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Supporting Information

Inhibition of ACE2-Spike Interaction by an ACE2 Binder Suppresses SARS-CoV-2 Entry

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< Supplementary Information >

Contents

Materials and Methods

Virus and cells

Vero monkey kidney epithelial cells were obtained from the American Type Culture Collection (ATCC CCL-81) and maintained at 37 ℃ with 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM; Welgene), supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1× antibiotic-antimycotic solution (Gibco). The ancestral SARS-CoV-2 and SARS-CoV-2 variants were provided by Korea Disease Control and Prevention Agency (KDCA) and were propagated in VeroE6 cells. Viral titers were determined by plaque assays in Vero cells. All experiments using SARS-CoV-2 were performed at Institut Pasteur Korea in compliance with the guidelines of the Korea National Institute of Health (KNIH), using enhanced biosafety level 3 (BSL3) containment procedures in laboratories approved for use by the KDCA.

Reagents

Lopinavir (LPV; S1380) was purchased from SelleckChem (Houston, TX), and remdesivir (HY-104077) was purchased from MedChemExpress (Monmouth Junction, NJ). Reagents were dissolved in dimethyl sulfoxide (DMSO) as stock solutions. Anti-SARS-CoV-2 N protein antibody was purchased from Sino Biological Inc. (Beijing, China). Alexa Fluor 488 goat anti-rabbit IgG secondary antibody and Hoechst 33342 were purchased from Molecular Probes (Eugene, OR). Paraformaldehyde (PFA) (32% aqueous solution) and normal goat serum were purchased from Electron Microscopy Sciences (Hatfield, PA) and Vector Laboratories, Inc. (Burlingame, CA), respectively.

ELISA-based RBD-ACE2 binding assay

The inhibitory effects of testing compounds on the interaction between RBD and ACE2 were measured using the RBD-hACE2 sandwich ELISA assay kit (RayBiotech, #CoV-SACE2) according to the manufacturer's instructions. Briefly, the RBD pre-coated wells were added with the solution of ACE2 and the testing compounds with the final concentration of 20 μM in 2% DMSO. The mixture was incubated overnight at 4 ℃, followed by washing steps and antibody incubations for the detection. To measure the binding between the mutant RBD (N501Y) and ACE2, each well in a 96-well clear plate was pre-coated with the mutant RBD (Arg319–Phe541 (N501Y), His-tag) (Sino biological, #40592-V08H82) in a carbonate buffer (100 mM, pH 9.6) by incubating overnight at 4 ℃. After washing with phosphate-buffered saline (PBS) with 0.05% Tween 20 (PBST) for 3 times, the wells were blocked with 5% bovine serum albumin in PBS solution, followed by PBST washing for 5 times. The wells were added with the solution of hACE2 (Gln18–Ser740, His-tag) (Raybiotech, #230-30165) and the testing compounds in 2% DMSO, and incubated overnight at 4 ℃. The anti-ACE2 primary antibody and HRP-conjugated anti-horse IgG secondary antibody were used for the colorimetric development by sequential addition of TMB-stop solutions, of which solutions were measured at 450 nm using the plate reader.

Surface plasmon resonance (SPR) assay

SPR experiments were performed using a Biacore T100 instrument (Cytiva). Recombinant hACE2 (Gln18–Ser740, His-tag) (Raybiotech, #230-30165) and RBD (Arg319–Phe541, His-tag) (Raybiotech, #230-301620) proteins were immobilized through amide bonds to a CM5 sensor chip (Cytiva) by activating the carboxyl group on the CM5 chip with 1:1 mixture of *N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide and *N*-hydroxysuccinimide. The protein immobilization reactions were carried out with 0.05% Tween 20 in PBS for hACE2 (pH 4.5) and RBD (pH 7.0). Bindings between the compounds and proteins were monitored by flowing various concentrations of compounds in PBS (pH 7.3) containing 2% DMSO and 0.05% Tween 20 at 25 ℃. Data were analyzed to calculate kinetic parameters by fitting sensorgrams with the 1:1 binding model using Biacore T100 Evaluation software (Cytiva).

hACE2 enzymatic activity assay

The peptidase activity of recombinant hACE2 was measured using the hACE2 activity test kit (Biovision, #K310) according to the manufacturer's instruction. Briefly, hACE2 solution was pre-incubated with the solution of testing compounds in 1% DMSO for 15 min. The solution was sequentially added with the fluorophore-labeled peptide substrate of hACE2 and incubated for 2.5–3 h at room temperature (r.t.). The resulting solution was measured at Ex/Em 320/420 nm to quantify the cleaved fluorophore from the hACE2 substrate peptide.

Immunofluorescence analysis (IFA) of virus infection

Infected Vero cells were subjected to the evaluation of cellular antiviral activity with an immunofluorescence-based imaging assay by labeling viral N protein of the SARS-CoV-2 within infected cells. Vero cells were seeded at 1.2×10^4 cells per well in DMEM, supplemented with 10% FBS and 1× antibiotic-antimycotic solution (Gibco) in black 384-well μClear plates (Greiner Bio-One) 24 h prior to the experiment. Ten-point dose-response curves (DRCs) were generated with compound concentrations ranging from 0.1 to 50 μM, and added to the cells 1 h prior to the viral infection. For the viral infections, plates were transferred into the BSL3 containment facility and SARS-CoV-2 was added at a multiplicity of infection (MOI) of 0.008. The cells were fixed at 24 h post-infection (hpi) with 4% PFA and analyzed by immunofluorescence. The acquired images (Operetta, Perkin Elmer) were analyzed using Columbus software (Perkin Elmer) to quantify cell numbers and infection ratios. The infection ratio of each well was normalized to that of no-infection control (mock) and that of infection control (DMSO) in each assay plate, which were set as 100% and 0% inhibition of infection, respectively. Cell viability was measured by counting nuclei in each well and normalizing it to the mock control. The plot for DRCs was generated using Prism 7 software (GraphPad, San Diego, CA). IC_{50} (half-maximal inhibitory concentration) and CC_{50} (half-maximal cytotoxic concentration) values were calculated using XLfit 4 software (IDBS), with the following equation: $Y = \text{bottom} + (\text{top} - \text{bottom})/[1 + (IC_{50}/X)^{\text{Hillslope}}]$. IC_{50} values were calculated from the normalized activity data set-fitted curves. All IC_{50} and CC_{50} values were

measured in duplicate, and the quality of each assay was controlled by Z'-factor and the coefficient of variation in percent (%CV).

Time-of-addition assay

Vero cells were seeded at 3.5×10^4 cells per well in DMEM, supplemented with 2% heat-inactivated FBS and 2% antibiotic-antimycotic solution (Gibco), in 96-well μ-Clear plates (Greiner Bio-One) at 24 h prior to the experiment. Subsequently, cells were infected with SARS-CoV-2 at an MOI of 3 at 4 ℃ for 1 h to synchronize the infection, followed by three washes with DPBS to remove the unbound virus. Compounds were added at a concentration higher than their IC_{90} value to Vero cells either 1 h prior to, or at 0, 1, 2, 3, and 4 h after infection with SARS-CoV-2. At 12 hpi, the infected cells were fixed with 4% PFA and quantified by IFA as previously described.

Drug combination study

Vero cells were seeded at 1.2×10^4 cells per well with DMEM (Welgene) supplemented with 2% heat-inactivated FBS and 2% antibiotic-antimycotic solution (Gibco) in a black, 384-well, μClear plates (Greiner Bio-One) 24 h before the experiment. Seven-point with a 2-fold serial dilution from $8 \times IC_{50}$, where were determined in separated single-drug experiments, were generated; 0.39 to 25 μM for SB27041 and SB27047, and 0.78 to 50 μM for remdesivir. At 24 hpi, the infected cells were fixed with 4% PFA, quantified by IFA, and the data were analyzed and normalized as previously described. The synergistic scores were calculated by synergyfinder R-package (v3.2.2) using the zero interaction potency (ZIP) model.[a,b]

[a] W. D. Jang, S. Jeon, S. Kim, S. Y. Lee, *PNAS*. **2021**, *118*, e2024302118.
[b] A. Ianevski, A. K. Giri, T. Aittokallio. *Nucleic Acids Res*. **2020**, 48, W488–W493.

Supplementary Figures

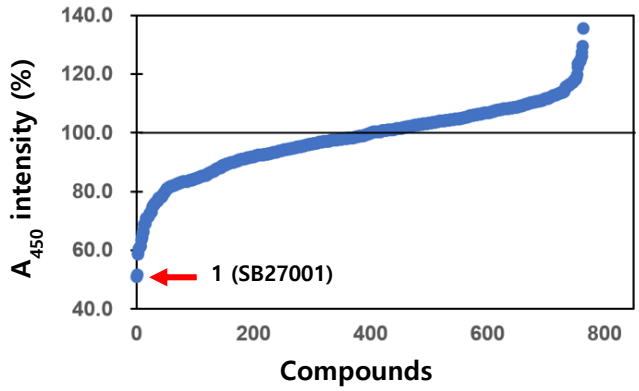


Figure S1. The screening data of the RBD-ACE2 sandwich ELISA assay.

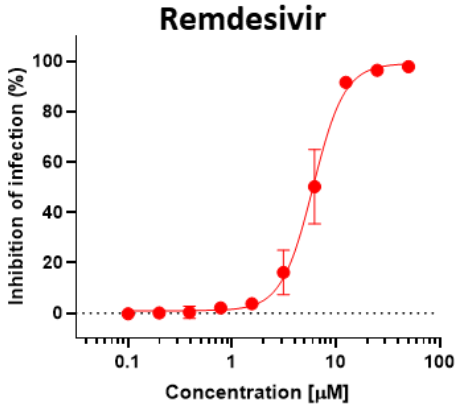


Figure S2. Dose-dependency data on the basis of immunofluorescence analysis with Vero cells infected by SARS-CoV-2 for 24 h upon remdesivir treatment. (IC_{50} = 6.0 μM)

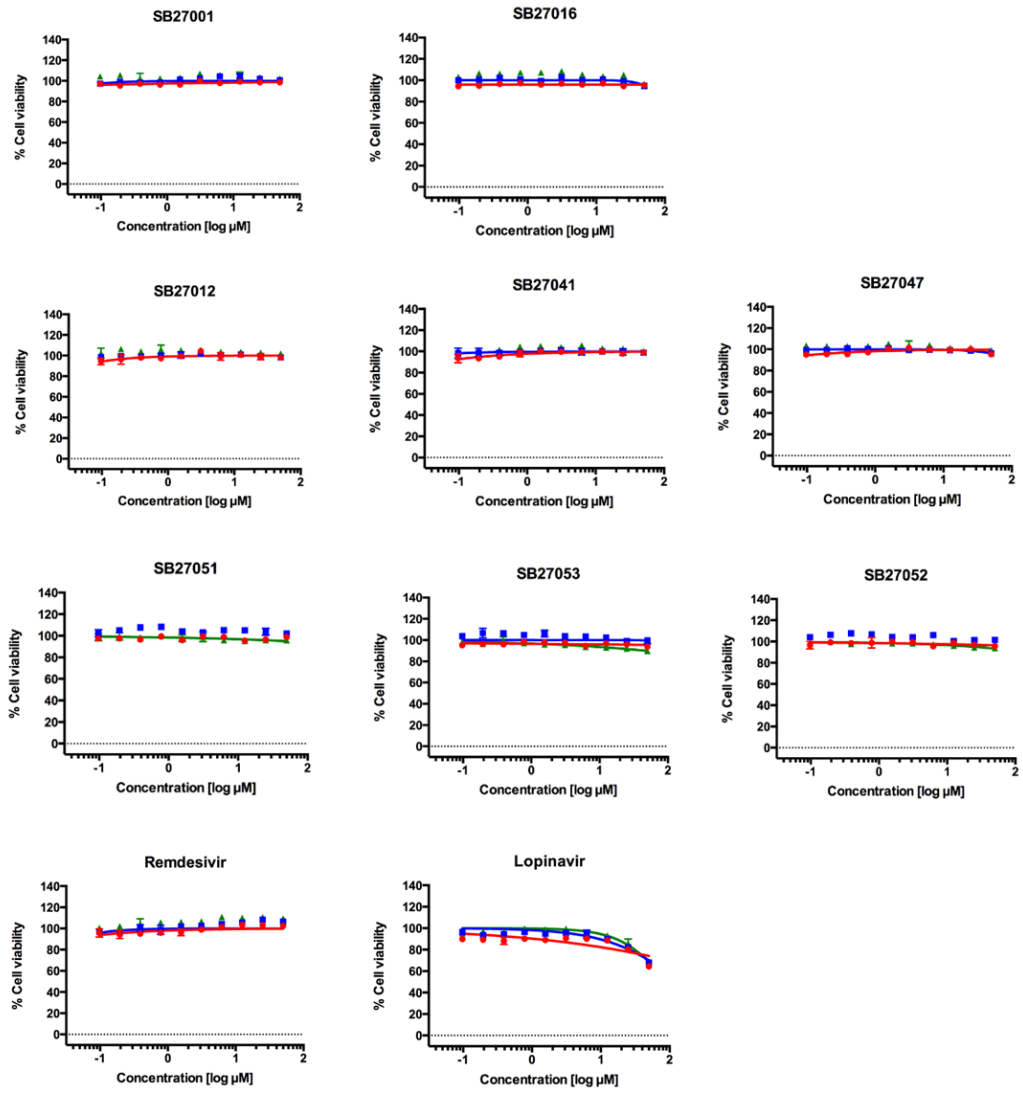


Figure S3. Viability data of Vero cells infected with ancestral (red, lineage A) and variants of SARS-CoV-2 from UK (blue, lineage B.1.1.7) and South Africa (green, lineage B.1.351) in the presence of testing compounds for 24 h.

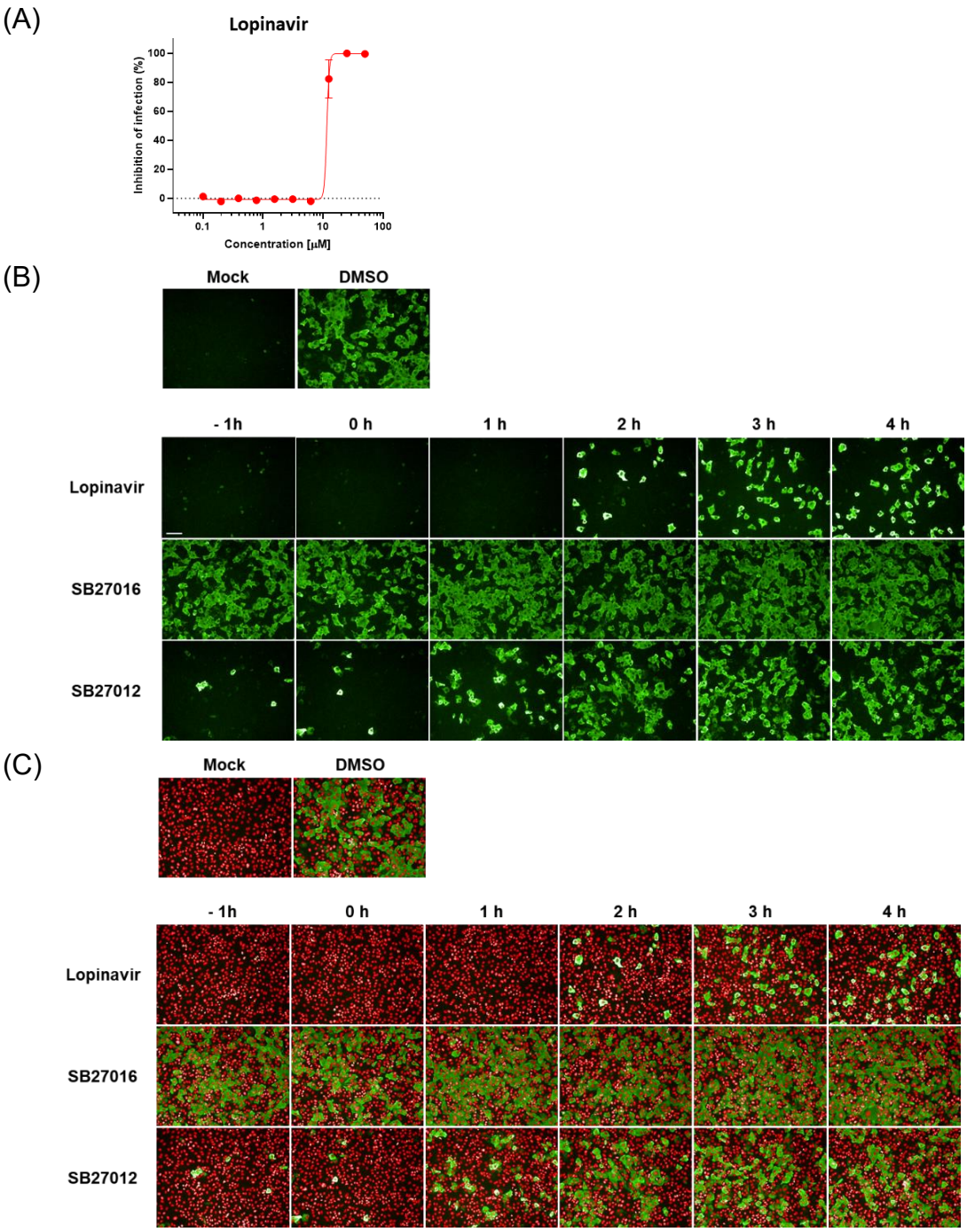


Figure S4. (A) Dose-dependency data for the immunofluorescence analysis of Vero cells infected by SARS-CoV-2 for 24 h upon lopinavir treatment (IC_{50} = 11.6 μM). (B, C) Immunofluorescence data from the time-of-addition study in Fig. 3B, showing SARS-CoV-2 N protein (green) without (B) and with (C) nuclei (red). Vero cells were treated with compounds (25 μM) at the indicated time points. (Scale bar = 100 μm)

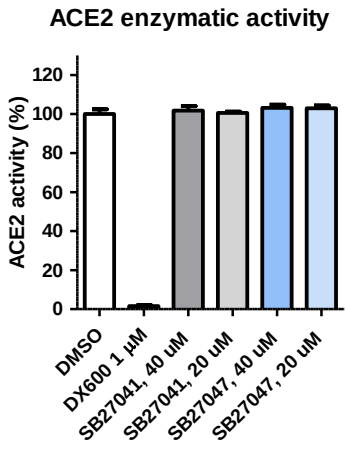


Figure S5. ACE2 enzymatic activity inhibition data. SB27041 and SB27047 did not inhibit the protease activity of ACE2. Data shown as Mean ± SEM (n = 2).

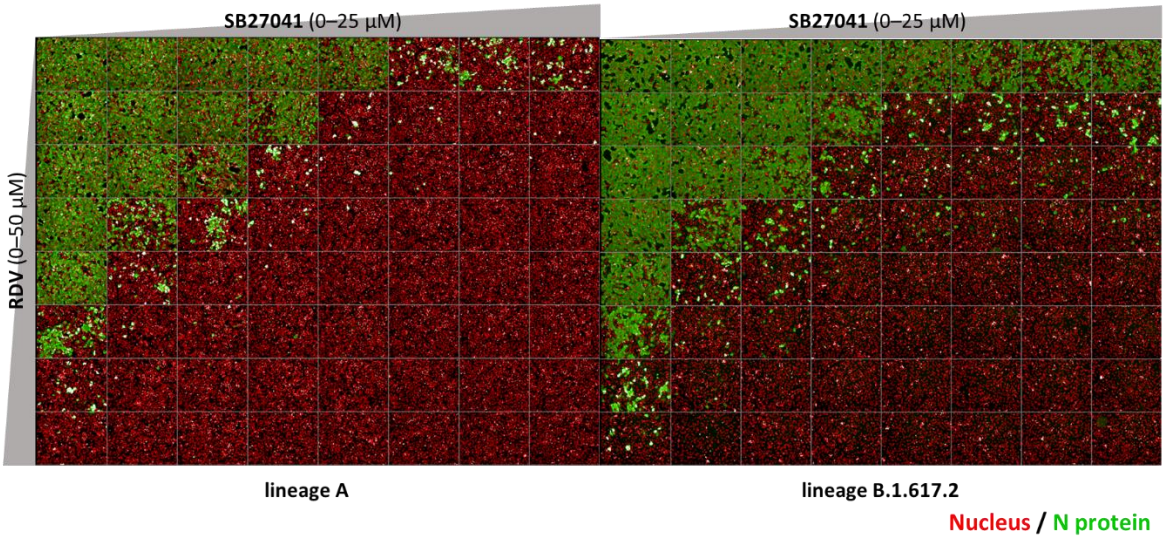


Figure S6. Immunofluorescence data from the drug combination study with remdesivir (RDV) and SB27041 in Vero cells infected with ancestral (left image) or delta variant (right image) of SARS-CoV-2.

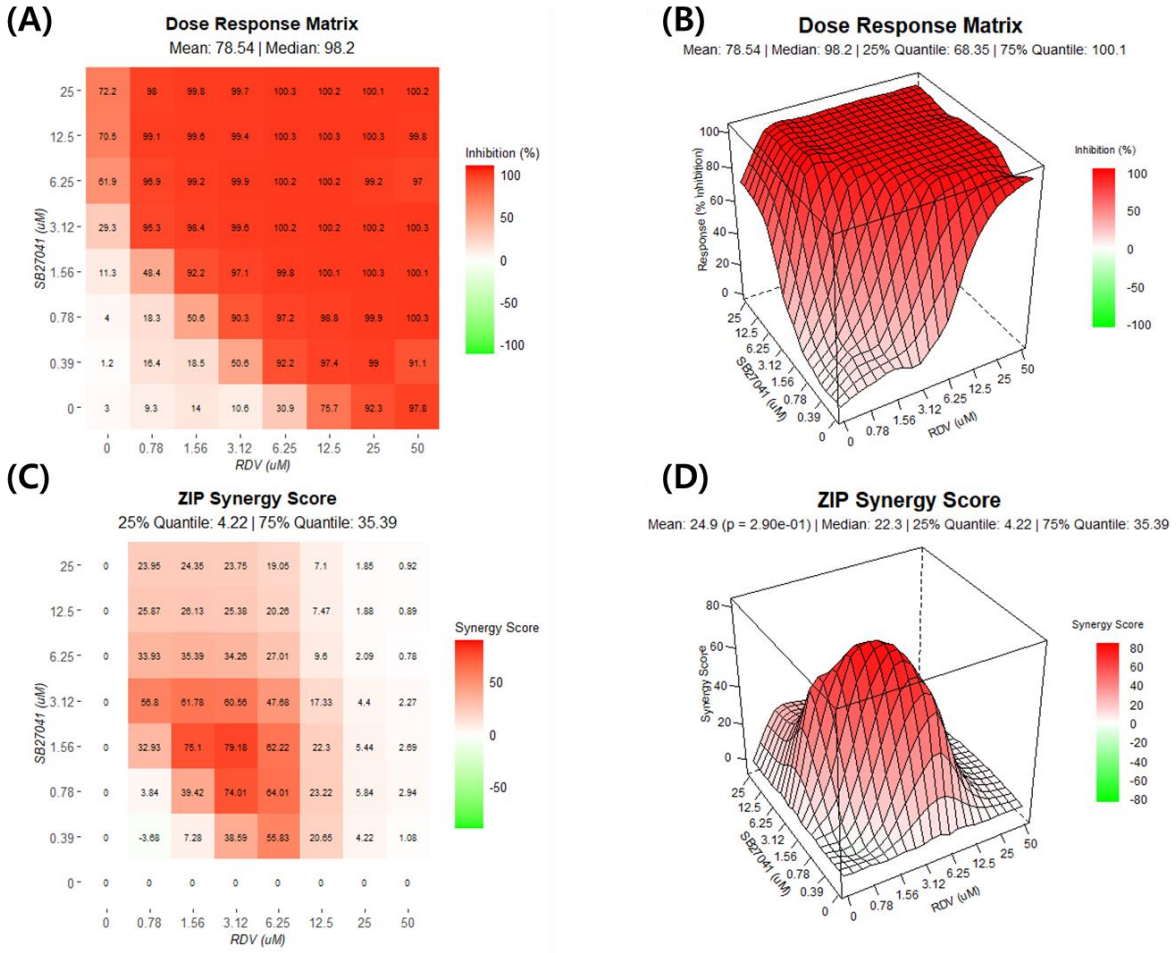


Figure S7. Quantitative analyses of the drug combination data with SB27041 and RDV in Vero cells infected with SARS-CoV-2 variant (lineage A, ancestral). Quantified dose-dependent data for the viral inhibition (%) are visualized in 2-D (A) and 3-D (B) manners. In addition, synergy score from SB27041 and RDV are visualized in 2-D (C) and 3-D (D) manners.

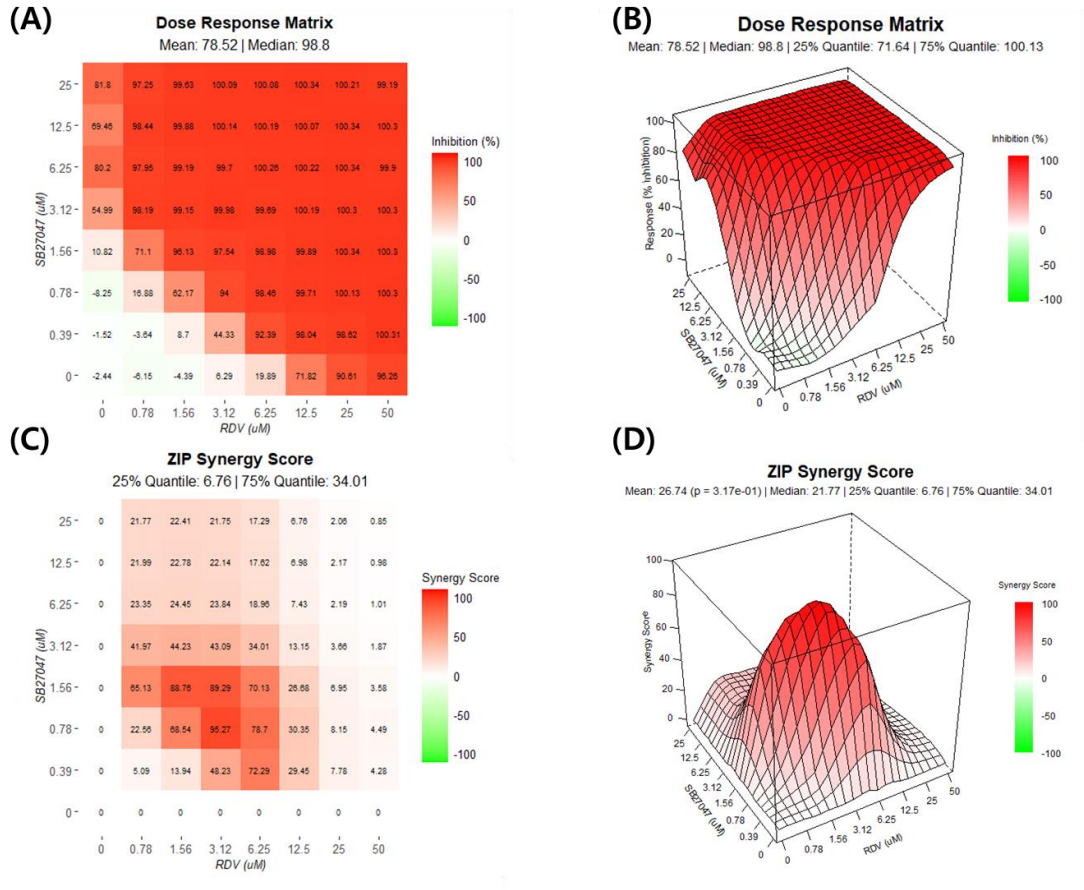


Figure S8. Quantitative analyses of the drug combination data with SB27047 and RDV in Vero cells infected with SARS-CoV-2 variant (lineage A, ancestral). Quantified dose-dependent data for the viral inhibition (%) are visualized in 2-D (A) and 3-D (B) manners. In addition, synergy score from SB27047 and RDV are visualized in 2-D (C) and 3-D (D) manners.

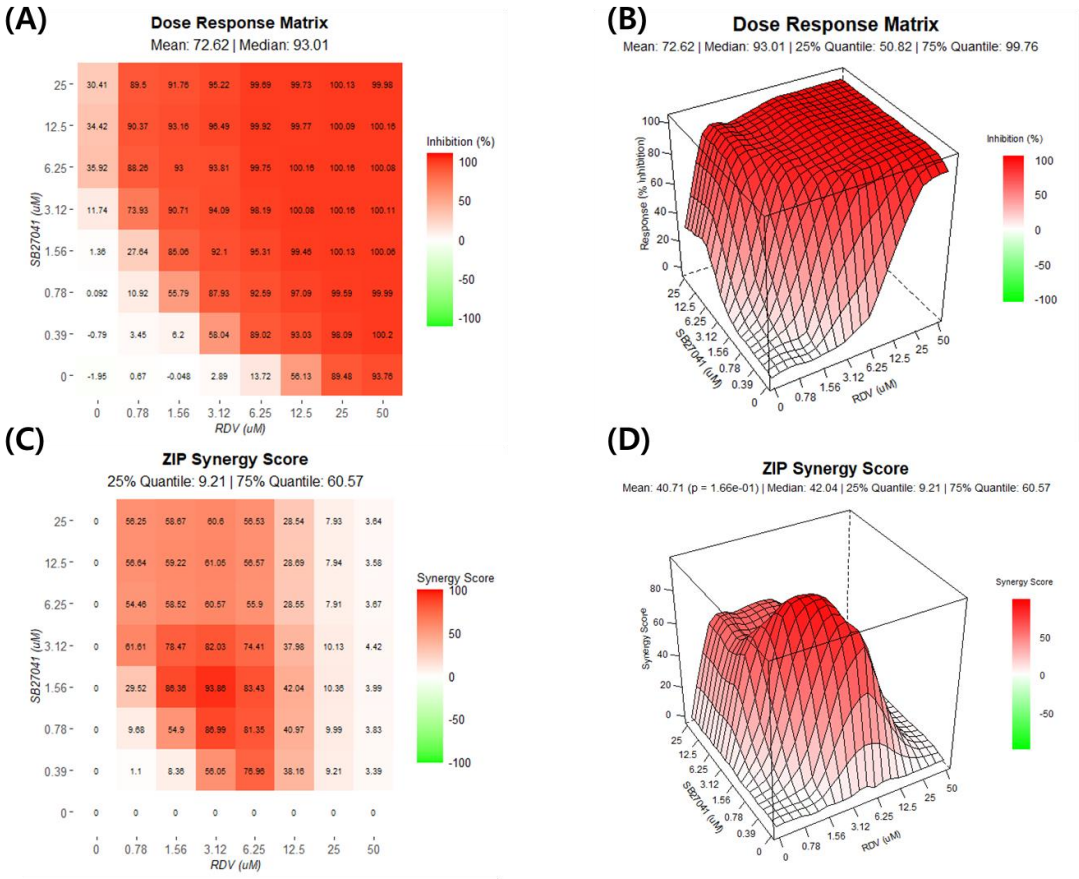


Figure S9. Quantitative analyses of the drug combination data with SB27041 and RDV in Vero cells infected with SARS-CoV-2 variant (lineage B.1.617.2, delta). Quantified dose-dependent data for the viral inhibition (%) are visualized in 2-D (A) and 3-D (B) manners. In addition, synergy scores from SB27041 and RDV are visualized in 2-D (C) and 3-D (D) manners.

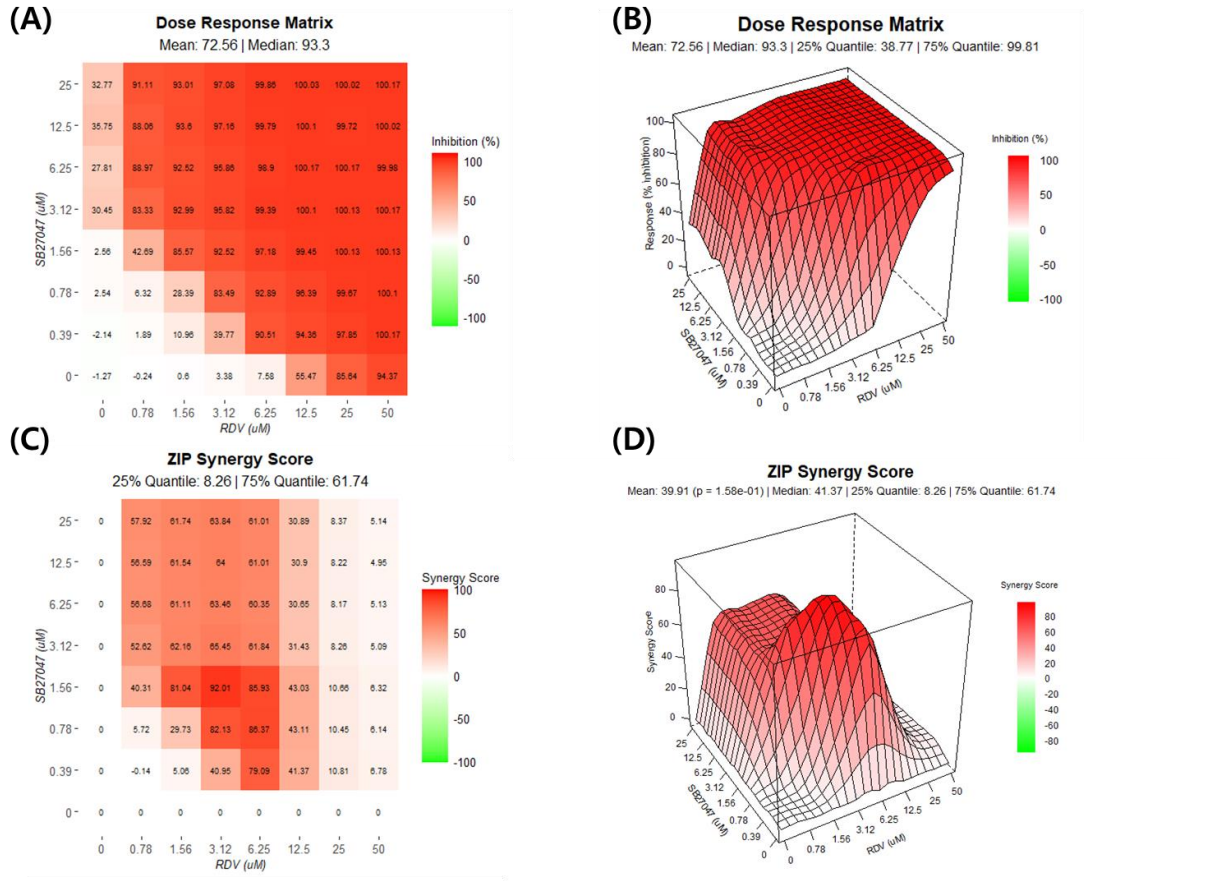


Figure S10. Quantitative analyses of the drug combination data with SB27047 and RDV in Vero cells infected with SARS-CoV-2 variant (lineage B.1.617.2, delta). Quantified dose-dependent data for the viral inhibition (%) are visualized in 2-D (A) and 3-D (B) manners. In addition, synergy score from SB27047 and RDV are visualized in 2-D (C) and 3-D (D) manners.

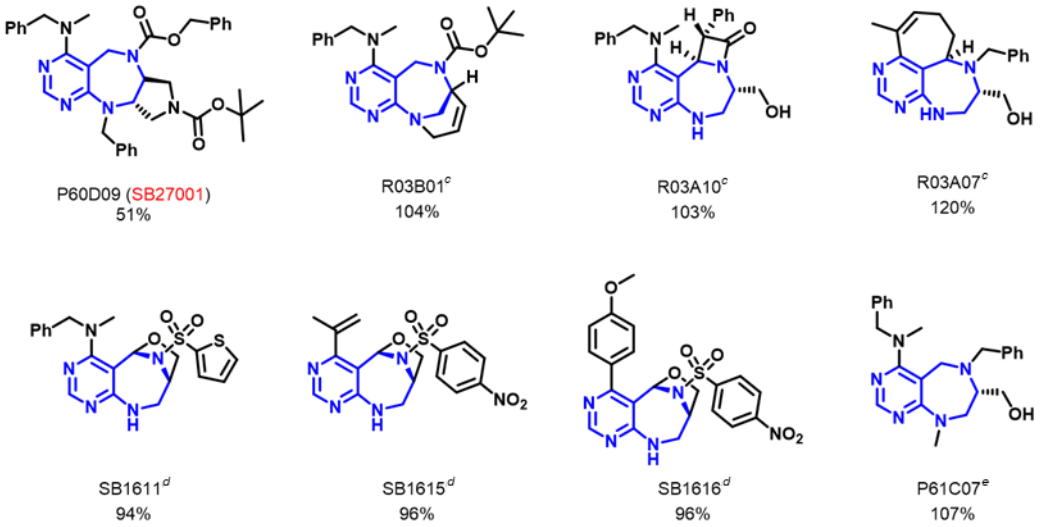


Figure S11. Pyrimidodiazepine-contained compounds inactive for the RBD-ACE2 ELISA assay. The compounds above are belong to our in-house pDOS library tested at 20 μM for the initial ELISA screening. The % values are ELISA data for the intensity of absorbance at 450 nm. 100% indicates the full binding between the RBD and ACE2. Only reported compounds are shown here.

[c] J. Kim, J. Jung, J. Koo, W. Cho, W. S. Lee, C. Kim, W. Park, S. B. Park, *Nat. Commun.* **2016**, *7*, 13196.
[d] Y. H. Shin, H. N. Cho, B. Y. Choi, J. Kim, J. Ha, S. W. Suh, S. B. Park, *Angew. Chem. Int. Edit.* **2021**, *60*, 1831–1838.
[e] J. Koo, J. Kim, S. B. Park, *Org. Lett.* **2017**, *19*, 344–347.

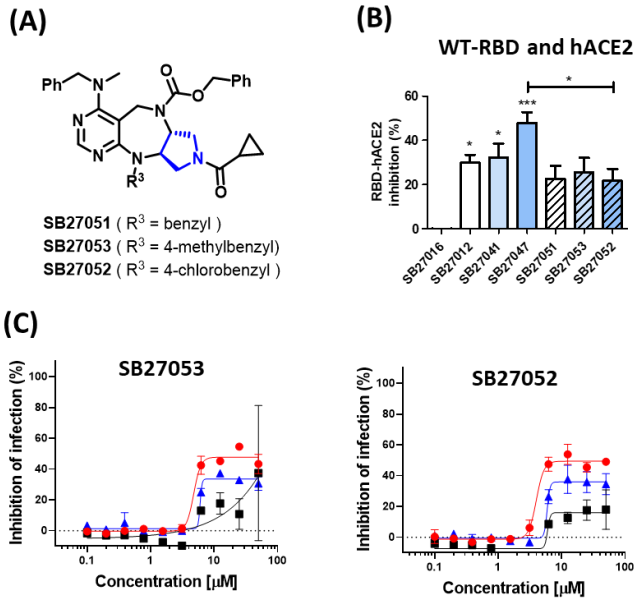


Figure S12. Activity analyses of SB27051, SB27053, and SB27052, enantiomers of SB27012, SB27041, and SB27047, respectively. (A) Chemical structure of enantiomers of SB27012, SB27041, and SB27047. (B) RBD-ACE2 sandwich ELISA data evaluating the inhibitory activity of SB compounds at 5 μM. Statistical differences between the compounds were analyzed using the one-way ANOVA test with Dunnett's post hoc analysis ($P > 0.05$, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$). (C) Dose-response curve analysis of the compounds in Vero cells infected with lineage A (red), lineage B.1.1.7 (black), and lineage B.1.351 (blue) SARS-CoV-2, respectively, for 24 h.

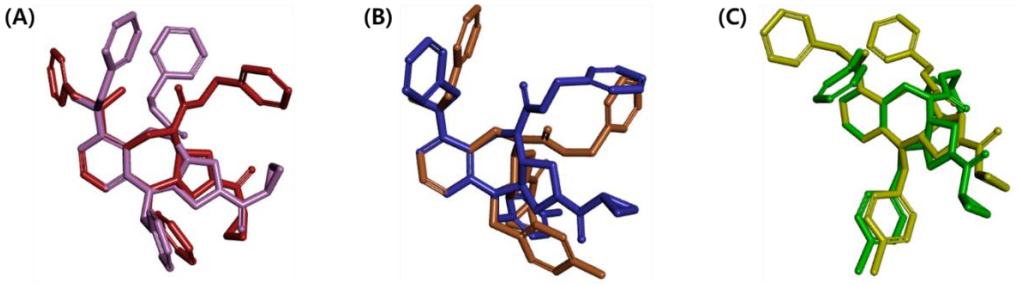


Figure S13. Overlay of energy-minimized structures of the original forms and the enantiomeric forms aligned with the pyrimidine substructure. (A) SB27012 (red) and SB27051 (purple). (B) SB27041 (brown) and SB27053 (blue). (C) SB27047 (yellow) and SB27052 (green).

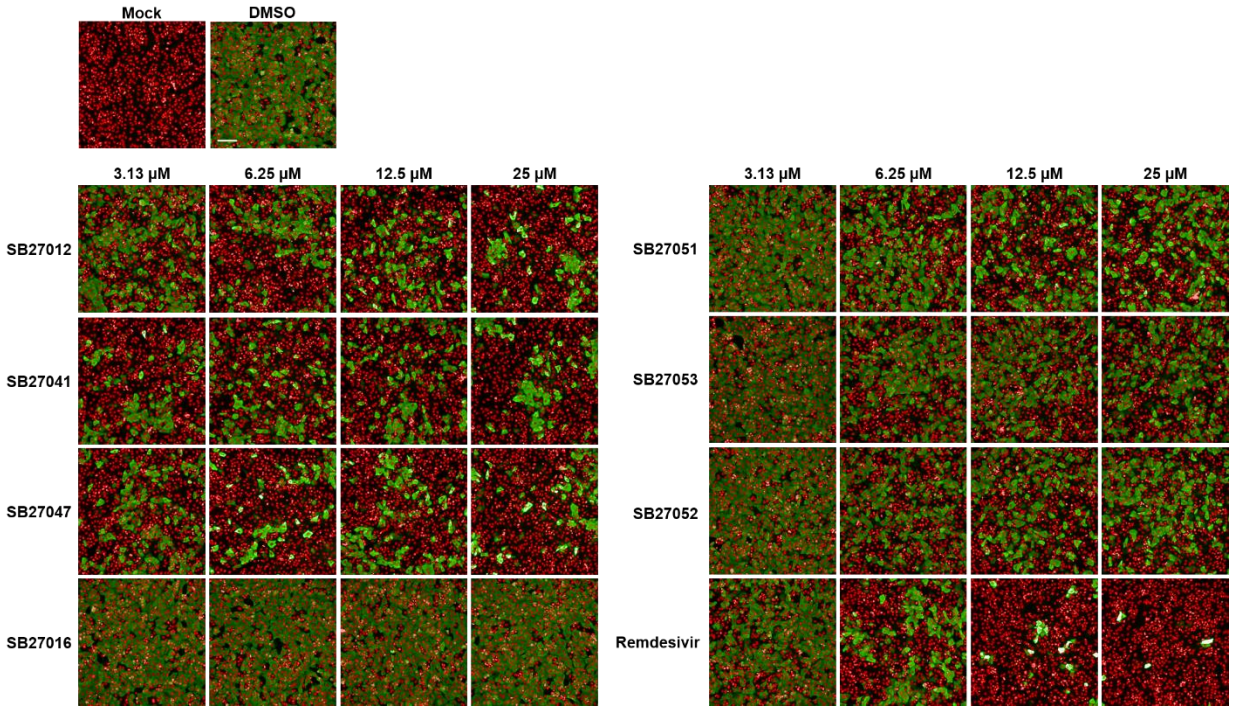
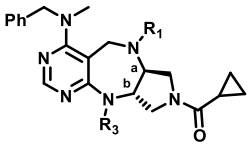
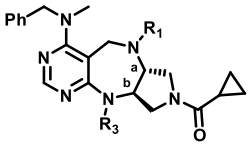


Figure S14. Immunofluorescence image data of Vero cells infected by SARS-CoV-2 for 24 h upon treatment with designated compounds. Infected cells were detected with anti-SARS-CoV-2 N protein antibody (green), and cell nuclei were stained with Hoechst 33342 (red). SB27051, SB27053, and SB27052 (the enantiomers of SB27012, SB27041, and SB27047, respectively) showed significantly decreased activities on the suppression of SARS-CoV-2 infection. (Scale bar = 100 μm)

Table S1. The RBD-ACE2 ELISA data converted as % inhibition for the second SAR study. Compounds were treated as 20 μM. Data were shown as Mean ± SD (n ≥ 3).



(S, S)



(R, R)

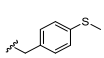
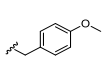
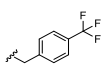
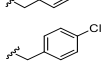
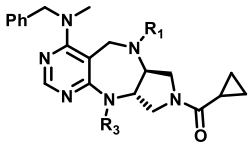
	R^1	R^3	(a, b)	RBD-hACE2 Inhibition (%)
6 (SB27012)			(S. S)	67.8 ± 5.9
14 (SB27018)			(S. S)	24.2 ± 10.1
15 (SB27019)			(S, S)	43.9 ± 6.0
16 (SB27020)			(S, S)	17.4 ± 6.2
17 (SB27021)			(S, S)	13.0 ± 14.4
18 (SB27023)			(S, S)	50.0 ± 11.8
19 (SB27024)			(S, S)	14.5 ± 5.5
20 (SB27025)			(S, S)	25.1 ± 14.7
21 (SB27026)			(S, S)	40.7 ± 9.2
22 (SB27028)			(S, S)	30.0 ± 4.7
23 (SB27030)			(S, S)	52.0 ± 0.6
24 (SB27031)			(S, S)	48.5 ± 9.1
25 (SB27032)			(S, S)	41.7 ± 1.4
26 (SB27041)			(S, S)	61.9 ± 4.2
27 (SB27043)			(S, S)	60.9 ± 8.4
28 (SB27044)			(S, S)	57.4 ± 1.5
29 (SB27045)			(S, S)	52.2 ± 6.7
30 (SB27048)			(S, S)	n.d.
31 (SB27047)			(S, S)	64.7 ± 3.3
32 (SB27049)			(S, S)	56.2 ± 0.9
33 (SB27050)			(S, S)	65.8 ± 0.3
34 (SB27051)			(R, R)	54.0 ± 1.2
35 (SB27053)			(R, R)	58.4 ± 2.2
36 (SB27052)			(R, R)	59.4 ± 0.3

Table S2. The RBD-ACE2 ELISA data converted as % inhibition for the enantiomeric compounds tested at 5 μM. Data were shown as Mean ± SD (n ≥ 3).



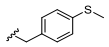
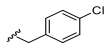
	R^1	R^3	RBD-hACE2 Inhibition (%)
6 (SB27012)			30.2 ± 9.0
26 (SB27041)			17.8 ± 4.0
27 (SB27043)			36.0 ± 5.9
28 (SB27044)			31.3 ± 1.1
31 (SB27047)			42.6 ± 4.9
32 (SB27049)			20.9 ± 3.7
33 (SB27050)			14.0 ± 4.3

Table S3. IC_{50} values and maximum inhibition percentages of the compounds about lineage A.

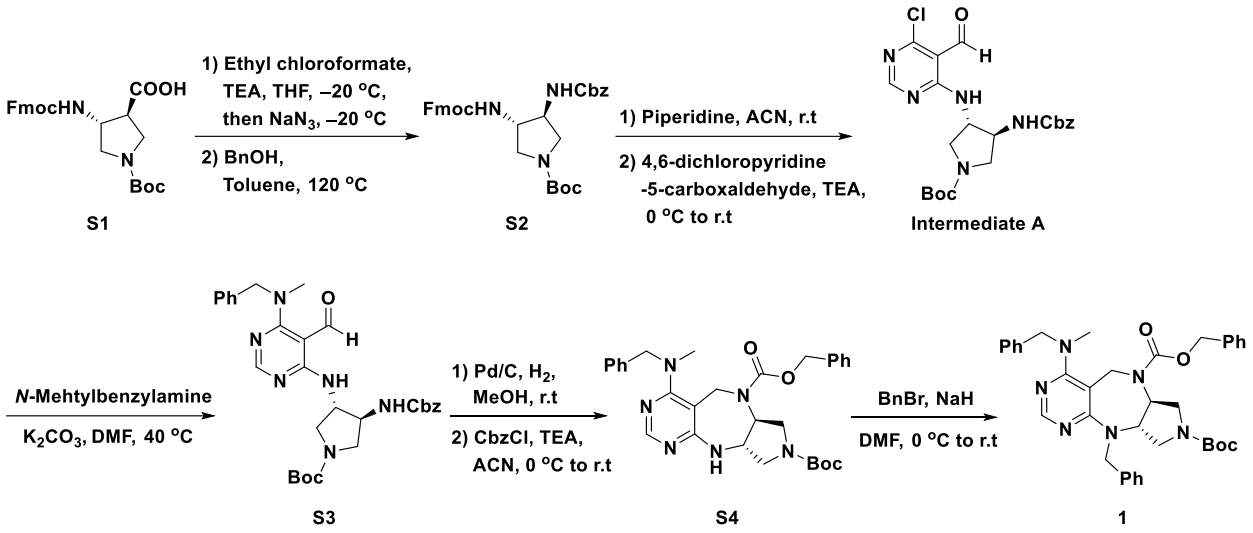
	IC_{50} (μM)	Maximum inhibition percentage (%)
SB27012	3.7	69
SB27041	2.2	72
SB27047	2.6	69
SB27051	8.1	53
SB27052	7.0	49
SB27053	7.8	48

Synthetic Procedures and Compound Characterization

General Information of Synthetic Protocols

NMR spectra were obtained on an Agilent 400-MR DD2 Magnetic Resonance System (400 MHz, Agilent, USA), JEOL ECX-400 (400 MHz, Jeol, Japan), Varian/Oxford Unity Inova-500 (500 MHz, Varian Assoc., Palo Alto, USA), VNMRS 500 (500 MHz, Varian Assoc., Palo Alto, USA) or Bruker Avance 600 MHz Cryo-NMR Spectrometer (600 MHz, Bruker, Germany). Chemical shift values were recorded as parts per million (δ), referenced to tetramethylsilane (TMS) as the internal standard, or to the residual solvent peak ($CDCl_3$, ^1H: 7.26, ^{13}C: 77.16, DMSO-d_6, ^1H: 2.50, ^{13}C: 39.52). Multiplicities were indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), p (pentet); m (multiplet); dd (doublet of doublet); dt (doublet of triplet); td (triplet of doublets); br s (broad singlet) and so on. Coupling constants were reported in hertz (Hz). Low-resolution mass spectrometry (LRMS) was conducted by LCMS-2020 (Shimadzu). High-resolution mass spectrometry (HRMS) of final compounds was confirmed by Thermo Scientific Orbitrap (ThermoFisher Scientific). Optical rotation was measured by JP/P-1030 (JASCO) using a sodium lamp (D line, 589 nm). HPLC was carried out on LCMS-2020 (Shimadzu) by using YMC-Triart C18 column (TA12S05-1546WT, 150×4.6 mmID, S-5 μm, 12 nm) under the following condition: gradient 10% B to 100% B in 20 min (buffer A = 0.1% HCOOH in water; buffer B = 0.1% HCOOH in acetonitrile). All commercially available reagents for organic synthesis were purchased from Sigma-Aldrich, Tokyo Chemical Industry Co., Ltd, or Thermo Fisher Scientific and used without further purification unless otherwise specified. Solvents were purchased from commercial vendors and used without further purification unless otherwise mentioned. Dry solvents were prepared using the ultimate solvent purification system CT-SPS-SA (Glass Contour). Analytical thin-layer chromatography (TLC) was performed using Merck Kiselgel 60 F_{254} plates, and the components were visualized by observation under UV light (254 and 365 nm) or by treating the plates with ninhydrin followed by thermal visualization. Flash column chromatography was performed on Merck Kieselgel 60 (230–400 mesh), and Prep-TLC was performed on Merck PLC Silica gel F_{254}, 1mm. All quantum mechanical calculations were performed in Gaussian09W. The ground state structures of synthesized core skeletons were optimized using density functional theory (DFT) at the B3LYP/6-31G* level.

Preparation of 6-benzyl 8-(*tert*-butyl) (6a*S*,9a*S*)-10-benzyl-4-(benzyl(methyl)amino)-5,6a,7,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4] diazepine-6,8-dicarboxylate (1)



The synthesis of the (3*S*,4*R*)-4-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-1-(tert-butoxycarbonyl)pyrrolidine-3-carboxylic acid (**S1**) was previously reported.[f]

[f] H. S. Lee, P. R. LePlae, E. A. Porter, S. H. Gellman, *J. Org. Chem*. **2001**, *66*, 3597–3599.

***tert*-Butyl (3*S*,4*S*)-3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)-amino)-4-(((benzyloxy)carbonyl)amino)pyrrolidine-1-carboxylate (S2)**. To a solution of **S1** (5.21 g, 11.51 mmol) and dry triethylamine (Et_3N; 3.21 mL, 23.03 mmol) in dry tetrahydrofuran (THF; 230.0 mL) under argon atmosphere was added ethyl chloroformate (1.64 mL, 17.27 mmol) dropwise over 10 min at –20 °C. The mixture was stirred at –20 °C for 30 min. A solution of sodium azide (NaN_3; 1.87 g, 28.78 mmol) in water (5.0 mL) was added to the resultant solution. After being stirred at –20 °C for 1 h, the reaction mixture was concentrated under reduced pressure. The resultant was diluted with dichloromethane (DCM), washed with 5% sodium carbonate (Na_2CO_3), 10% citric acid, and brine sequentially. The organic layer was dried over anhydrous sodium sulfate (Na_2SO_4), and filtered. The filtrate was condensed under reduced pressure, and the crude residue was dissolved in toluene (110.0 mL). Benzyl alcohol (3.0 mL, 28.85 mmol) was added to this solution, and the resulting mixture was stirred at 120 °C for 3 h. The resultant was condensed under reduced pressure, followed by silica-gel flash column chromatography to afford **S2** (5.03 g, 78% overall yield) as a white solid.

R_f = 0.57 (EtOAc/Hex = 1:1); $[\alpha]_D^{25}$ +22.8 (*c* = 0.3, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 7.89 (d, *J* = 7.5 Hz, 2H), 7.73–7.58 (m, 4H), 7.41 (t, *J* = 7.5 Hz, 2H), 7.38–7.25 (m, 7H), 5.02 (s, 2H), 4.38–4.27 (m, 2H), 4.26–4.19 (m, 1H), 4.03–3.88 (m, 2H), 3.59–3.49 (m, 2H), 3.12–3.03 (m,

2H), 1.40 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.9, 153.4, 143.9, 143.8, 140.8, 136.9, 128.3, 127.8, 127.6, 127.1, 125.2, 125.1, 120.1, 78.6, 65.5, 54.5, 53.7, 49.1, 48.6, 46.7, 28.1; LRMS(ESI+): Cacld for $C_{32}H_{35}N_3O_6Na^+$ $[M+Na]^+$ 580.24, found 580.10.

***tert*-Butyl (3*S*,4*S*)-3-((6-(benzyl(methyl)amino)-5-formyl-pyrimidin-4-yl)amino)-4-(((benzyloxy)carbonyl)amino)pyrrolidine-1-carboxylate (S3)**. To a solution of **S2** (1.50 g, 2.70 mmol) in acetonitrile (ACN; 55.0 mL) was added piperidine (5.33 mL, 54.0 mmol), and the resulting mixture was stirred at r.t. After completion of the reaction as indicated by TLC, the resultant was filtered through a short pad of silica gel with DCM/MeOH (10:1) to remove excess amount of piperidine. The filtrate was condensed under reduced pressure, and the crude residue was dissolved in chloroform ($CHCl_3$; 35.0 mL). Dry Et_3N (1.5 mL, 10.8 mmol) and 4,6-dichloropyridine-5-carboxaldehyde (0.62 g, 3.5 mmol) were sequentially added to this solution at 0 ℃, and the resulting mixture was left to stir and allowed to warm to r.t. After completion of the reaction as indicated by TLC, the resultant was quenched with saturated ammonium chloride (NH_4Cl) (aq) and extracted three times with DCM. The combined organic layer was dried over anhydrous Na_2SO_4(s) and filtered, followed by silica-gel flash column chromatography to afford the intermediate **A** (846 mg, 66% yield). To a solution of intermediate **A** (700 mg, 1.47 mmol) in dry *N,N*-dimethylformamide (DMF; 14.0 mL) were sequentially added potassium carbonate (K_2CO_3; 1.02 g, 7.35 mmol) and *N*-methyl benzylamine (0.57 mL, 4.41 mmol) at r.t, and the mixture was stirred at 40 ℃. After completion of the reaction as indicated by TLC, the reaction mixture was quenched with saturated NH_4Cl(aq) and extracted three times with ethyl acetate (EtOAc). The combined organic layer was dried over anhydrous Na_2SO_4(s), filtered and evaporated. Subsequent silica-gel flash column chromatography afforded **S3** (610 mg, 84% yield, 55% overall yield) as a pale yellow solid.

R_f = 0.30 (EtOAc/Hex = 1:1); ^{1}H NMR (400 MHz, DMSO-d_6, 100 ℃) δ 9.84 (s, 1H), 9.19 (d, J = 7.0 Hz, 1H), 8.11 (s, 1H), 7.38–7.22 (m, 11H), 5.09–4.97 (m, 2H), 4.88 (s, 2H), 4.58 (p, J = 6.9 Hz, 1H), 4.15 (p, J = 7.2 Hz, 1H), 3.79 (dd, J = 11.0, 7.0 Hz, 1H), 3.64 (dd, J = 11.1, 7.4 Hz, 1H), 3.22–3.15 (m, 2H), 3.14 (s, 3H), 1.41 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6, 100 ℃) δ 187.5, 165.4, 161.6, 158.6, 155.3, 153.1, 136.7, 136.6, 128.0, 127.7, 127.2, 127.0, 126.9, 126.7, 95.6, 78.3, 65.1, 54.9, 54.3, 53.8, 49.1, 48.5, 27.7; LRMS(ESI+): Cacld for $C_{30}H_{37}N_6O_5^+$ $[M+H]^+$ 561.28, found 561.35.

6-Benzyl 8-(*tert*-butyl) (6a*S*,9a*S*)-4-(benzyl(methyl)amino)-5,6a,7,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6,8-dicarboxylate (S4). To a solution of **S3** (0.51 g, 0.91 mmol) in methanol (MeOH; 20.0 mL) was carefully added 30 wt % Pd/C (0.15 g) at r.t and the mixture was vigorously stirred under H_2 atmosphere (1 atm). After completion of the reaction as

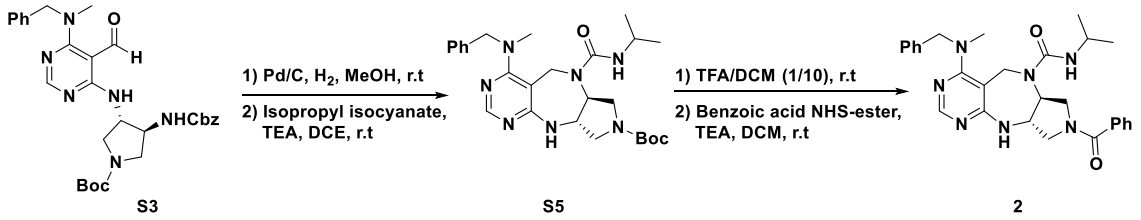
indicated by TLC, the reaction mixture was filtered through Celite® while washing with MeOH. The filtrate was condensed under reduced pressure, and the crude resultant was dissolved in dry ACN (15.0 mL). Dry Et_3N (0.63 mL, 4.52 mmol) and benzyl chloroformate (CbzCl; 0.44 mL, 3.15 mmol) were sequentially added to this solution at 0 ℃, and the resulting mixture was left to stir and allowed to warm to r.t. After completion of the reaction as indicated by TLC, the resultant was quenched with saturated NH_4Cl(aq) and extracted three times with DCM. The combined organic layer was dried over anhydrous Na_2SO_4(s) and filtered. The filtrate was condensed under reduced pressure, followed by silica-gel flash column chromatography to afford **S4** (432 mg, 88% overall yield) as a white solid.

R_f = 0.62 (EtOAc/MeOH = 10:1); ^{1}H NMR (400 MHz, DMSO-*d*$_6$, 100 ℃) δ 7.94 (s, 1H), 7.34–7.17 (m, 10H), 7.08 (br s, 1H), 5.13 (d, *J* = 12.7 Hz, 1H), 5.05 (d, *J* = 12.7 Hz, 1H), 4.89 (d, *J* = 16.2 Hz, 1H), 4.82–4.69 (m, 2H), 4.54 (d, *J* = 15.4 Hz, 1H), 4.22 (d, *J* = 15.3 Hz, 1H), 4.19–4.13 (m, 1H), 3.67 (dd, *J* = 10.0, 7.3 Hz, 1H), 3.61 (dd, *J* = 10.0, 7.8 Hz, 1H), 3.19 (t, *J* = 10.2 Hz, 1H), 3.07–3.00 (m, 1H), 2.71 (s, 3H), 1.40 (s, 9H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$, 100 ℃) δ 166.3, 162.4, 155.0, 154.3, 153.1, 137.9, 136.2, 127.8, 127.7, 127.3, 127.0, 126.8, 126.2, 97.7, 78.4, 66.2, 62.3, 56.1, 51.8, 47.2, 45.8, 39.8, 38.8, 27.7; LRMS(ESI+): Cacld for $C_{30}H_{37}N_6O_4^+$ $[M+H]^+$ 545.29, found 545.25.

6-Benzyl 8-(*tert*-butyl) (6a*S*,9a*S*)-10-benzyl-4-(benzyl(methyl)amino)-5,6a,7,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6,8-dicarboxylate (1). To a solution of **S4** (0.25 g, 0.46 mmol) in dry DMF (5.0 mL) under argon atmosphere was added sodium hydride (NaH; 60 % dispersion in mineral oil, 55 mg, 1.38 mmol) at 0 ℃. After being stirred at 0 ℃ for 30 min, benzyl bromide (0.11 mL, 0.92 mmol) was slowly added to the resultant suspension and the mixture was allowed to slowly warm to r.t. After the starting material was consumed as indicated by TLC, the reaction mixture was quenched with saturated NH_4Cl(aq) and extracted three times with EtOAc. The combined organic layer was dried over anhydrous Na_2SO_4(s) and filtered and evaporated. Subsequent silica-gel flash column chromatography afforded **1** (233 mg, 80% yield) as a white solid.

R_f = 0.67 (EtOAc/Hex = 1:1); ^{1}H NMR (400 MHz, DMSO-*d*$_6$, 100 ℃) δ 8.04 (s, 1H), 7.36–7.14 (m, 15H), 5.14 (d, *J* = 12.7 Hz, 1H), 5.03 (d, *J* = 12.8 Hz, 1H), 5.01–4.92 (m, 2H), 4.87 (d, *J* = 16.7 Hz, 1H), 4.80 (d, *J* = 16.4 Hz, 1H), 4.73–4.67 (m, 2H), 4.49 (ddd, *J* = 11.6, 9.9, 7.6 Hz, 1H), 4.32 (d, *J* = 15.4 Hz, 1H), 3.51 (dd, *J* = 10.0, 7.7 Hz, 1H), 3.39–3.28 (m, 2H), 3.04 (t, *J* = 10.0 Hz, 1H), 2.81 (s, 3H), 1.34 (s, 9H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$, 100 ℃) δ 166.3, 162.6, 154.9, 153.6, 153.1, 139.6, 138.0, 136.3, 127.8, 127.7, 127.7, 127.2, 127.1, 126.7, 126.3, 126.1, 125.8, 97.8, 78.4, 66.2, 57.9, 56.8, 55.8, 46.6, 45.4, 44.1, 40.6, 38.8, 27.6; HRMS(ESI+): Cacld for $C_{37}H_{43}N_6O_4^+$ $[M+H]^+$ 635.3341, found 635.3340, Δppm –0.16; HPLC purity: 93.8%, t_R = 17.6 min.

Preparation of (6a*S*,9a*S*)-8-benzoyl-4-(benzyl(methyl)amino)-*N*-isopropyl-6a,7,8,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6(5*H*)-carboxamide (2)



***tert*-Butyl (6a*S*,9a*S*)-4-(benzyl(methyl)amino)-6-(isopropyl-carbamoyl)-5,6a,7,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-8(6*H*)-carboxylate (S5)**. To a solution of **S3** (73 mg, 0.13 mmol) in MeOH (4.0 mL) was carefully added 30 wt % Pd/C (21 mg) at r.t and the mixture was vigorously stirred under H_2 atmosphere (1 atm). After completion of the reaction as indicated by TLC, the reaction mixture was filtered through Celite® while washing with MeOH. The filtrate was condensed under reduced pressure, and the crude resultant was dissolved in dry dichloroethane (DCE; 3.0 mL). Dry Et_3N (0.09 mL, 0.64 mmol) and isopropyl isocyanate (0.025 mL, 0.26 mmol) were sequentially added to this solution, and the resulting mixture was stirred at r.t. After the starting material was consumed as indicated by TLC, the reaction mixture was quenched with saturated NH_4Cl(aq) and extracted three times with DCM. The combined organic layer was dried over anhydrous Na_2SO_4(s) and filtered. The filtrate was condensed under reduced pressure, followed by silica-gel flash column chromatography to afford **S5** (48 mg, 75% overall yield) as a white solid.

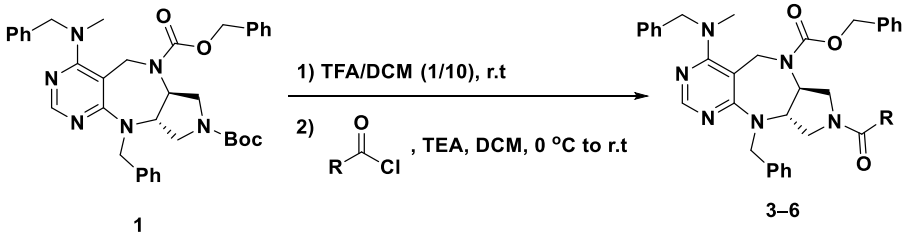
R_f = 0.34 (EtOAc/MeOH = 20:1); ^{1}H NMR (400 MHz, DMSO-d_6, 100 °C) δ 7.92 (s, 1H), 7.37–7.34 (m, 2H), 7.33–7.28 (m, 2H), 7.25–7.20 (m, 1H), 7.08 (br s, 1H), 5.54 (d, J = 7.4 Hz, 1H), 4.86 (d, J = 16.4 Hz, 1H), 4.77–4.66 (m, 1H), 4.59 (d, J = 7.7 Hz, 1H), 4.55 (d, J = 6.6 Hz, 1H), 4.28 (d, J = 15.3 Hz, 1H), 4.19–4.10 (m, 1H), 3.81–3.71 (m, 2H), 3.61 (dd, J = 9.9, 7.5 Hz, 1H), 3.17 (t, J = 10.0 Hz, 1H), 2.95–2.87 (m, 1H), 2.75 (s, 3H), 1.42 (s, 9H), 1.04 (dd, J = 16.1, 6.5 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6, 100 °C) δ 166.2, 162.7, 156.3, 154.1, 153.2, 137.9, 127.7, 127.3, 126.3, 98.7, 78.3, 61.4, 56.3, 52.7, 46.9, 45.9, 41.6, 38.9, 38.8, 27.8, 22.3; LRMS(ESI+): Cacld for $C_{26}H_{38}N_7O_3^+$ $[M+H]^+$ 496.30, found 496.35.

