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Dynamic boronic acid-mediated autoligation of DNA strands*

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Abstract: The single common feature of all biological systems is the dependence on self-assembly of molecular units to be morphed into well-defined functional architectures. Thanks to a dynamic equilibrium process, incorrect structural units are rejected with high levels of fidelity. The development of synthetic systems displaying similar attributes is an emerging field with wide applications from biotechnology to medicine. In this context, we developed a stimuli-responsive nucleic acid-based system relying on the reversible formation of cyclic boronate internucleosidic linkages. The dynamic assembly of this new borono-based helix has been accomplished through a DNA- and an RNA-templated autoligation process featuring a 5'-ended boronic acid oligonucleotide connecting to a 3'-ended ribonucleosidic oligonucleotide partner.

Keywords: autoligation; boronic acids; nucleic acids; self-assembly; template synthesis.

INTRODUCTION

Non-enzymatic DNA- and RNA-templated ligation reactions of oligonucleotides have been the focus of intense research over the past few decades. In these reactions, the ligation is triggered by hybridization through Watson–Crick base pairing of synthetic oligonucleotides to a DNA or RNA template. The binding of the reactants increases their effective molarity, thus promoting the ligation process. Pioneered by Orgel's work on the study of the origins of life on Earth (prebiotic chemistry) [1,2], many chemical systems have been designed to understand, mimic, and evolve the processes of storage and transfer of genetic information. Indeed, the non-enzymatic DNA- or RNA-templated ligation was found to tolerate a large variety of chemical reactions leading to the formation of natural [3,4] or synthetically modified backbones such as phosphorothioates [5,6], phosphoroselenoates [7], phosphoramidates [8], pyrophosphates [9], amines [10,11], amides [12,13], and various other metal-mediated reactions [14–16]. Among all these systems, the ones relying on autoligation reactions are particularly appealing, as no reagent needs to be added for the reaction to proceed. This feature was notably exploited for the detection of cellular RNAs through the formation of irreversible covalent junctions [15,17], but numerous efforts have also been devoted to the design of reversible DNA- or RNA-templated autoligation. Such approaches require molecular species to experience the reversible formation of covalent DNA linkages under mild conditions. Being reversible, these systems are therefore capable of responding to external factors such as temperature, pH, or molecular recognition events [18]. The main motivations behind these studies are: (1) testing the hypothesis that the early-life selection process might have taken

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advantage of reversible backbone linkages through a variety of possible combinations of information building blocks [10,19,20]; (2) evolving artificial biomolecular materials with higher levels of behavior such as healing, adaptation and response to external stimuli or environmental factors (heat, light, chemical additives, etc.) [21–25]. In this context, we recently devised a dynamic DNA- and RNA-templated autoligation system based on the reversible formation of five-membered cyclic boronate internucleosidic linkages. This parabiotic project (*next to the living*, *by modifying it*) aims at providing answers to prebiotic chemistry questions with the objective of developing a postbiotic (*bio-inspired and functional)* system.

DESIGN

Among the many existing reversible covalent reactions, boronate esters that are formed dynamically by the reaction of a boronic acid and a *cis*-diol have not been exploited so far in the field of nucleic acid analogues. Usually designed and evaluated as probes for carbohydrate detection, boronic acids can bind 1,2- or 1,3-*cis*-diol-containing molecules through the reversible formation of five- or six-membered cyclic boronate esters without chemical activation [26–29]. In the presence of water, there is an equilibrium between free boronic acids and boronate esters depending on various parameters: substitution of the diol moiety, substitution of the boronic acid, pH and temperature of the medium, presence of anions, etc. [30]. Indeed, the well-known Lewis acidity of the electron-deficient trigonal boron (sp2) species allows them to coordinate basic molecules. This coordination induces a rehybridization of boron from sp2 to sp3, leading to an anionic tetrahedral species having a carbon-like configuration. Thus, boronate esters are able to reversibly dissociate and associate and rearrange their molecular components depending on the media. Moreover, the recent discovery of the thermal stabilization of ribose by borate minerals and the demonstration that borate can be used as a phosphate mimic in enzymatic catalysis shed a new light on the potential prebiotic relevance of boron [31–34].

