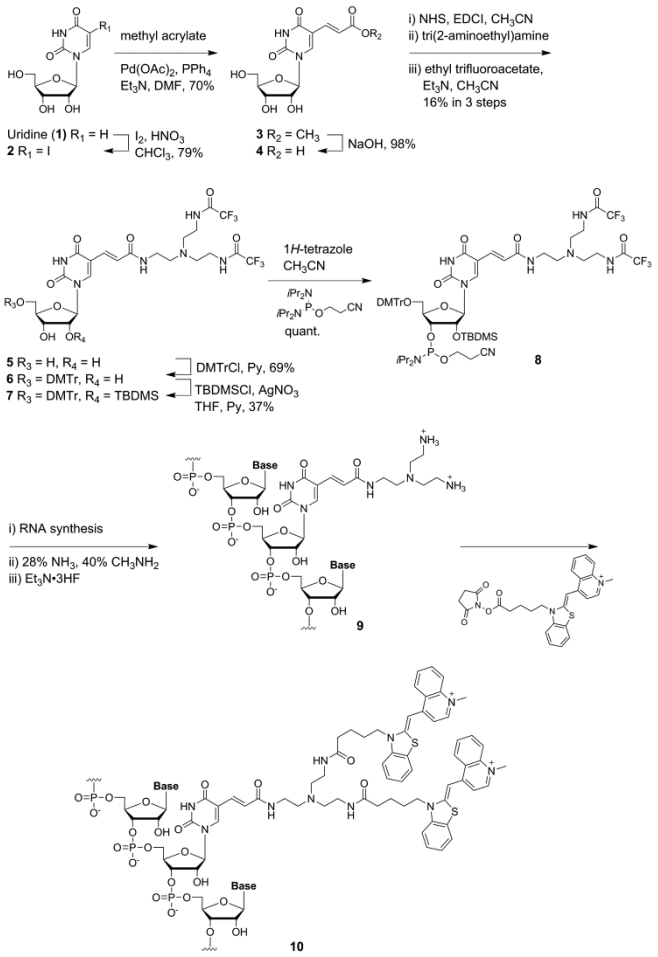
Electronic Supplementary Material (ESI) for ChemComm.

Fluorescence-switching RNA for detection of bacterial ribosome

Lihao Guo[a,b] and Akimitsu Okamoto[a,c,*]

Supporting Information

Table of Contents



Scheme S1. Synthesis of an exciton-controlled hybridization-sensitive fluorescent RNA.

Experimental Procedures

General. All chemicals were purchased from Sigma-Aldrich, Wako chemicals, and Tokyo Chemical Industry. ^{1}H and ^{13}C NMR spectra were measured with a Bruker Avance 600 (600 MHz). Electrospray ionization mass spectra ESI-MS spectra were recorded by a Bruker microTOF II-NAC. MALDI-TOF mass spectra were recorded by a Bruker microflex-NAC. RNA was synthesized on a NTS H-8 DNA/RNA synthesizer (Nihon Techno Service). RNA oligonucleotides were purified by HPLC system composed by GILSON Inc. and JASCO Inc. modules. Absorption and fluorescence spectra were recorded on Shimadzu UV-2550 spectrophotometer and RF-5300PC spectrofluorophotometer, respectively.

Compound 2. In a round bottom flask, uridine (**1**) (2.0 g, 8.2 mmol) and iodine powder (2.3 g, 9.0 mmol) were dissolved in a mixture of chloroform (110 mL) and 1 M nitric acid (20 mL). The reaction was heated at reflux (80 ^{o}C) for 5h. The reaction progress was monitored by TLC (30 % methanol in chloroform). After cooling of the reaction mixture to 0 ^{o}C, the precipitate was collected by filtration and dried under vacuum overnight to provide **2** as colorless needles (2.4 g, 79%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.70 (s, 1H), 8.50 (s, 1H), 5.72 (d, 1H, J = 4.4 Hz), 4.03 (t, 1H, J = 4.7 Hz), 3.99 (t, 1H, J = 4.4 Hz), 3.87 (m, 1H), 3.70 (q, 1H, J = 12.1 Hz), 3.59 (q, 1H, J = 12.0 Hz); ^{13}C NMR (150 MHz, DMSO-d_6) δ 161.3, 151.2, 146.0, 89.2, 85.6, 74.8, 70.3, 70.1, 61.1; ESI-MS $[M-H]^-$ $C_9H_{10}IN_2O_6$ 368.9584 (calcd.), 368.9589 (found).

Compound 3. To a solution of **2** (2.0 g, 5.6 mmol) in *N*,*N*-dimethylformamide (30 mL) was added palladium(II) acetate (60.0 mg, 0.36 mmol), triphenylphosphine (148.0 mg, 0.76 mmol), triethylamine (1.0 mL, 7.2 mmol) and methyl acrylate (0.9 mL, 10.8 mmol). The mixture was heated at 100 ^{o}C for 4 h. The mixture was filtered and the filtrate was concentrated *in vacuo*. The residue was recrystallized using methanol to afford **3** as a white solid (1.3 g, 70%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.68 (s, 1H), 8.53 (s, 1H), 7.34 (t, 1H, J =8.5 Hz), 6.85 (t, 1H, J = 8.5 Hz), 5.78 (s, 1H), 5.49 (s, 1H), 5.35 (s, 1H), 5.10 (s, 1H), 4.09 (s, 1H), 4.03 (s, 1H), 3.89 (s, 1H), 3.74 (s, 1H), 3.68 (s, 3H), 3.63 (s, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 168.0, 162.6, 150.3, 144.8, 138.7, 117.1, 109.1, 89.5, 85.5, 74.8, 69.9, 61.0, 52.1; ESI-MS $[M-H]^-$ $C_{13}H_5N_2O_8$ 327.0828 (calcd.), 327.0821 (found).

Compound 4. To 3.0 M sodium hydroxide in 1:1 water/ethanol (12 mL) was added **3** (1.3 g, 4.0 mmol) and this mixture was stirred at room temperature for 3 h. 1M HCl was poured into the solution on cooling ice bath to give white precipitate. The precipitate was filtered and washed with hexane. The product was dried *in vacuo* to yield white powder (1.2 g, 98%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.64 (s, 1H), 8.51 (s, 1H), 7.30 (d, 1H, J = 15.8 Hz), 6.79 (d, 1H, J = 15.8 Hz), 5.79 (d, 1H, J = 4.1 Hz), 4.11 (d, 1H, J = 4.4 Hz), 4.06 (t, 1H, J = 4.7 Hz), 3.90 (s, 1H), 3.75 (d, 1H, J = 11.7 Hz), 3.63 (d, 1H, J = 10.0 Hz); ^{13}C NMR (150 MHz, DMSO-d_6) δ 168.9, 162.6, 150.4, 144.3, 138.1, 118.7, 109.3, 89.5, 85.6 74.9, 70.0, 61.0; ESI-MS $[M-H]^-$ $C_{12}H_{13}N_2O_8$ 313.0672 (calcd.), 313.0677 (found).

Compound 5. *N*-Hydroxysuccinimide (732.6 mg, 6.4 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.2 g, 6.4 mmol) were added to a solution of **4** (1.0 g, 3.2 mmol) in acetonitrile (80 mL) and stirred at 25 ^{o}C for 24 h. Tris(2-aminoethyl)amine (4.8 mL, 32.0 mmol) was added to the suspension and was stirred at 25 ^{o}C for 2 h. Ethyl trifluoroacetate (19.0 mL, 160 mmol) and triethylamine (22.3 mL, 160 mmol) were added to the suspension and stirred at 25 ^{o}C for 48 h. The mixture was concentrated *in vacuo* and purified by silica gel column chromatography (5%−10% methanol/dichloromethane). The fraction containing **5** was concentrated *in vacuo*. The residue was dissolved in a small amount of acetone. A white powder precipitated from the mixture upon the addition of diethyl ether. The precipitate was filtered and washed with ether and then dried *in vacuo* to give **5** as a colorless oil (527.1 mg, 26%). ^{1}H NMR (600 MHz, CD_3OD) δ 8.49 (s, 1H), 7.26 (d, 1H, J = 15.6 Hz), 7.10 (d, 1H, J = 15.6 Hz), 5.95 (s, 1H), 4.23 (s, 2H), 4.07 (s, 1H), 3.96 (d, 1H, J = 12.0 Hz), 3.82 (d, 1H, J = 12.0 Hz), 3.38 (d, 6H, J = 6.2 Hz), 2.74 (t, 6H, J = 6.2 Hz); ^{13}C NMR (150 MHz, CD_3OD) δ 168.3, 162.7, 158.3, 158.0, 150.4, 142.9, 133.2, 121.1, 117.5, 115.6, 110.0, 90.1, 85.4, 75.1, 69.9, 60.9, 53.4, 52.9, 46.8, 38.0, 37.7, 8.3. ESI-MS $[M-H]^-$ $C_{22}H_{27}F_6N_6O_9$ 633.1744 (calcd.), 633.1737 (found).