(6a*S*,9a*S*)-8-Benzoyl-4-(benzyl(methyl)amino)-*N*-isopropyl-6a,7,8,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6(5*H*)-carboxamide (2). **S5** (38 mg, 0.076 mmol) was treated with 10% trifluoroacetic acid (TFA) in DCM (7.5 mL) and the resulting mixture was stirred at r.t. After the starting material was consumed as indicated by TLC, any excess TFA was removed by azeotropic evaporation with toluene under reduced pressure. To a solution of the resulting Boc-deprotected product in dry DCM (1.5 mL) were sequentially added dry Et_3N (0.053 mL, 0.382

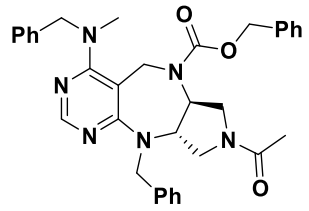
mmol) and benzoic acid *N*-hydroxysuccinimide ester (25 mg, 0.115 mmol) at r.t, and the mixture was left to stir. After completion of the reaction as indicated by TLC, the solvent was removed under reduced pressure, and the residue was purified through the prep-TLC (PLC) to afford **2** (28 mg, 73% overall yield) as a white solid.

R_f = 0.20 (EtOAc/MeOH = 20:1); ^{1}H NMR (400 MHz, DMSO-d_6) (as a mixture of rotamers) δ 7.93 (d, J = 14.7 Hz, 1H), 7.61–7.23 (m, 11H), 5.96 (dd, J = 36.7, 7.7 Hz, 1H), 4.99–4.74 (m, 2H), 4.62–4.49 (m, 2H), 4.30 (dd, J = 15.6, 11.0 Hz, 1H), 4.21 (td, J = 10.5, 7.1 Hz, 0.4H), 4.05 (qd, J = 10.7, 6.9 Hz, 1H), 3.85–3.73 (m, 1H), 3.74–3.63 (m, 1.2H), 3.59–3.50 (m, 0.7H), 3.48–3.39 (m, 1.3H), 3.13 (t, J = 10.7 Hz, 0.4H), 2.73 (d, J = 8.3 Hz, 3H), 1.10–0.87 (m, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) (as a mixture of rotamers) δ 168.8, 168.7, 166.5, 166.4, 162.8, 162.8, 156.4, 156.2, 154.5, 154.4, 138.3, 138.3, 136.0, 135.6, 130.4, 130.1, 128.5, 128.3, 128.3, 127.6, 127.6, 127.5, 126.9, 126.9, 98.7, 98.7, 62.0, 61.0, 56.6, 56.3, 53.3, 52.4, 50.1, 49.2, 47.1, 46.3, 42.0, 41.9, 39.2, 38.8, 23.0, 22.9, 22.9, 22.8; HRMS(ESI+): Cacld for $C_{28}H_{34}N_7O_2^+$ $[M+H]^+$ 500.2769, found 500.2768, Δppm –0.20; HPLC purity: 99.9%, t_R = 9.4 min.

General procedure for the preparation of 3–6



1 (38 mg, 0.059 mmol) was treated with 10% TFA in DCM (7.5 mL) at r.t, and the resulting mixture was stirred at r.t. After the starting material was consumed as indicated by TLC, any excess TFA was removed by azeotropic evaporation with toluene under reduced pressure. To a solution of the resulting Boc-deprotected crude product in dry DCM (3.0 mL) were sequentially added dry NEt_3 (0.033 μL, 0.24 mmol) and acid chloride (0.12 mmol) at 0 °C. The resulting mixture was left to stir and allowed to warm to r.t. After completion of the reaction as indicated by TLC, the solvent was removed under reduced pressure, and the residue was purified through the PLC to afford desired amide product.



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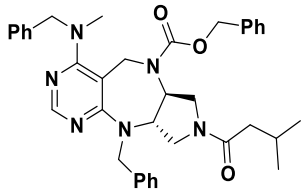
Benzyl (6a*S*,9a*S*)-8-acetyl-10-benzyl-4-(benzyl(methyl)amino)-6a,7,8,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6(5*H*)-carboxylate (3). A white solid; R_f = 0.16 (EtOAc/Hex = 2:1); 30 mg, 88% overall yield; ^{1}H NMR (400 MHz, DMSO-d_6, 100 ℃) (as a mixture of rotamers) δ 8.04 (d, J = 1.8 Hz, 1H), 7.34–7.13 (m, 15H), 5.13 (d, J = 12.8 Hz, 1H), 5.08–4.85 (m, 4H), 4.80 (d, J = 16.3 Hz, 1H), 4.75–4.65 (m, 2H), 4.56 (q, J = 10.2 Hz, 0.6H), 4.43 (q, J = 9.8 Hz, 0.4H), 4.32 (d, J = 15.5 Hz, 1H), 3.70–3.50 (m, 2.4H), 3.26 (dt, J = 17.4, 10.2 Hz, 1.2H), 3.03–2.95 (m, 0.4H), 2.81 (s, 3H), 1.82 (d, J = 22.9 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6, 100 ℃) (as a mixture of rotamers) δ 168.2, 166.3, 162.6, 154.9, 153.6, 139.5, 137.9, 136.2, 127.8, 127.8, 127.2, 127.1, 126.7, 126.3, 126.1, 125.9, 125.7, 97.8, 66.2, 58.4, 57.5, 57.4, 56.5, 55.8, 55.7, 46.6, 46.5, 45.3, 44.6, 43.3, 40.6, 38.8, 20.6; HRMS(ESI+): Cacld for $C_{34}H_{37}N_6O_3^+$ $[M+H]^+$ 577.2922, found 577.2921, Δppm –0.17; HPLC purity: 99.9%, t_R = 13.8 min. The product was synthesized with acetyl chloride.



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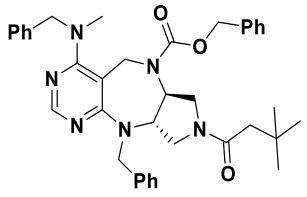
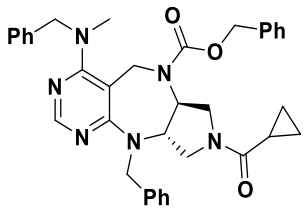
Benzyl (6a*S*,9a*S*)-8-acetyl-10-benzyl-4-(benzyl(methyl)amino)-6a,7,8,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6(5*H*)-carboxylate (4). A white solid; R_f = 0.36 (EtOAc/Hex = 2:1); 33 mg, 91% overall yield; ^{1}H NMR (400 MHz, DMSO-d_6, 70 ℃) (as a mixture of rotamers) δ 8.04 (d, J = 5.1 Hz, 1H), 7.37–7.14 (m, 15H), 5.14 (d, J = 12.8 Hz, 1H), 5.07–4.64 (m, 7H), 4.61–4.37 (m, 1H), 4.32 (dd, J = 15.5, 2.4 Hz, 1H), 3.71–3.48 (m, 2.3H), 3.26 (td, J = 10.3, 9.7, 3.8 Hz, 1.3H), 3.00 (t, J = 10.6 Hz, 0.4H), 2.80 (s, 3H), 2.10–1.87 (m, 3H), 0.90–0.79 (m, 6H); ^{13}C NMR (150 MHz, DMSO-d_6, 70 ℃) (as a mixture of rotamers) δ 170.3, 170.2, 166.4, 162.6, 162.6, 155.0, 153.8, 153.7, 139.7, 138.1, 136.4, 128.0, 128.0, 127.9, 127.4, 127.2, 127.1, 126.5, 126.3, 126.2, 126.1, 125.8, 97.8, 97.8, 66.3, 58.4, 57.5, 57.3, 56.3, 55.9, 55.7, 46.6, 44.9, 43.5, 41.6, 41.5, 40.7, 40.1, 39.0, 38.9, 24.3, 22.1, 22.1, 22.0; HRMS(ESI+): Cacld for $C_{37}H_{43}N_6O_3^+$ $[M+H]^+$ 619.3391, found 619.3391, Δppm 0.00; HPLC purity: 99.9%, t_R = 16.1 min. The product was synthesized with isovaleryl chloride.



5

Benzyl (6a*S*,9a*S*)-10-benzyl-4-(benzyl(methyl)amino)-8-(3,3-dimethylbutanoyl)-6a,7,8,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6(5*H*)-carboxylate (5). A white solid; R_f = 0.66 (EtOAc/Hex = 2:1); 35 mg, overall 94% yield; ^{1}H NMR (400 MHz, DMSO-d_6, 70 ℃) (as a mixture of rotamers) δ 8.05 (d, J = 7.6 Hz, 1H), 7.39–7.12 (m, 15H), 5.14 (d, J = 12.8 Hz, 1H), 5.10–4.62 (m, 7H), 4.60–4.35 (m, 1H), 4.32 (dd, J = 15.5, 2.4 Hz, 1H), 3.70 (dd, J = 9.5, 7.7 Hz, 0.6H), 3.66–3.50 (m, 1.7H), 3.25 (td, J = 10.3, 9.7, 7.6 Hz, 1.3H), 3.00 (t, J = 10.6 Hz, 0.4H), 2.80 (d, J = 2.1 Hz, 3H), 2.11–1.97 (m, 1H), 1.97 (s, 1H), 0.93 (d, J = 8.4 Hz, 9H); ^{13}C NMR (150 MHz, DMSO-d_6, 70 ℃) (as a mixture of rotamers) δ 169.7, 169.6, 166.4, 162.6, 162.5, 155.0, 153.8, 153.7, 139.7, 139.7, 138.1, 136.4, 136.3, 128.0, 128.0, 128.0, 127.9, 127.4, 127.2, 127.1, 126.8, 126.5, 126.3, 126.2, 126.1, 125.8, 97.9, 97.8, 66.3, 58.4, 57.5, 57.4, 56.2, 55.9, 55.8, 46.6, 45.5, 44.8, 44.6, 43.5, 40.7, 40.6, 39.0, 38.9, 30.3, 30.3, 29.3, 29.2; HRMS(ESI+): Cacld for $C_{38}H_{45}N_6O_3^+$ $[M+H]^+$ 633.3548, found 633.3547, Δppm –0.16; HPLC purity: 99.9%, t_R = 16.8 min. The product was synthesized with 3,3-dimethylbutyryl chloride.

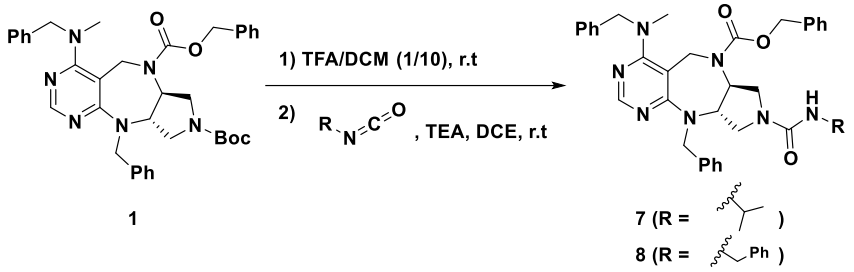


6

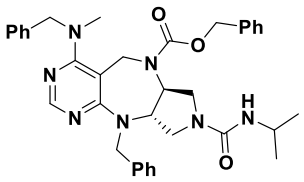
Benzyl (6a*S*,9a*S*)-10-benzyl-4-(benzyl(methyl)amino)-8-(cyclopropanecarbonyl)-6a,7,8,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6(5*H*)-carboxylate (6). A white solid; R_f = 0.24 (EtOAc/Hex = 2:1); 30 mg, 84% overall yield; $[\alpha]_D^{25}$ –95.5 (c = 0.3, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6, 70 ℃) (as a mixture of rotamers) δ 8.04 (s, 1H), 7.37–7.15 (m, 15H), 5.14 (d, J = 12.8 Hz, 1H), 5.08–4.67 (m, 7H), 4.65–4.41 (m, 1H), 4.33 (d, J = 15.5 Hz, 1H), 3.88 (t, J = 8.5 Hz, 0.65H), 3.77 (d, J = 9.2 Hz, 0.7H), 3.66–3.50 (m, 1H), 3.42 (t, J = 9.7 Hz, 0.65H), 3.28 (t, J = 10.8 Hz, 0.65H), 3.03 (t, J = 10.7 Hz, 0.35H), 2.81 (s, 3H), 1.69–1.62 (m, 0.65H), 1.52–1.43 (m, 0.35H), 0.77–0.60 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6, 70 ℃) (as a mixture of rotamers) δ 171.2, 166.4, 162.6, 155.0, 153.8, 139.7, 138.1, 136.4, 128.0, 128.0, 127.4, 127.2, 127.1, 126.8, 126.5, 126.3, 126.3, 126.0, 125.9, 97.8, 66.3, 58.3, 57.4, 57.2, 56.2, 55.9, 55.7, 55.7, 46.6, 44.7, 43.8, 40.7, 40.1, 38.9, 10.9, 6.7, 6.4; HRMS(ESI+): Cacld for $C_{36}H_{39}N_6O_3^+$ $[M+H]^+$ 603.3078, found 603.3077, Δppm –0.17; HPLC purity: 98.5%, t_R = 15.1 min. The product was

synthesized with cyclopropanecarbonyl chloride.

General procedure for the preparation of 7 and 8

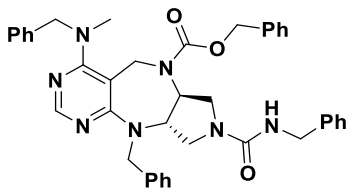


1 (49 mg, 0.077 mmol) was treated with 10% TFA in DCM (10.0 mL), and the resulting mixture was stirred at r.t. After the starting material was consumed as indicated by TLC, any excess TFA was removed by azeotropic evaporation with toluene under reduced pressure. To a solution of the resulting Boc-deprotected product in dry DCE (3.5 mL) were sequentially added dry NEt_3 (0.043 mL, 0.31 mmol) and isocyanate (0.12 mmol) at r.t. The resulting mixture was stirred at r.t. After completion of the reaction as indicated by TLC, the solvent was removed under reduced pressure, and the residue was purified through the PLC to afford desired urea product.



7

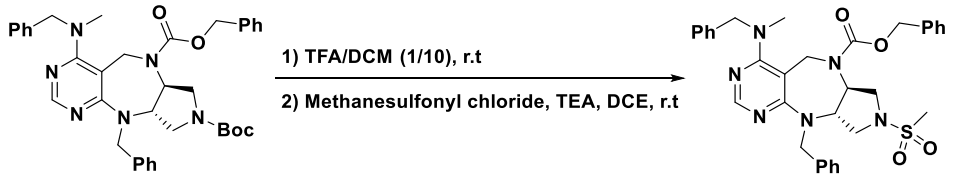
Benzyl (6a*S*,9a*S*)-10-benzyl-4-(benzyl(methyl)amino)-8-(isopropylcarbamoyl)-6a,7,8,9,9a,10 –hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6(5*H*)-carboxylate (7). A white solid; R_f = 0.27 (EtOAc/Hex = 2:1); 32 mg, 67% overall yield; ^{1}H NMR (400 MHz, DMSO-d_6, 100 ℃) δ 8.02 (s, 1H), 7.36–7.13 (m, 15H), 5.49 (d, J = 7.5 Hz, 1H), 5.11 (d, J = 12.8 Hz, 1H), 5.08–4.91 (m, 3H), 4.85–4.62 (m, 4H), 4.43 (ddd, J = 11.7, 9.8, 7.7 Hz, 1H), 4.31 (d, J = 15.4 Hz, 1H), 3.70 (dp, J = 7.6, 6.5 Hz, 1H), 3.53 (ddd, J = 9.5, 7.6, 2.9 Hz, 2H), 3.30 (t, J = 10.0 Hz, 1H), 3.02 (d, J = 9.6 Hz, 1H), 2.81 (s, 3H), 1.03 (d, J = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6, 100 ℃) δ 166.3, 162.6, 155.5, 154.9, 153.5, 139.5, 138.0, 136.3, 127.8, 127.7, 127.2, 127.1, 126.7, 126.3, 126.0, 125.8, 97.9, 66.2, 58.5, 57.0, 55.8, 46.6, 45.3, 43.8, 41.1, 40.6, 38.8, 22.4, 22.3; HRMS(ESI+): Cacld for $C_{36}H_{42}N_7O_3^+$ $[M+H]^+$ 620.3344, found 620.3343, Δppm –0.16; HPLC purity: 99.9%, t_R = 14.6 min. The product was synthesized with isopropyl isocyante.



8

Benzyl (6a*S*,9a*S*)-10-benzyl-4-(benzyl(methyl)amino)-8-(benzylcarbamoyl)-6a,7,8,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6(5*H*)-carboxylate (8). A white solid; R_f = 0.39 (EtOAc/Hex = 2:1); 37 mg, 72% overall yield; ^{1}H NMR (400 MHz, DMSO-d_6, 100 ℃) δ 8.02 (s, 1H), 7.36–7.12 (m, 20H), 6.45 (t, J = 5.9 Hz, 1H), 5.12 (d, J = 12.8 Hz, 1H), 5.07–4.94 (m, 3H), 4.86–4.76 (m, 2H), 4.76–4.67 (m, 2H), 4.48 (ddd, J = 11.7, 9.8, 7.6 Hz, 1H), 4.31 (d, J = 15.4 Hz, 1H), 4.19 (d, J = 5.9 Hz, 2H), 3.59 (ddd, J = 17.8, 9.4, 7.7 Hz, 2H), 3.36 (t, J = 10.0 Hz, 1H), 3.07 (t, J = 9.6 Hz, 1H), 2.81 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6, 100 ℃) δ 166.3, 162.6, 156.2, 154.9, 153.6, 140.4, 139.5, 138.0, 136.3, 127.8, 127.7, 127.5, 127.2, 127.1, 126.7, 126.6, 126.3, 126.0, 125.9, 125.8, 97.9, 66.2, 58.4, 57.0, 55.8, 46.7, 45.3, 43.9, 43.0, 40.6; HRMS(ESI+): Cacld for $C_{40}H_{42}N_7O_3^+$ $[M+H]^+$ 668.3344, found 668.3343, Δppm –0.15; HPLC purity: 99.9%, t_R = 15.5 min. The product was synthesized with benzyl isocyante.

Preparation of benzyl (6a*S*,9a*S*)-10-benzyl-4-(benzyl(methyl)amino)-8-(methylsulfonyl)-6a,7,8,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6(5*H*)-carboxylate (9)



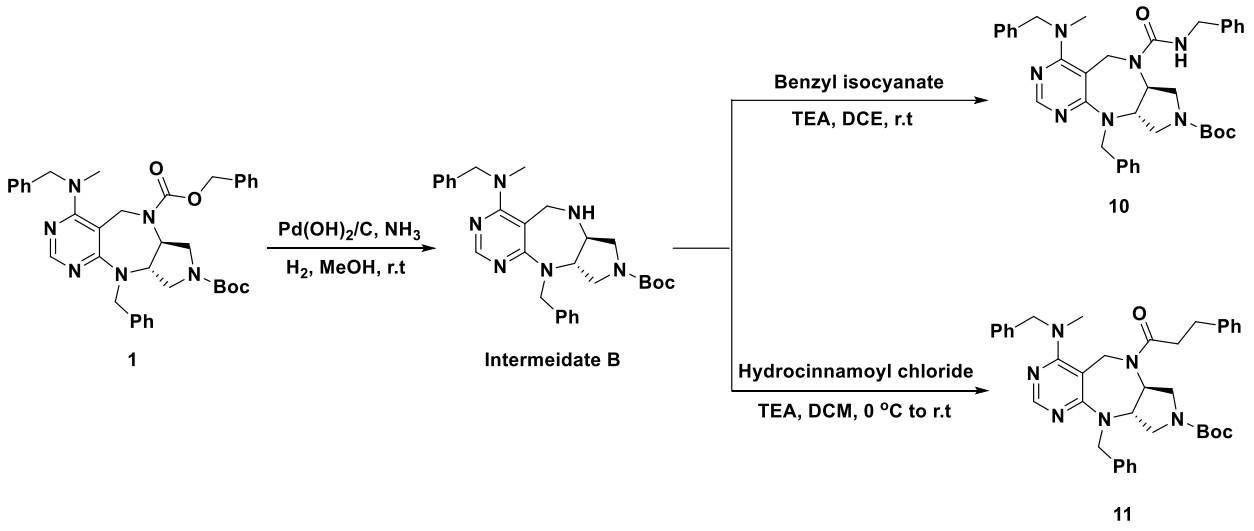
1 9

1 (38 mg, 0.059 mmol) was treated with 10% TFA in DCM (7.5 mL), and the resulting mixture was stirred at r.t. After the starting material was consumed as indicated by TLC, any excess TFA was removed by azeotropic evaporation with toluene under reduced pressure. To a solution of the resulting Boc-deprotected product in dry DCM (3.0 mL) were sequentially added dry NEt_3 (0.033 mL, 0.24 mmol) and methanesulfonyl chloride (0.012 mL, 0.12 mmol) at r.t. The resulting mixture was stirred at r.t. After completion of the reaction as indicated by TLC, the solvent was removed under reduced pressure, and the residue was purified through the PLC to afford **9** (30 mg, 83% overall yield) as a white solid.

R_f = 0.48 (EtOAc/Hex = 2:1); ^{1}H NMR (400 MHz, DMSO-d_6, 100 ℃) δ 8.04 (s, 1H), 7.39–7.11

(m, 15H), 5.13 (d, J = 12.7 Hz, 1H), 5.08–4.94 (m, 2H), 4.92 (s, 2H), 4.81 (d, J = 16.5 Hz, 1H), 4.75–4.66 (m, 2H), 4.54 (ddd, J = 11.4, 9.8, 7.7 Hz, 1H), 4.33 (d, J = 15.4 Hz, 1H), 3.47 (dd, J = 9.3, 7.7 Hz, 1H), 3.44–3.32 (m, 2H), 3.11 (t, J = 9.6 Hz, 1H), 2.86 (s, 3H), 2.81 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6, 100 °C) δ 166.3, 162.5, 154.8, 153.7, 139.4, 137.9, 136.2, 127.8, 127.8, 127.8, 127.3, 127.1, 126.7, 126.3, 126.1, 125.8, 97.9, 66.3, 58.1, 57.3, 55.7, 46.7, 46.2, 45.1, 40.6, 38.8, 34.6; HRMS(ESI+): Cacld for $C_{28}H_{34}N_7O_2^+$ $[M+H]^+$ 613.2592, found 613.2591, Δppm –0.16; HPLC purity: 99.9%, t_R = 15.1 min.

Preparation of 10 and 11



***tert*-Butyl (6a*S*,9a*S*)-10-benzyl-4-(benzyl-(methyl)amino)-6-(benzylcarbamoyl)-5,6a,7,9,9a, 10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-8(6*H*)-carboxylate (10)**. To a solution of **1** (58 mg, 0.091 mmol) in MeOH (3.0 mL) were carefully added 30 wt % $Pd(OH)_2/C$ (Pearlman`s catalyst; 17 mg) and ammonia (2 M in MeOH, 0.023 mL, 0.045 mmol) at r.t. The mixture was vigorously stirred under H_2 atmosphere (1 atm) at r.t. After completion of the reaction as indicated by TLC, the reaction mixture was filtered through Celite® while washing with MeOH. The filtrate was condensed under reduced pressure, followed by PLC to afford the intermediate **B** (43 mg, 93% yield). To a solution of intermediate **B** (43 mg, 0.086 mmol) in dry DCE (1.0 mL) were sequentially added NEt_3 (0.060 mL, 0.43 mmol) and benzyl isocyanate (0.021 mL, 0.17 mmol) and the resulting mixture was stirred at r.t. After completion of the reaction as indicated by TLC, the solvent was removed under reduced pressure, and the residue was purified through the PLC to afford **10** (42 mg, 77% yield, 72% overall yield) as a white solid.

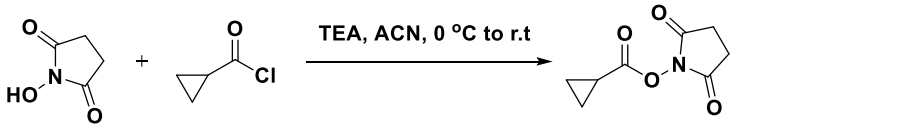
R_f = 0.43 (EtOAc/Hex = 2:1); ^{1}H NMR (400 MHz, DMSO-d_6, 100 °C) δ 7.99 (s, 1H), 7.39–7.32 (m, 2H), 7.32–7.15 (m, 13H), 6.50 (t, J = 5.7 Hz, 1H), 5.01–4.89 (m, 2H), 4.87 (d, J = 9.7 Hz, 1H), 4.82 (d, J = 9.4 Hz, 1H), 4.69 (d, J = 15.3 Hz, 1H), 4.55 (d, J = 16.4 Hz, 1H), 4.46–4.34 (m, 2H),

4.24 (qd, *J* = 15.2, 5.7 Hz, 2H), 3.69 (dd, *J* = 9.8, 7.5 Hz, 1H), 3.40 (dd, *J* = 9.9, 7.6 Hz, 1H), 3.24 (t, *J* = 10.3 Hz, 1H), 2.99 (t, *J* = 10.0 Hz, 1H), 2.82 (s, 3H), 1.35 (s, 9H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$, 100 ℃) δ 166.2, 162.8, 156.9, 153.3, 153.2, 140.2, 139.6, 138.1, 127.8, 127.7, 127.5, 127.3, 126.5, 126.3, 126.1, 125.9, 125.8, 98.5, 78.4, 57.6, 55.7, 46.8, 45.2, 44.2, 43.4, 39.9, 38.8, 27.6; HRMS(ESI+): Cacld for $C_{37}H_{44}N_7O_3^+$ $[M+H]^+$ 634.3500, found 634.3500, Δppm 0.00; HPLC purity: 99.9%, t_R = 15.2 min.

***tert*-Butyl (6a*S*,9a*S*)-10-benzyl-4-(benzyl-(methyl)amino)-6-(3-phenylpropanoyl)-5,6a,7,9,9a, 10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-8(6*H*)-carboxylate (11)**. To a solution of intermediate **B** (31 mg, 0.062 mmol) in dry DCM (1.0 mL) were sequentially added dry NEt_3 (0.043 mL, 0.31 mmol) and hydrocinnamoyl chloride (0.018 mL 0.124 mmol) at 0 ℃. The resulting mixture was left to stir and allowed to warm to r.t. After completion of the reaction as indicated by TLC, the solvent was removed under reduced pressure, and the residue was purified through the PLC to afford **11** (27 mg, 69% yield) as a white solid.

R_f = 0.47 (EtOAc/Hex = 1:1); ^{1}H NMR (600 MHz, DMSO-*d*$_6$) (as a mixture of rotamers) δ 8.15–7.85 (m, 1H), 7.44–7.33 (m, 2H), 7.33–7.10 (m, 12H), 7.06–6.98 (m, 1H), 5.15–4.82 (m, 3.6H), 4.80–4.62 (m, 2H), 4.54–4.34 (m, 1.4H), 4.35–4.19 (m, 1H), 3.57 (dd, *J* = 10.1, 7.6 Hz, 0.2H), 3.52–3.44 (m, 0.8H), 3.30–3.19 (m, 1.7H), 3.03–2.94 (m, 0.5H), 2.94–2.51 (m, 7.3H), 2.34–2.20 (m, 0.5H), 1.37–1.27 (m, 9H); ^{13}C NMR (150 MHz, DMSO-*d*$_6$) (as a mixture of rotamers) δ 172.3, 172.2, 171.5, 166.6, 166.3, 163.0, 162.9, 154.3, 153.8, 153.5, 153.4, 141.3, 140.8, 140.2, 139.9, 138.5, 138.3, 128.5, 128.4, 128.4, 128.4, 128.3, 128.3, 128.3, 128.2, 128.2, 128.0, 128.0, 127.7, 127.7, 127.5, 127.5, 126.9, 126.9, 126.7, 126.6, 126.4, 126.3, 126.2, 126.1, 125.9, 125.9, 97.9, 97.7, 78.9, 78.8, 58.2, 57.8, 57.7, 57.1, 56.9, 56.7, 56.4, 56.1, 55.9, 46.8, 46.6, 45.3, 45.3, 45.0, 44.7, 44.5, 44.0, 42.1, 40.1, 38.5, 34.5, 34.0, 33.9, 30.7, 30.5, 30.4, 28.0, 28.0, 28.0; HRMS(ESI+): Cacld for $C_{38}H_{45}N_6O_3^+$ $[M+H]^+$ 633.3548, found 633.3547, Δppm –0.16; HPLC purity: 97.4%, t_R = 17.2 min.

Preparation of 2,5-dioxopyrrolidin-1-yl cyclopropanecarboxylate



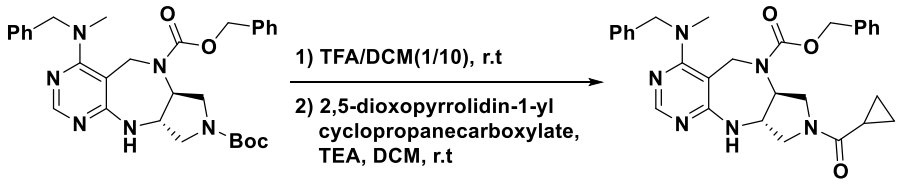
2,5-dioxopyrrolidin-1-yl cyclopropanecarboxylate

To a solution of *N*-hydroxysuccinimide (183 mg, 1.59 mmol) in dry ACN (20.0 mL) were sequentially added dry NEt_3 (0.67 mL, 4.78 mmol) and cyclopropanecarbonyl chloride (0.20 mL,

2.07 mmol) at 0 °C. The resulting mixture was left to stir and allowed to warm to r.t. After the starting material was consumed as indicated by TLC, the reaction mixture was quenched with saturated NH_4Cl(aq) and extracted three times with DCM. The combined organic layer was dried over anhydrous Na_2SO_4(s) and filtered. The filtrate was condensed under reduced pressure, followed by silica-gel flash column chromatography to afford 2,5-dioxopyrrolidin-1-yl cyclopropanecarboxylate (280 mg, 96% yield) as a white solid.

R_f = 0.45 (EtOAc/Hex = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.83 (s, 4H), 1.90 (tt, J = 7.8, 4.7 Hz, 1H), 1.32–1.06 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3, 169.4, 25.7, 10.7, 10.4.

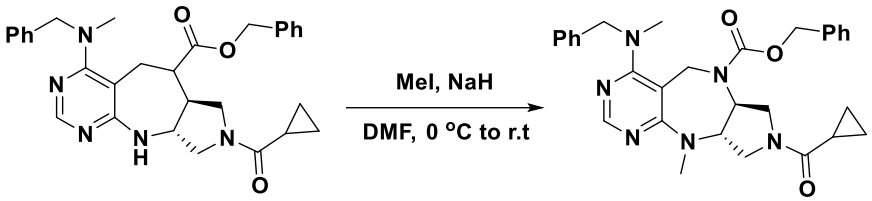
Preparation of benzyl (6a*S*,9a*S*)-4-(benzyl(methyl)amino)-8-(cyclopropanecarbonyl)-6a,7,8,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6(5*H*)-carboxylate (12)



S4 (95 mg, 0.174 mmol) was treated with 10% TFA in DCM (10.0 mL), and the resulting mixture was stirred at r.t. After the starting material was consumed as indicated by TLC, any excess TFA was removed by azeotropic evaporation with toluene under reduced pressure. To a solution of the resulting Boc-deprotected product in dry DCM (4.0 mL) were sequentially added dry Et_3N (0.073 mL, 0.523 mmol) and 2,5-dioxopyrrolidin-1-yl cyclopropanecarboxylate (48 mg, 0.262 mmol) and the resulting mixture was stirred at r.t. After the starting material was consumed as indicated by TLC, the reaction mixture was quenched with saturated NH_4Cl(aq) and extracted three times with DCM. The combined organic layer was dried over anhydrous Na_2SO_4(s) and filtered. The filtrate was condensed under reduced pressure, followed by silica-gel flash column chromatography to afford **12** (82 mg, 92% overall yield) as a white solid.

R_f = 0.38 (EtOAc/MeOH = 10:1); ^{1}H NMR (400 MHz, DMSO-d_6, 70 °C) (as a mixture of rotamers) δ 7.96 (s, 1H), 7.38–7.12 (m, 11H), 5.23–4.99 (m, 2H), 4.92–4.75 (m, 3H), 4.54 (dd, J = 15.5, 5.4 Hz, 1H), 4.34–4.14 (m, 2H), 4.06–3.96 (m, 1H), 3.86–3.71 (m, 1H), 3.55 (t, J = 9.8 Hz, 0.4H), 3.38 (t, J = 9.8 Hz, 0.6H), 3.17 (t, J = 10.7 Hz, 0.6H), 2.98 (t, J = 10.7 Hz, 0.4H), 2.72 (s, 3H), 1.74–1.56 (m, 1H), 0.86–0.58 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6, 70 °C) (as a mixture of rotamers) δ 171.1, 166.4, 162.5, 155.1, 154.5, 138.0, 136.3, 128.0, 127.9, 127.5, 127.1, 127.0, 126.5, 97.7, 66.4, 62.8, 61.7, 56.3, 52.3, 51.1, 47.7, 46.8, 46.3, 45.5, 11.0, 6.6, 6.4, 6.3; HRMS(ESI+): Cacld for $C_{29}H_{33}N_6O_3^+$ $[M+H]^+$ 513.2609, found 513.2607, Δppm –0.39; HPLC purity: 98.3%, t_R = 10.6 min.

Preparation of benzyl (6a*S*,9a*S*)-4-(benzyl(methyl)amino)-8-(cyclopropanecarbonyl)-10-methyl-6a,7,8,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6(5*H*)-carboxylate (13)

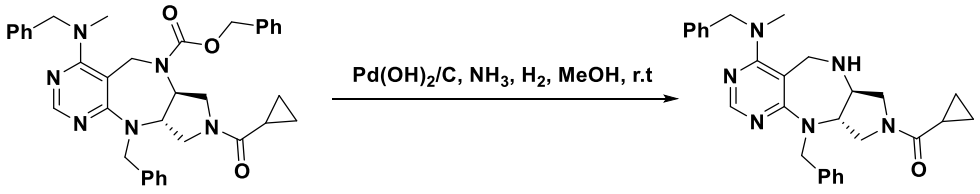


12 13

To a solution of **12** (30 mg, 0.059 mmol) in dry DMF (3.0 mL) under argon atmosphere was added NaH (60 % dispersion in mineral oil, 7 mg, 0.176 mmol) at 0 ℃. After being stirred at 0 ℃ for 30 min, methyl iodide (0.007 mL, 0.117 mmol) was slowly added, and the mixture was allowed to slowly warm to r.t. After the starting material was consumed as indicated by TLC, the reaction mixture was quenched with saturated NH_4Cl(aq) and extracted three times with EtOAc. The combined organic layer was dried over anhydrous Na_2SO_4(s) and filtered. The filtrate was condensed under reduced pressure, followed by PLC to afford **13** (29 mg, 94% yield) as a white solid.

R_f = 0.66 (DCM/MeOH = 10:1); ^{1}H NMR (600 MHz, DMSO-d_6, 70 ℃) (as a mixture of rotamers) δ 8.06 (s, 1H), 7.35–7.18 (m, 10H), 5.11 (d, *J* = 12.7 Hz, 1H), 5.04 (t, *J* = 13.4 Hz, 1H), 4.95 (td, *J* = 11.2, 7.8 Hz, 0.45H), 4.85 (td, *J* = 11.1, 7.7 Hz, 0.55H), 4.76–4.55 (m, 4H), 4.28 (d, *J* = 15.5 Hz, 1H), 4.10 (t, *J* = 8.4 Hz, 0.45H), 4.00–3.92 (m, 1H), 3.86 (dd, *J* = 10.6, 7.7 Hz, 0.55H), 3.72 (dd, *J* = 11.0, 7.7 Hz, 0.45H), 3.50 (dt, *J* = 27.0, 10.1 Hz, 1.1H), 3.10 (d, *J* = 19.2 Hz, 6.45H), 1.79 (tt, *J* = 7.9, 4.6 Hz, 0.45H), 1.70 (td, *J* = 7.6, 3.8 Hz, 0.55H), 0.82–0.67 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6, 70 ℃) (as a mixture of rotamers) δ 171.2, 166.2, 162.9, 155.1, 153.6, 138.1, 136.4, 128.0, 127.9, 127.4, 127.2, 127.1, 126.8, 126.5, 98.4, 98.3, 66.3, 57.5, 57.4, 56.3, 55.9, 55.8, 46.4, 45.5, 44.7, 43.7, 40.7, 38.9, 38.8, 30.4, 30.3, 11.0, 11.0, 6.7, 6.4; HRMS(ESI+): Cacld for $C_{30}H_{35}N_6O_3^+$ $[M+H]^+$ 527.2765, found 527.2764, Δppm –0.19; HPLC purity: 99.9%, t_R = 11.6 min.

Preparation of ((6a*S*,9a*S*)-10-benzyl-4-(benzyl(methyl)amino)-5,6a,7,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepin-8(6*H*)-yl)(cyclopropyl)methanone (Intermediate C)

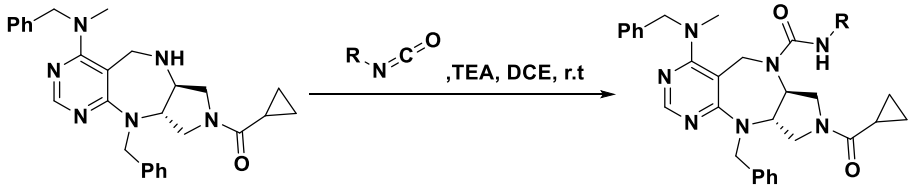


6 Intermediate C

To a solution of **6** (50 mg, 0.084 mmol) in MeOH (2.0 mL) were carefully added 30 wt % Pearlman`s catalyst (15 mg) and ammonia (2 M in MeOH, 0.021 mL, 0.042 mmol) at r.t. The mixture was vigorously stirred under H_2 atmosphere (1 atm) at r.t. After completion of the reaction as indicated by TLC, the reaction mixture was filtered through Celite® while washing with MeOH. The filtrate was condensed under reduced pressure, followed by silica-gel flash column chromatography to afford intermediate **C** (34 mg, 88% yield) as a white solid.

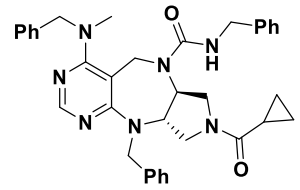
*R*f = 0.41 (DCM/MeOH = 10:1); ^{1}H NMR (600 MHz, DMSO-*d*$_6$) (as a mixture of rotamers) δ 8.09 (d, *J* = 7.4 Hz, 1H), 7.37–7.31 (m, 5H), 7.30–7.22 (m, 5H), 5.55–5.43 (m, 1H), 4.82 (dd, *J* = 15.9, 2.0 Hz, 1H), 4.70 (dd, *J* = 15.8, 3.2 Hz, 1H), 4.30 (dd, *J* = 9.9, 7.1 Hz, 0.4H), 4.22 (dd, *J* = 15.8, 6.7 Hz, 1H), 4.01–3.95 (m, 2H), 3.95–3.86 (m, 1H), 3.72 (t, *J* = 8.8 Hz, 0.6H), 3.66 (dd, *J* = 11.1, 8.1 Hz, 0.5H), 3.63–3.56 (m, 1.4H), 3.41–3.33 (m, 1.1H), 3.02–2.98 (m, 1H), 2.97 (s, 3H), 2.69–2.55 (m, 1H), 1.75 (tt, *J* = 7.6, 5.1 Hz, 0.4H), 1.64 (tt, *J* = 6.9, 5.4 Hz, 0.6H), 0.74–0.62 (m, 4H); ^{13}C NMR (150 MHz, DMSO-*d*$_6$) (as a mixture of rotamers) δ 170.9, 170.8, 165.5, 165.4, 165.2, 165.1, 154.7, 154.6, 139.0, 139.0, 138.7, 128.5, 128.5, 127.4, 127.2, 127.1, 127.0, 126.9, 126.8, 97.1, 97.0, 61.5, 60.3, 58.6, 57.2, 55.8, 55.7, 51.2, 50.6, 50.4, 49.8, 49.7, 49.3, 44.3, 40.1, 38.1, 38.0, 11.2, 11.1, 7.2, 7.1, 7.0; HRMS(ESI+): Cacld for $C_{28}H_{33}N_6O^+$ $[M+H]^+$ 469.2710, found 469.2710, Δppm 0.00.

General procedure for the preparation of 14–17



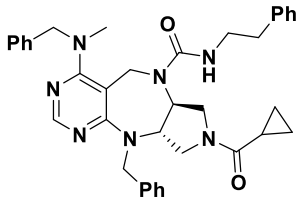
Intermediate C 14–17

To a solution of intermediate **C** (18 mg, 0.038 mmol) in dry DCE (1.5 mL) were sequentially added dry NEt_3 (0.027 mL, 0.192 mmol) and isocyanate (0.077 mmol) at r.t. The resulting mixture was stirred at r.t. After completion of the reaction as indicated by TLC, the solvent was removed under reduced pressure, and the residue was purified through the PLC to afford desired urea product.



14

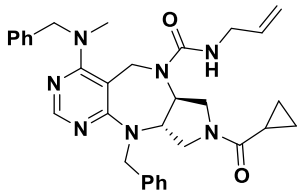
(6a*S*,9a*S*)-*N*,10-Dibenzyl-4-(benzyl(methyl)amino)-8-(cyclopropanecarbonyl)-6a,7,8,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6(5*H*)-carboxamide (14). A white solid; R_f = 0.28 (EtOAc/Hex = 2:1); 14 mg, 61% yield; ^{1}H NMR (600 MHz, DMSO-*d*$_6$, 70 ℃) (as a mixture of rotamers) δ 8.00 (s, 1H), 7.42–7.10 (m, 15H), 6.78–6.57 (m, 1H), 5.14–5.04 (m, 0.65H), 5.03–4.91 (m, 1.35H), 4.87 (dd, *J* = 16.6, 10.7 Hz, 1.65H), 4.79 (d, *J* = 16.7 Hz, 0.35H), 4.69 (dd, *J* = 15.4, 6.3 Hz, 1H), 4.61 (d, *J* = 16.3 Hz, 0.65H), 4.55 (d, *J* = 16.3 Hz, 0.35H), 4.49–4.30 (m, 2H), 4.30–4.15 (m, 2H), 4.09–4.06 (m, 0.65H), 3.90–3.82 (m, 0.7H), 3.67 (t, *J* = 9.9 Hz, 0.35H), 3.58 (dd, *J* = 10.8, 7.6 Hz, 0.65H), 3.38 (t, *J* = 9.6 Hz, 0.65H), 3.21 (t, *J* = 10.8 Hz, 0.65H), 2.97 (t, *J* = 10.5 Hz, 0.35H), 2.82 (d, *J* = 5.5 Hz, 3H), 1.71–1.62 (m, 0.65H), 1.52 (td, *J* = 7.2, 3.9 Hz, 0.35H), 0.77–0.60 (m, 4H); ^{13}C NMR (150 MHz, DMSO-*d*$_6$, 70 ℃) (as a mixture of rotamers) δ 171.3, 171.1, 166.3, 162.9, 156.9, 153.4, 140.4, 139.7, 138.2, 128.0, 127.9, 127.8, 127.7, 127.7, 127.4, 127.4, 126.7, 126.7, 126.5, 126.3, 126.2, 126.1, 125.9, 125.8, 98.5, 98.4, 58.3, 58.1, 57.1, 57.0, 55.8, 55.7, 46.8, 46.7, 45.8, 45.0, 44.0, 43.5, 43.5, 42.9, 40.1, 38.9, 38.9, 10.9, 6.8, 6.6, 6.5, 6.3; HRMS(ESI+): Cacld for $C_{36}H_{40}N_7O_2^+$ $[M+H]^+$ 602.3238, found 602.3238, Δppm 0.00; HPLC purity: 99.9%, t_R = 12.9 min. The product was synthesized with benzyl isocyante.



15

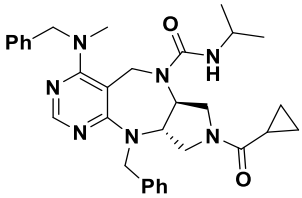
(6a*S*,9a*S*)-10-Benzyl-4-(benzyl(methyl)amino)-8-(cyclopropanecarbonyl)-*N*-phenethyl-6a,7,8,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6(5*H*)-carboxamide (15). A white solid; R_f = 0.25 (EtOAc/Hex = 2:1); 18 mg, 76% yield; ^{1}H NMR (600 MHz, DMSO-*d*$_6$, 70 ℃) (as a mixture of rotamers) δ 8.00 (s, 1H), 7.45–7.01 (m, 15H), 6.33–6.01 (m, 1H), 5.09–4.79 (m, 4H), 4.70 (d, *J* = 15.5 Hz, 1H), 4.61–4.46 (m, 1H), 4.43–4.32 (m, 1.65H), 4.24 (q, *J* = 9.8 Hz, 0.35H), 3.97 (t, *J* = 8.3 Hz, 0.65H), 3.89–3.76 (m, 0.7H), 3.65 (t, *J* = 9.9 Hz, 0.35H), 3.55 (dd, *J* = 10.8, 7.6 Hz, 0.65H), 3.36–3.15 (m, 3.3H), 2.93 (t, *J* = 10.4 Hz, 0.35H), 2.82 (d, *J* = 6.1 Hz, 3H), 2.76–2.65 (m, 2H), 1.64 (td, *J* = 8.0, 7.2, 4.4 Hz, 0.65H), 1.56–1.47 (m, 0.35H), 0.81–0.54 (m, 4H); ^{13}C NMR (150 MHz, DMSO-*d*$_6$, 70 ℃) δ 171.2, 166.2, 162.9, 156.6, 153.4, 139.7, 139.5, 138.2, 128.2, 128.2, 128.0, 128.0, 127.9, 127.4, 126.5, 126.3, 125.9, 125.8, 125.6, 98.6, 57.8, 57.1,

55.8, 46.8, 45.7, 45.0, 44.0, 41.6, 40.1, 38.9, 35.6, 35.5, 10.9, 6.8, 6.5; HRMS(ESI+): Cacld for $C_{37}H_{42}N_7O_2^+$ $[M+H]^+$ 616.3395, found 616.3394, Δppm –0.16; HPLC purity: 99.9%, t_R = 13.3 min. The product was synthesized with phenethyl isocyante.



16

(6a*S*,9a*S*)-*N*-Allyl-10-benzyl-4-(benzyl(methyl)amino)-8-(cyclopropanecarbonyl)-6a,7,8,9, 9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6(5*H*)-carboxamide (16). A white solid; R_f = 0.24 (EtOAc/Hex = 2:1); 12 mg, 57% yield; ^{1}H NMR (400 MHz, DMSO-d_6, 70 ℃) (as a mixture of rotamers) δ 7.99 (s, 1H), 7.45–7.10 (m, 10H), 6.36–6.12 (m, 1H), 6.00–5.54 (m, 1H), 5.15–4.76 (m, 6H), 4.71 (d, *J* = 15.8 Hz, 1H), 4.62–4.48 (m, 1H), 4.46–4.19 (m, 2H), 4.07 (t, *J* = 8.2 Hz, 0.65H), 3.85 (q, *J* = 8.0, 7.6 Hz, 0.65H), 3.71–3.62 (m, 2.35H), 3.58 (dd, *J* = 10.8, 7.6 Hz, 0.7H), 3.35 (t, *J* = 9.5 Hz, 0.65H), 3.21 (t, *J* = 10.8 Hz, 0.65H), 2.94 (t, *J* = 10.5 Hz, 0.35H), 2.83 (s, 3H), 1.68 (p, *J* = 6.5 Hz, 0.65H), 1.58–1.43 (m, 0.35H), 0.80–0.57 (m, 4H); ^{13}C NMR (150MHz, DMSO-d_6, 70 ℃) (as a mixture of rotamers) δ 171.3, 171.0, 166.3, 162.8, 156.7, 156.6, 153.4, 139.7, 138.2, 136.4, 128.0, 128.0, 127.4, 127.3, 126.5, 126.3, 125.9, 125.8, 114.2, 114.1, 98.5, 58.3, 58.0, 57.1, 57.0, 55.8, 55.7, 46.8, 46.7, 45.8, 45.0, 44.0, 42.4, 42.3, 40.1, 39.0, 38.9, 10.9, 6.8, 6.6, 6.5, 6.3; HRMS(ESI+): Cacld for $C_{32}H_{38}N_7O_2^+$ $[M+H]^+$ 552.3082, found 552.3081, Δppm –0.18; HPLC purity: 96.3%, t_R = 11.5 min. The product was synthesized with allyl isocyante.

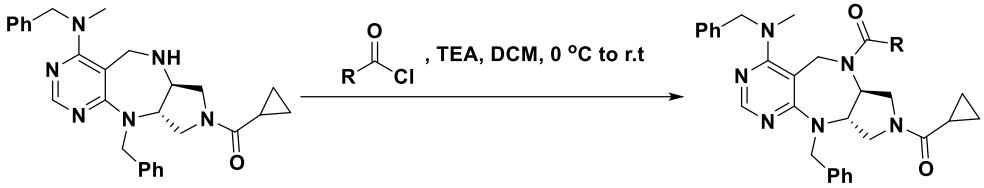


17

(6a*S*,9a*S*)-10-Benzyl-4-(benzyl(methyl)amino)-8-(cyclopropanecarbonyl)-*N*-isopropyl-6a,7,8,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6(5*H*)-carboxamide (17). A white solid; R_f = 0.25 (EtOAc/Hex = 2:1); 13 mg, 61% yield; ^{1}H NMR (400 MHz, DMSO-d_6, 70 ℃) (as a mixture of rotamers) δ 7.99 (s, 1H), 7.49–7.02 (m, 10H), 5.83–5.60 (m, 1H), 5.20–4.89 (m, 2H), 4.89–4.68 (m, 3H), 4.59–4.22 (m, 3H), 4.04 (t, *J* = 8.2 Hz, 0.65H), 3.93–3.70 (m, 1.7H), 3.71–3.55 (m, 1H), 3.33 (t, *J* = 9.5 Hz, 0.65H), 3.20 (t, *J* = 10.8 Hz, 0.65H), 2.92 (t, *J* = 10.4 Hz, 0.35H), 2.83 (s, 3H), 1.75–1.60 (m, 0.65H), 1.60–1.48 (m, 0.35H), 1.11–0.98 (m, 6H), 0.76–0.64 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6, 70 ℃) (as a mixture of rotamers) δ 171.3,

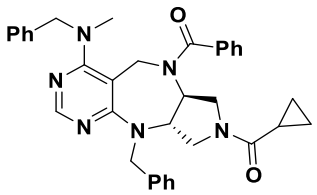
166.3, 162.8, 156.1, 153.4, 139.7, 138.2, 128.0, 128.0, 127.4, 127.3, 126.5, 126.3, 125.9, 125.7, 98.8, 98.7, 58.4, 58.0, 57.2, 56.9, 55.8, 55.7, 46.8, 46.7, 45.8, 45.0, 44.0, 41.7, 41.7, 40.1, 39.0, 38.9, 22.6, 22.6, 22.5, 10.9, 6.8, 6.6, 6.3; HRMS(ESI+): Cacld for $C_{32}H_{40}N_7O_2^+$ $[M+H]^+$ 554.3238, found 554.3237, Δppm –0.18; HPLC purity: 99.9%, t_R = 11.9 min. The product was synthesized with isopropyl isocyanate.

General procedure for the preparation of 18–22



Intermediate C 18–22

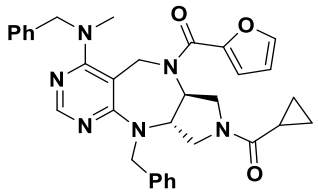
To a solution of intermediate **C** (18 mg, 0.038 mmol) in dry DCM (1.5 mL) were sequentially added dry NEt_3 (0.027 mL, 0.192 mmol) and acid chloride (0.077 mmol) at 0 °C. The resulting mixture was left to stir and allowed to warm to r.t. After completion of the reaction as indicated by TLC, the solvent was removed under reduced pressure, and the residue was purified through the PLC to afford desired urea product.



18

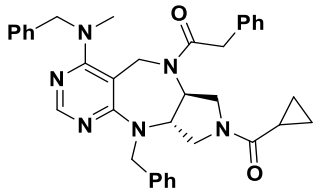
((6a*S*,9a*S*)-6-Benzoyl-10-benzyl-4-(benzyl(methyl)amino)-5,6a,7,9,9a,10-hexahydro-pyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepin-8(6*H*)-yl)(cyclopropyl)methanone (18). A white solid; R_f = 0.36 (EtOAc/Hex = 2:1); 15 mg, 68% yield; ^{1}H NMR (400 MHz, DMSO-d_6) (as a mixture of rotamers) δ 8.19 (br s, 1H), 7.62–7.06 (m, 12H), 6.99 (d, J = 7.2 Hz, 1.5H), 6.73–6.65 (m, 1.5H), 5.30–4.51 (m, 5.2H), 4.48–4.15 (m, 1.8H), 4.14–4.01 (m, 1.45H), 3.92–3.61 (m, 1H), 3.56–3.36 (m, 1.7H), 3.32–3.27 (m, 0.5H), 3.04 (dt, J = 41.1, 10.7 Hz, 0.35H), 2.88 (br s, 0.5H), 2.58 (d, J = 9.1 Hz, 2.5H), 1.78–1.67 (m, 0.65H), 1.55–1.36 (m, 0.35H), 0.81–0.55 (m, 4H); ^{13}C NMR (150MHz, DMSO-d_6) (as a mixture of rotamers) δ 171.6, 171.5, 171.4, 166.4, 163.0, 154.7, 140.0, 137.7, 135.5, 130.3, 128.4, 128.4, 128.3, 126.7, 126.7, 126.6, 126.3, 126.3, 126.1, 98.1, 57.9, 56.8, 56.0, 46.9, 46.8, 45.9, 44.8, 43.9, 43.9, 40.1, 37.9, 37.7, 11.2, 7.3, 6.9, 6.8; HRMS(ESI+): Cacld

for $C_{35}H_{37}N_6O_2^+$ $[M+H]^+$ 573.2973, found 573.2973, Δppm 0.17; HPLC purity: 99.0%, t_R = 14.0 min. The product was synthesized with benzoyl chloride.



19

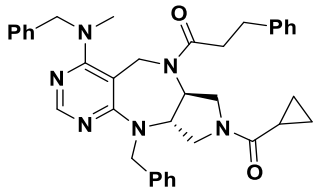
((6a*S*,9a*S*)-10-Benzyl-4-(benzyl(methyl)amino)-6-(furan-2-carbonyl)-5,6a,7,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepin-8(6*H*)-yl)(cyclopropyl)methanone (19). A white solid; R_f = 0.30 (EtOAc/Hex = 2:1); 16 mg, 74% yield; ^{1}H NMR (400 MHz, DMSO-d_6, 70 ℃) (as a mixture of rotamers) δ 8.06 (s, 1H), 7.75 (d, J = 10.8 Hz, 1H), 7.36–7.01 (m, 10H), 6.79 (br s, 1H), 6.66–6.53 (m, 1H), 5.29–5.06 (m, 1.4H), 5.05–4.57 (m, 5.2H), 4.55–4.35 (m, 1.4H), 4.09 (t, J = 8.5 Hz, 0.65H), 3.86–3.73 (m, 1H), 3.65–3.48 (m, 1.35H), 3.29 (t, J = 10.7 Hz, 0.65H), 3.21–3.13 (m, 0.35H), 2.84 (s, 3H), 1.71 (q, J = 6.4 Hz, 0.65H), 1.60–1.45 (m, 0.35H), 0.83–0.53 (m, 4H); ^{13}C NMR (150 MHz DMSO-d_6, 70 ℃) (as a mixture of rotamers) δ 171.2, 171.1, 166.3, 162.7, 159.7, 153.8, 146.3, 145.0, 144.9, 139.6, 138.0, 128.0, 127.3, 127.2, 126.5, 126.3, 125.9, 125.8, 111.1, 97.4, 57.6, 56.3, 56.0, 55.8, 46.4, 46.0, 45.3, 44.6, 43.6, 40.1, 38.7, 38.6, 11.0, 10.9, 6.8, 6.5, 6.4; HRMS(ESI+): Cacld for $C_{33}H_{35}N_6O_3^+$ $[M+H]^+$ 563.2765, found 563.2764, Δppm –0.18; HPLC purity: 99.9%, t_R = 12.8 min. The product was synthesized with 2-furoyl chloride.



20

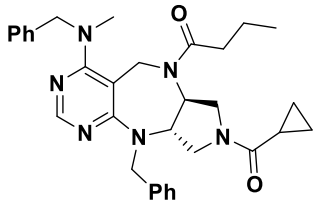
1-((6a*S*,9a*S*)-10-Benzyl-4-(benzyl(methyl)amino)-8-(cyclopropanecarbonyl)-6a,7,8,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepin-6(5*H*)-yl)-2-phenylethan-1-one (20). A white solid; R_f = 0.32 (EtOAc/Hex = 2:1); 11 mg, 49% yield; ^{1}H NMR (600 MHz, DMSO-d_6) (as a mixture of rotamers) δ 8.06–7.95 (m, 1H), 7.43–7.09 (m, 14H), 7.02–6.94 (m, 1H), 5.22–5.11 (m, 0.3H), 5.10–4.99 (m, 1.4H), 4.99–4.90 (m, 0.6H), 4.89–4.30 (m, 5.8H), 3.92 (ddd, J = 11.0, 9.3, 7.8 Hz, 0.6H), 3.81–3.38 (m, 4.3H), 3.28–3.24 (m, 0.3H), 3.17 (t, J = 10.8 Hz, 0.3H), 3.05 (t, J = 10.6 Hz, 0.2H), 2.92 (t, J = 10.6 Hz, 0.2H), 2.80 (dd, J = 19.8, 5.0 Hz, 3H), 1.76–1.57 (m, 0.7H), 1.51–1.41 (m, 0.3H), 0.77–0.56 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6) (as a mixture of rotamers) δ 171.5, 171.3, 170.5, 166.6, 166.1, 163.0, 162.9, 154.2, 153.9, 140.1, 139.9, 138.4, 138.2, 135.6, 134.6, 129.2, 129.2, 128.4, 128.4, 128.4, 128.3, 128.3, 128.3, 127.9, 127.8, 127.5, 127.4, 127.0, 126.9, 126.7, 126.6, 126.5, 126.4, 126.4, 126.2, 126.2, 126.1, 97.7, 58.7, 57.2, 56.8,

55.9, 55.8, 46.7, 45.9, 45.6, 44.0, 43.9, 42.6, 40.4, 40.1, 38.8, 11.2, 11.2, 7.4, 7.3, 7.1, 6.9; HRMS(ESI+): Cacld for $C_{36}H_{39}N_6O_2^+$ $[M+H]^+$ 587.3129, found 587.3128, Δppm –0.17; HPLC purity: 97.4%, t_R = 13.7 min. The product was synthesized with phenylacetyl chloride.



21

1-((6a*S*,9a*S*)-10-Benzyl-4-(benzyl(methyl)amino)-8-(cyclopropanecarbonyl)-6a,7,8,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepin-6(5*H*)-yl)-3-phenylpropan-1-one (21). A white solid; R_f = 0.32 (EtOAc/Hex = 2:1); 16 mg, 69% yield; ^{1}H NMR (600 MHz, DMSO-d_6) (as a mixture of rotamers) δ 8.14–7.95 (m, 1H), 7.43–7.34 (m, 2H), 7.32–7.12 (m, 12H), 7.06–6.97 (m, 1H), 5.21–4.97 (m, 2.1H), 4.95–4.80 (m, 1.9H), 4.80–4.66 (m, 1.6H), 4.57–4.22 (m, 2.6H), 3.96 (dd, *J* = 9.5, 7.7 Hz, 0.3H), 3.89 (dd, *J* = 9.3, 7.8 Hz, 0.3H), 3.79–3.65 (m, 0.7H), 3.59 (dd, *J* = 11.0, 7.7 Hz, 0.2H), 3.47 (td, *J* = 10.8, 7.6 Hz, 0.8H), 3.31–3.20 (m, 1H), 3.00 (t, *J* = 10.7 Hz, 0.2H), 2.92–2.53 (m, 6.5H), 2.49–2.43 (m, 0.3H), 2.29 (dddd, *J* = 15.3, 13.2, 8.7, 6.5 Hz, 0.5H), 1.73–1.58 (m, 0.7H), 1.52–1.37 (m, 0.3H), 0.83–0.48 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6) (as a mixture of rotamers) δ 172.3, 172.2, 171.6, 171.5, 171.4, 171.4, 166.7, 166.2, 166.2, 163.0, 163.0, 154.4, 153.9, 141.3, 141.3, 140.8, 140.8, 140.3, 140.2, 139.9, 139.9, 138.5, 138.3, 128.5, 128.4, 128.4, 128.4, 128.4, 128.3, 128.3, 128.3, 128.3, 128.2, 128.2, 128.0, 128.0, 127.8, 127.7, 127.5, 127.4, 126.9, 126.9, 126.8, 126.7, 126.6, 126.4, 126.2, 126.2, 126.1, 125.9, 125.9, 125.9, 125.8, 98.0, 97.9, 97.7, 97.6, 58.5, 58.0, 57.5, 57.1, 57.0, 56.9, 56.1, 55.9, 55.8, 46.9, 46.8, 46.6, 45.8, 45.7, 45.1, 44.8, 44.0, 42.1, 40.1, 39.1, 38.5, 34.5, 34.5, 34.0, 33.8, 30.7, 30.5, 30.4, 11.2, 11.1, 7.4, 7.3, 7.3, 7.2, 6.9; HRMS(ESI+): Cacld for $C_{37}H_{41}N_6O_2^+$ $[M+H]^+$ 601.3286, found 601.3285, Δppm –0.17; HPLC purity: 99.9%, t_R = 14.8 min. The product was synthesized with hydrocinnamoyl chloride.

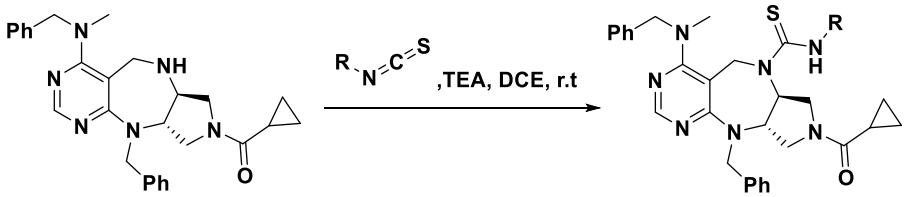


22

1-((6a*S*,9a*S*)-10-Benzyl-4-(benzyl(methyl)amino)-8-(cyclopropanecarbonyl)-6a,7,8,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepin-6(5*H*)-yl)butan-1-one (22). A white solid; R_f = 0.33 (EtOAc/Hex = 2:1); 14 mg, 68% yield; ^{1}H NMR (600 MHz, DMSO-d_6) (as a

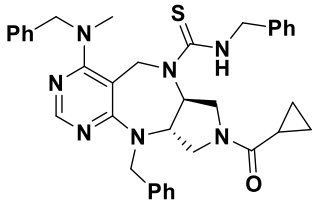
mixture of rotamers) δ 8.07–7.99 (m, 1H), 7.40–7.15 (m, 10H), 5.22–5.12 (m, 0.4H), 5.12–4.70 (m, 5H), 4.58–4.21 (m, 2.5H), 4.07 (dd, *J* = 9.4, 7.7 Hz, 0.3H), 3.88 (dd, *J* = 9.4, 7.8 Hz, 0.3H), 3.84–3.70 (m, 0.7H), 3.58 (dd, *J* = 10.9, 7.7 Hz, 0.2H), 3.54–3.39 (m, 1.3H), 3.31–3.24 (m, 0.9H), 3.04 (t, *J* = 10.7 Hz, 0.2H), 2.91 (t, *J* = 10.6 Hz, 0.2H), 2.88–2.81 (m, 3H), 2.31–2.15 (m, 1.2H), 2.11 (t, *J* = 7.8 Hz, 0.3H), 1.97–1.84 (m, 0.5H), 1.76–1.66 (m, 0.7H), 1.56–1.31 (m, 2.3H), 0.92–0.80 (m, 1.7H), 0.79–0.54 (m, 5.3H); ^{13}C NMR (150 MHz, DMSO-*d*$_6$) (as a mixture of rotamers) δ 172.9, 172.1, 171.6, 171.5, 171.5, 166.6, 166.3, 163.0, 163.0, 154.3, 153.8, 140.2, 139.9, 138.4, 128.5, 128.4, 128.4, 128.3, 127.7, 127.6, 127.5, 127.5, 127.0, 126.9, 126.7, 126.6, 126.5, 126.2, 126.2, 126.1, 97.9, 97.8, 58.6, 57.6, 57.0, 56.9, 55.9, 46.8, 46.6, 45.9, 45.7, 45.2, 44.8, 44.0, 42.1, 40.1, 38.4, 34.9, 34.9, 33.9, 33.8, 18.1, 18.1, 17.9, 17.9, 13.6, 13.6, 13.5, 11.2, 11.1, 7.4, 7.2, 7.2, 6.9; HRMS(ESI+): Cacld for $C_{32}H_{39}N_6O_2^+$ $[M+H]^+$ 539.3129, found 539.3127, Δppm –0.37; HPLC purity: 97.1%, t_R = 13.4 min. The product was synthesized with butyryl chloride.

General procedure for the preparation of 23–25



Intermediate C　　23–25

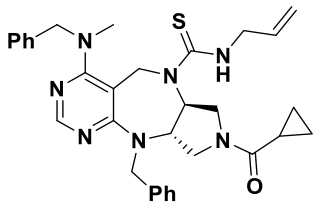
To a solution of intermediate **C** (18 mg, 0.038 mmol) in dry DCE (1.5 mL) at r.t were sequentially added dry NEt_3 (0.027 mL, 0.192 mmol) and isothiocyanate (0.077 mmol). The resulting mixture was left to stir. After completion of the reaction as indicated by TLC, the solvent was removed under reduced pressure, and the residue was purified through the PLC to afford desired thiourea product.



