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Nucleoside bearing boron clusters and their phosphoramidites – building blocks for modified oligonucleotide synthesis†

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This paper describes a general method for the synthesis of four canonical nucleosides T, dC, dA and dG and their phosphoramidites suitable for automated synthesis of DNA modified with a carborane cage. A boron cluster in the form of an electroneutral, lipophilic 1,2-dicarba-*closo*-dodecaborane ($C_2B_9H_{11}$) or negatively charged, redox-active 7,8-dicarba-*nido*-undecaborate ion ($C_2B_9H_{12}(-1)$) was used as a modifying unit. The method is based on the "click chemistry" type Huisgen–Sharpless–Meldal reaction. All boron cluster-nucleoside conjugates have been characterized electrochemically; they have shown different redox potentials allowing for selective electrochemical identification of individual nucleosides in the mixture. There is also the first description of the crystallographic structure of the boron cluster-nucleoside conjugate: N^3-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}methylenethymidine.

1. Introduction

Modified nucleic acids are widely used as suitable tools in molecular biology, biotechnology, and medicine. This, in turn, creates a continuous and increasing need for high quality modified oligonucleotides with unique or improved properties. Contemporary chemical synthesis of natural or modified DNA/RNA-oligonucleotides usually accommodates this demand. However, sometimes new physicochemical properties are needed to fulfill the needs of emerging technologies. Boron cluster modified nucleic acids are examples of such a case.

Arguably, the most important step in oligonucleotide synthesis is the coupling of the nucleotide units. The phosphoramidite method is the most widely used coupling chemistry due to its high efficacy, dependability and simplicity. The phosphoramidite method is usually coupled with synthesis in the solid phase, where the growing oligonucleotide chain is covalently anchored to a solid matrix in the column and reagents are washed down the reaction.[1] The standard nucleotide to be coupled to the growing oligomer chain is in the form of a phosphoramidite containing a diisopropylamine leaving group and a cyanoethyl and a 4,4′-dimethoxytrityl group as P–O and 5′-hydroxyl protecting units, respectively.

The same format was used for the herein presented nucleoside phosphoramidites modified with a 1,2-dicarba-*closo*-dodecaborane group (*ortho*-$C_2B_9H_{11}$) designed for automated DNA synthesis. Tethering of the boron cluster to a suitable nucleoside, a substrate for phosphoramidite preparation, is based on the azide–alkyne 1,3-dipolar cycloaddition catalyzed by copper(I).[2–5] Nucleosides bearing a terminal alkyne group as a boron cluster acceptor and boron-cluster donors containing a terminal azide group were used.[6–8]

Previously, synthesis of several types of carborane-modified oligonucleotides bearing modification at the thymidine or uridine residue has been described. The first contains a (*ortho*-carborane-1-yl)methylphosphonate modification within the internucleotide linkage (CBMP oligonucleotides).[9–11] The second encloses the modification within the nucleic acid base, 2′-deoxyuridine at the 5-position (CDU-modification).[12] The next type of carborane-modified oligonucleotide contains a (*ortho*-carboranyl-1-yl-methyl) group attached to a sugar residue at the 2′ position (2′-CBM modification).[13] Several advantageous properties of these modifications have been discovered, including: increased resistance to enzymatic digestion, increased lipophilicity and the ability to form a stable duplex with complementary templates.

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† Electronic supplementary information (ESI) available: ^{1}H-, ^{13}C-, ^{11}B-, ^{11}B{H BB}-, ^{31}P-NMR, DEPT-135, IR, MS spectra of 1,2-dicarba-*closo*-dodecaborane derivatives of thymidine: **20**, **21**, and **29**; 2′-deoxycytidine: **22**, **23**, and **30**; 2′-deoxyadenosine: **12–14**, **24**, **25**, and **31**; 2′-deoxyguanosine: **16**, **17**, **26–28**, and **32**; and conjugates with the open-cage 7,8-dicarba-*nido*-undecaborate ion **33–36**. CD spectra of **20**, **22**, **24**, and **27**, and MALDI-TOF spectra of oligonucleotides **38–41**. CCDC 1010195. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c4nj01096e

Recently, we described the physicochemical and biological properties of siRNA duplexes targeted against a BACE1 gene modified at various positions with a carborane cluster.[14] We also developed a method for the synthesis of DNA-oligomers modified simultaneously with both carborane and metallacarborane boron clusters and studied the physicochemical and biological properties of these bioorganic-inorganic constructs. The ability to silence the insulin receptor substrate 1 (IRS-1) gene was also addressed in our previous studies.[15]

However, the applicability of boron clusters for nucleic acid modification exceeds the presented examples. We have proved that boron clusters, carboranes as well as metallacarboranes, have the potential to be versatile redox labels useful for electrochemical detection of DNA.[16] We have described a metallacarborane-nucleoside phosphoramidite monomer and shown its usefulness in the synthesis of DNA-oligonucleotides targeted against a cytomegalovirus (HCMV) sequence and labeled with cobalt or iron bearing metallacarborane.[17,18] DNA with metallacarborane bearing iron located at the 5′ position of the DNA strand has been used for electrochemical determination of the DNA sequence derived from Avian Influenza Virus, type H5N1.[19]

The above results prompted us to expand the earlier developed methodology beyond pyrimidine nucleosides, thymidine or uridine, to all four canonical nucleosides: thymidine (T), 2′-deoxycytidine (dC), 2′-deoxyadenosine (dA) and 2′-deoxyguanosine (dG), labeled with a 1,2-dicarba-*closo*-dodecaborane group and their physicochemical characteristics. The resulting modified monomers allow for the synthesis of DNA bearing carborane modification at different designed locations.

2. Results and discussion

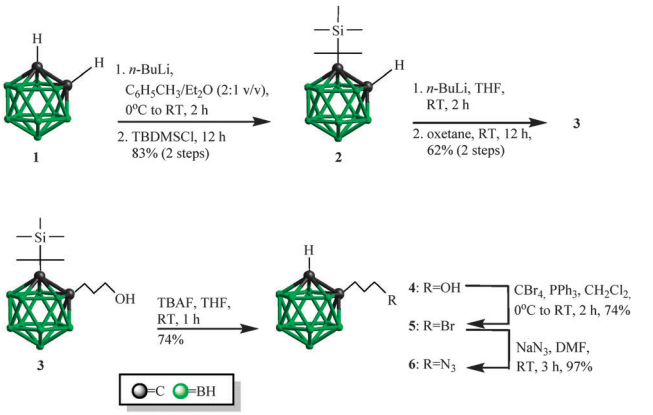
2.1 Synthesis of a boron-cluster donor

The synthesis of the boron-cluster donor 1-(3-azidopropyl)-1,2-dicarba-*closo*-dodecaborane (**6**) is depicted in Scheme 1.

The substrate for **6** synthesis, 1-(3-bromopropyl)-1,2-dicarba-*closo*-dodecaborane (**5**), was prepared according to the method by Ahrens *et al.*[20] First, one of the CH groups of 1,2-dicarba-*closo*-dodecaborane (**1**) was protected with the *tert*-butyldimethylsilyl (TBDMS) group. Then, 1-(*tert*-butyldimethylsilyl)-1,2-dicarba-*closo*-dodecaborane (**2**) was converted into 1-(*tert*-butyldimethylsilyl)-2-(3-hydroxypropyl)-1,2-dicarba-*closo*-dodecaborane (**3**). Next, the TBDMS group was removed to form deprotected 1-(3-hydroxypropyl)-1,2-dicarba-*closo*-dodecaborane (**4**).[21] The alcohol **4** was converted to 1-(3-bromopropyl)-1,2-dicarba-*closo*-dodecaborane (**5**) in an Appel reaction with carbon tetrabromide, triphenylphosphine.[20] The final product **6** was prepared from **5** in a reaction with sodium azide in DMF, analogously as described by us previously for *para*-carborane.[22] The reaction was completed after 3 h and provided **6** in a high yield.

2.2 Synthesis of a nucleoside-boron cluster acceptor

The synthesis of nucleoside acceptors of a boron cluster bearing terminal alkyne group was performed according to the literature procedures,[1,23–25] and new methods developed in our laboratory.



Scheme 1 Synthesis of boron-cluster donor **6**. TBDMSCl = *tert*-butyldimethylsilyl chloride, THF = tetrahydrofuran, TBAF = tetrabutylammonium fluoride, DMF = *N*,*N*-dimethylformamide, RT = room temperature.

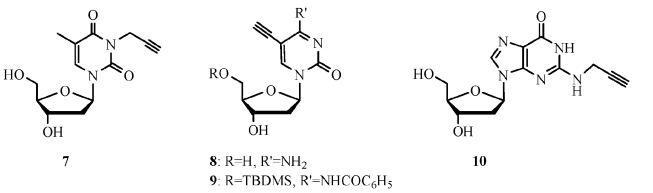


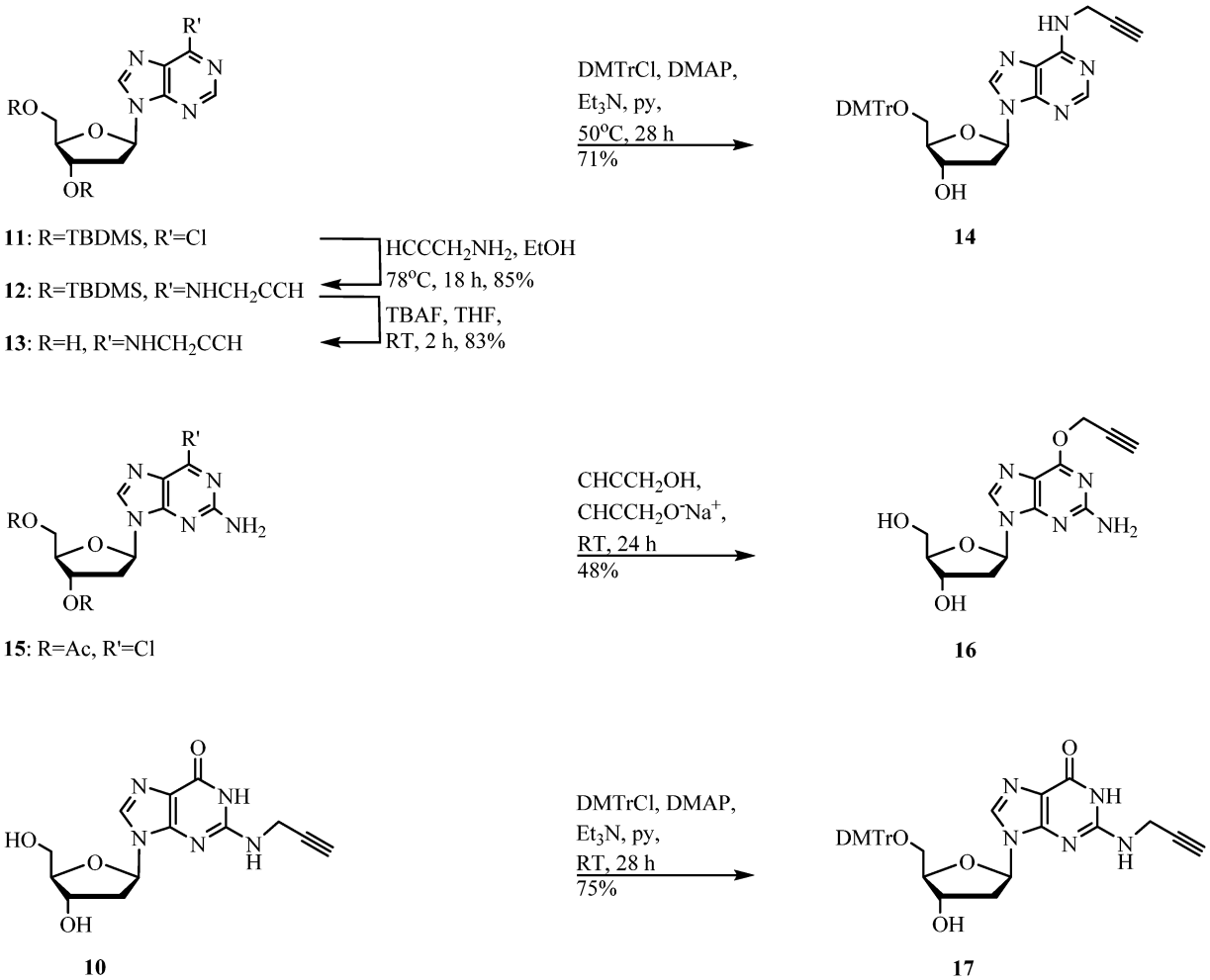
Fig. 1 Thymidine-(**7**), 2′-deoxycytidine-(**8**, **9**), 2′-deoxyguanosine-(**10**) boron cluster acceptors with a terminal triple bond.

N^3-(2-Propyn-1-yl)thymidine (**7**),[23] 5-ethynyl-2′-deoxycytidine (**8**)[24] and N^4-benzoyl-5′-*O*-*tert*-butyldimethylsilyl-5-ethynyl-2′-deoxycytidine (**9**),[1] N^2-(2-propyn-1-yl)-2′-deoxyguanosine (**10**)[25] were obtained according to described procedures (Fig. 1).

A new established method for the preparation of 2′-deoxyadenosine containing a triple bond at N-6 is based on nucleophilic substitution of chlorine in 9-[3,5-bis-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-β-D-*erythro*-pentofuranosyl]-6-chloropurine (**11**)[26] with propargylamine. This approach allowed for the introduction of a triple bond while restoring the amino function of 2′-deoxyadenosine (Scheme 2). The reaction was performed analogously to the reaction of 6-chloropurine nucleosides with other amines,[26,27] giving 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-N^6-(2-propyn-1-yl)-2′-deoxyadenosine (**12**) with an 85% yield. In the next step, the *tert*-butyldimethylsilyl protection was removed with TBAF to obtain N^6-(2-propyn-1-yl)-2′-deoxyadenosine (**13**) with a high yield.

A new 2′-deoxyguanosine-boron cluster acceptor with a terminal acetylenic group at oxygen O-6 (**16**) instead of at nitrogen N-2 (**10**) was also obtained. Nucleophilic displacement of the 6-chloro group in guanosine with a methoxide ion is known in the literature.[28]

Conversion of the 9-(3′,5′-*O*-diacetyl-2-deoxy-β-D-*erythro*-pentofuranosyl)-2-amino-6-chloropurine (**15**)[29] to O^6-(2-propyn-1-yl)-2′-deoxyguanosine (**16**) was achieved in the reaction of **15** with a freshly prepared sodium propargylate in anhydrous propargyl alcohol at room temperature for 24 h (48%) (Scheme 2). It is worth stressing that nucleophilic displacement of the 6-chloro group with a propargyl ion not only takes place with considerable



Scheme 2 Synthesis of 2′-deoxyadenosine-(**14**) and 2′-deoxyguanosine-(**16**, **17**) boron cluster acceptors with terminal alkyne group. TBDMS = *tert*-butyldimethylsilyl group, DMTrCl = dimethoxytriryl chloride, DMAP = 4-dimethylaminopyridine, py = pyridine.

ease, but that it is accompanied by the simultaneous removal of the acetate groups which is a desired process simplifying the synthetic procedure. Compound **16** has not been previously reported. A similar reaction between **15** and sodium propargylate in anhydrous methanol leads to the replacement of chlorine with methoxide instead of a propargyl ion, simultaneous deprotection of hydroxyl functions was also observed.

The synthesis of nucleoside–carborane phosphoramidite monomers requires protection of both the exocyclic amino group and the 5′-hydroxyl group.

The most common protecting groups for the nucleobases are a dimethylformamidyl or an isobutyryl group for guanine and a benzoyl group for adenine and cytosine. The benzoyl groups on adenine and cytosine are cleaved quickly in concentrated ammonium hydroxide, but the isobutyryl protection on guanine is much more resistant to alkaline hydrolysis; therefore, cleavage of the isobutyryl group from guanines is the key step that determines the rate of oligonucleotide deprotection. In the case of certain chemically modified oligonucleotides, prolonged incubation at elevated temperature in ammonia solution can lead to partial degradation of the oligomer, so a more labile guanine protection is required in such cases. The most popular more labile protection of guanine is the dimethylformamidyl group (dmf) which allows oligonucleotide deprotection to be carried out under milder conditions: concentrated ammonium hydroxide at 55 °C for 1 hour only.

The protected 2′-deoxycytidine derivative **9** was obtained by transient protection of the hydroxyl functions followed by benzoylation of the exocyclic amino function. The advantage of this method is that the silylation and benzoylation can be carried out in a single reaction vessel without isolation or purification of the intermediate silylated nucleoside.[1] Compound **9** was obtained with a 60% yield.

Due to the presence of a propargyl group at the N-2 position in **10** and N-6 in **13**, protection of the exocyclic amino group was not necessary. Synthesis of 2′-deoxyguanosine derivative **16** with a propargyl group at the O-6 position and a dmf group at exocyclic amino function was achieved by a simple stirring of **16** with *N*,*N*-dimethylformamidine dimethyl acetal and methanol.[1] Usually, the reaction is quantitative and isolation only requires partial removal of the solvents, dilution of the

residue with water, and filtration of the precipitated product. In the course of this study, it was found that the *N*-glycosidic bond in **16** was labile when the exocyclic amino function was protected with the dmf group. The same was observed for the isobutyryl group. Synthesis of derivative **16** with the protecting group at N-2 was unsuccessful and was abandoned.

