

In-Strand Metallated Nucleic Acids – Novel Bioinorganic Constructs

Edwin C. Constable^{a*}, Valérie Chaurin^a, Catherine E. Housecroft^a, and Amina Wirth^b

Abstract: Synthetic strategies have been developed for the preparation of in-chain metallated oligonucleotides, in which the oligonucleotide sequence is interrupted by a metal-containing motif. In our work, oligopyridine metal-binding domains are used and approaches are outlined using both labile and non-labile metal centres.

Keywords: Antisense · DNA · Oligopyridine · RNA · Ruthenium

Introduction

Nucleic acids are fundamental components of living systems that act as the genetic information pool for individuals and by extension for species and the entire biosphere. Apart from the inherent chemical interest in nucleic acids as supramolecules and the desire to understand their precise roles and regulation in the development of individual organisms, there are compelling medical and socio-economic reasons for their study. Numerous disease states are genetically linked, are associated with nucleic acid damage or are a direct consequence of invasion of the parent organism by other pathogenic organisms whose behaviour and action is controlled by their own nucleic acids. From a medical view-point, it is a high priority to develop methods for switching individual genes on or off, for detecting and

deleting damaged nucleic acid and for targeting the nucleic acids of specific pathogenic species. In a wider sense, DNA and RNA are information-storage molecules *par excellence*. They represent the proof of concept for molecule-based information storage systems and give a hint at the density of information which may be stored in molecule-based computing systems and clues as to the technologies that must be developed for read–write processes involving such soft storage devices [1].

Our interests in nucleic acids originate in our ongoing research in supramolecular chemistry and nanoscale devices and are expressed in a synthetic programme leading to the preparation of nucleic acids in which metal complexes are covalently linked to the oligonucleotide backbone. In this article, we present a brief overview of the literature relating to such species and some aspects of our own work in which metal complexes are incorporated into the backbone of the nucleotide to give in-chain metallated species.

Nucleic Acids and Metal Ions

In biology, nucleic acids are intimately associated with metal ions, whether they simply act as counterions or in more specific interactions with species such as magnesium and manganese in phosphate metabolism [2]. Recently, metal complexes which interact directly with nucleic acids and bind to them have been used as therapeutics, particularly in cancer chemotherapy [3]. Current and projected therapeutic applications of metal-containing drugs that target nucleic acids have a particular emphasis upon the recognition of specific sequences or genes, by covalently modifying

a site within the gene, impeding or altering the transcription of the nucleic acid, or by causing hydrolysis of the nucleic acid. It is a single step from targeting specific genes within a nucleic acid to developing an antisense methodology, in which single strand oligonucleotides or nucleic acids are actually used as therapeutics. Concurrent with the development and optimisation of therapeutics that target or are based upon nucleic acids, is the utilisation of information from the human genome project in general and the need to characterise specific nucleotide sequences in the context of PCR methods. These latter considerations have led us and others to consider unnatural bioconstructs in which metal centres are incorporated within an oligonucleotide chain [4]. We emphasise that this approach is fundamentally different to those based upon the interaction of discrete metal complexes with nucleic acids [5] or of that in which metal-containing functionality is appended to the backbone of a nucleotide or nucleic acid or is attached to a 3'- or 5'-terminal [4]. The strategy is conceptually similar to that which has been used for the metal-directed synthesis of artificial DNA-arrays, such as triangles, by the self assembly of self-complementary sequences functionalised with metal-binding domains [6].

Strategy

The basic strategy is presented in Fig. 1: formally, two nucleotide sequences are required, each of which is terminated with a metal-binding domain. The metal-binding domain of choice for our work is an oligopyridine such as 2,2':6,2''-terpyridine (tpy), 2,2'-bipyridine (bpy) or 1,10-phenanthroline (phen) (Fig. 2) which have well-

