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Preliminary Communication

The synthesis and NMR investigation on novel boron derivatives of stavudine

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ABSTRACT

Preparation and spectroscopic properties of novel boron-containing derivatives of anti-HIV agent stavudine are presented, The new compounds, (5′-*O*-(4,4,5,5-tetramethyl-1,3,2-dioxaboronate)-2′-3′-didehydro-2′-3′-dideoxythymidine and 5′-O-(dihydroxyboronate)-2′-3′-didehydro-2′-3′-dideoxythymidine), were prepared by direct reaction between stavudine and reagents containing B—H moieties – pinacolborane and borane–dimethylsulfide complexes, respectively. The boron coordination equilibrium of those compounds was analyzed by water titration monitored by NMR. Results of the DFT calculations and NMR experiments pointed to structural and electronic similarity of tetrahedral boron complexes to phosphate group.

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1. Introduction

Stavudine (2′,3′-didehydro-3′-deoxythymidine; D4T), a synthetic thymidine nucleoside analogue in which the 3′-hydroxyl group is replaced by a double bond between the 2′- and 3′-carbon atoms of the pentose ring, is a nucleoside reverse transcriptase inhibitor (NRTI) [1–3], approved for its use in combination with other antiretroviral compounds for the treatment of HIV infections in USA and European Union. The drug leads to inhibition of viral reverse transcription, as do didanosine (ddI), lamivudine (3TC), zalcitabine (ddC) and zidovudine (ZDV), also belonging to the family of NRTI [1–5], with stavudine 5′-triphosphate causing competitive *vs.* dTTP inhibition of HIV reverse transcriptase and termination of DNA polymerization (due to lack of 3′-OH in pentose ring necessary to form 3′-5′-phosphodiester linkage). As current anti-HIV agents show several drawbacks, including short- and long-term adverse effects, mitochondrial toxicity, antagonism with other antiretroviral and development of drug resistant or multi drug resistant HIV strains [6,7], numerous new stavudine analogues or derivatives were synthesized and tested as antiretroviral compounds, with only a few showing promising activity. For example (i) 4′-thiostavudine [8] and 4′-ethynylstavudine [8,9] showed inhibitory activity against HIV-1 comparable to that of stavudine, (ii) 5′—OH group of stavudine esterified with antimicrobial building blocks of selected agents (ciprofloxacin, norfloxacin, isoniazide, pyrazinamide, piperazine and dimetylamine acetic acid) led to prodrugs with broad spectrum chemotherapeutic activity [6,10], (iii) stampidine, a phosphoramidate derivative of stavudine, was a potential anti-HIV agent, some 100-fold more active than stavudine and twice as active as zidovudine [11] and (iv) stampidine, 5′-stavudine-[*p*-bromophenyl methoxyalaninylphosphate], with bromine at the phenyl moiety accelerating the compound's hydrolysis yielding *N*-alaninyl-stavudinemonophosphate [12,13].

In search of new derivatives of the drug, synthesis of boron analogue of stavudine 5′-phosphate group was undertaken. As a result, two compounds were obtained, with boron atom easily forming complexes in which an additional donor lone-pair, containing coordinates to boron atom, yields tetracoordinates, more or less distorted tetrahedral forms mimicking phosphate group.

Additionally, the pinacolborane stavudine derivative (**2**) will possibly improve cell wall transfer properties due to low polarity of pinacolborane anchor. The transferred compound would probably hydrolyze to free stavudine therefore improving overall stavudine – cell transfer.

2. Results and discussion

2.1. Synthesis and NMR properties of **1**, **2** and **3**

The synthesis of **1** (stavudine, d4t) was based on previously published work of Prusoff's group, modified and optimized towards the purity of the final product [14].

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Although relatively easy method of synthesis of alkoxyboron compounds by the reaction of boric acid and given alcohol is known, rapid equilibration could result in the formation of bis- and trisalkoxy species. Our borane approach, though more expensive and time-consuming, gives products of higher purity. Presented method of preparation of **2** and **3** is based on reaction environment without polar and protic solvents so equilibration between alkoxy and hydroxy forms is minimized.

