Iron Binding Dendrimers: A Novel Approach for the Treatment of Haemochromatosis

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A range of iron binding dendrimers terminated with hexadentate ligands formed from hydroxypyridinone, hydroxypyranone, and catechol moieties have been synthesized in order to investigate their potential as clinically useful iron(III)-selective chelators capable of removing dietary iron from the gastrointestinal tract and preventing the development of iron overload typical of haemochromatosis and thalassaemia intermedia. The iron chelating abilities of these molecules have been characterized by MALDI-TOF mass spectrometry and UV spectrometry. Hydroxypyridinone-terminated dendrimers were found to possess a high affinity and selectivity for iron(III). A hydroxypyridinone-based dendrimer was demonstrated to be highly efficient at reducing the absorption of iron(III) in rat intestine. This family of dendrimers may find an application in the treatment of iron overload.

Introduction

Transfusion-dependent patients such as those suffering from β-thalassaemia major develop a potentially fatal secondary haemosiderosis, and consequently, a selective iron chelator must be used to relieve such iron overload.[1] For this purpose, the design of an orally active iron chelator has been a major objective, resulting in a large number of iron chelators being synthesized. The orally active chelators deferiprone[2] and deferitrim[3] have been subjected to extensive clinical investigation. A method of preventing iron overload, which has been less extensively investigated, involves the reduction of iron absorption from the intestine by adminstering iron chelators which bind dietary iron irreversibly to form nontoxic, kinetically inert complexes that are not absorbed. Macromolecular chelators could, in principle, find application as nonabsorbable iron-selective additives. Such therapy is relevant for the treatment of haemochromatosis and thalassaemia intermedia.

Dendrimers[4] are unique synthetic macromolecules with highly branched structures and possess a number of applications in supramolecular chemistry,[5] nanoscience,[6] medicine,[7] and catalysis.[8] Surprisingly, dendritic metal scavengers have rarely been reported.[9] Considering the disadvantages of bidentate chelator-based macromolecules, we have designed a range of hexadentate ligand-terminated dendrimers. Extending our previous reports on preliminary studies on iron(III)-selective dendritic chelators,[10] we herein report the detailed synthesis of hexadentate-terminated dendrimers by adopting both divergent[11] and convergent synthetic approaches.[12] We demonstrate the iron chelating ability of these dendritic macromolecules by MALDI-TOF MS[a] and UV/Vis spectrometry. We have evaluated the iron(III) affinity of the hydroxypyridinone-based dendrimers by competition with a fluorescence probe. The ability of one of these hydropyridinone-based dendrimers to reduce intestinal absorption of iron(III) is reported.

Results and Discussion

Synthetic Strategy. (a) Divergent Approach. Initially, we synthesized four well-defined carboxyl group-terminated dendrimers, **1a**−**4a**, containing 9, 12, 18, and 27 carboxyl groups, respectively. Bidentate chelators, including hydroxypyridinones, hydroxypyranones, and catechols, were then conjugated to the carboxyl-terminated dendrimers via amide bonds using standard peptide synthetic methods.[13] Six dendritic chelators were synthesized using this method (Chart 1).

The synthetic route of the carboxyl-terminated dendrimers (**1a**−**4a**) is presented in Scheme 1. When amine **5**[14] is treated with 1,3,5-benzenetricarbonyltrichloride in the presence of triethylamine in dichloromethane, the three-directional nona-ester **6** was generated in excellent yield. Hydrolysis of **6** in formic acid afforded the first-generation carboxyl-terminated dendrimer **1a**. Coupling of the tetraacid **7** with amine **5** via treatment with DCCI/HOBt in DMF at room temperature provided dodeca-ester **9**, which was hydrolyzed in formic acid to give the four-directional first-generation carboxyl-terminated dendrimer **2a**. The second-generation 18-acid dendrimer **3a** was synthesized in four steps. The synthesis of second-generation 27-acid dendrimer **4a** was achieved via coupling of **1a** and amine **5** in the presence of DCCI and HOBt, followed by hydrolysis in formic acid. These carboxyl-terminated dendrimers have been characterized in detail.

Coupling of the polyacid dendrimers **1a**−**4a** with a protected bidentate chelator containing a free amino group such as 2-(aminomethyl)-3-(benzyloxy)-1,6-dimethylpyridin-4(1*H*)-one (**13**), 2-(aminomethyl)-3-(benzyloxy)-6-methyl-4*H*-pyran-4-one (**14**), and 2,3-dimethoxyphenyl methylamine (**15**) was carried out in the presence of DCCI and HOBt in DMF at room temperature. This yielded the corresponding protected dendritic chelators, which were isolated by chromatography on silica gel. Deprotection of the benzyl or methyl functions on the protected dendritic chelators was achieved by treatment with boron trichloride. It was found that the conjugation of the protected

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[a] Abbreviations: MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-light mass spectrometry; DCCI, 1,3-dicyclohexylcarbodiimide; HOBt, 1-hydroxybenzotriazole; DMF, *N*,*N*-dimethylformamide; MBT, 2-mercaptobenzothiazole; DCTB, *trans*-2-[3-{4-*tert*-butylphenyl}-2-methyl-2- propenylidene]malononitrile; ICP-MS, inductively coupled plasma mass spectrometry; TMS, tetramethylsilane; ESI MS, electrospray ionization mass spectrometry; CHCA, 4-hydroxy-alpha-cyano-cinnamic acid; THAP, trihydroxyacetophenone; NTA, nitrilotriacetic acid; MOPS, 3-(*N*-morpholino)propane sulphonic acid.

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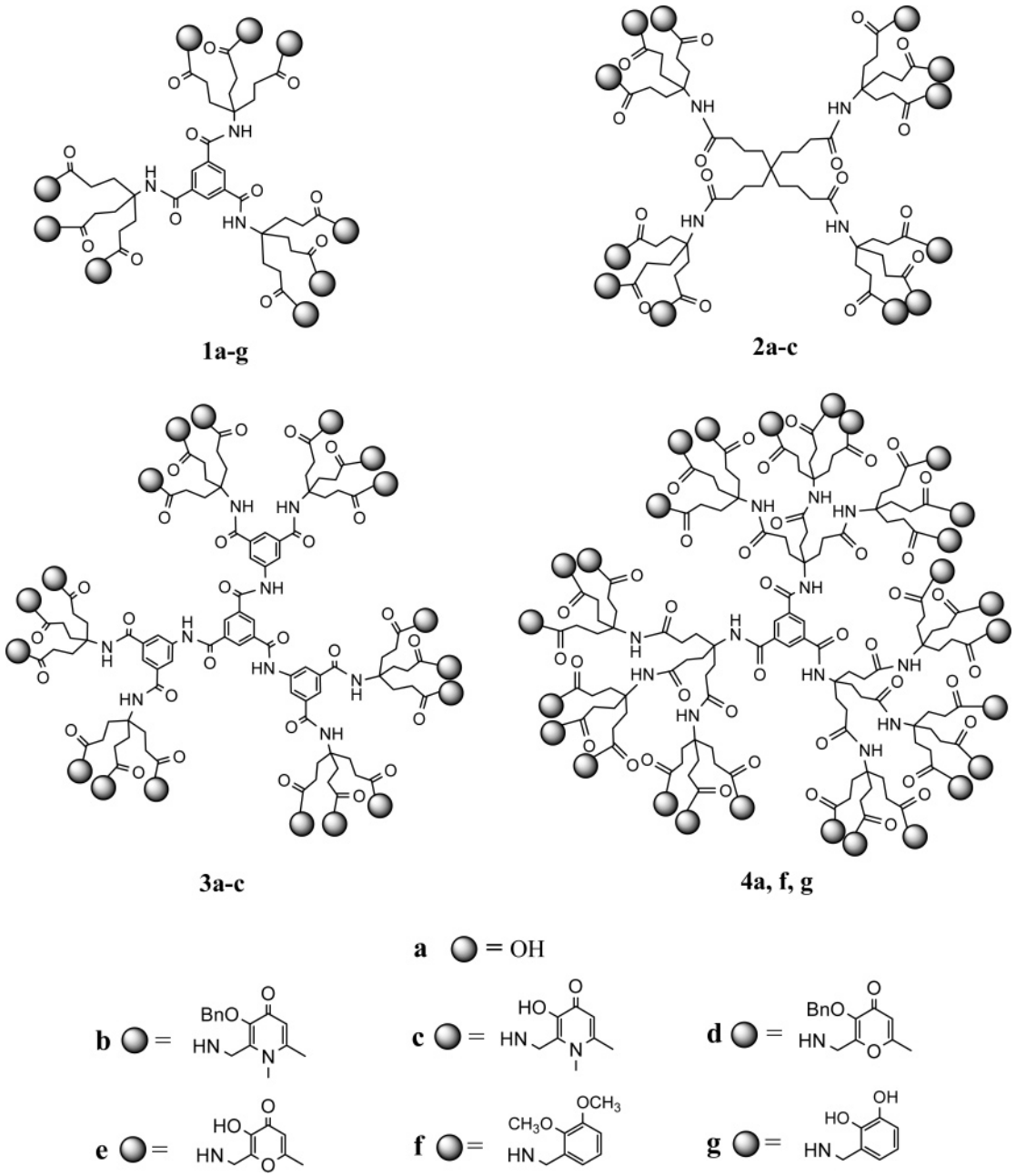
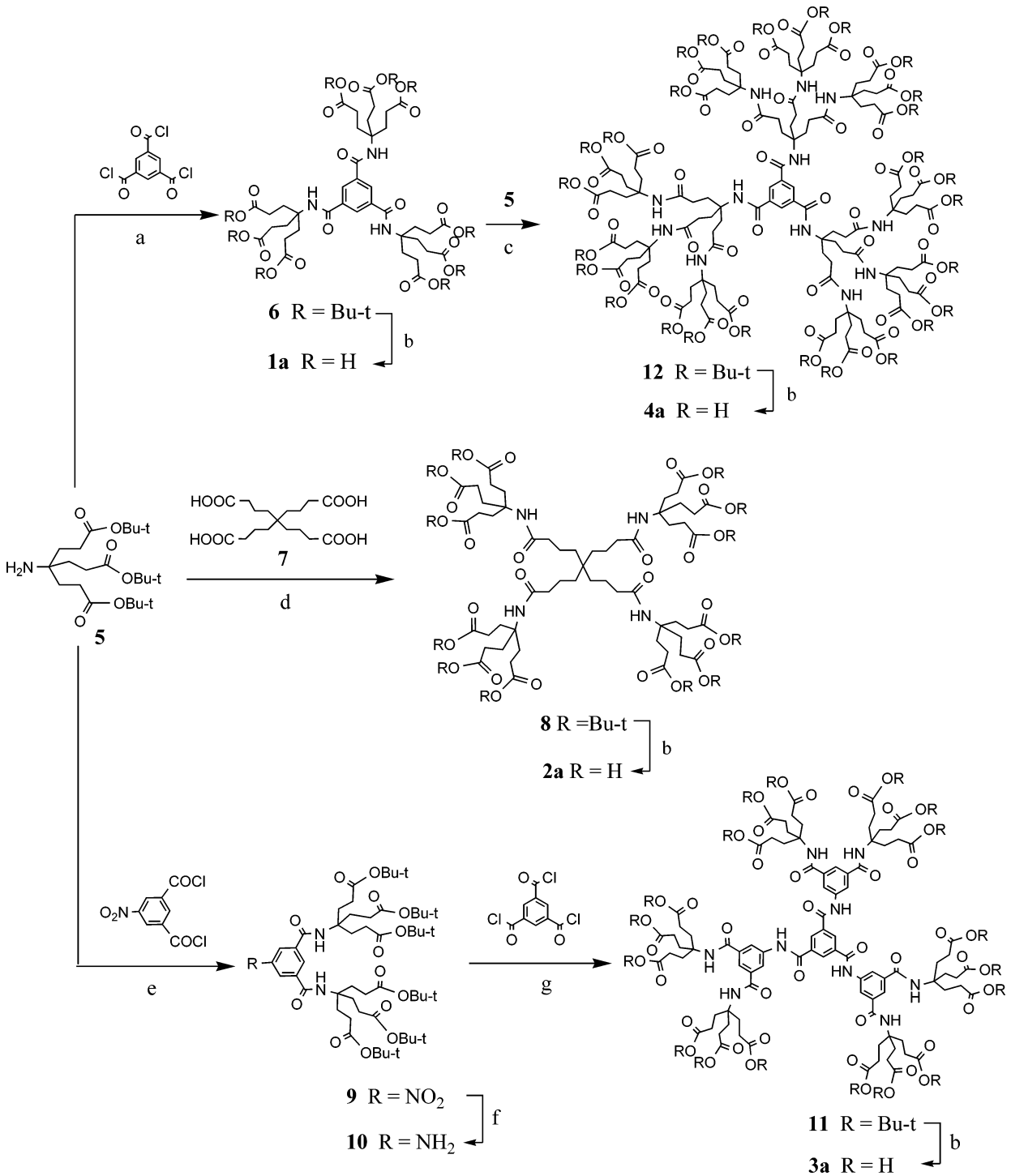
Chart 1. Polyacid Dendrimers, Protected and Deprotected Dendritic Chelators



