

Supporting Information

Two-dimensional barriers for probing conformational shifts in macrocycles

Shinya Kobori, Sungjoon Huh, Solomon D. Appavoo and Andrei K. Yudin*

Davenport Research Laboratories, Department of Chemistry, University of Toronto 80
St. George St., Toronto, ON, M5S 3H6, Canada

*Email: andrei.yudin@utoronto.ca

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1. General Information

General Information: Unless otherwise stated, acetonitrile (MeCN) was HPLC grade and all other solvents were of reagent grade quality. All reagents were purchased from commercial sources and used as received.

Chromatography: Flash column chromatography was carried out using Silicycle 230-400 mesh silica gel or Biotage SNAP® cartridge (KP-C18-HS) 30 g column on an ISCO Teledyne Combiflash Rf 200 Flash system or Biotage Isolera One. Thin-layer chromatography (TLC) was performed on TLC Silica gel 60 F₂₅₄ 25 Aluminum sheets (Merck) and visualized using a UV lamp (254 nm) or KMnO₄ stain.

Mass spectroscopy: High-resolution mass spectra were obtained on a VG 70-250S (double focusing) mass spectrometer at 70 eV or on an ABI/Sciex Qstar mass spectrometer with ESI source, MS/MS, and accurate mass capabilities or on a JEOL AccuTOF-DART instrument.

RP-HPLC/MS: Low-resolution mass spectra (ESI) were collected on an Agilent Technologies 1200 series HPLC paired to a 6130 Mass Spectrometer. Compounds were resolved on Phenomenex's Kinetex 2.6 μ C18 50 \times 4.6 mm column at room temperature with a flow of 1.0 mL/min. The gradient consisted of eluents A (0.1% formic acid in double distilled water) and B (0.1% formic acid in HPLC-grade acetonitrile). The run consisted of a linear gradient from 5% B to 95% B over 15 min at a flow rate of 1.0 mL/min.

Nuclear Magnetic Resonance Spectroscopy: NMR spectroscopic experiments were recorded at 298 K on Varian Mercury 300 MHz, 400 MHz, 500 MHz or 600 MHz spectrometers and an Agilent O500 500 MHz spectrometer. ¹H, ¹³C, gCOSY, zTOCSY, ROESY, ¹H,¹³C-gHSQC, and ¹H,¹³C-HMBC NMR experiments were recorded and used to confirm the cyclic topology of each substrate. ¹H NMR spectra were referenced to DMSO-*d*₆ (δ 2.50 ppm). ¹³C NMR spectra were referenced to DMSO-*d*₆ (δ 39.52 ppm). Mixing times for zTOCSY spectra were 80 ms, for ROESY spectra 150 ms. gHSQC spectra were recorded with a direct proton carbon coupling constant of 140 Hz, and gHMBC spectra with a long-range ¹H- ¹³C coupling constant of 7 Hz. 2D Spectra were apodized using exponential and square sine window functions.

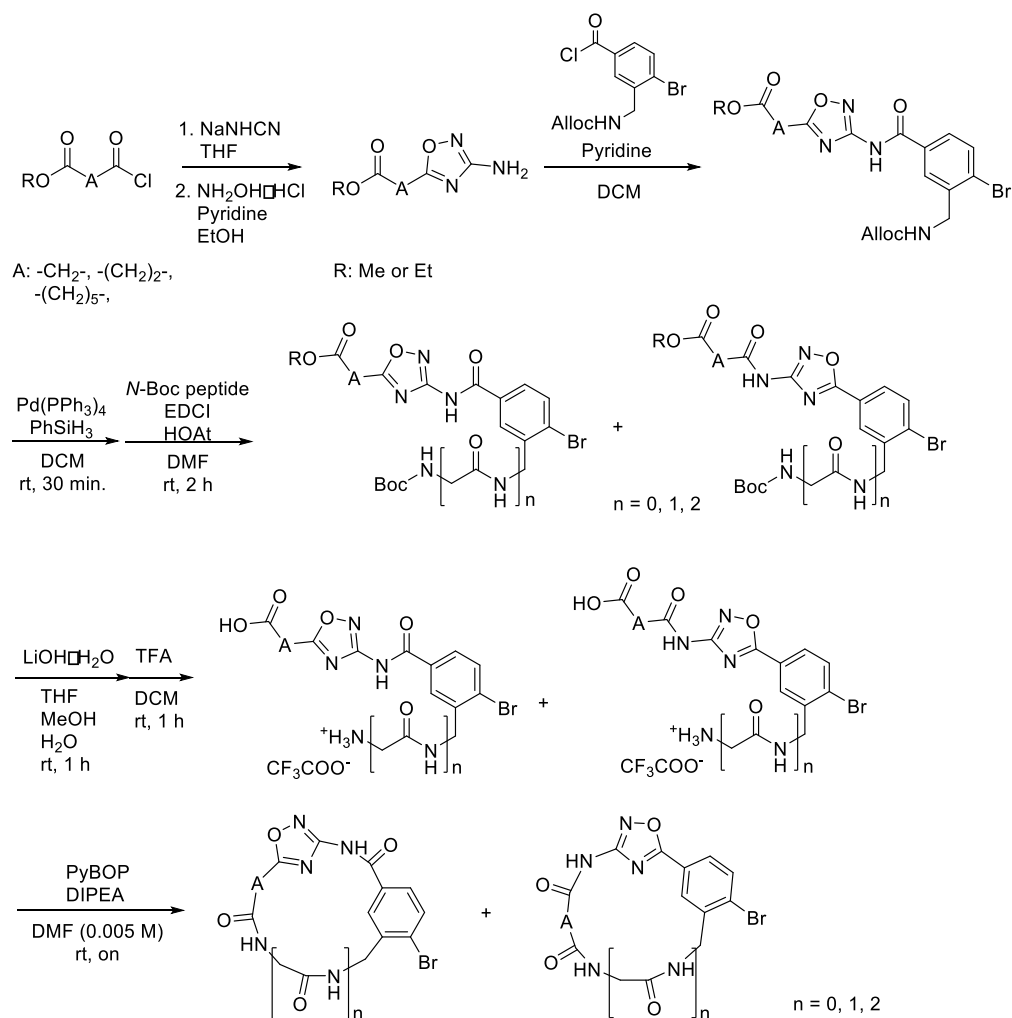
Variable temperature NMR: Variable temperature NMR was carried out to assess the temperature dependency of NH chemical shifts. ¹H-NMR spectra were acquired from

298 K to 323 K in 5 K increments in DMSO-*d*₆. Temperature shift coefficients below 4 ppb/K were selected to represent a strong intramolecular hydrogen bonding interaction and temperature shift coefficients above 4 ppb/K were attributed to solvent exposed protons¹.

Solution Structure Determination: The NMR structures were determined by a combination of NMR-derived distance information and molecular dynamics studies. ROESY spectra were integrated by using Mestrelab Research (v.12.0.0, Mestrelab Research S.L.) software. Integrated volumes of ROE cross-peaks were converted to proton interatomic distances using an inverse sixth power relationship. A reference integral was calculated as the average integral between sets of geminal protons which was then set to the calculated geminal interproton distance of 1.78 Å. The calculated distances were adjusted upwards and downwards by 10% to give upper and lower bounds to account for uncertainty in interproton distances. ³J coupling constants were recorded from the ¹H spectrum. NH-CαH ³J coupling constants of < 6 Hz were assigned phi dihedral values of -60° +/- 25°. NHCαH ³J coupling constants of > 8 Hz were assigned phi values of -120° +/- 25°. Crude structures of macrocycles were generated by a restrained Monte Carlo low mode molecular mechanics conformational search with an implicit solvent model (DMSO) in Macromodel (Schrodinger LLC, v11.0).

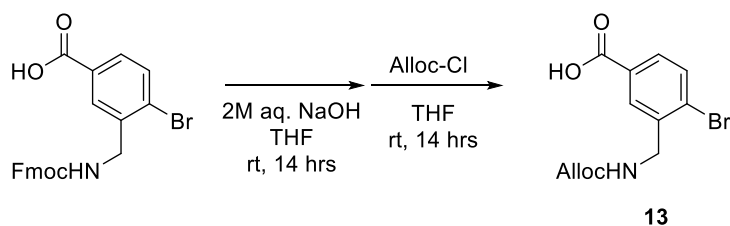
2. Experimental Details and Spectral Data for Products

General procedure for 3-acylamino-1,2,4-oxadiazole-containing macrocycles



Preparation of carboxylic acids

Carboxylic acids which were not available from commercial sources were prepared by the following methods.

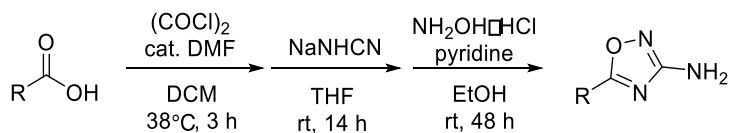


3-((((allyloxy)carbonyl)amino)methyl)-4-bromobenzoic acid (13)

3-((((9H-fluoren-9-yl) methoxycarbonyl)amino)methyl)-4-bromobenzoic acid (50 g, 110.5 mmol, 1.0 eq) prepared by the procedure described in WO2018/85921, was dissolved in THF (200 mL) and 2 M aqueous NaOH solution (200 mL) and stirred at room temperature for 14 h. The reaction solution was transferred to the separatory funnel by using EtOAc and the aqueous layer was collected into the round bottom flask. After adding THF (200 mL) into the flask, allyl chloroformate (1.2 eq) was slowly added into reaction solution and the reaction mixture was stirred at room temperature for 14 h. The resulting mixture was diluted with EtOAc and acidified with 2 M aqueous HCl solution at 0°C. The organic layer was washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure. The resulting white solids were washed with *n*-hexane and dried under reduced pressure. Finally, the title compound **1** was obtained in 92% yield (32.0 g, 101.9 mmol).

¹H NMR (400 MHz, DMSO-*d*₆) δ 13.13 (s, 1H), 7.91 (t, *J* = 6.1 Hz, 1H), 7.85 (s, 1H), 7.70 (d, *J* = 1.2 Hz, 2H), 5.90 (ddt, *J* = 17.3, 10.5, 5.3 Hz, 1H), 5.28 (dq, *J* = 17.3, 1.7 Hz, 1H), 5.15 (dq, *J* = 10.5, 1.6 Hz, 1H), 4.49 (dt, *J* = 5.4, 1.6 Hz, 2H), 4.24 (d, *J* = 6.1 Hz, 2H); ¹³C NMR (126 MHz, DMSO-*d*₆) δ 167.1, 156.6, 139.1, 134.0, 133.3, 130.7, 129.9, 129.4, 127.6, 117.5, 65.0, 44.5; HRMS (DART-MS) [M+H⁺] calculated for C₁₂H₁₃BrNO₄ = 314.0023, found = 314.0017.

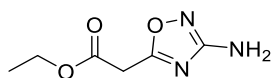
General procedure for 3-amino-1,2,4-oxadiazole intermediates



The corresponding acid (1.0 eq) was suspended in DCM (0.3 M) and then catalytic amount of DMF was added into the reaction solution. Oxalyl dichloride (1.5 eq) was slowly added into reaction solution at room temperature and the reaction mixture was stirred at 38°C in an oil bath for 3 h. After reaction flask was removed from an oil bath and cooled to room temperature, the resulting mixture was concentrated under reduced

pressure. The crude material was dried *in vacuo* and directly used to next reaction. The crude acid chloride was slowly added into the THF (0.3 M) solution of sodium hydrogen cyanamide (2.0 eq) at 0 °C and stirred at room temperature for 14 h. The resulting mixture was acidified to pH 2 with 2M aqueous HCl solution and extracted with ethyl acetate. The organic layer was washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure. The resulting crude materials were used to next reaction without further purification.

The crude *N*-cyanoamide derivative was dissolved in EtOH (0.5 M) and hydroxylamine hydrogen chloride (1.5 eq) and pyridine (4.0 eq) were added into reaction solution. After stirring at room temperature for 16 h, same amount of hydroxylamine hydrogen chloride and pyridine were added into the reaction mixture. After additionally stirring at room temperature for 20 h, the resulting mixture was concentrated *in vacuo*. The resulting crude materials were dissolved in ethyl acetate and resulting solution was adjusted to pH 10 with 2M aqueous NaOH solution. The organic layer was washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure. The crude products were purified by flash chromatography (SiO₂, *n*-hexane/EtOAc) to afford the corresponding 3-amino-1,2,4-oxadiazole intermediate.



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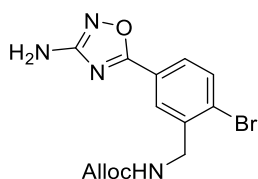
Ethyl 2-(3-amino-1,2,4-oxadiazol-5-yl)acetate (14)

The title compound was synthesized according to general procedure with malonyl chloride (4.7 g, 31.2 mmol, 1.0 eq) as a starting material and finally purified by flash chromatography (SiO₂, *n*-hexane/EtOAc 80: 20 to 60: 40).

[0.46 g, 2.69 mmol, 9 % over 3 steps]

¹H NMR and ¹³C NMR data were matched with previous reported values²

¹H NMR (500 MHz, DMSO-*d*₆) δ 6.26 (s, 2H), 4.12 (q, *J* = 7.1 Hz, 2H), 3.98 (s, 2H), 1.20 – 1.16 (m, 4H); ¹³C NMR (126 MHz, DMSO-*d*₆) δ 171.8, 169.0, 167.0, 61.8, 33.1, 14.4.



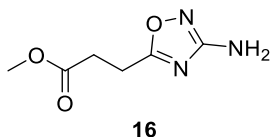
15

Allyl (5-(3-amino-1,2,4-oxadiazol-5-yl)-2-bromobenzyl)carbamate (15)

The title compound was synthesized according to general procedure with carboxylic acid (13) (5.0 g, 15.9 mmol, 1.0 eq) and finally purified by flash chromatography (SiO₂, *n*-hexane/EtOAc 80: 20 to 40: 60).

[2.78 g, 7.87 mmol, 49 % over 3 steps]

¹H NMR (500 MHz, DMSO-*d*₆) δ 7.98 (t, *J* = 6.1 Hz, 1H), 7.90 (d, *J* = 2.1 Hz, 1H), 7.82 (d, *J* = 8.2 Hz, 1H), 7.77 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.44 (s, 2H), 5.92 (ddt, *J* = 17.3, 10.6, 5.3 Hz, 1H), 5.38 – 5.14 (m, 2H), 4.59 – 4.43 (m, 3H), 4.29 (d, *J* = 6.1 Hz, 2H); ¹³C NMR (126 MHz, DMSO-*d*₆) δ 172.7, 169.5, 156.6, 140.0, 134.1, 134.0, 127.8, 127.2, 127.2, 123.9, 117.6, 65.1, 44.5; HRMS (DART-MS) [M+H⁺] calculated for C₁₃H₁₄BrN₄O₃ = 353.0249, found = 353.0256.

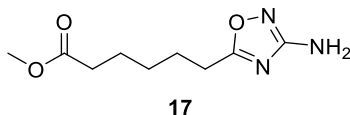


Methyl 3-(3-amino-1,2,4-oxadiazol-5-yl)propanoate (16)

The title compound was synthesized according to general procedure with 4-methoxy-4-oxobutanoic acid (18.5 g, 140 mmol, 1.0 eq) and the crude product was finally purified by washing with *n*-hexane and dried under reduced pressure.

[15.0 g, 87.6 mmol, 63 % over 3 steps]

¹H NMR (500 MHz, DMSO-*d*₆) δ 6.16 (s, 2H), 3.59 (s, 3H), 2.98 – 2.88 (m, 2H), 2.76 (dd, *J* = 7.5, 6.3 Hz, 2H); ¹³C NMR (126 MHz, DMSO-*d*₆) δ 177.3, 172.3, 168.7, 52.1, 30.0, 21.9; HRMS (DART-MS) [M+H⁺] calculated for C₆H₁₀N₃O₃ = 172.0717, found = 172.0715.



Methyl 6-(3-amino-1,2,4-oxadiazol-5-yl)hexanoate (17)

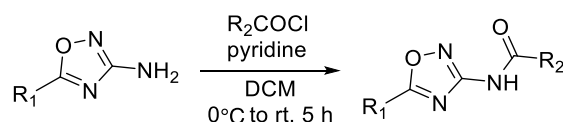
The title compound was synthesized according to general procedure with 7-methoxy-7-oxoheptanoic acid (5.45 g, 31.3 mmol, 1.0 eq, prepared by the reported procedure in *Chem. Eur. J.* **2017**, 23, 3107 – 3116) and finally purified by flash chromatography (SiO₂, *n*-hexane/EtOAc 75: 25 to 50: 50).

[2.66 g, 12.5 mmol, 40% over 3 steps]

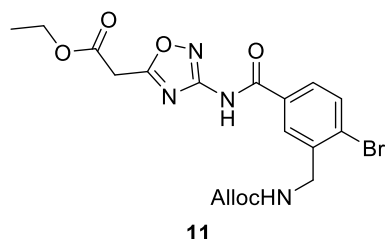
¹H NMR (400 MHz, DMSO-*d*₆) δ 6.10 (s, 2H), 3.54 (s, 3H), 2.66 (t, *J* = 7.4 Hz, 2H),

2.26 (t, $J = 7.4$ Hz, 2H), 1.66 – 1.58 (m, 2H), 1.54 – 1.48 (m, 2H), 1.31 – 1.23 (m, 2H); ^{13}C NMR (126 MHz, DMSO- d_6) δ 178.2, 173.7, 168.8, 51.6, 33.5, 28.2, 26.0, 26.00, 24.4; HRMS (DART-MS) $[\text{M}+\text{H}^+]$ calculated for $\text{C}_9\text{H}_{16}\text{N}_3\text{O}_3 = 214.1186$, found = 214.1193.

General procedure for 3-acylamino-1,2,4-oxadiazole intermediates



The corresponding 3-amino-1,2,4-oxadiazole (1.0 eq) was dissolved in DCM (0.3 M) and pyridine (2.0 eq) was added. Acid chloride (prepared by the corresponding acids with oxalyl chloride or thionyl chloride (2.0 eq) in DCM and catalytic amount of DMF at 38°C for 3 h) was slowly added into reaction solution at 0°C and the reaction mixture was stirred at room temperature for 5 h. The resulting mixture was quenched with aqueous NaHCO_3 solution and extracted with DCM. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude products were purified by flash chromatography (SiO_2 , n -hexane/ethyl acetate) to afford the corresponding 3-acylamino-1,2,4-oxadiazole derivative.



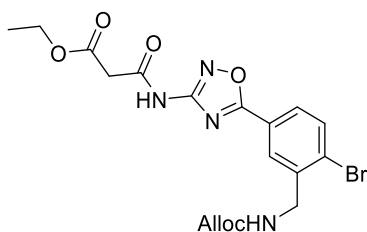
Ethyl 2-(3-(3-((((allyloxy)carbonyl)amino)methyl)-4-bromobenzamido)-1,2,4-oxadiazol-5-yl)acetate (11)

The title compound was synthesized according to general synthetic procedure with the compound (14) (0.38 g, 2.21 mmol, 1.0 eq) and finally purified by flash chromatography (SiO_2 , n -hexane/EtOAc 80: 20 to 50: 50).

[0.51 g, 1.09 mmol, 49 %]

^1H NMR (400 MHz, DMSO- d_6) δ 11.65 (s, 1H), 7.89 (d, $J = 2.2$ Hz, 1H), 7.87 – 7.78 (m, 2H), 7.75 (d, $J = 8.3$ Hz, 1H), 5.90 (ddt, $J = 16.0, 10.5, 5.3$ Hz, 1H), 5.36 – 5.09 (m, 2H), 4.50 (dt, $J = 5.5, 1.5$ Hz, 2H), 4.27 (d, $J = 6.0$ Hz, 2H), 4.24 (s, 2H), 4.13 (q, $J = 7.1$ Hz, 2H), 1.18 (t, $J = 7.1$ Hz, 3H); ^{13}C NMR (126 MHz, DMSO- d_6) δ 173.3, 166.7,

164.5, 163.8, 156.6, 139.1, 134.0, 133.1, 132.5, 128.8, 128.7, 127.3, 117.6, 65.1, 62.0, 44.6, 33.2, 14.4; HRMS (ESI+) $[M+H]^+$ calculated for $C_{18}H_{20}BrN_4O_6$ = 467.0561, found = 467.0560.



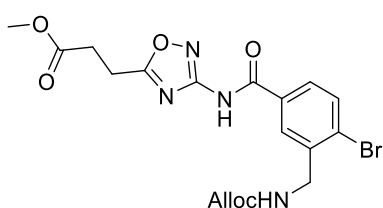
18

Ethyl 3-((5-(3-(((allyloxy)carbonyl)amino)methyl)-4-bromophenyl)-1,2,4-oxadiazol-3-yl)amino)-3-oxopropanoate (18)

The title compound was synthesized according to general synthetic procedure with (15) (2.0 g, 5.66 mmol, 1.0 eq) and finally purified by flash chromatography (SiO_2 , *n*-hexane/EtOAc 60: 40 to 40: 60).

[1.6 g, 3.42 mmol, 60 %]

1H NMR (500 MHz, $DMSO-d_6$) δ 11.52 (s, 1H), 8.04 – 7.94 (m, 2H), 7.86 (d, J = 2.5 Hz, 2H), 5.92 (ddt, J = 16.0, 10.5, 5.3 Hz, 1H), 5.41 – 5.11 (m, 2H), 4.60 – 4.43 (m, 2H), 4.31 (d, J = 6.1 Hz, 2H), 4.11 (q, J = 7.1 Hz, 2H), 3.57 (s, 2H), 1.18 (t, J = 7.1 Hz, 3H); ^{13}C NMR (126 MHz, $DMSO-d_6$) δ 173.7, 167.5 (2C), 163.5, 156.7, 140.3, 134.3, 134.0, 128.2, 128.0, 127.4, 123.1, 117.6, 65.2, 61.3, 44.5, 43.3, 14.5; HRMS (ESI+) $[M+H]^+$ calculated for $C_{18}H_{20}BrN_4O_6$ = 467.0561, found = 467.0560.



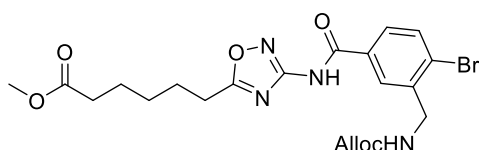
12

Methyl 3-(3-(3-(((allyloxy)carbonyl)amino)methyl)-4-bromobenzamido)-1,2,4-oxadiazol-5-yl)propanoate (12)

The title compound was synthesized according to general synthetic procedure with (16) (4.0 g, 23.4 mmol, 1.0 eq) and finally purified by flash chromatography (SiO_2 , *n*-hexane/EtOAc 67: 33 to 0: 100).

[6.0 g, 22.5 mmol, 55 %]

^1H NMR (500 MHz, DMSO- d_6) δ 11.59 (s, 1H), 7.90 (d, J = 2.2 Hz, 1H), 7.88 – 7.79 (m, 2H), 7.75 (d, J = 8.3 Hz, 1H), 5.92 (ddt, J = 17.3, 10.5, 5.3 Hz, 1H), 5.41 – 5.05 (m, 2H), 4.60 – 4.39 (m, 2H), 4.39 – 4.14 (m, 2H), 3.60 (s, 3H), 3.16 (dd, J = 7.4, 6.3 Hz, 2H), 2.87 (dd, J = 7.4, 6.3 Hz, 2H); ^{13}C NMR (126 MHz, DMSO- d_6) δ 178.7, 172.2, 164.5, 163.4, 156.6, 139.0, 134.0, 133.0, 132.6, 128.9, 128.7, 127.2, 117.6, 65.1, 52.1, 44.6, 29.9, 22.0; HRMS (ESI+) $[\text{M}+\text{H}^+]$ calculated for $\text{C}_{18}\text{H}_{20}\text{BrN}_4\text{O}_6$ = 467.0561, found = 467.0562.



19

Methyl 6-(3-(3-((((allyloxy)carbonyl)amino)methyl)-4-bromobenzamido)-1,2,4-oxadiazol-5-yl)hexanoate (19)

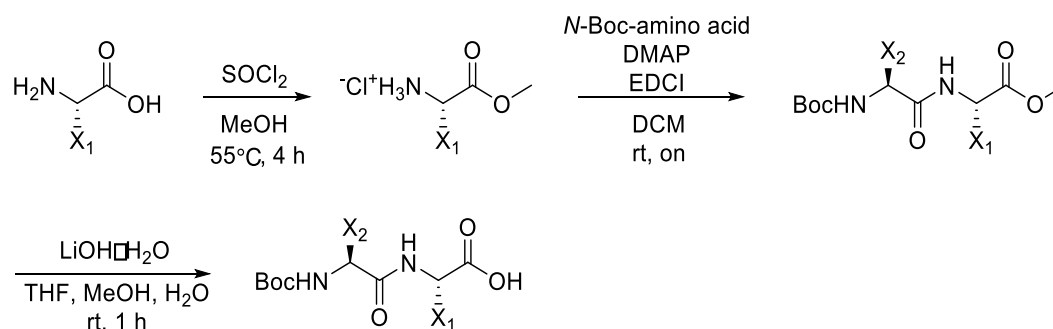
The title compound was synthesized according to general synthetic procedure with **17** (2.66 g, 12.5 mmol) and finally purified by flash chromatography (SiO_2 , *n*-hexane/EtOAc 50: 50 to 0: 100).

[2.73 g, 5.36 mmol, 43%]

^1H NMR (400 MHz, DMSO- d_6) δ 11.55 (s, 1H), 7.89 (s, 1H), 7.86 – 7.77 (m, 2H), 7.73 (d, J = 8.3 Hz, 1H), 5.90 (ddt, J = 15.8, 10.5, 5.4 Hz, 1H), 5.34 – 5.06 (m, 2H), 4.50 (d, J = 5.3 Hz, 2H), 4.27 (d, J = 5.9 Hz, 2H), 3.54 (s, 3H), 2.89 (t, J = 7.4 Hz, 2H), 2.28 (t, J = 7.4 Hz, 2H), 1.71 (p, J = 7.5 Hz, 2H), 1.53 (p, J = 7.4 Hz, 2H), 1.43 – 1.23 (m, 2H); ^{13}C NMR (126 MHz, DMSO- d_6) δ 179.6, 173.7, 164.5, 163.5, 156.6, 139.0, 134.0, 133.0, 132.6, 128.8, 128.7, 127.2, 117.6, 65.1, 51.6, 44.6, 33.5, 28.2, 26.1, 25.9, 24.4; HRMS (DART-MS) $[\text{M}+\text{H}^+]$ calculated for $\text{C}_{21}\text{H}_{26}\text{BrN}_4\text{O}_6$ = 509.1030, found = 509.1017.

Preparation of *N*-Boc protected dipeptide

N-Boc protected dipeptides were prepared by solution phase synthesis as shown below.



Thionyl chloride (2.0 eq) was slowly added into a methanol solution of a corresponding amino acid at room temperature and then the reaction mixture was heated at 55°C for 4 h. After removing from an oil bath and cooling to room temperature, the resulting mixture was evaporated under reduced pressure and dried *in vacuo*. The obtained crude material was suspended in dichloromethane. *N*-Boc amino acid (1.3 eq), *N,N'*-dimethylaminopyridine (DMAP) (4.0 eq), and *N*-Ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (1.5 eq) were added into reaction solution and reaction mixture was stirred at room temperature for 16 h. The resulting mixture was diluted with dichloromethane and washed with saturated aqueous NH_4Cl solution, saturated aqueous NaHCO_3 solution and brine. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The obtained *N*-Boc dipeptide methyl ester was dissolved in THF: methanol: water (4: 1: 1, 0.3 M) and lithium hydroxide monohydrate (2.0 eq) was added into reaction solution. The reaction mixture was stirred at room temperature for 1 h. The resulting mixture was diluted with chloroform and acidified to pH 2 with 2 M aqueous HCl solution. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The obtained *N*-Boc dipeptide was used to the condensation reaction without further purification.

Alloc deprotection and condensation with mono- or di-peptide

The corresponding 3-acylamino-1,2,4-oxadiazole intermediate (1.0 eq) was suspended in DCM (0.3 M) and then phenyl silane (3.0 eq) and tetrakis (triphenylphosphine) palladium (0) (0.05 eq) were added into reaction solution and the reaction mixture was stirred at room temperature for 30 min. The resulting mixture was concentrated under reduced pressure and diethyl ether was added to precipitate the solids. After removal of solvent, the obtained solids were dried under high vacuum and used in the next reaction. To a DMF (0.3 M) solution of the crude benzylamine derivative, *N*-Boc mono- or dipeptide (1.3 eq), diisopropylethylamine (DIPEA) (4.0 eq), EDCI (1.5 eq) and 1-Hydroxy-7-azabenzotriazole (HOAt) (1.5 eq) were added and the reaction mixture was stirred at room temperature for 2 h. The resulting mixture was added slowly into water

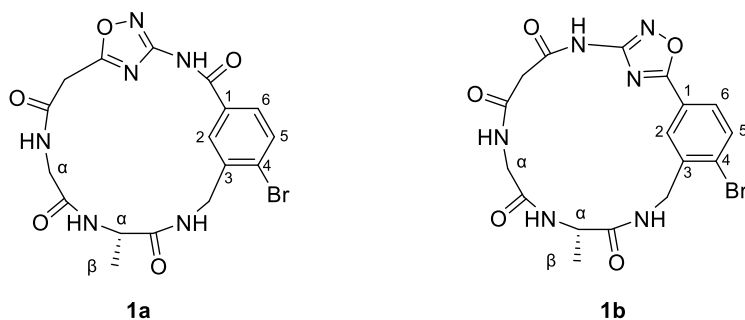
and then filtered. The filtrates were dissolved in ethyl acetate and washed with brine. The organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The obtained crude product was used to next step without further purification.

Hydrolysis and Boc deprotection

The obtained crude product which has mono- or dipeptide was dissolved in THF: MeOH: H₂O (4: 1: 1, 0.3 M) and lithium hydroxide monohydrate (2.0 eq) was added into reaction solution. After stirring at room temperature for 1 h, resulting solution was transferred to separatory funnel and diluted with diethyl ether and water. The aqueous layer was collected, acidified to around pH 2 with 2M aqueous HCl solution at 0°C and extracted with chloroform (if necessary, only a few amounts of MeOH was added). The organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The obtained crude material was dissolved in 20% trifluoroacetic acid in DCM (0.3 M) solution and stirred at room temperature for 1 h. The resulting mixture was concentrated under reduced pressure. Diethyl ether was added into the crude materials and the resulting mixture was sonicated to form the solids. After removal of the solvent, the obtained solids were washed with diethyl ether again and dried under reduced pressure. Finally, the trifluoroacetic acid salts of the linear precursor were obtained as mixture of non bi-aryl and bi-aryl compound.

Macrocyclization

To a DMF (0.01 M) solution of benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) (2.0 eq) and DIPEA (4.0 eq), trifluoroacetic acid salts of linear precursor (1.0 eq) in DMF (0.01 M) was slowly added at room temperature. The reaction mixture was stirred at room temperature for 18 h. After addition of Celite and toluene, the resulting mixture was concentrated under reduced pressure. The resulting crude materials were purified by reverse-phase chromatography to afford non bi-aryl and bi-aryl macrocycle, respectively.



The title compounds were synthesized according to general synthetic procedure using the

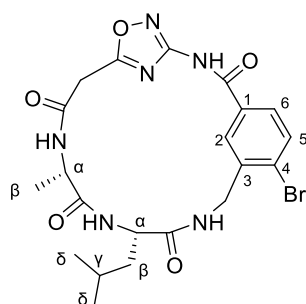
mixture of corresponding non bi-aryl and bi-aryl linear precursors (0.22 g, 0.36 mmol, 1.0 eq) and purified by reverse-phase chromatography with double distilled water and MeCN. The product containing fractions were collected and concentrated under reduced pressure. The obtained white solids were washed with diethyl ether and dried under reduced pressure.

Non bi-aryl macrocycle (1a) [30 mg, 0.064 mmol, 18%, white solid]

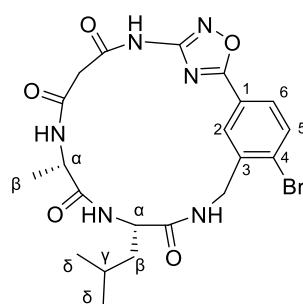
^1H NMR (500 MHz, DMSO- d_6) δ 11.15 (s, 1H, NH-ODZ), 8.77 (t, J = 5.0 Hz, 1H, NH-Gly), 8.22 (d, J = 7.3 Hz, 1H, NH-Ala), 7.71 (d, J = 8.2 Hz, 1H, H₅-Ph), 7.66 (dd, J = 6.8, 4.5 Hz, 1H, NH-Bn), 7.48 (dd, J = 8.2, 2.2 Hz, 1H, H₆-Ph), 7.13 (d, J = 2.2 Hz, 1H, H₂-Ph), 4.39 (dd, J = 15.2, 6.8 Hz, 1H, CHH-Ph), 4.19 (p, J = 7.2 Hz, 1H, CH α -Ala), 4.02 (dd, J = 15.2, 4.4 Hz, 1H, CHH-Ph), 3.98 – 3.84 (m, 2H, CH₂-ODZ), 3.70 (dd, J = 15.5, 4.8 Hz, 1H, CHH α -Gly), 3.51 (dd, J = 15.4, 5.3 Hz, 1H, CHH α -Gly), 1.26 (d, J = 7.2 Hz, 3H, CH₃ β -Ala); ^{13}C NMR (126 MHz, DMSO- d_6) δ 175.3 (O-C=N, ODZ), 172.2 (C=O, Ala), 168.9 (C=O, Gly or C=O, Ph), 168.9 (C=O, Gly or C=O, Ph), 166.6 (C=O, malonyl moiety), 164.6 (N-C=N in ODZ), 137.3 (C₃-Ph), 134.3 (C₁-Ph), 133.7 (C₅-Ph), 129.8 (C₆-Ph), 129.5 (C₂-Ph), 126.8 (C₄-Ph), 49.0 (C α -Ala), 44.3 (C α -Gly), 43.4 (CH₂-Ph), 34.1 (CH₂-ODZ), 17.8 (C β -Ala); HRMS (ESI-) [M-H]⁻ calculated for C₁₇H₁₆BrN₆O₅ = 463.0371, found = 463.0370.

Bi-aryl macrocycle (1b) [12 mg, 0.026 mmol, 7.2%, white solid]

^1H NMR (400 MHz, DMSO- d_6) δ 11.14 (s, 1H, NH-ODZ), 8.63 (t, J = 6.2 Hz, 1H, NH-Bn), 8.45 (d, J = 6.3 Hz, 1H, NH-Ala), 8.18 (s, 1H, NH-Gly), 7.97 (s, 1H, H₂-Ph), 7.85 (d, J = 8.1 Hz, 1H, H₅-Ph), 7.82 (dd, J = 8.3, 2.1 Hz, 1H, H₆-Ph), 4.45 – 4.28 (m, 2H, CH₂-Ph), 4.22 – 4.08 (m, 1H, CH α -Ala), 3.93 (d, J = 14.4 Hz, 1H, CHH in malonyl moiety), 3.87 (dd, J = 16.3, 5.0 Hz, 1H, CHH α -Gly), 3.77 (dd, J = 16.2, 5.2 Hz, 1H, CHH α -Gly), 3.39 (d, J = 14.1 Hz, 1H, CHH in malonyl moiety), 1.24 (d, J = 7.1 Hz, 3H, CH₃ β -Ala); ^{13}C NMR (126 MHz, DMSO- d_6) δ 175.0 (O-C=N, ODZ), 173.1 (C=O, Ala), 169.3 (C=O, Gly), 168.0 (C=O, malonyl-ODZ side), 165.9 (C=O, malonyl-Gly side), 164.7 (N-C=N, ODZ), 140.4 (C₃-Ph), 133.8 (C₅-Ph), 128.1 (C₂-Ph), 127.8 (C₄-Ph, C₆-Ph), 123.3 (C₁-Ph), 50.0 (C α -Ala), 44.1 (CH₂, C α -malonyl), 42.9 (C α -Gly), 42.4 (CH₂-Ph), 17.4 (C β -Ala); HRMS (ESI-) [M-H]⁻ calculated for C₁₇H₁₆BrN₆O₅ = 463.0371, found = 463.0368.



2a



2b

The title compounds were synthesized by modified procedure (HATU was used as a coupling reagent instead of PyBOP) using the mixture of corresponding non biaryl and biaryl linear precursors (0.05 g, 0.48 mmol, 1.0 eq) and purified by reverse-phase chromatography. The product containing fractions were collected respectively and concentrated under reduced pressure. Each product was washed with diethyl ether and dried under reduced pressure.