Compound **2**, 5′-*O*-(4,4,5,5-tetramethyl-1,3,2-dioxaboronate)-2′-3′-didehydro-2′-3′-dideoxythymidine, a 5′-pinacolborane derivative of stavudine was synthesized by direct reaction between a large excess (25-fold molar excess over **1**) of pinacolborane (4,4,5,5-tetramethyl-[1,3,2]dioxaborolane) and unprotected **1** (Fig. 1). The obtained hydrophobic boronic ester can be easily isolated from reaction mixture by a standard crystallization or extraction methods.

The reaction mechanism is typical for most of the borane complexes with B—H group and involves attack of boron-attached hydrogen atom onto 5′-hydroxyl group hydrogen atom with evolution of gaseous hydrogen, followed by an attack of resultant 5′-alcoholate oxygen onto boron atom. Compound **3** was prepared in a similar manner, with **1** being reacted with borane–dimethylsulphide complex (Fig. 1) at 0 °C, followed by hydrolysis of the resulting hydride compound 5′-CH_2—O—BH_2. The proposed reaction mechanism is similar to the one described for synthesis of **2**, with significant reactivity differences caused by lower electron density on the boron-attached hydrogen atom of pinacolborane, resulting from strong electron-withdrawing effect of the two pinacolborane oxygen atoms. It should be noted that no hydroboration reaction was observed under the reaction conditions used.

The ^{1}H NMR spectra of compounds **2** and **3** are similar to that of stavudine (**1**) with significant changes in the saccharide C(5′) region containing two magnetically nonequivalent hydrogen atoms (see Fig. 2).

Resonances of 5′-hydrogens of **1** form a multiplet at 3.67 ppm, whereas the hydrogen atoms at the corresponding position of compound **2** resonate at 4.05. In case of **3**, the steric and electronic differentiation between 5′-methylene group hydrogen atoms is large enough to form two distinct multiplets at 3.80 and 3.93 ppm. Such differentiation has not been observed for stavudine, the precursor of **3**. ^{11}B NMR spectra of **2** and **3** contain broad resonances at 22.4 and 17.8 ppm for **2** and **3**, respectively, in accord with previously published data [15–18].

2.2. DFT calculations

A possibility of tetrahedral boron group to mimic the geometric and electronic properties of phosphate group was tested with the aid of NMR and DFT methods. The close steric and electronic similarity of phosphates and esters of tetrahedral boric acid complexes (Fig. 3, bottom part of figure) might have interesting implications, such as a possibility to use boron analogs of nucleotides.

Coordination equilibrium of compounds **2** and **3** is presented in Fig. 3. Additional electron pair donors, e.g. water, thiol, amine, alcohol and also hydroxyl or carboxylate anion coordinates to positively polarized boron atom, creating a very stable eight-electron

