Chemical Ligation

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Dynamic and Programmable DNA-Templated Boronic Ester Formation**

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The Darwinian evolution of biomolecules is described as a continuous process of mutation, selection, and amplification.[1] According to a widely accepted hypothesis, the spontaneous prebiotic generation of nucleic acid oligomers evolved through a variety of possible combinations of information building blocks. Over the past few decades, several research groups have investigated various chemical systems able to promote nonenzymatic oligonucleotide ligation.[2] Among the hypotheses investigated, it has been postulated that the early-life selection process might have taken advantage of reversible backbone linkages, which provided a means to repair themselves or transform in response to their environment.[3] Indeed, reversible chemical connections in biomolecular species enable the development of adaptive dynamic systems capable of responding to external factors, such as temperature, the pH value, or molecular-recognition events.[4] So far, diverse dynamic nucleic acid based architectures with specific constitutions or activities have been reported,[5] but only a few reversible covalent reactions have been used to produce dynamic informational nucleic acid based oligomers.[3,6] Boronic ester formation is a reliable reaction for the generation of reversible covalent DNA linkages under enzyme-free conditions. Because of their well-known mild Lewis acidity, boronic acids have proved to be both sugar- and pH-responsive, and undergo a reversible transformation into cyclic esters in the presence of *cis* diols.[7] This ability has led to unique applications, such as self-healing materials, therapeutic agents, and sugar sensing.[8] Moreover, the 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 new light on the potential prebiotic relevance of boron.[9] In this context, our research group recently described the synthesis of the complete set of 2′-deoxybononucleotide analogues of natural nucleotide monophosphates and the reversible formation of the corresponding dinucleotides in the presence of uridine.[10] Following this study, we envisioned a dynamic and reversible DNA-templated ligation that would occur through the reaction of two oligodeoxynucleotides (ODNs), one with a boronic acid at its 5′ end, the other with a ribonucleotide at its 3′ end. The resulting joined duplex would differ from natural DNA in the replacement of a phosphodiester with a boronate internucleoside linkage. Herein, we report a new dynamic and programmable ligation of DNA sequences (Figure 1).

Our approach requires the synthesis of a oligodeoxynucleotide with a boronic acid at its 5′ end. This strand was prepared from dT^{bn} [10] by using standard phosphoramidite chemistry (see the Supporting Information). Our experimental design involves a 14 mer template (ODN**3**), the suitably modified 5′-boronic acid sequence (ODN**1**), and ODN**2**, a DNA sequence bearing a ribonucleoside residue at the 3′ end (Figure 1). The sequences exhibit purposefully major differences in affinity for the complementary strand ODN**3** (Table 1, entries 3 and 4; $T_m = 14.9\,^{\circ}C$ for ODN**3**/ODN**1** and $T_m = 48.5\,^{\circ}C$ for ODN**3**/ODN**2**).

In thermal-denaturation studies, we then examined the ability of the template to bring the two functions into close proximity. Control experiments were carried out with $(dT)_7$ (ODN**4**), an analogue of ODN**1** without the boronic acid modification. As expected, all curves featured a double sigmoidal transition corresponding to ODN**3**/ODN**1** and ODN**3**/ODN**2** half-duplexes. Our main goal was to explore whether the ligation between ODN**1** and ODN**2** occurred as a result of a selective recognition event. Since the boronic acid functionality is carried by the less stable half-duplex, we hypothesized that the formation of a novel boronate-linked full duplex would mainly influence the lower transition. Precisely this effect was observed, in support of our model hypothesis.

