Supporting Information

Diversity-Oriented Submonomer Synthesis of Azapeptide Mediated by Mitsunobu Reaction

Chuan Dai, Jun Ma, Min Li, Wen Wu, Xuefeng Xia,* Jinqiang Zhang*

Innovative Drug Research Centre (IDRC), Chongqing Key Laboratory of Natural Product Synthesis and Drug Research, School of Pharmaceutical Sciences, Chongqing University, Chongqing 401331, P. R. China

Table of contents

1. Experimental Section

1.1 General experimental procedures.

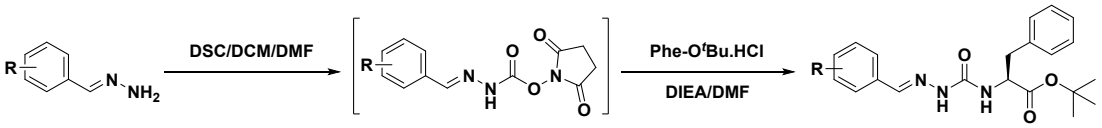
Chemicals were used as received from commercial sources without further purification unless stated otherwise. Wang resin (0.96 mmol/g) and Rink amide resin (0.67 mmol/g) was purchased from GL Biochem (Shanghai) Ltd, and the manufacturer's reported loading of the resin was used in the calculation of the yields of the final products. Reagents including 2-nitrobenzaldehyde, hydrazine hydrate, *N,N'*-Disuccinimidyl carbonate (DSC), *N,N*-diisopropylethylamine (DIEA), triphenylphosphine, diisopropyl azodicarboxylate (DIAD), sodium dithionite, tetrabutylammonium hydrogen sulfate, hydroxylamine hydrochloride, *m*-phenylenediamine, bistrichloromethyl carbonate (BTC), 2,4,6-collidine, *p*-hydroxybenzaldehyde, tert-butyldimethylsilyl chloride, imidazole, lithium aluminium hydride, 3-aminopropan-1-ol, di-*tert*-butyl dicarbonate piperidine, pyridine, 4-dimethylaminopyridine (DMAP), acetic anhydride, formic acid (FA), isopropanol, benzyl alcohol, cyclopropyl carbinol and anhydrous solvents tetrahydrofuran (THF) were purchased from Adamas-beta®. Fmoc amino acids and coupling reagents including HOBT and diisopropylcarbodiimide (DIC) were purchased from GL Biochem (Shanghai) Ltd. All solvents were obtained from Chronchem™. Analyses by LC-MS were performed on a Waters™ Acquity SQD Series instrument with ESI ion-source, single quadropole mass detection and positive and negative mode ionization. Analyses of crude peptide samples and purified peptide were determined with a Phenomenex Aeris™ C_{18} column (pore size: 100 Å, particle size: 2.6 μm; 75× 4.6 mm) with a flow rate of 0.5 mL/min using a proper linear gradient MeOH in water containing 0.1% FA or Phenomenex Aeris™ C_{18} column (pore size: 100 Å, particle size: 5 μm; 150 × 4.6 mm) with a flow rate of 0.8 mL/min using a proper linear gradient of CH_3CN or MeOH in water containing 0.1% FA. Preparative RP-HPLC was conducted on a Shimadzu™ LC-20AP instrument with a reverse-phase Phenomenex Aeris™ C_{18} column (pore size: 100 Å, particle size: 5μm; 150 × 21.2. mm) at a flow rate of 10 mL/min and monitored with a UV detector at 220 nm and 254 nm. Linear gradient of 10-60% of MeOH in water containing 0.1% FA was used for purification of the peptides. Chiral HPLC analyses of samples were determined with a Chiralcel IA-H with a flow rate of 1 mL/min or 0.5 mL/min using a proper isocratic elution i-PrOH in Hexane. HRMS of the purified peptides was performed on a Bruker™ solariX FTMS mass spectrometer with ESI ion-source.

Fmoc-based peptide syntheses were performed under standard conditions on an automated shaker using Wang resin (0.96 mmol/g) or Rink amide resin. Couplings of amino acids (3 equiv) were performed in DMF using HOBT (3 equiv) and DIC (3 equiv) as coupling reagent. Fmoc deprotections were performed by treating the resin with 20% piperidine in DMF for 30 min. Resin was washed after each coupling and deprotection step sequentially with DMF (3 × 10 mL), MeOH (3 × 10 mL), and DCM (3 × 10 mL).

Cleavage test of resin-bound peptide After Fmoc group removal, a sample of peptide bound resin (3-5 mg) was treated with a freshly made solution of TFA/H_2O/TES (95:2.5:2.5, v/v/v, 0.5 mL) for 1 h at room temperature. The cleavage mixture was filtered and then concentrated and crude peptide was precipitated with cold ether (1.5 mL). Crude peptide samples were agitated on a vortex shaker, and spun in a centrifuge. Decantation of the supernatant left a pellet, which was dissolved in 20% MeCN/H_2O (1 mg/mL) and subjected to LC-MS analysis.

Deprotection and cleavage of aza-peptide from the resin After Fmoc group removal, Wang resin-bound peptide was deprotected and cleaved from the support using a freshly made solution of TFA/H_2O/TES (95:2.5:2.5, v/v/v, 20 mL/g of peptide resin) at rt for 2 h. The resin was filtered and rinsed with 2 mL of TFA. The filtrate and rinses were concentrated until a crude oil persisted, from which a precipitate was obtained by addition of cold ether (10-15 mL). After centrifugation (1200 rpm for 10 min), the supernatant was removed and the crude peptide was taken up in aqueous acetonitrile (10% v/v) and freeze-dried to a solid prior to analysis and purification.

1.2 Synthesis of aza-dipeptide 1b-d.



1b-d

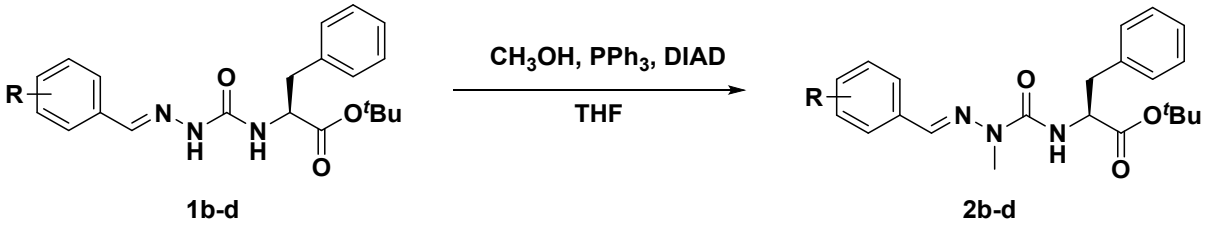
1b: R = *o*-NO_2
1c: R = *m*-NO_2
1d: R = *p*-NO_2

***Tert*-butyl (2-(2-nitrobenzylidene)hydrazine-1-carbonyl)-L-phenylalaninate 1b:** A solution of *N,N'*-disuccinimidyl carbonate (2.57 g, 10 mmol) in 40 mL of DMF/DCM (1:1, v/v) was treated dropwise with a solution of 2-nitrobenzylidene hydrazine (1.65 g, 10 mmol) in 10 mL of DCM over 15 min, stirred for 2 h at rt. Then L-Phe-O^{t}Bu·HCl (2.58 g, 10 mmol) was added. The mixture was treated with DIEA (4.96 mL, 30 mmol), stirred for 12 h at rt. After reaction completed, the mixture was washed sequentially with 1M $KHSO_4$, saturated $NaHCO_3$ and brine. The volatiles were evaporated under reduced pressure and the residue was purified by Al_2O_3 column chromatography (EtOAc/hexanes, 1:3 to 3:1, v/v). **1b** was obtained as yellow soild (2.00 g, 48%): mp 117.4-117.8 ℃; R_f = 0.26 (EtOAc/hexanes, 1:3, v/v); $[\alpha]_D^{20}$ +9.53 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.21 (s, 1H), 8.28 (s, 1H), 8.02 (dd, *J* = 8.2, 1.2 Hz, 1H), 7.94 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.62 (t, *J* = 7.7 Hz, 1H), 7.50 (ddd, *J* = 8.4, 7.3, 1.4 Hz, 1H), 7.34 – 7.21 (m, 5H), 6.59 (d, *J* = 8.3 Hz, 1H), 4.79 (dt, *J* = 8.1, 5.9 Hz, 1H), 3.25 – 3.12 (m, 2H), 1.42 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.83, 154.84, 147.90, 136.27, 133.25, 129.75, 129.64, 128.86, 128.35, 128.23, 126.87, 124.78, 82.27, 53.95, 38.72, 27.97; HRMS m/z calcd for $C_{21}H_{24}N_4NaO_5$ $[M+Na]^+$ 435.16389, found 435.16538.

***Tert*-butyl (2-(3-nitrobenzylidene)hydrazine-1-carbonyl)-L-phenylalaninate 1c:** was synthesized using DSC (2.57 g, 10 mmol), 3-nitrobenzylidene hydrazine (1.65 g, 10 mmol), L-Phe-O^{t}Bu·HCl (2.58 g, 10 mmol) and DIEA (4.96 mL, 30 mmol) according to the procedure described above for **1b**. The crude product was purified by Al_2O_3 column chromatography (EtOAc/hexanes, 3:1 to 1:3, v/v). **1c** was obtained as white solid (2.63 g, 64%): mp 159.8-160.3 ℃; R_f = 0.46 (EtOAc/hexanes, 1:1, v/v); $[\alpha]_D^{20}$ -15.3 (*c* 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.75 (d, *J* = 4.5 Hz, 1H), 8.37 (t, *J* = 2.0 Hz, 1H), 8.20 (ddd, *J* = 8.2, 2.4, 1.1 Hz, 1H), 7.89 (dt, *J* = 7.7, 1.3 Hz, 1H), 7.78 (s, 1H), 7.56 (t, *J* = 8.0 Hz, 1H), 7.39 – 7.25 (m, 5H), 6.58 (d, *J* = 8.3 Hz, 1H), 4.77 (dt, *J* = 8.3, 6.0 Hz, 1H), 3.27 – 3.14 (m, 2H), 1.45 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.65, 155.48, 148.63, 138.54, 136.14, 135.85, 132.09, 129.65, 129.52, 128.51, 127.11, 123.96, 121.45, 82.28, 54.05, 38.61, 27.99; HRMS m/z calcd for $C_{21}H_{24}N_4NaO_5$ $[M+Na]^+$ 435.16389, found 435.16517.

***Tert*-butyl (2-(4-nitrobenzylidene)hydrazine-1-carbonyl)-L-phenylalaninate 1d:** was synthesized using DSC (2.57 g, 10 mmol), 4-nitrobenzylidene hydrazine (1.65 g, 10 mmol), L-Phe-O^{t}Bu·HCl (2.58 g, 10 mmol) and DIEA (4.96 mL, 30 mmol) according to the procedure described above for **1b**. The crude product was purified by Al_2O_3 column chromatography (EtOAc/hexanes, 3:1 to 1:3, v/v). **1c** was obtained as yellow soild (1.98 g, 48%): mp 164.8-165.3 ℃, R_f = 0.46 (EtOAc/hexanes, 1:1, v/v); $[\alpha]_D^{20}$ +8.7 (c 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.90 (s, 1H), 8.19 (d, J = 8.3 Hz, 2H), 7.81 (s, 1H), 7.67 (d, J = 8.4 Hz, 2H), 7.25 (t, J = 10.1 Hz, 4H), 6.68 (d, J = 8.2 Hz, 1H), 4.76 (q, J = 6.5 Hz, 1H), 3.19 (tt, J = 13.9, 6.7 Hz, 2H), 1.43 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.64, 155.67, 148.04, 140.15, 138.56, 136.12, 129.58, 128.43, 127.21, 127.08, 123.98, 82.34, 53.94, 38.65, 27.99; HRMS m/z calcd for $C_{21}H_{24}N_4NaO_5$ $[M+Na]^+$ 435.16389, found 435.16550.

1.3 Synthesis of azadipeptide 2b-2d using Mitsunobu Reaction

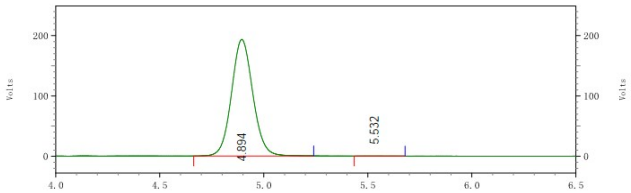


1b-d **2b-d**

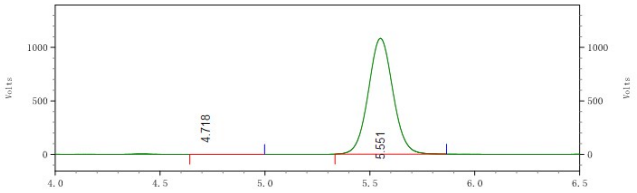
Representative synthesis of azadipeptide using Mitsunobu reaction in solution:

***Tert*-butyl (1-methyl-2-(2-nitrobenzylidene)hydrazine-1-carbonyl)-L-phenylalaninate 2b**: To a round-bottomed flask was added triphenylphosphine (525 mg, 2 mmol) in 3 mL of THF under nitrogen. The solution is cooled to 0 ℃ and sequentially added a solution of **1b** (413 mg, 1mmol) in THF (1mL), DIAD (394 μL, 2 mmol) in THF (500 μL), and Methanol (122 μL, 3mmol) in THF (500 μL). The mixture was stirred at rt for 5 h. The volatiles were evaporated under reduced pressure and the residue was purified by Al_2O_3 column chromatography (EtOAc/hexanes, 1:3 to 3:1, v/v). **2b** was obtained as yellow oil (305 mg, 71%): R_f = 0.45

(EtOAc/hexanes, 1:3, v/v); $[\alpha]_D^{20}$ +2.30 (*c* 0.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.05 (s, 1H), 8.01 (dd, *J* = 8.2, 1.3 Hz, 1H), 7.82 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.61 (td, *J* = 7.6, 1.3 Hz, 1H), 7.48 (ddd, *J* = 8.5, 7.4, 1.4 Hz, 1H), 7.32 – 7.26 (m, 2H), 7.25 – 7.20 (m, 3H), 6.99 (d, *J* = 8.3 Hz, 1H), 4.76 (dt, *J* = 8.3, 5.9 Hz, 1H), 3.38 (s, 3H), 3.21 (dd, *J* = 13.8, 5.6 Hz, 1H), 3.12 (dd, *J* = 13.8, 6.3 Hz, 1H), 1.42 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.94, 154.71, 148.03, 136.39, 133.26, 131.69, 129.60, 129.27, 128.39, 127.95, 126.85, 124.82, 82.05, 54.52, 38.59, 28.70, 27.99; HRMS m/z calcd for $C_{22}H_{26}N_4NaO_5$ $[M+Na]^+$ 449.17954, found 449.18130; Chiral HPLC analysis: Chiralcel IA-H (Hexane/*i*-PrOH) = 70:30, flow rate = 1.0 mL/min, wave length = 254 nm, t_R = 4.894 min (**2b**), t_R =5.551 min (enantiomer of **2b**).



UV-WL1 RetTime (min)	Width (min)	Height (Volts)	Area	Area (%)
4.894	0.58	773340	5504885	99.951
5.532	0.24	364	2718	0.049

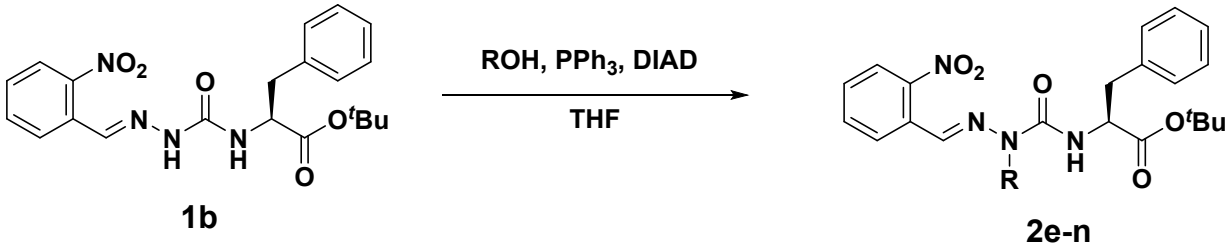


UV-WL1 RetTime (min)	Width (min)	Height (Volts)	Area	Area (%)
4.718	0.36	1110	9982	0.028
5.551	0.53	4331487	35291487	99.972

***Tert*-butyl (1-methyl-2-(3-nitrobenzylidene)hydrazine-1-carbonyl)-L-phenylalaninate 2c** was synthesized using azadipeptide **1c** (413 mg, 1mmol), triphenylphosphine (525 mg, 2 mmol), DIAD (394 µL, 2 mmol) and Methanol (122 µL, 3mmol) according to the procedure described above for **2b**. The crude product was purified by Al_2O_3 column chromatography (EtOAc/hexane, 3:1 to 1:3, v/v). **2c** was obtained as yellow oil (158 mg, 37%): R_f = 0.26 (EtOAc/hexanes, 1:3, v/v); $[\alpha]_D^{20}$ –11.0 (*c* 0.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.34 (t, *J* = 1.9 Hz, 1H), 8.21 – 8.14 (m, 1H), 7.85 (d, *J* = 7.7 Hz, 1H), 7.58 – 7.50 (m, 2H), 7.33 – 7.23 (m, 5H), 7.00 (d, *J* = 8.1 Hz, 1H), 4.73 (dt, *J* = 8.1, 6.0 Hz, 1H), 3.36 (s, 3H), 3.17 (qd, *J* = 13.8, 6.0 Hz, 2H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.91, 154.70, 148.67, 136.59, 136.34, 133.58, 131.71, 129.66, 129.49, 128.48, 126.97, 123.55, 121.58, 82.07, 54.67, 38.52, 28.54, 27.98; HRMS m/z calcd for $C_{22}H_{26}N_4NaO_5$ $[M+Na]^+$ 449.17954, found 449.18130.

***Tert*-butyl (1-methyl-2-(4-nitrobenzylidene)hydrazine-1-carbonyl)-L-phenylalaninate 2d** was synthesized using aza-dipeptide **1d** (413 mg, 1mmol), triphenylphosphine (525 mg, 2 mmol), DIAD (394 µL, 2 mmol) and Methanol (122 µL, 3mmol) according to the procedure described above for **2b**. The crude product was purified by Al_2O_3 column chromatography (EtOAc/hexanes, 3:1 to 1:3, v/v). **2d** was obtained as yellow oil (241 mg, 57%): R_f = 0.24 (EtOAc/hexanes, 1:3, v/v); $[\alpha]_D^{20}$ +6.00 (*c* 0.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.20 (d, *J* = 8.4 Hz, 2H), 7.62 (d, *J* = 8.4 Hz, 2H), 7.49 (s, 1H), 7.36 – 7.26 (m, 3H), 7.24 – 7.19 (m, 2H), 7.00 (d, *J* = 8.4 Hz, 1H), 4.74 (dt, *J* = 8.2, 5.7 Hz, 1H), 3.36 (s, 3H), 3.26 – 3.05 (m, 2H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.86, 154.59, 147.72, 140.77, 136.22, 133.44, 129.59, 128.48, 127.06, 127.02, 124.05, 82.18, 54.39, 38.44, 28.58, 28.00; HRMS m/z calcd for $C_{22}H_{26}N_4NaO_5$ $[M+Na]^+$ 449.17954, found 449.18072.

1.4 Synthesis of dipeptide 2e-n using Mitsunobu Reaction



***Tert*-butyl (1-isopropyl-2-(2-nitrobenzylidene)hydrazine-1-carbonyl)-L-phenylalaninate 2e** was synthesized using aza-dipeptide **1b** (100 mg, 0.24 mmol), triphenylphosphine (126 mg, 0.48 mmol), DIAD (85 µL, 0.48 mmol) and isopropanol (55 µL, 0.72 mmol) according to the procedure described above for **2b**. The crude product was purified by Al_2O_3 column chromatography (EtOAc/hexanes, 10:1 to 1:1, v/v). **2e** was obtained as yellow oil (78 mg, 71%): R_f = 0.55 (EtOAc/hexanes, 1:3, v/v); $[\alpha]_D^{20}$ –5.3 (*c* 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (s, 1H), 8.02 (dd, *J* = 8.2, 1.2 Hz, 1H), 7.73 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.58 (td, *J* = 7.7, 1.3 Hz, 1H), 7.46 (ddd, *J* = 8.5, 7.4, 1.5 Hz, 1H), 7.28 – 7.19 (m, 5H), 6.95 (d, *J* = 8.3 Hz, 1H), 5.06 (hept, *J* = 7.2 Hz, 1H), 4.71 (dt, *J* = 8.3, 5.9 Hz, 1H), 3.22 – 3.08 (m, 2H), 1.48 (d, *J* = 2.3 Hz, 3H), 1.46 (d, *J* = 2.3 Hz, 3H), 1.41 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.99, 154.91, 147.90, 136.51, 133.38, 131.76, 130.22, 129.62, 129.07, 128.34, 127.58, 126.77, 124.84, 81.90, 54.56, 45.83, 38.58, 27.98, 18.86; HRMS calcd for $C_{24}H_{30}N_4NaO_5$ $[M+Na]^+$ 477.21084, found 477.21208.

***Tert*-butyl (1-benzyl-2-(2-nitrobenzylidene)hydrazine-1-carbonyl)-L-phenylalaninate 2f** was synthesized using aza-dipeptide **1b** (100 mg, 0.24 mmol), triphenylphosphine (126 mg, 0.48 mmol), DIAD (85 µL 0.48 mmol) and benzyl alcohol (75 µL, 0.72 mmol) according to the procedure described above for **2b**. The crude product was purified by Al_2O_3 column chromatography (EtOAc/hexanes, 10:1 to 1:1, v/v). Propargylamine **2f** was obtained as yellow oil (74 mg, 61%): R_f = 0.56 (EtOAc/hexanes, 1:3, v/v); $[\alpha]_D^{20}$ +9.2 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (s, 1H), 7.93 (dd, *J* = 8.3, 1.3 Hz, 1H), 7.76 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.62 – 7.51 (m, 1H), 7.42 (td, *J* = 7.9, 7.4, 1.5 Hz, 1H), 7.37 – 7.21 (m, 10H), 7.10 (d, *J* = 8.4 Hz, 1H), 5.29 – 5.14 (m, 2H), 4.84 (dt, *J* = 8.5, 6.0 Hz, 1H), 3.27 – 3.19 (m, 2H), 1.46 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.94, 155.12, 147.86, 136.43, 135.00, 133.18, 132.85, 129.66, 129.44, 129.30, 128.87, 128.43, 127.77, 127.48, 126.95, 126.87, 124.76, 82.11, 54.72, 45.70, 38.64, 28.03; HRMS *m/z* calcd for $C_{28}H_{30}N_4NaO_5$ $[M+Na]^+$ 525.21084, found 525.21245.

***Tert*-butyl (1-(4-(benzyloxy)benzyl)-2-(2-nitrobenzylidene)hydrazine-1-carbonyl)-L-phenylalaninate 2g** was synthesized using aza-dipeptide **1b** (100 mg, 0.24 mmol), triphenylphosphine (126 mg, 0.48 mmol), DIAD (85 µL 0.48 mmol) and 4-(benzyloxy)phenyl)methanol (154 mg, 0.72 mmol) according to the procedure described above for **2b**. The crude product was purified by Al_2O_3 column chromatography (EtOAc/hexanes,

10:1 to 1:1, v/v). **2g** was obtained as yellow oil (93 mg, 64%): R_f = 0.29 (EtOAc/hexanes, 1:3, v/v); $[\alpha]_D^{20}$ +4.3 (*c* 0.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.95 (dd, *J* = 8.2, 1.3 Hz, 1H), 7.77 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.56 (td, *J* = 7.6, 1.3 Hz, 1H), 7.47 – 7.22 (m, 12H), 7.22 – 7.14 (m, 2H), 7.08 (d, *J* = 8.4 Hz, 1H), 6.98 – 6.89 (m, 2H), 5.20 (d, *J* = 16.2 Hz, 1H), 5.10 (d, *J* = 16.2 Hz, 1H), 5.03 (s, 2H), 4.84 (dt, *J* = 8.4, 6.0 Hz, 1H), 3.30 – 3.15 (m, 2H), 1.46 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.95, 158.19, 155.12, 147.87, 136.94, 136.45, 133.17, 132.64, 129.66, 129.50, 129.25, 128.53, 128.42, 127.90, 127.71, 127.47, 127.29, 126.86, 124.78, 115.24, 82.09, 70.04, 54.72, 45.11, 38.66, 28.03; HRMS m/z calcd for $C_{35}H_{36}N_4NaO_6$ $[M+Na]^+$ 631.25271, found 631.25319.

***Tert*-butyl (1-(3-((tert-butoxycarbonyl)amino)propyl)-2-(2-nitrobenzylidene)hydrazine-1-carbonyl)-L-phenylalaninate 2h** was synthesized using aza-dipeptide **1b** (100 mg, 0.24 mmol), triphenylphosphine (126 mg, 0.48 mmol), DIAD (85 μL, 0.48 mmol) and tert-butyl Boc-3-aminopropanol (126 mg, 0.72 mmol) according to the procedure described above for **2b**. The crude product was purified by Al_2O_3 column chromatography (EtOAc/hexanes, 10:1 to 1:1, v/v). **2h** was obtained as yellow oil (102 mg, 75%): R_f = 0.62 (EtOAc/hexanes, 1:3, v/v) ; $[\alpha]_D^{20}$ +5.3 (*c* 1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 8.02 (d, J = 8.2 Hz, 1H), 7.75 (d, J = 7.9 Hz, 1H), 7.61 (t, J = 7.6 Hz, 1H), 7.49 (t, J = 7.9 Hz, 1H), 7.23 (dd, J = 19.6, 6.2 Hz, 5H), 6.95 (d, J = 8.3 Hz, 1H), 5.45 (t, J = 6.3 Hz, 1H), 4.71 (dt, J = 8.3, 6.0 Hz, 1H), 4.03 (q, J = 6.4 Hz, 2H), 3.13 (q, J = 7.5, 6.4 Hz, 2H), 3.06 (q, J = 6.3 Hz, 2H), 1.74 (q, J = 6.0 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.89, 156.07, 155.46, 147.92, 136.27, 133.39, 132.10, 129.57, 129.54, 129.49, 128.42, 128.05, 126.91, 124.87, 82.13, 78.91, 54.60, 38.44, 38.15, 36.76, 28.42, 27.96, 25.51; HRMS *m/z* calcd for $C_{29}H_{40}N_5O_7$ $[M+H]^+$ 570.29223, found 570.29386.

***Tert*-butyl (1-(cyclopropylmethyl)-2-(2-nitrobenzylidene)hydrazine-1-carbonyl)-L-phenylalaninate 2i** was synthesized using aza-dipeptide **1b** (100 mg, 0.24 mmol), triphenylphosphine (126 mg, 0.48 mmol), DIAD (85 μL, 0.48 mmol) and cyclopropylmethanol (58 μL, 0.72 mmol) according to the procedure described above for **2b**. The crude product was purified by Al_2O_3 column chromatography (EtOAc/hexanes, 10:1 to 1:1, v/v). **2i** was obtained as yellow oil (61 mg, 54%): R_f = 0.69 (EtOAc/hexanes, 1:3, v/v); $[\alpha]_D^{20}$ +3.0 (*c* 0.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 8.02 (dd, *J* = 8.2, 1.3 Hz, 1H), 7.81 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.61 (td, *J* = 7.7, 1.3 Hz, 1H), 7.48 (ddd, *J* = 8.6, 7.3, 1.5 Hz, 1H), 7.32 – 7.20 (m, 5H), 6.98 (d, *J* = 8.3 Hz, 1H), 4.78 – 4.68 (m, 1H), 3.89 (d, *J* = 6.6 Hz, 2H), 3.16 (qd, *J* = 13.8, 5.9 Hz, 2H), 1.42 (s, 9H), 1.17 – 1.05 (m, 1H), 0.58 – 0.49 (m, 2H), 0.45 – 0.33 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.96, 154.96, 147.98, 136.46, 133.31, 131.51, 129.91, 129.62, 129.19, 128.36, 127.92, 126.81, 124.81, 81.96, 54.63, 45.21, 38.57, 27.98, 8.18, 4.20, 4.16; HRMS *m/z* calcd for $C_{25}H_{30}N_4NaO_5$ $[M+Na]^+$ 489.21084, found 489.21218.

***Tert*-butyl (1-allyl-2-(2-nitrobenzylidene)hydrazine-1-carbonyl)-L-phenylalaninate 2j** was synthesized using aza-dipeptide **1b** (100 mg, 0.24 mmol), triphenylphosphine (126 mg, 0.48 mmol), DIAD (85 μL, 0.48

mmol) and allyl alcohol (49 µL, 0.72 mmol) according to the procedure described above for **2b**. The crude product was purified by Al_2O_3 column chromatography (EtOAc/hexanes, 10:1 to 1:1, v/v). **2j** was obtained as yellow oil (88 mg, 81%): R_f = 0.54 (EtOAc/hexanes, 1:3, v/v); $[\alpha]_D^{20}$ +1.5 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (s, 1H), 7.98 (dd, *J* = 8.2, 1.2 Hz, 1H), 7.78 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.58 (t, *J* = 7.6 Hz, 1H), 7.50 – 7.41 (m, 1H), 7.27 (d, *J* = 6.9 Hz, 3H), 7.24 – 7.16 (m, 2H), 7.00 (d, *J* = 8.4 Hz, 1H), 5.74 (ddt, *J* = 17.3, 10.0, 4.8 Hz, 1H), 5.19 (dd, *J* = 34.4, 13.9 Hz, 2H), 4.75 (dt, *J* = 8.3, 5.9 Hz, 1H), 4.69 – 4.53 (m, 2H), 3.16 (qd, *J* = 13.8, 5.9 Hz, 2H), 1.42 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.89, 154.59, 148.00, 136.38, 133.25, 132.72, 130.22, 129.61, 129.55, 129.33, 128.38, 127.82, 126.85, 124.79, 117.62, 82.05, 54.53, 44.07, 38.55, 27.98; HRMS m/z calcd for $C_{24}H_{29}N_4O_5$ $[M+H]^+$ 453.21325, found 453.21438.

***Tert*-butyl (2-(2-nitrobenzylidene)-1-(prop-2-yn-1-yl)hydrazine-1-carbonyl)-L-phenylalaninate 2k** was synthesized using aza-dipeptide **1b** (100 mg, 0.24 mmol), triphenylphosphine (126 mg, 0.48 mmol), DIAD (85 µL, 0.48 mmol) and homopropargyl alcohol (55 µL, 0.72 mmol) according to the procedure described above for **2b**. The crude product was purified by Al_2O_3 column chromatography (EtOAc/hexanes, 10:1 to 1:1, v/v). **2k** was obtained as yellow oil (44 mg, 39%): R_f = 0.60 (EtOAc/hexanes, 1:3, v/v); $[\alpha]_D^{20}$ –1.5 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H), 8.02 (d, J = 8.2 Hz, 1H), 7.76 (d, J = 7.9 Hz, 1H), 7.61 (t, J = 7.6 Hz, 1H), 7.49 (t, J = 7.7 Hz, 1H), 7.26 – 7.18 (m, 5H), 6.93 (d, J = 8.3 Hz, 1H), 4.73 (dt, J = 8.4, 5.9 Hz, 1H), 4.17 (hept, J = 7.5 Hz, 2H), 3.15 (tt, J = 13.7, 6.5 Hz, 2H), 2.50 (td, J = 7.4, 2.6 Hz, 2H), 1.99 (d, J = 2.7 Hz, 1H), 1.41 (s, 9H).^{13}C NMR (100 MHz, $CDCl_3$) δ 170.85, 154.38, 147.96, 136.34, 133.40, 132.11, 129.61, 129.52, 128.72, 128.38, 128.14, 126.85, 124.85, 82.09, 80.37, 70.47, 54.49, 39.69, 38.55, 27.98, 16.11; HRMS *m/z* calcd for $C_{25}H_{28}N_4NaO_5$ $[M+Na]^+$ 487.19519, found 487.19659.

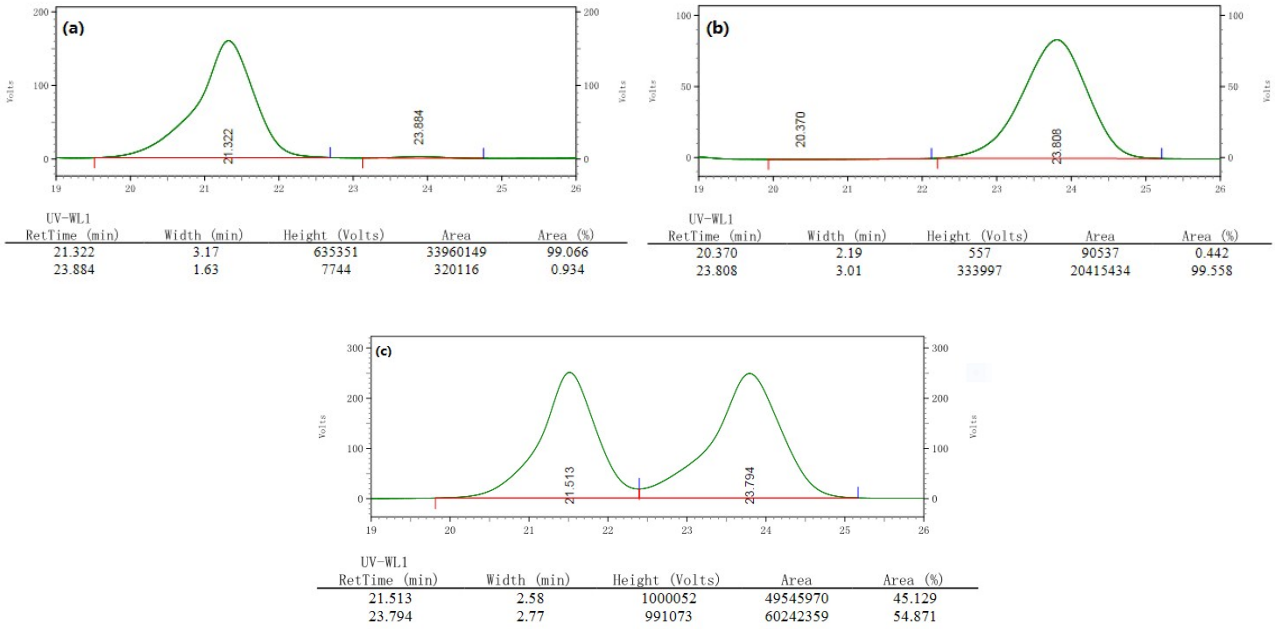
***Tert-butyl* (1-cyclohexyl-2-(2-nitrobenzylidene)hydrazine-1-carbonyl)-L-phenylalaninate 2l** was synthesized using aza-dipeptide **1b** (100 mg, 0.24 mmol), triphenylphosphine (126 mg, 0.48 mmol), DIAD (85 µL, 0.48 mmol) and 4-pentenol (74 µL, 0.72 mmol) according to the procedure described above for **2b**. The crude product was purified by Al_2O_3 column chromatography (EtOAc/hexanes, 10:1 to 1:1, v/v). **2l** was obtained as yellow oil (50 mg, 43%): R_f = 0.55 (EtOAc/hexanes, 1:3, v/v); $[\alpha]_D^{20}$ –7.0 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 1H), 8.00 (dd, *J* = 8.2, 1.4 Hz, 1H), 7.77 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.58 (td, *J* = 7.6, 1.3 Hz, 1H), 7.46 (ddd, *J* = 8.6, 7.3, 1.5 Hz, 1H), 7.27 (d, *J* = 6.1 Hz, 1H), 7.23 – 7.16 (m, 3H), 6.94 (d, *J* = 8.3 Hz, 1H), 5.83 (ddt, *J* = 16.8, 9.9, 6.6 Hz, 1H), 5.11 – 5.00 (m, 2H), 5.00 – 4.95 (m, 1H), 4.72 (dt, *J* = 8.3, 6.0 Hz, 1H), 3.96 (dd, *J* = 8.7, 6.5 Hz, 2H), 3.20 – 3.08 (m, 2H), 2.17 – 2.05 (m, 2H), 1.73 – 1.64 (m, 2H), 1.41 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.96, 154.70, 147.89, 137.32, 136.40, 133.37, 131.40, 129.79, 129.61, 129.26, 128.38, 127.88, 126.84, 124.87, 115.56, 82.01, 54.49, 40.62, 38.58, 30.88, 27.98, 24.39; HRMS *m/z* calcd for $C_{26}H_{33}N_4O_5$ $[M+H]^+$ 481.24455, found 481.24566.