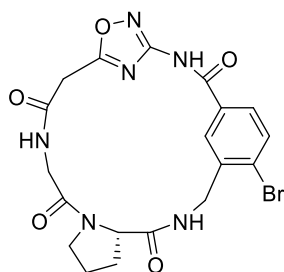
Non bi-aryl macrocycle (2a) [5 mg, 0.090 mmol, 19%]

^1H NMR (500 MHz, $\text{DMSO-}d_6$) *NH-ODZ peak was not observed due to broadening δ 8.78 (d, $J = 3.7$ Hz, 1H, NH-Ala), 7.75 – 7.69 (m, 2H, NH-Leu & H_5 -Ph), 7.50 (dd, $J = 8.2, 2.2$ Hz, 1H, H_6 -Ph), 7.17 (dd, $J = 7.7, 3.3$ Hz, 1H, NH-Bn), 7.06 (d, $J = 2.2$ Hz, 1H, H_2 -Ph), 4.58 (dd, $J = 14.8, 7.9$ Hz, 1H, CHH-Ph), 4.13 (q, $J = 8.5, 7.3$ Hz, 1H, $\text{CH}\alpha$ -Leu), 3.99 (d, $J = 16.1$ Hz, 1H, CHH in malonyl moiety), 3.89 (d, $J = 16.2$ Hz, 1H, CHH in malonyl moiety), 3.86 – 3.81 (m, 1H, $\text{CH}\alpha$ -Ala), 3.77 (dd, $J = 14.7, 3.3$ Hz, 1H, CHH-Ph), 1.64 – 1.54 (m, 3H, $\text{CH}\gamma$ -Leu & $\text{CH}_2\beta$ -Leu), 1.25 (d, $J = 7.3$ Hz, 3H, $\text{CH}_3\beta$ -Ala), 0.88 (d, $J = 6.1$ Hz, 3H, $\text{CH}_3\delta$ -Leu), 0.80 (d, $J = 6.2$ Hz, 3H, $\text{CH}_3\delta$ -Leu); ^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) δ 175.2, 172.6, 171.9, 169.0, 167.0, 164.7, 137.0, 134.1, 133.8, 130.1, 129.7, 127.13, 52.4, 52.0, 43.2, 34.2, 24.9, 23.4, 21.6, 17.5; HRMS (ESI+) $[\text{M}+\text{H}^+]$ calculated for $\text{C}_{21}\text{H}_{26}\text{BrN}_6\text{O}_5 = 521.1143$, found = 521.1141.

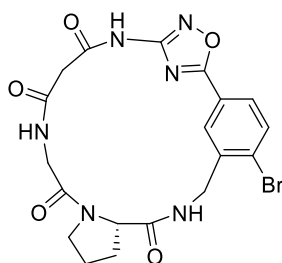
Bi-aryl macrocycle (2b) [3.6 mg, 0.083 mmol, 17 %]

^1H NMR (500 MHz, $\text{DMSO-}d_6$) *NH-ODZ peak was not observed due to broadening δ 8.94 (s, 1H, NH-Bn), 8.43 (d, $J = 6.0$ Hz, 1H, NH-Leu), 8.02 (s, 1H, NH-Ala), 7.91 (s, 1H, H_2 -Ph), 7.88 – 7.81 (m, 2H, H_6 -Ph & H_5 -Ph), 4.55 (dd, $J = 16.6, 6.4$ Hz, 1H, CHH-Ph), 4.43 (s, 1H, CHH in malonyl moiety), 4.33 – 4.19 (m, 3H, $\text{CH}\alpha$ -Ala & $\text{CH}\alpha$ -Leu & CHH-Ph), 3.35 (d, $J = 14.7$ Hz, 1H, CHH in malonyl moiety, partially overlapped with H_2O peak), 1.60 – 1.43 (m, 3H, $\text{CH}\gamma$ -Leu & $\text{CH}_2\beta$ -Leu), 1.24 (d, $J = 7.2$ Hz, 3H, $\text{CH}_3\beta$ -Ala), 0.90 (d, $J = 6.1$ Hz, 3H, $\text{CH}_3\delta$ -Leu), 0.86 (d, $J = 6.1$ Hz, 3H, $\text{CH}_3\delta$ -Leu); ^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) δ 174.3, 172.6, 172.4, 168.3, 166.0, 165.1, 140.4, 134.0, 127.9,

127.7 (2C), 123.3, 52.1, 49.7, 43.9, 42.3, 41.4, 24.8, 23.2, 22.6, 19.0; HRMS (ESI+) $[M+H]^+$ calculated for $C_{21}H_{26}BrN_6O_5 = 521.1143$, found = 521.1141.



3a



3b

(+conformer)

The title compounds were synthesized according to general synthetic procedure using the mixture of corresponding non bi-aryl and bi-aryl linear precursors (0.30 g, 0.48 mmol, 1.0 eq) and purified by reverse-phase chromatography. The product containing fractions were collected respectively and concentrated under reduced pressure. Each product was washed with mixed solvent of double distilled water/MeCN (1/1) and dried under reduced pressure.

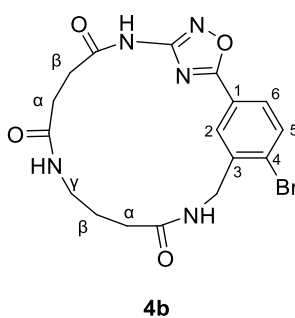
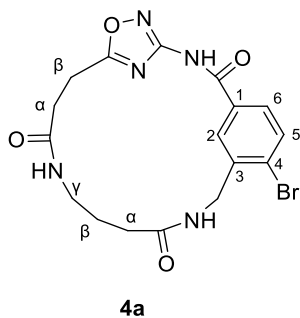
Non bi-aryl macrocycle (3a) [44 mg, 0.090 mmol, 19%]

1H NMR (500 MHz, DMSO- d_6) δ 11.30 (s, 1H, NH-ODZ), 8.49 (t, $J = 4.6$ Hz, 1H, NH-Gly), 8.08 (dd, $J = 7.7, 4.2$ Hz, 1H, NH-Bn), 7.70 (d, $J = 8.2$ Hz, 1H, H_5 -Ph), 7.45 (dd, $J = 8.1, 2.2$ Hz, 1H, H_6 -Ph), 7.38 (d, $J = 2.3$ Hz, 1H, H_2 -Ph), 4.55 (dd, $J = 16.2, 7.6$ Hz, 1H, CHH -Ph), 4.27 (dd, $J = 8.2, 4.8$ Hz, 1H, $CH\alpha$ -Pro), 4.01 – 3.86 (m, 4H, CHH -Ph & $CHH\alpha$ -Gly & CH_2 in malonyl moiety), 3.76 (dd, $J = 16.6, 4.3$ Hz, 1H, $CHH\alpha$ -Gly), 3.59 – 3.45 (m, 2H, $CH_2\beta$ -Pro), 2.13 – 2.02 (m, 1H, $CHH\gamma$ -Pro), 2.00 – 1.90 (m, 1H, $CHH\delta$ -Pro), 1.90 – 1.72 (m, 2H, $CHH\gamma$ -Pro & $CHH\delta$ -Pro); ^{13}C NMR (126 MHz, DMSO- d_6) δ 175.7, 171.9, 168.5, 167.5, 165.2, 164.3, 138.0, 134.9, 133.2, 128.8, 128.5, 125.9, 60.6, 46.6, 43.0, 42.7, 34.7, 29.7, 25.0; HRMS (ESI+) $[M+H]^+$ calculated for $C_{19}H_{20}BrN_6O_5 = 491.0673$, found = 491.0673.

Bi-aryl macrocycle (3b) [41 mg, 0.083 mmol, 17 %]

1H NMR (500 MHz, DMSO- d_6) δ 11.13 (s, 1H, NH-ODZ), 8.63 (t, $J = 6.0$ Hz, 1H, NH-Bn), 8.22 (s, 1H, NH-Gly), 8.12 (s, 1H, H_2 -Ph), 7.94 – 7.76 (m, 2H, H_6 -Ph & H_5 -Ph), 4.64 (dd, $J = 17.3, 7.4$ Hz, 1H, CHH -Ph), 4.17 (ddd, $J = 21.4, 12.7, 5.0$ Hz, 2H, $CH\alpha$ -Pro & $CHH\alpha$ -Gly), 4.07 (dd, $J = 17.4, 4.8$ Hz, 1H, CHH -Ph), 3.88 (dd, $J = 17.1, 4.2$ Hz, 1H, $CHH\alpha$ -Gly), 3.72 (t, $J = 14.7$ Hz, 1H, CHH in malonyl moiety), 3.66 – 3.58 (m, 1H, $CHH\beta$ -Pro), 3.51 (dd, $J = 11.4, 5.2$ Hz, 1H, $CHH\beta$ -Pro), 3.45-3.35 (m, 1H, CHH in malonyl moiety), 2.28 – 2.09 (m, 1H, $CHH\gamma$ -Pro), 1.97 (dt, $J = 13.0, 6.6$ Hz, 1H,

CHH δ -Pro), 1.85 (ddt, $J = 35.6, 12.4, 6.4$ Hz, 2H, CHH δ -Pro & CHH γ -Pro); ^{13}C NMR (126 MHz, DMSO- d_6) δ 175.5, 172.3, 167.9, 167.7, 165.6, 164.5, 140.5, 133.7, 128.3, 128.1, 127.8, 123.4, 61.4, 46.3, 44.2, 42.7, 42.5, 29.6, 25.1; HRMS (ESI+) $[M+H]^+$ calculated for $\text{C}_{19}\text{H}_{20}\text{BrN}_6\text{O}_5 = 491.0700$, found = 491.0700.



The title compounds were synthesized according to general synthetic procedure using the mixture of corresponding non bi-aryl and bi-aryl linear precursors (0.39 g, 0.70 mmol, 1.0 eq) and purified by reverse-phase chromatography with double distilled water and MeCN. The product containing fractions were collected and concentrated under reduced pressure. The obtained white solids were washed with double distilled water/MeCN (1/1) and dried under reduced pressure.

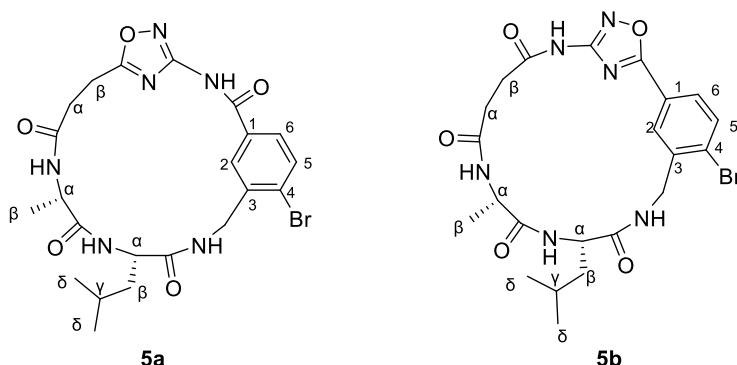
Non bi-aryl macrocycle (4a) [15 mg, 0.034 mmol, 5 %, white solid]

^1H NMR (500 MHz, DMSO- d_6) δ 11.50 (s, 1H, NH-ODZ), 8.35 (t, $J = 6.1$ Hz, 1H, NH-Bn), 7.94 (t, $J = 6.1$ Hz, 1H, NH-GABA), 7.73 (d, $J = 8.2$ Hz, 1H, H₅-Ph), 7.54 (dd, $J = 8.2, 2.2$ Hz, 1H, H₆-Ph), 7.46 (d, $J = 2.3$ Hz, 1H, H₂-Ph), 4.24 (d, $J = 6.0$ Hz, 2H, CH₂-Ph), 3.07 (dd, $J = 8.9, 4.4$ Hz, 4H, CH₂ γ -GABA & CH₂ β in succinyl moiety), 2.45 (t, $J = 6.3$ Hz, 2H, CH₂ α in succinyl moiety), 2.23 – 2.05 (m, 2H, CH₂ α -GABA), 1.90 – 1.69 (m, 2H, CH₂ β -GABA); ^{13}C NMR (126 MHz, DMSO- d_6) δ 178.7, 173.1, 170.1, 165.5, 163.1, 137.9, 133.5, 133.2, 128.5, 128.1, 126.1, 42.2, 39.0, 34.7, 32.4, 28.8, 22.5; HRMS (ESI+) $[M+H]^+$ calculated for $\text{C}_{17}\text{H}_{19}\text{BrN}_5\text{O}_4 = 436.0615$, found = 436.0606.

Bi-aryl macrocycle (4b) [15 mg, 0.034 mmol, 5 %, white solid]

^1H NMR (500 MHz, DMSO- d_6) δ 10.92 (s, 1H, NH-ODZ), 8.61 (t, $J = 6.2$ Hz, 1H, NH-Bn), 7.98 (t, $J = 6.1$ Hz, 1H, NH-GABA), 7.86 (dd, $J = 8.2, 0.5$ Hz, 1H, H₅-Ph), 7.83 (dd, $J = 8.2, 2.0$ Hz, 1H, H₆-Ph), 7.79 (d, $J = 2.0$ Hz, 1H, H₂-Ph), 4.26 (d, $J = 6.1$ Hz, 2H, CH₂-Ph), 3.08 (q, $J = 6.7$ Hz, 2H, CH₂ γ -GABA), 2.91 (s, 2H, CH₂ β in succinyl moiety), 2.33 (t, $J = 7.6$ Hz, 2H, CH₂ α in succinyl moiety), 2.26 (t, $J = 8.7$ Hz, 2H, CH₂ α -GABA), 1.75 (d, $J = 8.8$ Hz, 2H, CH₂ β -GABA); ^{13}C NMR (126 MHz, DMSO- d_6) δ 174.6, 173.2, 172.6, 172.1, 164.7, 140.1, 134.1, 127.5, 127.1, 127.0, 123.0, 42.3, 38.1,

33.5, 33.2, 32.3, 26.3; HRMS (ESI⁺) [M+H⁺] calculated for C₁₇H₁₉BrN₅O₄ = 436.0615, found = 436.0615.



The title compounds were synthesized according to general synthetic procedure using the mixture of corresponding non bi-aryl and bi-aryl linear precursors (0.10 g, 0.15 mmol, 1.0 eq) and purified by reverse-phase chromatography with 0.1 % formic acid containing double distilled water and 0.1 % formic acid containing MeCN. The product containing fractions were transferred to separatory funnel by using chloroform and then saturated aqueous NaHCO₃ solution was added to neutralize the aqueous layer. The organic layer was collected and dried over Na₂SO₄ and concentrated under reduced pressure. The obtained white solids were washed with diethyl ether and dried under reduced pressure.

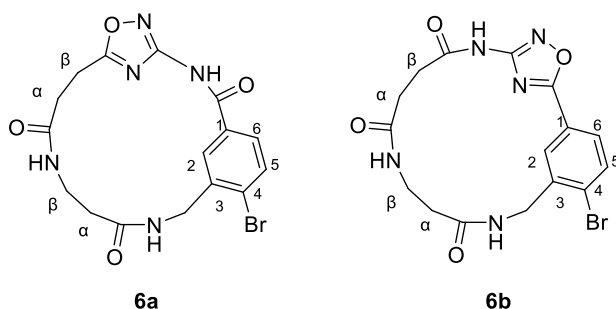
Non bi-aryl macrocycle (5a) [18 mg, 0.034 mmol, 23 %]

¹H NMR (500 MHz, DMSO-*d*₆) δ 10.75 (brs, 1H, NH-ODZ), 8.54 – 8.31 (m, 1H, NH-Bn), 8.01 (s, 1H, H₂-Ph), 7.98 (d, *J* = 4.4 Hz, 1H, NH-Leu), 7.95 (d, *J* = 6.8 Hz, 1H, NH-Ala), 7.78 (d, *J* = 8.2 Hz, 1H, H₆-Ph), 7.68 (d, *J* = 8.2 Hz, 1H, H₅-Ph), 4.69 (dd, *J* = 17.0, 8.7 Hz, 1H, CHH-Ph), 4.22 (p, *J* = 7.0 Hz, 1H, CHα-Ala), 4.08 (dt, *J* = 9.4, 5.0 Hz, 1H, CHα-Leu), 3.97 (dd, *J* = 16.7, 2.9 Hz, 1H, CHH-Ph), 3.13 – 2.99 (m, 2H, CH₂β in succinyl moiety), 2.47 – 2.28 (m, 2H, CH₂α in succinyl moiety), 1.74 (dt, *J* = 13.1, 6.5 Hz, 1H, CHγ -Leu), 1.56 (ddd, *J* = 14.5, 9.0, 5.9 Hz, 1H, CHHβ-Leu), 1.44 (dt, *J* = 13.4, 6.9 Hz, 1H, CHHβ-Leu), 1.16 (d, *J* = 7.1 Hz, 3H, CH₃β-Ala), 0.94 (d, *J* = 6.5 Hz, 3H, CH₃δ-Leu), 0.90 (d, *J* = 6.5 Hz, 3H, CH₃δ-Leu); ¹³C NMR (126 MHz, DMSO-*d*₆) δ 178.4, 173.2, 172.7, 170.7, 164.3, 163.1, 139.0, 133.4, 132.6, 128.9, 127.2, 126.6, 53.3, 48.7, 42.2, 40.7, 33.4, 24.7, 23.4, 22.9, 22.3, 18.9; HRMS (DART-MS) [M+H⁺] calculated for C₂₂H₂₈BrN₆O₅ = 535.1299, found = 535.1293.

Bi-aryl macrocycle (5b) [4.8 mg, 0.009 mmol, 6 %]

¹H NMR (400 MHz, DMSO-*d*₆) δ 10.98 (brs, 1H, NH-ODZ), 8.67 (s, 1H, NH-Bn), 8.06 (s, 1H, H₂-Ph), 8.00 - 7.87 (brs, 1H, NH-Leu), 7.83 (s, 2H, H₅-Ph&H₆-Ph), 7.75 – 7.54 (m, 1H, Nh-Ala), 4.70 – 4.55 (m, 1H, CHH-Ph), 4.23 (d, *J* = 6.4 Hz, 1H, CHα-

Leu), 4.14 (t, $J = 7.2$ Hz, 1H, CH α -Ala), 4.07 (dd, $J = 16.9, 4.6$ Hz, 1H, CHH-Ph), 3.20 – 2.93 (m, 1H, CHH β in succinyl moiety), 2.84 – 2.64 (m, 1H, CHH β in succinyl moiety), 2.55 – 2.25 (m, 2H, CH 2α in succinyl moiety), 1.58 (dd, $J = 13.0, 6.4$ Hz, 1H, CH γ -Leu), 1.50 (d, $J = 9.5$ Hz, 2H, CH 2β -Leu), 1.18 (d, $J = 7.1$ Hz, 3H, CH 3β -Ala), 0.90 (d, $J = 6.6$ Hz, 3H, CH 3δ -Leu), 0.85 (d, $J = 6.5$ Hz, 3H, CH 3δ -Leu); ^{13}C NMR (126 MHz, DMSO- d_6) *one carbonyl carbon peak is broadening δ 174.6, 173.1, 173.0, 172.5, 164.8, 140.3, 133.9, 128.0, 127.8, 127.6, 123.4, 52.5, 48.9, 42.8, 40.7, 33.3, 32.4, 24.6, 23.2, 22.5, 18.35; HRMS (DART-MS) $[\text{M}+\text{H}^+]$ calculated for $\text{C}_{22}\text{H}_{28}\text{BrN}_6\text{O}_5 = 535.1299$, found = 535.1290.



The title compounds were synthesized according to general synthetic procedure using the mixture of corresponding non bi-aryl and bi-aryl linear precursors (0.15 g, 0.27 mmol, 1.0 eq) and purified by reverse-phase chromatography with double distilled water and MeCN. The product containing fractions were collected respectively and concentrated under reduced pressure.

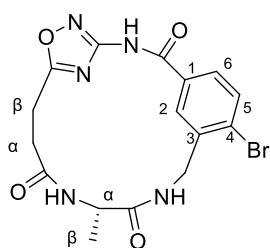
Non bi-aryl macrocycle (6a) [9.2 mg, 0.022 mmol, 8 %]

^1H NMR (500 MHz, DMSO- d_6) δ 11.01 (brs, 1H, NH-ODZ), 8.34 (t, $J = 6.1$ Hz, 1H, NH-Bn), 8.04 (t, $J = 5.8$ Hz, 1H, NH- β -Ala), 7.71 (d, $J = 8.2$ Hz, 1H, H $_5$ -Ph), 7.44 (dd, $J = 8.2, 2.2$ Hz, 1H, H $_6$ -Ph), 7.17 (d, $J = 2.1$ Hz, 1H, H $_2$ -Ph), 4.29 (d, $J = 6.1$ Hz, 2H, CH $_2$ Ph), 3.21 (q, $J = 5.7$ Hz, 2H, CH 2β in β -Ala), 3.10 – 3.02 (m, 2H, CH 2β in succinyl moiety), 2.51 (dd, $J = 6.0, 3.0$ Hz, 2H, CH 2α in succinyl moiety), 2.35 – 2.29 (m, 2H, CH 2α in β -Ala); ^{13}C NMR (126 MHz, DMSO- d_6) δ 179.5, 171.1, 170.5, 169.7, 164.2, 138.0, 134.1, 133.3, 129.3, 127.1, 125.7, 42.1, 35.1, 34.9, 31.8, 22.8; HRMS (DART-MS) $[\text{M}+\text{H}^+]$ calculated for $\text{C}_{16}\text{H}_{17}\text{BrN}_5\text{O}_4 = 422.0458$, found = 422.0450.

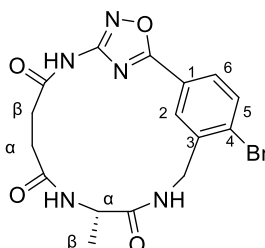
Bi-aryl macrocycle (6b) [2.1 mg, 0.005 mmol, 1.9 %]

^1H NMR (500 MHz, DMSO- d_6) δ *NH-ODZ peak was not observed due to broadening 8.68 (t, $J = 6.1$ Hz, 1H, NH-Bn), 8.13 (t, $J = 5.6$ Hz, 1H, NH- β -Ala), 7.87 (s, 3H, H $_2$, H $_5$, H $_6$ -Ph), 4.32 (d, $J = 6.0$ Hz, 2H, CH $_2$ Ph), 3.35 – 3.26 (m, 2H, CH 2β in β -Ala, overlapped with H $_2\text{O}$ peak), 3.01 – 2.90 (m, 2H, CH 2β in succinyl moiety), 2.48 – 2.44 (m, 2H, CH 2α

in β -Ala, *partially overlapped with solvent peak*), 2.44 – 2.37 (m, 2H, $\text{CH}_2\alpha$ in succinyl moiety); ^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) δ 174.7, 173.7, 171.7, 171.4, 164.7, 140.0, 134.0, 127.6, 127.3, 127.2, 123.1, 42.8, 35.6, 35.5, 33.7, 32.1; HRMS (DART-MS) $[\text{M}+\text{H}^+]$ calculated for $\text{C}_{16}\text{H}_{17}\text{BrN}_5\text{O}_4$ = 422.0458, found = 422.0467.



7a



7b

The title compounds were synthesized according to general synthetic procedure using the mixture of corresponding non bi-aryl and bi-aryl linear precursors (0.10 g, 0.18 mmol, 1.0 eq) and purified by reverse-phase chromatography with double distilled water and MeCN. The product containing fractions were collected respectively and concentrated under reduced pressure.

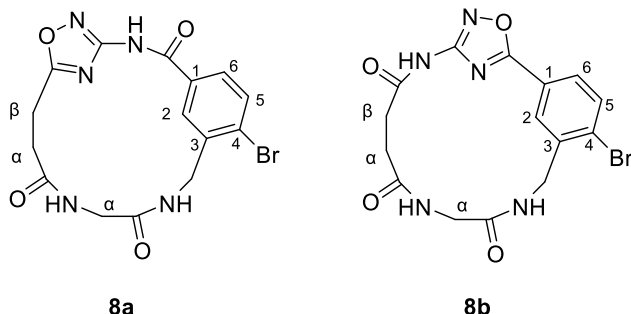
Non bi-aryl macrocycle (7a) [4.5 mg, 0.011 mmol, 6%]

^1H NMR (500 MHz, $\text{DMSO-}d_6$) δ 10.95 (s, 1H, NH-ODZ), 8.26 (d, J = 7.9 Hz, 1H, NH-Ala), 8.16 (dd, J = 6.9, 5.1 Hz, 1H, NH-Bn), 7.72 (d, J = 8.3 Hz, 1H, H_5 -Ph), 7.47 (dd, J = 8.2, 2.2 Hz, 1H, H_6 -Ph), 7.01 (d, J = 2.2 Hz, 1H, H_2 -Ph), 4.54 – 4.40 (m, 1H, CHH -Ph), 4.36 – 4.22 (m, 1H, $\text{CH}\alpha$ -Ala), 4.04 (dd, J = 15.8, 5.1 Hz, 1H, CHH -Ph), 3.08 (dp, J = 10.5, 5.3 Hz, 2H, $\text{CH}_2\beta$ in succinyl moiety), 2.72 (ddd, J = 15.1, 10.5, 4.7 Hz, 1H, $\text{CHH}\alpha$ in succinyl moiety), 2.54 – 2.50 (m, 1H, $\text{CHH}\alpha$ in succinyl moiety), 1.15 (d, J = 7.0 Hz, 3H, $\text{CH}_3\beta$ -Ala); ^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) δ 180.1, 172.8, 170.3, 169.4, 164.3, 137.9, 134.1, 133.5, 129.9, 127.5, 126.0, 48.6, 42.5, 32.2, 23.2, 17.6; HRMS (ESI+) $[\text{M}+\text{H}^+]$ calculated for $\text{C}_{16}\text{H}_{17}\text{BrN}_5\text{O}_4$ = 422.0458, found = 422.0465.

Bi-aryl macrocycle (7b) [2 mg, 0.005 mmol, 2.6 %]

^1H NMR (500 MHz, $\text{DMSO-}d_6$) δ 11.01 (brs, 1H, NH-ODZ), 8.72 (t, J = 6.1 Hz, 1H, NH-Bn), 8.39 (d, J = 8.4 Hz, 1H, NH-Ala), 7.94 – 7.73 (m, 3H, H_5 -Ph & H_6 -Ph & H_2 -Ph), 4.64 – 4.40 (m, 2H, $\text{CH}\alpha$ -Ala & CHH -Ph), 3.98 (dd, J = 17.1, 5.2 Hz, 1H, CHH -Ph), 3.45 – 3.27 (m, 1H, $\text{CHH}\beta$ in succinyl moiety), 2.78 – 2.61 (m, 1H, $\text{CHH}\alpha$ in succinyl moiety), 2.56 – 2.42 (m, 1H, $\text{CHH}\alpha$ in succinyl moiety), 2.33 (td, J = 12.1, 7.6 Hz, 1H, $\text{CHH}\beta$ in succinyl moiety), 1.19 (d, J = 6.9 Hz, 3H, $\text{CH}_3\beta$ -Ala); ^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) δ 174.6, 174.1, 172.5, 170.6, 164.8, 139.9, 134.0, 127.5, 127.5, 127.0,

122.9, 48.3, 42.3, 32.2, 31.3, 16.9; HRMS (ESI+) $[M+H^+]$ calculated for $C_{16}H_{17}BrN_5O_4$ = 422.0458, found = 422.0461.



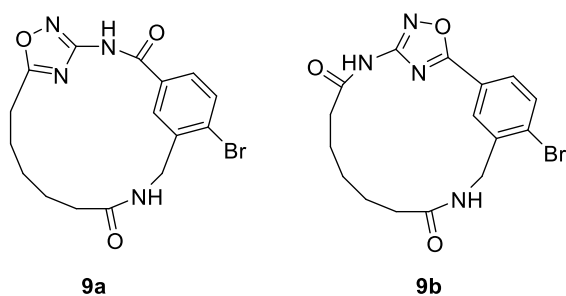
The title compounds were synthesized according to general synthetic procedure using the mixture of corresponding non bi-aryl and bi-aryl linear precursors (0.10 g, 0.19 mmol, 1.0 eq) and purified by reverse-phase chromatography with double distilled water and MeCN. The product containing fractions were collected respectively and concentrated under reduced pressure.

Non bi-aryl macrocycle (8a) [3.0 mg, 0.007 mmol, 4%]

1H NMR (500 MHz, DMSO- d_6) *odz-NH peak is not observed due to broadening δ 8.42 (t, J = 5.9 Hz, 1H, NH-Gly), 7.92 (t, J = 5.9 Hz, 1H, NH-Bn), 7.72 (d, J = 8.2 Hz, 1H, H_5 -Ph), 7.48 (dd, J = 8.2, 2.2 Hz, 1H, H_6 -Ph), 7.00 (d, J = 2.1 Hz, 1H, H_2 -Ph), 4.25 (d, J = 5.9 Hz, 2H, CH_2 -Ph), 3.66 (d, J = 6.0 Hz, 2H, $CH_2\alpha$ -Gly), 3.20 – 3.02 (m, 2H, $CH_2\beta$ in succinate moiety), 2.73 – 2.56 (m, 2H, $CH_2\alpha$ in succinate moiety); ^{13}C NMR (126 MHz, DMSO- d_6) δ 180.3, 171.3, 170.1, 169.4, 164.3, 137.6, 133.9, 133.6, 130.0, 127.5, 126.0, 43.8, 42.5, 32.2, 23.2; HRMS (DART-MS) $[M+H^+]$ calculated for $C_{15}H_{15}BrN_5O_4$ = 408.0302, found = 408.0298.

Bi-aryl macrocycle (8b) [1.8 mg, 0.004 mmol, 2%]

1H NMR (500 MHz, DMSO- d_6) *odz-NH peak is not observed due to broadening δ 8.66 (t, J = 6.1 Hz, 1H), 8.55 (t, J = 6.1 Hz, 1H), 7.92 – 7.77 (m, 3H), 4.26 (d, J = 6.1 Hz, 2H), 3.76 (d, J = 6.2 Hz, 2H), 2.83 (dd, J = 10.2, 6.3 Hz, 2H), 2.75 – 2.57 (m, 2H); ^{13}C NMR (126 MHz, DMSO- d_6) δ 174.6, 174.1, 171.3, 169.9, 164.8, 139.9, 133.9, 127.6, 127.5, 126.9, 122.8, 44.1, 42.2, 31.9, 31.2; HRMS (ESI+) $[M+Na^+]$ calculated for $C_{15}H_{14}BrN_5O_4Na$ = 430.0162, found = 430.0126.



Preparation of linear precursors

The starting material (18) (0.50 g, 0.98 mmol, 1.0 eq) was dissolved in DCM (4 mL). Triphenyl silane (3.0 eq) and tetrakis (triphenylphosphine) palladium (0) (0.05 eq) were added into reaction solution and the reaction mixture was stirred at room temperature for 30 min. The resulting mixture was concentrated under reduced pressure and diethyl ether was added to precipitate the solids. After removal of solvent, the obtained solids were dissolved in THF (3 mL) and 2M aqueous NaOH solution (3 mL). Di-*tert*-butyl dicarbonate (1.2 eq) was added into reaction solution and the reaction mixture was stirred at room temperature for 2 h. Resulting solution was transferred to separatory funnel and diluted with diethyl ether and water. The aqueous layer was collected, acidified to pH 2 with 2M aqueous HCl solution at 0°C and extracted with mixture of chloroform and only a few amounts of methanol. The organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The obtained crude material was dissolved in 20% trifluoroacetic acid in DCM (4.8 mL) solution and stirred at room temperature for 1 h. The resulting mixture was concentrated under reduced pressure. Diethyl ether was added into the crude materials and the resulting mixture was sonicated to form the solids. After removal of the solvent, the obtained solids were dried under reduced pressure. Finally, the trifluoroacetic acid salt of the linear precursor was obtained as mixture of non bi-aryl and bi-aryl compound.

Macrocyclization

Macrocyclization was carried out according to general synthetic procedure using the mixture of corresponding non bi-aryl and bi-aryl linear precursors (0.15 g, 0.29 mmol, 1.0 eq) and purified by reverse-phase chromatography with double distilled water and MeCN. The product containing fractions were collected respectively and concentrated under reduced pressure.

Non bi-aryl macrocycle (9a) [6.0 mg, 0.015 mmol, 5 %]

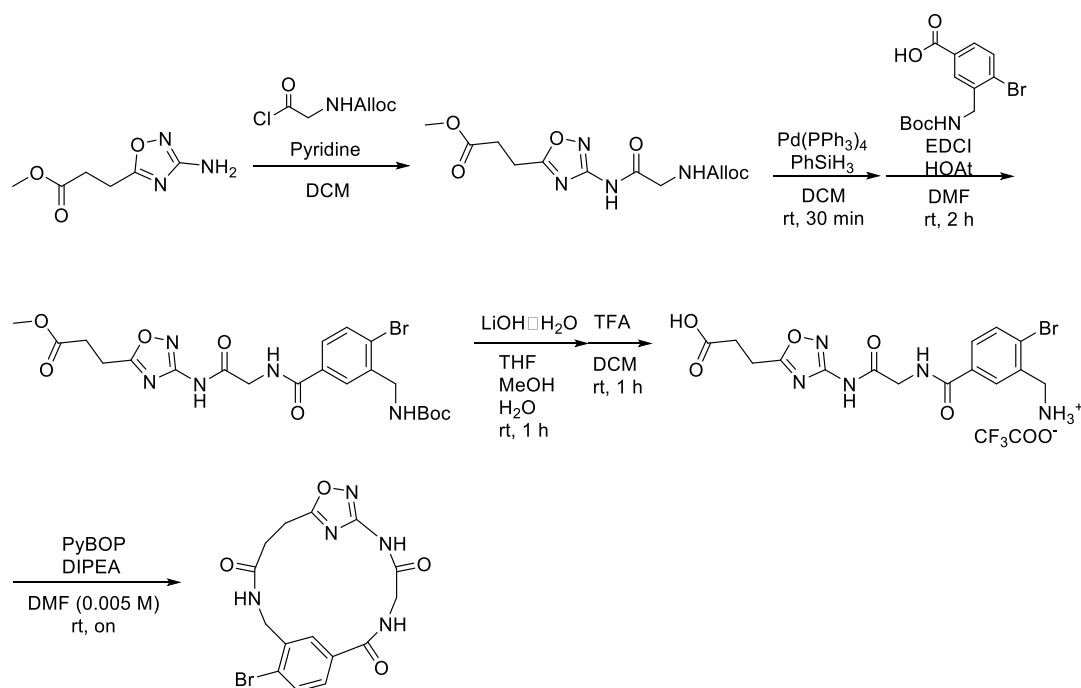
¹H NMR (500 MHz, DMSO-*d*₆) δ 11.05 (brs, 1H), 8.30 (t, *J* = 6.0 Hz, 1H), 7.71 (d, *J* = 8.2 Hz, 1H), 7.45 (dd, *J* = 8.2, 2.2 Hz, 1H), 7.15 (d, *J* = 2.1 Hz, 1H), 4.29 (d, *J* = 6.0 Hz,

2H), 2.93 – 2.72 (m, 2H), 2.14 – 1.98 (m, 2H), 1.72 – 1.49 (m, 2H), 1.40 (p, $J = 7.5$ Hz, 2H), 0.99 (p, $J = 7.6$ Hz, 2H); ^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) δ 181.0, 172.5, 169.7, 164.5, 138.4, 134.7, 133.5, 129.8, 127.9, 126.4, 42.2, 35.0, 26.8, 26.2, 25.9, 24.7; HRMS (DART-MS) $[\text{M}+\text{H}^+]$ calculated for $\text{C}_{16}\text{H}_{18}\text{BrN}_4\text{O}_3 = 393.0557$, found = 393.0553.

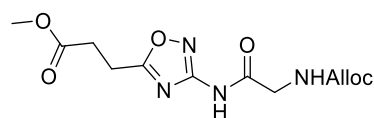
Bi-aryl macrocycle (9b) [2.2 mg, 0.006 mmol, 2 %]

^1H NMR (500 MHz, $\text{DMSO-}d_6$) δ 10.9 (brs, 1H), 8.68 (t, $J = 5.9$ Hz, 1H), 7.93 (s, 1H), 7.85 (t, $J = 6.8$ Hz, 2H), 4.27 (d, $J = 5.4$ Hz, 2H), 2.55 – 2.48 (m, 2H, *overlapped with solvent peaks), 2.39 – 2.18 (m, 2H), 1.77 – 1.64 (m, 4H), 1.58 – 1.48 (m, 2H); ^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) δ 174.7, 174.3, 173.7, 165.0, 140.0, 134.1, 127.4, 127.3, 127.0, 123.0, 42.6, 36.7, 36.6, 30.0, 27.3 (2C); HRMS (DART-MS) $[\text{M}+\text{H}^+]$ calculated for $\text{C}_{16}\text{H}_{18}\text{BrN}_4\text{O}_3 = 393.0557$, found = 393.0558.

Synthesis of Macrocycle 10a



Preparation of 3-acylamino-1,2,4-oxadiazole intermediate



Methyl 3-(3-(2-(((allyloxy)carbonyl)amino)acetamido)-1,2,4-oxadiazol-5-yl)propanoate (20)

Compound **16** (0.56 g, 3.27 mmol, 1.0 eq) was dissolved in DCM (0.3 M) and pyridine (2.0 eq) was added. Allyl (2-chloro-2-oxoethyl)carbamate (1.16 g, 6.54 mmol, 2 eq) was slowly added into reaction solution at 0°C and the reaction mixture was stirred at room temperature for 5 h. The resulting mixture was quenched with aqueous NaHCO₃ solution and extracted with DCM. The organic layer was washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure. The crude products were purified by flash chromatography (SiO₂, *n*-hexane/EtOAc 67: 33 to 0: 100).

[0.19 g, 0.61 mmol, 19 %]

¹H NMR (500 MHz, DMSO-*d*₆) δ 11.13 (s, 1H), 7.49 (t, *J* = 6.1 Hz, 1H), 5.89 (ddt, *J* = 17.2, 10.5, 5.3 Hz, 1H), 5.28 (dq, *J* = 17.2, 1.7 Hz, 1H), 5.16 (dq, *J* = 10.4, 1.5 Hz, 1H), 4.47 (dt, *J* = 5.3, 1.6 Hz, 2H), 3.82 (d, *J* = 6.1 Hz, 2H), 3.59 (s, 3H), 3.10 (t, *J* = 6.8 Hz, 2H), 2.83 (t, *J* = 6.8 Hz, 2H); ¹³C NMR (126 MHz, DMSO-*d*₆) δ 178.2, 171.8, 167.6, 162.3, 156.4, 133.5, 117.0, 64.6, 51.7, 43.7, 29.4, 21.5; HRMS (ESI+) [*M*+*H*⁺] calculated for C₁₂H₁₆N₄O₆ = 313.1070, found = 313.1071.