bidentate chelators **13**−**15** on the polyacid dendrimers **1a**−**3a** could be carried out smoothly and the anticipated fully coupled products were obtained in good yield. Conjugation of the protected bidentate chelators **13**−**15** with 27-acid dendrimer **4a** was also investigated. An acceptable result was obtained from the reaction of **15** and **4a**, and although the reaction of **14** and **4a** produced the fully coupled product as a major product, incompletely converted products were also formed. The reaction of **13** and **4a** led to appreciable amounts of incompletely converted products, in addition to a small quantity of the desired fully coupled product, as confirmed by the MALDI-TOF mass spectrum. These differences in behavior probably result from a gradation of steric congestion, which is minimal with **15** but greater with **14** and **13**.

(b) Convergent Approach. We first synthesized a benzyl protected hexadentate chelator containing the free amino group **16** and the polyacid cores **21**[15] and **23**. The protection of the amino group of β-analine was achieved by treatment with ethyl 1-methylene-3-oxoisoindoline-2-carboxylate to give **17**(Scheme 2). Coupling of **17** with amine **5** occurred in the presence of DCCI and HOBt in DMF to give a triester **18**, which generated the triacid **19** on hydrolysis in formic acid. Compound **20** was prepared by conjugating the bidentate ligand **13** with the triacid **19**. Deprotection of amino group in **20** by hydrazinelysis yielded **16**. The polyacid core **23** was synthesized in four steps (Scheme 3). Treatment of crude 5-nitroisophthaloyl dichloride with ethyl 3-aminopropanoate hydrochloride in the presence of triethylamine gave **24**, which was subjected to hydrogenation in the presence of T1-Raney Ni catalyst to produce **25**. Reaction of **25** with 1,3,5-benzenetricarbonyltrichloride afforded **26**, which on hydrolysis with dilute sodium hydroxide in methanol followed by acidification generated the corresponding 6-acid core **23**. Conjugation of **16** to the polyacid cores **21,** nitrilotriacetic acid (**22**) and **23** gave the corresponding protected dendritic chelators **27**, **29**, and **31** (Scheme 4). After the removal of the protective benzyl groups, the dendritic chelators **28**, **30,** and **32** were obtained in 93%, 89%, and 94% yields, respectively.

Scheme 1. Synthesis of the Carboxyl Group-Terminated Dendrimers **1a**−**4a**[a]

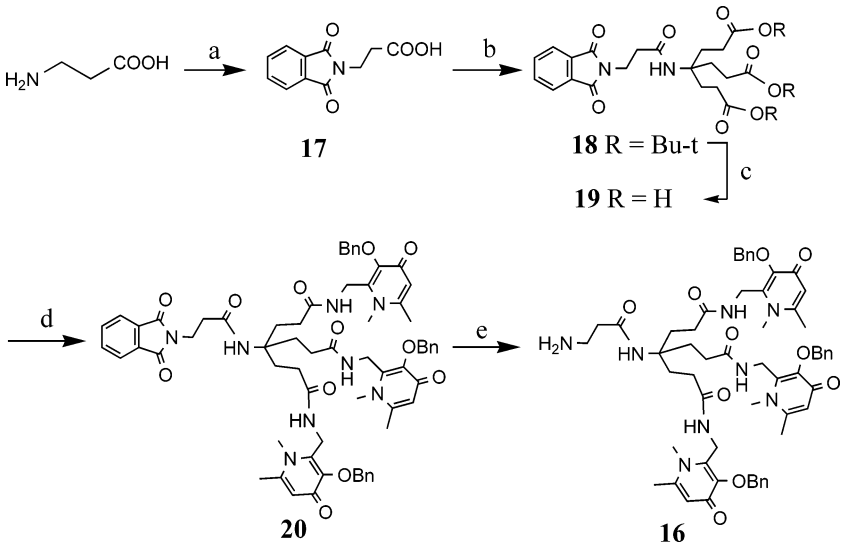


[a] (a) Et_3N, CH_2Cl_2, 95%; (b) 96% HCOOH, rt, nearly quantitative; (c) DCCI, HOBt, DMF, rt, 4d, 76%; (d) DCCI, HOBt, DMF, rt, 2d, 79%; (e) Et_3N, CH_2Cl_2, 92%; (f) H_2, T-1 Raney Ni, EtOH, 95%; (g) NEt_3, CH_2Cl_2, 65%.

All compounds synthesized were fully characterized using ^1H and ^{13}C NMR spectroscopy and mass spectrometry. The purity of these dendrimers could not be consistently characterized by elemental analysis, as they were hygroscopic. However, ^1H and ^{13}C NMR spectra provided strong qualitative evidence for the purity of these compounds.[16] NMR spectra demonstrated that the removal of benzyl and methyl protecting groups was complete. MALDI-TOF MS has previously proven to be a valuable technique for characterizing various dendrimers.[17] The efficiency of coupling reactions could be assessed by MALDI-TOF MS, and normally signals, as proton, sodium, or potassium adducts of the dendrimers were obtained. Occasionally, cesium chloride was added to promote the formation of cesium adducts of the dendrimers. A representative MALDI-TOF mass spectrum of the alkali metal adducts of dendrimer **4g** is presented in Figure 1. The peaks at *m*/*z* 6254.8, 6364.1, and 6497.3 correspond to sodium adduct ($[M + Na]^+$), cesium adduct ($[M + Cs]^+$), and dicesium adduct ($[M - H + 2Cs]^+$) of dendrimer **4g**, respectively.

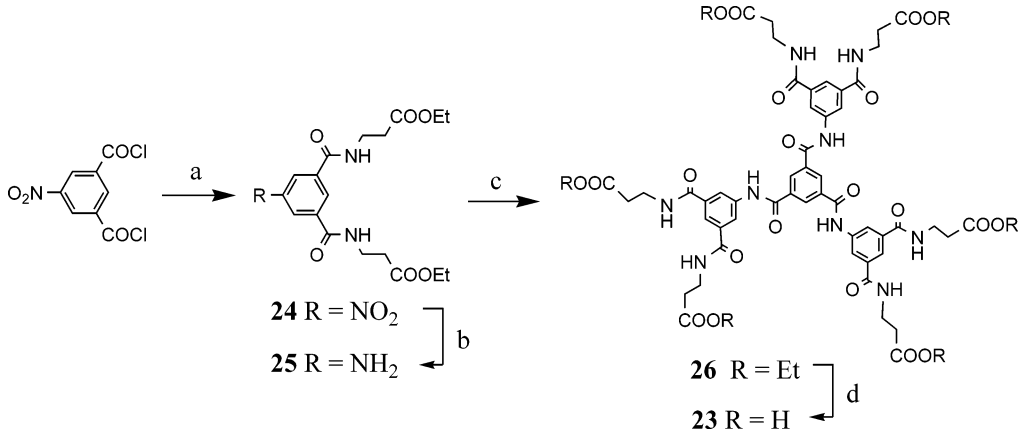
Iron Chelating Properties. Theoretically, three hydroxypyridinone ligands with ortho position groups relative to one of the chelating oxygen atoms attached to a suitable tripodal

Scheme 2. Synthesis of Protected Pyridinone Hexadentate Ligand Containing a Free Amino Group **16**[a]



[a] (a) *N*-Ethyloxycarbonylphthalimide, Na_2CO_3, H_2O, 72%; (b) HOBt, DCCI, DMF, 1d, 66%; (c) 96% HCOOH, rt, 97%; (d) HOBt, DCCI, DMF, 2d, 76%; (e) NH_2NH_2, EtOH, 96%.

