DNA Structures

DOI: 10.1002/anie.200804654

Discrete Self-Assembly of Iron(III) Ions inside Triple-Stranded Artificial DNA**

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DNA molecules provide an excellent structural motif for assembling functional building blocks in a programmable way. Well-established synthetic procedures for oligonucleotides[1] have made it possible to develop tools for discrete assembly of various kinds of functional molecules, such as dyes,[2] fluorophores,[3] and metal complexes,[4–7] along DNA scaffolds. We have recently reported homologous[5] and heterologous[6] metal arrays inside artificial DNA duplexes in which natural hydrogen-bonded base pairs were replaced by metal-mediated base pairs.[4–11] The multisite incorporation of hydroxypyridone-bearing nucleoside **H**[10] into DNA duplexes quantitatively provided one-dimensional discrete metal arrays, $[(Cu^{II})_n d(5'\text{-}G\mathbf{H}_nC\text{-}3')_2]$ ($n = 1$–5),[5] by self-assembly of a 2:1 square-planar Cu^{II}-mediated base pair, [**H**–Cu^{II}–**H**]. Furthermore, by combination with the pyridine-bearing nucleoside **P**,[11] which forms a 2:1 linear Hg^{II}-mediated base pair [**P**–Hg^{II}–**P**], heterologous arrays with both Cu^{II} and Hg^{II} were constructed within artificial DNA duplexes, $d(5'\text{-}G\mathbf{HPH}C\text{-}3')_2$ and $d(5'\text{-}G\mathbf{HHPHH}C\text{-}3')_2$.[6] Results suggest that artificial metallo-DNA is one of the most powerful ways to assemble metal ions in a programmable manner, especially for metal complexes with square-planar or linear coordination geometries, in accord with their constitutional similarity to natural base pairs.[7] Herein, we report discrete self-assembly of octahedral Fe^{III} ions as an alternative structural motif inside artificial DNA triplexes, possessing hydroxypyridone nucleobases as metal ligands (Figure 1). Since transition-metal ions with octahedral coordination geometry have attractive characteristics such as magnetic, optical, and redox properties, the alignment of such metal ions would provide unique metal-sequence-dependent functions.

3-Hydroxy-4-pyridone is known as a strong chelating ligand, not only for Cu^{II}[12] but also for Fe^{III}.[13,14] In this study, we have chosen octahedrally coordinated Fe^{III} ion to be incorporated into DNA scaffolds. Photometric titration experiments were conducted to pre-examine complexation between the hydroxypyridone-bearing nucleoside **H** and Fe^{III} ions, at pH 7.0 and 25 °C (Figure 2a). With an increase in Fe^{III} concentrations, an absorption band at around 460 nm, characteristic for Fe^{III} complexation,[13,14] appeared and its absorbance gradually increased. The spectra changed with two isosbestic points at 257 and 294 nm in proportion to the ratio $[Fe^{III}]/[\mathbf{H}]$ until it reached 0.33.[15] This result clearly indicates that the nucleoside **H** forms a complex with Fe^{III} in a 3:1 ratio to afford a novel Fe^{III}-mediated nonplanar "base-triplet". Electrospray-ionization time-of-flight (ESI-TOF) mass spectrometry also confirmed the formation of a neutral $[\mathbf{H}_3Fe^{III}]$ complex, with spontaneous deprotonation of the hydroxypyridone ligands (Figure 2b; calculated for $[M+Na]^+$ 799.18; found 799.21).

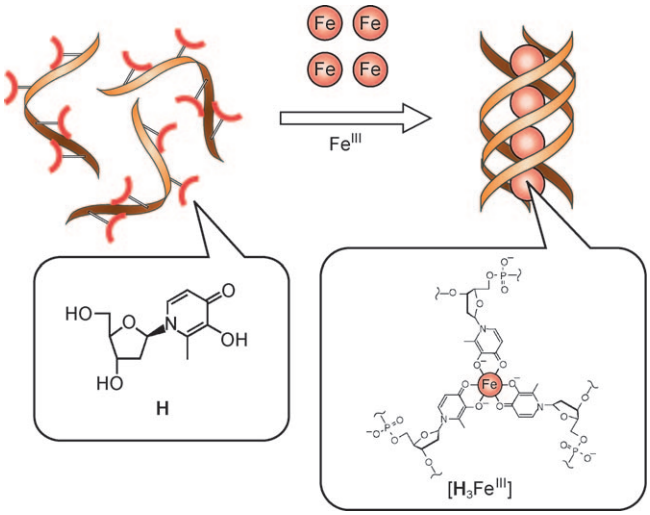

Figure 1. Schematic representation of the formation of a linear Fe^{III} array inside an artificial DNA triplex.

