Electronic Supplementary Material (ESI) for Organic Biomolecular Chemistry

Visualization of G-quadruplexes by Using a BODIPY-Labeled Macrocyclic Heptaoxazole

Masayuki Tera,[a] Keisuke Iida,[a] Kazunori Ikebukuro,[a] Hiroyuki Seimiya,[b]
Kazuo Shin-ya,[c] and Kazuo Nagasawa[*a]

a M. Tera, K. Iida, Prof. Dr. K. Ikebukuro. Prof. Dr. K. Nagasawa
Department of Biotechnology and Life Science, Faculty of Technology
Tokyo University of Agriculture and Technology
2-24-16 Nakamachi, Koganei Tokyo 184-8588 (Japan)
Fax: (+81) 42-388-7295
E-mail: knaga@cc.tuat.ac.jp

b Dr. H. Seimiya
Cancer Chemotherapy Center
Japanese Foundation for Cancer Research

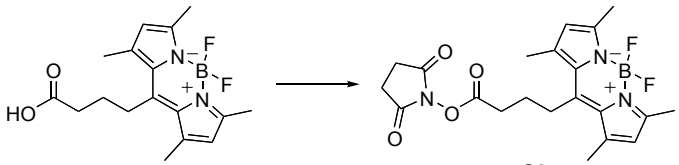
c Dr. K. Shin-ya
Biological Information Research Center
National Institute of Advanced Industrial Science and Technology

---

Table of Contents:

1. Synthesis of activated ester of BODIPY (**S2**).....2
2. HPLC chart for L1BOD-7OTD (**2**).....2
3. Copies of NMR spectra of compounds (**S2**, **2**).....3-6
4. Excitation and emission spectrum of L1BOD-7OTD (**2**).....7
5. UV melting analysis.....8
6. ESI-MS spectra.....9
7. Fluorescent polarization titration.....10

Synthesis of activated ester of BODIPY **S2**.

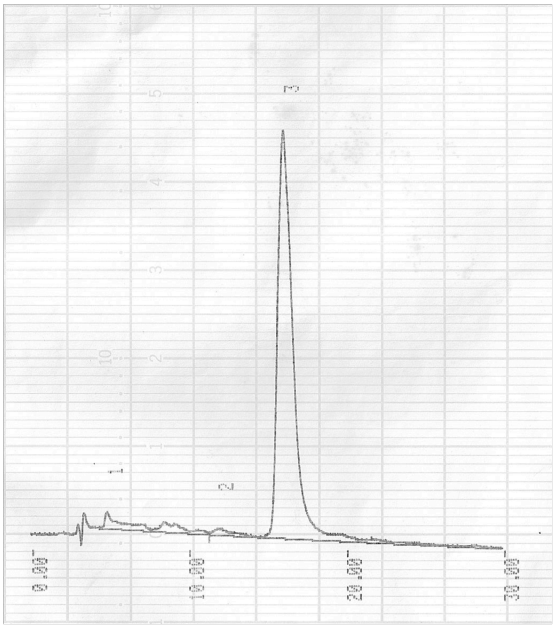


To a solution of **S1**[1] (450 mg, 1.35 mmol) in THF (10 mL), was added Et_3N (1.0 mL, 6.75 mmol), EDCI (776 mg, 6.75 mmol) N-hidroxylsuccinimide (466 mg, 4.05 mmol), and the mixture was stirred for 12 h at room temperature. To the reaction mixture was added H_2O and the organic layer was extracted with AcOEt, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was chromatographed on silica gel ($CHCl_3$-AcOEt = 20:1) to give activated ester of BODIPY **S2** as a dark red solid (284 mg, 50%). TLC R_f = 0.5 (6:1 $CHCl_3$/EtOAc)

Spectral data for **S2**: ^{1}H NMR (400 MHz, DMSO-d6) δ 6.24 (s, 2H), 3.07 (m, 2H), 2.95 (t, J = 6.9 Hz, 2H), 2.42 (s, 6H), 2.40 (s, 6H), 1.90 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d6) δ 170.2, 168.6, 153.5, 145.1, 141.1, 130.7, 121.9, 30.2, 26.6, 26.1, 25.4, 15.9, 14.1; HRMS (ESI, M+Na) calcd for $C_{21}H_{24}BF_2N_3O_4Na$ 454.1726, found 454.1715.

