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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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).

**19F 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, Japan) with excitation at 345 nm.

**Cell cultivation and fluorescence microscopic analysis:** Hela cells ($2.3 \times 10^4$ / chamber) in DMEM (10% FBS) were seeded on a 4-chamber glass-bottom dish (35 mm) and incubated for 24 h in a humidified chamber (37°C, 5% CO$_2$). After washing with FluoroBrite$^\text{TM}$ DMEM (Gibco, 300 µL × 2), a solution of 1 or bisbenzimide H 33258 (10 µM in FluoroBrite$^\text{TM}$ DMEM, 300 µL) was poured to the chambers and then incubated for 10 min in a humidified chamber (37°C, 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

![Image](image_url)

**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. $[1] = [\text{hpODN-CG}] = 10 \, \mu\text{M}$ in 50 mM Tris-HCl (pH 7.6) containing 100 mM NaCl and 10% (v/v) D$_2$O. 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 I and hpODNs having different stem length. 

$[I] = [\text{hpODN}] = 10 \, \mu\text{M}$ in 50 mM Tris-HCl (pH 7.6) containing 100 mM NaCl and 10% (v/v) D$_2$O. 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$_2$CO$_3$ (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 60°C 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™ 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 60°C). Lyophilization of the corrected peak at retention time 18 min afforded 1 as yellow powder (38 mg, 38 µmol, 47%).

**1H 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)

**13C NMR (125 MHz, DMSO-d6):** δ (ppm) 160.5, 158.2 (CF$_3$CO$_2$H, 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 (CF$_3$-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

**19F NMR (470 MHz, DMSO-d6):** δ (ppm) –61.19 (6F, s, (CF$_3$)$_2$-Ph), –74.12 (9F, s, 3(CF$_3$CO$_2$H))

**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-d$_6$)

![NMR spectrum]

Chemical shifts (ppm):
- 65.94
- 58.951
- 44.193
- 42.094
- 58.951
- 44.193
- 42.094
- 158.380
- 157.847
- 158.114
- 158.380

Structure:

![Chemical structure]

3(CF$_3$CO)$_2$H
\( ^{19}\text{F NMR (470 MHz in DMSO-d6)} \)

\[
\begin{array}{c}
\text{CF}_3 \\
\text{CF}_3 \\
3\text{(CF}_3\text{CO}_2\text{H)}
\end{array}
\]

\( -74.118 \)

\( -61.187 \)

\( 3.000 \)

\( 2.002 \)

\( \text{TFA} \)

\( \text{SI} = 65536 \)

\( \text{SF} = 470.5923770 \text{ MHz} \)

\( \text{WDW} = \text{EM} \)

\( \text{SSB} = 0 \)

\( \text{LB} = 0.30 \text{ Hz} \)

\( \text{PC} = 1.00 \)

S10
MALDI-TOF-MS (Matrix: DHBA)

[(M+H)⁺]
Calcd. 651.2307
Found 651.8114