Scheme 3. Synthesis of the [**6**]-Acid Core **23**[a]



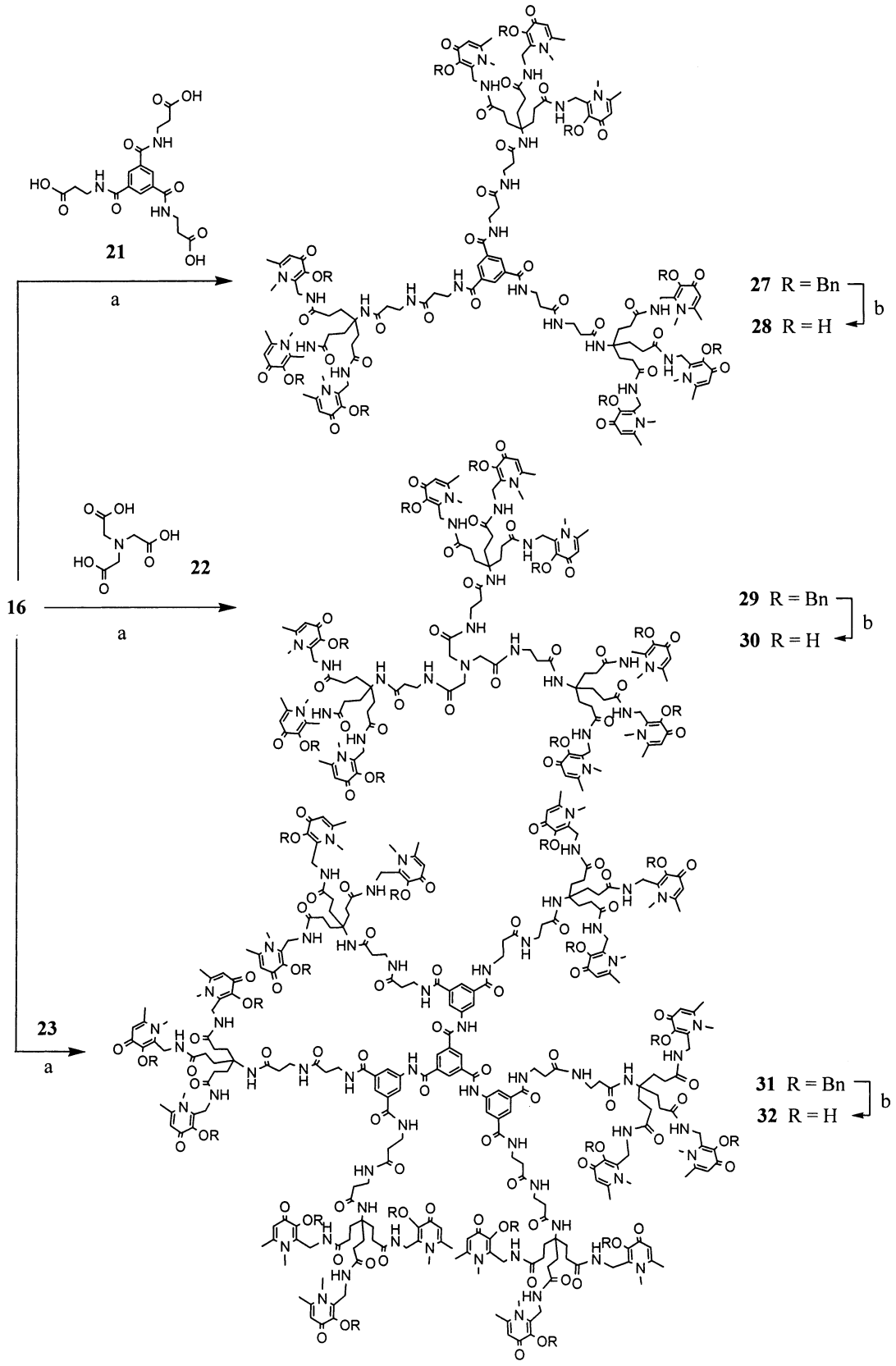
[a] (a) Ethyl β-analine hydrochloride, Et_3N, CH_2Cl_2, 78%; (b) H_2, T-1 Raney Ni, EtOH, 88%; (c) 1,3,5-benzenetricarbonyltrichlodide, Et_3N, CH_2Cl_2, 89%; (d) 2 M NaOH, MeOH and then 2 M HCl to pH 2, 84%.

molecule can form a high-affinity hexacoordination site.[18] Likewise, three catechol units linked to a suitable tripodal molecule can also form a hexadentate ligand.[19] Thus, it was reasoned that **1c**, **1e**, **1g**, **28**, and **30** effectively contain three hexadentate ligands, **2c** contains four hexadentate ligands, **3c** and **32** contain six hexadentate ligands, and **4g** contains nine hexadentate ligands. To evaluate the iron chelating properties of these dendrimers, both MALDI-TOF mass spectrometry and UV/Vis spectrometry were employed.

(a) Mass Spectrometry. These dendritic chelators were investigated for their iron chelating abilities using MALDI-TOF mass spectrometry. Direct evidence was obtained for the formation of iron complexes of pyridinone- and pyranone-terminated dendrimers (Table 1). The MALDI-TOF spectrum obtained from a one-to-three mixture of dendritic chelator **1c** and iron contains only one signal at *m*/*z* 2408.7, which corresponds to the proton adduct of one-to-three dendrimer−iron complex also annotated as $[M + (Fe^{III} - 3H)_3 + H]^+$.[10a] Evidently, three protons are released upon complexation of each iron(III). Similarly, the MALDI-TOF spectrum obtained from a one-to-four mixture of dendritic chelator **2c** and iron also contains only one signal at *m*/*z* 3290.0, which corresponds to the proton adduct of the one-to-four dendrimer−iron complex ($[M + (Fe^{III} - 3H)_4 + H]^+$). The MALDI-TOF spectrum obtained from the one-to-three mixture of dendritic chelator **28** and iron contains only one signal at *m*/*z* 2833.0, which corresponds to the proton adduct of the one-to-three dendrimer−iron complex also annotated as $[M + (Fe^{III} - 3H)_3 + H]^+$. The MALDI-TOF spectrum obtained from the one-to-three mixture of dendritic chelator **30** and iron contains two signals at *m*/*z* 2601.9 and 2623.9. These correspond to the proton adduct of the one-to-three dendrimer−iron complex ($[M + (Fe^{III} - 3H)_3 + H]^+$) and the sodium adduct of the one-to-three dendrimer−iron complex ($[M + (Fe^{III} - 3H)_3 + Na]^+$), respectively. The MALDI spectrum obtained from a one-to-six mixture of dendritic chelator **32** and iron also demonstrates complete iron binding; the signal at *m*/*z* 5949.6 corresponds to the proton adduct of the one-to-six dendrimer−iron complex also annotated as $[M + (Fe^{III} - 3H)_6 + H]^+$ (Figure 2). With these five pyridinone-based dendrimers, no evidence for the existence of iron-induced interdendrimer association was observed.

The MALDI-TOF spectrum obtained from a one-to-three mixture of dendritic chelator **1e** and iron contains a main signal at *m*/*z* 2312.3, which corresponds to the sodium adduct of the one-to-three dendrimer−iron complex also annotated as [M +

Scheme 4. Convergent Synthesis of the Dendritic Chelators **28**, **30**, and **32**[a]



[a] (a) DCCI, HOBt, DMF; (b) BCl_3, CH_2Cl_2.

$(Fe^{III} - 3H)_3 + Na]^+$, a minor peak at *m*/*z* 2290.2, corresponding to the proton adduct of the one-to-three dendrimer−iron complex ($[M + (Fe^{III} - 3H)_3 + H]^+$). However, another minor peak at *m*/*z* 2259.2, corresponding to the sodium adduct of the one-to-two dendrimer−iron complex ($[M + (Fe^{III} - 3H)_2 + Na]^+$, was also observed (Figure 3), which indicates **1e** did not chelate iron(III) completely and that pyranone-terminated dendrimers possibly possess a lower iron(III) affinity than the pyridinone-terminated dendrimers. The identification of a sodium adduct of the desired complex was due to the ubiquitous presence of

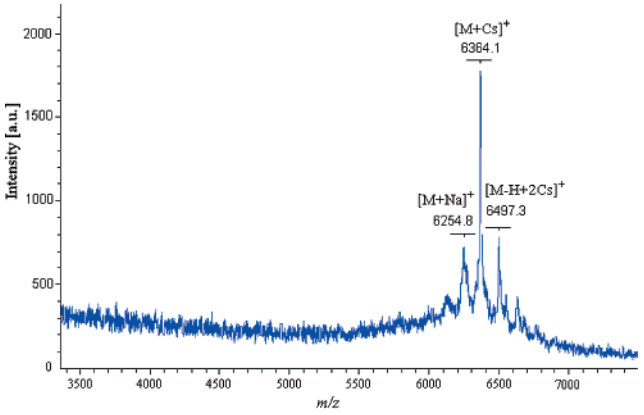


Figure 1. MALDI-TOF mass spectrum of dendrimer **4g**. Cesium chloride was added to the dendrimer preparation in order to encourage adduct formation. The MALDI matrix was CHCA.

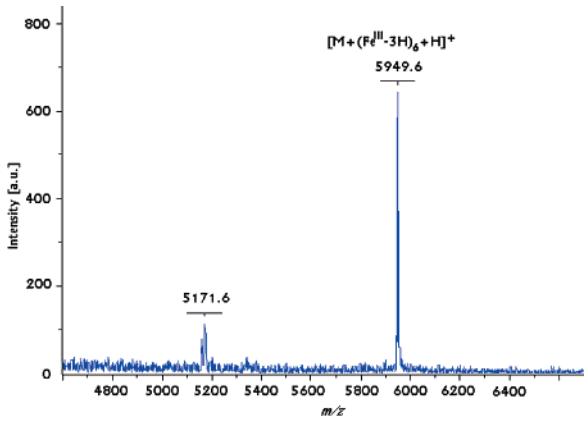


Figure 2. MALDI-TOF mass spectrum showing the proton adduct of the 1:6 dendrimer **32**−iron(III) complex $[M + (Fe^{III} - 3H)_6 + H]^+$. The peak at *m*/*z* 5171.6 shows the 1:5 dendrimer−iron(III) complex of the defect dendrimer, lacking one hexadentate branch. The spectrum was recorded from a DCTB matrix.

sodium in this dendrimer preparation and should not be misinterpreted as a specific chelation phenomenon.