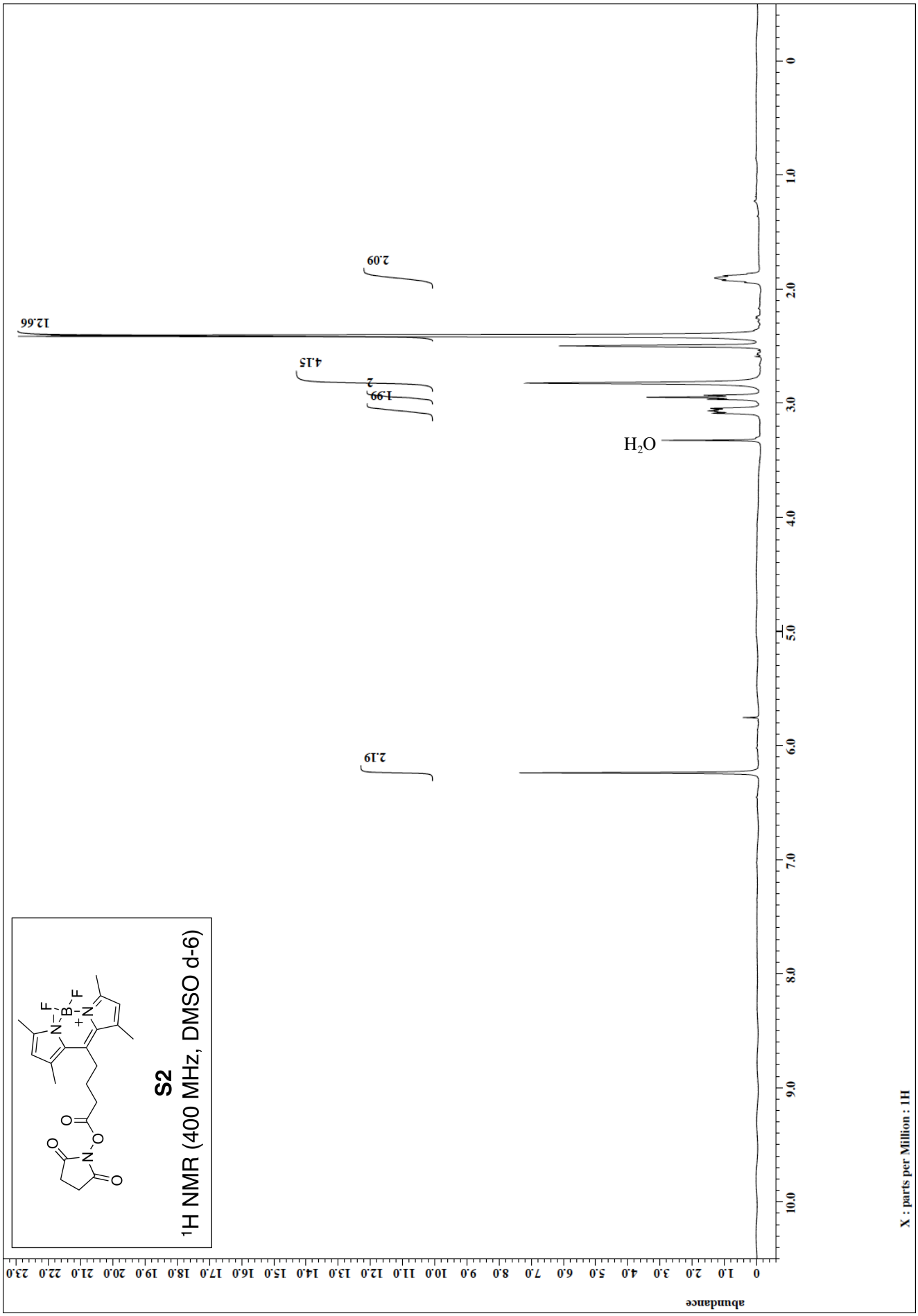
[1]Z. Li, E. Mintzer, R. Bittman, *J. Org. Chem*. 2006, **71**, 1718-1721.

