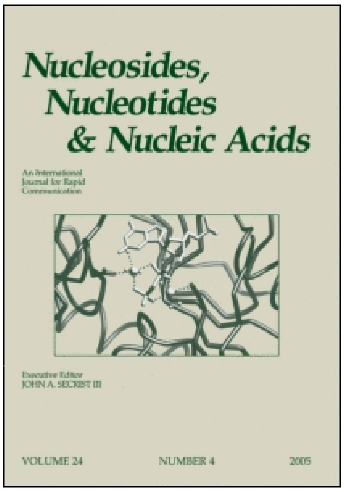

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HYDROXYL-FUNCTIONALIZED DNA: AN EFFICIENT ORTHOGONAL PROTECTING STRATEGY AND DUPLEX STABILITY

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□ *Tert-butyldiphenylsilyl (TBDPS) was found to be an effective orthogonal protecting strategy for the 5-substituted hydroxyl groups of de novo synthesized deoxyuridine analogues* ***1–3*** *and 7-(3-hydroxypropynyl)- of 8-aza-7-deazadeoxyadenosine* ***4*** *for their incorporation into oligodeoxynucleotides by phosphoramidite chemistry. It could be completely cleaved under normal and ultra-mild deprotection conditions applied to DNA synthesis, without extra cleaving operation. The new phosphoramidites were coupled as usual with high yields. The new modified oligodeoxynucleotides were characterized by MALDI-TOF and enzymatic cleavage analysis. The thermal stability and conformation of these hydroxyl-functionalized DNA duplexes were evaluated.*

Keywords Hydroxyl; TBDPS; nucleosides; DNA; functionalization

INTRODUCTION

Modification of nucleic acid residues with various functional groups provided an alternative choice for in vitro selection of new functional RNA or DNA, such as artificial ribozymes,[1] deoxyribozymes,[2] and aptamers.[3]When the basic base pairing of the modified residues was maintained, more interactions and functions could expect for functionalized DNA assemblies used in DNA nanotechnology and materials.[4] The functional groups selected for these purposes were mostly those actively involved in proton-transfer or hydrogen-bonding. For example, amino and imidazolyl groups were introduced to the nucleic acid libraries for

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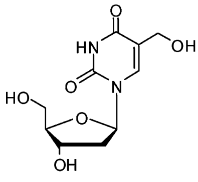
artificial ribozymes.[5,6] Several kinds of functional group-bearing residues have been tested as the substrates of polymerases for building functionalized DNA assemblies and libraries.[4,7] The hydroxyl group is known for hydrogen bonding, and it plays a critical role in the catalysis and interference of ribozymes and siRNA, respectively,[8,9] but it has been seldom used for the modification of oligonucleotides, partly because of the orthogonal protection for the extra hydroxyl group. 5-Hydroxymethyl-deoxyuridine (HMdU, **1**)[10,11] has been converted to its phosphoramidite for DNA solid-phase synthesis, with acetyl as the orthogonal protecting group for the 5-substituted hydroxyl group. However, this acetyl could not be applied for hydroxyl groups linked with other alkyl groups, such as in nucleoside analogues **2–3**.[12] In compound **1**, the 5-positioned hydroxyl group could be selectively protected by acetyl because it is more acidic than the 5′-hydroxyl group in the sugar moiety.[11] In compounds **2** and **3**, however, the two hydroxyl groups at 5- and 5′-positions hold almost the same reactivity toward acylation. Therefore, this orthogonal protection method was only specific for the 5-hydroxymethyl of compound **1**. The bulky *tert*-butyldimethylsilyl (TBDMS), which is often used for the protection of the 2′-hydroxyl of RNA residues as well as the 5-positioned hydroxyl group of 5-hydroxypropynyl-deoxyuridine,[13] was also used for compound **1**, producing a selective silylation with a big ratio between its 5-positioned hydroxyl and 5′-hydroxyl groups, but HPLC has to be used for the difficult separation.[10] It seems that these two protection groups were not feasible as a general orthogonal protection strategy for the hydroxyalkyl groups in **1–3**. Here we report an efficient orthogonal protection strategy for the incorporation of hydroxyl-bearing nucleoside analogues **1–3** into oligodeoxynucleotides with phosphoramidite chemistry. De novo synthesis of these nucleoside analogues was utilized to avoid the simultaneous presence of the 5-positioned hydroxyl and 5′-hydroxyl groups.

For the hydroxyl-containing purine nucleosides, we synthesized a deoxyadenosine analogue **4** to study the orthogonal protection method for its hydroxyl groups. To our best knowledge, 8-aza-7-deaza-deoxyadenosine (**5**)[14] possesses the same base-pairing ability as dA in the context of DNA duplex, its 7-substituent protrudes into the major groove, without much influence on the duplex conformation. Therefore, its 7-position is an ideal position for the introduction of various functional groups. Here, 3-hydroxypropynyl was introduced to its 7-position to obtain compound **4** by a cross-coupling reaction. This post-synthesis modification could allow the orthogonal protection between its two primary hydroxyl groups. The effect of these four hydroxyalkyl-containing nucleoside analogues on DNA duplex thermal stability was also evaluated.



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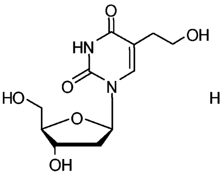

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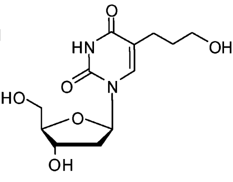

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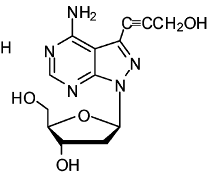

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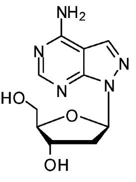

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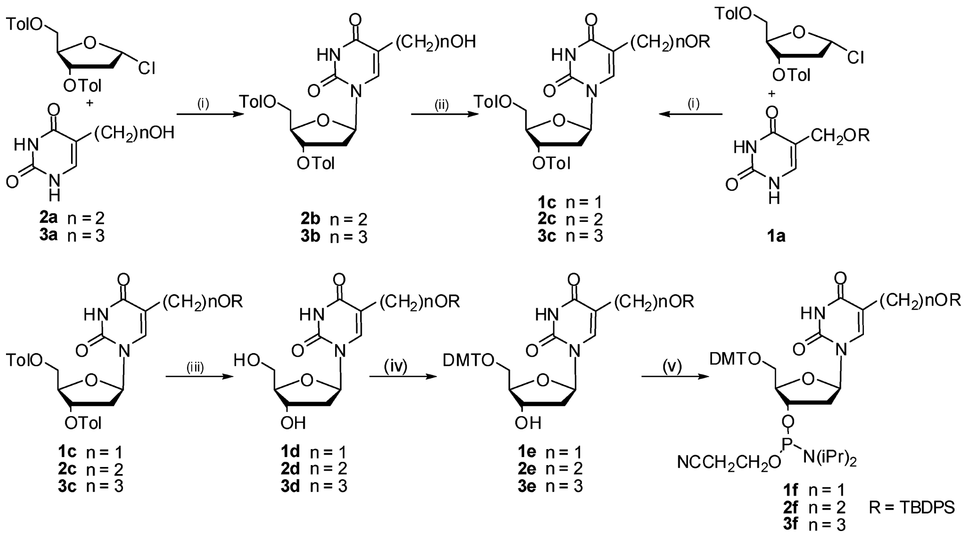
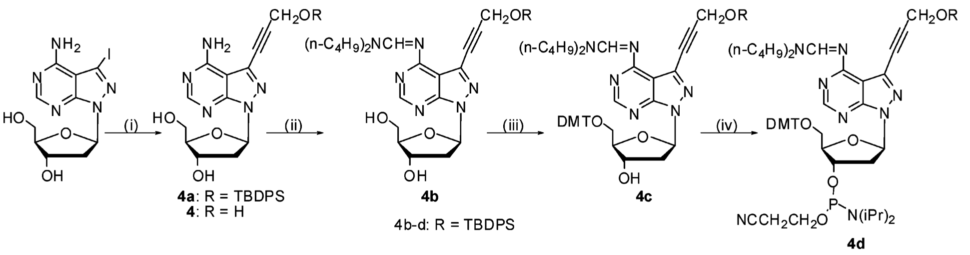
RESULTS AND DISCUSSION

