HIGHLY LIPOPHILIC *p*-CARBORANE-MODIFIED ADENOSINE PHOSPHATES+

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The method was developed for the synthesis of biologically important adenosine phosphates, AMP, cAMP and ATP modified with *p*-carborane cluster – a highly lipophilic pharmacophore. The adenosine phosphates modified with *p*-carborane are characterized by increased stability in human blood plasma and much more higher lipophilicity than that of the unmodified phosphates. ATP analog **4** bearing *p*-carborane cluster is not a Taq polymerase substrate and most probably not the polymerase inhibitor. These properties may have clinical implications.

Keywords: Carboranes; Pharmacophores; Nucleosides; Nucleotides; Adenosine phosphates; AMP; cAMP; ATP; Lipophilicity; PCR; Nucleoside triphosphates; Polymerase incorporation.

All three types of adenosine phosphates: adenosine 5′-monophosphate (AMP), adenosine cyclic-3′,5′-monophosphate (cAMP) and adenosine 5′-triphosphates (ATP) are important small biomolecules. To better understand the biological processes involving these molecules, a plethora of various modified analogs of AMP, cAMP and ATP have been prepared. cAMP analogs have been synthesized for mapping of cAMP binding proteins and as enzyme substrates or inhibitors[1]. They are also studied as prodrugs of antiviral or antitumor nucleosides and nucleotides. Modified ATP and other nucleoside triphosphates have received much attention in search for potential diagnostic and therapeutic agents, as well as compounds for studying numerous biochemical and pharmacological processes[1,2].

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Adenosine phosphates that serve as chemotherapeutics have a short half-life, unsatisfactory cell permeability and often unsatisfactory specificity and selectivity. Recently, there has been a growing interest in the application of carboranes (boron clusters containing carbon) as modifying entities for biologically active molecules[3,4]. Carboranes such as dicarba-*o*-dodecaborane (1,2-$C_2B_{10}H_{12}$) and dicarba-*p*-dodecaborane (1,12-$C_2B_{10}H_{12}$) are characteristic of spherical geometry, high chemical and biological stability and exceptional hydrophobic nature[3]. Therefore, they are used as hydrophobic pharmacophores in designs based on the hydrophobic nature of carboranes[3,4]. Several antiviral nucleoside drugs[5], DNA oligonucleotides[6], and inhibitors of some enzymes, which take part in the metabolism of steroid hormones have been modified with borane cages[7–9]. In this communication, we present a general method for the synthesis of cAMP, AMP and ATP modified with the lipophilic *p*-carboran-1-yl group at the 2′ position of the nucleoside unit and discuss some pertinent properties of these new molecules.

EXPERIMENTAL

Materials

Adenosine was purchased from Avocado Lancaster (Mühlheim am Main, Germany). Preparative column chromatography was performed on silica gel 230–400 mesh obtained from Sigma–Aldrich (Steinheim, Germany). TLC was performed on silica gel F254 plates purchased from Sigma–Aldrich (Steinheim, Germany). Acrylamide was purchased from Sigma Co. (Deisenhofen, Germany), and urea from POCh (Lublin, Poland). Thermus aquaticus (Taq) DNA polymerase was from Gibco BRL (Germany). Solvents were purchased in the highest available quality. UV measurements (λ, nm) were performed on a GBC Cintra10e UV-VIS spectrometer (Dandenong, Australia). ^{1}H and ^{31}P NMR spectra (δ, ppm; *J*, Hz) were recorded on a Bruker Avance DPX 250 spectrometer operating at 250.13 and 101.26 MHz, respectively. Tetramethylsilane and 85% H_3PO_4 were used as external standards for ^{1}H and ^{31}P NMR, respectively. Fast atom bombardment (FAB, 4-nitrobenzyl alcohol) mass spectra were recorded on a Finnigan MAT spectrometer (Bremen, Germany), Electrospray ionization (ESI, trihydroxyacetophenone/ammonium citrate) spectra were recorded on a ZQ Waters/micromass mass spectrometer (Manchester, U.K.). C18 reverse-phase column with Econosil C18 5 μm, 4.7 × 250 mm was obtained from Alltech Associates Applied Science Ltd. (Carnforth, U.K.). PCR was performed using thermal cycler UNO II (Biometra, Germany). Polyacrylamide gel electrophoresis was performed using a C.B.S. Scientific Co. apparatus (Del Mar (CA), U.S.A.). Molecular weight marker 100–1000 base pairs was from Promega (New England Biolabs, U.S.A.).