The stability of the resulting duplex was subsequently evaluated in the presence of various stimuli. It is well-known that the equilibrium of formation of boronic esters is dependent on the pH value.[11] Indeed, when the pH value is increased, a thermodynamically stable hydroxyboronate complex is formed, with a major release of angle tension as a result of the rehybridization of boron from sp^2 to sp^3. Thus, we envisioned that it should be possible to control the assembly of the boronate linkage by modulating the interaction between ODN**1** and ODN**2** through variations in the

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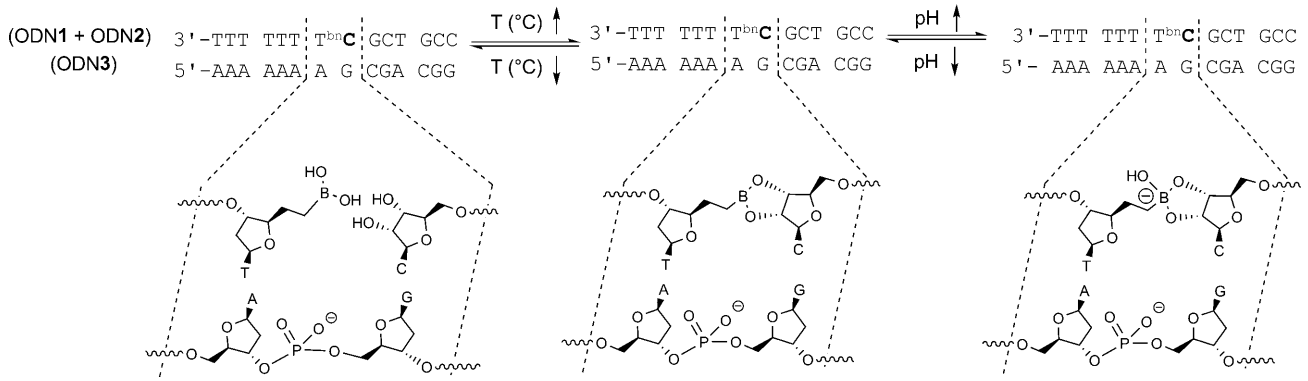

Figure 1. The dynamic and reversible DNA-templated ligation system investigated (T^{bn} refers to boronothymidine, and bold letters represent RNA residues). The ligation is reversible, whereby the direction of reaction depends on the temperature and the pH value.

Table 1: Results of UV thermal denaturation with ODN**3** as the template.[a]

Entry	Sequences[b]		T_m[c] [°C] pH 7.5	pH 8.5	pH 9.5
1	ODN**8**	3′-TTTTTTTCGCTGCC	63.5	63.5	62.7
2	ODN**4**	3′-TTTTTTT	15.1	15.1	14.9
3	ODN**2**	**C**GCTGCC-5′	48.5	48.5	47.8
4	ODN**1**	3′-TTTTTTT^{bn}	14.9	14.8	13.9
5[d]	ODN**4**+ODN**2**	3′-TTTTTTT **C**GCTGCC-5′	12.3	12.2	11.0
6[d]	ODN**1**+ODN**2**	3′-TTTTTTT^{bn} **C**GCTGCC-5′	19.1	23.8	26.7
7[d]	ODN**1**+ODN**5**	3′-TTTTTTT^{bn} CGCTGCC-5′	12.8	12.8	11.9
8[d]	ODN**1**+ODN**6**	3′-TTTTTTT^{bn} ***U***GCTGCC-5′	10.8	15.4	19.8
9[d]	ODN**1**+ODN**7**	3′-TTTTTTT^{bn} **C***GA*TGCC-5′	22.1	24.5	26.9
10[d,e]	ODN**1**+ODN**2**	3′-TTTTTTT^{bn} **C**GCTGCC-5′	30.3	–	–

[a] The template ODN**3** is 5′-AAAAAAAGCGACGG-3′. [b] T^{bn} refers to boronothymidine. Bold letters represent RNA residues, and mismatch bases are in italics. [c] Melting temperatures refer to the melting of the corresponding sequence(s) with ODN**3** and were obtained from the maximum of the first derivative of the melting curve (absorbance at 260 nm versus temperature) recorded in a buffer containing NaCl (1 M) and sodium cacodylate (10 mM). The concentration of each DNA strand was 3 μM. The curve-fit data were averaged from the fits of three denaturation curves. For values below 12 °C, uncertainty remains owing to the height of the low-temperature side of the baseline. [d] The T_m values indicated refer only to the lowest temperature-dependent transition. [e] Data were obtained in the presence of NaCN (3 mM).