The selection of the MALDI matrix was critical in ensuring desorption of intact metal complexes and matrixes such as MBT and DCTB often proved useful. Although these and several other matrixes were investigated for suitability in confirming the formation of iron−**3c** complex and iron−dendrimer functionalized with catechol (**1g**, **4g**) complexes, the corresponding peaks were not observed.

(b) UV/Vis Spectrometry. Several dendrimers were investigated by employing UV/ Vis spectrophotometric titration in order to evaluate the properties of the different binding groups (3-hydroxypyridin-4-one, pyranone, and catechol), dendrimer structures, and dendrimer generations that were synthesized.

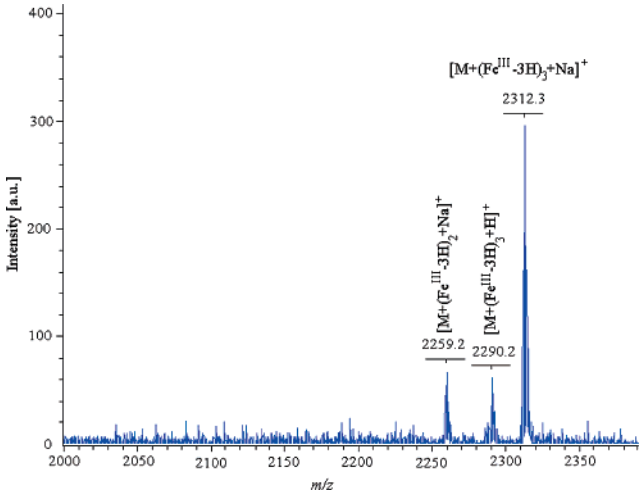


Figure 3. MALDI-TOF mass spectrum of a one-to-three mixture of dendritic chelator **1e** and iron. The peaks at *m*/*z* 2259.3, 2290.4, and 2312.3 correspond to the sodium adduct of a one-to-two dendrimer−iron complex $([M + (Fe^{III} - 3H)_2 + Na]^+$, the proton adduct of a one-to-three dendrimer−iron complex $([M + (Fe^{III} - 3H)_3 + H]^+)$, and the sodium adduct of a one-to-three dendrimer−iron complex $([M + (Fe^{III} - 3H)_3 + Na]^+)$, respectively. The spectrum was recorded from a DCTB matrix.

Titrations with iron(III) were undertaken on samples that were maintained at room temperature for at least 4 h in order to ensure the binding process was complete. In fact, kinetic studies indicated that pyridinone-terminated dendrimers bind iron(III) relatively quickly and complete acquisition within a few minutes (data not shown). The 3-hydroxypyridin-4-one-containing dendrimers **1c**, **2c**, **3c**, **28, 30**, and **32** all displayed similar spectral behavior. Addition of iron to hydroxypyridinone-terminated dendrimer-containing solutions resulted in the formation of iron−dendrimer complexes, which have a maximum absorbance at approximately 460 nm (Figure 4). In the case of dendrimers **1c**, **28**, and **30**, the intensity of absorbance at 460 nm increased until a 3:1 ratio of iron(III):dendrimer was obtained, after which the absorbance remained essentially constant, indicating the formation of a one-to-three ligand−iron(III) complex. With the iron(III) to dendrimer **1c**, **28**, and **30** ratios less than 3, the plot is linear, suggesting that the chelators **1c**, **28**, and **30** each possess a high affinity for iron(III) (as a sample, the data related to **28** is shown in Figures 4a and 4e). Likewise, in the case of dendrimer **2c**, the intensity of absorbance at 460 nm increased until a 4:1 ratio of iron(III):dendrimer was obtained (Figures 4b, 4f), indicating that **2c** binds 4 iron ions. For **3c** and **32,** the intensity of absorbance at 460 nm increased until a 6:1 ratio of iron(III):dendrimer was obtained (as a sample, the data related to **32** is shown in Figures 4c and 4g), indicating that both **3c** and **32** form 1:6 ligand−iron(III) complexes. These results

Table 1. MALDI Mass Spectrometric Data Showing the Saturated Iron Complexation of Dendritic Chelators

dendrimers	molecular formula	calculated MW for iron(III)−dendrimer complex	MALDI-TOF MS for iron(III)−dendrimer complex	matrix
1c	$C_{111}H_{141}N_{21}O_{30}$	2406.7 $[M + (Fe - 3H)_3 + H]^{+a}$	2407.6 $[M + (Fe - 3H)_3 + H]^+$	DCTB
1e	$C_{102}H_{114}N_{12}O_{39}$	2290.5 $[M + (Fe - 3H)_3 + H]^{+a}$	2290.2 $[M + (Fe - 3H)_3 + H]^+$	DCTB
		2312.5 $[M + (Fe - 3H)_3 + Na]^{+a}$	2312.3 $[M + (Fe - 3H)_3 + N a]^+$	
2c	$C_{153}H_{208}N_{28}O_{40}$	3290.2 $[M + (Fe - 3H)_4 + H]^{+a}$	3290.0 $[M + (Fe - 3H)_4 + H]^+$	MBT
3c	$C_{237}H_{291}N_{45}O_{63}$	5096.1$[M + (Fe - 3H)_6 + H]^{+b}$	not available	MBT, DCTB
28	$C_{129}H_{171}N_{27}O_{36}$	2834.0 $[M + (Fe - 3H)_3 + H]^{+a}$	2833.0 $[M + (Fe - 3H)_3 + H]^+$	MBT
30	$C_{117}H_{159}N_{25}O_{33}$	2601.9 $[M + (Fe - 3H)_3 + H]^{+a}$	2601.9 $[M + (Fe - 3H)_3 + H]^+$	MBT
		2623.9 $[M + (Fe - 3H)_3 + Na]^{+a}$	2623.9 $[M + (Fe - 3H)_3 + Na]^+$	
32	$C_{273}H_{351}N_{57}O_{75}$	5949.1 $[M + (Fe - 3H)_6 + H]^{+b}$	5949.6 $[M + (Fe - 3H)_6 + H]^+$	DCTB

[a] Monoisotopic molecular weight. [b] Average molecular weight.

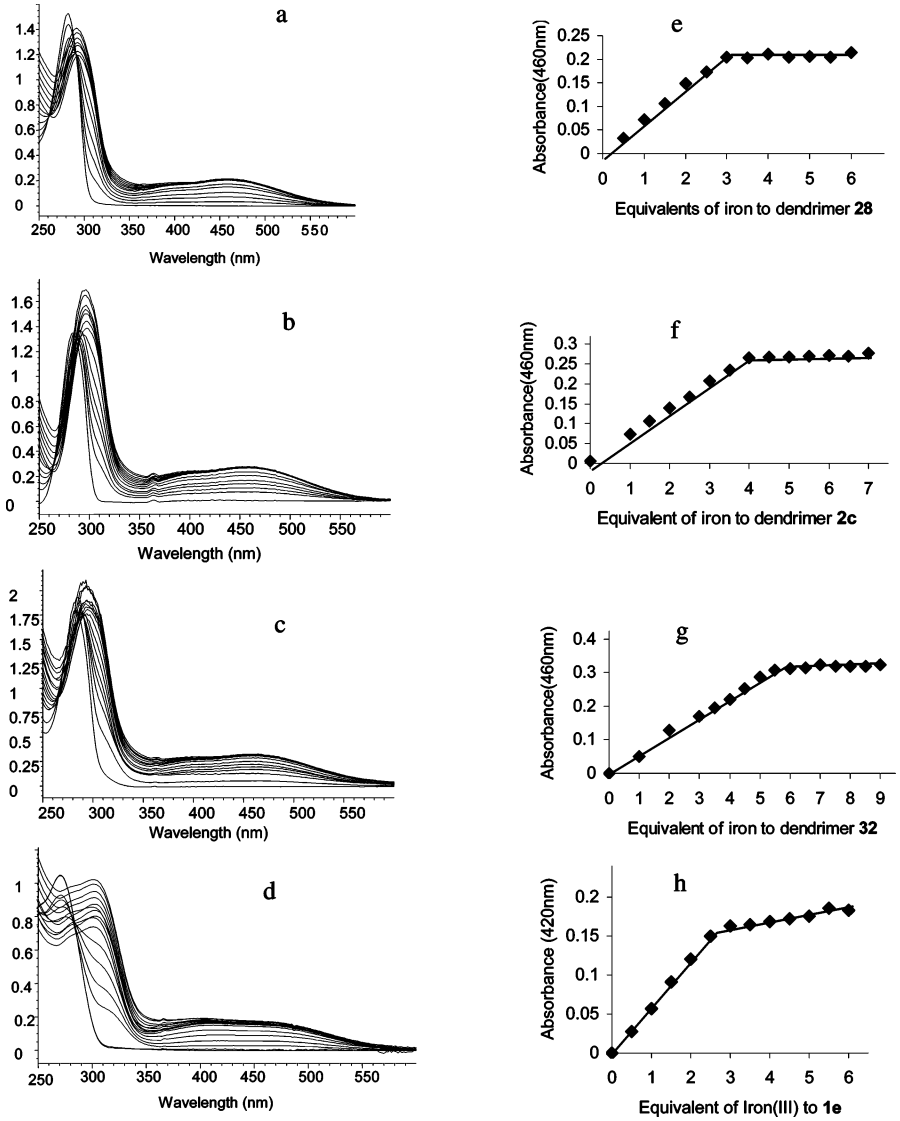


Figure 4. UV−Visible titration spectra of dendrimer **28** (a), **2c** (b), **32** (c), and **1e** (d) with iron(III) at pH 7.0. Plots of absorbance versus equivalent ratios of iron(III) to dendrimer **28** (e), **2c** (f), and **32** (g) at 460 nm and **1e** (h) at 420 nm. The concentrations of dendrimers **28**, **2c**, **32**, and **1e** were 14.3, 12.5, 10.0, and 14.3 μM. The solvent system was water for dendrimers **28** and **2c**, 5% methanol aqueous (v/v) for dendrimer **32**, and 7.1% methanol aqueous (v/v) for dendrimer **1e**.

suggest that each hexadentate branch binds one Fe^{3+}, as predicted, which is in agreement with the MALDI-TOF MS results.