*Correspondence: Prof. Dr. E.C. Constable^a

Tel.: +41 61 267 1001

Fax: +41 61 267 1015

E-Mail: edwin.constable@unibas.ch

^aDepartment of Chemistry

University of Basel

Spitalstrasse 51

CH-4056 Basel

^bFachhochschule beider Basel

Departement Industrie

Abteilung Chemie

Gründenstrasse 40

CH-4132 Muttenz

established coordination chemistry with almost every metal in the periodic table. These metal-binding domains have already been used for the attachment of pendant iron [7], europium [8], copper [9] and ruthenium [10] functionality. The approach is basically an antisense [11] or antigene [12] one, in which the metallated sequence is complementary to the target nucleotide or nucleic acid. We considered strategies involving both labile and non-labile metal ions. With labile metal ions (top route, Fig. 1), the two individual sequences are functionalised and allowed to react with the metal ion of choice, with a subsequent hybridisation step. The advantages and disadvantages of this strategy are presented later in this article. In our initial work, we adopted a methodology based upon kinetically inert ruthenium(II) centres which could be incorporated into an oligonucleotide sequence using standard solid phase methods (Fig. 1, lower route).

Synthesis of Bioconstructs with Kinetically Inert Metal Ions

We chose the kinetically inert $\{\text{Ru}(\text{tpy})_2\}^{2+}$ motif (tpy = 2,2':6',2''-terpyridine) for our initial studies as this is topologically linear and has well-defined electrochemical, spectroscopic and photophysical properties which will both allow facile detection of the new bioconstructs and might allow specific metal-mediated chemistry (hydrolysis, photocleavage *etc.*) within the complementary strand after hybridisation – in other words the site of incorporation of the metal-containing unit can be used to target a specific sequence or gene within the complementary DNA. Our synthetic method of choice was phosphoramidite chemistry at a solid support. The $\{\text{Ru}(\text{tpy})_2\}^{2+}$ unit could be incorporated in a single step, but this carries with it the necessity of preparing asymmetrically difunctionalised (one side protected, one side activated) complexes for incorporation into the solid-phase synthesis. Furthermore, to have a metallated strand complementary to a natural sequence, it is necessary to attach one of the tpy domains to the 3'-position of a nucleotide and the other to the 5'-position of the other nucleotide. Although we have used this route, we have found it to be low-yielding and very sensitive to environmental effects and have developed instead a highly efficient linear method (Fig. 3) in which the need for the difunctionalised complex is obviated. Using this route we obtain ruthenaoligonucleotides of generic structure 5'-S1COCH₂CH₂OCH₂CH₂Otpy R₂tpyOCH₂CH₂OCH₂CH₂OS2-3' (I: S1 = TTTTCTC S2 CTCTCT, II: S1 = TTCTTCT S2 CTCTCT, III: S1 = TTCTTC S2 CTCTCT) in yields of 90–95%. The new,

