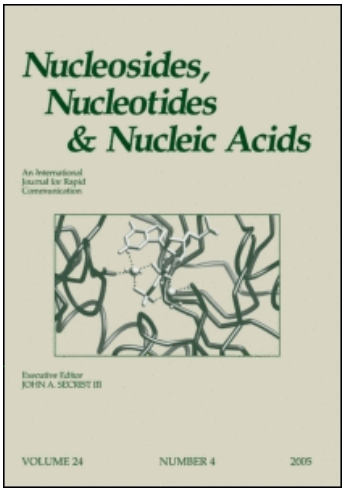

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HYDROXYL-FUNCTIONALIZED DNAS WITH DIFFERENT LINKERS AND THEIR COMPLMENTARY DUPLEX STABILITY

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□ *Tert-butyldiphenylsilyl (TBDPS) was testified to be an appropriate orthogonal protecting group for novel 7-hydroxyl-functionalized 8-aza-7-deaza-2′-deoxyadenosine analogues. It was stable in partial and complete hydrogenation reactions used for the different linker preparation. The corresponding phosphoramidites and hydroxyl-functionalized oligodeoxynucleotides were synthesized and identified. The thermal effect of the hydroxyl group with different linkers on DNA duplexes was evaluated. It provided a feasible strategy for the preparation of hydroxyl-functionalized DNAs for the nucleic acid research.*

Keywords *Tert*-butyldiphenylsilyl; 8-aza-7-deazaza-2′-deoxyadenosine; hydroxypropargyl; hydroxypropenyl; oligodeoxynucleotides; thermal stability

INTRODUCTION

As more and more attention has been focused on exploring new functions of nucleic acids, DNA and RNA modified with active functional groups—which are involved in protein functions such as amino, hydroxyl, and imidazolyl residues—have been used as an important approach for new functional nucleic acids.[1–3] In our previous article,[4] we reported an orthogonal protecting strategy for the synthesis of hydroxyl-containing oligodeoxynucleotides (ODNs) in which *tert*-butyldiphenylsilyl (TBDPS) group was used as the orthogonal protecting group of the extra hydroxyl

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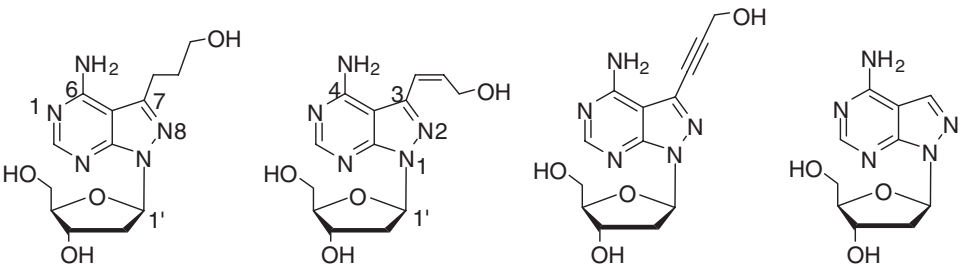
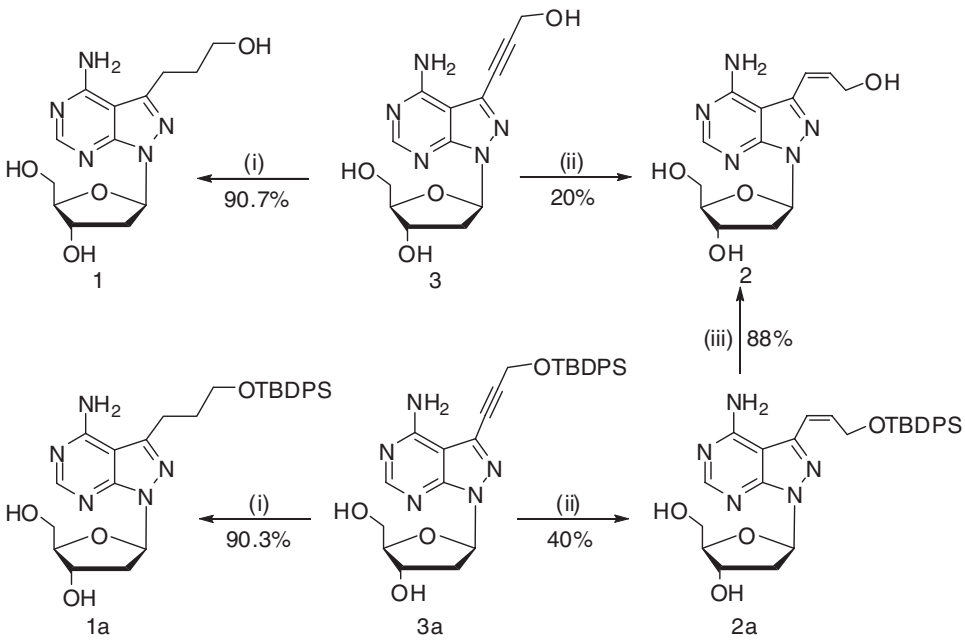


group linked to the position 5 of 2′-deoxyuridine or position 7 of 8-aza-7-deaza-2′-deoxyadenosine. Here, we continued our efforts for the synthesis of hydroxyl-functionalized ODNs with new 2′-deoxyadenosine analogues **1** and **2**, and their influence on DNA duplex stability was evaluated.

Nucleoside analogue **3** was synthesized from Pd (0)-catalyzed cross-coupling reaction between 7-iodo-8-aza-7-deaza-2′-deoxyadenosine and 3-hydroxypropyne.[4–6] Complete hydrogenation of the triple bond in nucleoside **3** with Pd/C (10%) was used to obtain the hydroxypropyl-containing nucleoside **1**. The partial hydrogenation of the triple bond in nucleoside **3** with $NiCl_2/NaBH_4$ as the catalyst[7] led to the *Z*-double bond linker in compound **2** (Scheme 1). The coupling constant of the hydrogen atoms attached to the double bond of compound **2** is around 12 Hz, which is consistent with other *Z*- alkenes.[8–10] However, the value for the hydrogen atoms of *E*-double bond is normally in the range of 15 to 18 Hz.[8–10] The chemical shifts of the alkenyl carbon atoms were 116.7 and 139.2 ppm, respectively.

