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General method for the synthesis of 2′-*O*-carboranyl-nucleosides

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Abstract—The carboranyl cage is a new modifying entity for nucleosides, DNA oligonucleotides, and other biomolecules. Herein, the first reliable method for the synthesis of nucleosides modified with a carborane cluster at the 2′-position is described.

The initial stimulus for the design and synthesis of boron-containing nucleosides was their potential application as boron-rich carriers for boron neutron capture therapy (BNCT).[1–4] More recently, it has been demonstrated that carboranes can be used to enhance hydrophobic interactions between pharmaceuticals and their receptors and to increase the in vivo stability of compounds that are normally rapidly metabolized. Carborane modification may also facilitate cellular uptake, increasing lipophilicity and penetration through cellular membranes of compounds bearing carboranes. Several antiviral nucleosides modified with carborane cages have been synthesized and their biological properties studied.[2] Carborane clusters have also been explored as new and versatile modifying entities for DNA oligonucleotides.[5,6] Thus, carborane-containing nucleosides are desirable precursors for the preparation of modified DNA oligomers. Nearly all the carborane-modified nucleosides described so far belong to the pyrimidine series and are thymidine or uridine derivatives containing the modification confined within the nucleic base.[3,7–9]

The only method for attachment of a carborane group to the 2′-position of a nucleoside unit is that described by Anisuzzaman for the synthesis of 3′-*O*- and 2′-*O*-(*o*-carboran-1-yl)methyluridine **4**.[10] The method is based on the reaction of 2′,3′-*O*-(dibutylstannylene)uridine with 3-bromopropyne, acetylation of the resulting mixture of 2′- and 3′-*O*-(3-propynyl)uridines, and then reaction with a bis(acetonitrile)decaborane adduct yielding a difficult to separate mixture of 3′,5′-di-*O*-acetyl-2′-*O*-(*o*-carboran-1-yl)methyluridine and 2′,5′-di-*O*-acetyl-3′-*O*-(*o*-carboran-1-yl)methyluridine. The applicability of this method of the synthesis of carborane-modified purine nucleosides was not reported.

Since RNA and DNA oligomers modified at the 2′-position have proven to be more valuable as biopharmaceuticals and molecular probes than those bearing modification at the 3′-position, finding a method that produces only one isomer is desirable.[11,12] Our initial approach to the site-specific synthesis of 2′-*O*-(*o*-carboran-1-yl)methyluridine **4** (Scheme 1) was based on the method for selective preparation of 2′-*O*-propargylnucleosides.[13,14a]

The propargyl group was introduced selectively at the 2′-position of compound **1**[14b] using 1 equiv of propargyl bromide and 2-*tert*-butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine (BEMP) providing **2**. However, we found that during the subsequent reaction, the tetraisopropyldisiloxane-1,3-diyl protection was unstable in the presence of the bis(acetonitrile)decaborane adduct and therefore the disilyl protection was removed and replaced by acetyl groups. Further reaction of the resultant

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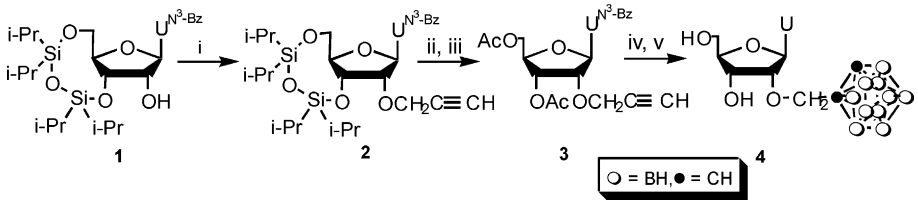
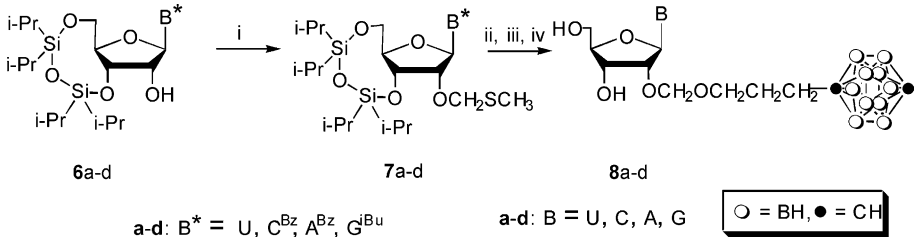


