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Anthracene based base-discriminating fluorescent oligonucleotide probes for SNPs typing: Synthesis and photophysical properties

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Abstract—2- and 9-Anthracenecarboxamide labeled 2′-deoxyuridines were synthesized and their photophysical properties were examined. These oligonucleonucleotide probes are capable of detecting adenine base on a target DNA sequence. It was also found that 2-anthracene based oligonucleotide probe is more efficient than the corresponding 9-anthracene based oligonucleotide in the application for DNA chip based SNP detection, due to its longer emission wavelength and high fluorescence intensity.

1. Introduction

Synthetic molecules that bind sequence specifically to DNA or recognize particular variation of single base alteration in a DNA sequence are legitimate targets in molecular biology and chemical genomics as a potential gene therapeutic agent and in gene diagnosis.[1] Oligonucleotides labeled with well-designed fluorophores are very useful to simplify DNA mutation assays if the fluorescence label exhibits a drastic change in its fluorescence intensity when the labeled probe is hybridized with a target sequence.[2]

Among numerous gene mutations, single nucleotide polymorphisms (SNPs) are the most common ones.[3] They are of medical and pharmacological interest in studies of disease susceptibility and drug response. Various techniques for SNP typing have been developed, such as 5′-exonuclease assay using TaqMan,[4] molecular beacon,[5] Invader assay,[6] as well as a DNA microarray method.[7] However, the single base discrimination in nearly all reported methods is directly or indirectly based on the different hybridization efficiency between matched and mismatched duplexes. Such DNA probes have inherent limitations of selectivity, since the differences in the hybridization efficiency vary with the sequence context and are often very small for the detection of a single base mismatch in a long target DNA. Particularly, to attain enough signal to noise (S/N) ratio when using DNA microarray method, the hybridization and washing conditions must be carefully controlled to minimize undesirable responses from the mismatched hybridization probes. Considering these limitations, alternative probes that do not rely on hybridization events are highly desirable.

Recently, we reported a new homogeneous assay to discriminate single base alteration by base-discriminating fluorescent (BDF) probes.[8] The concept of BDF probes is based on the fluorescence change of the BDF base itself in response to the bases on a complementary strand, not on whether the probe is hybridized. The working concept of BDF is based on the different microenvironment experienced by the fluorophore inside and outside the duplex, generating a different fluorescence signal. When hybridized with fully matched complementary target, the fluorophore has to reside outside the groove exposing itself to the more polar aqueous environment and as a result a strong fluorescence signal is observed. On the contrary, because of the lack of Watson–Crick base pairing in the mispaired position in the mismatched duplexes, the fluorophore remains inside the duplex facing highly hydrophobic environment, thereby exhibiting very weak fluorescence. The difficulty to use such BDF probes containing pyrenecarboxamide in SNP typing is their low fluorescence intensity and shorter wavelength emission. The probes whose emission wavelength lies

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in a visible region are more preferable in the effective application to SNP typing and in use for DNA microarray based SNP detection.

