Supplementary Material (ESI) for Chemical Communications

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G-Quadruplex Recognition by Macrocyclic Hexaoxazole (6OTD) Dimer: Greater Selectivity Than Monomer

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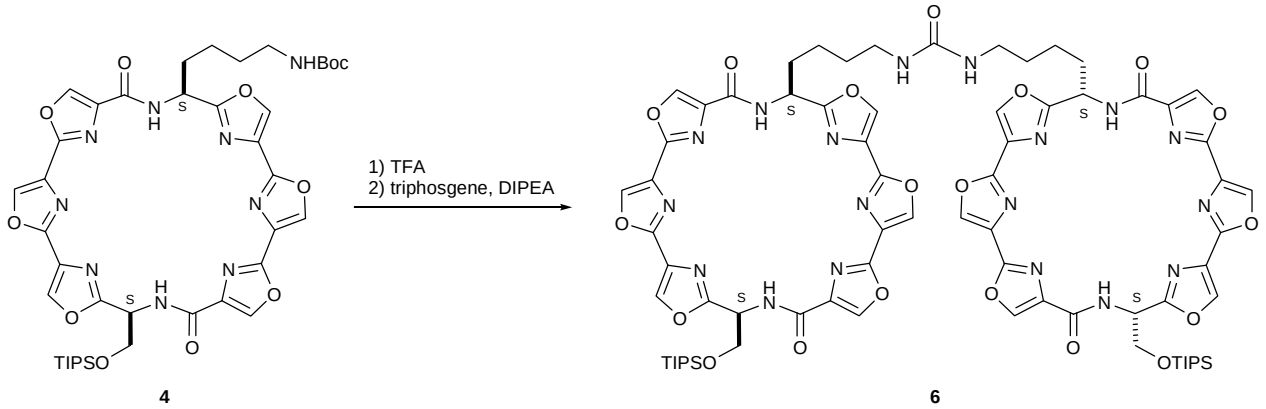
Table of Contents:

1. Synthesis

Flash chromatography was performed on Silica gel 60 (spherical, particle size 0.040 ~ 0.100 μm; Kanto). Optical rotations were measured on a JASCO DIP 1000 polarimeter, using the sodium D line. ^{1}H and ^{13}C NMR spectra were recorded on JEOL JNM-ECX 400. The spectra are referenced internally according to residual solvent signals of $CDCl_3$ (^{1}H NMR; δ = 7.26 ppm, ^{13}C NMR; δ = 77.0 ppm), $(CD_3)_2SO$ (^{1}H NMR; δ = 2.50 ppm, ^{13}C NMR; δ = 39.5 ppm). Data for ^{1}H NMR are recorded as follows: chemical shift (δ, ppm), multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad), integration, coupling constant (Hz). Data for ^{13}C NMR are reported in terms of chemical shift (δ, ppm). Mass spectra were recorded on JEOL JMS-T100X spectrometer with ESI-MS mode using methanol as solvent. Fluorescence was scanned with a phosphorimager (Typhoon 8600, Molecular Dynamics). All oligonucleotides purified were obtained from Sigma Genosys and dissolved in double-distilled water to be used without further purification. Fluorescence resonance energy transfer (FRET) melting assay were made with an excitation wavelength of 470 nm and a detection wavelength of 530 nm using a LightCycler® ST300 System RT-PCR machine (Roche).

2. Synthesis of 6OTD dimers

6OTD dimer **6**

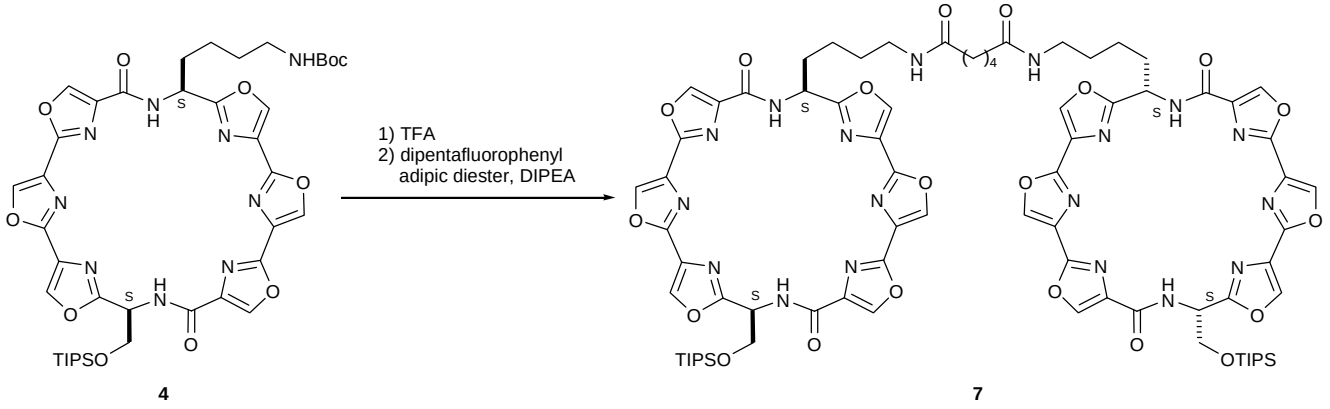


4 **6**

To a solution of **4** (400 mg, 458 μmol) in CH_2Cl_2 (20 mL), was added TFA (1 mL) and the mixture was stirred for 2 h. To the reaction mixture was added Amberlyst® A-26(OH) ionexchange resin. The resulting mixture was filtered through a cotton with MeOH, and the filtrates were concentrated *in vacuo* to give amine as a white solid. To the crude solution of amine in acetonitrile-dioxane = 1:1 (60 mL), was added DIPEA (390μL, 2.3 mmol), and triphosgene (20 mg, 69 μmol). The reaction mixture was stirred at 0 ºC. After stirring at rt for 18h, then evaporated. The residue was added 0.1 N HCl and H_2O and extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$, filtrated and concentrated *in vacuo*. The residue was purified on silica gel ($CHCl_3$-AcOEt-MeOH = 3:2:1) to give **6** (100 mg, 64 μmol 31 %).

Spectral data for **6**: $[\alpha]^{25}_D$ = 15.0 (*c* 1.4, $CHCl_3$-MeOH = 1:1); ^{1}H NMR (400 MHz, DMSO-d6) δ 9.20-9.05 (m, 8H), 8.97-8.87 (m, 4H), 8.46 (d, *J* = 7.3 Hz, 2H), 8.29 (d, *J* = 6.4 Hz, 2H), 5.68 (t, *J* = 5.5 Hz, 2H), 5.46-5.35 (m, 4H), 4.25 (dd, *J* = 4.1, 10.5 Hz, 2H), 4.21 (dd, *J* = 3.2, 10.5 Hz, 2H), 2.95-2.75 (m, 4H), 2.12-1.82 (m, 4H), 1.45-1.16 (m, 4H), 1.10-0.75 (m, 46H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.7, 164.0, 159.9, 158.6, 156.0, 155.9, 154.7, 154.6, 140.9, 139.3, 139.2, 138.6, 138.5, 136.7, 130.8, 129.7, 129.5, 77.2, 64.9, 50.2, 47.7, 40.0, 34.6, 29.6, 29.4, 22.0, 17.7, 11.7; HRMS (ESI, M+Na) calcd for $C_{73}H_{85}N_{18}O_{19}Na$ 1596.5675, found 1596.5711.

6OTD dimer **7**



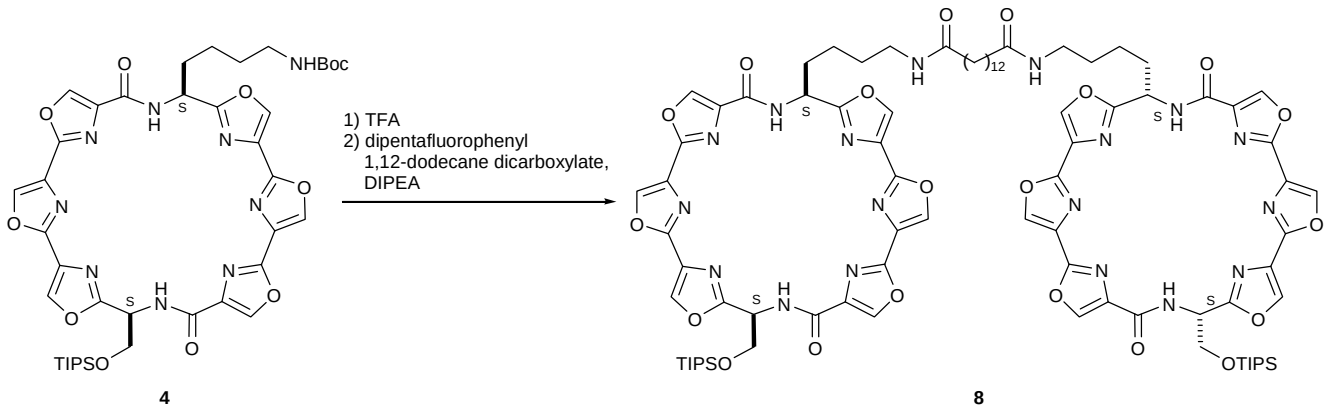
4 **7**

To a solution of **4** (200 mg, 229 μmol) in CH_2Cl_2 (19 mL), was added TFA (1 mL) and stirred for 2 h.

To the reaction mixture was added Amberlyst® A-26(OH) ionexchange resin. The resulting mixture was filtered through a cotton with MeOH, and the filtrates were concentrated *in vacuo* to give amine as a white solid. The crude amine was dissolved in acetonitrile solution (50 mL), was added DIPEA (389μL, 2.3 mmol), and dipentafluorophenyl adipic diester (44 mg, 92 μmol). The reaction mixture was refluxed for 3 h at 100 ºC, then evaporated. The residue was taken up in 0.1 N HCl and H_2O and extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$, filtrated and concentrated *in vacuo*. The residue was purified on silica gel ($CHCl_3$-AcOEt-MeOH = 3:2:1) to give **7** (102 mg, 62 μmol 67 %).

Spectral data for **7**: $[\alpha]^{25}_D$ = 5.9 (*c* 2.1, $CHCl_3$-MeOH = 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.54 (d, *J* = 7.7 Hz, 2H), 8.49 (d, *J* = 7.5 Hz, 2H), 8.35-8.09 (m, 12H), 6.12-5.98 (br, 2H), 5.51-5.38 (m, 4H), 4.27 (dd, *J* = 4.6, 9.5 Hz, 2H), 4.03 (dd, *J* = 6.8, 9.5 Hz, 2H), 3.32-3.09 (m, 4H), 2.25-1.87 (m, 8H), 1.76-1.48 (m, 8H), 1.30-0.83 (m, 46H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.8, 164.6, 164.0, 160.0, 159.9, 156.0, 154.8, 154.6, 141.0, 140.9, 139.3, 139.1, 138.5, 138.4, 136.8, 131.0, 130.9, 129.8, 129.6, 100.5, 77.2, 64.9, 50.3, 47.7, 39.2, 36.0, 34.7, 28.7, 24.9, 17.9, 17.8, 17.7, 11.7; HRMS (ESI, M+Na) calcd for $C_{78}H_{92}N_{18}O_{20}Na$ 1679.6172, found 1679.6123.

6OTD dimer **8**



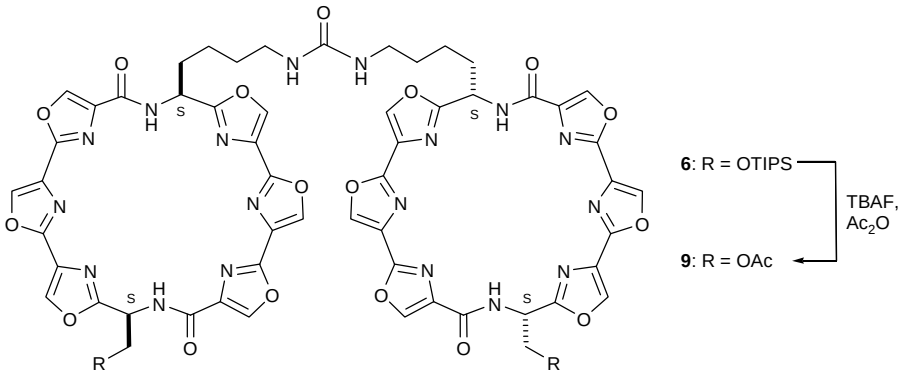
4 **8**

To a solution of **4** (200 mg, 229 μmol) in CH_2Cl_2 (19 mL), was added TFA (1 mL) and the mixture was stirred for 2 h. To the reaction mixture was added Amberlyst® A-26(OH) ionexchange resin. The resulting mixture was filtered through a cotton with MeOH, and the filtrates were concentrated *in vacuo* to give amine as a white solid. The crude amine was dissolved in acetonitrile solution (50 mL), was added DIPEA (389μL, 2.3 mmol), and dipentafluorophenyl 1,12-dodecanedicarboxylate (54 mg, 92 μmol). The reaction mixture was refluxed for 3 h at 100 ºC, then evaporated. The residue was taken up in 0.1 N HCl and H_2O and extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$, filtrated and concentrated *in vacuo*. The residue was purified on silica gel ($CHCl_3$-AcOEt-MeOH = 3:2:1) to give **8** (82 mg, 46 μmol 50 %).