Scheme 1. Reagents and conditions: (i) Propargyl bromide, BEMP; (ii) TBAF/THF; (iii) Ac_2O/Py; (iv) $B_{10}H_{14}{\cdot}CH_3CN$/toluene; (v) NH_3aq/CH_3CN.



Scheme 2. Reagents and conditions: (i) DMSO/AcOH/Ac_2O; (ii) TBABr/1-(3-hydroxypropyl)-*para*-carborane [**5**, $HO(CH_2)_3C_2B_{10}H_{11}$]/$CuBr_2$ in CH_2Cl_2; (iii) TBAF/THF; (iv) NH_3 in CH_3CN/H_2O.

3-*N*-benzoyl-3′,5′-di-*O*-acetyl-2′-*O*-propargyluridine **3** with the bis(acetonitrile)decaborane adduct provided fully protected 2′-*O*-(*o*-carboran-1-yl)methyluridine. Though this method, after deprotection, successfully provided **4**, an approach applying a similar procedure to other nucleosides failed. Instead of the desired 2′-*O*-isomer of carborane-modified cytosine, adenosine or guanosine, darkening of the reaction mixture was observed and a complex mixture of products was detected.

To avoid potential damage to the nucleoside component during carborane cage formation in the presence of the dodecaborane complex, we turned our attention to methods allowing attachment of the modifying unit bearing a preformed carborane cage. As the modifying entity, a *para*-carborane cage was chosen. Though *ortho*-carborane derivatives are easily available and less expensive than their *para*-counterparts, the closed *ortho*-carborane cage decomposes in basic conditions into its open cage, *nido*-form which is often associated with significantly increased toxicity.

The successful approach to the synthesis of 2′-*O*-[(*para*-carboran-1-yl)propyleneoxymethyl] derivatives of all four nucleosides **8a**–**d** is based on the formacetal linkage formation described by Matteucci et al.[15] and modified by Sawada and Ito.[16] The method involves nucleophilic substitution of the activated methylthiomethyl group in the fully protected 3′,5′-*O*,*O*-(tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-methylthiomethyl-nucleosides (**7a**–**d**) with a suitable alcohol bearing the carborane cage (Scheme 2). The key intermediates **7a**–**d** were obtained from the reaction of *N*-protected 3′,5′-*O*,*O*-(tetraisopropyldisiloxane-1,3-diyl)nucleosides **6a**–**d** with DMSO in a mixture of acetic acid/acetic anhydride.[17] The *N*-protected nucleosides **6a**–**d** are easily available and can be prepared according to the literature procedure from suitable nucleosides in high yields.[13]

Target compounds **8a**–**d** were obtained from **7a**–**d** in a three-step procedure without isolation or purification of the intermediate products. First, compounds **7a**–**d** were reacted with 1-(3-hydroxypropyl)-*para*-carborane (**5**) yielding the fully protected 3′,5′-*O*,*O*-TIPDSi-2′-*O*-[(*para*-carboran-1-yl)propyleneoxymethyl]nucleosides. Next, the disiloxane protection was removed using a solution of TBAF in THF yielding *N*-protected 2′-*O*-[(*para*-carboran-1-yl)propyleneoxymethyl]nucleosides. The *N*-acyl protection was removed using concentrated aqueous ammonia solution providing 2′-*O*-[(*para*-carboran-1-yl)propyleneoxymethyl]nucleosides **8a**–**d** (Scheme 2). Compounds **8a**–**d**[†] were obtained in yields of 40–

