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Journal of Organometallic Chemistry 648 (2002) 81–86

Journal of Organometallic Chemistry

www.elsevier.com/locate/jorganchem

Synthesis and electrochemistry of 5-ferrocene-glucosamide, 5-ferrocene-glucosamide phosphate and 5-ferrocene-amido-5-adenosine in aqueous solution

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Received 26 June 2001; received in revised form 16 October 2001; accepted 22 October 2001

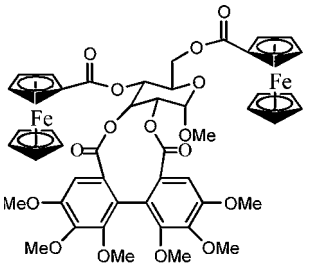
Abstract

The syntheses of new ferrocene-monosaccharide compounds, 5-ferrocene-glucosamide (**4**), 5-ferrocene-glucosamido-2′-phosphate (**5**), and 5′-ferrocene-amido-5′-deoxyadenosine (**6**), are reported starting from ferrocenoyl hydroxybenzotriazole ester and the corresponding amino sugars. All compounds are investigated spectroscopically. In addition, their redox properties are investigated by cyclic voltammetry in aqueous solution. These compounds show a reversible one-electron oxidation. This redox potential in the case of **4** exhibits a significant pH dependence.

Keywords: Bioconjugate; Ferrocene; Electrochemistry; Glucosamine; Amino sugar; Nucleoside

1. Introduction

The detection of glucose using a ferrocene–ferrocenium mediated glucose oxidase has been described in the literature [1]. However, the direct modification of sugar molecules by redox active groups such as ferrocene has been largely neglected until recently [2]. This is surprising since there is a great deal of interest in the redox modification of DNA for the purpose of detection of base–pair mismatches. Mirkin and co-workers have recently reported the synthesis of 5′-ferrocene-labeled DNA via a single phosphoamidate chemistry, which makes it amenable to automation [3]. However, the ferrocene group was linked to the 5′-PO_4-deoxyribose through a C_6 linker. Despite a well-behaved electrochemistry, the efficiency of the electronic communication in the system can be optimized by shortening the linker in the sugar or by attaching the redox group directly to the 5′-terminus. Itoh et al. prepared two ferrocenoyl-saccharide derivatives **1** and **2** [2], due to their general interest in medicinal applications of FcCO-glucoside derivatives, and their potential biological activities, in particular their antimalarial activity [2] and DNA binding abilities in cancer cells [4].



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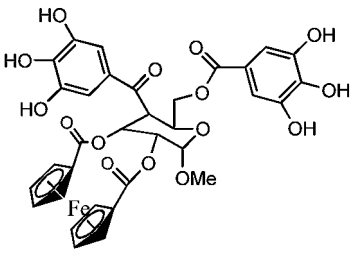

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0022-328X/02/$ - see front matter
PII: S0022-328X(01)01414-0

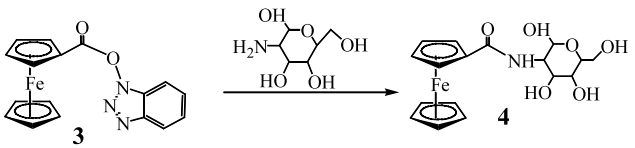
Donnio et al. reported the synthesis of new ferrocene-containing surfactants by reaction of ferrocenoyl chloride and 1-amino-1-deoxy-D-sorbitol in CH_2Cl_2 and DMF [5].

Our interest in the synthesis of new ferrocene amino saccharides stems from our interest in the modification of biological compounds with the ferrocene electrophore for electron transfer studies. We recently have developed a method to attach a ferrocene group to the N-terminus of amino acids and peptides under mild conditions using the carbodiimide protocol [6]. Here we report an extension of this synthetic methodology to include the coupling of ferrocene carboxylic acid to aminosaccharides in aqueous solution. Furthermore, we examine the electrochemical properties of the novel electroactive bioconjugates and present data on the effects of pH on the redox properties of 5-ferrocene-glucosamide in aqueous medium.

