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Iron(III)-selective dendritic chelators

Tao Zhou,[a] Robert C. Hider,[a,*] Zu D. Liu[a] and Hendrik Neubert[b]

[a]*Department of Pharmacy, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NN, UK*
[b]*Drug Control Center, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NN, UK*

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Abstract—Two novel iron(III)-selective hexadentate chelator-terminated dendrimers have been synthesized in high yields. MALDI-TOF mass spectra demonstrate that both dendritic chelators bind iron(III) efficiently. Preliminary studies show that these molecules possess a high affinity for iron(III).

Although iron is essential for the proper functioning of all living cells, it is toxic when present in excess. In the presence of molecular oxygen, 'loosely-bound' iron is able to redox cycle between the two most stable oxidation states iron(II) and iron(III) thereby generating oxygen-derived free radicals such as the toxic hydroxyl radical.[1] The accumulation of iron can be largely prevented by the use of iron-specific chelating agents and to this purpose we have designed and synthesized a series of orally active iron(III)-selective chelators centred on hydroxypyridinones.[2] An alternative method of relieving iron overload is to reduce the efficiency of iron absorption from the intestine by administering iron chelators, which can bind iron irreversibly to form non-toxic, kinetically inert complexes that are not absorbed and are therefore excreted in the faeces. High molecular weight chelators and in particular dendritic chelators could, in principle, find application as nonabsorbable iron-selective additives. The metal complexation of dendrimers has attracted much attention as these aggregates find widespread use primarily in catalysis.[3] However, there are a few reports describing the targeted sequestering of metal ions by dendritic structures.[4] In one such study, the iron(III) binding of commercially available poly(amidoamine) and poly(propyleneimine) dendrimers functionalized with bidentate chelators was investigated by UV/vis spectrophotometry.[4c]

Methods in dendrimer synthesis have been widely reported,[5] and to this end we have designed a range of hexadentate-terminated dendrimers based on a benzene tricarbonyl core. The selection of hexadentate moieties was guided by the desire to provide octahedral iron(III) coordination sites.[6] Hexadentate sites with a high affinity for iron(III) can be constructed by linking three 3-hydroxypyridin-4-one ligands via the 2- or 5-position to a suitable tripodal molecule. Herein, we report the synthesis of hexadentate-terminated dendrimers that are capable of chelating three or six iron(III) moieties by using both divergent[7] and convergent approaches.[8] Previously, the speciation profile of a 3-hydroxypyridin-4-one with iron(III) was studied using mass spectrometry.[9] In the present study the iron chelating abilities of the corresponding dendritic scavengers have been investigated using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

The divergent synthetic strategy was initially employed for the synthesis of a chelator-containing dendrimer. In this approach, carboxyl group-terminated dendrimers were firstly constructed and then hydroxypyridinones were conjugated via amide bond formation using standard peptide synthetic methodology.[10] The assembly of the first generation dendritic chelator is shown in Scheme 1. The protected dendritic chelator was isolated in 84% yield. Deprotection of the benzyl function was achieved in the presence of boron trichloride to generate the dendritic chelator **2**[11] (91% yield). MALDI-TOF mass spectrometric analysis confirmed the structure of **2** ($[M+H]^+$ 2248.7) (Fig. 1a). Using a similar method, the conjugation of 1,6-dimethyl-2-aminomethyl-3-benzyloxypyridin-4(1*H*)-one[2b] with a second generation

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* Corresponding author. Tel.: +44 207 848 4882; fax: +44 207 848 4800; e-mail: robert.hider@kcl.ac.uk

