Organic & Biomolecular Chemistry

Accepted Manuscript

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ARTICLE

Synthesis of oligonucleotides containing *N*,*N*-disubstituted 3-deazacytosine nucleobases by post-elongation modification and their triplex-forming ability with double-stranded DNA†

Cite this: DOI: 10.1039/x0xx00000x

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

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A phosphoramidite of a 2'-O,4'-C-methylene-bridged nucleoside, bearing 4-(2,4,6-triisopropylbenzenesulfonyloxy)pyridin-2-one as a nucleobase precursor, was synthesized and introduced into an oligonucleotide. Treatment with various secondary amines after elongating the oligonucleotide on an automated DNA synthesizer enabled facile and mild conversion of the precursor into the corresponding *N*,*N*-disubstituted 3-deazacytosine nucleobases. The evaluation of triplex-forming ability of the synthesized oligonucleotides with double-stranded DNA showed that the nucleobase possessing the (3*S*)-3-guanidinopyrrolidine moiety can recognize a CG base pair with high sequence-selectivity and binding-affinity.

Introduction

A triplex-forming oligonucleotide (TFO) that specifically binds to double-stranded DNA (dsDNA) to form triplex DNA can be used for genomic DNA-targeting technologies such as antigene methodology.[1] However, practical use of these technologies is difficult because the sequence of dsDNA that can be targeted by TFOs is limited to homopurine tracts. For example, T and protonated C (C^+H) in TFO recognize AT and GC base pairs in dsDNA to form T–AT and C^+H–GC base triplets, respectively (Fig. 1). In other words, no natural nucleobase can form a base triplet with a pyrimidine-purine (CG or TA) interruption. Thus, the development of TFOs bearing artificial nucleobases that are capable of recognizing CG or TA base pairs remains a challenge and is currently in great demand.[2]

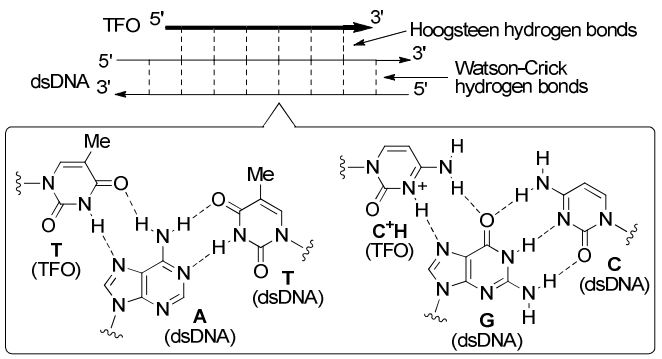


Fig. 1 Structures of triplex DNA and base triplets.

Promising nucleobases for CG base pair recognition have been recently reported by several groups.[3] We also recently explored a wide variety of *N*,*N*-disubstituted cytosine nucleobases that were synthesized using a post-elongation modification (PEM) strategy,[4,5] and found the 2′-*O*,4′-*C*-methylene-bridged nucleic acid bearing a 4-[(3*S*)-3-guanidinopyrrolidino]-5-methylpyrimidin-2-one nucleobase (GP^B, Fig. 2). The GP^B very strongly recognized the CG base pair with sequence-selectivity when the 5′ and 3′ sites adjacent to the CG base pair were AT base pairs.[4] However, when one of these sites was a GC base pair, the affinity of GP^B to the CG base pair was significantly decreased.

