

# CHEMBIOCHEM

## Supporting Information

### **Analysis of Interactions between Telomeric i-Motif DNA and a Cyclic Tetraoxazole Compound**

Shadi Sedghi Masoud<sup>+, [a]</sup> Yudai Yamaoki<sup>+, [b]</sup> Yue Ma,<sup>[a]</sup> Adrien Marchand,<sup>[c]</sup>  
Fernaldo Richtia Winnerdy,<sup>[d]</sup> Valérie Gabelica,<sup>[c]</sup> Anh Tuân Phan,<sup>[d]</sup> Masato Katahira,<sup>\*, [b]</sup> and  
Kazuo Nagasawa<sup>\*, [a]</sup>

cbic\_201800425\_sm\_miscellaneous\_information.pdf

# Supporting Information

## Table of contents

1. Experimental .....	S2
2. Synthesis of L2A2-4OTD ( <b>2</b> ) and Lin-L2H2-4OTD ( <b>3</b> ).....	S4
3. EMSA.....	S7
4. CD experiments.....	S10
5. NMR experiments.....	S11
6. NMR spectra of synthesized compounds.....	S12
7. Supplementary Information references.....	S16

## 1. Experimental

### General

Flash chromatography was performed on silica gel 60 (spherical, particle size 0.040-0.100 mm; Kanto, Tokyo, Japan). Optical rotation were measured on P-2200 polarimeter (JASCO, Tokyo, Japan), using the sodium lamp (589 nm).  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on AL300 instrument (JEOL, Tokyo, Japan). The spectra are referenced internally according to the residual solvent signals of DMSO- $d_6$  ( $^1\text{H}$  NMR;  $\delta = 2.50$  ppm,  $^{13}\text{C}$  NMR;  $\delta = 40.0$  ppm). Data for  $^1\text{H}$  NMR are recorded as follows; chemical shift ( $\delta$ , ppm), multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad), integration, and coupling constant (Hz). Data for  $^{13}\text{C}$  NMR are reported in terms of chemical shift ( $\delta$ , ppm). Mass spectra were recorded on a JMS-T100LC spectrometer (JEOL, Tokyo, Japan) with ESI-MS mode using MeOH.

### Oligonucleotides and compounds

The oligonucleotides were purchased from Sigma Genosys and Intergrated DNA Technologies and were dissolved as 1.0 mM stock solutions in MilliQ water to be used without further purification. Following dilutions were carried out in the relating buffers.

Synthesized compounds were dissolved as 10 mM stock solutions in DMSO.

**Table S1.** Oligonucleotides used in experiments.

	Sequences
h-teloC	CCCTAACCCCTAACCCCTAACCC
h-telo <sup>m</sup> C	CCCTAA <sup>5m</sup> CCCTAACCCUAACCCT
<i>bcl-2</i>	CAGCCCCGCTCCCGCCCCTTCCTCCCGCGCCCGCCCCT
<i>c-kit</i>	CCCTCCTCCCAGCGCCACCC
mutant	CTCTAACTCTAACTCTAACTC

## EMSA Experiments

Oligonucleotides diluted to 100  $\mu\text{M}$  in 50 mM sodium cacodylate buffer (pH 5.8) and heated at 95  $^{\circ}\text{C}$  for 2 min, then slowly cooled to room temperature. After addition of ligand, samples were mixed with 2  $\mu\text{L}$  of ficoll 400 solution (100 mg/mL) and incubated for 6 hours in 4  $^{\circ}\text{C}$ . The oligonucleotide final concentration is 10  $\mu\text{M}$  in 10  $\mu\text{L}$ . 2  $\mu\text{L}$  of each mixture was run on 20% non-denaturing polyacrylamide gel, (gel contained 50 mM sodium cacodylate buffer (pH 5.8 and pH 6.2)) at 100 V for 10 min, then 200 V for 40 min. Gel was stained with Gelstar<sup>®</sup> and scanned with phosphorimager (Typhoon 8600, GE Healthcare) using 580-640 band pass filter.

## CD titration experiments

CD spectra were recorded on J-720 spectropolarimeter (JASCO, TOKYO, JAPAN) using a quartz cell of 1 mm optical path length and instrument scanning speed of 500 nm  $\text{min}^{-1}$  with a response time of 1 s, over wavelength range of 220–320 nm. The CD spectra are representative of 5 averaged scans taken at 25  $^{\circ}\text{C}$ . Oligonucleotides were diluted to 10  $\mu\text{M}$  with sodium cacodylate buffer (pH 5.8, 50 mM). Solutions were annealed by heating at 95  $^{\circ}\text{C}$  for 2 min, then slowly cooled to room temperature. Ligands were added in 2 and 5 equivalent samples, incubated for 6 hours at room temperature and the total amount of 200 mL solutions spectra were measured in cell.