Based on such synthetic routes for compounds **1** and **2**, the preparation of their corresponding phosphoramidites used in hydroxyl-functionalized ODNs synthesis required that the 7-positioned hydroxyl group must be protected before it was introduced to the nucleosides. Therefore, the hydroxypropargyl group of compound **3** needs a proper protecting group, and the protecting group should be stable in the subsequent hydrogenation conditions. *Tert*-butyldiphenylsilyl was testified to be stable to go through the hydrogenation conditions. Compound **3a** was used as the key precursor, which was successfully converted to the two key intermediates **1a** and **2a** by partial and full hydrogenation, respectively (Scheme 1). Compound **2a** exhibited the same characteristic for the *Z*-alkenyl linker, the coupling constant of the hydrogen atoms attached to the *Z* double bond was about 12 Hz and the chemical shifts of the alkenyl carbon atoms were 117.5 and 136.6 ppm, respectively.[8–10] Therefore, orthogonal protection was smoothly realized with TBDPS between the two primary hydroxyl groups, the 7-positioned hydroxyl group and the 5′-hydroxyl of the sugar moiety in nucleoside analogues **1** and **2**. In addition, compound **1** and **2** could be prepared from



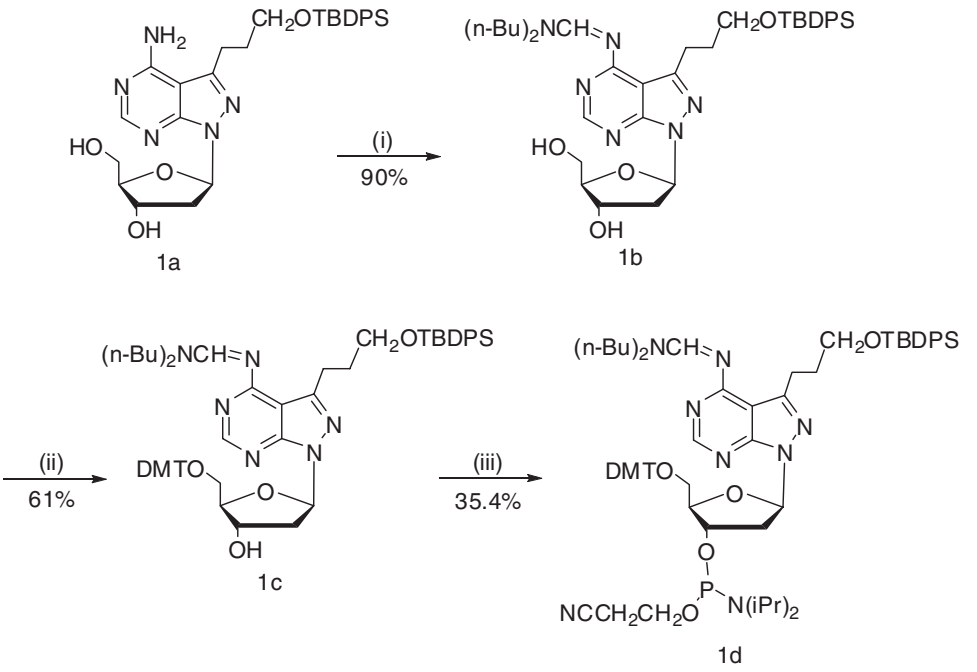
SCHEME 1 Synthesis of nucleosides **1–2** and their intermediates. Conditions: (i) H_2, Pd/C, room temperature at 3 hours; (ii) $NiCl_2/NaBH_4$, MeOH, −78°C; (iii) 1 M TBAF/THF, room temperature.

the deprotection of **1a** and **2a** with TBAF/THF, respectively. Especially, this route is a preferred choice for compound **2**, because yield of the partial hydrogenation of compound **3** was low (20%).

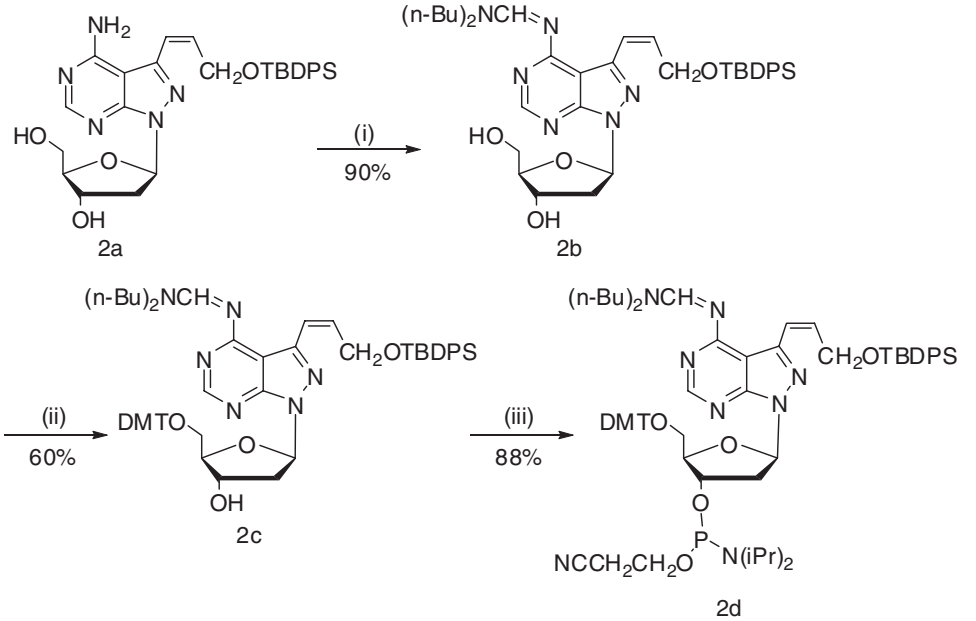
Further protection of the amino groups at position 4 in compounds **1a** and **2a** with *N*, *N*-di-n-butylformamide dimethyl acetal (**1b** and **2b**) and 5′-hydroxyl with DMT (**1c** and **2c**), and subsequent phosphitylation yielded the corresponding phosphoramidites **1d** (Scheme 2) and **2d** (Scheme 3), respectively.

For the synthesis of ODNs **7–9** containing **1**, the protected monomer **1d** with canonical residues was used. The normal deprotection condition, concentrated ammonia at 55–60°C for 18 hours, for DNA synthesis was capable of cleaving the *tert*-butyldiphenylsilyl group. For the synthesis of ODNs **10** and **11** containing **2**, mild deprotection was required because of the existence of the double bond linker. *Tert*-butyldiphenylsilyl could be cleaved by incubation in 1 M TBAF/THF overnight at room temperature.[4,8] Purification was carried out with denaturing PAGE. After desalting with a Sep-Pak Column (C18, Waters, Milford, MA, USA). MALDI-TOF was used for characterization (Table 1).

