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The use of 3,3-bis(2-imidazolyl) propionic acid (bip-OH) as a new chelating ligand for $Re(CO)_3$ and Ru complexes: Formation of organometallic PNA oligomers with (bip)$Re(CO)_3$ and their interaction with complementary DNA

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Abstract

We report the use of 3,3-bis(2-imidazolyl) propionic acid (bip-OH, **1**) as a new chelating bis(imidazole) ligand. The synthesis and full characterization of complexes Re(bip-O)$(CO)_3$ **2** and $[Ru(bpy)_2(bip\text{-}OH)]^{2+}$ **3** is reported. Both complexes show interesting spectroscopic properties, namely IR for compound **2** and ^1H NMR for **3**, respectively. The free carboxylic acid functionality of **1** may be used for the coupling to biomolecules. We have prepared two peptide nucleic acid (PNA) decamers to which the rhenium complex **2** is coupled. All reactions were carried out by solid phase synthesis methods. The Re–PNA oligomer conjugates $Re(CO)_3$-(bip– tgt cta gca a $–NH_2$) **4** and $Re(CO)_3$(bip– agg agc aac t-Lys–NH_2) **5** were obtained in good yield and high purity after HPLC purification and identified by their mass spectra. The interaction of **5** with complementary DNA yields a melting temperature of (53.9 ± 1) °C. This is the first DNA melting temperature reported for an organometallic metal–PNA conjugate.

Keywords: Bio-organometallic chemistry; Peptide nucleic acid; Rhenium compounds; Ruthenium compounds

1. Introduction

Bioorganometallic chemistry is a nascent field of research combining organometallic compounds and reactivity with aspects from biological, pharmaceutical and medicinal chemistry [1–4]. One prominent use has been the development of new immunoassays which do not rely on radioactive or fluorescent detection. Instead, the metal itself may be used for quantitative detection (metallo immuno assay, MIA) [5,6]. Alternatively, the strong C–O stretching vibrations in metal carbonyl compounds are used for selective detection (carbonyl metallo immuno assay, CMIA) [7–9]. The system has been implemented in clinical use for the simultaneous detection of several different anti-epileptic drugs directly from the blood of patients. Sequence-selective detection of RNA and DNA is another important area of analytical chemistry. Bioorganometallic systems have been devised for this purpose in which ferrocene serves as an electrochemical sensor molecule [10]. Sequence-selective binding is usually achieved through a complementary DNA strand, to which the ferrocene derivative is covalently bonded.

Peptide nucleic acid (PNA) oligomers are a synthetic DNA analogue which has gained a lot of attention because of its favourable properties, such as high stability

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in biological media in addition to strong and sequence-selective binding to RNA and DNA [11–17]. We have reported the first organometallic derivatives of PNA monomers and oligomers, namely chromium tricarbonyl and ferrocene derivatives [18,19]. In addition, Ruthenium tris(bipyridyl) derivatives were prepared and their interaction with complementary DNA was studied [19]. Subsequently, Maiorana et al. [20–22] prepared some interesting Fischer carbene derivatives of PNA monomers as well as dimers by Ugi four-component reactions.

In this paper, we present the application of a new ligand suitable for the preparation of (organometallic) bioconjugates, namely 3,3-bis(2-imidazolyl) propionic acid (bip-OH) **1**. The synthesis of **1** has been reported by Joseph et al. [23]. To the best of our knowledge, no coordination chemistry of **1** has been reported yet. It appeared to us that **1** should be a good ligand for various metal complexes through the imidazole nitrogen atoms. First, the imidazole ring in histidine is an excellent ligand for many transition metals in proteins [24]. Second, our group has recently reported various organometallic transition metal complexes with histidinate as a ligand to Mo, Tc and Re metal fragments [25–28]. These compounds did not only exhibit excellent stability and interesting spectrosocpic properties. They were also amendable for solid phase peptide synthesis protocols as shown recently for a Mo(allyl)(histidinate)$(CO)_3$ bioconjugate with the neuropeptide enkephalin [27]. We now report the synthesis and characterization of $Re(CO)_3$ and $Ru(bpy)_2$ complexes with **1**. In addition, the Re(bip)$(CO)_3$ fragment is also coupled to PNA decamers by solid phase synthesis. The resulting conjugates are unambigously characterized and their interaction with complementary DNA is studied.

