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Parallel RNA-strand recognition by 2′-amino-β-L-LNA

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ABSTRACT

A short synthetic route to the first β-L-*ribo* configured locked nucleic acid (LNA), that is, 2′-amino-β-L-LNA thymine phosphoramidite **6**, has been developed from bicyclic nucleoside **1**. Incorporation of 2′-amino-β-L-LNA thymine monomers into α-DNA strands results in probes forming stable duplexes with complementary RNA in parallel orientation.

Oligonucleotides (ONs) are widely explored as modulators of gene expression within the antisense regime to develop powerful research tools and therapeutics. Chemical modification of ONs is necessary to protect them adequately from enzymatic degradation by nucleases and to facilitate strong binding to complementary nucleic acid targets.[1] The use of ONs modified with conformationally restricted nucleotide building blocks has been a particularly successful approach toward this end.[2] LNA[3,4] (locked nucleic acid, β-D-*ribo* configuration), arguably the most promising member from this class of building blocks, exhibits greatly increased thermal affinity toward complementary antiparallel (ap) DNA/RNA and markedly improved stability toward nucleases.[5]

Similarly, α-DNA[6,7] (α-D-*ribo* configuration, Fig. 1) has been explored as a potential building block within the antisense strategy as it is highly resistant toward enzymatic degradation[8–10] and forms stable duplexes with complementary DNA and RNA. The two strands are aligned in parallel orientation to form a right handed helix held together by Watson–Crick base pairing.[11,12] More recently, analogs of α-DNA with C5-propynyl pyrimidines,[13,14] modified backbones including cationic phosphoramidate backbones[14–18] or conformationally restricted sugar moieties,[19–21] have been explored in order to optimize recognition of target oligonucleotide strands in parallel orientation.

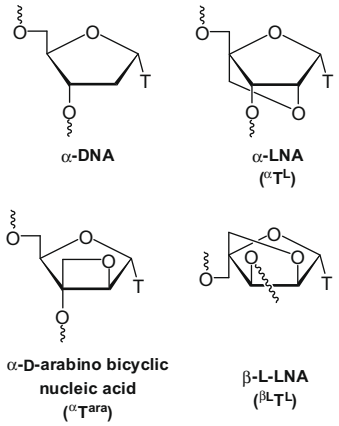

Figure 1. Building blocks utilized for parallel strand recognition.

As an example of the latter category, fully modified oligothymidylate,[21a] mixed pyrimidine,[21b] or mixed sequence[21c] α-LNA (α-D-*ribo* configuration, Fig. 1) exhibit markedly higher thermal affinity toward complementary parallel (p) RNA than α-DNA, while not leading to duplex formation with complementary pDNA, apDNA, or apRNA.[21a–c] However, α-LNA/α-DNA-mixmers, that is, α-DNA strands with interspersed incorporations of α-LNA monomers, exhibit lower affinity toward pDNA/pRNA than unmodified α-DNA.[21b] The functional incompatibility between these mono-

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mers has been attributed to the inability of α-DNA building blocks to adopt N-type[22] conformations imposed by α-LNA monomers.[21b] In contrast, mixmers between [3.2.0]bicyclic monomer $^{\alpha}T^{ara}$ (*E*-type,[22] Fig. 1) and α-DNA, display slightly higher affinity toward pRNA than unmodified α-DNA, but decreased affinity toward pDNA.[21d] Recent hybridization studies with fully modified 'α-LNA' and 'β-L-LNA' (*β*-L-*ribo* configuration, Fig. 1) studied in the mirror image world (i.e., α-L-LNA and β-D-LNA, respectively, against L-DNA/L-RNA targets), have suggested β-L-LNA as a possible structural and functional mimic of α-DNA.[21c] Thus, 'β-L-LNA' was found to form stable duplexes with both pRNA and pDNA but not with apRNA and apDNA. While the furanose conformation of β-L-LNA by definition is N-type due to its L-stereochemistry,[22] the furanose atoms overlay poorly with those of α-LNA (N-type). Studies evaluating β-L-LNA/α-DNA mixmers as probes for parallel strand recognition of nucleic acid targets have been precluded as the synthesis of β-L-LNA nucleotides has not been realized until now.

