Combined Biotin-Terpyridine Systems: A New Versatile Bridge between Biology, Polymer Science and Metallo-supramolecular Chemistry

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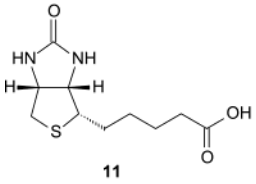
Biotin, a well-known binding unit for the proteins avidin and streptavidin, was combined with the chelating ligand terpyridine via polymeric and nonpolymeric spacers. An ω-amino-functionalized terpyridyl-poly(ethylene glycol) was prepared and utilized for complex formation with iron(II), nickel(II), and ruthenium(II) ions. The biocompatibility of the complex formation was investigated in aqueous media. Moreover, biotin was functionalized with a methoxy-poly(ethylene glycol) as a model system. The compounds were characterized by UV/vis and NMR spectroscopy as well as MALDI-TOF mass spectrometry. The systems represent a new combination of strong noncovalent binding units from both biology and synthetic supramolecular chemistry.

Introduction

Noncovalent interactions[1,2] are a major characteristic of biochemical systems and responsible for the highly organized secondary and tertiary structure of large biomolecules, such as in the case of proteins or DNA.[3,4] One of the most extensively studied noncovalent binding systems is represented by the coordination of biotin (also known as vitamin H) to the proteins avidin and streptavidin. Avidin consists of four identical subunits, each possessing one binding site for biotin (Figure 1, only one subunit displayed). A similar protein (also containing 4 biotin binding sites) is streptavidin. The resulting stable complexes with an association constant of 10^{15} mol^{-1} possess one of the strongest noncovalent interactions known and are based on multiple hydrogen-bonding and hydrophobic interactions:[5,6] the biotin unit exactly fits into the cavity of the avidin molecule like a key into a lock.

For many years, it has been the goal of synthetic chemistry to immobilize biological molecules, for detailed studies and for applications such as bio-chips and bio-sensors.[7] For this purpose, in particular water-soluble and bio-compatible spacers (oligomers and polymers) were connected to biotin-moieties. In this context, poly(ethylene glycol)s present a highly valuable class of compounds.[8,9]

The connection of biotin to synthetic supramolecular binding systems could open new avenues for the construction



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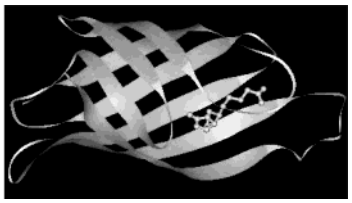

Figure 1. Left: schematic representation of biotin. Right: molecular modeling result of avidin[38] (one subunit) and its interaction with biotin.

of functional architectures or biosensor chips. In particular, metal complexes of bi- and terpyridines[10−12] are of special interest in this respect, since these complexes allow the reversible formation and cleavage of the metal coordinative bond by external stimuli, e.g., redox processes or the addition of competing ligands.[13−15] A recent contribution describes the synthesis of biotin-functionalized bipyridine and the subsequent formation of the corresponding iron(II) complex.[16] The complex can act as a "redox-biotin bridge" for potential biosensor applications: the addition of avidin to the complex could be monitored by cyclic voltammetry, revealing a change in the signal of the Fe(II)/(III) redox system. Moreover, the described system is characterized by a three-dimensionally orientated distribution of the biotin moieties, allowing the anchoring of multiple avidin units. An increased density of immobilized biomolecules could eventually lead to biosensors of enhanced sensitivity. Another powerful building block for metallo-supramolecular architectures is the ligand 2,2′:6′,2″-terpyridine,[17,18] which is known to form stable complexes with association constants ranging from 10^3 to 10^{15} or even higher, depending on the metal ions used.[19,20] Whereas zinc(II) forms rather weak complexes which exchange ligands in solution, iron(II) or ruthenium(II) complexes are stable in solution. The latter are stable even at higher temperatures. Moreover, the

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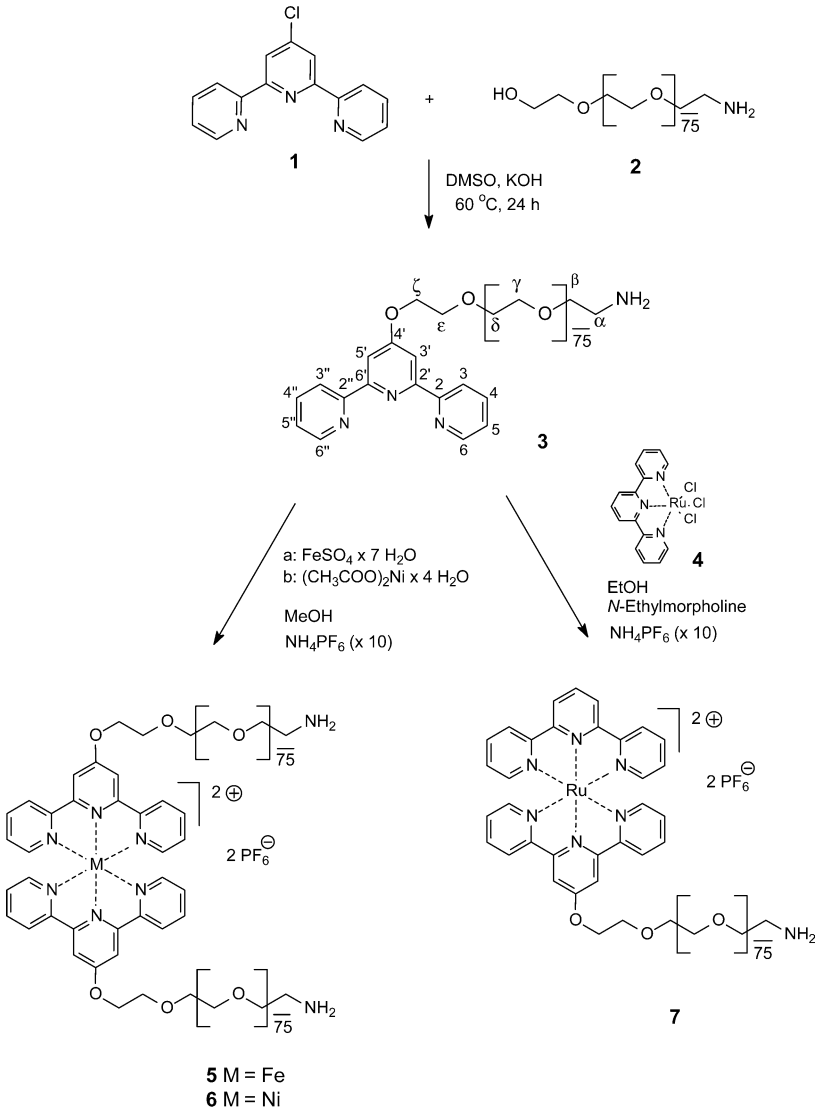
Scheme 1. Schematic Representation of the Synthesis of α-Amino-ω-(2,2′:6′,2″-terpyridin-4′-yl)-poly(ethylene glycol)$_{75}$ **3** and Complexes with Iron(II) (**5**), Nickel(II) (**6**), and Ruthenium(II) ions (**7**), Respectively



terpyridine-ruthenium(III)/(II) chemistry allows the build-up of defined metallo-supramolecular structures via the directed synthesis of asymmetric complexes.[21,22] Like a "LEGO system of building blocks", defined precursors make the construction of extended architectures possible. Among the examples are block[23] and graft[24] copolymers, which show the potential to form micelles.[14,25] The introduction of bioactive species into these systems could be useful for the construction of "nanoreactors". Smart "supramolecular glues" could even be utilized in the future on a molecular level for connecting molecular machines to an AFM tip.[26] The characteristic photophysical properties[27] of ruthenium complexes (metal-to-ligand charge transfer absorptions and low-temperature luminescence) are a suitable tool for analyzing the success and completeness of the complex formation. This is important especially for large structures where methods such as NMR or mass spectrometry reach their limits.