2. Results and discussion

2.1. Synthesis

Our synthetic approach is summarized in Scheme 1. Three yellow to orange compounds, 5-ferrocene-glucosamide (**4**), 5-ferrocene-glucosamido-2′-phosphate (**5**), and 5-ferrocene-amido-5′-adenosine (**6**), were readily prepared in good to moderate yields by the reaction of FcCO-OBt with suitable amino sugar derivatives in a mixture of THF–borate buffer (1:1) at pH 9. Compounds **4**–**6** are only sparingly soluble in non-polar organic solvents, but very soluble in water and DMSO and to a lesser extent methanol.



Scheme 1. Synthesis of 5-ferrocene-glucosamide (**4**) from FcCO-OBt (**3**) and glucosamine in THF–water (1:1) at pH 9.

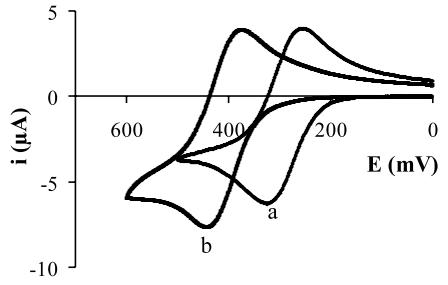
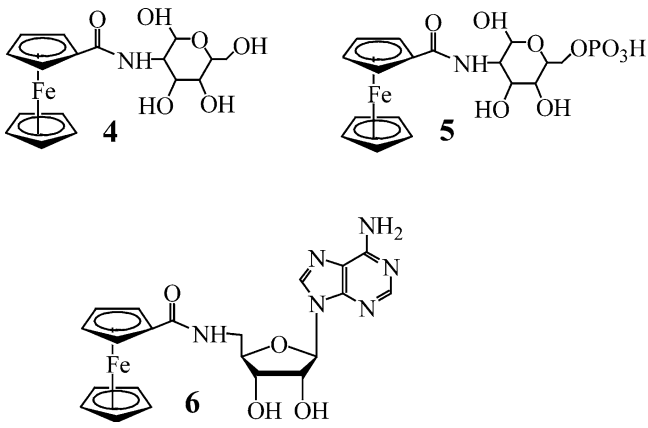


Fig. 1. Cyclic voltammograms of ferrocene carboxylic acid (**a**, $c = 2 \times 10^{-3}$ M) and of 5-ferrocene-glucosamide **4** (**b**, $c = 2.4 \times 10^{-3}$ M) in aqueous solution (borate buffer, pH 9). Scan rate $v = 100$ mV s^{-1}, gold working electrode, Pt counter electrode and Ag/AgCl reference electrode.

The ^{1}H-NMR spectra of compounds **4** and **5** are comparable to those of the glucosamine and glucosamine phosphate, respectively, showing signals of the cyclic pyranose and acyclic aldose form of glucosamine [7]. In addition, the characteristic signal pattern for monosubstituted ferrocenes are observed at δ 4.18 for the unsubstituted Cp, δ 4.20 for the two *meta*-H and at δ 4.43 for the two *ortho*-H of the glucosamine substituted Cp ring. Similarly, the ^{1}H-NMR spectrum of **6** has additional peaks due to the coupling of the FcCO group. The ^{13}C-NMR of **4** exhibits a new signal in the carbonyl region at δ 177.1 due to the FcCO-amide CO. Compound **5** exhibits a signal in the ^{31}P-NMR spectrum at $\delta = 6.8$, characteristic for a phosphate ester group [8]. The IR spectra of **4**–**6** clearly show the presence of amide I and II bands around 1650 and 1540 cm^{-1}, respectively. The amide A band (N–H) around 3200 cm^{-1} is masked by absorptions of the glucosamine hydroxyl groups. The visible spectra of **4**–**6**, exhibit single broad absorptions with low extinction coefficients due to the d–d transition of the ferrocene chromophore [9].