Compound 6. A solution of **5** (508.0 mg, 0.8 mmol) and 4,4'-dimethoxytrityl chloride (101.7 mg, 0.3 mmol) in pyridine (20 mL) was stirred at 25 ^{o}C for 16 h. Water (2 mL) was added to the reaction mixture and then the mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (5% methanol and 1% trimethylamine in dichloromethane). The fraction containing **6** was then concentrated. Saturated aqueous sodium bicarbonate was added to the residue, and then extracted with ethyl acetate, washed with brine, and dried *in vacuo* to give **6** as a white foam (516.7 mg, 69%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.69 (s, 1H), 9.33 (s, 1H), 7.96 (s, 1H), 7.40−6.89 (15H), 5.80 (s, 1H), 5.50 (s, 1H), 5.14 (s, 1H), 4.18 (s, 1H), 4.02 (s, 1H), 3.95 (s, 1H), 3.72 (s, 6H), 3.61 (s, 1H), 2.10 (m, 12H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 166.5, 162.6, 158.9, 157.3, 157.1, 150.3, 145.6, 144.1, 136.5, 136.4, 133.0, 130.6, 130.5, 128.6,

128.6, 127.5, 127.2, 122.9, 119.6, 117.8, 115.8, 114.1, 110.5, 90.2, 86.4, 83.6, 73.5, 70.7, 64.8, 55.8, 55.8, 53.8, 52.9, 38.3, 37.8, 31.5; ESI-MS $[M-H]^-$ $C_{43}H_{45}F_6N_6O_{11}$ 935.3051 (calcd.), 935.3049 (found).

Compound 7. A solution of **6** (468.0 mg, 0.5 mmol), silver nitrate (93.4 mg, 0.6 mmol) in pyridine (201 μL) and THF (4 mL) was stirred at 25 °C for 1 h, and then *tert*-butyldimethylsilyl chloride (82.9 mg, 0.6 mmol) was added. The solution was stirred at 25 °C for 24 h. The reaction mixture was concentrated *in vacuo* and purified by silica gel column chromatography (2% methanol and 1% trimethylamine in dichloromethane). The fraction containing **7** was concentrated. Saturated aqueous sodium bicarbonate was added to the residue, and then extracted with ethyl acetate, washed with brine, and dried *in vacuo* to give **7** as a white foam (194.3 mg, 37%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.98 (s, 1H), 7.87 (s, 1H), 7.48−6.53 (15H), 6.00 (s, 1H), 5.37 (s, 1H), 4.43(s, 1H), 4.16 (s, 2H), 3.79 (s, 6H), 3.67 (s, 1H), 3.62 (s, 1H), 3.35−2.07 (12H), 0.92 (9H), 0.15 (3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 167.0, 161.7, 159.2, 159.1, 158.4, 158.2, 149.6, 145.0, 140.0, 135.9, 135.8, 132.1, 130.4, 130.3, 128.6, 128.3, 127.6, 122.2, 117.2, 115.3, 113.9, 111.1, 89.6, 87.2, 84.3, 76.3, 71.2, 71.0, 63.67, 60.8, 55.7, 55.1, 53.5, 38.1, 26.0, 18.4; ESI-MS $[M-H]^-$ $C_{49}H_{59}F_6N_6O_{11}Si$ 1049.3915 (calcd.), 1049.3908 (found).

Compound 8. Acetonitrile (1 mL) and 2-cyanoethyl *N*,*N*,*N*',*N*'-tetraisopropylphosphordiamidite (155 μL, 0.49 mmol) were added to a mixture of **7** (171 mg, 0.16 mmol) and 1*H*-tetrazole (23 mg, 0.33 mmol) dried in a round-bottom flask. The mixture was stirred at 25 °C for 2 h. After identification of the end of the reaction by TLC, the reaction mixture was passed through a 0.45 μm filter and was used for automated RNA synthesis without further purification.

Succinimidylation of TO dyes.[21] *N*-Hydroxysuccinimide (4.6 mg, 40 μmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (7.7 mg, 40 μmol) were added to a solution of TO dye (20 μmol) in DMF (0.50 mL) and stirred at 25 °C for 16 h. The reaction mixture was used for the next reaction with RNA oligomers without further purification.

Synthesis of fluorescent RNA. RNA oligomers were synthesized by a conventional phosphoramidite method from **8** and commercially available A, G, C, and U phosphoramidites (Glen Research) using a NTS H-8 DNA/RNA synthesizer. The synthesized RNA oligomer was cleaved from the support by the hydrolysis in a solution of 1:1 (*v*/*v*) 28% aqueous ammonia/40% methylamine for 20 min, and then synthetic oligonucleotides were deprotected at 65 °C for 5 min. After concentration of the solution *in vacuo*, 2' silyl groups were removed with triethylamine trihydrofluoride at 65 °C for 2 h. After diluting with distilled water, RNA was purified by reversed-phase HPLC on a 5-ODS-H column (10 mm × 150 mm, elution with a solvent mixture of 0.1 M triethylammonium acetate (TEAA), pH 7.0, linear gradient over 30 min from 5% to 30% acetonitrile at a flow rate of 3.0 mL/min).

A solution of the succinimidyl ester of TO dye (100 *eq*. to an active amino group of RNA) in *N*,*N*-dimethylformamide was added to a solution of diamino-modified RNA in 100 mM sodium carbonate buffer (pH 9.0), and incubated at 25 °C for 1.5 h. The reaction mixture was diluted with ethanol. After centrifuging at −20 °C for 20 min, the supernatant was removed. The residue was dissolved in a small volume of water and the solution was passed through a 0.45 um filter. The product was purified by reversed-phase HPLC on a 5-ODS-H column (10 mm × 150 mm, elution with a solvent mixture of 0.1 M TEAA, pH 7.0, linear gradient over 30 min from 5% to 30% acetonitrile at a flow rate of 3.0 mL/min). The dye incorporation yields were about 30%. The concentration of the fluorescent RNA was determined at Nanodrop 1000 spectrophotometer. The fluorescent RNA was identified by MALDI–TOF mass spectrometry.

Absorption, fluorescence and circular dichroism spectra measurements. Absorption, fluorescence and circular dichroism spectra of the fluorescent probes of the fluorescent RNA samples (1−2.5 μM) were measured in 50 mM sodium phosphate buffer (pH 7.0) containing 100 mM sodium chloride using a cell with a 1 cm path length. The excitation and emission bandwidths were 1.5 nm.

Melting temperature (T_m) measurements. The T_m values of duplexes (2.5 μM) were measured in 50 mM sodium phosphate buffers (pH 7.0) containing 100 mM sodium chloride. The absorbance of the samples was monitored at 260 nm from 10 to 90 °C with a heating rate of 0.5 °C /min. From these absorbance profiles, the first derivatives were calculated to determine the value of T_m.

***E. coli* culture.** To test the relationship between fluorescent intensity of NeoAp1 and OD_{600} of *E. coli*, *E. coli* cells were cultured at 37 °C in LB medium. LB medium was purchased from Becton, Dickinson and Company and autoclaved at 120 °C for 20 min before use. A stock solution containing 40 nM NeoAp1, 400 μM neomycin B and 2× RNAsecure™ (Thermo Fisher Scientific) in 50 mM potassium phosphate buffer (pH 6.2) containing 100 mM potassium chloride was made in advance. The stock solution (20 μL) and the culturing solution with different OD_{600} (20 μL) were mixed for fluorescence intensity measurement. The excitation and emission bandwidths were 3 nm.

