Supporting Information

Palladium-Catalyzed Aminocarbonylation in Solid Phase Peptide Synthesis: A Method for Capping, Cyclization and Isotope Labeling

Anna Skogh,[†] Stig D. Friis,[‡] Troels Skrydstrup,[‡] and Anja Sandström*[,†]

[†]Department of Medicinal Chemistry, Organic Pharmaceutical Chemistry, BMC, Uppsala University, Box 574, SE-751 23 Uppsala, Sweden

[‡]Carbon Dioxide Activation Center (CADIAC), Interdisciplinary Nanoscience Center (iNANO) and Department of Chemistry, Aarhus University, Gustav Wieds Vej 14, 8000 Aarhus C, Denmark

*anja.sandstrom@orgfarm.uu.se

Table of Contents

General Information

Reagents and equipment

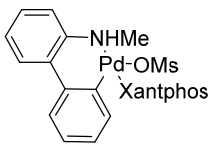
All reactions were set up under an ambient atmosphere (bench top) with subsequent flushing using argon or nitrogen (5-10 min). Reactions were set up using the commercially available COtube, with self-sealing PTFE/silicon septa. The septa were punctured twice using 21G needles. All reagents were acquired from commercial sources and used without further purification. Dry solvents were prepared according to standard literature procedures.[1] No special precautions were taken to keep other reagents dry or deoxygenated. Potassium fluoride was not dried prior to use. The Fmoc protected preloaded Wang PS resin **1a-h** (crosslinked with 1% DVB) was purchased from Sigma-Aldrich and the following loadings were used for calculating the isolated yields; Phe: 0.6 mmol/g; Pro: 0.6 mmol/g; Val: 0.7 mmol/g; Thr(tBu): 0.82 mmol/g; Tyr(tBu): 0.63 mmol/g; Asn(Trt): 0.62 mmol/g; Asp(OtBu): 0.52 mmol/g; Lys(Boc): 0.56 mmol/g. Peptides were synthesized on Biotage® Initiator+ Alstra™ Automated Microwave Peptide Synthesizer (Biotage AB, Uppsala, Sweden). Rink Amide PS resin was purchased from IRIS and the following loading was used for calculating the isolated yield for the peptides; 0.68 mmol/g. Preparative RP-HPLC was performed on a system equipped with a Macherey-Nagel Nucleodur C18 HTec (21 mm × 125 mm, particle size 5 µm), with a H_2O/MeCN gradient with 0.1% TFA as mobile phase and with UV detection at 220 or 254 nm. The purity of each of the peptides were determined with RP-HPLC using the columns Restek Alure biphenyl (50 mm × 4.6 mm, particle size 5µm) and Thermo Hypersil Fluophase RP (50 mm × 4.6 mm, particle size 5µm) with a H_2O/MeCN gradient with 0.1% TFA and UV detection at 220 nm.

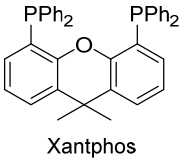
Analytical

^1H and ^{13}C NMR spectra were recorded at 400 and 101 MHz, respectively, using DMSO-*d6*, $CDCl_3$ or CD_3OD as a solvent. Chemical shifts are reported in ppm downfield to TMS ($\delta = 0$) and referenced via the residual solvent signal, using the following peak pattern abbreviation: br, broad; s, singlet; d, doublet; t, triplet; q, quartet; pent, pentet; sext, sextet; sept, septet; m, multiplet; dd, doublet of doublet; dt, doublet of triplets; ddd, doublet of doublet of doublets; ddt, doublet of doublet of triplets, and td, triplet of doublets. LC/MS was performed on an instrument equipped with a C18 column (50 × 3.0 mm, particle size 2.6 µm, pore size 100 Å). High resolution molecular mass (HRMS) was determined on a LC TOF (ES). Optical rotation was obtained on a Rudolph Research Analytical Autopol II Automatic Polarimeter and the concentration *c* is given as g/100 mL in the specified solvent. Exact mass for peptides **6c** and [^{13}C]-**6c*** were determinate with a Q-ToF instrument (Synapt G2-S, from Waters Corporation) equipped with an electrospray ion source used in positive ionization mode. For chromatographic separation, an Acquity C18 BEH column (50 × 2.1 mm, particle size 1.7 µm; Waters Corp.) was used, with a H_2O/MeCN gradient with 0.1% formic acid as mobile phase.

Experimental procedures and characterization data for compounds **2**, **3** and [^{13}C]-**3***

Palladacycle precatalyst (2)

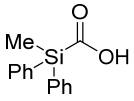




Xantphos

Prepared as described by Friis *et al* and Bruno *et al*.[2,3] Yellow solid (1.90 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, *J* = 7.7, 2H), 7.56 (d, *J* = 7.6, 1H), 7.50-6.81 (m, 28H), 6.73 (q, *J* = 7.2 Hz, 1H), 6.68-6.58 (m, 1H), 6.47 (d, *J* = 7.6, 1.2 Hz, 1H), 4.35 (s, 1H), 2.72 (s, 3H), 2.10 (d, *J* = 5.7 Hz, 3H), 1.69 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.3, 154.2, 140.4, 137.3, 135.8, 133.7, 133.4, 133.3, 132.5, 132.4, 131.1, 129.1, 128.9, 128.9, 128.4, 127.9, 126.7, 126.2, 125.2, 125.1, 120.2, 116.9, 40.2, 39.4, 36.1, 28.5 (Observed complexity due to P-C splitting and restricted rotation).

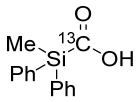
Methyldiphenylsilacarboxylic acid (3)



$C_{14}H_{14}O_2Si$
MW: 242.3

Prepared as described by Friis *et al.*[4] White solid (4.2 g, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72-7.68 (m, 4H), 7.50-7.39 (m, 6H), 0.86 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.3, 135.1, 131.6, 130.6, 128.3, -5.1. HRMS (ES) m/z calcd for [M–H+]: $C_{14}H_{13}O_2Si$ 241.0685 found 241.0686.

[^{13}C]-Methyldiphenylsilacarboxylic acid ([^{13}C]-3*)



$C_{13}{}^{13}CH_{14}O_2Si$
MW: 243.3

Prepared as described by Friis *et al.*[4] ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.71 (bs, 1H), 7.84-7.65 (m, 4H), 7.59-7.37 (m, 6H), 0.99-0.79 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.9 (^{13}C-enriched), 135.1, 131.6, 130.6, 128.3, -5.1. HRMS (ES) m/z calcd for [M–H+]: $C_{13}{}^{13}CH_{13}O_2Si$ 242.0718 found 242.0687.

Experimental procedures and characterization data for compounds **4a-h**

Procedure used in Table 1: Scope of amines

General procedure A: A 6 mL disposable syringe fitted with porous polyethylene filter was charged with 0.3 mmol preloaded Fmoc-Amino Acid Wang PS resin **1a-h** (crosslinked with 1 % DVB). The resin was allowed to swell in DMF for 30 min before treated with 20% Pip in DMF (2 × 3 mL, 2+10 min), and thereafter washed with DMF (3 × 5 mL), DCM (3 × 5 mL), MeOH (3 × 5 mL) and diethylether (3 × 5 mL). The resin was dried under vacuum to ensure complete removal of solvent. The Fmoc deprotected preloaded Wang-PS resin **1a-h** (1 equiv, 0.3 mmol) was transferred to a 8 mL vial charged with 4-iodoanisole (4 equiv, 1.2 mmol, 281 mg), $MePh_2SiCOOH$ **3** (2 equiv, 0.6 mmol, 145 mg), KF (2 equiv, 0.6 mmol, 35 mg), palladacycle precatalyst **2** (2 mol%, 0.006 mmol, 6 mg) and TEA (4 equiv, 1.2 mmol, 167 µL). The vial was sealed with a screw cap fitted with a Teflon® seal and evacuated, and backfilled with argon/nitrogen gas. Thereafter, dry DMF (4 mL) was added through the self-sealing septa by a syringe using a 21G needle. The needle was removed and the reaction was allowed to agitate for 15 h at r.t. Next, excess pressure was released using a needle and thereafter the resin was separated by filtration and washed with DMF (3 × 5mL), DCM (3 × 5mL) and MeOH (3 × 5mL). The resin was dried under vacuum and the final product was thereafter cleaved from the resin by treatment with 95% aqueous TFA (2 mL) and TES (100 µL) followed by agitation for 2 h at r.t. The resin was filtered off and washed with TFA (3 × 300µL). The filtrate was collected in a centrifuge tube and concentrated in a stream of argon/nitrogen to a volume of < 2 mL. The product was precipitated by the addition of cold diethylether (12 mL), collected by centrifugation, washed with diethylether (3 × 10 mL) and dried in a stream of argon/nitrogen and in vacuum overnight. The final products showed a purity between ca. 87-100% according to ^{1}H NMR analysis (mainly co-crystallized diethylether).

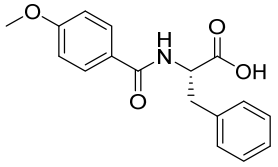
Procedure used in Table 1: Optical rotation measurements

General procedure B:

A 6 mL disposable syringe fitted with porous polyethylene filter was charged with preloaded Fmoc-Amino Acid Wang PS resin **1a-h** (crosslinked with 1 % DVB). The resin was allowed to swell in DMF for 30 min before treated with 20% Pip in DMF (2 × 3 mL, 2+10 min). The peptide coupling was performed in DMF (4 mL) using *p*-anisic acid (5 equiv, 1.5 mmol, 228 mg), HBTU (5 equiv, 1.5 mmol, 570 mg), and DIPEA (10 equiv, 30 mmol, 0.52 mL) followed by agitation for 30 min in r.t. The coupling was repeated twice to ensure complete coupling. The resin was washed with DMF (3 × 5mL), DCM (3 × 5mL), MeOH (3 × 5mL) and diethylether (3 × 5mL). The resin was dried under vacuum and the final product was thereafter cleaved from the resin by treatment with 95% aqueous TFA (2 mL) and TES (100 µL) followed by agitation for 2 h at r.t. The resin was filtered off and washed with TFA (3 × 300µL). The filtrate was collected in a centrifuge tube and concentrated in a stream of argon/nitrogen to a volume of < 2 mL. The product was precipitated by the addition of cold diethylether (12 mL), collected by centrifugation, washed with diethylether (3 × 10 mL) and dried in a stream of

argon/nitrogen and in vacuum overnight. Full conversion of the amino acid nucleophile was observed according to ^{1}H-NMR analysis.

(4-Methoxybenzoyl)-*L*-phenylalanine (4a)[5]



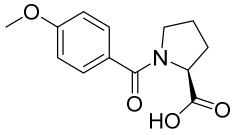
$C_{17}H_{17}NO_4$
MW: 299.3

Following General procedure A (here in detail): In a 6 mL disposable syringe fitted with porous polyethylene filter 500 mg of Fmoc-Phe Wang PS resin **1a** (1 equiv, 0.3 mmol, 0.6 mmol/g) was swelled in DMF (5 mL) for 30 min followed by Fmoc deprotection using 20% Pip in DMF (3 mL) for 2 min and a second time for 10 min. The resin was washed with DMF (3 × 5 mL), DCM (3 × 5 mL), MeOH (3 × 5 mL) and diethylether (3 × 5 mL). The resin was dried under vacuum to ensure complete removal of solvent.