Synthesis of the Phosphoramidites

Our strategy toward the phosphoramidites **1f–3f** was based on de novo synthesis of nucleoside analogues **1–3**.[12,15,16] as described in Scheme 1. The sugar moiety of compounds **2b** and **3b** was protected with toluoyl group after the glycosylation reaction of the hydroxyl-bearing nucleobases **2a** and **3a** with 1-chloro-3,5-ditoluoyl-2-deoxypentofuranose (chlorosugar).[17]The toluoyl group would be cleaved by basic treatment (0.1 M CH_3ONa/CH_3OH) in the next step. Therefore, the 5-positioned hydroxyl group must be protected before this deprotection step and its protecting group could go through such basic treatment. It is apparent that acetyl and toluoyl groups were inappropriate for the protection of the 5-positioned hydroxyl group. Although a selective silylation with TBDMS for **2b–3b** could be realized in this synthetic scheme, it was observed that TBDMS could be cleaved simultaneously with the 5′-toluoyl group by the basic treatment. Another silyl group, however, *tert*-butyldiphenylsilyl



SCHEME 1 Synthesis of phosphoramidites **1f–3f**. Reagents: (i) HMDS, CuI; (ii) TBDPSCl, imidazole; (iii) 0.1 M MeONa/MeOH; (iv) DMTCl, in pyridine; (v) $NC(CH_2)_2O(iPr)_2NPCl$, DIEA, in CH_2Cl_2.



SCHEME 2 Synthesis of phosphoramidite **4d**. Reagents: (i) 3-*tert*-butylediphenylsilyloxy-1-propyne or 3-hydroxy-1-propyne, $Pd(PPh_3)_4$, CuI, Et3N, in DMF; (ii) $(n\text{-Butyl})_2NCH(OMe)_2$, in MeOH, room temperature; (iii) DMTCl, in pyridine; (iv) $NC(CH_2)_2O(iPr)_2NPCl$, DIEA, in CH_2Cl_2.

(TBDPS) in **2c–3c** was stable enough to go through this basic treatment to the key intermediates **2d–3d**. More importantly, this protecting group could be cleaved efficiently by incubation in conc. aq. ammonia or 1 M TBAF/THF used in DNA/RNA synthesis.

After the determination of this orthogonal protecting group, 5-hydroxymethyluracil was silylated or protected with *tert*-butyldiphenylsilylchloride (TBDPS-Cl) in advance because of its poor solubility in the glycosylation reaction (Scheme 1) to obtain the silylated nucleobase **1a**. **1c** was obtained from the glycosylation reaction of **1a**, and then it was deprotected with 0.1 M CH_3ONa/CH_3OH to obtain **1d**. Compounds **1d–3d** were tritylated with DMT-Cl to offer **1e–3e**, followed by subsequent conversion to the phosphoramidites **1f–3f**.[18]

In compound **4**, the 3-hydroxypropynyl group was introduced by cross-coupling reaction to the 7-position of 8-aza-7-deaza- deoxyadenosine **5**[14] (Scheme 2). For the synthesis of phosphoramidite **4d**, the 7-positioned hydroxyl group was protected before it was introduced to the purine base. *Tert*-Butyldiphenylsilyl, *tert*-butyldimethylsilyl, and acetyl could be used in this route. The presence of the triple bond required a mild-fast deprotection condition for the 7-positioned hydroxyl and the 6-amino groups, for example, both concentrated aqueous ammonia at room temerpature for 2–4 hours and 1 M TBAF/THF were tolerable for the triple bond. TBDPS was used as the protecting group of the 7-positioned hydroxyl group (**4a**), because it could be cleaved completely by incubation with conc. aq. ammonia for 4 hours at room temperature, or 1 M TBAF/THF. The di-n-butylaminomethylidene group was applied to protect the 6-amino group (**4b**). **4b** was further protected with DMT (**4c**) and then converted to the phosphoramdite **4d**. Nucleoside **5** and its phosphoramidite were synthesized according to literature.[19]All the compounds were fully characterized by elemental analysis, ^{1}H NMR, ^{13}C NMR (Table 1), and/or UV data.

TABLE 1 ^{13}C NMR Chemical shifts of nucleoside derivatives thereof[a]

	C(2)[b,c] C(6)[c,d]	C(4)[b,c] C(7a)[c,d]	C(5)[b] C(3a)[d]	C(6)[b,c], C(4)[c,d]	C(3)[d]	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)
1a	151.2	163.4	111.2	138.2						
1c	150.0	162.2	112.9	136.4		81.4	36.7	74.6	85.2	64.3
1d	150.2	162.3	112.8	136.5		84.0	[e]	70.6	87.4	61.7
1e	150.2	162.2	113.2	135.1		83.9	[e]	70.6	85.6	64.2
2c	150.3	163.0	110.8	137.2		81.1	36.1	74.5	84.3	64.3
2d	150.4	163.3	110.2	137.6		83.8	[e]	70.7	87.4	61.8
2e	150.2	163.1	110.4	137.1		84.0	[e]	70.5	85.7	63.8
3c	150.2	163.2	113.9	135.6		81.2	36.2	74.7	84.5	64.2
3d	150.3	163.3	113.1	136.0		83.9		70.5	87.3	62.9
3e	150.2	163.2	113.7	135.6		83.8	[e]	70.6	85.5	63.7
4	157.7	156.8	100.8	153.7	126.7	84.0	37.9	71.0	87.8	62.4
4a	157.6	156.8	100.9	153.7	126.3	84.1	38.0	71.0	87.8	62.4
4b	157.1	155.4	106.1	155.3	127.8	83.8	37.9	71.3	87.6	63.0
4c	157.4	156.0	108.0	154.4	126.4	83.9	38.1	70.6	85.3	64.2

	alkyl, alkynyl, phenyl, and carboxyl groups
1a	18.8, 26.6, 58.9, 128.0, 130.0, 132.7, 135.0
1c	18.7, 21.2, 26.5, 58.8, 127.8, 129.2, 129.3, 129.4, 135.0, 143.8, 144.1, 165.2, 165.4
1d	18.8, 26.6, 59.0, 128.0, 130.0, 132.6, 132.7, 135.1
1e	18.7, 26.5, 59.0, 85.0, 126.7, 127.8, 127.9, 129..7, 129.9, 135.0, 144.8, 158.1
2c	18.6, 21.2, 26.5, 29.9, 61.7, 126.5, 127.7, 129.3, 129.4, 129.7, 133.1, 134.9, 143.9, 144.0, 165.2, 165.5
2d	18.8, 26.7, 30.2, 61.4, 127.8, 129.8, 133.2, 135.0
2e	18.7, 26.6, 30.0, 61.8, 85.3, 113.2, 127.6, 127.7, 127.8, 129.7, 133.1, 134.9, 144.8, 158.1
3c	18.7, 21.1, 21.2, 23.0, 26.6, 31.0, 63.0, 126.5, 127.9, 129.3, 129.4, 129.5, 129.8, 133.2, 135.0, 143.9, 144.1, 165.3, 165.5
3d	18.7, 22.9, 26.6, 30.6, 61.3, 127.8, 129.7, 133.2, 135.0
3e	18.7, 23.0, 26.6, 31.1, 63.1, 85.8, 113.2, 127.7, 127.8, 129.7, 129.8, 133.2, 135.0, 135.2, 135.3, 144.6
4	49.8, 75.3, 95.2
4a	18.8, 26.8, 53.2, 76.2, 93.2, 128.1, 130.1, 132.4, 135.1
4b	13.6, 13.7, 18.7, 19.1, 19.8, 24.8, 26.6, 28.6, 30.4, 31.0, 45.3, 51.4, 62.6, 127.8, 129.7, 133.2, 134.9, 146.9, 162.5
4c	13.6, 18.8, 19.1, 19.6, 26.5, 28.6, 30.4, 44.9, 51.1, 52.8, 54.9, 77.9, 85.2, 90.3, 113.0, 127.6, 128.0, 129.6, 129.7, 130.1, 132.2, 135.1, 145.1, 157.9, 162.2

[a] Measured in (D$_6$)DMSO at 303 K.

[b] Pyrimidine base numbering.

[c] Tentative.

[d] Systematic numbering for purine base.

[e] Superimposed by (D$_6$)DMSO.

