Supporting Information

Modulation of cell membrane functionalization by aggregates of oligodeoxynucleotides bearing alkyl chain-modified uridines

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Table S1. Oligodeoxynucleotides that Bind with ODN 1-5.

	Sequences[a]
ODN A	5'-*TAC GGA AGG AAG AGG GAG TC*-3'
F ODN A[b]	5'-F *TAC GGA AGG AAG AGG GAG TC*-3'
ODN B	5'-T AGT CGT CCC *TAC GGA AGG AAG AGG GAG TC*-3'
F ODN B[b]	5'-FT AGT CGT CCC *TAC GGA AGG AAG AGG GAG TC*-3'
ODN C	5'-TT TTT CTT ATT AGT CGT CCC *TAC GGA AGG AAG AGG GAG TC*-3'
F ODN C[b]	5'-FTT TTT CTT ATT AGT CGT CCC *TAC GGA AGG AAG AGG GAG TC*-3'
ODN D	5'-*TAC GGA AAG GAA AGA GGA GAG TC*-3'
F ODN D[b]	5'-F *TAC GGA AAG GAA AGA GGA GAG TC*-3'
ODN APT	5'-GAC GAC GAC ACA GGA TTA ATC TTA TTA GTC GTC CC *TAC GGA AGG AAG AGG GAG TC*-3'
F ODN APT[b]	5'-F GAC GAC GAC ACA GGA TTA ATC TTA TTA GTC GTC CC *TAC GGA AGG AAG AGG GAG TC*-3'

[a] Binding site of ODN with ^{A}U are shown in italic type.

[b] 'F' signifies a fluorophore (FAM).

Table S2. The Basic Properties of Aggregate Consisting of ODNs Used in This Study.

ODNs	Critical Aggregation Concentration (μM)	Size (d. nm)
ODN 1	44.6	97.5
ODN 1 / ODN A duplex	26.9	186.0
ODN 2	8.6	117.3
ODN 2 / ODN A duplex	4.8	100.5
ODN 3	5.1	59.4
ODN 3 / ODN A duplex	3.6	85.4
ODN 4	22.3	56.3
ODN 4 / ODN A duplex	9.0	107.9
ODN 5	3.8	N.D.[a]
ODN 5 / ODN D duplex	2.1	86.9
ODN 3 / ODN B duplex	2.3	N.D.[a]
ODN 3 / ODN C duplex	3.1	N.D.[a]
ODN 3 / ODN APT duplex	3.8	N.D.[a]

[a]No Data

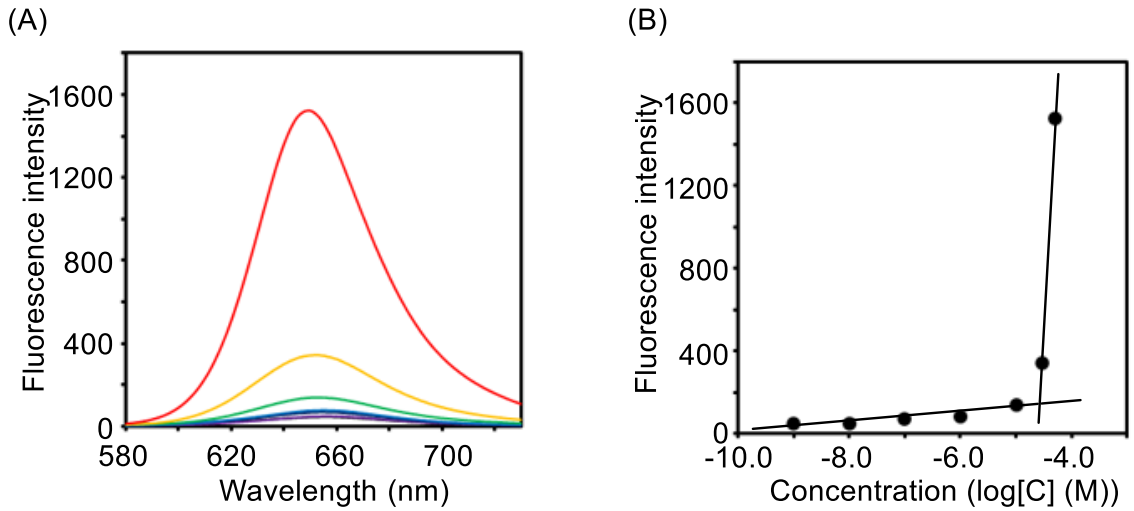


Figure S1. Formation of aggregates from ODN 1/ ODN A duplex and encapsulation of nile red. (A) Fluorescence spectra of nile red (10 μM) in sodium phosphate buffer (5 mM, pH 7.0) in the presence of ODN 1 / ODN A duplex (50 μM: red line, 30 μM: orange line, 10 μM: green line, 1 μM: blue line, 0.1 μM: dark blue line, 0.01 μM: purple line and 0.001 μM: black line). The fluorescence spectra were measured at 552 nm excitation. (B) Plot of fluorescence intensity of nile red vs concentration of ODN 1 / ODN A duplex to determine critical aggregation concentration (CAC).

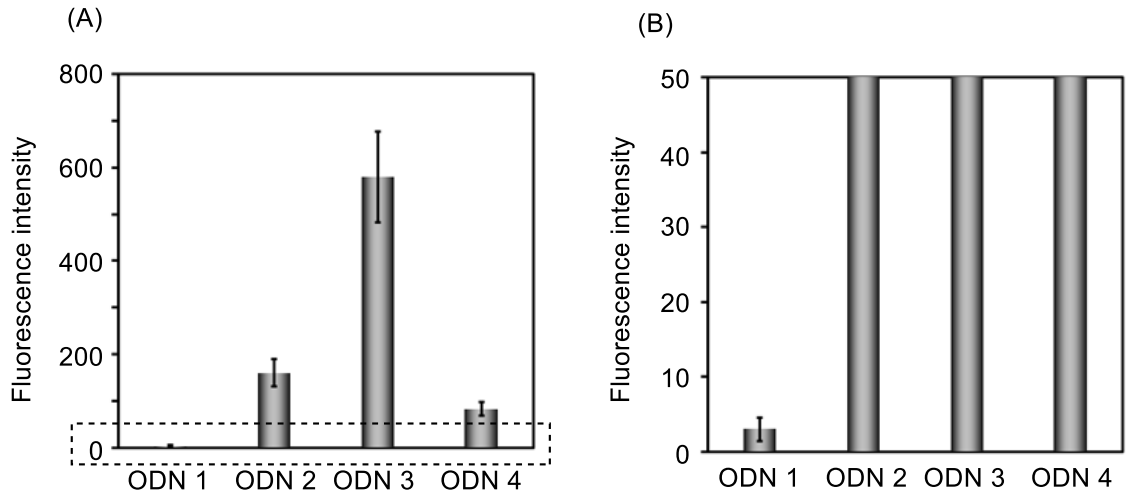


Figure S2. Fluorescence emission intensity obtained from Figure 3. (A) The emission intensity of FAM unit in F ODN A obtained from A549 cells that were incubated with duplexes ODN 1-ODN 4 / ODN A. (B) Enlarged view of the part of Figure S2A (Designated by dashed square).

