

Supporting Information  
**Synthesis and Biological Evaluation of *N*-Aminoimidazolidin-2-one Containing  
Angiotensin-(1-7) Peptidomimetics**

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**Table of contents**

1	Experimental Section	P2
	1.1 General experimental procedures	P2
	1.2 Synthesis of Aza-dipeptide <b>2a</b>	P3
	1.3 Synthesis of peptide Aid-dipeptide <b>5</b>	P4
	1.4 Synthesis of [Aid]-Ang-(1-7) analogs <b>12-17</b>	P7
2	Crystal structure of Aid-dipeptide <b>5</b>	P10
3	<sup>1</sup> H-NMR and <sup>13</sup> C-NMR spectrum	P11
4	Analytical LC-MS chromatograms of purified Aid-peptides <b>12-17</b>	P18
5	Biological evaluation of Aid-peptides <b>12-17</b>	P24
6	Analytical LC-MS chromatograms of Aid-peptides <b>12-17</b> after incubation with peptidase ACE or DPP3	P32

## 1. Experimental Section

### 1.1 General experimental procedures.

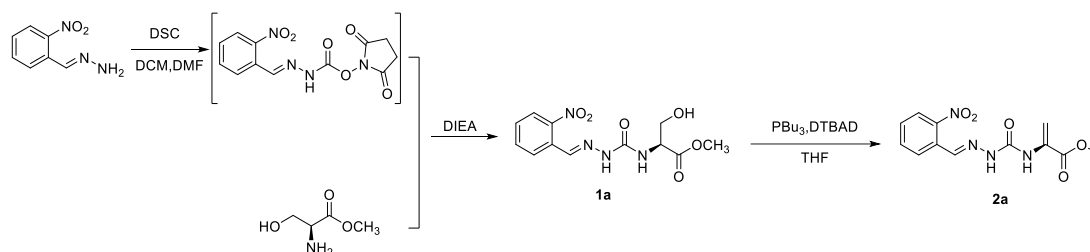
**Chemicals** were used as received from commercial sources without further purification unless stated otherwise. Rink amide resin (0.67 mmol/g) was purchased from GL Biochem (Shanghai) Ltd, and the manufacturer's reported loading of the resin was used in the calculation of the yields of the final products. Reagents including 2-nitrobenzaldehyde, hydrazine hydrate, *N,N'*-Disuccinimidyl carbonate (DSC), *N,N*-diisopropylethylamine (DIEA), triphenylphosphine, diisopropyl azodicarboxylate (DIAD), Di-*tert*-Butyl azodicarboxylate (DTBAD), sodium dithionite, tetrabutylammonium hydrogen sulfate (TBAHS), hydroxylamine hydrochloride, *m*-phenylenediamine, bistrichloromethyl carbonate (BTC), 2,4,6-collidine, piperidine, pyridine, 4-dimethylaminopyridine (DMAP), acetic anhydride, formic acid (FA) 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 quadrupole mass detection and positive and negative mode ionization. Analyses of crude peptide samples and purified peptide were determined with a Phenomenex Aeris™ C<sub>18</sub> column (pore size: 80 Å, particle size: 4 µm; 150 × 4.6 mm) with a flow rate of 0.5 mL/min using a proper linear gradient of CH<sub>3</sub>CN 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<sub>18</sub> 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 5-45% of MeOH in water containing 0.1% FA was used for purification of the peptides. Nuclear magnetic resonance (NMR) was performed on an Agilent™ 400MR DD2 spectrometer at 400 MHz for <sup>1</sup>H-NMR and at 100 MHz for <sup>13</sup>C-NMR. HRMS was performed on a Bruker™ Solarix Fourier Transform Mass Spectrometry with ESI ion-source and ion trap analyzer.

**Fmoc-based peptide syntheses** were performed under standard conditions on an automated shaker using Rink amide resin (0.67 mmol/g). Couplings of amino acids (3 equiv) were performed in DMF using HOBt (3 equiv) and HBTU (3 equiv) as coupling reagent and DIEA (6 equiv) as the base. 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<sub>2</sub>O/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 the 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<sub>2</sub>O (1 mg/mL) and subjected to LC-MS analysis.

**Deprotection and cleavage of the peptide from the resin** After Fmoc group removal, Rink amide resin-bound peptide was deprotected and cleaved from the support using a freshly made solution of TFA/H<sub>2</sub>O/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 the 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 2a

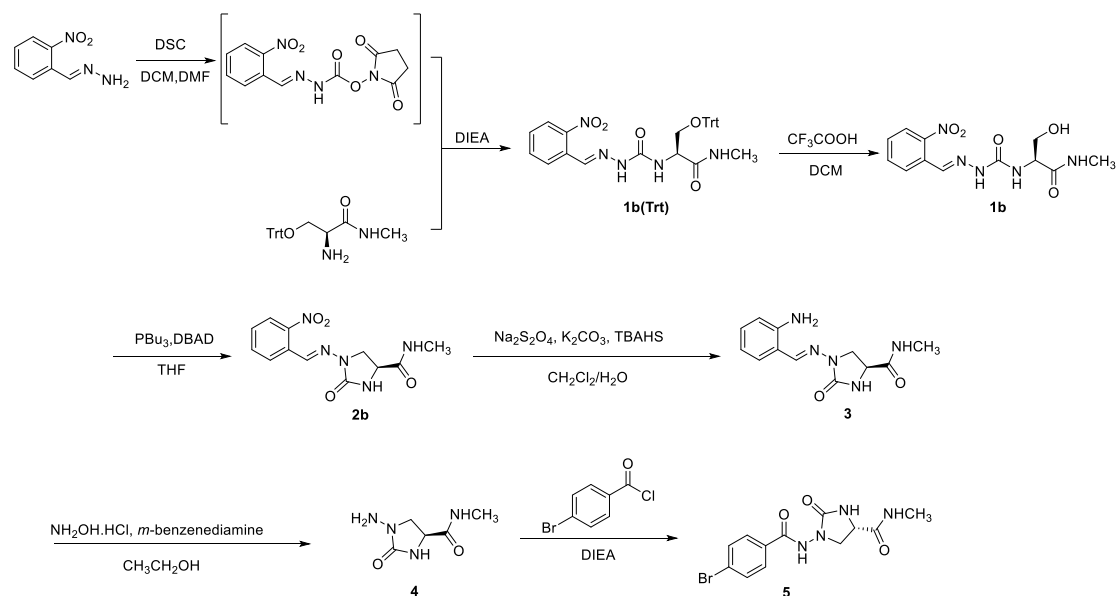


**Methyl (*E*)-(2-(2-nitrobenzylidene)hydrazine-1-carbonyl)-L-serinate 1a:** A solution of *N,N'*-disuccinimidyl carbonate (5.65 g, 22 mmol) in 80 mL of DMF/DCM (1:1, v/v) was treated dropwise with a solution of 2-nitrobenzylidene hydrazine (3.63 g, 22 mmol) in 20 mL of DCM over 15 min, stirred for 2 h at rt. Then L-Serine methyl ester hydrochloride (3.11 g, 20 mmol) was added. The mixture was treated with DIEA (6.61 mL, 40 mmol), stirred for 12 h at rt. After reaction completed, the mixture was washed sequentially with 1M KHSO<sub>4</sub>, saturated NaHCO<sub>3</sub> and brine. The volatiles were evaporated under reduced pressure and the residue was purified by silica column chromatography (DCM/MeOH, 1:0 to 1:1, v/v). **1a** was obtained as yellow solid (3.77 g, 61%); mp 145.9-146.3 °C; *R<sub>f</sub>* = 0.54 (DCM/MeOH, 9:1, v/v); [ $\alpha$ ]<sub>D</sub><sup>20</sup> -5.30 (*c* 0.1, DMSO); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  10.96 (s, 1H), 8.29 (s, 1H), 8.16 (dd, *J* = 7.9, 1.4 Hz, 1H), 8.02 (dd, *J* = 8.2, 1.2 Hz, 1H), 7.77 (td, *J* = 7.7, 1.3 Hz, 1H), 7.60 (ddd, *J* = 8.5, 7.4, 1.4 Hz,

1H), 7.06 (d,  $J = 8.3$  Hz, 1H), 5.20 (t,  $J = 5.8$  Hz, 1H), 4.36 (dt,  $J = 8.4, 4.2$  Hz, 1H), 3.86 – 3.68 (m, 2H), 3.64 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  172.0, 155.3, 148.3, 136.0, 133.9, 130.4, 129.1, 128.1, 125.1, 61.9, 55.5, 52.4; HRMS (ESI)  $m/z$  Calcd for  $\text{C}_{12}\text{H}_{14}\text{N}_4\text{NaO}_6$   $[\text{M}+\text{Na}]^+$  333.0806; Found 333.0801.

**Methyl (*E*)-2-(2-(2-nitrobenzylidene)hydrazine-1-carboxamido)acrylate **2a**:** To a round-bottomed flask was added tributylphosphine (100  $\mu\text{L}$ , 0.4 mmol) and DIAD (79  $\mu\text{L}$ , 0.4 mmol) in 3 mL of THF under nitrogen. The solution was cooled to 0  $^\circ\text{C}$  and a solution of **1a** (62 mg, 0.2 mmol) in THF (1 mL) was added. The mixture was stirred at rt for 5 h. The volatiles were evaporated under reduced pressure and the residue was purified by  $\text{Al}_2\text{O}_3$  column chromatography (DCM/MeOH, 1:0 to 1:1, v/v). **2a** was obtained as yellow solid (30 mg, 51%); mp 221.0–223.0  $^\circ\text{C}$ ;  $R_f = 0.73$  (DCM/MeOH, 9:1, v/v);  $[\alpha]_{\text{D}}^{20} +4.0$  ( $c$  0.1, DMSO);  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  11.27 (s, 1H), 8.64 (s, 1H), 8.33 (s, 1H), 8.06 (s, 2H), 7.78 (s, 1H), 7.61 (s, 1H), 6.12 (s, 1H), 5.60 (s, 1H), 3.78 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  164.6, 152.7, 148.3, 137.2, 134.1, 132.4, 130.7, 128.8, 127.9, 125.2, 106.2, 53.4; HRMS (ESI)  $m/z$  Calcd for  $\text{C}_{12}\text{H}_{12}\text{N}_4\text{NaO}_5$   $[\text{M}+\text{Na}]^+$  315.0700; Found 315.0696.

### 1.3 Synthesis of peptide Aid-dipeptide **5**



**(*S*, *E*)-*N*-(1-(methylamino)-1-oxo-3-(trityloxy)propan-2-yl)-2-(2-nitrobenzylidene)hydrazine-1-carboxamide **1b(Trt)**:** A solution of *N,N'*-disuccinimidyl carbonate (1.98 g, 7.7 mmol) in 40 mL of DMF/DCM (1:1, v/v) was treated dropwise with a solution of 2-nitrobenzylidene hydrazine (1.27 g, 7.7 mmol) in 10 mL of DCM over 15 min, stirred for 2 h at rt. Then (*S*)-2-amino-*N*-methyl-3-(trityloxy)propanamide (1.77 g, 7 mmol) was added. The mixture was treated with DIEA (2.55 mL, 15.4 mmol), stirred for 12 h at rt.

After reaction completed, the mixture was washed sequentially with 1M KHSO<sub>4</sub>, saturated NaHCO<sub>3</sub> and brine. The volatiles were evaporated under reduced pressure and the residue was purified by silica column chromatography (DCM/MeOH, 1:0 to 1:1, v/v). **1b(Trt)** was obtained as yellow solid (1.56 g, 40%): mp 131.0-132.0 °C; *R<sub>f</sub>* = 0.81 (DCM/MeOH, 9:1, v/v); [ $\alpha$ ]<sub>D</sub><sup>20</sup> -24.7 (*c* 0.1, DMSO); <sup>1</sup>H NMR (400 MHz, Chloroform-*d*)  $\delta$  10.38 (s, 1H), 8.40 (s, 1H), 7.94 (d, *J* = 8.1 Hz, 1H), 7.87 (d, *J* = 7.9 Hz, 1H), 7.47 – 7.18 (m, 17H), 7.11 (d, *J* = 7.8 Hz, 1H), 6.83 (q, *J* = 4.8 Hz, 1H), 4.60 (dt, *J* = 8.6, 4.8 Hz, 1H), 3.80 (dd, *J* = 8.9, 3.8 Hz, 1H), 3.23 (dd, *J* = 8.9, 5.5 Hz, 1H), 2.79 (d, *J* = 4.8 Hz, 3H); <sup>13</sup>C NMR (100 MHz, Chloroform-*d*)  $\delta$  171.0, 155.9, 147.8, 143.5, 137.2, 133.4, 129.7, 128.9, 128.5, 128.0, 127.9, 127.2, 124.8, 86.9, 63.5, 53.6, 26.4; HRMS (ESI) *m/z* [M+Na]<sup>+</sup> Calcd for C<sub>31</sub>H<sub>29</sub>N<sub>5</sub>NaO<sub>5</sub> 574.2061; Found 574.2054.

**(*S*, *E*)-*N*-(3-hydroxy-1-(methylamino)-1-oxopropan-2-yl)-2-(2-nitrobenzylidene)hydrazine-1-carboxamide **1b**:** A solution of methyl (*E*)-(2-(2-nitrobenzylidene)hydrazine-1-carbonyl)-L-serinate (1.54 g, 2.8 mmol) in 30 mL of DCM was treated dropwise with trifluoroacetic acid (2.08 mL, 28 mmol), stirred for 5 h at rt. After reaction completed, the mixture was washed sequentially with 1M KHSO<sub>4</sub>, saturated NaHCO<sub>3</sub> and brine. The volatiles were evaporated under reduced pressure and the residue was purified by silica column chromatography (DCM/MeOH, 1:0 to 1:1, v/v). **1b** was obtained as yellow solid (0.84 g, 97%): mp 202.0-204.0 °C; *R<sub>f</sub>* = 0.51 (DCM/MeOH, 9:1, v/v); [ $\alpha$ ]<sub>D</sub><sup>20</sup> -33.0 (*c* 0.1, DMSO); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  10.91 (s, 1H), 8.28 (s, 1H), 8.13 (d, *J* = 7.9 Hz, 1H), 8.01 (d, *J* = 8.2 Hz, 1H), 7.90 (q, *J* = 4.6 Hz, 1H), 7.77 (t, *J* = 7.6 Hz, 1H), 7.59 (t, *J* = 7.8 Hz, 1H), 6.92 (d, *J* = 7.9 Hz, 1H), 5.03 (t, *J* = 5.5 Hz, 1H), 4.19 (dt, *J* = 8.7, 4.9 Hz, 1H), 3.64 (ddt, *J* = 32.9, 10.6, 5.3 Hz, 2H), 2.59 (d, *J* = 4.5 Hz, 3H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  171.1, 155.1, 148.2, 135.6, 133.9, 130.3, 129.2, 128.0, 125.1, 62.4, 55.8, 26.1; HRMS (ESI) *m/z* [M+Na]<sup>+</sup> Calcd for C<sub>12</sub>H<sub>15</sub>N<sub>5</sub>NaO<sub>5</sub> 332.0965; Found 332.0959.

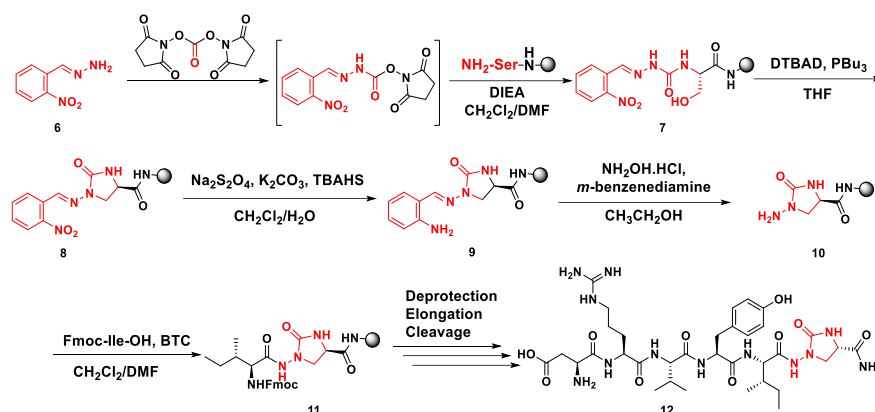
**(*S*, *E*)-*N*-methyl-1-((2-nitrobenzylidene)amino)-2-oxoimidazolidine-4-carboxamide **2b**:** To a round-bottomed flask was added tributylphosphine (500  $\mu$ L, 2 mmol) and DTBAD (461 mg, 2 mmol) in 4 mL of THF under nitrogen. The solution is cooled to 0 °C and sequentially added a solution of **1b** (309 mg, 1 mmol) in THF (4 mL). The mixture was stirred at rt for 5 h. The volatiles were evaporated under reduced pressure and the residue was purified by Al<sub>2</sub>O<sub>3</sub> column chromatography (DCM/MeOH, 1:0 to 1:1, v/v). **2b** was obtained as yellow solid (166 mg, 58%): mp 261.0-263.0 °C; *R<sub>f</sub>* = 0.62 (DCM/MeOH, 9:1, v/v); [ $\alpha$ ]<sub>D</sub><sup>20</sup> +4.0 (*c* 0.1, DMSO); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  8.13 (s, 1H), 8.00 (dd, *J* = 7.9, 5.5 Hz, 2H), 7.85 (s, 1H), 7.75 (t, *J* = 7.6 Hz, 1H), 7.70 (s, 1H), 7.61 – 7.54 (m, 1H), 4.22 (dd, *J* = 10.2, 4.9 Hz, 1H), 3.96 (t, *J* = 9.9 Hz, 1H), 3.62 (dd, *J* = 9.9, 5.0 Hz, 1H), 2.62 (d, *J* = 4.5 Hz, 3H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  171.3, 156.1, 148.3, 134.8, 134.0, 130.1, 129.8, 128.1, 125.1, 50.6, 46.5, 26.2; HRMS (ESI) *m/z*

$[M+Na]^+$  Calcd for  $C_{12}H_{13}N_5NaO_4$  314.0860; Found 314.0855.

