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A New Total Synthesis of Ferrioxamine E through Metal-templated Cyclic Trimerization

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Ferrioxamine E was synthesized in a one step cyclic trimerization of the ferric complex of *N*-5-aminopentyl-*N*-(hydroxy)-succinamic acid. This reaction was also performed using gallium (III) as the template. *N*-5-aminopentyl-*N*-(hydroxy)-succinamic acid was prepared according to methods described in the literature. Electrospray ionization mass spectrometry (ESI/MS) allows the detection of the pseudo-molecular ion peaks of Fe(III) and Ga(III) complexes of desferrioxamine E thus prepared. HPLC and ^{1}H and ^{13}C NMR data of the Ga(III)/desferrioxamine E complex are also reported.

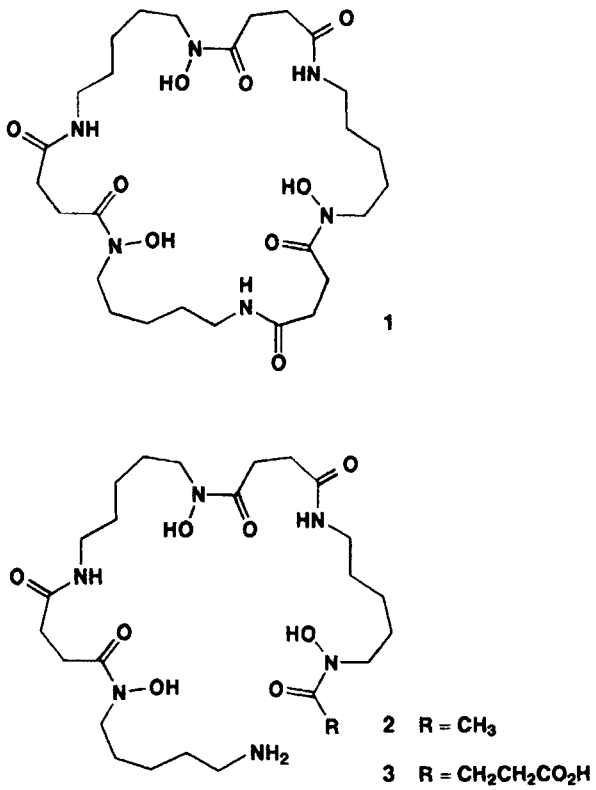
INTRODUCTION

Siderophores are natural iron (III) chelating agents produced by most micro-organisms. Their biological function is iron uptake from the environment into the cell *via* a specific protein transport system.[1] Desferrioxamine E** **1** (DFO E or nocardamine) is known as the hydroxamate type siderophore which has the highest affinity constant for iron (III).[2] It is produced by several *Streptomyces* and enterobacteria species.[3] The crystallographic structures of DFO E and ferrioxamine E (DFO E ferric complex) have already been established.[4,5]