23

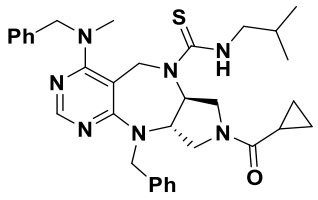
(6a*S*,9a*S*)-*N*,10-Dibenzyl-4-(benzyl(methyl)amino)-8-(cyclopropanecarbonyl)-6a,7,8,9,9a, 10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6(5*H*)-carbothioamide (23). A white solid; R_f = 0.42 (EtOAc/Hex = 2:1); 15 mg, 63% yield; ^{1}H NMR (600 MHz, DMSO-*d*$_6$,

70 ℃) (as a mixture of rotamers) δ 8.01 (s, 1H), 7.85–7.62 (m, 1H), 7.47–7.41 (m, 2H), 7.32–7.16 (m, 13H), 5.66 (d, *J* = 16.2 Hz, 1H), 5.30–5.21 (m, 0.35H), 5.19–5.10 (m, 1H), 5.02 (d, *J* = 16.8 Hz, 0.65H), 4.94–4.69 (m, 5.3H), 4.68–4.60 (m, 0.7H), 4.33 (dd, *J* = 15.4, 11.0 Hz, 1H), 4.17 (t, *J* = 8.3 Hz, 0.65H), 3.98–3.88 (m, 0.7H), 3.74–3.66 (m, 0.35H), 3.62 (dd, *J* = 10.8, 7.6 Hz, 0.65H), 3.43 (t, *J* = 9.6 Hz, 0.65H), 3.23 (t, *J* = 10.8 Hz, 0.65H), 3.01 (t, *J* = 10.5 Hz, 0.35H), 2.81 (d, *J* = 5.2 Hz, 3H), 1.68 (ddd, *J* = 12.5, 7.2, 3.8 Hz, 0.65H), 1.60–1.51 (m, 0.35H), 0.77–0.63 (m, 4H); ^{13}C NMR (150 MHz, DMSO-*d*$_6$, 70 ℃) (as a mixture of rotamers) δ 183.0, 171.3, 171.1, 166.5, 162.7, 153.6, 139.6, 139.0, 138.1, 128.1, 127.9, 127.8, 127.7, 127.5, 127.5, 126.9, 126.9, 126.5, 126.3, 126.3, 125.9, 125.8, 97.1, 97.0, 60.9, 60.0, 58.3, 57.1, 55.8, 55.7, 48.6, 48.5, 46.5, 45.1, 44.9, 44.6, 44.1, 43.7, 40.1, 39.0, 10.9, 10.9, 6.9, 6.7, 6.6, 6.4; HRMS(ESI+): Cacld for $C_{36}H_{40}N_7OS^+$ $[M+H]^+$ 618.3010, found 618.3008, Δppm –0.32; HPLC purity: 93.1%, t_R = 14.9 min. The product was synthesized with benzyl isothiocyanate.



24

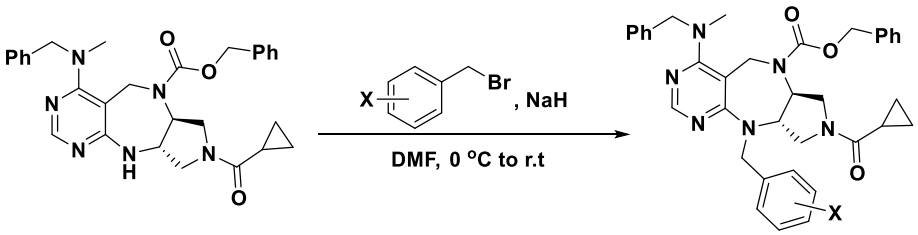
(6a*S*,9a*S*)-*N*-Allyl-10-benzyl-4-(benzyl(methyl)amino)-8-(cyclopropanecarbonyl)-6a,7,8,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6(5*H*)-carbothioamide (24). A white solid; R_f = 0.41 (EtOAc/Hex = 2:1); 17 mg, 78% yield; ^{1}H NMR (400 MHz, DMSO-*d*$_6$, 70 ℃) (as a mixture of rotamers) δ 8.00 (s, 1H), 7.50–7.15 (m, 11H), 5.96–5.76 (m, 1H), 5.72–5.59 (m, 1H), 5.28–4.99 (m, 4H), 4.93–4.70 (m, 3H), 4.70–4.48 (m, 1H), 4.33 (dd, *J* = 15.4, 5.2 Hz, 1H), 4.25–4.03 (m, 2.65H), 3.99–3.86 (m, 0.7H), 3.69 (t, *J* = 9.9 Hz, 0.35H), 3.62 (dd, *J* = 10.8, 7.6 Hz, 0.65H), 3.39 (t, *J* = 9.6 Hz, 0.65H), 3.23 (t, *J* = 10.7 Hz, 0.65H), 2.97 (t, *J* = 10.6 Hz, 0.35H), 2.84 (s, 3H), 1.73–1.63 (m, 0.65H), 1.60–1.53 (m, 0.35H), 0.78–0.63 (m, 4H); ^{13}C NMR (150 MHz, DMSO-*d*$_6$, 70 ℃) (as a mixture of rotamers) δ 182.5, 171.3, 171.1, 166.5, 162.6, 153.6, 139.6, 138.1, 134.9, 128.0, 127.9, 127.5, 127.5, 126.5, 126.3, 125.9, 125.7, 115.1, 115.0, 97.2, 97.1, 60.8, 59.9, 58.3, 57.1, 55.8, 55.7, 47.7, 47.6, 46.4, 45.1, 44.8, 44.6, 44.0, 43.7, 40.1, 10.9, 6.9, 6.7, 6.6, 6.4; HRMS(ESI+): Cacld for $C_{32}H_{38}N_7OS^+$ $[M+H]^+$ 568.2853, found 568.2852, Δppm –0.18; HPLC purity: 95.2%, t_R = 13.4 min. The product was synthesized with allyl isothiocyanate.



25

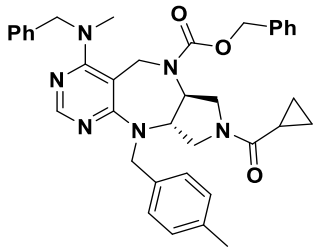
(6a*S*,9a*S*)-10-Benzyl-4-(benzyl(methyl)amino)-8-(cyclopropanecarbonyl)-*N*-isobutyl-6a,7,8,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6(5*H*)-carbothioamide (25). A white solid; R_f = 0.45 (EtOAc/Hex = 2:1); 16 mg, 71% yield; ^{1}H NMR (400 MHz, DMSO-*d*$_6$, 70 °C) (as a mixture of rotamers) δ 8.00 (s, 1H), 7.52–7.42 (m, 2H), 7.37–7.14 (m, 8H), 7.14–7.00 (m, 1H), 5.59 (d, *J* = 16.3 Hz, 1H), 5.32–4.61 (m, 6H), 4.34 (dd, *J* = 15.4, 5.5 Hz, 1H), 4.20–4.07 (m, 0.65H), 3.97–3.82 (m, 0.65H), 3.68 (t, *J* = 9.9 Hz, 0.35H), 3.61 (dd, *J* = 10.7, 7.5 Hz, 0.65H), 3.52–3.34 (m, 1.65H), 3.31–3.13 (m, 1.7H), 2.98 (t, *J* = 10.5 Hz, 0.35H), 2.84 (s, 3H), 1.94 (dq, *J* = 13.7, 6.8 Hz, 1H), 1.69 (p, *J* = 6.4 Hz, 0.65H), 1.61–1.50 (m, 0.35H), 0.86–0.78 (m, 6H), 0.77–0.63 (m, 4H); ^{13}C NMR (150 MHz, DMSO-*d*$_6$, 70 °C) (as a mixture of rotamers) δ 182.6, 171.3, 166.4, 162.7, 153.6, 139.6, 138.1, 128.0, 127.9, 127.6, 127.5, 126.5, 126.3, 125.9, 125.7, 97.0, 60.9, 58.3, 57.1, 55.9, 55.7, 52.8, 52.7, 46.6, 46.6, 44.9, 44.7, 44.1, 43.7, 40.1, 27.0, 19.8, 19.7, 10.9, 6.9, 6.7, 6.6, 6.4; HRMS(ESI+): Cacld for $C_{33}H_{42}N_7OS^+$ $[M+H]^+$ 584.3166, found 584.3166, Δppm 0.00; HPLC purity: 98.0%, t_R = 14.9 min. The product was synthesized with isopropyl isothiocyanate.

General procedure for the preparation of 26–33



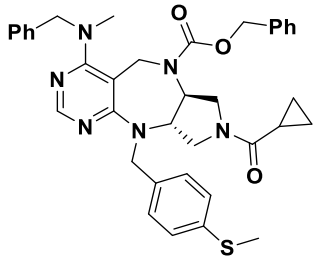
12 26–33

To a solution of **12** (20 mg, 0.039 mmol) in dry DMF (2.0 mL) under argon atmosphere was added NaH (60 % dispersion in mineral oil, 5 mg, 0.125 mmol) at 0 °C. After being stirred at 0 °C for 30 min, various benzyl bromide analog (0.078 mmol) was slowly added to the reaction suspension, and the mixture was allowed to slowly warm to r.t. After the starting material was consumed as indicated by TLC, the reaction mixture was quenched with saturated NH_4Cl(aq) and extracted three times with EtOAc. The combined organic layer was dried over anhydrous Na_2SO_4(s) and filtered. The filtrate was condensed under reduced pressure, followed by PLC to afford desired substituted product.



26

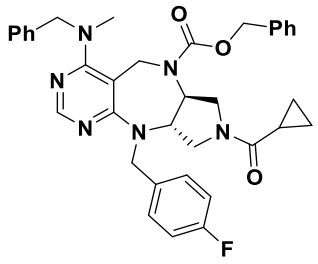
Benzyl (6a*S*,9a*S*)-4-(benzyl(methyl)amino)-8-(cyclopropanecarbonyl)-10-(4-methyl-benzyl)-6a,7,8,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6(5*H*)-carboxylate (26). A white solid; R_f = 0.38 (EtOAc/Hex = 2:1); 13 mg, 54% yield; $[\alpha]_D^{25}$ –91.5 (*c* = 0.3, $CHCl_3$); ^{1}H NMR (600 MHz, DMSO-*d*$_6$, 70 ℃) (as a mixture of rotamers) δ 8.03 (s, 1H), 7.36–7.03 (m, 14H), 5.15–4.67 (m, 8H), 4.63–4.39 (m, 1H), 4.32 (dd, *J* = 14.8, 4.3 Hz, 1H), 3.87 (t, *J* = 8.6 Hz, 0.65H), 3.84–3.74 (m, 0.7H), 3.63–3.52 (m, 1H), 3.42 (t, *J* = 9.6 Hz, 0.65H), 3.28 (t, *J* = 10.8 Hz, 0.65H), 3.02 (t, *J* = 10.6 Hz, 0.35H), 2.80 (s, 3H), 2.24 (s, 3H), 1.69–1.61 (m, 0.65H), 1.54–1.47 (m, 0.35H), 0.76–0.61 (m, 4H); ^{13}C NMR (151 MHz, DMSO-*d*$_6$, 70 ℃) (as a mixture of rotamers) δ 171.2, 171.1, 166.4, 162.6, 155.0, 153.8, 138.1, 136.6, 136.4, 135.3, 128.6, 128.0, 128.0, 127.4, 127.2, 127.1, 126.8, 126.5, 126.0, 125.8, 97.8, 97.7, 66.3, 58.3, 57.4, 57.3, 56.2, 55.9, 55.7, 46.3, 44.7, 43.8, 40.7, 40.0, 38.9, 20.2, 10.9, 6.7, 6.4; HRMS(ESI+): Cacld for $C_{37}H_{41}N_6O_3^+$ $[M+H]^+$ 617.3235, found 617.3234, Δppm –0.16; HPLC purity: 97.6%, t_R = 15.7 min. The product was synthesized with 4-methylbenzyl bromide.



27

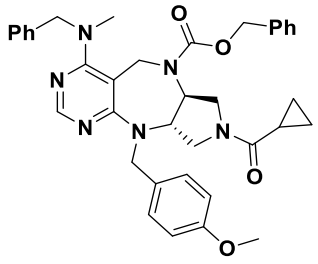
Benzyl (6a*S*,9a*S*)-4-(benzyl(methyl)amino)-8-(cyclopropanecarbonyl)-10-(4-(methylthio)benzyl)-6a,7,8,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6(5*H*)-carboxylate (27). A white solid; R_f = 0.28 (EtOAc/Hex = 2:1); 15 mg, 59% yield; ^{1}H NMR (400 MHz, DMSO-*d*$_6$, 70 ℃) (as a mixture of rotamers) δ 8.04 (s, 1H), 7.38–7.07 (m, 14H), 5.17–4.65 (m, 8H), 4.65–4.39 (m, 1H), 4.33 (d, *J* = 15.7 Hz, 1H), 3.97–3.70 (m, 1.35H), 3.66–3.51 (m, 1H), 3.42 (t, *J* = 9.6 Hz, 0.65H), 3.29 (t, *J* = 10.7 Hz, 0.65H), 3.03 (t, *J* = 10.6 Hz, 0.35H), 2.81 (s, 3H), 2.42 (s, 3H), 1.65 (p, *J* = 6.6 Hz, 0.65H), 1.56–1.46 (m, 0.35H), 0.81–0.59 (m, 4H); ^{13}C NMR (150 MHz, DMSO-*d*$_6$, 70 ℃) δ 171.2, 171.1, 166.4, 162.6, 155.0, 153.8, 138.1, 136.6, 136.4, 135.8, 128.0, 128.0, 127.4, 127.2, 127.1, 126.8, 126.6, 126.5, 126.2, 97.9, 97.8, 66.3, 58.3, 57.4, 57.3,

56.2, 55.9, 55.7, 46.2, 44.7, 43.8, 40.7, 38.9, 14.9, 11.0, 10.9, 6.7, 6.4; HRMS(ESI+): Cacld for $C_{37}H_{40}N_6O_3S^+$ $[M+H]^+$ 649.2956, found 649.2955, Δppm –0.15; HPLC purity: 95.1%, t_R = 16.0 min. The product was synthesized with 4-(methylthio)benzyl bromide.



28

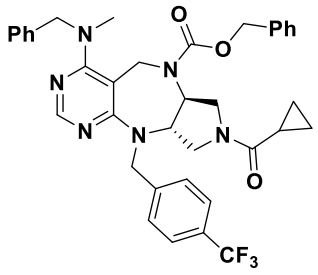
Benzyl (6a*S*,9a*S*)-4-(benzyl(methyl)amino)-8-(cyclopropanecarbonyl)-10-(4-fluorobenzyl)-6a,7,8,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6(5*H*)-carboxylate (28) A white solid; R_f = 0.23 (EtOAc/Hex = 2:1); 12 mg, 50% yield; ^{1}H NMR (400 MHz, DMSO-d_6, 70 ℃) (as a mixture of rotamers) δ 8.05 (s, 1H), 7.35–7.18 (m, 12H), 7.09–6.98 (m, 2H), 5.17–4.65 (m, 8H), 4.64–4.40 (m, 1H), 4.33 (d, *J* = 15.5 Hz, 1H), 3.88 (t, *J* = 8.5 Hz, 0.65H), 3.77 (d, *J* = 9.1 Hz, 0.7H), 3.65–3.58 (m, 0.35H), 3.53 (dd, *J* = 10.7, 7.7 Hz, 0.65H), 3.42 (t, *J* = 9.6 Hz, 0.65H), 3.29 (t, *J* = 10.8 Hz, 0.65H), 3.03 (t, *J* = 10.5 Hz, 0.35H), 2.81 (s, 3H), 1.69–1.62 (m, 0.65H), 1.54–1.46 (m, 0.35H), 0.76–0.62 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6, 70 ℃) δ 171.2, 166.4, 162.6, 160.9 (d, $^1J_{C,F}$ = 242.2 Hz), 155.0, 153.8, 138.1, 136.4, 135.8, 128.0, 128.0, 127.9 (d, $^3J_{C,F}$ = 8.0 Hz), 127.5, 127.2, 127.1, 126.5, 114.7(d, $^2J_{C,F}$ = 21.4 Hz), 97.8, 66.3, 58.2, 57.4, 57.1, 56.2, 55.8, 55.7, 46.0, 44.7, 43.8, 40.7, 40.1, 38.9, 10.9, 6.7, 6.4; HRMS(ESI+): Cacld for $C_{36}H_{38}FN_6O_3^+$ $[M+H]^+$ 621.2984, found 621.2983, Δppm –0.16; HPLC purity: 99.9%, t_R = 15.6 min. The product was synthesized with 4-fluorobenzyl bromide.



29

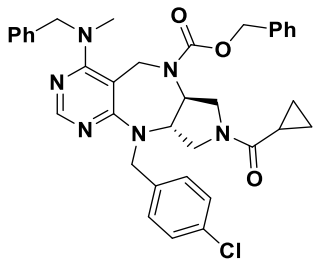
Benzyl (6a*S*,9a*S*)-4-(benzyl(methyl)amino)-8-(cyclopropanecarbonyl)-10-(4-methoxybenzyl)-6a,7,8,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6(5*H*)-carboxylate (29). A white solid; R_f = 0.24 (EtOAc/Hex = 2:1); 12 mg, 49% yield; ^{1}H NMR (400 MHz, DMSO-d_6, 70 ℃) (as a mixture of rotamers) δ 8.05 (s, 1H), 7.36–7.08 (m, 12H), 6.84–6.76 (m, 2H), 5.17–4.65 (m, 8H), 4.62–4.36 (m, 1H), 4.32 (d, *J* = 15.7 Hz, 1H), 3.87 (t, *J* = 8.6 Hz, 0.65H), 3.80 (d, *J* = 9.1 Hz, 0.7H), 3.70 (s, 3H), 3.65–3.49 (m, 1H), 3.41 (t, *J* = 9.7 Hz, 0.65H), 3.31 (t, *J* = 10.8 Hz,

0.65H), 3.02 (t, *J* = 10.6 Hz, 0.35H), 2.80 (s, 3H), 1.70–1.59 (m, 0.65H), 1.55–1.48 (m, 0.35H), 0.76–0.62 (m, 4H); ^{13}C NMR (150 MHz, DMSO-*d*$_6$, 70 ℃) (as a mixture of rotamers) δ 171.2, 166.4, 162.6, 157.9, 155.0, 153.8, 138.1, 136.4, 131.5, 128.0, 128.0, 127.4, 127.3, 127.2, 127.1, 127.1, 126.8, 126.5, 113.6, 97.7, 66.3, 58.3, 57.4, 57.2, 56.2, 55.9, 55.7, 54.8, 46.0, 44.7, 43.8, 40.7, 40.1, 38.9, 10.9, 6.7, 6.4; HRMS(ESI+): Cacld for $C_{37}H_{41}N_6O_4^+$ $[M+H]^+$ 633.3184, found 633.3184, Δppm 0.00; HPLC purity: 96.7%, t_R = 14.8 min. The product was synthesized with 4-methoxybnezyl bromide.



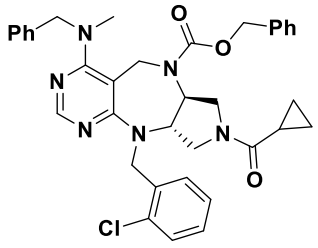
30

Benzyl (6a*S*,9a*S*)-4-(benzyl(methyl)amino)-8-(cyclopropanecarbonyl)-10-(4-(trifluoromethyl)benzyl)-6a,7,8,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6(5*H*)-carboxylate (30). A white solid; R_f = 0.35 (EtOAc/Hex = 2:1); 17 mg, 65% yield; ^{1}H NMR (400 MHz, DMSO-*d*$_6$, 70 ℃) (as a mixture of rotamers) δ 8.04 (s, 1H), 7.59 (d, *J* = 8.0 Hz, 2H), 7.46–7.15 (m, 12H), 5.18–4.45 (m, 9H), 4.34 (d, *J* = 15.7 Hz, 1H), 3.90 (t, *J* = 8.5 Hz, 0.65H), 3.83 (t, *J* = 8.3 Hz, 0.35H), 3.75 (t, *J* = 9.7 Hz, 0.35H), 3.68–3.60 (m, 0.35H), 3.54 (dd, *J* = 10.7, 7.7 Hz, 0.65H), 3.45 (t, *J* = 9.6 Hz, 0.65H), 3.26 (t, *J* = 10.7 Hz, 0.65H), 3.07–3.02 (m, 0.35H), 2.82 (s, 3H), 1.70–1.63 (m, 0.65H), 1.54–1.47 (m, 0.35H), 0.76–0.60 (m, 4H); ^{13}C NMR (150 MHz, DMSO-*d*$_6$, 70 ℃) (as a mixture of rotamers) δ 171.2, 166.4, 162.6, 155.1, 153.8, 144.8, 138.0, 136.4, 128.0, 128.0, 127.5, 127.2, 127.1, 126.7, 126.6, 126.5, 124.8 (q, $^3J_{C,F}$ = 3.7 Hz), 124.0 (q, $^1J_{C,F}$ = 270.5 Hz), 98.0, 98.0, 66.4, 58.2, 57.4, 57.2, 56.2, 55.8, 55.7, 46.6, 46.5, 44.6, 43.8, 40.7, 38.9, 10.9, 10.9, 6.8, 6.7, 6.4; HRMS(ESI+): Cacld for $C_{37}H_{38}F_3N_6O_3^+$ $[M+H]^+$ 671.2952, found 671.2952, Δppm 0.00; HPLC purity: 93.5%, t_R = 17.0 min. The product was synthesized with 4-(trifluoromethyl)benzyl bromide.



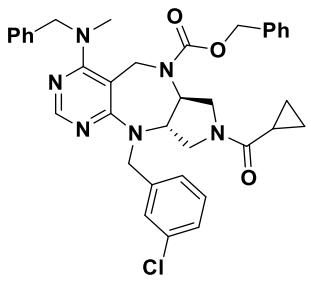
31

Benzyl (6a*S*,9a*S*)-4-(benzyl(methyl)amino)-10-(4-chlorobenzyl)-8-(cyclopropanecarbonyl)-6a,7,8,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6(5*H*)-carboxylate (31). A white solid; R_f = 0.36 (EtOAc/Hex = 2:1); 13 mg, 52% yield; $[\alpha]_D^{25}$ –84.3 (*c* = 0.3, $CHCl_3$); ^{1}H NMR (600 MHz, DMSO-*d*$_6$, 70 ℃) (as a mixture of rotamers) δ 8.05 (s, 1H), 7.37–7.17 (m, 14H), 5.15–4.68 (m, 8H), 4.64–4.44 (m, 1H), 4.33 (dd, *J* = 15.6, 4.1 Hz, 1H), 3.89 (t, *J* = 8.6 Hz, 0.65H), 3.84–3.72 (m, 0.7H), 3.62 (dd, *J* = 11.1, 7.7 Hz, 0.35H), 3.53 (dd, *J* = 10.5, 7.5 Hz, 0.65H), 3.43 (t, *J* = 9.7 Hz, 0.65H), 3.27 (t, *J* = 10.7 Hz, 0.65H), 3.03 (t, *J* = 10.6 Hz, 0.35H), 2.81 (s, 3H), 1.66 (tt, *J* = 8.1, 5.3 Hz, 0.65H), 1.50 (td, *J* = 7.9, 4.1 Hz, 0.35H), 0.79–0.60 (m, 4H); ^{13}C NMR (150 MHz, DMSO-*d*$_6$, 70 ℃) (as a mixture of rotamers) δ 171.2, 166.4, 162.6, 155.0, 153.8, 138.8, 138.1, 136.4, 130.9, 128.0, 128.0, 127.9, 127.8, 127.5, 127.2, 127.1, 126.5, 97.9, 66.4, 58.2, 57.4, 57.1, 56.2, 55.8, 55.7, 46.1, 44.7, 43.8, 40.7, 40.1, 39.0, 38.9, 10.9, 10.9, 6.8, 6.7, 6.4; HRMS(ESI+): Cacld for $C_{36}H_{38}ClN_6O_3^+$ $[M+H]^+$ 637.2689, found 637.2688, Δppm –0.16; HPLC purity: 97.5%, t_R = 16.5 min. The product was synthesized with 4-chlorobenzyl bromide.



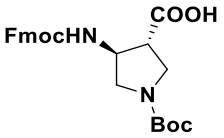
32

Benzyl (6a*S*,9a*S*)-4-(benzyl(methyl)amino)-10-(2-chlorobenzyl)-8-(cyclopropanecarbonyl)-6a,7,8,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6(5*H*)-carboxylate (32). A white solid; R_f = 0.47 (EtOAc/Hex = 2:1); 13 mg, 52% yield; ^{1}H NMR (400 MHz, DMSO-*d*$_6$, 70 ℃) (as a mixture of rotamers) δ 7.99 (s, 1H), 7.41 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.37–7.06 (m, 13H), 5.26–4.50 (m, 9H), 4.33 (d, *J* = 15.6 Hz, 1H), 3.91 (q, *J* = 9.3 Hz, 1H), 3.71–3.57 (m, 1.35H), 3.45 (t, *J* = 9.6 Hz, 0.65H), 3.20–3.11 (m, 0.65H), 3.08–3.02 (m, 0.35H), 2.82 (s, 3H), 1.70–1.63 (m, 0.65H), 1.53–1.44 (m, 0.35H), 0.76–0.62 (m, 4H); ^{13}C NMR (150 MHz, DMSO-*d*$_6$, 70 ℃) (as a mixture of rotamers) δ 171.2, 166.4, 162.5, 155.1, 153.8, 138.0, 136.4, 130.8, 128.9, 128.0, 128.0, 127.5, 127.2, 127.1, 127.1, 126.8, 126.5, 98.1, 66.4, 58.3, 57.3, 56.1, 55.9, 45.1, 44.5, 43.6, 40.7, 40.1, 38.9, 10.9, 6.7, 6.4; HRMS(ESI+): Cacld for $C_{36}H_{38}ClN_6O_3^+$ $[M+H]^+$ 637.2689, found 637.2687, Δppm –0.31; HPLC purity: 99.9%, t_R = 16.5 min. The product was synthesized with 2-chlorobenzyl bromide.



33

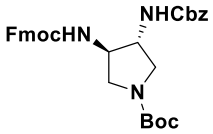
Benzyl (6a*S*,9a*S*)-4-(benzyl(methyl)amino)-10-(3-chlorobenzyl)-8-(cyclopropanecarbonyl)-6a,7,8,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6(5*H*)-carboxylate (33). A white solid; R_f = 0.41 (EtOAc/Hex = 2:1); 16 mg, 64% yield; ^{1}H NMR (400 MHz, DMSO-d_6, 70 ℃) (as a mixture of rotamers) δ 8.05 (s, 1H), 7.36–7.13 (m, 14H), 5.18–4.93 (m, 4H), 4.90–4.42 (m, 5H), 4.33 (d, J = 15.6 Hz, 1H), 3.90 (t, J = 8.6 Hz, 0.65H), 3.77 (d, J = 9.1 Hz, 0.7H), 3.68–3.60 (m, 0.35H), 3.53 (dd, J = 10.7, 7.7 Hz, 0.65H), 3.43 (t, J = 9.7 Hz, 0.65H), 3.27 (t, J = 10.7 Hz, 0.65H), 3.06–2.98 (m, 0.35H), 2.81 (s, 3H), 1.70–1.62 (m, 0.65H), 1.54–1.46 (m, 0.35H), 0.77–0.62 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6, 70 ℃) (as a mixture of rotamers) δ 171.2, 166.4, 162.6, 155.0, 153.8, 142.6, 138.0, 136.3, 132.8, 129.8, 128.0, 128.0, 127.5, 127.2, 127.1, 126.8, 126.5, 126.3, 126.0, 125.8, 124.7, 124.6, 98.0, 66.4, 58.1, 57.4, 57.1, 56.1, 55.8, 55.7, 46.4, 46.3, 44.7, 43.8, 40.7, 40.1, 39.0, 38.9, 11.0, 10.9, 6.7, 6.4; HRMS(ESI+): Cacld for $C_{36}H_{38}ClN_6O_3^+$ $[M+H]^+$ 637.2689, found 637.2687, Δppm –0.31; HPLC purity: 97.3%, t_R = 16.5 min. The product was synthesized with 3-chlorobenzyl bromide.



S6

(3*R*,4*S*)-4-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-1-(*tert*-butoxycarbonyl)pyrrolidine-3-carboxylic acid (S6). The synthesis of the **S6** was previously reported.[d]

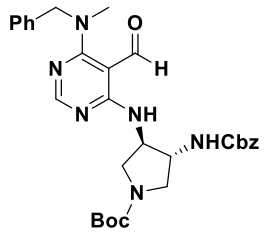
[d] H. S. Lee, P. R. LePlae, E. A. Porter, S. H. Gellman, *J. Org. Chem*. **2001**, *66*, 3597–3599.



S7

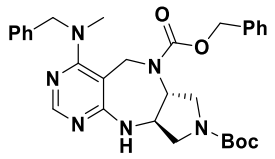
***tert*-Butyl (3*R*,4*R*)-3-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-4-(((benzyloxy)carbonyl)amino)pyrrolidine-1-carboxylate (S7)**. A white solid; R_f = 0.57 (EtOAc/Hex = 1:1); 2.18 g, 78%

overall yield; $[\alpha]_D^{25}$ –22.7 (*c* = 0.3, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 7.89 (d, *J* = 7.5 Hz, 2H), 7.73–7.58 (m, 4H), 7.42 (t, *J* = 7.4 Hz, 2H), 7.38–7.25 (m, 7H), 5.02 (s, 2H), 4.37–4.27 (m, 2H), 4.25–4.19 (m, 1H), 4.03–3.88 (m, 2H), 3.61–3.49 (m, 2H), 3.12–3.03 (m, 2H), 1.40 (s, 9H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 156.3, 153.8, 144.3, 144.3, 141.2, 137.4, 128.8, 128.3, 128.1, 127.5, 125.6, 125.6, 120.6, 79.1, 66.0, 55.0, 54.1, 49.5, 49.1, 47.2, 28.6; LRMS(ESI+): Cacld for $C_{32}H_{35}N_3O_6Na^+$ $[M+Na]^+$ 580.24, found 580.10. The product was synthesized according to the synthetic procedure for the preparation of **S2** from **S1**.



S8

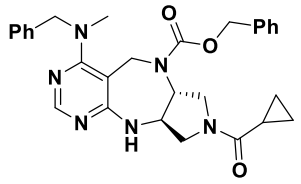
***tert*-Butyl (3*R*,4*R*)-3-((6-(benzyl(methyl)amino)-5-formyl-pyrimidin-4-yl)amino)-4-(((benzyloxy)carbonyl)amino)pyrrolidine-1-carboxylate (S8)**. A pale yellow solid; R_f = 0.30 (EtOAc/Hex = 1:1); 393 mg, 60% overall yield; ^{1}H NMR (400 MHz, DMSO-*d*$_6$, 100 ℃) δ 9.84 (s, 1H), 9.19 (d, *J* = 7.0 Hz, 1H), 8.11 (s, 1H), 7.38–7.18 (m, 11H), 5.09–4.94 (m, 2H), 4.88 (s, 2H), 4.59 (p, *J* = 6.9 Hz, 1H), 4.15 (p, *J* = 7.1 Hz, 1H), 3.80 (dd, *J* = 11.0, 7.0 Hz, 1H), 3.65 (dd, *J* = 11.1, 7.4 Hz, 1H), 3.22–3.15 (m, 2H), 3.14 (s, 3H), 1.41 (s, 9H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$, 100 ℃) δ 187.5, 165.4, 161.6, 158.6, 155.3, 153.1, 136.7, 136.6, 128.0, 127.7, 127.2, 127.0, 126.9, 126.7, 95.6, 78.3, 65.1, 54.9, 54.3, 53.8, 49.1, 48.5, 27.7; LRMS(ESI+): Cacld for $C_{30}H_{37}N_6O_5^+$ $[M+H]^+$ 561.28, found 561.35. The product was synthesized according to the synthetic procedure for the preparation of **S3** from **S2**.



S9

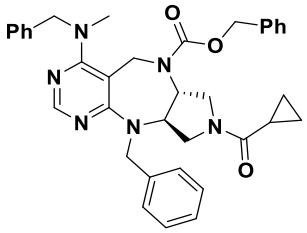
6-Benzyl 8-(*tert*-butyl) (6a*R*,9a*R*)-4-(benzyl(methyl)amino)-5,6a,7,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6,8-dicarboxylate (S9). A white solid; R_f = 0.62 (EtOAc/MeOH = 10:1); 178 mg, 76% overall yield; ^{1}H NMR (400 MHz, DMSO-*d*$_6$, 100 ℃) δ 7.95 (s, 1H), 7.37–7.15 (m, 10H), 7.09 (br s, 1H), 5.14 (d, *J* = 12.7 Hz, 1H), 5.05 (d, *J* = 12.7 Hz, 1H), 4.89 (d, *J* = 16.2 Hz, 1H), 4.83–4.68 (m, 2H), 4.54 (d, *J* = 15.4 Hz, 1H), 4.23 (d, *J* = 15.4 Hz, 1H), 4.23–4.11 (m, 1H), 3.68 (dd, *J* = 10.0, 7.3 Hz, 1H), 3.62 (dd, *J* = 10.0, 7.8 Hz, 1H), 3.20 (t, *J* = 10.1 Hz, 1H), 3.07–2.98 (m, 1H), 2.72 (s, 3H), 1.40 (s, 9H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$, 100 ℃) δ 166.3, 162.4, 155.0, 154.3, 153.2, 137.9, 136.2, 127.8, 127.7, 127.3, 127.1, 126.8, 126.3,

97.7, 78.4, 66.2, 62.3, 56.1, 51.8, 47.2, 45.8, 39.8, 38.8, 27.7; LRMS(ESI+): Cacld for $C_{30}H_{37}N_6O_4^+$ $[M+H]^+$ 545.29, found 545.25. The product was synthesized according to the synthetic procedure for the preparation of **S4** from **S3**.



S10

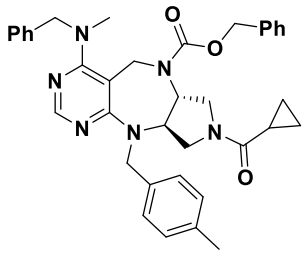
Benzyl (6a*R*,9a*R*)-4-(benzyl(methyl)amino)-8-(cyclopropanecarbonyl)-6a,7,8,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6(5*H*)-carboxylate (S10). A white solid; R_f = 0.38 (EtOAc/MeOH = 10:1); 64 mg, 97% overall yield; ^{1}H NMR (600 MHz, DMSO-d_6, 70 °C) (as a mixture of rotamers) δ 7.96 (s, 1H), 7.35–7.19 (m, 11H), 5.14 (dd, J = 12.7, 4.3 Hz, 1H), 5.07 (dd, J = 12.6, 9.9 Hz, 1H), 4.91–4.74 (m, 3H), 4.58–4.51 (m, 1H), 4.31–4.16 (m, 2H), 4.03–3.98 (m, 1H), 3.78 (ddd, J = 31.6, 10.9, 7.5 Hz, 1H), 3.55 (t, J = 9.8 Hz, 0.4H), 3.38 (t, J = 9.8 Hz, 0.6H), 3.17 (t, J = 10.7 Hz, 0.6H), 2.98 (t, J = 10.7 Hz, 0.4H), 2.72 (s, 3H), 1.71–1.62 (m, 1H), 0.81–0.69 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6, 70 °C) (as a mixture of rotamers) δ 171.1, 166.4, 162.5, 162.4, 155.1, 154.5, 138.0, 136.3, 128.0, 127.9, 127.5, 127.1, 127.0, 126.5, 97.7, 66.4, 62.8, 61.7, 56.3, 56.2, 52.3, 51.1, 47.7, 46.8, 46.3, 45.5, 38.9, 11.0, 6.7, 6.4, 6.3; LRMS(ESI+): Cacld for $C_{29}H_{33}N_6O_3^+$ $[M+H]^+$ 513.26, found 513.20. The product was synthesized according to the synthetic procedure for the preparation of **12** from **S4**.



34

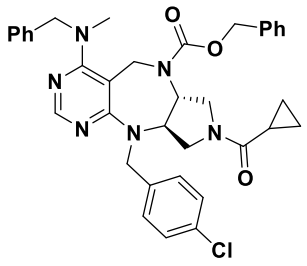
Benzyl (6a*R*,9a*R*)-10-benzyl-4-(benzyl(methyl)amino)-8-(cyclopropanecarbonyl)-6a,7,8,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6(5*H*)-carboxylate (34). A white solid; R_f = 0.24 (EtOAc/Hex = 2:1); 16 mg, 68% yield; $[\alpha]_D^{25}$ +96.1 (c = 0.3, $CHCl_3$); ^{1}H NMR (600 MHz, DMSO-d_6, 70 °C) (as a mixture of rotamers) δ 8.04 (s, 1H), 7.37–7.15 (m, 15H), 5.14 (d, J = 12.8 Hz, 1H), 5.11–4.66 (m, 7H), 4.65–4.41 (m, 1H), 4.33 (dd, J = 15.7, 4.7 Hz, 1H), 3.88 (t, J = 8.6 Hz, 0.65H), 3.77 (d, J = 9.2 Hz, 0.7H), 3.61 (dd, J = 11.0, 7.7 Hz, 0.35H), 3.54 (dd, J = 10.7, 7.6 Hz, 0.65H), 3.42 (t, J = 9.7 Hz, 0.65H), 3.28 (t, J = 10.8 Hz, 0.65H), 3.03 (t, J = 10.6 Hz, 0.35H), 2.81 (s, 3H), 1.68–1.62 (m, 0.65H), 1.50–1.44 (m, 0.35H), 0.76–0.61 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6, 70 °C) (as a mixture of rotamers) δ 171.2, 166.4, 162.6, 155.0, 153.8, 139.7,

138.1, 136.4, 128.0, 128.0, 127.4, 127.2, 127.1, 126.8, 126.5, 126.3, 126.3, 126.0, 125.9, 97.8, 66.3, 58.3, 57.4, 57.2, 56.2, 55.9, 55.7, 46.6, 44.7, 43.8, 40.7, 40.1, 38.9, 10.9, 6.7, 6.4; HRMS(ESI+): Cacld for $C_{36}H_{39}N_6O_3^+$ $[M+H]^+$ 603.3078, found 603.3078, Δppm 0.00; HPLC purity: 94.9%, t_R = 15.1 min. The product was synthesized according to the synthetic procedure for the preparation of **26** from **12** and benzyl bromide.



35

Benzyl (6a*R*,9a*R*)-4-(benzyl(methyl)amino)-8-(cyclopropanecarbonyl)-10-(4-methylbenzyl)-6a,7,8,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6(5*H*)-carboxylate (35). A white solid; R_f = 0.38 (EtOAc/Hex = 2:1); 18 mg, 73% yield; $[\alpha]_D^{25}$ +91.7 (*c* = 0.3, $CHCl_3$); ^{1}H NMR (600 MHz, DMSO-*d*$_6$, 70 °C) (as a mixture of rotamers) δ 8.04 (s, 1H), 7.37–7.01 (m, 14H), 5.16–4.65 (m, 8H), 4.64–4.39 (m, 1H), 4.32 (dd, *J* = 15.6, 4.6 Hz, 1H), 3.87 (t, *J* = 8.6 Hz, 0.65H), 3.83–3.74 (m, 0.7H), 3.63–3.51 (m, 1H), 3.42 (t, *J* = 9.7 Hz, 0.65H), 3.28 (t, *J* = 10.8 Hz, 0.65H), 3.02 (t, *J* = 10.6 Hz, 0.35H), 2.80 (s, 3H), 2.24 (s, 3H), 1.68–1.62 (m, 0.65H), 1.53–1.48 (m, 0.35H), 0.75–0.62 (m, 4H); ^{13}C NMR (150 MHz, DMSO-*d*$_6$, 70 °C) (as a mixture of rotamers) δ 171.2, 171.1, 166.4, 162.6, 155.0, 153.8, 138.1, 136.6, 136.4, 135.3, 128.6, 128.0, 128.0, 127.4, 127.2, 127.1, 126.8, 126.5, 126.0, 125.8, 97.8, 97.7, 66.3, 58.3, 57.5, 57.3, 56.2, 55.9, 55.7, 46.3, 44.7, 43.8, 40.7, 40.1, 38.9, 20.3, 10.9, 6.7, 6.4; HRMS(ESI+): Cacld for $C_{37}H_{41}N_6O_3^+$ $[M+H]^+$ 617.3235, found 617.3234, Δppm –0.16; HPLC purity: 99.9%, t_R = 16.5 min. The product was synthesized according to the synthetic procedure for the preparation of **26** from **12.**

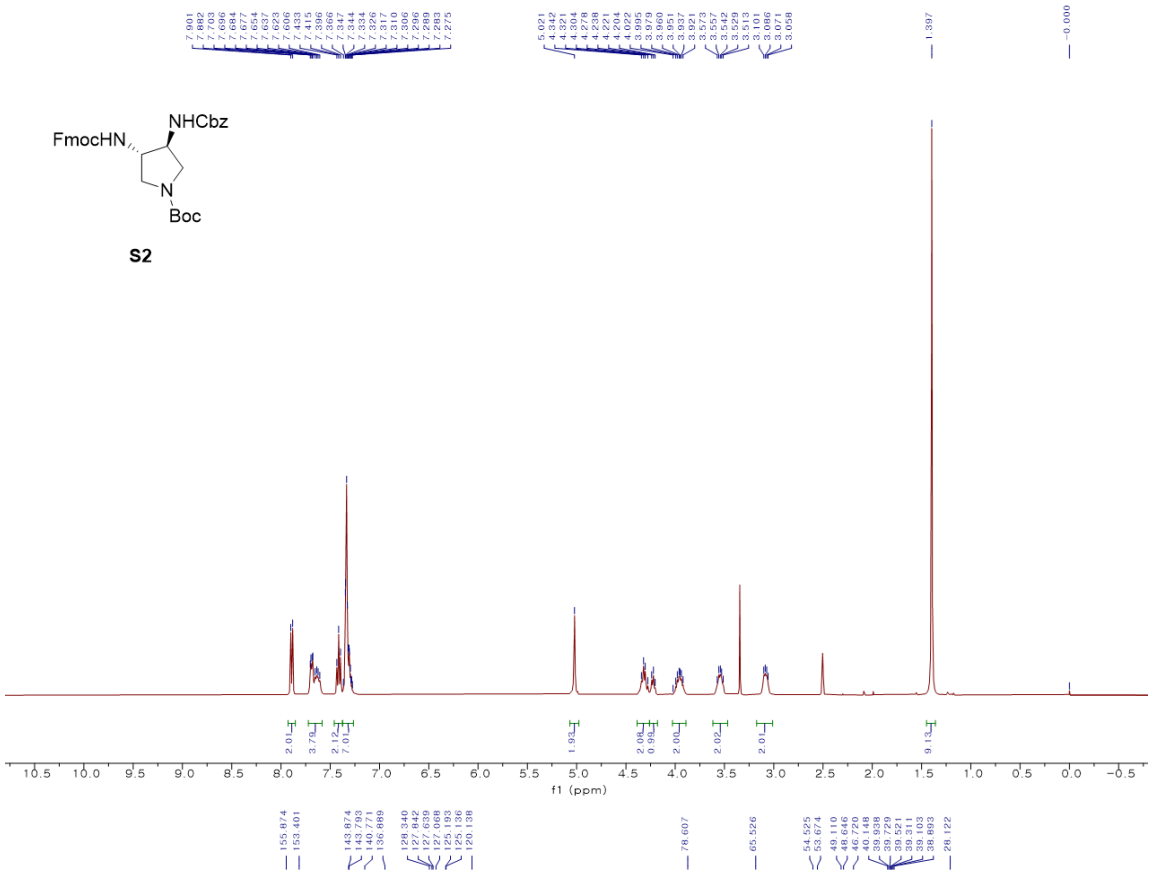


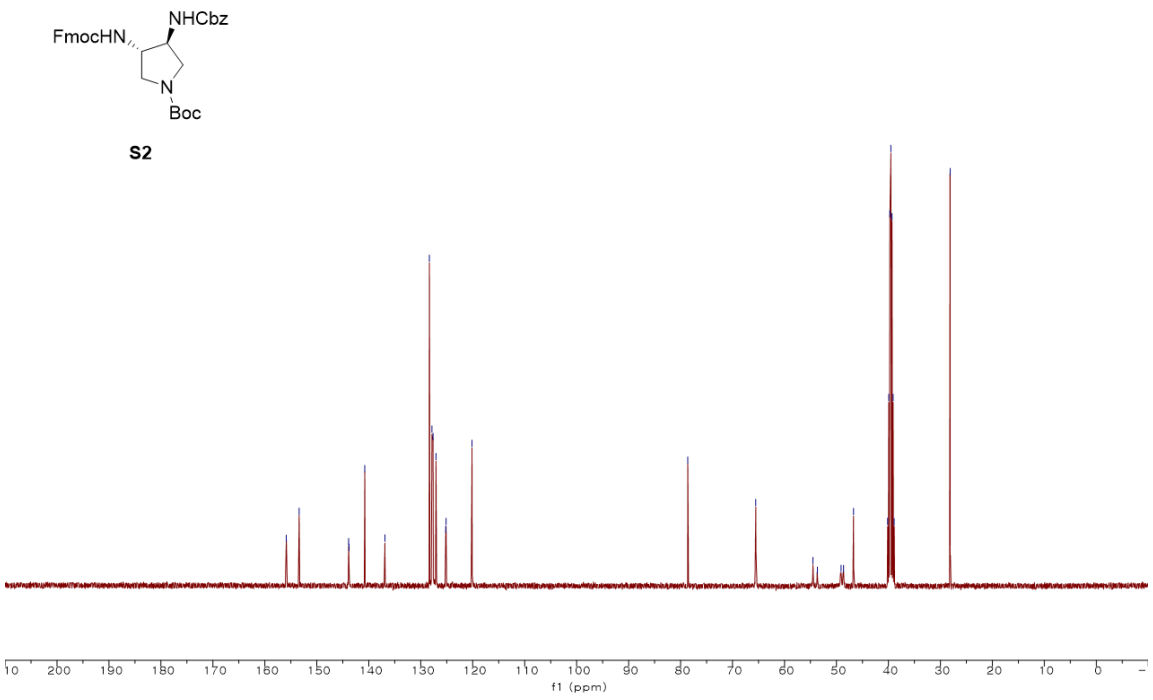
36

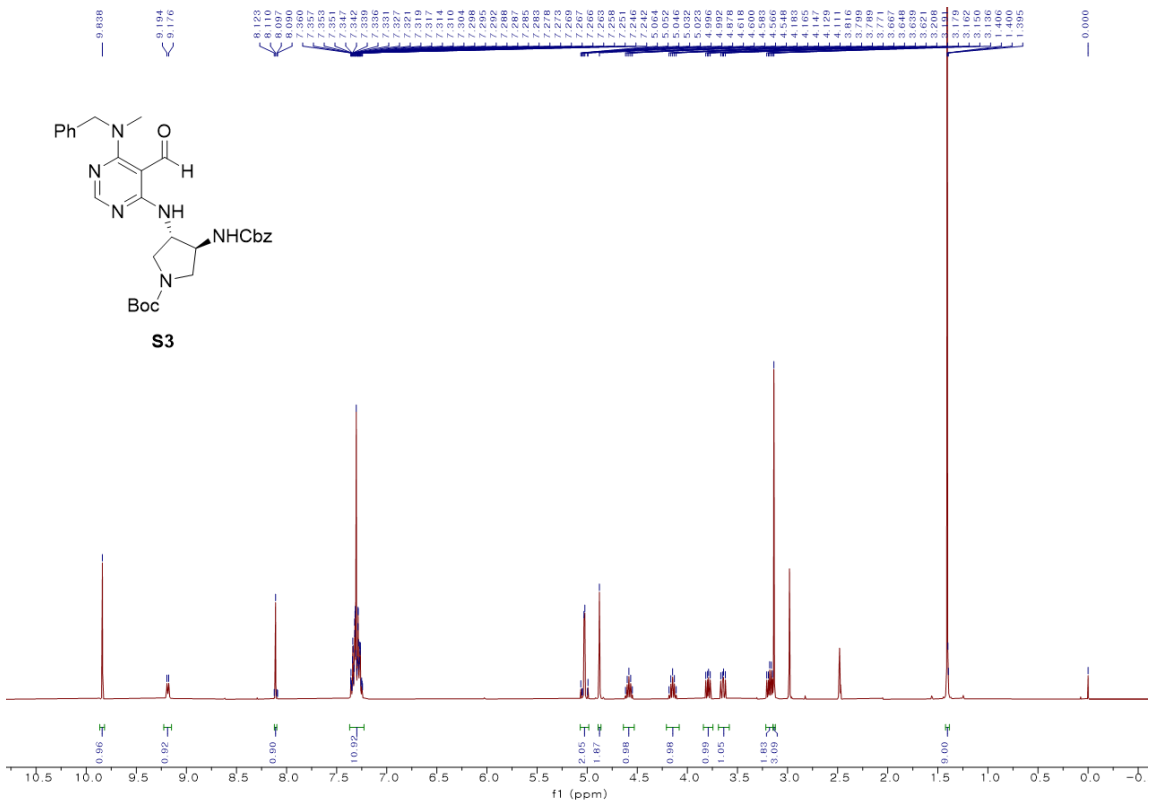
Benzyl (6a*R*,9a*R*)-4-(benzyl(methyl)amino)-10-(4-chlorobenzyl)-8-(cyclopropanecarbonyl)-6a,7,8,9,9a,10-hexahydropyrimido[4,5-*e*]pyrrolo[3,4-*b*][1,4]diazepine-6(5*H*)-carboxylate (36). A white solid; R_f = 0.36 (EtOAc/Hex = 2:1); 16 mg, 64% yield; $[\alpha]_D^{25}$ +86.7 (*c* = 0.3, $CHCl_3$); ^{1}H NMR (600 MHz, DMSO-*d*$_6$, 70 °C) (as a mixture of rotamers) δ 8.04 (s, 1H), 7.36–7.18 (m, 14H), 5.15–4.67 (m, 8H), 4.66–4.42 (m, 1H), 4.36–4.30 (m, 1H), 3.89 (t, *J* = 8.6 Hz, 0.65H), 3.82–

3.72 (m, 0.7H), 3.62 (dd, *J* = 11.1, 7.7 Hz, 0.35H), 3.53 (dd, *J* = 10.7, 7.6 Hz, 0.65H), 3.43 (t, *J* = 9.7 Hz, 0.65H), 3.27 (t, *J* = 10.7 Hz, 0.65H), 3.03 (t, *J* = 10.6 Hz, 0.35H), 2.81 (s, 3H), 1.69–1.63 (m, 0.65H), 1.53–1.49 (m, 0.35H), 0.78–0.60 (m, 4H); ^{13}C NMR (150 MHz, DMSO-*d*$_6$, 70 °C) (as a mixture of rotamers) δ 171.2, 166.4, 162.6, 155.0, 153.8, 138.8, 138.1, 136.4, 131.0, 128.1, 128.0, 127.9, 127.8, 127.5, 127.2, 127.1, 126.5, 97.9, 66.4, 58.2, 57.4, 57.1, 56.2, 55.8, 55.7, 46.1, 44.7, 43.8, 40.7, 40.1, 39.0, 38.9, 11.0, 10.9, 6.8, 6.7, 6.4; HRMS(ESI+): Cacld for $C_{36}H_{38}ClN_6O_3^+$ $[M+H]^+$ 637.2689, found 637.2689, Δppm 0.00; HPLC purity: 98.5%, t_R = 15.7 min. The product was synthesized according to the synthetic procedure for the preparation of **31** from **12.**

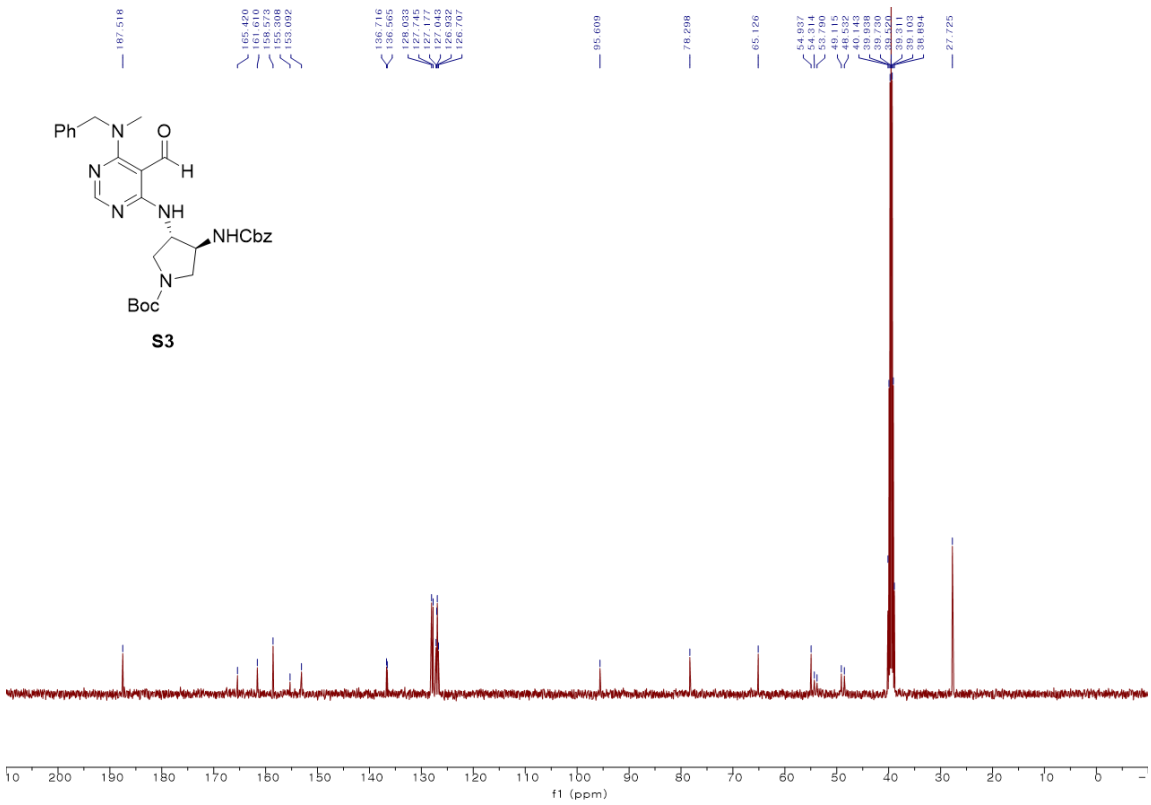
^{1}H and ^{13}C NMR Spectra



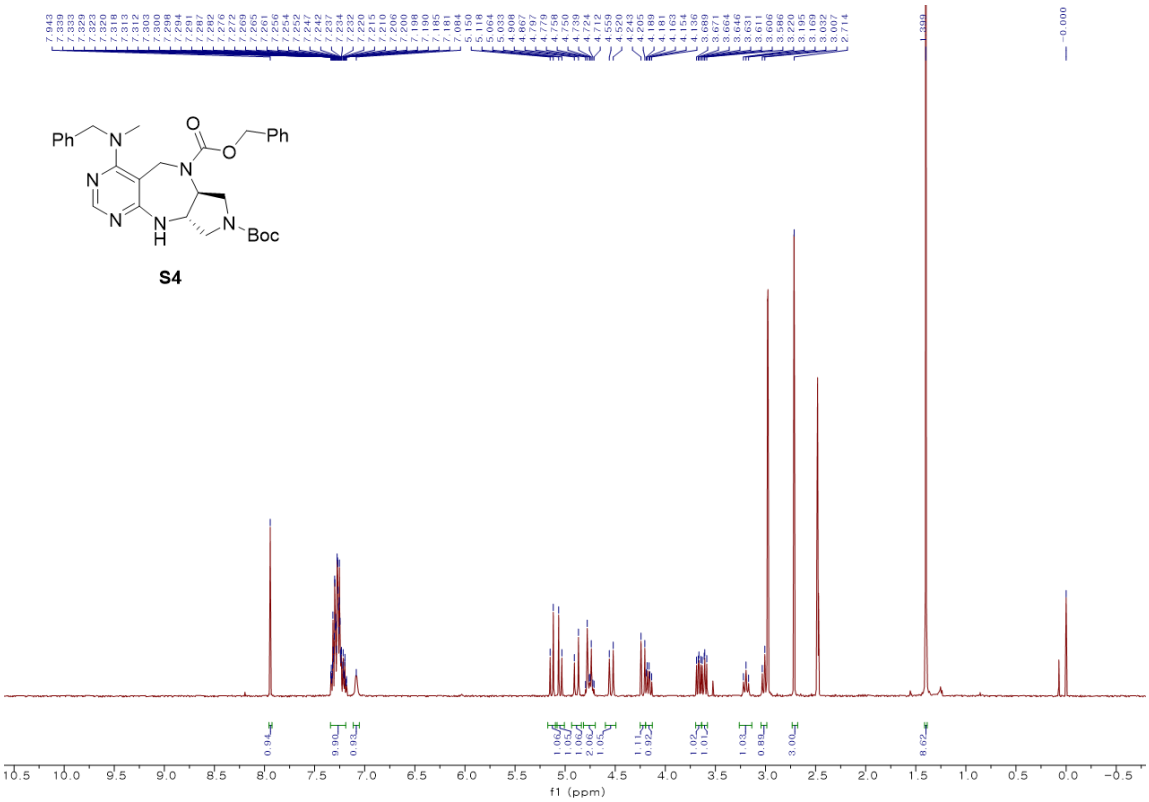




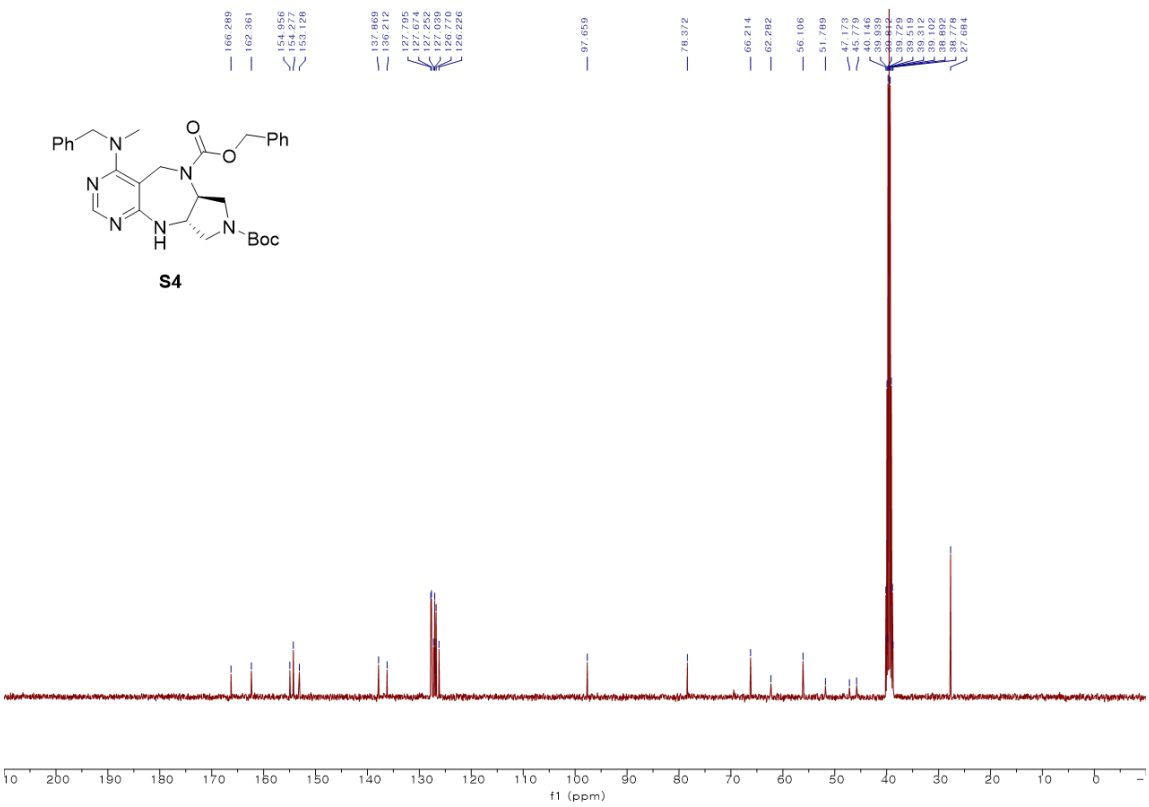
S3



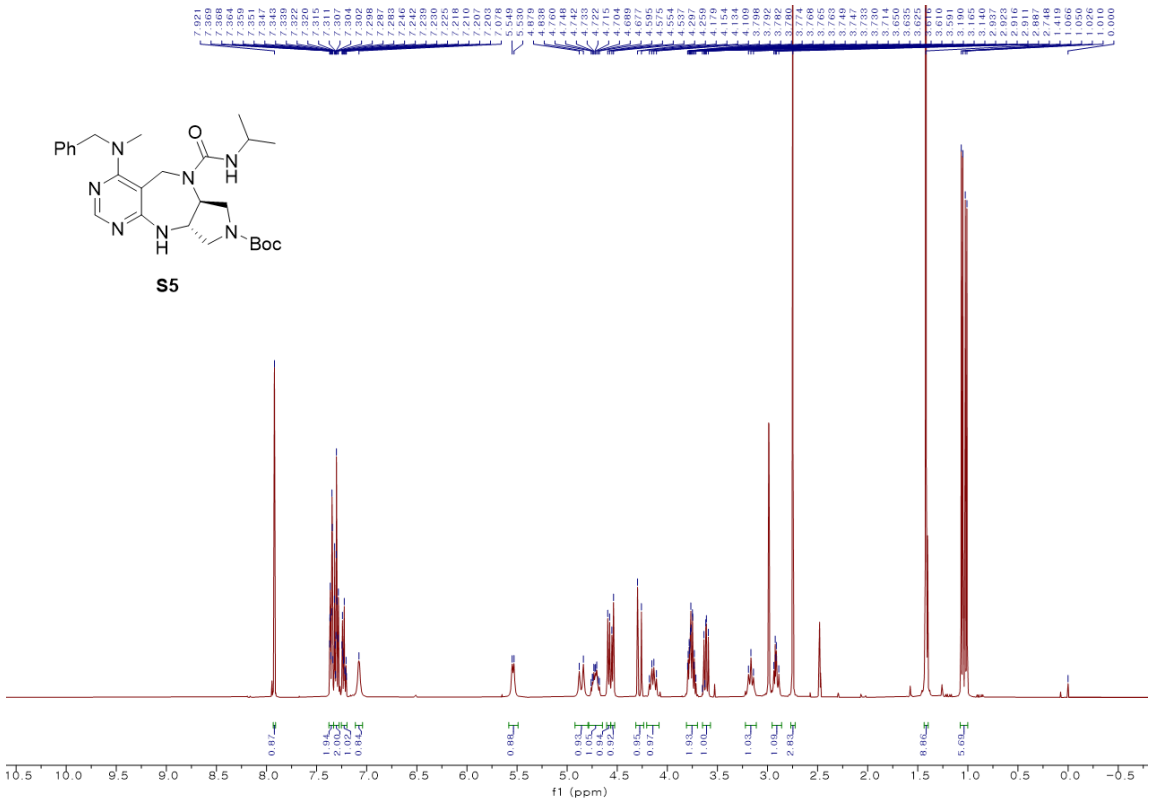
S3



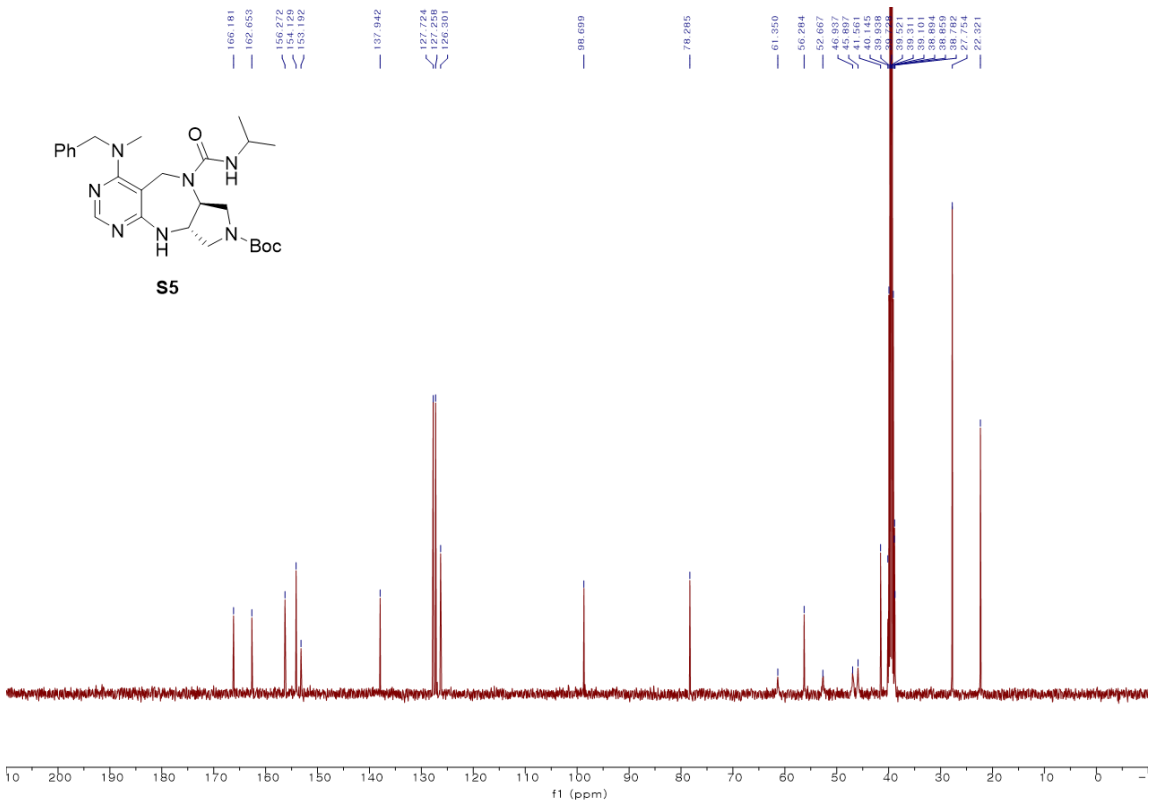
S4



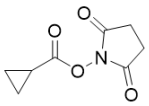
S4



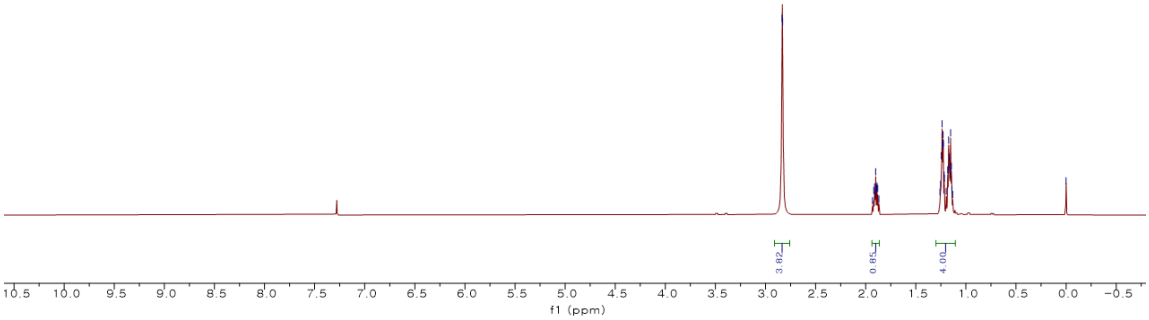
S5

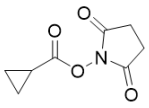


S5

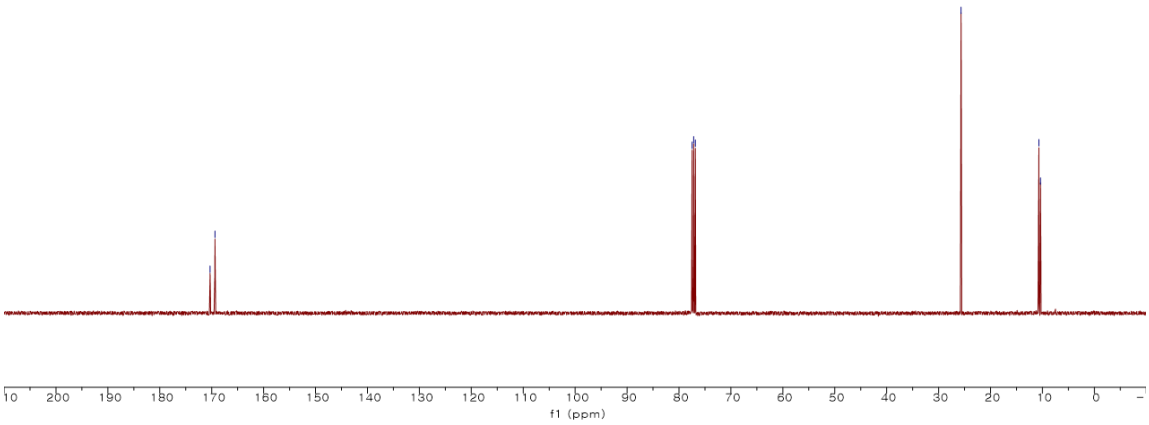


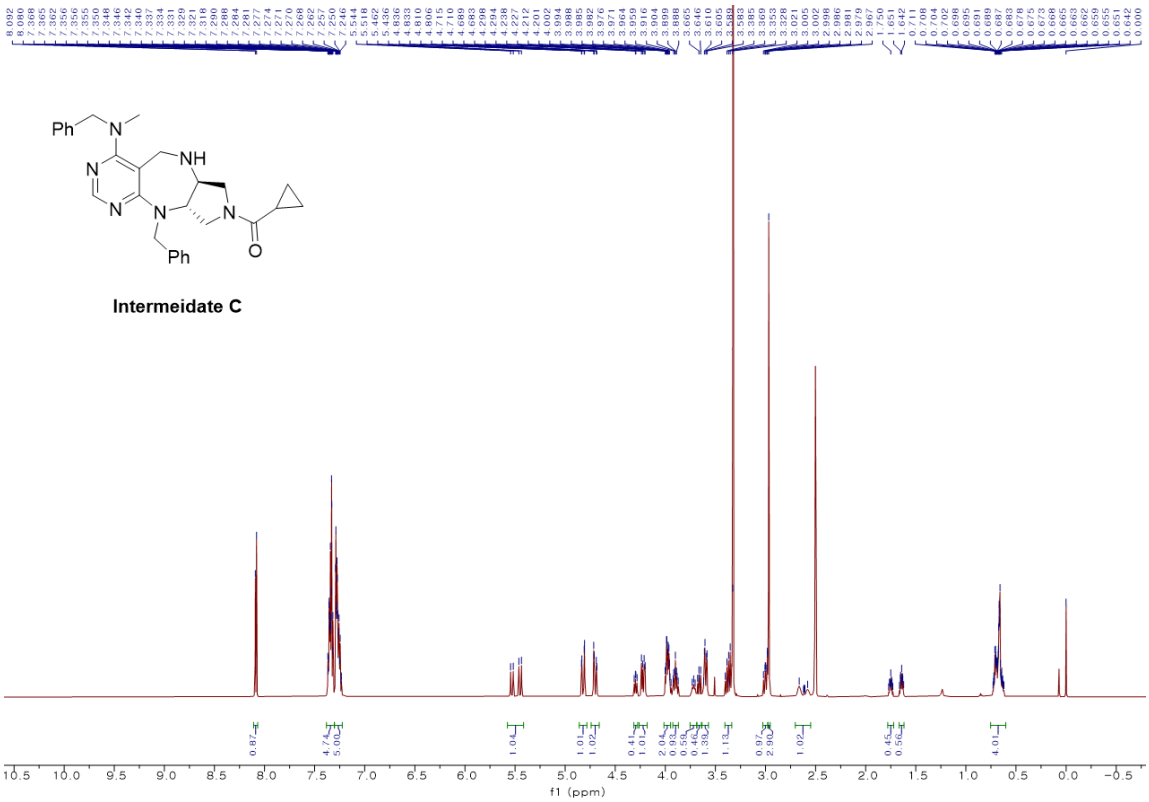
2,5-dioxopyrrolidin-1-yl cyclopropanecarboxylate



