Supporting information

Topology-Selective Photo-Crosslinking of G-Quadruplexes via Dual G-Quartet and Groove Recognition

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Materials and methods

Stock solution of 6OTD (**1**), 6OTD-Bp (**2**): **1** and **2** was dissolved in DMSO (10 mM). Further dilution was carried out from this solution. All experiments were performed with < 0.2% DMSO. All buffers used in this study were described as below.

Tris-KCl buffer (pH 7.5) : 50 mM Tris-HCl, 100 mM KCl (pH 7.5).
Cacodylate-LiCl buffer : 10 mM cacodylic acid-KOH, 90 mM LiCl (pH 7.2).
Exonuclease Ⅰ buffer : 10 mM Tris-HCl, 0.5 mM EDTA, 1 mM DTT (pH 7.5).
TBE buffer : 90 mM Tris-borate, 2 mM EDTA.

Non-labelled oligonucleotides were purchased from Eurofins (Japan) was used without further purification. Exonuclease I (Takara Bio, Japan) was purchased and used without purification. **1** were synthesized as previously reported.[1] All other reagents were obtained in the molecular biology grades from Sigma-Aldrich, Wako chemicals, or TCI (Japan). The fluorescent measurement was performed with an excitation wavelength of 300 nm and a detection wavelength of 320-500 nm using the FP-8600 (JASCO). PAGE analysis was carried out with Mini-Protean Gel system (Bio-Rad, USA). PAGE images were acquired on ChemiDoc XRS (Bio-Rad, USA). Image analysis was performed using ImageJ 1.47c (National Institutes of Health, USA). The molar mass of the samples was detected by MALDI-TOF MS measurement (Autoflex speed, Bruker Daltonics, USA). The measurement was performed in positive mode. 3-hydroxypicolinic acid was used as the matrix.

Thiazole Orange (TO) displacement assay

TO displacement assay was performed with 384-well plate by an excitation wavelength of 480 nm and a detection wavelength of 530 nm using the Spark (TECAN, Switzerland, Zurich). Oligonucleotides (purchased by Eurofin) were used in this protocol, and were dissolved as stock solutions (100 μM) in MilliQ water to be used without further purification. Further dilutions of the oligonucleotides were performed with 50 mM Tris-HCl buffer (pH 7.4) with 100 mM KCl, and this experiment was carried out with a 5.0 μM DNA solution. DNA solution was annealed by heating at 95 ℃ for 5 min, and then slowly cooled to room temperature. The compounds (**1**, **2** and TO) were prepared as DMSO stock solutions (10 mM). TO solution diluted to 1.0 μM and the **1**, **2** solutions diluted to 4.0 μM - 2 mM using 50 mM Tris-HCl buffer (pH 7.4) with 100 mM KCl. The TO solution (10.0 μM) was added into the annealing DNA solution, to give concentrations of the oligonucleotide (0.5 μM) and the compound solution (1.0 μM), and incubated for 30 min. Then, each solution containing each concentration of compound (1.0 μL) was added into the DNA samples (39.0 μL), and incubated at 25 ℃ for 1 h (Final concentration = DNA: 0.5 μM, TO: 1.0 μM, **1** and **2** : 0.1-50 μM).

Circular dichroism (CD) spectrometry

CD spectra were recorded on a J-720 spectropolarimeter (JASCO, Japan) using a quartz cell of 10 mm optical path length and an instrument scanning speed of 500 nm min^{-1} with a response time of 1 s, and over a wavelength range of 230-320 nm. CD spectra are representative of ten averaged scans taken at 37 °C. The nucleotide was dissolved in MilliQ water as a 100 μM stock solution to be used without further purification. Further dilution of the nucleotide was 100 mM KCl, 50 mM Tris-HCl buffer (pH 7.5) and CD spectra were performed with a 10 μM oligonucleotide solution. The solution was annealed by heating at 95 °C for 5 min, then slowly cooled to room temperature. 190 μL of the nucleotide solution and 10 μL of the compound solution (in 1% DMSO) were mixed and injected into a quartz cell.

Irradiation of the DNA-ligand complexes

The irradiations were performed with a LC-L1V3 UV-LED light source (HAMAMATSU, Japan) provided with a filter centered at 365 nm, a quartz light guide and a collimator lens. The apparatus was setup in a top-down fashion, exposing an ice-cooled uncapped 1.5 mL TreffLab tube to the UV beam at a distance of 30 mm measured from the collimator extremity to the tube top (at this distance, the irradiance is approximately 1556 mW/cm_2 at 365 nm). The reaction volume was 20 μL. Samples were prepared by heat denaturing for 5 min at 95 °C, 10 μM of G4 DNAs folding, in 50 mM Tris-HCl buffer (pH 7.5) and 100 mM KCl in a total volume of 15 μL; G4 folding was obtained by cooling the solutions at room temperature over a period of ~2 hours. Before irradiation, DNA was incubated with the **2** (100 μM) for overnight at room temperature to reach the total volume of 20 μL. After irradiation, the reaction mixture (2.0 μL) and urea (10 M, 8.0 μL) was mixed and heated to 80 ºC for 5 min then immediately loaded onto a denaturing polyacrylamide gel (7 M urea, 18% acrylamide, 2% bisacrylamide gel in TBE buffer) and the gels were run at 300 V × 20 min in 1 × TBE buffer at 60 ºC. The gels were stained with SYBR-gold. The gel was exposed to a storage phosphor screen and converted to a digital image with a ChemiDoc XRS (Bio-Rad, Japan). The yield of alkylation was defined as the ratio between the counts corresponding to the alkylation band and the sum of the counts corresponding to both the alkylation and the non-modified DNA bands, corrected by the background noise. The values given in % are the mean of at least three experiments.