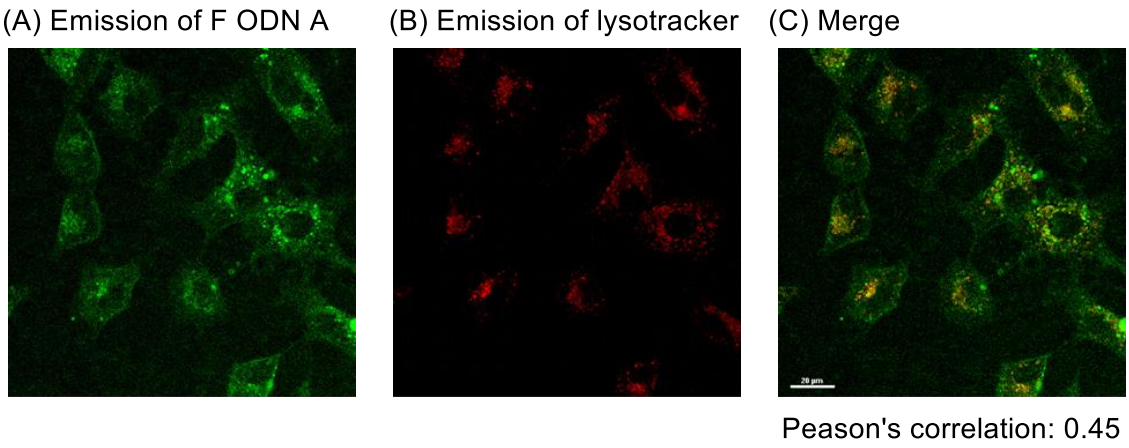


Figure S3. Localization of aggregate consisting of double stranded ODNs bearing hydrophobic units (ODN 1 / ODN A-F ODN A) in A549 cells. The cells were incubated with duplexes and then lysosome was stained by lysotracker. (A) Emission of F ODNs (Ex. 488 nm, Em. 558 nm). (B) Emission of lysotracker (Ex. 561 nm, Em. 567 nm). (C) Merged pictures.

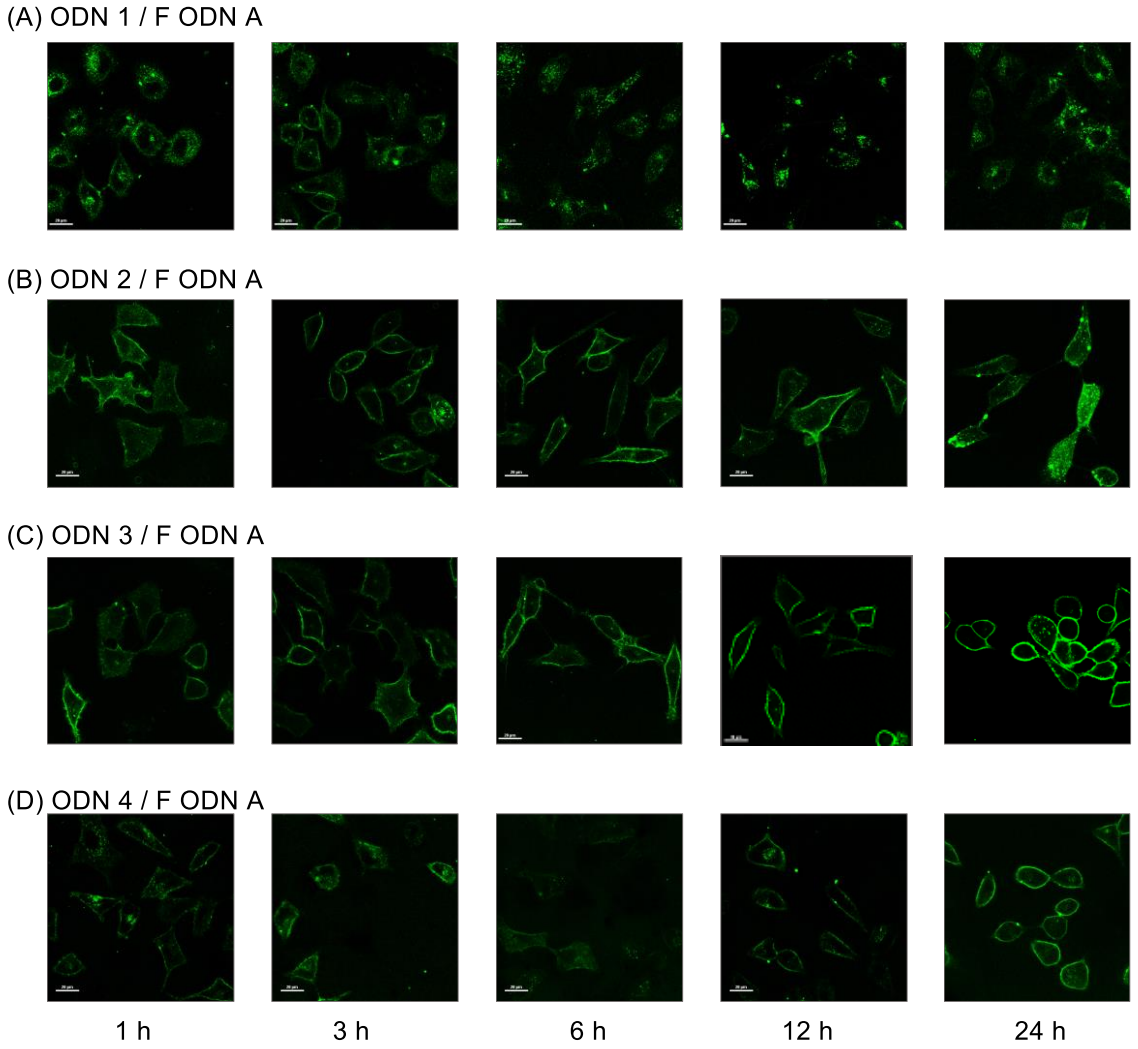


Figure S4. Localization of aggregate consisting of double stranded ODNs bearing hydrophobic units (ODN 1-4 / F ODN A). The cells were incubated with duplexes (ODN 1-4 / F ODN A) for 1, 3, 6, 12 and 24 h after administration of duplexes. Then the images were immediately taken by means of microscopy (excitation at 488 nm and emission at 558 nm).