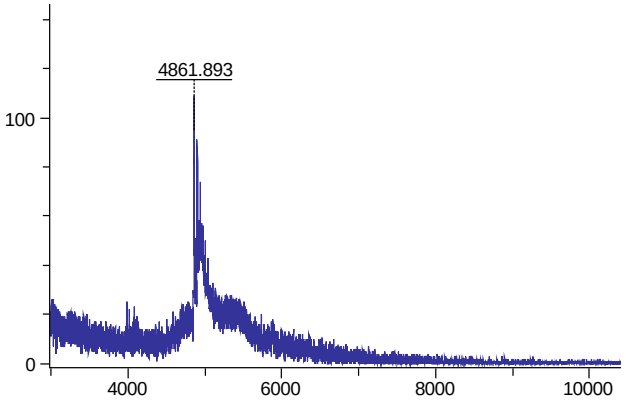
Bacteria population assessment. The experiment was performed in a 200 mL glass flask with synthetic food waste. The synthetic food waste was a mixture of fish food (Japan Pet Design Co., Ltd., Tokyo) and LB medium with a final solid content of approximately 50% (*w*/*w*). *E. coli* was cultured in the synthetic food waste at 4, 26, and 40 °C without shaking. 20 μL NeoAp1 stock solution (40 nM NeoAp1, 400 μM neomycin B and 2-times-diluted RNAsecure™ (Thermo Fisher Scientific) in 50 mM potassium phosphate buffer (pH 6.2) containing 100 mM potassium chloride) and 20 μL culturing slurry at different time points were mixed for fluorescence intensity measurements. The excitation and emission bandwidths were 3 nm.

Table S1. T_ms of artificial RNA-RNA duplexes.[a]

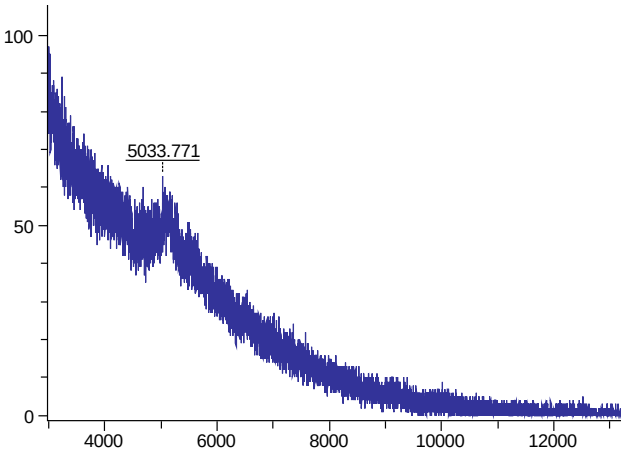
Duplexes	T_m (°C)
5′-UUUUUUDUUUUUU-3′ / 5′-AAAAAAAAAAAAA-3′ (ON1/ON1′)	41
5′-UUUUUUUUUUUUU-3′ / 5′-AAAAAAAAAAAAA-3′	27
5′-CGCAAUDUAACGC-3′ / 5′-GCGUUAAAUUGCG-3′ (ON2/ON2′)	55
5′-CGCAAUUUAACGC-3′ / 5′-GCGUUAAAUUGCG-3′	50
5′-UGAAGGGCUUDUGAACUCUG-3′ / 5′-CAGAGUUCAAAAGCCCUUCA-3′ (ON3/ON3′)	79
5′-UGAAGGGCUUUUGAACUCUG-3′ / 5′-CAGAGUUCAAAAGCCCUUCA-3′	76

[a] The T_m values of duplexes (2.5 μM) were measured in 50 mM sodium phosphate buffers (pH 7.0) containing 100 mM sodium chloride.

a ON1



b ON2



c ON3

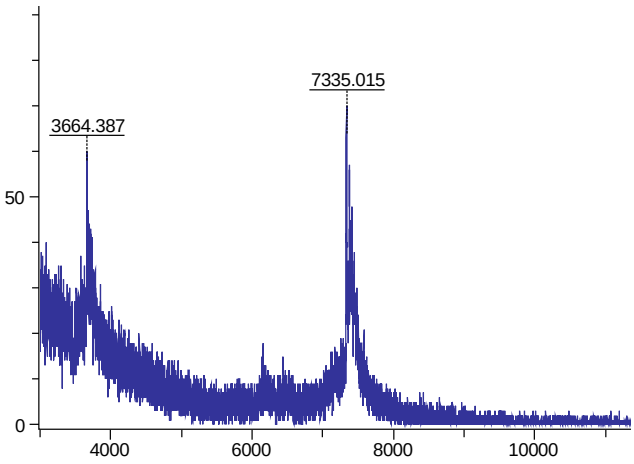


Figure S1. MALDI-TOF spectra of exciton-controlled hybridization-sensitive fluorescent RNA strands. $[M-H]^-$ (calcd.) (**a**) ON1 4861.4; (**b**) ON2 5033.8; (**c**) ON3 7334.1.

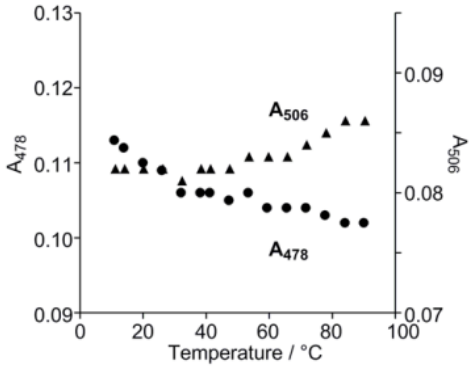


Figure S2. Changes of the absorbance at 478 and 506 nm of single-stranded ON1 at different temperatures. Spectra of ON1 (1 μM) were measured in 50 mM sodium phosphate (pH 7.0) containing 100 mM sodium chloride.

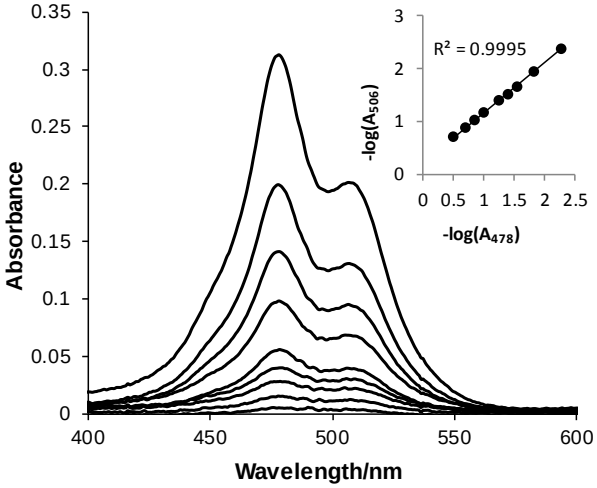


Figure S3. Absorption spectra of single-strand ON1 under different concentrations. Spectra of ON1 (0.5, 0.75, 1.0, 1.2, 1.5, 2.0, 2.5, 3.0, and 4.0 μM) were measured in 50mM sodium phosphate (pH 7.0) containing 100 mM sodium chloride at 25 °C. Inset: plot of $-\log(A_{506})$ against $-\log(A_{478})$.

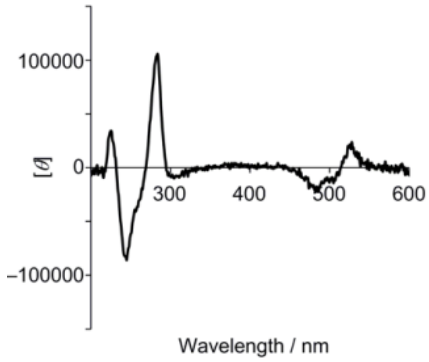


Figure S4. Circular dichroism spectrum of an ON1/ON1′ duplex. The duplex (2.5 μM) was measured in 50 mM sodium phosphate (pH 7.0) containing 100 mM sodium chloride at 25 °C. [θ] is shown in deg cm^2 dmol^{-1}.