The Fmoc deprotected H-Phe-Wang PS resin **1a** was transferred to a 8 mL vial charged with 281 mg 4-iodoanisole (4 equiv, 1.2 mmol), 145 mg $MePh_2SiCOOH$ **3** (2 equiv, 0.6 mmol), 35 mg KF (2 equiv, 0.6 mmol), 6 mg palladacycle precatalyst **2** (2 mol%, 0.006 mmol) and 167 µL TEA (4 equiv, 1.2 mmol). The vial was sealed with a screw cap fitted with a Teflon® seal and evacuated, and backfilled with argon/nitrogen gas. Thereafter, 4 mL dry DMF was added through the self-sealing septa by a syringe using a 21G needle. The needle was removed and the reaction was allowed to agitate for 15 h at r.t. Next, excess pressure was released using a needle and thereafter the resin was transferred to a 6 mL disposable syringe fitted with porous polyethylene filter and washed with DMF (3 × 5mL), DCM (3 × 5mL) and MeOH (3 × 5mL) and thereafter the resin was dried under vacuum.

The final product **4a** was cleaved from the resin by treatment with 2 mL 95% aqueous TFA and 100 µL TES for 2 h at r.t. After filtration the resin was washed with TFA (3 × 300µL). The filtrate was collected in a centrifuge tube and concentrated in a stream of argon/nitrogen to a volume of < 2 mL. The product was precipitated by the addition of cold diethylether (12 mL), collected by centrifugation, washed with diethylether (3 × 10 mL) and dried in a stream of argon/nitrogen and in vacuum overnight. The title compound **4a** (85 mg, 95%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.71 (s, 1H), 8.53 (d, J = 8.1 Hz, 1H), 7.86-7.74 (m, 2H), 7.33-7.29 (m, 2H), 7.26 (t, J = 7.5 Hz, 2H), 7.17 (t, J = 7.5 Hz, 1H), 7.01-6.94 (m, 2H), 4.60 (ddd, J = 10.6, 8.1, 4.5 Hz, 1H), 3.79 (s, 3H), 3.18 (dd, J = 13.7, 4.5 Hz, 1H), 3.06 (dd, J = 13.7, 10.6 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.4, 165.8, 161.7, 138.3, 129.2, 129.0, 128.2, 126.3, 126.1, 113.4, 55.3, 54.2, 36.3. HRMS (ES) m/z calcd for [M+H+]: $C_{17}H_{18}NO_4$ 300.1230 found 300.1229. Corresponding product was synthesized following general procedure B and the products had an value of $[\alpha]_D^{20}$ – 41.6 (*c* 1.0, MeOH) and $[\alpha]_D^{20}$ – 42.0 (*c* 1.0, MeOH), respectively.

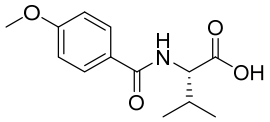
(4-Methoxybenzoyl)-*L*-proline (4b)[6]



$C_{13}H_{15}NO_4$
MW: 249.3

General procedure A was followed using Fmoc deprotected Pro-Wang PS resin **1b** (0.6 mmol/g, 1 equiv, 0.3 mmol) and 4-iodoanisole (4 equiv, 1.2 mmol, 281 mg). The title compound **4b** (71 mg, 94%) was obtained as a white solid and isolated as a 6:94 cis/trans rotamers mixture according to ^{1}H NMR analysis. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, *J* = 8.7 Hz, 2H), 6.90 (d, *J* = 8.7 Hz, 2H), 6.52 (s, 2H), 4.70-4.64 (m, 1H), 3.83 (s, 3H), 3.62 (t, *J* = 6.6 Hz, 2H), 2.33-2.13 (m, 2H), 2.06-1.96 (m, 1H), 1.92-1.81 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.8, 171.4, 161.7, 129.7, 127.2, 113.8, 77.2, 60.5, 55.5, 50.9, 28.4, 25.5. HRMS (ES) m/z calcd for [M+H+]: $C_{13}H_{16}NO_4$ 250.1074 found 250.1070. Corresponding product was synthesized following general procedure B and the products had an value of $[\alpha]_D^{20}$ – 57.4 (*c* 1.0, MeOH) and $[\alpha]_D^{20}$ – 56.9 (*c* 1.0, MeOH), respectively.

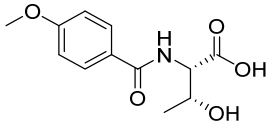
(4-Methoxybenzoyl)-*L*-valine (4c)[5]



$C_{13}H_{17}NO_4$
MW: 251.3

General procedure A was followed using Fmoc deprotected Val-Wang PS resin **1c** (0.7 mmol/g, 1 equiv, 0.3 mmol) and 4-iodoanisole (4 equiv, 1.2 mmol, 281 mg). The title compound **4c** (68 mg, 90%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.23 (d, *J* = 8.2 Hz, 1H), 7.95-7.83 (m, 2H), 7.09-6.92 (m, 2H), 4.26 (dd, *J* = 8.2, 7.0 Hz, 1H), 3.81 (s, 3H), 2.17 (q, *J* = 6.8 Hz, 1H), 0.96 (dd, *J* = 7.5, 6.8 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.9, 166.2, 161.7, 129.5, 126.3, 113.4, 58.3, 55.4, 29.5, 19.3, 18.9. HRMS (ES) m/z calcd for [M+H+]: $C_{13}H_{18}NO_4$ 252.1236 found 252.1249.

(4-Methoxybenzoyl)-*L*-threonine (4d)[7]

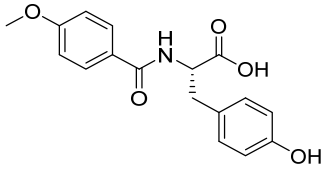


$C_{12}H_{15}NO_5$
MW: 253.3

General procedure A was followed using Fmoc deprotected Thr(tBu)-Wang PS resin **1d** (0.82 mmol/g, 1 equiv, 0.3 mmol) and 4-iodoanisole (4 equiv, 1.2 mmol, 281 mg). The title compound **4d** (72 mg, 95%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.94-7.81 (m, 3H), 7.03 (d, *J* = 8.8 Hz, 2H), 4.41 (dd, *J* = 8.5, 3.7 Hz, 1H), 4.23-4.14 (m, 1H), 3.82 (s, 3H), 1.14 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.3, 166.1, 161.8, 129.2, 126.2, 113.6, 66.6, 58.6, 55.4, 20.5. HRMS (ES) m/z calcd for [M+H+]: $C_{12}H_{16}NO_5$ 254.1028 found 254.1028. Corresponding product was synthesized following general procedure B and

both products had an equivalent value of $[\alpha]_D^{25}$ + 40.0 (*c* 1.0, Acetone).

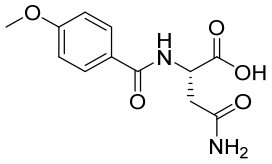
(4-Methoxybenzoyl)-*L*-tyrosine (4e)[5]



$C_{17}H_{17}NO_5$
MW: 315.3

General procedure A was followed using Fmoc deprotected Tyr(tBu)-Wang PS resin **1e** (0.63 mmol/g, 1 equiv, 0.3 mmol) and 4-iodoanisole (4 equiv, 1.2 mmol, 281 mg). The title compound **4e** (82 mg, 87%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 12.62 (bs, 1H), 9.16 (s, 1H), 8.45 (d, *J* = 8.1 Hz, 1H), 7.79 (d, *J* = 8.8 Hz, 2H), 7.08 (d, *J* = 8.4 Hz, 2H), 6.98 (d, *J* = 8.8 Hz, 2H), 6.63 (d, *J* = 8.4 Hz, 2H), 4.49 (ddd, *J* = 10.6, 8.1, 4.5 Hz, 1H), 3.04 (dd, *J* = 13.7, 4.5 Hz, 1H), 2.93 (dd, *J* = 13.7, 10.6 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 173.5, 165.8, 161.6, 155.8, 130.0, 129.2, 128.2, 126.2, 114.9, 113.4, 55.3, 54.5, 35.5. MS calcd for [M+H+]: $C_{17}H_{18}NO_5$ 316.1 found 316.2. Corresponding product was synthesized following general procedure B and the products had an value of $[\alpha]_D^{24}$ – 40.0 (*c* 1.0, MeOH) and $[\alpha]_D^{24}$ – 39.8 (*c* 1.0, MeOH), respectively.

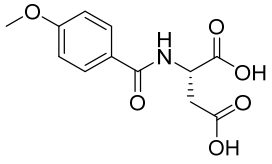
(4-Methoxybenzoyl)-*L*-asparagine (4f)



$C_{12}H_{14}N_2O_5$
MW: 266.3

General procedure A was followed using Fmoc deprotected Asn(Trt)-Wang PS resin **1f** (0.62 mmol/g, 1 equiv, 0.3 mmol) and 4-iodoanisole (4 equiv, 1.2 mmol, 281 mg). The title compound **4f** (74 mg, 93%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.48 (d, *J* = 7.8 Hz, 1H), 7.86-7.79 (m, 2H), 7.38 (s, 1H), 7.03-6.96 (m, 2H), 6.91 (d, *J* = 14.0 Hz, 1H), 4.70 (ddd, *J* = 14.0, 7.8, 5.6 Hz, 1H), 2.68 (dd, *J* = 15.4, 5.6 Hz, 1H), 2.59 (dd, *J* = 15.4, 7.8 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 173.6, 172.0, 166.0, 162.2, 129.6, 126.6, 114.0, 55.8, 50.0, 37.0. HRMS (ES) m/z calcd for [M+H+]: $C_{12}H_{15}N_2O_5$ 267.0975 found 267.0964.

(4-Methoxybenzoyl)-*L*-aspartic acid (4g)

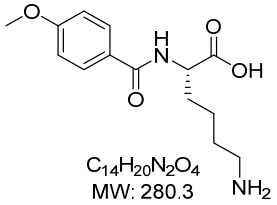


$C_{12}H_{13}NO_6$
MW: 267.2

General procedure A was followed using Fmoc deprotected Asp(OtBu)-Wang PS resin **1g** (0.67 mmol/g, 1 equiv, 0.3 mmol) and 4-iodoanisole (4 equiv, 1.2 mmol, 281 mg). The title compound **4g** (72 mg, 90%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 12.57 (bs,

2H), 8.57 (d, *J* = 7.8 Hz, 1H), 7.83 (d, *J* = 8.8 Hz, 2H), 7.01 (d, *J* = 8.8 Hz, 2H), 4.77-4.69 (m, 1H), 3.81 (s, 3H), 2.83 (dd, *J* = 16.4, 5.8 Hz, 1H), 2.72-2.65 (m, 1H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 172.7, 171.8, 165.5, 161.7, 129.2, 126.0, 113.5, 55.4, 49.3, 35.9. HRMS (ES) m/z calcd for [M+H+]: $C_{12}H_{14}NO_6$ 268.0821 found 268.0830. Corresponding product was synthesized following general procedure B and the products had an value of $[\alpha]_D^{21}$ – 4.2 (*c* 1.0, MeOH) and $[\alpha]_D^{21}$ – 4.5 (*c* 1.0, MeOH), respectively.

(4-Methoxybenzoyl)-*L*-lysine (4h)



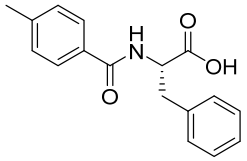
General procedure A was followed using Fmoc deprotected Lys(Boc)-Wang PS resin **1h** (0.56 mmol/g, 1 equiv, 0.3 mmol) and 4-iodoanisole (4 equiv, 1.2 mmol, 281 mg). The title compound **4h** (76 mg, 91%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.44 (d, *J* = 7.8 Hz, 1H), 7.89 (d, *J* = 8.8 Hz, 2H), 7.82 (s, 2H), 7.01 (d, *J* = 8.8 Hz, 2H), 4.42-4.32 (m, *J* = 8.6, 5.5 Hz, 1H), 3.82 (s, 3H), 2.90-2.65 (m, 2H), 1.88-1.73 (m, 2H), 1.63-1.52 (m, *J* = 13.6, 7.1 Hz, 2H), 1.48-1.34 (m, 2H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 173.9, 166.1, 161.7, 129.4, 126.2, 113.5, 55.4, 52.4, 38.7, 30.1, 26.6, 22.9. HRMS (ES) m/z calcd for [M+H+]: $C_{14}H_{20}N_2O_4$ 281.1496 found 281.1489. Corresponding product was synthesized following general procedure B and the products had an value of $[\alpha]_D^{25}$ – 7.1 (*c* 1.0, MeOH) and $[\alpha]_D^{25}$ – 7.4 (*c* 1.0, MeOH), respectively.

