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# Supplementary Information

# Fluorine-modified bisbenzimide derivative as a molecular probe for bimodal and simultaneous detection of DNAs by $^{19}$F NMR and fluorescence

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## Contents

## 1. Experimental Procedures

**ODNs and other reagents:** All hpODNs were purchased from Fasmac (Japan). Bisbenzimide H 33258 and 3,5-bis(trifluoromethyl)benzylbromide were purchased from Sigma-Aldrich (CA) and TCI (Tokyo, Japan), respectively. Other reagents and solvents for probe synthesis were purchased from Wako Pure Chemical Industry (Osaka, Japan) or Kanto Chemical (Tokyo, Japan).

**$^{19}$F NMR measurement:** $^{19}$F NMR spectra were recorded using AVANCE III 500 MHz NMR spectrometer (Bruker Biospin) equipped with a 5 mm probe head (PA BBO 500S2 BBF-H-D-05 Z, Bruker Biospin) at 470 MHz for $^{19}$F. The chemical shifts were corrected using TFA (–75.6 ppm) as an internal standard.

**Fluorescence measurement:** Fluorescence spectra were recorded using Varioskan fluorescence microplate reader (Thermo Scientific, MA) or FP-6500 spectrofluorophotometer (JASCO, Tokyo, Japn) with excitation at 345 nm.

**Cell cultivation and fluorescence microscopic analysis:** Hela cells ($2.3 \times 10^4$ / chambar) in DMEM (10% FBS) were seeded on a 4-chamber glass-bottom dish (35 mm) and incubated for 24 h in a humidified chamber (37ºC, 5% $CO_2$). After washing with FluoroBrite™ DMEM (Gibco, 300 μL × 2), a solution of **1** or bisbenzimide H 33258 (10 μM in FluoroBrite™ DMEM, 300 μL) was poured to the chambers and then incubated for 10 min in a humidified chamber (37ºC, 5% $CO_2$). Fluorescence microscopic analyses were performed by a fluorescence microscope (BZ-8000, Keyence, Japan) equipped with a filter box (Ex360/40, DM400, BA460/50) and an objective lens (PlanFluor ELWD 20×/0.45 Ph1 DM, Nikon, Japan).

## 2. Supporting Figures

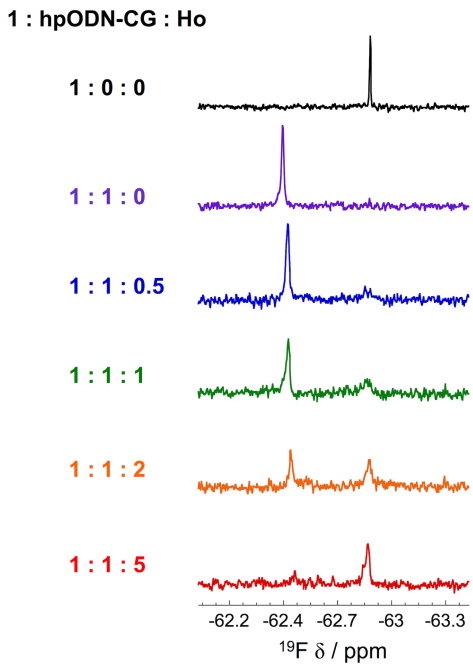



**Fig. S1** $^{19}$F NMR spectra of the mixture of **1**, hpODN-CG and various contents of bisbenzimide H 33258 (Ho). The molar ratio is indicated at the left of each spectrum. [**1**] = [hpODN-CG] = 10 μM in 50 mM Tris-HCl (pH 7.6) containing 100 mM NaCl and 10% (v/v) $D_2O$. Measurements were performed at 27°C.

