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Nucleosides and Nucleotides. Part 206: Introduction of Lipophilic Groups into 4′α-*C*-(2-Aminoethyl)thymidine-Containing Phosphodiester Oligodeoxynucleotides and Thermal Stabilities of the Duplexes☆

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Abstract—Synthesis and properties of the 4′α-*C*-(2-aminoethyl)thymidine (**1**)-containing oligodeoxynucleotides (ODNs), which have lipophilic groups such as palmitic acid, oleic acid, and cholesterol at the terminal of the aminoethyl linker of the compound **1**, are described. The ODNs were synthesized in a DNA synthesizer by using controlled pore glasses (CPGs) having the 4′α-*C*-[*N*-(palmitoyl), *N*-(oleoyl), and *N*-(cholesteryloxycarbonyl)aminoethyl]thymidine analogs (**9a**, **b**, and **c**). The stability of the duplexes formed by these ODNs and a complementary DNA **15** or RNA **16** was studied by thermal denaturation. It was found that the bulky functional group such as cholesterol thermally destabilizes the DNA/DNA duplexes, but that such thermal destabilization can be offset by the effects of the aminoethyl linker of **1**. Furthermore, these lipophilic groups do not largely influence the thermal stability of the DNA/RNA duplexes.

Introduction

Oligodeoxynucleotides (ODNs) that are capable of inhibiting cellular processes at the translational level by pairing with mRNA are known as antisense ODNs.[1] For ODNs to be effective as antisense molecules, they need to form stable Watson–Crick hybrids with complementary RNAs and be sufficiently resistant to degradation by ubiquitous nucleases. Since unmodified ODNs with natural phosphodiester linkages are good substrates for nucleases found in cell culture media and inside cells, many types of backbone-modified ODNs have been synthesized and used for antisense studies.[2]

However, we recently found that ODNs containing 4′α-*C*-(2-aminoethyl)thymidine (**1**; Fig. 1) were significantly resistant to both snake venom phosphodiesterase (3′-exonuclease) and DNase I (endonuclease), and were also very stable in human serum, even though they have natural phosphodiester linkages.[3] These ODNs also formed thermally stable duplexes with their complementary RNAs. Furthermore, the duplexes composed of these ODNs and their complementary RNAs were good substrates for *E. coli* and human RNase H. These results indicate that the ODNs containing **1** are good candidates for new antisense molecules.

In addition to the above-mentioned requirement for antisense molecules, antisense ODNs also need to be able to penetrate cellular membranes to reach their site of action.[1,4] Recently, it has been reported that the cellular uptake of ODNs was improved by covalent attachment of lipophilic groups such as cholesterol,[5] phospholipids,[6] palmitic acid,[7] and vitamin E,[8] which interact specifically with cell membranes.

In this paper, we wish to report the synthesis of the 4′α-*C*-(2-aminoethyl)thymidine-containing ODNs which have lipophilic groups such as palmitic acid, oleic acid, and cholesterol in order to increase the membrane permeability of the ODNs (Fig. 1). The thermal stabilities of the duplexes composed of these ODNs and a complementary DNA or RNA are also reported.

Results and Discussion

We introduced these lipophilic functional groups at the terminal of the aminoethyl linker of **1** that is positioned at the 3′-end of the strand, since nucleoside analogs with functional groups can be readily attached to the solid supports

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Keywords: antisense oligodeoxynucleotides; aminoethyl linker; lipophilic groups; melting temperature.

