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[8] Crystal structure determination: $C_{50}H_{63}Cl_3La_3O$ ($M_r = 1203.08$), $a = 12.299(3)$, $b = 18.734(3)$, $c = 23.954(5)$ Å, $\beta = 101.11(2)^\circ$, $V = 5416(2)$ Å^3 ($T = 220$ K), $\rho_{calcd} = 1.476$ g cm^{-1}, $F(000) = 2372$, $\lambda = 0.71073$ Å, monoclinic, $P2_1/n$, $Z = 4$, diffractometer: STOE-STADI 4, crystal dimensions: $0.30 \times 0.28 \times 0.22$ mm^3, ω-θ scans, 7409 measured reflections in the range $\theta = 1.69 - 25.02^\circ$, of which 7409 were independent and used for refinement. All C, O, Cl, and La layers were refined anisotropically, the H centers in calculated positions isotropically, *R* values: for $[F > 2\sigma(F)]$ $R1 = 0.0397$, $wR2 = 0.1413$. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-100445. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: int. code + (44) 1223-336033; e-mail: deposit@ccdc.cam.ac.uk).
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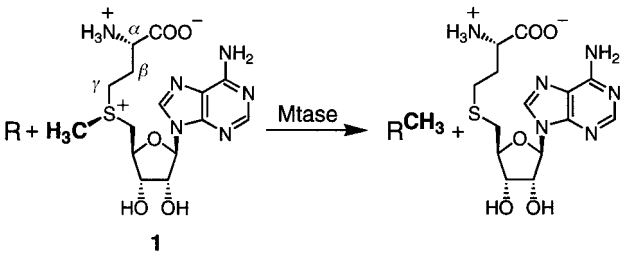
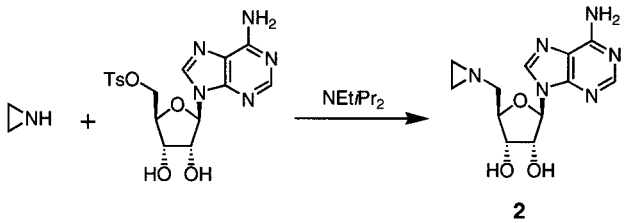
Coupling of a Nucleoside with DNA by a Methyltransferase**

Marc Pignot, Christoph Siethoff, Michael Linscheid, and Elmar Weinhold*

S-Adenosyl-L-methionine-dependent methyltransferases (Mtases) catalyse the transfer of the activated methyl group from the cofactor *S*-adenosyl-L-methionine (**1**) to sulfur, nitrogen, oxygen, and carbon acceptors (Scheme 1) of small molecules, phospholipids, proteins, RNA, and DNA with high specificty.[1] The transfer of larger chemical entities in a Mtase-catalyzed reaction has not been reported and thus represents an interesting challenge for bioorganic chemists. In principal, covalent linking of the activated methyl group with the γC atom of **1** would yield a three-membered thiiranium compound, which could lead to a coupling of the whole cofactor to the target substrate. Since thiiranium compounds are known to be unstable in nucleophilic solvents,[2] we concentrated on the more stable aziridine analogues, which can be activated as alkylating reagents upon protonation of their ring nitrogen atom.[3] *N*-Adenosylaziridine (**2**) was synthesized by nucleophilic substitution of the tosylate group of 5′-deoxy-5′-tosyladenosine (tosyl = Ts = toluene-4-sulfonyl) with aziridine (Scheme 2).



Scheme 1. Reaction catalyzed by *S*-adenosyl-L-methionine-dependent methyltransferases (Mtases). R = an acceptor with sulfur, nitrogen, oxygen, or carbon atoms to which the methyl groups become attached.



Scheme 2. Synthesis of *N*-adenosylaziridine (**2**).