Spectral data for **8**: $[\alpha]^{25}_D$ = 31.9 (*c* 1.0, $CHCl_3$-MeOH = 1:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.57 (d, *J* = 7.5 Hz, 2H), 8.50 (d, *J* = 7.5 Hz, 2H), 8.26-8.16 (m, 12H), 5.78-5.68 (br, 2H), 5.50-5.36 (m, 4H), 4.27 (dd, *J* = 4.6, 9.7 Hz, 2H), 4.03 (dd, *J* = 7.5, 9.7 Hz, 2H), 3.32-3.06 (m, 4H), 2.40-1.40 (m, 8H), 1.64-1.37 (m, 8H), 1.37-1.15 (m, 20H), 1.09-0.80 (m, 42H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.7, 164.0, 159.8, 156.0, 154.6, 140.9,

139.3, 139.2, 139.1, 138.4, 136.8, 130.9, 129.7, 129.6, 77.2, 64.9, 50.2, 47.7, 39.2, 36.8, 34.6, 29.4, 29.3, 29.2, 28.8, 25.8, 22.0, 17.8, 17.7, 11.7; HRMS (ESI, M+Na) calcd for $C_{86}H_{108}N_{18}O_{20}Na$ 1791.7424, found 1791.7413.

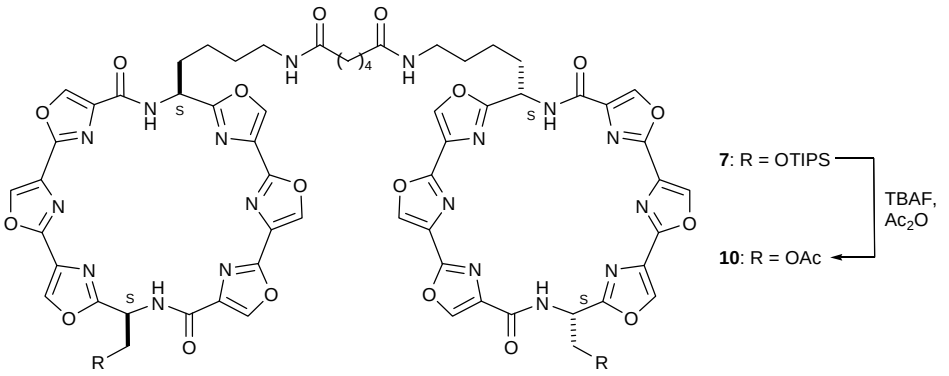
6OTD dimer **9**



To a solution of **6** (160 mg, 102 μmol) in THF (20 mL), was added TBAF (266 mg, 1.0 mmol) and the mixture was stirred for 5 min. To the reaction mixture was added Ac_2O (1 mL). After stirring at rt for 1h, then evaporated. The residue was added 0.1 N HCl and H_2O and extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$, filtrated and concentrated *in vacuo*. The residue was purified on silica gel ($CHCl_3$-AcOEt-MeOH = 3:2:2) to give **9** (40 mg, 30 μmol 29 %).

Spectral data for **9**: $[\alpha]^{25}_D$ = 25.0 (*c* 0.3, $CHCl_3$-MeOH = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (d, *J* = 6.9 Hz, 2H), 8.54 (d, *J* = 7.8 Hz, 2H), 8.28-8.18 (m, 12H), 5.64-5.57 (m, 2H), 5.45-5.36 (m, 2H), 4.80-4.69 (br, 2H), 4.64 (dd, *J* = 4.1, 11.5 Hz, 2H), 4.58 (dd, *J* = 3.2, 11.5 Hz, 2H), 3.23-3.98 (m, 4H), 2.10-1.80 (m, 14H), 1.58-1.38 (m, 4H), 1.36-1.22 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 164.8, 161.7, 160.0, 159.9, 158.3, 156.0, 155.8, 154.7, 141.1, 141.0, 139.8, 139.2, 138.7, 138.6, 136.8, 136.6, 130.9, 130.8, 129.8, 129.6, 77.2, 63.8, 47.7, 40.0, 34.7, 29.4, 22.0, 20.8; HRMS (ESI, M+Na) calcd for $C_{59}H_{48}N_{18}O_{21}Na$ 1367.3139, found 1367.3165.

6OTD dimer **10**

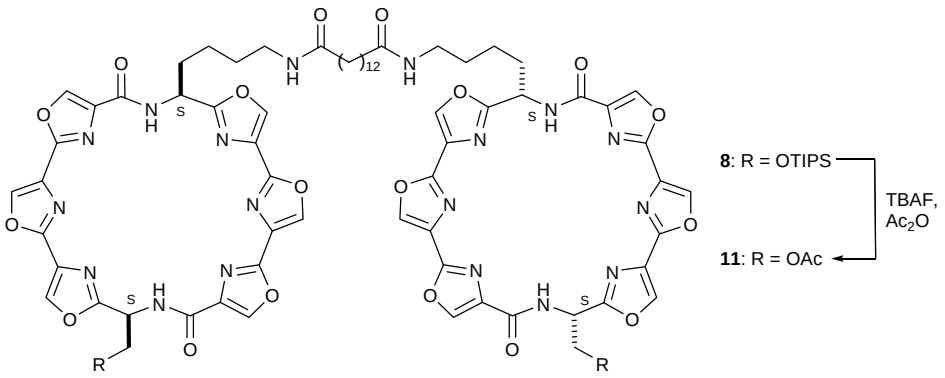


To a solution of **7** (102 mg, 62 μmol) in THF (10 mL), was added TBAF (160 mg, 615 μmol) and the

mixture was stirred for 30 min. To the reaction mixture was added Ac_2O (5 mL). After stirring at rt for 30 min, then evaporated. The residue was added H_2O and extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$, filtrated and concentrated *in vacuo*. The residue was purified on silica gel ($CHCl_3$-AcOEt-MeOH = 3:2:2) to give **10** (50 mg, 35 μmol 57 %).

Spectral data for **10**: $[\alpha]^{25}_D$ = 14.1 (*c* 0.3, $CHCl_3$-MeOH = 1:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.59 (d, *J* = 6.9 Hz, 2H), 8.58-8.49 (br, 2H), 8.29-8.18 (m, 12H), 6.12-5.92 (br, 2H), 5.63-5.58 (m, 2H), 5.47-5.37 (m, 2H), 4.63 (dd, *J* = 4.0, 11.5 Hz, 2H), 4.57 (dd, *J* = 3.4, 11.5 Hz, 2H), 3.30-3.10 (m, 4H), 2.20-1.90 (m, 14H), 1.67-1.38 (m, 8H), 1.33-1.20 (m, 4H); ^{13}C NMR (125 MHz, DMSO-d6) δ 171.7, 170.1, 164.5, 161.8, 159.0, 158.7, 155.6, 155.5, 154.6, 154.5, 142.7, 142.5, 142.3, 141.9, 141.2, 136.0, 135.8, 129.8, 129.7, 128.6, 128.4, 69.8, 63.1, 56.0, 47.5, 38.1, 35.1, 33.3. 28.7, 24.9, 21.2, 20.4, 18.6; HRMS (ESI, M+Na) calcd for $C_{64}H_{56}N_{18}O_{22}Na$ 1451.3714, found 1451.3720.

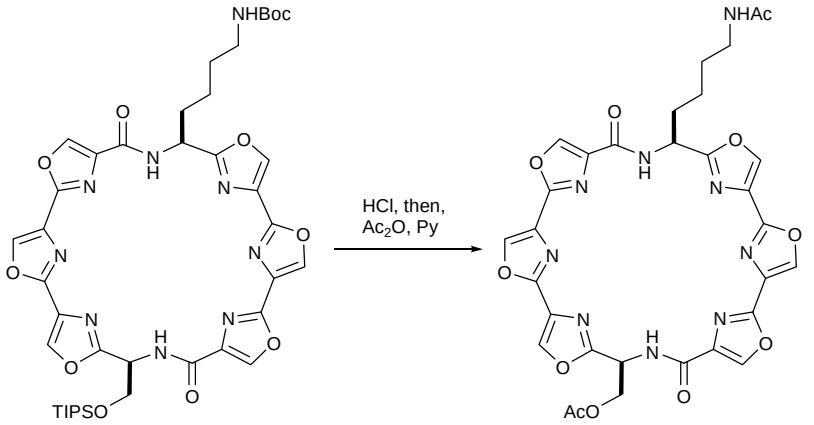
6OTD dimer **11**



To a solution of **8** (50 mg, 28 μmol) in THF (10 mL), was added TBAF (74 mg, 282 μmol) and the mixture was stirred for 5 min. To the reaction mixture was added Ac_2O (1 mL) and the mixture was stirred at rt for 1 h, then evaporated. The residue was added H_2O and extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$, filtrated and concentrated *in vacuo*. The residue was purified on silica gel ($CHCl_3$-AcOEt-MeOH = 3:2:2) to give **11** (27 mg, 17 μmol 61 %).

Spectral data for **11**: $[\alpha]^{25}_D$ = 82.6 (*c* 1.4, $CHCl_3$-MeOH = 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.59 (d, *J* = 7.0 Hz, 2H), 8.55 (d, *J* = 7.7 Hz, 2H), 8.26-8.18 (m, 12H), 5.74-5.64 (br, 2H), 5.63-5.56 (m, 2H), 5.49-5.38 (m, 2H), 4.64 (dd, *J* = 3.9, 11.4 Hz, 2H), 4.57 (dd, *J* = 3.5, 11.4 Hz, 2H), 3.32-3.09 (m, 4H), 2.20-1.90 (m, 14H), 1.70-1.38 (m, 8H), 1.37-1.14 (m, 18H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.2, 170.6, 164.7, 161.6, 160.0, 159.9, 156.0, 155.9, 154.7, 154.6, 141.1, 140.9, 139.8, 139.2, 138.5, 138.4, 136.9, 136.6, 131.0, 130.9, 129.9, 129.6, 77.2, 63.8, 47.7, 39.2, 36.8, 34.6, 29.5, 29.4, 29.3, 28.9, 25.8, 22.0, 20.8; HRMS (ESI, M+Na) calcd for $C_{72}H_{72}N_{18}O_{22}Na$ 1563.4966, found 1563.4941.

LSA2-6OTD (**3**)

