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SYNTHESIS AND CONFORMATIONAL STUDIES OF 3'-(2'-AMINOBORANE-2'-DEOXYURIDYL)-5'-THYMIDILYL HYDROGEN PHOSPHATE TO BE USED IN THE CONSTRUCTION OF OLIGONUCLEOTIDE SEQUENCES

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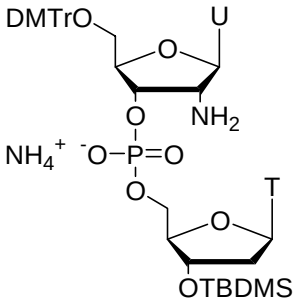
Abstract- The synthesis of the boronated dinucleotide (**2)** starting from commercially available building blocks was accomplished according to the phosphoramidite approach. The dimer, which can be used in the construction of oligonucleotide sequences, is itself interesting for its potential applications in Boron Neutron Capture Therapy and as a possible inhibitor of HIV-1 integrase. Computational studies were also performed showing its preferential C3'-*endo* (RNA-like) conformation.

In the last decade a great deal of effort has been directed toward the so called antisense approach in human therapy.[1] This approach is based on the binding, *via* Watson and Crick base pairing, of an oligoribo- or deoxyribonucleotide to a specific mRNA sequence associated with a diseased state and the subsequent inhibition of the translational event leading to a detrimental protein (antisense). The advantage of this concept over traditional small molecule protein targeting lies in the fact that only minimal structural knowledge about the target RNA (its base sequence) is required and that, in principle, an antisense oligodeoxynucleotide (ODN) can be designed to target any single gene within the entire human genome, potentially creating specific therapeutics for any disease in which the causative gene is known.
2'-Modified nucleosides are used in the development of antisense therapeutics and are important probes for the rapid screening of oligonucleotide sequences displaying high affinity toward protein targets.[2] Nucleosides containing a primary amine at 2' facilitate the incorporation of several reporter molecules

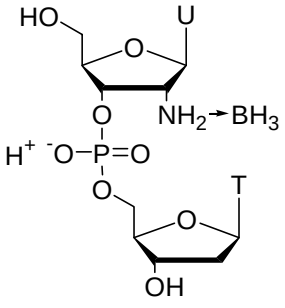
or labels, such as transition metal complex and fluorescent dyes, into oligonucleotides[2] and, more importantly, it has been shown that the presence of 2'-amino groups protects the molecule against degradation by RNase and alkaline cleavage increasing its stability *in vivo*.[1a]

Moreover, it is well established that sugar modifications that result in a preference for a C3'-*endo* or northern (N) conformation, typical of the ribose sugar in RNA duplexes, tend to result in oligos which form more stable A-type duplexes with complementary RNA. In RNA it is the presence of 2'-OH which drives the pseudorotational equilibrium of the ribose ring to the N-conformer, which is attributed to a *gauche effect* (GE) between 2'-OH and 4'-O (ring oxygen) combined with an *anomeric effect* (AE) which operates when the base is in a pseudoaxial position.[3] We therefore chose as our working hypotesis to mimic RNA-like or C3'-*endo*-like structures with nucleoside analogues in order to beneficially influence the RNA binding behaviour.

Considering the importance of 2'-modified nucleosides and the increasing evidence that boron containing biomolecules can play a much greater role in different areas of life science[4] (i.e. Boron Neutron Capture Therapy, a radiation therapy that can selectively destroy cells that have preferentially taken up boron),[5] our objective was to investigate the synthesis of two oligonucleotides sequences containing sugar modified units characterized by the presence of a 2' $NH_2 \rightarrow BH_3$ moiety and to study their behaviour in melting temperature (T_m) studies. This modification, which appears to possess sufficient chemical stability and no demonstrable toxicity, was supposed to shield the 3' position thus preventing degradation by nucleases[1e] and, at the same time, due to the low steric hindrance of BH_3 group,[4f] to allow the Watson-Crick hydrogen bonding scheme to be maintained. This had to be accomplished by introducing the $NH_2 \rightarrow BH_3$-containing nucleoside into an oligonucleotide using standard automated DNA synthesis. To this end, following the phosphoramidite approach, we first synthesised dimer (**1**) (Figure 1) which was the key intermediate for the preparation of the target compound (**2**) on which molecular modeling studies were performed in order to establish the preferential conformation adopted by the sugar moieties in the molecule.