CD melting assay

A solution of all DNA (5.0 μM) was prepared in 10 mM Tris–HCl buffer (pH 8.0). Subsequently, corresponding concentrations of **2** (12.5 μM) were added. Melting and annealing curves were obtained by monitoring the CD intensity at 265 nm on a J-720 spectropolarimeter (JASCO, Tokyo, Japan) by using a quartz cell of 1 mm optical path length; the temperature was changed as follows: 25 to 90 °C then 90 to 25 °C at 1.0 °C min^{-1}.

Exonuclease I digestion coupled with PAGE analyses

Oligonucleotides (10 μM), pre-annealed in Tris-KCl buffer, were mixed with ligand (25 μM) and incubated for 1 hour at 37 ºC. The mixture was then irradiated with ultraviolet light (365 nm) for a total of 150 seconds (5 seconds × 30 cycles). The resulting oligonucleotides (10 μL) were treated with exonuclease I (0.08 U/μL) in Tris-HCl buffer (100 mM, pH 8.0), supplemented with $MgCl_2$ (2 mM) and transfer RNA (0.5 mg/mL), for 30 minutes at 37 ºC (final concentrations: DNA, 4 μM; exonuclease I, 0.08 U/μL). The reaction mixture was then heated to 80 ºC for 15 minutes to inactivate exonuclease I.

For each adduct either treated or untreated samples were analyzed by denaturing 20% polyacrylamide gel electrophoresis, run at 300 V × 20 min in 1 × TBE buffer. The digest was filtered, freeze-dried, and purified by HLB cartridge (Oasis). The digested oligonucleotides were desalted with Zip-tip (Merck-Millipore, USA). The eluted oligonucleotides were mixed with matrix (360 μM 3-HPA, 442 μM AHC in MQ:ACN=1:1) on the metal plate and subjected to MALDI-MS measurement. Crosslinking bases at G11 and G16 in telo24 were detected by MALDI-MS at 5007.9 and 6583.20, respectively (calculated values: 5005.8 and 6585.9). Similarly, A10 in *bcl2,* T12 and G13 in VEGFR were detected at 4694.4, 5264.3 and 5593.4, respectively (calculated values: 4696.6, 5265 and 5594.2).

Computational analyses

For initial coordinates of **1** and **2**, ionization and energy minimization were performed with the OPLS3e force field in the LigPrep script in the Maestro (Schrödinger, LLC, New York, NY, USA). These minimized structures were employed as input structures for docking simulations. The human telomeric G4 structures with hybrid-type (PDB ID: 2MB3), were refined for docking simulations using constrained energy refinements in the OPLS3e force field (Schrödinger LLC). Docking simulations were performed using the Glide XP docking program (Schrödinger, LLC, New York, NY, USA).

Oligonucleotides sequences

Table S1. Sequences of oligonucleotides and PDB IDs for their G-quadruplex structures used in this study.

Oligonucleotides	Sequence	PDB ID
telo24	d[TTG GGT TAG GGT TAG GGT TAG GGA]	2MB3
bcl2	d[GGG CGC GGG AGG AAT TGG GCG GG]	2F8U
PARP1	d[TGG GGT CCG AGG CGG GGC TTG GG]	6AC7
VEGFR	d[GGG TAC CCG GGT GAG GTG CGG GGT]	5ZEV
c-myc	d[TGA GGG TGG GTA GGG TGG GTA A]	1XAV
c-kit	d[CGG GCG GGC ACG AGG GAG GGT]	2KQG
RET	d[GGG GCG GGG CGG GGC GGG GT]	2L88
TBA	d[GGT TGG TGT GGT TGG]	148D

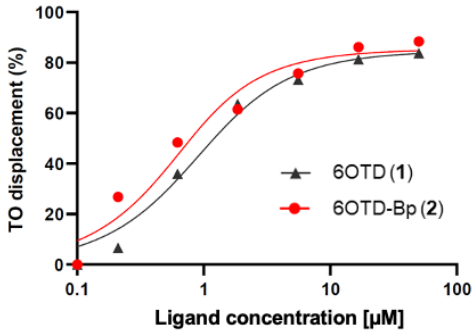


Fig. S1 TO displacement (1.0 μM) from telo24 (0.5 μM) by 6OTD (**1**) (black line) or 6OTD-Bp (**2**) (red line) (0.1–50 μM). Final concentration = DNA: 0.5 μM, TO: 1.0 μM, **1** and **2**: 0.1-50 μM.

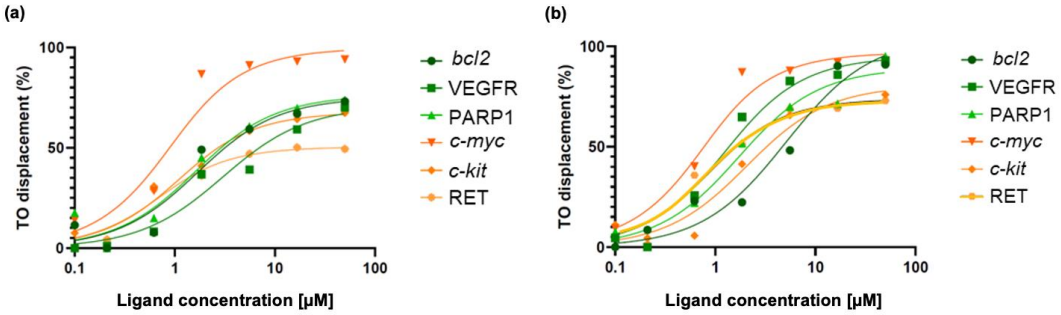


Fig. S2 TO displacement (1.0 μM) from G4 oligonucleotides (0.5 μM) by (a) 6OTD (**1**) or (b) 6OTD-Bp (**2**) (0.1–50 μM). Final concentration = DNA: 0.5 μM, TO: 1.0 μM, **1** and **2**: 0.1-50 μM.