**(*S, E*)-1-((2-aminobenzylidene)amino)-*N*-methyl-2-oxoimidazolidine-4-carboxamide 3:** A solution of sodium dithionite (992 mg, 5.70 mmol), potassium carbonate (1103 mg, 7.98 mmol), and TBAHS (194 mg, 0.57 mmol) in water (11 mL) and DCM (11 mL) was added to **2b** (166 mg, 0.57 mmol). The reaction was stirred at rt for 5 h. The mixture was evaporated under reduced pressure and the residue was purified by silica column chromatography (DCM/MeOH, 1:0 to 1:1, v/v). **3** was obtained as white solid (87 mg, 58%): mp 234.0-235.0 °C;  $R_f$  = 0.49 (DCM/MeOH, 9:1, v/v);  $[\alpha]_D^{20}$  -8.0 (*c* 0.1, DMSO);  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  8.10 (q,  $J$  = 4.5 Hz, 1H), 7.71 (s, 1H), 7.47 (s, 1H), 7.18 (d,  $J$  = 7.7 Hz, 1H), 7.00 (t,  $J$  = 7.6 Hz, 1H), 6.76 (s, 2H), 6.65 (d,  $J$  = 8.2 Hz, 1H), 6.51 (t,  $J$  = 7.4 Hz, 1H), 4.20 (dd,  $J$  = 10.3, 5.3 Hz, 1H), 3.94 (t,  $J$  = 9.7 Hz, 1H), 3.61 (dd,  $J$  = 9.5, 5.3 Hz, 1H), 2.62 (s, 3H);  $^{13}C$  NMR (101 MHz, DMSO- $d_6$ )  $\delta$  171.6, 156.9, 147.4, 143.8, 132.0, 129.7, 116.2, 115.4, 115.2, 50.9, 46.2, 26.2; HRMS (ESI)  $m/z$   $[M+Na]^+$  Calcd for  $C_{12}H_{15}N_5NaO_2$  284.1118; Found 284.1114.

**(*S*)-1-(4-bromobenzamido)-*N*-methyl-2-oxoimidazolidine-4-carboxamide 5:** compound **3** (70 mg, 0.27 mmol) was treated with a solution of  $NH_2OH \cdot HCl$  (94 mg, 1.35 mmol) and *m*-phenylenediamine (32 mg, 0.3 mmol) in EtOH (1.8 mL), and heated with sonication at 60 °C for 12 h. Without further purification, the mixture was evaporated under reduced pressure and treated with 4-bromobenzoyl chloride (755 mg, 2.7 mmol) and DIEA (929 mL, 5.4 mmol) in 4 mL of DCM. The reaction was stirred at rt for 12 h. The mixture was evaporated under reduced pressure and the residue was purified by silica column chromatography (DCM/MeOH, 1:0 to 1:1, v/v). **5** was obtained as white solid (33 mg, 36%): mp 241.0-243.0 °C;  $R_f$  = 0.38 (DCM/MeOH, 9:1, v/v);  $[\alpha]_D^{20}$  +45.0 (*c* 0.1, DMSO);  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  10.50 (s, 1H), 7.98 (q,  $J$  = 4.6 Hz, 1H), 7.76 (d,  $J$  = 8.7 Hz, 2H), 7.70 (d,  $J$  = 8.6 Hz, 2H), 7.26 (d,  $J$  = 1.7 Hz, 1H), 4.08 (ddd,  $J$  = 9.5, 5.4, 1.5 Hz, 1H), 3.80 (dd,  $J$  = 9.4, 7.8 Hz, 1H), 3.47 (dd,  $J$  = 7.8, 5.4 Hz, 1H), 2.62 (s, 3H);  $^{13}C$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  171.6, 165.0, 160.4, 132.0, 131.6, 129.9, 126.3, 51.1, 50.2, 26.2; HRMS (ESI)  $m/z$   $[M+Na]^+$  Calcd for  $C_{12}H_{13}BrN_4NaO_3$  363.0063; Found 363.0062.

## 1.4 Synthesis of [Aid]-Ang-(1-7) analogs 12-17



Rink amide resin supported  $\text{NH}_2\text{-Ser}$  was prepared by standard SPPS on Polystyrene Rink amide resin (0.67 mmol/g, typically ~299 mg per peptide).

### Synthesis of [Aid<sup>6-7</sup>]-Ang-(1-7) **12** as a representative for the synthesis of Aid-Ang-(1-7) analogs

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

***o*-Nitrobenzylidene semicarbazone resin **7**:** 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  $\text{NH}_2\text{-Ser}$  resin (~299 mg, 0.2 mmol). The resin mixture was treated with DIEA (207  $\mu\text{L}$ , 1.2 mmol), shaken on an automated shaker for 12 h. After filtration, the resin was washed sequentially with DMF ( $3 \times 10$  mL), MeOH ( $3 \times 10$  mL), THF ( $3 \times 10$  mL), and DCM ( $3 \times 10$  mL). Examination by LC-MS of a cleaved resin sample showed complete coupling and gave resin-bound semicarbazone **7** in good purity.

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

***o*-Nitrobenzylidene semicarbazone protected Aid resin **8**:** Vacuum dried semicarbazone resin **7** (~299 mg, 0.2 mmol) was suspended in anhydrous THF (3 mL) in a sealed flask under nitrogen and the suspension was cooled to 0 °C. Tributylphosphine (250  $\mu\text{L}$ , 1 mmol) in 2 mL of THF and DTBAD (230 mg, 1 mmol) in 2 mL of THF were sequentially added to the resin mixture. The mixture was shaken on an automated shaker for 5 h, and filtered. After filtration, the resin was washed sequentially with DMF ( $3 \times 10$  mL), MeOH ( $3 \times 10$  mL), THF ( $3 \times 10$  mL), and DCM ( $3 \times 10$  mL). Examination by LC-MS of a cleaved resin sample showed complete conversion and gave *o*-nitrobenzylidene semicarbazone protected Aid **8** in good

purity.

*Representative reduction of nitrobenzylidene semicarbazone to aminobenzylidene on solid phase:*

***o*-Aminobenzylidene semicarbazone resin 9:** 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 *o*-nitrobenzylidene **8** (~299 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/H<sub>2</sub>O (1:1, v/v, 10 mL), 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 *o*-aminobenzylidene semicarbazone **9** in good purity.

*Representative removal of aminobenzylidene on solid phase:*

**Aminoimidazolidin-2-one 10:** *o*-aminobenzylidene semicarbazone **9** (~299 mg, 0.2 mmol) was treated with a solution of NH<sub>2</sub>OH·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 syringe tube equipped with teflon™ filter, stopper and stopcock. The resin was filtered and washed with 10% DIEA in DMF (3 × 10 mL), DCM/H<sub>2</sub>O (1:1, v/v, 10 mL), 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 aminoimidazolidin-2-one **10** in good purity.

*Representative acylation of aminoimidazolidin-2-one on solid phase:*

**Aid-tripeptide 11:** Fmoc-Ile-OH (353 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 fume hood) 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 **10** (~299 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 yielded incomplete coupling. No further optimization was executed and the resin was used for the next peptide coupling.

**[Aid<sup>6-7</sup>]-Ang-(1-7) 12:** After Fmoc-removal of Aid-tripeptide **11**, peptide elongation using standard SPPS protocol provided **[Aid<sup>6-7</sup>]-Ang-(1-7) 12**. After the cleavage from the resin, the crude product was analyzed



by LC-MS with a crude purity of 15%. The crude product was purified by preparative RP-HPLC and the purity was ascertained by LC-MS.

[Aid]-Ang-(1-7) analogs **13-17** were synthesized using the same procedure as described for **12**.

**[Aid<sup>6-7</sup>]-Ang-(1-7) 12:** LC-MS (5-40% MeCN, 15 min) R.T. = 9.55 min; (10-70% MeOH, 15 min) R.T. = 9.57 min; HRMS (ESI) m/z [M+H]<sup>+</sup> Calcd for C<sub>34</sub>H<sub>55</sub>N<sub>12</sub>O<sub>10</sub> 791.4158; Found 791.4146.

**[Aid<sup>5-6</sup>]-Ang-(1-7) 13:** LC-MS (5-40% MeCN, 15 min) R.T. = 8.72 min; (10-70% MeOH, 15 min) R.T. = 8.87 min; HRMS (ESI) m/z [M+H]<sup>+</sup> Calcd for C<sub>33</sub>H<sub>51</sub>N<sub>12</sub>O<sub>10</sub> 775.3846; Found 775.3842.

**[Aid<sup>4-5</sup>]-Ang-(1-7) 14:** LC-MS (5-40% MeCN, 15 min) R.T. = 6.02 min; (10-40% MeOH, 15 min) R.T. = 5.35 min; HRMS (ESI) m/z [M+H]<sup>+</sup> Calcd for C<sub>30</sub>H<sub>49</sub>N<sub>14</sub>O<sub>9</sub> 749.3802; Found 749.3792.

**[Aid<sup>3-4</sup>]-Ang-(1-7) 15:** LC-MS (5-40% MeCN, 15 min) R.T. = 7.17 min; (10-40% MeOH, 15 min) R.T. = 7.02 min; HRMS (ESI) m/z [M+H]<sup>+</sup> Calcd for C<sub>31</sub>H<sub>51</sub>N<sub>14</sub>O<sub>9</sub> 763.3958; Found 763.3953.

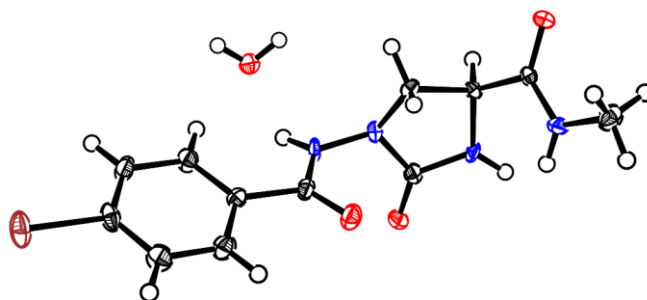
**[Aid<sup>2-3</sup>]-Ang-(1-7) 16:** LC-MS (5-40% MeCN, 15 min) R.T. = 9.82 min; (10-70% MeOH, 15 min) R.T. = 10.35 min; HRMS (ESI) m/z [M+H]<sup>+</sup> Calcd for C<sub>34</sub>H<sub>48</sub>N<sub>11</sub>O<sub>10</sub> 770.3580; Found 770.3571.

**[Aid<sup>1-2</sup>]-Ang-(1-7) 17:** LC-MS (5-40% MeCN, 15 min) R.T. = 11.93 min; (10-70% MeOH, 15 min) R.T. = 13.60 min; HRMS (ESI) m/z [M+H]<sup>+</sup> Calcd for C<sub>35</sub>H<sub>52</sub>N<sub>11</sub>O<sub>8</sub> 754.3995; Found 754.3980.

## 2. Crystal structure of Aid-dipeptide **5**

**Single crystals** were obtained from slow evaporation of a solution of Aid-dipeptide **5** (10 mg/mL) in 80% of methanol in water. A suitable crystal was selected and analyzed on a diffractometer (Agilent<sup>TM</sup> SuperNova). The crystal was kept at 150 K during data collection. Using Olex2, the structure was solved with the ShelXT structure solution program using Intrinsic Phasing and refined with the ShelXL refinement package using Least Squares minimization.

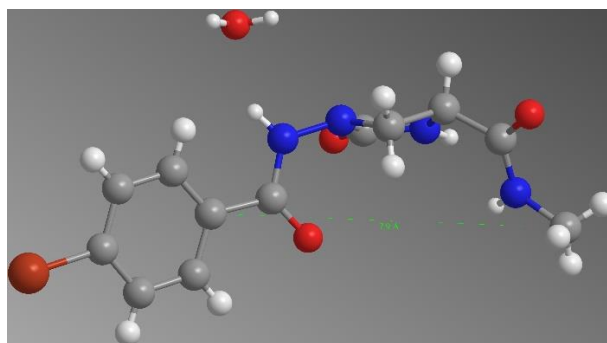
As shown in Table S1, the dihedral angles of Aid-dipeptide **5** are not fit in ideal turn structures due to the planar structure of *N*-aminoimidazolidin-2-one. However, the distance between the carbon of the benzene ring next to the carbonyl and the NHMe carbon (the two  $\alpha$ -carbon equivalents of a tetrapeptide) is 7.9 Å (Figure S2), which is close to the distance in normal  $\beta$ -turn (7.0 Å).



**Figure S1.** Crystal structure of Aid-dipeptide **5** (ellipsoid contour 50%)

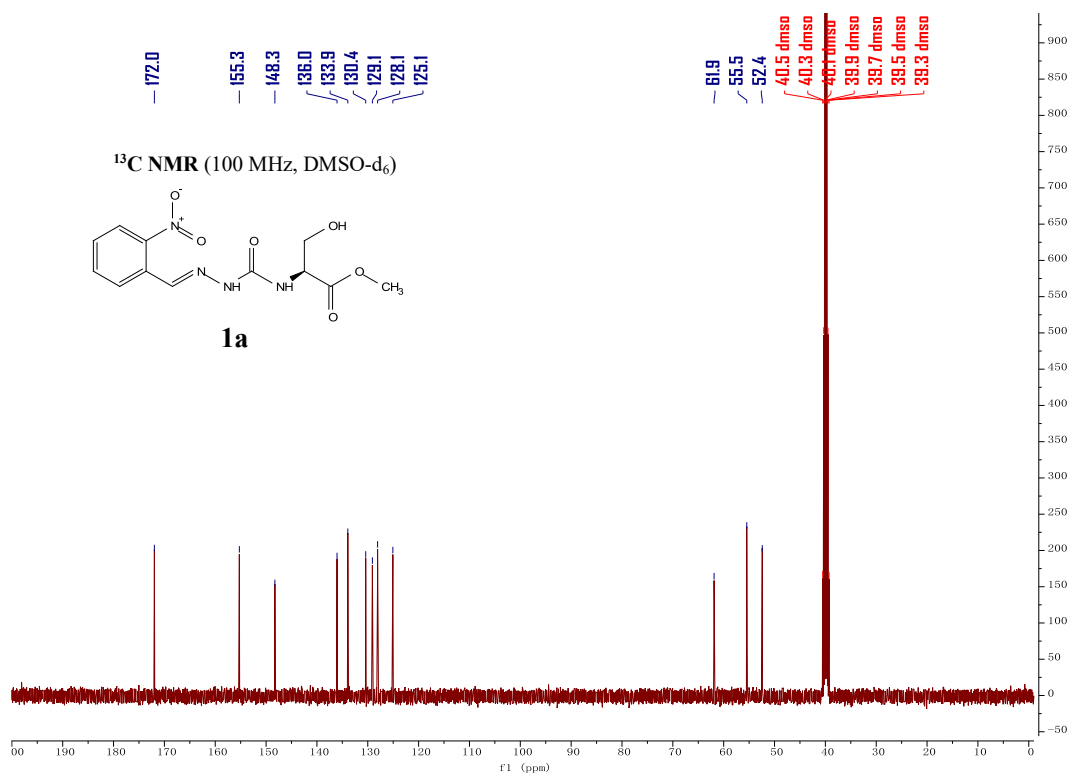
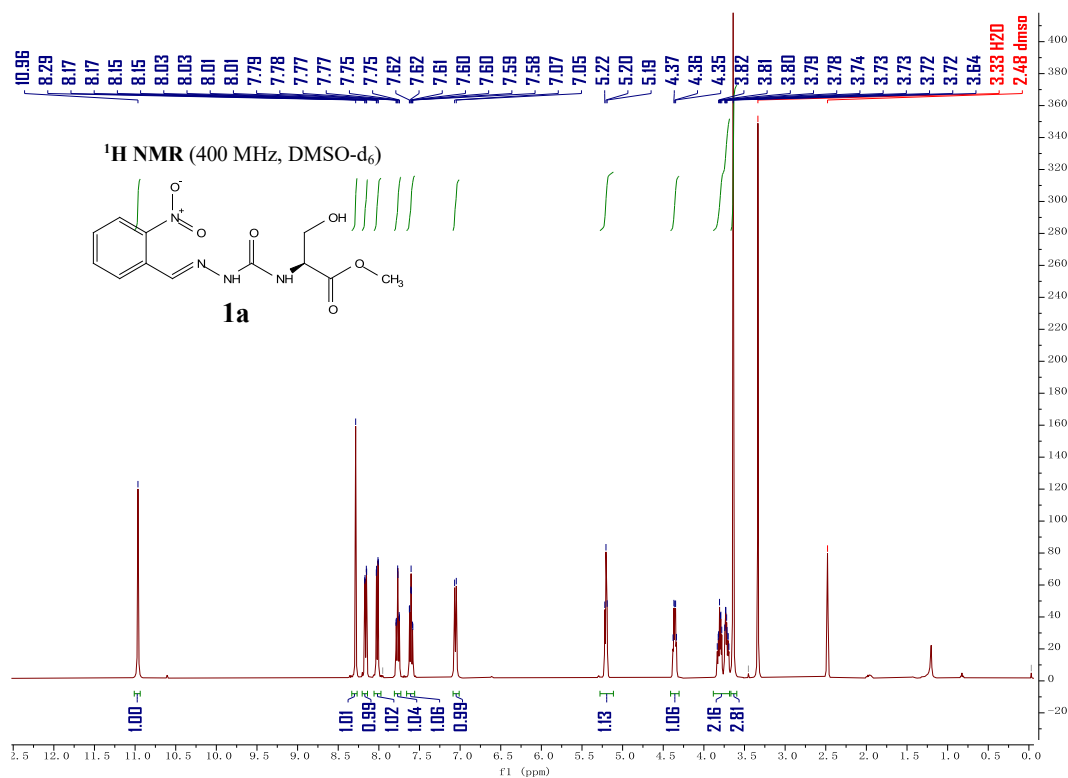
**Table S1.** Comparison of dihedral angles of **5** with ideal turns