Oligonucleotides

The complementary duplex, 5′-d (GCG CGA TAA GGC CG)-3′ (**6**), 5′-d (CGG CCT TAT CGC GC)-3′ (**7**), was used in this study. In the solid-phase synthesis of modified DNA sequences **8-19**, the coupling yields of the phosphoramidites **1f–3f** and **4d** (0.15– 0.2 M in acetonitrile) were all above 92%, with 4 minutes of coupling time, in the context

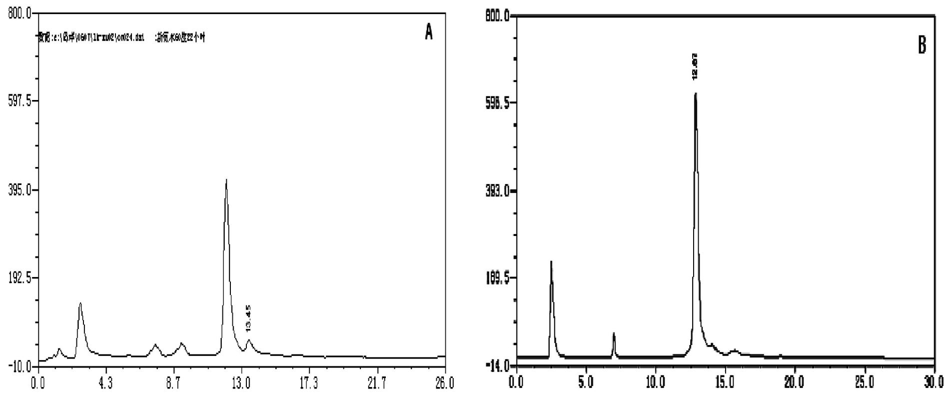


FIGURE 1 (A) HPLC purification profile of the DMT-on intermediate of sequence **8** after incubation in concentrated aqueous ammonia at 60°C for 18 hours. (B) HPLC purification profile of the DMT-on intermediate of sequence **10** after incubation in concentrated aqueous ammonia at 55°C for 19 hours

of the sequences. For the cleavage of TBDPS of compounds **1–4** in the oligodeoxynucleotides **8–16**, normal deprotection or mild deprotection methods were used. The normal deprotection procedure was conducted with the oligodeoxynucleotides containing **1–3**. For the sequences **8–9** containing nucleoside **1**, the optimum cleavage of TBDPS was performed at 60°C for 18 hours (**8**, (Figure 1A). For the sequences **10–13** containing **2** or **3**, 55°C and 19 hours in concentrated aqueous ammonia was enough for a complete cleavage (**10**, Figure 1B). 1 M TBAF/THF was also efficient for the cleavage of TBDPS in DMT-on or DMT-off sequences (such as **12**), which is compatible with ultra-mild DNA synthesis.[20] TBDPS has also been used for 5-hydroxymethyl pyrimidine in PNA synthesis, but its cleavage was conducted in a cold solution of trifluoroacetic acid and triethylsilane which is not suitable for DNA deprotection.[21] The cleavage of TBDPS in sequences **14–16** was also conducted in conc. aq. ammonia for 4 hours at room temperature, a complete deprotection was obtained (Figure 2). The sequences **6–19** were characterized with MALDI-TOF (Table 2) and some representative sequences were analyzed by enzymatic hydrolysis to be the expected hydroxyl-bearing oligodeoxynucleotides.

Thermal Stability of Hydroxyl-Functionalized DNA Duplexes

As presented in Table 3, the base pair **4**-dT in the duplex was more stable compared to the corresponding dA-dT and the unsubstituted **5**-dT,[22] and the increase of T_m was position dependent. More **4**-dT base pairs led to higher thermal stability of the modified duplexes. It is well known that the planar propynyl linker contributes to the helix stability. The functional group at the end of the linker also contributed to the duplex

TABLE 2 The measured molecular weight of the oligodeoxynucleotides **6–19** by MALDI-TOF

Sequences	MW (calc.)	MW (found)	Sequences	MW (calc.)	MW (found)
5′- d (GCG CGA TAA GGC CG)-3′ (6)	4312.8	4314.2	5′- d (CGG CC**3** **3**AT CGC GC)-3′ (13)	4302.8	4305.4
5′- d (CGG CCT TAT CGC GC)-3′ (7)	4214.7	4216.5	5′- d(GCG CG**4** TAA GGC CG)-3′ (14)	4366.9	4369.1
5′- d (CGG CC**1** TAT CGC GC)-3′ (8)	4230.7	4232.6	5′- d(GCG CGA T**44** GGC CG)-3′ (15)	4420.9	4424.1
5′- d (GCG CGA **1**AA GGC CG)-3′ (9)	4328.8	4330.1	5′- d(CGG CCT T**4**T CGC GC)-3′ (16)	4268.8	4270.8
5′- d (CGG CC**2** TAT CGC GC)-3′ (10)	4244.8	4244.0	5′- d(GCG CG**5** TAA GGC CG)-3′ (17)	4312.8	4314.9
5′- d (CGG CC**2** **2**AT CGC GC)-3′ (11)	4274.8	4274.5	5′- d(GCG CGA T**55** GGC CG)-3′ (18)	4312.8	4314.6
5′- d (CGG CCT TA**3** CGC GC)-3′ (12)	4258.8	4258.6	5′- d(CGG CCT T**5**T CGC GC)-3′ (19)	4214.7	4214.3

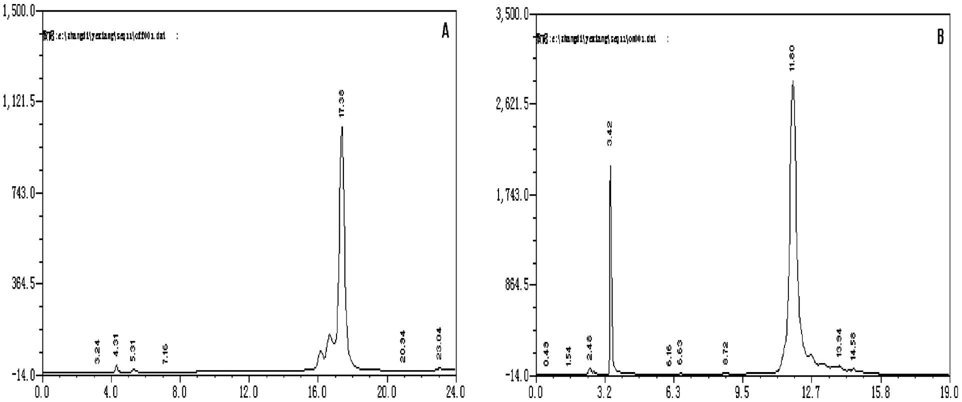


FIGURE 2 (A) HPLC purification profile of the DMT-on intermediate of sequence **14** after cleavage with concentrated aqueous ammonia at room temperature for 4 hours and (B) the target sequence **14** after detritylation.

stability. Compared to the amino group (of 3-aminopropynyl),[23,24] the hydroxyl group is a neutral group, which makes its contribution less than aminopropynyl to the duplex and triplex stability.[13]

When the hydroxyl group was linked to the nucleobase by alkyl chains, as shown in Table 3, the incorporation of the base pairs **1**-dA, **2**-dA, or **3**-dA in place of dT-dA, from one to three incorporations, led to the decrease of the T_m values, and more incorporations resulted in lower T_m. The effect of these hydroxyalkyl groups was contrary to that of the rigid planar 3-hydroxypropynyl, probably because these alkyl linkers were flexible and not contributable to base stacking, and their hydrophobic property was not compatible with the hydrophilic environment of the major groove. The duplexes containing **3**-dA were the least stable, because nucleoside **3** has the longest alkyl chain with the most hydrophobic and flexible properties.

All the melting curves of the duplexes exhibited a typical sigmoidal profile. The CD spectra of the control duplex and all the modified duplexes showed a positive band at around 280 nm and a negative band at around 250 nm that were attributable to B-like DNA conformation. It indicated that these hydroxyl-bearing base pairs did not result in significant deformation on the overall duplex conformation.