In this contribution, we describe a general strategy for the incorporation of biotin into terpyridine containing compounds. Organic molecules as well as polymeric spacers will be used for this purpose.

Results and Discussion

For the combination of biotin and terpyridine units, the biocompatibility as well as water-solubility of the connecting polymer is of major importance. In this respect, α-amino-ω-hydroxy-poly(ethylene glycol) **2** proved to be a well-suited starting material. The different functional groups at the chain ends allow a facile functionalization with two different functionalities, in the present case terpyridine and biotin.

As a first step, the hydroxy group of polymer **2** was functionalized with a terpyridine moiety through a nucleophilic aromatic substitution on 4′-chloroterpyridine **1** in DMSO in the presence of KOH for 24 h at 60 °C (Scheme 1). The resulting terpyridine-functionalized polymer **3**, which contains an amino group for further functionalization, was obtained in 88% yield after extraction with CH_2Cl_2, followed by precipitation in diethyl ether. The polymer was characterized by ^{1}H and ^{13}C NMR, UV/vis, as well as MALDI-TOF mass spectrometry. Subsequently, compound **3** was converted into a series of model complexes using iron(II), nickel(II), and ruthenium(II) ions. Iron(II) sulfate and the respective nickel(II) acetate solutions were added to a

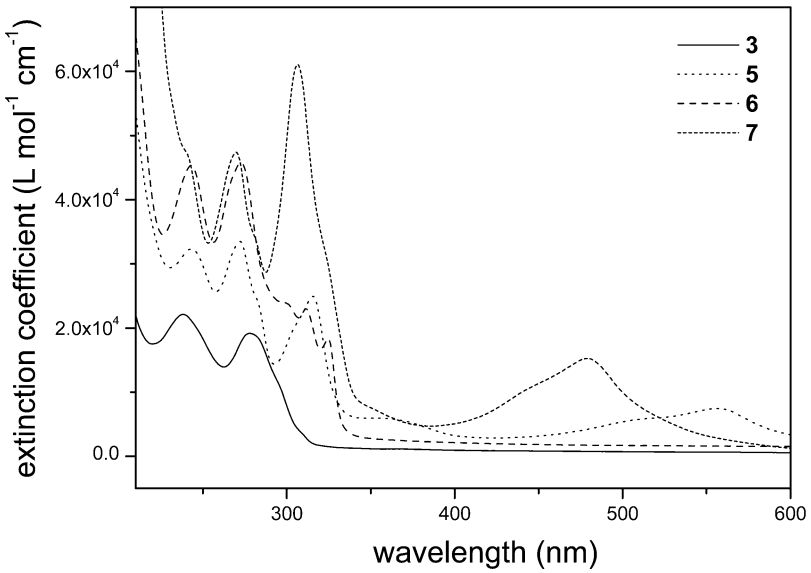


Figure 2. UV/vis spectra of **3** and the metal complexes **5**-**7** (in CH_3CN).

methanolic solution of polymer **3** to yield the symmetric complexes **5** and **6**. In the case of ruthenium, the asymmetric complex **7** was synthesized by reacting **3** with the ruthenium(III) trichloride complex **4** of a second (in this case unfunctionalized) terpyridine unit. During the reaction, the ruthenium(III) metal complex was reduced to ruthenium(II). All complexes could be isolated in good yields (74−83%) after exchange of the counterions to PF_6^- and preparative size exclusion chromatography (Bio Beads SX-1). The UV/vis spectra showed a bathochromic shift of the ligand-centered $\pi-\pi^*$ absorption bands around 270 and 310 nm for all complexes. In addition, the metal-to-ligand charge transfer (MLCT) band of the ruthenium(II) complex **7** at 475 nm and the iron(II) complex **6** at 556 nm was detected (Figure 2). The spectra are in accordance with UV−vis spectra of model complexes and PEG-containing complexes reported in the literature.[28] ^{1}H NMR spectroscopy showed the characteristic shifts of the aromatic protons, with the chemical shifts in accordance with model complexes. Especially, the upfield shift of the 6,6″-protons[29] is characteristic for this type of complexes (Figure 3). A comparison with similar compounds allowed the assignment of all signals of the ruthenium(II) complex.

Moreover, complex formation was proven by MALDI-TOF mass spectrometry. The polymeric complexes could be detected as unfragmented species (Figure 4).[30] Only singly charged species were detected (for this effect see also ref 31). A double molecular weight was found for the symmetric iron(II) complex **5**, and in the case of the ruthenium(II) complex **7**, a mass shift was detected that is in accordance with a terpyridine-ruthenium fragment.

Bioreactions and biocompatible reactions are usually carried out in aqueous solutions. Because proteins are often degrading at elevated temperatures, room temperature is usually applied to this kind of reaction. Thus, attempts were undertaken to modify the reaction procedure in order to make this reaction compatible to biomolecules by changing from alcoholic to aqueous solutions and avoiding the need of applying elevated temperatures.

For this purpose, water-soluble polymeric and nonpolymeric educts were utilized and the complex formation was monitored by means of UV−vis spectroscopy (Scheme 2). The intensity of the absorption band at 483 nm was used to obtain the rates of the complex formation (Figure 5). In these test reactions, a solution of 4′-(ω-methoxy-poly(ethylene glycol))-terpyridine ruthenium(III) trichloride **8** ($M_n = 3400$) and 4′-(methoxy di(ethylene glycol))-2,2′:6′,2″-terpyridine **9** in water was stirred at room temperature (Scheme 2). Three different reaction procedures were investigated: a. no more ingredients were added, the reaction was performed in pure water. b. 0.1 mL of ethanol was added. c. 0.1 mL of ethanol and 1 drop of *N*-ethylmorpholine were added.

In regular intervals, a sample was taken from the solution and a UV−vis spectrum was recorded. In all three reactions, a complexation to compound **10** took place. The characteristic bathochromic shift of the ligand-centered absorptions could be detected. Furthermore, the metal−ligand charge-transfer band characteristic for terpyridine ruthenium(II) complexes was observed. An increase of this band during the reaction was seen. The UV spectra are shown in Figure 5 (top) and the absorption of the MLCT band with time is plotted in Figure 5 (bottom). Even in pure water, the appearance of the complex bands can be observed. However, the reaction is very slow: after 6 days the conversion is about 65% (32% after 2 days). Addition of 5% ethanol, which acts as reducing agent for Ru(III), increases the reaction speed significantly. After 2 days 50% conversion is reached and after 8 days the reaction is completed. The most remarkable increase can be observed after addition of *N*-ethylmorpholine, which is supposed to act as a catalyst. In this case, 80% of the reaction is completed after 8 h and 100% conversion occurred after 48 h.