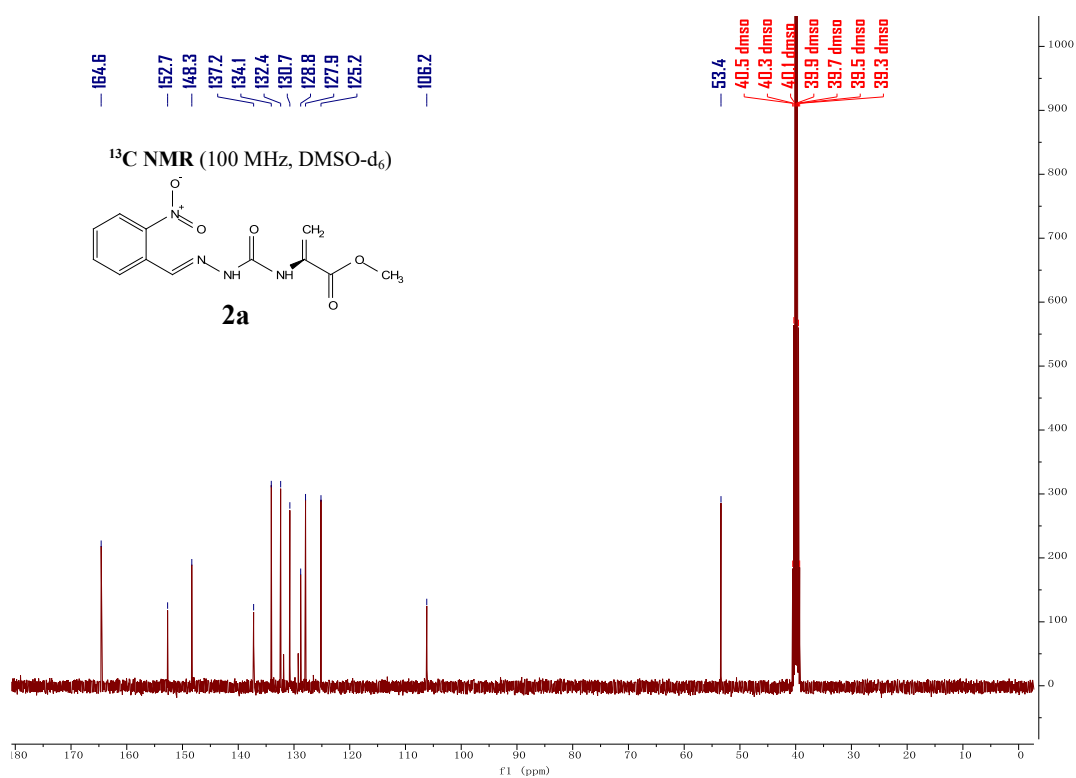
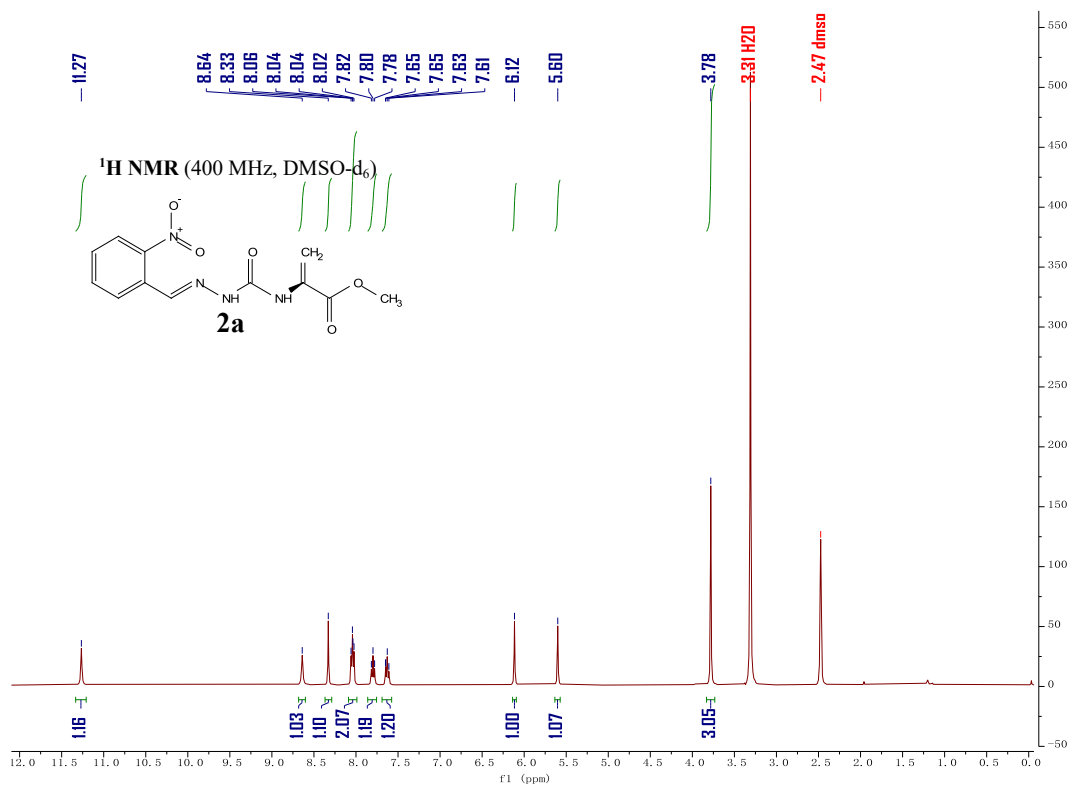
Type of turn	$\phi_i$	$\psi_i$	$\phi_{i+1}$	$\psi_{i+1}$
<b>5</b>	74	-159	143	-22
$\beta$ -II'	60	-120	-80	0
$\beta$ -II	-60	120	80	0
inverse $\gamma$	n/a	n/a	-70	60
classic $\gamma$	-	-	75	-65

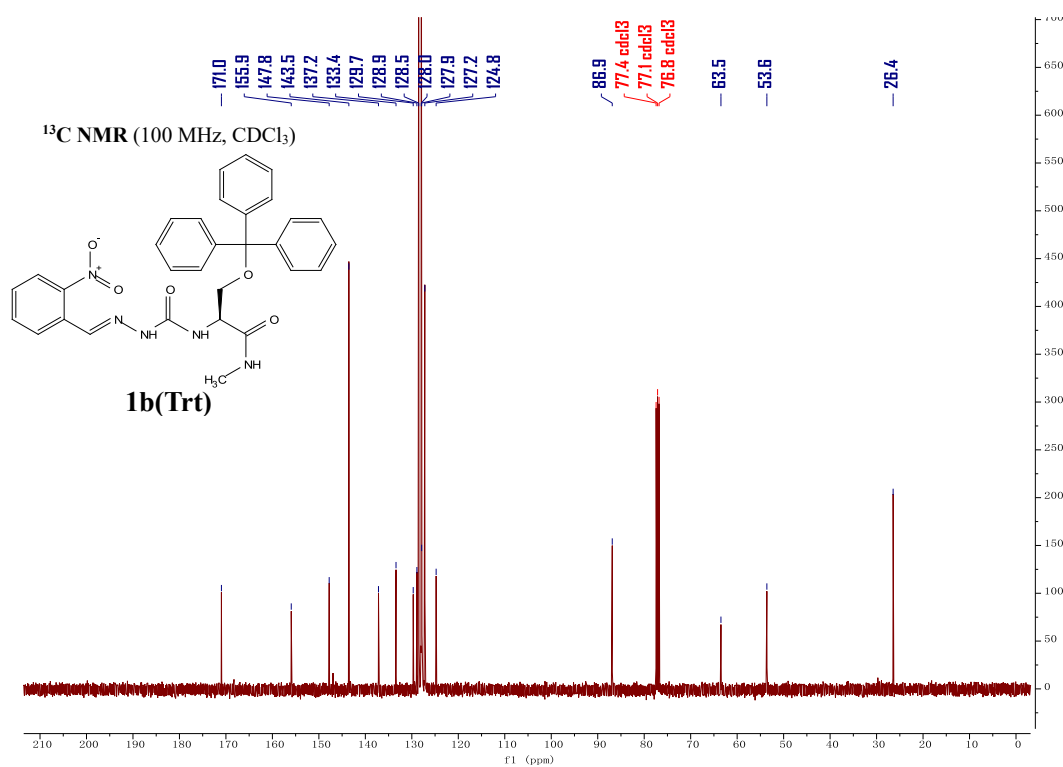
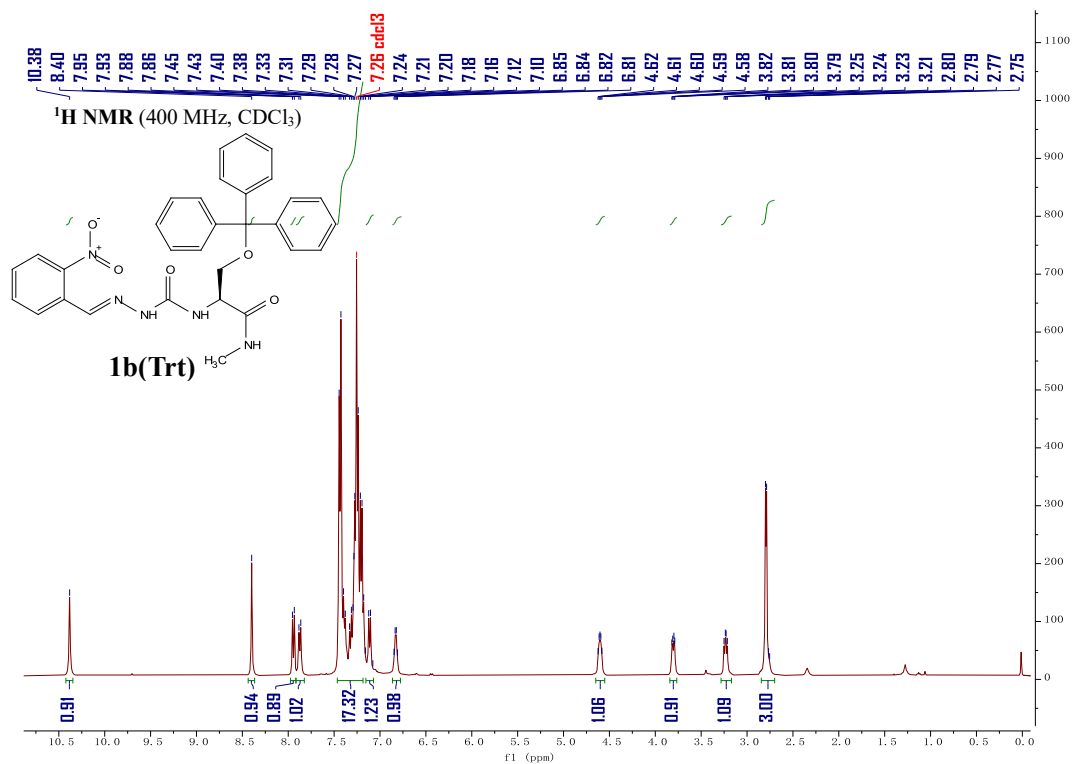


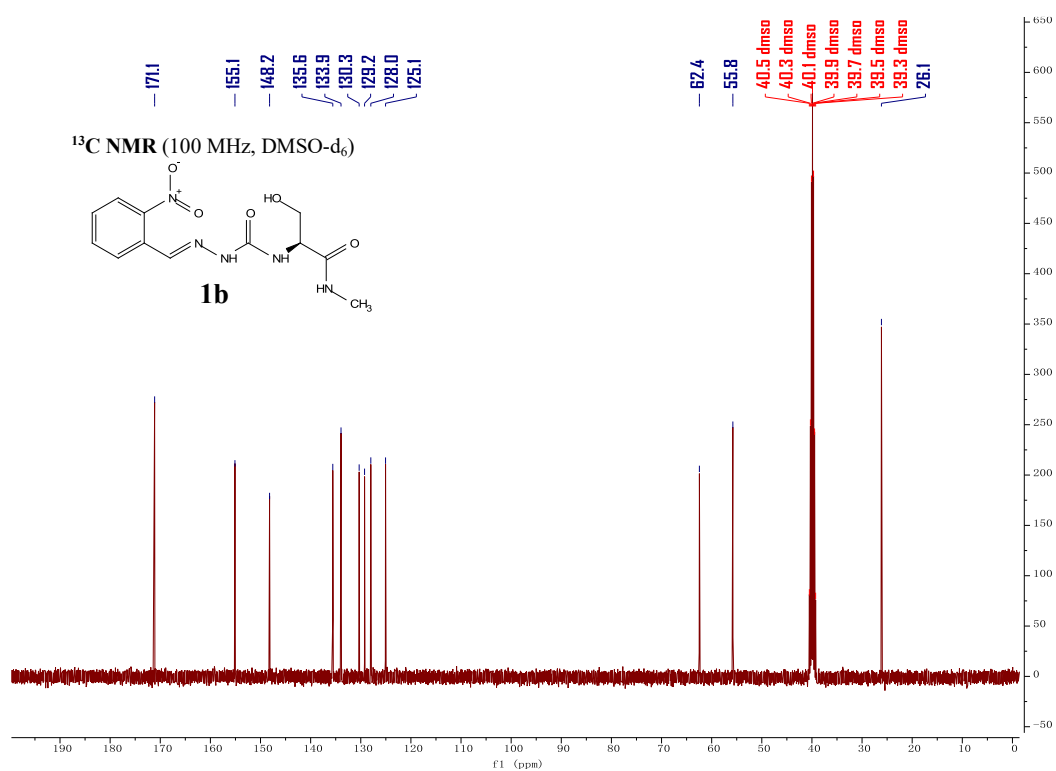
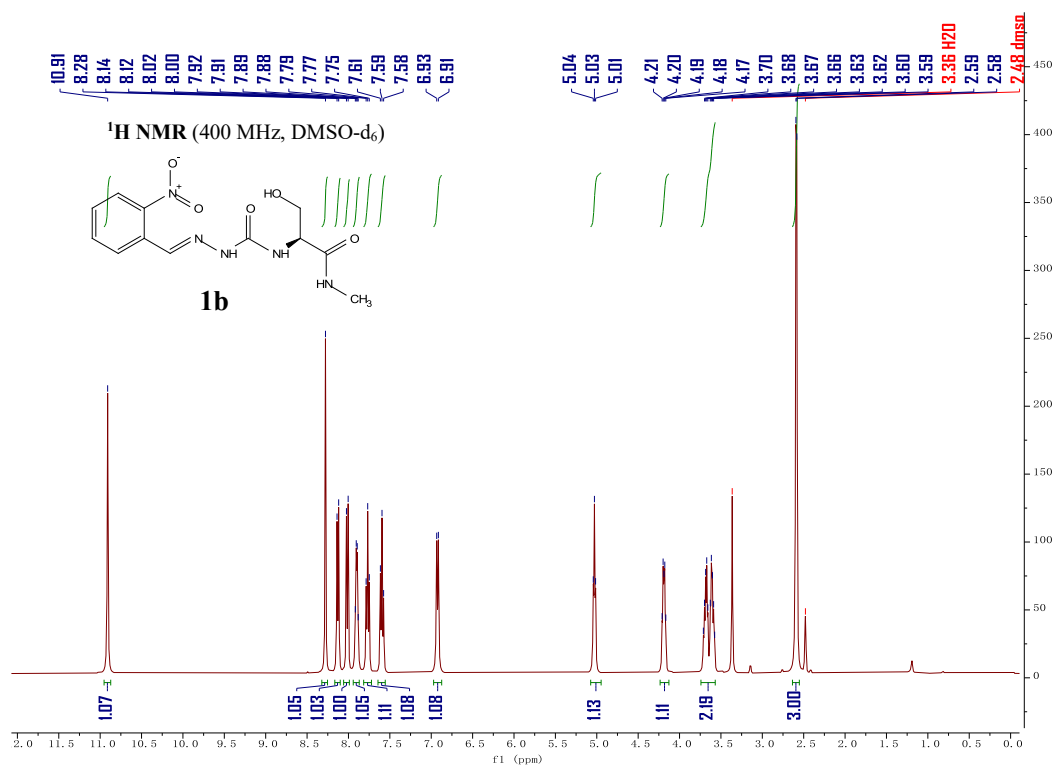
**Figure S2.** Distance between the carbons of the benzene ring next to the carbonyl and the NHMe carbon