***Tert-butyl* (2-(2-nitrobenzylidene)-1-(pyridin-3-ylmethyl)hydrazine-1-carbonyl)-L-phenylalaninate 2m** was synthesized using aza-dipeptide **1b** (100 mg, 0.24 mmol), triphenylphosphine (126 mg, 0.48 mmol), DIAD (85 μL, 0.48 mmol) and pyridin-3-ylmethanol (70 μL , 0.72 mmol) according to the procedure described above for **2b**. The crude product was purified by Al_2O_3 column chromatography (EtOAc/hexanes, 10:1 to 1:1, v/v). **2m** was obtained as yellow oil (65 mg, 54%): R_f = 0.42 (EtOAc/hexanes, 1:1, v/v); $[\alpha]_D^{20}$ +3.2 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (d, J = 2.3 Hz, 1H), 8.48 – 8.44 (m, 1H), 8.03 (d, J = 2.5 Hz, 1H), 7.90 (dt, J = 8.3, 2.5 Hz, 1H), 7.72 – 7.67 (m, 1H), 7.66 – 7.58 (m, 4H), 7.49 (tdd, J = 7.4, 5.5, 3.3 Hz, 4H), 7.43 – 7.37 (m, 5H), 7.27 (dd, J = 6.9, 2.1 Hz, 1H), 7.21 (d, J = 7.2 Hz, 3H), 7.05 (d, J = 8.4 Hz, 1H), 5.26 – 5.12 (m, 2H), 4.78 (dt, J = 8.1, 6.1 Hz, 1H), 3.18 (dd, J = 6.0, 3.7 Hz, 2H), 1.42 (d, J = 2.3 Hz, 9H).^{13}C NMR (100 MHz, $CDCl_3$) δ 170.81, 154.91, 148.98, 148.59, 147.74, 136.27, 134.85, 133.36, 133.03, 132.08, 130.75, 129.59, 129.09, 128.54, 127.89, 126.93, 124.86, 123.69, 82.22, 54.68, 43.05, 38.53, 28.00; HRMS *m/z* calcd for $C_{27}H_{29}N_5NaO_5$ $[M+Na]^+$ 526.20609, found 526.20757.

***Tert*-butyl (1-((S)-sec-butyl)-2-((E)-2-nitrobenzylidene)hydrazine-1-carbonyl)-L-phenylalaninate 2n** was synthesized using aza-dipeptide **1b** (100 mg, 0.24 mmol), triphenylphosphine (125.9 mg, 0.48 mmol), DIAD (84.7 μL 0.48 mmol) and R-(-)-2-Butanol (66.1μL , 0.72 mmol) according to the procedure described above for **2b**. The crude product was purified by Al_2O_3 column chromatography (EtOAc/hexanes, 10:1 to 1:1, v/v). **2n** was obtained as yellow oil (71 mg, 63%): R_f = 0.45 (EtOAc/hexanes, 1:5, v/v); $[\alpha]_D^{20}$ -4.1 (c 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) 1H NMR (400 MHz, Chloroform-d) δ 8.65 (s, 1H), 8.01 (dd, J = 7.9, 1.5 Hz, 1H), 7.88 (dd, J = 8.1, 1.3 Hz, 1H), 7.54 (t, J = 7.6 Hz, 1H), 7.42 (td, J = 7.8, 7.4, 1.5 Hz, 1H), 7.29 (dd, J = 7.8, 5.9 Hz, 2H), 7.24 – 7.18 (m, 3H), 6.39 (d, J = 8.5 Hz, 1H), 5.05 (q, J = 6.2 Hz, 1H), 4.45 (dt, J = 8.6, 6.2 Hz, 1H), 3.09 (dd, J = 6.2, 3.8 Hz, 2H), 1.72 – 1.59 (m, 2H), 1.22 (d, J = 6.2 Hz, 3H), 0.92 (t, J = 7.5 Hz, 3H).^{13}C NMR (100 MHz, Chloroform-d) δ 170.78, 161.34, 148.36, 145.54, 136.27, 132.44, 130.39, 129.60, 128.86, 128.45, 128.40, 126.88, 124.24, 82.01, 74.74, 55.11, 39.34, 28.88, 27.93, 19.32, 9.51; HRMS m/z calcd for $C_{25}H_{33}N_4O_5$ $[M+H]^+$ 469.24455, found 469.24584; Chiral HPLC analysis: Chiralcel IA-H (Hexane/*i*-PrOH) = 98:2, flow rate = 0.5 mL/min, wave length = 254 nm, t_R = 21.322 min.

***Tert*-butyl (1-((R)-sec-butyl)-2-((E)-2-nitrobenzylidene)hydrazine-1-carbonyl)-L-phenylalaninate** was synthesized using the same procedure as described for **2n**, but (S)-(+)-2-Butanol was used. The crude product was purified by Al_2O_3 column chromatography (EtOAc/hexanes, 10:1 to 1:1, v/v). The product was obtained as yellow oil (68 mg, 60%): R_f = 0.46 (EtOAc/hexanes, 1:5, v/v); $[\alpha]_D^{20}$ -9.5 (c 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (s, 1H), 8.01 (dd, J = 7.9, 1.5 Hz, 1H), 7.88 (dd, J = 8.1, 1.3 Hz, 1H), 7.54 (t, J = 7.6 Hz, 1H), 7.42 (td, J = 7.8, 7.4, 1.5 Hz, 1H), 7.29 (dd, J = 7.8, 5.9 Hz, 2H), 7.24 – 7.18 (m, 3H), 6.39 (d, J = 8.5 Hz, 1H), 5.05 (q, J = 6.2 Hz, 1H), 4.45 (dt, J = 8.6, 6.2 Hz, 1H), 3.09 (dd, J = 6.2, 3.8 Hz, 2H), 1.72 – 1.59 (m, 2H), 1.22 (d, J = 6.2 Hz, 3H), 0.92 (t, J = 7.5 Hz, 3H).^{13}C NMR (100 MHz, $CDCl_3$)δ 170.79, 161.39, 148.36, 145.59,

136.19, 132.44, 130.38, 129.57, 128.88, 128.46, 128.42, 126.92, 124.24, 81.97, 74.83, 55.17, 39.19, 28.90, 27.93, 19.41, 9.56.HRMS m/z calcd for $C_{25}H_{32}N_4NaO_5$ $[M+Na]^+$ 491.22649, found 491.22763; Chiral HPLC analysis: Chiralcel IA-H (Hexane/*i*-PrOH) = 98:2, flow rate = 0.5 mL/min, wave length = 254 nm, t_R =23.808 min.



UV-WL1

RetTime (min)	Width (min)	Height (Volts)	Area	Area (%)
21.322	3.17	635351	33960149	99.066
23.884	1.63	7744	320116	0.934

UV-WL1

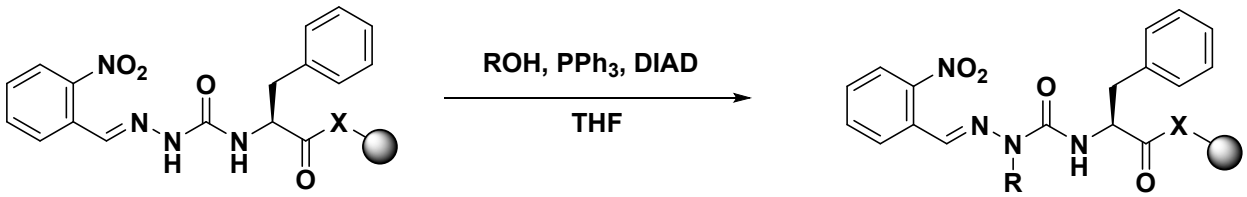
RetTime (min)	Width (min)	Height (Volts)	Area	Area (%)
20.370	2.19	557	90537	0.442
23.808	3.01	333997	20415434	99.558

UV-WL1

RetTime (min)	Width (min)	Height (Volts)	Area	Area (%)
21.513	2.58	1000052	49545970	45.129
23.794	2.77	991073	60242359	54.871

Chiral HPLC analysis of the Mitsunobu reaction product of azadipeptide **1b** with (a) (R)-*sec*-butanol, (b) (S)-*sec*-butanol, and (c) racemic *sec*-butanol.

1.5 Synthesis of dipeptide 3a-f and 4a-f using Mitsunobu Reaction



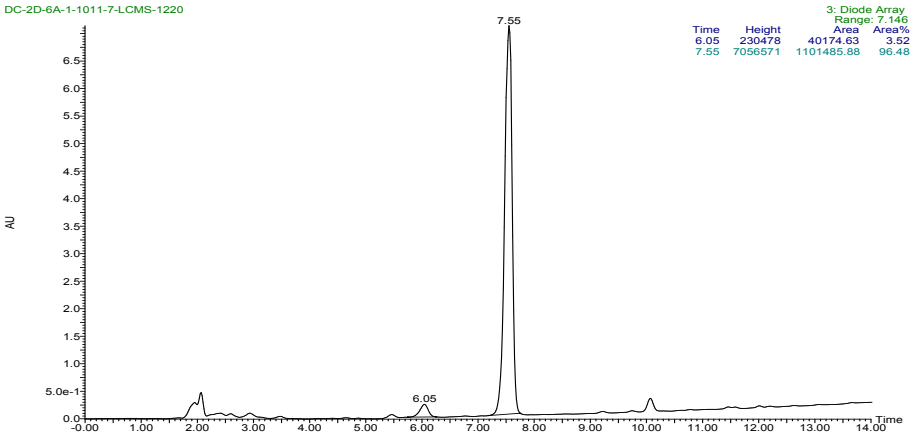
1e X = NH, Rink-amide resin
1f X = O, Wang-resin

3a-f
4a-f

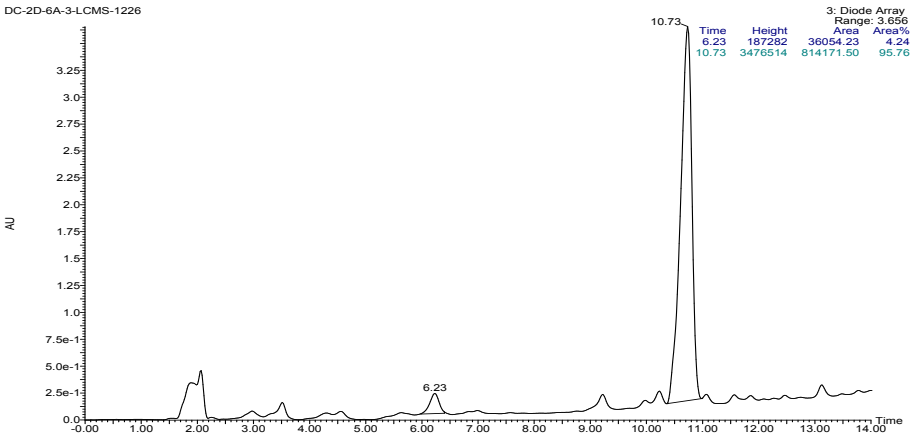
Representative synthesis of azadipeptide using Mitsunobu reaction on solid phase:

2-Nitrobenzylidene-azaAla-Phe-NH_2 3a: Vacuum dried semicarbazone resin **1e** (~208 mg, 0.2 mmol) was suspended in anhydrous THF (3 mL) in a sealed flask under nitrogen. Solutions of methanol (122 µL, 3 mmol) in anhydrous THF (1 mL), PPh_3 (525 mg, 2 mmol) in anhydrous THF (1 mL), and DIAD (394 µL, 2 mmol) in anhydrous THF (1 mL) were sequentially added to the resin mixture, which was shaken on an automated shaker for 5 h, and filtered. After filtration, the resin was washed sequentially with DMF (3 × 10 mL), MeOH (3 × 10 mL), THF (3 × 10 mL), and DCM (3 × 10 mL). Examination by LC-MS of a cleaved resin sample showed excellent conversion (96%);

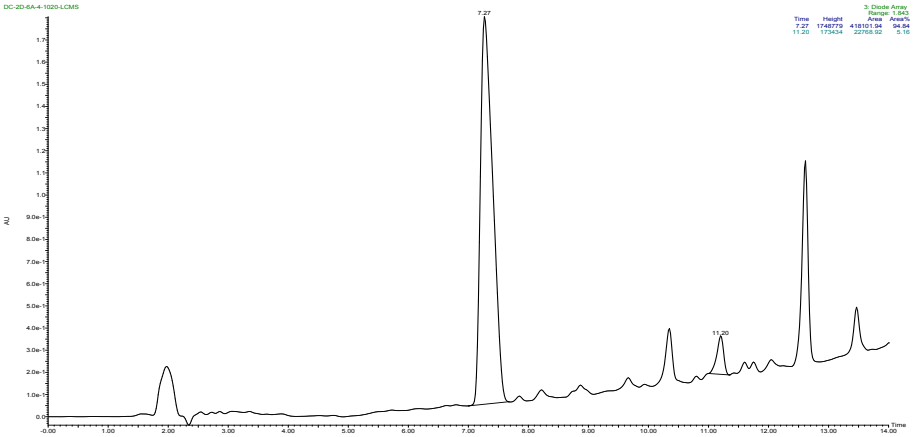
2-Nitrobenzylidene azadipeptide **3b-f** and **4a-f** were synthesized using the same protocol as described for **3a**.



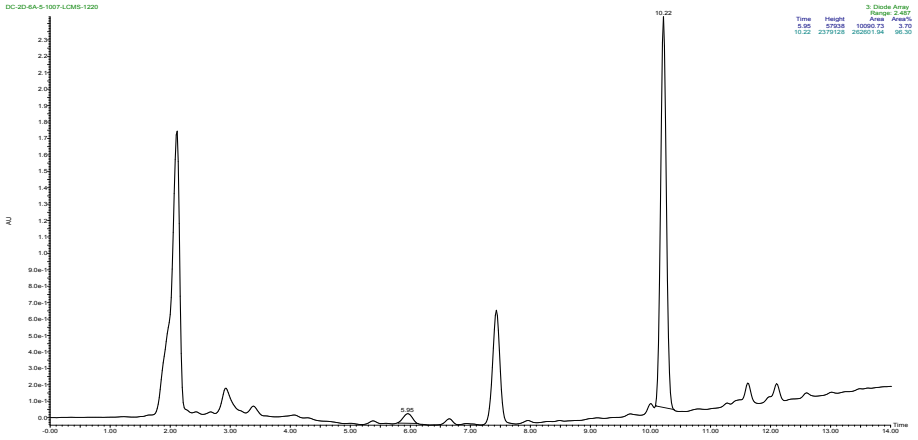
2-Nitrobenzylidene-azaAla-Phe-NH_2 3a: LC-MS (60-95% MeOH, 14 min) R.T. = 7.55min; Conversion 96% ; HRMS m/z calcd for $C_{18}H_{20}N_5O_4$ $[M+H]^+$370.15098, found 370.15206; LC-MS (60-95% MeOH, 14 min) R.T. = 7.55min;



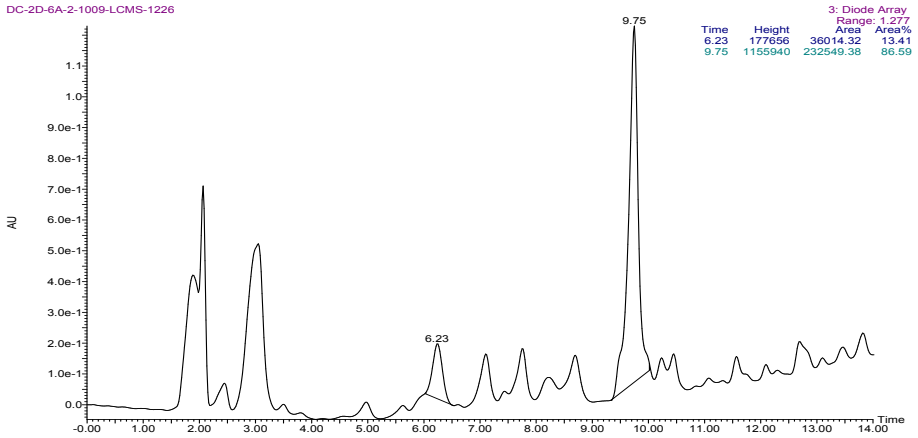
2-Nitrobenzylidene-azaPhe-Phe-NH_2 3b: LC-MS (60-95% MeOH, 14 min) R.T. = 10.73 min; Conversion 96%; HRMS m/z calcd for $C_{24}H_{24}N_5O_4$ $[M+H]^+$ 446.18228, found 446.18370.



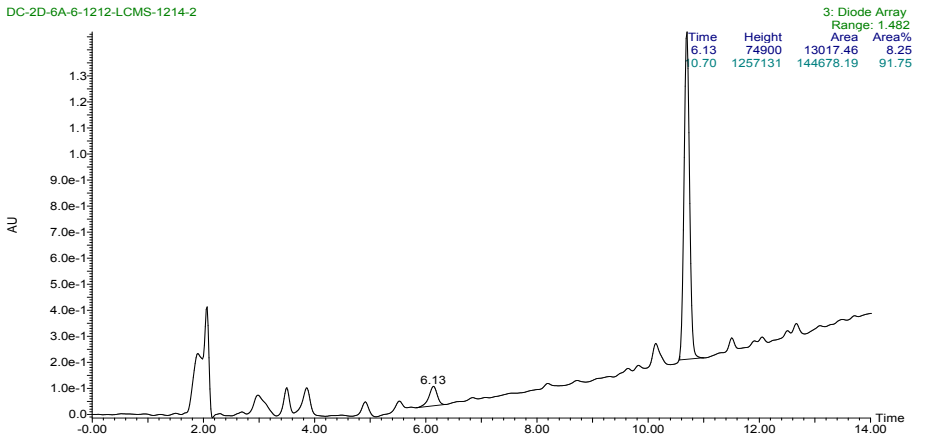
2-Nitrobenzylidene-azaOrn-Phe-NH_2 3c: LC-MS (40-95% MeOH, 14 min) R.T. = 7.27min; Conversion 95%; HRMS m/z calcd for $C_{20}H_{25}N_6O_4$ $[M+H]^+$ 413.19318, found 413.19426.



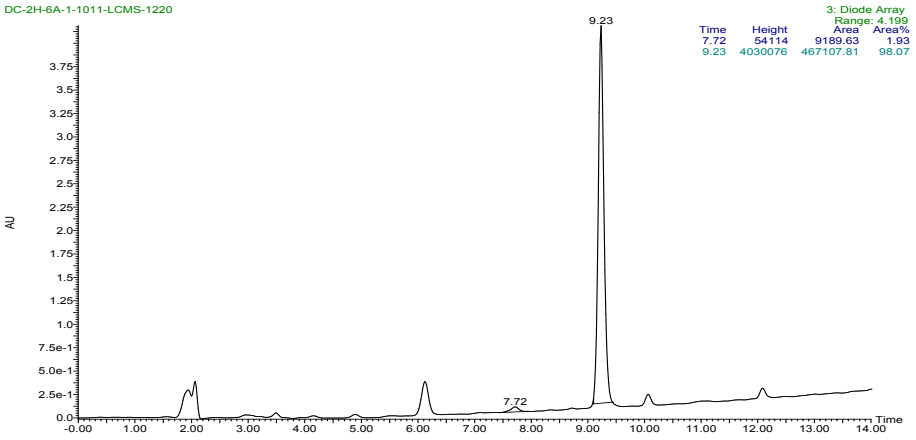
2-Nitrobenzylidene-cyclopropylmethyl-azaGly-Phe-NH_2 3d: LC-MS (60-95% MeOH, 14 min) R.T. = 10.22 min; Conversion 96%; HRMS m/z calcd for $C_{21}H_{24}N_5O_4$ $[M+H]^+$ 410.18228, found 410.18297.



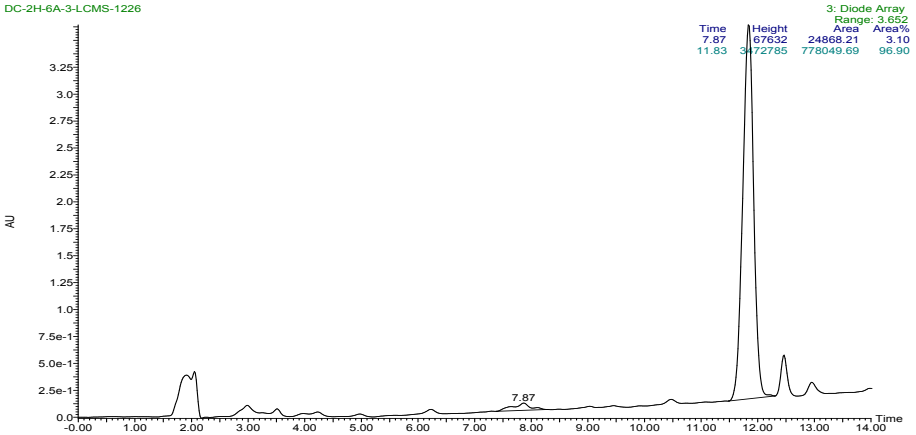
2-Nitrobenzylidene-azaVal-Phe-NH_2 3e: LC-MS (60-95% MeOH, 14 min) R.T. = 9.75 min; Conversion 87% (reaction was repeated for once); HRMS m/z calcd for $C_{20}H_{24}N_5O_4$ $[M+H]^+$ 398.18336, found 398.18228.



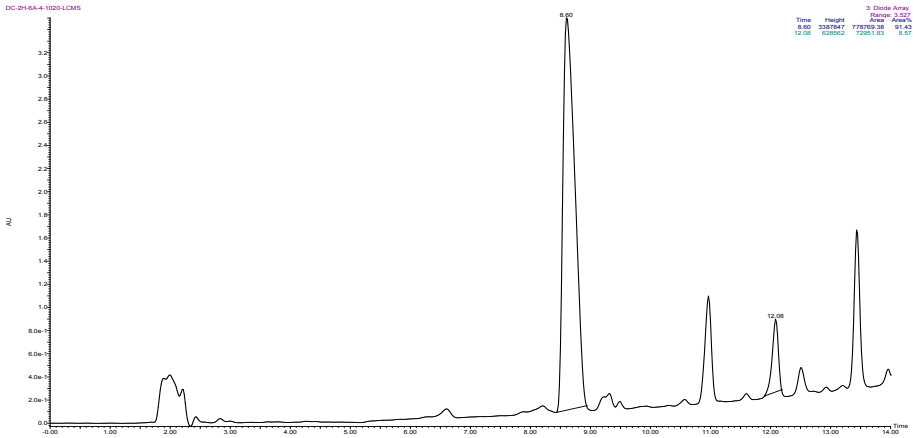
2-Nitrobenzylidene-azaIle-Phe-NH_2 3f: LC-MS (60-95% MeOH, 14 min) R.T. = 10.70 min; Conversion 92%; HRMS m/z calcd for $C_{21}H_{26}N_5O_4$ $[M+H]^+$ 412.19793, found 412.19877.



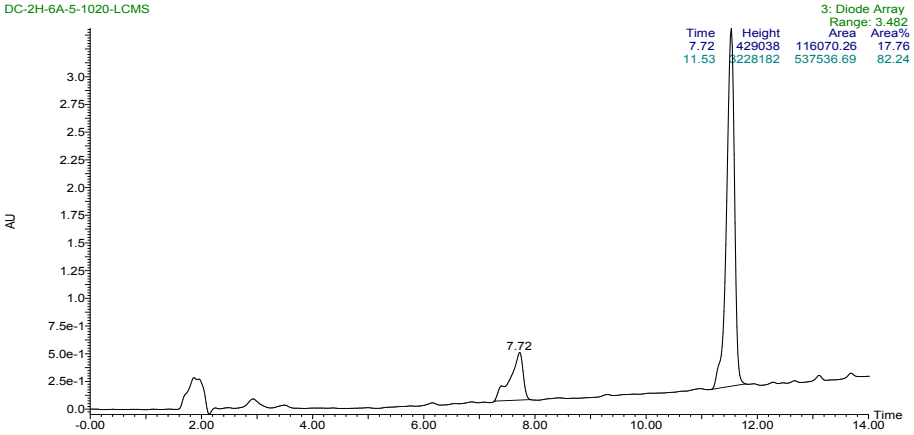
2-Nitrobenzylidene-azaAla-Phe-OH 4a: LC-MS (60-95% MeOH, 14 min) R.T. = 9.23 min; Conversion 98%; HRMS m/z calcd for $C_{18}H_{19}N_4O_5$ $[M+H]^+$ 371.13500, found 371.13568.



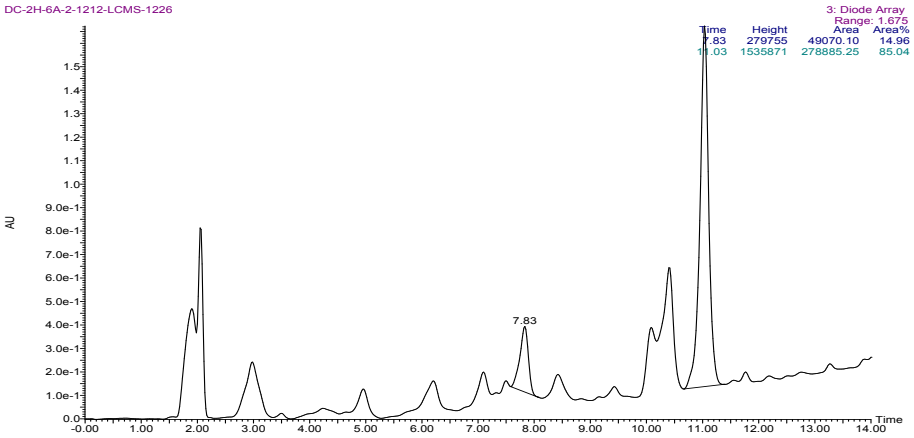
2-Nitrobenzylidene-azaPhe-Phe-OH 4b: LC-MS (60-95% MeOH, 14 min) R.T. = 11.83 min; Conversion 97%; HRMS m/z calcd for $C_{24}H_{23}N_4O_5$ $[M+H]^+$ 447.16630, found 447.16768.



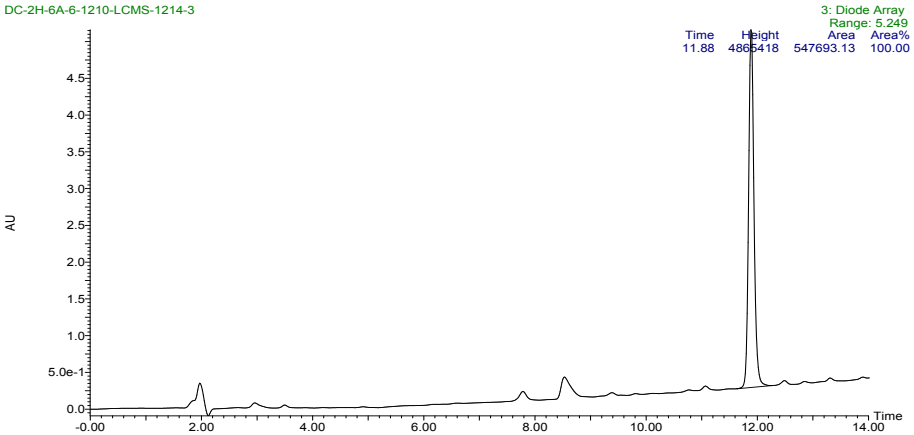
2-Nitrobenzylidene-azaOrn-Phe-OH 4c: LC-MS (40-95% MeOH, 14 min) R.T. = 8.60 min; Conversion 92%; HRMS m/z calcd for $C_{20}H_{24}N_5O_5$ $[M+H]^+$ 414.17720, found 414.17767.



2-Nitrobenzylidene-cyclopropylmethyl-azaGly-Phe-OH 4d: LC-MS (40-95% MeOH, 14 min) R.T. = 11.53min; Conversion 82%; HRMS m/z calcd for $C_{21}H_{23}N_4O_5$ $[M+H]^+$ 425.18195, found 425.18275.

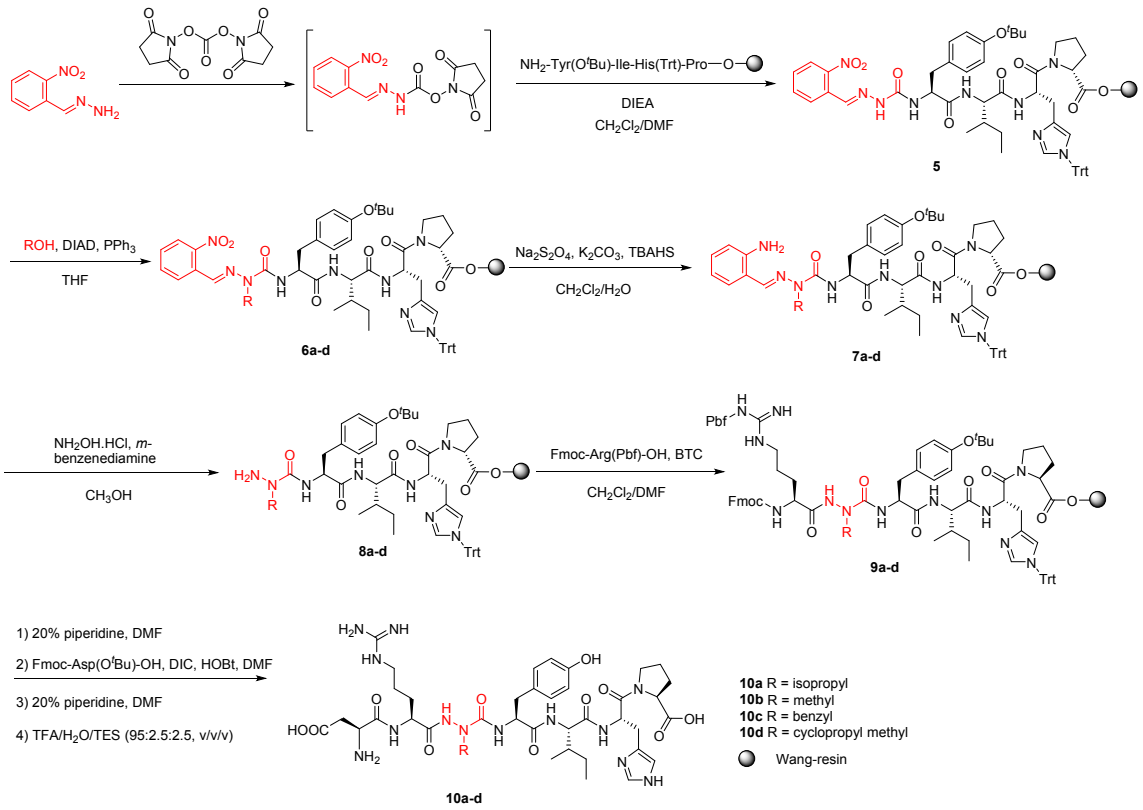


2-Nitrobenzylidene-azaVal-Phe-OH 4e: LC-MS (60-95% MeOH, 14 min) R.T. = 11.03 min; Conversion 85%(reaction was repeated for once); HRMS m/z calcd for $C_{20}H_{23}N_4O_5$ $[M+H]^+$ 399.16630, found 399.16764.



2-Nitrobenzylidene-azaIle-Phe-OH 4f: LC-MS (60-95% MeOH, 15 min) R.T. = 11.88 min; Conversion 100% ; HRMS m/z calcd for $C_{21}H_{25}N_4O_5$ $[M+H]^+$ 413.18195, found 413.18342.

1.6 Synthesis of [aza-Xaa^3]-Ang-(1-7) analogs 10a-d



Wang resin supported NH_2-Tyr(O^{t}Bu)-Ile-His(Trt)-Pro was prepared by standand SPPS on Polystyrene Wang resin (0.96 mmol/g, typically ~208 mg per peptide).

Synthesis of [aza-Val^3]-Ang-(1-7) 10a as a representative for the synthesis of [aza-Xaa^3]-Ang-(1-7) analogs using Mitsunobu reaction

Representative synthesis of nitrobenzylidene semicarbazone-protected aza-Gly on solid phase:

2-Nitrobenzylidene semicarbazone resin 5: A solution of *N,N'*-disuccinimidyl carbonate (205 mg, 0.8 mmol) in 3 mL of DMF/DCM (1:1, v/v) was treated dropwise with a solution of 2-nitrobenzylidene hydrazine (132 mg, 0.8 mmol) in 1 mL of DCM over 15 min, stirred for 2 h at rt, and then transferred to a syringe tube equipped with teflon™ filter, stopper and stopcock containing swollen NH_2-Tyr(O^{t}Bu)-Ile-His(Trt)-Pro resin (~208 mg, 0.2 mmol). The resin mixture was treated with DIEA (207 µL, 1.2 mmol), shaken on an automated shaker for 12 h. After filtration, the resin was washed sequentially with DMF (3 × 10 mL), MeOH (3 × 10 mL), THF (3 ×

10 mL), and DCM (3 × 10 mL). Examination by LC-MS of a cleaved resin sample showed complete coupling and gave semicarbazone **5** in good purity.

Representative alkylation of nitrobenzylidene semicarbazone-protected aza-Gly using Mitsunubu reaction on solid phase:

2-Nitrobenzylidene semicarbazone resin 6a: Vacuum dried semicarbazone resin **5** (~208 mg, 0.2 mmol) was suspended in anhydrous THF (3 mL) in a sealed flask under nitrogen. Solutions of isopropanol (229 μL, 3 mmol) in anhydrous THF (1 mL), PPh_3 (525 mg, 2 mmol) in anhydrous THF (1 mL), and DIAD (394 μL, 2 mmol) in anhydrous THF (1 mL) were sequentially added to the resin mixture, which was shaken on an automated shaker for 5 h, and filtered. After filtration, the resin was washed sequentially with DMF (3 × 10 mL), MeOH (3 × 10 mL), THF (3 × 10 mL), and DCM (3 × 10 mL). The above procedure was repeated for once. Examination by LC-MS of a cleaved resin sample showed complete conversion and gave semicarbazone **6a** in good purity. 2-Nitrobenzylidene semicarbazone **6b-d** were synthesized using the same procedure as described for **6a** using corresponding alcohols and the alkylation reaction does not need to be repeated.

Representative reduction of nitrobenzylidene semicarbazone to aminobenzylidene on solid phase:

2-Aminobenzylidene semicarbazone resin 7a: A solution of sodium dithionite (696 mg, 4 mmol), potassium carbonate (774 mg, 5.6 mmol), and TBAHS (136 mg, 0.4 mmol) in water (4 mL) and DCM (4 mL) was added to a syringe tube equipped with teflon™ filter, stopper and stopcock containing swollen semicarbazone resin **6a** (~208 mg, 0.2 mmol) and the reaction slurry was shaken at room temperature for 2 h. After filtration, the resin was washed sequentially with DCM/water (1:1, v/v), DMF (3 × 10 mL), THF (3 × 10 mL), MeOH (3 × 10 mL), and DCM (3 × 10 mL). Examination by LC-MS of a cleaved resin sample showed complete conversion and gave semicarbazone **7a** in good purity. 2-Aminobenzylidene semicarbazone **7b-d** were synthesized using the same procedure as described for **7a**.