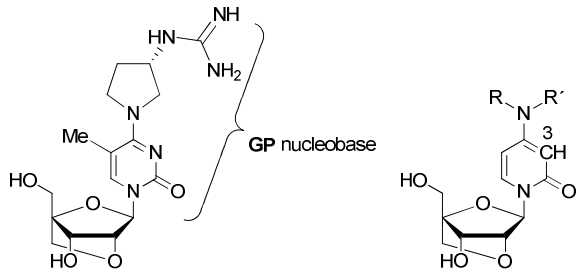


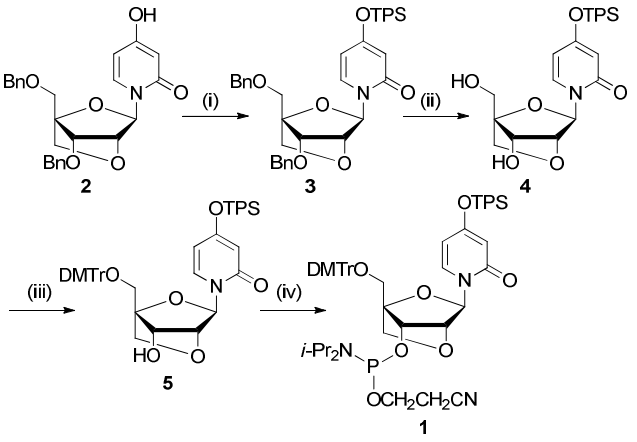
Fig. 2 Structures of the GP^B monomer and the 2′-*O*,4′-*C*-methylene-bridged nucleoside bearing *N*,*N*-disubstituted 3-deazacytosine designed in this study.

We previously demonstrated that a pyridin-2-one nucleobase, similar to thymine[6] and a 5-methylpyrimidin-2-one nucleobase (^{4H}T),[7] could form a single hydrogen bond with the C of a CG base pair to form triplex DNA.[8] Thus, we were interested in replacement of the pyrimidin-2-one unit of *N*,*N*-disubstituted

cytosines, like the GP nucleobase, by a pyridin-2-one unit for the development of a new nucleobase for CG base pair recognition. We therefore designed *N*,*N*-disubstituted 3-deazacytosine nucleobases containing a pyridine-2-one moiety (Fig. 2). Herein, we describe the results of our attempts to develop a method for the installation of various *N*,*N*-disubstituted 3-deazacytosine nucleobases on an oligonucleotide by PEM and to examine the recognition ability of the nucleobases for base pairs in triplex DNA formed with dsDNA.

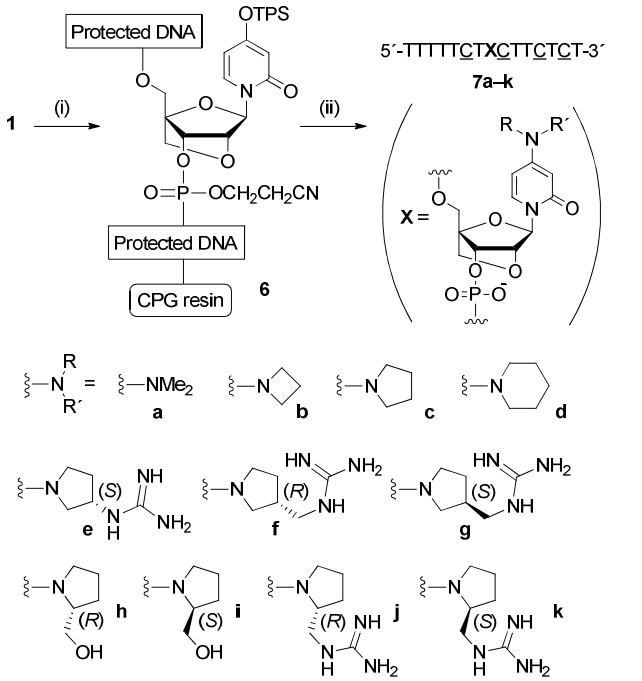
Results and discussion

To construct *N*,*N*-disubstituted 3-deazacytosine nucleobases by PEM, a pyridin-2-one nucleobase with a leaving group possessing high leaving ability at the 4-position is required. Therefore, 4-(2,4,6-triisopropylbenzenesulfonyloxy)pyridine-2-one (4-TPSO-pyridin-2-one) was chosen as the nucleobase precursor. The synthesis of a phosphoramidite **1**, a 2′-*O*,4′-*C*-methylene-bridged nucleoside bearing 4-TPSO-pyridin-2-one as a nucleobase, was carried out according to Scheme 1. The sulfonylation of **2**,[9] previously reported by us, using 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl) at –40 °C quantitatively afforded compound **3**, which was hydrogenolized with 20% $Pd(OH)_2$–C and cyclohexene to afford diol **4**. The primary alcohol of **4** was protected by the 4,4′-dimethoxytrityl (DMTr) group to yield **5**, which was converted by phosphitylation into the desired phosphoramidite **1**, a suitable building block for oligonucleotide synthesis.