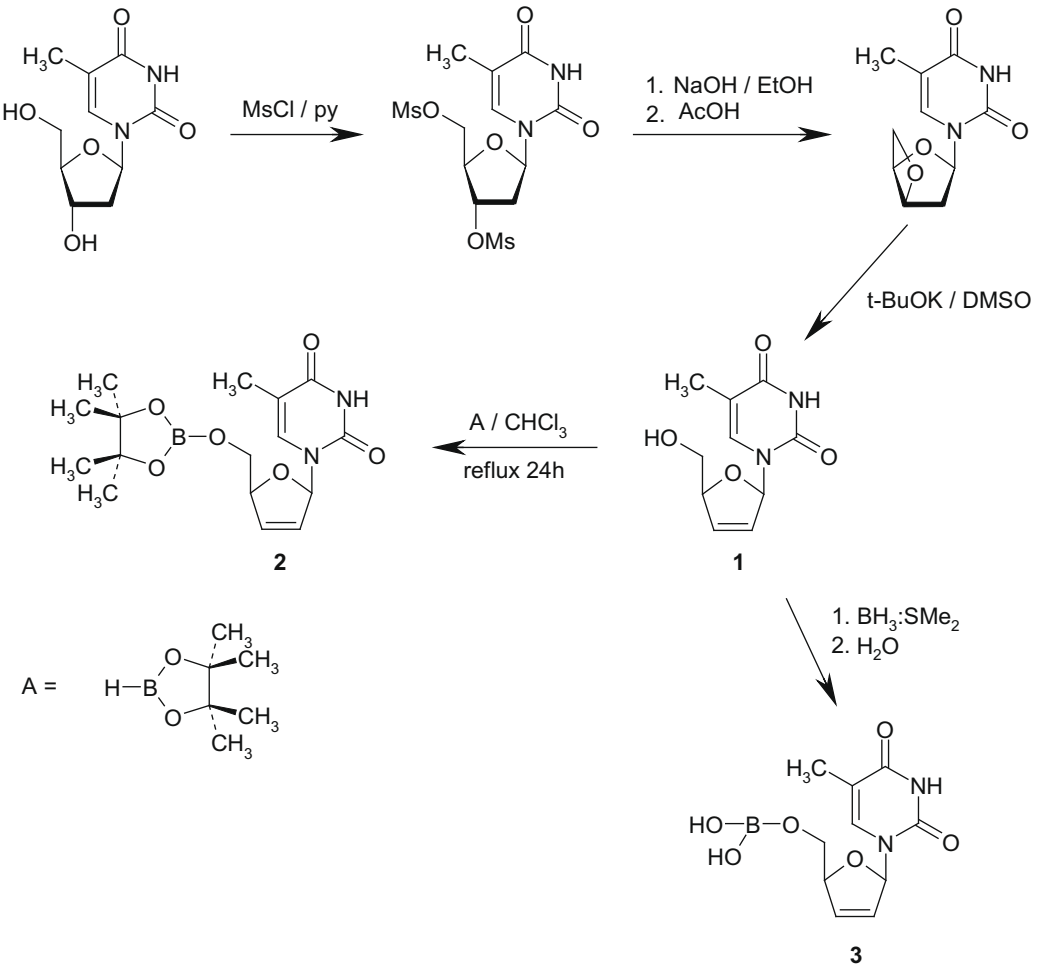

Fig. 1. Synthesis of **1**, **2** and **3**. A = 4,4,5,5-tetramethyl-[1,3,2]dioxaborolane.

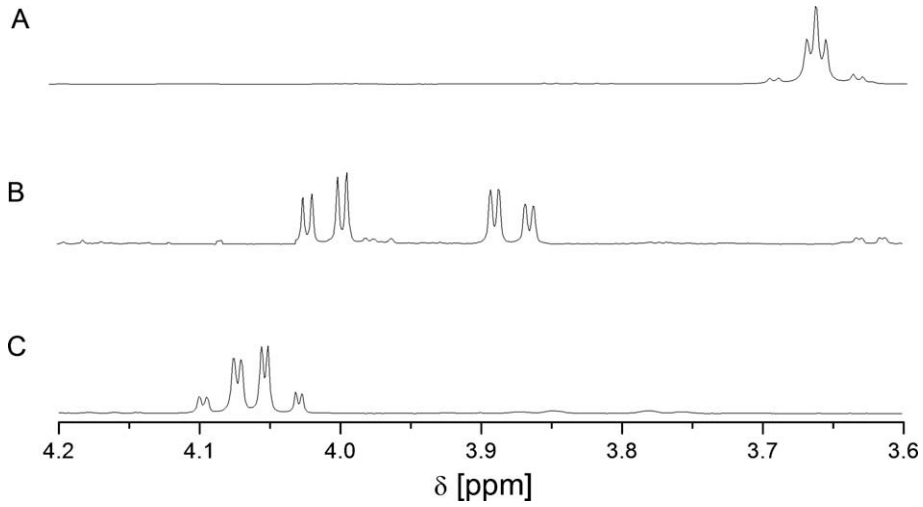

Fig. 2. ^{1}H NMR spectra of the saccharide C(5′) region of **1** (A), **2** (C) and **3** (B).

