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Synthesis and hybridization properties of 2′-*O*-(tetrazol-5-yl)ethyl-modified oligonucleotides

Hisao Saneyoshi [a,b], Keigo Tamaki [a,b], Akihiro Ohkubo [a,b], Kohji Seio [a,b], Mitsuo Sekine [a,b,*]

[a] *Department of Life Science, Tokyo Institute of Technology, Nagatsuta, Midoriku, Yokohama 226-8501, Japan*
[b] *CREST, JST (Japan Science and Technology Agency), Nagatsuta, Midoriku, Yokohama 226-8501, Japan*

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Abstract

2′-*O*-(1*H*-Tetrazol-5-yl)ethyladenosine was synthesized using 2′-*O*-cyanoethyladenosine derivative as a key intermediate. The 2′-*O*-(1*H*-tetrazol-5-yl)ethyl modifications exhibited intriguing properties such as the change in the structure of the tetrazole residue between a protonated and a deprotonated form. The T_m experiments of various oligodeoxynucleotides having a 2′-*O*-(1*H*-tetrazol-5-yl)ethyl-modified adenosine showed reduced hybridization affinity in comparison to the unmodified oligonucleotides toward their complementary oligodeoxynucleotides. The mechanism of the reduced hybridization affinity was discussed on the basis of the structure and the physicochemical properties of the tetrazole moiety.
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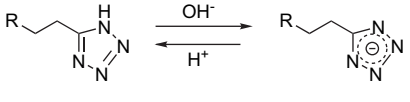
Keywords: 2′-*O*-Modified oligonucleotide; Cyanoethyl; (1*H*-Tetrazol-5-yl)ethyl; pH-dependent hybridization

1. Introduction

Chemically modified oligonucleotides have been utilized as indispensable materials for DNA chip technology,[1] gene therapy,[2] gene regulation,[3] and recent nanotechnology,[4] because of their hybridization affinity for target DNA or RNA molecules. Modification of their carbohydrate residues at the 2′-position has been studied as the principal strategy for improving hybridization affinity, nuclease resistance, and conjugation with functional molecules in nucleic acids.[5] We have recently reported that 2′-*O*-cyanoethyl RNAs have superior properties compared to simple 2′-*O*-alkylated RNAs with respect to their hybridization ability and nuclease resistance.[6] It is well known that the cyano group can be converted to other functional groups by a variety of chemical reactions. Therefore, 2′-*O*-cyanoethylated ribonucleosides may be useful precursors for the synthesis of further functionalized 2′-*O*-modified ribonucleosides.

In this study, we focused on the conversion of the cyanoethyl group into the (1*H*-tetrazol-5-yl)ethyl group that could be a new interaction site for the hybridization affinity. There are numerous studies that utilized the high versatility of tetrazole.[7–10] For example, in nucleic acid chemistry, tetrazoles have been used as a proton donor/nucleophile for activating the phosphoramidite derivatives,[11] a component of 3′-*N*-tetrazolylthymidine (AZT analogue),[12] water-soluble inter-nucleotidic bond mimics,[13] and as a *N*-tetrazolyl nucleobase for a metal-mediated base pair.[14] On the other hand, because of the acidity of the 5-carbon substituted tetrazole function (pK_a=5.5–5.6),[15] the tetrazole moiety is in the monoanionic form at the physiological conditions. This character could affect the hybridization property of oligonucleotides containing tetrazole residues (Fig. 1).

In this paper, we report the synthesis of oligonucleotides containing 2′-*O*-[(1*H*-tetrazol-5-yl)ethyl]adenosine (**At**) and



R = oligonucleotide

Figure 1. Interconversion between a neutral and a dissociated species of the tetrazole moiety.

* Corresponding author. Tel.: +81 45 924 5706; fax: +81 45 924 5772.
E-mail address: msekine@bio.titech.ac.jp (M. Sekine).

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2′-*O*-[(1-methyl-1*H*-tetrazol-5-yl)ethyl]adenosine (***Amt***) as a reference material and their hybridization affinity for the complementary DNA oligomers. In addition, 2′-*O*-[(1*H*-tetrazol-5-yl)ethyl]adenosine 3′-phosphoramidite building block and its methylated derivative were synthesized via 2′-*O*-cyanoethyladenosine previously reported by us as the precursors for the synthesis of the corresponding 3′-phosphoramidite building blocks and were successfully incorporated into oligonucleotides.

2. Results and discussion

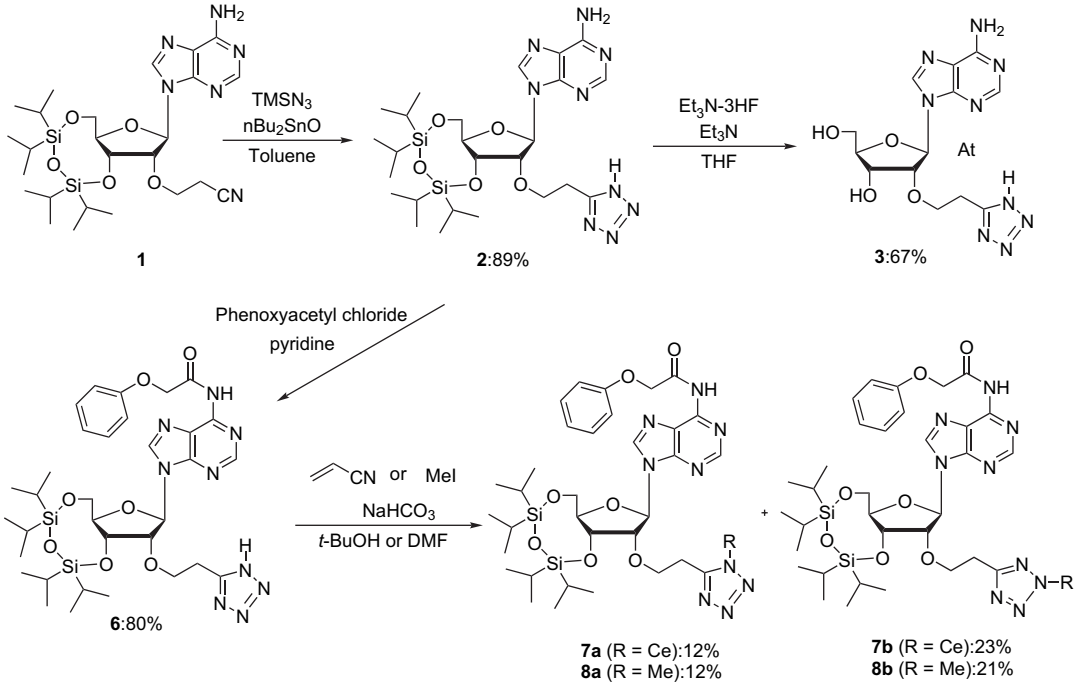
2.1. Synthesis of 2′-O-[(1H-tetrazol-5-yl)ethyl]adenosine derivatives and protected adenosine 3′-phosphoramidite derivatives

Conversion of the nitrile group of the 2′-*O*-cyanoethylated adenosine derivative (**1**)[16] to a tetrazole ring was studied. The combined use of sodium azide and ammonium chloride,[17] which have been widely used for tetrazole ring formation, resulted in the formation of complex mixtures. These reaction media are slightly acidic; therefore, an increase in the temperature of the reaction led to considerable loss of the 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl (TIPDS) group. However, the use of trimethylsilylazide ($TMSN_3$) in the presence of a catalytic amount of Bu_2SnO[18] produced an 89% yield of the desired product **2** without decomposition.