In a first experiment of linking biotin to a terpyridine ligand, a short spacer was utilized. A peptide coupling of the carboxylic group of the biotin **11** and the amino group of the 4′-aminopentoxy-terpyridine **12** was performed utilizing the DCC/HOBT method, yielding compound **13** in 52% yield (Scheme 3). The terpyridine containing biotin **13** was

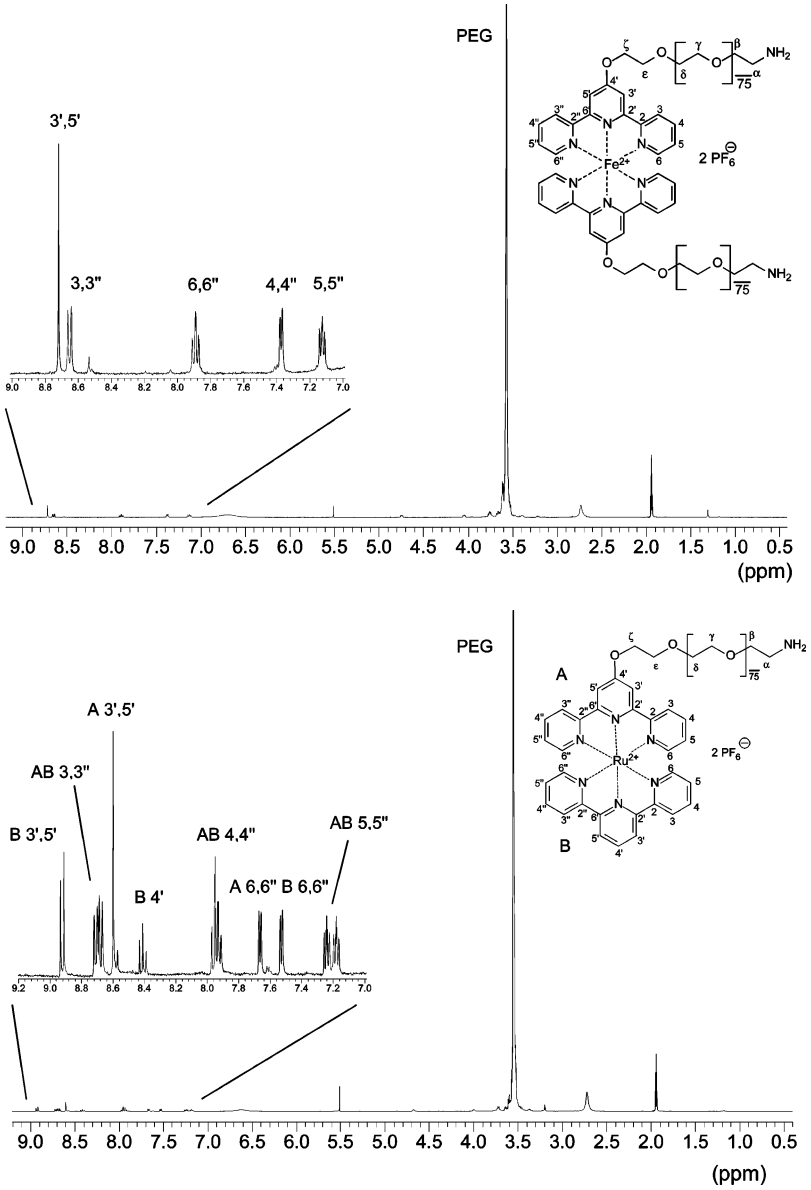


Figure 3. ^{1}H NMR spectra of the complexes **5** and **7** (in $CDCl_3$).

characterized using ^{1}H NMR, UV/vis, and MALDI-TOF MS (Figure 6, top). The addition of cobalt(II) acetate resulted in the formation of the complex **14**, which was isolated by exchange of the counterions with PF_6^- through addition of a 10-fold excess of ammoniumhexafluorophosphate, resulting in an immediate precipitation of the complex. UV/vis spectroscopy as well as MALDI-TOF mass spectrometry revealed the presence of the cobalt(II) complex **14** (Figure 6, bottom). The cation without counterions and an ion pair with one PF_6^- counterion could be detected. Again, all species were singly charged.[31] The isotopic pattern fits to the simulation (inset in Figure 6, bottom).

Subsequently, a polymeric spacer was introduced to connect the biotin moiety to the terpyridine unit in order to improve the biocompatibility and water-solubility. To explore the possibility to connect amino-functionalized poly(ethylene glycol) to biotin, an α-amino-ω-methoxy poly(ethylene glycol) was reacted with the biotin, applying again the DCC/HOBT method. However, this approach was unsuccessful to couple the biotin to the amino-functionalized polymer **3**. Therefore, a different method was employed: the carboxy group of the biotin was activated by the conversion into a succinimide ester group.[32,33] The activated intermediate **15** could be coupled successfully with the amino-group of the poly(ethylene glycol) **16** (M_w = 5000 daltons) within 48 h at room temperature (Scheme 4). After preparative size exclusion chromatography on a Bio Beads column, the biotin-functionalized polymer **17** was isolated in 80% yield. Proton NMR spectroscopy revealed a shift of the methylene protons adjacent to the carbonyl-carbon (H11) to 2.19 ppm, indicating a successful coupling of the biotin to the polymer (see also experimental part). A mass shift of approximately 230 units, corresponding to the biotin fragment, was found by comparing the MALDI-TOF mass spectrum of **17** with the starting polymer **16** (Figure 7, left).

Subsequently, the terpyridine-functionalized poly(ethylene glycol) **3** was treated with biotin in the same fashion, resulting in the α-biotinyl-ω-terpyridyl-poly(ethylene glycol) **18**, which could be isolated in 82% yield after a Bio Beads column. As described for the model system **17**, the successful

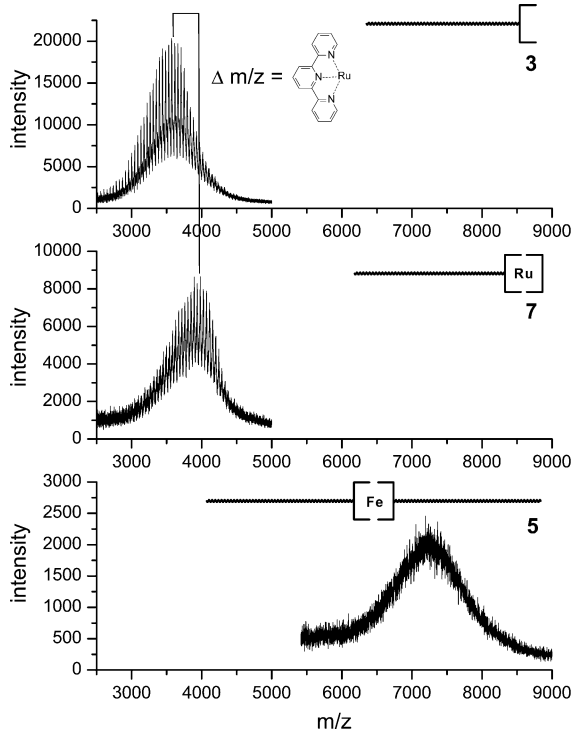


Figure 4. MALDI-TOF mass spectra of polymer **3** and the complexes **5** and **7**.

reaction could be shown by ^{1}H NMR spectroscopy. In addition to the characteristic shift of the H11 methylene protons, the signals of the terpyridine moiety were detected between 7.2 and 8.7 ppm. Moreover, MALDI-TOF mass spectrometry revealed the successful functionalization of the polymers **3** (Figure 7, right). The characteristic mass shift could also be detected for **18**, corresponding to the addition of the biotin moiety. Moreover, only a single distribution was found, indicating a complete functionalization of the precursors.