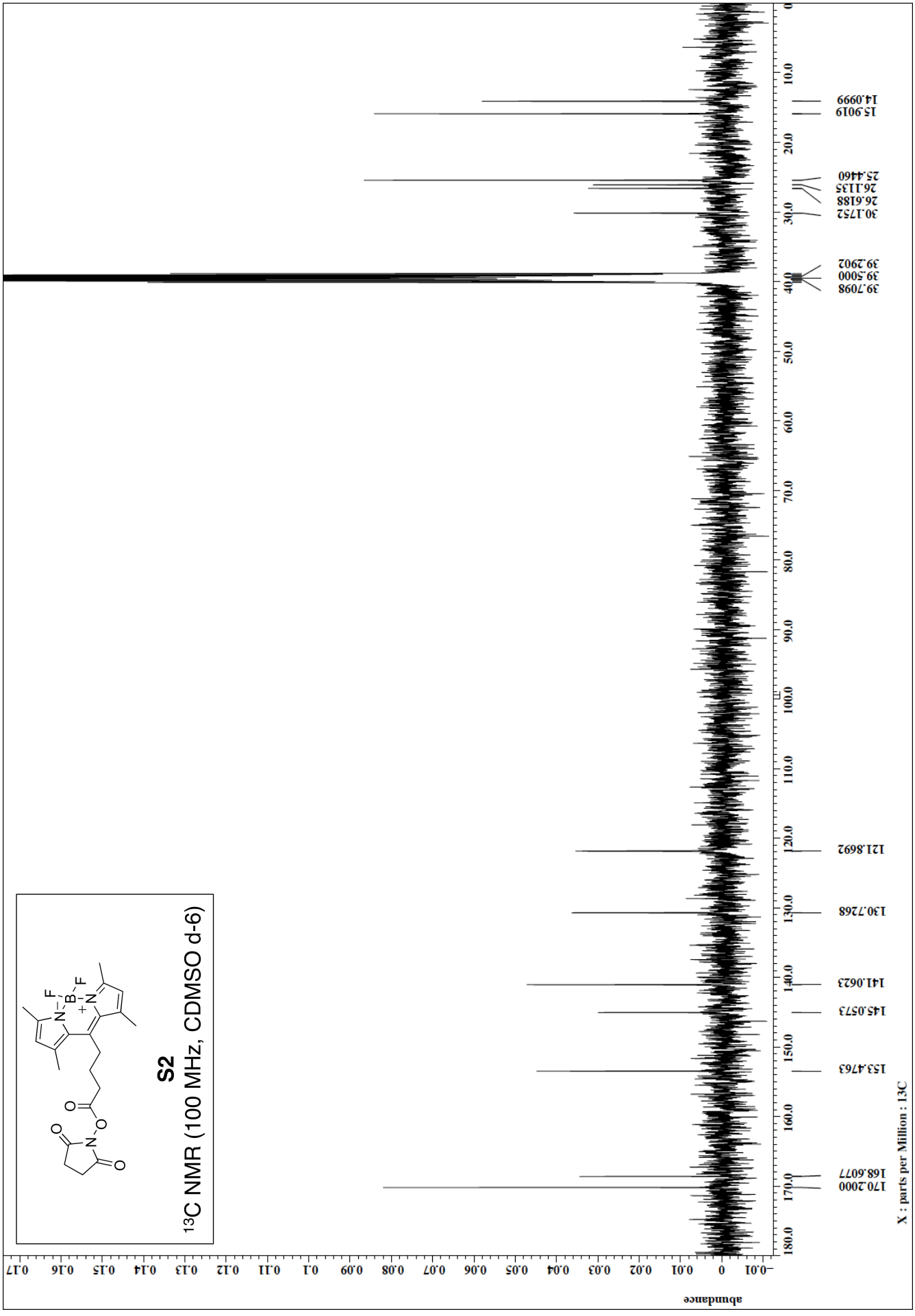
HPLC chart of L1BOD-7OTD (**2**).