pH value. At pH 7.5, the boronate-ligated duplex displayed a higher melting temperature than that of the nicked dsDNA ($T_m = 19.1$ versus 12.3 °C, $\Delta T_m = 6.8$ °C; Table 1, entries 5 and 6). Moreover, at pH 9.5, the melting temperature of ODN**3**/(ODN**1**+ODN**2**) was 15.7 °C higher than that of its nicked counterpart ($T_m = 26.7$ versus 11.0 °C, $\Delta T_m = 15.7$ °C; Table 1, entries 5 and 6). We hypothesized that this stabilization arises from the formation of a relaxed tetrahedral sp^3 boronate anion at pH > 7.5. This hypothesis was confirmed by molecular-dynamics (MD) simulations (see the Supporting Information). Control experiments performed in the presence of ODN**5**, the corresponding 3′-deoxyribonucleoside analogue of ODN**2**, showed no stabilization regardless of the pH value and thus confirmed the significance of the *cis*-diol functionality in the recognition event (Table 1, entry 7). The boronate junction is nevertheless destabilizing: an unmodified duplex has a much higher melting temperature ($T_m = 63.5$ versus 19.1 °C at pH 7.5; Table 1, entries 1 and 6). This destabilization might be a result of the distortion of the DNA backbone caused by perturbation of the ribose sugar pucker.[6a,10a] These results indicate that the DNA-templated pH-controlled system is highly effective and yields a completely novel thermodynamically stable architecture.

Considering the reversible nature of the boronate linkage, we assumed that the addition of a diol to the system might be another means of controlling the assembly of the duplex. To test this assumption, we added 1000 equivalents of fructose to a solution of the ligated assembled duplex at 4 °C. This experiment was carried out at pH 7.5, 8.5, and 9.5. Examination of the resulting thermal-denaturation curves revealed that fructose could not dismantle the ligated duplex. However, when fructose was added to a solution of ODN**1**, ODN**2**, and ODN**3** at 90 °C, the pH dependency was inverted. Whereas fructose had no effect at pH 7.5 ($T_m = 19.9$ °C for ODN**3**/(ODN**1**+ODN**2**), the T_m value decreased slightly to 19.7 °C at pH 8.5 and further to 10.2 °C at pH 9.5 (see the Supporting Information). Thus, the formation of a boronate ester between ODN**1** and fructose at pH 9.5 prevents the DNA-templated ligation of the probes.

This first set of results was confirmed by nondenaturing polyacrylamide gel electrophoresis (PAGE). Experiments were carried out at pH 8.5 in a Tris/borate/EDTA (TBE) buffer at 10 °C (Figure 2; Tris = 2-amino-2-hydroxymethyl-propane-1,3-diol, EDTA = ethylenediaminetetraacetic acid). These conditions do not permit the formation of a stable ODN**3**/ODN**1** half-duplex: the respective bands for ODN**1** and ODN**3** were clearly observed (Figure 2, lane 5). The addition of ODN**2** produced a new band corresponding to the ligated product, with the total disappearance of the bands for ODN**3** and ODN**1** (Figure 2a, lane 6). On the other hand, no ligated product was observed for the reaction of ODN**3** with

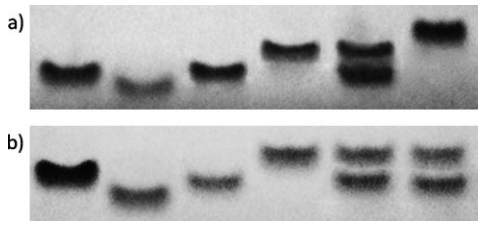

Figure 2. Analytical PAGE under nondenaturing conditions (UV shadowing). a) Lane 1: 14 mer control ODN**3**; lane 2: 7 mer control ODN**2**; lane 3: ODN**3**/ODN**2**; lane 4: ODN**1**; lane 5: ODN**3** and ODN**1**; lane 6, ODN**3**/(ODN**1**+ODN**2**). b) Lane 1: 14 mer control ODN**3**; lane 2: 7 mer control ODN**5**; lane 3: ODN**3**/ODN**5**; lane 4: ODN**1**; lane 5: ODN**3** and ODN**1**; lane 6: ODN**3**/ODN**5** and ODN**1**.