To assemble Fe^{III} ions within artificial DNA scaffolds, we designed a series of oligonucleotides, $d(5'\text{-}\mathbf{H}_n\text{-}3')$ ($n = 2$–4), possessing only hydroxypyridone nucleobases (**H**) as the template ligands. In light of the fact that the 3:1 complex of 1,2-dimethyl-3-hydroxy-4-pyridone with Fe^{III} is the facial (*fac*) isomer,[16] the hydroxypyridone-bearing oligonucleotides were expected to form a parallel triple-stranded structure with C_3 symmetry upon complexation with Fe^{III}. The artificial oligonucleotides were efficiently prepared using an automated DNA synthesizer with Universal Support II (Glen Research) as a solid support to incorporate **H** at the 3′ terminus. After cleavage from the support and deprotection, the products were purified by HPLC and then identified

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[**] This work is supported by a Grant-in-Aid for Scientific Research (S) to M.S. (No. 16105001), the Global COE Program for Chemistry Innovation, and a Grant-in-Aid for JSPS Fellows to Y.T. (No. 11921) from the Japan Society for the Promotion of Science.

Supporting Information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200804654.

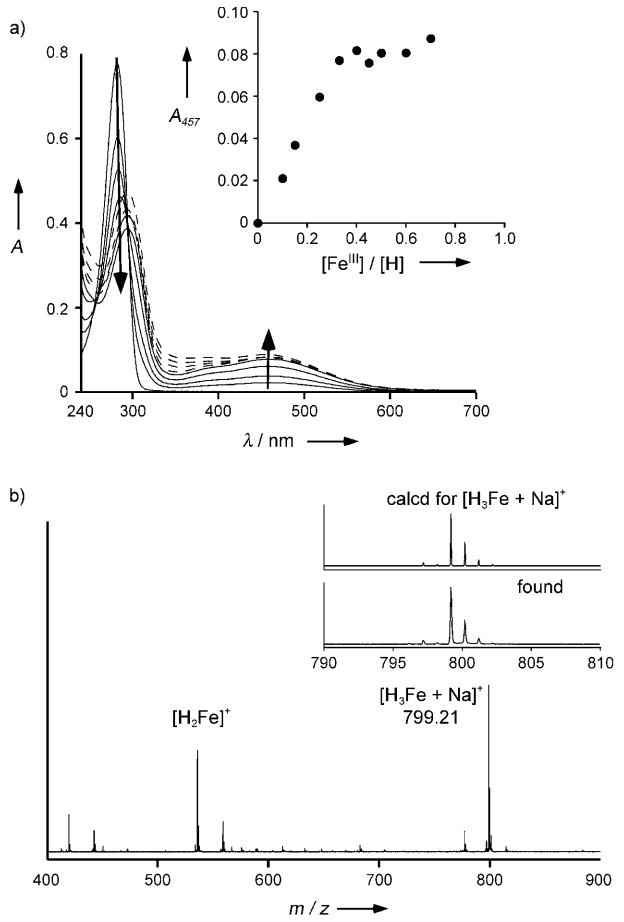

Figure 2. a) UV/Vis absorption spectra of nucleoside **H** at various concentrations of $Fe(NO_3)_3$: $[Fe^{III}]/[\mathbf{H}] = 0$, 0.10, 0.15, 0.25, 0.33 (——), 0.40, 0.45, 0.50, 0.60 (– – –). Inset: plot of absorbance at 457 nm against $[Fe^{III}]/[\mathbf{H}]$. Each sample was allowed to stand at room temperature for 1.5 days before the measurement. $[\mathbf{H}] = 250\ \mu M$ in 25 mM MOPS (3-(*N*-morpholino)propanesulfonic acid; pH 7.0) aqueous solution at 25 °C, $l = 0.2$ cm. b) ESI-TOF mass spectrum (positive mode) of a 3:3:1 mixture of nucleoside **H**, $NaHCO_3$, and $Fe(NO_3)_3$. $[\mathbf{H}] = 100\ \mu M$ in 1:1 H_2O/CH_3OH.