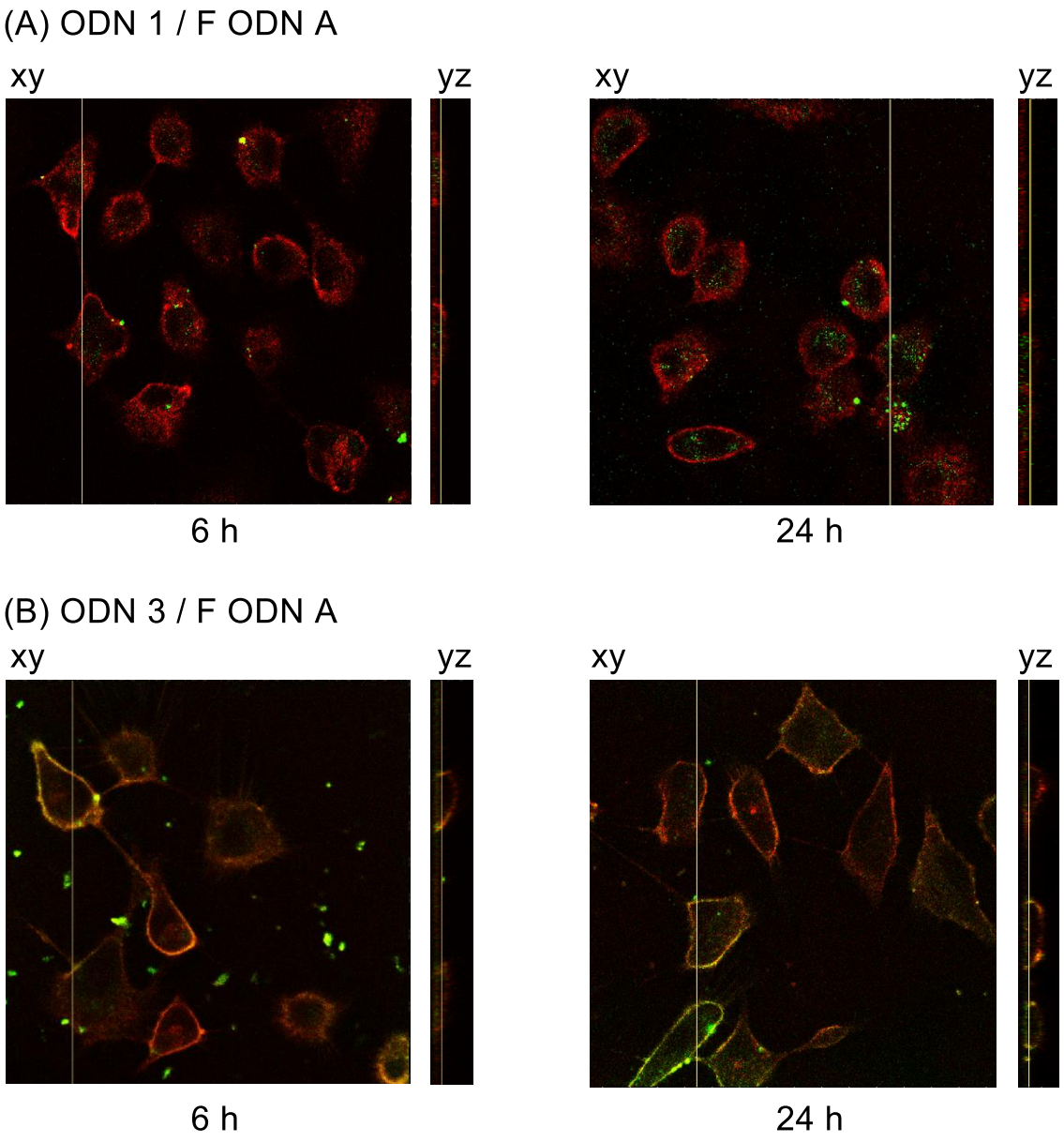


Figure S5. Fluorescent images of the cells with three-dimension. The cells were incubated with duplexes (A: ODN 1 / F ODN A, B: ODN 3 / F ODN A) for 6 and 24 h after administration of duplexes and then, cell membrane was stained by cell mask. Then the images were immediately taken by means of microscopy (FAM: excitation at 488 nm and emission at 558 nm (green), CellMask: excitation at 561 nm and emission at 567 nm (red)). The figures show merged images.

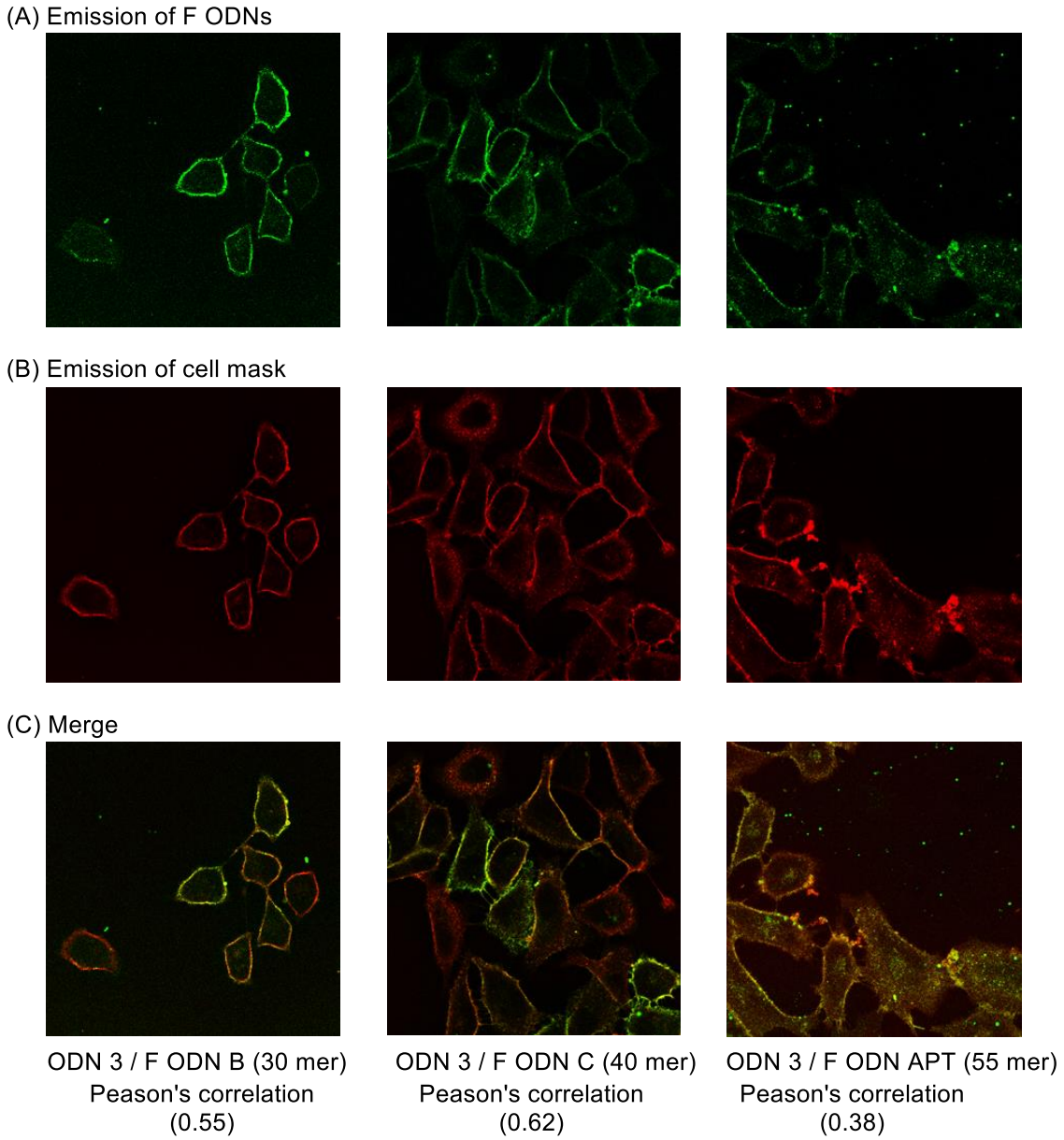


Figure S6. Localization of aggregate consisting of double stranded ODNs bearing hydrophobic units (ODN 3 / ODN A-F ODN A, ODN 3 / ODN B-F ODN B, ODN 3 / ODN APT-F ODN APT) in A549 cells. The cells were incubated with duplexes (ODN 1-4 / F ODN A) and then cell membrane was stained by cell mask. (A) Emission of F ODNs (Ex. 488 nm, Em. 558 nm). (B) Emission of cell mask (Ex. 561 nm, Em. 567 nm). (C) Merged pictures.

