Boron Clusters − A New Entity for DNA-Oligonucleotide Modification

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Dedicated to Professor Jaromir Plešek on the occasion of his 75th birthday

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The carboranyl cluster is a new and versatile modifying entity for nucleotides and nucleic acids. Three types of carboranyl ($-C_2B_{10}H_{11}$) group-containing DNA-oligonucleotides are described: (1) CBMP-oligonucleotides, consisting of the carborane cage within an internucleotide linkage; (2) CDU-oligonucleotides, containing the carborane cage attached to a nucleobase; and (3) 2′-CBM-oligonucleotides, with the carborane cage linked to a sugar residue at the 2′ position. The method of synthesis and the physicochemical and biochemical features of these novel modifications are discussed, together with structure–property relationships.

The carboranyl cluster-containing oligonucleotides form a crossover between (carba)borane chemistry and molecular biology. They are potentially useful as antisense agents for antisense oligonucleotide therapy (AOT) and boron carriers for boron neutron capture therapy (BNCT). The chemistry of carborane-modified nucleic acids has implications beyond BNCT and AOT. Owing to the unique properties of carborane clusters they have potential for further development as molecular probes for molecular medical diagnostics and bioinorganic material for emerging technologies.

Introduction

Oligonucleotides are short pieces of synthetic DNA or RNA with the ability to hybridize in a sequence-specific manner to the complementary fragment of another nucleic acid. The sequence of the nucleobases A,T,G,C in a single-stranded DNA chain encodes genetic information. Albeit, any information can be encoded as a four-bases sequence, similarly to the use of binary code in electronic information storage and computing. This fundamental property of nucleic acids as information-carrying molecules, together with the development of automated chemical methods for oligonucleotide synthesis and enzyme-assisted synthetic methods,[1] has allowed numerous applications of oligonucleotides as tools in molecular biology and medical diagnostics, and as prospective biotherapeutic agents.[2−4] Emerging technologies such as nanotechnology and biosensing show that synthetic DNA and RNA have implications beyond biology and medicine. Natural and modified nucleic acids and oligonucleotides find applications as components for nanoconstruction[5,6] and in the field of DNA biocomputing.[7] The range of technological applications of nucleic acids is expanding very rapidly.

Unmodified oligonucleotides are readily available; however, they often do not satisfy the requirements necessary

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Zbigniew J. Lesnikowski was born in Lodz, Poland, in 1950. He received his Ph.D. in Organic Chemistry at the Center of Molecular and Macromolecular Studies (CMMS) PAS in 1981 under Prof. Wojciech J. Stec. After postdoctoral studies at the University of Virginia (1981−1983) with Prof. Sidney M. Hecht, he continued his work at CMMS. He received a D.Sc. degree at the Technical University of Lodz in 1992, continuing his affiliation with CMMS as Assistant Professor till 1994. In 1992 he received a Fulbright Scholar Fellowship and moved to the Emory University in Atlanta. He worked at Emory University and Veterans Administration Medical Center in Atlanta with Prof. Raymond F. Schinazi till 1996. In 1997 he joined the Center of Microbiology and Virology (CMV) PAS in Lodz as a head of the Laboratory of Molecular Virology and Biological Chemistry, and since 2002 as an Associate Director and next Director of CMV. He is interested in the chemistry of nucleic acids and their applications in molecular diagnostics and chemotherapy with focus on boron-containing oligonucleotides as boron carriers for BNCT, antisense agents, and new bioinorganic materials. He is also one of the inventors of the stereoselective synthesis of P-chiral analogs of oligonucleotides.

MICROREVIEWS: This feature introduces the readers to the authors′ research through a concise overview of the selected topic. Reference to important work from others in the field is included.

to perform designed functions, and hence modified oligonucleotides are frequently used. There is an array of DNA- and RNA-oligonucleotide modifications designed and synthesized for specific applications. Essentially, all constituents of the oligonucleotide chain: nucleobases, sugar, and phosphate residue, can be modified.[8−10] Carborane ($-C_2B_{10}H_{11}$)-containing oligonucleotides are one of the most recently developed and versatile types of oligonucleotide derivatives.[11,12] Their potential applications range from use as boron-rich carriers for Boron Neutron Cancer Therapy (BNCT), antisense drugs, molecular probes for molecular diagnostics and molecular biology to new materials for emerging technologies. The carborane-modified oligomers form a junction between the bioorganic chemistry of nucleic acids and the inorganic chemistry of boron clusters. Some aspects of the chemistry and the physicochemical and biochemical characteristics of these novel oligonucleotide modifications are summarized in this overview.

Discussion

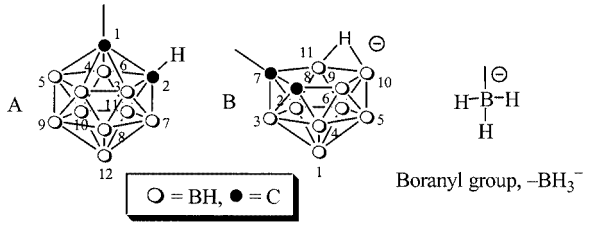
Boron-Containing Nucleic Acids

The search for DNA analogs useful as potential pharmaceuticals and materials for new technologies based on hybridization of nucleic acids and their ability to store (DNA, RNA), process (RNA), and transmit (DNA⟩RNA⟩protein) information has led to a wide range of DNA analogs. However, among these various derivatives only a few have found broader applications, leaving room for the development of new modifications.[13] Boron-containing oligonucleotides form a new branch of the modified oligonucleotides family with potential for application in many fields of science and practice.

At present there are two major types of boron-containing oligonucleotides described in the literature. The first type, modified with a borane ($-BH_3$)[14−18] or cyanoborane ($-BH_2CN$)[19,20] function, contains one boron atom per modification; the second type, bearing the highly lipophilic carboranyl group ($-C_2B_{10}H_{11}$) comprises ten boron atoms involved in a carboranyl cage structure (Figure 1). Discussion of oligonucleotides modified with a borane group is beyond the scope of the present outline. Interested readers are referred to recent reviews on this promising oligonucleotide modification.[21−26] The chemical, biophysical, and biological properties of oligonucleotides modified with a carborane cage are described below.

Carborane-Containing Oligonucleotides

Oligonucleotides (nucleic acids), in their primary chemical structure, are linear polymers of monomers called nucleotides. Nucleotides consist of a nucleoside and a phosphate group. Phosphate groups form phosphodiester linkages between nucleoside units. A nucleoside is built of a pentose sugar residue (deoxyribose in DNA, ribose in RNA) and a purine (A, G) or pyrimidine (C, T in DNA; C, U in RNA) nucleobase. Oligonucleotides can therefore be considered also as oligomers built of a phospho-sugar backbone and nucleobases attached to the sugar residues at carbon 1′ through an N-glycosidic linkage.



(A) 1,2-Dicarba-*closo*-decaborane-1-yl group ($-C_2B_{10}H_{11}$),
(B) 7,8-dicarba-*nido*-undecaborane-7-yl, group $(-C_2B_9H_{11})^-$.

Figure 1. Structure of carborane ($-C_2B_{10}H_{11}$) and borane ($-BH_3^-$) group

We developed versatile methods for modifying DNA-oligonucleotides with a carborane cage attached to a sugar, nucleobase, or phosphorus atom of the internucleotide linkage. This new modification allows modulation of important physicochemical and biological parameters of the oligomer.[12,27]

Boron-rich oligomeric phosphate diesters, described by Hawthorne and associates[28,29] as potential boron carriers for BNCT, have a skeleton resembling the phospho-sugar backbone of nucleic acids in which a pentose has been replaced with a simple acyclic diol, while the nucleobases are replaced with carborane cages (Figure 2).[30]