2.2. Electrochemical studies

Next, we examined the electrochemistry of compounds **4**–**6** by cyclic voltammetry (CV) in aqueous solution (borate buffer, pH 9), evaluated their diffusion coefficients at pH 9 and carried out a study of the influence of pH on their redox potential. All compounds exhibit a fully reversible one-electron oxidation with peak separations close to the reversible limit ($\Delta E = 60$–70 mV) and peak current ratios close to unity. Fig. 1 shows a typical CV curve of compound **4** (curve a) with a halfwave potential $E_{1/2} = 410 \pm 10$ mV. The CV curve of ferrocene carboxylic acid (FcCO-OH) is shown for comparison (curve b, $E_{1/2} = 300$ mV vs. Ag/AgCl). Since the acid functionality is more electron-withdrawing than the amide group, substitution of the

carboxylic acid group for an amide linkage is expected to cause a shift to higher redox potential [10]. This is in accord with theoretical results showing that amide groups will lower the energy level of the HOMO, making the ferrocene more difficult to oxidize [11]. The halfwave potentials of compounds **4**–**6** are similar (Table 1), indicating that the chemical environment of the amide groups are very similar.

Diffusion coefficients D_0 of **4**–**6** were obtained from the slope of plots of the anodic peak current versus the square-root of the scan rate, $v^{1/2}$, according to Eq. (1) (see Fig. 2) [12].

$$i_a = 0.4463nA(nF/RT)^{1/2} D_0^{1/2} v^{1/2} C^0 \quad (1)$$

with A, electrode surface (cm^2); D_0, diffusion coefficient ($cm^2\ s^{-1}$); v, scan rate ($V\ s^{-1}$); C, concentration (M); and n, number of electrons. In general the D_0's of compounds **4**–**6** are similar but an order of magnitude smaller than those of ferrocene carboxylic acid. This is understandable, since the hydroxyl groups in **4**–**6** will strongly interact with the water molecules, thereby reducing their mobility. And although FcCO-OH will also engage in H-bonding to the water, it will not be as effective as **4**–**6**, which have a higher number of groups able to engage in H-bonding. These diffusion coefficients compare well with those of ferrocene derivatives in SDS micelles [13]. For example, D_0 values for (ferrocenylmethyl)trimethyl-ammonium bromide in SDS–1-pentanol–dodecane–NaBr microemulsions vary from 2.8×10^{-7} to 5.6×10^{-7} $cm^2\ s^{-1}$ and for bis(pentamethyl)ferrocene from 2.2×10^{-7} to 19×10^{-7} $cm^2\ s^{-1}$. For comparison, the mobility of ferrocene in common organic solvents such as MeCN and DMF is signficantly higher, giving rise to D_0's of 2.37×10^{-5} and 1.07×10^{-5} $cm^2\ s^{-1}$, respectively [14].

Table 1
Electrochemical properties of ferrocene carboxylic acid (FcCO-OH), 5-ferrocene-glucosamide (**4**), 5-ferrocene-glucosamido-2′-phosphate (**5**), and 5′-ferrocene-amido-5′-deoxyadenosine (**6**)

Compound	i_a/i_c	$E_{1/2}$ (mV)	ΔE (mV)	D_0 ($cm^2\ s^{-1}$)
FcCO-OH	1.06	299 ± 10	66	4×10^{-6}
4	1.07	410 ± 10	66	4×10^{-7}
5	1.10	421 ± 10	69	1×10^{-7}
6	1.06	412 ± 5	70	2×10^{-7}

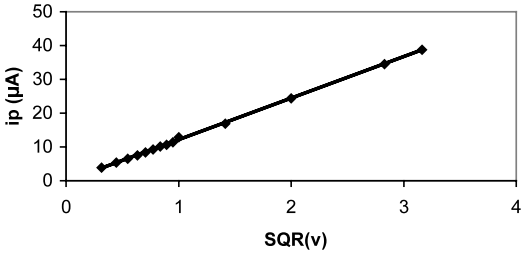


Fig. 2. Variation of peak current in case of 5-ferrocene-glucosamide **4** versus $v^{1/2}$.