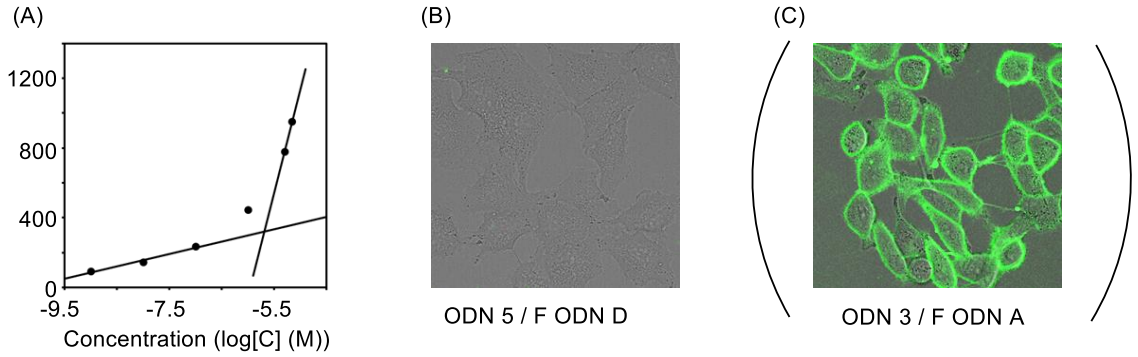


Figure S7. (A) Plot of fluorescence intensity of nile red vs concentration of ODN 5 / ODN D duplex to determine critical aggregation concentration (CAC). (B) Localization of aggregate consisting of double stranded ODNs bearing hydrophobic units (ODN 5 / ODN D + F ODN D). The cells were incubated with duplex (10 μM ODN 5 / 8 μM ODN D + 2 μM F ODN D) and then the cells were subjected confocal microscopy (Ex. 488 nm, Em. 558 nm). (C) Control experiment using ODN 3 / F ODN A duplex. The cells were incubated with duplex (10 μM ODN 3 / 8 μM ODN A + 2 μM F ODN A) and then the cells were subjected confocal microscopy (Ex. 488 nm, Em. 558 nm).

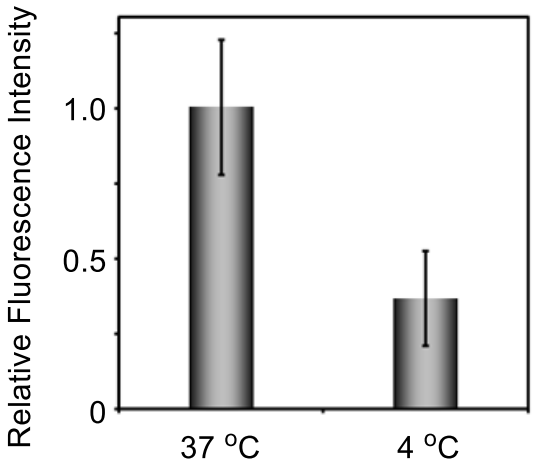


Figure S8. Cellular uptake of aggregates in A549 cells at 37 or 4 ˚C. The cells were incubated with ODN 3 / ODN A + F ODN A duplex (ODN 3: 10 μM, ODN A: 8 μM), F ODN A (2 μM) for 6 h at 37 or 4 ˚C, and then the cells were subjected to confocal microscopy (excitation at 488 nm and emission at 500–550 nm) to measure the fluorescence intensity.

