49. An Efficient Synthesis of Enantiomerically Pure Δ- and Λ-Ruthenium(II)-Labelled Oligonucleotides

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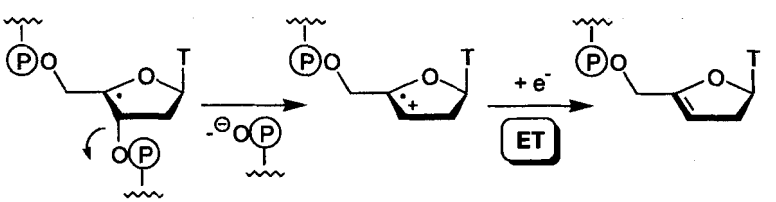
The synthesis of a novel tris(bidentate ligand)ruthenium(II) complex **7** and its efficient tethering to the 5′-end of oligonucleotides is described. The resulting Δ- and Λ-isomeric ruthenium(II)-labelled oligonucleotides **10a–c** and **11a–d** were separated either by reversed-phase HPLC or by polyacrylamide gel electrophoresis. The diastereoisomerically pure isomers were fully characterized by UV/VIS and CD spectroscopy, mass spectrometry, and enzymatic digestion with base analysis. We also investigated the thermal denaturation of the hybridized double strands.

1. Introduction. – Ruthenium(II) complexes covalently linked to synthetic oligonucleotides [1] [2a, b] have been recently described as nonradioactive label molecules for application in DNA probe technology [1] as well as for the investigation of the long-range photoinduced electron transfer (ET) through DNA [2] [3].

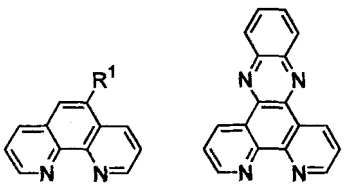
Recently, we have demonstrated that 4′-deoxyribonucleotide radicals can be generated selectively by photolytical cleavage of 4′-acylnucleotides [4a, c]. These radicals undergo heterolytic fragmentation with formation of radical cations, which can act as electron acceptors [4b] yielding enol ethers (*Scheme 1*).

Scheme 1



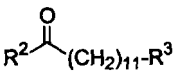
Our interest focused on the trapping of the radical-cation intermediate by ET using a Ru^{II} complex as electron donor. Here, we describe the synthesis of a Ru^{II} complex $[Ru(phen)(phen')(dppz)]^{2+}$ (**7**; phen = 1,10-phenanthroline, dppz = dipyridophenazine), its efficient attachment to oligonucleotides, separation of the isomerically pure adducts, and the formation of the corresponding double strands. The dppz ligand **2** of complex **7** is an excellent duplex DNA intercalator [5a] [6], and the alkyl spacer of ligand phen′ **1e** guarantees a well defined donor-acceptor distance in the Ru^{II}-labelled oligonucleotides.

2. Results and Discussion. – 2.1. *Synthesis of the Ru^{II} Complex* **7**. For the synthesis of the ligand **1e** (phen'), we attached an appropriate 12-hydroxylauric-acid derivative **3c** with 5-amino-1,10-phenanthroline (**1c**) *via* a stable amide bond followed by deacetylation with NH_3 saturated MeOH. The ligand **1c** was obtained from 5-nitro-1,10-phenanthroline (**1b**) [7] by transfer hydrogenation [8] with Pd/C and cyclohexene in EtOH. The synthesized 12-acetoxylauric-acid chloride (**3c**) occurred in two steps starting from 12-hydroxylauric acid (**3a**). Minor amounts of the dimeric side-product **3e** were removed upon *Kugelrohr* distillation in a rotary pump vacuum. Reaction of **1c** and **3c** yielded the AcO-protected phen' derivative **1d** which was deacetylated in NH_3-saturated MeOH. The crude ligand **1e** (phen') was purified by column chromatography over basic aluminium oxide.



1a R^1 = H (**phen**)
1b $R^1 = NO_2$
1c $R^1 = NH_2$
1d $R^1 = NHCO\text{-}(CH_2)_{11}\text{-}OAc$
1e $R^1 = NHCO\text{-}(CH_2)_{11}\text{-}OH$ (**phen'**)

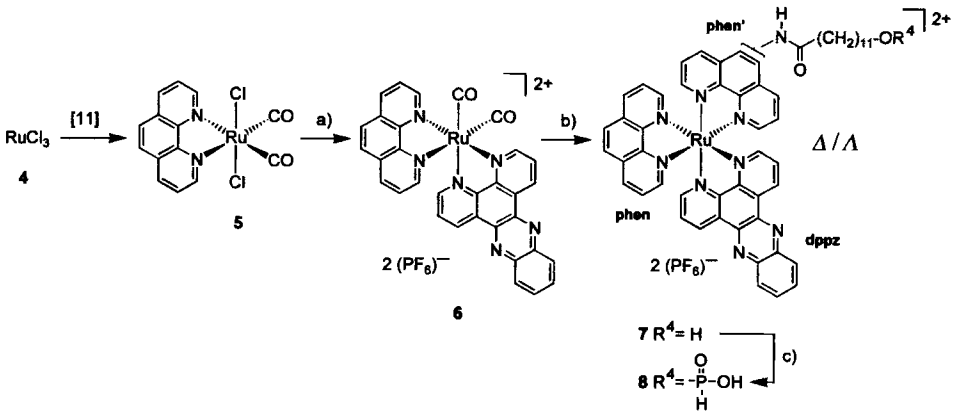
2 (**dppz**)



3a R^2 = OH, R^3 = OH
3b R^2 = OH, R^3 = OAc
3c R^2 = Cl, R^3 = OAc
3d R^2 = OH, $R^3 = OC(O)\text{-}(CH_2)_{11}\text{-}OAc$
3e R^2 = Cl, $R^3 = OC(O)\text{-}(CH_2)_{11}\text{-}OAc$

We performed the synthesis of the Ru^{II} complex **7** according to the method described by *Keene* and coworkers [9] by consecutive addition of the three bidentate ligands. Our synthesis started from the complex $[Ru(phen)(CO)_2Cl_2]$, which was obtained in two steps from $RuCl_3 \cdot xH_2O$ ($x \leq 1$) **4** [10]. For the introduction of the ligand **2**(dppz) [11], complex **5** was transformed into the corresponding triflato complex $[Ru(phen)(CO)_2(CF_3SO_3)_2]$ upon treatment with CF_3SO_3H at 120–130° for 2 h under Ar (*Scheme 2*). After removal of the solvent, refluxing in EtOH in the presence of an excess of the ligand **2** yielded the dication $[Ru(phen)(dppz)(CO)_2]^{2+}$ (**6**) which was isolated as PF_6^- salt upon addition of KPF_6. The introduction of the ligand **2** into the complex **5** led to the formation of a racemic mixture of **6**. The tris(bidentate ligand) complex $[Ru(phen)(phen')(dppz)]^{2+}$ (**7**) was formed by refluxing **6** with an excess of the third ligand **1e** (phen') in the presence of the decarbonylating reagent trimethylamine *N*-oxide. Complex **7** could be purified by column chromatography with $MeCN/H_2O$/sat. aqueous KNO_3 50:3:1 and isolated as the hexafluorophosphate upon treatment with KPF_6. Recrystallization from EtOH/MeCN yielded the desired red-orange target complex $[Ru(phen)(phen')(dppz)](PF_6)_2$ (**7**). The UV/VIS absorption spectrum of **7** showed the characteristic metal-to-ligand (MLCT) absorption band at $\lambda = 447$ nm and the inner ligand (IL, $\pi \rightarrow \pi^*$) transition band at $\lambda = 368$ nm. Corroborating evidence that the product was the desired complex **7** was obtained by fast-atom-bombardment mass spectrometry (FAB-MS) in a 3-nitrobenzyl-alcohol matrix [9a]. Here, the ion pair, $\{[Ru(phen)(phen')(dppz)](PF_6)\}^+$, and fragment ions corresponding to the loss of the second PF_6^- and sequential loss of the remaining ligands could be detected. The parent ion for **7** was not observed.