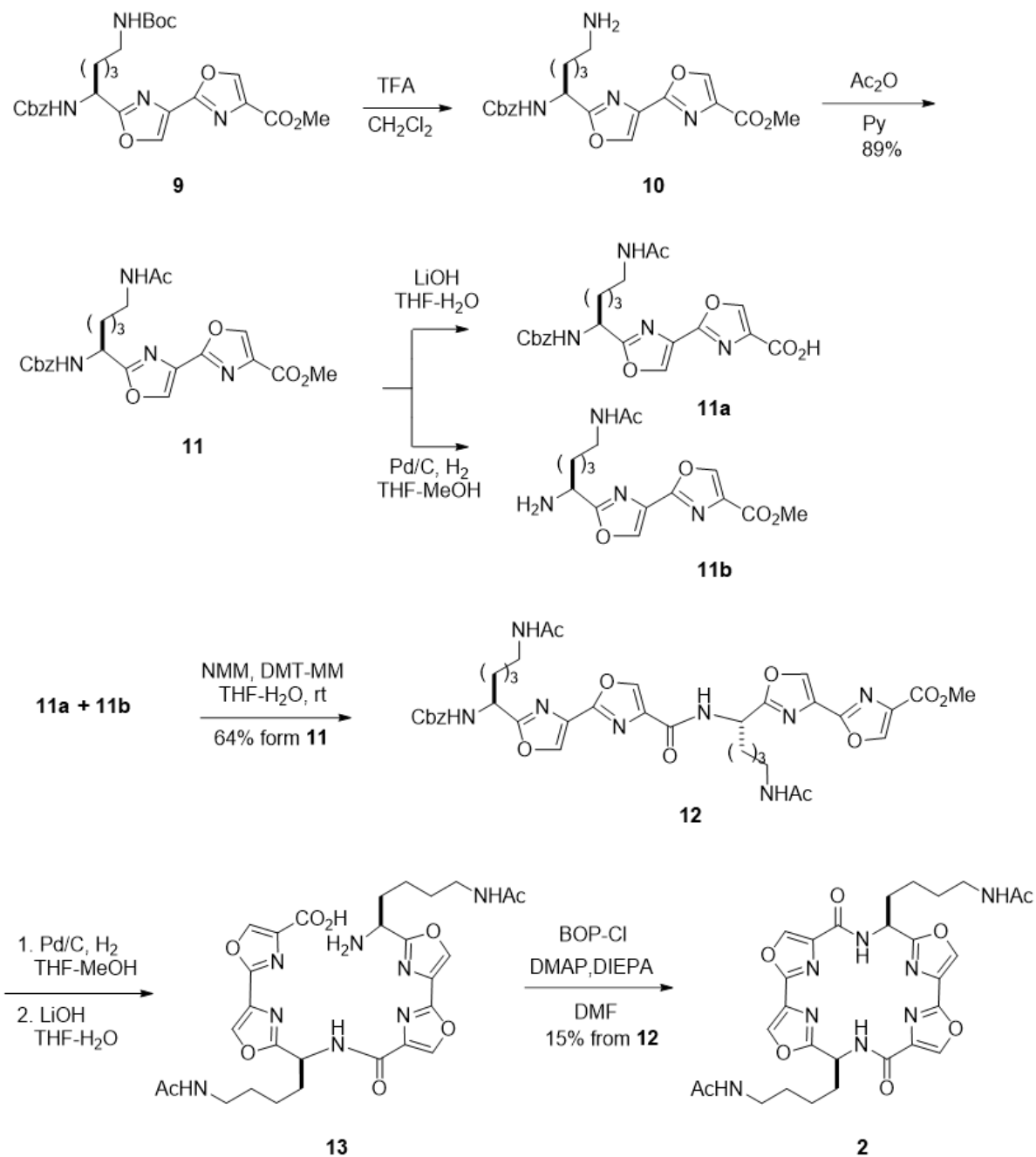
## NMR experiments

h-telo<sup>m</sup>C, 5'-CCCTAA<sup>5m</sup>CCCTAACCCUAACCCT-3', was dissolved in 50 mM sodium cacodylate buffer (pH 5.8) containing 5% D<sub>2</sub>O and 10  $\mu\text{M}$  2,2-dimethylsilapentane-5-sulfonic acid (DSS), to the final concentration of 100  $\mu\text{M}$  for the titration experiment and 400  $\mu\text{M}$  for the NOESY experiment, respectively. The solution was incubated at 95  $^{\circ}\text{C}$  for 2 min, and then slowly cooled to 25  $^{\circ}\text{C}$ . For the titration experiment, L2H2-4OTD (**1**) was added step by step. For the NOESY experiment, equimolar amount of L2H2-4OTD (**1**), 400  $\mu\text{M}$ , was added. NMR spectra were recorded at 25  $^{\circ}\text{C}$  using Bruker BioSpin DRX 600 and AVANCE III HD 600 spectrometers equipped with a cryogenic probe and a Z-gradient. DSS was used as an internal chemical shift reference.

## ESI-MS

ESI-MS spectra were carried out by using a LCT Premier mass spectrometer. The electrospray source was operated in the negative ion mode (capillary voltage at 2.2 kV). The source pressure was set to 45 mbar (measured using a Center Two probe, Oerlikon Leybold Vacuum, Cologne, Germany), the source temperature was 40  $^{\circ}\text{C}$ , the desolvation temperature was 60  $^{\circ}\text{C}$ , the ion guide 1 voltage was 5 V, and the sample cone voltage was 200 V. Samples were prepared with oligonucleotide (5  $\mu\text{M}$ ) and 5 or 10 equivalents of ligand in 10 mM trimethylammonium acetate buffer (pH 5.8). 100  $\mu\text{L}$  were injected at a flow rate of 4  $\mu\text{L}/\text{min}$ . In all experiment 2.5  $\mu\text{M}$  dT<sub>6</sub> (monoisotopic mass 1762.318 Da) was used as internal standard.

## 2. Synthesis of L2A2-4OTD (2) and Lin-L2H2-4OTD (3)



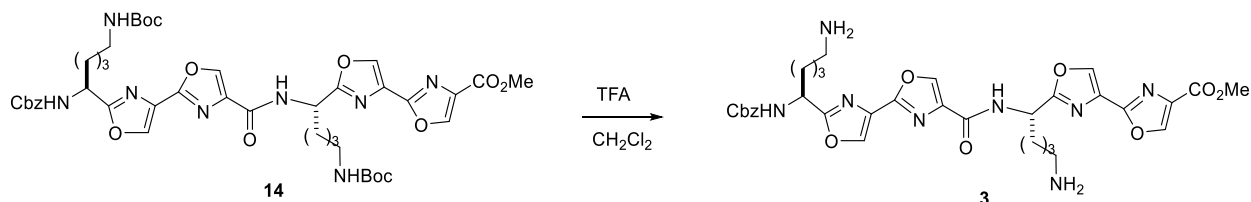
**Scheme S1.** Synthesis of L2A2-4OTD (2)

To a solution of **9**<sup>1</sup>) (1 g, 1.89 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1 mL), TFA (2 mL) was added and stirred at room temperature for 1 h. The reaction mixture was concentrated *in vacuo* to give **10** (0.813 g, 99%). Acetic anhydride (4 mL) and pyridine (4 mL) were added to **10** and the reaction mixture was stirred for 4 h. The volatiles were removed under vacuum conditions and the residue was purified by using silica gel column to give **11** (0.802 g, 89%) [ $\alpha$ ]<sub>D</sub><sup>25</sup> -40 (*c* 0.9, CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  8.29 (s, 1H), 8.28 (s, 1H), 7.34 (m, 5H), 5.67 (br, 1H), 5.55 (d, *J* = 9.0 Hz, 1H), 5.12 (m, 2H), 5.00 (dt, *J* = 6.0, 9.0 Hz, 1H), 3.93 (s, 3H), 3.28-3.12 (m, 2H), 2.06-1.81 (m, 5H), 1.59-1.48 (m, 2H), 1.45-1.32 (m, 2H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  170.2, 165.2, 161.2, 155.8, 155.4, 143.7, 139.5, 135.9, 134.2, 129.6, 128.4, 128.1, 127.9, 67.1, 52.2, 49.1, 38.8, 33.3, 28.7, 23.1, 22.3 ppm; HRMS (ESI- M+Na) calcd for C<sub>23</sub>H<sub>26</sub>N<sub>4</sub>O<sub>7</sub>Na 493.1699, found 493.1682.