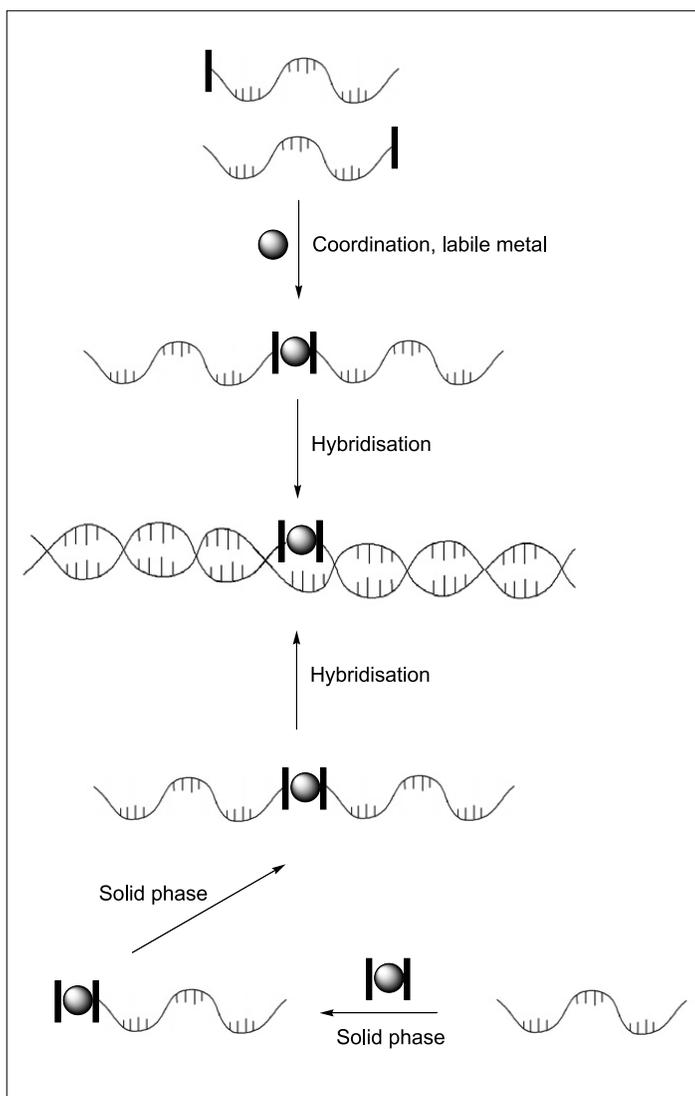


Fig. 1. Two approaches to the preparation of in-chain metallated oligonucleotides. In the upper route an oligonucleotide bearing a terminal metal-binding domain (black bar) is prepared and then reacted with a labile metal centre, whereas in the lower route a kinetically inert metal complex is inserted in a stepwise manner.

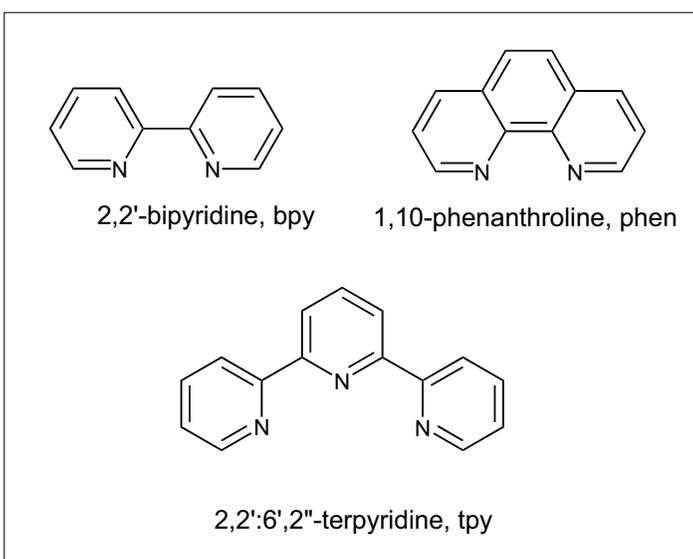


Fig. 2. Ligand structures

orange, oligonucleotides show the expected MLCT absorption at 484 nm, show parent ions in their MALDI-TOF mass spectra and are pure by HPLC and capillary electrophoresis analysis.

What properties do these new ruthenaoligonucleotides have? In general the melting points are lower than the best model native sequences, for example the three nucleotides **I**, **II**, and **III** in the presence of the complementary 3'-AAA-AAG-AGA-GAG-AGA-5' RNA have melting points of 34.3, 23.3 and 23.3 °C respectively, whereas wild-type 5'-TTT-TTC-TCT-CTC-TCT-3' shows a melting point of 50.4 °C. We considered that the most likely origin of the destabilisation was a mismatch resulting from the presence of the metal-containing unit and tested this in two ways. Firstly, we prepared a ruthenaoligonucleotide **IV** with a shorter OCH₂CH₂O spacer between the tpy and the first nucleotide and found for the sequence analogous to **I** a melting point of 34.2 °C – identical within experimental error. Secondly, we introduced a redundant triplet into the RNA complementary strand at the site of metallation using the sequence 3'-AAA-AAG-AGA-AAA-GAG-AGA-5' and found a slight stabilisation of **I** (35.1 °C) and a destabilisation of **IV** (30.2 °C). With a longer RNA complementary sequence 3'-AAA-AAG-AGA-AAAA-GAG-AGA-5' the melting points of both **I** and **IV** were slightly lowered.

We are currently investigating the photochemical and photophysical properties of these and related ruthenated systems.

Self-assembly of Bioconstructs with Labile Metal Ions

In the systems described above, we have used kinetically inert metal centres to construct stable metallated species which are stable both to biological and to chemical reaction conditions. A contrasting approach involves the use of labile metal ions to link together nucleic acids functionalised in the terminal positions to give new extended sequences. We are interested in this as a methodology for the construction of a complementary sequence for a target strand from a library of 'half-sequences' each functionalised with a metal-binding domain appropriate to the selected metal ion.