The iron−**1e** complex has a maximum absorbance at 420 nm. However, the changes in the absorbance at 420 nm increased linearly up to a iron(III):dendrimer **1e** ratio of 2.5:1, at which point the absorbance only marginally increased further with the addition of iron (Figures 4d and 4h). This indicates that **1e** failed to become fully iron saturated. Surprisingly, with the catechol-terminated dendrimers **1g** and **4g**, the addition of iron to the chelator initially resulted in the formation of a purple-red solution, which changed to dark-blue after a day and to brown after a further 3 days. This phenomenon is attributed to the catechol-mediated reduction of iron(III) to iron(II) (blue) which is then gradually oxidized by oxygen.[20]

Selectivity for Iron. The selectivity of the hydroxypyridinone dendrimers for Fe^{3+} compared with other metal ions, for instance, Cu^{2+}, Mg^{2+}, Zn^{2+}, and Ca^{2+}, was investigated using MALDI-TOF mass spectrometry and this analysis is illustrated using the dendritic chelator **1c**. The MALDI spectra recorded from 3:1 mixtures of any of the above metal ions excluding iron(III) and dendritic chelator **1c** yielded a strong signal for the free, "nonchelated" dendrimer and a variety of signals from numerous 1:1 complexes. This indicated that the dendrimers bind these other metal ions only very weakly. The example shown in Figure 5a illustrates the selectivity for iron(III) in comparison to that for Zn(II), whereby no higher order Zn(II) complexes were observed; in contrast, contaminating iron(III) ions were chelated. No higher order complexes could be observed other than mixed 1:1:1 iron(III)−zinc−**1c** complexes. In contrast, spectra obtained from 3:3:1 mixtures of iron, any of the above metal ions, and dendritic chelator **1c** provided evidence for the presence of only the 3:1 of iron and dendritic chelator **1c** complex. No other metal ion−dendrimer complexes were observed, indicating that dendrimer **1c** possesses a high selectivity for iron(III). As an example, a MALDI-TOF mass spectrum investigating the selectivity of **1c** between iron and zinc is presented in Figure 5b.

Affinity Constant of Iron Complex. To estimate the affinity of the hydroxypyridinone-terminated dendritic chelators for iron(III), the pK_a values of the analogous hexadentate ligand **33** (Chart 2) were investigated using an automated titration system;[21] the stability constant (log K_1) of hexadentate ligand **33** was determined spectrophotometrically by competition with

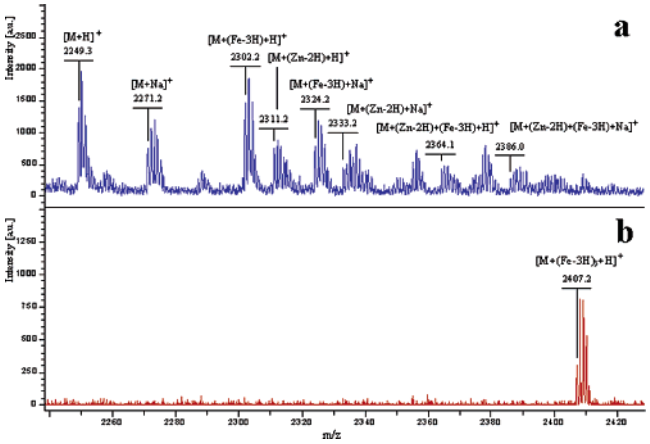
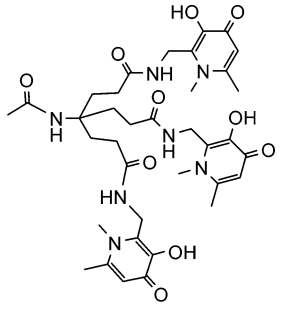


Figure 5. (a) MALDI mass spectrum obtained from the 3:1 mixture of Zn^{2+} and dendritic chelator **1c**; (b) MALDI mass spectrum obtained from the 3:3:1 mixture of Fe^{3+}, Zn^{2+}, and dendritic chelator **1c.**

Chart 2. Hexadentate Ligand **33**



33

EDTA. Hexadentate ligand **33** was found to possess a very high affinity for iron(III), namely, log $K_1 = 32.52$; $pFe^{3+} = 28.47$. The pFe^{3+} value of **33** is over 8 log units higher than that of the bidentate analogue 3-hydroxy-1,2-dimethylpyridin-4(1*H*)-one ($pFe^{3+} = 19.7$).

To evaluate the iron(III) affinity of hexadentate hydroxypyridinone-based dendrimers, a study associated with competition between a fluorescent chelator CP691[22] and hexadentate **33** or dendrimers (**1c**, **2c**, **3c**, **28**, **30**, and **32**) was performed. When CP691 is mixed with iron, fluorescence is quenched to a low level, but is gradually increased on the addition of a competing ligand (either hexadentate **33** or dendritic chelators)**.** When the competition reaches equilibrium, the relative fluorescence intensity of the corresponding solutions were found to be similar (Table 2), which indicates that the hexadentate moieties on the dendrimers possess a similar affinity for iron(III) as hexadentate **33**. As the hexadentate moieties on the dendrimers under investigation utilize the same pyridinone bidentate ligands as hexadentate **33,** it is assumed that these hexadentate moieties possess the same pK_a values as those of **33**. The iron(III) affinities of the hexadentate pyridinone moieties on these hydroxypyridinone-based dendrimers were estimated from the pK_a values and affinity constant of **33.** They are presented in Table 3. Essentially all the determined affinity constants are not significantly different from each other or from that of **33**. These findings indicate that the length of arm, nature of core, and generation of dendrimer do not greatly influence the iron binding affinities of the chelating end groups. Thus, there is no evidence for a dendritic effect typically associated with many dendritic binding phenomena.[23] This results from the maximum binding enhancement of iron already having been achieved in the design of the optimal hexadentate binding motifs.[24]

Table 3. Affinity Constants of 3-Hydroxypyridin-4-one-Terminated Dendrimers

	1c	28	30	2c	3c	32
log *K*	32.60	32.74	32.58	32.28	32.52	32.60
pFe^{3+}	28.55	28.69	28.53	28.24	28.47	28.55

In Vitro Intestinal Perfusion Study. To evaluate the ability of dendrimer **1c** to prevent gastointestinal iron(III) absorption, an in vitro rat intestinal perfusion method described by Schümann et al.[25] was adopted. ^{58}Fe is utilized in these experiments because ^{58}Fe presented to samples can be selectively measured by ICP-MS, thus avoiding interference from the existing iron in lumen. Two ^{58}Fe concentrations (50 and 10 μM) were used in the perfusion studies. The ratios of the calculated iron binding site numbers of dendrimer **1c** to ^{58}Fe were adjusted to either 1:1 or 2:1. Thus, in the case of 50 μM iron concentration, the concentration of dendrimer **1c** in perfusate was 16.7 or 33.3 μM, respectively, because 1 mol of dendrimer **1c** is able to bind 3 mol of iron. Similarly, in the case of 10 μM ^{58}Fe concentration, the concentration of dendrimer **1c** in perfusate was 3.33 or 6.67 μM, respectively. The samples (absorbates) were collected at the same time intervals as the control groups. The ^{58}Fe content in the samples was determined by ICP-MS. The results are presented in Figure 6. In the 50 μM ^{58}Fe concentration perfusion experiments, the accumulated iron contents in the absorbates of the 1:1 group and 2:1 group at 120 min were 123.9 and 48.0 pmol/cm of tissue, respectively, significantly less than that from the control groups (1826.5 pmol/cm of tissue) (Figure 6a). In the 10 μM ^{58}Fe concentration perfusion experiments, the accumulated iron contents in the absorbates of the 1:1 group and 2:1 group in 120 min are 68.5 and 31.5 pmol/cm of tissue, respectively (Figure 6b). Compared with the control groups (249.2 pmol/cm of tissue), the absorption of iron was again significantly reduced. Thus, dendritic chelators, by reducing iron absorption, may find application in iron overload therapy. Hydroxypyridinone-containing dendrimers have the advantage over tannic acid,[26] an iron chelator normally present in the diet, of being highly specific for iron(III); thus, they are predicted not to interfere with the gastrointesinal absorption of zinc and copper. With molecular weights in the range of 2000−6000, these iron-chelating dendrimers are

Table 2. Comparison of the Iron(III) Affinities of the Hexadentate Ligand **33** and Dendritic Chelators Using the Fluorescence Method[a]

	relative fluorescence intensity (%)						
time	CP691 + Fe(III) + **33**	CP691 + Fe(III) + **1c**	CP691 + Fe(III) + **28**	CP691 + Fe(III) + **30**	CP691 + Fe(III) + **2c**	CP691 + Fe(III) + **3c**	CP691 + Fe(III) + **32**
4 h	31.5	33.7	34.4	33.0	29.2	31.0	31.7
1 d	44.4	46.6	49.2	46.5	39.2	43.9	45.5
2 d	50.5	51.8	54.5	50.4	44.5	48.1	50.7
3 d	54.7	56.6	60.1	54.6	47.2	54.2	56.7
4 d	55.1	57.5	61.3	56.7	48.2	55.2	57.2
5 d	55.3	57.6	61.5	57.0	48.5	55.2	57.6

[a] Final concentration: [CP691] = 6 μM, [Fe(III)] = 6 μM, [**33**] = 6 μM, [**1c**] = [**28**] = [**30**] = 2 μM, [**2c**] = 1.5 μM, [**3c**] = [**32**] = 1 μM.

