SUPPORTING INFORMATION

# Design and synthesis of unsymmetric macrocyclic hexaoxazole compounds with ability to induce distinct G-quadruplex topologies in telomeric DNA

Mai Sakuma,[a] Yue Ma,[a] Yamato Tsushima,[a] Keisuke Iida,[b] Takatsugu Hirokawa[c] and Kazuo Nagasawa*[a]

[a]Department of Biotechnology and Life Science, Faculty of Technology, Tokyo University of Agriculture and Technology (TUAT), Koganei, Tokyo, 184-8588, Japan. E-mail: knaga@cc.tuat.ac.jp

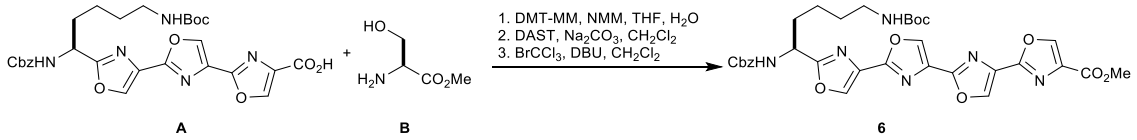
[b]Research Institute for Clinical Oncology, Saitama Cancer Center, Ina, Kitaadachi-gun, Saitama, 362-0806 , Japan

[c]Molecular Profiling Research Center for Drug Discovery, National Institute of Advanced Industrial Science and Technology, Koto-ward, Tokyo, 135-0064, Japan.

**Table of contents**

## 1. Synthesis of 6, 8, D, 10, 11

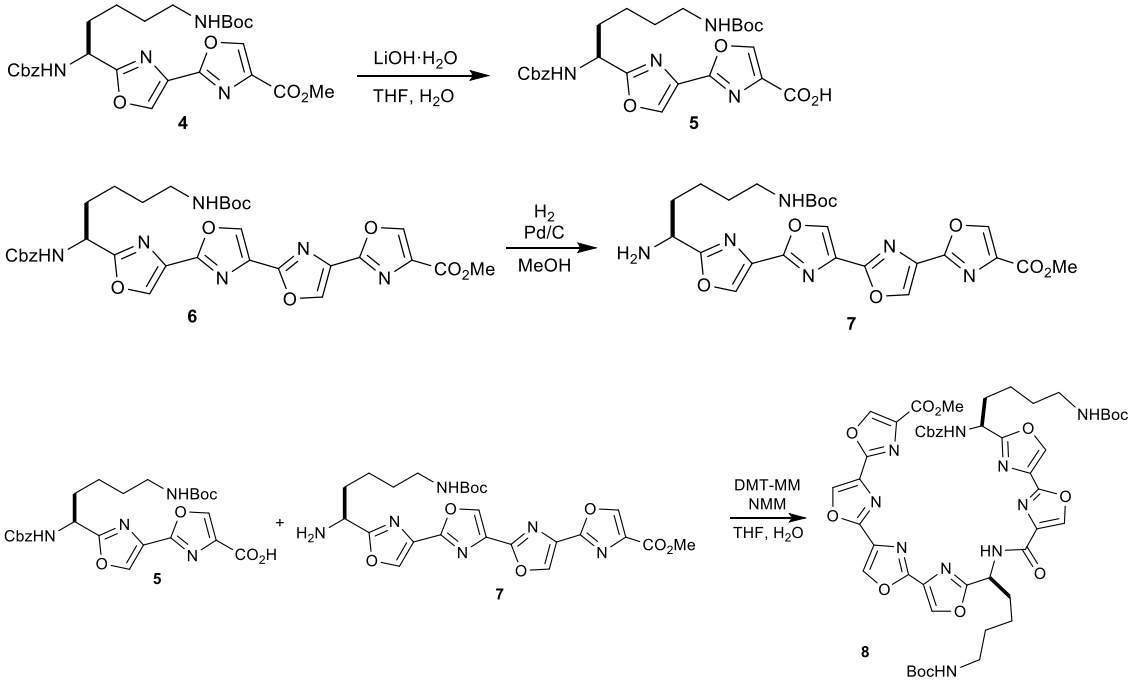
Synthesis of **6**




**A** **B** **6**

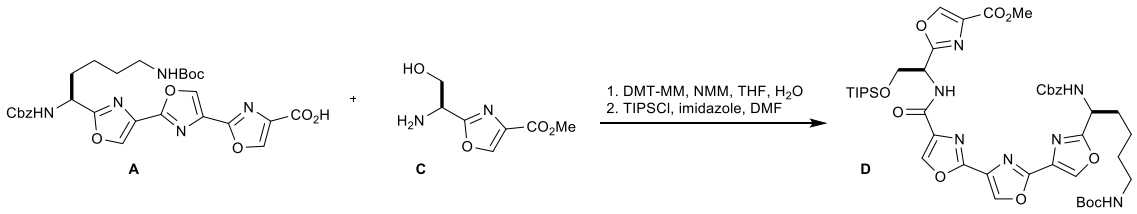
To a solution of carboxylic acid **A**[1] (300 mg, 0.504 mmol) in THF-$H_2O$ (1:1, 20 mL) was added NMM (0.22 mL, 2.02 mmol), DMT-MM (207 mg, 0.756 mmol) and serine methylester **B**[2] (78.4 mg, 0.504 mmol), and the mixture was stirred at room temperature. After being stirred for 3 h, to the reaction mixture was added $H_2O$, and the organic layer was extracted with $CHCl_3$, washed with 1.2 N HCl, dried over $MgSO_4$, and filtered. The filtrates were concentrated *in vacuo* to give β-hydroxy amide, which was used without further purification. To a solution of the β-hydroxyl amine in $CH_2Cl_2$ (15 mL), was added $Na_2CO_3$ (134 mg, 1.26 mmol) and DAST (61 μL, 0.46 mmol) at 0 °C under argon atmosphere. The reaction mixture was stirred for 10 min, quenched with $NaHCO_3$ aq to give a solution of oxazoline, which was used without further purification. To a solution of the crude oxazoline in $CH_2Cl_2$ (15 mL), was added $BrCCl_3$ (0.4 mL, 4.2 mmol) and DBU (0.7 mL, 4.2 mmol). The mixture was stirred at 0 °C under argon atmosphere. After being stirred for 24 h, to the reaction mixture was added $H_2O$ and the organic layer was extracted with $CHCl_3$, washed with 1.2 N HCl, dried over $MgSO_4$, and filtered. The filtrates were concentrated *in vacuo.* The residue was purified by silica gel column to give **6** (202 mg, 68%, 3 steps). $[\alpha]_D^{25}$ = -16.3 (*c* 1.6, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$) δ 8.45 (s, 1H), 8.42 (s, 1H), 8.33 (s, 1H), 8.31 (s, 1H), 7.35 (br, 5H), 5.56 (d, *J* = 9.0Hz, 1H), 5.12 (s, 2H), 5.05 (br, 1H), 4.55 (br, 1H), 3.95 (s, 3H), 3.09 (br, 2H), 2.04-1.90 (m, 3H), 1.64-1.41 (m, 10H), 1.27-1.21 (m, 2H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ 165.7, 163.7, 161.4, 156.9, 156.2, 155.4, 144.0, 139.6, 136.1, 134.5, 130.9, 129.8, 128.6, 128.2, 67.3, 52.4, 49.5, 29.7, 28.5, 22.5 ppm; HRMS (ESI, M+Na) calcd for $C_{32}H_{34}N_6O_{10}Na$ 685.2197, found 685.2234.

Synthesis of **8**



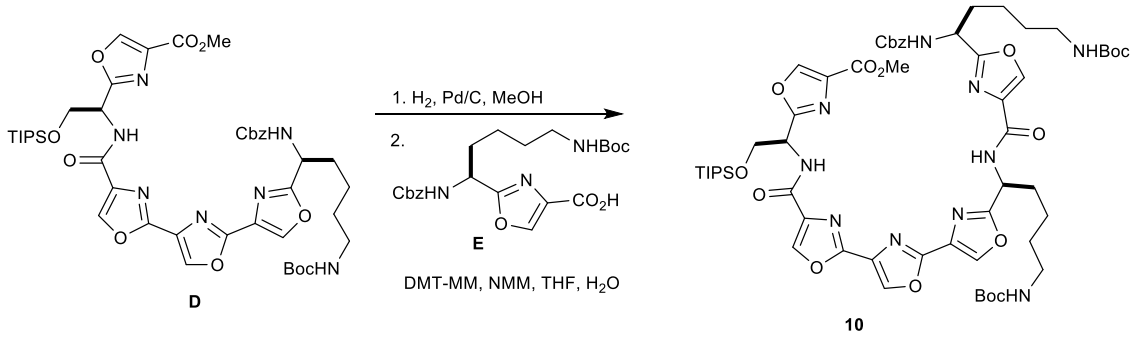

To a solution of **4**[2)] (125 mg, 189 μmol) in THF-$H_2O$ (3:1, 4 mL), was added LiOH·$H_2O$ (11.9 mg, 284 μmol) at room temperature. The mixture was stirred for 30 min, quenched with 3N HCl to give a solution of carboxylic acid **5**, which was used without further purification. To a solution of **6** (100 mg, 189 μmol) in MeOH (10 mL), was added 10% Pd/C (10 mg) at room temperature and mixture was stirred under hydrogen atmosphere for 2 h. The reaction mixture was filtered through a pad of Celite, and eluted with $CHCl_3$-MeOH (9:1). The filtrates were concentrated *in vacuo* to give amine **7**, which was used without further purification. To a solution of carboxylic acid **5** in THF-$H_2O$ (1:1, 10 mL) was added NMM (83 μL, 756 μmol), DMT-MM (77.7 mg, 284 μmol) and amine **7**, and the mixture was stirred at room temperature. After being stirred for 4 h, to the reaction mixture was added $H_2O$, and the organic layer was extracted with $CHCl_3$, washed with 1.2 N HCl, dried over $MgSO_4$, and filtered. The filtrates were concentrated *in vacuo* to give **8** (153 mg, 80%, 3 steps). $[\alpha]_D^{25}$ = +5.1 (*c* 2.6, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$) δ 8.46-8.14 (m, 6H), 7.55 (d, *J* = 9.0 Hz, 1H), 7.35 (s, 5H), 5.59 (br, 1H), 5.48 (dd, *J* = 9.0, 15.6 Hz, 2H), 5.12 (s, 2H), 4.58 (br, 2H), 3.94 (s, 3H), 3.10 (br, 6H), 2.05-1.40 (m, 28H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ 165.7, 164.7, 159.9, 156.2, 155.6, 154.6, 143.9, 141.8, 139.6, 136.8, 136.1, 134.4, 131.0, 129.8, 128.6, 128.2, 79.3, 67.3, 52.4, 46.9, 40.3, 40.0, 33.6, 29.7, 28.5, 22.8, 22.5 ppm; HRMS (ESI, M+Na) calcd for $C_{49}H_{56}N_{10}O_{15}Na$ 1047.3706, found 1047.3824.

Synthesis of **D**



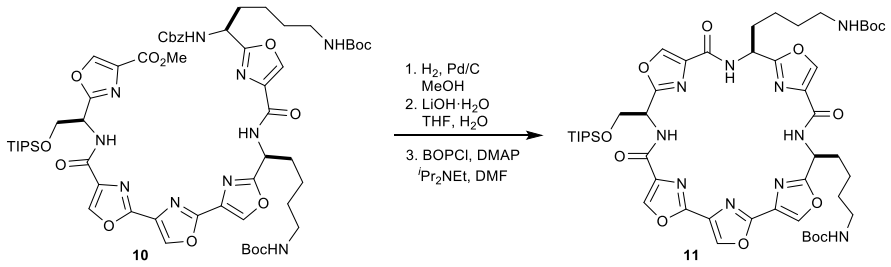

To a solution of carboxylic acid **A**[1] (2.93 g, 5.04 mmol) in THF-$H_2O$ (1:1, 30 mL) was added NMM (1.1 mL, 10.1 mmol), DMT-MM (2.07 g, 7.56 mmol) and amine **C**[3] (1.6 g, 5.04 mmol), and the mixture was stirred at room temperature. After being stirred for 8 h, to the reaction mixture was added $H_2O$, and the organic layer was extracted with $CHCl_3$, washed with 1.2 N HCl, dried over $MgSO_4$, and filtered. The filtrates were concentrated *in vacuo* to give β-hydroxy amide, which was used without further purification. To a solution of the β-hydroxyl amine in DMF (15 mL) was added TIPSCl (2.2 mL, 10.1 mmol) and imidazole (687 mg, 10.1 mmol), and the mixture was stirred at 0 °C under argon atmosphere. After being stirred for 24 h, to the reaction mixture was added $H_2O$, and the organic layer was extracted with $CHCl_3$, washed with $NH_4Cl$, dried over $MgSO_4$, and filtered. The filtrates were concentrated *in vacuo* to give **D** (1.04 g, 58%, 2steps). $[\alpha]_D^{25}$ = +8.0 (*c* 1.8, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$) δ 8.31-8.28 (m, 3H), 8.20 (m, 1H), 7.87 (d, *J* = 9.0 Hz, 2H), 7.34 (br, 5H), 5.54-5.48 (m, 2H), 5.12 (s, 2H), 4.55 (br, 2H), 4.31-4.26 (m, 1H), 4.16-4.11 (m, 1H), 3.90 (s, 3H), 3.08 (br, 2H), 2.06-1.92 (m, 3H), 1.50-1.40 (m, 11H), 1.03-0.99 (m, 21) ; $^{13}$C NMR (75 MHz, $CDCl_3$) δ 165.7, 163.1, 161.6, 160.0, 156.2, 154.5, 144.2, 141.6, 139.6, 139.1, 136.8, 136.2, 133.6, 131.1, 129.8, 128.6, 128.2, 79.2, 67.2, 64.8, 52.2, 49.4, 40.0, 33.6, 29.7, 28.5, 22.5, 17.9, 11.9 ppm; HRMS (ESI, M+Na) calcd for $C_{44}H_{59}N_7O_{12}SiNa$ 928.3890, found 928.3889.

