Biometallic Materials

A Nucleoside Conjugate Containing a Metallacarborane Group and Its Incorporation into a DNA Oligonucleotide**

*Agnieszka B. Olejniczak, Jaromir Plešek, Otomar Křiž, and Zbigniew J. Lesnikowski**

Nucleosides, nucleotides, and oligonucleotides site-specifically modified with metal complexes are of interest for analytical applications (hybridization probes, electrochemical sensors)[1,2] and therapeutic uses (anticancer agents and radiopharmaceuticals),[3,4] as novel materials for the assembly of nanostructures,[5,6] for structural and mechanistic studies (DNA-mediated electron and energy transfer),[7] and for the construction of artificial chemical nucleases.[8,9]

Metal-containing nucleosides, the precursors of the corresponding metal-derivatized nucleic acids, have been predominantly constructed by the synthesis of nucleoside–chelator conjugates followed by metal complexation (chelator-type metallanucleosides).[10] Another kind of metal-containing nucleoside is obtained by coupling the nucleoside moiety with a metallocene such as ferrocene (the π complex type of metallanucleoside).[11,12] Herein we describe a new type of metal-bearing nucleoside obtained by conjugation of the nucleoside with a metallacarborane moiety.

Metallacarboranes are a vast family of metallocene-type complexes consisting of at least one carborane cage ligand and one or more metal atoms. Carborane (borane) clusters are versatile and efficient ligands for metals like Al, Au, Co, Cr, Cu, Fe, Ir, Mn, Ni, Pt, and many others,[13,14] and potentially allow incorporation of an array of metals with different properties into a nucleic acid or its components. For

[*] Prof. Z. J. Lesnikowski
Center for Microbiology and Virology
Laboratory of Molecular Virology and Biological Chemistry
Polish Academy of Sciences
106 Lodowa St., 93-232 Lodz (Poland)
Fax: (+48) 42-677-1249
E-mail: zlesnik@cmiwpan.lodz.pl

Dr. A. B. Olejniczak
Center for Microbiology and Virology
Polish Academy of Sciences
106 Lodowa St., Lodz 93-232 (Poland)

Prof. J. Plešek, Dr. O. Křiž
Institute of Inorganic Chemistry
Academy of Sciences of the Czech Republic
250-68 Rez (Czech Republic)

[**] This work was supported by the Polish Committee for Scientific Research (KBN; Grant no. PBZ-KBN 059/T09/08). The authors thank Dr. Clara Viñas for her assistance in the cyclic voltametry measurements, Dr. Edyta Paradowska and Mrs. Slawomira Studzinska for the cytotoxicity assay, and Mrs Marzena Przepiorkiewicz for the polymerase chain reaction experiments. A.B.O thanks the Polish Academy of Sciences and Spanish Academy of Sciences for the award of a travel grant.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

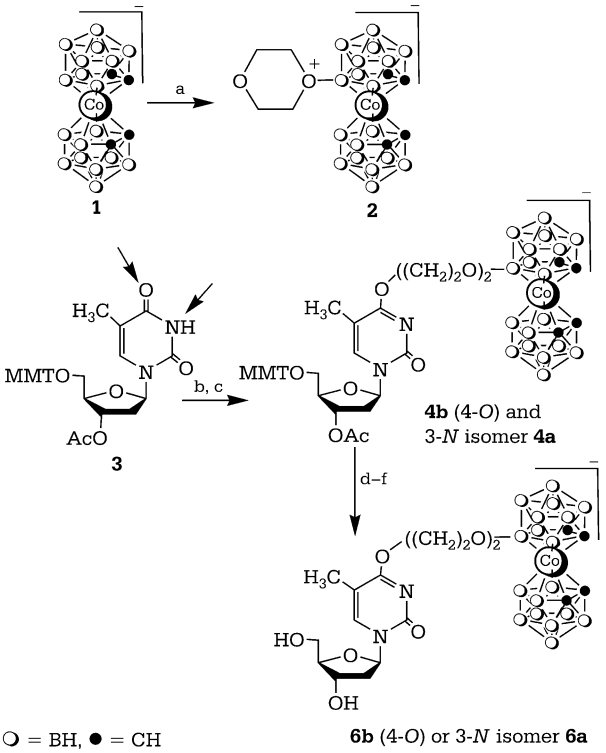
  DOI: 10.1002/ange.200352505

example, depending upon the radioisotope of the metal component of the metallacarborane cluster, the metal-containing nucleosides and oligonucleotides produced could potentially be used for different types of radiotherapy of tumors, such as alpha, beta, and Auger therapy.[4,15–17] In addition, the nucleoside–metallacarborane conjugates described herein contain twice as many boron atoms as nucleosides with a standard carborane modification, and up to twenty times more than nucleosides modified with boronic acid. Therefore, nucleoside–metallacarborane conjugates are potentially useful as boron carriers for boron neutron capture therapy (BNCT) of cancers.[18,19]

