Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron
Tetrahedron 63 (2007) 11299–11306

The oxime bond formation as an efficient tool for the conjugation of ruthenium complexes to oligonucleotides and peptides

Mathilde Villien,[a] Stéphanie Deroo,[b] Etienne Gicquel,[b] Eric Defrancq,[a,*] Cécile Moucheron,[b] Andrée Kirsch-De Mesmaeker[b] and Pascal Dumy[a]

[a]*Département de Chimie Moléculaire, UMR CNRS 5250, Université Joseph Fourier, BP 53, 38041 Grenoble Cedex 9, France*
[b]*Laboratoire de Chimie Organique et Photochimie, Université libre de Bruxelles (U.L.B.), B-1050 Bruxelles, Belgique*

Received 4 July 2007; revised 24 August 2007; accepted 28 August 2007
Available online 1 September 2007

Abstract—A convenient method for the conjugation of ruthenium complex on oligonucleotides and peptides through chemoselective oxime linkage is reported. Novel Ru(II) complexes sustaining an aminooxy containing ligand were prepared and efficiently coupled with the oligonucleotides and peptides functionalized with the complementary reactive aldehyde group. The method described herein could be a useful tool for preparing a broad range of metal complex–oligonucleotide and peptide conjugates.

1. Introduction

Conjugation of metal complexes to biomolecules such as nucleic acids and peptides shows an increasing interest. Indeed, the oligonucleotides (ODNs) are exploited as new therapeutic tools due to their recognition properties for their complementary single strand target ('antisense strategy') or double strand target ('antigene strategy') as well as for protein targeting ('aptamer strategy').[1] DNA represents also a promising candidate for the design of nanostructures in a bottom-up approach with respect to its selective and programmable self-assembly based on the complementary Watson–Crick base pairing.[2] On the other hand, the peptides can be used for their recognition properties to enhance cell penetration as well as to target a specific DNA sequence.[3] Various metal complexes have been attached to oligonucleotides in order to prepare luminescent oligonucleotide probes or light activated reagents.[4] Metal complexes have also been attached to oligonucleotides to create supramolecular nanoscale assemblies in which the metal complex and DNA duplex constitute the vertex and arm, respectively.[5] In this context, due to their photophysical and photochemical properties, a number of research investigations has focused on the use of Ru(II) complexes conjugated to biomolecules.[6]

Several methods have been reported for the attachment of Ru(II) complexes to the oligonucleotides (ODNs). The conjugates can be prepared either by 'on-support synthesis' or 'fragment solution coupling'. The on-support conjugation is usually carried out by incorporating Ru(II) complex into the ODNs as modified phosphoramidite.[7] Another on-support method consists in the support fragment conjugation strategy. Herein, the ODNs were assembled on solid support and the reactive functional moiety was introduced during the automated DNA synthesis inside the sequence or at extremities. The reporter group is then introduced while the ODNs are kept protected and support bound. The final ODN–metal complex conjugates were obtained after subsequent cleavage from the support, nucleobase deprotection, and purification. Barton et al. have used this strategy by reacting ruthenium and rhodium complexes modified with an activated acid with 5′-amino functionalized oligonucleotides.[8] A similar strategy using Sonogashira coupling reaction performed on support bound oligonucleotides has also been described.[9] However, the main disadvantage associated with the on-support approaches is that the metal complex needs to be stable under harsh basic conditions used for the final deprotection step of the oligonucleotides. On the other hand, the solution-phase conjugation is accomplished by incorporating mutually reactive functional groups into the ODN and the target molecule (i.e., metal complex) followed by their solution-phase coupling. In case of Ru(II) complexes, this strategy mainly involves the formation of an amide linkage by reaction between an amino-containing oligonucleotide with an activated acid sustained by the metal complex.[10] However, the reaction is not very efficient and a large excess of the complex is required leading to tedious purifications.

The attachment of ruthenium complexes on peptides has received less attention and is generally performed by using on-support strategy. Ruthenium complex bearing a carboxylic acid group on one of the ligands is introduced at N-terminal

* Corresponding author. E-mail: eric.defrancq@ujf-grenoble.fr

0040–4020/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2007.08.088

position of the peptide using standard coupling conditions. Cleavage from the resin and deprotection of the amino acid side chains were then achieved in acidic conditions (TFA).[11] Rhodium complexes have also been introduced on peptides using a similar strategy.[12] However, some limitations are associated with this approach due to the stability of the metal complex during various conditions used in SPPS. The final deprotection step of the peptide can be carried out in harsh acidic conditions (for example, removal of Mtr or Pmc protecting group of Arg). Moreover, the removal of commonly employed allyloxycarbamate (aloc) protecting group needs the use of Pd metal complex and others like Dde or Fmoc protecting groups require basic reagent such as hydrazine or piperidine. Consequently, the metal complex to be anchored must be stable in all these conditions for a general use of the on-support method.

Recent work of our laboratory has focused on the development of oxime linkages for the efficient preparation of oligonucleotide conjugates. It was shown that chemoselective oxime bond formation could be successfully employed to prepare various oligonucleotide conjugates bearing peptides, carbohydrates, phenanthroline at either 3′- or 5′-terminus of the oligonucleotides.[13] The earlier results from our group and others have shown that oxime bonds offer certain advantages over other types of linkages. For instance, the oxime bonds give high efficiency of coupling, do not require the use of any activation or stabilization step and do not suffer from the lack of regiospecific ligation as is the case with thio- or amine-based ligation. We were thus interested to apply this oxime strategy for the attachment of various ruthenium complexes to the oligonucleotides and peptides.

In this paper, we present the preparation of the two different complexes $[Ru(TAP)_2phen'']^{2+}$ **1** and $[Ru(TAP)_2TAP'']^{2+}$ **2** (phen=1,10-phenanthroline and TAP=1,4,5,8-tetraazaphenanthrene) bearing an aminooxy functionalized phen″ or TAP″ ligand (Fig. 1). These polypyridyl ruthenium(II) complexes are of great interest as it has been shown that Ru(II) complexes bearing at least two TAP ligands can lead to the formation of an irreversible photoadduct with the guanine residue of DNA.[14] Complexes **1** and **2** were efficiently conjugated to aldehyde containing oligonucleotides and peptides. The conjugation with oligonucleotides was achieved both at 5′- and 3′-ends and at N-terminus for peptides. Two different peptides of biological significance were used: (a) the Tat peptide GRKKRRQRRR, which is well known to enhance the cell penetration of conjugated molecule[15] and (b) the NLS peptide, a nuclear localizing signal sequence with basic peptide APKKKRKVED derived from the simian virus 40 antigen.[16]

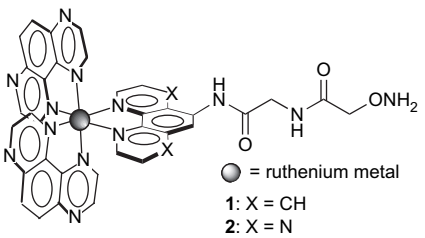


Figure 1. Structures of $[Ru(TAP)_2phen'']^{2+}$ **1** and $[Ru(TAP)_2TAP'']^{2+}$ **2** complexes.

