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Synthesis of *closo*-dodecaborate based nucleoside conjugates

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ABSTRACT

The first conjugates of *closo*-dodecaborate anion with nucleoside-thymidine were synthesized. The nucleophilic cleavage of dioxonium derivative of *closo*-dodecaborate by 3′,5′-bis(t-butyldimethylsilyl)-thymidine and "click" reaction between B_{12}-based azide and 3*N*-(4-pentyn-1-yl)thymidine were appeared as convenient approaches towards the synthesis of this new class of nucleoside-based boron cluster conjugates.

1. Introduction

One of the main aims of bioorganic chemistry of boron is design of boron carriers for Boron Neutron Capture Therapy of cancers (BNCT) with improved properties [1]. The successful treatment of tumors by BNCT requires selective delivery of the boron moiety into the tumor cells. One of ways to achieve this goal is attachment of boron-donor fragment to tumor-specific targeting molecules. Boronated nucleosides, like 5-(o-carborane-1-yl)-2′-deoxyuridine (CDU) [2], are considered to be potential BNCT candidates because they can accumulate in the tumor cells. There are many examples of the synthesis of boronated nucleosides [3]. The first example of such compound, the nucleoside with $B(OH)_2$-group (5-(dihydroxyboryl)-2′-deoxyuridine), was reported by Schinazi and Prusoff [4]. Later, a series of nucleoside-based boronic acids were prepared by Yamomoto via coupling reactions [5,6]. Preparation and activity studies of nucleosides, containing *o*-, *m*- and *p*-12-vertex carborane units were reviewed in details by Tjarks et al. recently [3]. Although a large number of compounds were prepared, the high hydrophobicity of carborane unit cause low solubility of nucleoside/boron cluster conjugates in water and requires further derivatization of these molecules to improve their hydrophilicity.

Synthesis and cleavage reactions of oxonium derivatives of various polyhedral boranes (*closo*-dodecaborate, *closo*-decaborate, dicarba-*nido*-undecaborate, cobalt-*bis*-dicarbolide) has made a real breakthrough in attaching of these boron rich modifications to different molecules [7]. Recently a series of boron-containing nucleosides have been prepared by the reactions of the 1,4-dioxane derivative of cobalt bis(dicarbollide) with all four canonical nucleosides like thymidine, 2-*O*-deoxycytidine, 2-*O*-deoxyadenosine, and 2-*O*-deoxyguanosine [8–10]. In the case of 3′,5′-protected thymidine (Scheme 1) and 2-*O*-deoxyguanosine the reactions resulted in a mixture of the one *N*- and two *O*-alkylated products **1**(**a**–**c**) which can be later separated by chromatographic methods [10].

However, till now there were no examples of boronated nucleosides that contain hydrophilic $B_{12}H_{12}^{2-}$ unit. In the present paper we report the synthesis and characteristics of this novel class of boronated nucleosides and suggest two methods for their preparation.

2. Experimental

2.1. Materials and equipment

Chemicals were reagent grade and were used as received from commercial vendors. Acetonitrile was distilled from P_2O_5 and then from CaH_2. Oxonium derivative **2**, azide **5** and 3*N*-(4-pentyn-1-yl)thymidine **6** were prepared according to the described methods [11–13]. The ^1H, ^{13}C and ^{11}B NMR spectra were recorded at 400.13,