Synthesis of **10**




To a solution of **D** (1.04 g, 1.15 mmol) in MeOH (15 mL) was added 10% Pd/C (100 mg) at room temperature and mixture was stirred under hydrogen atmosphere for 2 h. To the reaction mixture was filtered through a pad of Celite and eluted with $CHCl_3$-MeOH (9:1). The filtrates were concentrated *in vacuo* to give amine, which was used without further purification. To a solution of carboxylic acid **E**[2] was added NMM (0.3 mL, 2.30 mmol), DMT-MM (474 mg, 1.73 mmol) and amine, and the mixture was stirred at room temperature for 6 h. To the reaction mixture was added $H_2O$, and the organic layer was extracted with $CHCl_3$, washed with 1.2 N HCl, dried over $MgSO_4$, and filtered. The filtrates were concentrated *in vacuo* to give **10** (813 mg, 61%, 2 steps). $[\alpha]_D^{25}$ = -9.9 (*c* 1.6, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.33-8.15 (m, 5H), 7.81 (d, *J* = 6.0 Hz, 1H), 7.33 (s, 5H), 5.53-5.40 (m, 3H), 5.12 (s, 2H), 4.62 (br, 1H), 4.18 (dd, *J* = 3.3, 9.0 Hz, 4H), 3.9 (s, 3H), 3.09 (br, 1H), 1.52-1.46 (m, 30H), 0.87-0.83 (m, 25H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 165.1, 164.2, 163.1, 161.5, 160.3, 156.2, 156.1, 154.4, 144.2, 141.7, 139.8, 139.2, 136.7, 136.1, 135.4, 133.5, 131.0, 129.8, 128.5, 79.1, 67.2, 64.7, 52.2, 49.4, 47.0, 40.0, 33.4, 29.5, 28.4, 22.5, 17.8, 11.8 ppm; HRMS (ESI, M+Na) calcd for $C_{58}H_{80}N_{10}O_{16}SiNa$ 1223.5410, found 1223.5421.

Synthesis of **11**




To a solution of **10** (1.54 g, 1.33 mmol) in MeOH (40 mL), was added 10% Pd/C (154 mg) at room temperature and mixture was stirred under hydrogen atmosphere for 4 h. The reaction mixture was filtered through a pad of Celite and eluted with $CHCl_3$-MeOH (9:1). The filtrates were concentrated *in vacuo* and the residue was purified by silica gel column to give amine, which was used without further purification. To a solution of amine in THF-$H_2O$ (3:1, 20 mL), was added $LiOH{\cdot}H_2O$ (84.0 mg, 2.00 mmol) at room temperature for 2 h. To the reaction mixture was added 3N HCl, and volatiles were removed under vacuum conditions to give a carboxylic acid, which was used without further purification. To a solution of the crude carboxylic acid in dry DMF was added $^{i}Pr_2NEt$ (1.0 mL, 5.32 mmol), DMAP (16.2 mg, 1.33 mmol) and BOPCl (511 mg, 2.01 mmol). The mixture was stirred at room temperature under argon atmosphere. After being stirred for 24 h, to the resulting mixture was added $H_2O$ and the organic layer was extracted with $CHCl_3$, washed with 1.2 N HCl, dried over $MgSO_4$, and filtered. The filtrates were concentrated *in vacuo.* The residue was purified by silica gel column to give **11** (241 mg, 25%, 3 steps). $[\alpha]_D^{25}$ = -50.3 (*c* 1.1, $CHCl_3$); $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 9.18 (d, *J* = 9.0 Hz, 2H), 9.02-8.60 (m, 6H), 7.95 (d, *J* = 9.0 Hz, 1H), 6.83 (br, 2H), 5.38-5.31 (m, 3H), 4.10-3.99 (m, 4H), 2.91 (br, 4H), 2.10-1.98 (m, 8H), 1.44-1.35 (m, 22H), 1.03-0.73 (m, 18H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 166.1, 163.3, 162.3, 160.7, 159.9, 159.6, 156.6, 156.1, 154.7, 142.1, 141.9, 141.4, 139.4, 138.5, 136.7, 135.9, 130.7, 129.3, 79.3, 65.1, 49.8, 47.0, 45.2, 40.4, 33.9, 33.0, 22.9, 17.9, 11.8 ppm; HRMS (ESI, M+Na) calcd for $C_{49}H_{70}N_{10}O_{13}SiNa$ 1057.4781, found 1057.4791.

Reference

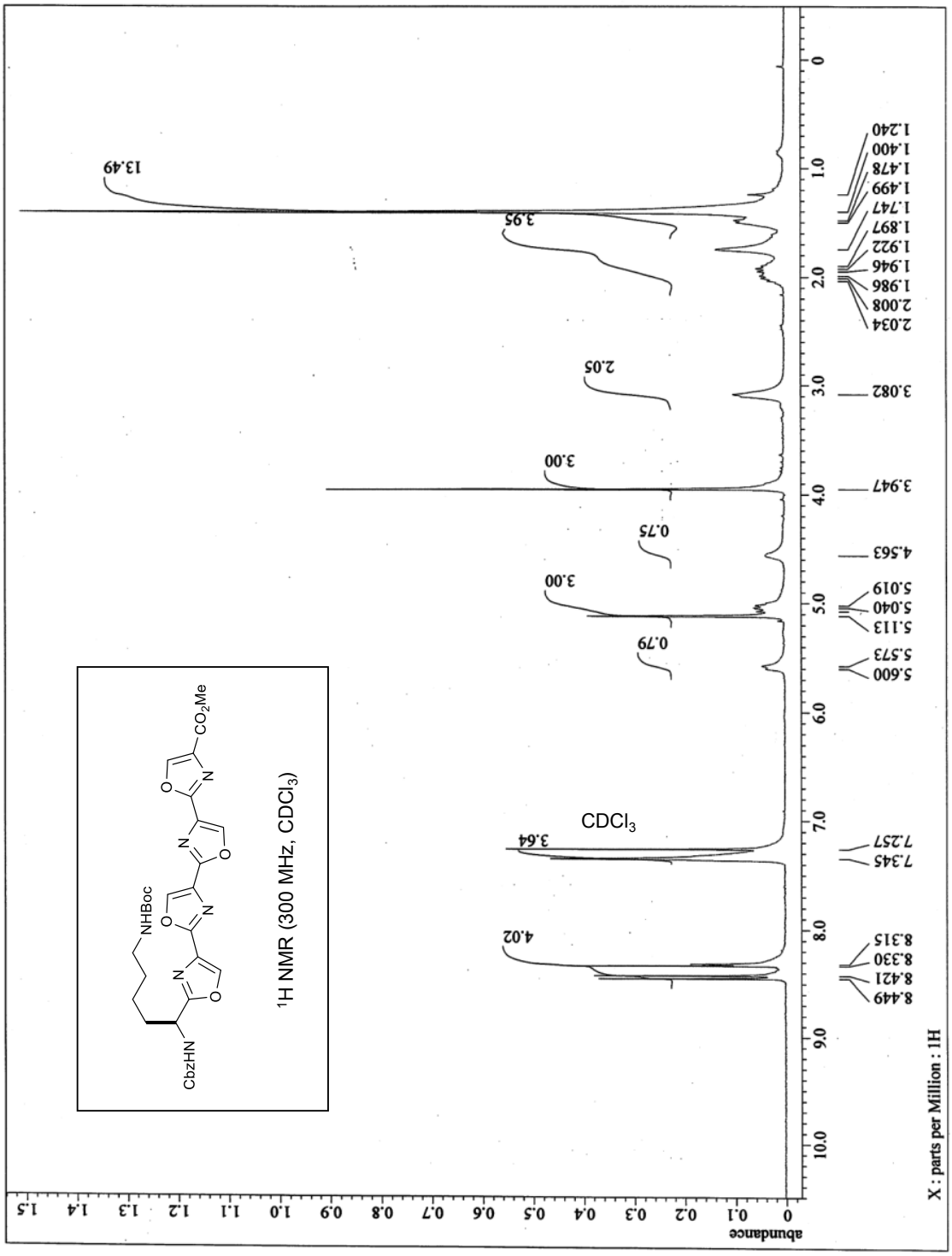
1. M. Tera, H. Ishizuka, M. Takagi, M. Suganuma, K. Shin-ya, K. Nagasawa, *Angew. Chem., Int. Ed.*, 2008, **47**, 5557–5560.

2. S. Sedghi Masoud, Y. Tsushima, K. Iida, K. Nagasawa, *Heterocycles,* 2015, **90**, 874-882.

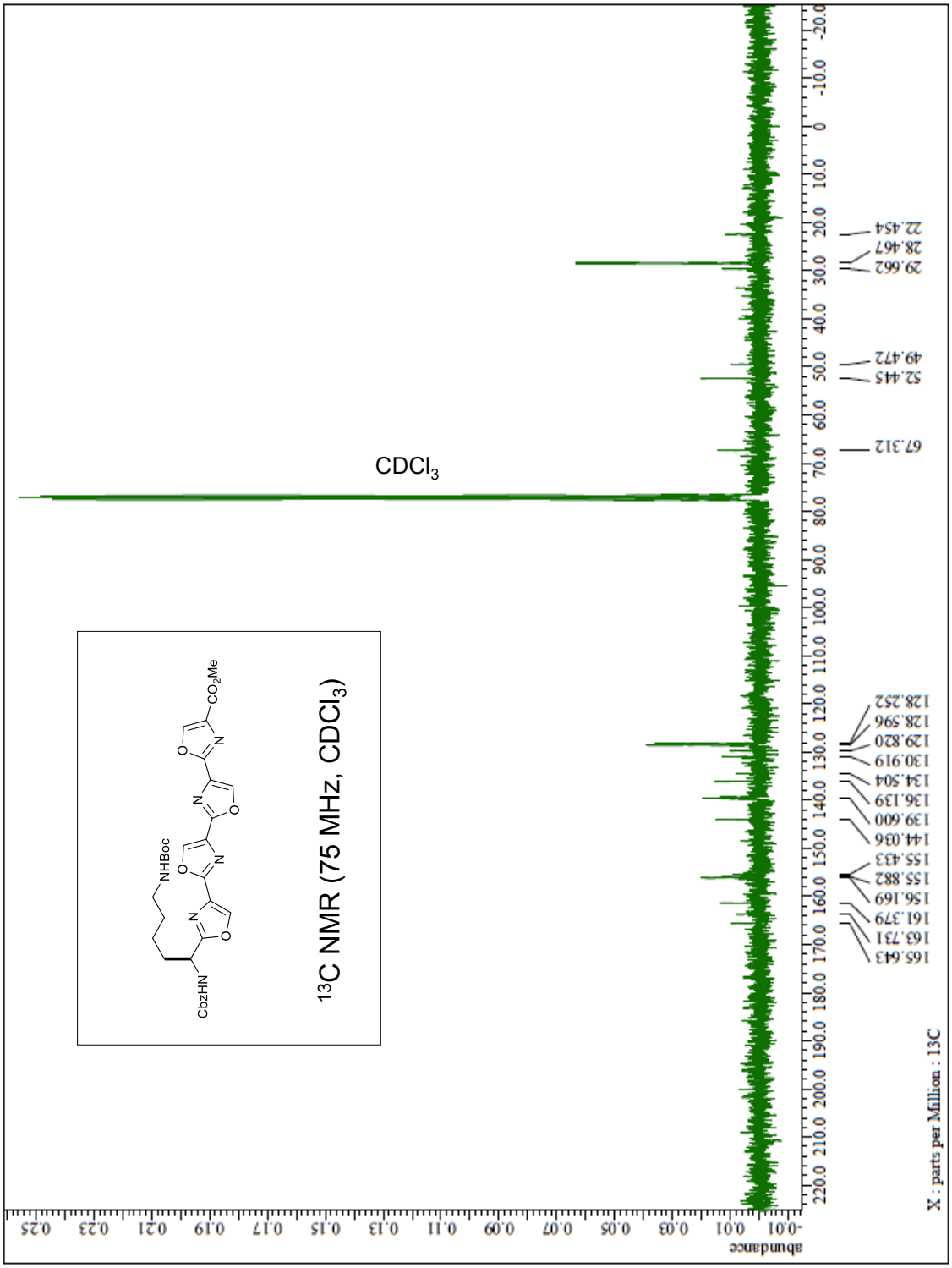
3. M. Tera, Y. Sohtome, H. Ishizuka, T. Doi, M. Takagi, K. Shin-ya, K. Nagasawa, *Heterocycles*, 2006, **69**, 505-514.