2. Experimental

2.1. General procedures

Reagents and solvents were obtained from commercial sources and used without further purification. NMR spectra were determined in deuterated solvents on a Brucker AM 360 spectrometer, ^1H operating at 360 MHz and ^{13}C operating at 95.56 MHz. Chemical shifts in both ^1H and ^{13}C are reported in ppm relative to TMS. Residual proton signals of the deuterated solvents were used as secondary standards. Coupling constants, J, are given in Hz. Elemental analyses were performed on a Foss Heraeus Vario EL Elementar Analysator in C, H, N mode by the departmental service. Infrared spectra were recorded at 20 °C on a Brucker Equinox55 FT-IR spectrometer as KBr discs with a spectral resolution of 2.0 cm^{-1}. Wavenumbers, v, are given in cm^{-1}. Mass spectra were measured on a Mat 8200 instrument in FAB mode (positive ions, glycerol matrix). For fragments containing metals only the isotopomer with highest intensity is given. HPL chromatograms were measured on a customized Varian Prostar instrument on reverse-phase Dynamax Microsorb 60-8 C_{18} columns. A linear gradient composed of A (0.1% TFA in water) and B (0.1% TFA in acetonitrile) was used for analytical and preparative HPLC. Analytical: Time 0 min: 5% B. Time 35 min: 50% B (250×4.6 mm, flow 1 ml min^{-1}). Preparative: Time 0 min: 5% B. Time 45 min: 40% B (250×10.0 mm, flow 8 ml min^{-1}).

2.2. Preparation of metal complexes

[Re(CO)$_3$ bip] (**2**): $Re(CO)_5Cl$ (180 mg, 0.5 mmol) and the (bip-O)-OH ligand **1** (113 mg, 0.55 mmol) were dissolved in dioxane–water (5 mL, 9:1 v/v). The reaction mixture was refluxed overnight. The solvent was reduced to small volume until a white substance precipitated. The precipitate was filtered off and suspended in boiling water for 2 h. The solution was cooled to room temperature and the product was collected by filtration. Complex **2** was obtained as a white powder (150 mg, 58%). MS (FAB): m/z 477 $(M - Cl + H)^+$; ^1H NMR (DMSO): δ 2.75 (d, 2H, $J = 3.8$ Hz, $–CH_2–$), 4.65 (t, 1H, $J = 3.8$ Hz, $–CH–CH_2–$), 7.28 (d, 2H, $J = 1.5$ Hz, CH^{im}), 7.35 (d, 2H, $J = 1.5$ Hz, CH^{im}), 13.12 (s, 2H, –NH–); ^{13}C NMR (DMSO): δ 33.5 ($–CH–CH_2–$), 45.0 ($–CH_2–$), 118.8 (CH^{im}), 131.8 (CH^{im}), 146.6 (C^{im}), 171.8 (COOH), 198.5 (CO); IR (KBr): 2022, 1900, 1863 (all metal CO); Elemental analysis for $C_{12}H_9N_4O_5Re \times H_2O$ Calc. C, 29.21; H, 2.25; N, 11.35. Found: C, 28.93; H, 2.49; N, 11.34%.

[Ru(bpy)$_2$bip-O]$^{2+}$ (**3**): $[Ru(bpy)_2Cl_2]$ [29] (145 mg, 0.30 mmol) and **1** (66 mg, 0.32 mmol) were dissolved in dioxane–water (10 ml, 9:1 v/v). The reaction mixture was refluxed for 20 h and a dark oily phase was formed in botumn of the reaction flask. The upper phase was decanted and the oil was dissolved in water (5 mL). The product was precipitated as PF_6 salt by slowly addition of saturated aqueous NH_4PF_6, collected by filtration and succesively washed with water and diethylether. Complex **3** was obtained as a red powder as **3**$(PF_6)_2$ (190 mg, 70%). MS (FAB): m/z 619 $(\mathbf{3})^+$; ^1H NMR (DMSO): δ 2.86 (dd, 1H, $J = 5.8$ Hz, $J = 17.2$ Hz), 3.09 (dd, 1H, $J = 4.9$ Hz, $J = 17.2$ Hz), 5.26 (t, 1H, $J = 5.4$ Hz, $–CH–CH_2–$), 5.6 (s, 2H, CH^{im}), 7.19 (d, 1H, $J = 1.1$ Hz, CH^{im}), 7.22 (d, 1H, $J = 1.1$ Hz, CH^{im}), 7.4–8.7 (several m, 16H, bpy); ^{13}C NMR (acetone-d_6): δ 35.8 ($–CH–CH_2–$), 44.6 ($–CH_2–$), 121.3, 121.6, 125.4, 125.5, 125.7, 128.2, 128.6, 128.8, 128.8, 128.9, 129.0, 138.4, 138.4, 138.5, 147.6, 147.9, 153.3, 153.5, 153.8, 154.1, 159.2, 159.5, 160.2, 160.2, 175.3 (COOH); Elemental analysis for $C_{29}H_{25}F_{12}N_8O_2P_2Ru \times 0.5(C_2H_5)_2O$: Calc. C, 39.37; H, 3.30; N, 11.84. Found: C, 39.78; H, 3.53; N, 12.32%.