[†] General procedure for the synthesis of **8a**–**d**: A mixture of solid 3′,5′-TIPDSi-2′-*O*-MTM (**7**) (1 mmol), tetrabutylammonium bromide (1.1 mmol) and 1-(3-hydroxypropyl)-*para*-carborane **5** (2.8 mmol) was dried under vacuum with 4 Å molecular sieves, and then dissolved in dry 1,2-dichlorethane (10 mL). The resultant solution was stirred at room temperature for 24 h and after this solid $CuBr_2$ (1.1 mmol) was added. After reaction completion (ca. 24 h), the reaction mixture was partitioned between dichloromethane and satd sodium bicarbonate solution. The organic phase was separated, dried over anhydrous magnesium sulfate, and the solvent was evaporated yielding the crude product. This material, without purification, was dissolved in THF (10 mL) and treated with TBAF/THF (0.5 M, 6 mL). Removal of the TIPDSi group (40 min) was followed by addition of pyridine/methyl alcohol/water (3:1:1, 10 mL) and ion exchange resin Dowex 50W × 8 (pyridinium form) (ca. 1 g wet weight). After 30 min, the ion exchange resin was filtered off and washed with pyridine/methyl alcohol/water (3 × 5 mL). The filtrate and washings were combined and evaporated to dryness. When appropriate, the remaining base protecting groups were removed by dissolving the evaporated material in acetonitrile (2 mL) and addition of (25%) aqueous ammonia solution (5 mL). After 2 h incubation at 55 °C, the solvent was evaporated and the product was dried by coevaporation with toluene. Crude compound **8** was purified by flash column chromatography.

70% and were fully characterized by chromatographic and spectroscopic methods.[‡]

Introduction of a large, bulky, and hydrophobic carborane unit to the sugar part of a nucleoside may be associated with substantial changes of the natural conformation of the nucleoside[18] that, in the worst scenario, could prevent the use of these modified compounds in many biological experiments.

The conformation of a nucleoside is determined by the rotation of the base about the single bond that joins it to the 1′-carbon of the furanose, the pucker of the atoms in the five-membered furanose ring, the rotation of the exocyclic CH_2OH group, and the rotation of the phosphate attached to this group. There are two general orientations of the base: *syn* and *anti*, either form is allowed although the *anti* conformation is more common physiologically due to both lower steric strain and to the fact that this conformation is required for nucleic acid structure. In addition, the *anti* form is favored for the pyrimidine nucleosides; this is due to a steric clash between the keto oxygen on the C-2 base with the ribose when in the *syn* conformation.[19] It was therefore important to compare the conformation of the derivatives obtained with their non-modified counterparts. We decided to use circular dichroism (CD), since these spectra are most affected by restricted rotation around the glycosidic bond.

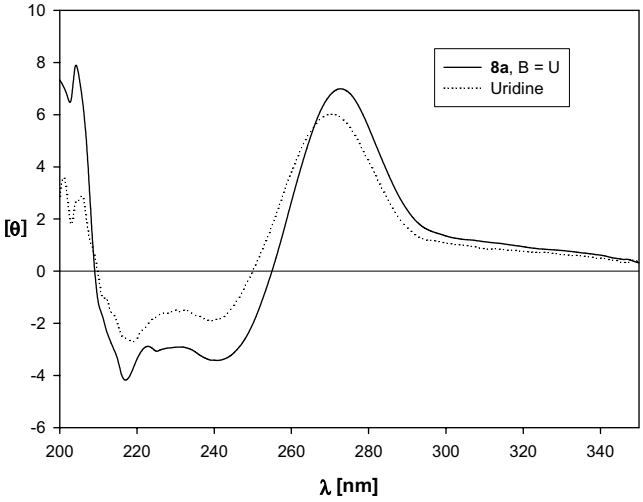


Figure 1. Circular dichroism spectra of 2′-*O*-[(*para*-carboran-1-yl)propyleneoxymethyl]uridine **8a** and unmodified uridine.