In our laboratory, we envisioned a DNA-templated ligation occurring through the reaction of two oligodeoxynucleotides (ODNs), one featuring a boronic acid group at its 5' end, while the other would carry a ribonucleotide at its 3' end. As such, only one chemically modified strand would be needed to achieve the replacement of the natural phosphodiester with a boronate internucleoside linkage. For this purpose, we designed a new set of 2'-deoxy-6'-boronoribonucleotide analogues **1–4** in which a 5'-linked C–B bond would mimic the monophosphate ester (Fig. 1).

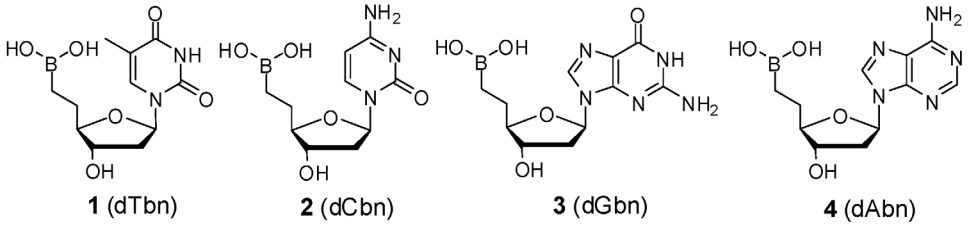


Fig. 1 2'-Deoxybororonucleotide analogues **1–4** of natural nucleotide monophosphates.

Molecular modeling of the electrostatic potentials of bororonucleotides **1–4** showed a very close distribution of charges as compared to the corresponding neutral nucleotide monophosphates, strongly suggesting that the boronic acid will not affect their base-pairing abilities (Fig. 2). Moreover, structural fit and bond measurements allowed us to demonstrate that the optimized structures of the borono-analogues fitted well with the natural molecules. Indeed, in all cases the differences in distance between the C4' and one of the oxygen of the acidic groups were comprised between 0.01 and 0.009 Å and no

