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# *N*-Hydroxyamide-Containing Heterocycles. Part 5.[1] Synthesis of Novel Hexadentate Ligands Composed of *N*-Hydroxy-2(1*H*)-pyrazinone, Aliphatic Diamine, and 1,1,1-Tris(carboxyethoxymethyl)ethane, and Properties of Their Ferric Complexes

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**Abstract**. Novel hexadentate ligands (**3HOPR$_n$CMe**) containing *N*-hydroxy-2(1*H*)-pyrazinone connected to tricarboxylic acid by an aliphatic diamine through amide bonds were synthesized. UV-vis spectra of the 1:1 molar mixtures of **3HOPR$_n$CMe** and ferric ion in aqueous solution and the mole ratio plot strongly supported the formation of intramolecular 1:1 ferric complexes. The relative stability constants (log K 20.6-21.7) of the complexes were affected by the spacer length in a molecule. Further, **3HOPR$_n$CMe** showed higher iron removal efficiency toward human transferrin than naturally occurring siderophore, desferrioxamine B.

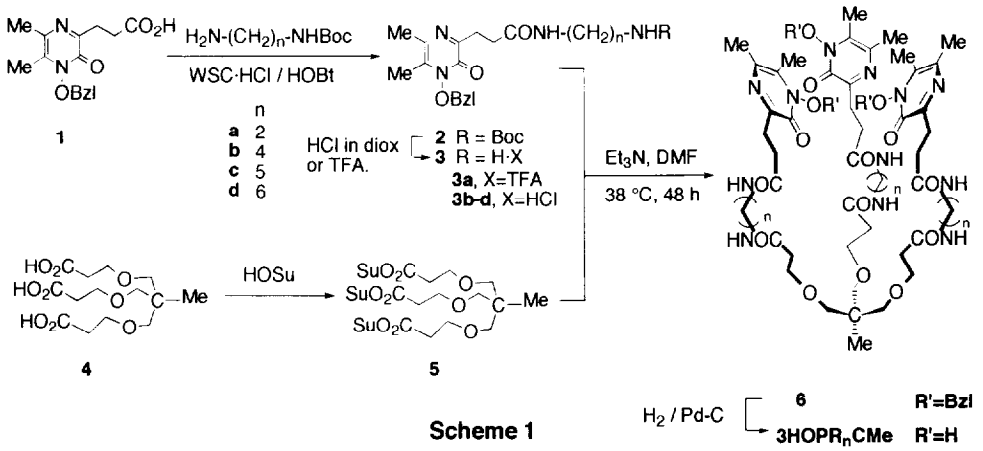
## Introduction

Because of the potential application in the treatment of iron overload disease, there is great interest in the development of new ligands for the effective complexation with iron. A currently used drug, methanesulfonate salt of desferrioxamine B (Desferal®: DFB), is derived from a natural trihydroxamate siderophore. However DFB is not orally active, though it is relatively effective and non toxic. Recently, considerable effort has been invested in developing hydroxy group-containing monoazines to search an effective ligand,[2-18] and several reports demonstrated the potential use of 1,2-dimethyl-3-hydroxy-4(1*H*)-pyridinone as an orally active drug.[4-6] For the purpose of the application of heterocycles to clinically useful chelators, we have recently investigated the synthesis and properites of a new tripodal compound **3-HOPR(X)**[1] possessing *N*-hydroxy-2(1*H*)-pyrazinone[19] as the binding moiety to iron, dipeptide as the spacer, and tris(2-aminoethyl)amine (TREN) as the anchor. As a result, **3-HOPR(X)** showed dramatically enhanced efficiency of iron removal from human transferrin compared to DFB. In order to clarify the influence of the molecular structure on iron chelation, we planned to synthesize novel hexadentate ligands, **3HOPR$_n$CMe**, in which each of three *N*-hydroxy-2(1*H*)-pyrazinones is linked to a $sp^3$ carbon by an aliphatic diamine through amide bonds. The $sp^3$ carbon at the bridgehead position would be expected to keep the rigid structure, in contrast to the fact that a tertiary amine easily undergoes the inversion of the lone-pair electron. Furthermore, the aliphatic diamine allows us to investigate the relationship between the spacer length of the free ligand and iron binding tendency. We describe here the synthesis of **3HOPR$_n$CMe**, their iron chelating properties in aqueous solution, and the effect of the spacer length on their chelating properties. Kinetic results on iron removal of these ligands from transferrin at physiological pH are also discussed.