Conjugation of the metallacarborane complex with a nucleoside unit was achieved by application of the nucleophilic reaction of a base-activated nucleoside with the dioxane group of the 8-dioxane-[(1,2-dicarba-*closo*-undecaborane)-3,3′-cobalt(−1)(1′,2′-dicarba-*closo*-undecaborane)] zwitterion {**2**; [3,3′-Co(8-$C_4H_8O_2$-1,2-$C_2B_9H_{10}$)(1′,2′-$C_2B_9H_{11}$)]}. The ring-opening of a tetrahydropyran or dioxane group attached to a [bis(1,2-dicarbollido)-3-cobalt(−1)]ate ion through attack by simple nucleophiles such as fluoride, chloride, or hydroxide anions,[20] phenolates and phosphorus-containing nucleophiles,[21] potassium phtalimide, diethyl acetamidomalonate, and cyanide ions,[22] or potassium pyrrolyl, indolyl, and carbazolyl compounds[23] has been described before.

Herein, we describe for the first time dioxane ring-opening of a dioxane–metallacarborane zwitterion by a biomolecule. We propose a novel type of metal-containing nucleoside with a metallacarborane modification, describe the incorporation of such a nucleoside into a DNA oligonucleotide, and present the results of studies on some of the physicochemical and biological properties of the modified products.

Nucleosides containing a metallacarborane complex were obtained by the synthetic procedure shown in Scheme 1. First, the [3,3′-Co(8-$C_4H_8O_2$-1,2-$C_2B_9H_{10}$)(1′,2′-$C_2B_9H_{11}$)] zwitterion (**2**; 8-dioxane-COSAN) was obtained by treatment of [Cs{3-Co-(1,2-$C_2B_9H_{11}$)$_2$}] (see **1**) with dioxane in the presence of dimethyl sulfate and sulfuric acid.[24] Alkylation of the thymine base in 5′-*O*-monomethoxytrityl-3′-*O*-acetylthymidine (**3**)[25] with metallacarborane derivative **2** was performed by activation of the 4-*O* (and competitively, the 3-*N*) atom in protected nucleoside **3** by treatment with sodium hydride.[26] The fully protected products of 3-*N* and 4-*O* alkylation, **4a** and **4b**, respectively, were separated by chromatography on silica gel. The 3-*N* or 4-*O* location of the bisethyleneoxy-8-[(1,2-dicarba-*closo*-undecaborane)-3,3′-cobalt(−1)(1′,2′-dicarba-*closo*-undecaborane)] moiety (BEMC) was determined by comparison of the UV spectra recorded for compounds **4a** and **4b** with those of 4-*O*- and 3-*N*-methylthymidine. The assignment is based on the characteristic bathochromic shift of the maximum in the spectra of the 4-*O*-alkylated derivatives.[27,28] The deacetylation of the 3′-OH group in **4b** (4-*O*)[29] yielded 5′-*O*-monomethoxytrityl-4-*O*-bisethyleneoxy-8-{(1,2-dicarba-*closo*-undecaborane)-3,3′-cobalt(−1)(1′,2′-dicarba-*closo*-undecaborane)]}thymidine (**5**), which was used in the synthesis of a metallacarborane-containing phosphoramidite monomer **7** and oligonucleotide **8**. Detritylation of the 5′-OH functional group in **5** under acidic conditions[30] gave the fully