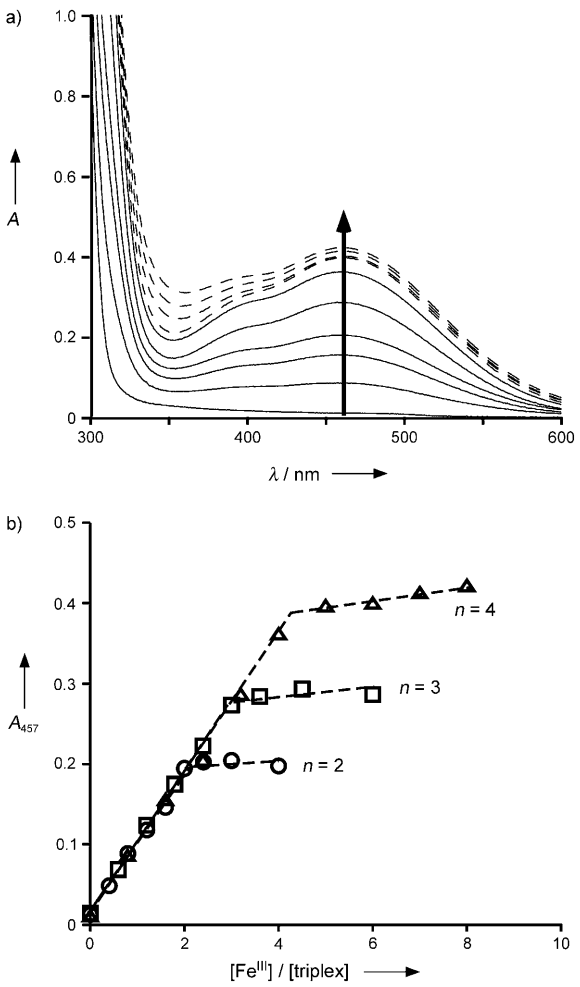

Figure 3. a) UV/Vis absorption spectra of tetranucleotide d(5′-**HHHH**-3′) at various concentrations of $Fe(NO_3)_3$: $[Fe^{III}]/[d(5'\text{-}\mathbf{HHHH}\text{-}3')_3] = 0$, 0.8, 1.6, 2.4, 3.2, 4.0 (——), 5.0, 6.0, 7.0, 8.0 (– – –). b) Plot of absorbance at 457 nm against $[Fe^{III}]/[d(5'\text{-}\mathbf{H}_n\text{-}3')_3]$ ($n = 2$–4). Each sample was allowed to stand at 85 °C for 2 days before the measurement. $[d(5'\text{-}\mathbf{H}_n\text{-}3')] = 50\ \mu M$ in 25 mM MOPS (pH 7.0) and 50 mM NaCl aqueous solution at 25 °C, $l = 1.0$ cm.

by ESI-TOF mass spectrometry (for example, for d(5′-**HHHH**-3′): calculated for $[M-H]^-$ 1149.24; found 1149.18).[17]