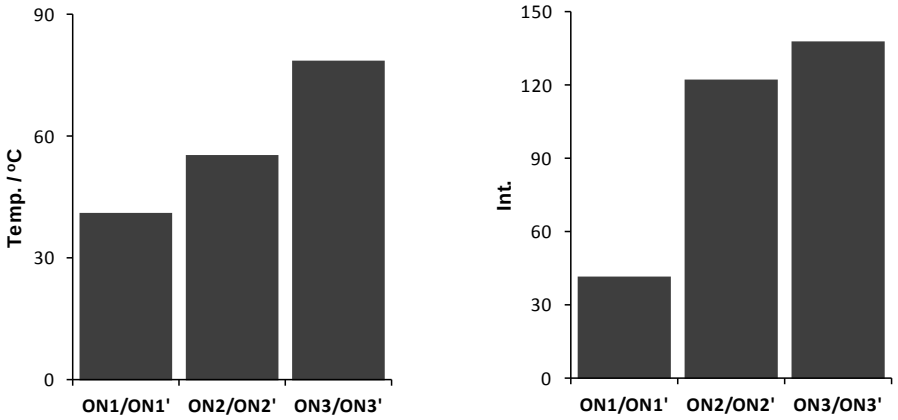


Figure S5. T_m values (left) and maximum fluorescent intensity (right) of ON1/ON1′, ON2/ON2′ and ON3/ON3′. The T_m values of the duplexes (1 μM, final duplex concentration) were measured in 50 mM sodium phosphate (pH 7.0) containing 100 mM sodium chloride. The absorbance of the samples was monitored at 260 nm from 10 to 90 °C with a heating rate of 0.5 °C/min. The data for maximum fluorescent intensities come from Figures 2, S6 and S7.

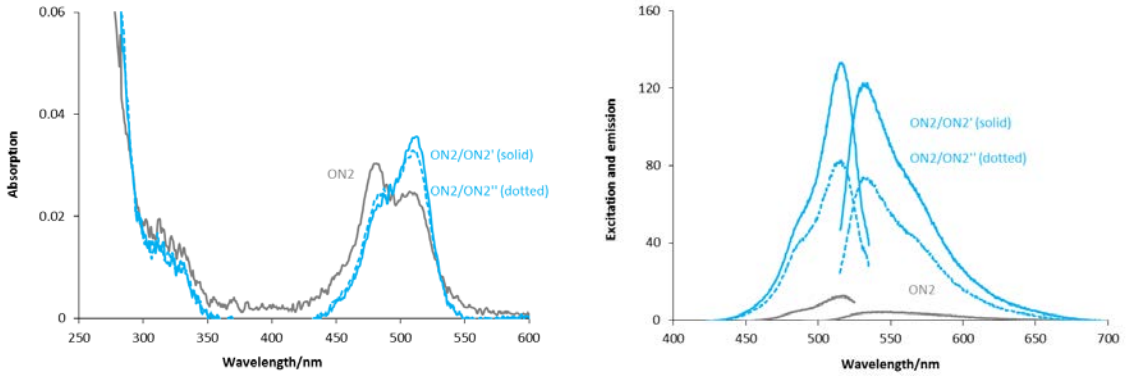


Figure S6. Photochemical behavior of ON2. (left) Absorption spectra; (right) Excitation and emission spectra. Spectra of ON2 (0.5 μM) were measured in 50 mM sodium phosphate (pH 7.0) containing 100 mM sodium chloride at 25 °C. Emission spectra were measured on excitation at 510 nm. Excitation spectra were measured for emission at 530 nm.

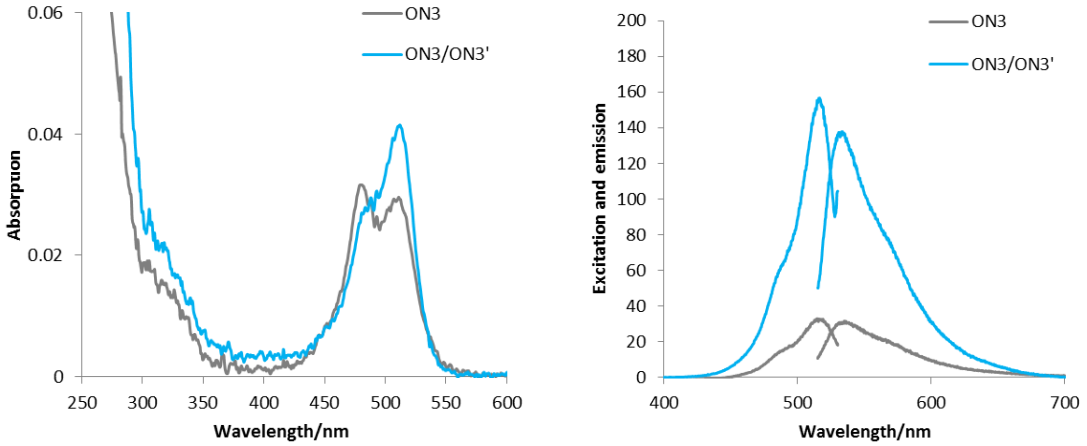
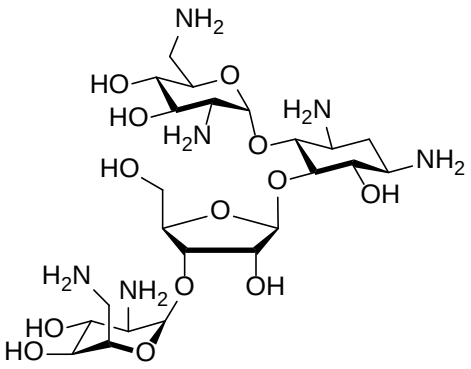
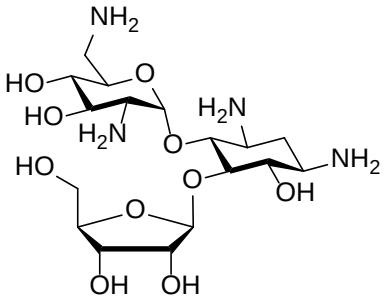


Figure S7. Photochemical behavior of ON3. (left) Absorption spectra; (right) Excitation and emission spectra. Spectra of ON3 (0.5 μM) were measured in 50 mM sodium phosphate (pH 7.0) containing 100 mM sodium chloride at 25 °C. Emission spectra were measured on excitation at 510 nm. Excitation spectra were measured for emission at 530 nm.

a



Neomycin B



Ribostamycin

b

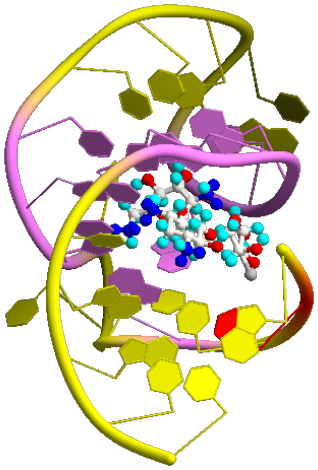
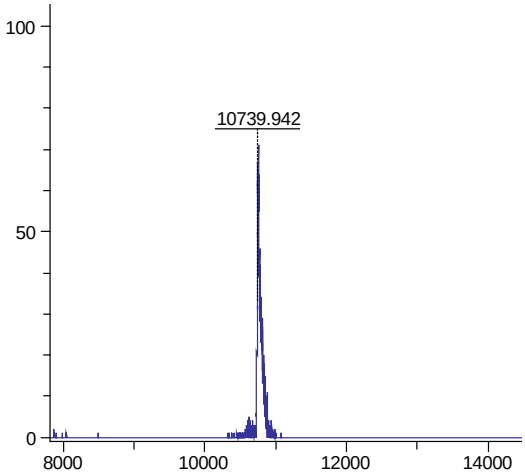
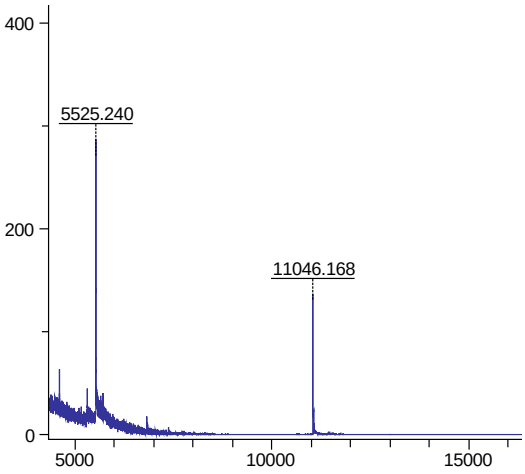