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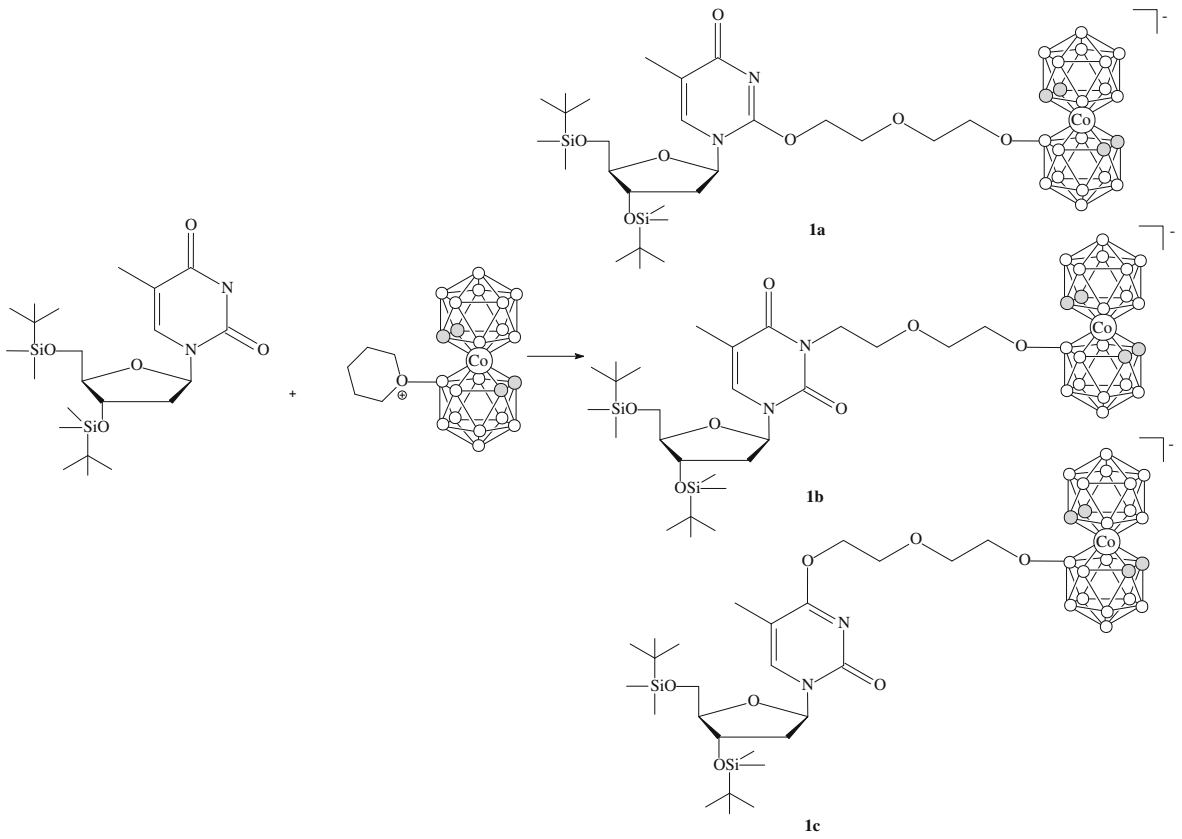


Scheme 1.

100.61 and 128.38 MHz, respectively, on a BRUKER-Avance-400 spectrometer in DMSO-d_6 (**3**, **4**, **7**) or in D_2O (**4a**). Tetramethylsilane and $BF_3/(C_2H_5)_2O$ were used as standards for ^1H and ^{13}C NMR, and ^{11}B NMR, respectively. All chemical shifts are reported in ppm (δ) relative to external standards. Elemental analysis was performed in Microanalytical laboratory of A.N. Nesmeyanov Institute of Organoelement Compounds.