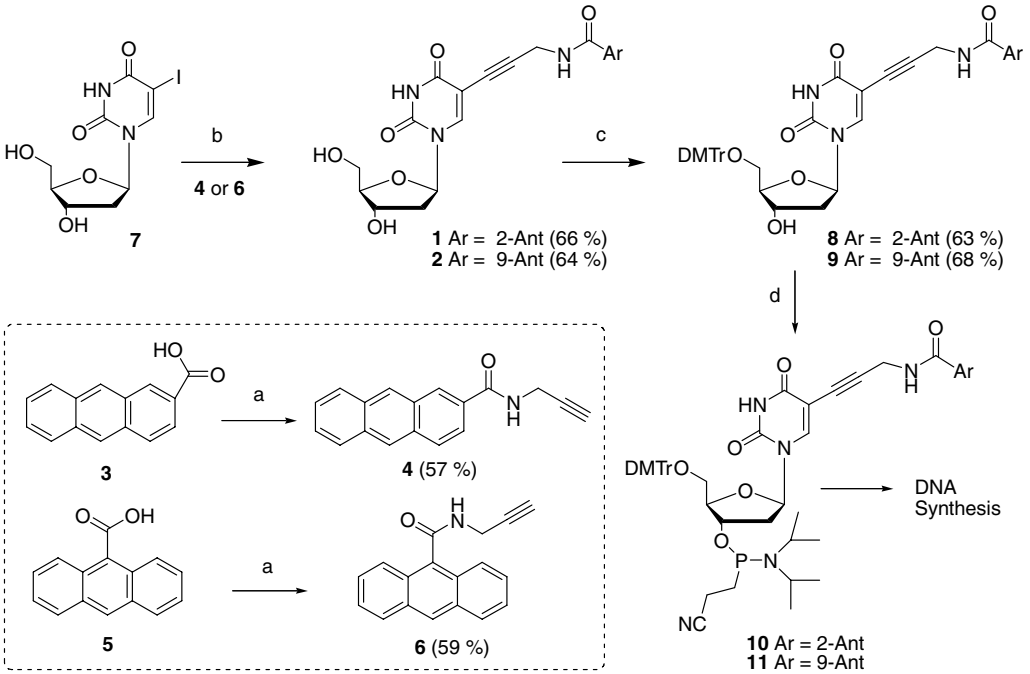
Anthracene is also a well-known intercalator for DNA duplex[9] and the fluorescence of 2-anthracenecarboxaldehyde and 2-acetylanthracene showed a strong dependence on solvent polarity. As the solvent polarity increases, the fluorescence intensity increases at a longer wavelength region. Thus, anthracene based fluorescent probe would be valuable in sensing the microenvironment inside and outside the DNA duplexes and they might be useful as a new BDF nucleoside for the detection of matched base opposite to the BDF base pair by a sharp fluorescence change. In order to know the generality for the solvofluorochromic anthracene derivatives as a chromophore of BDF nucleoside and in expecting high S/N ratio, we examined the design of novel anthracene labeled oligonucleotide probes.

2. Result and discussion

Here we report the efficiency of anthracene labeled oligonucleotide probes capable of detecting single base mismatch. Thus, we synthesized, 2- and 9-anthracenecarboxamide labeled uridine **1** and **2** via Sonogashira coupling[10] of 5-iodo-2′-deoxyuridine and the corresponding carboxamides bearing the rigid acetylenic linker (Scheme 1). The nucleosides **1** and **2** were incorporated into oligonucleotide sequences to afford **ODN 1** and **ODN 2** via a standard DNA synthesis and were characterized by MALDI-TOF mass spectrometry. These oligonucleotides were hybridized with their complementary strands containing matched and mismatched base pairs opposite the labeled uridine and the photophysical properties of these duplexes were studied (Table 1).

We first examined the photophysical properties of nucleosides **1** and **2** in organic solvents of different polarity. In the case of nucleoside **1**, it was clearly observed that both the emission intensity and the emission wavelength are markedly affected by solvent polarity. In less polar ethyl acetate, the nucleoside **1** displays relatively weak emission at 400 and 425 nm. As the solvent polarity increases, the shape of the emission band was changed. The intensity increased with a red shift. In water, a strong emission band appeared at around 475 nm. Solvents of intermediate polarity displayed an intermediate behavior (Fig. 1a). In contrast, in the case of 9-anthracenecarboxamide containing nucleoside **2**, the emission wavelength does not change and the intensity was decreased by increasing the solvent polarity (Fig. 1b).

Single-stranded oligodeoxynucleotide **ODN 1** labeled with 2-anthracenecarboxamide and mismatched duplexes **ODN 1/4**, **1/5**, and **1/6** showed weak fluorescence (Φ_F = 0.04, 0.06, and 0.10, respectively).[11] In contrast, only completely matched duplex **ODN 1/3** showed a strong fluorescence emission (Φ_F = 0.22) at 450 nm with a shoulder at visible region (575 nm) with a large Stokes shift of ca. 81 nm (Fig. 2a). Thus 2-anthracenecarboxamide labeled BDF oligonucleotide can be used for sensing adenine base. On the other hand, 9-anthracenecarboxamide labeled oligodeoxynucleotide **ODN 2**, when hybridized to its perfectly matched complementary sequence, emitted a relatively strong fluoresce (Φ_F = 0.03), although the intensity was much lower than



Scheme 1. Reagents and conditions: (a) Propargylamine, PyBop, DMF, rt, 17 h; (b) $Pd(PPh_3)_4$, CuI, DMF, Et_3N, rt, 5 h; (c) DMTrCl, pyridine, DMAP, Et_3N, rt, 16 h; (d) 2-cyanoethyl tetraisopropylphosphorodiamidite, 1*H*-tetrazole, CH_2Cl_2, rt, 1 h.