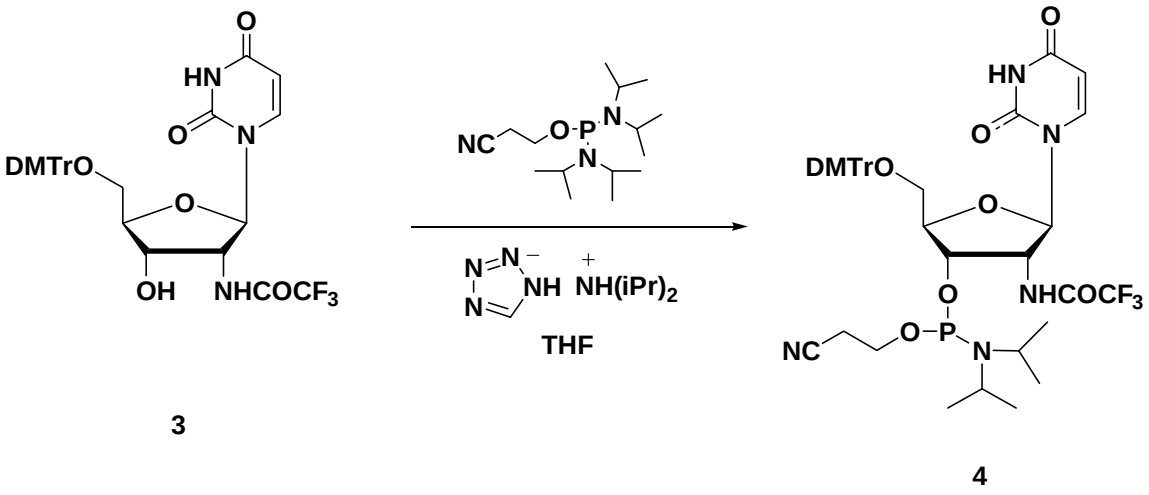
1



2

Figure 1

Commercially available uridine was used as starting material to synthesise the key intermediate (**3**) (Scheme 1) orthogonally protected at 5' and 2'.[6] Monomer (**3**) was then prepared for solid-phase-style coupling by converting it to its 3'-*O*-phosphoramidite (**4**) using 2-cyanoethyl-*N*,*N*,*N*',*N*'-tetraisopropylphosphorodiamidite under standard conditions.[3c]



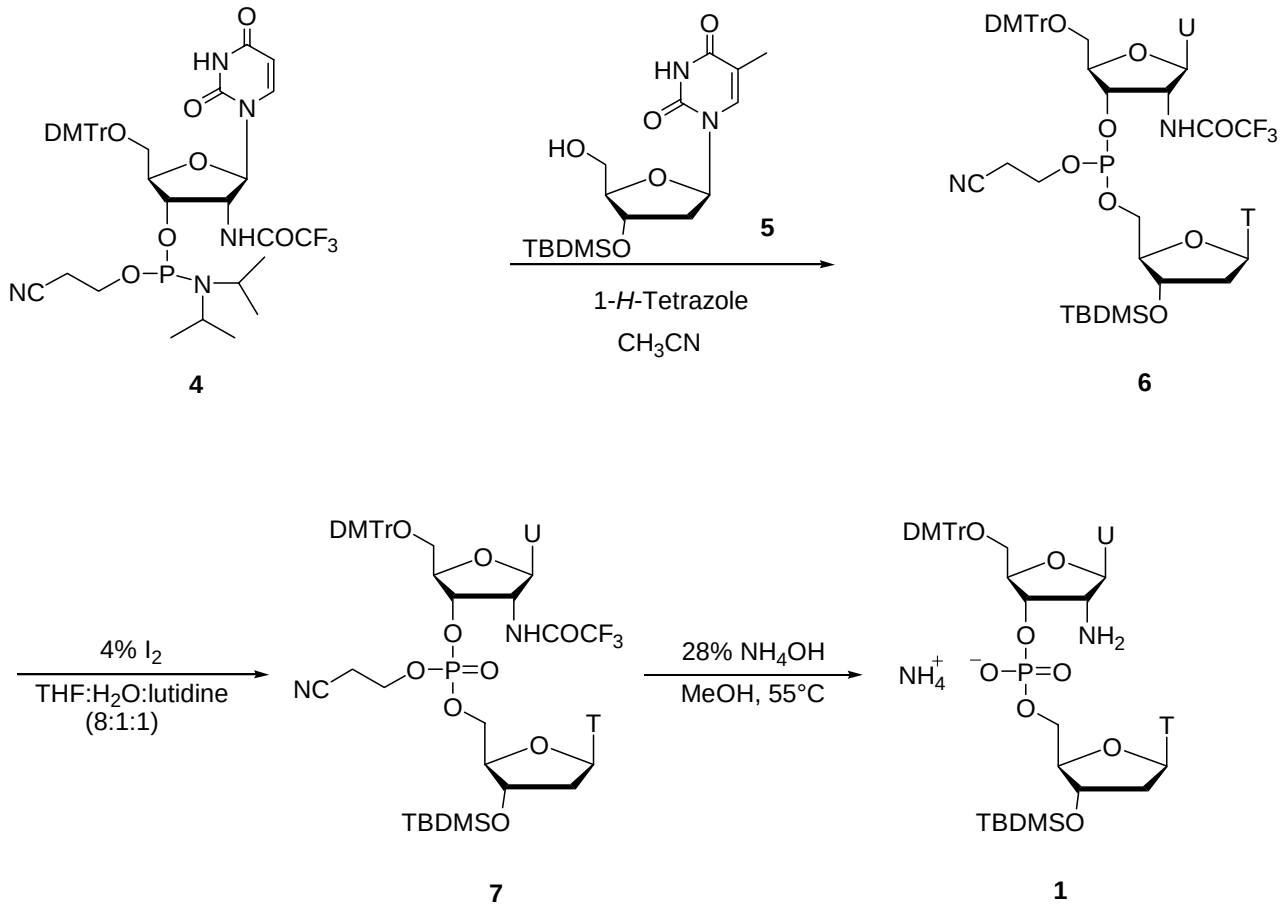
3 **4**

Scheme 1

On the other hand, monomer (**5**) (Scheme 2) was prepared starting from commercially available thymidine in three protection-deprotection steps.[7] Coupling of **4** with 3'-protected thymidine (**5**) in the presence of 1*H*-tetrazole gave the corresponding dimer (**6**) as a mixture of two diastereoisomers. The coupling reaction is catalysed by tetrazole, which protonates the *N*,*N*-diisopropylphosphoramidite, and converts the diisopropylamino moiety into a good leaving group. The protonated amino group is diplaced by the 5'-hydroxy group of the second monomer and the dimer is formed.[8] Rapid oxidation of phosphite (**6**) to the more stable phosphate (**7**) was accomplished by addition of a 4% solution (w/v) of I_2 in THF/2,6-lutidine/H_2O (8:1:1). Treatment of **7** with a methanolic ammonia solution allowed the simultaneous removal of cyanoethyl moiety and deprotection of the 2'-amino function giving **1** in quantitative yield after purification on Sephadex LH-20.