Scheme 2



a) 1. CF_3SO_3H, 1,2-dichlorobenzene, 120–130°, 2 h; 2. **2**, EtOH, reflux; 3. KPF_6; 48.5% **6**. b) 1. **1e**, Me_3NO, $MeO(CH_2)_2OH$, reflux; 2. KPF_6; 85.3% **7**. c) 1. tri(1*H*-imidazol-1-yl)phosphine, MeCN, Et_3N, 2 h; 2. 0.1M Et_3NHOAc (pH 7); 95% **8**

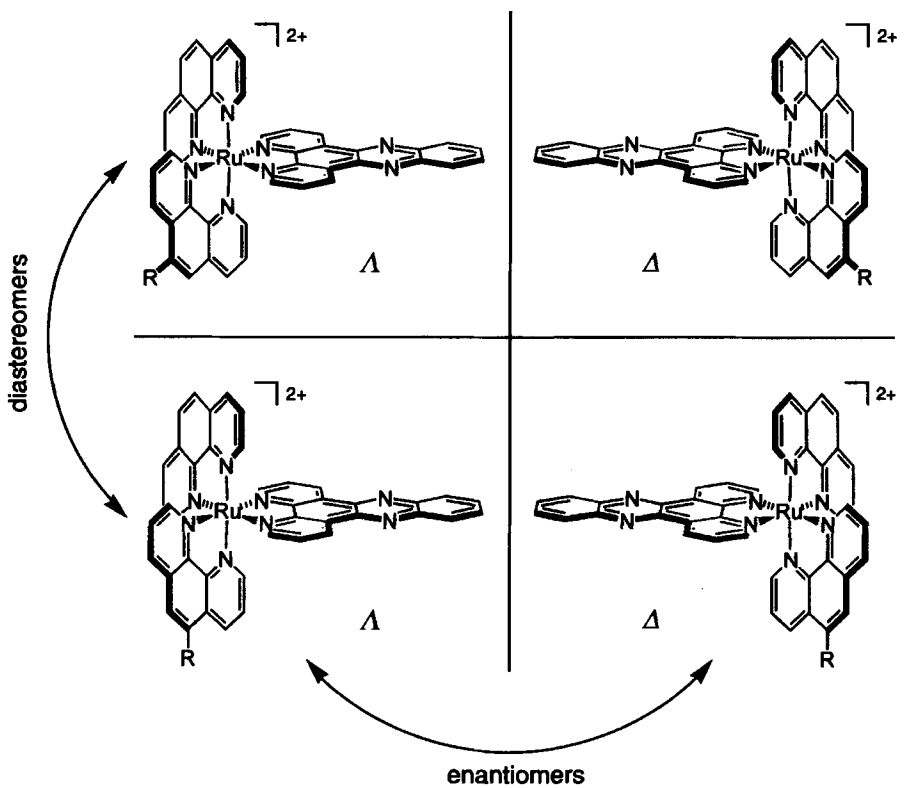


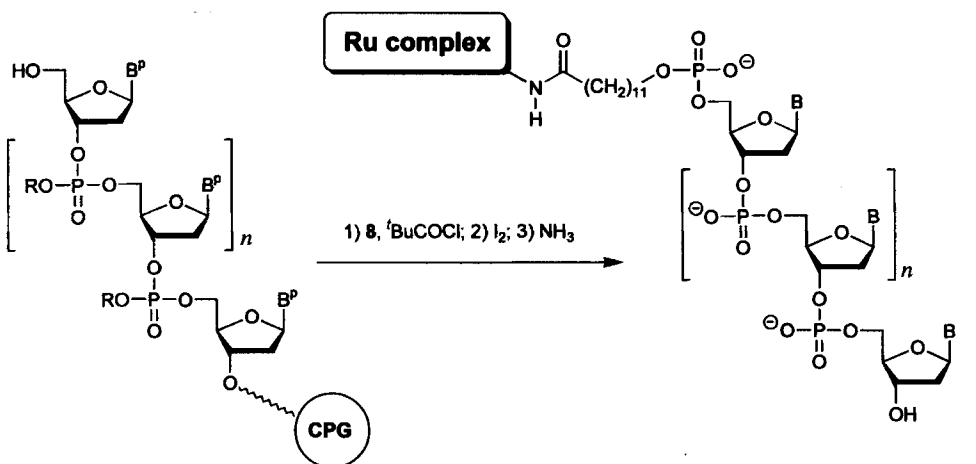
Fig. 1. *Structural representation of the four stereoisomers of [Ru(phen)(phen')(dppz)]*$^{2+}$ (**7**; R = $NHCO-(CH_2)_{11}-OP(O)HOH$)

Reversed-phase HPLC (*Vydac* C_4) of complex **7** showed only a single peak, although it exists as a racemic (Δ/Λ)-mixture of two diastereoisomers, based on the relative orientation of the hydroxyalkyl spacer of the ligand **1e** (*Fig. 1*). For both diastereoisomers two groups of different signals can be observed by ^{1}H-NMR spectroscopy. All four isomers of **7** were used without separation for the tethering reaction to oligonucleotides.

2.2. *Attachment of the Ru^{II} Complex* **7** *to Oligonucleotides*[1]). We coupled the complex $[Ru(phen)(phen')(dppz)]^{2+}$ (**7**) to the 5′-end of oligonucleotides *via* a phosphodiester bridge using the phosphonate approach [12]. For this purpose, the hydroxyalkyl group bearing Ru^{II} complex **7** was transformed into the corresponding phosphonate **8** upon reaction with tri(1*H*-imidazol-1-yl)phosphine according to the method described by *Bannwarth* and *Müller* [1d]. The crude **8** was characterized by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and used directly for the coupling reaction.

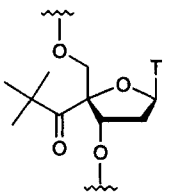
Activation with pivaloyl chloride and reaction with the 5′-OH group of the fully protected and solid-supported oligonucleotides was followed by oxidation with I_2 and deprotection with NH_3 and led to the Ru^{II}-labelled oligonucleotides (*Scheme 3*).