Experimental procedures and characterization data for compounds **5a-r**

Procedure used in Scheme 1: Scope of (hetero)aryl iodides

General procedure C: A 6 mL disposable syringe fitted with porous polyethylene filter was charged with Fmoc-Phe-Wang-PS resin **1a** (0.6 mmol/g, 1 equiv, 0.3 mmol, 500 mg, crosslinked with 1 % DVB). The resin was allowed to swell in DMF for 30 min before treated with 20% Pip in DMF (2 × 3 mL, 2+10 min), and thereafter washed with DMF (3 × 5 mL), DCM (3 × 5 mL), MeOH (3 × 5 mL) and diethylether (3 × 5 mL). The resin was dried under vacuum to ensure complete removal of solvent. The Phe-Wang-PS resin **1a** (1 equiv, 0.3 mmol) was transferred to a 8 mL vial charged with (hetero)aryl iodide (4 equiv, 1.2 mmol), $MePh_2SiCOOH$ **3** (2 equiv, 0.6 mmol, 145 mg or 3 equiv, 0.9 mmol, 218 mg), KF (2 equiv, 0.6 mmol, 35 mg or 3 equiv, 0.9 mmol, 52 mg), palladacycle precatalyst **2** (2 mol%, 0.006 mmol, 6 mg or 5 mol%, 0,015 mmol, 15 mg) and TEA (4 equiv, 1.2 mmol, 167 µL). The vial was sealed with a screw cap fitted with a Teflon® seal and evacuated, and backfilled with argon/nitrogen gas. Thereafter, dry DMF (4 mL) was added through the self-sealing septa by a syringe using a 21G needle. The needle was removed and the reaction was allowed to agitate for 15 h at r.t. or at 45°C using ultrasonication. Next, excess pressure was released using a needle and thereafter the resin was separated by filtration and washed with DMF (3 × 5 mL), DCM (3 × 5 mL) and MeOH (3 × 5 mL). The resin was dried under vacuum and the final product was thereafter cleaved from the resin by treatment with 95% aqueous TFA (2 mL) and TES (100 µL) followed by agitation for 2 h at r.t. The resin was filtered off and washed with TFA (3 × 300µL). The filtrate was collected in a centrifuge tube and concentrated in a stream of argon/nitrogen to a volume of < 2 mL. The product was precipitated by the addition of cold diethylether/*tert*-butylmehylether (12 mL), collected by centrifugation, washed with diethylether (3 × 10 mL) and dried in a stream of argon/nitrogen and in vacuum overnight. For those compounds that did not precipitated in diethylether/ *tert*-butyl mehyl ether, the filtrate was concentrated to completion and dissolved in H_2O/MeCN and lyophilized. The final products showed a purity between ca. 92-100% according to ^1H NMR analysis (mainly co-crystallized diethylether).

(4-Methylbenzoyl)-*L*-phenylalanine (5a)[8]



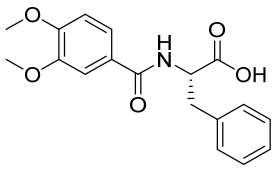
$C_{17}H_{17}NO_3$
MW: 283.3

Following General procedure C (here in detail): In a 6 mL disposable syringe fitted with porous polyethylene filter 500 mg of Fmoc-Phe Wang PS resin **1a** (1 equiv, 0.3 mmol, 0.6 mmol/g) was swelled in DMF (5 mL) for 30 min followed by Fmoc deprotection using 20% Pip in DMF (3 mL) for 2 min and a second time for 10 min. The resin was washed with DMF (3 × 5 mL), DCM (3 × 5 mL), MeOH (3 × 5 mL) and diethylether (3 × 5 mL). The resin was dried under vacuum to ensure complete removal of solvent.

The Fmoc deprotected H-Phe-Wang PS resin **1a** was transferred to a 8 mL vial charged with 262 mg 4-iodotoluene (4 equiv, 1.2 mmol), 145 mg $MePh_2SiCOOH$ **3** (2 equiv, 0.6 mmol), 35 mg KF (2 equiv, 0.6 mmol), 6 mg palladacycle precatalyst **2** (2 mol%, 0.006 mmol) and 167 µL TEA (4 equiv, 1.2 mmol). The vial was sealed with a screw cap fitted with a Teflon® seal and evacuated, and backfilled with argon/nitrogen gas. Thereafter, 4 mL dry DMF was added through the self-sealing septa by a syringe using a 21G needle. The needle was removed and the reaction was allowed to agitate for 15 h at r.t. Next, excess pressure was released using a needle and thereafter the resin was transferred to a 6 mL disposable syringe fitted with porous polyethylene filter and washed with DMF (3 × 5mL), DCM (3 × 5mL) and MeOH (3 × 5mL) and thereafter the resin was dried under vacuum.

The final product **5a** was cleaved from the resin by treatment with 2 mL 95% aqueous TFA and 100 µL TES for 2 h at r.t. After filtration the resin was washed with TFA (3 × 300µL). The filtrate was collected in a centrifuge tube and concentrated in a stream of argon/nitrogen to a volume of < 2 mL. The product was precipitated by the addition of cold diethylether (12 mL), collected by centrifugation, washed with diethylether (3 × 10 mL) and dried in a stream of argon/nitrogen and in vacuum overnight. The title compound **5a** (79 mg, 93%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 (d, J = 8.1 Hz, 1H), 7.70 (d, J = 8.1 Hz, 2H), 7.34-7.12 (m, 7H), 4.60 (ddd, J = 10.6, 8.1, 4.5 Hz, 1H), 3.18 (dd, J = 13.7, 4.5 Hz, 1H), 3.06 (dd, J = 13.7, 10.6 Hz, 1H), 2.34 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.3, 166.2, 141.2, 138.2, 131.1, 129.1, 128.8, 128.2, 127.4, 126.3, 54.2, 36.3, 21.0. HRMS (ES) m/z calcd for [M+H+]: $C_{17}H_{18}NO_3$ 284.1281 found 284.1281.

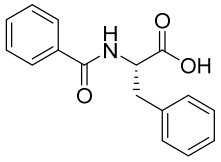
(3,4-Dimethoxybenzoyl)-*L*-phenylalanine (5b)[8]



$C_{18}H_{19}NO_5$
MW: 329.4

General procedure C was followed using Fmoc deprotected Phe-Wang PS resin **1a** (0.6 mmol/g, 1 equiv, 0.3 mmol, 500 mg) and 4-iodo-1,2-dimethoxybenzene (4 equiv, 1.2 mmol, 317 mg). The title compound **6b** (84 mg, 85%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (d, J = 8.2 Hz, 1H), 7.44 (dd, J = 8.2, 2.0 Hz, 1H), 7.36 (d, J = 2.0 Hz, 1H), 7.33-7.29 (m, 2H), 7.29-7.23 (m, 2H), 7.21-7.14 (m, 1H), 7.00 (d, J = 8.5 Hz, 1H), 4.64-4.55 (m, 1H), 3.79 (s, 3H), 3.78 (s, 3H), 3.18 (dd, J = 13.7, 4.6 Hz, 1H), 3.06 (dd, J = 13.7, 10.6 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.3, 165.8, 151.3, 148.1, 138.2, 129.1, 128.2, 126.3, 126.2, 120.6, 110.8, 110.8, 109.5, 55.6, 55.5, 54.2, 36.4. HRMS (ES) m/z calcd for [M+H+]: $C_{18}H_{20}NO_5$ 330.1341 found 330.1341.

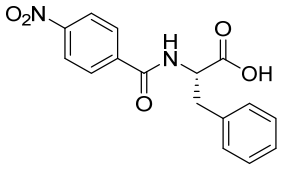
Benzoyl-*L*-phenylalanine (5c)[9]



$C_{16}H_{15}NO_3$
MW: 269.3

General procedure C was followed using Fmoc deprotected Phe-Wang PS resin **1a** (0.6 mmol/g, 1 equiv, 0.3 mmol, 500 mg) and iodobenzene (4 equiv, 1.2 mmol, 134 µL). The title compound **6c** (76 mg, 94%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) 8.69 (d, *J* = 8.1 Hz, 1H), 7.83-7.76 (m, 2H), 7.55-7.41 (m, 3H), 7.34-7.14 (m, 5H), 4.62 (ddd, *J* = 10.6, 8.1, 4.5 Hz, 1H), 3.19 (dd, *J* = 13.7, 4.5 Hz, 1H), 3.11-3.02 (m, 1H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 173.2, 166.4, 138.2, 133.9, 131.4, 129.0, 128.2, 128.2, 127.3, 126.3, 54.2, 36.2. HRMS (ES) m/z calcd for [M+H+]: $C_{16}H_{16}NO_3$ 270.1130 found 270.1134.

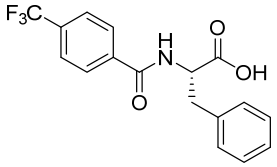
(4-Nitrobenzoyl)-*L*-phenylalanine (5d)



$C_{16}H_{14}N_2O_5$
MW: 314.3

General procedure C was followed using Fmoc deprotected Phe-Wang PS resin **1a** (0.6 mmol/g, 1 equiv, 0.3 mmol, 500 mg) and 1-iodo-4-nitrobenzene (4 equiv, 1.2 mmol, 299 mg). The title compound **5d** (78 mg, 83%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 9.09 (d, *J* = 8.1 Hz, 1H), 8.31 (d, *J* = 8.9 Hz, 2H), 8.01 (d, *J* = 8.9 Hz, 2H), 7.33-7.22 (m, 4H), 7.21-7.15 (m, 1H), 4.65 (ddd, *J* = 10.6, 8.1, 4.5 Hz, 1H), 3.22 (dd, *J* = 13.7, 4.5 Hz, 1H), 3.07 (dd, *J* = 13.7, 10.6 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 172.8, 164.8, 149.1, 139.5, 138.0, 129.0, 128.8, 128.2, 126.4, 123.6, 54.4, 36.2. HRMS (ES) m/z calcd for [M+H+]: $C_{16}H_{15}N_2O_5$ 315.0981 found 315.0983.

(4-(Trifluoromethyl)benzoyl)-*L*-phenylalanine (5e)[5]

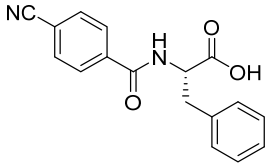


$C_{17}H_{14}F_3NO_3$
MW: 337.3

General procedure C was followed using Fmoc deprotected Phe-Wang PS resin **1a** (0.6 mmol/g, 1 equiv, 0.3 mmol, 500 mg) and 1-iodo-4-(trifluoromethyl)benzene (4 equiv, 1.2 mmol, 176 µL). The title compound **5e** (90 mg, 89%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.98 (d, *J* = 8.1 Hz, 1H), 7.98 (d, *J* = 8.2 Hz, 2H), 7.84 (d, *J* = 8.2 Hz, 2H), 7.33-7.23 (m, 4H), 7.22-7.15 (m, 1H), 4.67 (ddd, *J* = 10.6, 8.1, 4.5 Hz, 1H), 3.22 (dd, *J* = 13.7, 4.5 Hz, 1H), 3.08 (dd, *J* = 13.7, 10.6 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 173.0, 165.3,

138.1, 137.7, 131.3 (q, J_{CF_3} = 32.6 Hz), 129.1, 128.3, 128.2, 126.4, 125.4 (q, J_{CF_3} = 3.8 Hz), 124.0 (q, J_{CF_3} = 272.1 Hz), 54.32, 36.27. HRMS (ES) m/z calcd for [M+H+]: $C_{17}H_{15}F_3NO_3$ 338.1004 found 338.1015.