The treatment of the resulting product **2** with $Et_3N\cdot 3HF$ produced a 67% yield of 2′-*O*-[(1*H*-tetrazol-5-yl)ethyl]adenosine (**3**). The sugar conformation of **3** was analyzed by ^1H NMR and compared with that of 2′-*O*-methyladenosine (**4**) and 2′-*O*-(2-cyanoethyl)adenosine (**5**),[6] as shown in Table S1. The analyses revealed a slight increase in the population of the S-type (C2′-*endo*) conformation caused by the introduction of the tetrazolylethyl group.

To incorporate **3** into oligonucleotides, a suitable protecting group of the tetrazole moiety was required because the unprotected tetrazole moiety would accelerate the decomposition of the phosphoramidite unit. Protecting groups such as trityl, tetrahydropyranyl, benzyl, and cyanoethyl have been used for the NH function of the tetrazole residue.[7b] We chose the cyanoethyl group as the protecting group because it could be removed under the same conditions as those prescribed for the removal of base and phosphate.

Treatment of **3** with phenoxyacetyl chloride in pyridine produced an 80% yield of the *N*-protected derivative **6**. In a previous paper, we reported a new method for the cyanoethylation of the hydroxyl group using Cs_2CO_3 in *t*-BuOH.[6] Therefore, this method was initially used for the cyanoethylation of the tetrazole moiety of **1**. However, cyanoethylation at the base moiety occurred simultaneously. The basicity of Cs_2CO_3 seemed to be responsible for this result, because Cs_2CO_3 could abstract tetrazolic proton and the relatively acidic proton of the amide group on the base moiety too. Therefore, we used $NaHCO_3$ as a less basic reagent to avoid the side reaction. However, cyanoethylation occurred neither at the tetrazole moiety nor the base moiety. In this case, the $NaHCO_3$ as a base was too weak to activate tetrazole moiety at room temperature. Finally, we found that the reaction at 80 °C in *t*-BuOH produced a mixture of *N*-cyanoethylated regioisomers **7a** and **7b**. From the mixture, **7a** and **7b** were isolated with yields of 12 and 23%, respectively, and the cyanoethylated site was determined using HMBC spectra to confirm the structures shown in Scheme 1. Next, to observe the effect of the acidic proton of the tetrazole moiety on hybridization affinity, we synthesized the adenosine derivatives **8a** and



Scheme 1. Synthesis of 2′-*O*-[(1*H*-tetrazol-5-yl)ethyl]adenosine derivatives and its *N*-alkylated derivatives.

8b containing the *N*-methylated tetrazoyl group. Methylation of **6** with methyl iodide in DMF in the presence of $NaHCO_3$ produced a 12% yield of the desired compound **8a**. In this case, a 21% yield of the alkylated regioisomer **8b** was also obtained. The alkylated sites were determined from the HMBC spectra.

The 2′-*O*-(2-cyanoethyl-1*H*-tetrazol-5-yl)adenosine 3′-phosphoramidite derivative **11** was synthesized from compound **7b**, as shown in Scheme 2. Treatment of **7b** with $Et_3N\cdot 3HF$ produced a 99% yield of the 3′,5′-*O*-free derivative **9**. The usual tritylation of **9** produced a 73% yield of the 5′-*O*-protected derivative **10**. Phosphitylation of the resulting product **10** with $(i\text{-}Pr_2N)_2POCH_2CH_2CN$ produced a 71% yield of **11**. Similarly, the 2′-*O*-(1-methyl-1*H*-tetrazol-5-yl)adenosine 3′-phosphoramidite derivative **14** was synthesized from the compound **8a** via compounds **12** and **13**, as shown in Scheme 2.

2.2. Synthesis and properties of modified oligodeoxynucleotides

Next, to observe the effects of the tetrazole-type modifications on duplex stability when methylated, 2′-*O*-tetrazolyl-5-ylethyl-modified oligodeoxynucleotides **15**, **17**, and **19**, and 2′-*O*-methyltetrazol-5-ylethyl-modified **16**, **18**, and **20** with either one or four 2′-*O*-modified adenosine residues were synthesized by solid-phase synthesis, as summarized in Table 1. We noticed that the cyanoethyl groups on the tetrazole moieties in the oligonucleotides were not completely deprotected under NH_4OH at room temperature for 8 h (conditions b) in the case of **15**, **17**, and **19** according to the anion-exchange HPLC analysis. Therefore, the deprotection reactions were carried out in NH_4OH at 55 °C for 5 h (conditions a) for these oligonucleotides. The modified oligomers **15** and **16** have one-point modification with **At** and ***Amt***, respectively, at the central position. Oligomers **17** and **18** have four modified nucleosides at discontinuous positions. Oligomers **19** and **21** have a consecutive sequence of four modified nucleosides.

Hybridization affinities of unmodified 5′-d(CGTAGACTATGCTAT)-3′ (**21**), **15**, and **16** toward the complementary strand 3′-d(GCATCTGATACGATA)-5′ (**22**) were measured

Table 1
Modified oligodeoxynucleotides synthesized using **11** and **14**

	Oligonucleotide	MALDI-TOF MASS	
		Calcd.	Found
15	5′-d(CGTAG**At**CTATGCTAT)-3′[a]	4677.8	4679.1
16	5′-d(CGTAG***Amt***CTATGCTAT)-3′[b]	4691.9	4695.2
17	5′-d(CG**At**G**At**CT**At**TGCT**At**T)-3′[a]	5014.0	5015.7
18	5′-d(CG***Amt***G***Amt***CT***Amt***TGCT***Amt***T)-3′[b]	5070.0	5066.2
19	5′-d(CGTGC**At**A**t**A**t**A**t**TGTCTT)-3′[a]	5014.0	5018.8
20	5′-d(CGTGC***Amt***A***mt***A***mt***A***mt***TGTCTT)-3′[b]	5070.0	5075.1

[a] Deprotection conditions, NH_4OH, 55 °C, 5 h.
[b] Deprotection conditions, NH_4OH, rt, 8 h. **At**: 2′-*O*-[(1*H*-tetrazol-5-yl)-ethyl]adenosine, ***Amt***: 2′-*O*-[(1-methyl-1*H*-tetrazol-5-yl)ethyl]adenosine.