Preparation of linear precursor and macrocyclization

Alloc deprotection and condensation with benzoic acid

3-acylamino-1,2,4-oxadiazole intermediate **20** (0.19 g, 0.61 mmol, 1.0 eq) was suspended in DCM (0.3 M) and then phenyl silane (3.0 eq) and tetrakis (triphenylphosphine) palladium (0) (0.05 eq) were added into reaction solution and the reaction mixture was stirred at room temperature for 30 min. The resulting mixture was concentrated under reduced pressure and diethyl ether was added to precipitate the solids. After removal of solvent, the obtained solids were dried under high vacuum and used to next reaction.

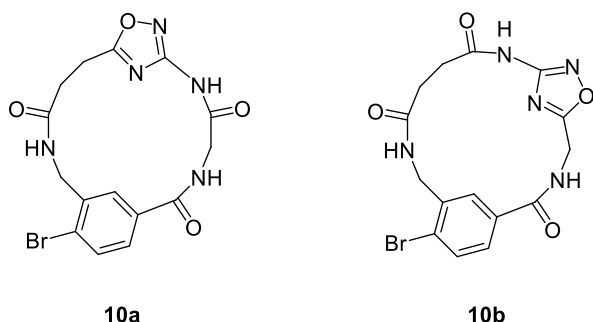
To a DMF (0.3 M) solution of the crude benzylamine derivative, 4-bromo-3-(((tert-butoxycarbonyl)amino)methyl)benzoic acid (1.3 eq), DIPEA (4.0 eq), EDCI (1.5 eq) and 1-Hydroxy-7-azabenzotriazole (HOAt) (1.5 eq) were added and the reaction mixture was stirred at room temperature for 2 h. The resulting mixture was added slowly into water and then filtered. The filtrates were dissolved in ethyl acetate and washed with brine. The organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The obtained crude product was used to next step without further purification.

Hydrolysis and Boc deprotection

The obtained crude product was dissolved in THF: MeOH: H₂O (4: 1: 1, 0.3 M) and lithium hydroxide monohydrate (2.0 eq) was added into reaction solution. After stirring at room temperature for 1 h, resulting solution was transferred to separatory funnel and diluted with diethyl ether and water. The aqueous layer was collected, acidified to around pH 2 with 2M aqueous HCl solution at 0°C and extracted with chloroform (if necessary, only a few amounts of MeOH was added). The organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The obtained crude material was dissolved in 20% trifluoroacetic acid in DCM (0.3 M) solution and stirred at room temperature for 1 h. The resulting mixture was concentrated under reduced pressure. Diethyl ether was added into the crude materials and the resulting mixture was sonicated to form the solids. After removal of the solvent, the obtained solids were washed with diethyl ether again and dried under reduced pressure. Finally, the trifluoroacetic acid salts of the linear precursor were obtained as a crude product. (In this case, rearranged product was not detected in the process of synthesis of linear precursor.)

Macrocyclization

To a DMF (0.01 M) solution of benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) (2.0 eq) and DIPEA (4.0 eq), trifluoroacetic acid salts of linear precursor (1.0 eq) in DMF (0.01 M) was slowly added at room temperature. The reaction mixture was stirred at room temperature for 18 h. After addition of Celite and toluene, the resulting mixture was concentrated under reduced pressure. The resulting crude materials were purified by reverse-phase chromatography to afford macrocycle **10a**.



Macrocycle **10a** [7.5 mg, 0.018 mmol, 3 %]

¹H NMR (500 MHz, DMSO-*d*₆) δ 10.46 (s, 1H, NH-ODZ), 8.64 (t, *J* = 5.9 Hz, 1H, NH-Bn), 8.58 (t, *J* = 5.3 Hz, 1H, NH-Gly), 7.64 (d, *J* = 8.2 Hz, 1H, H₅-Ph), 7.36 (dd, *J* = 8.2, 2.0 Hz, 1H, H₆-Ph), 6.82 (s, 1H, H₂-Ph), 4.23 (d, *J* = 5.8 Hz, 2H, CH₂-Ph), 4.05 (d, *J* = 5.4 Hz, 2H, CH₂α-Gly), 3.25 – 3.18 (m, 2H, CH₂β in succinyl moiety), 2.82 – 2.73 (m,

2H, CH₂α in succinyl moiety); ¹³C NMR (126 MHz, DMSO-*d*₆) δ 179.8, 170.9, 170.8, 168.2, 163.5, 138.1, 135.1, 132.9, 127.5, 126.0, 124.6, 45.6, 42.7, 31.9, 23.0; HRMS (DART-MS) [M+H⁺] calculated for C₁₅H₁₄BrN₅O₄ = 408.0229, found = 408.0225.

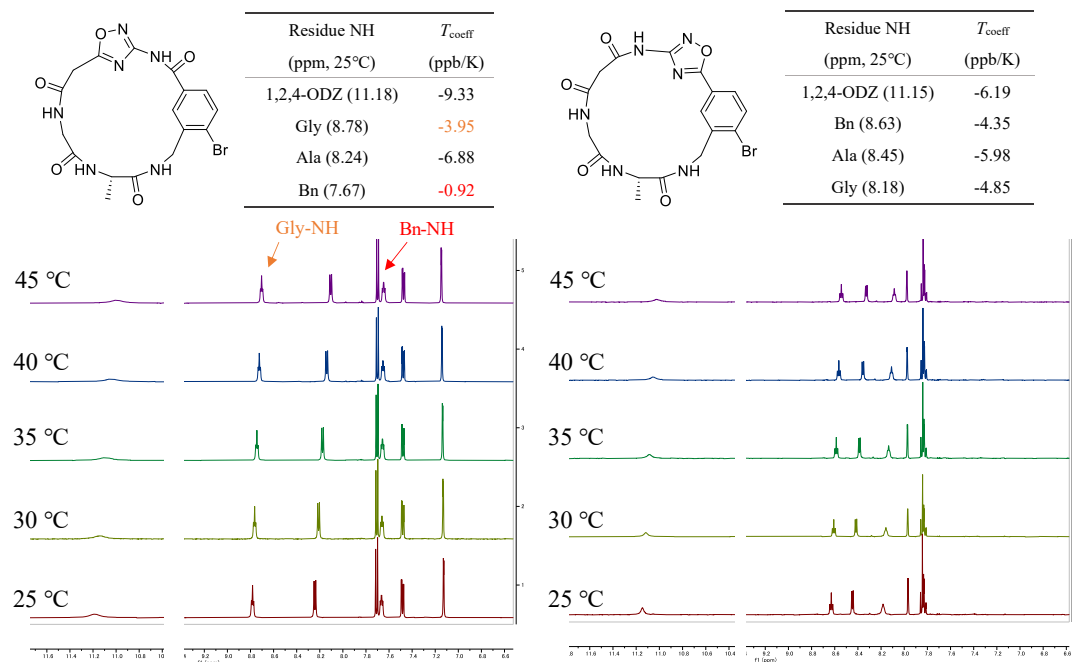
Macrocycle 10b [2.4 mg, 0.006 mmol, 1 %]

¹H NMR (500 MHz, DMSO-*d*₆) δ 10.98 (s, 1H, odz-NH), 9.12 (t, *J* = 5.8 Hz, 1H, NH-Bn), 8.64 (t, *J* = 6.1 Hz, 1H, NH-CH₂-odz), 7.69 (d, *J* = 8.1 Hz, 1H, H₅-Ph), 7.50 (d, *J* = 2.1 Hz, 1H, H₂-Ph), 7.37 (dd, *J* = 8.1, 2.1 Hz, 1H, H₆-Ph), 4.71 (d, *J* = 5.8 Hz, 2H, CH₂-Bn), 4.32 (d, *J* = 6.0 Hz, 2H, CH₂-odz), 3.24 – 3.17 (m, 2H, CH₂β in succinate moiety), 2.41 – 2.34 (m, 2H, CH₂α in succinate moiety).

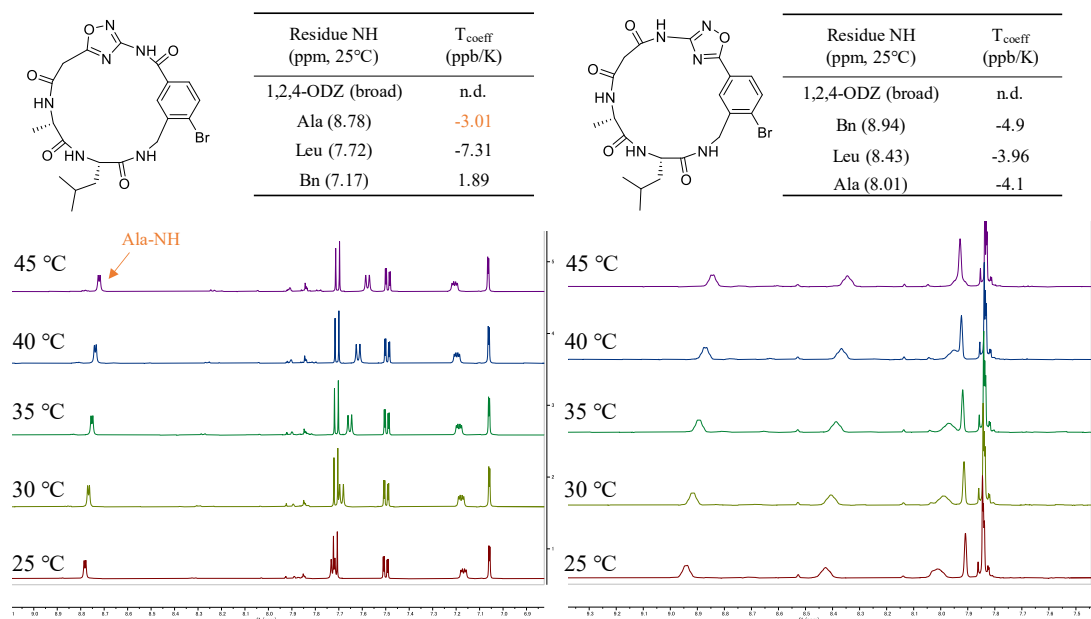
Carbon NMR and ESI MS were not obtained due to product reverting back to starting material

Intramolecular hydrogen bonding profile analysis in DMSO-*d*₆

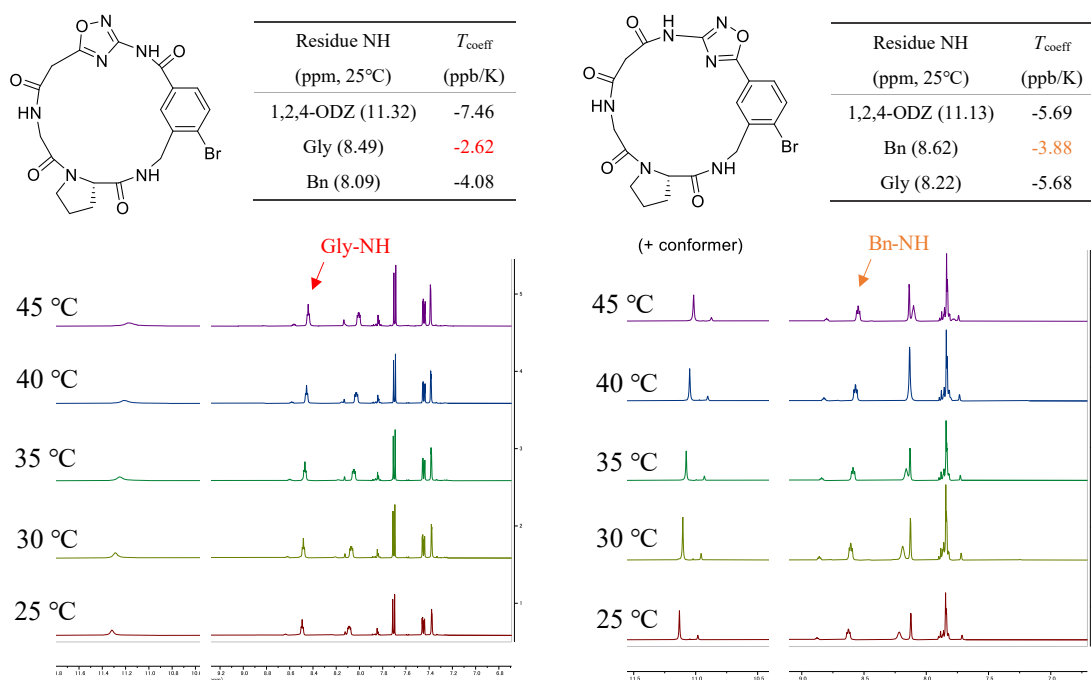
1. Macrocycle 1a and 1b



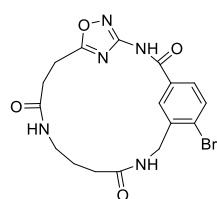
2. Macrocycle 2a and 2b



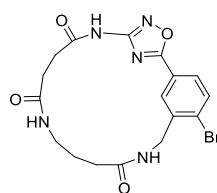
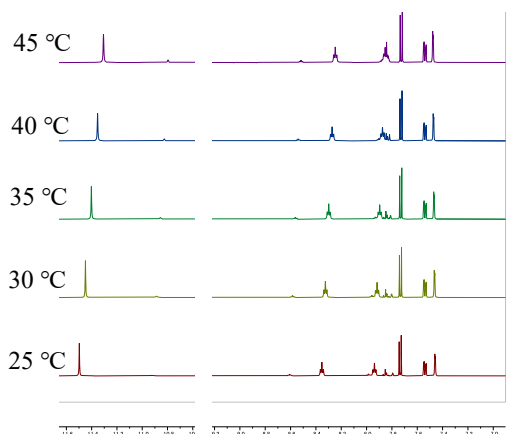
3. Macrocycle **3a** and **3b**



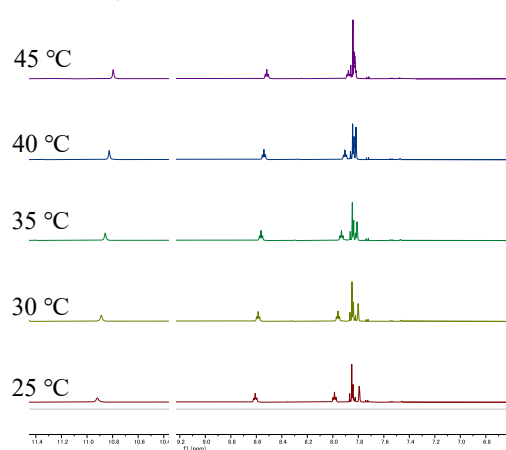
4. Macrocycle **4a** and **4b**



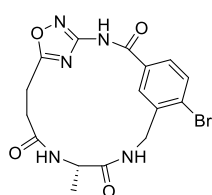
Residue NH (ppm, 25°C)	T_{coeff} (ppb/K)
1,2,4-ODZ (11.50)	-9.54
Bn (8.35)	-5.29
Alkyl (7.94)	-4.23



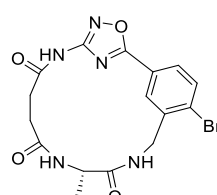
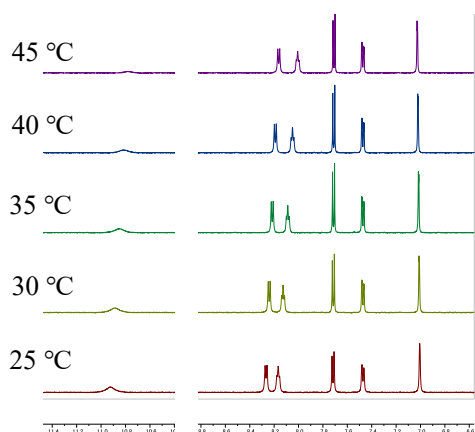
Residue NH (ppm, 25°C)	T_{coeff} (ppb/K)
1,2,4-ODZ (10.92)	-6.20
Bn (8.61)	-4.54
Alkyl (7.98)	-5.44



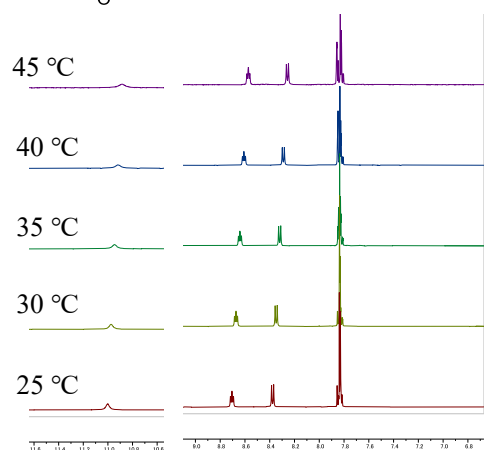
5. Macrocycle **7a** and **7b**



Residue NH (ppm, 25°C)	T_{coeff} (ppb/K)
1,2,4-ODZ (10.92)	-7.24
Ala (8.27)	-5.19
Bn (8.17)	-7.93



Residue NH (ppm, 25°C)	T_{coeff} (ppb/K)
1,2,4-ODZ (11.00)	-5.79
Bn (8.70)	-6.58
Ala (8.38)	-5.96



Kinetic Study by ¹H-NMR

In order to calculate kinetic and thermodynamic parameters of BK-R reaction in macrocycles and linear precursors, the reaction progress was monitored by using Varian 600 MHz spectrometer (probe: OneNMR600) at 75 °C or 110 °C. Kinetic data was

acquired using the array function in VNMR. A typical linear array **pad**, the pre-acquisition delay parameter, consisted of 500 values all with $\text{pad} = 5$ s (with the exception of the first value set to 0.5 s). For macrocycle 4a and 5a, pad consisted of 48 values with following setting; first value = 0.5 s, first 1 hour = 32 s, after 1 hour = 512 s.

Sample preparation

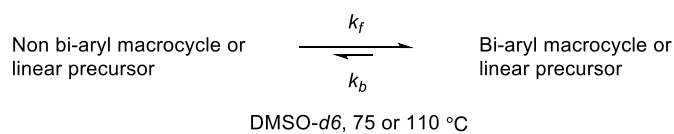
Each NMR sample was prepared with following procedure; 0.0064 mmol (1.0 eq) of substrate and 0.33 eq. of 1,3,5-trimethoxybenzene as an internal standard were dissolved in 0.2 mL of DMSO-*d*₆.

Spectral processing

After acquiring the linear arrayed kinetics data sets with time-stamp data, the NMR Reaction Monitoring wizard on MestReNova (v12.0.1-20560) was used to process the Fourier transformed spectra. The following methodology was used to process the spectra: 1) the free-induction decay was multiplied by a line-broadening exponential factor of 1 Hz, 2) automatic phase correction applied, 3) baseline correction made using the Bernstein 3rd order Polynomial Fit. The Data Analysis module was then used to select the internal reference signal (one aromatic proton at around 6.5 ppm) and was set to a number of 3 nuclides at a concentration of 0.011 mM. Using the Data Analysis module, a concentration graph was generated for the *independent proton peak* in the starting material and product (selected proton peak was depended on the substrate). The concentration versus time data was exported into excel sheets and processed using reaction progress kinetic analysis.

Calculation of kinetic and thermodynamic parameters

All kinetic and thermodynamic parameters were calculated in excel sheets by using the concentration versus time data. We first calculated each concentration value of non bi-aryl and bi-aryl compounds at equilibrium state as an average value during the equilibrium state and then obtained the equilibrium constant and standard Gibbs energy value (ΔG°) between both compounds. We calculated rate constant (k_{obs}) of first-order reaction by linear regressions from the graphical rate equations. The corresponding activation energy barriers (ΔG^\ddagger) were calculated from the rate constant of forwards reaction (k_f) using Eyring-Polanyi equation. Half-life of the reaction ($t_{1/2}$) was calculated from the k_{obs} value.



$$k_{obs} = k_f + k_b$$

$$\ln([A] - [A]_e) = -k_{obs} t + \ln([A]_0 - [A]_e)$$

$$k = \frac{k_B T}{h} \exp\left(\frac{\Delta G^\ddagger}{RT}\right) : \text{Eyring - Polanyi equation}$$

$$t_{1/2} = \ln 2 / k_{obs}$$

[A]: concentration of A at any given time
 [A]_e: concentration of A at equilibrium
 [A]₀: initial concentration of A
*k*_{obs}: the observed rate constant
*k*_f: the forwards rate constant
*k*_b: the backwards rate constant
*t*_{1/2}: half-life of the reaction

a.

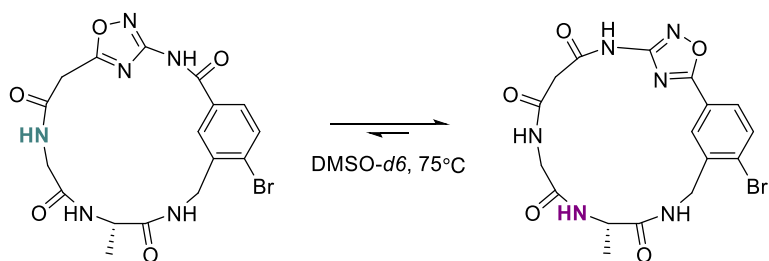
compound	A	R	ΔG° (kcal/mol)	ΔG^\ddagger (kcal/mol)	<i>k</i> _{obs} (s ⁻¹) [a]	<i>t</i> _{1/2} (h) [a]
malonamide linear precursor	-CH ₂ -	Et	-1.32	24.2	5.0 x 10 ⁻⁴	0.39
succinamide linear precursor	-(CH ₂) ₂ -	Me	-1.59	23.7	10.0 x 10 ⁻⁴	0.19

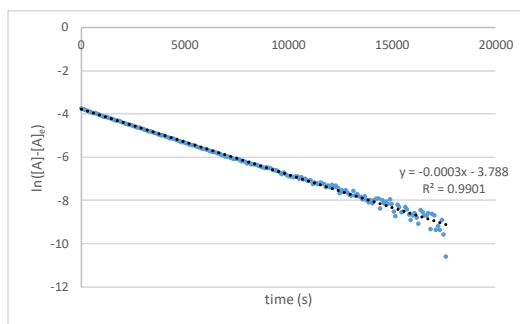
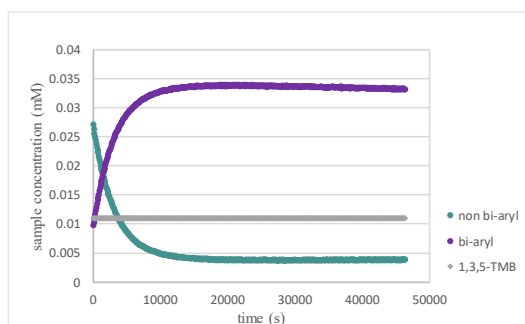
b.

Entry	A	n	Peptide sequence	Ring size	ΔG° (kcal/mol)	ΔG^\ddagger (kcal/mol)	<i>k</i> _{obs} (s ⁻¹) [a]	<i>t</i> _{1/2} (h) [a]
1	-CH ₂ -	2	Gly-Ala	18	-1.51	24.6	3.0 x 10 ⁻⁴	0.64
2	-CH ₂ -	2	Ala-Leu	18	-2.55	24.8	2.0 x 10 ⁻⁴	0.99
3	-CH ₂ -	2	Gly-Pro	18	-1.38	24.9	2.0 x 10 ⁻⁴	0.96
4	-(CH ₂) ₂ -	1	GABA	18	-1.43	24.8	2.0 x 10 ⁻⁴	0.96
5	-(CH ₂) ₂ -	2	Ala-Leu	19	-1.12	23.9	8.0 x 10 ⁻⁴	0.24
6	-(CH ₂) ₂ -	1	β-Ala	17	-4.00	25.7	17.0 x 10 ⁻⁴	0.11
7	-(CH ₂) ₂ -	1	Ala	16		No reaction		
8	-(CH ₂) ₂ -	1	Gly	16		No reaction		
9	-(CH ₂) ₅ -	0	-	16		No reaction		

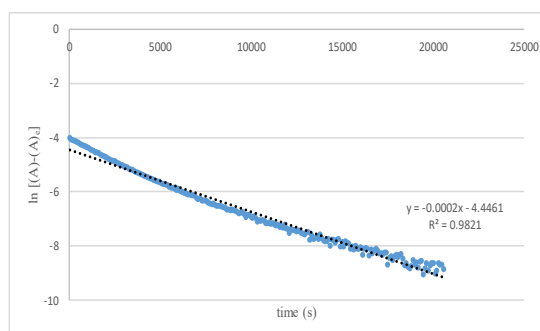
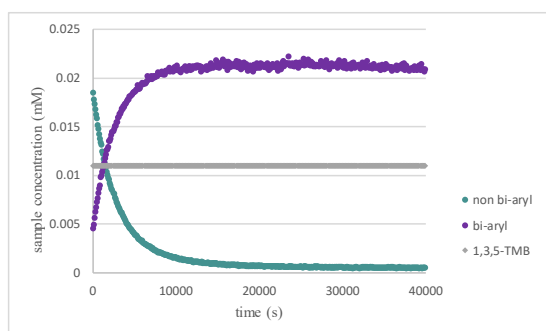
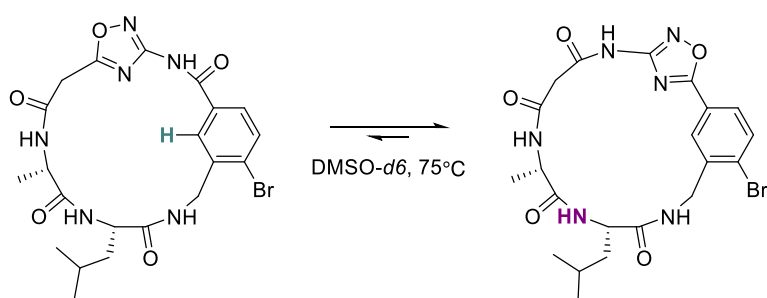
^a calculated at 75 °C except for entry 6 & 10 in which those were calculated at 110 °C.

1. Macrocycle **1a** and **1b**

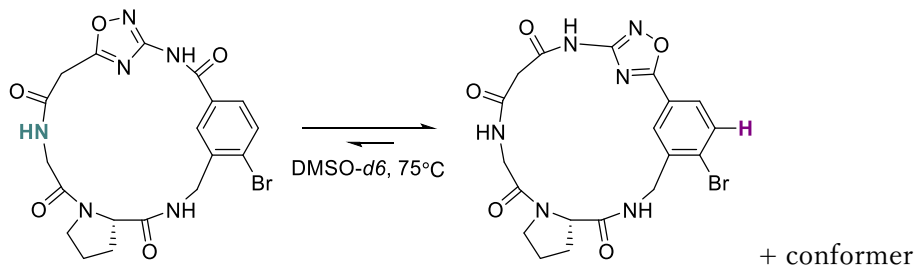


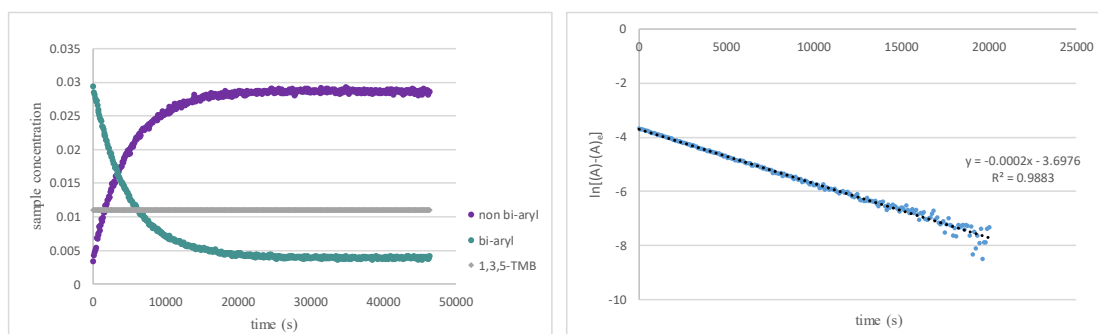


2. Macrocycle **2a** and **2b**

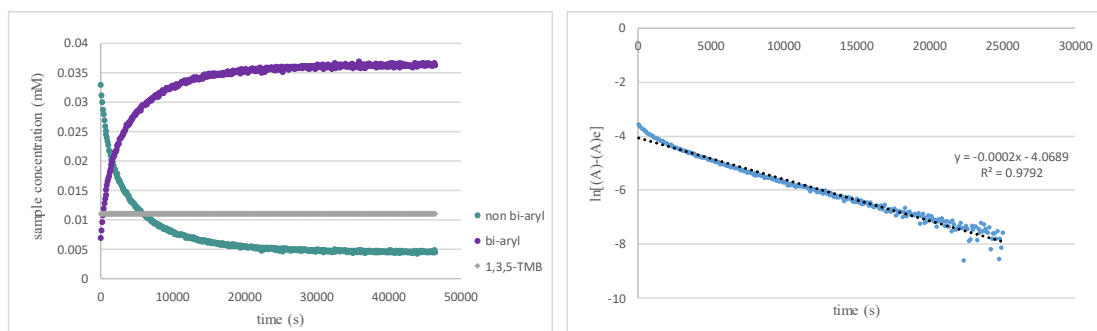
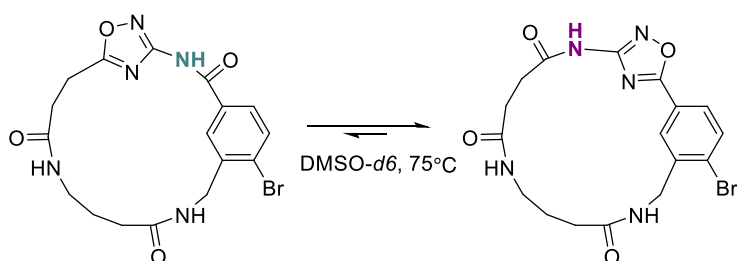


3. Macrocycle **3a** and **3b**

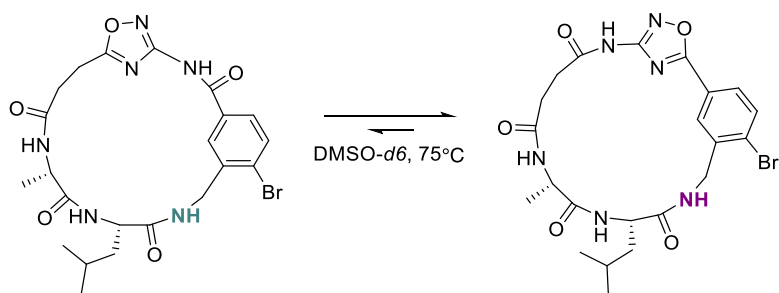


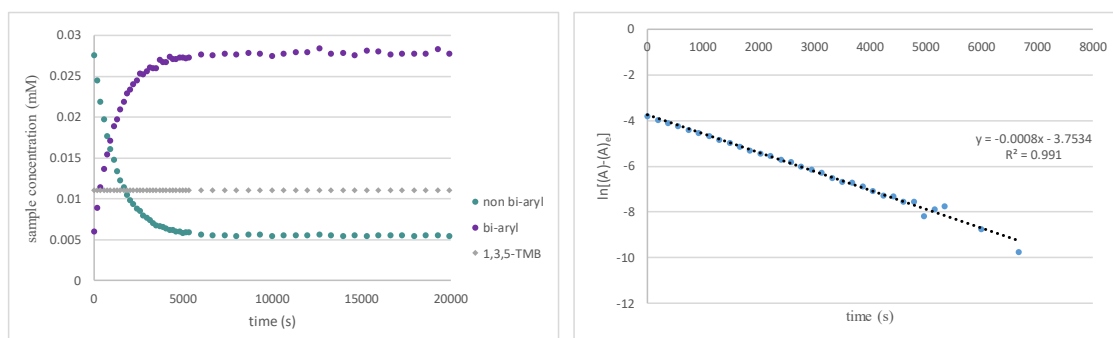


4. Macrocycle **4a** and **4b**

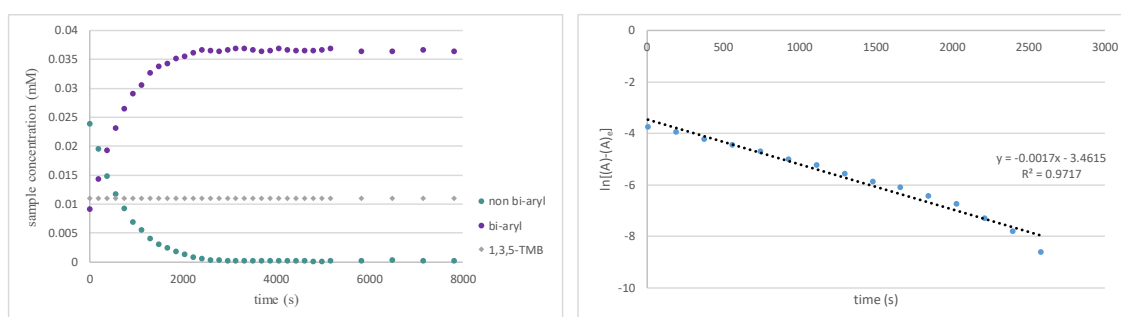
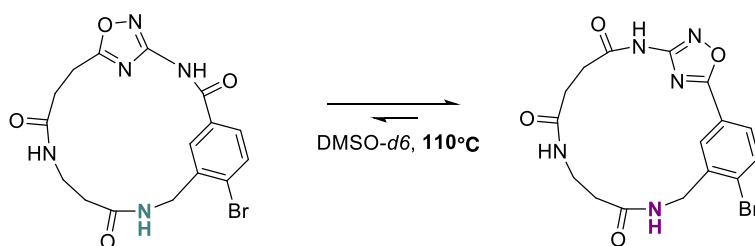


5. Macrocycle **5a** and **5b**

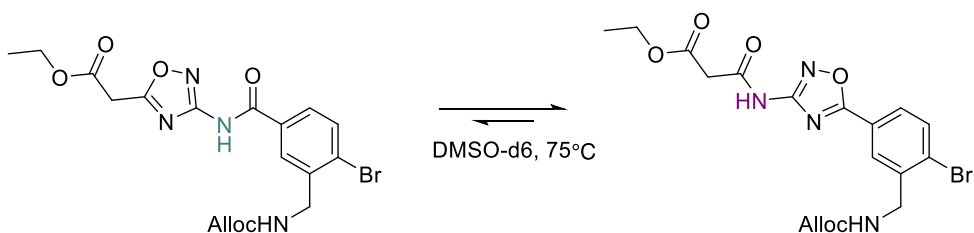


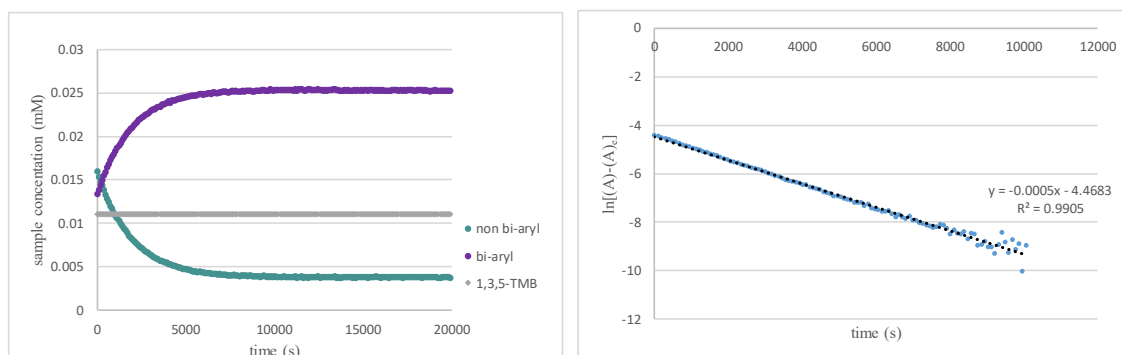


6. Macrocycle **6a** and **6b**

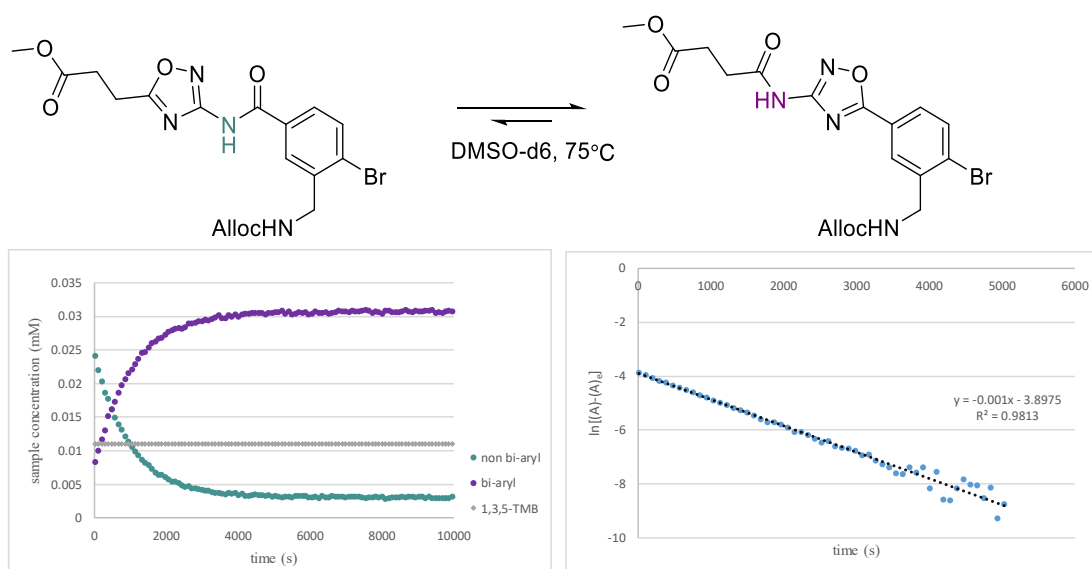


7. Malonamide linear precursor (**11**)





8. Succinamide linear precursor (12)



Solution Structure Determination: The NMR structures were determined by a combination of NMR derived distance information and molecular dynamics studies. ROESY spectra were integrated by using MestreNova (v.12.0.0, Mestrelab Research S.L.) software. Integrated volumes of ROE cross-peaks were converted to proton interatomic distances using an inverse sixth power relationship. A reference integral was calculated as the average integral between sets of geminal protons which was then set to the calculated geminal interproton distance of 1.78 Å. The calculated distances were adjusted upwards and downwards by 10% to give upper and lower bounds to account for uncertainty in interproton distances.³J coupling constants were recorded from the ¹H spectrum. NH-CαH ³J coupling constants of < 6 Hz were assigned phi dihedral values of -60° +/- 25°. NHCαH ³J coupling constants of > 8 Hz were assigned phi values of -120° +/- 25°. Crude structures of macrocycles were generated by a restrained Monte

Carlo low mode molecular mechanics conformational search with an implicit solvent model (DMSO) in MacroModel (Schrodinger LLC, v11.0). The lowest energy structure from the conformational search which satisfied the distance and angle restraints was used for molecular dynamics simulations. The restrained Monte Carlo searches for both macrocycles investigated during this indicated a *cis* amide-containing conformation was the lowest energy possibility that satisfied the distance and angle restraints. In order to investigate the possibility of a *trans* amide structure, an additional 180° torsional restraint was applied to the amide linkage to generate a *trans* amide structure which also satisfied the distance and torsional restraints. Both *cis* and *trans* possibilities were subjected to molecular dynamics and the final violations compared to identify the correct structure. Solvent explicit molecular dynamics simulations were carried out with the Desmond Molecular Dynamics software module (D.E. Shaw, v4.4) running inside Maestro (Schrodinger LLC, v2015- 2). The OPLS2005 force field was used for parameterization of the peptidomimetic macrocycle. The macrocycle representative structure was placed in an octahedral box solvent box (DMSO) with a minimum distance of 12 Å between solute atoms and the box boundary. The solvated box was minimized then brought to 300 K from 10 K using a restrained dynamics regime. Coulombic interactions were grouped into near- and far interactions with a near-interaction cutoff of 9 Å. Bonds were constrained with the SHAKE algorithm and an integration time step of 2 fs was used. The final MD production run was 100 ns in length with energy value recording every 1.2 ps and trajectory recording every 4.8 ps. The run trajectory was clustered using the Trajectory Clustering script within Maestro with a 0.4 Å RMSD cutoff for variation between backbone heavy atoms and a sampling frequency of 10%. The most populated cluster was taken as the “preferred” structure i.e. that conformation which the molecule spent most time in the dynamics run. The average interproton distances were measured, compared to the experimental NMR derived distances and the violations were tabulated below.