2.3. Synthesis of the PNA oligomer conjugates

A teflon syringe (2.5 mL) with a frit at the bottom was used as reactor for solid phase synthesis and all reactions were carried out on a vibrator at 600 vibrations per minute. HATU (14.4 mg, 38 μmol) was dissolved in DMF (300 μL) and transferred into an eppendorf tube containing the monomer (40 mmol). DIPEA (14 μL, 2.2 equiv) was added and the acid pre-activations were performed by vibration for 2 min (7 min for cytidine based monomer). Activated acids were transferred to the syringe containing the resin (50 mg) and the coupling was allowed to proceed for 40 min under vibration. The resin was washed with DMF and DCM successively. Quantitative coupling was determined by the Kaiser test and cappings were performed by acetic anhydride (5%), DIPEA (6%) in DMF (2 mL). Piperidine (20%) in DMF (3 + 2 min) was used for deprotection. Coupling of metal complex **2** was performed similar to the PNA monomers. To cleave the oligomer from the resin, the resin was washed successively with DCM and treated with 1ml of a mixture of anisol (30%) in TFA for 2 h. PNAs were precipitated by pouring the cleavage product into ice-cold diethyl ether. The precipitate was washed with cold diethyl ether (5×10 ml), centrifuged each time and collected. Crude products of **4** and **5** were purified by semi-preparative reversed-phase HPLC. The molecular mass was determined by MALDI-TOF, $m/z = 3187.1$ (**4**) and 3345.9 (**5**).

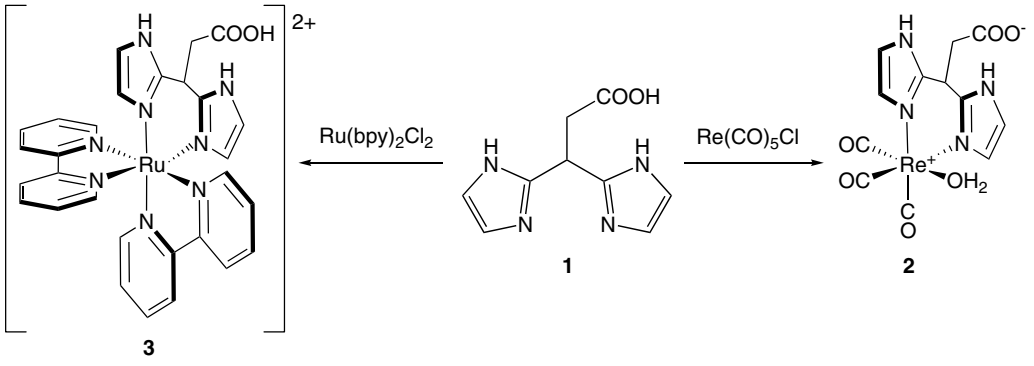
2.4. Melting temperatures

DNA was obtained from IBA, Germany, HPLC purified quality. Concentrations were determined from UV absorptions as described in the literature and were chosen around 1 OD/ml [30,19]. Equimolar amounts of **5** and complementary DNA (3′-TCC TCG TTG A-5′) were mixed in a UV cuvette in phosphate buffer (pH 7, about 0.1 M), which contained 0.1 M NaCl. The absorption was registered at 260 nm. Temperature profile: 5 min. incubation at 90 °C, then 30 min cooling to room temp., 60 min incubation at 4 °C, then from 10 to 90 °C and back to 10 °C in 0.5 °C/min. The melting temperature was determined as the maximum of the first derivative of the absorption vs. temperature curve after 9th order polynomal fitting between 15 and 90 °C.