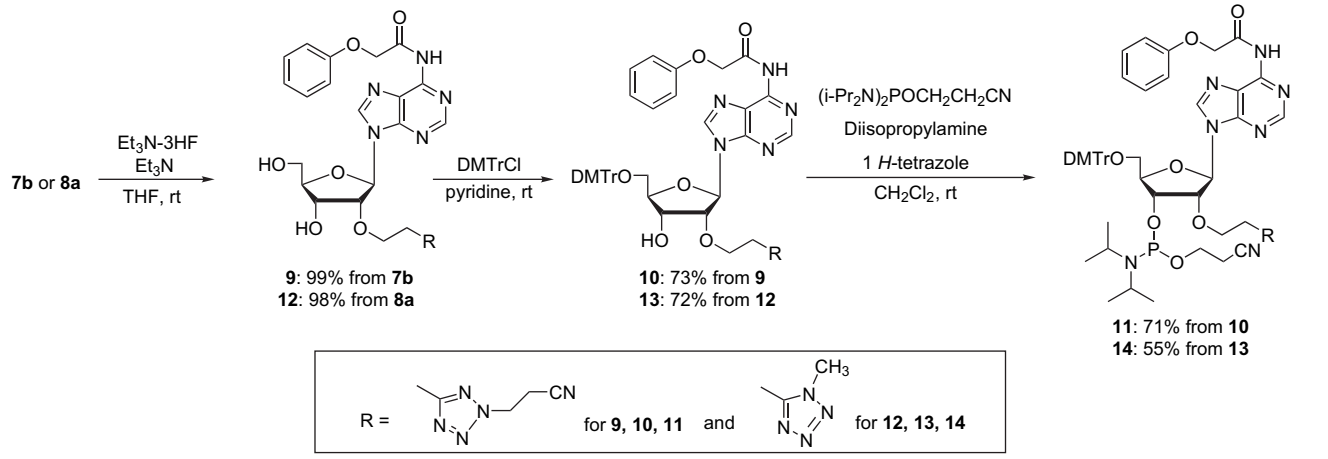
to evaluate the effect of the attachment of the 2′-substituent and the anionic modification. The T_m data are described in Table 2. As a results, the introduction of **At** (**15**) and ***Amt*** (**16**) reduced the hybridization affinities toward **22** in comparison with unmodified **21** by 2.9 and 2.0 °C, respectively. In addition, the stability of the **15/22** duplex was proved to be 0.9 °C lower than the **16/22** duplex, probably because the deprotonation of the tetrazole ring of **At** generated an additional negative charge and caused anion–anion repulsion with internucleotide phosphates.

Table 2
Effects of the 2′-substituents on the duplex stabilities[a]

	Oligonucleotide	T_m (°C)	ΔT_m (°C)
21	5′-d(CGTAGACTATGCTAT)-3′	48.5	—
15	5′-d(CGTAG**At**CTATGCTAT)-3′	45.6	−2.9
16	5′-d(CGTAG***Amt***CTATGCTAT)-3′	46.5	−2.0

[a] Conditions: 2.0 μM of **21**, **15**, or **16** and 2.0 μM of **22** in 10 mM sodium phosphate buffer (pH 7.0), 0.1 M NaCl, 0.1 mM EDTA.

The 2′-*O*-cationic modified oligonucleotides are known to stabilize duplexes due to the neutralization of phosphate anions in the case of a discontinuous sequence.[19] However, a consecutive sequence of modified nucleosides retains or decreases the hybridization affinity due to the cation–cation charge repulsions.[19] Therefore, we next examined the anionic repulsions between dissociated tetrazole moieties and phosphate anions in duplexes with four **At** or ***Amt*** modifications using



Scheme 2. Synthesis of protected adenosine 3′-phosphoramidite derivatives.

the duplexes **17/22**, **18/22**, **19/23**{3′-d(GCACGTTTTACAGAA)-5′}, and **20/23**.

As shown in Table 3, the introduction of the four **At** modifications into **17** and **19** significantly decreased the hybridization affinity as compared to their neutral counterparts **18** and **20** by 3.3 and 5.0 °C, respectively. As clarified by the comparison of the two ΔT_m values, the consecutively introduced **At** residues in **19** decreased the hybridization affinity more than the non-consecutively modified **17**. It should be noted that although the T_m of the **18/22** duplex became higher than that of the anionic **17/22** duplex, it was still much lower than that of the unmodified **21/22** duplex. Therefore, it seemed that not only the anionic charge of the **At** residue but also the steric and conformational effects of **At** and ***Amt*** residues contributed to the duplex destabilization. A similar trend was observed for the **19/23** and **20/23** duplexes in comparison with the unmodified duplex of 5′-d(CGTGCAAAATGTCTT)-3′ (**24**)**/23**, which gave the T_m of 55.7 °C.

Table 3
The sequence dependency of the duplex stabilities[a]

	Oligonucleotide	T_m (°C)	ΔT_m (°C)
17	5′-d(CGT**At**G**At**CT**At**TGCT**At**T)-3′	34.3	—
18	5′-d(CGT***Amt***G***Amt***CT***Amt***TGCT***Amt***T)-3′	37.6	+3.3[b]
19	5′-d(CGTGC**At**A**t**A**t**A**t**TGTCTT)-3′	43.6	—
20	5′-d(CGTGC***Amt****Amt****Amt****Amt***TGTCTT)-3′	48.6	+5.0[c]

[a] Conditions: 2.0 μM **17**, **18**, **19**, or **20** and 2 μM of **22** in 10 mM sodium phosphate buffer (pH 7.0), 0.1 M NaCl, 0.1 mM EDTA.
[b] ΔT_m=(T_m of **18/22** duplex)−(T_m of **17/22** duplex).
[c] ΔT_m=(T_m of **20/23** duplex)−(T_m of **19/23** duplex).

Finally, the thermodynamic parameters of the duplexes **17/22** and **18/22** were also estimated (Table 4). The ΔH^0 values of **17/22** and **18/22** duplexes were −81.5 and −86.8 kcal/mol, respectively. These data suggest that the less negative enthalpy value of **17** compared to **18** was the main reason for the lower hybridization affinity.

Table 4
Thermodynamic parameters of the duplexes containing **At** and ***Amt***[a]

Duplex	ΔH^0 (kcal/mol)	ΔS^0 (cal/K mol)	ΔG^0_{37} (kcal/mol)
17/22	−81.5	−237	−8.0
18/22	−86.8	−252	−8.7

[a] Conditions: 10 mM sodium phosphate buffer (pH 7.0), 100 mM NaCl, 0.1 mM EDTA and 60, 50, 30, 25, 15, 10, 5, 3, 2, 1.5, 1.0, 0.6, and 0.5 μM duplex, respectively. Eq: $1/T_m=\Delta S/\Delta H+R\ln(C_t/4)/\Delta H$.

