Novel Iron Sequestering Agents: Synthesis and Iron-Chelating Properties of Hexadentate Ligands Composed of 1-Hydroxy-2(1*H*)-pyrimidinone, *ω*-Amino Carboxylic Acid, and Tris(2-aminoethyl)amine

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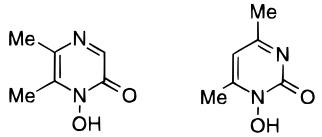
Novel heterocyclic hexadentate ligands (3HOPY$_n$: $n = 5-7$), in which three units of 1-hydroxy-2(1*H*)-pyrimidinone are linked to tris(2-aminoethyl)amine through an amide bond by an alkyl chain, were synthesized, and their iron-chelating properties were investigated. The stability of their iron complexes (25 to 27 in log *K*) was significantly larger than that of pyrazinone-containing ligands by virtue of higher pK_a values. On kinetic evaluation of iron removal from human transferrin, 3HOPY$_5$ showed remarkable efficiency over five times as much as commercially available desferrioxamine B. The conformational analysis of the corresponding Ga(III) complex of Fe(opy$_5$) by ^{1}H NMR and by MM and MD calculations are also discussed.

Introduction

Iron deficiency and overload are serious health problems. Considerable effort has been invested in the development of new chemotherapeutics for managing thalassemia.[1] Desferrioxamine B (DFO; Desferal), a microbial siderophore bearing three hydroxamate groups as iron binding sites, is still the only approved drug for treatment of patients suffering from acute iron intoxication or from chronic iron overload as a result of recurrent blood transfusions. However, DFO has short plasma half-life[2] and cannot be orally administered.[3] Furthermore, it possesses a number of side effects such as septicemia.[4] Therefore, the search for new nontoxic and orally active agents that would be expected to take the place of DFO has been one of the important issues. Recently, much attention has been focused on the application of nitrogen-containing heterocycles to iron chelators as therapeutic agents for the iron overload disease.[5] As for 3-hydroxy-4(1*H*)-pyridinones,[5d,5f−k] a number of clinical studies are presently ongoing,[6] although their clinical safety has not been established.

On the other hand, *N*-hydroxy amide-containing diazines can be regarded as cyclic hydroxamic acids. On chelating iron under physiological conditions, they would be expected to have several advantages: higher water solubility and acidity than those of monoazine, arising from their π-electron deficient ring system. Previously, we have reported the synthesis of 4,6-dimethyl-1-hydroxy-2(1*H*)-pyrimidinone (HOPY) and 5,6-dimethyl-1-hydroxy-2(1*H*)-pyrazinone (HOPR) and found them to be new iron-sequestering agents as bidentate ligands.[7] As



expected, both diazines showed high water solubility and acidity (pK_a = 6.1 for HOPY, 4.7 for HOPR). In order to develop more efficient chelators in low metal concentration, two types of hexadentate ligands composed of three HOPRs in a molecule have also been investigated.[8] It was noted that the hexadentate ligands showed remarkably high kinetic efficiency of iron removal from human transferrin, whereas the stability of iron complexes was

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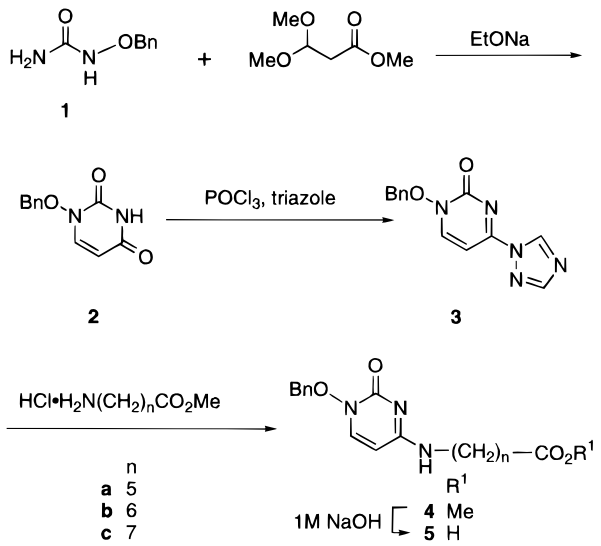
Scheme 1



very low due to their high acidity. The results prompted us to note the other bidentate ligand HOPY, which has higher pK_a value than that of HOPR. The pyrimidine bases consist of uracil, thymine, and cytosine which are regarded as 4-hydroxy-, 4-hydroxy-5-methyl-, and 4-amino-2(1*H*)-pyrimidinone, respectively, and thus 2(1*H*)-pyrimidinones seem to be attractive compounds from the viewpoint of biological interest. However, no multidentate ligand bearing 2(1*H*)-pyrimidinone has been reported, to the best of our knowledge. Thus, we designed novel hexadentate ligand 3HOPY$_n$ in which three units of 1-hydroxy-2(1*H*)-pyrimidinone were linked to tris(2-aminoethyl)amine (TREN) through an amide bond by an alkyl chain. In this paper, we report the synthesis and thermodynamic and kinetic results on iron-chelating of 3HOPY$_n$. The effect of the methylene chain length (n = 5−7) on the stability constant and the conformation of the iron complex is also discussed.

Results and Discussion

Synthesis. 1-(Benzyloxy)uracil (**2**) was prepared from the reaction of *N*-(benzyloxy)amine and sodium cyanate and subsequent cyclization of *N*-(benzyloxy)urea (**1**) with 3,3-dimethoxypropionic acid methyl ester in the presence of sodium ethoxide as shown in Scheme 1. Treatment of **2** with 1,2,4-triazole in the presence of phosphorus oxychloride[9] and Et_3N in dry MeCN gave 1-(benzyloxy)-4-(1,2,4-triazol-1-yl)-2(1*H*)-pyrimidinone (**3**) in 61% yield. In order to introduce a spacer group to the heterocyclic ring system, **3** was reacted with ω-amino carboxylic acid methyl ester (n = 5−7) to give **4a**−**c**. Two types of signals at 3.19 and 3.43 ppm (1:5 integrated intensity) assignable to CH_2N protons were observed in the ^{1}H NMR of **4a** in $CDCl_3$, suggesting that **4a** existed in a tautomeric equilibrium between 4-imino and 4-amino forms. The major peak at 3.43 ppm appeared as a quartet, indicating that **4a** predominantly exists in the 4-amino form. Two NH proton signals at δ 6.14 and 6.75 were D_2O-exchangeable, with changing of the quartet of CH_2N into a triplet. In DMSO-d_6 solution, **4a** exclusively existed in the 4-amino form. The hydrolysis of **4** with 1 M NaOH gave **5a**−**c**. Conversion to the corresponding *O*-succinimide esters **6**, followed by coupling with tris(2-aminoethyl)amine under mild conditions gave tripodal compounds **7a**−**c** as shown in Scheme 2. Finally, debenzylation of **7a**−**c** by the catalytic hydrogenation in MeOH under reflux afforded the desired hexadentate ligands, 3HOPY$_n$ (n = 5−7).