**NMR spectra**

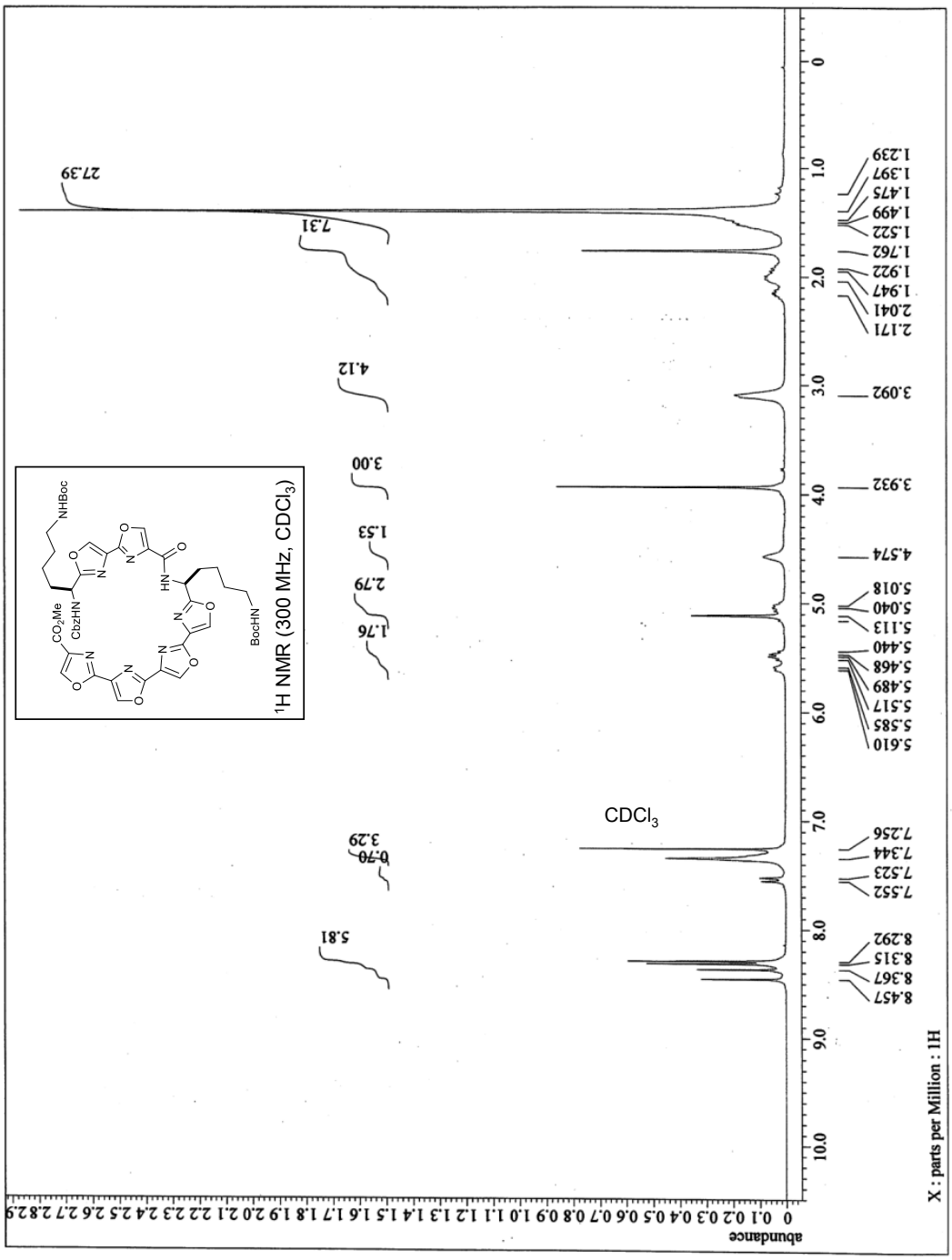
$^1$H NMR of **6**



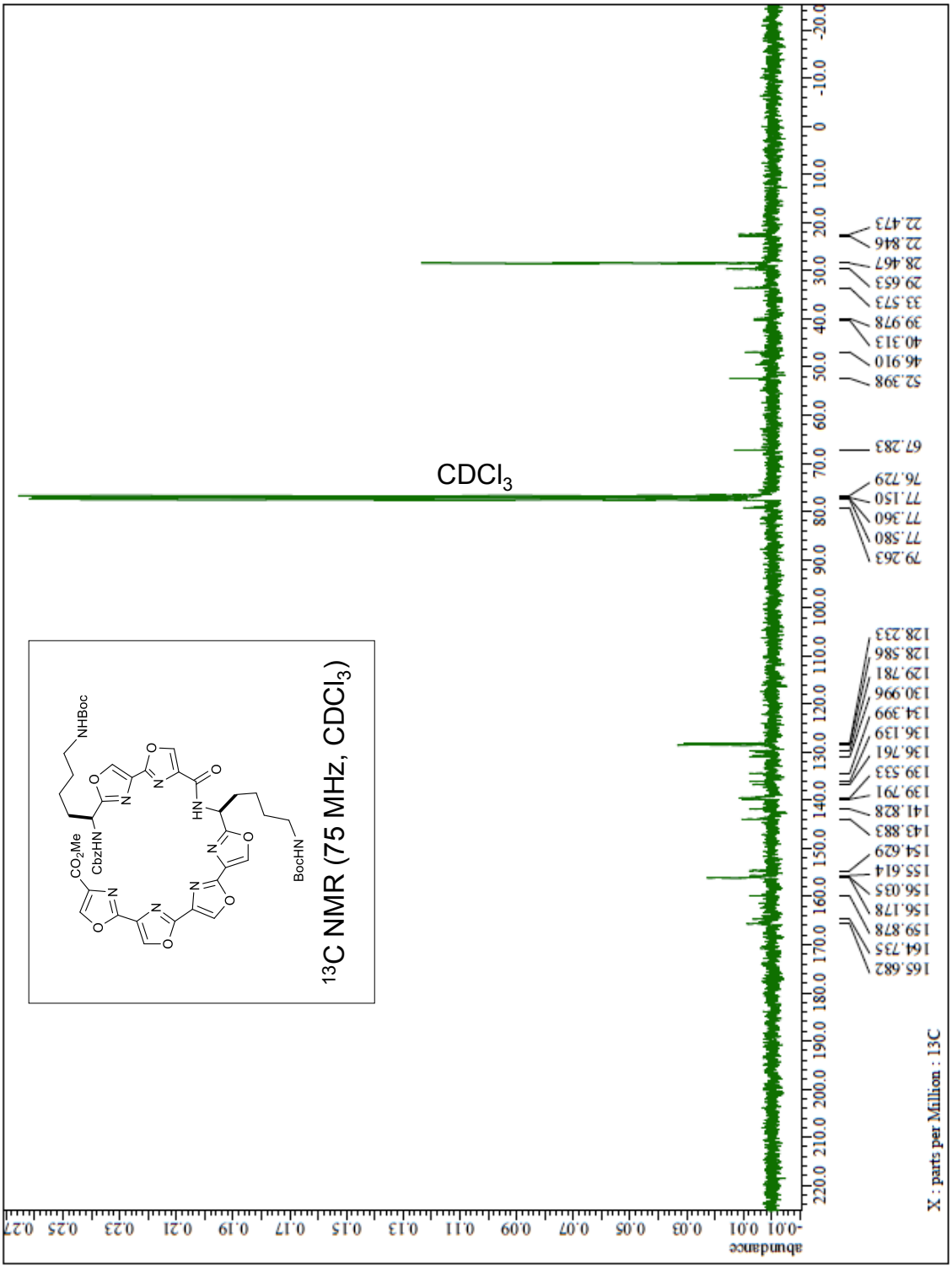
$^{13}$C NMR of **6**


CDCl3
13C NMR (75 MHz, CDCl3)
NHBoc
CbzHN
CO2Me
22.454
28.467
29.662
49.472
52.445
67.312
128.252
128.596
129.820
130.919
134.504
136.139
139.600
144.036
155.433
155.882
156.169
161.379
163.731
165.643
X : parts per Million : 13C
abundance