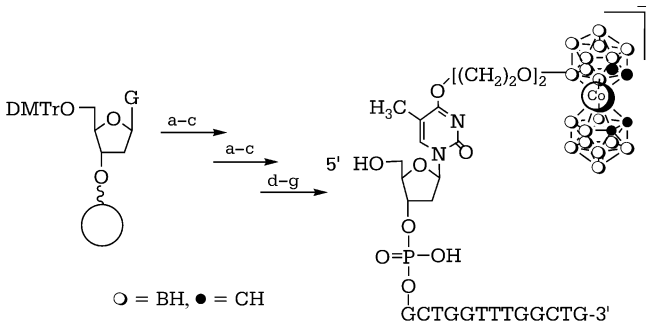


Scheme 1. Synthesis of 5′-*O*-monomethoxytrityl-3′-*O*-acetyl-3-*N*-(bisethyleneoxy-8-COSAN)thymidine (**4a**), 5′-*O*-monomethoxytrityl-3′-*O*-acetyl-4-*O*-(bisethyleneoxy-8-COSAN)thymidine (**4b**), and 4-*O*-(bisethyleneoxy-8-COSAN)thymidine (**6b**). a) Dioxane/Me_2SO_4, H_2SO_4; b) NaH/toluene; c) **2**/toluene; d) separation of **3**-*N* and **4**-*O* isomers; e) 80% CH_3COOH; f) aq NH_3. MMT = monomethoxytrityl.

deprotected metallacarborane–nucleoside conjugate 4-*O*-bisethyleneoxy-8-{(1,2-dicarba-*closo*-undecaborane)-3,3′-cobalt(−1)(1′,2′-dicarba-*closo*-undecaborane)]}thymidine (**6b**). The isomer **6a**, which has BEMC attached to *N*-3, was prepared from **4a** in an analogous manner.

Phosphitylation of **5** was performed according to the standard procedure[31,32] and led to modified monomer 5′-*O*-monomethoxytrityl-4-*O*-diethyleneoxy-8-{(1,2-dicarba-*closo*-undecaborane)-3,3′-cobalt(−1)(1′,2′-dicarba-*closo*-undecaborane)]}-3′-*O*-(*N,N*-diisopropyl-β-cyanoethyl)phosphoramidite (**7**). Synthesis of metallacarborane-modified oligonucleotide **8** [5′-d(^{BEMC}TGCTGGTTTGGCTG)-3′] was performed by using a β-cyanoethyl cycle on an automated DNA synthesizer[33] (Scheme 2). After completion of the synthesis of the unmodified part of the oligonucleotide, the metallacarborane-modified nucleoside was attached to the 5′-end of the oligomer chain manually by using monomer **7**.[34] In the oxidation step, *t*BuOOH was applied instead of I_2, which is routinely used as the oxidizing agent.[35,36] The capping step was omitted in the manual cycle. Complete deprotection of the nucleic acid bases was achieved by treatment with concentrated ammonia solution for 2 h at 50 °C.

The products were characterized by TLC, reverse-phase (RP) HPLC, UV and ^{1}H NMR spectroscopies, MS, and ^{11}B- and ^{31}P NMR spectroscopy where applicable. The purity of oligonucleotide **8** was checked by polyacrylamide gel electrophoresis and RP-HPLC; the molecular weight was confirmed



8

Scheme 2. Synthesis of a tetradecanucleotide [5′-d(^{BEMC}TGCTGGTTTGGCTG)-3′] (**8**) containing the modified base 4-*O*-(bisethyleneoxy-8-COSAN)thymidine (^{BEMC}T). a) Detritilation: 3% dichloroacetic acid in CH_2Cl_2; b) coupling: unmodified monomers; c) oxidation: 0.1 M I_2 in THF/pyridine/H_2O (13:6:1); capping: 1 M $(CH_3CO)_2O$ in THF/pyridine (1:8) and 0.5 M 4-dimethylaminopyridine in THF; d) coupling: modified monomer **7**; e) oxidation: 0.5 M *t*BuOOH in dodecane; d) cleavage from the support: 30% aq NH_3, 1 h, RT; f) base deprotection: 30% aq NH_3, 2 h, 50 °C. THF, tetrahydrofuran; DMTr, dimethoxytrityl.

by mass spectrometry. Experimental procedures and analytical data for compounds **5**–**8** are available in the Supporting Information.