CONCLUSIONS

The orthogonal protective group TBDPS was successfully applied for the incorporation of 5-hydroxyalkyl deoxyuridine analogues and 7-(3-hydroxypropynyl)-8-aza-7-deaza-deoxyadenosine to oligodeoxynucleotides. It was compatible with DNA solid-phase synthesis with either normal or ultra-mild deprotection method. Modification of DNA duplex with these

TABLE 3 The T_m values of duplexes containing compounds **1–4**[a]

Duplex	Tm (°C)	Duplex	Tm (°C)
5′- d(GCG CGA TAA GGC CG)-3′ (6·7) 3′- d(CGC GCT ATT CCG GC)-5′	68.1		
5′- d(GCG CG**4** TAA GGC CG)-3′ (14·8) 3′- d(CGC GCT ATT CCG GC)-5′	69.3	5′- d(GCG CG**5** TAA GGC CG)-3′ (17·7) 3′- d(CGC GCT ATT CCG GC)-5′	68.7
5′- d(GCG CGA TAA GGC CG)-3′ (6·16) 3′- d(CGC GCT **4**TT CCG GC)-5′	70.0	5′- d(GCG CGA TAA GGC CG)-3′ (6·19) 3′- d(CGC GCT **5**TT CCG GC)-5′	68.2
5′- d(GCG CGA T**44** GGC CG)-3′ (15·7) 3′- d(CGC GCT ATT CCG GC)-5′	69.6	5′- d(GCG CGA T**55** GGC CG)-3′ (18·7) 3′- d(CGC GCT ATT CCG GC)-5′	68.1
5′- d(GCG CG**4** TAA GGC CG)-3′ (14·16) 3′- d(CGC GCT **4**TT CCG GC)-5′	71.8	5′- d(GCG CG**5** TAA GGC CG)-3′ (17·19) 3′- d(CGC GCT **5**TT CCG GC)-5′	68.7
5′- d(GCG CGA T**44** GGC CG)-3′ (15·16) 3′- d(CGC GCT **4**TT CCG GC)-5′	72.4	5′- d(GCG CGA T**55** GGC CG)-3′ (18·19) 3′- d(CGC GCT **5**TT CCG GC)-5′	68.5
5′- d(GCG CGA **1**AA GGC CG)-3′ (9·7) 3′- d(CGC GCT ATT CCG GC)-5′	67.6	5′- d(GCG CGA TAA GGC CG)-3′ (6·10) 3′- d(CGC GCT AT**2** CCG GC)-5′	65.8
5′- d(GCG CGA TAA GGC CG)-3′ (6·8) 3′- d(CGC GCT AT**1** CCG GC)-5′	66.7	5′- d(GCG CGA TAA GGC CG)-3′ (6·11) 3′- d(CGC GCT A**22** CCG GC)-5′	65.8
5′- d(GCG CGA TAA GGC CG)-3′ (6·12) 3′- d(CGC GC**3** ATT CCG GC)-5′	65.2	5′- d(GCG CGA TAA GGC CG)-3′ (6·13) 3′- d(CGC GCT A**33** CCG GC)-5′	63.5

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

hydroxyl-bearing nucleoside analogues resulted in thermal stability changes which were dependent on the linker used for the hydroxyl group and the position of the modified nucleosides in the duplex, but had no significant influence on the whole duplex conformation. This kind of modification affords extra hydrogen bonding and reactivity for the modified oligodeoxynucleotides in the various applications of DNA in gene research and biotechnologies.

EXPERIMENTAL

General

Chemicals bought from ACRÖS were reagent grade or better and used without further purification. Chloroform was refluxed and distilled from P_2O_5, and pyridine was dried with calcium hydride. Silica gel TLC was carried out on HS GF_{254} (Yantai Institute of Chemical Industry, China) and spotted by UV shadowing. Silica gel (200–300 mesh, Qingdaohaiyang Chemicals. Co., China) was used for flash column chromatography. The ^1H and ^{31}P NMR were recorded on a JNM-ECA-400 spectrometer (JEOL, Japan). Chemical shifts (δ) were in p.p.m. downfield from internal TMS (^1H, ^{13}C) or external 85% H_3PO_4 (^{31}P). The J values were given in Hz, λ_{max} in nm, and ε in dm^3 mol^{-1} cm^{-1}. Microanalyses were performed by National Center of Biomedical Analysis (Beijing, China) on a Fisons-1108 (Fisons, Italy). Reversed phase (RP)-HPLC was performed on an SPD-10AT (Shimadzu, Japan) equipped with XTerra® MS column (C-18, 4.6 × 250 mm, 5 μm, Waters, USA). Solid-phase extraction was conducted on SEP-PAK Cartridge (Oasis MAX, C18, Waters, USA). The solvent gradient system for HPLC is A: 0.07 M $(Et_3NH)OAc$ (pH 7.0)/MeCN 95:5; B: MeCN. MALDI-TOF was performed on Kratos Axima-CFR-plus (Shimatzu, Japan) with THAP (2′,4′,6′-trihydroxyacetophenone) as the matrix.

Silylation of Nucleobase-bearing Hydroxyl Groups

5-Tert-butyldiphenylsiloxymethyl-1H,3H-pyrimidin-2,4-dione (1a)

To a suspension of 5-hydroxymethyluracil (1 g, 7.0 mmol) in dried THF (10 mL), TBDPS-Cl (2.3 g, 8.4 mmol) and imidazole (0.58 g, 8.4 mmol) were added. The mixture was stirred at room temperature until the reaction was finished. Methanol (6 mL) was added to the mixture. After stirring for 5 minutes, the solution was concentrated for purification by flash chromatography (CH_2Cl_2/CH_3OH from 80:1 to 40:1 as the eluent) to obtain **1a** as colorless solid (2.2 g, 84%). R_f (CH_2Cl_2/CH_3OH 40:1): 0.2. ^1H NMR (400 MHz, [D_6]DMSO): $\delta = 1.01$ (s, 9 H, *tert*-Bu), 4.36 (s, 2 H, CH_2), 7.27 (s, 1 H, 6-H), 7.41–7.66 (m, 10 H, arom. H), 10.74, 11.12 (2 s, 2 H, 2 NH). $C_{21}H_{24}N_2O_3Si$ (M 380.51): C, 66.29; H, 6.36; N, 7.36. Found: C, 66.71; H, 6.57; N, 7.02.

1-(2-Deoxy-3,5-di-O-p-toluoyl-β-D-erythro-pentofuranosyl)-5-(2-tert-butyldiphenylsilyloxyethyl)-1H,3H-pyrimidin-2,4-dione (2c)

To the solution of 3′,5′-di-O-*p*-toluoyl-5-(2-hydroxyethyl)-2′-deoxyuridine (1.0 g, 1.97 mmol) in CH_2Cl_2 (40 mL), TBDPS-Cl (0.32 g, 27.5 mmol), and imidazole (0.45 g, 6.62 mmol) were added. The solution was stirred at room temperature for 4 hr. Methanol (4 mL) was added to the solution. After stirring for 5 minutes, the solution was concentrated for flash chromatography (CH_2Cl_2/CH_3OH from 80:1 to 40:1 as the eluent). **2c** was obtained as colorless solid (1.4 g, 95.2%). R_f (CH_2Cl_2/CH_3OH 20:1): 0.63. UV (MeOH): λ_{max} 206 (46 000), 242 (33 000). ^1H NMR (400 MHz, [D_6]DMSO): $\delta = 0.92$ (s, 9 H, *tert*-Bu), 2.17–3.67 (m, 10 H, 2′-H, $\mathit{CH_2}CH_2$, 2 CH_3), 3.65 (m, 2 H, $CH_2\mathit{CH_2}$), 4.48–4.56 (m, 3 H, 4′-H, 5′-H), 5.60 (m, 1 H, 3′-H), 6.32 (t, $J =$ 7.3, 1 H, 1′-H), 7.29–7.54, 7.87–7.94 (m, 19 H, 6-H, arom. H), 11.40 (s, 1 H, NH). $C_{43}H_{46}N_2O_8Si$ (M 746.92): C, 69.15; H, 6.21; N, 3.75. Found: C, 68.92; H, 6.36; N, 3.96.