Scheme 3. *Coupling of the Ru^{II} Complex Phosphonate* **8** *to Synthetic Oligonucleotides*



We tethered the Ru^{II} complex **7** to a natural oligonucleotide **12** as well as to an oligonucleotide **13**, which was modified by a 4′-pivaloyl-substituted nucleotide **9** [4c,d] (*Fig. 2*). Complete formation of the Ru^{II}-labelled oligonucleotides **10** and **11** was observed by polyacrylamide gel electrophoresis (PAGE) and analytical reversed-phase HPLC. Both methods were used for isolation and purification of **10** and **11** in which the

[1]) The recently described synthesis of an oligonucleotide covalently linked to a $[Ru(phen)_2(dppz)]^{2+}$-type complex led to the desired adduct in poor yield ($\leq 1\%$), which was only characterized by UV/VIS and atom-absorption spectroscopy [2a,b].



9 (T*)

Ru-(5'-3')-d(A-G-A-G-C-A-C-A-A-C-T-A-G-C-A) **10 (10a-c)**

Ru-(5'-3')-d(A-C-G-G-C-T-T*-T-C-A-G-C-T-C-G) **11 (11a-d)**

(5'-3')-d(A-G-A-G-C-A-C-A-A-C-T-A-G-C-A) **12**

(5'-3')-d(A-C-G-G-C-T-T*-T-C-A-G-C-T-C-G) **13**

(5'-3')-d(T-G-C-T-A-G-T-T-G-T-G-C-T-C-T) **14**

(5'-3')-d(C-G-A-G-C-T-G-A-A-A-G-C-C-G-T) **15**

Ru = (Ru complex)-$(CH_2)_{11}$-O-P(=O)(O$^{\ominus}$)-{

Fig. 2. *Sequences of the synthesized oligonucleotides*

tethered Ru^{II} complex serves as a purification handle due to its high lipophilicity. Reversed-phase HPLC (*Vydac* C_4) was achieved with a linear gradient 10–(30–40)% MeCN in aqueous 0.05M triethylammonium acetate buffer (pH 7) over 40 min. The unreacted oligonucleotides were eluated at a relatively low gradient.

Both Ru^{II}-modified oligonucleotides **10** and **11** gave rise to different product fractions in reversed-phase HPLC as well as in PAGE (*Fig. 3, a* and *b*). Whereas the analytical HPLC of crude **10a–c** showed only three peaks with an integral of 1:1:2 (*Fig. 3, a*), the analytical HPLC of **11a–d** exhibited four peaks with a ratio of 1:1:1:1 (*Fig. 3, b*). The separated pure fractions of **10** (**10a–c**) as well as the separated pure fractions of **11** (**11a–d**) had identical MALDI-TOF-MS. This is in agreement with the existence of four diastereoisomeric conjugates. The resulting *Δ*- and *Λ*-isomeric Ru^{II}-labelled oligonucleotides **10a–c** and **11a–d** were separated either by reversed-phase HPLC or by PAGE. All four stereoisomers of the Ru^{II}-modified oligonucleotides **11a–d** which contained one 4'-pivaloyl-substituted nucleotide could be separated completely. To our knowledge, this is the first example for a complete separation of *Δ*- and *Λ*-diastereoisomeric Ru^{II}-labelled oligonucleotides [2]).

2.3. *Enzymatic Digestion and Base Analysis of the Ru^{II}-Labelled Oligonucleotides.* Oligonucleotides can be enzymatically degraded from their 3'-terminus to their constituent nucleotides with *snake venom phosphodiesterase* (*SVP*, 3'-exonuclease) which cleaves 3'–5'-internucleotide phosphate bonds from the 3'-terminus yielding 5'-monophosphate nucleosides. The analysis of the products by MALDI-TOF-MS is a powerful tool to determine sequence compositions [14].

Enzymatic digestion of **10c** by *SVP* was performed to confirm the specifically tethering of the Ru^{II} complex **7** to the 5'-end of the oligonucleotide **12**. To obtain an optimal distribution of cleavage products, aliquots were removed from the digestion mixture at time intervals of 1–10 min and directly analyzed by MALDI-TOF-MS (*Fig. 4*).

The sequence of **10c** can be identified by the differences in mass of adjacent oligonucleotide peaks representing the loss of one nucleotide each. The resulting mass fragments determined unequivocally the 5'-terminus tethering of the Ru^{II} complex **7**. Fragments

[2]) *Barton* and coworkers recently mentioned the separation of *Δ*- and *Λ*-$[Rh(phi)_2(bpy')]^{3+}$-labelled oligonucleotides [2f].