The CD spectra[§] of the 2′-*O*-(*o*-carboran-1-yl)-modified nucleosides under analogous conditions were almost identical in terms of their shape and molecular ellipticity values with unmodified nucleosides (Fig. 1), with the exception that a much higher molecular ellipticity of the maximum at 275 nm was observed for modified nucleoside **8b** than for unmodified cytidine. The CD measurement suggests, therefore, that the lipophilic and bulky *o*-carborane has little effect on the overall nucleoside conformation if linked through a propyleneoxymethyl linker to the 2′-position of the nucleoside.

The present approach provides a route to nucleoside conjugates modified with different types of carborane cages or other functional groups as long as a suitable alcohol terminated with the intended functional group is available. Our method provides an opening for the synthesis and study of nucleic acids modified with carborane clusters at desired locations[5,12] and of other biologically important derivatives of nucleosides.

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[‡]Selected data **8a**: Yield: 52%; TLC (CH_2Cl_2/MeOH, 9:1): $R_f = 0.4$; UV (96% C_2H_5OH): $\lambda_{min} = 231.2$ nm, 292.75 nm, $\lambda_{max} = 262.9$ nm; ^{1}H NMR, 250.131 MHz (CD_3OD): δ 1.00–3.34 (bm, 10H, BH-*p*-carborane), 1.35–1.75 (m, 4H, –OCH$_2$*CH$_2$CH$_2$*-*p*-carborane), 3.30–3.45 (m, 2H, –O*CH$_2$*CH$_2$CH$_2$-*p*-carborane), 3.81–4.02 (m, 2H, 2H-5′,5″), 4.10–4.29 (m, 1H, H-4′), 4.30–4.39 (m, 2H, H-3′, H-2′), 4.72–4.79 (m, 2H, –O*CH$_2$*O–), 5.74 (d, 1H, H-6, $J_{H6–H5} = 8.09$ Hz), 5.75 (d, 1H, H-1′, $J_{H1′–H2′} = 4.34$ Hz), 7.66 (d, 1H, H-5, $J_{H5–H6} = 8.14$ Hz), 8.02 (s, NH); FAB-MS (+ve, Gly) 458.1 [M]$^+$ (molecular formula: $C_{15}H_{30}B_{10}N_2O_7$, calculated exact mass = 457.333). IR (KBr): ν_{max} (B–H) = 2607 cm^{-1}. **8b** Yield: 71%; TLC (CH_2Cl_2/MeOH, 9:1): $R_f = 0.12$; UV (96% C_2H_5OH): $\lambda_{min} = 227.9$ nm, 254.0 nm, 304.2 nm, $\lambda_{max} = 242.0$ nm, 273.7 nm; ^{1}H NMR, 250.131 MHz (CD_3OD): δ 1.0–3.5 (bm, 10H, BH-*p*-carborane), 1.35–1.75 (m, 4H, OCH$_2$*CH$_2$CH$_2$*-*p*-carborane), 3.25–3.45 (m, 2H, O*CH$_2$*CH$_2$CH$_2$-*p*-carborane), 3.83–4.04 (m, 3H, H-5′,5″, H-4′), 4.22–4.30 (m, 2H, H-2′, H-3′), 4.77–4.86 (m, 2H, O*CH$_2$*O), 5.75 (s, 1H, H-6), 5.95 (d, 1H, $J_{H1′-H2′} = 7.70$ Hz), 7.98 (d, 1H, H-5, $J_{H5–H6} = 7.72$); FAB-MS (+ve, Gly): 459.2 [M+H]$^+$ (molecular formula: $C_{15}H_{31}B_{10}N_3O_6$; calculated exact mass 458.317); IR (KBr): ν_{max} (B–H) = 