involved in clinical trials (Figure 21). Other groups have confirmed the clinical potential of the THP conjugate Galliprost[®].^{190,191} In contrast to other ⁶⁸Ga-PSMA conjugates, the extremely simple radiolabelling procedures for ⁶⁸Ga-THP-PSMA require only a generator, a cold-kit vial to be reconstituted before use, a syringe, quality control facilities, and shielding. These results confirm the possibility of generating kit-based ⁶⁸Ga radiopharmaceuticals for use in hospitals and wider clinical contexts.



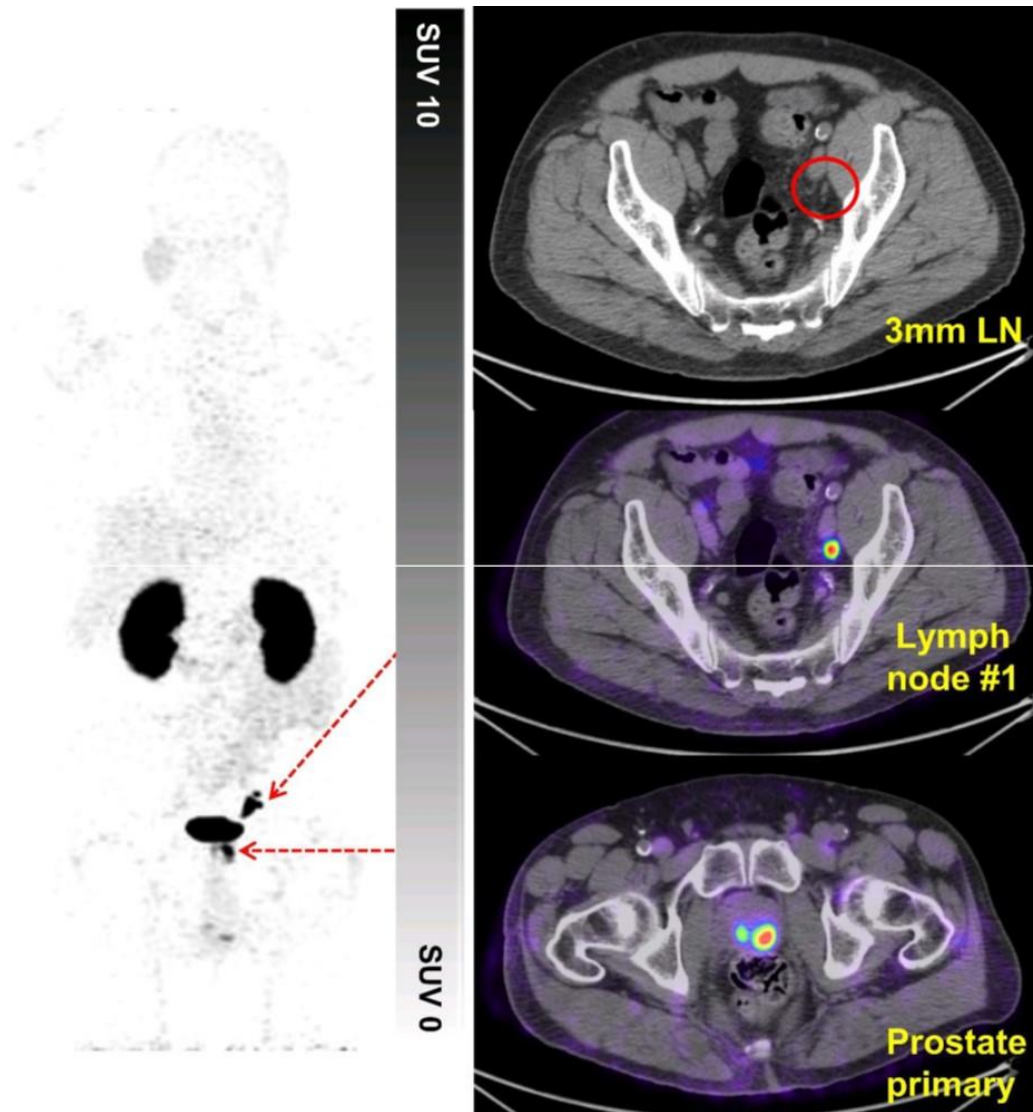


Figure 21. PET imaging of prostate cancer. Application of Galliprost® for patient with a prostate adenocarcinoma with high uptake in the primary prostate tumour and focal uptake in several left external iliac lymph nodes. This research was originally published in *JNM*. Hofman MS, Eu P, Jackson P, Hong E, Binns D, Iravani A, Murphy D, Mitchell C, Siva S, Hicks RJ, Young JD, Blower PJ, Mullen GE. Cold Kit for Prostate-Specific Membrane Antigen (PSMA) PET Imaging: Phase 1 Study of ^{68}Ga -Tris(Hydroxypyridinone)-PSMA PET/CT in Patients with Prostate Cancer. *J Nucl Med*. 2018;59:625-631. © SNMMI.¹⁸⁸

5.1.3.2. Imaging with zirconium-89

The larger radius of Zr^{4+} renders hexadentate ligands less suitable for tight chelation, although both DFO (**1**) and tripodal 3,4-HOPOs have been investigated.^{152,192,193} The stability of these complexes under *in vivo* conditions is not sufficient for imaging. As indicated in Section 4.4 tetrapodal 3,2-HOPOs and 1,2-HOPOs also lack sufficient *in vivo* stability.^{101,192} However the tetrapodal 3,4-HOPO (**40**) does largely overcome these problems.¹⁰⁵ There is quantitative labelling within 10 min at room temperature.¹⁰⁵ The ^{89}Zr -complex was found to be stable for over one week in human blood serum, stable *in vivo* and was excreted rapidly *via* urine in mice. Thus this structure would appear to be ideal for conjugation to targeting long-life circulating vectors such as immunoglobulins.

5.1.4. Magnetic Resonance Imaging (MRI)

As outlined in Section 4.5, complexes of Gd^{3+} behave as high-relaxivity MRI contrast agents. In particular a range of hexadentate HOPOs have been investigated in order to optimise solubility, proton relaxation and *in vivo* toxicity.^{83,194} However, to date none of these complexes have been introduced into regular clinical use, as further optimisation is required. Incorporation into dendrimers or attachment to macromolecules will slow molecular tumbling and this will increase rotational correlation times.¹⁹⁴ Such studies are currently in progress.

5.2. Therapeutic applications

5.2.1. Treatment of systemic metal overload

Poisoning due to metal overload can result from a number of causes; excess of metal that is essential for life (e.g. iron), excess of metal that is not essential for life (e.g. aluminum) and presence of a metal that is radioactive (e.g. plutonium).¹⁹⁵ HOPOs have been used therapeutically to remove excess metals belonging to each of these groups.

5.2.1.1. Iron chelation

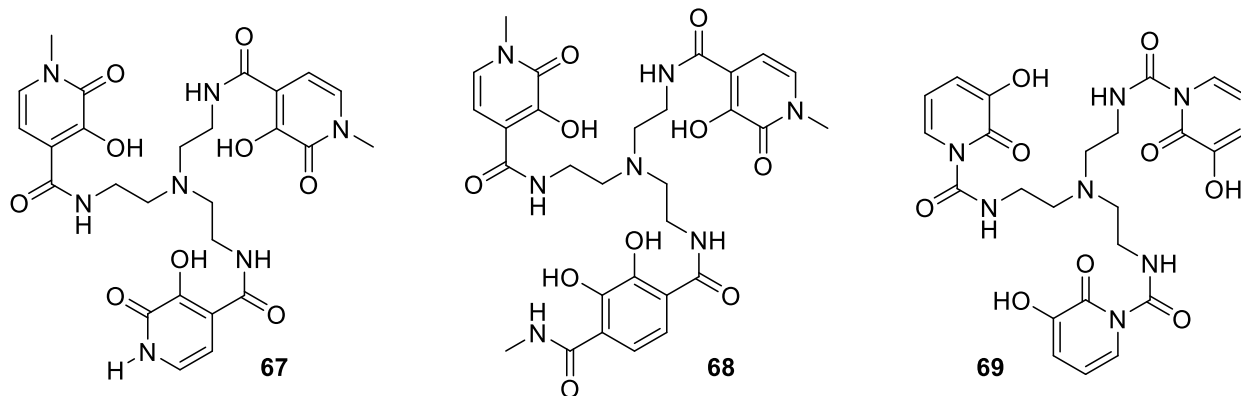
The therapeutic chelation of iron in iron-overload patients is comparatively wide spread, as regular blood transfusion which leads to iron overload, is an essential treatment for some diseases. This is an example of systemic iron overload and is associated with the treatment of thalassemia major, sickle cell disease and myelodysplastic syndromes.¹⁴¹ For many years DFO (**1**) was the only iron chelator in clinical use, but it suffered from the lack of oral activity and had to be administered parenterally. However a bidentate 3,4-HOPO was introduced in the clinic in 1987,¹⁹⁶ namely deferiprone (**3**) and since that time has been widely used to treat iron overload throughout the world.¹⁹⁶⁻²⁰¹ The molecular weight of the iron(III) complex of deferiprone (**3**) is less than 500 and by virtue of its non-charged nature, it can readily diffuse through membranes. Thus deferiprone can enter cells by simple diffusion, chelate labile iron and subsequently efflux from the cell (Figure 22). Deferiprone complies with Lipinski's guidelines¹⁷¹ and possesses neutral ligand and iron-complex forms at pH 7.4 (Figures 1 and 7). Bidentate 3-hydroxypyridin-4-ones possess distribution coefficients ($\log D_{\text{ligand}}$) which are largely dependent on the *N*-substituent. A biphasic linear relationship between the $\log D$ values of ligand and the corresponding iron-complex for 3-hydroxypyridin-4-ones exists (Figure 23) – ligands with $\log D_{\text{ligand}}$ greater than or equal to -1 possess a different linear

relationship (eq(1)) from those with $\log D_{\text{ligand}}$ less than -1 (eq(2)). These relationships can be used for ligand design in order to estimate the hydrophilicity of the iron complexes of bidentate and hexadentate analogues.

$$\log D_{\text{complex}} = 2.53 \log D_{\text{ligand}} - 0.08 \quad (1)$$

$$\log D_{\text{complex}} = 0.49 \log D_{\text{ligand}} - 2.45 \quad (2)$$

Hexadentate HOPOs have also been utilised for the scavenging and removal of toxic iron for instance **67**⁴³, **68**²⁰² and **69**.⁹¹ None of these hexadentate ligands have entered clinical trials.



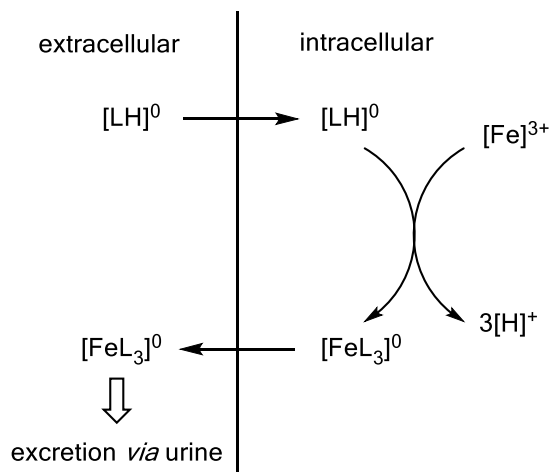


Figure 22. Mode of scavenging intracellular iron by 3,4-HOPOs, for example deferiprone (**3**).

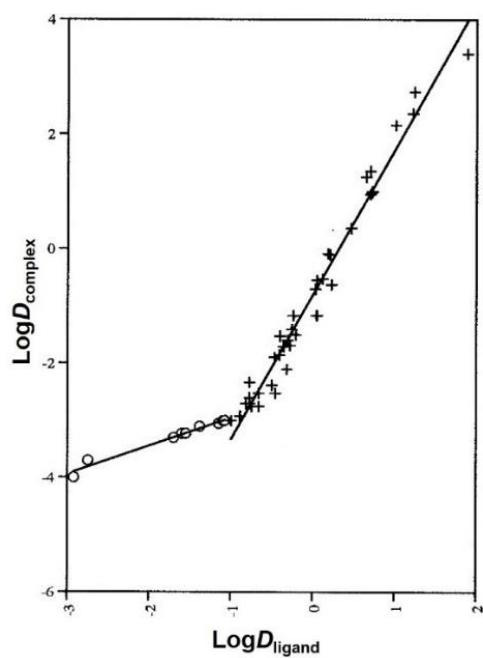


Figure 23. Relationship between the $\log D$ value of Fe complex ($\log D_{\text{complex}}$) and $\log D$ value of 3,4-HOPO ($\log D_{\text{ligand}}$). Distribution coefficients were determined using a MOPS buffer (50 mM, pH 7.4)/octanol system. Reproduced with permission from Ref.³⁸.