The compounds presented in this contribution open the avenue toward various supramolecular architectures that could lead eventually to new applications (Figure 8, see also introduction). Connecting a supramolecular binding moiety to biotin could lead to a biosensor-building block, which could be immobilized on a surface to create an activated layer of avidin, which specifically binds biotinylated molecules (e.g., proteins and deoxyribonucleotides). Instead of a flat surface, also nanoparticles or polymer matrixes could be used to bind biotin. Moreover, different functional groups ("X" in Figure 8) could be coupled to the biotin.

Replacing poly(ethylene glycol) by hydrophobic polymers would pave the way to giant amphiphile formation through the connection of the biotin unit to avidin or streptavidin. Such systems could form micelles that could be used as nanoreactors or for drug delivery.

Conclusion and Outlook

In this contribution, we have shown the possibility to combine one of the most prominent natural noncovalent binding units (biotin) with a widely used synthetic supramolecular system (terpyridine metal complexes). The metal terpyridine linker can be tuned regarding stability as well as kinetics and can be reversibly opened by external stimuli. A nonpolymeric as well as a polymeric poly(ethylene glycol) linker were used and the products were characterized by UV/vis, NMR, and MALDI-TOFMS. Further studies will include the reaction of the compounds with proteins such as avidin and streptavidin with and without metal-complexation and the construction of novel supramolecular architectures and giant amphiphiles from the obtained complexes. The immobilization of these systems on surfaces or nanoparticles will also be investigated.

Experimental Section

Materials and Instrumentation. All reagents were used without further purification. Biotin was purchased from Aldrich. 4-Chloro-(2,2′:6′,2″-terpyridine) **1** was synthesized according to ref 34. 1-Amino-5-(2,2′:6′,2″-terpyrid-4′-yloxy)-pentane **2**,[34,35] 2,2′:6,2″-terpyrid-4′-yl-ruthenium(III)-trichloride **4**,[36,37] 4′-(ω-methoxy-poly(ethylene glycol))-terpyridine ruthenium(III) trichloride **8**,[23] and 4′-(methoxy di(ethylene glycol))-2,2′:6′,2″-terpyridine **9**[18] were prepared according to the respective literature procedures. ^{1}H NMR spectra were obtained on a Varian Mercury VX 400 with TMS as internal standard. UV/vis spectra were taken on a Perkin-Elmer Lambda 45 UV/vis spectrometer. MALDI-TOF mass spectra were recorded on a BioSystems Perseptive Voyager 2000 instrument with dithranol as matrix.

α-Amino-ω-(2,2′:6′,2″-terpyrid-4′-yl) poly(ethylene glycol)$_{75}$ 3. α-Amino-ω-hydroxy-poly(ethylene glycol)$_{75}$ **2** (100 mg, 29.4 μmol) was added to a suspension of KOH (8.14 mg, 145.3 μmol) in 10 mL of DMSO. After 1 h at 60 °C, 4′-chloro-2,2′:6′,2″-terpyridine **1** (9.45 mg, 35.2 μmol) was added and stirring was continued for 24 h at 60 °C. After cooling to room temperature, the reaction mixture was poured into 100 mL of dichlormethane and washed with water (2 × 20 mL). The organic fraction was dried using sodium sulfate and the solvent was removed in vacuo. The remaining solid was dissolved in THF and precipitated into diethyl ether. Yield: 94.1 mg (25.8 μmol, 88%).

^{1}H NMR ($CDCl_3$): δ (ppm) = 2.92 (2H, t, $^3J_{HH}$ = 4.88 Hz, H_α), 3.33−3.89 (506H, m, $H_{\epsilon,\beta,\gamma,\delta}$), 4.34 (2H, t, $^3J_{HH}$ = 4.88 Hz, H_ξ), 7.28 (2H, d, $^3J_{HH}$ = 4.15 Hz, $H_{5,5''}$), 7.81 (2H, t, $^3J_{HH}$ = 4.85 Hz, $H_{4,4''}$), 7.98 (2H, s, $H_{3',5'}$), 8.55 (2H, d, $^3J_{HH}$ = 8.56 Hz, $H_{3,3''}$), 8.63 (2H, d, $^3J_{HH}$ = 3.9 Hz, $H_{6,6''}$).

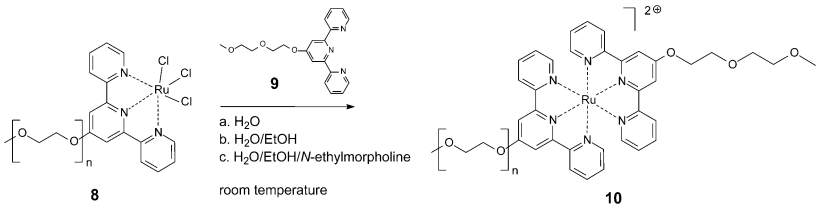
^{13}C NMR ($CDCl_3$): δ (ppm) = 41.2 (C_α), 61.7 (C_ξ), 67.9−76.8 ($C_{\beta,\delta,\gamma,\epsilon}$), 107.4 ($C_{55''}$), 121.3 ($C_{4,4''}$), 123.9 ($C_{3,3''}$), 136.8 ($C_{3',5'}$), 149.1 ($C_{6,6''}$), 156.0 ($C_{2,2''}$), 157.1 ($C_{2',6'}$), 167.0 ($C_{4'}$).

MS (MALDI-TOF): M_n = 3338, M_w = 3383, PDI = 1.01.

UV/vis (MeOH), λ_{max}/nm ($\epsilon/10^4$ L mol^{-1} cm^{-1}) = 275 (2.76), 239 (2.21).

Bis(α-amino-ω-(2,2′:6′,2″-terpyrid-4′-yl)-poly(ethylene glycol)$_{75}$)-iron(II) hexafluorophosphate 5. To a solution of α-amino-ω-(2,2′:6,2″-terpyrid-4′-yl)-poly(ethylene glycol)$_{75}$ **3** (20 mg, 5.49 μmol) in 5 mL of methanol was added iron(II)-sulfate-heptahydrate (0.763 mg, 2.74 μmol). The reaction mixture was stirred at 40 °C for 2 h, followed by the addition of ammonium hexafluorophosphate (8.9 mg, 55 μmol). From the purple reaction mixture, which was placed into a refrigerator, a purple precipitate formed within 12 h

Scheme 2. Schematic Representation of the Formation of the Ru(II) Terpyridine Complex **10** in Aqueous Solution