In order to clarify whether intramolecular hydrogen bonds exist in the free ligands or not, the temperature dependence of ^{1}H NMR chemical shifts was examined for the amide and amino protons of 3HOPY$_5$. ^{1}H NMR spectra of 3HOPY$_5$ in DMSO-d_6 at various temperatures from 23 to 90 °C exhibited one set of signals, indicating that 3HOPY$_5$ possesses the pseudo-C_3-symmetrical structure at a range of the temperatures. The plots of chemical shifts of these amide and amino protons versus the temperatures gave straight lines with the temperature coefficients -4.6×10^{-3} and -5.1×10^{-3} ppm/deg, respectively. These large values indicate that no particular hydrogen bond exists in DMSO-d_6 solution.[10]

Iron Complex Formation. Iron complexation ability of 3HOPY$_n$ in aqueous solution was demonstrated by means of spectrophotometric analysis. As for 3HOPY$_5$ shown in Figure 1, UV−vis spectra of a 1:1 molar mixture of ligand with ferric ion under various pH conditions showed the characteristic LMCT (ligand to metal charge transfer) band of a ferric complex around at 465 nm (ϵ 4550 at pH 5.6). The observed λ_{max} and ϵ values, which were comparable to those of Fe(3-opr(Me)) (ϵ 3000 at 450 nm),[8a] indicated the formation of an intramolecular 1:1 iron complex. The 1:1 stoichiometry was confirmed by the mole ratio plot. No apparent change in λ_{max} and absorbance was observed over a wide pH range, especially under acidic to neutral conditions, in contrast to the corresponding bidentate ligand, HOPY. This observation suggests that hexadentate ligand, 3HOPY$_5$, can form a stable 1:1 complex by virtue of its structural effect which is entropically superior to bidentate one.

Stability of Iron Complex. The stability constant of the complex of the hexadentate ligand with iron is defined by the following equilibrium:

$$Fe^{3+} + L^{3-} \overset{K}{\rightleftharpoons} FeL \qquad K = \frac{[FeL]}{[Fe^{3+}][L^{3-}]}$$

In order to estimate the stability constants of complexes of 3HOPY$_n$ with iron, the competitive reactions between EDTA and these ligands were carried out. Although three pK_a values of 3HOPY$_n$ are necessary for the calculation, measurement of these pK_a values was impossible due to experimental limitation. Consequently, 1-hydroxy-4-(butylamino)-2(1*H*)-pyrimidinone (**8**) was prepared as a model compound, and its pK_a value (7.5) was used for the calculation. This relatively higher pK_a than that of HOPY (6.1) may be attributable to the inductive effect of the electron-donating amino group at the C-4 position of the pyrimidinone ring. The competitive reaction of 3HOPY$_5$ and 3HOPY$_6$ with EDTA were carried out in water, while the measurement of stability of Fe(3opy$_7$) in water was precluded due to poor water solubility of 3HOPY$_7$. Thus, the reaction was also carried out in 40% aqueous MeOH in the same fashion of those in water. The results are summarized in Table 1. As expected, the stability constants (25 to 27 in log K) were

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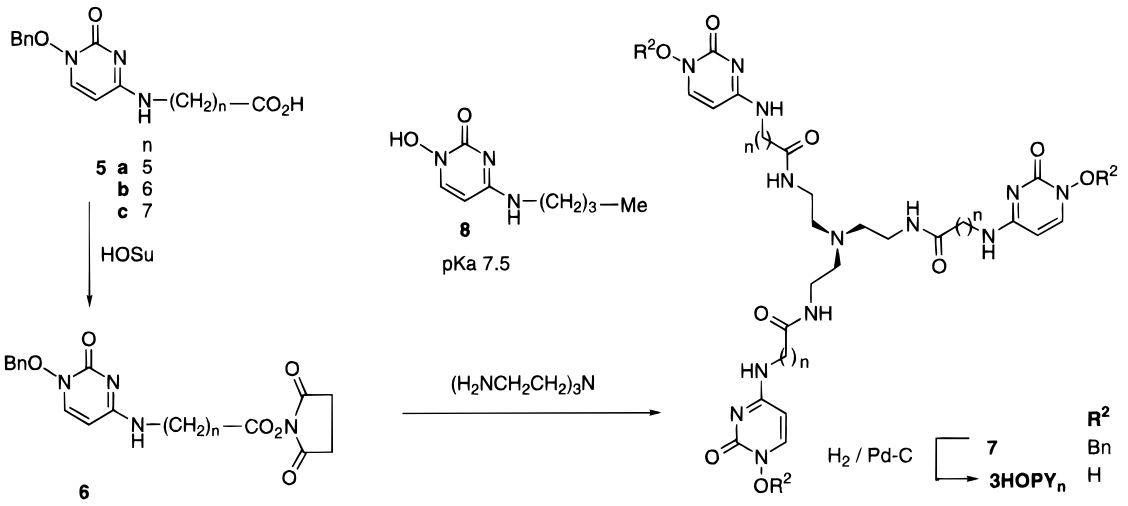
Scheme 2