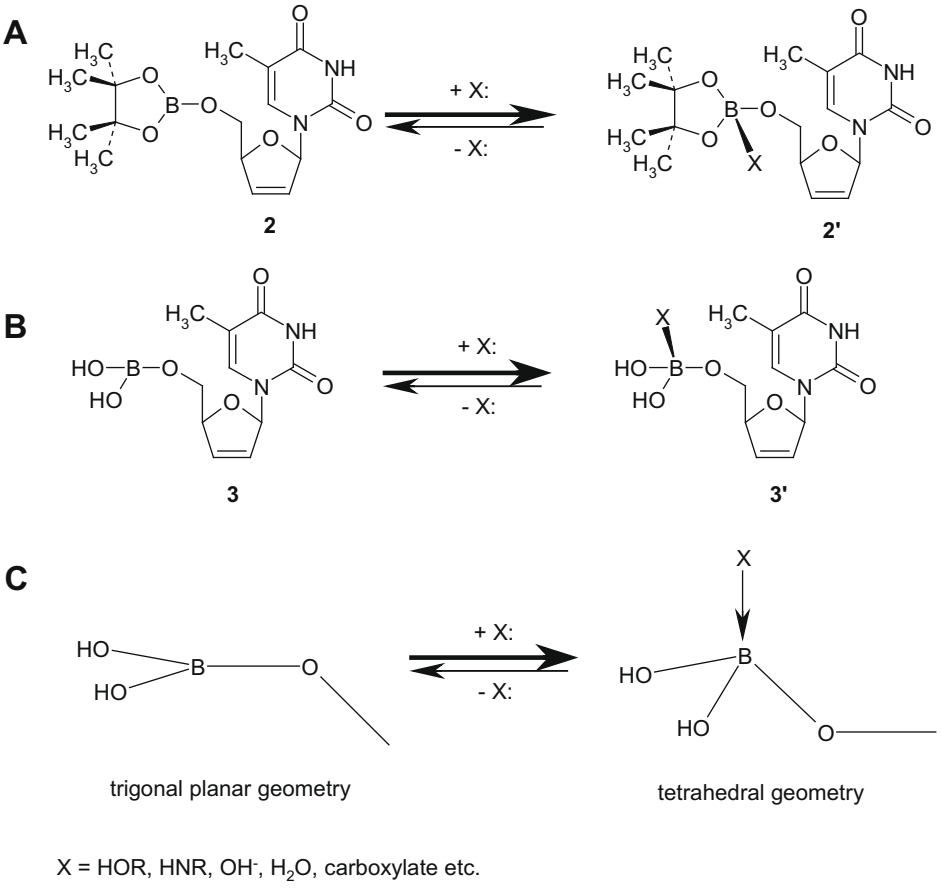

Fig. 3. Coordination equilibrium of trigonal planar and tetrahedral geometry of boron compounds with additional electron pair donor.

tetrahedral adduct. As a consequence of the high stability of eight-electron products, is the fact that vast majority of boron compounds contain only traces of trigonal form. Moreover, stable eight-electron tetrahedral complexes are often formed by intra- or extra-molecular interactions with bond lengths of 1.5–2 Å, found in X-ray diffraction data [19]. The stability of final products depends on steric and electronic properties of boron-attached groups [20].

Coordination equilibrium (*vide supra*) was confirmed with the use of the ^{11}B NMR method. The observed ^{11}B resonance of **2** was found at approx. 22.4 ppm in non-coordinating solvent. Addition of 50 μl of H_2O into NMR tube (approx. 20 mg of **2**) resulted in a significant *upfield* shift of broad ^{11}B resonance to −10.0 ppm, with additional 100 μl of H_2O shifting the described resonance even more *upfield*, up to −11.2 ppm. Titration of the sample with additional amounts of water did not result in further shifting of