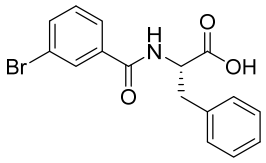
(4-Cyanobenzoyl)-*L*-phenylalanine (5f)



$C_{17}H_{14}N_2O_3$
MW: 294.3

General procedure C was followed using Fmoc deprotected Phe-Wang PS resin **1a** (0.6 mmol/g, 1 equiv, 0.3 mmol, 500 mg) and 4-iodobenzonitrile (4 equiv, 1.2 mmol, 275 mg). The title compound **5f** (79 mg, 90%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.01 (d, J = 8.2 Hz, 1H), 8.04-7.86 (m, 4H), 7.36-7.22 (m, 4H), 7.21-7.13 (m, 1H), 4.64 (ddd, J = 10.7, 8.2, 4.4 Hz, 1H), 3.22 (dd, J = 13.8, 4.4 Hz, 1H), 3.06 (dd, J = 13.8, 10.7 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.9, 165.0, 138.0, 137.8, 132.4, 129.0, 128.2, 128.2, 126.4, 118.3, 113.8, 54.4, 36.2. HRMS (ES) m/z calcd for [M+H+]: $C_{17}H_{15}N_2O_3$ 295.1083 found 295.1098.

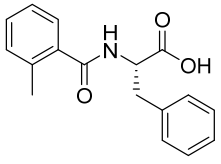
(3-Bromobenzoyl)-*L*-phenylalanine (5g)



$C_{16}H_{14}BrNO_3$
MW: 347.2

General procedure C was followed using Fmoc deprotected Phe-Wang PS resin **1a** (0.6 mmol/g, 1 equiv, 0.3 mmol, 500 mg) and 1-bromo-3-iodobenzene (4 equiv, 1.2 mmol, 153 µL). The title compound **5g** (86 mg, 82%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.87 (d, J = 8.1 Hz, 1H), 7.98 (s, 1H), 7.79 (d, J = 7.9 Hz, 1H), 7.72 (d, J = 7.9 Hz, 1H), 7.42 (dd, J = 7.9 Hz, 1H), 7.33-7.21 (m, 4H), 7.21-7.12 (m, 1H), 4.70-4.58 (m, 1H), 3.21 (dd, J = 13.7, 4.5 Hz, 1H), 3.06 (dd, J = 13.7, 10.6 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.0, 164.9, 138.1, 136.1, 134.2, 130.6, 130.0, 129.0, 128.2, 126.5, 126.4, 121.6, 54.3, 36.3. HRMS (ES) m/z calcd for [M+H+]: $C_{16}H_{15}BrNO_3$ 348.0235 found 348.0235.

(2-Methylbenzoyl)-*L*-phenylalanine (5h)

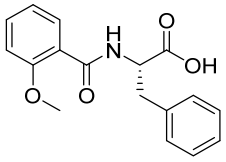


$C_{17}H_{17}NO_3$
MW: 283.3

General procedure C was followed using Fmoc deprotected Phe-Wang PS resin **1a** (0.6 mmol/g, 1 equiv, 0.3 mmol, 500 mg) and 2-iodotoluene (4 equiv, 1.2 mmol, 153 µL). The title compound

5h (68 mg, 80%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.51 (d, J = 8.3 Hz, 1H), 7.42-6.94 (m, 9H), 4.60 (ddd, J = 10.9, 8.3, 4.4 Hz, 1H), 3.16 (dd, J = 13.9, 4.4 Hz, 1H), 2.93 (dd, J = 13.9, 10.9 Hz, 1H), 2.12 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.1, 169.1, 138.1, 136.8, 135.2, 130.2, 129.2, 129.1, 128.1, 126.9, 126.3, 125.3, 53.6, 36.3, 19.1. HRMS (ES) m/z calcd for [M+H+]: $C_{17}H_{18}NO_3$ 284.1287 found 284.1301.

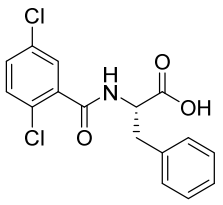
(2-Methoxybenzoyl)-*L*-phenylalanine (5i)



$C_{17}H_{17}NO_4$
MW: 299.3

General procedure C was followed using Fmoc deprotected Phe-Wang PS resin **1a** (0.6 mmol/g, 1 equiv, 0.3 mmol, 500 mg) and 2-iodoanisole (4 equiv, 1.2 mmol, 156 µL). The title compound **5i** (75 mg, 83%) was obtained as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.32 (d, J = 7.3 Hz, 1H), 7.81 (dd, J = 7.5, 1.9 Hz, 1H), 7.52-7.45 (m, 1H), 7.36-7.29 (m, 2H), 7.27-7.21 (m, 3H), 7.14 (dd, J = 8.5, 1.0 Hz, 1H), 7.07-7.00 (m, 1H), 4.69 (ddd, J = 13.8, 7.5, 5.1 Hz, 1H), 3.81 (s, 3H), 3.18 (dd, J = 13.8, 5.1 Hz, 1H), 3.10 (dd, J = 13.8, 7.5 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.7, 164.2, 157.3, 137.1, 132.9, 130.8, 129.2, 128.3, 126.7, 121.3, 120.7, 112.3, 56.0, 53.7, 36.5. HRMS (ES) m/z calcd for [M+H+]: $C_{17}H_{18}NO_4$ 300.1236 found 300.1254.

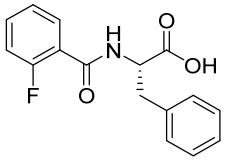
(2,5-Dichlorobenzoyl)-*L*-phenylalanine (5j)



$C_{16}H_{13}Cl_2NO_3$
MW: 337.2

General procedure C was followed using Fmoc deprotected Phe-Wang PS resin **1a** (0.6 mmol/g, 1 equiv, 0.3 mmol, 500 mg) and 1,4-dichloro-2-iodobenzene (4 equiv, 1.2 mmol, 162 µL). The title compound **5j** (85 mg, 84%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.89 (d, J = 8.3 Hz, 1H), 7.54-7.47 (m, 2H), 7.32-7.28 (m, 4H), 7.26-7.20 (m, 1H), 7.15 (dd, J = 2.1, 0.8 Hz, 1H), 4.61 (ddd, J = 10.4, 8.3, 4.7 Hz, 1H), 3.18 (dd, J = 13.9, 4.7 Hz, 1H), 2.94 (dd, J = 13.9, 10.4 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.5, 164.7, 137.7, 137.6, 131.5, 131.3, 130.6, 129.1, 128.9, 128.5, 128.1, 126.5, 53.7, 36.4. HRMS (ES) m/z calcd for [M+H+]: $C_{16}H_{14}Cl_2NO_3$ 338.0351 found 338.0355.

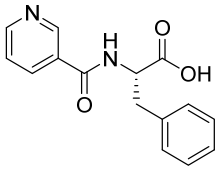
(2-Fluorobenzoyl)-*L*-phenylalanine (5k)



$C_{16}H_{14}FNO_3$
MW: 287.3

General procedure C was followed using Fmoc deprotected Phe-Wang PS resin **1a** (0.6 mmol/g, 1 equiv, 0.3 mmol, 500 mg) and 1-fluoro-2-iodobenzene (4 equiv, 1.2 mmol, 140 µL). The title compound **5k** (70 mg, 81%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.49 (dd, J = 7.9, 2.8 Hz, 1H), 7.58-7.42 (m, 2H), 7.31-7.18 (m, 7H), 4.62 (ddd, J = 9.8, 7.9, 4.7 Hz, 1H), 3.18 (dd, J = 13.8, 4.7 Hz, 1H), 3.02 (dd, J = 13.8, 9.8 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.7, 163.6, 159.19 (d, J_{CF} = 245.0 Hz), 137.7, 132.62 (d, J_{CF} = 7.8 Hz), 130.03 (d, J_{CF} = 3.2 Hz), 129.1, 128.2, 126.5, 124.40 (d, J_{CF} = 7.8 Hz), 123.37 (d, J_{CF} = 21.0 Hz), 116.13 (d, J_{CF} = 21.0 Hz). 54.1, 36.3. HRMS (ES) m/z calcd for [M-H+]: $C_{16}H_{13}FNO_3$ 286.0879 found 286.0889.

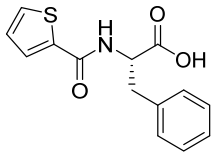
Nicotinoyl-*L*-phenylalanine (5l)



$C_{15}H_{14}N_2O_3$
MW: 270.3

General procedure C was followed using Fmoc deprotected Phe-Wang PS resin **1a** (0.6 mmol/g, 1 equiv, 0.3 mmol, 500 mg) and 3-iodopyridine (4 equiv, 1.2 mmol, 246 mg). The title compound **5l** (76 mg, 94%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.02 (d, J = 8.1 Hz, 1H), 8.97 (d, J = 1.7 Hz, 1H), 8.75 (dd, J = 5.0, 1.7 Hz, 1H), 8.24 (dt, J = 8.0, 1.7 Hz, 1H), 7.61 (dd, J = 8.0, 5.0 Hz, 1H), 7.35-7.22 (m, 4H),7.22-7.13 (m, 1H), 4.65 (ddd, J = 10.6, 8.1, 4.5 Hz, 1H), 3.22 (dd, J = 13.7, 4.5 Hz, 1H), 3.05 (dd, J = 13.7, 10.6 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.8, 164.4, 150.8, 147.3, 137.9, 136.5, 129.9, 129.1, 128.2, 126.4, 124.1, 54.2, 36.3. HRMS (ES) m/z calcd for [M+H+]: $C_{15}H_{15}N_2O_3$ 271.1083 found 271.1086.

(Thiophene-2-carbonyl)-*L*-phenylalanine (5m)

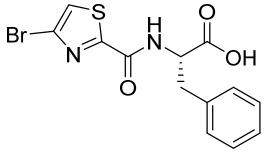


$C_{14}H_{13}NO_3S$
MW: 275.3

General procedure C was followed using Fmoc deprotected Phe-Wang PS resin **1a** (0.6 mmol/g, 1 equiv, 0.3 mmol, 500 mg,) and 2-iodothiophene (4 equiv, 1.2 mmol, 133 µL). The title

compound **5m** (73 mg, 88%) was obtained as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.73 (d, J = 8.1 Hz, 1H), 7.81 (dd, J = 3.7, 0.9 Hz, 1H), 7.74 (dd, J = 5.0, 0.9 Hz, 1H), 7.31-7.15 (m, 5H), 7.13 (dd, J = 5.0, 3.7 Hz, 1H), 4.57 (ddd, J = 10.6, 8.1, 4.5 Hz, 1H), 3.19 (dd, J = 13.7, 4.5 Hz, 1H), 3.03 (dd, J = 13.7, 10.6 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.1, 161.2, 139.3, 138.1, 131.1, 129.1, 128.6, 128.2, 127.9, 126.4, 54.1, 36.3. HRMS (ES) m/z calcd for [M+H+]: $C_{14}H_{14}NO_3S$ 276.0694 found 276.0703.