With TBDPS as an orthogonal protecting group, the synthesis of hydroxyl-functionalized ODNs with extra hydroxyl group in purine or pyrimidine bases was realized. TBDPS could be cleaved either with 1 M TBAF/THF meeting ultra-mild deprotection, or under the normal deprotection



SCHEME 2 Synthesis of phosphoramidite **1d**. Conditions: (i) $(C_4H_9)_2NCH(OCH_3)_2$, MeOH, 2 hours; (ii) DMTCl, in pyridine, at room temperature for 1 hour; (iii) $(NCCH_2CH_2O)(iPr)_2NPCl$, $(iPr)_2EtN$, in CH_2Cl_2, at room temperature for 30 minutes.



SCHEME 3 Synthesis of phosphoramidite **2d**. Conditions: (i) $(C_4H_9)_2NCH(OCH_3)_2$, MeOH, 2 hours; (ii) DMTCl, in pyridine, at room temperature for 1 hour; (iii) $(NCCH_2CH_2O)(iPr)_2NPCl$, $(iPr)_2EtN$, in CH_2Cl_2, at room temperature for 30 minutes.

TABLE 1 Molecular weights of oligodeoxynucleotides **5–11** determined by MALDI-TOF mass spectrometry

No.	Sequences	Molecular weight (calc.)	Molecular weight (found)
5	5′- d(GCG CGA TAA GGC CG)-3′	4313.8	4314.2
6	5′- d(CGG CCT TAT CGC GC)-3′	4214.7	4216.5
7	5′- d(GCG CG**1** TA**1** GGC CG)-3′	4430.0	4429.4
8	5′- d(GCG CGA T**11** GGC CG)-3′	4430.0	4429.1
9	5′- d(CGG CCT T**1**T CGC GC)-3′	4272.8	4271.3
10	5′- d(GCG CG**2** TAA GGC CG)-3′	4369.9	4369.5
11	5′- d(CGG CCT T**2**T CGC GC)-3′	4270.9	4270.4

condition (55°C conc. aq. ammonia). Therefore, TBDPS is a good choice for the synthesis of hydroxyl-functionalized DNAs.

Thermal Stability of Hydroxyl-Functionalized DNA Duplexes

It has been demonstrated that the propargyl linker at 5-position of 2′-deoxyuridine or the 7-position of 8-aza-7-deaza-2′-deoxyadenosine is favorable for the stability of corresponding base pairs and increases the thermal stability of duplexes and triplexes.[4,5,11–13] Hydroxylpropynyl of 8-aza-7-deaza-2′-deoxyadenosine (**3**) behaves similarly, as we have reported previously, the incorporation of compound **3** into DNA duplexes always leads to an increase of thermal stability.[4] When the linker was changed to propyl in compound **1**, a negative effect on duplex stability was observed (see Table 2) and more incorporations resulted in a lower T_m. The decrease of T_m was dependent on the number and positions of compound **1** in ODNs. For example, three incorporations of compound **1** in duplex **7·9** (see Table 2) gave a T_m of 67.3°C, while a lower T_m of 63.7°C was observed for the other duplex **8·9** with differently located three incorporations. The hydrophobicity and flexibility of the alkyl linker was supposed to be responsible for the negative effect. With the incorporation of compound **2** with a *Z*-propenyl linker, a similar position-dependent negative effect was observed. However, this negative effect could not exclude the positive contribution of the planar double bond linker to the base-stacking, as observed with the planar triple bond linker in compound **3**. It is probably that the geometry of the *Z*-propenyl linker buried the group deeply within the major groove, the regular duplex structure was disturbed and a negative effect on duplex stability was generated. This negative contribution was possibly greater than the positive base-stacking of the double bond. On the contrary, the triple bond extruded outward linearly; there was less agitation in the major groove and a positive effect was always obtained. The similar phenomenon was also observed with triplexes modified with 5-aminopropargyl and 5-(*Z*)-aminopropenyl-2′-deoxyuridines.[8] A T_m increase was observed for the triplexes containing the

TABLE 2 The T_m values of duplexes containing deoxyadenosine analogues **1–4**[a]

No.	Duplex	T_m (°C)
5·6	5′- d(GCG CGA TAA GGC CG)-3′ 3′- d(CGC GCT ATT CCG GC)-5′	68.1[b]
7·9	5′- d(GCG CG**1** TA**1** GGC CG)-3′ 3′- d(CGC GCT **1**TT CCG GC)-5′	67.3
5·9	5′- d(GCG CGA TAA GGC CG)-3′ 3′- d(CGC GCT **1**TT CCG GC)-5′	66.9
8·6	5′- d(GCG CGA T**11** GGC CG)-3′ 3′- d(CGC GCT ATT CCG GC)-5′	65.6
8·9	5′- d(GCG CGA T**11** GGC CG)-3′ 3′- d(CGC GCT **1**TT CCG GC)-5′	63.7
11·6	5′- d(GCG CG**2** TAA GGC CG)-3′ 3′- d(CGC GCT ATT CCG GC)-5′	66.8
5·12	5′- d(GCG CGA TAA GGC CG)-3′ 3′- d(CGC GCT **2**TT CCG GC)-5′	66.1
11·12	5′- d(GCG CG**2** TAA GGC CG)-3′ 3′- d(CGC GCT **2**TT CCG GC)-5′	66.0
15·6	5′- d(GCG CG**3** TAA GGC CG)-3′ 3′- d(CGC GCT ATT CCG GC)-5′	69.3[b]
15·13	5′- d(GCG CG**3** TAA GGC CG)-3′ 3′- d(CGC GCT **3**TT CCG GC)-5′	71.8[b]
5·13	5′-d(GCG CGA TAA GGC CG)-3′ 3′- d(CGC GCT **3**TT CCG GC)-5′	70.0[b]
14·6	5′- d(GCG CGA T**33** GGC CG)-3′ 3′- d(CGC GCT ATT CCG GC)-5′	69.6[b]
14·13	5′- d(GCG CGA T**33** GGC CG)-3′ 3′- d(CGC GCT **3**TT CCG GC)-5′	72.4[b]
16·6	5′- d(GCG CGA T**44** GGC CG)-3′ 3′- d(CGC GCT ATT CCG GC)-5′	68.1[b]
16·17	5′- d(GCG CGA T**44** GGC CG)-3′ 3′- d(CGC GCT **4**TT CCG GC)-5′	68.5[b]
18·17	5′- d(GCG CG**4** TAA GGC CG)-3′ 3′- d(CGC GCT **4**TT CCG GC)-5′	68.7[b]

[a]Measured in 100 mM NaCl, 10 mM $MgCl_2$, and 10 mM Na-cacodylate (pH 7.0) with 5 μM oligodeoxynucleotides.

