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# Synthesis and superoxide dismutase activity of novel iron complexes

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**Abstract**

We have previously shown that the Fe(II) tetrakis-*N*,*N*,*N*′,*N*′-(2-pyridylmethyl)ethylenediamine complex (Fe(II)TPEN) has high superoxide dismutase (SOD) activity, using the xanthine–xanthine oxidase–cytochrome *c* method (cyt. *c* method) [J. Biol. Chem. 264 (1989) 9243–9249]. X-ray analysis showed that Fe(II)TPEN has two different coordination structures, one in which Fe(II) is coordinated with six nitrogens of TPEN and one in which Fe(II) is coordinated with five nitrogens of TPEN and one oxygen of sulfate anion as the counter anion [Chem. Pharm. Bull. 48 (2000) 223–230]. To investigate the relationship between these two structures and SOD activity, we synthesized novel Fe(II) complexes of TPEN analogues and measured their SOD activity by the cyt. *c* method. The Fe(II) tetrakis-*N*,*N*,*N*′,*N*′-(2-pyridylmethyl)trimethylenediamine complex (Fe(II)TPTN) and the Fe(II) tris(2-pyridylmethyl)triazacyclononane complex (Fe(II)TPTCN) had no SOD activity ($IC_{50} = > 100$ μM), probably because these two complexes have undistorted steric structure with no easily substituted ligand. On the other hand, other Fe(II) complexes with unsaturated coordination or an easily substituted ligand had high SOD activity ($IC_{50} = 0.4–20$ μM). The results indicate that the substitution reaction of a ligand with superoxide or the coordination of superoxide is essential for Fe(II)TPEN analogue complexes to have SOD activity. Moreover, we examined the effect of steric hindrance of the ligands on the SOD activity and the stability of the iron complexes to oxygen. 

*Keywords:* Superoxide; Superoxide dismutase; Iron complex

## 1. Introduction

Superoxide ($O_2^-$), a product of cellular respiration, activates polymorphonuclear leukocytes and endothelial cells, and is involved in the pathology of many diseases, such as inflammation, ischemia-reperfusion injury and carcinogenesis. Superoxide dismutases (SODs) catalyze the conversion of superoxide to hydrogen peroxide plus dioxygen and protect cells from damage by $O_2^-$ [1]. Therefore, SODs may have potential as pharmaceuticals, and there have been reports of beneficial effects of SODs and modified SODs. However, there are problems intrinsic to their protein nature, such as cost, stability, membrane permeability and immunogenicity, which must be overcome in order to use SODs as pharmaceuticals. The use of SOD mimics is one possible approach, and many low-molecular metal complexes, mainly copper and manganese complexes, have been synthesized to examine their SOD activity in vitro and in vivo [2–17]. Some complexes have been reported to have SOD activity in vivo [13,15,18–24]. However, there are few reports about the structure–activity relationship (SAR) of SOD mimics. It is important to investigate SAR in order to develop novel SOD mimics as pharmaceuticals. We have previously shown that the Fe(II) tetrakis-*N*,*N*,*N*′,*N*′-(2-pyridylmethyl)ethylenediamine complex (Fe(II)TPEN) has high SOD activity, using the xanthine–xanthine oxidase–cytochrome *c* (cyt. *c*) method, and it protected *Escherichia coli* cells from paraquat toxicity [25]. We recently revealed that Fe(II)TPEN has two different structures, depending on the species of counter anion [26]. In the cases of pentafluorophosphine or perchlorate [27], which have no, or very low, ability to coordinate, Fe(II) is coordinated

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with six nitrogens of TPEN (Fig. 1(a)). However, in the case where the sulfate anion is the counter anion, one pyridine is replaced by the sulfate anion and Fe(II) is coordinated with five nitrogens of TPEN and one oxygen of the sulfate anion (Fig. 1(b)). This shows that Fe(II)TPEN has one easily substituted ligand.

A ligand which is readily substituted by an anionic species may be required for the SOD activity of Fe complexes. We synthesized some Fe(II) complexes of TPEN analogues and measured their SOD activity by the cyt. *c* method to elucidate the relationship between the structures of the Fe(II) complexes and the SOD activity.

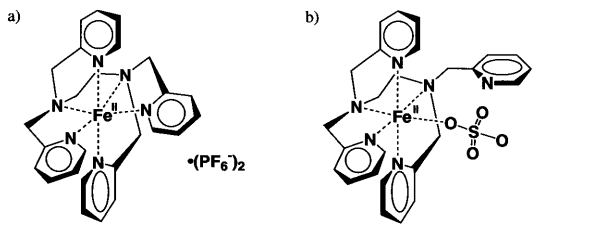



Fig. 1. Structures of Fe(II)TPEN$(PF_6)_2$ and Fe(II)TPEN$(SO_4)$.