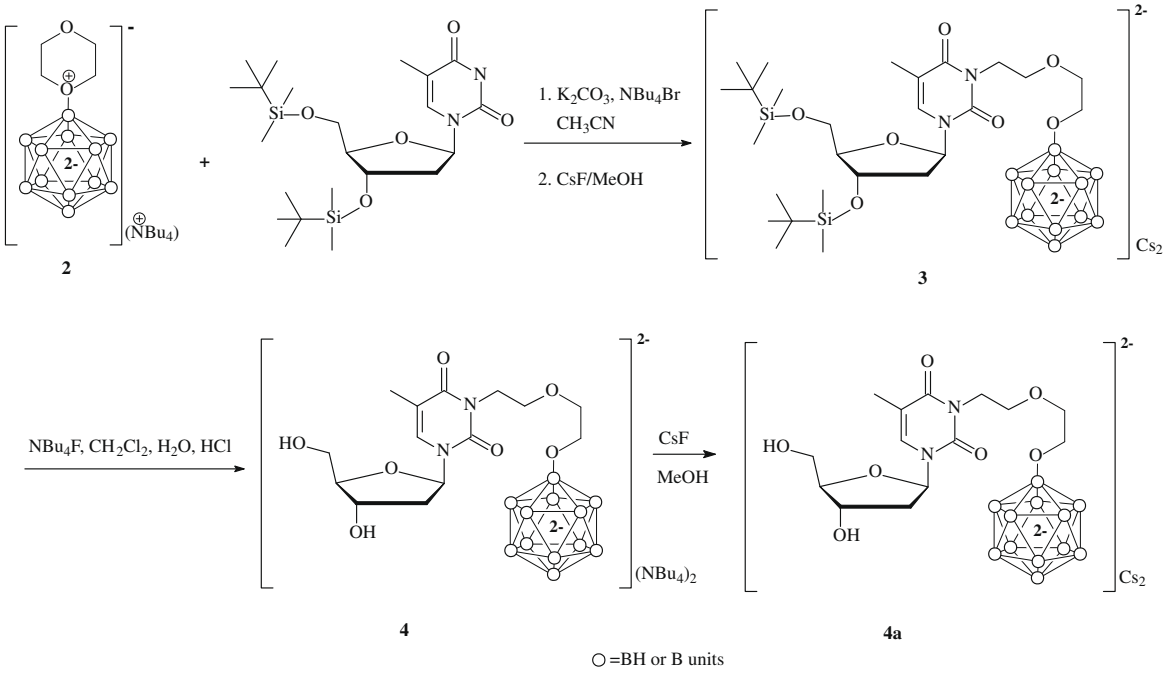
2.2. Synthesis of **3**

A mixture of 100 mg (0.213 mmol) of 3′,5′-bis(t-butyldimethylsilyl)-thymidine, 100 mg (0.213 mmol) of oxonium derivative **2**, 67 mg (0.213 mmol) of NBu_4Br and 200 mg of dry K_2CO_3 was refluxed in 3 ml of anhydrous CH_3CN for 1 h. After this period no more starting materials were detected by TLC (CH_2Cl_2/CH_3OH 20/1). Then the residue was filtered off and CH_3CN evaporated. The resulting oily residue was dissolved in 2 ml of CH_3OH and the product **3** was precipitated by the addition of 65 mg (0.426 mmol) of CsF in 1 ml of CH_3OH. It was filtered off and air dried. Yield: 140 mg (145 mmol, 68%). Anal: Calc. For $C_{26}H_{60}N_2B_{12}Cs_2O_7Si_2$: C, 32.38; H, 6.27; N, 2.90; B, 13.45. Found: C, 32.14; H, 6.29; N, 2.86; B, 13.49%. ^1H NMR (ppm): 7.49 (1H, s, H-6); 6.20 (1H, t, H-1′); 4.37 (1H, m, H-3′); 3.96 (2H, t, CH_2–N (spacer)); 3.81 (1H, m, H-4′); 3.72 (2H, m, H-5′, 5′); 3.47 (2H, t, CH_2–O (spacer)); 3.41 (4H, m, CH_2–O (spacer)); 2.17 (2H, m, H-2′), 1.83 (3H, s, 5-CH_3); 0.89 (18H, s, SiC(CH_3)$_3$; 0.09 (2s, 12H, $SiCH_3$); 1.9–0.1 (11H, broad m, BH). ^{11}B NMR (ppm): 6.3 (1B, s, B(1)); −16.8 (5B, d, B(2–6)); -18.2 (5B, d, B(7–11)); -22.5 (1B, d, B(12)). ^{13}C NMR (ppm): 163.1 (C-4); 150.8 (C-2); 134.7 (C-6); 109.1 (C-5); 87.3 (C-4′); 85.2 (C-1′); 72.4 (C-3′); 72.3, 67.4, 66.9 (CH_2O-spacer); 63.1 (C-5′); 40.2 (CH_2N-spacer), 38.8 (C-2′); 26.2 ((CH_3)$_3$CSi)); 18.5, 18.2 ((CH_3)$_3$*C*Si));13.4 (5-CH_3); −4.2, −4.5, −4.9,−5.0 (CH_3Si).