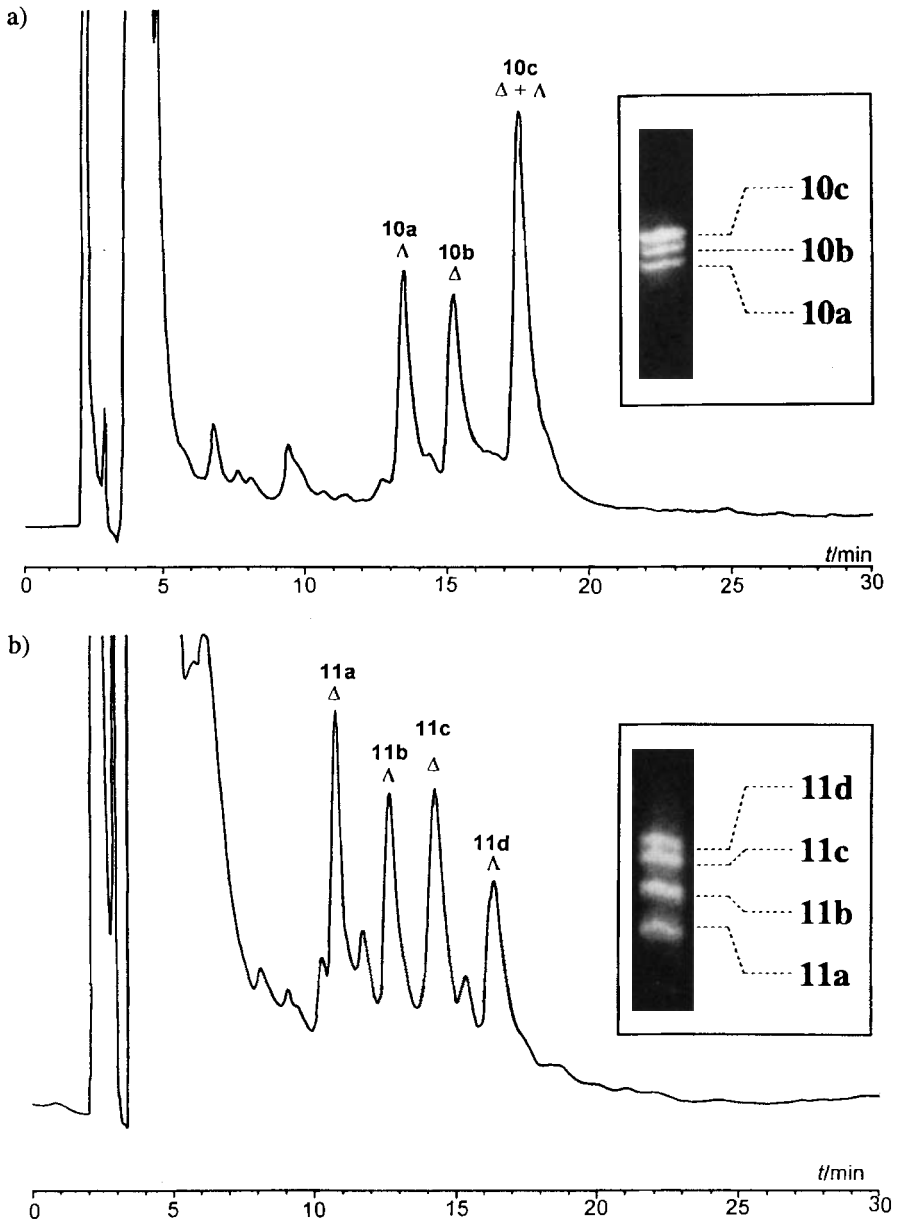


Fig. 3. *UV-Shadowing polyacrylamide gel* ($\lambda_{exc.}$ = 366 nm) *and reversed-phase HPLC of the crude mixtures of* Ru^{II}*-labelled oligonucleotides. a)* **10a–c** (0.05M Et_3NHOAc/MeCN 10–40% in 40 min), *b)* **11a–d** (0.05M Et_3NHOAc/MeCN 10–30% in 40 min). The minor side-peaks resulted from the one-base shortened Ru^{II}-labelled oligonucleotides

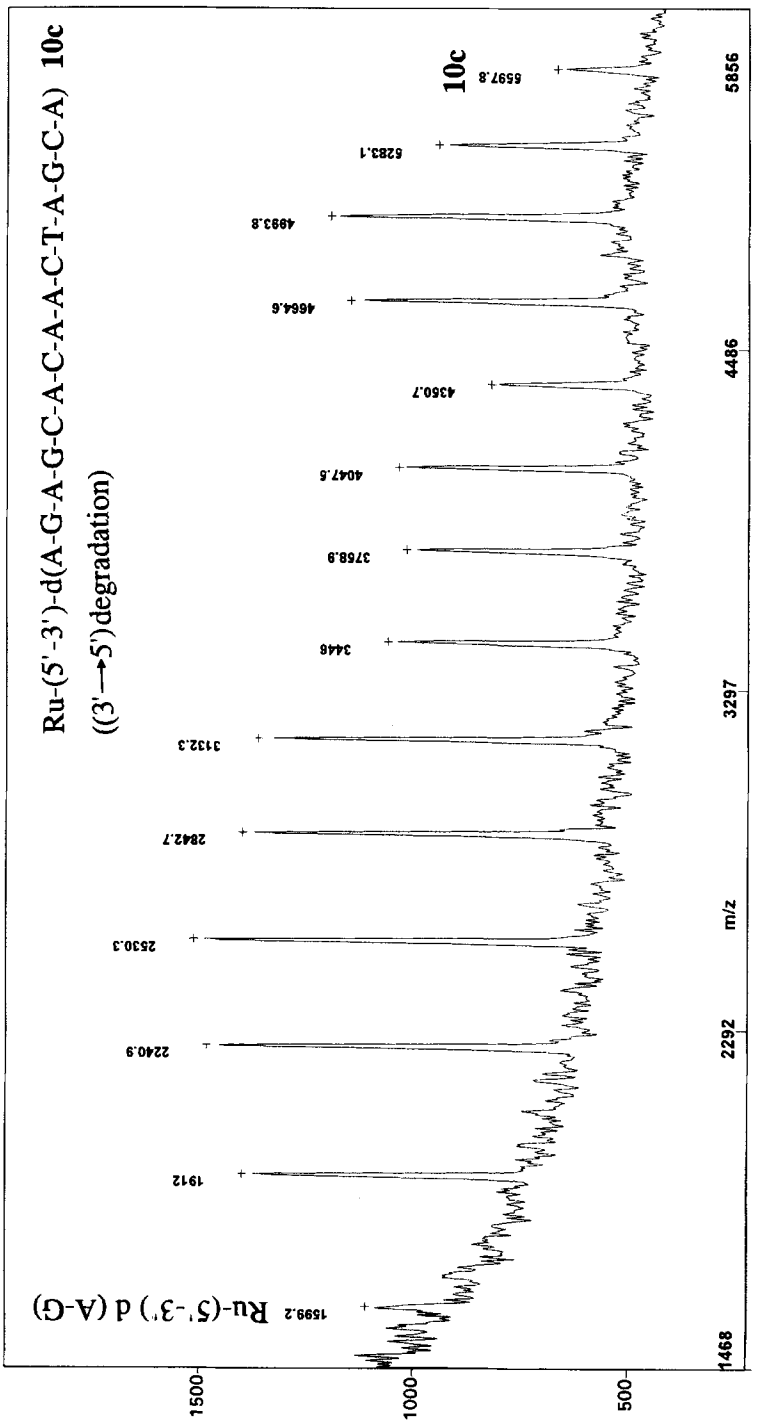


Fig. 4. *Negative-ion MALDI-TOF-MS* (25 kV, matrix: 2,4-dihydroxyacetophenone) *of the partial digestion of* **10c** *with snake venom phosphodiesterase after 2 min of digestion*

shorter than two nucleotides tethered with Ru^{II} complex were not observed. The MS at low mass numbers tended to be obscured by matrix and low-molecular-weight fragments. The relative scarcity of signal for very short fragments may also be enzyme-related.

2.4. *UV/VIS Spectroscopy of Ru^{II}-Labelled Oligonucleotides.* The Ru^{II}-labelled oligonucleotides **10a–c** and **11a–d** showed a characteristic MLCT absorption band of the complex $[Ru(phen)(phen')(dppz)]^{2+}$ (**7**) at $\lambda = 437$ nm and an IL transition band at $\lambda = 382$ nm [1c] [2a] [5c], whereas the Ru^{II}-complex-free oligonucleotides **12** and **13** did not absorb in this spectral region.