Scheme 1 Reagents and conditions: (i) 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl), DMAP, Et_3N, CH_2Cl_2, –40 °C, 11 h, quant.; (ii) 20% $Pd(OH)_2$–C, cyclohexene, EtOH, reflux, 1 h, 81%; (iii) 4,4′-dimethoxytrityl chloride (DMTrCl), pyridine, room temperature, 6 h, 89%; (iv) *i*-$Pr_2NP(Cl)OCH_2CH_2CN$, *i*-Pr_2NEt, CH_2Cl_2, 0 °C, 3 h, 92%.

The synthesis of TFOs containing *N*,*N*-disubstituted 3-deazacytosines was attempted. The sequence of TFO shown in Scheme 2 was chosen because GP^B demonstrates poor recognition of the CG base pair in the sequence. Initially, phosphoramidite **1** was employed for oligonucleotide synthesis using an automated DNA synthesizer for the preparation of TFO precursor **6** supported by the controlled pore glass (CPG) resin (Scheme 2). Then, using our previously reported PEM method for the synthesis of *N*,*N*-disubstituted cytosines,[3,4] transformations of the 4-TPSO-pyridin-2-one moiety to *N*,*N*-disubstituted 3-deazacytosines by using secondary amine solutions were performed (see Experimental for details of the procedure). The PEM proceeded successfully (Fig. S1, ESI†), and TFOs **7a**–**k** containing various *N*,*N*-disubstituted 3-deazacytosines were obtained.



Scheme 2 Reagents and conditions: (i) DNA synthesizer; (ii) 10–20% aq. secondary amines, room temperature, 2–3 h; then 28% aq. ammonia, room temperature, 3–4 h. CPG indicates controlled pore glass. C indicates 2′-deoxy-5-methylcytidine.

The triplex-forming ability of the synthetic TFOs was evaluated by UV melting experiments, and compared with those of TFOs **8** and **9** containing GP^B and T (Fig. 3). For triplex DNA formed with dsDNA containing a CG base pair, the T_m values obtained from the experiments are summarized in Fig. 3. The T_m values of TFOs **7a**–**d** with simple *N*,*N*-dialkylated 3-deazacytosine nucleobases were 32–33 °C, higher than that of the triplex formed by using TFO **9** (29 °C) and dsDNA containing a natural T–CG base triplet. Moreover, TFO **7e**, possessing the same (3*S*)-3-guanidinopyrrolidine structure as the GP^B in **8**, showed significantly improved affinity for the CG base pair, and the T_m value was 37 °C. TFOs **7f** and **7g** bearing a (3*R*)- and (3*S*)-3-guanidinomethylpyrrolidine unit, respectively, had a similar affinity to that of **7c** containing a simple pyrrolidine unit. On the other hand, among the 2-substituted pyrrolidines (**7h**–**k**) investigated as *N*,*N*-disubstituted amine moieties, only (2*R*)-2-guanidinomethyl analog **7j** showed significantly increased affinity for the CG base pair compared to **7c**. Interestingly, alike the *N*,*N*-disubstituted cytosines previously reported by us,[4] it was found that the stereochemistry of the substituent was essential as (*R*)-isomer **7k** had a 5 °C lower T_m value than its

stereoisomer **7j**. Furthermore, **7e** and **7j**, which showed improved affinity for the CG base pair by the introduction of a guanidine unit, had the same stereochemistry as our previous results for TFOs containing the GP^B unit. Therefore, the results obtained in this study strongly suggest that the guanidine groups in **7e** and **7j** could recognize the G of the CG base pair. In particular, the 3-deazacytosine analog **7e**, in contrast to **8**, containing the GP nucleobase, showed strong recognition for the CG base pair in the sequence in which the 3′-site adjacent to the CG base pair was a GC base pair.

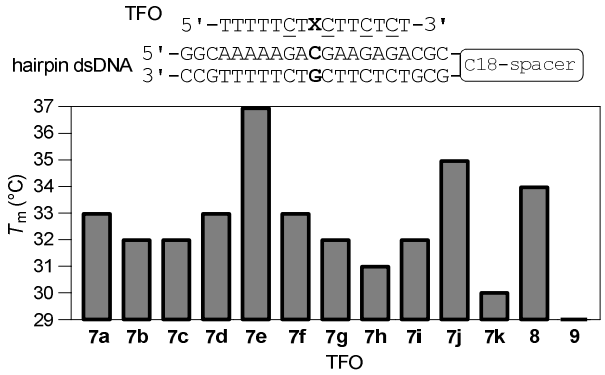


Fig. 3 T_m values of triplex DNA containing an X–CG base triplet. Conditions: 10 mM sodium cacodylate buffer (pH 6.8), 100 mM KCl and 50 mM $MgCl_2$. The concentration of each oligonucleotide used was 1.89 μM. TFOs **8** and **9** contain GP^B and T for X, respectively.[4] C18-spacer indicates a hexa(ethyleneglycol) linker.