To test whether the cofactor analogue **2** serves as a cofactor for a Mtase we used the DNA Mtase from *Thermus aquaticus* (M · *Taq*I). Naturally, M · *Taq*I catalyzes the transfer of the methyl group from the cofactor **1** to the exocyclic amino group of 2′-deoxyadenosine within the double-stranded 5′-TCGA-3′ DNA sequence (Scheme 3, top).[4] For our studies we used the short duplex oligodeoxynucleotide **3** · **4** (Scheme 3), in which strand **4** contains N^6-methyl-2′-deoxyadenosine (A^{Me}) at the target position, so that it cannot be further methylated by M · *Taq*I. The reaction of the cofactor analogue **2** with the duplex **3** · **4** was performed in the presence of a stoichiometric amount of M · *Taq*I and monitored by anion exchange chromatography. A new compound with a shorter retention time relative to **3** · **4** was formed during the reaction (Figure 1), and after 240 min the duplex **3** · **4** had reacted quantitatively. The shorter retention time of the product is consistent with the proposed structure **5** · **4** (Scheme 3, bottom), since **5** · **4** contains an additional aliphatic amino group that ought to be protonated under the conditions used to elute the reaction mixture (pH 7.6).

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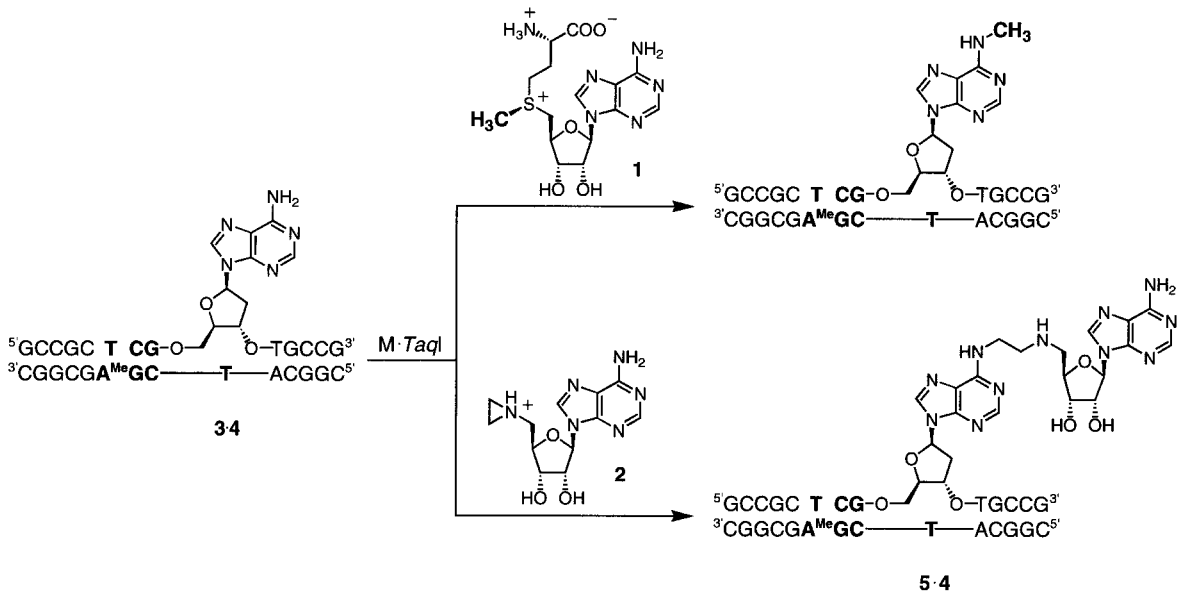

Scheme 3. Reactions catalyzed by the DNA methyltransferase M · *Taq*I. Top: Transfer of a methyl group by the natural cofactor *S*-adenosyl-L-methionine (**1**). Bottom: Coupling of protonated *N*-adenosylaziridine (**2**) to the duplex **3** · **4** ($A^{Me} = N^6$-methyl-2′-deoxyadenosine).