Representative removal of aminobenzylidene semicarbazone on solid phase:

Semicarbazide 8a: Semicarbazone **7a** (~208 mg, 0.2 mmol) was treated with a solution of $NH_2OH.HCl$ (278 mg, 4 mmol) and *m*-phenylenediamine (433 mg, 4 mmol) in EtOH (5.3 mL), and heated with sonication at 60 °C for 12 h in a sealed syrine tube equipped with teflon™ filter, stopper and stopcock. The resin was filtered and washed with 10% DIEA:DMF (3 × 10 mL), DCM/water (1:1, v/v), DMF (3 × 10 mL), THF (3 × 10 mL), MeOH (3 × 10 mL), and DCM (3 × 10 mL). Examination by LC-MS of a cleaved resin sample showed complete conversion and gave semicarbazide **8a** in good purity. Semicarbazide **8b-d** were synthesized using the same procedure as described for **8a.**

Representative amino acylation of semcarbazide on solid phase:

Azahexapeptide resin 9a: Fmoc-Arg(Pbf)-OH (649 mg, 1 mmol) in 6 mL of DCM was treated with BTC (99 mg, 0.33 mmol, CAUTION: BTC may liberate small quantities of highly toxic phosgene, and must be handled with extreme care in a fumehood) and 2,4,6-Collidine (684 µL, 4.6 mmol). After stirring for 5 min the mixture was transferred to a syringe tube equipped with teflon™ filter, stopper and stopcock containing swollen resin **8a** (~208 mg, 0.2 mmol). The resin was shaken on an automated shaker for 12 h. After filtration, the resin was washed sequentially with DMF (3 × 10 mL), MeOH (3 × 10 mL), THF (3 × 10 mL), and DCM (3 × 10 mL). Examination by LC-MS of a cleaved resin sample showed the coupling was incomplete. The coupling procedure was repeated for once and still yield incomplete coupling with only a conversion of 8%. No further optimization was executed and the resin was used for next peptide coupling. Azahexapeptide resin **9b-d** were synthesized using the same procedure as described for **9a**.

Asp-Arg-azaVal-Tyr-Ile-His-Pro-OH (10a): After Fmoc-removal of azahexapeptide **9a** and standard peptide coupling with Fmoc-Asp(O^{t}Bu)-OH, [aza-Val^3]-Ang-(1-7) **10a** was obtained. After final cleavage from resin, the crude product was analyzed by LC-MS with a crude purity of 2%. The crude product was purified by preparative RP-HPLC and the purity was ascertained by LC-MS. [Aza-Xaa^3]-Ang-(1-7) analogs **10b-d** were synthesized using the same procedure as described for **10a**.

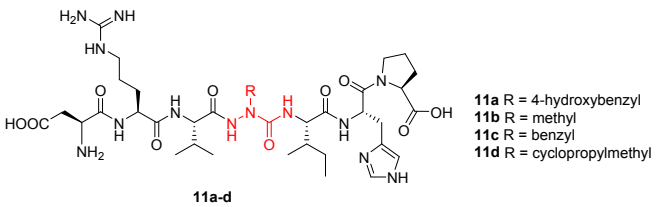
Asp-Arg-azaVal-Tyr-Ile-His-Pro-OH (10a): LC-MS (10-60% MeOH, 15 min) R.T. = 7.00 min; (5-50% MeCN, 15 min) R.T. = 5.07 min; HRMS *m/z* calcd for $C_{40}H_{62}N_{13}O_{11}$ $[M+H]^+$ 900.46863, found 900.47159

Asp-Arg-azaAla-Tyr-Ile-His-Pro-OH (10b) : LC-MS (10-60% MeOH, 15 min) R.T. = 5.78 min; (5-50% MeCN, 15 min) R.T. = 4.50 min; HRMS *m/z* calcd for $C_{38}H_{58}N_{13}O_{11}$ $[M+H]^+$ 872.43733, found 872.44010

Asp-Arg-azaPhe-Tyr-Ile-His-Pro-OH (10c) : LC-MS (10-60% MeOH, 15 min) R.T. = 9.77 min; (5-50% MeCN, 15 min) R.T. = 6.60 min; HRMS *m/z* calcd for $C_{44}H_{62}N_{13}O_{11}$ $[M+H]^+$ 948.46863, found 948.46939

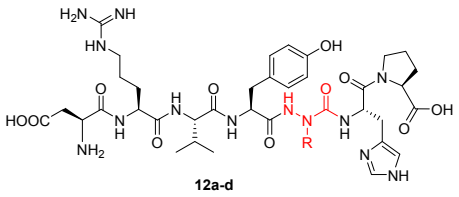
Asp-Arg-cyclopropylmethyl-azaGly-Tyr-Ile-His-Pro-OH (10d): LC-MS (10-60% MeOH, 15 min) R.T. = 7.60 min; (5-50% MeCN, 15 min) R.T. = 5.37 min; HRMS *m/z* calcd for $C_{41}H_{62}N_{13}O_{11}$ $[M+H]^+$ 912.46863, found 912.46706

1.7 Synthesis of [aza-Xaa^4]-Ang-(1-7) analogs 11a-d and [Aza-Xaa^5]-Ang-(1-7) analogs 12a-d



11a R = 4-hydroxybenzyl
11b R = methyl
11c R = benzyl
11d R = cyclopropylmethyl

11a-d



12a R = (S)-*sec*-butyl
12b R = methyl
12c R = benzyl
12d R = cyclopropylmethyl

12a-d

[Aza-Xaa^4]-Ang-(1-7) peptide analogs 11a-d and [Aza-Xaa^5]-Ang-(1-7) peptide analogs 12a-d were synthesized following the same procedures above as described for the synthesis of azapeptide 12a by varying the position of the aza-residue. The peptides were purified by preparative RP-HPLC and product purity was ascertained by LC-MS.

Asp-Arg-Val-azaTyr-Ile-His-Pro-OH (11a): LC-MS (10-60% MeOH, 15 min) R.T. = 6.60 min; (5-50% MeCN, 15 min) R.T. = 4.90 min; HRMS *m/z* calcd for $C_{40}H_{62}N_{13}O_{11}$ $[M+H]^+$ 900.46863, found 900.46869

Asp-Arg-Val-azaAla-Ile-His-Pro-OH (11b): LC-MS (10-60% MeOH, 15 min) R.T. = 5.25 min; (5-50% MeCN, 15 min) R.T. = 4.18 min; HRMS *m/z* calcd for $C_{34}H_{58}N_{13}O_{10}$ $[M+H]^+$ 808.44241, found 808.44373

Asp-Arg-Val-azaPhe-Ile-His-Pro-OH (11c): LC-MS (10-60% MeOH, 15 min) R.T. = 8.97 min; (5-50% MeCN, 15 min) R.T. = 6.07 min; HRMS *m/z* calcd for $C_{40}H_{62}N_{13}O_{10}$ $[M+H]^+$ 884.47371, found 884.47509

Asp-Arg-Val-cyclopropylmethyl-azaGly-Ile-His-Pro-OH (11d): LC-MS (10-60% MeOH, 15 min) R.T. = 6.87 min; (5-50% MeCN, 15 min) R.T. = 4.95 min; HRMS *m/z* calcd for $C_{37}H_{62}N_{13}O_{10}$ $[M+H]^+$ 848.47371, found 848.47498

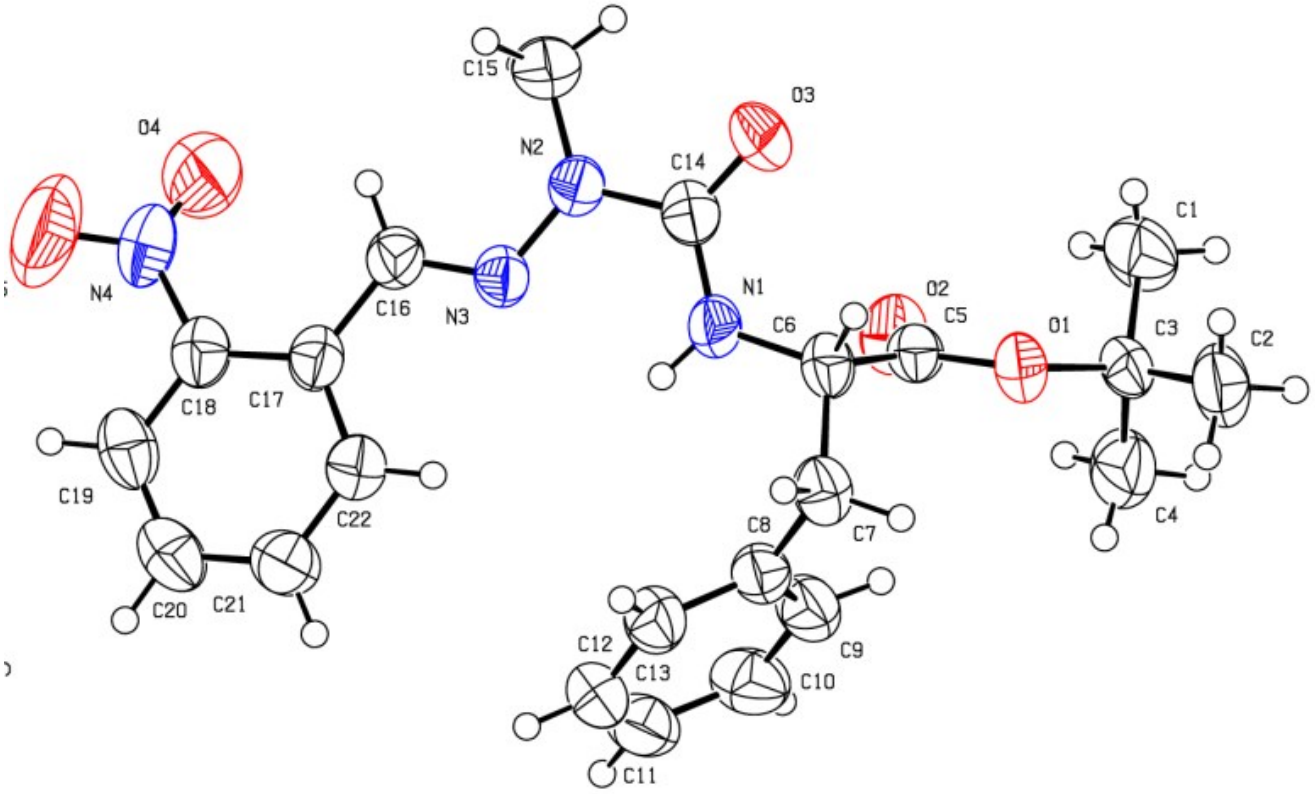
Asp-Arg-Val-Tyr-azaIle-His-Pro-OH (12a): LC-MS (10-60% MeOH, 15 min) R.T. = 6.12 min; (5-50% MeCN, 15 min) R.T. = 4.75 min; HRMS *m/z* calcd for $C_{40}H_{62}N_{13}O_{11}$ $[M+H]^+$ 900.46863, found 900.46706

Asp-Arg-Val-Tyr-azaAla-His-Pro-OH (12b): LC-MS (10-60% MeOH, 15 min) R.T. = 3.92 min; (5-50% MeCN, 15 min) R.T. = 3.62 min; HRMS *m/z* calcd for $C_{37}H_{56}N_{13}O_{11}$ $[M+H]^+$ 858.42168, found 858.42278

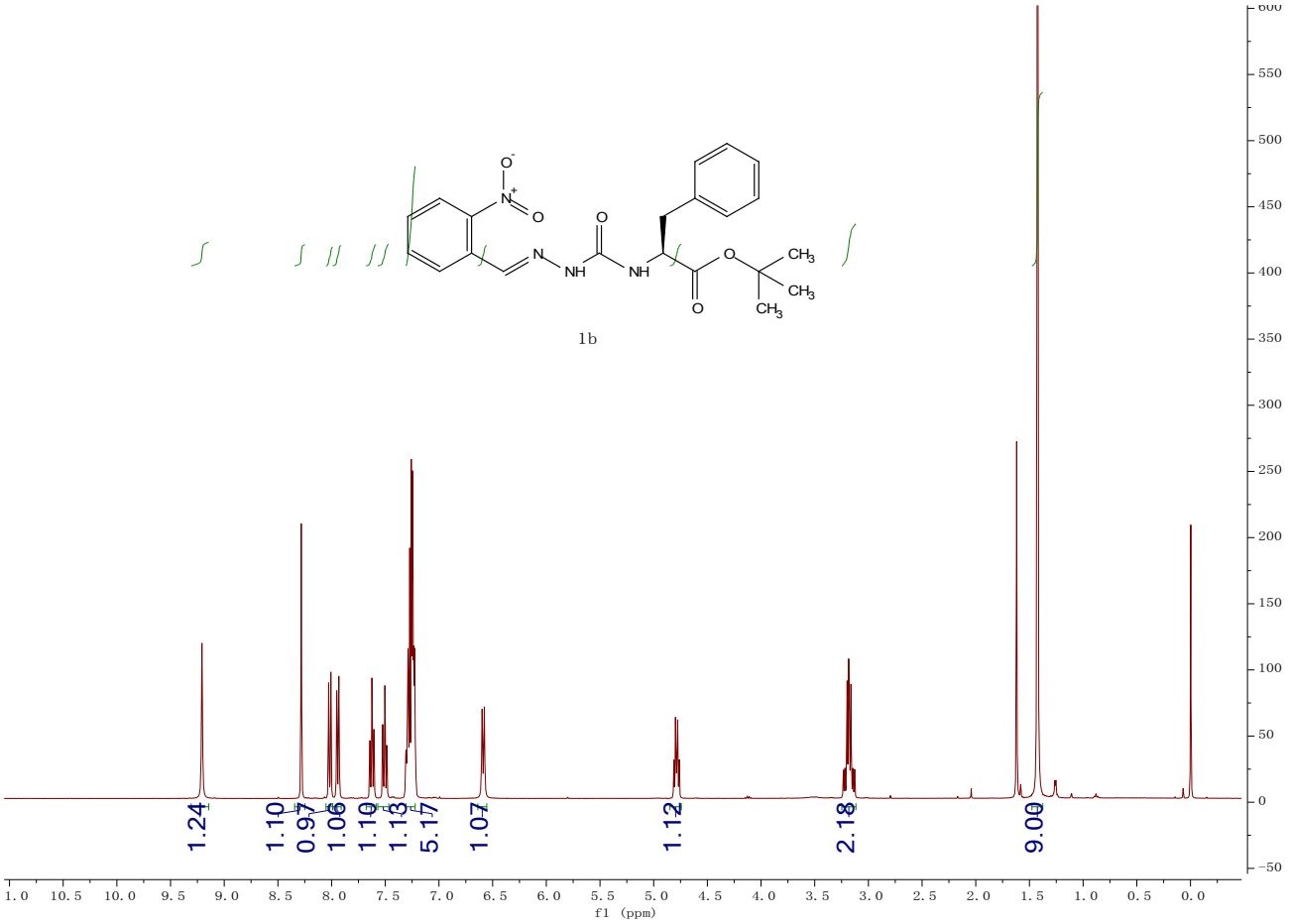
Asp-Arg-Val-Tyr-azaPhe-His-Pro-OH (12c): LC-MS (10-60% MeOH, 15 min) R.T. = 7.87 min; (5-50% MeCN, 15 min) R.T. = 5.75 min; HRMS *m/z* calcd for $C_{43}H_{60}N_{13}O_{11}$ $[M+H]^+$ 934.45298, found 934.45619

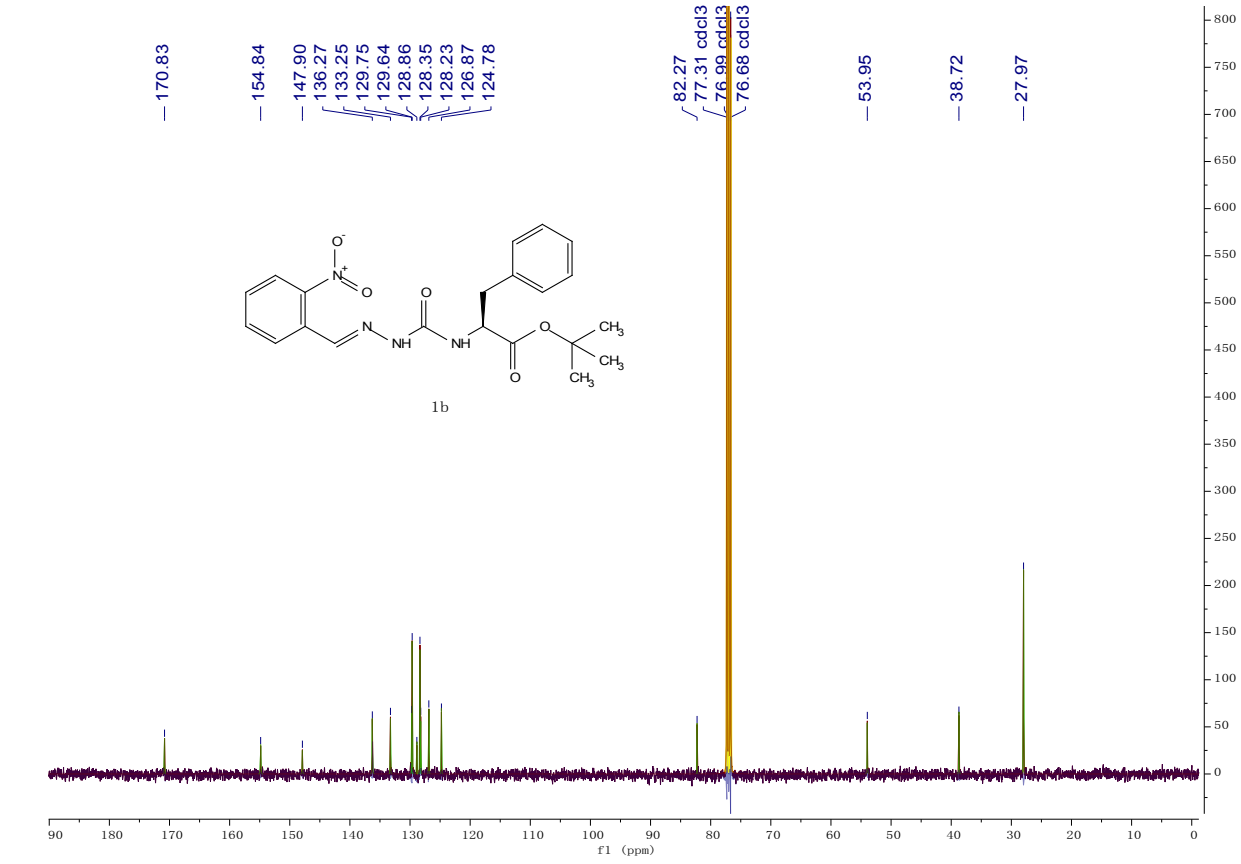
Asp-Arg-Val-Tyr-cyclopropylmethyl-azaGly-His-Pro-OH (12d): LC-MS (10-60% MeOH, 15 min) R.T. = 5.62 min; (5-50% MeCN, 15 min) R.T. = 4.52 min; HRMS *m/z* calcd for $C_{40}H_{60}N_{13}O_{11}$ $[M+H]^+$ 898.45298, found 898.45388

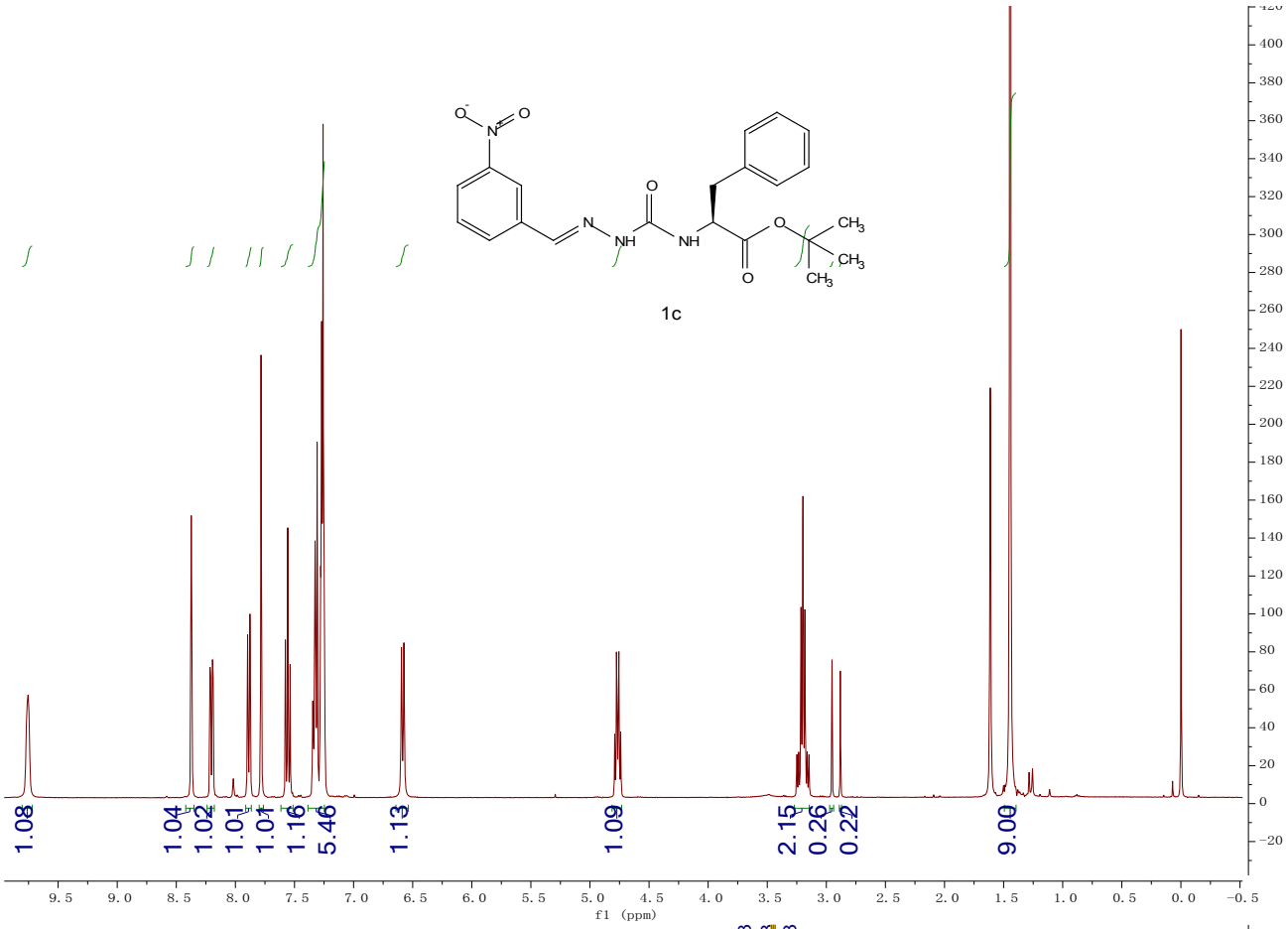
2. Crystal structure of azadipeptide 2b



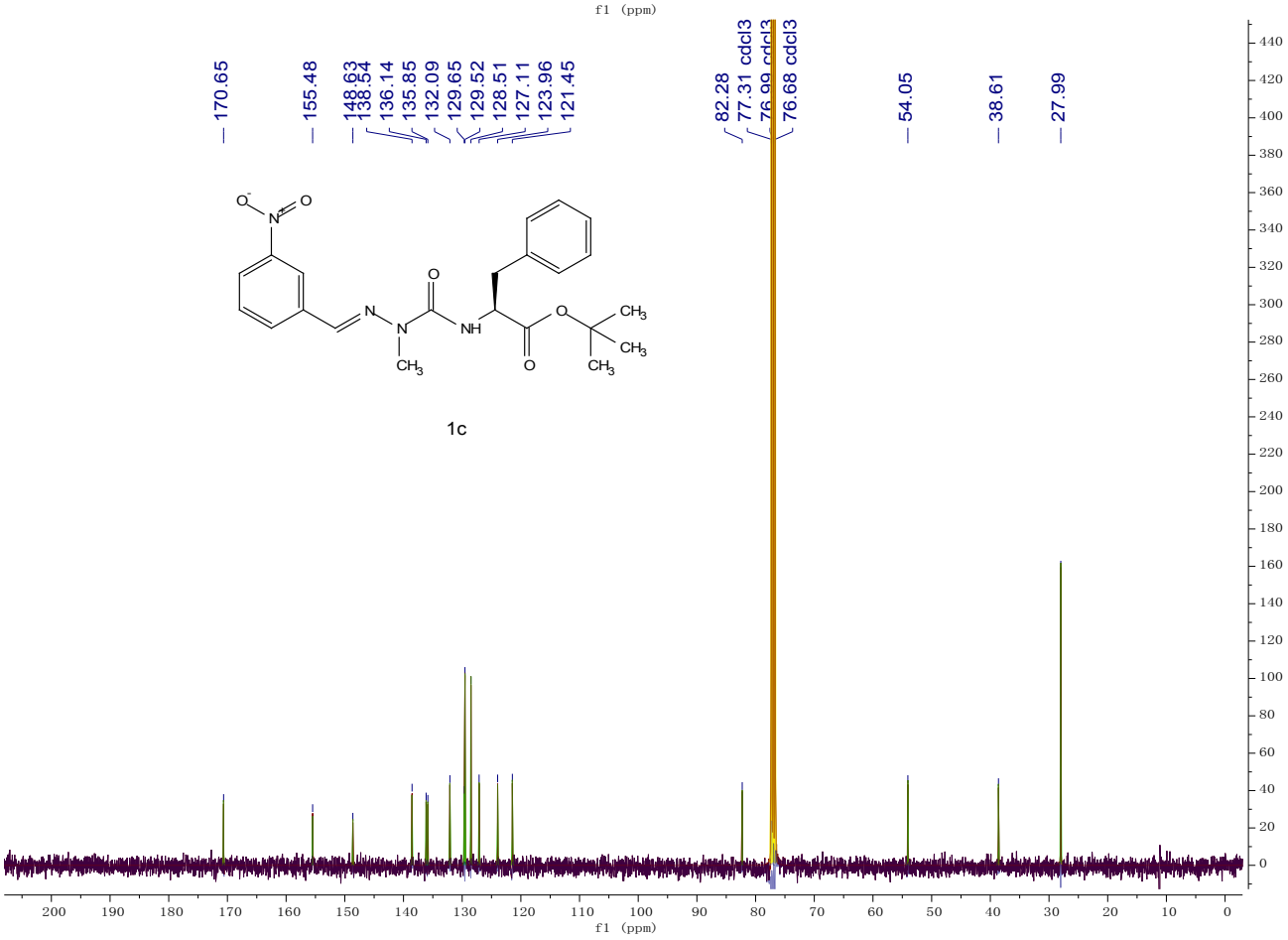
3. ^{1}H-NMR and ^{13}C-NMR spectra of compounds 1b-d, 2b-n



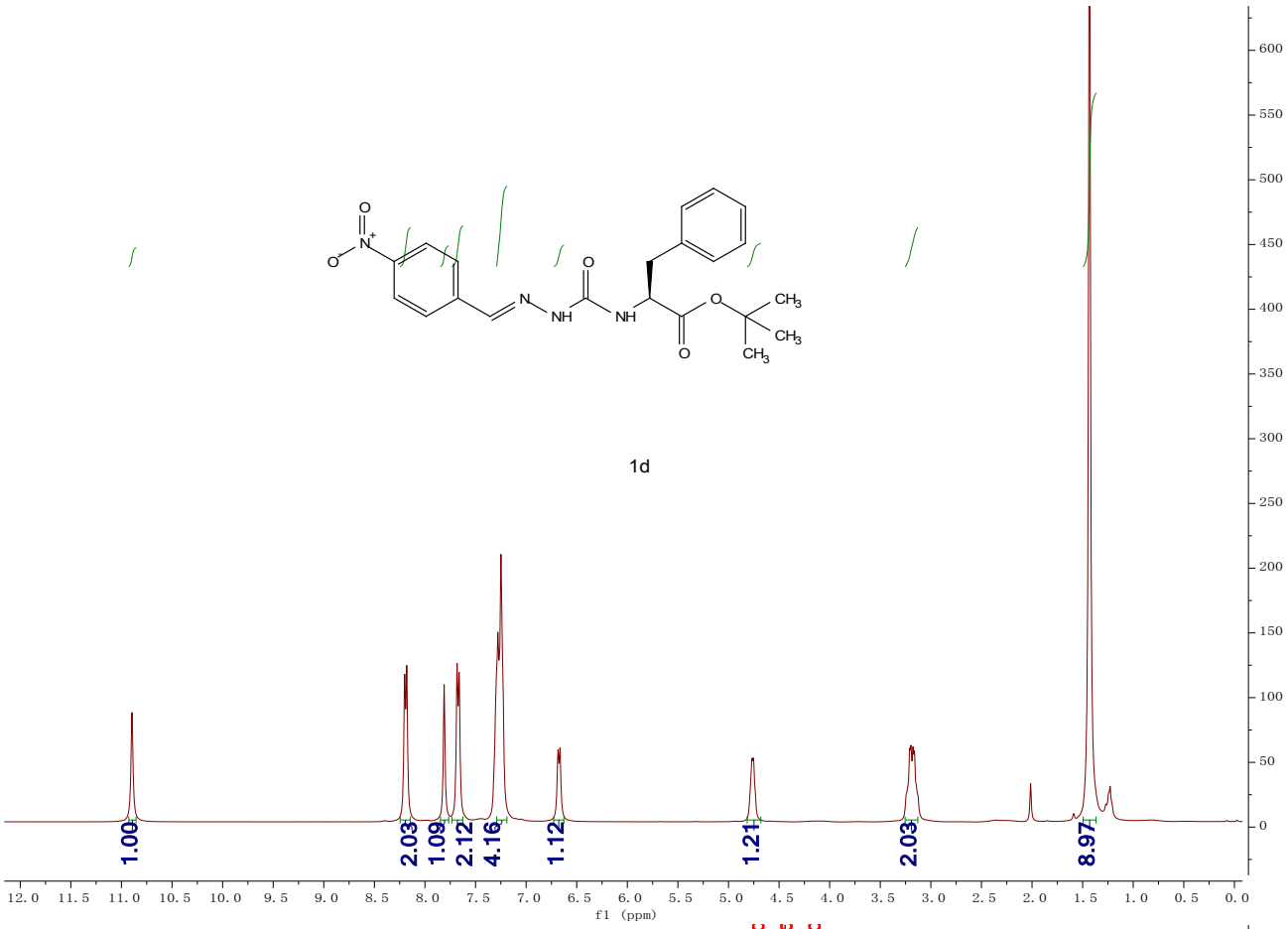


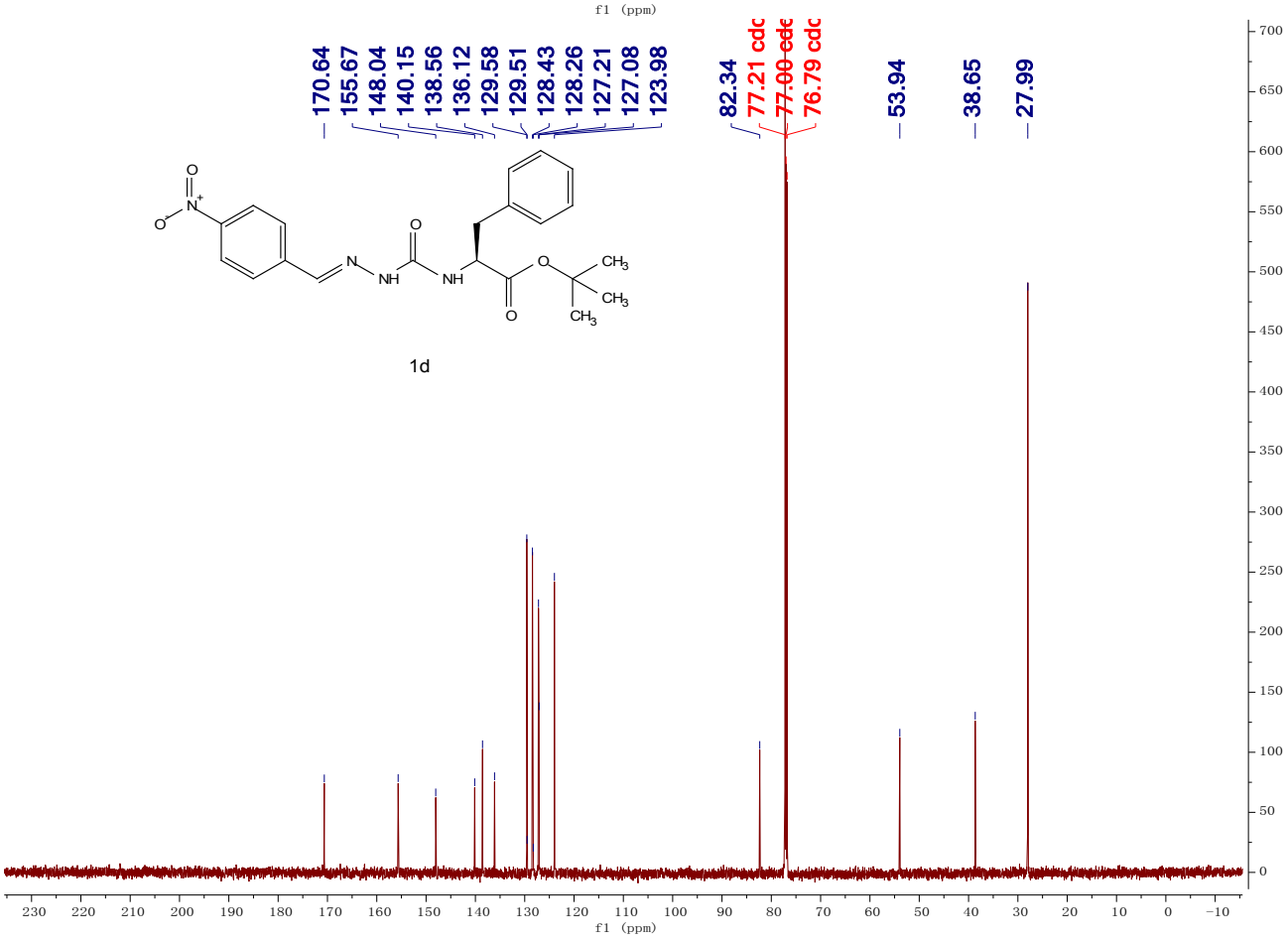

1.08
1.04
1.02
1.01
1.01
1.16
5.46
1.13
1.09
2.15
0.26
0.22
9.00
f1 (ppm)