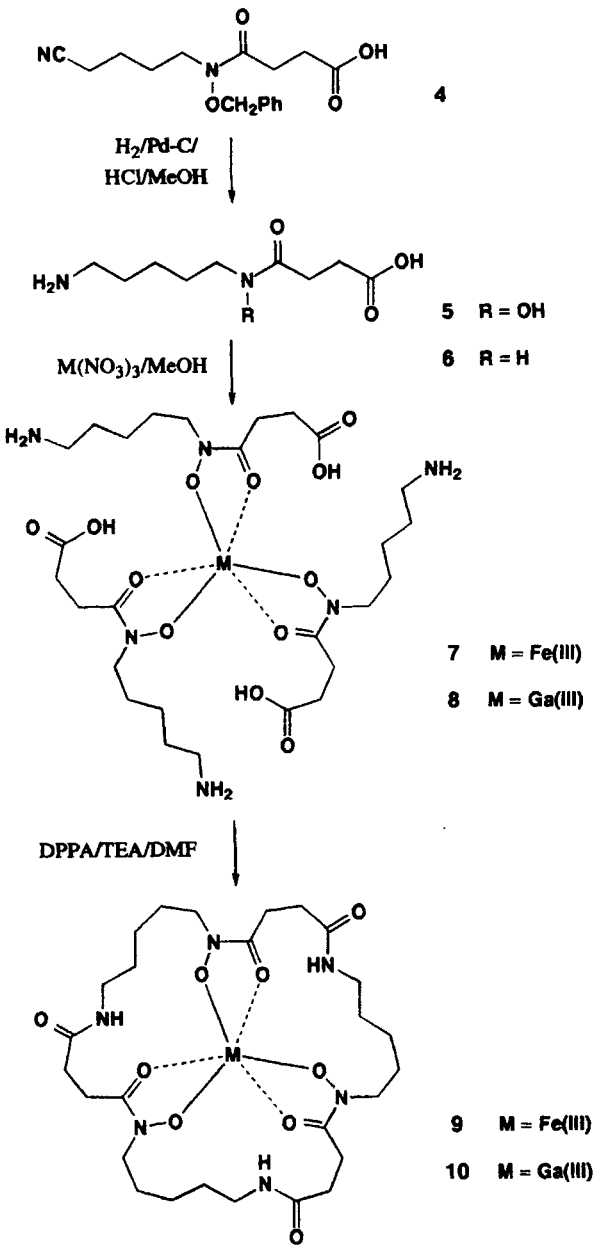
The methanesulfonate of DFO B **2** (Desferal®, Ciba-Geigy), a linear analog of DFO E, is actually used in the treatment of iron overload diseases.[6] There is a renewed interest in ferrioxamine E since this compound is a good tool for medical diagnosis, e.g. for salmonella detection.[7]

In the early sixties, Prelog has described the synthesis of ferrioxamine E starting from ferrioxamine G_1.[8] Indeed, this latter compound is the ferric complex of DFO G_1 **3**, the open chain equivalent of DFO E. The total synthesis of DFO G_1 was described in the same period.[9,10] More recently, Bergeron has published new versatile

*Corresponding author
**1,12,23-Trihydroxy-1,6,12,17,23,28-hexaazacyclotritriacontane-2,5,13,16,24,27-hexone.



SCHEME 1



SCHEME 2

syntheses of DFO G_1 and DFO E.[11] The novelty and advantage of our approach resides in the one step cyclic trimerization of *N*-5-aminopentyl-*N*-(hydroxy)-succinamic acid **5**, the fundamental unit of ferrioxamines, using Fe(III) or Ga(III) as templates (M) and diphenylphosphorylazide (DPPA) as the coupling reagent (Scheme 2). Prelog's and Bergeron's syntheses imply twelve and fourteen steps respectively, while our synthetic scheme provides ferrioxamine E in six steps.

RESULTS AND DISCUSSION

Synthesis of the Fundamental Unit of DFO E

DFO E is the cyclic trimer of *N*-5-aminopentyl-*N*-(hydroxy)-succinamic acid **5**. This latter compound is the fundamental unit of the ferrioxamine family of siderophores. The key to its synthesis is the regiospecific condensation of 1-amino-5-(*N*-hydroxyamino)-pentane with succinic acid. We have chosen the pathway starting from *O*-(benzyl)-hydroxylamine towards *N*-4-cyanobutyl-*N*-