ODN**1** and ODN**5** (Figure 2b, lane 6). These results are consistent with the data for UV thermal denaturation (compare entries 6 and 7 in Table 1). In the absence of a DNA template, no observable ligation product was formed between ODN**1** and ODN**2**, even at high concentration (see the Supporting Information).

We undertook a preliminary investigation into the sequence selectivity of the ligation and studied the formation of the duplex DNA with a boronate linkage in the presence of singly mismatched probes in which the position of the mismatch was at the 3′ end (in ODN**6**), corresponding to the 5′ side of the junction, or a CA mismatch was inside the 7 mer diol probe (in ODN**7**). Thermal-denaturation analysis revealed that the relative placement of the mismatch affects the ligation. Whereas the stability of the resulting duplex was mostly unaffected when the mismatch was inside the 7 mer probe, the ligation proceeded only at pH > 8.5 when the mismatch was located at the 3′ end of the mismatched probe and involved in the formation of the junction (Table 1, entries 8 and 9). These results suggest that the ligation based on boronic ester formation is able to display levels of discrimination.

Boronic acids have also been known to form tight and reversible complexes with cyanide ions.[12] Indeed, experiments performed in the presence of NaCN (3 mM) at pH 7.5 showed that the ligation proceeds efficiently ($T_m = 30.3\,^{\circ}C$). This outcome is significant, as it demonstrates the generation of stable tetrahedral boronate ions at a neutral pH level (Table 1, entry 10).

Finally, we evaluated the ligation in the presence of the RNA template ORN**9** (Table 2). Interestingly, control experiments revealed that the ORN**9**/ODN**1** half-duplex was slightly more stable than the analogous half-duplex ODN**3**/ODN**1**, with an increase in the T_m value of 3.4 °C at pH 7.5. Moreover, since an increase in the pH value had basically no effect on the ODN**3**/ODN**1** half-duplex, we were surprised to observe a pH-dependent stabilization of ORN**9**/ODN**1**. MD simulations performed to explain these results suggested the assistance of the RNA template in the formation of the tetrahedral boronate ester (see the Supporting Information). Surprisingly, at pH 7.5, the melting temperature of the RNA-templated ligated duplex was 14.0 °C higher than that of its DNA-templated counterpart ($T_m = 33.1$ versus 19.1 °C). The pH-dependant stabilization is, however, less marked than that observed for the DNA-templated system ($T_m = 35.6\,^{\circ}C$ at pH 9.5; Table 2, entry 5). To explain these results, we assume that in the junction environment, the ligated duplex may prefer an A-like over a B-like helical conformation. This hypothesis is in accordance with NMR spectroscopic studies performed on dinucleotides in which it was found that

Table 2: Data for UV thermal denaturation with ORN**9** as the template.[a]

Entry	Sequence[b]		T_m[c] [°C] pH 7.5	pH 8.5	pH 9.5
1	ODN**7**	3′-TTTTTTTCGCTGCC-5′	61.5	–	–
2	ODN**4**	3′-TTTTTTT-5′	12.0	12.0	11.5
3	ODN**1**	3′-TTTTTT^{bn}-5′	18.3	20.2	22.2
4[d]	ODN**4** + ODN**2**	3′-TTTTTTT **C**GCTGCC-5′	15.3	16.0	15.9
5[d]	ODN**1** + ODN**2**	3′-TTTTTT^{bn} **C**GCTGCC-5′	33.1	34.1	35.6