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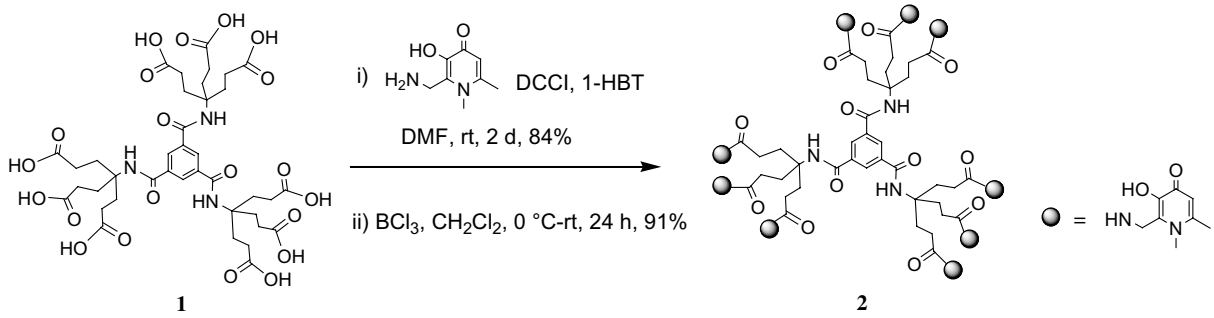


Scheme 1. Synthesis of dendritic chelator **2**.

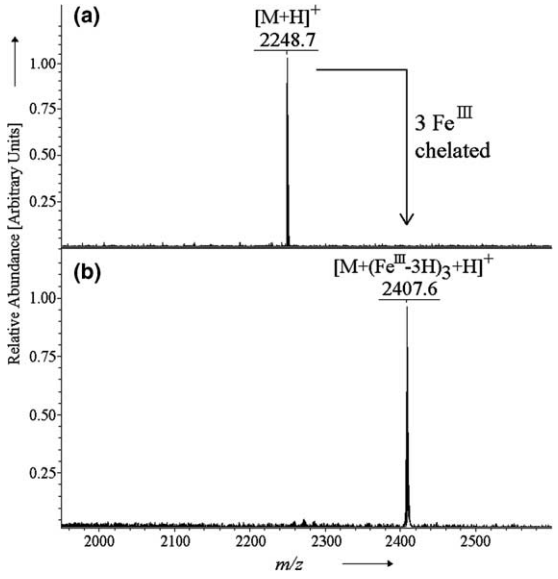


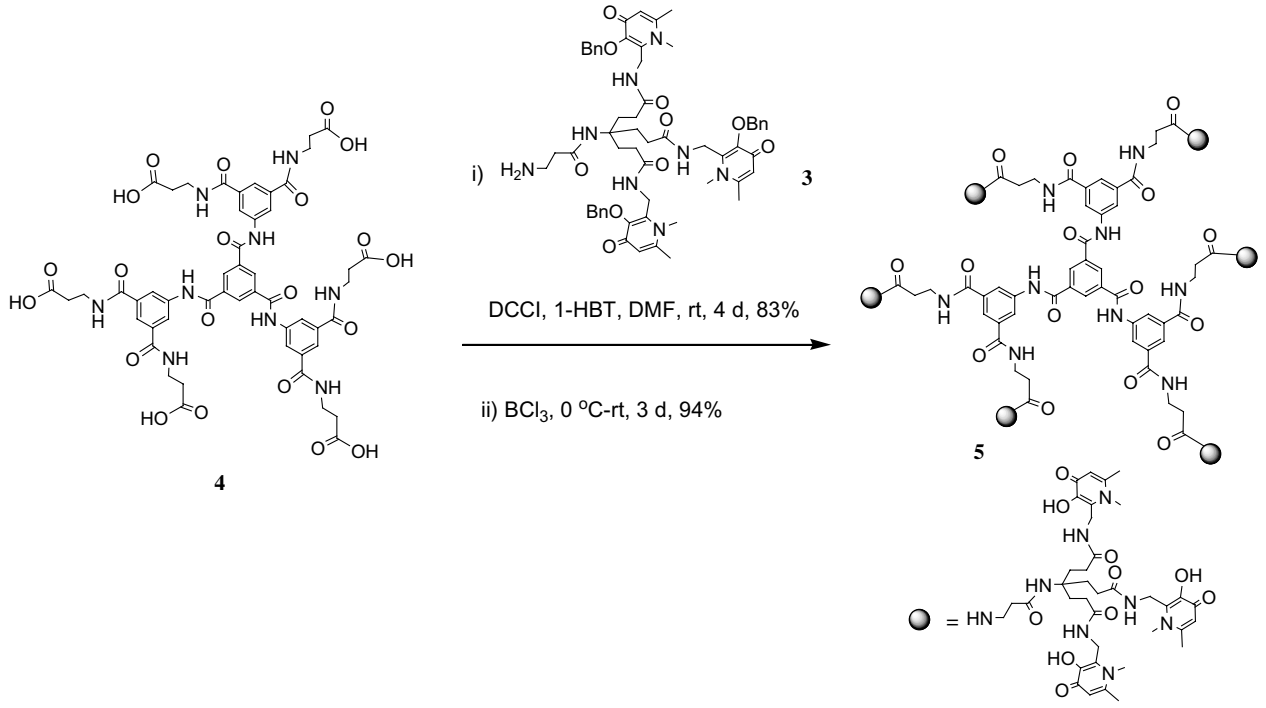
Figure 1. (a) MALDI-TOF mass spectrum of the proton adduct of dendrimer **2** $[M+H]^+$ recorded from α-cyano-4-hydroxycinnamic acid matrix (CHCA). (b) MALDI-TOF mass spectrum of the proton adduct of the 1:3 dendrimer-iron(III) complex $[M+(Fe^{III}-3H)_3+H]^+$ recorded from 2-(2*E*)-3-(4-*tert*-butylphenyl)-2-methylprop-2-enylidene malononitrile (DCTB) matrix.[13] The selection of the MALDI matrix was critical in ensuring desorption of intact metal complexes.

