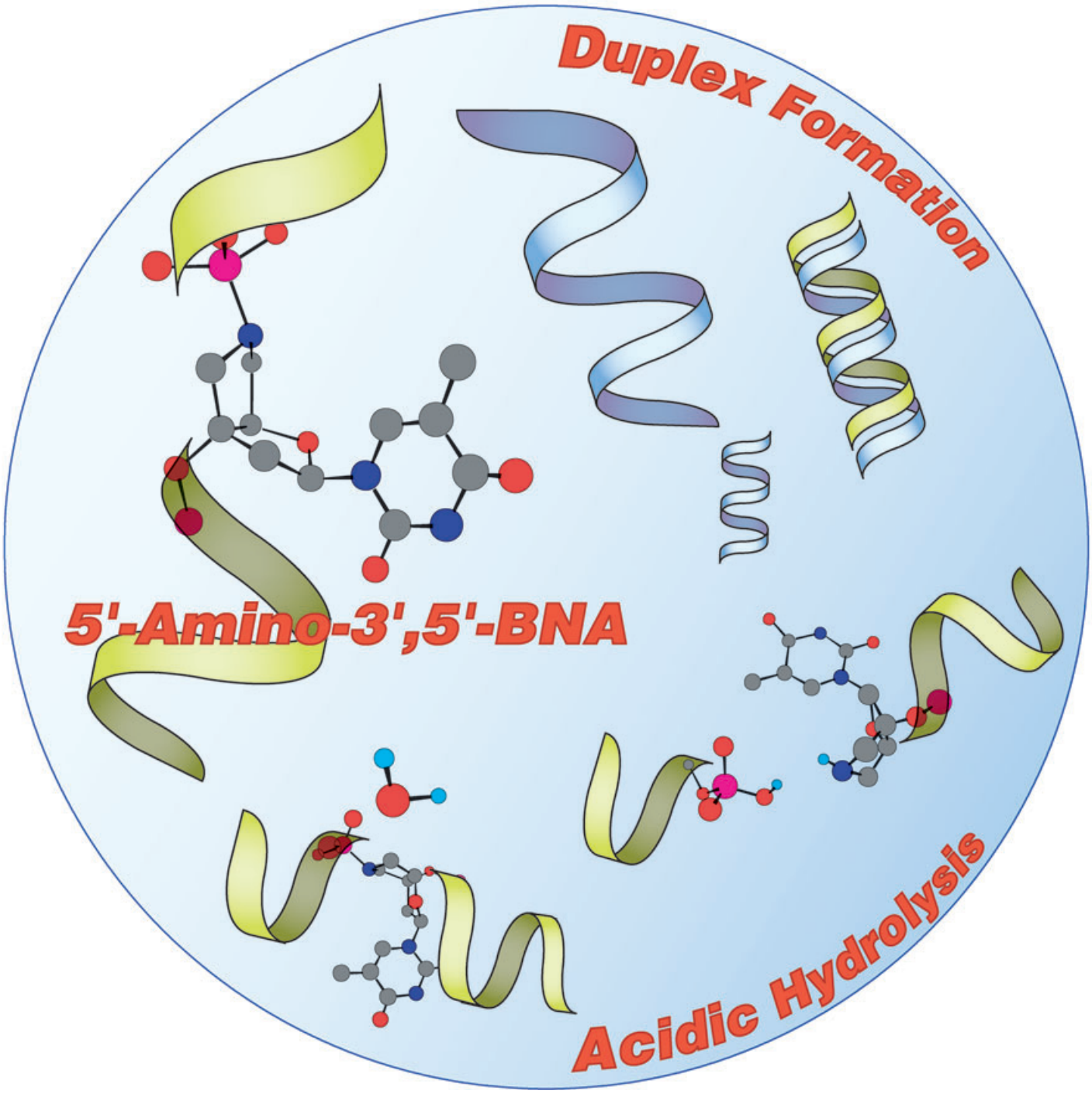
Communications



A methylene bridge between the C3′ and N5′ atoms adjusts the γ dihedral angle in 5′-amino-3′,5′-BNA. The bridged structure of this new type of oligonucleotide P3′→N5′ phosphoramidate greatly enhances duplex-forming ability with complementary strands and accelerates cleavage of the phosphoramidate linker under mild acidic conditions.