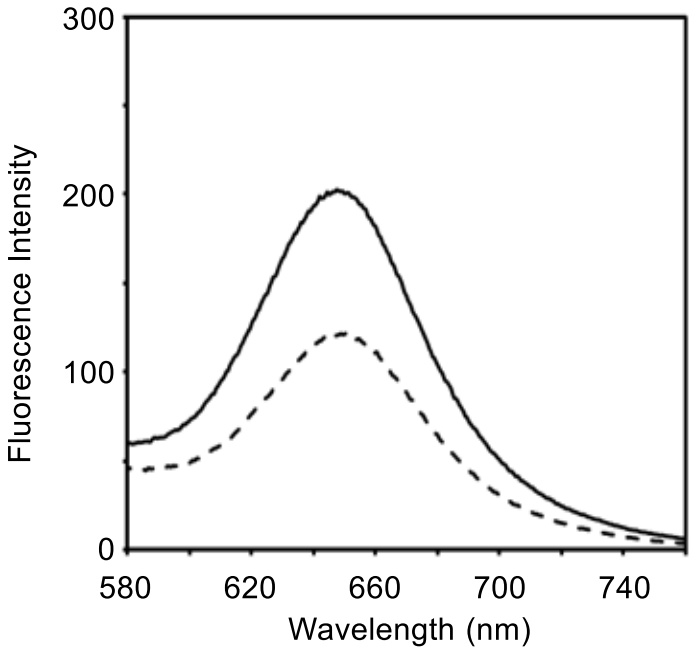
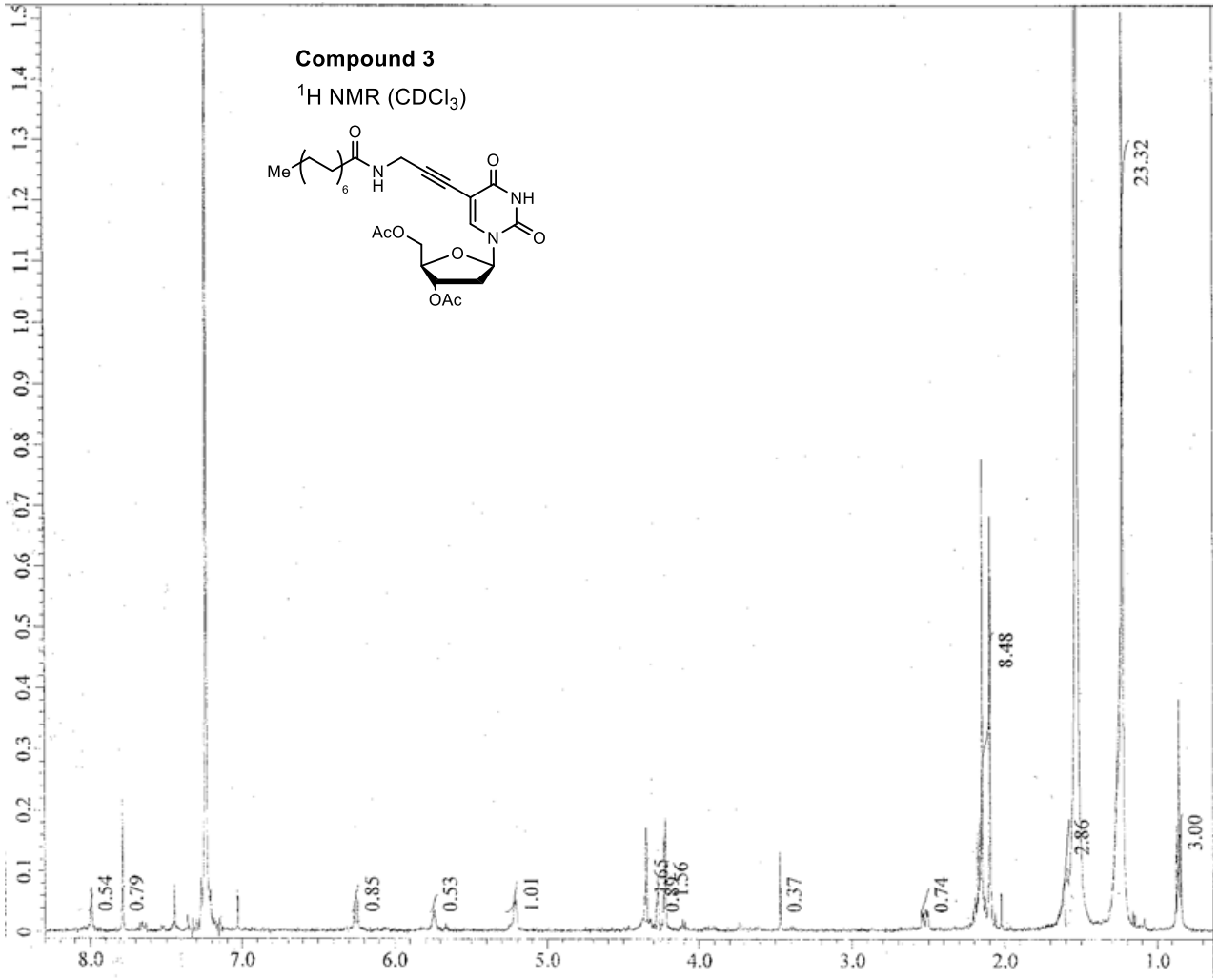
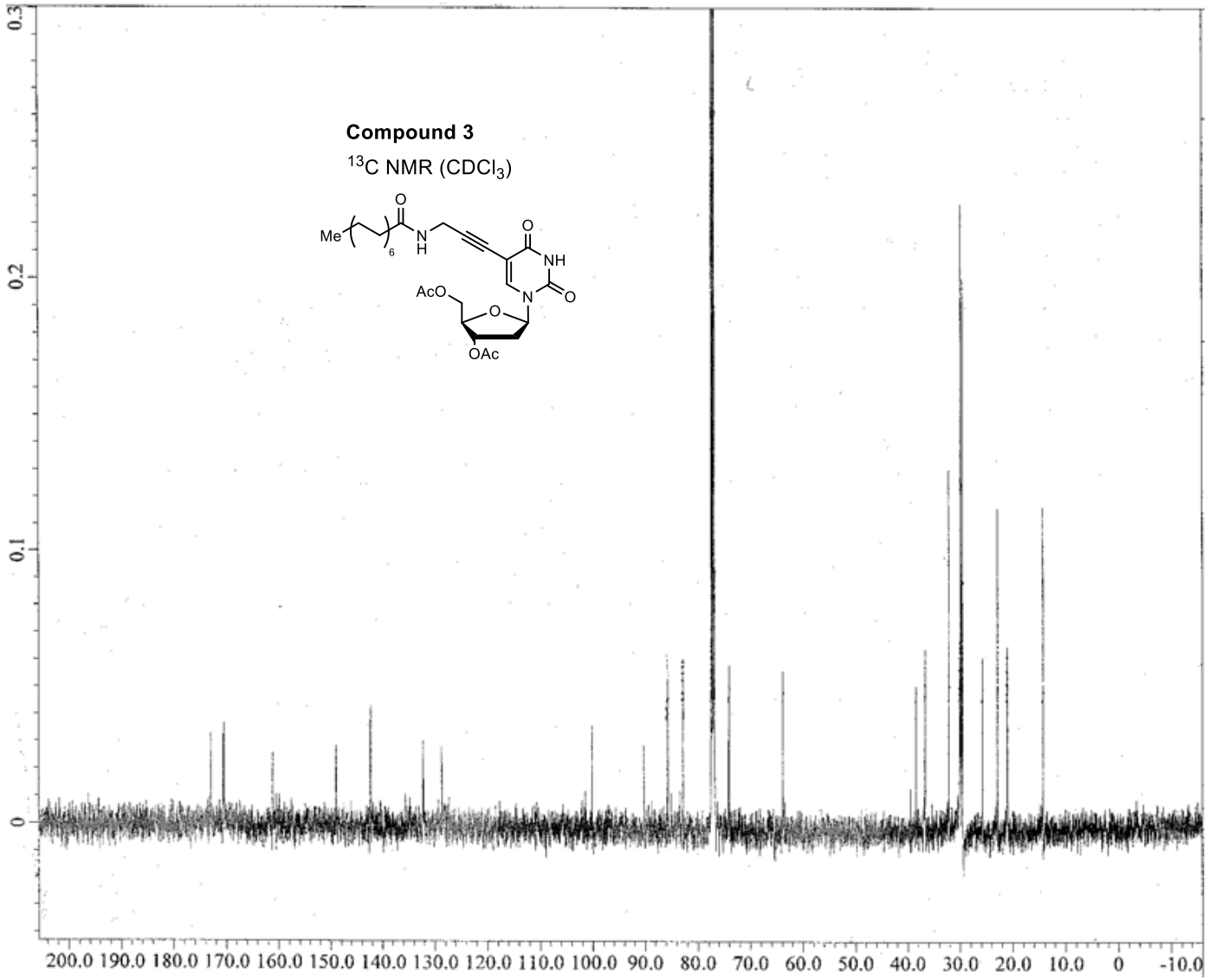
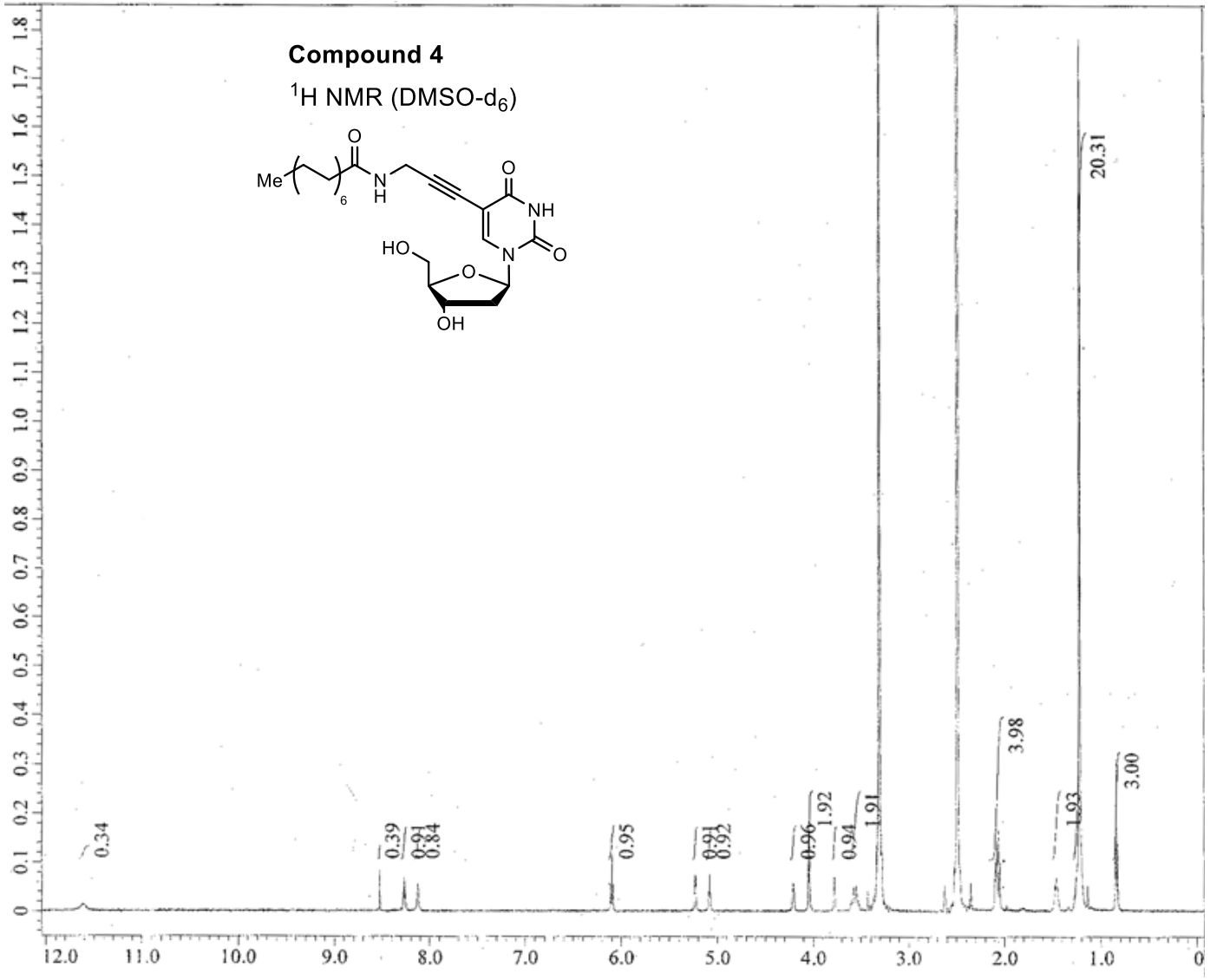


Figure S9. Emission of nile red (10 μM) encapsulated in ODN 3 / ODN APT aggregate (3 μM, solid line). The emission was decreased (dashed line) after addition of ThT (25 μM) due to the release of nile red from the aggregate. The fluorescence emission was obtained using excitation at 552 nm.