2.3. Synthesis of **4**

Ninety two milligram (0.095 mmol) of **3** was dissolved in 3 ml of CH_2Cl_2 followed by addition of 30 mg (0.190 mmol) of $NBu_4F \times 3H_2O$ and 1 drop of 12N HCl. The resulting mixture was stirred for 6 h. Then the mixture was dried over Na_2SO_4 and evaporation of CH_2Cl_2 followed by vacuum drying afforded **4** without further purification. Yield: 90 mg (0.093 mmol, 98%). M.P. = 140 °C (dec). Anal: Calc. For $C_{46}H_{104}N_4B_{12}O_7$: C, 57.85; H, 10.98; N, 5.87; B, 13.58. Found: C, 57.39; H, 10.93; N, 5.89; B, 13.61%. ^1H NMR (ppm): 7.73 (1H, s, H-6); 6.17 (1H, t, H-1′); 4.21 (1H, m, H-3′); 3.93 (2H, t, CH_2-N (spacer)); 3.73 (1H, m, H-4′); 3.58-3.45 (8H, m, H-5′, 5′ and 3 × CH_2-O (spacer)); 3.12 (16H, m, $NCH_2CH_2CH_2CH_3$); 2.07 (2H, m, H-2′), 1.79 (3H, s, 5-CH_3); 1.53 (16H, m, $NCH_2CH_2CH_2CH_3$); 1.28 (16H, m, $NCH_2CH_2CH_2CH_3$); 0.90 (24H, t, $NCH_2CH_2CH_2CH_3$); 1.9–0.1 (11H, broad m, BH). ^{11}B NMR (ppm): 6.3 (1B, s, B(1)); −16.8 (5B, d, B(2-6)); −18.2 (5B, d, B(7–11)); −22.5 (1B, d, B(12)). ^{13}C NMR (ppm): 163.1 (C-4); 150.8 (C-2); 135.3 (C-6); 108.9 (C-5); 87.8 (C-4′); 85.2 (C-1′); 71.1 (C-3′); 70.7, 68.1, 66.9 (CH_2O-spacer); 61.6 (C-5′); 58.0 ($NCH_2CH_2CH_2CH_3$); 41.2 (CH_2N-spacer); 39.9 (C-2′); 23.5 (NCH_2-$CH_2CH_2CH_3$); 19.7 ($NCH_2CH_2CH_2CH_3$); 13.9 ($NCH_2CH_2CH_2CH_3$); 13.4 (5-CH_3).

For the measurement of partition coefficient **4** was converted from NBu_4-salt to Cs-salt (**4a**). 20 mg (0.021 mmol) of **4** was dissolved in 0.1 ml of MeOH and the solution of 7 mg (0.042 mmol) of CsF in 0.1 ml of MeOH was added. The white precipitate was filtered and vacuum dried. Yield: 15 mg (0.021 mmol), 100%. ^1H NMR (ppm): 7.74 (1H, s, H-6); 6.19 (1H, t, H-1′); 4.20 (1H, m, H-3′); 3.95 (2H, t, CH_2-N (spacer)); 3.71 (1H, m, H-4′); 3.58–3.45 (8H, m, H-5′, 5′ and 3 × CH_2-O (spacer)); 2.07 (2H, m, H-2′), 1.79 (3H, s, 5-CH_3); 1.9–0.1 (11H, broad m, BH).