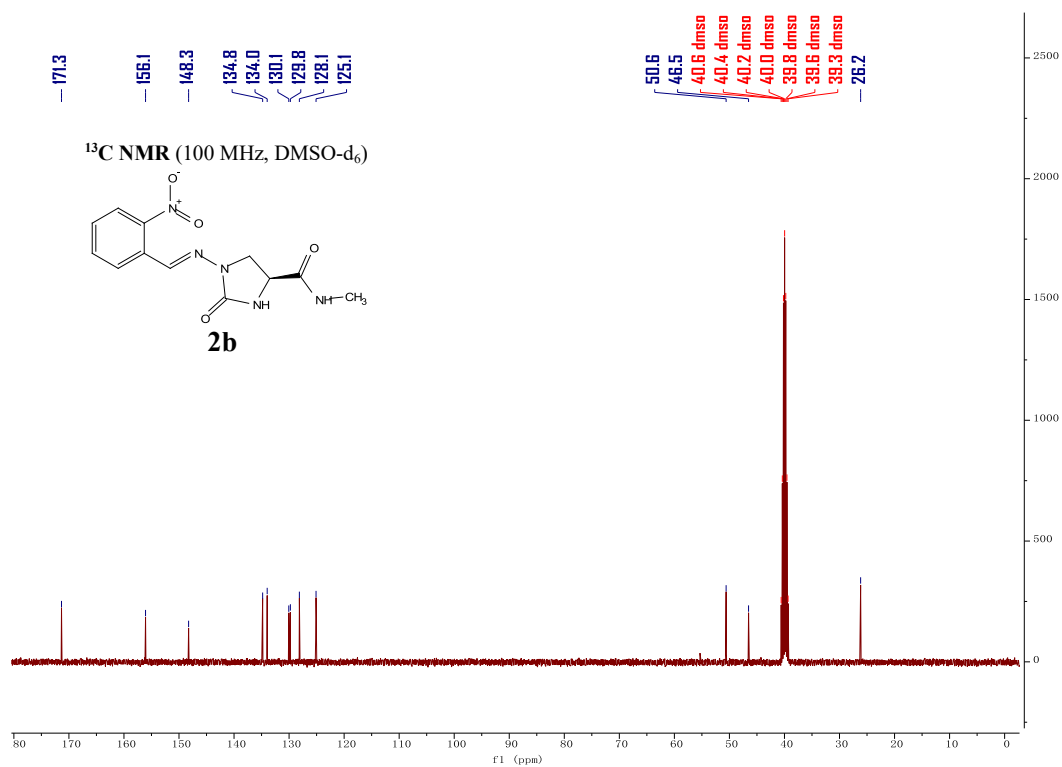
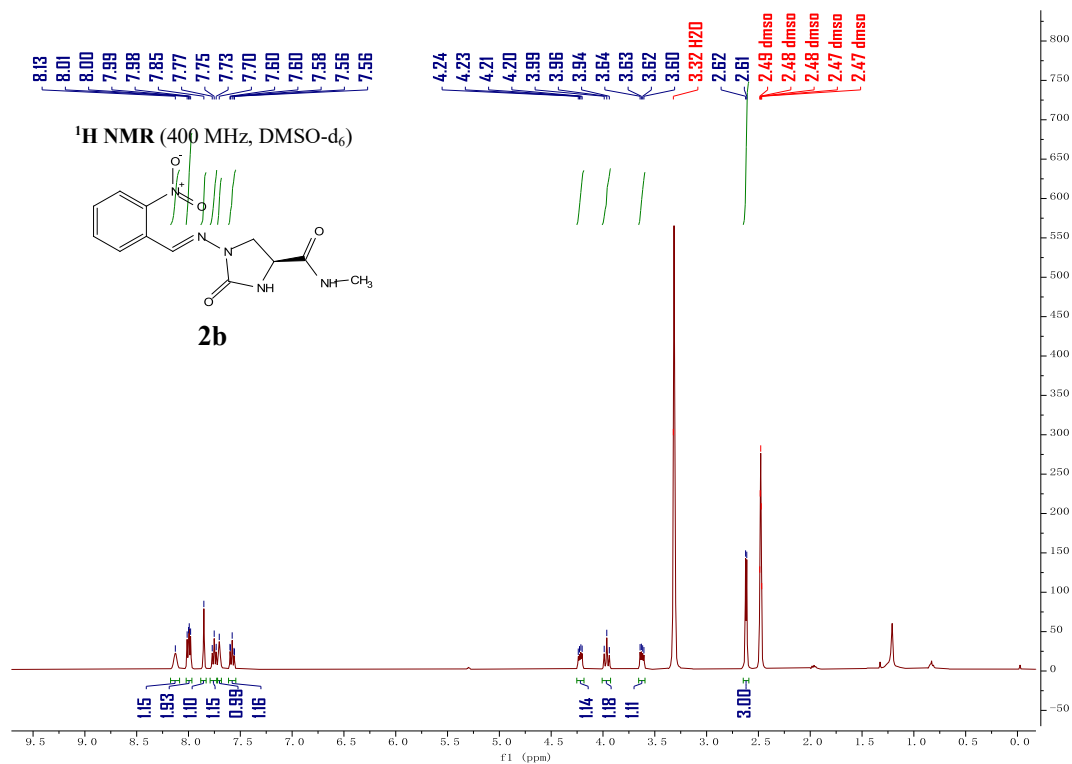
### 3. $^1\text{H}$ -NMR and $^{13}\text{C}$ -NMR spectrum

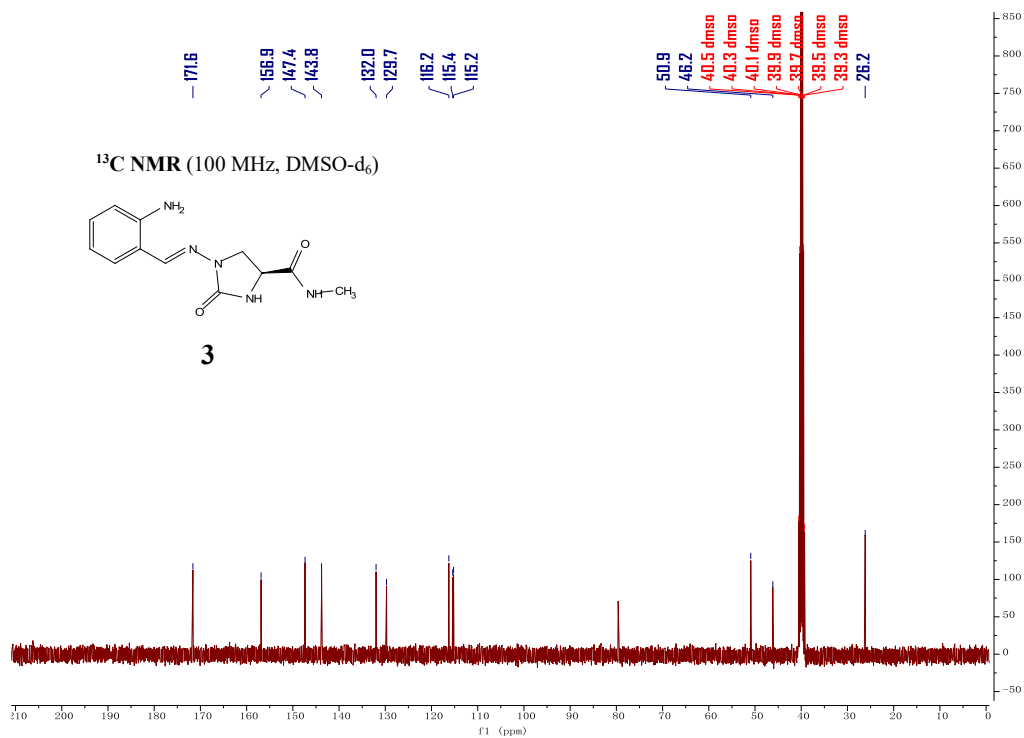
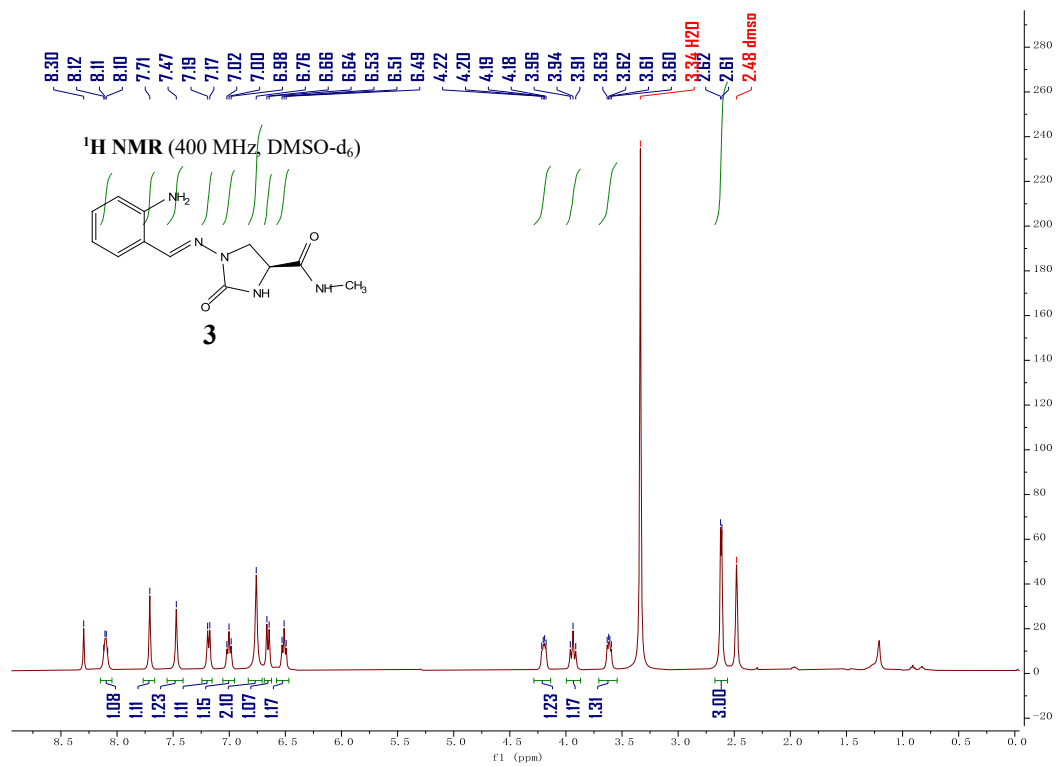




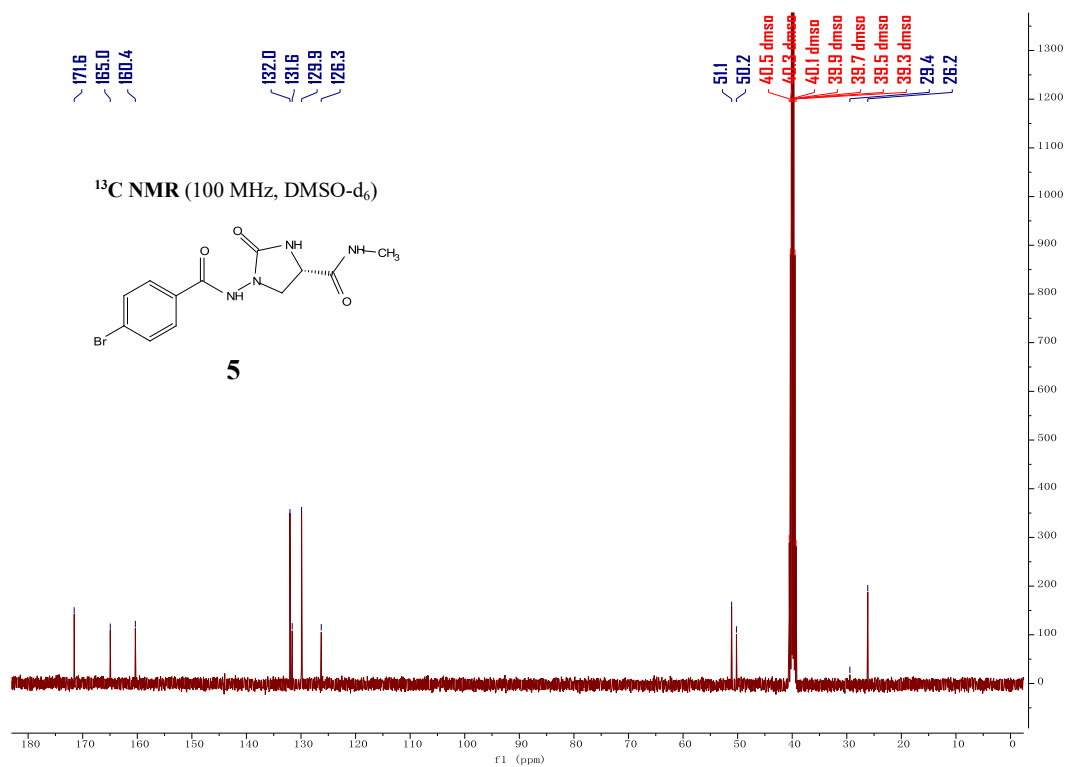
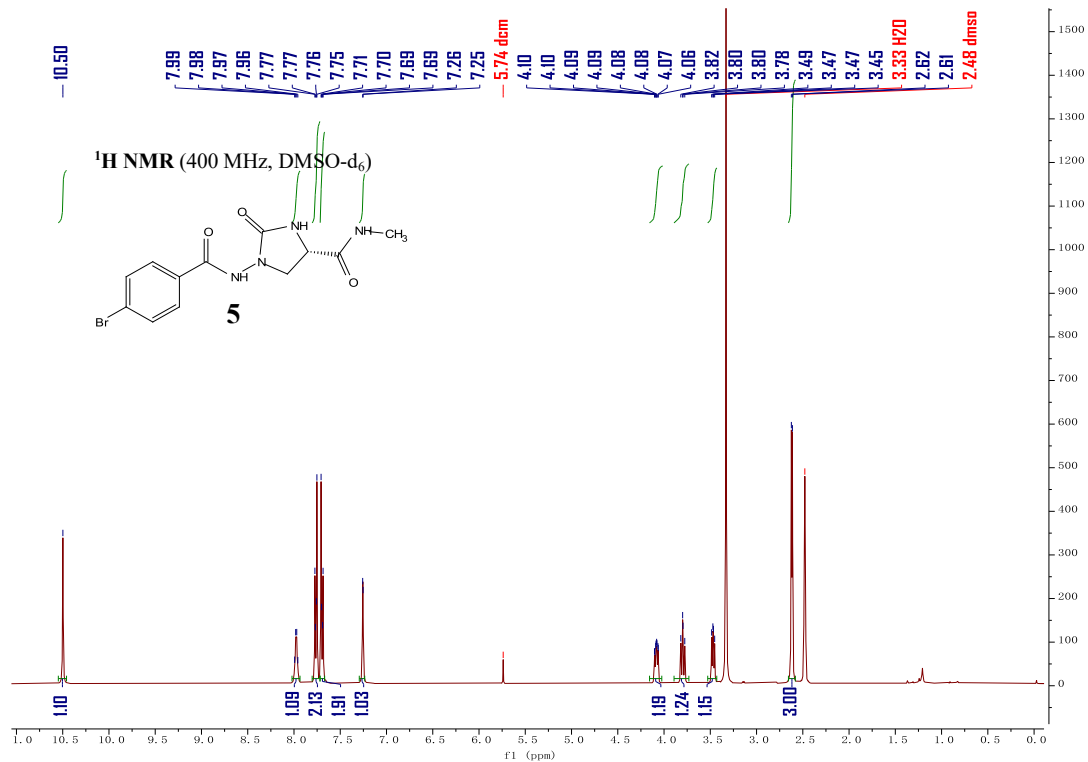