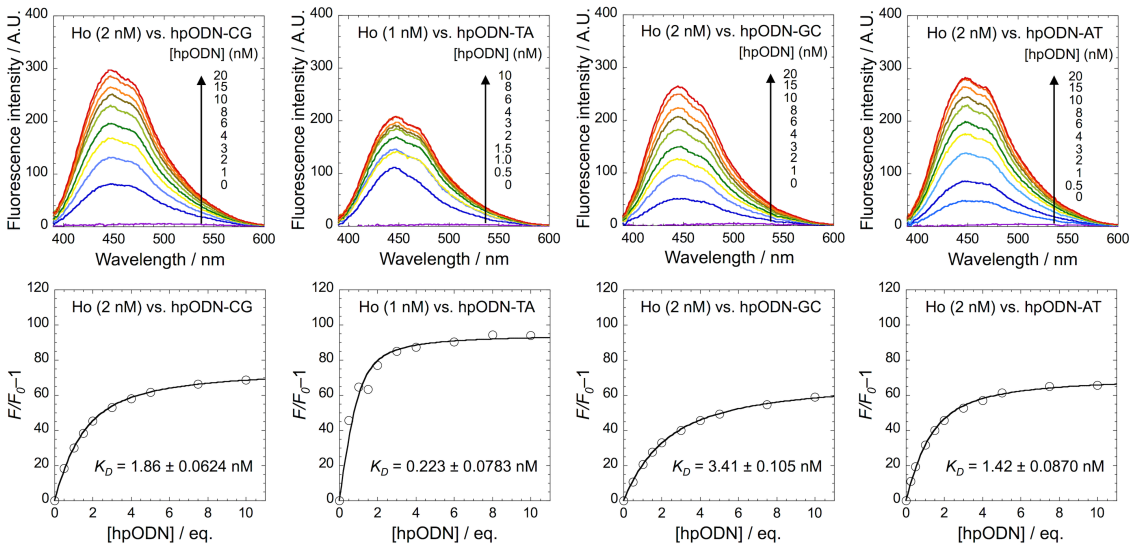



**Fig. S2** Fluorescence spectra and fluorescence titration curves of bisbenzimide H 33258 (Ho) versus hpODNs. [Ho] = 1 or 2 nM in 50 mM Tris-HCl (pH 7.6) containing 100 mM NaCl. Measurements were performed at 27°C. Fluorescence intensity at 460 nm was used for preparing titration curves and $K_D$ values were calculated with non-linear least square fitting.

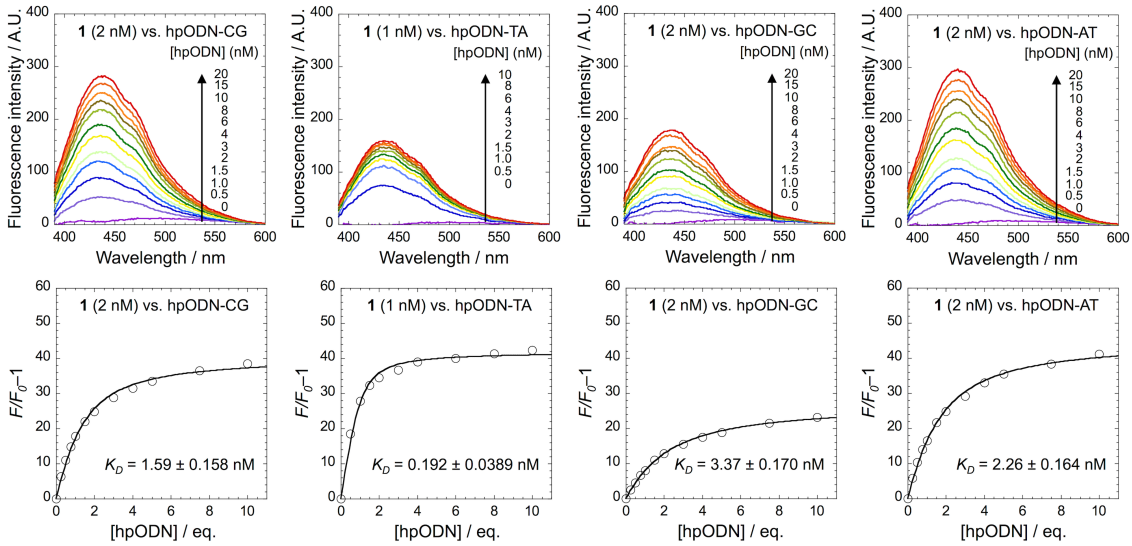



**Fig. S3** Fluorescence spectra and fluorescence titration curves of **1** versus hpODNs. [**1**] = 1 or 2 nM in 50 mM Tris-HCl (pH 7.6) containing 100 mM NaCl. Measurements were performed at 27°C. Fluorescence intensity at 460 nm was used for preparing titration curves and $K_D$ values were calculated with non-linear least square fitting.