To a solution of **11** (369 mg, 0.785 mmol) in THF-H<sub>2</sub>O (3:1, 20 mL), LiOH.H<sub>2</sub>O (49.4 mg, 1.177 mmol) was added at room temperature. The mixture was stirred for 1 h, quenched with 3N HCl to give the solution of carboxylic acid **11a** which was used without further purification. Again to a solution of **11** (433 mg, 0.921 mmol) in THF-MeOH (2:1, 30 mL), 10% Pd/C (45 mg) was added at room temperature and mixture was stirred under hydrogen atmosphere for 2 h. The reaction mixture was filtered through Celite pad and eluted with CHCl<sub>3</sub>-MeOH (9:1). The filtrate was concentrated *in vacuo* to give amine **11b**. To solution of **11a** in THF-H<sub>2</sub>O (1:1, 40mL), was added NMM (172  $\mu$ L, 1.57 mmol), DMT-MM (346 mg, 1.17 mmol) and **11b**, the mixture was stirred at room temperature for 5 h. The organic layer was extracted by CHCl<sub>3</sub>, washed with 1.2 N HCl, dried over MgSO<sub>4</sub> and filtered. The filtrates were concentrated *in vacuo* and the residue was purified by using silica gel column to give **12** (388 mg, 64%) [ $\alpha$ ]<sub>D</sub><sup>25</sup> -18 (*c* 1.2, CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  8.30 (s, 1H), 8.27 (s, 1H), 8.25 (s, 1H), 8.21 (s, 1H), 7.56 (d, *J* = 9.0 Hz, 1H), 7.33 (m, 5H), 5.81 (br, 1H), 5.74 (br, 1H), 5.67 (d, *J* = 6.0 Hz, 1H), 5.46 (dt, *J* = 6.0 Hz, 2H), 5.11 (m, 2H), 3.92 (s, 3H), 3.27-3.15 (m, 4H), 2.15-1.87 (m, 10H), 1.59-1.34 (m, 8H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  170.3, 170.2, 165.5, 164.5, 161.2, 159.9, 155.4, 154.7, 143.7, 141.5, 139.4, 136.4, 135.9, 129.7, 128.4, 128.1, 128.0, 67.1, 52.2, 46.6, 39.0, 38.8, 33.3, 33.1, 28.8, 28.7, 23.1, 22.6, 22.3 ppm; HRMS (ESI- M+Na) calcd for C<sub>37</sub>H<sub>42</sub>N<sub>8</sub>O<sub>11</sub>Na 797.2870, found 797.2836.

To solution of **12** (320 mg, 0.413 mmol), was added 10% Pd/C (40 mg) at room temperature and mixture was stirred under hydrogen atmosphere for 6 h. The reaction mixture was filtered through Celite pad and eluted with CHCl<sub>3</sub>-MeOH (9:1). The filtrates were concentrated *in vacuo* to give amine which was used without purification. To solution of amine in THF-H<sub>2</sub>O (3:1, 12 mL), was added LiOH.H<sub>2</sub>O (11.7 mg, 0.278 mmol) at room temperature for 1 h. 3 N HCl was added to reaction mixture and volatiles were removed under vacuum condition to give carboxylic acid, which was used without further purification. To solution of carboxylic acid in dry DMF (60 mL, 3mM) was added DIPEA (126.5  $\mu$ L, 0.744 mmol), DMAP (22.7 mg, 0.186 mmol) and BOP-Cl (71 mg, 279 mmol) at 0 °C. The mixture was stirred at room temperature for 1 h, H<sub>2</sub>O was added and organic layer was extracted with CHCl<sub>3</sub>, washed with 1.2 N HCL, fried over MgSO<sub>4</sub> and filtered. The filtrates were concentrated *in vacuo* and the residue was purified by silica gel column

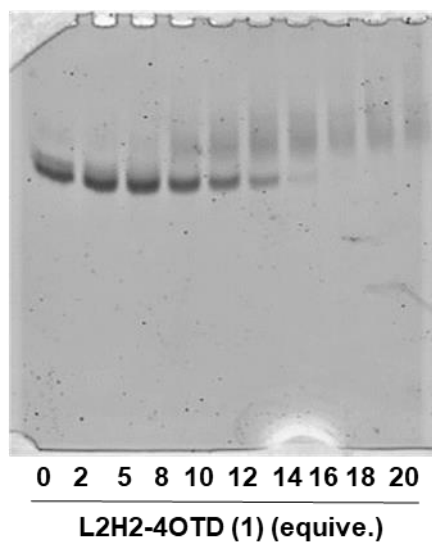
and preparative TLC (CHCl<sub>3</sub>/MeOH/EtOAc = 3:2:1) to give **2** (17mg, 15%, 3 steps). [ $\alpha$ ]<sub>D</sub><sup>25</sup> -15.7 (c 1.3, DMSO); <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  9.045 (s, 1H), 9.016 (s, 1H), 8.86 (s, 2H), 8.75 (s, 2H), 7.84 (m, 2H), 5.29 (m, 2H), 3.01 (m, 4H), 2.11-2.0 (m, 4H), 1.75 (s, 6H), 1.52-1.28 (m, 8H); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  169.6, 66.1, 160.5, 155.3, 141.7, 143.2, 137.04, 46.9, 38.8, 31.6, 23.4, 23.1 ppm; HRMS (ESI, M+Na) calcd for C<sub>28</sub>H<sub>32</sub>N<sub>8</sub>O<sub>8</sub>Na 631.2240, found 631.2275.