2607 cm^{-1}. **8c** Yield: 39%; TLC (CH_2Cl_2/MeOH, 9:1): $R_f = 0.26$; UV (96% C_2H_5OH): $\lambda_{min} = 239.6$ nm, 298.6 nm, $\lambda_{max} = 256.9$ nm, 313.6 nm; ^{1}H NMR, 250.131 MHz (CD_3OD): δ 0.96–3.30 (bm, 10H, BH-*p*-carborane), 0.97–1.10 (m, 2H, OCH$_2$*CH$_2$*CH$_2$-*p*-carborane), 1.34–1.41 (m, 2H, OCH$_2$CH$_2$*CH$_2$*-*p*-carborane), 2.41 (br s, 1H, 1H-C-*p*-carborane), 2.94–3.08 (m, 2H, O*CH$_2$*CH$_2$CH$_2$-*p*-carborane), 3.72 and 3.86 (dd, 1H, H-5′, $J_{H5′–H4′} = 2.41$, $J_{H5′–H5″} = 12.54$; dd, 1H, H-5″, *JH5″–H4′* = 2.34, $J_{H5″–H5′} = 12.62$), 4.16 (d, 1H, 1H-4′, $J_{H4′–H5″} = 2.24$), 4.35–4.45 (m, 1H, 1H-3′), 4.50–4.70 (m, 2H, O*CH$_2$*O; 1H, H-2′), 6.04 (d, 1H, H-1′, *JH1′–H2′* = 6.75), 8.20 (s, 1H, H-2), 8.29 (s, 1H, H-8); ^{11}B {1H} NMR (CD_3OD): δ −10.23 (s, 5B), −12.67 (s, 5B). FAB-MS (+ve, Gly) 483.4 [M$^+$] (molecular formula: $C_{17}H_{37}B_{10}N_5O_4$, calculated exact mass: 483.360); IR (KBr): ν_{max} (B–H) = 2607 cm^{-1}. **8d** Yield: 15–30%; UV (96% C_2H_5OH): $\lambda_{min} = 224.4$ nm, 299.0, $\lambda_{max} = 251.5$ nm, 274.6 nm; TLC (CH_2Cl_2/MeOH, 9:1): $R_f = 0.21$; ^{1}H NMR (CD_3OD): δ 0.88–3.5 (bm, 10H, BH-*p*-carborane), 1.01–1.50 (m, 4H, CH$_2$*CH$_2$CH$_2$-*p*-carborane), 3.05–3.14 (m, 2H, O*CH$_2$*CH$_2$CH$_2$-*p*-carborane), 3.68–3.86 (m, 2H, 2H-5′,5″), 4.06–4.09 (m, 1H, H-4′), 4.21 (d, 1H, H-2′, $J_{H2′–H1′} = 6.19$ Hz), 4.35 (dd, 1H, H-3′, $J_{H3′–H2′} = 4.93$, $J_{H3′–H4′} = 3.14$ Hz), 4.59–4.65 (m, 2H, O*CH$_2$*O), 5.89 (d, 1H, H-1′, $J_{H1′–H2′} = 6.15$ Hz), 7.93 (s, 1H, H-8); FAB-MS (+ve, Gly): 497.1 [M]$^+$, ($C_{16}H_{31}B_{10}N_5O_6$: 497.340); IR (Film): $\nu_{max} = 2608$ cm^{-1} (B–H).

[§]The absorption maxima and minima in circular dichroism (CD) spectra of modified nucleosides **4a**–**d** are as follows: **4a**: $\lambda_{max} = 271$ ($\Theta = 6.9$) and 232 nm ($\Theta = -2.8$), $\lambda_{min} = 242$ ($\Theta = -3.3$) and 218 nm ($\Theta = -4.1$); **4b**: $\lambda_{max} = 273$ nm ($\Theta = 7.4$), $\lambda_{min} = 223$ ($\Theta = -5.2$); **4c**: $\lambda_{max} = 292$ ($\Theta = 1.4$), 235 ($\Theta = 1.8$), 219 nm ($\Theta = 2.1$), $\lambda_{max} = 270.0$ nm ($\Theta = 0.0$), 227.0 nm ($\Theta = 1.4$); **4d**: $\lambda_{max} = 291$ ($\Theta = 1.1$), 218 ($\Theta = 2.2$), $\lambda_{min} = 245$ ($\Theta = 0.6$).

Supplementary data

IR, CD and MS spectra of **8a–d** are available. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tetlet.2005.04.046.

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