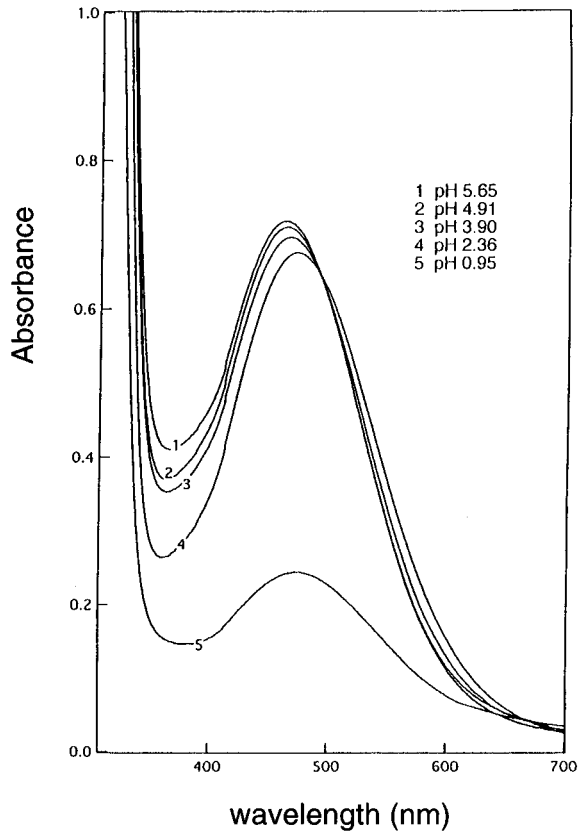


Figure 1. Spectral change of Fe(3opy$_5$) in aqueous solution under various pH conditions.

considerably greater than those of pyrazinone-containing hexadentate ligands, 3-HOPR(Me) (Figure 2),[8a] by at least 3 orders of magnitude. Interestingly, the longer spacer was slightly advantageous to the stable chelation with iron. This observation disagreed with the consideration that we have previously proposed. As for 3HOPR$_n$CMe (Figure 2), the stability of iron complex increased with decrease of spacer length, suggesting that the shorter spacer was suitable for stabilization entropically of the complex by virtue of the chelating effect.[8b] The different propensity of the effect of spacer length on the stability constant could be explained in terms of the difference of the spacer position in each heterocyclic ring system. As shown in Figure 2a, the pyrazinone derivatives were linked to the spacer group at the *meta* position

Table 1. Estimated Stability Constants of Iron(III) Complexes of 3HOPY$_n$[a]

	log K	
ligand	in H_2O	in 40% aqueous MeOH
3HOPY$_n$		
$n = 5$	25.7	25.1
$n = 6$	26.9	27.0
$n = 7$		27.1
3-HOPR(Me)[b]	22.5	
3HOPR$_n$CMe[c]	20.6−21.7	
HOPY[d]	22.1	

[a] Initial concentrations of Fe(3opy$_n$) and EDTA, 0.30 mM; in acetate buffer (pH 4.0) at 20 °C, μ = 0.04 with KNO_3 solution. [b] Reference 8a. [c] n = 2−6; reference 8b. [d] log β_3; reference 7.

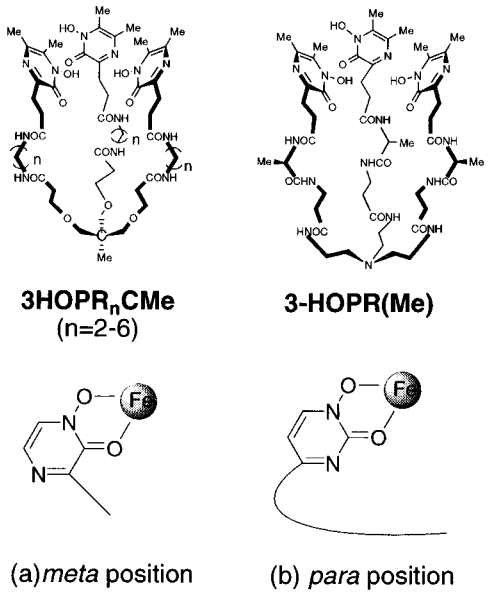


Figure 2.

toward the NOH moiety, and they could form an octahedral 3:1 iron complex with hydroxamate moieties in the molecule. In contrast, the pyrimidinone derivatives had the spacer group at the *para* position toward the NOH (Figure 2b), so that the shorter spacer should prevent complexation. Therefore, in the case of 3HOPY$_n$, the longer spacer would be favorable to form an octahedral complex.

Conformation of Gallium Complex. The examination using molecular model showed that the conformation