Table 1. Oligonucleotides used in this study

ODNs	Sequences	ODNs	*abl* gene sequences
ODN 1	5′-d(CGCAAC$^{2\text{-Ant}}$UCAACGC)-3′	5′-NH_2-ODN_{abl} ($^{2\text{-Ant}}$U)	
ODN 2	5′-d(CGCAAC$^{9\text{-Ant}}$UCAACGC)-3′	**ODN 8**	5′-NH_2-C_{12}-d(TGAAGGGCT$^{2\text{-Ant}}$UCTTCCAGATA)-3′
ODN 3	5′-d(GCGTTG **A** GTTGCG)-3′	ODN_{abl} (**N**) (**N** = **A**, **C**, **G**, or **T**)	
ODN 4	5′-d(GCGTTG **C** GTTGCG)-3′	**ODN 9**	5′-d(TATCTGGAAGAAGCCCTTCA)-3′
ODN 5	5′-d(GCGTTG **G** GTTGCG)-3′	**ODN 10**	5′-d(TATCTGGAAGCAGCCCTTCA)-3′
ODN 6	5′-d(GCGTTG **T** GTTGCG)-3′	**ODN 11**	5′-d(TATCTGGAAGGAGCCCTTCA)-3′
ODN 7	5′-d(CGCAAC **T** CAACGC)-3′	**ODN 12**	5′-d(TATCTGGAAGTAGCCCTTCA)-3′

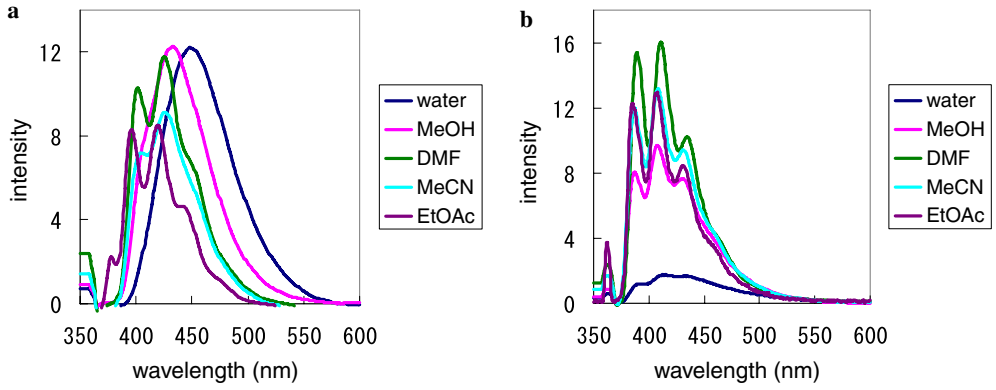


Figure 1. (a) Fluorescence spectra of $^{2\text{-Ant}}$U (**1**, 10 μM) in water, methanol, DMF, acetonitrile, and ethyl acetate. (b) Fluorescence spectra of $^{9\text{-Ant}}$U (**2**, 10 μM) in water, methanol, DMF, acetonitrile, and ethyl acetate.