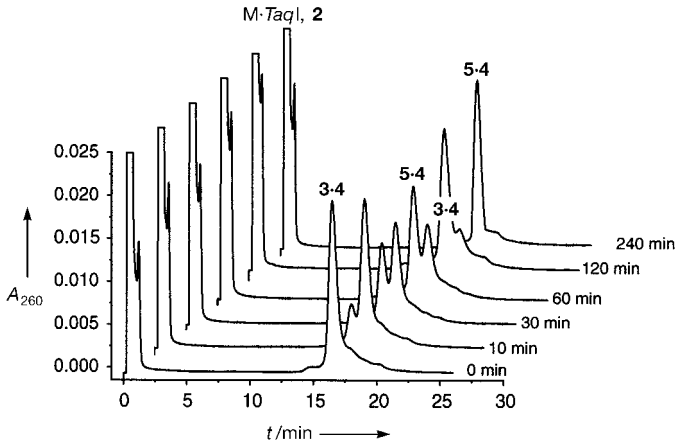

Figure 1. Analysis of the reaction between the duplex oligodeoxynucleotide **3** · **4** and the cofactor analogue **2** in the presence of M · *Taq*I by anion exchange chromatography. Aliquots (50 µL) of the reaction mixture were withdrawn after different incubation times, mixed with a urea solution (100 µL, 6 M) and injected onto an anion exchange column (Poros 10 HQ, 10 µm, 4.6 × 100 mm, PerSeptive Biosystems). Compounds were eluted with aqueous KCl (0.5 M for 5 min, followed by a linear gradient to 1 M in 30 min, 4 mL min^{-1}) in Tris-HCl buffer (10 mM, pH 7.6). A_{260} = absorption at 260 nm.

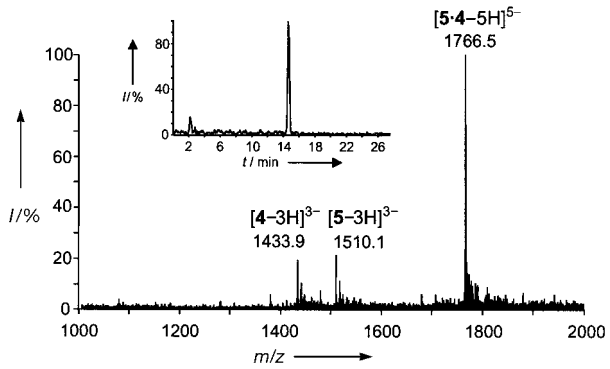

Figure 2. RP-HPLC/ESI mass spectrum of the product **5** · **4** performed with an ion-trap electrospray mass spectrometer (LCQ, Finnigan MAT) equipped with a micro HPLC system (M480 and M300, Gynkotek). Purified and desalted solutions of **5** · **4** were injected onto a capillary column (Hypersil-ODS, 3 µm, 0.3 × 150 mm, LC Packings) and eluted with a CH_3CN gradient (7–10% in 10 min, followed by 10–70% in 30 min, 150 µL min^{-1}) in triethylammonium acetate buffer (0.1 M, pH 7.0). The chromatogram obtained by observing the total ion current is given in the inset. *I* = relative intensity.

The reaction product **5** · **4** was isolated by anion exchange chromatography, and its structure was verified by reversed-phase HPLC-coupled electrospray ionisation mass spectrometry (RP-HPLC/ESI-MS). The duplex **5** · **4** eluted after 14.6 min and was detected as the fivefold negatively charged ion at *m/z* 1766.5 (Figure 2, Table 1). Some of the duplex **5** · **4** underwent dissociation in the mass spectrometer, and the single strands **4** and **5** were detected as their threefold negatively charged ions at *m/z* 1433.9 and 1510.1, respectively. No signal for a modification of strand **4** with *N*-adenosylaziridine was observed. The duplex **5** · **4** was further analyzed by ESI-MS with direct infusion (Table 1). In this case **5** · **4** was no longer detectable in its duplex form. The single strands **4** and **5** were detected as five-, six- (Figure 3), and sevenfold negatively charged ions. In addition, **4** and **5** were also detected as sodium, potassium, and copper adducts, which were not observed under the RP-HPLC/ESI-MS conditions. The sixfold negatively charged state of **5** is demonstrated directly by the mass differences of the isotope multiplet of the $[\mathbf{5} - 6\,H]^{6-}$ ion (expansion in Figure 3), and the derived monoisotopic relative molecular weight of 4530.9 is identical to the calculated value.

It is well known that aziridines react with the nucleobases of DNA. Several covalent adducts between antitumor drugs such as bis(2-chloroethyl)methylamine (nitrogen mustard),[5] 2,5-bis(1-aziridinyl)-1,4-benzoquinones,[6] mitomycin C,[7] and carzinophilin (azinomycin A)[8] and DNA have been charac-

Table 1. Characterization of oligodeoxynucleotides by negative-ion electrospray mass spectrometry.