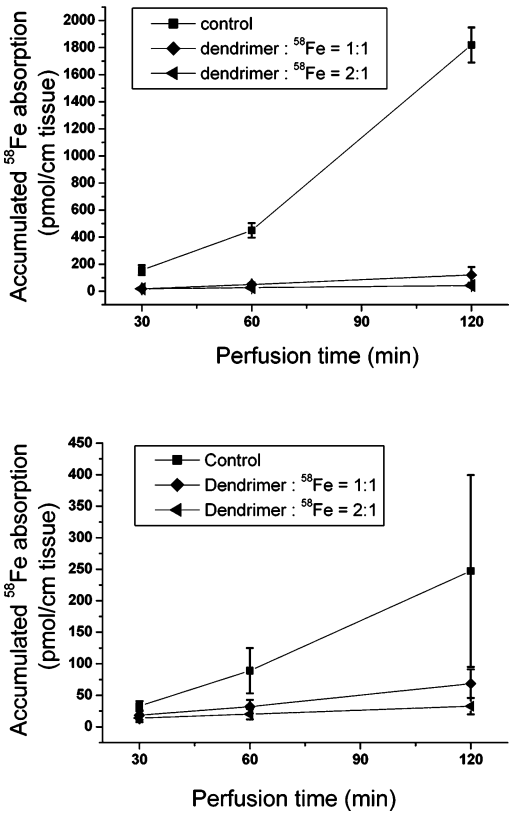


Figure 6. In vitro intestinal perfusion investigation. (a) Initial concentration of ^{58}Fe is 50 μM; (b) initial concentration of ^{58}Fe is 10 μM.

predicted to not be appreciably absorbed by the mammalian intestine. This prediction is currently under investigation using ^{14}C-labeled dendrimers.

Experimental Section

General. All chemicals were reagent grade and were used without further purification. Melting points were determined on an Electothermal IA 9100 Digital Melting Point Apparatus and were uncorrected. ^{1}H NMR spectra were recorded on a Bruker Avance 360 (360 MHz) or Bruker Avance 500 (500 MHz) spectrometer. Chemical shifts (δ) for ^{1}H spectra are given in parts per million (ppm) downfield from the internal standard TMS. ^{13}C NMR spectra (90 MHz) were recorded on a Bruker Avance 360 spectrometer. Chemical shifts (δ) for ^{13}C spectra are given in ppm relative to the signal of $CDCl_3$ (δ 77.16) or DMSO (δ 39.52). MALDI-TOF mass spectra were recorded on an Autoflex MALDI-TOF mass spectrometer (Brukes Daltonics, Coventry, UK). ESI mass spectra were obtained by infusing samples into an LCQ Deca XP ion trap instrument. HRMS were determined at the Strand campus, King's College London. UV spectra were recorded on a HP8453 UV spectrometer.

Nonaester 6. A solution of 1,3,5-benzenetricarbonyltrichloride (1.33 g, 5 mmol) in CH_2Cl_2 was slowly added to a stirred mixture of amine **5** (7.48 g, 18 mmol) and triethylamine (1.82 g, 18 mmol) in CH_2Cl_2 cooled by an ice bath. Stirring was continued overnight. The reaction mixture was washed successively with cold aqueous hydrochloric acid (10%), aqueous sodium hydrogen carbonate (10%), and water, dried over Na_2SO_4, and concentrated. The residue was subjected to column chromatography on silica gel (EtOAc/hexane 1:1) to give 6.67 g (95%) of **6** as a white solid.

General Procedure for Preparation of Polyacid Dendrimers (1a, 2a, 3a, 4a, and 19). A solution of polyester dendrimers (**6**, **8**, **11**, **12**, and **18**) in formic acid was stirred overnight. After concentration and the removal of residual formic acid, the crude product was obtained.

Compound 8. A mixture of tetraacid **7** (0.72 g, 2 mmol), amine **5** (3.99 g, 9.6 mmol), DCCI (1.98 g, 9.6 mmol), HOBt (1.30 g, 9.6 mmol), and DMF (30 mL) was stirred at room temperature for 2 d. After filtration and removal of the solvent under reduced pressure, the residue was chromatographed on silica gel using EtOAc/hexane (2:1) as an eluent to afford 3.08 g (79%) of **8** as a white solid.

Compound 9. 5-Nitroisophthalic acid (3.16 g, 15 mmol) was suspended in $SOCl_2$ (30 mL); the mixture was refluxed for 24 h. Excess of $SOCl_2$ was removed in vacuo, dry benzene (3 × 5 mL) was added, and the solution was concentrated in vacuo. The residue was dissolved in dry CH_2Cl_2 (60 mL). After cooling to 0 °C, amine **5** (13.71 g, 33 mmol) and Et_3N (3.33 g, 33 mmol) were added; the mixture was stirred at room temperature overnight. The reaction mixture was washed successively with aqueous sodium hydrogen carbonate (10%), water, cold aqueous hydrochloric acid (10%), and brine. The organic phase was dried over Na_2SO_4 and concentrated. The residue was subjected to column chromatography on silica gel (EtOAc/hexane 1:1) to give **9** (13.87 g, 92%) as a white solid.

Analogous procedures were used for the preparation of compound **24**.

Amino Ester 10. A solution of nitro ester **9** (10.10 g, 10 mmol) in anhydrous DMF (100 mL) with T-1 Raney Ni[27] (5.0 g) was hydrogenated at 50 psi and room temperature for 2 d. After the catalyst was cautiously filtered, the solvent was removed in vacuo, affording a solid, which was column-chromatographed on silica gel, eluting with $CHCl_3$/MeOH (9.5:0.5) to give **10** (8.68 g, 89%) as a white solid.

Analogous procedures were used for the preparation of compound **25**.

Compound 11. A solution of 1,3,5-benzenetricarbonyltrichloride (0.53 g, 2 mmol) in DCM was slowly added to a stirred mixture of amine **10** (7.03 g, 7.2 mmol) and triethylamine (7.27 g, 7.2 mmol) in DCM cooled by an ice bath. Stirring was continued overnight. With use of the workup procedure described above for **6**, compound **11** (4.96 g, 65%) was isolated as a white solid. Analogous procedures were used for the preparation of compound **26**.

General Procedure for Coupling of Bidentate or Hexadentate Ligand Containing a Free Amine Group with Polyacid. Illustrated for the preparation of protected dendritic chelator 1b. A mixture of nonaacid dendrimer **1a** (0.90 g, 1 mmol), 1,6-dimethyl-2-aminomethyl-3-benzyloxy-pyridin-4(1*H*)-one **13** (2.79 g, 10.8 mmol), DCCI (2.22 g, 10.8 mmol), HOBt (1.46 g, 10.8 mmol), and DMF (30 mL) was stirred for 2 d. After filtration and removal of solvent under reduced pressure, the residue was chromatographed in a silica gel column using MeOH/$CHCl_3$ (1:4) as eluent, followed by MeOH/$CHCl_3$/40% ammonium hydroxide aqueous (10:40:1) to afford **1b** (2.70 g, 88% yield) as a white solid.

Analogous procedures starting with other bidentate ligands or hexadentate ligand and polyacid gave protected dendritic chelators **1d**, **1f**, **2b**, **3b**, **4f**, **20**, **27**, **29**, and **31**.

General Procedure for the Deprotection of Benzyl Groups and Methyl Groups on Protected Dendritic Chelators. Illustrated for the Preparation of Dendritic Chelator 1b. In an atmosphere of nitrogen, 1 M boron trichloride in dichloromethane (18 mL, 18 mmol) was dropped slowly into an ice-bath-cooled suspension of solution of **1b** (2.45 g, 0.8 mmol) in CH_2Cl_2 (25 mL). The mixture was stirred at room temperature for 1 d. Methanol (20 mL) was added to quench the reaction. After the removal of the solvent, the residue was precipitated with methanol/acetone three times to afford the hydrochloric acid salt of **1c** as a white powder (1.88 g, 91%). ^{1}H NMR (360 MHz, DMSO-d_6): δ 2.01 (br, CH_2, 18H), 2.16 (br, CH_2, 18H), 2.56 (s, CH_3, 27H), 3.88 (s, CH_3, 27H), 4.55 (br, CH_2, 18H), 7.25 (s, pyridinone C-5H, 9H), 8.06 (br, ArH, 3H), 8.41 (s, NH, 3H), 8.99 (br, NH, 3H); ^{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), 39.4 (N*C*H_3), 113.1 (pyridinone *C*-5H), 140.1 (pyridinone *C*-2), 143.1 (pyridinone *C*-3), 148.9 (pyridinone *C*-6), 160.1 (pyridinone *C*-4), 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 $[M + H]^+$ (CHCA as matrix).

Analogous procedures for deprotection of other protected chelators (**1d**, **1f**, **2b**, **3b, 4f, 27**, **29**, and **31)** gave chelators **1e**, **1g**, **2c**, **3c**, **4g**, **28**, **30**, and **32**.

Dendritic Chelator 1e. After quenching of the reaction and the removal of solvent, the residue was precipitated with methanol/diethyl ether three times to afford **1e** (82%) as a white powder. ^{1}H NMR (DMDO-d_6) δ 2.02 (br, CH_2, 18H), 2.11 (br, CH_2, 18H), 2.20 (s, CH_3, 27H), 4.21 (d, J = 5.0 Hz, CH_2, 18H), 6.19 (s, pyranone C-5H, 9H), 7.88 (m, ArH, 3H), 8.33−8.37 (m, NH, 12H); ^{13}C NMR δ 19.6 (*C*H_3), 29.8 (C*C*H_2CH$_2$), 29.3 (CCH$_2$*C*H$_2$), 35.8 (NH*C*H$_2$), 111.6 (pyranone *C*-5H), 142.0 (pyranone *C*-2), 147.3 (pyranone *C*-3), 164.8 (pyranone *C*-6), 172.8 (*C*ONH), 173.9 (pyranone *C*-4). MALDI-TOF MS: Calculated for $C_{102}H_{114}N_{12}O_{39}$, 2130.7 (monoisotopic molecular weight M); found, 2131.7 [M + H]$^+$ (CHCA as matrix).