### Scheme S2. Synthesis of Lin-L2H2-4OTD (**3**)

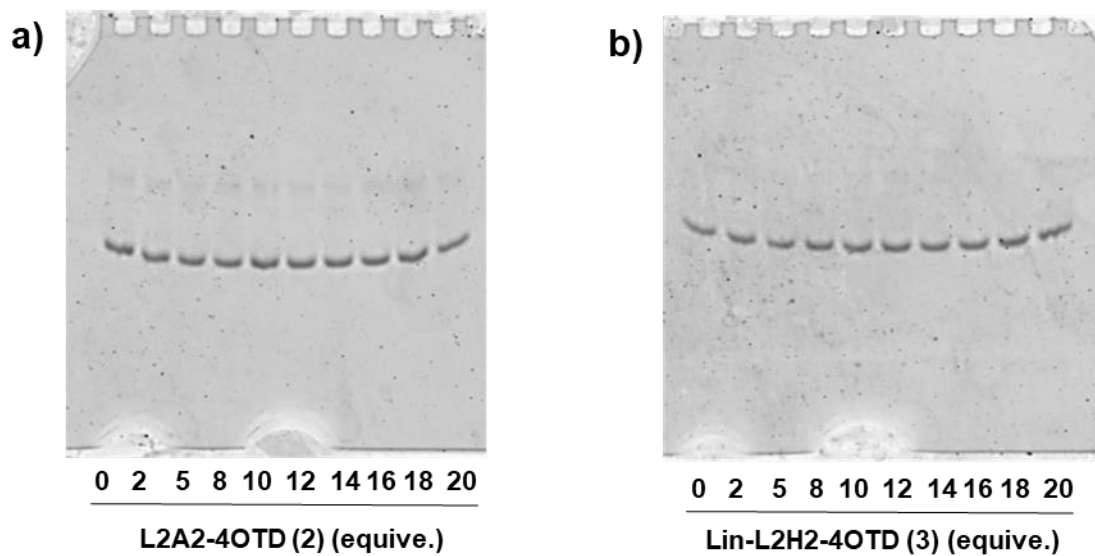
To a solution of **14**<sup>1)</sup> (30 mg, 0.033 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1 mL), TFA (1 mL) was added and stirred at room temperature for 1 h. The reaction mixture was concentrated *in vacuo* to give **10** (22.4 mg, 98%). [ $\alpha$ ]<sub>D</sub><sup>25</sup> -20.6 (c 0.98, MeOH); <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  9.05 (d, *J* = 6.0 Hz, 1H), 8.97 (s, 1H), 8.94 (s, 1H), 8.86 (s, 1H), 8.78 (s, 1H), 8.07 (d, *J* = 9.0 Hz, 1H), 7.78 (br, 4H), 7.37 (m, 5H), 5.26 (m, 1H), 5.06 (s, 2H), 4.77 (m, 1H), 3.83 (s, 3H), 2.79 (m, 4H), 2.11-1.85 (m, 4H), 1.62-1.52 (m, 4H), 1.46-1.32 (m, 4H); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  172.3, 168.2, 166.0, 165.5, 161.4, 160.4, 158.9, 158.4, 156.4, 155.7, 155.1, 146.0, 143.1, 141.5, 137.3, 137.0, 133.7, 129.4, 128.9, 128.3, 66.2, 55.0, 52.5, 49.3, 47.0, 32.0, 31.5, 29.1, 22.8, 22.7 ppm ; HRMS (ESI, M+Na) calcd for C<sub>33</sub>H<sub>38</sub>N<sub>8</sub>H<sub>1</sub>O<sub>9</sub>Na 713.2659, found 713.2659.

### 3. EMSA

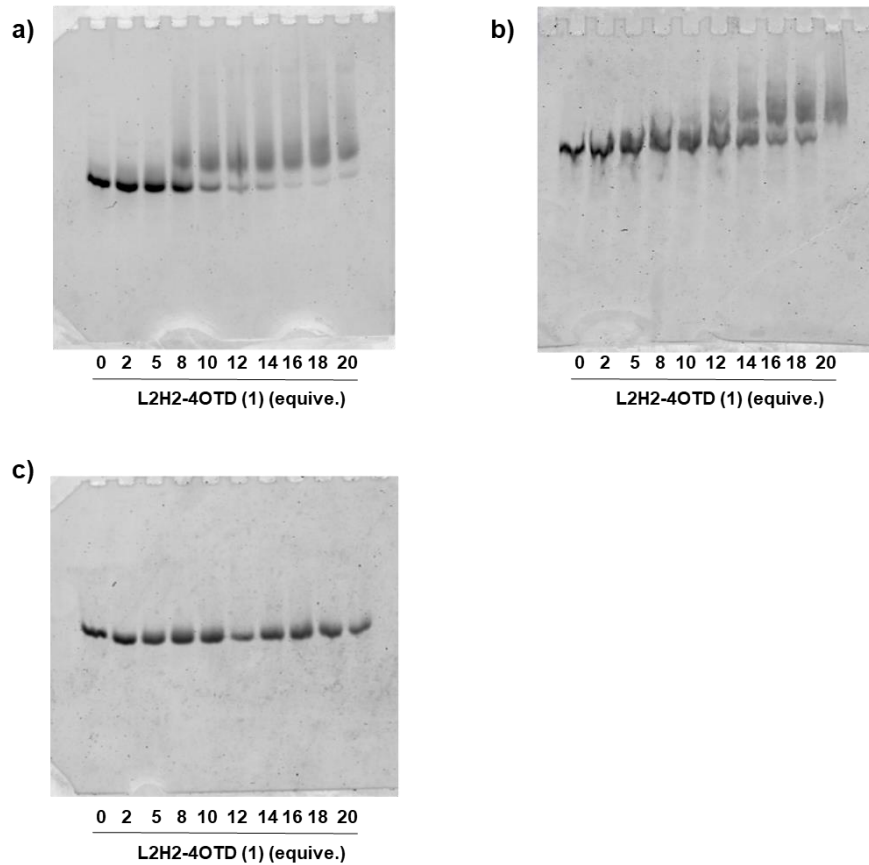


**Figure S1.** The uncut gel data for electrophoretic mobility shift assay of 10  $\mu$ M telomeric i-motif in 50 mM sodium cacodylate buffer (pH 5.8), by increasing the concentration of L2H2-4OTD (1).