3. Results and discussion

3.1. Synthesis and characterization of metal complexes

3,3-Bis(2-imidazolyl) propionic acid (**1**, bip-OH) was synthesized by the method of Joseph et al. [23] in our laboratory. All spectral data were in accordance to the described report. The bip-OH ligand reacted with rhenium pentacarbonyl chloride (Scheme 1) in dioxane only in presence of 10% water and $Re(bip\text{-}O)(CO)_3(H_2O)$ **2** was formed in good yield. Elemental analysis and mass spectral data are in agreement with the loss of hydrogen chloride and formation of an intermolecular salt in the mono-aquated complex **2**. The complex showed three CO stretching vibrations in the metal carbonyl region between 2000 and 1800 cm^{-1} in the IR spectrum, typical of Re(I) diimine tricarbonyl complexes with the carbonyls arranged in a facial configuration [31,32]. The ^{13}C NMR spectrum showed only a single signal for the metal carbonyl groups in DMSO solution. This might be due to free rotation of the $Re(CO)_3$ unit in the cationic $Re(bip)(CO)_3$ complex in solution. The ^1H NMR showed slight shifts of doublet and triplet signals belonging to the CH_2 and CH protons of the bip ligand compared to the free ligand **1**. The imidazole CH protons gave two doublets at 7.28 and 7.35 ppm (Fig. 1) due to complex formation and stabilization of one tautomeric form on the imidazole ring nitrogens. The bip-OH ligand **1** also was reacted with $[Ru(bpy)_2Cl_2]$ [29] in similar conditions used for the preparation of complex **2** and the complex $[Ru(bpy)_2(bip\text{-}OH)]^{2+}$ **3** was precipated as PF_6 salt. Mass spectrometry confirmed the proposed cation. ^1H NMR clearly showed an ABX



Scheme 1. Synthesis of compounds **2** and **3** starting from **1**.

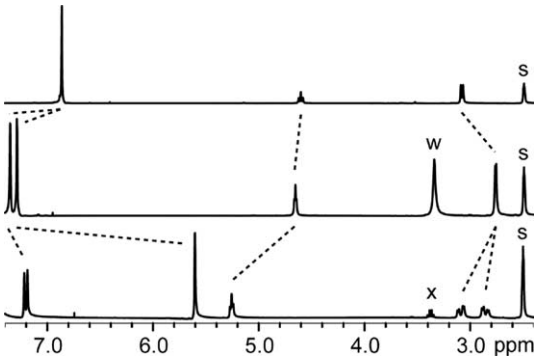


Fig. 1. NMR spectra of **1** (top), **2** (middle), and **3** (bottom). Only the signals from the bip ligand (excluding NH signals) are shown. "s" denotes solvent signals, "w" denotes the water signal and "x" an impurity.

system for CH_2 (AB part, 2.86 and 3.09 ppm) and CH protons (X part, 5.26 ppm) of the bip ligand owing to chirality of the octahedral ruthenium complex. Imidazole CH protons gave two doublets at 7.19 and 7.22, which integrated for two protons only. The other two protons were shifted to higher field by more than 1 ppm and gave a singlet at 5.6 ppm in DMSO. Two signals of intensity 1H each were observed in acetone at similar shift values. Molecular modelling suggests that these protons are shielded by the ring current of a bipyridine ligand.

3.2. PNA oligomer conjugate synthesis

Next, we explored the possibility to use the free carboxylic acid in **2** for the formation of metal bioconjugates. To this end, the rhenium complex **2** was added at the end of a peptide nucleic acid oligomer. A mixed 10-mer sequence (^{C}aac gat ctg t^{N}) was chosen and the PNA oligomer **4** was prepared on solid support utilizing Fmoc strategy (Scheme 2). Tentagel resin with PAL linker was used and the manual syringe method was applied for the oligomer assembly. A HATU/DIPEA mixture was used as coupling reagent. Complete coupling was determined by the Kaiser test. In case of incomplete couplings, the resin was capped with acetic anhydride. The rhenium complex **2** was attached to *N*-terminus of the PNA oligomer on the resin using the same procedure as applied for the coupling of PNA monomers. No major decomposition of the complex or metal detachment was detected after 2 h of deprotection and cleavage from the resin using anisole–TFA (3:7 v/v) as cleavage coctail. The PAL linker yields the *C*-terminal carboxamide. The crude metal–conjugated PNA **4** was purified by RP-HPLC and the pure substance was obtained in moderate yield (25% based on the amount of resin). The molecular mass of the Re-conjugated