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A range of neurodegenerative diseases are associated with the local accumulation of iron in different regions of the brain for instance, Alzheimer's disease,²⁰³ Parkinson's disease,^{204,205} multiple sclerosis,²⁰⁶ and pantothenate kinase-associated neurodegeneration (PKAN).²⁰⁷ Macular degeneration is associated with iron accumulation in retinal tissue.²⁰⁸ Post-mortem examination of the relevant tissue confirms the presence of elevated levels of iron, and also associated oxidative damage to lipids and proteins. Undoubtedly, this iron-induced redox damage to the tissue contributes to the progression of these diseases. Many bidentate 3-hydroxypyridin-4-one derivatives have been developed for the potential treatment of neurodegeneration.²⁰⁹ However, among the three iron chelators currently approved for clinical use, deferiprone is the only one that can readily cross the blood brain barrier.^{210,211} Consequently, deferiprone has been investigated in animal models of the various diseases and in some clinical studies. With Parkinson's disease, both animal models^{212,213} and clinical investigations have been centred on deferiprone.^{214,215} There is currently a Phase II clinical trial taking place in different European centres. Deferiprone has also been administered to Friedreich's ataxia patients with the treatment leading to improved clinical scores.^{216,217}

5.2.1.2. Aluminum chelation

The toxicity of aluminum is associated with anaemia, osteomalacia and some neurological disorders. Aluminum disrupts the homeostasis of the essential metals, iron and calcium. Bidentate HOPOs have been found to be effective at removing

aluminum,¹⁴⁹ as have tetradentate and hexadentate HOPOs (Figure 16). However to date none of these compounds have been used clinically.

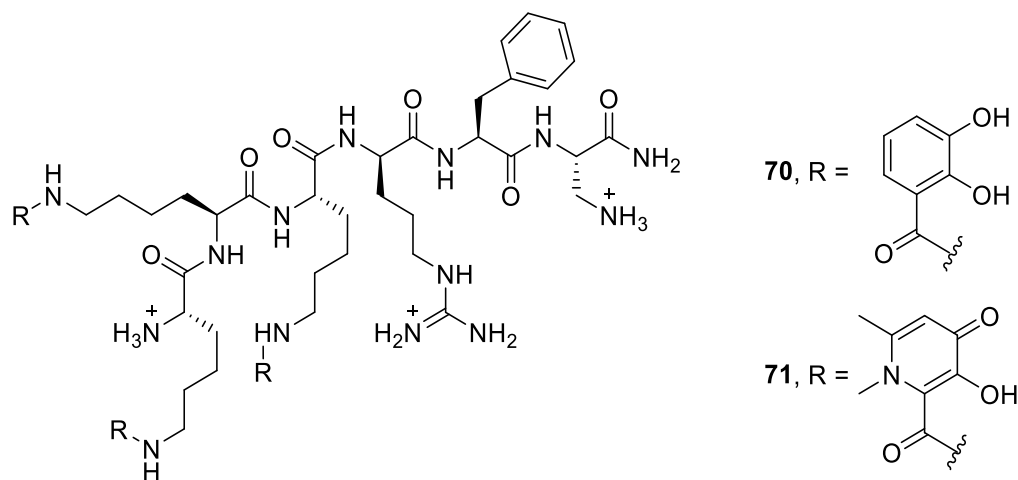
5.2.1.3. Chelation of plutonium and other actinides

The use of actinides in industry and defense has resulted in potential environmental and health issues as a large range of toxic radionucleotides, including plutonium and uranium, are being continuously generated. Controlled processing and the disposal of waste from the nuclear fuel cycle present many problems, as does nuclear weapon testing and potential terrorist use. By virtue of the similarities with ferric iron, plutonium is readily absorbed and distributed within living tissue by virtue of binding to iron transport proteins. It is retained in the liver, kidneys and bone.¹⁹⁵ A large range of chelators have been investigated for the potential of scavenging plutonium (and related actinides),¹⁶⁰ many of them being hydroxypyridinones (over 40 HOPOs are listed in ref¹⁶⁰). As with iron chelators, the aim is to design orally active, non-toxic chelators with a powerful scavenging activity. The octadentate ligand (**48**), a tetra 1,2-HOPO, is the most likely HOPO chelator to be used for this purpose, clinically. It has successfully completed safety and efficacy tests in three animal species and has been approved by FDA for first-in-human studies. Indeed, HOPOs can remove, not only plutonium, but also a range of different actinides from biological tissues. Compound **48** is particularly effective for the removal of plutonium and thorium, compound **47** for americium and compound **46** for neptunium,¹⁶⁰ thus there is the possibility of identifying ligand mixtures for particular accident situations.

5.2.2. Subcellular targeting of chelators

It is now possible to direct iron chelators to both mitochondrial and lysosomal compartments (Section 5.1.1.2). At the present time these are utilised as fluorescent probes for the quantification of intracellular pools of labile iron,^{124,125} but in principle they can also be used as therapeutic agents. Both a tricatechol hexadentate ligand (**70**)²¹⁸ and its tri 3,4-HOPO-analogue (**71**) have been demonstrated to provide high photoprotection against solar ultraviolet A radiation²¹⁸ of fibroblasts and thus could be a useful component of sunscreen formulations.

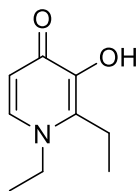
Some diseases, for instance Friedreich's ataxia lead to accumulation of iron specifically in the mitochondria.²¹⁶ This genetic disease is fatal. However it has been demonstrated that **71** is capable of removing elevated levels of iron from such mitochondria in FEK4 cell lines.



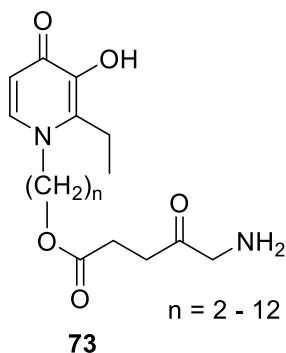
5.2.3. Photodynamic therapy

Photodynamic therapy (PDT) is based on the activation of exogenously applied or endogenously formed photosensitizers by visible light in the presence of molecular oxygen. This process leads to the formation of singlet oxygen, which is a powerful oxidant, leading to the damage of subcellular organelles and cell death.^{219,220} 5-

Aminolevulinic acid photodynamic therapy utilises the haem biosynthesis pathway to transiently produce elevated amounts of the natural endogenous photosensitizer, protoporphyrin IX. This is achieved by the addition of exogenous 5-aminolevulinic acid which enters the haem biosynthesis pathway.²²¹ The stage following protoporphyrin IX production is the insertion of iron(II) which is catalysed by ferrochelatase, thereby converting protoporphyrin XI into haem. Chelation of the intracellular labile iron pool leads to lower levels of ferrochelatase, further enhancing the accumulation of protoporphyrin IX. The bidentate 3,4-HOPO (CP94, **72**) has proved to be particularly effective in this role.²²² Developing this concept further, a series of prodrugs of 5-aminolevulinic acid (**73**) have been synthesised by conjugating 5-aminolevulinic acid to 3-hydroxypyridin-4-ones.²²³ These compounds have the additional advantage of rendering the extremely hydrophilic 5-aminolevulinic more lipophilic and hence capable of penetrating membranes. Once inside the cell these prodrugs are rapidly hydrolysed by cytosolic esterases to yield 5-aminolevulinic acid and a 3,4-HOPO which is capable of inhibiting ferrochelatase. The most effective compound for enhancing protoporphyrin XI levels in KB, MCF-7 and MCF-7R cells is compound **73** (n= 10).²²³ This compound is also more effective than the prodrugs of 5-aminolevulinic acid that are currently in clinical use.²²³



CP94, **72**



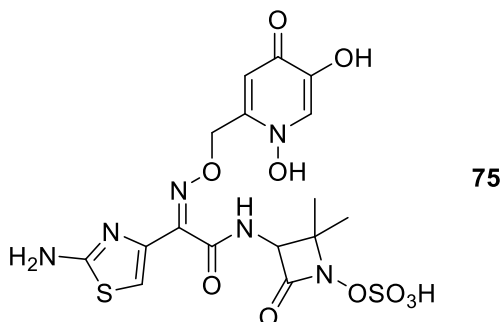
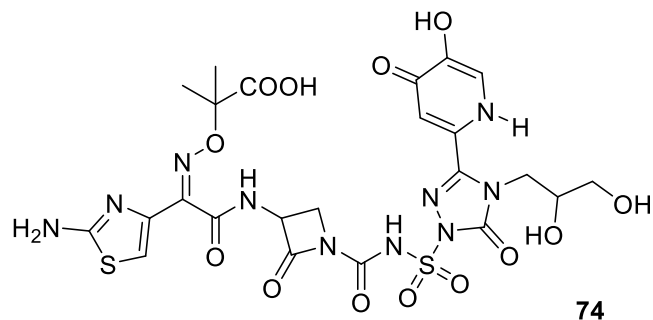
73

5.2.4. Antimicrobial applications

There are two major approaches for the design of chelator-based antimicrobial agents; one is centred on the exploitation of bacterial iron acquisition by the use of siderophore conjugates and the second is the deprivation of iron by competition with a powerful iron chelator.

5.2.4.1. The application of siderophore conjugates

One of the most widespread mechanisms for iron uptake by bacteria and fungi is the use of high affinity iron(III) chelators called siderophores.⁶ Siderophores are secreted in the environment where they scavenge iron. The resulting complexes are accumulated by the microorganism using an active transport system.²⁵ Most synthetic siderophore conjugates utilise hydroxamates or catechols,²³ although there are some that utilise 3,4-HOPOs. Thus the monobactam MC1 (**74**) has excellent activity against Gram-negative bacteria including *Pseudomonas aeruginosa* and offers superior protection in an *in vivo* model of respiratory tract infection.²²⁴ In similar fashion, BAL 30072 (**75**) possesses potent activity against multidrug-resistant *P. aeruginosa* and *Acinetobacter* sp., including many carbapenem – resistant strains.²²⁴



5.2.4.2. Iron scavenging action

Siderophore mediated transport of iron to microorganisms can be inhibited by the presence of chelators that possess a higher affinity for iron(III) than the endogenous siderophores. An essential requirement of this mechanism is that the competing chelator has no affinity for the iron-siderophore complex transporter. Indeed it would appear that many such hexadentate 3,4-HOPOs lack this property.²²⁵ Indeed hexadentate 3-hydroxypyridin-4-ones effectively inhibit the growth of both Gram-positive and Gram-negative bacteria, as well as methicillin resistant *Staphylococcus aureus* (MRSA).^{95,96,226-228} The clinical potential of the combination of 3,4-HOPOs with antibiotics has also been reported.¹⁴³

Chelators with strong antimicrobial activity, especially dendrimers²²⁹ and polymers¹¹⁹ have potential for use as external antimicrobial agents. For instance, they could find application in the treatment of wound healing, especially with ulcers in the elderly. They may also find application in cosmetics, such as shampoos and anti-perspirants. For these purposes, macromolecular iron chelators, such as dendrimers and polymers, are assumed to have more promising potential due to their non-absorbable nature *via* skin, leading to low toxicity.¹¹⁹

6. Conclusions

Over the past twenty years hydroxypyridinones have emerged as important ligands for a wide range of metals. Each of the three types of metal-chelating hydroxypyridinone, namely 1-hydroxypyridin-2-one (1,2-HOPO), 3-hydroxypyridin-2-one (3,2-HOPO) and 3-hydroxypyridin-4-one (3,4-HOPO), have found application in one or more of the following areas; analytical chemistry, environmental chemistry, diagnostic medicine and therapeutic medicine. The pyridinone ring is easily synthesised and readily converted into tetradentate, hexadentate and octadentate chelators. There is considerable potential for the control of the stereochemistry of the resulting metal complex and hence the properties of these multidentate molecules.