1-(2-Deoxy-3,5-di-O-p-toluoyl-β- D-erythro-pentofuranosyl)-5-(3-tert-butyldiphenylsilyloxyprop-1-yl)-1H,3H-pyrimidin-2,4-dione (3c)

As described for **2c**, from 3′, 5′-di-O-*p*-toluoyl-5-(3-hydroxypropyl)-2′-deoxyuridine (9.7 g, 18.56 mmol), TBDPS-Cl (16.5 ml, 63.02 mmol) and imidazole (4.27 g, 62.79 mmol) in CH_2Cl_2 (130 ml), **3c** was obtained as colorless foam (13.4 g, 95%). R_f (CH_2Cl_2/CH_3OH 20:1): 0.59. UV (MeOH): λ_{max} 207 (75 000), 270 (14 600). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 0.96 (s, 9 H, *tert*-Bu), 1.58 (m, 2 H, $CH_2\mathit{CH_2}CH_2$), 2.11 (t, J = 7.4, 2 H, $\mathit{CH_2}CH_2CH_2$), 2.29, 2.40 (2 s, 6 H, 2 CH_3), 2.57 (m, 2 H, 2′-H), 3.50 (t, J = 6.3, 2 H, $CH_2CH_2\mathit{CH_2}$), 4.50–4.63 (m, 3 H, 4′-H, 5′-H), 5.62 (m, 1 H, 3′-H), 6.30 (t, J = 7.10, 1 H, 1′-H), 7.24–7.94 (m, 19 H, 6-H, arom. H), 11.40 (s, 1 H, NH). $C_{44}H_{48}N_2O_8Si$ (M 760.95): C, 69.45; H, 6.13; N, 3.68. Found: C, 69.18; H, 6.11; N, 3.88.

1-(2-Deoxy-3,5-di-O-p-toluoyl-β-D-erythro-pentofuranosyl)-5-tert-butyldiphenylsilyloxymethyl-1H,3H-pyrimidin-2,4-dione (1c)

The suspension of **1a** (1.5 g, 3.96 mmol) in hexamethyldisilazane (HMDS) (10 mL, 48.0 mmol) was refluxed for 3 hours to a clear solution, with ammonium sulfate (0.052 g, 0.39 mmol) as the catalyst. Extra HMDS was evaporated off under vacuum, and the residue was dissolved in dried chloroform (100 mL). To the solution 1-chloro-2-deoxy-3,5-di-O-α-D-toluoylpentose (1.5 g, 3.87 mmol) and CuI (0.42 g, 2.19 mmol) were added. The mixture was stirred at room temperature overnight, and then poured to sat. aq. $NaHCO_3$. The organic layer was washed with water, dried with anhydr. Na_2SO_4, and concentrated for flash chromatography (ethyl acetate/petroleum ether from 15:1 to 8:1 as the eluent). The product was obtained as colorless solid (1.13 g, 41 %). R_f (ethyl acetate/petroleum ether 1:2): 0.6. UV (MeOH): λ_{max} 206 (44 600), 242 (14 600).^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 0.94 (s, 9 H, *tert*-Bu), 2.33, 2.40 (2 s, 6 H, CH_3), 2.55 (m, 2 H, 2′-H), 4.30 (m, 2 H, 5-CH_2), 4.54 (m, 3 H, 4′-H, 5′-H), 5.55 (m, 1 H, 3′-H), 6.27 (t, J = 7.0, 1 H, 1′-H), 7.25–7.94 (m, 19 H, 6-H, arom. H). $C_{42}H_{44}N_2O_8Si$ (M 732.89): C, 68.83; H, 6.05; N, 3.82. Found: C, 68.72; H, 5.95; N, 3.87.

Deprotection Procedure for Compounds 1c–3c

1-(2-Deoxy-β-D-erythro-pentofuranosyl)-5-tert-butyldiphenylsilyloxymethyl-1H,3H-pyrimidin-2,4-dione (1d)

The solution of **1c** (1.8 g, 2.46 mmol) in 0.1 M CH_3ONa/ CH_3OH (50 mL) was stirred at room temerpature for 2 hours. Then it was neutralized with glacial acetic acid. The solution was concentrated for flash chromatography (CH_2Cl_2/CH_3OH from 40:1 to 15:1 as the eluent) to obtain the product as colorless foam (0.94 g, 75.8%). R_f (CH_2Cl_2/CH_3OH 9:1): 0.50. UV (MeOH): λ_{max} 209 (49 000), 229 (50 000). ^1H NMR (400 MHz, [D_6]DMSO): $\delta = 1.02$ (s, 9 H, *tert*-Bu), 2.03 (m, 2 H, 2′-H), 3.51 (m, 2 H, 5′-H), 3.79 (m, 1 H, 4′-H), 4.22 (m, 1 H, 3′-H), 4.39 (s, 2 H, 5-CH_2), 4.92 (t, $J = 5.3$, 1 H, C5′-OH), 5.28 (d, $J = 4.2$, 1 H, 3′-OH), 6.19 (t, $J = 6.7$, 1 H, 1′-H), 7.42–7.66 (m, 10 H, arom. H), 7.74 (s, 1 H, 6-H), 11.39 (s, 1 H, NH). $C_{26}H_{32}N_2O_6Si$ (M 496.63): C, 62.88; H, 6.49; N, 5.64. Found: C, 62.48; H, 6.57; N, 5.39.

1-(2-Deoxy-β-D-erythro-pentofuranosyl)-5-(2-tert-butyldiphenylsilyloxyethyl)-1H,3H-pyrimidin-2,4-dione (2d)

As described for **1d**, **2c** (1.2 g, 1.6 mmol) was dissolved in 0.1 M CH_3ONa/ CH_3OH (50 mL), and the solution was stirred for 2 hours. After neutralization with glacial acetic acid, the solution was concentrated for flash chromatography (CH_2Cl_2/CH_3OH from 40:1 to 15:1 as the eluent) to obtain the product as colorless foam (0.78 g, 95%), R_f (CH_2Cl_2/CH_3OH 9:1): 0.54. UV (MeOH): λ_{max} 250 (12 900), 287 (14 200). ^1H NMR (400 MHz, [D_6]DMSO): $\delta = 0.97$ (s, 9 H, *tert*-Bu), 2.02 (m, 2 H, 2′-H), 2.50 (m, 2 H, $\mathit{CH_2}CH_2$), 3.56 (m, 2 H, $CH_2\mathit{CH_2}$), 3.72–3.79 (m, 3 H, 4′-H, 5′-H), 4.23 (m, 1 H, 3′-H), 5.01 (t, $J = 4.9$, 1 H, 5′-OH), 5.22 (d, $J = 3.9$, 1 H, 3′-OH), 6.18 (t, $J = 6.9$, 1 H, 1′-H), 7.75 (s, 1 H, 6-H), 7.38–7.58 (m, 10 H, arom. H), 11.28 (s, 1 H, NH). $C_{27}H_{34}N_2O_6Si$ (M 510.65): C, 63.50; H, 6.71; N, 5.49. Found: C, 63.06; H, 6.56; N, 5.40.

1-2-Deoxy-β- D-erythro-pentofuranosyl)-5-(3-tert-butyldiphenylsilyloxyprop-1-yl)-1H,3H-pyrimidin-2,4-dione (3d)

As described for **1d**, the solution of **3c** (14.6 g, 19.2 mmol) in 0.1 M CH_3ONa/ CH_3OH (500 mL) was stirred at room temperature for 2 hours. **3d** was obtained as colorless foam (10.5 g, 98%), R_f (CH_2Cl_2/CH_3OH 9:1): 0.68. UV (MeOH): λ_{max} 207 (47 200), 250 (12 900), 287 (14 200). ^1H NMR (400 MHz, [D_6]DMSO): $\delta = 0.99$ (s, 9 H, *tert*-Bu), 1.72 (m, 2 H, $CH_2\mathit{CH_2}CH_2$), 2.04 (m, 2 H, 2′-H), 2.30 (m, 2 H, $\mathit{CH_2}CH_2CH_2$), 3.54 (m, 2 H, $CH_2CH_2\mathit{CH_2}$), 3.64 (m, 2 H, 5′-H_2), 3.77 (m, 1 H, 4′-H), 4.22 (m, 1 H, 3′-H), 4.98 (t, $J = 5.2$, 1 H, 5′-OH), 5.22 (d, $J = 4.2$, 1 H, 3′-OH), 6.16 (t, $J = 6.9$, 1 H, 1′-H), 7.39–7.65 (m, 11 H, arom. H, 6-H), 11.26 (s, 1 H, NH). $C_{28}H_{36}N_2O_6Si.0.5H_2O$ (M 533.68): C, 62.96; H, 6.93; N, 5.25. Found: C, 62.70; H, 6.90; N, 5.09.