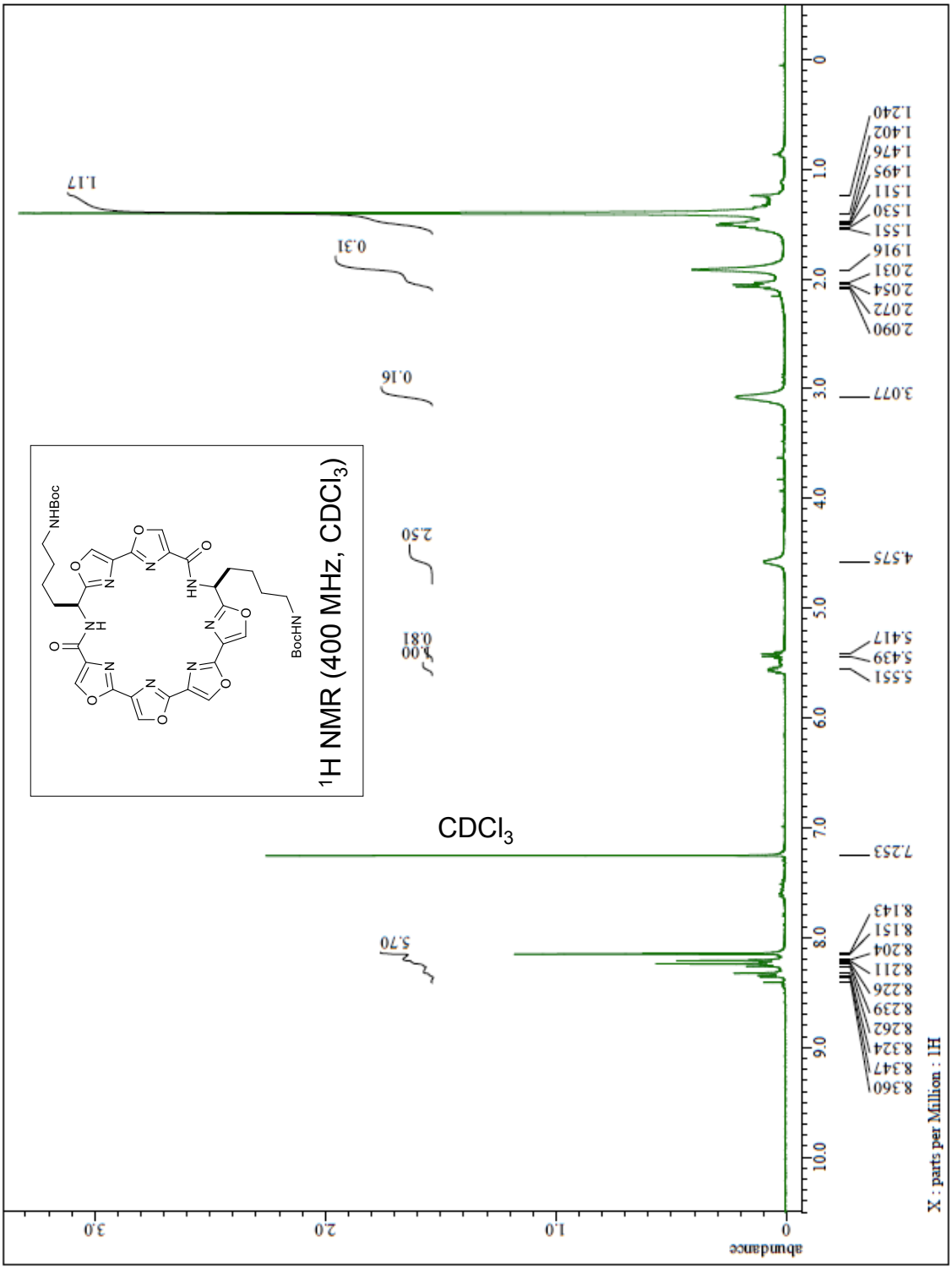
$^{1}$H NMR of **8**



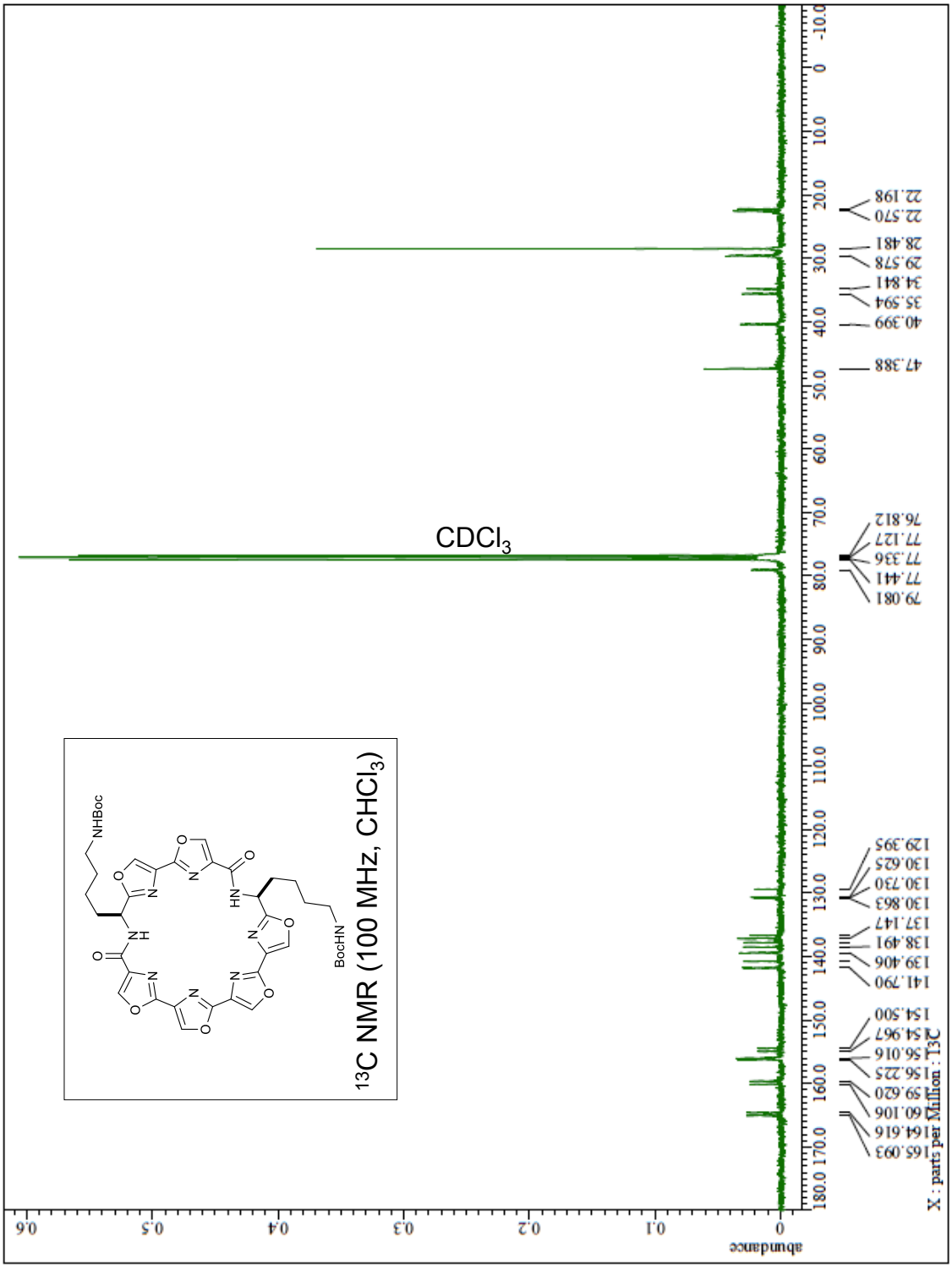
$^{13}$C NMR of **8**


CDCl3
13C NMR (75 MHz, CDCl3)
NHBoc
CO2Me
CbzHN
BocHN
HN
X : parts per Million : 13C
abundance

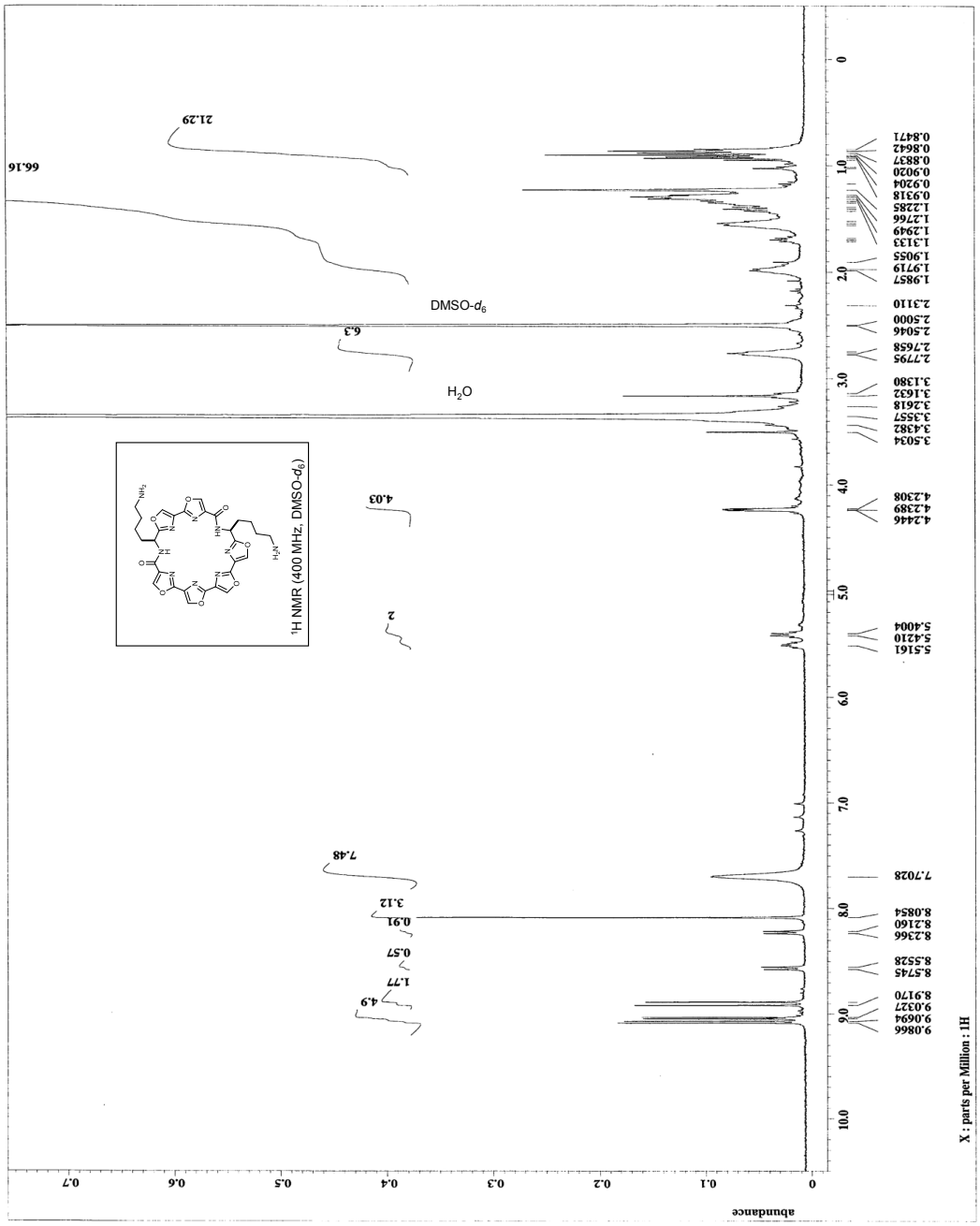
$^{1}$H NMR of *macrocyclic bisamide* ***9***



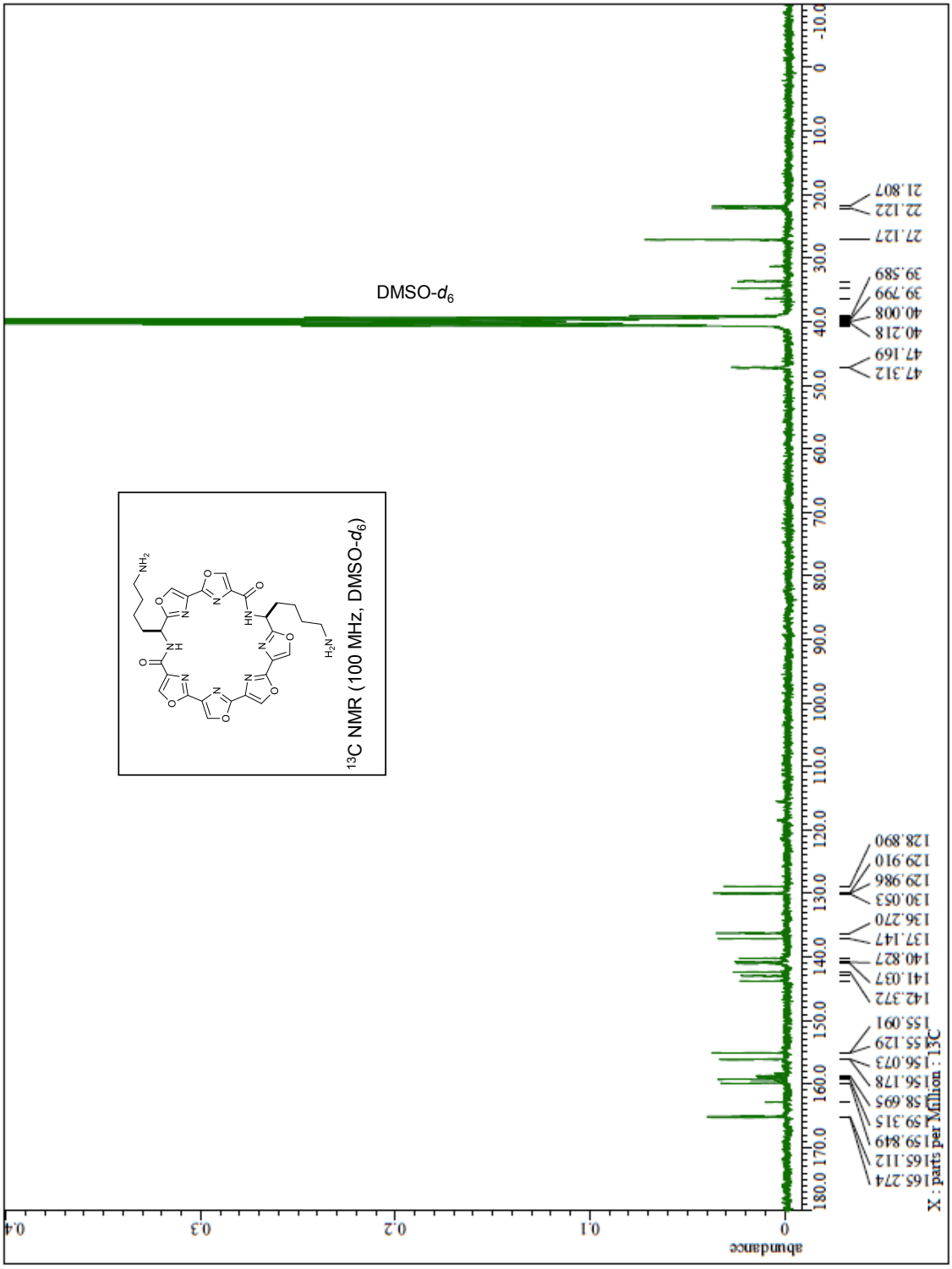
$^{13}$C NMR of *macrocyclic bisamide* ***9***