Figure S8. Aminoglycoside antibiotics neomycin B and ribostamycin. (**a**) Structures of neomycin B and ribostamycin; (**b**) Crystal structure of engineered neomycin B RNA aptamer binding with ribostamycin (PDB 2KXM). The interaction bases with ribostamycin are highlighted in purple, and the base modified in NeoAp1 is highlighted in red.

a NeoAp1



b NeoAp2



c NeoAp3

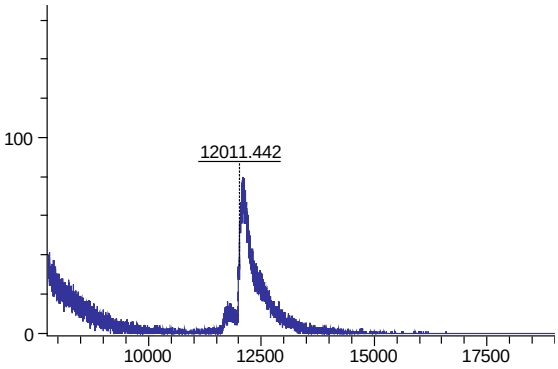


Figure S9. MALDI-TOF spectra of fluorescent neomycin B aptamers. $[M-H]^-$ (calcd.) (**a**) NeoAp1 10739.8; (**b**) NeoAp2 11046.0; (**c**) NeoAp3 12009.6

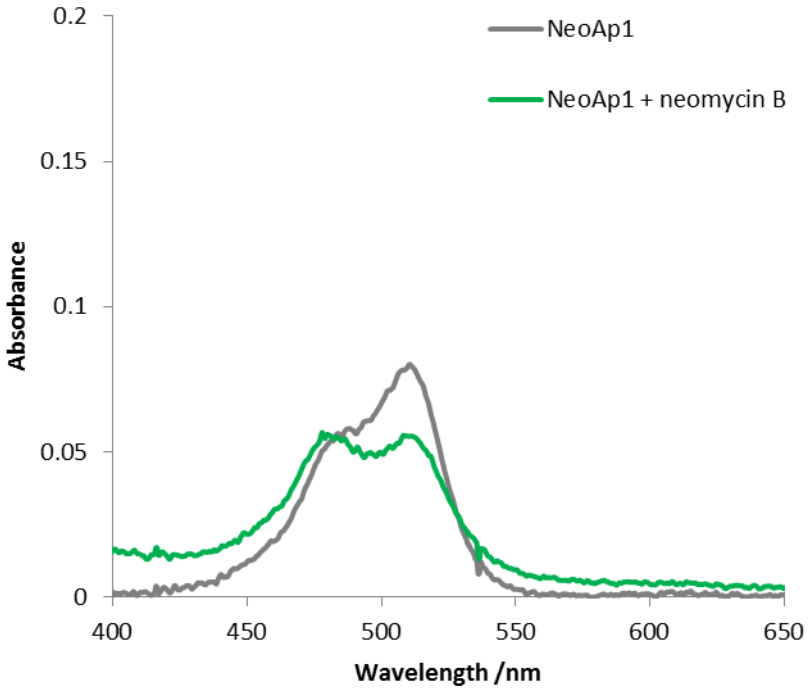


Figure S10. Absorption spectra of NeoAp1 in the presence/absence of Neomycin B. Spectra of NeoAp1 (1.5 µM) were measured in 50 mM potassium phosphate (pH 6.2) containing 200 µM neomycin B and 100 mM potassium chloride at 25 °C.

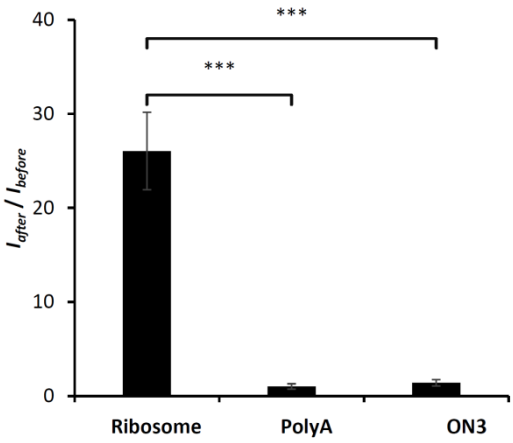
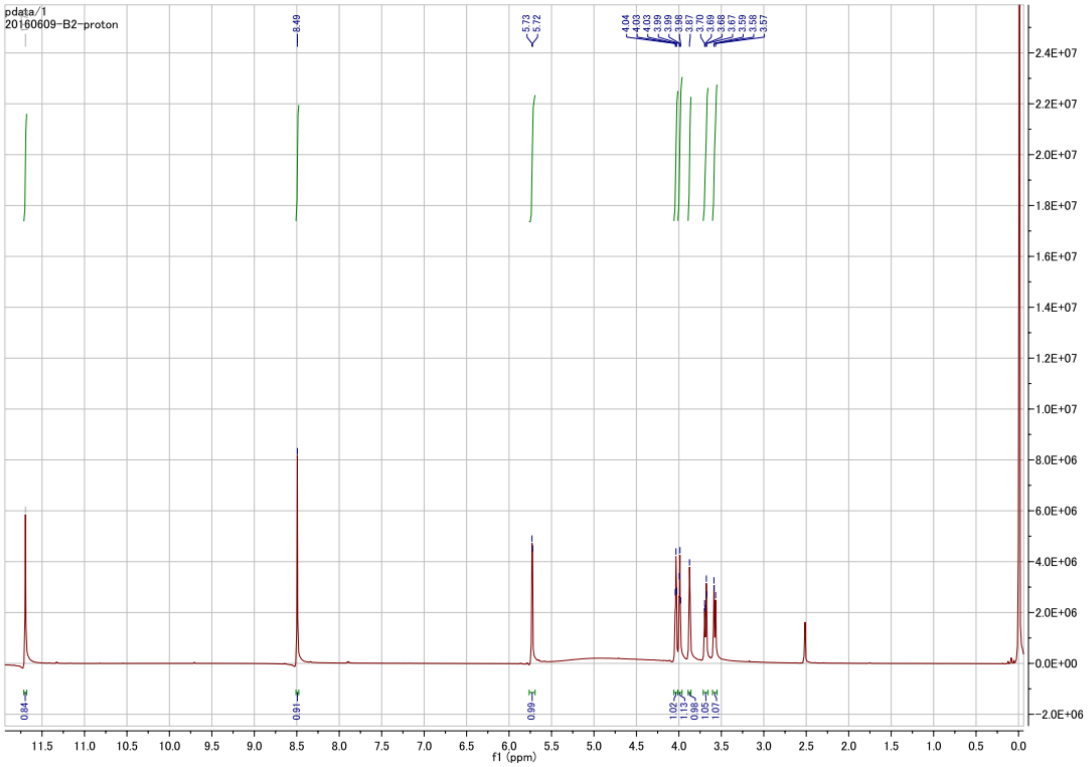
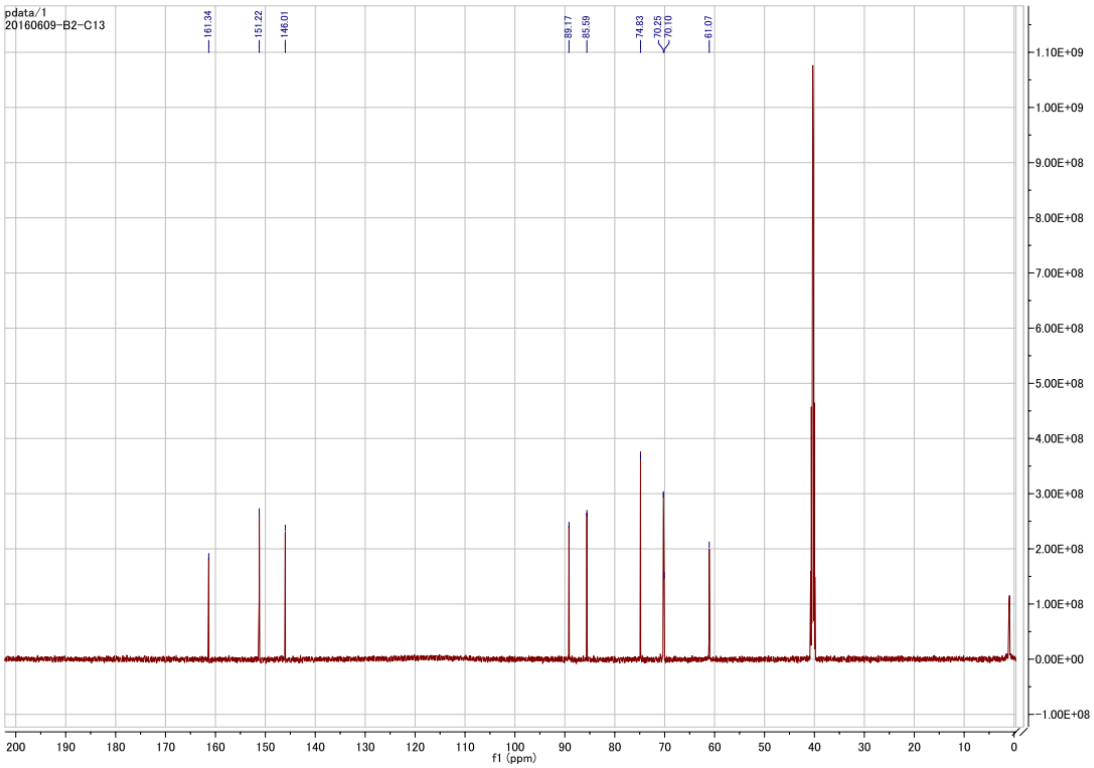