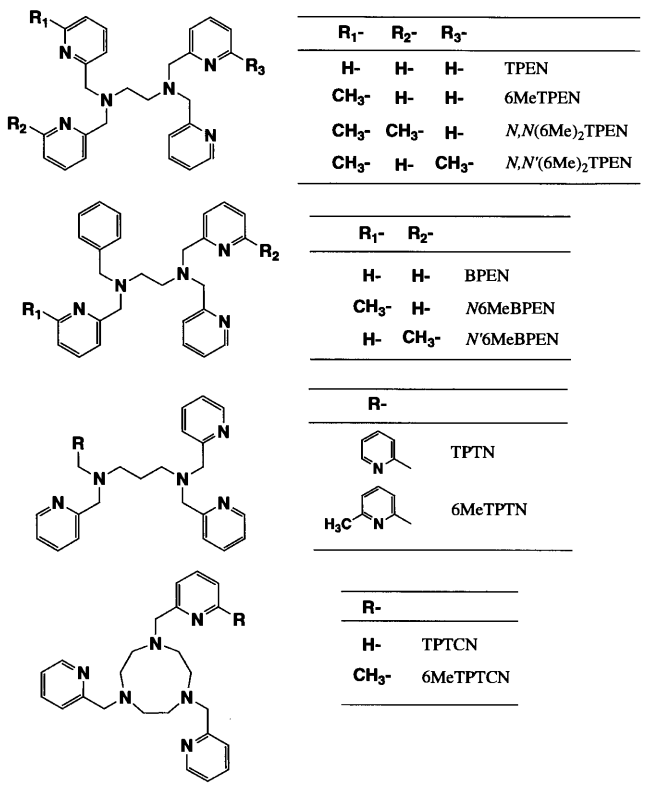



Fig. 2. Structures of chelators.

## 2. Results and discussion

### 2.1. Synthesis of chelators and Fe(II) complexes

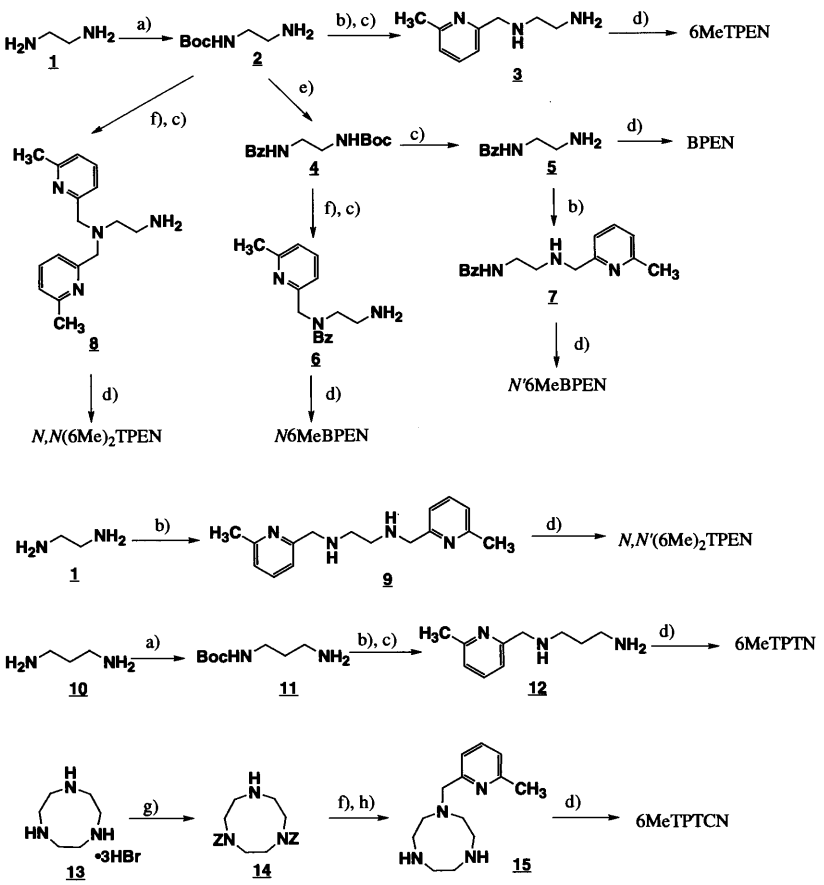
We synthesized 11 chelators consisting of pyridine and alkylamine, as shown in Fig. 2. The synthetic routes are shown in Scheme 1. TPEN, TPTN and TPTCN were synthesized by reacting the corresponding amine and 2-pyridylmethyl chloride hydrochloride at room temperature in alkaline aqueous solution. 6MeTPEN, BPEN, *N*,*N*(6Me)$_2$TPEN, *N*6MeBPEN and *N*′6MeBPEN, were synthesized via the common intermediate; *N*-*tert*-butoxycarbonylethylenediamine (**2**) by Schiff base formation with aldehyde, reduction of the Schiff base with $NaBH_4$, deprotection and *N*-alkylation. *N*,*N*′(6Me)$_2$TPEN was prepared by a method analogous to that used for 6MeTPEN. 6MeTPTN was synthesized analogously to 6MeTPEN from *N*-*tert*-butoxycarbonyltrimethylenediamine (**11**). 6MeTPTCN was synthesized via 1-(6-methyl-2-pyridylmethyl)triazacyclononane (**15**), which was prepared by *N*-6-methyl-picolylation and deprotection.

