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Supporting Information

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Cellular Uptake Quantification of Metallated Peptide and Peptide Nucleic Acid (PNA) Bioconjugates by Atomic Absorption Spectroscopy

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Experimental section

Figure S1

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General remarks. ESI mass spectra were measured on a Finnigan TSQ 700. MALDI-TOF MS were recorded on a Bruker Biflex III spectrometer with a sinapinic acid matrix. RP-HPLC was performed using a Varian Pro Star PDA detector (model 330), a Varian solvent delivery system (model 210) and an analytical C-18 Microsorb (4.6 mm x 250 mm, 60 Å / 8 µm; Dynamax, Varian) or semipreparative C-18 Microsorb (10 mm x 250 mm, 60 Å / 8 µm; Dynamax, Varian) column. Water (A) and acetonitrile (B) were used as solvents, both containing 0.1 % TFA. The flow rate was 1 ml min^{-1} (analytical) or 4 ml min^{-1} (semipreparative) and peaks were detected at 254 mm and 220 nm. UV melting curves were measured on a Varian CARY 100 instrument equipped with a multicell holder and a temperature control unit in 1 cm quarz Suprasil cells at 260 nm. DNA oligomers were obtained from IBA, Göttingen, Germany.

Ligands 1 - 3. The parent bpa ligand **1** is commercially available (Aldrich). Compounds **2** and **3** were prepared according to the published procedure.[1]

Metal complex 2_{Co}. The ligand **2** (86.8 mg, 0.25 mmol) was dissolved in methanol (5 ml) in a beaker, $Co(NO_3)_2 \times 6\ H_2O$ (72.8 mg, 0.25 mmol) was dissolved in methanol (5 ml) in another beaker and both were heated to boiling. The solution containing the ligand was poured into the solution with Co^{2+} ions. The reaction mixture was filtered in a vial (25 ml, which was silanized by shaking for 5 min with bis(trimethylsilyl)amine in order to avoid sticking of the crystals on the walls of the vial) and allowed to cool to room temperature. The vial was placed in a screw-capped container (250 ml) filled with diethyl ether (10 ml) and closed. After standing overnight at room temperature the precipitated product was collected by filtration and dried in air. Yield: 62 mg (47 %) of red crystals of X-ray quality. M_r ($C_{21}H_{21}N_5O_8$) = 530.36; MS (ESI-pos.): 468 $[M - NO_3]^+$.

Oligomers 4 - 6. The synthesis of oligomers **4** - **6** was performed manually in a syringe equipped with a porous filter (5 ml, Multisyntech) using Fmoc solid phase synthesis strategy with a Rink-amide resin (28.2 mg, 20.0 µmol, loading 0.71 mmol/g, Novabiochem), PNA monomers (Applied Biosystems), amino acid monomers (Novabiochem), bpa ligand **3** and amine free DMF (Roth) as solvent. Side chain protecting goups were Boc (Lys), Pbf (Arg) and Bhoc (a, c and g). Synthetic cycle: (A) Fmoc-deprotection using two times about 3 ml of

a 20 % piperidine solution in DMF, first 2 min and then 10 min, (B) DMF wash 5 × with about 3 ml DMF; (C) coupling using 1.05 ml of coupling mixture during 20 min (see below) and (D) DMF wash 5 × with about 3 ml DMF. The coupling mixture contained the corresponding monomer (0.1 mmol, 5 fold excess) and HATU (0.98 mmol, 4.9 fold excess) dissolved in DMF (1 ml), then DIPEA (0.05 ml, 0.3 mmol, 15 fold excess, activation period 1 min) was added. Workup: After the resin was dried (1 h, 10 mbar), final deprotection and cleavage from the resin was performed with a TFA mixture (TFA : H_2O : TIS = 95 : 2.5 : 2.5, 2 ml, 3 h). The suspension was filtered and the resin washed with TFA (1 × 1 ml). The combined TFA solutions were poured into cold ether (10 ml, -30 °C), and the suspension was centrifuged (8000 rpm, 5 min). After decanting the supernatant, the crude product was washed with cold ether (2 × 10 ml), dissolved in water, filtered and purified by semipreparative HPLC (5 % -> 40 % B in 30 min). A detailed synthetic procedure for manual SPPS including the use of metal complexes is available: "*Manual Solid Phase Peptide Synthesis*", S. I. Kirin, F. Noor, N. Metzler-Nolte, W. Mier, *J. Chem. Educ.* **2007**, *84*, 108-111.

bpa-CH_2-(*p*-C_6H_4)-C(O)-Ahx-Pro-Lys-Lys-Lys-Arg-Lys-Val-NH_2, 4. M_r ($C_{66}H_{107}N_{19}O_9$) = 1310.68, MS (ESI-pos.) = 655.7 $[M + 2 H]^{2+}$, 437.26 $[M + 3 H]^{3+}$.

bpa-CH_2-(*p*-C_6H_4)-C(O)-Ahx-tgttatcc-Lys-NH_2, 5. M_r ($C_{118}H_{151}N_{47}O_{30}$) = 2707.76, MS (MALDI) = 2707.1 $[M + H]^+$.

bpa-CH_2-(*p*-C_6H_4)-C(O)-Ahx-tgttatcc-Gly-Gly-Pro-Lys-Lys-Lys-Arg-Lys-Val-NH_2, 6. M_r ($C_{156}H_{221}N_{61}O_{38}$) = 3558.81, MS (MALDI) = 3558.2 $[M + H]^+$.