At first, boronation on the protected dimer (**1**) was attempted. To perform the reaction, borane-THF and borane-dimethyl sulfide complex were not taken into consideration because of their strong reducing ability which may cause base modification[9] and we decided to use a borane-trialkylamine complex with a low hydroborating ability.[10] **1** was dissolved in THF and reacted with 6 equiv of borane-*N*,*N*-diisopropylethylamine complex (DIPEA· BH_3) to give the corresponding boronated dinucleotide (**8**) (Scheme 3). Purification of **8** proved to be quite difficult. In fact, flash chromatography on silica gel afforded a partially degraded compound as demonstrated by ^{11}B-NMR spectrum which showed two

peaks: -20.771 ppm of $BH_3 \rightarrow NH_3$ and +18.412 ppm of $B(OH)_3$ deriving from the hydrolisis of the aminoborane group on silica gel; on the other hand, a simple filtration on Sephadex LH-20 prevented any degradation but afforded **8** still contaminated by the presence of DIPEA· BH_3 complex (quartet at -13.45 ppm).

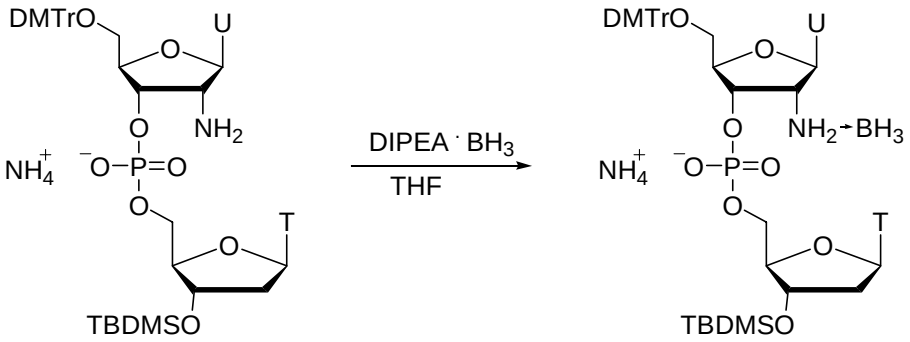


Scheme 2

As a result of these observations and considering the incompatibility of borano- and trityl groups during acid treatment,[4d, 11] we decided to perform the boronation directly on the deprotected dimer. Deprotection of 3' and 5' positions of the dimer (Scheme 4) was accomplished with TBAF and 80% CH_3COOH, respectively, giving the deprotected dinucleoside (**9**) in 88% yield after several washings with ethyl acetate. Due to the high polarity of the products, the reactions were monitored by analytical HPLC.

Treatment with a large excess of DIPEA· BH_3 in THF resulted only in a partial conversion of the starting material, probably due to solubility problems. Better results were obtained repeating the reaction in the more polar CH_3CN: in this case, using up to 24 eq. of the reagent, the HPLC analysis showed the

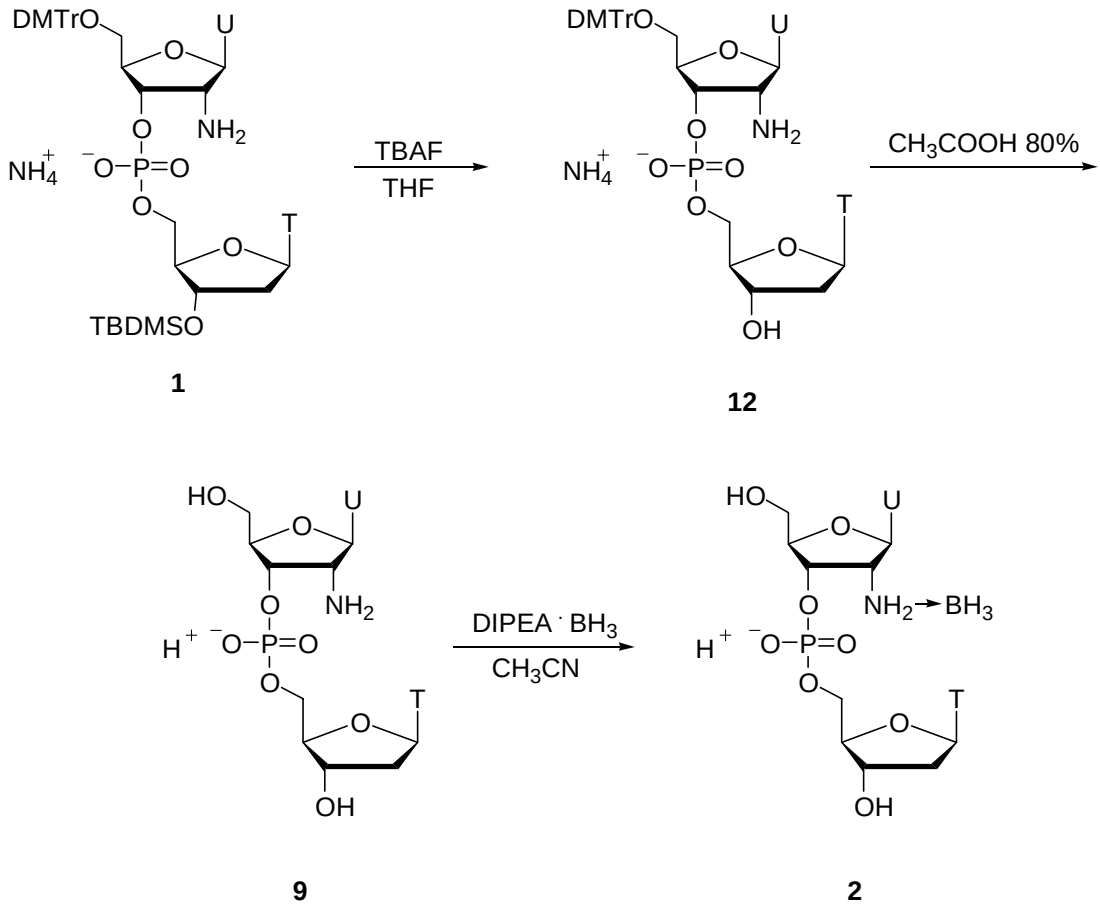
complete conversion of dimer (**9**) to the corresponding boronated compound (**2**) which was purified by semipreparative HPLC and identified by mass spectrometry analysis.