Compounds Data




Compound 3
13C NMR (CDCl3)
Me
6
N
H
O
NH
N
AcO
O
OAc
0.3
0.2
0.1
0
200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0 0 -10.0


Compound 4
^{1}H NMR (DMSO-d$_6$)
Me
6
N
H
O
NH
HO
OH
0.34
0.39
0.91
0.84
0.95
0.91
0.92
0.96
1.92
0.94
1.91
3.98
1.93
20.31
3.00
12.0
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4.0
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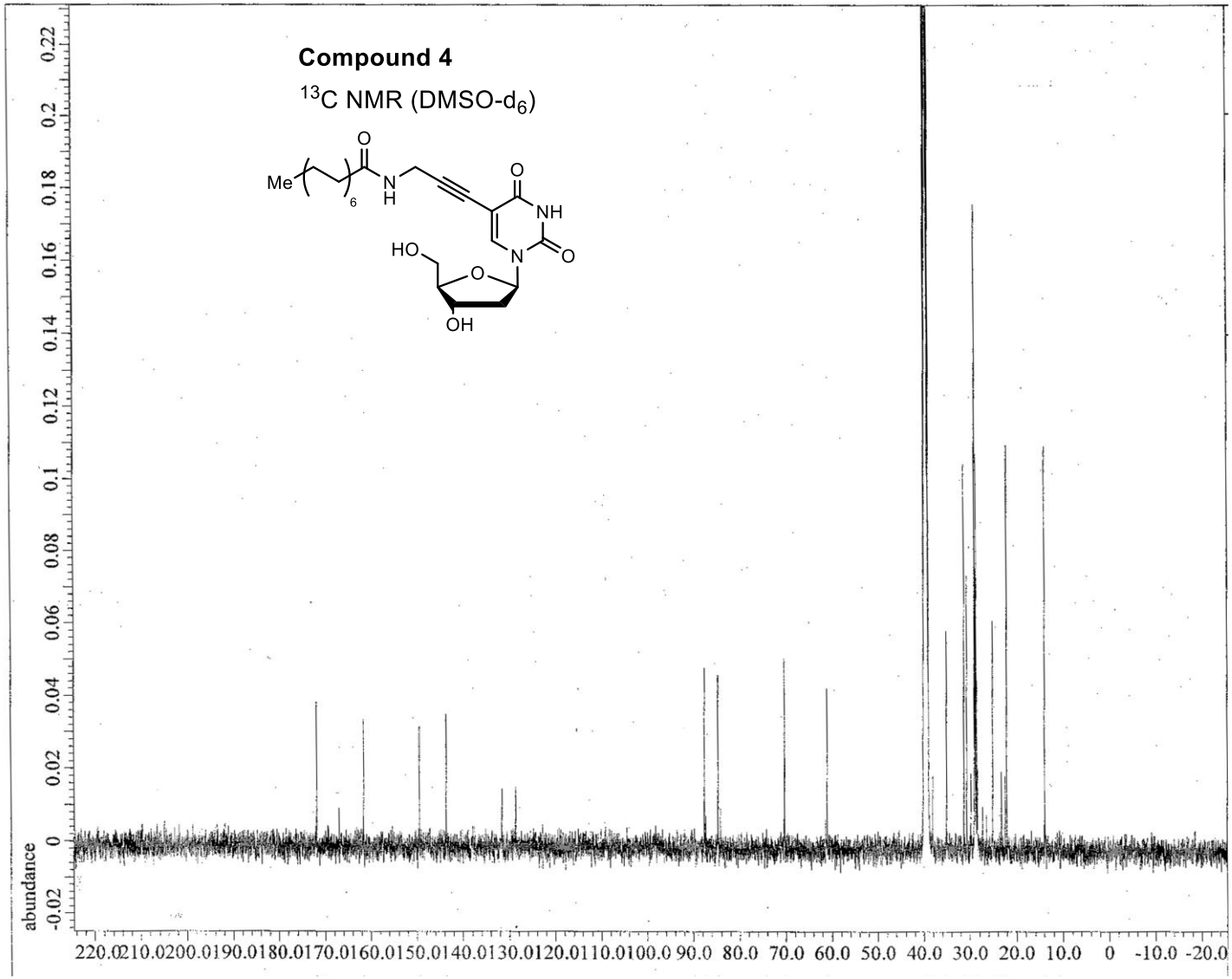
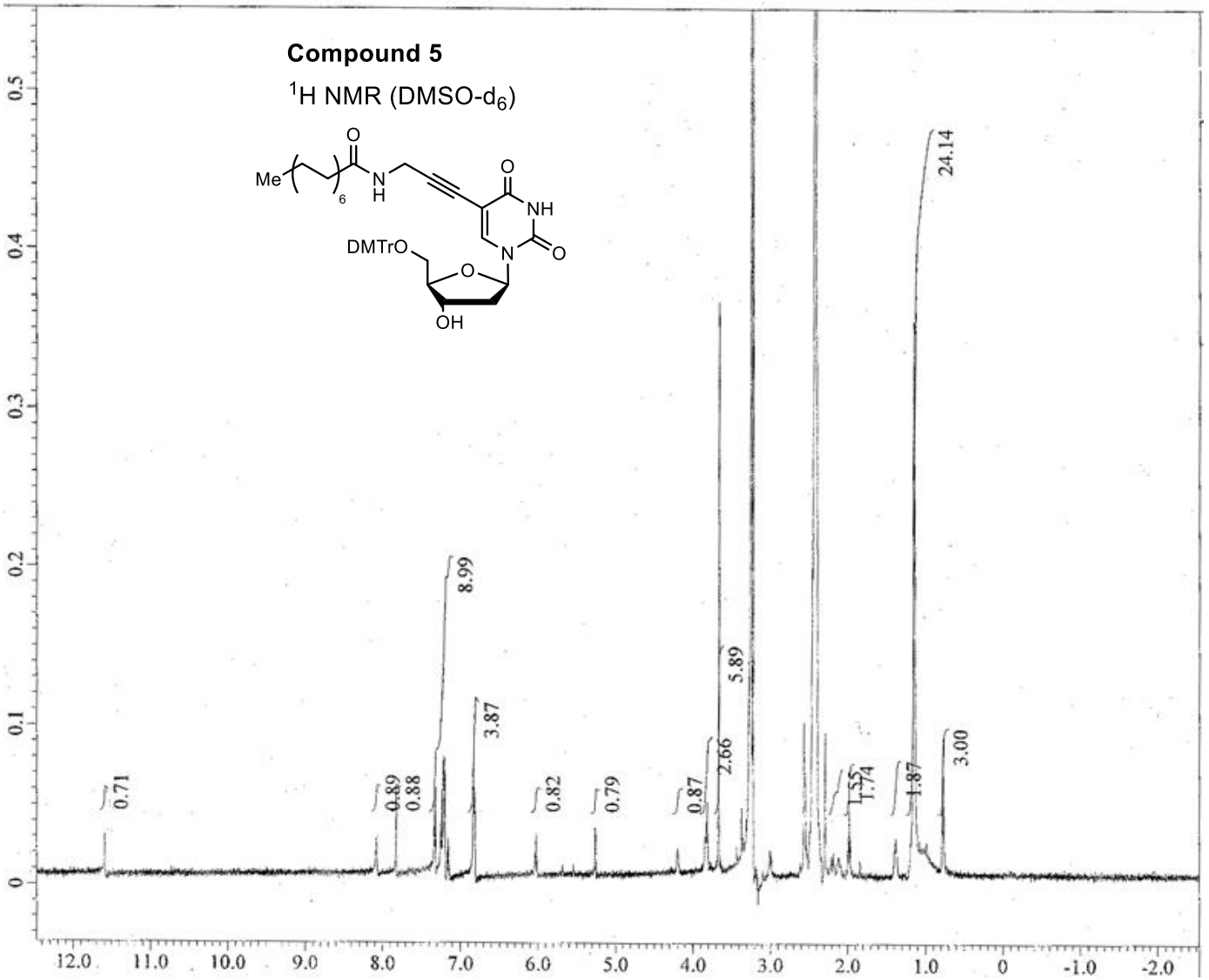
1.0
0