Ferrous complexes were prepared by reacting equimolar $FeSO_4 \cdot 7H_2O$ or $Fe(ClO_4)_2 \cdot 6H_2O$ and the chelators in MeOH at room temperature for several minutes, followed by evaporation in vacuo.

### 2.2. SOD activity of Fe(II) complexes

SOD activity of these ferrous complexes was determined by the xanthine–xanthine oxidase–cytochrome *c* method (Table 1) [28]. The $IC_{50}$ value is defined as the concentration of the complex that is required to inhibit the reduction rate of cytochrome *c* by 50%, and exerts SOD activity equivalent to 1 unit of native SOD. SOD activity of Fe(II)(6MeTPEN) and Fe(II)BPEN was equal to that of Fe(II)TPEN. The spectral data, especially molar extinction coefficients, show that Fe(II)(6MeTPEN) ($\varepsilon = 1670$, $\lambda_{max} = 363$ nm) and Fe(II)BPEN ($\varepsilon = 1760$, $\lambda_{max} = 396$ nm) are both five-coordinated Fe(II) complexes in a high spin state. Although Fe(II)TPEN ($\varepsilon = 10\,000$, $\lambda_{max} = 416$ nm) is a six-coordinated complex in a low spin state, one ligand can be easily replaced by $O_2^-$, due to the distorted structure. Thus, Fe(II)TPEN is considered to have structural characteristics resembling those of a five-coordinated complex.

The SOD activities of Fe(II)(*N*,*N*(6Me)$_2$TPEN), Fe(II)(*N*,*N*′(6Me)$_2$TPEN), Fe(II)(*N*6MeBPEN) and Fe(II)(*N*′6MeBPEN) were lower by one order of magnitude than that of Fe(II)TPEN. These complexes are five-coordinated, based on the spectral data ($\varepsilon = 1200$, 1550, 1540 and 1630, respectively). However, the coordination structures of these four complexes are different from that of five-coordinated Fe(II)BPEN. That is, the former four complexes are coordinated by four pyridi-




Scheme 1.

nes and one 6-methylpyridine, while Fe(II)BPEN is coordinated by five pyridines. In the case of Fe(II)(6MeTPEN), 6-methylpyridine is probably not coordinated to the Fe(II) ion, because the coordination ability of 6-methylpyridine is lower than that of pyridine. On the other hand, Fe(II)(*N*,*N*(6Me)$_2$TPEN), Fe(II)(*N*,*N*′(6Me)$_2$TPEN), Fe(II)(N6MeBPEN) and Fe(II)(N′6MeBPEN) are coordinated with one 6-methylpyridine. Thus, the 6-methylpyridine ligand should hinder the approach of $O_2^-$ to the sixth coordination site of the complexes, which may be the reason why the SOD activities of the four complexes are lower by one order of magnitude than those of Fe(II)-(6MeTPEN) and Fe(II)BPEN. In other words, the steric hindrance of the methyl group decreases the reaction rate at the step involving $O_2^-$.

Interestingly, Fe(II)TPTN and Fe(II)TPTCN showed no SOD activity, while Fe(II)(6MeTPTN) and Fe(II)(6MeTPTCN) had moderate SOD activity, as shown in Table 1. Fe(II)TPTN and Fe(II)TPTCN are undistorted, stable six-coordinated low-spin complexes [29], of which the $\varepsilon$ values are 11 000 and 12 000, respectively. $O_2^-$ cannot undergo a substitution reaction with Fe(II)TPTN and Fe(II)TPTCN. Fe(II)(6Me-TPTN) and Fe(II)(6MeTPTCN), which are the derivatives of Fe(II)TPTN and Fe(II)TPTCN with 6-methylpyridine as a ligand, should have unsaturated coordination or distorted structure, which leads to SOD activity. This characteristic structure allows $O_2^-$ to attack the complex, leading to one-electron oxidation of Fe(II) to Fe(III), followed by a disproportionation reaction of $O_2^-$.

Table 1
SOD activity of Fe(II) complexes [a]

| Fe(II) complex | $IC_{50}$ (μM) |
|---|---|
| Fe(II)TPEN | 0.5 |
| Fe(II)(6MeTPEN) | 0.4 |
| Fe(II)(*N*,*N*(6Me)$_2$TPEN) | 3.0 |
| Fe(II)(*N*,*N*′(6Me)$_2$TPEN) | 3.0 |
| Fe(II)BPEN | 0.4 |
| Fe(II)(*N*6MeBPEN) | 3.0 |
| Fe(II)(*N*′6MeBPEN) | 3.5 |
| Fe(II)TPTN | >100 |
| Fe(II)(6MeTPTN) | 2.0 |
| Fe(II)TPTCN | >100 |
| Fe(II)(6MeTPTCN) | 20.0 |

[a] $IC_{50}$ value is the concentration of Fe(II) complex required to inhibit by 50% the reduction rate of cytochrome *c* by superoxide. DMSO was used as a co-solvent.