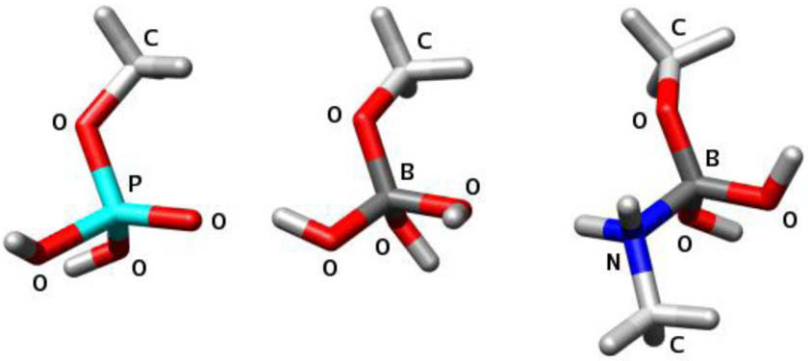

Fig. 4. The optimized geometries of stavudine-5′-CH_2—O—X models: methylphosphate (X = phosphate group, left) and its boron analogues: methylboronic acid–hydroxyl complex (X = —$B(OH)_3^-$, middle) and methylboronic acid–methylamine complex (X = —$B(OH)_2$:NH_2CH_3, right).

^{11}B resonance. Those observations confirm the equilibrium hypothesis (Fig. 3), assuming the formation of new compound (**2′**·H_2O) of tetrahedral geometry that dominates after addition of water. Similar behavior was also observed for **3**, apparently forming **3′**·H_2O. It should be noted that no significant changes were observed in the ^{1}H NMR spectrum during H_2O titration, indicating that no side reactions took place.

In accord, the ^{11}B chemical shift was previously observed to be strongly dependent on electronic environment of boron atom, with formation of tetrahedral complexes, resulting from coordination of additional lone-pair or anionic donor, shifting boron resonances *upfield* even by 20–40 ppm [15–18,20].

Considering tetrahedral boron moieties as potentially capable of mimicking the steric and also electronic properties of phosphate group, the geometries and partial atomic charges of phosphate and borane moieties were compared by theoretical calculations on three model molecules: methylphosphate, methylboronic acid–hydroxyl complex and methylboronic acid–methylamine complex (Fig. 4). The density functional method B3LYP/LANL2DZ was used to optimize geometries and to calculate Merz–Kollman ESP charges [21,22] and molecular volume.

All three molecules (Fig. 4) show tetrahedral geometry. The calculated Merz–Kollman charges for external (hydroxyl and P═O) oxygen atoms are compared in Table 1. For geometry comparisons, three parameters were used: Y—O bond lengths, O—Y—O bond angles and Y—O—C bond angles, where Y denotes P or B atom in model molecules. The bond angles are similar for all three molecules, whereas the B—O bond lengths are by about 0.2 Å smaller than P—O. Molecular volume was calculated for molecules 1 and 2. The obtained volumes were similar too, being 72.6 cm^3/mol for methylphosphate and 69.3 cm^3/mol for methylboronic acid–hydroxyl complex. The theoretical results suggest that local structure of phosphate and borane moieties should exhibit similar short-range (steric, determined by molecular volume, bond lengths and angles) as well as long-range (mainly electrostatic, determined by electronic density distribution roughly characterized by Merz–Kollman charges) intermolecular interactions with the environment. In consequence, one can expect a similar activity of phosphate and borane moieties in selected biochemical systems.

3. Conclusions

Preparation routes of novel boron-containing derivatives of anti-HIV agent stavudine (5′-*O*-(4,4,5,5-tetramethyl-1,3,2-dioxaboronate)-2′-3′-didehydro-2′-3′-dideoxythymidine and 5′-O-(dihydroxyboronate)-2′-3′-didehydro-2′-3′-dideoxythymidine) are presented. The new compounds, were prepared by one-step reaction between stavudine and commercially available pinacolborane or borane–dimethylsulfide complexes. Resonances of 5′-hydrogens of **1** form a multiplet at 3.67 ppm, whereas the hydrogen atoms at the corresponding position of compound **2** resonate at 4.05. In case of **3**, the steric and electronic differentiation between 5′-methylene group hydrogen atoms is large enough to form two distinct multiplets at 3.80 and 3.93 ppm. ^{11}B NMR spectra of **2** and **3** contain broad resonances at 22.4 and 17.8 ppm for **2** and **3**, respectively. Coordination equilibrium was analyzed with the use of the ^{11}B NMR method. The observed ^{11}B resonance of **2** was found at approx. 22.4 ppm in non-coordinating solvent. Addition of small amount of water into NMR tube with **2** or **3** solutions resulted in a significant *upfield* shift of broad ^{11}B resonances. Those observations confirm the equilibrium hypothesis assuming the formation of new compound of tetrahedral geometry that dominates after addition of water. The theoretical results suggest that local structure of phosphate and borane moieties should exhibit similar short-range as well as long-range intermolecular interactions with the environment.

