Electronic Supplementary Information

Live-Cell Imaging of Multiple Endogenous mRNAs Permits Direct Observation of RNA Granule Dynamics

Kenji Yatsuzuka,[a] Shin-ichi Sato,*[a] Kathleen Beverly Pe,[a] Yousuke Katsuda,‡[a]

Ippei Takashima,[a] Mizuki Watanabe §[a] and Motonari Uesugi *[ab]

[a]*Institute for Chemical Research and* [b]*Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University, Uji, Kyoto 611-0011, Japan*

Present Addresses

‡Division of Materials Science, Faculty of Advanced Science and Technology, Kumamoto University, Kumamoto 860-8555, Japan

§Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan

*Correspondence should be addressed to M.U. (uesugi@scl.kyoto-u.ac.jp), and S.S. (ssato@scl.kyoto-u.ac.jp).

Supplementary Contents

Supplementary Figures

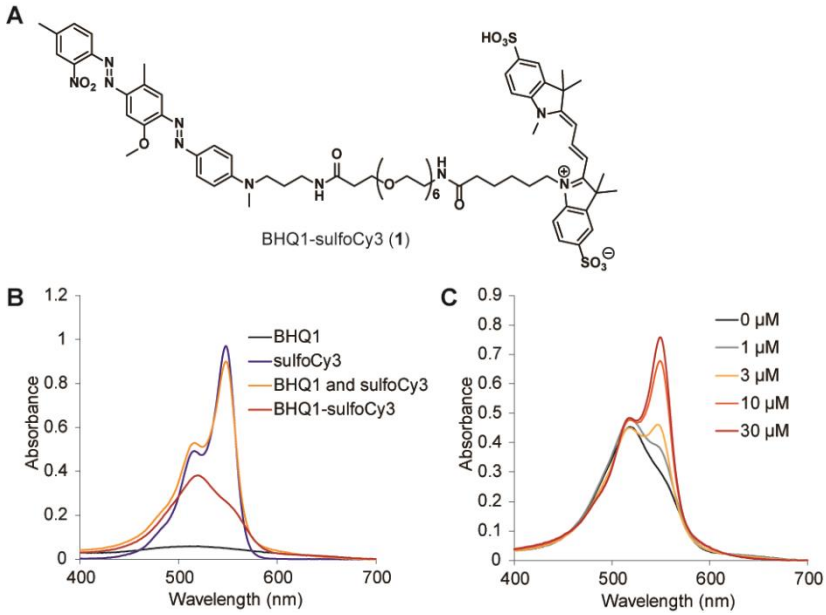


Figure S1. Chemical properties of probe **1**. (A) Chemical structure of probe **1**. (B) Absorption spectra of BHQ1, sulfoCy3, a 1:1 mixture of BHQ1 and sulfoCy3, and BHQ1-sulfoCy3 conjugate probe **1** [5 μM each] in a binding buffer (10 mM Tris-HCl, 100 mM KCl, 5 mM $MgCl_2$, pH 7.6). (C) Absorption spectra of probe **1** [5 μM] in the presence of various concentrations of A1 aptamer.

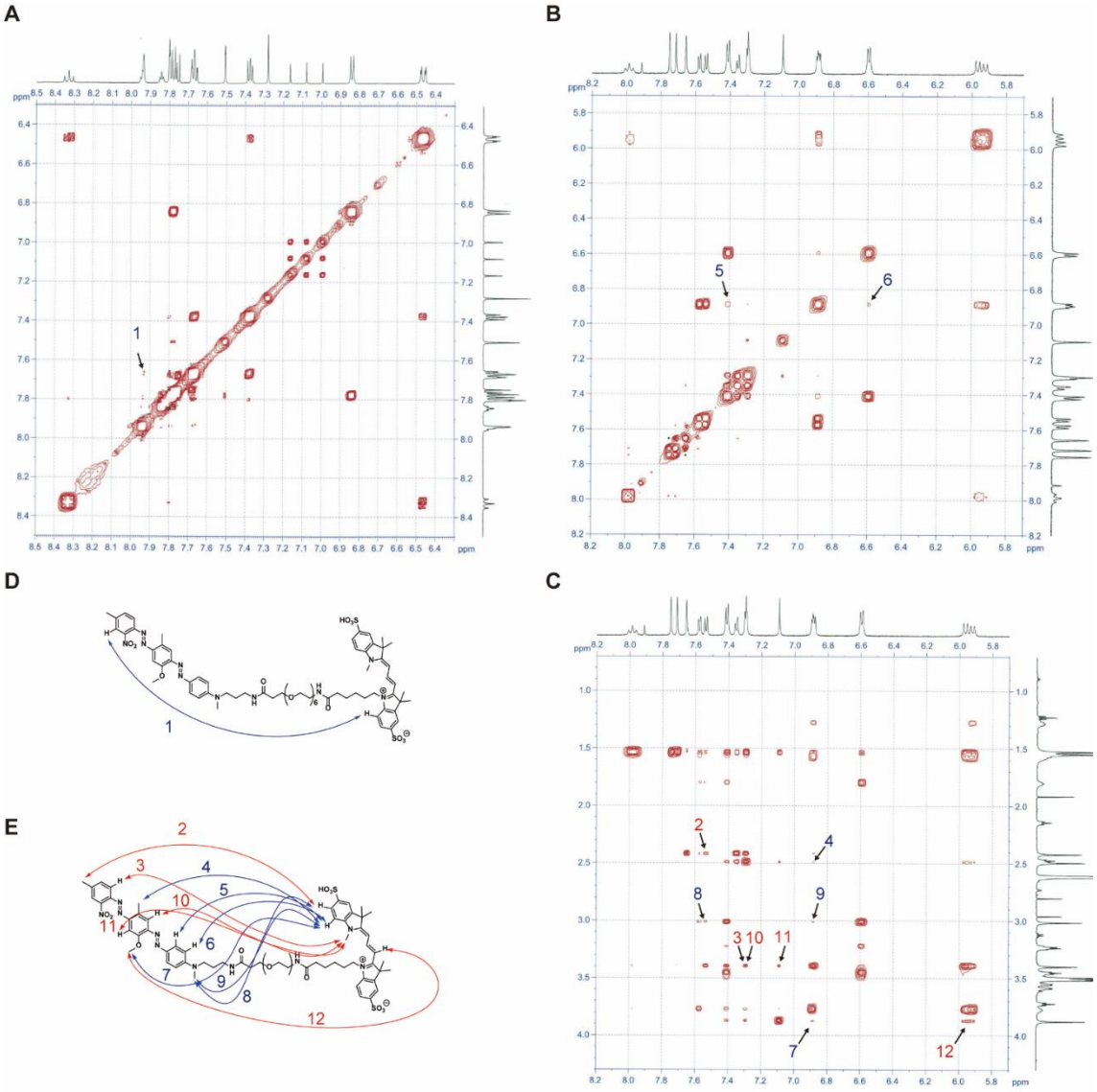


Figure S2. NOESY analysis of probe **1**. (A,B) Expanded aromatic regions of NOESY spectra of probe **1** in DMSO-d_6 (A) and in 50% (v/v) D_2O/DMSO-d_6 (B); (C) Expanded aromatic to aliphatic regions of NOESY spectra of probe **1** in 50% (v/v) D_2O/DMSO-d_6; (D,E) Observed NOE interactions between BHQ1 and sulfoCy3 in DMSO-d_6 (D) and in 50% (v/v) D_2O/DMSO-d_6 (E). Blue arrows and red arrows indicate locations of weak and strong NOEs, respectively.

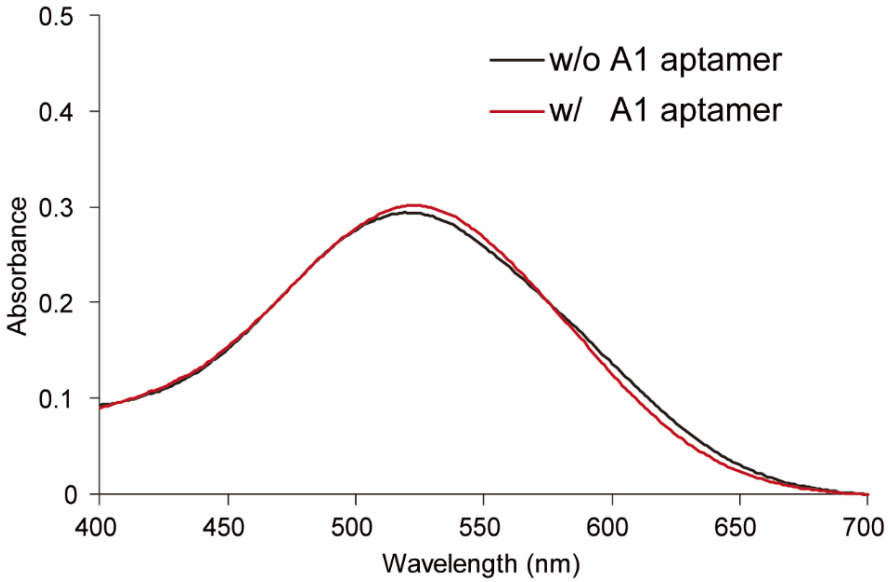


Figure S3. Absorption spectrum of BHQ1. Absorption spectra of BHQ1 [10 μM] in the absence (black) and presence (red) of A1 aptamer [30 μM] in binding buffer (10 mM Tris-HCl, 100 mM KCl, 5 mM $MgCl_2$, pH 7.6).

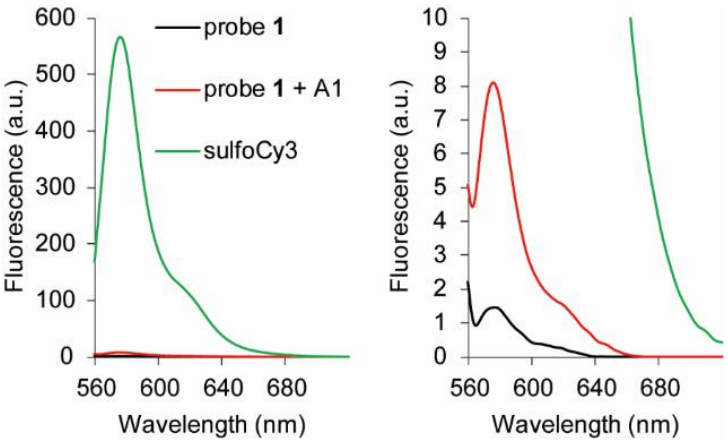


Figure S4. Full size (left) and enlargement (right) of fluorescence emission spectra of the probe **1** with (red) and without (black) the A1 aptamer, and sulfoCy3 (green) in binding buffer (10 mM Tris-HCl, 100 mM KCl, 5 mM $MgCl_2$, pH 7.6).

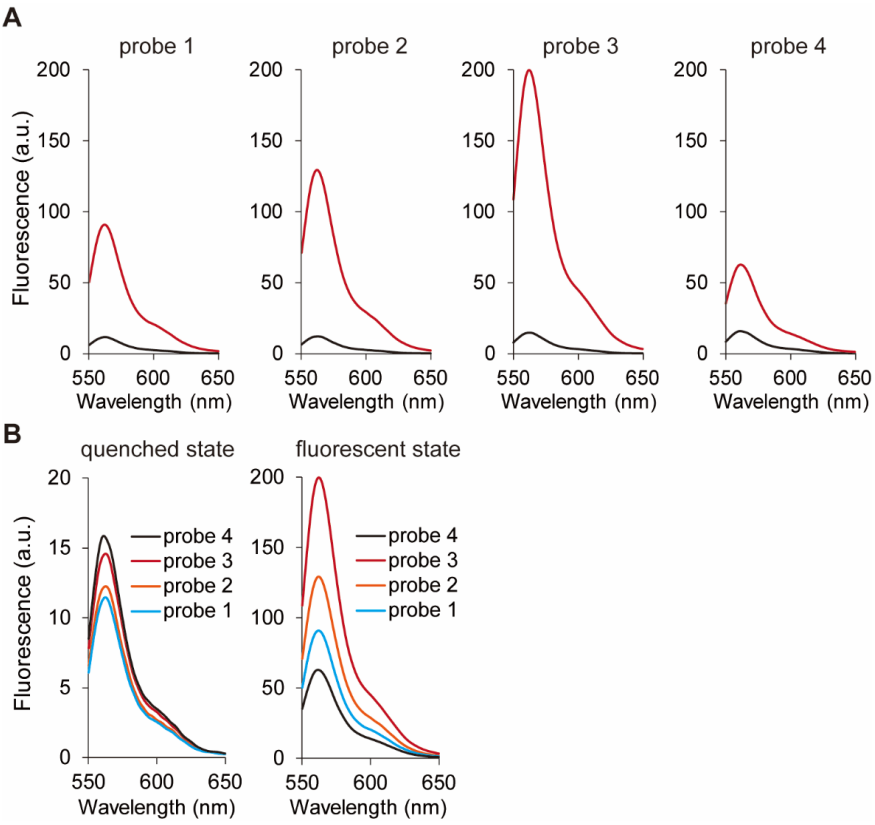


Figure S5. Fluorescence emission spectra of BHQ1-sulfoCy3 conjugate probes. (A) Fluorescence intensity of the probes [2 μM] with different linker lengths, in the absence (black) and presence (red) of an A1 aptamer [30 μM] in binding buffer (10 mM Tris-HCl, 100 mM KCl, 5 mM $MgCl_2$, pH 7.6). (B) Fluorescence intensity of each probe in quenched and fluorescent states.

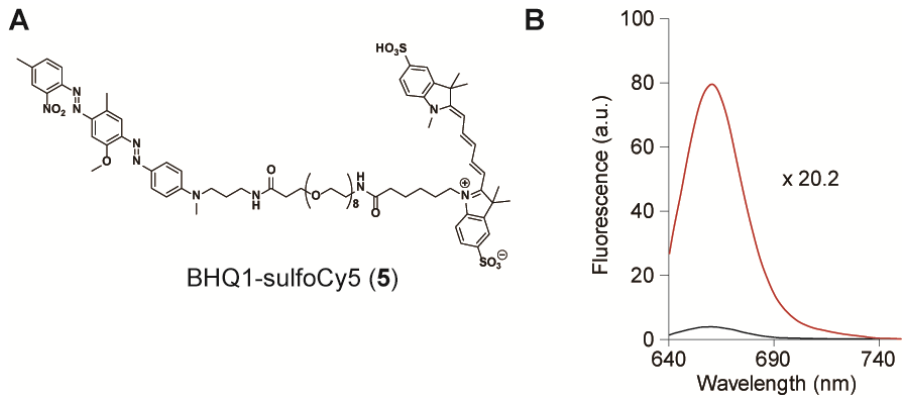


Figure S6. Probe **5**. (A) Chemical structure of BHQ1-sulfoCy5 conjugate probe **5**. (B) Fluorescence emission spectra of the probe in the absence (black) and presence (red) of A1 aptamer.

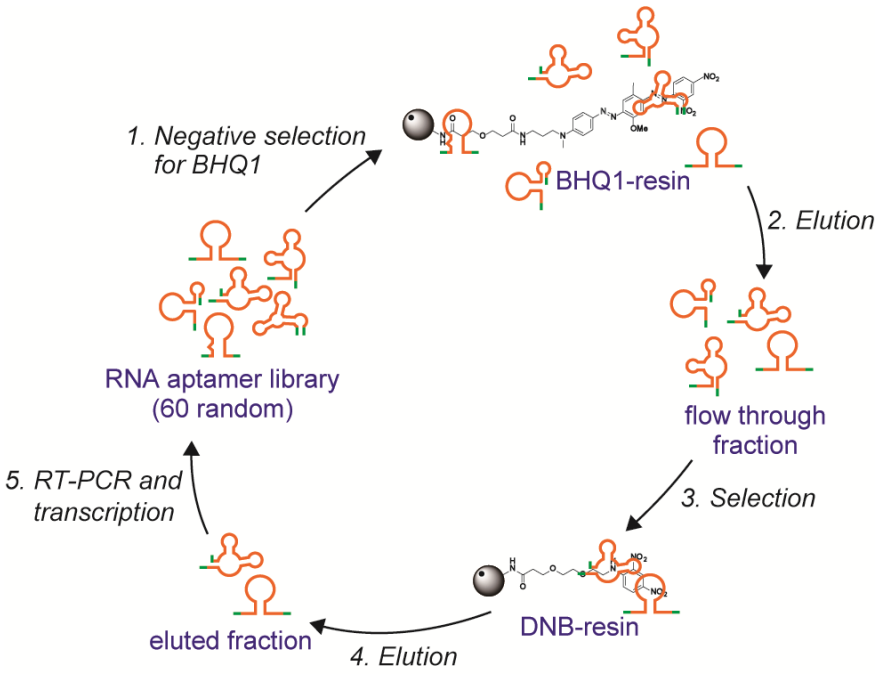


Figure S7. *In vitro* selection of DNB aptamer. To obtain an RNA aptamer for DNB, we carried out in vitro selection of an RNA library comprising 60-base random sequences. The sequences that potentially exhibit cross-responses to BHQ1 were removed from the library using BHQ1-immobilized resins (steps 1 and 2). The unbound RNAs were further treated with DNB-immobilized resins (step 3). After extensive washing, DNB-binding sequences were eluted with free DNB (step 4). The next-generation library of the collected RNAs was regenerated by subsequent reverse transcription, polymerase chain reaction, and transcription (step 5).

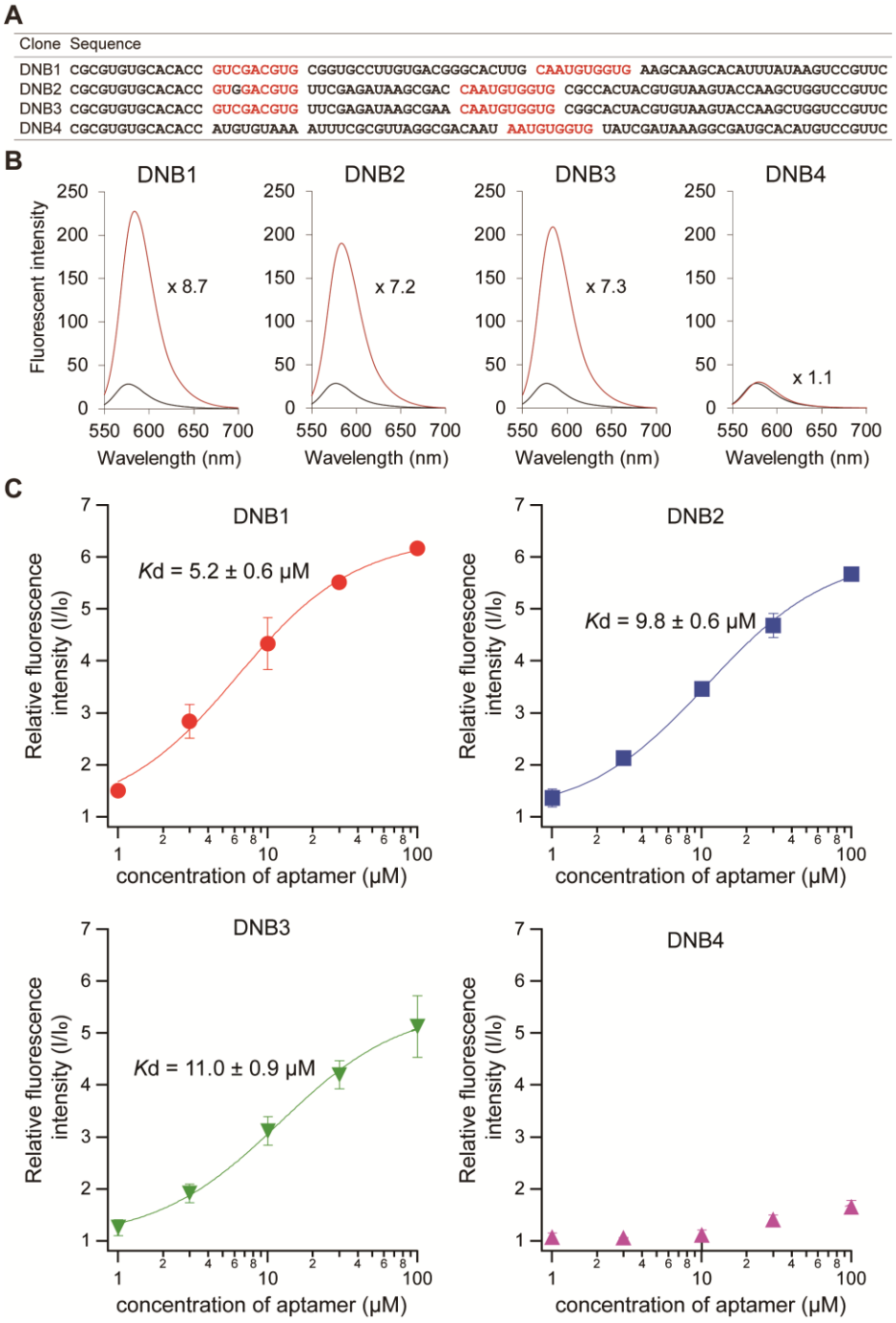


Figure S8. DNB aptamers. (A) Converged sequences from in vitro selection for DNB-binding aptamers (DNB1-4). (B) Fluorescence emission spectra of probe **6** in the presence of each DNB aptamer. (C) Fluorescence intensity changes following binding of clones DNB1–4 to probe **6** and *K*d value of each aptamer. Relative fluorescence intensity is means ± SD of at least three independent experiments.