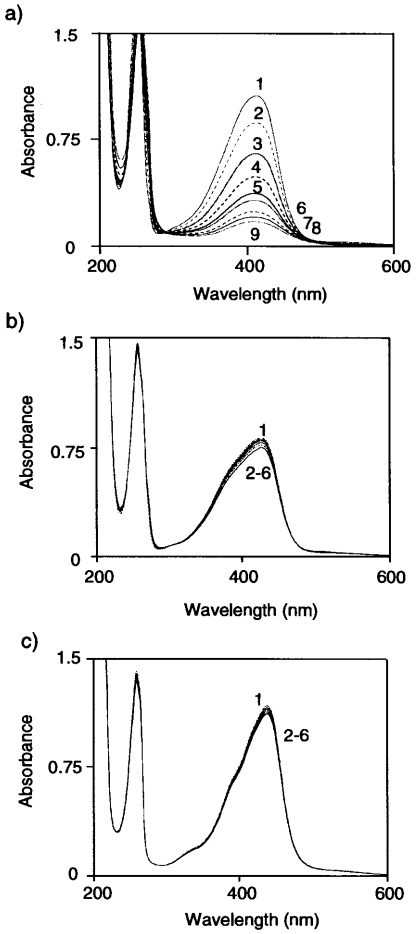



Fig. 3. Spectral changes of Fe(II)TPEN (a), Fe(II)TPTN (b) and Fe(II)TPTCN (c) by hydroxide anion. The spectra were measured in $H_2O$. The concentration of Fe(II) complex was 0.1 mM. (a) Line 1, no additive; line 2, 1.0 mM NaOH; line 3, 2.5 mM NaOH; line 4, 5.0 mM NaOH; line 5, 10 mM NaOH; line 6, 20 mM NaOH; line 7, 50 mM NaOH; line 8, 75 mM NaOH; line 9, 100 mM NaOH. (b) line 1, no additive; line 2, 1.0 mM NaOH; line 3, 2.0 mM NaOH; line 4, 3.0 mM NaOH; line 5, 4.0 mM NaOH; line 6 5.0 mM NaOH. (c) line 1, no additive; line 2, 1.0 mM NaOH; line 3, 2.0 mM NaOH; line 4, 3.0 mM NaOH; line 5, 4.0 mM NaOH; line 6 5.0 mM NaOH.

### 2.3. Spectral changes of Fe(II) complexes induced by hydroxide anion

Fe(II)TPEN has two different structures and one pyridine of Fe(II)TPEN can be replaced by another anion which has coordination ability. Fe(II)TPEN-$(ClO_4)_2$ and Fe(II)TPEN($SO_4$) give the same spectra in an aqueous solvent, while they show quite different spectra in MeOH (Fe(II)TPEN($ClO_4$); $\varepsilon = 10\,000$, $\lambda_{max} = 416$ nm, (Fe(II)TPEN($SO_4$); $\varepsilon = 3640$, $\lambda_{max} = 403$ nm). These observations suggest that the replacement of one pyridine of Fe(II) complexes by an anion such as sulfate or hydroxide anion changes the spectra. We measured the spectral change induced by hydroxide anion in $H_2O$. Fig. 3 shows the visible spectra of Fe(II)TPEN, Fe(II)TPTN and Fe(II)TPTCN in $H_2O$. The spectra of Fe(II)TPEN were changed and the absorbance was decreased by the addition of hydroxide anion. However, the addition of hydroxide anion had no effect on the spectra of Fe(II)TPTN and Fe(II)TPTCN. This indicates that Fe(II)TPTN and Fe(II)TPTCN do not have any easily replaced ligands and therefore should have no SOD activity.

To confirm this interpretation, Fe(II)(6MeTPTN) and Fe(II)(6MeTPTCN) were also examined in the presence of hydroxide anion, because these two complexes are expected to be easily substituted owing to the steric hindrance of the methyl functional group introduced at the 6-position of one pyridine ligand. As expected, the spectra of Fe(II)(6MeTPTN) and Fe(II)(6MeTPTCN), which have SOD activity, were changed like that of Fe(II)TPEN by the addition of hydroxide anion (Fig. 4). Large amounts of hydroxide anion were required to obtain a spectral change in the case of Fe(II)(6MeTPTCN) compared with Fe(II)-(6MeTPTN), which is consistent with the SOD activities of Fe(II)(6MeTPTN) ($IC_{50} = 2.0$ μM) and Fe(II)(6MeTPTCN) ($IC_{50} = 20.0$ μM). These results support the idea that it is essential for complexes to have an easily replaced ligand(s) in order to show SOD activity.