[a] The template ORN**9** is 5′-AAAAAAAGCGACGG-3′. [b] T^{bn} refers to boronothymidine, and bold letters represent RNA residues. [c] Melting temperatures refer to the melting of the corresponding sequence(s) with ORN**9** and were obtained from the maximum of the first derivative of the melting curve (absorbance at 260 nm versus temperature) recorded in a buffer containing NaCl (1 M) and sodium cacodylate (10 mM). The concentration of each DNA strand was 3 μM. The curve-fit data were averaged from the fits of three denaturation curves. For values below 12 °C, uncertainty remains owing to the height of the low-temperature side of the baseline. [d] The T_m values indicated refer only to the lowest temperature-dependant transition.

bononucleotides induce a favorable RNA-like conformation on the ribonucleoside partner through the formation of the internucleosidic linkage.[10b]

In summary, we have developed a new dynamic and programmable ligation system based on the reversible DNA-templated formation of a boronate internucleosidic linkage. This system has several significant features: 1) DNA-templated dynamic self-organization; 2) adaptative behavior in response to external triggers (temperature, pH value, diol concentration, or cyanide ions); and 3) dynamic selection of the optimal building blocks. From a broader perspective, the present results open the possibility to generate biologically and prebiotically relevant dynamic systems that may also find useful application in medicinal chemistry and the preparation of biocompatible materials.

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[1] G. P. Chiusoli, *Rend. Lincei-Sci. Fis.* **2009**, *20*, 199–209.
[2] a) L. E. Orgel, *Trends Biochem. Sci.* **1998**, *23*, 491–495; b) X. Y. Li, D. R. Liu, *Angew. Chem.* **2004**, *116*, 4956–4979; *Angew. Chem. Int. Ed.* **2004**, *43*, 4848–4870; c) A. P. Silverman, E. T.

Kool, *Chem. Rev.* **2006**, *106*, 3775 – 3789; d) C. Dose, S. Ficht, O. Seitz, *Angew. Chem.* **2006**, *118*, 5495 – 5499; *Angew. Chem. Int. Ed.* **2006**, *45*, 5369 – 5373; e) M. Röthlingshöfer, E. Kervio, T. Lommel, U. Plutowski, A. Hochgesand, C. Richert, *Angew. Chem.* **2008**, *120*, 6154 – 6157; *Angew. Chem. Int. Ed.* **2008**, *47*, 6065 – 6068; f) J. P. Schrum, A. Ricardo, M. Krishnamurthy, J. C. Blain, J. W. Szostak, *J. Am. Chem. Soc.* **2009**, *131*, 14560 – 14570.

[3] a) Y. Ura, J. M. Beierle, L. J. Leman, L. E. Orgel, M. R. Ghadiri, *Science* **2009**, *325*, 73 – 77; b) H. D. Bean, F. A. L. Anet, I. R. Gould, N. V. Hud, *Origins Life Evol. Biosphere* **2006**, *36*, 39 – 63.

[4] J. M. Lehn, *Chem. Soc. Rev.* **2007**, *36*, 151 – 160.

[5] a) A. Bugaut, K. Jantos, J. L. Wietor, R. Rodriguez, J. K. M. Sanders, S. Balasubramanian, *Angew. Chem.* **2008**, *120*, 2717 – 2720; *Angew. Chem. Int. Ed.* **2008**, *47*, 2677 – 2680; b) N. Sreenivasachary, D. T. Hickman, D. Sarazin, J. M. Lehn, *Chem. Eur. J.* **2006**, *12*, 8581 – 8588; c) A. Bugaut, J. J. Toulme, B. Rayner, *Angew. Chem.* **2004**, *116*, 3206 – 3209; *Angew. Chem. Int. Ed.* **2004**, *43*, 3144 – 3147; d) S. Lena, G. Brancolini, G. Gottarelli, P. Mariani, S. Masiero, A. Venturini, V. Palermo, O. Pandoli, S. Pieraccini, P. Samori, G. P. Spada, *Chem. Eur. J.* **2007**, *13*, 3757 – 3764; e) D. T. Hickman, N. Sreenivasachary, J. M. Lehn, *Helv. Chim. Acta* **2008**, *91*, 1 – 20; f) E. Buhler, N. Sreenivasachary, S. J. Candau, J. M. Lehn, *J. Am. Chem. Soc.* **2007**, *129*, 10058 – 10059; g) N. Sreenivasachary, J. M. Lehn, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 5938 – 5943.