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Bridged Nucleic Acids

Adjustment of the γ Dihedral Angle of an Oligonucleotide P3′→N5′ Phosphoramidate Enhances Its Binding Affinity towards Complementary Strands**

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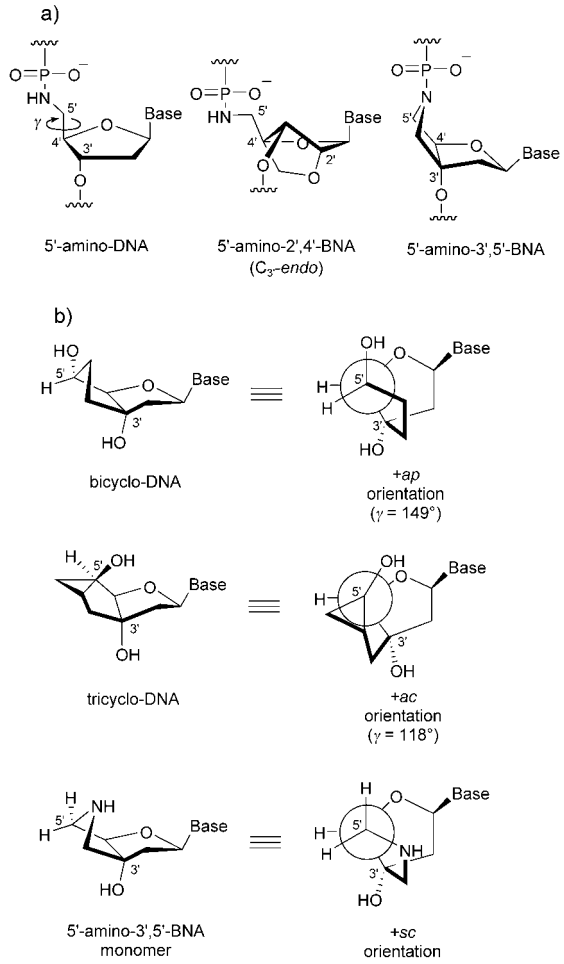
Chemical modification of oligodeoxynucleotides (ODNs) has been receiving increasing attention in the fields of gene therapeutics and genetic diagnosis.[1,2] One promising approach is an internucleoside linkage modification of the ODNs. An N3′→P5′-phosphoramidate-linked ODN, in which the 3′-oxygen atom is replaced with a nitrogen atom, forms a stable duplex structure with its DNA or RNA complement.[3] On the other hand, P3′→N5′-phosphoramidate-linked ODNs (5′-amino-DNA, Scheme 1 a), with a 5′-nitrogen atom instead of an oxygen atom, can be hydrolyzed at the phosphoramidate linkage under mild acidic conditions.[4] This property of 5′-amino-DNA has attracted much attention and has been applied to a DNA-sequence determination.[5,6] However, the 5′-amino-DNA modification of ODNs decreases the hybridizing ability with its complementary strand.[7,8] This disadvantage of 5′-amino-DNA may be caused by an inappropriate γ dihedral angle (N5′–C5′–C4′–C3′). ^{1}H NMR analysis of a 5′-amino-DNA dimer revealed that the orientation of the C4′–C5′ bond is predominantly $+ap$ ($\gamma \approx 180°$) or $-sc$ ($\gamma \approx -60°$), which is different from that in a typical DNA/DNA or RNA/RNA duplex ($+sc$, $\gamma \approx 60°$).[9]

One promising strategy for restricting the conformational flexibility of the nucleoside sugar moiety is to increase the binding affinity of the ODNs. We have developed a series of novel nucleic acid analogues bearing a conformationally restricted sugar moiety, bridged nucleic acids (BNAs), and have found that ODNs containing some kinds of BNA acquired extremely high binding affinity for their DNA or RNA complements.[10–12] One such nucleic acid analogue, 5′-amino-2′,4′-BNA (Scheme 1 a), in which the sugar puckering is exactly restricted to the C3′-*endo* conformation (a typical N-type conformation), exhibited high binding affinity with complementary strands, although this nucleic acid analogue has a P3′→N5′ phosphoramidate linkage.[12] Thus, the 5′-amino-2′,4′-BNA may be one example of how to overcome the drawback of 5′-amino-DNA; however, the effect of the γ dihedral angle of 5′-amino-DNA on its hybridizing properties is still unclear.