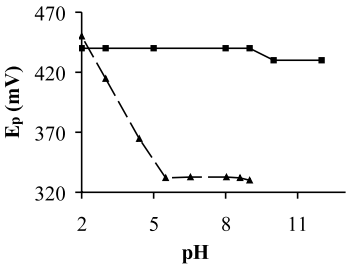


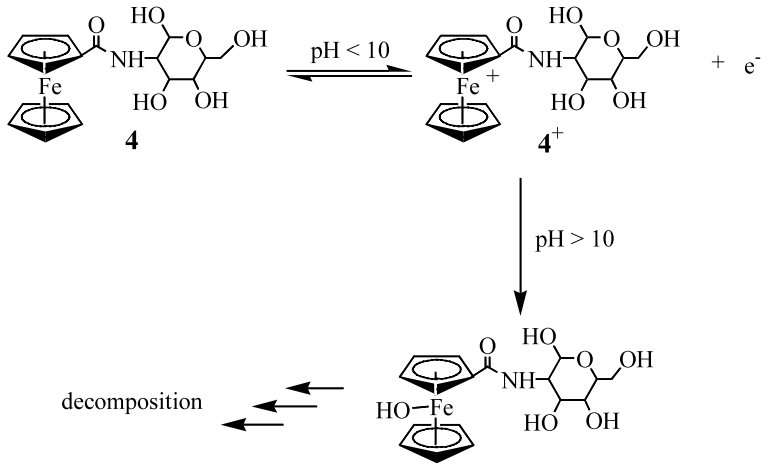
Fig. 3. Variation of the oxidation peak potentials associated with the oxidation of the ferrocene group in ferrocene carboxylic acid (▲) and 5-ferrocene-glucosamide **4** (■) with pH in aqueous solution at 23 °C.

The redox properties as a function of pH have been studied for ferrocene carboxylic (FcCO-OH), ferrocene acetic and ferrocene propionic acids by Fabbrizzi and co-workers in a MeCN–water mixtures. It was shown that the redox potential of the free acid FcCO-OH and the carboxylate $FcCO\text{-}O^-$ have significantly different E^0 values (FcCO-OH: $E^0 = 528$ mV; $FcCO\text{-}O^-$: $E^0 = 337$ mV all vs. *SCE*) [15]. The effects were much smaller for ferrocene acetic and ferrocene propionic acids, in which the charge is separated from the electrophore by methylene groups. Thus for 5-ferrocene-glucosamide (**4**), we would expect a small pH influence on redox properties due to potential deprotonation of the hydroxyl groups. Fig. 3 shows the variation of the oxidative peak potential with pH for FcCO-OH and compound **4** in aqueous solution.

At $pH < 6$ FcCO-OH remains protonated and its oxidation peak potential varies linearly with pH. When the solution pH is lower than 2, the ferrocene monocarboxylic acid precipitates. At $pH > 6$, the equilibrium between FcCO-OH and $FcCO\text{-}O^-$ is shifted toward the carboxylate and oxidation peak potential shifts to 333 mV (vs. Ag/AgCl). Throughout the titration, the redox behavior remained fully reversible. This behavior compares well with that reported by Fabbrizzi and co-workers [15]. In contrast to this, the 5-ferrocene-glucosamide (**4**), exhibits only a small variation (10 mV) in its oxidation peak potential over the entire pH range. Below pH 10, the oxidation wave was fully reversible and can be described by Scheme 2.

At $pH < 10$ the cation $\mathbf{4}^+$ is stable and no reaction with any species in the medium were observed. However, at $pH > 10$, the oxidation wave became irreversible at scan rates below 1 $V\ s^{-1}$. Above this scan rate, some reversibility was observed. In basic medium ($pH > 10$), the cation $\mathbf{4}^+$ reacts with the nucleophile species in the medium, most probably the OH^- ion (Fig. 4).

This is in line with the report by Prins et al. of the instability of the ferrocenium ion in nucleophilic solvents, such as DMSO, DMF and aqueous solutions at



Scheme 2. Oxidation of 5-ferrocene-glucosamide (**4**) in aqueous solution. At pH < 10, the redox reaction is fully reversible and at pH > 10, the reaction becomes irreversible.

high pH [16]. The nucleophile most likely attacks the Fe(III) center, followed by expulsion of the Cp rings. After acidification of the solution to a pH < 10, the reaction was again fully reversible.