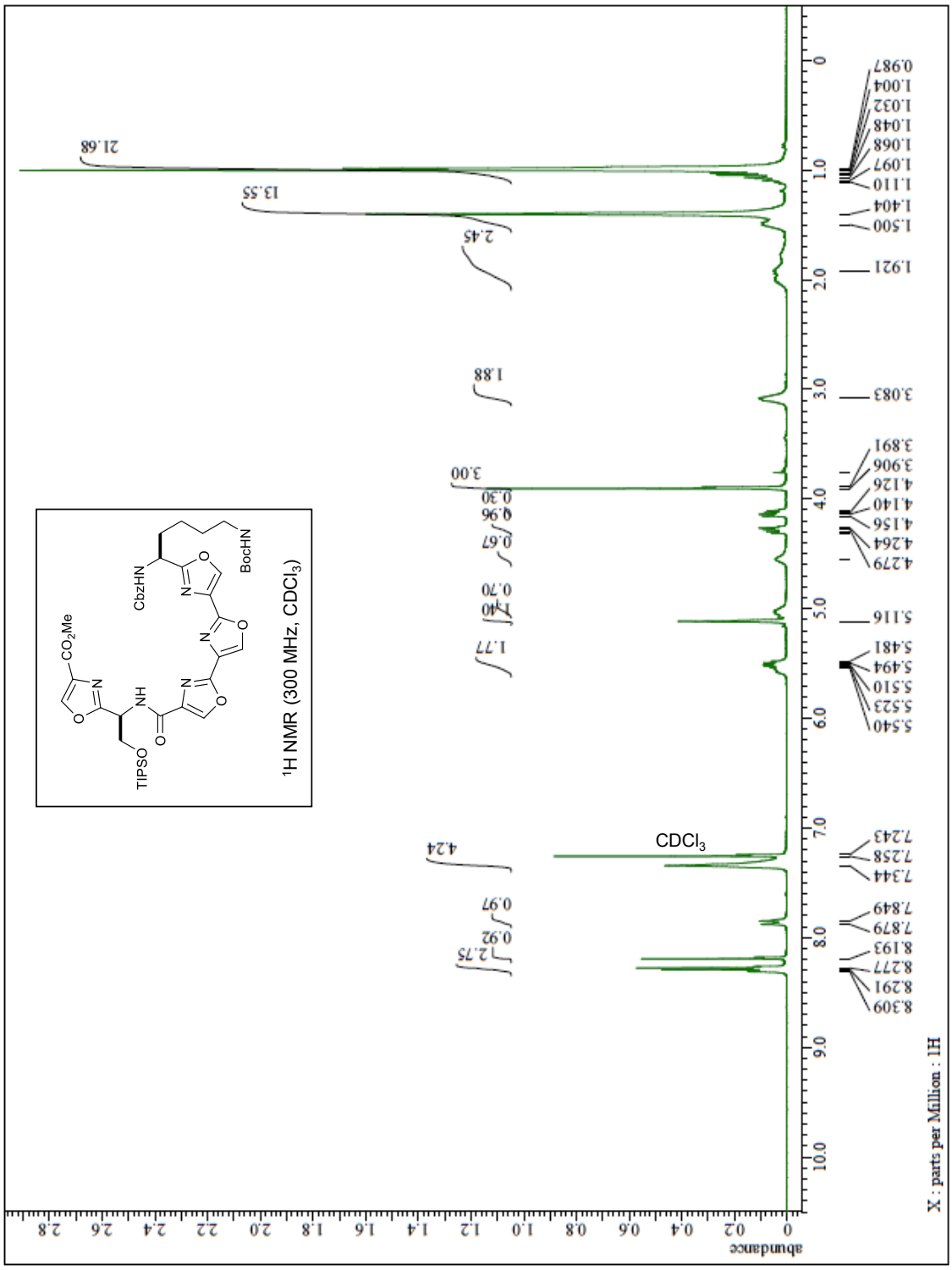
$^{1}$H NMR of *4,2-L2H2-6OTD (**2**)*



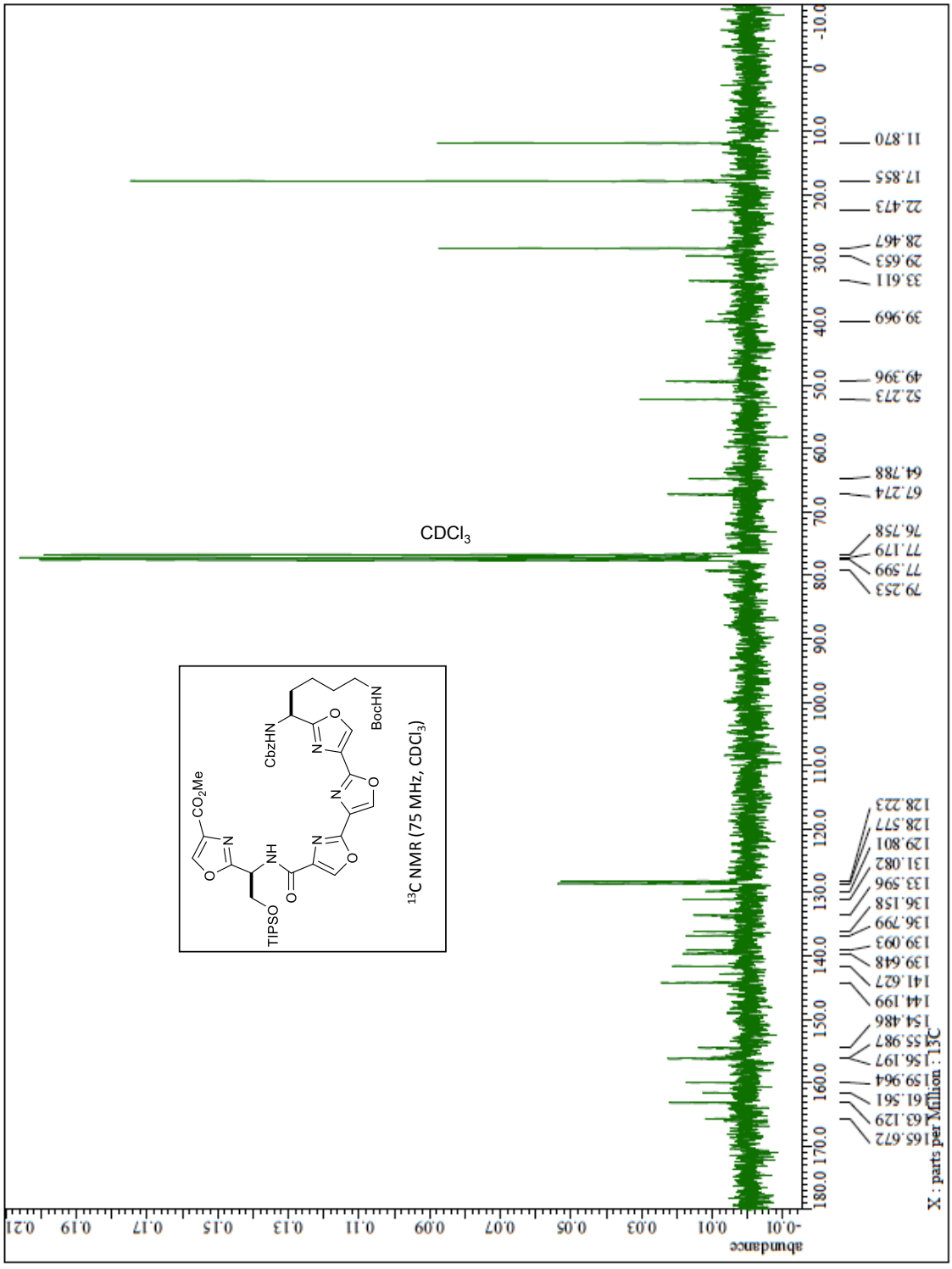
$^{13}$C NMR of *4,2-L2H2-6OTD* (***2***)



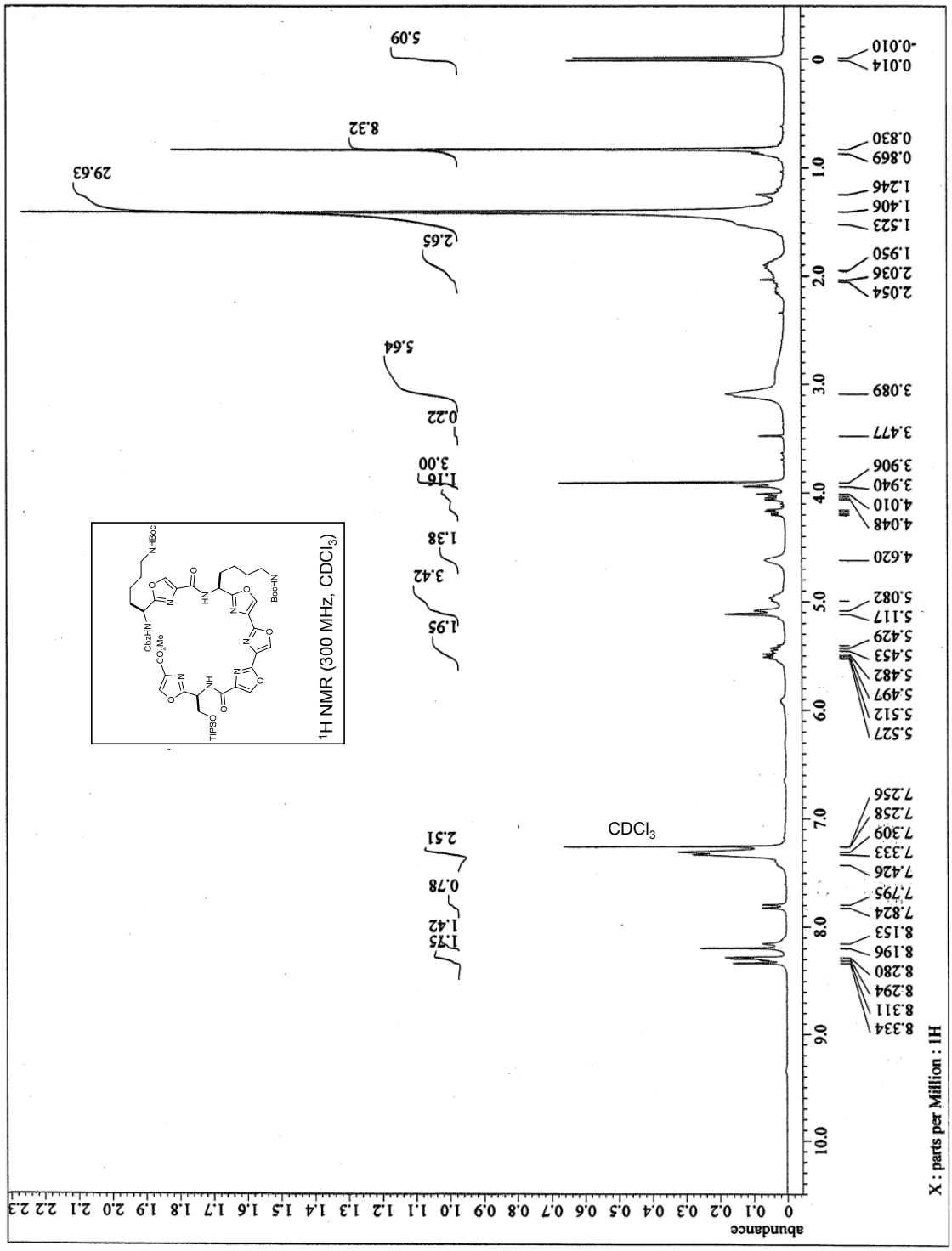
$^{1}$H NMR of **D**



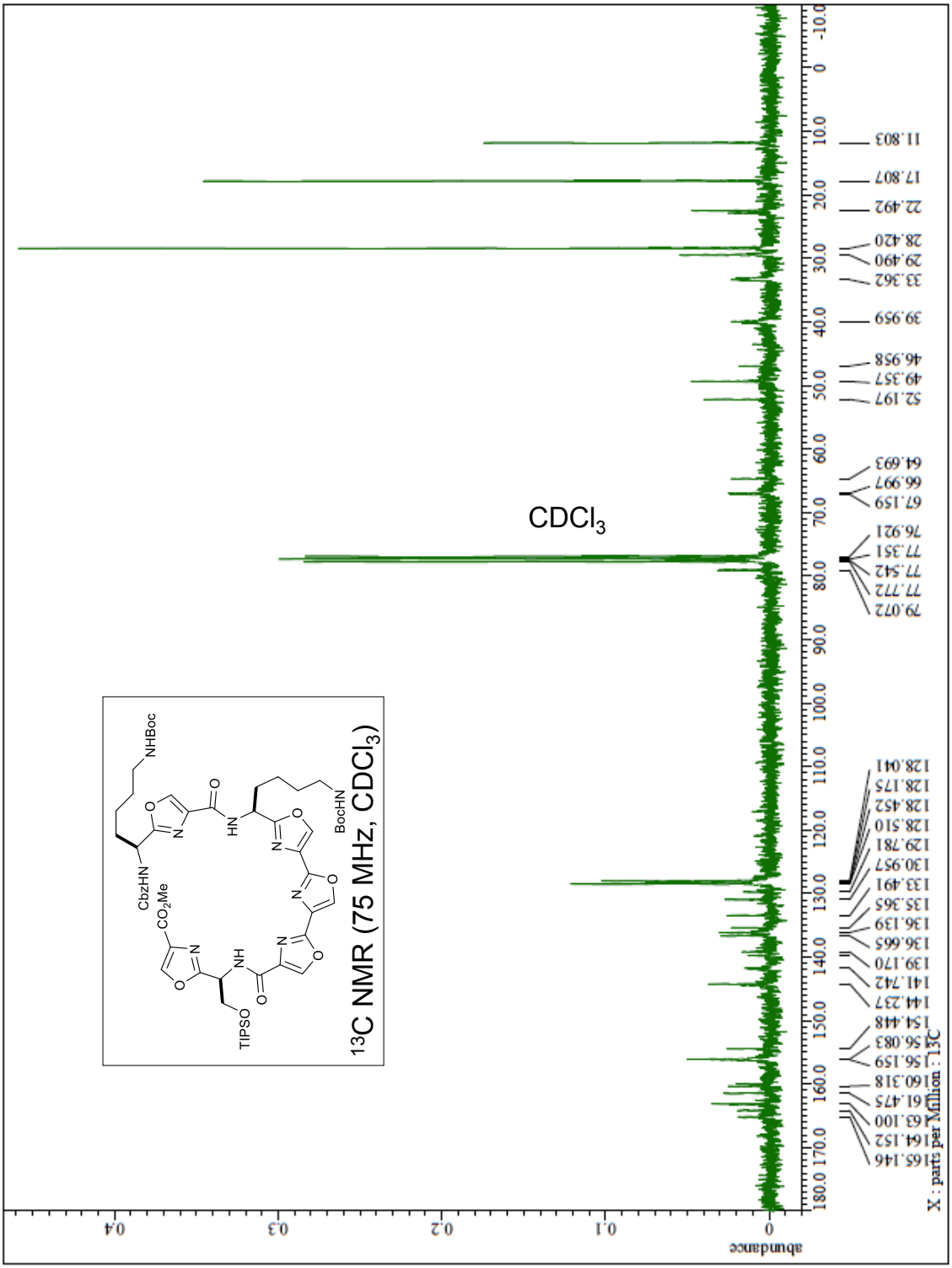
$^{13}$C NMR of **D**


CDCl3
13C NMR (75 MHz, CDCl3)
CbzHN
BocHN
CO2Me
NH
TIPSO
11.870
17.855
22.473
28.467
29.653
33.611
39.969
49.396
52.273
64.788
67.274
76.758
77.179
77.599
79.253
128.223
128.577
129.801
131.082
133.596
136.158
136.799
139.093
139.648
141.627
144.199
154.486
155.987
156.197
159.964
161.561
163.129
165.672
X : parts per Million : 13C
abundance