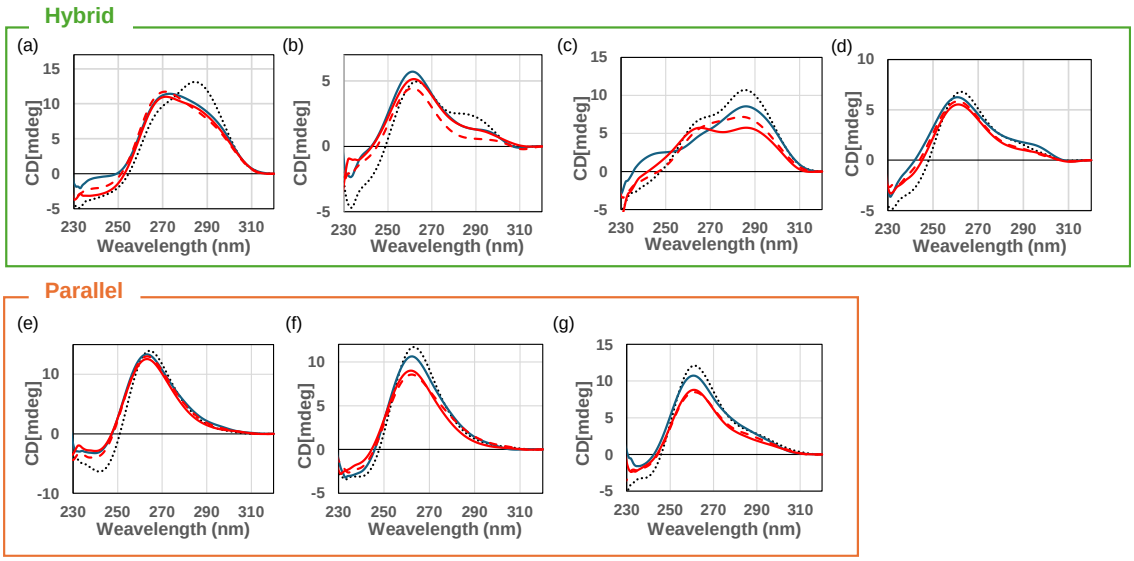


Fig. S3 CD spectrometry of oligonucleotides ((a) telo24 (b) *bcl2* (c) PARP1 (d) VEGFR (e) *c-myc* (f) *c-kit* (g) RET) (dotted black), in the presence of 6OTD (**1**) (solid blue), 6OTD-Bp (**2**) without irradiation (dashed red), and 6OTD-Bp (**2**) with irradiation (solid red).

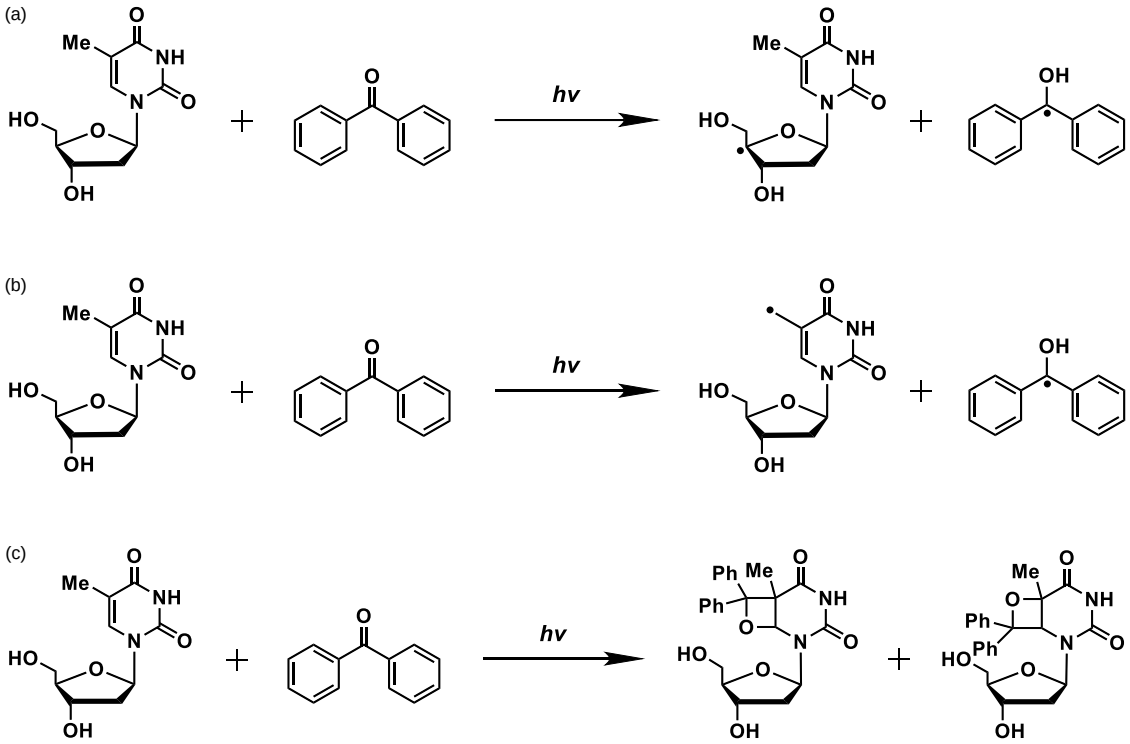


Fig. S4 Presumed crosslinking mechanism of Bp and DNA. (a) Deoxyribose at 4' position. (b) Methyl group in thymidine. (c) [2+2] Cycloaddition at the double group in thymidine.