3. Summary

Three ferrocene amido monosaccharides were prepared in aqueous solution at pH 9 using FcCO-OBt and aminosaccharide derivatives. The products are air-stable and water-soluble products, which exhibit a reversible one-electron oxidation. The FcCO redox potential is slighty influenced by the saccharide derivative. 5-Ferrocene-glucosamide exhibits a small dependence of its oxidation peak potential on pH.

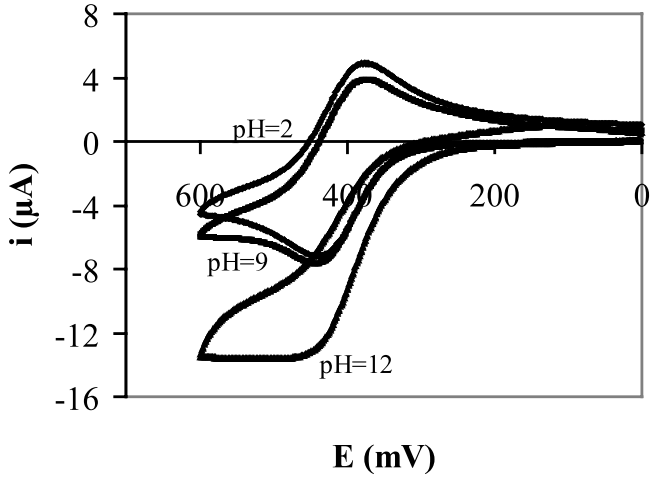


Fig. 4. 5-Ferrocene-glucosamide **4** in aqueous solution (20 mM H_3BO_3) at various pH. Please note the full reversibility of the oxidation at pH 2 and 9. At pH 12, the oxidation becomes irreversible. Scan rate: 100 mV s^{-1}, gold working electrode, Pt counter electrode, reference: Ag/AgCl.

Importantly, in basic solution, the oxidation becomes irreversible, most likely due to the reaction of the hydroxyl anion with the ferrocenium cation, followed by the decomposition of the complex.

4. Experimental

4.1. General procedure

D(+)-glucosamine hydrochloride, D(+)-glucosamine 6-phosphate and 5-amino-5′-adenosine *p*-toluenesulfonate salt, H_3BO_3, NaOH, KOH and KBr were purchased from commercial sources and used as received (SIGMA). FcCO-OBt was prepared according to the published procedure [17]. For column chromatography, a column with a width of 2.7 cm (ID) and a length of 45 cm was packed 18–22 cm high with 230–400 mesh silica gel (VWR). NMR spectra were recorded on a Bruker AMX-300 spectrometer operating at 300.135 MHz (^{1}H) and 75.478 MHz (^{13}C{^{1}H}). Peak positions in both ^{1}H and ^{13}C spectra are reported in ppm relative to TMS. Infrared spectra were obtained with a Perkin–Elmer model 1605 FT–IR. FcCO-OBt was prepared as described before using EDC instead of DCC. The pH was measured using a Fisher Accumet® pH meter Model 620 equipped with a glass electrode. MS spectra were obtained using a VG Analytical 70-VSE mass spectrometer. UV–vis data were collected on a CARY 100SCAN spectrometer using quartz cuvettes with a 1 cm pathlength.

4.2. General synthesis of 5-ferroceneamido-D-glucose (4)

D(+)-glucosamine hydrochloride (82 mg, 0.38 mmol) was dissolved in 5 ml borate buffer (20 mM; pH 9) and added to a solution of FcCO-OBt (187 mg, 0.57