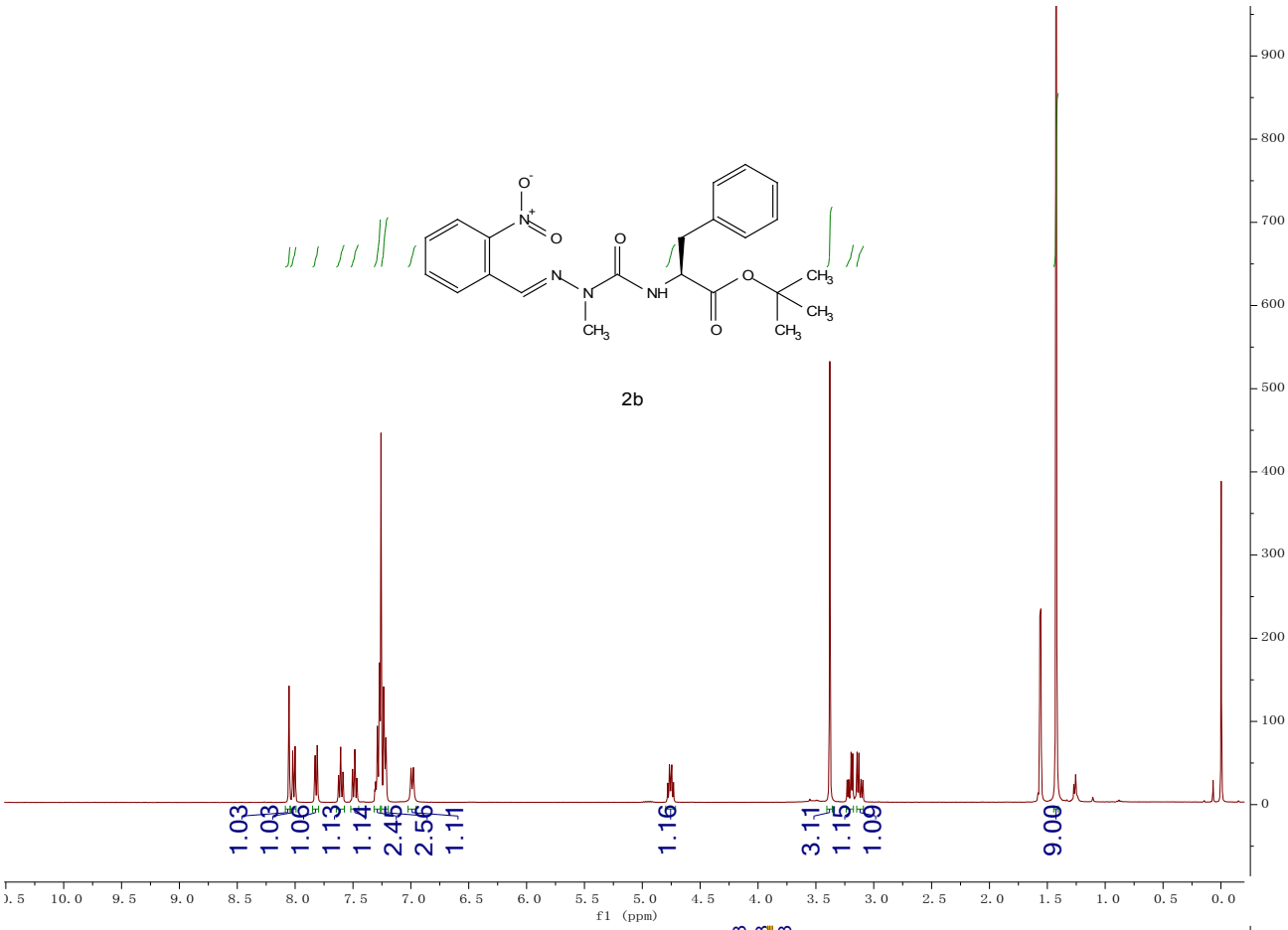
1c


170.65
155.48
148.63
138.54
136.14
135.85
132.09
129.65
129.52
128.51
127.11
123.96
121.45
82.28
77.31 cdcl3
76.99 cdcl3
76.68 cdcl3
54.05
38.61
27.99
f1 (ppm)

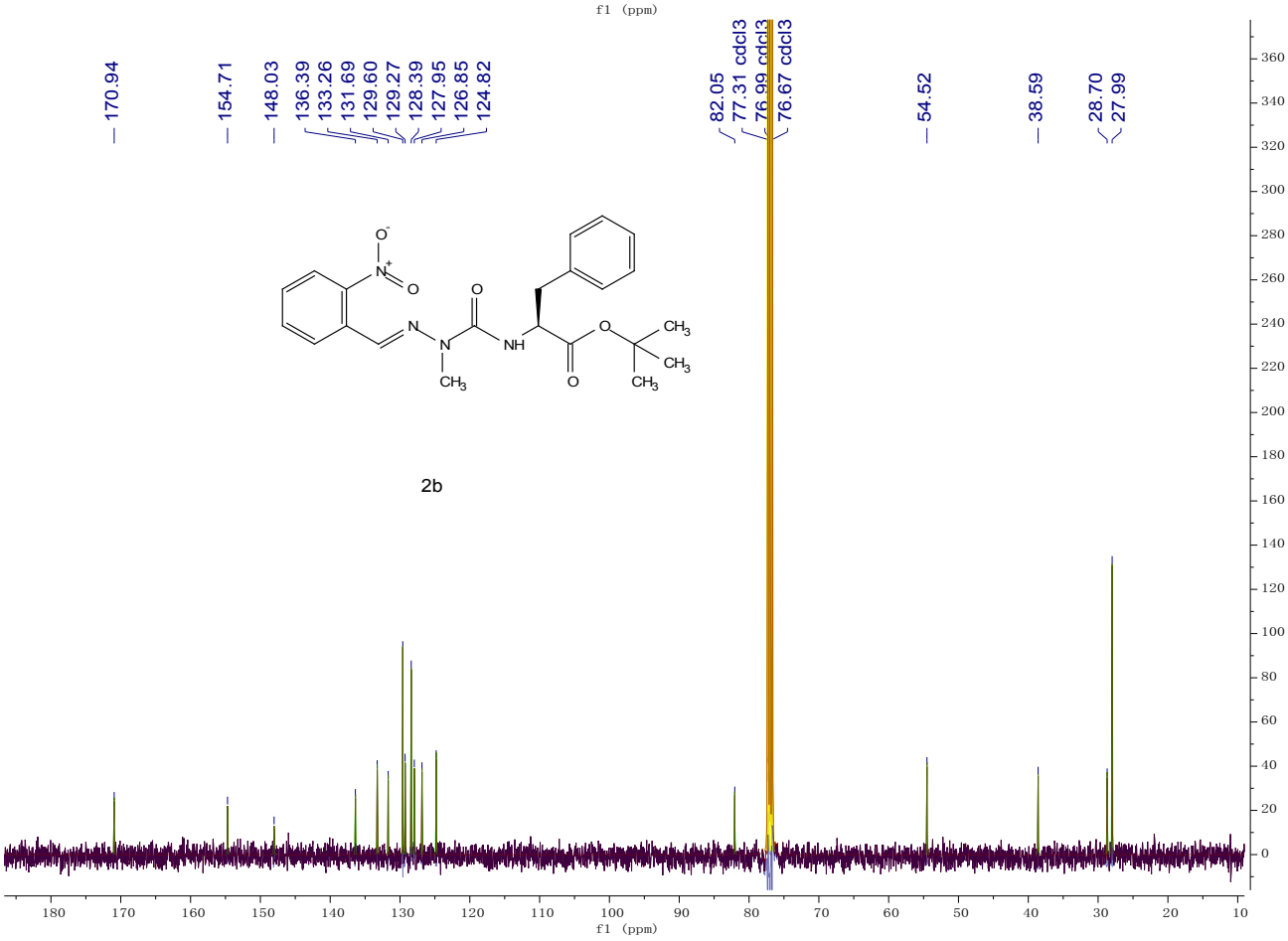
1c


1d
1.00
2.03
1.09
2.12
4.16
1.12
1.21
2.03
8.97
f1 (ppm)

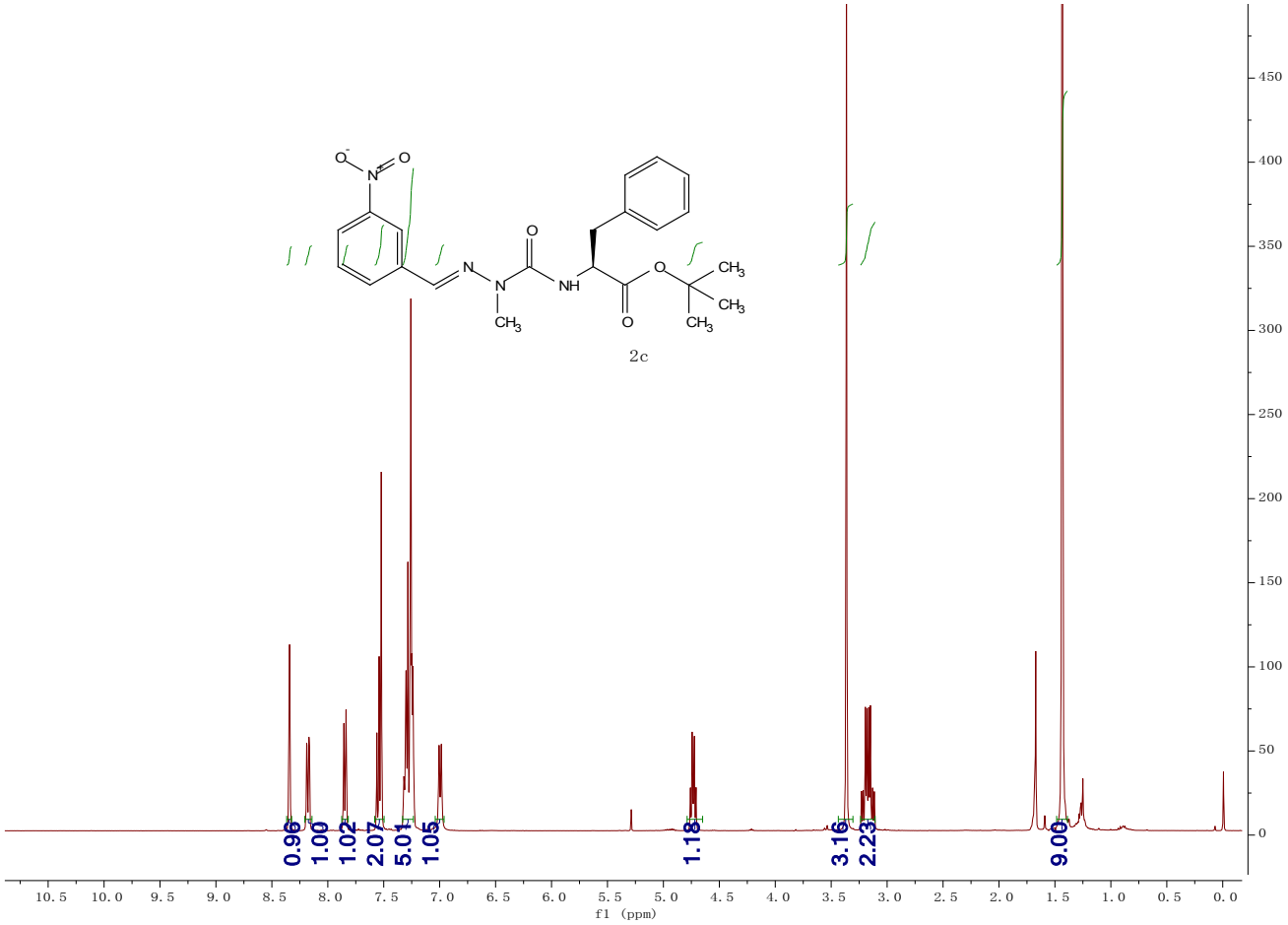

170.64
155.67
148.04
140.15
138.56
136.12
129.58
129.51
128.43
128.26
127.21
127.08
123.98
82.34
77.21 cdc
77.00 cdc
76.79 cdc
53.94
38.65
27.99
1d
f1 (ppm)

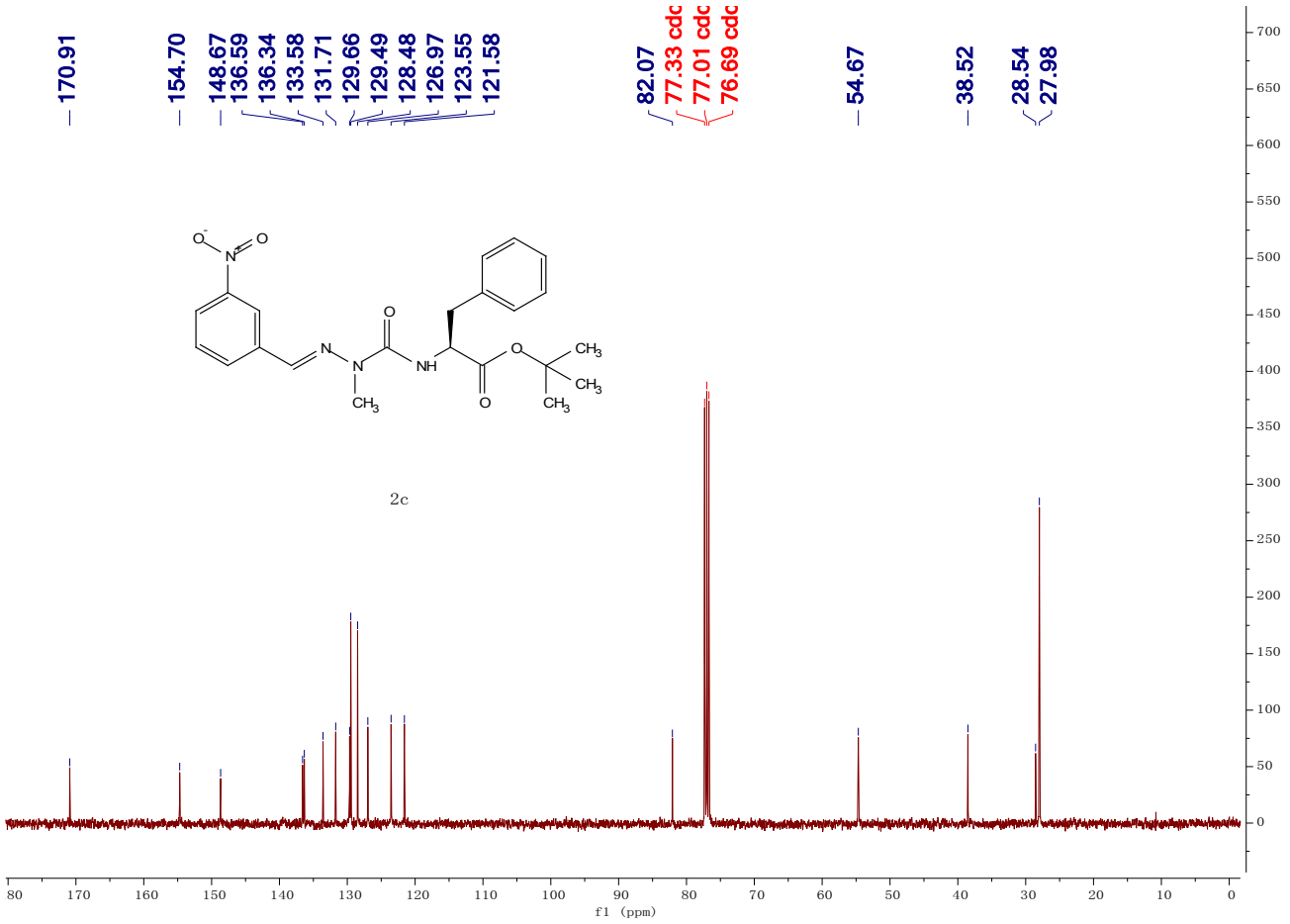

1.03
1.03
1.00
1.13
1.14
2.45
2.56
1.11
1.16
3.11
1.15
1.09
9.00
f1 (ppm)

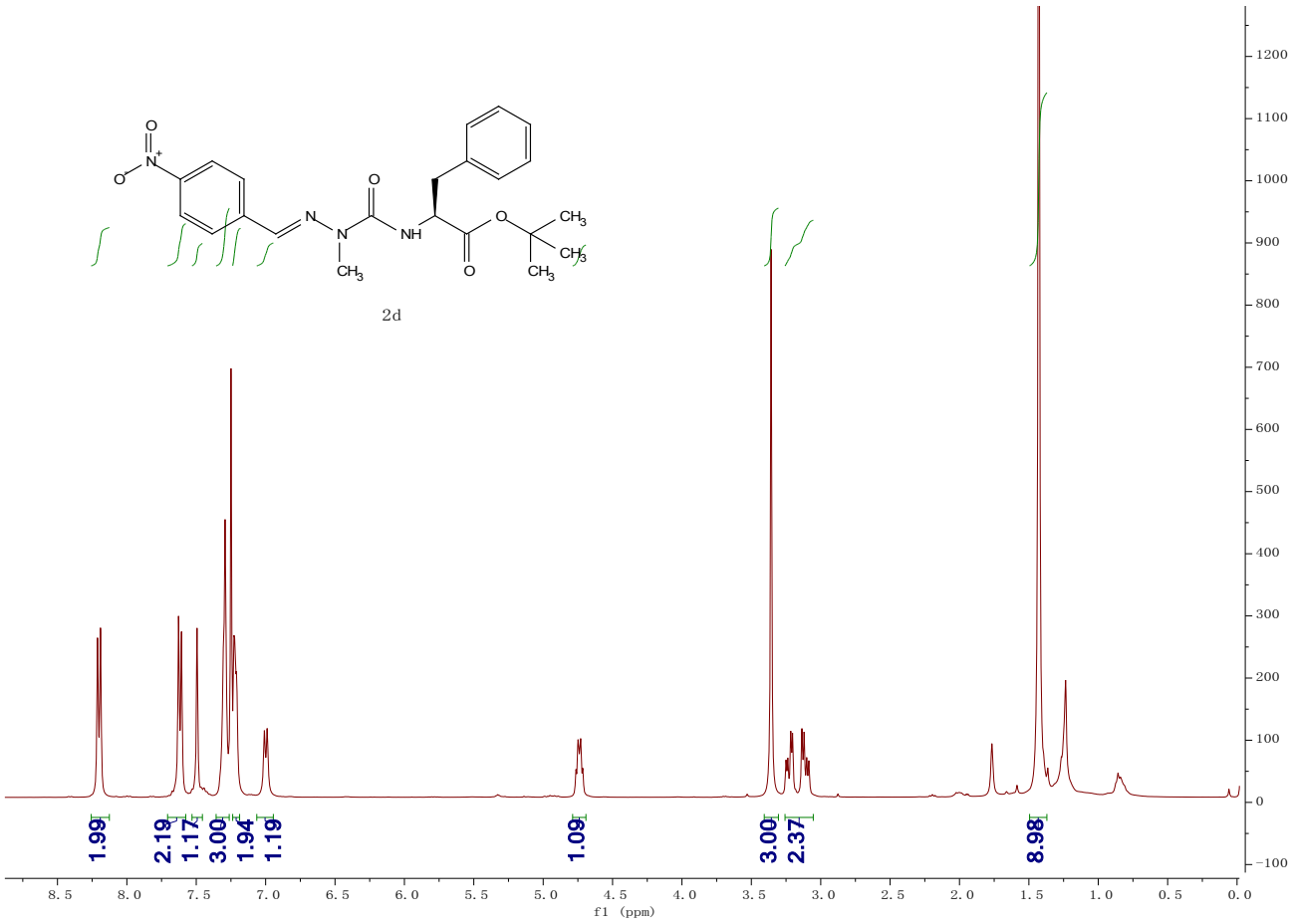
2b

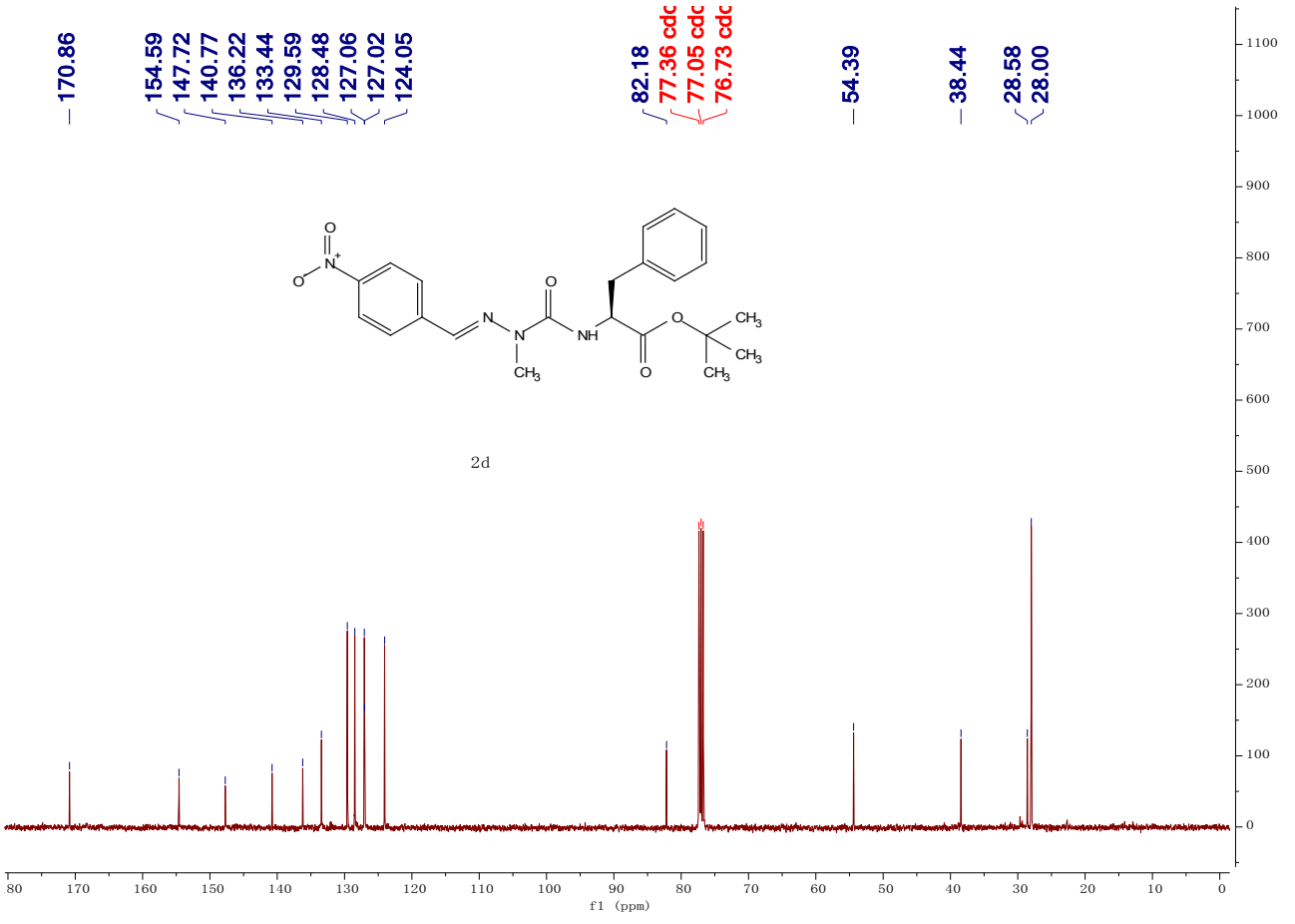

170.94
154.71
148.03
136.39
133.26
131.69
129.60
129.27
128.39
127.95
126.85
124.82
82.05
77.31 cdcl3
76.99 cdcl3
76.67 cdcl3
54.52
38.59
28.70
27.99
f1 (ppm)

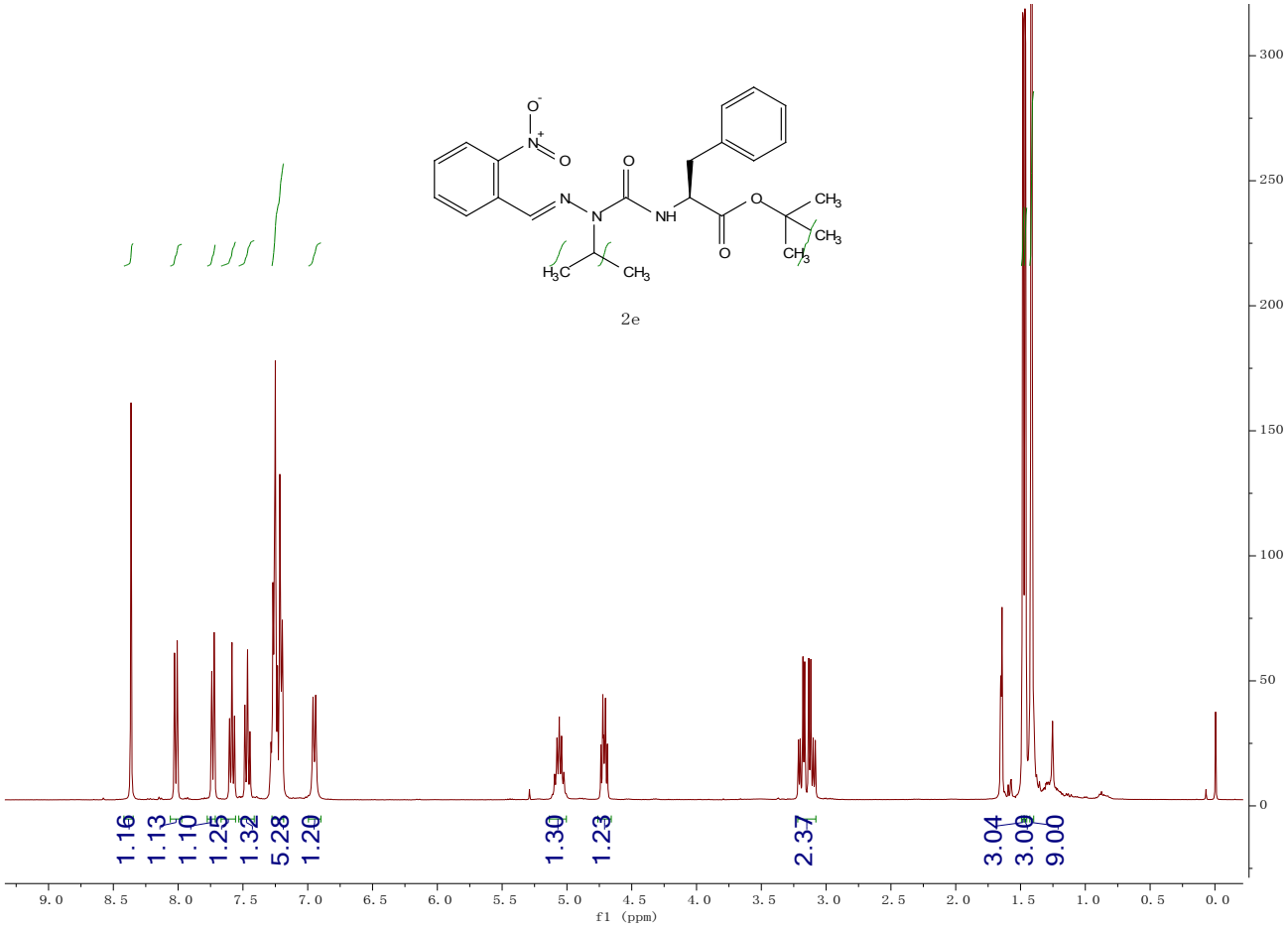
2b

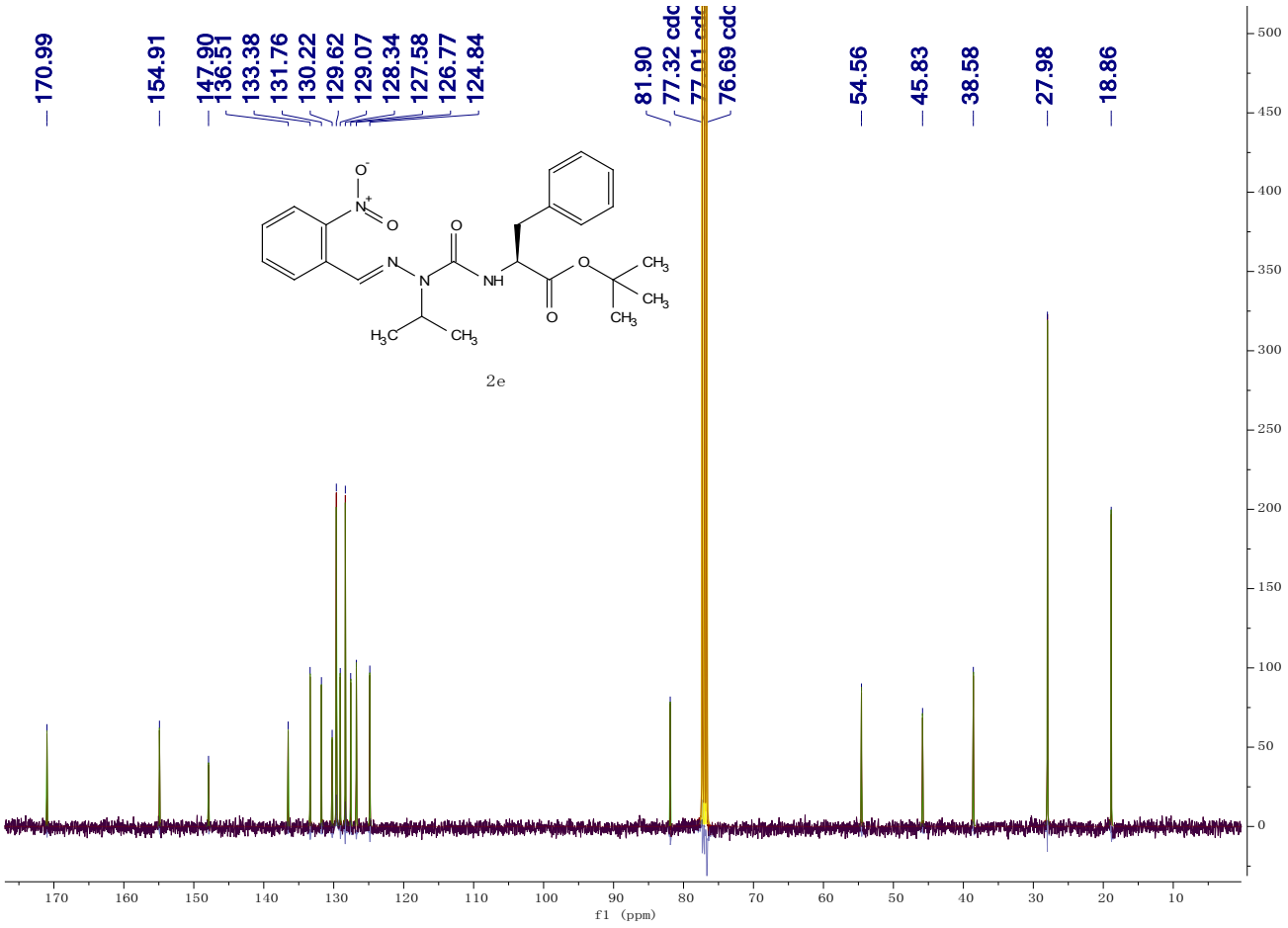

2c
0.96
1.00
1.02
2.07
5.01
1.05
1.18
3.16
2.23
9.00
f1 (ppm)

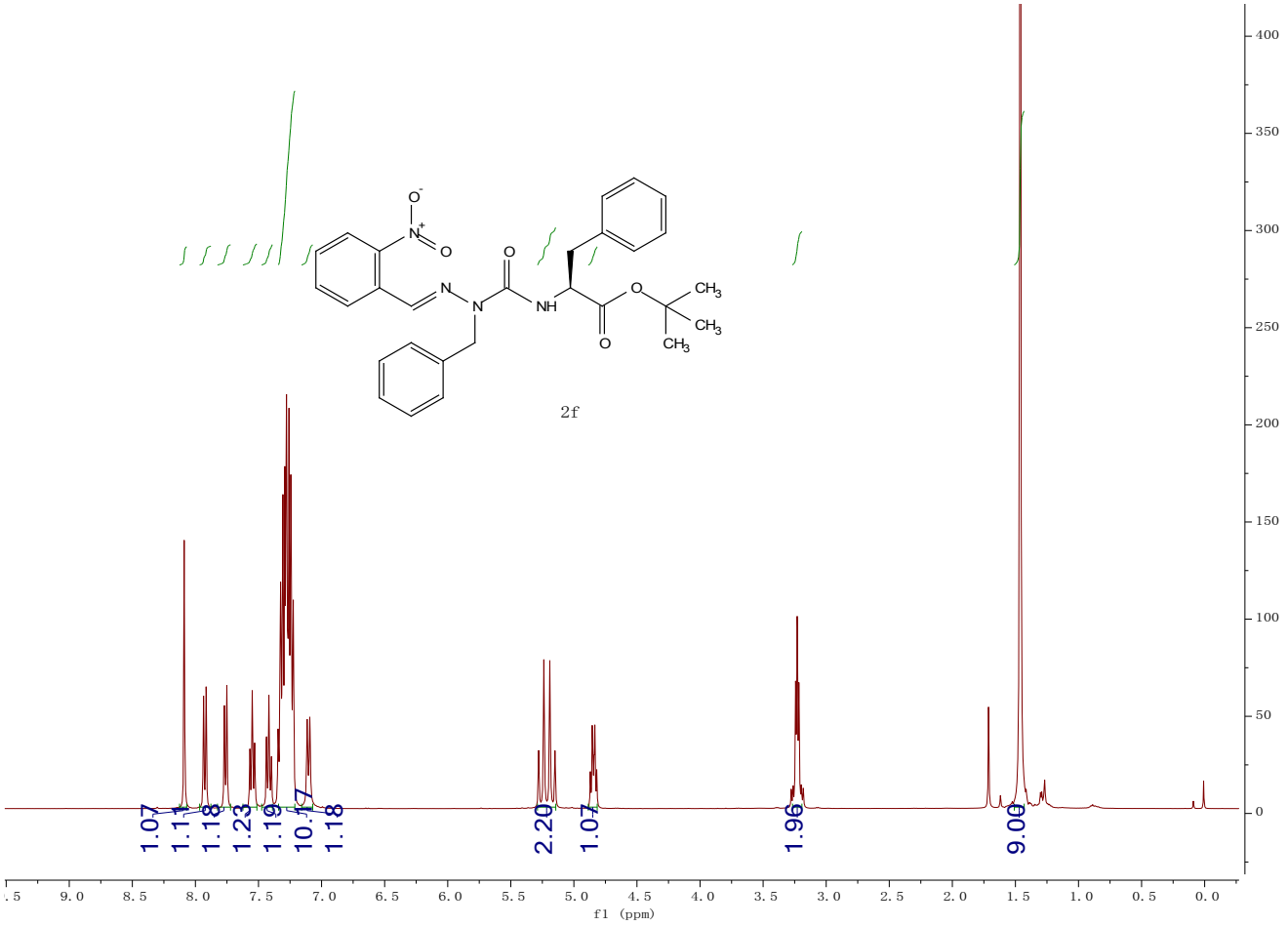

170.91
154.70
148.67
136.59
136.34
133.58
131.71
129.66
129.49
128.48
126.97
123.55
121.58
82.07
77.33 cdc
77.01 cdc
76.69 cdc
54.67
38.52
28.54
27.98
2c
f1 (ppm)

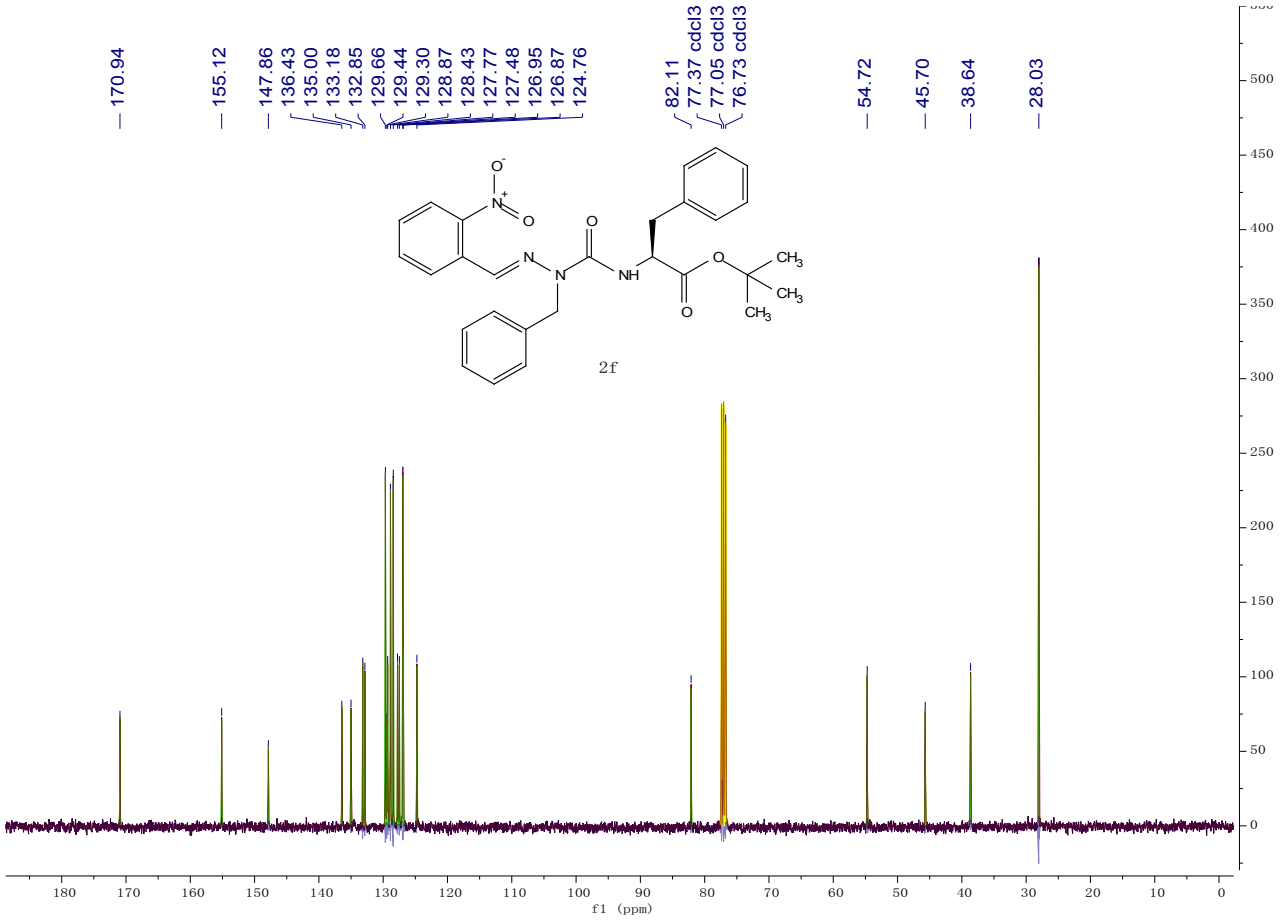

2d
1.99
2.19
1.17
3.00
1.94
1.19
1.09
3.00
2.37
8.98
f1 (ppm)