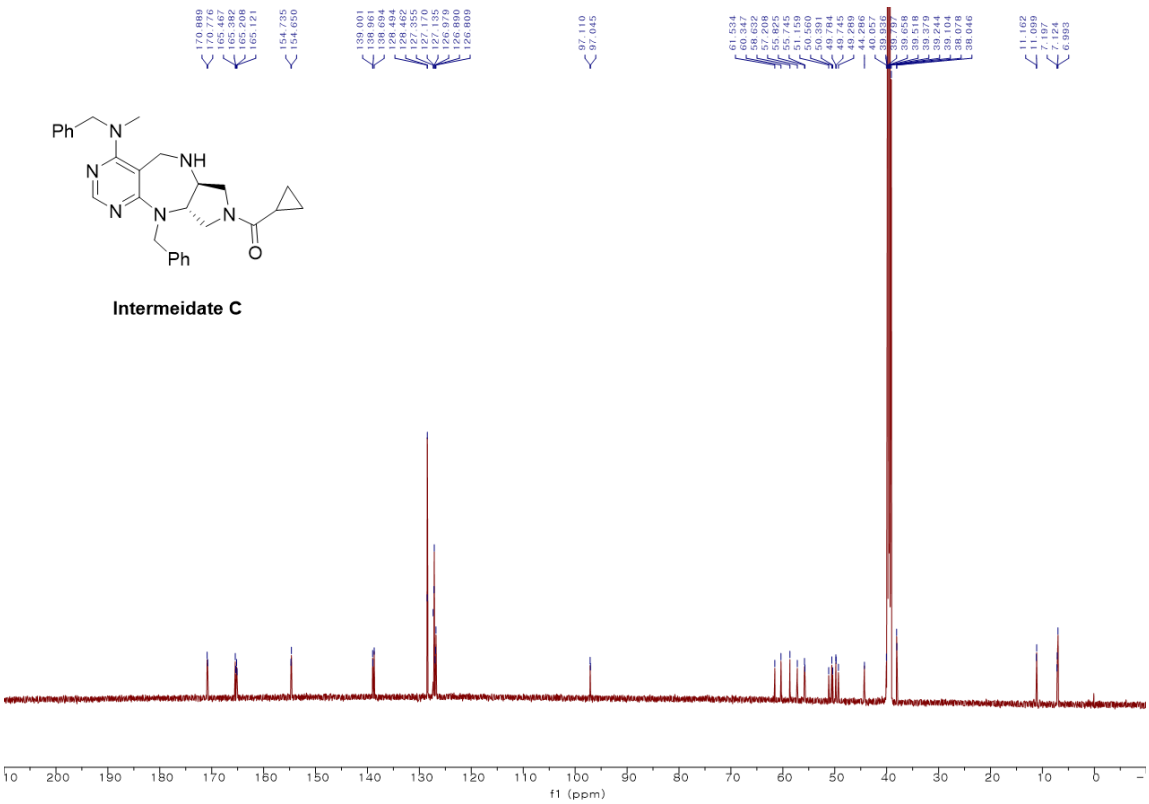


2,5-dioxopyrrolidin-1-yl cyclopropanecarboxylate

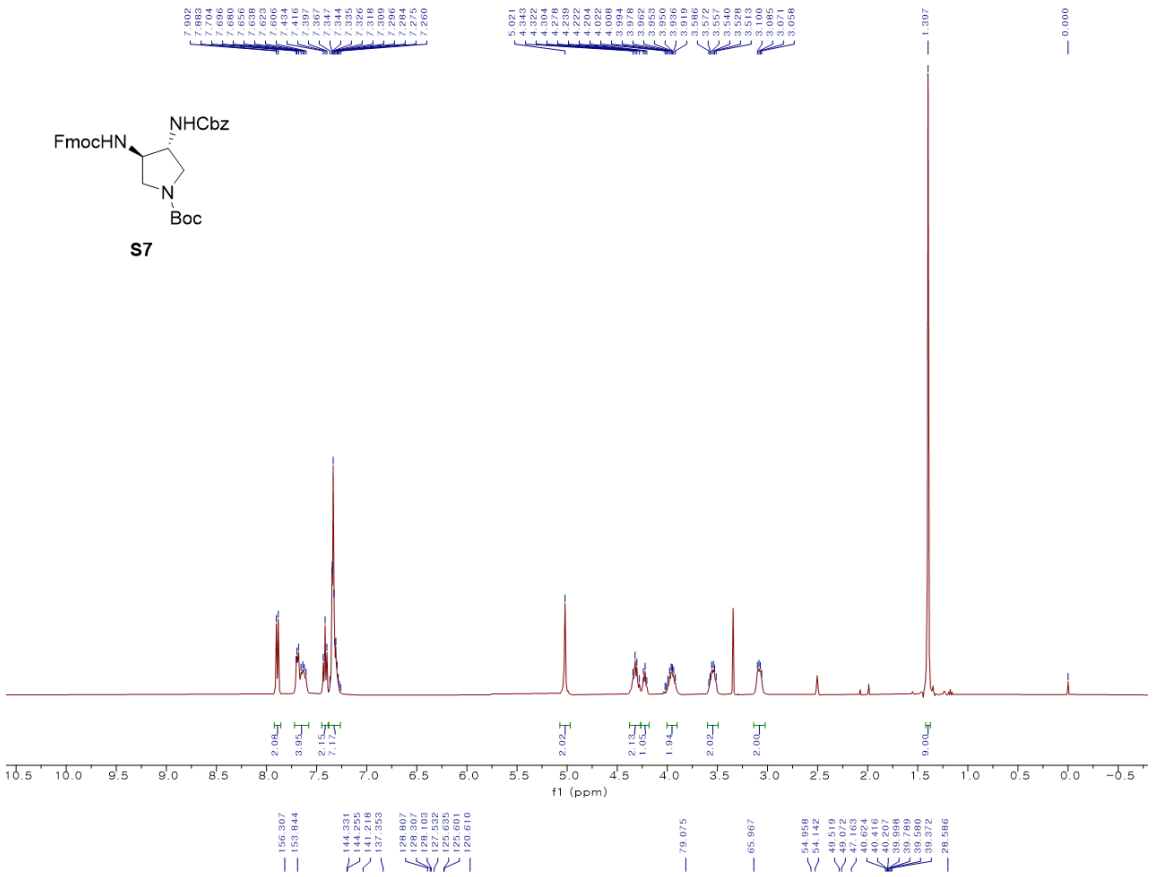




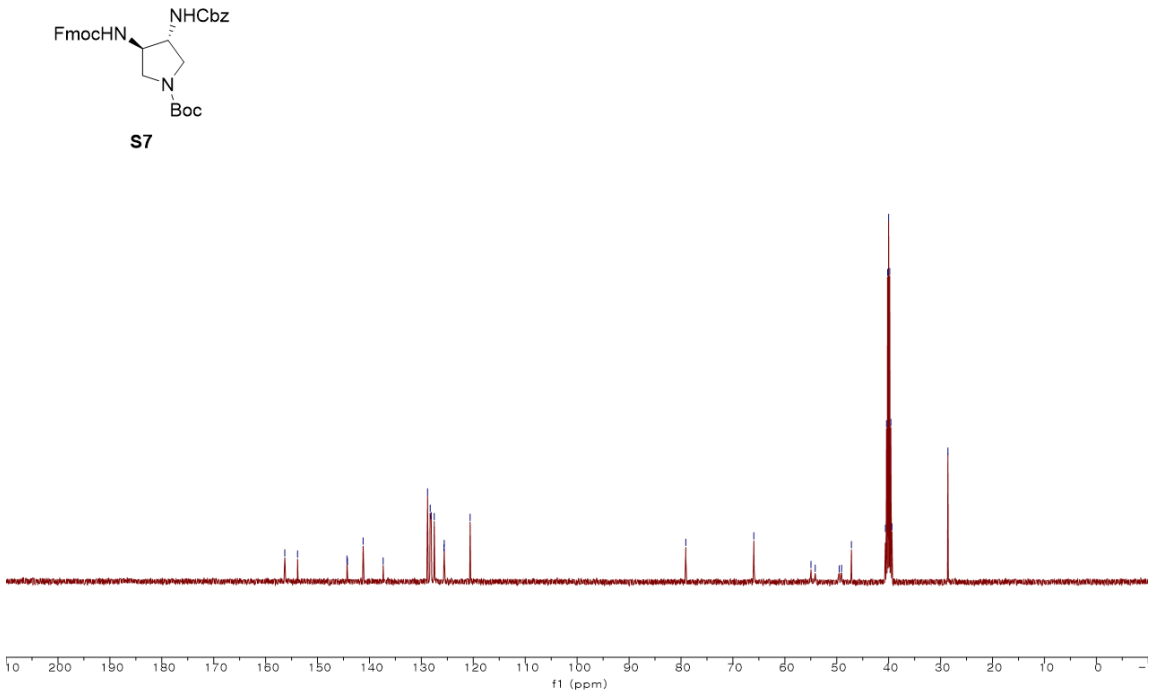
Intermeidate C



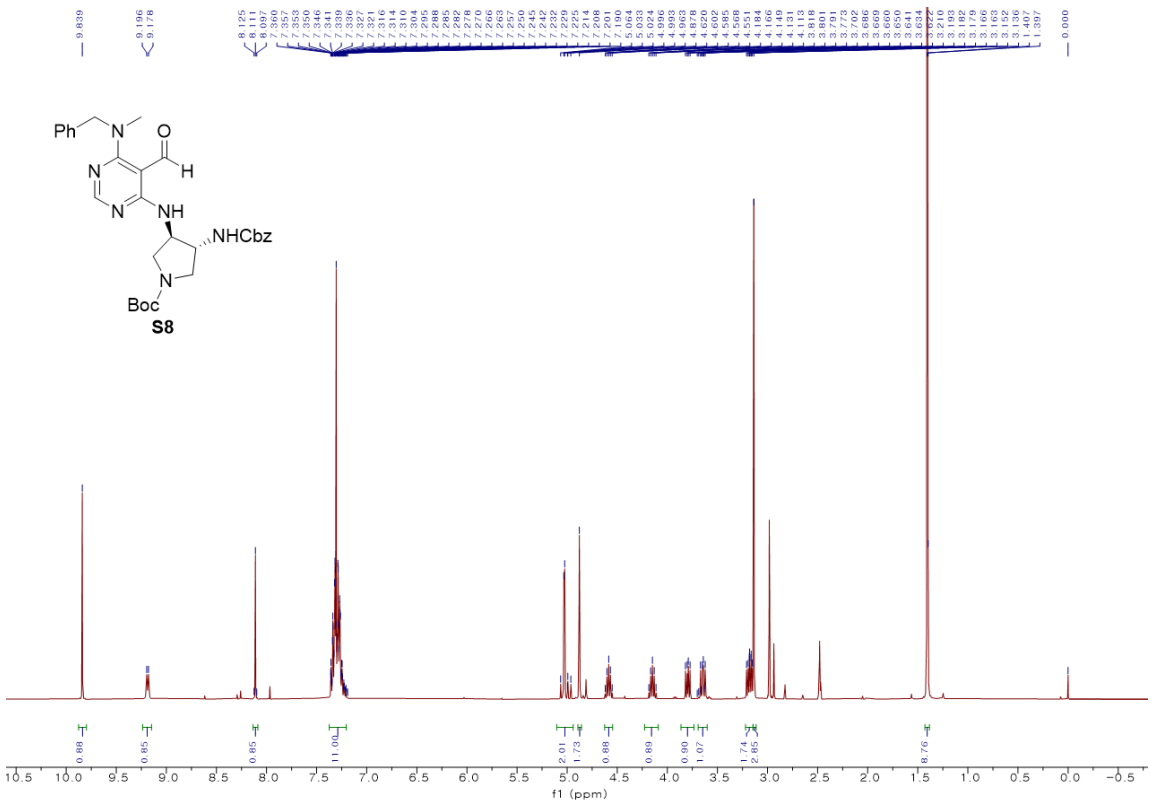
Intermeidate C



S7



S7


Ph
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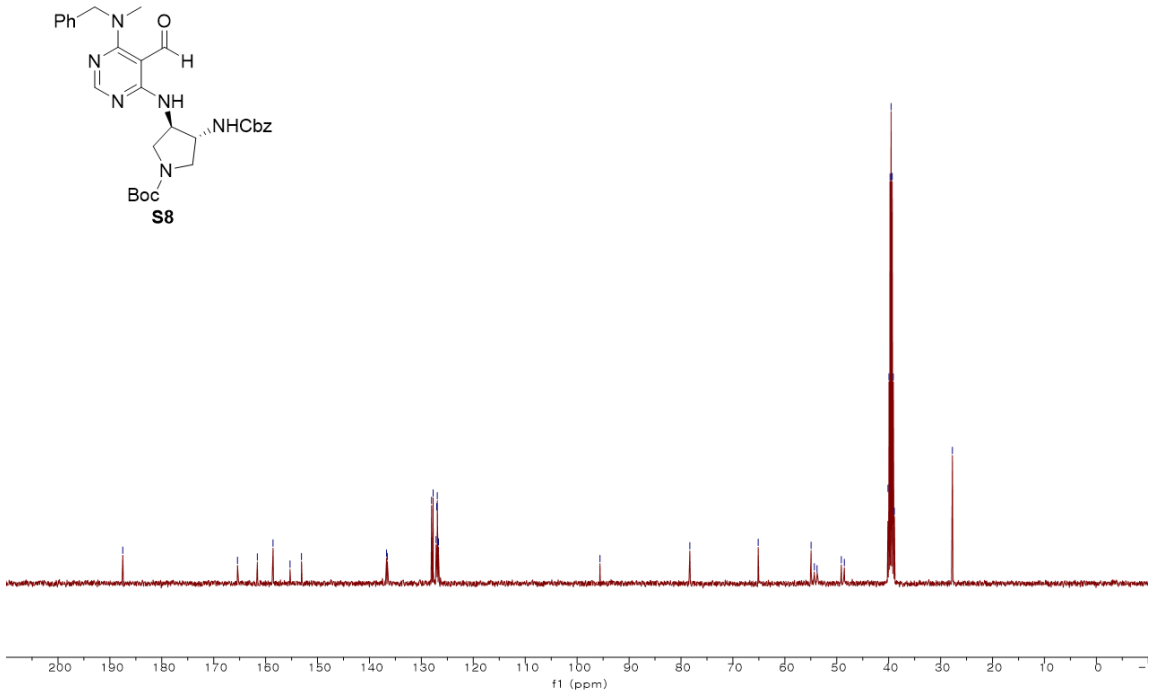
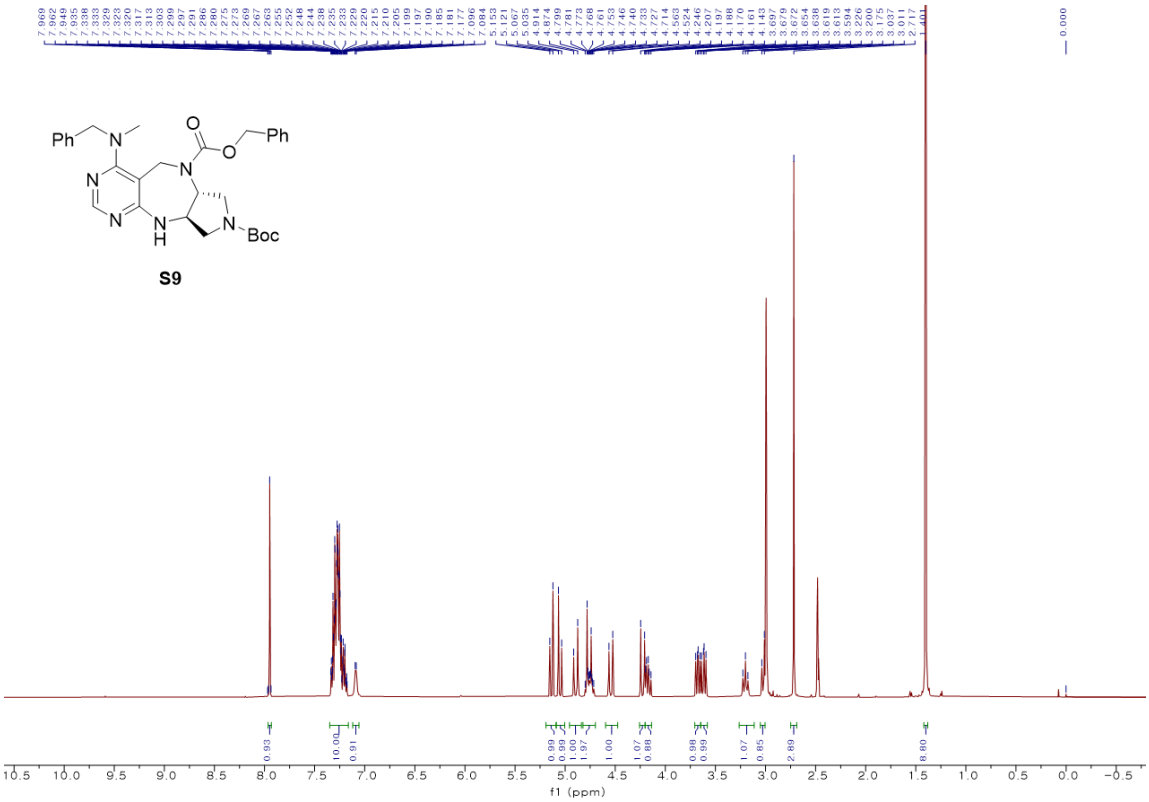
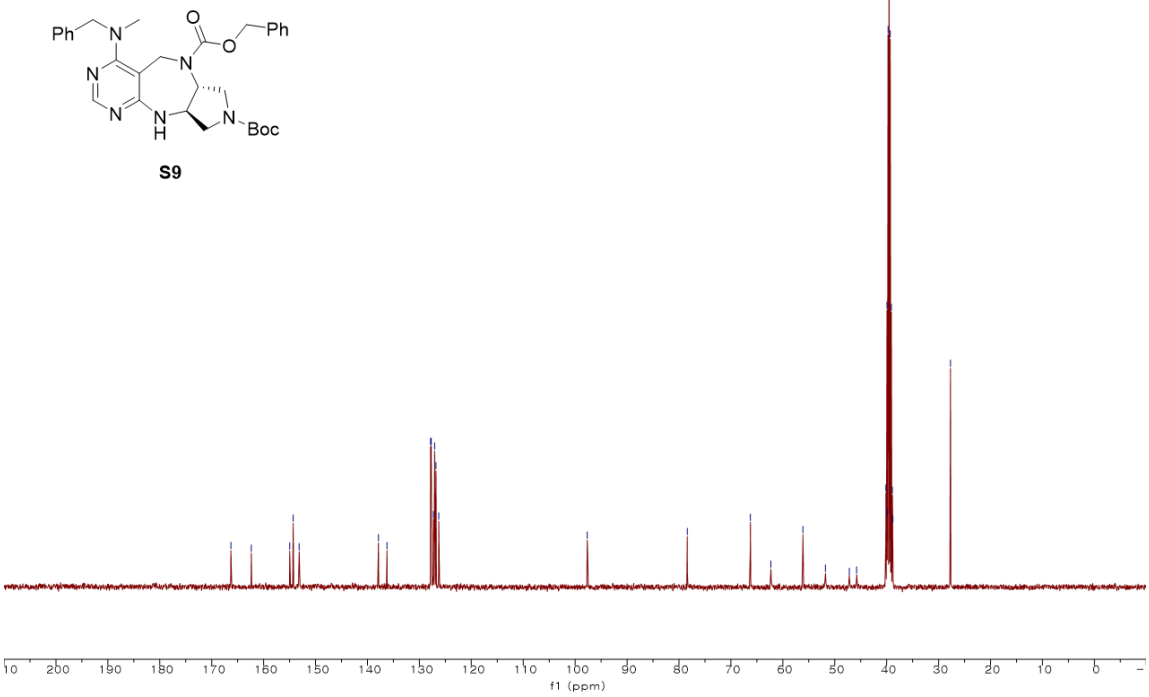
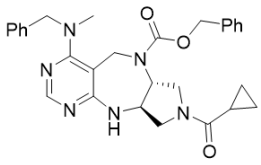
H
N
N
NH
NHCbz
N
Boc
S8
f1 (ppm)

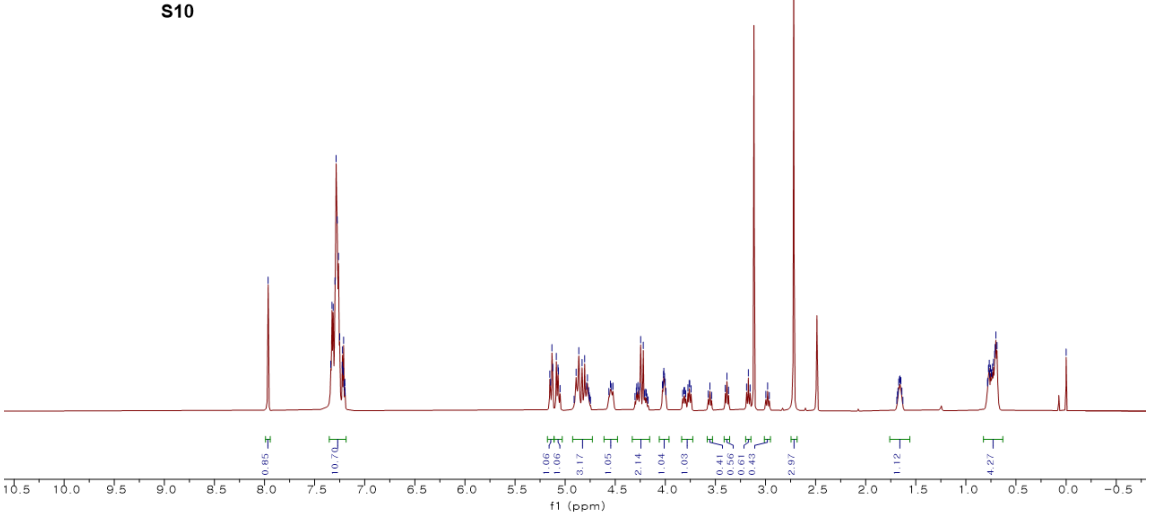

Ph
N
O
H
N
N
NH
NHCbz
N
Boc
S8
f1 (ppm)

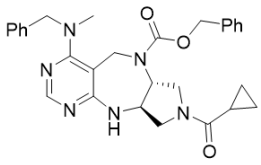

Ph
N
O
O
Ph
N
N
N
N
H
N
Boc
S9
f1 (ppm)


Ph
N
O
O
Ph
N
N
N
N
H
N
Boc
S9
f1 (ppm)

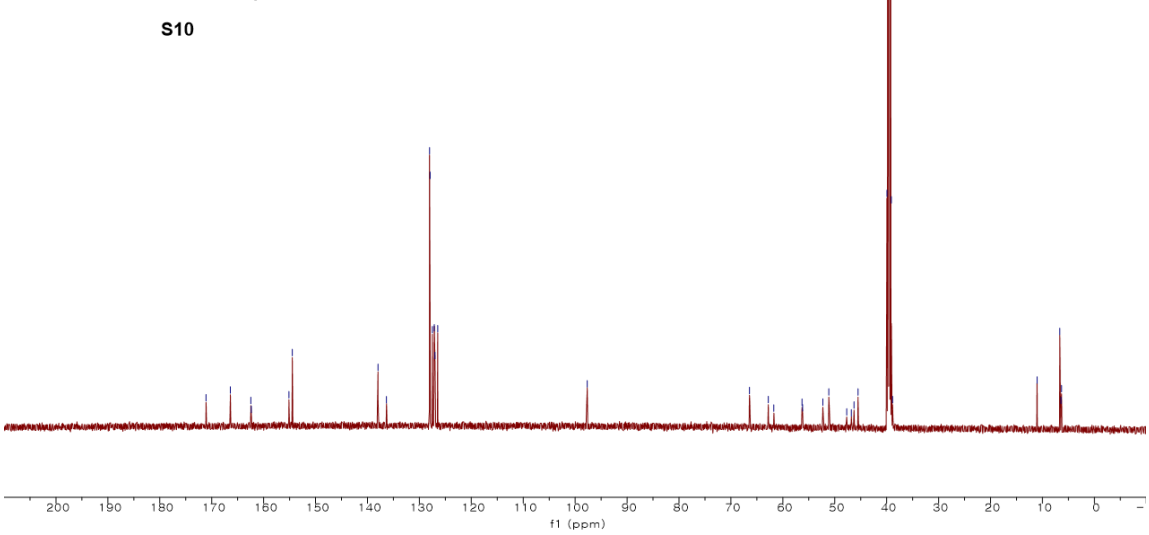


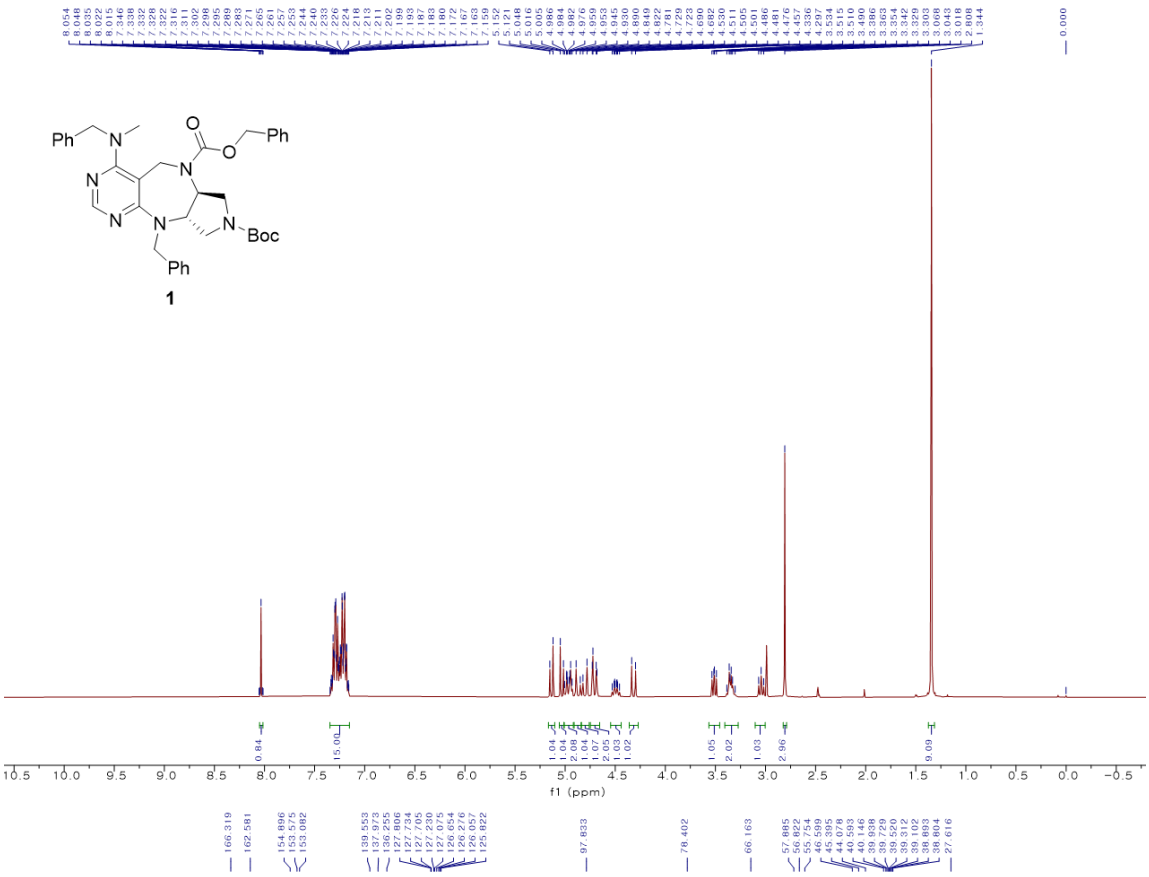
S10





S10




Ph
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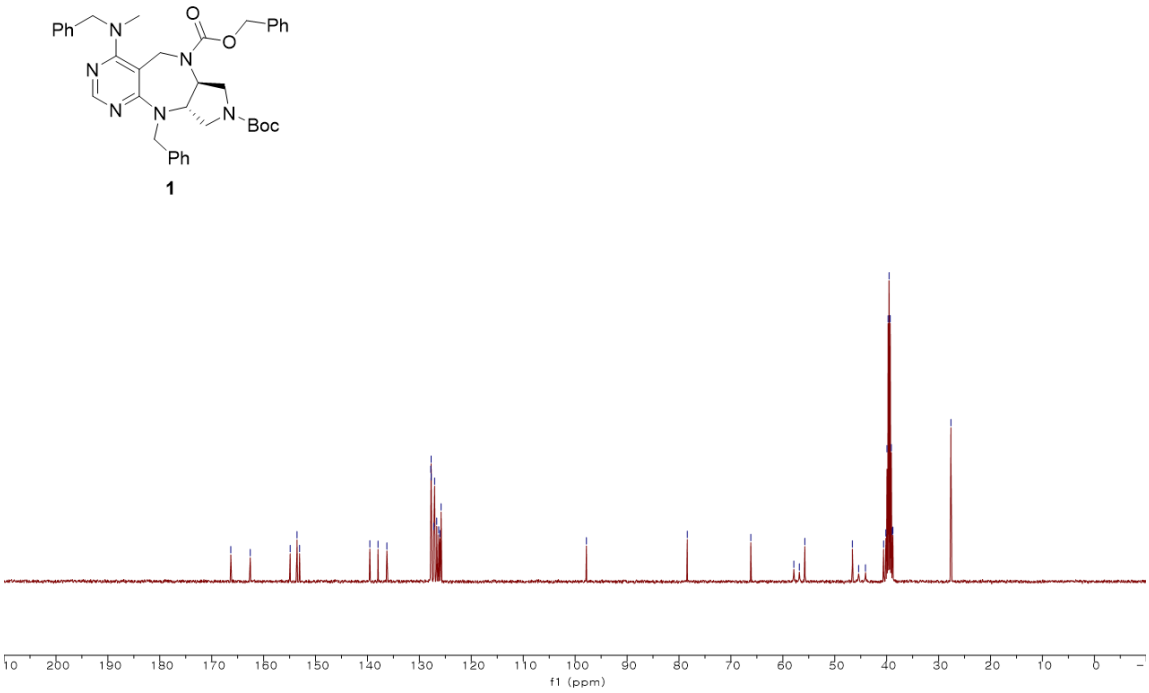
O
Ph
N
N
N
N
N
Boc
Ph
1
f1 (ppm)


Ph
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Ph
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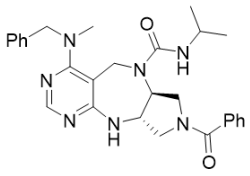
N
N
N
N
Boc
Ph
1
f1 (ppm)



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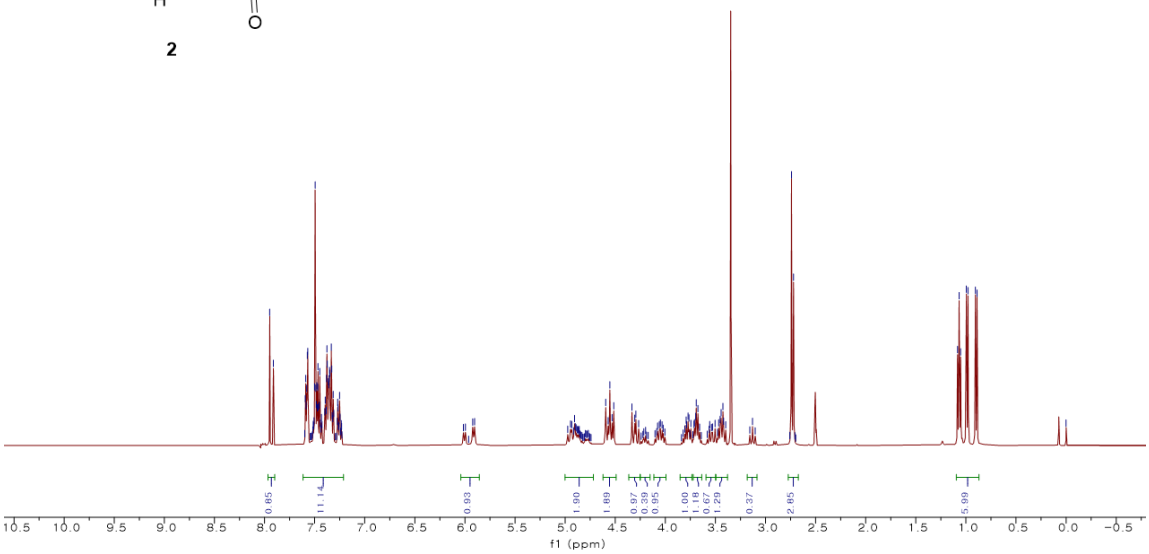
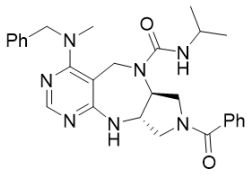
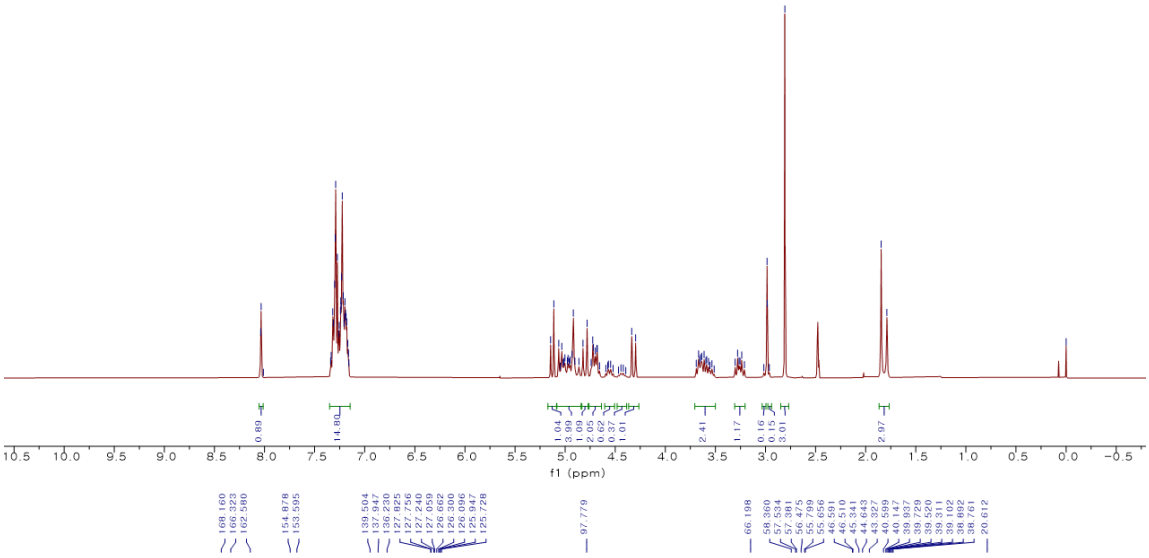
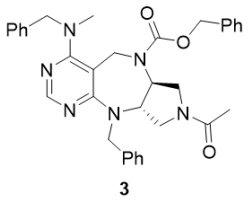




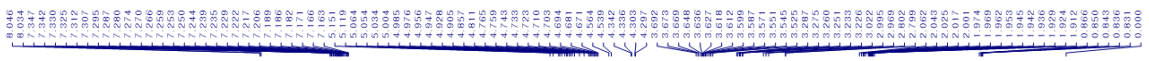
2

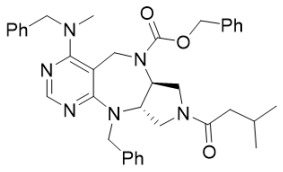
3



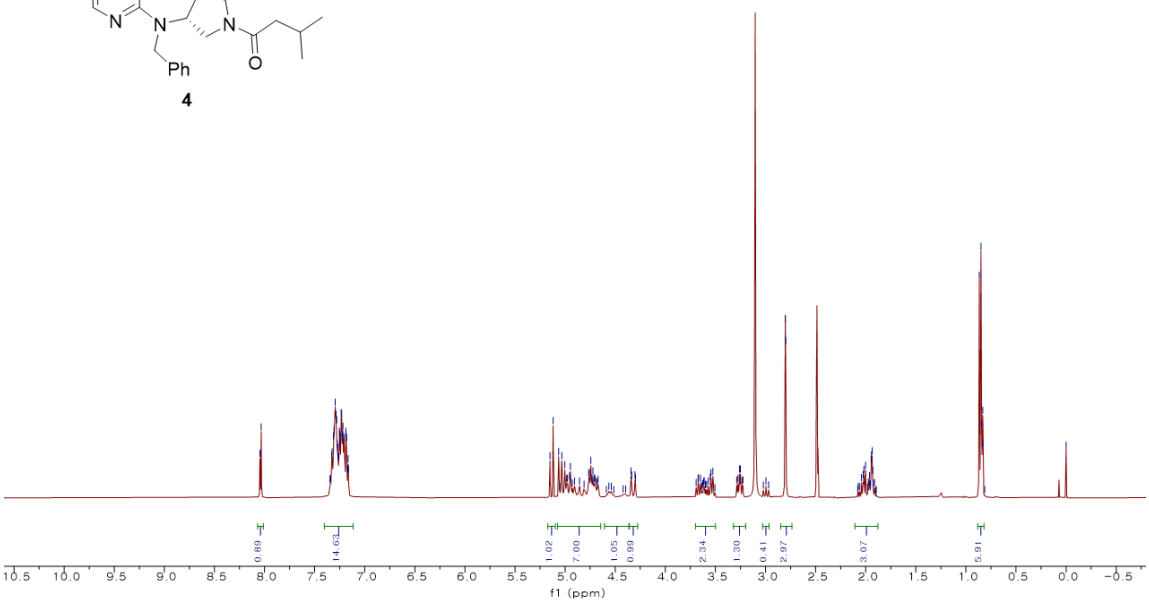


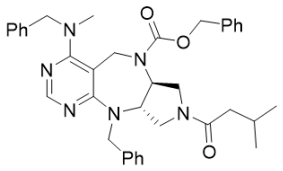
3



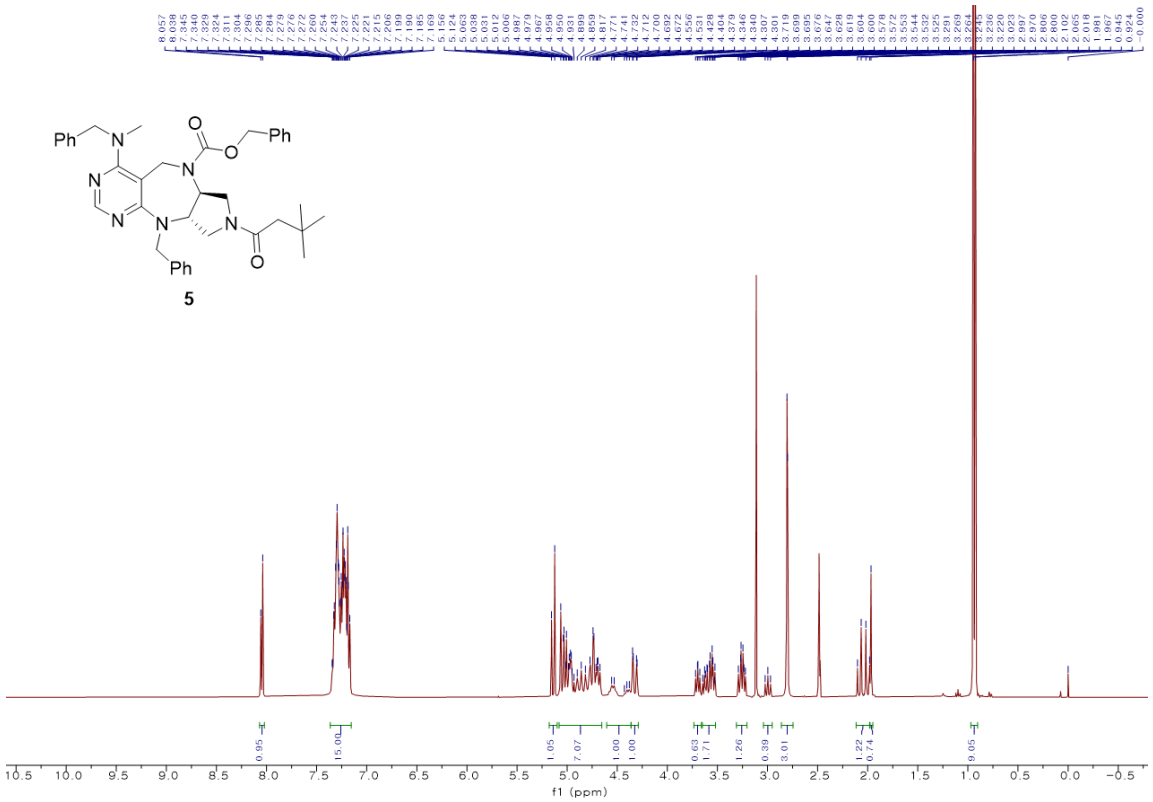


4





4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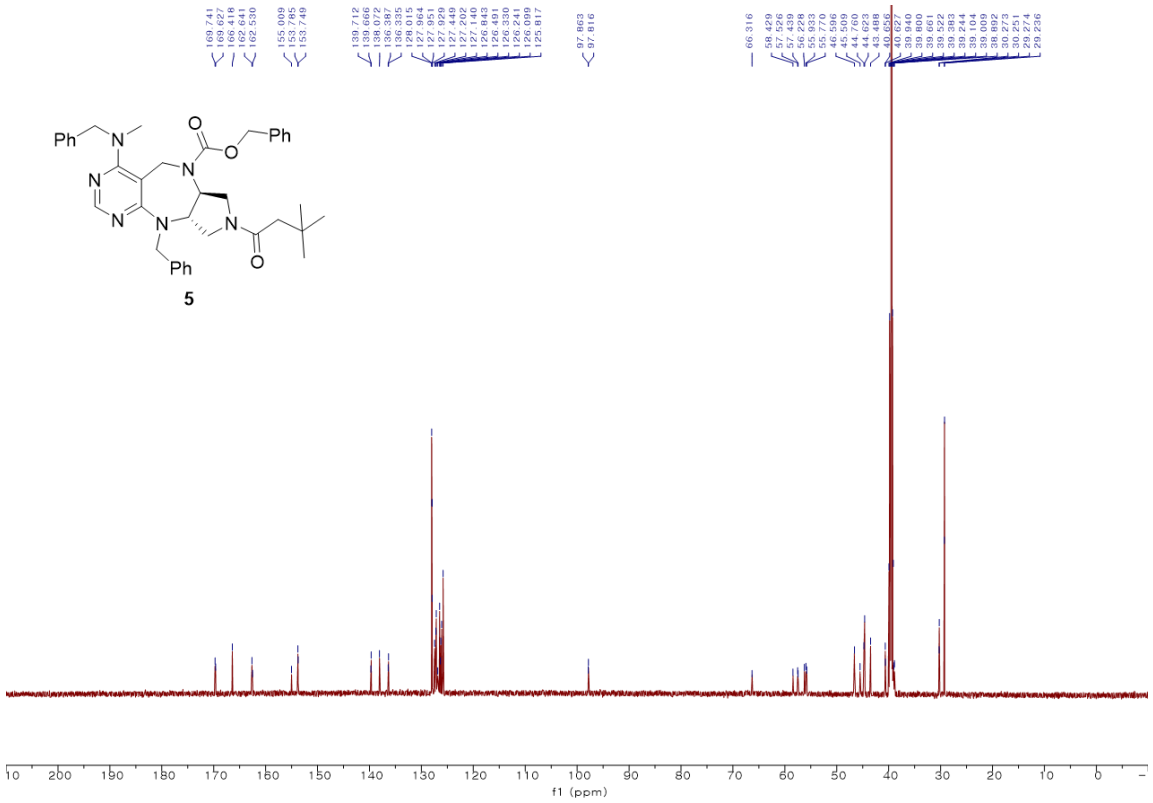
O
Ph
N
N
N
N
O
Ph
O
5
f1 (ppm)


Ph
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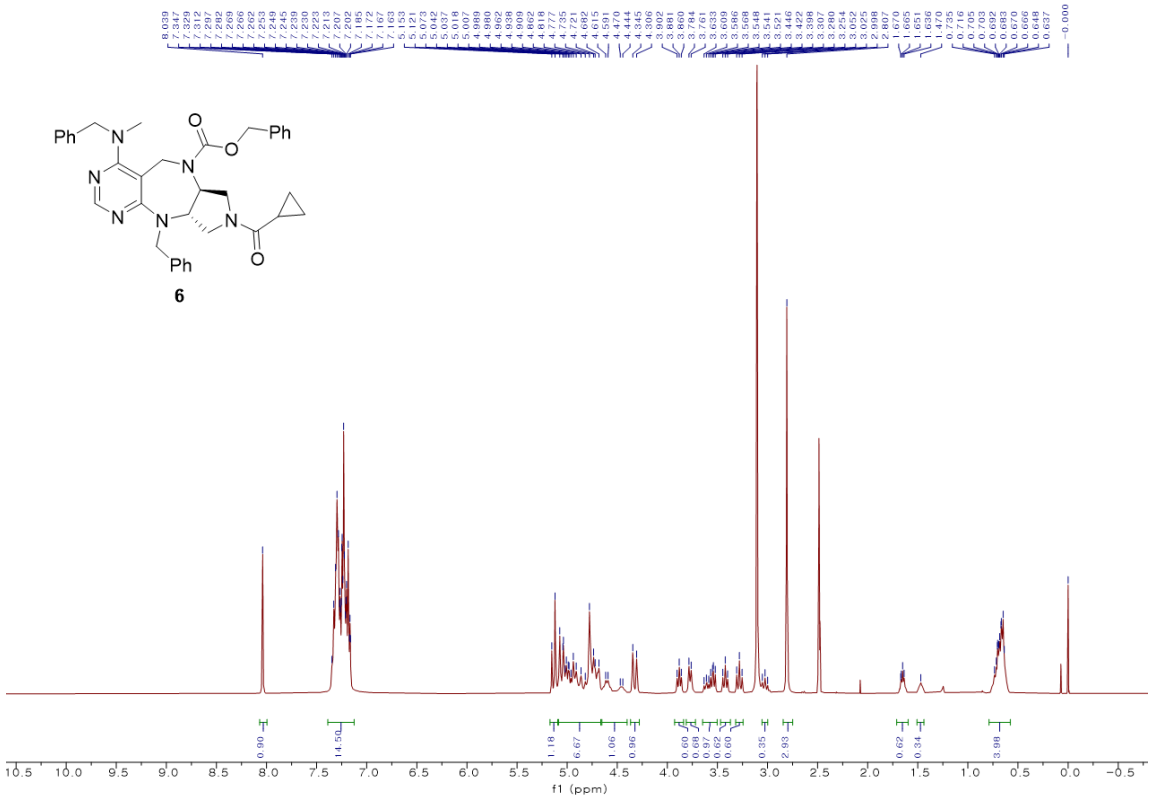
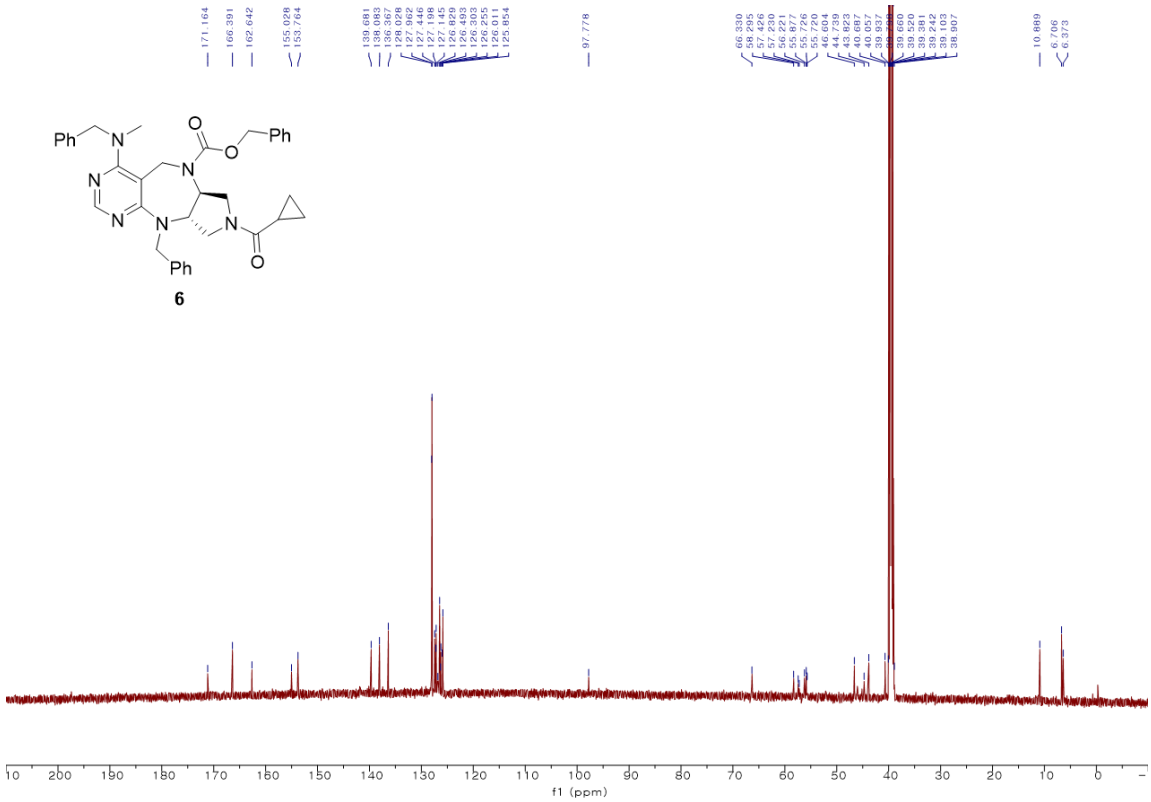
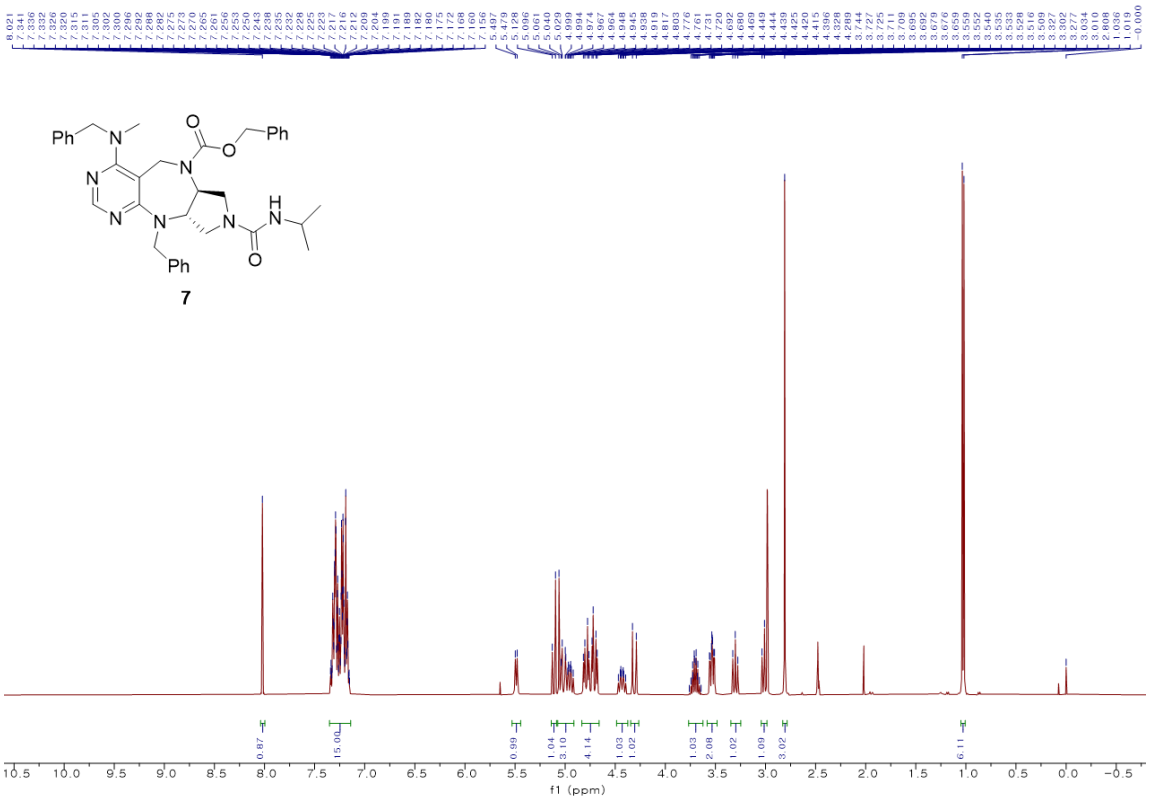
O
Ph
N
N
N
N
O
Ph
O
5
f1 (ppm)

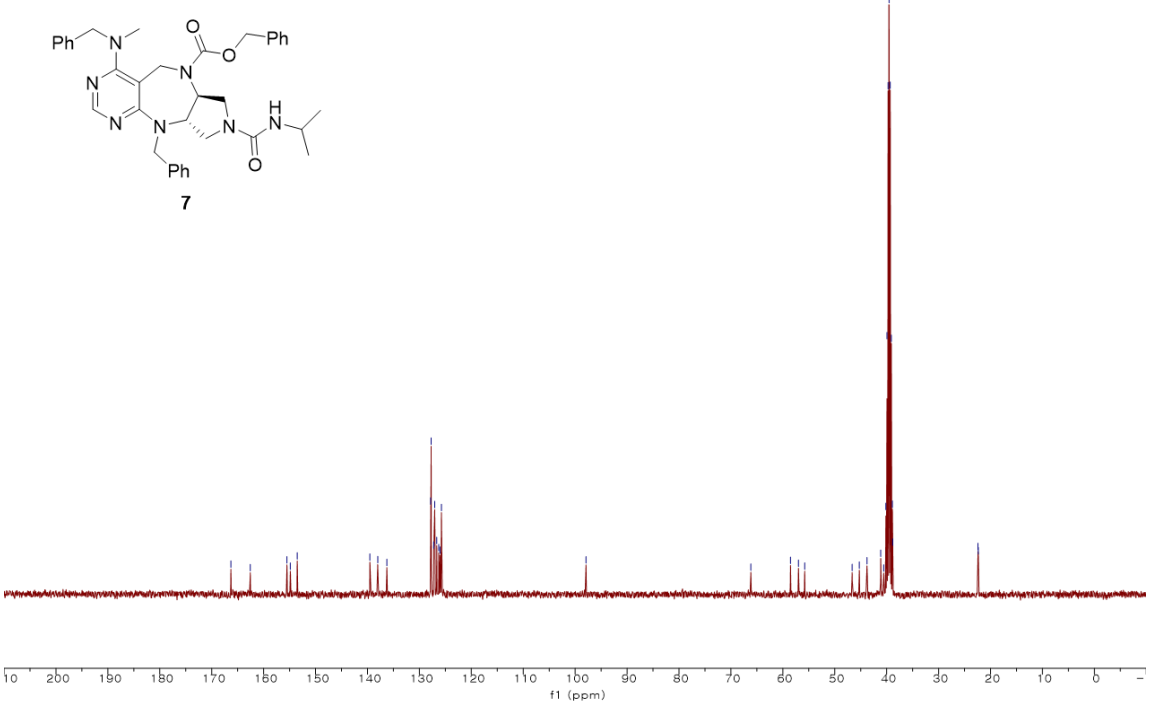

Ph
N
O
Ph
N
N
N
N
O
Ph
6

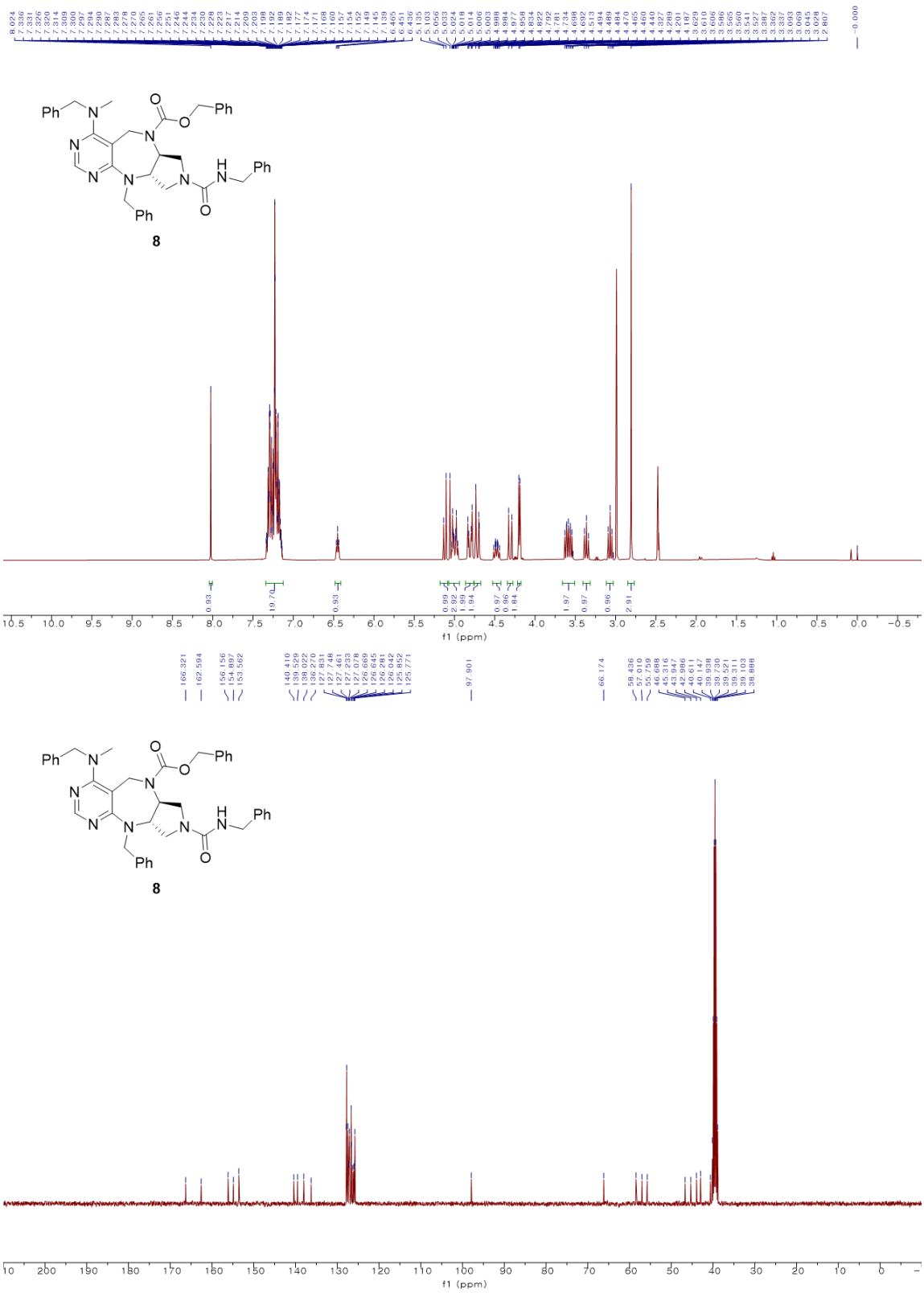

f1 (ppm)