## Results and Discussion

**Synthesis.** The synthetic procedure for **3HOPR$_n$CMe** is depicted in Scheme 1. In the present work, tricarboxylic acid **4**[20] was used as an anchor for constructing the tripodal compound. The coupling of 1-benzyloxy-5,6-dimethyl-2(1*H*)-pyrazinone derivative (**1**)[1] with $N^{\omega}$-*tert*-butoxycarbonyl (Boc)-substituted

aliphatic diamines by using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (water soluble carbodiimide: WSC·HCl) and 1-hydroxybenzotriazole (HOBt), followed by deprotection of the Boc group under appropriate acidic conditions, gave salts **3**. *O*-Succinimide ester **5**, which was derived from tricarboxylic acid, was coupled with **3** under mild conditions to afford tripodal compounds **6**. Finally, debenzylation of **6** by catalytic hydrogenation gave desired hexadentate ligands, **3HOPR$_n$CMe**. In this paper, a term "spacer length" is used for expression of length of NH-$(CH_2)_n$-NH.




**Scheme 1**

**Iron Complex Formation.** UV-vis spectroscopic features on octahedral 3:1 iron complexes of hydroxypyridinones (**HOPO**'s) have been well documented.[2,9,11] These complexes generally show a characteristic LMCT (ligand to metal charge transfer) band at 400-500 nm. The visible spectra of 1:1 molar mixtures of **3HOPR$_n$CMe** and ferric ion in aqueous solution showed an absorption maximum at 450 nm (**3HOPR$_4$CMe**: ε 3600 at pH 5.8). The observed $\lambda_{max}$ and ε values, which were comparable to those of Fe(**opr-Me**)$_3$ (ε 4237 at 425 nm),[19] suggested the formation of an intramolecular 1:1 complex. The complex formation was also confirmed by the mole ratio plot. The spectral change under various pH conditions of Fe(**3opr$_4$CMe**) is shown in Figure 1. The intensity of LMCT band is proportional to the number of ligands binding to iron. Further, the LMCT band increases in energy with crystal field strength of the overall ligand set.[2] In this case, no apparent change in $\lambda_{max}$ and absorbance was observed over a wide pH range, especially in acidic to neutral conditions. This observation indicated that hexadentate ligand, **3HOPR$_4$CMe,** could form a stable 1:1 complex compared to the corresponding bidentate ligand, *N*-hydroxy-5,6-dimethyl-2(1*H*)-pyrazinone (**HOPR-Me**).[19] In contrast, the absorbance decreased under basic conditions, which is attributable to the decomposition of the iron complex by attack of hydroxyl ion.

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

$$Fe^{3+} + L^{3-} \underset{}{\overset{K}{\rightleftharpoons}} FeL \qquad K = \frac{[FeL]}{[Fe^{3+}]\,[L^{3-}]} \qquad (1)$$

The stability constants of Fe(**3opr$_n$CMe**) in aqueous solution were determined by the competition reaction with EDTA.[21] Three proton dissociation constants of *N*-hydroxyamide groups are important parameters for this calculation. These values, however, were approximated by the pKa value of the corresponding bidentate ligand **HOPR-Me** (pKa 4.7)[19] since **3HOPR$_n$CMe** did not show enough solubility in water for titration. After

determination of an equilibrium point for the iron exchange reaction at pH~6, the relative stability constants were calculated from the acid dissociation constants[22] and the stability constant[23] of Fe(edta). The results are summarized in Table 1.