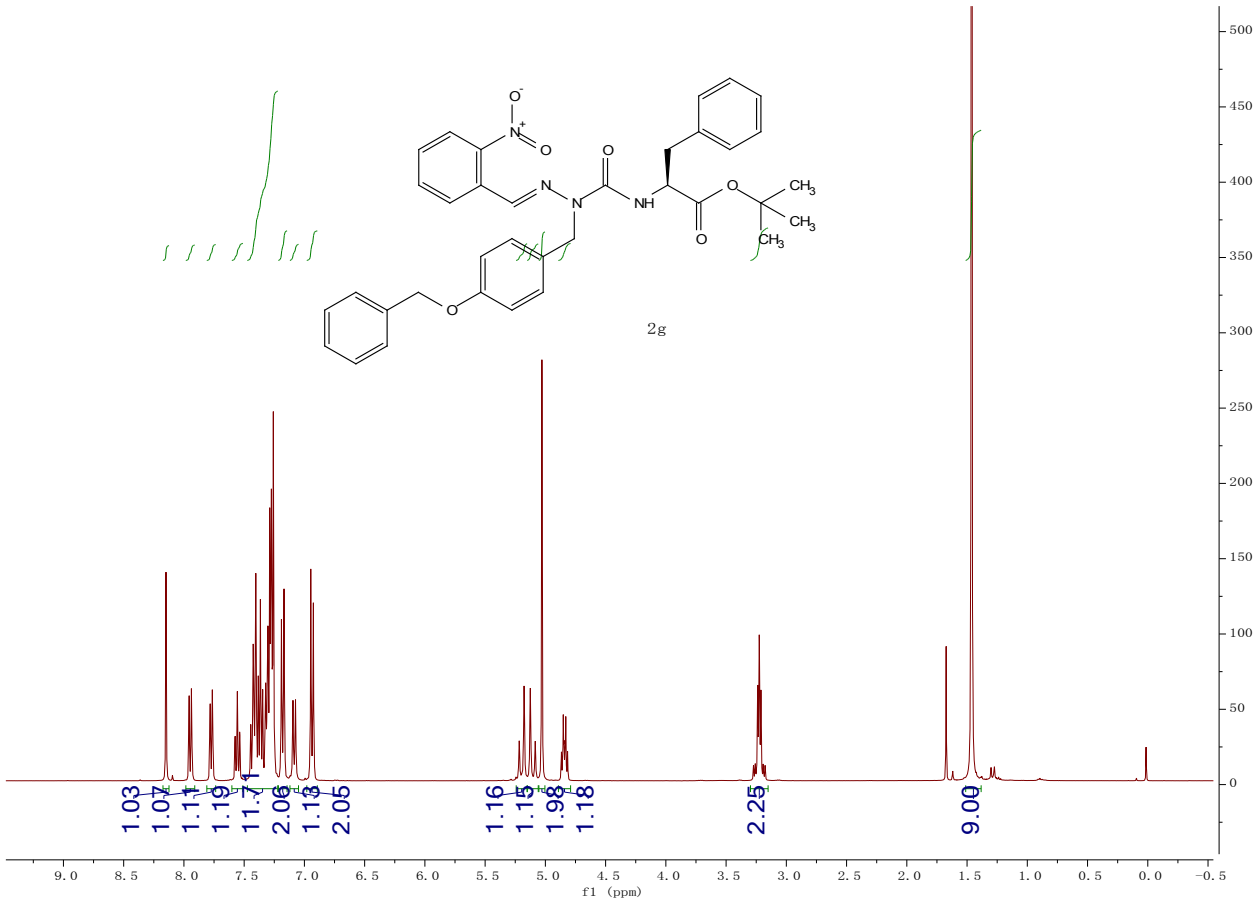

170.86
154.59
147.72
140.77
136.22
133.44
129.59
128.48
127.06
127.02
124.05
82.18
77.36 cdc
77.05 cdc
76.73 cdc
54.39
38.44
28.58
28.00
2d
f1 (ppm)

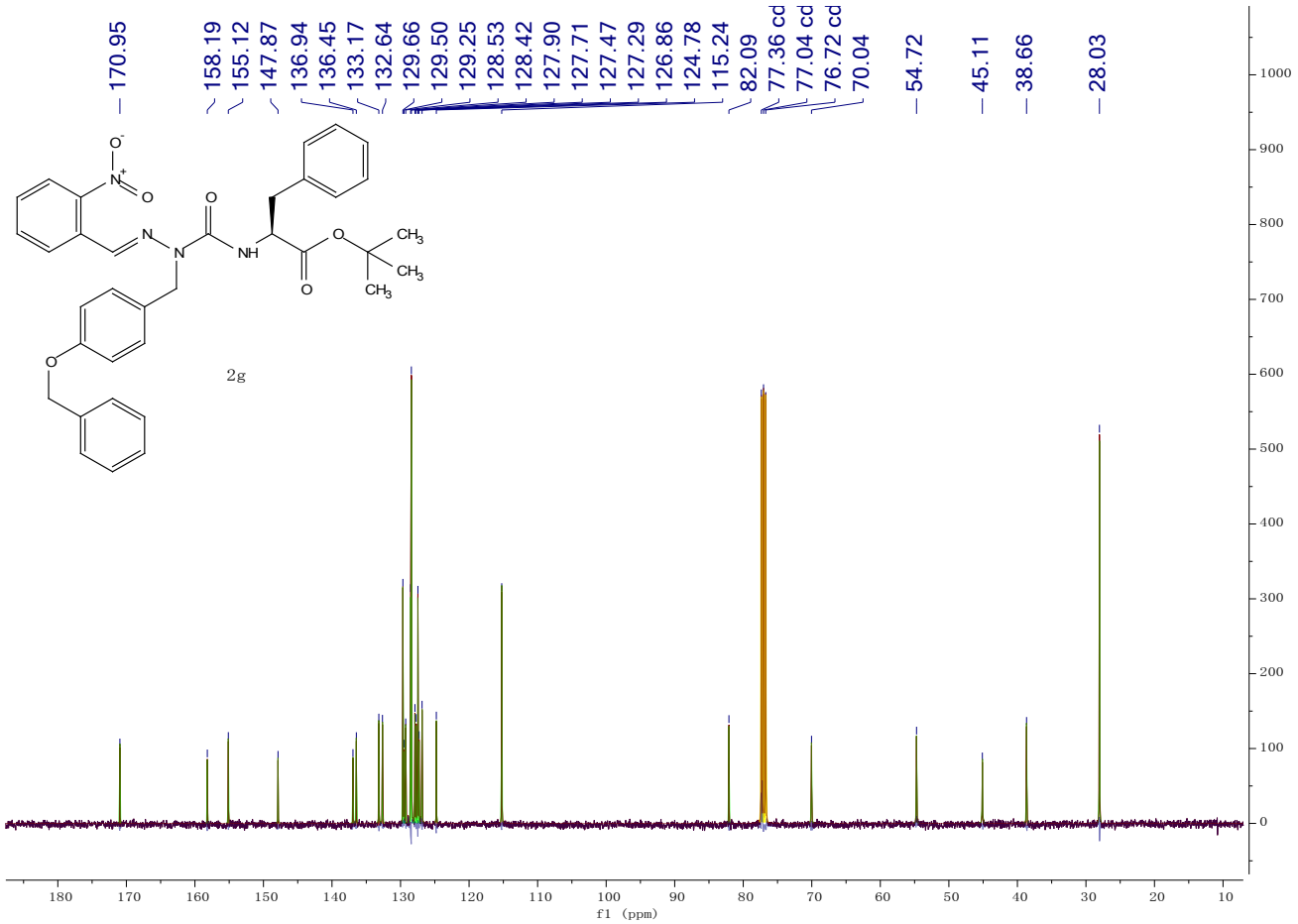

2e
1.16
1.13
1.10
1.25
1.32
5.28
1.20
1.30
1.23
2.37
3.04
3.00
9.00
f1 (ppm)

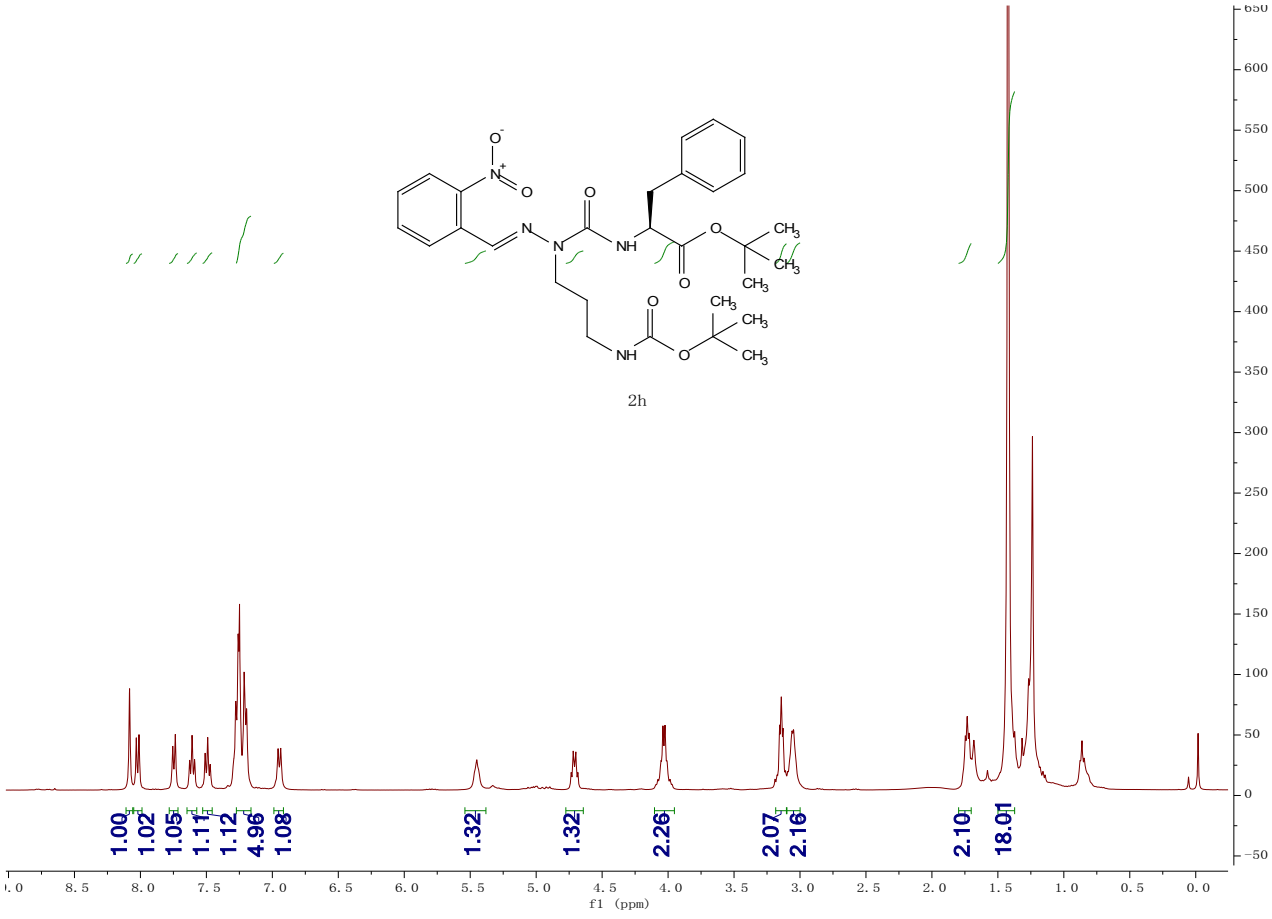

170.99
154.91
147.90
136.51
133.38
131.76
130.22
129.62
129.07
128.34
127.58
126.77
124.84
81.90
77.32 cdc
77.01 cdc
76.69 cdc
54.56
45.83
38.58
27.98
18.86
2e
f1 (ppm)

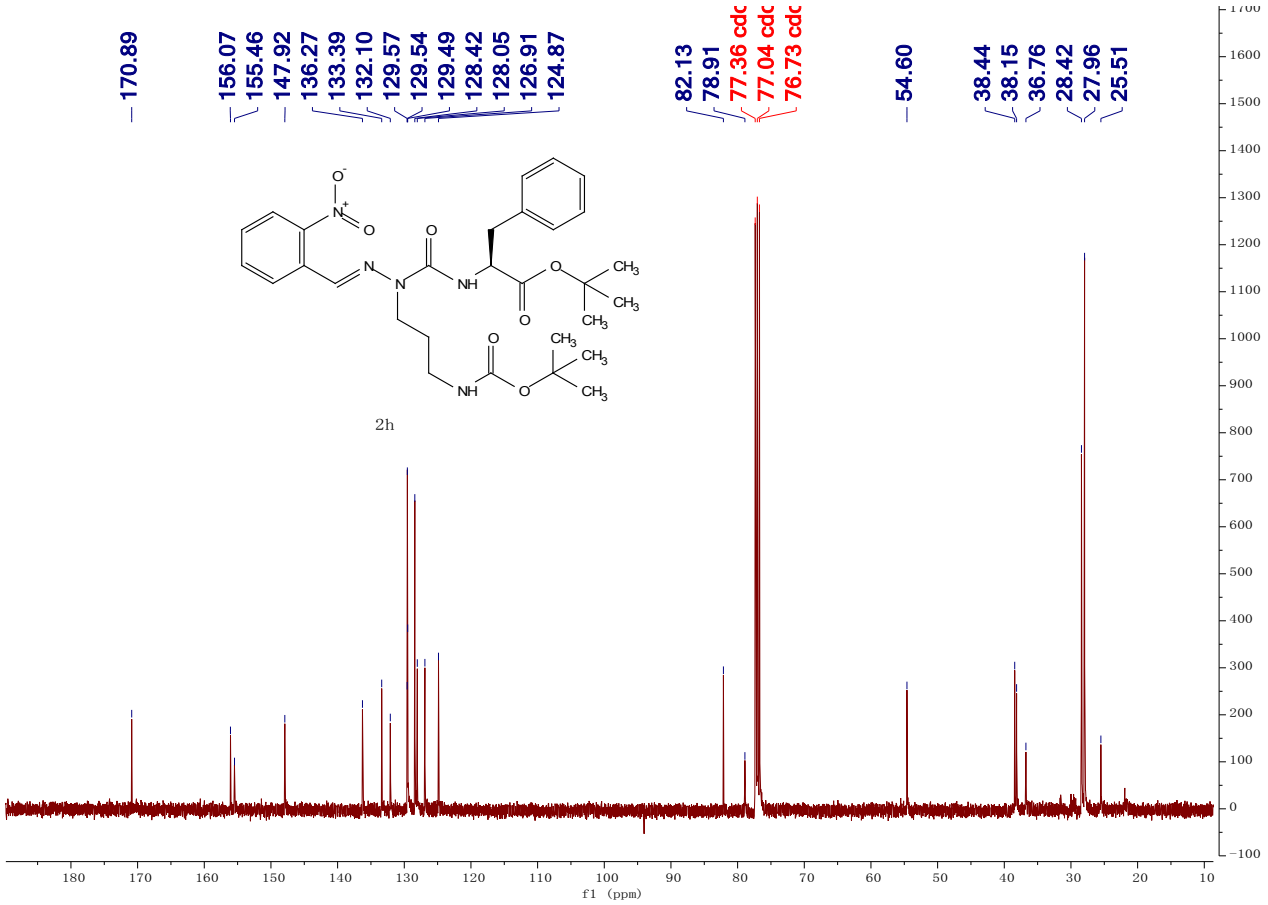

2f
1.07
1.11
1.18
1.23
1.19
10.17
1.18
2.20
1.07
1.96
9.00
f1 (ppm)

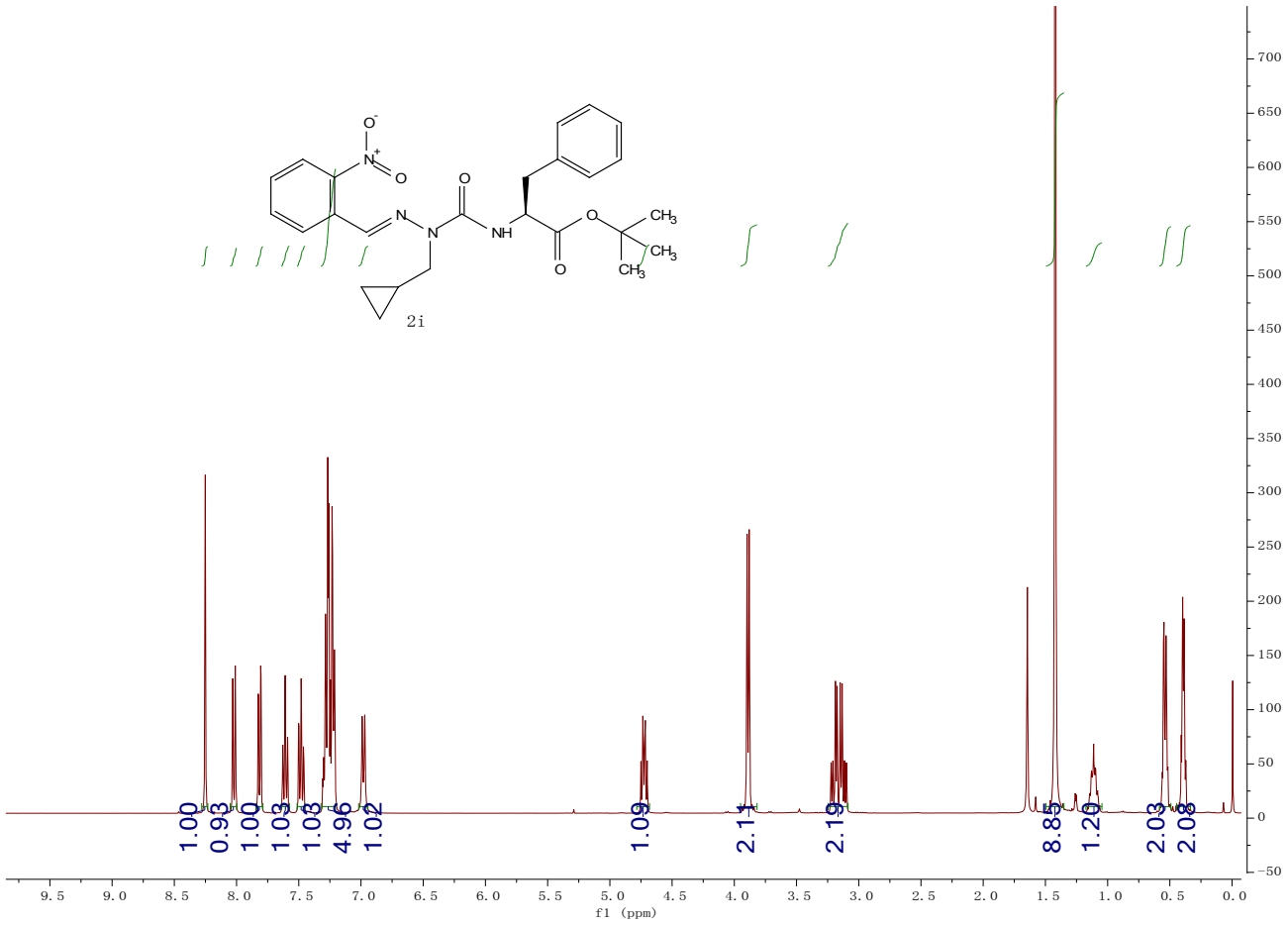

170.94
155.12
147.86
136.43
135.00
133.18
132.85
129.66
129.44
129.30
128.87
128.43
127.77
127.48
126.95
126.87
124.76
82.11
77.37 cdcl3
77.05 cdcl3
76.73 cdcl3
54.72
45.70
38.64
28.03
2f
f1 (ppm)

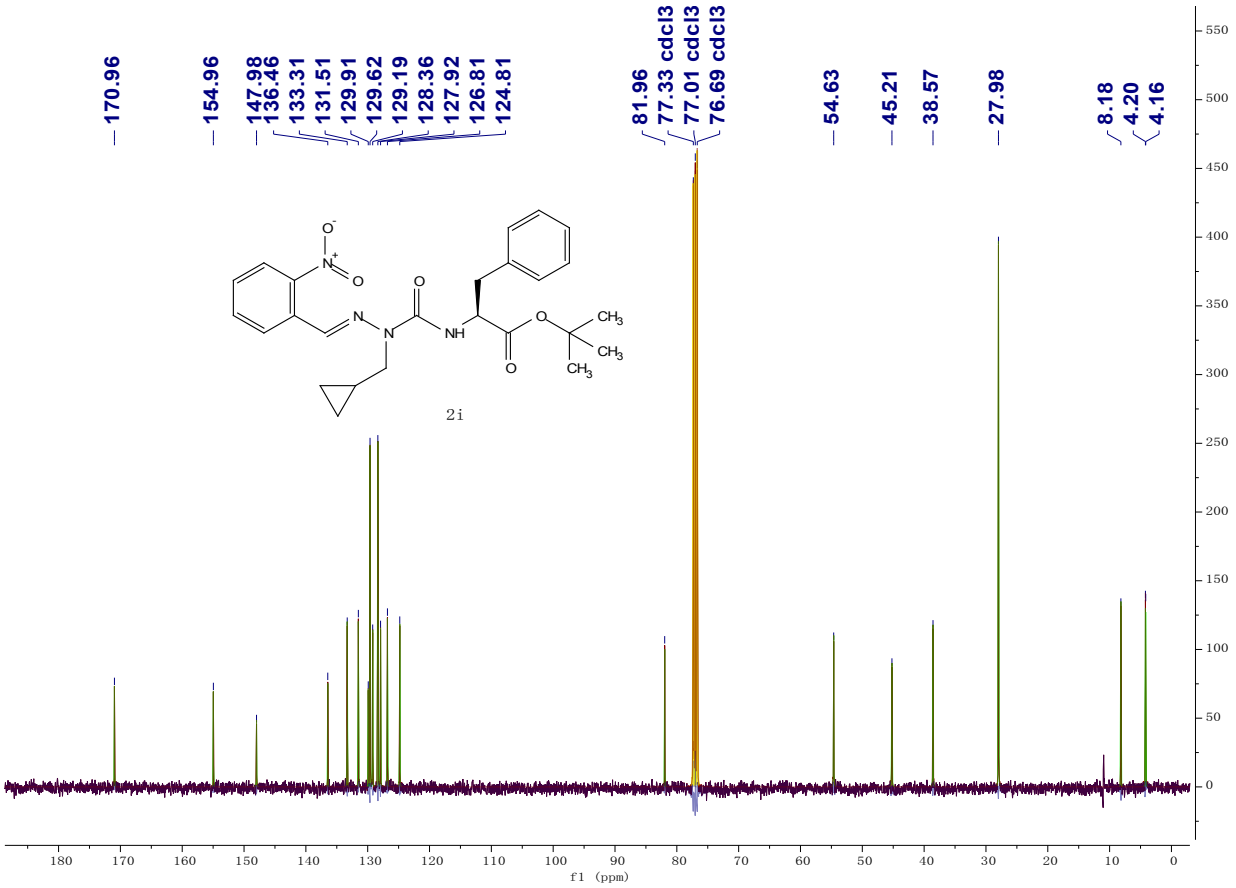

1.03
1.07
1.11
1.19
11.74
2.06
1.13
2.05
1.16
1.15
1.98
1.18
2.25
9.00
2g
f1 (ppm)

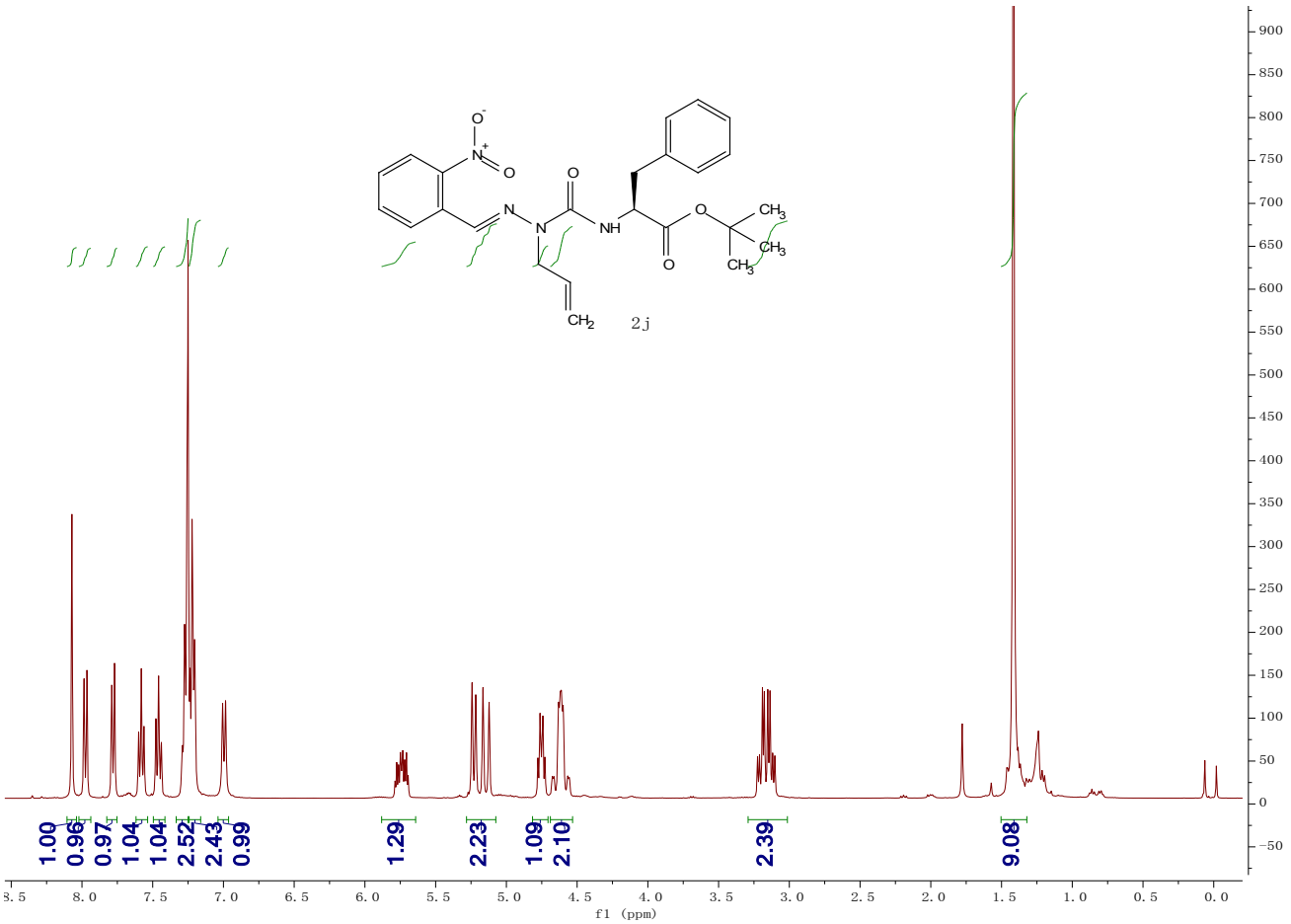

170.95
158.19
155.12
147.87
136.94
136.45
133.17
132.64
129.66
129.50
129.25
128.53
128.42
127.90
127.71
127.47
127.29
126.86
124.78
115.24
82.09
77.36 cd
77.04 cd
76.72 cd
70.04
54.72
45.11
38.66
28.03
2g
f1 (ppm)

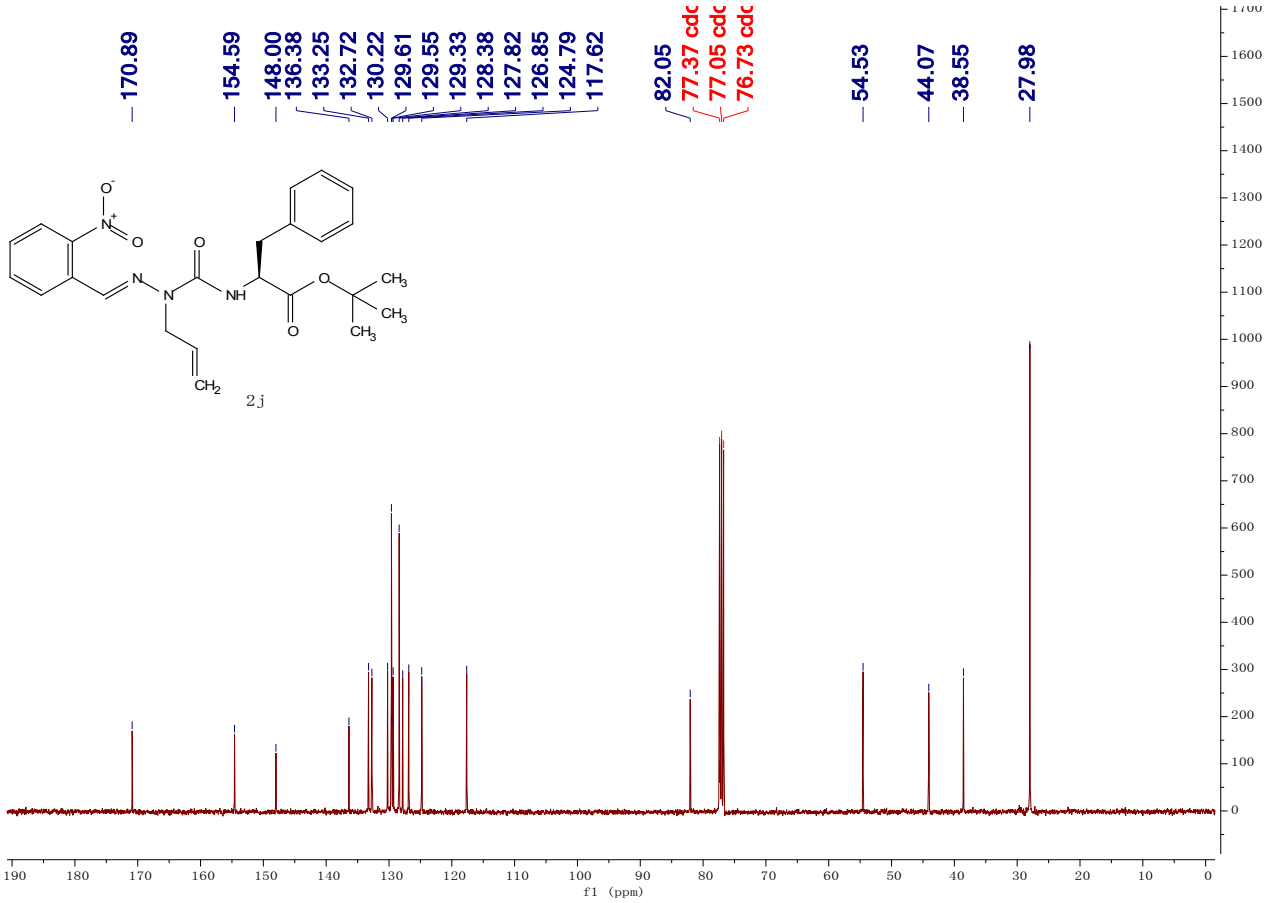

1.00
1.02
1.05
1.11
1.12
4.96
1.08
1.32
1.32
2.26
2.07
2.16
2.10
18.01
2h
f1 (ppm)


170.89
156.07
155.46
147.92
136.27
133.39
132.10
129.57
129.54
129.49
128.42
128.05
126.91
124.87
82.13
78.91
77.36 cdc
77.04 cdc
76.73 cdc
54.60
38.44
38.15
36.76
28.42
27.96
25.51
2h
f1 (ppm)

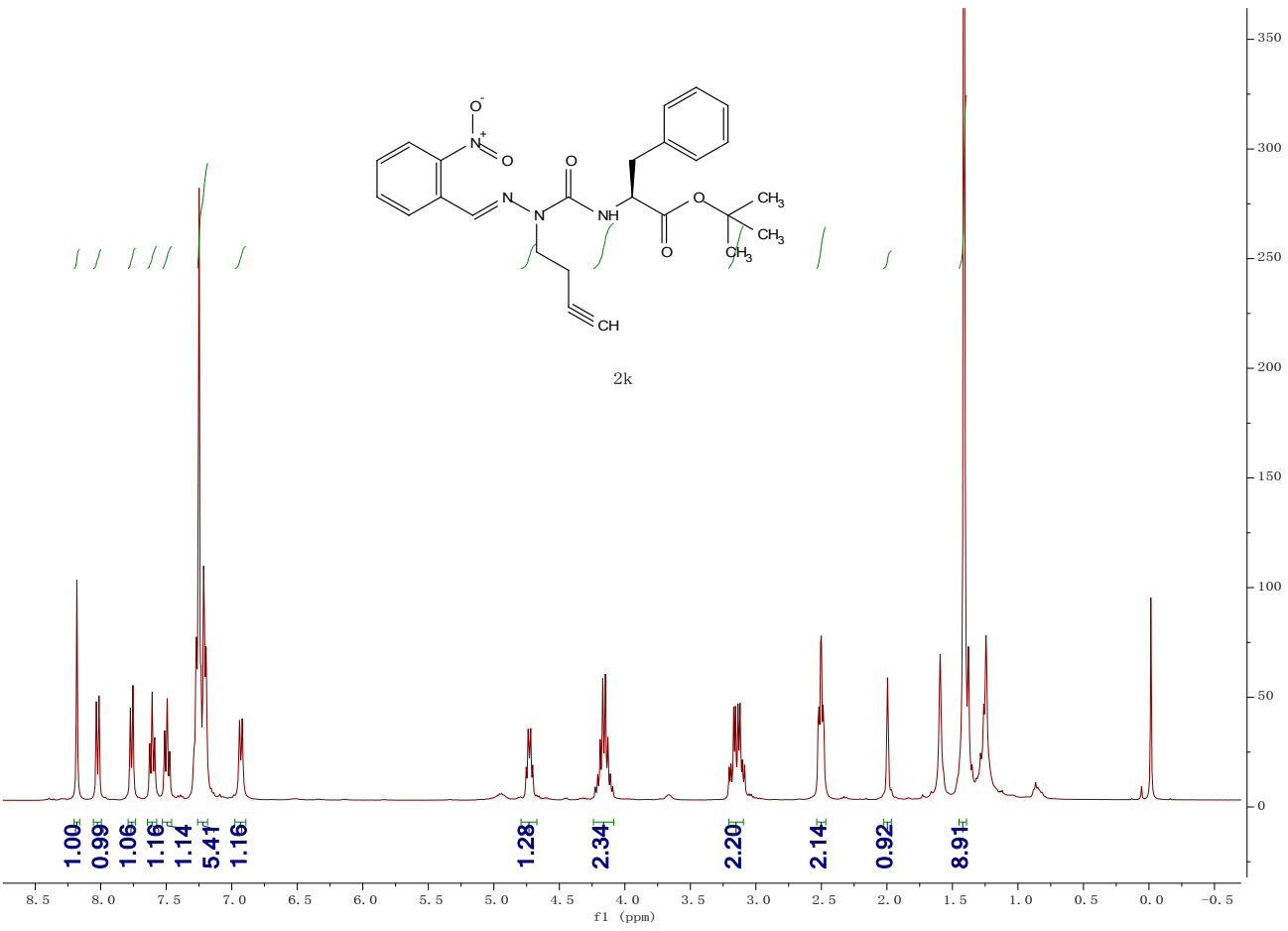

2i
1.00
0.93
1.00
1.03
1.03
4.96
1.02
1.09
2.11
2.19
8.85
1.20
2.03
2.08
f1 (ppm)