Tritylation of 5′-Hydroxyl Group of Compounds 1d–3d

1-[2-Deoxy-5-O-(4,4′-dimethoxytrityl)-β-D-erythro-pentofuranosyl]-5-tert-butyldiphenylsilyloxymethyl-1H,3H-pyrimidin-2,4-dione (1e)

Compound **1d** (1.4 g, 2.8 mmol) was firstly dried by repeated co-evaporation with anh. pyridine (3 × 5 mL), then it was dissolved in dried pyridine (4 mL). To the stirred solution DMT-Cl (1.15 g, 3.36 mmol) was added in portions. After stirring at room temperature for 4 hours, methanol (4 mL) was added to the mixture, and stirring was continued for 15 minutes. The mixture was concentrated and then co-evaporated with methanol (3 × 5 mL), the residue was dissolved in CH_2Cl_2 (3 mL) for flash chromatography (CH_2Cl_2/CH_3OH from 80:1 to 40:1 as the eluent) to obtain the product as colorless foam (1.8 g, 80%). R_f (CH_2Cl_2/CH_3OH 40:1): 0.3. UV (MeOH): λ_{max} 208 (57 500), 266 (11 500). ^{1}H NMR (400 MHz, [D_6]DMSO): $\delta = 0.88$ (s, 9 H, *tert*-Bu), 2.08, 2.21 (2 m, 2 H, 2′-H), 3.16 (m, 2 H, 5′-H), 3.68 (s, 6 H, 2 CH_3O), 3.89 (m, 1 H, 4′-H), 4.07 (m, 1 H, 3′-H), 4.27 (m, 2 H, CH_2), 5.42 (d, $J = 4.5$, 1 H, 3′-OH), 6.20 (t, $J = 6.3$, 1 H, 1′-H), 6.82–7.58 (m, 24 H, 6-H, arom. H). $C_{47}H_{50}N_2O_8Si$ (M 798.99): C, 70.65; H, 6.31; N, 3.51. Found: C, 70.31; H, 6.18; N, 3.57.

1-[2-Deoxy-5-O-(4,4′-dimethoxytrityl)-β-D-erythro-pentofuranosyl]-5-(2-tert-butyldiphenylsilyloxyethyl)-1H,3H-pyrimidin-2,4-dione (2e)

As described for **1e**, with **2d** (5.6 g, 11 mmol) and DMT-Cl (4.87 g, 14.37 mmol) in dried pyridine (15 mL), **2e** was obtained as colorless foam (8.86 g, 98 %) after purification with flash chromatography (CH_2Cl_2/CH_3OH from 80:1 to 40:1 as the eluent). R_f (CH_2Cl_2/CH_3OH 20:1): 0.44. UV (MeOH): λ_{max} 205 (52 000), 249 (37 800), 326 (49 500). ^{1}H NMR (400 MHz, [D_6]DMSO): $\delta = 0.91$ (s, 9 H, *tert*-Bu), 2.09 (m, 2 H, 2′-H), 2.27 (t, $J = 1.6$, 2 H, $C\mathit{H_2}CH_2$), 3.18 (m, 2 H, 5-$CH_2C\mathit{H_2}$), 3.65 (m, 2 H, 5′-H), 3.70 (s, 3 H, OCH_3), 3.88 (m, 1 H, 4′-H), 4.21 (m, 1 H, 3′-H), 5.34 (d, $J = 4.5$, 1 H, 3′-OH), 6.17 (t, $J = 6.7$, 1 H, 1′-H), 6.85–7.53 (m, 24 H, 6-H; arom. H), 11.34 (s, 1 H, NH). $C_{48}H_{52}N_2O_8Si$ (M 813.02): C, 70.91; H, 6.45; N, 3.45. Found: C, 70.83; H, 6.40; N, 3.58.

1-[2-Deoxy-5-O-(4,4′-dimethoxytrityl)-β-D-erythro-pentofuranosyl]-5-(3-tert-butyldiphenylsilyloxyprop-1-yl)-1H,3H-pyrimidin-2,4-dione (3e)

As described for **1e**, with **3d** (1 g, 1.91 mmol) and DMT-Cl (0.77 g, 2.3 mmol) in dried pyridine (2 mL), **3e** was obtained as colorless foam (1.3 g, 82.5%) after purification with flash chromatography (CH_2Cl_2/CH_3OH from 80:1 to 40:1 as the eluent). R_f (CH_2Cl_2/CH_3OH 20:1): 0.49. UV (MeOH): λ_{max} 208 (82 500), 284 (30 500), 329 (30 300). ^{1}H NMR (400 MHz, [D_6]DMSO): $\delta = 0.94$ (s, 9 H, *tert*-Bu), 1.51 (m, 2 H, $CH_2\mathit{CH_2}CH_2$), 1.92 (m, 2 H, $\mathit{CH_2}CH_2CH_2$), 2.16 (m, 2 H, 2′-H), 3.17 (m, 2 H, $CH_2CH_2\mathit{CH_2}$), 3.40 (m, 2 H, 5′-H), 3.67 (2 s, 6 H, 2 OCH_3), 3.88 (m, 1 H, 4′-H), 4.28 (m, 1 H, 3′-H), 5.32 (d, $J = 4.5$, 1 H, 3′-OH), 6.19 (t, $J = 6.9$, 1 H, 1′-H), 6.82–7.57 (m, 24 H, 6-H, arom.H), 11.32 (s,1 H, NH). $C_{49}H_{54}N_2O_8Si$ (M 827.05): C, 71.16; H, 6.58; N 3.39. Found: C, 71.30; H, 6.65; N, 3.22.

Phosphitylation Procedure for Compounds 1e–3e

*1-[2-Deoxy-5-O-(4,4′-dimethoxytrityl)-β-D-erythro-pentofuranosyl]-5-*tert-*butyldiphenylsilyloxymethyl-1H,3H-pyrimidin-2,4-dione 3′-[(2-cyanoethyl)* N,N-*diisopropylphosphoramidite] (1f)*

To a solution of **1e** (0.8 g, 1.00 mmol) and diisopropylethylamine (1 mL, 6.05 mmol) in CH_2Cl_2 (10 mL) was added 2-cyanoethyl diisopropylphosphoramido chloridite (0.45 mL, 2.0 mmol) until complete conversion. The solution was diluted with CH_2Cl_2 (20 mL), washed with 5% aqueous $NaHCO_3$ (30 mL) and sat. brine (30 mL), dried with anhydr. Na_2SO_4, and concentrated to an oily residue for flash chromatography (CH_2Cl_2/CH_3COCH_3 from 60:1 to 40:1 as the eluent). The product was obtained as a mixture of two isomers (0.8 g, 80%). R_f (CH_2Cl_2/CH_3COCH_3 20:1) 0.47, 0.64. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.99$ (s, 9 H, *tert*-Bu), 1.17 [m, 12 H, 2 N ($CH(C\mathit{H}_3)_2$)], 2.63, 2.76 (2 t, $J = 5.9$, 2 H, $NCCH_2CH_2O$), 3.20 (m, 2 H, 5′-H), 3.45–3.75 [m, 10 H, $NCCH_2CH_2O$, 2 CH_3O, 2 N ($CH(CH_3)_2$)], 4.02 (m, 1 H, 4′-H), 4.27 (m, 3 H, CH_2, 3′-H), 6.17 (m, 1 H, 1′-H), 6.81–7.59 (m, 24 H, 6-H; arom. H). ^{31}P NMR (400 MHz, $CDCl_3$): 148.18, 148.72.

1-[2-Deoxy-5-O-(4,4′-dimethoxytrityl)-β-D-erythro-pentofuranosyl]-5-(2-tert-butyldiphenylsilyloxyethyl)-1H,3H-pyrimidin-2,4-dione 3′-[(2-cyanoethyl) N,N-diisopropylphosphoramidite] (2f)

As described for **1f**, with **2e** (1.8 g, 2.21 mmol), diisopropylethylamine (2.5 mL, 15.13 mmol), and 2-cyanoethyl diisopropylphosphoramido chloridite (0.9 mL, 4.06 mmol) in CH_2Cl_2 (30 mL), **2f** was obtained as colorless foam (1.33 g, 59%). R_f (CH_2Cl_2/CH_3COCH_3 20:1): 0.65, 0.75. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.99$ (s, 9 H, *tert*-Bu), 1.08, 1.12 [m, 12 H, 2N ($CH(C\mathit{H}_3)_2$)], 2.27 (m, 2 H, 2′-H), 2.11–2.64 (m, 6 H, $C\mathit{H}_2CH_2$, $NCCH_2CH_2O$), 3.30–3.78 (m, 6 H, $CH_2C\mathit{H}_2$, $NCCH_2CH_2O$, 5′-H), 3.74 (2 s, 6 H, OCH_3), 4.17 (m, 1 H, 4′-H), 4.52 (m, 1 H, 3′-H), 6.28 (t, $J = 6.9$, 1 H, 1′-H), 6.79–7.56 (m, 24 H, 6-H; arom. H). ^{31}P NMR (400 MHz, $CDCl_3$): 149.06, 149.65.