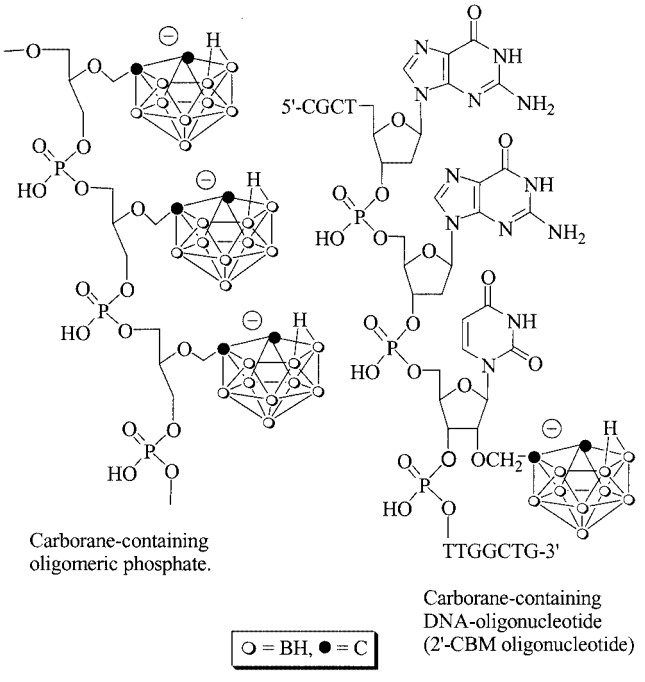


Figure 2. Example of oligomeric *nido*-carborane phosphate diester (refs.[28,29]) and oligonucleotide containing *nido*-carborane group (ref.[53])

Boron-containing oligomeric phosphates (“boron trailers”) can be synthesized using the same chemistry and

automated DNA-synthesizers as developed originally for the chemical synthesis of nucleic acids. The compatibility of the synthetic methods for oligonucleotides and oligomeric phosphates, together with the same or similar techniques for their purification, analysis, and labeling with radioactive or fluorescent groups make possible the synthesis of chimeric oligomers containing both oligonucleotide and oligomeric phosphate sections.[29]

Boron-containing oligonucleotides, in contrast to oligomeric phosphates, are derivatives of complete nucleic acids modified with a carborane cage attached to the nucleotide monomer. Because of this, they retain the unique features of nucleic acids and their capacity to encode and carry information.

Structure and Properties of Dicarba-*ortho*-dodecaborane Cage (1,2-$C_2B_{10}H_{12}$)

There are numerous reviews and books on all aspects of carborane theory, structure, and chemistry,[41,42,44] so discussion here will be limited to the fundamentals.

Carboranes, e.g. dicarba-*ortho*-dodecaborane (1,2-$C_2B_{10}H_{12}$), from the viewpoint of theory can be regarded as derivatives of boron hydrides in which B^- or BH groups have been replaced by isoelectronic carbon atoms. The capacity of boron to catenate and form self-bonded complex molecular networks such as carboranes is as extensive as any element except for carbon. The term "carboranes" is an abbreviation of the IUPAC name "carbaboranes" and includes both closed polyhedra (*closo*-carboranes, Gr. *closo* = cage) and open-cage structures (*nido*- and *arachno*-carboranes, Gr. *nido* = nest, Gr. *arachno* = web).[41] Like their parent boron hydride, borane, carboranes are electron-deficient compounds, which means that they have more valence orbitals than electrons. In other words, they contain too few electrons to coordinate fully all skeletal atoms using solely two-center two-electron bonds, Therefore they share the bonding electrons, forming three-center two-electron bonds.[42]

The icosahedral dicarba-*ortho*-dodecacarborane (1,2-$C_2B_{10}H_{12}$) has spherical geometry with 20 sides and 12 vertices, in which the carbon and boron atoms are hexacoordinate and participate in the heavily delocalized bonding (Table 1). This, together with its undergoing reactions typical of aromatic compounds, makes the carborane molecule a "pseudoaromatic" system. It is remarkable that the aromatic character of carboranes extends in three dimensions, in contrast to the two-dimensional aromaticity in planar polygonal hydrocarbons such as benzene.[43]

The electronic effects of a boron atom in the carborane cage changes according to the following sequence: the more remote the boron atom (electronegativity = 2.0) is from the carbon atoms (electronegativity = 2.6) in the cluster, the stronger is its electron-donating effect.[45] Consequently the electron densities in *ortho*-carborane decrease in the order 9 (12) >8 (10) > 4 (5,7,11) > 3 (6) > 1 (2).[46,47] On the other hand, the carborane group as a whole has a strong electron-withdrawing character.

Table 1. Comparison of bond lengths found in the dicarba-*ortho*-dodecaborane cage (1,2-$C_2B_{10}H_{12}$)[45−47,50]

	Bond distance [Å]	
	Dicarba-*ortho*-dodecaborane	Other compounds
C−C	1.62−1.70	1.53 (alkane)
B−C	1.70−1.75	1.60 (not cages)
B−B	1.70−1.75	1.77 (in B_2H_6)
B−H	1.10	1.19 (not bridging in B_2H_6)
C−H	1.10	1.10 (alkane)

One of the most important features of a carborane system is its ability to enter into substitution reactions at both the carbon and boron atoms without degradation of the carborane cage. Another is the transformation of the closed cage carborane *closo*-1,2-$C_2B_{10}H_{12}$ into the open cage form *nido*-7,8-$C_2B_9H_{11}(-1)$ ion under basic conditions, followed by the ability to form "sandwich"-type derivatives which are analogs of the transition metal cyclopentadienide derivatives.

The space occupied by dicarba-*ortho*-dodecaborane is about 50% larger than that of a rotating phenyl group.[48] Carboranes are characterized by such properties as high boron content, remarkable thermal (up to 400 °C) and chemical stability, and exceptional hydrophobic character similar to the adamantyl group.[49]

Nucleotide Monomers for Synthesis of Carborane-Containing Oligonucleotides

Three types of carboranyl group ($-C_2B_{10}H_{11}$) containing DNA-oligonucleotides have been reported so far: (1) (*o*-carboran-1-yl)methylphosphonate-oligonucleotides (CBMP-oligonucleotides), consisting of a carborane cage within an internucleotide linkage,[11,51] (2) 5-(*o*-carboran-1-yl)-2′-deoxyuridine-oligonucleotides (CDU-oligonucleotides) containing a carborane cage attached to a nucleobase,[52] and (3) 2′-*O*-(*o*-carboran-1-yl)methyl-oligonucleotides (2′-CBM-oligonucleotides) with a carborane cage linked to a sugar residue at the 2′ position.[53] Suitable nucleotide monomers have been developed (Figure 3) for the synthesis of each of these oligonucleotide modifications.

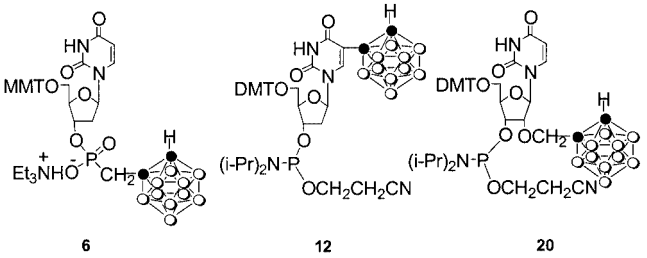


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Figure 3. Monomers for synthesis of CBMP-, CDU-, and 2′-CBM-oligonucleotides

The modified monomer for the synthesis of CBMP-oligonucleotides,[12] 5′-*O*-monomethoxytritylthymidine 3′-*O*-

(*o*-carboran-1-ylmethyl)phosphonate triethylammonium salt (**6**), was synthesized in a two-step procedure (Figure 4). First, the reaction of 5′-*O*-monomethoxytritylthymidine with the borophosphorylating agent, *O*-methyl (*o*-carboran-1-ylmethyl)phosphonate **4** was performed in the presence of triisopropylbenzenesulfonyl chloride as activating agent, 2,4,6-collidine and 1-methylimidazole,[54] yielding the intermediate 5′-*O*-monomethoxytritylthymidine 3′-*O*-(*O*-methyl-*o*-carboran-1-ylmethyl)phosphonate **5**. Selective demethylation of **5** with thiophenol/triethylamine/dioxane provided **6**. Borophosphorylating agent **4** was prepared in a three-step procedure.[11] In the first step, propargyl bromide was reacted with trimethyl phosphite using a Michaelis–Arbuzov-type reaction,[55] yielding *O*,*O*-dimethyl propargylphosphonate (**2**). In the second step, **2** was treated with decaborane in acetonitrile[56] to yield *O*,*O*-dimethyl (*o*-carboran-1-ylmethyl)phosphonate (**3**) which, under treatment with a mixture of thiophenol/triethylamine/dioxane,[57] was transformed into *O*-methyl (*o*-carboran-1-yl-methyl)phosphonate **4** obtained as a triethylammonium salt (Figure 4). The CBMP modification was incorporated into an oligonucleotide chain using the phosphotriester method of oligonucleotide synthesis,[54] and the rest of the oligomer was synthesized using standard automated amidophosphite chemistry on a solid support.[58]

Internucleotide linkage formation via the phosphotriester method, using monomers containing a four-coordinate phosphorus atom as in **6**, is in general less efficient than using phosphoramidite monomers containing a three-coordinate phosphorus atom as, for example, in **12** and **20**. This effect can arise from the properties of four- vs. three-coordinate phosphorus atoms themselves as well as from carboranyl cage bulkiness. Using spacers to link the carboranyl group to the phosphorus atom is a possible option to improve coupling of the boron-containing monomer by relieving some of the strain from the adverse steric interaction.