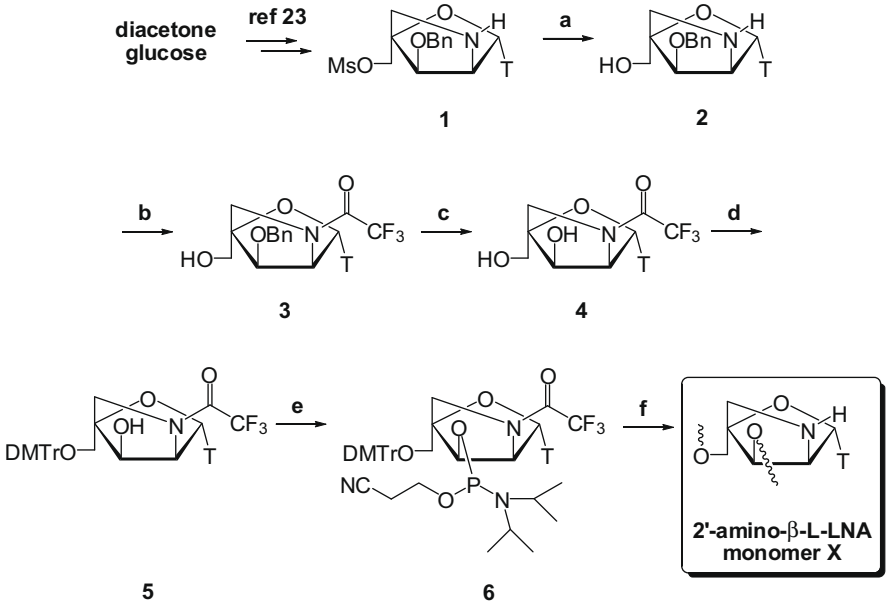
Herein, we have taken advantage of the recent synthetic availability of *β*-L-*ribo* configured LNA nucleosides obtained as prominent byproducts during our synthesis of 2′-amino-α-L-LNA monomers[23] and report: (a) the first synthesis of a *β*-L-*ribo* configured LNA phosphoramidite, (b) the automated solid-phase synthesis of fully modified 2′-amino-*β*-L-LNA and mixmers with DNA and α-DNA monomers, and (c) results from thermal denaturation experiments of complexes between these ONs and p/ap DNA/RNA targets.

The synthesis of 2′-amino-β-L-LNA phosphoramidite building block **6** initiates from *β*-L-*ribo* configured bicyclic nucleoside **1** (Scheme 1).[23] Nucleophilic displacement of the O5′-mesylate group of nucleoside **1** with a benzoate, and subsequent cleavage thereof using saturated methanolic ammonia afforded amino alcohol **2** (53% yield, two steps). Protection of the secondary amino group of **2** as a trifluoroacetamide furnished nucleoside **3** in 89% yield, which upon debenzylation using hydrogen and 20% $Pd(OH)_2/C$ in ethyl acetate gave diol **4** in 70% yield. Subsequent O5′-protection as the 4,4′-dimethoxytrityl (DMTr) ether afforded nucleoside **5** in 70% yield, which upon O3′-phosphitylation (56% yield) concluded this short sequence of protecting group manipulations to provide the desired 2′-amino-β-L-LNA phosporamidite building block **6** (Scheme 1). The identity of all reported compounds was fully ascertained by NMR (^1H, ^{13}C, COSY, and/or HETCOR) and MALDI-HRMS, while purity was verified by 1D NMR.[24]