2. Results and discussion

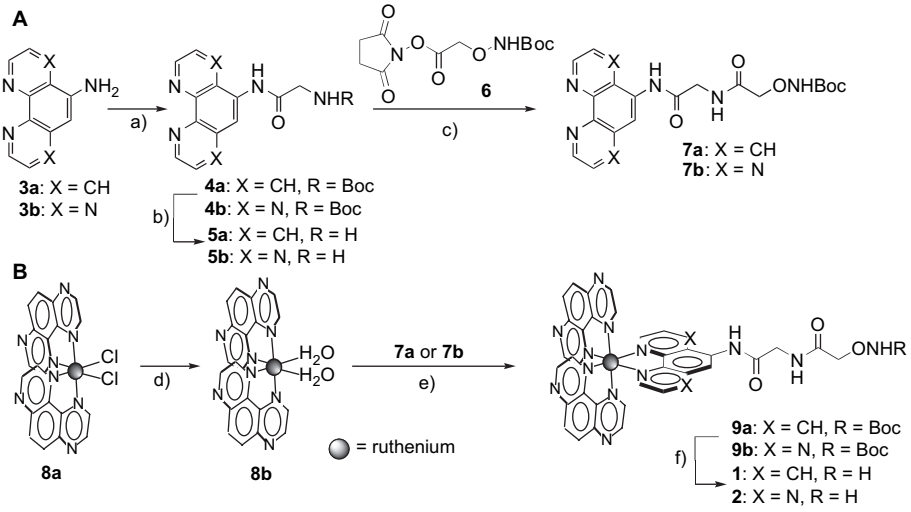
2.1. Syntheses of the Ru(II) complexes 1 and 2

The aminooxy modified phenanthroline (phen″) derivative used as ligand was prepared according to the procedure described previously.[17] The tetraazaphenanthrene (TAP″) ligand was thus synthesized by analogy using the same strategy. The main steps of the preparation of the two ligands are as follows and illustrated in Scheme 1A. The glycine linker was introduced into the amino-containing ligands **3a** and **3b** by reacting them with *N*-(*tert*-butoxycarbonyl)glycine anhydride. Due to inactivation of the exocyclic amine on the heterocyclic ring, an excess of anhydride has to be used for completion of the reaction. The protected phenanthroline **4a** and tetraazaphenanthrene **4b** were then purified by silica gel column chromatography. The *tert*-butoxycarbonyl group was subsequently removed by acidic treatment in CH_2Cl_2/TFA (50:50, v/v) at room temperature for 2 h to give the corresponding amino derivatives **5a** and **5b**, which were used in the next step without further purification. Introduction of the aminooxy group was achieved by coupling the activated ester of *N*-Boc-*O*-(carboxymethyl)-hydroxylamine **6** with the amino derivatives **5a** and **5b** in DMF in the presence of DIEA. The protected aminooxy derivatives **7a** and **7b** were purified by column chromatography and obtained as white powder.

The complexes $[Ru(TAP)_2phen'']^{2+}Cl_2$ **1** and $[Ru(TAP)_2TAP'']^{2+}Cl_2$ **2** were obtained by a straightforward synthesis illustrated in Scheme 1B. The precursor $Ru(TAP)_2Cl_2$ **8a** was synthesized from $RuCl_3$ and TAP ligands as previously described.[18] The precursor $Ru(TAP)_2Cl_2$ **8a** was first activated as $[Ru(TAP)_2(H_2O)_2]^{2+}$ **8b** by reacting with silver nitrate at room temperature. The aminooxy protected complexes **9a** and **9b** were obtained after substituting the two water molecules by the protected aminooxy phenanthroline **7a** or tetraazaphenanthrene **7b** ligands, respectively. The two different complexes **9a** and **9b** were purified by alumina column chromatography with 70% overall yield and characterized by NMR and mass data. The protecting Boc group of the aminooxy moiety was then removed by 1 N aqueous HCl solution at room temperature. The use of TFA for the deprotection step has to be avoided as it was observed that this ambident reagent could substitute one of the TAP ligands. Due to the high reactivity of the aminooxy group, the complexes **1** and **2** were used without further purification. Nevertheless, they have been characterized by ESI-MS analysis.

2.2. Coupling reaction of Ru(II) complexes with oligonucleotides through oxime linkage formation (Scheme 2)

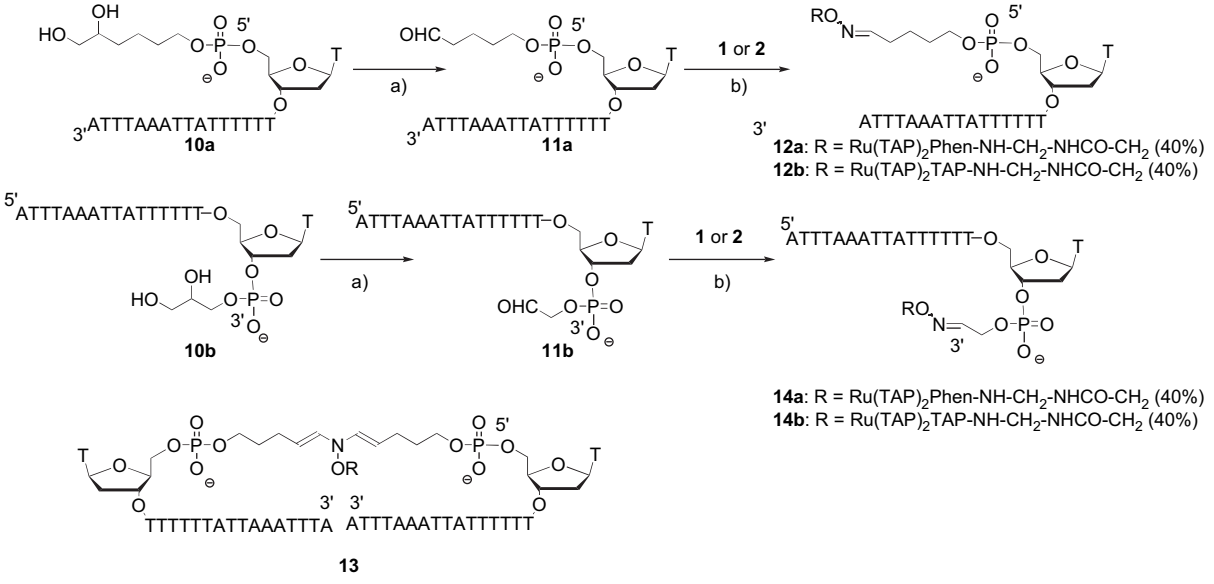
The two complexes **1** and **2** were conjugated at 5′- and 3′-end of oligonucleotides functionalized by the corresponding complementary aldehyde group. The oligonucleotides **11a** and **11b** bearing the aldehyde moiety at 5′- and 3′-end, respectively, were prepared according to the previous method of post-synthetic oxidation strategy.[12] Briefly, the oligonucleotides **10a** d(5′-XTTTTTTTATTAAATTTA-3′) and **10b** d(5′-ATTTAAATTATTTTTTTY-3′), in which X and Y represent the 5′- and 3′-diol linker, respectively, were prepared by automated DNA synthesis according to the standard