Another option for the automated synthesis of CBMP-oligonucleotides on a solid support allowing the phosphoramidite approach is to use dimer building blocks like 5′-*O*-dimethoxytrityl-2′-deoxynucleosidylyl-(3′,5′)-CBMP-2′-deoxynucleosidylyl-3′-*O*-[*N*,*N*-diisopropyl-*O*-(2-cyanoethyl)]phosphoramidite. Dinucleotide and trinucleotide phosphoramidite synthons are often used for the generation of oligonucleotide libraries or for the synthesis of backbone-modified oligomers.[59–61] Both this approach to the preparation of CBMP-oligonucleotides and the synthesis of three-coordinate phosphoramidite monomers are under investigation in our laboratory.

The modified monomer for the synthesis of CDU-oligonucleotides,[52] 5′-*O*-dimethoxytrityl-5-(*o*-carboran-1-yl)-2′-deoxyuridine 3′-*O*-[*N*,*N*-diisopropyl-*O*-(2-cyanoethyl)]phosphoramidite (**12**), was prepared from 5-(*o*-carboran-1-yl)-2′-deoxyuridine (CDU, **10b**) in a two-step procedure.[52,62,63] First, the 5′-hydroxyl function of CDU (see **10b**) was protected with the dimethoxytrityl group, yielding 5′-*O*-dimethoxytrityl-5-(*o*-carboran-1-yl)-2′-deoxyuridine (**11**). In the second step, compound **11** which contains a free 3′-hydroxy group, was reacted with (*N*,*N*-diisopropyl)-*O*-(2-cyanoethyl)chlorophosphoramidite, providing the phosphoramidite monomer **12** (Figure 5).

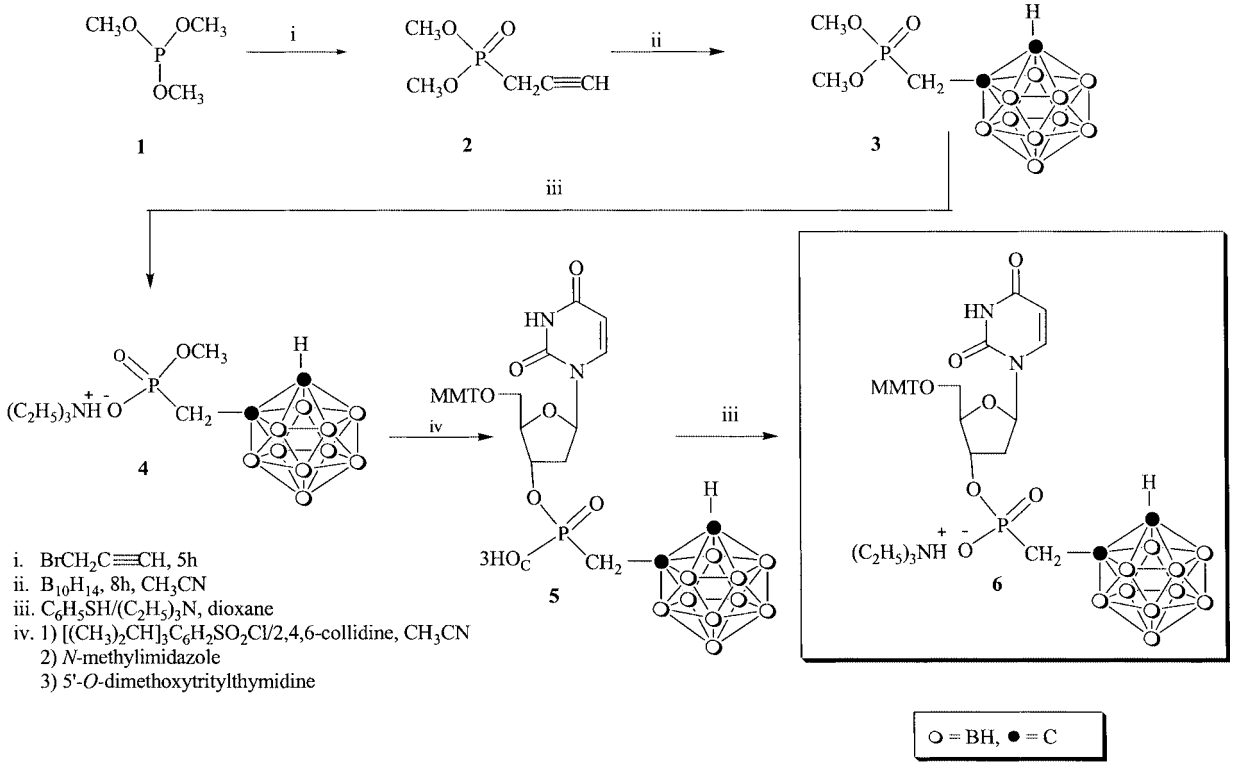


Figure 4. Synthesis of 5′-*O*-monomethoxytritylthymidine 3′-*O*-(*o*-carboran-1-yl-methyl)phosphonate (**6**) – monomer for preparation of CBMP-oligonucleotides

