Transition metal labels on peptide nucleic acid (PNA) monomers

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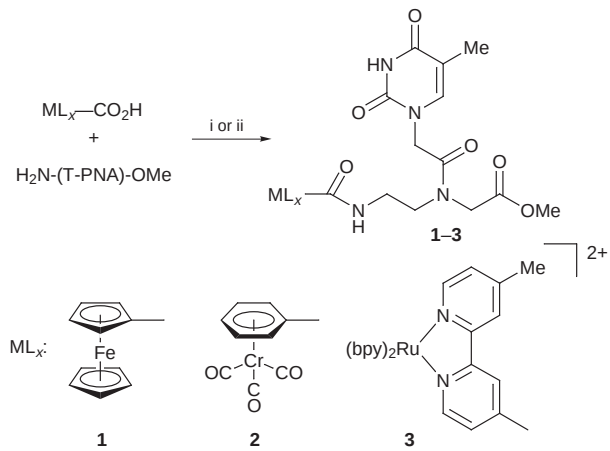
The synthesis, spectroscopic properties (including Mössbauer and electrochemistry) and self-association characteristics of thymine peptide nucleic acid (T–PNA) monomers with covalently linked transition metal markers are described.

Peptide nucleic acids (PNA) constitute a relatively young class of DNA analogues[1] for studies in structural biology[2–5] and applications in antisense-antigene technology.[6] PNA consists of a pseudopeptide backbone, to which the nucleobases are attached through a methylene-carbonyl spacer.[7,8] Among the favourable properties of PNA in the form introduced by Nielsen and Burchardt are high binding affinity to single- and double-stranded DNA with the formation of duplex and triplex helices and an increased hydrolytic stability compared to analogous DNA strands. For analytical purposes in molecular biology, however, PNA lacks a unique spectroscopic handle. Consequently, it would be highly desirable to have PNA attached to an independent and sensitive spectroscopic probe. To this end we have synthesized two organometallic PNA hybrids, namely ferrocene–PNA for electrochemical detection and (benzene)-chromiumtricarbonyl–PNA in which the carbonyl groups provide a sensitive probe for IR detection. Also, we present here the first synthesis and spectroscopic properties of a bipyridyl–ruthenium PNA derivative. Ruthenium derivatives of oligonucleotides have become invaluable tools for the study of electron transfer in DNA.

Ferrocene carboxylic acid thymine-PNA methyl ester (Fc–(T–PNA)–OMe, **1**) was originally prepared by the reaction of ferrocene carboxylic acid chloride, prepared *in situ* from ferrocene carboxylic acid and oxalylic chloride in CH_2Cl_2 solution, with thymine–PNA methyl ester H_2N–(T–PNA)–OMe (Scheme 1). For the synthesis of benzoic acid chromium-tricarbonyl thymine–PNA methyl ester (Cr–(T–PNA)–OMe, **2**), a HBTU (*O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate) mediated coupling between benzoic acid chromium tricarbonyl and H_2N–(T–PNA)–OMe was used.† After work-up, both compounds are obtained in 80 and 60% yield, respectively, as analytically pure yellow solids. Deeply orange–red coloured bis(bipyridyl) (4-methyl-4′-carboxylic acid bipyridyl) ruthenium(II) thymine–PNA methyl ester (Ru–(T–PNA)–OMe, **3**) was obtained by reaction of HBTU-activated $[(bpy)_2Ru(4\text{-Me-bpy-}4'\text{-}CO_2H)]^{2+}$ with H_2N–(T–PNA)–OMe and purified by chromatography on silica.

All metal–PNA hybrids exhibit characteristic NMR spectra which are essentially a combination of their components.‡ As an indication of a successful coupling, however, the signal of the newly introduced amide proton can be observed in aprotic solvents. There is a distinct solvent dependence of this resonance [for **1**: δ 7.24 in $CDCl_3$, δ 7.9 in $(CD_3)_2SO$] which is attributed to the formation of hydrogen bonds with the $(CD_3)_2SO$ solvent. On the other hand, the ^{15}N NMR resonance of the attached nitrogen atom, which can be detected by 2D indirect detection techniques [**1**: δ_N −276 in $CDCl_3$, δ_N −274 in $(CD_3)_2SO$ $^1J_{NH}$ = 90 Hz], remains almost unperturbed.[9] The existence of two isomers denoted major (ma) and minor (mi) in PNA monomers[7,10] and oligomers[11] is well documented. These isomers are also observed in **1**–**3** and we have used the good solubility and stability of the ferrocene derivative **1** in different solvents to shed further light on the nature of the two conformers. First, there is a distinct solvent dependence of the ma/mi ratio as shown in the ^1H NMR spectra of **1**. If all intermolecular hydrogen bonds are broken in CD_3OD solvent in favor of PNA–solvent interactions, the ma/mi ratio is 65 : 35. Changing to an aprotic solvent infers a further stabilisation of the ma isomer, possibly due to hydrogen bonding [ma/mi = 71 : 29 in $(CD_3)_2SO$, ma/mi = 79 : 21 in $CDCl_3$). Coalescence of all ma/mi signal pairs was observed by ^1H NMR spectroscopy in $(CD_3)_2SO$ between 40 and 100 °C. From these data a rotation barrier of 75 ± 0.5 kJ mol^{-1} is calculated, consistent with rotation about the tertiary amide bond.[7,10] There is also a distinct concentration dependence of some ^1H NMR signals of **1** in $CDCl_3$ (Fig. 1). Over a concentration range of 0.375–0.0027 mol l^{-1} the NH proton of the pyrimidine ring (a) shifts 1.5 ppm to higher field, consistent with the accepted model of T–T self association.[12,13] However, while the positions of most other signals remain almost constant and the