The melting temperature (T_m) of a given DNA double strand is a useful probe for its stability [13]. We measured the T_m values of the enantiomerically pure Ru^{II}-labelled oligonucleotides **10a,b** and **11a–d** in the presence of their complementary strands **14** and **15** and those of the Ru^{II}-complex-free double strands **12**/**14** and **13**/**15** (*Table*). We found,

Table. T_m *Values of the Enantiomerically Pure Δ- and Λ-[Ru(phen)(phen')(dppz)]$^{2+}$-Labelled Oligonucleotides* **10a,b** *and* **11a–d** *in the Presence of Their Complementary Strands* **14** *and* **15** *Compared to the* T_m *Values of the Ru^{II}-Free Double Strands* **12**/**14** *and* **13**/**15** (in 50 mM NaCl, 10 mM p_i (pH 7); $c = 1.1$ μM)

Double strand	T_m [°C]
10a/**14** (*Λ*-isomer)	59.1
10b/**14** (*Λ*-isomer)	59.8
11a/**15** (*Λ*-isomer)	58.6
11b/**15** (*Λ*-isomer)	58.9
11c/**15** (*Λ*-isomer)	58.5
11d/**15** (*Λ*-isomer)	59.0
12/**14**	51.7
13/**15**	52.9

that the Ru^{II}-labelled oligonucleotides showed a remarkable increase of the T_m values of 5.9–7.8° compared to those of the Ru^{II}-complex-free double strands. This points at a double-strand stabilization due to an intercalative interaction of the ligand **2** (dppz) of the Ru^{II} complex **7** with the DNA π-base stacks. This intercalative mode is in accordance with the UV/VIS spectra of the duplex conjugates in which a small red shift of the IL band (+ 2 nm) and a hypochromic effect of the IL and the MLCT band is observed [5c] [6c]. In contrast to the literature [2a], we observed a strong fluorescence for the double-stranded as well as for the single-stranded Ru^{II}-modified oligonucleotides.

2.5. *CD Spectroscopy of Ru^{II}-Labelled Oligonucleotides.* To determine the absolute configuration of the tethered Ru^{II} isomers, the circular dichroism (CD) [15] [16] of the separated HPLC fractions of **10a–c** and **11a–d** in the presence of their complementary strands **14** and **15** (*Fig. 5*) were measured and compared with the CD effects of the enantiomerically pure *Λ*-$[Ru(phen)_2(dppz)]^{2+}$ [5c].

For the Ru^{II}-labelled oligonucleotide fractions **10a**, **11b**, and **11d**, we assigned the *Λ*-configuration of the attached Ru^{II} complex **7** due to the identical course of the CD effects referred to *Λ*-$[Ru(phen)_2(dppz)]^{2+}$. The *Λ*-isomeric configuration for **10b**, **11a**, and **11c** was determined, since their CD spectra showed a mirrored course. No CD effects of **10c** were observed in the long-wavelength region above $\lambda = 380$ nm, which is

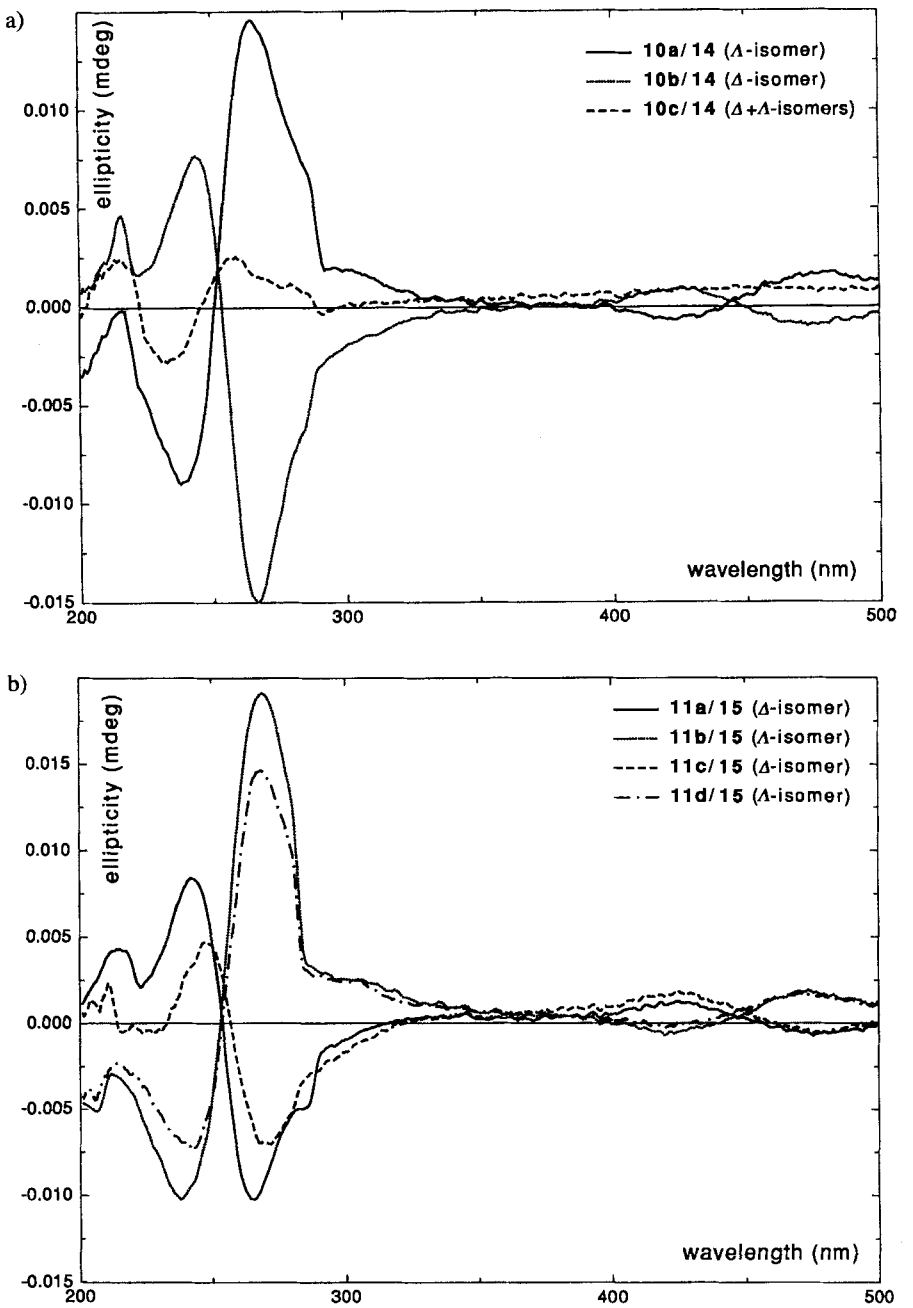


Fig. 5. *CD Spectra of the enantiomerically pure Ru^{II}-labelled oligonucleotides in the presence of their complementary strands* (in 50 mM NaCl, 10 mM p_i (pH 7); $c = 20-25$ μM). *a*) **10a–c/14**, *b*) **11a–d/15**.

characteristic for the Ru^{II} moiety of the adduct. Since we assigned for the Ru^{II}-labelled oligonucleotides **10a** and **10b** the *Λ*- and the *Δ*-isomeric configuration of the attached Ru^{II} complex **7**, respectively, fraction **10c** consists of a racemic 1:1 mixture of the two remaining (of the four possible) *Λ*- and *Δ*-enantiomers of $[Ru(phen)(phen')(dppz)]^{2+}$ (**7**). This led to a compensation of the Ru^{II} CD effects in **10c**. Measurement of the CD spectrum of **10c** led only to a small CD effect, which resulted from the DNA framework. The CD spectrum showed a positive band centered near $\lambda = 259$ nm, a negative band centered near $\lambda = 225$ nm, and a crossover around $\lambda = 252$ nm. These signature is typical for the B-form of DNA [16] and indicated that the end-specific attachment of the Ru^{II} complex **7** to synthetic oligonucleotides does not interfere with the double-helix formation. The Ru^{II}-complex-free double strands **12**/**14** and **13**/**15** also exists in the B-form under these conditions (CD spectra not shown).