Scheme 1. Preparation of the ligands **7a** and **7b** and the complexes **1** and **2**; (a) *N*-(*tert*-butoxycarbonyl)glycine anhydride, CH_3CN, rt overnight; (b) TFA/CH_2Cl_2 (50:50), rt, 2 h; (c) **6**, DIEA, DMF, rt, 1 h; (d) $AgNO_3$, H_2O, reflux, 2 h; (e) DMF, 100 °C, 1 h; (f) 1 N aq HCl, rt, 6 h.

β-cyanoethylphosphoramidite chemistry. After cleavage from the support and deprotection of bases using the standard protocol, the 1,2-diol containing oligonucleotides **10a** and **10b** were purified by reverse phase HPLC. Subsequent oxidative cleavage of the diol was carried out by using excess of $NaIO_4$, which lead to 5′- and 3′-aldehyde containing oligonucleotides **11a** and **11b**, respectively.

We first studied the conjugation of the $[Ru(TAP)_2$-phen″$]^{2+}Cl_2$ complex **1** with the 5′-aldehyde containing oligonucleotide **11a**. A slight excess of the aminooxy complex **1** was reacted with **11a** in ammonium acetate buffer solution at pH=4.5. Reactions were carried out in slightly acidic conditions as the optimal pH around 4–5 is necessary for efficient oxime bond formation. The course of the reaction was monitored by reverse phase HPLC and the reaction proceeded essentially to completion within 15 h to yield the oxime conjugate **12a**. Unfortunately, the retention time of the starting material **11a** and the conjugate **12a** were very close so that a clean purification by HPLC was excluded. The crude mixture was thus purified by 20% denaturing polyacrylamide gel electrophoresis (PAGE). Moreover, this method of purification revealed the presence of a by-product with a lesser electrophoretic mobility. The yield of this by-product rose up to 10% when only 1 equiv of the aminooxy complex **1** was used. According to its electrophoretic mobility, this by-product might correspond to the addition of two oligonucleotides **11a** to the complex **1**. The putative structure **13** was proposed on the basis of the similar bis reaction of one aminooxy group with two aldehydes as reported earlier.[19] Furthermore, a model reaction was performed by reacting the unprotected aminooxy phenanthroline ligand (1 equiv) with aldehyde containing oligonucleotide. The ESI-MS analysis revealed the presence of this



Scheme 2. Preparation of oligonucleotide–Ru(II) complex conjugates **12a**,**b** and **14a**,**b** and putative structure of by-product **13**; (a) $NaIO_4$ (20 equiv), H_2O, 1 h; (b) 0.4 M ammonium acetate buffer, rt, overnight.

by-product with a molecular weight corresponding to the addition of two oligonucleotides for one ligand. Nevertheless, the optimization of the coupling conditions was achieved and the yield of this by-product **13** became negligible if at least 2 equiv of the aminooxy compound **1** were used. The same protocol was then applied for the conjugation with $[Ru(TAP)_2TAP'']^{2+}Cl_2$ complex **2**. Oligonucleotide **11a** was reacted with the aminooxy complex **2** (2 equiv) in aqueous ammonium acetate buffer solution leading to the conjugate **12b** that was purified by PAGE. The 5′-conjugates **12a** and **12b** were obtained in almost 40% isolated yield. Importantly, it was noted that no degradation of the Ru(II) complexes was observed during the coupling reaction as well as during the purification.

Similarly, conjugation of the complexes **1** and **2** was achieved at the 3′-end by using the aldehyde containing oligonucleotide **11b** leading to the formation of conjugates **14a** and **14b**, respectively. Again the conjugates were purified by PAGE and obtained in almost 40% isolated yield. The conjugates **12a** and **12b** as well as **14a** and **14b** were characterized by MALDI-MS. In all cases, the experimentally determined molecular weights were in excellent agreement with the calculated values.

2.3. Coupling reaction with peptides through oxime linkage formation (Scheme 3)

The $[Ru(TAP)_2phen'']^{2+}Cl_2$ complex **1** was conjugated with two different peptides **16a** and **16b**. The reactive aldehyde moiety was introduced at N-terminal position by oxidative cleavage of a serine residue.[20] Actually, the 1,2-amino alcohol moiety of the serine was selectively oxidized using a slight excess of $NaIO_4$ leading to the exclusive formation of the corresponding glyoxylic aldehyde (CO–CHO) group. The coupling reaction was first studied using the NLS peptide **16a**. Reaction was carried out in ammonium acetate buffer at slightly acidic pH (4.6) using 3-fold excess of the complex **1** and monitored by reverse phase HPLC. The reaction proceeded to completion within 4 h to yield the conjugate **17a** as the major product. It was noted that the afore-mentioned bis reaction could not occur in this case as the glyoxylic aldehyde has no hydrogen at a position of the aldehydic function. The crude mixture was purified by C18 reverse phase HPLC and the conjugate **17a** was obtained in 45% isolated yield. Using the same procedure, complex **1** was further reacted with Tat peptide **16b** and the conjugate **17b** was obtained in 43% yield after HPLC purification. The conjugates **17a** and **17b** were characterized by ESI-MS analysis that showed an excellent agreement between the experimentally determined molecular weights and the calculated values.