The 5′-hydroxyl function in nucleosides is routinely protected as a dimethoxytrityl ether (DMTr). Due to steric hindrance, the trityl group finds specific applications and is used for selective protection of hydroxyls in different types of substrates, including nucleosides. The common procedure involves treatment of alcohol with DMTrCl in pyridine containing triethylamine. DMTrCl reacts almost exclusively with the primary 5′-hydroxyl function. We used this method for the synthesis of: 5′-*O*-(4,4′-dimethoxytrityl)-N^6-(2-propyn-1-yl)-2′-deoxyadenosine (**14**) (71%) (Scheme 2), 5′-*O*-(4,4′-dimethoxytrityl)-N^2-(2-propyn-1-yl)-2′-deoxyguanosine (**17**) (75%) (Scheme 2), 5′-*O*-(4,4′-dimethoxytrityl)-N^3-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}methylenethymidine (**21**) (73%) (Scheme 3). An attempt to protect the 5′-hydroxyl function in 5-ethynyl-2′-deoxycytidine (**8**) with a trityl group using DMTrCl in pyridine containing triethylamine or in triethylamine only used as a solvent, under microwave irradiation,[30] failed. We suppose that the steric clash between the bulky dimethoxytrityl group and the propargyl group at the nucleic base was the reason for the failure. Therefore, we decided to use a *tert*-butyldimethylsilyl group to protect the 5′- function in **8**. A *tert*-butyldimethylsilyl group reacts selectively with hydroxyl functions in nucleosides, preferentially with the 5′-hydroxyl. The *tert*-butyldimethylsilyl group is removed under conditions which do not affect other commonly used acid or base labile protecting groups. This allows for the synthesis of a variety of protected deoxynucleosides.[31]

2.3 Synthesis of nucleoside–carborane conjugates

The target nucleoside–carborane conjugates (Scheme 3) were obtained in a convenient, one-step procedure using copper(I)-catalyzed Huisgen–Meldal–Sharpless 1,3-dipolar cycloaddition of azides and alkynes to give triazoles (*i.e.* ''click chemistry'').[2–5] This is a very efficient and popular method used to label biomolecules, especially nucleosides and oligonucleotides.[4] The Cu(I) catalyzed ''click'' reaction is virtually quantitative under proper conditions, very robust, versatile and orthogonal, ensuring its broad use including *in vivo* tagging. The azide and alkyne functions are convenient to introduce independently, are stable, and do not react with common organic reagents or functional groups in biomolecules. The triazole formed during the ''click'' process is chemically resistant to a variety of reaction conditions, *e.g.* oxidation, reduction, and hydrolysis.[5]

The standard procedure for the synthesis of nucleoside–carborane conjugates *via* ''click chemistry'' involves the following steps. First, a suitable nucleoside acceptor bearing a terminal acetylenic group (**7–10, 13, 14, 16, 17**) was dissolved in a mixture of THF and water (1 : 1, v/v) together with 1.1 eq. of a boron-cluster donor **6** equipped with a terminal azide group. Catalytic amounts of $CuSO_4 \times 5H_2O$ (0.1 eq.) and sodium ascorbate (0.2 eq.) were added to the obtained solution at 0 °C. Reactions were performed at room temperature during 2–18 h with TLC control. After reaction completion, the solvents were evaporated under vacuum and the crude products were purified by column chromatography on silica gel with a gradient of methanol in dichloromethane as an eluent. The yields of purified products usually ranged 70–98%. All the nucleoside–carborane conjugates **20–28** were characterized by ^{1}H-, ^{13}C-, ^{11}B-NMR, FT-IR, MS, TLC, HPLC and CD techniques (**20**, **22**, **24**, **27**).

Compounds **7**, **8**, **10**, **13**, **16**, without protection of the exocyclic amino group and 5′-hydroxyl function were used for the synthesis of the T, dC, dA and dG bearing *ortho*-carborane modification in *closo*-form tethered *via* a triazole containing linker: N^3-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}methylenethymidine (**20**), 5-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}-2′-deoxycytidine (**22**), N^6-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}methylene-2′-deoxyadenosine (**24**), O^6-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}methylene-2′-deoxyguanosine (**26**), and N^2-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}methylene-2′-deoxyguanosine (**27**). The resultant conjugates **20**, **22**, **24**, **27**, after the transformation of the electroneutral *closo–ortho*-carborane into their negatively charged *nido*-form, were subject to the electrochemical studies described below.

Other boron-cluster-acceptors: **9**, **14**, **17**, with protected exocyclic amino and 5′ hydroxyl groups were substrates for the synthesis of conjugates: N^4-benzoyl-5′-*O*-*tert*-butyldimethylsilyl-5-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}-2′-deoxycytidine (**23**), 5′-*O*-(4,4′-dimethoxytrityl)-N^6-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}methylene-2′-deoxyadenosine (**25**), and 5′-*O*-(4,4′-dimethoxytrityl)-N^2-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}methylene-2′-deoxyguanosine (**28**). These compounds were used for the synthesis of the corresponding phosphoramidites.

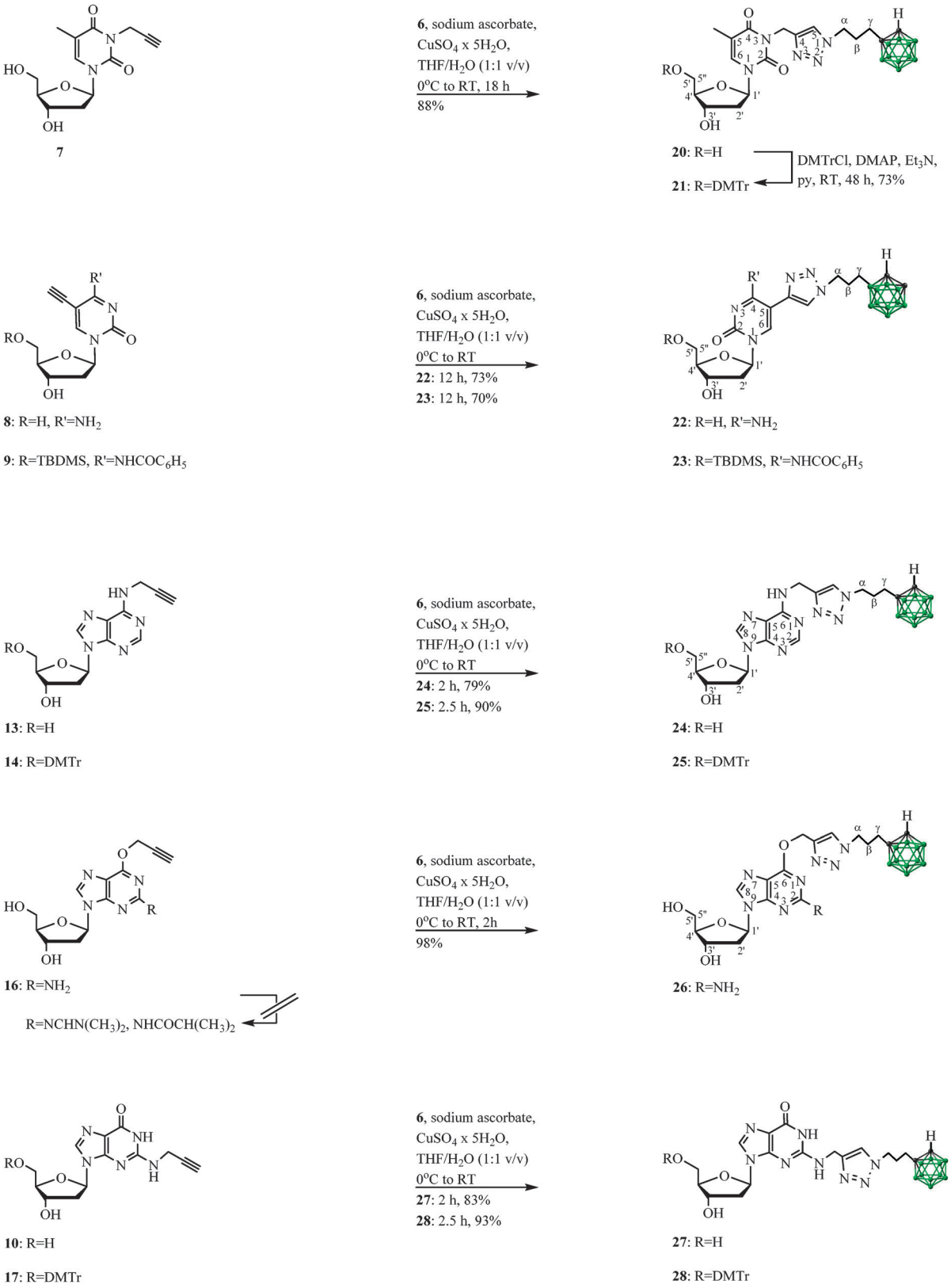
It is of interest that the synthesis of nucleoside–carborane conjugates using protected nucleosides **9**, **14**, **17** as boron-cluster acceptors afforded the expected products **23**, **25**, **28** with high yields (70–93%), while the reversed pathway based on protection of nucleoside–carborane conjugates **22**, **24**, **27** obtained in the preceding step yielded conjugates **23**, **25**, **28** with low yields only (5–10%). Therefore, the synthesis of the corresponding protected conjugates **23**, **25**, **28** was performed using the appropriate protected nucleosides **9**, **14**, **17**. This may suggest that the low yield of the reaction is probably due to the presence of a boron cluster which causes steric hindrance and prevents the introduction of blocking groups. We did not get a similar result for conjugate **20**. Compound 5′-*O*-(4,4′-dimethoxytrityl)-N^3-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}methylenethymidine (**21**) was obtained with a 73% yield, using **20** as a substrate.

2.4 Synthesis of nucleoside–carborane phosphoramidite monomers

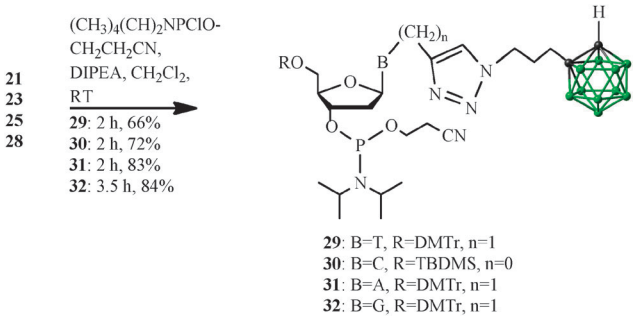
The general synthetic pathway for the synthesis of nucleoside–carborane phosphoramidites **29–32** is presented in Scheme 4.

The phosphoramidite monomers were prepared in a simple one step reaction of 5′-protected conjugates bearing boron clusters **21**, **23**, **25**, **28** with (β-cyanoethyl)(*N*,*N*-diisopropyloamino)chlorophosphine in the presence of DIPEA. The nucleoside–carborane

phosphoramidites **29–32** were isolated after purification by flash chromatography and precipitation as a mixture of P-diastereomers in 66–84% yield.[1,32] They were fully characterized by ^{1}H-, ^{13}C-, ^{11}B- and ^{31}P-NMR, FT-IR, MS and TLC.



Scheme 3 New nucleoside–carborane conjugates.



Scheme 4 Synthesis of nucleoside–carborane phosphoramidites. DIPEA = *N*,*N*-diisopropylethylamine, T = thymine, C = cytidine, A = adenine, G = guanine.

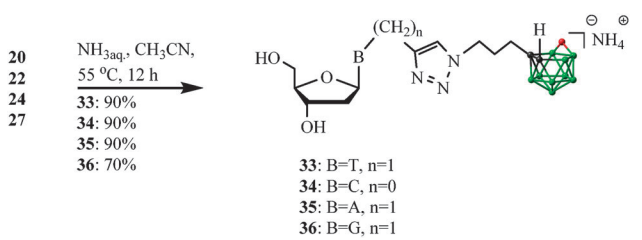
2.5 Transformation of electroneutral *closo*-carborane in 20, 22, 24, and 27 into negatively charged *nido*-carborane bearing derivatives 33–36

The *nido*-monoanion 7,8-$C_2B_9H_{12}(-1)$ was first obtained by alcoholic base degradation of *ortho*-carborane and substituted derivatives by Hawthorne.[33] Other deboronation reagents such as tertiary amines, hydrazine, ammonia, piperidine, pyrrolidine and fluoride ions are also used.[34] Removal of BH vertex occurs regiospecifically, at the most electropositive BH vertex, which in 1,2-$C_2B_{10}H_{11}$ is B(3) or B(6).[8]

It should be mentioned that after automated DNA synthesis oligonucleotide products are cleaved from the solid support *via* 1 h incubation with concentrated aqueous ammonia solution at room temperature; then, base deprotection is achieved by incubation of the resultant solution at 55 °C for another 18 h. Such conditions promote simple *closo*-to *nido*-transformation of the *ortho*-carborane cluster. Mass spectrometry analysis proved that the *closo*–*ortho*-carborane modification in DNA is in *nido*-status.[13,15]

To achieve electrochemical activity of the boron cluster label, the redox inactive *closo*-form of *ortho*-carborane (1,2-$C_2B_{10}H_{11}$) in conjugates **20**, **22**, **24**, **27** was transformed into the redox active opened-cage *nido*-form yielding **33–36**, respectively (Scheme 5).

The *closo*/*nido* transformation in conjugates **33–36** was performed with TLC control using concentrated aqueous ammonia in acetonitrile, at 55 °C overnight. After reaction completion, the solvents were evaporated and the crude product purified by column chromatography using a gradient of methanol in dichloromethane as the eluting solvent system. The yields of purified products were 85–90% for **33–35** and 70% for **36**. ^{1}H-NMR (the broad singlet signal at $\delta = -2.8$ ppm indicates the



Scheme 5 Conjugates of canonical nucleosides and *nido*-carborane.

B–H–B bridging hydrogen of the *nido*-carborane moiety) and MS spectra confirmed the structure of compounds **33–36**.

2.6 Synthesis of DNA oligonucleotides modified with the boron cluster

Synthesis of DNA oligonucleotides using modified phosphoramidite monomer **29**, as example, was performed. Four modified DNA oligonucleotides **38–41** differing in length and sequence of nucleobases, bearing one carborane modification at the 5′-end (position 16 or 23 counting from the 3′-end), and additionally amino linker C6 for oligonucleotides **40**, **41** (Scheme 6, Table 1) were obtained by solid phase synthesis using a standard β-cyanoethyl cycle.[32] The oligonucleotides were purified by a two-step trityl-on/trityl-off HPLC procedure.

The molecular mass of all DNA oligonucleotides was confirmed by MALDI-TOF mass spectrometry (Table 1, the MS spectra are shown in ESI,† Fig. S120–S123). Mass spectrometry analysis proved that the carborane cage is in *nido*-form. The availability of the methods for attachment of boron clusters to DNA oligonucleotides facilitate the study on use of the carborane label for electrochemical detection of DNA[16] and for other applications. Use of oligonucleotides **38–41** and similar constructs for electrochemical detection of DNA sequences derived from the cytomegalovirus (HCMV), analogous to the approach reported by us previously for the metallacarborane redox labels,[18,19] is ongoing in our Laboratory.

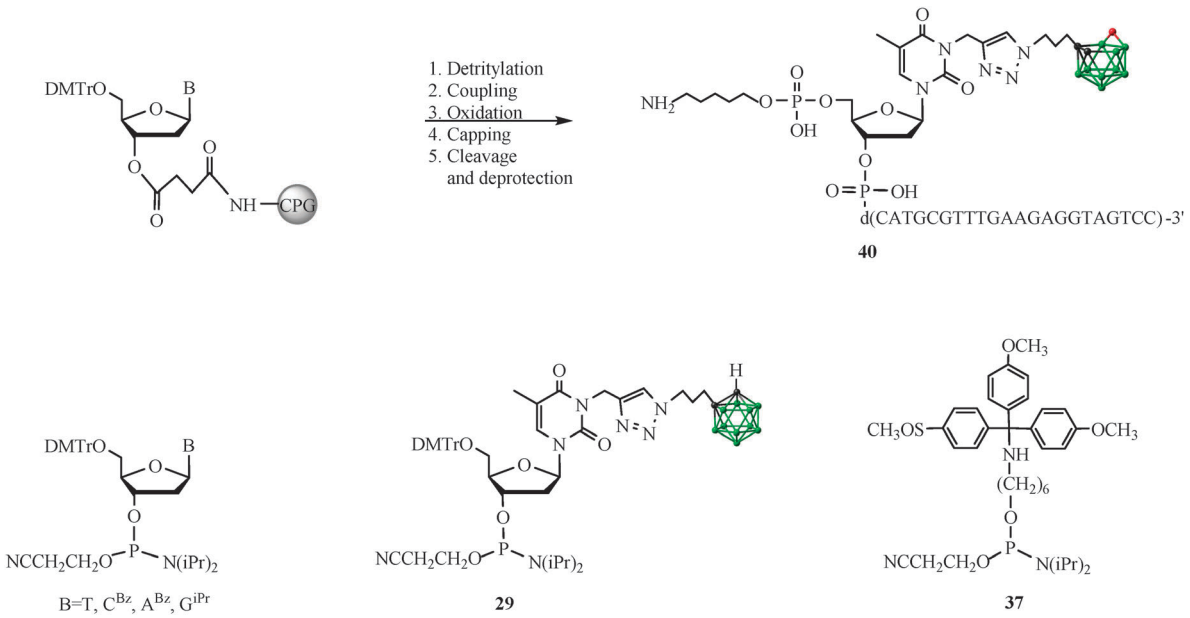
2.7 X-ray structure analysis

X-ray crystallography is the main method for characterizing the atomic structure of new materials and biological molecules. It is also an extremely useful technique in drug discovery. A detailed analysis of crystal structures allows the study of the specific interactions of a particular drug with its protein target at the atomic level and consequent improvement of the drug's chemistry and pharmacology.[35]

Unfortunately, this is not the case for nucleoside-boron cluster conjugates. Although the chemistry of nucleosides modified with boron clusters has a long history, no X-ray structure for this type of modification has so far been published. This, in spite of the importance of nucleoside derivatives as chemotherapeutics, limits the study of nucleoside-boron cluster conjugates as drug candidates. Herein, we present the first X-ray structure of this type of molecule (Table 2).

The crystal structure of the thymidine bearing *closo*-carborane cluster attached to the nucleobase *via* a linker containing a triazole moiety, **20**, is shown in Fig. 2.

The large substituent terminated with a carborane cluster is covalently attached to the endocyclic nitrogen atom N-3 (N24 in the crystallographic structure) at the Watson–Crick edge of the base ring. Difficulties in obtaining a crystal of sufficient quality is one of the major reasons for the slow progress in nucleoside-boron cluster conjugate crystallography. Crystallization of **20** was achieved after several attempts from aqueous ethanolic solutions. Unfortunately, this condition does not support crystallization of other conjugates, therefore the work on the



Scheme 6 Synthesis of example modified oligonucleotide **40**. CPG = Controlled Pore Glass.