Our preliminary work has centred upon 6,6'-disubstituted 2,2'-bipyridine or 2,9-difunctionalised 1,10-phenanthroline ligands which are optimised for the binding and stabilisation of copper(I) in aqueous conditions. The choice of copper(I) is predicated upon the easy detection (spectrophotometric, luminescence, electrochemical) of the newly formed {Cu^IL₂} motif. The initial concept is simple: a library of functionalised sequences A, B, ..., n_i will react with copper(I) to form a new library of complex-

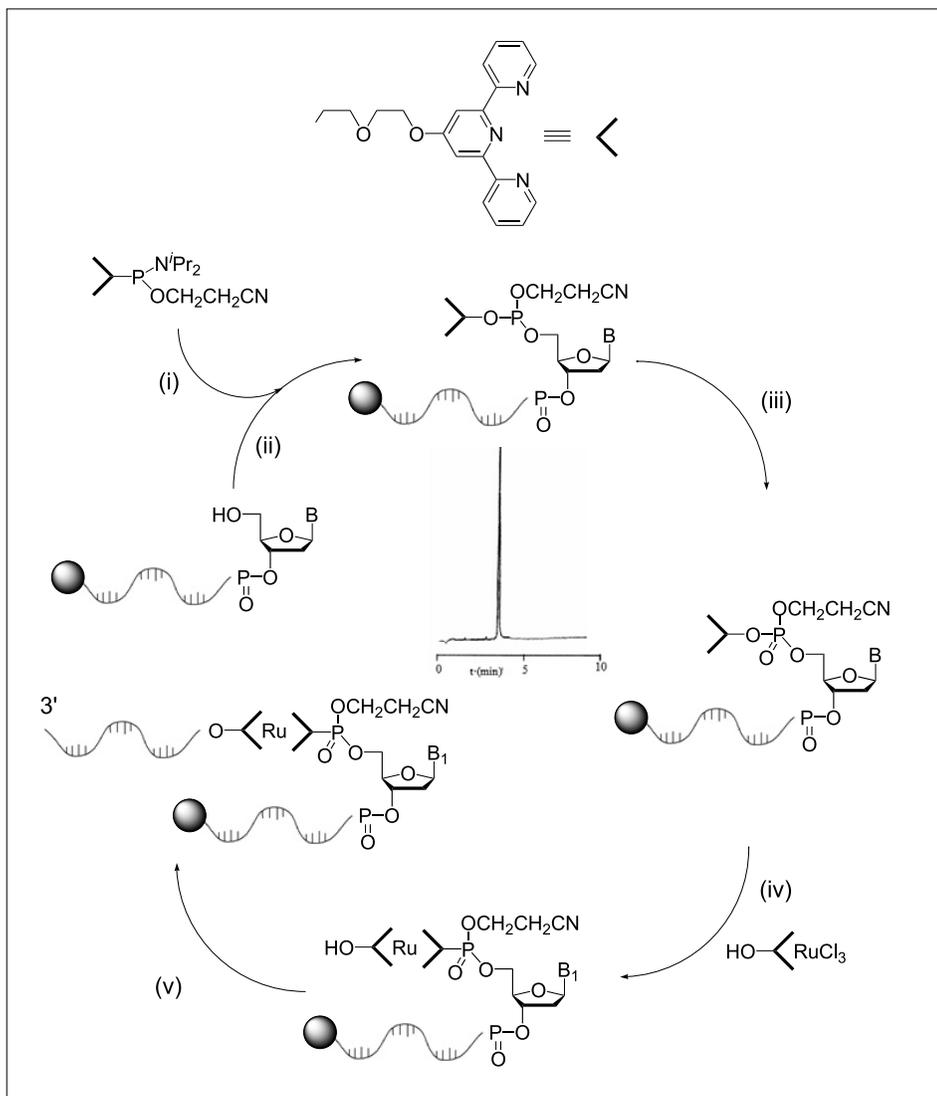


Fig. 3. The synthesis of in-chain ruthenaoligonucleotides at a polystyrene/long chain alkylamine (35–40 μmol/g) solid phase: i) tetrazole; ii) coupling on support; iii) *tert*-butyl hydroperoxide; iv) HOCH₂CH₂OH, N-ethylmorpholine, acetic acid, pH 7; v) deprotection of next residue (DMT-protected, CCl₃CO₂H). The final sequence is removed from the support by treatment with aqueous ammonia solution. The inset shows a typical capillary electrophoresis trace for HPLC purified material.