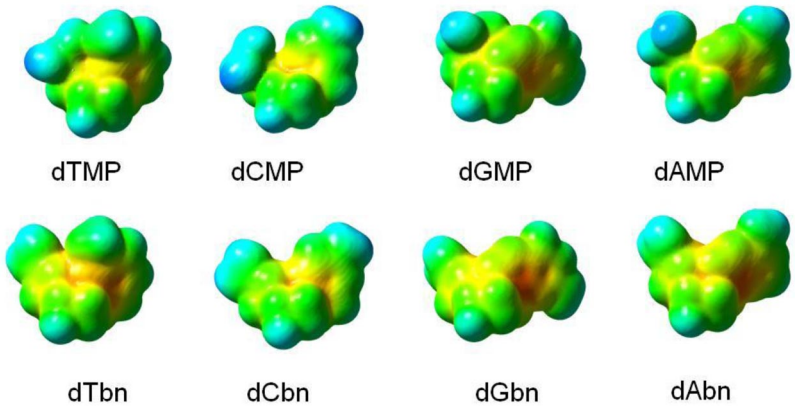
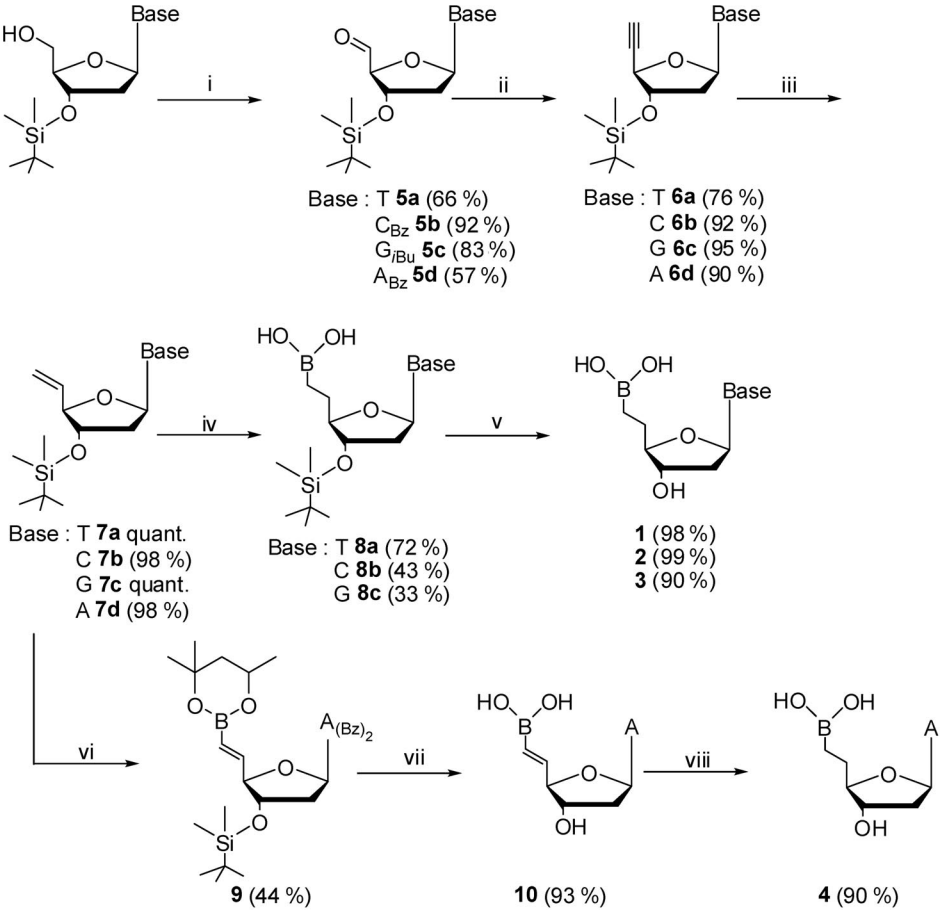


Fig. 2 Mapped electrostatic potentials of the borononucleotides (bottom) compared with the natural nucleotide monophosphates (top).

strong variation of the sugar pucker could be detected [35]. These new bio-inspired analogues appeared, therefore, as good mimic of their natural counterparts.

SYNTHESIS

The synthesis of a set of four 2'-deoxyborononucleotide analogues started from the respective 3'-*O*-TBDMS-5'-aldehydic nucleosides, which were obtained after oxidation of the corresponding 5'-hydroxy function through a slightly modified Moffatt oxidation procedure (Scheme 1) [36]. In all cases, homologation of the aldehydic function using the Bestmann–Ohira reagent (dimethyl-1-diazo-2-oxopropylphosphonate) [37–40] under mild basic conditions efficiently yielded the corresponding alkynes with concomitant protecting groups removal on C, G, and A nucleobases. Then, the alkynes were readily reduced with H_2 in the presence of Lindlar catalyst to give alkenes **7a–d** in quasi-quantitative yields. The key step of these synthesis was the introduction of the boronic acid function. For alkenes **7a–c**, this was achieved via a hydroboration reaction using diisopinocampheylborane followed by an acetaldehyde-promoted oxidative dealkylation leading to boronic acids **8a–c**. Subsequent desilylation of these latter compounds allowed us to obtain the first members of the boronucleotide family, namely, dTbn, dCbn, and dGbn [35,41]. However, we were unable to reach acceptable yields for the hydroboration of 5'-methylideneadenosine **7d** whatever the hydroborating agent used. To access the target compound, we thus envisioned a cross-metathesis reaction between the 5'-methylidene double bond and various vinyl boronates. After 6-*N*,*N*-dibenzoylation of the adenine ring, the best yields were achieved with vinyl 2-methyl-2,4-pentanediol boronate in the presence of 10 % of the second-generation Hoveyda–Grubbs catalyst. Removal of the 6-*N*-benzoyl groups on boronic ester **9** followed by treatment with $Et_3N{\cdot}3HF$ in THF led directly to vinyl boronic acid **10**, which was then hydrogenated in the presence of catalytic amounts of Pd/C to give target compound dAbn. With these new artificial nucleotides analogues in hand, the stage was set to study their autoligation ability.