Results of MD simulation for macrocycle **1a**

Atom1	Atom2	NOE derived distance	Upper bound	Lower bound	Cis isomer	Trans isomer
Gly-NH	Ala-NH	3.20	3.52	2.88	3.13	3.95
Ala-NH	Bn-NH	2.66	2.93	2.40	2.34	4.41
Ala-NH	Ala-α	3.05	3.35	2.74	3.01	3.01

Bn-NH	Ala- α	2.94	3.23	2.64	3.00	2.09
Bn-NH	Ph-2	3.20	3.52	2.88	3.50	2.90

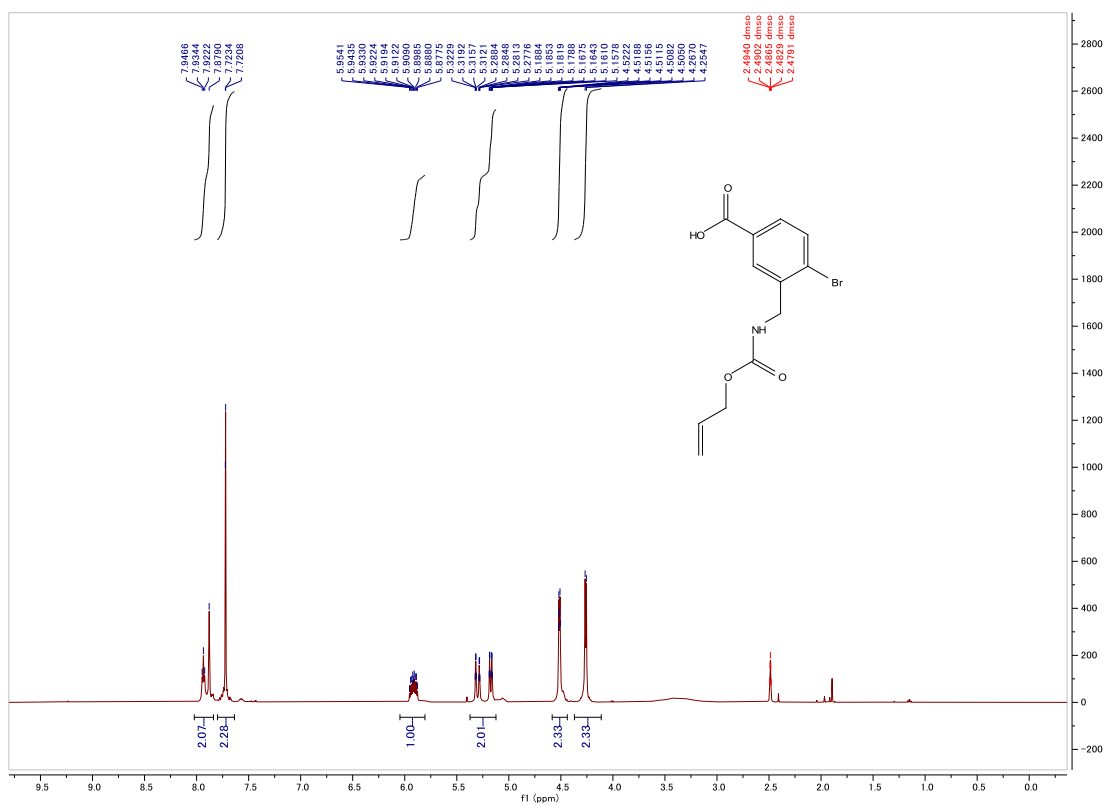
Results of MD simulation for macrocycle **1b**

Atom1	Atom2	NOE derived distance	Upper bound	Lower bound	Cis isomer	Trans isomer
Bn-NH	Ph-2	2.65	2.92	2.38	2.51	3.29
Bn-NH	Ala-NH	2.84	3.12	2.55	2.59	4.71
Bn-NH	Ala- α	2.61	2.87	2.35	2.62	2.21
Ala-NH	Ala- α	2.93	3.22	2.63	2.82	2.87

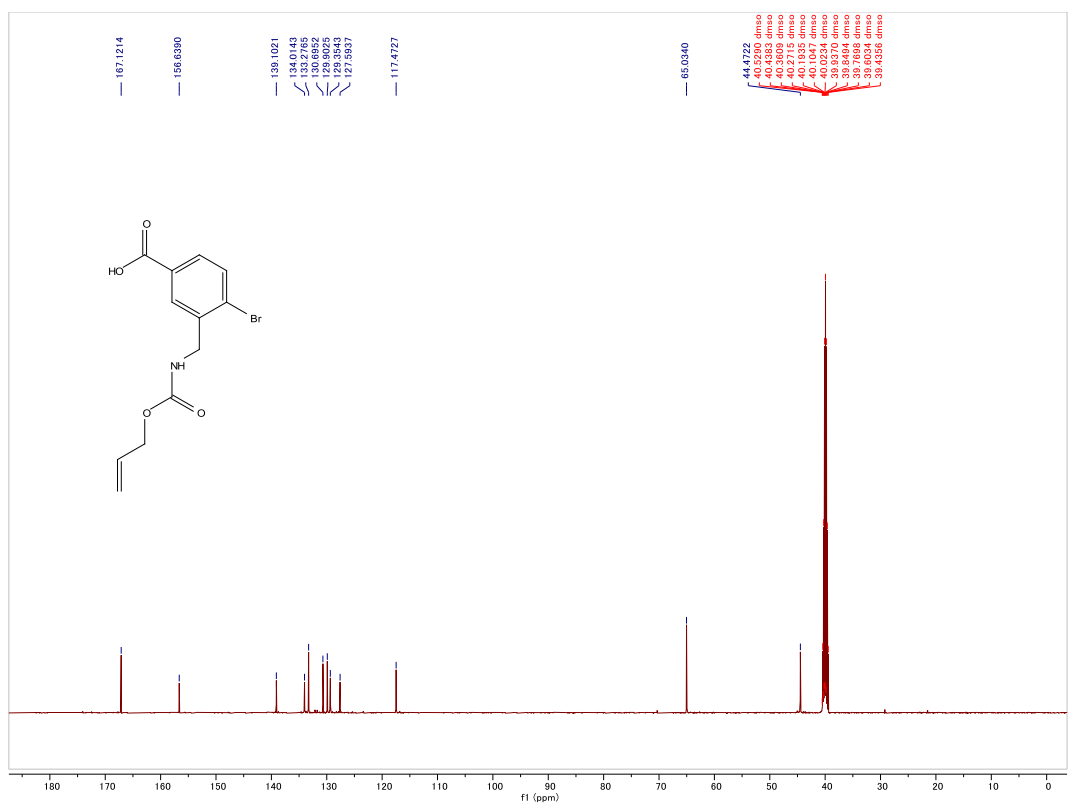
¹H NMR and ¹³C NMR spectra

3-((((allyloxy)carbonyl)amino)methyl)-4-bromobenzoic acid (13)

¹H NMR (500 MHz, DMSO-*d*₆)

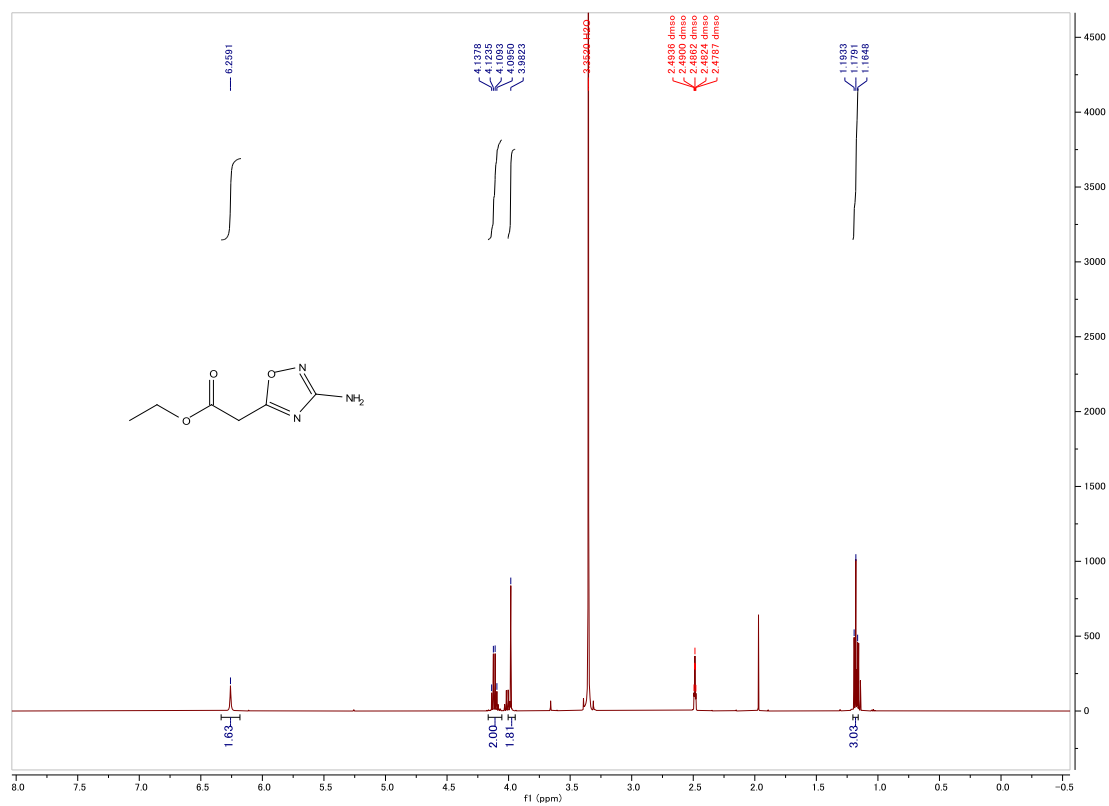


¹³C NMR (126 MHz, DMSO-*d*₆)

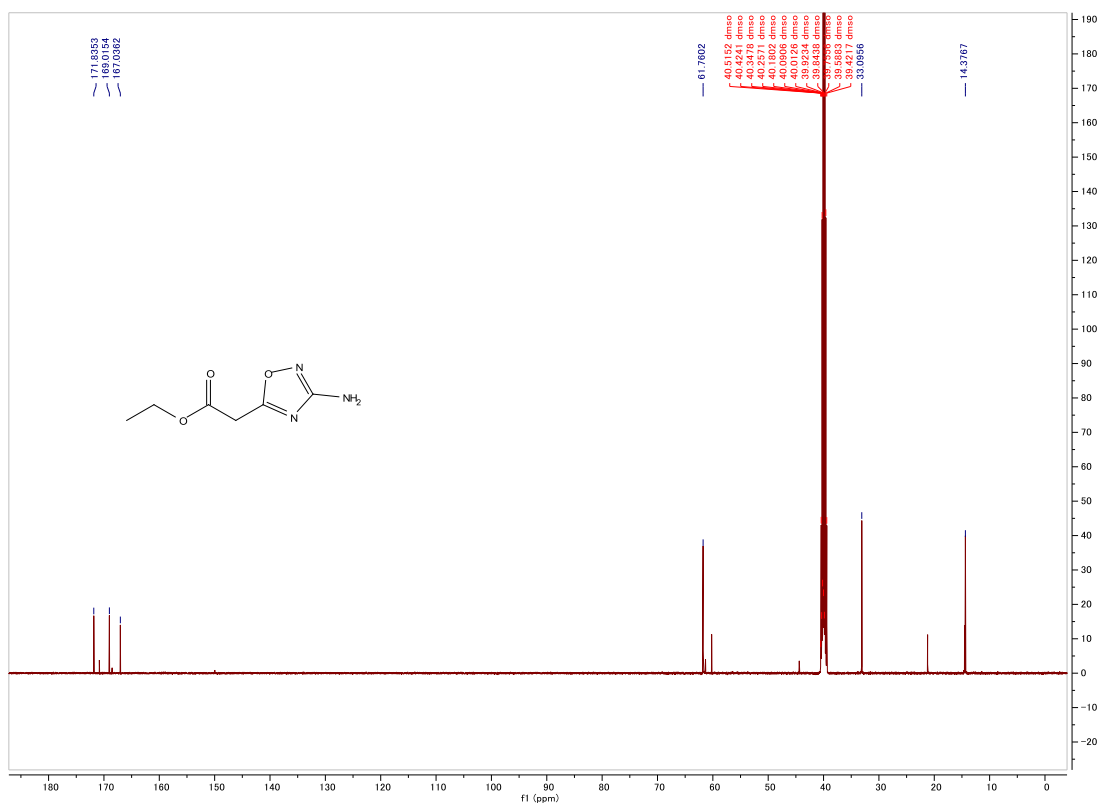


Ethyl 2-(3-amino-1,2,4-oxadiazol-5-yl)acetate (14)

^1H NMR (500 MHz, $\text{DMSO}-d_6$)

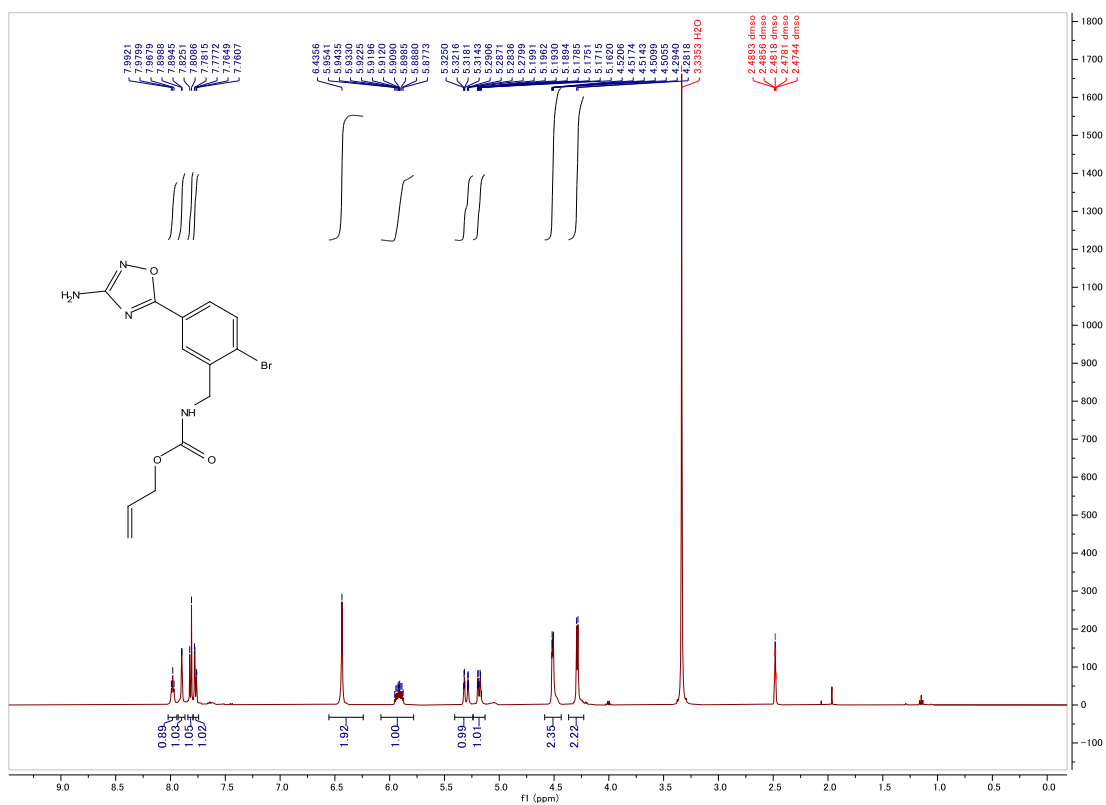


^{13}C NMR (126 MHz, $\text{DMSO}-d_6$)

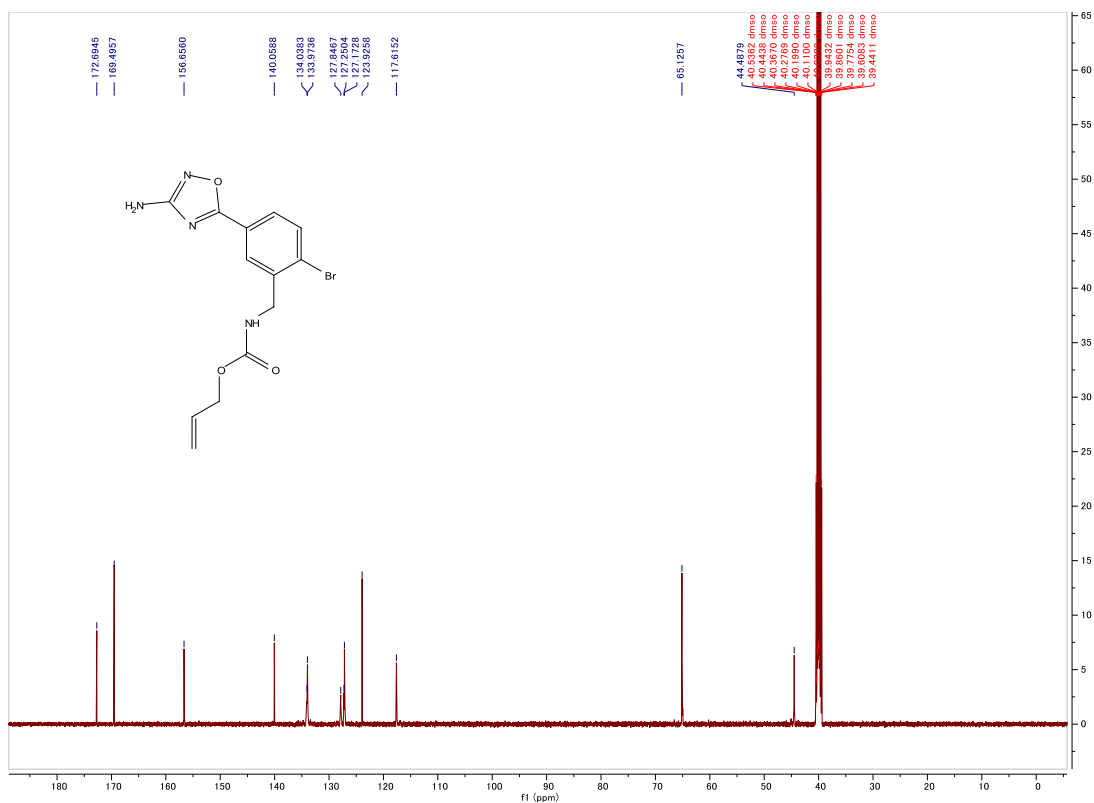


Allyl (5-(3-amino-1,2,4-oxadiazol-5-yl)-2-bromobenzyl)carbamate (15)

¹H NMR (500 MHz, DMSO-*d*₆)

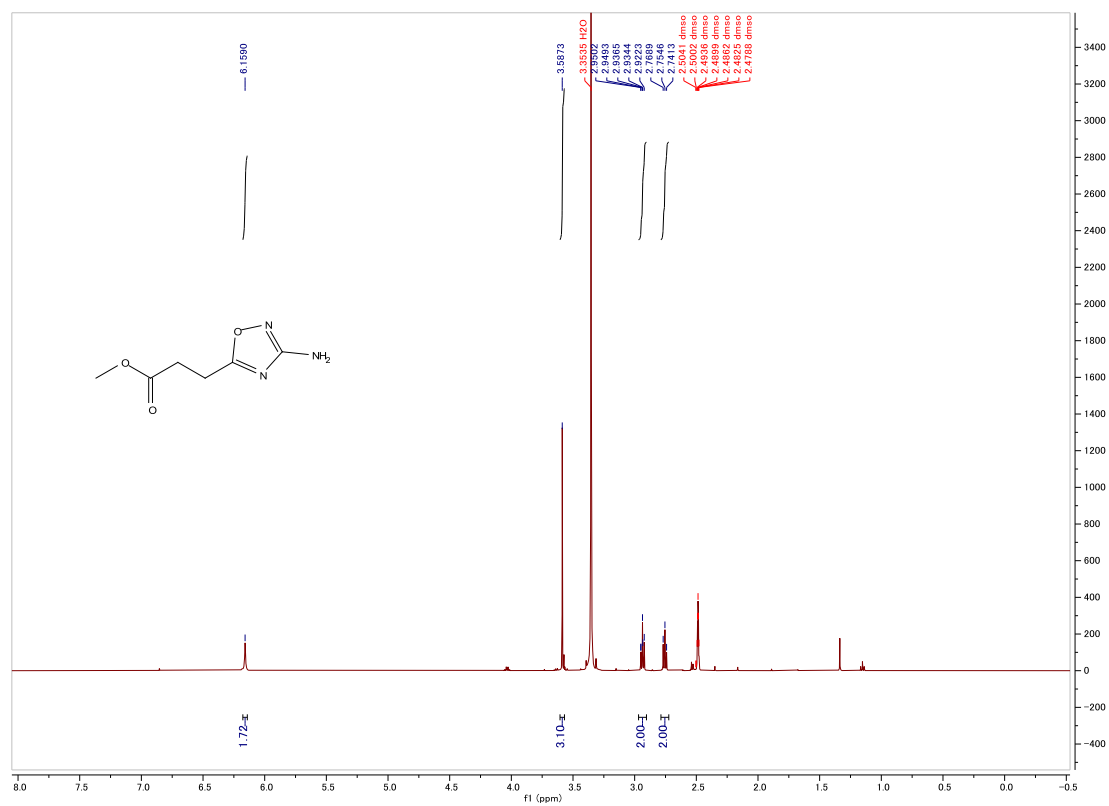


¹³C NMR (126 MHz, DMSO-*d*₆)

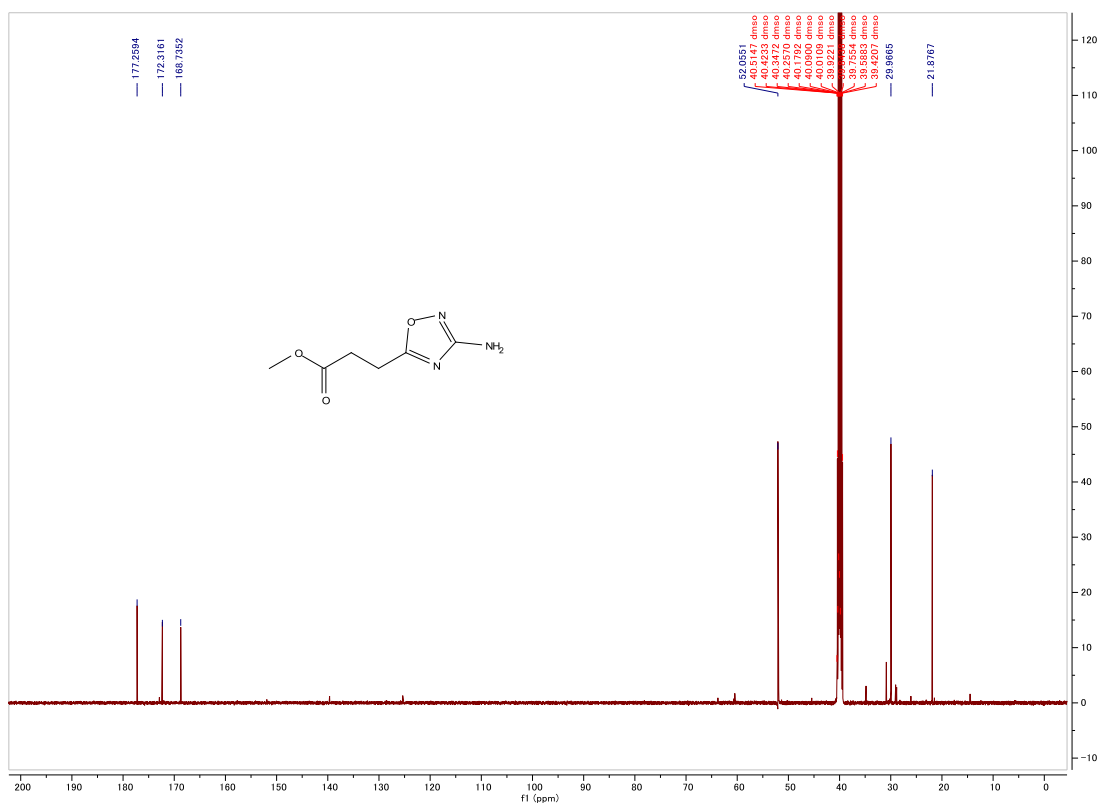


Methyl 3-(3-amino-1,2,4-oxadiazol-5-yl)propanoate (16)

^1H NMR (500 MHz, $\text{DMSO}-d_6$)

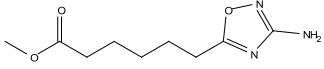
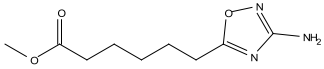


^{13}C NMR (126 MHz, $\text{DMSO}-d_6$)



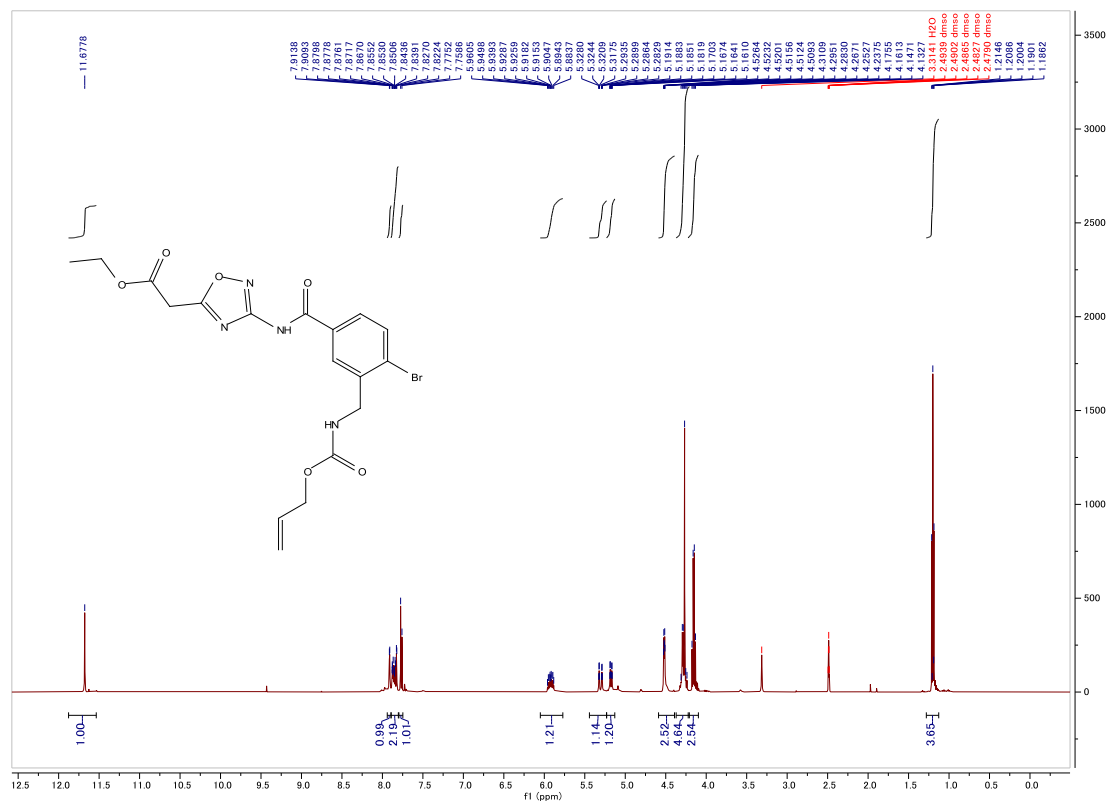
Methyl 6-(3-amino-1,2,4-oxadiazol-5-yl)hexanoate (17)

¹H NMR (400 MHz, DMSO-*d*₆)

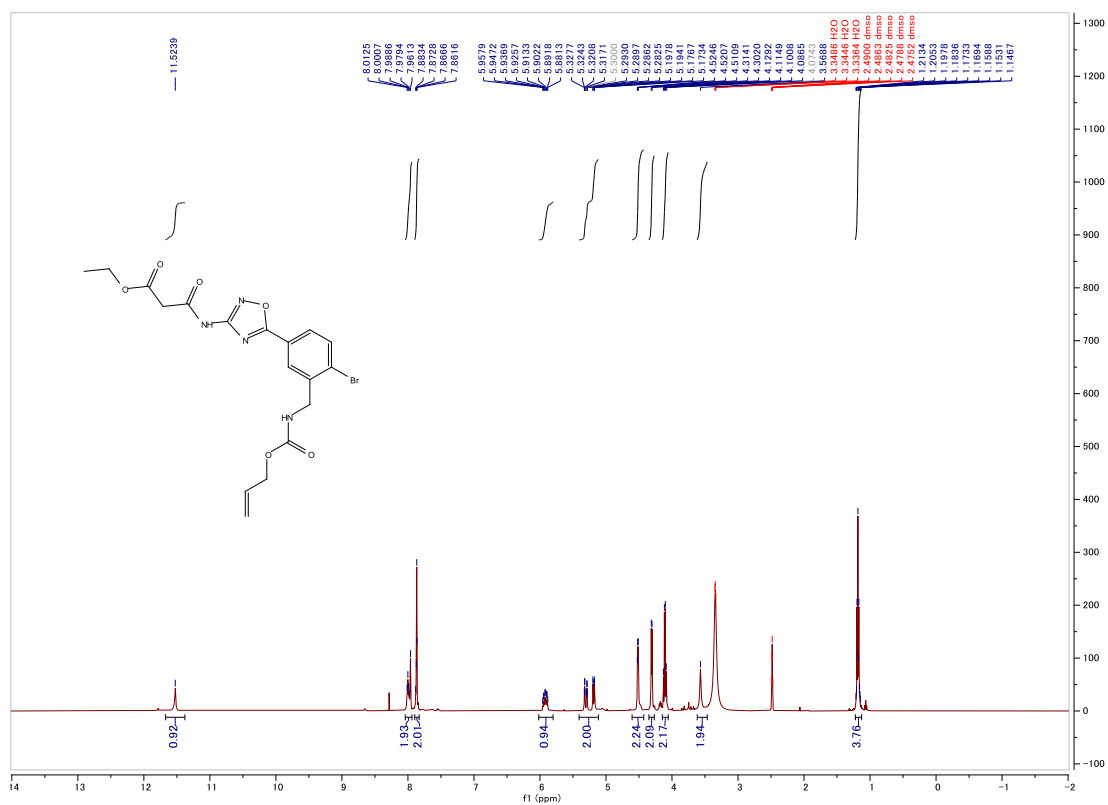


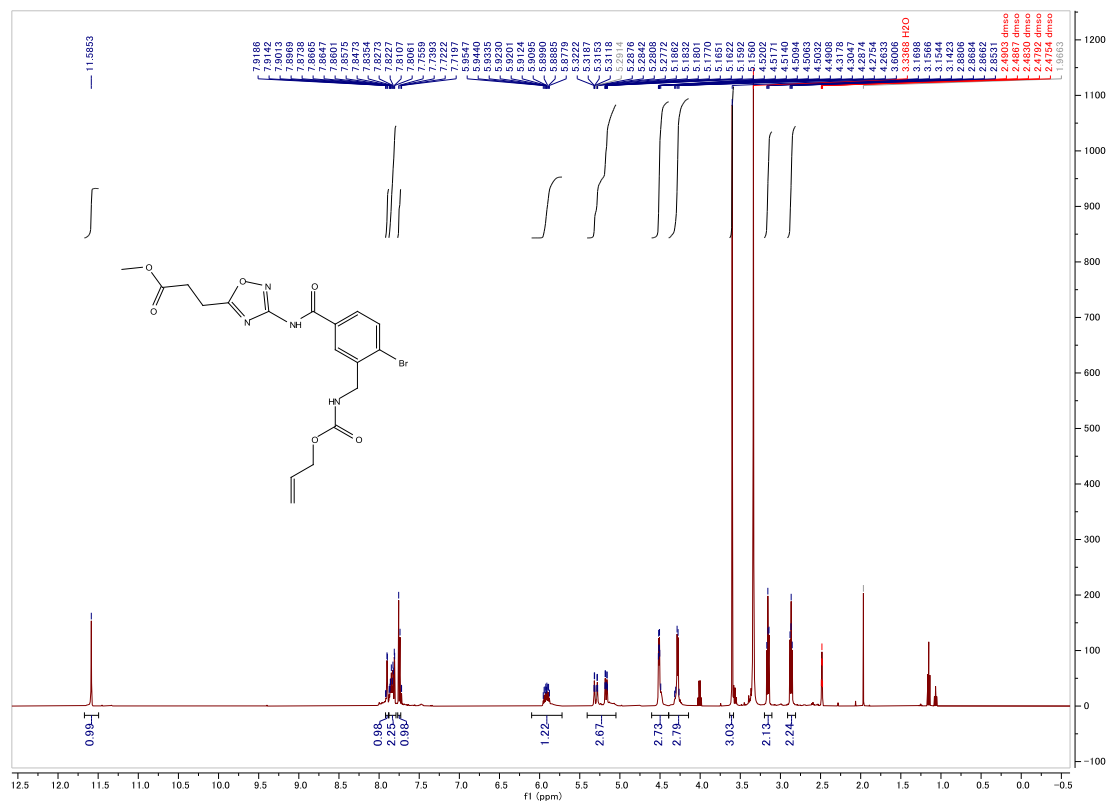
Ethyl 2-(3-(3-((((allyloxy)carbonyl)amino)methyl)-4-bromobenzamido)-1,2,4-oxadiazol-5-yl)acetate (11)