Scheme 1. a) Structures of 5′-amino-DNA, 5′-amino-2′,4′-BNA, and 5′-amino-3′,5′-BNA. b) Structures and γ dihedral angle orientation of bicyclo-DNA, tricyclo-DNA, and the 5′-amino-3′,5′-BNA monomer.

In this study, we have focused on the adjustment of the γ dihedral angle of 5′-amino-DNA. As DNA derivatives having a restricted γ dihedral angle, bicyclo-DNA[13] and tricyclo-DNA,[14] developed by Leumann et al., are well known. These DNA analogues showed interesting duplex- and triplex-forming properties, and the tricyclo-DNA was found to be useful even as an antisense oligonucleotide.[15] However, the γ dihedral angles of bicyclo-DNA and tricyclo-DNA were observed to be 149° and 118°, respectively (Scheme 1 b). These γ angles are beyond the range of those for typical DNA/DNA and RNA/RNA duplexes. To adjust the γ angle of 5′-amino-DNA to an appropriate value for stable duplex formation, we have designed a novel bridged nucleic acid, 5′-amino-3′,5′-BNA, which has a methylene linkage between the 3′-carbon and 5′-nitrogen atoms (Scheme 1 a). The orientation of the C4′–C5′ bond of the 5′-amino-3′,5′-BNA was fully expected to be $+sc$ by comparison with the structure of bicyclo-DNA (Scheme 1 b). Herein we describe the synthesis

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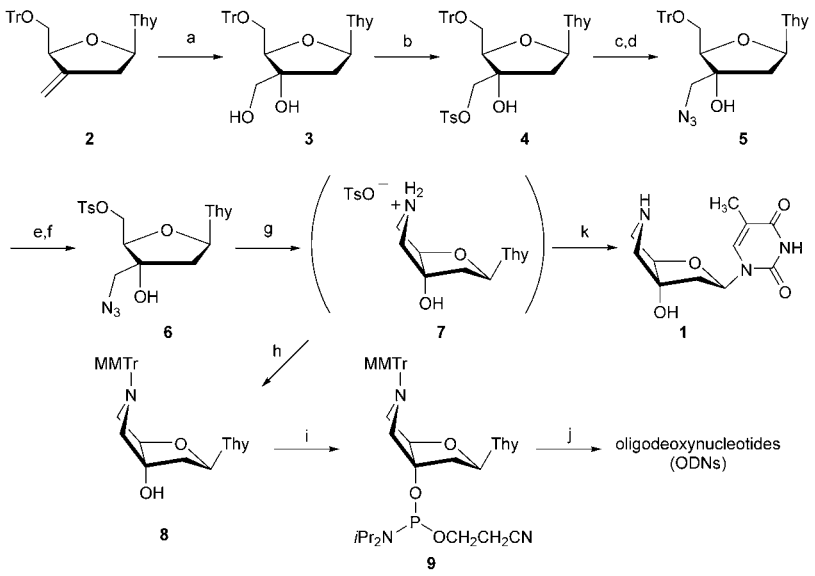
and conformation of the 5′-amino-3′,5′-BNA monomer and some interesting properties of its ODN derivatives.

For the synthesis of the 5′-amino-3′,5′-BNA, we chose 3′-deoxy-3′-*C*-methylene-5′-*O*-triphenylmethylthymidine (**2**)[16] as the starting material (Scheme 2). Stereoselective oxidation



Scheme 2. Synthesis of 5′-amino-3′,5′-BNA-thymine monomer **1** and its phosphoramidite derivative **9**. a) OsO_4 (cat.), NMO, pyridine, H_2O, *t*BuOH, 76 °C, 69 %; b) TsCl, nBu_2SnO, Et_3N, CH_2Cl_2, RT, 72 %; c) K_2CO_3, MeOH, RT; d) NaN_3, DMF, 90 °C, 98 % from **4**; e) CSA, CH_2Cl_2/MeOH, RT; f) TsCl, pyridine, 50 °C, 53 % from **5**; g) 10 % Pd/C (wet), H_2, MeOH, RT; h) MMTrCl, pyridine, RT, 69 % from **6**; i) 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, iPr_2NEt, CH_2Cl_2, RT, 86 %; j) DNA synthesizer (ABI Expedite 8909); k) Et_3N, MeOH, RT, then purified by reversed-phase HPLC. CSA = (+)-camphorsulfonic acid, DMF = *N*,*N*-dimethylformamide, MMTr = monomethoxytrityl, NMO = 4-methylmorpholine *N*-oxide, Thy = thymin-1-yl, Tr = triphenylmethyl = trityl, Ts = toluene-4-sulfonyl = tosyl.