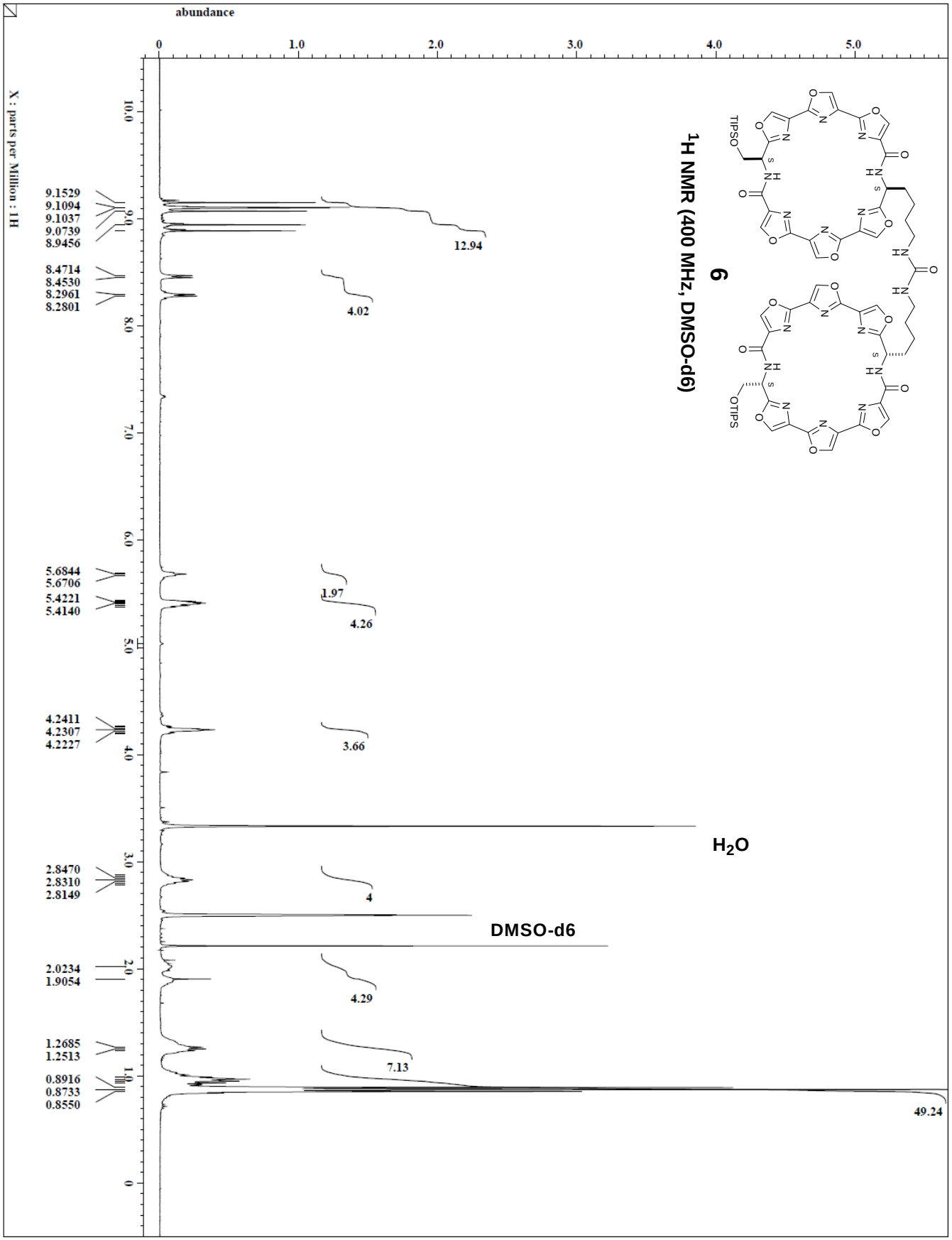


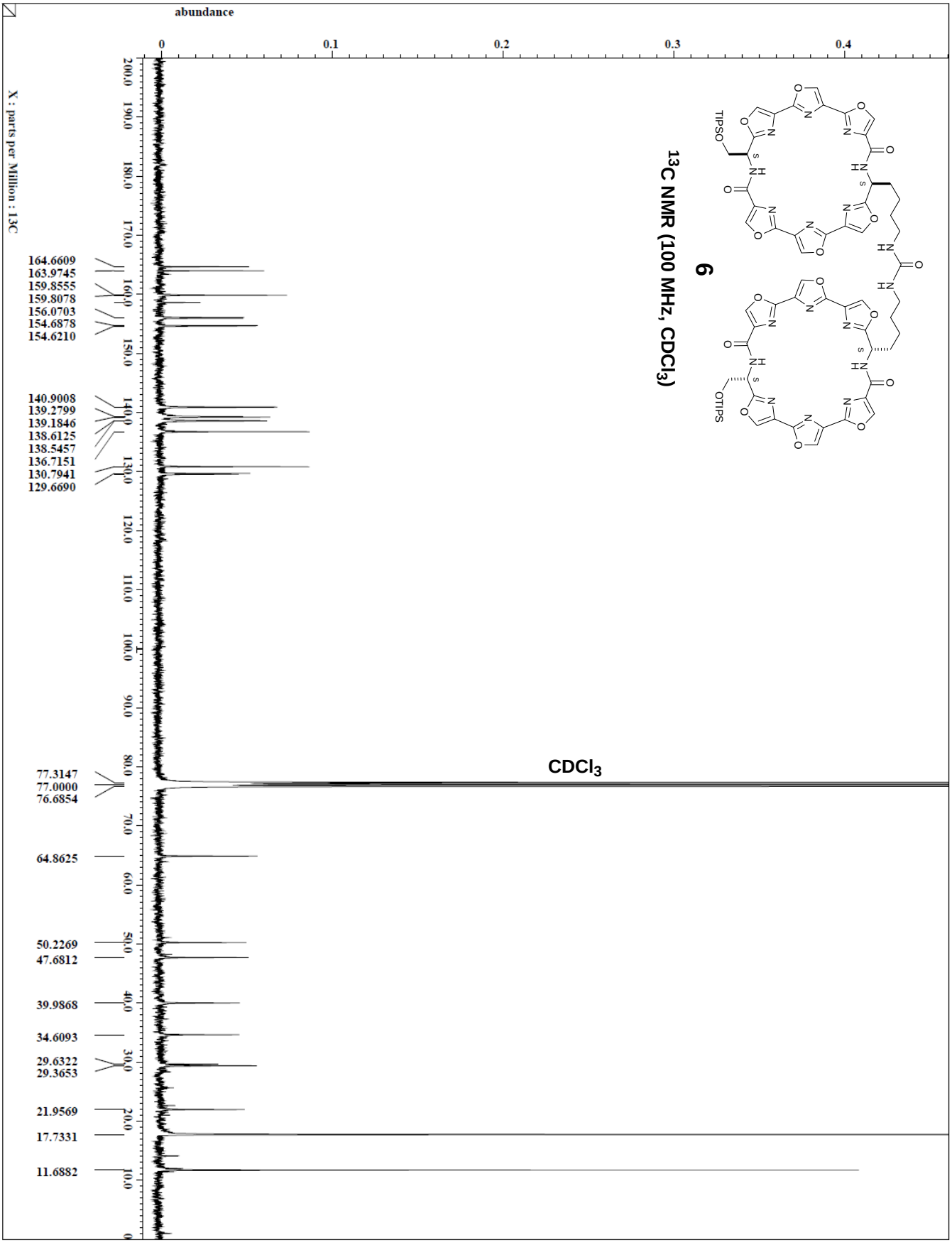
4 **3**

To a solution of **4** (41 mg, 46 μmol) in acetonitrile-12N HCl = 11:1 solution (2 mL) and the mixture was stirred for 0.5h, then evaporated. The residue was dissolved in pyridine (10 mL), was added Ac_2O (3 mL) and the reaction mixture was stirred at rt. After stirring at 70 ºC for 1h, then evaporated. The residue was purified on silica gel ($CHCl_3$-AcOEt-MeOH = 3:2:1) to give **3** (30 mg, 43 μmol 93 %).

Spectral data for **3**: $[\alpha]^{25}_D$ = 5.71 (*c* 1.0, $CHCl_3$-MeOH = 1:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.70-8.50 (m, 2H), 8.28-8.18 (m, 6H), 5.88-5.78 (br, 1H), 5.63-5.56 (m, 1H), 5.48-5.40 (m, 1H), 4.63 (dd, *J* = 4.0, 11.5 Hz, 1H), 4.57 (dd, *J* = 3.4, 11.5 Hz, 1H), 3.28-3.10 (m, 2H), 2.20-1.90 (m, 8H), 1.62-1.40 (m, 2H), 1.33-1.20 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.6, 170.2, 164.7, 161.7, 160.0, 156.0, 155.8, 154.7, 154.6, 141.2, 141.1, 140.9, 140.8, 139.8. 139.7. 139.3, 139.2, 138.5, 136.8. 136.6, 130.9, 130.8, 129.8, 129.5, 63.8, 47.7, 39.3, 34.5, 28.7, 23.3, 21.9, 20.7; HRMS (ESI, M+Na) calcd for $C_{31}H_{27}N_9O_{11}Na$ 724.1728, found 724.1721