The sequence selectivity of **7e** and **7j** in triplex DNA formation was explored by UV melting experiments using dsDNA targets with four natural base pairs for YZ (Table 1 and Fig. S2, ESI†). Although the GP^B in TFO **8** had higher selectivity (≥17 °C) for the CG base pair than that shown by T (≥14 °C) for the AT base pair in the triplex containing the stable, natural T–AT base triplet for X–YZ, the modified nucleotides in **7e** and **7j** were much superior to the GP^B in **8** regarding the selectivity for the CG base pair.

Table 1 T_m values (°C) of triplexes formed between TFOs and dsDNA targets[a]

TFO 5'-TTTTTCTXCTTCTCT-3'
hairpin dsDNA 5'-GGCAAAAAGAYGAAGAGACGC- / 3'-CCGTTTTTCTZCTTCTCTGCG- C18-spacer

		YZ			
TFO	ΔT_m[b]	CG	TA	GC	AT
7e	≥21 (CG)	37	16	13	14
7j	≥21 (CG)	35	11	14	13
8	≥17 (CG)	34	8	13	17
9	≥14 (AT)	29	11	nd[c]	43

[a] See the caption of Fig. 3. [b] Difference between the T_m value in targeting base pairs shown in parentheses and those in targeting other base pairs. [c] nd indicates not determined; triplex DNA was too unstable to be observed.

The results depicted in Fig. 3 and Table 1 show that when the 3′-flanking site of the CG base pair was a GC base pair, 2′-*O*,4′-*C*-methylene-bridged nucleotide possessing (3*S*)-3-guanidinopyrrolidine-containing 3-deazacytosine in **7e** showed significantly improved affinity and selectivity for the CG base pair compared to GP^B in **8**. This finding may allow the sequence of dsDNA that is targetable by TFO to be expended. In the future, we plan to develop a new nucleotide by this strategy using a PEM method for the efficient synthesis of various TFO derivatives to further reduce the limitations of the targetable sequence of dsDNA.

Conclusions

In this study, we developed a new PEM method for enhancing the efficiency of the synthesis of oligonucleotides containing various *N*,*N*-disubstituted 3-deazacytosines. This method allowed us to successfully find a 2′-*O*,4′-*C*-methylene-bridged nucleotide with the (3*S*)-3-guanidinopyrrolidine-containing 3-deazacytosine capable of stably and selectively recognizing the CG base pair within dsDNA that comprises a GC base pair at its flanking 3′ site. By using this nucleotide and GP^B previously found by us, a wider sequence range of dsDNA might be targetable. This result, in conjunction with our previous results, demonstrates that PEM methods are powerful for the exploration of nucleobases for their effective recognition of a base pair in the formation of triplex DNA. Moreover, this PEM method developed can be applied to the construction of *N*-unsubstituted or *N*-mono-substituted 3-deazacytosines in oligonucleotides, which might contribute to the development of 3-deazacytosines with a new functionality.

Experimental

General

Melting points are uncorrected. All moisture-sensitive reactions were carried out in well-dried glassware under a N_2 atmosphere. ^{1}H NMR (400 MHz), ^{13}C NMR (101 MHz), and ^{31}P NMR (162 MHz) were recorded on JEOL JNM-ECS-400 spectrometers. Chemical shifts are reported in parts per million downfield from an internal standard [tetramethylsilane (0.00 ppm) for ^{1}H NMR, CD_3OD (49.00 ppm) or $CDCl_3$ (77.00 ppm) for ^{13}C NMR], or an external standard [H_3PO_4 (0.00 ppm) for ^{31}P NMR]. IR spectra were recorded on a JASCO FT/IR-4200 spectrometer. Optical rotations were recorded on a JASCO P-2200 instrument. Mass spectra were measured on a JEOL JMS-600, JEOL JMS-700, Bruker Daltonics Autoflex II TOF/TOF or JEOL JMS-S3000 mass spectrometer. For silica gel flash column chromatography, Fuji Silysia PSQ-100B and FL-100D were used.

