Supporting Information

DNA templated nucleophilic aromatic substitution reactions for fluorogenic sensing of oligonucleotides

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Contents

1 **Table S1.** The quantum yields of the compound **3**[a]

	AMCA	Compound **3**
Φ[b]	0.641	0.009

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3 [a] All measurements were done in 0.1 M phosphate buffer (pH 10). Compounds were

4 excited at 375 nm.

5 [b] Quantum yields are determined by using 4-methylumbelliferone (0.63) as a standard.

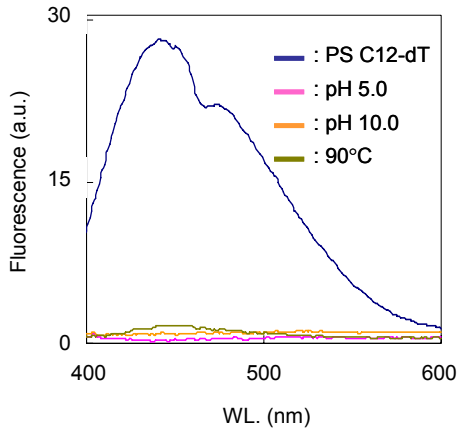


Figure S1. Stability of DNs-AMCA probe under various conditions.

The stability of the DNs-AMCA probe was tested at pH 5.0, 10.0, and high temperature (90 °C) conditions. DNs-AMCA probe was incubated at 20 mM Tris-borate (pH 5.0) or 20 mM Tris-HCl buffer (10.0) for 30 min at 37 °C or in 20 mM Tris-HCl buffer (pH 7.2) for 30 min at 90 °C. No significant fluorescence with excitation of 375 nm was observed in these conditions by comparison with the spectra after the treatment of thymidine 3'-*O*-(12-(phosphoryloxy)dodecyl *O,S*-dihydrogen phosphorothioate) (PS C12-dT).