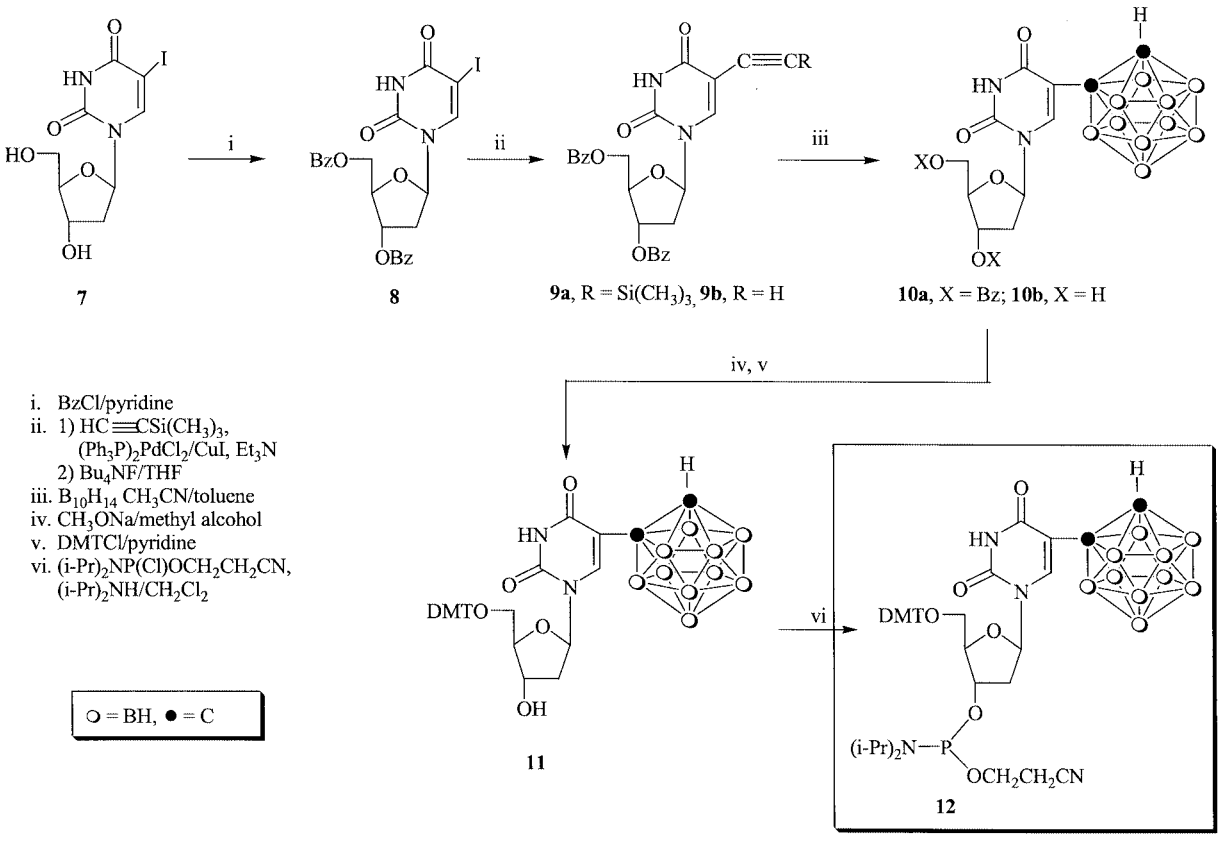


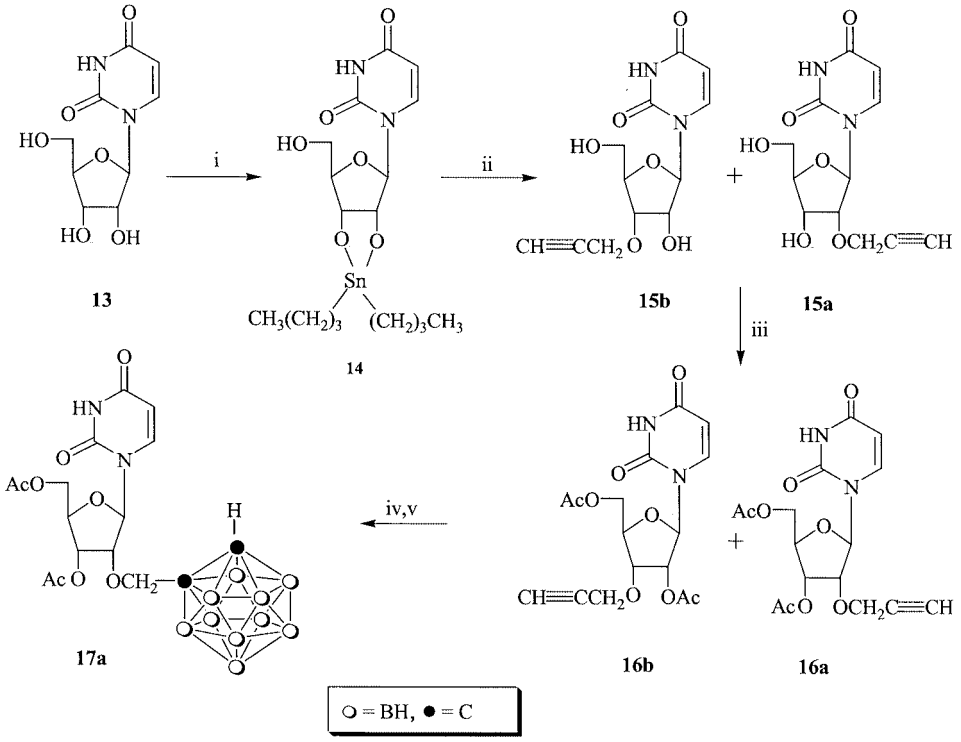
Figure 5. Synthesis of 5′-*O*-dimethoxytrityl-5-(*o*-carboran-1-yl)-2′-deoxyuridine 3′-*O*-[*N,N*-diisopropyl-*O*-(2-cyanoethyl)]phosphoramidite (**12**) monomer for preparation of CDU-oligonucleotides

Compound **10b** was prepared according to the literature procedure starting from commercially available 5-iodo-2′-deoxyuridine (**7**) (Figure 5).[68,69] Thus, the protected nucleoside precursor 5-iodo-3′,5′-(*O,O*-dibenzoyl)-2′-deoxyuridine (**8**) was prepared from **7** by treatment with benzoyl chloride, then **8** was coupled with trimethylsilylacetylene in the presence of bis(triphenylphosphane)palladium(II) chloride/CuI yielding 5-trimethylsilylethynyl-3′,5′-(*O,O*-dibenzoyl)-2′-deoxyuridine (**9a**). The trimethylsilyl group protecting the ethynyl function in **9a** was removed with tetrabutylammonium fluoride in tetrahydrofuran, yielding 5-ethynyl-3′,5′-(*O,O*-dibenzoyl)-2′-deoxyuridine (**9b**). Hydroboration of the ethynyl residue in **9b** with decaborane is performed analogously as described above for **2**, yielding 5-(*o*-carboran-1-yl)-3′,5′-(*O,O*-dibenzoyl)-2′-deoxyuridine (**10a**). Debenzoylation of the 3′- and 5′-hydroxyl functions in compound **10a** under alkaline conditions provided CDU.

A nucleoside precursor for the synthesis of 2′-CBM-oligonucleotides, 2′-*O*-(*o*-carboran-1-yl)methyluridine (**18**), was synthesized from uridine in a five-step procedure, as described by Soloway and co-workers (Figure 6).[70] Thus, 2′- and 3′-oxygen functionalities in the uridine **13** were activated with dibutyltin oxide yielding the intermediate 2′,3′-*O,O*-dibutylstannyleneuridine (**14**).[71] Alkylation of **14** with propargyl bromide gave a mixture of 2′-*O*-(3-prop-1-ynyl)uridine (**15a**) and 3′-*O*-(3-prop-1-ynyl)uridine (**15b**). Next, a mixture of regioisomers **15a** and **15b** was acetylated with acetic anhydride, resulting in a mixture of 3′-*O*-acetyl-2′-*O*-(3-prop-1-ynyl)uridine (**16a**) and 2′-*O*-acetyl-3′-*O*-(3-prop-1-ynyl)uridine (**16b**). Compounds **16a** and **16b** after hydroboration with the bis(acetonitrile)decaborane complex as described above for **2** and **9b** were transformed into a mixture of 3′-*O*-acetyl-2′-*O*-(*o*-carboran-1-yl)methyluridine (**17a**) and 2′-*O*-acetyl-3′-*O*-(*o*-carboran-1-yl)methyluridine (**17b**) which were separated into individual species by column chromatography.

The modified monomer for the synthesis of 2′-CBM-oligonucleotides,[53] 5′-*O*-dimethoxytrityl-2′-*O*-(*o*-carboran-1-yl)methyluridine 3′-*O*-[*N,N*-diisopropyl-*O*-(2-cyanoethyl)]-phosphoramidite (**20**), was obtained in a two-step procedure (Figure 7). First, the 5′-hydroxyl function of 2′-*O*-(*o*-carboran-1-yl)methyluridine (**18**) obtained from 3′,5′-diacetyl-2′-*O*-(*o*-carboran-1-yl)methyluridine (**17a**), was protected with the dimethoxytrityl group and next the resultant, partially protected 5′-*O*-dimethoxytrityl-2′-*O*-(*o*-carboran-1-yl)methyluridine (**19**), was treated with the phosphitylating agent (*N,N*-diisopropyl)-*O*-(2-cyanoethyl)-chlorophosphane yielding the phosphoramidite monomer **20**.[62−64]

The monomers, phosphonate **6** and phosphoramidites **12**, **20**, are derivatives of pyrimidine nucleosides. Work is in progress in our laboratory on conjugates of purine nucleosides containing a carborane cage. Purine nucleosides such as adenosine and guanosine, as well as their counterparts in the deoxyribonucleoside series, play an important role in cellular metabolism. Until now, however, less attention has



i. $(n\text{-}Bu_2SnO)_n/CH_3OH$, ii. $BrCH_2C{\equiv}CH$/DMF, iii. Ac_2O/pyridine, iv. $B_{10}H_{14}$ CH_3CN/toluene,
v. Separation of 2'- and 3'-isomers **17a** and **17b**

Figure 6. 3′,5′-diacetyl-2′-*O*-(*o*-carboran-1-yl)methyluridine (**17a**)

been given to this class of carborane-modified nucleosides because of difficulties in their preparation. We have developed recently a method for the synthesis of adenosine with a *para*-carborane cage attached to the sugar residue at the 2′-position.[72]

The successful approach to the synthesis of 2′-*O*-alkylcarborane-adenosine (2′-CBA, **24**) is based on the nucleophilic substitution of the activated thiomethyl group in 3′,5′-*O*,*O*-(tetraisopropyldisiloxane-1,3-diyl)-6-*N*-benzoyl-2′-*O*-methylthiomethyladenosine (**21**) with a suitable alcohol bearing a carborane cage. The key intermediate **21** was obtained in the reaction of 3′,5′-*O*,*O*-(tetraisopropyldisiloxane-1,3-diyl)-6-*N*-benzoyladenosine with DMSO in a mixture of acetic acid/acetic anhydride according to the literature procedure (Figure 8).