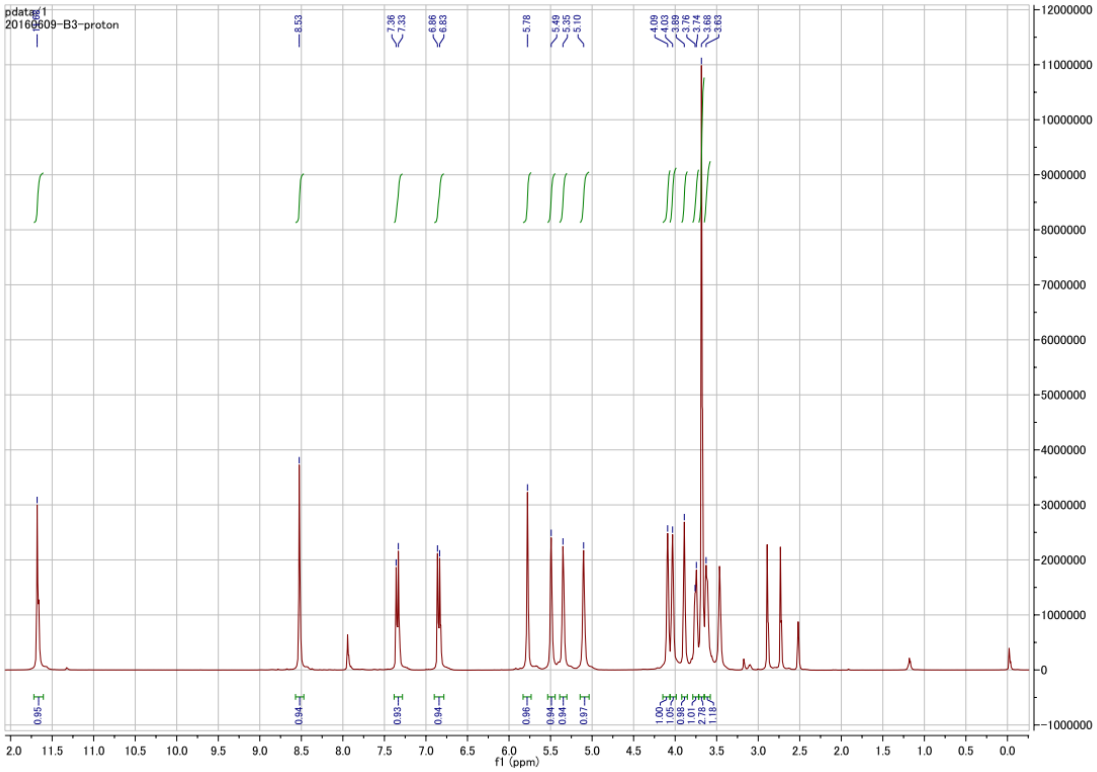
Figure S11. Fluorescence changes of NeoAp1 in the presence of competitors. Spectra of NeoAp1 (20 nM) were measured in 50 mM potassium phosphate (pH 6.2) containing 640 nM competitors, 200 µM neomycin B and 100 mM potassium chloride at 25 °C. The emission spectra were measured with excitation at 510 nm. The mean values of triplicate samples are given. Error bars, mean ± standard deviation. Stars indicate significance in Student's t-test, *P < 0.05, **P < 0.01, ***P < 0.001.

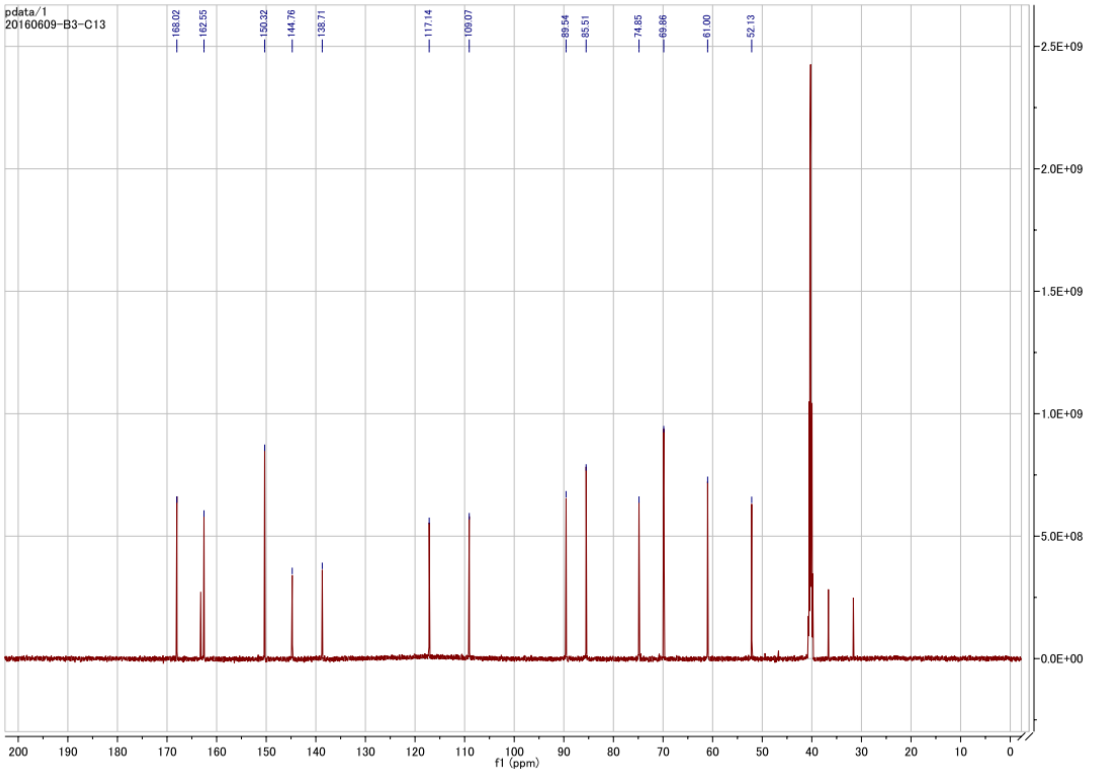
^{1}H and ^{13}C NMR charts of Compound **2**