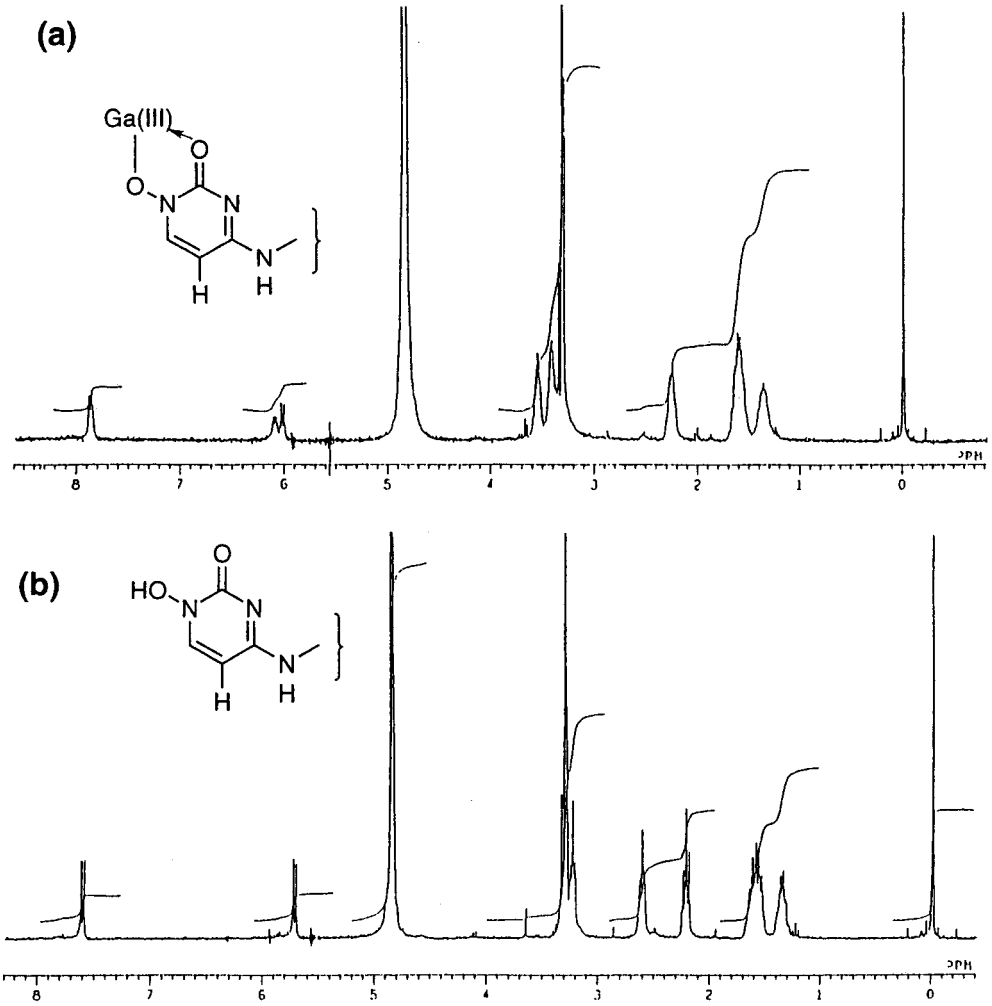


Figure 3. ^{1}H NMR spectra of (a) Ga(3opy$_5$) complex and (b) free ligand (3HOPY$_5$) in CD_3OD.

of Fe(3opy$_n$) should possess the C_3 symmetry. In order to understand the conformation in a solution, a Ga(III) complex was prepared from 3HOPY$_5$ and Ga$(NO_3)_3$, and the ^{1}H NMR spectrum of the complex was measured in CD_3OD at room temperature. The spectrum is shown in Figure 3 together with that of free ligand, 3HOPY$_5$. Interestingly, two doublet peaks at δ 6.0−6.2 assignable to the 5-H of the pyrimidinone ring were observed, while the other protons showed only simple sets of signals. In general, two isomers are possible for a Ga(III) complex, *cis* and *trans* forms. On the basis of the molecular model examination, it is evident that simultaneous existence of both *cis* and *trans* forms is possible by virtue of rather long spacers in the molecule. Thus, the existence of *cis* and *trans* isomers would reflect the separation of the 5-H doublet.

MM Calculation. In order to confirm the hypothesis described above, MM calculation of the iron complex was carried out. The conformation of *cis* and *trans* isomers of Fe(3opy$_5$) was optimized by MM2 and MD calculations.[11] The X-ray crystallographic data of Fe(1,2-opo)$_3$[5m] were used for the Cartesian coordinate of each atom around iron. The optimized structure of the *cis* form completely possessed C_3 symmetry, while that of the *trans* form was slightly distorted (Figure 4). The heats of formation were −96.34 and −92.23 kcal/mol for *cis* and *trans* isomers, respectively. Energy gap of 4 kcal/mol between the isomers indicated that the *cis* isomer is superior to *trans* one at least in vacuum system. However, when other factors that determine the stability of the complex in solution are considered, such as the interaction with the solvent, the difference in heat of formation is regarded to be not enough to allow the formation of the *cis* isomer predominantly in solution.

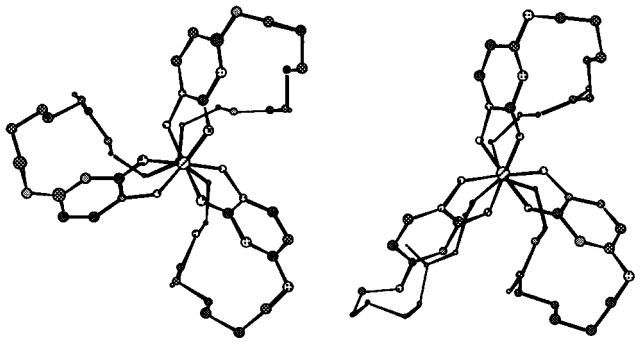


Figure 4. Optimized structures of Fe(3opy$_5$) by MM calculation (a view from the top of the complex: all hydrogens were excluded for clarity).

(11) The MM2 and MD calculations were performed on Macintosh Quadra 950 with coprocessor CXP40M using MM2 in CAChe (Ver. 3.6) argumented MM2 force field (CAChe Scientific) and MD in CAChe (Ver. 3.6).

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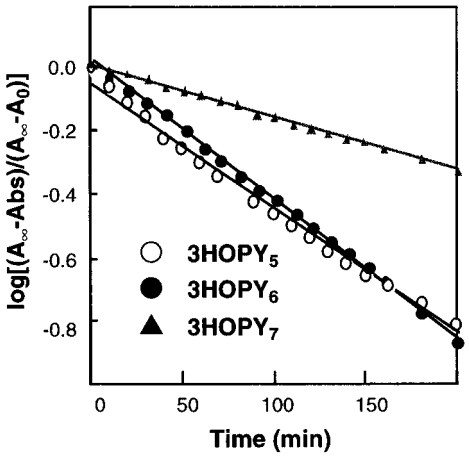

Figure 5. Plots of $\log[(A_\infty - \text{Abs})]/[(A_\infty - A_0)]$ vs time on iron removal of $3HOPY_n$ from $Tf_{Fe2.0}$.

Table 2. Iron Removal from Transferrin at pH 7.4

ligand $3HOPY_n$, n =	$[L]/[Tf_{Fe2.0}]$[a]	k_{obs} ($\times 10^{-3}$ min^{-1})	% Fe removed[b]
5	60	4.5	27
6	60	4.0	24
7	60	1.7	11
DFO	100	0.66	5 (5)[c]

[a] $[Tf_{Fe2.0}]_0 = 0.02$ mM, $Tf_{Fe2.0}$ was prepared from human serum apotransferrin (Sigma). [b] At a point 30 min after the reaction was initiated. [c] Reference 12.

Therefore, the two isomers might be in the equilibrium in the system, as observed in the ^{1}H NMR spectrum.

Iron Removal from Transferrin. There is great interest to the potential application of $3HOPY_n$ to a chemotherapeutic agent for the iron overload. High kinetic efficiency of iron removal from transferrin is one of the quite important requisites of a nontoxic drug. Therefore, kinetic evaluation of the iron removal ability of each ligand from human transferrin was carried out in physiological conditions. As shown in Figure 5, there are good linear relationships on the plots of $\log[(A_\infty - \text{Abs})/(A_\infty - A_0)]$ as a function of time, indicating that the iron removal from transferrin by $3HOPY_n$ proceeded in the pseudo-first-order kinetics. From the slope of the straight line, k_{obs} was obtained. The kinetic results are summarized in Table 2 together with data of DFO. It is noteworthy that $3HOPY_n$, especially $3HOPY_5$, efficiently removed iron from transferrin over five times as much as commercially available DFO did even at a lower concentration of the ligand than that of DFO. This difference is apparently observed in the percentages of iron removal after the reactions were performed for 30 min (27% for $3HOPY_5$; 5% for DFO).