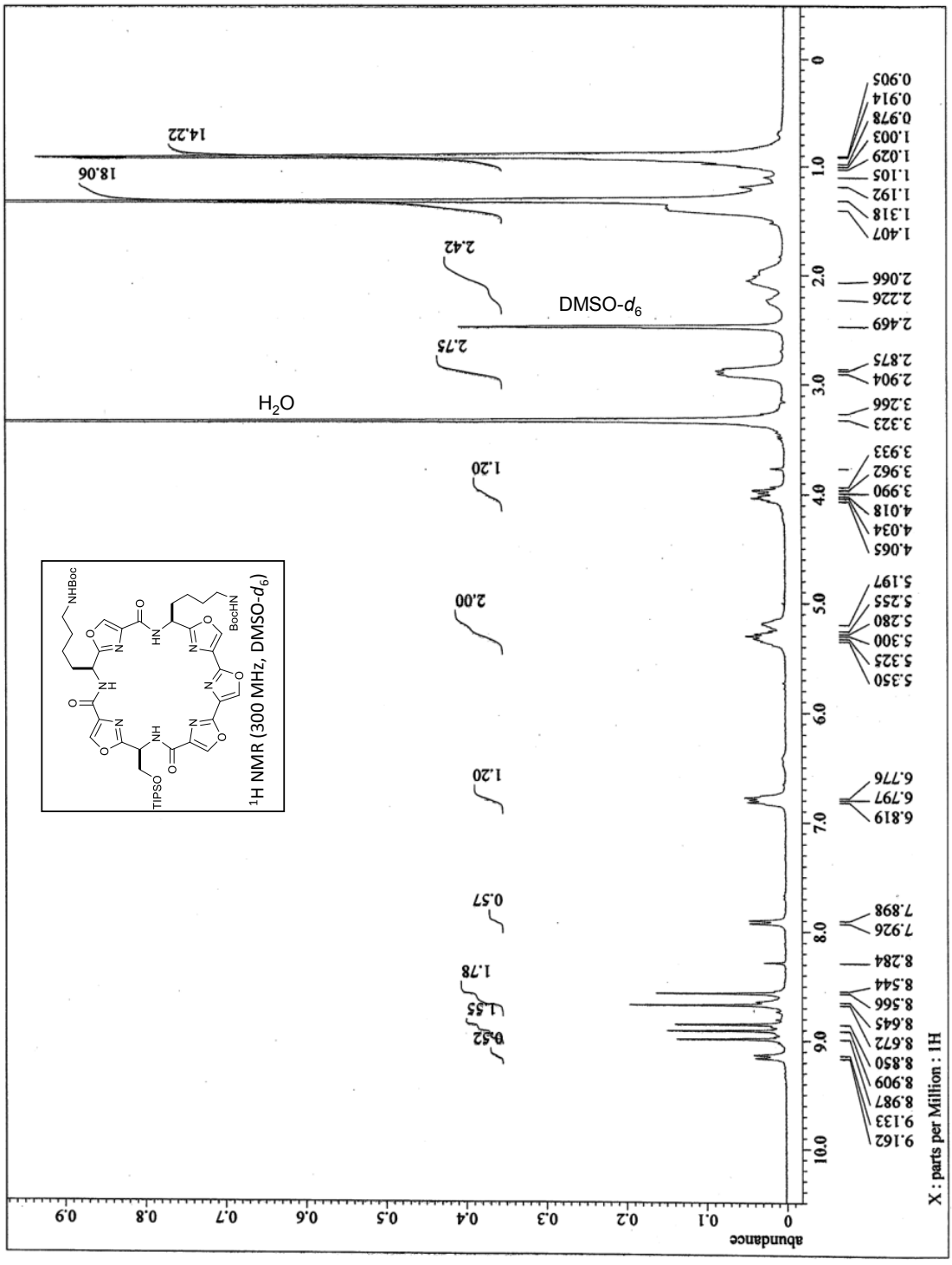
$^{1}$H NMR of **10**



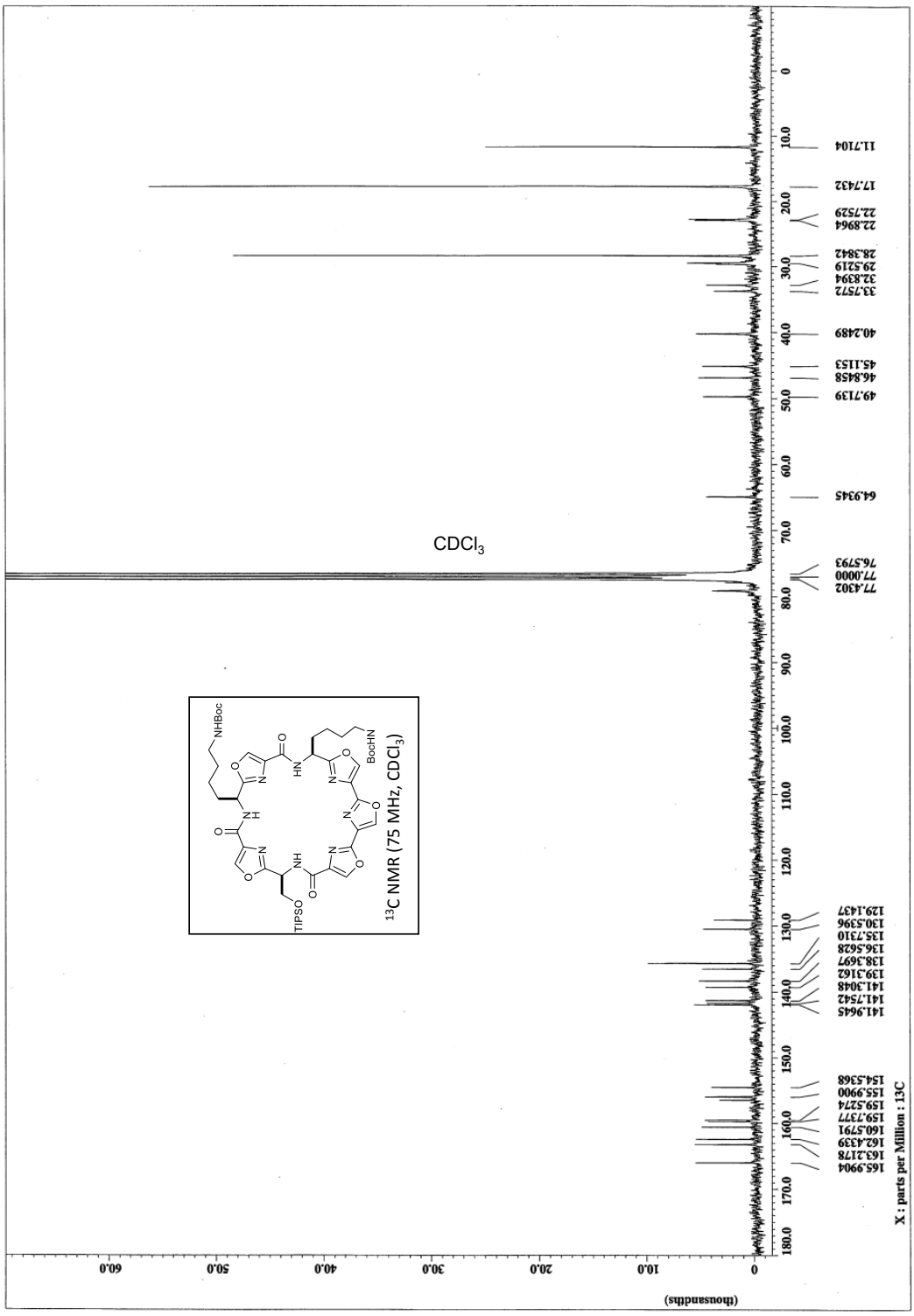
$^{13}$C NMR of **10**


$^{13}$C NMR (75 MHz, $CDCl_3$)
$CDCl_3$
NHBoc
BocHN
CbzHN
$CO_2Me$
TIPSO
HN
NH
O
N
abundance
X : parts per Million : 13C

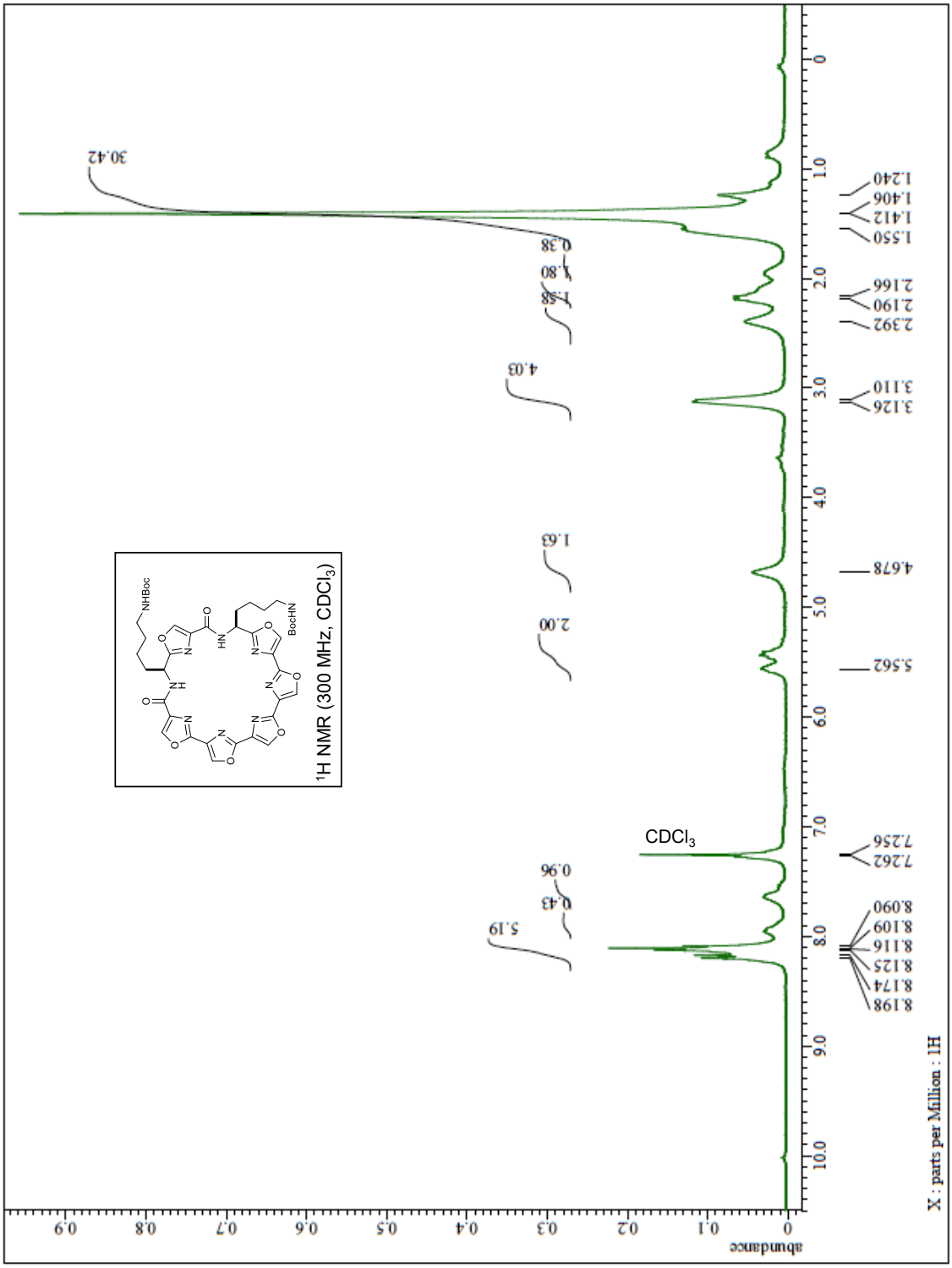
$^{1}$H NMR of **11**


DMSO-$d_6$
$H_2O$
$^1$H NMR (300 MHz, DMSO-$d_6$)
NHBoc
BocHN
TIPSO
X : parts per Million : 1H
abundance