Conclusion. – We have synthesized a novel complex $[Ru(phen)(phen')(dppz)]^{2+}$ (**7**) and described its specific attachment to the 5'-end of oligonucleotides **12** and **13** *via* the ligand **1e** (phen') which contains an alkyl spacer. The tethering reaction was performed in the course of a solid-phase DNA synthesis and led to the formation of stereoisomerically *Δ*- and *Λ*-Ru^{II}-labelled oligonucleotides **10a–c** and **11a–d**. In contrast to the previously described methods for the cumbersome separation of *Δ*- and *Λ*-enantiomerically pure Ru^{II} complexes [5a], we readily separated the four stereoisomers of **7** after their tethering to oligonucleotides **12** and **13** during the purification process by means of reversed-phase HPLC and polyacrylamide gel-electrophoresis (PAGE). The isolated diastereoisomerically pure Ru^{II}-labelled oligonucleotides **10a–c** and **11a–d** were characterized by UV/VIS and CD spectroscopy, mass spectrometry, and enzymatic digestion with base analysis. Furthermore, we have shown that Ru^{II}-labelled oligonucleotides hybridized specifically to single-stranded complementary sequences and increased the melting temperatures of the resulting double strands significantly. The so formed modified oligonucleotides should be useful systems for the investigation of the electron transfer through DNA and could be extremely valuable in the development of novel hybridization probes both for heterogeneous and homogenous assays [2a].

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Experimental Part

General. All reagents are commerically available and used without further purification *1,10-phenanthroline* (phen, **1a**) was purchased from *Fluka* as monohydrate. The dipyridophenazine ligand (dppz, **2**) was synthesized as described in [10]. The solvents were purified and dried according to standard procedures. The reactions were carried out in carefully dried apparatus under Ar. *Snake venom phosphodiesterase* (SVP, 3'-exonuclease from *crotalus durissus*) was purchased from *Boehringer Mannheim* (Germany). DNA-Synthesis: *PerSeptive Biosystems EXPEDITE 8909* synthesizer (1-μmol scale, 500-Å CPG support). All hybridizations and double-strand investigations were carried out in phosphate buffer saline (50 mM NaCl, 10 mM p_i (pH 7)). The double-strand concentrations were used in the range of 20 to 25 μM for the CD investigations and 1.1 μM for the T_m measurements. The probes were heated at 80° for 5 min and cooled down slowly to r.t. before measurements. Polyacrylamide gel electrophoresis (PAGE): 20% under denaturating conditions using *Accu Gel 40*™ from *National Diagnostics*. TLC: *Merck* precoated silica gel sheets F_{254}, *Macherey-Nagel Alugram* silica gel *C18* sheets *RP-18W/UV*$_{254}$. Flash chromatography (FC): *Merck* silica gel *60* (40–63 μm). Column chromatography (CC): *Fluka* basic aluminium oxide. HPLC: *Hewlett-Packard* HPLC system series *1050* using a *Vydac* C_4 column *214TP10415* (10 μm).

M.p. *Büchi* 530, temp. are uncorrected. UV/VIS: *Perkin-Elmer* UV/VIS spectrometer *Lambda 2*; λ_{max} (ε) in nm. UV-Absorption melting curves: *Perkin-Elmer* UV/VIS spectrometer *Lambda 2* with peltier temp. programmer *PTP-6*. CD: *CARY 61* spectropolarimeter; in mdeg. IR: *Perkin-Elmer 1600 FTIR* spectrophotometer; in cm^{-1}. NMR: *Varian Gemini 300* (^1H at 300 MHz, ^{13}C at 75.5 MHz); (δ) in ppm relative to internal TMS for ^1H ($\delta = 0.00$ ppm) and $CDCl_3$ for ^{13}C ($\delta = 77.5$ ppm); (J) in Hz. FAB-MS: *VC-70 250* (matrix: nitrobenzyl alcohol (NBA) and NBA + KCl); in *m/z* (rel. %). MALDI-TOF-MS: *Vestec Benchtop II* (matrix: 2,4-dihydroxyacetophenone, 2,4,6-trihydroxyacetophenone, or sinapic acid and ammonium tartrate; 15–25 kV acceleration voltage; laser wavelength $\lambda = 337$ nm). Microanalyses were performed at the Mikroanalytisches Labor at the University of Basel.

5-Amino-1,10-phenanthroline (**1c**). *5-Nitro-1,10-phenanthroline* [7] **1b** (1 g, 4.44 mmol) was suspended with 1.5 g of Pd/C (10% Pd, *Fluka*) and cyclohexene (2.5 g, 30.44 mmol) and refluxed in 50 ml of EtOH under Ar for 8 h. The hot soln. was filtered through *Celite* followed by several washings with hot EtOH. After removal of the solvent, the remaining yellow solid **1c** (700 mg, 80.8%) was used without further purification. The spectroscopic data are in accordance with those described in [17].

11-(Chlorocarbonyl)undecyl Acetate (**3c**). *12-Hydroxydodecanoic acid* (**3a**; 1.5 g, 6.93 mmol) was dissolved in 400 ml of anh. pyridine. After dropwise addition of Ac_2O (20 ml) at 0°, the mixture was stirred for 12 h at r.t. The reaction was quenched upon addition of H_2O (100 ml) and stirred for further 3 h at r.t. Then, 200 ml of 2N aq. HCl was added, and the mixture was extracted three times with CH_2Cl_2. The org. phase was dried by filtration through hot cotton wool and evaporated. Traces of Ac_2O and AcOH were co-evaporated with toluene. The crude oily or waxy residue was purified by CC (silica gel, pentane/AcOEt 3:1) yielding *12-acetoxydocecanoic acid* (**3b**; 1.70 g, 94.9%) together with a minor amount of the dimeric ester **3d**, which can be removed in the course of the further reaction.

To a soln. of **3b** (4 g, 15.48 mmol) in benzene (35 ml) was added, under Ar, oxalyl chloride (3.5 ml, 40.72 mol). After a few min, a vigorous gas formation started and the mixture became darker within the next 2.5 h. The solvent and excess of oxalyl chloride was evaporated, and the dark residue was distilled twice by means of a *Kugelrohr* at 180° in a rotary pump vacuum: 3.08 g (71.9%) of **3c** were obtained as colorless liquid, which can be stored at −20° under Ar. The spectroscopic data are in accordance with those described in [18].