3. Conclusion

In this paper, a new class of artificially 2′-modified ribonucleosides were synthesized using a 2′-*O*-cyanoethylated adenosine derivative (**1**) as a precursor. Chemical conversion of the nitrile function of the cyanoethyl group into the 1*H*-tetrazol-5-yl group was carried out using trimethylsilylazide in the presence of a catalytic amount of dibutyltin oxide without losing the 3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl) group. The NH function of the tetrazole moiety was protected with the cyanoethyl group by a Michael reaction. Selective N-protection was achieved for preparing the phosphoramidite derivative **11**. The 2′-*O*-(1*H*-tetrazol-5-yl)-ethyl modification provided a new property different from that of 2′-*O*-cyanoethylated oligonucleotides. The oligonucleotides incorporating **At** having a 1*H*-tetrazol-5-yl group showed reduced hybridization affinity toward the complementary strands probably due to the anionic charges of the tetrazole moiety as clarified by the comparative T_m analyses by use of the oligonucleotides incorporating ***Amt*** having a 1-methyl-1*H*-tetrazol-5-yl group. Although the methylation of the acidic NH group slightly improved the hybridization properties, the T_m values of the duplexes modified with ***Amt*** were still much lower than those of the unmodified duplexes. Therefore, not only the anionic charges of the tetrazolide moiety but also the 2′-*O*-(1*H*-tetrazol-5-yl)ethyl skeleton itself seemed to contribute to the duplex instability. Although such duplex destabilize, this ionizable modification would make it possible to interact with a metal–cation, proton and/or protonated amine for the regulation of the hybridization affinities. It should be noted, however, although the pK_a of 1*H*-tetrazole (5.5–5.6) supported the deprotonation of the tetrazole moiety of **At** in the oligonucleotides, the structure of the tetrazole moiety in the oligonucleotides has not yet been determined directly. Detailed physicochemical experiments such as NMR and UV titration of the oligonucleotides are necessary to clarify this point. The studies on the structures and the applicability of the oligonucleotides modified with the (1*H*-tetrazol-5-yl)ethyl are underway and will be reported elsewhere.

4. Experimental section

4.1. General remarks

^{1}H NMR spectra were recorded at 500 MHz and the chemical shifts were measured from the solvent peak as an internal standard, and ^{13}C NMR spectra were recorded at 500 MHz and the chemical shifts were measured from the solvent peak used as an internal standard and dioxane (D_2O) used as an external standard. ^{31}P NMR spectra were recorded at 109 MHz and the chemical shifts were measured from 85% H_3PO_4 as an external standard. Pyridine was distilled twice from *p*-toluenesulfonylchloride and CaH_2 after being refluxed for several hours and stored over molecular sieves 4 Å. TLC was performed on Merck Kieselgel 60 F_{254} precoated glass plates. Column chromatography was performed with silica gel C-200, C-300 (Wako Co., Ltd.), 60 N (Kanto Chemical, Co., Inc.), and NH (Fuji Silysia Chemical Ltd.), and a minipump for a goldfish bowl was conveniently used to attain sufficient pressure for rapid chromatographic separation. Recycling preparative HPLC was performed by use of an apparatus from Japan Analytical Industry Co., Ltd. Anion-exchange HPLC was performed using the following systems. System A: anion-exchange HPLC was performed on a Waters Aliance system with a Waters 3D UV detector and a Gen-Pak FAX column (Waters, 4.6×100 mm). A linear gradient (0–60%) starting from 25 mM sodium phosphate buffer (pH 6.0) and applying 25 mM sodium phosphate buffer (pH 6.0) containing 1 M NaCl (pH 6.0) was used at a flow rate of 1 mL/min for

45 min at 50 °C. High resolution ESI mass spectrometry was performed by use of a Mariner (PerSeptive Biosystems, Inc.).

4.1.1. 3′,5′-O-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2′-O-[2-(tetrazol-5-yl)ethyl]adenosine (**2**)

Compound **1** (23.3 g, 41.4 mmol) was dissolved in anhydrous toluene (200 mL). To the solution were added trimethylsilyilazide (27.5 mL, 207 mmol) and *n*-Bu_2SnO (2.06 g, 8.28 mmol). After being stirred at 110 °C for 23 h, the mixture was evaporated in vacuo. The residue was chromatographed on a column of silica gel with hexane/$CHCl_3$/MeOH (1:0:0/1:1:0/0:1:0/0:98:2, v/v/v) to give compound **2** (22.4 g, 89%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.94−1.13 (28H, m), 3.36−3.40 (2H, m), 4.06−4.10 (2H, m), 4.20 (1H, d, J=8.6 Hz), 4.24 (1H, d, J=4.6 Hz), 4.30 (1H, d, J=13.6 Hz), 4.38−4.41 (1H, m), 4.67−4.70 (1H, m), 5.92 (2H, br), 6.09 (1H, s), 8.17 (1H, s), 8.39 (1H, s); ^{13}C NMR ($CDCl_3$) δ 12.3, 12.9, 13.1, 13.6, 16.9, 17.2, 17.4, 17.5, 24.4, 59.4, 68.1, 69.2, 81.8, 82.6, 88.4, 120.5, 138.0, 148.5, 153.3, 153.8, 155.8. HRMS calcd for $C_{25}H_{43}N_9O_5Si_2$ (M+H$^+$) 606.2999, found 606.3051.

4.1.2. 2′-O-[2-(Tetrazol-5-yl)ethyl]adenosine (**3**)

Compound **2** (151 mg, 0.25 mmol) was dissolved in anhydrous THF (2.5 mL). To the solution were added triethylamine trihydrogen fluoride (141 μl, 0.88 mmol) and triethylamine (62 μl, 0.45 mmol). After being stirred at room temperature for 1 h, the mixture was evaporated in vacuo. The residue was chromatographed on a column of silica gel with hexane/ethyl acetate (3:1/1:1, v/v) and passed through Dowex (H$^+$) to give compound **3** (61 mg, 67%). ^{1}H NMR (D_2O, 500 MHz) δ 3.13−3.17 (2H, m), 3.82−3.90 (3H, m), 4.17−4.22 (1H, m), 4.28−4.30 (1H, m), 4.47−4.54 (1H, m), 4.43−4.54 (1H, m), 4.73−4.85 (1H, m), 5.93 (1H, d, J=7.1 Hz), 8.17 (1H, s), 8.19 (1H, s); ^{13}C NMR (D_2O) δ 24.5, 62.2, 68.0, 69.7, 82.4, 86.9, 87.5, 119.5, 141.2, 148.5, 152.0, 155.4, 155.8. HRMS calcd for $C_{13}H_{17}N_9O_4$ (M+H$^+$) 364.1476, found 364.1483.