Name	Sequence
Tg	GGGAAUUCUCCGUCGACAUUAACAAGCUUGGAUCCC
RT1	GGGAUCCAAGCUUGUUUCACCGUCGACGUGCGGUGCCUUGUGACGGGCACUUGCAAUGUGGUGUUCGACGGAGAAUUC
RT2	GGGAUCCAAGCUUGUUUACCGUCGACGUGCGGUGCCUUGUGACGGGCACUUGCAAUGUGGUUUCGACGGAGAAUUC
RT3	GGGAUCCAAGCUUGUUUCCGUCGACGUGCGGUGCCUUGUGACGGGCACUUGCAAUGUGGUUCGACGGAGAAUUC
RT4	GGGAUCCAAGCUUGUUUUCGUCGACGUGCGGUGCCUUGUGACGGGCACUUGCAAUGUGUUCGACGGAGAAUUC
RT5	GGGAUCCAAGCUUGUUUACCGUCGACGUGCGGUGCUGUGACGCACUUGCAAUGUGGUUUCGACGGAGAAUUC
RT6	GGGAUCCAAGCUUGUUUACCGUCGACGUGCGGUUGUGACACUUGCAAUGUGGUUUCGACGGAGAAUUC
RT7	GGGAUCCAAGCUUGUUUACCGUCGACGUGCGGUGUGACCUUGCAAUGUGGUUUCGACGGAGAAUUC

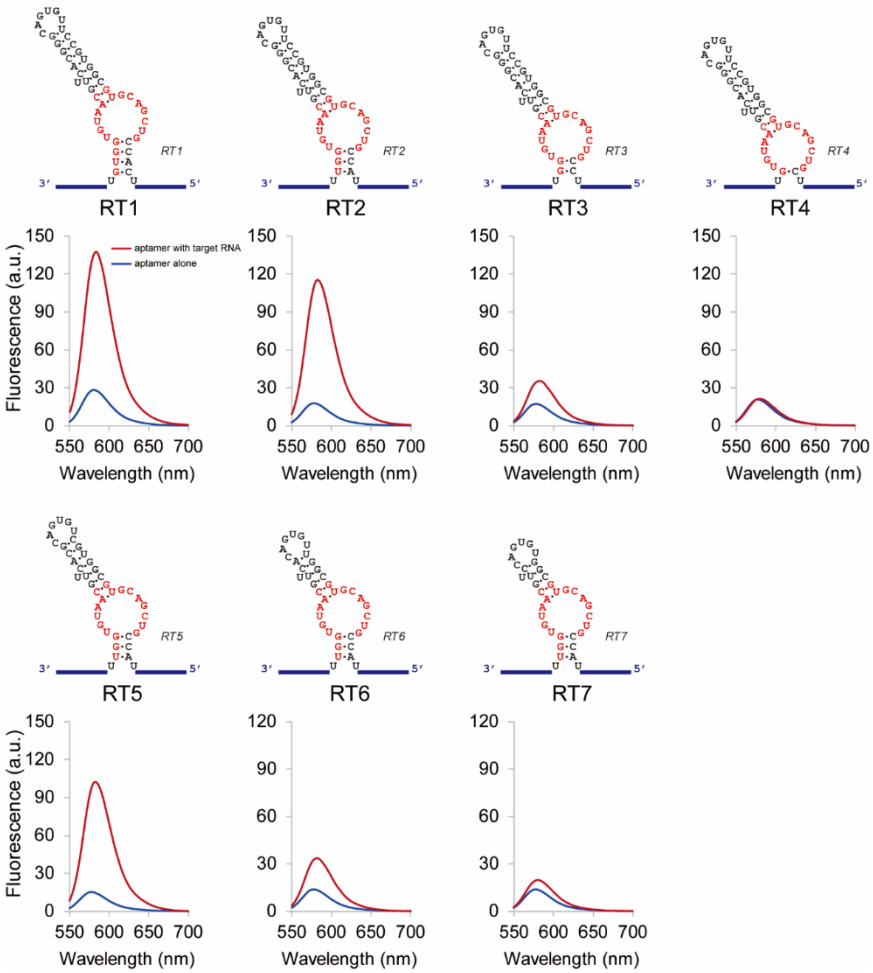


Figure S9. Restoration of the fluorescence ability of DNB-RT aptamers. Fluorescence intensity of probe **6** with each aptamer in the absence and presence of the target RNA.

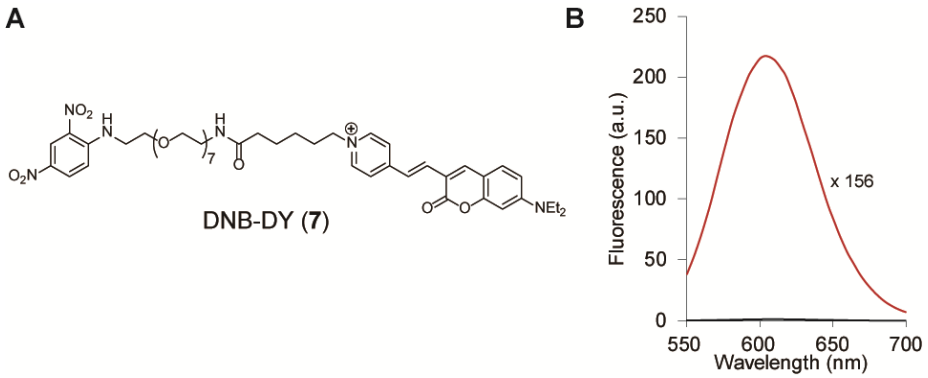


Figure S10. Probe **7**. (A) Chemical structure of DNB-DY conjugate probe **7**. (B) Fluorescence emission spectra of probe **7** in the absence (black) and presence (red) of the DNB1 aptamer.

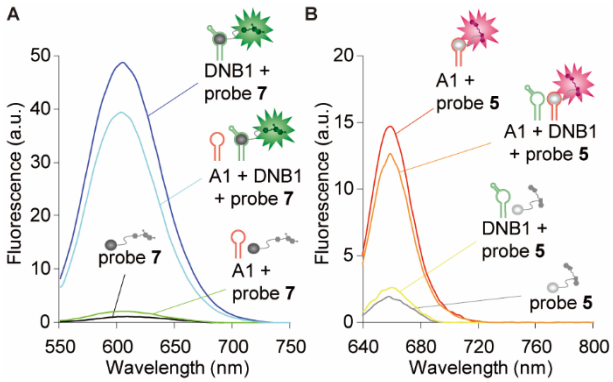


Figure S11. Estimation of the cross response of aptamers to fluorescent probes. (A) Cross response of probe **7** to each aptamer. (B) Cross response of probe **5** to each aptamer. A1 aptamer and DNB1 aptamer have no cross response to the BHQ1-conjugated probe (**5**) and the DNB-conjugated probe (**7**), respectively.

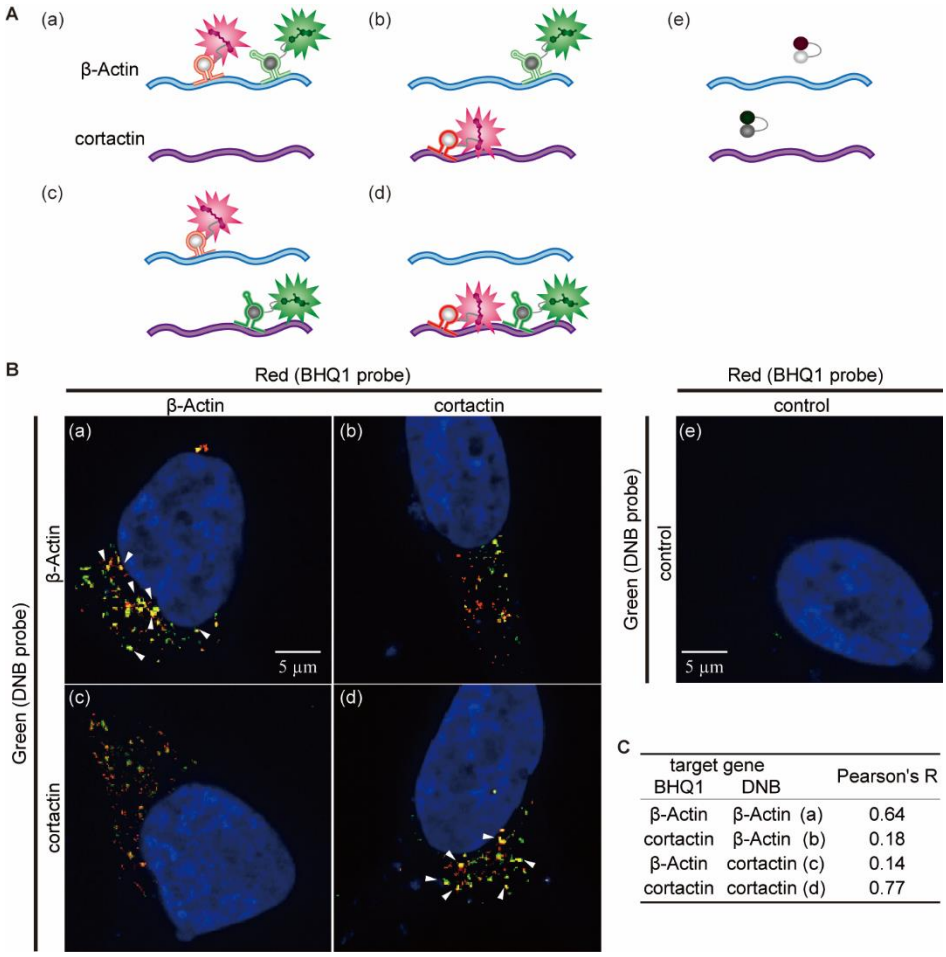


Figure S12. Multicolor imaging of endogenous β-actin mRNA and cortactin mRNA in fixed HeLa cells. (A) Schematic illustration of the experiments. (B) Real experimental images. (a) Both probe **5** (red dots) and probe **7** (green dots) were targeted to β-actin mRNA by their corresponding aptamers; (b, c) probe **5** (red dots) and probe **7** (green dots) were targeted to different mRNAs; (d) both probe **5** (red dots) and probe **7** (green dots) were targeted to cortactin mRNA. (e) A control experimental image. Cells were treated with probe **5** and probe **7** and without aptamers. Staining of the nuclei with DAPI is shown in blue. (C) Assessment of colocalization between green and red fluorescent dots. Pearson's correlation coefficient values (Pearson's R) were quantified by ImageJ.

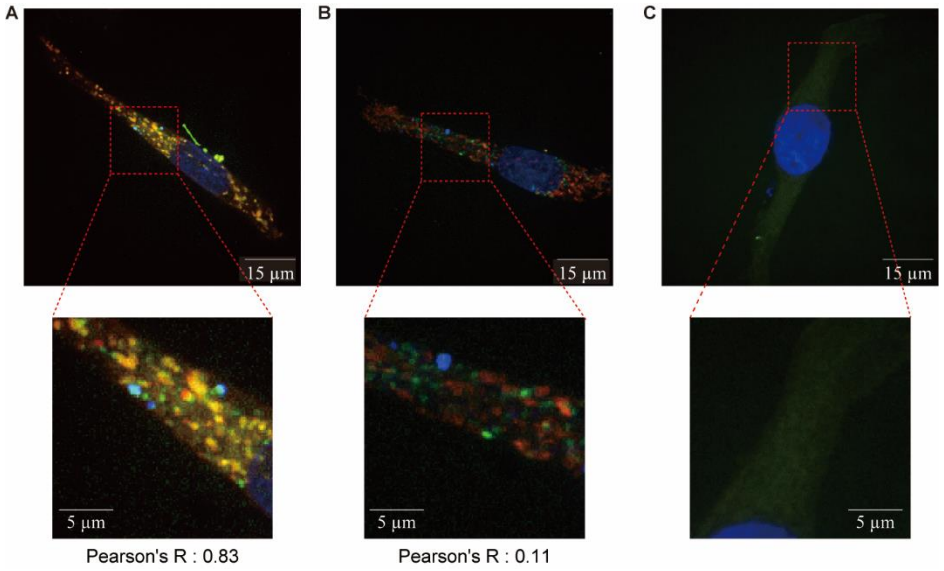


Figure S13. Multicolor imaging of endogenous mRNAs in living HeLa cells. (A) β-actin mRNA with BHQ1 aptamer paired with BHQ1-Cy5 probe **8** (red dots), and DNB aptamer paired with DNB-DY probe **7** (green dots). (B) β-actin mRNA with BHQ1 aptamer paired with BHQ1-Cy5 probe **8** (red dots); cortactin mRNA with DNB aptamer paired with DNB-DY probe **7** (green dots). (C) Background fluorescence of BHQ1-Cy5 probe **8** (red dots) and DNB-DY probe **7** (green dots) without aptamer expression. Staining of the nuclei with Hoechst 33342 is shown in blue. Co-localisation between BHQ1-Cy5 probe **8** (red dots) and DNB-DY probe **7** was quantified by ImageJ. Pearson's correlation coefficient values (Pearson's R) are shown at the bottom.

aptamer	aptamer sequences	target sequences
BHQ aptamar for β-Actin	GGAGCAAUGAUGGCCUAGAUAAAUUCGGUGCUUGAUCUUCAUU	AAUGAAGAUCAAGAUCAUUGCUCC
DNB aptamar for β-Actin	AUAGGAAUCCUACCGUCGACGUGCGGUGCUGUGACGCACUUGCAAUGUGGUUGACCCAUGCC	GGCAUGGGUCAGAAGGAUUCCTAU
BHQ aptamar for cortactin	UACUGUCUUCUGGCCUAGAUAAAUUCGGAGCUUAGGCAGAGGA	UCCUCUGCCUACCAGAAGACAGUA
DNB aptamar for cortactin	AAGGAUGAUGUACCGUCGACGUGCGGUGCTGUGACGCACUUGCAAUGUGGUUAAUGCACCUC	GAGGUGCAUUUUCUCAUCAUCCUU

β-Actin mRNA

accgccgagaccgcgucccgccccgcgagcacagagccucgccuuugccgauccgccgcccguccacacccgccgccagcucaccauggaugaugauaucg
ccgcgcucgucgucgacaacggcuccggcaugugcaaggccggcuucgcgggcgacgaugccccccgggccgucuuccccuccaucgugggggcgccccag
gcaccagggcgugauggugGGCAUGGGUCAGAAGGAUUCCUAUgugggcgacgaggcccagagcaagagaggcauccucacccugaaguaccccaucgag
cacggcaucgucaccaacugggacgacauggagaaaaucuggcaccacaccuucuacaaugagcugcguguggcuccccgaggagcaccccgugcugcuga
ccgaggccccccugaaccccaaggccaaccgcgagaagaugacccagaucauguuugagaccuucaacaccccagccauguacguugcuauccaggcugu
gcuaucccuguacgccucuggccguaccacuggcaucgugauggacuccggugacggggucacccacacugugcccaucuacgagggguaugcccucccc
caugccauccugcgucuggaccuggcuggccgggaccugacugacuaccucaugaagauccucaccgagcgcggcuacagcuucaccaccacggccgagc
gggaaaucgugcgugacauuaaggagaagcugugcuacgucgcccuggacuucgagcaagagauggccacggcugcuuccagcuccuccccuggagaagag
cuacgagcugccugacggccaggucaucaccauuggcaaugagcgguuccgcugcccugaggcacucuuccagccuuccuuccugggcauggaguccugu
ggcauccacgaaacuaccuucaacuccaucaugaagugugacguggacauccgcaaagaccuguacgccaacacagugcugucuggcggcaccaccaugu
acccuggcauugccgacaggaugcagaaggagaucacugcccuggcacccagcacAAUGAAGAUCAAGAUCAUUGCUCCuccugagcgcaaguacuccgu
guggaucggcggcuccauccuggccucgcuguccaccuuccagcagauguggaucagcaagcaggaguaugacgaguccggccccuccaucguccaccgc
aaaugcuucuaggcggacuaugacuuaguugcguuacacccuuucuugacaaaaccuaacuugcgcagaaaacaagaugagauuggcauggcuuuauuug
uuuuuuuuguuuuguuuugguuuuuuuuuuuuuuuggcuugacucaggauuuaaaaacuggaacggugaaggugacagcagucgguuggagcgagcauc
ccccaaaguucacaauguggccgaggacuuugauugcacauuguuguuuuuuuaauagucauuccaaauaugagaugcguuguuacaggaaguccccuugc
cauccuaaaagccacccccacuucucucuaaggagaauggcccaguccucucccaaguccacacaggggaggugauagcauugcuuucguguaaauuaugu
aaugcaaaauuuuuuuaaucuucgccuuaaauacuuuuuuauuuuguuuuauuuugaaugaugagccuucgugccccccuucccccuuuuuugucccca
acuugagauguaugaaggcuuuuggucucccugggaguggguggaggcagccagggcuuaccuguacacugacuugagaccaguugaauaaaagugcaca
ccuuaaaaaugaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa

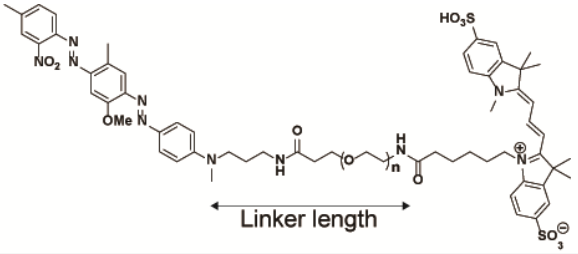
cortactin mRNA

gaugcggccccgccccgcccucggaaccggaaguagagccuggugccugggagcggcuggcgcggcggaauccagggccgacccgggccggaccgaccc
caggcggcgacggaaucaguccccaaugccuggaaauucccucauuggauuacuguguuuuaaacagaauuucgugaacagccuuuuaucuccaagcggaa
agaaagAUGuggaaagcuucagcaggccacgcuguguccaucgcccaggaugacgcgggggccgaugacugggagaccgacccugauuuugugaaugaug
ugagugagaaggagcaaagauggggugccaagacggugcagggcuccgggcaccaggagcauauccaacauacacaagcugagggagaaugucuuucaaga
gcaucagacccuuaaggagaaggaacuugaaacaggaccaaaagcuucccauggcuauggagggaaauuuGGUGUGGAACaagaccgaauggauaaguca
gcugucggccacgaauaucagucgaaacuuuccaagcacugcucgcaggugacucggucccguggcuucggaggcaaguuuggugucccagauggacagag
uugaucagucugcuguaggcuuugaauaccaggggaagacugagaagcaugccucccagaaagacuacuccaguggguuuuggcggcaaguauggcgugca
ggccgaccgaguagacaagagcgcggugggcuucgacuaccagggcaagacggagaagcacgagucacagagagauuacuccaaagguuucggcggcaaa
uacgguaucgacaaggacaaaguggauaagagcgccguuggcuuugaguaucaaggcaaaacggagaagcacgagucccagaaagacuaugugaaagggu
uuggaggaaaauuuggugugcagacagacagacaagacaaaugugcccuuggcugggaucaccaggagaaauugcagcugcaugaaucccaaaaagacua
cuccaaaggauucggcgggaaguauggggugcagaaggaucggauggauaagaaugcgucaaccuuugaggaugucacccaggugUCCUCUGCCUACCAG
AAGACAGUAccugucgaagcugugaccagcaaaacaaguaacaucagagcuaacuuugaaaaccucgcuaaggagaaagagcaggaggacaggcggaagg
cggaggcggagagagcccagcggauggccaaggagcggcaggagcaggaagaggccaggaggaagcuggaggagcaagccagagccaaaacgcaaacgcc
cccuguucgcccgcaccucagccaaccgaggagaggcugcccucgagccccgucuaugaggaugcggcuuccuucaaggcagagcugagcuacagaggc
ccugugagugggacggagccggagcccguguacagcauggaggccgcugacuaccgagaggccagcagccagcagggccuggccuaugccacagaggcug
ucuaugaaagcgcagaggccccgggccacuaucccgcagaggacagcaccuacgaugaguacgagaacgaucuggggaucacagccgucgcccuguacga
cuaccaggcugcgggcgaugaugagaucucauuugacccugaugacaucaucaccaacaucgagaugauugacgacggcuggugggcgcggggugugcaag
ggccgcuucagggagcucgcauucucuugugguucguguugcccucgugcccaucaagugcagucgggaccucccaggacaagcacgaggccucaggucgg
cccugugggcggguaggcaggaaggacugucccagacgaggggcuuccucuagagucucacugcuggggaggagaggacugggccugaugaaguuaaccc
ggagcuaagucacccagagcacaggagcugccaugucagaugggaaaucugccuaugucauaccgugacagcccgcaggaucaggaaacucaucuccuuc
cugaggagccgggaggcuggaccagucccgucgugcagucaggugggcgguguucuuuccagaaggucacguggaaaugucucgggacuuggguccccgg
agugcccgugaagcguguuuuugcuccuGAGGUGCAUUUUCUCAUCAUCCUUgcuuuaccacaaugagcaaugaggucgggguuuuauaugcaacuuauug
uaucugaauuccuguagcacaccucauagguaugauuuuuuuaaauuaaagaauucagaauaaacauuuuuugauccacuugcgugaaaaaaaaaaaaaa
aaaaaaaaaaaaaaaaaaaaaaa

Figure S14. Nucleotide sequences of aptamers, mRNAs, and target sites for RNA detection. Target sequences for BHQ1-based aptamers are shown in red. Target sequences for DNB-based aptamers are shown in green.

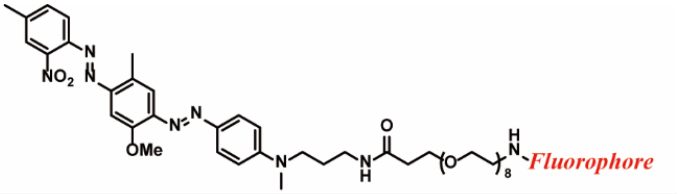
Supplementary Tables

Table 1. Signal-to-background ratios of BHQ1-sulfoCy3 conjugate probes with different linker lengths. Probes [2 μM] and A1 aptamer [30 μM] in binding buffer (10 mM Tris-HCl, 100 mM KCl, 5 mM $MgCl_2$, pH 7.6).



probe	n =	Length (Å)	signal-to-background ratio
1	6	38	7.9
2	8	45	10.5
3	12	59	13.7
4	4	30	4.0

Table S2. Signal-to-background ratio of BHQ1-conjugated probes with different fluorophores. Probes [2 μM] and A1 aptamer [30 μM] in binding buffer (10 mM Tris-HCl, 100 mM KCl, 5 mM $MgCl_2$, pH 7.6).



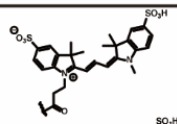
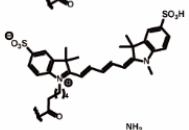
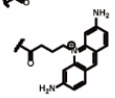
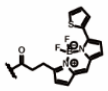
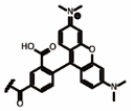
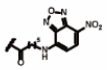
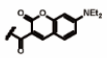
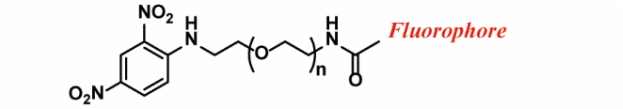
Fluorophore		signal-to-background ratio
sulfoCy3 (**3**)		10.5
sulfoCy5 (**5**)		20.2
ATTO465 (**S4**)		2.4
BODIPY 558/568 (**S5**)		1
TAMRA (**S6**)		2.2
NBD-X (**S7**)		3.6
DEAC (**S8**)		4.4

Table S3. Signal-to-background ratio of DNB-conjugated probes with different fluorophores. Probes [2 μM] and DNB1 aptamer [30 μM] in binding buffer (10 mM Tris-HCl, 100 mM KCl, 5 mM $MgCl_2$, pH 7.6).



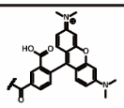
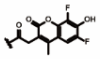
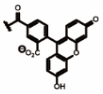
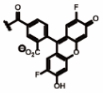
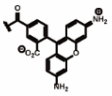
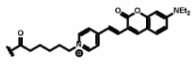
Fluorophore		n	signal-to-background ratio
TAMRA (**6**)		2	11.0
DEAC (**S9**)		2	2.4
Marina Blue (**S10**)		2	3
fluorescein (**S11**)		2	1.5
Oregon Green 488 (**S12**)		2	1.5
Rhodamine Green (**S13**)		2	5.7
DY (**S14**)		2	52.4
DY (**7**)		7	85.5

Table S4. DNA oligonucleotides.

For in vitro selection of DNB aptamer	
template	GCTAATACGACTCACTATAGGGAATTCCGCGTGTGCACACC-N60-GTCCGTTGGGATCCTCATGG
forward primer for selection	GCTAATACGACTCACTATAGGGAATTCCGCGTGTGCACACC
reverse primer for selection	CCATGAGGATCCGAACGGAC
For evaluation of probe-binding activity for short versions of DNB aptamer	
forward primer for DNB short	GCTAATACGACTCACTATAGTGTGCACACCGTCGACGTG
reverse primer for DNB short	ATGTGCTTGCTTCACCACAT
DNB short mut template1 forward	GTCTCCACACCGTCGACGTGCGGTGCCTTGTGACGGGCACTTG
DNB short mut template1 reverse	CAAGTGCCCGTCACAAGGCACCGCACGTCGACGGTGTGGAGAC
DNB short mut template2 forward	TGCCTTGTGACGGGCACTTGCAATGTGGTGAAGCAACCACAT
DNB short mut template2 reverse	ATGTGGTTGCTTCACCACATTGCAAGTGCCCGTCACAAGGCA
forward primer for DNB short mut	GCTAATACGACTCACTATAGGGTCTCCACACCGTCGACGTG
reverse primer for DNB short mut	ATGTGGTTGCTTCACCACAT
For conversion of DNB binding aptamer to RNA-responsive form	
RNA target forward	GCTAATACGACTCACTATAGGGAATTCTCCGTCGACATTAACAAGCTTGGATCCC
RNA target reverse	GGGATCCAAGCTTGTTAATGTCGACGGAGAATTCCCTATAGTGAGTCGTATTAGC
RT1 template forward	GGGATCCAAGCTTGTTTCACCGTCGACGTGCGGTGCCTTGTGACGGGCACTTGCAATGTGGTGTTCGACGGAGAATTC
RT1 template reverse	GAATTCTCCGTCGAACACCACATTGCAAGTGCCCGTCACAAGGCACCGCACGTCGACGGTGAAACAAGCTTGGATCCC
RT2 template forward	GGGATCCAAGCTTGTTTACCGTCGACGTGCGGTGCCTTGTGACGGGCACTTGCAATGTGGTTTCGACGGAGAATTC
RT2 template reverse	GAATTCTCCGTCGAAACCACATTGCAAGTGCCCGTCACAAGGCACCGCACGTCGACGGTAAACAAGCTTGGATCCC
RT3 template forward	GGGATCCAAGCTTGTTTCCGTCGACGTGCGGTGCCTTGTGACGGGCACTTGCAATGTGGTTCGACGGAGAATTC
RT3 template reverse	GAATTCTCCGTCGAACCACATTGCAAGTGCCCGTCACAAGGCACCGCACGTCGACGGAAACAAGCTTGGATCCC
RT4 template forward	GGGATCCAAGCTTGTTTCGTCGACGTGCGGTGCCTTGTGACGGGCACTTGCAATGTGTTCGACGGAGAATTC
RT4 template reverse	GAATTCTCCGTCGAACACATTGCAAGTGCCCGTCACAAGGCACCGCACGTCGACGAAACAAGCTTGGATCCC
RT5 template forward	GGGATCCAAGCTTGTTTACCGTCGACGTGCGGTGCTGTGACGCACTTGCAATGTGGTTTCGACGGAGAATTC
RT5 template reverse	GAATTCTCCGTCGAAACCACATTGCAAGTGCGTCACAGCACCGCACGTCGACGGTAAACAAGCTTGGATCCC
RT6 template forward	GGGATCCAAGCTTGTTTACCGTCGACGTGCGGTTGTGACACTTGCAATGTGGTTTCGACGGAGAATTC
RT6 template reverse	GAATTCTCCGTCGAAACCACATTGCAAGTGTCACAACCGCACGTCGACGGTAAACAAGCTTGGATCCC
RT7 template forward	GGGATCCAAGCTTGTTTACCGTCGACGTGCGGTGTGACCTTGCAATGTGGTTTCGACGGAGAATTC
RT7 template reverse	GAATTCTCCGTCGAAACCACATTGCAAGGTCACACCGCACGTCGACGGTAAACAAGCTTGGATCCC
forward primer for RT	GCTAATACGACTCACTATAGGGATCCAAGCTTGTTT
reverse primer for RT	GGGAATTCTCCGTCGAA

Table S4–Continued

For preparation of RNA aptamers that are responsive to RNA targets	
template for BHQ1 binding aptamer to β-actin forward	TAATACGACTCACTATAGGGGGAGCAATGATGGCCTAGATAAATTCGGAGCTTGATCTTCATT
template for BHQ1 binding aptamer to β-actin reverse	AATGAAGATCAAGCTCCGAATTTATCTAGGCCATCATTGCTCCCCCTATAGTGAGTCGTATTA
template for DNB binding aptamer to β-actin forward	TAATACGACTCACTATAGGGATAGGAATCCTACCGTCGACGTGCGGTGCTGTGACGCACTTGCAATGTGGTTGACC CATGCC
template for DNB binding aptamer to β-actin reverse	GGCATGGGTCAACCACATTGCAAGTGCGTCACAGCACCGCACGTCGACGGTAGGATTCCTATCCCTATAGTGAGTC GTATTA
template for BHQ1 binding aptamer to cortactin forward	TAATACGACTCACTATAGGGTACTGTCTTCTGGCCTAGATAAATTCGGAGCTTAGGCAGAGGA
template for BHQ1 binding aptamer to cortactin reverse	TCCTCTGCCTAAGCTCCGAATTTATCTAGGCCAGAAGACAGTACCCTATAGTGAGTCGTATTA
template for DNB binding aptamer to cortactin forward	TAATACGACTCACTATAGGGAAGGATGATGTACCGTCGACGTGCGGTGCTGTGACGCACTTGCAATGTGGTTAATG CACCTC
template for DNB binding aptamer to cortactin reverse	GAGGTGCATTAACCACATTGCAAGTGCGTCACAGCACCGCACGTCGACGGTACATCATCCTTCCCTATAGTGAGTC GTATTA
For preparation of aptamer expression plasmids	
insert for BHQ1 binding aptamer to β-actin forward	GATCCCCGGAGCAATGATGGCCTAGATAAATTCGGAGCTTGATCTTCATTTTTTTA
insert for BHQ1 binding aptamer to β-actin reverse	AGCTTAAAAAAATGAAGATCAAGCTCCGAATTTATCTAGGCCATCATTGCTCCGGG
insert for DNB binding aptamer to β-actin forward	CCCCGGAGCAATGATACCGTCGACGTGCGGTGCTGTGACGCACTTGCAATGTGGTTGATCTTCATTTTT
insert for DNB binding aptamer to β-actin reverse	AAAAATGAAGATCAACCACATTGCAAGTGCGTCACAGCACCGCACGTCGACGGTATCATTGCTCCGGGG
insert for BHQ1 binding aptamer to cortactin forward	GATCCCCTACTGTCTTCTGGCCTAGATAAATTCGGAGCTTAGGCAGAGGATTTTTA
insert for BHQ1 binding aptamer to cortactin reverse	AGCTTAAAAATCCTCTGCCTAAGCTCCGAATTTATCTAGGCCAGAAGACAGTAGGG
insert for DNB binding aptamer to cortactin forward	GATCCCCAAGGATGATGTACCGTCGACGTGCGGTGCTGTGACGCACTTGCAATGTGGTTAATGCACCTCTTTTTA
insert for DNB binding aptamer to cortactin reverse	AGCTTAAAAAGAGGTGCATTAACCACATTGCAAGTGCGTCACAGCACCGCACGTCGACGGTACATCATCCTTGGG

Supplementary Movies

Movie S1. Time-lapse multicolor images of β-actin mRNA granules (green and red dots) in live cells. Images were captured every 1 minutes for 7 minutes; playback is at 2 frames per second. Staining of the nuclei with Hoechst 33342 is shown in blue.

Movie S2. Time-lapse multicolor images of β-actin mRNA granules (green and red dots) in live cells. Images were captured every 1 minutes for 15 minutes; playback is at 2 frames per second. Staining of the nuclei with Hoechst 33342 is shown in blue.

Movie S3. Time-lapse multicolor images of β-actin (red dots) and cortactin (green dots) mRNA granules in live cells. Images were captured every 30 seconds for 15 minutes; playback is at 2 frames per second. Staining of the nuclei with Hoechst 33342 is shown in blue.

Movie S4. Time-lapse multicolor images of β-actin (red dots) and cortactin (green dots) mRNA granules in live cells. Images were captured every 30 seconds for 5 minutes; playback is at 2 frames per second. Staining of the nuclei with Hoechst 33342 is shown in blue.

Movie S5. Time-lapse multicolor images of β-actin (red dots) and cortactin (green dots) mRNA granules in live cells. Images were captured every 1 minutes for 15 minutes; playback is at 2 frames per second. Staining of the nuclei with Hoechst 33342 is shown in blue.

Movie S6. Time-lapse multicolor images of β-actin (red dots) and cortactin (green dots) mRNA granules in live cells under a cellular stress condition. The cells shown in **Movie S3** were further treated with $NaAsO_2$ (500 μM). Images were captured every 15 seconds for 6 minutes; playback is at 2 frames per second. Staining of the nuclei with Hoechst 33342 is shown in blue.

Movie S7. Time-lapse multicolor images of β-actin (red dots) and cortactin (green dots) mRNA granules in live cells under cellular stress induced by adding $NaAsO_2$ (500 μM). Images were captured every 12 seconds for 10 minutes; playback is at 2 frames per second. Staining of the nuclei with Hoechst 33342 is shown in blue.

Movie S8. Time-lapse multicolor images of β-actin (red dots) and cortactin (green dots) mRNA granules in live cells under cellular stress induced by adding $NaAsO_2$ (500 μM). Images were captured every 30 seconds for 13 minutes; playback is at 2 frames per second. Staining of the nuclei with Hoechst 33342 is shown in blue.

Movie S9. Time-lapse images without any aptamers in live cells. Images were captured every 12 seconds for 6 minutes; playback is at 2 frames per second. Staining of the nuclei with Hoechst 33342 is shown in blue.

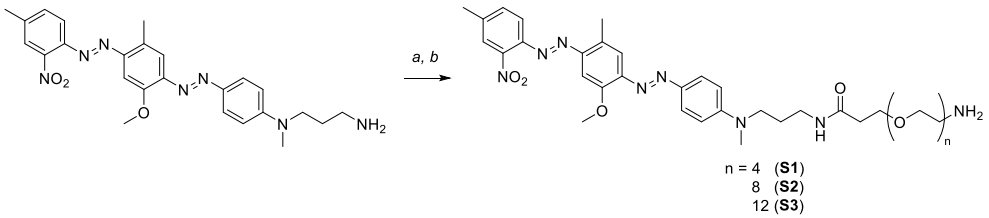
Supplementary Methods

Chemical Synthesis

General. The solvents and chemicals for chemical syntheses were used as purchased, with no further purification. Synthetic method of probe **1** was described in our previous research[1]. Methoxytrityl-*N*-dPEG®$_n$-TFP ester reagents were purchased from Quanta BioDesign, Ltd. (Ohio, USA). SulfoCy3-NHS-ester Cy5-NHS-ester and sulfoCy5-NHS ester were purchased from Lumiprobe Co. (Florida, USA). TAMRA-SE was purchased from AAT Bioquest, Inc. (California, USA). ATTO465 NHS was purchased from ATTO-TEC GmbH (Siegen, Germany). DEAC-SE was purchased from AnaSpec, Inc. (California, USA). NDB-X-SE was purchased from Setareh Biotech, LLC. (Oregon, USA). 5-Carboxyfluorescein-*N*-hydroxysuccinimide ester was purchased from Toronto Research Chemicals (Ontario, Canada). BODIPY 558/568 NHS, Marina Blue SE and Oregon Green 488 carboxylic acid were purchased from Thermo Fisher Scientific Inc. (Massachusetts, USA). BHQ-1 amine[2], Rhodamine Green-5-carboxylic acid[3], and DY (deSO$_3$-DY520-XL)[4] were synthesized according to the previous reports. All other solvents and chemicals were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), Watanabe Chemical Industries, Ltd. (Hiroshima, Japan), Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan) or Nacalai Tesque, Inc. (Kyoto, Japan).

High-performance liquid chromatography (HPLC) was performed with Shimadzu Prominence HPLC system. The synthetic molecules were purified by reversed-phase HPLC. HPLC conditions were as follows: GL science Inertsil® ODS-3 column (5 μm, 20 × 100 mm); solvent gradient, A, 0.1% trifluoroacetic acid (TFA) in H_2O; B, 0.1% TFA in acetonitrile with gradient indicated below; flow rate, 4.0 mL/min; detector, 220/254/350/480/550/650 nm. HPLC grade reagents were used. NMR spectra were recorded on Bruker Avance III 600 (600 MHz), or JEOL JNM-ECP300 (300 MHz) NMR systems. High-resolution mass spectra were obtained using a Bruker FT-ICR MS Solarix in ESI mode.

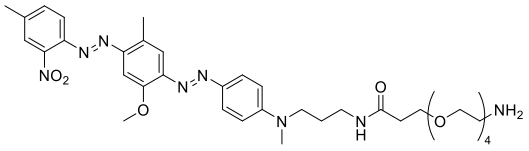
General procedure for synthesis of BHQ1-peg$_n$-NH$_2$ intermediates S1–S3.