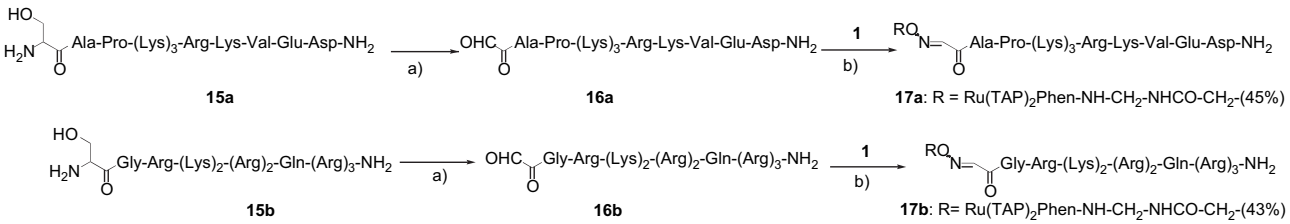
3. Conclusion

In conclusion, we present a very convenient and facile strategy to prepare oligonucleotide conjugates sustaining a ruthenium complex at the 3′- or 5′-end. This strategy is also applicable to the preparation of peptide–ruthenium complex conjugates. The whole procedure involves the preparation of ruthenium complexes bearing an aminooxy containing ligand, which was introduced by the conventional method during the course of complexes syntheses. After cleavage of the protecting group on the reactive aminooxy moiety, the conjugation with reactive aldehyde containing oligonucleotides and peptides is performed under mild conditions that are compatible with the stability of the Ru(II) complexes. It should be noted that cautions have to be used during the oligonucleotide conjugation due to the fact that two aliphatic aldehyde containing oligonucleotides can be attached to one aminooxy reacting group. The results reported herein therefore open up new prospects in the preparation and availability of a large variety of metal complex–oligonucleotide and peptide conjugates. The studies of the different oligonucleotide–ruthenium complex and peptide–ruthenium complex conjugates are currently under investigation.

4. Experimental

4.1. General

All solvents and reagents used were of highest purity available. *N*-Boc-*O*-(carboxymethyl)-hydroxylamine was purchased from Fluka. The solid support 3-[(4,4′-dimethoxytrityl)-glyceryl-1-succinyl] long chain alkylamino CPG) was purchased from Eurogentec. The diol containing oligonucleotides **10a** and **10b** were purified on a μ-Bondapak C-18 column (Macherey-Nagel Nucleosil: 10×250 mm, 7 μm) using the following system of solvents: solvent A, 20 mM ammonium acetate/CH_3CN, 95:5 (v/v); solvent B (CH_3CN); flow rate, 4 mL min^{-1}; a linear gradient from 0 to 30% B in 20 min was applied. The purity of the product was assessed on analytical column using the same gradient at a flow rate of 1 mL min^{-1}. The oligonucleotide conjugates **12a**,**b** and **14a**,**b** were purified by denaturing polyacrylamide gel electrophoresis (PAGE). The peptide conjugates **17a**,**b** were purified on a Delta Pak™ C-18 column (Waters: 25×200 mm, 15 μm). Following system of solvents was used: solvent A, H_2O/TFA 99.9:0.1 (v/v); solvent B, CH_3CN/H_2O/TFA, 90:10:0.1 (v/v/v); flow rate 22 mL min^{-1}; a linear gradient from 5 to 100% B in 30 min was applied. Mass spectra were measured on a Polarisq (Thermo-Finnigan) for EI, on an Esquire 3000 (Bruker) for ESI and on a MALDI-TOF (Bruker). The analysis was performed in the positive mode for the peptide conjugates and in negative mode for



Scheme 3. Preparation of peptide–Ru(II) complex conjugates **17a**,**b**; (a) $NaIO_4$ (3 equiv), H_2O, 1 h; (b) 0.4 M ammonium acetate buffer, rt, overnight.

oligonucleotides using 50% aqueous acetonitrile as eluent. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Spectrospin spectrometer.

4.2. Syntheses of ligands

The 5-amino-1,10-phenanthroline **3a** has been prepared from commercially available 5-nitro-1,10-phenanthroline by reduction with Pd/C/N_2H_4.[21] The preparation of 9-amino-1,4,5,8-tetraazaphenanthrene **3b** has been reported previously.[22] The *N*-hydroxy succinimide activated ester **6** was prepared from *N*-Boc-*O*-(carboxymethyl)-hydroxylamine by using standard protocol.

4.2.1. 5-(*N*-(*tert*-Butoxycarbonyl)glycinamido)-1,10-phenanthroline (4a). A solution of commercial *N*-(*tert*-butoxycarbonyl)glycine (2.7 g, 15 mmol) and DCC (1.6 g, 7.8 mmol) in acetonitrile (30 mL) was stirred at room temperature for 1 h and filtered to remove the DCU precipitate. The so-formed anhydride was used without further purification and added to a solution of 5-amino-1,10-phenanthroline **3a** (304 mg, 1.55 mmol) in acetonitrile (30 mL). The solution was stirred at room temperature for 20 h under argon and the solvent was removed under reduced pressure. The ligand **4a** (445 mg, 81%) was obtained as a pale yellow powder after purification by silica gel column chromatography using 2–6% MeOH in CH_2Cl_2 as eluent. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.43 (s, 9H, *t*-Bu), 3.94 (d, J=5.5 Hz, 2H, CH_2NH), 7.26 (t, J=5.5 Hz, NH), 7.74 (dd, J=8.0 Hz, J=4.0 Hz, 1H, H–Ar), 7.80 (dd, J=8.0 Hz, J=4.0 Hz, 1H, H–Ar), 8.14 (s, 1H, H–Ar), 8.46 (dd, J=8.0 Hz, J=1.5 Hz, 1H, H–Ar), 8.64 (dd, J=8.0 Hz, J=1.5 Hz, 1H, H–Ar), 9.04 (dd, J=4.0 Hz, J=1.5 Hz, 1H, H–Ar), 9.12 (dd, J=4.0 Hz, J=1.5 Hz, 1H, H–Ar), 10.28 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 28.1 (CH_3), 43.8 (CH_2), 78.1 (Cq), 120.1 (CH), 122.7 (CH), 123.5 (CH), 124.7 (Cq), 128.0 (Cq), 131.7 (Cq), 131.8 (CH), 135.7 (CH), 143.7 (Cq), 145.7 (Cq), 149.3 (CH), 149.8 (CH), 156.0 (Cq), 169.6 (Cq). Anal. Calcd for $C_{19}H_{20}N_4O_3\cdot 1H_2O$: C, 61.61; H, 5.80; N, 14.79. Found: C, 62.06; H, 5.80; N, 14.79. HRMS (ESI) *m*/*z* 353.1603 (M+H$^+$ $C_{19}H_{21}N_4O_3$ requires 353.1608).

4.2.2. 9-(*N*-(*tert*-Butoxycarbonyl)glycinamido)-1,4,5,8-tetraazaphenanthrene (4b). The ligand **4b** was prepared from 9-amino-1,4,5,8-tetraazaphenanthrene **3b** (150 mg, 0.76 mmol) by using the same protocol as mentioned above for **4a**. Purification by silica gel column chromatography (eluent 0–2% MeOH in CH_2Cl_2) gave the ligand **4b** (164 mg, 61%) as pale yellow powder. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.46 (s, 9H, *t*-Bu), 3.97 (d, J=6.0 Hz, 2H, CH_2NH), 7.61 (t, J=6.0 Hz, 1H, NH), 9.07 (m, 2H, H–Ar), 9.10 (d, J=2.0 Hz, 1H, H–Ar), 9.20 (d, J=2.0 Hz, 1H, H–Ar), 9.33 (d, J=2.0 Hz, 1H, H–Ar), 10.53 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 28.1 (CH_3), 44.9 (CH_2), 78.70 (Cq), 114.9 (CH), 134.9 (Cq), 135.7 (Cq), 137.0 (Cq), 140.2 (Cq), 143.8 (CH), 144.4 (Cq), 145.0 (CH), 146.4 (CH), 147.1 (CH), 156.0 (Cq), 169.7 (Cq). Anal. Calcd for $C_{17}H_{18}N_6O_3$: C, 57.62; H, 5.12; N, 23.72. Found: C, 57.78; H, 5.26; N, 23.30. HRMS (ESI) *m*/*z* 377.1327 (M+Na$^+$ $C_{17}H_{18}N_6O_3Na$ requires 377.1333).