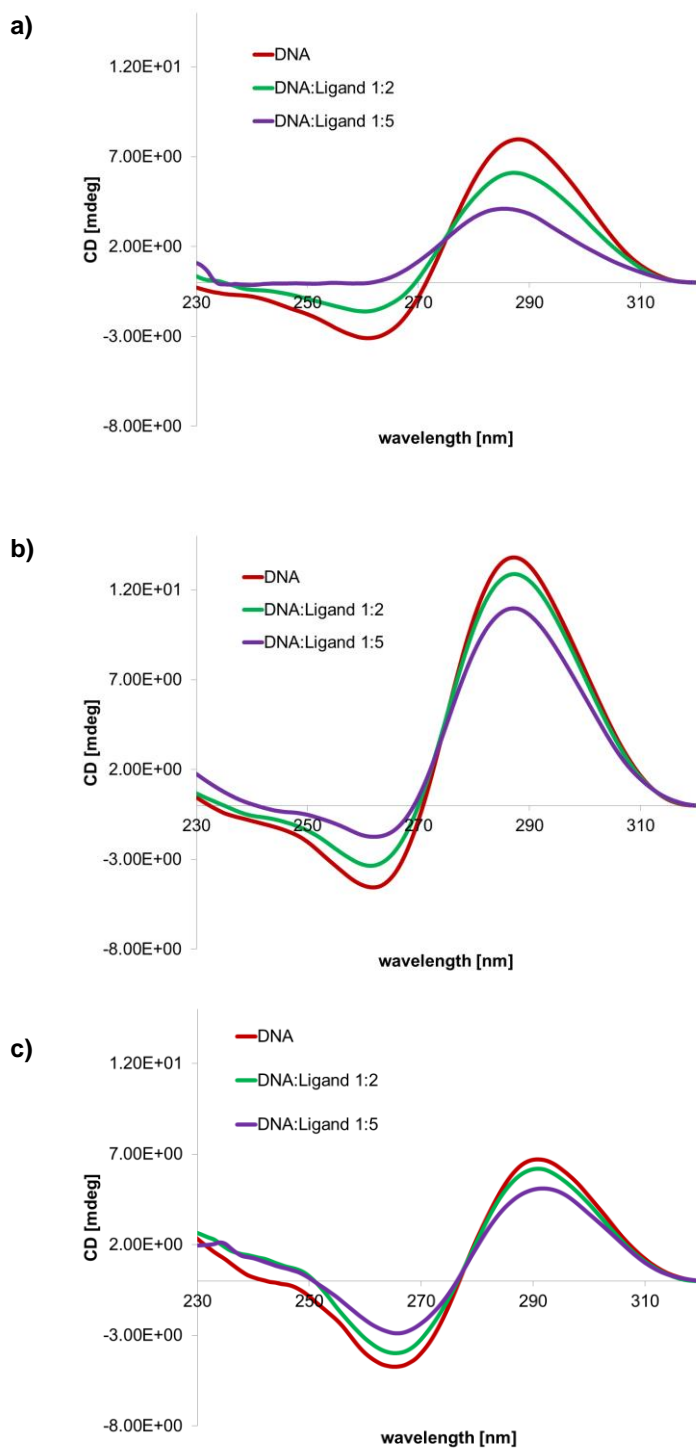
**Figure S2.** The uncut gel data for electrophoretic mobility shift assay of 10  $\mu$ M telomeric i-motif by increasing concentration of a) L2A2-4OTD (**2**) and b) Lin-L2H2-4OTD (**3**) in 50 mM sodium cacodylate buffer (pH 5.8). Significant interaction were not observed in the case of 4OTD with N-Ac group in the side chains and the linear analog.



**Figure S3.** Electrophoretic mobility shift assay of 10  $\mu$ M a) *c-kit* i-motif, b) *bcl-2* i-motif in 50 mM sodium cacodylate buffer (pH 6.2) and c) telomeric i-motif mutant in 50 mM sodium cacodylate buffer (pH 5.8) by increasing the concentration of L2H2-4OTD (**1**).

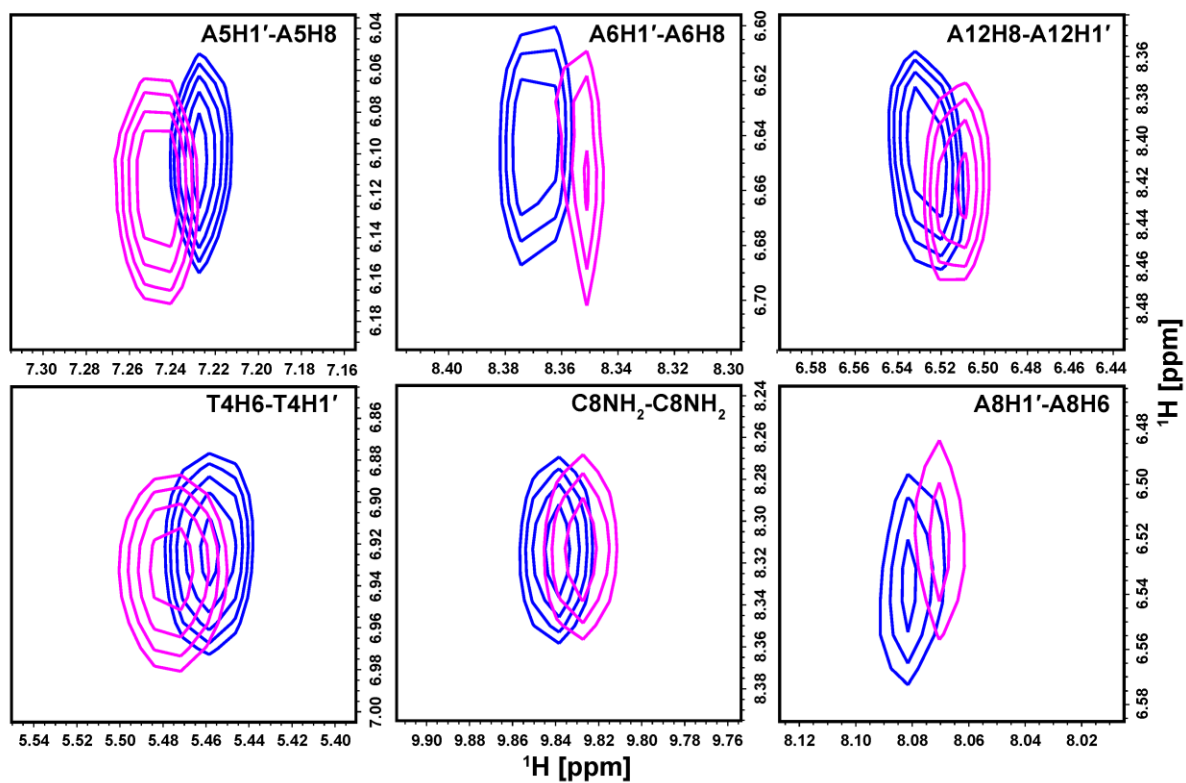
Comment: In these experiments, we observed smear bands in Fig S1-S3. This probably due to the effects of high concentration of unlabeled DNA sequences. Actually, when we used TAMRA-labeled DNA with four times diluted conditions, clear bands are observed (data not shown). However to avoid the hypothesis of the interaction effects of fluorescent probe (TAMRA) in DNA with L2H2-4TOD (**1**), we need to use the unlabeled DNA and the staining procedure by Gelstar.