[b]Data from reference 4.

triple bond linker, while no T_m increase could be observed for the triplexes appended with the *Z*-double bond linker. The distinct influence of the linkers on thermal stability of DNA helix was supposed to be closely related to their inherent properties.[11,14] In our study, the stabilities of base pairs **3**-dT > **4**-dT > **1**-dT ~ **2**-dT, reflected the contribution of the 7-positioned linkers of 8-aza-7-deaza-2′-deoxyadenosine in the order of propargyl > H > alkyl ~ (*Z*)-alkenyl.

Computer aided calculation on the duplexes **5·9**, **5·12**, **5·13** was conducted with AMBER 8.0 (University of California, San Francisco, CA, USA), employing the Amber 2003 force field (15–17) on a SGI Altix 350 workstation (SGI, Freemont, CA, USA). The calculation results showed that total energy of these three duplex systems was very similar to each other. The

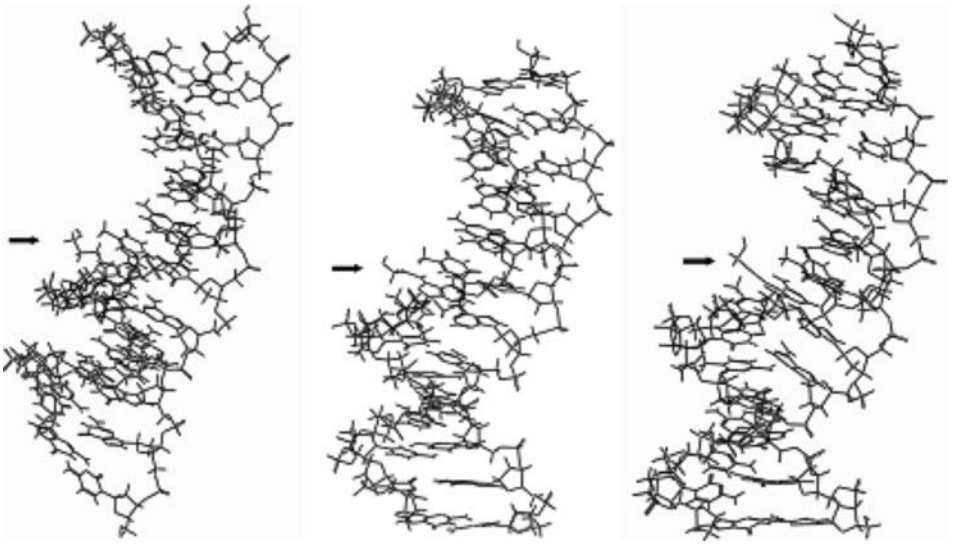


FIGURE 1 Calculated averaged conformations of duplex **5·9** (left), **5·12** (middle), **5·13** (right), with arrows pointing to the 7-positioned substituents of nucleoside analogues **1**, **2**, and **3**, respectively.

three 7-substitutents located in the major groove (Figure 1), but with a different extending mode for each of them, depending on the geometry of the linkers. The propyl link is flexible, the propargyl extends straight outsides fully, and the propenyl linker bends a little towards the duplex. Thus, the local environment was affected differently, and different T_m values were observed for them, respectively.

In summary, our research provided the approach for the synthesis of hydroxyl-functionalized ODNs, with TBDPS as the orthogonal protecting group. These primary results demonstrated that the influence of hydroxyl group on the thermal stability of duplexes is closely related to the properties of the linker. The oligodeoxynucleotides with hydroxyl group might be applied in the finding of new functional nucleic acids or other biotechnologies.

MATERIALS AND METHODS

General

Commercially available reagents were used without further purification. Pyridine was dried and distilled over calcium hydride. Thin layer chromatography (TLC) and flash column chromatography were run on HS GF_{254} (Qingdao, China) and silica gel (200–300 mesh, Qingdao, China), respectively. All ^{1}H, ^{13}C, and ^{31}P-NMR were recorded on a JNM-ECA-400 spectrometer (JEOL, Tokyo, Japan), chemical shifts (δ) are in ppm downfileld from internal TMS (^{1}H, ^{13}C) or external 85% H_3PO_4 (^{31}P), and the J values were

given in Hz. Melting points were obtained using a RY-1 apparatus and are uncorrected (Tianjin, China). Ultraviolet (UV) spectra were recorded on a Cary-100 Bio UV-visible spectrophotometer, λ_{max} in nm, ε in $dm^3mol^{-1}cm^{-1}$. Microanalyses were performed on a Fisons-1108 (Fisons, Milan, Italy).

4-Amino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3-(3-hydroxyprop-1-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (1). 10% Pd/C (0.6 g) was added to the methanolic solution (50 ml) of compound **3** (0.5 g, 1.64 mmol). The mixture was sealed in a stainless oven under hydrogen (5 Kg pressure) and stirred at 30°C. After complete hydrogenation, the catalyst was filtered off and the product was crystallized from the concentrated filtrate as a colorless solid (0.46 g, 90.7%), m.p. 150–152°C. R_f (CH_2Cl_2/CH_3OH 5:1) 0.52. UV (MeOH): λ_{max} 209 (30600), 259 (8100), 278 (8700). ^{1}H-NMR (DMSO-d_6): δ 1.79 (dt, $J = 7.00$, 2 H, 5-CH_2CH_2C$H_2$$CH_2$OH), 2.20 (m, 1 H, C2′-$H_\alpha$), 2.78 (m, 1 H, C2′-$H_\beta$), 2.94 (m, 2 H, 5-C$H_2$$CH_2CH_2$OH), 3.28–3.56 (m, 4 H, C5′-H, 5-CH_2CH_2CH_2OH), 3.78 (m, 1 H, C4′-H), 4.42 (m, 1 H, C3′-H), 4.66 (t, $J = 5.0$, 1 H, 5-$CH_2CH_2CH_2$O*H*), 4.80 (t, $J = 5.9$, 1 H, C5′-OH), 5.22 (d, $J = 4.5$, 1 H, C3′-OH), 6.50 (t, $J = 6.6$, 1 H, C1′-H), 7.32, 8.15 (br, 2 H, NH_2), 8.16 (s, 1 H, C2-H). ^{13}C-NMR (DMSO-d_6): δ 24.2, 31.6, 37.9, 59.7, 62.6, 71.2, 83.6, 87.5, 98.9, 145.8, 154.8, 155.9, 158.2. Anal. Calcd for $C_{13}H_{19}N_5O_4$ (M 309.32): C, 50.48; H, 6.19; N, 22.64. Found: C, 50.54; H, 6.10; N 22.55.