Experimental Section

General. Melting points were recorded on a Mel-Temp apparatus in open capillaries and are uncorrected. IR spectra were recorded on a JASCO IR-700 infrared spectrometer. UV spectra were recorded on a JASCO Ubest V-550 UV/vis Spectrophotometer. ^{1}H NMR were recorded on JEOL GX-270 NMR spectrometer in $CDCl_3$, DMSO-d_6, D_2O, and CD_3OD. Chemical shifts were reported in ppm (δ) downfield from internal tetramethylsilane (TMS) or 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). Thin layer chromatography (TLC) analyses were performed on silica gel 60F-254 with a 0.2 mm layer thickness. Column chromatography was carried out with Merck kieselgel 60 (230−400 mesh). Combustion analyses were performed on a Yanaco MT-3 CHN CORDER and Perkin-Elmer Series II 2400 CHNS Analyzer. FAB mass spectra were taken on a JEOL DX303 with a DA 5000 data system by using a Xe beam at 6 keV and a nitrobenzyl alcohol matrix. *N*-(Benzyloxy)urea (**1**) was prepared by the same procedure described in the literature.[13]

Distilled deionized water was used at all times. For all experiments, observed pH was measured as $-\log [H^+]$ with a calibrated Horiba combination electrode (Horiba-6378) using a Horiba F-12 meter.

1-(Benzyloxy)uracil (2). *N*-(Benzyloxy)urea (9.97 g, 0.06 mol) was added to a solution of sodium (1.5 g, 0.07 mol) in absolute EtOH (90 mL) at 30 °C under a nitrogen atmosphere. After addition of 3,3-dimethoxypropionic acid methyl ester (9.78 g, 0.07 mol), the reaction mixture was stirred for 3 h at 20 °C, then refluxed for another 17 h, and finally cooled to 10 °C and maintained at the temperature for 4 h. The precipitated sodium salt was collected by filtration and dissolved in H_2O (120 mL). The pH of the aqueous solution was adjusted to 4 with AcOH. The resulting precipitate was collected by filtration and then recrystallized from an EtOH−H_2O (1:1) mixture to give the product **2**; 1.52 g (67%): mp 180−183 °C (lit.[14] mp 185 °C); IR (KBr) 3234, 1633, 738, 699 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.18 (s, 2H), 5.40 (d, J = 9 Hz, 1H), 6.98 (d, J = 9 Hz, 1H), 7.41 (s, 5H).

1-(Benzyloxy)-4-(1,2,4-triazol-1-yl)-2(1*H*)-pyrimidinone (3). A solution of 1,2,4-triazole (6.22 g, 90 mmol) in dry MeCN (25 mL) was treated with $POCl_3$ (2.5 mL, 27 mmol) and Et_3N (12.5 mL, 90 mmol) at 0 °C. A solution of **2** (1.96 g, 9 mmol) in dry MeCN (100 mL) was added to the mixture at 0 °C. The pH of the solution was adjusted to around 9 by addition of Et_3N (ca. 5 mL), and the reaction mixture was stirred for 24 h at room temperature. Et_3N (12.5 mL) and H_2O (5 mL) were successively added to the reaction mixture in order to quench the reaction, and MeOH was added to the emulsion until the solution became clear. After removal of the solvents, the residue was washed with cold water (2 × 40 mL) and recrystallized from EtOH to give the product **3**; 1.47 g (61%): mp 206−209 °C; IR (KBr) 1680, 740, 695 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.36 (s, 2H), 6.75 (d, J = 8 Hz, 1H), 7.40 (s, 5H), 7.55 (d, J = 8 Hz, 1H), 8.07 (s, 1H), 9.22 (s, 1H). Anal. Calcd for $C_{13}H_{11}N_5O_2$: C, 57.99; H, 4.12; N, 26.01. Found: C, 57.75; H, 4.36; N, 25.77.

General Procedure for the Coupling of 3 with Methyl ω-Amino Carboxylate Hydrochloride 4a−c. A Typical Example: 4-[(5-(Methoxycarbonyl)pentyl)amino]-1-(benzyloxy)-2(1*H*)-pyrimidinone (4a). Methyl 6-aminohexanoate hydrochloride (2.12 g, 11.7 mmol) and Et_3N (1.18 g, 11.7 mmol) were added to a solution of **3** (2.7 g, 10 mmol) in dry THF (60 mL). The reaction mixture was refluxed for 44 h and then concentrated. H_2O (30 mL) was added to the residue, and the aqueous solution was extracted with $CHCl_3$ (5 × 50 mL). The combined organic layers were dried over anhydrous $MgSO_4$. After evaporation of the solvent, the resulting residual solid was recrystallized from EtOH to give the product **4a**; 2.41 g (70%): mp 144−146 °C; IR (KBr) 3020, 1670, 1630, 750, 700 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.30−1.45 (m, 2H), 1.55−1.70 (m, 4H), 2.32 (t, J = 8 Hz, 2H), 3.19 (br s, 1/5H), 3.43 (q, J = 8 Hz, 4/5H), 3.65 (s, 3H), 5.20 (s, 2H), 5.41 (br s, 1/5H), 5.56 (d, J = 8 Hz, 4/5H), 6.14 (br s, 4/5H), 6.75 (br s, 1/5H), 6.90 (d, J = 8 Hz, 4/5H), 7.07 (d, J = 8 Hz, 1/5H), 7.38 (s, 5H); (DMSO-d_6) δ 1.25−1.38 (m, 2H), 1.40−1.60 (m, 4H), 2.3 (t, J = 8 Hz, 2H), 3.18 (q, J = 8 Hz, 2H), 3.58 (s, 3H), 5.05 (s, 2H), 5.45 (d, J = 8 Hz, 1H),7.35−7.46 (m, 5H), 7.55 (d, J = 8 Hz, 1H), and 7.67 (br s, 1H). Anal. Calcd for $C_{18}H_{23}N_3O_4$: C, 62.59; H, 6.71; N, 12.17. Found: C, 62.61; H, 6.74; N, 12.22.