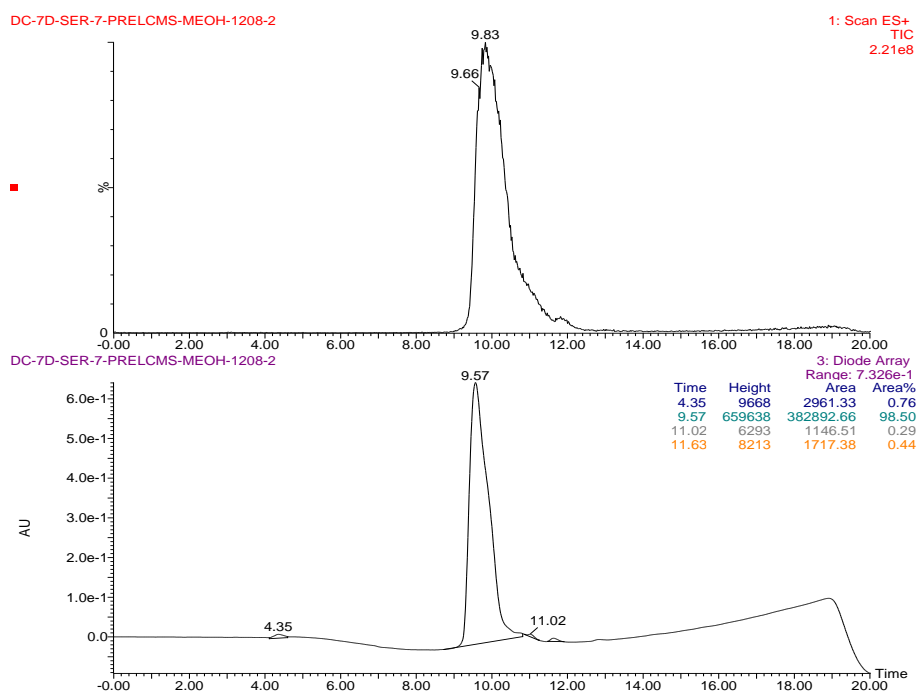




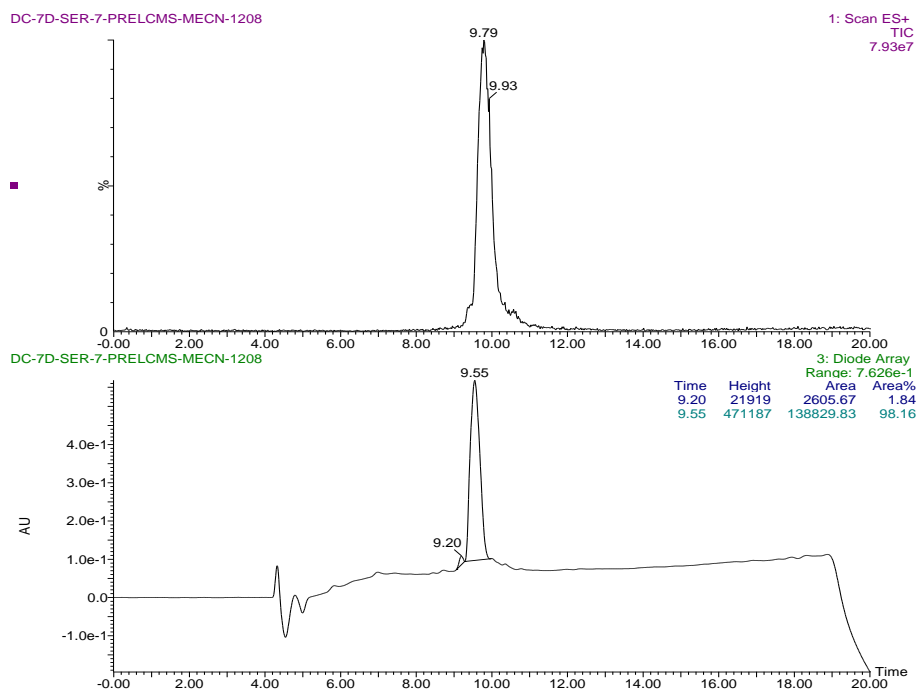




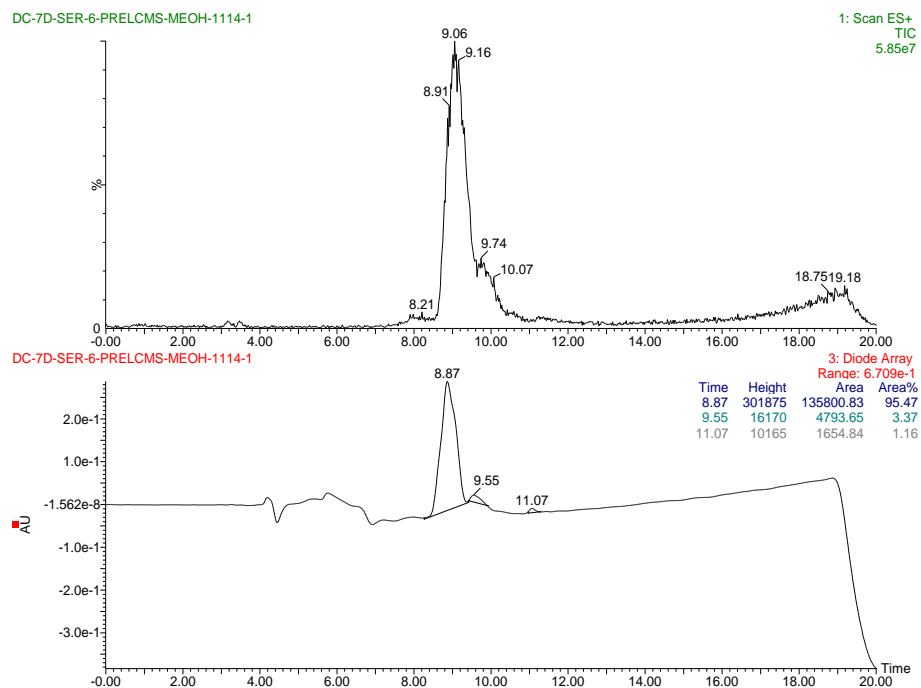
#### 4. Analytical LC-MS chromatograms of purified Aid-peptides 12-17



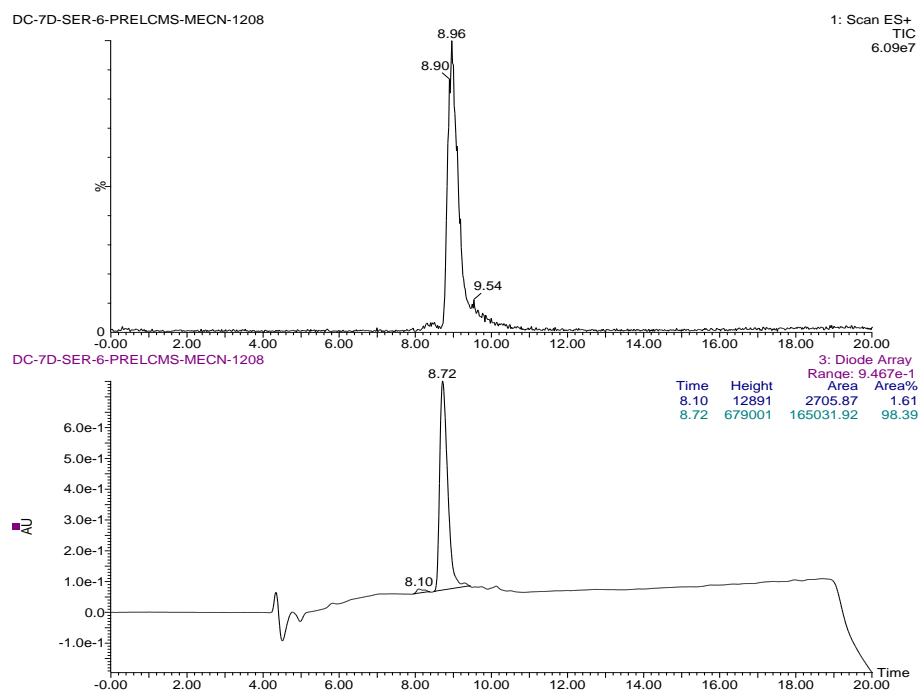
LC-MS chromatogram of **12** in a linear gradient of 10-70% of MeOH in H<sub>2</sub>O containing 0.1% FA over 15 min, R.T. = 9.57 min



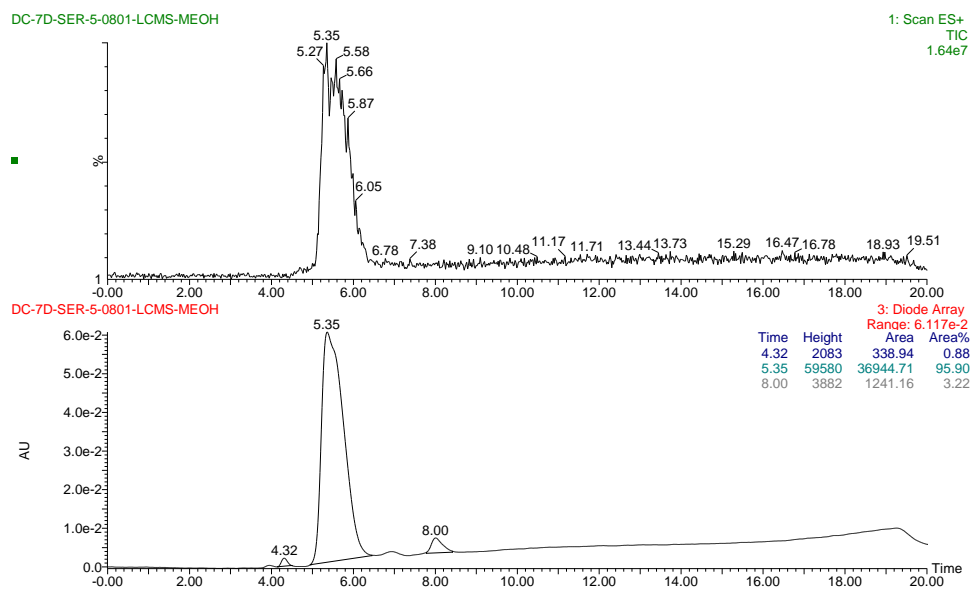
LC-MS chromatogram of **12** in a linear gradient of 5-40% of MeCN in H<sub>2</sub>O containing 0.1% FA over 15 min, R.T. = 9.55 min



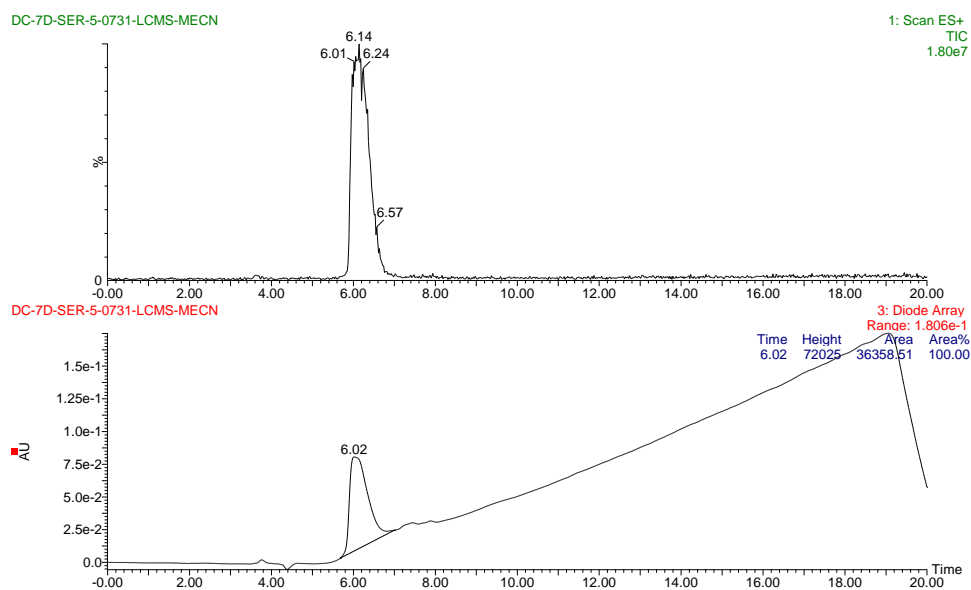
LC-MS chromatogram of **13** in a linear gradient of 10-70% of MeOH in H<sub>2</sub>O containing 0.1% FA over 15 min, R.T. = 8.87 min



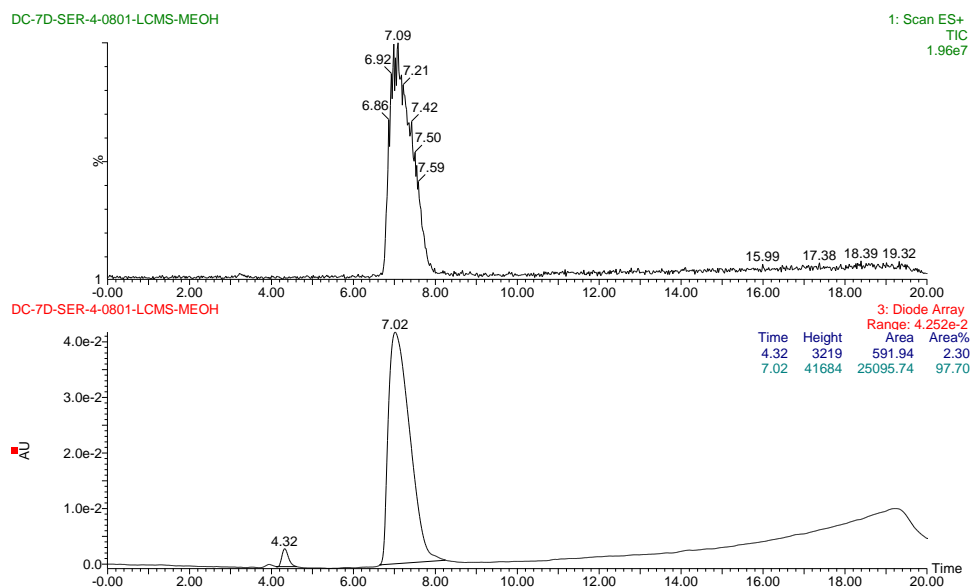
LC-MS chromatogram of the crude of **13** in a linear gradient of 5-40% of MeCN in H<sub>2</sub>O containing 0.1% FA over 15 min, R.T. = 8.72 min



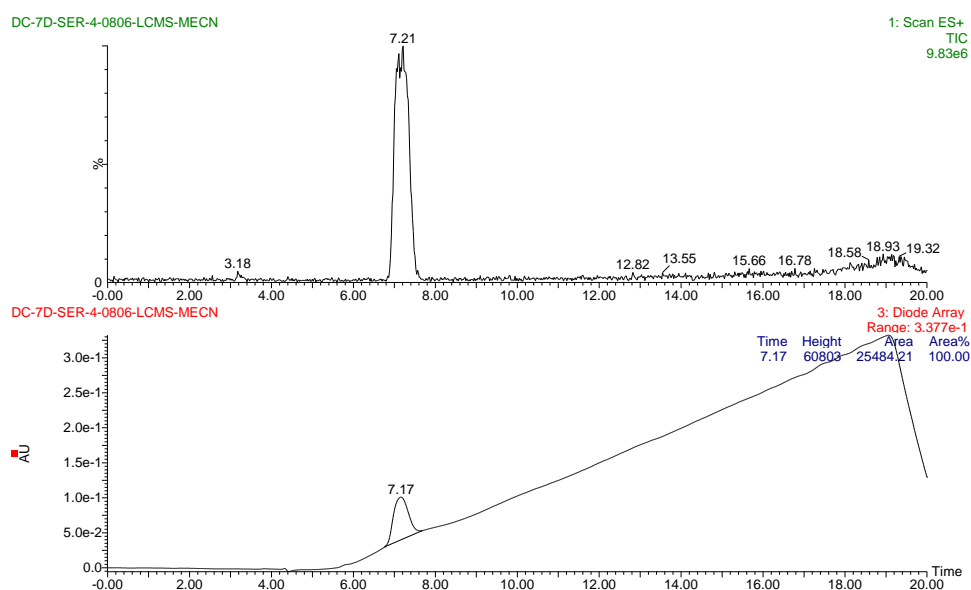
LC-MS chromatogram of **14** in a linear gradient of 10-40% of MeOH in H<sub>2</sub>O containing 0.1% FA over 15 min, R.T. = 4.50 min