4.2.3. 5-(Glycinamido)-1,10-phenanthroline (5a). The protected phenanthroline derivative **4a** (102 mg, 0.29 mmol) was stirred in a CH_2Cl_2/TFA solution (10 mL, 50:50, v/v) at room temperature for 2 h. The solvent was then removed under vacuum and the product **5a** was obtained in quantitative yield and was used without further purification. ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.08 (s, 2H, CH_2NH), 7.85 (dd, J=8.0 Hz, J=4.0 Hz, 1H, H–Ar), 7.91 (dd, J=8.0 Hz, J=4.0 Hz, 1H, H–Ar), 8.23 (s, 1H, H–Ar), 8.34 (br s, 2H, NH_2), 8.60 (dd, J=8.0 Hz, J=1.5 Hz, 1H, H–Ar), 8.75 (dd, J=8.0 Hz, J=1.5 Hz, 1H, H–Ar), 9.09 (dd, J=4.0 Hz, J=1.5 Hz, 1H, H–Ar), 9.17 (dd, J=4.0 Hz, J=1.5 Hz, 1H, H–Ar), 10.80 (br s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 41.0 (CH_2), 119.8 (CH), 123.5 (CH), 124.2 (CH), 124.6 (Cq), 128.2 (Cq), 131.3 (Cq), 132.7 (CH), 137.8 (CH), 141.7 (Cq), 144.0 (Cq), 148.7 (CH), 150.0 (CH), 166.4 (Cq). MS (EI) *m*/*z* 252 (M)$^+$.

4.2.4. 9-(Glycinamido)-1,4,5,8-tetraazaphenanthrene (5b). The derivative **5b** was obtained from protected derivative **4b** (164 mg, 0.46 mmol) by using the same protocol as discussed for **5a** and was obtained in quantitative yield and used without further purification. ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.19 (s, 2H, CH_2NH), 8.30 (br s, 2H, NH_2), 9.11 (m, 3H, H–Ar), 9.24 (d, J=2.0 Hz, 1H, H–Ar), 9.34 (d, J=2.0 Hz, 1H, H–Ar), 11.11 (br s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 41.7 (CH_2), 116.5 (CH), 135.2 (Cq), 136.1 (Cq), 137.2 (Cq), 140.2 (Cq) 144.2 (CH+Cq), 145.0 (CH), 146.3 (CH), 147.2 (CH), 168.9 (Cq). MS (EI) *m*/*z* 254 (M)$^+$.

4.2.5. Protected phenanthroline ligand (7a). To a suspension of the deprotected phenanthroline derivative **5a** (73 mg, 0.29 mmol) in anhydrous DMF (15 mL), the activated ester of *N*-Boc-*O*-(carboxymethyl)-hydroxylamine **6** (112 mg, 0.39 mmol) and DIEA (0.167 mL, 0.94 mmol) were added. The mixture was stirred at room temperature for 1 h under argon. The solvent was then removed under reduced pressure and the product was purified by silica gel column chromatography (eluent 5–8% MeOH in CH_2Cl_2). The protected ligand **7a** (68 mg, 55%) was obtained as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.40 (s, 9H, *t*-Bu), 4.18 (d, J=5.5 Hz, 2H, CH_2NH), 4.30 (s, 2H, CH_2–O), 7.75 (dd, J=8.0 Hz, J=4.0 Hz, 1H, H–Ar), 7.82 (dd, J=8.0 Hz, J=4.0 Hz, 1H, H–Ar), 8.14 (s, 1H, H–Ar), 8.43 (t, J=5.5 Hz, 1H, NH), 8.46 (dd, J=8.0 Hz, J=1.5 Hz, 1H, H–Ar), 8.62 (dd, J=8.0 Hz, J=1.5 Hz, 1H, H–Ar), 9.05 (dd, J=4.0 Hz, J=1.5 Hz, 1H, H–Ar), 9.13 (dd, J=4.0 Hz, J=1.5 Hz, 1H, H–Ar), 10.22 (br s, 1H, NH), 10.31 (br s, 1H, O–NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 27.9 (CH_3), 42.3 (CH_2), 74.7 (CH_2), 80.5 (Cq), 120.5 (CH), 122.8 (CH), 123.5 (CH), 124.7 (Cq), 127.9 (Cq), 131.4 (Cq), 131.7 (CH), 135.8 (CH), 143.9 (Cq), 145.8 (Cq), 149.4 (CH), 149.8 (CH), 156.6 (Cq), 168.6 (Cq). Anal. Calcd for $C_{21}H_{23}N_5O_5\cdot 0.5\ H_2O$: C, 58.06; H, 5.57; N, 16.12. Found: C, 58.10; H, 5.98; N, 16.08. HRMS (ESI) *m*/*z* 426.1764 (M+H$^+$ $C_{21}H_{24}N_5O_5$ requires 426.1772).

4.2.6. Protected tetraazaphenanthrene ligand (7b). The protected ligand **7b** was obtained in 76% yield (149 mg, 0.35 mmol) from tetraazaphenanthrene derivative **5b** (117 mg, 0.46 mmol) after purification by silica gel column chromatography (eluent 0–5% MeOH in CH_2Cl_2) using the same protocol as above. ^{1}H NMR (300 MHz, DMSO-d_6):

δ 1.40 (s, 9H, *t*-Bu), 4.27 (d, *J*=6.0 Hz, 2H, CH_2NH), 4.34 (s, 2H, CH_2–O), 8.61 (t, *J*=6.0 Hz, 1H, NH), 9.02 (m, 1H, H–Ar), 9.05 (d, *J*=2.0 Hz, 1H, H–Ar), 9.08 (d, *J*=2.0 Hz, 1H, H–Ar), 9.20 (d, *J*=2.0 Hz, 1H, H–Ar), 9.32 (d, *J*=2.0 Hz, 1H, H–Ar), 10.33 (s, 1H, NH), 10.58 (br s, 1H, O–NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 27.8 (CH_3), 43.5 (CH_2), 74.5 (CH_2), 80.6 (Cq), 115.3 (CH), 135.2 (Cq), 135.8 (Cq), 137.0 (Cq), 140.1 (Cq) 143.9 (CH), 144.4 (Cq), 145.0 (CH), 146.3 (CH), 147.1 (CH), 156.7 (Cq), 168.7 (Cq), 168.8 (Cq). HRMS (ESI) *m/z* 450.1488 ($M+H^+$ $C_{19}H_{21}N_7O_5Na$ requires 450.1496).