Availability of this type of nucleoside conjugate will provide an opening for the synthesis and study of adenosine and its biologically important phosphates such as AMP, ATP, and cAMP modified with lipophilic carborane pharmacophores. The same method can be applied for the synthesis of guanosine with a carborane modification at the 2′-position.

The bioorganic chemistry of boron clusters usually exploits derivatives of dicarba-*ortho*-dodecaborane (1,2-$C_2B_{10}H_{12}$). There are, however, other boron hydride cage systems such as dicarba-*para*-dodecaborane (1,7-$C_2B_{10}H_{12}$) or the dodecahydro-*closo*-dodecaborate anion [$(B_{12}H_{12})^{2-}$] which possess advantageous properties. Dicarba-*para*-dodecaborane is more lipophilic than dicarba-*ortho*-dodecaborane and does not undergo transformation from an electrically neutral, *closo*-cage into an electrically charged, open *nido*-form cage under basic conditions. This is an advantage since *nido*-carborane cages are often associated with increased toxicity. Salts of the dodecahydro-*closo*-dodecaborate anion on the other hand are hydrophilic and highly water soluble. The above mentioned adenosine conjugate with a *para*-carborane cage attached at the 2′-position takes advantage of the high lipophilicity and chemical stability of the dicarba-*para*-dodecaborane system. The synthesis of adenosine analogs modified with hydrophilic dodecahydro-*closo*-dodecaborate anion is under study in our laboratory.

Synthesis of Oligonucleotides

CBMP-, CDU-, and 2′-CBM-oligonucleotides modified with carborane cages were obtained by solid-phase automatic syntheses using a standard β-cyanoethyl phosphoramidite cycle.[8,9,73] Modified monomers **12** and **20**, used for the synthesis of CDU- and 2′-CBM-oligonucleotides respectively, were inserted into the oligonucleotide chain automatically or manually (Figure 9). Manual step phosphotriester chemistry was used for insertion of the modified monomer **6**, bearing the CBMP group.

To study the effect on oligomer properties of the carborane cage and its location within the oligonucleotide chain, several CDU- and CBMP-dodecathymidylic acids and 2′-CBM-heterooligonucleotides bearing modification at the

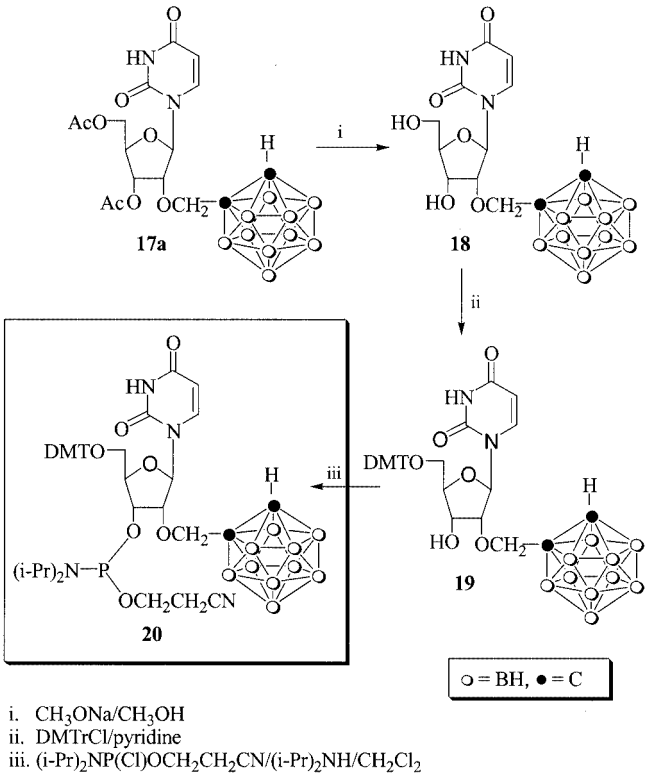


Figure 7. Synthesis of 5′-*O*-dimethoxytrityl-2′-*O*-(*o*-carboran-1-yl)methyluridine 3′-*O*-[*N*,*N*-diisopropyl-*O*-(2-cyanoethyl)]phosphoramidite (**20**) monomer for preparation of 2′-CBM-oligonucleotides

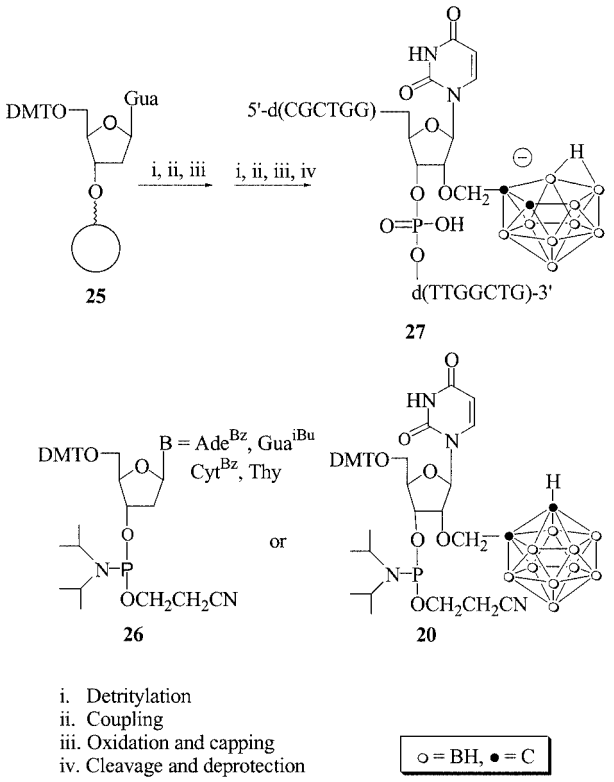


Figure 9. Solid phase, automated synthesis of carborane-modified oligonucleotide (2′-CBM-oligonucleotide, **27**) using standard phosphoramidite chemistry

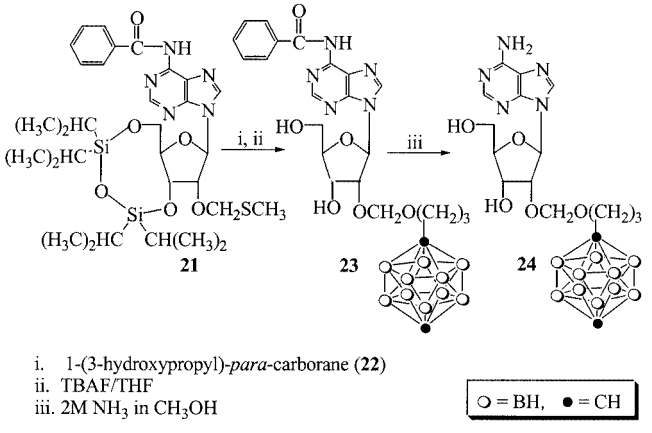


Figure 8. Synthesis of 2′-*O*-[(*para*-carboran-1-yl)propyleneoxymethyl]adenosine (**24**)

3′- or 5′-end, or in the middle of oligonucleotide, were synthesized.[12,52,53,74]

Owing to the predisposition of *ortho*-carboranes (1,2-$C_2B_{10}H_{12}$) to transform from closed to opened cage (*closo*/*nido* transformation), CBMP- and CDU-oligonucleotides were obtained as mixtures containing both *closo*- [*closo*-1,2-$C_2B_{10}H_{12}$], and *nido*-forms [*nido*-7,8-$C_2B_9H_{11}$] of the carborane cluster. Oligonucleotides were detached from the solid support then, following removal of the 5′-dimethoxytrityl group, they were purified and separated into species bearing *nido*- and *closo*-forms of the carborane cluster by reversed-phase high-pressure liquid chromatography (RP-HPLC). In the case of 2′-CBM-oligonucleotides, prolonged exposure to the alkaline conditions required for nucleic base deprotection meant that the carborane cage was completely transformed into the *nido*-form.