3. Copies of NMR spectra of compounds **6-11** and **3**.

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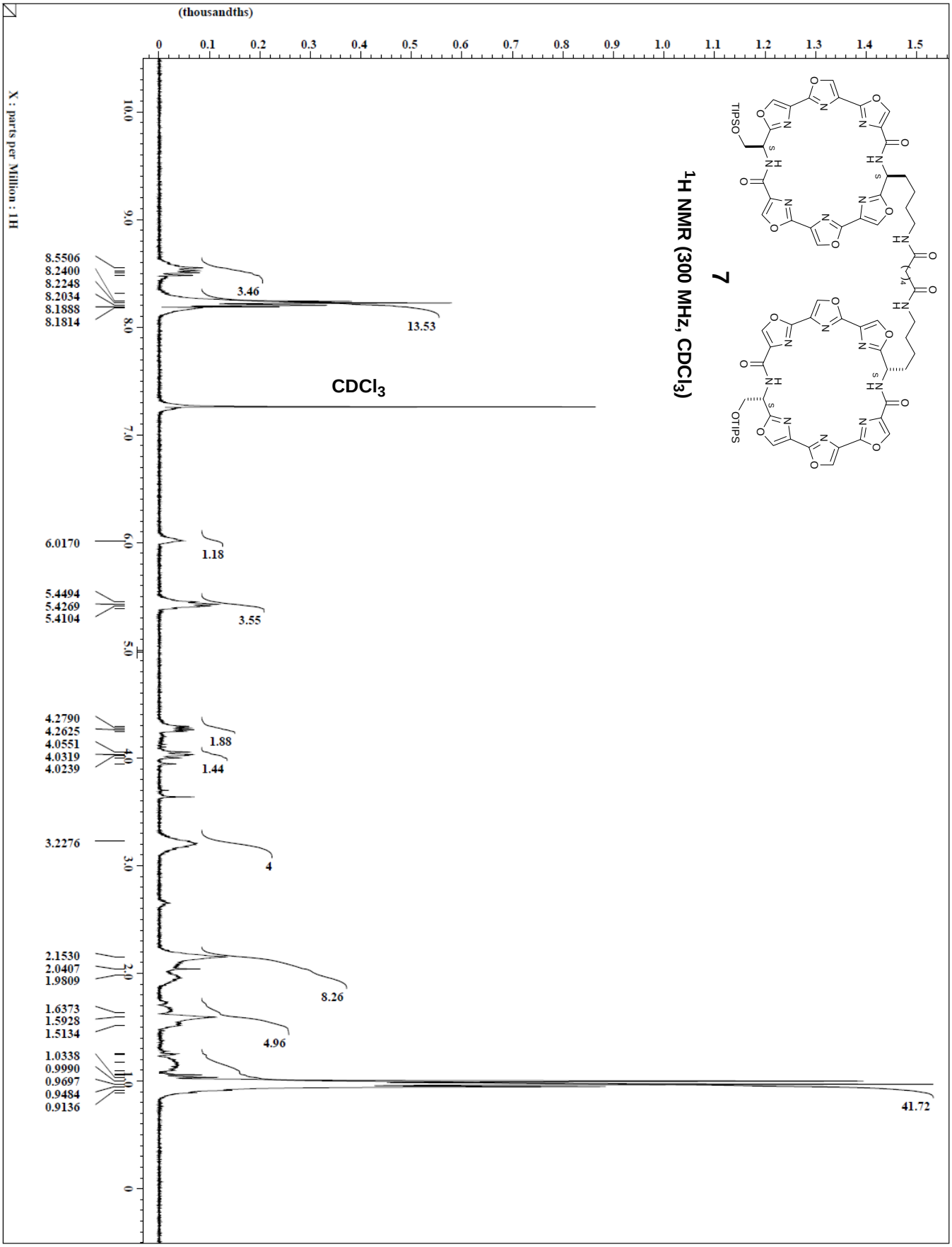
6
TIPSO
OTIPS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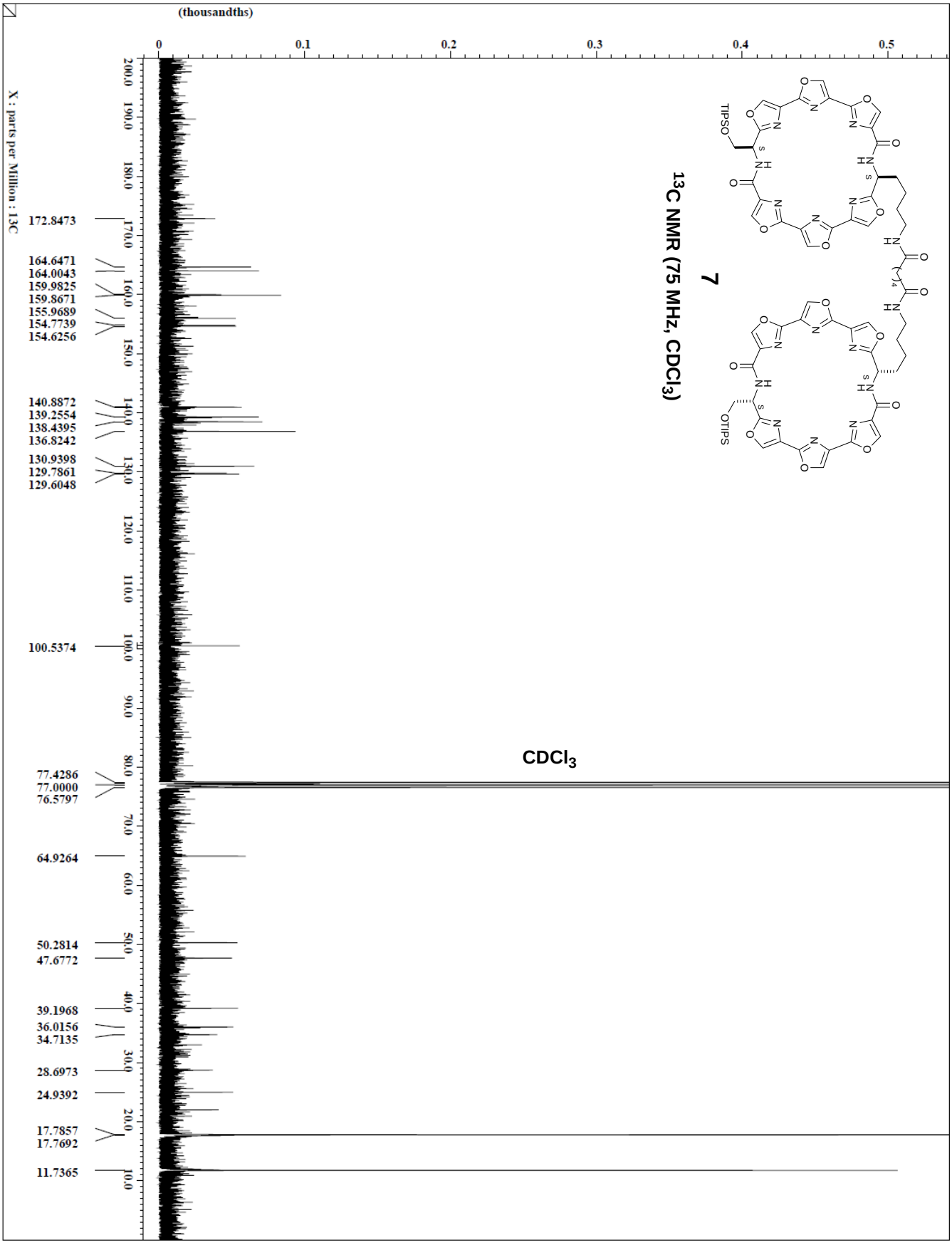
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7
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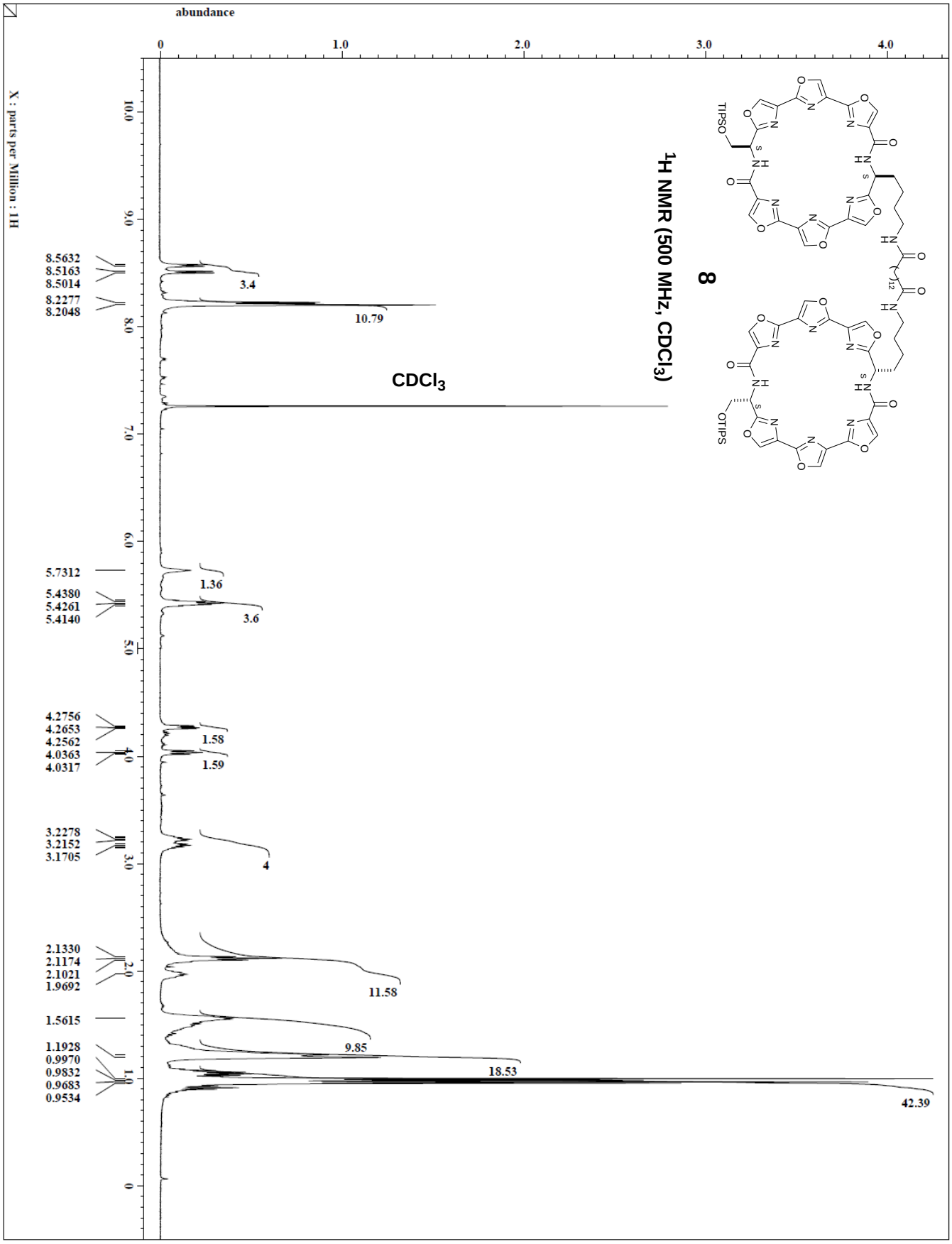
TIPSO
OTIPS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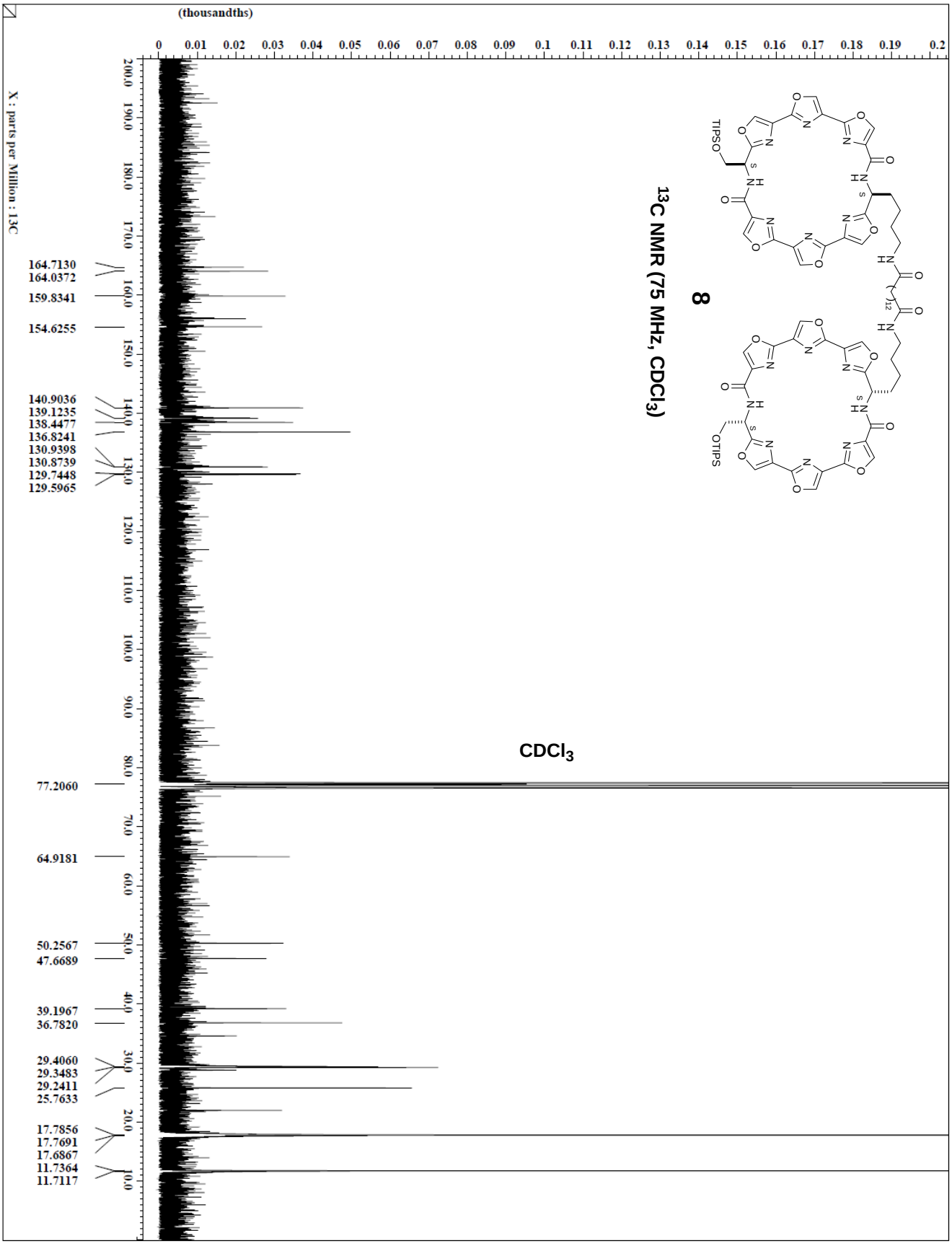
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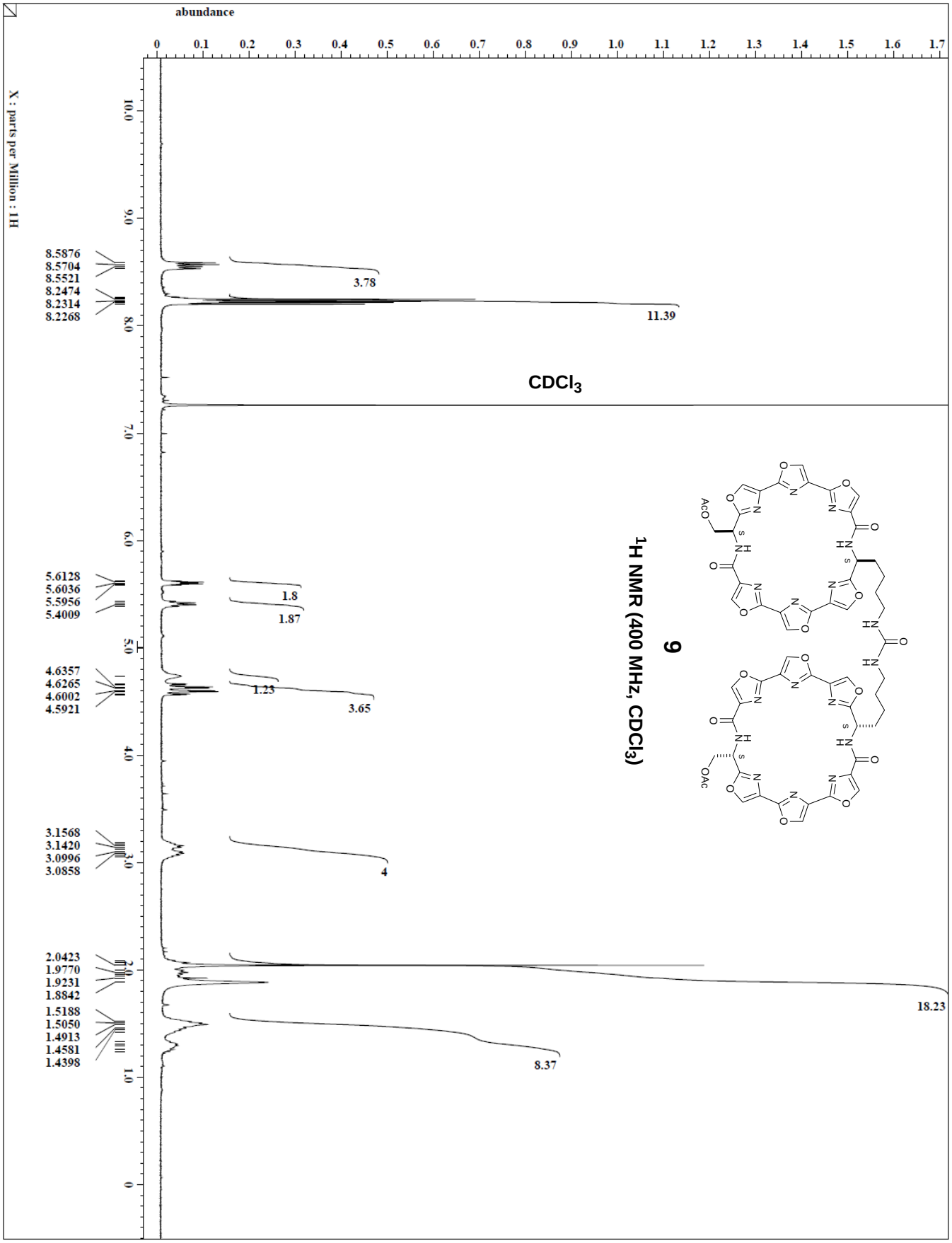
TIPSO
OTIPS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3.78
11.39
CDCl3
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5.6036
5.5956
5.4009
1.8
1.87
4.6357
4.6265
4.6002
4.5921
1.23
3.65
3.1568
3.1420
3.0996
3.0858
4
2.0423
1.9770
1.9231
1.8842
1.5188
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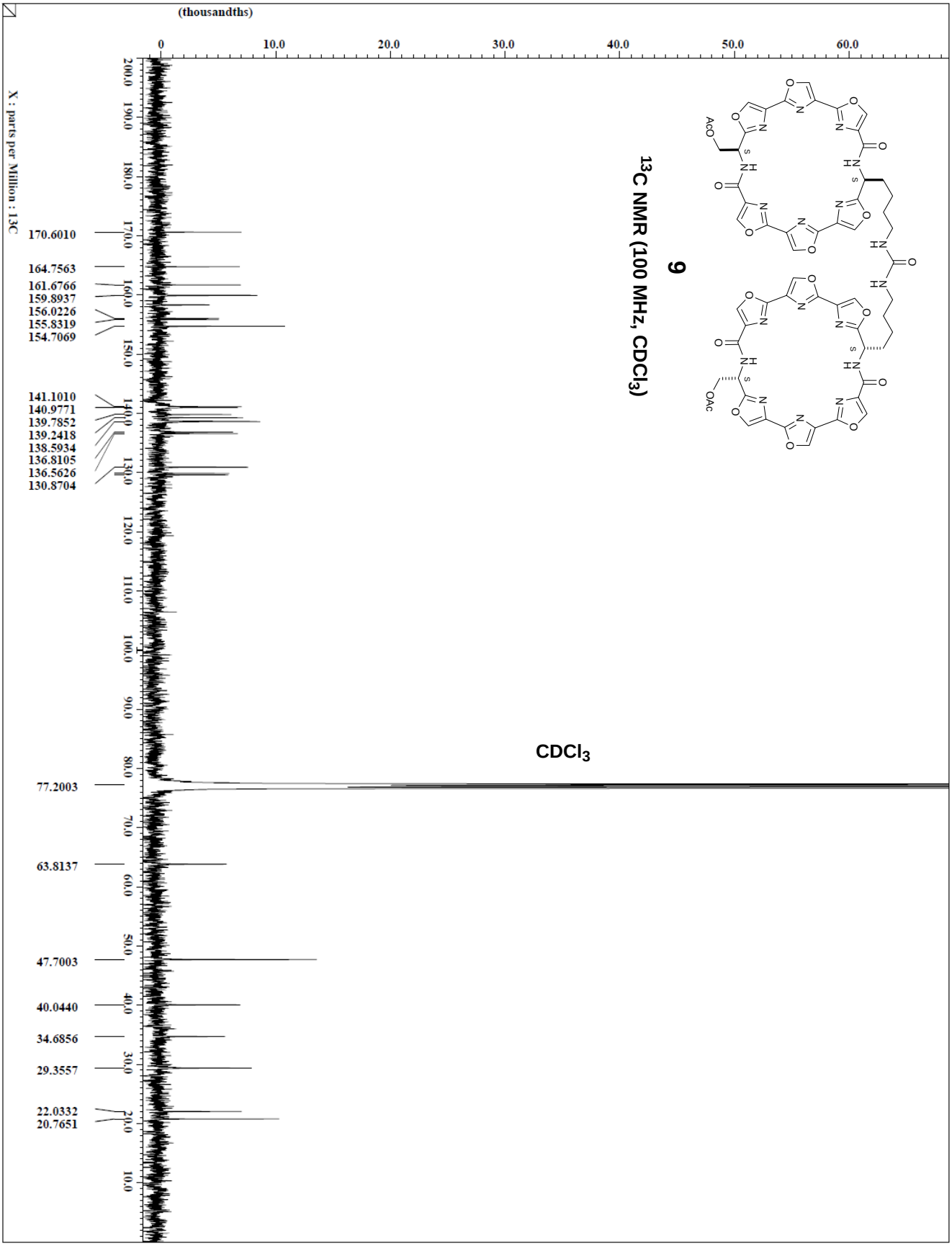
1.4398
18.23
8.37
9
1H NMR (400 MHz, CDCl3)
AcO
OAc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139.7852
139.2418
138.5934
136.8105
136.5626
130.8704
77.2003
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40.0440
34.6856
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22.0332
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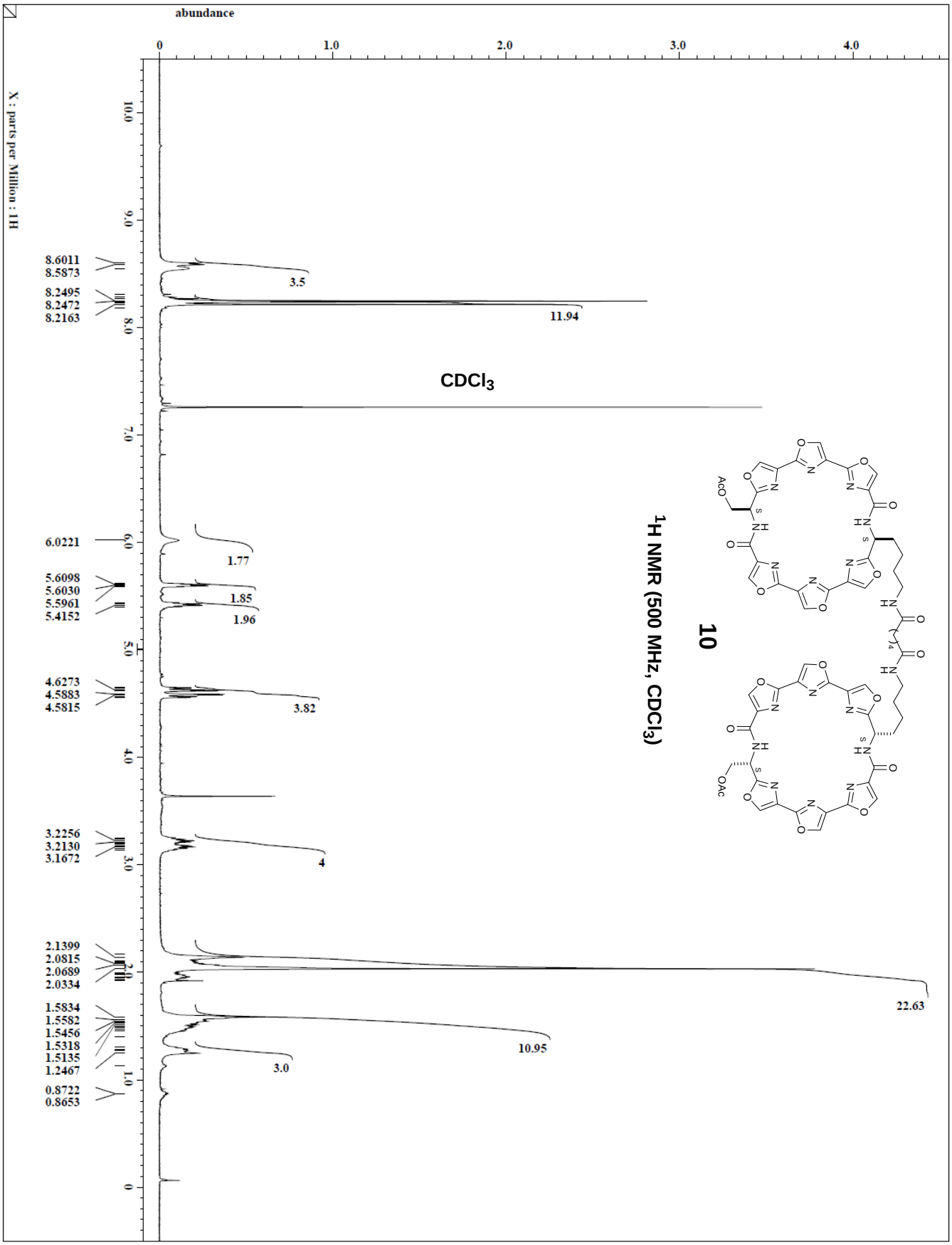
9
13C NMR (100 MHz, CDCl3)
CDCl3