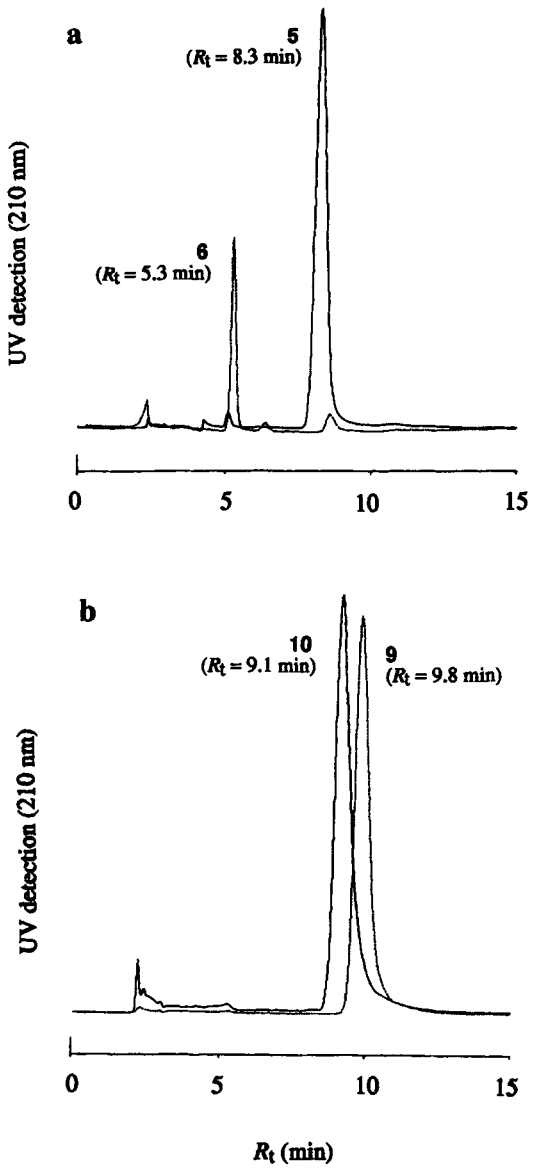


FIGURE 1 HPLC (C_{18}) of **5** and **6** using a mixture of methanol/water (4:96) as eluant (**a**), and of **9** and **10** using a mixture of acetonitrile/water (1:9) as eluant (**b**) (c = 0.5 10^{-3} g.mL^{-1}, inj. vol. = 20 µL).

(benzyloxy)-succinamic acid **4**, recently applied successfully to the total syntheses of natural products like bisucaberin (the cyclic dimer of **5**), DFO B, G_1 and E.[11-14]

The hydrogenation of **4** with catalytic amounts of palladium on carbon (Pd-C) and hydrochloric acid (HCl) allows the reduction, in one step, of the benzyloxy and cyano groups, leading to **5** (Scheme 2). However, the presence of HCl, which is necessary for the reduction of the cyano group, leads also to the partial reduction of the *N*-hydroxy group, providing *N*-5-aminopentyl-*N*-succinamic acid **6**. The kinetics of this reaction have been studied previously in the course of the total synthesis of DFO B.[14] In our case, the reaction time leading to the highest yield (40%) of **5** was 1 h 30, necessitating nevertheless purification by preparative HPLC over a reverse-phase silica gel column (C_{18}). Indeed, on an analytical column and using a mixture of methanol/water (4:96) as eluant, the difference in the retention time between **5** and **6** is 3 minutes (Figure 1a).

Metal(ligand)$_3$ Complexes and Condensation Reactions

Fe(III) and Ga(III) offer an octahedral coordination sphere and similar ionic radii (0.64 and 0.62 Å, respectively). Their affinity constant towards a given ligand are also very close.[2] Contrary to Fe(III), Ga(III) is diamagnetic, a property which allows standard NMR analyses of its complexes.

The Fe(**5**)$_3$ **7** and Ga(**5**)$_3$ **8** complexes were respectively prepared with Fe(III) and Ga(III) nitrates in methanol solution. The condensation reactions were performed in dimethylformamide (DMF) with diphenylphosphorylazide (DPPA) as the coupling reagent and triethylamine as base (Scheme 2).[15] While several traditional coupling reagents (dicyclohexylcarbodiimide, carbonyldiimidazole and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide) were tested unsuccessfully. In order to prevent intermolecular condensation reactions, complexes **7** and **8** were highly diluted (10^{-3} g.mL^{-1}). Ferrioxamine E **9** and Ga(DFO E) **10** thus obtained were then purified by reverse-phase silica gel (C_{18}) chromatography, ion exchange chromatography (Dowex 1 × 8) and silica gel chromatography, successively. No significant difference in the yield of the cyclization (10%) was observed between Fe(III) and Ga(III) as templates.