12-Acetoxy-N-(1,10-phenanthrolin-5-yl)dodecanamide (**1d**). Compound **1a** (500 mg, 2.77 mmol) was dissolved in 50 ml of abs. CH_2Cl_2 and 5 ml of Et_3N. Over a period of 100 min, **3c** (720 mg, 2.6 mmol) in 5 ml of abs. CH_2Cl_2 was added dropwise at 0°. After stirring at r.t. for 3 h, the mixture was cooled again to 0°, and the process was repeated in the same manner and with the same amounts of **3c**, followed by stirring at r.t. overnight. Then, 100 ml of MeOH were added to the clear red-brown soln. and stirred for 1 h at r.t., followed by addition of 100 ml of 5% $NaHCO_3$ soln. A white slime precipitated, and the mixture was extracted three times with CH_2Cl_2. The org. phase was dried by filtration through hot cotton wool and evaporated. The remaining crude residue was purified by CC (basic aluminium oxide; CH_2Cl_2/MeOH 80:1 to 10:1) yielding 1.15 g of **1d** (95.3%). A sample was recrystallized for characterization from CH_2Cl_2/pentane. M.p. 101–103°. TLC (*RP-18*, MeOH): R_f 0.71. UV/VIS (EtOH): 310 (sh), 270(28090), 233(33380), 225(33640). IR (KBr): 3396*m*, 2924*s*, 2849*s*, 1700*s* (C=O), 1534*s*, 1413*sm*, 1273*s*, 1167*m*, 1038*w*, 886*w*, 812*w*, 744*m*. ^1H-NMR ($CDCl_3$): 1.30(*m*, H–C(4–10)); 1.63, 1.82(2*m*, H–C(3), H–C(11)); 2.04(*s*, Me); 2.55(*m*, H–C(2)); 4.04(*t*, $^3J = 7.1$, H–C(12)); 7.58, 8.17, 8.3, 9.11(4*m*, H–C(aryl)); 7.99(br. *s*, NH). The chemical shifts strongly depend on the concentration due to the aggregation behavior of **1d**. MS: 436(100, $[M + H]^+$). Anal. calc. for $C_{26}H_{33}N_3O_3 \cdot 0.05\,CH_2Cl_2$ (439.82): C 71.14, H 7.59, N 9.55, O 10.91; found: C 70.96, H 7.67, N 9.47, O 11.35.

12-Hydroxy-N-(1,10-phenanthrolin-5-yl)dodecanamide (**1e**). Compound **1d** (497 mg, 1.14 mmol) was dissolved under Ar in 200 ml of NH_3 sat. MeOH by means of ultrasonic and stirred for 4 d at r.t. After the solvent was removed, the residue was dried *in vacuo* and taken up in a minimum amount of CH_2Cl_2/MeOH 1:1. CC (basic aluminium oxide; CH_2Cl_2/MeOH 80:1) yielded 382 mg (85.1%) of **1e**, which slowly crystallized as a pale-yellow solid. A sample was recrystallized from CH_2Cl_2/pentane for characterization. M.p. 122–124.5°. TLC (*RP-18*, MeOH): R_f 0.61. UV/VIS (EtOH): 310 (sh), 270(23930), 232(28180), 225(28690). IR (KBr): 3266*s* (OH), 2918*s*, 2847*s*, 1661*s* (C=O), 1540*s* (C=O), 1421*m*, 738*m*. ^1H-NMR ($CDCl_3$): 1.30(*m*, H–C(4–10)); 1.54, 1.80(2*m*, H–C(3), H–(11)); 2.52(*m*, H–C(2)); 3.60(*t*, $^3J = 6.6$, H–C(12)); 7.61, 8.18, 8.25, 9.10, 9.18(5*m*, H–C(aryl)). The chemical shifts strongly depend on the concentration due to the aggregation behavior of **1e**. MS: 394(100, $[M + H]^+$). Anal. calc. for $C_{24}H_{31}N_3O_2 \cdot 0.05\,CH_2Cl_2$ (397.78): C 72.62, H 7.88, N 10.56; found: C 72.60, H 8.04, N 10.42.

[Ru(phen)(dppz)(CO)$_2$](PF6)$_2$ (**6**). [Ru(phen)$(CO)_2Cl_2$] (**5** [11]; 1 g, 2.45 mmol) was dissolved in 300 ml of CH_2Cl_2 and flushed with Ar for 30 min. After dropwise addition of CF_3SO_3H (1 ml, 11.3 mmol) within 45 min, the mixture was heated for 2 h to 120–130°. The mixture was cooled to r.t., and the pale-yellow soln. of the

corresponding triflato species was evaporated to dryness in a rotary pump vacuum. After addition of abs. EtOH (50 ml, Ar-flushed) and *dipyridophenazine* (**2** [10]; 1.5 g, 5.31 mmol), the mixture was refluxed for 3 h. For workup, the dark-green suspension was evaporated, and 150 ml of H_2O were added. After refluxing for 30 min, the mixture was cooled and filtered. The complex **6** was isolated as PF_6^- salt upon addition of an excess of KPF_6. Precipitation was completed at 4° overnight. The crude product **6** was filtered and dried. Recrystallization from acetone and EtOH yielded 1.08 g (48.5%) of pale-yellow **6**. UV/VIS (EtOH): 203(174110), 225 (sh), 274(98730), 326(22200), 362(19940), 382 (sh). IR (KBr): 3103*w*, 2109*s* (CO), 2050*s* (CO), 1636(br.)., 1500*m*, 1431*m*, 1362*w*, 1083*w*, 1058*w*, 839(br.) (PF), 728*m*, 558*s*. ^{1}H-NMR ($CDCl_3$): 7.82(*dd*, J = 5.3, 8.3 Hz, H–C(8) of phen); 7.98(*dd*, J = 5.4, 8.3, H–C(7) of dppz); 8.21–8.25, 8.43–8.59(2*m*, H–C(6,10,13) of dppz, H–C(3,5,6) of phen); 8.31(*dd*, J = 1.3, 5.3, H–C(9) of phen); 8.68(*dd*, J = 5.4, 8.3, H–C(2) of dppz); 8.94(*dd*, J = 1.3, 8.3, H–C(7) of phen); 9.32(*dd*, J = 1.25, 8.4, H–C(4) of phen); 9.87(*dd*, J = 1.4, 8.3, H–C(8) of dppz); 10.06(*dd*, J = 1.3, 5.4, H–C(2) of phen); 10.10(*dd*, J = 1.4, 5.4, H–C(3) of dppz); 10.25(*dd*, J = 1.4, 8.4, H–C(1) of dppz). MS: 765(45, $[M - PF_6]^+$ for ^{102}Ru), 639(24), 611(25), 592(32, $[M - 2PF_6 - CO]^+$ for ^{102}Ru), 583(35), 564(70, $[M - 2PF_6 - 2CO]^+$ for ^{102}Ru), 384(27), 356(14), 310(21), 296(20), 283(54, $[dppz + H]^+$), 181(49, $[phen + H]^+$), 107(33), 89(77), 77(100), 65(55), 63(63). Anal. calc. for $C_{32}H_{18}F_{12}N_6O_2P_2Ru$ (909.53): C 42.26, H 1.99, N 9.24; found: C 41.97, H 2.15, N 9.33.