Originally the bidentate hydroxypyridinones were investigated for their potential as orally active iron chelators⁸ and indeed one of them, deferiprone (**3**), a 3,4-HOPO, is currently used world-wide as an therapeutic agent.²⁰¹ The upsurge of interest in the association of the inappropriate accumulation of metals in the brain (particularly iron) with a range of neurodegenerative diseases has led to the design of novel chelators based on the

HOPO structure; already deferiprone (**3**) is in phase 2 clinical trials for the treatment of Parkinson's disease.²¹⁴

It has gradually emerged that oligomeric hydroxypyridinones have considerable potential as aluminum, gallium, zirconium, gadolinium, europium and plutonium chelators. This diversity of metals which are bound tightly to hydroxypyridinones, has led to many applications. Thus HOPO's have found a role not only in scavenging iron, but also in scavenging aluminum and plutonium.²³⁰ Indeed, HOPOs can remove a range of different actinides from biological tissue and this finding has led to the possibility of identifying ligand mixtures for particular accident situations.¹⁶⁰ There is a requirement for improved knowledge in this area, so that we will be better able to treat persons exposed to multiple actinides.

Preformed HOPO complexes of gallium-68 and zirconium-89 have potential as positron emission tomography (PET) probes. Indeed successful clinical trials are currently in progress with ⁶⁸Ga-THP-PSMA (Galliprost[®], **66**) being used to monitor patients with prostate cancer.¹⁸⁸ This progress is likely to lead to the diagnosis of other cancers, for instance breast and pancreas. Furthermore, complexes of gallium-67, by virtue of the production of four gamma photons on decay, have the potential for cancer therapy.

Preformed complexes of europium have found application in high-throughput screening and preformed complexes of gadolinium have potential for improving the signal to noise ratio in magnetic resonance imaging (MRI). The coupling of fluorescent moieties to bidentate 3,4-HOPOs has led to the creation of iron-sensitive fluorescent probes which have been utilised to monitor the labile iron pool in the cytosol, lysosome and mitochondria.¹⁷³ Attachment of fluorescent labelled HOPOs to polymeric beads has

facilitated the quantification of toxic non transferrin-bound iron in thalassemia patients. Preliminary investigations of dendrimeric and polymeric HOPO's have indicated their potential for environmental, cosmetic and clinical applications.

These many new discoveries have demonstrated the tremendous potential of hydroxypyridinones as ligands for a relatively large number of hard metals. Our aim in preparing this review is to assist researchers in identifying suitable synthetic routes to both simple bidentate and multidentate HOPOs and to indicate the potential of these molecules in the fields of medicine, analytical science and environmental science.

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8. Abbreviations

AHMP = 1-(β -acrylamidoethyl)-3-hydroxy-2-methyl-pyridin-4-one

AIBN = azobisisobutyronitrile

ANPD = 3-(2-aminoethyl)-3-nitro-1,5-pentanediamine

C2A = C2A domain of complement protein C2

CFB = chelating fluorescent bead

CPBD = 2-cyano-2-propyl benzodithioate

DCC = *N,N'*-dicyclohexylcarbodiimide

DCM = dichloromethane

DFO = desferrioxamine

DIC = 1,3-diisopropylcarbodiimide

DMAA = dimethyl acetamide

DMF = dimethyl formamide

DIPEA = *N,N*-diisopropylethylamine

DTPA = diethylenetriaminepentaacetic acid

DU145 = Duke University 145, human prostate cancer cell line

EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide

EDTA = ethylenediaminetetraacetic acid (usually as $\text{CaNa}_2\text{-EDTA}$)

EBA = *N,N*-ethylene-*bis*-acrylamide

EGDMA = ethylene glycol dimethylacrylate

FACS = fluorescence-activated cell sorting, a specialized type of flow cytometry

FEK4 = human dermal fibroblast cell line

Fl = fluorophore

Fmoc = fluorenylmethyloxycarbonyl

GMA = glycidyl methacrylate

HATU = 1-*[bis(dimethylamino)methylene]*-1H-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate

HEA = 2-hydroxyethyl acrylate

HEMA = hydroxyethylmethacrylate

HOBt = hydroxybenzotriazole

HOPO = hydroxypyridinone

1,2-HOPO = 1-hydroxy-2-pyridinone

3,2-HOPO = 3-hydroxy-2-pyridinone

3,4-HOPO = 3-hydroxy-4-pyridinone

KEMP = cis,cis-1,3,5-triamino-cis,cis-1,3,5-trimethylcyclohexane

LDA = lithium diisopropylamide

log D = distribution coefficient of a molecule between buffer (i.e. specific pH) and lipophilic phases (e.g. *n*-octanol)

LTMP = lithium tetramethylpiperidide

mCPBA = *meta*-chloroperoxybenzoic acid

MRI = magnetic resonance imaging

MRSA = methicillin-resistant *Staphylococcus aureus*

NHS = *N*-hydroxysuccinimide

NMM = *N*-methylmorpholine

NTBI = non-transferrin-bound serum iron

L-Orn = L-ornithine

PAMAM = polyamidoamine

PDI = polydispersity index

PDT = photodynamic therapy

PGMA = poly(glycidyl methacrylate)

PKAN = pantothenate kinase-associated neurodegeneration

PPA = polyphosphoric acid

PSMA = prostate-specific membrane antigen

PyOxP = O-[(1-cyano-2-ethoxy-2-oxoethylidene)amino]-oxytri(pyrrolidin-1-yl)
phosphonium hexafluorophosphate

RAFT = reversible addition-fragmentation chain transfer

SPPS = solid-phase peptide synthesis

TATE = tyrosine-3-octreotate

TBTU = 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylammonium tetrafluoroborate

THP = hexadentate *tris*(3-hydroxypyridin-4-one) ligand

TMDM = *N,N,N',N'*-tetramethyldiaminomethane

TR-LRET = time-resolved luminescence resonance energy transfer

TRAM = 1,3,5-*tris*(aminomethyl)benzene

TREN = *tris*(2-aminoethyl)amine

TsOH = *para*-toluenesulfonic acid (tosylic acid)

9. References

- (1) Crichton, R. *Iron metabolism: from molecular mechanisms to clinical consequences*; John Wiley & Sons: London, 2016.
- (2) Halliwell, B.; Gutteridge, J. M. *Free radicals in biology and medicine*; Oxford University Press: Oxford, 2015.
- (3) Hershko, C.; Konijn, A. M.; Link, G. Iron chelators for thalassaemia. *Br. J. Haematol.* **1998**, *101*, 399-406.
- (4) Pearson, R. G. Hard and soft acids and bases. *J. Am. Chem. Soc.* **1963**, *85*, 3533-&.
- (5) Harris, D. C.; Aisen, P. Facilitation of Fe (II) antoxidation by Fe (III) complexing agents. *Biochim. Biophys. Acta* **1973**, *329*, 156-158.
- (6) Hider, R. C.; Kong, X. Chemistry and biology of siderophores. *Nat. Prod. Rep.* **2010**, *27*, 637-657.

- (7) Fagerholm, U.; Nilsson, D.; Knutson, L.; Lennernas, H. Jejunal permeability in humans in vivo and rats in situ: investigation of molecular size selectivity and solvent drag. *Acta Physiol. Scand.* **1999**, *165*, 315-324.
- (8) Hider, R.; Kontoghiorghes, G.; Silver, J. Patent GB-2118176, 1982; *Brit. UK Pat. Appl.* **1983**, 13.
- (9) Avdeef, A.; Sofen, S. R.; Bregante, T. L.; Raymond, K. N. Coordination chemistry of microbial iron transport compounds. 9. Stability-constants for catechol models of enterobactin. *J. Am. Chem. Soc.* **1978**, *100*, 5362-5370.
- (10) Hider, R. C.; Ma, Y. M. *The properties of therapeutically useful iron chelators*. In *Iron Physiology and Pathophysiology in Humans*; Springer, 2012; 567-590.
- (11) Liu, Z. D.; Hider, R. C. Design of iron chelators with therapeutic application. *Coord. Chem. Rev.* **2002**, *232*, 151-171.
- (12) Hider, R. C.; Mohd-Nor, A. R.; Silver, J.; Morrison, I. E.; Rees, L. V. Model compounds for microbial iron-transport compounds. Part 1. Solution chemistry and Mössbauer study of iron (II) and iron (III) complexes from phenolic and catecholic systems. *J. Chem. Soc., Dalton Trans.* **1981**, 609-622.
- (13) Zhou, T.; Ma, Y.; Kong, X.; Hider, R. C. Design of iron chelators with therapeutic application. *Dalton Trans.* **2012**, *41*, 6371-6389.
- (14) Grady, R. W.; Graziano, J. H.; White, G. P.; Jacobs, A.; Cerami, A. The development of new iron-chelating drugs. II. *J. Pharmacol. Exp. Ther.* **1978**, *205*, 575-565.
- (15) Silva, A. M.; Kong, X.; Hider, R. C. Determination of the pKa value of the hydroxyl group in the alpha-hydroxycarboxylates citrate, malate and lactate by ¹³C NMR: implications for metal coordination in biological systems. *Biometals* **2009**, *22*, 771-778.
- (16) Thielen, A.; Winkelmann, G. Rhizoferrin: a complexone type siderophore of the morales and entomophthorales (Zygomycetes). *FEMS Microbiol. Lett.* **1992**, *94*, 37-41.
- (17) Liu, Z. D.; Hider, R. C. Design of clinically useful iron(III)-selective chelators. *Med. Res. Rev.* **2002**, *22*, 26-64.
- (18) Nurchi, V. M.; Crisponi, G.; Pivetta, T.; Donatoni, M.; Remelli, M. Potentiometric, spectrophotometric and calorimetric study on iron(III) and copper(II) complexes with 1,2-dimethyl-3-hydroxy-4-pyridinone. *J. Inorg. Biochem.* **2008**, *102*, 684-692.
- (19) Clarke, E. T.; Martell, A. E.; Reibenspies, J. Crystal-structure of the tris 1,2-dimethyl-3-hydroxy-4-pyridinone (Dmhp) complex with the Fe(III) ion. *Inorg. Chim. Acta* **1992**, *196*, 177-183.
- (20) Merkofer, M.; Kissner, R.; Hider, R. C.; Koppenol, W. H. Redox properties of the iron complexes of orally active iron chelators CP20, CP502, CP509, and ICL670. *Helv. Chim. Acta* **2004**, *87*, 3021-3034.
- (21) Harris, W. R.; Carrano, C. J.; Cooper, S. R.; Sofen, S. R.; Avdeef, A. E.; McArdle, J. V.; Raymond, K. N. Coordination chemistry of microbial iron transport compounds. 19. Stability constants and electrochemical behavior of ferric enterobactin and model complexes. *Journal of the American Chemical Society* **1979**, *101*, 6097-6104.
- (22) Bergeron, R. J. Synthesis and solution structure of microbial siderophores. *Chem. Rev.* **1984**, *84*, 587-602.