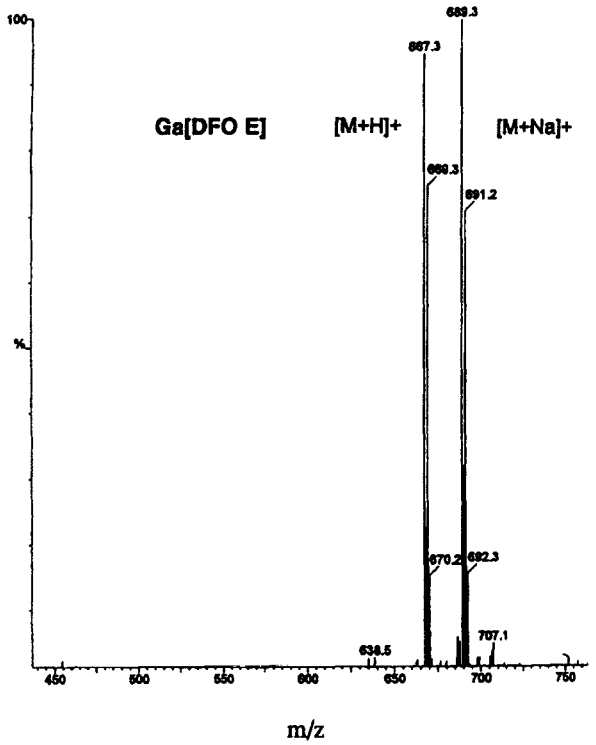


FIGURE 2 ESI/MS spectrum of the Ga(DFO E) complex **10**.

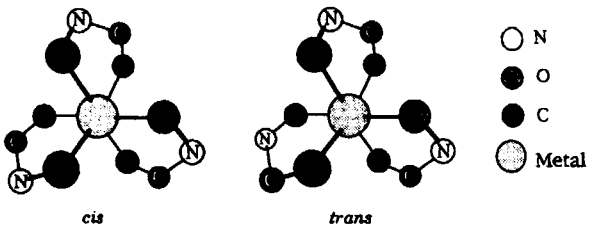


FIGURE 3 Theoretical stereoisomers of the M(**5**)$_3$ and M(DFO E) complexes (only the δ absolute configurations are represented).

Over an analytical HPLC reverse-phase silica gel column (C_{18}) and using a mixture of acetonitrile/water (1:9) as eluant, the difference in the retention time between **9** and **10** was 40 seconds (Figure 1b).[16]

Fast Atom Bombardment (FAB/MS) and Electrospray Ionization Mass Spectrometry (ESI/MS) have already been applied to hydroxamate containing siderophores.[17,18] In a previous study, we reported that ESI/MS was an efficient technique for the detection of Fe(III) complexes of diverse natural desferrioxamines.[3] Herein, we describe the detection of pseudo-molecular and sodium-adduct peaks of synthetic Fe(III) and Ga(III) complexes of DFO E (m/z 654 $[MH]^+$, 676 $[MNa]^+$ and m/z 667 $[MH]^+$, 689 $[MNa]^+$, respectively) (Figure 2). ESI/MS allowed also the detection of the other reaction products (using iron as template): the complexes of the cyclic dimer bisucaberin (m/z 454 $[M\text{-}2H + Fe]^+$), the linear dimer (m/z 472 $[M\text{-}2H + Fe]^+$) and DFO G_1 (m/z 672 $[M\text{-}2H + Fe]^+$). The spectrum of Fe(**5**)$_3$ shows the pseudo-molecular peak of **5** (m/z 219 $[MH]^+$) but also two peaks of lower intensity corresponding to the complex itself (m/z 708 $[MH]^+$, 730 $[MNa]^+$). The equivalent peaks of Ga(**5**)$_3$ were not detected, suggesting that the stability of this complex is too weak for its detection by ESI/MS.

NMR Spectroscopy of the Ga(III) Complexes

^1H and ^{13}C chemical shifts (D_2O) of **5**, Ga(**5**)$_3$, Ga(DFO E) and DFO E are reported in Table I. The correlations between ^1H and ^{13}C chemical shifts (DMSO) have been reported previously for DFO E.[21] Herein, these correlations were confirmed by 2D NMR experiments and the most important coordination induced shifts were observed at the carbonyl and the methylene groups at the α positions of the *N*-hydroxy group. In the case of the Ga(DFO E) complex, the coordination to the metal induces a differentiation of the methylenic protons.