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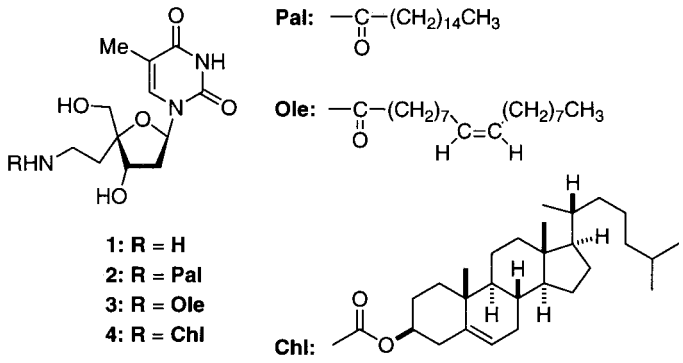
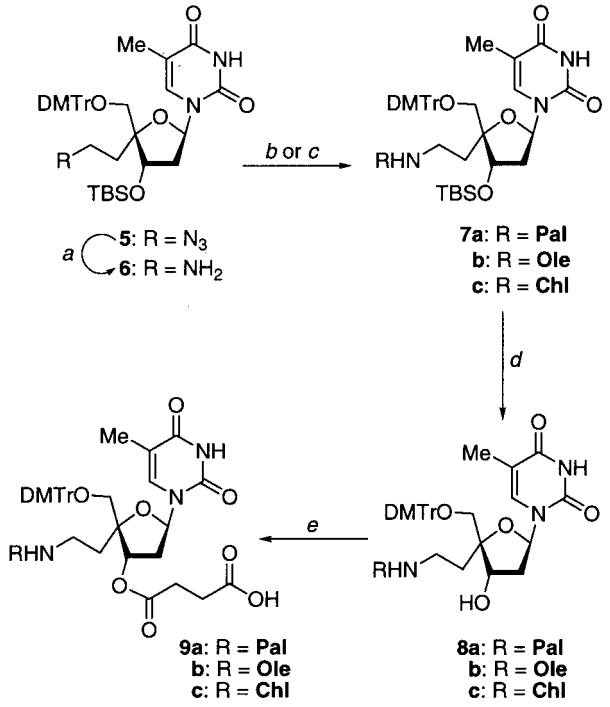


Figure 1. Structures of the modified nucleoside analogs.

before DNA synthesis. Furthermore, we expected that such bulky functional groups at their 3′-ends of the strands will enhance the nuclease-resistant property of the ODNs against 3′-exonuclease and in serum.[1,9] 4′α-*C*-(Azidoethyl)-3′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(dimethoxytrityl)thymidine (**5**), which was prepared by the procedure reported previously,[3] was hydrogenated to produce the 4′α-*C*-aminoethyl derivative **6** in 95% yield (Scheme 1). Palmitic acid and oleic acid were introduced into **6** in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSCI) to yield the *N*-functionalized nucleosides **7a** and **7b** in 80 and 83% yield, respectively. On the other hand, cholesterol was converted into its *O*-carbonylimidazolide, which was then reacted with **6** to give the cholesterol-modified nucleoside **7c** in 70% yield. After desilylation of **7a**, **7b**, and **7c** by TBAF, **8a**, **8b**, and **8c** were modified to the corresponding 3′-succinates **9a**, **9b**, and **9c**, which are reacted with controlled pore glass (CPG) to give a solid support containing **9a** (35 μmol/g), **9b** (41 μmol/g), and **9c** (36 μmol/g), respectively.



Scheme 1. Conditions: (a) H_2, Pd–C, MeOH, 95%; (b) palmitic acid or oleic acid, WSCI, CH_2Cl_2, 80% for **7a** and 83% for **7b**; (c) cholesterol, *N*,*N*′-carbonyldiimidazole, CH_2Cl_2, 70% for **7c**; (d) TBAF, THF, 94% for **8a**, 88% for **8b**, and 75% for **8c**; (e) succinic anhydride, DMAP, pyridine, 98% for **9a**, 83% for **9b**, and 97% for **9c**.

Three 21-mer ODNs[10] having **2**, **3**, or **4** at their 3′-ends were synthesized in a DNA synthesizer by using the CPGs containing **9a**, **9b**, or **9c** (Table 1). The fully protected ODNs (each 1 μmol scale) linked to the solid supports were treated with concentrated NH_4OH at 55°C for 16 h. Purification by C-18 column chromatography followed by detritylation with 80% CH_3CO_2H gave ODNs **12**, **13**, and **14** in 84, 36, and 64 A_{260} units, respectively. These ODNs were analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS), and the observed molecular weights supported their structures.

The stability of the duplexes formed by these ODNs and a complementary DNA **15** or RNA **16** was studied by thermal denaturation. One transition was observed in the melting profile of each duplex. Melting temperatures (T_ms) are listed in Table 2. When the DNA **15** was used as a complementary strand, the T_m for the unmodified ODN **10** was 35.6°C whereas the T_m for the ODN **11** containing the five molecules of **1** was 43.1°C. The T_ms of the ODNs **12**, **13**, or **14** containing the nucleoside analogs **2**, **3**, or **4** at their 3′-ends were lower than that for the ODN **11**, but still greater than that of the unmodified ODN **10**. The order of the T_m values for the ODNs with the lipophilic groups was as follows: the ODN **12** containing **2** (T_m=42.2°C)>the ODN **13** containing **3** (T_m=41.0°C)>the ODN **14** containing **4** (T_m=37.7°C).