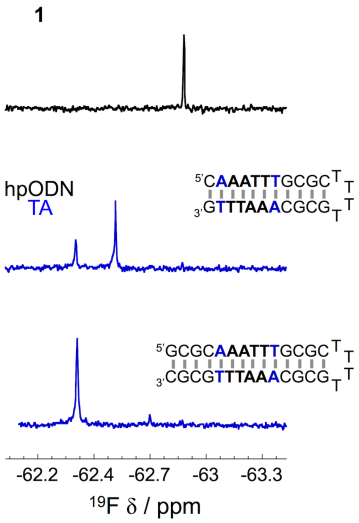
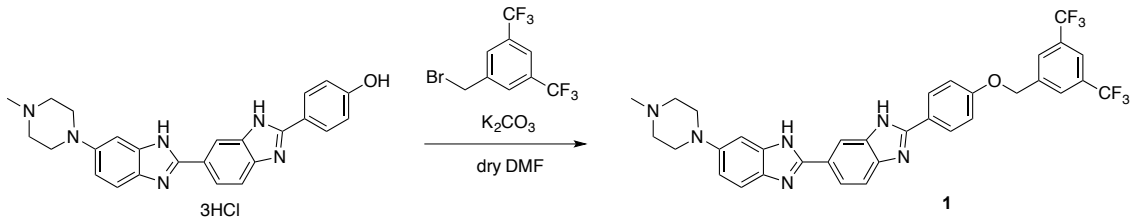



**Fig. S4** $^{19}$F NMR spectra of the mixture of **1** and hpODNs having different stem length. [**1**] = [hpODN] = 10 μM in 50 mM Tris-HCl (pH 7.6) containing 100 mM NaCl and 10% (v/v) $D_2O$. Measurements were performed at 27°C.

## 3. Synthetic Procedures




**Synthesis of Compound 1:** To a suspension of bisbenzimide H 33258 (43 mg, 81 µmol) and $K_2CO_3$ (34 mg, 243 µmol) in dry DMF (1 mL), 3,5-bis(trifluoromethyl)benzylbromide (38 mg, 122 µmol) was added and stirred for 38 h at 60ºC under nitrogen atmosphere. After cooling down to ambient temperature, supernatant was diluted with 0.1 % (v/v) aqueous solution of TFA (1 mL) and MeOH (2 mL) and then purified by a reversed-phase HPLC (JASCO PU-980, HG-980-31, DG-980-50, UV-970 system equipped with an InertSustain$^{TM}$ C18 column (GL Science, 5 µm, 10 × 150 mm), 0 to 70% acetonitrile containing 0.1% v/v TFA in 0.1% aqueous TFA over 30 min (flow rate: 3 mL/min) at 60ºC). Lyophilization of the corrected peak at retention time 18 min afforded **1** as yellow powder (38 mg, 38 µmol, 47%).

**$^{1}$H NMR (500 MHz, DMSO-d6):** δ (ppm) 8.44 (1H, s), 8.37 (3H, s), 8.10 (2H, d, J = 8.75 Hz), 8.08 (1H, d, J = 8.0 Hz), 7.87 (1H, d, J = 8.5 Hz), 7.72 (1H, d, J = 9.0 Hz), 7.32 (1H, d, J = 9.0 Hz), 7.28 (1H, s), 7.00 (2H, d, J = 8.7 Hz), 4.95 (2H, s), 3.88 (2H, d, J = 14.0 Hz), 3.78 (2H, dd, 10.0 Hz), 3.60 (2H, d, 12.2 Hz), 3.49 (2H, dd, 11.1 Hz), 3.19 (3H, s)