Scheme 2.

2.4. Synthesis of **7**

The 3*N*-(4-pentyn-1-yl)thymidine (**6**) (12 mg, 0.039 mmol) and 1-(5-azido-3-oxa-pentoxy)-*closo*-dodecaborate (bis tetra-*n*-butylammonium salt) (**5**) (30 mg, 0.040 mmol), were dissolved in a mixture of *tert*-butanol and water (1:1, 1 ml). To the resultant mixture a solution of $CuSO_4{\cdot}5H_2O$ (0.0019 mmol, 5% mol, 19.5 μl of 100 mM solution) was added followed by addition of solution of sodium ascorbate (0.0039 mmol, 10% mol, 39 μl of 100 mM solution). The mixture was stirred at 70 °C temperature until TLC monitoring (CH_2Cl_2:CH_3OH, 9:1) showed complete conversion of the nucleoside starting material (ca. 24 h). Then, the solvent was evaporated to dryness under vacuum and the crude product was dissolved in methanol (1 ml) and 12 mg of CsF was added. The white precipitate was recovered by centrifugation (5 min. 12000 rpm) and washed with methanol (2 × 300μl). Yield: 26.2 mg, (0.031 mmol, 80%). Anal: Calc. For $C_{19}H_{39}N_5B_{12}O_7Cs_2$: C, 27.00; H, 4.65; N, 8.29; B, 15.35. Found: C, 26.83; H, 4.63; N, 8.24; B, 15.44%. ^{1}H NMR (ppm): 7.87 (1H, s, CH of triazole); 7.72 (1H, s, H-6); 6.18 (1H, t, H-1′); 5.20 (1H, s, OH-3′); 5.01 (1H, s, OH-5′); 4.42 (2H, m, N–C*H*$_2$CH$_2$O); 4.20 (2H, m, H-3′); 4.06 (1H, m, H-4′); 3.84 (2H, t, N(3)–CH$_2$); 3.71 (2H, m, OCH$_2$-spacer); 3.53 (2H, m, OCH$_2$-spacer); 3.31 (2H, m, OCH$_2$-spacer); 3.14 (2H, d, H-5′, 5′); 3.45 (2H, broad s, OH); 2.58 (2H, t, N(3)–CH$_2$CH$_2$C*H*$_2$); 2.08 (2H, m, H-2′); 1.82 (2H, m, N(3)–CH$_2$C*H*$_2$CH$_2$); 1.79 (3H, s, H-5); (11H, broad m, BH). ^{11}B NMR (ppm): 6.3 (1B, s, B(1)); −16.8 (5B, d, B(2-6)); −18.2 (5B, d, B(7-11)); −22.5 (1B, d, B(12)). ^{13}C NMR (ppm): 163.1 (C-4); 151.0 (C-2); 146.3 (C-triazole); 135.2 (C-6); 123.0 (CH triazole); 108.9 (C-5); 87.8 (C-4′); 85.2 (C-1′); 72.4 (C-3′); 70.7, 69.1, 67.8 (CH$_2$O); 61.6 (C-5′), 49.7 (NCH$_2$CH$_2$O); 40.8 (N(3)–*C*H$_2$-CH$_2$CH$_2$); 40.5 (C-2′); 27.5 (N(3)CH$_2$CH$_2$*C*H$_2$); 23.2 (N(3)CH$_2$*C*H$_2$-CH$_2$); 13.5 (5-CH$_3$).

2.5. Partition coefficient (K) measurement of ***4a***

Measurement of the partition coefficient of **4a** was performed as previously described [9].