## 4. CD experiments



**Figure S4.** CD spectra of 10  $\mu$ M a) *c-kit* i-motif and b) *bcl-2* i-motif and c) h-telo<sup>m</sup>C, upon titration of L2H2-4OTD (1) resulted in a decrease in the positive signal at 288 nm.

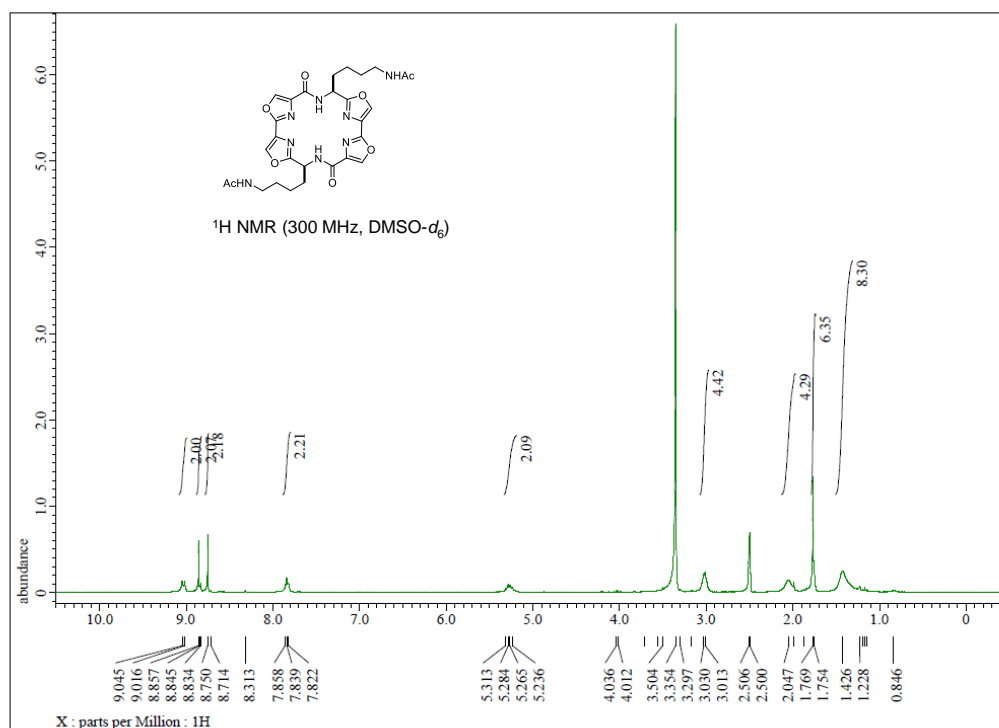
## 5. NMR experiments



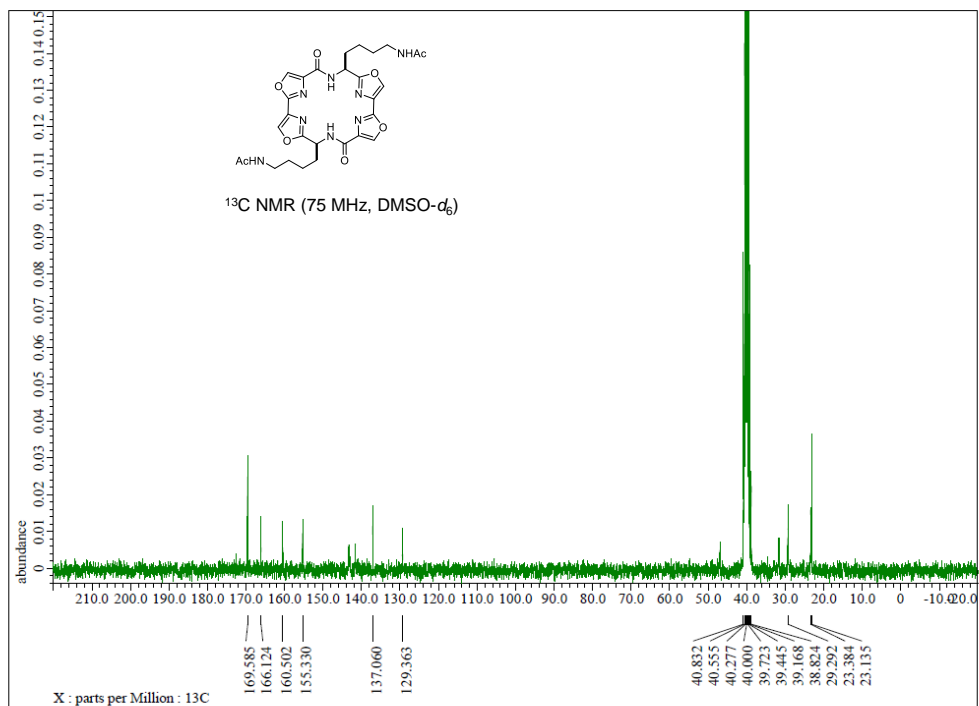
**Figure S5.** NOESY spectra of h-telo<sup>m</sup>C in the absence (blue) and presence (magenta) of L2H2-4OTD (1). The signals that exhibited the large CSPs are indicated.