mmol) in THF (5 ml). The pH was maintained at pH 9 by the addition of a 0.1 M solution of KOH. The reaction mixture was stirred overnight and then evaporated to dryness. The crude product was purified by flash chromatography on silica gel (EtOAc–MeOH: 80/20) and a band with the $R_f = 0.6$ was collected to give orange compound **4** in 80% yield (116 mg). MS (FAB^+): Calc. for $C_{17}H_{21}FeNO_6$: 391. Found: 414 [$(M + Na)^+$], 391 $[M]^+$, 213 ($M^+ - 178$, FcCO). HRMS (FAB^+): Calc. 391.1970. Found: 391.2236. FT–IR (KBr, in cm^{-1}): 1640 (C=O amide I), 1536 (C=O amide II). ^{1}H-NMR (δ in ppm, H_2O): 5.19 (d, 3.0 Hz), 4.43 (2H, *ortho*-H), 4.20 (2H, *meta*-H), 4.18 (5H, unsubstituted Cp), 4.01–3.91 ($C_6H_{12}O_6$), 3.83–3.65 ($C_6H_{12}O_6$), 3.43–3.36 ($C_6H_{12}O_6$). ^{13}C-NMR (δ in ppm, H_2O): 177.1 (FcCO-CO), 97.5, 93.6, 78.5, 76.2 (substituted Cp), 75.8 (substituted Cp), 74.2, 72.8 (overlapping 5 C of unsubstituted Cp and glucosamime), 71.2 (substituted Cp), 63.2 (br), 59.2, 56.7. vis (H_2O, λ in nm (ε in l mol^{-1} cm^{-1}): 441 (253).

4.3. Synthesis of 5-ferrocene-glucosamide phosphate (5)

The procedure is identical to that described for compound **4**. Amounts used: glucosamine phosphate (39 mg, 0.15 mmol), FcCO-OBt (110 mg, 0.32 mmol). The purification of **5** was achieved by passing the reaction mixture over a silica pad (5 cm), washing with EtOAc, followed by elution of the reaction product with MeOH. Yield: 70% (50 mg). EIMS (+ve ion mode). Accurate mass: Calc. for $C_{17}H_{22}FeNO_9P$: 471.1807. Found: 472 $[M]^+$. FT–IR (KBr, in cm^{-1}): 1660 (C=O amide I band), 1556 (C=O amide II). vis (H_2O, λ in nm (ε in l mol^{-1} cm^{-1}): 434 (205). ^{31}P-NMR (δ in ppm, H_2O): 6.8 (s).

4.4. Synthesis of 5-ferroceneamido-adenosine (6)

The procedure is identical to that described for compounds **4**. Amounts used: 5-amino-adenosine *p*-toluenesulfonate (20 mg, 0.045 mmole), FcCO-OBt (37 mg, 0.11 mmol). The product was very polar and thus the purification of **6** was achieved by passing the reaction mixture over a short silica pad (5 cm). By-products were washed away with THF and the product was eluted using a mixture of THF–water (1:1). After evaporation, the product was obtained as a red solid. Yield: 80% (17 mg). MS (FAB^+): m/z Calc. for $C_{21}H_{22}FeN_6O_4$: 478. Found: 478 $[M]^+$, 371 ($[M - 107]^+$, fragmentation of adenine). HRMS (FAB): m/z Calc. for $C_{21}H_{22}FeN_6O_4$: 478.297. Found: 478.105 $[M + 1]^+$. FT–IR (KBr, in cm^{-1}): 1640 (C=O amide I band), 1536 (C=O amide II). ^{1}H-NMR (δ in ppm, H_2O): 8.25 (m, 1H, CH aromatic), 7.39 (m, NH amide), 6.74 (br s, 2H, NH_2 of adenine), 5.97 (d, $J = 6$ Hz, CH aromatic), 4.35 (m, 1H, diastereotopic H of HN–CH_2), 4.26 (m, 1H, diastereotopic H of HN–CH_2), 4.17 (s, 5H, Cp unsubstituted), 4.35 (2H, *m*-H substituted Cp), 4.83 (s, 1H, *o*-H, substituted Cp), 4.87 (s, 1H, *o*-H, substituted Cp), 4.04 (m, 1H), 3.91 (m, 2H), 3.53 (m, 1H). vis (MeOH, λ in nm (ε in l mol^{-1} cm^{-1}): 433 (103).

4.5. Electrochemical studies

The electrochemical experiments were carried out using a CV-50W Voltammetric Analyzer (BAS) at room temperature (r.t.) (22 ± 2 °C). No special precautions were taken to exclude oxygen. The CV was performed with a three-electrode configuration. A glassy carbon (BAS, diameter 3 mm) or gold-disk (BAS, diameter 1.5 mm) working electrode and a platinum wire counter electrode were used. The reference electrode was a Ag/AgCl electrode (BAS). IR compensation was applied. Borate buffer (20 mM; pH 9) was used as supporting electrolyte. The pH was adjusted to pH 9 by the addition of NaOH.