Reagents and conditions: a) Methoxytrityl-*N*-dPEG®$_n$-TFP ester, *i*Pr$_2$NEt, DMSO, rt; b) TFA, rt.

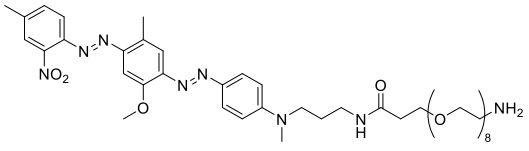
Methoxytrityl-*N*-dPEG®$_n$-TFP ester (15 μmol) was added to a solution of BHQ1 amine (4.75 mg, 10 μmol) and *i*Pr$_2$NEt (6.80 μL, 40 μmol) in DMSO (50 μL). After stirring for 3 h at room temperature, H_2O (300 μL) and hexane (500 μL) were added to the mixture, and the resulting mixture was centrifuged (21,500 ×g for 10 min). After removal of supernatant, residual solid or oil was washed with H_2O (500 μL). The residue was dried *in vacuo* and used for next step without further purification.

The dried residue was dissolved in TFA (400 μL). After stirring for 2 h at room temperature, the mixture was dried *in vacuo*. Resulting crude mixture was purified by preparative HPLC.



S1

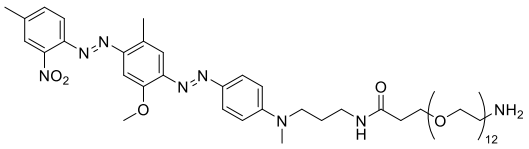
BHQ1-peg$_4$-NH$_2$ (S1). Methoxytrityl-*N*-dPEG®$_4$-TFP ester (10.3 mg, 15 μmol) converted according to the general procedure described above. Purification by HPLC (0–3 min: B, 55%; 3–15 min: B, 55–61% at RT, t_R = 11.9 min) gave **S1** as a TFA salt (60% yield). ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 7.95–7.92 (2H, m), 7.79 (2H, d, *J* = 9.0 Hz), 7.76 (1H, d, *J* = 7.8 Hz), 7.71 (3H, brs), 7.70–7.67 (1H, m), 7.51 (1H, d, *J* = 0.6 Hz), 7.29 (1H, s), 6.85 (2H, d, *J* = 9.0 Hz), 3.92 (3H, s), 3.62 (2H, t, *J* = 6.6 Hz), 3.58 (2H, t, *J* = 4.8 Hz), 3.57–3.52 (4H, m), 3.51–3.46 (10H, m), 3.13 (2H, q, *J* = 6.6 Hz), 3.06 (3H, s), 3.00–2.94 (2H, m), 2.63 (3H, s), 2.54 (3H, s), 2.34 (2H, t, *J* = 6.6 Hz), 1.72 (2H, quint, *J* = 7.2 Hz). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 170.02, 157.60, 157.40, 154.25, 151.68, 150.04, 146.29, 144.60, 143.43, 142.53, 142.19, 133.81, 132.12, 125.39, 124.18, 119.98, 118.39, 111.36, 99.10, 69.622, 69.616, 69.58, 69.53, 69.50, 69.42, 66.77, 66.54, 55.85, 49.30, 38.53, 38.21, 36.12, 36.07, 26.44, 20.58, 16.17. MS (ESI) calculated for $C_{36}H_{51}N_8O_8$ $[M+H]^+$ 723.3824, observed 723.3821.



S2

BHQ1-peg$_8$-NH$_2$ (S2). Methoxytrityl-*N*-dPEG®$_8$-TFP ester (12.9 mg, 15 μmol) converted according to the general procedure described above. Purification by HPLC (0–3 min: B, 55%; 3–23 min: B, 55–65% at RT, t_R = 20.6 min) gave **S2** as a TFA salt (57% yield). ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 7.95–7.93 (1H, m), 7.92 (1H, t, *J* = 6.0 Hz), 7.90 (2H, d, *J* = 9.6 Hz), 7.76 (1H, d, *J* = 8.4 Hz), 7.71 (3H, brs), 7.70–7.67 (1H, m), 7.51 (1H, d, *J* = 0.6 Hz), 7.29 (1H, s), 6.85 (2H, d, *J* = 9.6 Hz), 3.92 (3H, s), 3.62 (2H, t, *J* = 6.6 Hz), 3.59 (2H, t, *J* = 4.8 Hz), 3.57–3.46 (30H, m), 3.13 (2H, q, *J* = 6.6 Hz), 3.06 (3H, s), 2.97 (2H, q, *J* = 6.0 Hz), 2.63 (3H, d, *J* = 0.6 Hz), 2.54 (3H, s), 2.34 (2H, t, *J* = 6.6

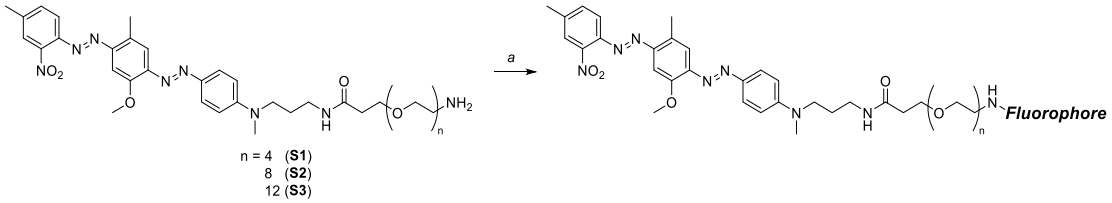
Hz), 1.72 (2H, quint, J = 7.2 Hz). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 169.99, 154.25, 151.68, 150.02, 146.29, 144.62, 143.43, 142.52, 142.19, 133.81, 132.12, 125.39, 124.18, 119.98, 118.38, 111.35, 99.09, 69.65, 69.63, 69.61, 69.58, 69.55, 69.51, 69.43, 66.79, 66.56, 55.84, 49.30, 39.97, 38.20, 36.12, 36.11, 26.44, 20.58, 16.16. MS (ESI) calculated for $C_{44}H_{67}N_8O_{12}$ $[M+H]^+$ 899.4873, observed 899.4873.



S3

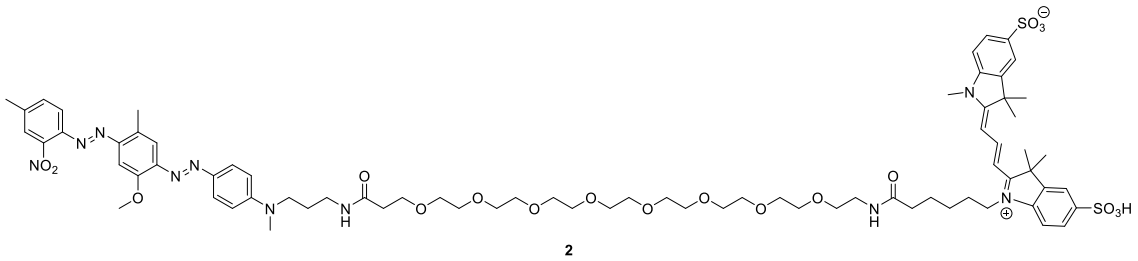
BHQ1-peg$_{12}$-NH$_2$ (S3). Methoxytrityl-*N*-dPEG®$_{12}$-TFP ester (15.6 mg, 15 μmol) converted according to the general procedure described above. Purification by HPLC (0–3 min: B, 60%; 3–17 min: B, 60–67% at RT, t_R = 14.9 min) gave **S3** as a TFA salt (49% yield). ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 7.94–7.93 (1H, m), 7.92 (1H, t, J = 6.0 Hz), 7.90 (2H, d, J = 9.0 Hz), 7.76 (1H, d, J = 7.8 Hz), 7.73 (3H, brs), 7.70–7.67 (1H, m), 7.51 (1H, d, J = 0.6 Hz), 7.29 (1H, s), 6.85 (2H, d, J = 9.0 Hz), 3.92 (3H, s), 3.62 (2H, t, J = 6.6 Hz), 3.59 (2H, t, J = 5.4 Hz), 3.58–3.54 (4H, m), 3.52–3.47 (42H, m), 3.13 (2H, q, J = 6.6 Hz), 3.06 (3H, s), 2.98 (2H, q, J = 6.0 Hz), 2.63 (3H, d, J = 0.6 Hz), 2.54 (3H, s), 2.34 (2H, t, J = 6.6 Hz), 1.72 (2H, quint, J = 7.2 Hz). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 169.99, 157.83, 157.62, 154.26, 151.68, 150.02, 146.30, 144.62, 143.43, 142.51, 142.19, 133.81, 132.13, 125.39, 124.17, 119.98, 118.39, 111.36, 99.08, 69.65, 69.64, 69.62, 69.59, 69.56, 69.52, 69.44, 66.80, 66.56, 55.84, 49.30, 43.67, 38.54, 38.20, 36.13, 36.11, 26.44, 20.58, 16.16. MS (ESI) calculated for $C_{52}H_{83}N_8NaO_{16}$ $[M+H+Na]^{2+}$ 549.2907, observed 549.2908.

General procedure for synthesis of BHQ1-fluorophore conjugated probes 2–5, 8, S4–S8.



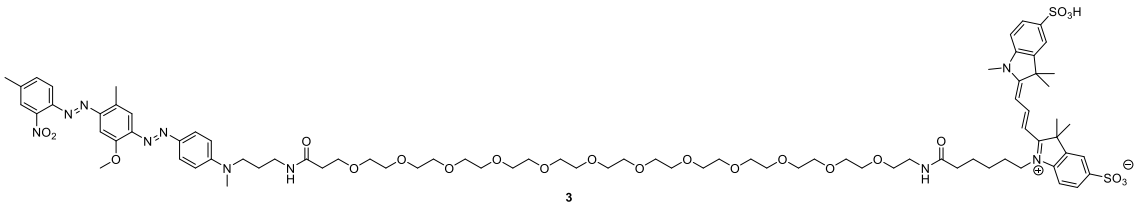
Reagents and conditions: a) fluorophore-NHS ester, *i*Pr$_2$NEt, DMSO, rt.

Fluorophore-NHS ester (1.1–1.5 eq.) was added to a solution of BHQ1-peg$_n$-NH$_2$ (1 eq.) and *i*Pr$_2$NEt (4 eq.) in DMSO (20 μL). After stirring for 3–12 h at room temperature, the mixture was diluted with acetonitrile/H$_2$O/TFA (600 μL, 47.5:47.5:5 solution) and the mixture was purified by preparative HPLC.



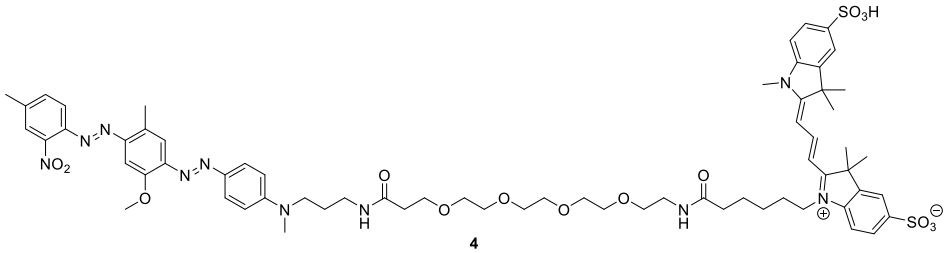
2

BHQ1-peg$_8$-sulfoCy3 (2). BHQ1-peg$_8$-NH$_2$ (**S2**) (0.50 μmol) and sulfoCy3-NHS (0.551 mg, 0.75 μmol) were converted according to the general procedure described above. Purification by HPLC (0–3 min: B, 45%; 3–20.5 min: B, 45–58% at RT, t_R = 22.0 min) gave **2** (22% yield). MS (ESI) calculated for $C_{74}H_{100}N_{10}Na_2O_{19}S_2$ $[M+2Na]^{2+}$ 771.3196, observed 771.3183.



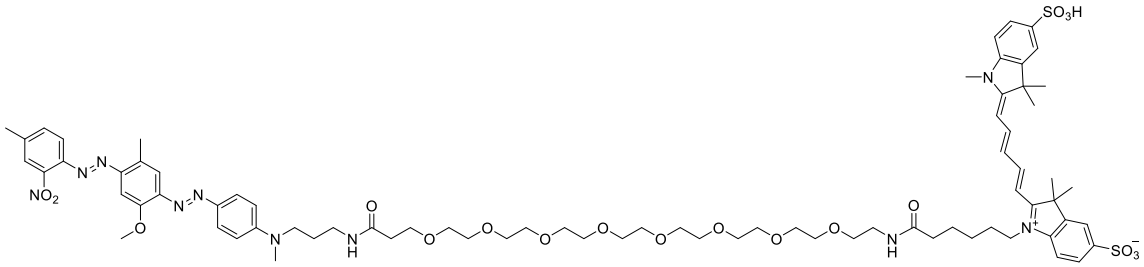
3

BHQ1-peg$_{12}$-sulfoCy3 (3). BHQ1-peg$_{12}$-NH$_2$ (**S3**) (0.50 μmol) and sulfoCy3-NHS (0.551 mg, 0.75 μmol) were converted according to the general procedure described above. Purification by HPLC (0–3 min: B, 45%; 3–28 min: B, 45–55% at RT, t_R = 24.9 min) gave **3** (16% yield). MS (ESI) calculated for $C_{82}H_{116}N_{10}Na_2O_{23}S_2$ $[M+2Na]^{2+}$ 859.3720, observed 859.3703.



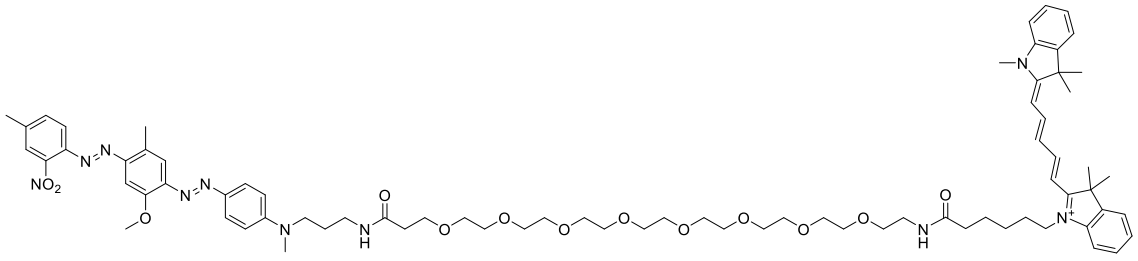
4

BHQ1-peg$_4$-sulfoCy3 (4). BHQ1-peg$_4$-NH$_2$ (**S1**) (0.25 μmol) and sulfoCy3-NHS (0.276 mg, 0.38 μmol) were converted according to the general procedure described above. Purification by HPLC (0–3 min: B, 50%; 3–23 min: B, 50–70% at RT, t_R = 18.2 min) gave **4** (19% yield). MS (ESI) calculated for $C_{66}H_{84}N_{10}Na_2O_{15}S_2$ $[M+2Na]^{2+}$ 683.2672, observed 683.2670.



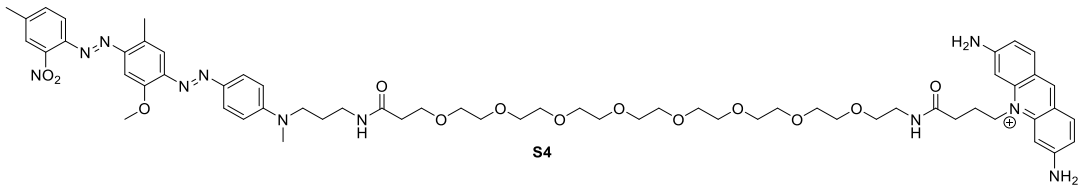
5

BHQ1-peg$_8$-sulfoCy5 (5). BHQ1-peg$_8$-NH$_2$ (**S2**) (0.25 μmol) and sulfoCy5-NHS (0.248 mg, 0.33 μmol) were converted according to the general procedure described above. Purification by HPLC (0–3 min: B, 45%; 3–21 min: B, 45–61% at RT, t_R = 19.2 min) gave **5** (79% yield). ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 8.34 (2H, dd, 13.2, 13.2), 7.94–7.90 (2H, m), 7.82–7.78 (1H, m), 7.81 (1H, s), 7.78 (2H, d, *J* = 9.0 Hz), 7.76 (1H, d, *J* = 8.4 Hz), 7.69–7.66 (1H, m), 7.66–7.62 (2H, m), 7.50 (1H, d, *J* = 0.6 Hz), 7.33–7.28 (2H, m), 7.28 (1H, s), 6.84 (2H, d, *J* = 9.0 Hz), 6.55 (1H, dd, *J* = 13.2, 13.2 Hz), 6.29 (1H, d, *J* = 13.2 Hz), 6.24 (1H, d, *J* = 13.2 Hz), 4.06 (2H, t, *J* = 7.2 Hz), 3.91 (3H, s), 3.61 (2H, t, *J* = 6.6 Hz), 3.58 (3H, s), 3.53–3.46 (32H, m), 3.40–3.30 (16H, m) 3.19–3.11 (4H, m), 3.05 (3H, s), 2.62 (3H, s), 2.33 (2H, t, *J* = 6.6 Hz), 2.05 (2H, t, *J* = 7.2 Hz), 1.75–1.65 (2H, m), 1.53 (2H, quint, *J* = 7.2 Hz), 1.37–1.29 (2H, m), 1.28–1.23 (2H, m). MS (ESI) calculated for $C_{76}H_{102}N_{10}NaO_{19}S_2$ $[M+Na]^{2+}$ 784.3274, observed 784.3270. Quantum yield (φ): 0.31%. Molar extinction coefficient (ε): 271000.



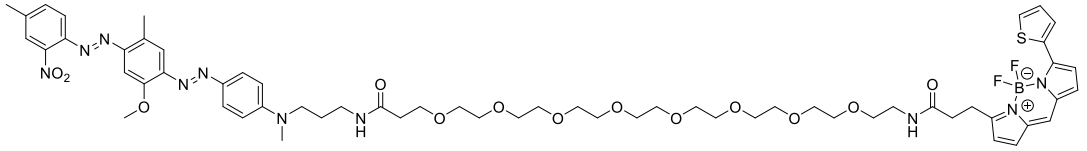
8

BHQ1-peg$_8$-Cy5 (8). BHQ1-peg$_8$-NH$_2$ (**S2**) (0.50 μmol) and Cy5-NHS (0.370 mg, 0.60 μmol) were converted according to the general procedure described above. Purification by HPLC (0–3 min: B, 55%; 3–33 min: B, 55–80% at RT, t_R = 29.1 min) gave **5** (76% yield). MS (ESI) calculated for $C_{76}H_{103}N_{10}NaO_{13}$ $[M+Na]^{2+}$ 693.3796, observed 693.3791. Quantum yield (φ): 0.29%. Molar extinction coefficient (ε): 250000.



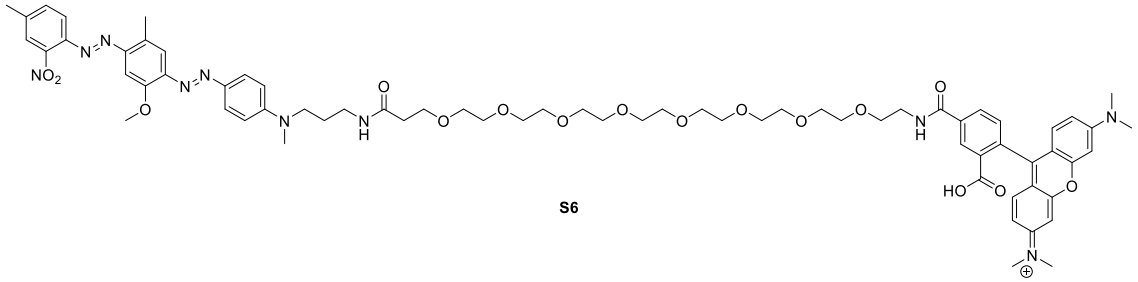
S4

BHQ1-peg$_8$-ATTO465 (S4). BHQ1-peg$_8$-NH_2 (**S2**) (0.50 μmol) and ATTO465-NHS (0.271 mg, 0.55 μmol) were converted according to the general procedure described above. Purification by HPLC (0–3 min: B, 50%; 3–23 min: B, 50–60% at RT, t_R = 21.2 min) gave **S4** (83% yield). MS (ESI) calculated for $C_{61}H_{83}N_{11}O_{13}$ $[M+H]^{2+}$ 588.8080, observed 588.8081.