Several observations directly or indirectly support the suggested *β*-L-*ribo* configuration of bicyclic phosphoramidite **6**: (a) key ^1H NMR Nuclear Overhauser Enhancements (NOE) between H6 and H2′/H3′, and between H1′ and H5″ in starting material **1** have previously been identified and discussed,[23] (b) *β*-L-*ribo* configured nucleoside **2** exhibits specific rotation of identical magnitude but opposite sign relative to the known enantiomer[25] (Table S1),[24] and (c) $^1H/^{13}C$ NMR data of *β*-L-nucleosides **2** and **4**–**6** are identical to those of the corresponding enantiomers.[24–26]

Automated synthesis of ONs was performed on a 0.2 μmol scale using universal CPG solid supports. 2′-Amino-β-L-LNA monomer **X** was incorporated into DNA and α-DNA strands, and was moreover oligomerized to a fully modified ON. The corresponding phosphoramidite building blocks for the incorporation of α-DNA thymine and 5-methylcytosine monomers were synthesized as previously described.[27,28] Standard procedures were applied for ON synthesis except for extended coupling time (25 min) and the use of 1*H*-tetrazole during incorporation of 2′-amino-β-L-LNA phosphoramidite building block **6**. The stepwise coupling yield was >95% for α-DNA phosphoramidites and ~99% for the phosphoramidite building block **6**. Following standard workup and purification protocols,[24] the composition and purity (>80%) of all modified ONs were verified by MALDI-MS (Table S4) and ion-exchange HPLC, respectively.[24] The hybridization properties of these ONs with DNA/RNA targets in parallel/antiparallel orientation were studied by thermal denaturation measurements (A_{260} vs T) using a neutral medium salt phosphate buffer.

First, monomer **X** was incorporated once or thrice into mixed sequence 9-mer DNA strands (**ON1** and **ON2**). Singly modified **ON1** formed a significantly destabilized duplex with complementary apRNA, while transitions were not even observed with the other mixtures, attesting detrimental effects of monomer **X** on du-



Scheme 1. Reagents and conditions: (a) (i) NaOBz, 15-crown-5, DMF, Δ; (ii) satd NH_3/MeOH, rt, 53% over two steps; (b) $(CF_3CO)_2O$, pyridine, CH_2Cl_2, 0 °C to rt, 89%; (c) H_2, $Pd(OH)_2/C$, EtOAc, rt, 70%; (d) DMTrCl, DMAP, pyridine, rt, 70%; (e) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, *N*,*N*-diisopropylethylamine, CH_2Cl_2, rt, 56%; (f) DNA synthesizer; T = thymin-1-yl; DMTr = 4,4′-dimethoxytrityl.

Table 1
Thermal denaturation temperatures of duplexes between 2′-amino-β-L-LNA and complementary apDNA/apRNA[a]

ON	Sequence	T_m (°C)	
		+ apDNA	+ apRNA
ON1	5′-d(GTG A**X**A TGC)	<10	18.0
ON2	5′-d(G**X**G A**X**A **X**GC)	<10	<10
ON3	5′-d(GTG ATA TGC)	28.5	26.5

[a] Thermal denaturation temperatures [T_m values/°C] measured as the maximum of the first derivative of the melting curve (A_{260} vs T) recorded in medium salt buffer ([Na^+] = 110 mM, [Cl^-] = 100 mM, pH 7.0 (NaH_2PO_4/Na_2HPO_4)), using 1.0 μM concentration of each strand. T_m-values are averages of at least two measurements. See Scheme 1 for the structure of monomer **X**.