abundance
X : parts per Million : 1H
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5.5961
5.4152
4.6273
4.5883
4.5815
3.2256
3.2130
3.1672
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2.0815
2.0689
2.0334
1.5834
1.5582
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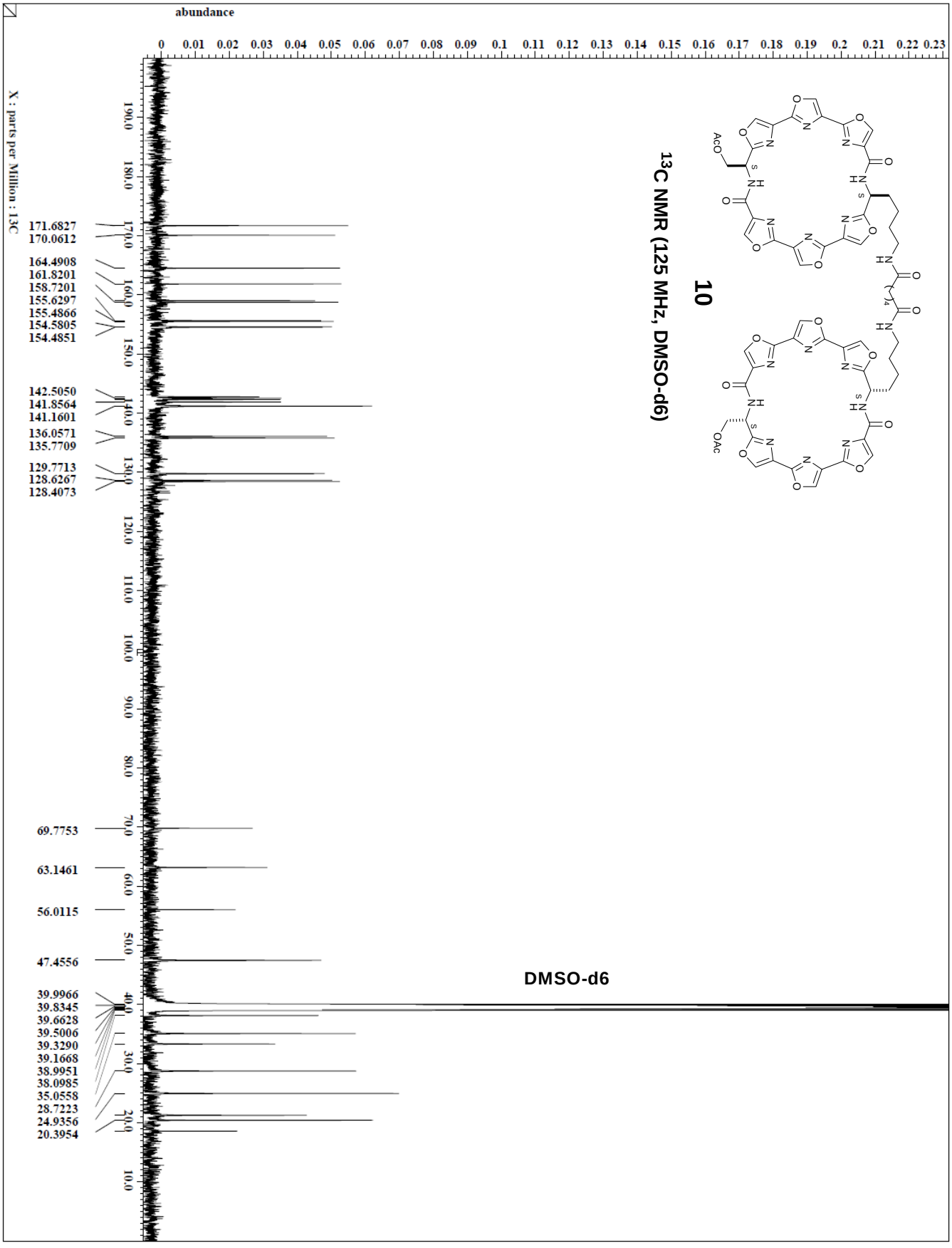
0.8722
0.8653
3.5
11.94
1.77
1.85
1.96
3.82
4
22.63
10.95
3.0
CDCl3
1H NMR (500 MHz, CDCl3)
10
AcO
OAc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X : parts per Million : 13C
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170.0612
164.4908
161.8201
158.7201
155.6297
155.4866
154.5805
154.4851
142.5050
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128.4073
69.7753
63.1461
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47.4556
39.9966
39.8345
39.6628
39.5006
39.3290
39.1668
38.9951
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35.0558
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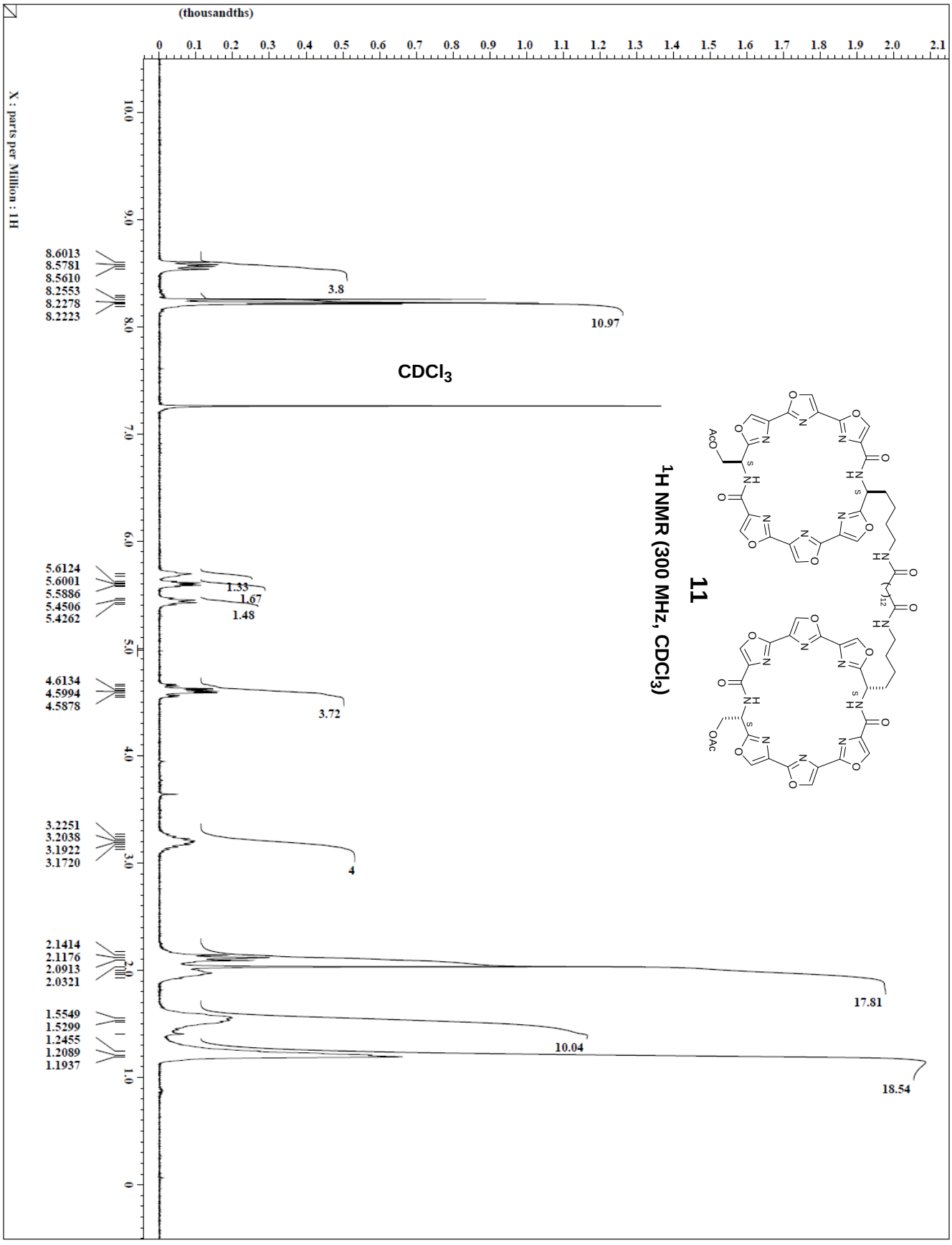
20.3954
DMSO-d6
13C NMR (125 MHz, DMSO-d6)
10
AcO
OAc