Compound 4
^{13}C NMR (DMSO-d$_6$)
Me
6
N
H
O
NH
HO
O
N
OH
abundance
0.22
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
-0.02
220.0 210.0 200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0 0 -10.0 -20.0


Compound 5
^{1}H NMR (DMSO-d$_6$)
Me
6
N
H
O
NH
N
O
DMTrO
O
OH
0.71
0.89
0.88
8.99
3.87
0.82
0.79
0.87
2.66
5.89
1.35
1.74
1.87
24.14
3.00
0
0.1
0.2
0.3
0.4
0.5
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
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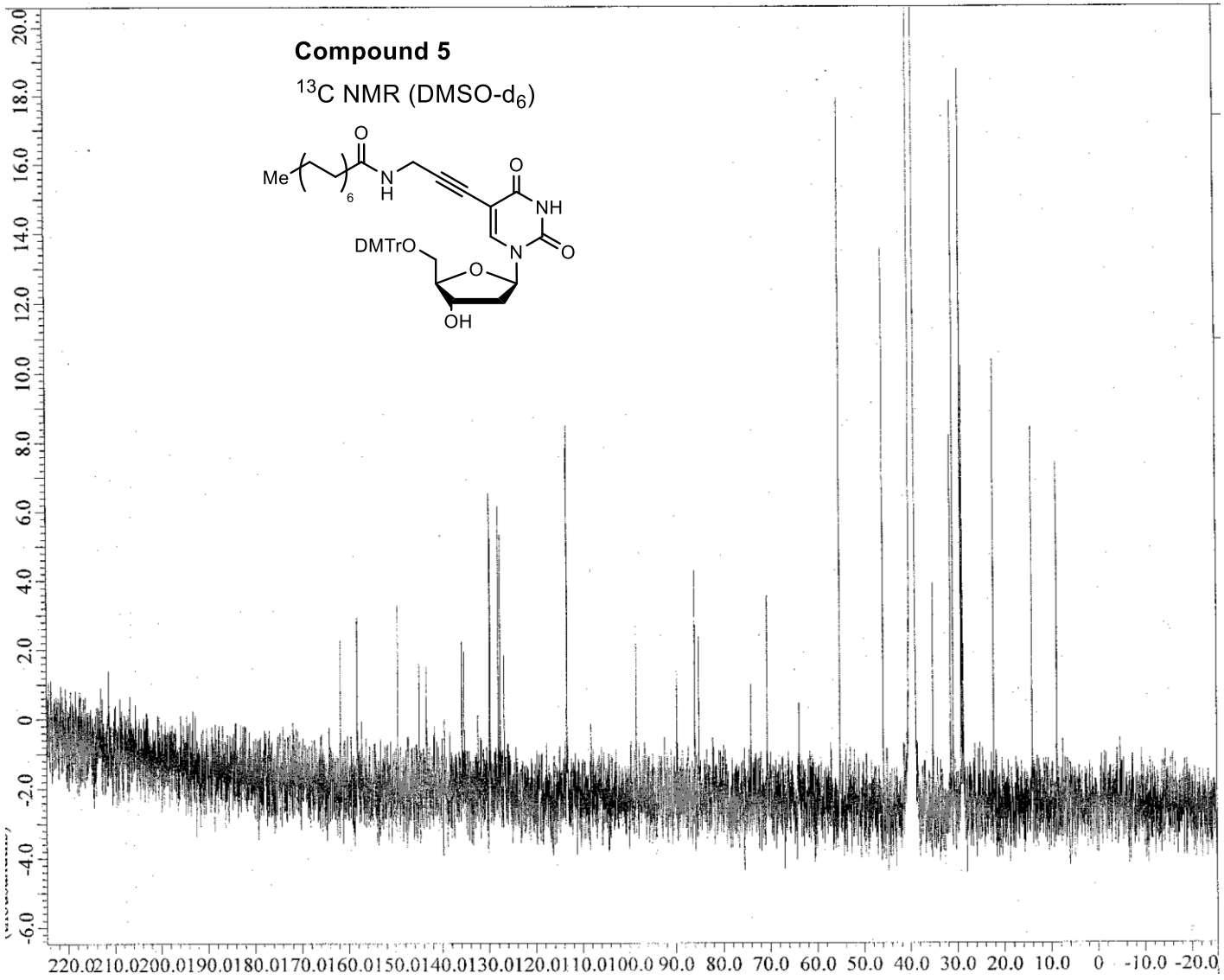
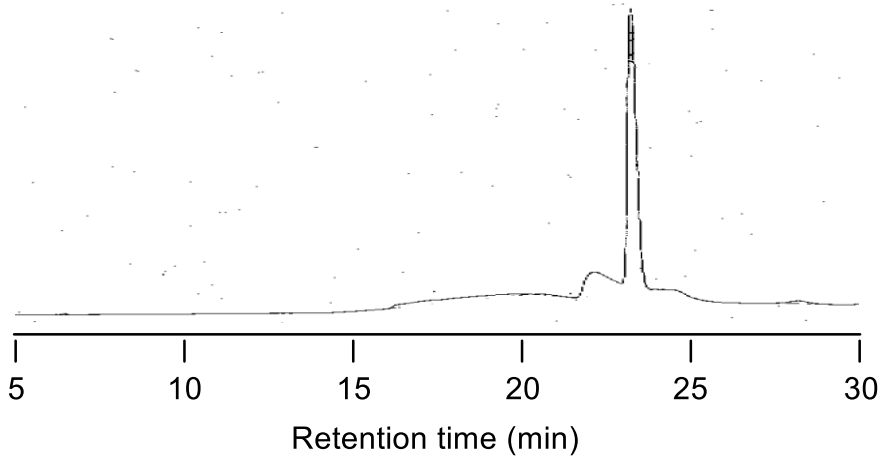
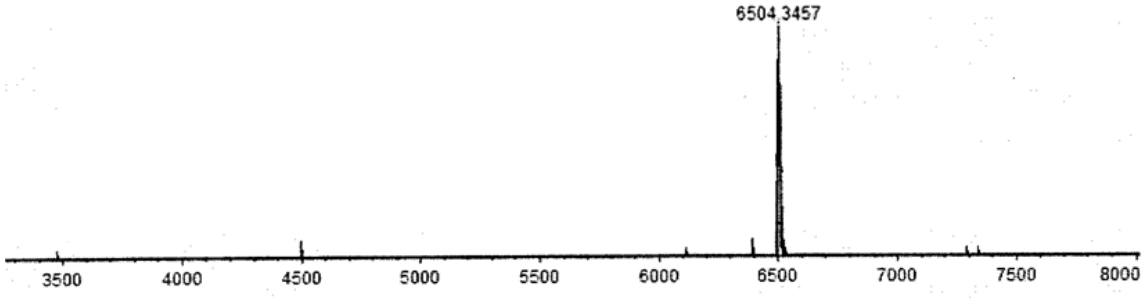
-2.0