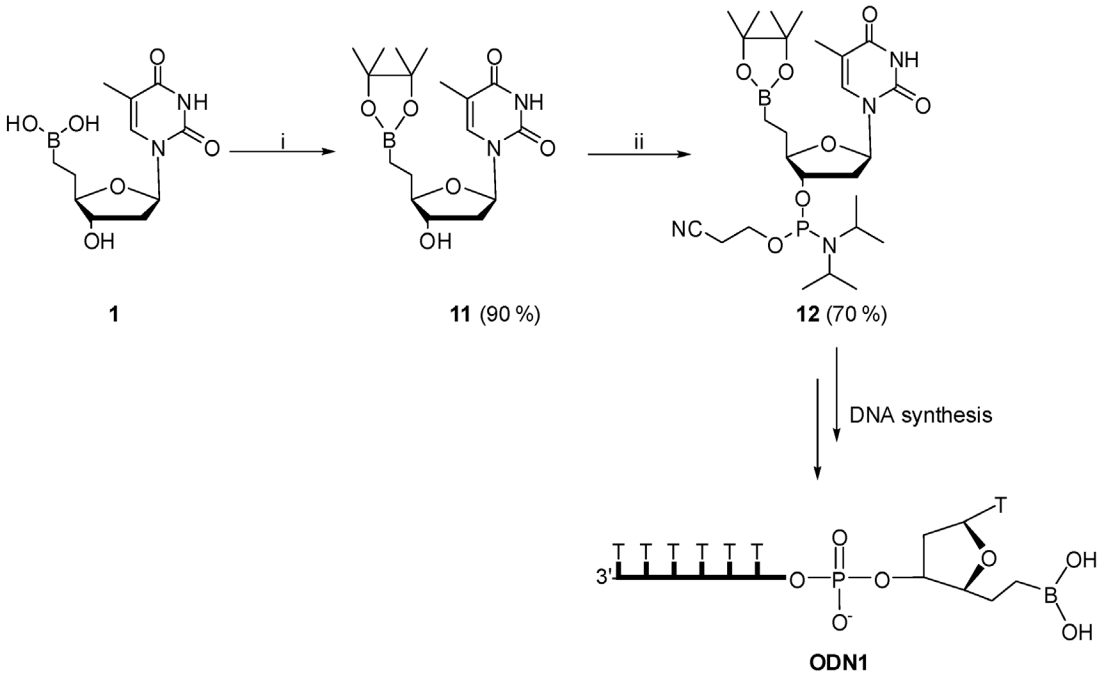


Scheme 1 Synthesis of the bononucleotides. (i) Dichloroacetic acid, DCC, DMSO; (ii) dimethyl-1-diazo-2-oxopropylphosphonate, K_2CO_3, MeOH, rt; (iii) H_2, Lindlar catalyst 15 %, MeOH, rt; (iv) (a) diisopinocampheylborane, THF, rt, (b) acetaldehyde, rt; (v) HCl 3 M, rt (**1**, **2**, and **4**), TBAF (**3**); (vi) (a) BzCl, pyridine, (b) vinyl 2-methyl-2,4-pentanediol boronate, Hoveyda–Grubbs II catalyst 10 mol %, CH_2Cl_2, rt; (vii) (a) K_2CO_3, MeOH, (b) $Et_3N{\cdot}3HF$, rt, 24 h; (viii) H_2, Pd/C 10 %, MeOH, rt.