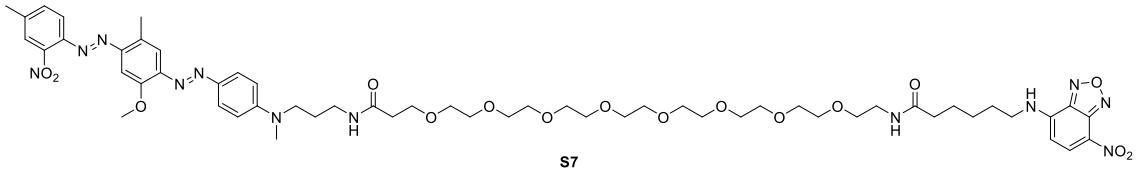
S5

BHQ1-peg$_8$-BODIPY 558/568 (S5). BHQ1-peg$_8$-NH_2 (**S2**) (0.50 μmol) and BODIPY 558/568-NHS (0.266 mg, 0.60 μmol) were converted according to the general procedure described above. Purification by HPLC (0–3 min: B,70%; 3–23 min: B, 70–78% at RT, t_R = 20.1 min) gave **S5** (4.7% yield). MS (ESI) calculated for $C_{60}H_{77}BF_2N_{10}O_{13}S$ $[M+H]^{2+}$ 613.2721, observed 613.2720.



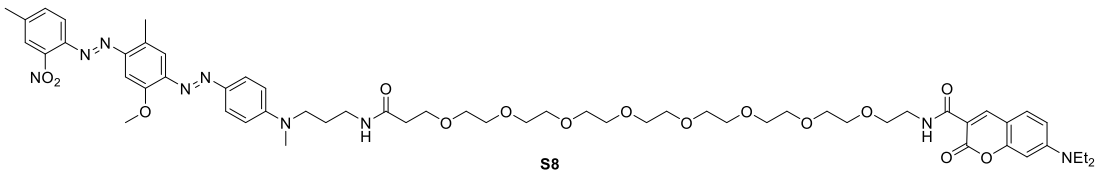
S6

BHQ1-peg$_8$-TAMRA (S6). BHQ1-peg$_8$-NH_2 (**S2**) (0.50 μmol) and TAMRA-SE (0.271 mg, 0.55 μmol) were converted according to the general procedure described above. Purification by HPLC (0–3 min: B, 50%; 3–23 min: B, 50–60% at RT, t_R = 23.7 min) gave **S6** (quant.). MS (ESI) calculated for $C_{69}H_{87}N_{10}NaO_{16}$ $[M+Na]^{2+}$ 667.3094, observed 667.3090.



S7

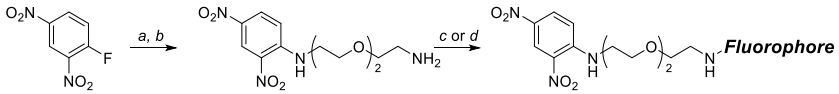
BHQ1-peg$_8$-NBD-X (S7). BHQ1-peg$_8$-NH_2 (**S2**) (0.25 μmol) and NBD-X-SE (0.147 mg, 0.38 μmol) were converted according to the general procedure described above. Purification by HPLC (0–3 min: B, 60%; 3–28 min: B, 60–85% at RT, t_R = 26.5 min) gave **S7** (14% yield). MS (ESI) calculated for $C_{56}H_{80}N_{12}O_{16}$ $[M+2H]^{2+}$ 588.2902, observed 588.2904.



S8

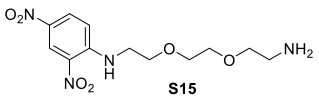
BHQ1-peg$_8$-DEAC (S8). BHQ1-peg$_8$-NH$_2$ (**S2**) (0.25 μmol) and DEAC-SE (0.134 mg, 0.38 μmol) were converted according to the general procedure described above. Purification by HPLC (0–3 min: B, 70%; 3–27 min: B, 70–86% at RT, t_s = 9.54 min) gave **S7** (12% yield). MS (ESI) calculated for $C_{58}H_{79}N_9NaO_{15}$ $[M+Na]^+$ 1164.5588, observed 1164.5580.

Synthesis of DNB-peg$_2$-fluorophore conjugated probes 6, **S9**–**S14.**



S15 S16

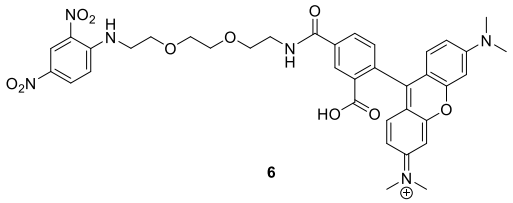
Reagents and conditions: a) BocNH-PEG$_2$-NH$_2$, *i*Pr$_2$NEt, CH$_2$Cl$_2$, rt; b) 4 M HCl/AcOEt, rt; c) fluorophore-NHS ester, *i*Pr$_2$NEt, DMSO, rt; d) fluorophore-CO$_2$H, HATU, HOAt, *i*Pr$_2$NEt, DMSO, rt.



S15

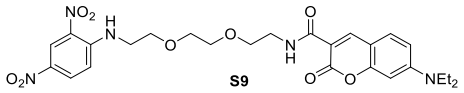
DNB-peg$_2$-NH$_2$ (S15). 2,4-dinitrofluorobenzen (106 μL 0.85 mmol) was added to a solution of BocNH-PEG$_n$-NH$_2$ (233 mg, 0.94 mmol) and *i*Pr$_2$NEt (288 μL 1.7 mmol) in CH$_2$Cl$_2$ (5 mL). After stirring for 2 h at room temperature, 0.2 M HCl aq. (20 mL) was added to the mixture, and the resulting mixture was extracted with CH$_2$Cl$_2$ (10 mL × 3). The combined organic layer was washed with brine (10 mL), dried over Na$_2$SO$_4$, filtered, and evaporated. The residue was purified by silica gel column chromatography using hexane/AcOEt (77:23 to 19:81) as eluent, to give DNB-peg$_2$-NHBoc (322mg, 92% yield). ^{1}H-NMR (300 MHz, CDCl$_3$) δ: 9.15 (1H, d, *J* = 2.8 Hz), 8.82 (1H, brs) 8.29 (1H, dd, *J* = 2.8, 9.3), 6.94 (1H, d, *J* = 9.3), 4.97 (1H, brs), 3.84 (2H, t, *J* = 5.4), 3.75–3.50 (m, 8H), 3.33 (2H, q, *J* = 4.8), 1.43 (9H, s).

DNB-peg$_2$-NHBoc (322 mg, 0.78 mmol) was dissolved in 4 M HCl/AcOEt (5 mL) and stirred at room temperature for 3 h. The resulting solid was collected by filtration to give **S15** as a HCl salt (236 mg, 86% yield). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 8.87 (1H, d, *J* = 2.4 Hz), 8.87–8.83 (1H, m), 8.28 (1H, dd, *J* = 2.4, 9.6 Hz), 7.97 (3H, brs), 7.30 (1H, d, *J* = 9.6 Hz), 3.75–3.66 (4H, m), 3.65–3.55 (6H, m), 2.94 (2H, q, *J* = 5.4 Hz). MS (ESI) calculated for $C_{12}H_{19}N_4O_6$ $[M+H]^+$ 315.1299, observed 315.1299.



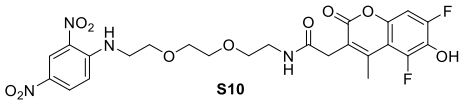
6

NB-peg$_2$-TAMRA (6). TAMRA-SE (0.158 mg, 0.30 μmol) was added to a solution of DNB-peg$_2$-NH$_2$ (**S15**) (0.0875 mg, 0.25 μmol) and *i*Pr$_2$NEt (0.170 μL 1.0 μmol) in DMSO (20 μL). After stirring for 12 h at room temperature, the mixture was diluted with acetonitrile/H$_2$O/TFA (600 μL, 47.5:47.5:5 solution) and the mixture was purified by preparative HPLC (0–3 min: B, 40%; 3–21 min: B, 40–49% at RT, t_R = 20.9 min) gave **6** (84% yield). ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 13.31 (1H, brs), 8.92–8.87 (1H, m), 8.85 (1H, t, *J* = 5.4 Hz), 8.83 (1H, d, *J* = 2.4 Hz), 8.63 (1H, brs), 8.26 (1H, d, *J* = 7.8 Hz), 8.23 (1H, ddd, *J* = 0.6, 2.4, 9.6 Hz), 7.51 (1H, brs), 7.26 (1H, d, *J* = 9.6 Hz), 7.23–6.64 (5H, m), 6.53 (1H, m), 3.72 (2H, t, *J* = 5.4 Hz), 3.65 (2H, t, *J* = 5.4 Hz), 3.65–3.59 (4H, m), 3.59 (2H, t, *J* = 6.0 Hz), 3.53–3.45 (2H, m), 3.22 (12H, brs). MS (ESI) calculated for $C_{37}H_{39}N_6O_{10}$ $[M]^+$ 727.2722, observed 727.2714.



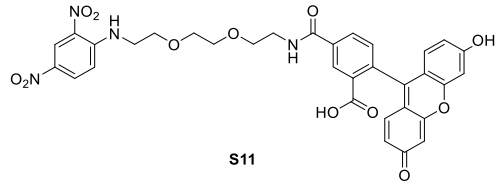
S9

DNB-peg$_2$-DEAC (S9). DEA-SE (0.128 mg, 0.30 μmol) was added to a solution of DNB-peg$_2$-NH$_2$ (**S15**) (0.0875 mg, 0.25 μmol) and *i*Pr$_2$NEt (0.170 μL 1.0 μmol) in DMSO (20 μL). After stirring for 10 h at room temperature, the mixture was diluted with acetonitrile/H$_2$O/TFA (600 μL, 47.5:47.5:5 solution) and the mixture was purified by preparative HPLC (0–3 min: B, 45%; 3–33 min: B, 45–75% at RT, t_R = 32.0 min) gave **S9** (41% yield). MS (ESI) calculated for $C_{26}H_{31}N_5NaO_9$ $[M+Na]^+$ 580.2014, observed 580.2014.



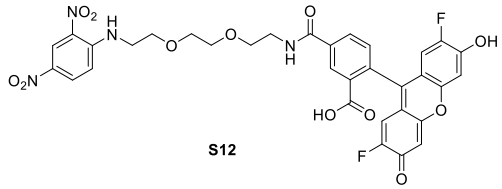
S10

DNB-peg$_2$-Marina Blue (S10). Marina Blue-SE (0.110 mg, 0.30 μmol) was added to a solution of DNB-peg$_2$-NH$_2$ (**S15**) (0.0875 mg, 0.25 μmol) and *i*Pr$_2$NEt (0.170 μL 1.0 μmol) in DMSO (20 μL). After stirring for 10 h at room temperature, the mixture was diluted with acetonitrile/H$_2$O/TFA (600 μL, 47.5:47.5:5 solution) and the mixture was purified by preparative HPLC (0–3 min: B, 45%; 3–28 min: B, 45–70% at RT, t_R = 18.5 min) gave **S10** (41% yield). MS (ESI) calculated for $C_{24}H_{24}F_2N_4NaO_{10}$ $[M+Na]^+$ 589.1353, observed 589.1353.



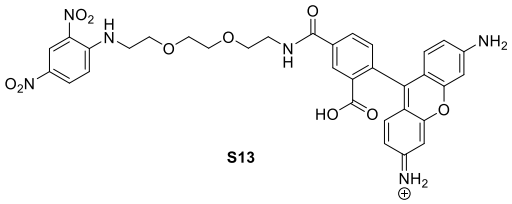
S11

DNB-peg$_2$-fluorescein (S11). 5-Carboxyfluorescein-N-hydroxysuccinimide Ester (0.142 mg, 0.30 μmol) was added to a solution of DNB-peg$_2$-NH$_2$ (**S15**) (0.0875 mg, 0.25 μmol) and *i*Pr$_2$NEt (0.170 μL 1.0 μmol) in DMSO (20 μL). After stirring for 10 h at room temperature, the mixture was diluted with acetonitrile/H$_2$O/TFA (600 μL, 47.5:47.5:5 solution) and the mixture was purified by preparative HPLC (0–3 min: B, 45%; 3–28 min: B, 45–70% at RT, t_R = 20.2 min) gave **S11** (53% yield). MS (ESI) calculated for $C_{33}H_{28}N_4NaO_{12}$ $[M+Na]^+$ 695.1596, observed 695.1594.



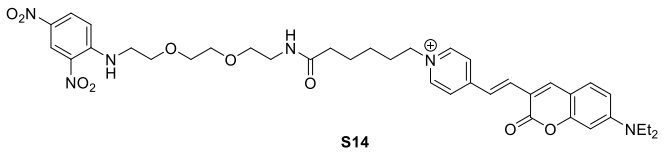
S12

DNB-peg$_2$-Oregon Green 488 (S12). *i*Pr$_2$NEt (0.170 μL 1.0 μmol) was added to a solution of Oregon Green 488 carboxylic acid (0.124 mg, 0.30 μmol), HATU (0.190 mg, 0.50 μmol), HOAt (0.0681 mg, 0.50 μmol) and DNB-peg$_2$-NH$_2$ (**S15**) (0.0875 mg, 0.25 μmol) in DMSO (20 μL). After stirring for 10 h at room temperature, the mixture was diluted with acetonitrile/H$_2$O/TFA (600 μL, 47.5:47.5:5 solution) and the mixture was purified by preparative HPLC (0–3 min: B, 45%; 3–28 min: B, 45–70% at RT, t_R = 21.9 min) gave **S12** (25% yield). MS (ESI) calculated for $C_{33}H_{26}F_2N_4NaO_{12}$ $[M+Na]^+$ 731.1407, observed 731.1406.



S13

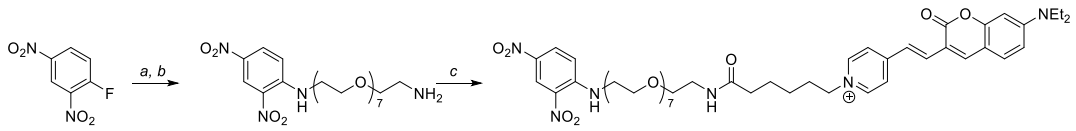
DNB-peg$_2$-Rhodamine Green (S13). *i*Pr$_2$NEt (0.170 μL 1.0 μmol) was added to a solution of Rhodamine Green-5-carboxylic acid (0.147 mg, 0.30 μmol), HATU (0.190 mg, 0.50 μmol), HOAt (0.0681 mg, 0.50 μmol) and DNB-peg$_2$-NH$_2$ (**S15**) (0.0875 mg, 0.25 μmol) in DMSO (20 μL). After stirring for 10 h at room temperature, the mixture was diluted with acetonitrile/H$_2$O/TFA (600 μL, 47.5:47.5:5 solution) and the mixture was purified by preparative HPLC (0–3 min: B, 40%; 3–28 min: B, 40–65% at RT, t_R = 11.9 min) gave **S13** (22% yield). MS (ESI) calculated for $C_{33}H_{31}N_6O_{10}$ $[M]^+$ 671.2096, observed 671.2093.



S14

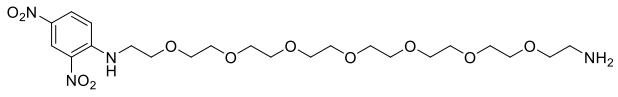
DNB-peg$_2$-DY (S14). *i*Pr$_2$NEt (0.170 μL 1.0 μmol) was added to a solution of deSO$_3$-DY520-XL (0.163 mg, 0.30 μmol), HATU (0.190 mg, 0.50 μmol), HOAt (0.0681 mg, 0.50 μmol) and DNB-peg$_2$-NH$_2$ (**S15**) (0.0875 mg, 0.25 μmol) in DMSO (20 μL). After stirring for 16.5 h at room temperature, the mixture was diluted with acetonitrile/H$_2$O/TFA (600 μL, 47.5:47.5:5 solution) and the mixture was purified by preparative HPLC (0–3 min: B, 50%; 3–23 min: B, 50–60% at RT, t_R = 15.0 min) gave **S14** (62% yield).

Synthesis of DNB-peg$_7$-DY conjugated probe 7.



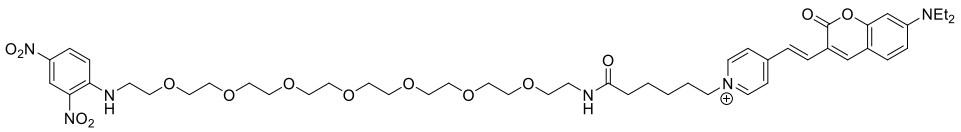
S17 7

Reagents and conditions: a) BocNH-PEG$_7$-NH$_2$, *i*Pr$_2$NEt, CH$_3$CN, rt; b) 4 M HCl/AcOEt/AcOEt (3:1), rt; c) deSO$_3$-DY520-XL, HATU, HOAt, *i*Pr$_2$NEt, DMSO, rt.



S17

DNB-peg$_7$-NH$_2$ (S17). 2,4-dinitrofluorobenzen (0.628 μL 5.0 μmol) was added to a solution of BocNH-PEG$_n$-NH$_2$ (2.81 mg, 6.0 μmol) and *i*Pr$_2$NEt (1.70 μL 10 μmol) in CH$_3$CN (50 μL). After stirring for 2 h at room temperature, 0.2 M HCl aq. (200 μL) was added to the mixture, and the resulting mixture was extracted with CH$_2$Cl$_2$ (200 μL × 4). The combined organic layer was washed with brine (100 μL), dried over Na$_2$SO$_4$, filtered, and evaporated. The residue was dissolved in 4 M HCl/AOEt (300 μL) / AcOEt (100 μL) and stirred at room temperature for 2 h. Hexane (2 mL) was added to the mixture, and the resulting mixture was centrifuged (21,500 ×g for 10 min). After removal of supernatant, residual oil was used for next step without further purification. to give **S17** as a HCl salt. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 8.87 (1H, d, J = 3.0 Hz), 8.86–8.84 (1H, m), 8.27 (1H, ddd, J = 0.6, 3.0, 9.6 Hz), 7.74 (3H, brs), 7.29 (1H, d, J = 9.6 Hz), 3.71–3.65 (4H, m), 3.60–3.47 (26H, m), 2.97 (2H, t, J = 5.4 Hz). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 148.29, 134.81, 129.79, 129.58, 123.47, 115.60, 69.70, 69.69. 69.65, 69.64, 69.62, 69.55, 69.51, 42.57, 38.53. MS (ESI) calculated for C$_{22}$H$_{39}$N$_4$O$_{11}$ [M+H]$^+$ 535.2610, observed 535.2607.