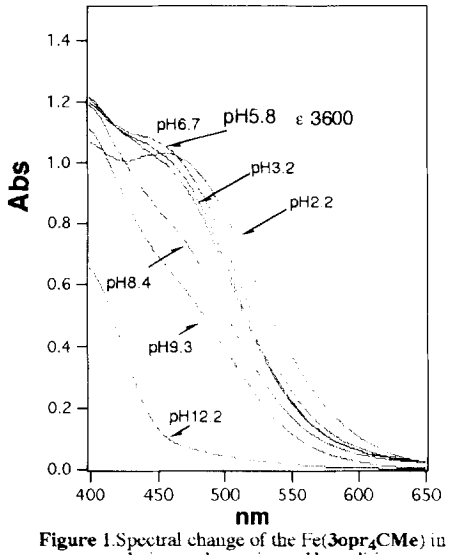



**Figure 1**.Spectral change of the Fe(**3opr$_4$CMe**) in aqueous solution under various pH conditions.

**Table 1. The Relative Stability Constants of 3HOPR$_n$CMe-Ferric Complexes**[a]

| n | Keq[b] | log K |
|---|---|---|
| **2** | 0.062 | 21.5 |
| **4** | 0.042 | 21.7 |
| **5** | 0.452 | 20.7 |
| **6** | 0.168 | 20.6 |
| **DFB**[c] | - | 30.5 |

[a] T=20 °C, μ=0.04 with $KNO_3$ solution, pH 6.0 (Mcvallin's buffer). $C_{Fe(III)}$ 0.04-0.20 mM, $C_L$ 0.04-0.20 mM, $C_{EDTA}$ 0.08-0.40 mM. [b] Keq is the equilibrium constant for the reaction Fe(**3opr$_n$CMe**) + $H_2$**EDTA**$^{2-}$ + $H^+ \rightleftharpoons$ Fe**EDTA**$^-$ + **3HOPR$_n$CMe**. [c] Ref.22

The stability constant of **3HOPR$_2$CMe** was comparable to those of **3-HOPR(X)** (logK 22.5 for X=Me and 21.7 for X=Bu$^t$).[1] This is responsible for the fact that the number of atoms from the bridge head atom to the C-3 of pyrazinone ring of **3HOPR$_2$CMe** is close to that of **3-HOPR(X)**. On the other hand, the stability constants of **3HOPR$_n$CMe** were in the range from $10^{20.6}$ to $10^{21.7}$. It was found that the stability constant slightly increased with decreasing spacer length. The stability constant of **3HOPR$_2$CMe** was almost 1 order greater than that of **3HOPR$_6$CMe**. It was suggested that the shorter spacer was advantageous for stabilizing the complex by virtue of the increased chelating effect. It is worth noting, however, that the stability constants of their iron complexes were far below that of DFB, since the pKa value of *N*-hydroxy-2(1*H*)-pyrazinone was considerably lower than that of hydroxamic acid.

**Iron Removal from Transferrin.** The iron removal efficiency of **3HOPR$_n$CMe** from iron transport protein, transferrin, was evaluated under physiological conditions. In this study, diferric human transferrin (**Tf**$_{Fe2.0}$) was prepared from commercially available human apotransferrin (apo**Tf**) according to the literature method.[10, 24-26]

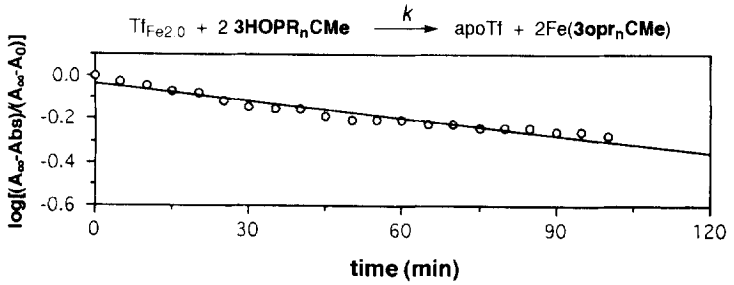



**Figure 2**. The plots of $\log[(A_\infty\text{-Abs}) / (A_\infty\text{-}A_0)]$ vs time on iron removal of **3HOPR$_6$CMe** from **Tf**$_{Fe2.0}$.

