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DNA-Dinucleotides Bearing a 3',3'-Cobalt- or 3',3'-Iron-1,2,1',2'-dicarbollide Complex

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Summary: The synthesis of mononucleoside and dinucleoside phosphates and H-phosphonates bearing boron cluster complexes of cobalt or iron is described. The proposed method provides key synthons for the preparation of bioinorganic DNA/metallacarborane conjugates for a variety of applications.

There is an array of DNA- and RNA-oligonucleotide modifications designed and synthesized for specific biological and technical use. The incorporation of metal centers into nucleic acids offers exceptional opportunities to create new types of materials merging the ability of nucleic acids to store, transfer, and process information with electronic, magnetic, or optical properties characteristic of metals and their complexes. Metal- and metalloid-containing nucleic acids exhibit a wide range of biological and physicochemical activities, some of which have been employed in molecular medicine, diagnostics, and technology, providing many useful advantages. Applications of metal-labeled nucleic acids include their use as electrochemically detectable probes, fluorescent probes, artificial “chemical nucleases”, probes for DNA-mediated electron transfer, and “molecular wires” for bioelectrochemical devices.¹ The study of the mononucleosides and their mono-, di-, and triphosphate derivatives bearing carborane or metallacarborane modification as potential antivirals, boron carriers for boron neutron capture therapy of cancers (BNCT),^{2,3} or modulators of purinergic receptors represents another fascinating field of research. The recent emergence of applications of carboranes as materials for nanotechnology⁴ and pharmacophores in drug design^{5,6} has expanded the potential use of boron clusters in practice.

Metal centers are usually attached to the nucleoside unit or to an internucleotide or a terminal phosphate group of DNA in the form of their metal complexes. In the majority of cases chelators are used as vectors for the metal ions.^{7,8} One of the few exceptions is the metallocene type of complex, with the

best known example being ferrocene.^{8,9} The only other examples of nucleoside/metallocene type conjugate reported so far are nucleoside/metallacarborane modifications.^{10,11} Recently we described a general method for the synthesis of conjugates of all four canonical nucleosides, T, dC, dA, and dG, and metallacarboranes containing cobalt.¹⁰ Incorporation of thymidine conjugate into a DNA oligomer via the phosphoramidite method was also shown.¹¹

Herein we report the synthesis of a nucleoside/metallacarborane conjugate bearing iron, transformation of the cobalt- and iron-containing conjugates into H-phosphonate synthons, and synthesis of metalated dinucleotide blocks bearing a metallacarborane complex. As a metal carrier a 2'-deoxyadenosine (dA) derivative was used.

The metallacarborane/2'-deoxyadenosine conjugate containing cobalt (**5Co**) was obtained according to the original procedure described recently.¹⁰ The conjugate bearing iron (**5Fe**) was obtained analogously. Briefly, first the 5'- and 3'-hydroxyl functions of 2'-deoxyadenosine (**1**) were protected. For that purpose *tert*-butyldimethylsilyl protection was used for both hydroxyl groups, providing 3',5'-*O,O*-bis(*tert*-butyldimethylsilyl)-2'-deoxyadenosine (**2**).¹²

In the second step **2** was activated with a 2.5 mol excess of sodium hydride and then treated with a 1.5 mol excess of the 8-dioxane-(3,3'-iron-1,2,1',2'-dicarbollide) adduct [8-O(CH₂CH₂)₂O-1,2-C₂B₉H₁₀-3,3'-Fe-1',2'-C₂B₉H₁₁] (**3Fe**) or alternatively with the adduct containing cobalt (**3Co**). The compound **3Co** was prepared as described,¹³ and the compound **3Fe** was synthesized from 3,3'-iron-1,2,1',2'-dicarbollide¹⁴ using the same procedure as for **3Co**.

Alkylation of **2** with **3** gave 3',5'-*O,O*-bis(*tert*-butyldimethylsilyl)-6-*N*-{5-[(3,3'-iron-1,2,1',2'-dicarbollide)-8-yl]-3-oxapentoxo}-2'-deoxyadenosine (**4Fe**) or its cobalt counterpart **4Co** in good yield. In the third step, the *tert*-butyldimethylsilyl protective groups in **4** were removed with tetrabutylammonium fluoride, providing the metallacarborane/nucleoside conjugate 6-*N*-{5-[(3,3'-iron-1,2,1',2'-dicarbollide)-8-yl]-3-oxapentoxo}-2'-deoxyadenosine (**5Fe**) or the cobalt-containing adduct **5Co** (Scheme 1). It is worth noting that though alkylation of 2'-deoxyadenosine with **3Fe** proceeded analogously as with **3Co**,