es {Cu^I(L₁)(L₂)} comprising $\sum_{i=1}^n n_i$ components in statistical amounts. If the sequences A, B ... n_i are complementary to A*, B* ... n_i* then the target sequence, say A*C*, will only be complementary to one component of the library of complexes, {Cu^IAC}. As copper(I) complexes are labile, the formation of the duplex [A*C*•{Cu^IAC}] will result in a redistribution of the library to concentrate the A and C components in {Cu^IAC} – in other words, this is a metal-centred dynamic combinatorial library. The practice is complicated by the fact that the oligonucleotides can be functionalised at the 3' or 5' ends, e.g. 3'-A or 5'-A (where 3'-A refers to the site of attachment of the metal-binding domain) and only the resulting chimera complex {Cu^I(3'-A)(5'-C)} will be complementary to 3'-A*C*-5'.

This latter constraint has a number of interesting consequences. Firstly, by taking any single sequence, it is possible in a single

self-assembly step to generate an unnatural palindromic sequence such as {Cu^I(3'-A)(3'-C)} which has no natural complementary Watson-Crick sequence (Fig. 4). Only by preparing unnatural nucleic acids with a direction inversion (3'-A*C*-3' with a 5',5' linkage of the A* and C* sections) could a genuinely complementary nucleotide sequence be achieved. However, a much more interesting possibility exists that we are currently exploring. Consider a library of complexes prepared from the complementary functionalised sequences 3'-A, 5'-A*: this will give a library of three complexes {Cu^I(3'-A)(3'-A)}, {Cu^I(3'-A)(5'-A*)}, {Cu^I(5'-A*)(5'-A*)}, which are expected to give just two complementary hybrids, [{Cu^I(3'-A)(3'-A)}•{Cu^I(5'-A*)(5'-A*)}] and [{Cu^I(3'-A)(5'-A*)}•{Cu^I(5'-A*)(3'-A)}] in a statistical ratio of 1:4.

Of more direct relevance to applications of this methodology, is the binding of one

sequence to a support. If an A sequence is, for example, attached to the support through the 3' end, then we effectively have a surface-immobilised S-5'-A fragment. It is only necessary to have copper(I) and 3'-functionalised solution species to give surface immobilised species that run uniquely in a non-palindromic 3' to 5' direction. This is the basis of our current approach to surface tethered species.

An example of strategy in action is presented in Fig. 4. The asymmetric ligand 2-hydroxymethyl-9-methyl-1,10-phenanthroline I is prepared by the conversion of 2,9-dimethyl-1,10-phenanthroline to its N-oxide and subsequent heating with acetic anhydride and hydrolysis of the intermediate 2-acetoxymethyl-9-methyl-1,10-phenanthroline. This was converted to the phosphoramidite II in 55% yield under standard conditions which was then coupled with 3'-CGCCGAGTTATT-5' attached to Universal Solid Support II by the 3' end under standard reaction conditions to give the conjugate IV. The resultant conjugate exhibits a parent ion at m/z 4287 in its MALDI-TOF mass spectrum corresponding to {IV + 2Na⁺}. The palindromic complex [Cu(IV)₂]¹²⁺ is obtained directly by the reaction of [Cu(MeCN)₄]⁺ salts with IV in aqueous acetonitrile.

We are currently investigating the preparation of artificial dynamic combinatorial libraries of self-assembled metalloligand-oligonucleotides and developing surface tethered species for use in DNA-chip methodology.

Conclusions

The modification of nucleotides through the covalent attachment of metal complexes or of metal-binding domains is a powerful technique for the construction of bioinorganic conjugates with novel and designed redox, chemical or photophysical properties. A judicious choice of metal and oxidation state allows the strategy to be based upon self-assembly at labile metal species or upon linear synthesis utilising kinetically inert metal centres.