Table 1 Sequence, HPLC, MALDI-TOF MS data for modified oligonucleotides[a]

Oligomer	Sequence	t_R [min]	Calculated molecular mass	MALDI-TOF *m/z*
38	3′-d(CCTGATGGAGAAGTTTGCGTACT$^{nido\text{-}C2B9H11}$)-5′	10.49	7348.11	7352.3
39	3′-d(GGTGGCGTGACTCCTT$^{nido\text{-}C2B9H11}$)-5′	11.21	5157.72	5156.4
40	3′-d(CCTGATGGAGAAGTTTGCGTACT$^{nido\text{-}C2B9H11}$$(CH_2)_6NH_2$)-5′	10.45	7527.27	7523.0
41	3′-d(GGTGGCGTGACTCCTT$^{nido\text{-}C2B9H11}$$(CH_2)_6NH_2$)-5′	10.75	5336.88	5338.2

[a] d = deoxyribooligonucleotide.

Table 2 Crystallographic data

Formula	$C_{18}H_{33}B_{10}N_5O_5$
M_r	507.59
System	Orthorhombic
Space group	$P2_12_12_1$
T [K]	130 K
a [Å]	7.32385(13)
b [Å]	13.0124(2)
c [Å]	26.9145(5)
V [Å]	2564.63(8)
Z	4
λ [Å]	1.54184
θ range [°]	3.28–76.74
Absorption coefficient	0.692
Reflns measured	29049
Independent reflns	5351
Reflection [$I > 2\sigma(I)$]	5086
R_{int}	0.0350
$R[F^2 > 2\sigma(F^2)]$	0.0390
w$R(F^2)$	0.0946
S	1.048
$\Delta\rho_{max}$ [e]	0.175
$\Delta\rho_{min}$ [e]	−0.194

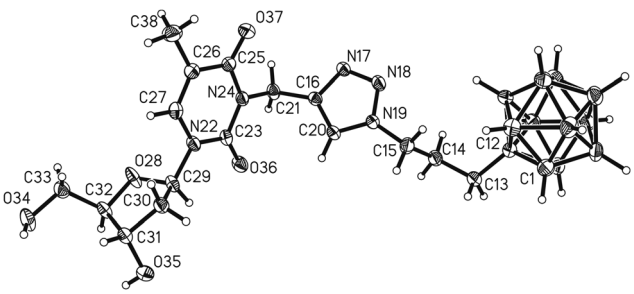


Fig. 2 An ORTEP representation of conjugate **20** in the asymmetric unit. Displacement ellipsoids are drawn at the 50% probability level; H atoms are depicted as spheres of arbitrary radius.

preparation of X-ray analysis-ready crystals of other nucleoside-boron cluster conjugates is ongoing in our laboratory.

The asymmetric unit consists of one molecule which is arranged in a crystal lattice in a specific manner. Along the *c*-axis the carborane heads of two neighboring layers interdigitate and form one line. The nucleotide part is arranged in a similar way and forms another parallel line (Fig. 3).

The overall conformation of the nucleoside residue is typical for B-DNA (Tables 3 and 4). The ribose ring shows the C2′-*endo* puckering with a pseudo-rotation phase angle P of 147.11° (S-type). The values of χ (rotation around the *N*-glycosidic bond, N22–C29) and δ (rotation of C31–C32 bond) torsion angles correspond well with the conformational range found in B-DNA. The χ angle is −107.7° and corresponds to

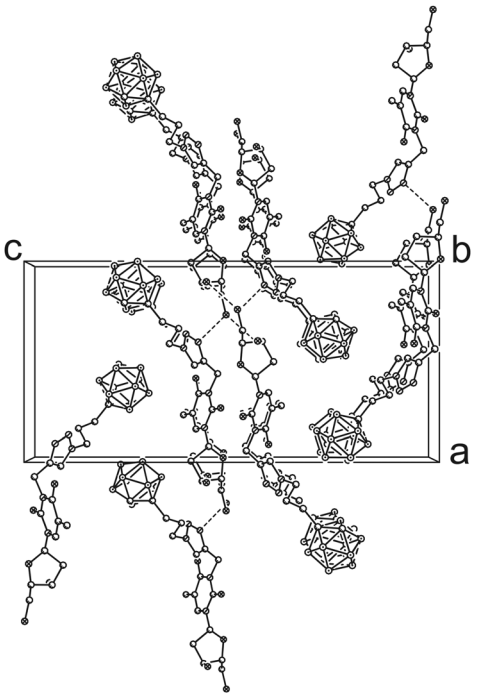


Fig. 3 The crystal packing of N^3-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}methylenethymidine (**20**) in the $P2_12_12_1$ lattice. The hydrogen bonds between symmetry related molecules are drawn with dashed lines.

Table 3 The values of torsion angles of the nucleoside residue

Torsion angle	Atom names	Values of the torsion angle (°)	B-DNA standard[a] value (°)
γ	C31–C32–C33–O34	−80.3	38
δ	O35–C31–C32–C33	141.4	139
χ	C23–N22–C29–O28	−107.7	−102

[a] Standard values according to X-ray fibre diffraction data.[36]

Table 4 The values of torsion angles of the ribose ring residue

Torsion angles	Atom names	Values of the torsion angle (°)
v0	C32–O28–C29–C30	−30.93
v1	O28–C29–C30–C31	39.32
v2	C32–C31–C30–C29	−32.41
v3	C30–C31–C32–O28	15.35
v4	C29–O28–C32–C31	9.59

the −*gauche* − *sc* arrangement, while the δ angle of 141.1° is in the +*ac* range.

The γ angle (rotation of C32–C33 bond) is equal to −80.3° (−*sc*, −*gauche*) and differs from the standard value of 38°. However, the C2′-*endo* puckering allows more conformational freedom than C3′-*endo* for the γ angle; thus, the −*sc* position is observed in many crystal structures of nucleosides.[35] This could also be a result of hydrogen interaction in which O35 is involved.

The carborane group is relatively large compared to the nucleoside residue. It leans on the 3′ side of the nucleoside,

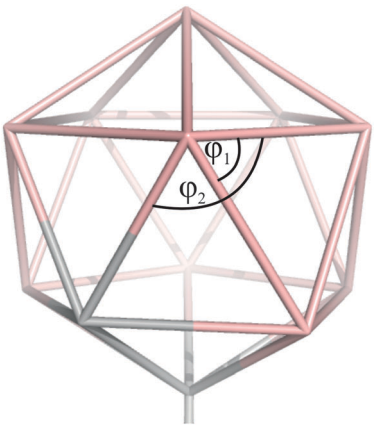


Fig. 4 The stick representation of a carborane cluster. The boron atoms are pink and carbon atoms are grey. Angles φ_1 and φ_2 are marked.

nearly perpendicular to the base ring plane, as indicated by the angle of 113.08° measured between the N24–C21–C16 atoms. The carborane cluster is linked to the base by a long linker and is located more than 8 Å away from the nucleotide (the distance between C21–C12 is 8.14 Å). The cluster consists of two carbon atoms and ten boron atoms forming an icosahedron. The average bond distance between the atoms forming the cluster is 1.757 ± 0.035 Å and the calculated volume is approx. 11.83 Å^3.[37]

Two types of angles can be distinguished within the icosahedron (Fig. 4). The first (φ_1) is between atoms forming the triangular faces. Since an icosahedron is built of 20 equilateral triangles φ_1 is 60 ± 1.2°. The second (φ_2) angle is between two edges of neighboring faces. The average value is 108 ± 2.7°. In the case when the angle involves at least one carbon atom the standard deviation is higher (s.d. = 4.13° and average value = 107.95°) than for angles involving only boron atoms (s.d. = 0.3°, average value = 108°).

Two hydrogen bonds are observed in the crystal structure (Fig. 3 and Table 5). The first is formed between the O35–H35···O34^{i} [symmetry code: (i) x + 1/2, $-y$ + 1/2, $-z$ − 1] atoms of the ribose. The second H-bond is between O34–H34···N17^{ii} [symmetry code: (ii) x, y + 1, z]. The N17 atom is within the triazol ring of the linker, while O34–H34 is the hydroxyl group at the 5′end of the sugar ring.

Recently, we tested several thymidine derivatives modified with carborane or metallacarborane as substrates for human deoxynucleoside kinases. Thymidine derivatives with modification attached to N3 *via* a linker containing the triazole moiety were efficiently phosphorylated by cytosolic thymidine kinase 1 and mitochondrial thymidine kinase 2.[38] The presented crystallographic structure of **20** and more crystallographic data on nucleoside-boron cluster conjugates which will be obtained in

Table 5 Hydrogen bond geometry

D–H···A	D–H (Å)	H···A (Å)	D···A (Å)	D–H···A (°)
O35–H35···O34^{i}	0.86(2)	1.88(2)	2.739(2)	174(3)
O34–H34···N17^{ii}	0.81(3)	1.98(3)	2.780(2)	165(3)

Symmetry codes: (i) x + 1/2, $-y$ + 1/2, $-z$ − 1, (ii) x, y + 1, z.

future will facilitate the study of the interactions of nucleoside-boron cluster conjugates with biological targets.

2.8 Circular dichroism of nucleoside–carborane conjugates

The *syn–anti* conformer equilibrium of nucleobases with respect to the sugar ring is of fundamental importance in the determination of the conformations of nucleoside and nucleic acid derivatives in solution. The majority of X-ray structure determinations of nucleosides, nucleotides, and oligonucleotides show that the rings of purine and pyrimidine bases exist in the *anti* conformation.[39] The *syn* conformation is generally observed when the base ring contains a bulky substituent (at C-8 for purine and C-6 for pyrimidine) or due to stabilization by N-3–OH-5′ intramolecular hydrogen bonding in purine derivatives. These results indicate that the *anti* conformers of both purine and pyrimidine nucleosides are more stable than the *syn* conformers.[39]

It was of interest to test whether carborane modification affects the conformation of the nucleoside–carborane conjugates, especially with respect to the rotation of the base around the *N*-glycosidic bond and the *syn/anti* preferences. Circular dichroism (CD) was used to compare the nucleobase conformation in *ortho*-nucleoside–carborane conjugates **20**, **22**, **24**, **27** and in their unmodified counterparts. In all cases, the CD spectra of unprotected conjugates **20**, **22**, **24**, **27** were recorded under the same conditions as for the unmodified nucleosides (ESI,† Fig. S116–S119).

In the case of compound **20** and a comparison with unmodified thymidine, the CD spectra revealed no effect of carborane modification on thymidine conjugate conformation. In terms of the shape of the spectrum and molecular ellipticity values in the recorded range 195–350 nm, only minor differences have been detected. The absorption maxima and minima in the CD spectrum of modified thymidine **20** are as follows: λ_{max} = 232 nm (Θ = −1.16) and 273 nm (Θ = 1.90), λ_{min} = 218 nm (Θ = −2.13), 240 nm (Θ = −1.45). For unmodified thymidine, the following values have been recorded: λ_{max} = 230 nm (Θ = −1.47) and 272 nm (Θ = 1.54), λ_{min} = 218 nm (Θ = −2.44), 241 nm (Θ = −1.62). The maxima and minima for **20** are almost the same as for the unmodified nucleoside. The CD spectra of **20** and thymidine change as they are flat, with Θ near to zero, in the range 295–350 nm. This suggests the same base conformation around the glycosidic bond in conjugate **20** and in unmodified thymidine.

In contrast, the CD spectra for carborane-2′-deoxycytidine conjugate **22** and its unmodified counterpart revealed a strong effect of the modification on the conjugate conformation in terms of the spectral shape and molecular ellipticity [Θ] in the recorded range 210–340 nm. The absorption maxima and minima in the CD spectra of modified nucleoside **22** are as follows: λ_{max} = 217 nm (Θ = 1.43), 256 nm (sh) (Θ = 0.11) and 294 nm (Θ = 0.47), λ_{min} = 237 nm (Θ = −0.40). The values recorded for 2′-deoxycytidine are as follows: λ_{max} = 236 nm (sh) (Θ = 0.01) and 279 nm (Θ = 1.06), λ_{min} = 217 nm (Θ = −1.28). The CD spectrum of **22** shows strong, positive absorption with a maximum at 217 nm. In the same range, 2′-deoxycytidine revealed strong, negative absorption. Both compounds demonstrate positive absorption in the range 235–335 nm, but with lower molecular ellipticity and a red shift of a maximum for **22** at 294 nm. This suggests a contrasting base conformation around the glycosidic bond.

CD analysis of carborane-2′-deoxyadenosine conjugate **24** in comparison with unmodified nucleoside also revealed a clear effect of modification on conjugate conformation in the recorded range 205–325 nm. The absorption maxima and minima in the CD spectrum of modified 2′-deoxyadenosine **24** are as follows: λ_{max} = 215 nm (Θ = 3.33) and 252 nm (Θ = 0.03), λ_{min} = 237 nm (Θ = −0.87) and 283 nm (Θ = −0.57). The following values have been recorded for 2′-deoxyadenosine: λ_{max} = 216 nm (Θ = 0.31) and 294 nm (Θ = 0.05), λ_{min} = 263 (Θ = −0.77). The most characteristic difference between **24** and 2′-deoxyadenosine is a decrease in the molecular ellipticity at 215 nm. The CD of **24** reveals a minimum at 237 nm, in the range 225–245 nm. The CD spectrum of 2′-deoxyadenosine is almost flat with Θ near to zero. The CD spectra of modified 2′-deoxyadenosine **24** and unmodified 2′-deoxyadenosine between 245 and 325 nm are almost mirror images. This suggests a contrasting base conformation around the glycosidic bond in conjugate **24** compared to that of unmodified 2′-deoxyadenosine.

The CD spectra for carborane-2′-deoxyguanosine **27** and 2′-deoxyguanosine, in the range of 220–290 nm, revealed the effect of carborane modification on 2′-deoxyguanosine conformation. The absorption maxima and minima of **27** are as follows: λ_{max} = 212 nm (Θ = 1.08) and 243 (sh) nm (Θ = 0.43), λ_{min} = 232 nm (Θ = 0.42) and 279 (Θ = −0.28). The following values have been recorded for 2′-deoxyguanosine: λ_{max} = 212 nm (Θ = 1.12) and 275 nm (sh) (Θ = −0.12), λ_{min} = 249 (Θ = −0.43). The CD spectrum of **27** shows strong, positive absorption with a maximum at 212 nm and similar molecular ellipticity, as well as unmodified 2′-deoxyguanosine. The spectra of **27** and dG between 230–270 nm, with λ_{max} around 245 nm, change drastically and revealed positive and negative absorption, respectively. The CD spectra of both compounds in the range 270–290 nm show a minimum for **27** and a shoulder for 2′-deoxyguanosine. In the range 290–340 nm, the CD spectra are almost flat.

In summary, the CD analysis revealed a strong effect of boron cluster modification 2′-deoxynucleosides (dC, dA, dG) with the exception of thymidine (T). The bulkiness and electronic properties of the cluster may contribute to the observed changes. It is worth recalling that an effect of the carborane substituent on the conformational *syn/anti* preferences of **22**, **24** and **27** can be additionally amplified by the presence of a long, triazole bearing linker between the nucleobase and carborane modification.

2.9 HDV characteristics of nucleoside–carborane conjugates

The electrochemical properties of boron clusters and their complexes with metals have been the focus of interest since the discovery of boron clusters. Morris *et al.*[40] analyzed the electrochemical properties of simple boron compounds as well as boranes, metallaboranes, carboranes, and metallacarboranes.

We have studied the electrochemical properties of nucleosides bearing boron cluster modifications[17,41,42] and proposed an electrochemical DNA sensor based on a 7,8-dicarba-*nido*-undecaborate group[16] and metallacarboranes as efficient redox labels.[18,19]

In this work, we established and optimized high-performance liquid chromatography coupled with an electrochemical detection (HPLC-ED) format for highly sensitive, rapid, and parallel determination of all four canonical nucleosides: T, dC, dA and dG labeled with a 7,8-dicarba-*nido*-undecaborate group, **33–36**. A standard CoulArray electrochemical detector (Model 5600A, ESA, Chelmsford, MA, USA) equipped with two low volume flow-through analytical cells (Model 6210, ESA) with four carbon porous working electrodes, a palladium electrode as a reference electrode and auxiliary electrodes was used. A coulometric detector is an apparatus which measures the electrical charge required to oxidize or reduce the total amount of a compound while it passes a cell in the detector. A useful and important feature of coulometric detection in a multichannel system lies in its compatibility with the gradient elution used in HPLC analysis and provides complete voltammetric differentiation of the analytes according to their reaction potential. Another advantage of the coulometric method is the larger working surface of a cell which is made of porous graphite allowing for more than 90% of the oxidation–reduction of the analyte compared to a conventional working electrode.[43]

The optimal potentials of a CoulArray detector working electrode had to be found to achieve the most sensitive determination of the studied compounds. Initially, a Hypersil Gold C18 reversed phase column (4.6 × 250 mm, 5 μm particle size) and gradient elution with an eluent containing 2% to 60% acetonitrile were used for chromatographic separation of the compounds **33–36**. A relatively high final concentration of acetonitrile has to be applied in the eluting solvent system, because of the high lipophilicity of boron clusters and boron cluster containing derivatives. Contents of acetonitrile below 60% negatively influenced the chromatographic separation and elution of the analyte. The potentials 300, 375, 450, 525, 600, 675, 750, and 825 mV were applied on working electrodes. A full scan of all the studied compounds is shown in Fig. 5A. The resulting hydrodynamic voltammogram (HDV) of the conjugates **33–36** is presented in Fig. 5B.

The signals were evaluated as the cumulative response – the sum of the signals measured at all electrodes according to the literature.[44] The most suitable analytical potential for simultaneous determination of **33–36** on a working electrode is 750 mV for **33**, 675 mV for **34**, **35**, and 600 mV for **36**. These and our previous results[16] show the high electrochemical activity of a 7,8-dicarba-*nido*-undecaborate group as a redox label.

The results prove the usefulness of the 7,8-dicarba-*nido*-undecaborate group as a boron cluster based electrochemical active and efficient redox label for nucleosides. The signals generated for individual nucleosides are characterized by differentiated redox potentials and are well separated and developed. The coupled HPLC-ED approach provides a useful methodology for the electrochemical detection of molecules labeled with this redox active tag.