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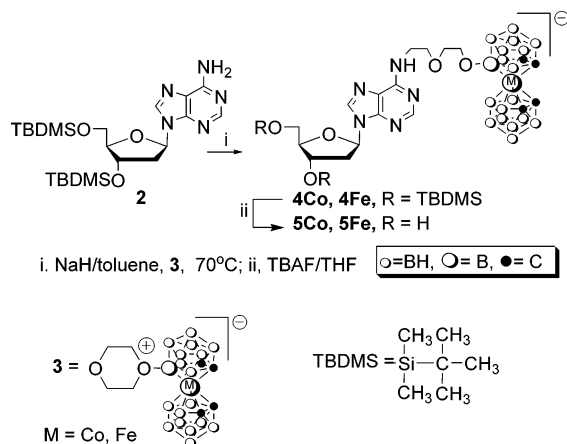
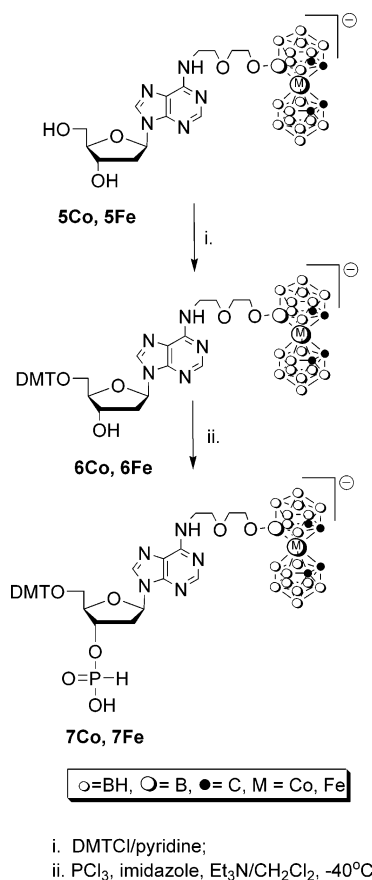
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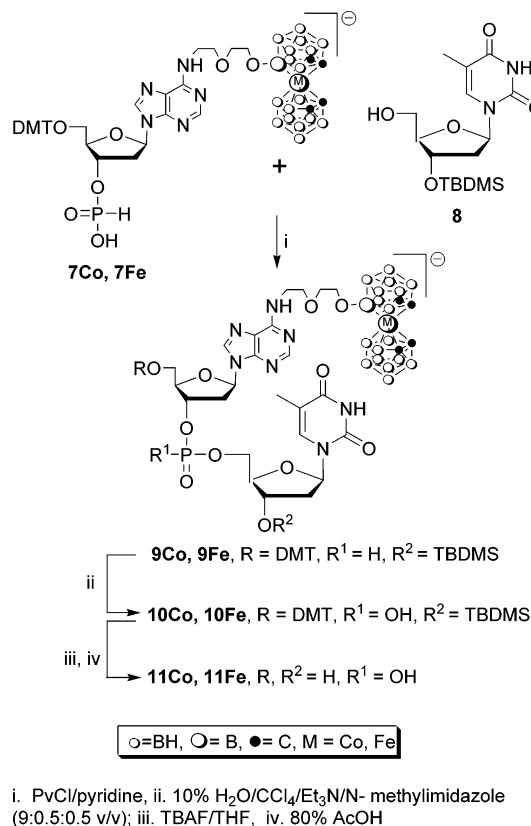
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Scheme 1. Synthesis of Metallocarborane/2'-Deoxyadenosine Conjugates Containing Iron and Cobalt**Scheme 2. Synthesis of H-phosphonates of Nucleoside/Metallocarborane Conjugates**

the NMR characterization of **5Fe** and its derivatives was more difficult than for **5Co**, due to the paramagnetic properties of iron(III).

The key intermediates for the synthesis of metalated dinucleotide blocks via the H-phosphonate method, 5'-*O*-dimethoxytrityl 6-*N*-{5-[(3,3'-cobalt-1,2,1,2'-dicarbollide)-8-yl]-3-oxapentoxyl}-2'-deoxyadenosine 3'-*O*-(H-phosphonate) (**7Co**) and 5'-*O*-dimethoxytrityl 6-*N*-{5-[(3,3'-iron-1,2,1,2'-dicarbollide)-8-yl]-3-oxapentoxyl}-2'-deoxyadenosine 3'-*O*-(H-phosphonate) (**7Fe**), were obtained by the two-step procedure shown in Scheme 2.