- (23) Miller, M. J.; Zhu, H.; Xu, Y.; Wu, C.; Walz, A. J.; Vergne, A.; Roosenberg, J. M.; Moraski, G.; Minnick, A. A.; McKee-Dolence, J. et al. Utilization of microbial iron assimilation processes for the development of new antibiotics and inspiration for the design of new anticancer agents. *Biometals* **2009**, *22*, 61-75.
- (24) Raymond, K. N.; Müller, G.; Matzanke, B. F. *Complexation of iron by siderophores a review of their solution and structural chemistry and biological function*. In *Structural Chemistry. Topics in Current Chemistry*; Springer: Berlin, Heidelberg, 1984; Vol. 123; p 49-102.
- (25) Ji, C.; Juarez-Hernandez, R. E.; Miller, M. J. Exploiting bacterial iron acquisition: siderophore conjugates. *Future Med. Chem.* **2012**, *4*, 297-313.
- (26) Armit, J. W.; Nolan, T. J. CCCCXIX.—Derivatives of kojic acid. *J. Chem. Soc.* **1931**, 3023-3031.
- (27) Kleipool, R.; Wibaut, J. The preparation of some 3-hydroxy-4-pyridones substituted on the nitrogen atom. *Recl. Trav. Chim. Pays-Bas* **1950**, *69*, 1041-1047.
- (28) Heyns, K.; Vogelsang, G. Über γ -pyrone und γ -pyridone, I. Mitteil.: zur konstitution der oxy- γ -pyrone und oxy- γ -pyridone. *Chem. Ber.* **1954**, *87*, 13-18.
- (29) Berson, J. A.; Jones, W. M.; Ocallaghan, L. F. Spectra as a guide to structure in the hydroxypyrene and hydroxypyridone series. *J. Am. Chem. Soc.* **1956**, *78*, 622-623.
- (30) Adams, R.; Jones, V. V.; Johnson, J. L. The structure of leucenol. IV. *J. Am. Chem. Soc.* **1947**, *69*, 1810-1812.
- (31) Harris, R. L. N. Potential wool growth-inhibitors - improved syntheses of mimosine and related 4(1h)-pyridones. *Aust. J. Chem.* **1976**, *29*, 1329-1334.
- (32) Spenser, I. D.; Notation, A. D. A synthesis of mimosine. *Can. J. Chem.* **1962**, *40*, 1374-&.
- (33) Yasue, M.; Kawamura, N.; Sakakiba, J. Synthesis of N-substituted-3-glucosyloxy-2-methyl-4-pyridones and their aglycones. *Yakugaku Zasshi* **1970**, *90*, 1222-&.
- (34) Jones, M. M.; Singh, P. K.; Lane, J. E.; Rodrigues, R. R.; Nasset, A.; Suarez, C. C.; Bogitsh, B. J.; Carter, C. E. Inhibition of *Trypanosoma cruzi* epimastigotes in vitro by iron chelating agents. *Arzneimittelforschung* **1996**, *46*, 1158-1162.
- (35) Singh, P. K.; Jones, M. M.; Lane, J. E.; Nasset, A.; Zimmerman, L. J.; RibeiroRodrigues, R.; Richter, A.; Stenger, M. R.; Carter, C. E. Synthesis and in vitro trypanocidal activity of some novel iron chelating agents. *Arzneimittelforschung* **1997**, *47*, 311-315.
- (36) Dobbin, P. S.; Hider, R. C.; Hall, A. D.; Taylor, P. D.; Sarpong, P.; Porter, J. B.; Xiao, G.; van der Helm, D. Synthesis, physicochemical properties, and biological evaluation of N-substituted 2-alkyl-3-hydroxy-4(1H)-pyridinones: orally active iron chelators with clinical potential. *J. Med. Chem.* **1993**, *36*, 2448-2458.
- (37) Jakopčić, K.; Mintas, M.; Isufi, I. Preparation of 3-hydroxy-and 3-methoxy-N-heteroaryl-2-methyl-4-pyridones. *J. Heterocycl. Chem.* **1993**, *30*, 429-433.
- (38) Rai, B. L.; Dekhordi, L. S.; Khodr, H.; Jin, Y.; Liu, Z.; Hider, R. C. Synthesis, physicochemical properties, and evaluation of N-substituted-2-alkyl-3-hydroxy-4(1H)-pyridinones. *J. Med. Chem.* **1998**, *41*, 3347-3359.
- (39) Zbinden, P. EU Patent EP0768302A2, 1997; *Eur. Pat. Appl.* **1997**, 41.

- (40) Tam, T. F.; Spino, M.; Li, W.; Wang, Y.; Zhao, Y.; Shah, B. H. Patent WO2005049609 A1, 2008; *PCT Int. Appl.* **2005**, 77.
- (41) Hider, R. C., Liu, Z.D., Yu, Y. And Li, Z. EU Patent EP2692724 A1, 2011; *PCT Int. Appl.* **2012**, 33.
- (42) Liu, S. EU Patent EP1474396B9, 2004; *PCT Int. Appl.* **2003**, 52.
- (43) Bartulin, J.; Belmar, J.; Gallardo, H.; Leon, G. Syntheses of 2-acetyl-3-hydroxy-1-n-propylpyrrole from isomaltol and 1-n-alkyl-3-hydroxy-2-methyl-4-pyridones from maltol. *J. Heterocycl. Chem.* **1992**, 29, 1017-1019.
- (44) Barral, K.; Balzarini, J.; Neyts, J.; De Clercq, E.; Hider, R. C.; Camplo, M. Synthesis and antiviral evaluation of cyclic and acyclic 2-methyl-3-hydroxy-4-pyridinone nucleoside derivatives. *J. Med. Chem.* **2006**, 49, 43-50.
- (45) Barral, K.; Hider, R. C.; Balzarini, J.; Neyts, J.; De Clercq, E.; Camplo, M. Synthesis and antiviral evaluation of 3-hydroxy-2-methylpyridin-4-one dideoxynucleoside derivatives. *Bioorg. Med. Chem. Lett.* **2003**, 13, 4371-4374.
- (46) Liu, G.; Bruenger, F. W.; Barrios, A. M.; Miller, S. C. Synthesis of 2-alkyl-3-hydroxy-4-pyridinone-ribonucleosides, potential oral iron chelators. *Nucleosides Nucleotides Nucleic Acids* **1995**, 14, 1901-1904.
- (47) Patel, M. K.; Fox, R.; Taylor, P. D. Directed aminomethylation of 3-hydroxy-2(1H)-pyridinones and 3-hydroxy-4(1H)-pyridinones: Synthesis of iso-deferiprone. *Tetrahedron* **1996**, 52, 1835-1840.
- (48) Aytemir, M. D.; Calis, U.; Ozalp, M. Synthesis and evaluation of anticonvulsant and antimicrobial activities of 3-hydroxy-6-methyl-2-substituted 4h-pyran-4-one derivatives. *Arch. Pharm.* **2004**, 337, 281-288.
- (49) Liu, Z. D.; Khodr, H. H.; Liu, D. Y.; Lu, S. L.; Hider, R. C. Synthesis, physicochemical characterization, and biological evaluation of 2-(1'-hydroxyalkyl)-3-hydroxypyridin-4-ones: novel iron chelators with enhanced pFe³⁺ values. *J. Med. Chem.* **1999**, 42, 4814-4823.
- (50) Piyamongkol, S.; Liu, Z. D.; Hider, R. C. Novel synthetic approach to 2-(1'-hydroxyalkyl)-and 2-amido-3-hydroxypyridin-4-ones. *Tetrahedron* **2001**, 57, 3479-3486.
- (51) Li, J.; Lu, Z.; Kong, X.; Ma, Y.; Zhang, X.; Bansal, S. S.; Abbate, V.; Hider, R. C. Design and synthesis of novel pegylated iron chelators with decreased metabolic rate. *Future Med. Chem.* **2015**, 7, 2439-2449.
- (52) Moridani, M. Y.; Tilbrook, G. S.; Khodr, H. H.; Hider, R. C. Synthesis and physicochemical assessment of novel 2-substituted 3-hydroxypyridin-4-ones, novel iron chelators. *J. Pharm. Pharmacol.* **2002**, 54, 349-364.
- (53) Liu, Z. D.; Piyamongkol, S.; Liu, D. Y.; Khodr, H. H.; Lu, S. L.; Hider, R. C. Synthesis of 2-amido-3-hydroxypyridin-4(1H)-ones: Novel iron chelators with enhanced pFe(3+) values. *Bioorg. Med. Chem.* **2001**, 9, 563-573.
- (54) Piyamongkol, S.; Ma, Y. M.; Kong, X. L.; Liu, Z. D.; Aytemir, M. D.; van der Helm, D.; Hider, R. C. Amido-3-hydroxypyridin-4-ones as Iron (III) Ligands. *Chem. Eur. J.* **2010**, 16, 6374-6381.
- (55) Fassihi, A.; Abedi, D.; Saghaie, L.; Sabet, R.; Fazeli, H.; Bostaki, G.; Deilami, O.; Sadinpour, H. Synthesis, antimicrobial evaluation and QSAR study of some 3-hydroxypyridine-4-one and 3-hydroxypyran-4-one derivatives. *Eur. J. Med. Chem.* **2009**, 44, 2145-2157.

- (56) Chen, Y. L.; Chen, J.; Ma, Y. M.; Hider, R. C. The synthesis of 5-functional 3-hydroxypyridin-4-ones and their impact on the chelating properties of the ligands. *Chem. Lett.* **2015**, *44*, 515-517.
- (57) Aytemir, M. D.; Hider, R. C.; Erol, D. D.; Ozalp, M.; Ekizoglu, M. Synthesis of new antimicrobial agents; Amide derivatives of pyranones and pyridinones. *Turkish J. Chem.* **2003**, *27*, 445-452.
- (58) Li, M. S.; Gu, L. Q.; Huang, Z. S.; Xiao, S. H.; Ma, L. Synthesis of 6-substituted-3-hydroxy-4-(1H)-pyridinones: Oxidation-Michael addition of 3-hydroxy-4(1H)-pyridinones. *Tetrahedron* **1999**, *55*, 2237-2244.
- (59) Ma, Y.; Roy, S.; Kong, X.; Chen, Y.; Liu, D.; Hider, R. C. Design and synthesis of fluorinated iron chelators for metabolic study and brain uptake. *J. Med. Chem.* **2012**, *55*, 2185-2195.
- (60) Ma, Y. M.; Hider, R. C. Design and synthesis of fluorine-substituted 3-hydroxypyridin-4-ones. *Tetrahedron Lett.* **2010**, *51*, 5230-5233.
- (61) Xie, Y.-Y.; Lu, Z.; Kong, X.-L.; Zhou, T.; Bansal, S.; Hider, R. Systematic comparison of the mono-, dimethyl- and trimethyl 3-hydroxy-4 (1H)-pyridones—Attempted optimization of the orally active iron chelator, deferiprone. *Eur. J. Med. Chem.* **2016**, *115*, 132-140.
- (62) Belmont, A.; Kwiatkowski, J. L. Deferiprone for the treatment of transfusional iron overload in thalassemia. *Expert. Rev. Hematol.* **2017**, *10*, 493-503.
- (63) Lott, W.; Shaw, E. Analogs of aspergillitic acid. II. Various antibacterial heterocyclic hydroxamic acids. *J. Am. Chem. Soc.* **1949**, *71*, 70-73.
- (64) Mizukami, S.; Hirai, L.; Morimoto, M. A new series of pyridine-1-oxides *Annu. Rep. Shionogi Res. Lab* **1966**, *16*, 29-36.
- (65) den Hertog, H. J.; van Ammers, M. The directive influence of the N-oxide group during the nitration of derivatives of pyridine-N-oxide (III). The nitration of 2- and 3-methoxypyridine-N-oxide. *Recl. Trav. Chim. Pays-Bas* **2010**, *74*, 1160-1166.
- (66) Finger, G.; Starr, L. D. Aromatic fluorine compounds. IX. 2-fluoropyridines. *J. Am. Chem. Soc.* **1959**, *81*, 2674-2675.
- (67) Talik, Z. O niektórych reakcjach N-tlenkow-2-chlorowco-4-nitropirydyn. *Rocz. Chem.* **1961**, *35*, 475-488.
- (68) Gilman, H.; Spatz, S. M. Pyridyllithium compounds. *J. Org. Chem.* **1951**, *16*, 1485-1494.
- (69) Scarrow, R. C.; Riley, P. E.; Abudari, K.; White, D. L.; Raymond, K. N. Ferric ion sequestering agents. 13. Synthesis, structures, and thermodynamics of complexation of cobalt(III) and iron(II) tris complexes of several chelating hydroxypyridinones. *Inorg. Chem.* **1985**, *24*, 954-967.
- (70) Lohaus, G.; Dittmar, W. U.S. Patent US05499988, 1978; *Ger. Offen.* **1973**, 17.
- (71) Möhrle, H.; Weber, H. Zur kenntnis der 1-methyl-3-hydroxypyridone-(2) und-(6): Über pyridone—II. *Tetrahedron* **1970**, *26*, 3779-3785.
- (72) Streater, M.; Taylor, P. D.; Hider, R. C.; Porter, J. Novel 3-hydroxy-2(1H)-pyridinones. Synthesis, iron(III)-chelating properties, and biological activity. *J. Med. Chem.* **1990**, *33*, 1749-1755.
- (73) Xu, J.; Kullgren, B.; Durbin, P. W.; Raymond, K. N. Specific sequestering agents for the actinides. 28. Synthesis and initial evaluation of multidentate 4-carbamoyl-