170.96
154.96
147.98
136.46
133.31
131.51
129.91
129.62
129.19
128.36
127.92
126.81
124.81
81.96
77.33 cdcl3
77.01 cdcl3
76.69 cdcl3
54.63
45.21
38.57
27.98
8.18
4.20
4.16
2i
f1 (ppm)

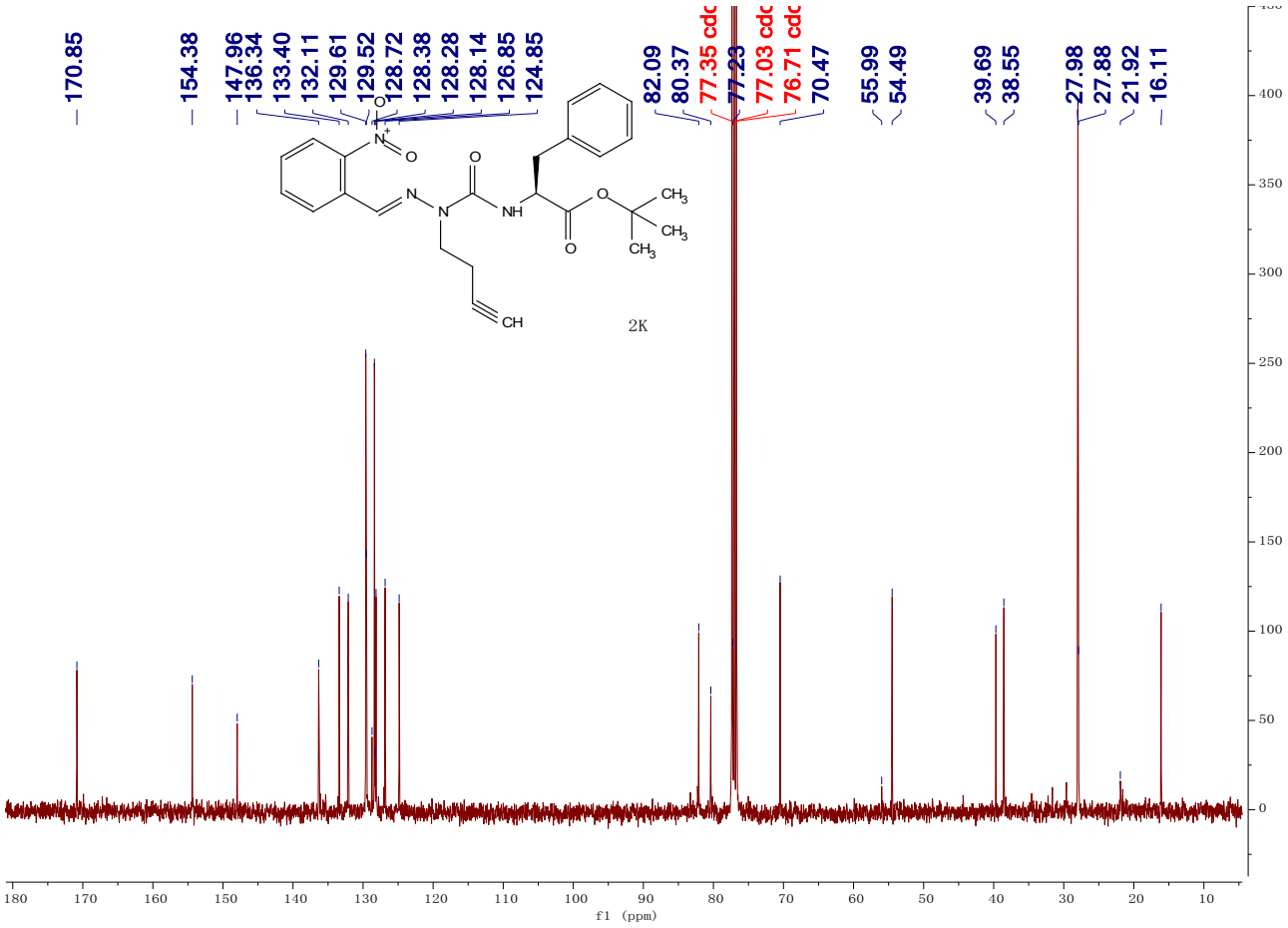

1.00
0.96
0.97
1.04
1.04
2.52
2.43
0.99
1.29
2.23
1.09
2.10
2.39
9.08
2j
f1 (ppm)

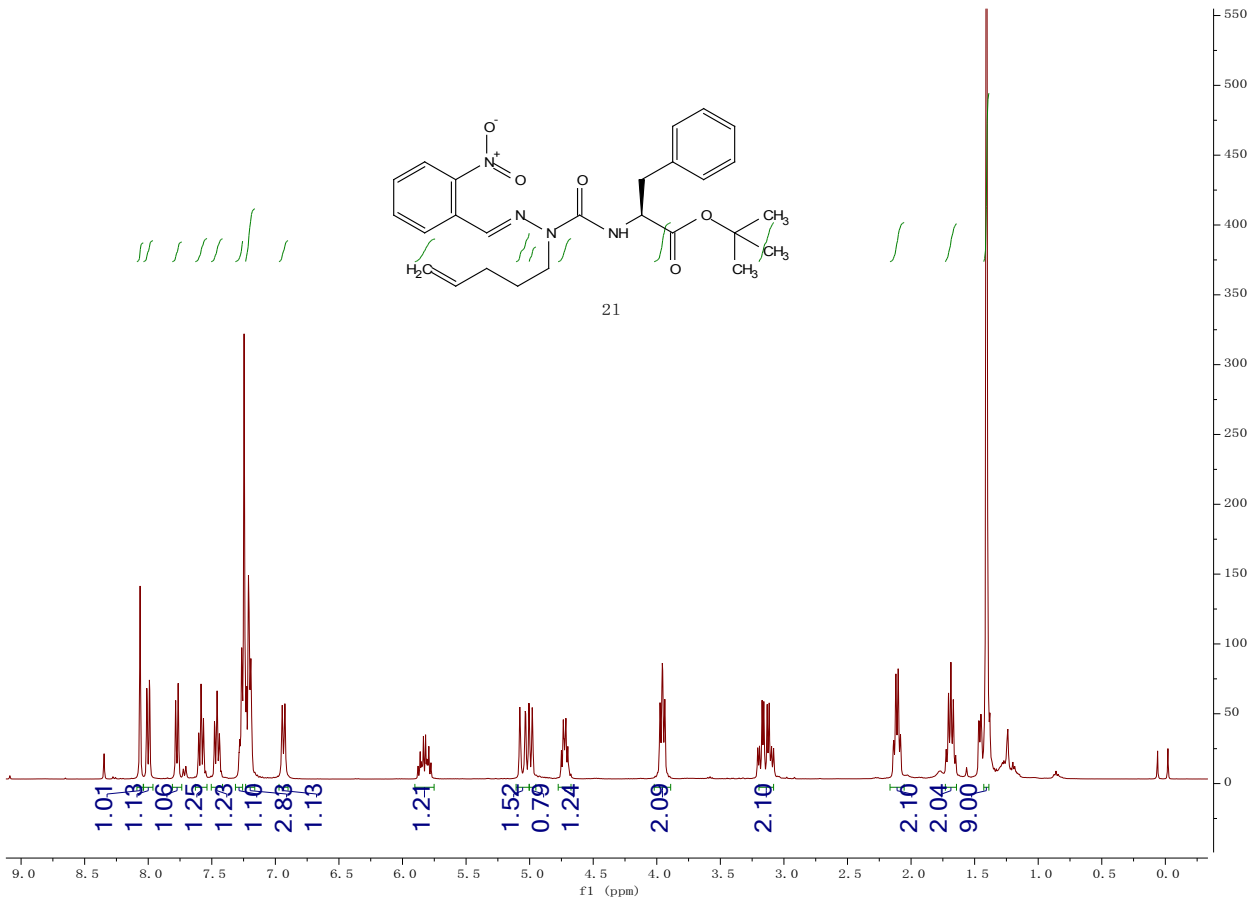

170.89
154.59
148.00
136.38
133.25
132.72
130.22
129.61
129.55
129.33
128.38
127.82
126.85
124.79
117.62
82.05
77.37 cdc
77.05 cdc
76.73 cdc
54.53
44.07
38.55
27.98
f1 (ppm)

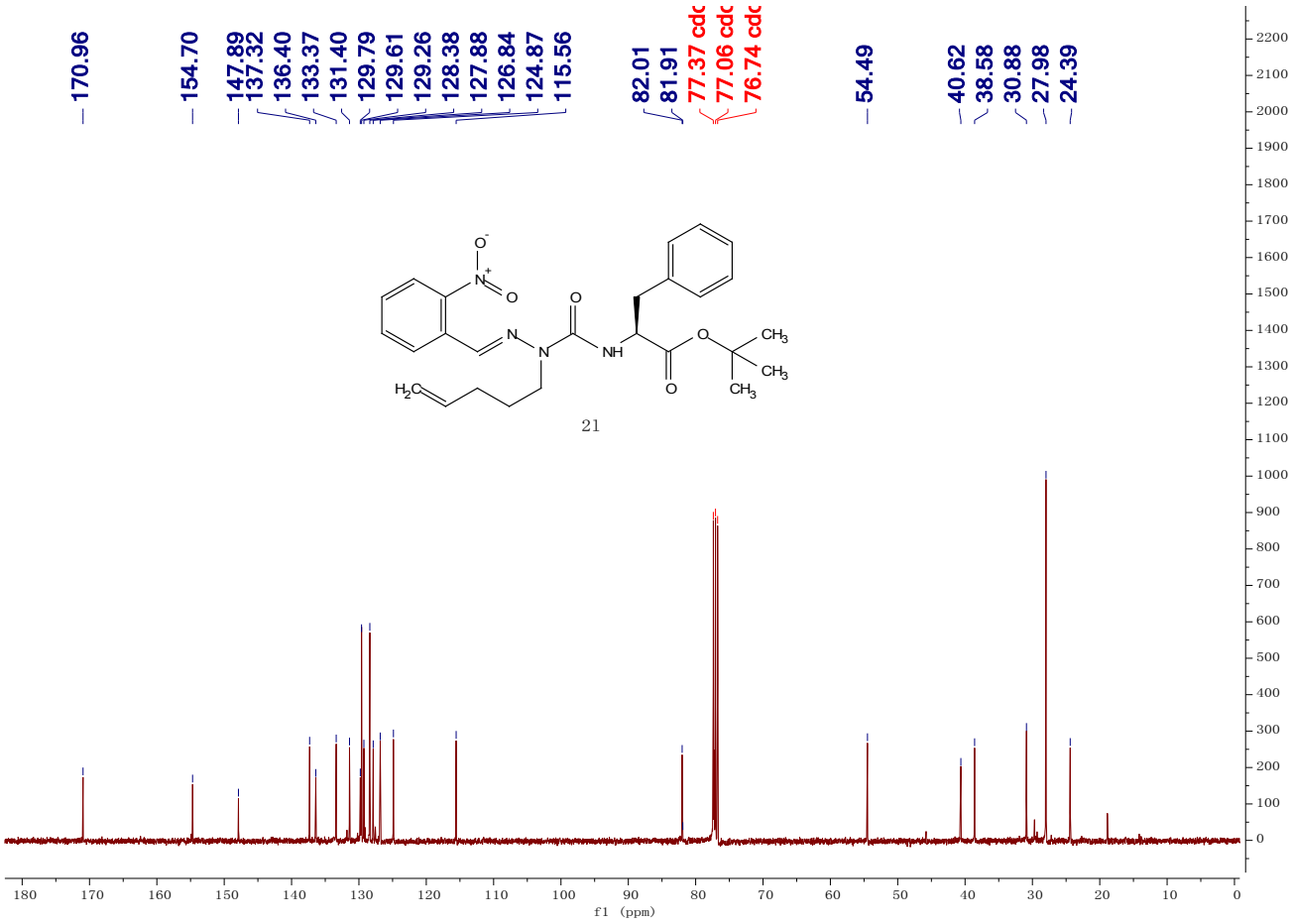
2j


1.00
0.99
1.06
1.16
1.14
5.47
1.16
1.28
2.34
2.20
2.14
0.92
8.91
f1 (ppm)

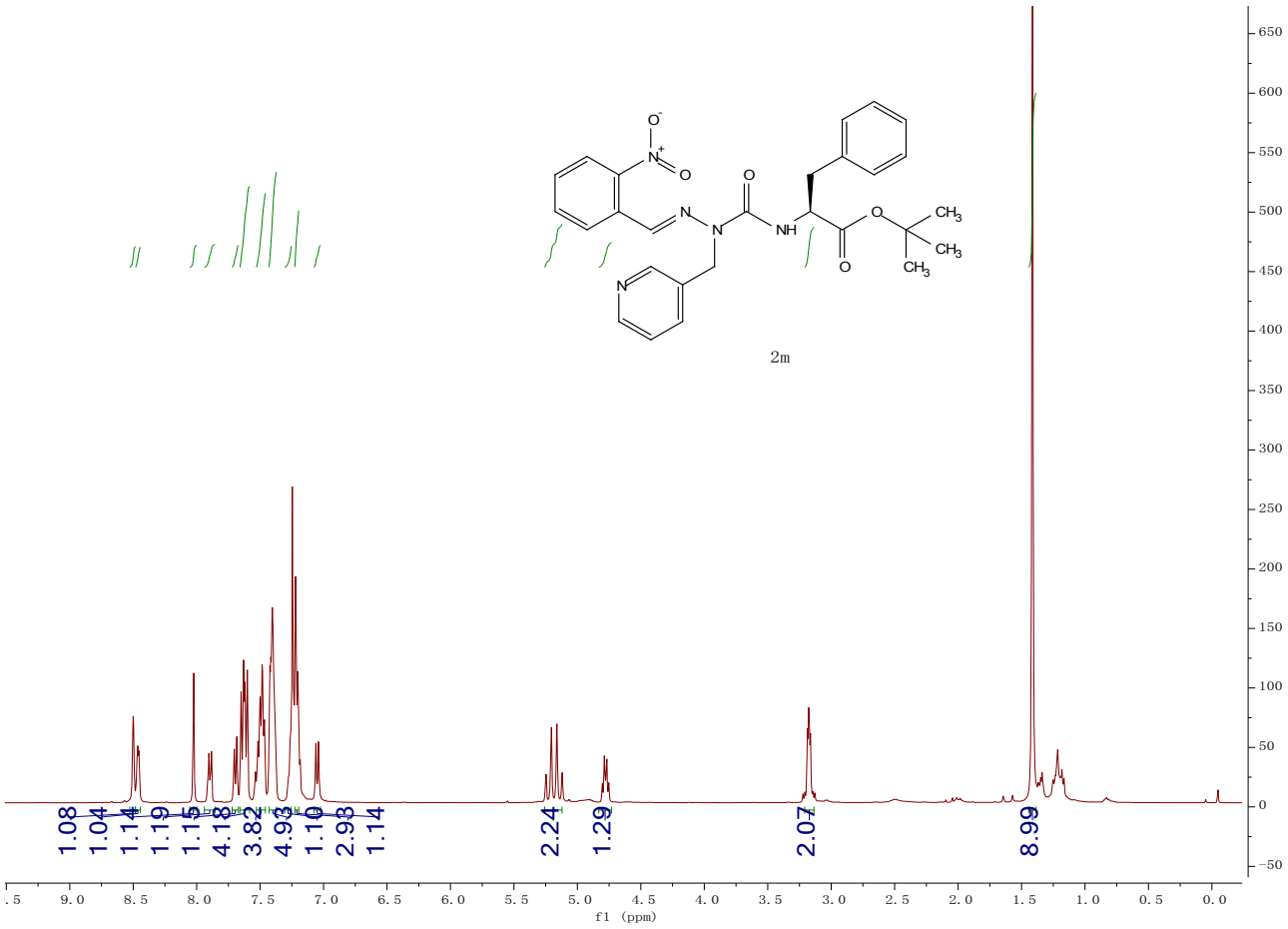
2k

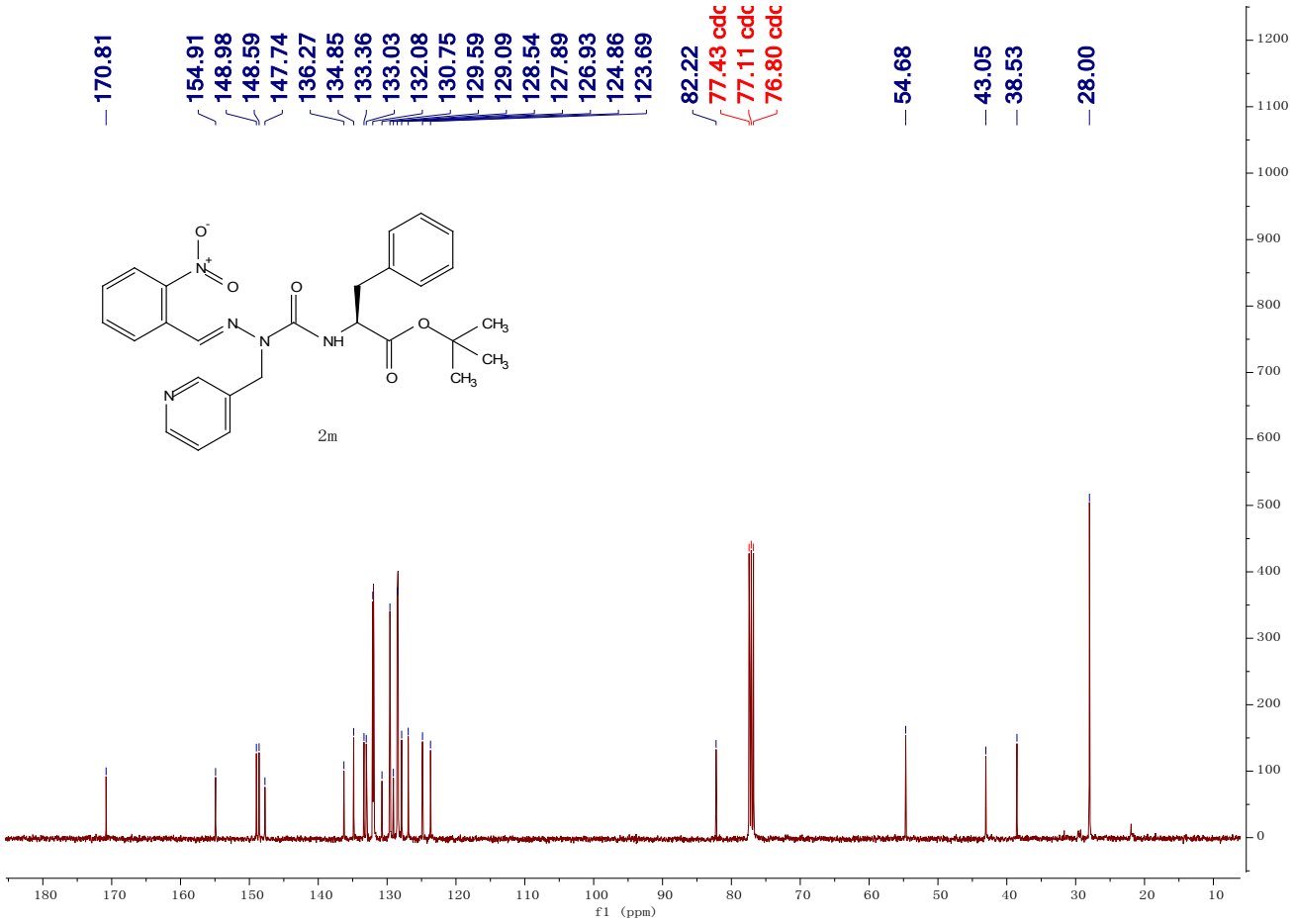

170.85
154.38
147.96
136.34
133.40
132.11
129.61
129.52
128.72
128.38
128.28
128.14
126.85
124.85
82.09
80.37
77.35 cdc
77.23
77.03 cdc
76.71 cdc
70.47
55.99
54.49
39.69
38.55
27.98
27.88
21.92
16.11
2K
f1 (ppm)

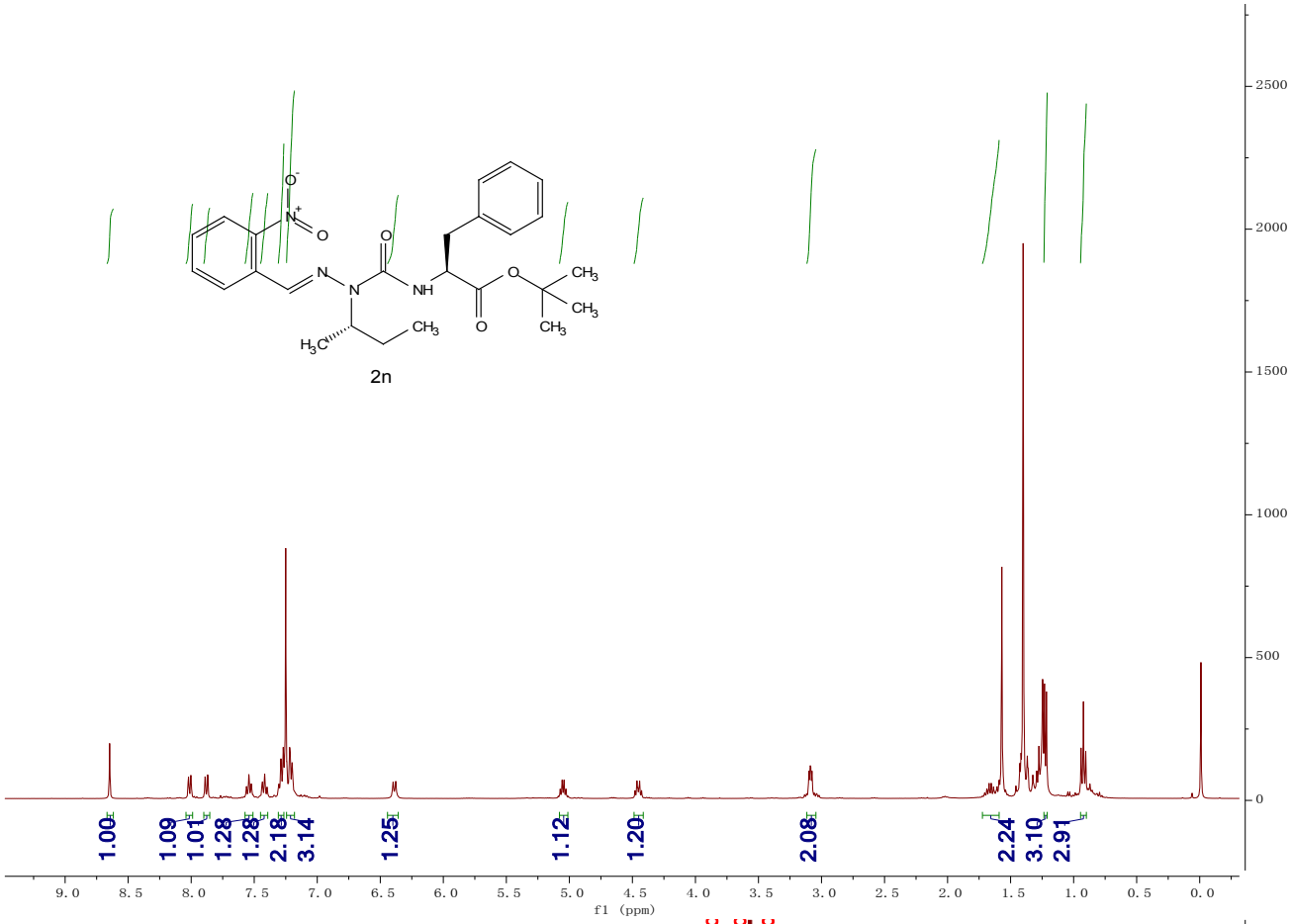

21
1.01
1.13
1.06
1.25
1.23
1.10
2.83
1.13
1.21
1.52
0.79
1.24
2.09
2.10
2.10
2.04
9.00
f1 (ppm)


170.96
154.70
147.89
137.32
136.40
133.37
131.40
129.79
129.61
129.26
128.38
127.88
126.84
124.87
115.56
82.01
81.91
77.37 cdc
77.06 cdc
76.74 cdc
54.49
40.62
38.58
30.88
27.98
24.39
f1 (ppm)

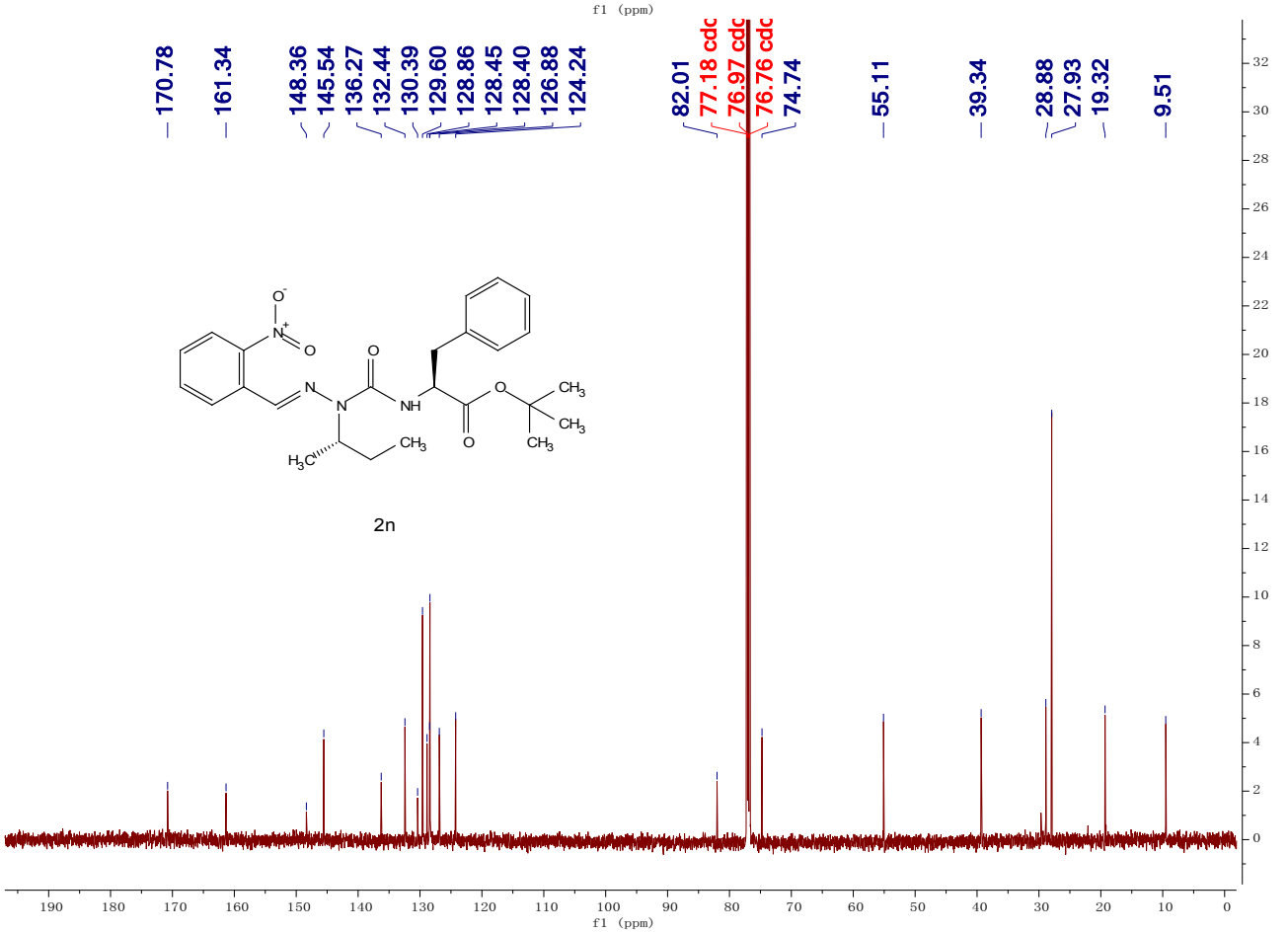
21


2m
1.08
1.04
1.14
1.19
1.15
4.13
3.82
4.93
1.10
2.98
1.14
2.24
1.29
2.07
8.99
f1 (ppm)

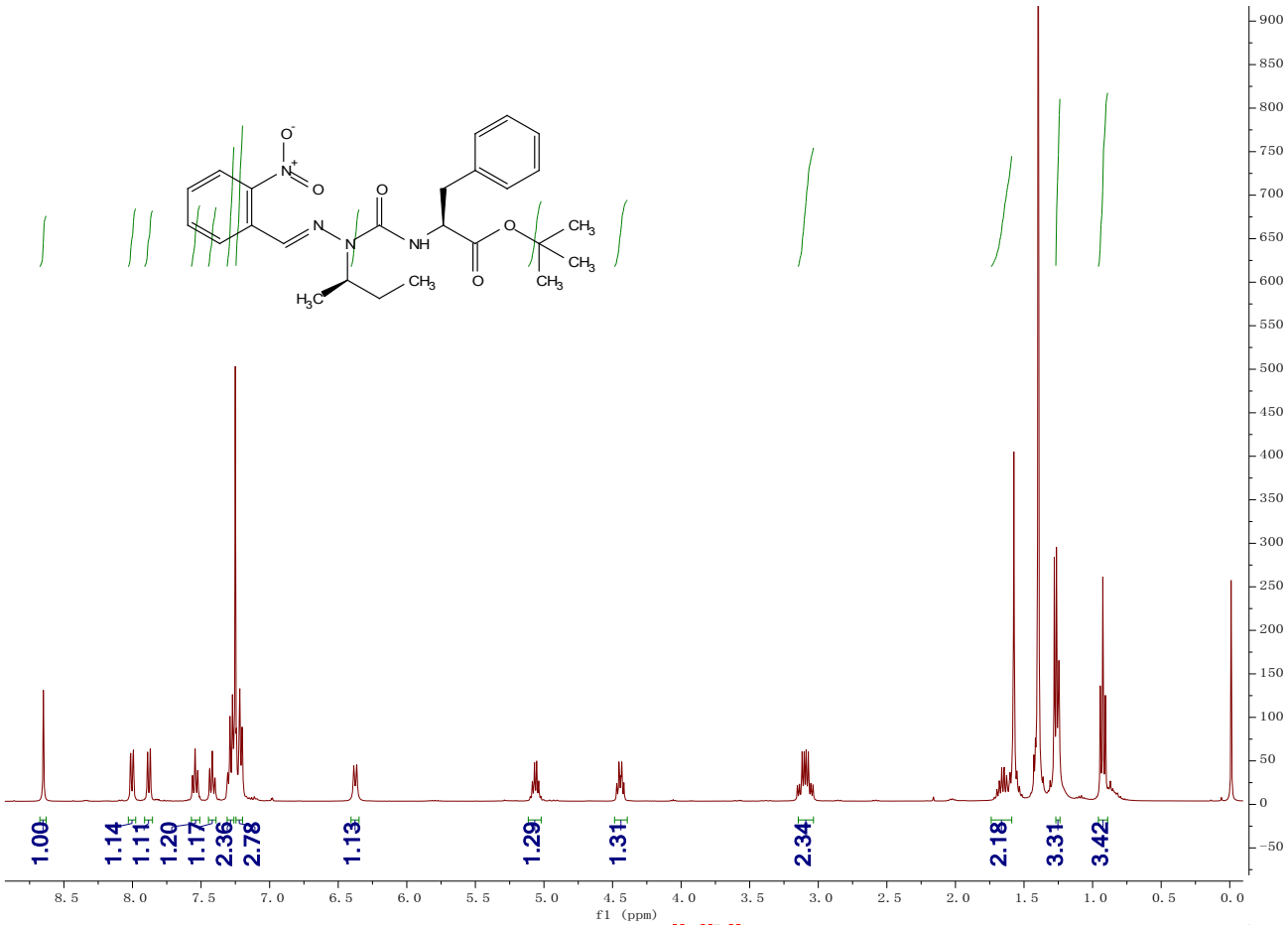

170.81
154.91
148.98
148.59
147.74
136.27
134.85
133.36
133.03
132.08
130.75
129.59
129.09
128.54
127.89
126.93
124.86
123.69
82.22
77.43 cdc
77.11 cdc
76.80 cdc
54.68
43.05
38.53
28.00
2m
f1 (ppm)

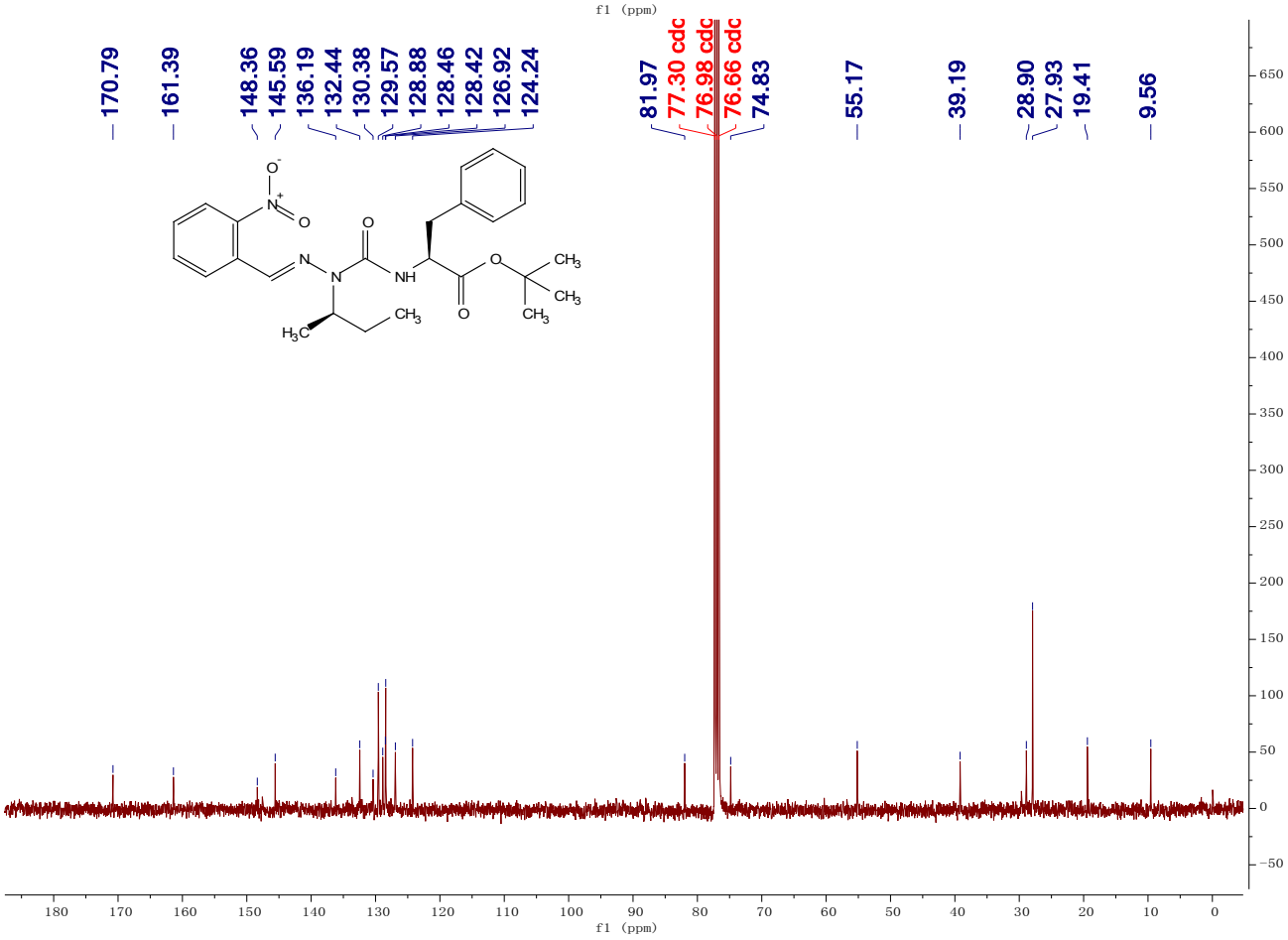

1.00
1.09
1.01
1.28
1.28
2.18
3.14
1.25
1.12
1.20
2.08
2.24
3.10
2.91
f1 (ppm)