Compd	Charge	$(m/z)_{expt}$	M_{expt}	M_{calcd}
RP-HPLC/ESI-MS[a]				
5·**4**[b]	5 –	1766.5	8837.5	8836.9
5[b]	3 –	1510.1	4533.3	4533.1
4[b]	3 –	1433.9	4304.7	4303.8
3·**4**[c]	5 –	1708.0	8545.0	8544.6
3[c]	3 –	1412.7	4241.1	4240.8
ESI-MS by direct infusion[d]				
5[c]	7 –	646.4	4531.9	4533.1
5[e]	6 –	754.5	4533.1	4533.1
5[c]	5 –	905.6	4533.0	4533.1
4[c]	7 –	613.7	4303.0	4303.8
4[e]	6 –	716.1	4302.7	4303.8
4[c]	5 –	859.9	4304.5	4303.8
3[c]	7 –	604.8	4240.6	4240.8
3[c]	6 –	705.7	4240.3	4240.8
3[c]	5 –	847.2	4241.0	4240.8

[a] See legend in Figure 2. [b] Data shown in Figure 2. [c] Data not shown in Figures 2 or 3. [d] See legend to Figure 3. [e] Data shown in Figure 3.

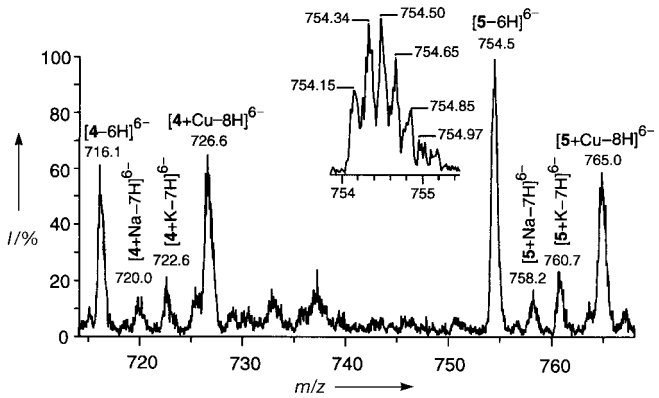

Figure 3. ESI mass spectrum of the product **5**·**4** obtained by direct infusion with a double focussing sector field mass spectrometer (MAT 90, Finnigan MAT) equipped with an electrospray ion source (ESI II). Desalted **5**·**4** (aqueous solution) and a liquid sheath flow (2-propanol) were delivered with a syringe pump (Harvard apparatus). The expansion shows the signal of the $[\mathbf{5}-6\mathrm{H}]^{6-}$ ion with isotopic resolution. I = relative intensity.

terized. However, no product of a nonenzymatic reaction was observed under the conditions used in this study. A covalent adduct between **2** and the duplex **3**·**4** was only formed in the presence of M·*Taq*I. In this adduct strand **3**, which contains an adenine at the target position within the recognition sequence of M·*Taq*I, is modified exclusively. Our observation that strand **4**, which contains N^6-methyladenine at the other target position and an adenine outside the recognition sequence, is not modified gives strong evidence that the base and sequence specificity of M·*Taq*I is not altered with the new cofactor **2**. This result also provides indirect structural evidence that the target adenine of strand **3** is the site of modification.[13]

This reaction represents the first example of a Mtase-catalyzed formation of a covalent bond between a group larger than a methyl group and the substrate for a Mtase. Since cofactor binding as observed in the three-dimensional structure of M·*Taq*I[9] is very similar to that of other DNA, RNA, protein, or small molecule Mtases,[10] **2** could also serve as a cofactor for other Mtases that act on a wide variety of substrates. In addition, it is conceivable that the new cofactor **2** in combination with M·*Taq*I could be used as a delivery system for fluorescent or other reporter groups. By the covalent attachment of such groups to the 8-position, which is directed towards the solvent in the crystal structure of M·*Taq*I in the complex with the natural cofactor **1**,[9] site-specific labeled DNA may be obtained. DNA fragments labeled in this way could find interesting applications in molecular biology.