- 3-hydroxy-1-methyl-2(1H)-pyridinone ligands for in-vivo plutonium(IV) chelation. *J. Am. Chem. Soc.* **1995**, *38*, 2606-2614.
- (74) Carrano, C. J.; Raymond, K. N. Coordination chemistry of microbial iron transport compounds. 10. Characterization of the complexes of rhodotorulic acid, a dihydroxamate siderophore. *J. Am. Chem. Soc.* **1978**, *100*, 5371-5374.
- (75) Scarrow, R. C.; White, D. L.; Raymond, K. N. Ferric ion sequestering agents. 14. 1-Hydroxy-2 (1H)-pyridinone complexes: properties and structure of a novel iron-iron dimer. *J. Am. Chem. Soc.* **1985**, *107*, 6540-6546.
- (76) Moore, E. G.; Jocher, C. J.; Xu, J.; Werner, E. J.; Raymond, K. N. An octadentate luminescent Eu(III) 1,2-HOPO chelate with potent aqueous stability. *Inorg. Chem.* **2007**, *46*, 5468-5470.
- (77) D'Aléo, A.; Moore, E. G.; Szigethy, G.; Xu, J.; Raymond, K. N. Aryl bridged 1-hydroxypyridin-2-one: effect of the bridge on the Eu (III) sensitization process. *Inorg. Chem.* **2009**, *48*, 9316-9324.
- (78) Daumann, L. J.; Werther, P.; Ziegler, M. J.; Raymond, K. N. Siderophore inspired tetra- and octadentate antenna ligands for luminescent Eu(III) and Tb(III) complexes. *J. Inorg. Biochem.* **2016**, *162*, 263-273.
- (79) Xu, J.; Raymond, K. N. Uranyl sequestering agents: correlation of properties and efficacy with structure for UO_2^{2+} complexes of linear tetradentate 1-methyl-3-hydroxy-2 (1H)-pyridinone ligands. *Inorg. Chem.* **1999**, *38*, 308-315.
- (80) Szigethy, G.; Raymond, K. N. The influence of linker geometry in bis(3-hydroxy-N-methyl-pyridin-2-one) ligands on solution phase uranyl affinity. *Chem. Eur. J.* **2011**, *17*, 1818-1827.
- (81) Santos, M. A.; Gama, S.; Pessoa, J. C.; Oliveira, M. C.; Tóth, I.; Farkas, E. Complexation of molybdenum (VI) with bis (3-hydroxy-4-pyridinone) amino acid derivatives. *Eur. J. Inorg. Chem.* **2007**, 1728-1737.
- (82) Zhou, T.; Hider, R. C.; Kong, X. Mode of iron(III) chelation by hexadentate hydroxypyridinones. *Chem. Commun.* **2015**, *51*, 5614-5617.
- (83) Datta, A.; Raymond, K. N. Gd-hydroxypyridinone (HOPO)-based high-relaxivity magnetic resonance imaging (MRI) contrast agents. *Acc. Chem. Res.* **2009**, *42*, 938-947.
- (84) Werner, E. J.; Kozhukh, J.; Botta, M.; Moore, E. G.; Avedano, S.; Aime, S.; Raymond, K. N. 1, 2-Hydroxypyridonate/terephthalamide complexes of gadolinium (III): Synthesis, stability, relaxivity, and water exchange properties. *Inorg. Chem.* **2008**, *48*, 277-286.
- (85) Weizman, H.; Shanzer, A. 1,2-Hopobactin: a hydroxamate analog of enterobactin. *Chem. Comm.* **2000**, 2013-2014.
- (86) Seitz, M.; Pluth, M. D.; Raymond, K. N. 1,2-HOIQO--a highly versatile 1,2-HOPO analogue. *Inorg. Chem.* **2007**, *46*, 351-353.
- (87) Xu, J.; O'Sullivan, B.; Raymond, K. N. Hexadentate hydroxypyridonate iron chelators based on TREN-Me-3, 2-HOPO: variation of cap size. *Inorg. Chem.* **2002**, *41*, 6731-6742.
- (88) Werner, E. J.; Avedano, S.; Botta, M.; Hay, B. P.; Moore, E. G.; Aime, S.; Raymond, K. N. Highly soluble tris-hydroxypyridonate Gd(III) complexes with increased hydration number, fast water exchange, slow electronic relaxation, and high relaxivity. *J. Am. Chem. Soc.* **2007**, *129*, 1870-1871.

- (89) Meyer, M.; Telford, J. R.; Cohen, S. M.; White, D. J.; Xu, J.; Raymond, K. N. High-yield synthesis of the enterobactin trilactone and evaluation of derivative siderophore analogs. *J. Am. Chem. Soc.* **1997**, *119*, 10093-10103.
- (90) Harrington, J. M.; Chittamuru, S.; Dhungana, S.; Jacobs, H. K.; Gopalan, A. S.; Crumbliss, A. L. Synthesis and iron sequestration equilibria of novel exocyclic 3-hydroxy-2-pyridinone donor group siderophore mimics. *Inorg. Chem.* **2010**, *49*, 8208-8221.
- (91) Rai, B. L.; Khodr, H.; Hider, R. C. Synthesis, physico-chemical and iron(III)-chelating properties of novel hexadentate 3-hydroxy-2(1H)pyridinone ligands. *Tetrahedron* **1999**, *55*, 1129-1142.
- (92) Pailloux, S. L.; Nguyen, S.; Zhou, S.; Hom, M. E.; Keyser, M. N.; Smiles, D.; Raymond, K. N. Synthesis and chemical reactivity of a 6-Me-3,2-hydroxypyridinone dithiazolidine with primary amines: a route to new hexadentate chelators for hard metal(III) ions. *J. Heterocycl. Chem.* **2016**, *53*, 1065-1073.
- (93) Clarke Jurchen, K. M.; Raymond, K. N. Linear hexadentate ligands as iron chelators. *J. Coord. Chem.* **2005**, *58*, 55-80.
- (94) Piyamongkol, S.; Zhou, T.; Liu, Z. D.; Khodr, H. H.; Hider, R. C. Design and characterisation of novel hexadentate 3-hydroxypyridin-4-one ligands. *Tetrahedron Lett.* **2005**, *46*, 1333-1336.
- (95) Xu, B.; Kong, X. L.; Zhou, T.; Qiu, D. H.; Chen, Y. L.; Liu, M. S.; Yang, R. H.; Hider, R. C. Synthesis, iron(III)-binding affinity and in vitro evaluation of 3-hydroxypyridin-4-one hexadentate ligands as potential antimicrobial agents. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6376-6380.
- (96) Zhang, M. X.; Zhu, C. F.; Zhou, Y. J.; Kong, X. L.; Hider, R. C.; Zhou, T. Design, synthesis, and antimicrobial evaluation of hexadentate hydroxypyridinones with high iron(III) affinity. *Chem. Biol. Drug. Des.* **2014**, *84*, 659-668.
- (97) Grazina, R.; Gano, L.; Sebestik, J.; Amelia Santos, M. New tripodal hydroxypyridinone based chelating agents for Fe(III), Al(III) and Ga(III): Synthesis, physico-chemical properties and bioevaluation. *J. Inorg. Biochem.* **2009**, *103*, 262-273.
- (98) Chaves, S.; Capelo, A.; Areias, L.; Marques, S. M.; Gano, L.; Esteves, M. A.; Santos, M. A. A novel tripodal tris-hydroxypyrimidinone sequestering agent for trivalent hard metal ions: synthesis, complexation and in vivo studies. *Dalton Trans.* **2013**, *42*, 6033-6045.
- (99) Chaves, S.; Marques, S. M.; Matos, A. M.; Nunes, A.; Gano, L.; Tuccinardi, T.; Martinelli, A.; Santos, M. A. New tris(hydroxypyridinones) as iron and aluminium sequestering agents: synthesis, complexation and in vivo studies. *Chem. Eur. J.* **2010**, *16*, 10535-10545.
- (100) D'Aléo, A.; Moore, E. G.; Xu, J.; Daumann, L. J.; Raymond, K. N. Optimization of the sensitization process and stability of octadentate Eu (III) 1, 2-HOPO complexes. *Inorg. Chem.* **2015**, *54*, 6807-6820.
- (101) Deri, M. A.; Ponnala, S.; Zeglis, B. M.; Pohl, G.; Dannenberg, J.; Lewis, J. S.; Francesconi, L. C. Alternative chelator for ⁸⁹Zr radiopharmaceuticals: radiolabeling and evaluation of 3, 4, 3-(LI-1, 2-HOPO). *J. Med. Chem.* **2014**, *57*, 4849-4860.

- (102) Daumann, L. J.; Tatum, D. S.; Snyder, B. E.; Ni, C.; Law, G.-I.; Solomon, E. I.; Raymond, K. N. New insights into structure and luminescence of Eulll and Smlll complexes of the 3, 4, 3-LI (1, 2-HOPO) ligand. *J. Am. Chem. Soc.* **2015**, *137*, 2816-2819.
- (103) Hou, Z.; Whisenhunt Jr, D. W.; Xu, J.; Raymond, K. N. Potentiometric, spectrophotometric, and ¹H NMR study of four desferrioxamine B derivatives and their ferric complexes. *J. Am. Chem. Soc.* **1994**, *116*, 840-846.
- (104) Allott, L.; Da Pieve, C.; Meyers, J.; Spinks, T.; Ciobota, D. M.; Kramer-Marek, G.; Smith, G. Evaluation of DFO-HOPO as an octadentate chelator for zirconium-89. *Chem. Commun.* **2017**, *53*, 8529-8532.
- (105) Buchwalder, C.; Rodriguez-Rodriguez, C.; Schaffer, P.; Karagiozov, S. K.; Saatchi, K.; Hafeli, U. O. A new tetrapodal 3-hydroxy-4-pyridinone ligand for complexation of (89)zirconium for positron emission tomography (PET) imaging. *Dalton. Trans.* **2017**, *46*, 9654-9663.
- (106) Cohen, S. M.; Petoud, S.; Raymond, K. N. Synthesis and metal binding properties of salicylate-, catecholate-, and hydroxypyridinonate-functionalized dendrimers. *Chemistry* **2001**, *7*, 272-279.
- (107) Zhou, T.; Neubert, H.; Liu, D. Y.; Liu, Z. D.; Ma, Y. M.; Kong, X. L.; Luo, W.; Mark, S.; Hider, R. C. Iron binding dendrimers: a novel approach for the treatment of haemochromatosis. *J. Med. Chem.* **2006**, *49*, 4171-4182.
- (108) Zhou, T.; Liu, Z. D.; Neubert, H.; Kong, X. L.; Ma, Y. M.; Hider, R. C. High affinity iron(III) scavenging by a novel hexadentate 3-hydroxypyridin-4-one-based dendrimer: synthesis and characterization. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5007-5011.
- (109) O'Sullivan, B.; Xu, J.; Raymond, K. N. *New multidentate chelators for iron*. In *Iron Chelators: New Development Strategies*; Saratoga group, 2000; Vol. -; p 177-208.
- (110) Grazina, R.; Santos, M. A. New hydroxypyridinone-functionalized Sepharoses as sorbing agents for hard metal ions. *J. Hazard Mater.* **2011**, *186*, 1902-1908.
- (111) Feng, M.; Van Der Does, L.; Bantjes, A. Iron (III) chelating resins II. 3-Hydroxy-4 (1H)-pyridinones-Sepharose gels. *J. Biomater. Sci. Polym. Ed.* **1993**, *4*, 145-154.
- (112) Feng, M.; Van Der Does, L.; Bantjes, A. Iron (III) chelating resins. V. Cross-linked copolymers of 1-(β-acrylamidoethyl)-3-hydroxy-2-methyl-4 (1H) pyridinone (AHMP) and N, N-dimethylacrylamide (DMAA) for iron (III) chelation studies. *J. Appl. Polym. Sci.* **1994**, *52*, 21-28.
- (113) Feng, M.-H.; van der Does, L.; Bantjes, A. Iron (III) chelating resins IV. Crosslinked copolymer beads of 1-(β-acrylamidoethyl)-3-hydroxy-2-methyl-4 (1H)-pyridinone (AHMP) with 2-hydroxyethyl methacrylate (HEMA). *Eur. Polym. J.* **1994**, *30*, 941-947.
- (114) Li, J. P.; Olaleye, E. D.; Kong, X. L.; Zhou, T.; Ma, Y. M.; Jurach, J.; Al Rugaie, O.; Hider, R. C.; Zhang, G. Q.; Alsam, S. et al. Macromolecular iron-chelators via RAFT-polymerization for the inhibition of methicillin-resistant *Staphylococcus aureus* growth. *Polymer* **2016**, *87*, 64-72.
- (115) Moggia, F.; Fages, F.; Brisset, H.; Chaix, C.; Mandrand, B.; Levillain, E.; Roncali, J. Design, synthesis and electrochemical properties of a thiophene derivative