1 **8**

Scheme 3

Today, almost all synthetic oligonucleotides are prepared by solid phase phosphoramidite techniques.[12]

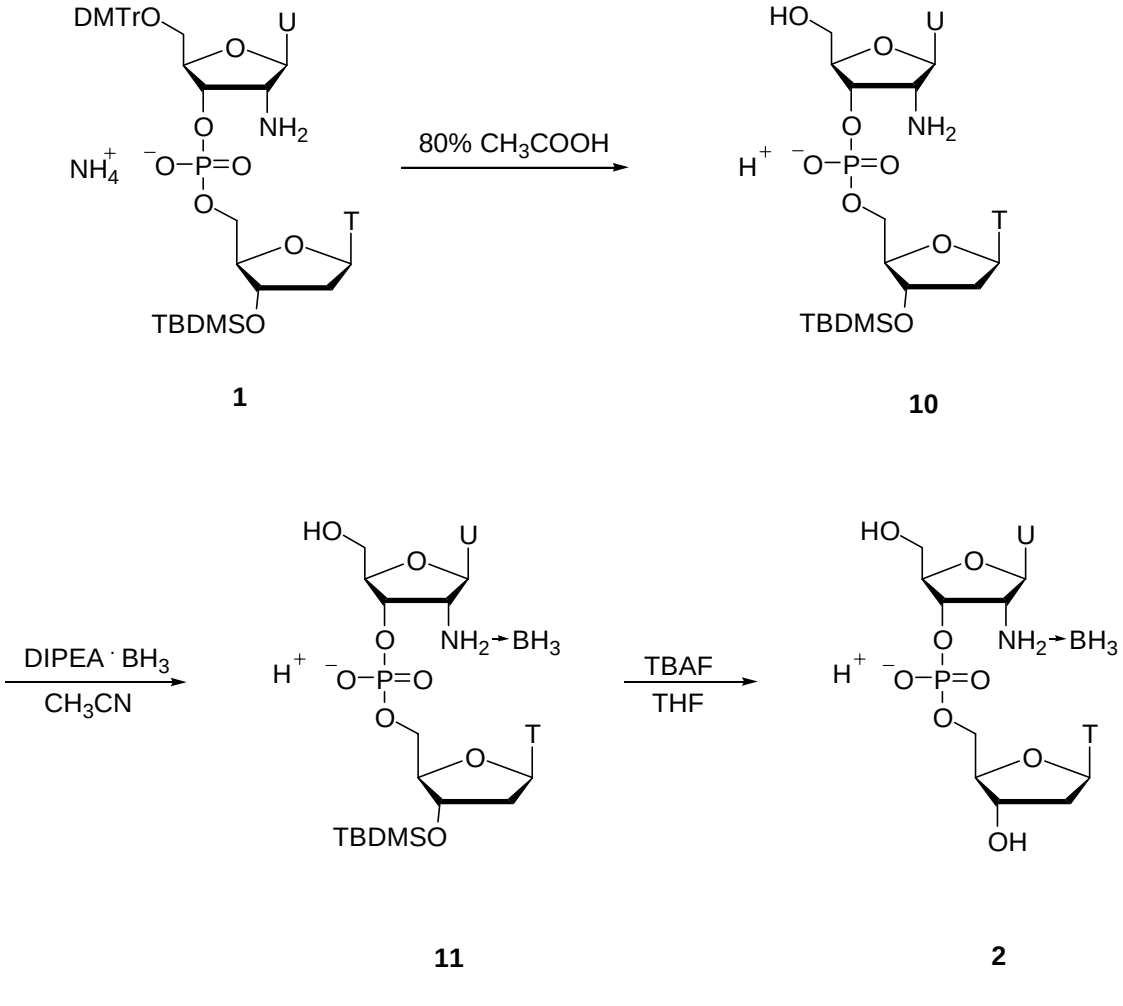


1 **12**

9 **2**

Scheme 4

In order to have a synthetic sequence that could be applied to the solid phase synthesis of oligonucleotides, another approach to the preparation of **2** was attempted.