Scheme 1 Synthesis metal–PNA conjugates. *Reagents and conditions*: i, $(COCl)_2$, CH_2Cl_2 then NEt_3, THF (**1**), (ii) HBTU, MeCN (**2** and **3**, see Experimental).

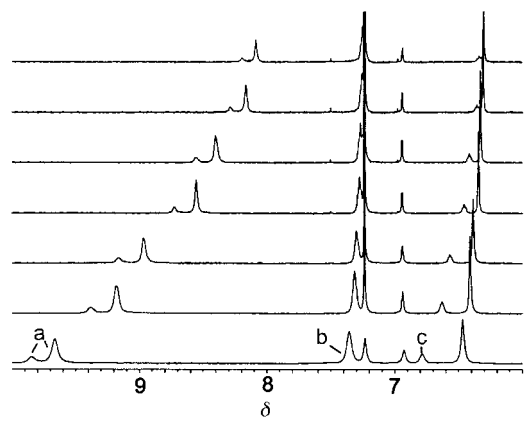


Fig. 1 ^1H NMR spectra of **1** at different concentrations between 375 mmol l^{-1} (bottom trace) and 2.7 mmol l^{-1} (top trace) in $CDCl_3$ at 27 °C. Marked signals are thymine NH(both isomers, a), amide NH (ma, b) and amide NH (mi, c).

FcCON*H* proton of the ma isomer (b) moves < 0.1 ppm, the amide proton signal of the mi isomer (c) shifts also by 0.5 ppm. This suggests that an additional intermolecular interaction, which may be impossible in the ma isomer on steric grounds, might stabilize the mi isomer.§

In the chromium derivative **2**, which is generally less soluble in organic solvents than **1**, the ma/mi ration is 25 : 10 in $(CD_3)_2SO$. In the IR spectrum of **2**, the two strongest bands occur at 1973 and 1898 cm^{-1} and can be assigned to the A and E modes of the $Cr(CO)_3$ moiety. Jaouen and coworkers have used the very strong bands of organometallic carbonyl compounds for the detection of biogenic amines with excellent sensitivity.[14,15] In an elegant application, this carbonyl metallo immuno assays (CMIA), has been applied to study the binding of polyclonal antibodies to cortisol and testosterone.[16]

To confirm the possibility of electrochemical detection of metal–PNA hybrids the ferrocene and ruthenium derivatives **1** and **3** were investigated by cyclic voltammetry. **1** can be reversibly oxidized at a potential of +169 mV *vs.* Fc/Fc^+ (CH_2Cl_2 solution with 0.1 mol l^{-1} NBu_4PF_6) a potential which can be easily achieved in a biological environment without destruction of the biological matrix.[17,18] For **3**, a reversible one-electron oxidation occurs at 897 mV *vs.* Fc/Fc^+, consistent with a Ru^{II}/Ru^{III} redox couple. Ligand centred redox waves were observed at −1.63, −1.90 and −2.15 V. The optical spectrum of **3** exhibits several overlapping bands around 450–430 nm which are assigned to metal-to-ligand charge transfer transitions (MLCT),[19] while two weak bands at 359 and 327 nm result from metal centred transitions and mostly ligand centred π–π* transitions are observed around 290 nm.[20] We have also recorded a ^{57}Fe Mössbauer spectrum for **1**. The isomer shift (0.52 mm s^{-1}) and quadrupole splitting (2.32 mm s^{-1}) are very similar to the value for ferrocene (0.53 mm s^{-1} and 2.37 mm s^{-1}),21 suggesting that there is little influence of the overall solid state structure of **1** on the electric field gradient of the iron nucleus.

We have reported the straightforward synthesis and full characterization of transition metal derivatives of T–PNA monomers and we are confident that this chemistry can be extended to PNA oligomers. As shown in this communication, these new compounds show unique spectroscopic features which in principle permit their sensitive detection even in complex biological matrices such as intact cells. Encouraged by these results, we are further developing the chemistry of metal–PNA hybrids towards applications in structural biology and antisense / antigene technology.