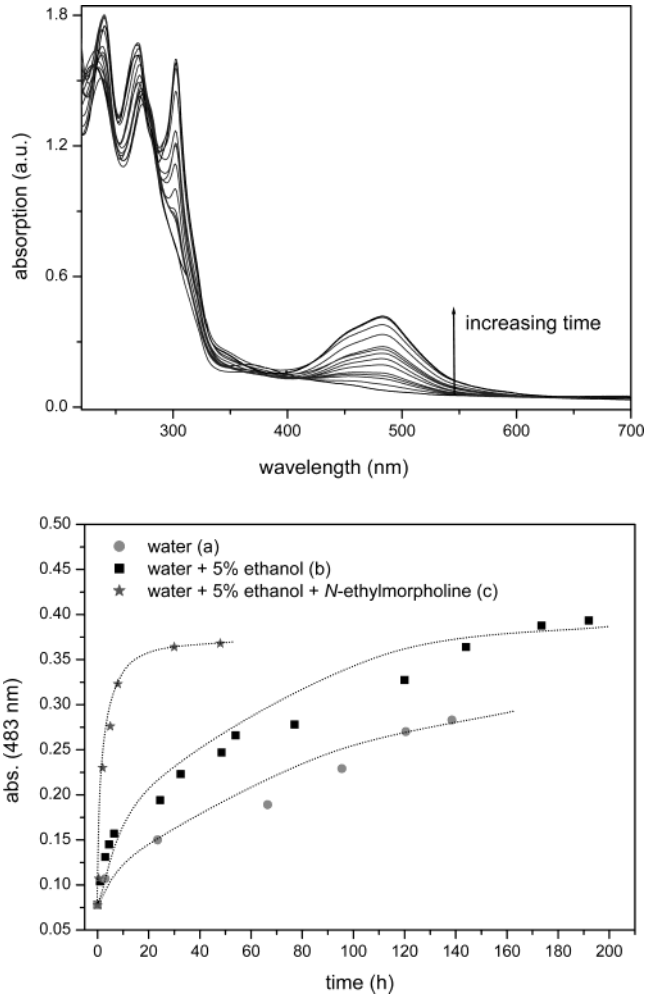


Figure 5. Top: UV−vis spectra of the monitoring of the formation of PEG-terpyridine ruthenium(II) complexes in aqueous solution. Bottom: kinetic curves of the complexation under different reaction conditions.

at 4 °C, which was collected by filtration and dried under reduced pressure. Yield: 15.6 mg (2.05 μmol, 74%).

^{1}H NMR ($CDCl_3$): δ (ppm) = 2.92 (4H, t, $^3J_{HH}$ = 4.88 Hz, H_α), 3.33−3.89 (506H, m, $H_{\epsilon,\beta,\gamma,\delta}$), 4.74 (4H, t, $^3J_{HH}$ = 4.56 Hz, H_ξ), 7.20 (4H, m, $H_{5,5''}$), 7.42 (4H, m, $H_{6,6''}$), 8.01 (4H, m, $H_{4,4''}$), 8.76 (4H, d, $^3J_{HH}$ = 7.45 Hz, $H_{3,3''}$), 8.82 (4H, m, $H_{3',5'}$).

MS (MALDI-TOF): M_n = 7383, M_w = 7469, PDI = 1.01.

UV/vis (CH_3CN): λ_{max}/nm (ϵ/10^4 L mol^{-1} cm^{-1}) = 556 (0.5), 315 (1.99), 272 (2.68), 242 (2.58).

Bis(α-amino-ω-(2,2′:6′,2″-terpyrid-4′-yl)-poly(ethylene glycol)$_{75}$)-nickel(II) hexafluorophoshate 6. To a solution of α-amino-ω-(2,2′:6,2″-terpyrid-4′-yl)-poly(ethylene glycol)$_{75}$ **3** (20 mg, 5.5 μmol) in 5 mL of methanol was added nickel(II)-acetate-tetrahydrate (0.691 mg, 2.77 μmol). The reaction mixture was stirred at 70 °C for 12 h. Subsequently, ammonium hexafluorophosphate (8.9 mg, 55 μmol) was added to the hot reaction solution and stirring continued for 10 min. A white precipitate formed within 12 h at 4 °C, which was collected by filtration and dried in vacuo. Yield: 16.34 mg (2.14 μmol, 78%).

MS (MALDI-TOF): M_n = 7100, M_w = 7182, PDI = 1.01.

UV/vis (CH_3CN): λ_{max}/nm (ϵ/10^4 L mol^{-1} cm^{-1}) = 324 (1.69), 311 (2.13), 272 (4.58), 243 (4.35).

[α-Amino-ω-(2,2′:6′,2″-terpyrid-4′-yl-poly(ethylene glycol)$_{75}$] [2,2′:6,2″-terpyridine]-ruthenium(II)-hexafluorophosphate 7. To a solution of α-amino-ω-(2,2′:6,2″-terpyrid-4′-yl)-poly(ethylene glycol)$_{75}$ **3** (20 mg, 5.55 μmol) in 5 mL of absolute ethanol were added 2,2′:6,2″-terpyrid-4′-yl-ruthenium(III)-trichloride **4** (2.95 mg, 6.66 μmol) and 30 μL *N*-ethylmorpholine and refluxed for 4 h. Subsequently, NH_4PF_6 (9 mg, 55.2 μmol) in 2 mL of ethanol was added into the hot solution. After cooling to room temperature and keeping the solution for 12 h at −20 °C, the brown solid was collected by filtration and washed with cold ethanol and dried in vacuo. Finally, the product was purified by preparative size exclusion chromatography (Bio Beads SX 1, in CH_2Cl_2). Yield: 18 mg (4.5 μmol, 83%).

^{1}H NMR (aceton-d_6): δ (ppm) = 2.88 (2H, m, H_α), 3.3−3.8 (506H, m, $H_{\beta,\gamma,\delta}$), 4.11 (2H, m, H_ϵ), 4.78 (2H, t, $^3J_{HH}$ = 4.39 Hz, H_ξ), 7.29 (2H, m, $H_{B5,5''}$), 7.35 (2H, m, $H_{A5,5''}$), 7.64 (2H, d, $^3J_{HH}$ = 4.76 Hz, $H_{B6,6''}$), 7.77 (2H, d, $^3J_{HH}$ = 4.76 Hz, $H_{A6,6''}$), 8.05 (4H, m, $H_{AB,4,4''}$), 8.52 (1H, t, $^3J_{HH}$ = 8.42 Hz, $H_{B4'}$), 8.71 (2H, s, $H_{A3',5'}$), 8.81 (4H, m, $H_{AB3,3''}$), 9.03 (2H, d, $^3J_{HH}$ = 8.06 Hz, $H_{B3',5'}$).

MS (MALDI-TOF): M_n = 3824, M_w = 3890, PDI = 1.02.

UV/vis (CH_3CN): λ_{max}/nm (ϵ/10^4 L mol^{-1} cm^{-1}) = 266 (5.61), 304 (6.34), 475 (1.83).

Studies of the Complex Formation Rates in Aqueous Media. In all three reactions (a−b), 5 mg of 4′-(ω-methoxy-poly(ethylene glycol))-terpyridine ruthenium(III) trichloride **8** (M_n = 3400) and 0.5 mg of 4′-(methoxy di(ethylene glycol))-2,2′:6′,2″-terpyridine **9** were dissolved in 2 mL of water and stirred at room temperature.

(a) No more ingredients were added, the reaction was performed in pure water.