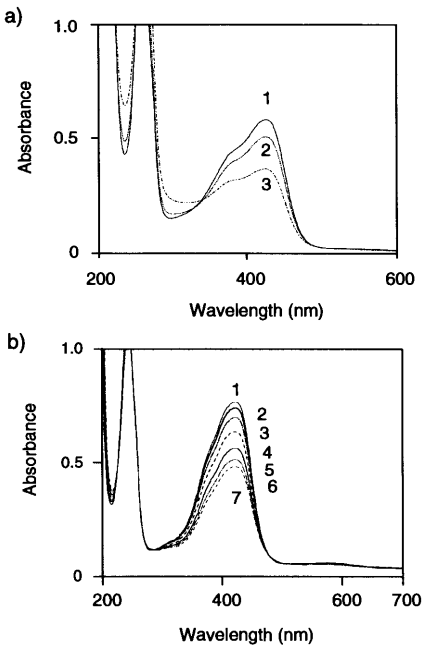



Fig. 4. Spectral changes of Fe(II)(6MeTPTN) (a) and Fe(II)(6MeTPTCN) (b) by hydroxide anion. The spectra were measured in $H_2O$. The concentration of Fe(II) complex was 0.1 mM. (a) line 1, no additive; line 2, 0.1 mM NaOH; line 3, 0.2 mM NaOH; line 4, 0.3 mM NaOH. (b) line 1, no additive; line 2, 5.0 mM NaOH; line 3, 25 mM NaOH; line 4, 50 mM NaOH; line 5, 100 mM NaOH; line 6, 150 mM NaOH; line 7, 200 mM NaOH.

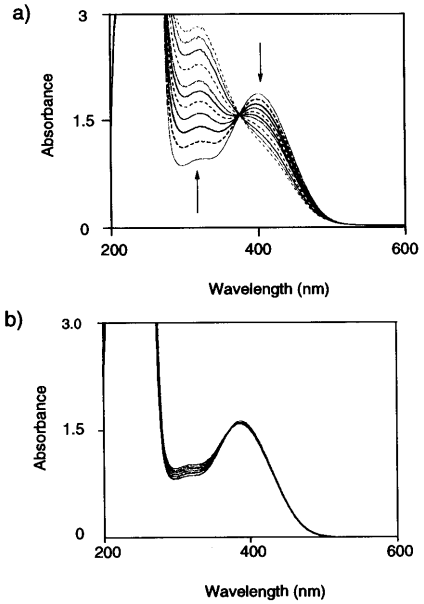



Fig. 5. Spectral change of Fe(II)BPEN (a) and Fe(II)(*N*′6MeBPEN) (b) in the presence of $O_2$. The spectra were measured in 50 mM potassium phosphate buffer (pH 7.4). The concentration of Fe(II) complex was 1.0 mM. (a) The spectra were measured after 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6 and 7 h. (b) The spectra were measured after 0, 1, 2, 3, 4, 5, 6, 7, 8 and 9 h.

### 2.4. Stability of Fe(II) complexes to oxygen

In order to investigate the steric effect of a methyl group, the stability of Fe(II)BPEN and Fe(*N*′6MeBPEN) to oxygen was examined. Fig. 5 shows that Fe(II)BPEN was auto-oxidized, while Fe(*N*′6MeBPEN) was stable, which indicates that the methyl functional group increased the stability of the complex to oxygen. The methyl group of 6-methylpyridine can apparently block the approach of oxygen to the Fe(II) complex.

Fe(II)TPEN was stable in the presence of oxygen for more than 1 month, and Fe(II)TPTN and Fe(II)-TPTCN were also non-reactive with oxygen. These three complexes are considered not to react with oxygen because of their saturated coordination structure.

In summary, we synthesized 11 chelators and measured the SOD activity of their Fe(II) complexes in order to clarify the relationship between the two structures of Fe(II)TPEN and SOD activity. It is important for an Fe(II) complex to have an easily replaced ligand in order to exhibit SOD activity. Moreover, coordination of 6-methylpyridine to Fe(II) makes the complex stable to oxygen, owing to steric hindrance.

We are now searching for more active complexes than Fe(II)TPEN by introducing an electron-donating group such as a dimethylamino, methoxy or methyl group at the 4-position of pyridine of TPEN. In addition, further studies on biological applications of these complexes are in progress using biological assay systems such as paraquat toxicity in *E. coli*.

## 3. Experimental

### 3.1. Materials

Catalase, cytochrome *c* (type III), and xanthine sodium salt were purchased from Sigma. Xanthine oxidase was purchased from Boehringer Mannheim GmbH. The other chemicals were all reagent-grade products from Aldrich, Tokyo Kasei Kogyo Ltd, and Wako Ltd.