The Fe^{III}-mediated triple-strand formation was monitored by changes in the UV/Vis absorption at different concentrations of Fe^{III}. Each complexation took 2 days at 85 °C to go to completion. During titration of the tetranucleotide d(5′-**HHHH**-3′) with Fe^{III}, an absorption around 457 nm increased linearly in the range of $[Fe^{III}]/[d(5'\text{-}\mathbf{HHHH}\text{-}3')_3]$ from 0.0 to 4.0 (Figure 3a), indicating that four Fe^{III} ions were quantitatively assembled inside the DNA triplexes through Fe^{III}-mediated base-triplet $[\mathbf{H}_3Fe^{III}]$ formation. Similar changes occurred for the oligonucleotides d(5′-**HH**-3′) and d(5′-**HHH**-3′) (see the Supporting Information). Changes in the absorption at 457 nm were plotted as a function of the ratio of Fe^{III} to the triplex, $d(5'\text{-}\mathbf{H}_n\text{-}3')_3$ (Figure 3b). In all cases, intermolecular oligonuclear complexes of the form $[(Fe^{III})_n d(5'\text{-}\mathbf{H}_n\text{-}3')_3]$ ($n = 2$–4) were quantitatively formed, in which 2–4 Fe^{III}-mediated base triplets $[\mathbf{H}_3Fe^{III}]$ were aligned in the DNA triplexes. These structures were also evidenced by ESI-TOF mass spectrometry (for example, $[(Fe^{III})_4 d(5'\text{-}\mathbf{HHHH}\text{-}3')_3]$, Figure 4: calculated for $[M-7H+2Na]^{5-}$ 740.47; found 740.43).[17] Overall, di-, tri-, and tetranuclear Fe^{III} complexes were quantitatively formed within the triple-stranded artificial oligonucleotides through self-assembly processes according to the number of the **H** ligands incorporated into the strands. In addition, the absorbance of the metallo-triplexes was proportional to the number of the base triplets, suggesting that the resulting Fe^{III} complexes have no significant intermolecular interactions.

Given that the octahedral Fe^{III} complex of these bidentate hydroxypyridone-nucleosides possibly adopts a *fac* structure, in analogy with *N*-methylhydroxypyridone,[16] each Fe^{III} center should be in the Δ or Λ form within three strands arranged parallel to one another. Circular dichroism (CD) analysis was carried out to determine the helical configuration of the Fe^{III}-assembled DNA triplexes. The monomeric com-

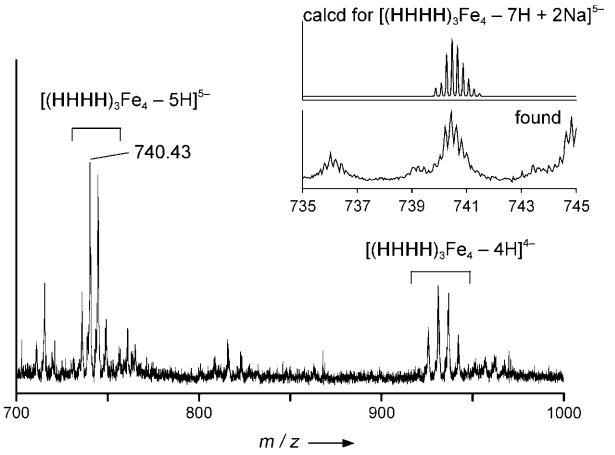

Figure 4. ESI-TOF mass spectrum (negative mode) of a 3:27:4 mixture of tetranucleotide d(5′-**HHHH**-3′), $NaHCO_3$, and $Fe(NO_3)_3$. [d(5′-**HHHH**-3′)] = 100 μM in 1:1 H_2O/CH_3OH.

plex [$\mathbf{H}_3Fe^{III}$] ([**Fe-1**]) did not show any characteristic signals in the visible region (dotted line in Figure 5). This result suggests that nearly the same amount of two diastereomers,

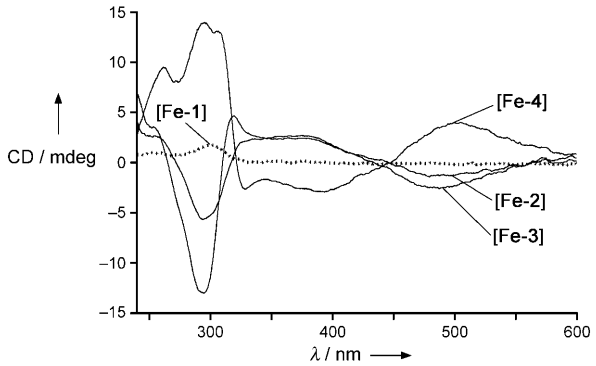

Figure 5. CD spectra of Fe^{III}-assembled complexes, [$(Fe^{III})_n$d(5′-$\mathbf{H}_n$-3′)$_3$] (n = 1–4) (abbreviated as [**Fe-*n***]). [d(5′-$\mathbf{H}_n$-3′)] = 50 μM in 25 mM MOPS (pH 7.0) and 50 mM NaCl at 25 °C, l = 1.0 cm. Each sample was allowed to stand at 85 °C for 2 days before the measurement.