On the other hand, when the RNA **16** was used as a complementary strand, the T_ms for the unmodified ODN **10** and the ODN **11** containing the five molecules of **1** were 44.3 and 44.0°C, respectively. Although the ODN **14** containing **4** only slightly decreased the thermal stability of the ODN/RNA duplex as compared with the ODNs **10** and **11**, the T_ms

Table 1. Sequences of oligonucleotides

No.	
10	5′-d[TAATTCATATTTTTTTAACAT]-3′
11	5′-d[**1**AAT**1**CATATT**1**TTT**1**AACA**1**]-3′
12	5′-d[**1**AAT**1**CATATT**1**TTT**1**AACA**2**]-3′
13	5′-d[**1**AAT**1**CATATT**1**TTT**1**AACA**3**]-3′
14	5′-d[**1**AAT**1**CATATT**1**TTT**1**AACA**4**]-3′
15	5′-d[ATGTTAAAAAAATATGAATTA]-3′
16	5′-r[AUGUUAAAAAAAUAUGAAUUA]-3′

Table 2. Hybridization data (Experimental conditions are described in the Experimental)

	T_m (°C)	
ODN	ODN/DNA[a]	ODN/RNA[b]
10	35.6	44.3
11	43.1	44.0
12	42.2	44.4
13	41.0	44.5
14	37.7	42.5

[a] The complementary DNA: **15**.
[b] The complementary RNA: **16**.

for the ODNs containing **2** and **3** were almost identical to those for the ODNs **10** and **11**.

The ODNs **12** or **13** containing the nucleoside analogs **2** or **3** at their 3′-ends slightly decreased the thermal stability of the ODN/DNA duplexes as compared with the ODN **11**. The ODN **11** has the five molecules of the aminoethyl linker, which enhances the thermal stability of the ODN/DNA duplex, whereas the ODNs **12** or **13** have the four molecules of the aminoethyl linker. Thus, the T_m values for the ODNs **12** or **13** seemed to reflect the difference of the number of the aminoethyl linker. On the other hand, the ODN **14** containing the nucleoside analog **4** largely reduced the thermal stability of the ODN/DNA duplex as compared with the ODN **11**. The 4′-position of the sugar moiety of the nucleoside is located at the edge of the minor groove in a DNA/DNA duplex. The minor groove of the DNA/DNA duplex is narrower and deeper than its major groove.[12] Thus, the bulky functional group such as cholesterol may not be well accommodated in the minor groove of the duplex. However, it is worthwhile to note that the T_m value for the ODN **14** is still greater than that for the unmodified ODN **10**. It was found that thermal destabilization of the DNA/DNA duplex by such bulky functional group can be adequately compensated by the effect of the aminoethyl linker of **1**.

On the other hand, when the RNA was used as a complementary strand, the ODN **14** containing **4** only slightly decreased the thermal stability of the ODN/RNA duplex. It is known that the minor groove of A-type duplexes (DNA/RNA and RNA/RNA duplexes) is shallower and flatter than that of DNA/DNA duplexes.[12] Thus, the bulky functional group introduced at the terminal of the aminoethyl linker of **1** may not largely influence the thermal stability of the DNA/RNA duplex.

Conclusions

In this paper, we reported the synthesis of the 4′α-*C*-(2-aminoethyl)thymidine-containing phosphodiester ODNs that have lipophilic groups at the terminal of the aminoethyl linker of **1**. It was found that the bulky functional group introduced thermally destabilizes the DNA/DNA duplex, but that such thermal destabilization can be offset by the effects of the aminoethyl linker of **1**. Furthermore, it was found that the functional groups do not largely influence the thermal stability of the DNA/RNA duplexes. Applications of these ODNs as antisense ODNs, especially the membrane permeability of ODNs containing **2**, **3**, and **4**, are currently being studied.

Experimental

General remarks

NMR spectra were recorded at 270 or 400 MHz (^{1}H), and are reported in ppm downfield from TMS. *J* values are given in Hertz. Mass spectra were obtained by fast atom bombardment (FAB) method. Thin-layer chromatography was done on Merck coated plates $60F_{254}$. The silica gel used for column chromatography was Merck silica gel 5715.