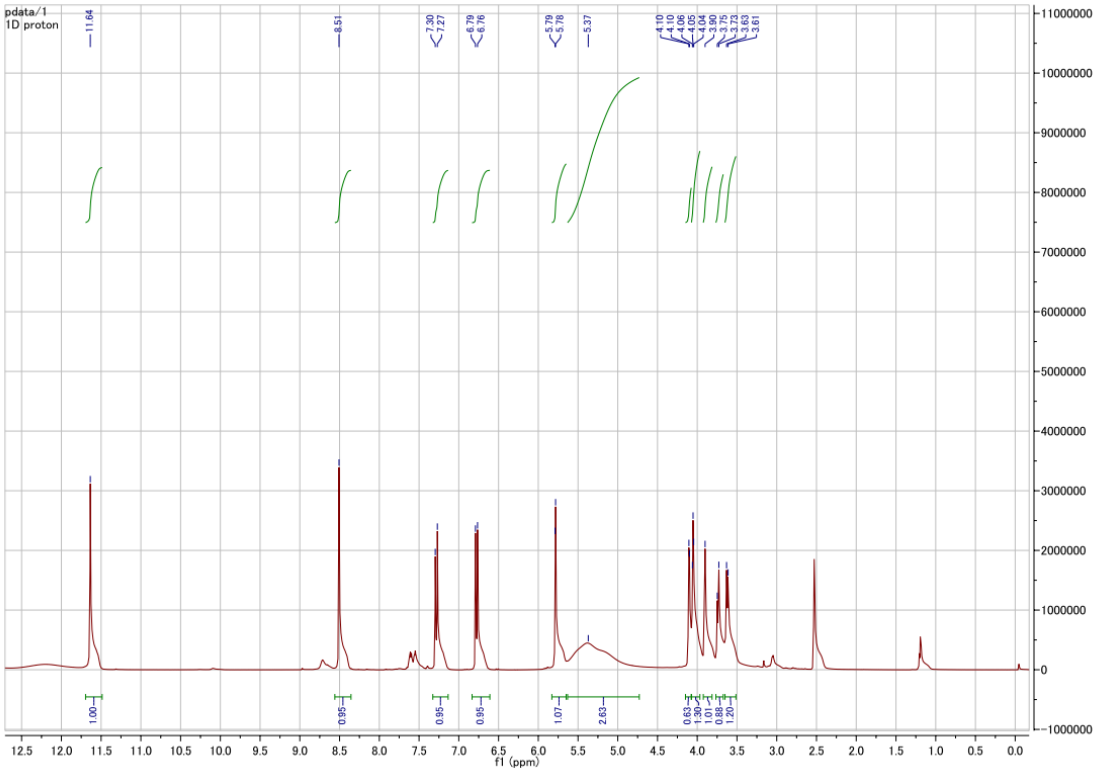


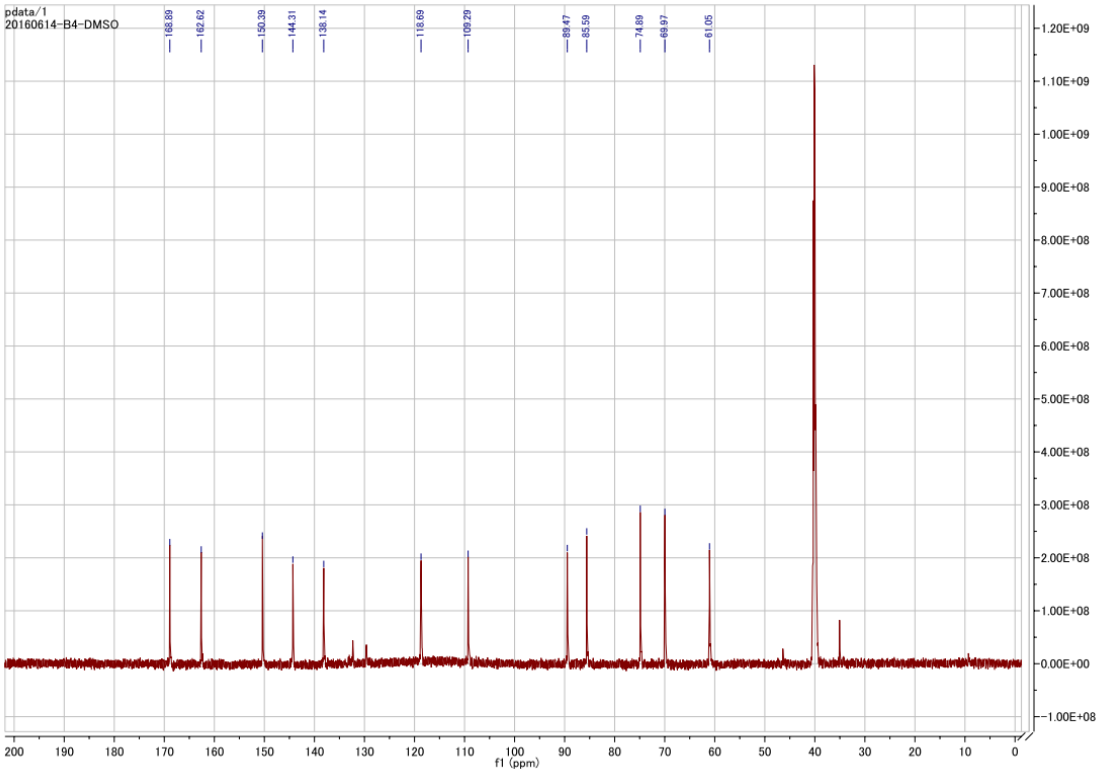
^{1}H and ^{13}C NMR charts of Compound **3**