1 **10**

11 **2**

Scheme 5

To this end, **1** was selectively deprotected at 5' using 80% CH_3COOH to give the partially protected dimer (**10**) in quantitative yield (Scheme 5). Considering that the basic chemical step that results in oligonucleotide chain extension involves the reaction of an activated monomer in solution with the free 5'-OH of the support-bound oligonucleotide, **10** represents a good model for the oligonucleotide in elongation.

10 was then dissolved in THF and treated with a large excess of DIPEA· BH_3 to afford the partially protected boronated dimer (**11**). Deprotection at 3' gave the final compound (**2**) in good yield.

Conformational analysis on compound (8)

In order to perform a conformational analysis of compounds (**8**) and (**2**), a preliminary parameterization study of the methylaminoborane moiety was carried out by *ab initio* methods. In fact no molecular mechanics force field within those included into the MacroModel package ver. 7.2[13] is able to model compounds (**8**) and (**2**) because there are several missing bonding parameters pertinent to the boron atom type in the methylaminoborane moiety. The force field considered for the parameterization and the conformational study is AMBER* all atom notation because of the well-known applicability in nucleic acid chemistry.[14]

The methylaminoborane moiety was fully optimized with Jaguar[15] with 6-31G** basis set, RHF method and Mulliken charge distribution. The optimized structure was considered for deriving the missing parameters of the AMBER* force field.

B-H, B-N and N-C stretching and B-N-C, H-B-H and H-B-C bending parameters were biased using the geometry of the optimized *ab initio* structure. The Mulliken charge distribution was reproduced adding 3 dipole moments to the new stretching parameters. Following our previously reported approach[16] the torsional energy profiles around the bond B-N and the N-C bond were left under Van der Waals and electrostatic control.

All the implemented parameters are reported in Table 1.

Bond Stretching / Bending	**Equilibrium distance in Å / angle in deg**	**Force constant in kcal/mol**	**Dipole moment**
H-B	0.841	331.0	0.68
B-N	1.20	300.0	-1.00
N-C	1.40	300.0	0.35
H-B-H	110.8	50.0	-
H-B-N	113.7	50.0	-
B-N-C	107.7	50.0	-

Table 1: missing AMBER* parameters implemented for the methylaminoborane moiety

For non-bonding terms the Boron Van der Waals radius and epsilon were respectively set to 1.700 Å and 0.12 Kcal/mol.

In order to avoid the charge problem when new substructures are added to the AMBER* force field,[17] we simply introduced a special substructure taking into account the presence of the boron atom (Table 1), while the original force field parameters of the carbohydrate were maintained. Checking the total charge of the system we found the expected -1 due to the phosphate moiety of compounds (**8**) and (**2**). The AMBER* modified force field was used for performing the conformational search of the dinucleotide (**8**) containing the aminoborane chemical group in position 2' of one sugar moiety. The large flexibility of **8**, with 19 rotatable bonds, prompted us to carry out a long Monte Carlo simulation generating 20,000 conformers with MacroModel package.[13] The simulation was carried *in vacuo* setting the dielectric constant to 4.8, the value of the chloroform medium where the NMR spectral experiment was performed. In order to compare the theoretical results with the NMR spectral data the analysis of the C1'-C2'-C3'-C4' torsional bond of the aminoborane sugar was carried out on the large ensemble of 3,810 conformers found within 12.5 kcal/mol above the global minimum by using the Boltzmann's probability computed at room temperature.

Details about the six conformers found within 1 kcal/mol above the global minimum reveal the tendency for positive values (C3' *endo* sugar conformation), equal to 44.29 % versus negative values (C2' *endo* sugar conformation) equal to 18.01. This theoretical result is in agreement with the NMR spectral data which showed low $J_{1'-2'}$ coupling costants (range 6.8-7.9 Hz) for **8** which are very indicative of a 3'-*endo* conformation.

In Figure 2 the global minimum of **8** is reported.

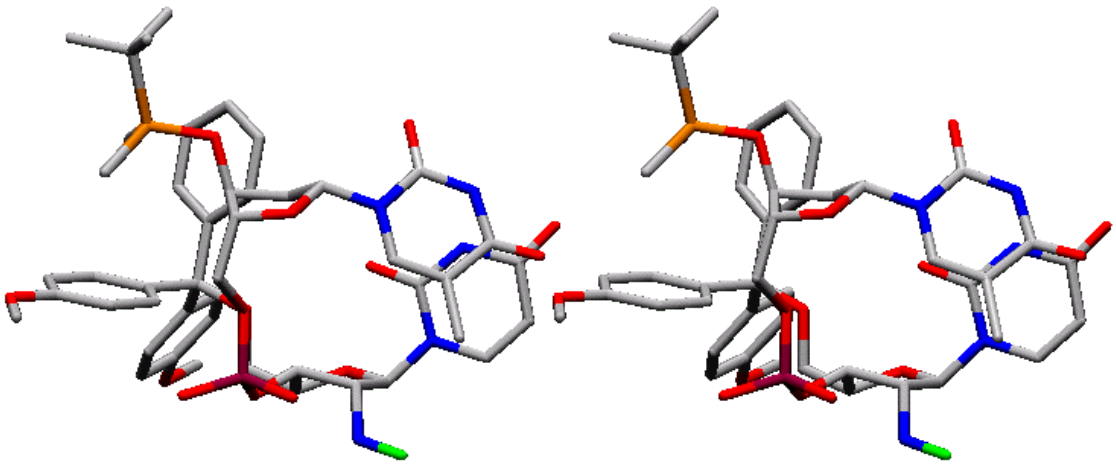


Figure 2: Stereoview of the global minimum of **8**.