- functionalized with a siderophore-like chelator. *J. Electroanal. Chem.* **2009**, *626*, 42-46.
- (116) Harmatz, P.; Grady, R. W.; Dragsten, P.; Vichinsky, E.; Giardina, P.; Madden, J.; Jeng, M.; Miller, B.; Hanson, G.; Hedlund, B. Phase Ib clinical trial of starch-conjugated deferoxamine (40SD02): a novel long-acting iron chelator. *Br. J. Haematol.* **2007**, *138*, 374-381.
- (117) Hallaway, P. E.; Eaton, J. W.; Panter, S. S.; Hedlund, B. E. Modulation of deferoxamine toxicity and clearance by covalent attachment to biocompatible polymers. *Proc. Natl. Acad. Sci. U S A* **1989**, *86*, 10108-10112.
- (118) Zhou, T.; Kong, X. L.; Liu, Z. D.; Liu, D. Y.; Hider, R. C. Synthesis and iron(III)-chelating properties of novel 3-hydroxypyridin-4-one hexadentate ligand-containing copolymers. *Biomacromolecules* **2008**, *9*, 1372-1380.
- (119) Zhou, Y. J.; Kong, X. L.; Li, J. P.; Ma, Y. M.; Hider, R. C.; Zhou, T. Novel 3-hydroxypyridin-4-one hexadentate ligand-based polymeric iron chelator: synthesis, characterization and antimicrobial evaluation. *Medchemcomm* **2015**, *6*, 1620-1625.
- (120) Carter, K. P.; Young, A. M.; Palmer, A. E. Fluorescent sensors for measuring metal ions in living systems. *Chem. Rev.* **2014**, *114*, 4564-4601.
- (121) Que, E. L.; Domaille, D. W.; Chang, C. J. Metals in neurobiology: probing their chemistry and biology with molecular imaging. *Chem. Rev.* **2008**, *108*, 1517-1549.
- (122) Bennett, F. A.; Barlow, D. J.; Dadoo, A. N. O.; Hider, R. C.; Lansley, A. B.; Lawrence, M. J.; Marriott, C.; Bansal, S. S. L-(6,7-dimethoxy-4-coumaryl) alanine: an intrinsic probe for the labelling of peptides. *Tetrahedron Lett.* **1997**, *38*, 7449-7452.
- (123) Ma, Y.; Luo, W.; Quinn, P. J.; Liu, Z.; Hider, R. C. Design, synthesis, physicochemical properties, and evaluation of novel iron chelators with fluorescent sensors. *J. Med. Chem.* **2004**, *47*, 6349-6362.
- (124) Fakhri, S.; Podinovskaia, M.; Kong, X.; Collins, H. L.; Schaible, U. E.; Hider, R. C. Targeting the lysosome: fluorescent iron(III) chelators to selectively monitor endosomal/lysosomal labile iron pools. *J. Med. Chem.* **2008**, *51*, 4539-4552.
- (125) Abbate, V.; Reelfs, O.; Hider, R. C.; Pourzand, C. Design of novel fluorescent mitochondria-targeted peptides with iron-selective sensing activity. *Biochem. J.* **2015**, *469*, 357-366.
- (126) Telpoukhovskaia, M. A.; Cawthray, J. F.; Rodriguez-Rodriguez, C.; Scott, L. E.; Page, B. D.; Patrick, B. O.; Orvig, C. 3-Hydroxy-4-pyridinone derivatives designed for fluorescence studies to determine interaction with amyloid protein as well as cell permeability. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 3654-3657.
- (127) Ma, Y.; Luo, W.; Camplo, M.; Liu, Z.; Hider, R. C. Novel iron-specific fluorescent probes. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3450-3452.
- (128) Ma, Y. M.; Kong, X. L.; Abbate, V.; Hider, R. C. Synthesis and characterization of novel iron-specific bicyclic fluorescent probes. *Sens. Actuators B-Chem.* **2015**, *213*, 12-19.
- (129) Ma, Y. M.; Hider, R. C. The selective quantification of iron by hexadentate fluorescent probes. *Bioorg. Med. Chem.* **2009**, *17*, 8093-8101.

- (130) Nunes, A.; Podinovskaia, M.; Leite, A.; Gameiro, P.; Zhou, T.; Ma, Y.; Kong, X.; Schaible, U. E.; Hider, R. C.; Rangel, M. Fluorescent 3-hydroxy-4-pyridinone hexadentate iron chelators: intracellular distribution and the relevance to antimycobacterial properties. *J. Biol. Inorg. Chem.* **2010**, *15*, 861-877.
- (131) Sebastiani, G.; Pantopoulos, K. NTBI unveiled by chelatable fluorescent beads. *Biochem. J.* **2014**, *463*, e7-9.
- (132) Chen, Y. L.; Barlow, D. J.; Kong, X. L.; Ma, Y. M.; Hider, R. C. Prediction of 3-hydroxypyridin-4-one (HPO) log K₁ values for Fe(III). *Dalton Trans.* **2012**, *41*, 10784-10791.
- (133) Crumbliss, A. L.; Harrington, J. M. Iron sequestration by small molecules: thermodynamic and kinetic studies of natural siderophores and synthetic model compounds. *Adv. Inorg. Chem.* **2009**, *61*, 179-250.
- (134) Smith, R.; Martell, A. *Hydroxide ion*. In *Critical stability constants, Inorganic complexes*, 1976; Vol. 4; p 7.
- (135) Ma, R.; Reibenspies, J. J.; Martell, A. E. Stabilities of 1,2-Diethyl-3-Hydroxy-4-Pyridinone Chelates of Divalent and Trivalent Metal-Ions. *Inorg. Chim. Acta* **1994**, *223*, 21-29.
- (136) Clarke, E. T.; Martell, A. E. 1-Methyl-3-hydroxy-2-pyridinone and 1,4-dihydroxy-2-pyridinone complexes of the trivalent metal-ions of Fe(III), Ga(III), Al(III), In(III) and Gd(III) - potentiometric and spectrophotometric determination of stabilities. *Inorg. Chim. Acta* **1992**, *196*, 185-194.
- (137) Yue, J. L.; Martell, A. E. Potentiometric and spectrophotometric determination of stabilities of the 1-hydroxy-2-pyridinone complexes of trivalent and divalent metal ions. *Inorg. Chim. Acta* **1993**, *214*, 103-111.
- (138) Eigen, M. Fast elementary steps in chemical reaction mechanisms. *Pure Appl. Chem.* **1963**, *6*, 97-116.
- (139) Martell, A. E.; Hancock, R. D. *Metal complexes in aqueous solutions*; Springer Science & Business Media: New York, 1996.
- (140) Pearson, R. G.; Songstad, J. Application of the principle of hard and soft acids and bases to organic chemistry. *J. Am. Chem. Soc.* **1967**, *89*, 1827-1836.
- (141) Ma, Y.; Zhou, T.; Kong, X.; Hider, R. C. Chelating agents for the treatment of systemic iron overload. *Curr. Med. Chem.* **2012**, *19*, 2816-2827.
- (142) Pangjit, K.; Banjerdpongchai, R.; Phisalaphong, C.; Fucharoen, S.; Xie, Y. Y.; Lu, Z. D.; Hider, R. C.; Srichairatanakool, S. Characterisation of a novel oral iron chelator: 1-(N-acetyl-6-aminoethyl)-3-hydroxy-2-methylpyridin-4-one. *J. Pharm. Pharmacol.* **2015**, *67*, 703-713.
- (143) Zhu, C. F.; Qiu, D. H.; Kong, X. L.; Hider, R. C.; Zhou, T. Synthesis and in-vitro antimicrobial evaluation of a high-affinity iron chelator in combination with chloramphenicol. *J. Pharm. Pharmacol.* **2013**, *65*, 512-520.
- (144) Clevette, D. J.; Lyster, D. M.; Nelson, W. O.; Rihela, T.; Webb, G. A.; Orvig, C. Solution chemistry of gallium and indium 3-hydroxy-4-pyridinone complexes in vitro and in vivo. *Inorg. Chem.* **1990**, *29*, 667-672.
- (145) Santos, M. A.; Gil, M.; Marques, S.; Gano, L.; Cantinho, G.; Chaves, S. N-Carboxyalkyl derivatives of 3-hydroxy-4-pyridinones: synthesis, complexation with Fe(III), Al(III) and Ga(III) and in vivo evaluation. *J. Inorg. Biochem.* **2002**, *92*, 43-54.

- (146) Berry, D. J.; Ma, Y.; Ballinger, J. R.; Tavare, R.; Koers, A.; Sunassee, K.; Zhou, T.; Nawaz, S.; Mullen, G. E.; Hider, R. C. et al. Efficient bifunctional gallium-68 chelators for positron emission tomography: tris(hydroxypyridinone) ligands. *Chem. Commun.* **2011**, *47*, 7068-7070.
- (147) Santos, M. A.; Gama, S.; Gano, L.; Farkas, E. Bis(3-hydroxy-4-pyridinone)-EDTA derivative as a potential therapeutic Al-chelating agent. Synthesis, solution studies and biological assays. *J. Inorg. Biochem.* **2005**, *99*, 1845-1852.
- (148) Thompson, K. H.; Barta, C. A.; Orvig, C. Metal complexes of maltol and close analogues in medicinal inorganic chemistry. *Chem. Soc. Rev.* **2006**, *35*, 545-556.
- (149) Yokel, R. A.; Meurer, K. A.; Skinner, T. L.; Fredenburg, A. M. The 3-hydroxypyridin-4-ones more effectively chelate aluminum in a rabbit model of aluminum intoxication than does desferrioxamine. *Drug Metab. Dispos.* **1996**, *24*, 105-111.
- (150) Gambhir, S. S. Molecular imaging of cancer with positron emission tomography. *Nat. Rev. Cancer* **2002**, *2*, 683-693.
- (151) Tinianow, J. N.; Pandya, D. N.; Pailloux, S. L.; Ogasawara, A.; Vanderbilt, A. N.; Gill, H. S.; Williams, S.-P.; Wadas, T. J.; Magda, D.; Marik, J. Evaluation of a 3-hydroxypyridin-2-one (2, 3-HOPO) based macrocyclic chelator for $^{89}\text{Zr}^{4+}$ and its use for immunoPET imaging of HER2 positive model of ovarian carcinoma in mice. *Theranostics* **2016**, *6*, 511-521.
- (152) Ma, M. T.; Meszaros, L. K.; Paterson, B. M.; Berry, D. J.; Cooper, M. S.; Ma, Y.; Hider, R. C.; Blower, P. J. Tripodal tris(hydroxypyridinone) ligands for immunoconjugate PET imaging with $(^{89}\text{Zr}^{4+})$: comparison with desferrioxamine-B. *Dalton Trans.* **2015**, *44*, 4884-4900.
- (153) Bhatt, N. B.; Pandya, D. N.; Wadas, T. J. Recent advances in zirconium-89 chelator development. *Molecules* **2018**, *23*, 638.
- (154) Wadas, T. J.; Wong, E. H.; Weisman, G. R.; Anderson, C. J. Coordinating radiometals of copper, gallium, indium, yttrium, and zirconium for PET and SPECT imaging of disease. *Chem. Rev.* **2010**, *110*, 2858-2902.
- (155) Caravan, P.; Ellison, J. J.; McMurry, T. J.; Lauffer, R. B. Gadolinium(III) chelates as MRI contrast agents: Structure, dynamics, and applications. *Chem. Rev.* **1999**, *99*, 2293-2352.
- (156) Kumar, K.; Tweedle, M. F. Macrocyclic polyaminocarboxylate complexes of lanthanides as magnetic resonance imaging contrast agents. *Pure Appl. Chem.* **1993**, *65*, 515-520.
- (157) Xu, J.; Franklin, S. J.; Whisenhunt, D. W.; Raymond, K. N. Gadolinium complex of tris[(3-hydroxy-1-methyl-2-oxo-1,2-didehydropyridine-4-carboxamido)ethyl]amine - a new class of gadolinium magnetic-resonance relaxation agents. *J. Am. Chem. Soc.* **1995**, *117*, 7245-7246.
- (158) Helm, L.; Merbach, A. E. Water exchange on metal ions: experiments and simulations. *Coord. Chem. Rev.* **1999**, *187*, 151-181.
- (159) Mendonça, A. C.; Martins, A. F.; Melchior, A.; Marques, S. M.; Chaves, S.; Villette, S.; Petoud, S.; Zanonato, P. L.; Tolazzi, M.; Bonnet, C. S. New tris-3, 4-HOPO lanthanide complexes as potential imaging probes: complex stability and magnetic properties. *Dalton Transactions* **2013**, *42*, 6046-6057.