of **2** by using osmium tetroxide gave diol **3**. A *p*-toluenesulfonyl group was introduced at the primary hydroxy group of **3** to afford **4**. Epoxidation with potassium carbonate and subsequent treatment with sodium azide gave **5**. After removing the 5′-*O*-triphenylmethyl (trityl) group, the obtained diol was treated with *p*-toluenesulfonyl chloride to give **6**. The azide group of **6** was reduced with palladium on carbon under a hydrogen atmosphere and subsequent pyrrolidine ring formation afforded the desired 5′-amino-3′,5′-BNA monomer **7** as a salt of *p*-toluenesulfonic acid. Without any purification at this stage, **7** was treated with monomethoxytrityl chloride to give **8**, and the phosphoramidite building block **9** was obtained by phosphitilation of **8**. Alternatively, the salt **7** was treated with triethylamine and was then purified by reversed-phase HPLC to afford the 5′-amino-3′,5′-BNA monomer **1** in a free form. X-ray crystallograpic analysis of **1** (Figure 1) showed that the γ dihedral angle of **1** is 28.3° (+*sc* orientation).[17] This value is quite different from that found in the 5′-amino-DNA dimer[9] and would be appropriate for stable duplex formation. It was also observed that the furanose ring of **1** has the C1′-*exo*-O4′-*endo* conformation (pseudorotational phase angle $P = 115.4°$), which is neither an N-type nor an S-type conformation, but an in-between conformation.

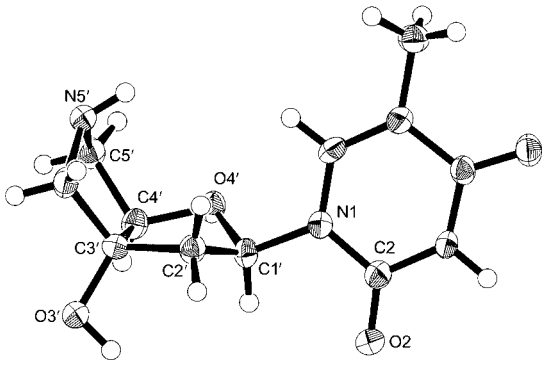


Figure 1. X-ray crystal structure of 5′-amino-3′,5′-BNA monomer **1**.

The phosphoramidite **9** was incorporated into 12-mer DNA strands **11**–**13** by using an automated DNA synthesizer.[18] To improve coupling and oxidizing efficiency, 4,5-dicyanoimidazole[19] and *t*BuOOH[20,21] were used instead of 1*H*-tetrazole and I_2/pyridine/H_2O, respectively. The coupling efficiency for **9**, estimated from monitoring the free trityl groups, was approximately 90 %.

Duplex-forming ability of the ODNs with complementary DNA and RNA was evaluated by means of UV melting experiments. The differences in melting temperatures (ΔT_m values) between the duplexes containing 5′-amino-3′,5′-BNA or 5′-amino-DNA[22] and the natural DNA/DNA or DNA/RNA duplexes are summarized in Figure 2.[23] As previously reported,[7,8] the duplexes comprising the 5′-amino-DNA, **14**–**16**, showed a decrease in T_m value compared with the corresponding DNA/DNA and DNA/RNA duplexes. ODN **16**, in particular, containing six con-