Experimental Section

2: Dry aziridine[11] (360 µl, 7.2 mmol) was added slowly to a suspension of 5′-deoxy-5′-tosyladenosine (100 mg, 0.24 mmol, Aldrich) and NEt_iPr_2 (125 µL, 0.7 mmol) under argon, and the resulting solution was stirred at room temperature for three days. Any aziridine remaining was removed under reduced pressure, and the crude reaction product was dissolved in H_2O (1 mL) and neutralized with acetic acid. The solution (100 µL at a time) was injected onto a reversed-phase HPLC column (Hypersil-ODS, 5µm, 120 Å, 250 × 10 mm, Bischoff), and the product was eluted with a linear gradient of CH_3CN (7 → 10 % in 30 min, 2 mL min^{-1}) in triethylammonium hydrogencarbonate buffer (0.1M, pH 8.4). Fractions containing product (retention time 11.3 min) were combined, concentrated by lyophilization to 5.5 mL (10.5 mM with $\lambda = 260$, $\varepsilon = 15400$ of adenosine) and stored at −80 °C. Yield: 0.058 mmol (24 %). For characterization an aliquot was completely lyophilized to afford **2** as a white solid. ^{1}H NMR (500 MHz, D_2O): $\delta = 8.36$ (s, 1H; 2-H), 8.30 (s, 1H; 8-H), 6.13 (d, ^3J = 5.0 Hz, 1H; 1′-H), 4.84 (dd = t, ^3J = 5.3 Hz, 1H; 2′-H), 4.46 (dd = t, ^3J = 5.1 Hz, 1H; 3′-H), 4.35 (ddd = dt, ^3J = 4.6, 4.6, 6.7 Hz, 1H; 4′-H), 2.74 and 2.68 (AB part of ABX-spectrum, ^3J = 4.3, 6.6 Hz, ^2J = 13.3 Hz, 2H; 5′-Ha, 5′-Hb), 1.85 – 1.74 (m, 2H; aziridine-H), 1.49 – 1.40 (m, 2H; aziridine-H); FAB-MS (thioglycolic acid): *m/z* (%): 293 (100) $[M^+ + H]$, 250 (4) $[M^+ - C_2H_4N]$, 178 (11) $[B^+ + C_2H_4O]$, 167 (34), 165 (5), 164 (5) $[B^+ + CH_2O]$, 158 (36) $[M^+ - B]$, 149 (78), 136 (91) $[BH_2^+]$, 102 (23); B = deprotonated adenine.

Enzymatic reaction: Synthesis, purification, and annealing of oligodeoxynucleotides as well as preparation of M·*Taq*I were performed as descibed before.[12] The enzymatic reaction was carried out in a mixture (500 µL) of M·*Taq*I (10 µM), **3**·**4** (10 µM), **2** (1 mM) and Tris-OAc (20 mM, pH 6.0; Tris = tris(hydroxymethyl)aminomethane), KOAc (50 mM), $Mg(OAc)_2$ (10 mM) and Triton X-100 (0.01 %) at 37 °C.

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[13] The site of modification was further proven by enzymatic fragmentation of the duplex **5**·**4** with phosphodiesterase from *Crotalus durissus*, phosphodiesterase from calf spleen, DNase I, and alkaline phosphatase. Reverse-phase HPLC analysis of the digest revealed besides dC, dG, T, dA, and dA^{Me} an additional compound. This was isolated and detected as positively charged ion at *m*/*z* 544.6 in an ESI-MS experiment. The observed mass is identical with the calculated molecular mass of a protonated 2′-deoxyadenosine modified with *N*-adenosylaziridine.

Metallosupramolecular Thin Polyelectrolyte Films**

Markus Schütte, Dirk G. Kurth,* Matthew R. Linford, Helmut Cölfen, and Helmut Möhwald

The manufacture and investigation of supramolecular devices is an active area of research in chemistry and materials science.[1] Supramolecular devices open new avenues to functional materials with potential applications in electronics, photonics, and catalysis.[2] The realization of such materials will depend on improving existing methods and finding new routes for integrating supramolecular units in ordered, structurally coherent macroscopic assemblies.