1-[2-Deoxy-5-O-(4,4′-dimethoxytrityl)-β-D-erythro-pentofuranosyl]-5-(3-tert-butyldiphenylsilyloxyprop-1-yl)-1H,3H-pyrimidin-2,4-dione 3′-[(2-cyanoethyl) N,N-diisopropylphosphoramidite] (3f)

As described for **1f**, with **3e** (1 g, 1.21 mmol), diisopropylethylamine (0.6 mL, 2.7 mmol), and 2-cyanoethyl diisopropylphosphoramido chloridite (1.2 mL, 5.4 mmol) in CH_2Cl_2 (20 mL), **3f** was obtained as colorless foam (0.65 g, 52.4%). TLC (CH_2Cl_2/CH_3COCH_3 20:1) R_f 0.60, 0.63. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.98$ (s, 9 H, *tert*-Bu), 1.21, 1.29 [m, 6 H, $N(CH(\mathit{CH_3})_2)$], 1.46–2.73 [m, 14 H, 2 $N(C\mathit{H}(CH_3)_2)$, 5-$CH_2\mathit{CH_2}CH_2$, 2′-H], 2.41 (t, $J = 6.5$, 2 H, $\mathit{CH_2}CH_2CH_2$), 3.30–3.68 (m, 6 H, $CH_2CH_2\mathit{CH_2}$, $NCCH_2\mathit{CH_2}O$, 5′-H), 3.71 (2 s, 6 H, 2 OCH_3), 4.17 (m, 1 H, C4′-H), 4.60 (m, 1 H, 3′-H), 6.35 (m, 1 H, 1′-H), 6.77–7.61 (m, 24 H, 6-H, arom. H). ^{31}P NMR (400 MHz, $CDCl_3$): 149.06, 149.53.

1-(2-Deoxy-β-D-erythro-pentofuranosyl)-3-(prop-1-ynyl)-1H-pyrazolo[3, 4-d]pyrimidin-4-amine (4)

To the solution of compound 7-iodo-8-aza-7-deaza-2′-deoxyadenosine (1.95 g, 5.17 mmol) in DMF (10 ml) was added Pd $[P(C_6H_5)_3]_4$ (300 mg, 0.25 mmol) and CuI (200 mg, 1.04 mmol), the mixture was stirred under N_2 at room temperature 3-hydroxypropyne (0.28 ml, 5 mmol) and triethylamine (2.0 ml, 11.48 mmol) were added to the mixture subsequently. The stirring was continued overnight. The solvent was evaporated off under vacuum and the residue was subjected to flash chromatography, yielding the product as colorless solid (1.45 g, 92%). TLC (CH_2Cl_2/CH_3OH 5:1) R_f 0.58. UV (MeOH): λ_{max} 207 (24 500), 249 (11 400), 286 (13 000). ^1H NMR (400 MHz, $[D_6]DMSO$): $\delta = 2.22$ (m, 1 H, C2′-H_α), 2.72 (m, 1 H, C2′-H_β), 3.25–3.48 (m, 2 H, C5′-H), 3.81 (m, 1 H, C4′-H), 4.38–4.43 (d, $J = 6.16$, 3 H, 5-C≡CCH_2OH, C3′-H), 4.76 (t, $J = 5.9$, 1 H, C5′-OH), 5.27 (d, $J = 4.48$, 1 H, C3′-OH), 5.47 (t, $J = 6.02$, 2 H, 5-C≡CCH_2OH), 6.50 (t, $J = 6.44$, 1 H, C1′-OH), 6.66, -8.09 (br, 2 H, NH_2). $C_{13}H_{15}N_5O_4$ (M 305.29): C, 51.14; H, 4.95; N, 22.94. Found: C, 51.18; H, 4.86; N, 22.92.

1-(2-Deoxy-β-D-erythro-pentofuranosyl)-3-(3-tert-butyldiphenylsilyloxyprop-1-ynyl)-1H-pyrazolo[3, 4-d]pyrimidin-4-amine (4a)

The coupling reaction of 7-iodo-8-aza-7-deaza-2′-deoxyadenosine (0.38 g, 1.0 mmol) with 3-*tert*-butyldiphenylsilyloxypropyne (0.57 g, 2.0 mmol) was conducted with the procedure described for compound **4**, with $Pd[P(C_6H_5)_3]_4$ (100 mg, 0.09 mmol) and CuI (60 mg, 0.33 mmol) as the catalysts and triethylamine (0.5 ml, 2.87mmol) as the base in DMF (5 ml). After purification by flash chromatography, the product was yielded as colorless solid (0.45 g, 84%). TLC (CH_2Cl_2/CH_3OH 9:1) R_f 0.58. UV (MeOH): λ_{max} 206 (44 100), 250 (12 800), 287 (13 700). ^1H NMR (400 MHz, $[D_6]DMSO$): $\delta = 1.0$ (s, 9 H, *tert*-Bu), 2.29 (m, 1 H, C2′-H_α), 2.80 (m, 1 H, C2′-H_β), 3.56, 3.41 (2 m, 2 H, C5′-H), 3.85 (m, 1 H, C4′-H), 4.47 (m, 1 H, C3′-H), 4.73 (s, 2 H, 5-C≡CCH_2), 4.81 (t, $J = 5.8$, 1 H, C5′-OH), 5.32 (d, $J = 4.4$, 1 H, C3′-OH), 6.58 (t, $J = 6.4$, 1H, C1′-H), 7.72–7.44 (2m, 10 H, arom. H), 8.26 (s, 1 H, C2-H). $C_{29}H_{33}N_5O_4Si$ (M 543.69): C, 64.06; H, 6.12; N, 12.88. Found: C, 63.56; H, 6.06; N 12.76.

1-(2-Deoxy-β-D-erythro-pentofuranosyl)-4-{[(di-n-butylamino)methylidene]amino}-3-(3-tert-butyldiphenylsilyloxyprop-1-ynyl)-1H-pyrazolo[3, 4-d]pyrimidine (4b)

To the solution of compound **4a** (1.16 g, 2.19 mmol) in methanol (10 ml) *N, N*-di-n-butylformamide dimethyl acetal (0.45 g, 2.2 mmol) was added. After being stirred at 40°C for 4 h, the reaction mixture was evaporated under vacuum, and the residue was subjected to flash chromatography. The product was yielded as colorless foam (1.3 g, 91%). TLC ($\mathrm{CH_2Cl_2/CH_3OH}$ 15:1) R_f 0.57. UV (MeOH): λ_{max} 218 (47 000), 329 (27 700). ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 0.76, 0.90 [2 t, 6 H, $\mathrm{N = CHN(CH_2CH_2CH_2C}H_3\mathrm{)_2}$]. 1.03 (s, 9 H, *tert*-Bu), 1.16, 1.25, [2 m, 4 H, $\mathrm{N = CHN(CH_2CH_2C}H_2\mathrm{CH_3)_2}$], 1.51 [m, 4 H, $\mathrm{N = CHN(CH_2C}H_2\mathrm{CH_2CH_3)_2}$], 2.29 (m, 1 H, C2′-$H_\alpha$), 2.82 (m, 1 H, C2′-$H_\beta$), 3.50 [m, 6 H, $\mathrm{N = CHN(C}H_2\mathrm{CH_2CH_2CH_3)_2}$, C5′-H], 3.84 (m, 1 H, C4′-H), 4.46 (m, 1 H, C3′-H), 4.64 (s, 2 H, 5-$\mathrm{C{\equiv}CC}H_2$), 4.79 (t, *J* = 5.7, 1 H, C5′-OH), 5.32 (d, *J* = 4.5,1 H, C3′-OH), 6.61 (t, *J* = 6.3, 1 H, C1′-H), 7.70~7.42 (m, 10 H, arom. H), 8.47 (s, 1 H, C2-H), 8.95 [s, 1 H, $\mathrm{N = C}H\mathrm{N(CH_2CH_2CH_2CH_3)_2}$]. $\mathrm{C_{38}H_{50}N_5O_4Si}$ (M 682.93): C, 66.83; H, 7.38; N, 12.31. Found: C, 66.57; H, 7.53; N, 11.98.