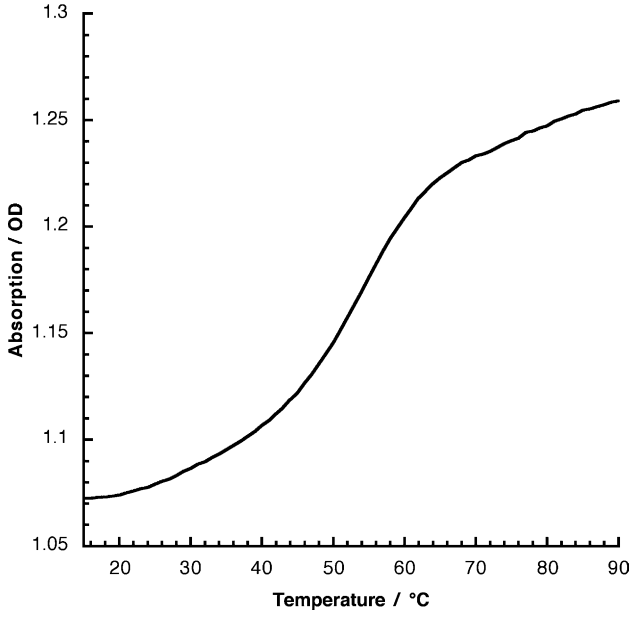
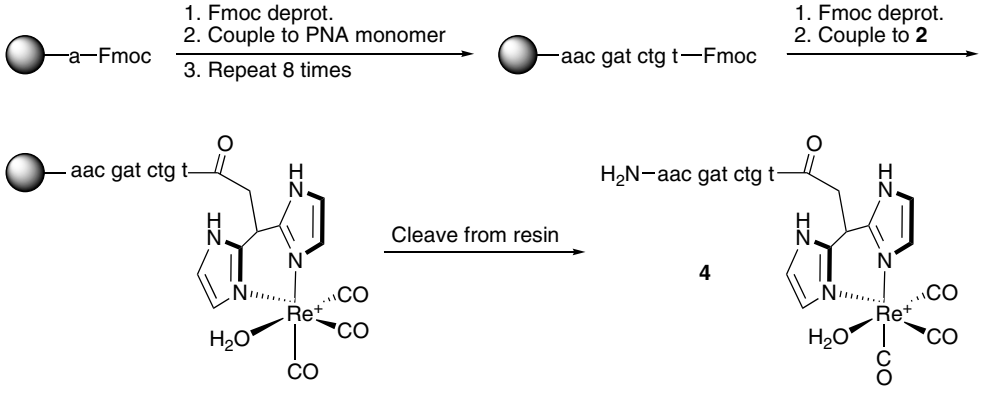


Fig. 2. UV melting curve of **5** · DNA. The melting temperature is (53.9 ± 1) °C. See text and Section 2 for details.



Scheme 2. Synthesis of metal–PNA oligomer conjugate **4** by solid phase synthesis. See Section 2 for synthesis details. Small letters denote PNA monomer units, the same nomenclature as for DNA bases is employed.

PNA oligomer **4** was confirmed by MALDI-TOF. A strong signal was observed at $m/z = 3187.1$, which is the correct mass for the monocation $(\mathbf{4} + H)^+$. A second metal–PNA conjugate with different sequence and a *C*-terminal lysine (H_2N–Lys-tca acg agg a –(bip)$Re(CO)_3$, **5**) was also prepared, purified by preparative HPLC and satisfactorily characterized by its MALDI-TOF mass spectrum. The interaction of **5** with a complementary DNA decamer was studied by temperature-dependent UV spectroscopy. A well-defined sigmoidal curve was obtained on both melting and annealing (Fig. 2). The melting temperature (UV–T_m) was determined as the maximum of the first derivative of the melting curve to be (53.9 ± 1) °C.