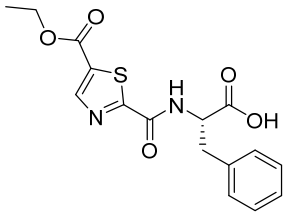
(4-Bromothiazole-2-carbonyl)-*L*-phenylalanine (5n)



$C_{13}H_{11}BrN_2O_3S$
MW: 354.2

General procedure C was followed using Fmoc deprotected Phe-Wang PS resin **1a** (0.6 mmol/g, 1 equiv, 0.3 mmol, 500 mg,) and 2,4-dibromothiazole (4 equiv, 1.2 mmol, 291 mg). The title compound **5n** (90 mg, 85%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.08 (d, J = 8.4 Hz, 1H), 8.17 (s, 1H), 7.32-7.22 (m, 4H), 7.19-7.10 (m, 1H), 4.65 (ddd, J = 9.2, 8.4, 5.4 Hz, 1H), 3.26-3.06 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.1, 163.8, 158.0, 137.8, 129.0, 128.2, 126.4, 124.8, 124.6, 53.9, 35.6. HRMS (ES) m/z calcd for [M+H+]: $C_{13}H_{12}BrN_2O_3S$ 354.9752 found 354.9768.

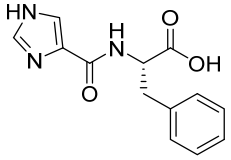
(5-(Ethoxycarbonyl)thiazole-2-carbonyl)-*L*-phenylalanine (5o)



$C_{16}H_{16}N_2O_5S$
MW: 348.4

General procedure C was followed using Fmoc deprotected Phe-Wang PS resin **1a** (0.6 mmol/g, 1 equiv, 0.3 mmol, 500 mg,) and ethyl 2-bromothiazole-5-carboxylate (4 equiv, 1.2 mmol, 283 mg). The title compound **5o** (90 mg, 86%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.00 (d, J = 8.4 Hz, 1H), 8.75 (d, J = 0.6 Hz, 1H), 7.25 (d, J = 3.9 Hz, 4H), 7.19-7.13 (m, 1H), 4.83-4.64 (m, 1H), 4.43-4.25 (m, 2H), 3.23 (d, J = 7.2 Hz, 2H), 1.32 (t, J = 7.2, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.1, 163.9, 160.3, 158.6, 146.8, 137.8, 133.9, 129.0, 128.2, 126.4, 61.1, 53.8, 35.7, 14.2. HRMS (ES) m/z calcd for [M+H+]: $C_{16}H_{17}N_2O_5S$ 349.0858 found 349.0865.

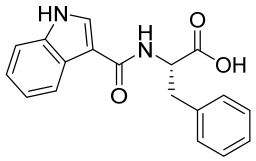
(1*H*-Imidazole-4-carbonyl)-*L*-phenylalanine (5p)



$C_{13}H_{13}N_3O_3$
MW: 259.3

General procedure C was followed using Fmoc deprotected Phe-Wang PS resin **1a** (0.6 mmol/g, 1 equiv, 0.3 mmol, 500 mg,) and 4-iodo-1-trityl-1*H*-imidazole (4 equiv, 1.2 mmol, 524mg). The title compound **5p** (62 mg, 80%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.91 (d, J = 8.2 Hz, 1H), 8.81 (s, 1H), 8.10 (s, 1H), 7.29-7.23 (m, 4H), 7.20 – 7.14 (m, 1H), 4.65 (ddd, J = 10.0, 8.2, 4.6 Hz, 1H), 3.20 (dd, J = 13.9, 4.6 Hz, 1H), 3.01 (dd, J = 13.9, 10.0 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.8, 158.1, 137.7, 136.3, 129.2, 128.8, 128.5, 126.7, 120.6, 53.8, 36.6. HRMS (ES) m/z calcd for [M+H+]: $C_{13}H_{14}N_3O_3$ 260.1035 found 260.1044.

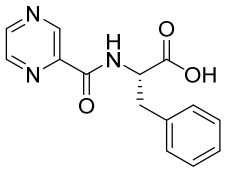
(1*H*-Indole-3-carbonyl)-*L*-phenylalanine (5q)



$C_{18}H_{16}N_2O_3$
MW: 308.3

General procedure C was followed using Fmoc deprotected Phe-Wang PS resin **1a** (0.6 mmol/g, 1 equiv, 0.3 mmol, 500 mg,) and *tert*-butyl 3-iodo-1*H*-indole-1-carboxylate (4 equiv, 1.2 mmol, 281 mg). The title compound **5q** (80 mg, 86%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.57 (s, 1H), 8.12-7.99 (m, 3H), 7.41 (d, J = 8.1 Hz, 1H), 7.33 (d, J = 7.3 Hz, 2H), 7.26 (dd, J = 14.4, 6.7 Hz, 2H), 7.20-7.01 (m, 3H), 4.65 (ddd, J = 10.6, 8.1, 4.5 Hz, 1H), 3.17 (dd, J = 13.7, 4.5 Hz, 1H), 3.05 (dd, J = 13.7, 10.6 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.8, 164.5, 138.3, 136.1, 129.1, 128.2, 128.2, 126.3, 126.1, 121.9, 120.9, 120.4, 111.8, 110.0, 53.5, 36.6. HRMS (ES) m/z calcd for [M+H+]: $C_{18}H_{17}N_2O_3$ 309.1239 found 309.1234.

(Pyrazine-2-carbonyl)-*L*-phenylalanine (5r)[9]



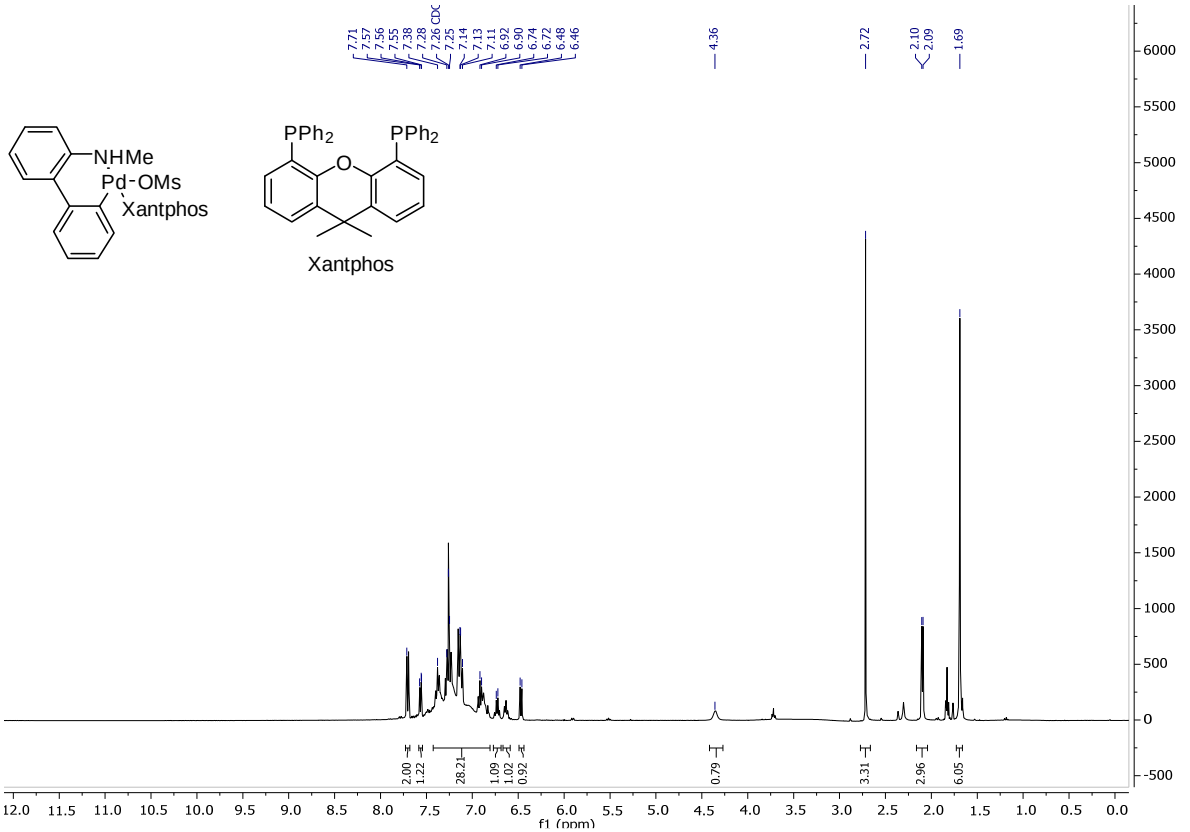
$C_{14}H_{13}N_3O_3$
MW: 271.3

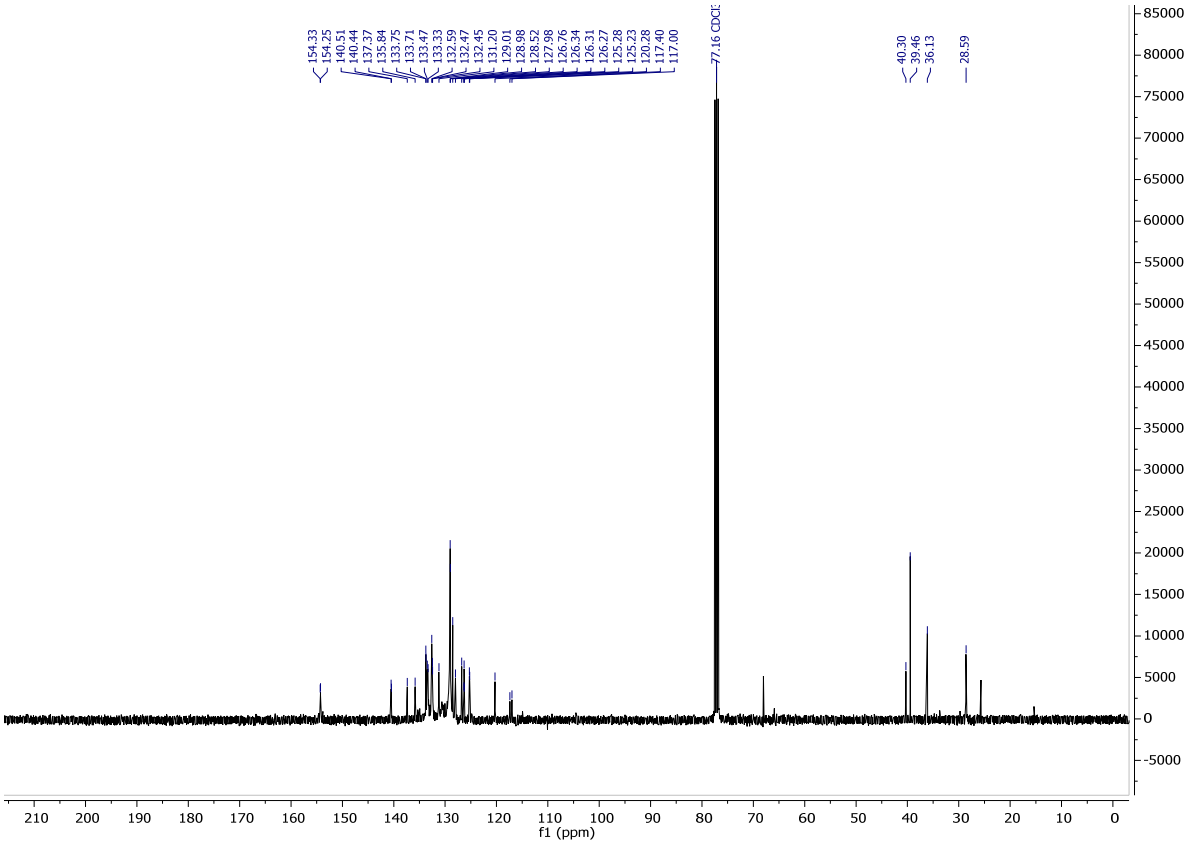
General procedure C was followed using Fmoc deprotected Phe-Wang PS resin **1a** (0.6 mmol/g, 1 equiv, 0.3 mmol, 500 mg,) and 2-iodopyrazine (4 equiv, 1.2 mmol, 118 μL). The title compound **5r** (67 mg, 82%) was obtained as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.12 (d, J = 1.5 Hz, 1H), 8.86 (d, J = 2.5 Hz, 1H), 8.83 (d, J = 8.2 Hz, 1H), 8.71 (dd, J = 2.5, 1.5 Hz, 1H), 7.25-7.20 (m, 4H), 7.20-7.12 (m, 1H), 4.77-4.69 (m, 1H), 3.27-3.14 (m, 2H). ^{13}C

NMR (101 MHz, DMSO-d_6) δ 172.3, 162.6, 147.8, 144.1, 143.5, 143.5, 137.5, 129.1, 128.2, 126.5, 53.4, 36.1. MS calcd for [M+H+]: $C_{14}H_{14}N_3O_3$ 272.1 found 272.2.