4-Amino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3-(3-*tert*-butyldiphenyl silyloxyprop-1-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (1a). With the same procedure described for compound **1**, compound **1a** was prepared from **3a** (2.9 g, 3.07 mmol) and 10% Pd/C (0.52 g) in methanol (50 ml) as a colorless solid (2.7 g, 90.3%), R_f (CH_2Cl_2/CH_3OH 9:1) 0.53. UV (MeOH): λ_{max} 207 (24450), 259 (7900), 278 (8000). ^{1}HNMR (DMSO-d_6): δ 0.98 (s, 9 H, *t*Bu), 1.94 (m, 2 H, 5-CH_2C$H_2$$CH_2$OH), 2.16 (m, 1 H, C2′-$H_\alpha$), 2.73(m, 1 H, C2′-$H_\beta$), 3.02 (m, 2 H, 5-C$H_2$$CH_2CH_2$OH), 3.36, 3.51 (2 m, 2 H, C5′-H), 3.76 (m, 3 H, C4′-H, 5-CH_2CH_2CH_2OH), 4.41 (m, 1 H, C3′-H), 4.81 (t, $J = 6.4$, 1 H, C5′-OH), 5.22 (d, $J = 4.5$, 1 H, C3′-OH), 6.49 (t, $J = 6.3$, 1 H, C1′-H), 7.10–7.70 (2 m, 12 H, arom. H, NH_2), 8.16 (s, 1 H, C2-H). ^{13}C-NMR (DMSO-d_6): δ 18.8, 24.0, 26.7, 31.1, 37.9, 62.6, 71.3, 83.7, 87.6, 98.9, 127.8, 127.9, 129.8, 133.3, 135.0, 145.4, 154.8, 155.8, 158.2. Anal. Calcd for $C_{29}H_{37}N_5O_4Si$. 0.5 H_2O (M 556.73), C, 62.56; H, 6.88; N, 12.58. Found: C, 62.72; H, 6.77; N, 12.28.

1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-4-{[(di-n-butylamino)methylide ne]amino}-3-(3-*tert*-butyldiphenylsilyloxyprop-1-yl)-1*H*-pyrazolo[3,4-*d*]pyrimi dine (1b). *N*, *N*-di-n-butylformamide dimethyl acetal (0.58 g, 2.82 mmol) was added to the methanolic solution (50 ml) of compound **1a** (1.50 g, 2.81 mmol). The solution was stirred at 40°C for 2 hours, and then concentrated for flash chromatography (CH_2Cl_2/CH_3OH 40:1 as the eluent). The product was obtained as a colorless syrup (1.70 g, 90%), R_f (CH_2Cl_2/CH_3OH 15:1) 0.57. UV (MeOH): λ_{max} 214 (22500), 319 (21500).

^{1}HNMR (DMSO-d_6): δ 0.83, 0.90 [2 t, 6 H, N=CHN($CH_2CH_2CH_2CH_3$)$_2$], 0.96 (s, 9 H, *t*Bu), 1.26 [m, 4 H, N=CHN($CH_2CH_2CH_2CH_3$)$_2$], 1.56 [m, 4 H, N=CHN($CH_2CH_2CH_2CH_3$)$_2$], 2.06 (m, 2 H, 5-$CH_2CH_2CH_2OH$), 2.19 (m, 1 H, C2′-H_α), 2.77 (m, 1 H, C2′-H_β), 3.07 (m, 2 H, 5-$CH_2CH_2CH_2OH$), 3.37, 3.49 [2 m, 6 H, N=CHN($CH_2CH_2CH_2CH_3$)$_2$, C5′-H], 3.72 (t, J = 6.4, 2 H, 5-$CH_2CH_2CH_2OH$), 3.81 (m, 1 H, C4′-H), 4.43 (m, 1 H, C3′-H), 4.80 (t, J = 5.9, 1 H, C5′-OH), 5.25 (d, J = 4.5, 1 H, C3′-OH), 6.53 (t, J = 6.6, 1 H, C1′-H), 7.34–7.58 (m, 10 H, arom. H), 8.42 (s, 1 H, C2-H), 8.97 [s, 1 H, N=C*H*N($CH_2CH_2CH_2CH_3$)$_2$]. ^{13}C-NMR (DMSO-d_6): δ 13.5, 13.6, 18.7, 19.1, 19.7, 24.7, 26.7, 28.6, 30.4, 31.0, 37.9, 45.3, 51.3, 62.6, 63.0, 71.2, 83.8, 87.5, 106.1, 127.7, 129.7, 133.2, 134.9, 146.9, 155.3, 155.4, 157.1, 162.4. Anal. Calcd for $C_{38}H_{54}N_6O_4Si$ (M 686.96): C, 66.44; H, 7.92; N, 12.23. Found: C, 66.68; H, 8.06; N, 11.92.