1-[2-Deoxy-5-O-(4,4′-dimethoxytriphenylmethyl)-β-D-erythro-pentofuranosyl]-4-″[(di-n-butylamino)methylidene]amino″-3-(3-tert-butyldiphenylsilyloxyprop-1-ynyl)-1H-pyrazolo[3, 4-d]pyrimidin-4-amine (4c)

As described for **1e,** with compound **4b** (1.6 g, 2.39 mmol) and 4, 4′-dimethyltriphenylchloride (DMT-Cl) (0.97 g, 2.87 mmol) in dried pyridine (5ml), the product **4c** was obtained as colorless foam (1.46 g, 63%), TLC ($\mathrm{CH_2Cl_2/CH_3OH}$ 20:1) R_f 0.39. UV (MeOH): λ_{max} 206 (82 300), 328 (29 800). ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 0.73, 0.90 [2 t, 6 H, $\mathrm{N = CHN(CH_2CH_2CH_2C}H_3\mathrm{)_2}$], 1.01 (s, 9 H, *tert*-Bu), 1.14, 1.25 [2 m, 4 H, $\mathrm{N = CHN(CH_2CH_2C}H_2\mathrm{CH_3)_2}$], 1.50 [m, 4 H, $\mathrm{N = CHN(CH_2C}H_2\mathrm{CH_2CH_3)_2}$], 2.33 (m, 1 H, C2′-$H_\alpha$), 2.82 (m, 1 H, C2′-$H_\beta$), 3.06 (m, 2 H, C5′-H), 3.47 [m, 4 H, $\mathrm{N = CHN(C}H_2\mathrm{CH_2CH_2CH_3)_2}$], 3.66, 3.65 (2 s, 6 H, 2 $\mathrm{OCH_3}$), 3.94 (m, 1 H, C4′-H), 4.55 (m, 1 H, C3′-H), 4.63 (s, 2 H, 5-$\mathrm{C{\equiv}CC}H_2$), 5.35 (d, *J* = 4.8, 1 H, C3′-OH), 6.63 (m, 1 H, C1′-H), 7.70~6.70 (m, 23 H, arom. H), 8.49 (s, 1 H, C2-H), 8.95 [s, 1 H, $\mathrm{N = C}H\mathrm{N(CH_2CH_2CH_2CH_3)_2}$]. ^{13}C NMR (400 MHz, [D_6]DMSO): 13.6, 18.8, 19.1, 19.6, 26.5, 28.6, 30.4, 38.1, 44.9, 51.1, 52.8, 54.9, 64.2, 70.6, 77.9, 83.9, 85.2, 85.3, 90.3, 108.0, 113.0, 126.4, 127.6, 128.0, 129.6, 129.7, 130.1, 132.2, 135.1, 145.1, 154.4, 156.0, 157.4, 157.9, 162.2. $\mathrm{C_{59}H_{68}N_6O_6Si}$ (M 985.29): C, 71.92; H, 6.96; N, 8.53. Found: C, 71.91; H, 7.01; N, 8.40.

1-[2-Deoxy-5-O-(4,4′-dimethoxytriphenylmethyl)-β-D-erythro-pentofuranosyl]-4-{[(di-n-butylamino)methylidene]amino}-3-(3-tert-butyldiphenylsilyloxyprop-1-ynyl)-1H-pyrazolo[3, 4-d]pyrimidine 3′-[(2-cyanoethyl) N,N-diisopropylphosphoramidite] (4d)

As described for **1f**, this phosphoramidite was prepared from the reaction of compound **4c** (1.0 g, 1.03 mmol) with 2-cyanoethyl *N*, *N*′-(diisopropyl)-phosphoramidochloridite (0.5 g, 2.1 mmol) in dried dichloromethane (10 ml) in the presence of diisopropylethylamine (1 ml, 6.05 mmol). The product **4d** was obtained as colorless foam (0.57 g, 47.3%), TLC (CH_2Cl_2/CH_3OH 30:1) R_f 0.66, 0.75. ^1H NMR (400 MHz, $CDCl_3$): $\delta =$ 0.82, 0.95 [2 t, 6 H, N = $CHN(CH_2CH_2CH_2CH_3)_2$], 1.01 (s, 9 H, *tert*-Bu), 1.10–1. 57 [m, 14 H, $NCH(CH_3)_2$, N = $CHN(CH_2CH_2CH_2CH_3)_2$], 2.63, 2.45 (2 m, 2 H, C2′-H), 3.15–3.74 [m, 10 H, N = $CHN(CH_2CH_2CH_2CH_3)_2$, C5′-H, OCH_2CH_2CN], 3.72, 3.71 (2 s, 6 H, 2 OCH_3), 4.23 (m, 1 H, C4′-H), 4.59 (s, 2 H, 5-C≡CCH_2), 4.80 (t, 1 H, C3′-H), 7.75~6.71 (m, 24 H , arom. H, C1′-H), 8.50 (s, 1 H, C2-H), 8.84 (s, 1 H, N = $CHN(CH_2CH_2CH_2CH_3)_2$). ^{31}P NMR (400 MHz, $CDCl_3$): 149.06, 148.90.

Synthesis and Purification of Oligodeoxynucleotides

The oligodeoxynucleotide synthesis was carried out on an ABI 392 DNA/RNA synthesizer (Applied Biosystems, USA) with 1 μmol scale, according to the User Protocol for phosphoramidite chemistry with DMT-on or DMT-off mode. For the sequences containing compounds **1-3**, normally protected phosphoramidites were used. The sequences containing nucleoside **1** were deprotected in conc. aq. ammonia at 60°C for 18 hours; and 19 hours at 55°C for the sequences containing nucleoside **2** or **3**. The DMT-on sequences **14–16** containing compound **4** were synthesized with tac(*tert*-butylphenoxyacetyl)-protected phosphoramidites. TBDPS could be cleaved by incubation in conc. aq. ammonia at room temperature for 4 hours.

The TBDPS of the DMT-on or DMT-off sequences containing **1–4** was also cleaved with 1 M TBAF/THF efficiently. After the DMT-on or DMT-off sequence was cleaved from CPG, the ammonic soln. was concentrated and dried; the residue was then dissolved in 1 M TBAF/THF and incubated at room temperature overnight. The soln. was desalted with SEP-PAK for HPLC purification.

The DMT-on oligodeoxynucleotide was purified by HPLC with the Gradient One (0–20 minutes, 10–50% B in A with a flow rate of 1.0 ml/min) and pooled together; the solution was concentrated and extracted with SEP-PAK column. After washing the column with water, 2.5% trifluoroacetic acid was eluted through the column to remove the DMT group. The DMT-off oligodeoxynucleotide was then eluted with methanol/water (70:30, V/V) for further purification by HPLC with gradient two (0–20 minutes, 0–16 % B in A, with a flow rate of 1 ml/min).

The purified oligodeoxynucleotides were desalted on SEP-PAK columns, lyophilized and stored at −18°C. The homogeneous product was characterized by MALDI-TOF and enzymatic hydrolysis[17] with snake venom phosphodiesterase (EC 3.1.15.1, *Crotallus adamanteus*) and alkaline phosphatase (EC 3.1.3.1, *E. coli*) (Sigma, USA). The following extinction coefficients ε_{260} were used: dA 15400, dG 11700, dT 8800, and dC 7300, the extinction coefficients of compounds **1–4** were assumed to be the same as that of canonical nucleosides.

T_m Measurement

The T_m measurement of the complementary oligodeoxynucleotides was performed with a Cary-100 Bio UV-Visible spectrophotometer (Varian, USA) equipped with a Cary temperature controller. The buffer used for the measurement consisted of 100 mM NaCl, 10 mM $MgCl_2$ and 10 mM sodium cacodylate (pH 7.0)[17] with 5 μM of each oligodeoxynucleotide. The two complementary oligodeoxynucleotides were mixed in the buffer (1 ml), and the solution was heated to 85°C. After halting for 10 minutes, it was cooled to 35°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.

Circular Dichroism Measurement

The CD experiments were conducted on a Jasco J-810 spectropolarimeter equipped with a Jasco PTC-423S temperature controller. The duplex solution was the same as that for T_m measurement. Before the measurement, the sample was cooled to 5°C in a cuvette with a path length of 10 mm. The ellipticities of duplexes were recorded from 220 to 350 nm. The spectra were the averages of three accumulations and smoothed using a 25-point adaptive smoothing algorithm.

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