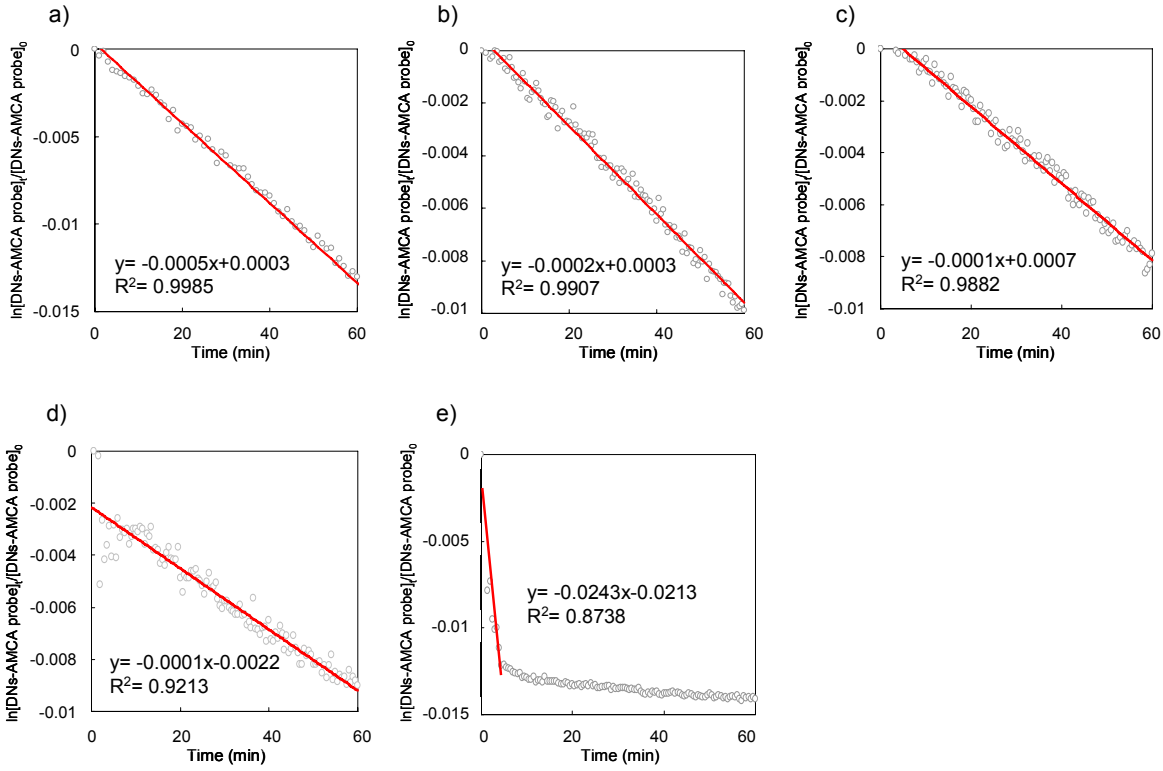


Figure S2. Plots of $\ln[\text{DNs-AMCA probe}]_t/[\text{DNs-AMCA probe}]_0$ versus time for 500 nM DNs-AMCA probe and 500 μM thiol compound in 20 mM Tris-HCl (pH 7.2) containing 100 mM $MgCl_2$. The thiol compound: (a) cysteine; (b) dithiothreitol; (c) 2-mercaptoethanol; (d) PS-dT; (e) PS C12-dT.

3'-*PS C12*-T TGG GTC-5': **PS probe**

DNs-AMCA probe: 3'-G CAC TTC TCC CTT TG-*DNs-AMCA*-5'

5'-CTA ACG TCC GTC GTG AAG AGG GAA AC $(T)_n$ A ACC CAG ACC GCC AGC TAA GGT CCC A-3'

23S-C: n= 0, **23S-C5T**: n= 5, **23S-C10T**: n= 10

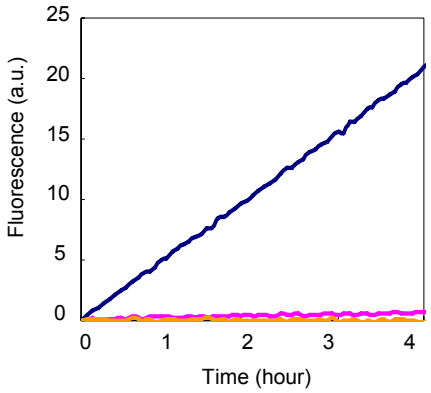


Figure S3. Time course of the fluorescence intensity in the reaction between 250 nM DNs-AMCA probe and 500 nM PS probe with 250 nM of 23S-C(blue), 23S-C5T(red), 23S-C10T(orange) in 20 mM Tris-HCl buffer (pH7.2) containing 10 mM $MgCl_2$. Reaction was monitored by excitation at 375 nm and emission at 450 nm.

Experimental details

Measurement of quantum yield.[S1] A 1 mM DMF stock solution of each compound was prepared. Absorption spectra were obtained with a 0.1 M phosphate buffer (pH 10) solution of each compound at the desired concentration (Abs at 375nm< 0.02), adjusted by appropriate dilution of the 1 mM DMF stock solution. For determination of the quantum efficiency of fluorescence (Φ_{fl}), 4-methylumbelliferone in 0.1 M phosphate buffer (pH 10) was used as a fluorescence standard ($\Phi = 0.63$). The quantum efficiency of fluorescence was obtained with the following equation (F denotes fluorescence intensity at each wavelength and Σ [F] was calculated by summation of fluorescence intensity).

$$\Phi_{fl}^{\ sample} = \Phi_{fl}^{\ standard}\ Abs^{\ standard}\ \Sigma\ [F^{\ sample}] / Abs^{\ sample}\ \Sigma\ [F^{\ standard}]$$

Synthesis of 5'- DNs-protected AMCA -linked oligonucleotide (DNs-AMCA probe). DNs-protected AMCA NHS ester was reacted with 5'-amino-modified oligonucleotide. 5'-aminomodifier C6 (Glen Research) was used to prepare 5'-aminomodified oligonucleotide. A DMF solution containing DNs-AMCA **3** (0.2 M), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (0.2 M), and N-hydroxysuccinimide (0.2 M) was incubated for 11 h at room temperature in the dark. The reaction was carried out by intensively stirring a mixed solution comprising 16 mM DNs-AMCA NHS ester, 100 mM sodium tetraborate (pH 8.5) and 200 μM 5'-amino-modified oligonucleotides solution at room temperature for 7 h (DMF concentration in the reaction solution: 46%). The reacted products were collected by ethanol precipitation. Next, the collected products were purified by reverse-phase HPLC (0-60% acetonitrile/50 mM triethylammonium acetate gradient). The probe structure was

confirmed by MALDI-TOF mass spectrometry. DNs-AMCA probe: Calcd for $C_{155}H_{177}N_{42}O_{63}P_8S$ 3915.2, Found 3929.0.