First, the 5'-hydroxyl function of conjugates **5Fe** and **5Co** was protected with a dimethoxytrityl group under standard conditions,¹⁵ yielding **6Co** and **6Fe**. Next, the 5'-protected conjugates **6Co** and **6Fe** were transformed into the correspond-

Scheme 3. Synthesis of Dinucleotides Bearing Cobalt and Iron bis(carborane) Complexes

ing 3'-*O*-(H-phosphonates) **7Fe** and **7Co** according to the PCl_3 /triazole method.¹⁶ Thus, to a cooled (to -40 °C) and stirred solution of imidazole, triethylamine, and phosphorus trichloride in methylene chloride was added a solution of **6Fe** or **6Co** in the same solvent. After completion of the reaction a solution of tetraethylammonium bicarbonate (TEAB) buffer was added and the resulting mixture warmed to room temperature spontaneously. After a standard workup the product was isolated by silica gel column chromatography using a linear gradient of CH_3OH in CH_2Cl_2 as the eluting solvent system. Deoxyadenosine/ferracarborane (**5Fe**) and deoxyadenosine/cobaltacarborane (**5Co**) conjugates were fully characterized by UV, ^1H , ^{31}P , and ^{11}B NMR, and mass spectroscopy.

The DNA-dinucleotides **11Fe** and **11Co**, bearing cobalt or iron bis(dicarbollide) complexes, were obtained via the H-phosphonate method in solution,¹⁶ by condensation reactions of the H-phosphonate nucleotide components **7Co** and **7Fe** with 3'-*O*-(*tert*-butyldimethylsilyl)thymidine (**8**). The fully protected dinucleotide intermediates **9Co** and **9Fe** were next oxidized to transform the H-phosphonate internucleotide linkage into the natural phosphate one, yielding **10Co** and **10Fe**. Subsequently, the 3'-*O*-bis(*tert*-butyldimethylsilyl) group and the 5'-*O*-dimethoxytrityl group was removed with TBAF and 80% acetic acid, respectively, yielding the final dinucleotide conjugates **11Co** and **11Fe** (Scheme 3).

The deprotected metalated dinucleotides **11Co** and **11Fe** were characterized by HPLC, by UV and ^{31}P NMR spectroscopy, and by FAB-MS. Due to the presence of an amphiphilic

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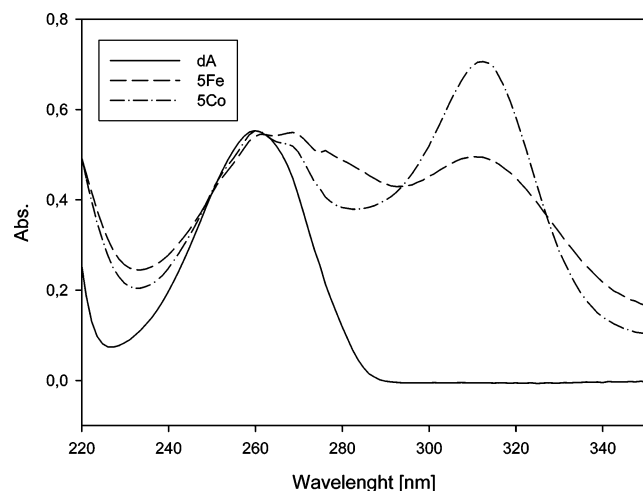


Figure 1. UV spectra of 2'-deoxyadenosine (dA) and its deprotected derivatives **5Fe** and **5Co**, containing a metallacarborane substituent attached to the nucleobase.

metallacarborane group, both dinucleotides showed highly increased lipophilicity, as measured by their retention times (R_t) on an RP-HPLC column. The usefulness and diagnostic value of the UV spectra in the case of nucleoside/metallacarborane conjugates should be pointed out. In addition to nucleic base absorptions a characteristic absorption of the metallacarborane moiety around λ_{max} 310 nm for all nucleoside/metallacarborane conjugates was observed (Figure 1).

In addition, enzymatic digestion of dinucleotides **11Co** and **11Fe** with snake venom phosphodiesterase (svPDE) was

performed. HPLC analysis of the digests showed the presence of both modified and unmodified nucleoside components, as expected.

In conclusion, we have shown the applicability of nucleoside alkylation with 8-dioxane-(3,3'-metal-1,2,1',2'-dicarbollide) adducts for incorporation of iron as well as cobalt¹⁰ into nucleoside molecules.

The work on incorporation of other metals is in progress. An efficient method for the formation of internucleotide bonds between natural and metallacarborane-modified nucleosides using H-phosphonate chemistry was shown for the first time. The development of a metallacarborane-based method for DNA metalation may open the way for more widespread use of metal-labeled nucleic acids for a variety of applications.^{1,17}

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Supporting Information Available: Text and figures giving experimental details and procedures for **4Fe**, **5Fe**, **6Co**, **6Fe**, **7Fe**, **7Co**, **9Fe**, **9Co**, **10Fe**, **10Co**, **11Fe**, and **11Co** and their UV, ¹H, ³¹P, and ¹¹B NMR, and MS spectra. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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