Acknowledgements

The authors wish to thank Dr. Jonathan Hall (Novartis) and Prof. Robert Häner (Universität Bern) for invaluable and ongoing collaboration in this work and Novartis, the Swiss National Science Foundation, the University of Basel and ESF (CERC3) for financial support. We also thank Dr. Maria Schwarz for assistance with the capillary electrophoresis.

Received: September 20, 2005

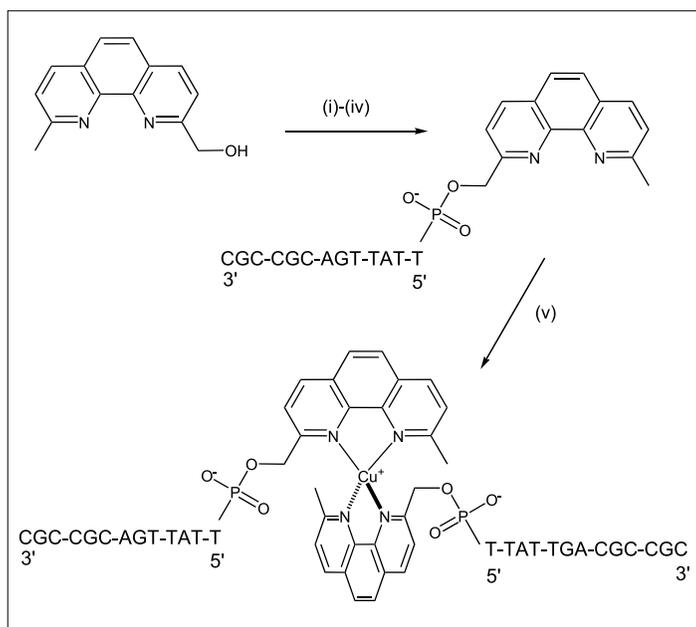


Fig. 4. Synthesis of a palindromic oligonucleotide by self assembly. i) CEP-Cl (299 μ l, 1.338 mmol) and a solution of DIEA (174 μ l, 1.784 mmol) in anhydrous dichloromethane; ii) 3-CGC-CGC-AGT-TAT-T-ODMT 5' on Universal solid support II, tetrazole, MeCN; iii) I₂, pyridine, THF, H₂O; iv) NH₃; v) [Cu(MeCN)₄][PF₆].