Selected physicochemical and biochemical properties of conjugates **6a** and **6b** and metallacarborane-containing oligonucleotide **8** were studied. To check the applicability of the metallacarborane group as a redox label for nucleosides, the electrochemical properties of conjugate **6b** and its counterpart with the metallacarborane complex attached to *N*-3, **6a**, were studied by cyclic voltammetry. A three-electrode cell with acetonitrile as the solvent, 0.1 M $[NBu_4]ClO_4$ as the supporting electrolyte, and platinum as the working electrode was used.[37–39] The *E* values representing the average of the forward and return peak potentials were: **6b** (4-*O*): $E° = -964$ mV; 3-*N* isomer **6a**: $E° = -1007$ mV. Under the same conditions, 3,3′-Co(8-[bis-(C_2H_4O)]OH]-1,2-$C_2B_9H_{10}$)(1′,2′-$C_2B_9H_{11}$) and thymidine (taken as standards), had E° values of −964 and 0 mV, respectively (cyclic voltammetry plots are available in the Supporting Information). These observations illustrate the potential usefulness of metallacarborane complexes as redox labels for nucleosides.

To test the susceptibility of the oligonucleotide labeled with the metallacarborane cage to elongation by the polymerase chain reaction (PCR), the oligonucleotide 5′-d(^{BEMC}TGCTGGTTTGGCTG)-3′ (**8**) was used as one of two primers in PCR amplification of a fragment of human cytomegalovirus (HCMV) DNA, strain AD 169. The other primer was unmodified nucleotide P12 (5′-AAACGGCG-CAGCCACATAAGG-3′). Part of gene US14 and gene US13 were amplified to yield a 812-bp product, as expected. This result proves the susceptibility of the oligonucleotide with the metallacarborane modification at its 5′ end to amplification by Taq DNA polymerase.

In addition, studies were carried out on the cytotoxicity of compound **6a** and its 4-*O* isomer **6b** in two cell lines, Vero and A549, by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.[40] The toxicity of nucleoside **6a**, measured as the 50% inhibitory concentration (IC_{50}) value, was found to be 94 μM and 82 μM in the Vero and A549 cell lines, respectively; for the 4-*O* isomer **6b**, toxicity was detected at concentrations higher than 100 μM for both cell lines. The toxicities of nucleoside conjugates **6a** and **6b** to Vero cells are lower than that of 5-carboranyl-2′-deoxyuridine, whose IC_{50} value is 17 μM,[41,42] but more than an order of magnitude higher than the toxicity of L-4-(dihydroxyboryl)phenylalanine in the TIG-1–20 cell line ($IC_{50} = 2.2 \times 10^3$ μM).[43] These findings show that both nucleoside–metallacarborane conjugates **6a** and **6b** are low enough in toxicity to be considered as a potential new type of boron carrier for BNCT.

In summary, we have described a new class of metal-containing nucleosides and shown their application in the synthesis of oligonucleotide–metal conjugates, as well as the ability of a metallacarborane-modified oligonucleotide to serve as a primer for Taq DNA polymerase in PCR amplification of HCMV DNA. The applicability of metallacarboranes as redox labels for nucleosides was also demonstrated. In addition, the nucleoside–metallacarborane conjugates were found to have low toxicity, which could lead to their application as a new type of boron-rich carrier for BNCT of tumors.