4.3. Synthesis of complexes

The synthesis and purification of the complex [Ru(TAP)$_2$Cl$_2$] **8a** was reported previously.[18]

4.3.1. Protected [Ru(TAP)$_2$phen″]$^{2+}$ complex (9a). The activated [Ru(TAP)$_2$(H$_2$O)$_2$]$^{2+}$ complex **8b** was prepared by adding $AgNO_3$ (32 mg, 0.19 mmol) in an aqueous suspension of [Ru(TAP)$_2$Cl$_2$] **8a** (52 mg, 0.095 mmol). The solution was stirred and refluxed for 2 h followed by centrifugation to eliminate the formed AgCl precipitate. The solvent was removed under reduced pressure and the crude mixture was added to a solution of phenanthroline ligand **7a** (30 mg, 0.071 mmol) in DMF (15 mL). The reaction mixture was stirred at 100 °C under argon for 1 h. The DMF was then removed under reduced pressure and the crude mixture was purified by basic alumina column chromatography (eluent 0–10% H_2O in CH_3CN). The protected aminooxy complex **9a** (60 mg, 85%) was obtained as an orange solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.40 (s, 9H, *t*-Bu), 4.23 (d, *J*=6.0 Hz, 2H, CH_2NH), 4.28 (s, 2H, CH_2O), 7.74 (dd, *J*=8.0 Hz, *J*=5.0 Hz, 1H, H–Ar), 7.82 (dd, *J*=8.0 Hz, *J*=5.0 Hz, 1H, H–Ar), 8.17 (d, *J*=5.0 Hz, 1H, H–Ar), 8.23 (dd, *J*=3.0 Hz, 2H, H–Ar), 8.30 (d, *J*=5.0 Hz, 1H, H–Ar), 8.47 (m, 3H, H–Ar and NH), 8.62 (s, 1H, H–Ar), 8.65 (s, 4H, H–Ar), 8.79 (d, *J*=8.0 Hz, 1H, H–Ar), 8.99 (d, *J*=8.0 Hz, 1H, H–Ar), 9.04 (m, 4H, H–Ar), 10.32 (s, 1H, NH), 10.71 (s, 1H, O–NH). ^{13}C NMR (75 MHz, DMSO-d_6) 27.9 (CH_3), 42.6 (CH_2), 74.5 (CH_2), 80.5 (Cq), 119.4 (CH), 125.6 (CH), 126.4 (CH), 130.2 (Cq), 132.3 (CH), 133.5 (CH), 136.9 (Cq), 137.3 (CH), 141.8 (Cq), 141.9 (Cq), 144.1 (Cq), 144.4 (Cq), 146.7 (Cq), 148.6 (CH), 149.5 (CH), 149.6 (CH), 152.8 (CH), 154.0 (CH), 156.6 (Cq), 167.0 (Cq), 168.6 (Cq). MS (ESI) *m/z* 445.6 $(M)^{2+}$, 926.2 $(M+Cl)^+$.

4.3.2. Protected [Ru(TAP)$_2$TAP″]$^{2+}$ complex (9b). The ruthenium complex **9b** was obtained as an orange solid in 69% yield (65 mg, 0,067 mmol) from the TAP containing ligand **7b** (41 mg, 0,096 mmol) by the same protocol as mentioned above. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.41 (s, 9H, *t*-Bu), 4.29 (d, *J*=6.0 Hz, 2H, CH_2NH), 4.30 (s, 2H, CH_2O), 8.35 (d, *J*=3.0 Hz, 1H, H–Ar), 8.52 (m, 4H, H–Ar), 8.58 (d, *J*=3.0 Hz, 1H, H–Ar), 8.67 (s, 4H, H–Ar), 8.72 (t, *J*=6.0 Hz, NH), 8.99 (d, *J*=3.0 Hz, 1H, H–Ar), 9.04 (d, *J*=3.0 Hz, 1H, H–Ar), 9.09 (m, 4H, H–Ar), 9.26 (s, 1H, H–Ar), 10.38 (s, 1H, NH), 10.89 (s, 1H, O–NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 27.9 (CH_3), 43.2 (CH_2), 74.4 (CH_2), 80.5 (Cq), 115.4 (CH), 132.2 (CH), 137.1 (Cq), 137.4 (Cq), 138.0 (Cq), 141.3 (Cq), 141.7 (Cq), 144.4 (Cq), 145.2 (Cq), 147.4 (CH), 147.9 (CH), 149.7 (CH), 149.9 (CH), 150.2 (CH), 156.6 (Cq), 168.8 (Cq), 169.3 (Cq). MS (ESI) *m/z* 446.6 $(M)^{2+}$, 928.2 $(M+Cl)^+$.

4.3.3. [Ru(TAP)$_2$phen″]$^{2+}$Cl$_2$ complex (1). The protected complex **9a** (8 mg, 8.3 μmol) was dissolved in 1 N HCl aqueous solution and the reaction mixture was stirred at room temperature for 6 h. The solvent was then evaporated under vacuum to obtain the aminooxy containing [Ru(TAP)$_2$phen″]$^{2+}$Cl$_2$ complex **1** (3.2 mg, 4 μmol), which was then used for the coupling reaction with peptides and oligonucleotides without further purification. MS (ESI) *m/z* 395.5 $(M)^{2+}$, 826.0 $(M+Cl)^+$.

4.3.4. [Ru(TAP)$_2$TAP″]$^{2+}$Cl$_2$ complex (2). The complex **2** (3.8 mg, 4.72 μmol) was obtained from protected complex **9b** (9.9 mg, 10.2 μmol) using the similar protocol. MS (ESI) *m/z* 396.4 $(M)^{2+}$.