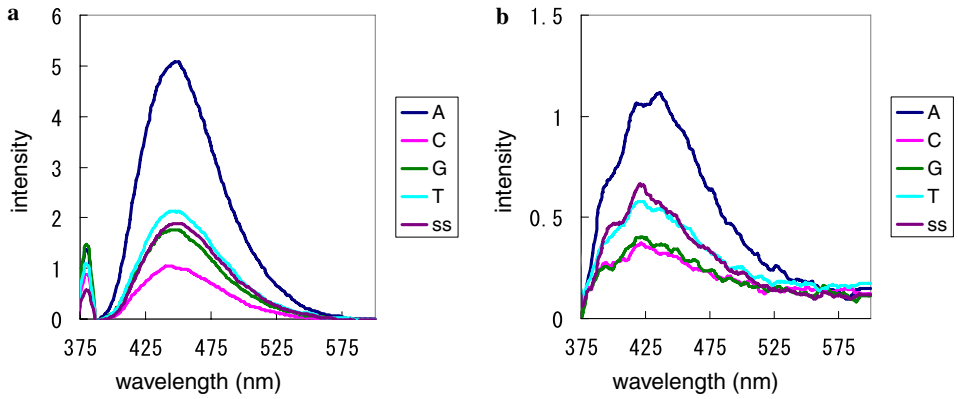


Figure 2. (a) Fluorescence spectra of **ODN 1** hybridized with 2.5 μM **ODN 3** (N = A, full much), **ODN 4** (N = C), **ODN 5** (N = G), or **ODN 6** (N = T) and single-stranded **ODN 1**. Excitation wavelength was at 381 nm. (b) Fluorescence spectra of **ODN 2** hybridized with 2.5 μM **ODN 3** (N = A, full much), **ODN 4** (N = C), **ODN 5** (N = G), or **ODN 6** (N = T) and single-stranded **ODN 2** (50 mM sodium phosphate, 0.1 M sodium chloride, pH 7.0, room temperature). Excitation wavelength was at 369 nm.

that for 2-anthracene analogue. **ODN 2** showed a similar behavior with other three mismatched sequences as shown by 2-anthracene analogue (Fig. 2b).

We also examined the thermal stability of the duplexes containing **1** and **2** (Table 2). The melting temperature measurement revealed that the duplexes containing **ODNs 1** and **2** are almost as stable as unmodified duplexes (T_m = 56.1, 57.3, and 59.8 °C for duplexes **ODN 1/3**, **2/3**, and **7/3**, respectively). It was evident that $^{2\text{-Ant}}$U-containing **ODN 1** is able to sense the type of the base opposite to $^{2\text{-Ant}}$U on the complementary strand. Thus, $^{2\text{-Ant}}$U-containing ODN would be very useful for SNP typing and also can be used for DNA microarray. For such a test, we have synthesized $^{2\text{-Ant}}$U-containing oligodeoxynucleotide **ODN 8** [5′-NH_2-ODN_{abl}($^{2\text{-Ant}}$U)] containing *abl* gene sequence and followed up the fluorescence change hybridization

Table 2. Melting temperature (T_m) and fluorescence quantum yield of oligonucleotides

Duplexes	T_m (°C)	λ_{em} (nm)	Φ	Duplexes	T_m (°C)	λ_{em} (nm)	Φ
ODN 1	—	450	0.09	**ODN 2**	—	425	0.012
ODN 1/3	56.1	452	0.22	**ODN 2/3**	57.3	438	0.034
ODN 1/4	53.4	446	0.04	**ODN 2/4**	50.9	426	0.011
ODN 1/5	54.2	449	0.06	**ODN 2/5**	53.8	426	0.014
ODN 1/6	54.1	450	0.10	**ODN 2/6**	53.1	425	0.016
ODN 7/3	59.8	Unmodified A/T base pair		**ODN 7/3**	59.8	Unmodified A/T base pair	