Experimental Section

Synthesis of key intermediates 5′-*O*-monomethoxytrityl-3′-*O*-acetyl-3-*N*-{bisethyleneoxy-8-[(1,2-dicarba-closo-undecaborane)-3,3′-cobalt-(−1)(1′,2′-dicarba-closo-undecaborane)]}thymidine (**4a**) and 5′-*O*-monomethoxytrityl-3′-*O*-acetyl-4-*O*-{bisethyleneoxy-8-[(1,2-dicarba-closo-undecaborane)-3,3′-cobalt(−1)(1′,2′-dicarba-closo-undecaborane)]}thymidine (**4b**): The procedure was performed under a positive pressure of argon. 5′-*O*-Monomethoxytrityl-3′-*O*-acetylthymidyne (**3**; 0.9 g, 1.6 mmol) and 8-dioxane-COSAN (**2**; 1.4 g, 3.3 mmol) were mixed together then dried over P_2O_5 under high vacuum for 24 h. NaH (60% suspension in mineral oil, 80 mg, 3.3 mmol) was added, followed by anhydrous toluene (18 mL). The reaction mixture was stirred at 70 °C in an oil bath. After 8 h, excess NaH was removed by centrifugation and the supernatant added dropwise to hexane (135 mL). The resultant precipitate was separated by centrifugation and the sediment was dried under vacuum to yield crude **4** (2.4 g) as a mixture of 3-*N* and 4-*O* isomers (**4a** and **4b**, respectively). Crude **4** was purified by silica gel column chromatography (70 g silica gel, 230–400 mesh) with 12% CH_3OH in $CHCl_3$ containing 1% Et_3N as the eluting solvent system. Yield: **4a**, 221 mg; **4b**, 450 mg. **4a**: TLC ($CH_3CN/CHCl_3$, 1:2): $R_f = 0.14$; UV/Vis (CH_3CN): $\lambda_{min} = 227.46$, 249.18, 287.30 nm, $\lambda_{max} = 234.43$, 261.89, 313.93 nm; ^{1}H NMR (500 MHz, $[D_3]CHCl_3$, 25 °C, tetramethylsilane (TMS)): $\delta = 1.8$–3.5 (br m, 21 H; BH-COSAN), 1.58 (s, 3 H; CH_3CO), 1.71 (s, 3 H; CH_3–5), 2.54 (m, 2 H; H-2′), 2.95–3.08 (m, 4 H; 4 × CH-COSAN), 3.28–3.41 (br s, 4 H; 2 × OCH_2), 3.43 (s, 3 H; CH_3O), 3.46–3.52 (q, 2 H; H-5′,5″), 3.92 (br s, 2 H; OCH_2), 3,96 (s, 1 H; H-4′), 4.48–4.59 (m, 2 H; OCH_2), 5.36 (s, 1 H; H-3′), 6.31 (s, 1 H; H-1′), 6.87 (d, 2 H; α-arom. in CH_3OPh), 7.28–7.60 (m, 12 H; H arom. in MMTr), 8.11 (s, 1 H; H-6) ppm; ^{13}C NMR (125 MHz, $[D_3]CHCl_3$, 25 °C, TMS): $\delta = 13.62$ (CH_3-5), 20.99 (CH_3 in CH_3CO), 39.76 (C-2′), 48.10, 51.94 (C-COSAN), 55.72 (CH_3O), 64.61 (C-5′), 68.73, 73.48 (OCH_2), 75.67 (C-3′), 86.41 (C-4′), 87.46 (C-1′), 88.74 (C-methylidene in MMTr), 114.65,

129.23, 131.40, 144.85, 145.06 (MMTr), 135.92 (C-6), 156.91 (C-2), 160.24 (δ-C in CH_3OPh), 170.30 (CO in CH_3CO), 176.38 (C-4) ppm; ^{11}B NMR (160 MHz, $[D_3]CHCl_3$, 25 °C, $BF_3/(C_2H_5)_2O$): $\delta = 25.4$ (s, 1B), 20.0 to −35.0 ppm (brs, max. at 8.0, −5.3 and −16.7, 17B) ppm; FAB-MS (negative, nitrobenzylalcohol): m/z: 966.9 [$M-1$]. **4b**: TLC ($CH_3CN/CHCl_3$, 1:2): $R_f = 0.41$; UV/Vis (CH_3CN): $\lambda_{min} = 252.87$ nm, $\lambda_{max} = 283.91$, 311.84 nm; ^{1}H NMR (500 MHz, $[D_3]CHCl_3$, 25 °C, TMS): $\delta = 1.50$–4.00 (brm, 21H; BH-COSAN), 1.48 (s, 3H; CH_3–5), 1.62 (s, 3H; CH_3CO), 2.55 (m, 2H; H-2′), 2.77–3.07 (m, 4H; 4 × CH-COSAN), 3.21 (brs, 4H; 2 × OCH_2), 3.37 (s, 3H; CH_3O), 3.45–3.51 (q, $J(5', 5'') = 9.04$ Hz, 2H; H-5′,5″), 3.88 (brs, 2H; OCH_2), 4.08 (s, 1H; H-4′), 4.37–4.43 (m, 2H; OCH_2), 5.43 (s, 1H; H-3′), 6.64 (s, 1H; H-1′), 6.82 (d, 2H; α-H arom. in CH_3OPh), 7.06–7.52 (m, 12H; H arom. in MMTr), 8.20 ppm (s, 1H; H-6) ppm; ^{13}C NMR (125 MHz, $[D_3]CHCl_3$, 25 °C, TMS): $\delta = 12.33$ (CH_3-5), 21.09 (CH_3-acetyl), 40.22 (C-2′), 48.12, 51.85, 51.95 (C-COSAN), 55.60 (CH_3O), 64.43 (C-5′), 68.27, 71.68, 73.26 (OCH_2), 75.53 (C-3′), 85.97 (C-4′), 87.75 (C-1′), 88.56 (C-methylidene in MMTr), 114.55, 129.14, 129.23, 129.55, 131.41, 142.33, 144.94, 145.09 (MMTr), 135.95 (C-6), 158.93 (C-2), 160.21 (C-4 in 4-CH_3OPh), 170.58 (C-4), 172.52 (CH_3-acetyl) ppm; ^{11}B NMR (160 MHz, $[D_3]CHCl_3$, 25 °C, $BF_3/(C_2H_5)_2O$): 25.6 (s, 1B), 20.0 to −30.0 (brs, max at 9.0, −5.1 and −15.8, 17B) ppm; FAB-MS (negative, nitrobenzylalcohol): m/z: 966,7 [$M-1$].