Syntheses

2′-O-{[3-(p-Carboran-1-yl)propoxy]methyl}adenosine (**1**) was synthesized as previously described in[10,11].

2′-O-{[3-(p-Carboran-1-yl)propoxy]methyl}adenosine 5′-monophosphate (**2**)[12]. 2′-*O*-{[3-(*p*-Carboran-1-yl)propoxy]methyl}adenosine (**1**; 50 mg, 0.1 mmol) was dissolved in freshly distilled triethyl phosphate (1 ml). The resulting solution was cooled to 0 °C and then $POCl_3$ (23 μl, 0.25 mmol) was added. Then the mixture was stirred at 0 °C. The reaction progress was monitored by TLC (isopropyl alcohol/water/aqueous ammonia, 7:1:2). After completion of the reaction (usually 1.5–2 h), 1 M triethylammonium hydrogencarbonate buffer (TEAB, pH 7.5, 2 ml) was added and the mixture was concentrated by evaporation. The crude product **2** was purified by FPLC using a HiPrep 16/10 DEAE FF column (Et_3HN^+ form, Pharmacia®), equilibrated with TEAB. Chromatography was performed with a linear gradient from 0.05 to 0.1 M of TEAB. The fractions containing the product were combined, concentrated under vacuum and then co-evaporated with ethanol (3 × 3 ml) to remove traces of buffer. Yield of **2**: 29%. UV-VIS (H_2O), λ: 260. ^{31}P NMR (101 MHz, D_2O): 3.96. ^1H NMR (250 MHz, D_2O): 8.59 (s, 1 H, H-8); 8.25 (s, 1 H, H-2); 6.15 (d, 1 H, J = 7.0, H-1′); 4.75 (s, 2 H, OCH_2O); 4.34–4.39 (m, 2 H, OCH_2); 4.33 (s, 1 H, NH_2); 3.89–4.04 (m, 2 H); 3.95–4.01 (m, 1 H, H-2′); 3.15–3.24 (m, 1 H, H-3′); 2.98–3.07 (m, 1 H, H-4′); 2.88–2.96 (m, 1 H, H-5′); 1.61–1.82 (bs, 1 H, OH); 1.15–1.41 (m, 6 H); 0.93 (m, 2 H, $OCH_2CH_2CH_2$); 0.62 (m, 2 H, $OCH_2CH_2CH_2$). FAB-MS, *m/z*: 560.4 [M – H]. $C_{16}H_{32}B_{10}N_5O_8P$ (calculated 561.5). FPLC-ion exchange: R_t 74 min; RP-HPLC: R_t 41 min; TLC (isopropyl alcohol/water/aqueous ammonia, 7:1:2): R_F 0.62.

2′-O-{[3-(p-Carboran-1-yl)propoxy]methyl}adenosine 3′,5′-cyclic monophosphate (**3**)[13]. Nucleoside **1** (50 mg, 0.1 mmol) was dissolved in freshly distilled triethyl phosphate (1 ml). The resulting solution was cooled to 0 °C, then $POCl_3$ (23 μl, 0.25 mmol) was added, and the mixture was stirred at 0 °C. The reaction was monitored by TLC (isopropyl alcohol/water/aqueous ammonia, 7:1:2). After completion of the reaction (usually 1.5–2 h), the reaction mixture was added to a stirred solution of 0.08 M KOH in H_2O/MeCN (4:6, 90 ml) at 0 °C. The mixture was immediately neutralized with 0.1 M HCl and then evaporated. The crude product **3** was purified by FPLC using a HiPrep 16/10 DEAE FF column (Et_3HN^+ form, Pharmacia®), equilibrated with TEAB (pH 7.5). Chromatography was performed with a linear gradient from 0.05 to 0.1 M of TEAB. Fractions containing the product were combined, concentrated under vacuum and then co-evaporated with ethanol (3 × 3 ml) to remove traces of buffer. Yield of **3**: 22%. UV-VIS (H_2O), λ: 259. ^{31}P NMR (101 MHz, D_2O): –1.83. ^1H NMR (250 MHz, D_2O): 8.20 (s, 1 H, H-2); 6.13 (s, 1 H, H-1′); 4.78 (s, 2 H, OCH_2O); 3.53 (bs, 1 H, H-2′); 3.39–3.31 (m, 1 H, H-3′); 3.23–3.15 (m, 1 H, H-4′); 3.10–3.02 (m, 2 H, $OCH_2CH_2CH_2$); 2.92, 2.89 (2d, 2 H, J = 7.25, 8.25, H-5′); 1.61 (bs, 1 H, OH); 1.33–1.24 (m, 6 H); 0.95–0.92 (m, 2 H, $OCH_2CH_2CH_2$); 0.66–0.59 (m, 2 H, $OCH_2CH_2CH_2$). FAB-MS, *m/z*: 542 [M – H]. $C_{16}H_{30}B_{10}N_5O_7P$ (calculated 543.5). FPLC-ion exchange (the conditions as above): R_t 83 min; RP-HPLC: R_t 46 min; TLC (isopropyl alcohol/water/aqueous ammonia, 7:1:2): R_F 0.75.