4.4. Aldehyde functionalized oligonucleotides

The oligonucleotides **11a** and **11b** functionalized at 5′ or 3′-ends by an aldehyde group have been prepared following the previously reported protocol of a post-synthetic oxidation of a diol moiety.[13] Automated synthesis of oligodeoxynucleotides (ODNs) was carried out on an Expedite DNA synthesizer (Perkin–Elmer) by using standard β-cyanoethyl nucleoside phosphoramidite protocols at 1 μM scale. The automated synthesis was carried out on a modified solid support, 3-[(4,4′-dimethoxytrityl)-glyceryl-1-succinyl] long chain alkylamino controlled pore glass for the introduction of the 3′-protected diol group. For the incorporation of 5′-diol, the corresponding phosphoramidite linker bearing 1,2 protected diol was used during the last step of DNA synthesis. After, ODNs were cleaved from the solid support and released into the solution by treatment with 28% ammonia (1.5 mL) for 2 h followed by final deprotection by incubating the ammonia solution for 16 h at 55 °C. The oligonucleotides **10a** and **10b** were obtained after HPLC purification and treatment with 80% aqueous acetic acid for 1 h at room temperature (standard procedure). The ODNs **10a** and **10b** bearing 1,2-diol moiety were subjected to oxidative cleavage with aqueous sodium-*m*-periodate to obtain ODNs **11a** and **11b** with aldehyde functionality after C18 reverse phase purification. MS (ESI) **10a**: *m/z* calcd 5350.6, found 5349.4 $(M-H)^-$; **10b**: *m/z* calcd 5308.5, found 5307.9 $(M-H)^-$; **11a**: *m/z* calcd 5318.6, found 5318.2 $(M-H)^-$; **11b**: *m/z* calcd 5276.5, found 5276.1 $(M-H)^-$.

4.5. Aldehyde functionalized peptides

Peptides **16a** and **16b** were prepared by using a post-synthetic oxidation strategy of N-terminal serine residue by the method previously described.[13] Peptides **15a** and **15b** bearing the serine residue at N-terminal were prepared by SPPS using Fmoc/*t*-Bu chemistry with Rink amide MBHA resin (loading 0.91 mmol g^{-1}). The coupling reactions were performed for 25 min by using a 2.3-fold excess of *N*-Fmoc protected amino acid, PyBOP, and DIEA in DMF. The *N*-Fmoc protecting groups were removed by three consecutive treatments with piperidine/DMF solution (1:4 v/v 10 mL/g resin) for 10 min. Cleavage from the resin and deprotection of the side chain were achieved using TFA/H_2O/TIS (95:2.5:2.5) for 2 h. Subsequent oxidation of the

serine residue with $NaIO_4$ (3 equiv) in water for 1 h gave the aldehyde containing peptide **16a** and **16b,** which were purified by HPLC. MS (ESI) **15a**: *m/z* calcd 1284.5, found 1284.4 $(M+H)^+$; **15b**: *m/z* calcd 1482.8, found 1482.6 $(M+H)^+$; **16a**: *m/z* calcd 1253.5, found 1253.8 $(M+H)^+$; **16b**: *m/z* calcd 1451.7, found 1451.6 $(M+H)^+$.

4.6. General procedure for coupling reaction between oligonucleotides and Ru(II)-complexes

To a solution of aldehyde containing oligonucleotides in ammonium acetate buffer (pH=4.5), a solution of complex **1** or **2** in water (2 equiv) was added and the resultant reaction mixture (final concn of oligonucleotide=5 mM) was stirred at room temperature for overnight. The solvent was then evaporated under vacuum and the crude product was dissolved in 7 M aqueous urea solution. The conjugates were purified by denaturing PAGE using 150 mL acrylamide/bis-acrylamide (19:1), 30 mL TBE buffer, 150 g urea, 10.5 mL H_2O, 20 mL TEMED, and 2 mL 10% APS. The bands containing the conjugates were visualized on the gel by UV shadowing, removed from the gel, crushed, and extracted with water. Then, the conjugates were desalted by using C18 reverse phase chromatography. The conjugates were obtained in 35–50% yield and characterized by MALDI-TOF mass analysis. **12a**: *m/z* calcd 6091.2, found 6090.4 $(M-H)^-$; **12b**: *m/z* calcd 6093.5, found 6090.9 $(M-H)^-$; **14a**: *m/z* calcd 6049.1, found 6049.3 $(M-H)^-$; **14b**: *m/z* calcd 6051.3, found 6051.6 $(M-H)^-$.

4.7. General procedure for coupling reaction with peptides

To a solution of peptide **16a** or **16b** in ammonium acetate buffer (pH=4.5), a solution of complex **1** in water (3 equiv) was added and the resultant reaction mixture (final concn of peptides=0.02 M) was stirred at room temperature for overnight. The crude product was purified by reverse phase HPLC to afford the conjugates **17a** and **17b** in 45% yield. MS (ESI) **17a**: *m/z* (ESI) calcd 2026.3, found 2023.4 $(M+H)^+$; **17b**: *m/z* calcd 2224.5, found 2223.3 $(M+H)^+$.

Acknowledgements

The authors wish to thank the Centre National de la Recherche Scientifique (CNRS-France), the Fonds National de la Recherche Scientifique (FNRS-Belgium) through a Laboratoire Européen Associé (L.E.A.) and the ARC program 2002–2007 (Action de Recherche Concertée, Belgium) for financial support. The COST D35 is gratefully acknowledged. M.V. and S.D. wish to thank the Région Rhône-Alpes and the FRIA (Fonds pour la Recherche dans l'Industrie et l'Agriculture), respectively, for fellowships.