4. Conclusions

In this work, we have shown that the bip-OH ligand **1** coordinates with rhenium and ruthenium in good yields and forms stable chelates. We report an organometallic Re complex **2**, as well as a $Ru(bpy)_2$ derivative **3** with a chiral metal centre. The carboxylic acid arm on the bip ligand offers the opportunity to conjugate the ligand **1** or metal complexes thereof to biomolecules such as PNA via a peptide bond. The PNA–rhenium conjugates **4** and **5** are stable in chemical solid phase synthesis and purification and can be obtained in good yield. Their properties can be compared to the first metal–PNA oligomer conjugate prepared in our group [19]. Indeed, the $Re(CO)_3$ core is frequently introduced as an organometallic marker because it serves as a non-radioactive surrogate for the $^{99m}Tc(CO)_3$ radiolabel, which has become very popular recently [33–35,28]. The melting temperature for **5** · DNA was determined to be (53.9 ± 1) °C. This is the first PNA · DNA melting temperature reported for a metal–PNA conjugate.

Metal–PNA conjugates are of interest for the detection of complementary DNA or RNA due to the excellent hybridization properties of PNA, coupled to the methods for detection which are peculiar to the organometallic complexes. Cais et al. [5,6] suggested the use of metal AAS for metal–estrogen conjugates. More recently, IR spectroscopy was introduced as an alternative detection method in immunoassays (carbonyl metallo immuno assay) [7,36,9,8]. Complexes like **2** seem ideally suited to extend the idea of metal carbonyl detection by IR spectroscopy to PNA [20,21]. This may also be possible in an array format as demonstrated for DNA chips [37]. Wang et al. [38–40] have established PNA biosensors, some of which use freely diffusing metal complexes to enhance electrochemical detection. In contrast, the metal complex is covalently linked to the PNA oligomer in conjugates like **4** and **5**. We are currently exploring the potential of conjugates such as **4** and **5** in biological applications.

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References

[1] A.D. Ryabov, Angew. Chem. 103 (1991) 945; Angew. Chem., Int. Ed. Engl. 30 (1991) 931.
[2] G. Jaouen, A. Vessières, I.S. Butler, Acc. Chem. Res. 26 (1993) 361.
[3] K. Severin, R. Bergs, W. Beck, Angew. Chem. 110 (1998) 1722; Angew. Chem., Int. Ed. Engl. 37 (1998) 1634.
[4] R.H. Fish, G. Jaouen, Organometallics 22 (2003) 2166.
[5] M. Cais, S. Dani, Y. Eden, O. Gandolfi, M. Horn, E.E. Isaacs, Y. Josephy, Y. Saar, E. Slovin, L. Snarsky, Nature 270 (1977) 535.
[6] M. Cais, E. Slovin, L. Snarsky, J. Organomet. Chem. 160 (1978) 223.
[7] M. Salmain, A. Vessières, P. Brossier, I.S. Butler, G. Jaouen, J. Immunol. Meth. 148 (1992) 65.
[8] A. Vessières, M. Salmain, P. Brossier, G. Jaouen, J. Pharm. Biomed. Anal. 21 (1999) 625.
[9] M. Salmain, A. Vessières, A. Varenne, P. Brossier, G. Jaouen, J. Organomet. Chem. 589 (1999) 92.
[10] T.S. Zatsepin, S.Y. Andreev, T. Hianik, T.S. Oretskaya, Russ. Chem. Rev. 72 (2003) 537.
[11] P.E. Nielsen, M. Egholm, R.H. Berg, O. Buchardt, Science 254 (1991) 1497.
[12] M. Egholm, O. Buchardt, L. Christensen, C. Behrens, S.M. Freier, D.A. Driver, R.H. Berg, S.K. Kim, B. Norden, P.E. Nielsen, Nature 365 (1993) 566.
[13] K.L. Dueholm, M. Engholm, C. Behrens, L. Christensen, H.F. Hansen, T. Vulpius, K.H. Petersen, R.H. Berg, P.E. Nielsen, O. Buchardt, J. Org. Chem. 59 (1994) 5767.
[14] P.E. Nielsen, G. Haaima, Chem. Soc. Rev. 96 (1997) 73.
[15] H.J. Larsen, T. Bentin, P.E. Nielsen, Biochim. Biophys. Acta 1489 (1999) 159.
[16] P.E. Nielsen, Acc. Chem. Res. 32 (1999) 624.
[17] E. Uhlmann, A. Peyman, G. Breipohl, D.W. Will, Angew. Chem. 110 (1998) 2954; Angew. Chem., Int. Ed. Engl. 37 (1998) 2796.
[18] A. Hess, N. Metzler-Nolte Soc., J. Chem. Soc., Chem. Commun. (1999) 885.
[19] J.C. Verheijen, G.A. van der Marel, J.H. van Boom, N. Metzler-Nolte, Bioconjugate Chem. 11 (2000) 741.
[20] C. Baldoli, S. Maiorana, E. Licandro, G. Zinzalla, D. Perdicchia, Org. Lett. 4 (2002) 4341.
[21] S. Maiorana, E. Licandro, D. Perdicchia, C. Baldoli, B. Vandoni, C. Giannini, M. Salmain, J. Mol. Catal. A 204–205 (2003) 165.
[22] C. Baldoli, C. Giannini, E. Licandro, S. Maiorana, G. Zinzalla, Synlett (2004) 1044.
[23] M. Joseph, T. Leigh, M.L. Swain, Synthesis (1977) 459.
[24] S.J. Lippard, J.M. Berg, Principles of Bioinorganic Chemistry, University Science Books, Mill Valley, CA, 1994.
[25] D.R. van Staveren, E. Bothe, T. Weyhermüller, N. Metzler-Nolte, J. Chem. Soc., Chem. Commun. (2001) 131.
[26] D.R. van Staveren, E. Bill, E. Bothe, M. Bühl, T. Weyhermüller, N. Metzler-Nolte, Chem. Eur. J. 8 (2002) 1649.
[27] D.R. van Staveren, N. Metzler-Nolte, J. Chem. Soc., Chem. Commun. (2002) 1406.