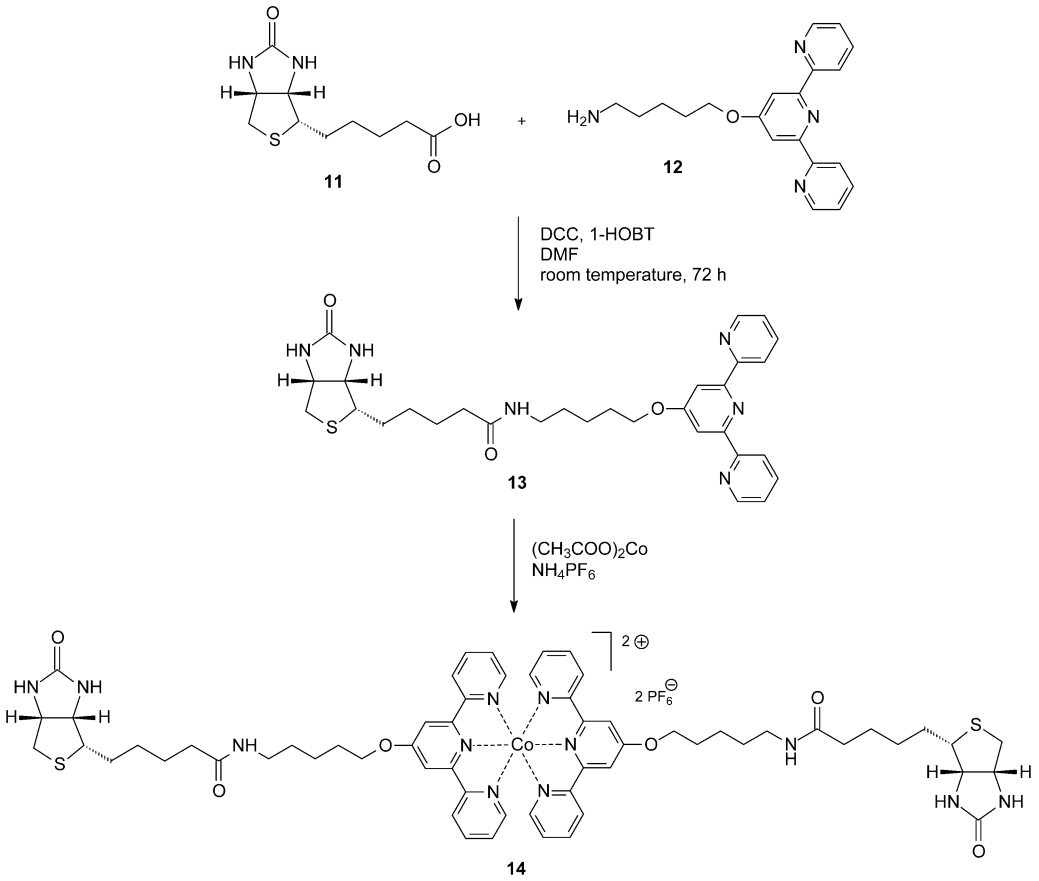
(b) 0.1 mL of ethanol was added.

(c) 0.1 mL of ethanol and 1 drop of *N*-ethylmorpholine were added.

In regular intervals, a sample was taken from the solution, diluted to a concentration of 5 × 10^{-5} and a UV−vis spectrum was recorded.

Terpyridine-Functionalized Biotin 13. To a solution of biotin **11** (50 mg, 0.205 mmol) in 5 mL DMF was added 1-hydroxybenzotriazol (1-HOBT, 40 mg, 0.307 mmol). After stirring for 10 min at room temperature, *N*,*N*′-dicyclohexylcarbodiimide (DCC, 63 mg, 0.307 mmol) was added.

Scheme 3. Schematic Representation of the Synthesis of the Biotin-functionalized Terpyridine **13**, Separated by a Short Spacer, and Complexation with Cobalt(II) Ions (**14**)



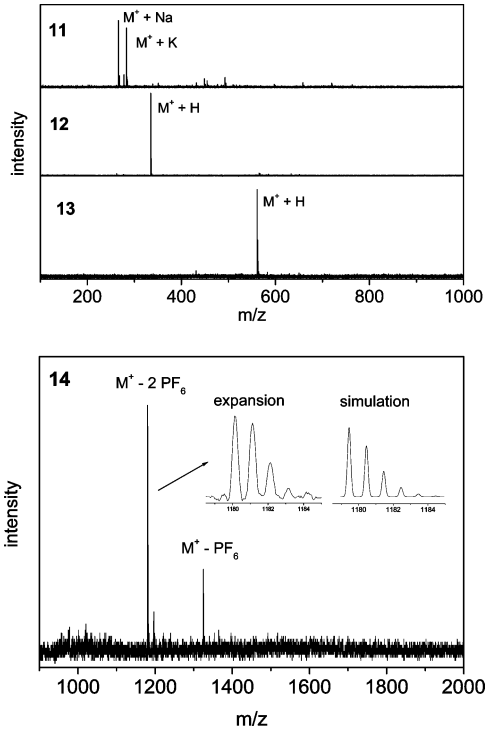


Figure 6. MALDI-TOF mass spectra of compounds **11**, **12** and the biotinyl-terpyridine **13** as well as the corresponding cobalt(II) complex **14**.

Subsequently, 72 mg 1-amino-5-(2,2′:6′,2″-terpyrid-4′-yl-oxy)pentane **12** (0.218 mmol) were added after an additional stirring for 4 h at room temperature. The mixture was stirred for 72 h at room temperature and the formed precipitate was collected by filtration and dried in vacuo. Yield: 61 mg (0.106 mmol, 52%), mp: 191.5 °C.

^{1}H NMR (DMSO, 300 MHz): δ (ppm) = 1.01−1.82 (14H, m, CH_2), 2.05 (3H, t, $^3J_{HH}$ = 7.26 Hz, CH_2N), 2.76 (2H, m, CH_{biotin}), 3.06 (3H, m, CHS; CH_2S), 4.21 (2H, m, CH_2O), 6.16 (1H, s, NH), 6.23 (1H, s, NH), 7.34 (2H, m, $H_{5,5''}$), 7.80 (2H, s, $H_{3',5'}$), 7.83 (2H, dt, $^3J_{HH}$ = 7.83 Hz, $^4J_{HH}$ = 1.74 Hz, $H_{4,4''}$), 8.45 (2H, d, $^3J_{HH}$ = 8.01 Hz, $H_{3,3'}$), 8.55 (2H, d, $^3J_{HH}$ = 3.99 Hz, $H_{6,6''}$).

^{13}C NMR (DMSO-d_6): δ (ppm) = 23.21 ($C_{9'}$), 25.68 ($C_{17'}$), 28.39 ($C_{15',16'}$), 29.21 ($C_{8'}$), 33.69 ($C_{10'}$), 35.59 ($C_{14'}$), 47.86 ($C_{11'}$), 55.79 ($C_{19',23'}$), 59.52 ($C_{18'}$), 61.38 ($C_{24'}$), 58.27 ($C_{7'}$), 107.08 ($C_{5,5''}$), 121.21 ($C_{4,4''}$), 124.82 ($C_{3,3''}$), 137.68 ($C_{3',5'}$), 149.57 ($C_{6,6''}$), 155.23 ($C_{2,2''}$), 157.02 ($C_{2',6'}$), 163.02 ($C_{13'}$), 167.0 ($C_{4'}$), 172.16 ($C_{21'}$).

MS (MALDI-TOF): m/z = 561.03 (M^++H, 100%).

UV/vis (DMF): λ_{max}/nm ($\epsilon/10^4$ L mol^{-1} cm^{-1}) = 275 (1.51).