1-[2-Deoxy-5-O-(4,4′-dimethoxytriphenylmethyl)-β-D-*erythro*-pentofuranosyl]-4-{[(di-n-butylamino)methylidene]amino}-3-(3-*tert*-butyldiphenylsilyloxyprop-1-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (1c). Compound **1b** (1.20 g, 1.78 mmol) was coevaporated with dried pyridine for three times, dissolved in dried pyridine (2 ml). DMT-Cl (0.76 g, 2.23 mmol) was added to the solution in portions with stirring at room temerpature. Then methanol was added to the solution, and the solution was concentrated for flash chromatography (CH_2Cl_2/CH_3OH from 80:1 to 40:1 as the eluent). The product was obtained as a colorless solid (1.06 g, 61%). R_f (CH_2Cl_2/CH_3OH 20:1) 0.46. UV (MeOH): λ_{max} 205 (77000), 318 (30400). ^{1}HNMR (DMSO-d_6): δ 0.80, 0.91 [t, 6 H, N=CHN($CH_2CH_2CH_2CH_3$)$_2$], 0.95 (m, 9 H, *t*Bu), 1.20 [m, 4 H, N=CHN($CH_2CH_2CH_2CH_3$)$_2$], 1.55 [m, 4 H, N=CHN($CH_2CH_2CH_2CH_3$)$_2$], 1.83 (m, 2 H, 5-$CH_2CH_2CH_2OH$), 2.27 (m, 1 H, C2′-H_α), 2.71 (m, 1 H, C2′-H_β), 3.00 (m, 4 H, C5′-H, 5-$CH_2CH_2CH_2OH$), 3.47 [m, 4 H, N=CHN($CH_2CH_2CH_2CH_3$)$_2$], 3.64 (m, 8 H, 2 OCH_3, 5-$CH_2CH_2CH_2OH$), 3.95 (m, 1 H, C4′-H), 4.50 (m, 1 H, C3′-H), 5.28 (d, J = 5.0, 1 H, C3′-OH), 6.58–7.58 (m, 24 H, arom. H, C1′-H), 8.44 (s, 1 H, C2-H), 8.98 [s, 1 H, N=C*H*N($CH_2CH_2CH_2CH_3$)$_2$]. ^{13}C-NMR (DMSO-d_6): δ 13.6, 18.7, 19.1, 19.7, 24.8, 26.6, 28.6, 30.4, 31.0, 38.3, 45.2, 51.3, 54.8, 63.2, 64.9, 71.4, 83.4, 85.1, 85.5, 106.1, 112.8, 112.9, 126.3, 127.5, 127.6, 127.7, 127.8, 129.5, 129.6, 129.7, 133.2, 134.9, 135.6, 145.1, 147.0, 155.3, 155.4, 157.0, 157.8, 157.9, 162.4. Anal. Calcd for $C_{59}H_{72}N_6O_6Si$ (M 989.33): C, 71.63; H, 7.34; N, 8.49. Found: C, 71.31; H, 7.50; N, 8.30.

1-[2-Deoxy-5-O-(4,4′-dimethoxytriphenylmethyl)-β-D-*erythro*-pentofuranosyl]-4-{[(di-n-butylamino)methylidene]amino}-3-(3-*tert*-butyldiphenylsilyloxyprop-1-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidine 3′-[(2-Cyanoethyl) N,N-diisopropylphosphoramidite] (1d). Compound **1c** (0.85 g, 0.87 mmol) was dissolved in dried dichloromethane (10 ml), DIEA (1 ml, 5.75 mmol) and diisopropylphosphoramido chloridite (0.25 g, 1.0 mmol) were added to the solution. After stirring at room temperature for 2 hours, the solution was diluted with dichloromethane (20 ml), washed with

cold aq. 5% $NaHCO_3$ and brine, dried with anhydr. Na_2SO_4, and concentrated for flash chromatography (CH_2Cl_2/CH_3COCH_3 20:1 as the eluent). The product was obtained as colorless foam (0.35 g, 35.4%). R_f (CH_2Cl_2/CH_3OH 30:1) 0.61, 0.71. ^1H NMR($CDCl_3$): δ 0.86, 0.98 [t, 6 H, N=CHN($CH_2CH_2CH_2CH_3$)$_2$], 1.01 (m, 9 H, tBu), 1.14–1.40 [m, 10 H, NCH(CH_3)$_2$, N=CHN($CH_2CH_2CH_2CH_3$)$_2$], 1.60 [m, 4 H, N=CHN($CH_2CH_2CH_2CH_3$)$_2$], 1.94–2.17 (m, 3 H, 5-$CH_2CH_2CH_2OH$, C2′-H_α), 2.36–2.63 (m, 3 H, 5-$CH_2CH_2CH_2OH$, C2′-H_β), 2.92–3.91 [m, 18 H, C5′-H, 5-$CH_2CH_2CH_2OH$, N=CHN($CH_2CH_2CH_2CH_3$)$_2$, 2 OCH_3, OCH_2CH_2CN], 4.23 (m, 1 H, C4′-H), 4.80 (m, 1 H, C3′-H), 6.66–7.64 (m, 24 H, arom. H, C1′-H), 8.48 (s, 1 H, C2-H), 8.86 [s, 1 H, N=CHN($CH_2CH_2CH_2CH_3$)$_2$]. ^{31}P NMR ($CDCl_3$): 148.75, 148.49.

4-Amino-1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-3-[3-*tert*-butyldiphenylsilyloxy-(*Z*)-prop-1-enyl]-1*H*-pyrazolo[3,4-*d*]pyrimidine (2a). The methanolic solution of compound **3a** (2 g, 3.68 mmol) and $NiCl_2.6H_2O$ (0.9 g, 3.78 mmol) was cooled to −78°C. $NaBH_4$ (0.21 g, 5.56 mmol) was added in portions to the solution. After stirring for 30 minutes, the reaction mixture was warmed naturally until it turned black. The mixture was concentrated for flash chromatography (CH_2Cl_2/CH_3OH from 40:1 to 20:1 as the eluent). The product was obtained as colorless needles (0.8 g, 40%), m.p. 150–153°C (recrystallized from methanol). R_f (CH_2Cl_2/CH_3OH 10:1) 0.45. UV (MeOH): λ_{max} 208 (37800), 251 (13800). ^1H NMR (DMSO-d_6): δ 1.02 (s, *t*Bu), 2.04 (m, 1 H, C2′-H_α), 2.44 (m, 1 H, C2′-H_β), 3.18, 3.28 (2 m, 2 H, C5′-H), 3.75 (m, 1 H, C4′-H), 4.22 (m, 1 H, C3′-H), 4.64 (t, J = 5.6, 1 H, C5′-OH), 4.67–4.86 (ddd, J = 2.2, 4.5, 16.2, 2 H, CH = CHCH_2), 5.18 (d, J = 4.5, 1 H, C3′-OH), 6.11 (dt, J = 5.0, 11.5, 1 H, CH=$CH$$CH_2$), 6.48 (t, J = 6.7, 1 H, C1′-H), 6.93 (dt, J = 11.8, 1 H, CH=$CHCH_2$), 7.35–7.63 (m, arom., NH_2), 8.17 (s, 1 H, C2-H). ^{13}C NMR (DMSO-d_6): δ 18.8, 26.7, 37.4, 62.6, 63.1, 71.2, 83.3, 87.7, 98.6, 117.5, 127.8, 129.8, 134.9, 136.6, 140.9, 154.4, 156.0, 158.0. Anal. Calcd for $C_{29}H_{35}N_5O_4Si$ (M 545.7): C, 63.83; H, 6.46; N, 12.83. Found: C, 63.62; H, 6.35; N 12.60.