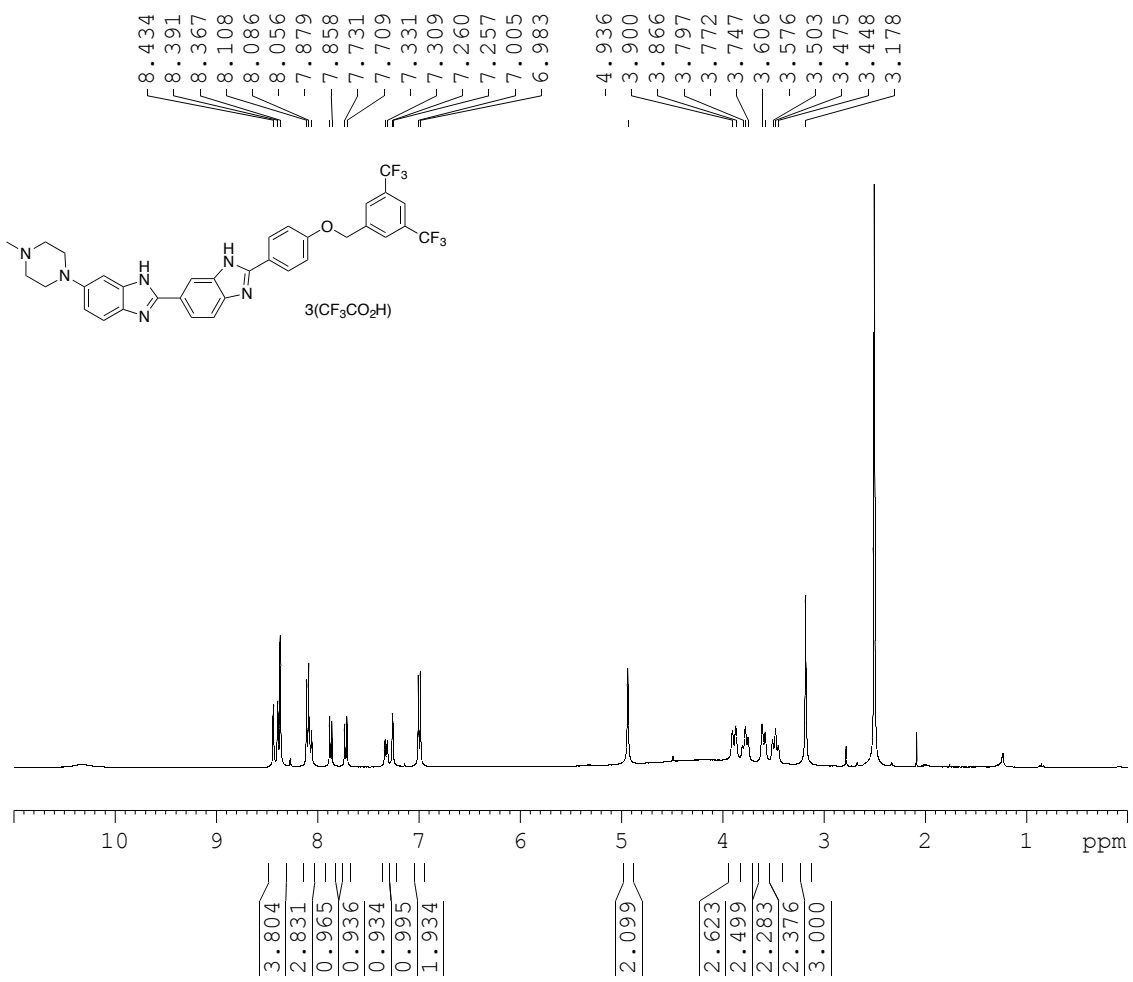
**$^{13}$C NMR (125 MHz, DMSO-d6):** δ (ppm) 160.5, 158.2 ($CF_3\mathit{C}O_2H$, q, $^{2}J_{FC}$ = 33 Hz), 154.1, 149.6, 147.9, 133.9, 132.0, 130.8 ($CF_3$-Ph, q, $^{2}J_{FC}$ = 33 Hz), 130.5, 129.1, 127.8, 124.4, 123.1 ($\mathit{C}F_3$-Ph, q, $^{1}J_{FC}$ = 275 Hz), 118.6, 117.9, 116.1, 116.0, 115.3, 114.6, 114.1, 99.4, 66.0, 59.0, 44.2, 42.8, 42.1