dendrimer containing 27 carboxyl groups was also investigated. However the reaction led only to a small quantity of the desired dendrimer and considerable amounts of defect dendritic structures as inferred from the MALDI-TOF mass spectrum. As a consequence of this finding, a convergent approach for the synthesis of a second-generation dendritic chelator was explored. The benzyl-protected hexadentate ligand containing a free amino group **3** was synthesized and coupled to the first generation polyacid dendrimer **4** to afford the desired protected dendritic chelator (83% yield). In order to reduce the possibility of steric congestion, dendrimer **4** contained the reactive sites at the termini of elongated chains. Removal of the benzyls protecting groups afforded the dendritic chelator **5**[12] in 94% yield (Scheme 2). The molecular weight of **5** ($[M+H]^+$ 5631.5) was confirmed by MALDI-TOF mass spectrometric analysis and a small amount of defect dendrimer lacking one hexadentate unit ($*_1$, $[M+H]^+$ 4908.3) was also detected (Fig. 2a).

The dendritic chelator **2** contains effectively three hexadentate ligands, each comprising three equivalent bidentate 3-hydroxypyridin-4-one units conjugated to the same building block. Likewise, the dendritic chelator **5** contains six equivalent hexadentate ligands. These two structures were probed for their iron-chelating abilities using MALDI-TOF mass spectrometry. Direct evidence for the formation of a dendrimer-iron complex was obtained. The MALDI spectrum recorded from the one-to-three mixture of dendritic chelator **2** with iron had a peak at *m*/*z* 2408.7, which corresponds to the proton adduct of a one-to-three dendrimer-iron complex, also annotated as $[M+(Fe^{III}-3H)_3+H]^+$ (Fig. 1b). Evidently, three protons are released upon complexation of each iron(III). Similarly, the MALDI spectrum obtained from a one-to-six mixture of dendritic chelator **5** with iron demonstrated complete iron binding (Fig. 2b). The main mass peak is that of the proton adduct of the one-to-six dendrimer-iron complex at *m*/*z* 5949.6, which is also annotated as $[M+(Fe^{III}-3H)_6+H]^+$. A defect dendritic structure lacking one hexadentate unit was also found to bind iron completely, as confirmed by the presence of the signal at *m*/*z* 5171.6, corresponding to the proton adduct of the one-to-five dendrimer-iron complex ($*_2$).