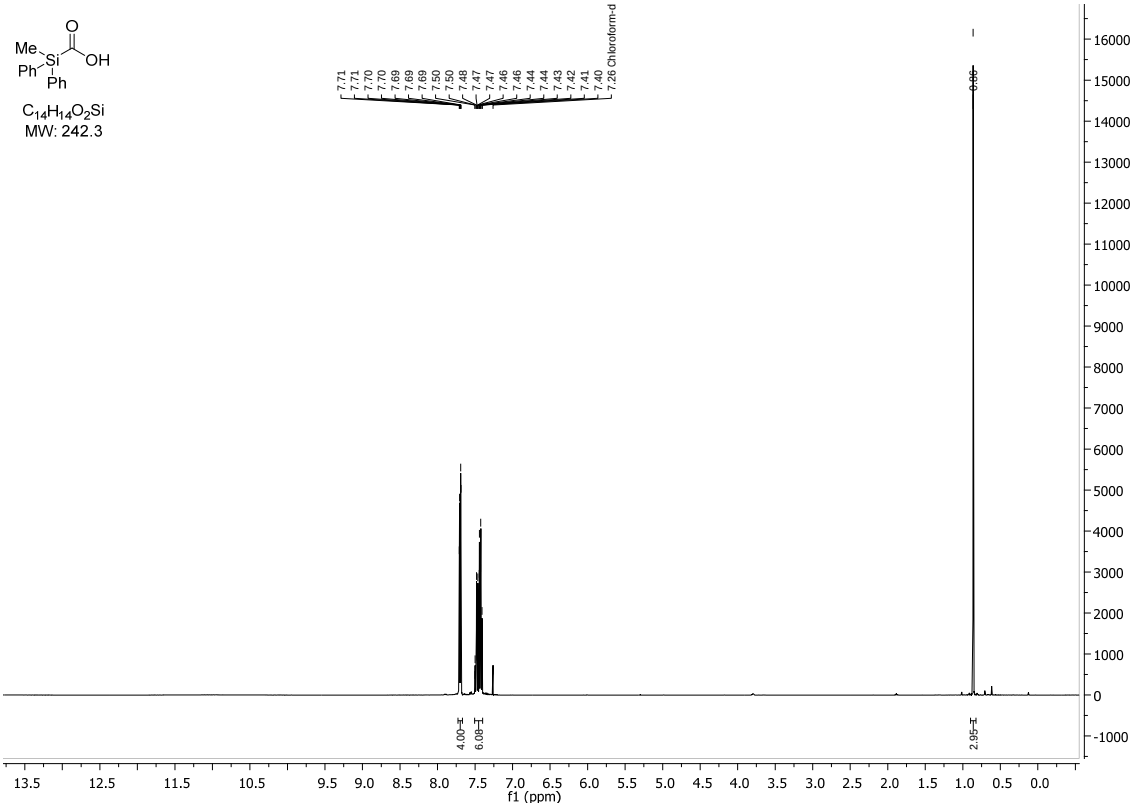
NMR Spectra for compounds **2**, **3**, **[^{13}C]-3***, **4a-h** and **5a-r**