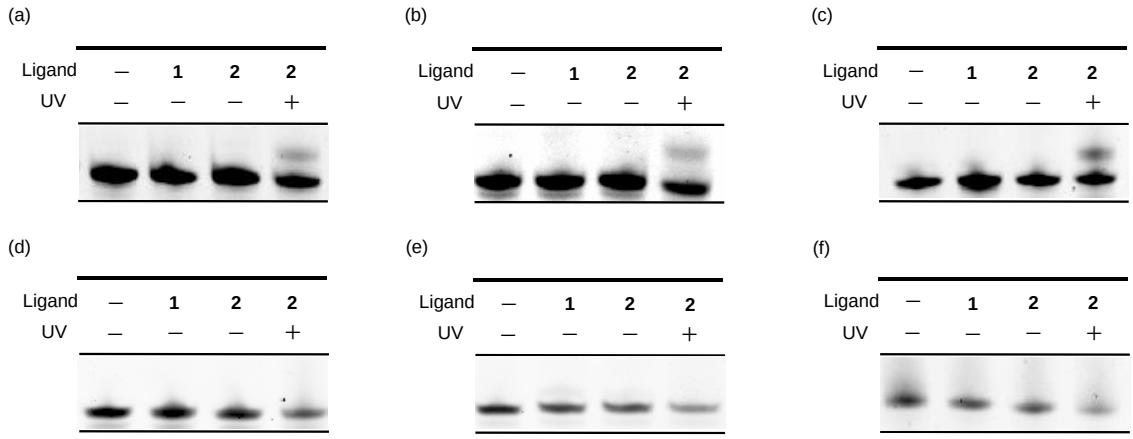


Fig. S5 Denaturing PAGE analysis for oligonucleotides (10 μM), in the presence of 6OTD (**1**) (25 μM), in the presence of 6OTD-Bp (**2**) (25 μM) and crosslinked with 6OTD-Bp (**2**). Oligonucleotides (10 μM) were incubated with ligand (25 μM) and the mixture was irradiated with ultraviolet light (365 nm) for 150 seconds (5 seconds × 30 times), were analyzed by denaturing 20% polyacrylamide gel electrophoresis, run at 300 V × 20 min in 1 × TBE buffer at 60 ºC. The gels were stained with SYBR-gold. (a) *bcl2* (b) PARP1 (c) VEGFR (d) *c-myc* (e) *c-kit* (f) RET

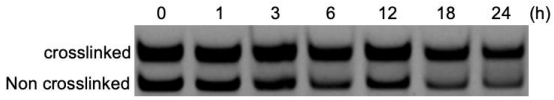


Fig. S6 Denaturing PAGE analysis for exonuclease I-treated PARP1 (2.0 μM) crosslinked with 6OTD-Bp (**2**) (20 μM).

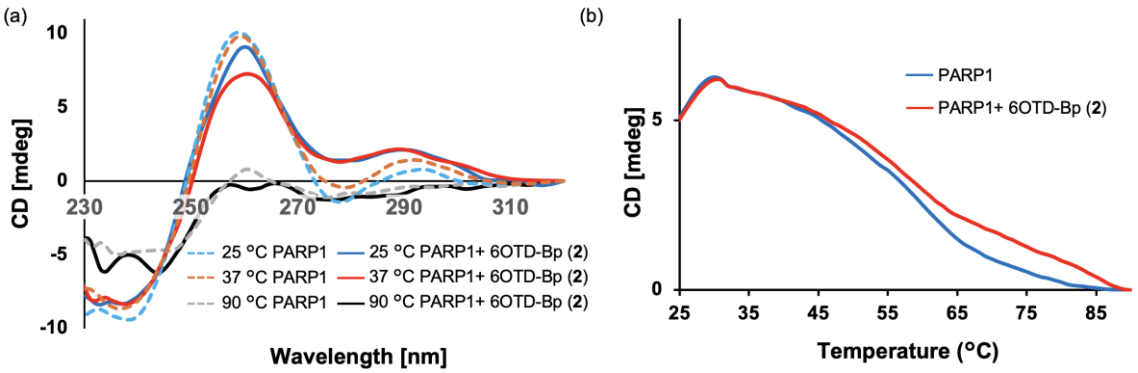


Fig. S7 CD analysis of the crosslinked adduct between 6OTD-Bp (**2**) and PARP1 G4. PARP1 G4 (5.0 μM) and 6OTD-Bp (**2**) (12.5 μM) was dissolved in 10 mM Tris-HCl (pH 8.0). After photo-irradiation (365 nm) for 150 seconds (5 seconds × 30 times), the samples were subjected to CD analysis. (a) CD spectra of PARP1 (the dotted line) and crosslinked adduct (the solid line) at 25°C (light blue and blue), 37°C (orange and red), and 90°C (gray and black). (b) Melting analysis of the crosslinked adduct at 265 nm; the temperature was changed as follows: 25 to 90 °C.

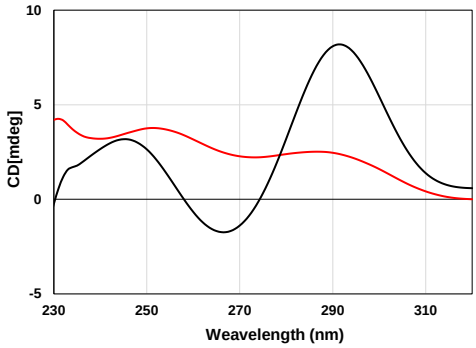


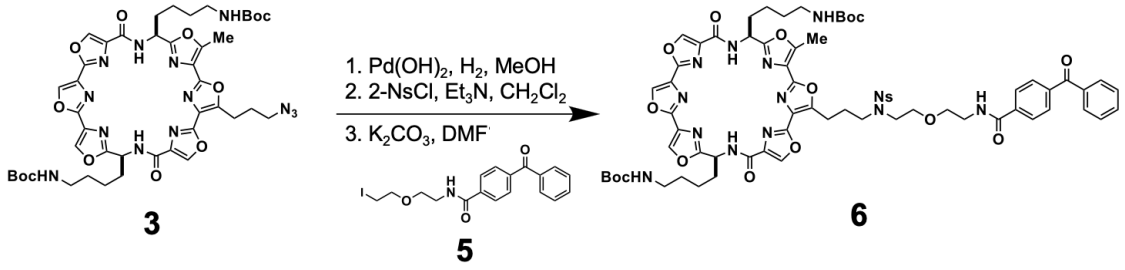
Fig. S8 CD analysis of the TBA (2.0 μM) (black) and in the presence of 6OTD-Bp (**2**) (20 μM) (red).