Cobalt Complex of the Terpyridine-Functionalized Biotin 14. The biotin-functionalized terpyridine **13** (23 mg, 0.041 mmol) and cobalt(II) acetate tetrahydrate (5.1 mg, 0.021 mmol) were dissolved in 2 mL of DMF and stirred for 2 h at 70 °C, followed by the addition of 200 mg of ammoniumhexafluorophosphate. After evaporation of the solvent, water was added (2 × 20 mL), and the raw product was subjected to ultrasonification and subsequently filtrated. This procedure was repeated with 20 mL of diethyl ether. The complex was dried in vacuo and further purified by

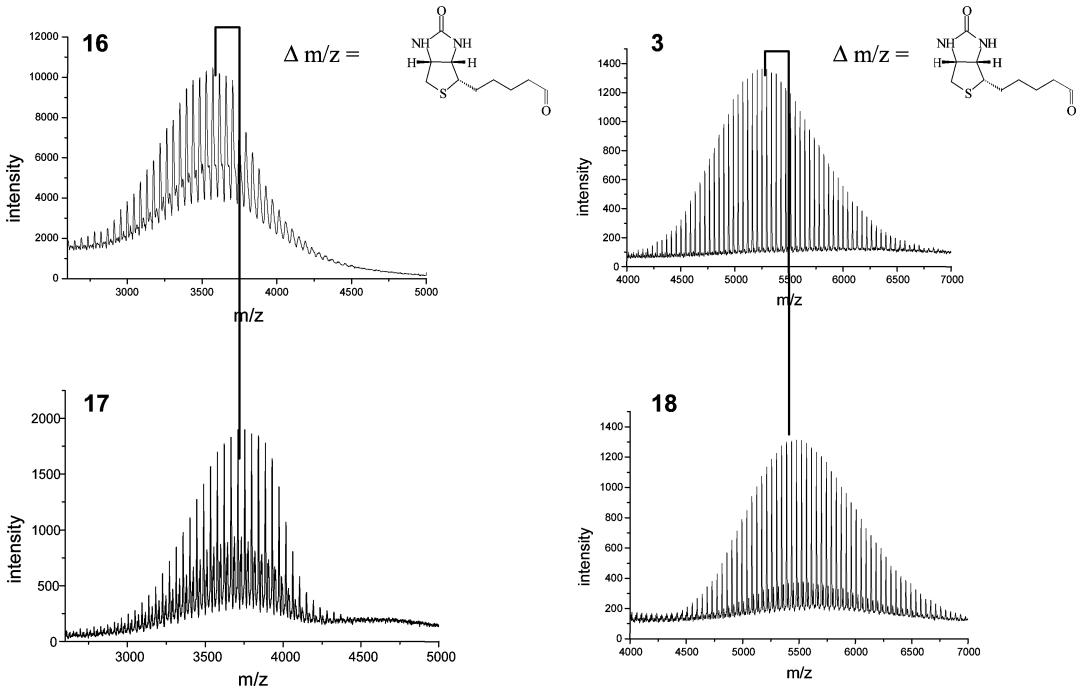
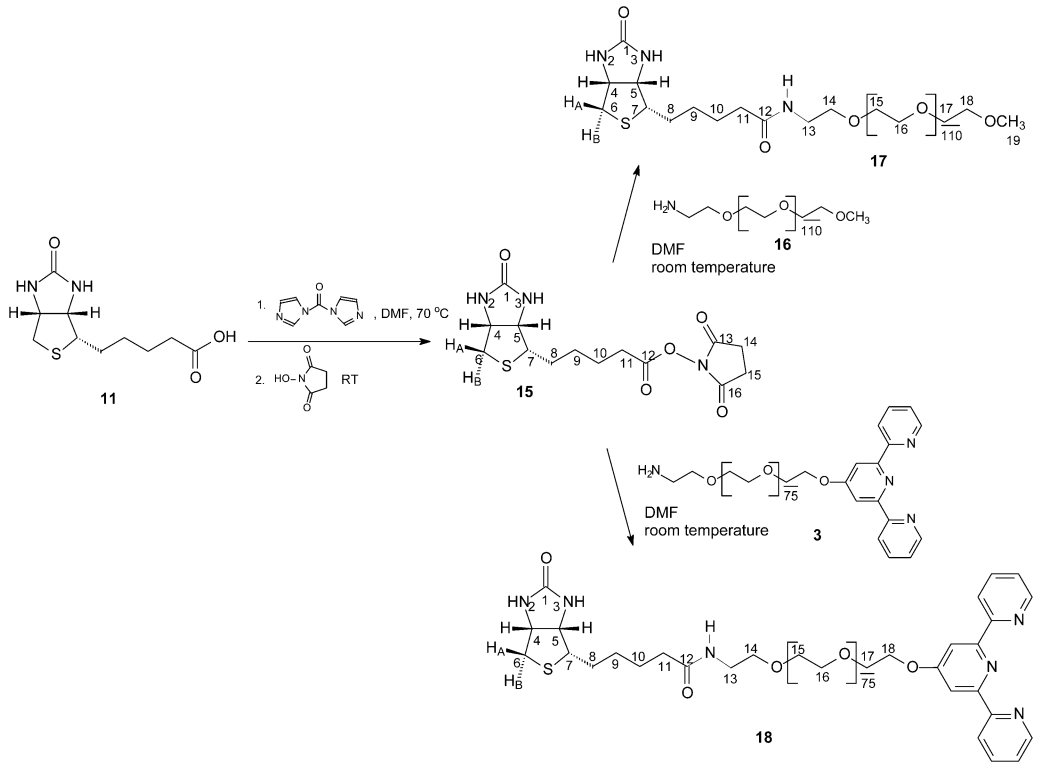


Figure 7. MALDI-TOF mass spectra of the biotin-functionalized poly(ethylene glycol)s **17** (without terpyridine, bottom left) and **18** (terpyridine-functionalized, bottom right) in comparison to their precursors **16** and **3** (top left and right, respectively).

Scheme 4. Schematic Representation of the Synthesis of the α-Methoxy-ω-(2,2′:6′,2″-terpyridin-4′-yl)-poly(ethylene glycol)$_{110}$ **17** and α-Biotinyl-ω-(2,2′:6′,2″-terpyridin-4′-yl)-poly(ethylene glycol)$_{75}$ **18**



preparative size exclusion chromatography (BioBeads, eluent DMF). Yield: 13.67 mg (9.30 μmol, 45%).

MS (MALDI-TOF): m/z = 1324.86 (M^+-PF_6, 40%), 1180.15 ($M^+-2\ PF_6$, 100%).

UV/vis (DMF): λ_{max}/nm ($\epsilon/10^4$ L mol^{-1} cm^{-1}) = 274 (3.99), 306 (1.67), 319 (1.27).

Biotin-*N*-hydroxy-succinimide-ester 15. To a solution of biotin **11** (250 mg, 1.02 mmol) in 10 mL of DMF was added 166 mg of *N*,*N*′-cabonyldiimidazole (1.02 mmol) at 78 °C. The reaction mixture was stirred for 2 h at room temperature, yielding a white precipitate. 115.7 mg of *N*-hydroxysuccinimide (1.02 mmol) was added and stirring was continued for 1 h. The solvent was evaporated in vacuo and the residue crystallized from 2-propanol and DMF/diethyl ether consecutively. Yield: 261.1 mg (0.76 mmol, 75%), mp: 209 °C.