**Table 2. Iron Removal from Transferrin at pH 7.4**

| **3HOPR$_n$CMe** n | [L]/[**Tf**$_{Fe2.0}$][a] | $k_{obs}$ ($\times 10^{-3}$ min$^{-1}$) | % Fe removed[b] |
|---|---|---|---|
| **2** | 40 | 4.18 | 25 |
| **4** | 40 | 5.56 | 30 |
| **5** | 40 | 3.20 | 21 |
| **6** | 40 | 4.22 | 25 |
| **DFB** | 100 | 0.66 | 5 (5)[c] |

[a] [**Tf**$_{Fe2.0}$]$_0$=0.05 mM, **Tf**$_{Fe2.0}$ was prepared from human serum apotransferrin (Sigma). [b] At a point 30 min after the reaction was initiated. [c] Ref.27.

After mixing a buffered solution of **Tf**$_{Fe2.0}$ with 40 times excess of **3HOPR$_6$CMe**, change in absorbance at 500 nm was monitored. The plots of log[(A$\infty$-Abs)/(A$\infty$-A$_0$)] as a function of time gave a good linear relationship as shown in Figure 2, indicating that the iron removal from transferrin by **3HOPR$_6$CMe** proceeded in the pseudo-first-order-kinetics. From the slope of the straight line, $k_{obsd}$ was obtained. The kinetic results are summarized in Table 2 together with data of DFB. The rates of **3HOPR$_n$CMe** were in the range of 5.56-3.20$\times 10^{-3}$ min$^{-1}$, indicating that there is no remarkable influence of the spacer length on the kinetic efficiency of iron removal from transferrin. At least 40 times excess of **3HOPR$_n$CMe** to transferrin was required in order to monitor the iron exchange reaction, whereas **3-HOPR(X)** was in only 5 times excess.[1] It is noteworthy, however, that **3HOPR$_n$CMe** efficiently removed iron over 4 times as much iron from transferrin as DFB did even at a small excess of the ligand to transferrin at 30 min after the reaction was initiated.

## Experimental Section

**General Methods.** Melting points were determined on a Mel-Temp apparatus in open capillaries and are uncorrected. IR and UV-vis spectra were recorded on a JASCO A-100 infrared and on a JASCO Ubest V-550 spectrophotometers, respectively. $^1$H NMR spectra were obtained on a JEOL GX-270 spectrometer in $CDCl_3$, $CD_3OD$, and DMSO-$d_6$ solutions. Chemical shifts are reported in ppm ($\delta$) downfield from internal TMS. Thin layer chromatography (TLC) was performed on silica gel 60 F-254 with a 0.2 mm layer thickness. Column chromatography was carried out with Merck Kieselgel 60 (230-400 mesh). HPLC was carried out on a JASCO 880-PU and a 875-UV equipped with a JASCO IT integrator by using a column packed with a Finepak SIL $C_{12}S$. Combustion analyses were performed on a YANACO MT-3 CHN corder. *N*-Benzyloxy-3-carboxyethyl-5,6-dimethyl-2(1*H*)-pyrazinone (**1**)[1] and 1,1,1-tris(carboxyethoxymethyl)ethane (**4**)[20] was prepared according to the literature.

**General Procedure for coupling of Compound (1) and $N^{\omega}$-Boc-substituted Diamine. A Typical Example: *N*-(*tert*-Butoxycarbonyl)-*N*'-3-(1-benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyldiaminoethane, 2a.** WSC·HCl (479 mg, 2.5 mmol) in $CH_2Cl_2$ (8 mL) was added to a mixture of compound **1** (756 mg, 2.5 mmol), *N*-Boc-ethylenediamine (400 mg, 2.5 mmol), and HOBt (308 mg, 2.5 mmol) in DMF (4 mL) at -10 °C. The mixture was stirred overnight at room temperature. After removal of DMF under reduced pressure, the residue was dissolved in $CHCl_3$ (200 mL). The organic layer was washed with water, 5% $NaHCO_3$, 10% citric acid, brine, and dried ($MgSO_4$). Evaporation of the

solvent, followed by recrystallization of the residual solid from AcOEt-hexane mixture gave the product, 760 mg (85%): mp 149-151 °C; $^1$H NMR ($CDCl_3$) δ 1.43 (s, 9H), 2.20 (s, 3H), 2.24 (s, 3H), 2.66 (t, *J* 7 Hz, 2H), 3.17 (t, *J* 7 Hz, 2H), 3.28 (m, 2H), 3.36 (m, 2H), 5.00 (br s, 1H), 5.25 (s, 2H), 6.57 (br s, 1H), 7.39-7.50 (m, 5H). Anal. Calcd for $C_{23}H_{32}N_4O_5$: C, 62.14; H, 7.26; N, 12.60. Found: C, 61.91; H, 7.05; N, 12.57.