X-ray Crystallography. Crystal data for $\mathbf{2_{Co}}$: Pink crystals of $\mathbf{2_{Co}}$ were obtained by diffusion of diethylether into an ethanol solution at room temperature. $C_{21}H_{21}CoN_5O_8$, *M* = 530.36 g mol^{-1}, 0.12 x 0.12 x 0.06 mm^3, a = 9.8665(6) Å, b = 14.2680(7) Å, c = 16.7896(8) Å, β = 106.13(1)º, V = 2270.5(2) $Å^3$, Mo-K_α radiation (λ = 0.71073 Å), μ(Mo-K_α) = 6.45 mm^{-1}, ρ = 1.551 gcm^{-3}, monoclinic, space group $P2_1/c$, Z = 4, Siemens-Smart CCD-diffractometer, T = 100(2) K, 11041 reflections measured, 3386 uniq refl. (R_{int} = 0.043), 3212 obs. refl. (Fo > 4 σ(Fo)), $2\Theta_{max}$ = 62.85°, R = 0.0897 (Fo > 4 σ(Fo)), R = 0.0946 (all data), wR = 0.1661 (Fo > 4 σ(Fo)), 311 params., refinement against F^2, ShelXTL 6.14 Bruker AXS program suite. Crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Centre, CCDC 648421.

UV melting curves. DNA stock solutions were used as received from IBA. PNA stock solutions were prepared by dissolving the lyophilisate of the pure HPLC fractions in

phosphate buffer (100 mM, pH = 7.0) containing NaCl (100 mM). The stock concentrations were determined at 260 nm and 25 °C (DNA) or 70 °C (PNA). Extinction coefficients, ε, of the oligomers were calculated according to Lambert-Beer's law using increments obtained from literature. (a) For DNA 13.7 ($\mathbf{e_A}$), 6.6 ($\mathbf{e_C}$), 11.7 ($\mathbf{e_G}$) and 8.6 ($\mathbf{e_T}$) cm^2 μmol^{-1} were taken.[2] ; (b) For PNA 15.3 ($\mathbf{e_a}$), 7.6 ($\mathbf{e_c}$), 12.2 ($\mathbf{e_g}$) and 8.7 ($\mathbf{e_t}$) cm^2 μmol^{-1} were taken.[3] The ε of the free bpa ligand **3** was determined to be ε_{bpa} = 9.2 cm^2 μmol^{-1}. **Procedure**: Equimolar mixtures of the two particular oligonucleotides were mixed to concentrations of 3 μM in phosphate buffer (0.1 M, pH = 7.0) containing NaCl (100 mM). Prior to recording a melting profile, the solution was heated in a water bath to 70 °C for 3 min. in order to completely dehybridize the sample, supersonicated and cooled to room temperature. Then, the mixture was cooled to 5 °C in the instrument at a rate of 1 °C per minute and kept at 5 °C for 15 min. A nitrogen flow was used in order to prevent steaming up of the cuvettes at low temperatures. The mixture was then heated to 80 °C at a rate of 0.5 °C while A_{260} was recorded against temperature. The T_m values were determined as the maxima of the first derivative plots of absorbance *vs* temperature. Each T_m was determined as the average of four melting experiments.

Uptake into HT-29 cells and nuclei. HT-29 human colon carcinoma cells were maintained in Eagle's MEM (Sigma, Germany) supplemented with $NaHCO_3$ (2.2 g/L), sodium pyruvate (110 mg/L), gentamycin (50 mg/L) and 10% (V/V) fetal calf serum according to standard procedures. For uptake studies the cells were grown in 75 cm^2 culture flasks at 37 °C in 5% CO_2 / 95% air atmosphere until at least 70% confluency. The medium was replaced with 10 mL medium containing the freshly prepared complexes in a concentration of 50 μM. Solutions of the metal complexes were prepared by mixing equimolar amounts of compounds **4** – **6** and $Co(NO_3)_2$ in water and diluting them to the desired concentration with MEM. After further incubation for 6 h or 24 h hours the cells were detached by trypsinization, re-suspended in 10 mL phosphate buffered saline pH 7.4 (PBS), pelleted by centrifugation (2000 rpm, 5 min) and washed twice with PBS. For cell uptake studies the isolated pellets were resuspened in 1.0 mL twice distilled water and lysed by means of a sonotrode. The cobalt content and protein content of the lysates was determined according to a reported procedure.[4] The molar cellular concentrations were calculated according to the literature.[5] For quantification of the uptake into the nuclei the isolated pellets were treated as previously described.[6]

Cytotoxicity experiments. Cell growth inhibitory effects were evaluated as previously described.[5]

References

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Figure S1. Melting curves of **5 • 7** and **6 • 7**. UV absorbance spectra were recorded at 260 nm in a 1 cm path lenght at heating rate 0.5 °C / min in phosphate buffer (pH = 7) and at [NaCl] = 0.1 M. Oligomer concentrations were [PNA] = [DNA] = 3 μM. The melting temperature T_m (maximum of the first derivative) is indicated by an arrow.

Figure S1