Table 2
Hybridization data of fully modified 2′-amino-β-L-LNA $\mathbf{X_{10}}$ and previously reported oligonucleotides[a]

ON	Sequence	Descriptor	T_m (°C)		Ref.
			+ dA_{14}	+ rA_{14}	
ON4	5′-$\mathbf{X}_{10}$	2′-Amino-β-L-LNA	71.0	83.0	—
ON5	5′-T_{10}	DNA	22.0	20.0	21a
ON6	5′-αT_{10}	α-DNA	17.5	35.0	21d
ON7	5′-$(^{\alpha}T^{L})_{10}$	α-LNA	<10	45.0	21a
ON8	5′-$(^{\alpha}T^{ara})_{10}$	[3.2.0]bicyclic NA	<10	22.0	21d
ON9	5′-d$(T^{L})_9$T	LNA/DNA	80.0	70.5	3
ON10[b]	5′-β-L-d$(^{\beta L}T^{L})_9$T	β-L-LNA/β-L-DNA	nd[c]	52.0	32

[a] For conditions see Table 1.
[b] Studied in the mirror image world.
[c] nd = not determined.

plex stability. This is in excellent agreement with previous observations where introduction of L-nucleotides into DNA strands results in large decreases in thermal affinity toward p/ap DNA/RNA.[29–31]

In stark contrast, fully modified decameric 2′-amino-β-L-LNA **ON4** exhibits very pronounced thermal affinity toward DNA and RNA targets (T_m = 71.0 °C and 83.0 °C, respectively, Table 2) compared with the previously reported unmodified DNA **ON5**,[21a] fully modified α-DNA **ON6**,[21d] α-LNA **ON7**,[21a] or [3.2.0]bicyclic nucleic acid **ON8**,[21d] or the highly modified LNA **ON9**[3] or 'β-L-LNA' **ON10**.[32] Interestingly, the UV-mixing curve (i.e., Job plots)[33] between **ON4** and complementary RNA strongly suggests the formation of triplexes with a 2:1 stoichiometry (Fig. S2).[24] This is noteworthy as the thermal denaturation curves between **ON4** and complementary DNA/RNA only exhibit smooth monophasic transitions (Fig. S1).[24] Unfortunately, Job plots have not been determined for the related **ON5**–**ON10** preventing a direct comparison with **ON4**. Importantly, triplexes between **ON4** and DNA/RNA strands with a central mismatch were significantly less stable [T_m(**ON4** + 5′-d(A_6CA_7)) = no cooperative transition, T_m(**ON4** + 5′-r(A_6CA_7)) = 74.0 °C].

2′-Amino-β-L-LNA **X** monomers were subsequently incorporated into a decameric non-selfcomplementary mixed pyrimidine α-DNA that has been used to study parallel recognition of DNA/RNA targets by α-LNA[21b] and [3.2.0]bicyclic nucleic acid.[21d] As previously reported,[21d] unmodified α-DNA **ON11** forms stable duplexes with complementary pDNA and pRNA but not with complementary apDNA/apRNA (Table 3).[34] Introduction of a single 2′-amino-β-L-LNA monomer **X** into α-DNA (**ON12**) only resulted in a mild destabilization of the duplex with pRNA (ΔT_m = −1.0 °C; Table 3), while a substantial decrease in thermal affinity toward complementary pDNA was observed (ΔT_m = −7.5 °C, Table 3). Incorporation of three or six **X** monomers into α-DNA further substantiated these trends (see ΔT_m values for **ON13**–**ON14**, Table 3). Importantly, excellent discrimination of singly mismatched targets was observed for 2′-amino-β-L-LNA [T_m(**ON12**:5′-d(GAG G**C**A GAA A)) = 28.0 °C; T_m(**ON12**:5′-r(GAG G**C**A GAA A)) = 13.0 °C; no transitions for **ON14** with these mismatched pDNA/pRNA targets]. All parallel duplexes with reported T_m-values exhibited smooth monophasic denaturation profiles (Fig. S3).[24] Two experimental observations underline that mixmers between 2′-amino-β-L-LNA and α-DNA (**ON12**–**ON14**) indeed form duplexes with pDNA/pRNA: (a) identical T_m-values for **ON12**:pDNA were observed in pH 6 and pH 7 thermal denaturation medium salt buffers (i.e., T_m = 35.5 °C), suggesting a lack of Hoogsteen base pairs typically observed in triplexes, and (b) the Job plot between **ON12** and pDNA strongly suggests a 1:1 stoichiometry (Fig. S4).[24] Duplexes with antiparallel targets were generally not observed except for a weak duplex between the heavily modified **ON14** and apRNA. This is more likely reflecting the formation of a parallel structure rather than an antiparallel duplex, as five complementary Watson–Crick base pairs between **ON14** and apRNA can be formed if the sequences align in parallel orientation. Direct comparison of the hybridization properties of **ON12**–**ON14** with the corresponding mixmers between α-DNA and α-LNA[21b] or [3.2.0]bicyclic nucleic acid[21d] (Table S5),[24] reveals that the thermal affinity toward pDNA as well as pRNA decreases in the order [3.2.0]bicyclic nucleic acid > 2′-amino-β-L-LNA > α-LNA. This clearly suggests that the conformationally restricted 2′-amino-β-L-LNA nucleotides are adequately tolerated in combination with α-DNA nucleotides in duplexes with pRNA, but less well than the *E*-type [3.2.0]bicyclic nucleic acid nucleotides.