***N*-(*tert*-Butoxycarbonyl)-*N*'-3-(1-benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyldiaminobutane, 2b.** 76%: mp 118-119 °C; $^1$H NMR ($CDCl_3$) δ 1.44 (s, 9H), 1.49 (m, 4H), 2.20 (s, 3H), 2.24 (s, 3H), 2.65 (t, *J* 7 Hz, 2H), 3.16 (m, 4H), 3.26 (q, *J* 6 Hz, 2H), 4.60 (br s, 1H), 5.25 (s, 2H), 6.32 (br s, 1H), 7.41-7.49 (m, 5H). Anal. Calcd for $C_{25}H_{36}N_4O_5 \cdot 0.1H_2O$: C, 63.30; H, 7.69; N, 11.81. Found: C, 63.16; H, 7.45; N, 11.71.

***N*-(*tert*-Butoxycarbonyl)-*N*'-3-(1-benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyldiaminopentane, 2c.** The residual oil was purified by silica gel column chromatography with $CHCl_3$-acetone-EtOH (100:10:2) mixture to give the product, 56%: mp 60-65 °C; $^1$H NMR ($CDCl_3$) δ 1.43 (s, 9H), 1.49 (m, 9H), 2.20 (s, 3H), 2.24 (s, 3H), 2.64 (t, *J* 7 Hz, 2H), 3.12-3.22 (m, 6H), 4.70 (br s, 1H), 5.24 (s, 2H), 6.50 (br s, 1H), 7.40-7.46 (m, 5H). Anal. Calcd for $C_{26}H_{38}N_4O_5$: C, 64.18; H, 7.87; N, 11.51. Found: C, 64.00; H, 8.03; N, 11.77.

***N*-(*tert*-Butoxycarbonyl)-*N*'-3-(1-benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyldiaminohexane, 2d.** The residual oil was purified by silica gel column chromatography with $CHCl_3$-acetone-EtOH (100:10:2) mixture to give the product, 75%: mp 97-100 °C; $^1$H NMR ($CDCl_3$) δ 1.32 (m, 4H), 1.44 (s, 13H), 2.20 (s, 3H), 2.24 (s, 3H), 2.65 (t, *J* 7 Hz, 2H), 3.09 (q, *J* 7 Hz, 3H), 3.16 (t, *J* 7 Hz, 2H), 3.23 (q, *J* 7 Hz, 2H), 4.50 (br s, 1H), 5.24 (s, 2H), 6.25 (br s, 1H), 7.41-7.47 (m, 5H). Anal. Calcd for $C_{27}H_{40}N_4O_5 \cdot 0.1H_2O$: C, 64.54; H, 8.06; N, 11.15. Found: C, 64.14; H, 8.03; N, 11.04.

***N*-3-(1-benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyldiaminoethane hydrochloride, 3a.** To a solution of compound **2a** (226 mg, 0.48 mmol) in $CH_2Cl_2$ (4 mL) was added $CF_3CO_2H$ (TFA) (4 mL) dropwise at 0 °C. The reaction mixture was stirred for 30 min at 0 °C, and then the solvent was evaporated. Dry benzene was added to the residue and evaporated. Addition and evaporation of benzene were repeated three times to give the product (**3a**) (ca. 100%), which was directly used for the next reaction.

**General Procedure for Deprotection of the Boc Group of Compounds (2b-d). A Typical Example:*N*-3-(1-benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyldiaminobutane hydrochloride, 3b.** To a solution of compound **2b** (308 mg, 0.65 mmol) in dry dioxane (6 mL) was added dropwise 4N HCl in dioxane (3 mL) at 0 °C. The reaction mixture was stirred for 20 min at 0 °C, and then the solvent was evaporated. Dry benzene was added to the residue and evaporated. Addition and evaporation of benzene were repeated three times to give the product (**3b**) (ca. 100%), which was directly used for the next reaction.