### 3.2. Assay of SOD activity

SOD activity was measured according to the method of Ref. [28], except for omitting EDTA and adding catalase. The reaction mixture was prepared with 50 mM potassium phosphate buffer (pH 7.4) containing 10 μM cytochrome *c*, 50 μM xanthine and catalase (1200 U $ml^{-1}$). The reaction was started by adding xanthine oxidase at 25°C, and reduction of cytochrome *c* was monitored at 550 nm. The amount of xanthine oxidase added was adjusted so as to give a rate of $A_{550nm} = 0.025$ $min^{-1}$ in the absence of a SOD mimic. SOD activity ($IC_{50}$) was determined as the concentration which inhibits by 50% the reduction rate of cytochrome *c*.

### 3.3. Synthesis

#### 3.3.1. *N*,*N*,*N*′,*N*′-Tetrakis(2-pyridylmethyl)-ethylenediamine (TPEN)

TPEN was synthesized according to Ref. [30]. 2-Pyridylmethyl chloride hydrochloride (31.0 g, 189 mmol) was dissolved in 10 ml $H_2O$. To this solution, NaOH solution (5 N 100 ml), ethylenediamine (2.86 g, 47 mmol) and cetyltrimethylammonium chloride (323 mg) were added. The reaction mixture was stirred at room temperature (r.t.) under Ar for 24 h and the crude product was extracted with $CH_2Cl_2$. The $CH_2Cl_2$ phase was dried over anhydrous $K_2CO_3$ and evaporated. The residue was chromatographed on alumina and recrystallized from benzene to afford TPEN (45.3%). M.p. 115–117°C. $^1$H-NMR (400 MHz, $CDCl_3$/TMS) 2.76 (s, 4H), 3.78 (s, 8H), 7.11 (dd, 4H), 7.45 (d, 4H), 7.57 (ddd, 4H), 8.48 (d, 4H); MS 424 [$M^+$]; Anal. Calc. for $C_{26}H_{28}N_6$: C, 73.56; H, 6.65; N, 19.80. Found: C, 73.55; H, 6.70; N, 19.60%.

#### 3.3.2. *N*,*N*,*N*′,*N*′-Tetrakis(2-pyridylmethyl)-trimethylenediamine (TPTN)

TPTN was prepared analogously to TPEN from trimethylenediamine (59.6%). M.p. 84.5–86.0°C (cyclohexane). $^1$H-NMR (400 MHz, $CDCl_3$/TMS) 1.81 (m, 2H), 2.55 (t, 4H, $J = 7.6$ Hz), 3.75 (s, 8H), 7.11 (ddd, 4H), 7.41 (d, 4H), 7.58 (ddd, 4H), 8.48 (dd, 4H); MS 438 [$M^+$]; Anal. Calc. for $C_{27}H_{30}N_6$: C, 73.94; H, 6.89; N, 19.16. Found: C, 74.04; H, 7.00; N, 19.01%.

*3.3.3. 1,4,7-Tris(2-pyridylmethyl)trimethylenediamine (TPCTN)*

TPTCN was prepared from 1,4,7-triazacyclononane (quant). $^1$H-NMR (400 MHz, $CDCl_3$/TMS) 2.90 (s, 12H), 3.84 (s, 6H), 7.15 (dd, 3H), 7.51 (d, 3H), 7.65 (ddd, 3H), 8.51 (d, 3H); MS 402 [$M^+$].

*3.3.4. N-tert-Butoxycarbonylethylenediamine (2)*

To EtOH 20 ml containing ethylenediamine **1** (16 ml, 239 mmol) and triethylamine (1.03 g, 10.2 mmol), di-*tert*-butyl dicarbonate (2.22 g, 10.2 mmol) was added dropwise at 0°C. After having been stirred at r.t. for 1 h, the reaction mixture was concentrated. The residue was dissolved in $CH_2Cl_2$ and the product was extracted with 1 N AcOH aqueous solution. The aqueous phase was basified with NaOH aqueous solution and the product was again extracted with $CH_2Cl_2$. The $CH_2Cl_2$ phase was dried over anhydrous $K_2CO_3$ and evaporated to afford **2**. $^1$H-NMR ($CDCl_3$/TMS) 1.22 (s, 2H), 1.42 (s, 9H), 2.50–2.85 (m, 2H), 2.95–3.20 (m, 2H), 5.60 (brs, 1H).

*3.3.5. N-(6-Methyl-2-pyridylmethyl)ethylenediamine (3)*

To MeOH solution containing **2** (5.394 g, 33.7 mmol) and 3 Å molecular sieves, 6-methylpyridine-2-carboxaldehyde (4.083 g, 33.8 mmol) in MeOH was added dropwise under Ar. The reaction mixture was refluxed for 1.5 h. After removal of the molecular sieves by filtration, $NaBH_4$ (3.93 g, 103.4 mmol) was added and the whole was stirred overnight at r.t. MeOH was evaporated and 2 N $Na_2CO_3$ aqueous solution was added to the residue. The crude product was extracted with $CH_2Cl_2$. The $CH_2Cl_2$ phase was dried over anhydrous $K_2CO_3$ and evaporated. The residue was chromatographed on alumina to afford *N*-*tert*-butoxycarbonyl-*N*′-(6-methyl-2-pyridylmethyl)ethylenediamine (30.1%). $^1$H-NMR ($CDCl_3$/TMS) 1.33 (s, 9H), 2.07 (s, 1H), 2.54 (s, 3H), 2.65–2.95 (m, 2H), 3.07–3.35 (m, 2H), 3.85 (s, 2H), 5.20 (brs, 1H), 7.13 (dd, 2H, $J = 7$ Hz, 3 Hz), 7.54 (t, 1H, $J = 7$ Hz).