IR spectra analysis of the free and bound dendritic ligands[14] indicated that both the dendritic chelator **2**-Fe complex and the dendritic chelator **5**-Fe complex possess a strong peak, which can be assigned to the Fe–O vibration, at 542 and 544 cm^{-1}, respectively.

In order to estimate the affinity of the dendritic chelators for iron(III), an analogous hexadentate ligand **6** (Fig. 3) was synthesized and its physico-chemical properties were determined using an automated titration system.[15] Hexadentate ligand **6** has a very high affinity for iron(III) namely $\log K_1 = 32.6$; $pFe^{3+} = 27.4$.[16] The pFe^{3+} value of **6** is over 7 log units higher than that of the bidentate analogue 3-hydroxy-1,2-dimethylpyridin-4(1*H*)-one ($pFe^{3+} = 19.7$). Thus, the dendritic chelators **2** and **5**, which both contain similar hexadentate units to that of **6** are predicted to possess extremely high affinities for iron(III). In fact, the absolute stability constant



Scheme 2. Synthesis of dendritic chelator **5**.

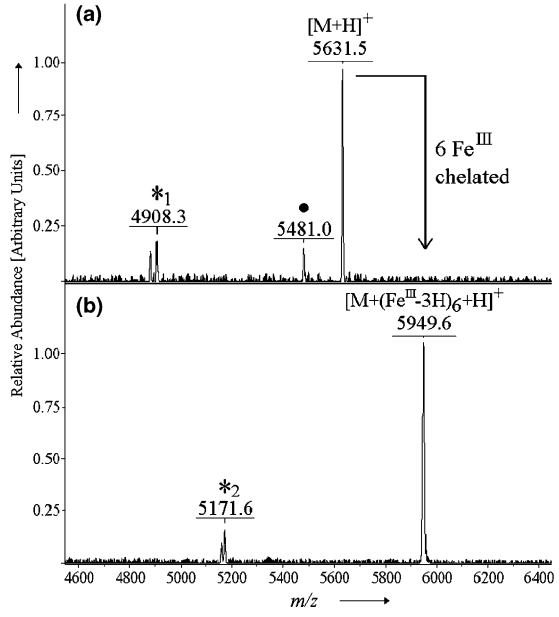


Figure 2. (a) MALDI-TOF mass spectrum of the proton adduct of dendrimer **5** $[M+H]^+$. A signal for a defect dendrimer lacking one hexadentate branch is annotated with ($*_1$). The signal annotated as (●) shows a fragment of dendrimer **5** lacking one terminal 3-hydroxypyridin-4-one group possibly resulting from MS-induced fragmentation. (b) MALDI-TOF mass spectrum of the proton adduct of the 1:6 dendrimer-iron(III) complex $[M+(Fe^{III}-3H)_6+H]^+$. ($*_2$) shows to the 1:5 dendrimer-iron(III) complex of the defect dendrimer lacking one hexadentate branch. Both spectra were recorded from DCTB matrix.

($\log K$) of the iron(III) complex of dendritic chelator **2** was determined to be 35.3 ± 1.3 by the spectrophotometric competition method[17] with the well characterized

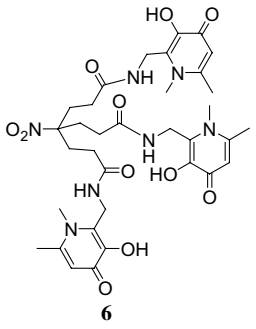


Figure 3. Hexadentate ligand **6**.

hexadentate ligand *N*,*N*′-di(2-hydroxybenzyl)ethylenediamine-*N*,*N*′-diacetic acid (HBED).