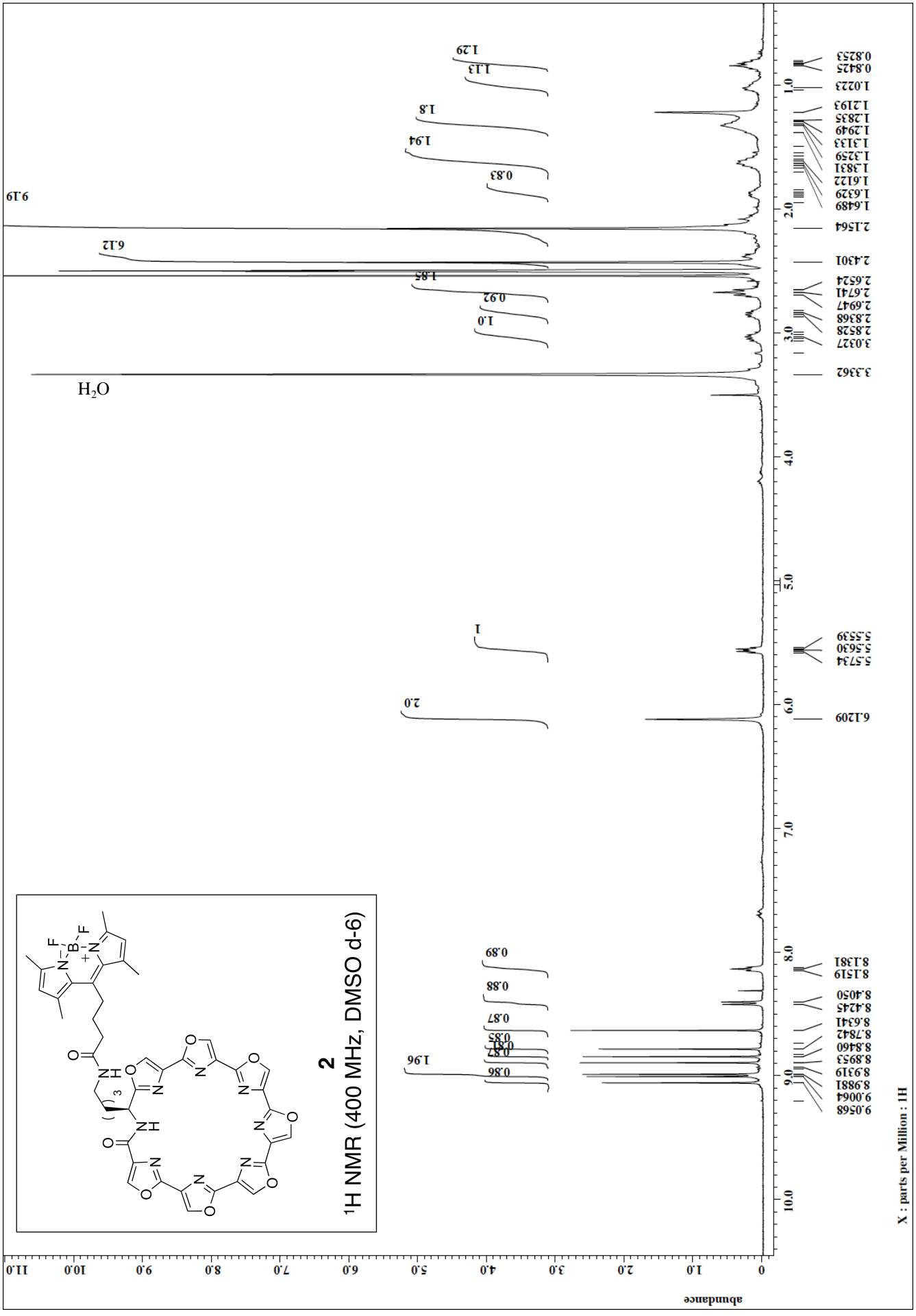


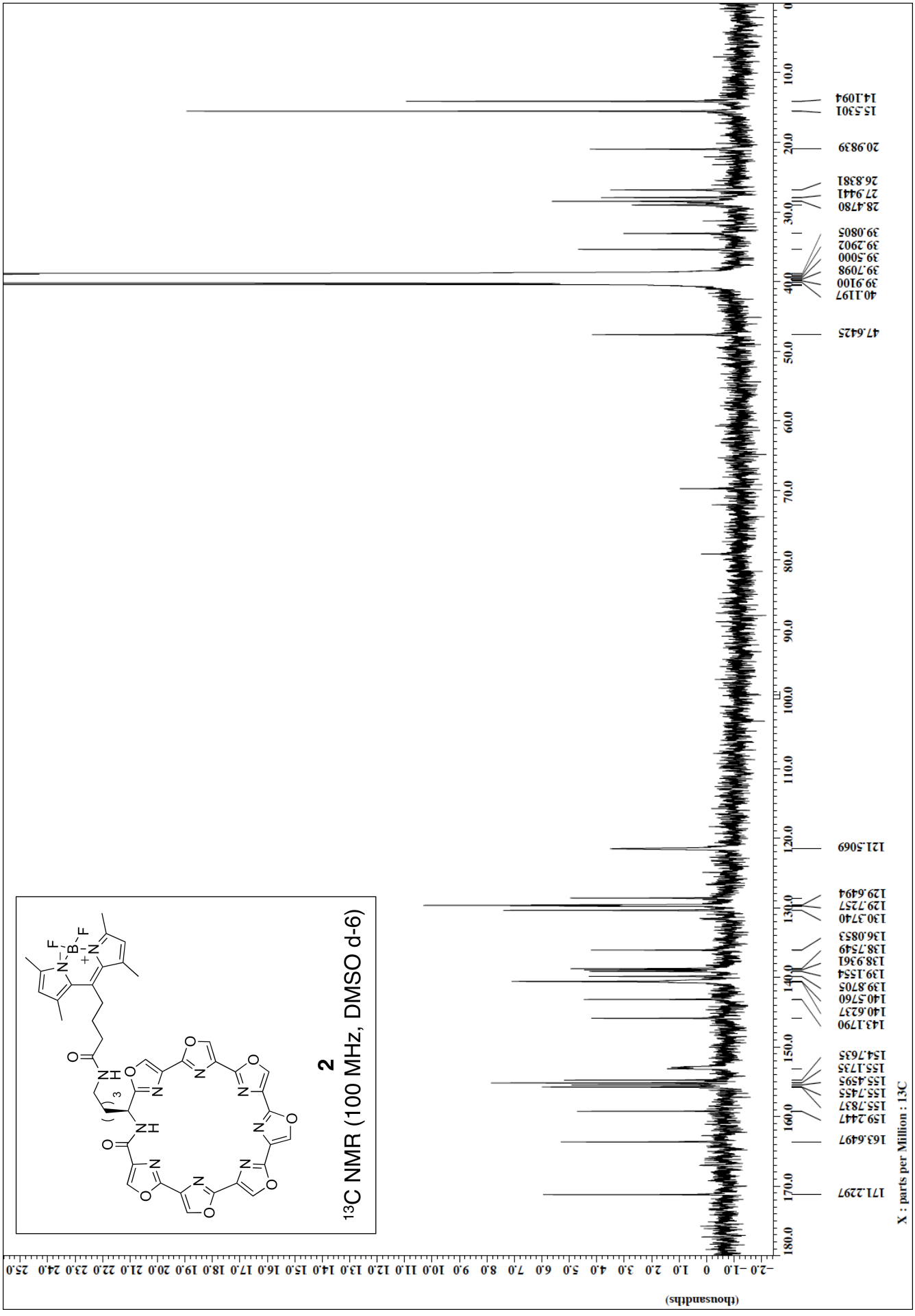
No'	Retention Time (min)	Peak Area (%)
1	4.861	6.81
2	11.885	1.66
3	16.081	91.53

Copies of NMR spectra of compounds **S2** and **2**.




S2
^{13}C NMR (100 MHz, CDMSO d-6)
X : parts per Million : 13C
abundance
170.2000
168.6077
153.4763
145.0573
141.0623
130.7268
121.8692
39.7098
39.5000
39.2902
30.1752
26.6188
26.1135
25.4460
15.9019
14.0999


2
^{1}H NMR (400 MHz, DMSO d-6)
H_2O
abundance
X : parts per Million : 1H


2
13C NMR (100 MHz, DMSO d-6)
X : parts per Million : 13C
(thousandths)
171.2297
163.6497
159.2447
155.7837
155.7455
155.4595
155.1735
154.7635
143.1790
140.6237
140.5760
139.8705
139.1554
138.9361
138.7549
136.0853
130.3740
129.7257
129.6494
121.5069
47.6425
40.1197
39.9100
39.7098
39.5000
39.2902
39.0805
28.4780
27.9441
26.8381
20.9839
15.5301
14.1094

Excitation and emission spectrum of L1BOD-7OTD (**2**)