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Notes and references

† *Typical experimental procedure using* HBTU: to a solution of 1 mmol of the transition metal complex in MeCN was added 1 equiv. of HBTU and NEt_3. After stirring for 15 min. (15 h for **3**) brine was added, the solution extracted with EtOAc ($MeNO_2$ for **3**), and the organic phase washed with 2 M HCl, water, aq. $NaHCO_3$, and water, dried over $MgSO_4$ and the solvent removed to yield the desired metal–PNA hybrid.

‡ *Spectroscopic data*: NMR spectra (500 MHz or 400 MHz for ^1H) in $CDCl_3$ (**1**), $(CD_3)SO$ (**2**, **3**), ma/mi where observed, all signals are s except when stated otherwise. **1**: δ_H 8.2/8.08 (1H, NH_T), 7.24/6.32 (br, 1H, NH), 6.8/6.3 (1H, C=CH), 4.76/4.62 (app. t, 2H, Cp), 4.39 (2H, CH_2), 4.39/4.30 (app. t, 2H, Cp), 4.23/4.17 (5H, Cp), 4.04 (2H, CH_2), 3.81/3.78 (3H, OCH_3), 3.54 (m, 4H, CH_2CH_2), 1.88/1.71 (3H, $CH_{3,\,T}$). δ_C 171.3 (CO_2Me), 170.7 (Cp-*C*O), 167.4 (CO), 164.5 (CO_T), 164.4 (CO_T), 151.3 (*C*=CH), 141.0 (C=*C*H), 75.4 ($Cp_{quat.}$), 70.8 (Cp), 68.2 (Cp), 69.8 (5C, Cp), 52.9 (OCH_3), 49.1 (CH_2), 48.3 (CH_2), 48.1, 37.4 (CH_2CH_2), 12.1 ($CH_{3,\,T}$). δ_N −273 (CONH), −269, −253 ($N_{1,\,T}$), −225 ($N_{3,\,T}$). IR (KBr): 1671 br cm^{-1}, UV-VIS [λ/nm (ε/l $mol^{-1}cm^{-1}$] 440 (156), 266 (25028). MS (EI, 70 eV): *m/z* 510 (100%, $M^{+\cdot}$), 445 (25%, M − Cp). **2**: δ_H 11.25 (NH_T), 8.65 / 8.45 (NH), 7.3 / 7.2 (s, 1H, C=CH), 6.2/6.1 (2H), 5.8 (1H), 5.8/5.7 (2H, CH_{Ar}), 4.69/4.48 (CH_2), 4.35/4.1 (CH_2), 3.7/3.6 (OCH_3), 3.4/3.3 (CH_2CH_2), 1.7 ($CH_{3,\,T}$). δ_C 232.7 [$Cr(CO)_3$], 170.0 (CO_2Me), 167.4, 164.3 (both CON), 150.9 (*C*=CH), 142.1 (C=*C*H), 98.0 ($C_{Ar,\,quat}$), 96.3, 94.0, 93.2 (all CH_{Ar}), 51.8 (OCH_3), 47.8, 47.7 (both CH_2), 46.5, 37.0 (CH_2CH_2), 11.9 ($CH_{3,\,T}$). IR (KBr): 1973 vs, 1898 vs, 1648 br cm^{-1}. MS (ESI^+, MeOH): *m/z* 561 (M + Na^+), 425 [M − $Cr(CO)_3$ + Na^+]. **3**: δ_H 11.2 (NH_T), 9.15/8.9 (br, NH), 9.05/8.95 (s), 8.82, 8.76/8.65 (s), 8.16, 7.87, 7.75, 7.72, 7.56, 7.52, 7.40 (all bpy), 7.30/7.2 (C=CH), 4.71/4.48 (2H, CH_2), 4.38/4.13 (2H, CH_2), 3.68/3.61 (3H, OCH_3), 3.58 (4H, CH_2CH_2), 2.53 ($CH_{3,\,bpy}$) 1.69/1.59 (3H, $CH_{3,\,T}$). δ_C 170 (CO_2Me), 169/167.5 (CO), 165 (CO_T), 152 (*C*=CH), 143 (C=*C*H), 120–160 (bpy), 52 (OCH_3), 48.5/49.2 (CH_2), 48.8 (CH_2), 47, 38 (CH_2-CH_2), 21.2 ($CH_{3,\,bpy}$), 12.4 ($CH_{3,\,T}$). UV–VIS [λ/nm, (ε/l $mol^{-1}cm^{-1}$) 455 (14 000), 290 (57 000). MS (ESI^+, Me_2CO): 1053 (M − PF_6^-).

§ Further investigations on the self association and A–T base pairing properties of transition metal PNA compounds are in progress in our group and will be reported comprehensively in due course.

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