4.1.3. 6-N-Phenoxyacetyl-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-O-[2-(tetrazol-5-yl)ethyl]adenosine (**6**)

Compound **3** (4.85 g, 8 mmol) was co-evaporated once with anhydrous pyridine and dissolved in anhydrous pyridine (40 mL). To the solution was added phenoxyacetyl chloride (1.22 mL, 8.8 mmol). After being stirred at room temperature for 4 h, the reaction mixture was quenched with H_2O and evaporated in vacuo. The residue was diluted with $CHCl_3$ and washed with brine and aq $NaHCO_3$. The organic phase was dried over Na_2SO_4 and filtrated. The residue was chromatographed on a column of silica gel with hexane/$CHCl_3$/MeOH (50:50/0:100:0/95:5, v/v) to give compound **6** (4.73 g, 80%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.34−3.40 (1H, m), 3.43−3.47 (1H, m), 3.98−4.03 (1H, m), 4.07−4.10 (1H, m), 4.25−4.27 (1H, m), 4.31−4.33 (2H, m), 4.51−4.54 (1H, m), 4.77 (1H, dd, J=4.6, 4.9 Hz), 4.85 (2H, br), 6.18 (1H, s), 7.04 (3H, m), 7.34−7.38 (2H, m), 8.36 (1H, s), 8.79 (1H, s), 9.47 (1H, br); ^{13}C NMR ($CDCl_3$) δ 12.1, 12.9, 13.0, 13.5, 16.8, 17.1, 17.3, 17.4, 17.5, 24.6, 59.2, 68.1, 68.2, 68.9, 81.9, 83.1, 88.1, 115.1, 122.6, 123.5, 130.0, 141.1, 148.6, 150.7, 152.8, 153.8, 157.1, 166.8. HRMS calcd for $C_{33}H_{49}N_9O_7Si_2$ (M+H$^+$) 740.3366, found 740.3330.

4.1.4. 2′-O-[(1-N-Cyanoethyltetrazol-5-yl)ethyl]-6-N-phenoxyacetyl-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine (**7a**) and its regioisomer (**7b**)

Compound **6** (0.5 g, 0.676 mmol) was dissolved in *t*-BuOH (3.4 mL). To the solution were added acrylonitrile (221 μl, 3.4 mmol) and $NaHCO_3$ (57 mg, 0.680 mmol). After being stirred at 80 °C for 23 h, the reaction mixture was evaporated in vacuo. The residue was diluted with ethyl acetate and washed with brine. The organic phase was dried over Na_2SO_4 and filtrated. The solution was evaporated in vacuo. The residue was chromatographed on a column of silica gel with $CHCl_3$/MeOH (99.7:0.3, v/v) to give compounds **7a** (66 mg, 12%) and **7b** (125 mg, 23%) was eluted with $CHCl_3$/MeOH (99.6:0.4, v/v). ^{1}H NMR of **7a** ($CDCl_3$, 500 MHz) δ 0.92−1.10 (28H, m), 3.10−3.14 (2H, m), 3.32−3.37 (2H, m), 3.97 (2H, d, J=11.0 Hz), 4.18−4.22 (2H, m), 4.25−4.30 (2H, m), 4.65−4.67 (2H, m), 4.71−4.80 (2H, m), 4.86 (2H, br), 6.00 (1H, s), 7.03−7.06 (3H, m), 7.32−7.35 (2H, m), 8.27 (1H, s), 8.74 (1H, s), 9.42 (1H, br); ^{13}C NMR of **7a** ($CDCl_3$) δ 12.5, 12.9, 13.0, 13.4, 16.9, 17.1, 17.1, 17.2, 17.4, 17.4, 17.5, 18.9, 24.8, 42.9, 59.7, 68.2, 69.3, 69.6, 81.8, 83.1, 88.3, 115.1, 116.1, 122.6, 123.4, 130.0, 141.5, 148.5, 150.8, 152.7, 153.8, 157.1, 166.8. HRMS of **7a** calcd for $C_{36}H_{52}N_{10}O_7Si_2$ (M+H$^+$) 793.3632, found 793.3548.

^{1}H NMR of **7b** ($CDCl_3$, 500 MHz) δ 0.98−1.10 (28H, m), 3.09−3.11 (2H, t, J=6.8 Hz), 3.23−3.35 (2H, m), 3.98−4.02 (1H, m), 4.09−4.20 (3H, m), 4.32−4.33 (1H, d, J=4.6 Hz), 4.39−4.43 (1H, m), 4.77 (1H, q, J=4.6 Hz), 4.83−4.86 (4H, m), 6.00 (1H, s), 7.05−7.08 (3H, m), 7.33−7.37 (2H, m), 8.28 (1H, s), 8.75 (1H, s), 9.41 (1H, br); ^{13}C NMR of **7b** ($CDCl_3$) δ 12.7, 12.9, 13.0, 13.5, 17.0, 17.1, 17.2, 17.3, 17.4, 17.5, 18.3, 26.9, 48.0, 59.9, 68.2, 69.1, 69.8, 81.5, 82.4, 88.9, 115.1, 115.6, 122.5, 123.4, 129.9, 142.2, 148.4, 151.0, 152.6, 157.1, 165.0, 166.8. HRMS of **7b** calcd for $C_{36}H_{52}N_{10}O_7Si_2$ (M+H$^+$) 793.3632, found 793.3656.

4.1.5. 2′-O-[(1-N-Methyltetrazol-5-yl)ethyl]-6-N-phenoxyacetyl-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-adenosine (**8a**) and its regioisomer (**8b**)

Compound **6** (0.5 g, 0.676 mmol) was dissolved in anhydrous DMF (3.4 mL). To the solution were added $NaHCO_3$ (57 mg, 0.680 mmol) and methyl iodide (210 μl, 3.4 mmol). After being stirred at room temperature for 19 h, the mixture was evaporated in vacuo. The residue was diluted with ethyl acetate and washed with H_2O three times. The organic layer was dried over Na_2SO_4, and filtered. The solution was evaporated in vacuo. The residue was chromatographed on a column of silica gel with $CHCl_3$ to give **8b** (107 mg, 21%). Compound **8a** (59 mg, 12%) was eluted with $CHCl_3$/MeOH (99.6:0.4, v/v). ^{1}H NMR of **8a** ($CDCl_3$, 500 MHz) δ 0.92−1.11 (28H, m), 3.22−3.31 (2H, m), 3.96−4.00 (2H, m), 4.12 (3H, s), 4.18−4.19 (1H, m), 4.21 (1H, s), 4.25−4.27 (2H, m), 4.64−4.67 (1H, m), 4.86 (2H, s), 6.00 (1H, s), 7.03−7.06 (3H, m), 7.32−7.36 (2H, m), 8.28 (1H, s), 8.74 (1H, s), 9.41 (1H, br);

^{13}C NMR of **8a** ($CDCl_3$) δ 12.5, 12.9, 13.0, 13.4, 16.9, 17.1, 17.2, 17.4, 17.5, 24.7, 34.0, 59.7, 68.3, 69.2, 69.5, 81.8, 83.0, 88.3, 115.1, 122.6, 123.4, 130.0, 141.5, 148.5, 150.8, 152.7, 153.5, 157.1, 166.8. HRMS of **8a** calcd for $C_{34}H_{52}N_9O_7Si_2$ ($M+Na^+$) 776.3348, found 776.3300.