Dendritic Chelator 1g. The reaction time was 2 d. 78% yield. ^{1}H NMR (DMDO-d_6) δ 2.02 (br, CH_2, 18H), 2.13 (br, CH_2, 18H), 4.13 (d, J = 5.3 Hz, CH_2, 18H), 6.53−6.58 (m, ArH, 18H), 6.66 (m, ArH, 9H), 7.88 (br, ArH, 3H), 8.39 (br, NH, 3H), 8.44 (m, NH, 9H); ^{13}C NMR δ 29.9 (C*C*H$_2$CH$_2$), 30.6 (CCH$_2$*C*H$_2$), 38.4 (NH*C*H$_2$), 114.8 (*C*H in Ar), 119.2 (*C*H in Ar), 119.8 (*C*H in Ar), 126.3 (*C* in Ar), 143.3 (*C* in Ar), 145.7 (*C* in Ar), 173.5 (*C*ONH). MALDI-TOF MS: Calculated $C_{102}H_{114}N_{12}O_{30}$, 1986.8 (monoisotopic molecular weight); found, 1987.9 [M + H]$^+$, 2009.9 [M + Na]$^+$ (CHCA as matrix).

Dendritic Chelator 2c Hydrochloride. 91% yield. ^{1}H NMR (DMDO-d_6) δ 0.99 (br, CH_2, 8H), 1.22 (br, CH_2, 8H), 1.79 (br, CH_2, 24H), 1.97 (br, CH_2, 8H), 2.08 (br, CH_2, 24H), 2.55 (s, CH_3, 36H), 3.87 (s, CH_3, 36H), 4.55 (br, CH_2, 24H), 7.23 (pyridinone C-5H, 12H), 8.94 (br, NH, 12H); ^{13}C NMR δ 21.0 (*C*H_3), 29.7 (C*C*H$_2$CH$_2$), 30.3 (CCH$_2$*C*H$_2$), 35.1 (NH*C*H$_2$), 39.4 (N*C*H$_3$), 113.1 (pyridinone *C*-5H), 140.0 (pyridinone *C*-2), 143.1 (pyridinone *C*-3), 149.0 (pyridinone *C*-6), 160.0 (pyridinone *C*-4), 174.0 (*C*ONH). MALDI-TOF MS (CHCA as matrix): Calculated for $C_{153}H_{208}N_{28}O_{40}$, 3077.5 (monoisotopic molecular weight M); found, 3078.6 [M + H]$^+$ (CHCA as matrix).

Dendritic Chelator 3c Hydrochloride. 88% yield. ^{1}H NMR (MeOD-d_4) δ 2.20 (br, CH_2, 36H), 2.43 (br, CH_2, 36H), 2.63 (s, CH_3, 54H), 4.03 (s, CH_3, 54H), 4.74 (br, CH_2, 36H), 7.11 (s, pyridinone C_5−H, 18H), 7.99 (m, ArH, 3H), 8.52 (m, ArH, 6H), 9.01 (m, ArH, 3H); ^{13}C NMR (MeOD-d_4) δ 21.9 (*C*H_3), 31.1 (C*C*H$_2$CH$_2$), 31.4 (CCH$_2$*C*H$_2$), 37.0 (NH*C*H$_2$), 40.7 (N*C*H$_3$), 114.7 (pyridinone *C*-5H), 141.3 (pyridinone *C*-2), 145.0 (pyridinone *C*-3), 151.4 (pyridinone *C*-6), 161.5 (pyridinone *C*-4), 177.2 (*C*ONH). MALDI-TOF MS: Calculated for $C_{237}H_{291}N_{45}O_{63}$, 4775.1 (monoisotopic molecular weight M); found, 4776.3 [M + H]$^+$ (CHCA as matrix).

Dendritic Chelator 4g. The reaction time was 4 d. White solid, 73% yield. ^{1}H NMR (DMDO-d_6) δ 1.85 (br, CH_2, 72H), 2.10 (br, CH_2, 72H), 4.13 (br, CH_2, 54H), 6.53 (m, ArH, 54H), 6.65 (m, ArH, 27H), 7.34 (br, NH, 12H), 7.91 (br, ArH, 3H), 8.45 (br, NH, 27H); ^{13}C NMR δ 29.7 (C*C*H$_2$CH$_2$), 30.3 (CCH$_2$*C*H$_2$), 38.5 (CONH*C*H$_2$), 57.1 (*C*CH$_2$CH$_2$), 114.8 (*C*H in Ar), 119.3 (*C*H in Ar), 120.0 (*C*H in Ar), 126.2 (*C* in Ar), 143.3 (*C* in Ar), 145.7 (*C* in Ar), 173.9 (CONH). MALDI-TOF MS: Calculated for $C_{318}H_{375}N_{39}O_{93}$, 6231.7 (average molecular weight M); found, 6354.8 [M + Na]$^+$, 6364.1 [M + Cs]$^+$, 6497.3 [M − H + 2Cs]$^+$ (CHCA as matrix).

3-(1,3-Dioxo-1,2-dihydro-isoindol-2-yl)-propionic Acid 17. A vigorously stirred solution of β-analine (4.46 g, 50 mmol) and sodium carbonate (5.35 g, 50.5 mmol) was treated with *N*-ethyloxycarbonylphthalimide (11.51 g, 52.5 mmol). After 30 min almost all the reagent dissolved. The solution was filtered from a small amount of unreacted starting material and acidified to pH 2−3 with 6 M HCl. Filtration was followed by washing with water and drying in air. Compound **17** (7.89 g, 72% yield) was collected on a filter as white crystals. mp 150−152 °C (lit:[28] 152−153 °C).

4-(2-*tert*-Butoxycarboxy-ethyl)-4-[3-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-propionylamino]-heptanedioic Acid Di-*tert*-butyl Ester (18). A mixture of 17 (2.19 g, 10 mmol), amine 5 (4.57 g, 11 mmol), DCCI (2.27 g, 11 mmol), HOBt (1.49 g, 11 mmol), and DMF (60 mL) was stirred at room temperature for 1 d. After filtration and solvent removal under reduced pressure, the residue was purified on a silica gel column using EtOAc/MeOH (9.5:0.5) as an eluent to afford **18** (4.08 g, 66%). mp 144−145 °C.

Compound 16. To a solution of **20** (3.51 g, 3 mmol) in ethanol (40 mL) was added 5.5% aqueous hydrazine (3 mL). After being refluxed for 3 h, the reaction mixture was cooled to 0 °C, acidified to pH 1 with concentrated HCl, and filtered. The filtrate was concentrated in vacuo, and the residue was dissolved in distilled water (40 mL), adjusted to pH 12 with 10 M NaOH, and extracted with chloroform (5 × 100 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 under reduced pressure to furnish **16** (2.99 g, 96%) as a white solid.

[6]-Acid Core 23. To an ice-bath-cooled solution of **26** (2.59 g, 2 mmol) in methanol (15 mL) was added 2 M NaOH (50 mL); the mixture was left to stir for 3 h. Neutralized with 2 M HCl to pH 2 and the precipitate collected, washed with water several times, and dried in air, compound **23** (1.89 g, 84%) was obtained as a white solid.

Dendritic Chelator 28 Hydrochloride. 93% yield, a white powder. ^{1}H NMR (360 MHz, DMSO-d_6) δ 1.81 (br, CH_2, 18H), 2.09 (br, CH_2, 18H), 2.25 (m, CH_2, 6H), 2.41 (m, CH_2, 6H), 2.56 (s, CH_3, 27H), 3.22 (m, CH_2, 6H), 3.45 (m, CH_2, 6H), 3.89 (s, CH_3, 27H), 4.57 (d, J = 3.9 Hz, CH_2, 18H), 7.28 (s, pyridinone C-5H, 9H), 7.35 (br, NH, 3H), 8.10 (m, NH, 3H), 8.49 (s, NH, 3H), 8.94 (m, NH, 9H; Ar, 3H); ^{13}C NMR (90 MHz, DMSO-d_6) δ 20.9 (*C*H_3), 29.6 (C*C*H$_2$CH$_2$CO), 30.3 (CCH$_2$*C*H$_2$CO), 35.1 (NH*C*H$_2$), 39.5 (N*C*H$_3$), 57.2 (NH*C*), 113.1 (*C-5*H in pyridinone), 140.4 (*C-2* in pyridinone), 143.0 (*C-3* in pyridinone), 148.9 (*C-6* in pyridinone), 159.8 (*C-4* in pyridinone), 170.7 (*C*ONH), 173.8 (*C*ONH). MS: Calculated for $C_{129}H_{171}N_{27}O_{36}$, 2674.2 (monoisotopic molecular weight M); found, MALDI-TOF MS 2674.7 [M + H]$^+$ (MBT as matrix).

Dendritic Chelator 30 Hydrochloride. 89% yield, a white solid. ^{1}H NMR (360 MHz, DMSO-d_6) δ 1.79 (br, CH_2, 18H), 2.08 (br, CH_2, 18H), 2.30 (br, CH_2, 6H), 2.57 (s, CH_3, 27H), 3.25 (br, CH_2, 6H), 3.89 (s, CH_3, 27H; and buried CH_2, 6H), 4.56 (br, CH_2, 18H), 7.30 (s, pyridinone C-5H, 9H), 7.43 (br, NH, 3H), 8.82 (br, NH, 3H), 9.02 (br, NH, 3H), 10.73 (br, HN$^+$); ^{13}C NMR (90 MHz, DMSO-d_6) δ 21.0 (*C*H_3), 29.7 (C*C*H$_2$CH$_2$CO), 30.3 (CCH$_2$*C*H$_2$-CO), 35.1 (NH*C*H$_2$), 39.6 (N*C*H$_3$), 57.3 (NCH$_2$CO), (NH*C*), 113.1 (*C-5*H in pyradinone), 140.3 (*C-2* in pyridinone), 143.0 (*C-3* in pyridinone), 148.9 (*C-6* in pyridinone), 159.8 (*C-4* in pyridinone), 170.2 (*C*ONH), 173.8 (*C*ONH). MS: Calculated for $C_{117}H_{159}N_{25}O_{33}$, 2442.15 (monoisotopic molecular weight M); found, MALDI-TOF MS 2443.01 [M + H]$^+$, 2465.02 [M + Na]$^+$, 2481.02 [M + K]$^+$ (MBT as matrix).