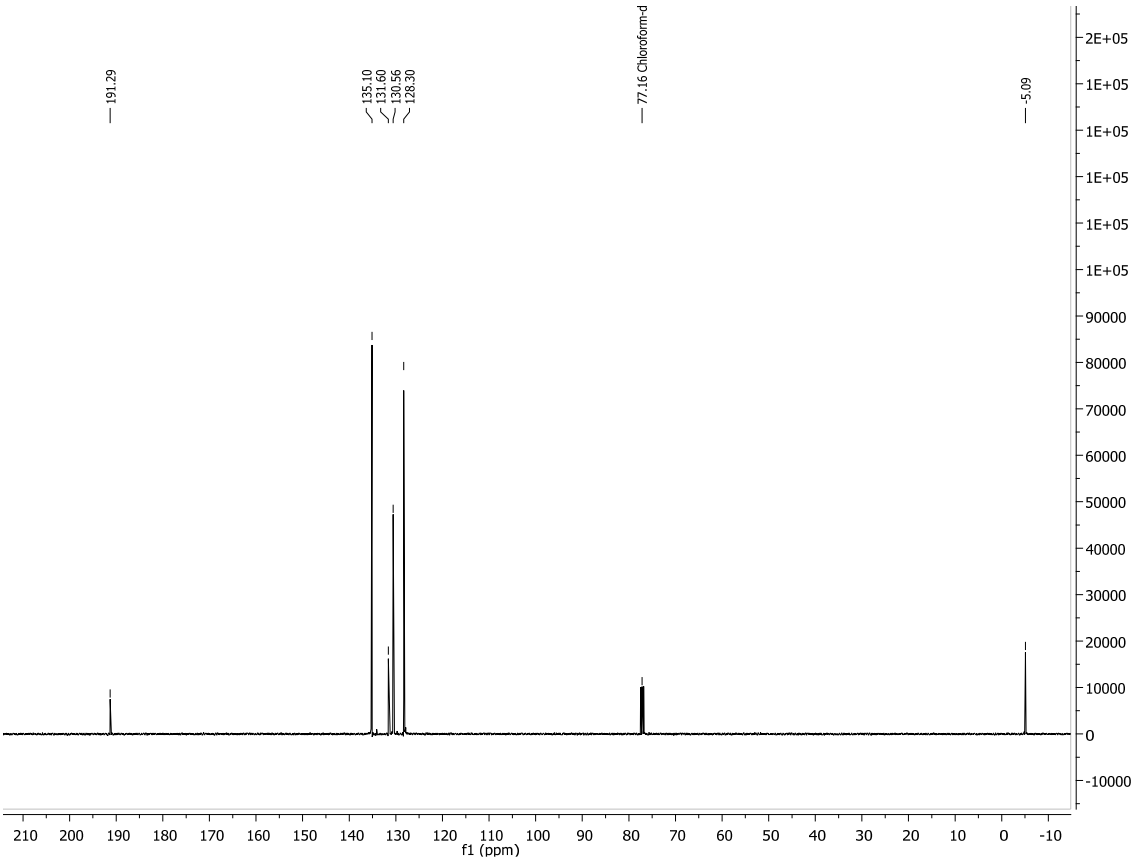
Compound **2**



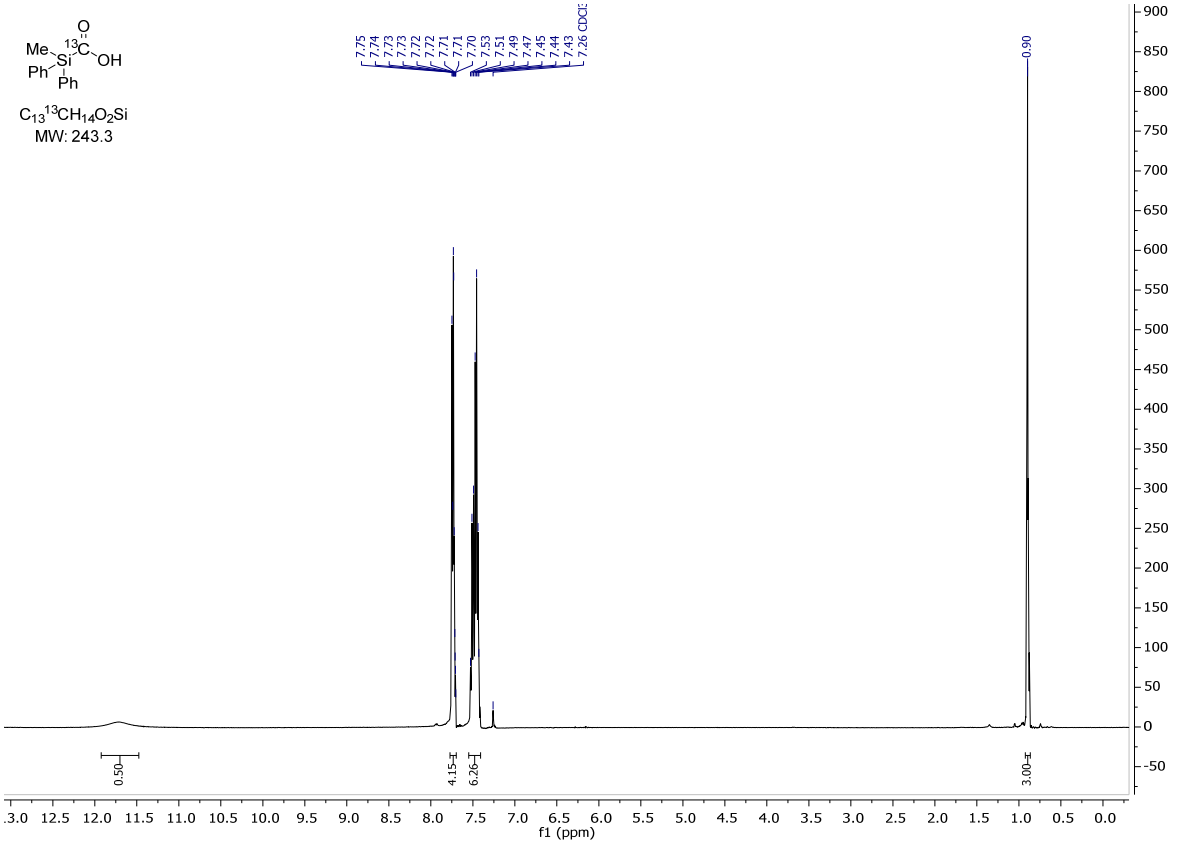


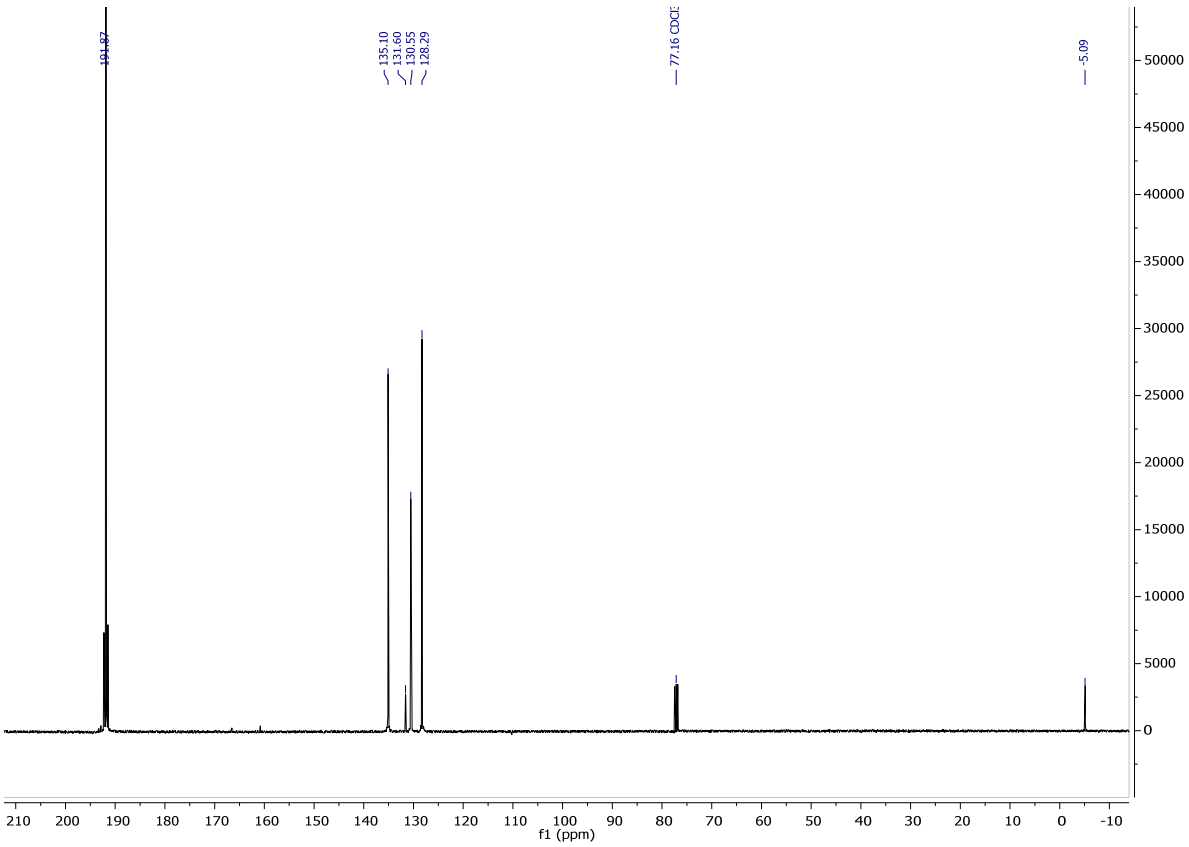
Compound **3**


Me
Si
Ph
Ph
O
OH
C14H14O2Si
MW: 242.3
7.71
7.71
7.70
7.70
7.69
7.69
7.69
7.50
7.50
7.48
7.47
7.47
7.46
7.46
7.44
7.44
7.43
7.42
7.41
7.40
7.26 Chloroform-d
0.00
4.00
6.08
2.95
f1 (ppm)

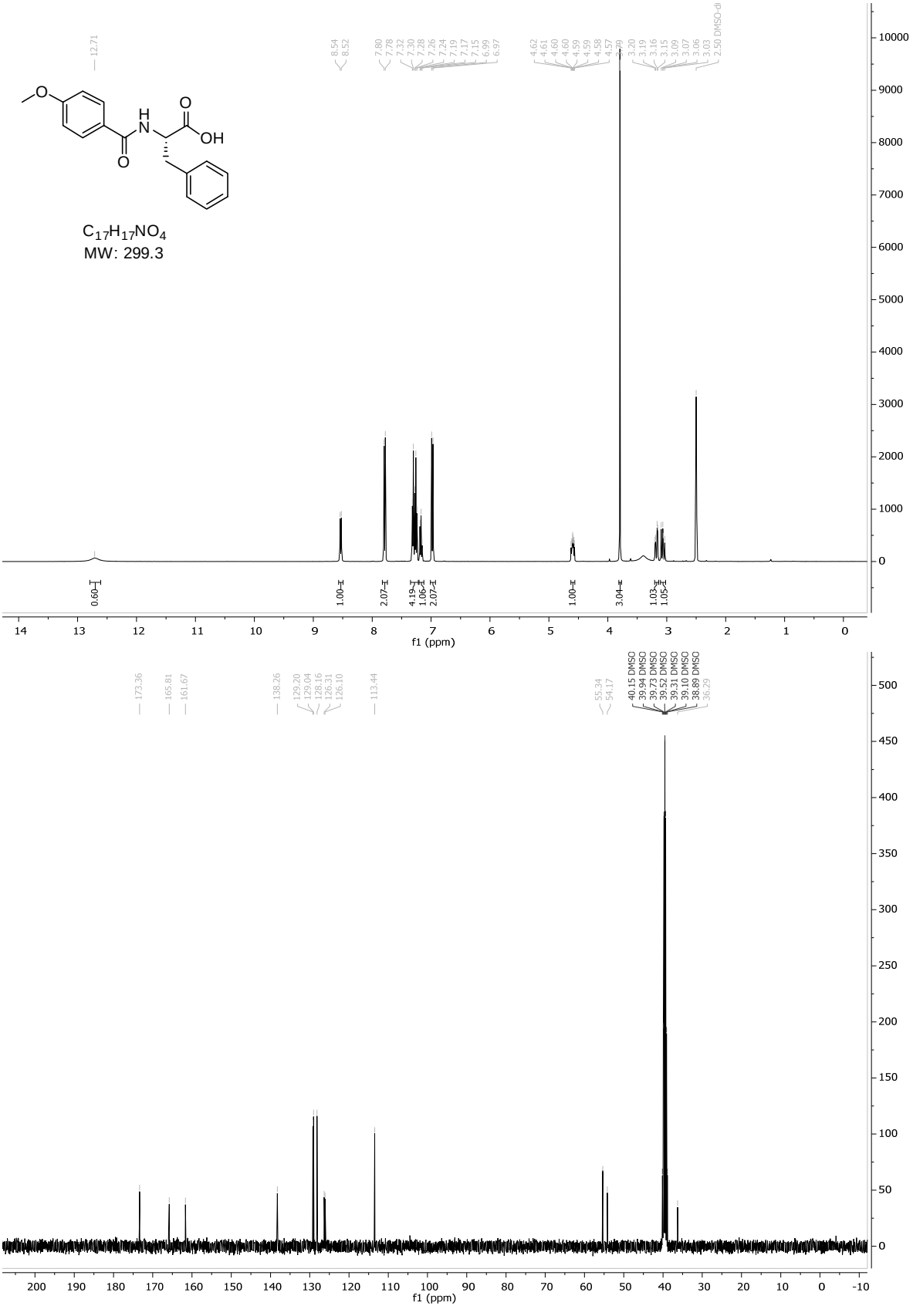

191.29
135.10
131.60
130.56
128.30
77.16 Chloroform-d
-5.09
f1 (ppm)