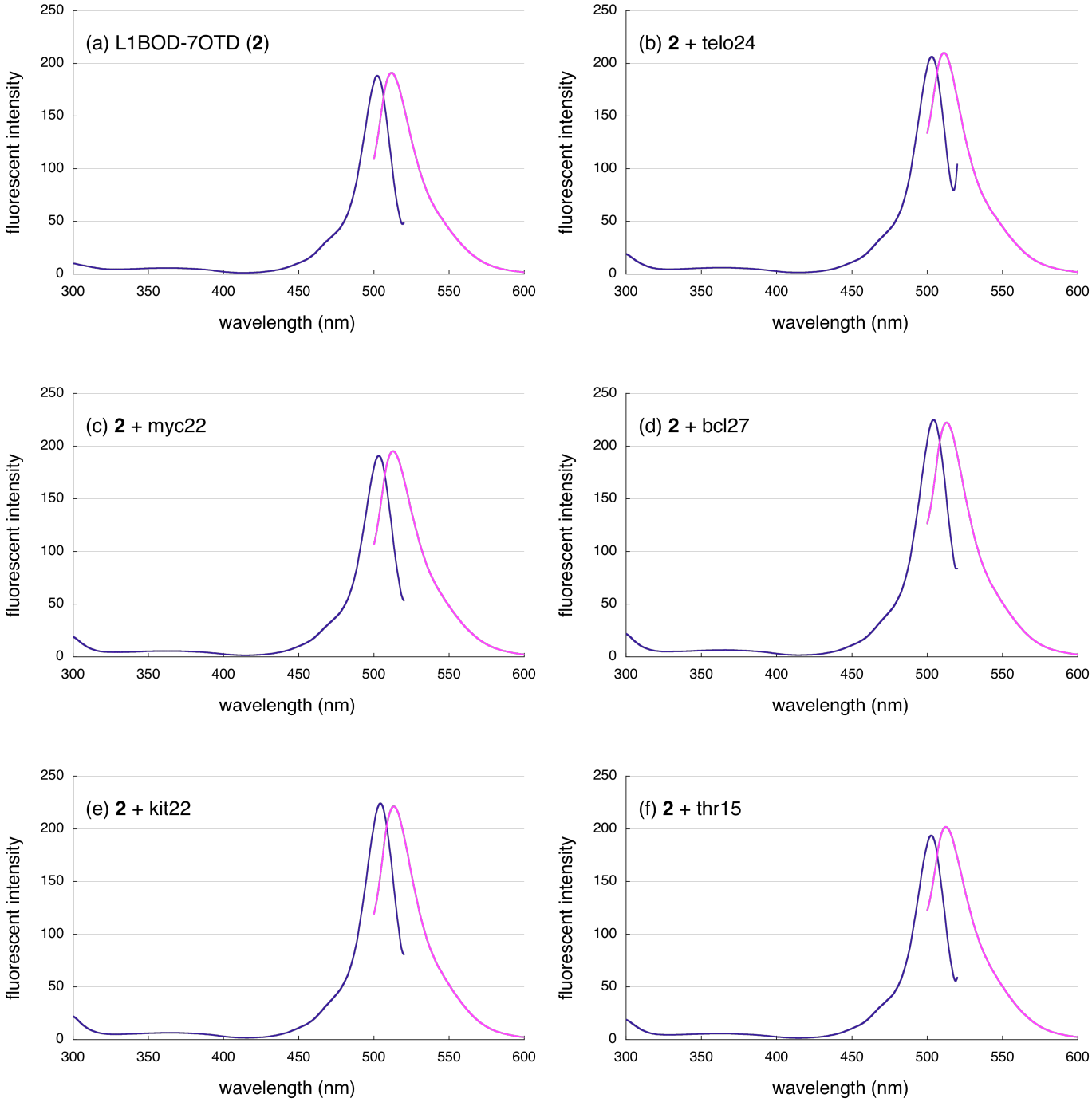


Fig. S1 Excitation and emission spectra of **2** (0.7 μM) in the absence (a) or presence (b-f) of GFOs (4 μM). All samples were diluted by 10 mM Tris-HCl buffer, pH7.3. The spectra are representative of three averaged scans taken at 25 ºC.

UV melting analysis of L1BOD-7OTD (**2**) in the presence of double-stranded DNA.