DNA- AND RNA-TEMPLATED AUTOLIGATION

Incorporation into oligonucleotides

Incorporation of dTbn at the 5'-end of an oligonucleotidic sequence was performed following standard oligonucleotide synthesis protocols. Thus, the 5'-boronic acid function was first protected as a pinacol ester, while the 3'-OH group was phophitylated to afford the desired dTbn-phosphoramidite **12** in very good yield (Scheme 2). Then, the first 5'-boronic acid oligonucleotide (**ODN1**) was synthesized using standard automatized phosphoramidite chemistry and the incorporation **12** was achieved with a good coupling yield. At the end of the elongation, treatment of the support with aqueous ammonia provided oligothymidylate **ODN1** after in situ deprotection of the pinacol ester.

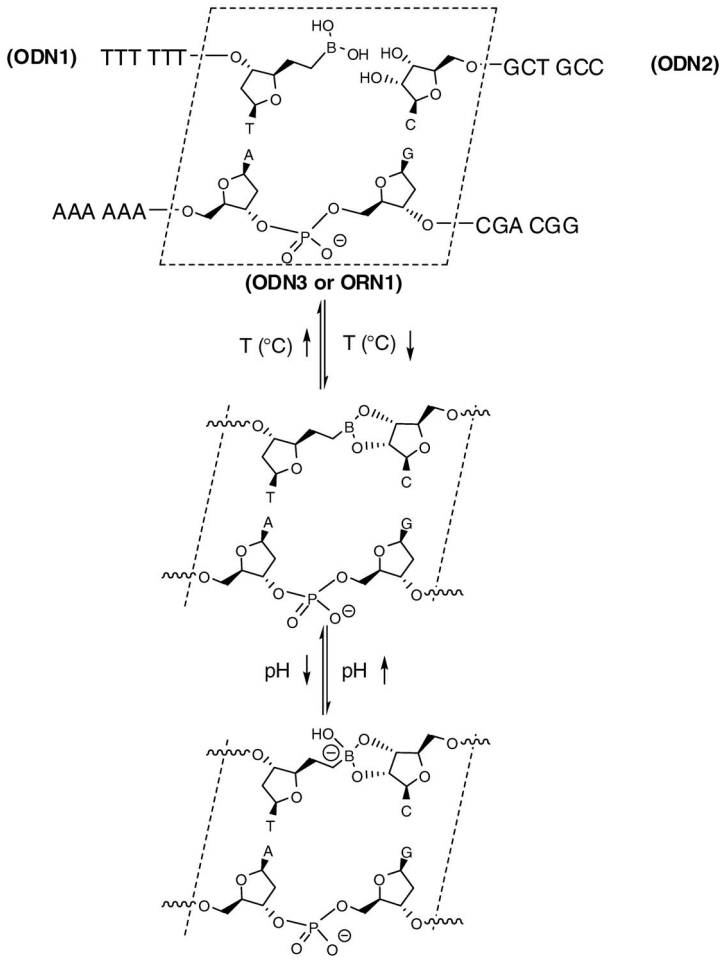


1 **11** (90 %) **12** (70 %)

Scheme 2 Synthesis of a borono-oligonucleotide. (i) Pinacol, THF; (ii) 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, DIPEA, CH_2Cl_2.