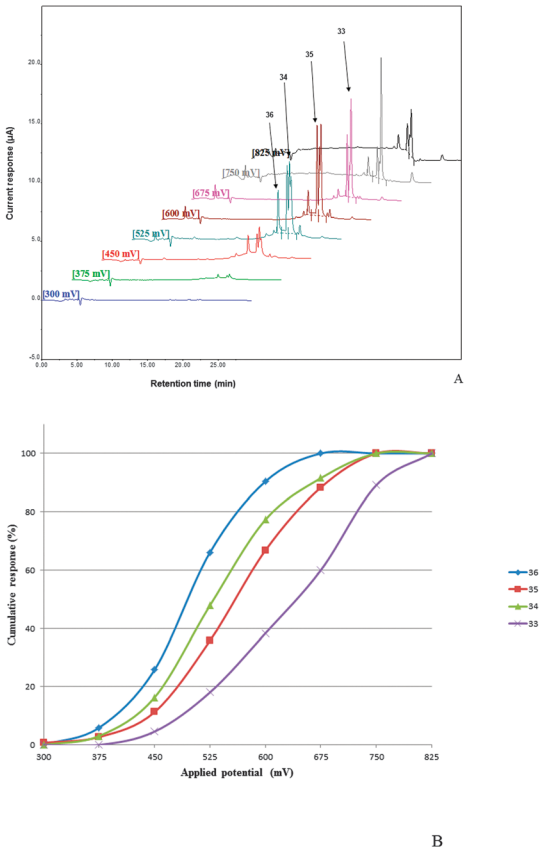


Fig. 5 (A) HPLC-ED full scan chromatograms of **33–36**. Dependence of the current response of **33–36** on detector electrode potential (300, 375, 450, 525, 600, 675, 750, 825 mV): **36** = 20.76 min, **35** = 22.03 min, **34** = 22.35 min, **33** = 22.62 min. (B) Hydrodynamic voltammogram of the analyzed conjugates **33–36**. The chromatographic conditions: mobile phase A: acetonitrile/water (2 : 98 v/v) containing ammonium acetate (0.05 M, pH 5.5) and B: acetonitrile/water (60 : 40 v/v) containing ammonium acetate (0.05 M, pH 5.5). HPLC conditions: 20 minutes from 0% B to 100% B, 5 minutes 100% B, 5 minutes from 100% B to 0% B. Flow rate 1 mL min^{-1}. Time for each analysis 30 minutes. The concentration of the analyzed compounds was 2.19 μg mL^{-1}. The sample was injected manually.

3. Experimental section

3.1 General information

All experiments that involved water-sensitive compounds were conducted under rigorously dry conditions and under an argon atmosphere. Most chemicals were obtained from Sigma-Aldrich (Sigma-Aldrich Sp. z o.o. Poznan, Poland) and used without further purification unless otherwise stated. Nucleosides (deoxyadenosine, deoxyguanosine, deoxycytidine, thymidine) were purchased from Pharma-Waldhof (Düsseldorf, Germany). Acetic anhydride and 4,4′-dimethoxytrityl chloride were from Avocado Research Chemicals Ltd (Karlsruhe, Germany). Propargyl bromide (80% in toluene), acetyl chloride, and sodium, *tert*-butyldimethylsilyl chloride were purchased from Lancaster (Morecambe, United Kingdom). *N*,*N*-Dimethylpiperidin-4-amine was from ABCR GmbH&Co. KG (Karlsruhe, Germany). Carborane was purchased from KATCHEM spol. s r.o. (Reź/Prague, Czech Republic). Flash column chromatography was performed on

silica gel 60 (230–400 mesh, Sigma-Aldrich). R_f refer to analytical TLC performed using pre-coated silica gel 60 F254 plates purchased from Sigma-Aldrich (Steinheim, Germany) and developed in the solvent system indicated. Compounds were visualized by use of UV light (254 nm) or a 0.5% acidic solution of $PdCl_2$ in HCl/methanol by heating with a heat gun for boron-containing derivatives. The yields are not optimized.

^{1}H-NMR, ^{13}C-NMR, ^{11}B-NMR, ^{31}P-NMR spectra were recorded mostly on a Bruker Avance III 600 MHz spectrometer equipped with a direct ATM probe. The spectra for ^{1}H-, ^{13}C-, ^{11}B-, and ^{31}P- were recorded at 600.26 MHz, 150.94 MHz, 192.59 MHz, and 242.99 MHz, respectively. Deuterated solvents were used as standards. All chemical shifts (δ) are quoted in parts per million (ppm) relative to the external standards. For NMR following solvents were used: D_2O (δ_H = 3.5 ppm), $CDCl_3$ (δ_H = 7.25, δ_C = 77.00 ppm), CD_3OD (δ_H = 3.35, δ_C = 50.00 ppm), DMSO-d_6 (δ_H = 2.50, δ_C = 39.70 ppm), CD_2Cl_2 (δ_H = 5.35, δ_C = 54.25 ppm). The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, dd = doublet of doublets, ddd = doublet of doublets of doublets, t = triplet, dt = doublet of triplets, q = quartet, quin = quintet, bs = broad singlet, m = multiplet. *J* values are given in Hz.

IR spectra were recorded using a Thermo Scientific Nicolet™ 6700 FT-IR spectrometer equipped with a Smart Orbit Attenuated Total Reflectance (ATR) accessory, and diamond crystal.

FAB mass spectra were recorded with a Finningan MAT 95 spectrometer (Bremen, Germany) with glycerin (Gly) or *m*-nitrobenzyl alcohol (NBA) as the matrix. Calculation of the theoretical molecular mass for compounds was performed using the "Show Analysis Window" option in the ChemDrawUltra 12.0 program. The calculated *m/z* corresponds to the average mass of the elements consisting of natural isotopes.

The MALDI-TOF MS spectra of oligonucleotides **38–41** were recorded on a Voyager Elite mass spectrometer (PerSeptive Biosystems Inc., Framingham, MA) equipped with a nitrogen laser (337 nm). Typically, oligonucleotide stock solutions (1 μL) in water (0.01 optical density units ODU_{A260} per μL H_2O) were mixed with 3-hydropicolinic acid (HPA, 50 mg mL^{-1} in 50% CH_3CN/H_2O) containing ammonium citrate dibasic (AC, 50 mg mL^{-1} in H_2O), HPA : AC – 8 : 1 v/v. Spectra were obtained in the linear, negative ion mode at the acceleration voltage of 20 kV. RP-HPLC analysis was performed on a Hewlett-Packard 1050 system equipped with a UV detector, and Hypersil Gold C18 column (4.6 × 250 mm, 5 μm particle size, Thermo Scientific, Runcorn, UK). UV detection was conducted at λ = 268 nm. The flow rate was 1 mL min^{-1}. All analyses were run at ambient temperature. The gradient elution profile was as follows: 20 min. from 0 to 100% B, 5 min. at 100% B, and 5 min. from 100 to 0% B. Buffer A contained 0.1 M TEAB (triethylammonium bicarbonate), pH 7.0, in acetonitrile : water (2 : 98), and buffer B contained 0.1 M TEAB, pH 7.0, in acetonitrile : water (60 : 40).

3.2 Syntheses

3.2.1 Synthesis of 1-(3-azidopropyl)-1,2-dicarba-*closo*-dodecaborane (6). 1-(3-Bromopropyl)-1,2-dicarba-*closo*-dodecaborane (**5**) (0.77 g, 2.91 mmol) was dissolved in dry DMF (8.8 mL) and NaN_3 (0.22 g, 3.38 mmol) was added. The mixture was stirred at RT for 3 h and concentrated. The crude product was purified by column chromatography on silica gel (230–400 mesh), with a gradient of CH_2Cl_2 (0–25%) in *n*-pentane as the eluent to give product **5** (0.64 g, 97%) as a colorless oil. R_f = 0.58 (CH_2Cl_2/*n*-hexane 1 : 1). ^{1}H-NMR (600.26 MHz, $CDCl_3$) δ 3.57 (br s, 1H, CH-carborane), 3.32 (t, ^3J(CH_2-α-CH_2-β) = 6 Hz, 2H, CH_2-α), 2.75–1.50 (m, 10H, $B_{10}H_{10}$), 2.31–2.28 (m, 2H, CH_2-γ), 1.78–1.75 (m, 2H, CH_2-β) ppm.

3.2.2 Synthesis of 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-N^6-(2-propyn-1-yl)-2′-deoxyadenosine (12). To a solution of 9-[3,5-bis-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-β-D-*erythro*-pentofuranosyl]-6-chloropurine (**11**) (0.85 g, 1.35 mmol) in absolute EtOH (22 mL), propargylamine (0.074 g, 1.35 mmol, 0.086 mL) was added dropowise. The reaction mixture was refluxed for 18 h. The reaction progress was monitored by TLC using CH_2Cl_2/CH_3OH (98 : 2) as an eluting solvent system. After 18 h additional propargylamine (0.074 g, 1.35 mmol, 0.086 mL) was added, and the reaction mixture was stirred overnight. The solvent was evaporated under reduced pressure and the resulting crude product was purified by column chromatography on silica gel (230–400 mesh) with a gradient of CH_3OH (0–3%) in CH_2Cl_2 as the eluent. Product **12** was obtained (0.60 g, 85%) as a white solid. R_f = 0.63 (CH_2Cl_2/CH_3OH 98 : 2). ^{1}H-NMR (600.26 MHz, DMSO-d_6) δ 8.32 (s, 1H, H-8), 8.26 (d, 1H, H-2), 8.11 (br s, 1H, NH), 6.35 (t, ^3J(H1′–H2′) = 6.6 Hz, 1H, H-1′), 4.64–4.63 (m, 1H, H-3′), 4.27 (br s, 2H, NCH_2), 3.86–3.82 (m, 1H, H-4′), 3.81–3.80 (m, 1H, H-5′), 3.67 (dd, ^3J(H5″–H4′) = 4.8 Hz, ^2J(H5″–H5′) = 11.4 Hz, 1H, H-5″), 2.99 (t, ^4J(HC≡CNCH_2) = 2.4 Hz, 1H, HC≡C), 2.95–2.90 (m, 1H, H-2′), 2.32 (ddd, ^3J(H2′–H3′) = 3.6 Hz, ^3J(H2′–H1′) = 6.6 Hz, ^2J(H2′–H2″) = 10.8 Hz, 1H, H-2″), 0.91 (s, 9H, $(CH_3)_3$CSi), 0.85 (s, 9H, $(CH_3)_3$CSi), 0.12 (s, 6H, $(CH_3)_2$Si), 0.03 (s, 3H, $(CH_3)_2$Si), 0.01 (s, 3H, $(CH_3)_2$Si) ppm.

3.2.3 Synthesis of N^6-(2-propyn-1-yl)-2′-deoxyadenosine (13). 3′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-N^6-(2-propyn-1-yl)-2′-deoxyadenosine (**12**) (0.52 g, 1.03 mmol) was dissolved in THF (15 mL), and TBAF (1 M in THF, 0.81 g, 3.10 mmol, 0.90 mL) was added. The reaction mixture was stirred at RT for 2 h whilst being protected against the introduction of moisture. After completion, the solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica gel (230–400 mesh) with a gradient of CH_3OH (1–10%) in CH_2Cl_2 as the eluent to give pure **13** (0.25 g, 83%) as a white solid. R_f = 0.18 (CH_2Cl_2/CH_3OH 96 : 4). ^{1}H-NMR (600.26 MHz, DMSO-d_6) δ 8.37 (s, 1H, H-8), 8.27 (s, 1H, H-2), 8.13 (br s, 1H, NH), 6.37 (dd, ^3J(H1′–H2′) = 7.2 Hz, ^3J(H1′–H2″) = 6.6 Hz, 1H, H-1′), 5.27 (d, ^3J(OH(C-3′)-H3′) = 4.7 Hz, 1H, OH(C-3′)), 5.12 (t, ^3J(OH(C-5′)-H5′) = 4.7 Hz, 1H, OH(C-5′)), 4.42 (m, 1H, H-3′), 4.28 (br s, 2H, NCH_2), 3.89–3.88 (m, 1H, H-4′), 3.64–3.62 (m, 1H, H-5′), 3.54–3.53 (m, 1H, H-5″), 3.00 (t, ^4J(HC≡CNCH_2) = 1.8 Hz, 1H, HC≡C), 2.73–2.71 (m, 1H, H-2′), 2.28 (ddd, ^3J(H2′–H3′) = 3 Hz, ^3J(H2′–H1′) = 6.6 Hz, ^2J(H2′–H2″) = 9, 1H, H-2″) ppm.

3.2.4 Synthesis of 5′-*O*-(4,4′-dimethoxytrityl)-N^6-(2-propyn-1-yl)-2′-deoxyadenosine (14). N^6-(2-Propyn-1-yl)-2′-deoxyadenosine (**13**) (0.10 g, 0.35 mmol) was evaporated with anhydrous pyridine (3 × 5 mL), then dissolved in the same solvent (4 mL). To the resultant solution DMAP (0.0026 g, 0.021 mmol),

TEA (0.07 g, 0.70 mmol, 0.097 mL), and DMTrCl (0.24 g, 0.70 mmol) were added. After stirring for 28 h at ambient temperature, the reaction was quenched with H_2O (10 mL) at 0 °C dropwise, and then extracted with Et_2O (3 × 6 mL). The organic phase was separated, dried over $MgSO_4$, filtered, and evaporated to dryness. The residue was purified by column chromatography on silica gel (230–400 mesh) using a gradient of CH_3OH (0–5%) in CH_2Cl_2 (+1% TEA) as the eluent to give product **14** (0.13 g, 71%) as a white foam. R_f = 0.38 (CH_2Cl_2/CH_3OH 95 : 5). ^{1}H-NMR (600.26 MHz, DMSO-d_6) δ 8.27 (s, 1H, H-8), 8.21 (s, 1H, H-2), 8.11 (br s, 1H, NH), 7.33–7.32 (m, 4H, α-H-arom of the DMT group), 7.22–7.20 (m, 7H, H-arom of the DMT group), 6.82–6.78 (m, 2H, H-arom of the DMT group), 6.38 (t, ^3J(H1′–H2′) = 6.6 Hz, 1H, H-1′), 5.33 (d, ^3J(OH(C-3′)-H3′) = 4.8 Hz, 1H, OH(C-3′)), 4.49–4.48 (m, 1H, H-3′), 4.27 (br s, 2H, NCH_2), 3.99 (q, ^3J(H4′–H3′) = 4.8 Hz, 1H, H-4′), 3.72 (2 × s, 6H, 2 × OCH_3), 3.19–3.18 (m, 2H, H-5′, H-5″), 3.00 (t, ^4J(HC≡C-NCH_2) = 2.4 Hz, 1H, HC≡C), 2.89–2.87 (m, 1H, H-2′), 2.34–2.30 (m, 1H; H-2″) ppm. ^{13}C-NMR (150.94 MHz, DMSO-d_6) δ 158.12 (s, C-arom), 153.97 (s, C-6), 152.36 (s, C-2), 148.59 (s, C-4), 144.96 (s, C-arom), 139.88 (s, C-8), 135.76, 135.66, 129.77, 127.78, 126.68, 113.19, (s, C-arom), 119.91 (s, C-5), 85.94 (s, C-methylidene of the DMT group), 85.54 (s, C-4′), 83.56 (s, C-1′), 81.96 (s, ≡C–), 72.38 (s, HC≡), 70.80 (s, C-3′), 64.17 (s, C-5′), 55.12 (s, OCH_3 of the DMT group), 38.81 (s, C-2′) ppm. FT-IR (cm^{-1}) 3279 (NH), 2932, 2910, 2834 (CH alkyl), 1616 (C=C arom). MS (NBA, FAB, +VE) *m/z* (%): 592.4 (100) $[M + H]^+$, (NBA, FAB, −VE) *m/z* (%): 590.3 (100) $[M - H]^-$, 744.5 (80) $[M + NBA]^-$, calcd for $C_{34}H_{33}N_5O_5$ = 591.25.

3.2.5 Synthesis of O^6-(2-propyn-1-yl)-2′-deoxyguanosine (16). 9-(3′,5′-*O*-Diacetyl-2-deoxy-β-D-*erythro*-pentofuranosyl)-2-amino-6-chloropurine (**15**) (0.36 g, 0.97 mmol) was dissolved in anhydrous propargyl alcohol (10 mL), and freshly prepared sodium propargylate (0.46 g, 5.86 mmol) was added. The solution was stirred at RT. After 24 h the reaction mixture was concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (230–400 mesh) using a gradient of CH_3OH (0–3%) in CH_2Cl_2 as an eluent to give the product **16** (0.14 g, 48%) as a white solid. R_f = 0.57 (CH_2Cl_2/CH_3OH 9 : 1). ^{1}H-NMR (600.26 MHz, DMSO-d_6) δ 8.11 (s, 1H, H-8), 6.46 (br s, 1H, NH_2), 6.22 (dd, ^3J(H1′–H2′) = 6 Hz, ^3J(H1′–H2″) = 7.2 Hz, 1H, H-1′), 5.23 (br s, 1H, OH(C-3′)), 5.11 (d, ^4J(OCH_2C≡CH) = 2.4 Hz, 2H, OCH_2), 4.93 (br s, 1H, OH(C-5′)), 4.36–4.35 (m, 1H, H-3′), 3.83–3.82 (m, 1H, H-4′), 3.57–3.54 (m, 1H, H-5′), 3.52 (t, ^4J(HC≡COCH_2) = 2.4 Hz, 1H, HC≡C), 3.52–3.51 (m, 1H, H-5″), 2.58–2.57 (m, 1H, H-2′), 2.24–2.21 (m, 1H; H-2″) ppm. ^{13}C-NMR (150.94 MHz, DMSO-d_6) δ 159.64 (s, C-6), 159.51 (s, C-2), 154.32 (s, C-4), 138.30 (s, C-8), 113.95 (s, C-5), 87.77 (s, C-4′), 83.15 (s, C-1′), 79.29 (s, ≡C–), 79.22 (s, HC≡), 70.88 (s, C-3′), 61.86 (s, C-5′), 53.08 (s, OCH_2), 39.28 (s, C-2′) ppm. FT-IR (cm^{-1}) 3335 (≡C–H), 3279 (OH), 3213 (NH), 2923, 2869 (CH alkyl), 2123 (C≡C), 1578 (C=C arom), 1234 (C–O). MS (Gly, FAB, +VE) *m/z* (%): 306.2 (40) $[M + H]^+$ 673.3 (35) $[M + 4Gly]^+$, (Gly, FAB, −VE) *m/z* (%): 304.2 (20) $[M - H]^-$, 396.2 (20) $[M + Gly]^-$, 488.3 (50) $[M + 2Gly]^-$, calcd for $C_{13}H_{15}N_5O_4$ = 305.11.