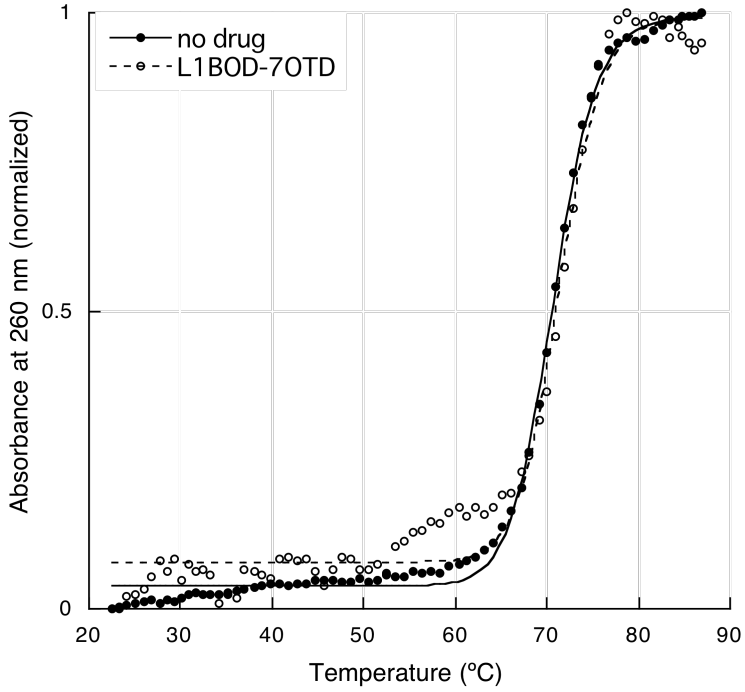


Fig. S2 UV melting curves of double-stranded DNA (salmon sperm DNA) in the absence and presence of L1BOD-7OTD (**2**). The salmon sperm DNA was dissolved in Tris-HCl buffer (10 mM, pH 7.2) containing 50 mM KCl. L1BOD-7OTD was dissolved in DMSO. The salmon sperm DNA was used at a base pair concentration of 15 μM and L1BOD-7OTD also used the same concentration. The melting curves were acquired on a Beckman Coulter UV/Visible spectrophotometer (DU 800) using quarts cell of 10-mm optical path length. The heating rate was 1.0 ºC/min and the samples were allowed to equilibrate for 1.0 min at each temperature setting.

ESI-MS spectra of GFOs (telo24, myc22, bcl27, kit22 and thr15)