2n

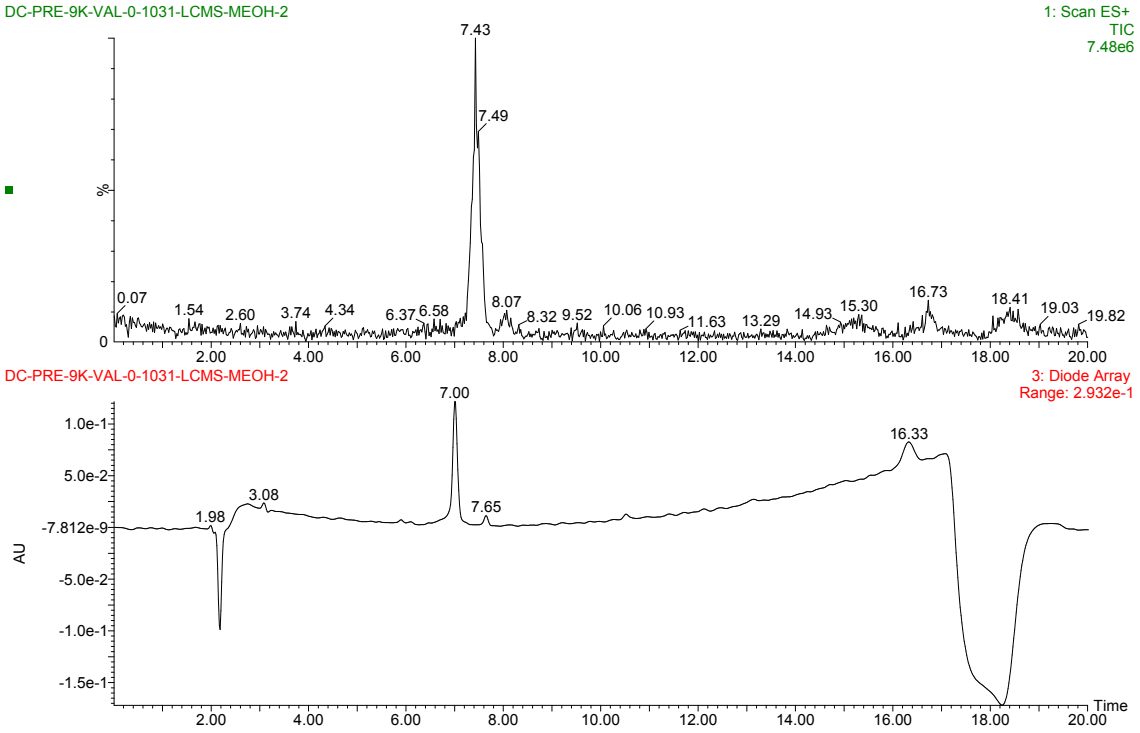

170.78
161.34
148.36
145.54
136.27
132.44
130.39
129.60
128.86
128.45
128.40
126.88
124.24
82.01
77.18 cdc
76.97 cdc
76.76 cdc
74.74
55.11
39.34
28.88
27.93
19.32
9.51
f1 (ppm)

2n

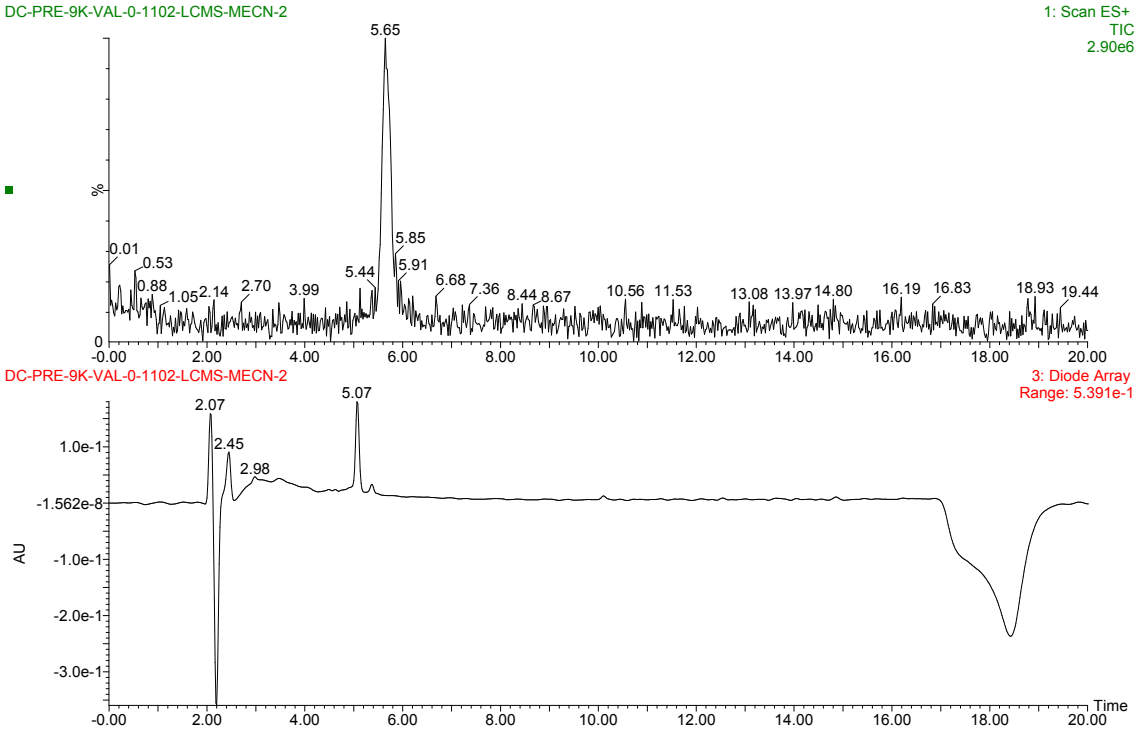

1.00
1.14
1.11
1.20
1.17
2.36
2.78
1.13
1.29
1.31
2.34
2.18
3.31
3.42
f1 (ppm)


170.79
161.39
148.36
145.59
136.19
132.44
130.38
129.57
128.88
128.46
128.42
126.92
124.24
81.97
77.30 cdc
76.98 cdc
76.66 cdc
74.83
55.17
39.19
28.90
27.93
19.41
9.56
f1 (ppm)

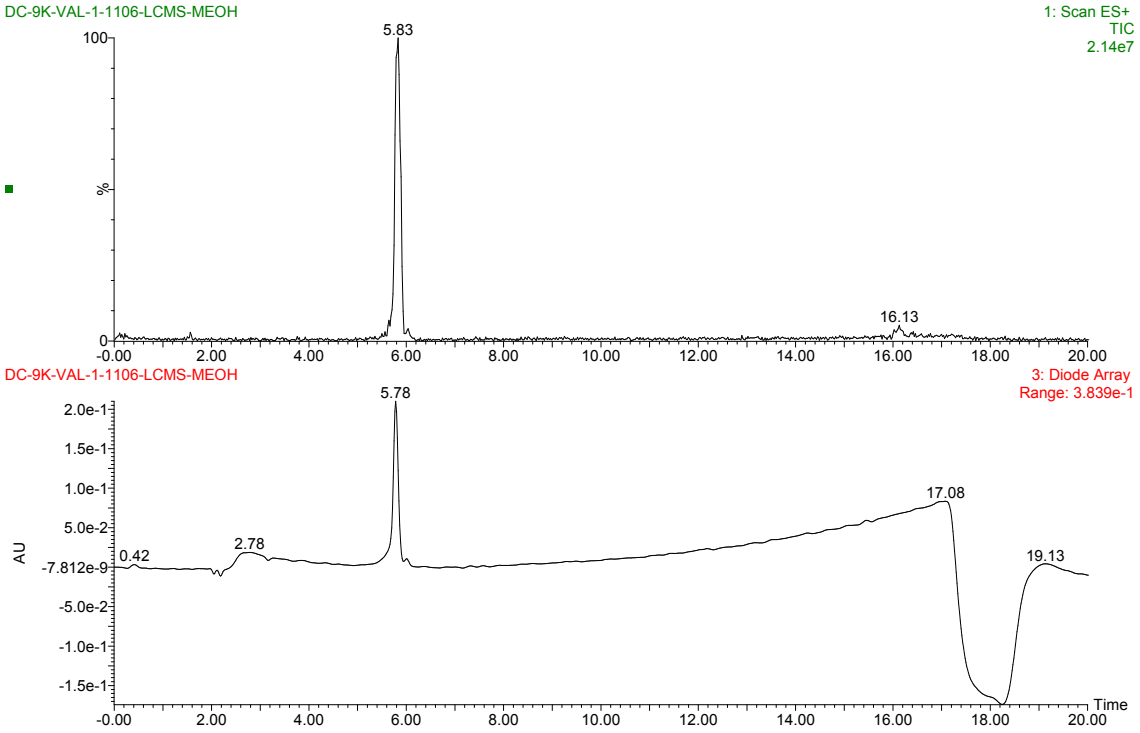
4. Analytical LC-MS chromatograms of purified azapeptides 10a-d, 11a-d and 12a-d



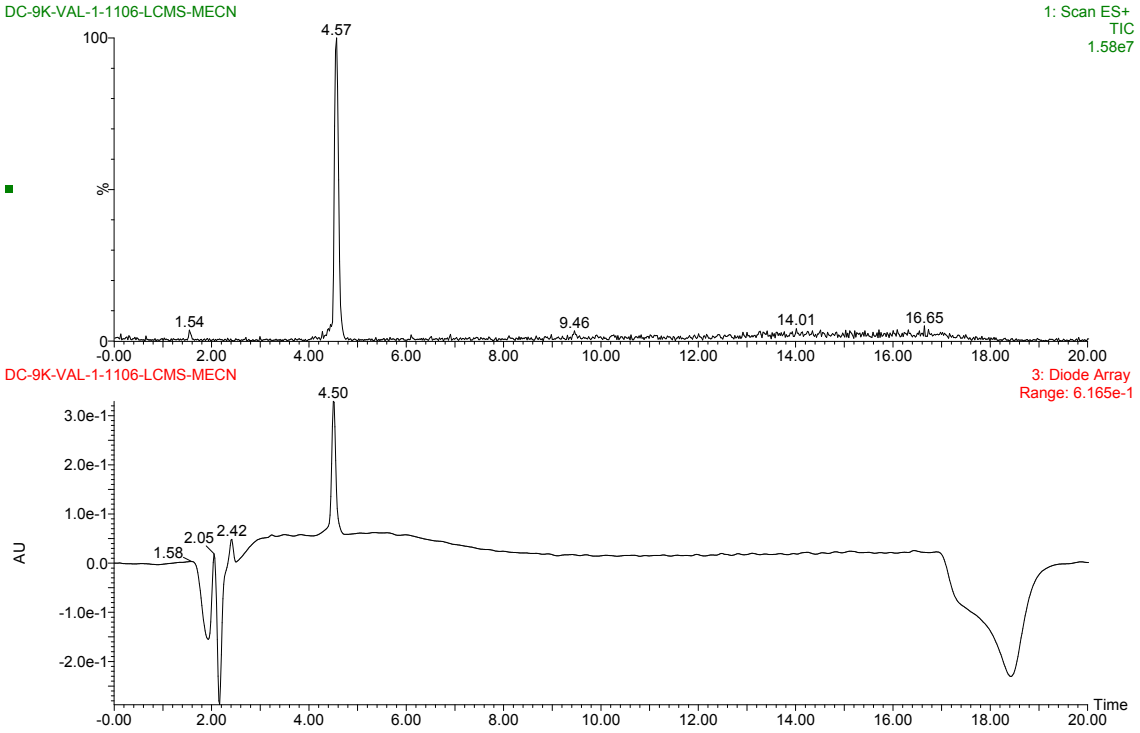
LC-MS chromatogram of [azaVal^3]-Ang-(1-7) **10a** in a linear gradient of 10-60% of MeOH in H_2O containing 0.1% FA over 15 min, R.T. = 7.00 min



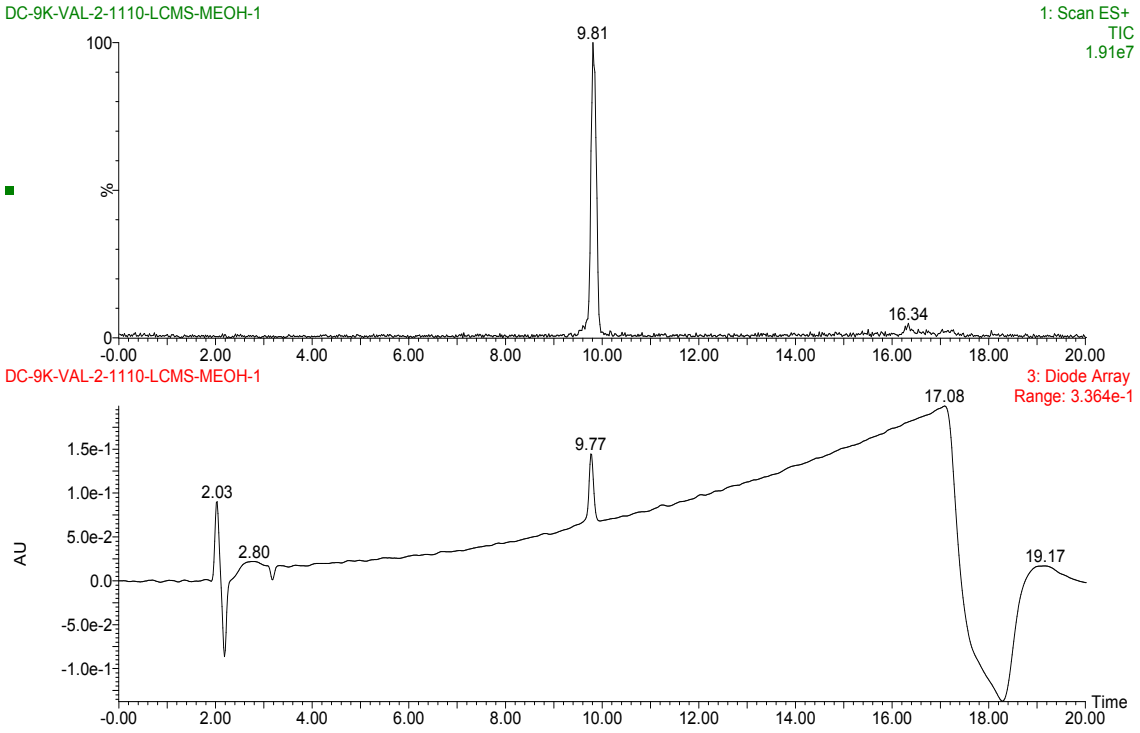
LC-MS chromatogram of [azaVal^3]-Ang-(1-7) **10a** in a linear gradient of 5-50% of MeCN in H_2O containing 0.1% FA over 15 min, R.T. = 5.07 min



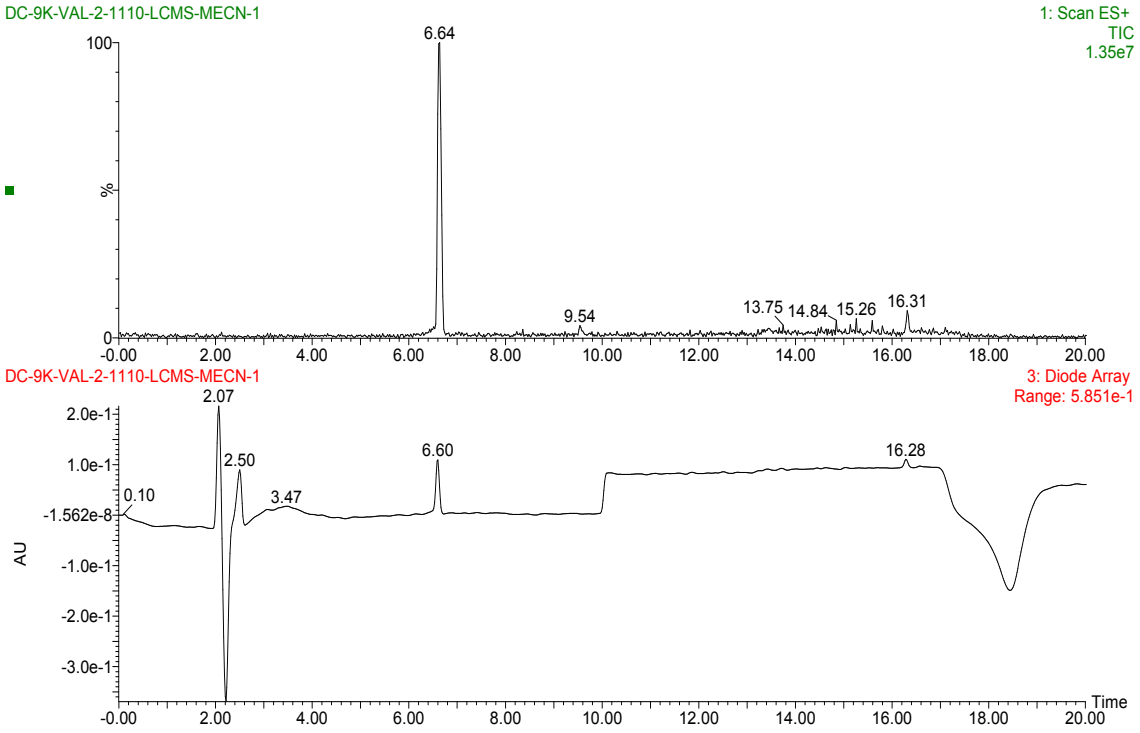
LC-MS chromatogram of [azaAla^3]-Ang-(1-7) **10b** in a linear gradient of 10-60% of MeOH in H_2O containing 0.1% FA over 15 min, R.T. = 5.78 min



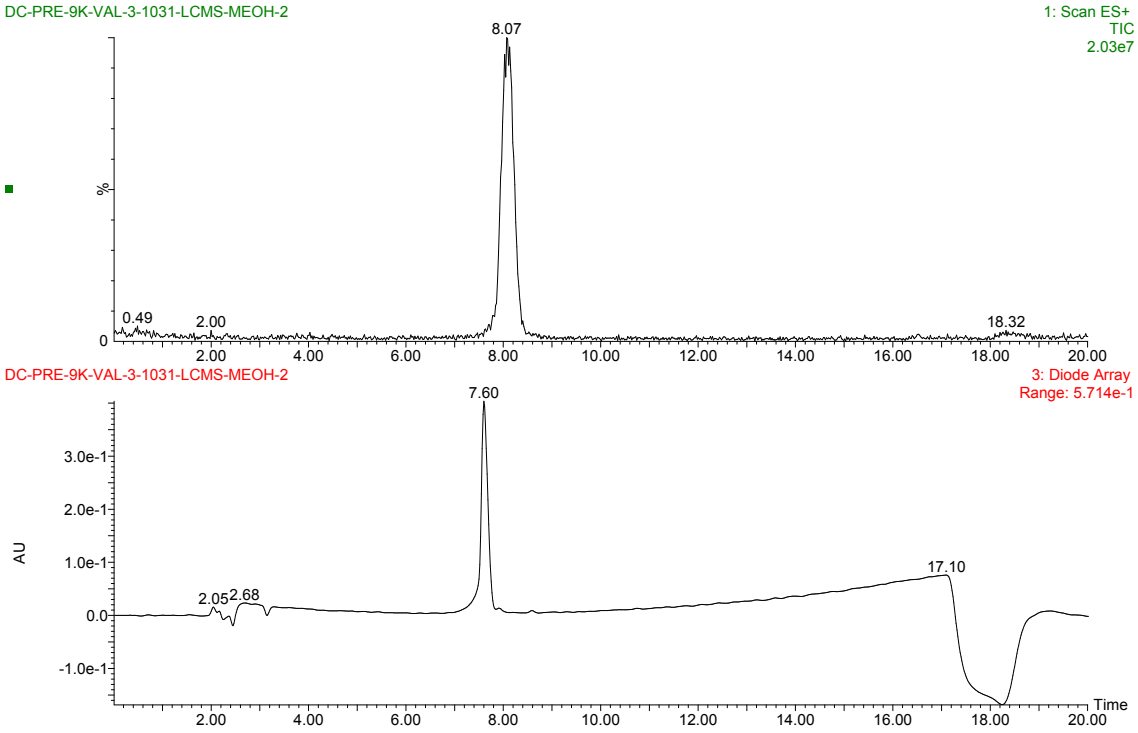
LC-MS chromatogram of [azaAla^3]-Ang-(1-7) **10b** in a linear gradient of 5-50% of MeCN in H_2O containing 0.1% FA over 15 min. LC-MS (5-50% MeCN, 15 min) R.T. = 4.50 min



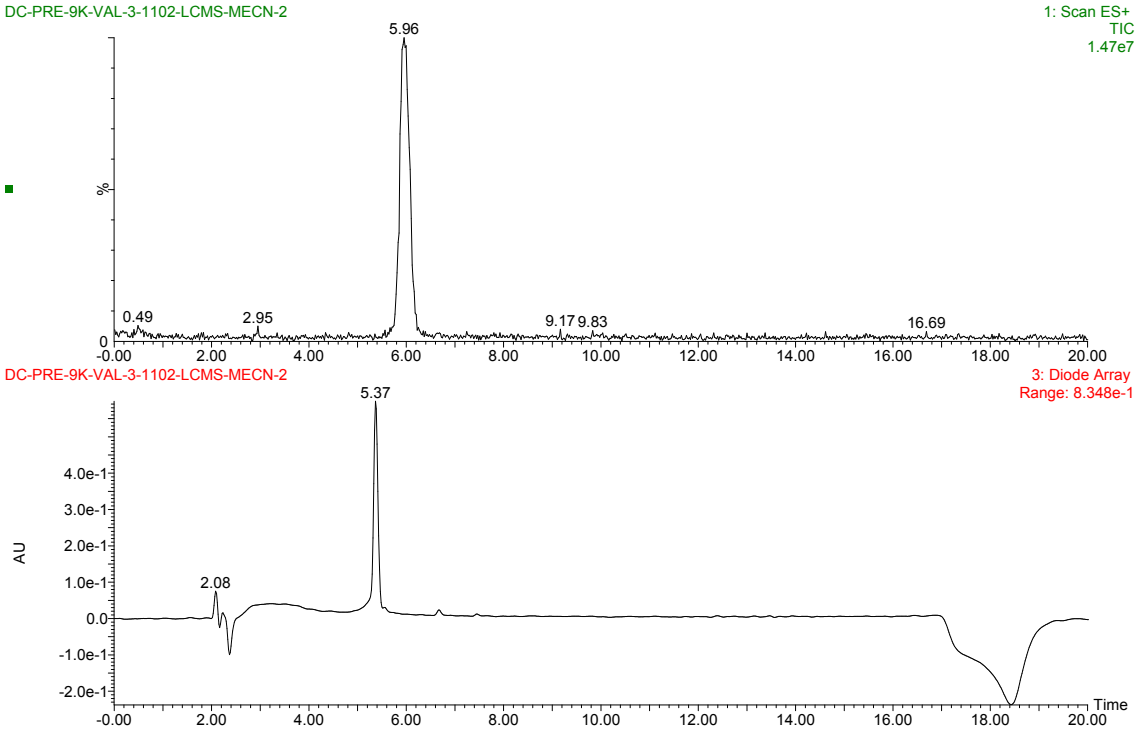
LC-MS chromatogram of [azaPhe^3]-Ang-(1-7) **10c** in a linear gradient of 10-60% of MeOH in H_2O containing 0.1% FA over 15 min, R.T. = 9.77 min



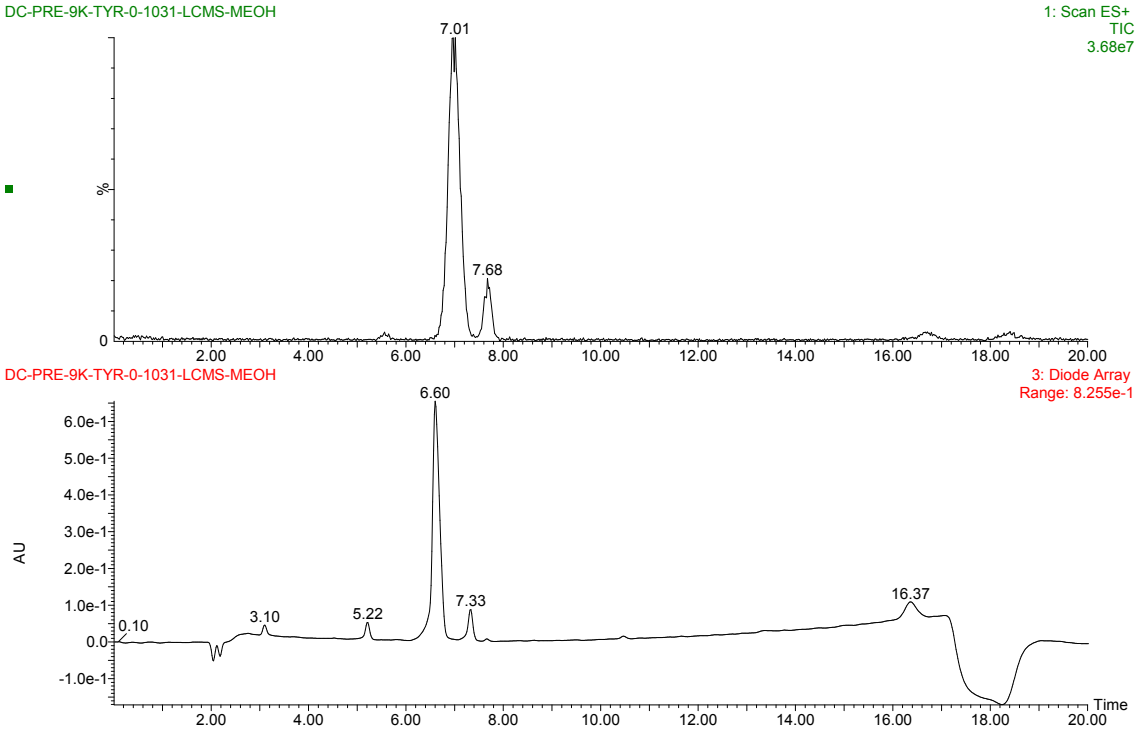
LC-MS chromatogram of [azaPhe^3]-Ang-(1-7) **10c** in a linear gradient of 10-60% of MeCN in H_2O containing 0.1% FA over 15 min, R.T. = 6.60 min



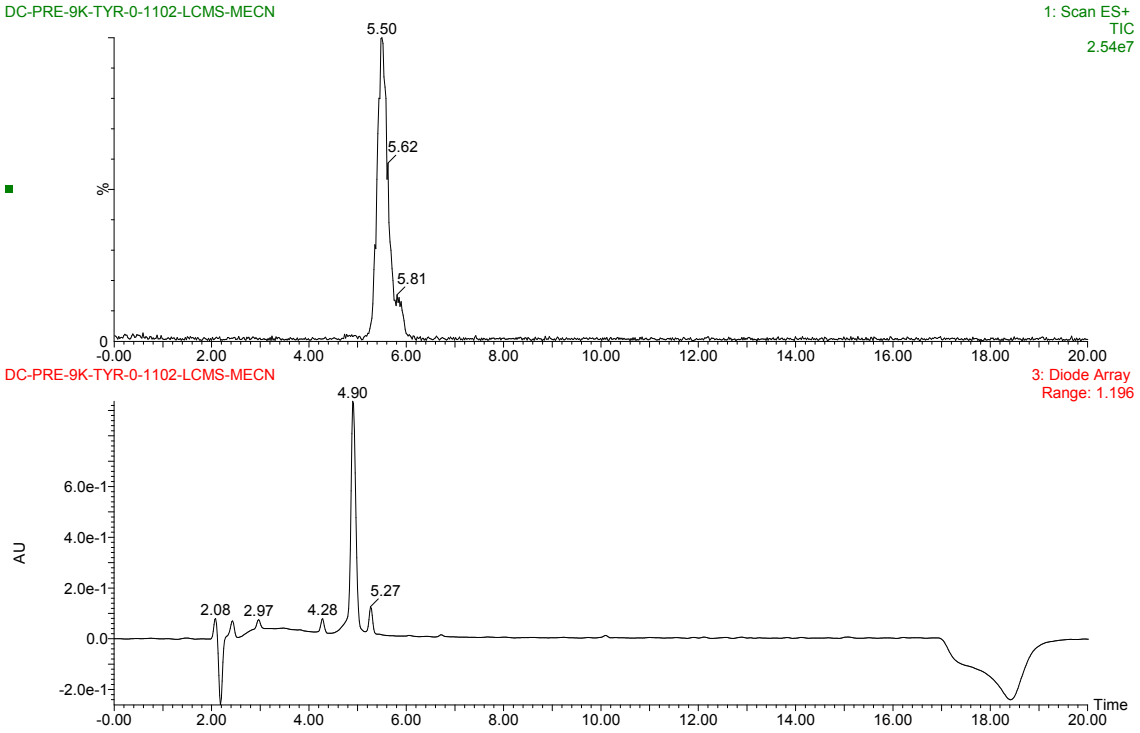
LC-MS chromatogram of [cyclopropylmethyl-azaGly^{3}]-Ang-(1-7) **10d** in a linear gradient of 10-60% of MeOH in H_2O containing 0.1% FA over 15 min, R.T. = 7.60 min



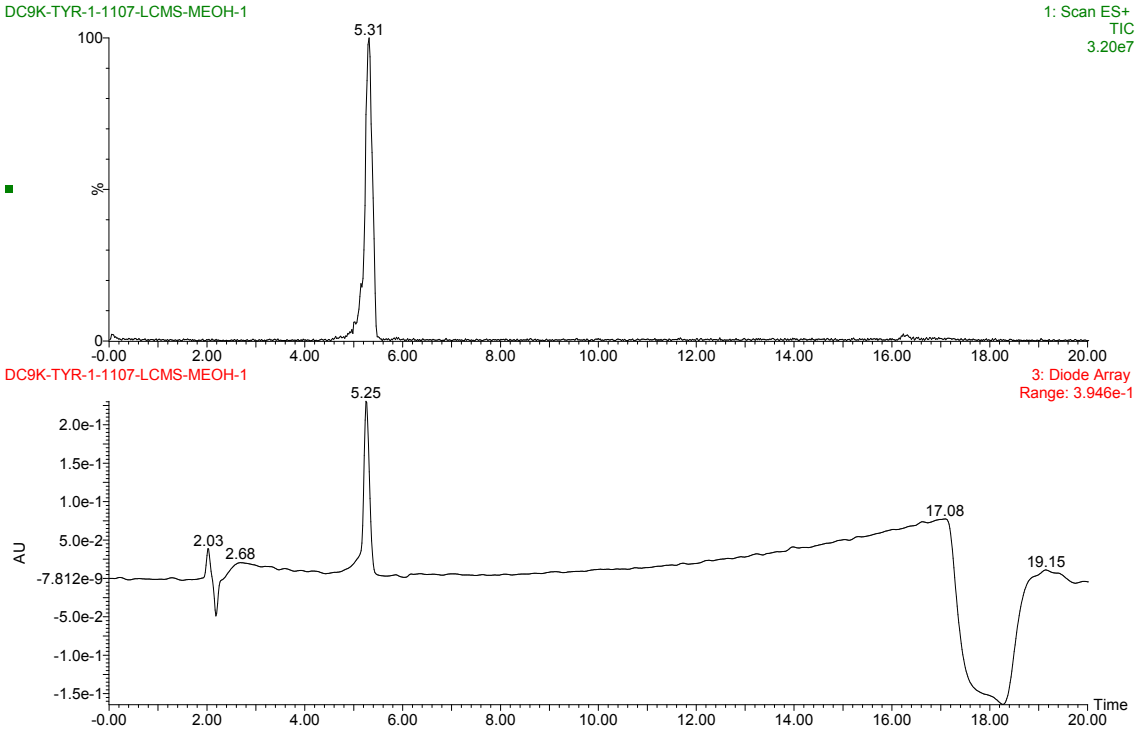
LC-MS chromatogram of [cyclopropylmethyl-azaGly^{3}]-Ang-(1-7) **10d** in a linear gradient of 5-50% of MeCN in H_2O containing 0.1% FA over 15 min, R.T. = 5.37 min



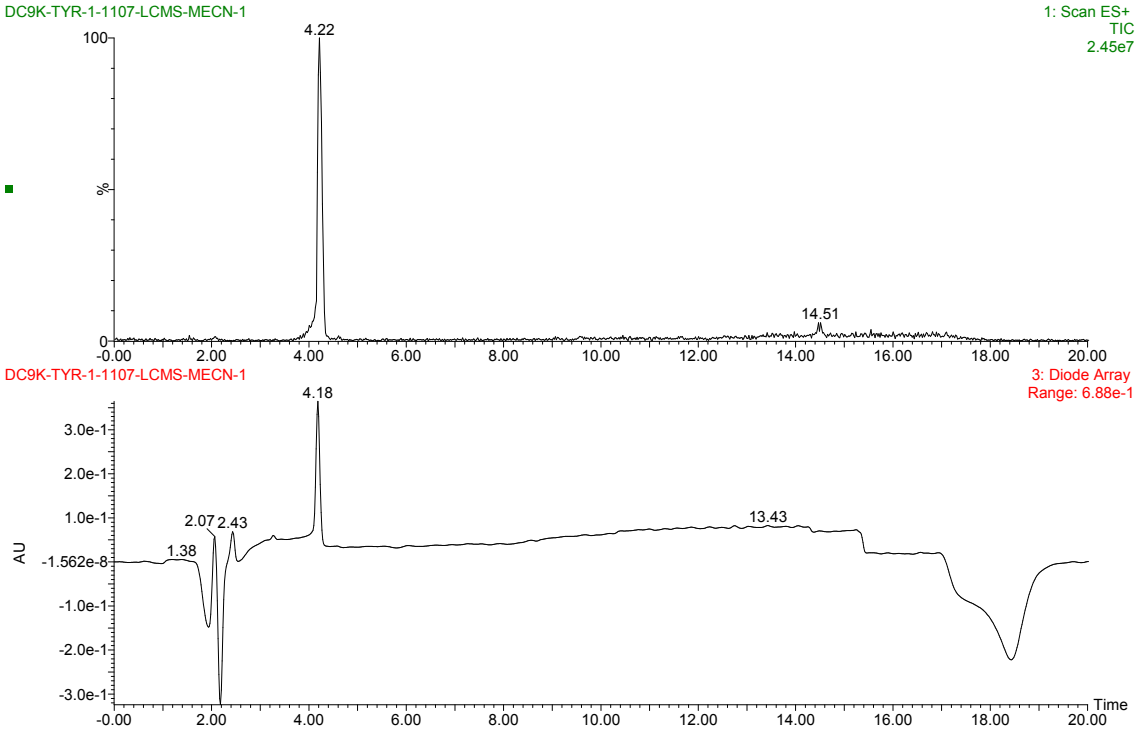
LC-MS chromatogram of [azaTyr^4]-Ang-(1-7) **11a** in a linear gradient of 10-60% of MeOH in H_2O containing 0.1% FA over 15 min, R.T. = 6.60 min