The *closo*-carboranyl cage is achiral (though prochiral), while the *nido*-form is chiral and can exist as two enantiomers.[12,75,76] The nucleosides are themselves chiral; the absolute configuration of chirality centers is the same for every nucleoside unit. Consequently an unmodified natural DNA or RNA molecule is homochiral (tactic) and can be considered as a single diastereomer. Because of the stereochemistry of the *ortho*-carboranyl cage, oligonucleotides containing a single modification exist as one species for the achiral *closo*-form of the cage, and as two diastereomers for the enantiomeric *nido*-form of the carboranyl residue. In the case of oligonucleotides containing two or more carborane groups, the number of possible oligonucleotide diastereomers is defined by the formula m^n, where m is the number of forms in which the carboranyl cage can exist (*closo*- and/or two *nido*-) and n is the number of carboranyl cages. The situation is more complicated in the case of oligonucleotides bearing a CBMP modification, which carries an additional center of chirality at the phosphorus of modified internucleotide linkage. In same cases diastereomers of carborane oligonucleotides, which were diastereomeric owing to the chirality of the *nido*-carborane substituent, were

separable by HPLC, and their properties were studied independently (Tables 2−4).[52,74]

Physicochemical Characteristics

The carborane-containing oligomers were characterized by RP-HPLC, ultraviolet spectroscopy (UV), and circular dichroism (CD). Their integrity was confirmed by electrospray ionization mass spectrometry (ESIMS). The effect of carborane modification on the stability of the duplexes formed between modified oligonucleotides and complementary DNA and RNA sequence was noted. The stabilities of these modified duplexes were compared by melting temperature (T_m) measurements to those formed between unmodified oligomers and the template. The effect of the carborane cage on the lipophilicity of oligonucleotides as measured by affinity to the reversed phase of the RP-HPLC column (R_t)[49] and the stability of the duplexes is discussed below.

The carboranyl cages are characterized by extremely high lipophilicity and therefore they can be used as the hydrophobic component (pharmacophore) in biologically active molecules, providing them with the capacity to interact hydrophobically with other molecules such as proteins or lipids in cellular membranes, improving their ability to penetrate lipid bilayers and potentially increasing the cellular uptake.[77,78] In the present overview the affinities of CBMP-, CDU-, and 2′-CBM-oligonucleotides to a lipophilic stationary phase were compared with unmodified counterparts by co-injection experiments under the same HPLC conditions using a C_{18} reversed-phase column.

Conclusions on carborane oligonucleotide lipophilicity based on the data presented in Table 2 must be drawn with some care, since homo-oligonucleotides for CBMP- and CDU-modification are compared with hetero-oligonucleotides in the case of 2′-CBM-modification. However, some general remarks can be proposed: (1) As anticipated, the lipophilicity of the *closo* derivatives, as measured by the retention time (R_t) was found to be higher than that of the *nido*-counterpart and in both cases it was significantly higher than that of unmodified oligomers. (2) The affinity towards the C_{18} reversed phase increases in the order CBMP-oligonucleotides > CDU-oligonucleotides > 2′-CBM-oligonucleotides. The high R_t shown by CBMP-oligo-

Table 2. HPLC characteristics of carborane-modified CBMP-, CDU-, and 2′-CBM-oligonucleotides

Oligomer	Status of carboranyl cage	Location of modification	R_t (min)	R_t, % of unmodified oligomer
	–	–	7.4	100[a] [b]
5′-d(T_{CBMP}TTTTTTTTTT)-3′	*closo-Fast*[a]	5′-end	13.7	185[a] [b] [c]
	closo-Slow[a]		14.0	189[a] [b] [c]
5′-d(TTTTTT$_{CBMP}$TTTTTT)-3′	*closo-*	middle	13.7	185[a] [b] [c]
5′-d(TTTTTTTTTT$_{CBMP}$T)-3′	*closo-Fast*[a]	3′-end	17.4	235[a] [b] [c]
	closo-Slow[a]	3′-end	18.6	251[a] [b] [c]
5′-d(T_{CBMP}TTTTTTTTTT)-3′	*nido-*	5′-end	12.2	165[a] [b] [c]
5′-d(TTTTTT$_{CBMP}$TTTTTT)-3′	*nido-*	middle	9.4	127[a] [b] [c]
5′-d(TTTTTTTTTT$_{CBMP}$T)-3′	*nido-Fast*[a]	3′-end	13.3	178[a] [b] [c]
	nido-Slow[a]		14.3	193[a] [b] [c]
5′-d(TTTTTTTTTTT)-3′	–	–	15.5	100[d] [e]
5′-d[(CDU)TTTTTTTTTT)]-3′	*closo-*	5′-end	25.8	166[d] [e] [f]
5′-d[(TTTTTT(CDU)TTTTTT)]-3′	*closo-*	middle	23.1	149[d] [e] [f]
5′-d[(TTTTTTTTTT(CDU)T]]-3′	*closo-*	3′-end	32.8	212[d] [e] [f]
5′-d[(CDU)TTTTTTTTTT)]-3′	*nido-1*[f]	5′-end	21.0	135[d] [e] [f]
	nido-2[f]		21.5	139[d] [e] [f]
5′-d[(TTTTTT(CDU)TTTTTT)]-3′	*nido-1*[f]	middle	19.9	128[d] [e] [f]
	nido-2[f]		20.3	131[d] [e] [f]
5′-d[(TTTTTTTTTT(CDU)T]]-3′	*nido-1*[f]	3′-end	25.0	161[d] [e] [f]
	nido-2[f]		25.5	165[d] [e] [f]
5′-d(CGCTGGTTTGGCTG)-3′	–	–	12.3	100[g] [h]
5′-d[CGC($U_{2'\text{-CBM}}$)GGTTTGGCTG)-3′	*nido-*[g]	5′-end	14.6	119[g] [h] [i]
5′-d(CGCTGG($U_{2'\text{-CBM}}$)TTGGCTG)-3′	*nido-*[g]	middle	14.4	117[g] [h] [i]
5′-d(CGCTGGTTTGGC($U_{2'\text{-CBM}}$)G]-3′	*nido-*[g]	3′-end	15.7	128[g] [h] [i]

[a] Separation of the diastereomers due to the presence of center of chirality at phosphorus atom of internucleotide linkage. [b] Z. J. Lesnikowski, R. M. Lloyd Jr., R. F. Schinazi, unpublished results. [c] HPLC conditions: buffer A: 0.05 M triethylammonium acetate (TEAA, pH 7.0); buffer B: CH_3CN/H_2O (1:1, v/v) containing 0.05 M TEAA (pH, 7.0); 5 min from 10% B to 40% B, 23 min 60% B, 2 min 60% B; Whatman Partisphere C_{18} 5 μm, 4.7 × 235 mm, flow rate of 1.0 mL/min. [d] Ref.[76] [e] HPLC conditions: buffer A: 0.05 M triethylammonium acetate (TEAA, pH 7.0); buffer B: CH_3CN/H_2O (1:1, v/v) containing 0.05 M TEAA (pH, 7.0); 20 min from 5% B to 20% B, 5 min from 20% to 30% B, 3 min 30% B; Whatman Partisphere C_{18} 5 μm, 4.7 × 235 mm, flow rate of 1.0 mL/min. [f] Separation of the diastereomers due to chirality of *nido*-carborane cage. [g] Ref.[53] [h] HPLC: buffer A, CH_3CN/H_2O (2:98, v/v) containing 0.1 M triethylammonium hydrogencarbonate (TEAB, pH 7.0); buffer B, CH_3CN/H_2 O (40:60, v/v) containing 0.1 M TEAB (pH, 7.0), 20 min from 0% to 100% B, 5 min from 100% B to 0% B, Econosil C_{18} 5 μm, 4.7 × 250 mm, flow rate of 1.0 mL/min. [i] Oligonucleotides diastereomeric due to chirality of *nido*-carborane cage, no separation of the diastereomers was detected.