Δ-D and Λ-D (D arises from D-ribose), were formed upon complexation with Fe^{III}. In contrast, in all cases of oligonucleotide complexes, [$(Fe^{III})_n$d(5′-$\mathbf{H}_n$-3′)$_3$] (n = 2–4, abbreviated as [**Fe-*n***]), distinct Cotton effects[18] were detected around 460 nm, which could be attributed to the asymmetric Fe^{III} centers. In addition, the intensity of the Cotton effects became larger as the number of Fe^{III}-mediated base triplets was increased. Although the details remain unclear, these results suggest that the helicity state of each triple-stranded DNA assembly may induce a specific configuration of the octahedral Fe^{III} center. Interestingly, it appears that the helicity of the tetranuclear complex [$(Fe^{III})_4$d(5′-**HHHH**-3′)$_3$] is opposite to those of the dinuclear and trinuclear Fe^{III}-mediated triplexes. Further investigation on the detailed configurational isomerism of the Fe^{III}-mediated DNA triplexes is currently underway.

In summary, we successfully assembled Fe^{III} ions, with octahedral coordination geometry, inside artificial DNA triplexes, in which 2–4 Fe^{III}-mediated octahedral "base triplets" [$\mathbf{H}_3Fe^{III}$] were arranged. Since the number of metal ions assembled in the triplex could be predetermined by changing the number of ligand-type oligonucleotides, such a DNA triplex with a new structural motif would provide a novel tool for systematic arrays of octahedral transition-metal complexes in a programmable fashion.[19] Furthermore, oligonucleotides with more than two ligand-type nucleobases would allow heterologous metal arrays inside the DNA triplex, in a manner similar to the DNA duplex.[6] Thus, arranging a series of octahedral metal ions in predetermined numbers and orders[20] in a DNA triplex provides an excellent way to construct rows of metal centers with tunable, metal-sequence-dependent functions, such as magnetic and conductive properties.

Experimental Section

Artificial oligonucleotides were synthesized by the standard β-cyanoethyl phosphoramidite chemistry utilizing an automated DNA synthesizer, Applied Biosystems 394. All reagents except artificial nucleoside derivatives were purchased from Applied Biosystems. The phosphoramidite derivative of hydroxypyridone-bearing nucleoside (**H**) was prepared according to a previously reported procedure.[10] DNA syntheses were performed on a 1.0 μmol scale trityl-off mode, according to the manufacturer's protocol. Universal Support II (Glen Research) was used as the solid support to incorporate the artificial nucleoside at the 3′ terminus of the strand. The reaction conditions were the same as those for syntheses of natural DNA oligomers, except that the coupling time was prolonged to 15 min. Cleavage from the support and the terminal dephosphorylation were accomplished by treating the oligonucleotides with 2 M NH_3/CH_3OH for 1 h at room temperature. Subsequent treatment with aqueous NH_3 for 12 h at 55 °C provided deprotected oligonucleotides. The crude oligomers were purified by HPLC (Waters XTerra MS C_{18} column) using 0.1 M tetraethylammonium acetate (TEAA, pH 7.0) in 2–30% CH_3CN/H_2O as eluent. Fractions containing a desired oligomer were lyophilized to remove the buffer. Concentrations of the DNA solutions were determined by UV absorption spectroscopy at 277 nm on the assumption that the molar extinction coefficients of the oligonucleotides ($\varepsilon = 2.80\times10^4$, 4.20×10^4, and $5.60\times10^4\,M^{-1}cm^{-1}$ for d(5′-**HH**-3′), d(5′-**HHH**-3′), and d(5′-**HHHH**-3′), respectively) are sums of that of the mononucleoside **H** ($\varepsilon = 1.40\times10^4\,M^{-1}cm^{-1}$).