## 6. NMR spectra of synthesized compound

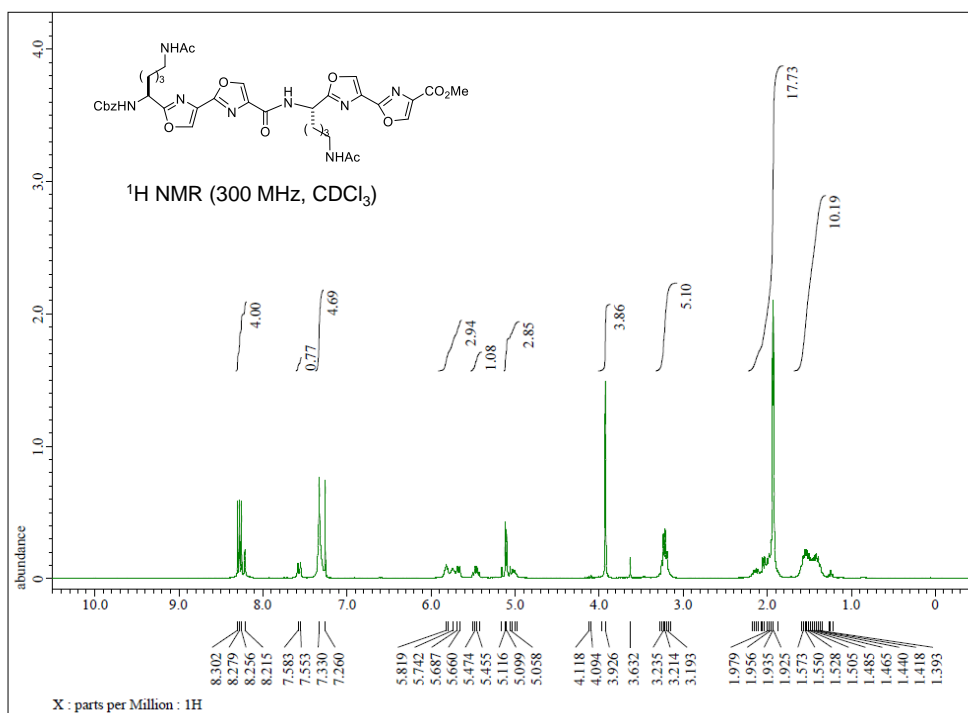
### $^1\text{H}$ NMR of L2A2-4OTD (2)



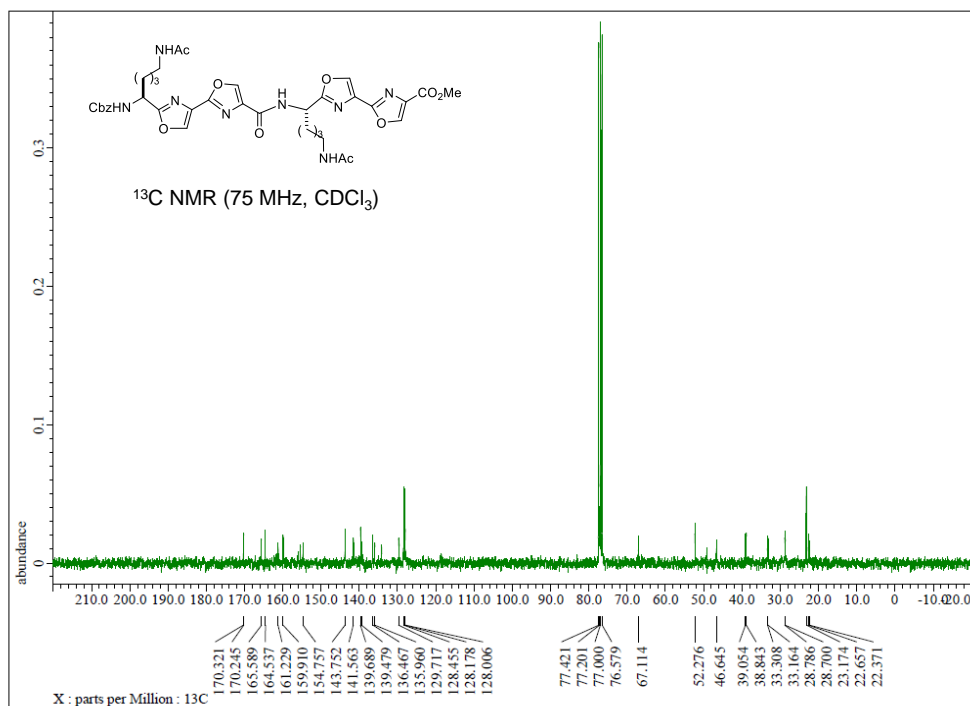
### $^{13}\text{C}$ NMR of L2A2-4OTD (2)