Autoligation experiments

The spontaneous and reversible organization of these new structural units into ordered constructs was evaluated as a function of various stimuli. The success of this process rests on its ability to form double helices with high specificity in sequence recognition. Results obtained with the system represented in Scheme 3 demonstrated the validity of this concept [42]. Thermal denaturation studies were performed with a 14mer DNA template (**ODN3**), the 5'-boronic acid sequence **ODN1** and a 7mer carrying a ribonucleoside at its 3' end (**ODN2**). **ODN1** and **ODN2** respective sequences were designed as to display large differences in affinity for the template (T_m 14.9 °C for **ODN3/ODN1** and 48.5 °C for **ODN3/ODN2**, Table 1, entries 1 and 2). Since the boronic acid function is carried by the less stable sequence, we hypothesized that the formation of a novel boronate-linked duplex will mainly influence the less stabilized transition. This was precisely observed, thereby supporting our three-state model hypothesis. Our main goal was to explore whether the self-assembly of **ODN1** and **ODN2** occurs as a result of a selective recognition event, and the results were compared with an unmodified nicked DNA [**ODN4** d(T_7); Table 1, entry 3]. In all experiments, melting curve resolution allowed the extraction of independent melting transitions. It is well known that boronate ester can be switched on and off by changing the pH value. Considering that the stability of the boronate-diol complex is largely dependent on the ionization constants, at physiological pH, the equilibrium should be shifted toward trigonal boronate species. We were pleased to find out that at pH 7.5 the boronate-based non-natural helix displayed a higher melting temperature relative to the nicked DNA [Fig. 3a, T_m = 19.1 (filled circle) vs. 12.3 °C (filled square) respectively]. At higher pHs, the duplex stability continues to increase, reaching a T_m value 15.7 °C higher than the unmodified heptathymidilate d(T_7) **ODN4** at pH 9.5 (Fig. 3a, open circle; Table 1, entries 4 and 5). We hypothesize that the added stability of these helical complexes arose from the formation of a stabilized tetrahedral sp3 boronate anion at pH > 8. Boronic acids have also been known to form tight and reversible complexes with cyanide ions [43]. Indeed, experiments per-



Scheme 3 Dynamic DNA- or RNA-templated autoligation system investigated.

formed in the presence of 3 mM of NaCN at pH 7.5 showed that the ligation proceeds efficiently (T_m = 30.3 °C). This represents a significant outcome as it allows the generation of stable tetrahedral boronate ions at neutral pH (Fig. 3a, filled triangle; Table 1, entry 6).

RNA-templated autoligation experiments were also performed in the presence of **ORN1**, the RNA analogue of **ODN3**. These experiments showed a high stabilization of the RNA-templated ligated duplex at pH 7.5 (Fig. 3b, filled circle) compared to its DNA-templated counterpart (T_m = 33.1 vs. 19.1 °C; Table 1, entries 7–10). On the other hand, the stabilization of the boronate junction from pH 7.5 to pH 9.5 was found to be lower than the DNA-templated system (Fig. 3b, filled circle and open circle, respectively). Finally, as with the DNA-templated system, a high level of stabilization could also be achieved at neutral pH in the presence of 3 mM of sodium cyanide (Fig. 3b, filled triangle; Table 1, entry 11) [42]. These results reward our efforts directed to the elaboration of a stimuli-responsive nucleic acid-based system and will unambiguously allow the emergence of new functional artificial nucleic acids.

Table 1 UV thermal denaturation data of DNA[a]- and RNA[b]-templated autoligation.

Entry	Sequences[c]			T_m[d] [°C]		
				pH 7.5	pH 8.5	pH 9.5
1[a]	ODN**1**	3'-TTTTTTT^{bn}		14.9	14.8	13.9
2[a]	ODN**2**		**C**GCTGCC-5'	48.5	48.5	47.8
3[a]	ODN**4**	3'-TTTTTTT		15.1	15.1	14.9
4[a,e]	ODN**4** + ODN**2**	3'-TTTTTTT	**C**GCTGCC-5'	12.3	12.2	11.0
5[a,e]	ODN**1** + ODN**2**	3'-TTTTTTT^{bn}	**C**GCTGCC-5'	19.1	23.8	26.7
6[a,e,f]	ODN**1** + ODN**2**	3'-TTTTTTT^{bn}	**C**GCTGCC-5'	30.3	–	–
7[b]	ODN**1**	3'-TTTTTTT^{bn}		18.3	20.2	22.2
8[b]	ODN**4**	3'-TTTTTTT		12.0	12.0	11.5
9[b,e]	ODN**4** + ODN**2**	3'-TTTTTTT	**C**GCTGCC-5'	15.3	16.0	15.9
10[b,e]	ODN**1** + ODN**2**	3'-TTTTTTT^{bn}	**C**GCTGCC-5'	33.1	34.1	35.6
11[b,e,f]	ODN**1** + ODN**2**	3'-TTTTTTT^{bn}	**C**GCTGCC-5'	34.6	–	–