3.2.6 Synthesis of 5′-*O*-(4,4′-dimethoxytrityl)-N^2-(2-propyn-1-yl)-2′-deoxyguanosine (17). N^2-(2-Propyn-1-yl)-2′-deoxyguanosine (**10**) (0.08 g, 0.3 mmol) was dried by coevaporation with dry pyridine (3 × 4 mL) and then dissolved in dry pyridine (2 mL). DMAP (0.0018 g, 0.015 mmol), TEA (0.03 g, 0.3 mmol, 0.042 mL) and DMTrCl (0.15 g, 0.45 mmol) were added. After stirring for 18 h at ambient temperature, the reaction was quenched with H_2O (10 mL) at 0 °C. The reaction mixture was stirred for 18 h under argon atmosphere. To the reaction mixture H_2O (10 mL) was added at 0 °C, then extracted with CH_2Cl_2 (3 × 6 mL). The organic phase was separated, dried over $MgSO_4$, filtered, and evaporated to dryness. The residue was purified by column chromatography on silica gel (230–400 mesh) using a gradient of CH_3OH (0–6%) in CH_2Cl_2 (+0.1% TEA). The residue was purified by column chromatography with a mixture of CH_2Cl_2 and CH_3OH (0–6% + 0.1% TEA) as the eluent to give product **17** (0.12 g, 75%) as a yellow foam. R_f = 0.5 (CH_2Cl_2/CH_3OH 9 : 1). ^{1}H-NMR (700 MHz, DMSO-d_6) δ 10.70 (br s, 1H, NH), 7.83 (s, 1H, H-8), 7.37–7.34 (m, 2H, H-arom of the DMT group), 7.25–7.21 (m, 7H, H-arom of the DMT group), 6.84–6.80 (m, 4H, α-H-arom of the DMT group), 6.69 (br s, 1H, N*H*-CH_2), 6.21 (t, ^3J(H1′–H2′) = 7 Hz, 1H, H-1′), 5.32 (d, ^3J(OH(C-3′)-H3′) = 4.7 Hz, 1H, OH(C-3′)), 4.42–4.39 (m, 1H, H-3′), 3.96–3.94 (m, 3H, NCH_2, H-4′), 3.73 (2 × s, 6H, 2 × OCH_3), 3.21–3.20 (m, 1H, H-5′), 3.12–3.11 (m, 2H, H-5″, HC≡C), 2.77–2.75 (m, 1H, H-2′), 2.27–2.26 (m, 1H, H-2″) ppm.

3.2.7 General procedure for the synthesis of N^3-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}methylenethymidine (20), 5-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}-2′-deoxycytidine (22), N^4-benzoyl-5′-*O*-*tert*-butyldimethylsilyl-5-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}-2′-deoxycytidine (23), N^6-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}methylene-2′-deoxyadenosine (24), 5′-*O*-(4,4′-dimethoxytrityl)-N^6-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}methylene-2′-deoxyadenosine (25), O^6-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}methylene-2′-deoxyguanosine (26), N^2-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}methylene-2′-deoxyguanosine (27), and 5′-*O*-(4,4′-dimethoxytrityl)-N^2-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}methylene-2′-deoxyguanosine (28). N^3-(2-Propyn-1-yl)thymidine (**7**), 5-ethynyl-2′-deoxycytidine (**8**), N^4-benzoyl-5′-*O*-*tert*-butyldimethylsilyl-5-ethynyl-2′-deoxycytidine (**9**), N^6-(2-propyn-1-yl)-2′-deoxyadenosine (**13**), 5′-*O*-(4,4′-dimethoxytrityl)-N^6-(2-propyn-1-yl)-2′-deoxyadenosine (**14**), O^6-(2-propyn-1-yl)-2′-deoxyguanosine (**16**), N^2-(2-propyn-1-yl)-2′-deoxyguanosine (**10**), 5′-*O*-(4,4′-dimethoxytrityl)-N^2-(2-propyn-1-yl)-2′-deoxyguanosine (**17**) (0.05–0.36 g, 0.085–0.98 mmol) was dissolved in THF/H_2O (1.2–2 mL, 1 : 1 v/v), and cooled to 0 °C. Next, $CuSO_4$ × $5H_2O$ (0.1 eq.), sodium ascorbate (0.2 eq.), and then 1-(3-azidopropyl)-1,2-dicarba-*closo*-dodecaborane (**6**) (1.1 eq.) were added. The mixture was allowed to warm to RT, then continued to react for 2–18 h. The reaction was quenched by evaporation of the solvent. The crude compounds were purified by column chromatography on silica gel (230–400 mesh) with a gradient of CH_3OH (0–10%) in CH_2Cl_2 as the eluent.

*N^3-{[(o-Carboran-1-yl)propyl]-1N-1,2,3-triazol-4-yl}methylenethymidine (**20**).* White solid, yield 0.16 g, 88%. R_f = 0.39 ($(CH_3)_2CO$/CH_2Cl_2 3 : 2). ^{1}H-NMR (600.26 MHz, DMSO-d_6) δ 7.93 (s, 1H, H-triazole), 7.80 (d, ^4J(H6-CH_3(C-5)) = 1.2 Hz, 1H, H-6), 6.21 (t, ^3J(H1′–H2′) = 6 Hz, 1H, H-1′), 5.22 (d, ^3J(OH(C-3′)-H3′) = 4.2 Hz, 1H, OH(C-3′)), 5.16 (br s, 1H, CH-carborane),

5.04–5.01 (m, 3H, NCH_2, OH(C-5′)), 4.29–4.24 (m, 3H, CH_2-α, H-3′), 3.78–3.77 (m, 1H, H-4′), 3.60–3.54 (m, 2H, H-5′, H-5″), 2.39–1.50 (m, 10H, $B_{10}H_{10}$), 2.28–2.25 (m, 2H, CH_2-γ), 2.12–2.10 (m, 2H, H-2′, H-2″), 1.99–1.84 (m, 2H, CH_2-β), 1.84 (d, ^4J(CH_3(C-5)-H6) = 1.2 Hz, 3H, CH_3(C-5)) ppm. ^{13}C-NMR (150.94 MHz, DMSO-d_6) δ 162.43 (s, C-2), 150.37 (s, C-triazole), 142.84 (s, C-4), 135.15 (s, C-6), 123.55 (s, CH-triazole), 108.64 (s, C-5), 87.54 (s, C-4′), 84.96 (s, C-1′), 75.69 (s, C-carborane), 70.40 (s, C-3′), 63.02 (s, CH-carborane), 61.35 (s, C-5′), 48.25 (s, CH_2-α), 36.08 (s, NCH_2), 33.71 (s, CH_2-γ), 29.50 (s, CH_2-β), 12.97 (s, CH_3(C-5)) ppm. ^{11}B-NMR {^{1}H BB} (192.59 MHz, DMSO-d_6) δ −3.23 (s, 2B), −6.27 (s, 2B), −9.83 (s, 2B), −11.73 to −13.08 (m, 4B) ppm. ^{11}B-NMR (192.59 MHz, DMSO-d_6) δ −3.23 (d, 2B), −5.97 (s, 2B), −9.46 to −12.11 (m, 6B) ppm. FT-IR (cm^{-1}) 3366 (OH), 3143, 3041, 2930 (CH arom), 2583 (BH), 1696 (C═O), 1631 (C═C arom). RP-HPLC: t_R = 20.85 min. MS (Gly, FAB, +VE) *m/z* (%): 508.5 (100) $[M + H]^+$, MS (Gly, FAB, −VE) *m/z* (%): 507.5 (100) $[M]^-$, calcd for $C_{18}H_{33}B_{10}N_5O_5$ = 507.35.

*5-{[(o-Carboran-1-yl)propyl]-1N-1,2,3-triazol-4-yl}-2′-deoxycytidine (**22**).* White solid, yield 0.11 g, 73%. R_f = 0.64 (CH_2Cl_2/CH_3OH 95 : 5). ^{1}H-NMR (600.26 MHz, CD_3OD) δ 8.71 (br s, 1H, H-6), 8.18 (br s, 1H, H-triazole), 6.31 (br s, 1H, H-1′), 4.58 (br s, 1H, CH-carborane), 4.49–4.47 (m, 3H, CH_2-α, H-3′), 4.01–3.96 (m, 2H, H-4′, H-5′), 3.84 (d, ^2J(H5″–H5′) = 10.8 Hz, 1H, H-5″), 2.70–1.40 (m, 10H, $B_{10}H_{10}$), 2.49–2.42 (m, 1H, H-2′), 2.41–2.39 (m, 2H, CH_2-γ), 2.30–2.28 (m, 1H, H-2″), 2.21–2.18 (m, 2H, CH_2-β) ppm. ^{13}C-NMR (150.94 MHz, CD_3OD) δ 165.47 (s, C-4), 157.97 (s, C-2), 144.85 (s, C-triazole), 142.16 (s, C-6), 122.83 (s, CH-triazole), 90.00 (s, C-4′), 88.96 (s, C-1′), 77.07 (s, C-5), 72.09 (s, C-3′), 65.76 (s, C-carborane), 64.90 (s, CH-carborane), 63.21 (s, C-5′), 51.38 (s, CH_2-α), 43.55 (s, C-2′), 36.68 (s, CH_2-γ), 31.84 (s, CH_2-β) ppm. ^{11}B-NMR {^{1}H BB} (192.59 MHz, CD_3OD) δ −2.71 (s, 2B), −5.83 (s, 2B), −9.50 (s, 2B), −11.70 to −12.99 (m, 4B) ppm. ^{11}B-NMR (192.59 MHz, CD_3OD) δ −2.73 (d, 2B), −5.83 (d, 2B), −9.55 (d, 2B), −11.30 to −13.40 (m, 4B) ppm FT-IR (cm^{-1}) 3305 (OH), 3061, 2934 (CH alkyl), 2582 (BH), 1651 (C═O), 1507, 1487, 1454 (C═C arom). RP-HPLC: t_R = 20.26 min. MS (Gly, FAB, +VE) *m/z* (%): 479.4 (5) $[M + H]^+$, MS (Gly, FAB, −VE) *m/z* (%): 478.4 (100) $[M]^-$, 540.3 (20) $[M + Cu]^-$, 605.3 (100) $[M + 2Cu]^-$, calcd for $C_{16}H_{30}B_{10}N_6O_4$ = 478.33.

*N^4-Benzoyl-5′-O-tert-butyldimethylsilyl-5-{[(o-carboran-1-yl)propyl]-1N-1,2,3-triazol-4-yl}-2′-deoxycytidine (**23**).* White solid, yield 0.05 g, 70%. R_f = 0.26 (CH_2Cl_2/CH_3OH 95 : 5). ^{1}H-NMR (600.26 MHz, DMSO-d_6) δ 11.27 (br s, 1H, NH), 9.43 (br s, 1H, NH), 8.61 (s, 1H, H-6), 8.34 (br s, 1H, H-triazole), 8.03 (d, ^3J(H–H) = 7.2 Hz, 2H, H-benzoyl), 7.64–7.54 (m, 3H, H-benzoyl), 6.23 (t, ^3J(H1′–H2′) = 6.6 Hz, 1H, H-1′), 5.31 (d, ^3J(OH(C-3′)-H3′) = 4.2, 1H, OH(C-3′)), 5.17 (br s, 1H, CH-carborane), 4.41 (t, ^3J(CH_2-α-CH_2-β) = 6.6 Hz, 2H, CH_2-α), 4.24–4.23 (m, 1H, H-3′), 4.01–4.00 (m, 1H, H-4′), 3.85 (dd, ^3J(H5′–H4′) = 3 Hz, ^2J(H5′–H5″) = 11.4 Hz, 1H, H-5′), 3.79 (dd, ^3J(H5″–H4′) = 3 Hz, ^2J(H5″–H5′) = 12 Hz, 1H, H-5″), 2.55–1.40 (m, 10H, $B_{10}H_{10}$), 2.41–2.38 (m, 1H, H-2′), 2.33–2.30 (m, 2H, CH_2-γ), 2.05–1.98 (m, 3H, H-2″, CH_2-β), 0.77 (s, 9H, $(CH_3)_3CSi$), 0.03 (s, 3H, $(CH_3)_2Si$), −0.02 (s, 3H, $(CH_3)_2Si$) ppm. ^{13}C-NMR (150.94 MHz, DMSO-d_6) δ 168.09 (s, C-2), 164.31 (s, C═O of the benzoyl group), 157.64 (s, C-4), 140.76 (C-triazole), 139.12 (s, C-6), 135.53 (O═C–*C* of the benzoyl group), 131.86, 128.70, 128.02 (s, C of the benzoyl group), 123.67 (s, CH-triazole), 106.96 (s, C-5), 87.85 (s, C-4′), 86.72 (s, C-1′), 75.69 (s, C-carborane), 71.00 (s, C-3′), 63.47 (s, C-5′), 63.10 (s, CH-carborane), 48.29 (s, CH_2-α), 41.44 (s, C-2′), 33.67 (s, CH_2-γ), 29.95 (s, CH_2-β), 25.88 (s, $(CH_3)_3CSi$), 18.05 (s, $(CH_3)_3CSi$), −5.49 (s, $(CH_3)Si$), −5.54 (s, $(CH_3)Si$) ppm. ^{11}B-NMR {^{1}H BB} (192.59 MHz, DMSO-d_6) δ −3.20 (s, 2B), −6.09 (s, 2B), −9.83 to −12.92 (m, 6B) ppm. ^{11}B-NMR (192.59 MHz, DMSO-d_6) δ −3.23 (d, 2B), −6.05 (s, 2B), −9.47 to −12.29 (m, 6B) ppm. FT-IR (cm^{-1}) 3307 (OH), 3061 (NH), 2928, 2856 (CH alkyl), 2582 (BH), 1653 (C═O). MS (Gly, FAB, +VE) *m/z* (%): 696.7 (100) $[M + H]^+$, (Gly, FAB, −VE) *m/z* (%): 695.4 (100) $[M - 2H]^-$, calcd for $C_{29}H_{48}B_{10}N_6O_5Si$ = 697.44.

*N^6-{[(o-Carboran-1-yl)propyl]-1N-1,2,3-triazol-4-yl}methylene-2′-deoxyadenosine (**24**).* White solid, yield 0.14 g, 79%. R_f = 0.48 ($(CH_3)_2CO$). ^{1}H-NMR (600.26 MHz, DMSO-d_6) δ 8.36 (br s, 1H, H-8,), 8.24 (br s, 2H, NH, H-triazole), 7.89 (s, 1H, H-2), 6.36 (dd, ^3J(H1′–H2′) = 6.6 Hz, ^3J(*H*1′–H2″) = 7.8 Hz, 1H, H-1′), 5.29 (br s, 1H, OH(C-3′)), 5.18–5.15 (m, 2H, CH-carborane, OH(C-5′)), 4.73 (br s, 2H, NCH_2), 4.42–4.41 (m, 1H, H-3′), 4.27 (t, ^3J(CH_2-α-CH_2-β) = 6.6 Hz, 2H, CH_2-α), 3.89–3.88 (m, 1H, H-4′), 3.62 (dd, ^3J(H5′–H4′) = 4.2 Hz, ^2J(H5′–H5″) = 12 Hz, 1H, H-5′), 3.52 (dd, ^3J(H5″–H4′) = 4.2 Hz, ^2J(H5″–H5′) = 12 Hz, 1H, H-5″), 2.75–2.71 (m, 1H, H-2′), 2.70–1.54 (m, 10H, $B_{10}H_{10}$), 2.26–2.23 (m, 3H, H-2′, CH_2-γ), 1.94–1.91 (m, 2H, CH_2-β) ppm. ^{13}C-NMR (150.94 MHz, DMSO-d_6) δ 154.39 (s, C-6), 152.37 (s, CH-triazole), 148.00 (s, C-triazole), 145.68 (s, C-4), 139.73 (s, C-8), 122.94 (s, C-2), 120.00 (s, C-5), 85.18 (s, C-4′), 84.13 (s, C-1′), 75.60 (s, C-carborane), 71.12 (s, C-3′), 63.04 (s, C-carborane), 62.04 (s, C-5′), 48.21 (s, CH_2-α), 39.27 (s, C-2′), 35.00 (s, NCH_2), 33.73 (s, CH_2-γ), 29.61 (s, CH_2-β) ppm. ^{11}B-NMR {^{1}H BB} (192.59 MHz, DMSO-d_6) δ −3.25 (s, 2B), −6.24 (s, 2B), −9.86 (s, 2B), −11.76 to −13.12 (m, 4B) ppm. ^{11}B-NMR (192.59 MHz, DMSO-d_6) δ −3.29 (d, 2B), −6.02 (s, 2B), −9.48 to −12.16 (m, 6B) ppm. FT-IR (cm^{-1}) 3261 (OH), 3143, 3039, 2928, (CH aromat.), 2867 (CH alkyl), 2578 (BH), 1613 (C═C arom). RP-HPLC: t_R = 20.62 min. MS (Gly, FAB, +VE) *m/z* (%): 517.4 (100) $[M]^+$, MS (Gly, FAB, −VE) *m/z* (%): 516.4 (100) $[M - H]^-$, calcd for $C_{18}H_{32}B_{10}N_8O_3$ = 517.36.