Synthesis of 3'-phosphorothioate C12-Linked Oligonucleotide (PS probe). 3'-phosphate CPG and Spacer C12 CE Phosphoramidite (Glen Research) were used to prepare 3'-phosphorothioate C12-linked oligonucleotide. The 3'-phosphate CPG was sulfurized by the sulfurizing reagent (Glen Research) after the Spacer C12 was added. Deprotection and cleavage from the CPG support was carried out by the standard method. The probe structure was confirmed by MALDI-TOF mass spectrometry. PS probe: Calcd for $C_{81}H_{114}N_{24}O_{49}P_8S$ 2486.5, Found 2487.6.

Fluorescence Measurement. Reactions on the DNA template were performed in 1.2 mL of Tris-HCl buffer (20 mM, pH 7.2) containing 100 mM $MgCl_2$ with template DNA (0 or 500 nM), DNs-AMCA probe (500 nM), and PS probe (500 nM) at 37 °C. The increase of fluorescence intensity produced by deprotection of DNs group on DNs-AMCA probe was continuously monitored at time intervals. Reactions were observed by fluorescence spectrometry (FP-6500; JASCO). Fluorescence spectra were measured under the following conditions: excitation, 375 nm, emission, 450 nm.

Synthesis of Methyl 7-amino-4-methylcoumarin-3-acetate 1. Sulfuric acid (5 drops) was added to a solution of 7-Amino-4-methyl-3-coumarinyl- acetic acid (43.1 mg, 0.18 mmol) in methanol (40 mL). The reaction mixture was stirred under reflux for 1 h. The reaction was cooled and then quenched with sat $NaHCO_3$ aq. The mixture was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and evaporated in vacuo. The residue was purified by flash chromatography to give the compound **1** (34.4 mg, 0.14 mmol, 75%).

^{1}H-NMR (400 MHz, DMSO-*d*6) :δ7.47-7.45(1H, d, *J*= 8.0 Hz, Ar), 6.58-6.56(1H, dd, *J*= 8.0 Hz, Ar), 6.40(1H, ds, Ar), 6.06(2H, s, NH_2), 3.59(2H, s, CH_2CO), 3.30(3H, s, CH_3), 2.27(3H, s, CH_3); ^{13}C-NMR (99.5 MHz, DMSO-*d*6) :δ170.91, 161.24, 153.99, 152.52, 149.94, 126.39, 112.00, 111.32, 108.88, 98.23, 51.63, 32.10, 14.70; HR-ESI-MS *m/z*: Calcd for $C_{13}H_{13}NNaO_4^+$ ($[M+Na]^+$) 270.0737, Found 270.0745.

Synthesis of Methyl 7-(2,4-dinitrophenylsulfonamido)-4-methylcoumarin-3-acetate 2. To a solution of **1** (29.6 mg, 0.12 mmol) in pyridine/CH_2Cl_2 (=1:1, 1.2 ml) was added 2,4-dinitrobenzensulfonyl chloride (52.5 mg, 0.20 mmol, 1.6 equiv). After 8 h, the reaction mixture was diluted with $CHCl_3$, washed with H_2O. The organic layer was dried over Na_2SO_4 and evaporated in vacuo. The residue was purified by PLC to give the compound **2** (33.6 mg, 0.07 mmol, 56%).