Dendritic Chelator 32 Hydrochloride. 94% yield, a white solid. ^{1}H NMR (500 MHz, CD_3OD): δ 1.91 (br, CH_2, 36H), 2.22 (br, CH_2, 36H), 2.41 (br, CH_2, 12H), 2.57 (br, CH_2, 12H), 2.60 (s, CH_3, 54H), 3.44 (br, CH_2, 12H), 3.66 (br, CH_2, 12H), 4.00 (s, CH_3, 54H), 4.71 (s, CH_2, 36H), 4.91 (br, OH), 7.08 (s, pyridinone C-5H, 18H), 8.04 (s, ArH, 3H), 8.49 (s, ArH, 6H), 8.95 (s, ArH, 3H); ^{13}C NMR (90 MHz, CD_3OD): δ 21.8 (*C*H_3), 31.2 (C*C*H$_2$CH$_2$), 31.5 (CCH$_2$*C*H$_2$), 36.9 (NH*C*H$_2$-pyridinone), 40.6 (N*C*H$_3$), 59.6 (NH*C*), 114.6 (*C-5*H 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). MS: Calculated for $C_{273}H_{351}N_{57}O_{75}$, 5631.1 (average molecular weight); found, MALDI-TOF MS, 5631.5 for [M + H]$^+$ (DCTB as matrix).

UV/Vis Spectrophotometry for Iron Chelation. Batch spectrophotometric determinations were performed on a UV spectrophotometer scanning from 250 to 600 nm. A range of solutions with different ratios of dendrimer and iron were prepared by adding iron solution (100 μM, 1 mM NTA, 50 mM NH_4HCO_3) to 0.5 mL of dendrimer (100 μM in water or 50% methanol aqueous) followed by the addition of water to yield a certain volume (3.5 mL for dendrimer **1c**, **28**, **30**, and **1e**; 4 mL for dendrimer **2c**, 5 mL for dendrimer **3c** and **32**). The final pH of the solution was 7.0−7.2. All samples were equilibrated at room temperature for at least 4 h before spectral acquisition.

Sample Preparation for Iron Selectivity Studies. One hundred microliters of dendrimer **1c** (100 μM) was mixed with 300 μL of metal salt such as $CuSO_4$, $MgSO_4$, $ZnSO_4$, and $CaCl_2$ solutions. The final pH was about 6 and was not adjusted to pH 7.0 because the dendrimer binds these metals only very weakly and the free dendrimer tends to precipitate at pH 7.0. The mixture was used for MALDI-TOF MS determination 4 h after preparation. To the mixture of 300 μL of metal ion solution (Cu^{2+}, Mg^{2+}, Zn^{2+}, Ca^{2+}) and 300 μL of iron solution (pH 3.0), 100 μL of dendrimer **1c** (100 μM) was added followed by 25 μL of NH_4HCO_3 (50 mM) (final pH 7.2). After 4 h, the mixture was used for probing iron selectivity by MALDI-TOF MS.

Determination of Iron Affinities of the Dendritic Chelators Using the Fluorescence Method. The fluorescent probe CP691 was selected for this purpose. Fe(III) (12 μM) prepared from ferric chloride in the presence of 4-fold NTA solution was added to MOPS buffer (50 mM) containing CP691 (12 μM). After a few minutes, the competing chelator at a concentration yielding an equal amount number of iron binding units [**33** (12 μM), **1c**, **28**, and **30** (4 μM), **2c** (3 μM), **3c**, and **32** (2 μM)] in DMSO was mixed with the iron−probe complex at the same volume. The time course of the fluorescence intensity of the samples was measured by a spectrofluorometer. The iron-free CP691 fluorescence was set at 100% probe fluorescence intensity, and the fluorescence intensity of CP691 in the presence of an equimolar amount of iron(III) was set at 0%. The percentage of fluorescence intensity of the mixture of the iron−probe complex with various competing ligands could be thus calculated based on their measured arbitrary units of fluorescence.

Calculation of Iron Affinities of the Dendritic Chelators by Competition with a Fluorescent Chelator, The fluorescent probe CP691, the hexadentate ligand **33**, and the hexadentate branches on the dendritic chelator form 1:1 iron(III)−ligand complexes as indicated above. The stability constants of CP691, **33**, and the hexadentate ligands on the dendritic structures can be represented as follows:

$$\mathrm{Fe} + \mathrm{Flu} \rightleftharpoons \mathrm{FeFlu};\ K_{\mathrm{Flu}} = \frac{[\mathrm{FeFlu}]}{[\mathrm{Fe}][\mathrm{Flu}]} \tag{1}$$

$$\mathrm{Fe} + \mathrm{Hex} \rightleftharpoons \mathrm{FeHex};\ K_{\mathrm{Hex}} = \frac{[\mathrm{FeHex}]}{[\mathrm{Fe}][\mathrm{Hex}]} \tag{2}$$

$$\mathrm{Fe} + \mathrm{Den} \rightleftharpoons \mathrm{FeDen};\ K_{\mathrm{Den}} = \frac{[\mathrm{FeDen}]}{[\mathrm{Fe}][\mathrm{Den}]} \tag{3}$$

where Flu is the fluorescent probe CP691, Hex is the hexadentate ligand **33**, and Den is a hexadentate ligand on the dendrimer. For the sake of brevity, all charges have been omitted. In the equilibrium situation, the system can be represented in the following way:

$$\mathrm{FeFlu} + \mathrm{Hex} \rightleftharpoons \mathrm{FeHex} + \mathrm{Flu}$$

$$K_{\mathrm{q1}} = \frac{[\mathrm{FeHex}][\mathrm{Flu}]}{[\mathrm{FeFlu}][\mathrm{Hex}]} = \frac{K_{\mathrm{Hex}}}{K_{\mathrm{Flu}}} \tag{4}$$

$$\mathrm{FeFlu} + \mathrm{Den} \rightleftharpoons \mathrm{FeDen} + \mathrm{Flu}$$

$$K_{\mathrm{q2}} = \frac{[\mathrm{FeDen}][\mathrm{Flu}]'}{[\mathrm{FeFlu}]'[\mathrm{Den}]} = \frac{K_{\mathrm{Den}}}{K_{\mathrm{Flu}}} \tag{5}$$

From eqs 4 and 5, the following equation can be obtained:

$$K_{\mathrm{Den}} = \frac{[\mathrm{FeFlu}][\mathrm{Hex}][\mathrm{FeDen}][\mathrm{Flu}]'}{[\mathrm{FeHex}][\mathrm{Flu}][\mathrm{FeFlu}]'[\mathrm{Den}]} K_{\mathrm{Hex}} \tag{6}$$

The same total concentration of iron, fluorescent probe CP691, hexadentate ligand **33**, and dendrimer-based hexadentate ligand was studied. In the calculation, we assume that the hexadentate ligands on the investigated dendrimers possess the same pK_a value as that of **33**. With this assumption, the stability constant K of hexadentate ligand on the dendrimers could be calculated using eq 6. As an example, $K_{\mathbf{1c}}$ was calculated as follows:

$$K_{\mathbf{1c}} = \frac{(1 - 0.553)(1 - 0.553)\ 0.576 \cdot 0.576}{0.553 \cdot 0.553(1 - 0.576)(1 - 0.576)}$$

$$\log K_{\mathbf{1c}} = \log 1.2K_{\mathrm{Hex}} = 32.60$$

The pFe^{3+} value was calculated by HYSS[29] based on the affinity constants determined and assuming identical pK_a values for the hexadentate units. The conditions for the calculation were pH = 7.4, $[Fe^{3+}] = 10^{-6}$ M, [Ligand] = 10^{-5} M.

In Vitro Intestinal Perfusion. ^{58}Fe was purchased from Chemgas (Boulogne, France) in metal piece form. This product contains ^{56}Fe 0.35%, ^{57}Fe 6.50%, and ^{58}Fe 93.13%. It was dissolved in nitric acid (1 M) to contain 170 mM ^{58}Fe and diluted to 25 mM or 5 mM ^{58}Fe using NTA solution (pH 7.0). The molecular ratio of ^{58}Fe:NTA in these two ^{58}Fe solutions was kept at 1:5. Male Wistar rats (230−250 g) were purchased from Harlan UK Ltd. (Oxon, England) and housed in the Biological Service Unit, King's College London, for at least 3 days before experiments. The animals were maintained at a temperature between 20 and 23 °C, with food and water ad libitum. The experiments carried out were authorized by the Secretary of State (England) under Animals Act 1986.

The experiments were undertaken using an in vitro intestinal perfusion model described by Schümann et al.[25] The animals were terminally anaesthetized by a combined i.p. application of Hypnorm and Hypnovel. Then 10−15 cm of the jejunal section was removed from body and perfused for periods up to 2 h using 50 mL of Tyrode solution at 37 °C, pH 7.0, saturated with carbogen (95% O_2 and 5% CO_2). Tyrode solution contains NaCl (137 mM), KCl (2.7 mM), $NaHCO_3$ (11.9 mM), NaH_2PO_4 (0.4 mM), $CaCl_2 \cdot 2H_2O$ (0.14 mM), $MgCl_2 \cdot 6H_2O$ (0.05 mM), and d-glucose (15 mM).

For the control group, 10 min after the start of perfusion, 100 μL of ^{58}Fe solution (25 or 5 mM) was added to perfusate (50 mL). The perfusion time was reset and the samples (absorbates) were collected over the periods 0−30, 30−60, and 60−120 min. For the test groups, 5 min after the start of perfusion, the dendrimer **1c** (10 mM in water) was added to the perfusate. After 10 min of perfusion, 100 μL of ^{58}Fe solution (25 or 5 mM) was added to the perfusate (50 mL). The ratios of the calculated iron binding site numbers of dendrimer **1c** to ^{58}Fe were adjusted to either 1:1 or 2:1. The samples were collected in the same periods as the control groups.

^{58}Fe Analysis. A quadrupole-based ICP-MS Spectrometer (Perkin-Elmer SCIEX ELAN 6100 DRC) with a dynamic reaction cell (DRC) was used in the present study (PerkinElmer SCIEX, Concord, Ontario, Canada). A quartz cyclonic spray chamber with Meinhard nebulizer was used for sample introduction. The dynamic reaction cell gas used was 5% hydrogen in argon (BOC Special Gases, Guildford, Surrey). A summary of the analytical parameters is shown in the Supporting Information. Samples (200 μL) were diluted to 1.5 mL using 0.5% HNO_3 containing indium (10 mg/L as internal standard) before being subjected to ICP-MS analysis.

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Supporting Information Available: Routine spectroscopic data and ICP-MS operating parameters for the analysis of 58Fe in the perfusion samples. This material is available free of charge via the Internet at http://pubs.acs.org.

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