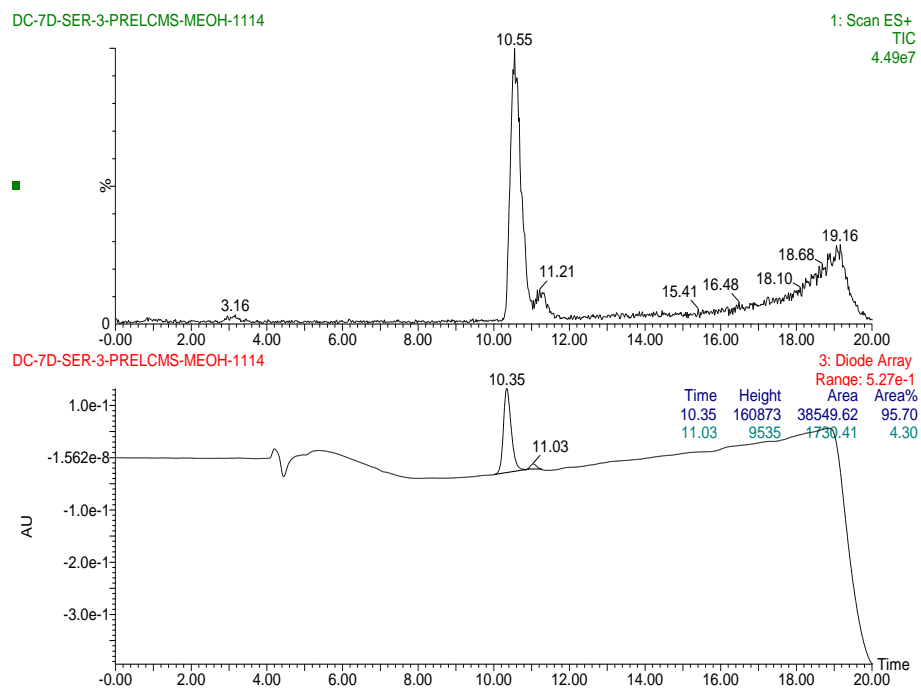
LC-MS chromatogram of **14** in a linear gradient of 5-40% of MeCN in H<sub>2</sub>O over 15 min, R.T. = 4.28 min



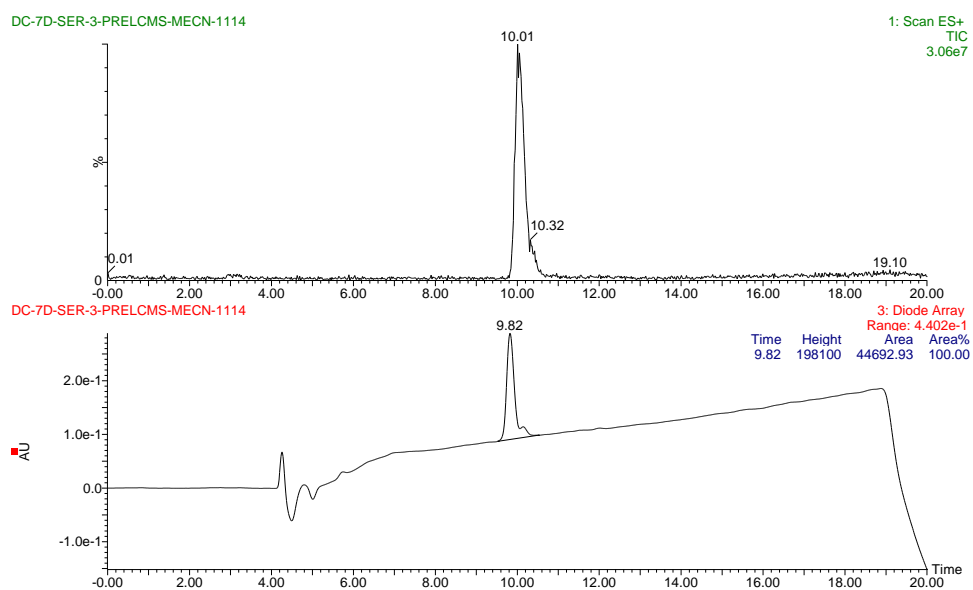
LC-MS chromatogram of **15** in a linear gradient of 10-40% of MeOH in H<sub>2</sub>O containing 0.1% FA over 15 min, R.T. = 5.80 min



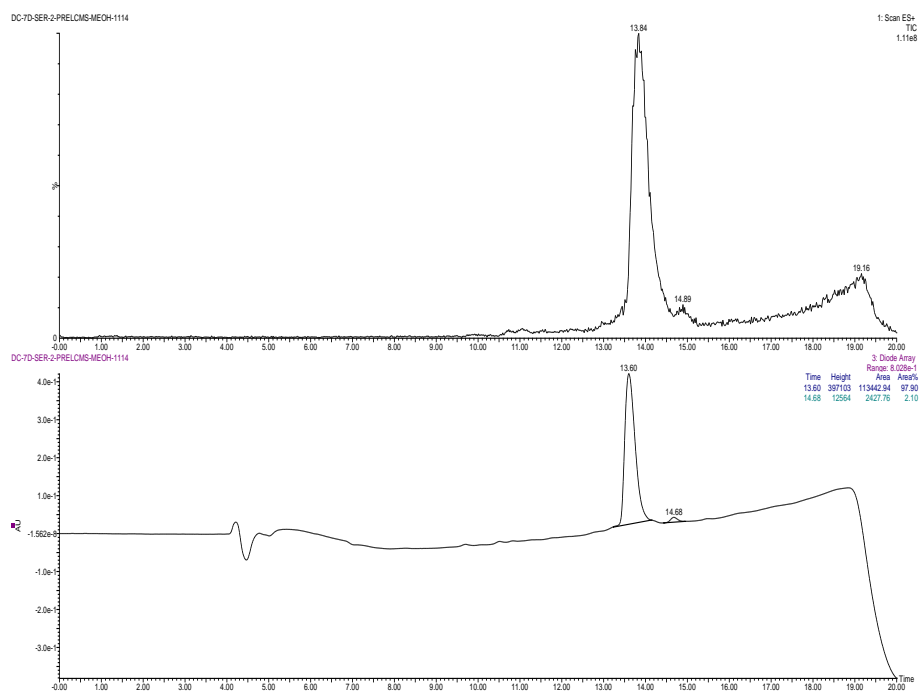
LC-MS chromatogram of **15** in a linear gradient of 5-40% of MeCN in H<sub>2</sub>O over 15 min, R.T. = 6.03 min



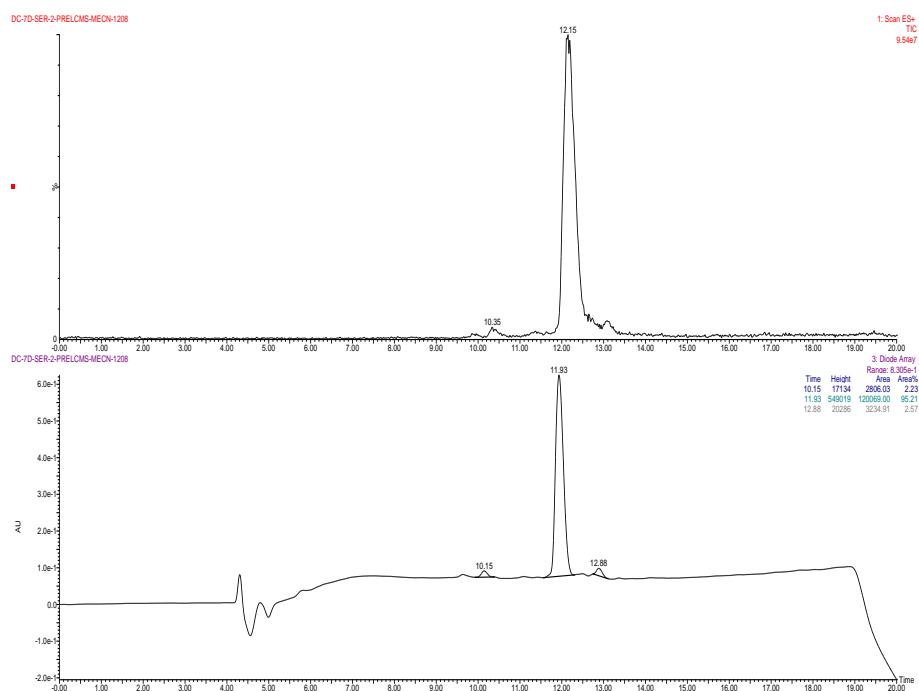
LC-MS chromatogram of **16** in a linear gradient of 10-70% of MeOH in H<sub>2</sub>O containing 0.1% FA over 15 min, R.T. = 10.35 min



LC-MS chromatogram of **16** in a linear gradient of 5-40% of MeCN in H<sub>2</sub>O containing 0.1% FA over 15 min, R.T. = 9.82 min



LC-MS chromatogram of **17** in a linear gradient of 10-70% of MeOH in H<sub>2</sub>O containing 0.1% FA over 15 min, R.T. = 13.60 min



LC-MS chromatogram of **17** in a linear gradient of 5-40% of MeCN in H<sub>2</sub>O containing 0.1% FA over 15 min, R.T. = 11.93 min

## 5. Biological evaluation of Aid-peptides 12-17

### Proteolytic stability :

The stability of the six purified Aid analogs was assessed against human ACE and DPP3. Ang-(1–7) was obtained from GL Biochem (Shanghai) Ltd. Ang-(1-7)-NH<sub>2</sub> was synthesized using standard SPPS protocol and Fmoc-chemistry on Rink-amide resin. The peptides (500 nmol) were incubated with human ACE (0.5 µg, R&D Systems) for 360 min or human DPP 3 (0.5 µg, R&D Systems) for 240 min, respectively, at 37 °C in 0.1 mL of the HEPES buffer (21 mM HEPES, 137 mM NaCl, 10 mM KCl, 6 mM Glucose, 0.4 mM NaHPO<sub>4</sub>·12H<sub>2</sub>O, pH 7.4). The quantity of remaining intact peptide was determined every 60 min using LC-MS equipped with a Phenomenex Aeris™ C<sub>18</sub> column (pore size: 80 Å, particle size: 4 µm; 150× 4.6 mm) with a flow rate of 0.5 mL/min using a proper linear gradient of CH<sub>3</sub>CN in water.

### Effects of [Aid]-Ang-(1–7) analogs on the production of NO and the expression of inflammatory cytokines in RAW264.7 macrophages:

The murine RAW264.7 (ATCC #TIB-71) macrophage cells were obtained from American Type Cell Collection (Virginia, USA), seeded at 2 × 10<sup>5</sup> cells/well in DMEM (Biological Industries, 06-1055-57-1A, Kibbutz Beit-Haemek, Israel) culture media supplemented with 10% fetal bovine serum (Biological Industries, 04-001-1A, Kibbutz Beit-Haemek, Israel) on a 24-well plate, and cultured at 37°C with 5% CO<sub>2</sub>. After 24 h, the culture media was refreshed with DMEM supplemented with 10% fetal bovine serum. RAW264.7 cells were treated first with LPS at a concentration of 20 ng/mL for 30 min, and then incubated with peptides at a concentration of 100 µM for 24 h. Culture supernatants were collected and the production of NO and the expression of IL-6 were determined by the Griess reaction and ELISA assay, respectively.

1) For NO, briefly, collected supernatant fraction was mixed with an equal volume of Griess reagent according to the manufacturer's protocol, and samples were incubated at room temperature for 10 min. Nitrite production was measured by determining the absorbance at 540 nm, and the concentration was calculated using a standard curve generated with NaNO<sub>2</sub>.

2) The expression of IL-6 in the culture medium were measured by ELISA kit (Dakewe, #1210602, Shenzhen, China) according to the manufacturer's protocol. The content of IL-6 was determined using standard curves. The absorbance of standard and samples was measured at 450 nm.

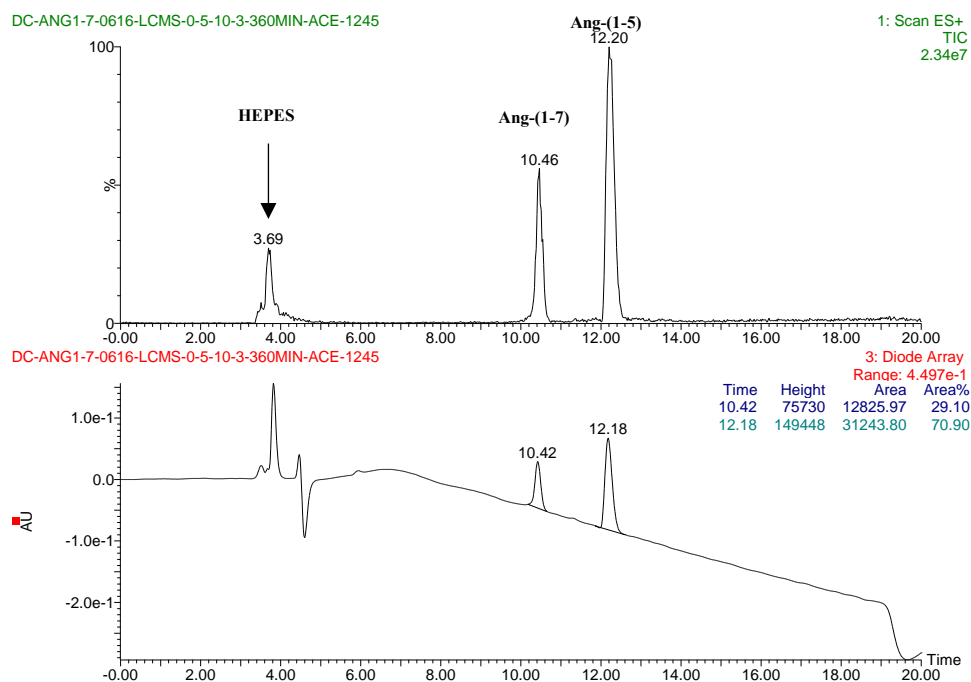
Each test was reproduced independently for three times in three replicates. Statistical tests of data were examined by unpaired t test with the Graph Pad® Prism software package (version 6.02). P<0.05 was considered statistically significant. \* denotes p < 0.05, \*\* denotes p < 0.01, \*\*\* denotes p < 0.001 and \*\*\*\*



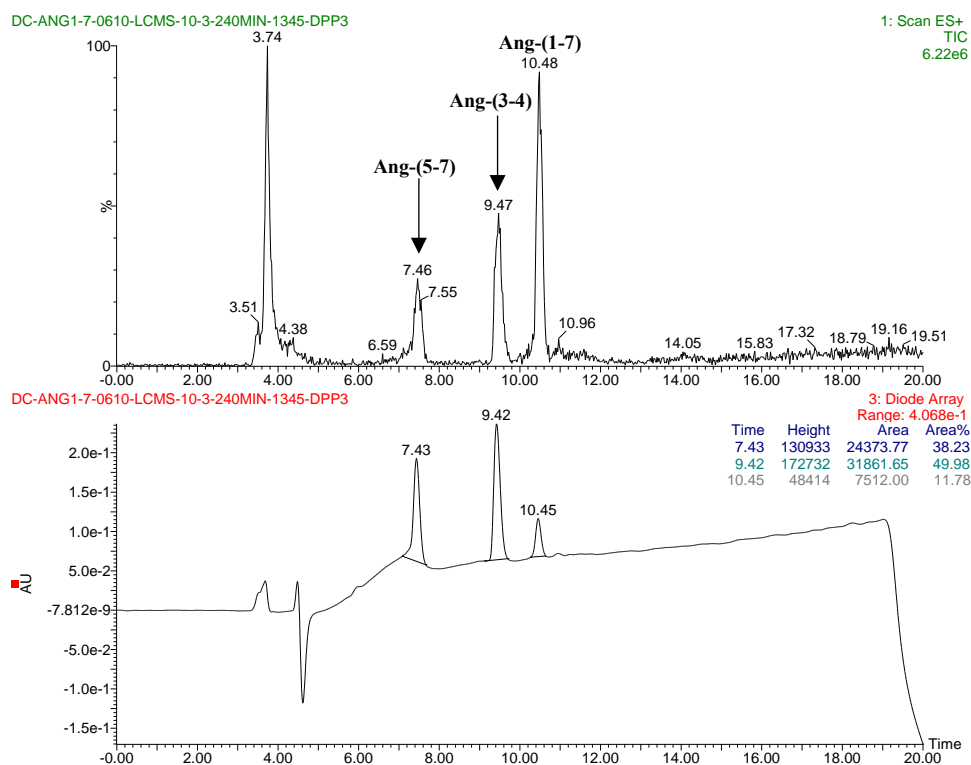
denotes  $p < 0.0001$ .