As shown in Figure 2, compound (**8**) prefers the C3' *endo* conformation probably driven by the Van der Waals interactions between the DMT protective group and the silanoyl moiety as well as by the partial stacking of the two pyrimidine bases. Interestingly, the natural sugar ring in 3' was found, in this simulation, with a 3'-*exo* probability larger than 90%, indicating that the modification due to the presence of the aminoborane at 2' can strongly influence the 5-member ring pucker conformation.

The same simulation was repeated with the deprotected compound (**2**). Using the chloroform dielectric constant we observed a larger C3'-*endo* conformation of the modified sugar (about 30%) with respect to the natural one in 3' (about 9%). The same experiment carried out with dielectric constant equal to 80 could not lead to the same conclusion probably due to the low approximation of the solvating method. In order to get more accurate simulations in these modified nucleotides we are currently going on with the parameterization of boron in the GB/SA implicit model of solvation.

Conclusions

We report herein the synthesis of a 2'-modified nucleosidic dimer containing a $NH_2 \rightarrow BH_3$ functionality. It is anticipated that this dimer will ultimately be used in the construction of two oligonucleotide sequences in order to evaluate the influence of this modification on the melting temperature of the duplex. Computational studies were also performed on **8** and **2** showing that, in agreement with NMR spectral data, the C3'-*endo* (RNA-like) conformation is preferential for molecule (**8**). In the case of **2**, such a conformation, though not the preferred one, is much more populated with respect to the corresponding not boronated compound. This aspect could be particulary interesting as far as the RNA binding affinity of the corresponding oligo is concerned.

EXPERIMENTAL

General methods. All reactions were carried out under an argon atmosphere. Reagents were obtained from commercial suppliers and used without further purifications. Solvents were dried before use (CH_2Cl_2 over CaH_2, CH_3CN over P_2O_5 and CaH_2, CH_3OH over Mg, THF over Na).

Melting points are uncorrected. ^{1}H-NMR spectra on the monomers were measured at 200 MHz. Chemical shifts are reported relative to $CDCl_3$ at 7.24 ppm and TMS at 0.00 ppm. ^{1}H-NMR, ^{31}P-NMR and ^{11}B-NMR spectra on the dimers were measured at 600 MHz. Chemical shifts are reported relative to $CDCl_3$, 85% H_3PO_4 and $BF_3(OC_2H_5)_2$ respectively. EI low-resolution MS spectra were recorded with an electron beam of 70 eV. HPLC was conducted using an Agilent 1100 liquid chromatograph equipped with a variable-wavelenght UV detector recording at 254 nm. Analysis were performed on a Zorbax Eclipse

XDB-C8 reverse-phase column, using a gradient of 20→80% CH_3OH in H_2O over 25 min, with a flow rate of 1 ml/min. Purifications were performed on a ODS Semi-Prep reverse-phase column, using a gradient of 0→30% CH_3CN in triethylammonium acetate (pH 6.5) over 20 min, with a flow rate of 2 mL/min.

The elementary analyses were obtained from a Perkin-Elmer 1600 Elemental Analyzer 240L.

5'-*O*-Dimethoxytrityl-3'-*O*-[(2-cyanoethyl)-*N*,*N*-diisopropyl]phosphoramidite-2'-trifluoroacetamido-2'-deoxyuridine (4)

To a solution of **3** (625 mg, 0.98 mmol) in dry THF (3.5 mL) (diisopropyl) bis ammoniumtetrazolide (110 mg, 0.62 mmol) and 2-cyanoethyltetraisopropylphosphorodiamidite (470 μL, 1.48 mmol) were added and the reaction mixture was stirred at rt for 16 h. After removal of the solvent at reduced pressure, the residue was dissolved in $AcOC_2H_5$ and washed with a saturated solution of $NaHCO_3$, brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a white solid which was purified by flash chromatography (eluent CH_2Cl_2/CH_3OH, 9.5/0.5) affording 550 mg (67%) of **4** as a white foam. ^{1}H-NMR ($CDCl_3$): δ 1.08-1.21 (m, 12H), 2.42 (t, *J* = 6.3 Hz, 2H), 2.58 (t, *J* = 6.4Hz, 2H), 3.43-3.49 (m, 2H), 3.53-3.72 (m, 8H), 3.76 (s, 6H), 4.03-4.41 (m, 1H), 4.24-4.29 (m, 1H), 4.42-4.47 (m, 1H), 5.41 (d, *J* = 8.0 Hz, 1H), 6.20 (d, *J* = 6.0 Hz, 1H), 6.25 (d, *J* = 6.1 Hz, 1H), 6.81-7.40 (m, 13H), 7.62 (d, *J* = 8.1 Hz, 1H), 7.68 (d, *J* = 8.0 Hz, 1H). mp 240-243 °C (ethanol). Anal. Calcd for $C_{41}H_{47}N_5O_9F_3P$: C, 58.50; H, 5.63; N, 8.32. Found C, 58.70; H, 5.61; N, 8.33.