**1,1,1-Tris(succinimideoxycarbonylethoxymethyl)ethane, 5.** To a solution of 1,1,1-tris(carboxyethoxymethyl)ethane **4** (104 mg, 0.31 mmol), HOSu (150 mg, 1.30 mmol) in THF (10 mL) was added WSC·HCl (190 mg, 0.99 mmol) in $CH_2Cl_2$ (10 mL) at -10 °C. After stirring for 24 h at room temperature, the solvent was evaporated, and the residue was dissolved in AcOEt (300 mL). The organic layer was washed with $H_2O$, 5% $NaHCO_3$, brine, and dried ($MgSO_4$). Evaporation of the solvent gave the *O*-succinimide ester (**5**) as colorless oil, which was used for the next reaction without further purification, 147 mg (76%); IR (neat) 1812, 1788, 1744 $cm^{-1}$.

**General Procedure for Tripodal Compounds (6a-d). A Typical Example: 1,1,1-Tris[2-[2-[3-[1-(benzyloxy)-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl]propanamide]ethylaminocarbonyl]ethyloxymethyl]ethane, 6a.** A solution of compound **3a** (490 mg, 1.1 mmol), compound **5** (200 mg, 0.32 mmol) and $NEt_3$ (121 mg, 1.2 mmol) in DMF (8 mL) was stirred for 48 h at 38 °C. After removal of the solvent, $CHCl_3$ (200 mL) was added to the residue. The organic layer was washed with $H_2O$, 5% $NaHCO_3$, 10% citric acid, and brine, and dried ($MgSO_4$). Purification by column chromatography on

silica gel with $CHCl_3$-MeOH (6:1) mixture and subsequent gel chromatography on TOYOPEARL HW-40 with MeOH as eluent gave the product as an amorphous solid (**6a**), 217 mg (51%); $^1H$ NMR ($CDCl_3$) δ 0.86 (s, 3H), 2.18 (s, 9H), 2.22 (s, 9H), 2.42 (t, *J* 7 Hz, 6H), 2.64 (t, *J* 7Hz, 6H), 3.13 (t, *J* 7 Hz, 6H), 3.24 (m, 6H), 3.37 (s, 12H), 3.64 (t, *J* 7 Hz, 6H), 5.22 (s, 6H), 7.08 (br s, 3H), 7.40-7.55 (m, 18H). Anal. Calcd for $C_{68}H_{90}N_{12}O_{15}\cdot 2H_2O$: C, 60.43; H, 7.04; N, 12.44. Found: C, 60.32; H, 7.34; N, 12.88.

**1,1,1-Tris[2-[2-[3-[1-(benzyloxy)-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl]propanamide]butylaminocarbonyl]ethyloxymethyl]ethane, 6b.** 82%: mp 104-109 °C (decomp.); $^1H$ NMR ($CDCl_3$) δ 0.87 (s, 3H), 1.53 (m, 12H), 2.19 (s, 9H), 2.23 (s, 9H), 2.42 (t, *J* 7 Hz, 6H), 2.65 (t, *J* 7 Hz, 6H), 3.14 (t, *J* 7 Hz, 6H), 3.25 (m, 18H), 3.64 (t, *J* 7 Hz, 6H), 5.22 (s, 6H), 6.66 (br s, 3H), 6.90 (br s, 3H), 7.39-7.47 (m, 15H). Anal. Calcd for $C_{76}H_{102}N_{12}O_{15}\cdot 4H_2O$: C, 61.03; H, 7.41; N, 11.24. Found: C, 60.85; H, 7.29; N, 11.49.

