Bononucleotides: synthesis, and formation of a new reversible boronate internucleosidic linkage†

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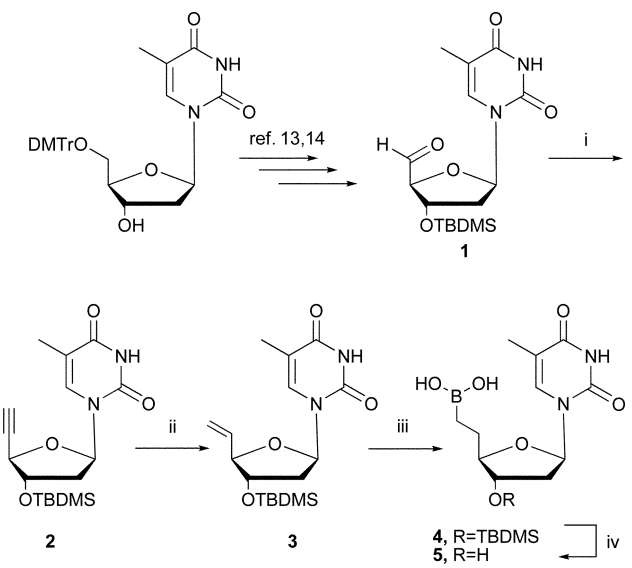
The synthesis of a bononucleotide analogue of thymidine and its association towards the formation of new borono-linked dimers is described.

Boronic acids are versatile and valuable functional groups that are increasingly used in synthetic, biological and medicinal chemistry. Most notably, major advances include the development of novel materials,[1,2] carbohydrate sensors and receptors,[3] and inhibitors of hydrolytic enzymes.[4] The broad interest in boronic acid-containing substrates results from various interesting features such as their relatively low mammalian toxicity, the electronic structure of boron and their ability to form reversible cyclic esters in the presence of *cis*-diols under physiological conditions. These properties prompted several research groups, including ours, to either synthesize boronic acid-based nucleosides[5] and nucleotides[6–8] for their pharmaceutical potential, or to use the 2′,3′-*cis*-diol function of nucleosides for their specific detection with boronic acid sensors.[9] Whilst a large number of chemically modified oligonucleotides have been introduced,[10,11] the replacement of the phosphodiester linkage by a reversible covalent binding interaction would be an ideal building material for the programmed assembly of dynamic, highly ordered nano-structures. Moreover, the borate-dependent ribose synthesis pathway recently described by Ricardo *et al.* confirmed the structure-directing function of boron-containing molecules and brought evidence of their dynamic ability to amplify stabilized structures.[12] Here, we wish to report the synthesis of the first bononucleotide isostere of 5′-monophosphate thymidine (TMP) along with its affinity in water towards various nucleosides and diols.

The boronic acid analogue of TMP (**5**) was synthesized from 5′-*O*-dimethoxytritylthymidine (5′-*O*-DMTr-T) in seven steps with 31% overall yield as shown in Scheme 1. Hence, 5′-*O*-DMTr-T was converted in three steps to the 3′-*O*-TBDMS-5′-aldehyde derivative **1**.[13,14] Homologation of the aldehyde function using dimethyl-1-diazo-2-oxopropylphosphonate (Bestmann–Ohira reagent)[15] gave alkyne **2** which was quantitatively reduced to the corresponding terminal alkene **3**[16] by catalytic hydrogenation. Hydroboration of the latter was next achieved using diisopinocampheylborane to give 3′-*O*-TBDMS boronic acid **4**. Desilylation under acidic conditions yielded bononucleotide analogue **5** (Scheme 1).‡

The reversible formation of the corresponding dinucleotide between **5** and uridine, which is characterized by a boronate internucleosidic linkage, was first studied by ^{1}H NMR in DMSO-d_6 (Fig. 1).

Indeed, NMR was shown to be a valuable tool to study the reversible covalent bonds created by the formation of boronic esters.[17] The dynamic process of binding was shown to be slow at the NMR time scale, since both free and complexed forms of uridine were distinguishable (Fig. 2). One thus noticed the appearance of peaks at 11.40 (ESI†) and 7.71 ppm assigned respectively to the NH and H_6 protons of complexed uridine. As a general trend, significant changes occur to all the exchangeable protons of uridine. Indeed, in wet DMSO the labile protons of free uridine give rise to broad resonances due to chemical exchanges. The addition of boronic acid **5**, even in a weak proportion, leads to a drastic reduction of the peak widths corresponding to the hydroxy and amine protons, indicating a decrease of the exchange kinetics in the course of the boronate formation. When the boronic acid **5** is in excess, the two signals at 5.37 and 5.09 ppm, assigned to the



Scheme 1 Synthesis of bononucleotide analogue of thymidine. *Reagents and conditions*: i. dimethyl-1-diazo-2-oxopropylphosphonate, K_2CO_3, MeOH, rt, 76%; ii. H_2, Lindlar catalyst 15%, MeOH, rt, quant; iii. (a) diisopinocampheylborane, THF, rt; (b) acetaldehyde, rt; (c) HCl 0.1 M, 72%; iv. HCl 3 M, rt, 98%.