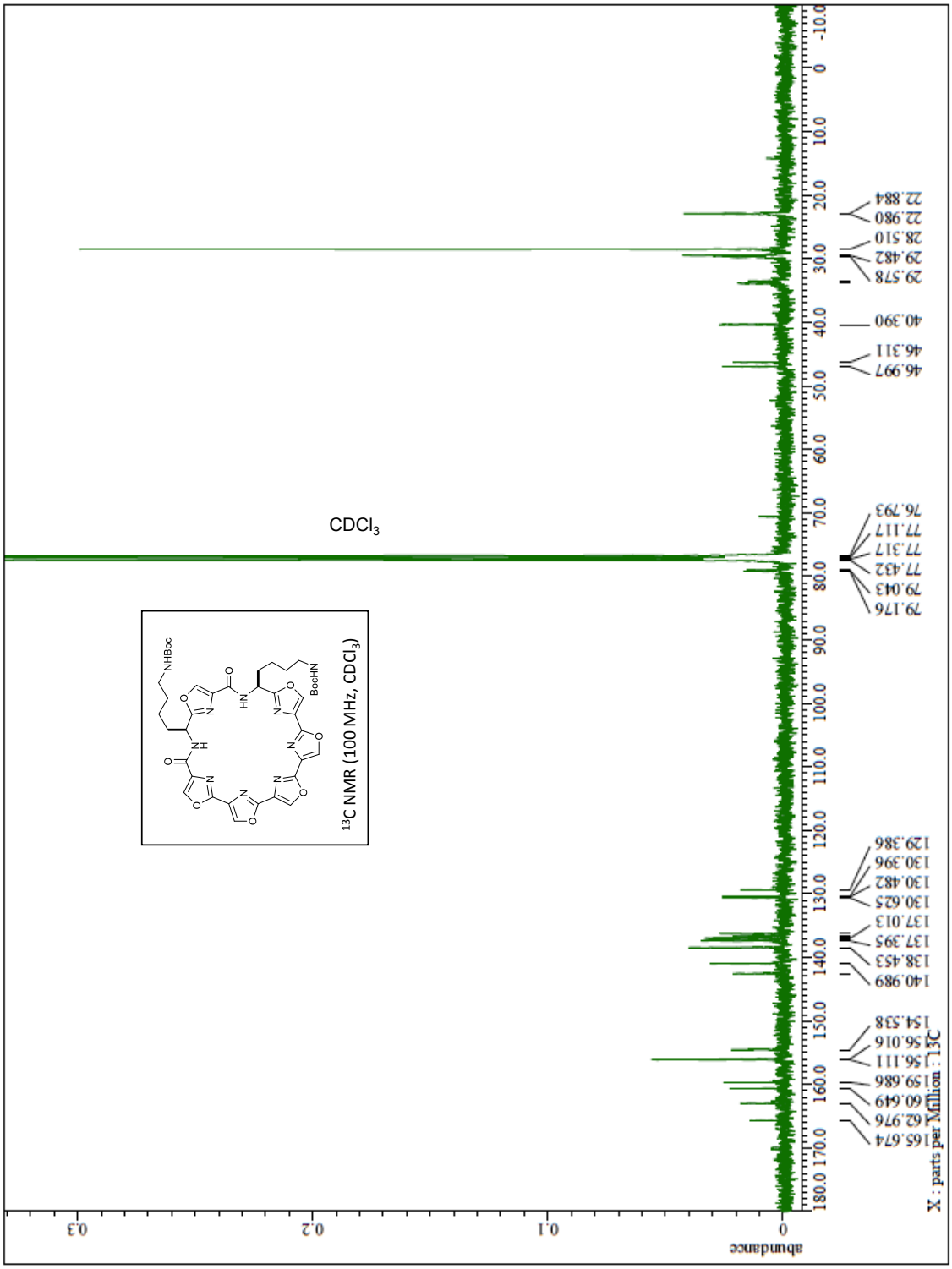
$^{13}$C NMR of **11**


CDCl3
11.7104
17.7432
22.7529
22.8964
28.3842
29.5219
32.8394
33.7572
40.2489
45.1153
46.8458
49.7139
64.9345
76.5793
77.0000
77.4302
129.1437
130.5396
135.7310
136.5628
138.3697
139.3162
141.3048
141.7542
141.9645
154.5368
155.9900
159.5274
159.7377
160.5791
162.4339
163.2178
165.9904
X : parts per Million : 13C
(thousandths)
NHBoc
BocHN
TIPSO
13C NMR (75 MHz, CDCl3)

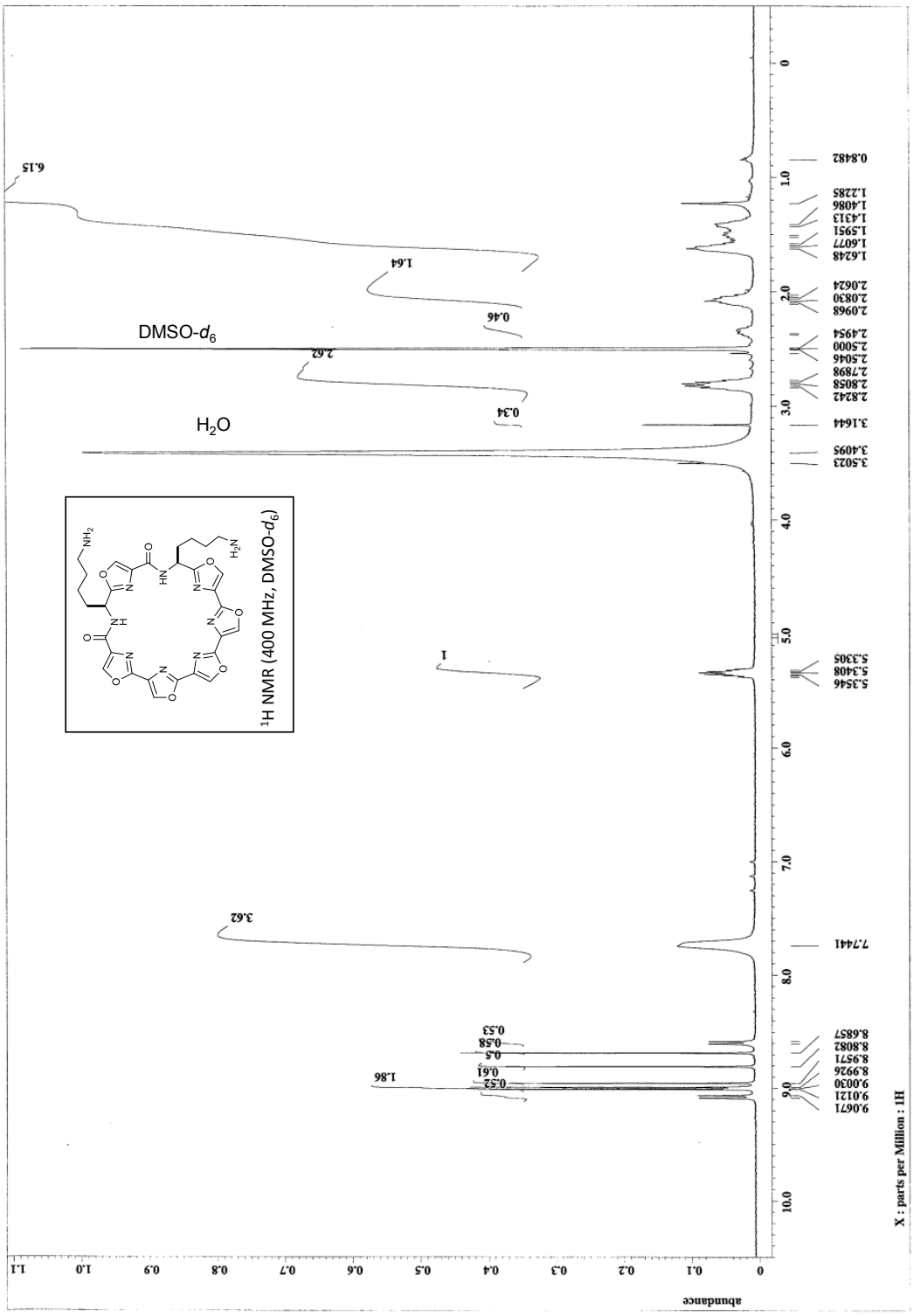
$^{1}$H NMR of *macrocyclic bisamide* ***12***



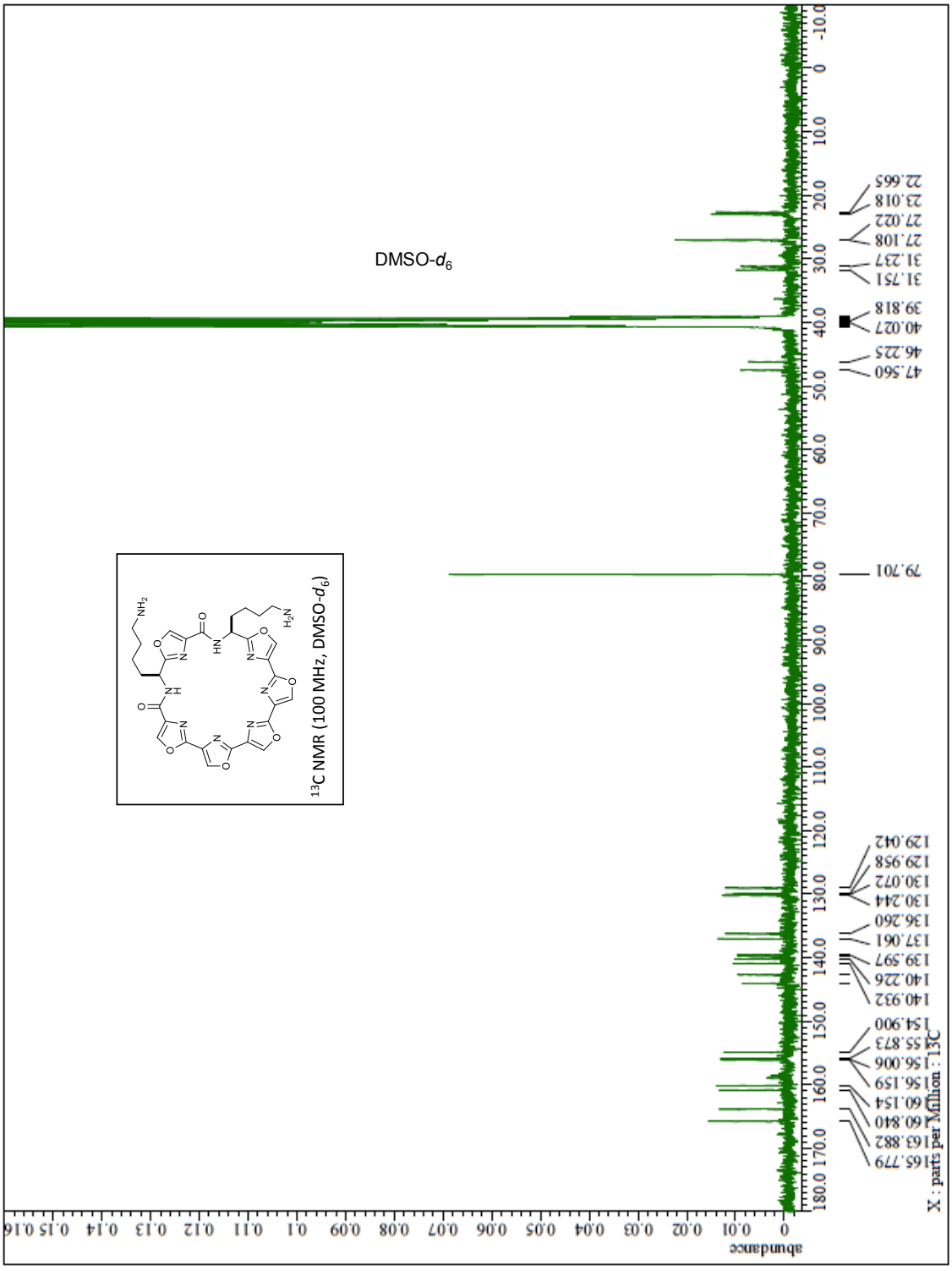
$^{13}$C NMR of *macrocyclic bisamide* ***12***



$^{1}$H NMR of *5,1- L2H2-6OTD (**3**)*



$^{13}$C NMR of *5,1-L2H2-6OTD* (***3***)



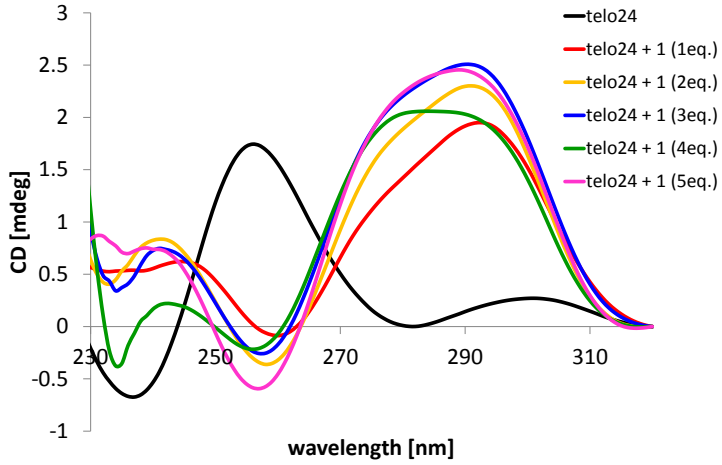
## 2. FRET melting assay of G4 forming DNA sequences

Table S1 $\Delta T_m$ (°C) values in FRET melting assay of G4 forming DNA sequences in the presence of ligands **1**-**3**.