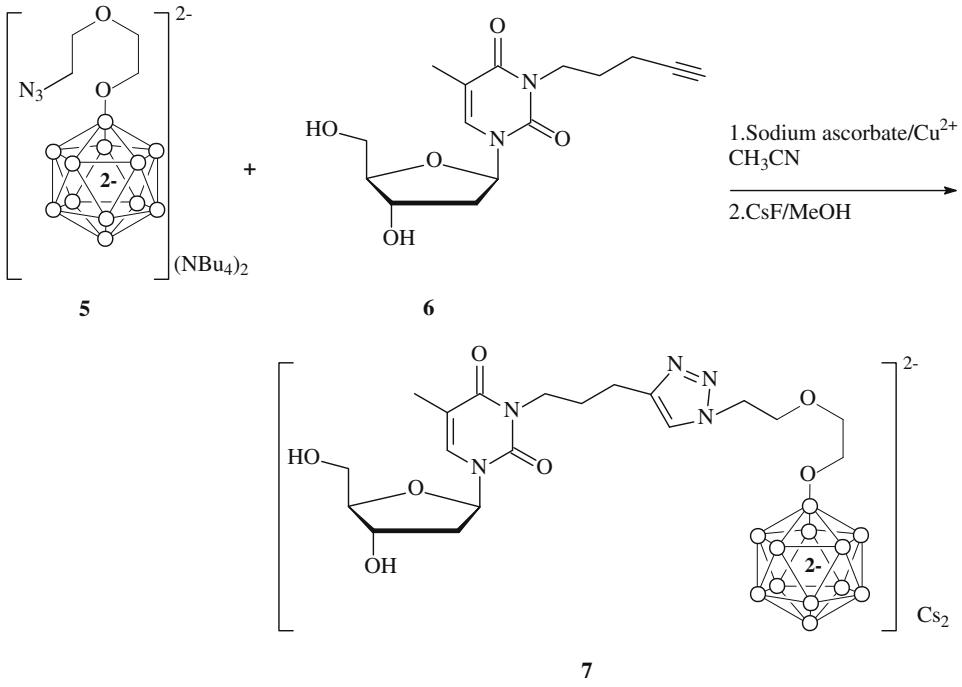
3. Results and discussion

Our attempt to introduce **2** into the reaction with protected thymidine under the same conditions as for the oxonium salt of cobald-bisdicarbolide using NaH as a base resulted in a mixture of products that could not be isolated. Thus, for the synthesis of **3**, activation of nucleobase with K_2CO_3 was applied as described earlier for the alkylation of thymidine with alkyne bromides or alkyne tosylates [14]. We have found that dioxonium derivative **2** of *closo*-dodecaborate reacts with 3′,5′-bis(t-butyldimethylsilyl)-thymidine in presence of K_2CO_3 leading to cleavage product **3**. Removing of the protection groups followed by purification resulted in the very first B_{12}- modified thymidine **4** (Scheme 2) with high yield.

It is to be emphasized that the N-alkylated product was formed regioselectively under these conditions. The structure of **3** and **4** was proved by high-resolution NMR-technique. In the ^{11}B NMR of **3** the signal of the substituted boron atom is shifted ca 2 ppm downfield compared to the starting material **2** [11]. This is typical for the transformation of BO$^+$R$_2$ system to B-OR. The ^{1}H and ^{13}C NMR data perfectly match the structure of **3** as well. The signals were assigned using ^{1}H–^{1}H-COSY and ^{13}C–^{1}H-HMQC-gs experiments. The ^{13}C spectrum of **3** was additionally compared with this for O- and N-alkylated metallocarborane derivatives of thymidine **1**(**a–c**), reported earlier [10]. Thus, the signal of N(3)–CH$_2$ group of **3** was observed at δ = 40.2 ppm. The same value was observed for the N(3)-alkylated Co-carborane derivative **1b** (42.3 ppm). Contrary, in O-alkylated products **1(a–c)** the signals of O(2)–CH$_2$ and O(4)–CH$_2$ appeared at ca 70 ppm. Also the δ-values of pyrimidine ring carbon atoms are similar to **1b** and differ from **1a** and **1c** (Table 1). The

Table 1
^{13}C NMR data of CH$_2$–X (X = O, N) groups and thymidine rings carbons of **3** and **1(a–c)**.