**1,1,1-Tris[2-[2-[3-[1-(benzyloxy)-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl]propanamide]pentylaminocarbonyl]ethyloxymethyl]ethane, 6c.** An amorphous solid, 57%: $^1H$ NMR ($CDCl_3$) δ 0.88 (s, 3H), 1.32 (m, 6H), 1.50 (m, 12H), 2.20 (s, 6H), 2.34 (s, 6H), 2.41 (t, *J* 7 Hz, 6H), 2.65 (t, *J* 7 Hz, 6H), 3.12-3.24 (m, 18H), 3.65 (t, *J* 7 Hz, 6H), 5.24 (s, 6H), 6.62 (t, *J* 6 Hz, 3H), 6.85 (t, *J* 6 Hz, 3H), 7.41-7.53 (m, 15H). Anal. Calcd for $C_{79}H_{108}N_{12}O_{15}\cdot 7H_2O$: C, 59.61; H, 7.72; N, 10.56. Found: C, 59.69; H, 7.80; N, 10.75.

**1,1,1-Tris[2-[2-[3-[1-(benzyloxy)-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl]propanamide]hexylaminocarbonyl]ethyloxymethyl]ethane, 6d.** 54%: mp 115-112 °C (decomp.); $^1H$ NMR ($CDCl_3$) δ 0.88 (s, 3H), 1.32 (m, 12H), 1.49 (12H, m), 2.20 (s, 9H), 2.23 (s, 9H), 2.40 (t, *J* 7 Hz, 6H), 2.64 (t, *J* 7 Hz, 6H), 3.12-3.23 (m, 24H), 3.64 (t, *J* 7 Hz, 6H), 5.23 (s, 6H), 6.59 (br s, 3H), 6.79 (br s, 3H), 7.39-7.49 (m, 15H). Anal. Calcd for $C_{82}H_{114}N_{12}O_{15}\cdot 3H_2O$: C, 63.06; H, 7.74; N, 10.76. Found: C, 62.92; H, 7.94; N, 10.78.

**General Procedure for Target Compounds (3HOPR$_n$CMe). A Typical Example: 1,1,1-Tris[2-[2-[3-[1-hydroxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl]propanamide]ethylaminocarbonyl]ethyloxymethyl]ethane, 3HOPR$_2$CMe.** A suspension of 10% Pd-C (19 mg) suspended in MeOH (10 mL) was prehydrogenated with $H_2$ for 0.5 h. To the suspension was added a solution of compound **6a** (106 mg, 0.08 mmol) in MeOH (20 mL). After hydrogenation with $H_2$ under atmospheric pressure for 30 min at room temperature, the catalyst was removed by filtration. The filtrate was evaporated to give the residue, which was purified by gel chromatography on Shephadex LH-20 with MeOH to afford the product (**3HOPR$_2$CMe**), 82 mg (100%): $^1H$ NMR ($CD_3OD$) δ 0.86 (s, 3H), 2.32 (s, 9H), 2.39 (m, 15H), 2.60 (t, *J* 7 Hz, 6H), 3.04 (t, *J* 7 Hz, 6H), 3.20-3.40 (m, 18H), 3.62 (t, *J* 7 Hz, 6H). Anal. Calcd for $C_{46}H_{72}N_{12}O_{15}\cdot 7H_2O$: C, 48.18; H, 7.40; N, 14.35. Found: C, 48.49; H, 6.94; N, 14.00.

**1,1,1-Tris[2-[2-[3-[1-hydroxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl]propanamide]-butylaminocarbonyl]ethyloxymethyl]ethane, 3HOPR$_4$CMe.** 100%: $^1H$ NMR ($CD_3OD$) δ 0.85 (s, 3H), 1.50 (m, 12H), 2.31 (s, 9H), 2.38 (m, 15H), 2.58 (t, *J* 7 Hz, 6H), 3.03 (t, *J* 7 Hz, 6H), 3.17 (m, 12H), 3.24 (s, 6H), 3.61 (t, *J* 7 Hz, 6H). Anal. Calcd for $C_{53}H_{84}N_{12}O_{15}\cdot 2H_2O$: C, 54.63; H, 7.61; N, 14.42. Found: C, 54.33; H, 7.50; N, 14.23.