Synthesis and characterization

Instrumentation

Flash chromatography was performed on Silica gel 60 (spherical, particle size 40 ~ 100 μM; Kanto). ^{1}H and ^{13}C NMR spectra was recorded on JEOL JNM-ECX 500 spectrometer. 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), DMSO-d_6 (^{1}H NMR; δ = 2.50 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 content (Hz). Data for ^{13}C NMR are reported in terms of chemical shift (δ, ppm). Mass spectra were recorded on a JEOL JMS-T100X spectrometer with ESI-MS mode using methanol as solvent.

Synthesis of **6**

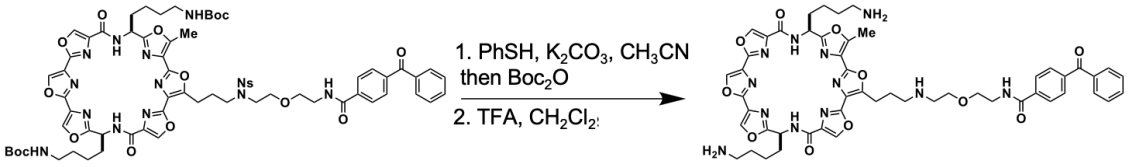


To a solution of **3** [2] (50.0 mg, 52.3 μmol) in MeOH (8.0 mL) was added Pd $(OH)_2$ (50.0 mg) at room temperature and mixture was stirred under hydrogen atmosphere. The reaction mixture was filtered through a pad of Celite® and eluted with $CHCl_3$-MeOH (9:1). The filtrates were concentrated *in vacuo*, which was used without further purification. To a solution of the crude macrocyclic amine in CH_2Cl_2 (4mL) was added triethylamine (43.8 μL, 312 μmol) and 2-NsCl (41.6 mg, 188 μmol) at room temperature and mixture was stirred under argon atmosphere for 4 h. The reaction mixture was added H_2O, and organic layer was extracted with $CHCl_3$. The extracts were washed with 1.2 N HCl, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give 2-Nitrobenzenesulfonyl protected (Ns) amine, which was used without further purification. To a solution of the crude protected amine in dry DMF (2 mL) was added K_2CO_3 (20.1 mg, 146 μmol) and a solution of **5** (36.9 mg, 87.2 μmol) in DMF (2 mL). After stirring at room temperature for 12 h, the reaction mixture was added H_2O, and organic layer was extracted with $CHCl_3$. The extracts were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to give Ns protected 6OTD (**6**) (35.3 mg, 25.0 μmol, 48% in 3 steps).

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.11 (s, 1H), 9.08 (s, 1H), 8.90 (s, 1H), 8.85 (s, 1H), 8.70 (t, J = 5.4 Hz, 1H), 8.31-8.27 (m, 2H), 7.98 (d, J = 7.4 Hz, 2H), 7.81-7.66 (m, 5H), 7.57-7.54 (m, 2H), 6.76 (t, J = 5.2 Hz, 2H), 5.42 (dt, J = 6.9, 5.7 Hz, 1H), 5.32 (dt, J = 5.2, 3.7 Hz, 1H), 3.56-

3.51 (m, 4H), 3.45-3.42 (m, 2H), 3.11-3.03 (m, 2H), 2.83 (m, 4H), 2.70 (s, 3H), 2.04-1.89 (m, 8H), 1.40-1.27 (m, 37H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 195.3, 165.5, 164.5, 162.0, 158.8, 158.7, 155.6, 155.5, 155.4, 154.9, 154.5, 153.2, 151.4, 147.6, 139.1, 137.6, 136.6, 136.1, 135.9, 134.5, 133.0, 129.7, 128.7, 128.4, 127.3, 124.2, 79.1, 77.2, 68.6, 47.3, 46.9, 33.3, 29.1, 28.1, 21.0, 20.9, 11.4; HRMS(ESI, M+H$^+$) calcd. for $C_{67}H_{80}N_{12}NaO_{17}$ 1347.5702, found 1347.5662.