**$^{19}$F NMR (470 MHz, DMSO-d6):** δ (ppm) –61.19 (6F, s, $(C\mathit{F}_3)_2$-Ph), –74.12 (9F, s, $3(C\mathit{F}_3CO_2H)$)

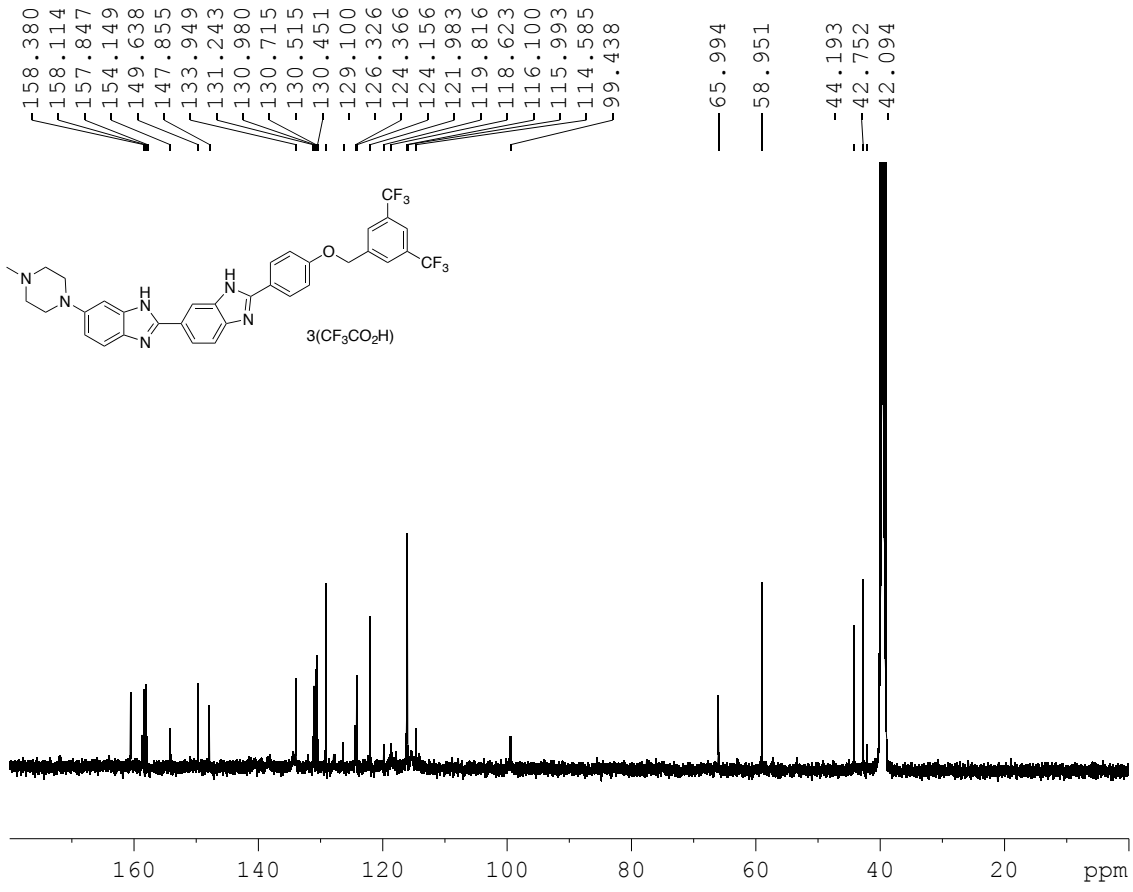
**MALDI-TOF-MS (Matrix, DHBA):** 651.23 calcd. for [$(M+H)^+$], found 651.81.