2′-O-{[3-(p-Carboran-1-yl)propoxy]methyl}adenosine 5′-triphosphate (**4**)[14]. Nucleoside **1** (10 mg, 0.02 mmol) was dissolved in freshly distilled triethyl phosphate (400 μl) and molecular sieve (4Å) was added. The solution was cooled to 0 °C and then $POCl_3$ (4.6 μl, 0.05 mmol) was added, and the mixture was stirred at 0 °C for 2 h. The reaction was monitored by TLC (isopropyl alcohol/water/aqueous ammonia, 7:1:2). To the reaction mixture was then added a solution of tributylammonium diphosphate (500 μl, 0.2 mmol) in DMF followed by

tributylamine (400 μl). After 1 min, the reaction was terminated by addition of aqueous 1 M TEAB (8 ml, pH 7.5). The resulting mixture was concentrated under vacuum then transferred into an ion exchange column. The crude product was purified by FPLC using a HiPrep 16/10 DEAE FF column (Et_3HN^+ form, Pharmacia®), equilibrated with TEAB (pH 7.5). Chromatography was performed with linear gradient from 0.05 to 0.1 M of TEAB. Fractions containing the product were combined, concentrated under vacuum and then co-evaporated with ethanol (3 × 3 ml) to remove traces of buffer. Yield of **4**: 33%. UV-VIS (H_2O), λ: 260. ^{31}P NMR (101 MHz, D_2O): –22.70 (d, H-β); –11.10 (s, H-α); –10.30 (d, H-γ). ^1H NMR (250 MHz, D_2O): 8.50 (s, 1 H, H-8); 8.25 (s, 1 H, H-2); 5.80 (s, 1 H, H-1′); 4.75 (s, 2 H, OCH_2O); 3.27–3.35 (m, 1 H, H-4′); 3.12–3.24 (m, 2 H, $OCH_2CH_2CH_2$); 2.98–3.04 (m, 1 H, H-5′); 1.60 (bs, 1 H, OH); 1.23–1.26 (m, 2 H, $OCH_2CH_2CH_2$); 0.88–0.93 (m, 2 H, $OCH_2CH_2CH_2$). ESI-MS (negative mode): 719.9 $[M - 2H]^{2-}$. $C_{16}H_{34}B_{10}N_5O_{14}P_3$ (calculated 721.5). FPLC-ion exchange: R_t 121 min; RP-HPLC: R_t 38 min; TLC (isopropyl alcohol/water/aqueous ammonia 7:1:2): R_F 0.08.

Methods

HPLC analysis of 2′-O-{[3-(p-carboran-1-yl)propoxy]methyl}adenosine phosphates **2**–**4** *and unmodified AMP* (**2a**)*, cAMP* (**3a**) *and ATP* (**4a**). HPLC analysis was performed in a Hewlett–Packard 1050 system, using Altech Econosil C18 5 μm, 4.7 × 250 mm column. All analyses were run at room temperature. HPLC conditions were as follows: 5 min A, 50 min from 0 to 100% B, 5 min from 100 to 0% B. Buffer A contained 0.1 M TEAB (pH 7.0) in a mixture of acetonitrile/water (1:49, v/v), buffer B contained 0.1 M TEAB (pH 7.0) in a mixture of acetonitrile/water (3:2, v/v). Flow rate 0.5 ml/min, UV detection at λ 266 nm.

Partition coefficient (P) measurement[15]. To 1 ml of a 100 μM solution of compound **2**–**4** and **2a**–**4a** in deionized H_2O containing 5% of CH_3OH in 2-ml Eppendorf tube, octan-1-ol (1 ml) was added. The resulting mixture was shaken vigorously at room temperature (20–22 °C) for 1 h and then the mixture was left standing for 1 h for phase separation. Each sample was subsequently centrifuged at 13,000 rpm for 5 min, then 0.9 ml of H_2O or organic solution was transferred into 1-ml UV cell and the UV absorption at λ 260 nm was measured.