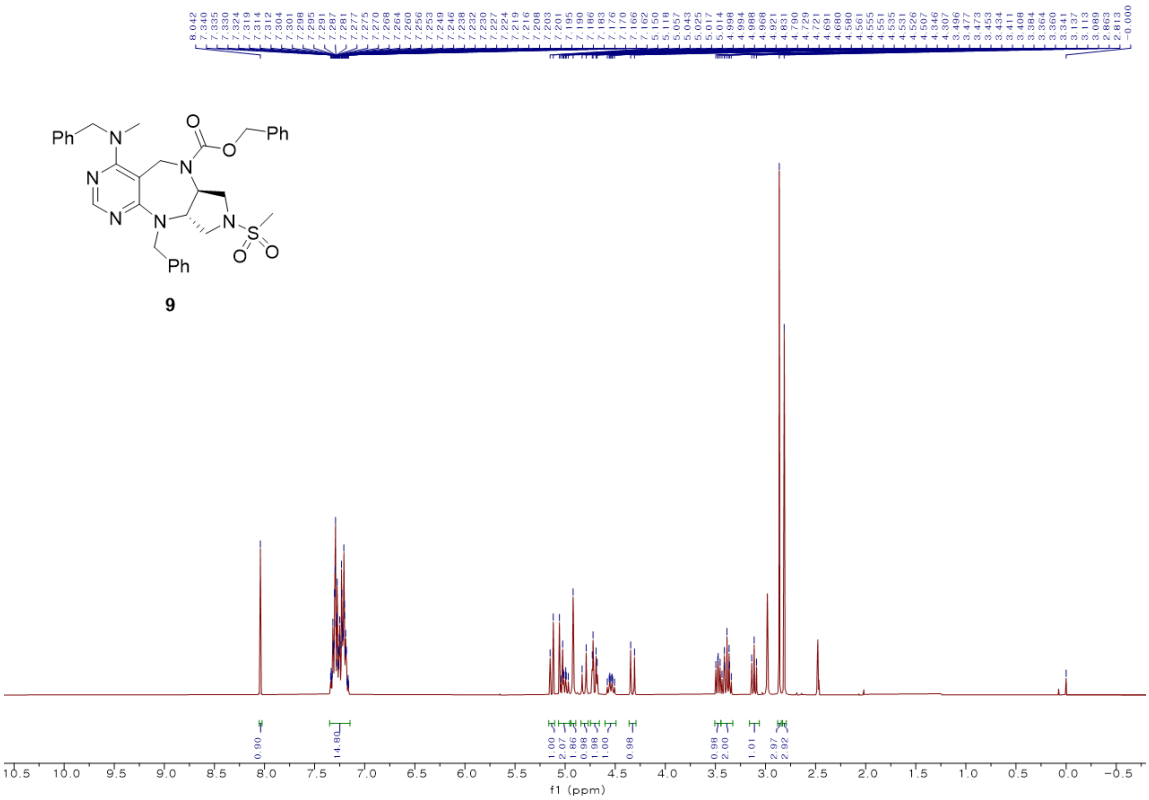
f1 (ppm)

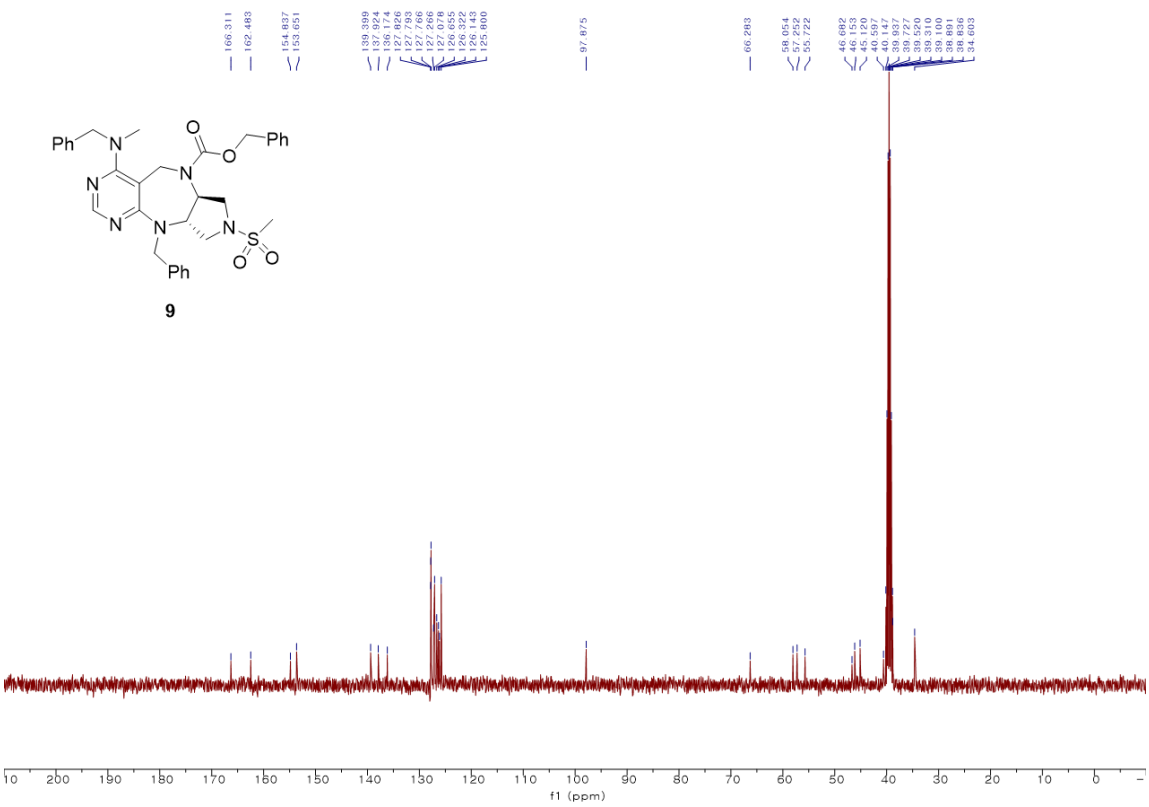
Ph
N
O
Ph
N
N
N
N
O
Ph
6

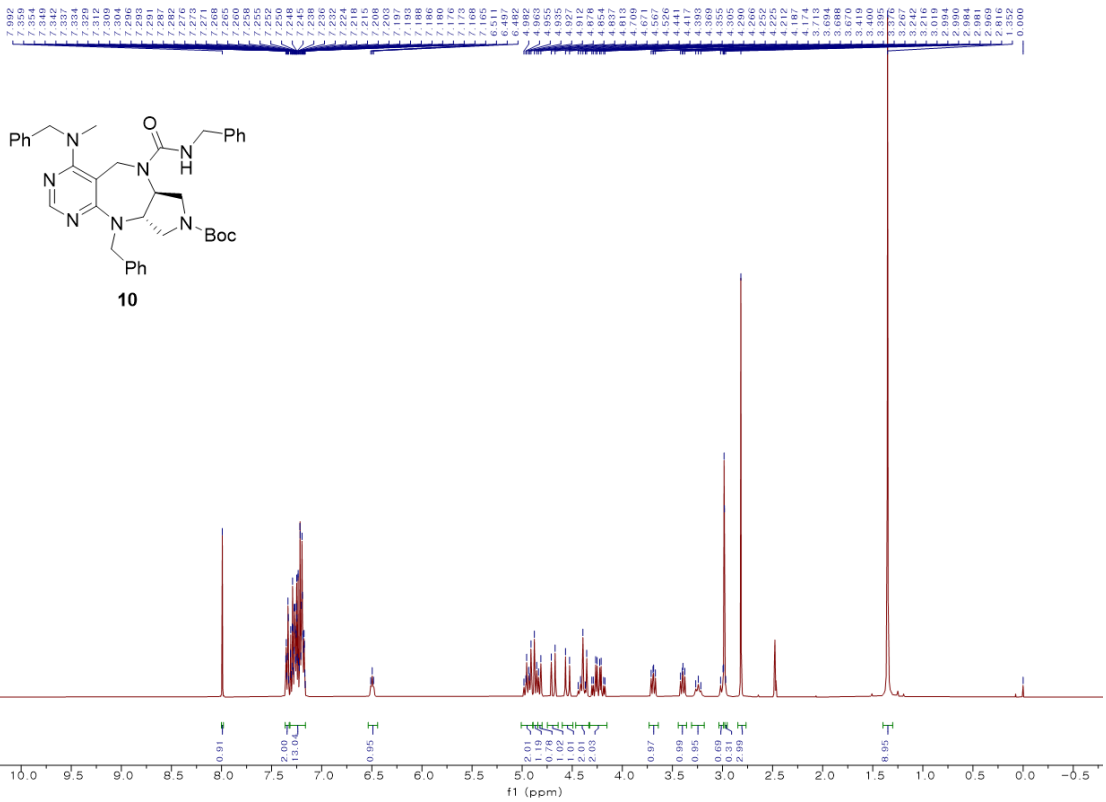

Ph
N
O
O
Ph
N
N
N
N
H
N
O
Ph
7
f1 (ppm)

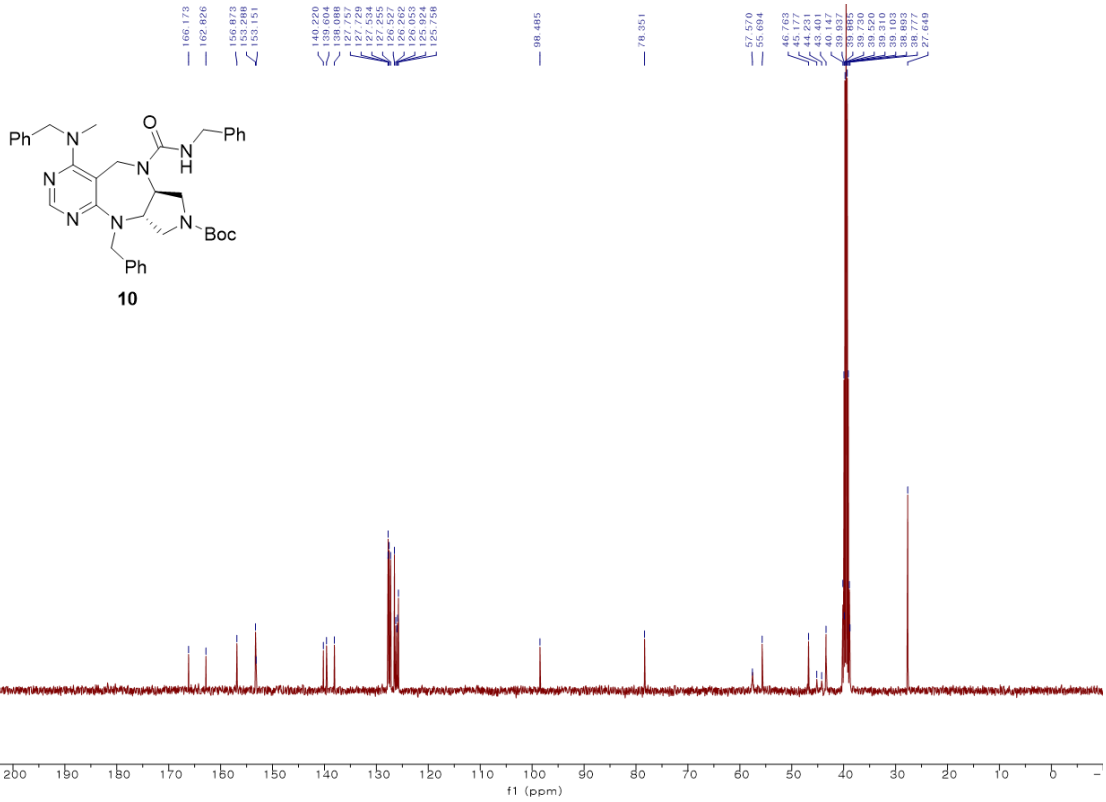

Ph
N
O
O
Ph
N
N
N
N
H
N
O
Ph
7
f1 (ppm)

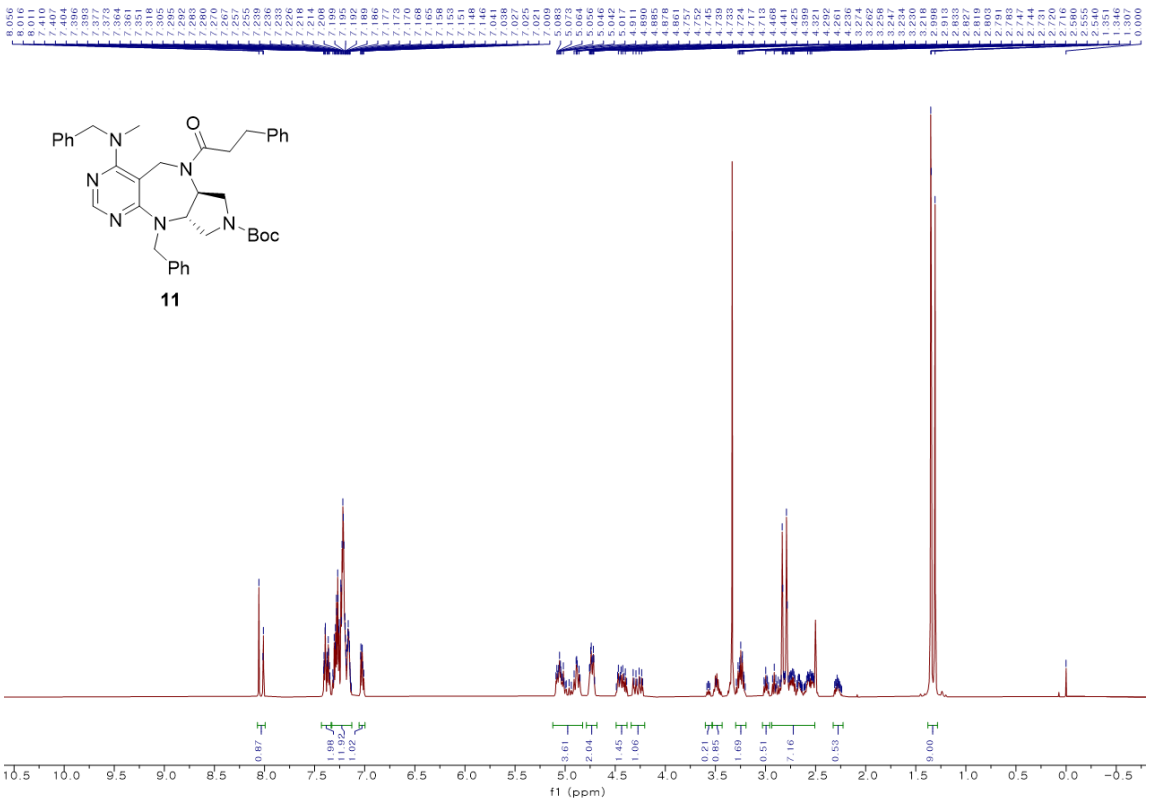

8
f1 (ppm)
8
f1 (ppm)

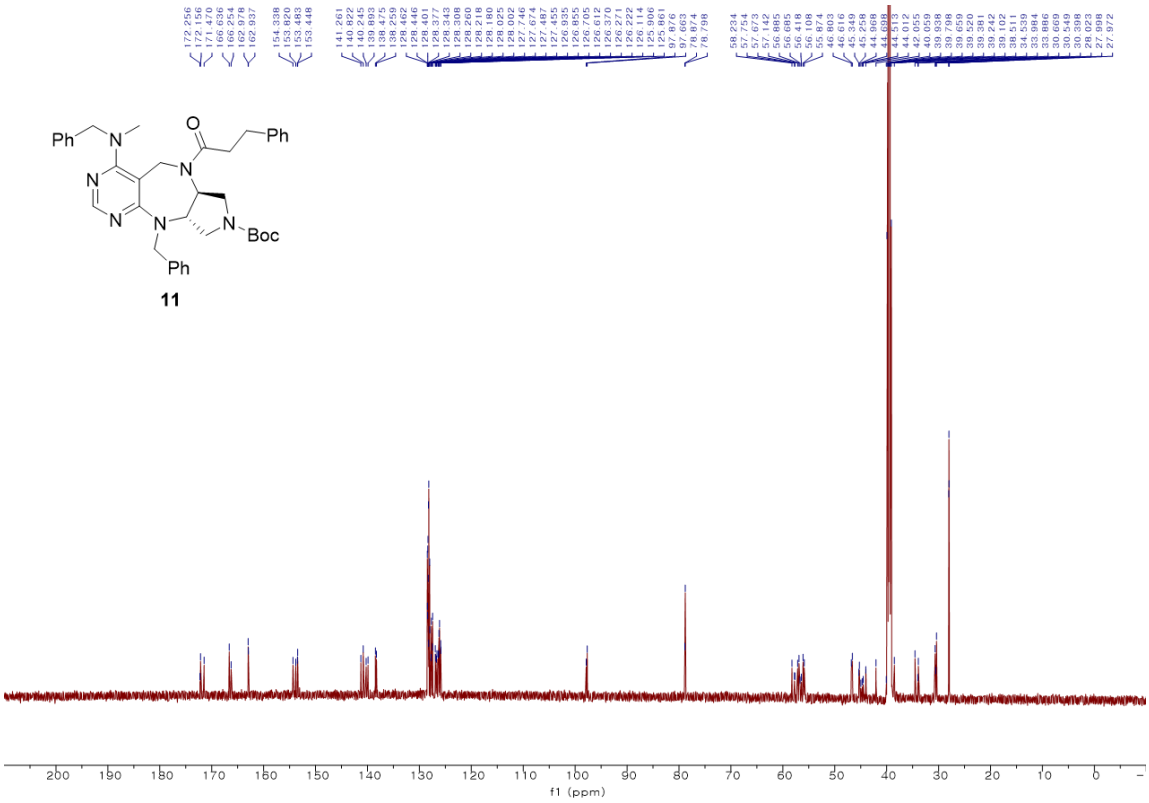

Ph
N
O
O
Ph
N
N
N
N
N
S
O
O
Ph
9
f1 (ppm)

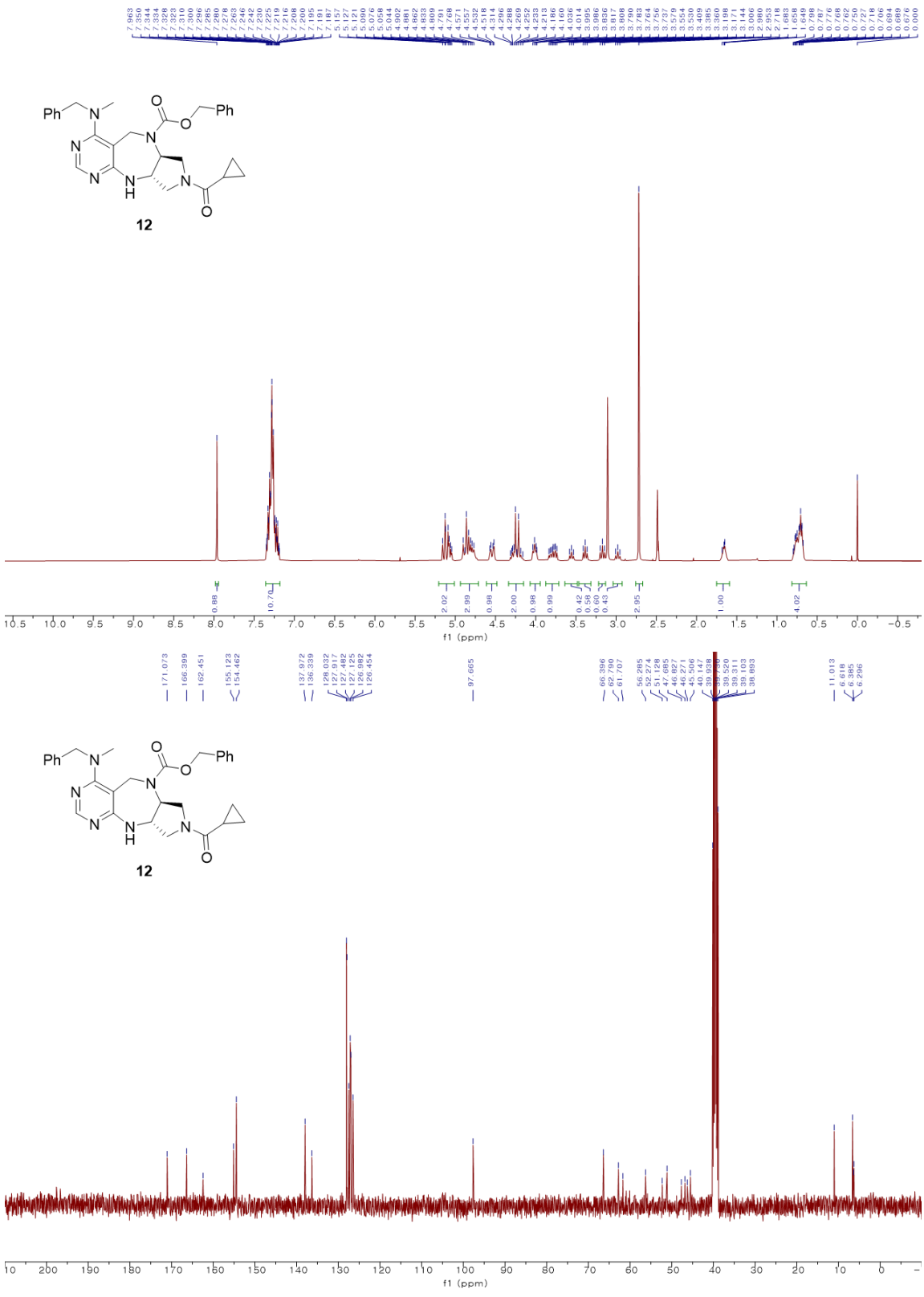

Ph
N
O
O
Ph
N
N
N
N
N
S
O
O
Ph
9
f1 (ppm)

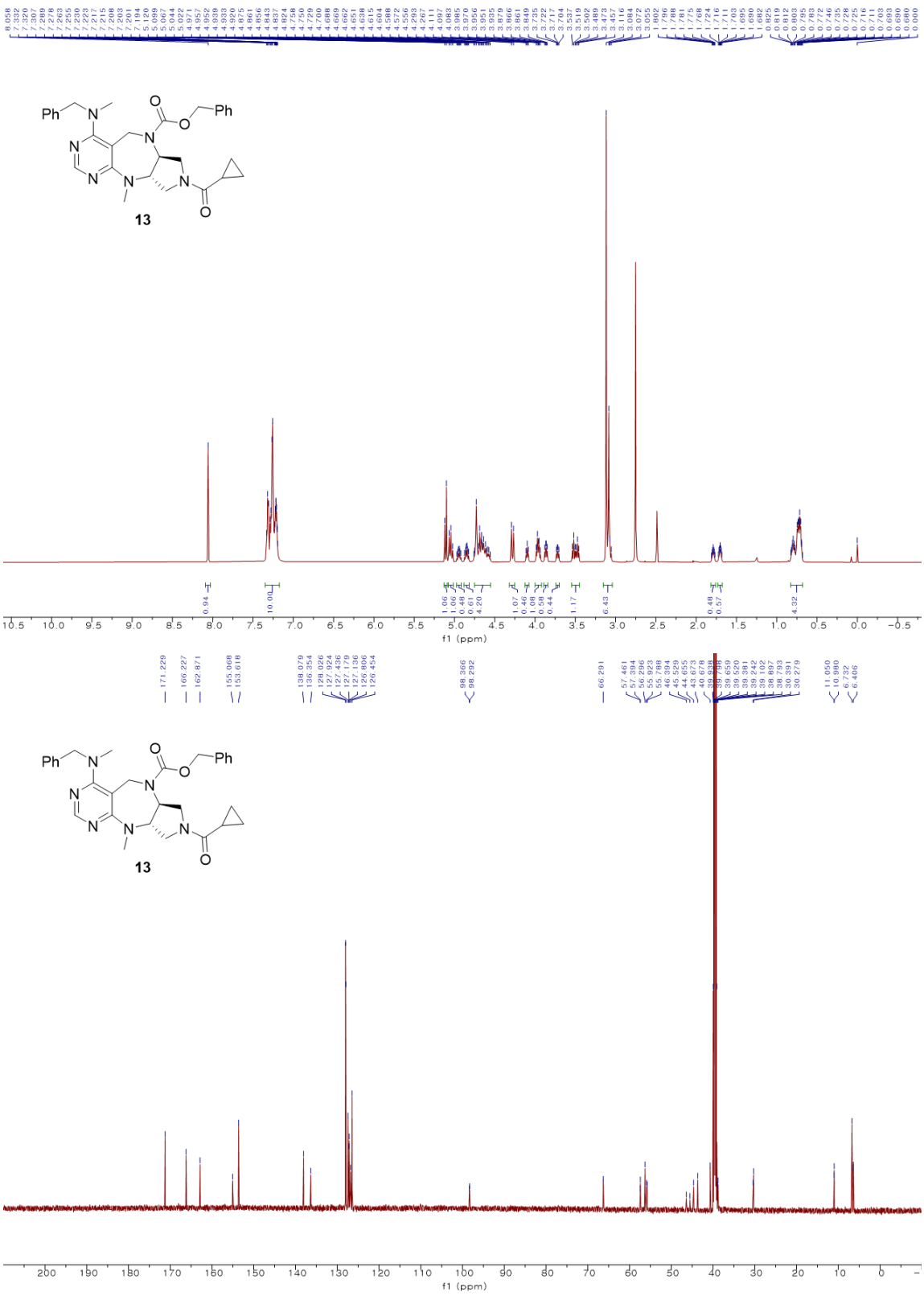

Ph
N
O
N
H
Ph
N
N
N
N
N
Boc
Ph
10
f1 (ppm)

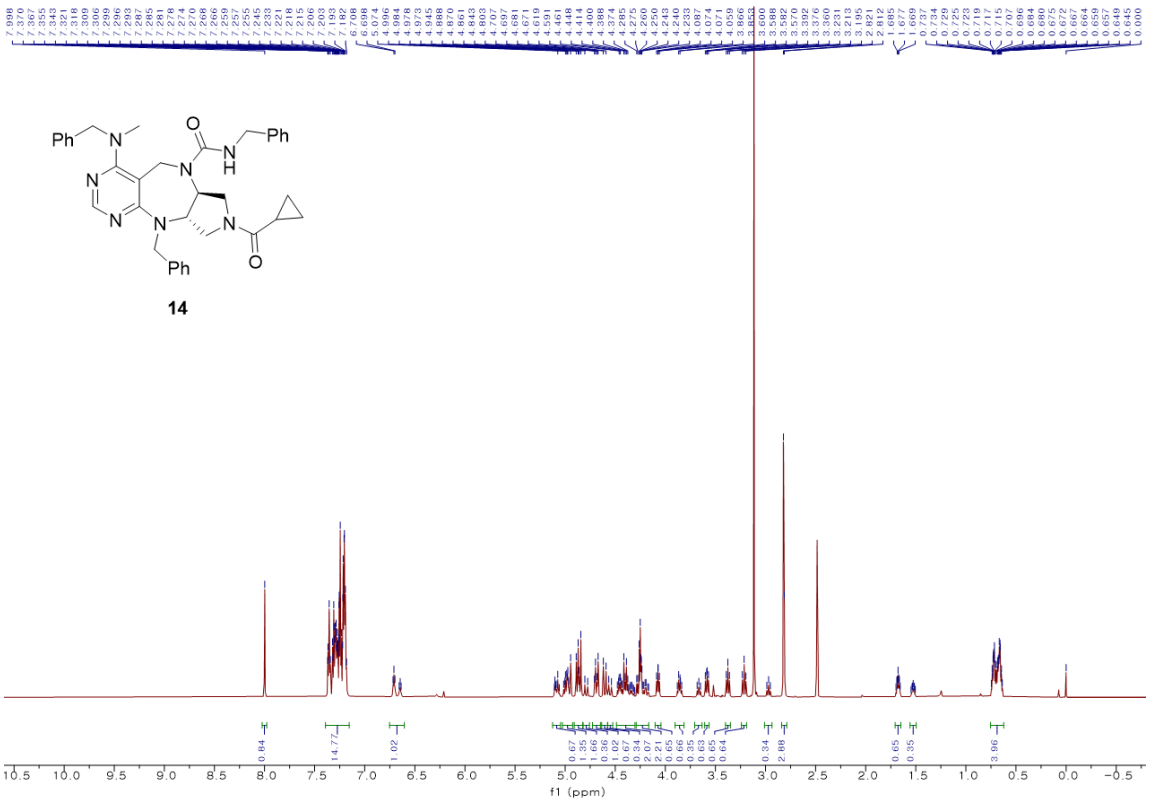

Ph
N
O
N
H
Ph
N
N
N
N
N
Boc
Ph
10
f1 (ppm)

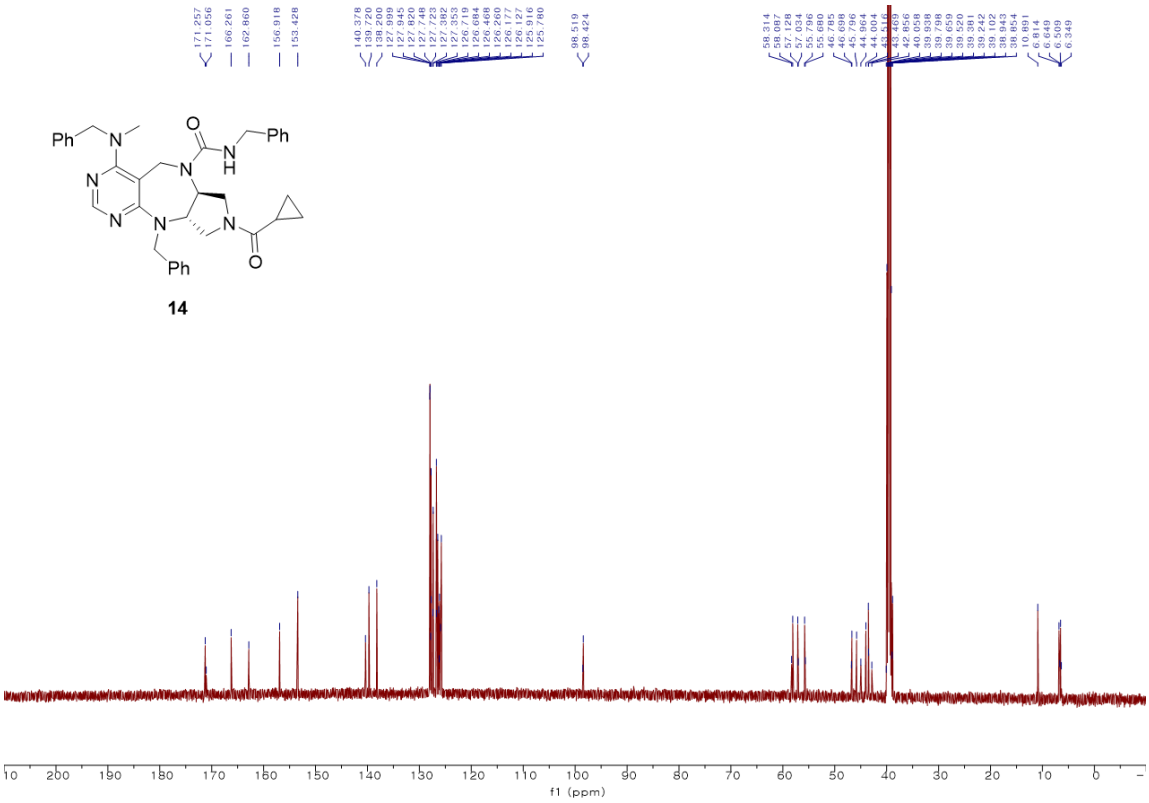

Ph
N
O
Ph
N
N
N
N
N
Boc
Ph
11
f1 (ppm)

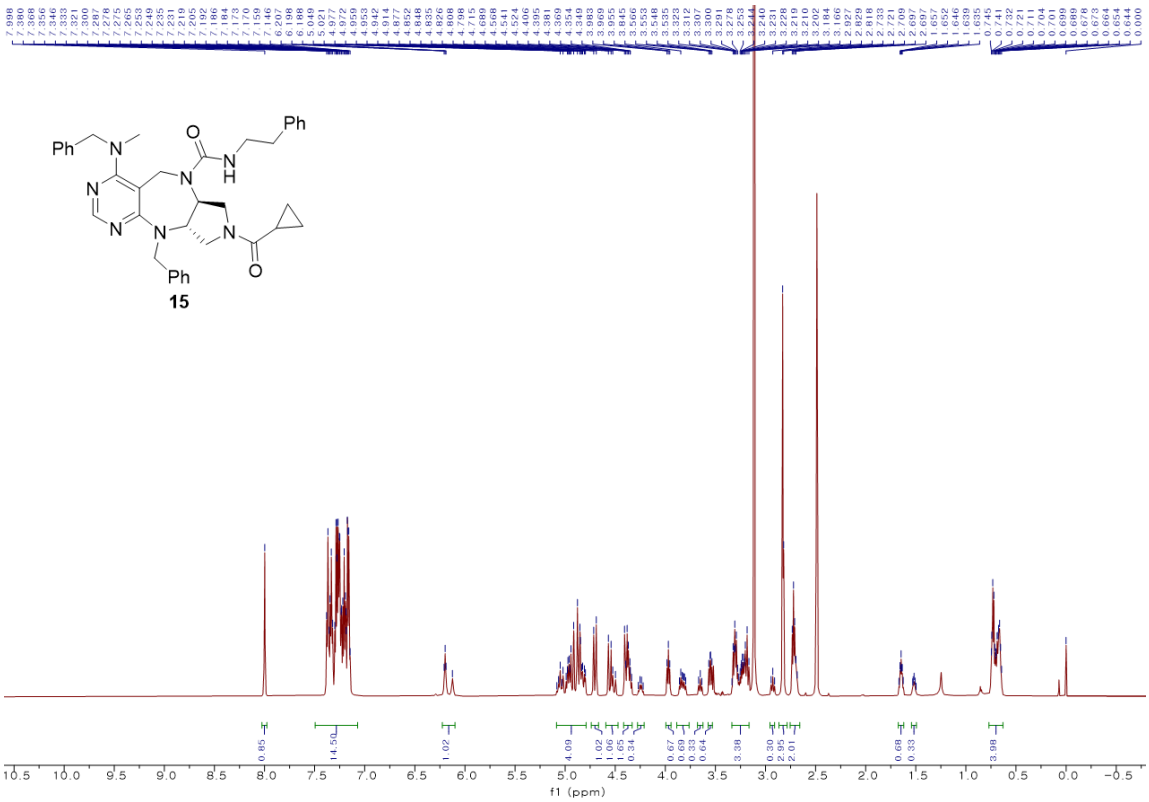

Ph
N
O
Ph
N
N
N
N
N
Boc
Ph
11
f1 (ppm)


12
f1 (ppm)
12
f1 (ppm)


13
f1 (ppm)
13
f1 (ppm)


Ph
N
O
N
H
Ph
N
N
N
N
N
O
Ph
14
f1 (ppm)


Ph
N
O
N
H
Ph
N
N
N
N
N
O
Ph
14
f1 (ppm)


Ph
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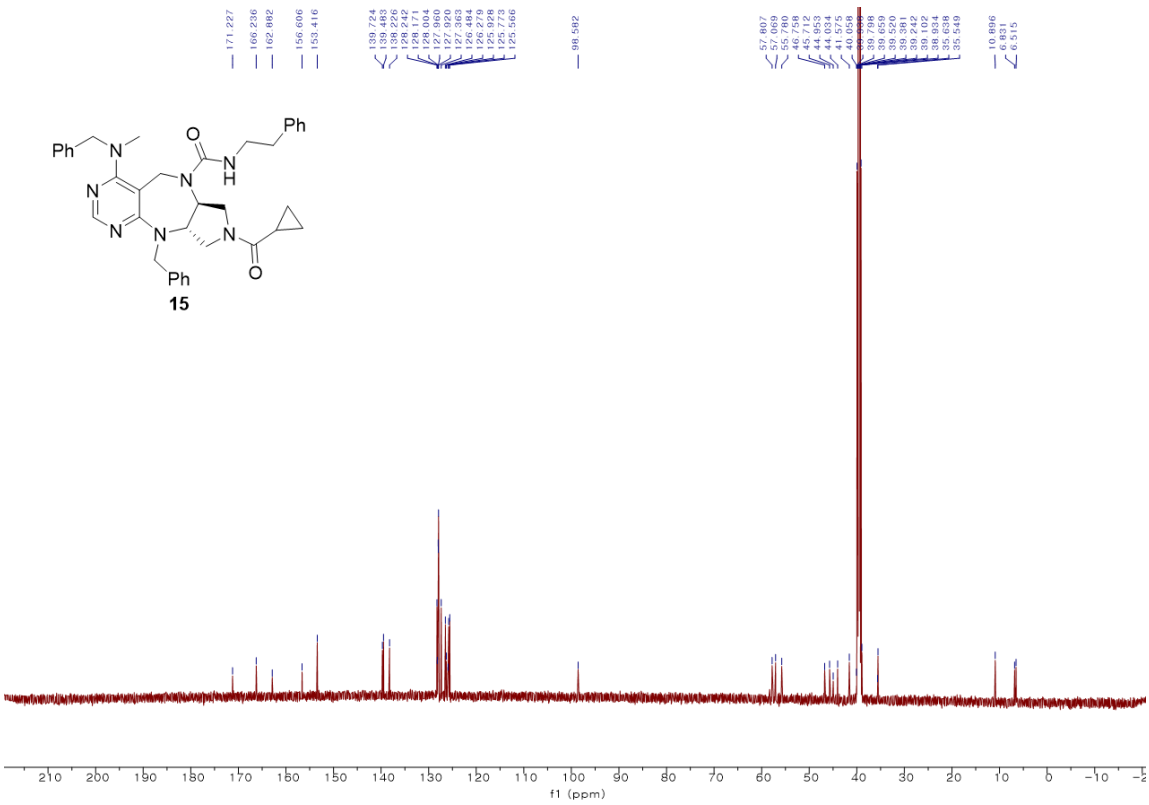
O
N
H
Ph
N
N
N
N
N
O
Ph
15
f1 (ppm)


Ph
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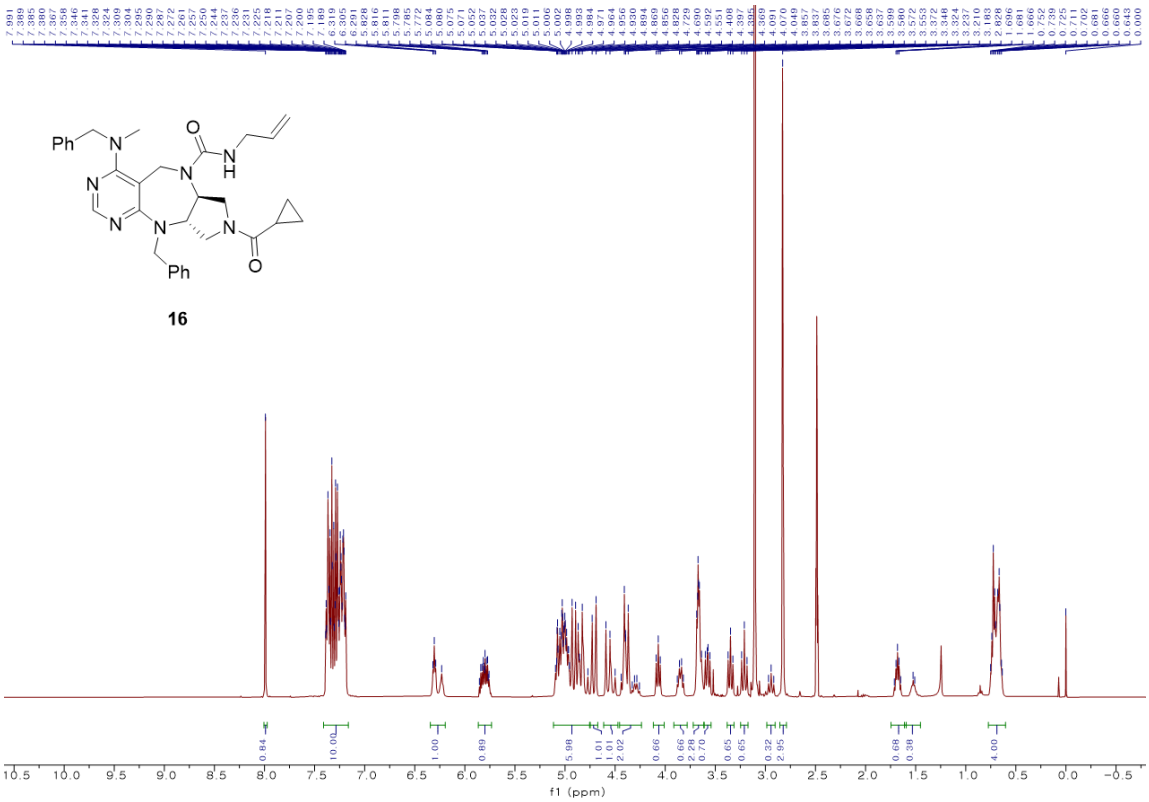
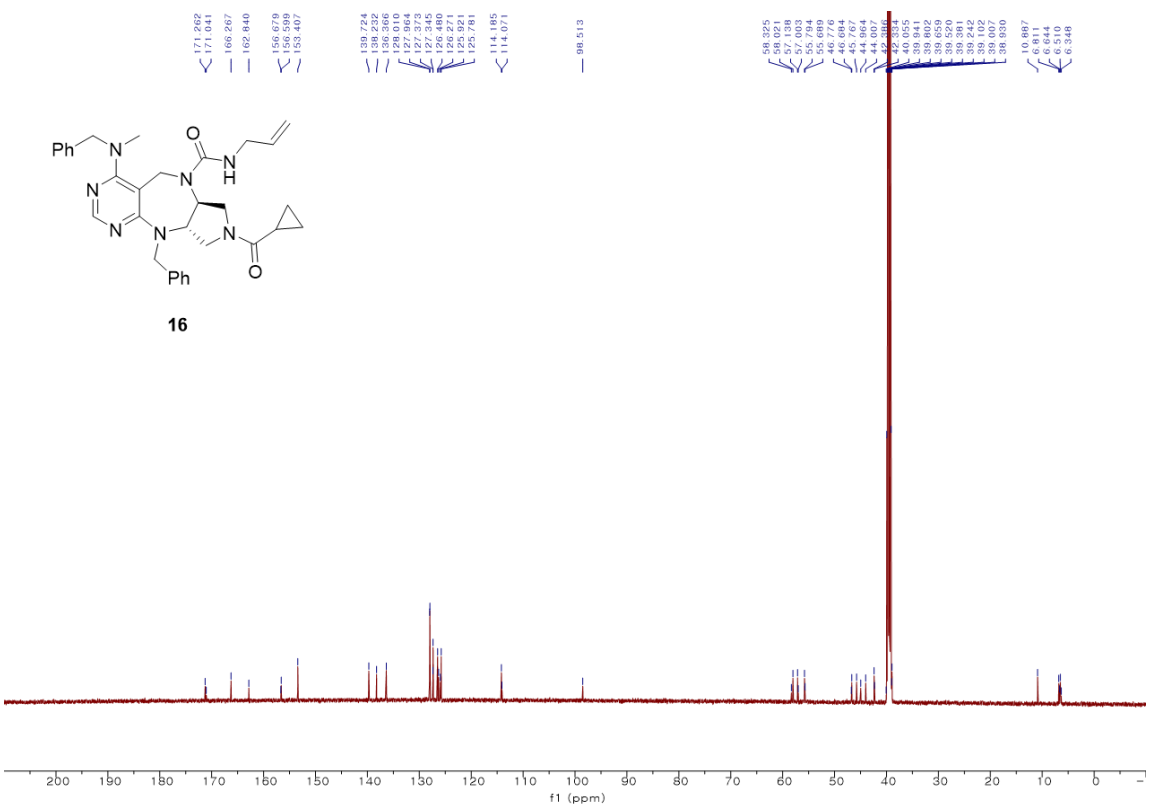
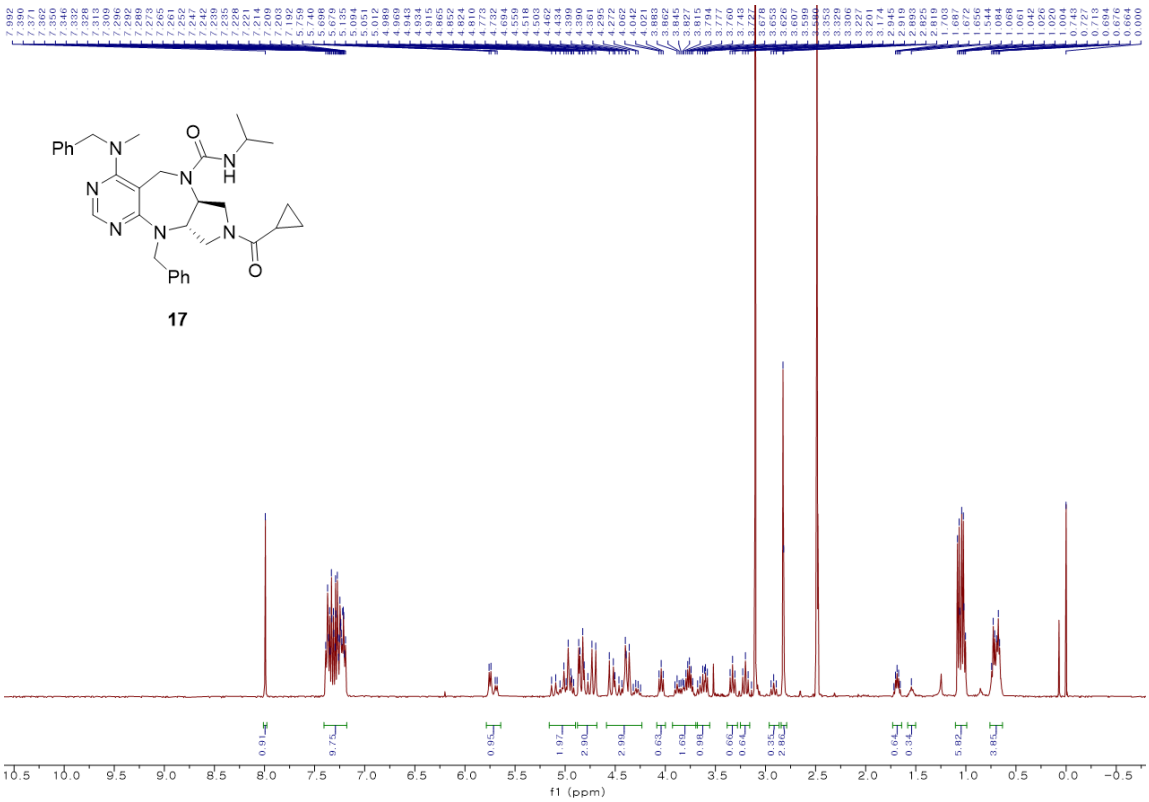
O
N
H
Ph
N
N
N
N
N
O
Ph
15
f1 (ppm)



16



16


Ph
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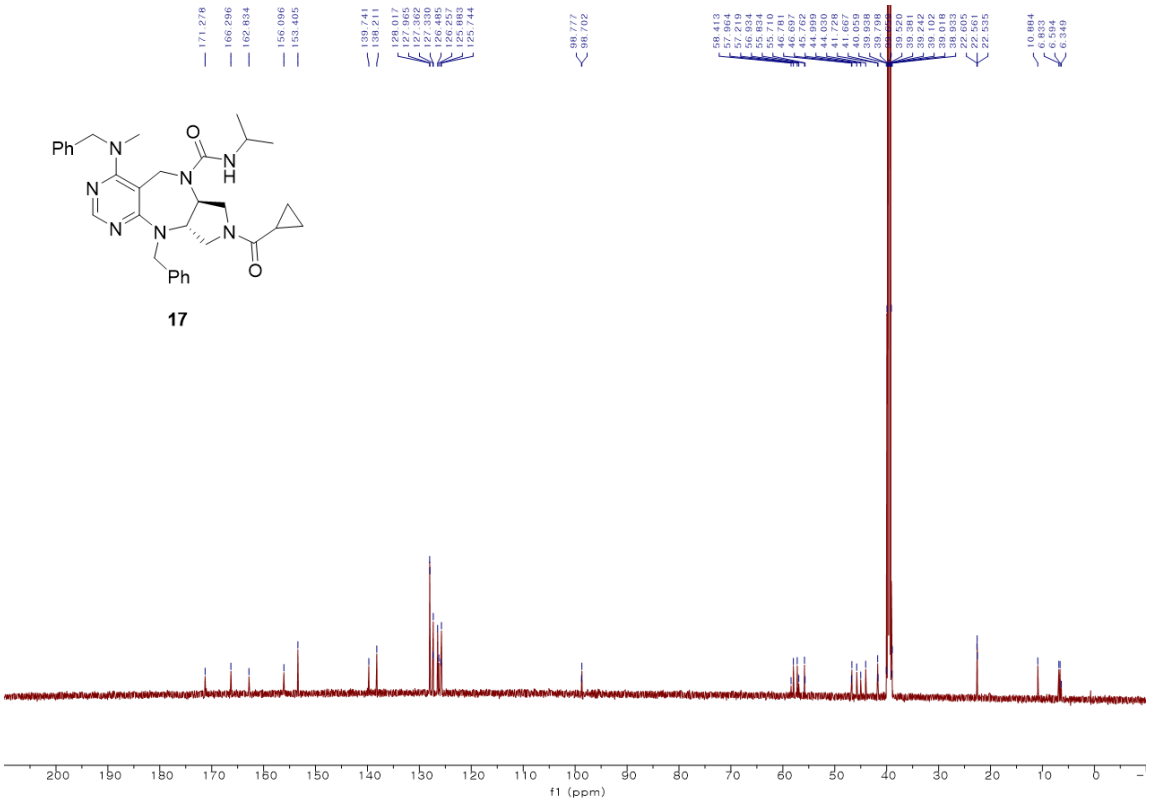
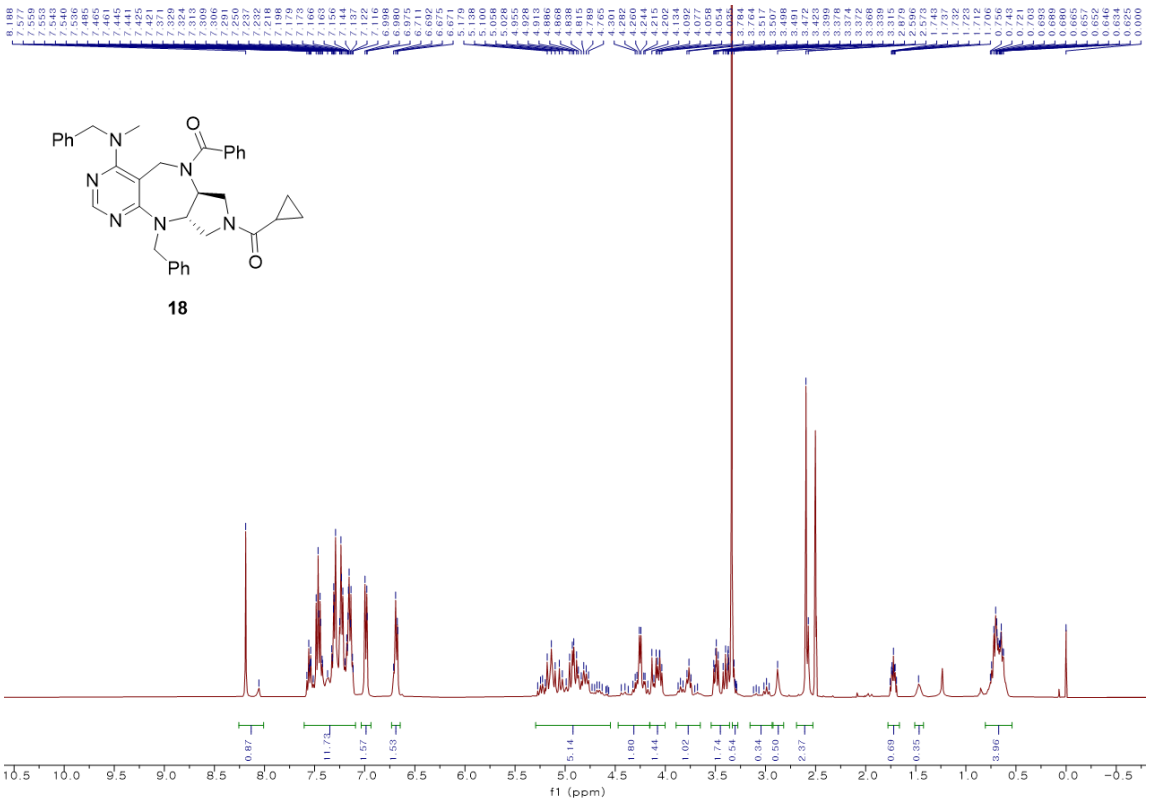
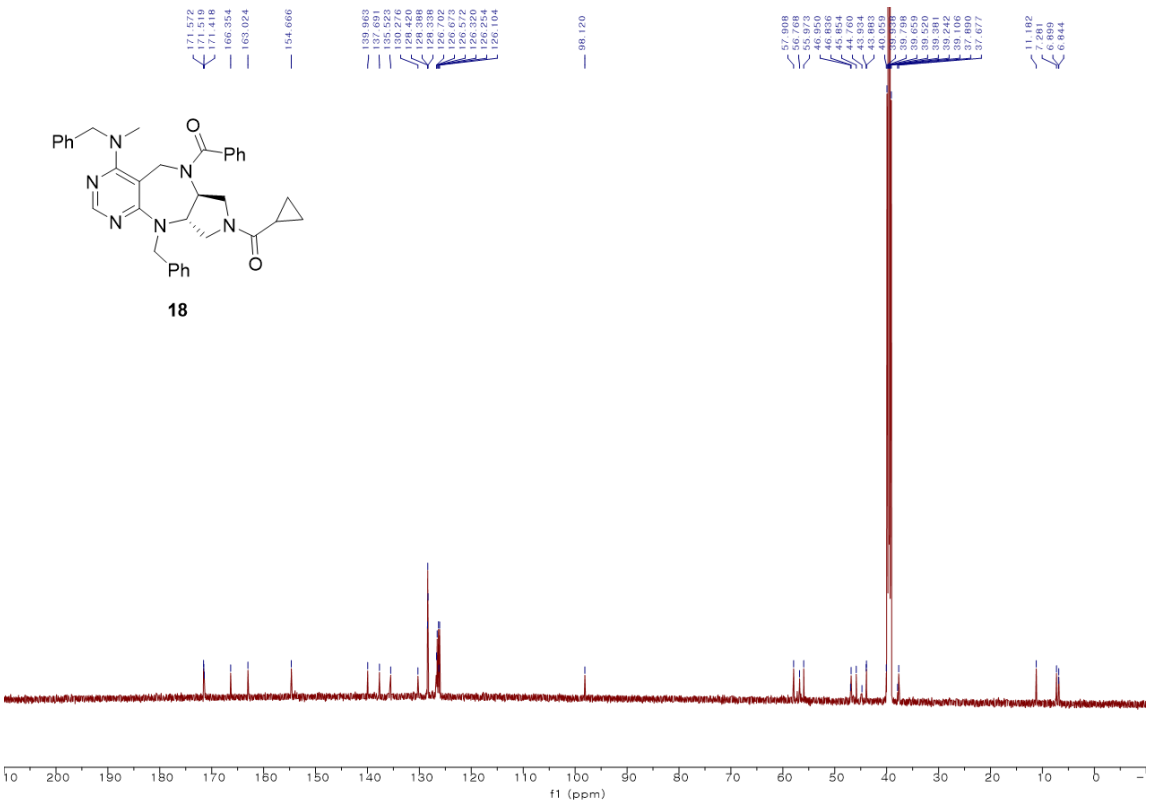
O
N
H
N
N
N
N
N
O
Ph
17
f1 (ppm)

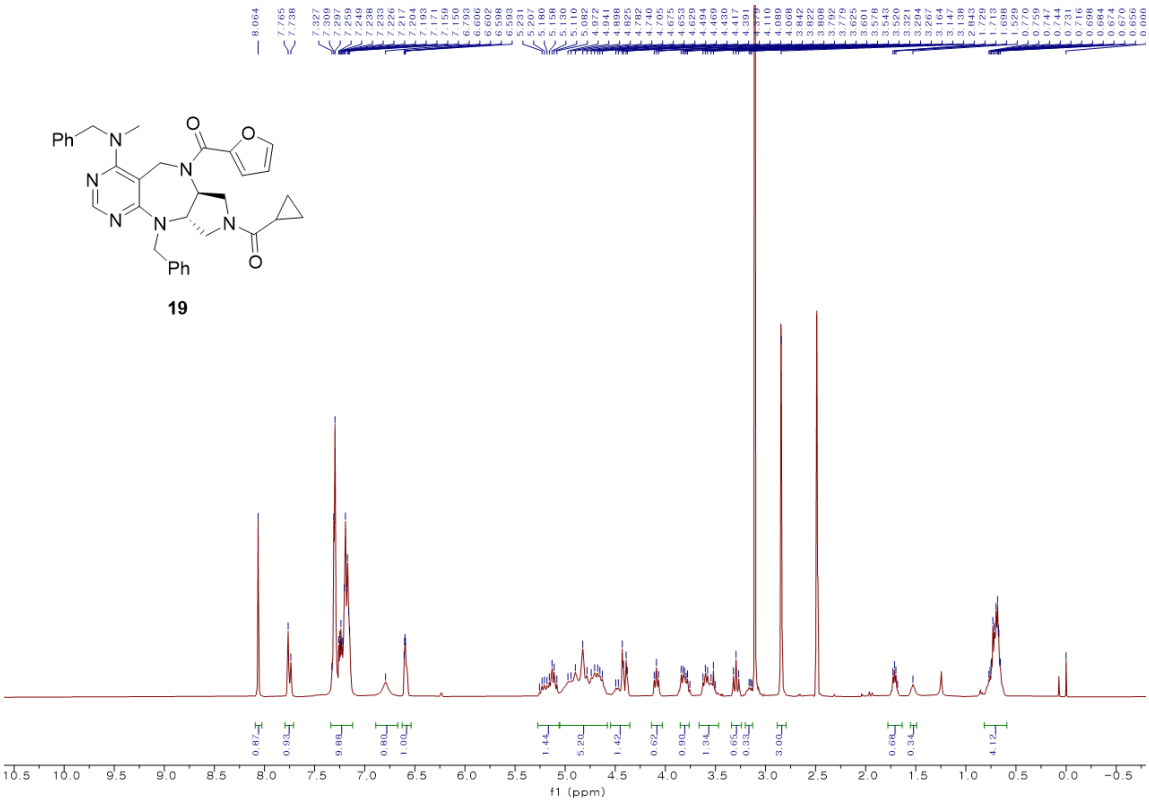

Ph
N
O
N
H
N
N
N
N
N
O
Ph
17
f1 (ppm)



18



18


Ph
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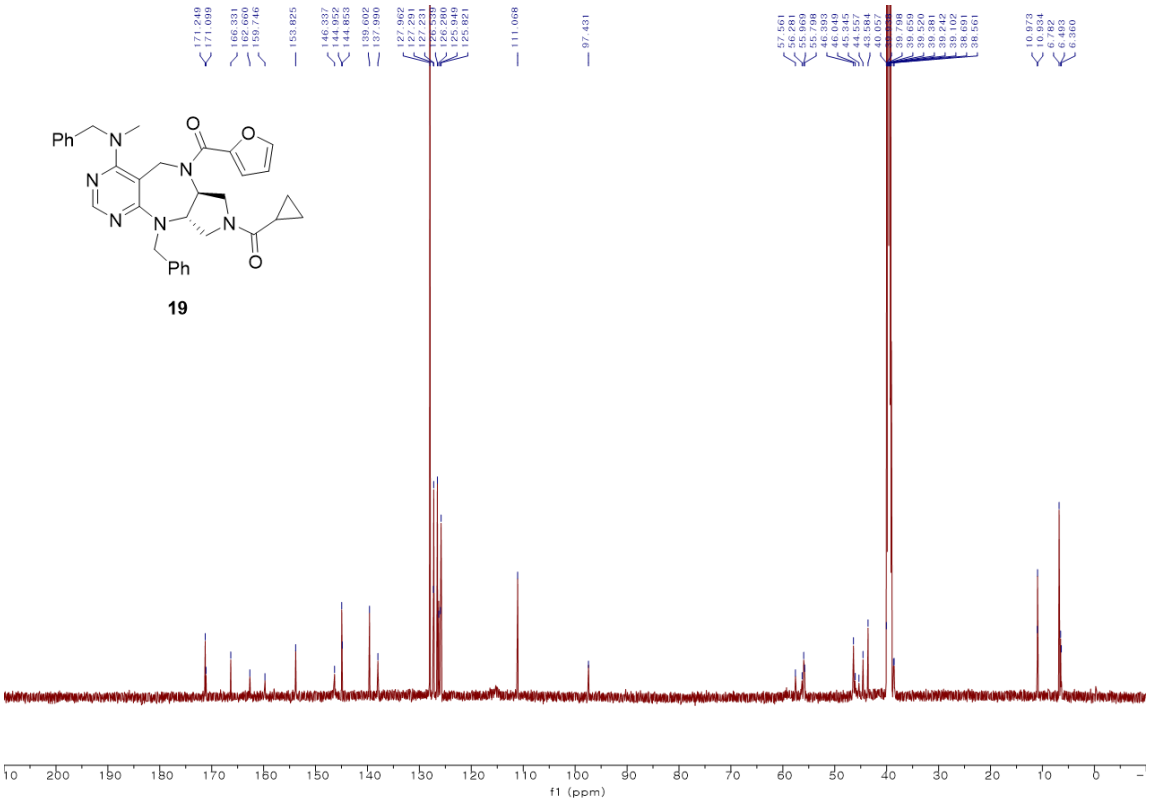
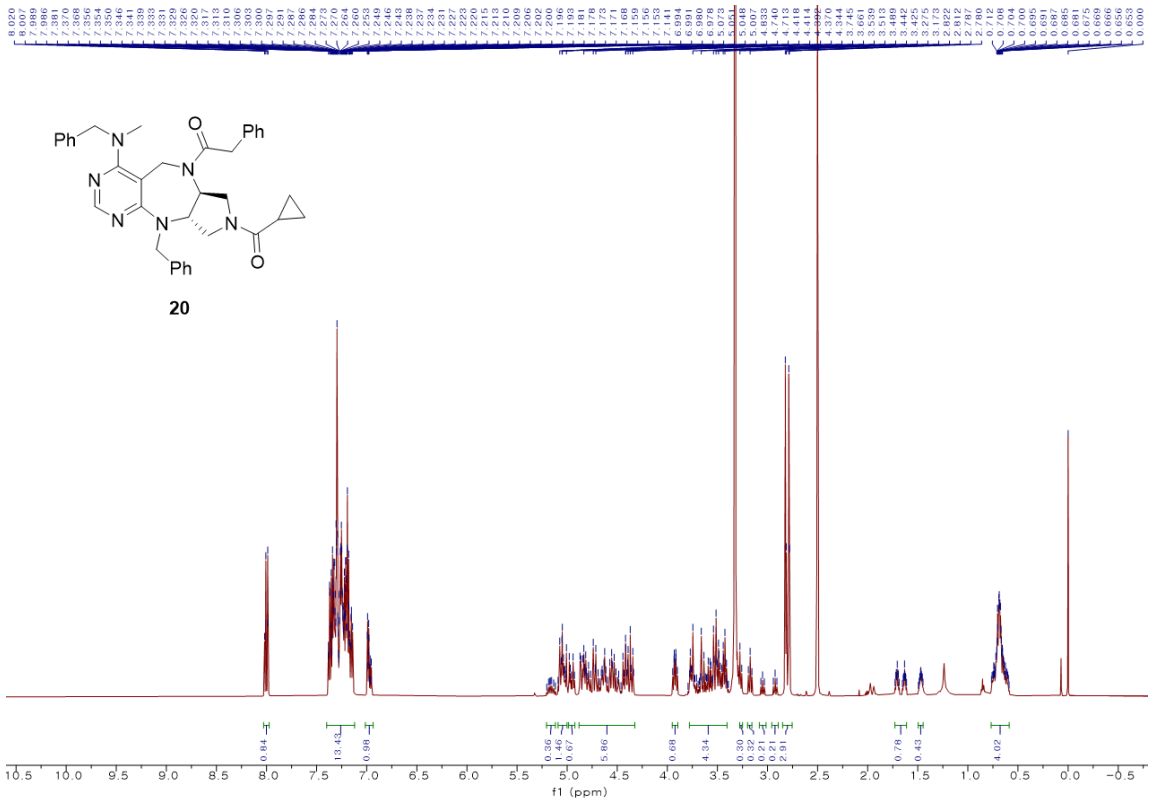
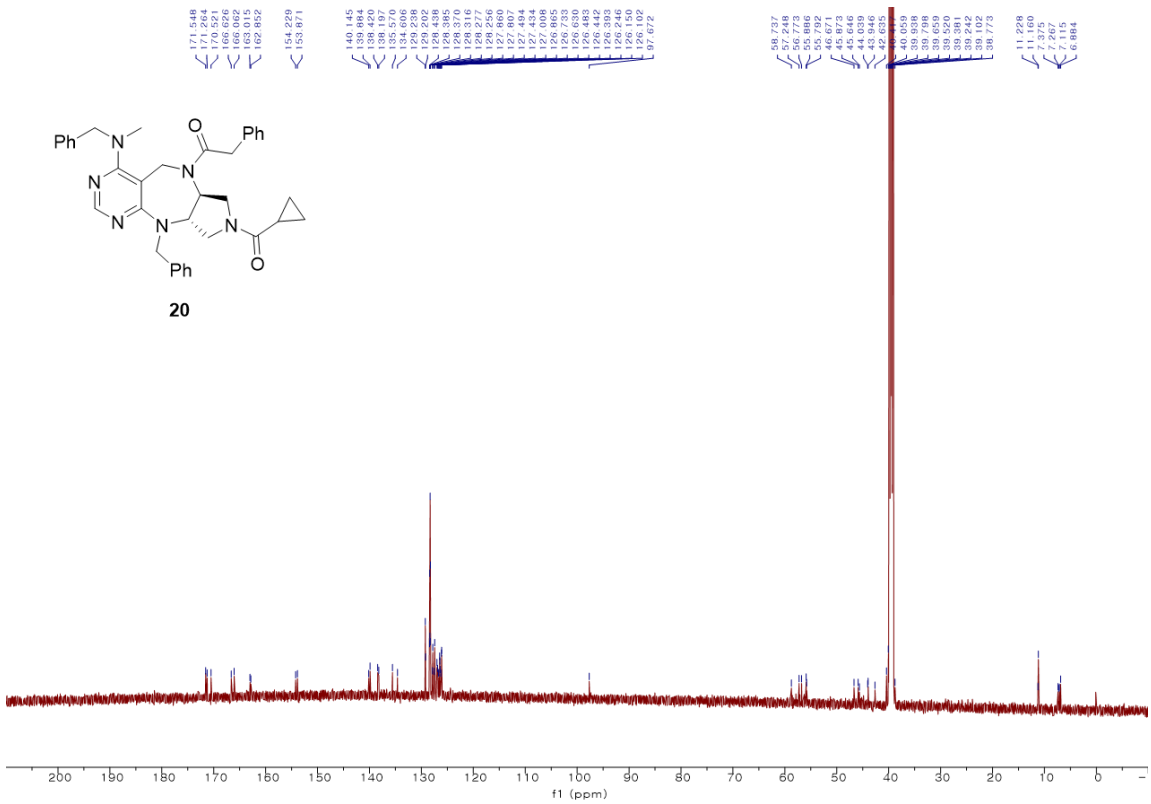
N
N
N
O
O
N
O
N
Ph
19
f1 (ppm)