4′-*C*-(2-Aminoethyl)-3′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(dimethoxytrityl)thymidine (6). A mixture of 4′-*C*-(azidoethyl)-3′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(dimethoxytrityl)thymidine (**5**)[3] (790 mg, 1.09 mmol) and Pd–C (10%, 70 mg) in MeOH (20 mL) was stirred under atmospheric pressure of H_2 at room temperature. After 24 h, the catalyst was filtered off with Celite. The filtrate was evaporated under reduced pressure and dried in vacuo to give **6** (726 mg, 95% as a white foam): FAB-MS *m/z* 702 (MH^+); ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.57 (s, 1H), 7.37–6.77 (m, 13H), 6.20 (t, *J*=6.6 Hz, 1H), 4.60 (t, *J*=6.6 Hz, 1H), 3.75 (s, 6H), 3.32 (d, *J*=9.9 Hz, 1H), 3.05 (d, *J*=9.9 Hz, 1H), 2.72 (m, 1H), 2.58 (m, 1H), 2.29 (dt, *J*=6.6, 13.9 Hz, 1H), 2.46 (dt, *J*=6.6, 13.9 Hz, 1H), 1.79 (m, 1H), 1.40 (m, 1H), 1.40 (s, 3H), 0.81 (s, 9H), −0.04 (s, 3H), −0.07 (s, 3H). HRMS (FAB) calcd for $C_{39}H_{52}O_7N_3Si$ (MH^+): 702.3580. Found: 702.3562.

3′-*O*-(*tert*-Butyldimethylsilyl)-5′-*O*-(dimethoxytrityl)-4′-*C*-[*N*-(palmitoyl)aminoethyl]thymidine (7a). A mixture of **6** (100 mg, 014 mmol), palmitic acid (54 mg, 0.21 mmol), and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (WSCI) (40 mg, 0.21 mmol) in CH_2Cl_2 (2 mL) was stirred at room temperature. After 1 h, the mixture was diluted with $CHCl_3$. The organic layer was washed with aqueous $NaHCO_3$ (saturated) and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 40–60% EtOAc in hexane) to give **7a** (106 mg, 80% as a white foam): FAB-MS *m/z* 962 (MNa^+); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 7.26 (s, 1H), 7.40–6.82 (m, 13H), 6.26 (t, *J*=6.6 Hz, 1H), 5.78 (t, *J*=5.1 Hz, 1H), 4.55 (dd, *J*=6.1, 4.6 Hz, 1H), 3.79 (s, 6H), 3.40 (m, 1H), 3.34 (d, *J*=10.3 Hz, 1H), 3.19 (m, 1H), 3.12 (d, *J*=10.3 Hz, 1H), 2.25 (m, 2H), 2.06 (t, *J*=7.6 Hz, 2H), 1.90 (dt, *J*=7.3, 14.4 Hz, 1H), 1.63 (m, 3H), 1.53 (m, 2H), 1.48 (s, 3H), 1.25 (m, 22H), 0.88 (t, *J*=7.3 Hz, 3H), 0.85 (s, 9H), 0.04 (s, 3H), −0.03 (s, 3H). HRMS (FAB) calcd for $C_{55}H_{81}O_8N_3SiNa$ (MNa^+): 962.5691. Found: 962.5690. Anal. Calcd for $C_{55}H_{81}O_8N_3Si\cdot 1/5H_2O$: C, 69.98; H, 8.69; N, 4.45. Found: C, 69.83; H, 8.73; N, 4.37.

3′-*O*-(*tert*-Butyldimethylsilyl)-5′-*O*-(dimethoxytrityl)-4′-*C*-[*N*-(oleoyl)aminoethyl]thymidine (7b). Compound **6** (100 mg, 0.14 mmol) was oleoylated by using oleic acid (67 μL, 0.21 mmol) as described in the preparation of **7a** to give **7b** (112 mg, 83% as a white foam): FAB-MS *m/z* 988 (MNa^+); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (s, 1H),