Circular dichroism (CD) of compounds **2**–**4** *and their unmodified counterparts AMP* (**2a**)*, cAMP* (**3a**) *and ATP* (**4a**). CD spectra were recorded on a CD6 dichrograph (Jabin–Yvon, Longjumeau, France) using cell with 5-mm path length, 2-nm bandwidth, and 1–2 s integration time. The same molar absorption coefficients for all compounds **2**–**4** and **2a**–**4a** as for AMP was assumed[16]: $\varepsilon = 15.4 \times 10^3$. Samples for CD measurements were prepared by mixing 50 μl aliquots of concentrated stock solutions in methanol with 0.95 ml of 4 mM phosphate buffer (pH 7) containing 0.9 mM KCl, 100 mM NaCl, 0.5 mM EDTA (1 ml) to give final concentration of compounds **2**–**4** and **2a**–**4a** from 6.2×10^{-5} to 1.1×10^{-4} mol l^{-1} and 5% CH_3OH. The spectra (200–350 nm) were recorded at 25 °C. Each spectrum was smoothed with a 15- or 25-point algorithm (included in the manufacturer software, version 2.2.1).

Assay for enzymatic stability of 2′-O-{[3-(p-carboran-1-yl)propoxy]methyl}adenosine phosphates **2**–**4** *in human blood plasma and its comparison with unmodified AMP* (**2a**)*, cAMP* (**3a**) *and ATP* (**4a**)[17]. Plasma was separated from human blood containing 0.38% sodium citrate by centrifugation at 16,000 rpm for 5 min. Plasma aliquots were carefully withdrawn, leaving the untouched packed cells. The samples of compounds **2**–**4** and **2a**–**4a** were incubated in 95% plasma (total volume 400 μl) at final concentration of the compounds 0.5 mM (total amount of compound 0.2 μmol). Thus, 20 μl of 10 mM stock solution of a compound was added to 380 μl of 100% human blood serum and then the whole was incubated at 37 °C. At various

times (usually 0, 0.25, 0.5, 1, 2, 4, and 8 h), 50 μl aliquots were withdrawn (0.025 μmol of the compound per aliquot), diluted with 50 μl of water. Then the enzymatic reaction was quenched by addition 230 μl of methanol, and cooled at –20 °C for 30 min. The samples were centrifuged at 12,000 rpm for 10 min, and the supernatant was concentrated to 50 μl. Then 10 μl aliquots were taken and analyzed by TLC; as eluting solvent system i-PrOH/aqueous NH_3/H_2O (7:1:2) and isobutyric acid/H_2O/aqueous NH_3 (66:33:1) were used for compounds **2**, **3**, **2a**, **3a** and **4**, **4a**, respectively. The TLC plates were visualized at 302 nm and analyzed using a FluorChem™ Imaging System (Alpha Innotech Co., San Leandro (CA), U.S.A.) and FluorChem 8800 computer program. The extent of hydrolysis was assessed by measuring the amount of the starting compound.