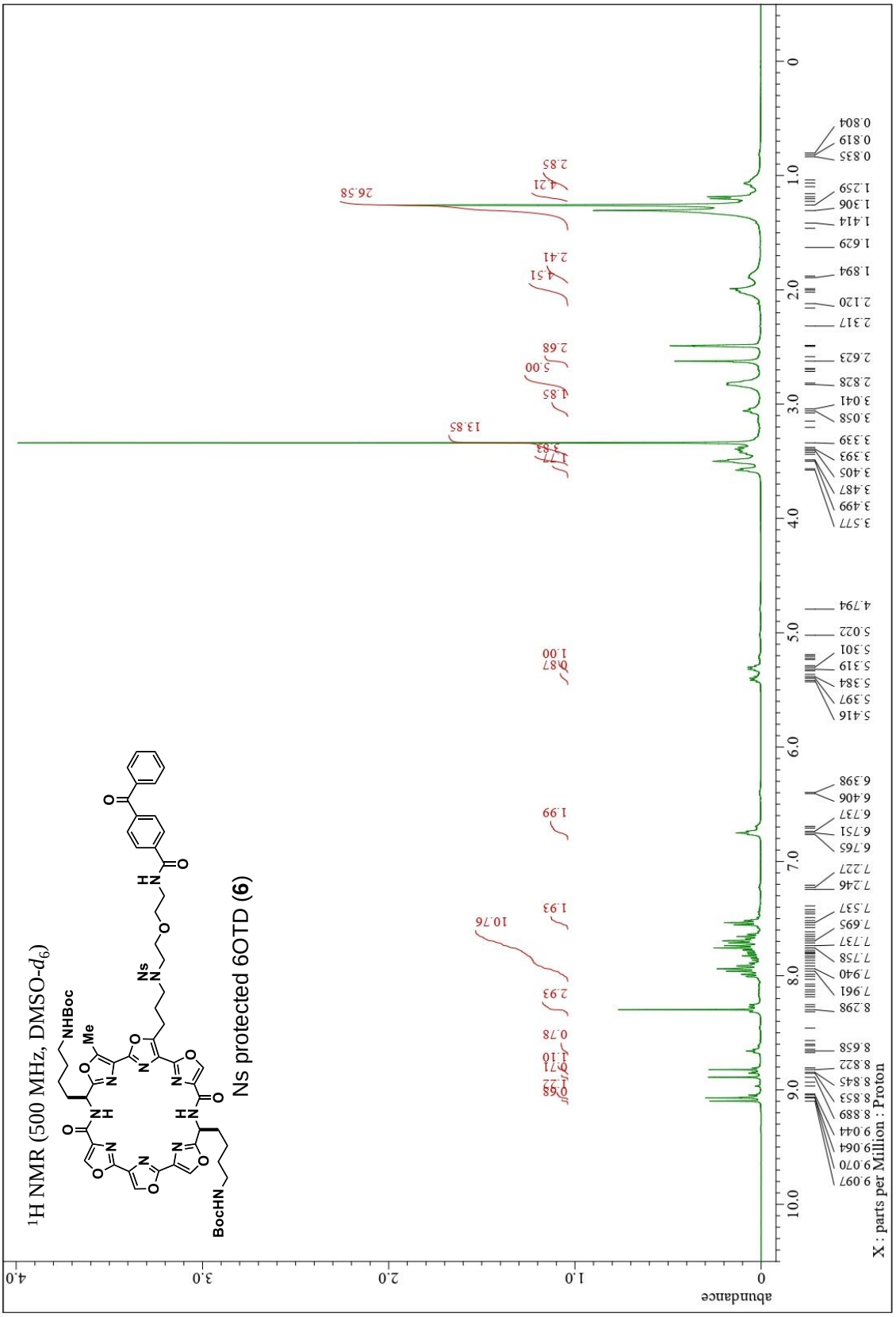
Synthesis of **2**



6 **2**

To a solution of Ns protected Benzophenone 6OTD (**6**) (35.3 mg, 25.0 μmol) in CH_3CN (4 mL) was added K_2CO_3 (17.3 mg, 125 μmol) and Thiophenol (14.7 μL, 125 μmol), and mixture was stirred at 65 ˚C under argon atmosphere for 2 h. To the reaction mixture was added Boc_2O (54.6 mg, 250 μmol), and mixture was stirred at 65 °C under argon atmosphere for 3 h. To the reaction mixture was added H_2O, and organic layer was extracted with $CHCl_3$. The extracts were dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give Boc protected amine, which was used without further purification. To a solution of the crude Boc protected 6OTD in CH_2Cl_2 (2 mL), was added TFA (3 mL) and stirred at room temperature for 1 h. The reaction mixture was concentrated *in vacuo* to give 6OTD-Bp (**2**) (10.1 mg, 9.83 μmol, 57% in 2 steps).