*N* - *tert* - Butoxycarbonyl - *N*′ - (6 - methyl - 2 - pyridylmethyl)ethylenediamine (616 mg, 2.32 mmol) in $CH_2Cl_2$ was added dropwise to trifluoroacetic acid 20 ml at 0°C. The mixture was stirred at r.t. for 1.5 h, then concentrated. The residue was taken up in 2 N $Na_2CO_3$ aqueous solution and the crude product was extracted with $CH_2Cl_2$. The $CH_2Cl_2$ phase was dried over anhydrous $K_2CO_3$ and evaporated. The residue was used in the next reaction without further purification. $^1$H-NMR ($CDCl_3$/TMS) 1.78 (s, 3H), 2.55 (s, 3H), 2.68–2.88 (m, 4H), 3.85 (s, 2H), 5.20 (brs, 1H), 6.98 (d, 1H, $J = 7$ Hz), 7.08 (d, 1H, $J = 7$ Hz), 7.50 (t, 1H, $J = 7$ Hz).

*3.3.6. N-(6-Methyl-2-pyridylmethyl)-N,N′,N′-tris-(2-pyridylmethyl)ethylenediamine (6MeTPEN)*

*N*-(6-Methyl-2-pyridylmethyl)ethylenediamine (399 mg, 1.56 mmol) and 2-pyridylmethyl chloride hydrochloride (768 mg, 4.68 mmol) were added to 5 N NaOH aqueous solution 10 ml and the mixture was stirred at r.t. for 4 days. $H_2O$ (10 ml) was added and the crude product was extracted with $CH_2Cl_2$. The $CH_2Cl_2$ phase was dried over anhydrous $K_2CO_3$ and evaporated. The residue was chromatographed on alumina and recrystallization from *n*-hexane afforded 6MeTPEN (43.9%). M.p. 86.0–87.0°C. $^1$H-NMR (400 MHz, $CDCl_3$/TMS) 2.49 (s, 3H), 2.76 (s, 4H), 3.75 (s, 2H), 3.77 (s, 6H), 6.97 (d, 1H), 7.11 (ddd, 3H), 7.28 (d, 1H), 7.44–7.48 (m, 4H), 7.56 (ddd, 3H), 8.48–8.49 (m, 3H); MS 438 [$M^+$]; Anal. Calc. for $C_{27}H_{30}N_6$: C, 73.94; H, 6.89; N, 19.16. Found: C, 74.12; H, 6.94; N, 19.09%.

*3.3.7. N-Benzyl-N,N′,N′-tris(2-pyridylmethyl)-ethylenediamine (BPEN)*

BPEN was prepared analogously to 6MeTPEN from **2**. M.p. 84.0–87.0°C (*n*-hexane). $^1$H-NMR (400 MHz, $CDCl_3$/TMS) 2.70–2.74 (m, 4H), 3.58 (s, 2H), 3.71 (s, 2H), 3,77 (s, 4H), 7.09–7.13 (m, 3H), 7.20–7.30 (m, 5H), 7.43 (d, 2H), 7.46 (d, 1H), 7.54–7.59 (m, 3H), 8.47–8.49 (m, 3H); MS 423 [$M^+$]; Anal. Calc. for $C_{27}H_{29}N_5$: C, 76.56; H, 6.90, N, 16.53. Found: C, 76.70; H, 6.80; N, 16.40%.

*3.3.8. N,N-Bis(6-methyl-2-pyridylmethyl)-N′N′-bis(2-pyridylmethyl)ethylenediamine (N,N(6Me)$_2$TPEN)*

*N*,*N*(6Me)$_2$TPEN was prepared analogously to 6MeTPEN from *N*,*N*-bis(2-pyridylmethyl)ethylenediamine. M.p. 85.5–87.0°C (*n*-hexane). $^1$H-NMR (400 MHz, $CDCl_3$/TMS) 2.49 (s, 6H), 2.75 (s, 4H), 3.75 (s, 4H), 3.77 (s, 4H), 6.96 (d, 2H, $J = 7.2$ Hz), 7.10 (t, 2H, $J = 7.2$ Hz), 7.29 (d, 2H, $J = 7.2$ Hz), 7.45 (t, 2H, $J = 7.6$ Hz), 7.56 (ddd, 2H, $J = 5.0$, 2.0 Hz), 8.48 (d, 2H, $J = 8.0$ Hz); MS 452 [$M^+$].