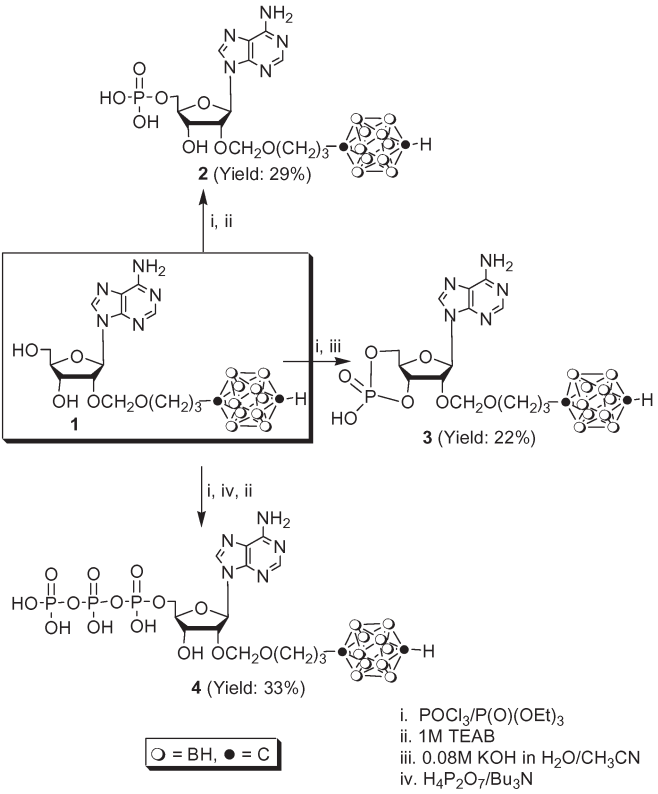
PCR and PAGE analysis. The DNA samples were amplified in 50 μl of the reaction mixture containing 0.5 μg DNA isolated from MRC-5 cells infected with human cytomegalovirus (HCMV) AD169, 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 2.5 mM of each dNTP, 50 pmol of primers P12 and M16 targeted a fragment (526 bp) of US14 genes of HCMV AD 169 [18] and 2.5 units of Taq DNA polymerase. DNA was amplified in a thermal cycler UNO II using 30 cycles as follows: initial denaturation of the template at 95 °C for 150 s, denaturation at 94 °C for 30 s, annealing at 55 °C for 120 s, and extension at 72 °C for 120 s. The PCR experiment was performed with three sets of dNTP mixtures differing in contents of 2′-*O*-{[3-(*p*-carboran-1-yl)propoxy]methyl}adenosine 5′-triphosphate (**4**): A molecular weight marker; B dATP, dTTP, dCTP, dGTP; C modified dATP (2′-*O*-[3-(*p*-carboran-1-yl)-propoxy]methyl (2′-CBPM) ATP: **4**) and unmodified dTTP, dCTP, dGTP; D 1:1 modified dATP (2′-CBPM ATP: **4**) and unmodified dATP and unmodified dTTP, dCTP, dGTP (Fig. 1). An aliquote (10 μl) of the postamplification mixture was analyzed and identified by 7% polyacrylamide gel electrophoresis after staining with ethidium bromide (3%) then followed by photography using C.B.C. Scientific CO (U.S.A.) and Visioneer Paperport program, version 3.0. The length of the amplicons was established by comparison with the molecular weight marker 100–1000 base pairs.

RESULTS AND DISCUSSION

Most of the currently described carborane-modified nucleosides belong to the pyrimidine series[5,9]. Though purine nucleosides, such as adenosine and guanosine, play an important role in the cellular metabolism, they have received less attention due to the difficulties in the synthesis of their conjugates with boron clusters. We have recently developed a method for the synthesis of the adenosine derivative **1** with a *p*-carborane cage attached to a sugar residue at 2′ position[10]. This approach formed a basis for the synthesis of adenosine phosphates **2**–**4** modified with borane cage and for the development of general method of preparing nucleosides bearing carborane pharmacophore at the 2′ position[11].

Synthesis of the 2′-*O*-{[3-(*p*-carboran-1-yl)propoxy]methyl}adenosine (**1**; 2′-CBPM A) mono- and triphosphates was performed utilizing standard Yoshikawa's procedure for unprotected nucleoside phosphorylation with phosphorus oxychloride (Scheme 1)[12]. Chlorophosphate intermediate ob-

tained following the phosphorylation step was hydrolyzed, cyclized or transformed into triphosphate of nucleoside **1**. Briefly, modified nucleoside **1** was dissolved in triethyl phosphate, cooled and then treated with an excess of phosphorus oxychloride[12]. After completion of the reaction, further procedure was performed in a different manner for each modified nucleoside **2**–**4**. 2′-*O*-{[3-(*p*-Carboran-1-yl)propoxy]methyl}adenosine 5′-monophosphate (**2**) was obtained by simple hydrolysis of the phosphorus-oxychloride-treated nucleoside with TEAB. In order to obtain 2′-*O*-{[3-(*p*-carboran-1-yl)propoxy]methyl}adenosine 3′,5′-cyclic monophosphate (**3**), the phosphorus-oxychloride-treated nucleoside was added to a stirred solution of potassium hydroxide in a mixture of water and acetonitrile at 0 °C.



SCHEME 1
Synthesis of 2′-*O*-{[3-(*p*-carboran-1-yl)propoxy]methyl}adenosine 5′-mono-, cyclic-3′,5′-mono- and 5′-triphosphates (**2**–**4**)

The mixture was immediately neutralized with aqueous HCl [13]. 2′-*O*-{[3-(*p*-Carboran-1-yl)propoxy]methyl}adenosine 5′-triphosphate (**4**) was obtained after direct reaction between the phosphoryl-oxychloride treated nucleoside and a solution of tributylammonium diphosphate in anhydrous dimethylformamide followed by tributylamine[14]. The reaction was terminated by addition of aqueous TEAB. Crude products **2**–**4** were then purified by FPLC in an ion exchange column. Nucleoside monophosphate **2** was formed as a major side-product during synthesis of both **3** and **4**. The purity of the 2′-CBPM adenosine phosphates **2**–**4** was checked by HPLC (Table I) and ^{31}P NMR, and the integrity of the obtained products was confirmed by mass spectrometry.