Received: September 22, 2008
Published online: December 29, 2008

Keywords: DNA structures · helical structures · iron · oligonucleotides · self-assembly

[1] D. E. Bergstrom in *Current Protocols in Nucleic Acid Chemistry* (Eds.: S. L. Beaucage, D. E. Bergstorm, G. D. Glick, R. A. Jones), Wiley, New York, **2001**, Unit 1.4.
[2] a) H. Kashida, H. Asanuma, M. Komiyama, *Angew. Chem.* **2004**, *116*, 6684–6687; *Angew. Chem. Int. Ed.* **2004**, *43*, 6522–6525; b) H. Kashida, M. Tanaka, S. Baba, T. Sakamoto, G. Kawai, H. Asanuma, M. Komiyama, *Chem. Eur. J.* **2006**, *12*, 777–784.
[3] a) E. T. Kool, *Acc. Chem. Res.* **2002**, *35*, 936–943; b) J. N. Wilson, Y. N. Teo, E. T. Kool, *J. Am. Chem. Soc.* **2007**, *129*, 15426–15427.

[4] a) K. Tanaka, M. Shionoya, *Coord. Chem. Rev.* **2007**, *251*, 2732 – 2742; b) M. Shionoya, K. Tanaka, *Curr. Opin. Chem. Biol.* **2004**, *8*, 592 – 597; c) G. H. Clever, C. Kaul, T. Carell, *Angew. Chem.* **2007**, *119*, 6340 – 6350; *Angew. Chem. Int. Ed.* **2007**, *46*, 6226 – 6236; d) K. Tanaka, M. Shionoya, *Chem. Lett.* **2006**, *35*, 695 – 699.

[5] K. Tanaka, A. Tengeiji, T. Kato, N. Toyama, M. Shionoya, *Science* **2003**, *299*, 1212 – 1213.

[6] K. Tanaka, G. H. Clever, Y. Takezawa, Y. Yamada, C. Kaul, M. Shionoya, T. Carell, *Nat. Nanotechnol.* **2006**, *1*, 190 – 194; see also: J. Müller, *Nature* **2006**, *444*, 698.

[7] G. H. Clever, T. Carell, *Angew. Chem.* **2007**, *119*, 254 – 257; *Angew. Chem. Int. Ed.* **2007**, *46*, 250 – 253. In this case, the assembled Mn^{III} ions inside the DNA duplex were considered six-coordinate, but the detailed geometry remains unknown.

[8] a) K. Tanaka, M. Shionoya, *J. Org. Chem.* **1999**, *64*, 5002 – 5003; b) H. Cao, K. Tanaka, M. Shionoya, *Chem. Pharm. Bull.* **2000**, *48*, 1745 – 1748; c) K. Tanaka, M. Tasaka, H. Cao, M. Shionoya, *Eur. J. Pharm. Sci.* **2001**, *13*, 77 – 83; d) M. Tasaka, K. Tanaka, M. Shiro, M. Shionoya, *Supramol. Chem.* **2001**, *13*, 671 – 675; e) K. Tanaka, M. Tasaka, H. Cao, M. Shionoya, *Supramol. Chem.* **2002**, *14*, 255 – 261; f) M. Shionoya, K. Tanaka, *Bull. Chem. Soc. Jpn.* **2000**, *73*, 1945 – 1954.