4-[(6-(Methoxycarbonyl)hexyl)amino]-1-(benzyloxy)-2(1*H*)-pyrimidinone (4b); 50%: mp 110−111 °C; IR (KBr) 3252, 1737, 1645, 702 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.25−1.35 (m, 4H), 1.52−1.64 (m, 4H), 2.33 (t, 2H), 3.15 (br s, 2/5H), 3.40 (m, 8/5H), 3.65 (s, 3H), 5.20 (s, 2H), 5.55 (d, J = 8 Hz, 1H), 6.41 (br s, 1H), 6.68 (br s, 1/5H), 6.89 (d, J = 8 Hz, 4/5H), 7.10

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(d, J = 8 Hz, 1/5H), 7.37 (s, 5H). Anal. Calcd for $C_{19}H_{25}N_3O_4$: C, 63.49; H, 7.01; N, 11.69. Found: C, 63.46; H, 7.21; N, 11.66.

4-[(7-(Methoxycarbonyl)heptyl)amino]-1-(benzyloxy)-2(1*H*)-pyrimidinone (4c). The crude product was purified by column chromatography on silica gel (eluent: $CHCl_3$−acetone−EtOH = 100:10:2) to afford the pure product **4c**; 60%: mp 132−135 °C; IR (KBr) 3250, 1737, 1671, 1642, 749, 702 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.25−1.35 (m, 6H), 1.53−1.65 (m, 4H), 2.32 (t, 2H), 3.16 (br s, 2/5H), 3.41 (m, 8/5H), 3.66 (s, 3H), 5.19 (s, 2H), 5.40 (d, J = 8 Hz, 1/5H), 5.56 (d, J = 8 Hz, 4/5H), 6.41 (br s, 4/5H), 6.68 (br s, 1/5H), 6.88 (d, J = 8 Hz, 4/5H), 7.10 (d, J = 8 Hz, 1/5H), 7.37 (s, 5H). Anal. Calcd for $C_{20}H_{27}N_3O_4$: C, 64.32; H, 7.29; N, 11.25. Found: C, 64.26; H, 7.30; N, 11.02.

General Procedure for Preparation of 5a−c. A Typical Example: 4-[(5-Carboxypentyl)amino]-1-(benzyloxy)-2(1*H*)-pyrimidinone (5a). To a solution of compound **4a** (2.41 g, 6.98 mmol) in MeOH (100 mL) was added 1M NaOH (7.7 mL, 7.7 mmol), and the reaction mixture was stirred for 1 h at room temperature. Additional 1 M NaOH (15.4 mL, 15.4 mmol) was added to the mixture, and the reaction mixture was stirred for another 14 h at room temperature. After evaporation of the solvent, the residue was dissolved in H_2O (40 mL). The pH of the aqueous solution was adjusted to 3 with 5% HCl. The precipitated crude solid was recrystallized from EtOH to give the product **5a**; 2.16 g (93%): mp 192−193 °C; IR (KBr) 3200−2800, 1710, 1642, 752, 701 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.18−1.39 (m, 2H), 1.43−1.61 (m, 4H), 2.19 (t, 2H), 3.17−3.30 (m, 2H), 5.17 (s, 2H), 5.51 (d, J = 7 Hz, 1H), 7.32−7.47 (m, 6H), 7.67 (br s, 1H). Anal. Calcd for $C_{17}H_{21}N_3O_4\cdot 0.3H_2O$: C, 60.80; H, 6.58; N, 12.45. Found: C, 60.63; H, 6.46; N, 12.45.

4-[(6-Carboxyhexyl)amino]-1-(benzyloxy)-2(1*H*)-pyrimidinone (5b); 69%: mp 134−137 °C; IR (KBr) 3200−2700, 1716, 1673, 739, 700 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.28−1.48 (m, 8H), 2.20 (t, 2H), 5.08 (s, 2H), 5.63 (d, J = 8 Hz, 1H), 7.40 (s, 5H), 7.68 (d, J = 8 Hz, 1H). Anal. Calcd for $C_{18}H_{23}N_3O_4$: C, 56.68; H,7.13; N, 11.02. Found: C, 56.73; H, 6.77; N, 11.19.

4-[(7-Carboxyheptyl)amino]-1-(benzyloxy)-2(1*H*)-pyrimidinone (5c); 80%: IR (KBr) 3200−2700, 1739, 1636, 739, 703 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.28−1.46 (m, 10H), 2.19 (t, 2H), 3.13−3.18 (m, 2H), 5.06 (s, 2H), 5.50 (d, J = 8 Hz, 1H), 7.42 (s, 5H), 7.55 (d, J = 8 Hz, 1H), 7.68 (br s, 1H), 11.95 (br s, 1H). Anal. Calcd for $C_{19}H_{25}N_3O_4\cdot 0.5H_2O$: C, 61.94; H,7.11; N, 11.41. Found: C, 62.07; H, 7.07; N, 11.15.

General Procedure for Preparation of *O*-Succinimide Esters 6a−c. A Typical Example: 4-[(5-Carboxypentyl)-amino]-1-(benzyloxy)-2(1*H*)-pyrimidinone *O*-Succinimide Ester (6a). To a solution of compound **5a** (700 mg, 2.11 mmol) and HOSu (486 mg, 4.22 mmol) in dry DMF (10 mL) was added a solution of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (WSC·HCl; 810 mg, 4.22 mmol) in CH_2Cl_2 (20 mL) at −10 °C. The reaction mixture was stirred for 14 h at room temperature, the solvent was evaporated, and then the resulting solid was dissolved in $CHCl_3$ (150 mL). The organic layer was washed with H_2O (2 × 50 mL) and dried over anhydrous Na_2SO_4. After removal of the solvent, the *O*-succinimide ester **6a** was obtained and used for the next reaction without further purification; 740 mg (82%): IR ($CDCl_3$) 1810, 1780, 1720, 1620, 740, 690 cm^{-1}.

4-[(6-Carboxyhexyl)amino]-1-(benzyloxy)-2(1*H*)-pyrimidinone *O*-succinimide ester (6b); 100%: IR ($CDCl_3$) 1810, 1770, 1720 cm^{-1}.

4-[(7-Carboxyheptyl)amino]-1-(benzyloxy)-2(1*H*)-pyrimidinone *O*-succinimide ester (6c); 100%: IR ($CDCl_3$) 1808, 1772, 1720 cm^{-1}.