7

DNB-peg$_7$-DY (7). *i*Pr$_2$NEt (0.170 µL 1.0 µmol) was added to a solution of deSO$_3$-DY520-XL (0.163 mg, 0.30 µmol), HATU (0.190 mg, 0.50 µmol), HOAt (0.0681 mg, 0.50 µmol) and DNB-peg$_2$-NH$_2$ (**S15**) (0.0875 mg, 0.25 µmol) in DMSO (20 µL). After stirring for 16.5 h at room temperature, the mixture was diluted with acetonitrile/H$_2$O/TFA (600 µL, 47.5:47.5:5 solution) and the mixture was purified by preparative HPLC (0–3 min: B, 50%; 3–23 min: B, 50–60% at RT, t_R = 15.7 min) gave **S14** as a TFA salt (62% yield). ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 8.87–8.82 (4H, m), 8.25 (1H, ddd *J* = 0.6, 2.4, 9.6 Hz), 8.21 (1H, s), 8.16 (2H, d, *J* = 9.6 Hz), 7.85–7.81 (2H, m), 7.68 (1H, d, *J* = 16.2 Hz), 7.54 (1H, d, *J* = 9.0 Hz), 7.27 (1H, d, *J* = 9.6 Hz), 6.80 (1H, dd, *J* = 2.4, 9.0 Hz), 6.60 (1H, d, *J* = 2.4 Hz), 6.53 (1H, brs), 4.45 (2H, t, *J* = 7.2 Hz), 3.70–3.63 (4H, m), 3.59–3.56 (2H, m), 3.54–3.44 (26H, m), 3.17 (2H, q, *J* = 6.0 Hz), 2.07 (2H, t, *J* = 7.2 Hz), 1.89 (2H, quint, *J* = 7.2 Hz), 1.53 (2H, quint, *J* = 7.2 Hz), 1.28–1.18 (4H, m), 1.15 (6H, t, *J* = 7.2 Hz). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 171.83, 159.48, 156.22, 153.22, 151.90, 148.26, 145.32, 143.83, 136.87, 134.78, 130.65, 129,76, 129.53, 123.43, 123.19, 122.49, 115.55, 113.52, 109.95, 108.25, 96.12, 69.69, 69.65, 69.64, 69.63 69.60, 69.44, 69.03, 59.20, 44.27, 38.3234.74, 30.12, 24.86, 24.35, 12.26. MS (ESI) calculated for $C_{48}H_{67}N_6O_{14}$ $[M]^+$ 951.4710, observed 951.4707. Quantum yield (φ): 6.7%. Molar extinction coefficient (ε): 49244.

General procedure

UV-vis absorption spectra in a binding buffer (10 mM Tris-HCl, 100 mM KCl, 5 mM $MgCl_2$, pH 7.6) were measured using a U-3010 spectrophotometer (Hitachi High-Technologies Corp.). Fluorescence spectra were recorded using an LS 55 Fluorescence spectrometer (PerkinElmer Co., Ltd.). The live-cell and fixed cell imaging were performed using a CellVoyager™ CV1000 Confocal Scanner Box (Yokogawa Electric Corp.), equipped with an ultra-sensitive EMCCD C_9100 13 camera (512 × 512 pixels) and an Olympus UPLSAPO 60XO 1.35 NA oil objective. The excitation laser wavelength and emission filter were as follows: excitation 405 and emission BP477/60 nm for detection of Hoechst signal; excitation 488 and emission BP617/73 nm for detection of probe **7** (DY); excitation 561 and emission BP617/73 nm for detection of probes **5** (sulfoCy5) and **8** (Cy5). The images were analyzed using CellVoyager™ CV1000 measurement software and NIH ImageJ, and presented as maximum-intensity projection (MIP) or the best focus single plane.

The BHQ1-binding aptamer A1 was prepared as described in a previous report[1,5]. The dsDNAs for T7 transcription of DNB-binding aptamer were PCR amplified using corresponding pUC19 plasmids as a template, with primer sets described in **Table S4** (see Method for *in vitro* selection procedures for DNB-binding aptamer). The dsDNA templates for the DNB-binding aptamer and DNB-short aptamers were prepared by PCR, amplified using pUC19-DNB1 plasmid as a template, with the primer set described in **Table S4**. The dsDNA template for the mutated version of DNB-short aptamer (DNB short mut) was prepared by PCR, amplified using dsDNA from annealing of two synthetic ssDNAs (**Table S4**) as a template, with the primer set described in **Table S4**. The dsDNA templates for the RNA aptamers and target RNAs were prepared by annealing of two synthetic ssDNAs (**Table S4**). RNAs for *in vitro* experiments were prepared by *in vitro* transcription reaction at 37°C for 12 h using T7 MegaScript kit (invitrogen), and purified using an NAP-5 column to remove unincorporated NTPs. The dsDNA templates of the RNA aptamers that were responsive to β-actin or cortactin were prepared by annealing two synthetic ssDNAs (**Table S4**). The dsDNAs were subcloned into the *Bgl* II and *Hind* III sites in pSuper.neo vector. Synthetic ssDNAs were purchased from Thermo Fisher Scientific Inc. (Massachusetts, USA).

Fluorescence measurement. Fluorescence spectra of each probe (2 μM) were obtained in binding buffer (10 mM Tris-HCl, 100 mM KCl, 5 mM $MgCl_2$, pH 7.6), in the absence or presence of RNA aptamer and target RNA (annealed before mixing with probe). The excitation wavelengths and fluorescence emissions (Ex/Em) monitored for each conjugates were 520/540-700 nm for probes **1**–**4**, **6**, **S5**, and **S6**; 620/640-800 nm for probe **5**; 430/450-600 nm for probes **S4** and **S7**; 390/420-600 nm for probes **S8**–**S10**; 480/500-650 nm for probes **S11**–**S13**; and 500/550-800 nm for probes **7** and **S14**.

***In vitro* selection procedures for DNB binding aptamer (Figure S7).** BHQ1-immobilized affinity resin was prepared as described in a previous report[1,5]. DNB-peg$_2$-NH$_2$ (**S15**) was immobilized to EAH Sepharose™ 4B resin, linked by diglycolic acid to give DNB-immobilized affinity resin. The dsDNA library for templates of the 1st round RNA pool was prepared by Klenow reaction of template and reverse primer (**Table S4**). The dsDNA template was converted to an RNA pool by *in vitro* transcription reaction at 37°C for 12 h, using T7 MegaScript kit (Invitrogen), and purified using an NAP-5 column. The resulting RNA pool was annealed in binding buffer (200 µL for 1st round, 100 µL for 2nd and additional rounds, 10 mM Tris-HCl, 100 mM KCl, pH 7.6). After addition of 1 µL of $MgCl_2$ solution (final concentration, 5 mM), the solution was incubated on the BHQ1-immobilized affinity resin for 15 minutes at 4ºC. The unbound RNA species were drained, and the pass-through faction was incubated on the DNB-immobilized affinity resin for 30 minutes at 4ºC. The unbound RNA species were drained, and the resin was washed 8 times with a binding buffer to remove the non-binding species. Bound RNA species were eluted using binding buffer saturated with free DNB-peg$_2$-NH$_2$ solution (1 mM), 3 times for 10 min each. The eluted fractions were pooled and precipitated with ethanol. The selected RNAs were reverse-transcribed using ReverTra Ace® (Toyobo Co., Ltd.), and the resulting cDNA was PCR-amplified with the forward and the reverse primers in **Table S4**. The DNA templates were transcribed *in vitro*, and the resulting RNAs were subjected to the next round of selection. After eight rounds of selection, enriched RNAs were reverse-transcribed, and converted to dsDNA by PCR. The resulting DNAs were ligated into pUC19 at the *Eco*RI and *Bam*HI sites, and transformed to stellar competent cells of an *E. coli* strain (Takara Bio Inc.). The resulting clones were isolated and sequenced.

***K*d determination.** Probe **6** (2 µM) was dissolved in the binding buffer in the presence of RNA (0, 1, 3, 10, 30 or 100 µM), and fluorescence intensity was measured using an MTP-800 multi microplate reader (CORONA) with an Ex/Em = 550/610 nm filter set. The *K*d values of DNB1-DNB4 were estimated from the fluorescence titration data, using the equation:

$$F\text{obs} = A((([\mathbf{6}]_T + [\text{aptamer}]_T + K\text{d}) - (([\mathbf{6}]_T + [\text{aptamer}]_T + K\text{d})^2 - 4[\mathbf{6}]_T[\text{aptamer}]_T)^{1/2})/2[\mathbf{6}]_T)$$

where A is the increase in fluorescence at saturating DNB1 concentration (F max - F min), Kd is the dissociation constant, and $[\mathbf{6}]_T$ and $[\text{aptamer}]_T$ are the total concentrations of probe **6** and aptamer DNB1-DNB4, respectively.

Cell culture and DNA transfection. HeLa cells were maintained in Dulbecco's modified Eagle medium (Gibco®), supplemented with 100 units/mL penicillin, 100 µg/mL streptomycin sulfate, and 10% (v/v) fetal bovine serum (Biowest), at 37°C and 5% CO_2 in a humidified incubator. The cells

were transfected with the aptamer expression plasmids using FuGENE HD transfection reagent (Promega), according to the manufacturer's protocol. To induce stress, 0.05 M sodium arsenite solution (Sigma-Aldrich Co., LLC) was added (final concentration, 500 μM). The stress-induced cells were used immediately for live-cell experiments, and after 1 h incubation for fixed-cell experiments.

Fixed cell imaging. HeLa cells were seeded on a Poly-D-lysine coated glass-bottom ViewPlate®-96F microplate (PerkinElmer, Inc.) at 3.0–5.0 × 10^3 cells per well. After day 1, cells were fixed with 4% paraformaldehyde solution (Muto Pure Chemicals Co., Ltd) at room temperature for 15 min, washed twice with PBS, and then permeabilized with methanol overnight at -20°C. The cells were soaked in 2× SSC buffer (300 mM NaCl, 30 mM sodium citrate, pH 7.0) containing 50% formamide at 50°C for 15 min. The cells were then incubated at 4°C for 24 h with a 2× SSC buffer (50 μL), containing 10 μM aptamers, 10% dextran sulfate, 0.02% RNase-free BSA, and 40 μg *E. coli* tRNA. Excess aptamers were washed out twice with 2× SSC buffer. The cells were then treated at 4°C for 5 min with probes **5** (2 μM) and **7** (2 μM), and DAPI (2 μg/mL) in 2× SSC buffer (30 μL). Excess probes were washed out with 2× SSC buffer, and the cells were observed on CV1000 microscope. The cell images were taken with a vertical range of 2 μm, and each image stack was then projected onto a single plane.

Live-cell imaging. On day 0, HeLa cells were seeded on a 12-well plate at 5.0 × 10^4 cells per well. On day 1, the cells were transfected with pSuper.neo vectors encoding each aptamer. On day 2, the cells were detached with 0.25% trypsin, and re-seeded at 1.0 × 10^4 cells per cell on CELLVIEW™ glass bottom cell culture dishes (Greiner Bio One International GmbH, 4 compartments, 35 mm, pre-coated with advanced TC) in the complete growth medium, then incubated at 37°C for 10 min in a humidified 5% CO_2 incubator. After the cells attached to the plate, they were stained with probes **7** (5 μM) and **8** (5 μM), and Hoechst 33342 (1 μg/mL) in the complete growth medium (100 μL), and incubated at 37°C for 5 min in a humidified 5% CO_2 incubator. The medium was then changed to Medium 199, Hank's Balanced Salts (Gibco®), and the cells were observed on a CV1000 microscope. Live-cell images were collected as 4 μm of vertical z-stack range (~11 focal planes), and presented as MIP. Time-lapse images were taken at 0-30 s intervals for up to 10 minutes.

Supplementary References

(1) Murata, A.; Sato, S.; Kawazoe, Y.; Uesugi, M. *Chem. Commun.* **2011**, *47*, 4712–4714.

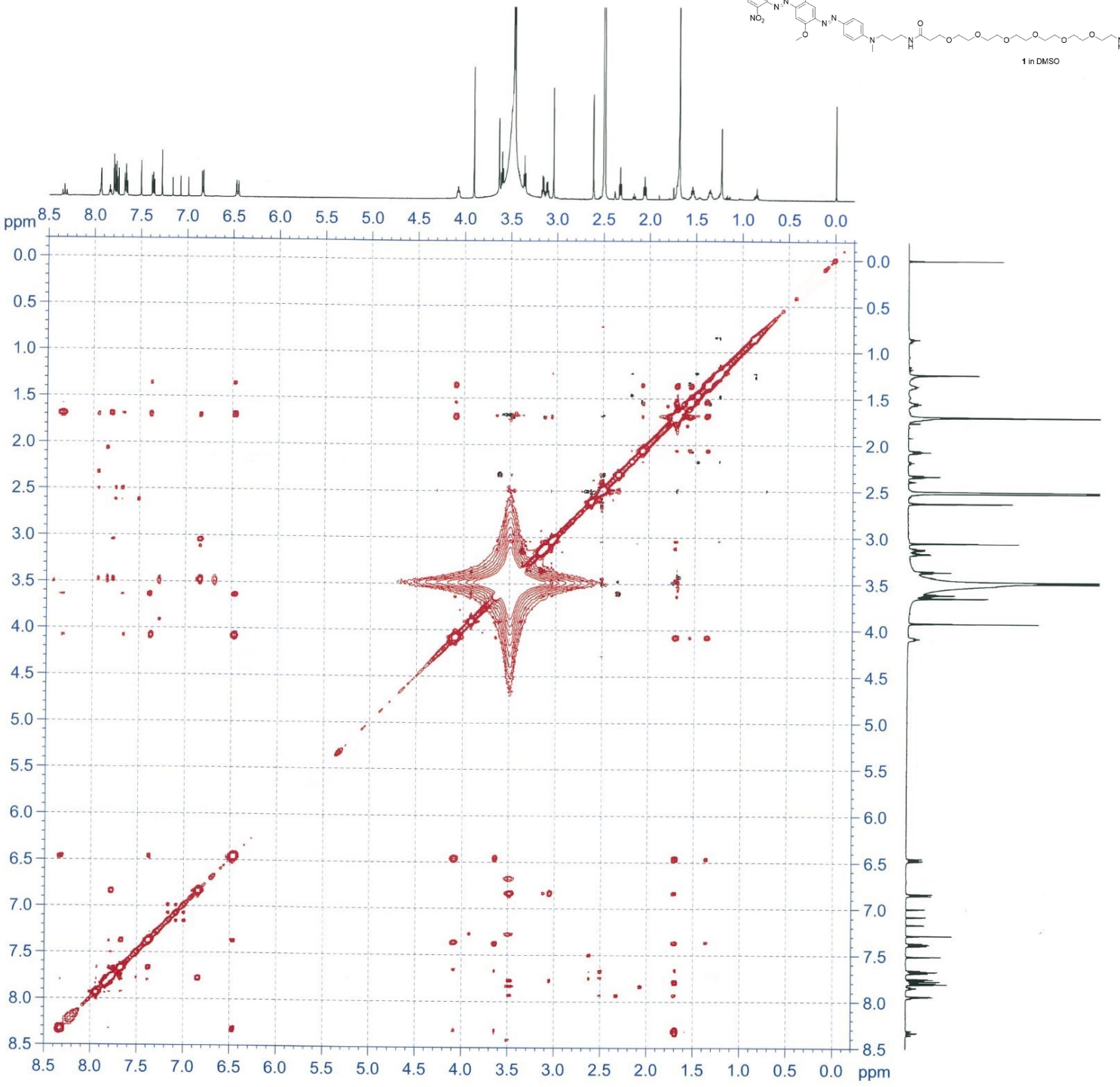
(2) Jing, C.; Cornish, V. W. *ACS Chem. Biol.* **2013**, *8*, 1704–1712.

(3) Grimm, J. B.; Lavis, L. D. *Org. Lett.* **2011**, *13*, 6354–6357.

(4) Peter Czerney, Matthias Wenzel, Bernd Schweder, F. L. US20040260093A1, 2004.

(5) Sato, S.; Yatsuzuka, K.; Katsuda, Y.; Uesugi, M. *Methods Mol. Biol.* **2018**, *1649*, 305–318.

peg6-slufoCY3;H-H NOESY



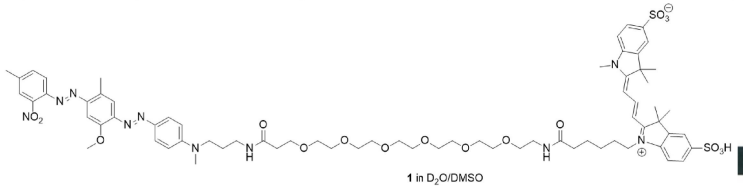
BRUKER

NAME	yatsuzuka peg6-slufoCy3
EXPNO	5
PROCNO	1
Date_	20151209
Time	22.39
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	noesyph
TD	4096
SOLVENT	DMSO
NS	40
DS	4
SWH	6024.096 Hz
FIDRES	1.470727 Hz
AQ	0.3400180 sec
RG	161
DW	83.000 usec
DE	8.00 usec
TE	298.2 K
D0	0.00006517 sec
D1	2.00000000 sec
D8	0.50000000 sec
IN0	0.00016600 sec

======== CHANNEL f1 ========

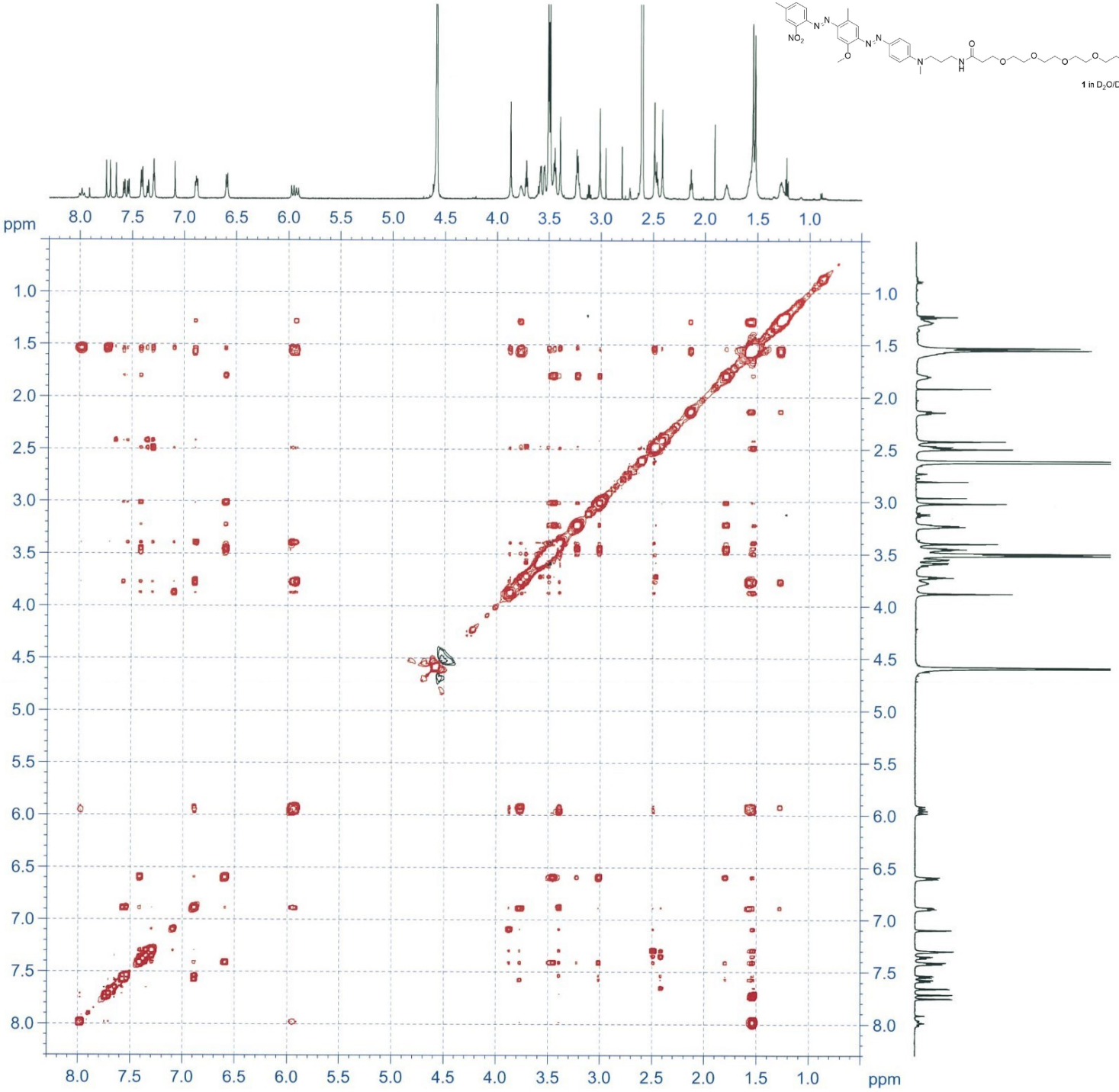
NUC1	1H
P1	14.00 usec
PL1	-0.48 dB
PL1W	13.88689613 W
SFO1	600.1325011 MHz
ND0	1
TD	512
SFO1	600.1325 MHz
FIDRES	11.765813 Hz
SW	10.038 ppm
FnMODE	States-TPPI
SI	2048
SF	600.1300055 MHz
WDW	QSINE
SSB	2
LB	0.00 Hz
GB	0
PC	1.00
SI	2048
MC2	States-TPPI
SF	600.1300055 MHz
WDW	QSINE
SSB	2
LB	0.00 Hz
GB	0