^{1}H NMR of **8b** ($CDCl_3$, 500 MHz) δ 0.97–1.14 (28H, m), 3.22–3.31 (2H, m), 4.00–4.03 (1H, m), 4.11–4.22 (3H, m), 4.27 (3H, s), 4.33–4.34 (1H, m), 4.37–4.39 (1H, m), 4.75–4.81 (1H, m), 4.89 (2H, s), 6.02 (1H, s), 7.04–7.08 (3H, m), 7.32–7.37 (2H, m), 8.31 (1H, s), 8.75 (1H, s), 9.58 (1H, br); ^{13}C NMR of **8b** ($CDCl_3$) δ 12.7, 12.8, 13.0, 16.9, 17.0, 17.1, 17.2, 17.3, 17.5, 26.8, 39.2, 59.8, 68.2, 69.3, 69.7, 81.5, 82.3, 88.9, 115.0, 122.4, 123.3, 129.8, 142.1, 148.4, 150.9, 152.5, 157.1, 164.2, 166.9. HRMS of **8b** calcd for $C_{34}H_{52}N_9O_7Si_2$ ($M+Na^+$) 776.3348, found 776.3493.

*4.1.6. 2′-O-[(2-N-Cyanoethyltetrazol-5-yl)ethyl)]-6-N-phenoxyacetyladenosine (**9**)*

Compound **7a** (5.02 g, 6.35 mmol) was dissolved in anhydrous THF (32 mL). To the solution were added $Et_3N\cdot 3HF$ (3.6 mL, 22.2 mmol) and triethylamine (1.6 mL, 11.4 mmol). After being stirred at room temperature for 3 h, the mixture was evaporated in vacuo. The residue was chromatographed on a column of silica gel with hexane/$CHCl_3$/MeOH (100:0:0/0:98:2, v/v/v) to give compound **9** (3.46 g, 99%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.07–3.22 (4H, m), 3.78–3.87 (2H, m), 3.99–4.05 (2H, m), 4.41 (1H, s), 4.57 (1H, br), 4.69–4.70 (1H, d, J=4.2 Hz), 4.86–4.91 (5H, m), 5.92–5.94 (1H, d, J=7.6 Hz), 6.01 (1H, d, J=10.5 Hz), 7.05–7.09 (3H, m), 7.34–7.38 (2H, m), 8.02 (1H, s), 8.80 (1H, s), 9.46 (1H, br). ^{13}C NMR ($CDCl_3$) δ 18.3, 26.2, 48.3, 63.5, 66.9, 68.2, 70.7, 80.2, 81.6, 88.5, 89.5, 115.1, 115.5, 122.7, 124.7, 130.1, 143.9, 149.3, 150.8, 152.2, 157.1, 165.1, 166.7. HRMS calcd for $C_{24}H_{26}N_{10}O_6$ ($M+H^+$) 551.2110, found 551.2127.

*4.1.7. 2′-O-[(1-N-Methyltetrazol-5-yl)ethyl]-6-N-phenoxyacetyladenosine (**12**)*

^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.89–2.93 (1H, m), 3.04–3.10 (1H, m), 3.80 (1H, m), 3.88–3.91 (1H, m), 3.96–4.03 (4H, m), 4.12–4.17 (1H, m), 4.41 (1H, s), 4.78 (1H, d, J=4.2 Hz), 4.84–4.88 (3H, m), 5.67 (1H, br), 5.94 (1H, d, J=7.8 Hz), 6.01 (1H, br), 7.03–7.06 (3H, m), 7.32–7.36 (2H, m), 8.07 (1H, s), 8.78 (1H, s), 9.57 (1H, br); ^{13}C NMR ($CDCl_3$) δ 24.1, 33.5, 63.4, 64.9, 68.2, 70.3, 81.5, 88.5, 89.2, 115.0, 122.6, 124.6, 130.0, 144.0, 149.2, 150.8, 152.1, 153.3, 157.1, 166.9. HRMS calcd for $C_{22}H_{25}N_9O_6$ ($M+Na^+$) 534.1820, found 534.1859.

*4.1.8. 2′-O-[(2-N-Cyanoethyltetrazol-5-yl)ethyl]-5′-O-(4,4′-dimethoxyltrityl)-6-N-phenoxyacetyladenosine (**10**)*

Compound **9** (3.46 g, 6.28 mmol) was co-evaporated four times with anhydrous pyridine and dissolved in anhydrous pyridine (31 mL). To the solution was added 4,4-dimethoxytrityl chloride (2.55 g, 7.54 mmol). The mixture was stirred at room temperature for 165 min and quenched with H_2O. The mixture was evaporated in vacuo and diluted with ethyl acetate. The solution was washed with brine and aq $NaHCO_3$. The organic layer was dried over $MgSO_4$ and filtered. The solution was evaporated in vacuo. The residue was chromatographed on a column of silica gel with hexane/ethyl acetate (1:1/0:1, v/v) containing 0.5% triethylamine to give compound **10** as white foam (5.15 g, 73%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.10 (2H, m), 3.20–3.21 (1H, m), 3.24 (1H, dd, J=4.2, 5.1 Hz), 3.41–3.44 (1H, m), 3.51–3.54 (1H, m), 3.78 (6H, s), 4.11–4.14 (1H, m), 4.16–4.19 (2H, m), 4.26 (1H, dd, J=4.2, 3.7 Hz), 4.54 (1H, q, J=4.9 Hz), 4.75 (1H, t, J=4.6 Hz), 4.85–4.88 (4H, m), 6.16 (1H, d, J=4.6 Hz), 6.80–6.83 (4H, m), 7.05–7.08 (3H, m), 7.20–7.38 (9H, m), 7.43–7.45 (2H, m), 8.22 (1H, s), 8.71 (1H, s), 9.41 (1H, br); ^{13}C NMR ($CDCl_3$) δ 18.2, 26.3, 48.2, 55.3, 63.2, 67.5, 68.2, 69.9, 81.9, 84.4, 86.7, 87.0, 113.3, 115.1, 115.5, 122.6, 123.5, 127.1, 128.0, 128.3, 130.0, 130.2, 135.7, 142.4, 144.6, 148.4, 151.7, 152.6, 157.1, 158.7, 165.0, 166.7. HRMS calcd for $C_{45}H_{44}N_{10}O_8$ ($M+Na^+$) 875.3236, found 875.3234.