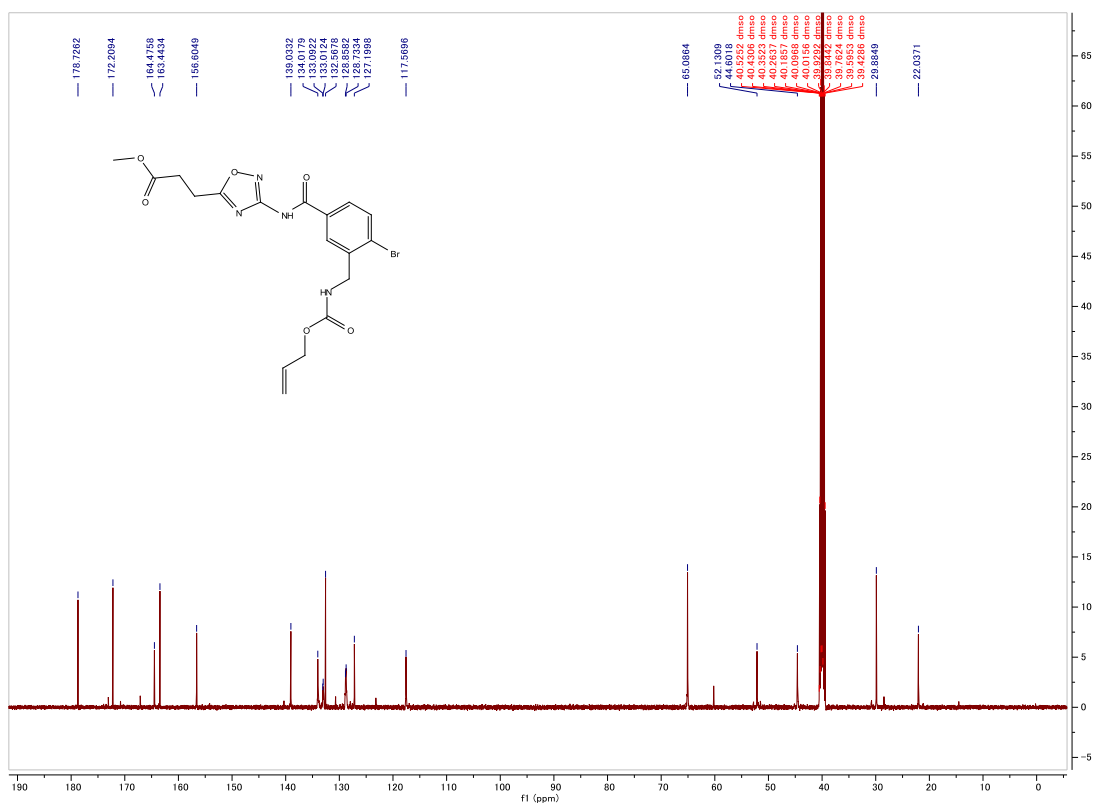
^1H NMR (500 MHz, $\text{DMSO}-d_6$)

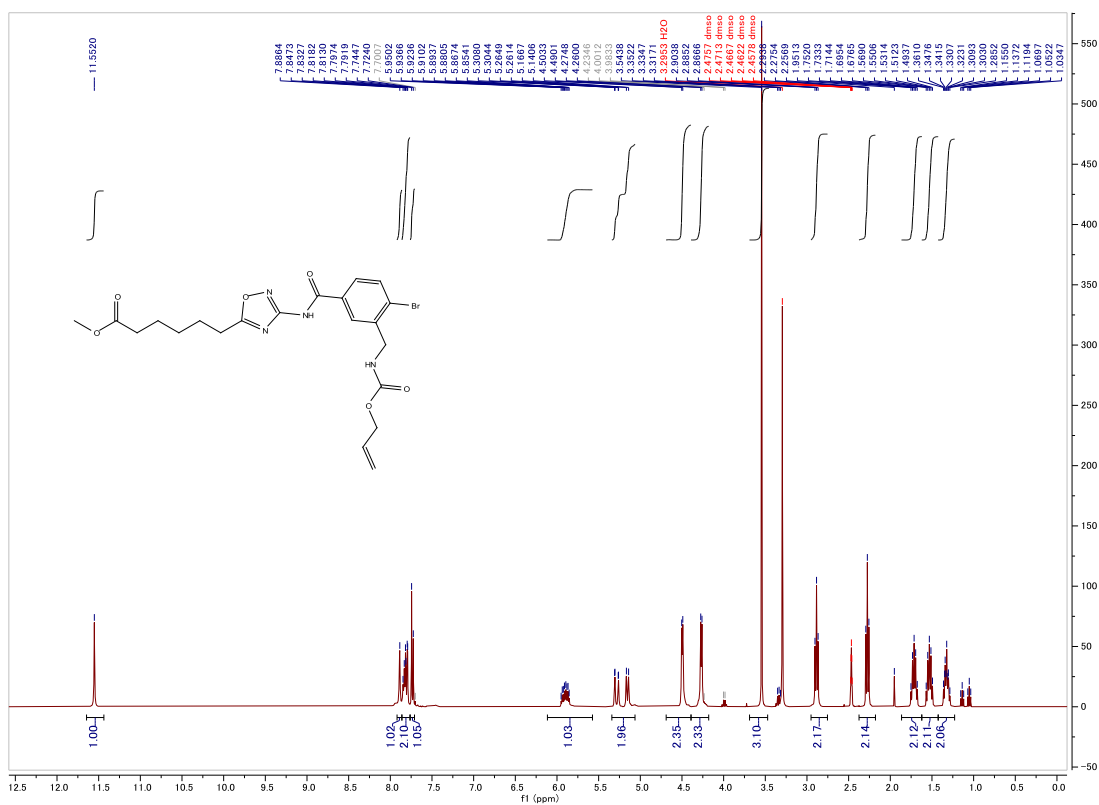


^{13}C NMR (126 MHz, $\text{DMSO}-d_6$)

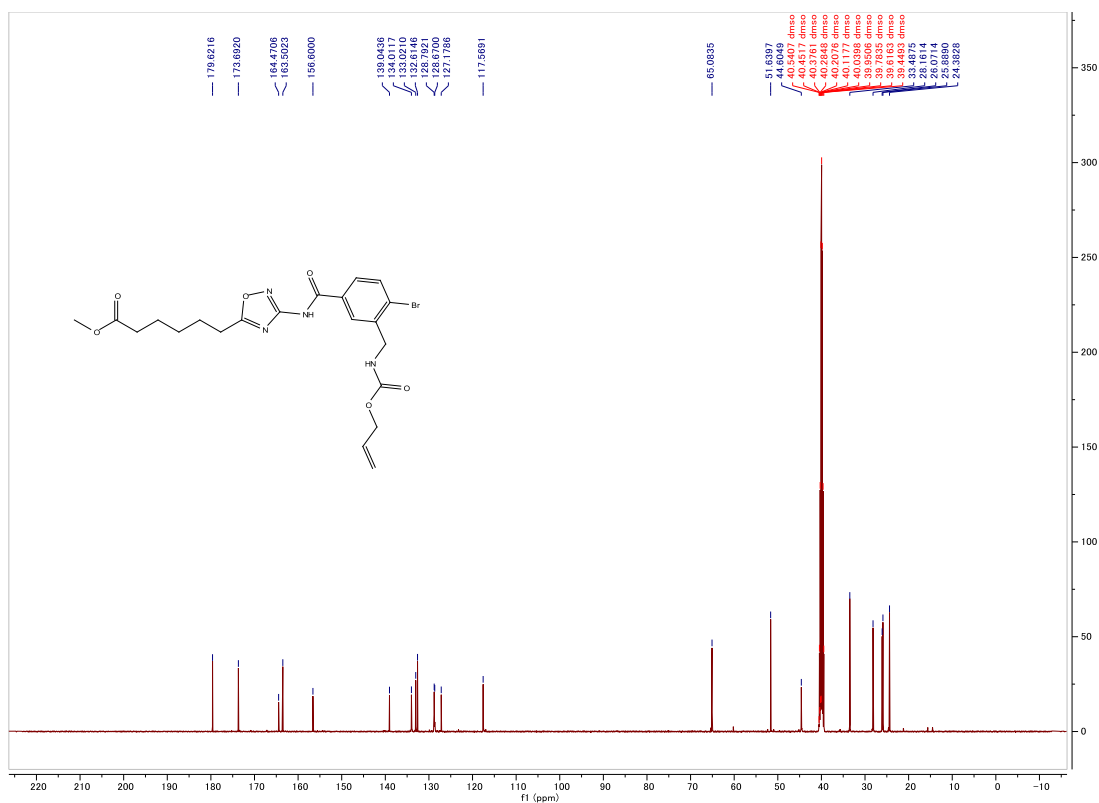


¹H NMR (500 MHz, DMSO-*d*₆) ^{13}C NMR (126 MHz, DMSO- d_6)



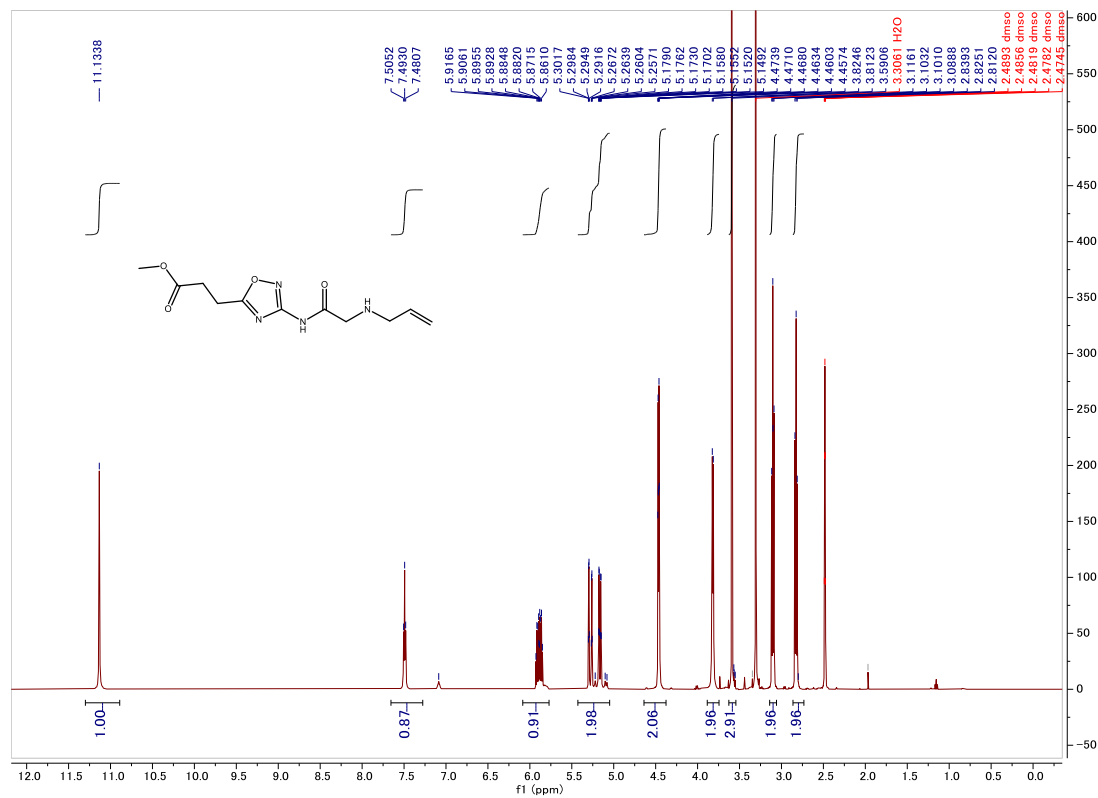


¹³C NMR (126 MHz, DMSO-*d*₆)

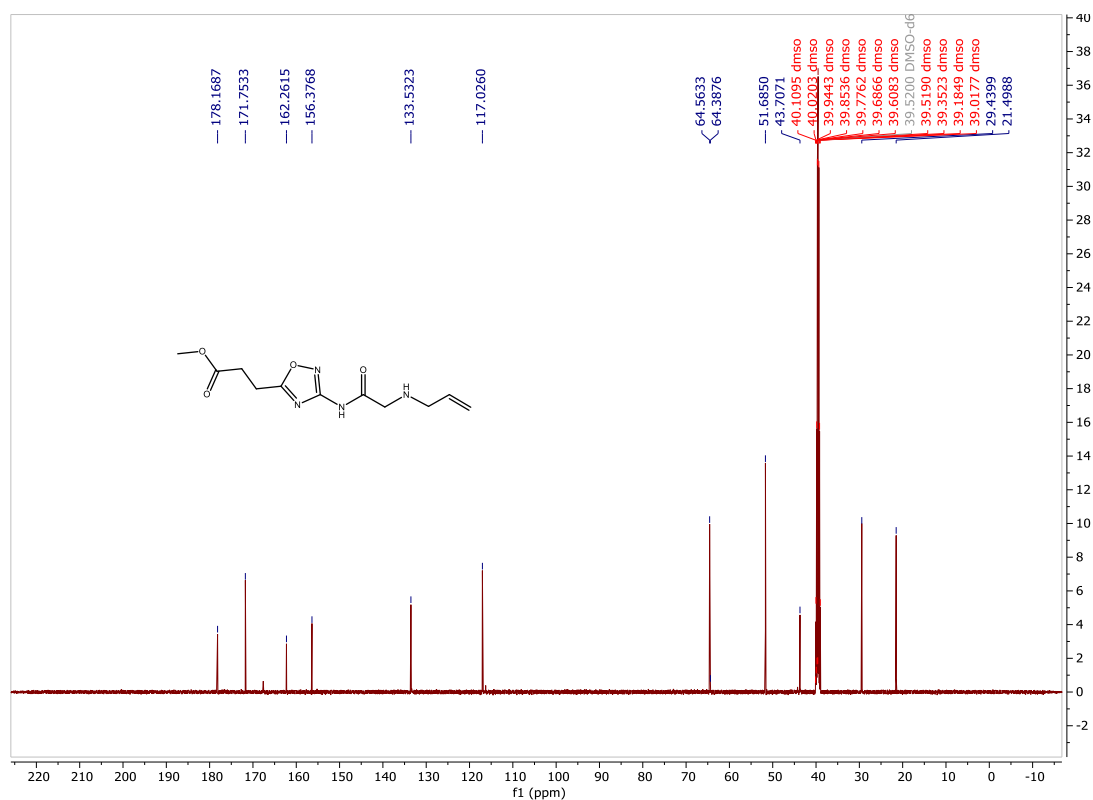


Methyl 3-(3-(2-(((allyloxy)carbonyl)amino)acetamido)-1,2,4-oxadiazol-5-yl)propanoate (20)

^1H NMR (500 MHz, $\text{DMSO}-d_6$)

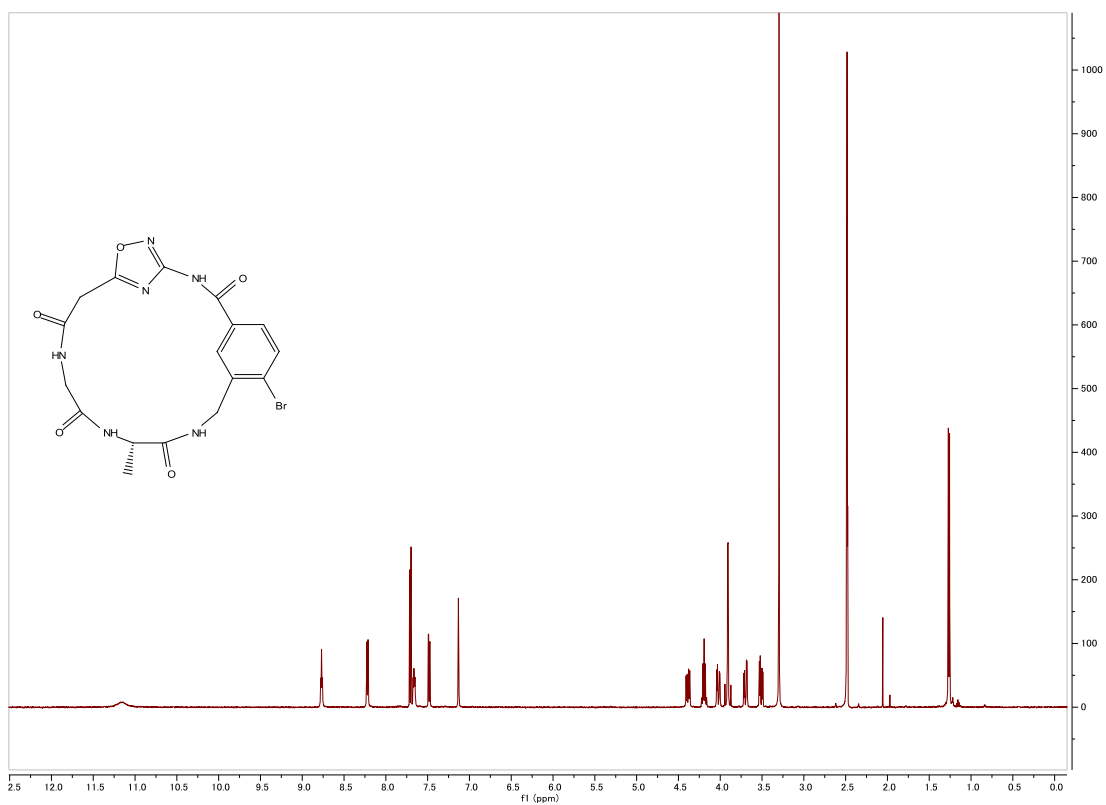


^{13}C NMR (126 MHz, $\text{DMSO}-d_6$)

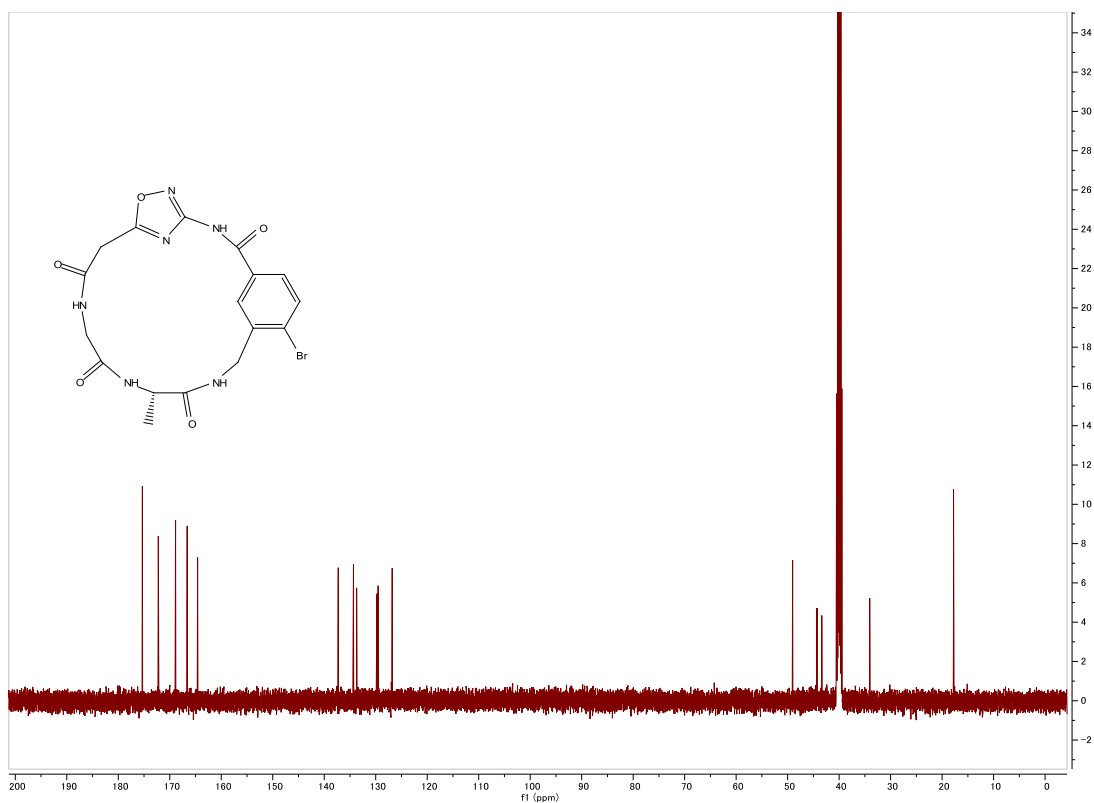


Non bi-aryl macrocycle (1a)

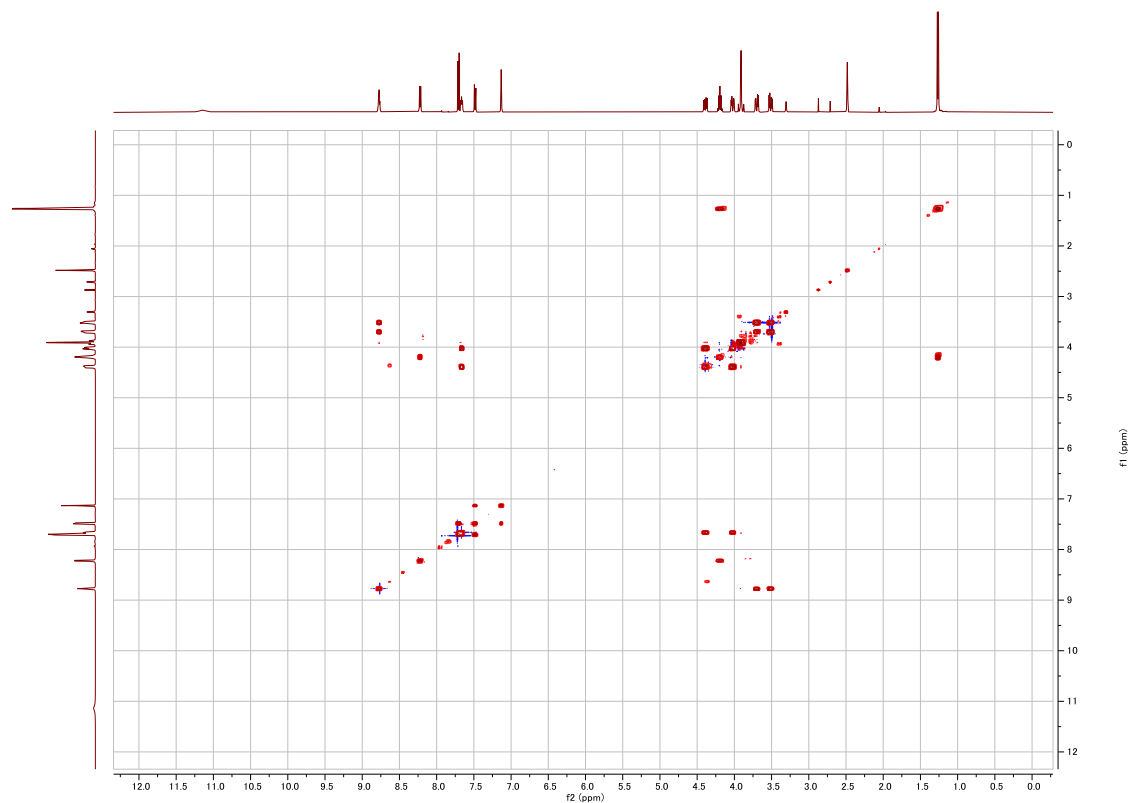
¹H NMR (500 MHz, DMSO-*d*₆)



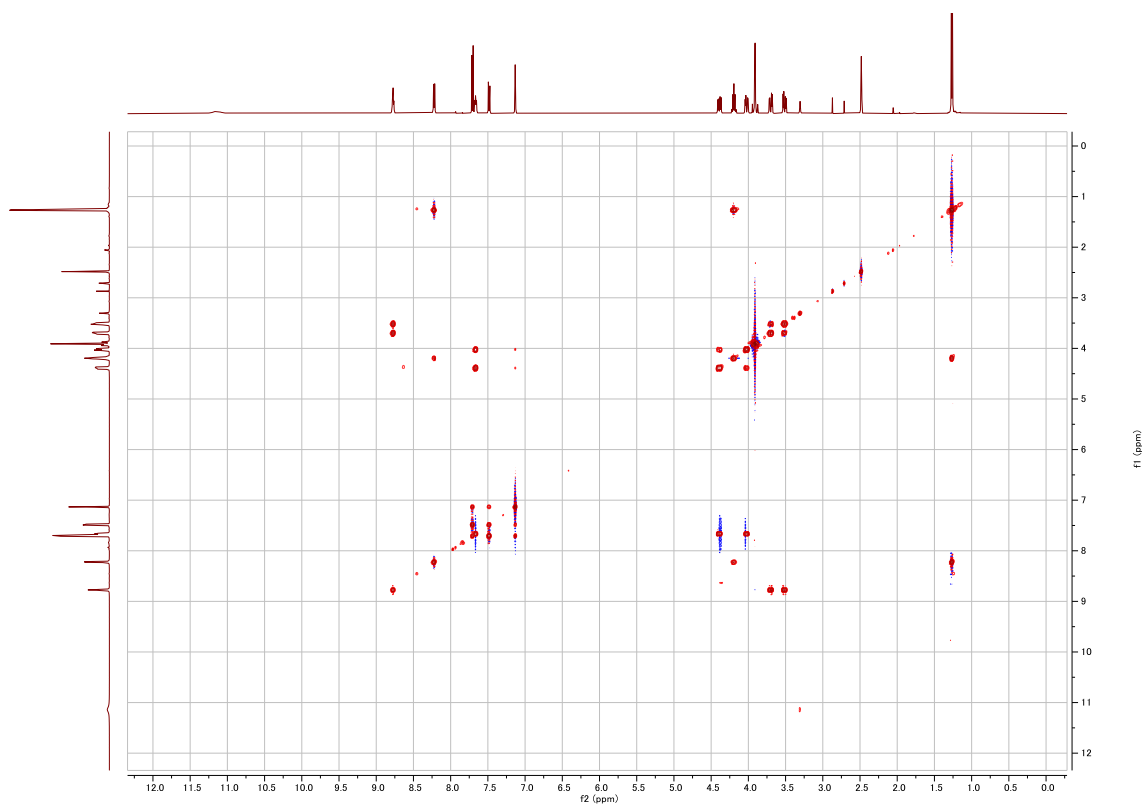
^{13}C NMR (126 MHz, $\text{DMSO}-d_6$)



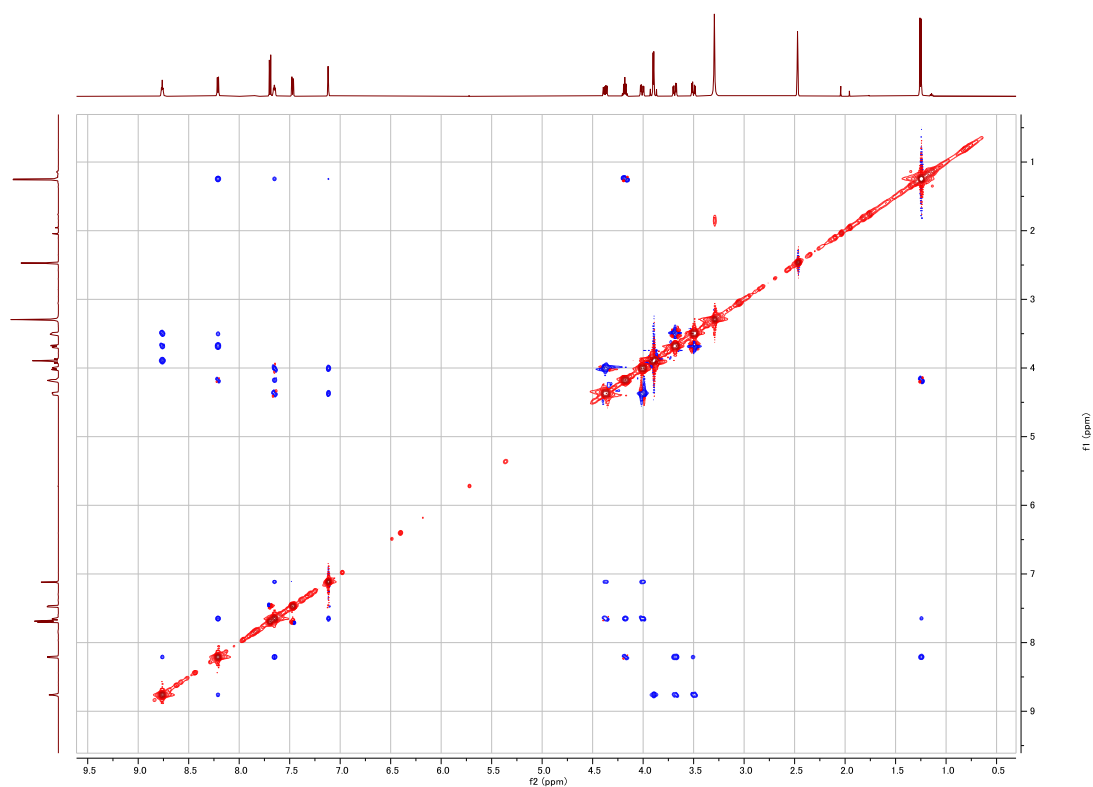
gCOSY (500 MHz, DMSO-*d*₆)



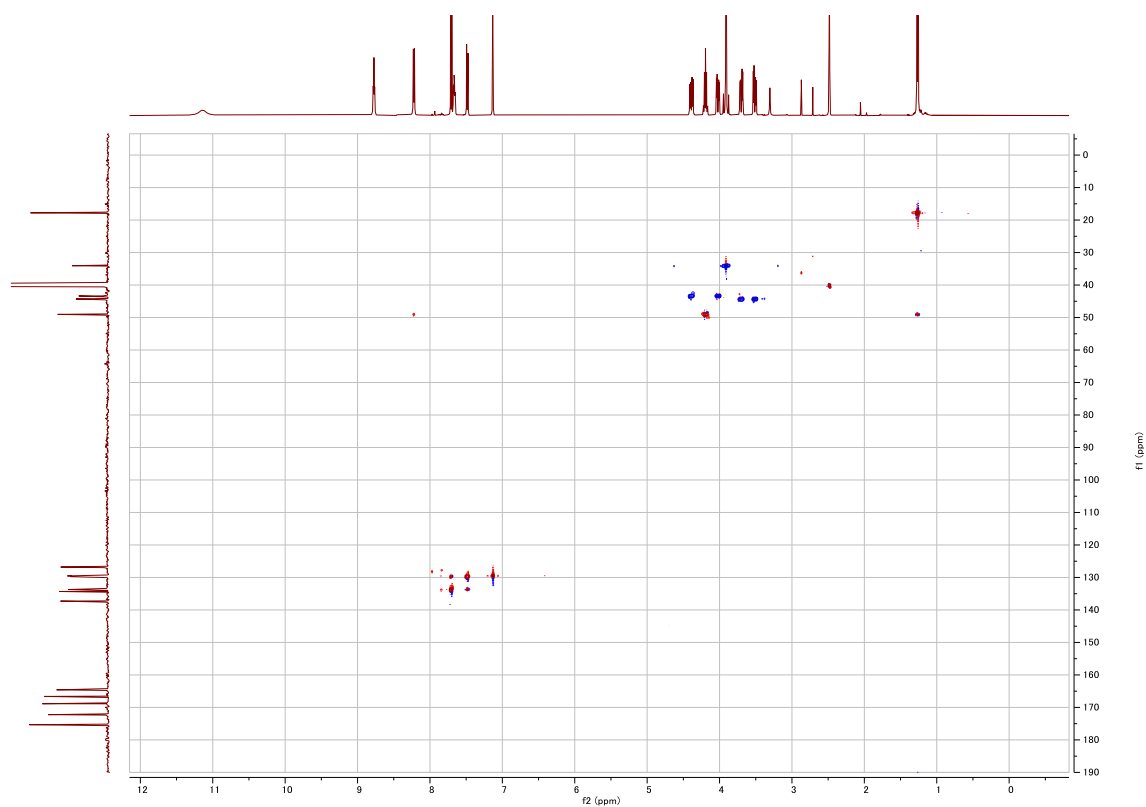
zTOCSY (500 MHz, DMSO-*d*₆)



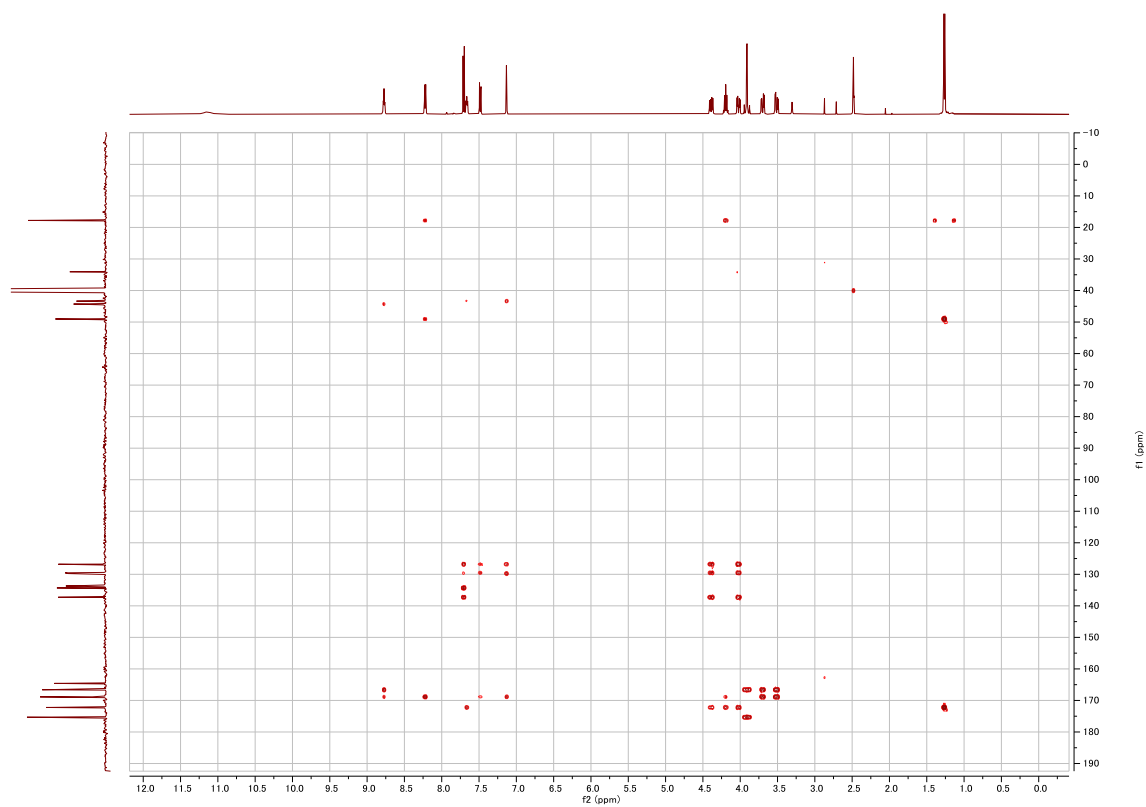
ROESY (600 MHz, DMSO-*d*₆, 150 ms mixing time)



HSQC (500 MHz, DMSO-*d*₆)

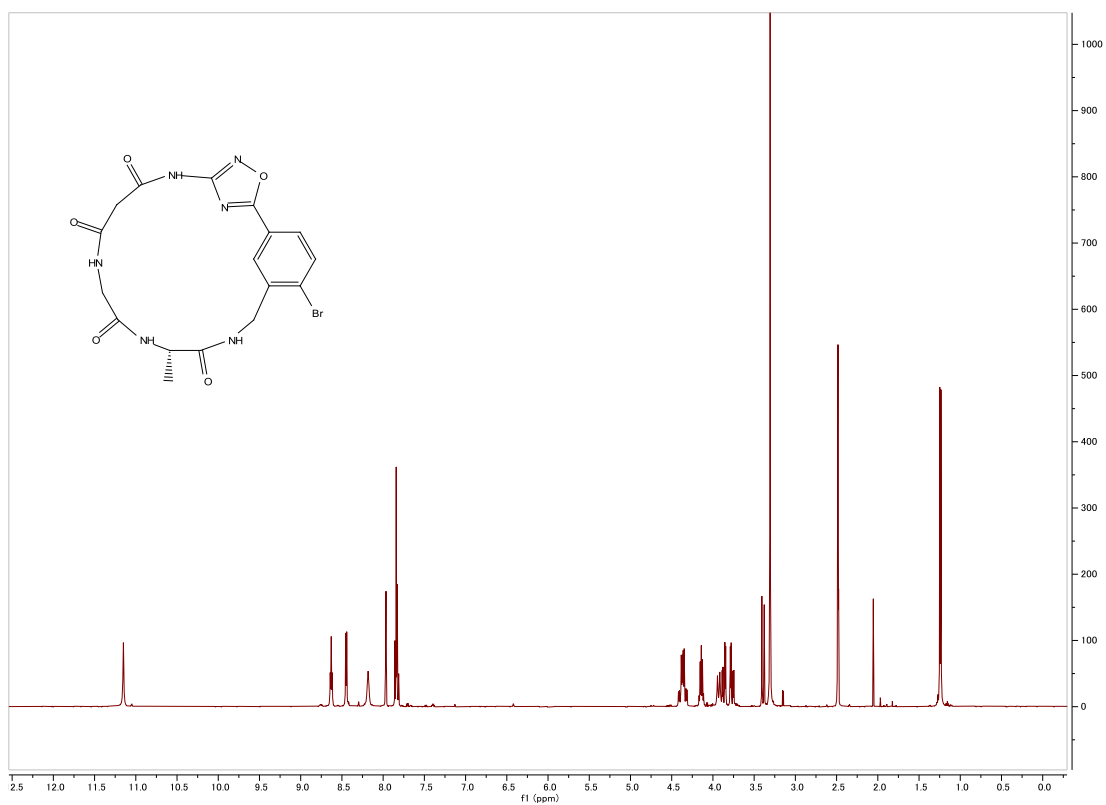


HMBC (500 MHz, DMSO-*d*₆)