Ferrioxamine E presents, theoretically, a mixture of four stereoisomers (*cis/trans* and Δ/Λ) (Figure 3). According to X-ray diffraction analysis, the structure of natural ferrioxamine E is the racemate *cis* and one of the targets of the synthesis of Ga(DFO E) **10** was to check if this isomer prevails also in solution.[5] Indeed, a spectroscopic investigation of the Ga(DFO B) complex reported

TABLE I ^{13}C and ^{1}H chemical shifts in D_2O of **5**, $Ga(\mathbf{5})_3$ **8**, Ga(DFO E) **10** and DFO E **1** using dioxane (67.4 ppm) and HDO (4.60 ppm) as internal standards

C position		*10*	*9*	*8*	*7*	*6*	*4*	*3*	*2*	*1*
5	^{13}C (1C)	40.3	27.1	23.5	26.1	48.3	175.4	28.6	31.6	181.1
	^{1}H (2H)	2.74	1.44	1.12	1.42	3.40	—	2.50	2.25	—
$Ga(\mathbf{5})_3$	^{13}C (3C)	40.1	27.2	23.4	26.6	51.4	165.2	26.6	32.4	180.0
	^{1}H (6H)	2.75	1.43	1.14	1.48	3.51	—	2.47	2.29	—
Ga(DFO E)	^{13}C (3C)	38.7	28.0	22.2	26.4	51.5	164.1	25.6	31.0	174.7
	^{1}H (3H)	2.71	1.27	0.93	1.27	3.31	—	2.26	2.26	—
	^{1}H (3H)	3.20	1.40	0.93	1.54	3.82	—	2.52	2.84	—
DFO E	^{13}C (3C)	40.0	28.7	23.7	26.2	48.6	175.6	28.5	31.5	174.6
	^{1}H (6H)	2.93	1.40	1.05	1.27	3.38	—	2.26	2.57	—

that its ^{13}C NMR spectrum shows a splitting of several signals, which was interpreted as evidence for various isomers (for the asymmetric trihydroxamate complexes, the number of theoretical isomers increases to sixteen);[19] in the same study, the two main isomers, which exhibit differences in their ^{13}C spectra, were isolated by ion exchange chromatography and identified as *N-cis-cis* and *C-trans-trans* (Δ and Λ).[19] These results suggest that the eventual *trans* Ga(DFO E) isomers should be detectable by ^{13}C NMR spectroscopy. Under our conditions, the ^{13}C NMR spectrum of the Ga(DFO E) complex shows only a single set of peaks without any apparent signal splitting.

The $M(\mathbf{5})_3$ complexes present also, theoretically, a mixture of the four stereoisomers. Statistically, the relative amount of the *cis* isomers (Δ and Λ) is 25%, without taking into account kinetic and thermodynamic considerations. Since the condensation reaction on the *trans* isomers leads to M(bisucaberin) and to *trans* M(DFO G_1), and since *trans* M(DFO G_1) should not be cyclized into *trans* M(DFO E) because of apparent structural incompability, the yield of the cyclic trimerization reaction should depend on the relative amount of the *cis* isomers of $M(\mathbf{5})_3$ in solution. As no splitting of the ^{13}C NMR signals of the $Ga(\mathbf{5})_3$ complex was observed, that can be explained with the absence of sterical constraint and/or the presence of fast chemical exchanges ("rhombic twist") into the ML_3 complexes, which certainly prevent also the isolation of the *cis* and *trans* isomers.[20]

CONCLUSION

The X-ray diffraction analysis of ferrioxamine E **9** and the ^{13}C NMR spectrum of the Ga(DFO E) complex **10** (D_2O) strongly suggest that the stereochemistry of the M(DFO E) complexes in solution is the racemate *cis*.[5] Consequently, the cyclic trimerization should depend on the relative amount of the *cis* $M(\mathbf{5})_3$ complexes in solution. The observed yield for this reaction was 10% using Fe(III) or Ga(III) as templates. Calculated from the common starting material (**4**), our overall yield is twice higher than the most recent preparation of DFO E.[11,12] In spite of the rather low yield of the final step, this new pathway towards ferrioxamine E (and of DFO E after deferration) presents an efficient example of the synthesis of a natural compound *via* a supramolecular device. Finally, ESI/MS revealed to be an efficient method for the detection of the various products of the metal-templated reaction.