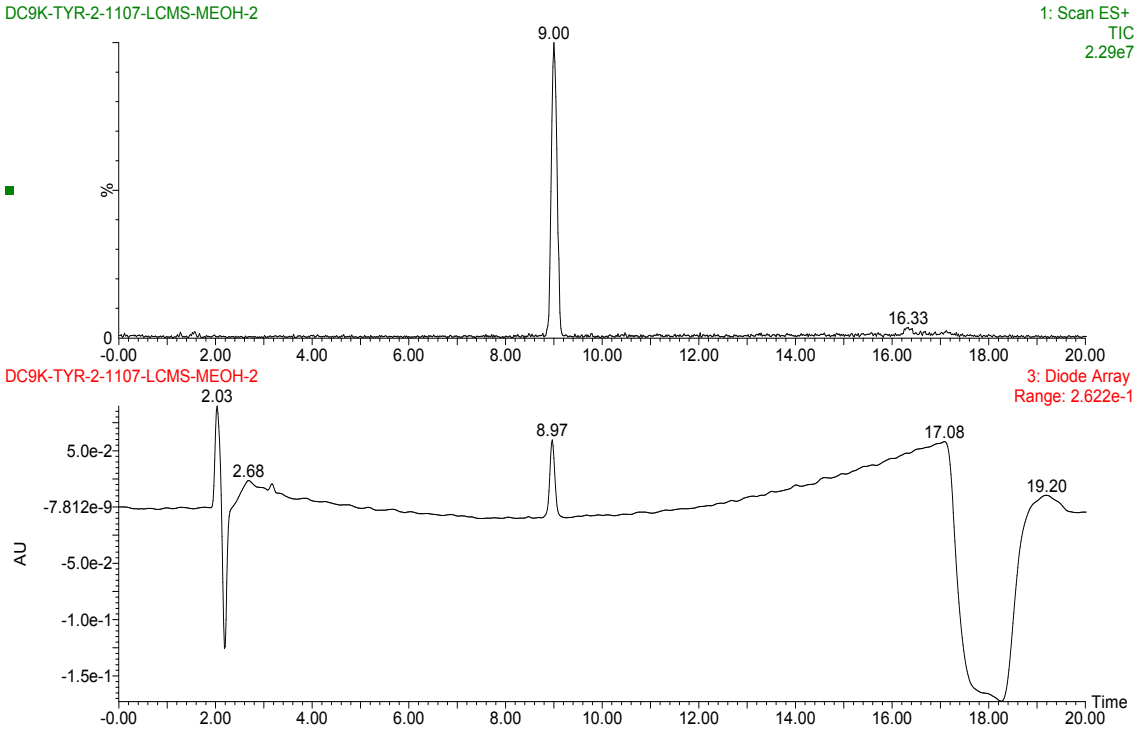
LC-MS chromatogram of [azaTyr^4]-Ang-(1-7) **11a** in a linear gradient of 5-50% of MeCN in H_2O containing 0.1% FA over 15 min, R.T. = 4.90 min



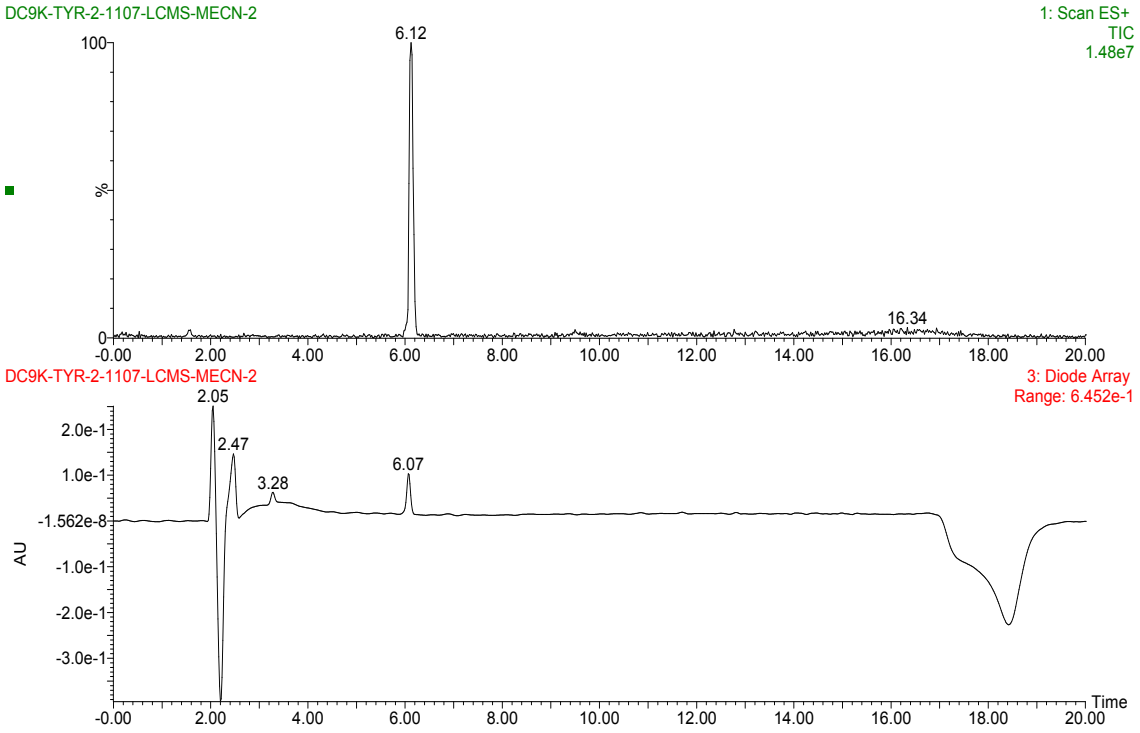
LC-MS chromatogram of [azaAla^4]-Ang-(1-7) **11b** in a linear gradient of 10-60% of MeOH in H_2O containing 0.1% FA over 15 min, R.T. = 5.25 min



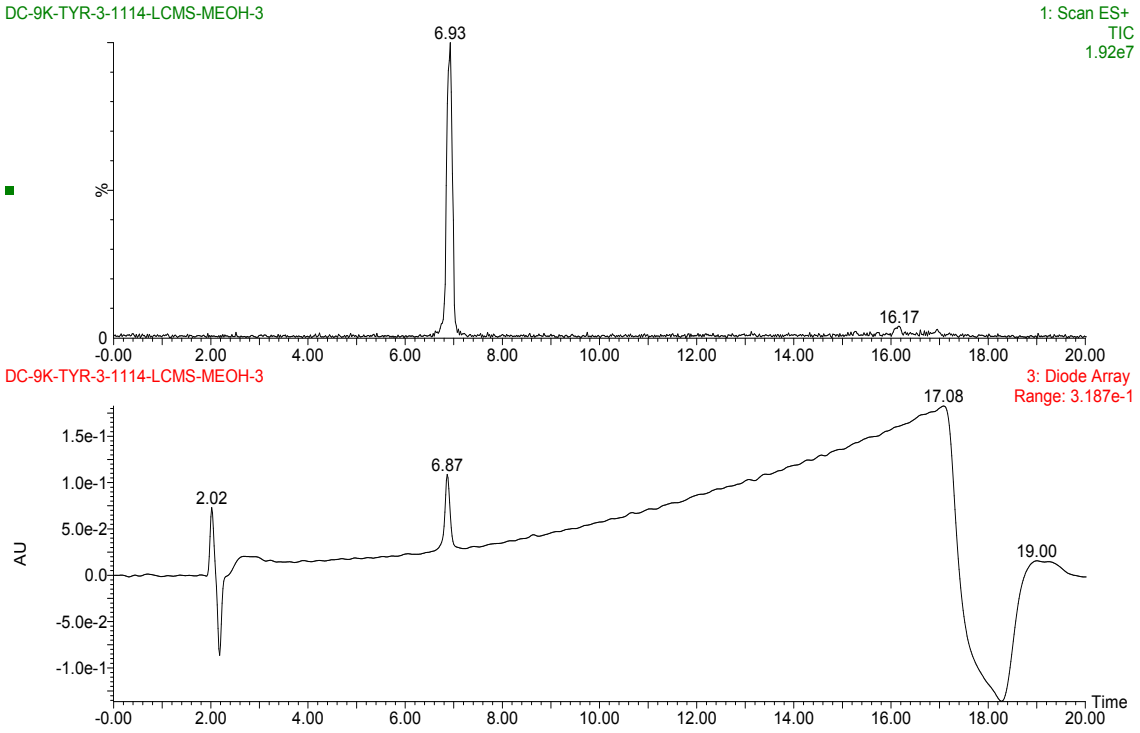
LC-MS chromatogram of [azaAla^4]-Ang-(1-7) **11b** in a linear gradient of 5-50% of MeCN in H_2O containing 0.1% FA over 15 min, R.T. = 4.18 min



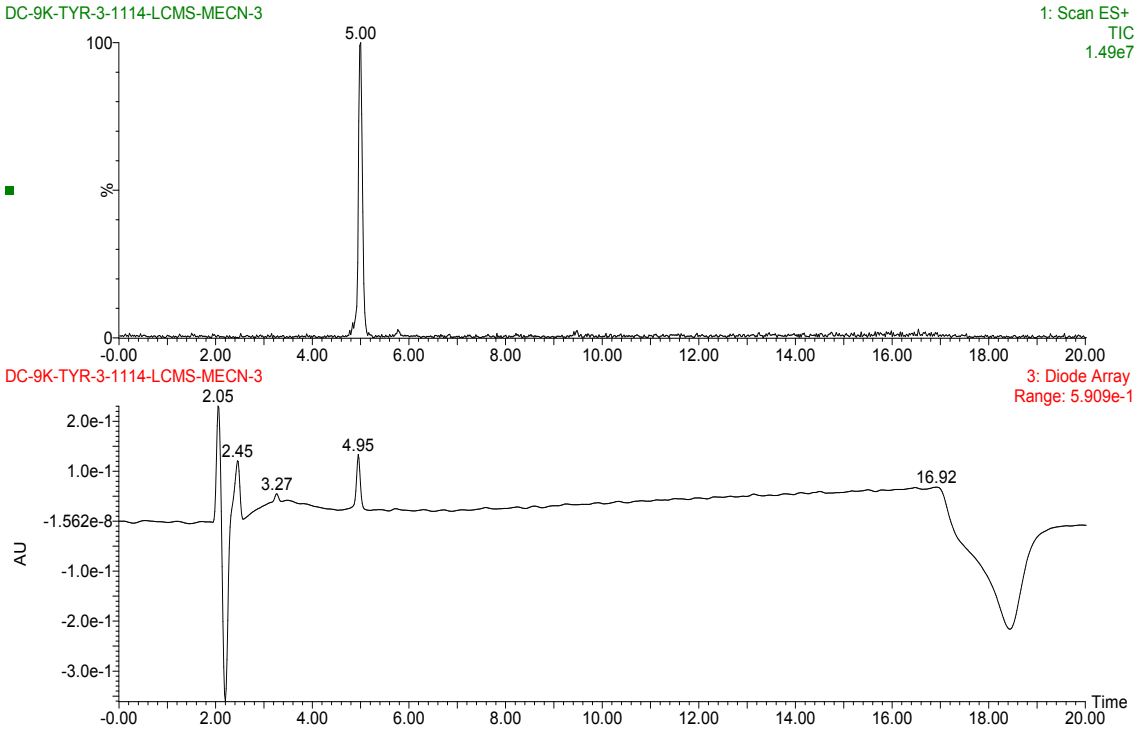
LC-MS chromatogram of [azaPhe^4]-Ang-(1-7) **11c** in a linear gradient of 10-60% of MeOH in H_2O containing 0.1% FA over 15 min, R.T. = 8.97 min



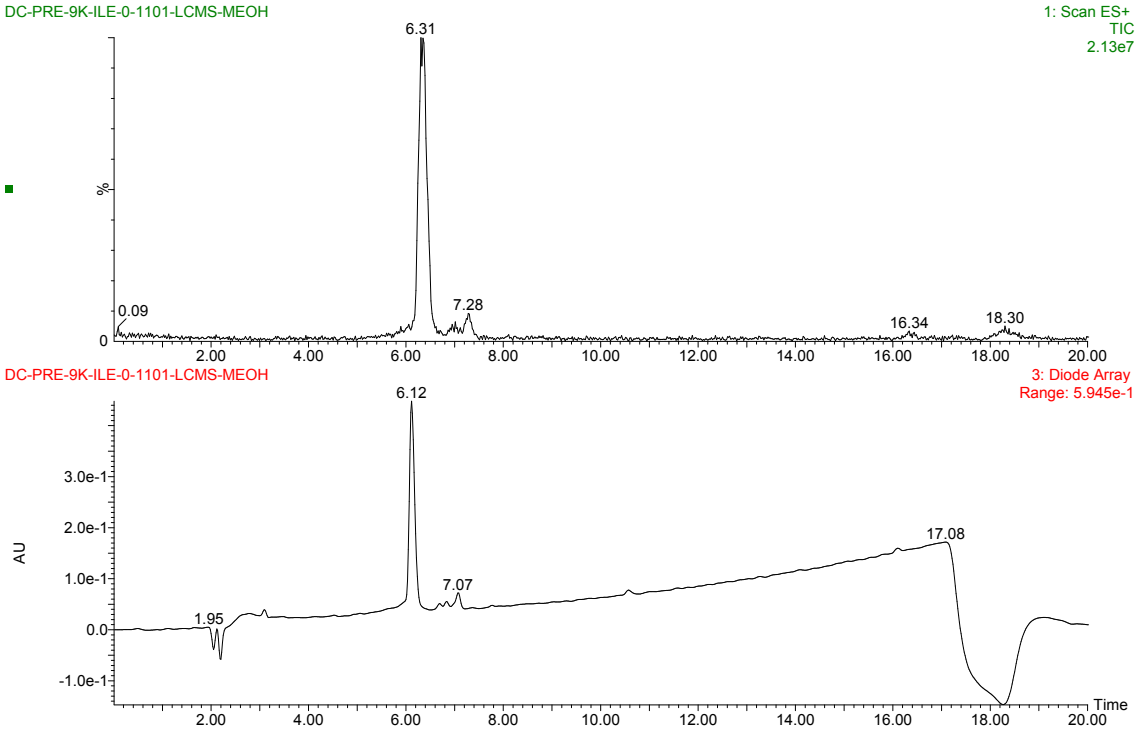
LC-MS chromatogram of [azaPhe^4]-Ang-(1-7) **11c** in a linear gradient of 5-50% of MeCN in H_2O containing 0.1% FA over 15 min, R.T. = 6.07 min



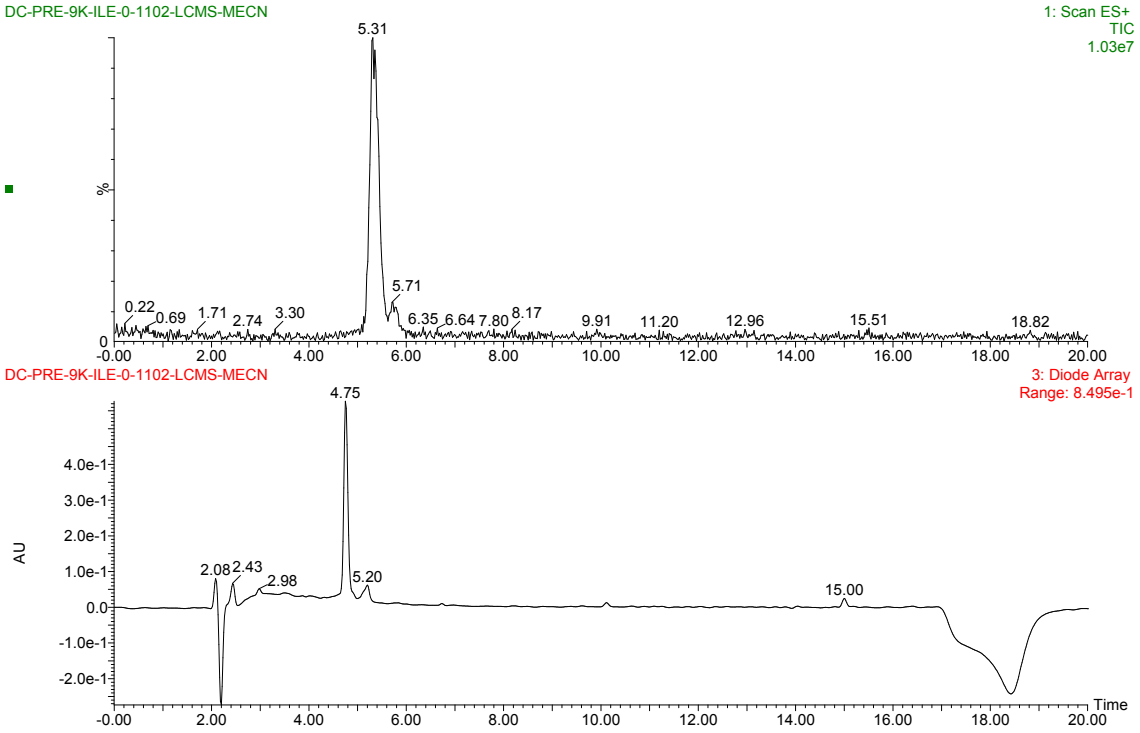
LC-MS chromatogram of [cyclopropylmethyl-azaGly^4]-Ang-(1-7) **11d** in a linear gradient of 10-60% of MeOH in H_2O containing 0.1% FA over 15 min, R.T. = 6.87 min



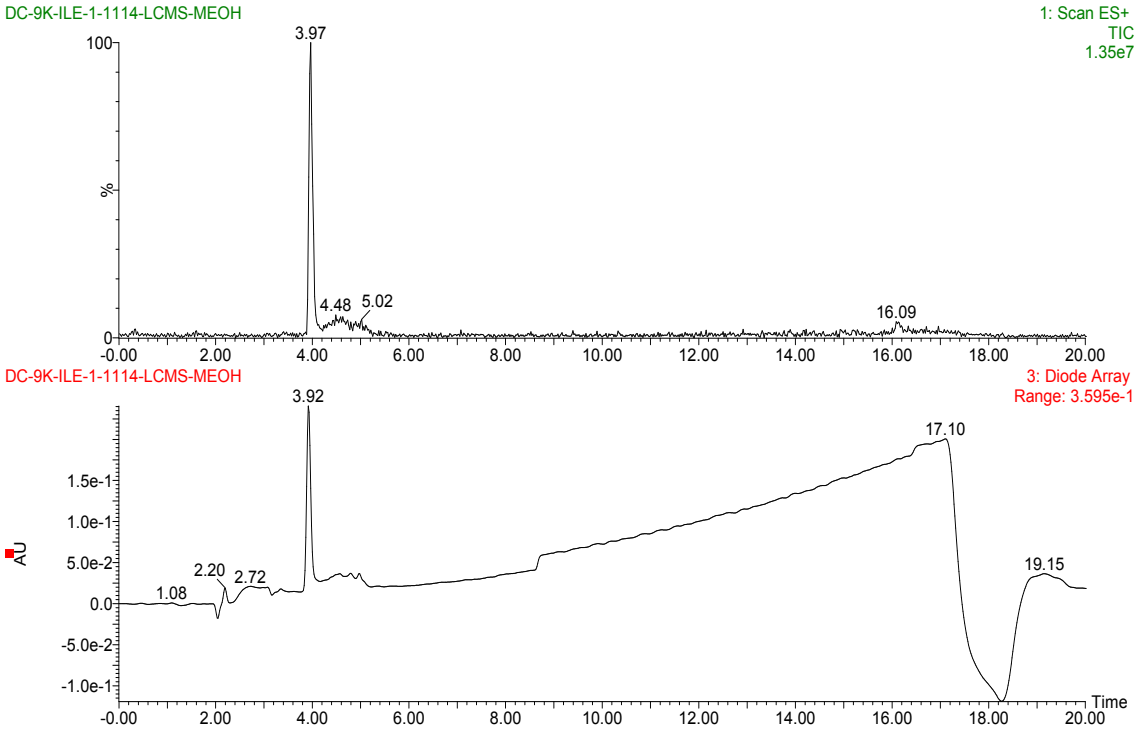
LC-MS chromatogram of [cyclopropylmethyl-azaGly^4]-Ang-(1-7) **11d** in a linear gradient of 10-60% of MeCN in H_2O containing 0.1% FA over 15 min, R.T. = 4.95 min



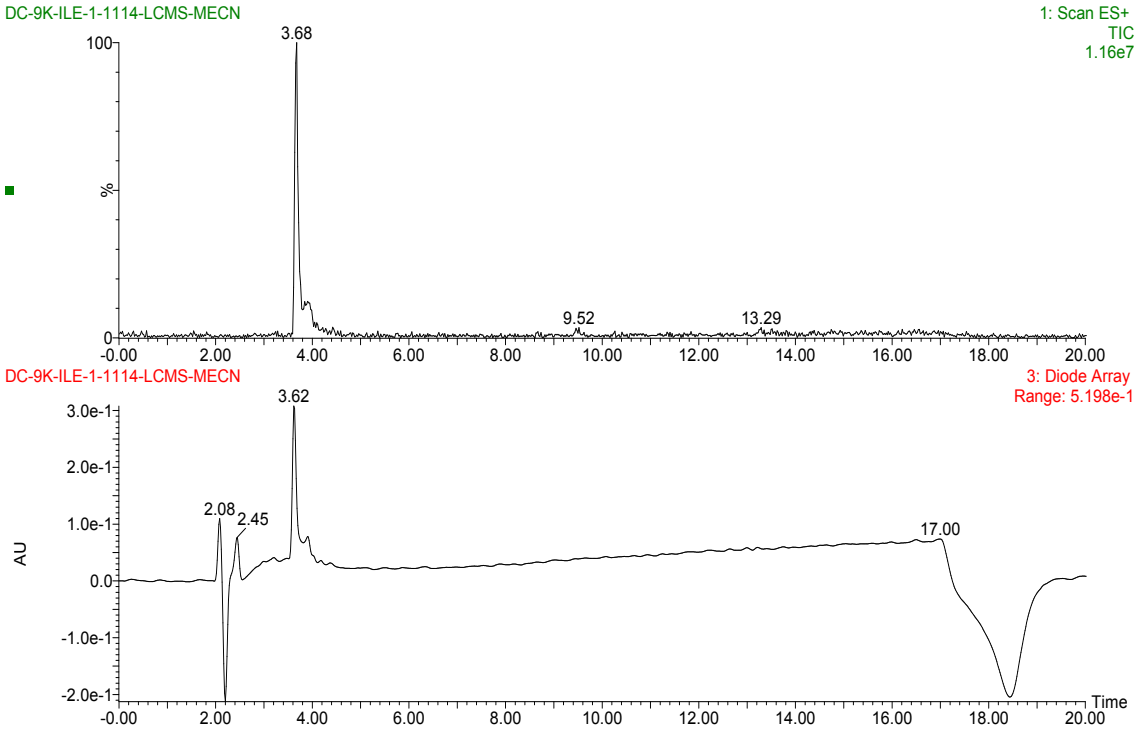
LC-MS chromatogram of [azaIle^5]-Ang-(1-7) **12a** in a linear gradient of 10-60% of MeOH in H_2O containing 0.1% FA over 15 min, R.T. = 6.12 min



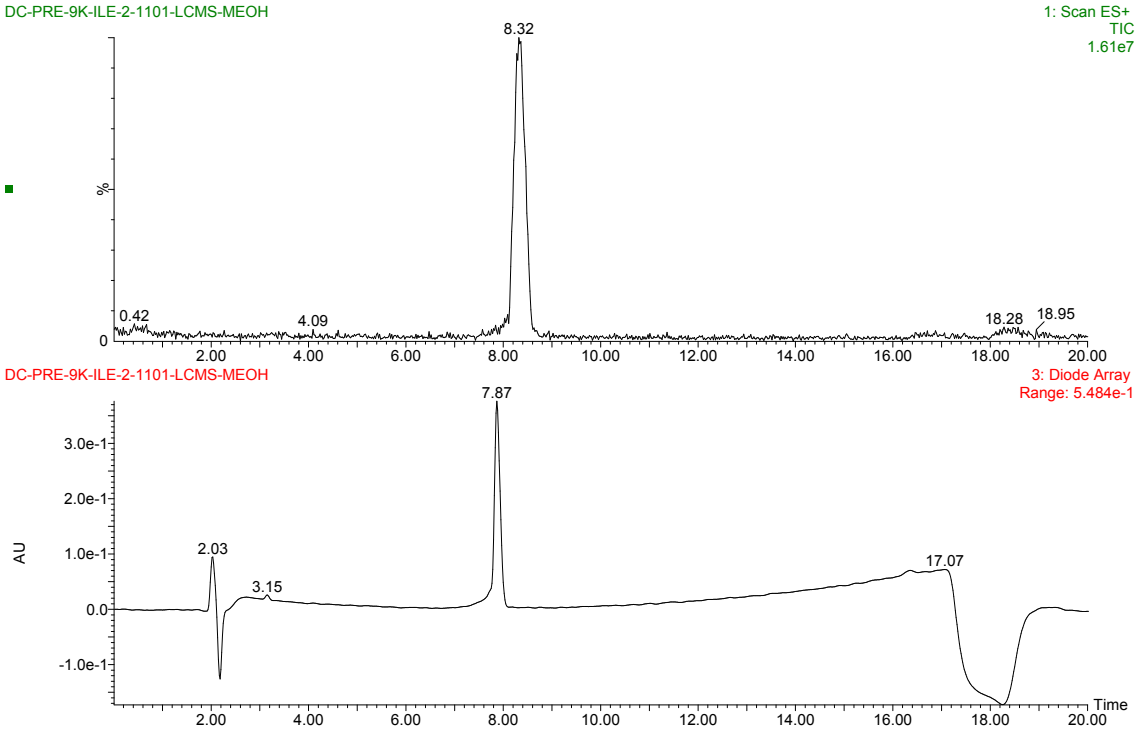
LC-MS chromatogram of [azaIle^5]-Ang-(1-7) **12a** in a linear gradient of 5-50% of MeCN in H_2O containing 0.1% FA over 15 min, R.T. = 4.75 min



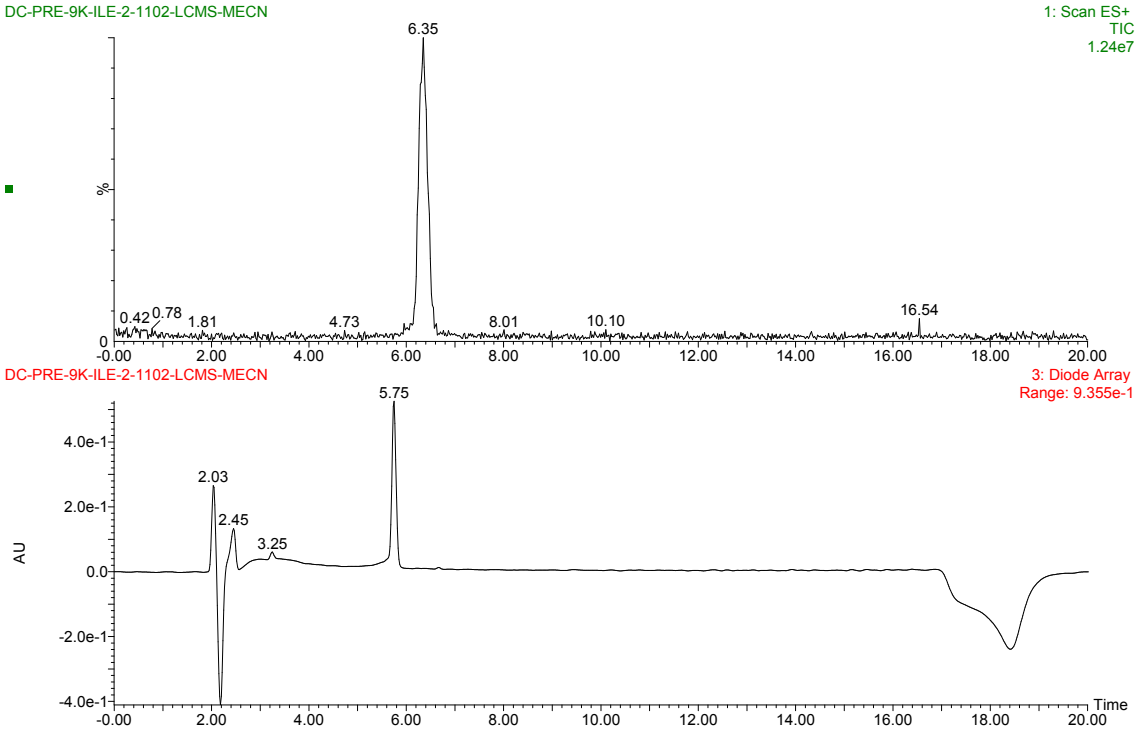
LC-MS chromatogram of [azaAla^5]-Ang-(1-7) **12b** in a linear gradient of 10-60% of MeOH in H_2O containing 0.1% FA over 15 min, R.T. = 3.92 min



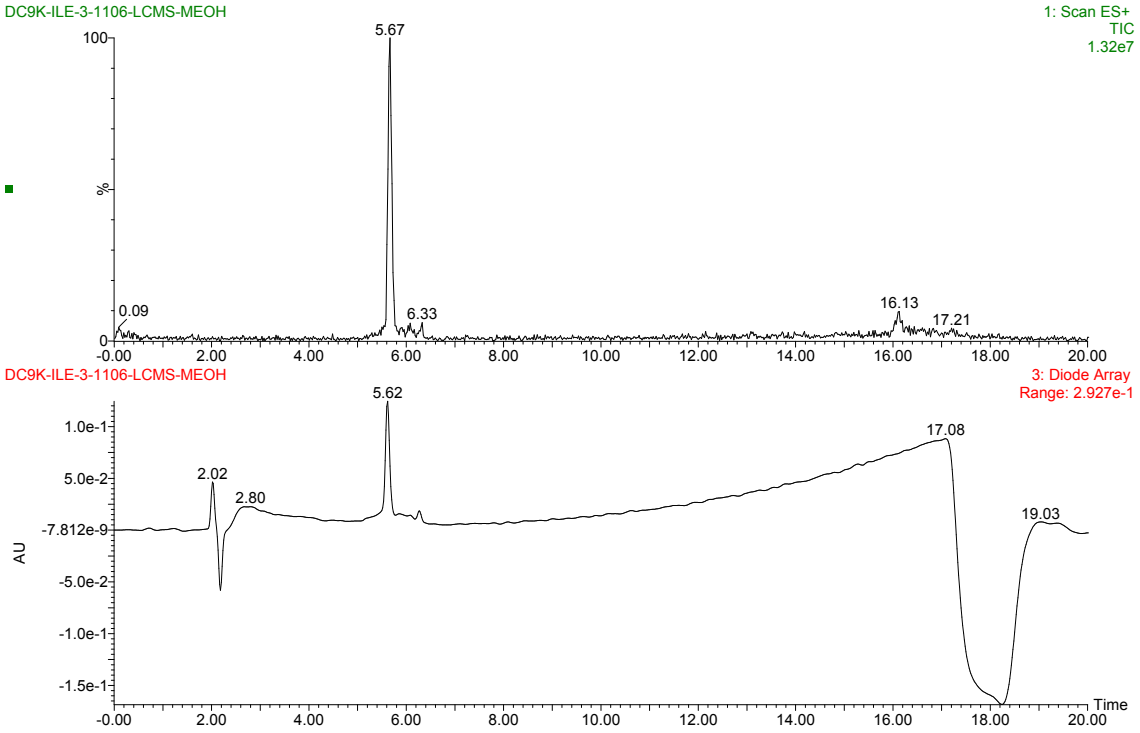
LC-MS chromatogram of [azaAla^5]-Ang-(1-7) **12b** in a linear gradient of 5-50% of MeCN in H2O containing 0.1% FA over 15 min, R.T. = 3.62 min



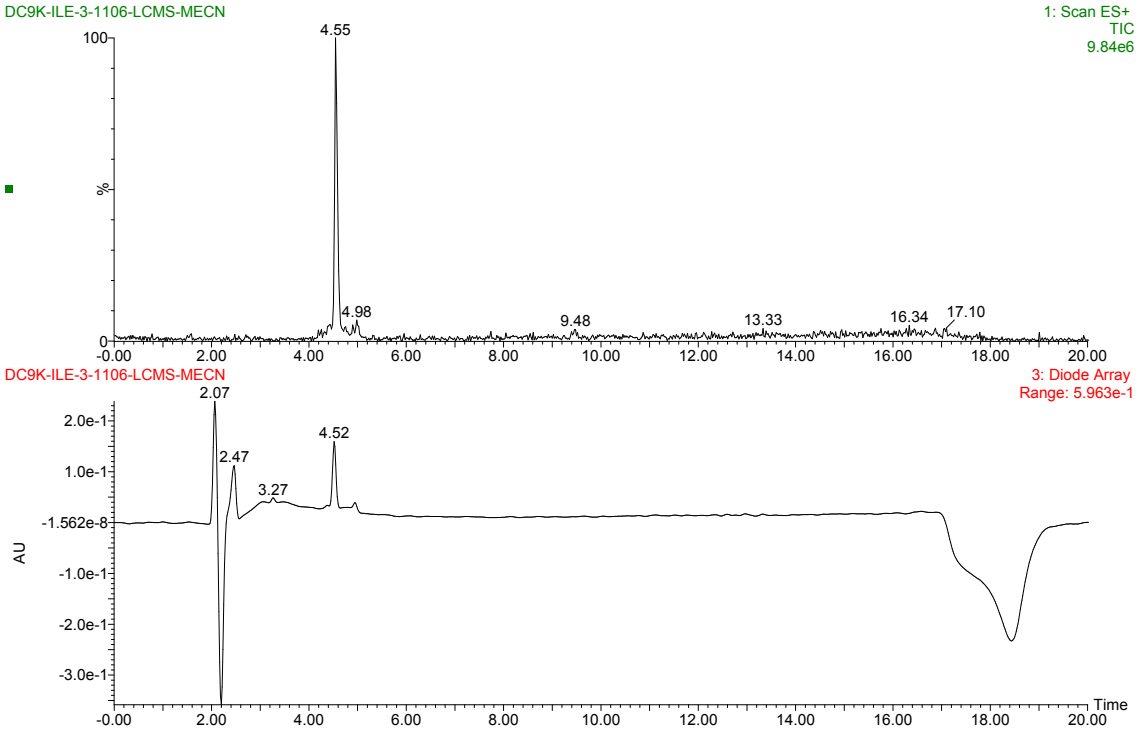
LC-MS chromatogram of [azaPhe^5]-Ang-(1-7) **12c** in a linear gradient of 10-60% of MeOH in H2O containing 0.1% FA over 15 min, R.T. = 7.87 min



LC-MS chromatogram of [azaPhe^5]-Ang-(1-7) **12c** in a linear gradient of 5-50% of MeCN in H2O containing 0.1% FA over 15 min, R.T. = 5.75 min



LC-MS chromatogram of [cyclopropylmethyl-azaGly^5]-Ang-(1-7) **12d** in a linear gradient of 10-60% of MeOH in H_2O containing 0.1% FA over 15 min, R.T. = 5.62 min



LC-MS chromatogram of [cyclopropylmethyl-azaGly^5]-Ang-(1-7) **12d** in a linear gradient of 5-50% of MeCN in H_2O containing 0.1% FA over 15 min, R.T. = 4.52 min