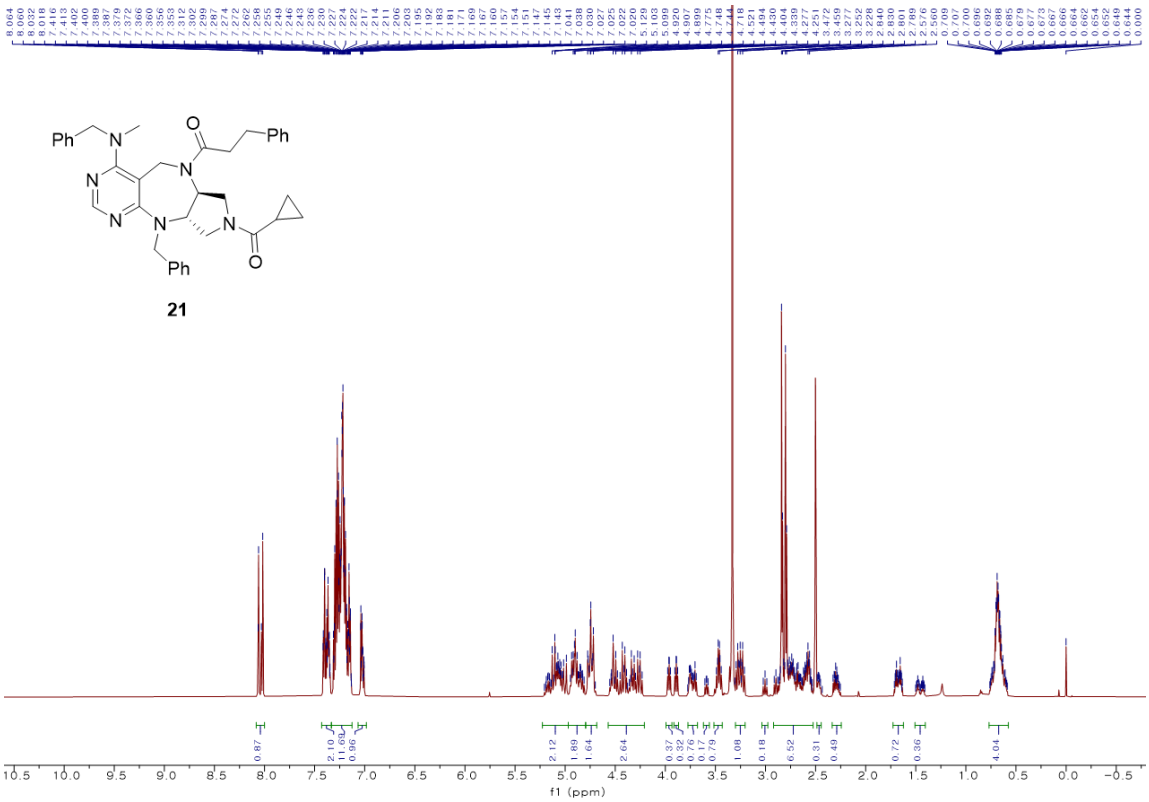

Ph
N
N
N
N
O
O
N
O
N
Ph
19
f1 (ppm)



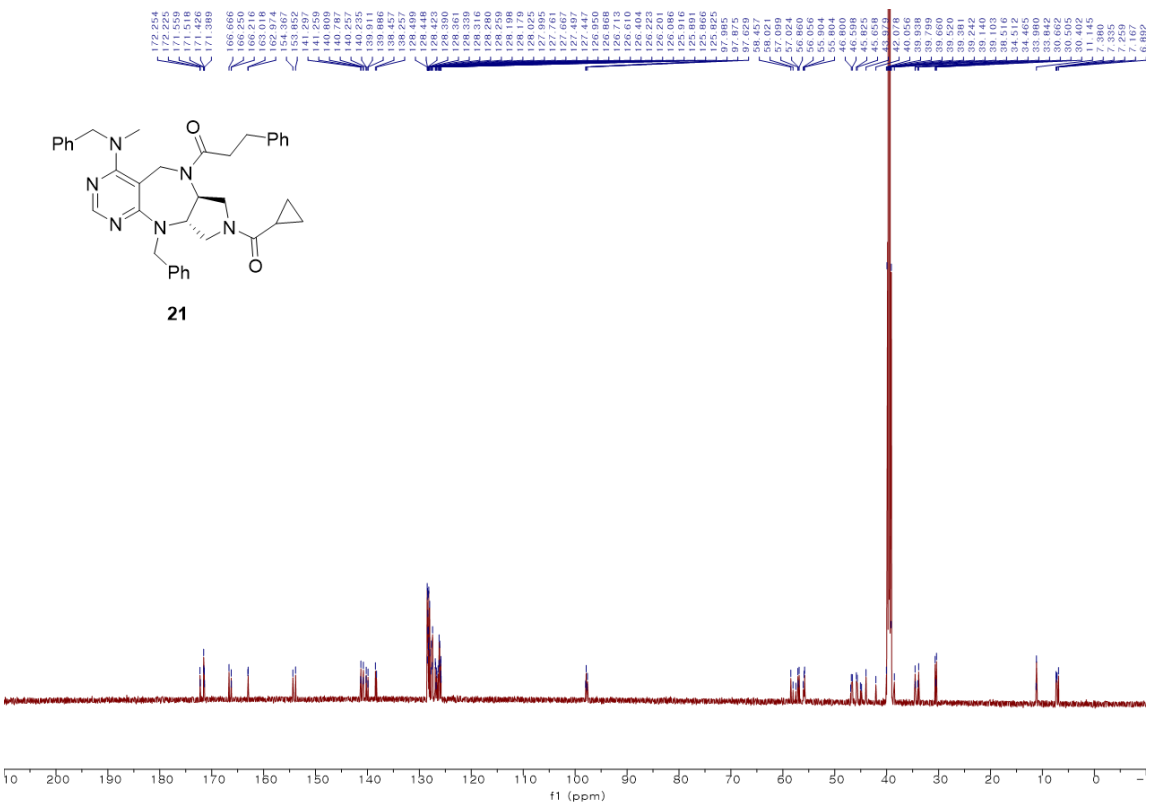
20



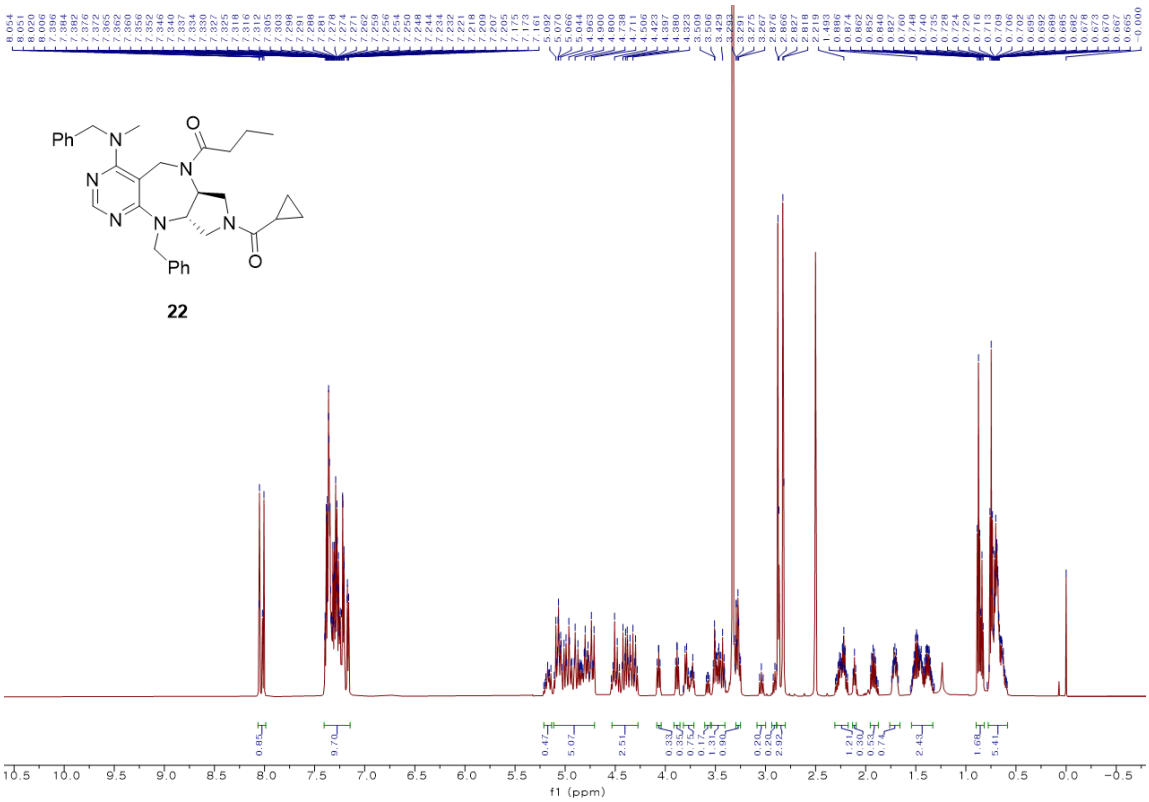
20



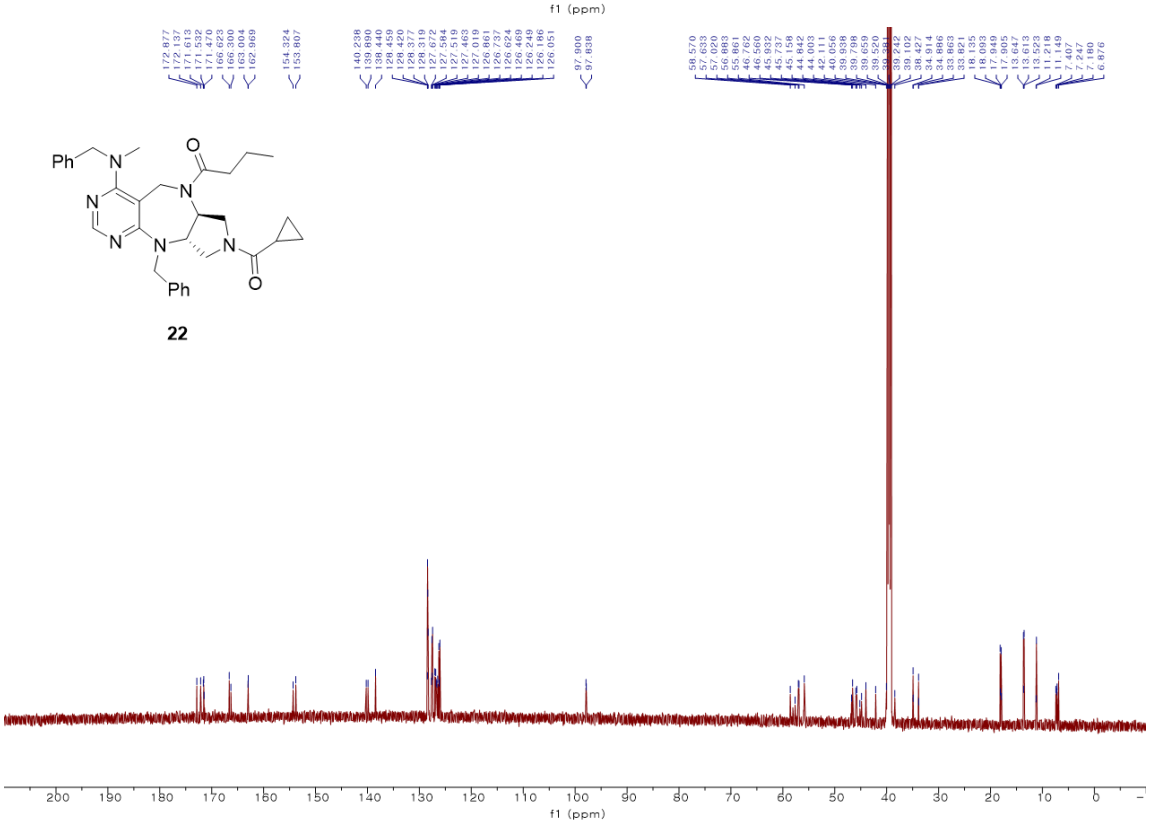
21



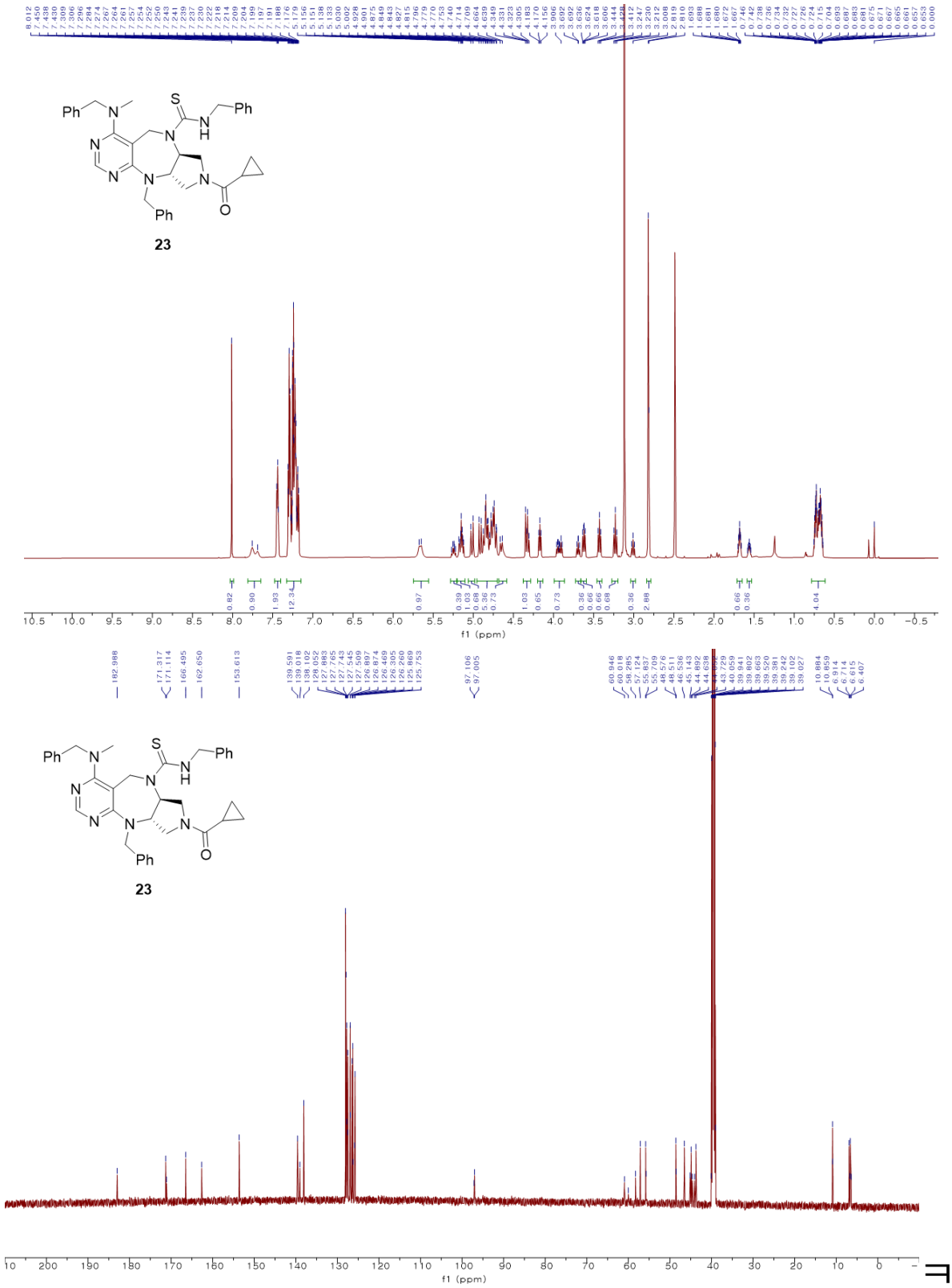
21

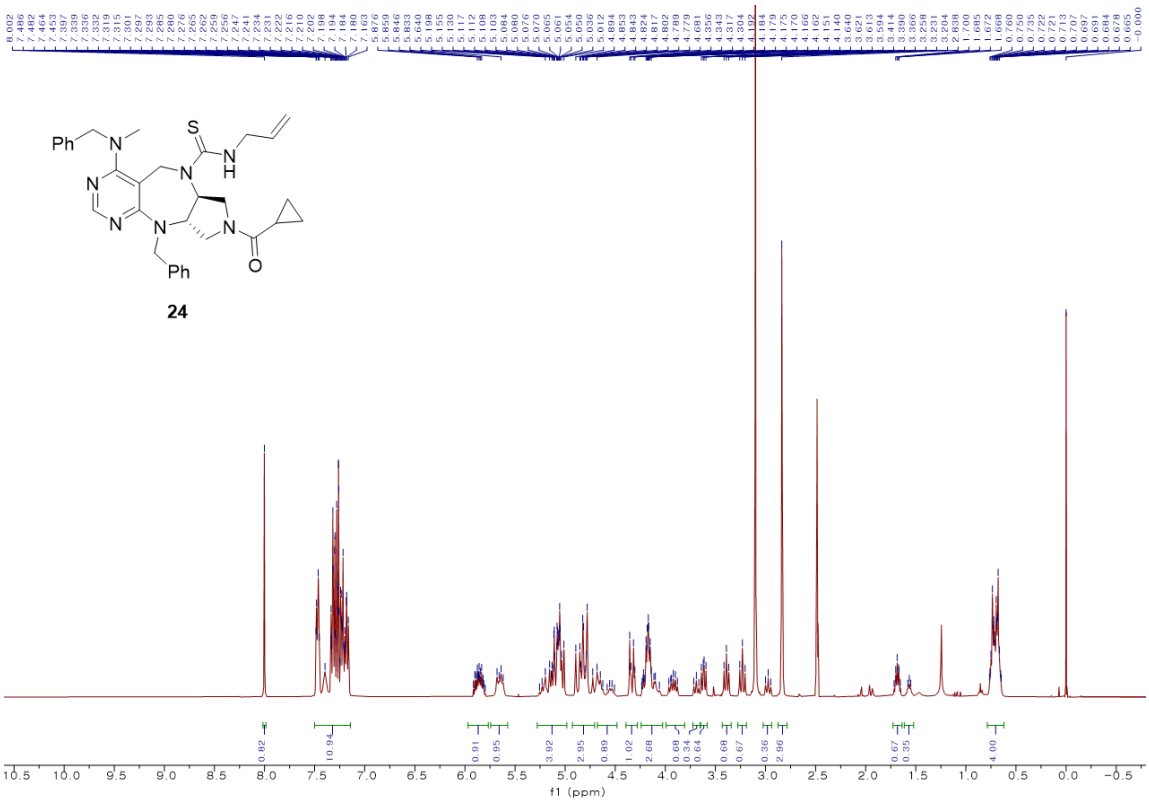


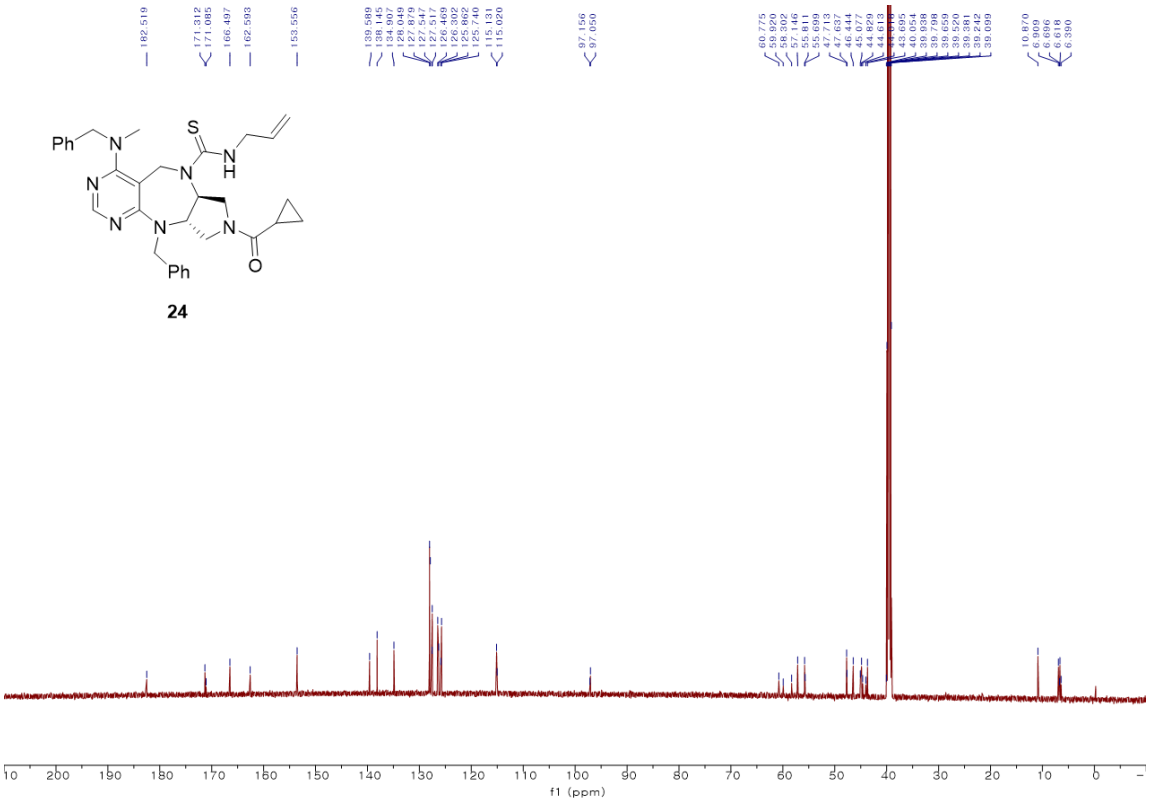
22

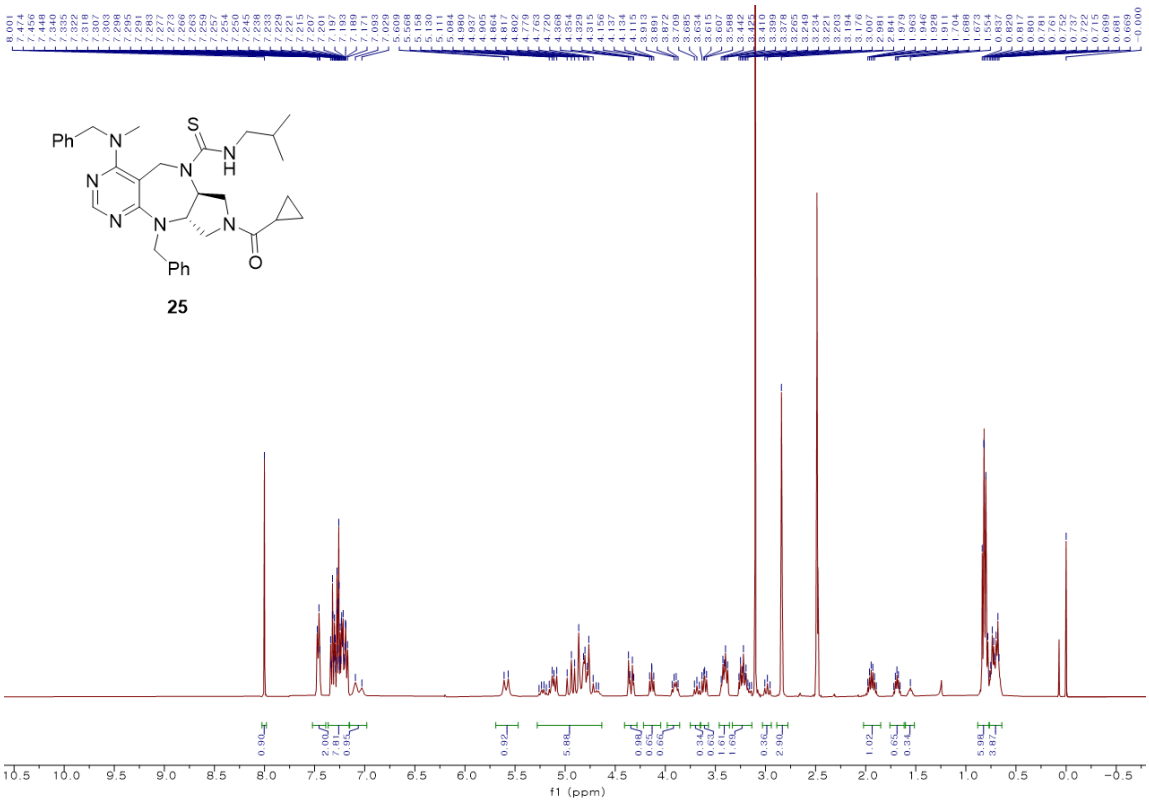


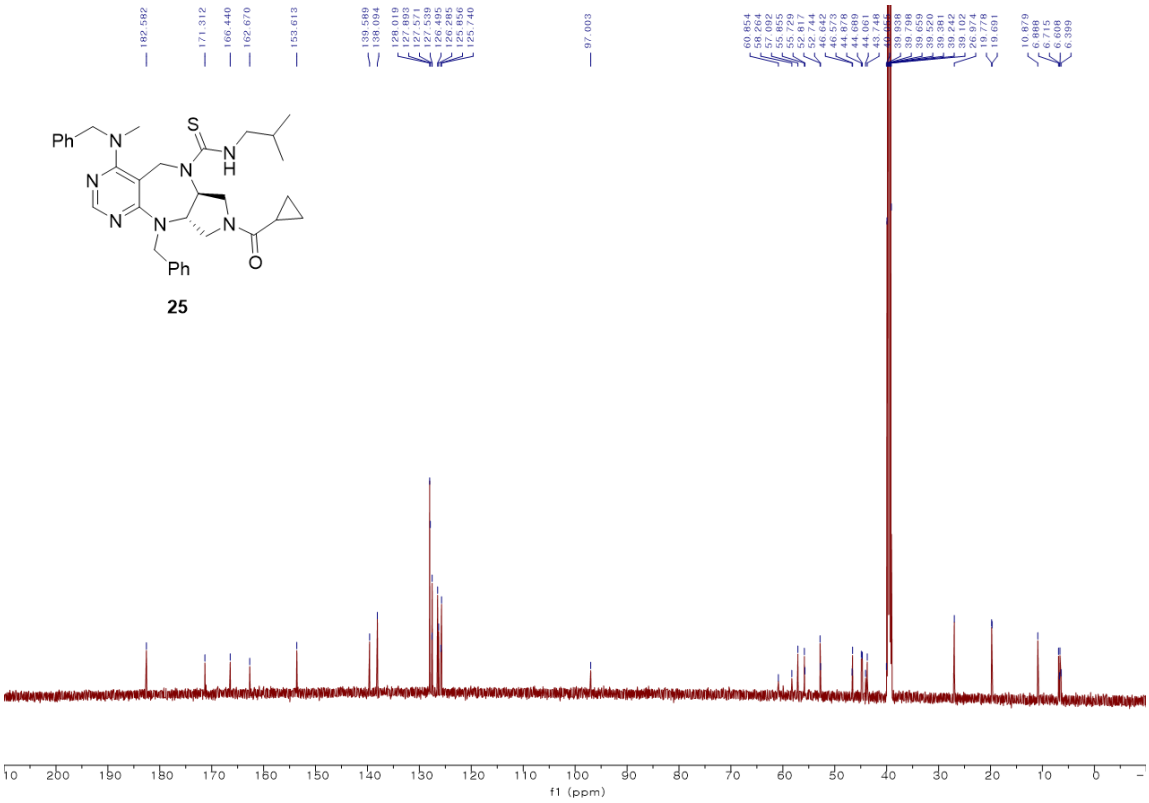
22

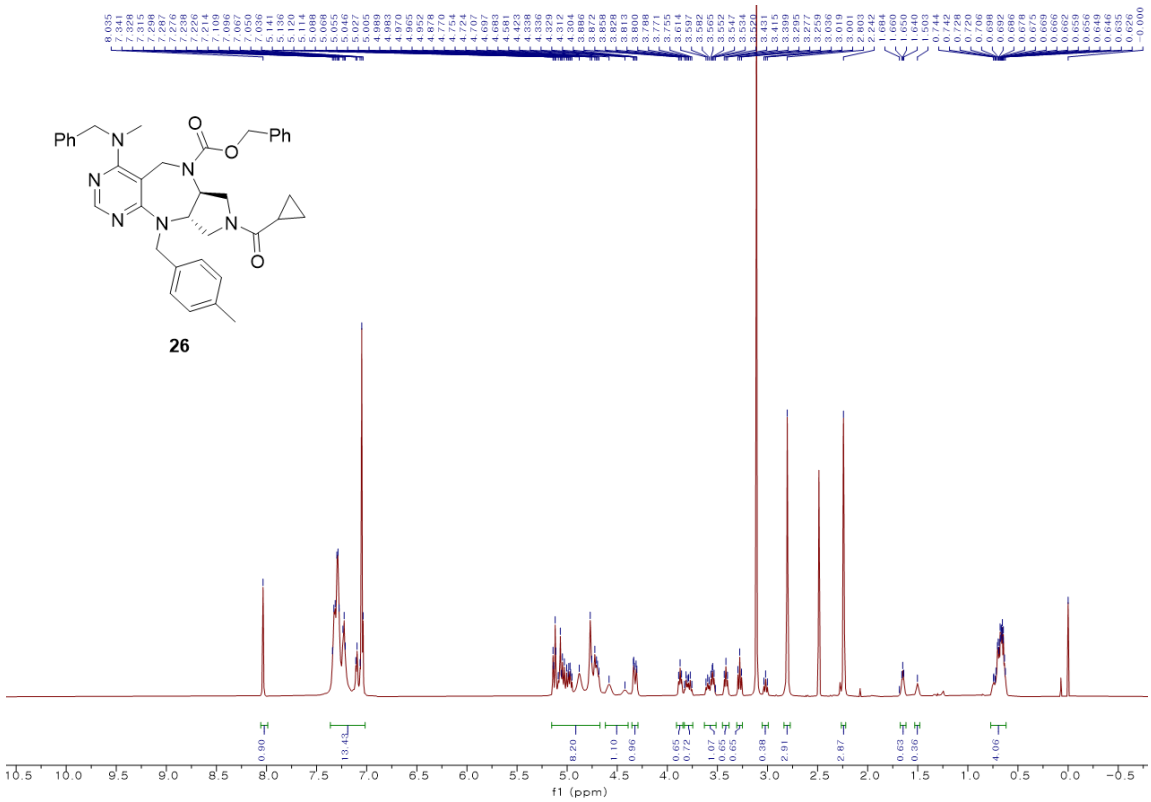

Ph
N
S
N
Ph
N
N
N
N
N
O
Ph
23
f1 (ppm)
Ph
N
S
N
Ph
N
N
N
N
N
O
Ph
23
f1 (ppm)

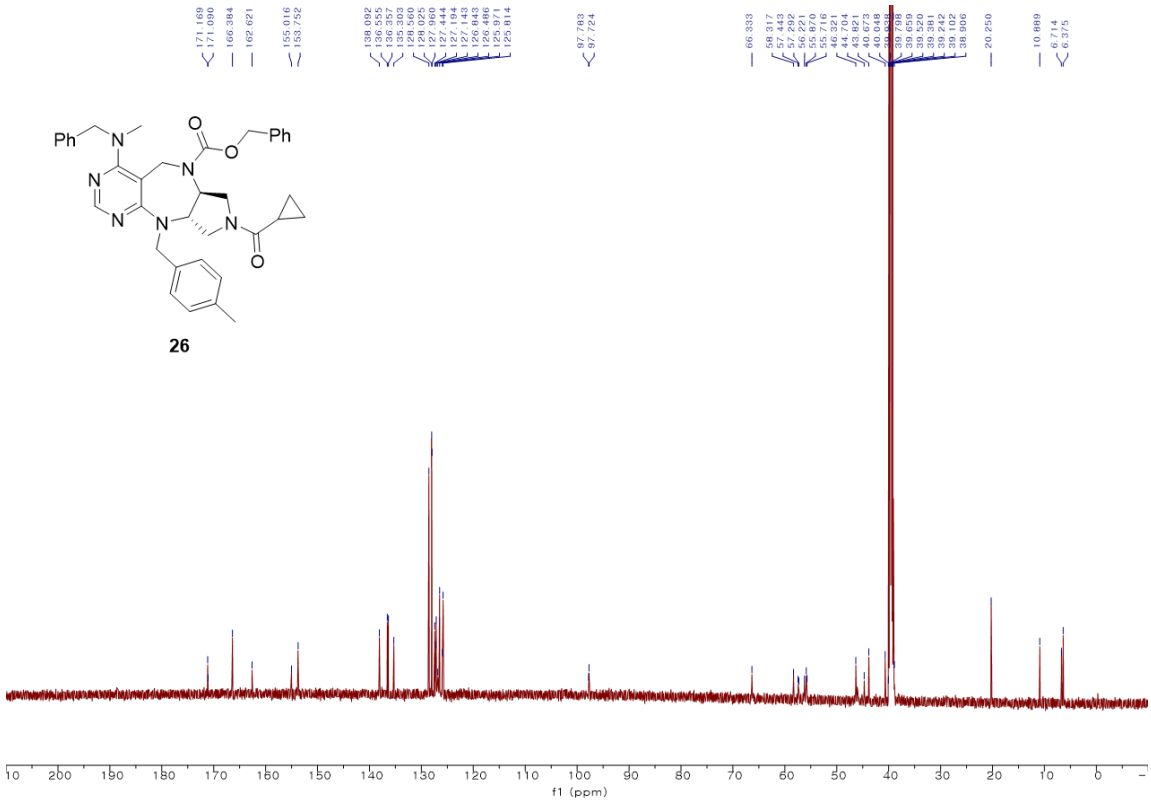

Ph
N
S
N
H
N
N
N
N
N
O
Ph
24
f1 (ppm)

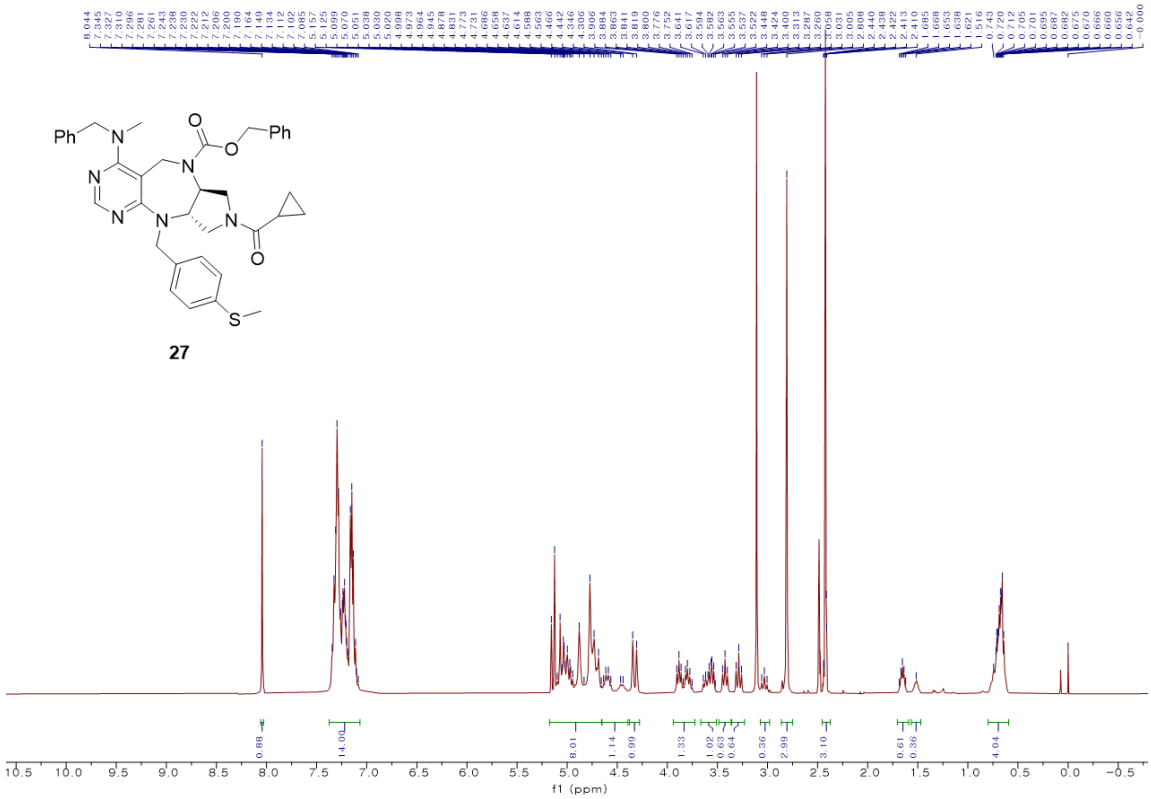

Ph
N
S
N
H
N
N
N
N
N
O
Ph
24
f1 (ppm)


Ph
N
S
N
H
N
N
N
N
N
O
Ph
25
f1 (ppm)

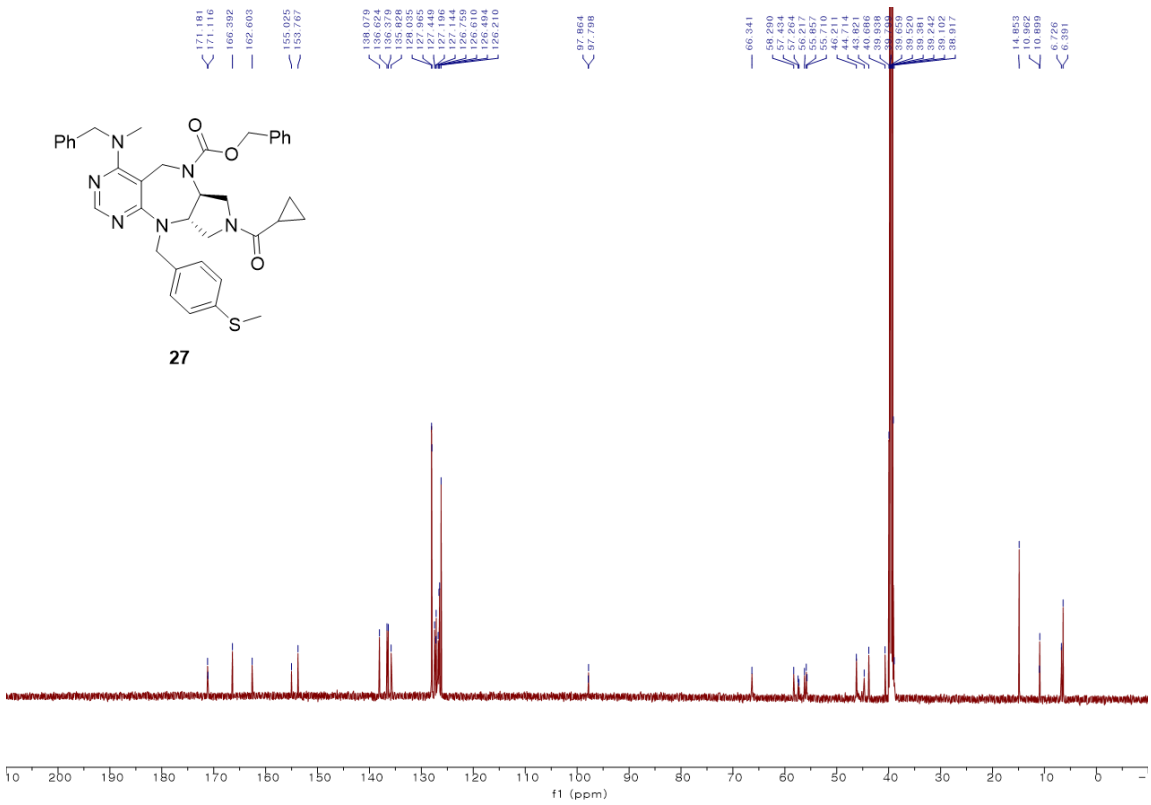

Ph
N
S
N
H
N
N
N
N
N
O
Ph
25
f1 (ppm)


Ph
N
O
Ph
N
N
N
N
N
O
O
26
f1 (ppm)

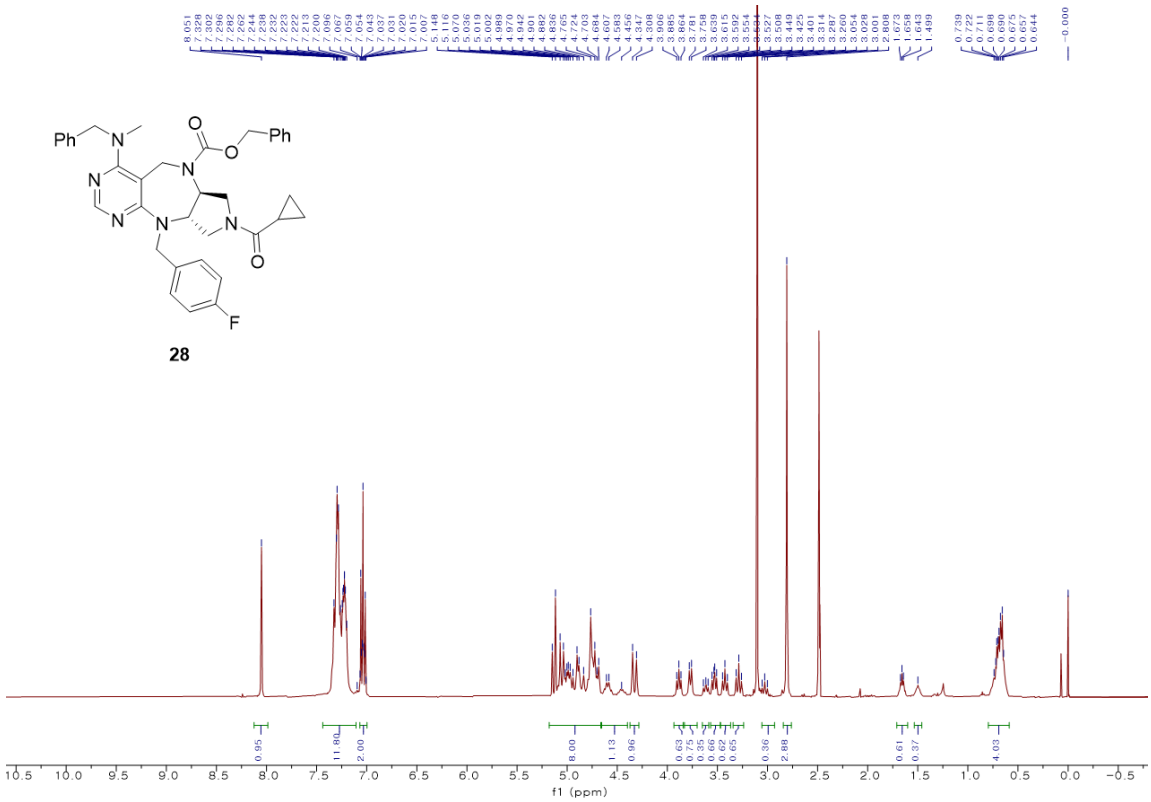

Ph
N
O
Ph
N
N
N
N
N
O
O
26
f1 (ppm)



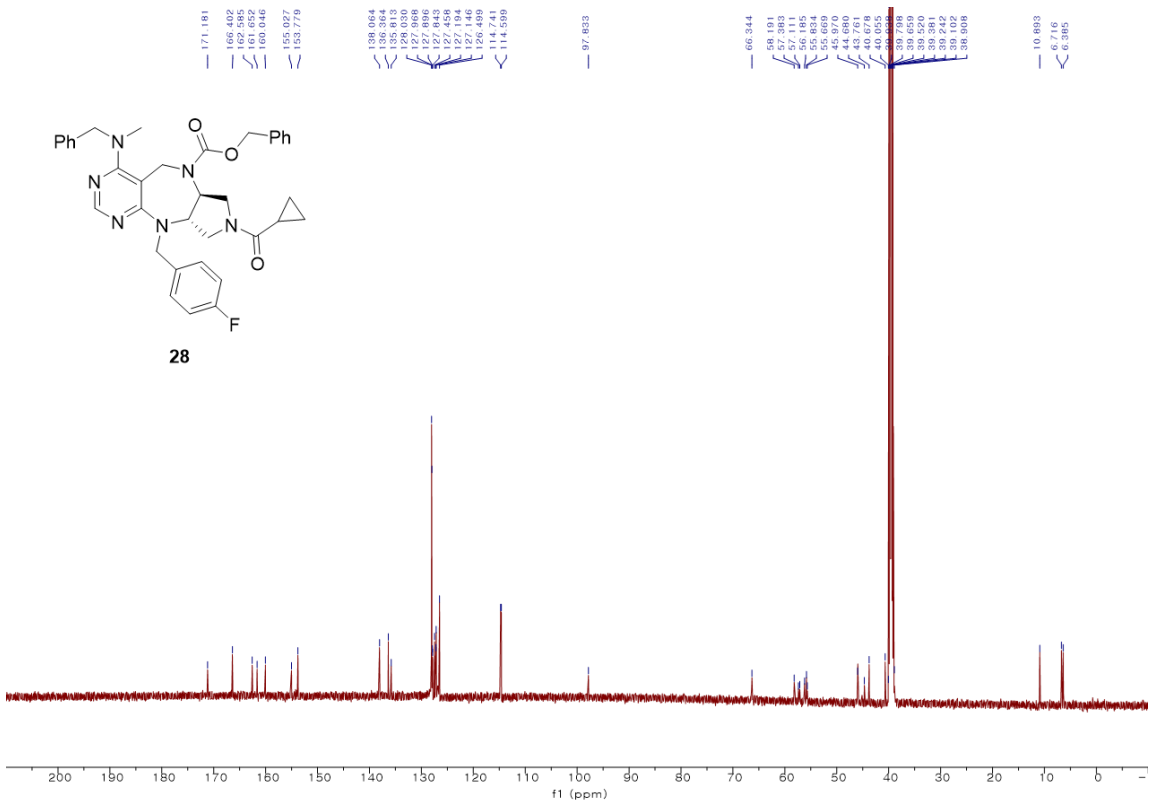
27



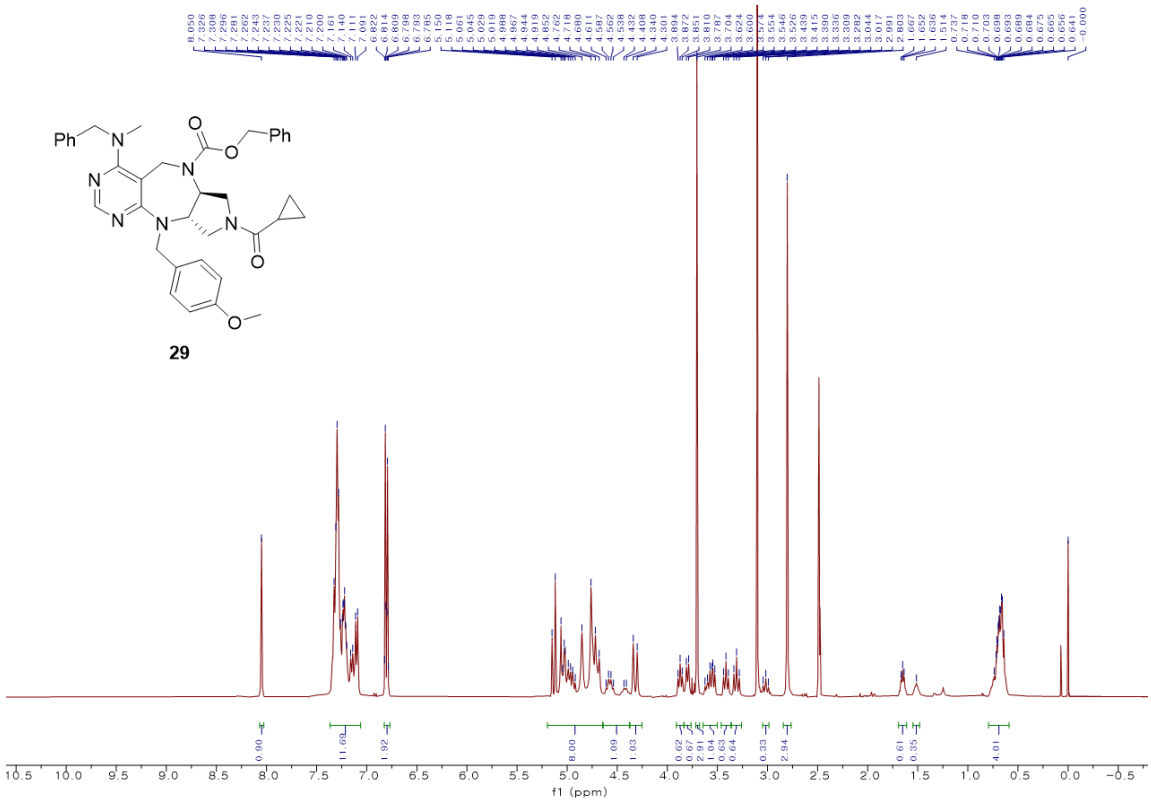
27

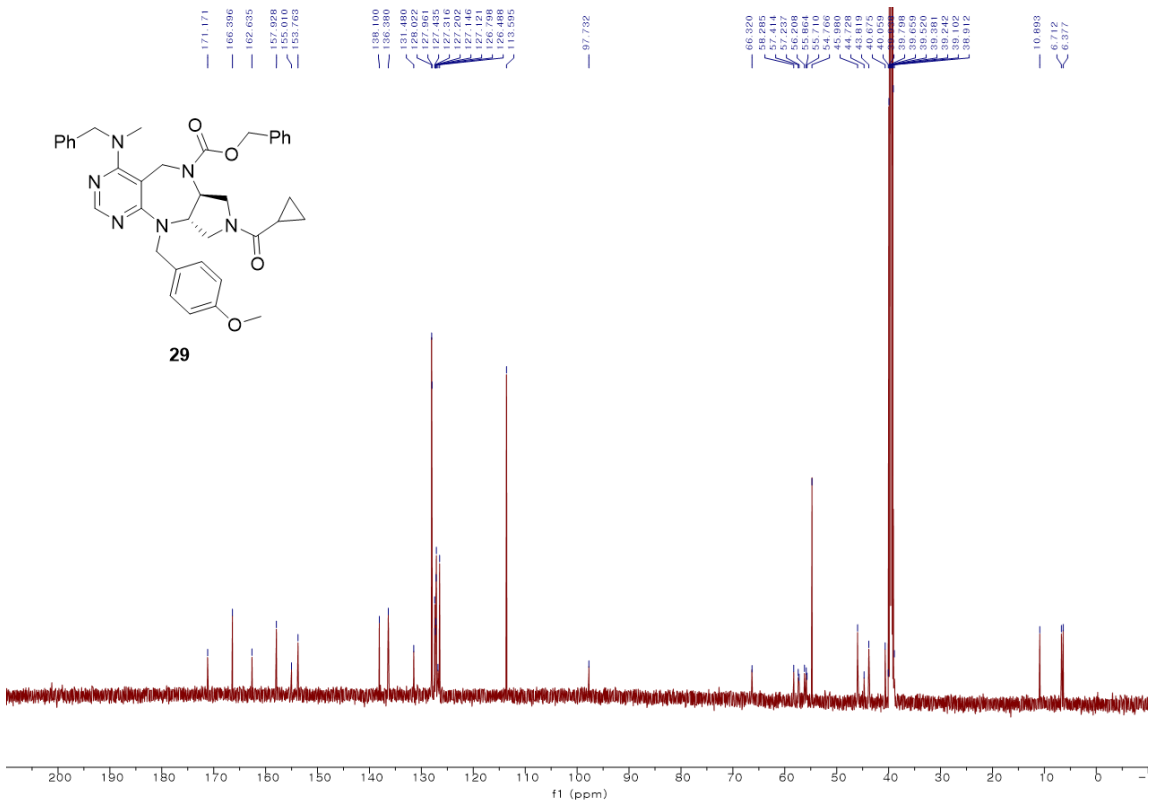


28



28


Ph
N
O
Ph
N
N
N
N
N
O
O
O
29
f1 (ppm)


Ph
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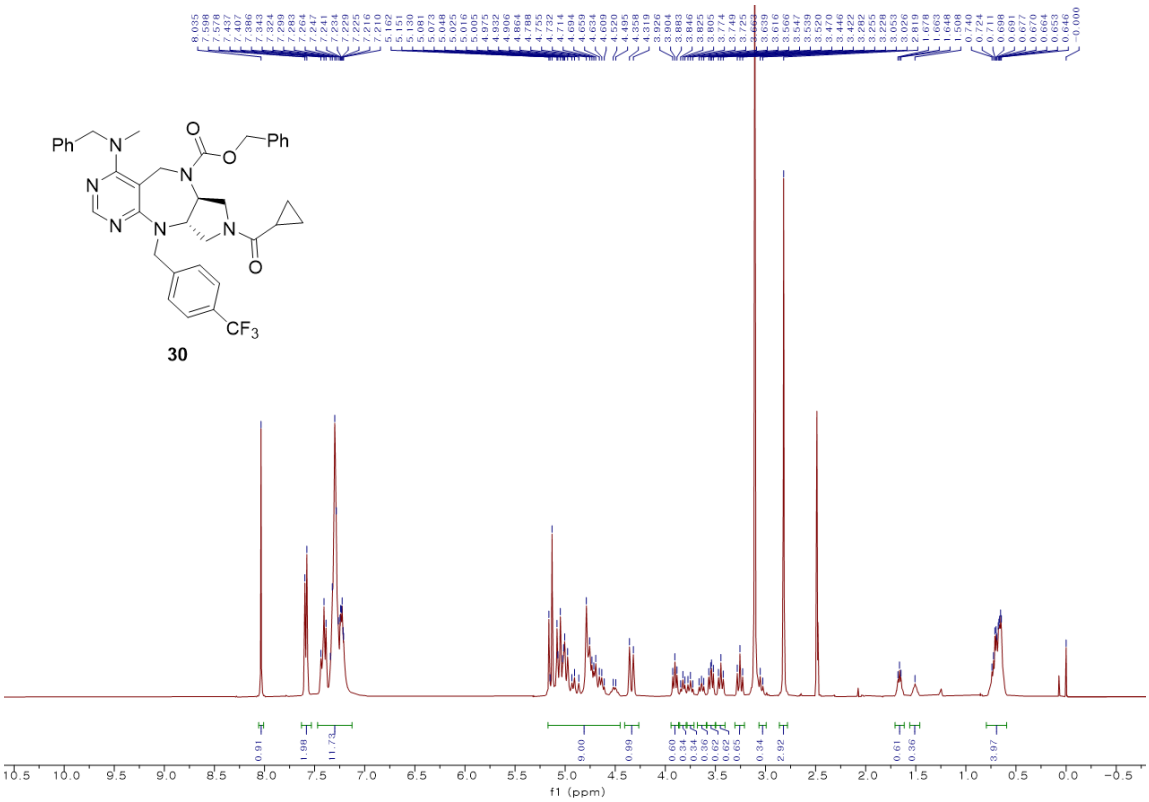
Ph
N
N
N
N
N
O
O
O
29
f1 (ppm)


Ph
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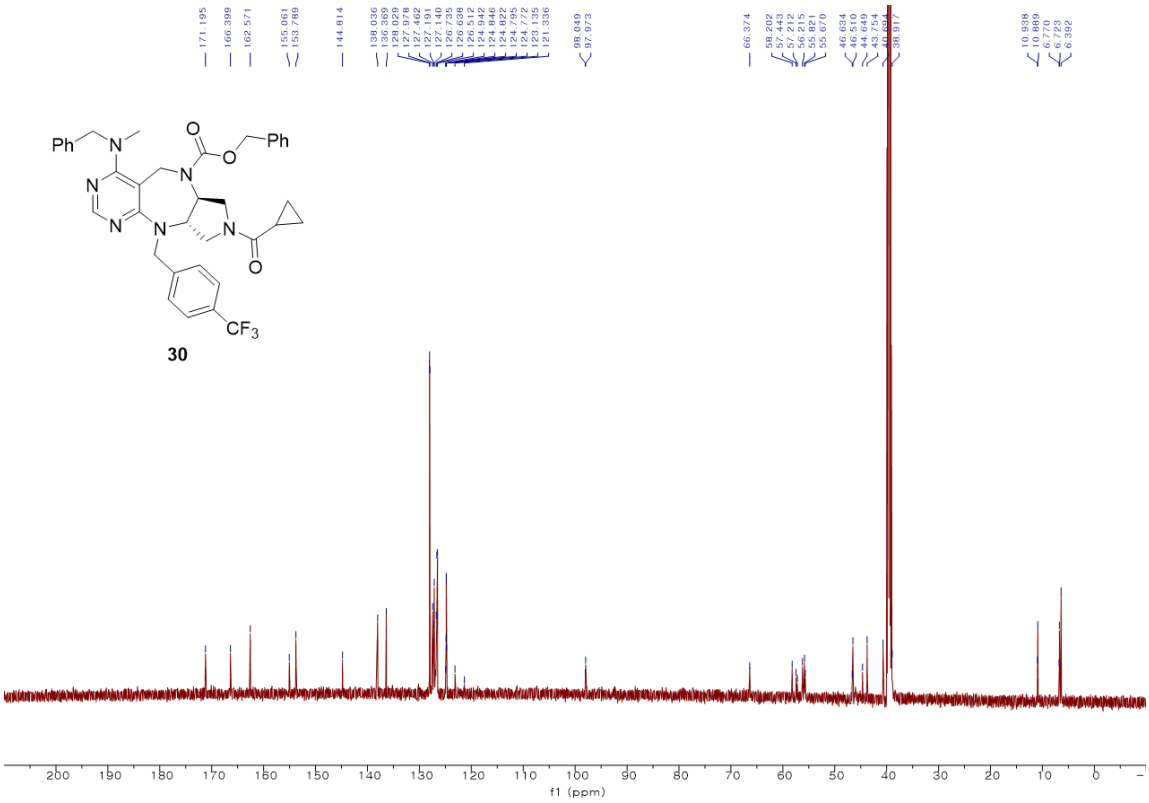
O
Ph
N
N
N
N
O
CF3
30
f1 (ppm)


Ph
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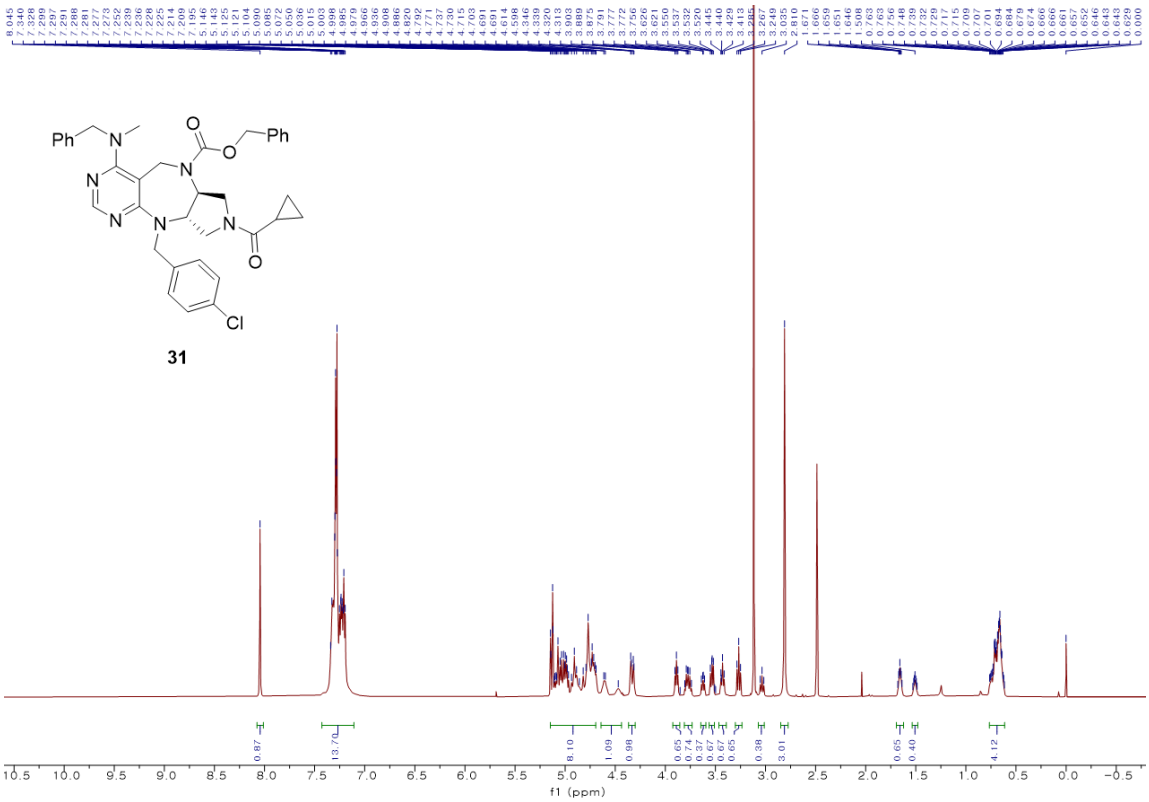
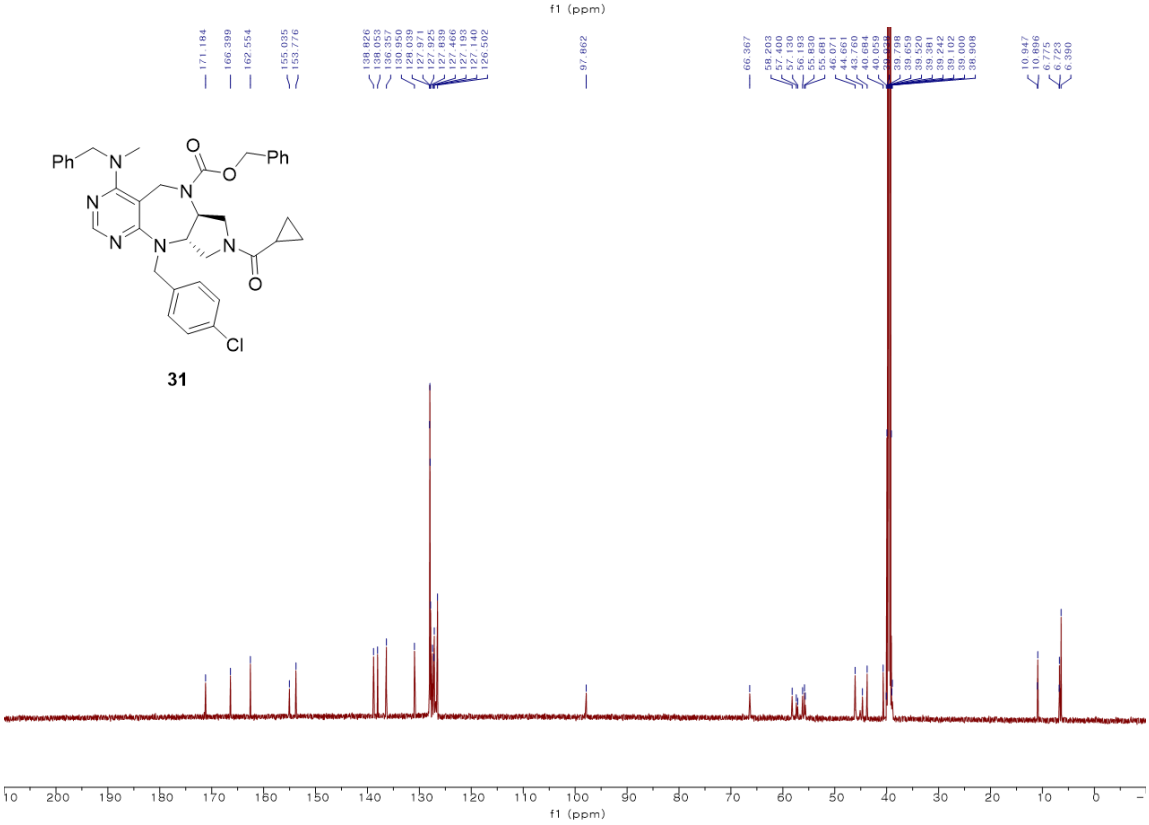
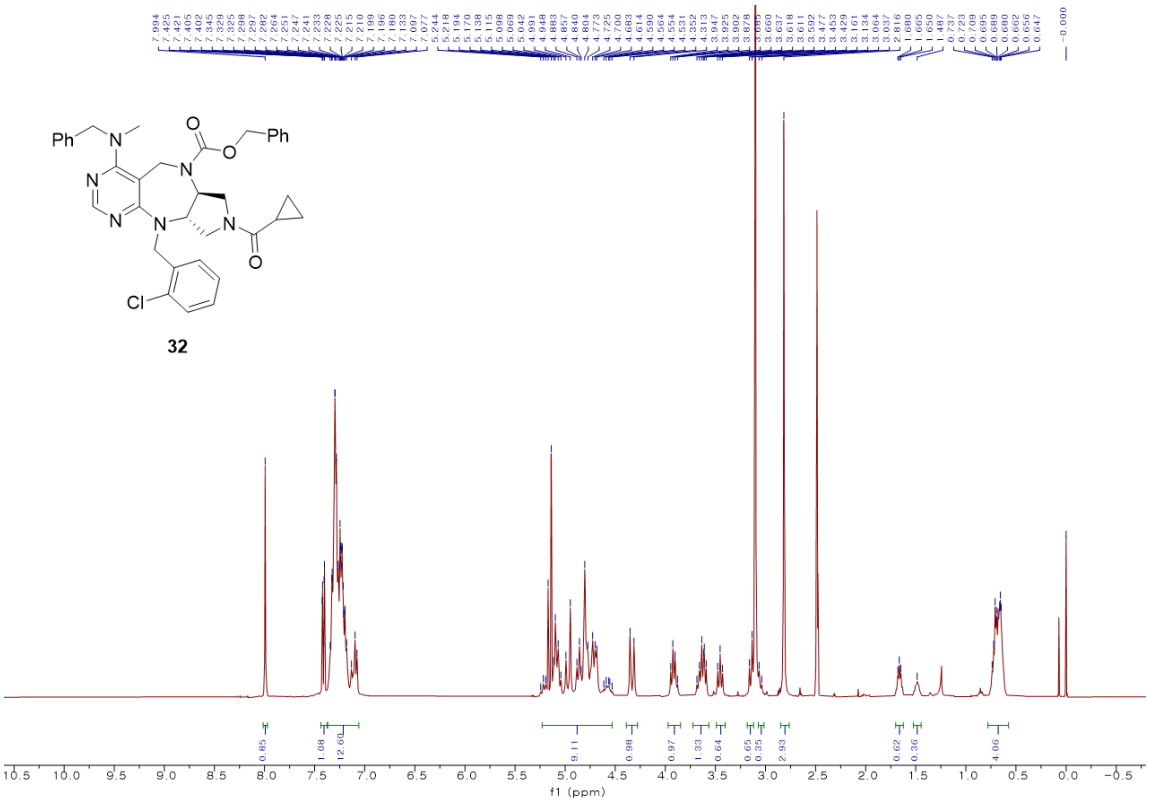
O
O
Ph
N
N
N
N
O
CF3
30
f1 (ppm)