The carboranyl modification can provide the original compounds with the ability to interact hydrophobically with other molecules such as proteins or lipids of cellular membranes. This assumption is in accord with the observation of a strong effect of carboranyl modification on the affinity of modified phosphates **2**–**4** for the HPLC C18 reverse phase and on partition coefficient.

The RP-HPLC retention time (R_t) under the same conditions was two- to fourfold higher for 2′-*O*-(*p*-carboran-1-yl)-modified adenosine phosphates **2**–**4** than for unmodified compounds. The lipophilicity of adenosine phosphates **2**–**4** measured as partition coefficient (*P*) values increased as ex-

TABLE I
Comparison of lipophilicity of 2′-*O*-{[3-(*p*-carboran-1-yl)propoxy]methyl}adenosine phosphates

Compound	HPLC[a] R_t, min	Partition coefficient[b] log *P*
2′-CBPM AMP (**2**)	41.0	–1.09 (±0.03)
AMP (**2**)	9.1	–4.20[c]
2′-CBPM cAMP (**3**)	46.0	–0.30 (±0.08)
cAMP (**3**)	21.6	–3.80[c]
2′-CBPM ATP (**4**)	38.0	–1.29 (±0.07)
ATP (**4**)	12.2	–4.48[c]

[a] Altech Econosil C18 5 μm, 4.6 × 250 mm column, at room temperature, gradient elution: 5 min A, 50 min 0→100% B, 5 min 100→0% B. Buffer A: 0.1 M TEAB (pH 7.0) in acetonitrile/water (1:49, v/v), buffer B: 0.1 M TEAB (pH 7.0) in acetonitrile/water (3:2, v/v). Flow rate 0.5 ml/min, UV detection was performed at λ 266 nm. [b] The ratio of the concentration of the compound present in octan-1-ol to that in the aqueous phase. [c] Calculated: **2a**, –3.10; **3a**, –2.5; **4a**, –5.5 (http://www.vcclab.org/).

pected in the following order: **4** [ATP 2′-CBPM, $P = 5.0 \times 10^{-2}$ (± 0.01)] < **2** [AMP 2′-CBPM, $P = 8.0 \times 10^{-2}$ (± 0.01)] < **3** [cAMP 2′-CBPM, $P = 5.0 \times 10^{-1}$ (± 0.07)]. In all cases it was by three orders of magnitude higher than that of unmodified counterparts (**2a**, $P = 5.9 \times 10^{-5}$; **3a**, $P = 1.6 \times 10^{-4}$; **4a**, $P = 3.3 \times 10^{-5}$). Table I collects the retention times (R_t) and partition coefficients (P) for adenosine phosphates **2**–**4** and **2a**–**4a**. The curves shown in Fig. 2 illustrating log P/R_t relationship may serve for assigning lipophilicity to structurally similar boron-cluster-modified nucleoside phosphates based on HPLC mobilities[19].

Physicochemical properties and biological activities of the compounds are affected by their chemical structure, stereochemistry, including absolute configuration of their chiral centers and conformation of the molecule. Since the absolute configuration at chiral centers of the nucleoside unit in modified phosphates **2**–**4** has been considered the same as in unmodified counterparts **2a**–**4a**, it was of interest to know whether the conformation of the adenosine phosphates containing the 2′-CBPM group, in particular syn/anti nucleobase orientation, was affected by the modification. Either form is allowed although the anti configuration is more common for pyrimidine nucleosides. Purine nucleosides are in syn and anti conformation equilibrium[20].