To conclude, significant improvement of parallel strand recognition by incorporation of conformationally restricted nucleotide building blocks into α-DNA strands, remains largely elusive despite the development of numerous building blocks with differently conformationally restricted sugar moieties.[19–21] The 2′-amino-β-L-LNA monomer reported herein, however, offers the prospect of additional N2′-functionalization in an equivalent manner as for 2′-amino-LNA[35] or 2′-amino-α-L-LNA.[36] It is envisioned that the hybridization properties thereby can be fine-tuned to facilitate more efficient recognition of complementary parallel DNA/RNA targets.

Table 3
Thermal denaturation temperatures of duplexes between α-DNA strands modified with monomer **X** and complementary parallel and antiparallel DNA/RNA[a]

ON	Sequence	T_m (ΔT_m/mod)			
		pDNA	pRNA	apDNA	apRNA
ON11[b]	5′-α-D-(^{Me}CT^{Me}C ^{Me}CTT ^{Me}CTT T)	43.0	32.0	<10	<10
ON12	5′-α-D-(^{Me}CT^{Me}C ^{Me}CT**X** ^{Me}CTT T)	35.5 (−7.5)	31.0 (−1.0)	<10	<10
ON13	5′-α-D-(^{Me}C**X**^{Me}C ^{Me}CT**X** ^{Me}C**X**T T)	22.5 (−7.2)	29.0 (−1.0)	<10	<10
ON14	5′-α-D-(^{Me}C**X**^{Me}C ^{Me}C**XX** ^{Me}C**XX X**)	<10	23.5 (−1.4)	<10	17.5

[a] For conditions see Table 1. ΔT_m/mod values for **ON12**–**ON14** are given relative to **ON11**. For the structure of monomer **X** and α-DNA see Figure 1 and Scheme 1; pDNA: 5′-d(GAG GAA GAA A); pRNA: 5′-r(GAG GAA GAA A); apDNA: 3′-d(GAG GAA GAA A); apRNA: 3′-r(GAG GAA GAA A).
[b] T_m-values previously reported in Ref. 21b.

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Supplementary data

General experimental section; preparation and characterization of nucleosides **2**–**6**; protocols for determination of specific rotation and synthesis of ONs; representative RP-HPLC (Table S1) and ion-exchange HPLC gradients (Table S2); MALDI-MS of synthesized ONs (Table S3); protocol for thermal denaturation studies; representative thermal denaturation profiles (Figs. S1 and S3); UV-mixing curves (Figs. S2 and S4); comparison of T_m-values for α-DNA with incorporations of monomer **X**, $^{\alpha}\mathbf{T}^{\mathbf{L}}$ and $^{\alpha}\mathbf{T}^{\mathbf{ara}}$ (Table S5); NMR spectra of nucleosides **2**–**6**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.03.079.