4-Amino-1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-3-[3-hydroxy-(*Z*)-prop-1-enyl]-1*H*-pyrazolo[3,4-*d*]pyrimidine (2). To the anhydrous THF solution (20 ml) of compound **2a** (0.6 g, 1.1 mmol), TBAF (0.48 g, 1.5 mmol) was added, the solution was stirred at room temperature for 10 minutes. TLC indicated a complete deprotection reaction. The reaction mixture was concentrated for flash chromatography (CH_2Cl_2/CH_3OH 10:1 as the eluent), and the product was obtained as colorless solid (0.3 g, 88%), m.p. 170–173°C. R_f (CH_2CH_2:CH_3OH 5:1) 0.4. UV (MeOH): λ_{max} 209 (22600), 250 (14000), 282 (10200). ^1H NMR (DMSO-d_6): δ 2.27 (m, 1 H, C2′-H_α), 2.76 (m, 1 H, C2′-H_β), 3.38, 3.54 (2 m, 2 H, C5′-H), 3.81 (m, 1 H, C4′-H), 4.47 (m, 3 H, C3′-H, CH=CHCH_2OH), 4.77 (br, C5′-OH), 4.95 (br, 1 H, CH=$CHCH_2O$*H*), 5.29 (d, J = 4.5, C3′-OH), 6.02 (2 t, J = 5.1, J = 12.1, 1 H, CH=$CH$$CH_2$OH), 6.60 (t, J = 6.2, 1 H, C1′-H), 6.87, 6.90 (2 t,

J = 2.2, 11.8, 1 H, C*H*=CHCH$_2$OH), 7.50 (br, 2 H, NH$_2$), 8.18 (s, 1 H, C2-H). ^{13}C NMR (DMSO-d_6): δ 37.9, 60.2, 62.6, 71.1, 83.4, 87.6, 98.6, 116.7, 139.2, 141.3, 154.3, 155.9, 158.0. Anal. Calcd for $C_{13}H_{17}N_5O_4$.(M 307.31): C, 50.81; H, 5.58; N, 22.79. Found: C, 51.20; H, 5.53; N 22.33.

1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-4-{[(di-n-butylamino)methylidene]amino}-3-[3-*tert*-butyldiphenylsilyloxy-(*Z*)-prop-1-enyl]-1*H*-pyrazolo[3,4-*d*]pyrimidine (2b). Compound **2a** (1.50 g, 2.81 mmol) was dissolved in methanol (50 ml), followed by the addition of *N*, *N*-di-n-butylformamide dimethyl acetal (0.58 g, 2.82 mmol). The solution was stirred at 40°C for 2 hours, and then was concentrated for flash chromatography (CH_2Cl_2/CH_3OH 40:1 as the eluent). The product was obtained as a colorless syrup (1.70 g, 90%), R_f (CH_2Cl_2/CH_3OH 15:1) 0.57. UV (MeOH): λ_{max} 205 (20500), 316 (28500). ^{1}HNMR (DMSO-d_6): δ 0.96, 1.00 [2 t, 6 H, N=CHN(CH$_2$CH$_2$CH$_2$C*H*$_3$)$_2$], 1.07 (s, 9 H, *t*Bu), 1.39 [2 m, 4 H, N=CHN(CH$_2$CH$_2$C*H*$_2$CH$_3$)$_2$], 1.67 [2 m, 4 H, N=CHN(CH$_2$C*H*$_2$CH$_2$CH$_3$)$_2$], 2.13 (m, 1 H, C2′-H$_\alpha$), 2.58 (m, 1 H, C2′-H$_\beta$), 3.24, 3.34 (2 m, 2 H, C5′-H), 3.61 [m, 4 H, N=CHN(C*H*$_2$CH$_2$CH$_2$CH$_3$)$_2$], 3.82 (m, 1 H, C4′-H), 4.31 (m, 1 H, C3′-H), 4.71 (t, J = 5.8, 1 H, C5′-OH), 4.81, 4.95 (2 m, 2 H, CH=CHC*H*$_2$), 5.27 (d, J = 4.5, 1 H, 3′-OH), 6.14 (m, 1 H, CH=C*H*CH$_2$), 6.58 (t, J = 6.4, 1 H, C1′-H), 7.23 (m, 1 H, C*H*=CHCH$_2$), 7.41–7.69 (2 m, 10 H, arom. H), 8.48 (s, 1 H, C2-H), 9.02 [s, 1 H, N=C*H*N(CH$_2$CH$_2$CH$_2$CH$_3$)$_2$]. ^{13}C-NMR (DMSO-d_6): δ 13.5, 13.6, 18.8, 19.1, 19.8, 26.7, 28.5, 30.4, 37.5, 45.6, 51.5, 62.6, 63.1, 71.2, 83.5, 87.7, 105.9, 117.8, 127.7, 127.8, 129.7, 133.1, 134.9, 142.5, 154.8, 155.6, 157.0, 162.4. Anal. Calcd for $C_{38}H_{52}N_6O_4Si.0.25H_2O$ (M 689.45): C, 66.20; H, 7.68; N, 12.19. Found: C, 66.39; H, 7.88; N, 11.70.