Received: July 29, 2003 [Z52505]

Keywords: DNA · metallacarboranes · molecular probes · nucleosides · redox chemistry

[1] T. Ihara, M. Nakayama, M. Murata, K. Nakano, M. Maeda, *Chem. Commun.* **1997**, 1609 – 1610.
[2] *Probing of Nucleic Acids by Metal Ion Complexes of Small Molecules, Vol. 33* (Eds.: A. Siegel, H. Siegel), Marcel Dekker, New York, **1996**, pp. 1 – 678.
[3] C. K. Younes, R. Boisgard, B. Tavitian, *Curr. Pharm. Des.* **2002**, *8*, 1451 – 1466.
[4] A. I. Kassis, S. J. Adelstein, G. Mariani, *Q. J. Nucl. Med.* **1996**, *40*, 301 – 319.
[5] J. J. Storhoff, C. A. Mirkin, *Chem. Rev.* **1999**, *99*, 1849 – 1862.
[6] N. C. Seeman, *Synlett* **2000**, 1536 – 1548.
[7] B. Giese, *Curr. Opin. Chem. Biol.* **2002**, *6*, 612 – 618.
[8] D. M. Perrin, A. Mazumder, D. S. Sigman, *Progr. Nucl. Acid Res. Mol. Biol.* **1996**, *52*, 123 – 151.
[9] J. A. Cowan, *Curr. Opin. Chem. Biol.* **2001**, *5*, 634 – 642.
[10] R. Haner, J. Hall, *Antisense Nucleic Acid Drug Dev.* **1997**, *7*, 423 – 430.
[11] C. J. Yu, H. Yowanto, Y. Wan, T. J. Meade, Y. Chong, M. Strong, L. H. Donilon, J. F. Kayyem, M. Gozim, G. F. Blackburn, *J. Am. Chem. Soc.* **2000**, *122*, 6767 – 6768.
[12] M. Nakayama, T. Ihara, K. Nakano, M. Maeda, *Talanta* **2002**, *56*, 857 – 866.
[13] A. K. Saxena, N. S. Hosmane, *Chem. Rev.* **1993**, *93*, 1081 – 1124.
[14] R. N. Grimes, *Coord. Chem. Rev.* **2000**, *200*, 773 – 811.
[15] R. N. Hanson, *Curr. Pharm. Des.* **2000**, *6*, 1457 – 1468.
[16] M. T. Ercan, M. Caglar, *Curr. Pharm. Des.* **2000**, *6*, 1085 – 1121.
[17] R. Schibili, P. A. Schubiger, *Eur. J. Nucl. Med. Mol. Imaging* **2002**, *29*, 1529 – 1542.
[18] Z. J. Lesnikowski, J. Shi, R. F. Schinazi, *J. Organomet. Chem.* **1999**, *581*, 156 – 169, and references cited therein.
[19] J. F. Valliant, K. J. Guenther, A. S. King, P. Morel, P. Schaffer, O. O. Sogbein, K. A. Stephenson, *Coord. Chem. Rev.* **2002**, *232*, 173 – 230.
[20] T. Peymann, K. Kück, D. Gabel, *Inorg. Chem.* **1997**, *36*, 5138 – 5139.
[21] J. Plesek, B. Grüner, J. Baca, P. Selucky, J. Rais, N. V. Šistkova, B. Casensky, *Proc. 6th Int. Inform. Exch. Meet. (Madrid, 2000)*, NEA OECD Publications, Paris Cedex 16, France, **2001**, paper N43, p. 659.
[22] I. B. Sivaev, Z. A. Starikova, S. Sjöberg, V. I. Bregadze, *J. Organomet. Chem.* **2002**, *649*, 1 – 8.
[23] J. Llop, C. Masalles, C. Viñas, F. Teixidor, R. Sillanpää, R. Kivekäs, *J. Chem. Soc. Dalton Trans.* **2003**, 556 – 561.