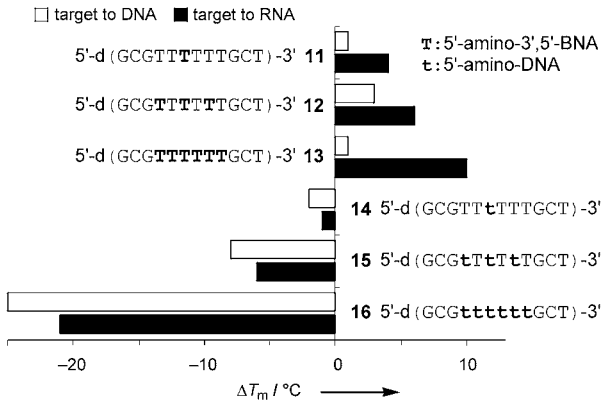


Figure 2. Differences in melting temperatures (ΔT_m values) between the modified oligonucleotides **11**–**16** and the reference oligonucleotide **10**, 5′-d(GCGTTTTTTGCT)-3′. The T_m values of duplexes of **10** with complementary DNA and RNA were 50 °C and 45 °C, respectively. The T_m values obtained from the maxima of the first derivatives of the melting curves (absorbance at 260 nm versus temperature) were recorded in a medium salt buffer (10 mM sodium phosphate, 100 mM NaCl, pH 7.2) with 4 μM complementary strands. The sequence of target DNA and RNA complement is 5′-AGCAAAAAACGC-3′.

secutive 5′-amino-DNA monomers, displayed a drastically decreased binding affinity with its DNA and RNA complements. On the other hand, the 5′-amino-3′,5′-BNA ODNs **11**–**13** achieved stable duplex formation with complementary strands. An increase in the T_m values by 1–3 °C and 4–10 °C was observed when the ODNs **11**–**13** formed duplexes with complementary DNA and RNA, respectively. It is noteworthy that the difference between the T_m values of **13**/RNA and **16**/RNA hybrids was over 30 °C. Thus, the 5′-amino-3′,5′-BNAs have strong duplex-forming ability. This result indicates that the methylene bridge between the C3′ and N5′ atoms successfully restricts the conformation around the γ dihedral angle in an appropriate form for duplex formation.

Next, we investigated the effect of the methylene bridge on acid-mediated hydrolysis of the P3′→N5′-phosphoramidate linkage. The 5′-amino-3′,5′-BNA ODN **11** was treated with buffer (pH 3.0 or pH 7.0) to be hydrolyzed, and the amount of intact **11** was determined by reversed-phase HPLC analysis (Figure 3). Under pH 3.0 conditions, 50 % of **11** was

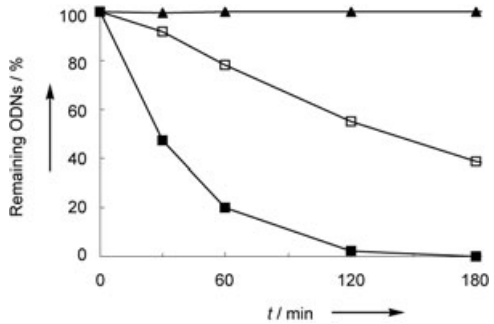


Figure 3. Hydrolytic cleavage of the P3′→N5′-phosphoramidate bond in modified ODNs: ■: 5′-amino-3′,5′-BNA ODN **11** at pH 3.0; ▲: 5′-amino-3′,5′-BNA ODN **11** at pH 7.0; □: 5′-amino-DNA ODN **14** at pH 3.0. The reaction was carried out at 30 °C with 1 nmol of ODN in buffer (100 μL). The sequence of oligonucleotides **11** and **14** is shown in Figure 2.

hydrolyzed at the P3′→N5′-phosphoramidate linkage within 30 min and 95 % was cleaved at 120 min, while no hydrolysis was observed at pH 7.0. The 5′-amino-DNA **16** was also cleaved at pH 3.0; however, hydrolysis is much slower than for 5′-amino-3′,5′-BNA. Thus, the additional methylene bridge between N5′ and C3′ atoms accelerates the hydrolysis of phosphoramidate linkage probably due to its electron-donating property.

We have synthesized a novel 5′-amino-DNA analogue, 5′-amino-3′,5′-BNA, with a γ dihedral angle that is well adjusted by the methylene bridge between the C3′ and N5′ atoms. We have also found that the methylene bridge effectively elicits not only a strong hybridizing ability but also the rapid hydrolysis of the P3′→N5′-phosphoramidate linkage of 5′-amino-3′,5′-BNA. This feature of 5′-amino-3′,5′-BNA would be applicable to a variety of genome technologies, such as a novel sequence determination or single-nucleotide-polymorphism analysis.