mers is due to the replacing of a charged natural phosphodiester internucleotide linkage with the CBMP modification bearing neutral *closo*-carboranyl cages, and by virtue of the lipophilic property of the carboranyl cage itself. This is illustrated not only by the higher R_t values of carboranyl-modified oligonucleotides compared to unmodified ones, but also by the observation that their lipophilicity is substantially higher then that of methylphosphonate oligonucleotides bearing modifications in the same location of the oligonucleotide chain.[51] (3) Furthermore, oligonucleotides bearing the charged, low electron density, *nido*-carboranyl group are more lipophilic than the unmodified $d(T)_{12}$ despite the same number of formal negative charges. (4) The affinity of the modified oligomers for the C_{18} reversed phase of the HPLC column is dependent upon the location of the carborane group within the oligonucleotide chain. In all cases oligomers having the carborane cage at the 3′-end exhibited the highest value of R_t. It is worth pointing out that this phenomenon is independent of the *closo*-/*nido*-status of the carborane cage, the sequence of the oligomer (homo-oligonucleotide vs. hetero-oligonucleotide), and the type of carborane modification (CBMP-, CDU-, or 2′-CBM).[51−53,74,76]

This finding may reflect the polar nature of nucleic acids (the 3′-end differs from the 5′-end) and their tacticity. There are three centers of chirality at carbons C1′(*R*), C3′(*R*), and C4′(*R*) of β-D-2′-deoxyriboses in DNA and four centers of chirality in the β-D-riboses of RNA [carbons C1′(*R*), C2′(*R*), C3′(*R*), and C4′(*R*)]. The chirality is the same for every nucleotide unit. Consequently an unmodified natural DNA or RNA molecule can be considered as a single, stereoregular macromolecular diastereomer.

The above observations may have practical implications because they suggest that, in spite of their flexibility, single-stranded oligomers may interact with lipophilic structures such as cellular membranes preferentially from one terminus.

Similarly, a relationship has been found between the thermostabilty (T_m) of the duplexes formed by the carborane-containing oligonucleotides with a natural, deoxy- or ribo-

Table 3. Melting temperatures (T_m) of carborane-modified CBMP-, CDU and 2′-CBM-oligonucleotides

Oligomer	Status of carboranyl cage	Location of modification	T_m (°C)	T_m, % of unmodified oligomer
5′-d(TTTTTTTTTTTT)-3′	–	–	25	100[a] [b]
5′-d(T_{CBMP}TTTTTTTTTT)-3′	*closo-1/2*[a]	5′-end	38	152[a] [b] [c]
5′-d(TTTTTT$_{CBMP}$TTTTTT)-3′	*closo-*	middle	27	108[a] [b] [c]
5′-d(TTTTTTTTTT$_{CBMP}$T)-3′	*closo-1/2*[a]	3′-end	n.d.	–
5′-d(T_{CBMP}TTTTTTTTTT)-3′	*nido-*	5′-end	30	120[a] [b] [c]
5′-d(TTTTTT$_{CBMP}$TTTTTT)-3′	*nido-*	middle	23	92[a] [b] [c]
5′-d(TTTTTTTTTT$_{CBMP}$T)-3′	*nido-1*[a]	3′-end	27	108[a] [b] [c]
	nido-2[a]		31	124[a] [b] [c]
5′-d(TTTTTTTTTTTT)-3′	–	–	29	100[d] [e]
5′-d[(CDU)TTTTTTTTTTT)]-3′	*closo-*	5′-end	26	90[d] [e]
5′-d[(TTTTTT(CDU)TTTTTT)]-3′	*closo-*	middle	15	52[d] [e]
5′-d[(TTTTTTTTTT(CDU)T]]-3′	*closo-*	3′-end	21	72[d] [e]
5′-d[(CDU)TTTTTTTTTTT)]-3′	*nido-1*[f]	5′-end	21	72[d] [e] [f]
	nido-2[f]		22	76[d] [e] [f]
5′-d[(TTTTTT(CDU)TTTTTT)]-3′	*nido-1*[f]	middle	20	69[d] [e] [f]
	nido-2[f]		20	69[d] [e] [f]
5′-d[(TTTTTTTTTT(CDU)T]]-3′	*nido-1*[f]	3′-end	25	66[d] [e] [f]
	nido-2[f]		26	90[d] [e] [f]
5′-d(CGCTGGTTTGGCTG)-3′	–	–	56	100[g]
5′-d[CGC($U_{2′\text{-CBM}}$)GGTTTGGCTG)-3′	*nido-*[g]	5′-end	49	88[g] [h]
5′-d(CGCTGG($U_{2′\text{-CBM}}$)TTGGCTG)-3′	*nido-*[g]	middle	45	80[g] [h]
5′-d(CGCTGGTTTGGC($U_{2′\text{-CBM}}$)G]-3′	*nido-*[g]	3′-end	52	93[g] [h]

[a] Separation of the diastereomers due to the presence of center of chirality at phosphorus atom of internucleotide linkage. [b] Z. J. Lesnikowski, R. M. Lloyd Jr., R. F. Schinazi, unpublished results. [c] T_m: samples for T_m measurements were prepared by mixing of the $d(T)^{12}$ or (*o*-carboran-1-yl-methyl)phosphonate modified d(T) and poly r(A) to give a 1:1 base ratio (40 μM per base) in 4 m M phosphate buffer (pH, 7.0) containing 0.9 mM KCl, 100 mM NaCl, and 0.5 mM EDTA. Change of Cotton effect at 248 nm was followed as a function of temperature using a JASCO J720 spectrometer. [d] Ref.[52] [e] T_m: samples for T_m measurements were prepared by mixing of the $d(T)^{12}$or (*o*-carboran-1-yl-methyl)phosphonate modified d(T) and $d(A)_{12}$ to give a 1:1 base ratio (40 μM per base) in 10 mM PIPES buffer (pH, 7.0) containing 100 mM NaCl, and 1.0 mM EDTA. Change of UV absorbance at 260 nm was followed as a function of temperature using a Cary 4 UV spectrometer. [f] Separation of the diastereomers due to chirality of *nido*-carborane cage. [g] T_m: samples for T_m measurements were prepared by mixing of the 5′-d(CGCTGGTTTGGCTG)-3′ or (*o*-carboran-1-yl-methyl)phosphonate modified oligonucleotide and d(CAGCCAAACCAGCG) to give a 1:1 base ratio (ca. 3.5 μM per oligonucleotide) in 4 mM phosphate buffer containing 0.9 mM KCl, 100 mM NaCl, and 0.5 mM EDTA. Change of UV absorbance at 260 nm was followed as a function of temperature using a GBC Cintra 916 UV/Vis spectrometer. [h] Oligonucleotides diastereomeric due to chirality of *nido*-carborane cage, no separation of the diastereomers was detected. n.d. = not determined.

oligonucleotide complementary strand. Significant effects of CBMP-, CDU-, and 2′-CBM-modification on T_m were noted, depending on the location of the modification within the oligonucleotide chain, and the *closo*- or *nido*-status of the carboranyl cage (Table 3). All oligonucleotides bearing a carborane cage in the *nido*-status located at the 3′-end of the modified oligomer displayed a melting temperature higher than the oligonucleotide with the modification located at the 5′-end. Interestingly, the *closo*-status of the carborane cage had the inverse effect on the duplex stability of CDU-oligonucleotides. Thus, CDU-oligomers bearing a carborane cage in the *closo*-status at the 3′-end of the oligonucleotide chain displayed melting temperatures lower than those with the modification located at the 5′-end. Oligonucleotides with a centrally located modification were always characterized by lower T_m values, indicating a lower affinity of the modified oligomers for the template. The above observation is valid independently of the sequence of the oligomer (homo-oligonucleotide vs. hetero-oligonucleotide) and the type of carborane modification (CBMP-, CDU-, or 2′-CBM).[51−53,74,76] It may reflect a conformational characteristic of the duplex and a strong steric clash with the duplex components for a neutral carborane cage in a 3′-substituted double-stranded structure as compared to a 5′-substituted one, but a weaker electrostatic repulsion between a negatively charged *nido*-cage and a negatively charged internucleotide linkage at the same location. However, more study is needed for a better understanding of this interdependence.

Biochemical Characteristics

CBM-, CDU-, and 2′-CBM-oligonucleotides bearing *nido*-carborane cages are chemically stable at physiological pH. Under the same conditions an *ortho*-carborane cage in the *closo*-form attached to the oligonucleotide chain is slowly transformed into the *nido*-form.