7.49 (s, 1H), 7.36–6.79 (m, 13H), 6.22 (t, J=6.6 Hz, 1H), 5.74 (m, 1H), 5.30 (m, 2H), 4.55 (dd, J=6.4, 4.6 Hz, 1H), 3.76 (s, 6H), 3.35 (m, 1H), 3.30 (d, J=10.3 Hz, 1H), 3.17 (m, 1H), 3.09 (d, J=10.3 Hz, 1H), 2.22 (m, 2H), 2.03 (t, J=7.6 Hz, 2H), 1.96 (m, 4H), 1.84 (d, J=7.3, 14.7 Hz, 1H), 1.58 (m, 3H), 1.51 (m, 2H), 1.44 (s, 3H), 1.23 (m, 18H), 0.84 (t, J=7.3 Hz, 3H), 0.81 (s, 9H), 0.04 (s, 3H), −0.06 (s, 3H). HRMS (FAB) calcd for $C_{57}H_{83}O_8N_3SiNa$ (MNa^+): 988.5847. Found: 988.5836. Anal. Calcd for $C_{57}H_{83}O_8N_3Si$: C, 70.84; H, 8.66; N, 4.35. Found: C, 70.60; H, 8.74; N, 4.24.

3′-*O*-(*tert*-Butyldimethylsilyl)-5′-*O*-(dimethoxytrityl)-4′-*C*-[*N*-(cholesteryloxycarbonyl)aminoethyl]thymidine (7c). A mixture of cholesterol (165 mg, 0.43 mmol) and *N*,*N*′-carbonyldiimidazole (69 mg, 0.43 mmol) in CH_2Cl_2 (3 mL) was stirred at room temperature. After 1 h, **6** (150 mg, 0.21 mmol) was added to the mixture, and the whole was stirred at room temperature. After two days, the mixture was concentrated in vacuo. The residue was purified by column chromatography (SiO_2, 25–40% EtOAc in hexane) to give **7c** (162 mg, 70% as a white foam): FAB-MS m/z 1136 (MNa^+); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (s, 1H), 7.57 (s, 1H), 7.42–6.82 (m, 13H), 6.23 (t, J=6.6 Hz, 1H), 5.35 (m, 1H), 4.84 (m, 1H), 4.57 (dd, J=6.4, 4.4 Hz, 1H), 4.45 (m, 1H), 3.79 (s, 6H), 3.34 (d, J=10.0 Hz, 1H), 3.20 (m, 2H), 3.11 (d, J=10.0 Hz, 1H), 2.30 (m, 2H), 1.88 (m, 1H), 1.65 (m, 1H), 2.24–0.67 (m, 53H), 0.04 (s, 3H), −0.03 (s, 3H). HRMS (FAB) calcd for $C_{67}H_{95}O_9N_3SiNa$ (MNa^+): 1136.6736. Found: 1136.6750.

5′-*O*-(Dimethoxytrityl)-4′-*C*-[*N*-(palmitoyl)aminoethyl]thymidine (8a). A mixture of **7a** (88 mg, 90 μmol) and TBAF (1 M in THF, 0.19 mL, 0.19 mmol) in THF (1 mL) was stirred at room temperature. After 30 min, the mixture was concentrated in vacuo. The residue was purified by column chromatography (SiO_2, 60–100% EtOAc in hexane) to give **8a** (70 mg, 94% as a white foam): FAB-MS m/z 825 (M^+); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (s, 1H), 7.52 (s, 1H), 7.40–6.82 (m, 13H), 6.37 (t, J=6.6 Hz, 1H), 5.89 (t, J=5.4 Hz, 1H), 4.52 (m, 1H), 3.95 (d, J=3.17 Hz, 1H), 3.80 (s, 6H), 3.27 (m, 1H), 3.24 (d, J=10.3 Hz, 1H), 3.19 (d, J=10.3 Hz, 1H), 3.00 (m, 1H), 2.40 (m, 2H), 2.12 (t, J=7.3 Hz, 1H), 1.91 (m, 2H), 1.64 (m, 2H), 1.56 (m, 2H), 1.50 (s, 3H), 1.25 (m, 22H), 0.88 (t, J=7.1 Hz, 3H). HRMS (FAB) calcd for $C_{49}H_{67}O_8N_3$: 825.4928 (M^+). Found: 825.4948. Anal. Calcd for $C_{49}H_{67}O_8N_3$: C, 71.24; H, 8.17; N, 5.09. Found: C, 71.21; H, 8.22; N, 5.06.