[9] a) J. Müller, D. Böhme, P. Lax, M. M. Cerda, M. Roitzsch, *Chem. Eur. J.* **2005**, *11*, 6246 – 6253; b) H. Weizman, Y. Tor, *J. Am. Chem. Soc.* **2001**, *123*, 3375 – 3376; c) L. Zhang, E. Meggers, *J. Am. Chem. Soc.* **2005**, *127*, 74 – 75; d) C. Switzer, S. Sinha, P. H. Kim, B. D. Heuberger, *Angew. Chem.* **2005**, *117*, 1553 – 1556; *Angew. Chem. Int. Ed.* **2005**, *44*, 1529 – 1532; e) C. Switzer, D. Shin, *Chem. Commun.* **2005**, 1342 – 1344; f) E. Meggers, P. L. Holland, E. B. Tolman, F. E. Romesberg, P. G. Schultz, *J. Am. Chem. Soc.* **2000**, *122*, 10714 – 10715; g) S. Atwell, E. Meggers, G. Spraggon, P. G. Schultz, *J. Am. Chem. Soc.* **2001**, *123*, 12364 – 12367; h) N. Zimmermann, E. Meggers, P. G. Schultz, *Bioorg. Chem.* **2004**, *32*, 13 – 25; i) D. Shin, C. Switzer, *Chem. Commun.* **2007**, 4401 – 4403; j) B. D. Heuberger, D. Shin, C. Switzer, *Org. Lett.* **2008**, *10*, 1091 – 1094; k) N. Zimmermann, E. Meggers, P. G. Schultz, *J. Am. Chem. Soc.* **2002**, *124*, 13684 – 13685; l) G. H. Clever, K. Polborn, T. Carell, *Angew. Chem.* **2005**, *117*, 7370 – 7374; *Angew. Chem. Int. Ed.* **2005**, *44*, 7204 – 7208; m) G. H. Clever, Y. Söltl, H. Burks, W. Spahl, T. Carell, *Chem. Eur. J.* **2006**, *12*, 8708 – 8718; n) C. Brotschi, C. J. Leumann, *Nucleosides Nucleotides Nucleic Acids* **2003**, *22*, 1195 – 1197; o) F.-A. Polonius, J. Müller, *Angew. Chem.* **2007**, *119*, 5698 – 5701; *Angew. Chem. Int. Ed.* **2007**, *46*, 5602 – 5604.

[10] K. Tanaka, A. Tengeiji, T. Kato, N. Toyama, M. Shiro, M. Shionoya, *J. Am. Chem. Soc.* **2002**, *124*, 12494 – 12498.

[11] K. Tanaka, Y. Yamada, M. Shionoya, *J. Am. Chem. Soc.* **2002**, *124*, 8802 – 8803.

[12] A. El-Jammal, P. L. Howell, M. A. Turner, N. Li, D. M. Templeton, *J. Med. Chem.* **1994**, *37*, 461 – 466.

[13] a) R. C. Scarrow, P. E. Riley, K. Abu-Dari, D. L. White, K. N. Raymond, *Inorg. Chem.* **1985**, *24*, 954 – 967; b) R. J. Motekaitis, A. E. Martell, *Inorg. Chim. Acta* **1991**, *183*, 71 – 80.

[14] Hydroxypyridone-bearing ribonucleoside is also known as an iron chelator: a) G. Liu, F. W. Bruenger, A. M. Barrios, S. C. Miller, *Nucleosides Nucleotides* **1995**, *14*, 1901 – 1904; b) G. Liu, F. W. Bruenger, S. C. Miller, A. M. Arif, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3077 – 3080.

[15] The molar extinction coefficient of $[\mathbf{H}_3Fe^{III}]$ at 457 nm ($\varepsilon = 4.62 \times 10^3\,M^{-1}cm^{-1}$) was in good agreement with that of a 3:1 complex of the ribonucleoside reported in ref. [14] ($\varepsilon = 4.48 \times 10^3\,M^{-1}cm^{-1}$).

[16] a) J. Charalambous, A. Dodd, M. McPartlin, S. O. C. Matondo, N. D. Pathirana, H. R. Powell, *Polyhedron* **1988**, *7*, 2235 – 2237; b) E. T. Clarke, A. E. Martell, J. Reibenspies, *Inorg. Chim. Acta* **1992**, *196*, 177 – 183.

[17] *M* represents d(5′-**HHHH**-3′) or $[(Fe^{III})_4\,d(5'\text{-}\mathbf{HHHH}\text{-}3')_3]$ in which the phosphodiester moieties are fully protonated.

[18] R. D. Peacock, B. Stewart in *Comprehensive Coordination Chemistry II* (Eds.: J. A. McCleverty, T. J. Meyer), Elsevier, Oxford, 2004, Section 2.5.

[19] B. Hasenknopf, J.-M. Lehn, *Helv. Chim. Acta* **1996**, *79*, 1643 – 1650.

[20] a) N. C. Fletcher, R. T. Brown, A. P. Doherty, *Inorg. Chem.* **2006**, *45*, 6132 – 6134; b) P. Cucos, M. Pascu, R. Sessoli, N. Avarvari, F. Pointillart, M. Andruh, *Inorg. Chem.* **2006**, *45*, 7035 – 7037.