Since CD is most affected by the restricted rotation around the glycosidic bond, the technique has been used to determine whether 2′-*O*-(*p*-carboran-1-yl)-modified nucleosides have the same or different conformation as their un-

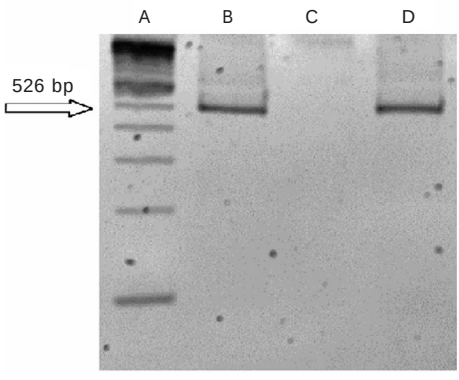


FIG. 1
PAGE analysis of PCR products: A molecular weight marker 100–1000 bp; B dATP, dTTP, dCTP, dGTP; C **4** and unmodified dTTP, dCTP, dGTP; D 1:1, **4** and unmodified dATP dTTP and dCTP, dGTP. 7% polyacrylamide denaturating gel containing urea (7 M). The samples were visualized by staining with ethidium bromide

modified counterparts. CD spectrum of 2′-*O*-{[3-(*p*-carboran-1-yl)propoxy]-methyl}adenosine (**1**) recorded under the same conditions is similar to unmodified adenosine **1a** [11] in terms of its shape and molecular ellipticity values in the range below 240 and above 280 nm. In contrast, the spectra of modified compounds **2**–**4** between 240 and 280 nm with maximum λ around 260 nm were mirror-images of the spectra of corresponding unmodified adenosine phosphates **2a**–**4a** (Fig. 3). Since CD spectra of AMP (**2a**), cAMP (**3a**) and ATP (**4a**) recorded in pH 7.0 buffer are identical, their conformation in this solvent must be the same[21]. Consistently, such reasoning leads to the conclusion that conformation of the 2′-*O*-(*p*-carboran-1-yl)-modified adenosine phosphates **2**–**4** (or population of conformers) is opposite to that of the unmodified counterparts. This feature, together with a chemical modification itself and its lipophilic characteristics, may be responsible for different biochemical properties of modified and unmodified adenosine phosphates **2**–**4** and **2a**–**4a**.

To test this hypothesis, a preliminary study on stability of nucleotides **2**–**4** and **2a**–**4a** in human plasma was performed. A noticeable effect of the 2′-*O*-(*p*-carboran-1-yl)-modification on AMP, cAMP and ATP resistance to degradation was observed. After 8 h of incubation in 95% plasma, the unmodified nucleotides **2a** and **4a** were hydrolyzed approximately with 70–80% conversion with $T_{1/2}$ = 4.4 and 6.4 h, respectively. The unmodified

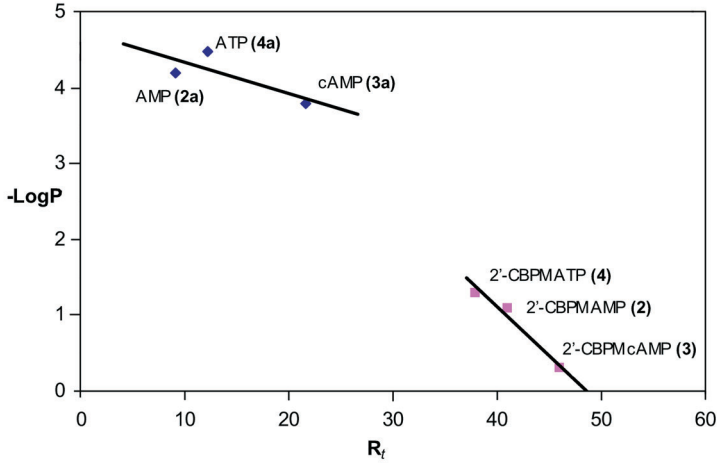


FIG. 2
Log P/R_f relationship for 2′-*O*-[3-(*p*-carboran-1-yl)propoxy]methyl modified (**2**–**4**) and unmodified (**2a**–**4a**) adenosine 5′-mono-, cyclic-3′,5′-mono- and 5′-triphosphates

nucleotide **3a** appeared to be more resistant to degradation, as after 8 h incubation it was degraded to 10% only, with $T_{1/2}$ = 27.5 h. The modified nucleotides **2** and **4** have appeared to be much more stable than the unmodified counterparts; after 8 h of incubation, less than 5% were hydrolyzed, with $T_{1/2}$ = 54.1 and 45.9 h, respectively. The $T_{1/2}$ for modified nucleotide **3** was 41.5 h and it was only about twice higher than that for unmodified nucleotide **3a**. The nucleotide stability in human plasma increased in the order **2a** (unmodified AMP) < **4a** (unmodified ATP) << **3a** (unmodified cAMP) <<< **3** (2′-CBPM cAMP) < **4** (2′-CBPM ATP) < **2** (2′-CBPM AMP). The observed shielding effect of 2′-CBPM modification on degradation of adenosine phosphates and on an increase in lipophilicity is noteworthy and may have practical implications.