[24] J. Plesek, S. Hermanek, A. Franken, I. Cisarova, C. Nachtigal, *Collect. Czech. Chem. Commun.* **1997**, *62*, 47 – 56.
[25] H. Schaller, G. Weiman, B. Lerch, H. G. Khorana, *J. Am. Chem. Soc.* **1963**, *85*, 3821 – 3827.
[26] A. Shabarova, A. Bogdanov, *Advanced Organic Chemistry of Nucleic Acids*, Wiley-VCH, Weinheim, **1994**, pp. 413 – 414.
[27] P. D. Lawley, D. J. Orr, S. A. Shah, P. B. Farmer, M. Jarman, *J. Biochem.* **1973**, *135*, 193 – 201.
[28] K. Takenuki, H. Itoh, A. Matsuda, T. Ueda, *Chem. Pharm. Bull.* **1990**, *38*, 2947 – 2952.
[29] J. P. H. Verheyden, J. G. Moffat in *Synthetic Procedure in Nucleic Acid Chemistry* (Eds.: W. W. Zorbach, R. S. Tipson), Wiley-Interscience, New York, **1968**, pp. 383 – 387.
[30] H. Weber, H. G. Khorana, *J. Mol. Biol.* **1972**, *72*, 219.
[31] T. Atkinson, M. Smith in *Oligonucleotide Synthesis: A Practical Approach* (Ed.: M. Gait), **1984**, IRL, Oxford, pp. 35 – 81.
[32] S. L. Beaucage in *Methods in Molecular Biology, Vol. 20, Protocols for Oligonucleotides and Analogs. Synthesis and Properties* (Ed.: S. Agrawal), Humana, Totowa, **1993**, pp. 33 – 61.
[33] *Oligo 1000 DNA Synthesizer Operating Instructions*, Beckman Instruments Inc., Fullerton, CA, **1992**.
[34] A. B. Olejniczak, M. Koziołkiewicz, Z. J. Leśnikowski, *Antisense Nucleic Acid Drug Dev.* **2002**, *12*, 79 – 94.
[35] Y. Hayakawa, M. Uchiyama, R. Noyori, *Tetrahedron Lett.* **1986**, *27*, 4191 – 4194.
[36] Y. Hayakawa, S. Wakabayashi, H. Kato, R. Noyori, *J. Am. Chem. Soc.* **1990**, *112*, 1691 – 1696.
[37] M. F. Hawthorne, D. C. Young, T. D. Andrews, D. V. Howe, R. L. Pilling, A. D. Pitts, M. Reintjes, L. F. Warren, P. A. Wegner, *J. Am. Chem. Soc.* **1968**, *90*, 879 – 895.
[38] C. Viñas, J. Pedrajas, J. Bertran, F. Teixidor, R. Kivekäs, R. Sillanapää, *Inorg. Chem.* **1997**, *36*, 2482 – 2486.
[39] W. G. Geiger Jr. , *Metal Interactions with Boron Clusters* (Ed.: R. N. Grimes), **1982**, Plenum, New York, pp. 239 – 267.
[40] T. Mosmann, *J. Immunol. Methods* **1983**, *65*, 55 – 63.
[41] Y. Yamamoto, T. Seko, H. Nakamura, H. Niemoto, H. Hojo, N. Mukai, Y. Hashimoto, *J. Chem. Soc. Chem. Commun.* **1992**, 157 – 158.
[42] R. F. Schinazi, N. M. Goudgaon, G. Fulcrand, Y. El Kattan, Z. J. Lesnikowski, J. Moravek, D. C. Liotta, *Intl. J. Radiat. Oncol. Biol. Phys.* **1994**, 28, 1113 – 1120.
[43] H. Nemoto, J. Cai, N. Asao, S. Iwamoto, Y. Yamamoto, *J. Med. Chem.* **1995**, *38*, 1673 – 1678.