*5′-O-(4,4′-Dimethoxytrityl)-N^6-{[(o-carboran-1-yl)propyl]-1N-1,2,3-triazol-4-yl}methylene-2′-deoxyadenosine (**25**).* White solid, yield 0.062 g, 90%. R_f = 0.55 ($(CH_3CH_2)_2O$/C_2H_5OH 97 : 3). ^{1}H-NMR (600.26 MHz, DMSO-d_6) δ 8.26 (br s, 1H, H-8,), 8.19 (br s, 2H, NH, H-triazole), 7.88 (s, 1H, H-2), 7.35–7.34 (m, 2H, H-arom. of the DMT group), 7.24–7.18 (m, 7H, H-arom of the DMT group), 6.84–6.80 (m, 4H, α-H-arom of the DMT group), 6.38 (t, ^3J(H1′–H2′) = 6.6 Hz, 1H, H-1′), 5.35 (d, ^3J(OH(C-3′)-H3′) = 4.2 Hz, 1H, OH(C-3′)), 5.15 (br s, 1H, CH-carborane), 4.75 (br s, 2H, NCH_2), 4.49–4.48 (m, 1H, H-3′), 4.27 (t, ^3J(CH_2-α-CH_2-β) = 6.6 Hz, 2H, CH_2-α), 4.00–3.99 (m, 1H, H-4′), 3.73 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3), 3.20–3.19 (m, 2H, H-5′, H-5″), 2.87–2.84 (m, 1H, H-2′), 2.44–1.40 (m, 10H, $B_{10}H_{10}$), 2.34–2.30 (m, 1H, H-2″), 2.28–2.25 (m, 2H, CH_2-γ), 1.95–1.92 (m, 2H, CH_2-β) ppm. ^{13}C-NMR

(150.94 MHz, DMSO-d_6) δ 158.13, 158.11 (s, C-arom), 154.00 (s, C-6), 153.73, 144.94, 135.68, 129.77, 129.73, 127.78, 126.67, 113.19, 113.17 (s, C-arom), 152.44 (s, C-triazole) 139.56 (s, C-8, CH-triazole) 122.86 (s, C-2), 85.92, (s, C-methylidene of the DMT group), 85.55 (s, C-5′), 83.50 (s, C-1′), 75.65 (s, C-carborane), 70.84 (s, C-3′), 64.17 (s, C-5′), 63.00 (s, CH-carborane), 55.08 (s, OCH_3 of the DMT group), 48.16 (s, CH_2-α), 38.81 (s, C-2′), 35.10 (s, NCH_2), 33.71 (s, CH_2-γ), 29.55 (CH_2-β) ppm. ^{11}B-NMR {^{1}H BB} (192.59 MHz, DMSO-d_6) δ −3.22 (s, 2B), −6.22 (s, 2B), −9.85 (s, 2B), −11.68 to −13.03 (m, 4B) ppm. ^{11}B-NMR (192.59 MHz, DMSO-d_6) δ −3.24 (d, 2B), −6.21 (s, 2B), −9.48 to −12.15 (m, 6B) ppm. FT-IR (cm^{-1}) 3305 (NH), 3050 (OH) 2930, 2873, 2834 (CH alkyl), 2579 (BH), 1611 (C═C arom). MS (NBA, FAB, +VE) *m/z* (%): 819.6 (100) $[M]^+$, (NBA, FAB, −VE) *m/z* (%): 818.4 (100) $[M - H]^-$, calcd for $C_{39}H_{50}B_{10}N_8O_5$ = 819.49.

*O^6-{[(o-Carboran-1-yl)propyl]-1N-1,2,3-triazol-4-yl}methylene-2′-deoxyguanosine (**26**).* White solid, yield 0.085 g, 98%. R_f = 0.40 ($(CH_3)_2CO/CH_2Cl_2$ 8 : 2). ^{1}H-NMR (600.26 MHz, DMSO-d_6) δ 8.29 (s, 1H, H-triazole), 8.08 (s, 1H, H-8), 6.51 (br s, 1H, NH_2), 6.21 (dd, ^3J(H1′–H2″) = 6 Hz, ^3J(*H*1′–H2′) = 7.2 Hz, 1H, H-1′), 5.51 (s, 2H, OCH_2) 5.25 (br s, 1H, OH(C-3′)), 5.17 (br s, 1H, CH-carborane), 4.97 (br s, 1H, OH(C-5′)), 4.37–4.34 (m, 2H, H-3′, CH_2-α), 3.84–3.82 (m, 1H, H-4′), 3.57 (dd, ^3J(H5′–H4′) = 4.8 Hz, ^2J(H5′–H5″) = 12 Hz, 1H, H-5′), 3.51 (dd, ^3J(H5″–H4′) = 4.8 Hz, ^2J(H5″–H5′) = 12 Hz, 1H, H-5″), 2.57–2.52 (m, 1H, H-2′), 2.53–1.40 (m, 10H, $B_{10}H_{10}$), 2.31–2.28 (m, 1H, CH_2-γ), 2.24–2.21 (ddd, ^3J(H2″–H3′) = 3 Hz, ^3J(H2″–H1′) = 6 Hz, ^2J(H2″–H2′) = 9 Hz, 1H, H-2′), 2.02–1.96 (m, 2H; CH_2-β) ppm. ^{13}C-NMR (150.94 MHz, DMSO-d_6) δ 159.79 (s, C-6), 159.72 (s, C-2), 154.20 (s, C-4), 142.45 (s, C-triazole), 138.06 (s, C-8), 125.55 (s, CH-triazole), 114.00 (s, C-5), 87.78 (s, C-4′), 83.01 (s, C-1′), 75.68 (s, C-carborane), 70.92 (s, C-3′), 63.09 (s, CH-carborane), 61.89 (s, C-5′), 58.72 (s, OCH_2), 48.42 (s, CH_2-α) 39.20 (s, C-2′), 33.75 (s, CH_2-γ), 29.58 (s, CH_2-β) ppm. ^{11}B-NMR {^{1}H BB} (192.59 MHz, DMSO-d_6) δ −3.23 (s, 2B), −6.12 (s, 2B), −9.83 (s, 2B), −11.71 to −13.08 (m, 4B) ppm. ^{11}B-NMR (192.59 MHz, DMSO-d_6) δ −3.22 (d, 2B), −6.01 (s, 2B), −9.45 to −12.15 (m, 6B) ppm. FT-IR (cm^{-1}) 3322 (OH), 3209 (NH), 2921, 2851 (CH alkyl) 2577 (BH), 1581 (C═C arom). RP-HPLC: t_R = 20.53 min. MS (Gly, FAB, +VE) *m/z* (%): 533.3 (20) $[M + H]^+$, 596.3 $[M + Na + K]^+$ (40), (Gly, FAB, −VE) *m/z* (%): 532.4 (100) $[M - H]^-$, calcd for $C_{18}H_{32}B_{10}N_8O_4$ = 532.35.

*N^2-{[(o-Carboran-1-yl)propyl]-1N-1,2,3-triazol-4-yl}methylene-2′-deoxyguanosine (**27**).* White solid, yield 0.05 g, 83%. R_f = 0.34 ($CH_3COOC_2H_5/CH_3OH$ 8 : 2). ^{1}H-NMR (600.26 MHz, DMSO-d_6) δ 10.61 (br s, 1H, NH), 8.01 (s, 1H, H-8), 7.92 (s, 1H, H-triazole), 6.88 (t, ^3J(NH–CH_2) = 5.4 Hz, 1H, N*H*-CH_2), 6.18 (t, ^3J(H1′–H2′) = 6.6 Hz, 1H, H-1′), 5.26 (d, ^3J(OH(C-3′)-H3′) = 4.2, 1H, OH(C-3′)), 5.15 (br s, 1H, CH-carborane), 4.84 (t, ^3J(OH(C-5′)-H5′) = 5.4 Hz, 1H, OH(C-5′)), 4.54 (t, ^3J(CH_2-α-CH_2-β) = 6 Hz, 2H, CH_2-N), 4.36–4.31 (m, ^3J(CH_2-α-CH_2-β) = 7.2 Hz, 3H, H-3′, CH_2-α), 3.83–3.81 (m, 1H, H-4′), 3.58–3.54 (m, 1H, H-5′), 3.51–3.46 (m, 1H, H-5″), 2.63–2.59 (m, 1H, H-2′), 2.55–1.25 (m, 10H, $B_{10}H_{10}$), 2.30–2.27 (m, 2H, CH_2-γ), 2.23–2.20 (ddd, ^3J(H2′–H3′) = 3 Hz, ^3J(H2′–H1′) = 6 Hz, ^2J(H2′–H2″) = 9.6 Hz, 1H, H-2″), 1.98–1.95 (m, 2H, CH_2-β) ppm. ^{13}C-NMR (150.94 MHz, DMSO-d_6) δ 156.73 (s, C-6), 152.36 (s, C-4), 150.38 (s, C-triazole), 144.42 (s, C-2), 135.98 (s, CH-triazole), 123.03 (s, C-8), 117.31 (s, C-5), 87.79 (s, C-4′), 83.00 (s, C-1′), 75.67 (s, C-carborane), 70.95 (s, C-3′), 63.09 (s, CH-carborane), 61.95 (s, C-5′), 48.32 (s, CH_2-α), 36.29 (s, NCH_2), 33.72 (s, CH_2-γ), 31.39 (s, CH_2-β) ppm. ^{11}B-NMR {^{1}H BB} (192.59 MHz, DMSO-d_6) δ −3.23 (s, 2B), −6.14 (s, 2B), −9.83 (s, 2B), −11.73 to −13.09 (m, 4B) ppm. ^{11}B-NMR (192.59 MHz, DMSO-d_6) δ −3.23 (d, 2B), −6.02 (br s, 2B), −9.46 to −12.24 (m, 6B) ppm. FT-IR (cm^{-1}) 3225 (OH), 3128, 2926 (CH alkyl), 2585 (BH), 1682 (C═O), 1609, 1520, 1563 (C═C arom). MS (Gly, FAB, +VE) *m/z* (%): 534.4 (80) $[M + H]^+$, 596.3 (100) $[M + Na + K]^+$; MS (Gly, FAB, −VE) *m/z* (%): 531.2 (100) $[M - 2H]^-$, $C_{18}H_{32}B_{10}N_8O_4$ = 533.35.

*5′-O-(4,4′-Dimethoxytrityl)-N^2-{[(o-carboran-1-yl)propyl]-1N-1,2,3-triazol-4-yl}methylene-2′-deoxyguanosine (**28**).* Yellowish solid, yield 0.025 g, 93%. R_f = 0.3 (CH_2Cl_2/CH_3OH 95 : 5). ^{1}H-NMR (600.26 MHz, DMSO-d_6) δ 10.64 (br s, 1H, NH), 7.98 (s, 1H, H-8), 7.82 (s, 1H, H-triazole), 7.34–7.33 (m, 2H, H-arom of the DMT group), 7.24–7.17 (m, 7H, H-arom of the DMT group), 6.86 (t, ^3J(NH–CH_2) = 5.4 Hz, 1H, N*H*-CH_2), 6.81–6.79 (m, 4H, α-H-arom of the DMT group), 6.23 (t, ^3J(H1′–H2′) = 6.6 Hz, 1H, H-1′), 5.32 (d, ^3J(OH(C-3′)-H3′) = 4.9 Hz, 1H, OH(C-3′)), 5.15 (br s, 1H, CH-carborane), 4.47 (dd, ^3J(CH–NH) = 5.5 Hz, ^3J(CH–CH) = 15 Hz, 1H, HC-N), 4.42–4.35 (m, 2H, HC-N, H-3′), 4.29 (t, ^3J(CH_2-α-CH_2-β) = 7.2 Hz, 3H, CH_2-α), 3.96–3.93 (m, 1H, H-4′), 3.73 (2 × s, 6H, 2 × OCH_3), 3.21 (dd, ^3J(H5′–H4′) = 6.6 Hz, ^3J(H5′–H5″) = 10.2, 1H, H-5′), 3.13 (dd, ^3J(H5″–H4′) = 6.6 Hz, ^2J(H5″–H5′) = 10.2 Hz, 1H, H-5″), 2.73–2.68 (m, 1H, H-2′), 2.55–1.40 (m, 10H, $B_{10}H_{10}$), 2.29–2.26 (m, 3H, H-2″, CH_2-γ), 1.98–1.94 (m, 2H, CH_2-β) ppm. ^{13}C-NMR (150.94 MHz, DMSO-d_6) δ 158.15, 158.13 (s, C-arom), 156.72 (s, C-6), 152.27 (s, C-4), 150.32 (s, C-triazole), 145.00 (s, C-2), 135.73, 135.65, 129.78, 129.75, 127.81, 126.71, 113.18 (C-arom) 135.98 (s, CH-triazole), 122.99 (s, C-5), 85.96 (s, C-methylidene of the DMT group), 85.54 (s, C-4′), 83.03 (s, C-1′), 75.63 (s, C-carborane), 70.82 (s, C-3′), 64.44 (s, CH-carborane), 63.09 (s, C-5′), 55.11 (s, OCH_3 of the DMT group), 40.42 (s, CH_2-α), 36.32 (s, NCH_2), 33.71 (s, CH_2-γ), 29.59 (s, CH_2-β) ppm. ^{11}B-NMR {^{1}H BB} (192.59 MHz, DMSO-d_6) δ −3.24 (s, 2B), −6.08 (s, 2B), −9.86 (s, 2B), −11.83 to −13.10 (m, 4B) ppm. ^{11}B-NMR (192.59 MHz, DMSO-d_6) δ −3.22 (d, 2B), −6.07 (br s, 2B), −8.47 to −12.36 (m, 6B) ppm. FT-IR (cm^{-1}) 3216 (OH), 3048, 2931 (CH alkyl), 2581 (BH), 1699 (C═O), 1604, 1507, 1463 (C═C arom). MS (Gly, FAB, +VE) *m/z* (100): 836.7 (100) $[M + 2H]^+$, (Gly, FAB, −VE) *m/z* (100): 833.5 (100) $[M - H]^-$, calcd for $C_{39}H_{50}B_{10}N_8O_6$ = 834.49.

3.2.8 Synthesis of 5′-*O*-(4,4′-dimethoxytrityl)-N^3-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}methylenethymidine (21). N^3-{[(*o*-Carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}methylenthymidine (**20**) (0.133 g, 0.262 mmol) was evaporated with anhydrous pyridine (3 × 5 mL), and then dissolved in the same solvent (3 mL). To the resultant solution, DMAP (0.0016 g, 0.0131 mmol), TEA (0.04 g, 0.4 mmol, 0.056 mL), and DMTrCl (0.131 g, 0.393 mmol) were added. After stirring for 48 h at RT, the reaction was quenched with H_2O (10 mL) at 0 °C, then extracted with Et_2O (3 × 6 mL). The organic phase was separated, dried over $MgSO_4$,

filtered, and evaporated to dryness. The residue was purified by column chromatography on silica gel (230–400 mesh) using a gradient of CH_3OH (0–20%) in CH_2Cl_2 (+1% TEA) as the eluent to give product **10** (0.17 g, 88%) as a white foam. R_f = 0.40 ($(CH_3)_2CO/CH_2Cl_2$ 95 : 5). ^{1}H-NMR (600.26 MHz, DMSO-d_6) δ 7.94 (s, 1H, H-triazole), 7.59 (d, ^4J(H6–CH_3(C-5)) = 0.6 Hz, 1H, H-6), 7.39–7.38 (m, 2H, H-arom of the DMT group), 7.31–7.25 (m, 7H, H-arom of the DMT group), 6.90–6.88 (m, 4H, α-H-arom of the DMT group), 6.26 (t, ^3J(H1′–H2′) = 7.2 Hz, 1H, H-1′), 5.31 (d, ^3J(OH(C-3′)-H3′) = 4.2 Hz, 1H, OH(C-3′)), 5.15 (br s, 1H, CH-carborane), 5.07–5.01 (m, 2H, NCH_2), 4.34–4.33 (m, 1H, H-3′), 4.28 (t, ^3J(CH_2-α-CH_2-β) = 7.2 Hz, 2H, CH_2-α), 3.91 (q, ^3J(H4′–H3′) = 3.6 Hz, 1H, H-4′), 3.74 (2 × s, 6H, 2 × OCH_3 of the DMT group) 3.25–3.19 (m, 2H, H-5′, H-5″), 2.46–1.60 (m, 10H, $B_{10}H_{10}$), 2.28–2.25 (m, 2H, CH_2-γ), 2.21–2.17 (m, 2H, H-2′, H-2″), 1.96–1.93 (m, 2H, CH_2-β), 1.50 (s, ^4J(CH_3(C-5)-H6) = 1.2 Hz, 3H, CH_3(C-5)) ppm. ^{13}C-NMR (150.94 MHz, DMSO-d_6) δ 162.34 (s, C-2), 158.29, 144.76, 135.55, 135.39, 129.83, 127.99, 127.81, 126.90 (s, C-arom), 150.27 (s, C-triazole), 142.75 (s, C-4), 134.62 (s, C-6), 123.59 (s, CH-triazole), 108.90 (s, C-5), 86.02 (s, C-4′), 85.76 (s, C-methylidene of the DMT group), 84.90 (s, C-1′), 75.67 (s, C-carborane), 70.51 (s, C-3′), 63.78 (s, C-5′), 63.00 (s, CH-carborane), 55.17 (s, OCH_3 of the DMT group), 48.23 (s, CH_2-α), 36.11 (s, NCH_2), 33.70 (s, CH_2-γ), 29.47 (s, CH_2-β), 12.41 (s, CH_3(C-5)) ppm. ^{11}B-NMR {^{1}H BB} (192.59 MHz, DMSO-d_6) δ −3.23 (s, 2B), −6.20 (s, 2B), −9.82 (s, 2B), −11.71 to −13.10 (s, 4B) ppm. ^{11}B-NMR (192.59 MHz, DMSO-d_6) δ −3.24 (d, 2B), −5.84 (s, 2B), −9.47 to −12.24 (m, 6B) ppm. FT-IR (cm^{-1}) 3211 (OH), 2928, 2871 (CH arom), 2583 (BH), 1622 (C═C arom). MS (NBA, FAB, +VE) *m/z* (%): 810.4 (100%) $[M]^+$, MS (NBA, FAB, −VE) *m/z* (%): 809.4 (100) $[M - H]^-$, calcd for $C_{39}H_{51}B_{10}N_5O_7$ = 810.48.