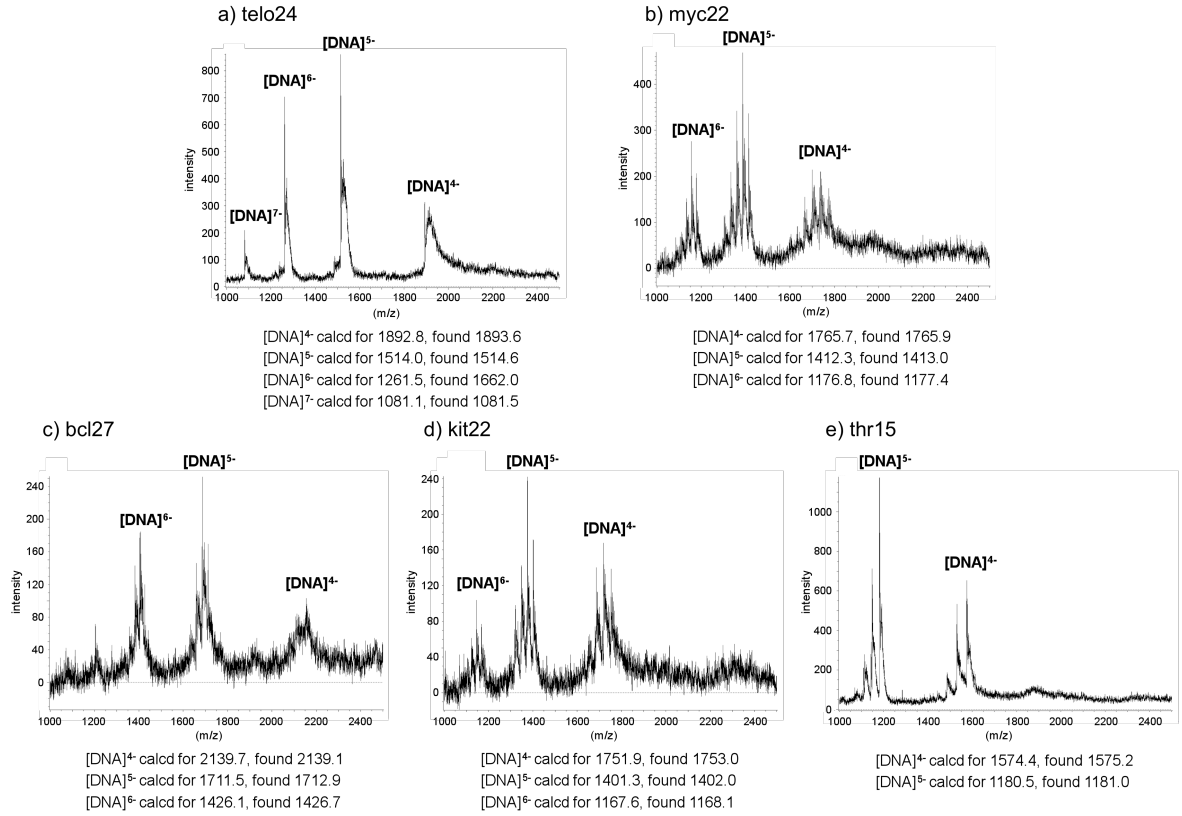


Fig. S3 ESI-MS spectra of 10 μM GFOs without L1BOD-7OTD: (a) telo24, (b) myc22, (c) bcl27, (d) kit22 and (e) thr15. The measurement conditions and the sample preparation procedures were as follows: capillary needle voltage, -2.0 kV; ring lens voltage, -15 V; orifice 1 voltage, -75 V; orifice 2 voltage, 0 V; orifice 1 temperature, 80 °C; desolvation temperature, 80 °C; sample flow rate, 5 μL/min; All experiments were performed in 20 mM NH_4OAc containing 10 μM of GFOs. Methanol (10%) was added just before injection. The role of methanol is to increase ion signals.

Fluorescent polarization (FP) titration using telomut24 and ds-telo24.

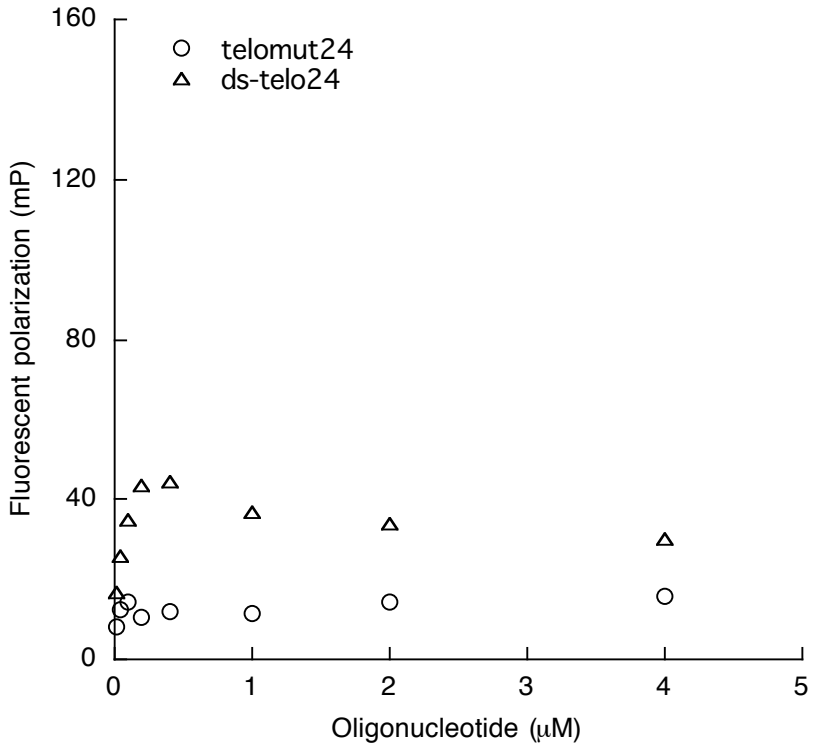


Fig. S4 FP titrations of telomut24 and ds-telo24. L1BOD-7OTD (**2**) (0.7 μM) was incubated with various concentrations (0.02-4 μM) of the oligonucleotides (open circle: telomut24, open triangle: ds-telo24) at 25 ºC for 12 h in the presence of 50 mM KCl, 5 mM Tris-HCl, pH 7.0 and 50% DMSO (v/v). The polarization associated with the emission of **2** was measured at 530 nm (excitation at 500 nm). The fluorescent polarization values are representative of five scans taken at 25ºC. All plots result from duplicate assays.