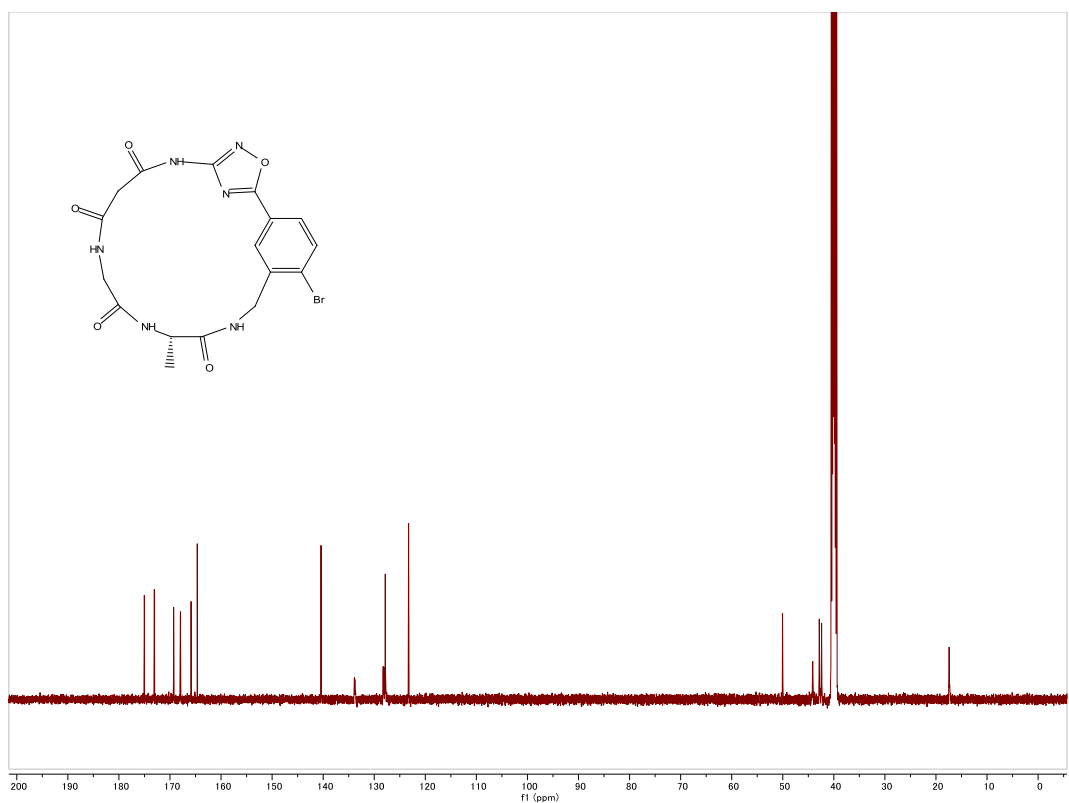


Bi-aryl macrocycle (1b)

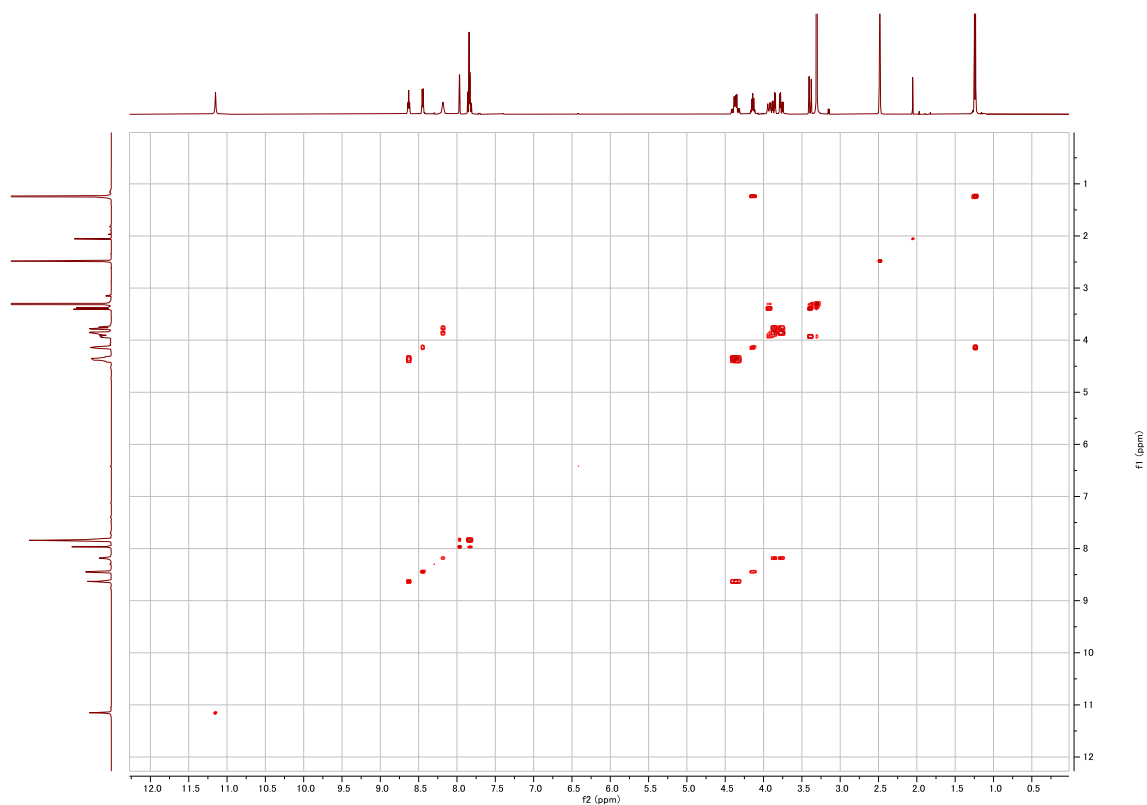
¹H NMR (400 MHz, DMSO-*d*₆)



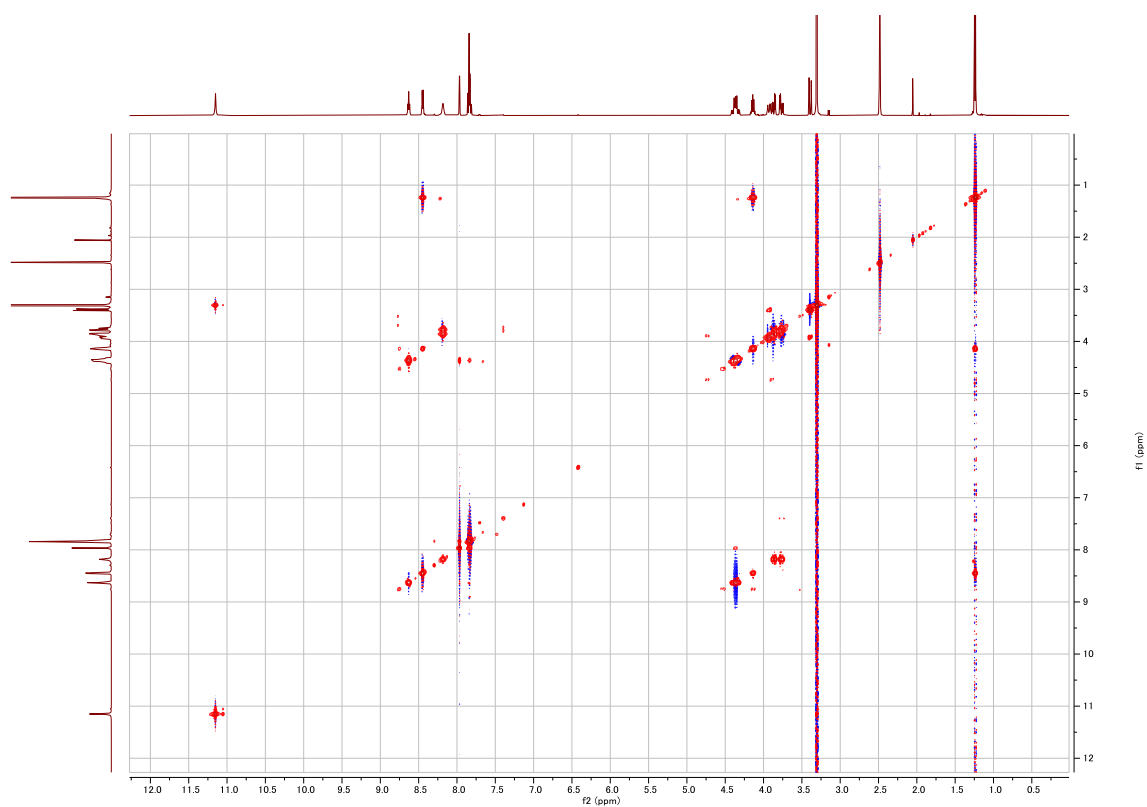
^{13}C NMR (126 MHz, $\text{DMSO}-d_6$)



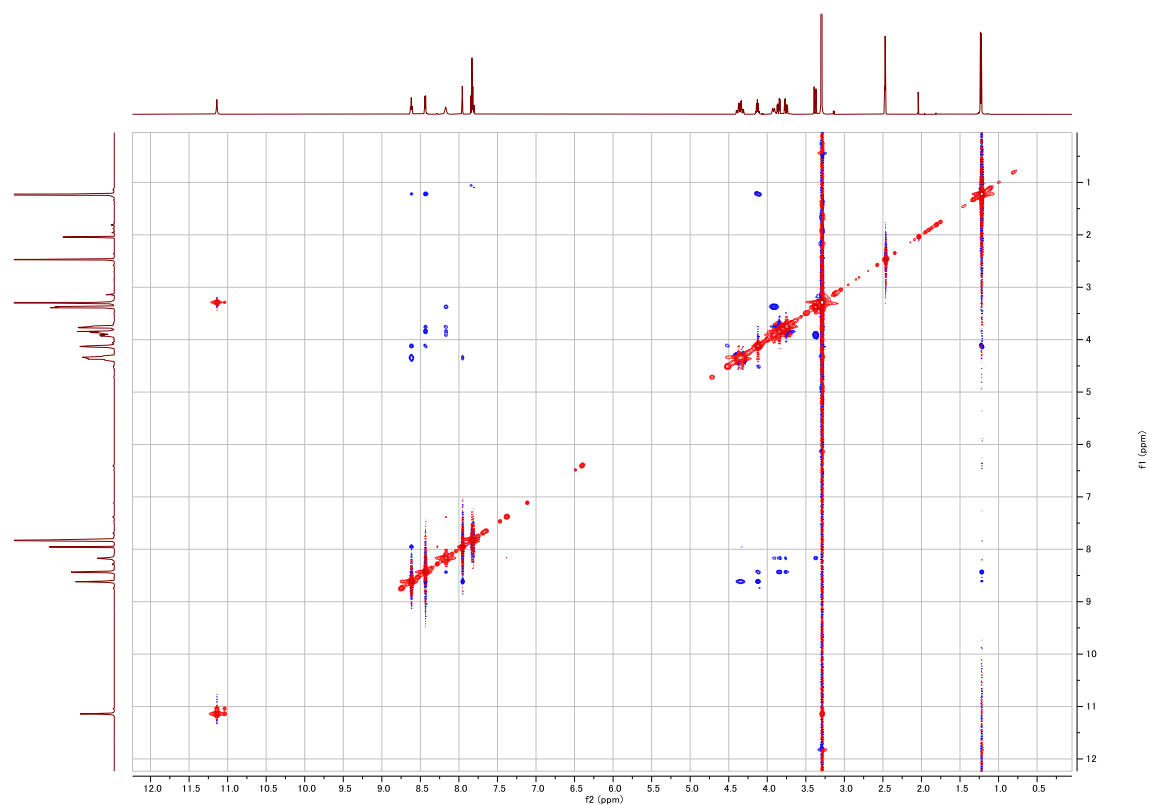
gCOSY (500 MHz, DMSO-*d*₆)



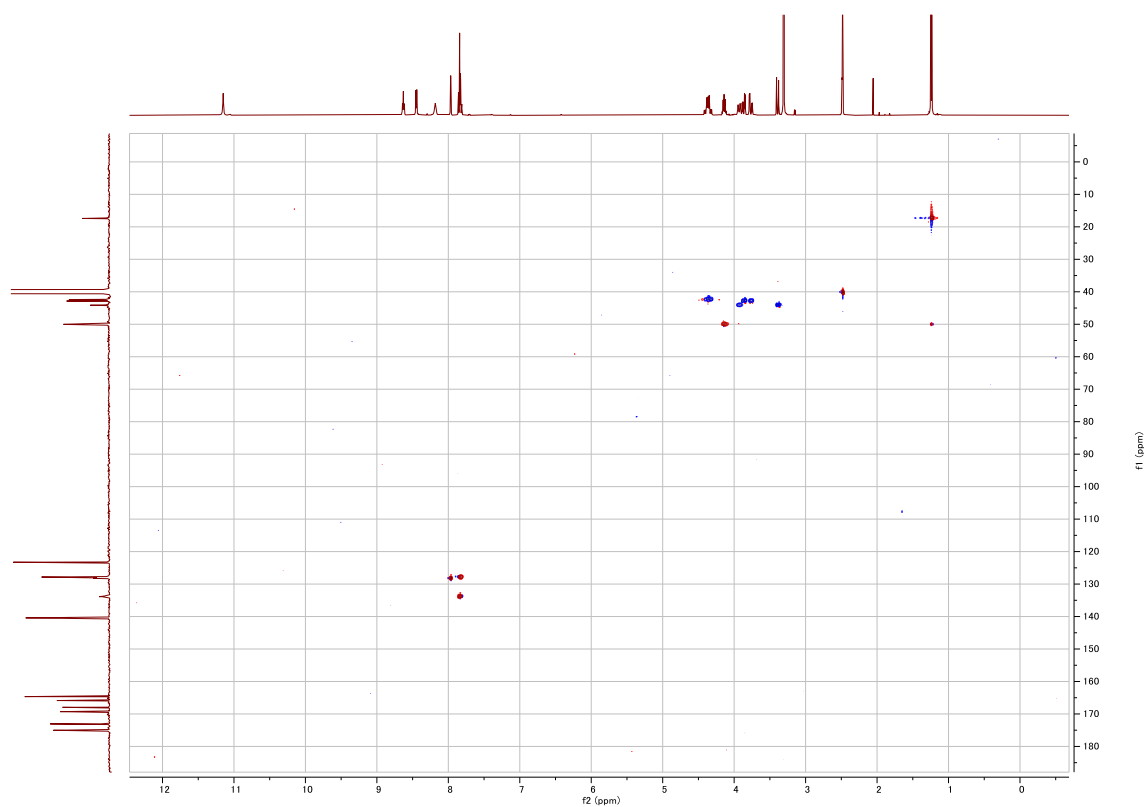
zTOCSY (500 MHz, DMSO-*d*₆)



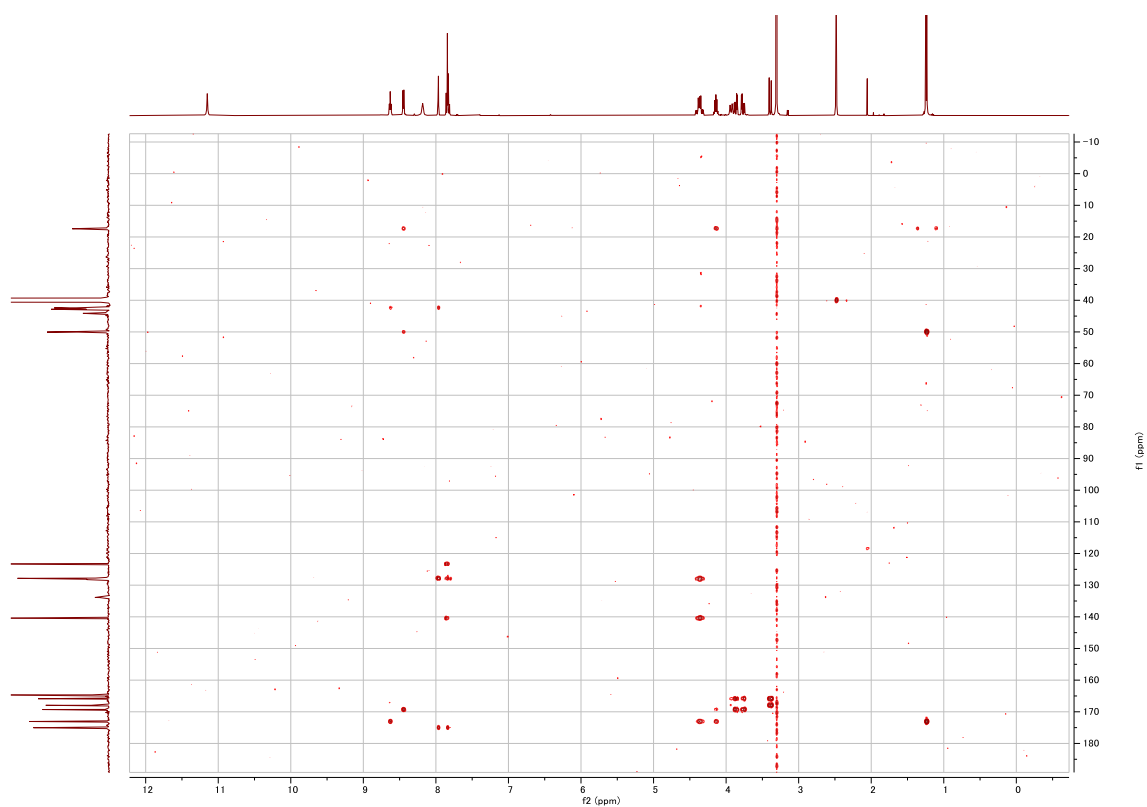
ROESY (600 MHz, DMSO-*d*₆, 150 ms mixing time)



HSQC (500 MHz, DMSO-*d*₆)

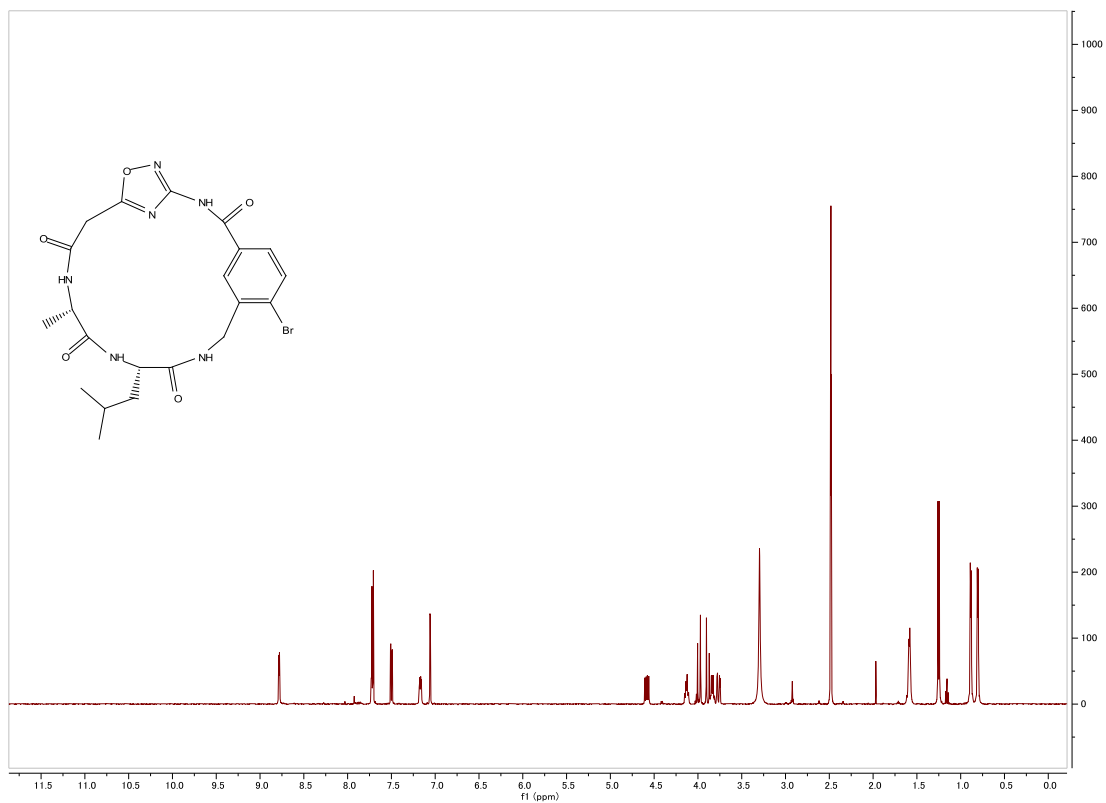


HMBC (500 MHz, DMSO- d_6)

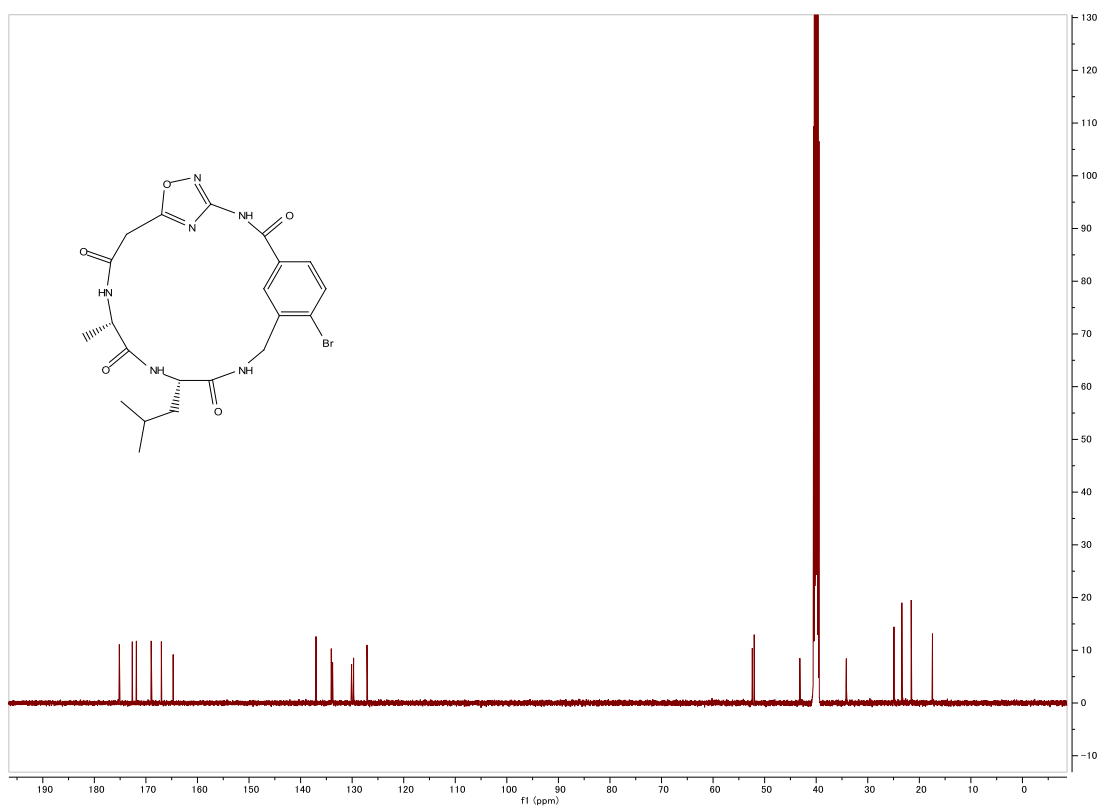


Non bi-aryl macrocycle (2a)

^1H NMR (500 MHz, $\text{DMSO}-d_6$)



^{13}C NMR (126 MHz, $\text{DMSO}-d_6$)

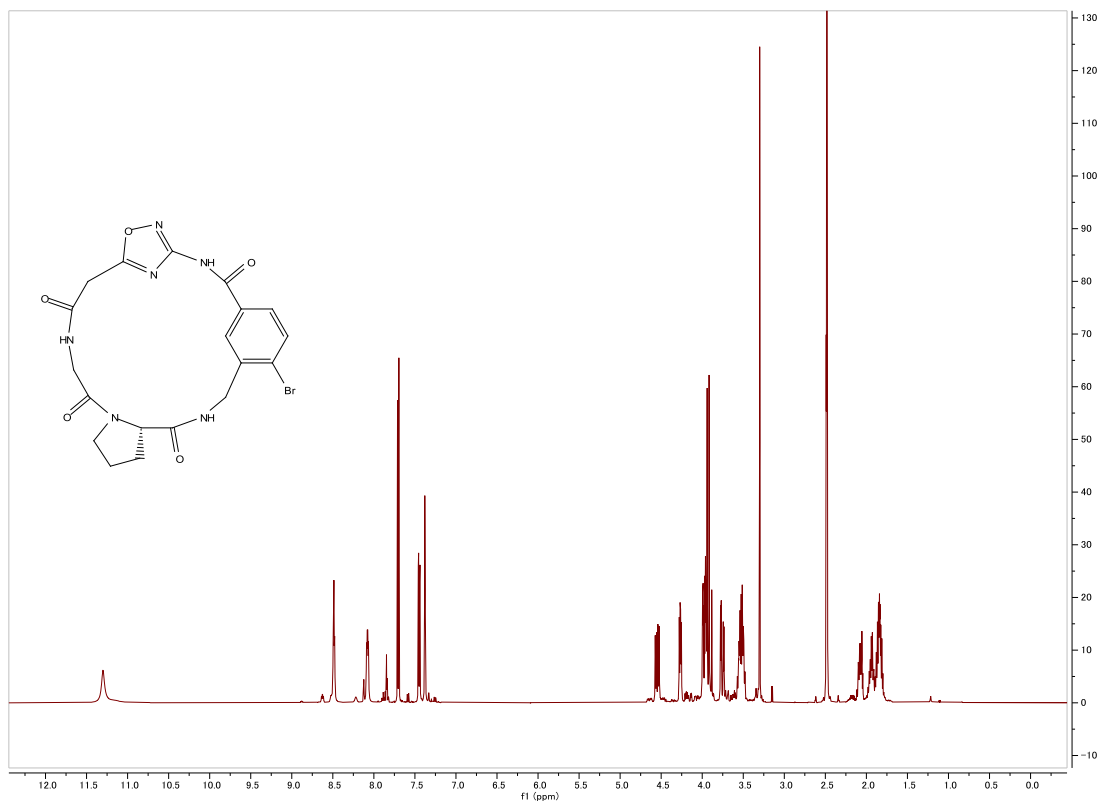


Bi-aryl macrocycle (2b)

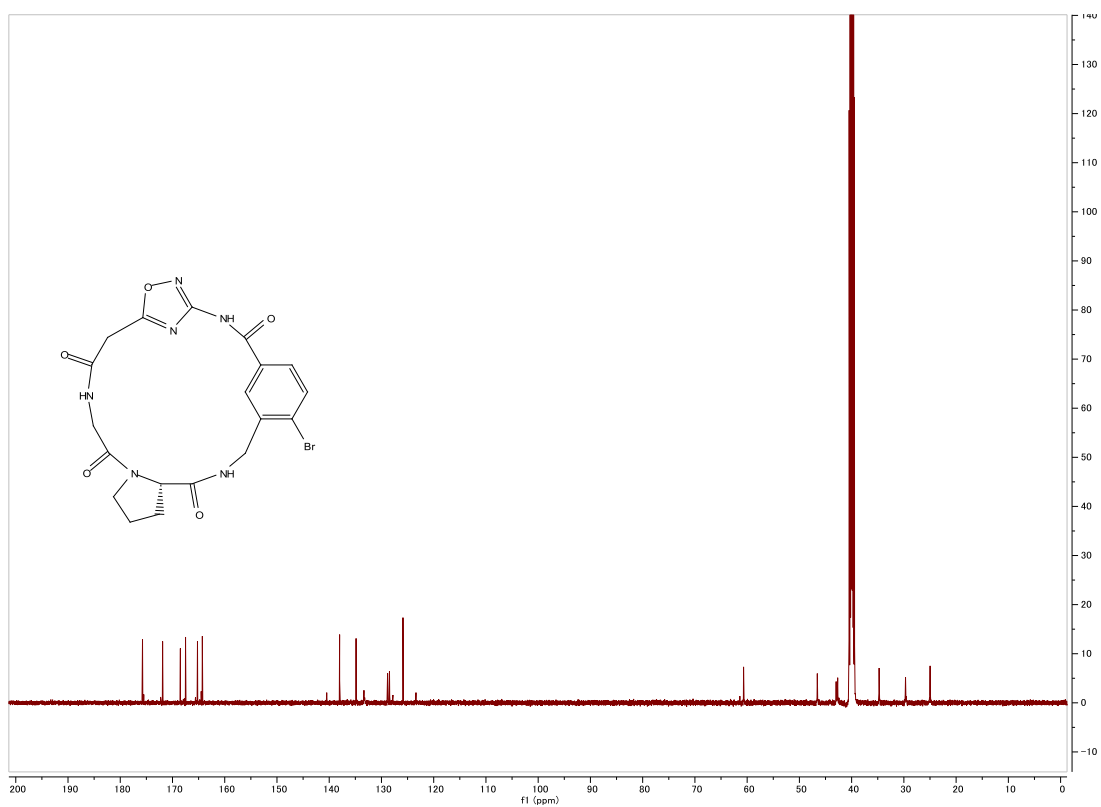
^1H NMR (500 MHz, $\text{DMSO-}d_6$)

Non bi-aryl macrocycle (3a)

^1H NMR (500 MHz, $\text{DMSO}-d_6$)

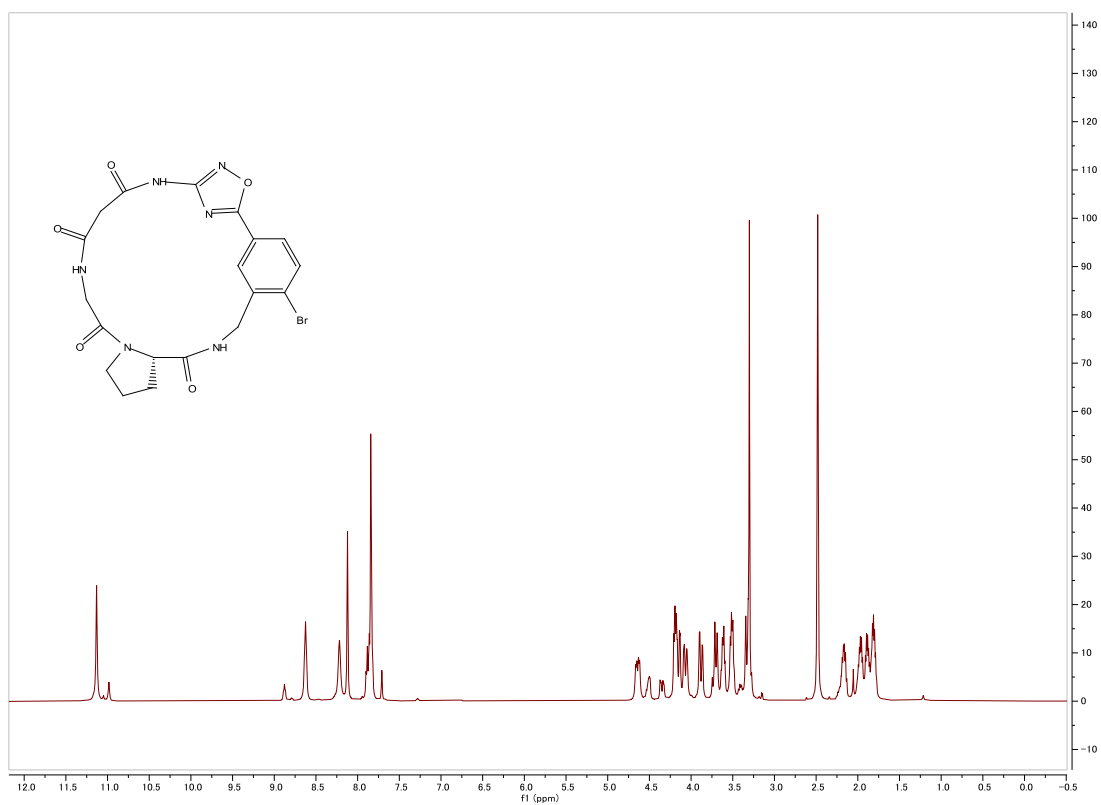


^{13}C NMR (126 MHz, $\text{DMSO}-d_6$)

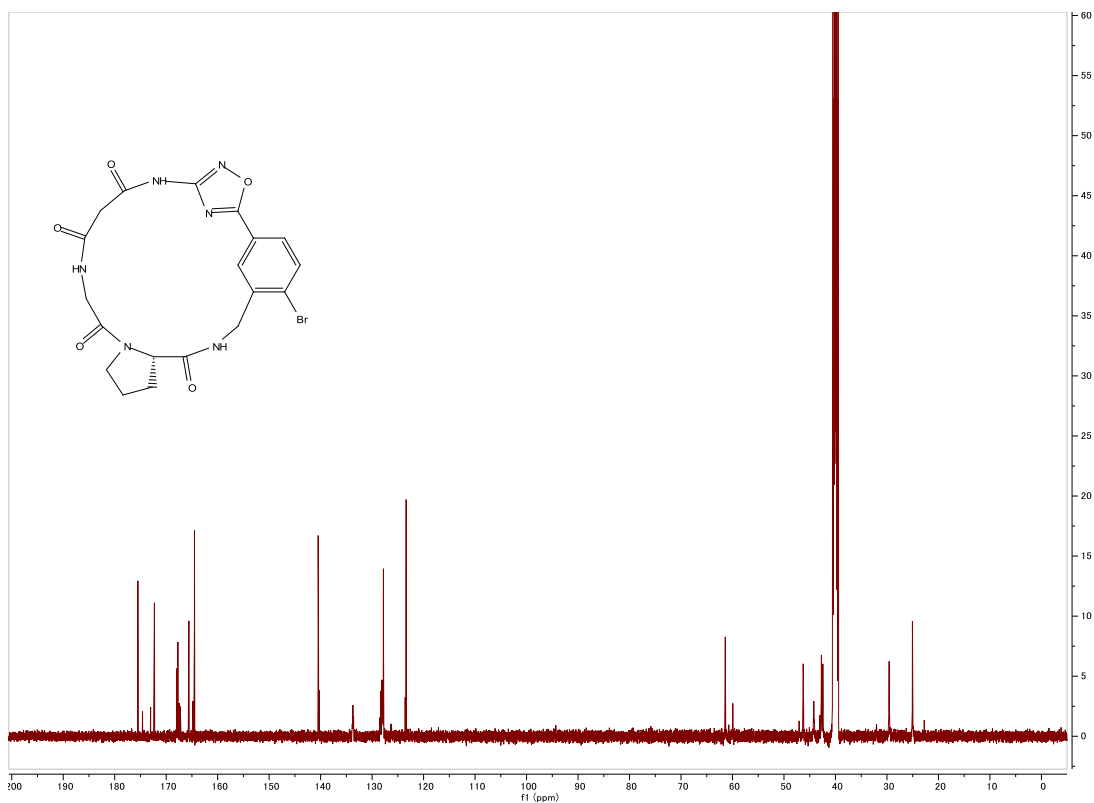


Bi-aryl macrocycle (3b)

^1H NMR (500 MHz, $\text{DMSO}-d_6$)

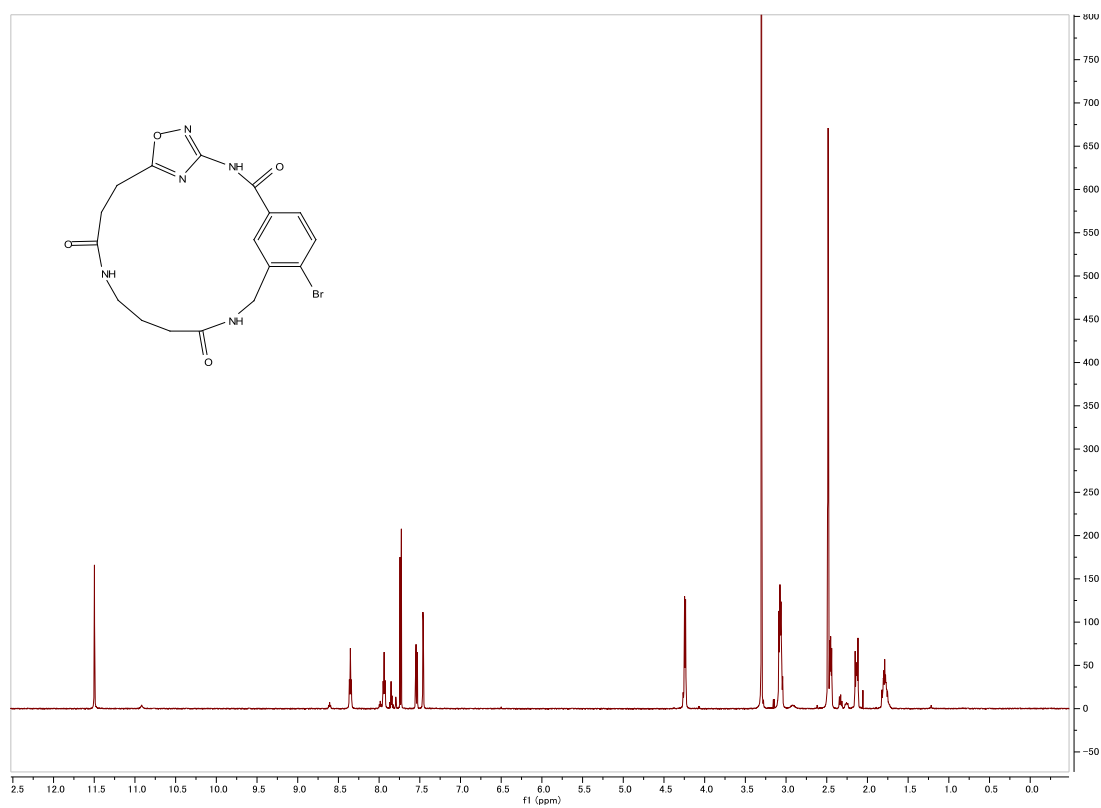


¹³C NMR (126 MHz, DMSO-*d*₆)