[28] D.R. van Staveren, S. Mundwiler, U. Hoffmanns, J. Kyoung Pak, B. Spingler, N. Metzler-Nolte, R. Alberto, Org. Biomol. Chem. (2004) 2593.
[29] B.P. Sullivan, D.J. Salmon, T.J. Meyer, Inorg. Chem. 17 (1978) 3334.
[30] A. Holmén, B. Nordén, in: P.E. Nielsen, M. Egholm (Eds.), Peptide Nucleic Acids, Horizon Scientific Press, Wymondham, 1999, p. 87.
[31] G. Sprintschnik, H.W. Sprintschnik, P.P. Kirsch, D.G. Whitten, J. Am. Chem. Soc. 98 (1976) 2337.
[32] D.R. Cary, N.P. Zaitseva, K. Gray, K.E. O'Day, C.B. Darrow, S.M. lane, T.A. Peyser, J.H. Satcher, W.P. van Antwerp, A.J. Nelson, J.G. Reynolds, Inorg. Chem. 41 (2002) 1662.
[33] R. Alberto, R. Schibli, A. Egli, A.P. Schubiger, U. Abram, T.A. Kaden, J. Am. Chem. Soc. 120 (1998) 7987.
[34] R. Alberto, R. Schibli, A.P. Schubiger, U. Abram, H.J. Pietzsch, B. Johannsen, J. Am. Chem. Soc. 121 (1999) 6076.
[35] R. Alberto, R. Schibli, R. Waibel, U. Abram, A.P. Schubiger, Coord. Chem. Rev. 192 (1999) 901.
[36] A. Varenne, S.M. Vessières, S. Durand, P. Brossier, G. Jaouen, Anal. Biochem. 242 (1996) 172.
[37] S. Matysiak, S. Würtz, N.C. Hauser, H. Gausepohl, J.D. Hoheisel, in: P.E. Nielsen, M. Egholm (Eds.), Peptide Nucleic Acids, Horizon Scientific Press, Wymondham, 1999, p. 119.
[38] J. Wang, E. Palecek, P.E. Nielsen, G. Rivas, X.H. Cai, H. Shiraishi, N. Dontha, D.B. Luo, P.A.M. Farias, J. Am. Chem. Soc. 118 (1996) 7667.
[39] J. Wang, Biosens. Bioelectron. 13 (1998) 757.
[40] J. Wang, in: P.E. Nielsen, M. Egholm (Eds.), Peptide Nucleic Acids, Horizon Scientific Press, Wymondham, 1999, p. 155.