5′-*O*-(Dimethoxytrityl)-4′-*C*-[*N*-(oleoyl)aminoethyl]thymidine (8b). Compound **7b** (96 mg, 0.10 mmol) was de-silylated as described in the preparation of **8a** to give **8b** (75 mg, 88% as a white foam): FAB-MS m/z 851 (M^+); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H), 7.47 (s, 1H), 7.40–6.82 (m, 13H), 6.37 (t, J=6.6 Hz, 1H), 5.87 (dd, J=5.9, 5.4 Hz, 1H), 5.34 (m, 2H), 4.52 (m, 1H), 3.89 (m, 1H), 3.79 (s, 6H), 3.25 (m, 1H), 3.24 (d, J=10.0 Hz, 1H), 3.19 (d, J=10.0 Hz, 1H), 2.99 (m, 1H), 2.40 (m, 2H), 2.12 (t, J=7.3 Hz, 2H), 1.99 (m, 4H), 1.89 (m, 2H), 1.58 (m, 4H), 1.51 (s, 3H), 1.27 (m, 18H), 0.88 (t, J=7.1 Hz, 3H). HRMS (FAB) calcd for $C_{51}H_{69}O_8N_3$: 851.5085 (M^+). Found: 851.5100. Anal. Calcd for $C_{51}H_{69}O_8N_3Si{\cdot}1/4H_2O$: C, 71.51; H, 8.22; N, 4.84. Found: C, 71.52; H, 8.22; N, 4.91.

5′-*O*-(Dimethoxytrityl)-4′-*C*-[*N*-(cholesteryloxycarbonyl)aminoethyl]thymidine (8c). Compound **7c** (141 mg, 0.13 mmol) was de-silylated as described in the preparation of **8a** to give **8c** (95 mg, 75% as a white foam): FAB-MS m/z 1000 (MH^+); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (s, 1H), 7.52 (s, 1H), 7.40–6.83 (m, 13H), 6.34 (t, J=6.6 Hz, 1H), 5.36 (m, 1H), 4.92 (t, J=5.6 Hz, 1H), 4.51 (m, 1H), 4.45 (m, 1H), 3.79 (s, 6H), 3.24 (m, 3H), 3.11 (m, 2H), 2.39 (m, 2H), 1.93 (m, 2H), 2.24–0.67 (m, 44H). HRMS (FAB) calcd for $C_{61}H_{82}O_9N_3$ (MH^+): 1000.6051. Found: 1000.6060.

5′-*O*-(Dimethoxytrityl)-4′-*C*-[*N*-(palmitoyl)aminoethyl]-3′-*O*-(succinyl)thymidine (9a). A mixture of **8a** (57 mg, 69 μmol), succinic anhydride (21 mg, 0.21 mmol), and DMAP (3 mg, 21 μmol) in pyridine (1 mL) was stirred at room temperature. After two days, the mixture was partitioned between EtOAc and H_2O. The organic layer was washed with aqueous $NaHCO_3$ (saturated) and brine, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by column chromatography (SiO_2, 2–10% MeOH in $CHCl_3$) to give **9a** (63 mg, 98% as a white foam): FAB-MS m/z 925 (M^+); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (s, 1H), 7.38–6.80 (m, 13H), 6.27 (m, 1H), 6.17 (m, 1H), 5.48 (m, 1H), 3.76 (s, 6H), 3.23 (m, 3H), 3.11 (m, 1H), 2.60 (m, 4H), 2.48 (m, 1H), 2.41 (m, 1H), 2.10 (m, 2H), 1.91 (m, 1H), 1.79 (m, 1H), 1.53 (m, 2H), 1.43 (s, 3H), 1.24 (m, 24H), 0.88 (t, J=7.1 Hz, 3H). HRMS (FAB) calcd for $C_{53}H_{71}O_{11}N_3$ (M^+): 925.5089. Found: 925.5060.

5′-*O*-(Dimethoxytrityl)-4′-*C*-[*N*-(oleoyl)aminoethyl]-3′-*O*-(succinyl)thymidine (9b). Compound **8b** (64 mg; 75 μmol) was succinylated as described in the preparation of **9a** to give **9b** (59 mg, 83% as a white foam): FAB-MS m/z 951 (M^+); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (s, 1H), 7.38–6.81 (m, 13H), 6.29 (m, 1H), 6.06 (m, 1H), 5.53 (m, 1H), 5.33 (m, 2H), 3.78 (s, 6H), 3.25 (m, 3H), 3.14 (m, 1H), 2.64 (m, 4H), 2.49 (m, 1H), 2.41 (m, 1H), 2.10 (m, 2H), 1.99 (m, 4H), 1.90 (m, 1H), 1.71 (m, 1H), 1.54 (m, 2H), 1.42 (s, 3H), 1.26 (m, 20H), 0.87 (t, J=6.6 Hz, 3H). HRMS (FAB) calcd for $C_{55}H_{73}O_{11}N_3$ (M^+): 951.5245. Found: 951.5232.