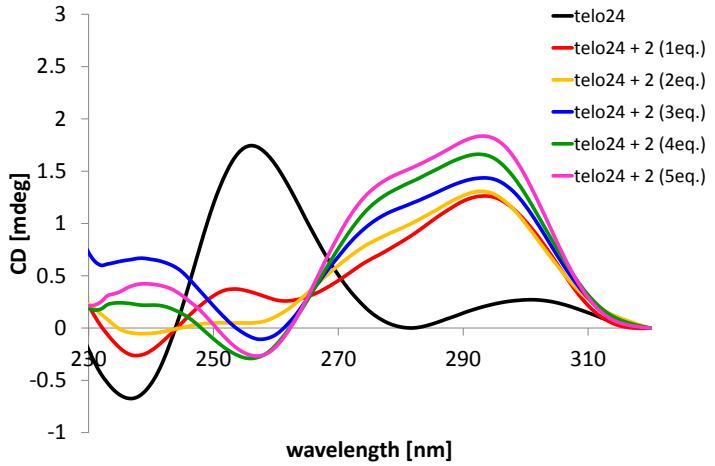
| Ligands | $\Delta T_m$ values (°C) | | | | | |
|---|---|---|---|---|---|---|
| | *bcl-2* | *c-myc* | *c-kit1* | *K-ras* | TBA | dsDNA |
| **1** (control) | 14.9 ± 0.1 | 9.1 ± 0.0 | 16.6 ± 0.4 | 16.5 ± 0.1 | 8.6 ± 0.1 | 0.3 ± 0.0 |
| **2** | 9.1 ± 0.2 | 7.7 ± 0.2 | 10.1 ± 0.6 | 5.5 ± 0.1 | 3.0 ± 0.2 | 0.3 ± 0.0 |
| **3** | 6.5 ± 0.1 | 4.5 ± 0.0 | 8.9 ± 0.0 | 2.8 ± 0.2 | 0.8 ± 0.2 | 0.0 ± 0.0 |

## 3. CD titration experiments

a)




b)




c)

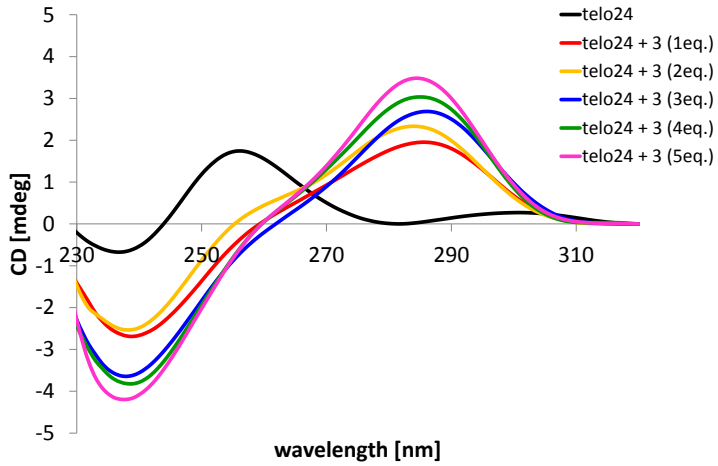
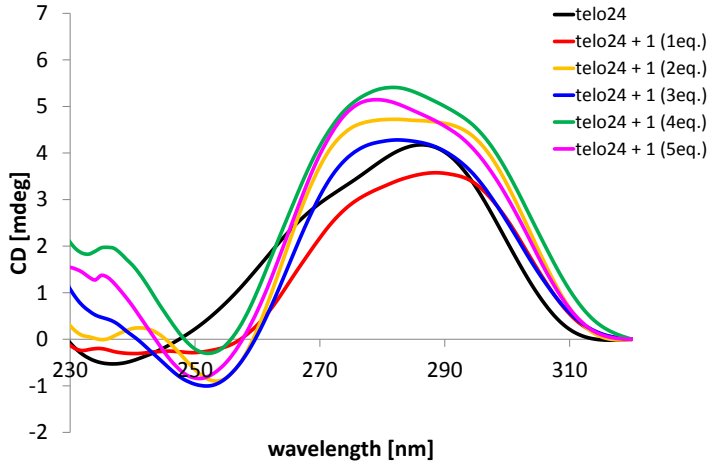
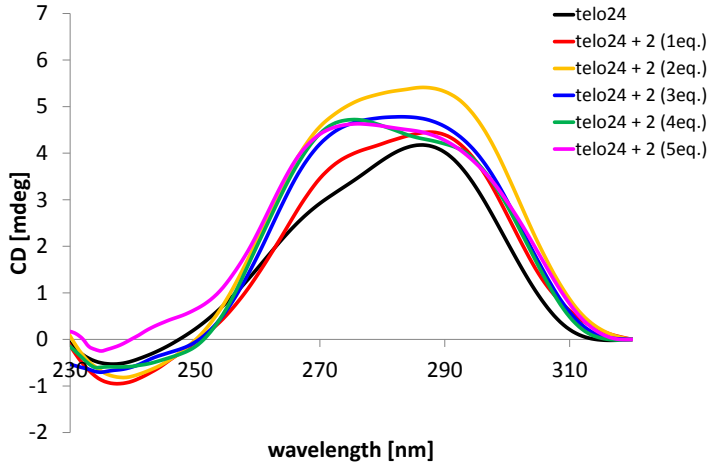



Fig. S1 CD titration spectra of ss-telo24 (10 μM) in 50 mM Tris-HCl buffer (without ion, pH 7.6) with **1**, **2** and **3** (0–5 eq.). a) 3,3-L2H2-6OTD (**1**), b) 4,2-L2H2-6OTD (**2**), c) 5,1-L2H2-6OTD (**3**).

a)




b)




c)

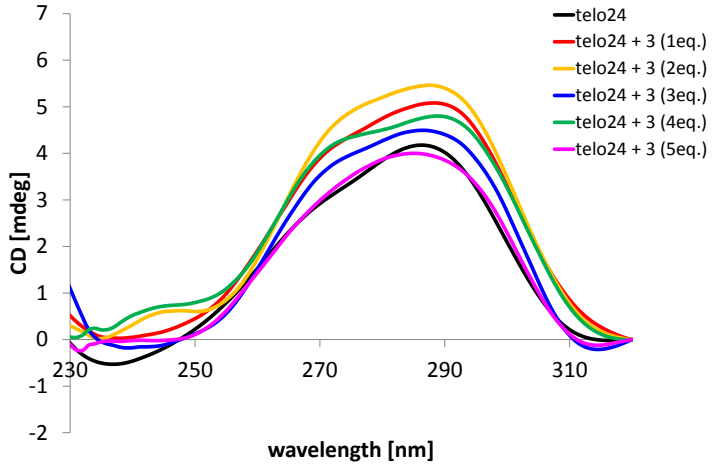
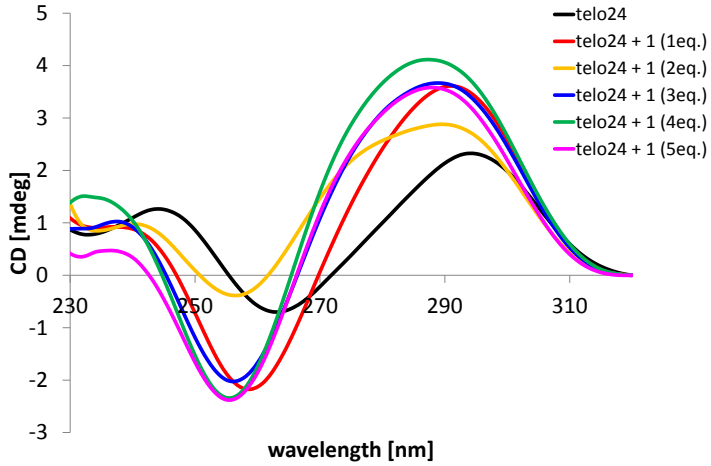
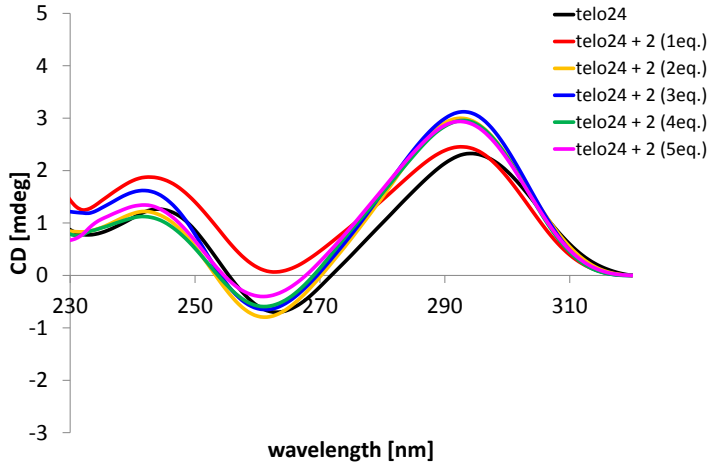



Fig. S2 CD titration spectra of ss-telo24 (10 μM) in 50 mM Tris-HCl buffer (100 mM KCl, pH 7.6) with **1**, **2** and **3** (0–5 eq.). a) 3,3-L2H2-6OTD (**1**), b) 4,2-L2H2-6OTD (**2**), c) 5,1-L2H2-6OTD (**3**).

a)




b)




c)

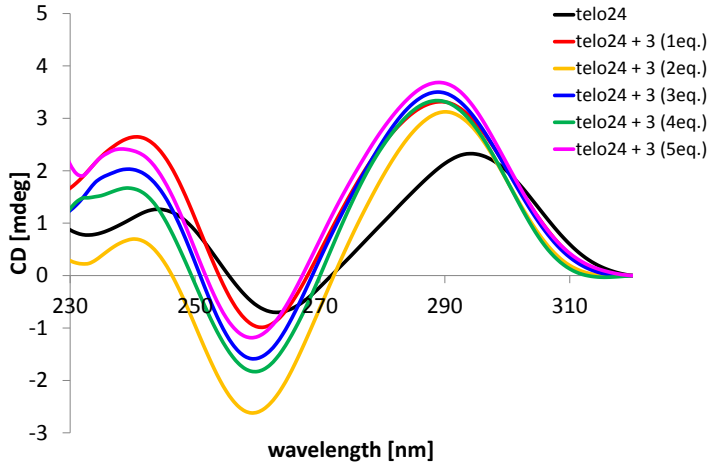
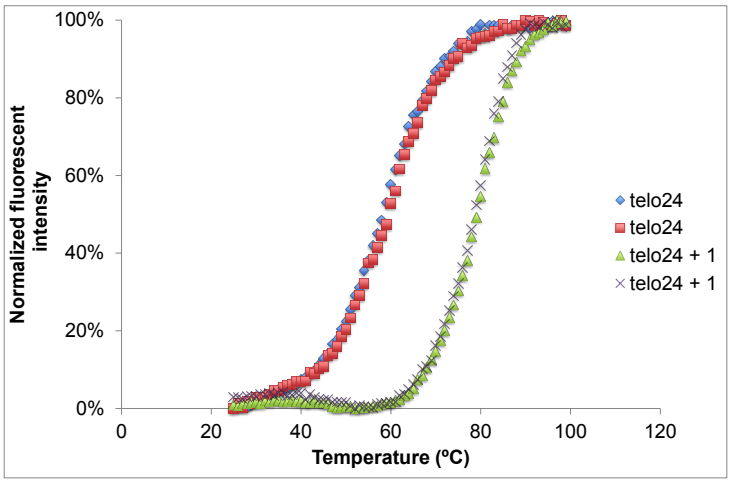


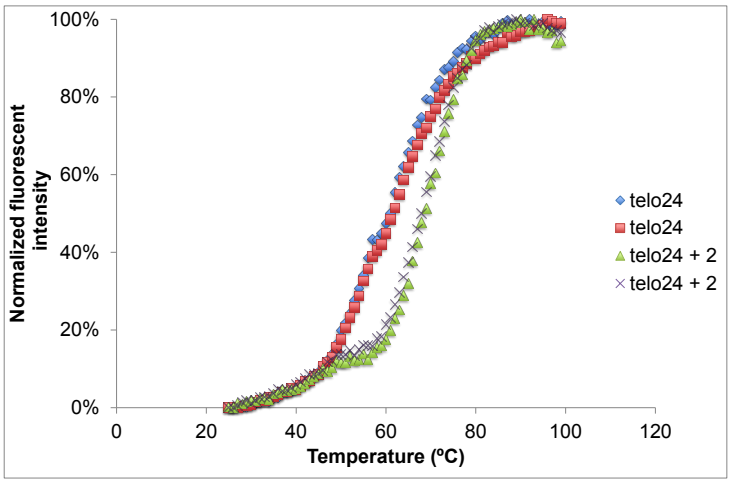

Fig. S3 CD titration spectra of ss-telo24 (10 μM) in 50 mM Tris-HCl buffer (100 mM NaCl, pH 7.6) with **1**, **2** and **3** (0–5 eq.). a) 3,3-L2H2-6OTD (**1**), b) 4,2-L2H2-6OTD (**2**), c) 5,1-L2H2-6OTD (**3**).

## 5. FRET melting curves

a)




b)




c)

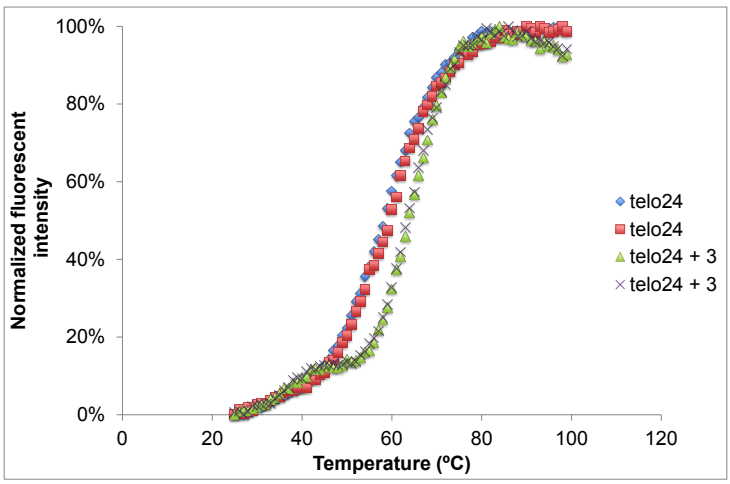



Fig. S4 FRET melting curves of 0.4 μM Flu-ss-telo24 with 2.0 μM **1**, **2** and **3**. a) 3,3-L2H2-6OTD (**1**), b) 4,2-L2H2-6OTD (**2**), c) 5,1-L2H2-6OTD (**3**).