(thousandths)
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5.5886
5.4506
5.4262
1.33
1.67
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4.5878
3.72
3.2251
3.2038
3.1922
3.1720
4
2.1414
2.1176
2.0913
2.0321
17.81
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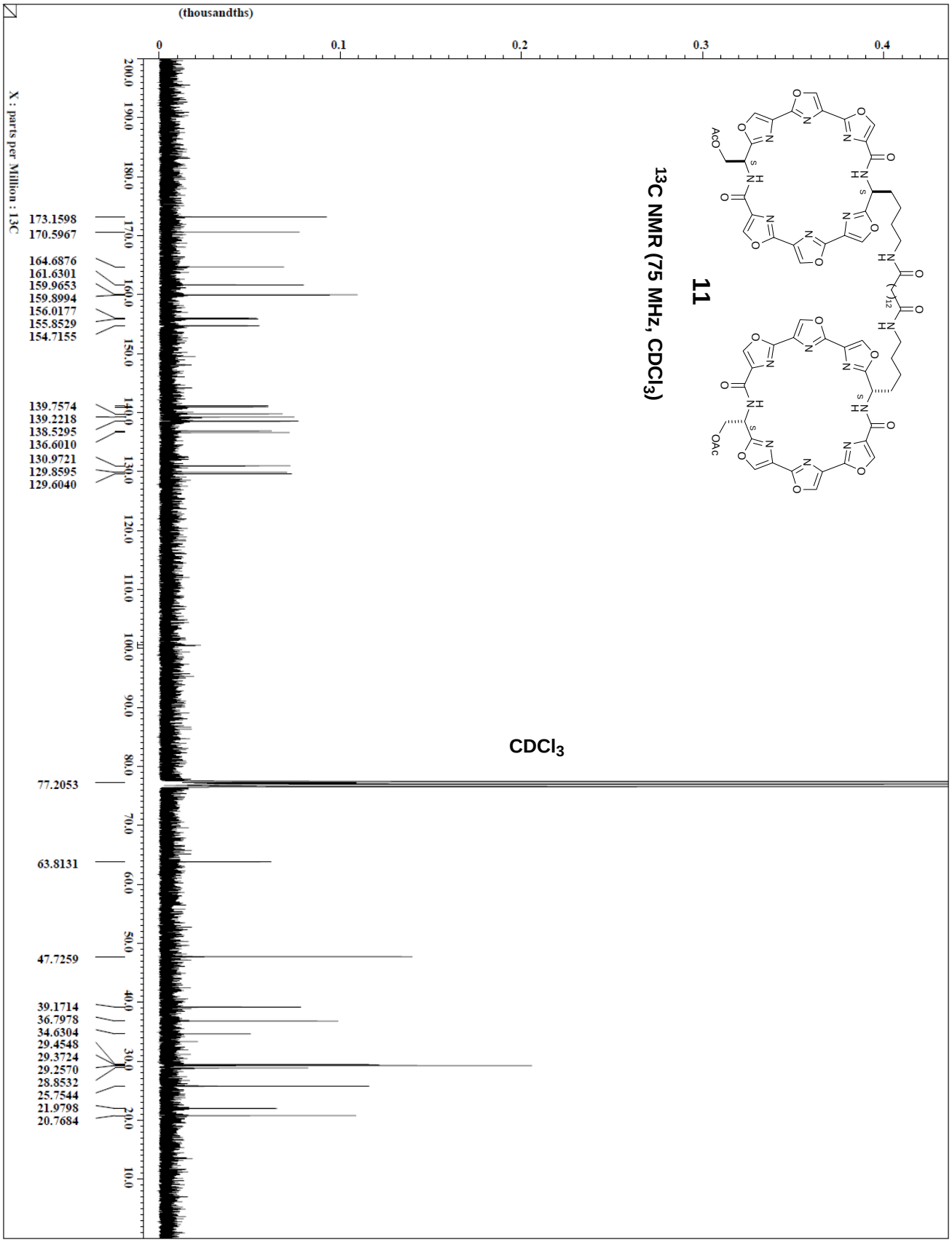
18.54
1H NMR (300 MHz, CDCl3)
11
AcO
OAc


(thousandths)
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0.2
0.3
0.4
X : parts per Million : 13C
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180.0
170.0
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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
173.1598
170.5967
164.6876
161.6301
159.9653
159.8994
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155.8529
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139.7574
139.2218
138.5295
136.6010
130.9721
129.8595
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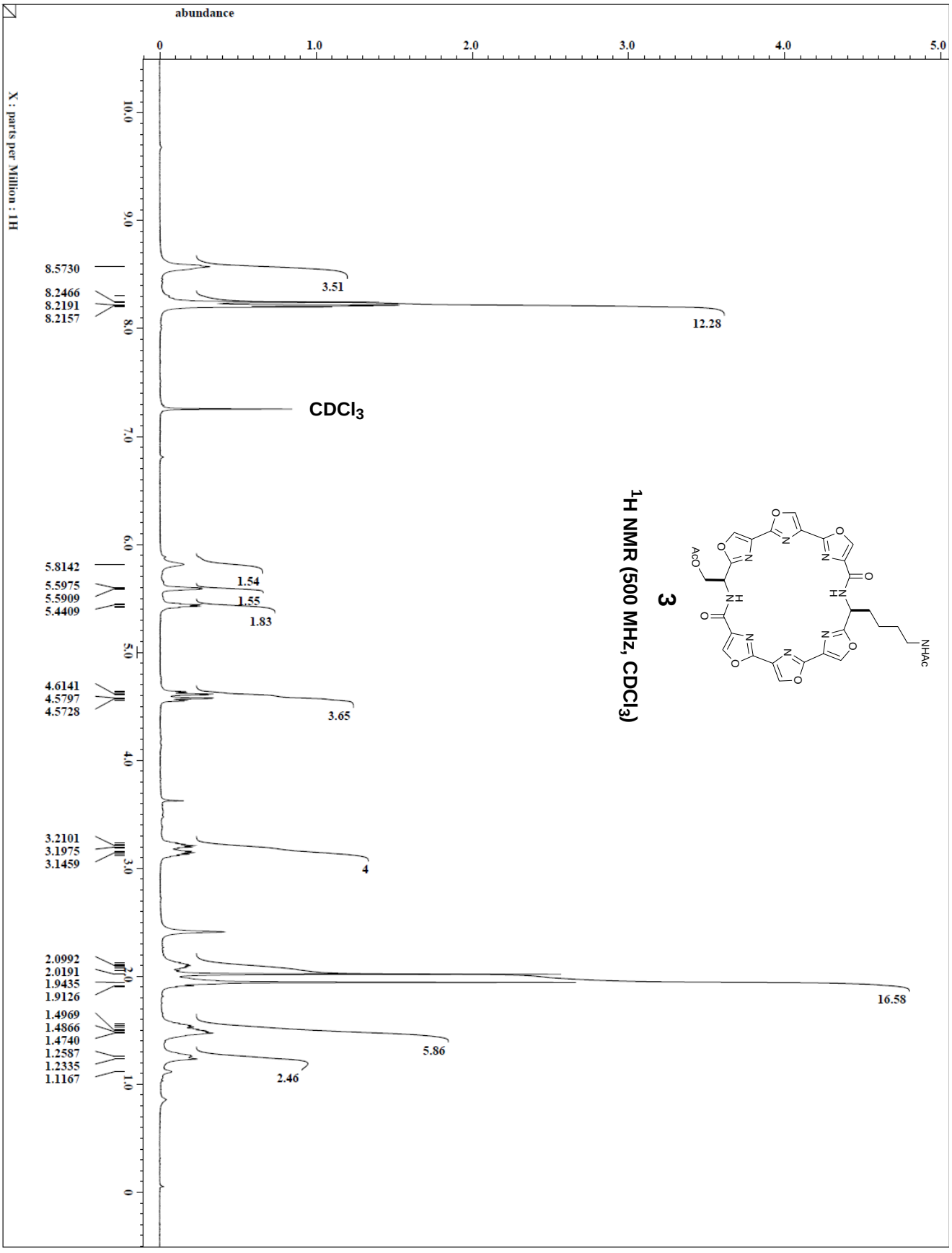
77.2053
63.8131
47.7259
39.1714
36.7978
34.6304
29.4548
29.3724
29.2570
28.8532
25.7544
21.9798
20.7684
^{13}C NMR (75 MHz, CDCl$_3$)
11
AcO
OAc
CDCl$_3$


abundance
X : parts per Million : 1H
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3.1975
3.1459
2.0992
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1.9435
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1.4740
1.2587
1.2335
1.1167
3.51
12.28
CDCl3
1.54
1.55
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16.58
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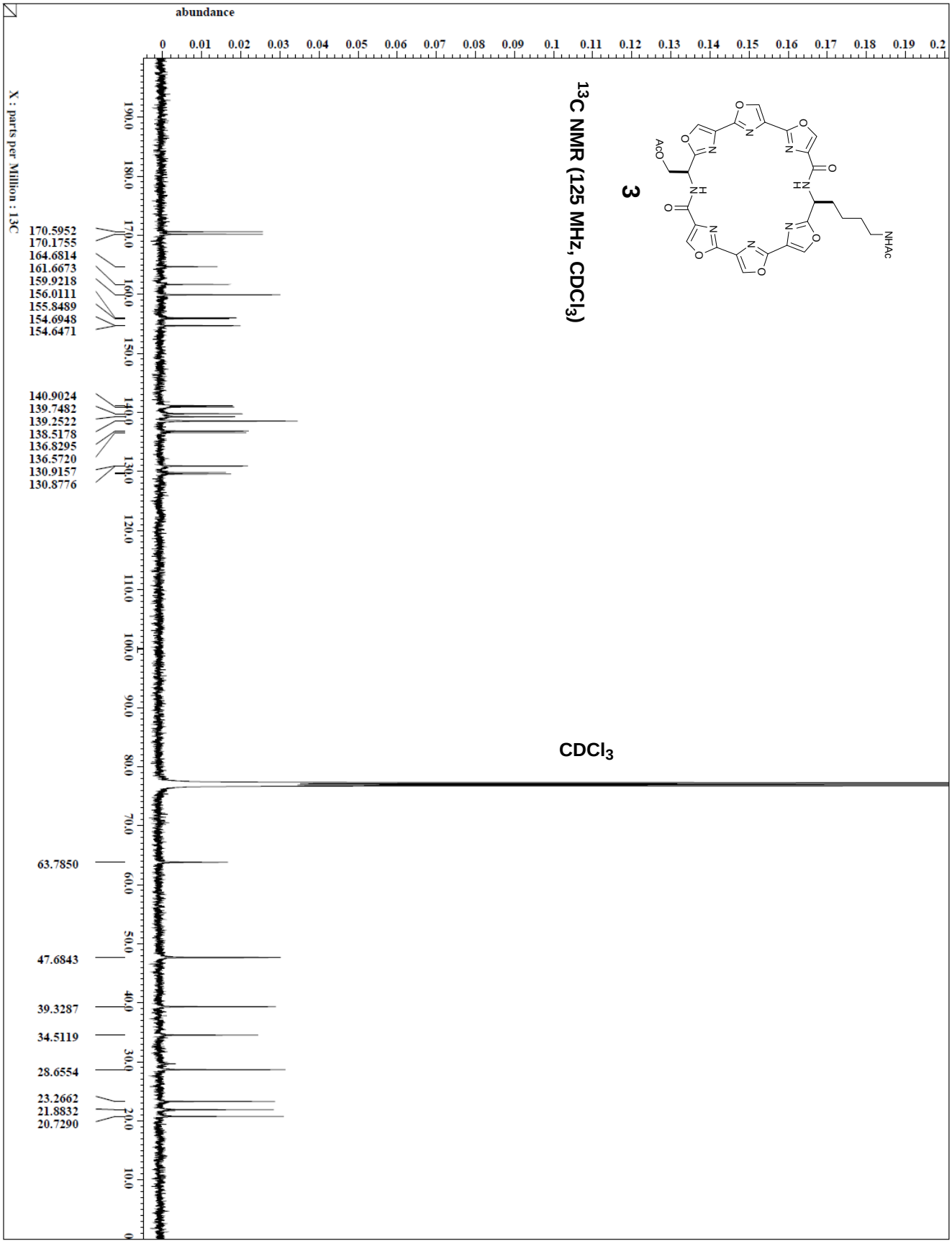
2.46
1H NMR (500 MHz, CDCl3)
3
AcO
NHAc