^{1}H-NMR (400 MHz, CD_3OD) :δ8.51(1H, ds, Ar), 8.38-8.35(1H, dd, *J*= 12.0, 4.0 Hz, Ar), 8.19-0.17(1H, d, *J*= 8.0 Hz, Ar), 7.52-7.50(1H, d, *J*= 8.0 Hz, Ar), 7.01-6.98(1H, dd, *J*= 12.0, 4.0 Hz, Ar), 6.95(1H, ds, Ar), 3.58(2H, s, CH_2CO), 2.27(3H, s, CH_3), 1.19(3H, s, CH_3); ^{13}C-NMR (99.5 MHz, CD_3OD) :δ172.82, 163.68, 154.51, 151.50, 151.02, 150.00, 133.37, 127.17, 127.03, 124.46, 120.96, 120.39, 119.39, 117.70, 116.30, 108.47, 52.64, 33.38, 15.23; HR-ESI-MS *m/z*: Calcd for $C_{19}H_{14}N_3O_{10}S^-$ ($[M-H]^-$) 476.0405, Found 476.0415.

Synthesis of 7-(2,4-dinitrophenylsulfonamido)-4-methyl-3-coumarinylacetic acid 3.[S2] An aqueous solution of lithium hydroxide monohydrate (28.7 mg, 0.68 mmol in 4.5 mL) was added to a solution of **2** (43.5 mg, 0.09 mmol) in THF (4.5 mL) cooled to 0°C. The reaction mixture was stirred at 0°C for 3 h and allowed to warm to room temperature for 2 h until all starting product had disappeared. The mixture was acidified with 5% HCl and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and

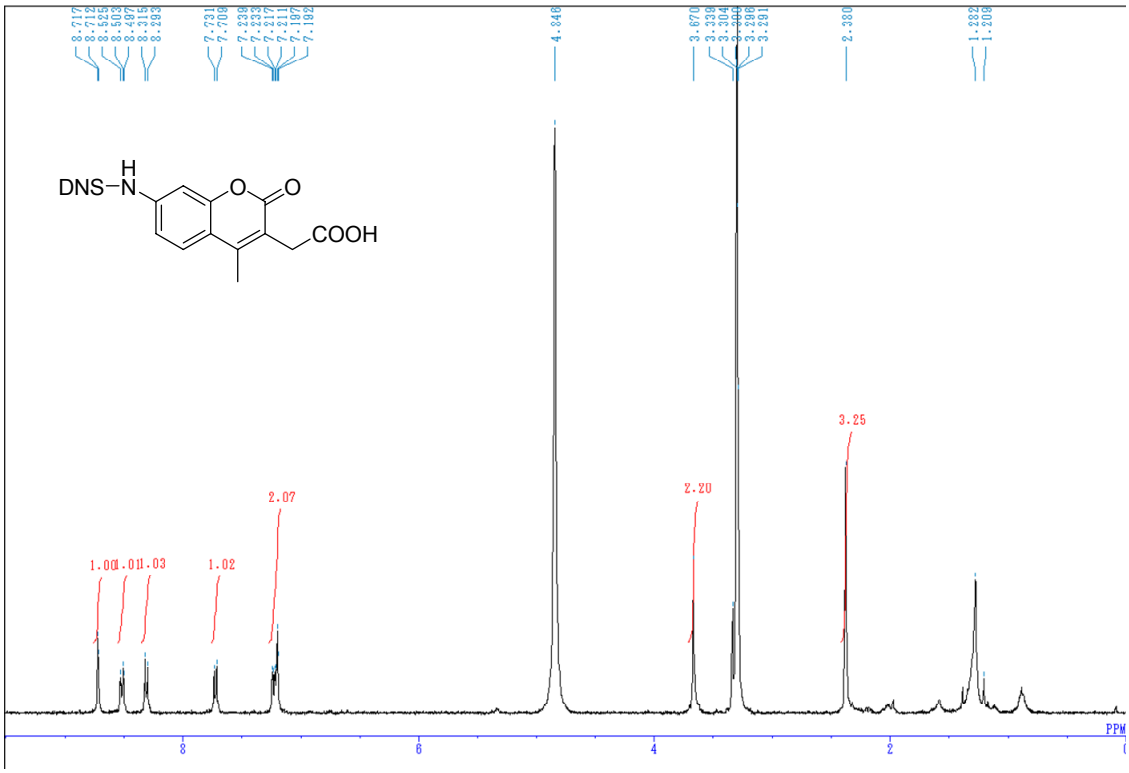
evaporated in vacuo. The residue was purified by flash column chromatography to give the compound **3** (41.8 mg, 0.09 mmol, 99%).