^{1}H NMR (DMSO-d_6): δ (ppm) = 1.43−1.7 (6H, m, H_{8-10}), 2.58 (1H, d, $^3J_{HH}$ = 12.3 Hz, H_{6B}), 2.67 (2H, t, $^3J_{HH}$

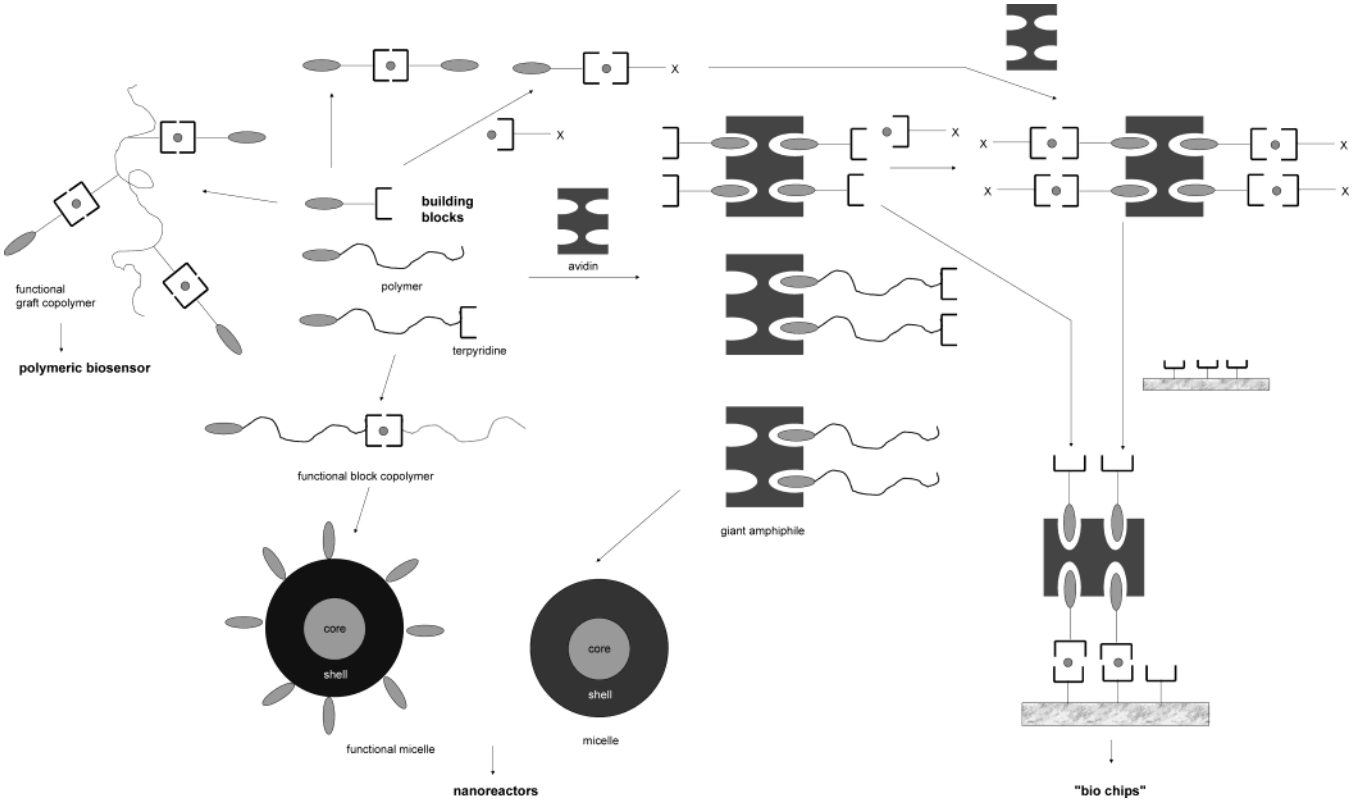


Figure 8. Schematic overview of the potential applications of the biotin-functionalized terpyridine systems.

= 7.41 Hz, H_{11}), 2.83 (1H, m, H_{6A}), 2.81 (4H, s, $H_{14,15}$), 3.08 (1H, m, H_7), 4.15 (1H, m, H_5), 4.31 (1H, m, H_4), 6.37 (1H, s, H_3), 6.44 (1H, s, H_2).

^{13}C NMR (DMSO-d_6): δ (ppm) = 24.5 (C_{10}), 25.6 ($C_{14,15}$), 27.8 (C_8), 28.0 (C_9), 30.2 (C_{11}), 40.6 (C_6), 55.4 (C_7), 59.3 (C_4), 61.2 (C_5), 163.1 (C_1), 169.2 (C_{12}), 170.6 ($C_{13,16}$).

MS (MALDI-TOF): m/z = 341.92 (M^+), 363.90 ($M+Na^+$), 379.87 ($M+K^+$).

α-Biotinyl-ω-methoxy-poly(ethylene glycol)$_{110}$ 17. To a solution of 100 mg (20 μmol) of α-amino-ω-methoxy-poly-(ethylene glycol)$_{75}$ **16** in 3 mL of DMF was added 10.3 mg (30 μmol) of the activated biotin-NHS-ester **15** (30 μmol) in 1 mL of DMF. The reaction mixture was stirred for 24 h at room temperature. The solvent was evaporated, and the residue was redissolved in toluene, filtered, and further purified utilizing size exclusion chromatography (SX-1, toluene). Yield: 83.1 mg (16 μmol, 80%).

^{1}H NMR ($CDCl_3$): δ (ppm) = 4.49 (1H, m, H_4), 4.32 (1H, m, H_5), 3.32 (3H, s, H_{19}), 3.39−3.78 (530H, m, H_{13-18}), 3.11 (1H, m, H_7), 2.84 (1H, m, H_{6A}), 2.52 (1H, m, H_{6B}), 2.19 (2H, t, $^3J_{HH}$ = 7.7 Hz, H_{11}), 1.41−1.74 (6H, m, H_{8-10}).

MS (MALDI-TOF): M_n = 5608, M_w = 5698, PDI = 1.02.

α-Biotinyl-ω-(2,2′:6′,2″-terpyrid-4′-yl)-poly(ethylene glycol)$_{75}$ 18. To a solution of 20 mg (5.5 μmol) of α-amino-ω-(2,2′:6′,2"-terpyrid-4′-yl) poly(ethylene glycol)$_{75}$ **3** in 3 mL of DMF was added 2.81 mg (30 μmol) of the activated biotin-NHS-ester **15** (30 μmol) in 1 mL of DMF, and the mixture was stirred for 36 h at room temperature. The solvent was evaporated in vacuo and the residue redissolved in toluene, filtered and further purified utilizing size exclusion chromatography (Bio Beads SX-1, toluene). Yield: 17.5 mg (4.5 μmol, 82%).

^{1}H NMR ($CDCl_3$): δ (ppm) = 1.39−1.71 (6H, m, H_{8-10}), 2.19 (2H, t, $^3J_{HH}$ = 7.7 Hz, H_{11}), 3.05 (1H, m, H_7), 2.78 (1H, m, H_{6A}), 2.51 (1H, m, H_{6B}), 3.33−3.89 (359H, m, H_{13-17}), 4.34 (2H, t, $^3J_{HH}$ = 4.88 Hz, H_{18}), 7.28 (2H, d, $^3J_{HH}$ = 4.15 Hz, $H_{5,5''}$), 7.81 (2H, t, $^3J_{HH}$ = 4.85 Hz, $H_{4,4''}$), 7.98 (2H, s, $H_{3',5'}$), 8.55 (2H, d, $^3J_{HH}$ = 8.56 Hz, $H_{3,3''}$), 8.63 (2H, d, $^3J_{HH}$ = 3.9 Hz, $H_{6,6''}$).

MS (MALDI-TOF): M_n = 3585, M_w = 3649, PDI = 1.02.

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