3.2.9 General procedure for the synthesis of 5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-(*N*,*N*-diisopropyl-β-cyanoethyl)-N^3-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}methylenethymidine phosphoramidite (29), N^4-benzoyl-5′-*O*-*tert*-butyldimethylsilyl-3′-*O*-(*N*,*N*-diisopropyl-β-cyanoethyl)-5-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}-2′-deoxycytidine phosphoramidite (30), 5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-(*N*,*N*-diisopropyl-β-cyanoethyl)-N^3-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}methylene-2′-deoxyadenosine phosphoramidite (31), and 5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-(*N*,*N*-diisopropyl-β-cyanoethyl)-N^2-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}methylene-2′-deoxyguanosine phosphoramidite (32). All procedures were performed under positive pressure of argon. 5′-*O*-(4,4′-dimethoxytrityl)-N^3-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}methylenethymidine (**21**), N^4-benzoyl-5′-*O*-*tert*-butyldimethylsilyl-5-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}-2′-deoxycytidine (**23**), 5′-*O*-(4,4′-dimethoxytrityl)-N^6-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}methylene-2′-deoxyadenosine (**25**), and 5′-*O*-(4,4′-dimethoxytrityl)-N^2-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}methylene-2′-deoxyguanosine (**28**) (0.02 g, 0.024–0.025 mmol) were dried under high vacuum for 24 h, then dissolved in anhydrous CH_2Cl_2 freshly distilled over CaH_2 (1.1 mL), and next DIPEA (5 eq.) was added. To the resultant solution, phosphitylating agent, (β-cyanoethyl) (*N*,*N*-diisopropylamino) chlorophosphine (3 eq.) was added. The reaction progress was monitored by TLC using CH_2Cl_2/CH_3OH (9 : 1) as an eluting solvent system. After stirring for 2–4 h under argon at RT, the reaction was quenched with anhydrous CH_3OH (0.4 mL). The resultant solution was diluted with CH_2Cl_2 (10 mL) and washed with $NaHCO_3$ (5% w/v, 3 × 2 mL). The organic layer was dried over $MgSO_4$, then the drying agent was filtered off and washed with CH_2Cl_2 containing 1% TEA. Filtrate and washings were combined, and evaporated to dryness under a vacuum. The crude product (*ca.* 200 mg) was immediately purified by column chromatography on silica gel (230–400 mesh) using a gradient of CH_3OH (0–3%) in CH_2Cl_2 (+1% TEA) as an eluting solvent system. Solvents were evaporated to dryness under a vacuum. The glassy residue was dissolved in anhydrous CH_2Cl_2 (1 mL), and cooled to −20 °C. The resultant solution was added to a vigorously stirred, cooled to −50 °C, anhydrous petroleum ether (10 mL). A precipitate was isolated by centrifugation. The product was then degassed under high vacuum, and stored at −20 °C.

*5′-O-(4,4′-Dimethoxytrityl)-3′-O-(N,N-diisopropyl-β-cyanoethyl)-N^3-{[(o-carboran-1-yl)propyl]-1N-1,2,3-triazol-4-yl}methylenethymidine phosphoramidite (**29**).* Colorless foam, yield 0.017 g, 66%. R_f = 0.48 (CH_2Cl_2/CH_3OH 95 : 5). ^{1}H-NMR (600.26 MHz, DMSO-d_6) δ 7.94 (s, 1H, H-triazole), 7.61 (2 × s, 1H, H-6), 7.40–7.38 (m, 2H, H-arom of the DMT group), 7.32–7.23 (m, 7H, H-arom of the DMT group), 6.90–6.88 (m, 4H, α-H-arom of the DMT group), 6.27 (q, ^3J(H1′–H2′) = 7.2 Hz, 1H, H-1′), 5.17 (br s, 1H, CH-carborane), 5.08–5.01 (m, 2H, NCH_2), 4.57–4.56 (m, 1H, H-3′), 4.28 (t, ^3J(HCH_2-α-CH_2-β) = 7.2 Hz, 2H, CH_2-α), 4.09–4.08, 4.03–4.01 (2 × m, 1H, H-4′), 3.73 (2 × s, 6H, 2 × OCH_3 of the DMT group), 3.64–3.49 (m, 4H, OCH_2 of $POCH_2CH_2CN$ group, $N[CH(CH_3)_2]_2$), 3.29–3.21 (m, 2H, H-5′, H-5″), 2.77, 2.63 (2 × t, ^3J(OCH_2–CH_2CN) = 6 Hz, 2H, CH_2CN of the $POCH_2CH_2CN$ group), 2.60–1.60 (m, 10H, $B_{10}H_{10}$), 2.43–2.38 (m, 1H, H-2′), 2.27–2.24 (m, 2H, CH_2-γ), 1.97–1.92 (m, 2H, CH_2-β), 1.54 (s, 3H, CH_3(C-5)), 1.52 (s, 3H, CH_3(C-5)), 1.20–0.99 (several d, 12H, $N[(CH(CH_3)_2]_2$) ppm. ^{13}C-NMR (150.94 MHz, DMSO-d_6) δ 162.33 (s, C-2), 128.00, 127.82, 127.77, 126.97, 113.37 (s, C-arom), 150.25 (s, C-triazole), 142.75 (s, C-4), 134.64 (s, C-6), 123.60 (s, CH-triazole), 119.00, 118.81 (s, CN), 109.11, 119.05 (s, C-5), 86.19, 86.12 (s, C-methylidene of the DMT group), 84.89, 84.82 (s, C-1′), 84.71 (s, C-4′), 75.68 (s, C-carborane), 73.07, 72.80 (s, C-3′), 70.00, 69.00 (s, CH of the $N[CH(CH_3)_2]_2$ group), 63.29, 63.18 (s, C-5′), 63.02 (CH-carborane), 55.18 (OCH_3 of the DMT group), 48.24 (s, CH_2-α), 42.81, 42.73 (s, OCH_2 of the $POCH_2CH_2CN$ group), 38.75, 38.67 (s, C-2′), 36.15 (s, NCH_2), 33.70 (s, CH_2-γ), 29.49 (s, CH_2-β), 24.46, 24.41, 24.27 (s, $N[CH(CH_3)_2]_2$), 19.97, 19.89, 19.85 (s, C-aliphatic of the CH_2CN group), 12.45 (CH_3(C-5)) ppm. ^{11}B-NMR {^{1}H BB} (192.59 MHz, DMSO-d_6) δ −3.22 (s, 2B), −6.24 (s, 2B), −9.84 (s, 2B), −11.68 to −13.00 (m, 4B) ppm. ^{11}B-NMR (192.59 MHz, DMSO-d_6) δ −3.31 (d, 2B), −5.98 (s, 2B),−9.47 to −12.25 (m, 6B) ppm. ^{31}P-NMR (242.99 MHz, DMSO-d_6) δ 147.71, 147.43 ppm (mixture of P-diastereomers). FT-IR (cm^{-1}) 2965, 2932, 2873, 2834 (CH alkil), 2572 (BH), 1701(C═O), 1644, 1605, 1504 (C═C arom). MS (NBA, FAB, +VE) *m/z* (%): 1009.7 (15) $[M]^+$, 1073.7 (15) $[M + Na + K]^+$, (NBA, FAB, −VE) *m/z* (%): 1009.50 (35) $[M]^-$, calcd $C_{48}H_{68}B_{10}N_7O_8P$ = 1009.59.

*N^4-Benzoyl-5′-O-tert-butyldimethylsilyl-3′-O-(N,N-diisopropyl-β-cyanoethyl)-5-{[(o-carboran-1-yl)propyl]-1N-1,2,3-triazol-4-yl}-2′-deoxycytidine phosphoramidite (**30**).* Colorless foam, yield 0.023 g, 72%. R_f = 0.80 (CH_2Cl_2/CH_3OH 98 : 2). ^{1}H-NMR (600.26 MHz, $(CD_3)_2CO$) δ 11.84 (br s, 1H, NH), 9.33 (br s, 1H, NH), 8.83 (s, 1H, H-6), 8.45 (br s, 1H, H-triazole), 8.14 (d, ^3J(H–H) = 7.2 Hz, 2H, benzoyl group), 7.64–7.56 (m, 3H, benzoyl group), 6.41–6.38 (m, 1H, H-1′), 4.69–4.67 (m, 2H, CH-carborane, H-3′), 4.51 (t, ^3J(CH_2-α-CH_2-β) = 7.2 Hz, 2H, CH_2-α), 4.29–4.28 (m, 1H, H-4′), 4.04–3.88 (m, 3H, N[C*H*$(CH_3)_2]_2$, H-5′, H-5″), 3.76–3.72 (m, 2H, OCH_2 of the $POCH_2CH_2CN$ group), 3.00–1.60 (m, 10H, $B_{10}H_{10}$), 3.10–2.80 (m, 1H, H-2′), 2.82–2.76 (m, 2H, CH_2CN of the $POCH_2CH_2CN$ group), 2.49–2.47 (m, 2H, CH_2-γ), 2.35–2.29 (m, 1H, H-2″), 2.23–2.18 (m, 2H, CH_2-β), 1.31–1.21 (several d, 12H, 2 × CH_3 of the N[$CH(CH_3)_2]_2$ group), 0.86 (s, 9H, $(CH_3)_3$CSi), 0.14 (s, 3H, $(CH_3)_2$Si), 0.10 ppm (s, 3H, $(CH_3)_2$Si) ppm. ^{13}C-NMR (150.94 MHz, $(CD_3)_2CO$) δ 169.45 (s, C-2), 166.23, 166.19 (s, C═O of the benzoyl group), 155.08, 154.97 (s, C-4), 142.22, 142.20 (s, C-triazole), 139.85, 139.73 (s, C-6), 137.18 (s, O═C–*C* of the benzoyl group), 132.52, 129.52, 129.39, 128.86 (s, C of the benzoyl group), 124.36 (s, CH-triazole), 118.89, 118.85 (s, CN), 108.69, 108.64 (s, C-5), 88.32 (s, C-4′), 88.49 (s, C-1′), 76.31 (s, C-carborane), 75.44, 75.40 (s, C-3′), 64.39, 64.31 (s, C-5′), 63.49 (s, CH-carborane), 59.69, 59.57 (N[*C*H$(CH_3)_2]_2$), 49.41 (s, CH_2-α), 44.08, 43.99 (s, OCH_2 of the $POCH_2CH_2CN$ group), 41.85, 41.81 (s, C-2′), 35.28 (s, CH_2-γ), 30.96 (s, CH_2-β), 26.43 (s, (*C*$H_3)_3$CSi), 24.91, 24.86 (s, N[CH(*C*$H_3)_2]_2$), 20.80, 20.75 (s, *C*H_2CN), 18.92, 18.87 (s, $(CH_3)_3$*C*Si), −5.17 (s, (CH_3)Si), −5.33 (CH_3Si) ppm. ^{11}B-NMR {^{1}H BB} (192.59 MHz, $(CD_3)_2CO$) δ −2.83 (s, 2B), −5.98 (s, 2B), −9.61 to −13.01 (m, 6B) ppm. ^{11}B-NMR (192.59 MHz, $(CD_3)_2CO$) δ −2.81 (d, 2B), −5.99 (d, 2B), −9.22 to −13.45 (m, 6B) ppm. ^{31}P-NMR (242.99 MHz, $(CD_3)_2CO$) δ 148.19, 147.80 ppm (mixture of P-diastereomers). FT-IR (cm^{-1}) 3054, 2961, 2929, 2857 (CH alkyl), 2582 (BH), 1659, 1623, 1599 (C═O), 1493, 1447 (C═C arom) ppm. MS (NBA, FAB, −VE) *m/z* (%) 895.4 (100) $[M - H]^-$, calcd for $C_{38}H_{65}B_{10}N_8O_6PSi$ = 896.55.

*5′-O-(4,4′-Dimethoxytrityl)-3′-O-(N,N-diisopropyl-β-cyanoethyl)-N^3-{[(o-carboran-1-yl)propyl]-1N-1,2,3-triazol-4-yl}methylene-2′-deoxyadenosine phosphoramidite (**31**).* Colorless foam, yield 0.020 g, 83%. R_f = 0.38 (CH_2Cl_2/CH_3OH 9 : 1). ^{1}H-NMR (600.26 MHz, DMSO-d_6) δ 8.28, 8.27 (2 × s, 1H, H-8), 8.21 (br s, 1H, NH), 8.15 (br s, 1H, H-triazole), 7.87 (s, 1H, H-2), 7.33–7.31 (m, 2H, H-arom of the DMT group), 7.23–7.18 (m, 7H, H-arom of the DMT group), 6.82–6.78 (m, 4H, α-H-arom of the DMT group), 6.38 (q, ^3J(H1′–H2′) = 6 Hz, 1H, H-1′), 5.13 (br s, 1H, CH-carborane), 4.80–4.75 (m, 3H, H-3′, NCH_2), 4.29–4.20 (m, 2H, CH_2-α), 4.15–4.13, 4.12–4.09 (2 × m, 1H, H-4′), 3.78–3.65 (m, 2H, N[C*H*$(CH_3)_2]_2$), 3.70 (2 × s, 6H, 2 × OCH_3 of the DMT group), 3.60–3.51 (m, 2H, OCH_2 gr. $POCH_2CH_2CN$ group), 3.21–3.18 (m, 2H, H-5′, H-5″), 3.10–3.02 (m, 1H, H-2′), 2.76, 2.67 (2 × t, ^3J(OCH_2–CH_2CN) = 6.0 Hz, 2H, CH_2CN of the $POCH_2CH_2CN$ group), 2.56–1.40 (m, 10H, $B_{10}H_{10}$), 2.55–2.43 (m, 1H, H-2″), 2.25–2.23 (m, 2H, CH_2-γ), 1.93–1.91 (m, 2H, CH_2-β), 1.20–1.04 (several d, 12H; N[$(CH(CH_3)_2]_2$) ppm. ^{13}C-NMR (150.94 MHz, DMSO-d_6) δ 158.16, 158.16 (s, C-arom), 154.00 (s, C-6), 152.41 (s, C-triazole), 145.66 (s, C-4), 139.97 (s, C-8, CH-triazole), 144.87, 135.63, 135.60, 135.56, 129.79, 129.75, 127.80, 127.74, 126.72, 113.20, 113.17 (s, C-arom), 122.89 (s, C-2), 120.00 (s, C-5), 119.03, 118.85 (s, CN), 85.70 (s, C-methylidene of the DMT group), 84.90, 84.01 (s, C-4′), 83.74, 83.69 (s, C-1′), 75.66 (s, C-carborane), 73.60, 72.95 (s, C-3′), 63.62, 63.48 (s, C-5′), 63.02 (s, CH-carborane), 55.12 (OCH_3 of the DMT group), 48.19 (s, CH_2-α), 42.83 (s, OCH_2 of the $POCH_2CH_2CN$ group), 37.58 (s, C-2′), 35.90 (s, NCH_2), 33.72 (s, CH_2-γ), 29.58 (s, CH_2-β), 24.50–24.29 (m, 2 × CH_3 of the N[$CH(CH_3)_2]_2$ group), 19.95–19.93 (m, C-aliphatic of the CH_2CN group) ppm. ^{11}B-NMR {^{1}H BB} (192.59 MHz, DMSO-d_6) δ −3.27 (s, 2B), −6.15 (s, 2B), −9.86 (s, 2B), −11.75 to −13.06 (m, 4B) ppm. ^{11}B-NMR (192.59 MHz, DMSO-d_6) δ −3.26 (d, 2B), −6.01 (s, 2B),−9.50 to −12.39 (m, 6B) ppm. ^{31}P-NMR (242.99 MHz, DMSO-d_6) δ 147.75, 147.22 ppm (mixture of P-diastereomers). FT-IR (cm^{-1}) 3137 (NH), 3045 (=CH), 2965, 2930, 2869, 2834 (C–H alkyl), 2581 (BH), 2250 (C≡N), 1611 (C═C arom). MS (NBA, FAB, +VE) *m/z* (%): 1019.70 (100) $[M + H]^+$, (NBA, FAB, −VE) *m/z* (%) 1018.60 (100) $[M]^-$, calcd for $C_{48}H_{67}B_{10}N_{10}O_6P$ = 1018.60.

*5′-O-(4,4′-Dimethoxytrityl)-3′-O-(N,N-diisopropyl-β-cyanoethyl)-N^2-{[(o-carboran-1-yl)propyl]-1N-1,2,3-triazol-4-yl}methylene-2′-deoxyguanosine phosphoramidite (**32**).* Colorless foam, yield 0.026 g, 84%. R_f = 0.45 (CH_2Cl_2/CH_3OH 95 : 5). ^{1}H-NMR (600.26 MHz, $(CD_3)_2CO$) δ 7.94 (s, 1H, H-8), 7.84 (s, 1H, H-triazole), 7.47–7.43 (m, 2H, H-arom of the DMT group), 7.34–7.16 (m, 8H, H-arom of the DMT group, N*H*-CH_2), 6.84–6.79 (m, 4H, α-H-arom. of the DMT group), 6.44–6.39 (m, 1H, H-1′), 4.88–4.87 (m, 1H, H-3′), 4.72–4.64 (m, 2H, CH-carborane, *H*CN), 4.54–4.50 (m, 1H, *H*CN), 4.37–4.32 (m, 2H, CH_2-α), 4.28–4.26, 4.24–4.22 (2 × m, 1H, H-4′), 3.93–3.89, 3.87–3.81 (2 × m, 1H, 2H of the N[C*H*$(CH_3)_2]_2$ group), 3.75, 3.74 (2 × s, 6H, OCH_3), 3.69–3.63 (m, 2H, OCH_2 of the $POCH_2CH_2CN$ group), 3.53–3.34 (m, 2H, H-5′, H-5″), 3.04–3.01 (m, 1H, H-2′), 3.01–1.50 (m, 10H, $B_{10}H_{10}$), 2.82–2.76 (m, 2H, CH_2CN of the $POCH_2CH_2CN$ group), 2.78–2.76 (m, 3H, H-2″, CH_2CN of the $POCH_2CH_2CN$ group), 2.39–2.36 (m, 2H, CH_2-γ), 2.11–2.07 (m, 2H, CH_2-β), 1.35–1.12 (several d, 12H of the N[$(CH(CH_3)_2]_2$ group) ppm. ^{13}C-NMR (150.94 MHz, $(CD_3)_2CO$) δ 159.62, 159.60 (s, C-arom), 158.66 (s, C-6), 153.63 (s, C-4), 151.80 (s, C-triazole), 146.08 (s, C-2), 130.97, 130.92, 129.06, 129.01, 128.58, 128.57, 127.57, 113.91 (s, C-arom), 136.79–136.76 (m, CH-triazole), 123.62 (s, C-8), 119.03, 118.82 (s, CN), 118.69 (s, C-5), 87.05 (s, C-4′), 86.48–86.25 (m, C-methylidene of the DMT group), 84.81–84.76 (m, C-1′), 76.24 (s, C-3′), 75.10–74.76 (m, C-carborane), 64.91–64.87 (m, C-5′), 63.53 (s, CH-carborane), 59.60–59.52 (m, N[$CH(CH_3)_2]_2$), 55.53, 55.52 (s, OCH_3 of the DMT group), 49.47 (s, CH_2-α), 44.09,44.01 (s, OCH_2 of the $POCH_2CH_2CN$ group), 39.45 (s, C-2′), 37.74 (s, NCH_2), 35.28 (s, CH_2-γ), 30.65 (s, CH_2-β), 24.98–24.83 (m, 2 × CH_3 of the N[$CH(CH_3)_2]_2$ group), 21.00–20.79 (m, C-aliphatic of the CH_2CN group) ppm. ^{11}B-NMR {^{1}H BB} (192.59 MHz, $(CD_3)_2CO$) δ −2.85 (s, 2B), −6.01 (s, 2B), −9.66 (s, 2B), −11.70 to −13.05 (m, 4B) ppm. ^{11}B-NMR (192.59 MHz, $(CD_3)_2CO$) δ −2.85 (d, 2B), −5.98 (d, 2B), −9.27 to −13.42 (m, 6B) ppm. ^{31}P-NMR (242.99 MHz, $(CD_3)_2CO$) δ 148.53, 148.41 ppm (mixture of P-diastereomers). FT-IR (cm^{-1}) 2966 (CH alkyl), 2584 (BH), 1688 (C═O), 1603,

1502, 1462 (C=C arom). MS (NBA, FAB, +VE) *m/z* (%): 1035.5 (100) $[M + H]^+$, (NBA, FAB, −VE) *m/z* (%): 1034.5 (100) $[M]^-$, calcd for $C_{48}H_{67}B_{10}N_{10}O_7P$ = 1034.59.