MATERIALS AND METHODS

General

High-performance Liquid Chromatography

Preparative and analytical HPLC were performed on Inertsil PREP-ODS (10 μ) 250 × 20 mm (GL Sciences Inc.) (flow rate: 10 mL· min^{-1}) and Inertsil-OD2 (10 μ) 250 × 4.6 mm (Interchrom) (flow rate: 1 mL· min^{-1}) columns, respectively. Eluates were monitored at 210 nm using a Perkin Elmer Diode Array detector.

Thin-layer Chromatography

Silica gel and reverse-phase silica gel (C_{18}) TLC were performed on $60F_{254}$ and RP-$18F_{254}$s (Merck) thin layers, respectively.

Electrospray Ionization Mass Spectrometry

Positive ion electrospray mass spectra were obtained on a PLATFORM quadrupole mass spectrometer (VG Instruments). The analyzer was calibrated from m/z 100 to 1000 (Da/e) with cesium iodide. The samples were dissolved in a mixture of acetonitrile/formic acid 0.2% (1:1) at a concentration of 100 ng·μL^{-1}. A 10 μL volume was injected into a Rheodyne valve and introduced into the mass spectrometer at a flow rate of 5 μL· min^{-1}.

Nuclear Magnetic Resonance Spectroscopy

^1H and ^{13}C NMR (D_2O) spectra were recorded on Bruker AM 400 and ARX 400 spectrometers using HDO (^1H, 4.60 ppm) and/or dioxane (^{13}C, 67.4 ppm) as internal standards. Chemical shifts are given in ppm.

Synthesis

N-4-cyanobutyl-N-(benzyloxy)-succinamic acid 4

Compound **4** was prepared according to Bergeron and McManis;[12] CID/MS (NH_3), m/z 305 $[MH]^+$; ^1H NMR ($CDCl_3$), δ 1.57–1.81 (m, 4H), 2.34 (t, 2H), 2.55–2.75 (m, 4H), 3.67 (t, 2H), 4.83 (s, 2H), 7.35 (s, 5H); IR (KBr), ν (cm^{-1}) 2244 (CN); Anal. calcd. for $C_{16}H_{20}N_2O_4$: C (63.14), H (6.62), N (9.20); found: C (63.25), H (6.59), N (9.19).

N-5-aminopentyl-N-(hydroxy)-succinamic acid hydrochloride 5

A mixture of 131 mL of methanol, 5.4 mL of 0.1 N hydrochloric acid, and 0.54 g of 5% palladium on carbon was prehydrogenated for 0.5 h. Compound **4** (0.12 g in 5 mL of methanol) was added and the hydrogenation was carried out at 1 atm of hydrogen for 1.5 h. The solution was filtered, the solvents were removed and the residue was washed with cold methanol and chloroform. The mixture of products was dissolved in 2 mL of water and compound **5** was purified by preparative HPLC over a reverse-phase (C_{18}) silica gel column (4 injections of 0.5 mL) using a mixture of methanol/water (4:94) as eluant to give 40 mg (40%) of a pale yellow solid; analytical HPLC, R_t = 8 min with a mixture of methanol/water (4:96) as eluant; TLC, R_f = 0.2 with methanol as eluant; TLC (C_{18}), R_f = 0.8 with a mixture of methanol/water (1:1) as eluant; CID/MS (NH_3), m/z 219 $[MH]^+$; ^1H NMR (D_2O), δ 1.05–1.19 (m, 2H), 1.34–1.52 (m, 4H), 2.25 (t, 2H), 2.50 (t, 2H), 2.74 (t, 2H), 3.40 (t, 2H); ^{13}C NMR (D_2O), δ 23.5, 26.1, 27.1, 28.6, 31.6, 40.3, 48.3, 175.4, 181.1; Anal. calcd. for $C_9H_{19}N_2O_4Cl$: C (42.44), H (7.46), N (11.00); found: C (42.32), H (7.68), N (11.47).