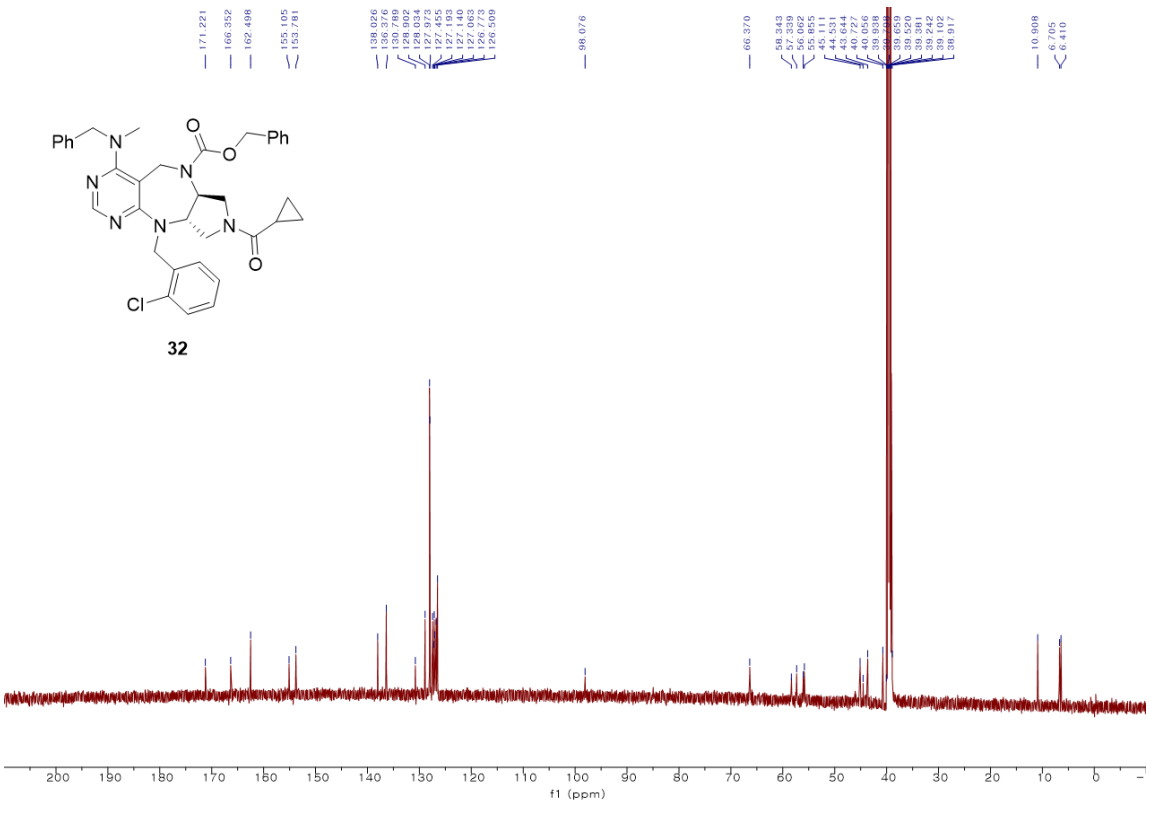
31



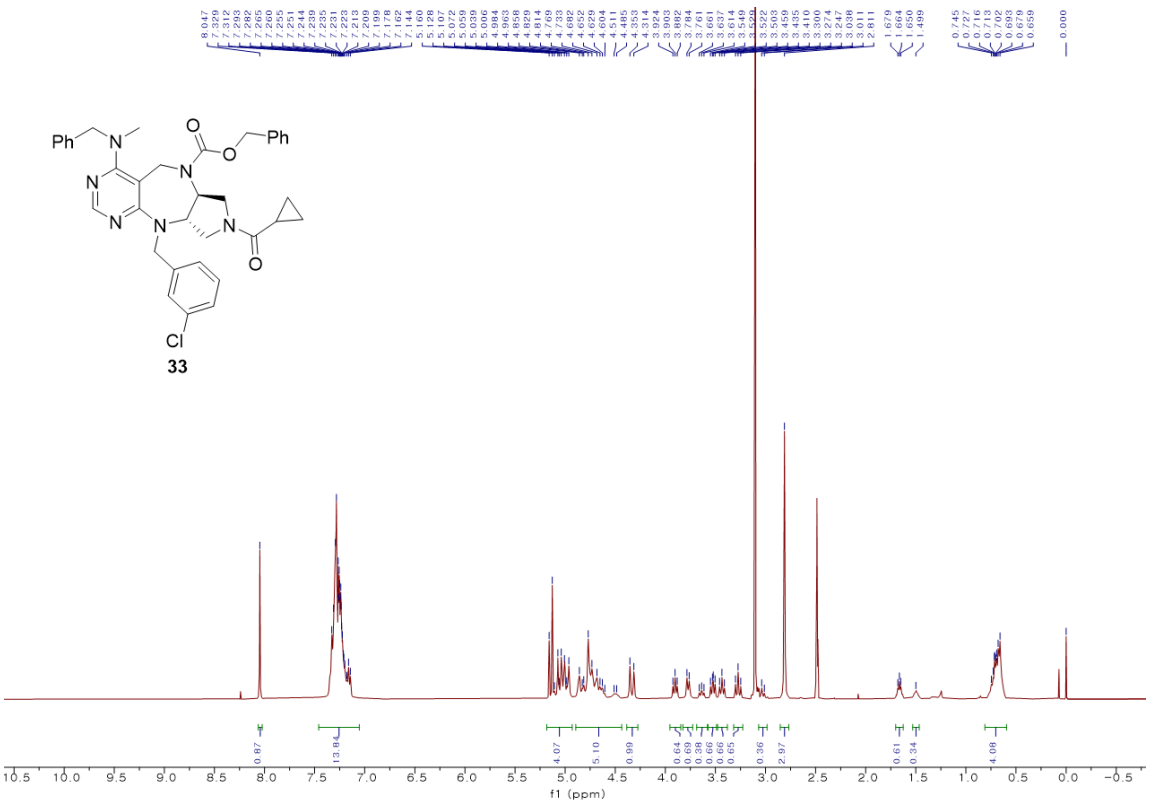
31

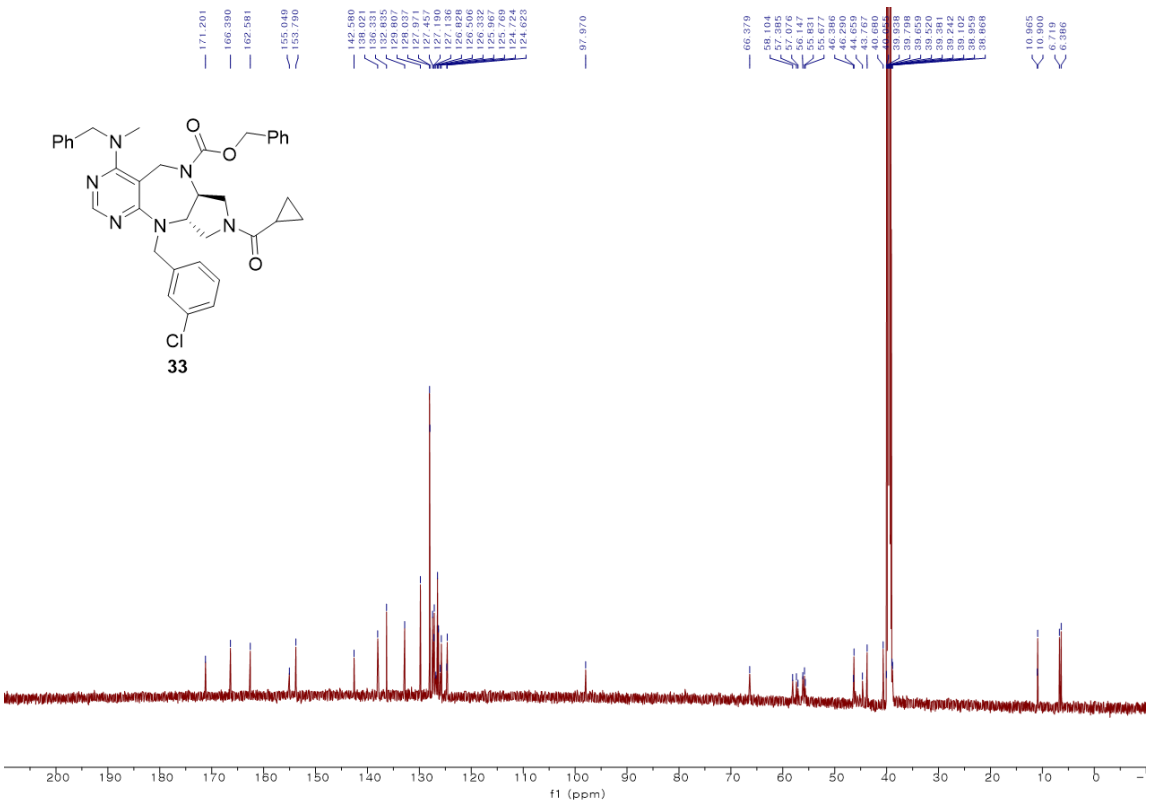


32



32


Ph
N
O
O
Ph
N
N
N
N
N
O
Cl
33
f1 (ppm)


Ph
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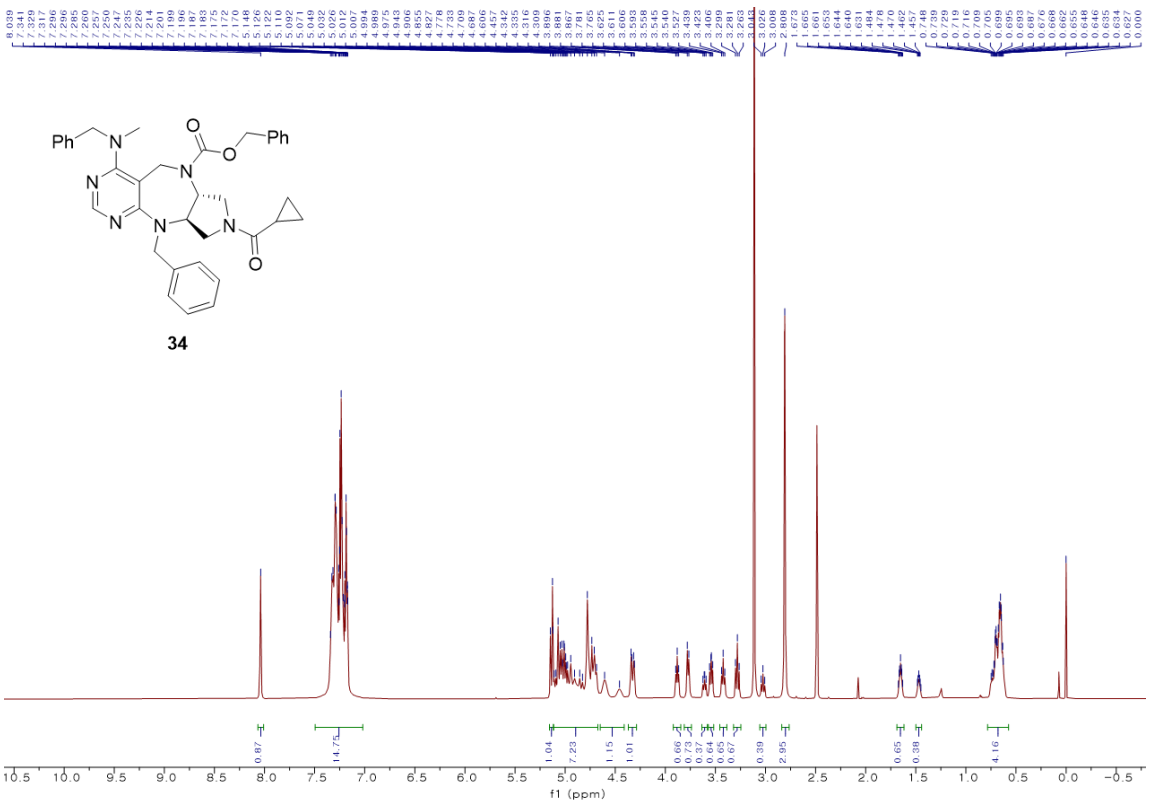
O
O
Ph
N
N
N
N
N
O
Cl
33
f1 (ppm)


Ph
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O
Ph
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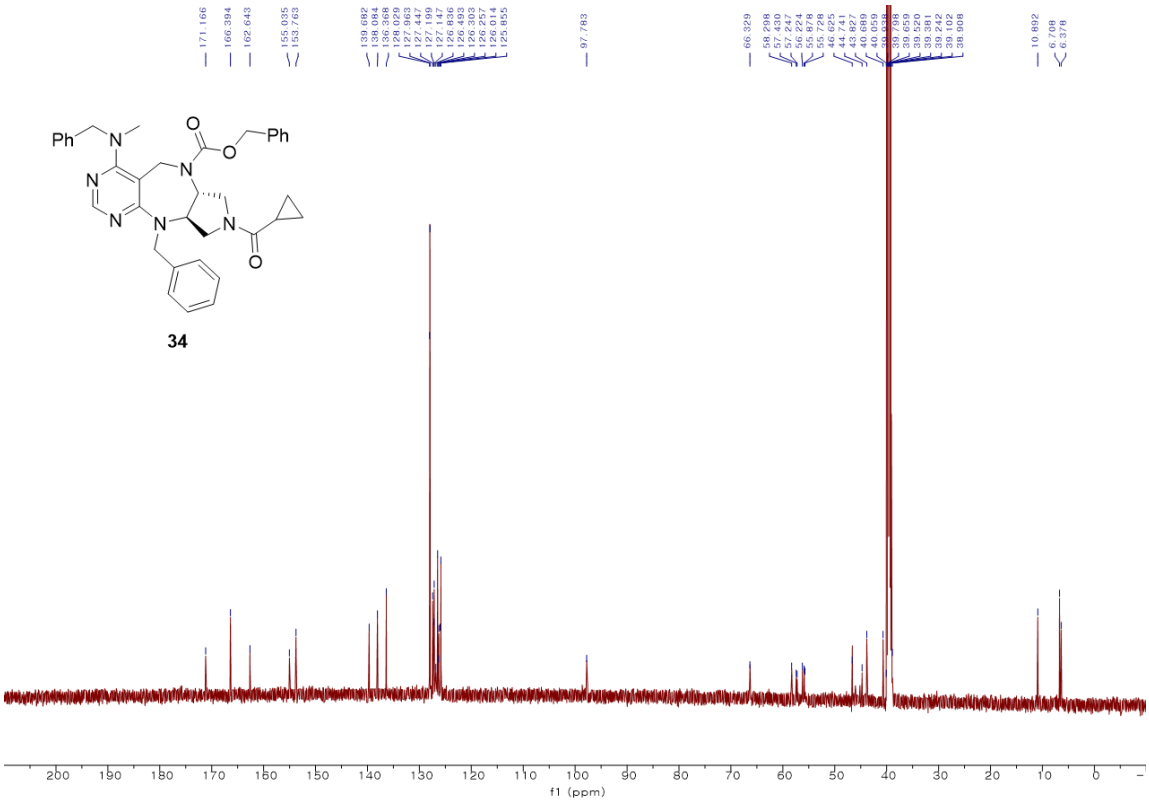
N
N
N
N
O
34
f1 (ppm)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Ph
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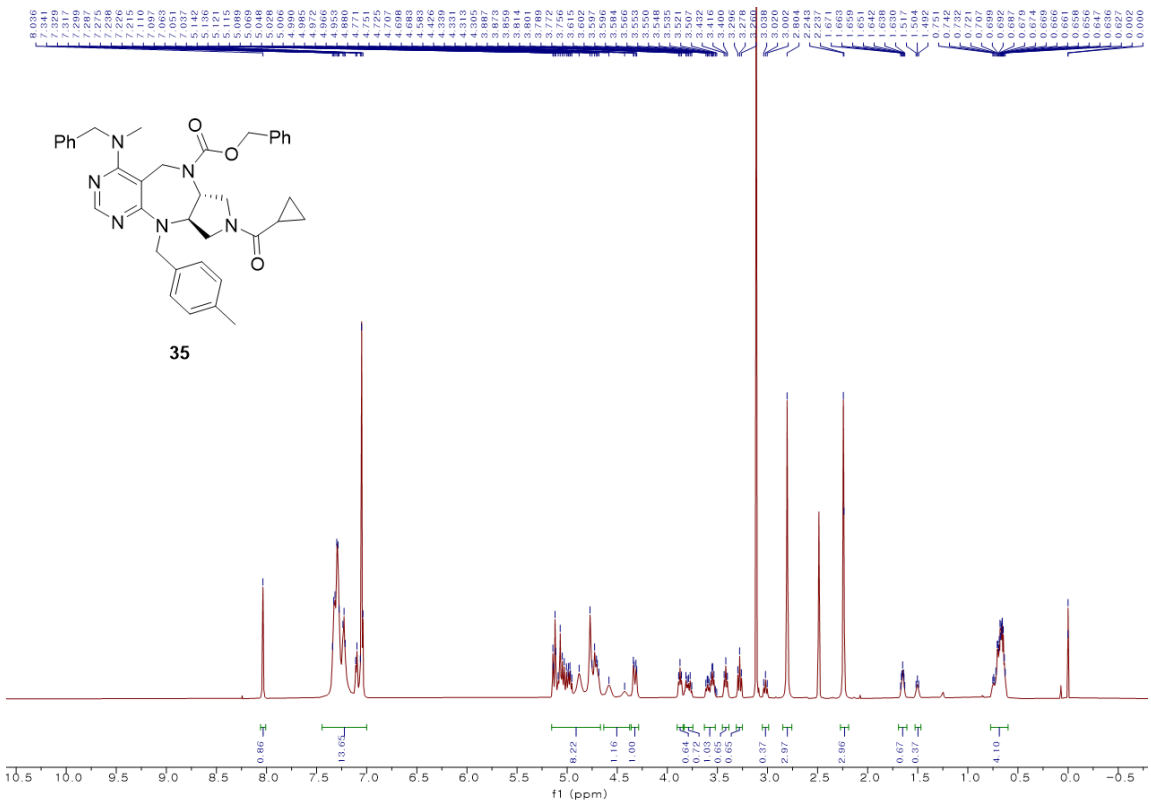
N
N
N
N
O
34
f1 (ppm)



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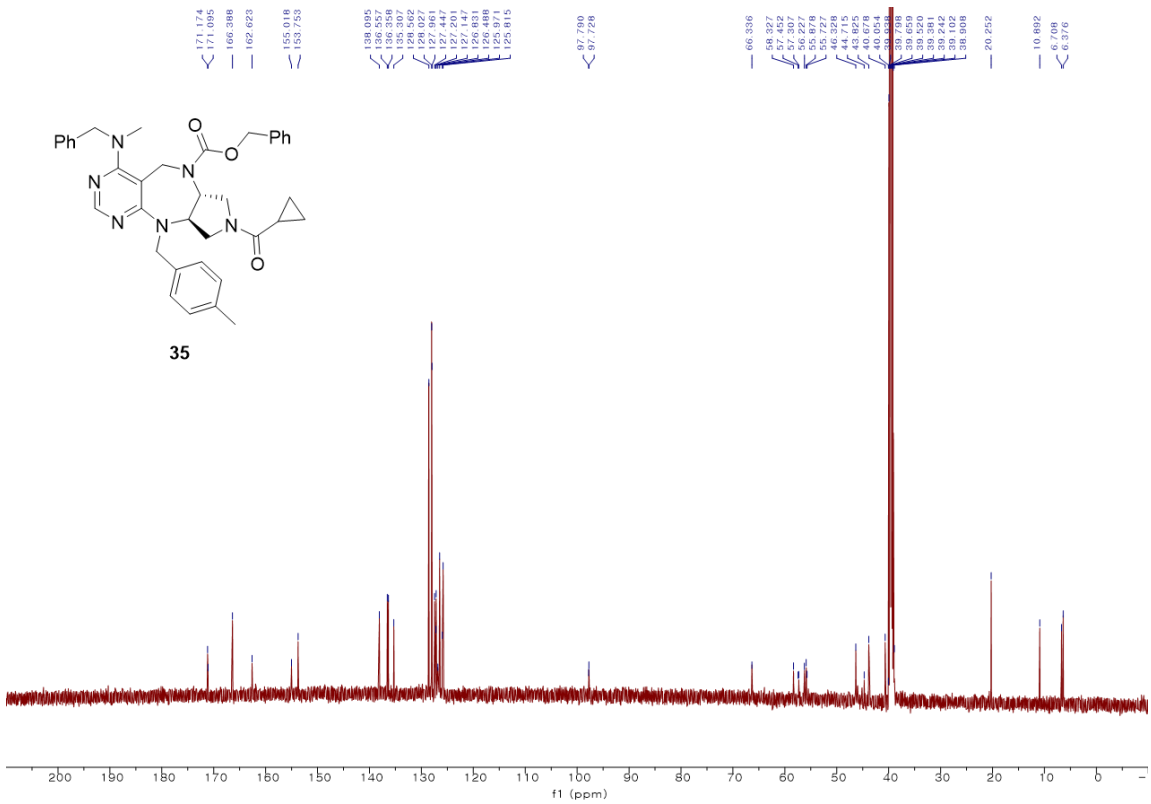
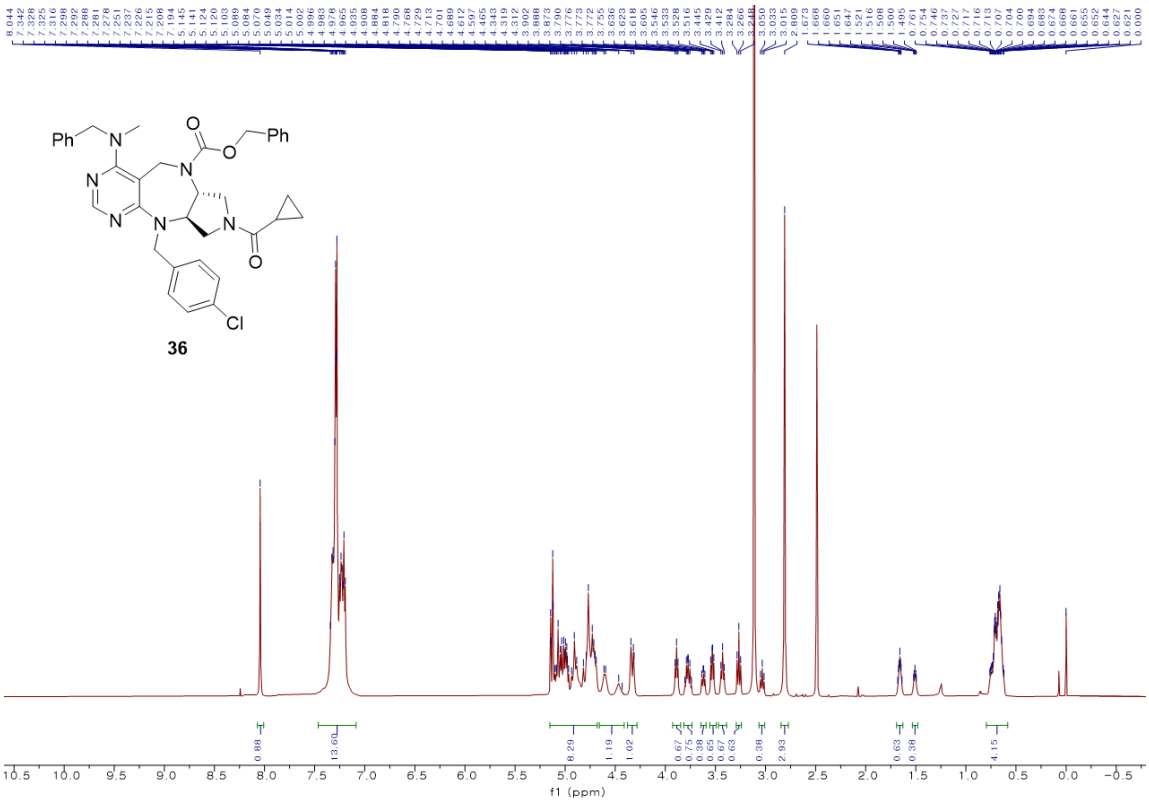


35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Ph
N
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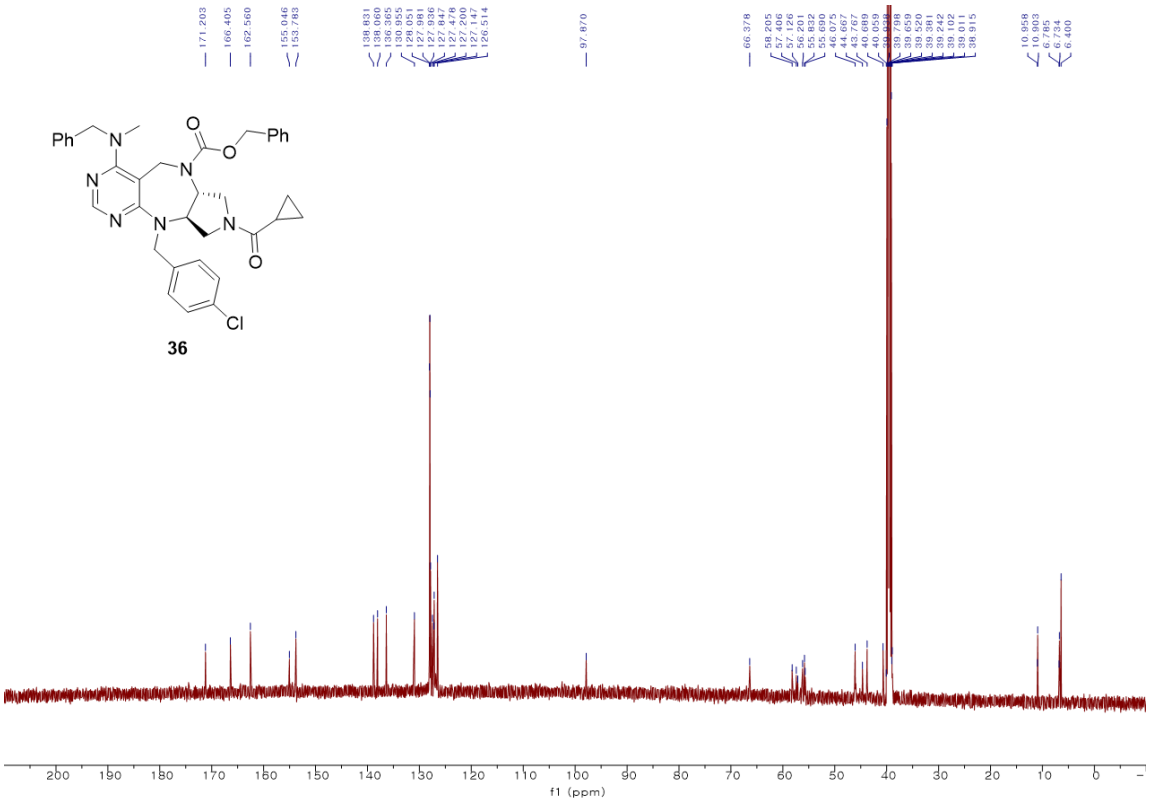
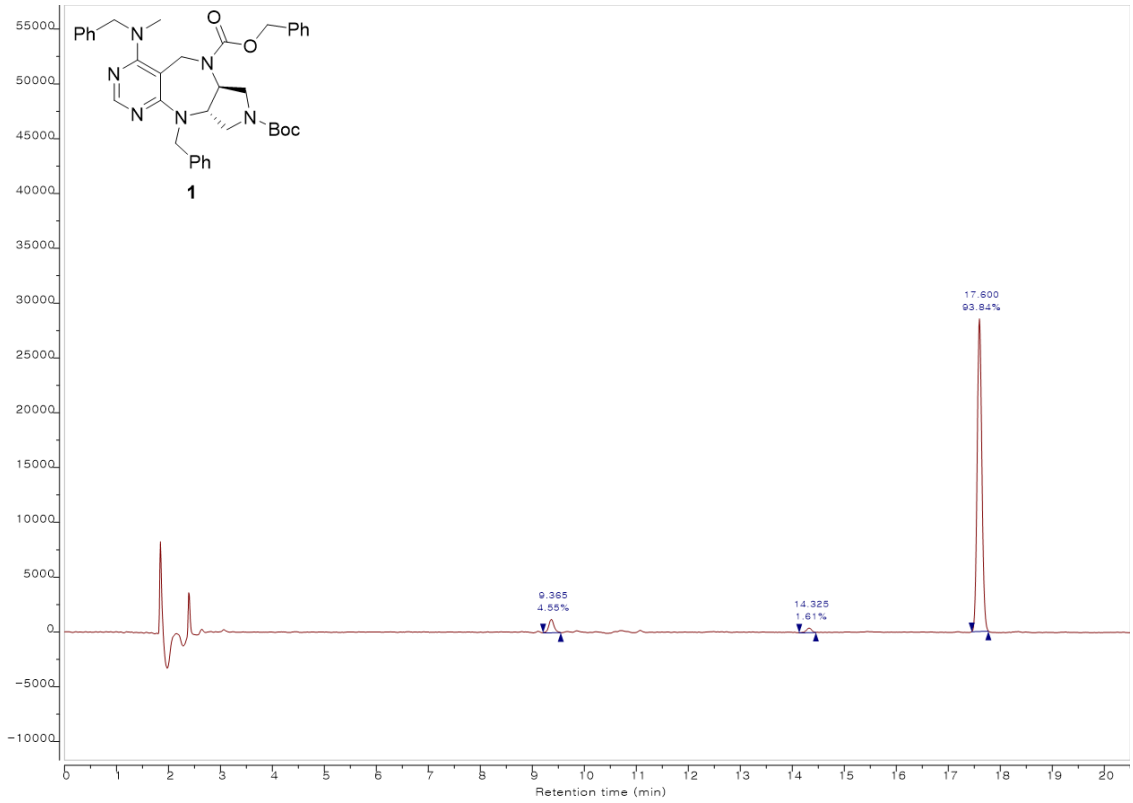
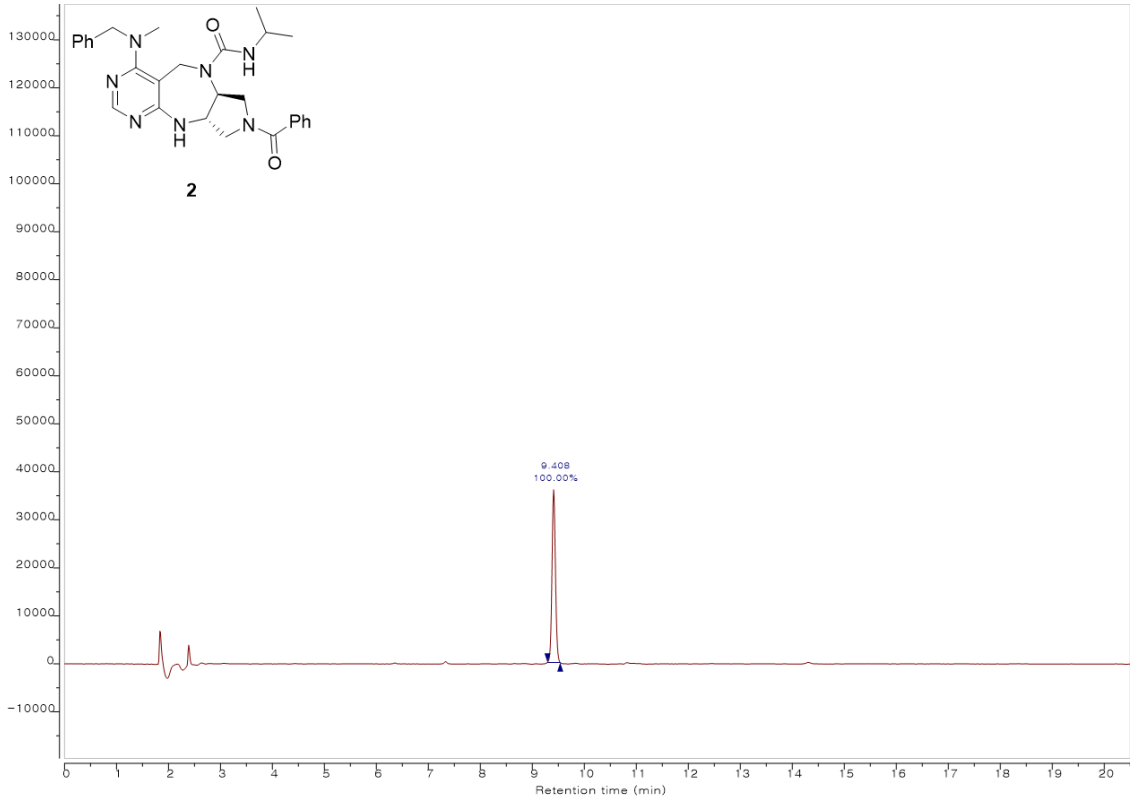
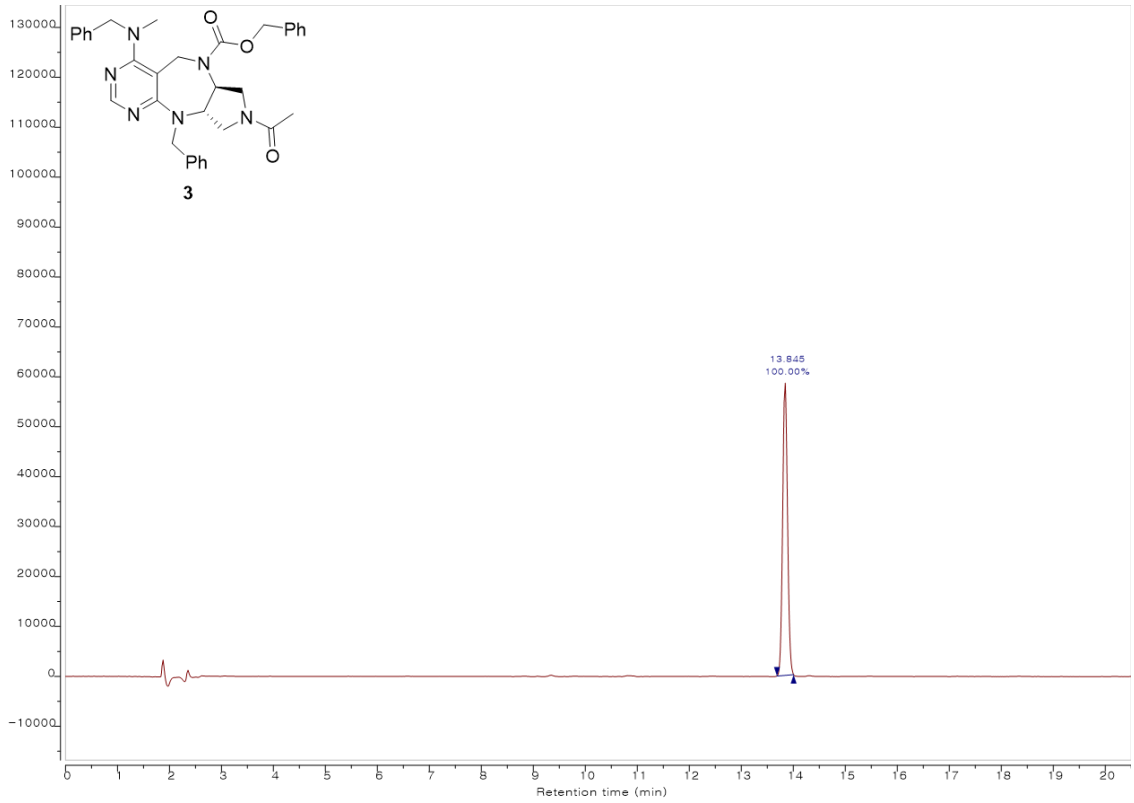
N
N
N
O
Cl
36
f1 (ppm)


Ph
N
O
O
Ph
N
N
N
N
N
O
Cl
36
f1 (ppm)

HPLC Spectra






3
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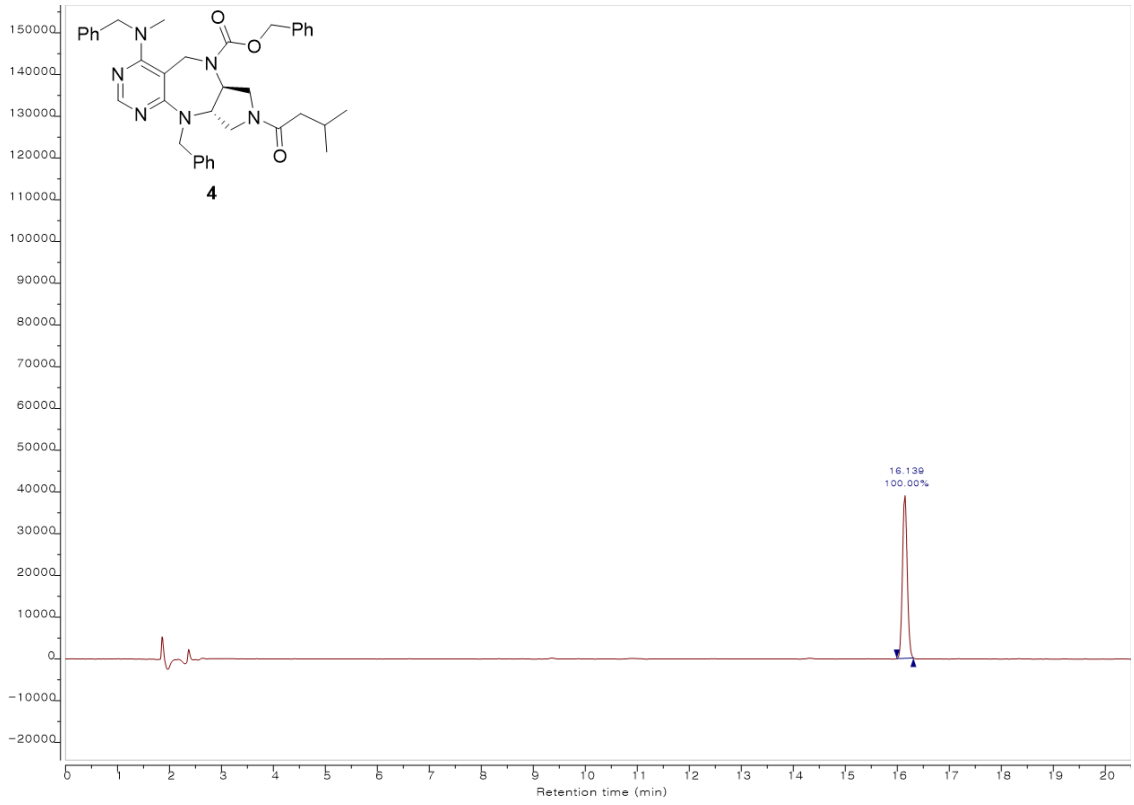
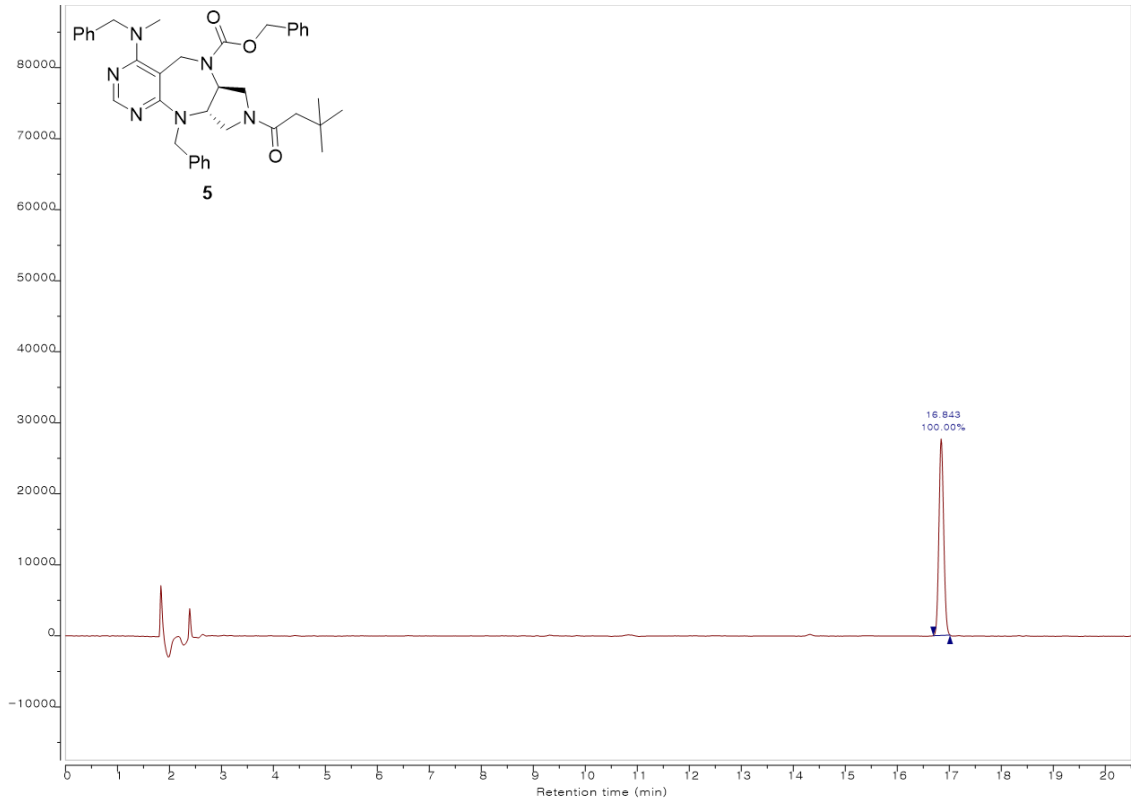
100.00%
Retention time (min)


4
16.139
100.00%
Retention time (min)


Ph
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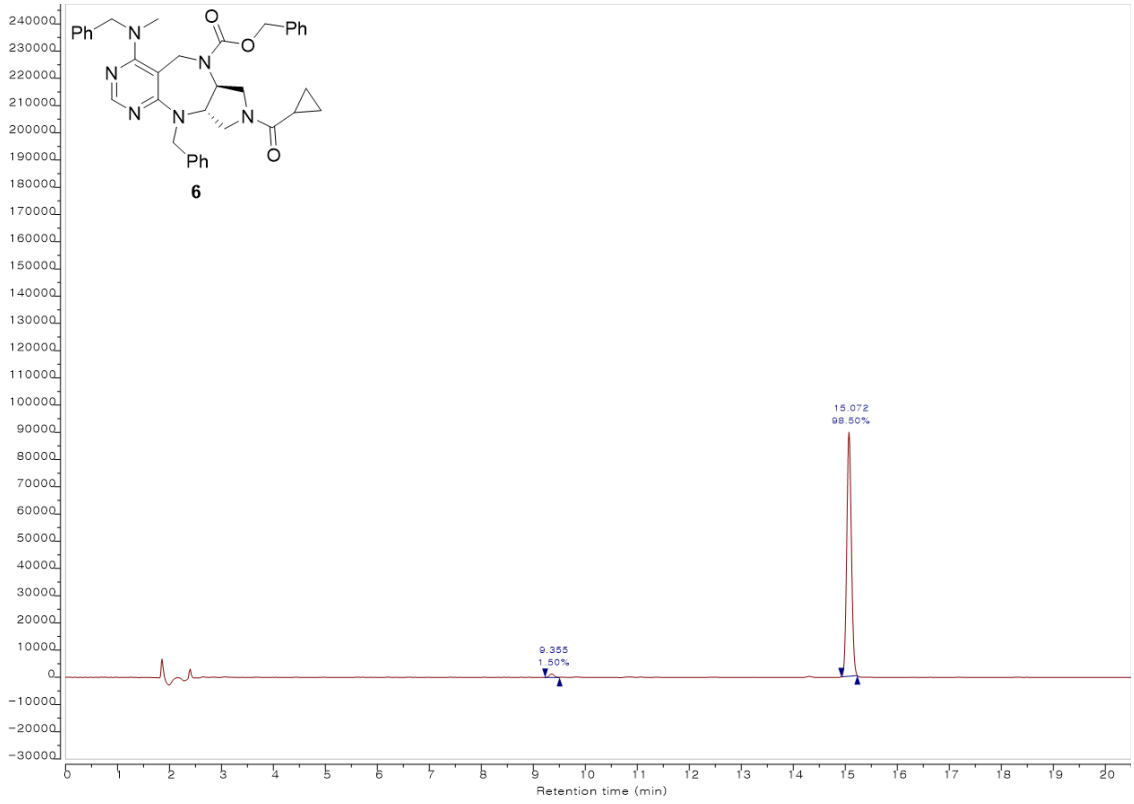
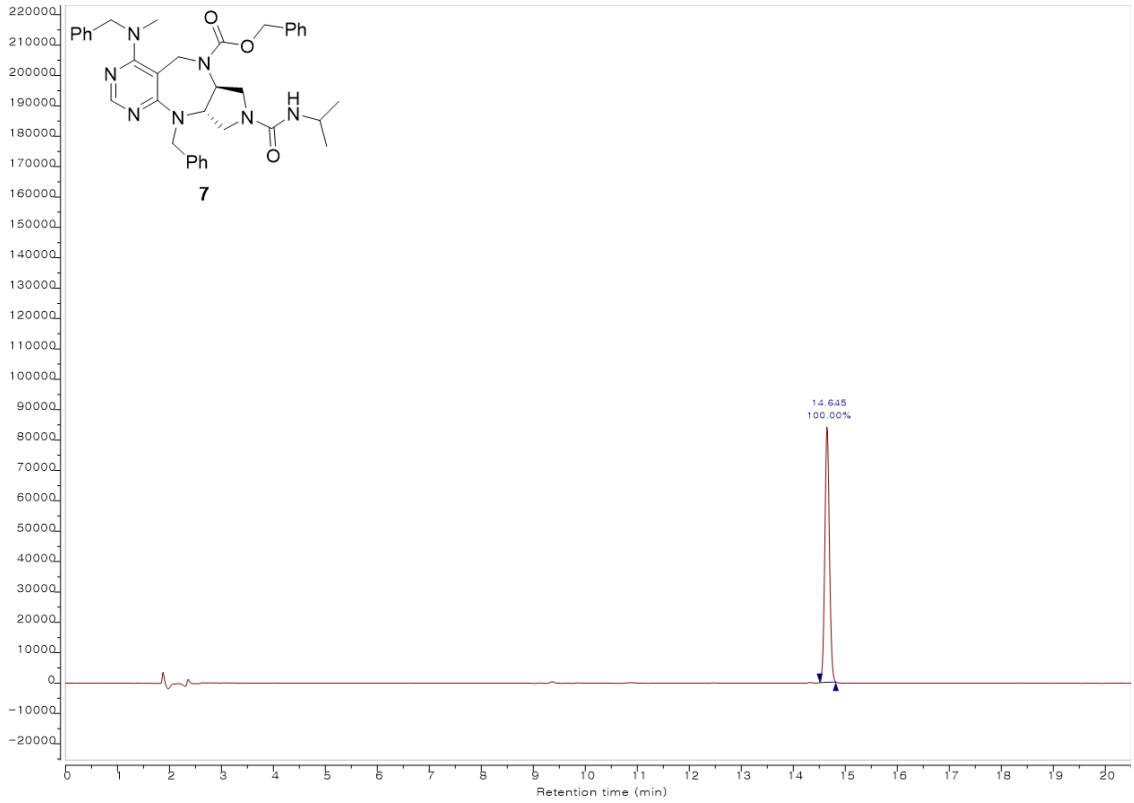
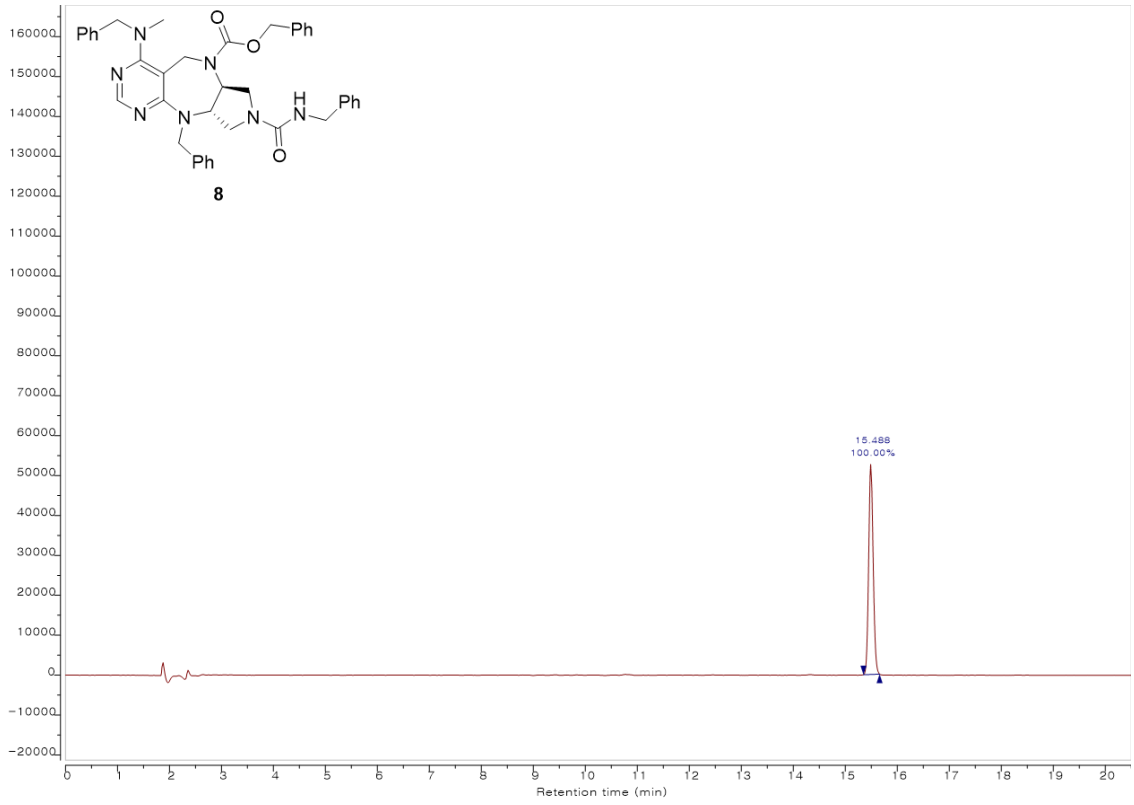
O
O
Ph
N
N
N
N
N
O
Ph
5
16.843
100.00%
Retention time (min)


Ph
N
O
O
Ph
N
N
N
N
N
O
Ph
6
15.072
98.50%
9.355
1.50%
Retention time (min)


7
14.645
100.00%
Retention time (min)


8
15.488
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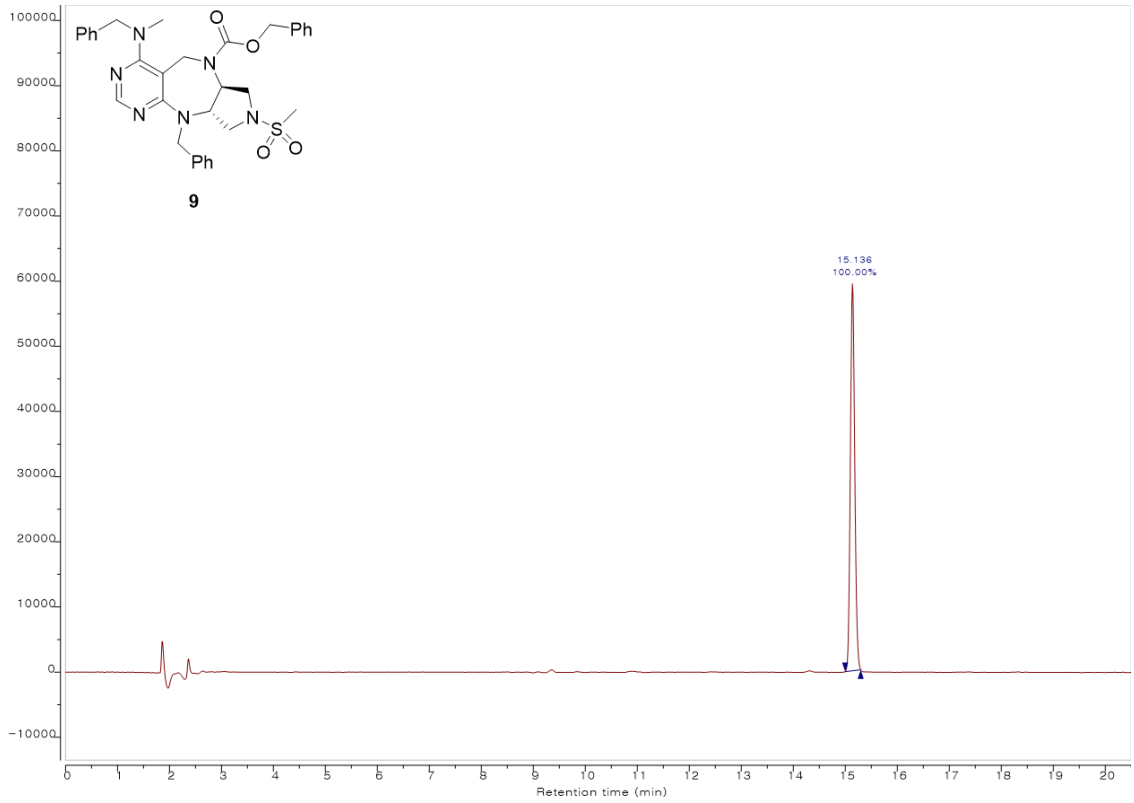
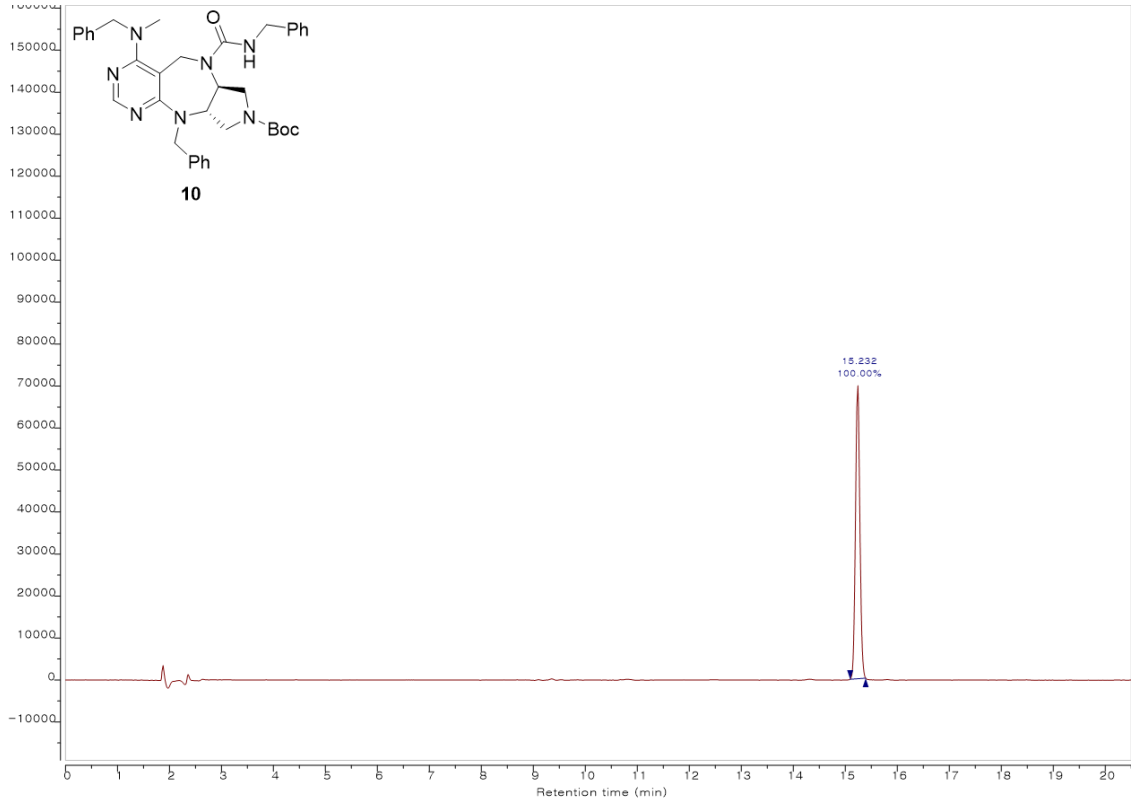
Retention time (min)


9
15.136
100.00%
Retention time (min)


10
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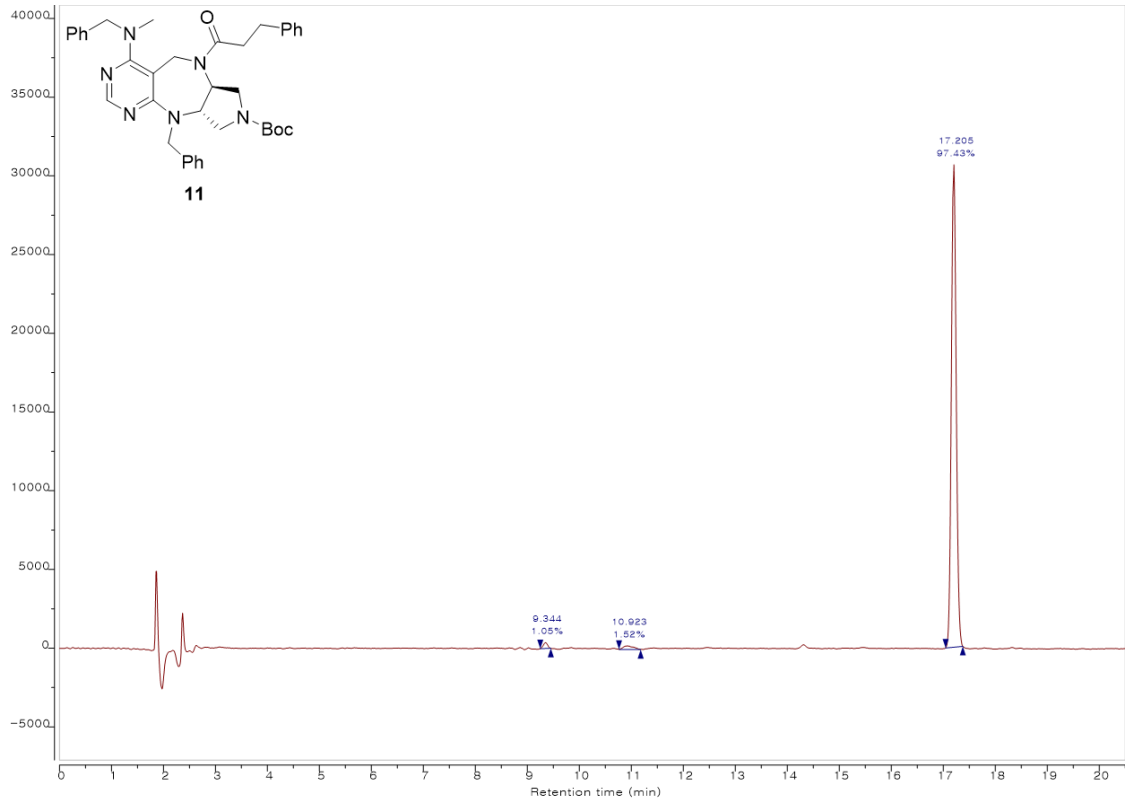
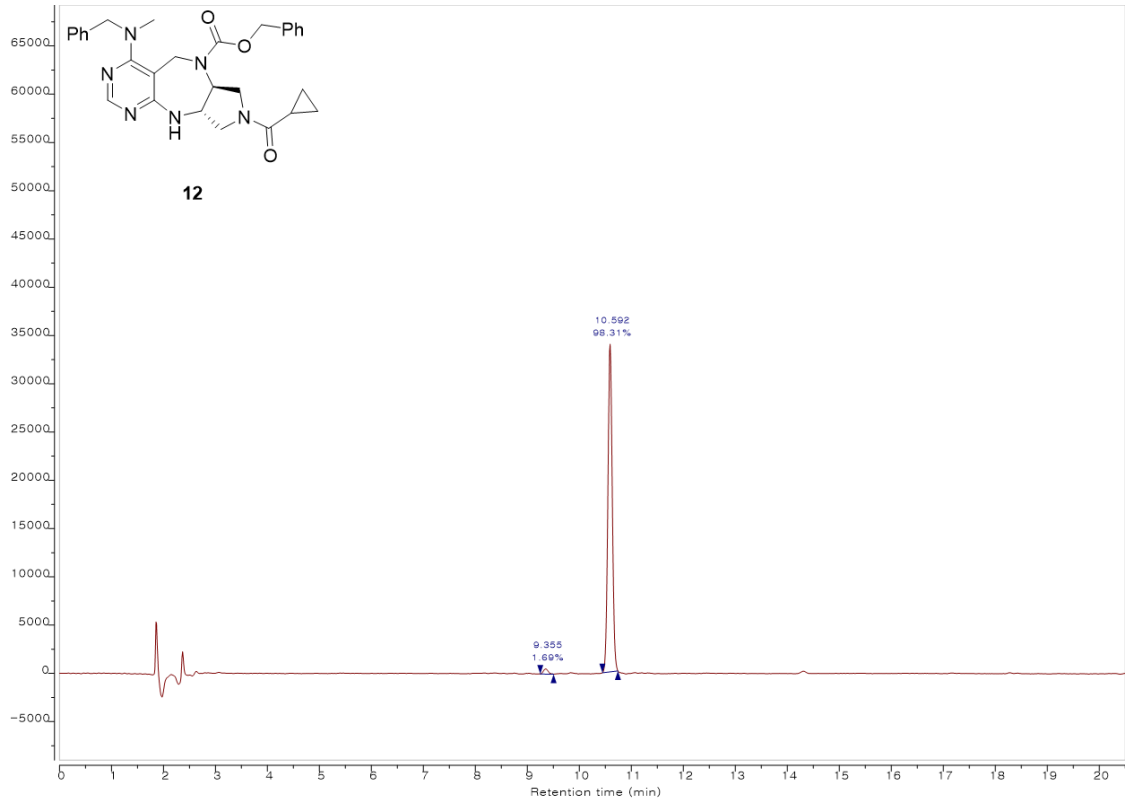
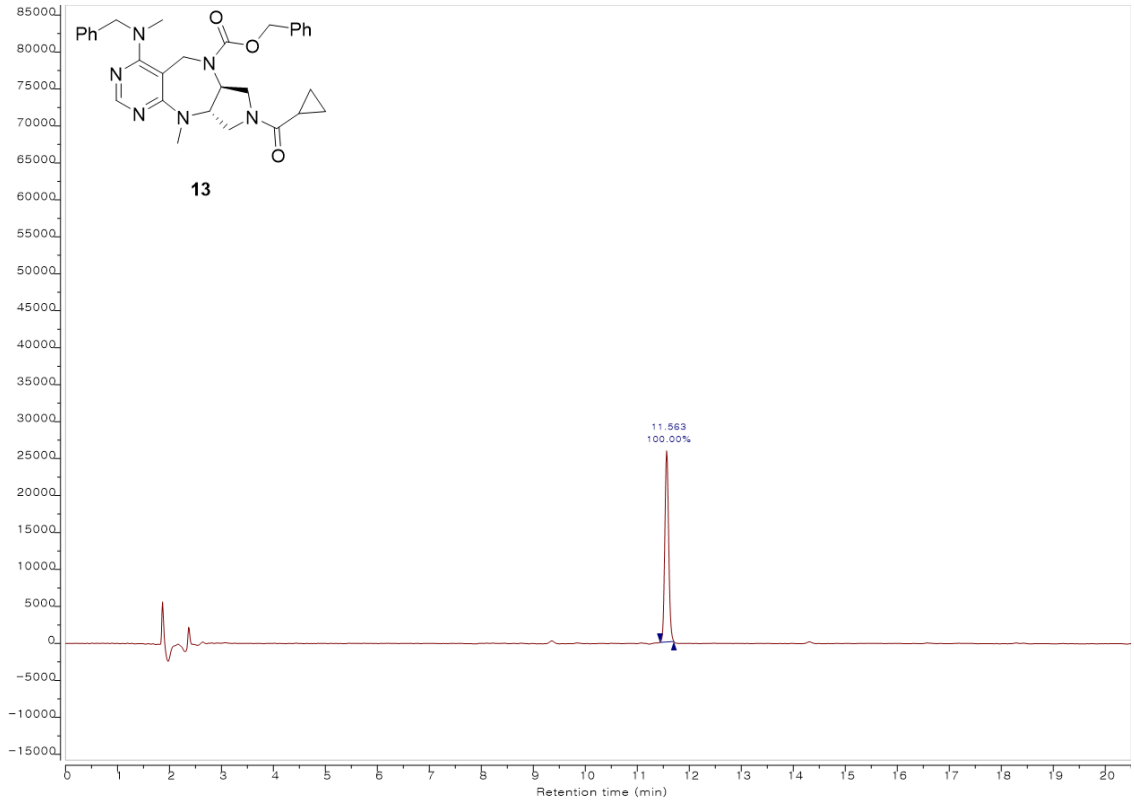
Retention time (min)


11
17.205
97.43%
9.344
1.05%
10.923
1.52%
Retention time (min)


12
10.592
98.31%
9.355
1.69%
Retention time (min)