abundance
X : parts per Million : 13C
^{13}C NMR (125 MHz, CDCl$_3$)
3
AcO
NHAc
CDCl$_3$
170.5952
170.1755
164.6814
161.6673
159.9218
156.0111
155.8489
154.6948
154.6471
140.9024
139.7482
139.2522
138.5178
136.8295
136.5720
130.9157
130.8776
63.7850
47.6843
39.3287
34.5119
28.6554
23.2662
21.8832
20.7290

4. Molecular dynamics simulation experiment for **9**, **10** and **11**

Initial structures of **9**, **10** and **11** were constructed using the Molecular Builder module in Maestro (Schrödinger LLC.). Molecular dynamics simulation was carried out using AMBER9 with the gaff (for compounds) and ff99SB (for water) force field. The compound was surrounded with a 20Å layer of TIP3PBOX water molecules. After minimization, heating and equilibration, the production phase was carried out at 300K for 10ns, using the NTV ensemble and PME algorithm. All simulations were performed on a IBM BlueGene/L supercomputer at the Computational Biology Research Center (CBRC).

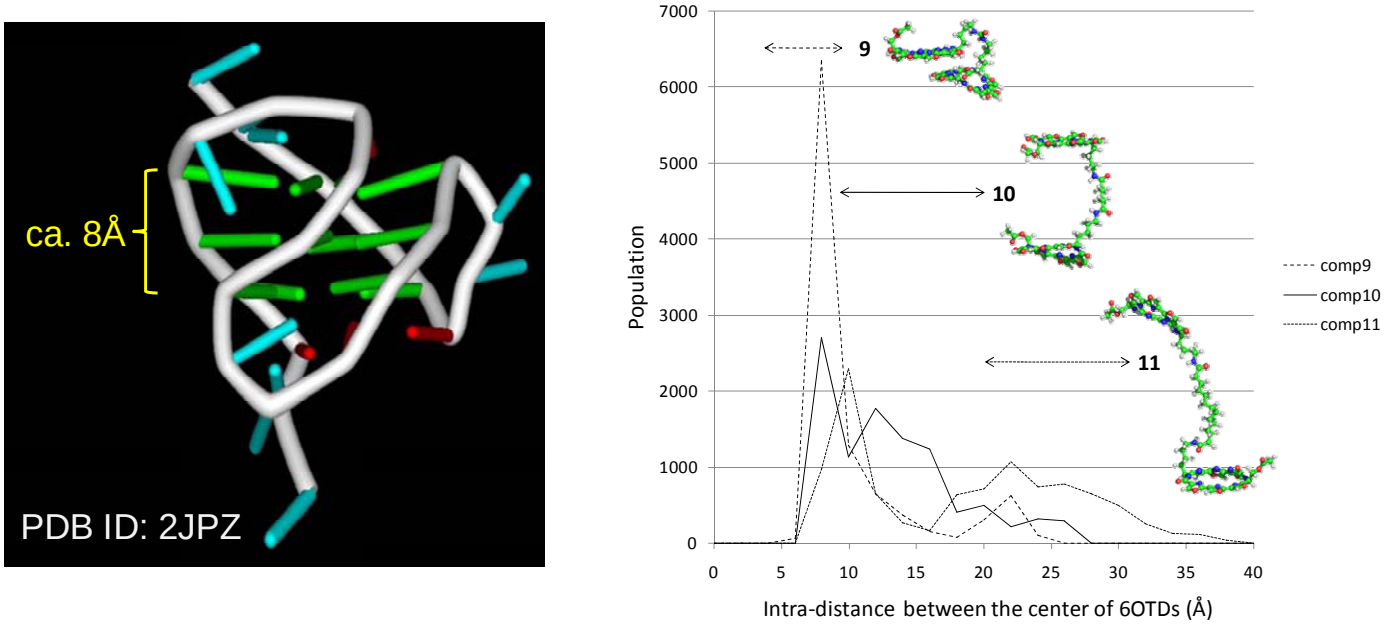


Fig. S1 Telomeric G-quadruplex structure and distribution of the intra-distance between the center of 6OTDs. Distribution of the intra-distance between the center of 6OTDs in three 6OTD dimers, **9** (dash line), **10** (solid line) and **11** (dotted line) derived from MD simulation in water. Ball and Stick representation of compounds on distribution diagram are the average conformations of **9**, **10** and **11** in each characteristic distribution ranges of distance: 5-10Å for **9**, 10-20Å for **10** and 20-30Å for **11**.

5. FRET melting assay

FRET melting assays were performed as reported methods.[S1] Oligonucleotides were initially dissolved as a 100 μM stock solution in MilliQ water; further dilutions were carried out in 60 mM potassium cacodylate buffer, pH 7.4 and FRET experiments were carried out with a 200 nM oligonucleotide solution. The dual fluorescently labeled oligonucleotides Flu-ss-telo21 5'-FAM-[GGG TTA GGG TTA GGG TTA GGG]-TAMRA-3' and Flu-ds-26mer 5'-FAM-[$(TA)_2GC(TA)_2T_6(TA)_2GC(TA)_2$]-TAMRA-3' were used in this protocol. The donor fluorophore was 6-carboxyfluorescein, FAM, and the acceptor fluorophore was 6-carboxytetramethylrhodamine, TAMRA. Dual-labeled DNA was annealed at a concentration of 400 nM by heating at 94 ºC for 10 min followed by cooling to rt. 96-well plates were prepared by addition of 10 μL of the annealed DNA solution to each well, followed by 10 μL of a solution of the respective molecule at an appropriate concentration. Fluorescence melting curves were determined in a LightCycler® ST300 System RT-PCR machine (Roche), using a total reaction volume of 20 μL.

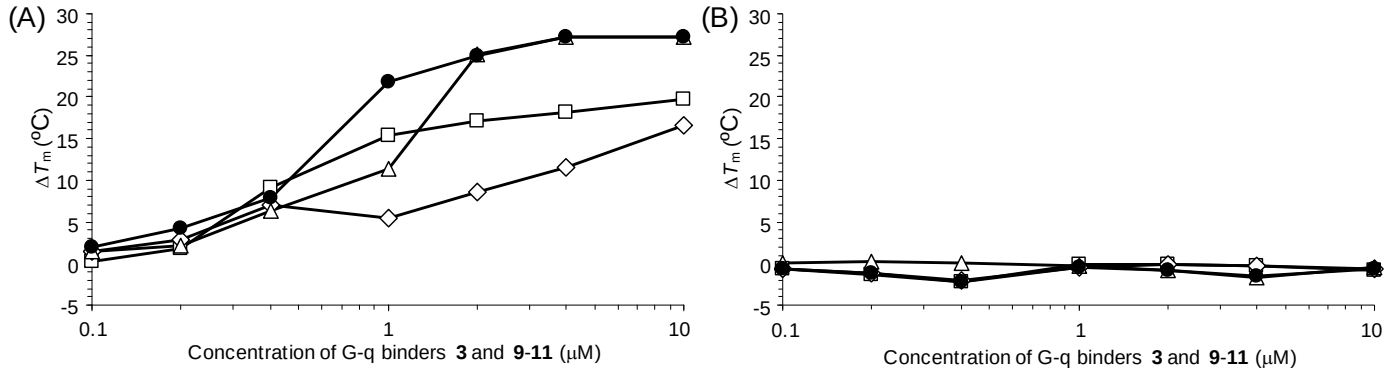


Fig. S2 ΔT_m of 0.2 μM ss-telo21 (A) or 0.2 μM Flu-ds-26mer (B) in the presence of ligands **3** and **9**-**11**.(●: monomer **3**, □: dimer **9**, △: dimer **10**, ◇: dimer **11**)

S1) S. Muller, G. D. Pantos, R. Rodriguez and S. Balasubramanian, *Chem. Commun.*, 2009, 80.

6. PCR stop assays

PCR stop assays were performed as previously reported. Oligonucleotides ss-telo24 and ss-telo24-mut, the complementary sequence of ss-telo24 d[TCT CGT CTT CCC TAA] were used. The chain-extension reaction was performed in 1×PCR buffer containing 0.2 mM dNTP, 5 U Taq polymerase, 7.5 pmol oligonucleotides and various concentrations of **3** and **9**-**11**. The mixtures were incubated in a thermocycler under the following conditions: 94ºC for 2 min, followed by 30 cycles of 94 ºC for 30 s, 47 ºC for 30 s, and 72 ºC for 30 s. Amplified PCR products were resolved on 12% native polyacrylamide gels in 0.5xTBE buffer and stained with ethidium bromide. The IC_{50} values were calculated based on the fluorescence intensity scanned with a phosphorimager (Typhoon 8600, Molecular Dynamics).

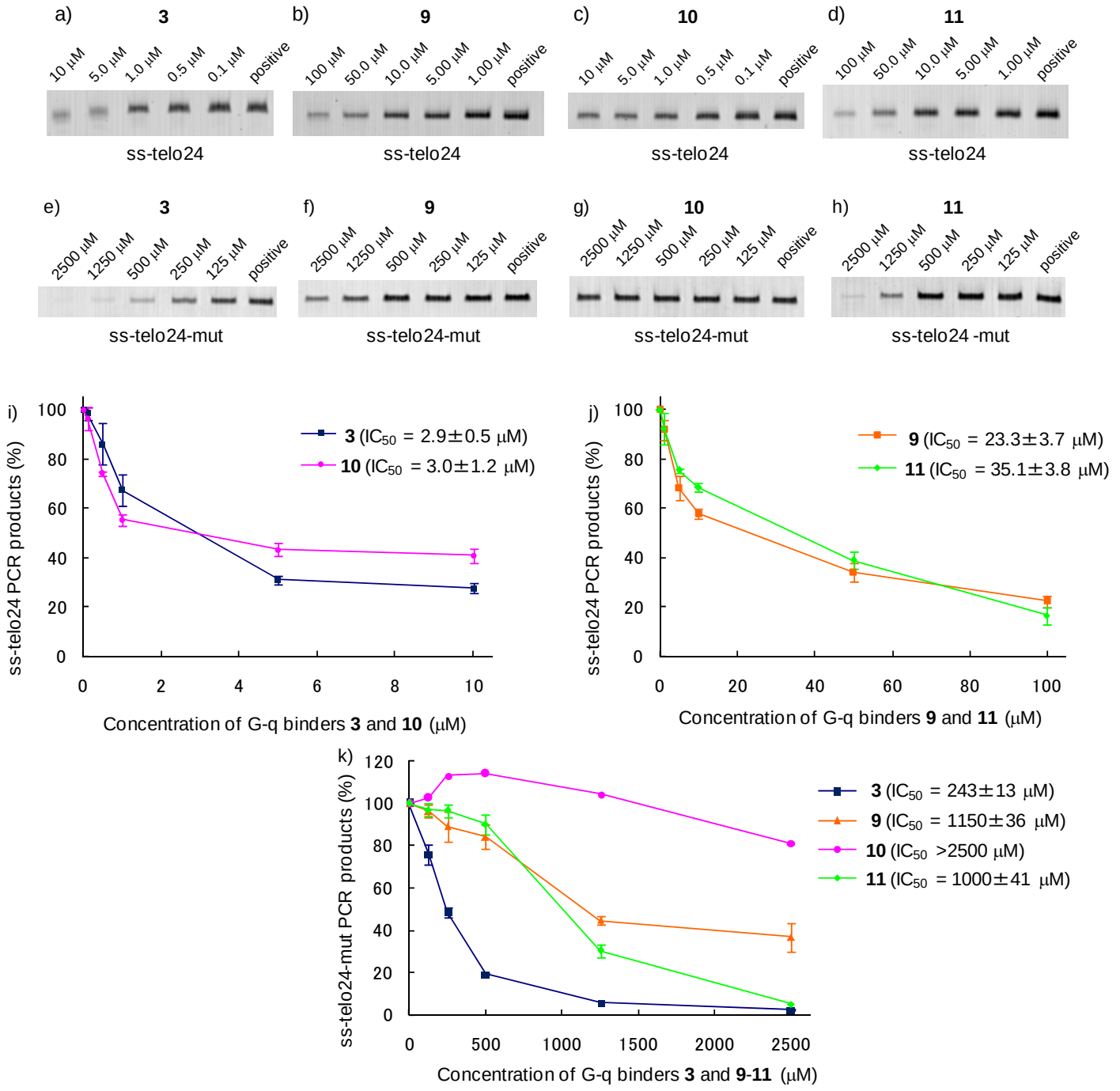
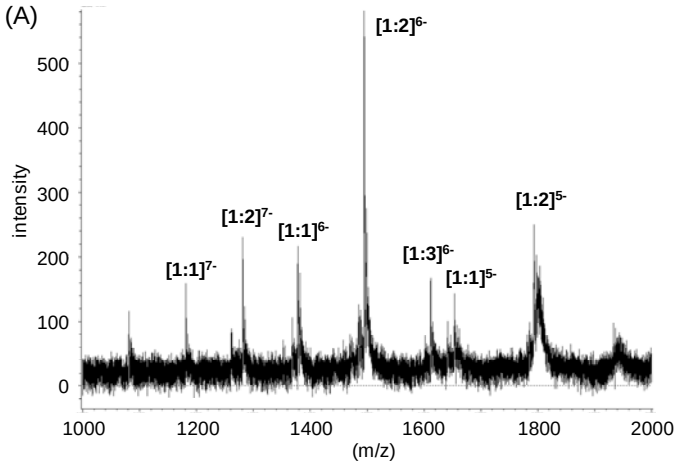