*3.3.9. N,N′-Bis(6-methyl-2-pyridylmethyl)-N′,N′-bis(2-pyridylmethyl)ethylenediamine (N,N′(6Me)$_2$TPEN)*

*N*,*N*′(6Me)$_2$TPEN was prepared analogously to 6MeTPEN. M.p. 93.5–94.5°C (*n*-hexane). $^1$H-NMR (400 MHz, $CDCl_3$/TMS) 2.49 (s, 6H), 2.75 (s, 4H), 3.75 (s, 4H), 3.77 (s, 4H), 6.97 (d, 2H), 7.11 (dd, 2H), 7.29 (d, 2H), 7.44–7.47 (m, 4H), 7.56 (ddd, 2H), 8.48 (d, 2H); MS 452 [$M^+$]; Anal. Calc. for $C_{28}H_{32}N_6$: C, 74.31; H, 7.13; N, 18.57. Found: C, 74.09; H, 7.26; N, 18.37%.

*3.3.10. N-Benzyl-N-(6-methyl-2-pyridylmethyl)-N′,N′-bis(2-pyridylmethyl)ethylenediamine (N6MeBPEN)*

*N*6MeBPEN was prepared analogously to 6MeTPEN from *N*-benzyl-*N*-(6-methyl-2-pyridylmethyl)-ethylenediamine. $^1$H-NMR (400 MHz, $CDCl_3$/TMS) 2.49 (s, 3H), 2.69–2.74 (m, 4H), 3.58 (s, 2H), 3.69 (s,

2H), 3.76 (s, 4H), 6.96 (d, 1H, $J = 7.2$ Hz), 7.09–7.59 (m, 13H), 8.48–8.50 (m, 1H).

### 3.3.11. *N*-Benzyl-*N*′-(6-methyl-2-pyridylmethyl)-*N*′,*N*′-bis(2-pyridylmethyl)ethylenediamine (*N*′6MeBPEN)

*N*′6MeBPEN was prepared analogously to 6MeTPEN from *N*-benzyl-*N*′-(6-methyl-2-pyridylmethyl)-ethylenediamine. M.p. 86.5–88.0°C (*n*-hexane). $^1$H-NMR (400 MHz, $CDCl_3$/TMS) 2.50 (s, 6H), 2.69–2.74 (m, 4H), 3.58 (s, 2H), 3.71 (s, 2H), 3.74 (s, 4H), 3.75 (s, 2H), 3.76 (s, 4H), 6.97 (d, 1H), 7.09–7.12 (m, 2H), 7.19–7.29 (m, 6H), 7.43–7.48 (m, 3H), 7.56 (d, 2H), 8.46–8.49 (m, 2H); MS 437 [$M^+$]; Anal. Calc. for $C_{28}H_{31}N_5$: C, 76.86; H, 7.14; N, 16.00. Found: C, 76.80; H, 7.12; N, 15.81%.

### 3.3.12. *N*-(6-Methyl-2-pyridylmethyl)-*N*,*N*′,*N*′-tris(2-pyridylmethyl)trimethylenediamine (6MeTPTN)

6MeTPTN was prepared analogously to 6MeTPEN. $^1$H-NMR (400 MHz, $CDCl_3$/TMS) 1.80 (m, 2H), 2.50 (s, 3H), 2.55 (t, 4H, $J = 6.8$ Hz), 3.73 (s, 2H), 3.75 (s, 6H), 6.96 (d, 1H, $J = 7.6$ Hz), 7.11 (ddd, 3H, $J = 6.4$, 1.2 Hz), 7.43 (d, 1H, $J = 7.6$ Hz), 7.42 (m, 3H), 7.47 (t, 1H, $J = 7.6$ Hz), 7.58 (ddd, 3H, $J = 7.6$, 1.6 Hz), 8.48 (m, 3H); MS 452 [$M^+$].

### 3.3.13. 1-(6-Methyl-2-pyridylmethyl)-4,7-bis(2-pyridylmethyl)triazacyclononane (6MeTPTCN)

6MeTPTCN was also prepared analogously from 1-(6-methyl-2-pyridylmethyl)triazacyclononane (**15**). $^1$H-NMR (300 MHz, $CDCl_3$/TMS) 2.52 (s, 3H), 2.85–2.90 (m, 12H), 3.80 (s, 2H), 3.83 (s, 4H), 7.00 (d, 1H, $J = 7.53$ Hz), 7.11–7.16 (m, 2H), 7.26 (d, 1H, $J = 7.68$ Hz), 7.50–7.56 (m, 3H), 7.62–7.67 (m, 2H), 8.49–8.52 (m, 2H); MS 416 [$M^+$].

### 3.3.14. Formation of ferrous complexes

Ferrous complexes were prepared by reacting equimolar $FeSO_4 \cdot 7H_2O$ or $Fe(ClO_4)_2 \cdot 6H_2O$ and the chelators in MeOH at r.t. for several minutes, evaporating the solvent and drying the product in vacuo.

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