- (160) Gorden, A. E.; Xu, J.; Raymond, K. N.; Durbin, P. Rational design of sequestering agents for plutonium and other actinides. *Chem. Rev.* **2003**, *103*, 4207-4282.
- (161) Durbin, P. W.; Kullgren, B.; Xu, J.; Raymond, K. N. Multidentate hydroxypyridinonate ligands for Pu (IV) chelation in vivo: comparative efficacy and toxicity in mouse of ligands containing 1, 2-HOPO or Me-3, 2-HOPO. *Int. J. Radiat. Biol.* **2000**, *76*, 199-214.
- (162) Gray, S. A.; Stradling, G. N.; Pearce, M. J.; Wilson, I.; Moody, J. C.; Burgada, R.; Durbin, P. W.; Raymond, K. N. Removal of plutonium and americium from the rat using 3,4,3-Lihopo and Dtpa after simulated wound contamination - Effect of delayed administration and mass of plutonium. *Radiat. Prot. Dosim.* **1994**, *53*, 319-322.
- (163) Sahoo, S. K.; Sharma, D.; Bera, R. K.; Crisponi, G.; Callan, J. F. Iron(III) selective molecular and supramolecular fluorescent probes. *Chem. Soc. Rev.* **2012**, *41*, 7195-7227.
- (164) Fan, L. J.; Zhang, Y.; Murphy, C. B.; Angell, S. E.; Parker, M. F. L.; Flynn, B. R.; Jones, W. E. Fluorescent conjugated polymer molecular wire chemosensors for transition metal ion recognition and signaling. *Coord. Chem. Rev.* **2009**, *253*, 410-422.
- (165) Formica, M.; Fusi, V.; Giorgi, L.; Micheloni, M. New fluorescent chemosensors for metal ions in solution. *Coord. Chem. Rev.* **2012**, *256*, 170-192.
- (166) Luo, W.; Ma, Y. M.; Quinn, P. J.; Hider, R. C.; Liu, Z. D. Design, synthesis and properties of novel iron(III)-specific fluorescent probes. *J. Pharm. Pharmacol.* **2004**, *56*, 529-536.
- (167) Ma, Y.; de Groot, H.; Liu, Z.; Hider, R. C.; Petrat, F. Chelation and determination of labile iron in primary hepatocytes by pyridinone fluorescent probes. *Biochem. J.* **2006**, *395*, 49-55.
- (168) Waldron, K. J.; Rutherford, J. C.; Ford, D.; Robinson, N. J. Metalloproteins and metal sensing. *Nature* **2009**, *460*, 823-830.
- (169) Ma, Y.; Xie, Y.; Hider, R. C. A novel fluorescence method for determination of pFe³⁺. *Analyst* **2013**, *138*, 96-99.
- (170) Ma, Y.; Zhou, T.; Hider, R. C. pFe(3+) determination of multidentate ligands by a fluorescence assay. *Analyst* **2015**, *140*, 3603-3606.
- (171) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* **1997**, *23*, 3-25.
- (172) Ma, Y.; Liu, Z.; Hider, R. C.; Petrat, F. Determination of the labile iron pool of human lymphocytes using the fluorescent probe, CP655. *Anal. Chem. Insights* **2007**, *2*, 61-67.
- (173) Ma, Y.; Abbate, V.; Hider, R. C. Iron-sensitive fluorescent probes: monitoring intracellular iron pools. *Metallomics* **2015**, *7*, 212-222.
- (174) Abbate, V.; Reelfs, O.; Kong, X.; Pourzand, C.; Hider, R. C. Dual selective iron chelating probes with a potential to monitor mitochondrial labile iron pools. *Chem. Commun.* **2016**, *52*, 784-787.

- (175) Fakih, S.; Podinovskaia, M.; Kong, X.; Schaible, U. E.; Collins, H. L.; Hider, R. C. Monitoring intracellular labile iron pools: A novel fluorescent iron(III) sensor as a potential non-invasive diagnosis tool. *J. Pharm. Sci.* **2009**, *98*, 2212-2226.
- (176) Hider, R. C.; Kong, X. Iron speciation in the cytosol: an overview. *Dalton Trans.* **2013**, *42*, 3220-3229.
- (177) Ma, Y.; Podinovskaia, M.; Evans, P. J.; Emma, G.; Schaible, U. E.; Porter, J.; Hider, R. C. A novel method for non-transferrin-bound iron quantification by chelatable fluorescent beads based on flow cytometry. *Biochem. J.* **2014**, *463*, 351-362.
- (178) Vogel, K. W.; Vedvik, K. L. Improving lanthanide-based resonance energy transfer detection by increasing donor-acceptor distances. *J. Biomol. Screen.* **2006**, *11*, 439-443.
- (179) Bergendahl, V.; Heyduk, T.; Burgess, R. R. Luminescence resonance energy transfer-based high-throughput screening assay for inhibitors of essential protein-protein interactions in bacterial RNA polymerase. *Appl. Environ. Microbiol.* **2003**, *69*, 1492-1498.
- (180) Moore, E. G.; Samuel, A. P.; Raymond, K. N. From antenna to assay: lessons learned in lanthanide luminescence. *Acc. Chem. Res.* **2009**, *42*, 542-552.
- (181) Blower, P. J. A nuclear chocolate box: the periodic table of nuclear medicine. *Dalton Trans.* **2015**, *44*, 4819-4844.
- (182) Hofman, M. S.; Kong, G.; Neels, O. C.; Eu, P.; Hong, E.; Hicks, R. J. High management impact of Ga-68 DOTATATE (GaTate) PET/CT for imaging neuroendocrine and other somatostatin expressing tumours. *J. Med. Imaging Radiat. Oncol.* **2012**, *56*, 40-47.
- (183) Afshar-Oromieh, A.; Zechmann, C. M.; Malcher, A.; Eder, M.; Eisenhut, M.; Linhart, H. G.; Holland-Letz, T.; Hadaschik, B. A.; Giesel, F. L.; Debus, J. et al. Comparison of PET imaging with a (68)Ga-labelled PSMA ligand and (18)F-choline-based PET/CT for the diagnosis of recurrent prostate cancer. *Eur. J. Nucl. Med. Mol. Imaging.* **2014**, *41*, 11-20.
- (184) Cusnir, R.; Imberti, C.; Hider, R. C.; Blower, P. J.; Ma, M. T. Hydroxypyridinone chelators: from iron scavenging to radiopharmaceuticals for PET imaging with gallium-68. *Int. J. Mol. Sci.* **2017**, *18*, 116.
- (185) Ma, M. T.; Cullinane, C.; Imberti, C.; Baguna Torres, J.; Terry, S. Y.; Roselt, P.; Hicks, R. J.; Blower, P. J. New tris (hydroxypyridinone) bifunctional chelators containing isothiocyanate groups provide a versatile platform for rapid one-step labeling and PET imaging with 68Ga3+. *Bioconjug. Chem.* **2015**, *27*, 309-318.
- (186) Ma, M. T.; Cullinane, C.; Waldeck, K.; Roselt, P.; Hicks, R. J.; Blower, P. J. Rapid kit-based (68)Ga-labelling and PET imaging with THP-Tyr(3)-octreotate: a preliminary comparison with DOTA-Tyr(3)-octreotate. *Eur. J. Nucl. Med. Mol. Imaging* **2015**, *5*, 52.
- (187) Young, J. D.; Abbate, V.; Imberti, C.; Meszaros, L. K.; Ma, M. T.; Terry, S. Y. A.; Hider, R. C.; Mullen, G. E.; Blower, P. J. (68)Ga-THP-PSMA: a PET imaging agent for prostate cancer offering rapid, room-temperature, 1-step kit-based radiolabeling. *J. Nucl. Med.* **2017**, *58*, 1270-1277.
- (188) Hofman, M. S.; Eu, P.; Jackson, P.; Hong, E.; Binns, D.; Iravani, A.; Murphy, D.; Mitchell, C.; Siva, S.; Hicks, R. J. et al. Cold kit PSMA PET imaging: phase I study

- of ⁶⁸Ga-THP-PSMA PET/CT in patients with prostate cancer. *J. Nuc. Med.* **2018**, *59*, 625-631.
- (189) Eder, M.; Schafer, M.; Bauder-Wust, U.; Hull, W. E.; Wangler, C.; Mier, W.; Haberkorn, U.; Eisenhut, M. ⁶⁸Ga-complex lipophilicity and the targeting property of a urea-based PSMA inhibitor for PET imaging. *Bioconjug. Chem.* **2012**, *23*, 688-697.
- (190) Derlin, T.; Schmuck, S.; Juhl, C.; Teichert, S.; Zörgiebel, J.; Wester, H.-J.; Schneefeld, S. M.; Walte, A. C.; Thackeray, J. T.; Ross, T. L. Imaging characteristics and first experience of [⁶⁸ Ga] THP-PSMA, a novel probe for rapid kit-based Ga-68 labeling and PET imaging: comparative analysis with [⁶⁸ Ga] PSMA I&T. *Mol. Imaging Biol.* **2018**, 1-9.
- (191) Derlin, T.; Schmuck, S.; Juhl, C.; Zörgiebel, J.; Schneefeld, S. M.; Walte, A. C.; Hueper, K.; von Klot, C. A.; Henkenberens, C.; Christiansen, H. PSA-stratified detection rates for [⁶⁸ Ga] THP-PSMA, a novel probe for rapid kit-based ⁶⁸ Ga-labeling and PET imaging, in patients with biochemical recurrence after primary therapy for prostate cancer. *Eur. J. Nucl. Med. Mol. Imaging* **2018**, 1-10.
- (192) Deri, M. A.; Ponnala, S.; Kozlowski, P.; Burton-Pye, B. P.; Cicek, H. T.; Hu, C.; Lewis, J. S.; Francesconi, L. C. p-SCN-Bn-HOPO: A superior bifunctional chelator for ⁸⁹Zr immunoPET. *Bioconj. Chem.* **2015**, *26*, 2579-2591.
- (193) Evans, M. J.; Holland, J. P.; Rice, S. L.; Doran, M. G.; Cheal, S. M.; Campos, C.; Carlin, S. D.; Mellinshoff, I. K.; Sawyers, C. L.; Lewis, J. S. Imaging tumor burden in the brain with ⁸⁹Zr-transferrin. *J. Nucl. Med.* **2013**, *54*, 90-95.
- (194) Werner, E. J.; Datta, A.; Jocher, C. J.; Raymond, K. N. High-relaxivity MRI contrast agents: where coordination chemistry meets medical imaging. *Angew. Chem. Int. Ed.* **2008**, *47*, 8568-8580.
- (195) Crichton, R. R.; Ward, R. J.; Hider, R. C. *Metal chelation in medicine*; Royal Society of Chemistry: Cambridge, UK, 2016.
- (196) Hoffbrand, A. V.; F, A. L.-R.; Davis, B.; Siritanakatkul, N.; Jackson, B. F.; Cochrane, J.; Prescott, E.; Wonke, B. Long-term trial of deferiprone in 51 transfusion-dependent iron overloaded patients. *Blood* **1998**, *91*, 295-300.
- (197) Maggio, A.; D'Amico, G.; Morabito, A.; Capra, M.; Ciaccio, C.; Cianciulli, P.; Di Gregorio, F.; Garozzo, G.; Malizia, R.; Magnano, C. et al. Deferiprone versus deferoxamine in patients with thalassemia major: a randomized clinical trial. *Blood Cells Mol. Dis.* **2002**, *28*, 196-208.
- (198) Cohen, A. R.; Galanello, R.; Piga, A.; De Sanctis, V.; Tricta, F. Safety and effectiveness of long-term therapy with the oral iron chelator deferiprone. *Blood* **2003**, *102*, 1583-1587.
- (199) Pepe, A.; Meloni, A.; Capra, M.; Cianciulli, P.; Prossomariti, L.; Malaventura, C.; Putti, M. C.; Lippi, A.; Romeo, M. A.; Bisconte, M. G. et al. Deferasirox, deferiprone and desferrioxamine treatment in thalassemia major patients: cardiac iron and function comparison determined by quantitative magnetic resonance imaging. *Haematologica* **2011**, *96*, 41-47.
- (200) Baksi, A. J.; Pennell, D. J. Randomized controlled trials of iron chelators for the treatment of cardiac siderosis in thalassaemia major. *Front. Pharmacol.* **2014**, *5*, 217.