3'-[5'-*O*-Dimethoxytrityl-2'-trifluoroacetamido-2'-deoxyuridyl]-5'-[3'-*O*-*tert*-butyldimethylsilyl-thymidilyl]-2-cyanoethyl phosphate (7)

To a solution of **4** (250 mg, 0.30 mmol) in dry CH_3CN (6 mL), **5** (171 mg, 0.48 mmol) and 1-*H*-tetrazole 0.45 M in CH_3CN (5.3 mL, 2.40 mmol) were added and the reaction mixture was stirred at rt for 1 h. A 4% (w/v) solution of I_2 in a THF/H_2O/lutidine, 8/1/1 system was added dropwise to the mixture until a deep red color persisted. After 1 h the solution was diluted with CH_2Cl_2 and washed with 10% $Na_2S_2O_4$ solution. The organic layer was extracted with CH_2Cl_2 and the combined organic layers were washed with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a white solid which was purified on Sephadex LH-20 (eluent CH_2Cl_2/CH_3OH, 9.5/0.5) affording 300 mg (90%) of **7** as a white foam. ^{1}H-NMR ($CDCl_3$): δ 0.05 (ps, 12H), 0.86 (ps, 18H), 1.80 (s, 6H), 2.14-2.32 (m, 2H), 2.65-2.70 (m, 2H), 3.50-3.52 (m, 2H), 3.77 (s, 6H), 4.01-4.10 (m, 2H), 4.15-4.35 (m, 3H), 4.45-4.50 (m, 1H), 4.97-5.09 (m, 2H), 5.42 (d, *J* = 8.6 Hz, 1H), 5.72 (t, *J* = 7.3 Hz, 1H), 5.85 (t, *J* = 6.6 Hz, 1H), 6.34 (d, *J* = 8.3 Hz, 1H),

6.79-7.47 (m, 13H), 7.57 (s, 1H), 7.62(d, *J* = 8.2 Hz, 1H), 8.72-8.76 (m, 1H), 8.83-8.86 (m, 1H), 9.6 (br s, 1H), 9.90 (br s, 1H). ^{31}P-NMR ($CDCl_3$): δ –1.6225 (s), –2.5455 (s). mp 282-284 °C (ethanol). Anal. Calcd for $C_{51}H_{60}N_6O_{15}F_3PSi$: C, 55.03; H, 5.43; N, 7.55. Found C, 55.12; H, 5.42; N, 7.53.

3'-[5'-*O*-Dimethoxytrityl-2'-amino-2'-deoxyuridyl]-5'-[3'-*O*-*tert*-butyldimethlsilylthymidilyl]-phosphate ammonium salt (1)

To a solution of **7** (300 mg, 0.27 mmol) in CH_3OH (13 mL), 30% NH_4OH (13 mL) was added and the reaction mixture was kept at 58°C for 24 h. After evaporation of the solvent, the residue was purified on Sephadex LH-20 (eluent CH_2Cl_2/CH_3OH, 7/3) affording 260 mg (98%) of **1** as a white solid. ^{1}H-NMR ($CDCl_3$): δ -0.10 (s, 6H), 0.72 (s, 9H), 1.73 (s, 3H), 2.04-2.08 (m, 2H), 3.21-3.25 (m, 4H), 3.46-3.48 (m, 2H), 3.63 (s, 6H), 4.18-4.25 (m, 2H), 4.69-4.71 (m, 1H), 5.17 (d, *J* = 8.0 Hz, 1H), 5.73 (d, *J* = 7.0 Hz, 1H), 6.07 (t, *J* = 6.3 Hz, 1H), 6.67-7.24 (m, 13H), 7.39 (s, 1H), 7.54 (d, *J* = 8.0 Hz, 1H). mp >270 °C (ethanol). ESI-MS (M^-): *m/z* 962.3. HPLC retention time 18.4 min. Anal. Calcd for $C_{46}H_{61}N_6O_{14}PSi$: C, 56.32, H, 6.27, N, 8.57. Found C, 56.24; H, 6.29, N, 8.57.

3'-[5'-*O*-Dimethoxytrityl-2'-aminoborane-2'-deoxyuridyl]-5'-[3'-*O*-*tert*-butyldimethlsilyl-thymidilyl]phosphate ammonium salt (8)

To a solution of **1** (100 mg, 0.104 mmol) in dry THF (6 mL), the complex DIPEA: BH_3 (108 μL, 0.62 mmol) was added and the reaction mixture was stirred for 6 h at rt. Removal of the solvent gave a white residue which was filtered on Sephadex LH-20 (eluent CH_2Cl_2/CH_3OH, 8.5/1.5) to give 110 mg of **8** (still contaminated with DIPEA:BH_3) as a white foam. ^{1}H-NMR ($CDCl_3$): δ 0.01 (s, 6H), 0.83 (s, 9H), 1.82 (s, 3H), 2.00-2.12 (m, 2H), 2.94-3.05 (m, 3H) 3.21-3.51 (m, 2H), 3.74 (s, 6H), 3.96-4.06 (m, 4H), 4.31-4.38 (m, 1H), 4.51-4.58 (m, 1H), 5.01-5.12 (m, 1H), 5.32 (d, *J* = 8.0 Hz, 1H), 6.21 (t, *J* = 6.8 Hz, 1H), 6.31 (d, *J* = 7.9 Hz, 1H), 6.70-7.45 (m, 15H). ^{11}B-NMR ($CDCl_3$) δ -19.471 (bs), -13.740 (q). mp >270 °C (ethanol). Anal. Calcd for $C_{46}H_{64}N_6O_{14}BPSi$: C, 55.53; H, 6.48; N, 8.45. Found C, 55.49; H, 6.50; N, 8.42.