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.14 (d, J = 7.2 Hz, 2H), 8.93 (s, 1H), 8.89 (s, 1H), 8.78-8.74 (m, 3H), 8.32-8.27 (m, 3H), 8.00-7.94 (m, 2H), 7.78-7.67 (m, 9H), 7.57-7.54 (m, 2H), 5.44 (dt, J = 5.2, 3.4 Hz, 1H), 5.35 (dt, J = 5.7, 3.4 Hz, 1H), 3.70-3.49 (m, 4H), 3.24-3.09 (m, 6H), 2.75 (m, 4H), 2.69 (s, 3H), 2.13-2.04 (m, 4H), 1.92-1.87 (m, 2H), 1.53-1.42 (m, 6H), 1.23-1.20 (m, 4H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 196.0, 166.3, 165.0, 162.7, 159.5, 156.3, 156.0, 155.3, 155.1, 153.4, 152.3, 143.2, 142.5, 139.8, 138.3, 137.2, 136.7, 136.6, 133.7, 130.3, 130.1, 129.3, 129.1,128.0, 125.5, 124.4, 70.43, 69.5, 66.0, 48.0, 47.8, 47.0, 46.8, 40.3, 40.2, 34.0, 27.3, 24.3, 23.2, 21.6, 12.2; HRMS(ESI, M+H$^+$) calcd. for $C_{52}H_{57}N_{12}O_{11}$ 1025.42834, found 1025.42697.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Ns protected 6OTD (6)
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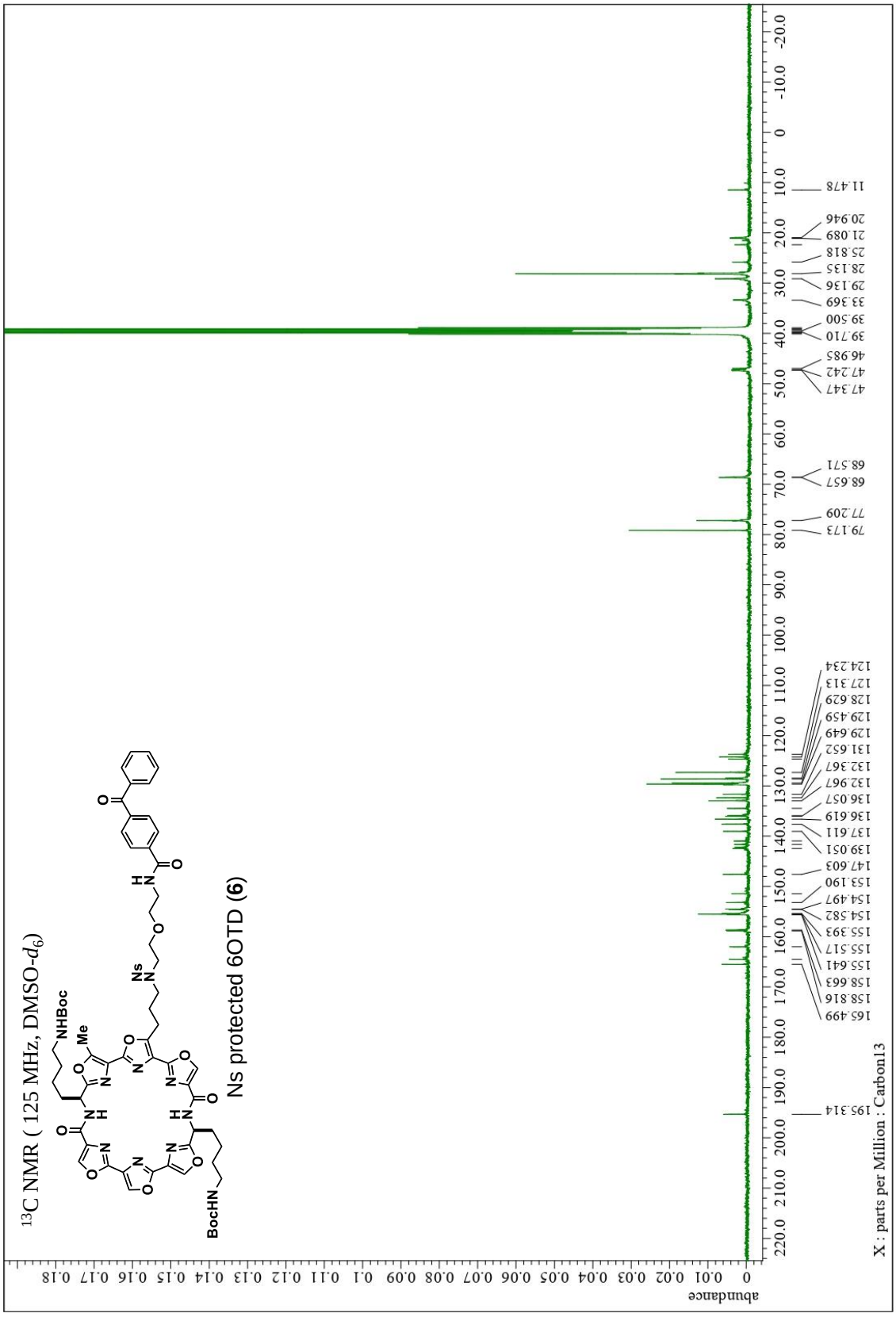
Me
Ns
BocHN
abundance
X : parts per Million : Proton