BHQ1-peg6-sulfoCy3;50%DMSOinD2O high;H-H NOESY



1 in D_2O/DMSO

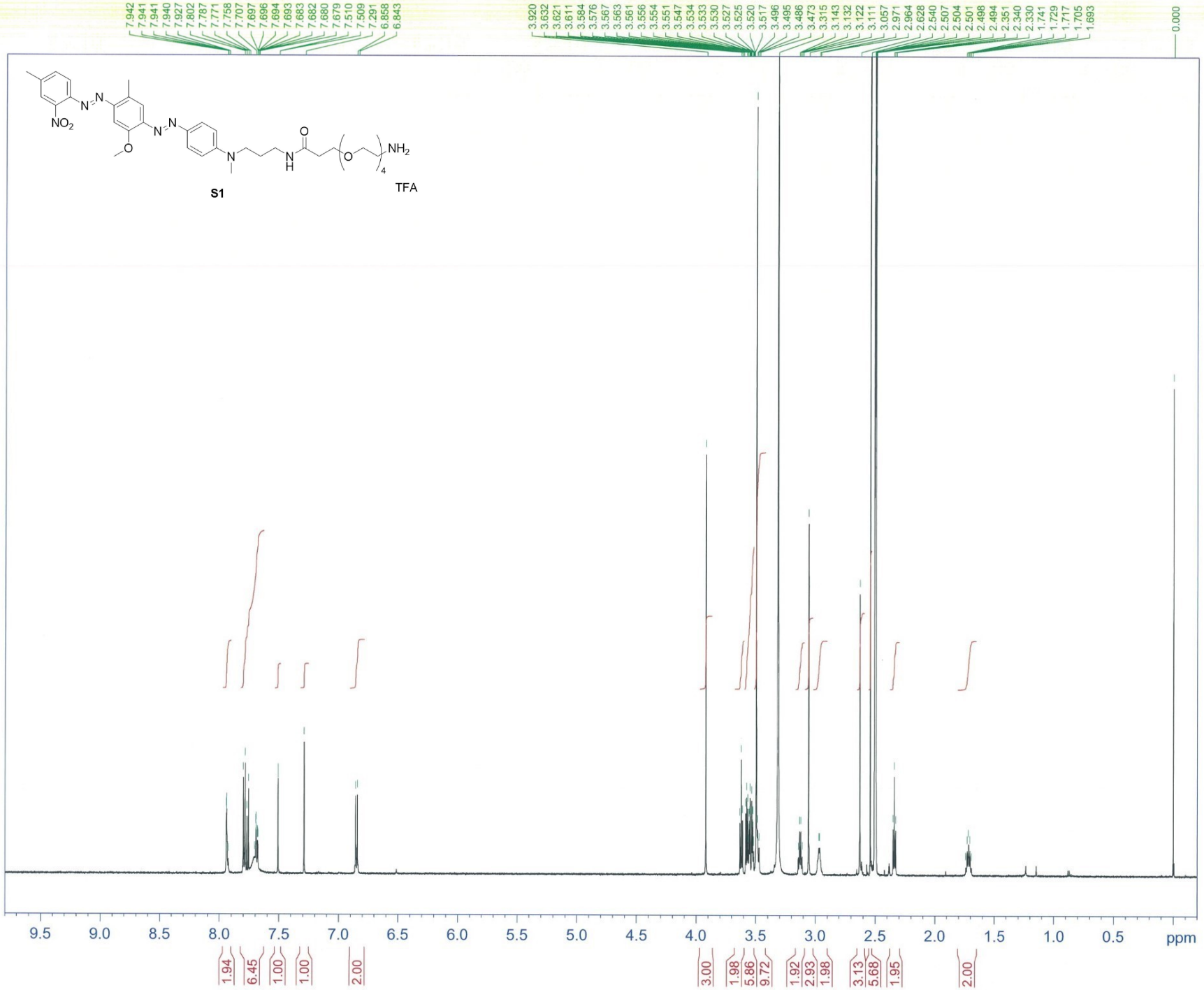
BRUKER




```
NAME        yatsuzuka BHQ1-peg6-sulfoCy3;50%DMSOinD2O high
EXPNO                5
PROCNO               1
Date_         20160211
Time              4.05
INSTRUM           spect
PROBHD    5 mm PABBO BB-
PULPROG          noesyph
TD                2048
SOLVENT           DMSO
NS                 144
DS                   4
SWH          5952.381 Hz
FIDRES       2.906436 Hz
AQ           0.1720820 sec
RG                 144
DW            84.000 usec
DE             8.00 usec
TE             298.2 K
D0           0.00006617 sec
D1           2.00000000 sec
D8           0.50000000 sec
IN0          0.00016800 sec

======== CHANNEL f1 ========
NUC1               1H
P1             14.00 usec
PL1            -0.48 dB
PL1W     13.88689613 W
SFO1     600.1323089 MHz
ND0                1
TD               256
SFO1     600.1323 MHz
FIDRES     23.251488 Hz
SW           9.918 ppm
FnMODE     States-TPPI
SI               1024
SF       600.1299407 MHz
WDW            QSINE
SSB                2
LB          0.00 Hz
GB                 0
PC              1.00
SI               1024
MC2        States-TPPI
SF       600.1299407 MHz
WDW            QSINE
SSB                2
LB          0.00 Hz
GB                 0
```

BHQ1-peg4;1H



BRUKER

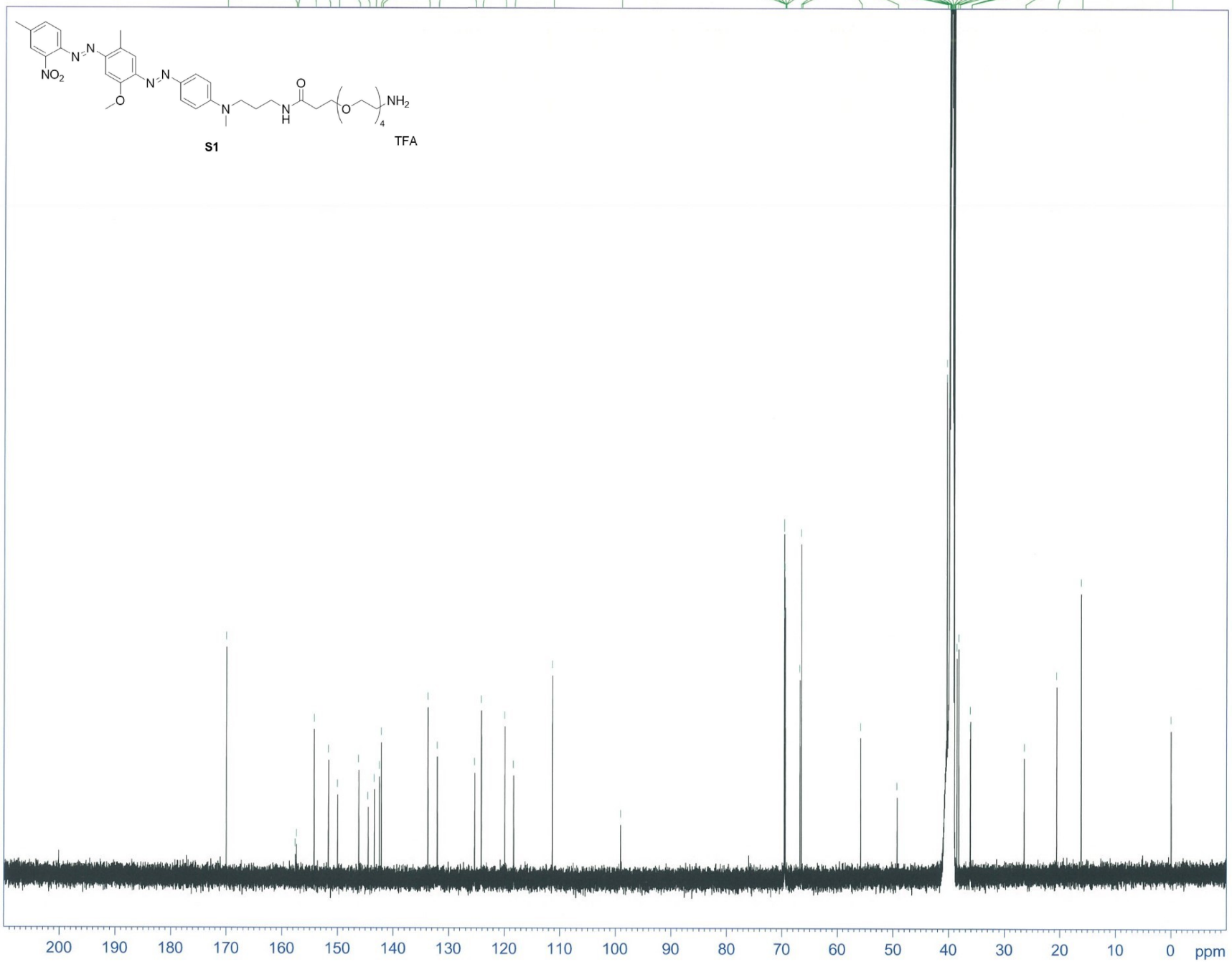
NAME yatsuzuka BHQ1-peg4
EXPNO 1
PROCNO 1
Date_ 20180205
Time 14.36
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 131072
SOLVENT DMSO
NS 128
DS 0
SWH 12335.526 Hz
FIDRES 0.094113 Hz
AQ 5.3128352 sec
RG 203
DW 40.533 usec
DE 8.00 usec
TE 298.2 K
D1 5.00000000 sec
TD0 1000

======== CHANNEL f1 ========
NUC1 1H
P1 14.00 usec
PL1 -2.80 dB
PL1W 23.69219017 W
SFO1 600.1337060 MHz
SI 65536
SF 600.1300066 MHz
WDW EM
SSB 0
LB 0.00 Hz
GB 0
PC 1.00

^{13}C … 後で測定予定

H
HH cosy
HSQC あり

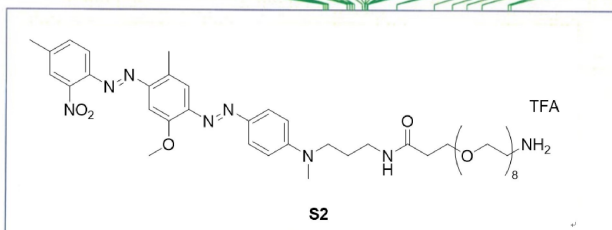
BHQ1-peg4;13C



BRUKER

NAME	yatsuzuka BHQ1-peg4
EXPNO	2
PROCNO	1
Date_	20180209
Time	18.52
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zgpg45
TD	131072
SOLVENT	DMSO
NS	92336
DS	4
SWH	48076.922 Hz
FIDRES	0.366798 Hz
AQ	1.3631988 sec
RG	203
DW	10.400 usec
DE	8.00 usec
TE	298.2 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	10000
======== CHANNEL f1 ========	
NUC1	13C
P1	9.00 usec
PL1	0.17 dB
PL1W	84.78267670 W
SFO1	150.9178988 MHz
======== CHANNEL f2 ========	
CPDPRG2	waltz16
NUC2	1H
PCPD2	70.00 usec
PL2	-2.80 dB
PL12	11.18 dB
PL13	13.00 dB
PL2W	23.69219017 W
PL12W	0.94755661 W
PL13W	0.62316805 W
SFO2	600.1324005 MHz
SI	131072
SF	150.9028969 MHz
WDW	EM
SSB	0
LB	0.20 Hz
GB	0
PC	1.40

BHQ1-peg8;1H



S2

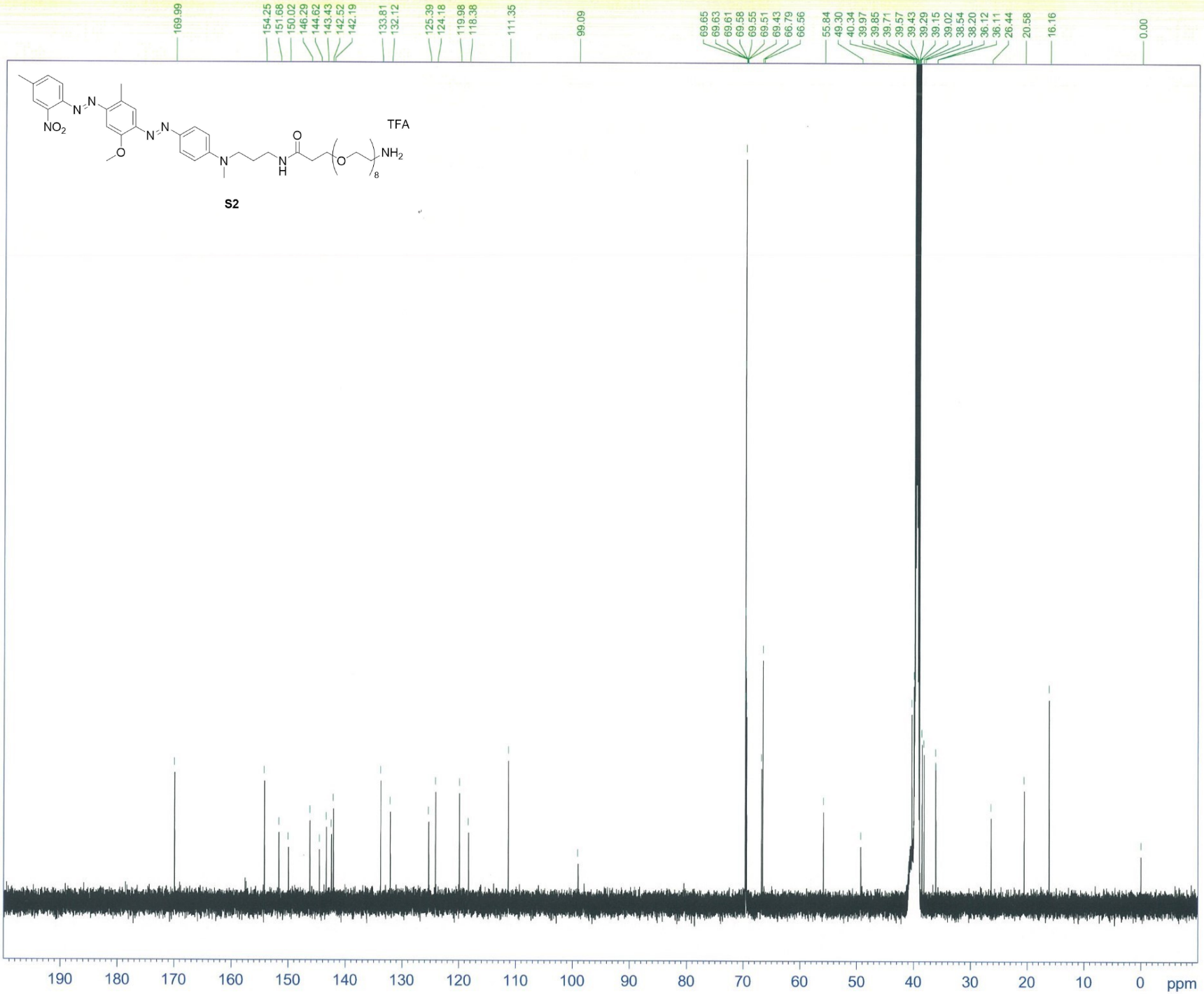
BRUKER

NAME yatsuzuka BHQ1-peg8
EXPNO 1
PROCNO 1
Date_ 20180206
Time 14.30
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 131072
SOLVENT DMSO
NS 128
DS 0
SWH 12335.526 Hz
FIDRES 0.094113 Hz
AQ 5.3128352 sec
RG 203
DW 40.533 usec
DE 8.00 usec
TE 298.2 K
D1 5.00000000 sec
TD0 1000

======== CHANNEL f1 ========
NUC1 1H
P1 14.00 usec
PL1 -2.80 dB
PL1W 23.69219017 W
SFO1 600.1337060 MHz
SI 65536
SF 600.1300065 MHz
WDW EM
SSB 0
LB 0.00 Hz
GB 0
PC 1.00

^{1}H
^{13}C
DEPT135

BHQ1-peg8;13C

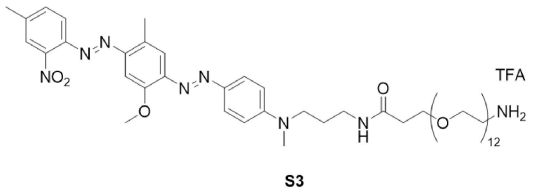


NAME yatsuzuka BHQ1-peg8
EXPNO 2
PROCNO 1
Date_ 20180206
Time 18.23
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg45
TD 131072
SOLVENT DMSO
NS 17200
DS 4
SWH 48076.922 Hz
FIDRES 0.366798 Hz
AQ 1.3631988 sec
RG 203
DW 10.400 usec
DE 8.00 usec
TE 298.2 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 10000

======== CHANNEL f1 ========
NUC1 13C
P1 9.00 usec
PL1 0.17 dB
PL1W 84.78267670 W
SFO1 150.9178988 MHz

======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 70.00 usec
PL2 -2.80 dB
PL12 11.18 dB
PL13 13.00 dB
PL2W 23.69219017 W
PL12W 0.94755661 W
PL13W 0.62316805 W
SFO2 600.1324005 MHz
SI 131072
SF 150.9028969 MHz
WDW EM
SSB 0
LB 0.20 Hz
GB 0
PC 1.40