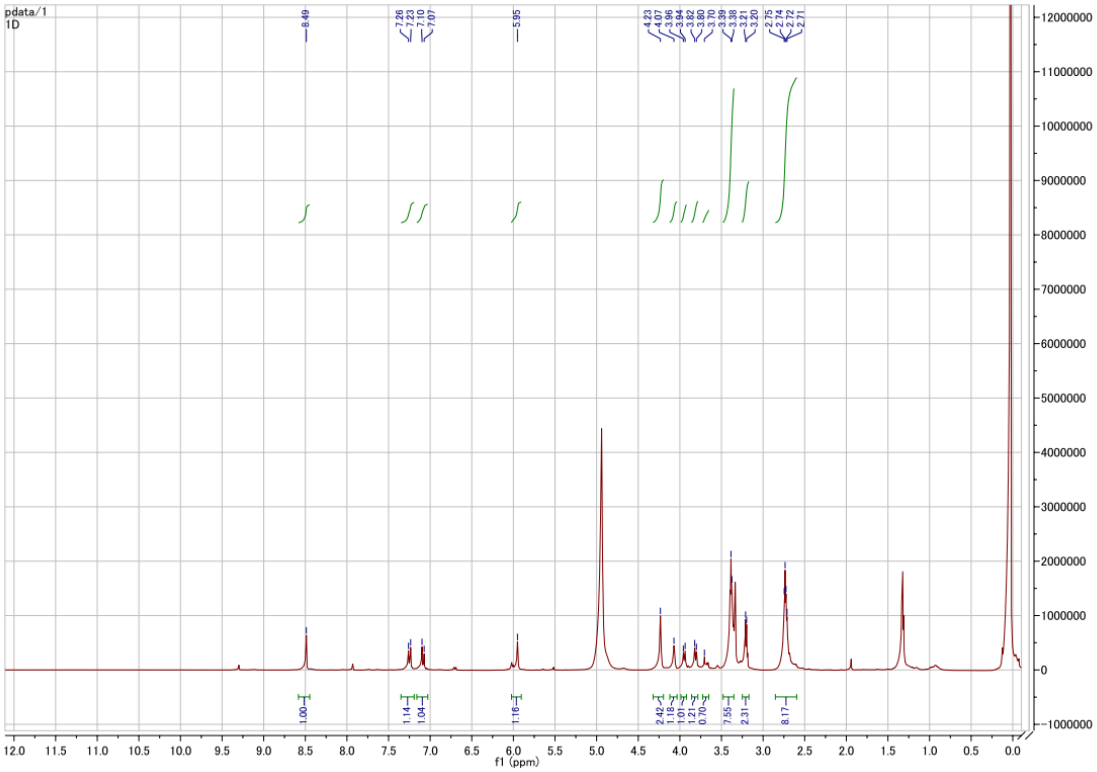


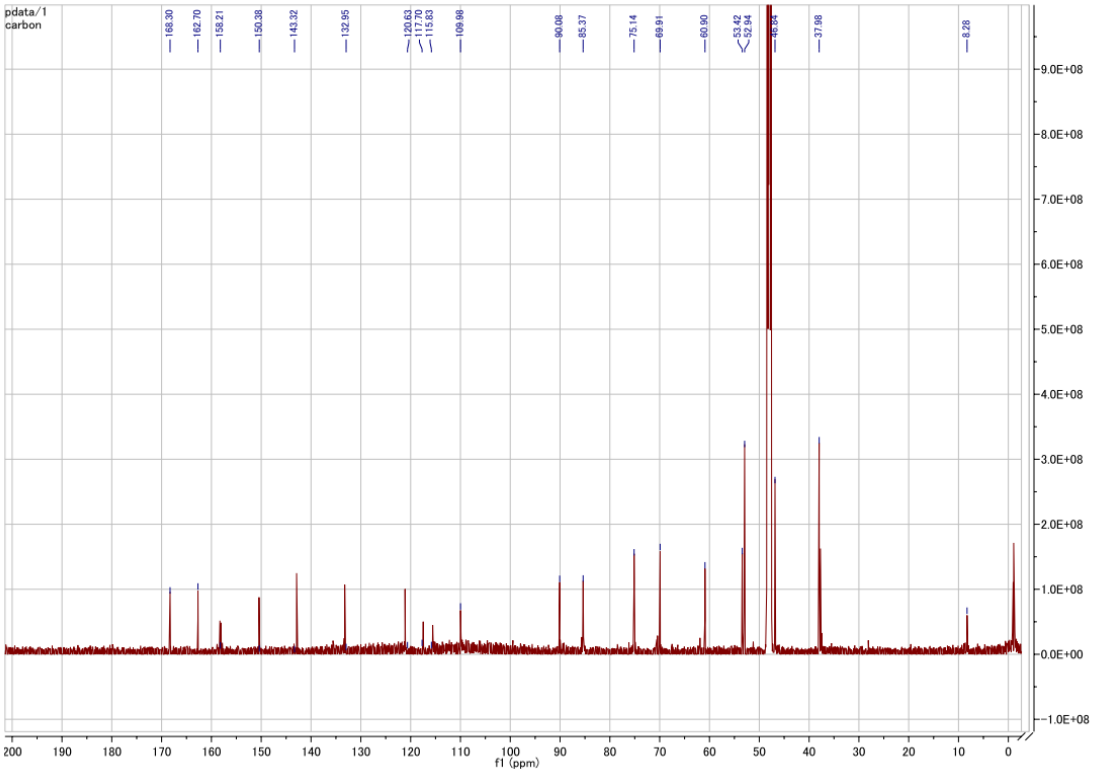
^{1}H and ^{13}C NMR charts of Compound **4**



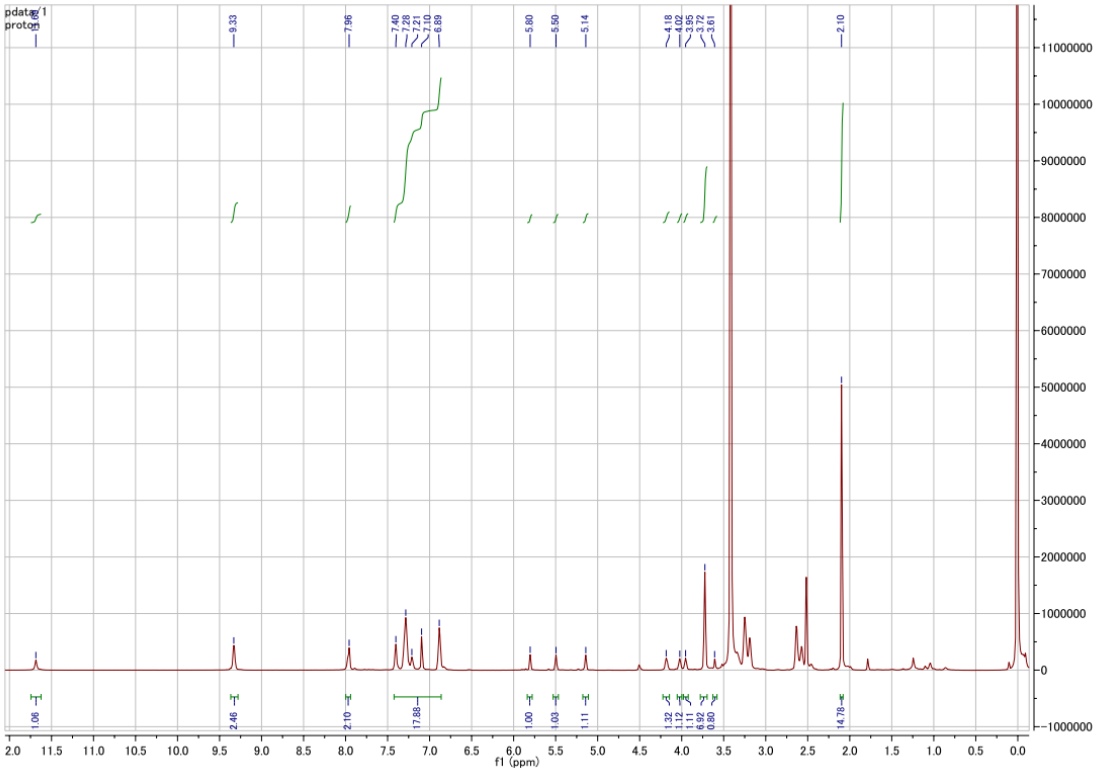


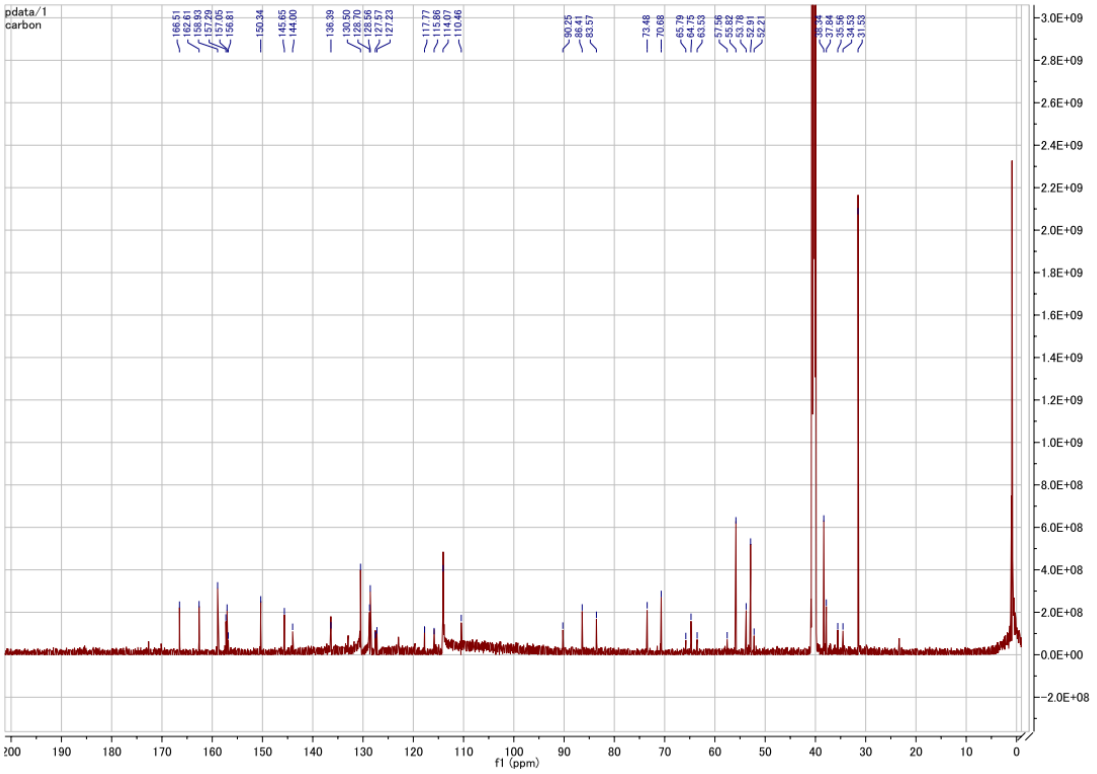
^{1}H and ^{13}C NMR charts of Compound **5**





^{1}H and ^{13}C NMR charts of Compound **6**





^{1}H and ^{13}C NMR charts of Compound **7**