3.2.10 General procedure for the synthesis of N^3-{[(7,8-dicarba-*nido*-udecaboranyl)propyl]-1*N*-1,2,3-triazol-4-yl}methylenethymidine (33), 5-{[(7,8-dicarba-*nido*-udecaboranyl)propyl]-1*N*-1,2,3-triazol-4-yl}-2′-deoxycytidine (34), N^6-{[(7,8-dicarba-*nido*-udecaboranyl)propyl]-1*N*-1,2,3-triazol-4-yl}methylene-2′-deoxyadenosine (35), and N^2-{[(7,8-dicarba-*nido*-udecaboranyl)propyl]-1*N*-1,2,3-triazol-4-yl}methylene-2′-deoxyguanosine (36). N^3-{[(*o*-Carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}methylenethymidine (**20**), 5-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}-2′-deoxycytidine (**22**), N^6-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}methylene-2′-deoxyadenosine (**24**), and N^2-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}methylene-2′-deoxyguanosine (**27**) (0.01 g, 0.019–0.021 mmol) were dissolved in acetonitrile (0.2 mL) and NH_{3aq} (30%, 1 mL) was added. The solution was incubated at 55 °C overnight. The reaction was quenched by evaporation of the solvent. The crude product was purified by column chromatography on silica gel (230–400 mesh) with a gradient of CH_3OH (0–25%) in CH_2Cl_2 as the eluent.

N^3-{[(7,8-Dicarba-nido-udecaboranyl)propyl]-1N-1,2,3-triazol-4-yl}methylenethymidine (33). White solid, yield 0.009 g, 90%. R_f = 0.45 (CH_2Cl_2/CH_3OH 8 : 2). ^{1}H-NMR (600.26 MHz, $(CD_3)_2CO$) δ 7.85 (2 × s, 2H, H-triazole, H-6), 6.34 (t, ^3J(H1′–H2′) = 6 Hz, 1H, H-1′), 5.17 (s, 2H, NCH_2), 4.50–4.49 (m, 1H, H-3′), 4.31 (t, ^3J(CH_2-α-CH_2-β) = 7.2 Hz, 2H, CH_2-α), 3.94 (q, ^3J(H3′–H4′) = 3 Hz, 1H, H-4′), 3.80 (dd, ^3J(H5′–H4′) = 3 Hz, ^2J(H-5′–H5″) = 11.4 Hz, 1H, H-5′), 3.75 (dd, ^3J(H5″–H4′) = 3 Hz, ^2J(H5″–H5′) = 11.4 Hz, 1H, H-5″), 2.26–2.23 (m, 2H, H-2′, H-2″), 2.00–0.60 (m, 9H, B_9H_9), 1.85 (d, ^4J(CH_3(C-5)-H6) = 1.2 Hz, 3H, CH_3(C-5)), 1.67–1.62 (m, 2H, CH_2-γ), 1.60 (br s, 1H, CH-carborane), 1.50–1.45 (m, 2H, CH_2-β), −2.78 (br s, 1H, BHB) ppm. MS (Gly, FAB, −VE) *m/z* (%): 497.4 (100) $[M - NH_4]^-$, calcd for $C_{18}H_{33}B_9N_5O_5$ = 497.34.

5-{[(7,8-Dicarba-nido-udecaboranyl)propyl]-1N-1,2,3-triazol-4-yl}-2′-deoxycytidine (34). White solid, yield 0.009 g, 90%. R_f = 0.30 (CH_2Cl_2/CH_3OH 8 : 2). ^{1}H-NMR (600.26 MHz, DMSO-d_6) δ 8.40 (s, 1H, H-6), 8.24 (s, 1H, H-triazol), 7.89 (br s, 1H, NH), 7.70 (br s, 1H, NH), 6.18 (t, ^3J(H1′–H2′) = 6.6 Hz, 1H, H-1′), 5.20 (d, ^3J(OH(C-3′)-H3′) = 4.2 Hz, 1H, OH(C-3′)), 5.13 (t, ^3J(OH(C-5′)-H5′) = 4.8 Hz, 1H, OH(C-5′)), 4.31 (t, ^3J(CH_2-α-CH_2-β) = 6.6 Hz, 2H, CH_2-α), 4.27–4.26 (m, 1H, H-3′), 3.82–3.81 (m, 1H, H-4′), 3.70–3.68 (m, 1H, H-5′), 3.63–3.61 (m, 1H, H-5″), 2.50–0.50 (m, 9H, B_9H_9), 2.21–2.18 (m, 1H, H-2′), 2.13–2.12 (m, 1H, H-2″), 1.96–1.91 (m, 2H, CH_2-γ), 1.67 (br s, 1H, CH-carborane), 1.61–1.56 (m, 1H, CH-β), 1.48–1.43 (m, 1H, CH-β), −2.84 (br s, 1H, BHB) ppm. MS (Gly, FAB, −VE) *m/z* (%): 468.4 (100) $[M - NH_4]^-$, calcd for $C_{16}H_{30}B_9N_6O_4$ = 468.32.

N^6-{[(7,8-Dicarba-nido-udecaboranyl)propyl]-1N-1,2,3-triazol-4-yl}methylene-2′-deoxyadenosine (35). White solid, yield 0.009 g, 90%. R_f = 0.40 (CH_2Cl_2/CH_3OH 8 : 2). ^{1}H-NMR (600.26 MHz, $(CD_3)_2CO$) δ 8.24 (br s, 1H, H-8,), 8.17 (s, 1H, H-triazole), 7.89 (s, 1H, H-2), 7.37 (br s, 1H, NH), 6.43 (dd, ^3J(H1′–H2′) = 5.4 Hz, ^3J(H1′–H2″) = 8.4 Hz, 1H, H-1′), 5.58 (br s, 1H, OH(C-3′)), 4.93 (br s, 1H, NCH_2), 4.64–4.63 (m, 1H, OH(C-5′)), 4.40 (d, ^3J(H3′–H4′) = 3 Hz, 1H, H-3′), 4.31 (t, ^3J(CH_2-α-CH_2-β) = 7.8 Hz, 2H, CH_2-α), 4.09–4.08 (m, 1H, H-4′), 3.80 (dd, ^3J(H5′–H4′) = 3 Hz, ^2J(H5′–H5″) = 12.6 Hz, 1H, H-5′), 3.80 (dd, ^3J(H5″–H4′) = 3 Hz, ^2J(H5″–H5′) = 12.6 Hz, 1H, H-5″), 2.92–2.89 (m, 1H, H-2′), 2.36–2.33 (m, 1H, H-2″), 2.00–0.60 (m, 10H, B_9H_{10}), 1.67–1.64 (m, 2H, CH_2-γ), 1.60 (br s, 1H, CH-carborane), 1.60–1.45 (m, 2H, CH_2-β), −2.73 (br s, 1H, BHB) ppm. MS (Gly, FAB, −VE) *m/z* (%): 506.1 (100) $[M - NH_4]^-$, calcd for $C_{18}H_{32}B_9N_8O_3$ = 506.35.

N^2-{[(7,8-Dicarba-nido-udecaboranyl)propyl]-1N-1,2,3-triazol-4-yl}methylene-2′-deoxyguanosine (36). White solid, yield 0.007 g, 70%. R_f = 0.23 (CH_2Cl_2/CH_3OH 8 : 2). ^{1}H-NMR (600.26 MHz, DMSO-d_6) δ 10.70 (br s, 1H, NH), 8.08 (s, 1H, H-8), 8.00 (s, 1H, H-triazole), 7.00 (s, 1H, N*H*-CH_2), 6.19 (t, ^3J(H1′-H2′) = 6 Hz, 1H, H-1′), 4.54–4.52 (m, 2H, CH_2-N), 4.37–3.36 (m, 1H, H-3′), 4.25 (t, ^3J(CH_2-α-CH_2-β) = 6 Hz, 2H, CH_2-α), 3.83–3.82 (s, 1H, H-4′), 3.57 (dd, ^3J(H5′–H4′) = 6 Hz, ^2J(H5′–H5″) = 12 Hz, 1H, H-5′), 3.51–3.46 (dd, ^3J(H5″–H4′) = 6 Hz, ^2J(H5″–H5′) = 12 Hz, 1H, H-5″), 2.64–2.51 (m, 1H, H-2′), 2.55–0.75 (m, 9H, B_9H_9), 2.25–2.23 (m, 1H, H-2′), 1.90–1.87 (m, 2H, CH_2-γ), 1.63 (br s, 1H, CH-carborane), 1.55–1.50 (m, 2H, CH_2-β), −2.86 (br s, 1H, BHB) ppm. MS (Gly, FAB, −VE) *m/z* (%): 522.4 (100) $[M - NH_4]^-$, calcd for $C_{18}H_{32}B_9N_8O_4$ = 522.34.

3.2.11 Synthesis and purification of DNA oligonucleotides 38–41. The unmodified part of the oligonucleotides **38–41** was synthesized using an ABI 39000 DNA synthesizer (Applied Biosystems). Columns loaded with controlled pore glass functionalized with 5′-*O*-dimethoxytrityl nucleosides (0.2 μmol) were used as a solid support. Suitable 5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxynucleoside-3′-*O*-(*N*,*N*-diisopropyl-β-cyanoethyl) phosphoramidites (B = C^{Bz}, G^{Pr}, A^{Bz}, T) were prepared as a 0.5 g/10 mL solution in anhydrous acetonitrile. Elongation of the oligomers with natural nucleotides was performed using the phosphoramidite approach[32] without changes in condensation time. Coupling of the 5′-terminal monomer 5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-(*N*,*N*-diisopropyl-β-cyanoethyl)-N^3-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}methylenethymidine phosphoramidite (**29**) and 6-(bis(4-methoxyphenyl)(4-(methylsulfinyl)phenyl)methylamino)hexyl *N*,*N*-diisopropyl-β-cyanoethyl phosphoramidite (**37**) (Metkinen), capping as well as the oxidation step were performed manually. After coupling cycles, detritylation and washing with acetonitrile, the column was detached from the DNA synthesizer and dried under vacuum (10 min). Modified monomer **29** (16 mg, 0.015 mmol) was dissolved in anhydrous acetonitrile (42 μL) followed by addition of tetrazole (0.5 M, 2.72 mg, 77.6 μL). Monomer **37** (13 mg, 0.019 mmol) was dissolved in anhydrous acetonitrile (25 μL) followed by addition of tetrazole (0.5 M, 3.33 mg, 95 μL). A solution of activated monomers was applied to the column and the coupling reaction was performed for 30 min. The column was washed with anhydrous acetonitrile (2 × 5 mL) followed by drying under high vacuum. The oxidation step was performed using a standard oxidizing solution containing iodine in tetrahydrofuran/pyridine/water (13 : 6 : 1) (0.1 M, 1 mL) for 30 seconds, followed by washing with acetonitrile (2 × 5 mL) and drying with a stream of argon. The capping step

was done with a standard capping mixture of 1 M acetic anhydride in tetrahydrofuran/pyridine (1 : 8 v/v) (0.5 mL) and 0.5 M 4-dimethylaminopyridine in tetrahydrofuran (0.5 mL) for 30 seconds, followed by washing of the column with acetonitrile (1 × 5 mL) and drying under high vacuum (5 min). Oligonucleotides were then cleaved from the support by 1 h incubation with concentrated aqueous ammonia solution (30%, 1 mL) at room temperature then the base deprotection was achieved by incubation of the resultant solution at 55 °C for 18 h. The solution of crude 5′-*O* dimethoxytrityl/dimethoxymethylsulfinyltrityl protected oligonucleotides **38–41** was degassed with a stream of argon, and evaporated to dryness under vacuum, and then re-dissolved in water. The resultant solutions of crude oligonucleotides were purified using a HPLC C18 reverse phase column (PR-HPLC) under conditions described in RP-HPLC analysis. Fractions containing the desired product were collected, and the buffer was evaporated under vacuum. The residue was co-evaporated with 95% ethyl alcohol to remove triethylammonium bicarbonate (TEAB), then detritylation was performed using a 80% acetic anhydride (1 mL) at room temperature for 20 min. Next the acetic solution was evaporated to dryness under vacuum and the totally deprotected oligonucleotides were purified by RP-HPLC under the same conditions as above. Fractions containing the desired product were collected, and the buffer was evaporated under vacuum. The residue was co-evaporated with 95% ethyl alcohol to remove TEAB then was dissolved in deionized water and lyophilized. All oligonucleotides were stored as a dry solid at −20 °C. When needed, they were re-dissolved in water, stored as frozen solution, and re-lyophilized as soon as possible. The molecular mass of the oligomers was confirmed by MALDI-TOF mass spectrometry (MALDI-TOF MS, Table 1).

3.3 Circular dichroism (CD) measurements

CD Spectra were recorded with a CD6 dichrograph (Jobin Yvon, Longjumeau, France) in a cell with a 0.5 mm path length, 2 nm bandwith, and 1–2 s integration time. Samples for CD measurements were prepared by dissolving the tested compounds in ethyl alcohol (95%, 1 mL) to give a solution of 1.5 mM (for T, dC, dA, **20**, **22**, **24**) and 0.3 mM (for dG, **27**). The spectra (195–350 nm) were recorded at 25 °C. Each spectrum was smoothed with a 15 or 25-point algorithm (included in the manufacturer's software version 2.2.1).

3.4 X-ray crystallography

Crystals of N^3-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}-methylenethymidine (**20**) were obtained by slow evaporation from its aqueous ethanolic solutions. The measurements of the crystal were performed on a SuperNova (Agilent) diffractrometer with Atlas CCD detector. Data reduction was performed using a DENZO/SCALEPACK.[45] The structure was solved by direct methods using SHELXS97[46] and refined using a SHELXL97.[47] H atoms were treated by a mixture of independent and constrained refinement. Summary of the X-ray data and refinement is given in Table 2. Crystallographic data for the structure reported in this paper have been deposited at the Cambridge Crystallographic Data Centre and allocated with the deposition number: CCDC 1010195.

3.5 High-performance liquid chromatography using a CoulArray electrochemical detector

An HPLC-EC system consisted of two solvent delivery pumps operating in the range of 0.001−9.999 mL min^{-1} (Model P580 Dionex, Germering, Germany), a Hypersil Gold reversed-phase column (250 × 4.6 mm, 5 μm particle size; Thermo Scientific, Runcorn, UK) and a CoulArray electrochemical detector (Model 5600A, ESA, Chelmsford, MA, USA). The electrochemical detector includes two low volume flow-through analytical cells (Model 6210, ESA). Each analytical cell consisted of four carbon porous working electrodes, a palladium electrode as a reference electrode and auxiliary electrodes. Analyses were run at room temperature. The mixture of four conjugates **33–36** (18.3 μL) was injected manually. Mobile phase consisted of A: acetonitrile/water (2 : 98 v/v) containing ammonium acetate (0.05 M, pH 5.5) and B: acetonitrile/water (60 : 40 v/v) containing ammonium acetate (0.05 M, pH 5.5). HPLC conditions: 20 minutes from 0% B to 100% B, 5 minutes 100% B, 5 minutes from 100% B to 0% B. Flow rate 1 mL min^{-1}. The potentials 300, 375, 450, 525, 600, 675, 750, 825 mV were applied on porous graphite working electrodes.

4. Conclusions

We developed a general method for the synthesis of four canonical nucleosides: T, dC, dA and dG, modified with an *ortho*-carborane cluster in both electroneutral *closo*- and electronegative, redox active *nido*-forms. The circular dichroism and electrochemical characteristics of the conjugates were assessed. Circular dichroism spectra revealed the effect of the carborane cluster on the nucleoside conformation in conjugates **22**, **24**, and **27** with the exception of thymidine–carborane conjugate **20** compared to the corresponding unmodified nucleosides. High-performance liquid chromatography coupled with electrochemical detection (CoulArray) was used for rapid, sensitive, and parallel determination of all four nucleosides **33–36** labeled with a 7,8-dicarba-*nido*-undecaborate group. The X-ray structure of the thymidine–carborane conjugate **20** was established providing the first example of X-ray analysis of a nucleoside-boron cluster conjugate. The conjugates **21**, **23**, **25**, **28** were then transformed into nucleoside phosphoramidite derivatives suitable for modified DNA oligonucleotides. Several DNA oligomers containing *nido*-carborane cluster (**38–41**) have been obtained using modified monomer **29** as an example. Boron cluster modification can affect properties of the DNA and RNA oligomers in such qualities as solubility, charge (in the case of a negatively charged 7,8-dicarba-*nido*-undecaborate group), binding affinity towards a complementary DNA/RNA strain, resistance to nucleolytic digestion or add new properties such as specific redox activity or infrared absorption (B–H stretching) in the range where the organic part of the molecule is transparent. These properties facilitate application of oligonucleotide conjugates bearing

boron clusters as molecular probes in medical diagnostics, in therapeutics, as potential therapeutic nucleic acids or prospective new, DNA-based materials for nanotechnology.

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