- (201) Di Maggio, R.; Maggio, A. The new era of chelation treatments: effectiveness and safety of 10 different regimens for controlling iron overloading in thalassaemia major. *Br. J. Haematol.* **2017**, *178*, 676-688.
- (202) Cohen, S. M.; O'Sullivan, B.; Raymond, K. N. Mixed hydroxypyridinonate ligands as iron chelators. *Inorg. Chem.* **2000**, *39*, 4339-4346.
- (203) Duce, J. A.; Bush, A. I. Biological metals and Alzheimer's disease: implications for therapeutics and diagnostics. *Prog. Neurobiol.* **2010**, *92*, 1-18.
- (204) Dexter, D. T.; Wells, F. R.; Agid, F.; Agid, Y.; Lees, A. J.; Jenner, P.; Marsden, C. D. Increased nigral iron content in postmortem parkinsonian brain. *Lancet* **1987**, *2*, 1219-1220.
- (205) Dexter, D. T.; Wells, F. R.; Lees, A. J.; Agid, F.; Agid, Y.; Jenner, P.; Marsden, C. D. Increased nigral iron content and alterations in other metal ions occurring in brain in Parkinson's disease. *J. Neurochem.* **1989**, *52*, 1830-1836.
- (206) Lynch, S. G.; Fonseca, T.; LeVine, S. M. A multiple course trial of desferrioxamine in chronic progressive multiple sclerosis. *Cell Mol. Biol.* **2000**, *46*, 865-869.
- (207) McNeill, A.; Chinnery, P. F. Neuroferritinopathy: update on clinical features and pathogenesis. *Curr. Drug Targets* **2012**, *13*, 1200-1203.
- (208) Hadziahmetovic, M.; Song, Y.; Wolkow, N.; Iacovelli, J.; Grieco, S.; Lee, J.; Lyubarsky, A.; Pratico, D.; Connelly, J.; Spino, M. The oral iron chelator deferiprone protects against iron overload-induced retinal degeneration. *Invest. Ophthalmol. Vis. Sci.* **2011**, *52*, 959-968.
- (209) Chaves, S.; Piemontese, L.; Hiremathad, A.; Santos, M. A. Hydroxypyridinone derivatives: a fascinating class of chelators with therapeutic applications-an update. *Curr. Med. Chem.* **2018**, *25*, 97-112.
- (210) Habgood, M. D.; Dehkordi, L. S.; Khodr, H. H.; Abbott, J.; Hider, R. C. Investigation into the correlation between the structure of hydroxypyridinones and blood-brain barrier permeability. *Biochem. Pharmacol.* **1999**, *57*, 1305-1310.
- (211) Hider, R. C.; Roy, S.; Ma, Y. M.; Le Kong, X.; Preston, J. The potential application of iron chelators for the treatment of neurodegenerative diseases. *Metallomics* **2011**, *3*, 239-249.
- (212) Ward, R. J.; Dexter, D.; Florence, A.; Aouad, F.; Hider, R.; Jenner, P.; Crichton, R. R. Brain iron in the ferrocene-loaded rat: its chelation and influence on dopamine metabolism. *Biochem. Pharmacol.* **1995**, *49*, 1821-1826.
- (213) Dexter, D. T.; Statton, S. A.; Whitmore, C.; Freinbichler, W.; Weinberger, P.; Tipton, K. F.; Della Corte, L.; Ward, R. J.; Crichton, R. R. Clinically available iron chelators induce neuroprotection in the 6-OHDA model of Parkinson's disease after peripheral administration. *J. Neural. Transm.* **2011**, *118*, 223-231.
- (214) Devos, D.; Moreau, C.; Devedjian, J. C.; Kluza, J.; Petrault, M.; Laloux, C.; Jonneaux, A.; Ryckewaert, G.; Garçon, G.; Rouaix, N. Targeting chelatable iron as a therapeutic modality in Parkinson's disease. *Antioxid. Redox Signaling* **2014**, *21*, 195-210.
- (215) Martin-Bastida, A.; Ward, R. J.; Newbould, R.; Piccini, P.; Sharp, D.; Kabba, C.; Patel, M. C.; Spino, M.; Connelly, J.; Tricta, F. et al. Brain iron chelation by deferiprone in a phase 2 randomised double-blinded placebo controlled clinical trial in Parkinson's disease. *Sci. Rep.* **2017**, *7*, 1398.

- (216) Boddaert, N.; Le Quan Sang, K. H.; Rotig, A.; Leroy-Willig, A.; Gallet, S.; Brunelle, F.; Sidi, D.; Thalabard, J. C.; Munnich, A.; Cabantchik, Z. I. Selective iron chelation in Friedreich ataxia: biologic and clinical implications. *Blood* **2007**, *110*, 401-408.
- (217) Kakhlon, O.; Manning, H.; Breuer, W.; Melamed-Book, N.; Lu, C.; Cortopassi, G.; Munnich, A.; Cabantchik, Z. I. Cell functions impaired by frataxin deficiency are restored by drug-mediated iron relocation. *Blood* **2008**, *112*, 5219-5227.
- (218) Reelfs, O.; Abbate, V.; Hider, R. C.; Pourzand, C. A powerful mitochondria-targeted iron chelator affords high photoprotection against solar ultraviolet A radiation. *J. Invest. Dermatol.* **2016**, *136*, 1692-1700.
- (219) Juzeniene, A.; Peng, Q.; Moan, J. Milestones in the development of photodynamic therapy and fluorescence diagnosis. *Photochem. Photobiol. Sci.* **2007**, *6*, 1234-1245.
- (220) De Rosa, F. S.; Bentley, M. V. Photodynamic therapy of skin cancers: sensitizers, clinical studies and future directives. *Pharm. Res.* **2000**, *17*, 1447-1455.
- (221) Kennedy, J. C.; Marcus, S. L.; Pottier, R. H. Photodynamic therapy (PDT) and photodiagnosis (PD) using endogenous photosensitization induced by 5-aminolevulinic acid (ALA): mechanisms and clinical results. *J. Clin. Laser Med. Surg.* **1996**, *14*, 289-304.
- (222) Curnow, A.; McIlroy, B. W.; Postle-Hacon, M. J.; Porter, J. B.; MacRobert, A. J.; Bown, S. G. Enhancement of 5-aminolaevulinic acid-induced photodynamic therapy in normal rat colon using hydroxypyridinone iron-chelating agents. *Br. J. Cancer* **1998**, *78*, 1278-1282.
- (223) Battah, S.; Hider, R. C.; MacRobert, A. J.; Dobbin, P. S.; Zhou, T. Hydroxypyridinone and 5-aminolaevulinic acid conjugates for photodynamic therapy. *J. Med. Chem.* **2017**, *60*, 3498-3510.
- (224) Han, S.; Zaniewski, R. P.; Marr, E. S.; Lacey, B. M.; Tomaras, A. P.; Evdokimov, A.; Miller, J. R.; Shanmugasundaram, V. Structural basis for effectiveness of siderophore-conjugated monocarbams against clinically relevant strains of *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. U S A* **2010**, *107*, 22002-22007.
- (225) Zhou, T.; Winkelmann, G.; Dai, Z. Y.; Hider, R. C. Design of clinically useful macromolecular iron chelators. *J. Pharm. Pharmacol.* **2011**, *63*, 893-903.
- (226) Qiu, D. H.; Huang, Z. L.; Zhou, T.; Shen, C.; Hider, R. C. In vitro inhibition of bacterial growth by iron chelators. *FEMS Microbiol. Lett.* **2011**, *314*, 107-111.
- (227) Xie, Y. Y.; Liu, M. S.; Hu, P. P.; Kong, X. L.; Qiu, D. H.; Xu, J. L.; Hider, R. C.; Zhou, T. Synthesis, physico-chemical properties, and antimicrobial evaluation of a new series of iron(III) hexadentate chelators. *Med. Chem. Res.* **2013**, *22*, 2351-2359.
- (228) Zhou, Y. J.; Liu, M. S.; Osamah, A. R.; Kong, X. L.; Alsam, S.; Battah, S.; Xie, Y. Y.; Hider, R. C.; Zhou, T. Hexadentate 3-hydroxypyridin-4-ones with high iron(III) affinity: design, synthesis and inhibition on methicillin resistant *Staphylococcus aureus* and *Pseudomonas* strains. *Eur. J. Med. Chem.* **2015**, *94*, 8-21.
- (229) Zhou, Y. J.; Zhang, M. X.; Hider, R. C.; Zhou, T. In vitro antimicrobial activity of hydroxypyridinone hexadentate-based dendrimeric chelators alone and in combination with norfloxacin. *FEMS Microbiol. Lett.* **2014**, *355*, 124-130.

- (230) Durbin, P. W.; Kullgren, B.; Ebbe, S. N.; Xu, J.; Raymond, K. N. Chelating agents for uranium(VI): 2. Efficacy and toxicity of tetradentate catecholate and hydroxypyridinonate ligands in mice. *Health Phys.* **2000**, *78*, 511-521.

Biographies

Agostino Cilibrizzi – Agostino received his Ph.D. degree in Chemistry and Pharmaceutical Technology from the University of Florence in 2011. He is a Medicinal Chemist and joined the Department of Pharmaceutical Science at King's College London in 2016 to work with Prof R. Hider and Dr V. Abbate. Prior to this he worked at the University of Florence, University of Cambridge and Imperial College London. His research focuses on the synthesis and medical applications of biologically active agents, metal chelators and optical probes for receptors and enzymes, including the development of bio-imaging techniques.

Vincenzo Abbate – Vincenzo Abbate completed his Pharm. D. in 2004 with full marks (110/110 cum laude) at Federico II University of Naples. He then moved to the UK to undertake a PhD in Chemistry and Analytical Sciences at The Open University. In 2008 he was awarded a prestigious Maplethorpe Fellowship of the University of London for the promotion of teaching and research in the pharmaceutical area. Abbate is currently a Lecturer in Analytical Toxicology at King's College London (KCL) where he leads a research group (8 personnel) together with Professor Hider working on the design and synthesis of small molecules and bioactive peptides for medical applications. He is also the Head of R&D in the Drug Control Centre, King's College London, where he runs a group of 5 scientists. He is the Director of the MSc Analytical Toxicology at KCL. Abbate is a Titular Member of the IUPAC Division of Chemistry and Human Health. He has

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Yu-Lin Chen – Yu-Lin was born in Taiwan and received B.Eng. degree in Bio-Industrial Mechatronics Engineer from the National Taiwan University. After military service, he moved to King's College London, where he completed his M.Sc and Ph.D. in Pharmaceutical Science. After a 2-year postdoctoral research with Yongmin Ma in Hangzhou, China, he returned to London. He has conducted research on various aspects of metal-chelating agents, prediction/determination of metal affinity and chemical lipophilicity and synthesis of hydroxypyridinones.

Yongmin Ma – Professor Yongmin Ma completed his PhD degree in Medicinal Chemistry in 2005 under the supervision of Professor Robert Hider at King's College London. He continued to work as a research fellow in Professor Hider's group until 2013. He was then awarded "1000-talents plan" from Zhejiang China and was appointed Professor at Zhejiang Chinese Medical University. His research interests include the design of iron chelators for the treatment of iron overload diseases and neurodegenerative diseases, the design of iron-specific fluorescent probes for detecting intracellular iron and non-transferrin bound iron.

Tao Zhou – Tao Zhou received his Ph.D. degree in Organic Chemistry from Zhejiang University in 2001. He worked as a postdoctoral research fellow under the supervision of Professor Hider in King's College London over the period January 2002 – April 2008.

He is currently a Professor of Medicinal Chemistry at Zhejiang Gongshang University, China and retains a strong research link with King's College London. He has published over 90 scientific papers and 10 patents. His research interest is mainly centred on Medicinal Chemistry (especially design and synthesis of clinically useful metal chelators) and Food Science.

Robert C. Hider – Robert is Professor of Medicinal Chemistry at King's College London, where he has worked since 1987. Prior to this he was a Lecturer in Biological Chemistry at Essex University. He has worked with siderophore-based iron uptake processes in microorganisms and the absorption of iron by mammalian cells. His work on membrane structure and transport mechanisms has led to the development of novel oral iron chelators for the treatment of iron overload. As a result, hydroxypyridinones have been identified as possessing potential for clinical application. Deferiprone is now used worldwide for the treatment of iron overload.

TOC