**Effects of different Ang-(1–7) analogs on the cell viability of cancer cells:** HT-1080 cells (#TCHu170), A549 cells (#TCHu150) or 4T1 cells (#TCM32) were obtained from Cell Bank of the Chinese Academy of Sciences (Shanghai, China), seeded at  $2 \times 10^3$  cells/well in DMEM or RPMI1640 (Biological Industries, Kibbutz Beit-Haemek, Israel) culture media supplemented with 10% fetal bovine serum (Biological Industries, 04-001-1A, Kibbutz Beit-Haemek, Israel) into a 96-well plate, and cultured at 37°C with 5% CO<sub>2</sub>. After 24h, the culture medium of adhered cells was changed to DMEM or RPMI1640 culture media supplemented with 1% FBS and the rapidly proliferating cells were incubated with peptides at a concentration of 10 µM for 24h. The cell viability per well was measured using MTT cell proliferation and cytotoxicity assay. Briefly, MTT stock solution (5 mg/ml, 10 µL) was added to each well containing culture medium (100 µL) and incubated for 4h. At the end of the incubation, the supernatants were removed and 150 µL DMSO was added to dissolve the formazan crystals. The absorbance was recorded at 490 nm by Microplate Reader (Molecular Devices, SpectraMax i3x, California, USA) at ambient temperature. The cell viability was calculated by the following equation: cell viability (%) =  $(A_{\text{treatment}} / A_{\text{control}}) \times 100\%$ . Statistical tests of data were examined by unpaired t test with the Graph Pad<sup>®</sup> Prism software package (version 6.02).  $P < 0.05$  was considered statistically significant. \* denotes  $p < 0.05$ , \*\* denotes  $p < 0.01$ , \*\*\* denotes  $p < 0.001$  and \*\*\*\* denotes  $p < 0.0001$ .

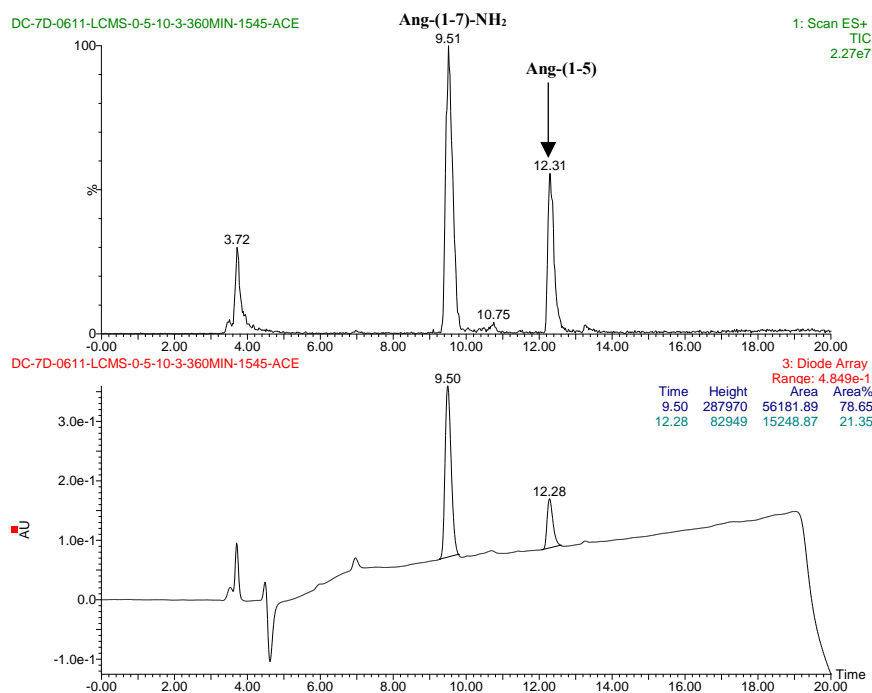
## 6. Analytical LC-MS chromatograms of Aid-peptides 12-17 after incubation with peptidase ACE or DPP3



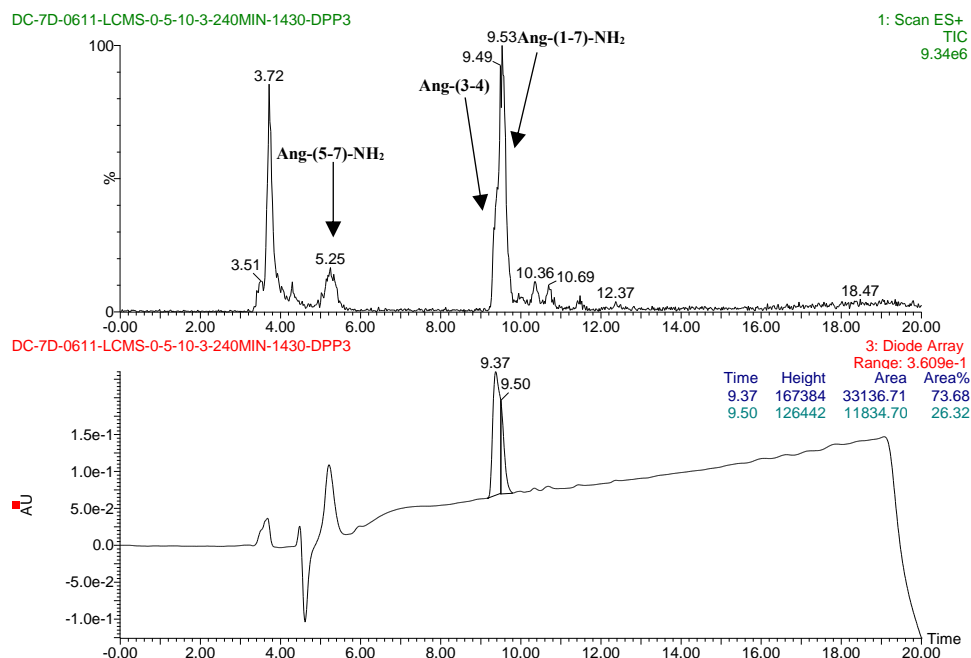
LC-MS chromatogram of **Ang-(1-7)** after incubation with ACE at 37 °C for 360 min in a linear gradient of 5-40% of MeCN in H<sub>2</sub>O containing 0.1% FA over 15 min.



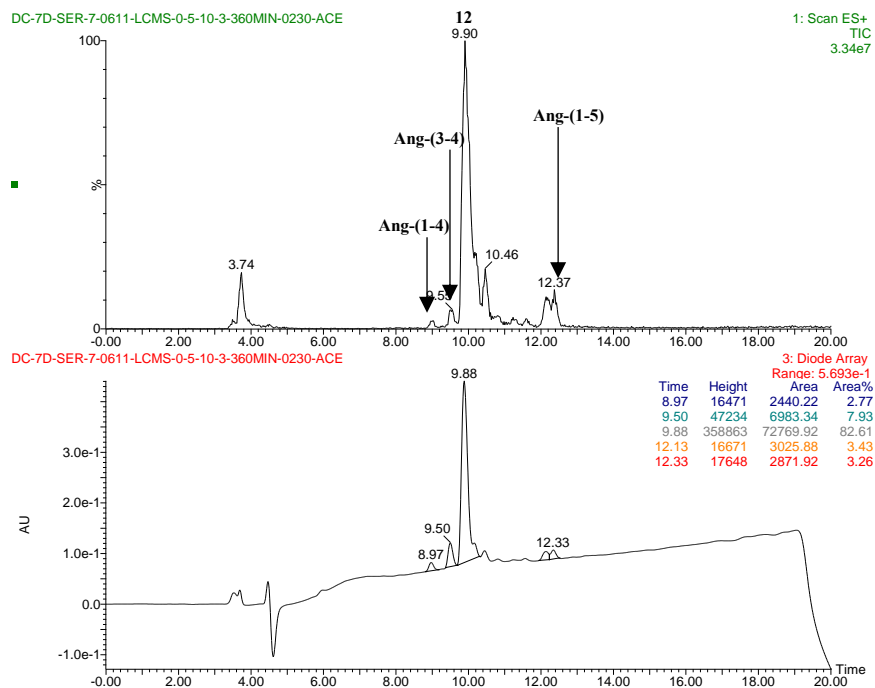
LC-MS chromatogram of **Ang-(1-7)** after incubation with DPP3 at 37 °C for 240 min in a linear gradient of 5-40% of MeCN in H<sub>2</sub>O containing 0.1% FA over 15 min.



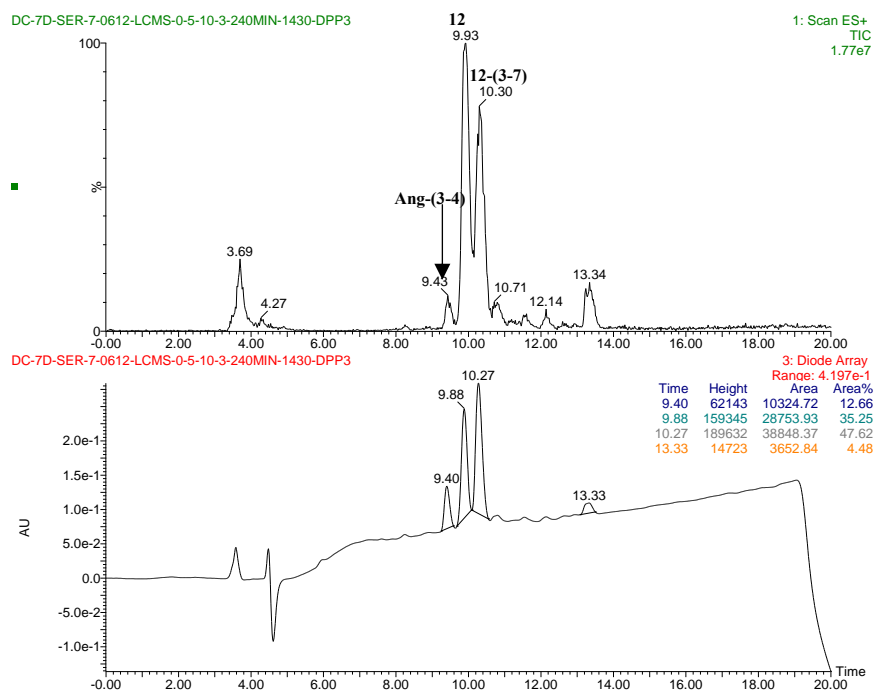
LC-MS chromatogram of **Ang-(1-7)-NH<sub>2</sub>** after incubation with ACE at 37 °C for 360 min in a linear gradient of 5-40% of MeCN in H<sub>2</sub>O containing 0.1% FA over 15 min.



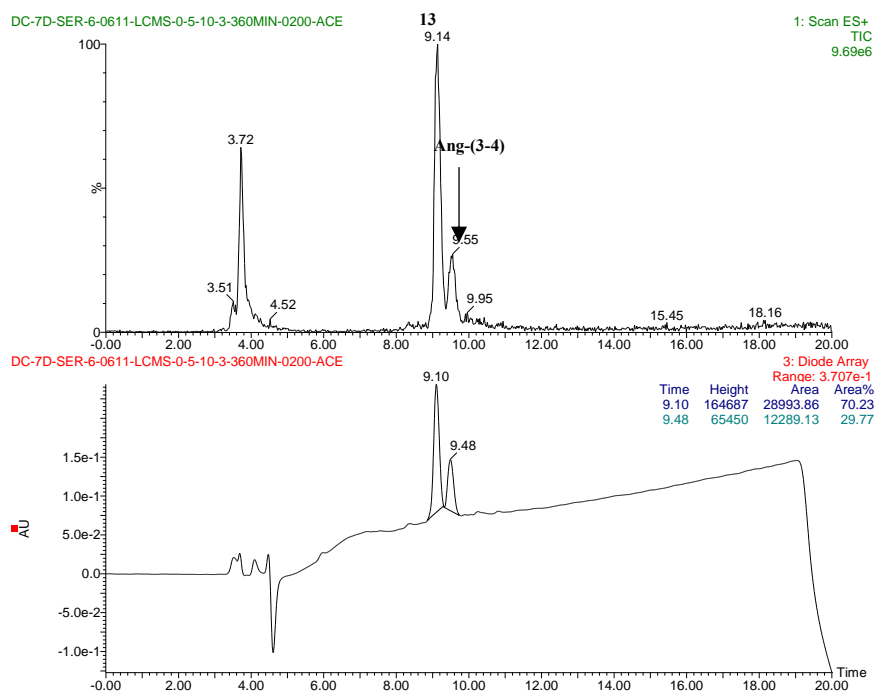
LC-MS chromatogram of **Ang-(1-7)-NH<sub>2</sub>** after incubation with DPP3 at 37 °C for 240 min in a linear gradient of 5-40% of MeCN in H<sub>2</sub>O containing 0.1% FA over 15 min.



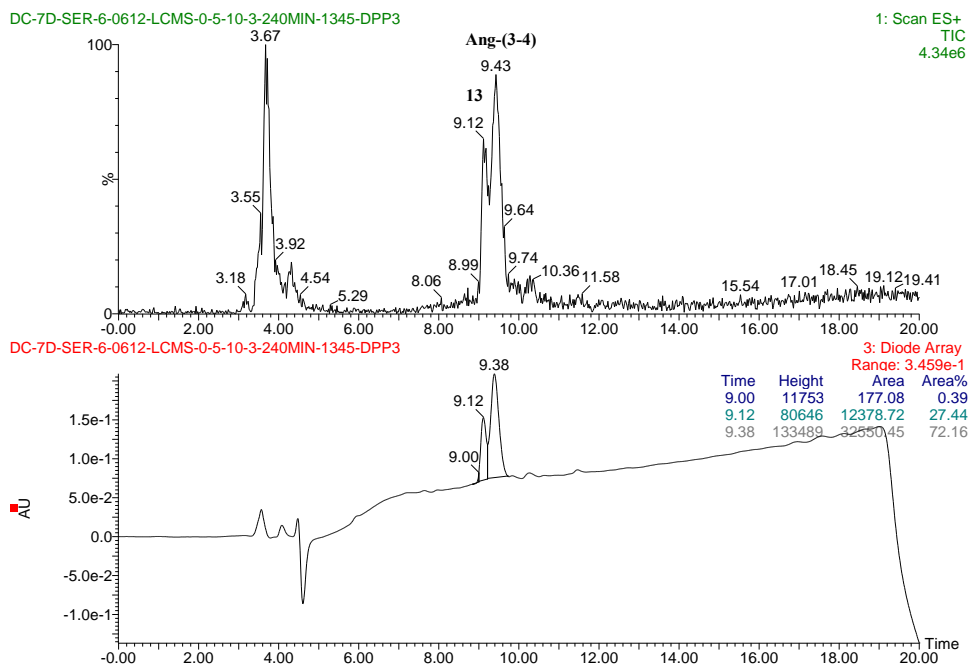
LC-MS chromatogram of **12** after incubation with ACE at 37 °C for 360 min in a linear gradient of 5-40% of MeCN in H<sub>2</sub>O containing 0.1% FA over 15 min.