[6] a) R. J. Jones, S. Swaminathan, J. F. Milligan, S. Wadwani, B. C. Froehler, M. D. Matteucci, *J. Am. Chem. Soc.* **1993**, *115*, 9816 – 9817; b) Z. Y. J. Zhan, D. G. Lynn, *J. Am. Chem. Soc.* **1997**, *119*, 12420 – 12421; c) Z. Y. Li, Z. Y. J. Zhang, R. Knipe, D. G. Lynn, *J. Am. Chem. Soc.* **2002**, *124*, 746 – 747.

[7] a) N. Christinat, E. Croisier, R. Scopelliti, M. Cascella, U. Rothlisberger, K. Severin, *Eur. J. Inorg. Chem.* **2007**, 5177 – 5181; b) W. J. Niu, M. D. Smith, J. J. Lavigne, *J. Am. Chem. Soc.* **2006**, *128*, 16466 – 16467; c) N. Iwasawa, H. Takahagi, *J. Am. Chem. Soc.* **2007**, *129*, 7754 – 7755; d) N. Nishimura, K. Kobayashi, *Angew. Chem.* **2008**, *120*, 6351 – 6354; *Angew. Chem. Int. Ed.* **2008**, *47*, 6255 – 6258; e) D. Roy, J. N. Cambre, B. S. Sumerlin, *Chem. Commun.* **2008**, 2477 – 2479.

[8] a) K. Severin, *Dalton Trans.* **2009**, 5254 – 5264; b) N. Fujita, S. Shinkai, T. D. James, *Chem. Asian J.* **2008**, *3*, 1076 – 1091; c) S. Jin, Y. Cheng, S. Reid, B. Wang, *Med. Res. Rev.* **2010**, *30*, 171 – 257; d) D. Luvino, D. Gasparutto, S. Reynaud, M. Smietana, J.-J. Vasseur, *Tetrahedron Lett.* **2008**, *49*, 6075 – 6078; e) D. Luvino, M. Smietana, J. J. Vasseur, *Tetrahedron Lett.* **2006**, *47*, 9253 – 9256.

[9] a) R. Scorei, V. M. Cimpoiasu, *Origins Life Evol. Biosphere* **2006**, *36*, 1 – 11; b) B. E. Prieur, *C. R. Acad. Sci.* **2001**, *4*, 667 – 670; c) A. Ricardo, M. A. Carrigan, A. N. Olcott, S. A. Benner, *Science* **2004**, *303*, 196; d) M. Sugiyama, Z. Hong, L. J. Whalen, W. A. Greenberg, C.-H. Wong, *Adv. Synth. Catal.* **2006**, *348*, 2555 – 2559; e) S. A. Gabel, R. E. London, *J. Biol. Inorg. Chem.* **2008**, *13*, 207 – 217.

[10] a) D. Luvino, C. Baraguey, M. Smietana, J. J. Vasseur, *Chem. Commun.* **2008**, 2352 – 2354; b) A. R. Martin, K. Mohanan, D. Luvino, N. Floquet, C. Baraguey, M. Smietana, J. J. Vasseur, *Org. Biomol. Chem.* **2009**, *7*, 4369 – 4377.

[11] J. Yan, G. Springsteen, S. Deeter, B. Wang, *Tetrahedron* **2004**, *60*, 11205 – 11209.

[12] a) R. Badugu, J. R. Lakowicz, C. D. Geddes, *Dyes Pigm.* **2005**, *64*, 49 – 55; b) E. Galbraith, T. D. James, *Chem. Soc. Rev.* **2010**, *39*, 3831 – 3842.