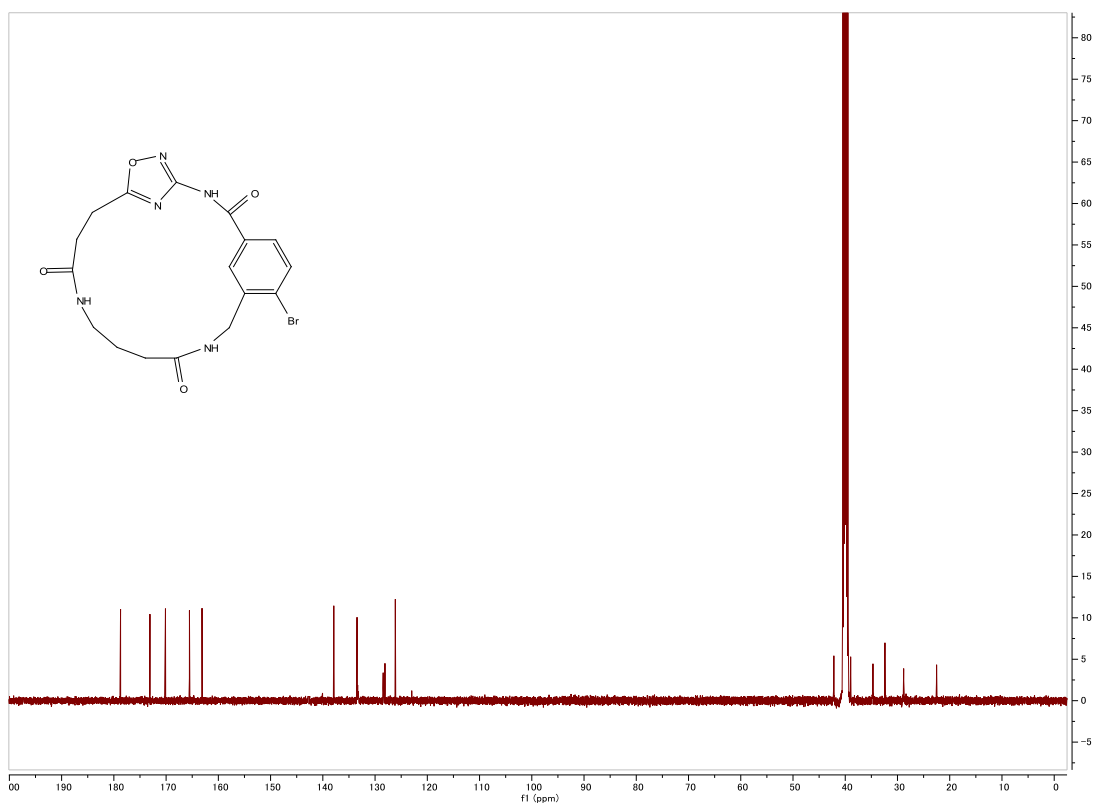


Non bi-aryl macrocycle (4a)

^1H NMR (500 MHz, $\text{DMSO}-d_6$)

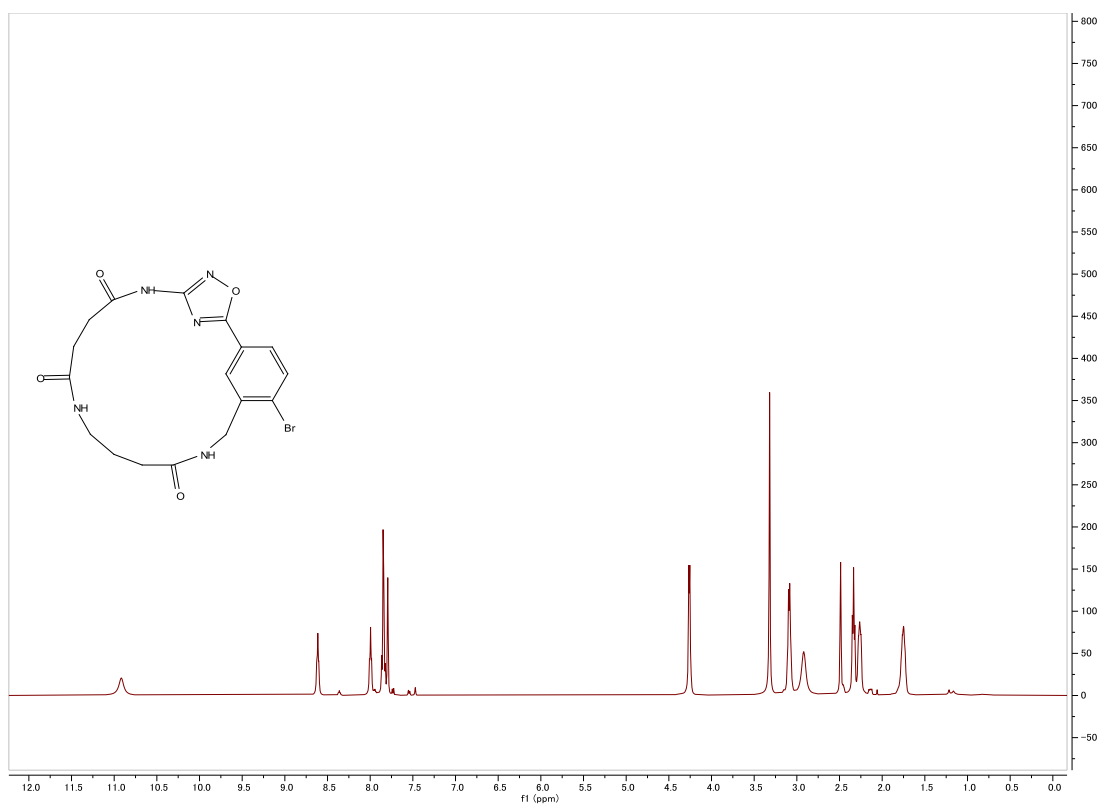


^{13}C NMR (126 MHz, $\text{DMSO}-d_6$)

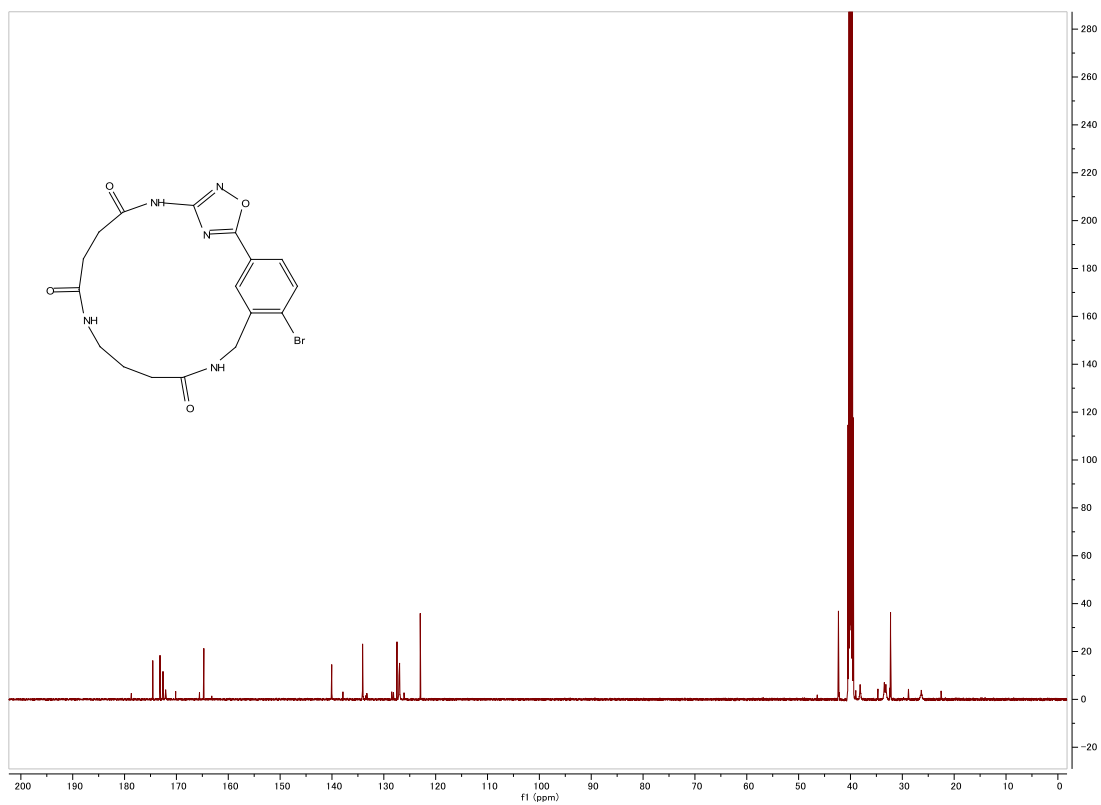


Bi-aryl macrocycle (4b)

¹H NMR (500 MHz, DMSO-*d*₆)

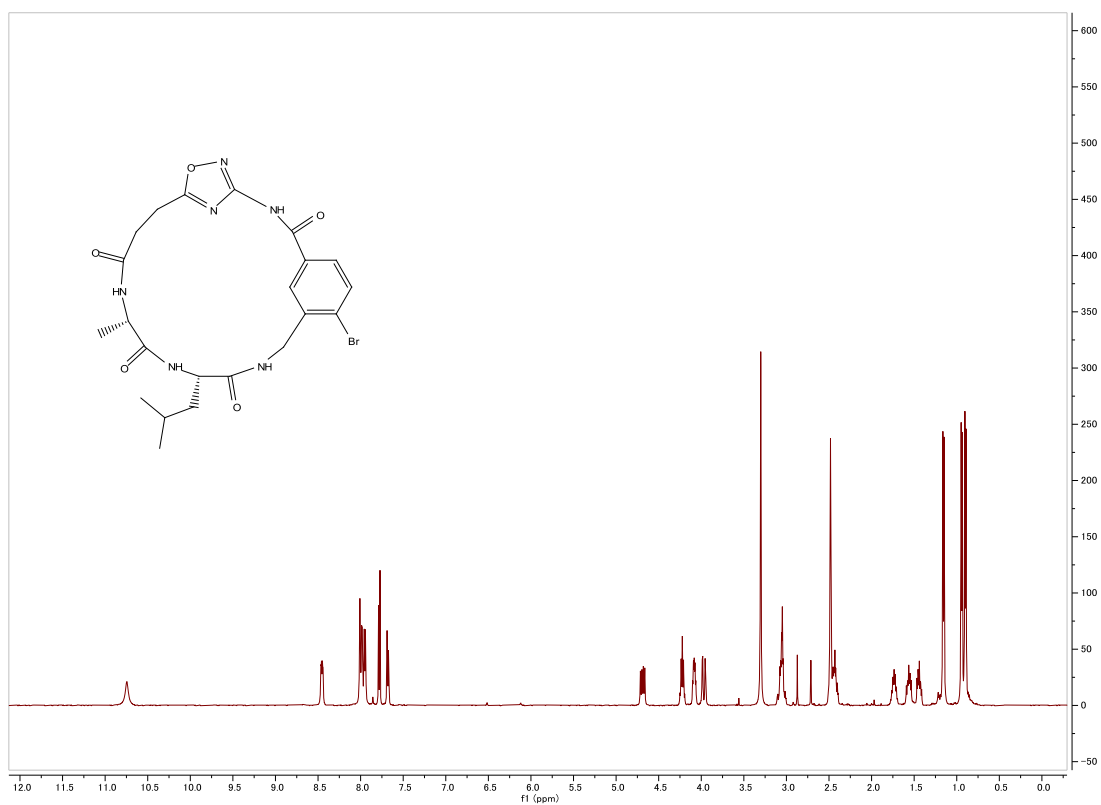
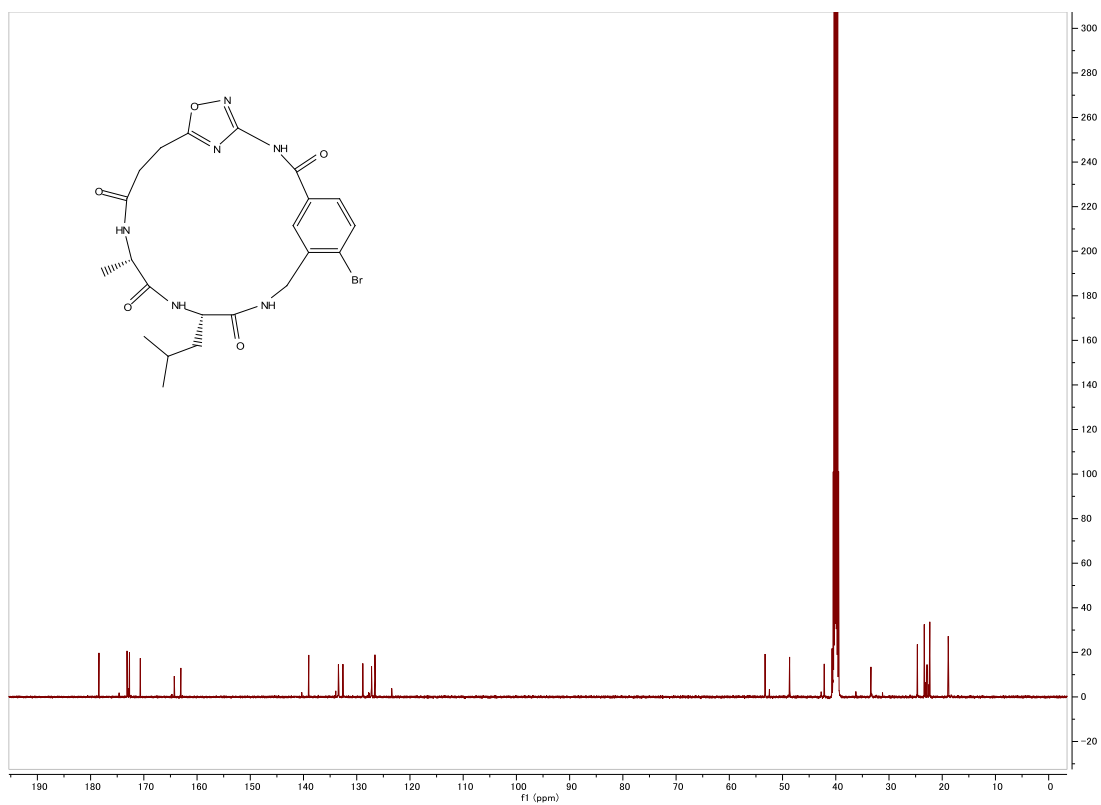


^{13}C NMR (126 MHz, $\text{DMSO-}d_6$)



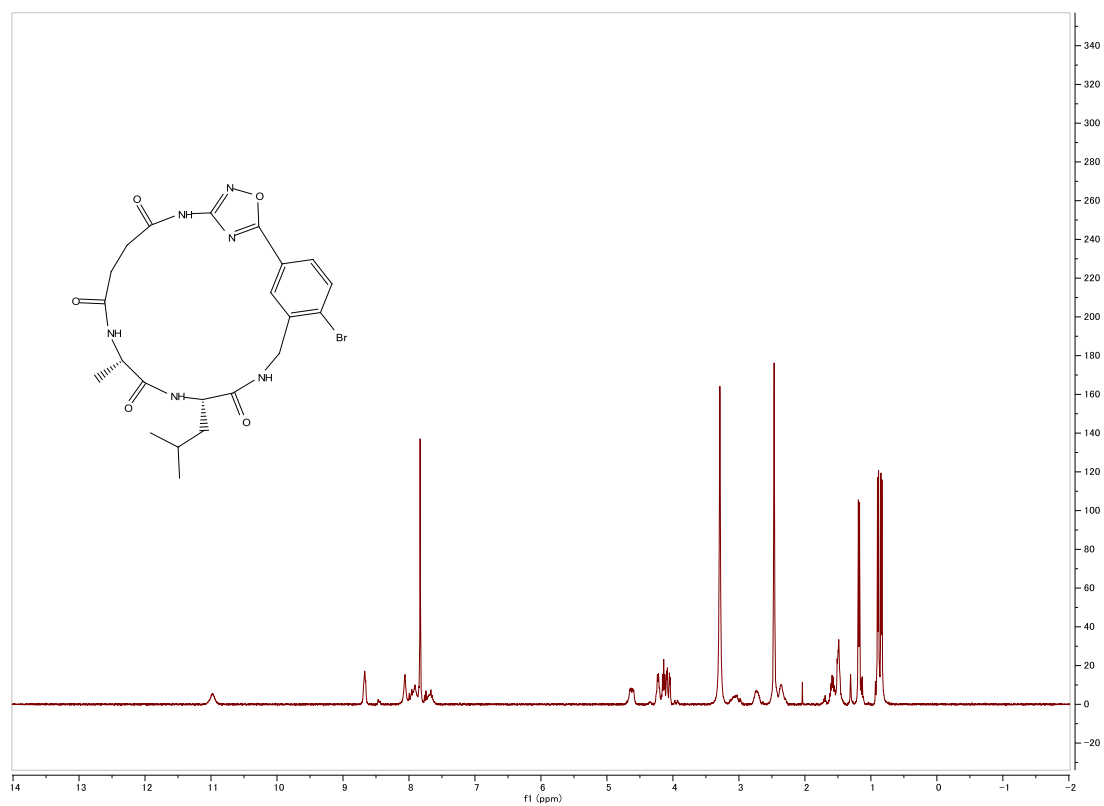
Non bi-aryl macrocycle (5a)

^1H NMR (500 MHz, $\text{DMSO-}d_6$)

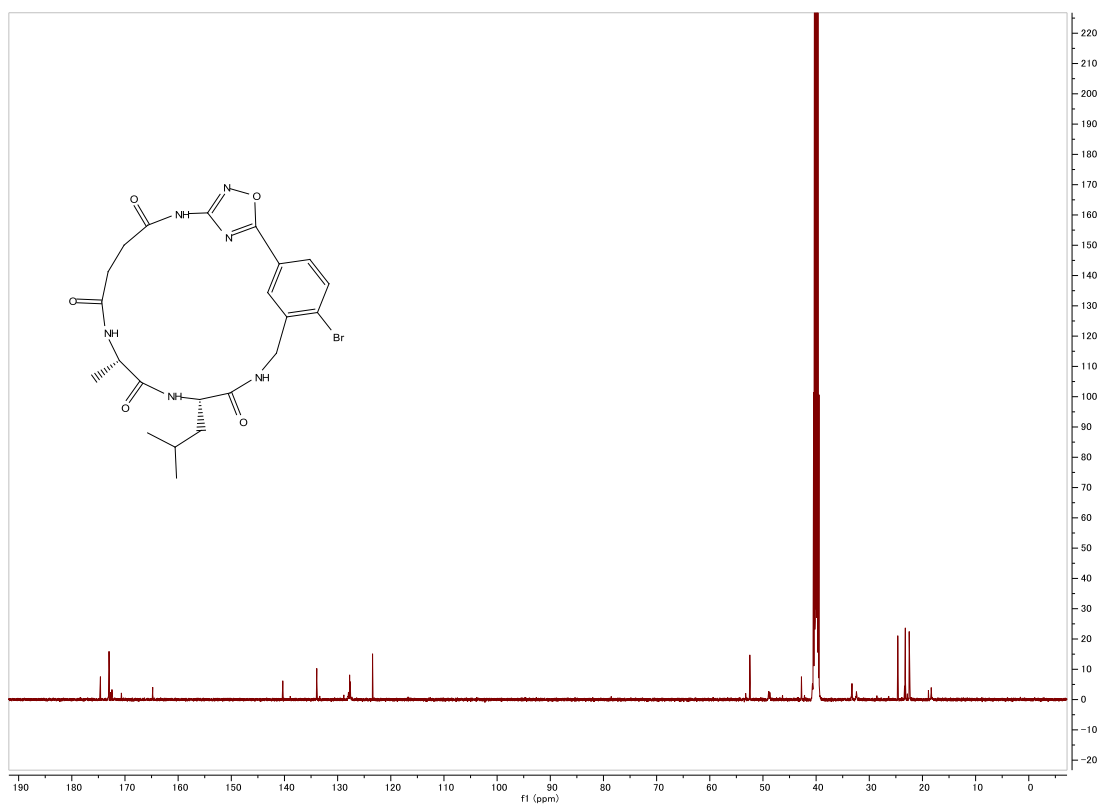
 ^{13}C NMR (126 MHz, DMSO- d_6)

Bi-aryl macrocycle (5b)

^1H NMR (500 MHz, $\text{DMSO}-d_6$)

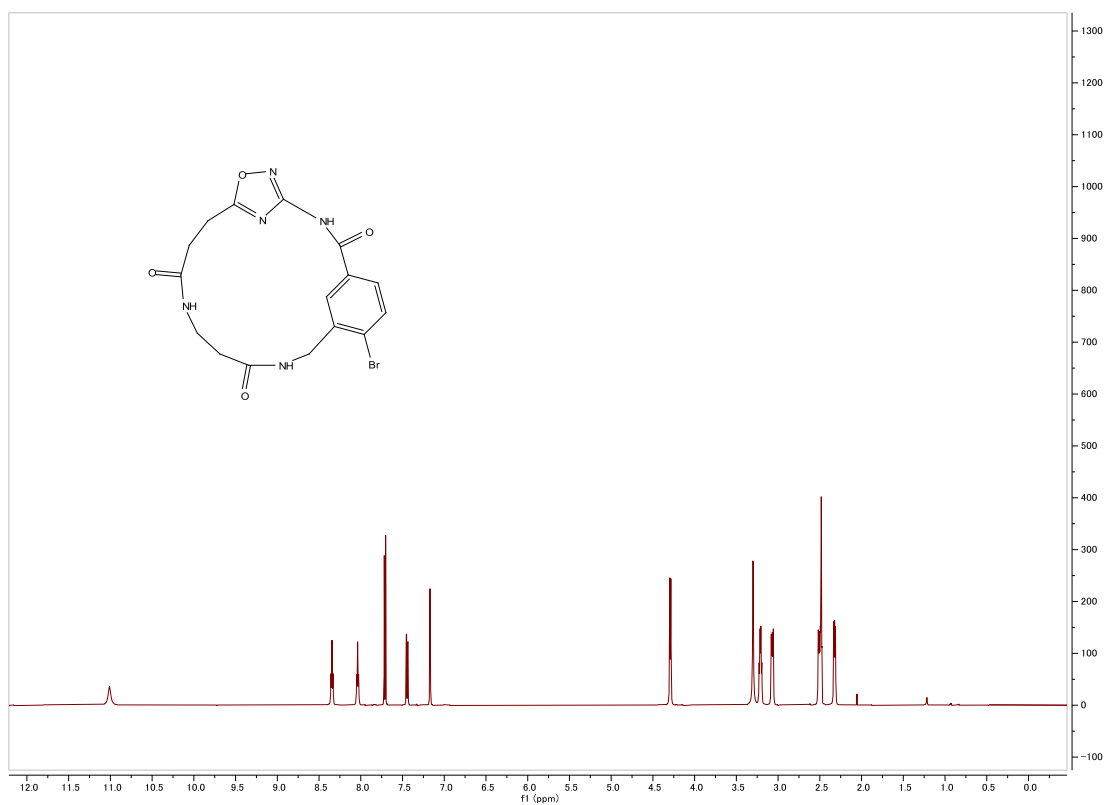


^{13}C NMR (126 MHz, $\text{DMSO}-d_6$)

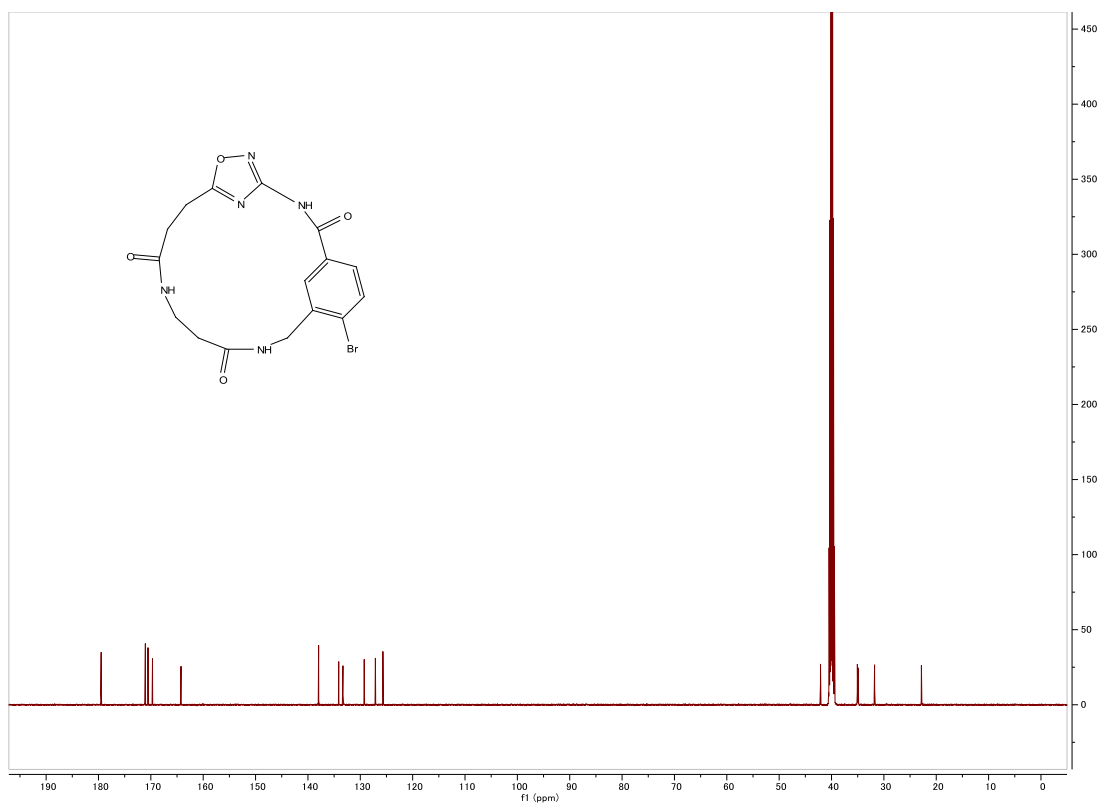


Non bi-aryl macrocycle (6a)

^1H NMR (500 MHz, $\text{DMSO}-d_6$)

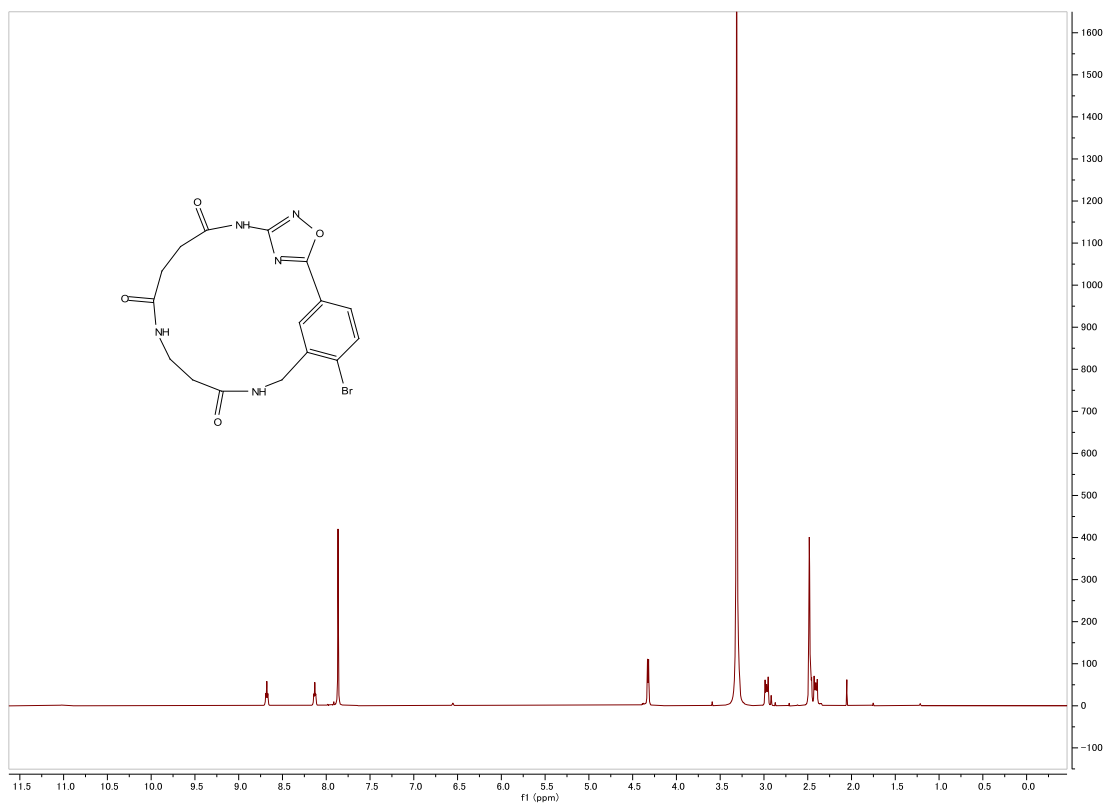


¹³C NMR (126 MHz, DMSO-*d*₆)

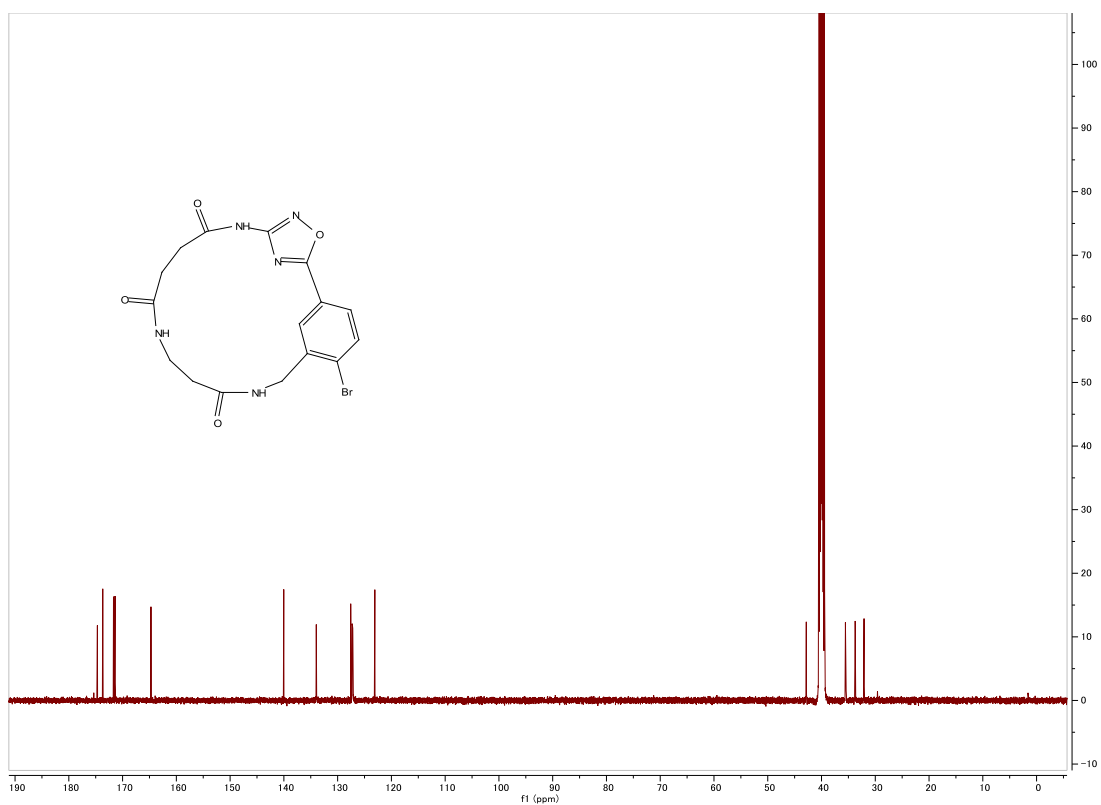


Bi-aryl macrocycle (6b)

^1H NMR (500 MHz, $\text{DMSO-}d_6$)

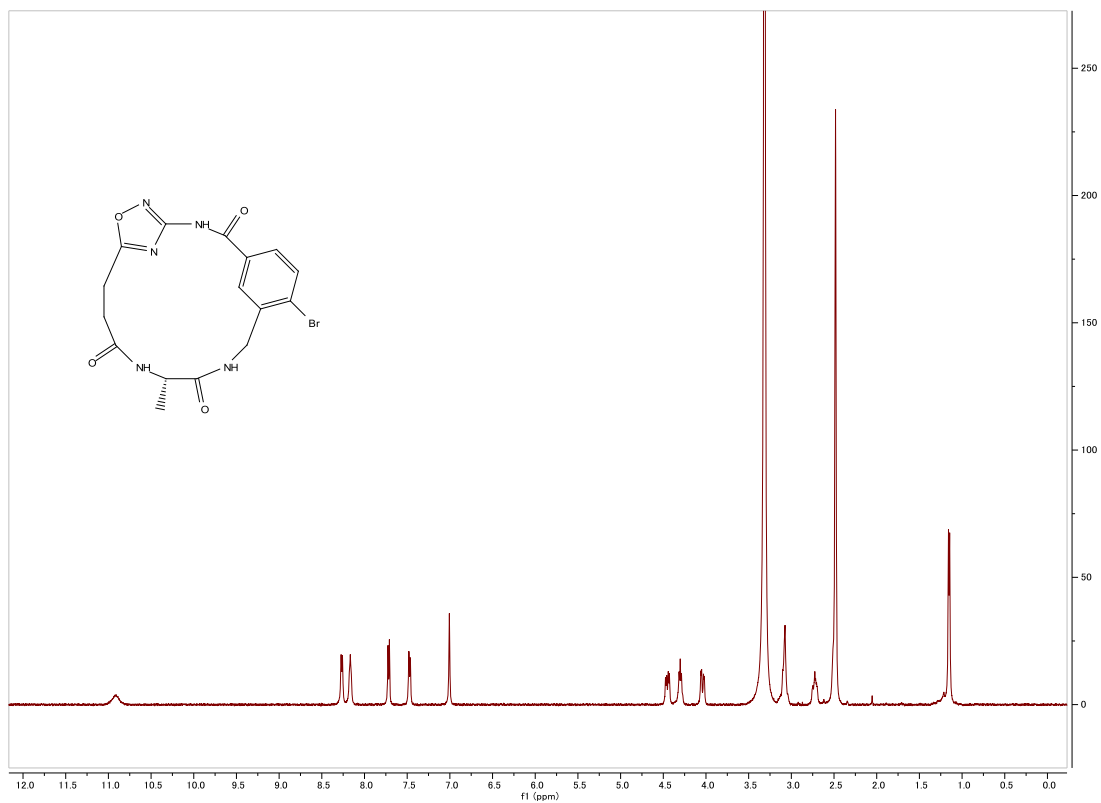


^{13}C NMR (126 MHz, $\text{DMSO-}d_6$)

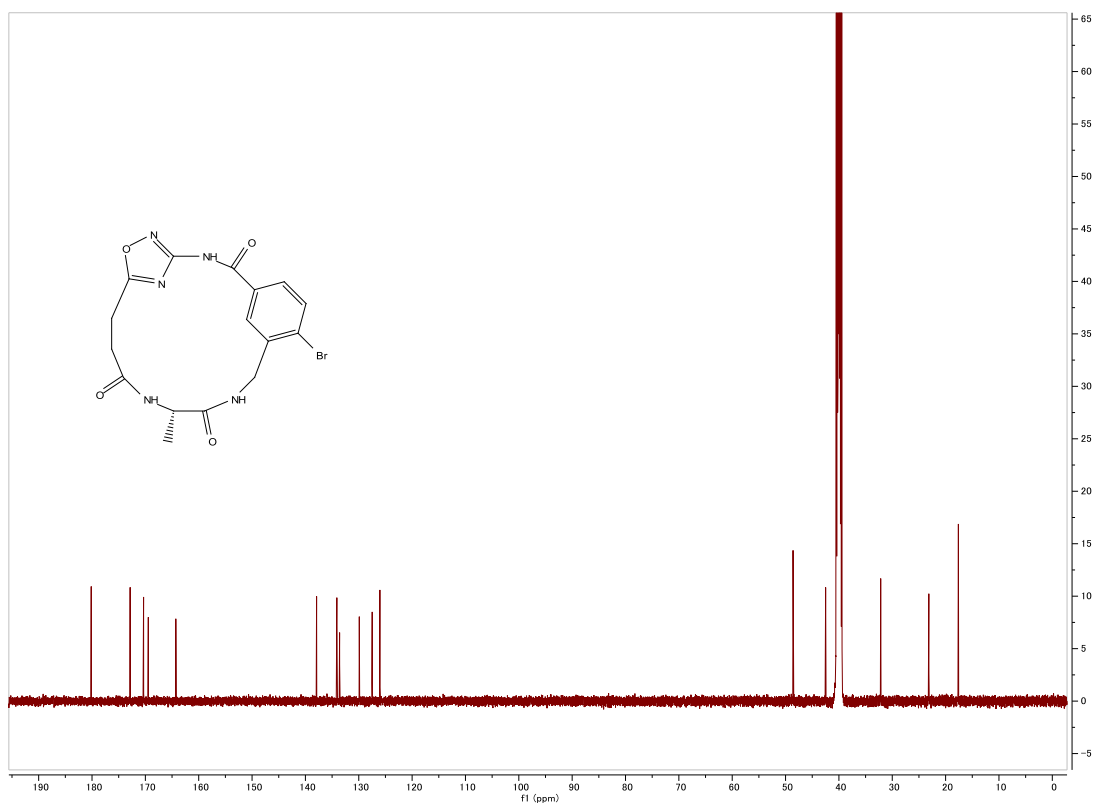


Non bi-aryl macrocycle (7a)

^1H NMR (500 MHz, $\text{DMSO}-d_6$)