[a]Template ODN**3** is 5'-d(AAAAAAAGCGACGG)-3'.
[b]Template ORN**1** is 5'-AAAAAAAGCGACGG-3'.
[c]T^{bn} refers to boronothymidine, and bold letters represent RNA residues.
[d]Melting temperatures refer to the melting of the corresponding sequence(s) with ODN**3** or ORN**1** and are obtained from the maxima of the first derivatives of the melting curve (A260 vs. temperature) recorded in a buffer containing 1 M NaCl and 10 mM of sodium cacodylate, DNA concentration 3 μM of each stand. Curve fit data were averaged from fits of three denaturation curves.
[e]T_m values indicated refer only to the lowest temperature-dependent transition.
[f]Data were obtained in the presence of 3 mM of NaCN.

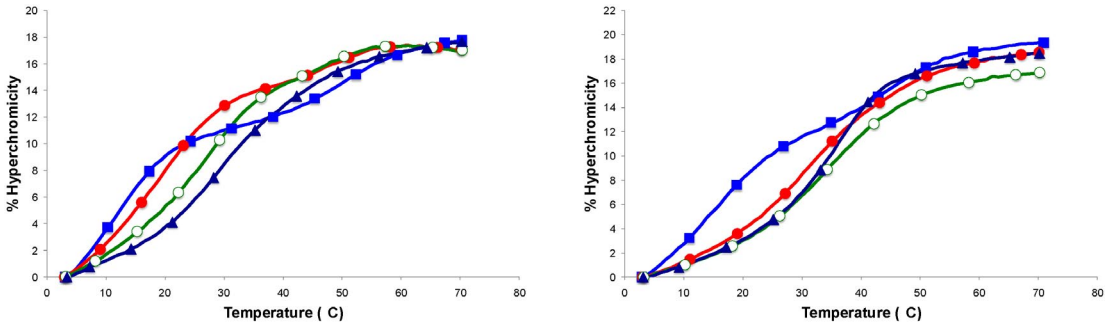


Fig. 3 (a) DNA-templated autoligation: **ODN3**/(**ODN4** + **ODN2**), pH 7.5, (control experiment with a nicked DNA, filled square); **ODN3**/(**ODN1** + **ODN2**), pH 7.5 (filled circle); **ODN3**/(**ODN1** + **ODN2**), pH 9.5 (open circle); **ODN3**/(**ODN1** + **ODN2**), pH 7.5, 3 mM NaCN (filled triangle). (b) RNA-templated autoligation: **ORN1**/(**ODN4** + **ODN2**), pH 7.5, (control experiment with a nicked DNA, filled square); **ORN1**/(**ODN1** + **ODN2**), pH 7.5, (filled circle); **ORN1**/(**ODN1** + **ODN2**), pH 9.5 (open circle); **ORN1**/(**ODN1** + **ODN2**), pH 7.5 3 mM NaCN (filled triangle).

SUMMARY

Besides its biological importance for the storage of genetic information, DNA can also be used to encode molecularly assembled systems. The understanding of the relationship between information storage and self-organized architectures is a requirement for the development of new biomaterials, for the comprehension of biological processes, and eventually to the study of the origin and evolution of life. In this context, reversible covalent bonds ensure the formation of well-defined molecules with high

level of complexity as well as proofreading control. In this context, we described the completion of the set of four 2'-deoxy-5'-bononucleotide (dAbn, dTbn, dGbn, and dCbn) analogues of natural nucleotide monophosphates. These derivatives were designed to exploit the reversible formation of five-membered cyclic boronate esters in the presence of diol partners and were further derivatized for direct incorporation at the 5'-end of an oligonucleotide sequence. DNA- and RNA-templated autoligation experiments showed that a new boronate internucleosidic linkage can be generated through the reaction between this 5'-end boronic acid oligonucleotide and a 3'-ribonucleotide oligonucleotide partner. Moreover, the autoligation process was found to be controlled reversibly by various external stimuli such as variations of pH, temperature, or by the presence of anions. We are now studying extensively the characteristics of this artificial system, focusing on the assembly of smart architectures.

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