Acknowledgements

This work was supported by NSERC by grants to H.-B. K. and J.S.L.

References

[1] (a) G. Tao, E. Katz, I. Willner, Chem. Commun. (1997) 2073;
(b) S. Marx-Tibbon, E. Katz, I. Willner, J. Am. Chem. Soc. 117 (1995) 9925;
(c) Y. Degani, A. Heller, J. Phys. Chem. 91 (1987) 1285;
(d) Y. Degani, A. Heller, J. Am. Chem. Soc. 110 (1988) 2615;
(e) V. Tegoulia, B.B. Gnedenko, A.D. Ryabov, Biochem. Mol. Biol. Int. 31 (1993) 769;
(f) A.E. Cass, G. Davis, G.D. Francis, H.A. Hill, W.J. Aston, I.J. Higgins, E.V. Plotkin, L.D. Scott, A.P. Turner, Anal. Chem. 56 (1984) 667;
(g) J.E. Frew, H.A. Hill, Philos. Trans. R. Soc. Lond. B Biol. Sci. 316 (1987) 95.
[2] (a) T. Itoh, S. Shirakami, N. Ishida, Y. Nakao, T. Yoshida, Chem. Lett. (1998) 979;
(b) T. Itoh, S. Shirakami, N. Ishida, Y. Yamashita, T. Yoshida, H.-S. Kim, Y. Wataya, Bioorg. Med. Chem. Lett. 10 (2000) 1677.
[3] R.C. Mucic, M.K. Herrlein, C.C. Mirkin, R.L. Letsinger, Chem. Commun. (1996) 555.
[4] H. Tamura, M. Miwa, Chem. Lett. (1977) 1177.
[5] B. Donnio, J.-M. Seddon, R. Deschenaux, Organometallics 19 (2000) 3077.
[6] (a) P. Saweczko, H.-B. Kraatz, Coord. Chem. Rev. 190–192 (1999) 185–198;
(b) P. Saweczko, G.D. Enright, H.-B. Kraatz, Inorg. Chem. 40 (2001) 4409.
[7] C.J. Pouchet, The Aldrich Library of NMR Spectra, vol. 2, second ed. Aldrich Chemical Company Inc, Milwaukee, WI, 1983.
[8] D.P. Smith, E. Kohen, M.F. Maestre, R.H. Fish, Inorg. Chem. 32 (1993) 4119.

[9] (a) A. Hess, O. Brosch, T. Wyehermuller, N. Metzler-Nolte, J. Organomet. Chem. 589 (1999) 75;
(b) A.D. Ryabov, V.N. Goral, E.V. Ivanova, M.D. Reshetova, A. Hradsky, B. Bildstein, J. Organomet. Chem. 589 (1999) 85.
[10] (a) H.-B. Kraatz, D.M. Leek, A. Houmam, G.D. Enright, J. Lusztyk, D.D.M. Wayner, J. Organomet. Chem. 589 (1999) 38;
(b) M.V. Baker, H.-B. Kraatz, J.W. Quail, New J. Chem. 25 (2001) 427.
[11] (a) L. Lin, A. Berces, H.-B. Kraatz, J. Organomet. Chem. 556 (1998) 11;
(b) J.C. Green, Struct. Bond. 43 (1981) 37.
[12] A.J. Bard, L.R. Faulkner, Electrochemical Methods. Fundamentals and Applications, 2nd ed., Wiley, New York, 2001 Chapter 6.
[13] D.M. Shah, K.M. Davies, A. Hussam, Langmuir 13 (1997) 4729.
[14] S.R. Jacob, Q. Hong, B.A. Coles, R.G. Compton, J. Phys. Chem. B 103 (1999) 2963.
[15] G. De Santis, L. Fabbrizzi, M. Lichelli, P. Pallavicini, Inorg. Chim. Acta 225 (1994) 239.
[16] R. Prins, A.R. Korswagen, A.G.T.G. Kortbeek, J. Organomet. Chem. 39 (1972) 335.
[17] H.-B. Kraatz, J. Lusztyk, G.D. Enright, Inorg. Chem. 36 (1997) 2400.