General Procedure for Preparation of Tripodal Compounds 7a−c. A Typical Example: Tris(2-(6-(1-(1-(benzyloxy)-2-oxo-1,2-dihydropyrimidi-4-yl)amino)hexanamido)ethyl)amine (7a). A solution of tris(2-aminoethyl)-amine (76 mg, 0.52 mmol) in DMF (5 mL) was added to a solution of compound **6a** (740 mg, 0.52 mmol) in DMF (15 mL), and then the mixture was stirred for 48 h at 38 °C. After removal of the solvent, the residue was purified by column chromatography on silica gel with a $CHCl_3$−MeOH (5:1) mixture, followed by gel chromatography on Toyopearl HW-40 with MeOH to give the pure product **7a**; 296 mg (52%): IR (KBr) 3500, 2940, 2860, 1760, 1630, 750, and 700 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.18−1.32 (m, 6H), 1.45−1.65 (m, 12H), 2.15−2.25 (m, 6H), 2.55−2.70 (m, 6H), 3.25−3.40 (m, 12H), 5.15 (s, 6H), 5.65 (d, J = 8 Hz, 3H), 6.90 (d, J = 8 Hz, 3H), 7.25 (s, 15H), 7.72 (br s, 3H). Anal. Calcd for $C_{57}H_{75}N_{13}O_9\cdot 3H_2O$: C, 60.04; H, 7.16; N, 15.97. Found: C, 60.22; H, 6.98; N, 15.71.

Tris(2-(6-(1-(1-(benzyloxy)-2-oxo-1,2-dihydropyrimidi-4-yl)amino)heptanamido)ethyl)amine (7b); 86%: IR (KBr) 3284, 1636, 753, 702 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.18−1.32 (m, 12H), 1.48−1.65 (m, 12H), 2.22 (m, 6H), 2.58−2.68 (m, 6H), 3.20−3.35 (m, 12H), 5.12 (s, 6H), 5.71 (d, J = 8 Hz, 3H), 6.92 (d, J = 8 Hz, 3H), 7.36 (s, 15H), 7.51 (br s, 3H), 7.71 (br s, 3H). Anal. Calcd for $C_{60}H_{81}N_{13}O_9\cdot 3H_2O$: C, 60.94; H, 7.42 N, 15.40. Found: C, 61.14; H, 7.61; N, 15.46.

Tris(2-(6-(1-(1-(benzyloxy)-2-oxo-1,2-dihydropyrimidi-4-yl)amino)octanamido)ethyl)amine (7c); 71%: IR (KBr) 3284, 1636, 753, 703 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.20−1.32 (m, 18H), 1.45−1.65 (m, 12H), 2.22 (m, 6H), 2.48−2.55 (m, 6H), 3.20−3.40 (m, 12H), 5.12 (s, 6H), 5.72 (d, J = 8 Hz, 3H), 6.93 (d, J = 8 Hz, 3H), 7.35 (s, 15H), 7.64 (br s, 3H), 7.71 (br s, 3H). Anal. Calcd for $C_{63}H_{87}N_{13}O_9\cdot 0.5H_2O$: C, 64.16; H, 7.52 N, 15.44. Found: C, 63.99; H, 7.50; N, 15.58.

1-Hydroxy-4-(*N*-butylamino)-2(1*H*)-pyrimidinone (8). A solution of **3** (0.63 g, 2.34 mmol) and butylamine (0.21 g, 2.87 mmol) in dry THF (20 mL) was refluxed for 4 h. The solvent was evaporated, and H_2O (10 mL) was added to the residue. The aqueous solution was extracted with $CHCl_3$ (4 × 50 mL), and then the combined organic layers were dried over anhydrous Na_2SO_4. After removal of the solvent, the crude product was recrystallized from EtOH to give 1-(benzyloxy)-4-(*N*-butylamino)-2(1*H*)-pyrimidinone; 75%: mp 160−162 °C; IR (KBr) 3246, 1644, 747, 699 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.94 (t, J = 8 Hz, 3H), 1.3−1.41 (m, 2H), 1.50−1.60 (m, 2H), 3.10−3.50 (m, 2H), 5.22 (s, 3H, Bn and 5-H), 6.90 (d, J = 8 Hz, 1H), 7.40 (s, 5H). Anal. Calcd for $C_{15}H_{19}N_3O_2$: C, 65.91; H, 7.01; N, 15.37. Found: C, 65.80; H, 7.18; N, 15.46.

A suspension of 10% Pd−C (25 mg) in distilled MeOH (10 mL) was prehydrogenated with H_2 for 0.5 h. A solution of 1-(benzyloxy)-4-(*N*-butylamino)-2(1*H*)-pyrimidinone (252 mg, 0.92 mmol) in distilled MeOH (20 mL) was added to the suspension. After hydrogenation with H_2 under reflux for 2 h, the catalyst was removed by filtration. The filtrate was evaporated to give the product **8**; 100%: mp 120−123 °C. IR (KBr) 3300−2700, 3284, and 1636 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.85 (t, J = 7 Hz, 3H), 1.25−1.51 (m, 4H), 3.20 (q, J = 7 Hz, 2H), 5.55 (d, J = 7 Hz, 1H), 7.51 (br s, 1H) 7.65 (d, J = 7 Hz, 1H), and 10.85 (br s, 1H). Anal. Calcd for $C_8H_{13}N_3O_2\cdot 0.2H_2O$: C 51.44, H 7.23, N 22.49. Found: C 51.62, H 7.09, N 22.75.