3'-[5'-*O*-Dimethoxytrityl-2'-amino-2'-deoxyuridyl]-5'-thymidilylphosphate ammonium salt (12)

To a solution of **1** (100 mg, 0.104 mmol) in dry THF (5 mL) 1 M TBAF in THF (400 μL, 0.40 mmol) was added and the reaction mixture was stirred at rt for 12 h. Evaporation of the solvent gave a yellow oil which was purified by column chromatography (eluent $AcOC_2H_5/CH_3OH/NH_4OH$, 7/2.5/0.5) affording 75 mg of **12** (85%) as a white solid. ^{1}H-NMR ($CDCl_3$): δ 1.81 (s, 3H), 2.11-2.14 (m, 1H), 2.19-2.23 (m, 1H), 3.30-3.34 (m, 4H), 3.57-3.60 (m, 2H), 3.71 (s, 6H), 3.89-3.91 (m, 1H), 3.96-3.98 (m, 1H), 4.72-4.74

(m, 1H), 5.19 (d, *J* = 8.2 Hz, 1H), 5.85 (d, *J* = 7.2 Hz, 1H), 6.16 (t, *J* = 6.1 Hz, 1H), 6.75-7.29 (m, 13H), 7.49 (s, 1H), 7.65 (d, *J* = 8.1 Hz, 1H). mp >270 °C (ethanol). ESI-MS (M^-): *m/z* 848.3. HPLC retention time 15.1 min. Anal. Calcd for $C_{40}H_{47}N_6O_{14}P$: C, 55.43; H, 5.47; N, 9.70. Found C, 55.57; H, 5.46; N, 9.72.

3'-(2'-Amino-2'-deoxyuridyl)-5'-thymidilyl hydrogen phosphate (9)

12 (30 mg, 0.035 mmol) was dissolved in 80% CH_3COOH (900 μL) and the mixture was stirred at rt for 2 h. Evaporation of the solvent gave a yellow solid which was dissolved in water and extracted with AcOEt. The aqueous layer was evaporated to give 17 mg (88%) of **9** as a white solid. ^{1}H-NMR (CD_3OD): δ 1.84 (s, 3H), 2.15-2.19 (m, 1H), 2.21-2.26 (m, 1H), 3.66 (dd, *J* = 2.8, 12.1 Hz, 2H), 3.73 (dd, *J* = 2.64, 12.1 Hz, 2H), 3.90-3.94 (m, 2H), 4.25-4.28 (m, 1H), 4.42-4.46 (m, 1H), 4.52-4.56 (m, 1H), 5.64 (d, *J* = 8.0 Hz, 1H), 5.84 (d, *J* = 7.8 Hz, 1H), 6.26 (t, *J* = 7.2 Hz, 1H), 7.72 (s, 1H), 7.95 (d, *J* = 8.1 Hz, 1H). mp >270 °C (ethanol). ESI-MS (M^-): *m/z* 546.2. HPLC (semipreparative) retention time 6.9 min. Anal. Calcd for $C_{19}H_{26}N_5O_{12}P$: C, 41.69; H, 4.79; N, 12.79. Found C, 41.81; H, 4.80; N, 12.80.

3'-(2'-Aminoborane-2'-deoxyuridyl)-5'-thymidilyl hydrogen phosphate (2)

To a solution of **9** (17 mg, 0.031 mmol) in dry CH_3CN (5 mL), the complex DIPEA-BH_3 (130 μL, 0.75 mmol) was added and the reaction mixture was stirred at rt for 24 h. Evaporation of the solvent gave a pale yellow solid which was dissolved in water. The aqueous solution was extracted several times with $AcOC_2H_5$ and then the organic layer was evaporated to give a white solid which was purified by semi-preparative HPLC affording 10 mg (60%) of **2** as a white solid. ^{1}H-NMR (CD_3OD): δ 2.01 (s, 3H), 2.20-2.24 (m, 1H), 2.26-2.30 (m, 1H), 3.69-3.72 (m, 2H), 3.75-3.77 (m, 2H), 3.99-4.02 (m, 2H), 4.13-4.18 (m, 1H), 4.32-4.35 (m, 1H), 4.48-4.52 (m, 1H),5.71 (d, *J* = 8.0 Hz, 1H), 6.14 (d, *J* = 7.8 Hz, 1H), 6.32 (dd, *J* = 6.1, 7.4 Hz, 1H), 7.72 (s, 1H), 7.91 (d, *J* = 8.1 Hz, 1H), 8.55 (s, 1H). ^{11}B-NMR (CD_3OD): δ -18.0--19.0 (m). mp >270 °C (ethanol). ESI-MS (M^-): *m/z* 560.1. HPLC (semipreparative) retention time 7.7 min. Anal. Calcd for $C_{19}H_{29}N_5O_{12}BP$: C, 40.66; H, 5.21; N, 12.48. Found C, 40.72; H, 5.21; N, 12.52.

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