**1,1,1-Tris[2-[2-[3-[1-hydroxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl]propanamide]-pentylaminocarbonyl]ethyloxymethyl]ethane, 3HOPR$_5$CMe.** 70%: $^1H$ NMR ($CD_3OD$) δ 0.86 (s, 3H), 1.47 (m, 18H), 2.30 (s, 9H), 2.37 (m, 15H), 2.57 (t, *J* 7 Hz, 6H), 3.02 (t, *J* 7 Hz, 6H), 3.16 (m, 12H), 3.24 (s, 6H), 3.62 (t, *J* 7 Hz, 6H). Anal. Calcd for $C_{56}H_{90}N_{12}O_{15}\cdot 1.5H_2O$: C, 60.80; H, 8.05; N, 10.91. Found: C, 60.99; H, 7.75; N, 10.56.

**1,1,1-Tris[2-[2-[3-[1-hydroxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl]propanamide]-hexylaminocarbonyl]ethyloxymethyl]ethane, 3HOPR$_6$CMe.** 98%: mp 135-143 °C (decomp); $^1H$ NMR (DMSO-$d_6$) δ 0.77 (s, 3H), 1.23-1.37 (m, 24H), 2.23 (s, 9H), 2.28 (m, 15H), 2.42 (t, *J* 7 Hz, 6H),

2.87 (t, *J* 8 Hz, 6H), 3.02 (m, 12H), 3.14 (s, 6H), 3.52 (t, *J* 7 Hz, 6H), 7.79 (br s, 6H). Anal. Calcd for $C_{59}H_{96}N_{12}O_{15}\cdot 3H_2O$: C, 55.91; H, 8.11; N, 13.26. Found: C, 56.19; H, 8.23; N, 12.91.

**Measurement of UV-vis spectra of Fe(3opr4CMe) under various pH Conditions.** A sample (3 mg) of **3HOPR4CMe** was mixed with an equimolar amount of the stock ferric nitrate solution (3.28 mM), and diluted to 10 mL with deionized water (0.28 mM). The pH of the solution was adjusted to an appropriate value with 0.1 or 0.01 N NaOH before measurement.

**Iron Binding Ratio.** A sample (3 mg) of each hexadentate ligand was dissolved in deionized water (5.0 mL). The solution (0.5 mL) was mixed with an appropriate amount of standard aqueous ferric nitrate solution (0.25-2.25 mL, 0.33 mM) and 0.5 mL of 0.4 M $KNO_3$. The pH of the mixture was adjusted to 6.0 with 0.01 or 0.1 N NaOH and diluted to 5.0 mL with McIlvaine's buffer (pH 6.0), and the visible spectra were measured.

**Iron Exchange Reaction.** Each ferric complex solution (0.04 mM) of hexadentate ligand was prepared by mixing the stock ligand solution (1.0 mM), an equimolar amount of ferric nitrate solution (3.28 mM), 0.5 mL of 0.4 M $KNO_3$, and then diluting to 5.0 mL with McIlvaine's buffer solution. EDTA in the buffer solution was prepared by dissolving $EDTA{\cdot}2Na^{+}{\cdot}2H_2O$ in McIlvaine's buffer solution (ionic strength 0.04, pH 6.0) to give a concentration of 15.4 mM. The iron exchange reaction was initiated by mixing of complex solution (2 mL) with EDTA solution (64 μl), and followed by monitoring the decrease of absorbance at 500 nm. The relative stability constant of ferric complex was calculated by using the stability constant of Fe(edta),[23] the pKa of the **HOPR-Me**[19] and an equilibrium point at pH 6.0 at 20 °C.

**Iron Removal from Transferrin.** A commercially available human serum apotransferrin (98%, Sigma) was used. $Fe_{2.0}$**Tf** was prepared according to the literature reported in detail by Raymond.[10, 26] The stock solutions of **3HOPR$_n$CMe** (0.22 mL, 20 mM, pH 7.4) and $Fe_{2.0}$**Tf** (2 mL, 0.05 mM) in Tris buffer were combined, and then the absorbance of the solution was monitored at 500 nm. The pseudo-first-order-rate constant ($k_{obsd}$) was calculated from the slope of the plots of log $[(A_\infty\text{-Abs})/(A_\infty\text{-A0})]$ as a function of time.

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