In summary, it has been demonstrated that conjugation of the iron chelator 3-hydroxypyridin-4-one to carboxyl group-terminated dendrimers with a benzene tricarbonyl core can be achieved via facile amide bond formation in the presence of DCCI and 1-hydroxybenzotriazole (1-HBT). Two novel iron(III)-selective hexadentate chelator-terminated dendrimers were successfully synthesized in high yields by divergent and convergent approaches. The convergent strategy has the advantage of using a specifically designed hexadentate iron chelator wedge for the final attachment to a polyacid dendrimer core. MALDI-TOF mass spectrometry has been vital in ascertaining that these two dendritic chelators bind iron efficiently. Preliminary studies demonstrate that dendritic chelators possess a high affinity constant for iron(III). The detailed metal ion binding characteristics of these molecules are currently under investigation.

Acknowledgements

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11. Data for hydrochloric acid salt of dendrimer **2**: IR (KBr): ν = 3398, 3243, 3065, 1636, 1540, 1484, 1430, 1359, 1292, 1129, 1041, 951, 873 cm^{-1}. ^1H NMR (360 MHz, DMSO-d_6): δ = 2.01 (br, 18H; CH_2), 2.16 (br, 18H; CH_2), 2.56 (s, 27H; CH_3), 3.88 (s, 27H; CH_3), 4.55 (br, 18H; CH_2), 7.25 (s, 9H; pyridinone C–5H), 8.06 (br, 3H; ArH), 8.41 (s, 3H; NH), 8.99 (br, 9H; NH). ^{13}C NMR (90 MHz, DMSO-d_6): δ = 21.0 (*C*H_3), 30.1 (C*C*H_2*C*H_2), 35.1 (NH*C*H_2-pyridinone), 39.4 (N*C*H_3), 113.1 (*C*–5H in pyridinone), 140.1 (*C*-2 in pyridinone), 143.1 (*C*-3 in pyridinone), 148.9 (*C*-6 in pyridinone), 160.1 (*C*-4 in pyridinone), 173.8 (*C*ONH). MALDI-TOF MS: calculated for $C_{111}H_{141}N_{21}O_{30}$: 2248.0 (monoisotopic molecular weight), found: 2248.7 for $[M+H]^+$ (matrix: CHCA).
12. Data for hydrochloric acid salt of dendrimer **5**: IR (KBr): ν = 3262, 3064, 1642, 1542, 1489, 1441, 1364, 1296, 1129, 1039, 951, 867 cm^{-1}. ^1H NMR (500 MHz, CD_3OD): δ = 1.91 (br, 36H; CH_2), 2.22 (br, 36H; CH_2), 2.41 (br, 12H; CH_2), 2.57 (br, 12H; CH_2), 2.60 (s, 54H; CH_3), 3.44 (br, 12H; CH_2), 3.66 (br, 12H; CH_2), 4.00 (s, 54H; CH_3), 4.71 (s, 36H; CH_2), 4.91 (br, OH), 7.08 (s, 18H, pyridinone C–5H), 8.04 (s, 3H; ArH), 8.49 (s, 6H; ArH), 8.95 (s, 3H; ArH). ^{13}C NMR (90 MHz, CD_3OD): δ = 21.8 (*C*H_3), 31.2 (C*C*H_2*C*H_2), 31.5 (C*C*H_2*C*H_2), 36.9 (NH*C*H_2-pyridinone), 40.6 (N*C*H_3), 59.6 (NH*C*CH_2CH_2), 114.6 (*C*–5H in pyridinone), 141.4 (*C*-2 in pyridinone), 145.1 (*C*-3 in pyridinone), 151.3 (*C*-6 in pyridinone), 161.4 (*C*-4 in pyridinone), 177.1 (*C*ONH). MALDI-TOF MS: calculated for $C_{273}H_{351}N_{57}O_{75}$: 5631.1 (average molecular weight), found: 5631.5 for $[M+H]^+$ (matrix: DCTB).
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