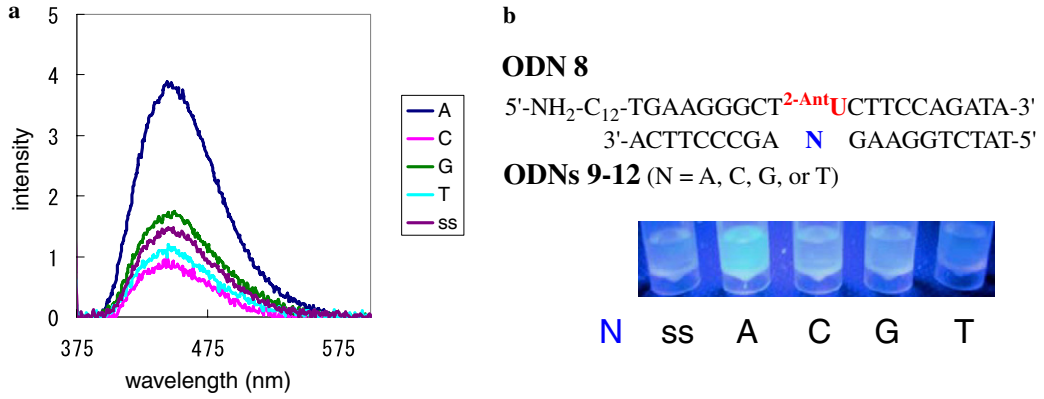


Figure 3. (a) Fluorescence spectra of **ODN 8** hybridized with 2.5 μM **ODN 9** (N = A, full much), **ODN 10** (N = C), **ODN 11** (N = G), or **ODN 12** (N = T) and single-stranded **ODN 8**. Excitation wavelength was at 371 nm. (b) Comparison of the fluorescence for the bases opposite $^{2\text{-Ant}}$**U** (50 mM sodium phosphate, 0.1 M sodium chloride, pH 7.0, room temperature). "ss" denotes a single-stranded BDF probe. The sample solutions were illuminated with a 365 nm transilluminator.

with **ODNs 9–12**. **ODN 8** is designed for the immobilization on a DNA microarray via the free terminal amino group. We simply mixed $^{2\text{-Ant}}$**U**-containing BDF probe (**ODN 8**) with **ODNs 9–12** [ODN$_{abl}$ (**N**), (**N** = **A**, **T**, **G**, or **C**)] and observed a strong emission only for the fully matched complementary strand, that is for **ODN 8/9** (**N** = **A**). For mismatched duplexes **ODNs 8/10**, **8/11**, and **8/12** (**N** = **T**, **G**, or **C**), the emission was negligible (Fig. 3). Thus, $^{2\text{-Ant}}$**U**-containing BDF probe was shown to be more effective for sensing adenosine on a target DNA by a drastic fluorescence change at longer wavelength than that for previously reported pyrenecarboxamide-labeled BDF probe.[8g] It is also important to note that the fluorescence emission of $^{2\text{-Ant}}$**U**-containing BDF probes does not depend on the flanking base pairs. For example, when BDF probes containing –T $^{2\text{-Ant}}$**U** T– sequence were used, a similar fluorescence change was observed (data not shown). However, the sensing efficiency was not so high as compared with the BDF probe containing –C $^{2\text{-Ant}}$**U** C– sequence, possibly because of the lack of the stabilization by franking C/G base pair.[8g] Thus, the newly designed BDF probe was shown to be useful for the gene detection and can be used in DNA microarray for SNPs typing.

3. Conclusion

We have synthesized two new BDF nucleosides, $^{2\text{-Ant}}$**U** (**1**) and $^{9\text{-Ant}}$**U** (**2**), and examined their fluorescence behavior upon hybridization with their target oligonucleotide sequences. Among these two BDF nucleosides, $^{2\text{-Ant}}$**U** (**1**) showed a stronger fluorescence dependency on solvent polarity and was more suitable for sensing DNA microenvironment, consistent with our hypothesis. The BDF oligonucleotide probes containing $^{2\text{-Ant}}$**U** (**1**) selectively emit fluorescence only when the base opposite to BDF base is adenine. Of particular importance is that $^{2\text{-Ant}}$**U**, a newly designed BDF nucleoside, is promising for homogeneous SNPs typing due to the fluorescence enhancement at a longer wavelength. Currently, SNPs typing on a DNA microarray using $^{2\text{-Ant}}$**U** as a BDF probe is underway.

4. Experimental

4.1. General

^{1}H NMR spectra were measured with Varian Mercury 400 (400 MHz) and. ^{13}C NMR spectra were measured with Bruker Avance 400F (100 MHz) spectrometer. Coupling constant (J value) is reported in hertz. The chemical shifts are shown in ppm downfield from tetramethylsilane, using residual chloroform (δ = 7.24 in ^{1}H NMR, δ = 77.0 in ^{13}C NMR) and methanol (δ = 3.30 in ^{1}H NMR, δ = 49.0 in ^{13}C NMR) as an internal standard. FAB masses were recorded on a JEOL JMS HX-110A spectrometer.