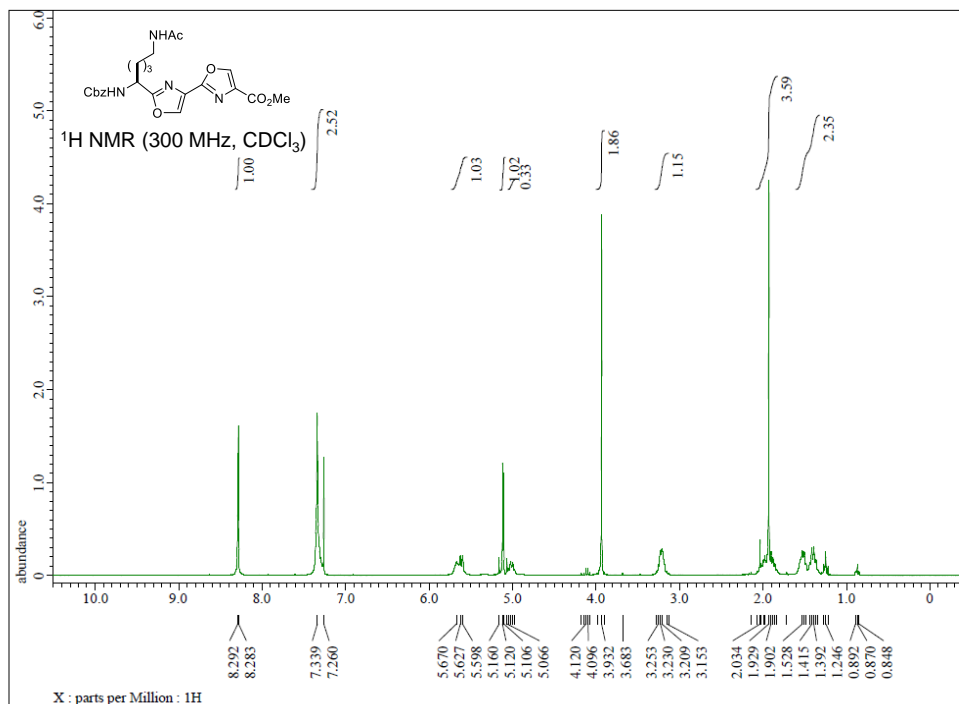
# <sup>1</sup>H NMR of 12



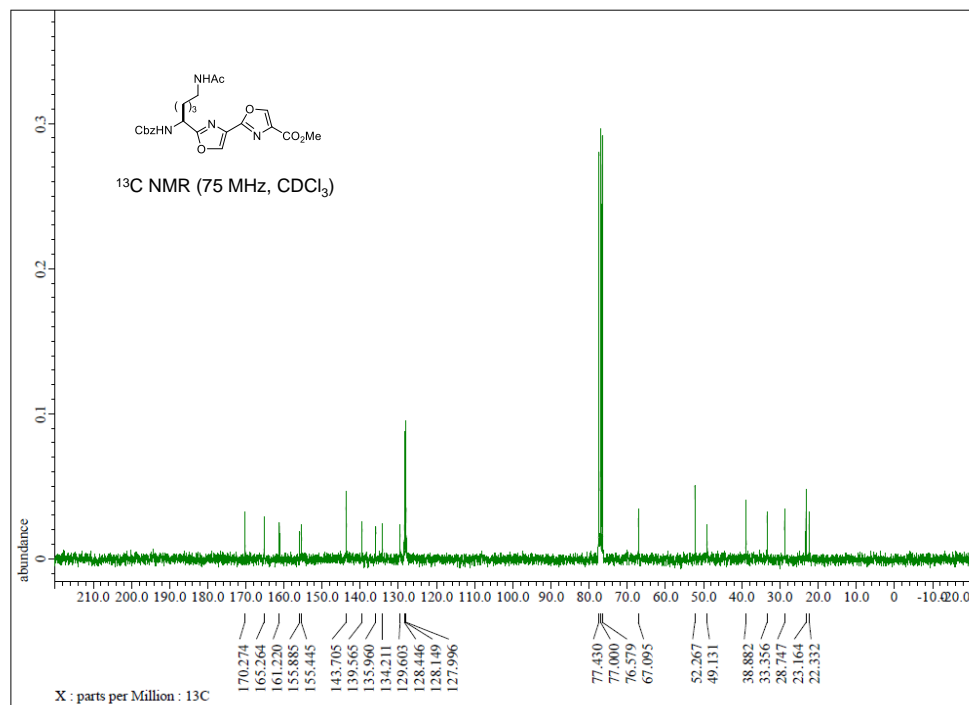
# <sup>13</sup>C NMR of 12



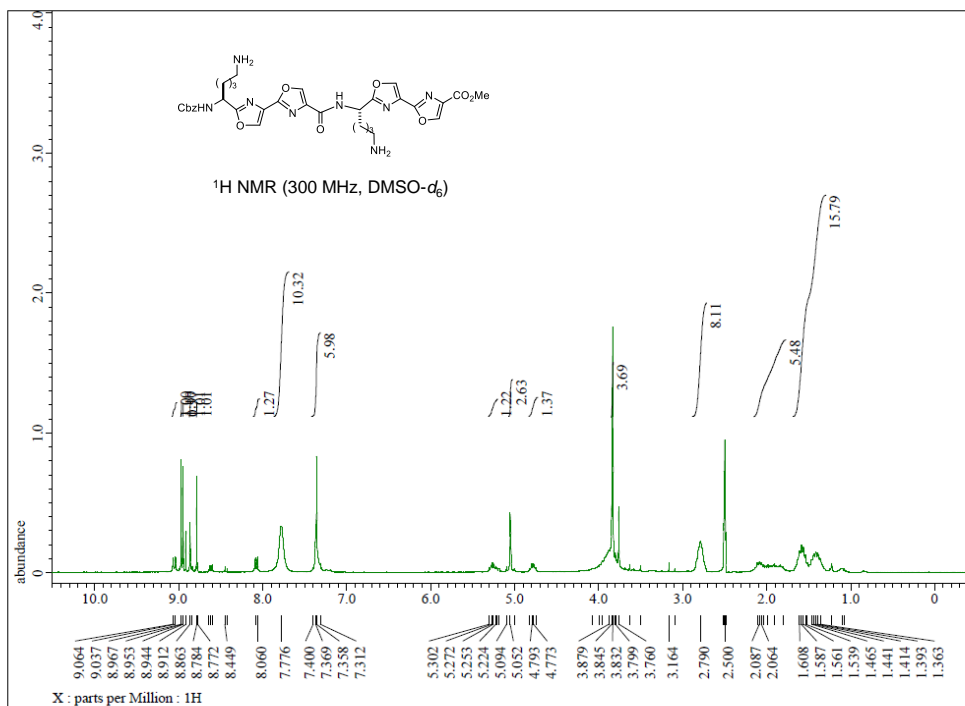
# <sup>1</sup>H NMR of 11



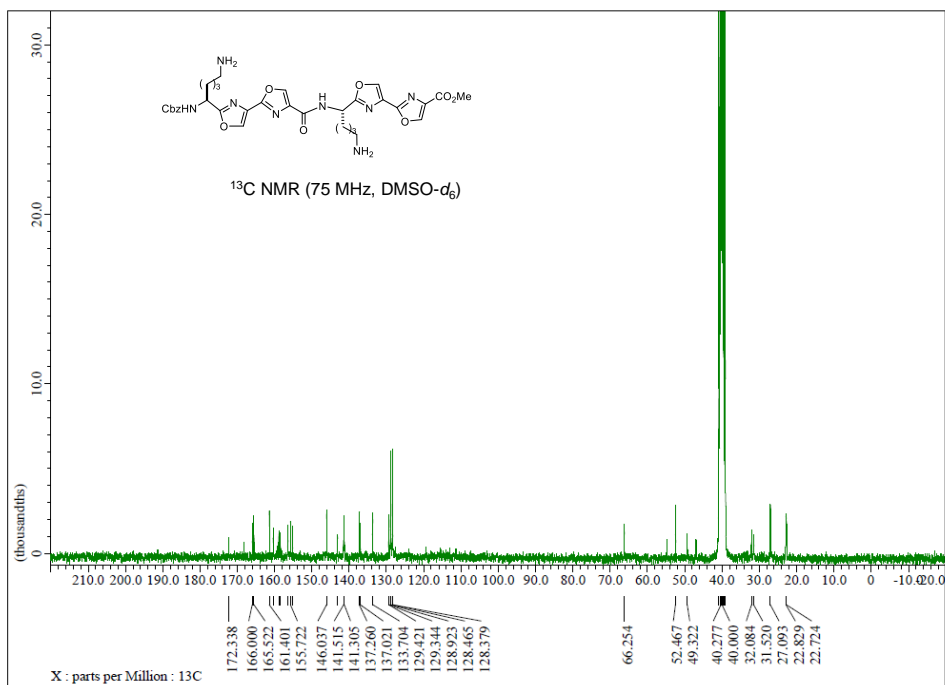
# <sup>13</sup>C NMR of 11



# <sup>1</sup>H NMR of Lin-L2H2-4OTD (3)



# <sup>13</sup>C NMR of Lin-L2H2-4OTD (3)





## 7. Supplementary Information references

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