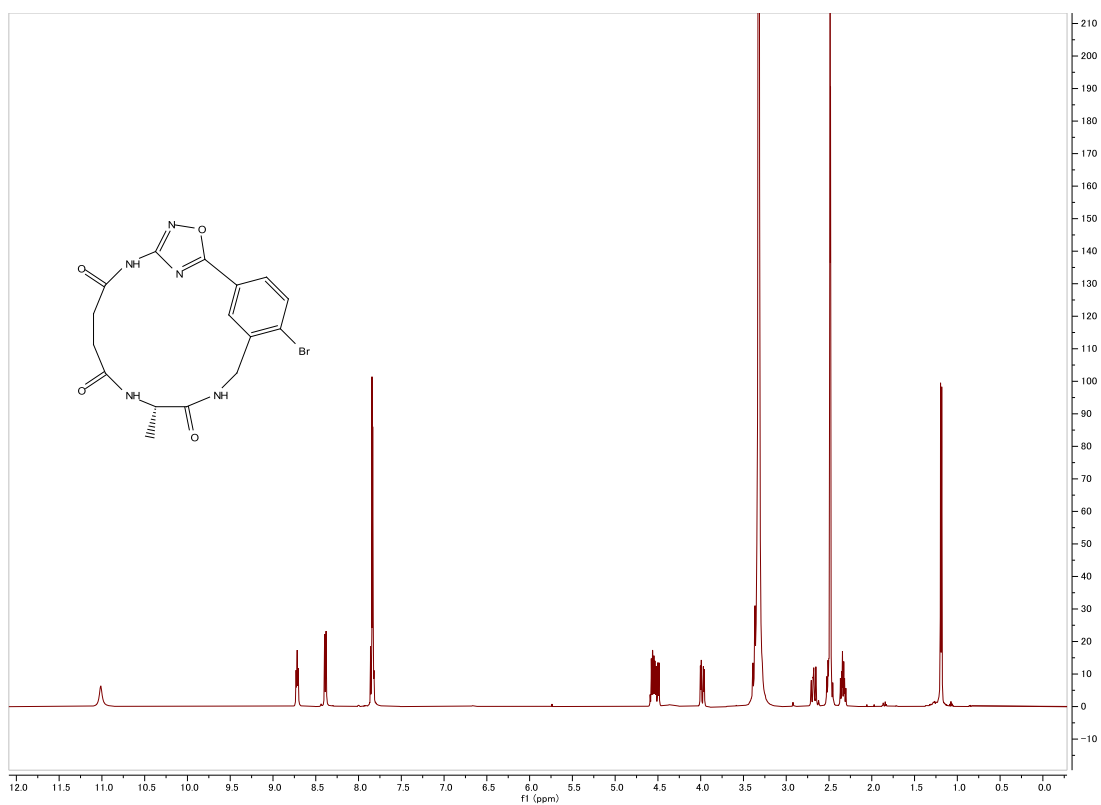


^{13}C NMR (126 MHz, $\text{DMSO}-d_6$)

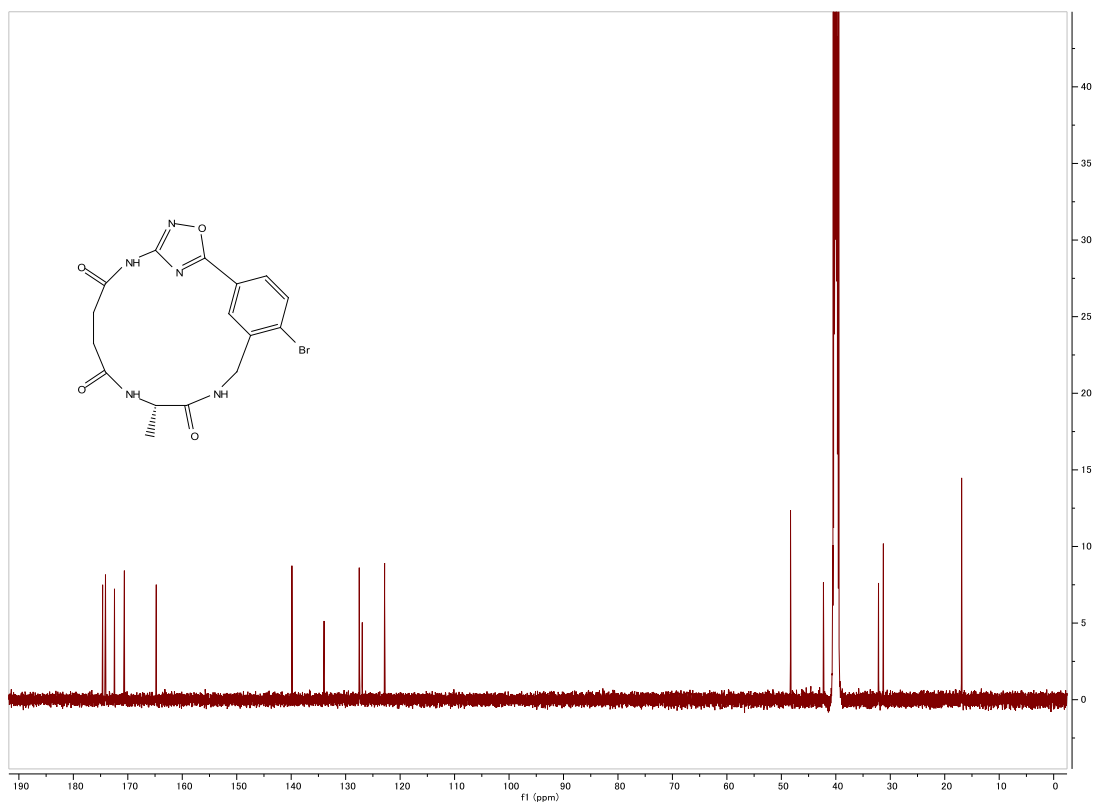


Bi-aryl macrocycle (7b)

¹H NMR (500 MHz, DMSO-*d*₆)

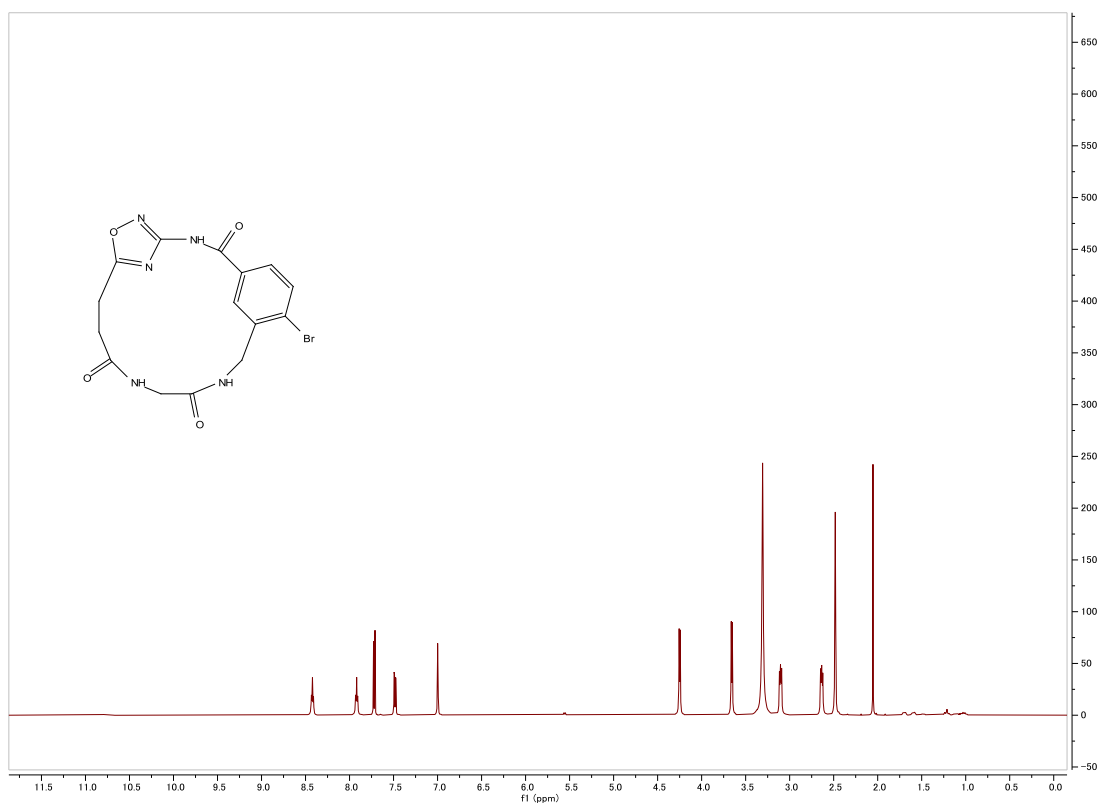


^{13}C NMR (126 MHz, $\text{DMSO-}d_6$)

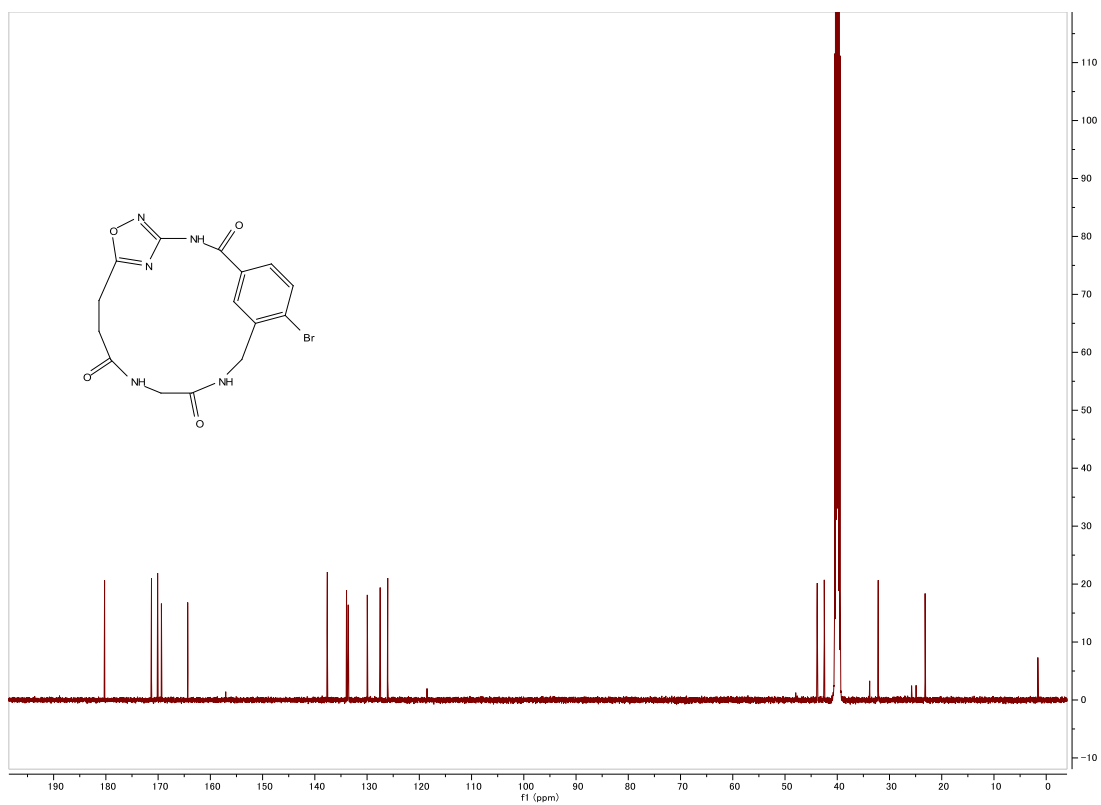


Non bi-aryl macrocycle (8a)

^1H NMR (500 MHz, $\text{DMSO-}d_6$)

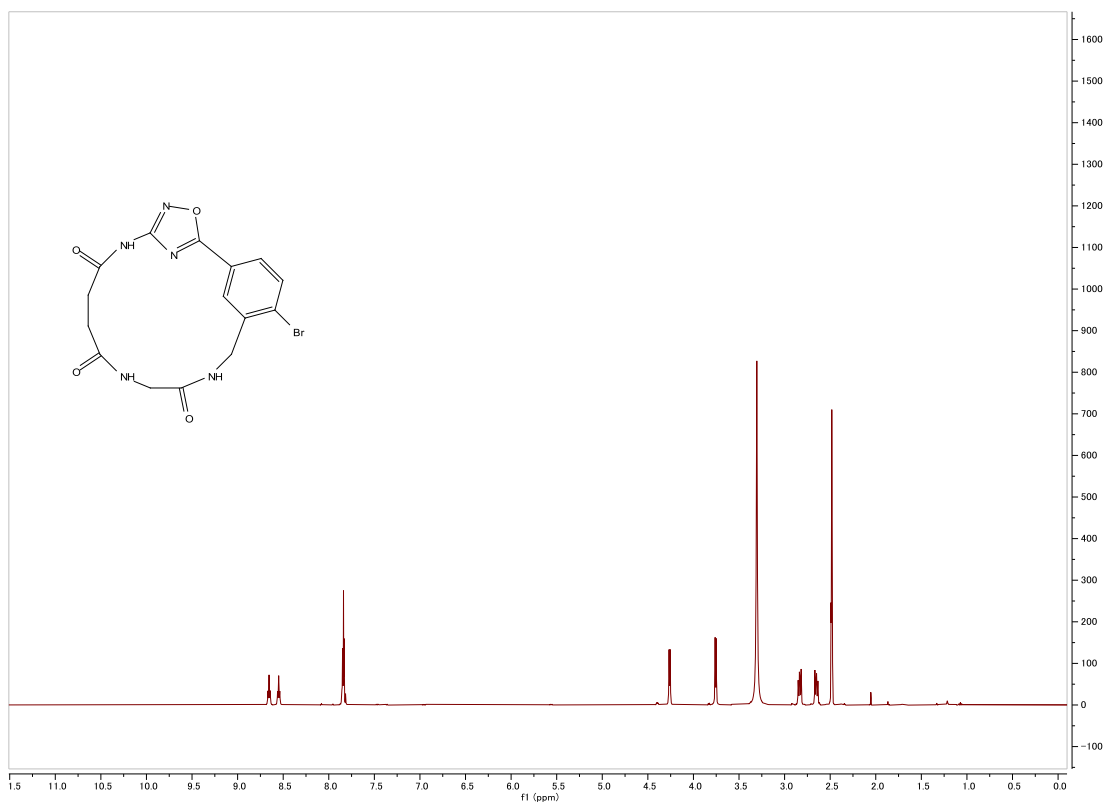


¹³C NMR (126 MHz, DMSO-*d*₆)

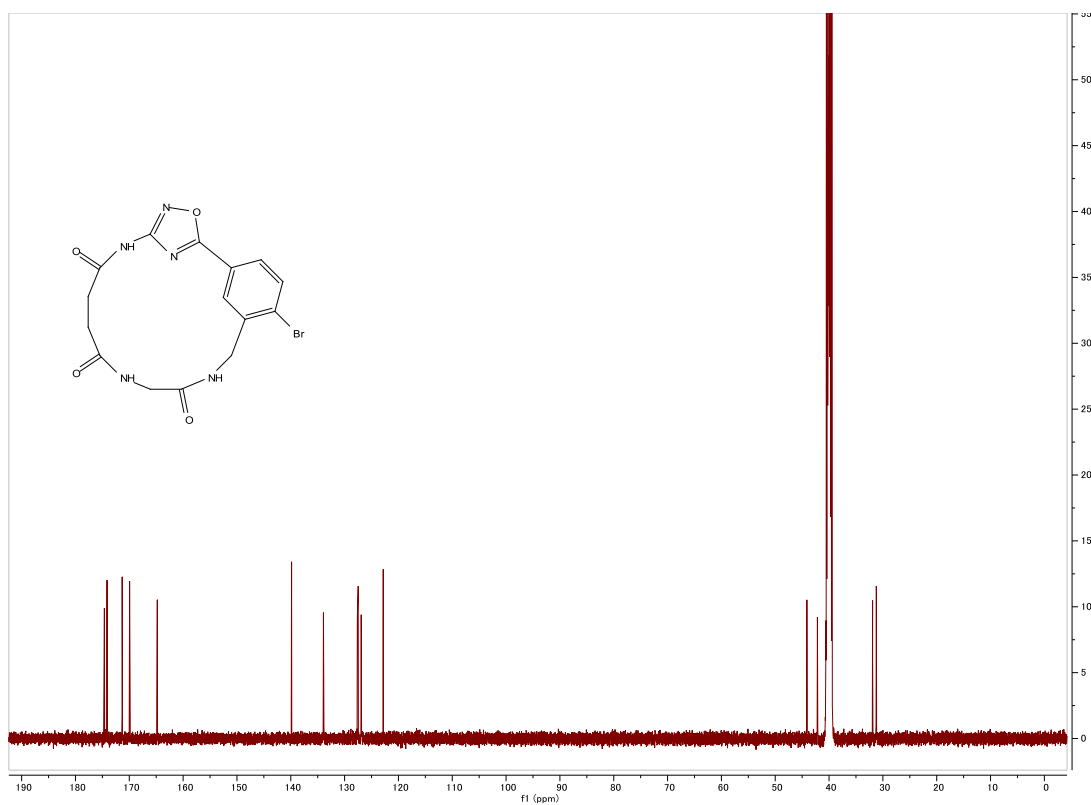


Bi-aryl macrocycle (8b)

^1H NMR (500 MHz, $\text{DMSO}-d_6$)

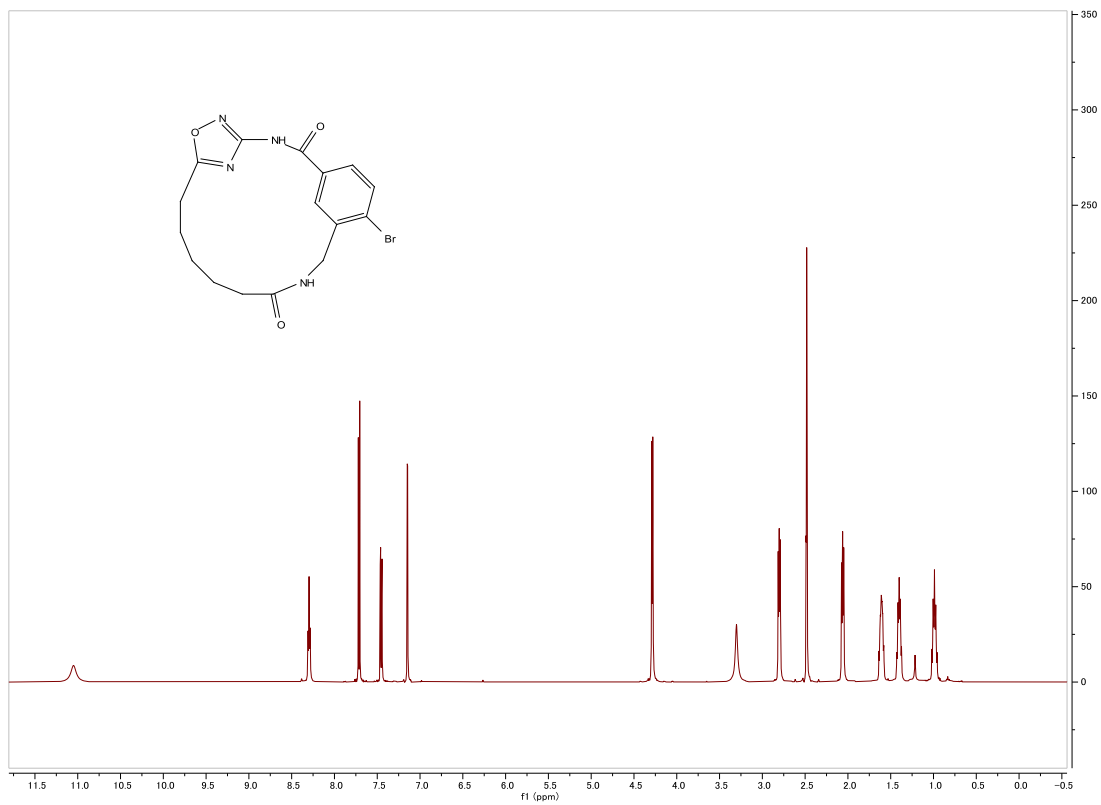


^{13}C NMR (126 MHz, $\text{DMSO}-d_6$)

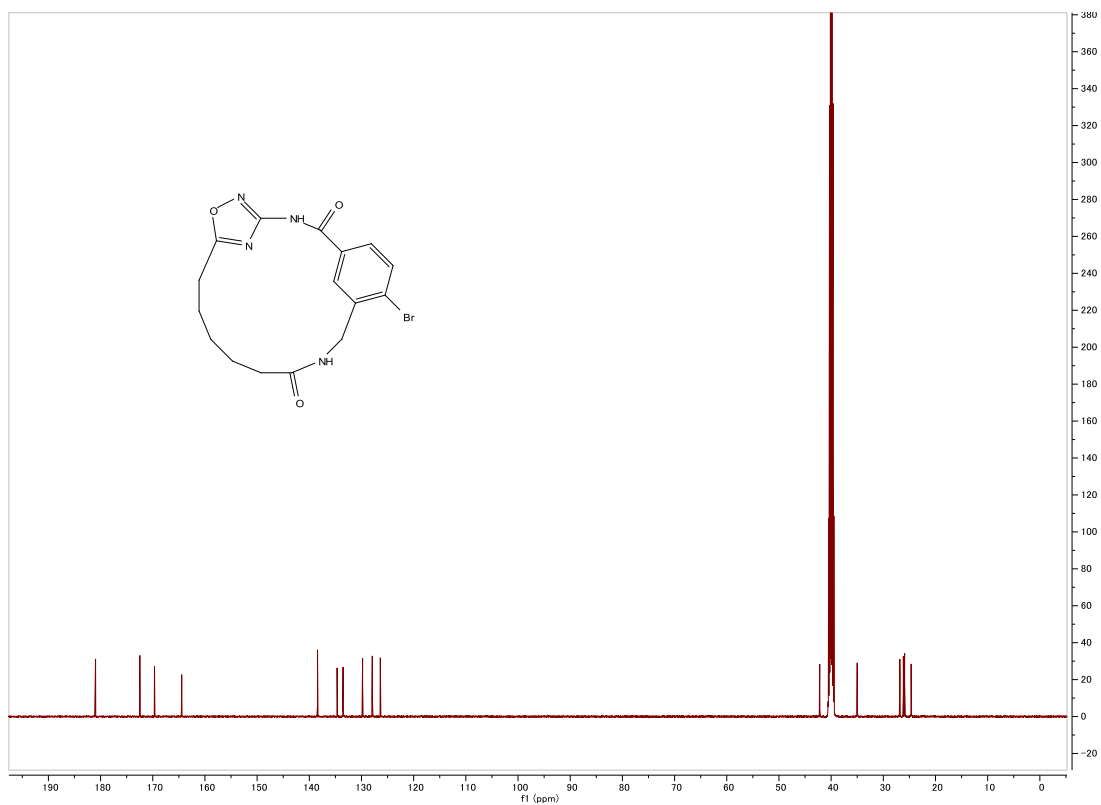


Non bi-aryl macrocycle (9a)

^1H NMR (500 MHz, $\text{DMSO}-d_6$)

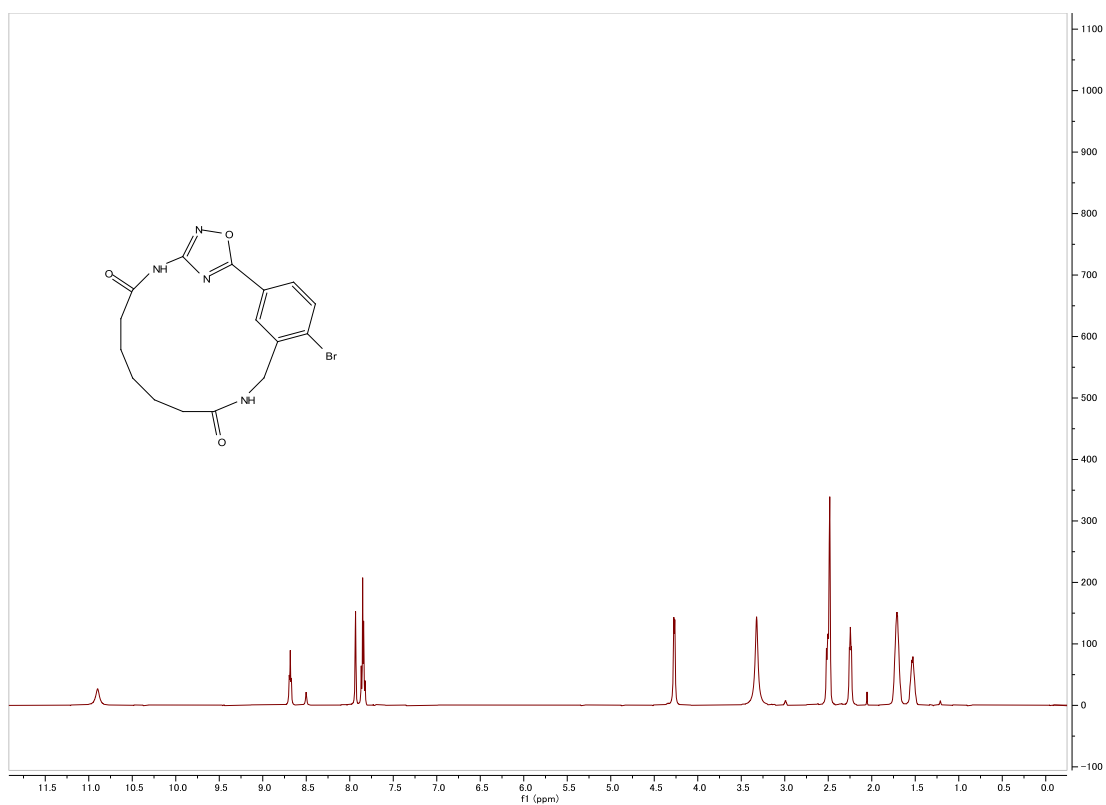


^{13}C NMR (126 MHz, $\text{DMSO}-d_6$)

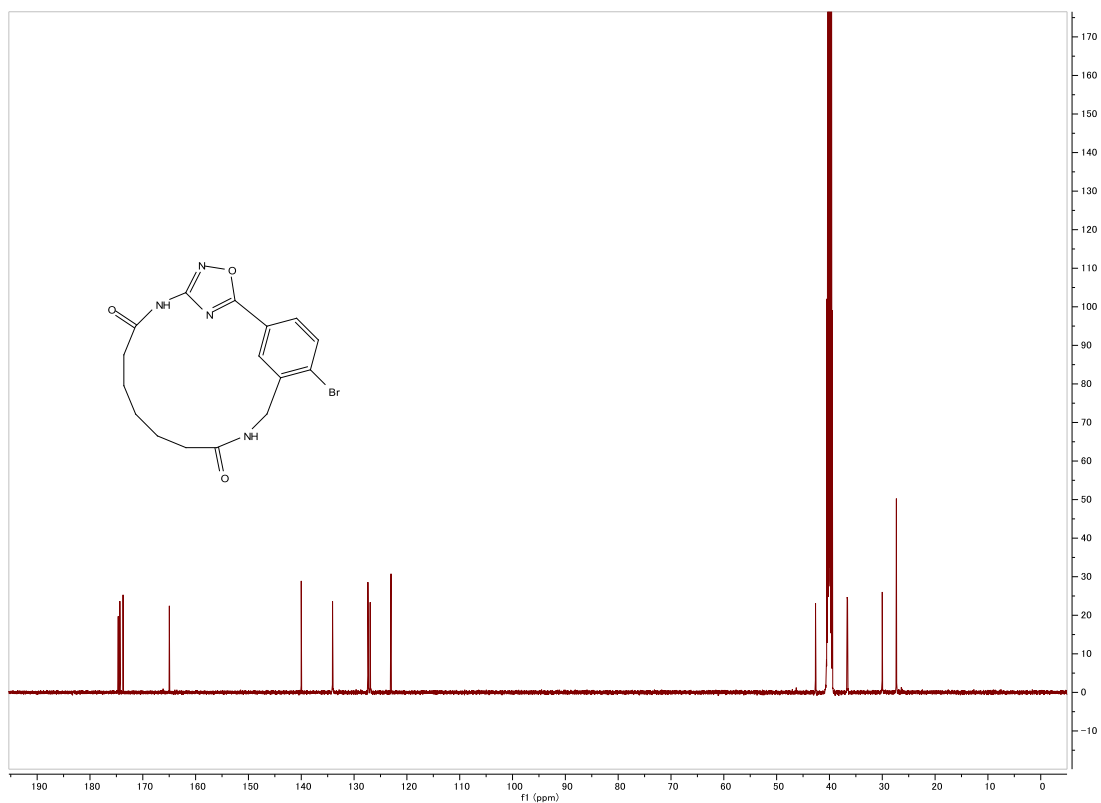


Bi-aryl macrocycle (9b)

¹H NMR (500 MHz, DMSO-*d*₆)

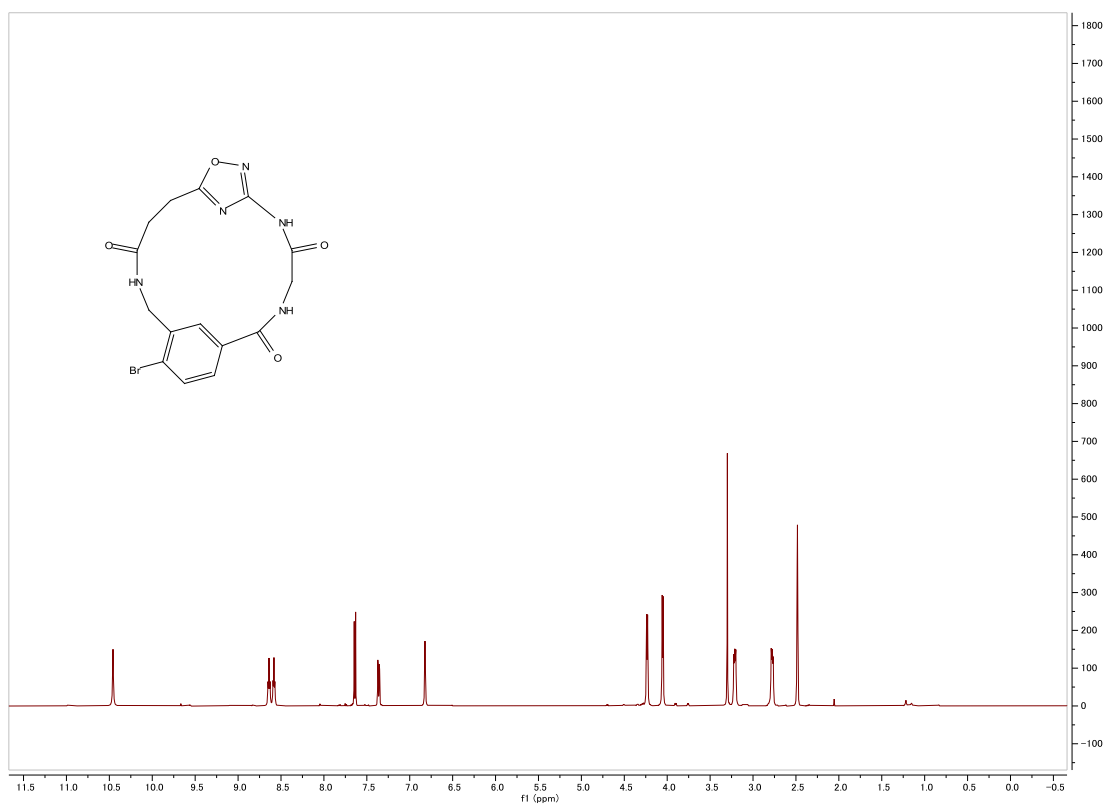


^{13}C NMR (126 MHz, $\text{DMSO-}d_6$)

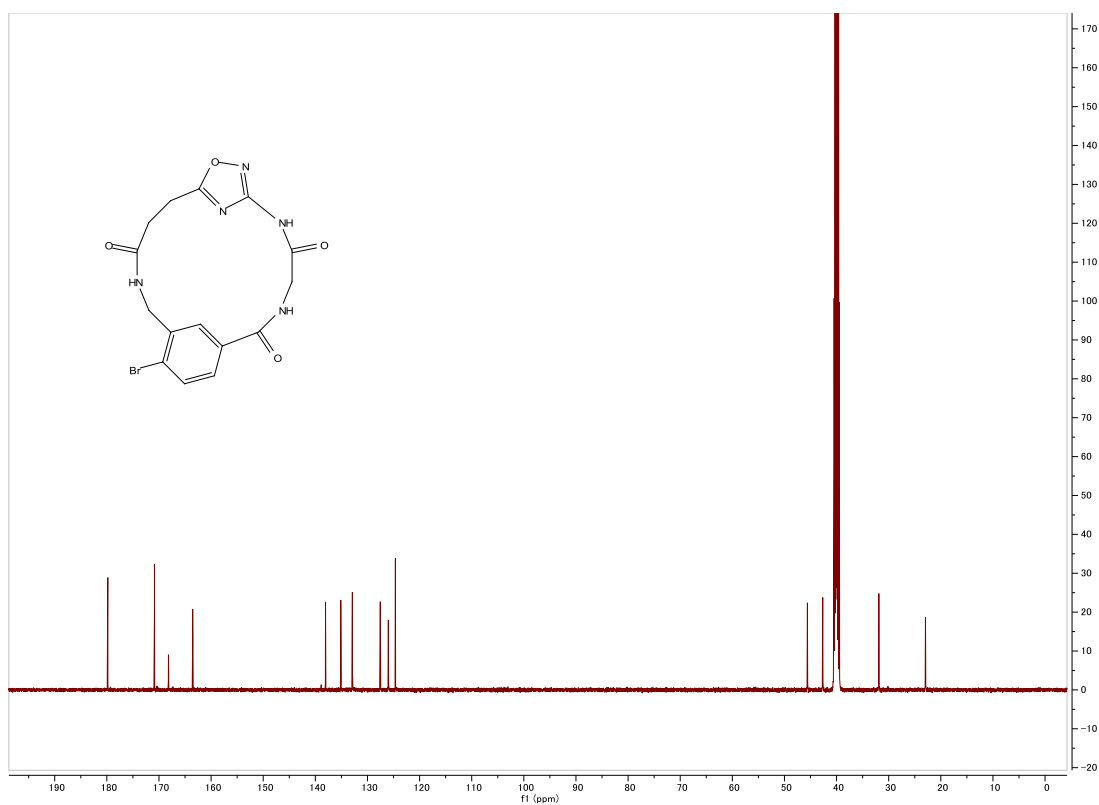


Macrocycle (10a)

^1H NMR (500 MHz, $\text{DMSO-}d_6$)

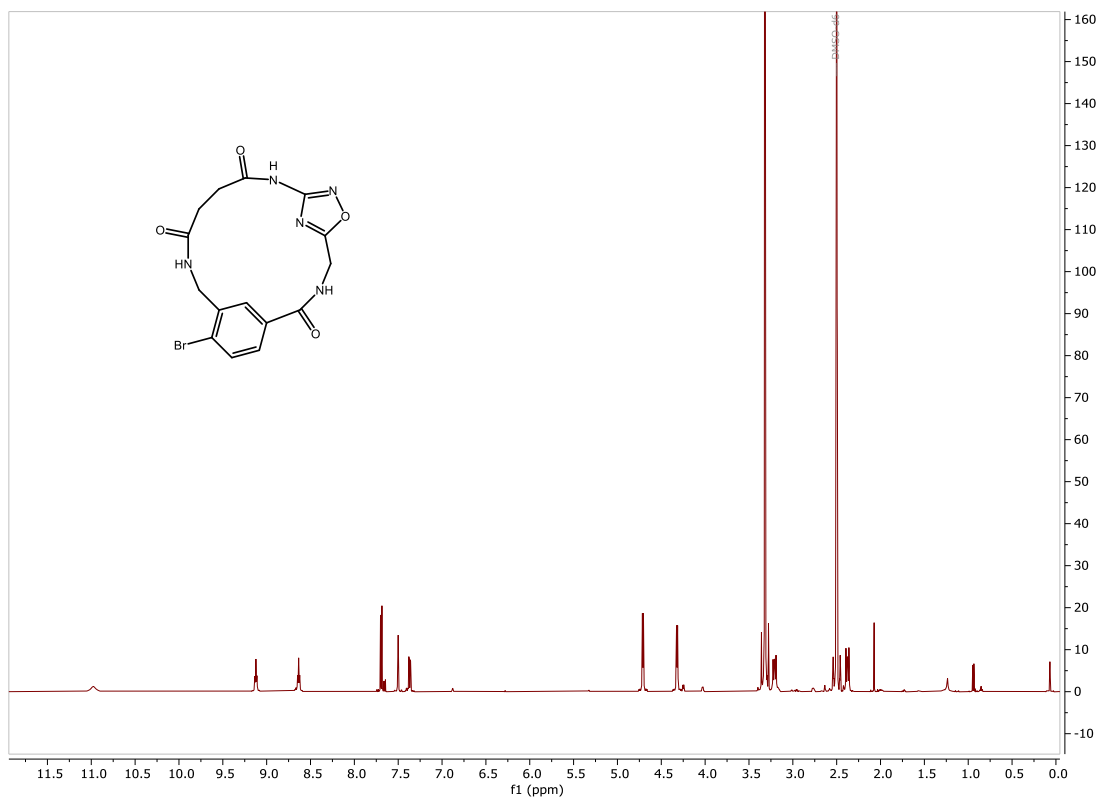


^{13}C NMR (126 MHz, $\text{DMSO-}d_6$)



Macrocycle (10b)

^1H NMR (500 MHz, $\text{DMSO-}d_6$)



¹ H. Kessler, *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 512 - 523.

² Y. Tang, C. He, G. H. Imler, D. A. Parrish, J. M. Shreeve, *Chem. Eur. J.* **2017**, *23*, 16401 – 16407.