The reagents for DNA synthesis were purchased from Glen Research. Mass spectra of oligodeoxynucleotides were determined with a MALDI-TOF MS (Shimadzu AXIMA-LNR, acceleration voltage 20 kV, positive

mode) with 2′,3′,4′-trihydroxyacetophenone as a matrix. Calf intestinal alkaline phosphatase (Promega), Crotalus adamanteus venom phosphodiesterase I (USB), and *Penicillium citrinum* nuclease P1 (Roche) were used for the enzymatic digestion of ODNs. All aqueous solutions utilized purified water (Millipore, Milli-Q sp UF). Reversed-phase HPLC was performed on CHEMCOBOND 5-ODS-H columns (10×150 mm, 4.6×150 mm) with a JASCO Chromatograph, Model PU-2080, using a UV detector, Model UV-2075 plus, at 254 nm.

4.1.1. *N*-Propargyl-2-anthracenecarboxamide (4). A solution of 2-anthracenecarboxylic acid (**3**, 150.0 mg, 0.675 mmol) and PyBOP (421.4 mg, 0.810 mmol) in anhydrous DMF (6.0 mL) was stirred at room temperature. After 60 min, propargylamine (44.6 mg, 0.810 mmol) was added, and the mixture was stirred at room temperature for 17 h. The resulting mixture was concentrated in vacuo and diluted with chloroform. The solution was washed with sat. NH_4Cl solution and brine, dried over Na_2SO_4, filtered off, and evaporated. The crude product was purified by silica gel column chromatography ($CHCl_3$/MeOH = 20:1) to yield **4** (57%, 99.6 mg) as a pale yellow solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 8.53–8.45 (m, 3H), 8.08–8.02 (m, 3H), 7.79 (d, 1H, J = 8.8 Hz), 7.54–7.51 (m, 2H), 4.35 (dd, 2H, J = 2.8, 5.2 Hz), 2.33 (t, 1H, J = 2.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ = 167.2, 132.9, 132.2, 132.2, 130.4, 130.4, 129.0, 128.5, 128.4, 128.2, 128.1, 126.4, 126.3, 126.0, 122.6, 79.5, 72.1, 30.0; HRFABMS calcd. For $C_{18}H_{14}NO$ ($[M+H]^+$) 260.1075, found 260.1086.

4.1.2. 5-[3-(2-Anthracenecarboxamido)propynyl]-2′-deoxyuridine (1). To a solution of 5-iodo-2′-deoxyuridine (**7**, 95.6 mg, 0.270 mmol), **4** (70.0 mg, 0.270 mmol), and triethylamine (41.0 mg, 0.405 mmol) in 3.0 mL of anhydrous DMF were added tetrakis(triphenylphosphine)palladium(0) (62.4 mg, 0.054 mmol) and copper(I) iodide (10.3 mg, 0.054 mmol) under nitrogen. The mixture was stirred at room temperature for 5 h. The resulting mixture was concentrated in vacuo and the residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 20:1) to yield **1** (66%, 86.4 mg) as a pale yellow solid: ^{1}H NMR (DMSO, 400 MHz) δ = 8.72 (s, 1H), 8.68 (s, 1H), 8.64 (s, 1H), 8.32 (s, 1H), 8.11–8.20 (m, 3H), 7.93 (dd, 1H, J = 1.6, 9.2 Hz), 7.56–7.59 (m, 3H), 6.12 (t, 1H, J = 7.2 Hz), 4.38 (d, 2H, J = 4.2 Hz), 4.21–4.25 (m, 1H), 3.79–3.81 (m, 1H), 3.53–3.62 (m, 2H), 2.08–2.14 (m, 2H); ^{13}C NMR (DMSO, 100 MHz) δ = 165.7, 149.3, 143.6, 132.1, 131.5, 130.5, 130.0, 128.4, 128.2, 128.0, 127.8, 126.3, 125.91, 125.87, 123.3, 98.1, 87.5, 84.6, 70.1, 60.9, 45.6 HRFABMS calcd. For $C_{27}H_{24}N_3O_6$ ($[M+H]^+$) 486.1665, found 486.1669.