*4.1.9. 5′-O-(4,4′-Dimethoxyltrityl)-2′-O-[(1-N-methyltetrazol-5-yl)ethyl]-6-N-phenoxyacetyladenosine (**13**)*

^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.95–3.00 (1H, m), 3.14–3.16 (1H, m), 3.42 (1H, dd, J=4.2, 6.6 Hz), 3.51 (1H, dd, J=3.2, 7.6 Hz), 3.78 (6H, m), 4.00 (3H, s), 4.15–4.17 (1H, m), 4.28 (1H, t, J=3.9 Hz), 4.30 (1H, d, J=8.8 Hz), 4.65 (1H, q, J=4.6 Hz), 4.78–4.80 (1H, m), 4.85 (2H, br), 5.20 (1H, d, J=5.1 Hz), 6.14 (1H, J=5.1 Hz), 6.81–6.83 (4H, m), 7.05–7.08 (3H, m), 7.20–7.29 (3H, m), 7.32–7.38 (6H, m), 7.43–7.45 (2H, m), 8.24 (1H, s), 8.71 (1H, s), 9.41 (1H, br); ^{13}C NMR ($CDCl_3$) δ 24.3, 33.5, 55.4, 63.3, 65.7, 68.2, 70.1, 82.0, 84.5, 86.8, 87.1, 113.3, 115.1, 122.6, 123.5, 127.1, 128.1, 128.3, 130.0, 130.2, 135.8, 142.5, 144.7, 148.4, 151.8, 152.6, 153.2, 157.1, 158.7, 166.7. HRMS calcd for $C_{43}H_{43}N_9O_8$ ($M+Na^+$) 836.3127, found 836.3192.

*4.1.10. 2′-O-[(2-N-Cyanoethyltetrazol-5-yl)ethyl]-5′-O-(4,4′-dimethoxyltrityl)-6-N-phenoxyacetyladenosine 3′-(2-cyanoethyl N,N-diisopropylphosphoramidite) (**11**)*

Compound **10** (341 mg, 0.4 mmol) was co-evaporated four times with anhydrous toluene and once with anhydrous CH_2Cl_2, and dissolved in anhydrous CH_2Cl_2 (2 mL) under argon atmosphere. To the solution were added 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (152 μl, 0.48 mmol) and diisopropylammonium tetrazolide (27 mg, 0.16 mmol). And further 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (62 μl, 0.20 mmol) and diisopropylammonium tetrazolide (20 mg, 0.12 mmol) were added. After being stirred at room temperature for 6 h, the mixture was diluted with ethylacetate. The solution was washed with brine and aq $NaHCO_3$. The organic layer was dried over Na_2SO_4 and filtered. The solution was evaporated in vacuo. The residue was purified by use of recycling preparative HPLC eluted with ethyl acetate to give compound **11** as white foam (298 mg, 71%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.10–1.31 (12H, m), 2.40–2.42 (1H, m), 2.65–2.68 (1H, m), 3.05–3.08 (2H, m), 3.12–3.22 (2H, m), 3.35–3.39 (1H, m), 3.53–3.73 (4H, m), 3.81 (6H, m), 3.84–4.01 (2H, m), 4.13–4.22 (1H, m), 4.36–4.43 (1H, m), 4.60–4.67 (1H, m), 4.78–5.00 (1H, m), 6.12–6.13 (1H, m), 6.82–6.85

(4H, m), 7.08–7.11 (3H, m), 7.22–7.47 (11H, m), 8.18–8.22 (1H, m), 8.69–8.72 (1H, m), 9.40 (1H, br); ^{13}C NMR ($CDCl_3$) δ 18.1, 20.2, 20.3, 20.5, 20.6, 24.7, 24.8, 26.3, 43.2, 43.3, 43.4, 43.5, 48.0, 55.3, 57.9, 58.1, 58.8, 58.9, 62.8, 63.1, 67.9, 68.2, 68.4, 70.9, 71.0, 71.4, 71.5, 80.2, 80.5, 84.0, 84.2, 86.7, 86.8, 87.0, 87.1, 113.3, 115.4, 115.7, 115.8, 117.5, 117.9, 122.5, 123.6, 127.1, 128.0, 128.3, 128.4, 130.0, 130.2, 135.7, 135.8, 142.7, 142.9, 144.5, 144.6, 148.4, 152.0, 152.5, 152.6, 157.2, 158.7, 164.4, 164.5, 166.8. ^{31}P NMR ($CDCl_3$) δ 151.1, 151.8. HRMS calcd for $C_{54}H_{61}N_{12}O_9P$ ($M+H^+$) 1053.4538, found 1052.4427.

*4.1.11. 5′-O-(4,4′-Dimethoxyltrityl)-2′-O-[(1-N-methyl-tetrazol-5-yl)ethyl]-6-N-phenoxyacetyladenosine 3′-2-cyano-ethyl N,N-diisopropylphosphoramidite (**14**)*

^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.03–1.16 (12H, m), 2.38–2.40 (1H, m), 2.61–2.66 (1H, m), 3.12–3.20 (2H, m), 3.33–3.37 (1H, m), 3.47–3.66 (5H, m), 3.78–3.79 (6H, m), 3.99–4.00 (3H, m), 4.01–4.32 (2H, m), 4.34 (1H, m), 4.55–4.70 (2H, m), 4.86 (2H, br), 6.13–6.17 (1H, m), 6.80–6.83 (4H, m), 7.05–7.08 (3H, m), 7.21–7.47 (11H, m), 8.21–8.27 (1H, m), 8.71–8.73 (1H, m), 9.40 (1H, br); ^{13}C NMR ($CDCl_3$) δ 7.21, 20.3, 20.4, 20.5, 24.4, 24.5, 24.6, 24.7, 24.8, 33.9, 43.2, 43.3, 43.4, 55.3, 55.4, 58.0, 58.1, 58.2, 58.3, 62.5, 63.0, 68.2, 68.4, 70.9, 71.0, 71.2, 71.3, 81.8, 83.8, 83.9, 86.8, 86.9, 87.1, 87.2, 113.3, 115.1, 117.5, 118.0, 122.5, 123.3, 127.1, 128.0, 128.3, 130.0, 130.2, 130.3, 135.5, 135.6, 142.0, 144.4, 144.5, 148.5, 151.6, 151.7, 152.6, 152.7, 153.1, 153.2, 157.2, 158.7, 158.8, 166.8. ^{31}P NMR ($CDCl_3$) δ 150.8, 151.2. HRMS calcd for $C_{52}H_{60}N_{11}O_9P$ ($M+H^+$) 1014.4385, found 1014.4324.