4. Experimental

4.1. Analytical methods

All NMR spectra were obtained with 500 MHz Bruker Avance II spectrometer operating in the quadrature mode. The residual peaks of deuterated solvents were used as internal standards. Structures of **1**, **2** and **3** were also confirmed by several 2-dimensional spectra: ^{1}H–^{13}C Heteronuclear Single Quantum Coherence (HSQC); ^{1}H–^{1}H Correlation spectroscopy, (COSY) and Distortionless

Table 1
Selected atomic charges and geometric properties of stavudine-5′-CH_2—O—X models. Y = P or B atom.

Partial atomic charges	Methylphosphate	Methylboronic acid–hydroxyl complex	Methylboronic acid–methylamine complex
O (external O—H or ═O)	−0.9/−0.8/−0.7 (for ═O)	−0.9/−1.0/−1.0	−1.0/−0.92
Geometry			
Distances Y—O	1.7/1.7/1.69/1.58 (for P═O)	1.5/1.5/1.5/1.5	1.46/1.45/1.46/1.68 (for B—N)
Angles O—Y—O	106.2/99.5/115.2	105.5/114.4/108.13	115.1/116.2/99.9
Angle Y—O—C	120.5	117.0	121.3

Enhancement by Polarization Transfer (DEPT) experiments. All ^{11}B NMR spectra were recorded using pure quartz 5 mm NMR tube.

Elemental analysis was performed using Elementar Vario EL-3 analyzer. All other reagents and deuterated solvents of the highest commercially available grade were purchased from Aldrich and used without further purification. Rubber septa joints were also purchased from Aldrich. All procedures, including preparation of samples for the NMR measurements, were carried out under nitrogen atmosphere. All the computations were performed with the Gaussian 03 program [23] on the computer cluster of ICM UW, Warsaw.

4.2. Modified synthetic route for 2′-3′-didehydro-2′-3′-dideoxythymidine (stavudine, D4T, **1**)

Thymidine (5 g, 20.6 mmol) was added at −15 °C to solution of methylsulfonyl chloride (MsCl, 3.52 ml, 45.4 mmol) in pyridine (43 ml). After 60 min. of vigorous stirring, water (1–2 °C, 600 ml) was added to the reaction mixture. Resulting mixture was stirred at 4 °C for another 24 h, and filtered off. White product was then washed with three 10 ml rations of water (cooled to 0 °C) and dried under high vacuum.

Reaction product was dissolved in sodium hydroxide solution (400 ml of 1.06 M solution) and refluxed for 2 h. Acetone (300 ml) was added to the reaction mixture cooled to 4 °C over a 30 min period. Precipitate that formed was filtered out, washed twice with acetone (2 × 20 ml, cooled to 4 °C) and vacuum dried. The product, in the form of white/grayish crystals, was used in the following reaction step without further purification. The crystals (0.94 g) were dissolved in anhydrous DMSO (5 ml) and a solution of potassium *tert*-butoxide (1.02 g in 2 ml of DMSO) was added dropwise. After the mixture was allowed to react for 2 h at room temperature, glacial acetic acid (1:1 v/v in ethanol) was used to neutralize it to pH ≈ 7, and the product was extracted with hexane (2 × 20 ml). Hexane layer was separated and dried under high vacuum. The resulting compound **1**, obtained with 42% yield, showed 99.5% purity, as judged from the ^1H NMR spectrum. Elemental analysis: C 57.62% (calculated 57.69%) H 5.87% (calculated 5.81%); N 13.42% (calculated 13.45%). ^1H NMR ($CDCl_3$, δ [ppm]): 7.50 (m, J = 1.3 Hz, 1H, C(6)H); 6.82 (m, J = 1.6 Hz, 1H, C(1′)H); 6.35 (m, 1H, C(2′)H); 5.86 (m, 1H, C(3′)H); 4.87 (m, 1H, C(4′)H); 3.67 (m, 2H, C(5′)H); 1.74 (d, J = 0.9 Hz, 3H, Me-C(5)).