4.1.3. 5-[3-(2-Anthracenecarboxamido)propynyl]-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine (8). A solution of **1** (86.5 mg, 0.178 mmol), 4-dimethylaminopyridine (catalytic amount), and 4,4′-dimethoxytrityl chloride (66.4 mg, 0.196 mmol) in dry pyridine (3.0 mL) was stirred at room temperature for 16 h. After concentration of the solution to dryness, the residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 20:1) to yield **8** (63%, 88.1 mg) as a pale yellow solid: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.35–8.32 (m, 2H), 8.17 (s, 1H), 7.96–7.89 (m, 2H), 7.64 (d, 1H, J = 9.2 Hz), 7.50–7.44 (m, 1H), 7.38 (d, 1H, J = 7.6 Hz), 7.29–6.76 (m, 13 H), 6.32 (t, 1H, J = 6.8 Hz), 4.55 (m, 2H), 4.17 (dd, 1H, J = 5.2, 8.4 Hz), 4.09–4.01 (m, 1H), 3.67 (s, 3H), 3.66 (s, 3H), 3.25 (m, 2H), 2.56–2.54 (m, 1H), 2.30–2.27 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ = 160.2, 158.7, 150.0, 144.3, 144.1, 135.43, 135.4, 135.3, 130.1, 130.04, 129.96, 128.3, 128.1 128.0, 127.9, 127.1, 122.8, 113.4, 87.1, 86.3, 85.4, 77.3, 77.2, 77.0, 76.7, 72.4, 68.7, 63.4, 55.3, 46.1, 41.4, 11.2; HRFABMS calcd. for $C_{48}H_{42}N_3O_8$ ($[M+H]^+$) 788.2972, found 788.2974.

4.1.4. 5-[3-(2-Anthracenecarboxamido)propynyl]-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine 3′-*O*-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (10). To a solution of **8** (47.0 mg, 0.060 mmol) and 1*H*-tetrazole in anhydrous acetonitrile (0.078 mmol) was added 2-cyanoethyltetraisopropylphosphorodiamidite (24.8 mL, 0.078 mmol) under nitrogen. The mixture was stirred at room temperature for 1 h. The mixture was filtered off and used for oligodeoxynucleotide synthesis without further purification.

4.1.5. *N*-Propargyl-9-anthracenecarboxamide (6). Compound **6** was prepared from 9-anthracenecarboxylic acid (95.0 mg, 0.427 mmol), PyBOP (288.8 mg, 0.555 mmol), anhydrous DMF (3.0 mL), and propargylamine (35.3 mg, 0.640 mmol) by the method described for **4**. Purification by silica gel column chromatography ($CHCl_3$/MeOH = 10:1) gave **6** (59%, 65.2 mg) as a pale yellow solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 8.41 (s, 1H), 8.01–7.94 (m, 4H), 7.50–7.42 (m, 4H), 4.40 (dd, 2H, J = 2.4, 5.6 Hz), 2.31 (t, 1H, J = 2.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ = 169.3, 131.0, 130.7, 128.6 (2×), 128.5 (2×), 128.1 (2×), 126.8 (2×), 125.5 (2×), 124.8 (2×), 79.1, 72.1, 29.7; HRFABMS calcd. for $C_{18}H_{14}NO$ ($[M+H]^+$) 260.1075, found 260.1077.

4.1.6. 5-[3-(9-Anthracenecarboxamido)propynyl]-2′-deoxyuridine (2). Compound **2** was prepared from 5-iodo-2′-deoxyuridine (49.0 mg, 0.138 mmol), **6** (35.9 mg, 0.138 mmol), triethylamine (21.0 mg, 0.208 mmol), anhydrous DMF (3.0 mL), tetrakis(triphenylphosphine)palladium(0) (32.0 mg, 0.028 mmol), and copper(I) iodide (5.3 mg, 0.028 mmol) by the method described for **1**. Purification by silica gel column chromatography ($CHCl_3$/MeOH = 10:1) gave **2** (64%, 43.0 mg) as a pale yellow solid: ^{1}H NMR (CD_3OD, 400 MHz) δ = 8.49 (s, 1H), 8.30 (s, 1H), 7.99–7.97 (m, 4H), 7.49–7.39 (m, 4H), 6.16 (dd, 1H, J = 6.4, 6.8 Hz), 4.48 (s, 2H), 4.31 (ddd, 1H, J = 3.2, 4.0, 6.4 Hz), 3.85 (dt, 1H, J = 3.2, 3.2 Hz), 3.71 (dd, 1H, J = 3.2, 12.0 Hz), 3.63 (dd, 1H, J = 3.2, 12.0 Hz), 2.23 (ddd, 1H, J = 4.0, 6.4, 13.6 Hz), 2.14 (ddd, 1H, J = 6.4, 6.8, 13.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ = 172.2, 164.6, 151.2, 145.5, 132.6, 132.4, 129.6 (2×), 129.4 (2×), 129.4 (2×), 127.9 (2×), 126.6 (2×), 126.1 (2×), 100.0, 89.8, 89.2, 87.1, 75.6, 72.1, 62.6, 41.8, 31.0; HRFABMS

calcd. for $C_{27}H_{24}N_3O_6$ ($[M+H]^+$) 486.1665, found 486.1670.