References and notes

1. (a) Ma, D. D.; Rede, T.; Naqui, T. A.; Cook, P. D. *Biotechnol. Annu. Rev.* **2000**, *5*, 155–196; (b) Tuschl, T. *ChemBioChem* **2001**, *2*, 239–245; (c) Praseuth, D.; Guieysse, A. L.; Helene, C. *Biochim. Biophys. Acta* **1999**, *1489*, 181–206; (d) Hermann, T.; Patel, D. J. *Science* **2000**, *287*, 820–825.
2. (a) Seeman, N. C. *Angew. Chem., Int. Ed.* **1998**, *37*, 3220–3238; (b) Park, S. H.; Pistol, C.; Ahn, S. J.; Reif, J. H.; Lebeck, A. R.; Dwyer, C.; LaBean, T. H. *Angew. Chem., Int. Ed.* **2006**, *45*, 735–739; (c) Chandra, M.; Keller, S.; Gloeckner, C.; Bornemann, B.; Marx, A. *Chem.—Eur. J.* **2007**, *13*, 3558–3564; (d) Feldkamp, U.; Niemeyer, C. M. *Angew. Chem., Int. Ed.* **2006**, *45*, 1856–1876.
3. (a) Fischer, R.; Fotin-Mleczek, M.; Hufnagel, H.; Brock, R. *ChemBioChem* **2005**, *6*, 2126–2142; (b) Venkatesan, N.; Kim, B. H. *Chem. Rev.* **2006**, *106*, 3712–3761; (c) Vives, E.; Richard, J. P.; Lebleu, B. *Nucleic Acid Therapeutics in Cancer*; Gewirtz, A. M., Ed.; Humana: Totowa, NJ, 2004; pp 115–127; (d) Valkna, A.; Soomets, U.; Langel, U. *Cell-Penetrating Peptides*; Langel, U., Ed.; CRC LLC: Boca Raton, FL, 2002; pp 347–363.
4. (a) Giese, B. *Acc. Chem. Res.* **2000**, *33*, 631–636; (b) Lewis, F. D.; Letsinger, R. L.; Wasielewski, M. R. *Acc. Chem. Res.* **2001**, *34*, 159–170; (c) Beilstein, A. E.; Tierney, M. T.; Grinstaff, M. W. *Comments Inorg. Chem.* **2000**, *22*, 105–127; (d) Zatsepin, T. S.; Andreev, S. Y.; Hianik, T.; Oretskaya, T. S. *Russ. Chem. Rev.* **2003**, *72*, 537–554; (e) Lentzen, O.; Defrancq, E.; Constant, J.-F.; Schumm, S.; Garcia-Fresnadillo, D.; Moucheron, C.; Dumy, P.; Kirsch-De Mesmaeker, A. *J. Biol. Inorg. Chem.* **2004**, *9*, 100–108.
5. (a) Mitra, D.; Di Cesare, N.; Sleiman, H. F. *Angew. Chem., Int. Ed.* **2004**, *43*, 5804–5808; (b) Stewart, K. M.; Rojo, J.; Mc Laughlin, L. W. *Angew. Chem., Int. Ed.* **2004**, *43*, 5808–5811; (c) Choi, J. S.; Kang, C. W.; Jung, K.; Yang, J. W.; Kim, Y.-G.; Han, H. *J. Am. Chem. Soc.* **2004**, *126*, 8606–8607; (d) Aldaye, F. A.; Sleiman, H. F. *Angew. Chem., Int. Ed.* **2006**, *45*, 2204–2209.
6. (a) Núñez, M. E.; Barton, J. K. *Cur. Opin. Chem. Biol.* **2000**, *4*, 199–206; (b) Zhang, C. X.; Lippard, S. J. *Cur. Opin. Chem. Biol.* **2003**, *7*, 481–489; (c) Elias, B.; Kirsch-De Mesmaeker, A. *Coord. Chem. Rev.* **2006**, *250*, 1627–1641.
7. (a) Ossipov, D.; Pradeepkumar, P. I.; Holmer, M.; Chattopadhyaya, J. *J. Am. Chem. Soc.* **2001**, *123*, 3551–3562; (b) Khan, S. I.; Beilstain, A. E.; Sykora, M.; Smith, G. D.; Hu, X.; Grinstaff, M. W. *Inorg. Chem.* **1999**, *38*, 3922–3925; (c) Bannwarth, W.; Schmidt, D. *Tetrahedron Lett.* **1989**, *30*, 1513–1516; (d) Rack, J. J.; Krider, E. S.; Meade, T. J. *J. Am. Chem. Soc.* **2000**, *122*, 6287–6288.
8. Holmlin, E. R.; Dandliker, P. J.; Barton, J. K. *Bioconjugate Chem.* **1999**, *10*, 1122–1130.
9. Khan, S. I.; Grinstaff, M. W. *J. Am. Chem. Soc.* **1999**, *121*, 4704–4705.
10. (a) Murphy, C. J.; Arkin, M. R.; Jenkins, Y.; Ghatlia, N. D.; Bossmann, S. H.; Turro, N. J.; Barton, J. K. *Science* **1993**, *262*, 1025–1029; (b) Ortmans, I.; Content, S.; Boutonnet, N.; Kirsch-De Mesmaeker, A.; Bannwarth, W.; Constant, J.-F.; Defrancq, E.; Lhomme, J. *Chem.—Eur. J* **1999**, *5*, 2712–2721; (c) Crean, C. W.; Kavanagh, Y. T.; O'Keeffe, C. M.; Lawler, M. P.; Stevenson, C. R.; Davies, J. H.; Boyle, P. H.; Kelly, J. M. *Photochem. Photobiol. Sci.* **2002**, *1*, 1024–1033.
11. (a) Peek, B. M.; Ross, G. T.; Edwards, S. W.; Meyer, G. J.; Meyer, T. J.; Erickson, B. W. *Int. J. Pept. Protein Res.* **1991**, *38*, 114–123; (b) Myari, A.; Hadjiliadis, N.; Garoufis, A. *J. Inorg. Biochem.* **2005**, *99*, 616–626.
12. (a) Copeland, K. D.; Fitzsimons, M. P.; Houser, R. P.; Barton, J. K. *Biochemistry* **2002**, *41*, 343–356; (b) Brunner, J.; Barton, J. K. *J. Am. Chem. Soc.* **2006**, *128*, 6772–6773.
13. (a) Forget, D.; Boturyn, D.; Defrancq, E.; Lhomme, J.; Dumy, P. *Chem.—Eur. J.* **2001**, *7*, 3976–3984; (b) Singh, Y.; Renaudet,

O.; Defrancq, E.; Dumy, P. *Org. Lett.* **2005**, *7*, 1359–1362; (c) Edupuganti, O. P.; Singh, Y.; Defrancq, E.; Dumy, P. *Chem.—Eur. J.* **2004**, *10*, 5988–5995.
14. (a) Lentzen, O.; Constant, J.-F.; Defrancq, E.; Prevost, M.; Schumm, S.; Moucheron, C.; Dumy, P.; Kirsch-De Mesmaeker, A. *ChemBioChem* **2003**, *4*, 195–202; (b) Uji-i, H.; Foubert, P.; De Schryver, F.; De Feyter, S.; Gicquel, E.; Etoc, A.; Moucheron, C.; Kirsch-De Mesmaeker, A. *Chem.—Eur. J.* **2006**, *12*, 758–762.
15. Lee, H. J.; Pardridge, W. M. *Bioconjugate Chem.* **2001**, *12*, 995–999.
16. Bremner, K. H.; Seymour, L. W.; Logan, A.; Read, M. L. *Bioconjugate Chem.* **2004**, *15*, 152–161.
17. Deroo, S.; Defrancq, E.; Moucheron, C.; Kirsch-De Mesmaeker, A.; Dumy, P. *Tetrahedron Lett.* **2003**, *44*, 8379–8382.
18. Kirsch-De Mesmaeker, A.; Jacquet, L.; Nasielski, J. *Inorg. Chem.* **1988**, *27*, 4451–4458.
19. Tronchet, J. M. J.; Zosimo-Landolfo, G.; Balkadjian, M.; Ricca, A.; Zsély, M.; Barbalat-Rey, F.; Cabrini, D.; Lichtle, P.; Geoffroy, M. *Tetrahedron Lett.* **1991**, *32*, 4129–4132.
20. Liu, C. F.; Tam, J. P. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 6584–6588.
21. Lecomte, J. P.; Kirsch-De Mesmaeker, A.; Demeunynck, M.; Lhomme, J. *J. Chem. Soc., Faraday Trans.* **1993**, *89*, 3261–3269.
22. Nasielski-Hinkens, R.; Benedek-Vamos, M.; Maetens, D. *J. Heterocycl. Chem.* **1980**, *17*, 873–876.