^{13}C NMR (125 MHz, DMSO-d_6)
Ns protected 6OTD (6)
NHBoc
Me
Ns
BocHN
abundance
X : parts per Million : Carbon13
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20.946
21.089
25.818
28.135
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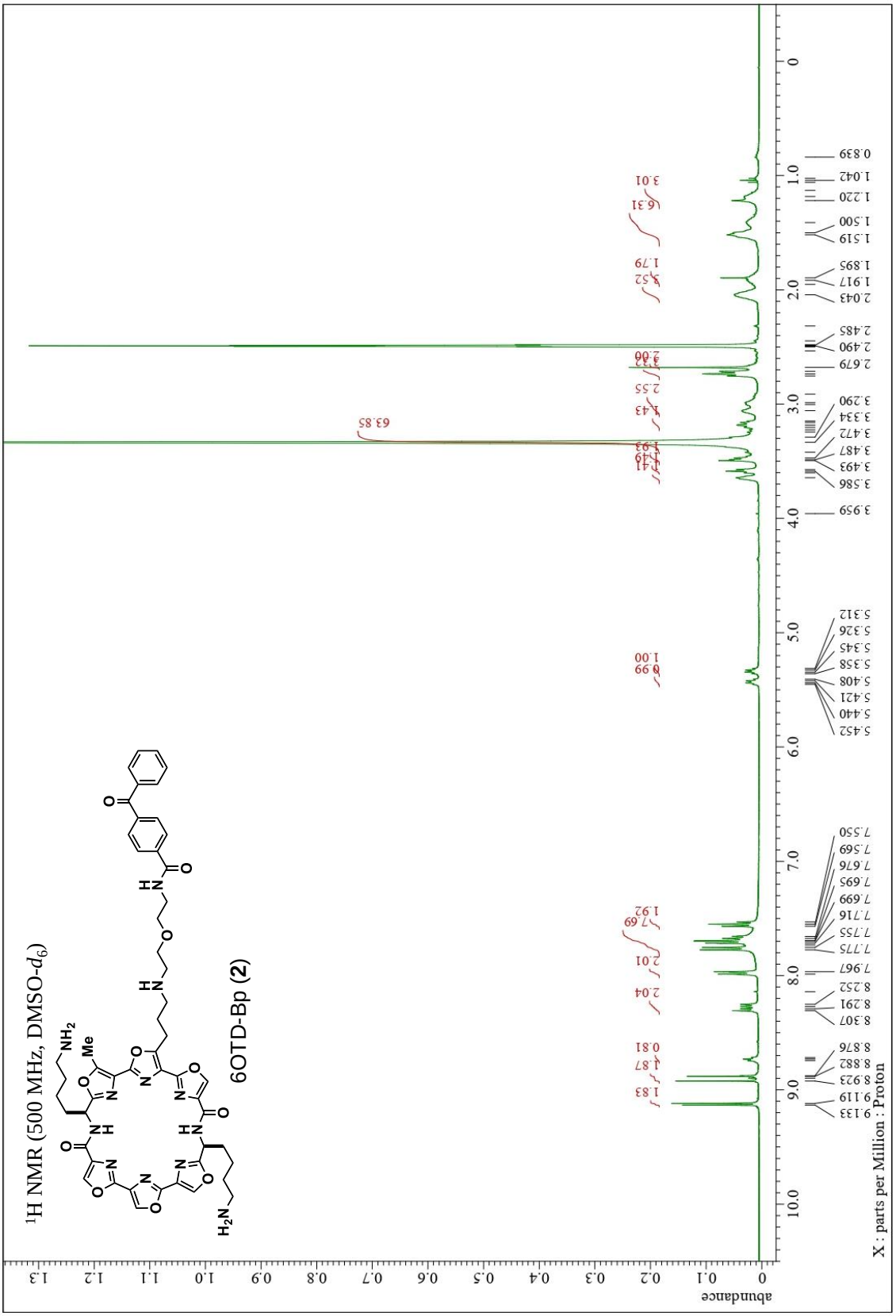
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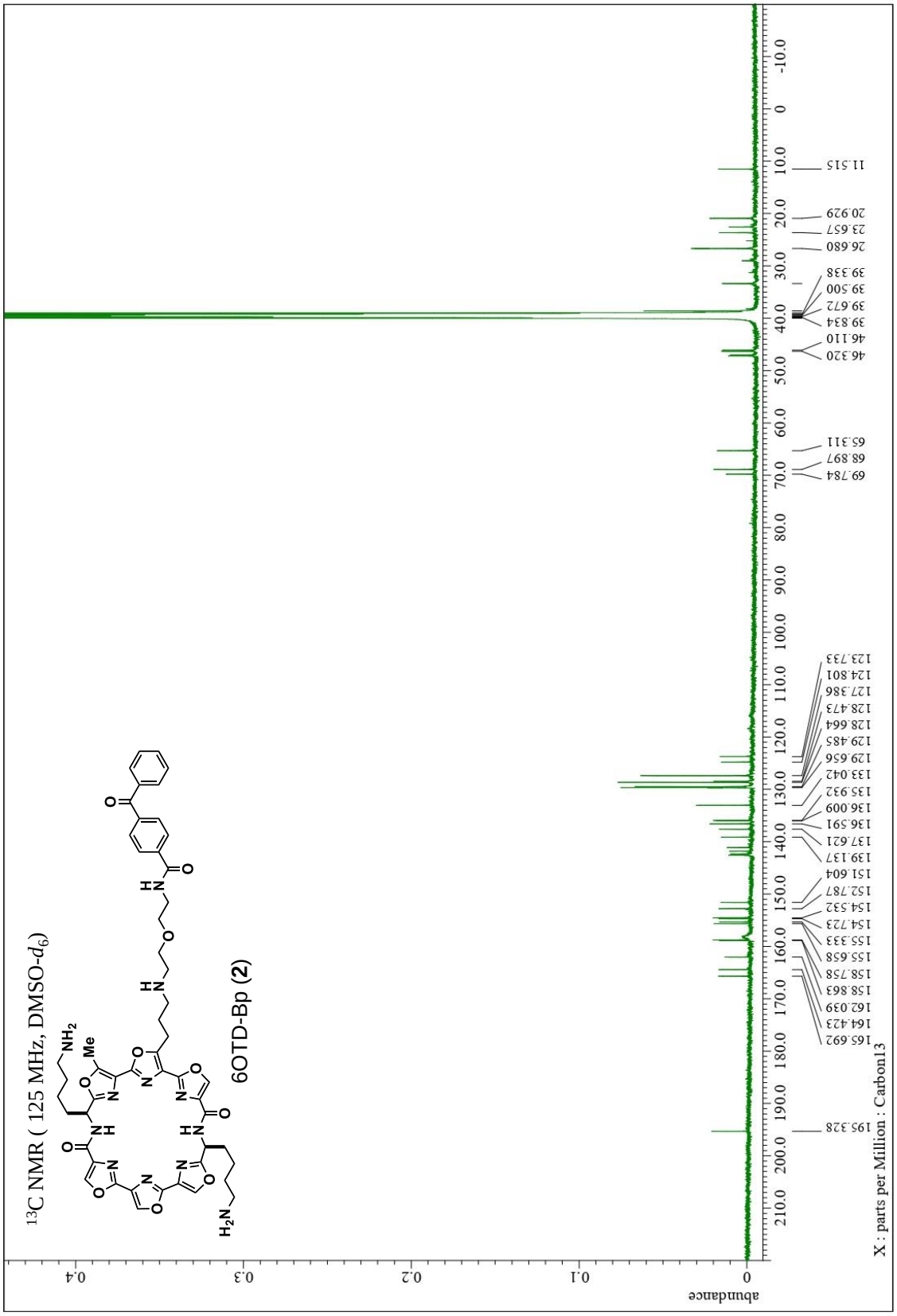
6OTD-Bp (2)
X : parts per Million : Proton
abundance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6OTD-Bp (2)
abundance
X : parts per Million : Carbon13
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20.929
23.657
26.680
39.338
39.500
39.672
39.834
46.110
46.320
65.311
68.897
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123.733
124.801
127.386
128.473
128.664
129.485
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154.532
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155.333
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158.758
158.863
162.039
164.423
165.692
195.328

Supplementary Reference

［1］ Majima. S. Tera. M, Iida. K, Shin-ya. K, Nagasawa. K, *Heterocycles.,* 2011, **82**, 1345−1357.

［2］ J. A. Punnoose, Y. Ma, M. E. Hoque, Y. Cui, S. Sasaki, A. H. Guo, K. Nagasawa, H. Mao, *Biochemistry.*, 2018, **57**, 6946–6955.