5′-*O*-(Dimethoxytrityl)-4′-*C*-[*N*-(cholesteryloxycarbonyl)aminoethyl]-3′-*O*-(succinyl)thymidine (9c). Compound **8c** (64 mg, 75 μmol) was succinylated as described in the preparation of **9a** to give **9c** (86 mg, 97% as a white foam): FAB-MS m/z 1100 (MH^+); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (s, 1H), 7.94 (s, 1H), 7.38–6.81 (m, 13H), 6.27 (m, 1H), 5.94 (m, 1H), 5.33 (m, 1H), 4.42 (m, 1H), 3.77 (s, 6H), 3.25 (m, 3H), 3.10 (m, 2H), 2.62 (m, 2H), 2.41 (m, 2H), 1.87 (m, 2H), 1.83 (m, 1H), 2.31–0.67 (m, 44H). HRMS (FAB) calcd for $C_{65}H_{86}O_{12}N_3$ (MH^+): 1100.6211. Found: 1100.6230.

Synthesis of the controlled pore glass support with 9a, 9b or 9c

Aminopropyl controlled pore glass (158 mg, 14.2 μmol, 89.8 μmol/g, CPG Inc.) was added to a solution of **9a** (53 mg, 57 μmol) and WSCI (11 mg, 57 μmol) in DMF (1 mL), and the mixture was kept at room temperature for two days. After the resin was washed with pyridine, 1 mL of a capping solution (0.1 M DMAP in pyridine/Ac_2O=9:1) was added, and the whole was kept at room temperature

for 14 h. The resin was washed with EtOH and acetone, and was dried under vacuum. The amount of loaded nucleoside **9a** to the solid support is 35 μmol/g from the calculation of released dimethoxytrityl cation by a solution of 70% $HClO_4$/EtOH (3:2, v/v). In a similar manner, the solid supports with **9b** and **9c** were obtained in 41 and 36 μmol/g of loading amounts.

Synthesis of ODNs

ODNs were synthesized on a DNA/RNA synthesizer (Applied Biosystem Model 392) by the phosphoramidite method. The fully protected ODNs were then deblocked and purified by the same procedure as for the purification of normal ODNs.[13] That is, each ODN linked to the resin was treated with concentrated NH_4OH at 55°C for 16 h, and the released ODN protected by a DMTr group at the 5′-end was chromatographed on a C-18 silica gel column (1×10 cm, Waters) with a linear gradient of MeCN from 10 to 80% in 0.1 M TEAA buffer (pH 7.0). The fractions were concentrated, and the residue was treated with aqueous 80% AcOH at room temperature for 20 min, then the solution was concentrated, and the residue was coevaporated with H_2O. The residue was dissolved in H_2O and the solution was washed with Et_2O, then the H_2O layer was concentrated to give a deprotected ODN **12** (84), **13** (36), and **14** (64). The yields are indicated in parentheses as OD units at 260 nm starting from 1 μmol scale.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

Spectra were obtained on a Voyager Elite reflection time-of-flight mass spectrometry (PerSeptive Biosystems, Inc., Framingham, MA) equipped with a nitrogen laser (337 nm, 3-ns pulse) in the negative ion mode. 3-Hydroxypicolinic acid (HPA), dissolved in 50% MeCN to give a saturated solution at room temperature, was used as the matrix. Time-to-mass conversion was achieved by calibration by using the peak representing the C^+ cation of the charged derivative to be analyzed. ODN **12**: calculated mass, 6808.5; observed mass, 6808.2. ODN **13**: calculated mass, 6834.5; observed mass, 6835.9. ODN **14**: calculated mass, 6982.6; observed mass, 6982.4.

Thermal denaturation

Each solution contains each ODN (3 μM) and the complementary DNA **15** (3 μM) in 0.01 M sodium cacodylate containing 0.01 M NaCl or RNA **16** (3 μM) in 0.01 M sodium cacodylate containing 0.1 M NaCl. The solution containing each ODN was heated at 90°C for 5 min, then cooled gradually to an appropriate temperature, and used for the thermal denaturation studies. Thermal-induced transitions of each mixture were monitored at 260 nm on a Perkin–Elmer Lambda2S. Sample temperature was increased 0.5°C/min.

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