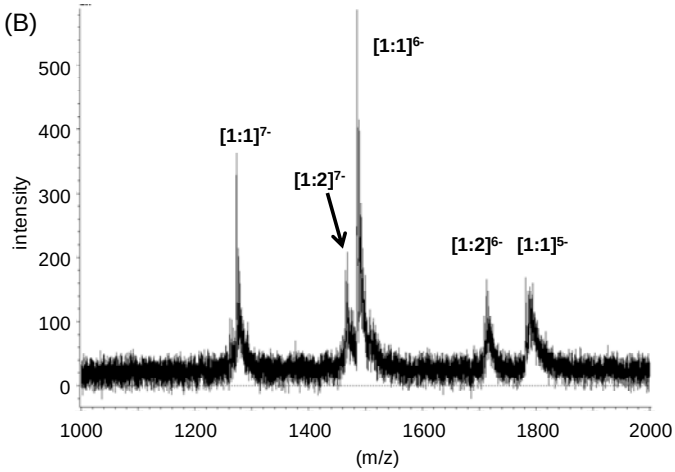
Fig. S3 PCR stop assay of **3** and **9-11**. Top panel presented the PCR product; a) **3**-ss-telo24, b) **9**-ss-telo24, c) **10**-ss-telo24, d) **11**-ss-telo24, e) **3**-ss-telo24-mut, f) **9**-ss-telo24-mut, g) **10**-ss-telo24-mut, h) **11**-ss-telo24-mut. Lower panel presented the quantification of the fluorescent intensity by using phosphorimager; i) (**3** and **10**)-ss-telo24, j) (**9** and **11**)-ss-telo24, k) (**3** and **9-11**)-ss-telo24-mut. Results represent means +/-SD of three independent experiments. PCR inhibitory activities were calculated by the following equation. (intensity of the band in the presence of ligands) / (intensity of positive control band).

7. ESI mass spectrometry

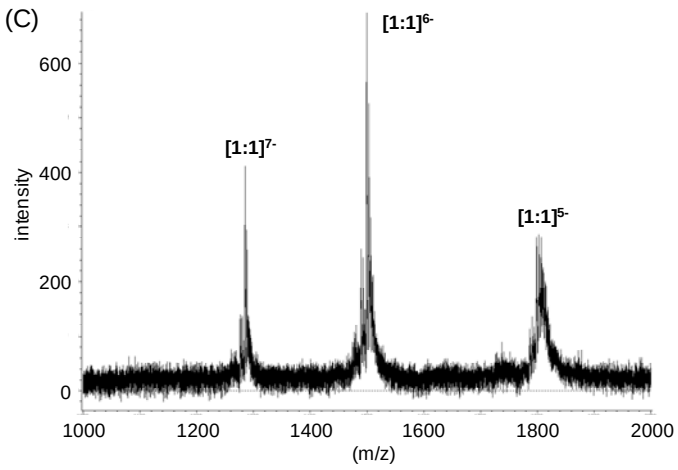
ESI mass spectra were obtained in the negative-ion mode with JEOL JMS-T100X spectrometer. The direct-infusion flow rate was 5.0 μL min^{-1}. All experiments were performed in 20 mM NH_4OAc containing 10 μM of ss-telo24 and 40 μM of **3** and **9**-**11**. 15% methanol was added just before injection.



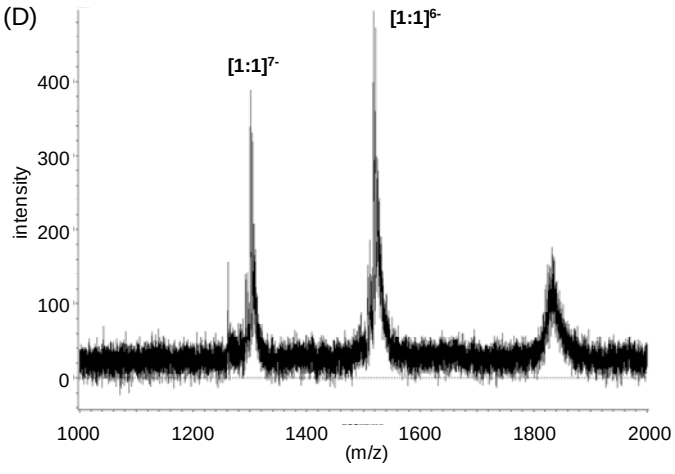
Exact mass of ss-telo24: 7571.27
Exact mass of **3**: 701.18
$[1:2]^{5-}$ calcd for 1793.37 found 1793.41
$[1:1]^{5-}$ calcd for 1653.49 found 1653.76
$[1:3]^{6-}$ calcd for 1611.47 found 1611.91
$[1:2]^{6-}$ calcd for 1494.61 found 1494.90
$[1:1]^{6-}$ calcd for 1377.74 found 1378.31
$[1:2]^{7-}$ calcd for 1280.95 found 1281.51
$[1:1]^{7-}$ calcd for 1180.78 found 1181.45



Exact mass of ss-telo24: 7571.27
Exact mass of **9**: 1344.32
$(1:1)^{5-}$ calcd for 1782.12 found 1781.73
$(1:2)^{6-}$ calcd for 1708.99 found 1712.70
$(1:1)^{6-}$ calcd for 1484.93 found 1485.29
$(1:2)^{7-}$ calcd for 1464.70 found 1468.16
$(1:1)^{7-}$ calcd for 1272.66 found 1273.16



Exact mass of ss-telo24: 7571.27
Exact mass of **10**: 1428.38
$[1:1]^{5-}$ calcd for 1798.93 found 1798.57
$[1:1]^{6-}$ calcd for 1498.94 found 1499.21
$[1:1]^{7-}$ calcd for 1284.66 found 1285.21



Exact mass of ss-telo24: 7571.27
Exact mass of **11**: 1540.51
$(1:1)^{6-}$ calcd for 1517.63 found 1518.19
$(1:1)^{7-}$ calcd for 1300.68 found 1301.12

Fig. S4 ESI mass spectra of 10 μM ss-telo24 with 40 μM ligand (A) **3**, (B) **9**, (C) **10**, (D) **11**

Telomestatin (TMS) and its derivatives 6OTDs have been reported to bind to telomeric G-quadruplex with two molecules of them and strongly stabilize the structure. Based on a docking study of TMS and telomeric G-quadruplex, an end-stacked binding mode was proposed, i.e., interaction of the terminal G-quartet flat surfaces through π-π stacking.[S2] We consider that 6OTD interact to telomeric G-quadruplex as the similar binding mode with TMS. From the above ESI-mass spectra, 6OTD dimers **9-11** were revealed to form the complex with ss-telo24 in a ratio with 1:1. We believe these interactions involve at least two types of complexations, i.e., type-A complex (one 6OTD moiety in dimer responsible for the interaction with ss-telo24) and type-B complex (both of two 6OTD moieties in dimer responsible for the interaction with ss-telo24), and one of these possible complexations for each types was shown in Fig. S5. In cases of dimers **9** and **11**, the type-A complex is thought to form predominantly because of the steric hindrances of their inappropriate length of the linkers. This speculation was supported by the instability of their complex with ss-telo24 compared to the monomer **3** (2:1 complexation with DNA), which were shown from the FRET melting analysis and PCR stop assay (Table 1). Actually, the dimer **9**, which has "less hinder linker" among the three dimers, forms 2:1 complex with DNA, although as a minor complexation (Fig. S4). This observation also supports the possibility of type-A complex.

On the contrary, dimer **10** is thought to form type-B complex with ss-telo24, since it stabilize the G-q structure more potently than the other dimers **9** and **11** (Table 1). However, stabilizing ability of **10** was the same level as the monomer **3**. We believe dimer **10** forms the complex in a mixture of type-A and B. Molecular dynamics result (Fig. S1) shows that dimer **10** has some intra-distances between the center of 6OTD, which may support this possibility.

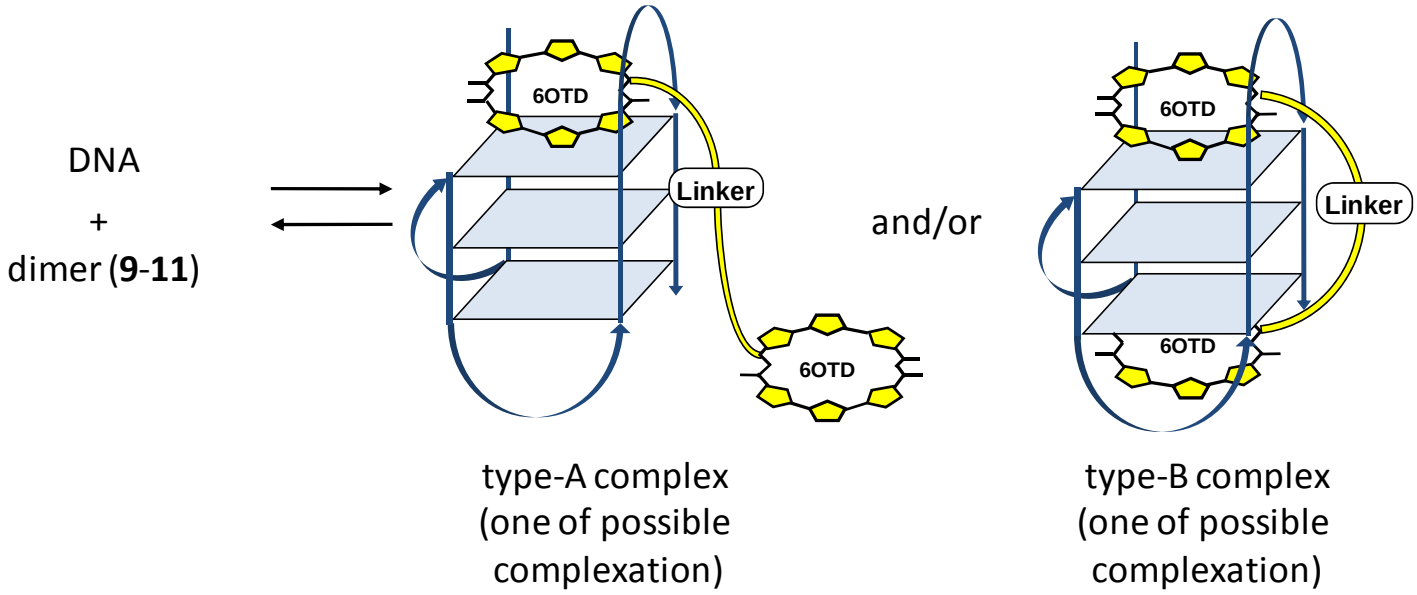


Fig. S5 Possible binding modes of 6OTD dimers **9-11** with G-quadruplex.

S2) M.-Y. Kim, H. Vankayalapati, K. Shin-ya, K. Wierzba, and L. H. Hurley, *J. Am. Chem. Soc.*, 2002, **124**, 2098.