1-(3,5-Di-*O*-benzyl-2-*O*,4-*C*-methylene-β-D-ribofuranosyl)-4-(2,4,6-triisopropylbenzenesulfonyloxy)pyridin-2-one (3)

Under a N_2 atmosphere, TPSCl (104 mg, 0.344 mmol) was added to a solution of compound **2**[9] (100 mg, 0.230 mmol), DMAP (2.8 mg, 0.023 mmol), and Et_3N (96.0 μL, 0.689 mmol) in anhydrous CH_2Cl_2 (10 mL); the resulting mixture was stirred at −40 °C for 11 h. After addition of saturated aqueous $NaHCO_3$ solution, the reaction mixture was extracted with CH_2Cl_2, washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel

flash column chromatography (*n*-hexane/AcOEt = 3/1) to give compound **3** (160 mg, quant.) as a colorless oil. $[\alpha]_D^{25}$ +126 (*c* 1.0, $CHCl_3$). IR ν_{max} (KBr) 3092, 3065, 3028, 2961, 2936, 2876, 1665, 1600, 1544, 1465, 1383, 1359, 1317, 1268, 1192, 1136, 1085, 1052 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.25 (12H, d, *J* = 6.9 Hz), 1.28 (6H, d, *J* = 6.9 Hz), 2.94 (1H, sept, *J* = 6.9 Hz), 3.78–3.86 (3H, m), 3.92 (1H, s), 4.02–4.11 (3H, m), 4.44 (1H, AB, *J* = 11.5 Hz), 4.56–4.62 (4H, m), 5.80 (1H, s), 6.04 (1H, d, *J* = 2.3 Hz), 6.10 (1H, dd, *J* = 2.3 and 7.8 Hz), 7.21–7.38 (12H, m), 7.82 (1H, d, *J* = 7.8 Hz). ^{13}C NMR ($CDCl_3$) δ 23.38, 24.49, 29.75, 34.17, 64.34, 71.98, 72.05, 73.55, 75.43, 76.08, 87.28, 87.98, 102.03, 109.55, 124.12, 127.36, 127.58, 127.87, 127.95, 128.34, 128.42, 129.32, 150.97, 154.84, 159.01, 162.03. MS (FAB) *m/z* 702 ($M+H^+$). HRMS (FAB): Calcd for $C_{40}H_{48}NO_8S$ ($M+H^+$), 702.3095; found, 702.3096.

1-(2-*O*,4-*C*-Methylene-β-D-ribofuranosyl)-4-(2,4,6-triisopropylbenzenesulfonyloxy)pyridin-2-one (4)

Compound **3** (100 mg, 0.143 mmol) and cyclohexene (0.722 mL, 7.12 mmol) were added to a suspension of 20% $Pd(OH)_2$ on carbon (100 mg) in EtOH (5.0 mL) and the resulting mixture was refluxed for 1 h. The reaction mixture was filtered and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography (AcOEt) to give compound **4** (58.4 mg, 81%) as a yellow oil. $[\alpha]_D^{30}$ +63.9 (*c* 1.0, MeOH). IR ν_{max} (KBr) 3366, 2962, 2930, 2872, 1721, 1655, 1597, 1546, 1466, 1426, 1383, 1351, 1321, 1270, 1193, 1141, 1081, 1052 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.24 (12H, d, *J* = 6.9 Hz), 1.27 (6H, d, *J* = 6.9 Hz), 2.94 (1H, sept, *J* = 6.9 Hz), 3.79 (1H, d, *J* = 8.0 Hz), 3.95–4.14 (6H, m), 4.32 (1H, brs), 4.45 (1H, s), 4.52 (2H, brs), 5.76 (1H, s), 6.27 (1H, d, *J* = 2.3 Hz), 6.34 (1H, dd, *J* = 2.3 and 7.4 Hz), 7.23 (2H, s), 7.99 (1H, d, *J* = 7.5 Hz). ^{13}C NMR ($CDCl_3$) δ 23.41, 24.50, 29.82, 34.25, 38.65, 56.87, 68.16, 69.12, 87.78, 89.29, 103.28, 108.84, 124.19, 128.74, 129.16, 130.89, 132.33, 134.35, 151.07, 155.11, 159.68, 167.86. MS (FAB) *m/z* 522 ($M+H^+$). HRMS (FAB): Calcd for $C_{26}H_{36}NO_8S$ ($M+H^+$), 522.2156; found, 522.2160.