4.1.7. 5-[3-(9-Anthracenecarboxamido)propynyl]-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine (9). Compound **9** was prepared from **2** (61.2 mg, 0.126 mmol), 4,4′-dimethoxytrityl chloride (49.1 mg, 0.145 mmol), 4-dimethylaminopyridine (catalytic amount), and anhydrous pyridine (3.0 mL) by the method described for **8**. Purification by silica gel column chromatography ($CHCl_3$/MeOH = 10:1) gave **9** (68%, 67.4 mg) as a pale yellow solid: ^{1}H NMR (CD_3OD, 400 MHz) d = 8.48 (s, 1H), 8.19 (s, 1H), 7.99 (d, 2H, 7.6 Hz), 7.90 (d, 2H, 6.4 Hz), 7.39–7.47 (m, 4H), 7.17–7.33 (m, 9H), 6.83 (m, 4H), 6.28 (t, 1H, 7.2 Hz), 4.32 (d, 2H, 4.8 Hz, CH_2), 4.00 (m, 1H), 3.59 (s, 3H), 3.56 (s, 3H), 3.31–3.37 (m, 1H), 3.24 (dd, 2H, 3.2, 10.8 Hz), 2.43 (ddd, 1H, 5.6, 7.2, 14.0 Hz), 2.26 (ddd, 1H, 6.0 Hz, 7.2, 14.0 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ = 169.0, 162.0, 158.7, 158.5, 149.4, 144.3, 144.0, 143.2, 135.4, 135.2, 131.0, 130.1, 130.08, 130.04, 129.9, 129.8, 128.4, 128.1, 128.0, 127.9, 127.8, 126.9, 126.7, 125.5, 125.1, 113.4, 113.2, 99.4, 88.6, 86.9, 86.5, 86.2, 85.6, 85.4, 77.3, 77.2, 77.0, 76.7, 75.1, 72.3, 72.1, 63.4, 55.3, 55.09, 55.07, 46.1, 41.7, 41.4, 30.8, 10.1; HRFABMS calcd. for $C_{48}H_{42}N_3O_8$ ($[M+H]^+$) 788.2972, found 788.2972.

4.1.8. 5-[3-(9-Anthracenecarboxamido)propynyl]-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine 3′-*O*-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (11). Compound **11** was prepared from **9** (52.1 mg, 0.066 mmol), 1*H*-tetrazole in anhydrous acetonitrile (0.45 M, 190.7 μL, 0.086 mmol), and 2-cyanoethyl tetraisopropylphosphorodiamidite (27.3 μL, 0.086 mmol) by the method described for **10**.

4.1.9. Melting temperature (T_m) measurements. All T_ms of the ODNs (2.5 mM, final duplex concentration) were measured in 50 mM sodium phosphate buffers (pH 7.0) containing 100 mM sodium chloride. Absorbance vs temperature profiles were measured at 260 nm using a Shimadzu UV-2550 spectrophotometer equipped with a Peltier temperature controller using 1 cm path length cell. The absorbance of the samples was monitored at 260 nm from 4 to 90 °C with a heating rate of 1 °C/min. From these profiles, first derivatives were calculated to determine T_m values.

4.1.10. Fluorescence experiments. ODN solutions were prepared as described in T_m measurement experiments. Fluorescence spectra were obtained using a Shimadzu RF-5300PC spectrophotometer at 25 °C using 1 cm path length cell. The excitation bandwidth was 1.5 nm. The emission bandwidth was 1.5 nm. The fluorescence quantum yields (Φ_F) were determined using 9,10-diphenylanthracene as a reference with the known Φ_F (0.95) in ethanol.[11]

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