Compound 5
^{13}C NMR (DMSO-d$_6$)
Me
6
N
H
O
O
NH
N
O
DMTrO
O
OH
20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0
-2.0
-4.0
-6.0
220.0 210.0 200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0 0 -10.0 -20.0

ODN 1 HPLC



ODN 1 MALDI TOF MS



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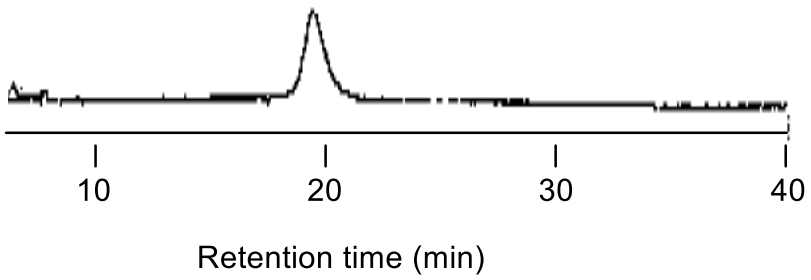

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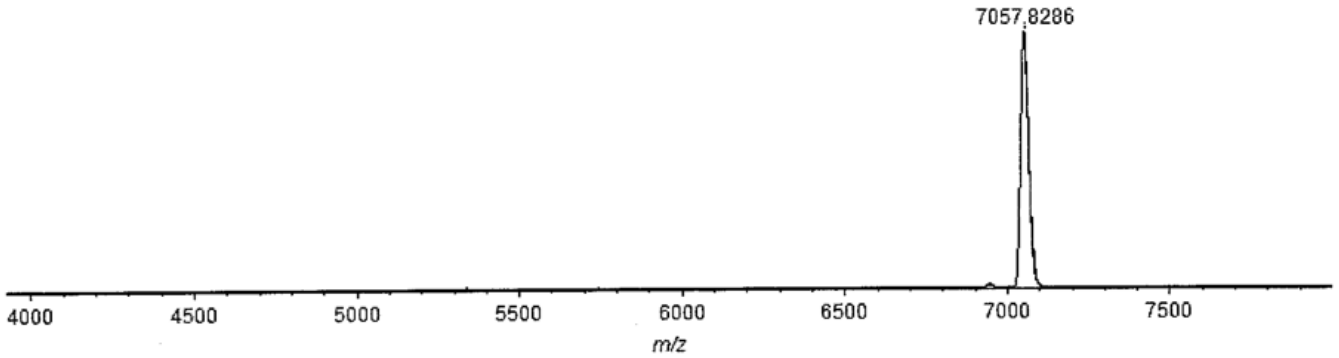

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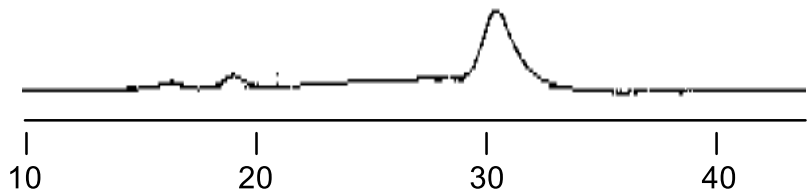
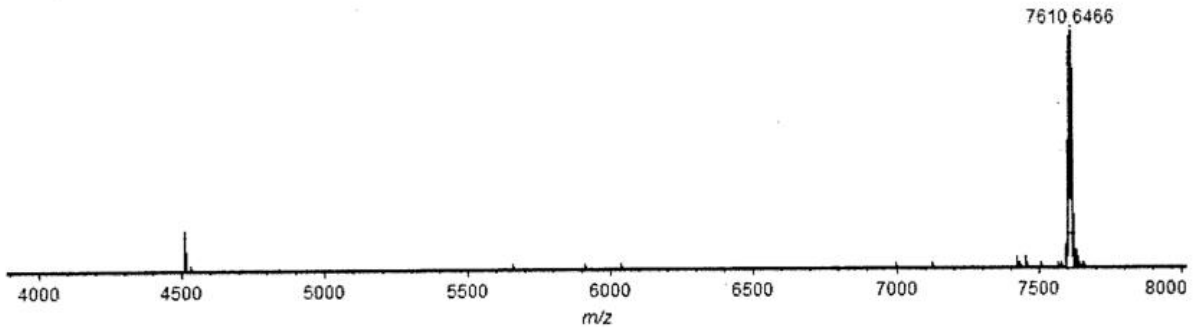


ODN 3 MALDI TOF MS



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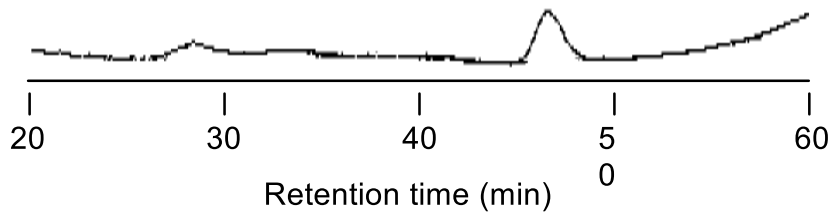

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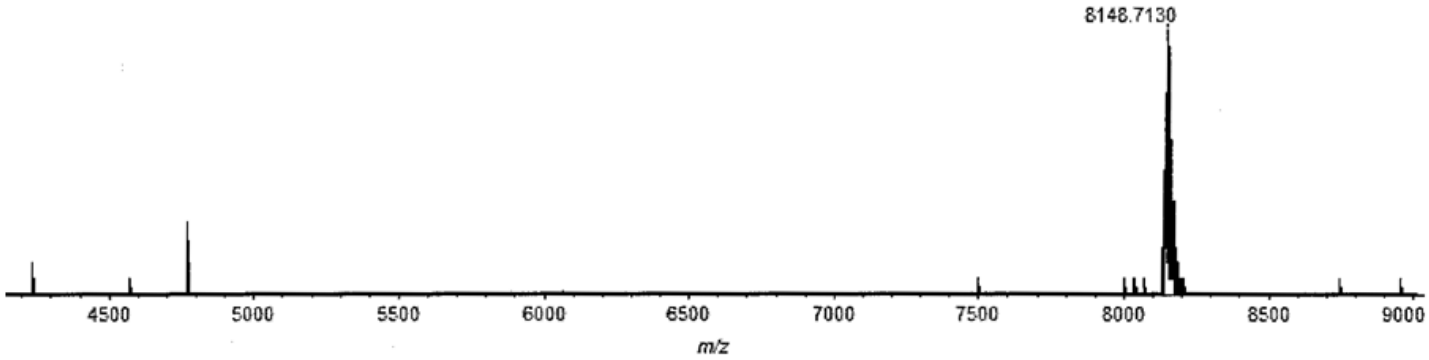

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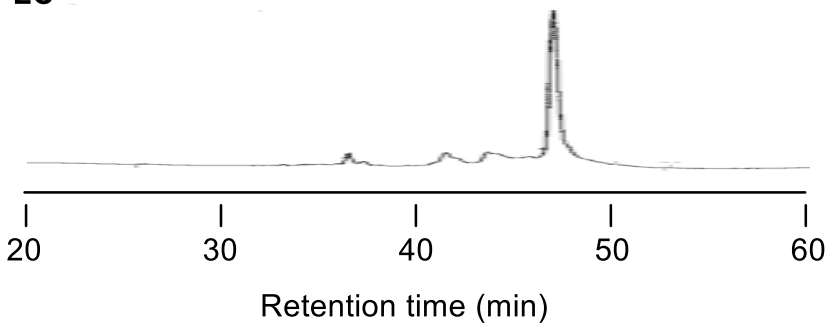

ODN 5 MALDI TOF MS