Molecular units can be integrated in ordered arrays in Langmuir–Blodgett films or in self-assembled monolayers.[3] Decher et al. developed a versatile approach to assembled multilayer structures by alternating adsorption of oppositely charged polyelectrolytes.[4] This method has been applied to a number of polyelectrolytes (colloids, enzymes, etc.). Mallouck et al. investigated lamellar inorganic structures based, for example, on zirconium phosphates.[5]

Here we present the first integration of metallosupramolecular functional units in structurally coherent, ultrathin polyelectrolyte interfaces.[6] We employed a two-step self-assembly process: first, metal ions react with polytopic ligands to form a charged metallosupramolecular coordination polyelectrolyte,[7] then alternating adsorption with negatively charged polyelectrolytes generates molecular films.[8]

The synthesis of coordination polyelectrolytes is shown in Scheme 1a. Reaction of 1,4-bis(2,2′,6′,2″-terpyrid-4′-yl)benzene (**1**) with metal ions in a 1:1 ratio in solution gives the coordination polyelectrolyte **2**. With most metal ions terpyridine (tpy) forms stereochemically defined octahedral complexes with D_{2d} symmetry.[9] It therefore seems reasonable to assume that **2** has a linear structure in which the positive charge lies along the molecular axis.

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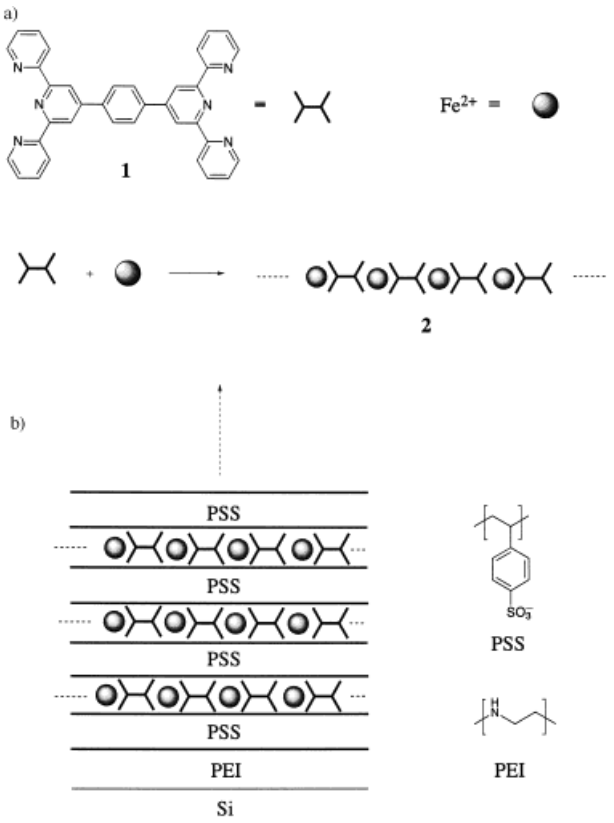

Scheme 1. a) Ligand **1** and metal ions in a 1:1 ratio form the coordination polyelectrolyte **2**. The counterions are omitted for clarity. b) Assembly of multilayers by sequential deposition of oppositely charged polyelectrolytes. PEI = polyethyleneimine, PSS = poly(styrene sulfonate).

A comparison of the UV/Vis and ^{1}H NMR spectra of **2** and $[Fe(me\text{-}ph\text{-}tpy)_2]Br_2$ (**3**; me-ph-tpy = 4′-*p*-tolyl-2,2′,6′,2″-terpyridine) confirms the presence of a coordination compound. Formation of a metal complex results in a characteristic metal-to-ligand charge-transfer (MLCT) transition in the visible region and a downfield shift of the ^{1}H NMR signals. The polymeric nature of **2** leads to broadening of the ^{1}H NMR signals and a red shift of the MLCT band relative to that of **3**.[10]

The molecular mass and polydispersity of **2** in methanol were determined by analytical ultracentrifugation. The sedimentation coefficient ($s = 1.60$ S) and an estimated diffusion coefficient ($D \leq 5.259 \times 10^{-7}$ cm^2 s^{-1}) were determined from the sedimentation velocity at 25 °C. Since the polydispersity leads to additional broadening of the sedimentation profile, the value of the diffusion coefficient will be too large. From the Swedberg formula, the lower limit of the molecular mass is 14900. For a dissociated polyelectrolyte with a molecular mass per repeat unit of 596.5, this corresponds to 25 repeat units. The width at half height of the particle size distribution function is 40%, that is, coordination polyelectrolytes of different chain length are present in solution. The fragment $[Fe(\mathbf{1})_2]^{2+}$ was observed in the electrospray (ES) mass spectra; oligomeric structures were not detected. Investigations by time-of-flight MS with matrix-assisted laser-desorption ionization (MALDI-TOF-MS) gave no further indications for the molecular weight of **2**.

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