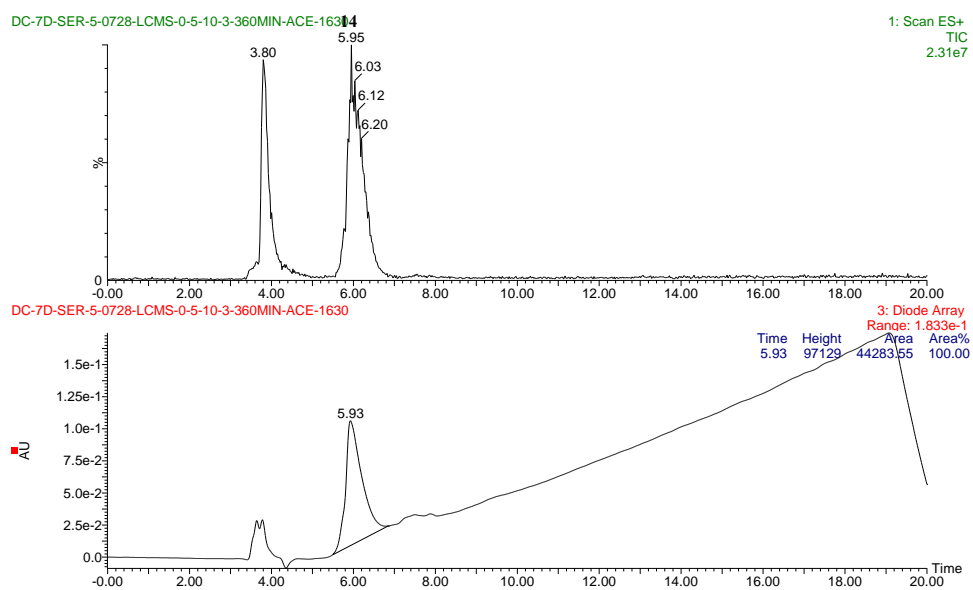
LC-MS chromatogram of **12** after incubation with DPP3 at 37 °C for 240 min in a linear gradient of 5-40% of MeCN in H<sub>2</sub>O containing 0.1% FA over 15 min.



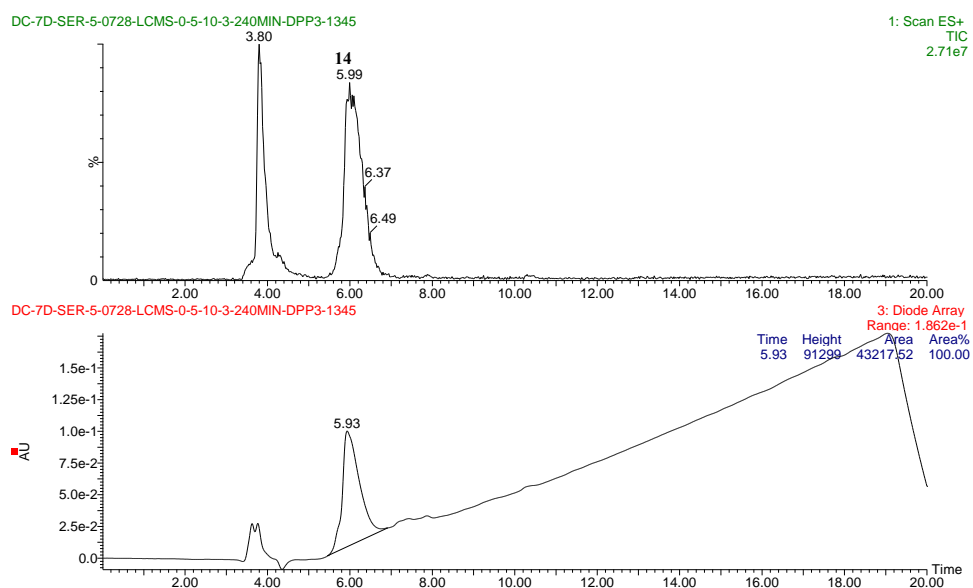
LC-MS chromatogram of **13** after incubation with ACE at 37 °C for 360 min in a linear gradient of 5–40% of MeCN in H<sub>2</sub>O containing 0.1% FA over 15 min.



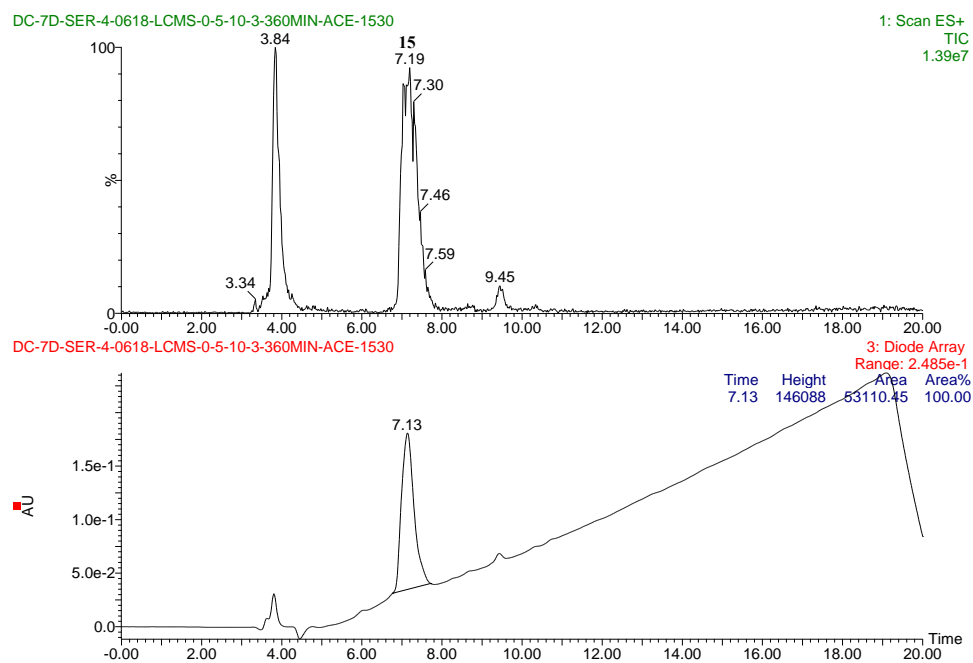
LC-MS chromatogram of **13** after incubation with DPP3 at 37 °C for 240 min in a linear gradient of 5–40% of MeCN in H<sub>2</sub>O containing 0.1% FA over 15 min.



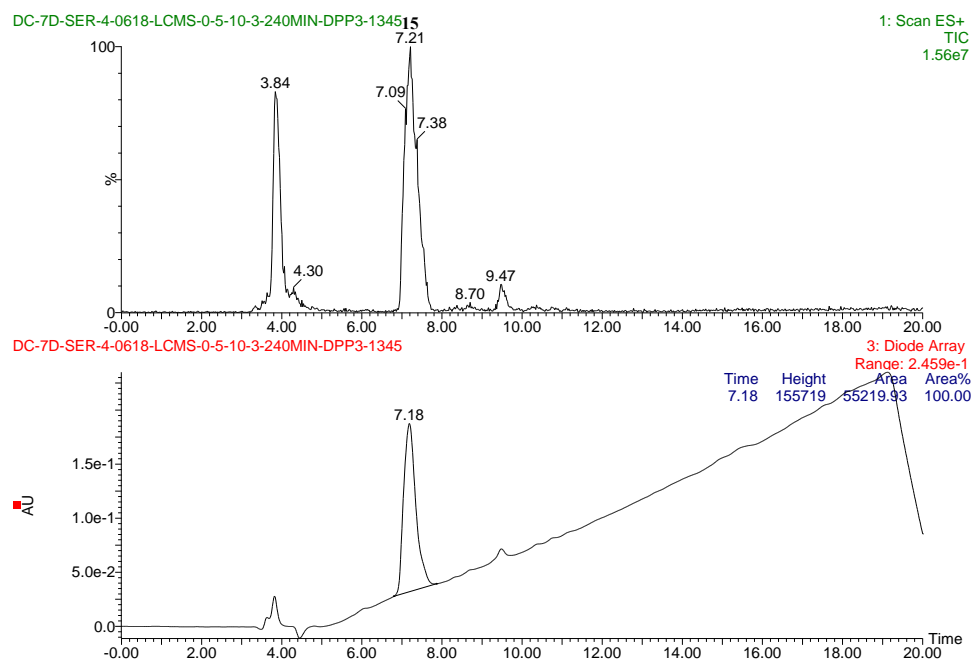
LC-MS chromatogram of **14** after incubation with ACE at 37 °C for 360 min in a linear gradient of 5-40% of MeCN in H<sub>2</sub>O over 15 min.



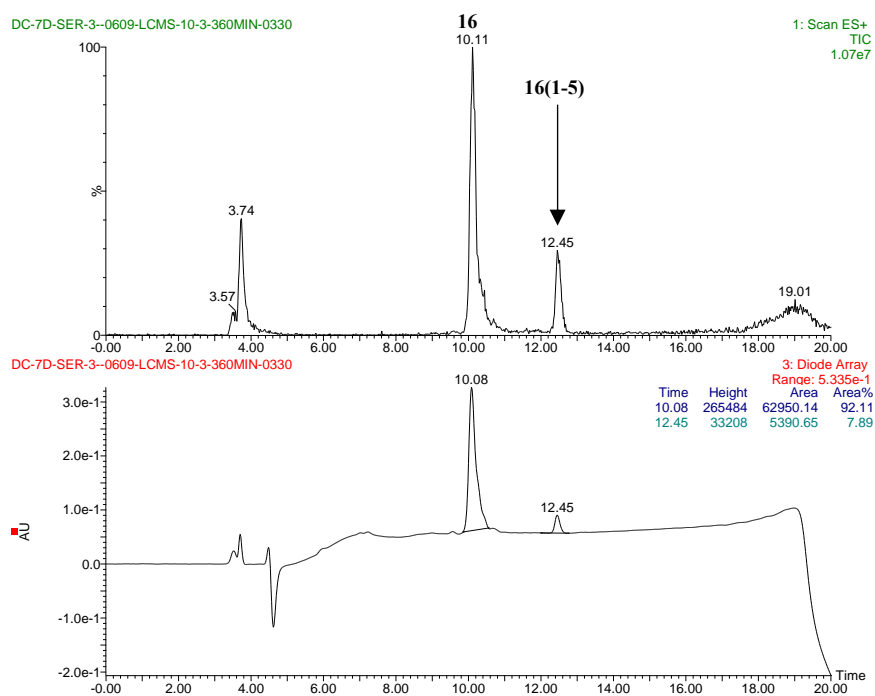
LC-MS chromatogram of **14** after incubation with DPP3 at 37 °C for 240 min in a linear gradient of 5-40% of MeCN in H<sub>2</sub>O over 15 min.



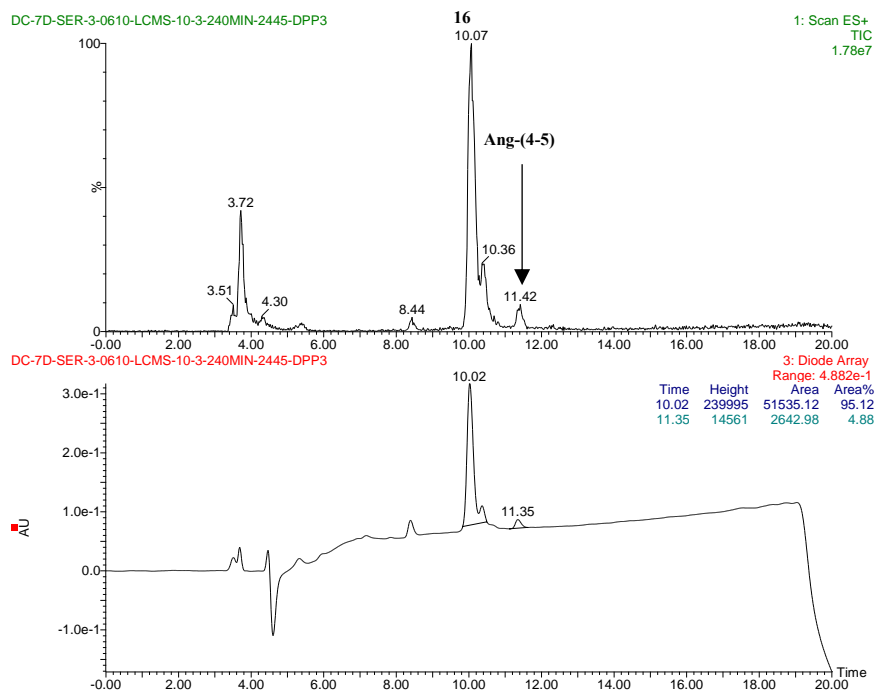
LC-MS chromatogram of **15** after incubation with ACE at 37 °C for 360 min in a linear gradient of 5-40% of MeCN in H<sub>2</sub>O over 15 min.



LC-MS chromatogram of **15** after incubation with DPP3 at 37 °C for 240 min in a linear gradient of 5-40% of MeCN in H<sub>2</sub>O over 15 min.

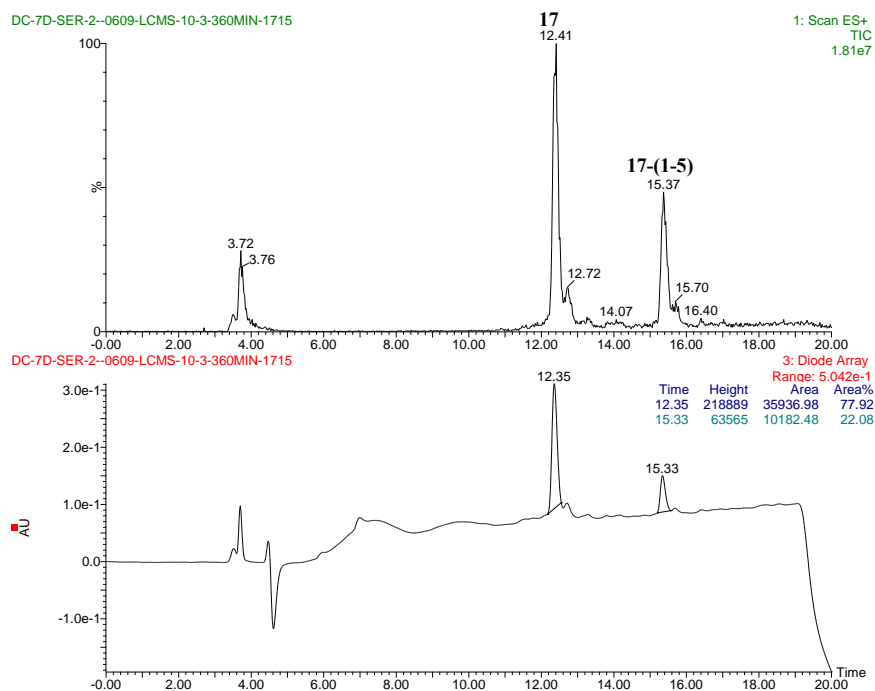


LC-MS chromatogram of **16** after incubation with ACE at 37 °C for 360 min in a linear gradient of 5-40% of MeCN in H<sub>2</sub>O containing 0.1% FA over 15 min.

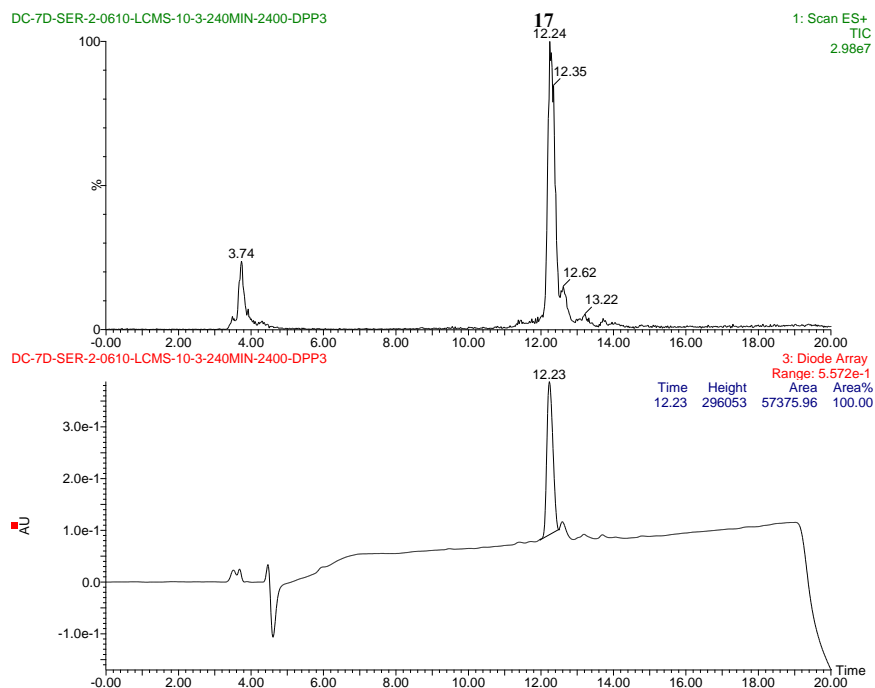


LC-MS chromatogram of **16** after incubation with DPP3 at 37 °C for 240 min in a linear gradient of 5-40% of MeCN in H<sub>2</sub>O containing 0.1% FA over 15 min.





LC-MS chromatogram of **17** after incubation with ACE at 37 °C for 360 min in a linear gradient of 5-40% of MeCN in H<sub>2</sub>O containing 0.1% FA over 15 min.



LC-MS chromatogram of **17** after incubation with DPP3 at 37 °C for 240 min in a linear gradient of 5-40% of MeCN in H<sub>2</sub>O containing 0.1% FA over 15 min.