General Procedure for Preparation of Hexadentate Ligands (3HOPY$_n$). A Typical Example: Tris(2-(6-(1-(1-hydroxy-2-oxo-1,2-dihydropyrimidi-4-yl)amino)hexanamido)ethyl)amine (3HOPY$_5$). A suspension of 10% Pd−C (35 mg) in MeOH (20 mL) was prehydrogenated with H_2 for 0.5 h. To the suspension was added a solution of compound **7a** (300 mg, 0.28 mmol) in MeOH (10 mL). After hydrogenation with H_2 under atmospheric pressure for 1 h under reflux, the catalyst was removed by filtration. The filtrate was concentrated to give the residue, which was purified by gel chromatography on Sephadex LH-20 with MeOH to afford the product 3HOPY$_5$; 150 mg (66%): IR (KBr) 3500−3200, 3282, 1636 cm^{-1}: ^{1}H NMR (DMSO-d_6) δ 1.20−1.30 (m, 6H), 1.41−1.53 (m, 12H), 2.08 (t, J = 7 Hz, 6H), 2.40−2.52 (m, 6H), 3.05−3.22 (m, 12H), 5.55 (d, J = 8 Hz, 3H), 7.55 (br s, 3H), 7.65 (d, J = 8 Hz, 3H), 7.73 (br s, 3H). Anal. Calcd for $C_{36}H_{57}N_{13}O_9\cdot 0.5H_2O$: C, 52.42; H, 7.09; N, 22.07. Found: C, 52.29; H, 7.37; N, 22.23.

Tris(2-(6-(1-(1-hydroxy-2-oxo-1,2-dihydropyrimidi-4-yl)amino)heptanamido) ethyl)amine (3HOPY$_6$); 66%: IR (KBr) 3500−3200, 3284, 1635 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.21−1.32 (m, 12H), 1.40−1.55 (m, 12H), 2.10 (t, J = 7 Hz, 6H), 2.39−2.50 (m, 6H), 3.05−3.25 (m, 12H), 5.55 (d, J = 8 Hz, 3H), 7.55 (br s, 3H), 7.65 (d, J = 8 Hz, 3H), 7.70 (br s, 3H), 10.90 (br s, 3H, OH); MS (FAB) m/z 858 (M^+). Anal.

Calcd for $C_{39}H_{63}N_{13}O_9 \cdot 1.8H_2O$: C, 52.61; H, 7.54; N, 20.45. Found: C, 52.73; H, 7.73; N, 20.25.

Tris(2-(6-(1-(1-hydroxy-2-oxo-1,2-dihydropyrimidi-4-yl)amino)octanamido)ethyl)amine (3HOPY$_7$); 61%: IR (KBr) 3500−3200, 3284, 1636 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.16−1.30 (m, 18H), 1.40−1.55 (m, 12H), 2.08 (t, J = 7 Hz, 6H), 2.41−2.51 (m, 6H), 3.05−3.25 (m, 12H), 5.57 (d, J = 8 Hz, 3H), 7.56 (br s, 3H), 7.65 (d, J = 8 Hz, 3H), 7.65 (br s, 3H), 10.86 (br s, 3H, OH); MS (FAB) m/z 900 (M^+). Anal. Calcd for $C_{42}H_{69}N_{13}O_9 \cdot 2H_2O$: C, 53.89; H, 7.86; N, 19.45. Found: C, 53.83; H, 7.85; N, 19.39.

Measurement of pK_a Value of 8. Compound **8** (30 mg) was dissolved in deionized water (30 mL). The pH of the solution was measured after every 0.1 mL addition of a 0.08 M NaOH solution at room temperature under an Ar atmosphere. The pK_a value was calculated from the pH value at the midpoint of neutralization.

General Procedure for Spectral Measurement of 1:1 Mixtures of Iron and Hexadentate Ligands. A sample (13−15 mg) of each hexadentate ligand was dissolved in deionized water (5.0 mL). The sample solution (1.0 mL) was mixed with an equimolar amount of ferric nitrate solution (3.28 mM) and diluted to 10.0 mL (0.3 mM). The pH of the solution was adjusted to an appropriate value with 0.1 or 0.01 M NaOH or 0.1 or 0.01 M HNO_3 before spectral measurement.

Iron Exchange Reaction. An iron complex solution (0.30 mM) of the hexadentate ligands (3HOPY$_5$ and 3HOPY$_6$) was prepared by mixing a stock solution of the ligand (3.28 mM) with an equimolar amount of ferric nitrate solution (3.28 mM) and 1.0 mL of 0.4 M KNO_3 and then diluting the solution to 10.0 mL with acetate buffer (pH 4.0). An EDTA solution was prepared by dissolving $(EDTA)^{2-} \cdot 2Na^+ \cdot 2H_2O$ in acetate buffer solution (ionic strength 0.04, pH 4.0) to give a concentration of 0.30 mM. The competitive reaction was initiated by combination the iron complex solution (2.0 mL) with the EDTA solution (2.0 mL). The iron exchange reaction was checked by monitoring the decrease of absorbance at 450 nm. The relative stability constants of the iron complexes were calculated by using the stability constant of Fe(edta) (log K 25.1),[15] the pK_a of the corresponding bidentate ligand (pK_a 7.5 of **8** for 3HOPY$_n$), and the pH of the solution at an equilibrium point at 20 °C. The experiments for all ligands were carried out by using 40% MeOH in an H_2O solution in the same fashion as described above, except for using MeOH to dilute to 10 mL in the preparation of the iron complex solution. The reaction solution after being combined with the same amount of EDTA stock solution totally contained MeOH in 40%. The apparent pH value of the solution was used for the calculation without corrections.

Gallium Complex Formation. 3HOPY$_5$ (6 mg) and $Ga(NO_3)_3$ (4 mg) were dissolved in 10% CD_3OD/D_2O (1:9; 0.5 mL). The pD was adjusted to 6 with freshly prepared 0.4% NaOD in D_2O.[16] The ^{1}H NMR spectrum was measured at room temperature. Ga(3opy$_5$): δ 1.35 (m, 6H), 1.60 (m, 12H), 2.25 (m, 6H), 3.40 (m, 6H), 3.55 (m, 6H), 6.00 and 6.12 (d each, J = 8 Hz each, 3H), 7.87 (d, J = 8 Hz, 3H).

Kinetics of Iron Removal from Tf$_{Fe_{2.0}}$ by 3HOPY$_n$. A Typical Example. A commercially available human serum transferrin (98%, Sigma) was used. $Fe_{2.0}$Tf was prepared according to the literature reported in detail by Raymond.[17] The stock solution of 3HOPY$_5$ (1.0 mL, 1.52×10^{-3} M) and Tf$_{Fe_{2.0}}$ (1.0 mL, 3.886×10^{-5} M) were prepared in Tris chloride buffer and combined. The absorbance of the solution was monitored at 460 nm. The pseudo-first-order rate constant (k_{obs}) was obtained from the slope of the plots of $\log[(A_\infty - \text{Abs})/(A_\infty - A_0)]$ against time (min).

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