13
11.563
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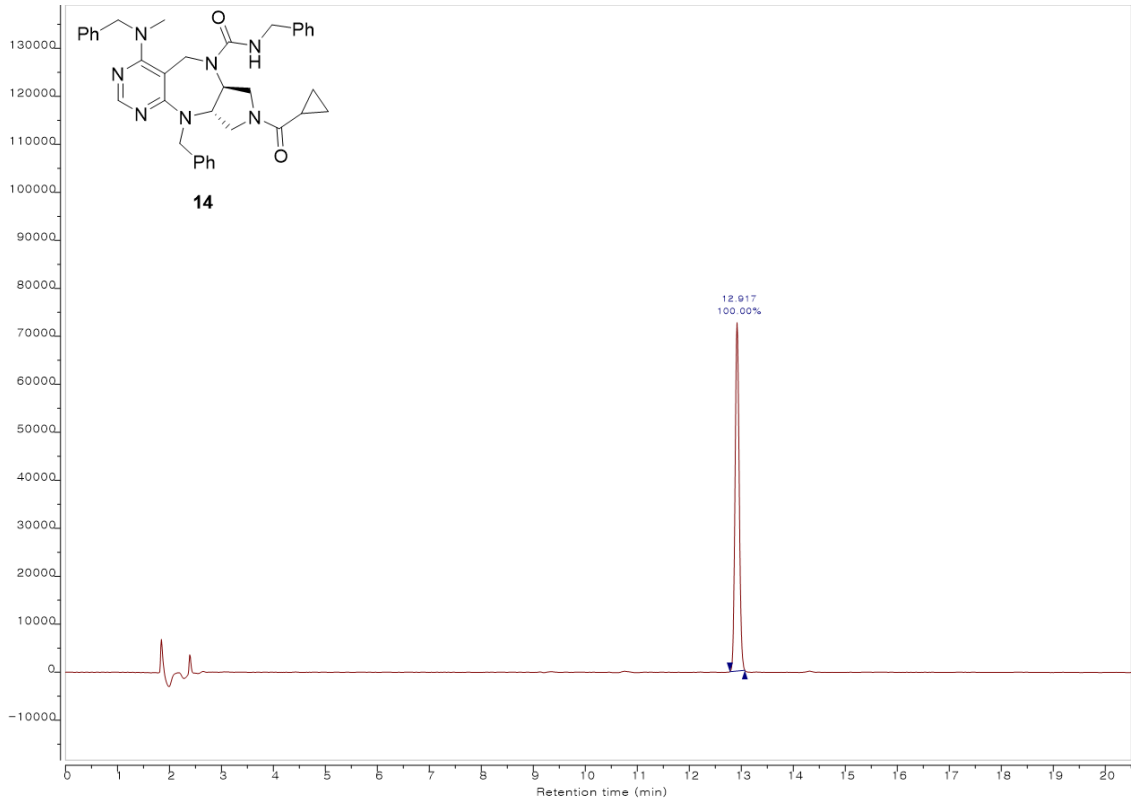
Retention time (min)


14
12.917
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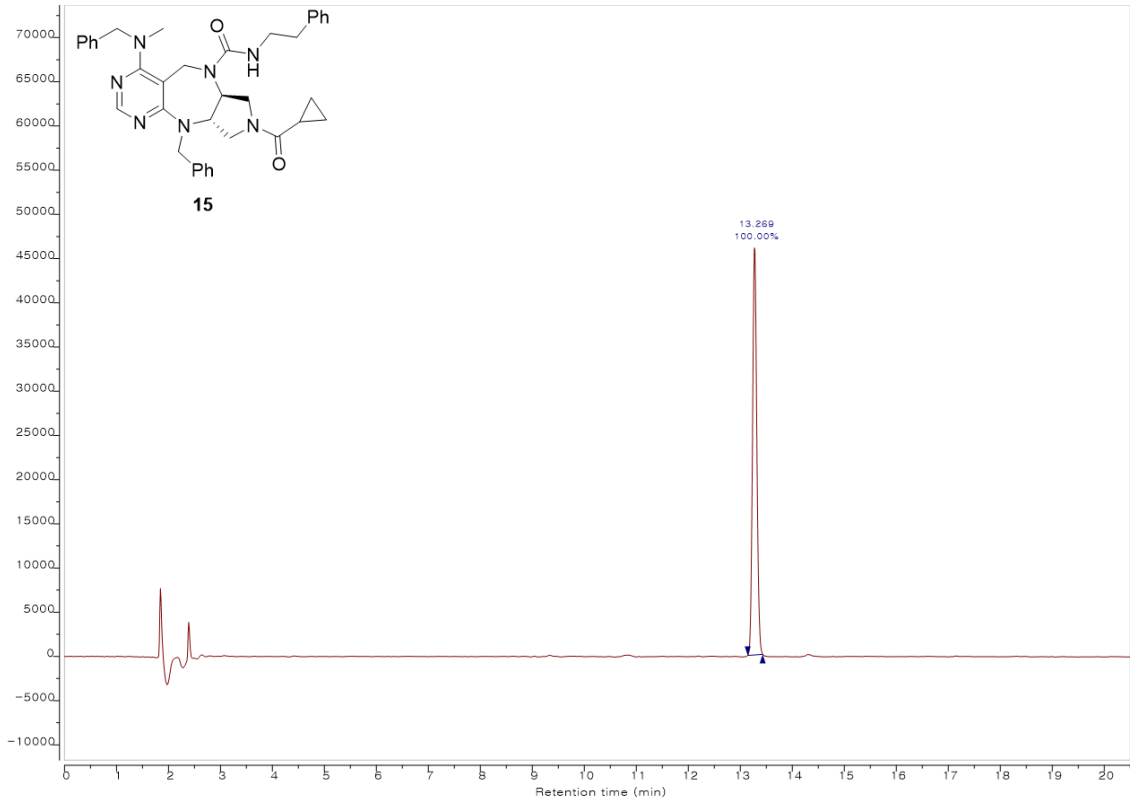
Retention time (min)


15
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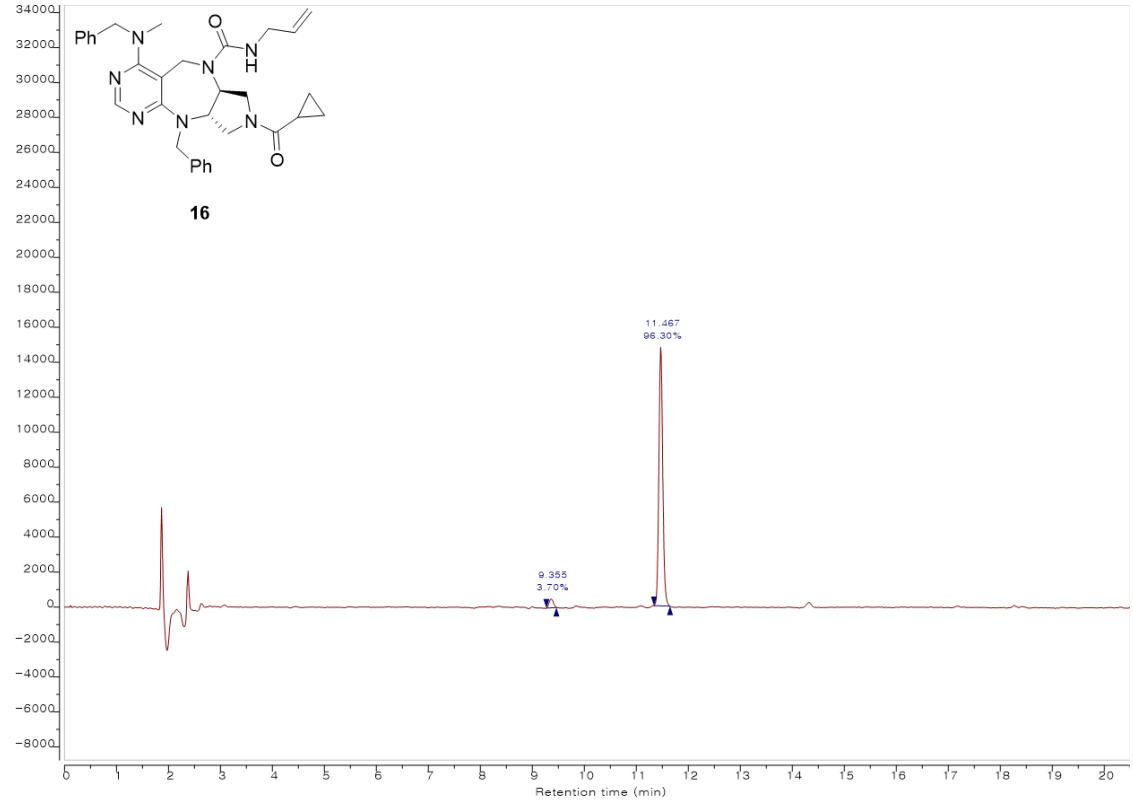
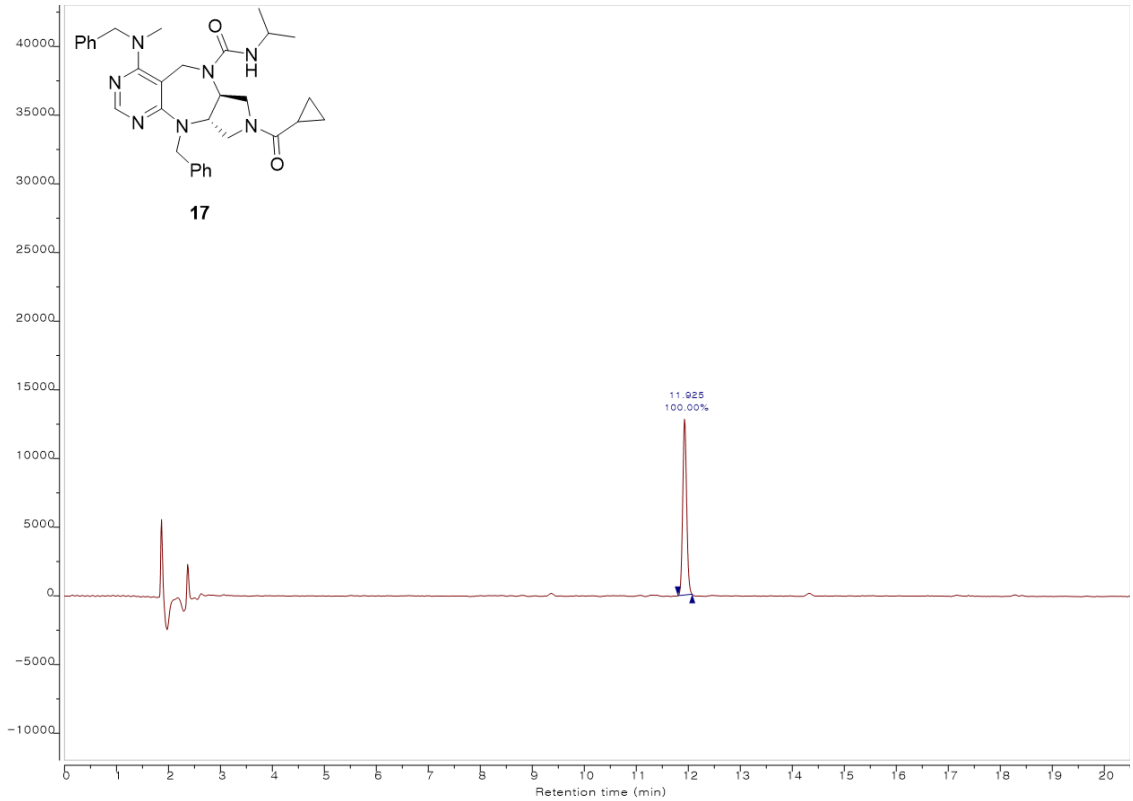
Retention time (min)


16
11.467
96.30%
9.355
3.70%
Retention time (min)


17
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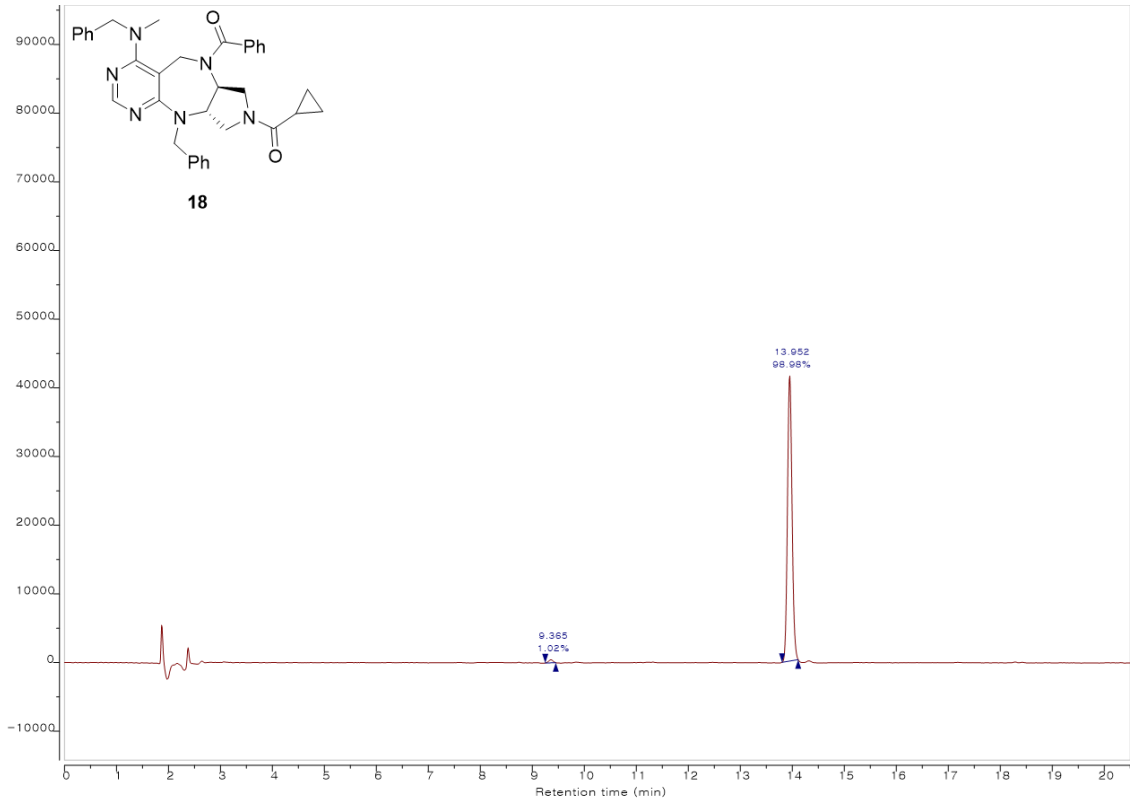
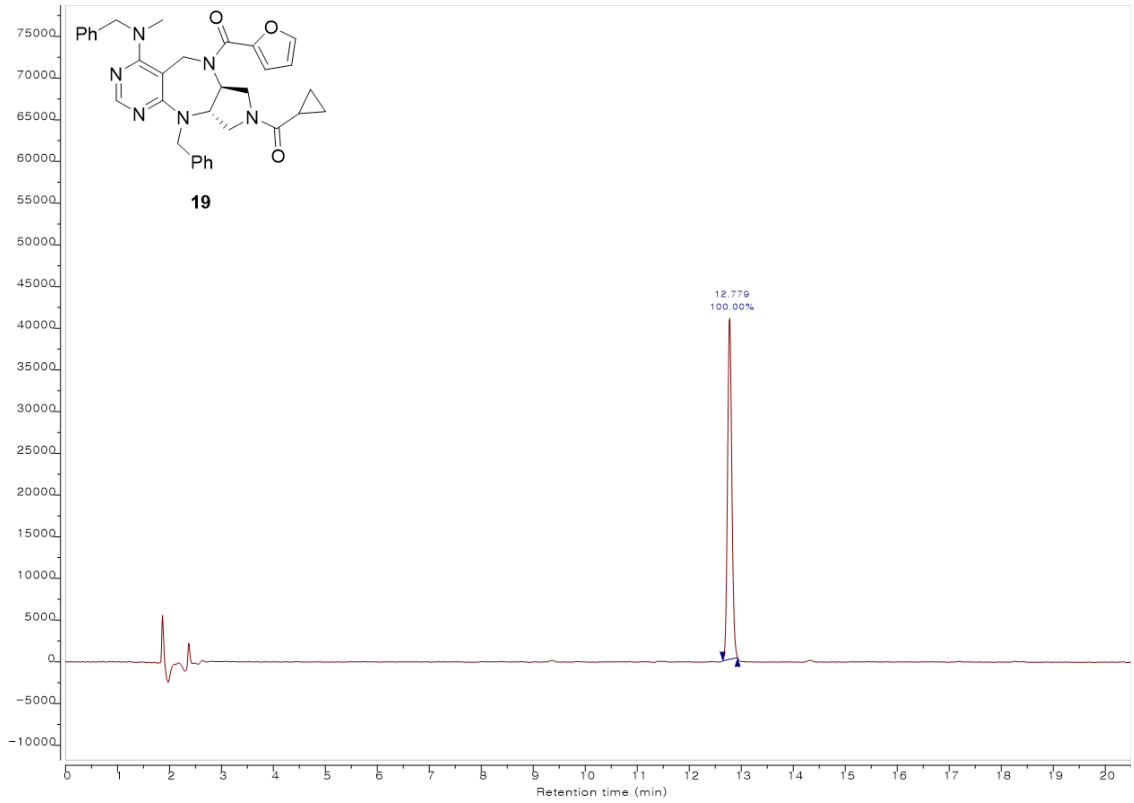
Retention time (min)


18
13.952
98.98%
9.365
1.02%
Retention time (min)


19
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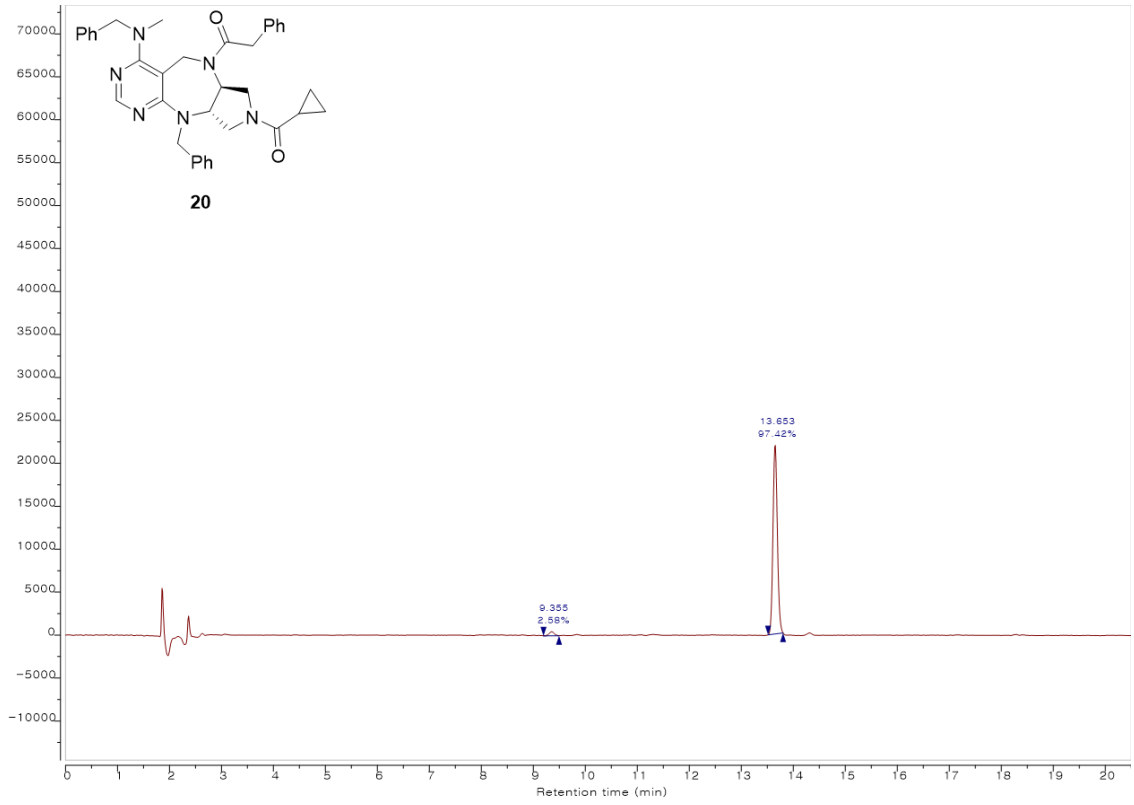
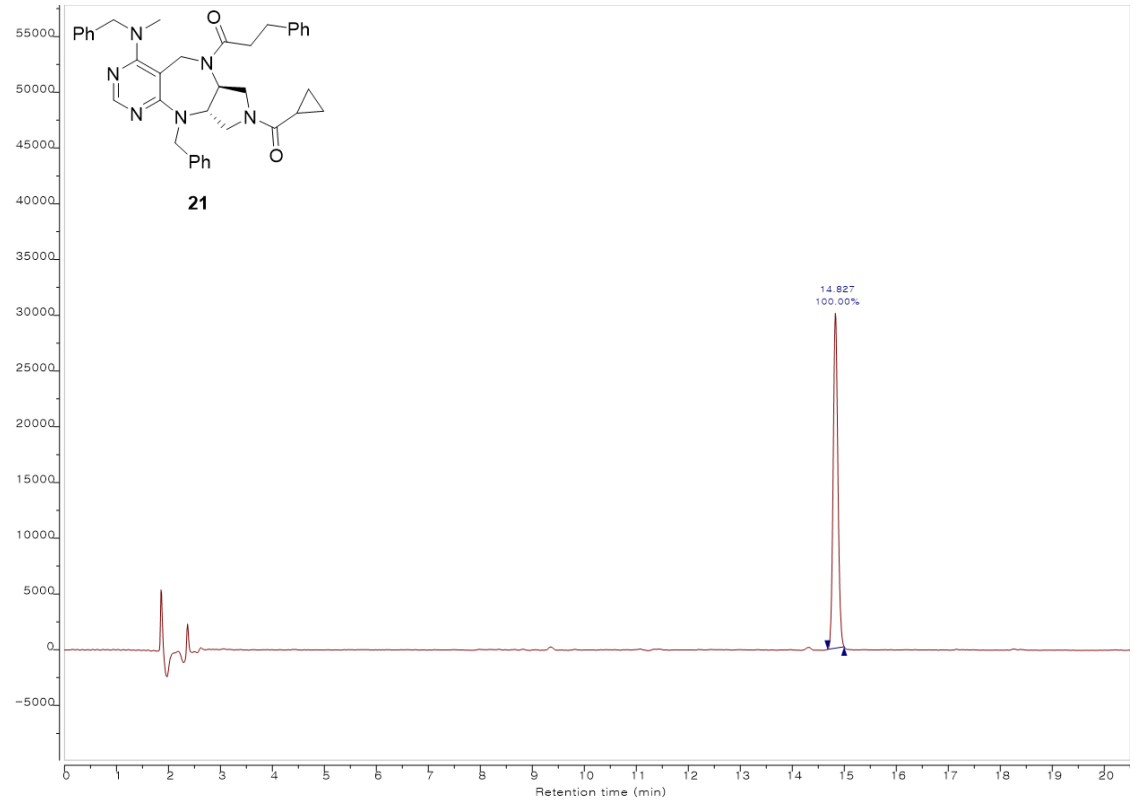
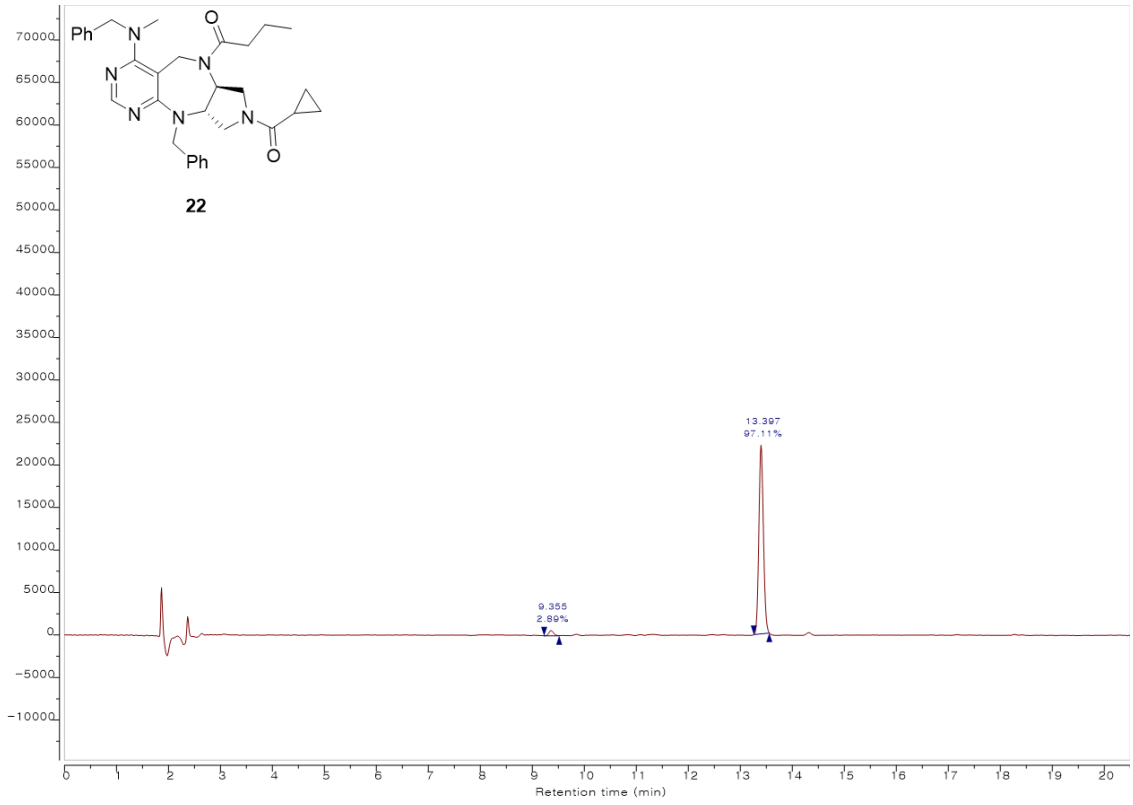
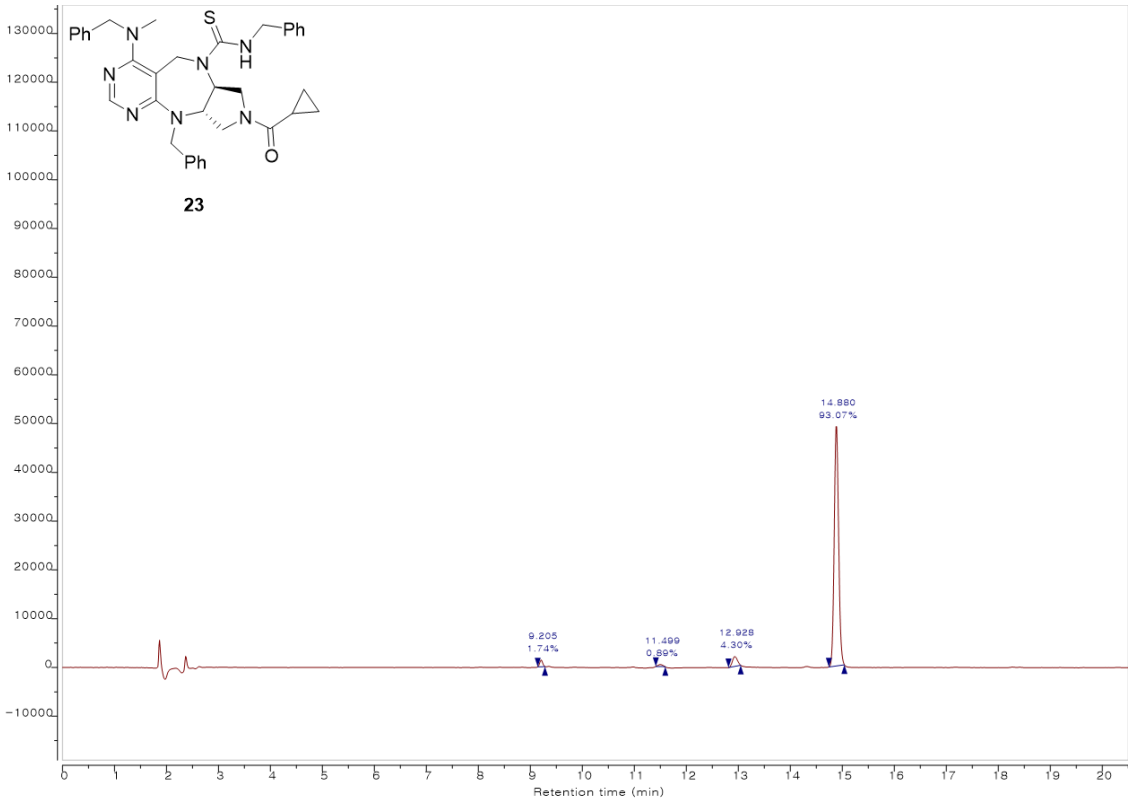
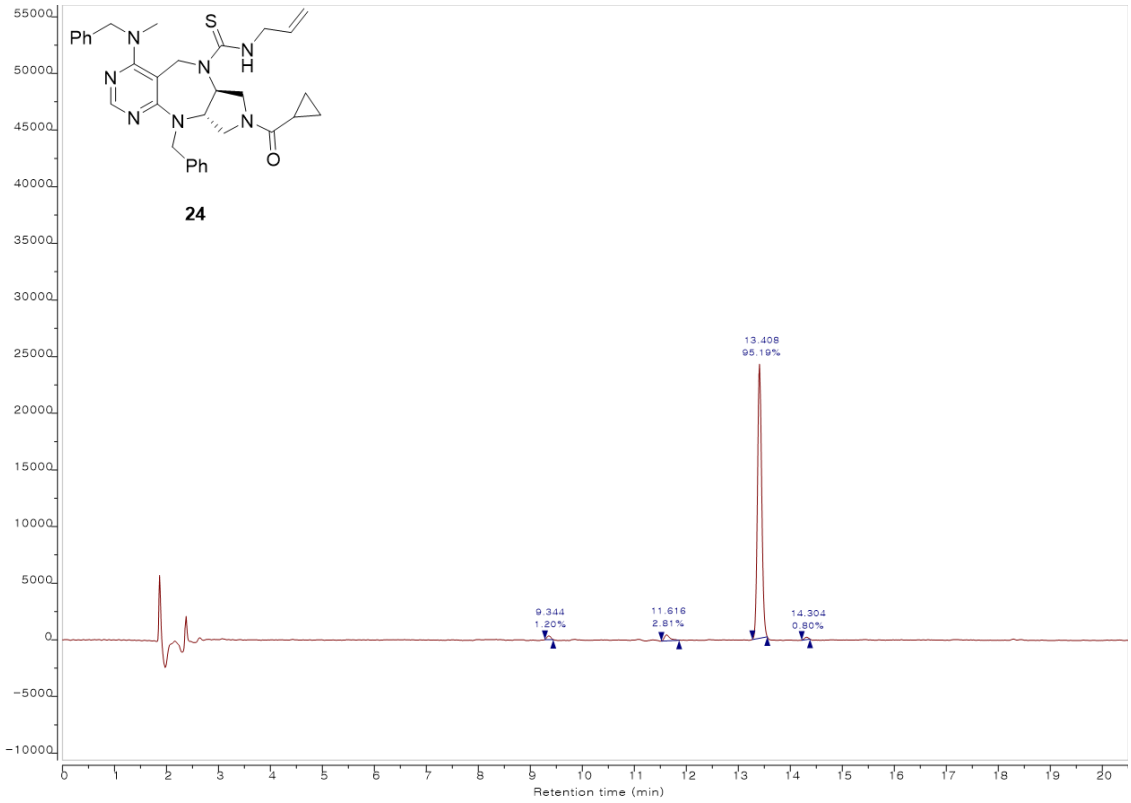

20
13.653
97.42%
9.355
2.58%
Retention time (min)


21
14.827
100.00%
Retention time (min)


22
13.397
97.11%
9.355
2.89%
Retention time (min)


Ph
N
S
N
H
Ph
N
N
N
N
N
O
Ph
23
14.880
93.07%
9.205
1.74%
11.499
0.89%
12.928
4.30%
Retention time (min)


Ph
N
S
N
H
N
N
N
N
O
Ph
24
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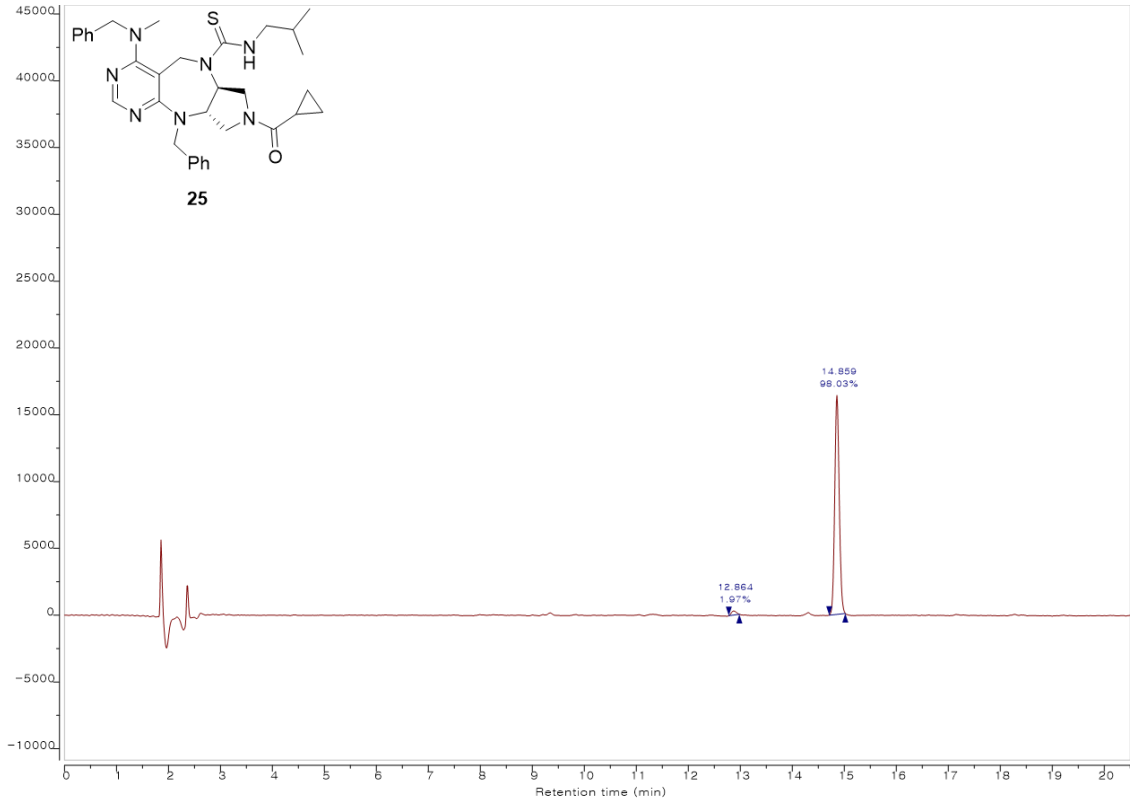
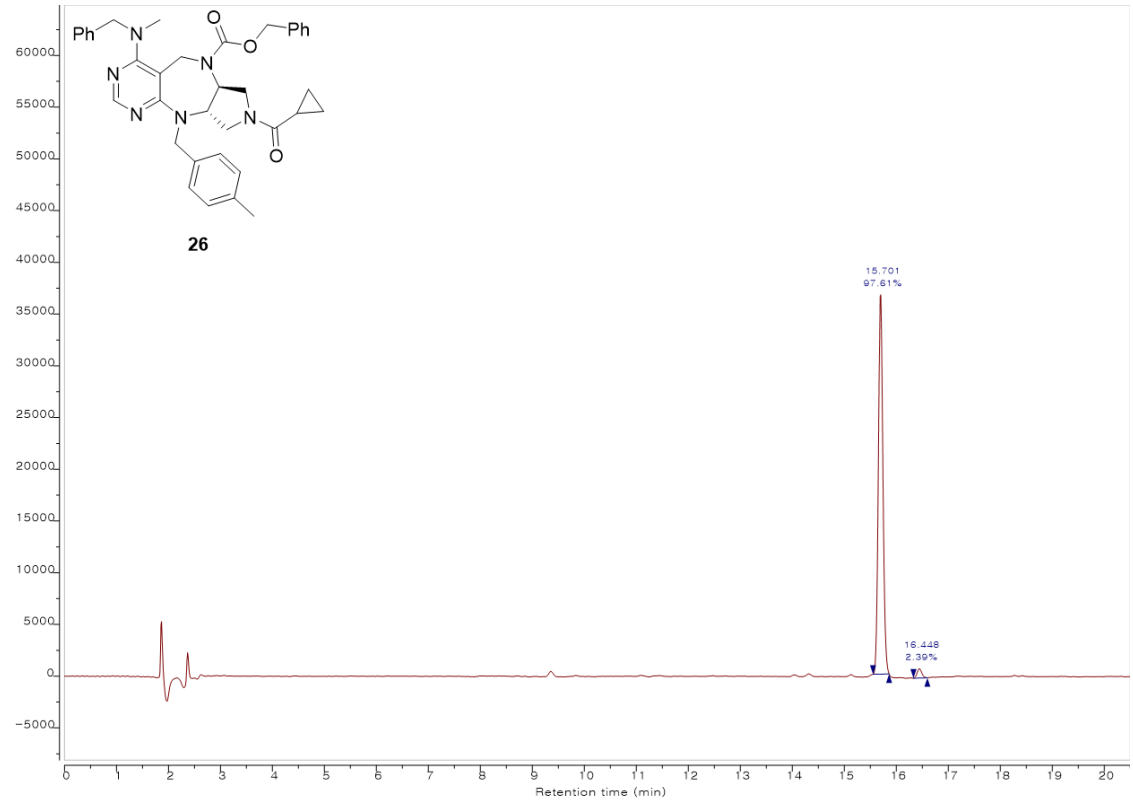
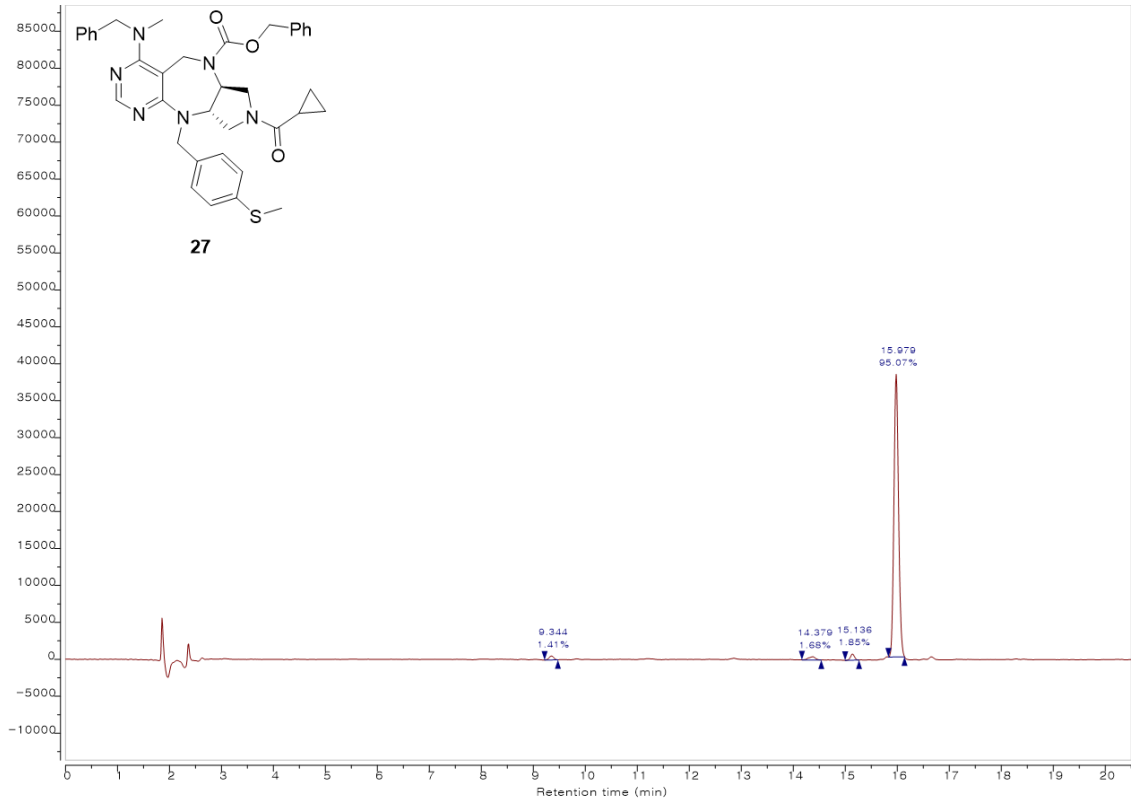
95.19%
9.344
1.20%
11.616
2.81%
14.304
0.80%
Retention time (min)


25
14.859
98.03%
12.864
1.97%
Retention time (min)


26
15.701
97.61%
16.448
2.39%
Retention time (min)


27
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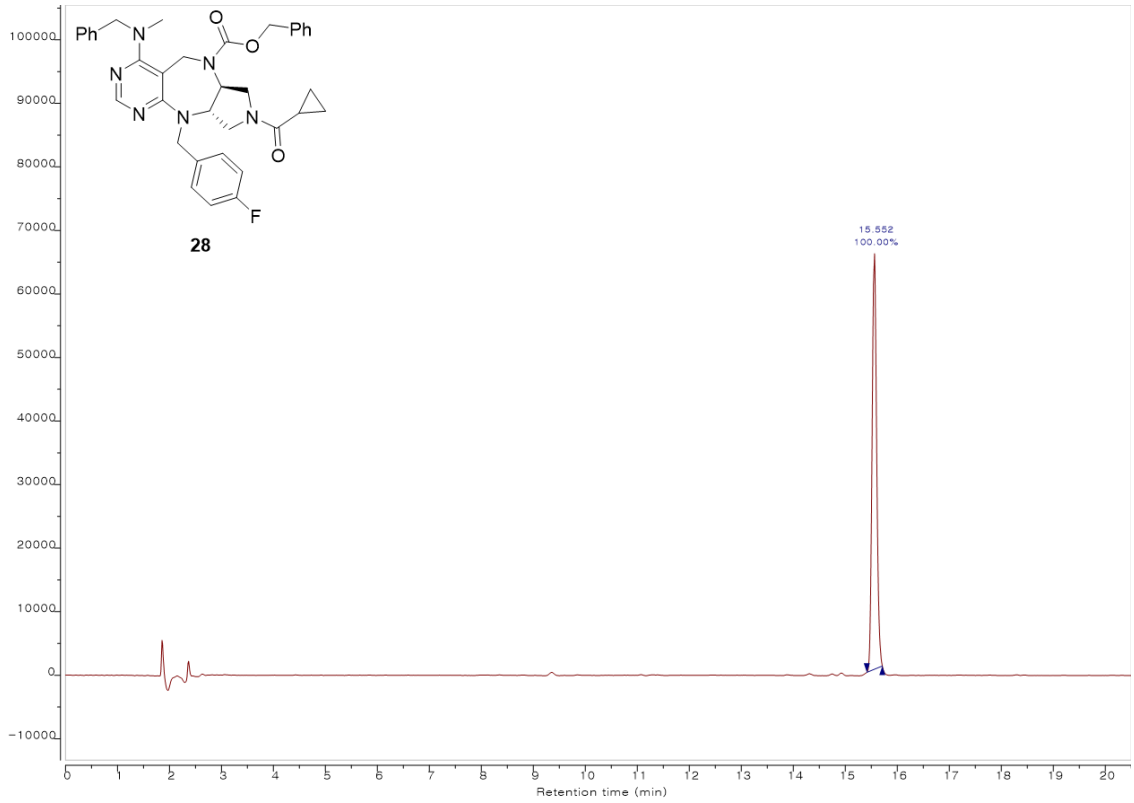
95.07%
9.344
1.41%
14.379
1.68%
15.136
1.85%
Retention time (min)


28
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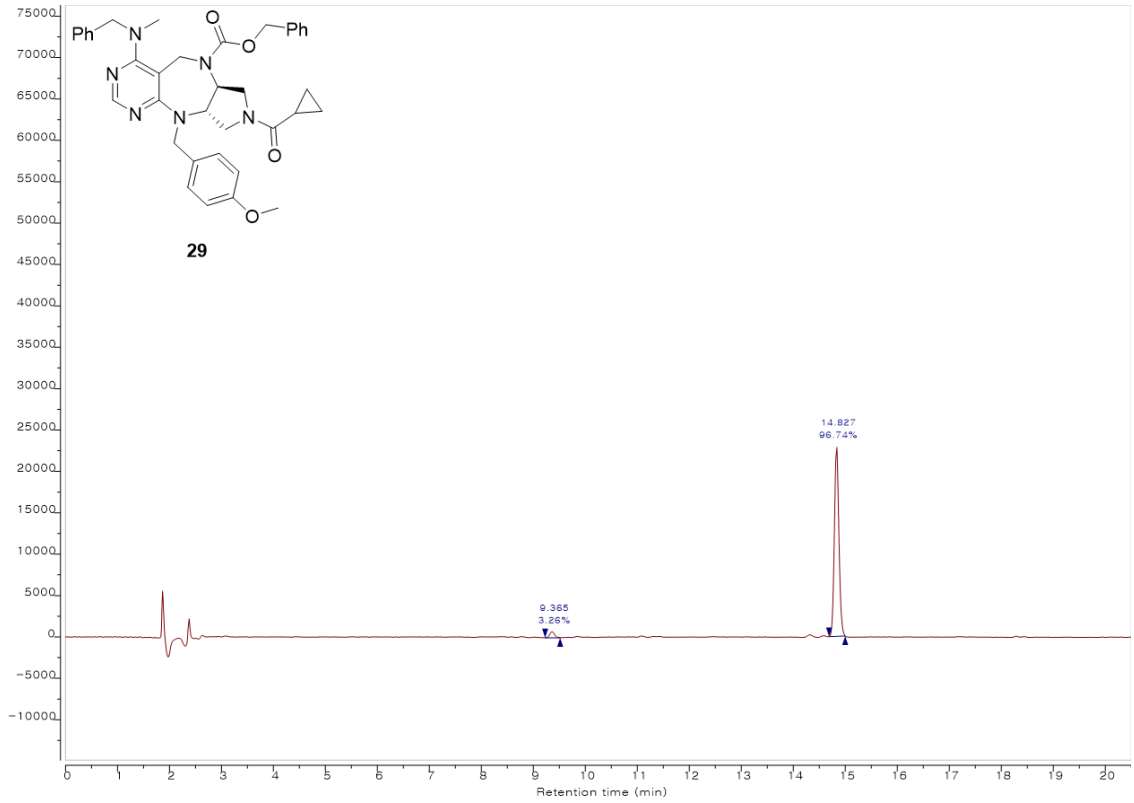
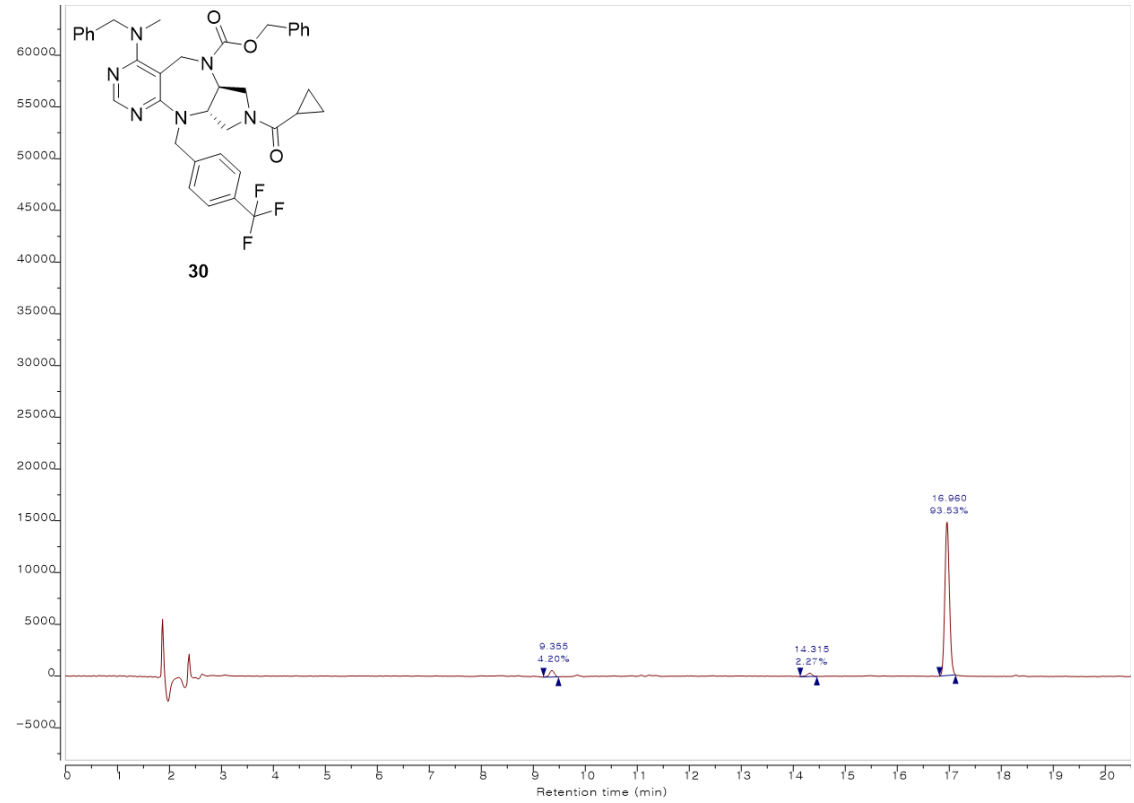
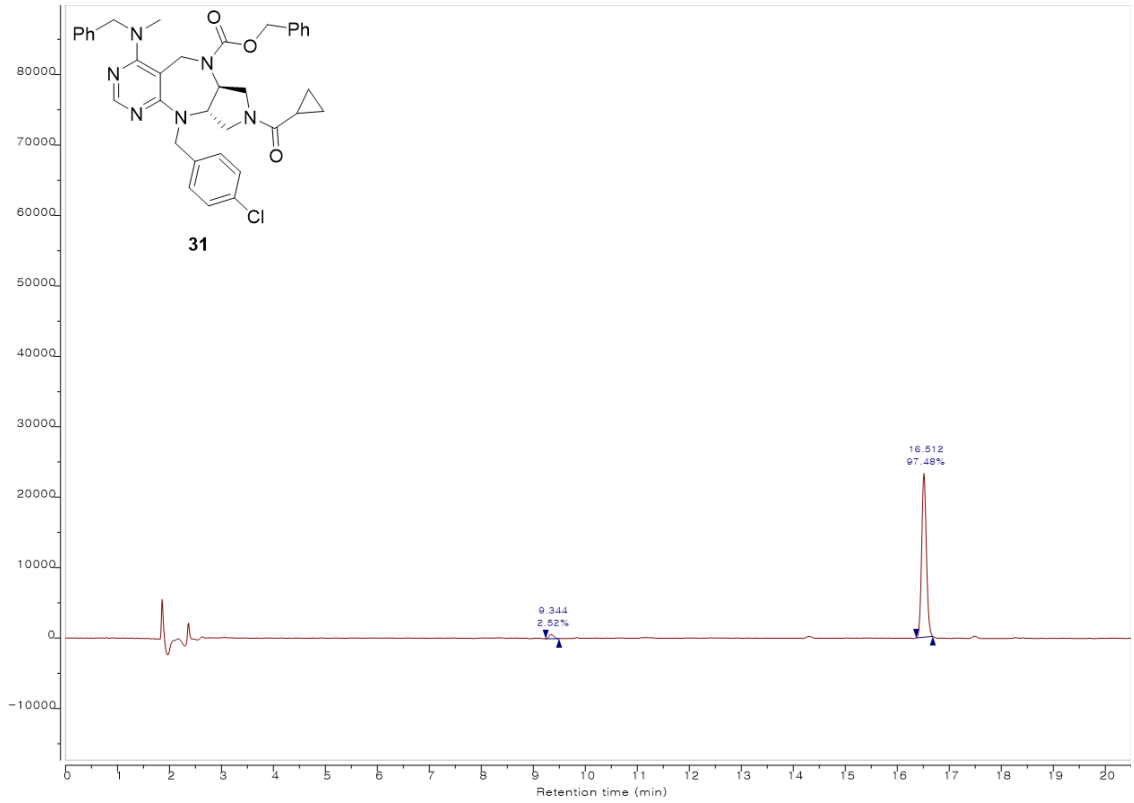
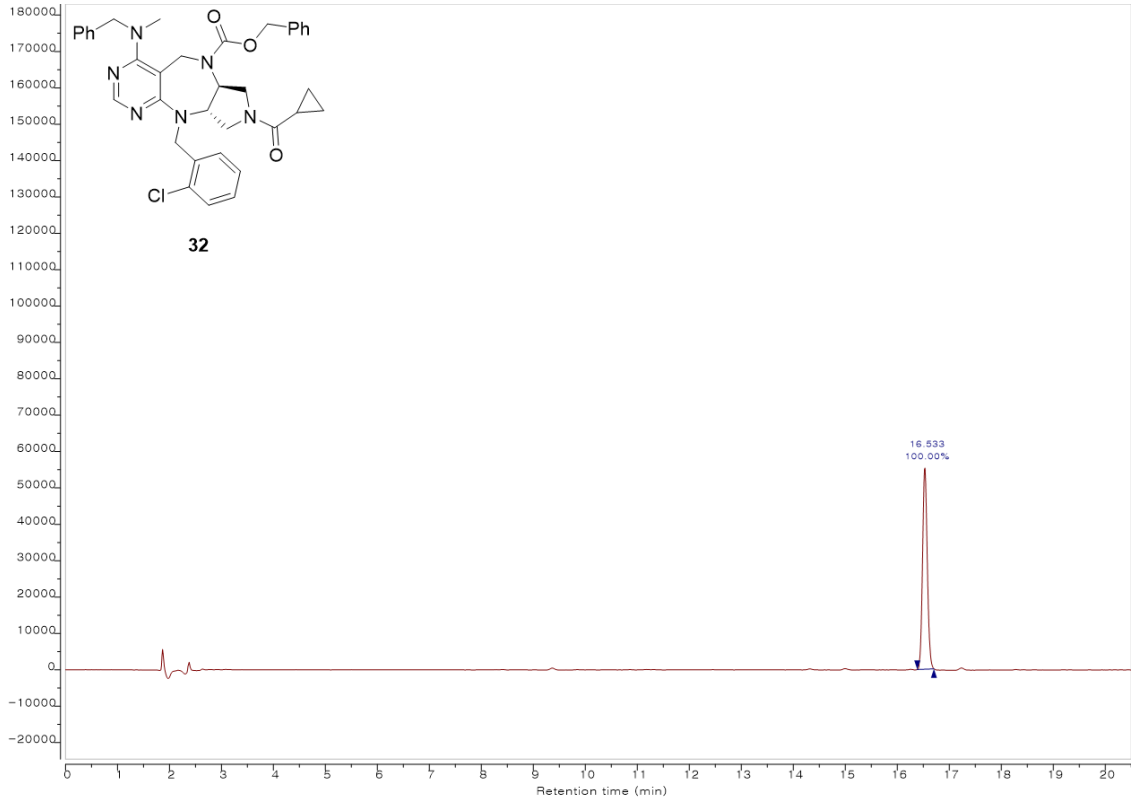
Retention time (min)


29
14.827
96.74%
9.365
3.26%
Retention time (min)


30
16.960
93.53%
9.355
4.20%
14.315
2.27%
Retention time (min)


Ph
N
O
O
Ph
N
N
N
N
N
O
Cl
31
16.512
97.48%
9.344
2.52%
Retention time (min)


Ph
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O
O
Ph
N
N
N
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N
O
Cl
32
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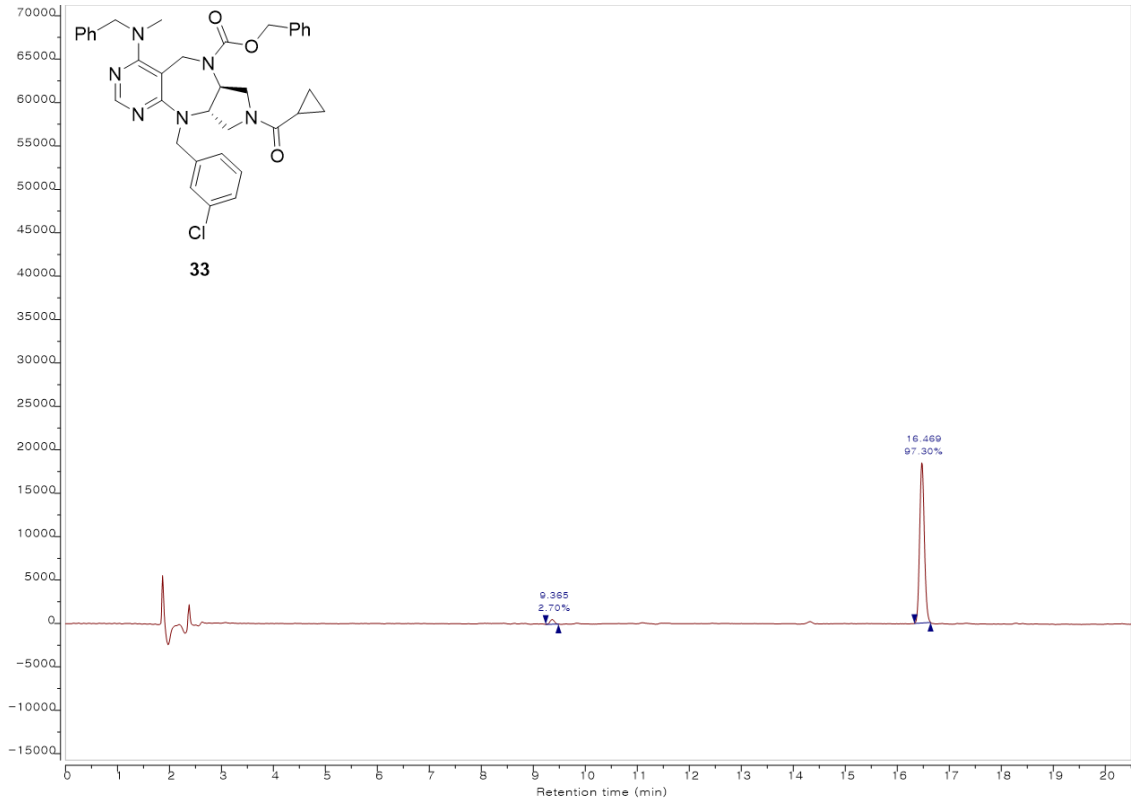
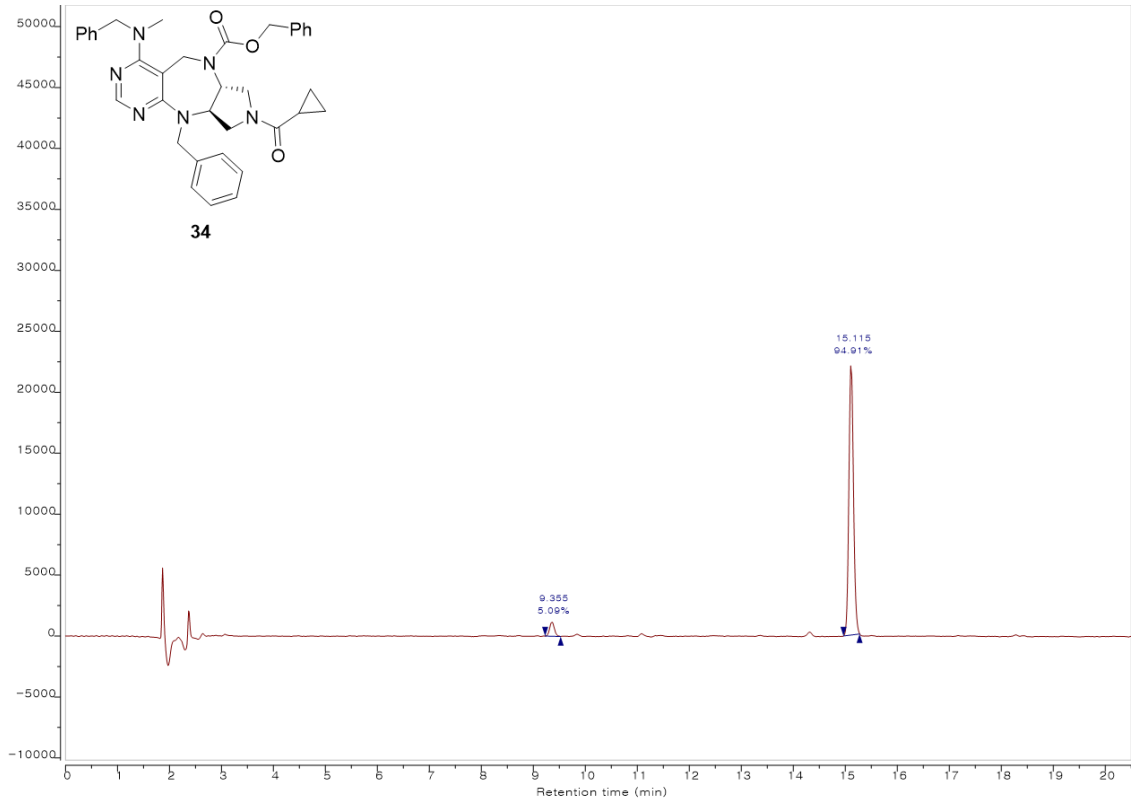
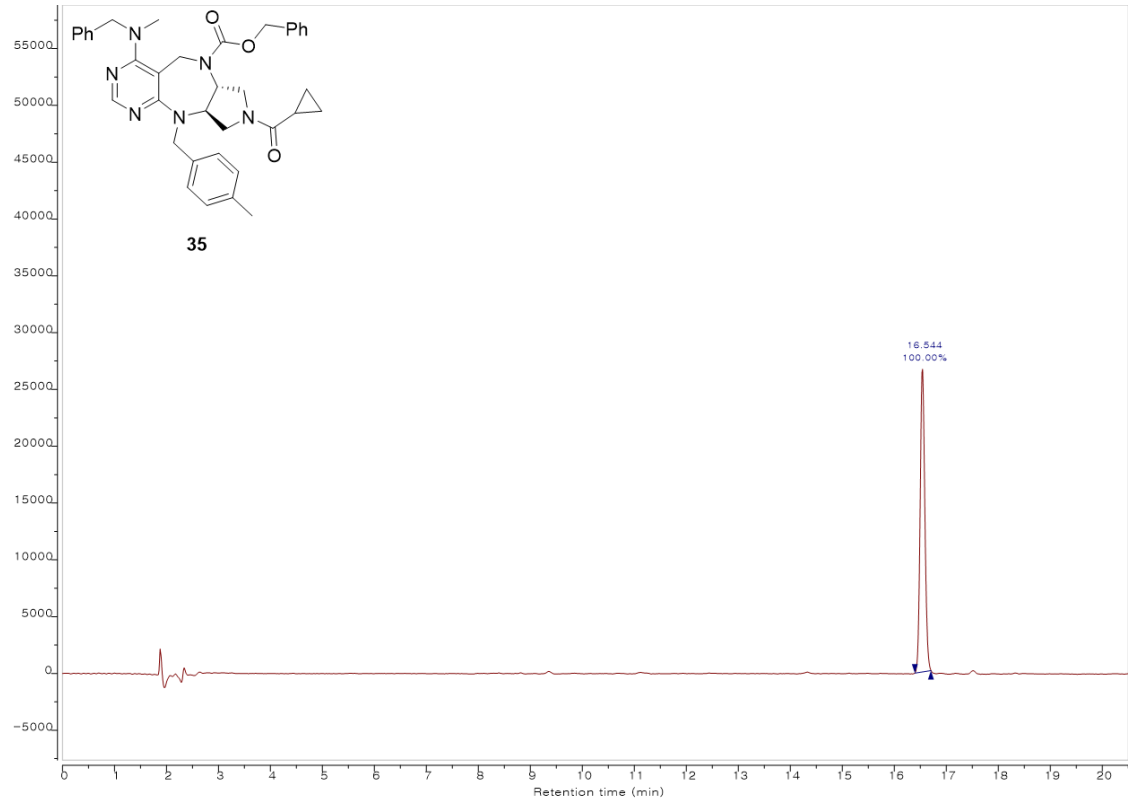
Retention time (min)


Ph
N
O
O
Ph
N
N
N
N
N
O
Cl
33
16.469
97.30%
9.365
2.70%
Retention time (min)


Ph
N
O
O
Ph
N
N
N
N
N
O
34
15.115
94.91%
9.355
5.09%
Retention time (min)


35
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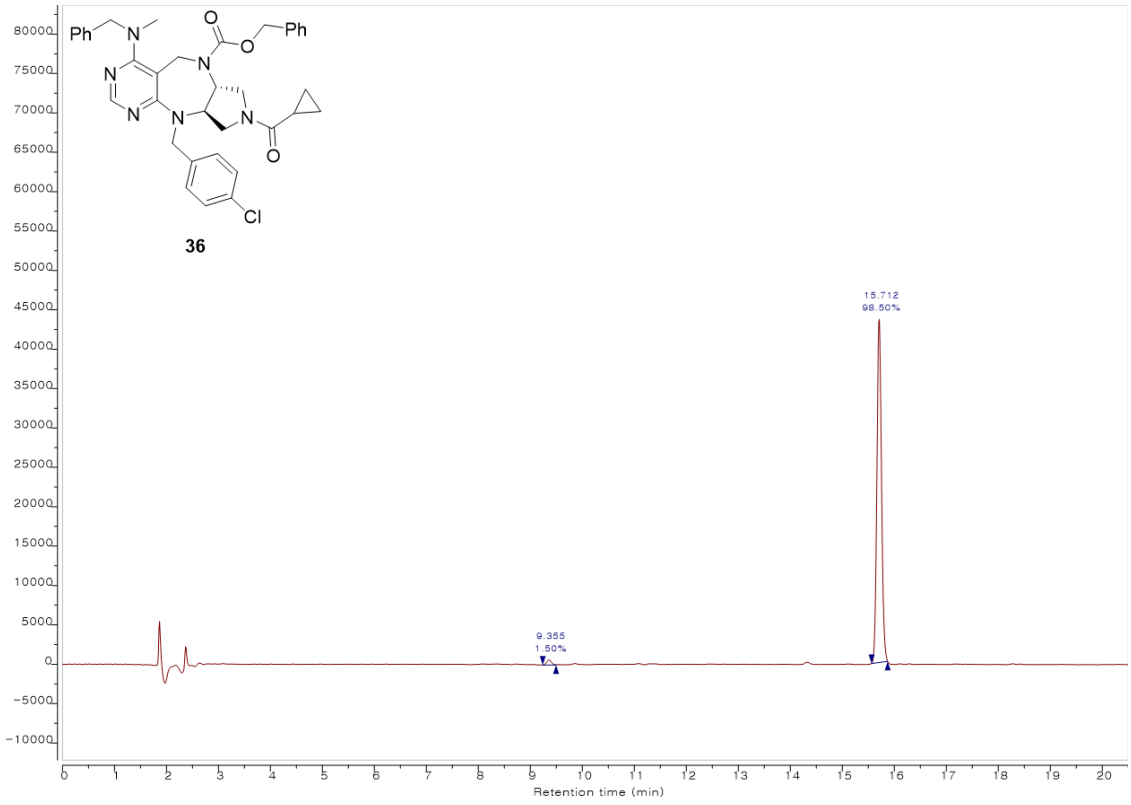
Retention time (min)


36
15.712
98.50%
9.355
1.50%
Retention time (min)