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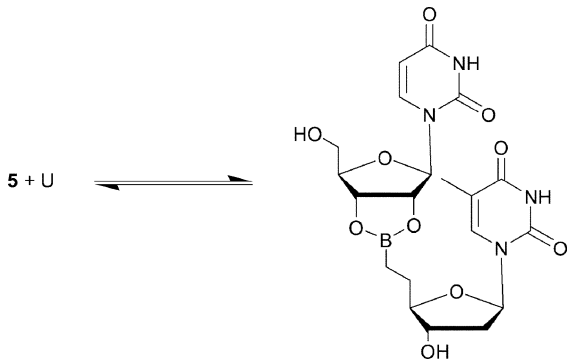

Fig. 1 Reversible formation of a dinucleotide modified by a boronate linkage.

hydroxylic protons 2′-OH and 3′-OH respectively, have vanished, leaving only the triplet signal of 5′-OH which overlapped with 3′-OH. In addition, the spectrum of the free boronic acid **5** showed a signal at 7.50 ppm for the two hydroxy groups borne by the boron atom, that also disappeared when increasing the quantity of uridine. The addition of molecular sieves (4 Å) to a stoichiometric mixture of uridine and **5** shifted totally the equilibrium towards the boronic ester, thus indicating that the dinucleotide can be easily amplified. A similar study was realized in D_2O and has shown that the boronate linkage also exists in aqueous conditions (ESI†).

The furanose ring occurs in two possible conformations referred to as the C2′-*endo* (south) and the C3′-*endo* (north) conformations. A change from one conformation to the other leads to major structural variations in the nucleoside. Hence, one can reasonably expect that the formation of the boronic ester will have a consequence on the sugar's puckering. The sugar conformation of uridine in the dinucleotide was therefore analyzed using the theory of Altona and co-workers,[18,19] and Obika *et al*.[20] Free uridine is in equilibrium between the two conformations, with a slight prevalence for the C2′-*endo* conformation (about 60% according to the coupling constant $^3J_{1'-2'}$ = 5.3 Hz). The complexation of uridine with boronic acid **5** leads to a lowering of the coupling constant $^3J_{1'-2'}$ = 2.8 Hz, indicative of a noteworthy change in the ring pucker, since this value is representative of about 75% of the north conformation. It thus appears that the boronate linkage promotes the RNA-like behaviour of the dinucleotide.

The affinities of **5** for carbohydrates were next analyzed in aqueous solution using Springsteen and Wang's colorimetric assay based on the competitive release of alizarin Red S (ARS).[21] Although K_a measurements are sensitive to the method and conditions employed, the values obtained are useful for comparative purposes. To our great satisfaction, encouraging binding of **5** with various diol systems was observed. On examination of the results shown in Table 1, it appears as expected that the *cis*-diol function is essential for an efficient binding (*cf*. entries 1 and 2). Steric factors also seem to play a key role as the binding of **5** to U is lowered by a factor of 2 with the α-anomer (*cf*. entries 1 and 3), while the binding with rC was even more favorable (entry 4). As other nucleosides are less soluble in aqueous media, we also measured the affinity of **5** with all four ribonucleotide monophosphates. The binding of pyrimidines is significantly amplified with their monophosphate derivatives, presumably because of a better hydration of the resulting dinucleotide 5′-monophosphate. The assay also revealed a higher selectivity for pyrimidine monophosphates over purine monophosphates (entries 5–8). As pyrimidine nucleosides and nucleotides are, in comparison with their purine counterparts, characterized by a higher conformational bias towards north-like conformations due to the stronger anomeric effects of cytosine and uracil,[22] these results confirmed the geometrical preferences of the resulting boronic esters. The importance of the ribose conformation for efficient binding with **5** was further demonstrated by comparing the binding of several saccharides (entries 9–11). As the bindings of **5** with saccharides are quite low, the complexes formations with ribonucleosides may also benefit entropically from internal stacking interactions. As G and C bases have