## 4. NMR and Mass Spectra of Compound 1

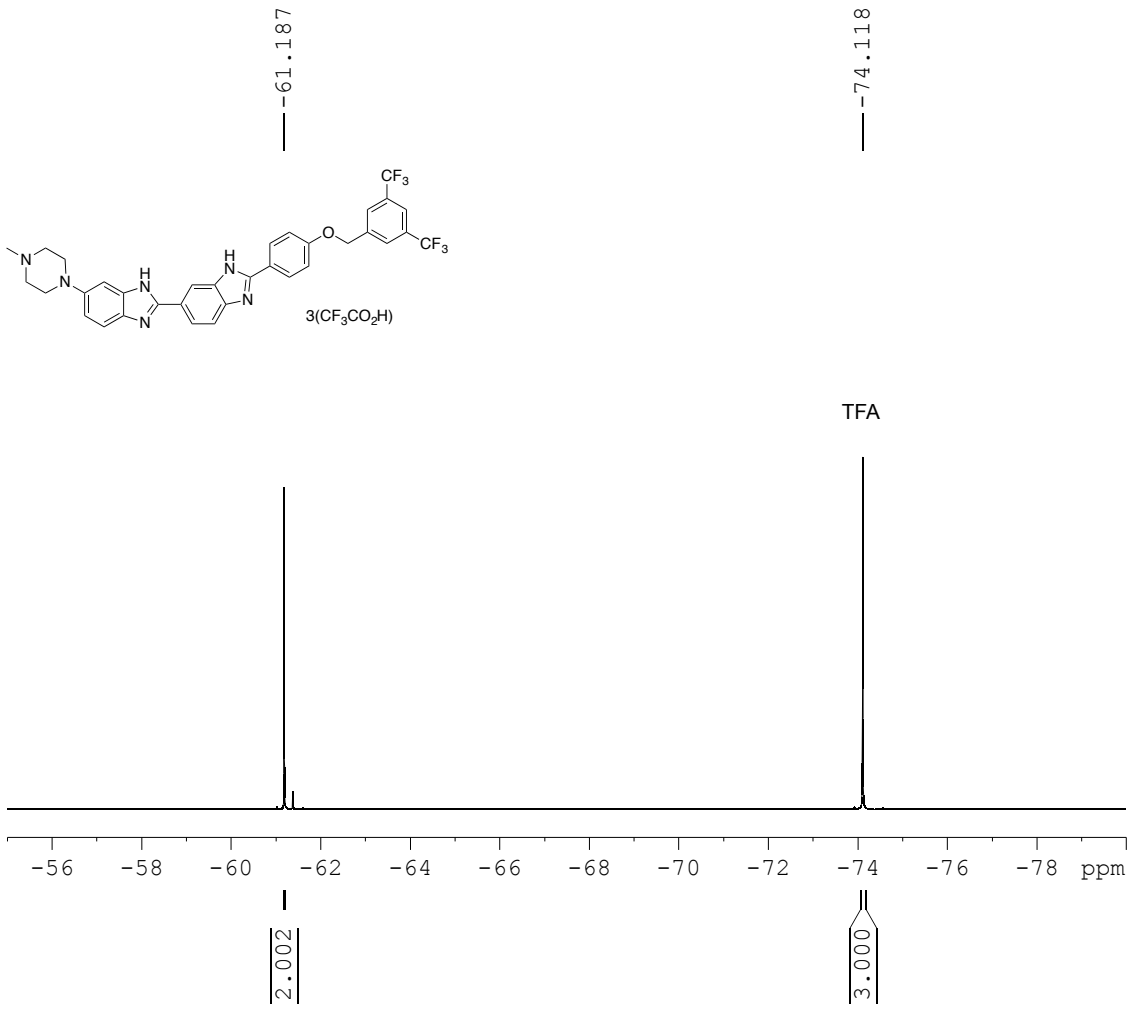
### $^1$H NMR (500 MHz in DMSO-d6)



**$^{13}$C NMR (125 MHz in DMSO-d6)**



**$^{19}$F NMR (470 MHz in DMSO-d6)**



**MALDI-TOF-MS (Matrix: DHBA)**