4.3. Synthesis of 5′-O-(4,4,5,5-tetramethyl-1,3,2-dioxaboronate)-2′-3′-didehydro-2′-3′-dideoxythymidine (**2**)

1 (25 mg) was dissolved in anhydrous DMSO (50 μl) and added dropwise to the solution of 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (384 μl in 10 ml of anhydrous $CHCl_3$). The reaction mixture was refluxed for 24 h, followed by removal of solvents under vacuum. The resulting material was left for crystallization in tetrahydrofuran (5 ml, 72 h, 4 °C), with crystals filtered off and liquid fraction dried under high vacuum. Thick oil obtained from THF fraction was containing almost pure **2**. Compound **2** was obtained with 95% yield and showed 96% purity, as judged from the ^1H NMR spectrum. Elemental analysis: C 54.85 (calculated 54.88%); H 6.69 (calculated 6.62%); N 7.99% (calculated 8.00%). ^1H NMR ($CDCl_3$, δ [ppm]): 9.48 (s, 1H, NH); 7.54 (m, J = 1.3 Hz, 1H, C(6)H); 7.00 (m, J = 1.9 Hz, 1H, C(1′)H); 6.26 (m, 1H, C(2′)H); 5.79 (m, 1H, C(3′)H); 4.88 (m, 1H, C(4′)H); 4.05 (m, 2H, C(5′)H); 1.89 (d, J = 1 Hz, 3H, Me-C(5)); 1.20 (s, 12H, $(CH_3)_4$). ^{13}C NMR ($CDCl_3$, δ [ppm]): 164.64, 151.35 ($C_{C=O}$); 137.02 (C(6)); 133.95 (C(2′); 126.97 (C(3′)); 89.34 (C(1′)); 86.02 (C(4′)); 75.14 (O-CMe_2); 65.11 (C(5′)); 29.69 ($(CH_3)_4$); 12.09 (C(5)-Me). ^{11}B NMR ($CDCl_3$, δ [ppm]): 22.37.

4.4. Synthesis of 5′-O-(dihydroxyboronate)-2′-3′-didehydro-2′-3′-dideoxythymidine (**3**)

1 (25 mg) was dissolved in anhydrous DMSO (50 μl) and added dropwise to the solution of borane–dimethylsulfide complex (120 μl in 2 ml of anhydrous CH_2Cl_2). The reaction mixture was stirred for 24 h (0 °C), followed by removal of solvents under vacuum. Deoxygenated water (69 μl) in THF (2 ml) was added dropwise to the obtained liquid material and the mixture allowed to react for 2 h, then extracted with water/chloroform (10 + 10 ml) system. Organic solvents from chloroform phase were vacuum-evaporated and the resulting solid dried under high vacuum. Compound **3** was obtained with 95% yield and showed 96% purity, as judged from the ^1H NMR spectrum in. Elemental analysis: C 44.75 (calculated 44.81%); H 4.92% (calculated 4.89%); N 10.40% (calculated 10.45%). ^1H NMR ($CDCl_3$, δ [ppm]): 8.33 (s, 1H, NH); 7.47 (m, J = 1.3 Hz, 1H, C(6)H); 7.01 (m, J = 1.9 Hz, 1H, C(1′)H); 6.32 (m, 1H, C(2′)H); 5.84 (m, 1H, C(3′)H); 4.91 (m, 1H, C(4′)H); 3.93 + 3.80 (m, 2H, C(5′)H); 1.86 (d, J = 1 Hz, 3H, Me-C(5)). ^{13}C NMR ($CDCl_3$, δ [ppm]): 163.62 ($C_{C=O}$); 136.58 (C(6)); 134.50 (C(2′); 125.53 (C(3′)); 89.92 (C(1′)); 87.08 (C(4′)); 63.42 (C(5′)); 12.43 (C(5)-Me). ^{11}B NMR ($CDCl_3$, δ [ppm]): 17.76.

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