Compound	δ_{CH2X}, ppm	$\delta_{C(2)}$, ppm	$\delta_{C(4)}$, ppm	$\delta_{C(5)}$, ppm	$\delta_{C(6)}$, ppm
3(N(3)CH$_2$)	40.2	150.8	163.1	109.1	134.7
1a(O(2)CH$_2$)	72.4	155.8	174.5	117.7	135.7
1b(N(3)CH$_2$)	42.3	151.5	165.8	110.6	135.6
1c(O(4)CH$_2$)	73.1	157.8	171.5	106.7	141.3



Scheme 3.

formation of N(3) derivative is typical for alkylation reactions of thymidines, when a weak bases are used [14,15]. The other factor could be related to the HSAB rules, and relative softness and hardness of the nucleophilic centers in nucleobase and a carbon atom in dioxane ring of $B_{12}H_{11}^-$vs.$C_2B_9H_{12}^-$ of metallacarborane.

Compound **4** was converted to Cs-salt **4a** and then partition coefficient (*K*) for it was established [6]. We found that *K* for conjugate **4a**, bearing *closo*-dodecaborate modification was as expected, slightly lower than natural thymidine with value 0.016 ± 0.002, proving very good water solubility of **4a**. For comparison, 5-o-carborane-1-yl-thymidine (CDU) bearing fat soluble *closo*-carborane residue has been characterized by partition coefficient 57.04 ± 11.90 [6]. Thus, applicability of *closo*-dodecaborate modification for synthesis of boron rich, water soluble nucleoside conjugates was confirmed.

Our attempts to react **2** with purine nucleosides like 3′,5′-O,O-di(t-butyldimethylsililyl)-2′-deoxyguanosine under the same conditions using K_2CO_2 activation failed. Unindintified products that could not be separated were formed. The reaction with 3′,5′-O,O-di(t-butyldimethylsililyl)-2′-deoxyadenosine resulted in the recovery of starting materials. Thus, we worked up the other way of preparation of B_{12}-modified nucleosides via, click"-reaction.

The "click" concept has been introduced into chemistry and biology by Sharpless and others with great success [16–18]. Recently a general method was proposed for the synthesis of nucleoside libraries bearing carborane or metallacarborane modification for biochemical screening using Huisgen 1,3-dipolar cycloadition of azides and terminal alkynes, a "click" chemistry approach [13]. It was found that also B_{12}-based azides undergo Cu^I-catalyzed "click" reactions with various alkynes leading to corresponding triazoles in high yields [12]. Herein we show expansion of the "click" chemistry approach towards synthesis of nucleosides bearing *closo*-dodecaborate modification. Thus, we have prepared alkynylated thymidine derivative **6** and introduced into the reaction with azide **5**. This reaction leads to novel B_{12} containing thymidine derivative, linked via triazole ring **7** (Scheme 3). The structure of **7** was proved using microanalysis and NMR-spectroscopy (cf. experimental). Thus, in ^{1}H NMR of **7** despite the typical signals of thymidine unit and two spacers, the signal of triazole proton at 7.87 ppm was observed. In ^{13}C NMR of the signals of triazole carbons at 146.3 ppm (C) and 123.0 ppm (CH) were observed as well.

4. Conclusion

In conclusion, the very first conjugates of *closo*-dodecaborate and thymidine were prepared by two versatile and effective methods. They can be easily expanded for other nucleoside derivatives and works in this direction are ongoing in our laboratories. Partition coefficient measurement confirmed applicability of *closo*-dodecaborate modification for synthesis of boron rich, water soluble nucleoside conjugates.

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