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[1] N. Venkatesan, S. J. Kim, B. H. Kim, *Curr. Med. Chem.* **2003**, *10*, 1973 – 1991.
[2] J. Kurreck, *Eur. J. Biochem.* **2003**, *270*, 1628 – 1644.
[3] S. M. Gryaznov, *Biochim. Biophys. Acta* **1999**, *1489*, 131 – 140.
[4] W. Bannwarth, *Helv. Chim. Acta* **1988**, *71*, 1517 – 1527.
[5] M. S. Shchepinov, M. F. Denissenko, K. J. Smylie, R. J. Wörl, A. L. Leppin, C. R. Cantor, C. P. Rodi, *Nucleic Acids Res.* **2001**, *29*, 3864 – 3872.
[6] J. L. Wolfe, B. H. Wang, T. Kawate, V. P. Stanton, Jr., *J. Am. Chem. Soc.* **2003**, *125*, 10500 – 10501.
[7] S. M. Gryaznov, R. L. Letsinger, *Nucleic Acids Res.* **1992**, *20*, 3403 – 3409.
[8] E. Viazovkina, K.-L. Min, A. Galarneau, M. J. Damha, *Nucleosides Nucleotides Nucleic Acids* **2003**, *22*, 1335 – 1338.
[9] E. M. Nottoli, J. B. Lambert, R. L. Letsinger, *J. Am. Chem. Soc.* **1977**, *99*, 3486 – 3491.
[10] T. Imanishi, S. Obika, *Chem. Commun.* **2002**, 1653 – 1659.
[11] a) S. Obika, D. Nanbu, Y. Hari, K. Morio, Y. In, T. Ishida, T. Imanishi, *Tetrahedron Lett.* **1997**, *38*, 8735 – 8738; b) S. Obika, K. Morio, D. Nanbu, T. Imanishi, *Chem. Commun.* **1998**, 1643 – 1644; c) S. Obika, J. Andoh, T. Sugimoto, K. Miyashita, T. Imanishi, *Tetrahedron Lett.* **1999**, *40*, 6465 – 6468; d) S. Obika, J. Andoh, M. Onoda, O. Nakagawa, A. Hiroto, T. Sugimoto, T. Imanishi, *Tetrahedron Lett.* **2003**, *44*, 5267 – 5270.
[12] S. Obika, O. Nakagawa, A. Hiroto, Y. Hari, T. Imanishi, *Chem. Commun.* **2003**, 2202 – 2203.
[13] M. Tarköy, M. Bolli, B. Schweizer, C. Leumann, *Helv. Chim. Acta* **1993**, *76*, 481 – 510.
[14] R. Steffens, C. Leumann, *Helv. Chim. Acta* **1997**, *80*, 2426 – 2439.
[15] a) D. Renneberg, E. Bouliong, U. Reber, D. Schümperli, C. J. Leumann, *Nucleic Acids Res.* **2002**, *30*, 2751 – 2757; b) D. Ittig, S. Liu, D. Renneberg, D. Schümperli, C. J. Leumann, *Nucleic Acids Res.* **2004**, *32*, 346 – 353.
[16] M. Sharma, M. Bobek, *Tetrahedron Lett.* **1990**, *31*, 5839 – 5842.
[17] CCDC-247780 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
[18] The composition of the oligonucleotides **11**–**13** was confirmed by MALDI-TOF MS in negative-ion mode. A molecular-ion peak $[M-H]^-$ was detected in all cases, except for oligonucleotide **12**. See the Supporting Information.
[19] C. Vargeese, J. Carter, J. Yegge, S. Krivjansky, A. Settle, E. Kropp, K. Peterson, W. Pieken, *Nucleic Acids Res.* **1998**, *26*, 1046 – 1050.
[20] C. Scheuer-Larsen, B. M. Dahl, J. Wengel, O. Dahl, *Tetrahedron Lett.* **1998**, *39*, 8361 – 8364.
[21] Y. Hayakawa, M. Uchiyama, R. Noyori, *Tetrahedron Lett.* **1986**, *27*, 4191 – 4194.
[22] Synthesis of 5′-amino-DNA oligonucleotides **14**–**16** was performed according to ref. [4].
[23] The T_m profiles of duplexes containing the oligonucleotides **11**–**13** can be found in the Supporting Information.