4.2. Oligonucleotide synthesis

Oligodeoxynucleotides were synthesized on an Applied Biosystems 392 oligonucleotide synthesizer in 1 μmol, using pac phosphoramidite building blocks. A 0.1 M solution of each 2′-*O*-tetrazol-5-ylethyl adenosine phosphoramidite was used and the time for coupling was set to be 15 min. 5-Benzylthio-1*H*-tetrazole (0.25 M) was used as the reaction activator. Release of full modified oligonucleotide and deprotection of protecting group at phosphate, base, and tetazole-5-yl was carried out by use of NH_4OH at room temperature for 8 h or 55 °C for 5 h. The crude oligonucleotides were purified with standard DMTr-on protocol by using C-18 cartridge and further purified by anion-exchange HPLC.

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Supplementary data

The ^{1}H, ^{13}C, and ^{31}P NMR data of all new products are provided. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.02.075.

References and notes

1. (a) Raddatz, S.; Mueller-Ibeler, J.; Kluge, J.; Wäss, L.; Burdinski, G.; Havens, J. R.; Onofrey, T. J.; Wang, D.; Schweitzer, M. *Nucleic Acids Res.* **2002**, *30*, 4793–4802; (b) Dogan, Z.; Paulini, R.; Stutz, J. A. R.; Narayanan, S.; Richert, C. *J. Am. Chem. Soc.* **2004**, *126*, 4762–4763; (c) Yim, S. C.; Park, H. G.; Chang, H. N.; Cho, D. Y. *Anal. Biochem.* **2005**, *337*, 332–337.
2. (a) Bumcrot, D.; Manoharan, M.; Koteliansky, V.; Sah, D. W. Y. *Nat. Chem. Biol.* **2006**, *12*, 711–719; (b) Davis, S.; Lollo, B.; Freier, S.; Esau, C. *Nucleic Acids Res.* **2006**, *34*, 2294–2304.
3. (a) Kim, D. H.; Behlke, M. A.; Rose, S. D.; Chang, M. C.; Choi, S.; Rossi, J. J. *Nat. Biotechnol.* **2005**, *23*, 222–226; (b) Lina Cekaite, L.; Furset, G.; Hovig, E.; Sioud, M. *J. Mol. Biol.* **2007**, 90–108.
4. (a) Wengel, J. *Org. Biomol. Chem.* **2004**, *2*, 277–280; (b) Shu, W.; Liu, D.; Watari, M.; Riener, C. K.; Strunz, T.; Welland, M. E.; Balasubramanian, S.; McKendry, R. A. *J. Am. Chem. Soc.* **2005**, *127*, 17054–17060; (c) Liu, D.; Bruckbauer, A.; Abell, C.; Balasubramanian, S.; Kang, D.-J.; Klenerman, D.; Zhou, D. *J. Am. Chem. Soc.* **2006**, *128*, 2067–2071.
5. (a) Manoharan, M. *Biochim. Biophys. Acta* **1999**, *1489*, 117–130; (b) Manoharan, M. *Curr. Opin. Chem. Biol.* **2004**, *8*, 570–579.
6. Saneyoshi, H.; Seio, K.; Sekine, M. *J. Org. Chem.* **2005**, *70*, 10453–10460.
7. (a) Goldgur, Y.; Craigie, R.; Cohen, G. H.; Fujiwara, T.; Yoshinaga, T.; Fujishita, T.; Sugimoto, H.; Endo, T.; Murai, H.; Davies, D. R. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 13040–13043; (b) Herr, R. J. *Bioorg. Med. Chem.* **2002**, *10*, 3379–3393; (c) Biot, C.; Bauer, H.; Schirmer, R. H.; Davioud-Charvet, E. *J. Med. Chem.* **2004**, *24*, 5972–5983; (d) De Clercq, E. *J. Med. Chem.* **2005**, *5*, 1297–1313; (e) Tominey, A. F.; Docherty, P. H.; Rosair, G. M.; Quenardelle, R.; Kraft, A. *Org. Lett.* **2006**, *7*, 1279–1282.
8. (a) Cobb, A. J. A.; Longbottom, D. A.; Shaw, D. M.; Ley, S. V. *Chem. Commun.* **2004**, 1808–1809; (b) Torii, H.; Nakadai, M.; Ishihara, K.; Saito, S.; Yamamoto, H. *Angew. Chem., Int. Ed.* **2004**, *43*, 1983–1986.
9. Qu, Z.; Zhao, H.; Xi-Sen Wang, X.; Li, Y.; Song, Y.; Liu, Y.; Ye, Q.; Xiong, R.; Brendan, F.; Abrahams, B. F.; Xue, Z.; You, X. *Inorg. Chem.* **2003**, *42*, 7710–7712.
10. Tominey, A.; Andrew, D.; Oliphant, L.; Rosair, G. M.; Dupré, J.; Kraft, A. *Chem. Commun.* **2006**, 2492–2494.
11. Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, *20*, 1859–1862.
12. (a) Ostrocskii, V. A.; Studentsov, E. P.; Poplavskii, V. S.; Ivanova, N. V.; Gurskaya, G. V.; Zavodnik, V. E.; Jasko, M. V.; Semizarov, D. G.; Krayevsky, A. A. *Nucleosides Nucleotides* **1995**, *14*, 1289–1300; (b) Malin, A. A.; Ostrovskii, V. A. *Russ. J. Org. Chem.* **2001**, *6*, 759–780.
13. Filicheva, V. V.; Alexander, A.; Malin, A. A.; Ostrovskii, V. A.; Pedersen, E. B. *Helv. Chim. Acta* **2002**, *85*, 2847–2855.
14. Müller, J.; Bchme, D.; Lax, P.; Cerd, M. M.; Roitzsch, M. *Chem.—Eur. J.* **2005**, *11*, 6246–6253.
15. Murlowska, K.; Sadlej-Sosnowska, N. *J. Phys. Chem. A* **2005**, *109*, 5590–5595.
16. Saneyoshi, H.; Ando, K.; Seio, K.; Sekine, M. *Tetrahedron* **2007**, *45*, 11195–11203.
17. Rajasekaran, A.; Thampi, P. P. *Eur. J. Med. Chem.* **2004**, *39*, 273–279.
18. Wittenberger, S. J.; Donner, B. G. *J. Org. Chem.* **1993**, *58*, 4139–4141.
19. (a) Prakash, T. P.; Manoharan, M.; Fraser, A. S.; Kawasaki, A. M.; Lesnik, E. A.; Owens, S. R. *Tetrahedron Lett.* **2000**, *41*, 4855–4859; (b) Prakash, T. P.; Püschl, A.; Lesnik, E.; Mohan, V.; Tereshko, V.; Egli, M.; Manoharan, M. *Org. Lett.* **2004**, *6*, 1971–1974.