1-[5-*O*-(4,4′-Dimethoxytrityl)-2-*O*,4-*C*-methylene-β-D-ribofuranosyl]-4-(2,4,6-triisopropylbenzenesulfonyloxy)pyridin-2-one (5)

Under a N_2 atmosphere, DMTrCl (147 mg, 0.435 mmol) was added to a solution of compound **4** (200 mg, 0.396 mmol) in pyridine (20 mL) and the resulting mixture was stirred at room temperature for 6 h. After addition of water, the reaction mixture was extracted with AcOEt, washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography (*n*-hexane/AcOEt = 2/1) to give compound **5** (283 mg, 89%) as a white amorphous powder. Mp 86–89 °C. $[\alpha]_D^{23}$ +49.5 (*c* 1.0, $CHCl_3$). IR ν_{max} (KBr) 3330, 2961, 2936, 2871, 2839, 1658, 1602, 1545, 1510, 1465, 1383, 1252, 1180, 1138, 1079, 1040 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.24 (12H, d, *J* = 6.4 Hz), 1.27 (6H, d, *J* = 6.4 Hz), 2.94 (1H, sept, *J* = 6.4 Hz), 3.49 (1H, AB, *J* = 11.0 Hz), 3.56 (1H, AB, *J* = 11.0 Hz), 3.79–3.88 (8H, m), 4.08 (2H, sept, *J* = 6.4 Hz), 4.23–4.24 (1H, m), 4.45 (1H, s), 5.80 (1H, s), 6.08 (1H, d, *J* = 2.3 Hz), 6.24 (1H, dd, *J* = 2.3 and 7.8 Hz), 6.86 (4H, d, *J* = 8.3 Hz), 7.23–7.46 (11H, m), 8.05 (1H, d, *J* = 7.8 Hz). ^{13}C NMR ($CDCl_3$) δ 23.42, 24.54, 29.83, 34.25, 55.19, 57.93, 69.88, 71.50, 78.71, 86.70, 87.65, 87.94, 102.33, 109.60, 113.27, 124.18, 127.08, 127.96, 128.01, 129.25, 129.97, 129.99, 134.01, 135.28, 135.37, 144.35, 151.09, 154.93, 158.66, 159.15, 162.21. MS (FAB) *m/z* 846 ($M+Na^+$). HRMS (FAB): Calcd for $C_{47}H_{57}NNaO_{10}S$ ($M+Na^+$), 846.3282; found, 846.3300.

1-{3-*O*-[2-Cyanoethoxy(diisopropylamino)phosphino]-5-*O*-(4,4′-dimethoxytrityl)-2-*O*,4-*C*-methylene-β-D-ribofuranosyl}-4-(2,4,6-triisopropylbenzenesulfonyloxy)pyridin-2-one (1)

Under a N_2 atmosphere, *i*-$Pr_2NP(Cl)OCH_2CH_2CN$ (0.226 mL, 1.30 mmol) was added to a solution of compound **5** (350 mg, 0.433 mmol) and *i*-Pr_2NEt (0.226 mL, 1.30 mmol) in anhydrous CH_2Cl_2 (5 mL) at 0 °C and the resulting mixture was stirred at room temperature for 3 h. After addition of saturated aqueous $NaHCO_3$ solution, the reaction mixture was extracted with CH_2Cl_2, washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography (*n*-hexane/AcOEt = 3/1) to give compound **1** (409 mg, 92%) as a white amorphous powder. Mp 63–65 °C. ^1H NMR ($CDCl_3$) δ 0.93 (3H, d, *J* = 6.8 Hz), 1.03 (3H, d, *J* = 6.8 Hz), 1.11 (3H, d, *J* = 6.8 Hz), 1.13 (3H, d, *J* = 6.8 Hz), 1.27–1.28 (18H, m), 2.36 (1H, t, *J* = 6.4 Hz), 2.52 (1H, t, *J* = 6.4 Hz), 2.94 (1H, sept, *J* = 6.8 Hz), 3.40–3.88 (7H, m), 4.04–4.12(2H, m), 4.22 (1H, d, *J* = 6.4 Hz), 4.35 (0.5H, d, *J* = 9.2 Hz), 4.57 (0.5 H, s), 4.68 (0.5H, s), 5.84 (0.5H, s), 5.86 (0.5 H, s), 6.07 (0.5H, d, *J* = 2.3 Hz), 6.08 (0.5H, d, *J* = 2.3 Hz), 6.20 (0.5H, dd, *J* = 2.3 and 7.8 Hz), 6.21 (0.5H, dd, *J* = 2.3 and 7.8 Hz), 6.84–6.88 (4H, m), 7.23–7.45 (11H, m), 8.08 (0.5H, d, *J* = 7.8 Hz), 8.12 (0.5H, d, *J* = 7.8 Hz). ^{31}P NMR ($CDCl_3$) δ 148.65, 149.31. MS (FAB) *m/z* 1024 ($M+H^+$). HRMS (FAB): Calcd for $C_{56}H_{71}N_3O_{11}S$ ($M+H^+$), 1024.4541; found, 1024.4558.