$Ga(5)_3$ complex 8

A mixture of compound **5** (10 mg) and $Ga(NO_3)_3 \cdot 9H_2O$ (5.5 mg, 1/3 eq.) in 5 mL of methanol was stirred for 1 h at room temperature. After removal of the solvent, the product was dissolved in 1 mL of water and chromatographed over a Sephadex G-10 column (10 g) with water as eluant. Water was removed and the product was dried over P_2O_5 for 16 h under vacuum to give 13.5 mg of a

pale yellow solid; ^{1}H NMR (D_2O), δ 1.04–1.26 (m, 6H), 1.34–1.62 (m, 12H), 2.29 (t, 6H), 2.47 (t, 6H), 2.75 (t, 6H), 3.51 (t, 6H); ^{13}C NMR (D_2O), δ 23.4, 26.6, 26.7, 27.2, 32.4, 40.1, 51.4, 165.2, 180.0.

Ferrioxamine E 9

Compound **5** (0.1 g) was dissolved in 50 mL of methanol, $Fe(NO_3)_3 \cdot 9H_2O$ (53 mg, 1/3 eq.) was added and the mixture was stirred for 1 h at room temperature. The solvent was removed and the product was dried over P_2O_5 for 16 h under vacuum. 100 mL of anhydrous DMF, 0.2 mL of diphenylphosphorylazide (DPPA, 2.4 eq.) and 0.13 mL of triethylamine (2.4 eq.) were added and the mixture was stirred at room temperature under argon for 7 days. After removal of the solvent, the reaction products were dissolved in 20 mL of a mixture of methanol/water (4:6) and the solution was filtered and flash-chromatographed over reverse-phase (C_{18}) silica gel (50 g) using a mixture of methanol/water (4:6) as eluant. After solvent removal, the fraction containing compound **9** was diluted in water (20 mL) and chromatographed over a strongly basic ion exchange resin (Dowex 1 × 8) (50 g) with water as eluant; this procedure allows an efficient elimination of diphenyl-phosphate (DPPA derivative). The final purification of **9** was achieved by chromatography over a silica gel column with a mixture of dichloromethane/methanol/water (80:18:2) as eluant to give 9 mg (10%) of a red solid; analytical HPLC, R_t = 9.1 min with a mixture acetonitrile/water (1:9) as eluant; TLC, R_f = 0.7 with a mixture of dichloromethane/methanol/water (72:24:4) as eluant; TLC (C_{18}), R_f = 0.4 with a mixture of methanol/water (1:1) as eluant; ESI/MS, m/z 676 $[MNa]^+$; ^{1}H NMR of DFO E (D_2O), δ 0.98–1.12 (m, 6H), 1.15–1.50 (m, 12H), 2.24 (t, 6H), 2.49 (t, 6H), 2.94 (t, 6H), 3.40 (t, 6H); ^{13}C NMR of DFO E (D_2O) δ 23.7, 26.2, 28.5, 28.7, 31.5, 40.0, 48.6, 174.6, 175.6 (for the 8-hydroxyquinoline deferration method, see ref. 22); high resolution mass spectrum (FAB/MS), calcd. for $C_{27}H_{46}N_6O_9Fe$ $[MH]^+$: 654.2675; obsd. 654.2661.

Ga(DFO E) Complex 10

Compound **10** was prepared along the same procedure, using $Ga(NO_3)_3 \cdot 9H_2O$ (55 mg, 1/3 eq.), to give 9.5 mg (10%) of a pale yellow solid; analytical HPLC, R_t = 9.8 min with a mixture of acetonitrile/water (1:9) as eluant; ESI/MS, m/z 689 $[MNa]^+$; ^{1}H NMR (D_2O), δ 0.88–1.02 (m, 6H), 1.18–1.52 (m, 9H), 1.52–1.68 (m, 3H), 2.18–2.35 (m, 6H), 2.52 (t, 3H), 2.66–2.77 (m, 3H), 2.84 (t, 3H), 3.15–3.26 (m, 3H), 3.26–3.37 (m, 3H), 3.82 (t, 3H); ^{13}C NMR (D_2O), δ 22.2, 25.6, 26.4, 28.0, 31.0, 38.7, 51.5, 164.1, 174.7.

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