- [2] a) 'Interactions of Metal Ions with Nucleotides, Nucleic Acids, and Their Constituents', Eds. A. Sigel, H. Sigel, Marcel Dekker, New York, Basel, Hong Kong; **1996**; b) 'Probing of Nucleic Acids by Metal Ion Complexes of Small Molecules', Eds. A. Sigel, H. Sigel, Marcel Dekker, New York, **1996**.
- [3] 'Uses of Inorganic Chemistry in Medicine', Ed. N.P. Farrell, Royal Society of Chemistry, Cambridge, **1999**.
- [4] 'Macromolecules Containing Metal and Metal-like Elements, Biomedical Applications, Vol. 3', Eds. A.S. Abd-El-Aziz, C.E. Carraher, C.U. Pittman, M. Zeldin, J.E. Sheats, Wiley, New York, **2004**.
- [5] See for example: K.E. Erkkila, D.T. Odom, J.K. Barton, *Chem. Rev.* **1999**, *99*, 2777.
- [6] J.S. Choi, C.W. Kang, K. Jung, J.W. Yang, Y.G. Kim, H.Y. Han, *J. Am. Chem. Soc.* **2004**, *126*, 8606.
- [7] D.E. Bergstrom, J.Y. Chen, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2211.
- [8] R. Häner, J. Hall, G. Rihs, *Helv. Chim. Acta* **1997**, *80*, 487.
- [9] J.T. Hwang, F.E. Baltasar, D.L. Cole, D.S. Sigman, C.H.B. Chen, M.M. Greenberg, *Bioorg. Med. Chem.* **2003**, *11*, 2321.
- [10] a) C.W. Crean, Y.T. Kavanagh, C.M. O'Keefe, M. P. Lawler, C. Stevenson, R. J. H. Davies, P.H. Boyle, J.M. Kelly, *Photochem. Photobiol. Sci.* **2002**, *1*, 1024; b) D. Ossipov, S. Gohil, J. Chattopadhyaya, *J. Am. Chem. Soc.* **2002**, *124*, 13416; c) T.E. Schutzbank, J. Smith, *J. Clin. Microbiol.* **1995**, *33*, 2036; d) Y. Jenkins, J.K. Barton, *J. Am. Chem. Soc.* **1992**, *114*, 8736; e) X. Hu, G.D. Smith, M. Sykora, S.J. Lee, M.W. Grinstaff, *Inorg. Chem.* **2000**, *39*, 2500; f) D.J. Hurley, Y. Tor, *J. Am. Chem. Soc.* **1998**, *120*, 2194; g) E. Meggers, D. Kusch, B. Giese, *Helv. Chim. Acta.* **1997**, *80*, 640; h) D. Ossipov, P.I. Pradeepkumar, M. Holmer, J. Chattopadhyaya, *J. Am. Chem. Soc.* **2001**, *123*, 3551; i) W. Bannwarth, D. Schmidt, R. L. Stallard, C. Hornung, R. Knorr, F. Müller, *Helv. Chim. Acta.* **1988**, *71*, 2085; j) J. Teller, A. Cruickshank, K.S. Schanze, T. L. Netzel, *J. Am. Chem. Soc.* **1989**, *111*, 7221; k) G.N. Grimm, A.S. Boutorine, P. Lincoln, B. Nordén, C. Hélène, *ChemBioChem.* **2002**, *3*, 324; l) D. García-Fresnadillo, N. Boutonnet, S. Schumm, C. Moucheron, A. Kirsch-De Mesmaeker, E. Defrancq, J.F. Constant, J. Lhomme, *Biophys. J.* **2002**, *82*, 978; m) S. Schumm, M. Prévost, D. García-Fresnadillo, O. Lentzen, C. Moucheron, A. Kirsch-De Mesmaeker, *J. Phys. Chem. B* **2002**, *106*, 2763; n) C.G. Coates, J.J. McGarvey, P.L. Callaghan, M. Colletti, J.G. Hamilton, *J. Phys. Chem. B* **2001**, *105*, 730; o) J.M. Kelly, A.B. Tossi, D.J. McConnell, C. OhUigin, C. Hélène, T. le Doan, in 'Free Radicals, Metal Ions and Biopolymers', Ed. P.C. Beaumont, D.J. Deeble, B.J. Parsons, C. Rice Evans, Richelieu Press, London, **1989**, p. 143; p) P.K. Bhattacharya, J.K. Barton, *J. Am. Chem. Soc.* **2001**, *123*, 8649; q) I. Ortmans, S. Content, N. Boutonnet, A. Kirsch-De Mesmaeker, W. Bannwarth, J-F. Constant, E. Defrancq, J. Lhomme, *Chem. Eur. J.* **1999**, *5*, 2712; r) W. Bannwarth, D. Schmidt, *Tetrahedron Lett.* **1989**, *30*, 1513; s) M.R. Arkin, E.D. Stemp, S.C. Pulver, J.K. Barton, *Chem. Biol.* **1997**, *4*, 389; t) K. Wiederholt, L.W. McLaughlin, *Nucleic Acids Res.* **1999**, *27*, 2487; u) S.I. Khan, A.E. Beilstein, M. Sykora, G.D. Smith, X. Hu, M.W. Grinstaff, *Inorg. Chem.* **1999**, *38*, 3922; v) J.J. Rack, E.S. Krider, T.J. Meade, *J. Am. Chem. Soc.* **2000**, *122*, 6287; w) I. Vargas-Baca, D. Mitra, H.J. Zulyniak, J. Banerjee, H.F. Sleiman, *Angew. Chem., Int. Ed.* **2001**, *40*, 4629.
- [11] A. de Mesmaeker, R. Häner, P. Martin, H.E. Moser, *Acc. Chem. Res.* **1995**, *28*, 366.
- [12] N.T. Thuong, C. Hélène, *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 666.

[1] J.-U. Fuhrhop, C. Endisch, 'Molecular and Supramolecular Chemistry of Natural Products and Their Model Compounds', Marcel Dekker, New York, **2000**.