References and notes

1. Kurreck, J. *Eur. J. Biochem.* **2003**, *270*, 1628.
2. Recent representative include: (a) Albæk, N.; Petersen, M.; Nielsen, P. *J. Org. Chem.* **2006**, *71*, 7731; (b) Sabatino, D.; Damha, M. J. *J. Am. Chem. Soc.* **2007**, *129*, 8259; (c) Abdur Rahman, S. M.; Seki, S.; Obika, S.; Yoshikawa, H.; Miyashita, K.; Imanishi, T. *J. Am. Chem. Soc.* **2008**, *130*, 4886; (d) Zhou, C.; Liu, Y.; Andaloussi, M.; Badgujar, N.; Plashkevych, O.; Chattopadhyaya, J. *J. Org. Chem.* **2009**, *74*, 118.
3. Singh, S. K.; Nielsen, P.; Koshkin, A. A.; Wengel, J. *Chem. Commun.* **1998**, 455.
4. Obika, S.; Nanbu, D.; Hari, Y.; Andoh, J.; Morio, K.; Doi, T.; Imanishi, T. *Tetrahedron Lett.* **1998**, *39*, 5401.
5. Kaur, H.; Babu, B. R.; Maiti, S. *Chem. Rev.* **2007**, *107*, 4672.
6. Morvan, F.; Rayner, B.; Imbach, J.-L.; Thenet, S.; Bertrand, J.-R.; Malvy, C.; Paoletti, C. *Nucleic Acids Res.* **1987**, *15*, 3421.
7. Gagnor, C.; Rayner, B.; Leonetti, J. P.; Imbach, J. L.; Lebleu, B. *Nucleic Acids Res.* **1989**, *17*, 5107.
8. Thuong, N. T.; Asseline, U.; Roig, V.; Takasugi, M.; Helene, C. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 5129.
9. Bacon, T. A.; Morvan, F.; Rayner, B.; Imbach, J. L.; Wickstrom, E. *J. Biochem. Biophys. Methods* **1988**, *16*, 311.
10. Morvan, F.; Porumb, H.; Degols, G.; Lefebvre, I.; Pompon, A.; Sproat, B. S.; Rayner, B.; Malvy, C.; Lebleu, B.; Imbach, J.-L. *J. Med. Chem.* **1993**, *36*, 280.
11. Morvan, F.; Rayner, B.; Imbach, J. L.; Lee, M.; Hartley, J. A.; Chang, D. K.; Lown, J. W. *Nucleic Acids Res.* **1987**, *15*, 7027.
12. Lancelot, G.; Guesnet, J. L.; Vovelle, F. *Biochemistry* **1989**, *28*, 7871.
13. Morvan, F.; Zeidler, J.; Rayner, B. *Tetrahedron* **1998**, *54*, 71.
14. Deglane, G.; Morvan, F.; Debart, F.; Vasseur, J.-J. *Biorg. Med. Chem. Lett.* **2007**, 951.
15. Laurent, A.; Naval, M.; Debart, F.; Vasseur, J.-J.; Rayner, B. *Nucleic Acids Res.* **1999**, *27*, 4151.
16. Michel, T.; Martinand-Mari, C.; Debart, F.; Lebleu, B.; Robbins, I.; Vasseur, J.-J. *Nucleic Acids Res.* **2003**, *31*, 5282.
17. Deglane, G.; Abes, S.; Michel, T.; Prevot, P.; Vives, E.; Debart, F.; Barvik, I.; Lebleu, B.; Vasseur, J.-J. *ChemBioChem* **2006**, *7*, 684.
18. Pongracz, K.; Gryaznov, S. M. *Nucleic Acids Res.* **1998**, *26*, 1099.
19. Keller, B. M.; Leumann, C. J. *Synthesis* **2002**, 789.
20. Scheidegger, S. P.; Leumann, C. J. *Chem. Eur. J.* **2006**, *12*, 8014.