1-[2-Deoxy-5-O-(4,4′-dimethoxytriphenylmethyl)-β-D-*erythro*-pentofuranosyl]-4-{[(di-n-butylamino)methylidene]amino}-3-[3-*tert*-butyldiphenylsilyloxy-(*Z*)-prop-1-enyl]-1*H*-pyrazolo[3,4-*d*]pyrimidine (2c). Compound **2b** (0.35 g, 0.51 mmol) was coevaporated with dried pyridine for three times firstly, then it was dissolved in dried pyridine (2 ml), DMT-Cl (0.2 g, 0.59 mmol) was added in portions to the solution. After stirring at room temperature for 2 hours, methanol (5 ml) was added, and the solution was concentrated for flash chromatography (CH_2Cl_2/CH_3OH from 60:1 to 40:1 as the eluent). The product was obtained as a colorless solid (0.3 g, 60%). R_f (CH_2Cl_2/CH_3OH 20:1) 0.45. UV (MeOH): λ_{max} 206 (61700), 322 (29400). ^{1}HNMR (DMSO-d_6): δ 0.92, 0.97 [2 t, 6 H, N=CHN(CH$_2$CH$_2$CH$_2$C*H*$_3$)$_2$], 0.99 (s, 9 H, *t*Bu), 1.34 [2 m, 4 H, N=CHN(CH$_2$CH$_2$C*H*$_2$CH$_3$)$_2$], 1.63 [2 m, 4 H, N=CHN(CH$_2$C*H*$_2$CH$_2$CH$_3$)$_2$], 2.16 (m, 1 H, C2′-H$_\alpha$), 2.47 (m, 1 H, C2′-H$_\beta$), 2.95 (2 m, 2 H, C5′-H), 3.50, 3.59 [2 m, 4 H, N=CHN(C*H*$_2$CH$_2$CH$_2$CH$_3$)$_2$], 3.66, 3.67 (2 s, 2 OCH$_3$), 3.93 (m, 1 H, C4′-H), 4.30 (m, 1 H, C3′-H), 4.38, 4.87 (2 m, CH=CHC*H*$_2$), 5.21 (d, J = 5.0, 1 H, C3′-OH), 6.05 (m, 1 H, CH=C*H*CH$_2$), 6.57 (m, 1 H, C1′-H), 6.63–7.58 (4 m, 24 H, C*H*=CHCH$_2$, arom. H), 8.46 (s, 1 H, C2-H), 8.98

[s, 1 H, N=C*H*N($CH_2CH_2CH_2CH_3$)$_2$]. ^{13}C-NMR (DMSO-d_6): δ 13.6, 18.8, 19.1, 19.8, 26.7, 28.6, 30.5, 37.8, 45.6, 51.5, 54.8, 54.9, 63.2, 64.9, 71.3, 83.6, 85.1, 85.9, 106.0, 112.9, 126.4, 127.5, 127.7, 127.8, 129.7, 134.9, 142.4, 145.0, 156.9, 157.8, 157.9, 162.4. Anal. Calcd for $C_{59}H_{70}N_6O_6Si$ (M 987.31): C, 71.77; H, 7.15; N, 8.51. Found: C, 71.88; H, 7.20; N, 8.43.

1-[2-Deoxy-5-O-(4,4′-dimethoxytriphenylmethyl)-β-D-*erythro*-pentofuranosyl]-4-{[(di-n-butylamino)methylidene]amino}-3-[3-*tert*-butyldiphenylsilyloxy-(*Z*)-prop-1-enyl]-1*H*-pyrazolo[3,4-*d*]pyrimidine 3′-[(2-Cyanoethyl) N,N-diisopropylphosphoramidite] (2d). To the solution of compound **2c** (0.21 g, 0.2 mmol) in dichloromethane (6 ml), DIEA (0.6 ml, 2.8 mmol) and diisopropylphosphoramido chloridite (0.1 g, 0.4 mmol) were added. After stirring at room temperature for 1 hour, the solution was diluted with dichloromethane (10 ml), and it was washed with cold aq. 5% $NaHCO_3$ and brine. The solution was then dried with anhydr. Na_2SO_4, and concentrated for flash chromatography (CH_2Cl_2/CH_3COCH_3 from 30:1 to 15:1 as the eluent). The product **2d** was obtained as colorless foam (0.22 g, 88%). R_f (CH_2Cl_2/CH_3OH 30:1) 0.61, 0.71. ^{1}H NMR($CDCl_3$): δ 0.89–1.68 [m, 29 H, N=CHN($CH_2CH_2CH_2CH_3$)$_2$, *t*Bu, N($CH(CH_3)_2$], 2.23–3.07 [m, 8 H, C2′-H, C5′-H,, N=CHN($CH_2CH_2CH_2CH_3$)$_2$], 3.50–4.15 (m, 11 H, 2 OCH_3, OCH_2CH_2CN, C4′-H), 4.53 (m, 2 H, CH=CHCH_2), 4.89 (m, 1 H, C3′-H), 6.03 (1 H, CH=C*H*CH_2), 6.57 (m, 1 H, C1′-H), 6.65, 7.08–7.60 (4 m, 24 H, C*H*=$CHCH_2$, arom. H), 8.46 (s, 1 H, C2-H), 8.98 [s, 1 H, N=C*H*N($CH_2CH_2CH_2CH_3$)$_2$]. ^{31}P NMR ($CDCl_3$): 148.30, 148.57.

Synthesis and Purification of Oligonucleotides

Deoxyoligonucleotides were synthesized on an ABI 392 DNA/RNA synthesizer (Applied Biosystems, USA) on a 1 μmol scale by standard protocol for phosphoramidite chemistry with DMT-off mode, the coupling yields of unnatural phosphoramidites were all above 95% with extending coupling time to 2 minutes. The deoxyoligonucleotides containing monomer **1** were synthesized with normal phosphoramidites and deprotected in conc. aq. ammonia at 55°C for 18 hours. The ammoniac solution was concentrated for further purification. Tac-protected naturally phosphoramidites were used for the synthesis of deoxyoligonucleotides containing monomer **2**, and the deprotection was conducted in conc. aq. ammonia overnight at room temperature. The solution was dried and the residue was immersed in 1 M TBAF/THF overnight at room temperature. 1 M (Et_3NH)OAc was added to the solution, and the mixture was desalted with Sep-Pak column. After washing thoroughly with water, the DMT-off oligonucleotide was eluted with methanol/water (70:30, V/V), and concentrated for further purification. The concentrated residues were purified with 20% polyacrylamide /7 M urea denaturing gel, extracted with 0.3 M sodium acetate. The solution was desalted on Sep-Pak. The homogeneous product was characterized by

MALDI-TOF (Table 1) performed on Kratos Axima-CFR-plus (Shimatzu, Kyoto, Japan) with BHB as matrix.

T_m Measurements

T_m of the DNA duplexes was performed with a Cary-100 Bio UV-Visible spectrophotometer (Varian, Palo Alto, CA, USA) equipped with a Cary temperature controller. A buffer consisting of 100 mM NaCl, 10 mM $MgCl_2$ and 10 mM Na-cacodylate (pH 7.0)[4,13] with 5 μM of each oligonucleotide was used. The solution was heated to 85°C, after halting for 10 minutes, it was cooled to 25°C at a rate of 0.5°C/min. The UV absorbance was recorded at 260 nm during the cooling process. The T_m values were obtained from the melting curves. Each melting curve was fit to a non-self-complementary two-state model.

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