[Ru(phen)(phen')(dppz)](PF$_6$)$_2$ (**7**). Complex **6** (100 mg, 0.11 mmol) and **1e** (75 mg, 0.19 mmol) were dissolved in 20 ml of 2-methoxyethanol and flushed with Ar for 30 min. After addition of trimethylamine *N*-oxide dihydrate (40 mg, 0.36 mmol), the mixture was refluxed under Ar for 7 h. The solvent was removed *in vacuo*, and the dark red oily residue was dried in a rotary pump vacuum. The residue was purified by FC (silica gel; MeCN/MeOH 4:1) to remove the excess of **1e**, and **7** was eluted with MeCN/H_2O/sat. aq. KNO_3 50:3:1. After removal of the solvent, MeCN was added and **7** was isolated as PF_6^- salt upon addition of an excess of KPF_6 (20 ml half-conc. soln.). Precipitation was completed at 4° overnight. The crude product **7** was filtered and washed by H_2O and Et_2O. The complex was dissolved in MeCN and filtered once again to remove impurities. Precipitation followed upon treatment with KPF_6. Recrystallization from MeCN and EtOH yielded 117 mg (85.3%) of pure **7**. The purity was determined by means of reversed-phase HPLC (*Vydac C$_4$*) using as eluent a linear gradient of 20–50% MeCN in aq. 0.05M Et_3NHOAc buffer (pH 7) over 60 min (t_R 56 min). UV (MeCN): 203(104500), 221 (sh), 266 (sh), 275(119160), 317 (sh), 359 (sh), 368(20060), 447(20780). IR (KBr): 3423(br.), 3101*w*, 2925*m*, 2852*m*, 1686*m*, 1629*m*, 1522*m*, 1459*m*, 1425*m*, 1358*m*, 842*s*, 722*m*, 557*m*. ^{1}H-NMR (CD_3CN): 1.2–1.5(br., H–C(4–11)); 1.75(*quint.*, H–C(3)); 2.6(2*t*, H–C(2)); 3.4(*m*, H–C(12)); 7.5–7.8(*m*, 6H–C(aryl)); 7.9–8.3(*m*, 10H–C(aryl)); 8.4–8.7(*m*, 7H–C(aryl)); 8.8(br. *s*, NH); 9.65(*m*, 2H–C(aryl)). MS: 1102(26, $[M - PF_6]^+$ for ^{102}Ru), 972(7), 956(18, $[M - 2PF_6 - H]^+$ for ^{102}Ru), 775(3), 675(5), 577(8), 564(21), 551(6), 479(14), 458(9), 398(7), 394(8, $[phen' + H]^+$), 384(12), 357(6), 296(12), 282(16, $[dppz]^+$), 196(15), 181(21, $[phen + H]^+$), 136(23), 107(21), 89(48), 77(54), 69(38), 55(100). Anal. calc. for $C_{54}H_{49}F_{12}N_9O_2P_2Ru \cdot H_2O$ (1265.05): C 51.27, H 4.06, N 9.96; found: C 51.15, H 3.90, N 10.05.

Phosphonate **8** *of the [Ru(phen)(phen')(dppz)]$^{2+}$ Complex* (**7**). 1*H*-Imidazole (0.9 g, 13.22 mol) was three times co-evaporated with 3 ml of abs. MeCN. After addition of abs. MeCN (50 ml) and Et_3N (1.9 ml), 0.35 ml of PCl_3 were added dropwise under Ar within 5 min by means of a syringe. After a few min, a white salt precipitated. The mixture was stirred for 45 min at r.t. The complex **7** (110 mg, 88.2 μmol) was twice co-evaporated with anh. MeCN and dissolved in 8.8 ml of anh. MeCN under Ar. The previously prepared soln. of tris(1*H*-imidazol-1-yl)phosphine (10 ml) was added, and the mixture was stirred for 2 h at r.t. TLC (silica gel; MeCN/H_2O/sat. aq. KNO_3 10:1:0.5) showed complete conversion. After the mixture was poured into 90 ml of 0.1M Et_3NHOAc (pH 7.0) and extracted three times with CH_2Cl_2 (15 ml), the combined org. layers were dried through hot cotton wool and evaporated. The residue was digerated in Et_2O, washed with Et_2O, and dried: 118 mg (95%) of **8** · Et_3N was obtained and used directly for the coupling reaction to the oligonucleotides. The phosphonate **8** can be stored without decomposition. MALDI-TOF-MS (pos. mode, 15 kV, matrix: sinapic acid): 1021.71 (calc. 1021.09).

Coupling of **8** *to Synthetic Oligonucleotides.* Compound **8** (12 mg, 8.5 μmol) was co-evaporated twice from anh. MeCN and taken up under Ar in 0.5 ml of anh. pyridine. From this soln., 0.25 ml were added at the same time as 0.25 ml of a pivaloyl chloride/MeCN soln. (200 μl of freshly dist. pivaloyl chloride in 6.8 ml of abs. MeCN) to 1 μmol of the oligonucleotide sequences d(A-G-A-G-C-A-C-A-A-C-T-A-G-C-A) (**12**) and d(A-C-G-G-C-T-T*-T-C-A-G-C-T-C-G) {T* = **9**[4c,d]} (**13**), which were synthesized in the trityl-off modus and still attached in the completely protected form to the solid support. After a condensation time of 20 min, excess of **8** and pivaloyl chloride was removed with MeCN. The condensation process was repeated in the same way and with the same amounts, followed by oxidation with 0.5 ml of 0.2M I_2/THF and 0.5 ml of Et_3N/H_2O/THF 1:1:8(*v*/*v*). After washing with MeCN and Et_2O, the support was treated with 1000 μl of conc. NH_3 for 2.5 h at 67° for deprotection. Polyacrylamide gel electrophoresis (PAGE, 20%) showed complete conversion of the starting oligonucleo-

tides into the desired Ru^{II}-labelled DNA fragments **10a–c** and **11a–d**. Purification and isolation on reversed-phase (*Vydac* C_4) was achieved with a linear gradient 10–(30–40)% MeCN in aq. 0.05M tiethylammonium acetate buffer (pH 7) over 40 min and led to the diastereoisomerically and enantiomerically pure isomers.

Enzymatic Digestion and Base Analysis. The 3′–5′ degradation of **10c** was carried out by time dependent enzymatic digestion with snake venom phosphodiesterase (SVP, 3′-exonuclease) in H_2O without the addition of salts and buffer. 1 µl of SVP (1 mg/0.5 ml) was diluted with 5 µl of H_2O. To 25 µl ($c \approx 5$ µM) of **10c** in H_2O 1 µl of the enzyme soln. was added at 0° and incubated. Samples of 1 µl were taken every min and added to 1 µl of matrix soln. (2,4-dihydroxyacetophenone and ammonium tartrate in $MeCN/H_2O$ 1:1) directly to the MALDI sample plate. The samples were measured directly after drying (25 kV).

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