It was found that CDU- and 2′-CBM-oligonucleotides, independently of the location of the modification, are good substrates for T4 polynucleotide kinase (PNK, an enzyme catalyzing addition of phosphate to the 5′-terminus of nucleic acids) and are phosphorylated efficiently. Enzymatic phosphorylation of CBMP-oligonucleotides with T4 PNK was observed for all oligonucleotides studied, with the exception of the oligonucleotide bearing a CBMP group at the 5′-end of the oligomer.[51,52,74,76]

CDU-oligonucleotides were primers for *Escherichia coli* polymerase I and human immunodeficiency virus type 1

Table 4. Resistance to enzymatic digestion of carborane modified CBMP-, CDU-, and 2′-CBM-oligonucleotides

Oligomer	Status of carboranyl cage	Location of modif.	$t_{1/2}$ [min] (% of unmodified oligonucleotide) bsPDE	svPDE
5′-d(TTTTTTTTTTTT)-3′	–	–	7.1 (100)[b,c]	1.5 (100)[b,d,e]
5′-d(T_{CBMP}TTTTTTTTTT)-3′	*closo-1/2*[a]	5′-end	> 40 (> 560)[a,b,c]	[a,b,d,e]
5′-d(TTTTTT$_{CBMP}$TTTTTT)-3′	*closo-*	middle	> 40 (> 560)[b,c]	[b,d,e]
5′-d(TTTTTTTTTTT$_{CBMP}$T)-3′	*closo-1*[a]	3′-end	4.8 (68)[b,c]	1.4[a,b,d]
	closo-2[a]		9.6 (135)[b,c]	1.6[a,b,d]
5′-d(T_{CBMP}TTTTTTTTTT)-3′	*nido-*	5′-end	>40(>560)	[a,b,e]
5′-d(TTTTTT$_{CBMP}$TTTTTT)-3′	*nido-*	middle	14.8 (208)	1.0[a,b,c]
5′-d(TTTTTTTTTTT$_{CBMP}$T)-3′	*nido-1*[a]	3′-end	23.0 (324)	0.2[a,b,c]
	nido-2[a]		42.5 (598)	1.0[a,b,c]
5′-d(TTTTTTTTTTTT)-3′	–	(100)[c,i]	0.5 (100)[d,e,h]	
5′-d[(CDU)TTTTTTTTTTT)]-3′	*closo*/*nido*[f]	5′-end	(>>100)[c,i]	n.d.
5′-d[(TTTTTT(CDU)TTTTTT)]-3′	*closo*/*nido*[f]	middle	(>100)[c,i]	n.d.
5′-d[(TTTTTTTTTTT(CDU)T]]-3′	*closo*/*nido*[f]	3′-end	(≈100)[c,i]	n.d.
5′-d[(TTTTTTTTTTT(CDU)T]]-3′	*nido-1*[g]	3′-end	n.d.	6.7 (134)[d,e,h]
5′-d(CGCTGGTTTGGCTG)-3′	–	–	175 (100)[j,k]	1.5 (100)[j,l]
5′-d[CGC($U_{2′\text{-}CBM}$)GGTTTGGCTG)-3′	*nido-*[g]	5′-end	58 (33)[j,k]	2.2 (147)[j,l]
5′-d(CGCTGG($U_{2′\text{-}CBM}$)TTGGCTG)-3′	*nido-* [g]	middle	39 h (1340)[j,k]	3.8 (253)[j,l]
5′-d(CGCTGGTTTGGC($U_{2′\text{-}CBM}$)G]-3′	*nido-* [g]	3′-end	100 (57)[j,k]	4.4 (293)[j,l]

[a] Separation of the diastereomers due to due to the presence of center of chirality at phosphorus atom of internucleotide linkage. [b] Z. J. Lesnikowski, R. M. Lloyd, Jr., R. F. Schinazi, unpublished. [c] Digestion with bsPDE: to 0.1 A_{260} ODU of each oligonucleotide in 200 mL of 100 mM NaOAc buffer (pH, 4.8) containing 1 mM $ZnSO_4$ and 0.1 A_{260} ODU of dC standard, $0.75{\cdot}10^{-3}$ unit of bsPDE (EC, 3.1.16.1) was added, the reaction was run at 37 °C. [d] Digestion with svPDE: to 0.1 A_{260} ODU of each oligonucleotide in 200 mL of 100 mM Tris·HCl buffer (pH, 8.9) containing 20 mM $MgSO_4$ and 0.1 A_{260} ODU of dC standard, $3{\cdot}10^{-3}$ unit of svPDE (EC, 3.1.4.1) was added, the reaction was run at 37 °C. [e] Digestion was too rapid to be determined. [f] A mixture of *closo-*/*nido*-oligomers was used, without separation into diastereomers due to *nido*-cage chirality. [g] Pure diastereomer *nido*-1 was used. [h] Ref.[52] [i] Ref.[74] [j] Ref.[73] [k] Digestion with bsPDE: to 0.75 A_{260} ODU of each oligonucleotide in 234 mL of 100 mM NaOAc buffer (pH, 6.6) containing 1 mM $ZnSO_4$ and 0.2 A_{260} ODU of uracil standard, $20{\cdot}10^{-2}$ unit of bsPDE (EC, 3.1.16.1) was added, the reaction was run at 38 °C. [l] Digestion with svPDE: to 0.75 A_{260} ODU of each oligonucleotide in 234 mL of 100 mM Tris·HCl buffer (pH, 8.9) containing 20 mM $MgSO_4$ and 0.2 A_{260} ODU of uracil standard, $11{\cdot}10^{-3}$ unit of svPDE (EC, 3.1.4.1) was added, the reaction was run at 38 °C.

(HIV-1) reverse transcriptase, but not for human DNA polymerase α and β. Oligonucleotides bearing a CDU modification at the 5′-end were elongated more efficiently than oligonucleotides modified at the 3′- end.[74]

All CDU-modified dodecathymidylates formed RNA−DNA complexes with a poly r(A) template, and were recognized as substrates for *E. coli* RNase H. RNase H digested these heteroduplexes in a fashion similar to the digestion of the unmodified duplex formed by dodecathymidylic acid.[74]

The resistance of modified oligonucleotides to nucleolytic digestion by the enzymes present in the blood and cytoplasm is one of the most important requirements for their biological applications. The resistance of CBMP-, CDU-, and 2′-CBM-oligonucleotides against snake venom (svPDE) and bovine spleen (bsPDE) exonucleases was studied. In the case of 2′-CBM-oligonucleotides their stability in human serum was also tested (Table 4).

The presence of a CBMP- or CDU-modification at the 5′-end of the oligonucleotide protected the modified oligomers against digestion by bsPDE (digestion from oligonucleotide 5′-end). Oligonucleotides with other than 5′-end locations of these modifications were digested, though at a significantly lower rate.[51,74] The behavior of 2′-CBM-oligomers is more complex since both protection of the oligomer against digestion and its faster decomposition were detected, depending upon the location of the modification.[53,79] This may suggest atypical interactions of the modified oligomer with the enzyme due to the presence of carborane cage, conformational changes of the oligomer, or formation of aggregates impeding enzymatic digestion.

Even more important than the resistance to 5′-exonucleases is resistance of the oligonucleotides to enzymatic digestion from the 3′-end because most of the nucleases present in the blood are 3′-exonucleases. Although no complete protection was achieved, CBMP-, CDU-, and 2′-CBM-modification had a pronounced effect on oligonucleotide resistance towards svPDE (digestion from oligonucleotide 3′-end).[51,53,72,73] The resistance was dependent upon the carborane cage location within the oligonucleotide chain. As expected, the 3′-end location provided better protection against svPDE than a middle or 5′-end position of the modification in the oligomer.

Prospects

The carboranyl cluster is a new modifying entity for oligonucleotides, potentially useful as antisense agents for antisense oligonucleotide therapy (AOT) and as molecular probes for molecular diagnostics of infectious and genetic disease based on hybridization technology. Though the practical application of biological molecules modified with carborane cages as boron carriers for BNCT and other biopharmaceuticals is often hindered by the exceptionally high hydrophobicity of carborane clusters which cause a poor water solubility[30] it is, in contrast, not a problem for the carborane-modified nucleic acids (and other oligophosphates).[28,29] Because of their polyanionic character, these molecules retain good solubility in water in spite of the hydrophobic carborane modification.

The range of technological applications of nucleic acids will expand rapidly in coming years. The chemistry of carborane-modified nucleic acids has implications beyond BNCT and AOT.[80] Emerging technologies such as nanotechnology, biosensing, and biocomputing are opening new avemes for their potential practical use.

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