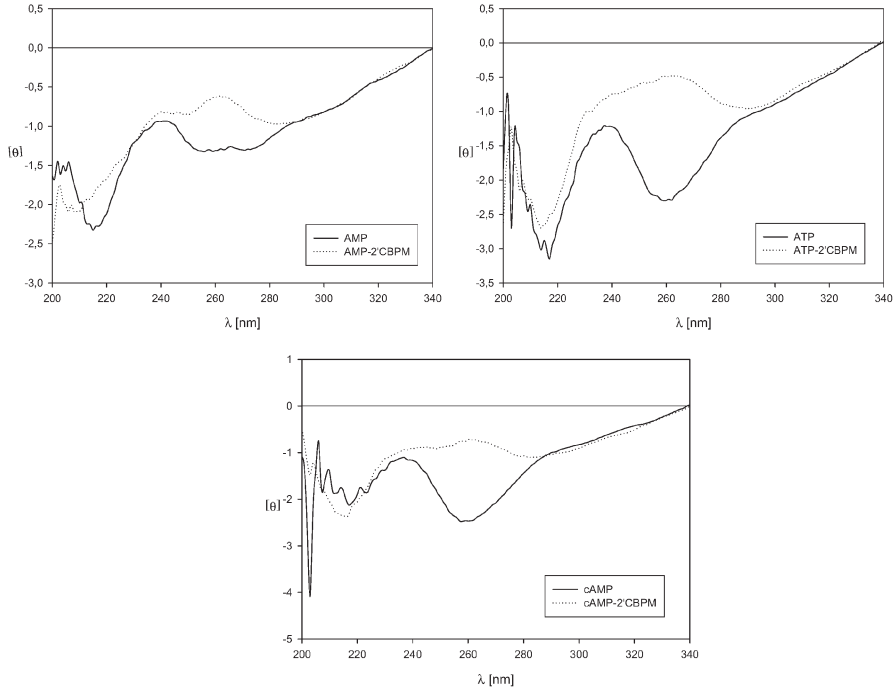


FIG. 3

CD spectra of 2′-*O*-{[3-(*p*-carboran-1-yl)propoxy]methyl}adenosine 5′-monophosphate (**2**); 2′-*O*-{[3-(*p*-carboran-1-yl)propoxy]methyl}adenosine cyclic-3′,5′-monophosphate (**3**), 2′-*O*-{[3-(*p*-carboran-1-yl)propoxy]methyl}adenosine 5′-triphosphate (**4**) and unmodified AMP (**2a**), cAMP (**3a**) and ATP (**4a**)

In addition, further biological properties of 2′-CBPM-modified ATP (**4**) were evaluated due to its ability to serve as a substrate for DNA Taq polymerase in PCR. After 30 cycles, no amplification of target 526 base pairs fragment of US14 genes of HCMV AD 169 was detected by PAGE for modified dATP used instead of natural ATP in solutions of four nucleotides containing unmodified dATP, dTTP, dCTP and dGTP. In control experiments with commercial solutions of four nucleotides (dATP, dTTP, dCTP and dGTP) or solutions containing modified dATP and unmodified dATP, dTTP, dCTP and dGTP (1:1), unrestrained amplification was observed (Fig. 1) which shows that **4** is most probably not Taq polymerase inhibitor. Steric hindrance caused by the bulkiness of the [(*p*-carboran-1-yl)propoxy]methyl group, its hydrophobic nature and the linking moieties between the carborane cage and sugar, may affect the incorporation of **4** into DNA. In addition, potential modifying group enzyme interactions have to be considered[22].

In conclusion, a method for modification of biologically important adenosine nucleotides, AMP, cAMP and ATP with carborane group at the 2′ position was proposed. The carborane cages are characterized by extremely high lipophilicity and therefore can be used as hydrophobic components in biologically active molecules rendering them able to interact hydrophobically with other molecules such as proteins or lipids of cellular membranes, thus improving potentially their ability to penetrate lipid bilayers and cellular uptake[23]. Indeed, 2′-*O*-(*p*-carboran-1-yl)-modified adenosine phosphates are characterized by dramatically increased lipophilicity and markedly higher stability in human serum. The proposed method forms a basis for general approach toward synthesis of polar nucleoside phosphates with increased lipophilicity. Work on methods for attachment of lipophilic boron clusters at the 6, 8 and 2 positions of adenosine is in progress in our laboratory[24]. The data shown in this paper represent the initial studies of indepth of the approach that might have clinical implications.

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