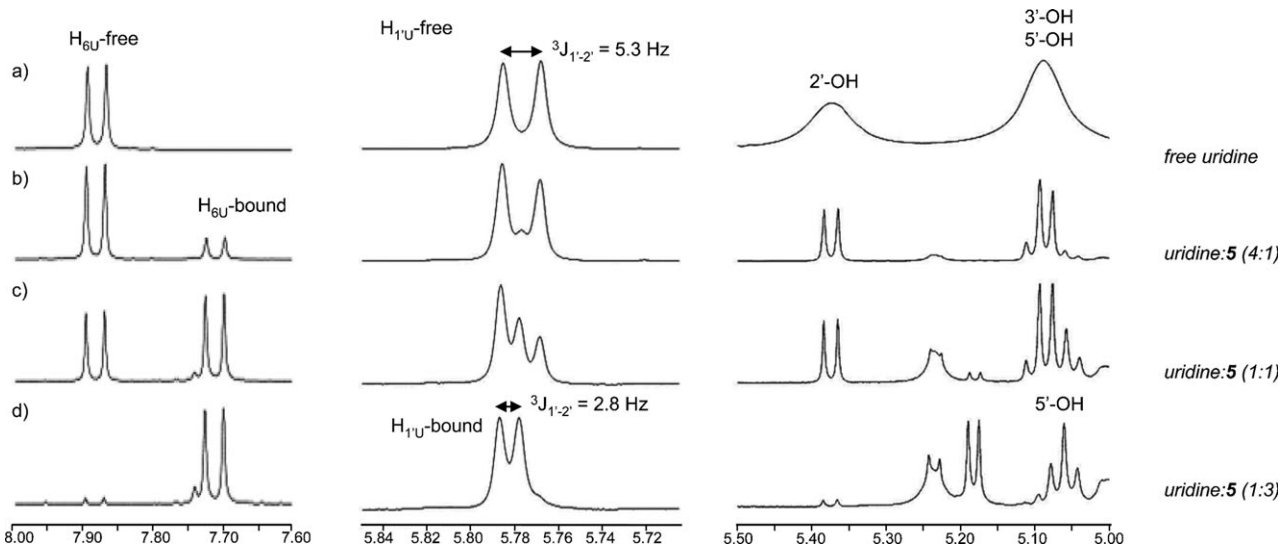

Fig. 2 Selected areas of the ^{1}H NMR spectra of (a) 23 mM of uridine, (b) 92 mM of uridine and 23 mM of boronic acid **5**, (c) 23 mM of uridine and 23 mM of **5**, (d) 23 mM of uridine and 69 mM of **5**. The samples were dissolved in DMSO-d_6 and the spectra were acquired at 300.13 MHz on a Bruker AM300 spectrometer locked on the deuterium frequency. The solvent residual peak was used as reference (2.49 ppm).

Table 1 Binding constants of **5**[a]

Entry	Diol	K_a/M^{-1}[b]
1	U	95
2	dU	6
3	α-U	44
4	rC	200
5	UMP	235
6	CMP	324
7	AMP	120
8	GMP	215
9	Fructose	10
10	Glucose	1
11	Ribose	32

[a] Conditions: pH 7.6, 0.1 M phosphate buffer. [b] Average of at least two experiments.

stronger stacking interactions than A and U bases, respectively, the stacking interactions provide a rationale to explain the differences observed between pyrimidines (compare UMP and CMP, entries 5 and 6) and purines (compare AMP and GMP, entries 7 and 8).[23] This assumption was further emphasized when we compared the affinities of **5** and *n*-butylboronic acid with U (K_a = 95 and 14 M^{-1}, respectively).

In conclusion, a new type of internucleosidic linkage has been achieved through the synthesis of the borononucleotide analogue of thymidine. The formation of this novel reversible boronic ester backbone appeared to be dependent on (i) the presence of a *cis*-diol system, (ii) a preorganized north-like sugar conformation of the diol moiety, and (iii) stacking interactions. Given that the rules of complementarity have manipulative value in creating artificial genetic systems, replacement of the phosphate-sugar backbone by a reversible boronate-sugar one could be relevant for the design of dynamic oligonuleotidic receptors and sensors. Moreover, as borate has been shown to stabilize ribose against degradation,[12] the novel backbone presented here might help us to understand the chemical etiology of nucleic acid structure.

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Notes and references

‡ Selected data for (**5**). ^{1}H NMR (D_2O): δ 0.87 (2 H, m), 1.75 (2 H, m), 1.89 (3 H, s), 2.33 (2 H, m), 3.87 (1 H, m), 4.30 (1 H, m), 6.25 (1 H, t, *J* 6.9 Hz), 7.43 (1 H, s); ^{13}C NMR: δ 12.2, 28.2, 38.5, 73.9, 85.3, 88.8, 112.2, 137.9, 152.4, 167.1; ^{11}B NMR: δ 33.5 (br); MS (ESI^+) *m*/*z* 285.1 ($[M + H]^+$, 100%); HMRS-ESI^+ *m*/*z* calc. for $C_{11}H_{18}BO_6N_2$ $[M + H]^+$ 285.1258; Found 285.1265.

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