21. (a) Nielsen, P.; Dalskov, J. K. *Chem. Commun.* **2000**, 1179; (b) Nielsen, P.; Christensen, N. K.; Dalskov, J. K. *Chem. Eur. J.* **2002**, *8*, 712; (c) Christensen, N. K.; Bryld, T.; Sørensen, M. D.; Arar, K.; Wengel, J.; Nielsen, P. *Chem. Commun.* **2004**, 282; (d) Sharma, P. K.; Petersen, M.; Nielsen, P. *J. Org. Chem.* **2005**, *70*, 4918.
22. For further information regarding furanose conformations see: Saenger, W. *Principles of Nucleic Acid Structure*; Springer: Berlin, 1984.
23. Kumar, T. S.; Madsen, A. S.; Wengel, J.; Hrdlicka, P. J. *J. Org. Chem.* **2006**, *71*, 4188.
24. See Supplementary data.
25. Rosenbohm, C.; Christensen, S. M.; Sørensen, M. D.; Pedersen, D. S.; Larsen, L. E.; Wengel, J.; Koch, T. *Org. Biomol. Chem.* **2003**, *1*, 655.
26. Singh, S. K.; Kumar, R.; Wengel, J. *J. Org. Chem.* **1998**, *63*, 10035.
27. Chassignol, M.; Thuong, N. T. *C.R. Acad. Sci. Paris Ser. II* **1987**, *305*, 1527.
28. Kurfürst, R.; Roig, V.; Chassignol, M.; Asseline, U.; Thuong, N. T. *Tetrahedron* **1993**, *49*, 6975.
29. Anderson, D. J.; Reischer, R. J.; Taylor, A. J.; Wechter, W. J. *Nucleosides Nucleotides* **1984**, *3*, 499.
30. Damha, M. J.; Giannaris, P. A.; Marfey, P. *Biochemistry* **1994**, *33*, 7877.
31. Urata, H.; Shimizu, H.; Hiroaki, H.; Kohda, D.; Akagi, M. *Biochem. Biophys. Res. Commun.* **2003**, *309*, 79.
32. Rajwanshi, V. K.; Håkansson, A. E.; Sørensen, M. D.; Pitsch, S.; Singh, S. K.; Kumar, R.; Nielsen, P.; Wengel, J. *Angew. Chem., Int. Ed.* **2000**, *39*, 1656.
33. Job, P. *Anal. Chim. Acta* **1928**, *9*, 113.
34. The corresponding DNA strand, that is, 5′-β-D(CTC CTT CTT T) forms duplexes with complementary apDNA/apRNA of comparable stability under these conditions (T_m = 29.0 °C and 36.5 °C, respectively).
35. For example: (a) Sørensen, M. D.; Petersen, M.; Wengel, J. *Chem. Commun.* **2003**, 2130; (b) Hrdlicka, P. J.; Babu, B. R.; Sørensen, M. D.; Harrit, N.; Wengel, J. *J. Am. Chem. Soc.* **2005**, *127*, 13293; (c) Kalek, M.; Madsen, A. S.; Wengel, J. *J. Am. Chem. Soc.* **2007**, *129*, 9392.
36. For example: (a) Hrdlicka, P. J.; Kumar, T. S.; Wengel, J. *Chem. Commun.* **2005**, 4279; (b) Kumar, T. S.; Wengel, J.; Hrdlicka, P. J. *ChemBioChem* **2007**, *8*, 1122; (c) Kumar, T. S.; Madsen, A. S.; Østergaard, M. E.; Wengel, J.; Hrdlicka, P. J. *J. Org. Chem.* **2008**, *73*, 7060; (d) Kumar, T. S.; Madsen, A. S.; Østergaard, M. E.; Sau, S. P.; Wengel, J.; Hrdlicka, P. J. *J. Org. Chem.* **2009**, *74*, 1070.