BHQ1-peg12;1H



S3

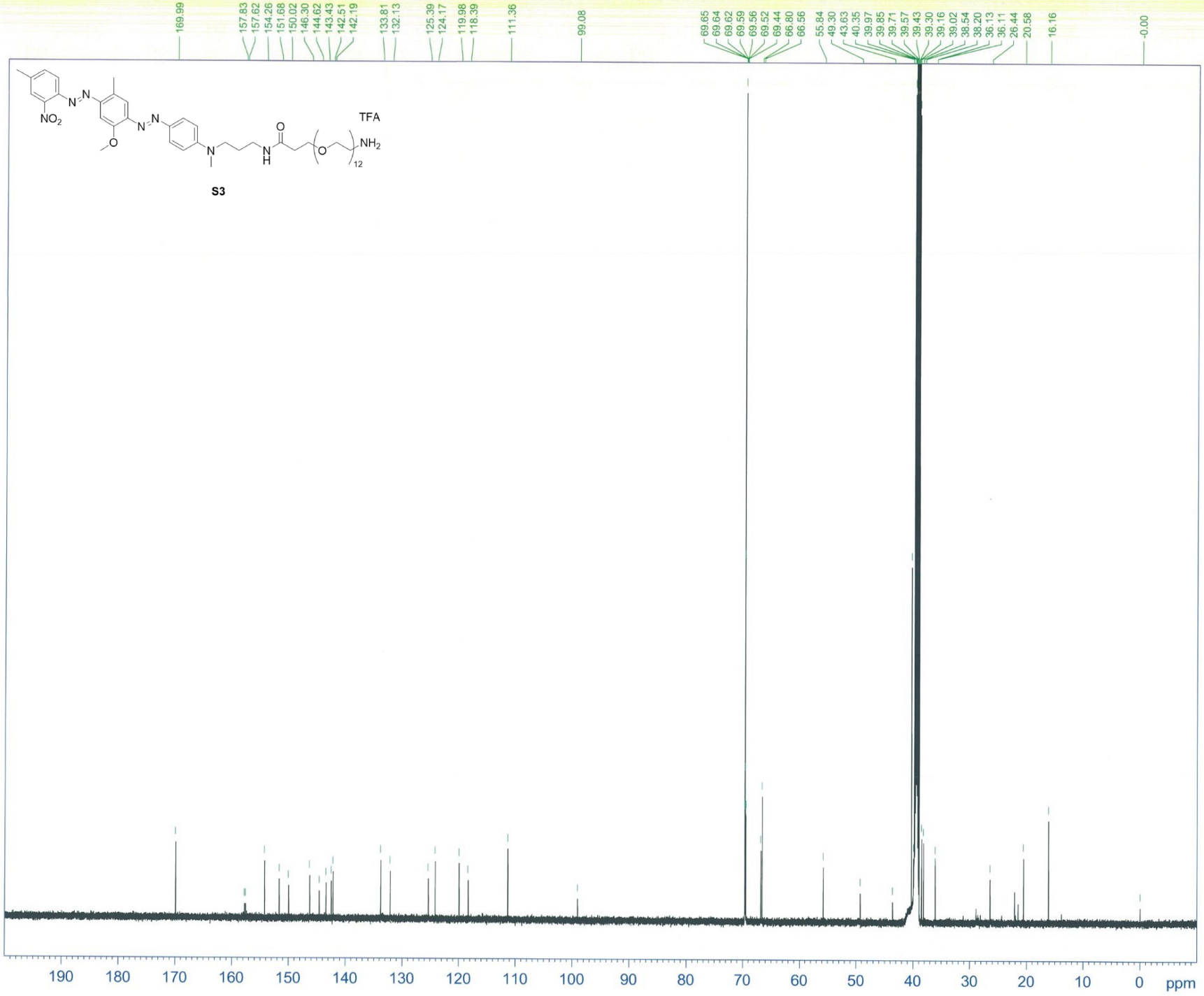
BRUKER

NAME yatsuzuka BHQ1-peg12
EXPNO 1
PROCNO 1
Date_ 20180207
Time 14.05
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 131072
SOLVENT DMSO
NS 112
DS 0
SWH 12335.526 Hz
FIDRES 0.094113 Hz
AQ 5.3128352 sec
RG 203
DW 40.533 usec
DE 8.00 usec
TE 298.2 K
D1 5.00000000 sec
TD0 1000

======== CHANNEL f1 ========
NUC1 1H
P1 14.00 usec
PL1 -2.80 dB
PL1W 23.69219017 W
SFO1 600.1337060 MHz
SI 65536
SF 600.1300061 MHz
WDW EM
SSB 0
LB 0.00 Hz
GB 0
PC 1.00

後で 13C
DEPT 135
測定予定

BHQ1-peg12;13C



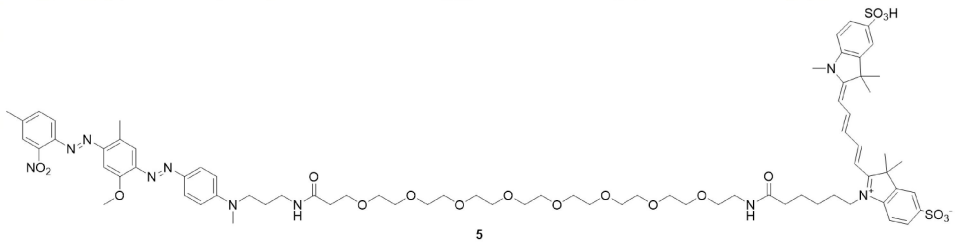
BRUKER

NAME yatsuzuka BHQ1-peg12
EXPNO 2
PROCNO 1
Date_ 20180207
Time 18.23
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg45
TD 131072
SOLVENT DMSO
NS 16976
DS 4
SWH 48076.922 Hz
FIDRES 0.366798 Hz
AQ 1.3631988 sec
RG 203
DW 10.400 usec
DE 8.00 usec
TE 298.3 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 10000

======== CHANNEL f1 ========
NUC1 13C
P1 9.00 usec
PL1 0.17 dB
PL1W 84.78267670 W
SFO1 150.9178988 MHz

======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 70.00 usec
PL2 -2.80 dB
PL12 11.18 dB
PL13 13.00 dB
PL2W 23.69219017 W
PL12W 0.94755661 W
PL13W 0.62316805 W
SFO2 600.1324005 MHz
SI 131072
SF 150.9028963 MHz
WDW EM
SSB 0
LB 0.20 Hz
GB 0
PC 1.40

BHQ1-sulfoCy5;1H



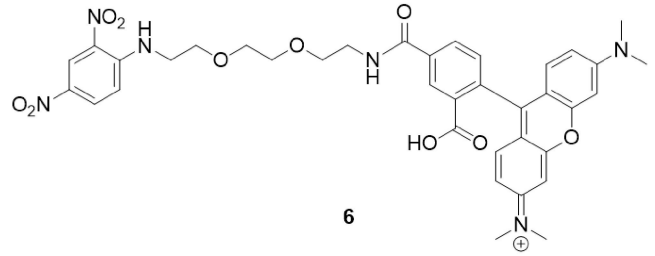
BRUKER

NAME yatsuzuka BHQ1-sulfoCy5
EXPNO 1
PROCNO 1
Date_ 20180207
Time 10.50
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 131072
SOLVENT DMSO
NS 208
DS 0
SWH 12335.526 Hz
FIDRES 0.094113 Hz
AQ 5.3128352 sec
RG 203
DW 40.533 usec
DE 8.00 usec
TE 298.2 K
D1 5.00000000 sec
TD0 1000

======== CHANNEL f1 ========
NUC1 1H
P1 14.00 usec
PL1 -2.80 dB
PL1W 23.69219017 W
SFO1 600.1337060 MHz
SI 65536
SF 600.1300063 MHz
WDW EM
SSB 0
LB 0.00 Hz
GB 0
PC 1.00

H
HH COSY) 97

DNB-TAMRA;1H



6

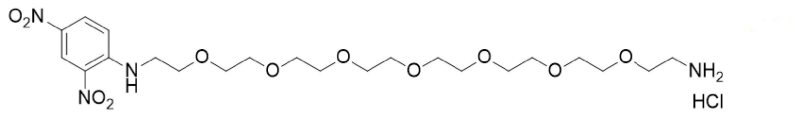
BRUKER

NAME yatsuzuka DNB-TAMRA
EXPNO 1
PROCNO 1
Date_ 20180201
Time 14.42
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 131072
SOLVENT DMSO
NS 320
DS 0
SWH 12335.526 Hz
FIDRES 0.094113 Hz
AQ 5.3128352 sec
RG 203
DW 40.533 usec
DE 8.00 usec
TE 298.2 K
D1 5.00000000 sec
TD0 1000

======== CHANNEL f1 ========
NUC1 1H
P1 14.00 usec
PL1 -2.80 dB
PL1W 23.69219017 W
SFO1 600.1337060 MHz
SI 65536
SF 600.1300059 MHz
WDW EM
SSB 0
LB 0.00 Hz
GB 0
PC 1.00

H 9 24

DNB-peg7;1H



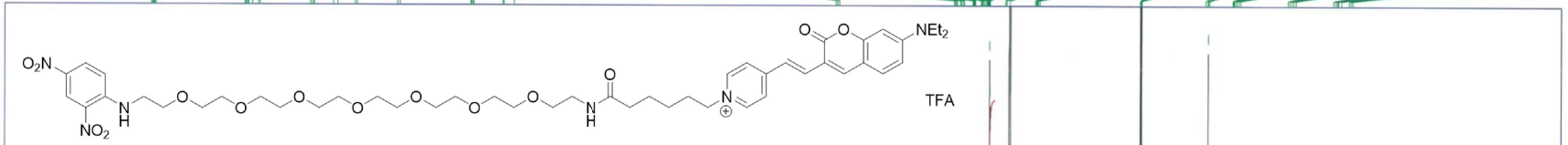
S17

BRUKER

NAME yatsuzuka DNB-peg7
EXPNO 1
PROCNO 1
Date_ 20180202
Time 16.42
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 131072
SOLVENT DMSO
NS 128
DS 0
SWH 12335.526 Hz
FIDRES 0.094113 Hz
AQ 5.3128352 sec
RG 203
DW 40.533 usec
DE 8.00 usec
TE 298.2 K
D1 5.00000000 sec
TD0 1000

======== CHANNEL f1 ========
NUC1 1H
P1 14.00 usec
PL1 -2.80 dB
PL1W 23.69219017 W
SFO1 600.1337060 MHz
SI 65536
SF 600.1300062 MHz
WDW EM
SSB 0
LB 0.00 Hz
GB 0
PC 1.00

DNB-DY;1H



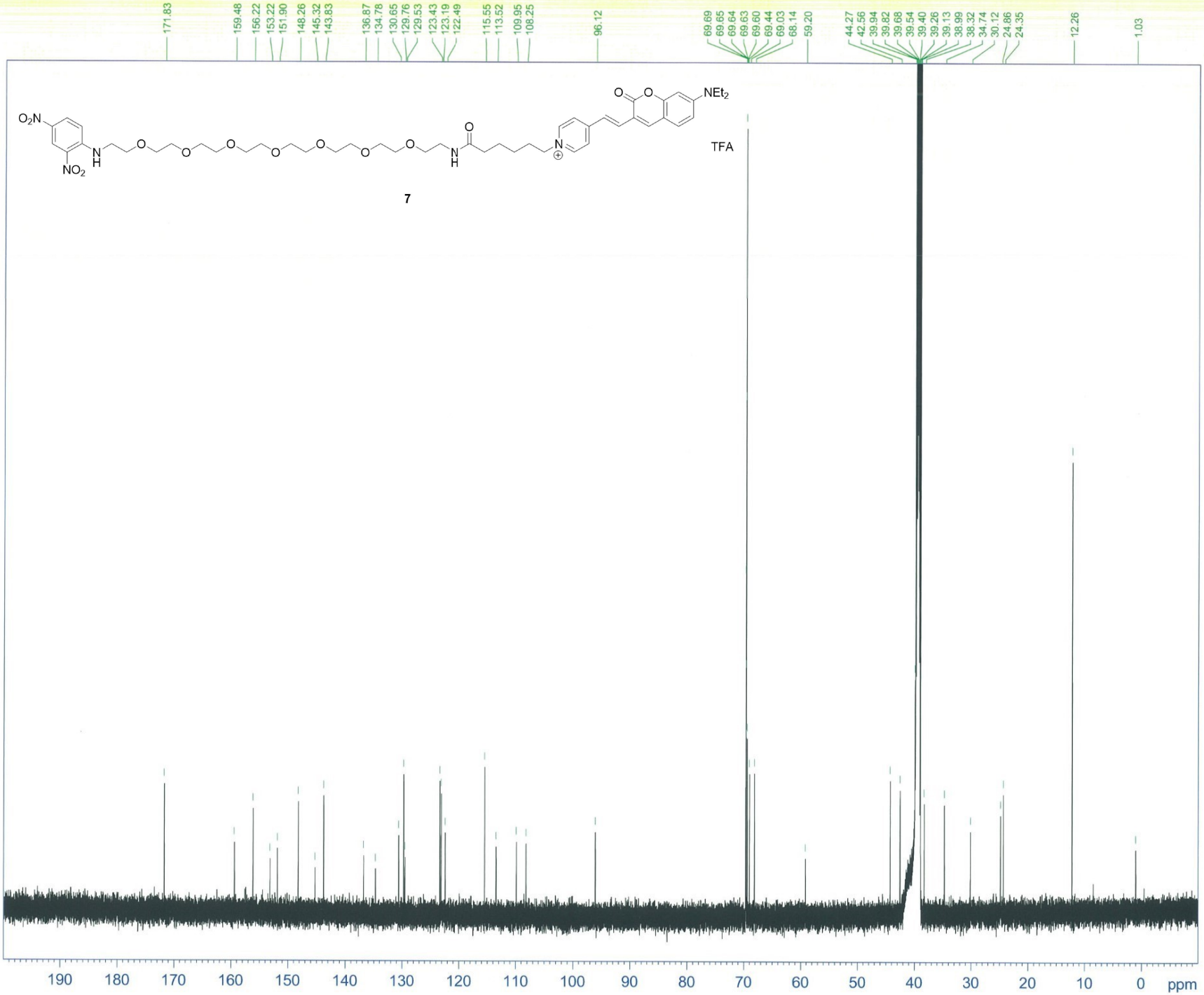
7

BRUKER

NAME yatsuzuka DNB-DY
EXPNO 1
PROCNO 1
Date_ 20180201
Time 15.45
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 131072
SOLVENT DMSO
NS 128
DS 0
SWH 12335.526 Hz
FIDRES 0.094113 Hz
AQ 5.3128352 sec
RG 203
DW 40.533 usec
DE 8.00 usec
TE 298.2 K
D1 5.00000000 sec
TD0 1000

======== CHANNEL f1 ========
NUC1 1H
P1 14.00 usec
PL1 -2.80 dB
PL1W 23.69219017 W
SFO1 600.1337060 MHz
SI 65536
SF 600.1300059 MHz
WDW EM
SSB 0
LB 0.00 Hz
GB 0
PC 1.00

DNB-DY;13C

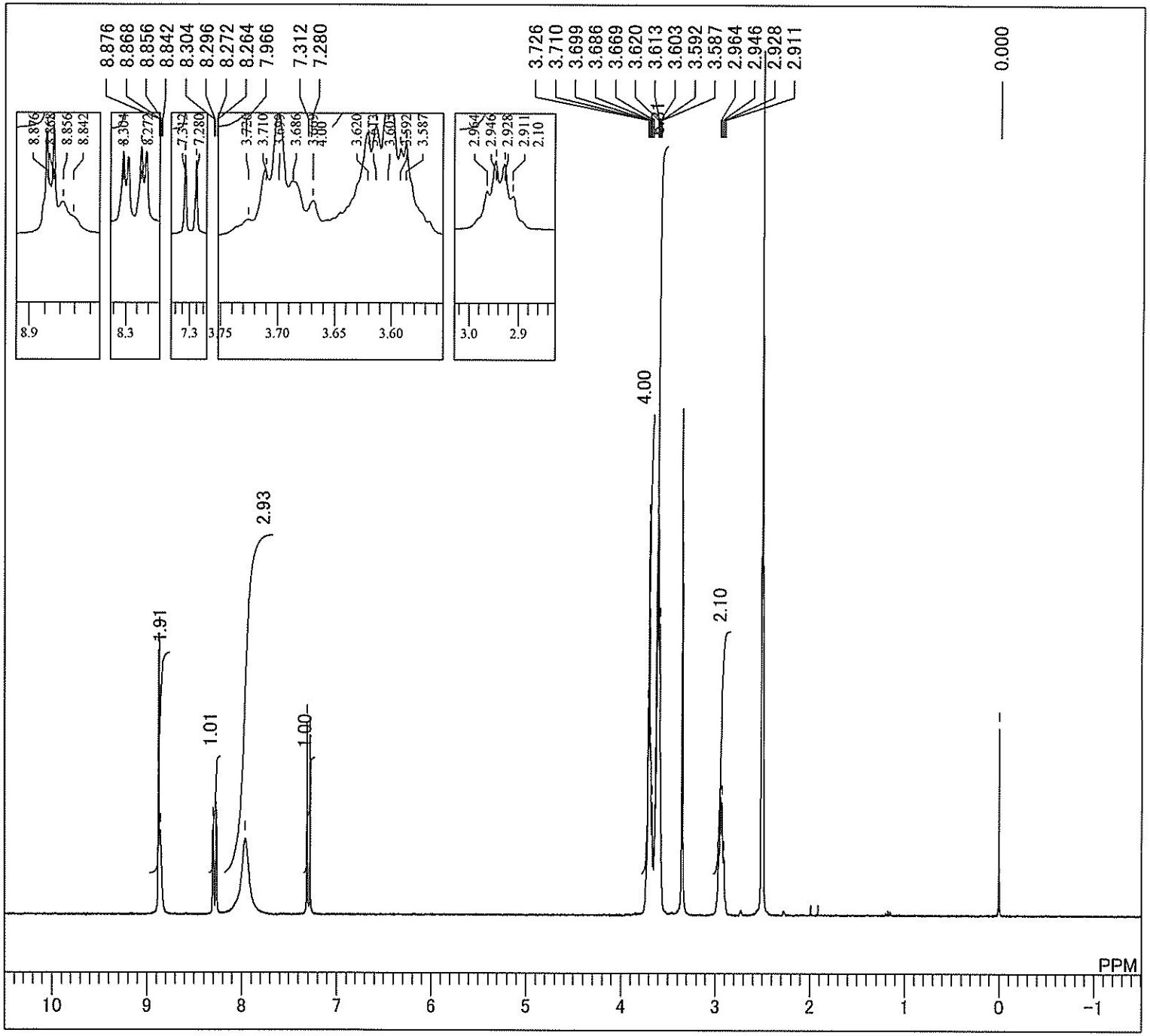


BRUKER

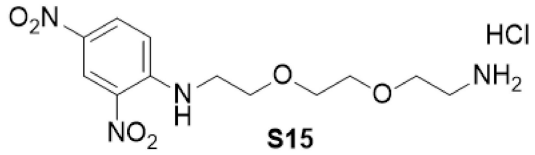
NAME yatsuzuka DNB-DY
EXPNO 2
PROCNO 1
Date_ 20180201
Time 20.17
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg45
TD 131072
SOLVENT DMSO
NS 14272
DS 4
SWH 48076.922 Hz
FIDRES 0.366798 Hz
AQ 1.3631988 sec
RG 203
DW 10.400 usec
DE 8.00 usec
TE 298.2 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 10000

======== CHANNEL f1 ========
NUC1 13C
P1 9.00 usec
PL1 0.17 dB
PL1W 84.78267670 W
SFO1 150.9178988 MHz

======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 70.00 usec
PL2 -2.80 dB
PL12 11.18 dB
PL13 13.00 dB
PL2W 23.69219017 W
PL12W 0.94755661 W
PL13W 0.62316805 W
SFO2 600.1324005 MHz
SI 131072
SF 150.9028976 MHz
WDW EM
SSB 0
LB 0.20 Hz
GB 0
PC 1.40



DFILE	yatsuzuka150917-1-83-deBoc2.al
COMNT	Single Pulse Experiment
DATIM	17-09-2015 11:53:58
OBNUC	1H
EXMOD	single_pulse.exp
OBFRQ	300.53 MHz
OBSET	1.15 KHz
OBFIN	8.57 Hz
POINT	16384
FREQU	4508.57 Hz
SCANS	32
ACQTM	3.6340 sec
PD	4.0000 sec
PW1	5.50 usec
IRNUC	
CTEMP	22.2 c
SLVNT	DMSO
EXREF	0.00 ppm
BF	0.12 Hz
RGAIN	15



S15