Synthesis of TFOs 7a–k by using the PEM method

TFOs **7a–k** were synthesized on a 0.2 μmol scale on an automated DNA synthesizer (GeneDesign nS-8) using the common phosphoramidite protocol (Synthesis mode: DMTr-ON). The CPG resin-supported oligonucleotides were treated with 10% aqueous secondary amine solutions at room temperature for 2 h for conversion of the 4-TPSO-pyridin-2-one nucleobase into the desired *N*,*N*-disubstituted 3-deazacytosine nucleobases. Then, additional treatment with 28% aqueous NH_3 solution at room temperature for 5–6 h resulted in complete removal of the acetyl groups of the 5-methylcytosine bases and complete cleavage of the oligonucleotides from the CPG resin. After the solvent was removed *in vacuo*, the crude TFOs obtained were purified with Nap™-10 columns (GE Healthcare) for the removal of excess amine and then treated with Sep-Pak® Plus C_{18} cartridges (Waters) followed by reversed-phase HPLC (Waters XBridge® MS C_{18} 2.5 μm, 10 × 50 mm). The composition of the TFOs was confirmed by MALDI-TOF-MS analysis. MALDI-TOF-MS data ($[M-H]^-$)

for TFOs **7a–k**: **7a**, found 4536.29 (calcd 4536.05); **7b**, found 4547.23 (calcd 4548.06); **7c**, found 4561.89 (calcd 4562.09); **7d**, found 4576.25 (calcd 4576.11); **7e**, found 4619.87 (calcd 4619.14); **7f**, found 4633.39 (calcd 4633.17); **7g**, found 4633.66 (calcd 4633.17); **7h**, found 4592.99 (calcd 4592.11); **7i**, found 4591.89 (calcd 4592.11); **7j**, found 4633.40 (calcd 4633.17); **7k**, found 4633.62 (calcd 4633.17).

UV melting experiments (T_m measurements)

UV melting experiments were carried out on a Shimadzu UV-1650PC spectrophotometer equipped with a T_m analysis accessory. The UV melting profiles were recorded in 10 mM sodium cacodylate buffer (pH 6.8), 100 mM KCl and 50 mM $MgCl_2$ from 5 °C to 90 °C at a scan rate of 0.5 °C/min at 260 nm. The final concentration of each oligonucleotide used was 1.89 μM. A two-point average method was used to obtain the T_m values and the final values were determined by averaging three independent measurements which were accurate to within 1 °C.

Acknowledgements

This work was supported in part by a Grant-in-Aid for Scientific Research on Innovative Areas (No. 22136006) from the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT); a Grant-in-Aid for JSPS Fellows of the Japan Society for the Promotion of Science (JSPS); and a Grant-in-Aid from Nissan Chemical Industry, Ltd. Award in Synthetic Organic Chemistry, Japan.

Notes and references

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† Electronic Supplementary Information (ESI) available: Full experimental details of new secondary amines used for the PEM method; representative HPLC charts of crude TFOs in PEM; representative UV-melting curves of triplexes; ^{1}H, ^{13}C, and ^{31}P spectra of all new compounds; and HPLC charts and MALDI-TOF-MS spectra of new oligonucleotides are shown. See DOI: 10.1039/b000000x/

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