^{1}H-NMR (400 MHz, CD_3OD) :δ8.72-8.71(1H, ds, *J*=2.0 Hz, Ar), 8.53-8.50(1H, dd, *J*= 11.2, 2.4 Hz, Ar), 8.32-8.29(1H, d, *J*= 8.8 Hz, Ar), 7.73-7.71(1H, d, *J*= 8.8 Hz, Ar), 7.24-7.21(1H, dd, *J*= 11.2, 2.4 Hz, Ar), 7.20(1H, s, Ar), 3.67(2H, s, CH_2CO), 2.38(3H, s, CH_3); ^{13}C-NMR (99.5 MHz, CD_3OD) :δ173.92, 163.04, 154.22, 151.92, 150.63, 149.81, 140.58, 138.05, 134.05, 127.78, 127.74, 121.68, 120.68, 118.68, 118.02, 108.82, 33.51, 15.30; HR-ESI-MS *m/z*: Calcd for $C_{18}H_{13}N_3O_{10}S^-$ ($[M-H]^-$) 476.0405, Found 476.0415.

Reference

S1. W. C. Sun, K. R. Gee and R. P. Haugland, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 3107-3110.

S2. S. Niwayama, *J. Org. Chem.*, 2000, **65**, 5834-5836.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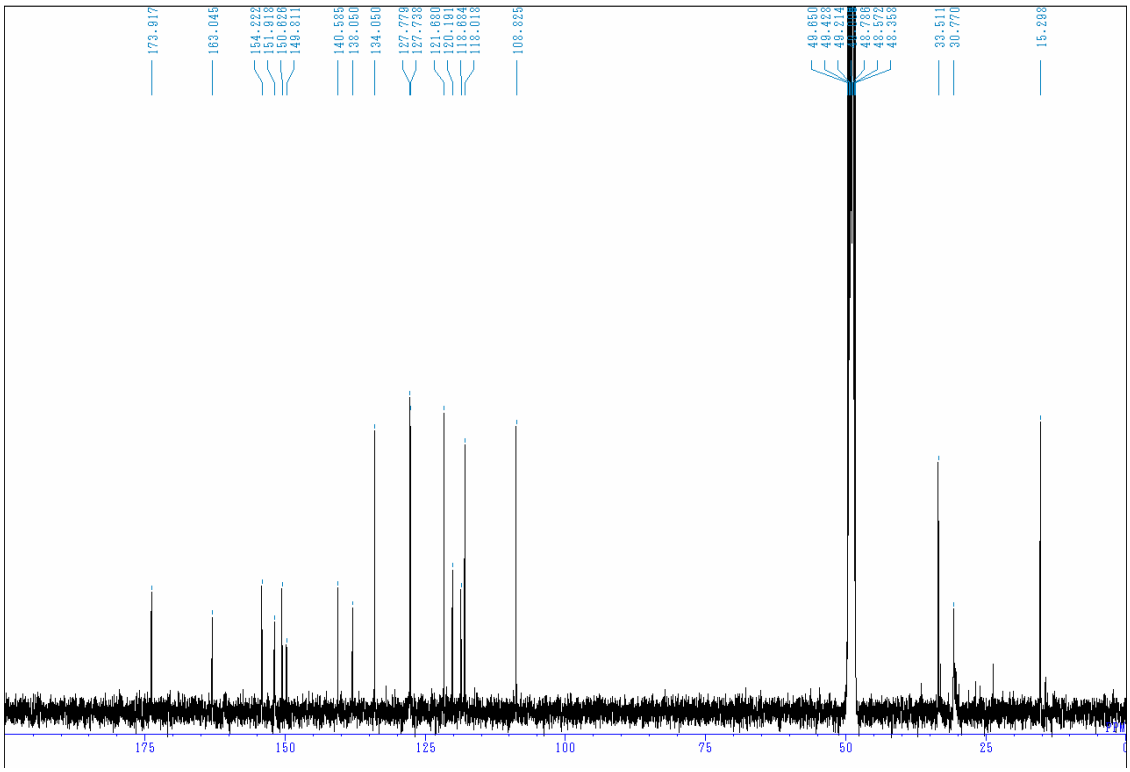
1

2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3

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