Compound [^{13}C]-**3***

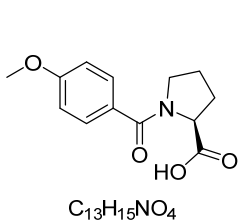




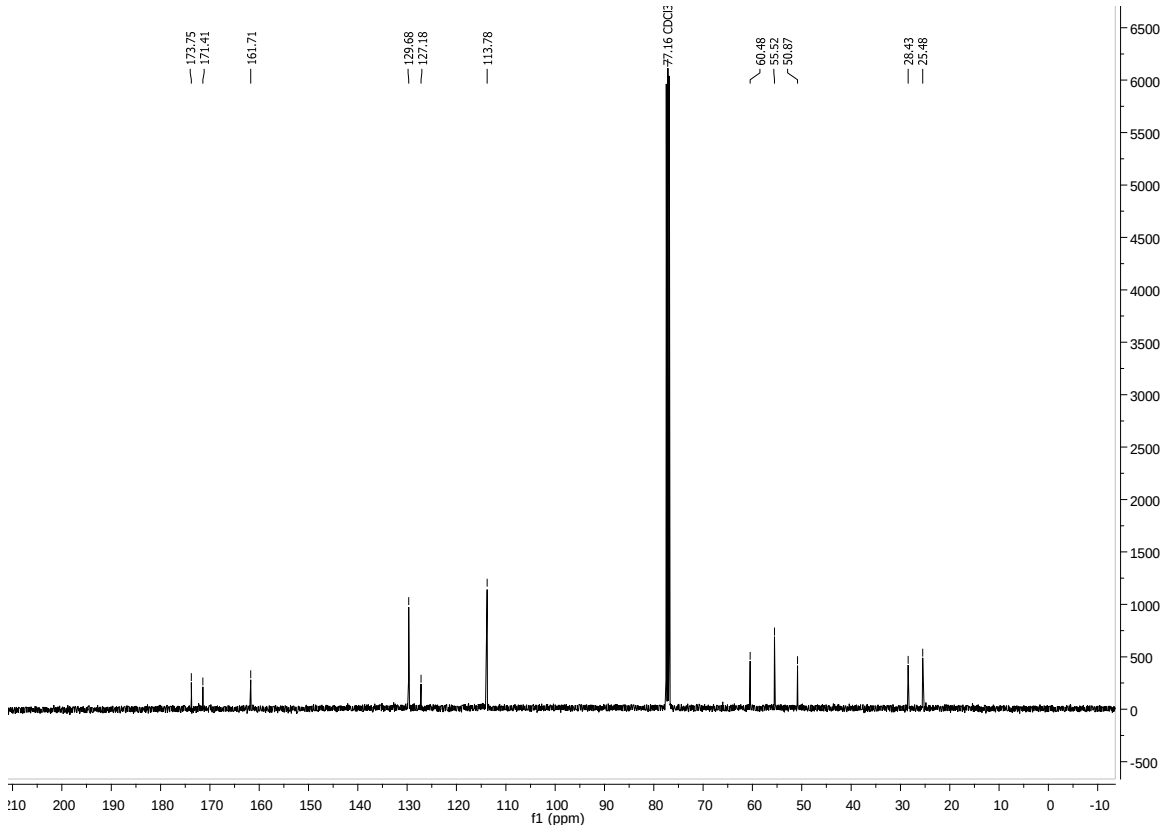
Compound **4a**



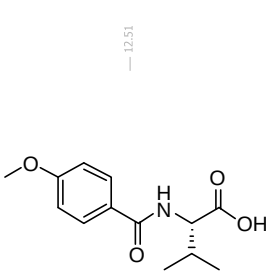
Compound **4b**



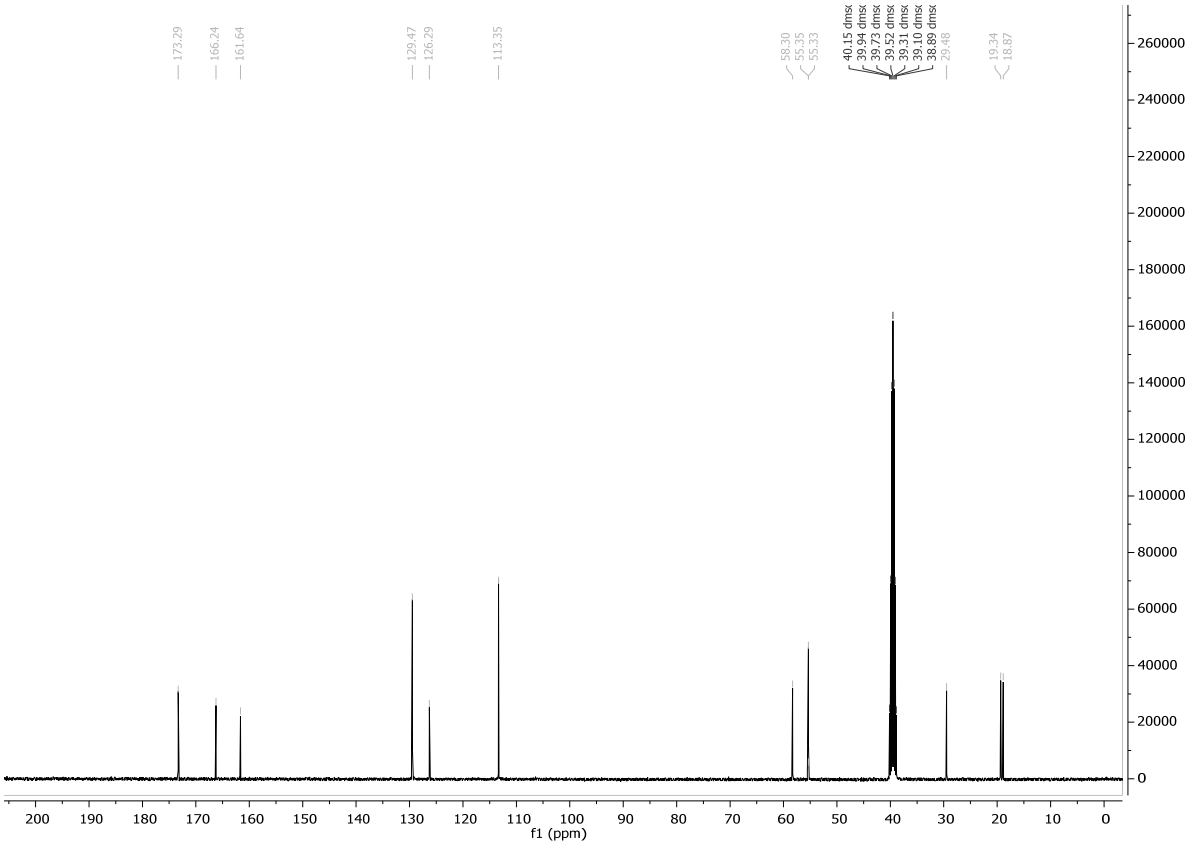
$C_{13}H_{15}NO_4$
MW: 249.3



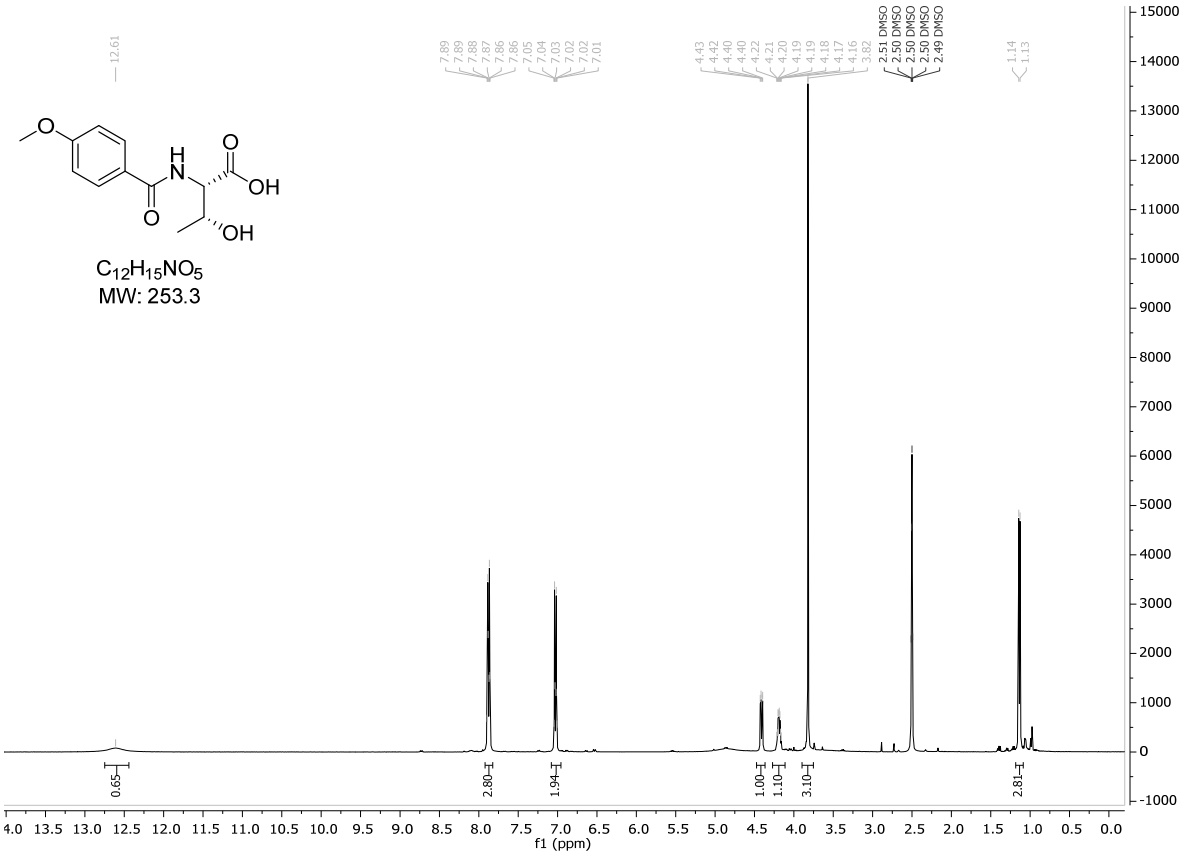
Compound **4c**

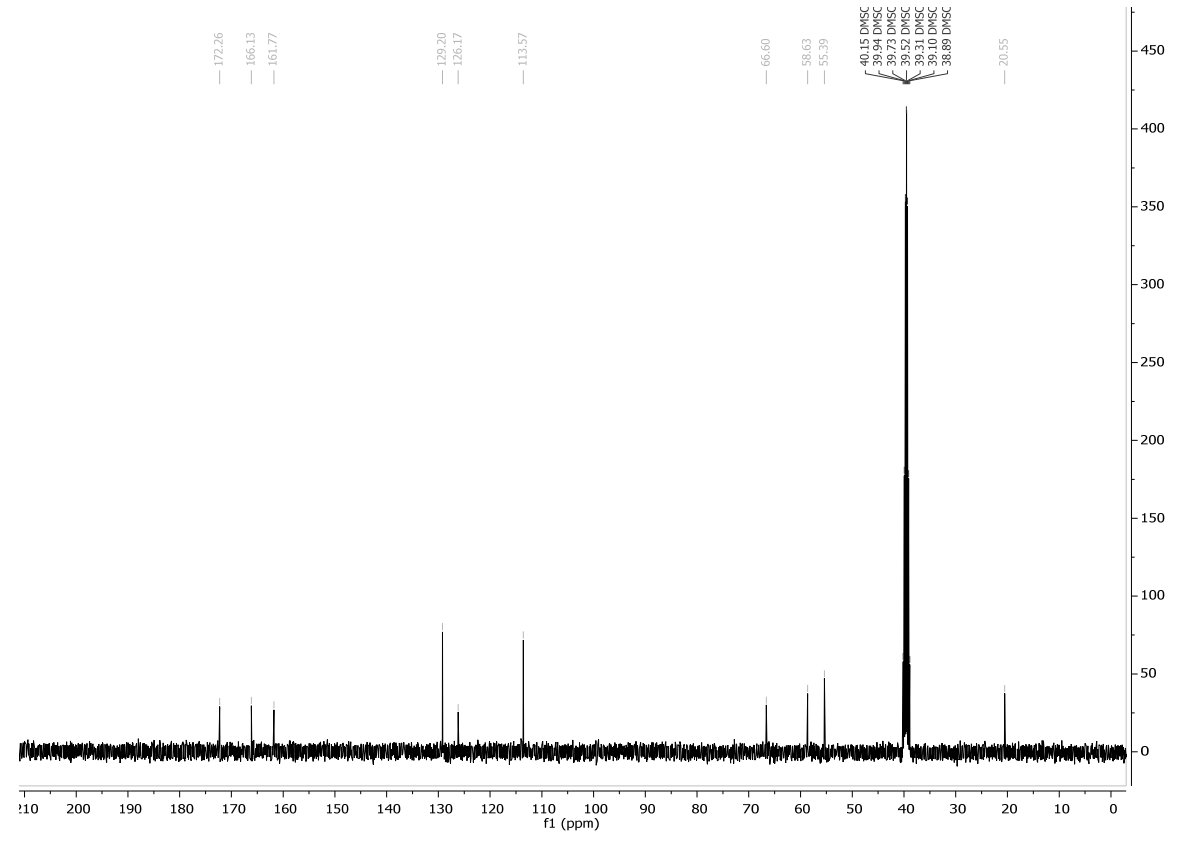


$C_{13}H_{17}NO_4$
MW: 251.3

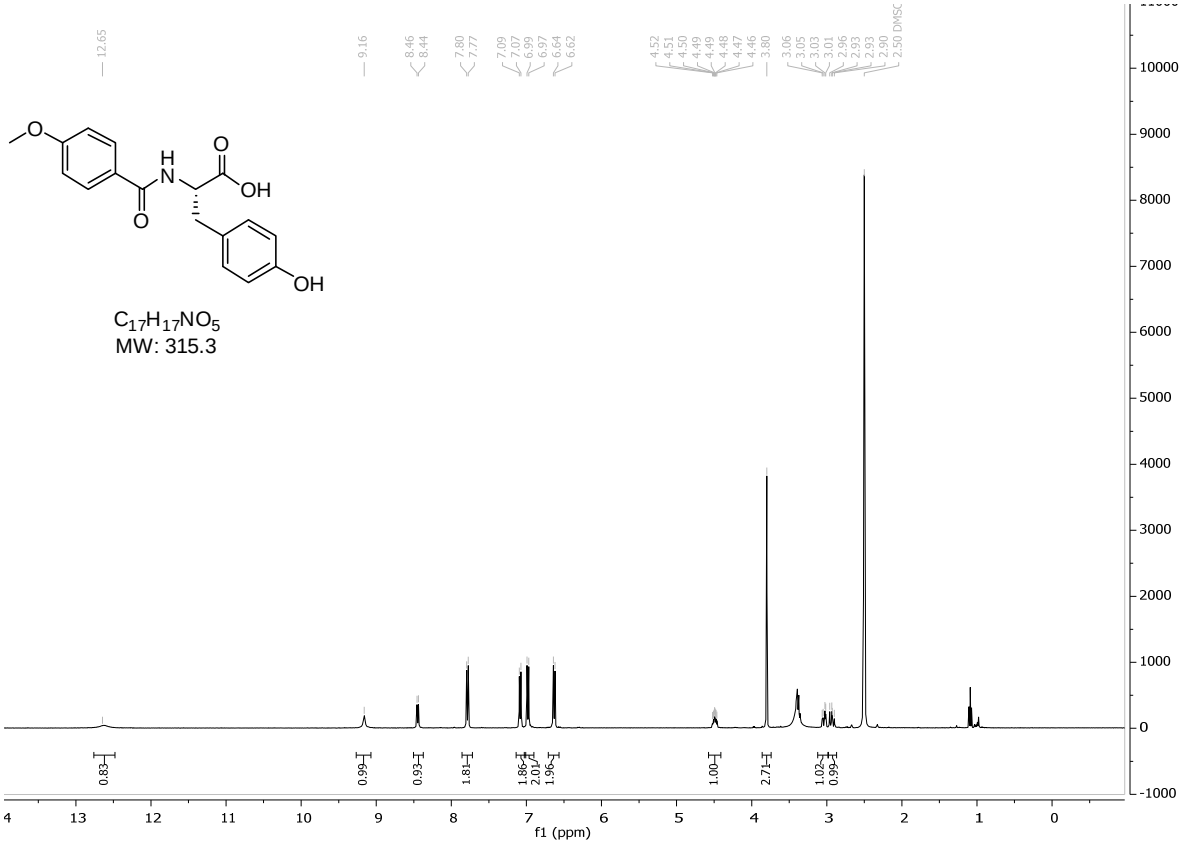


Compound **4d**

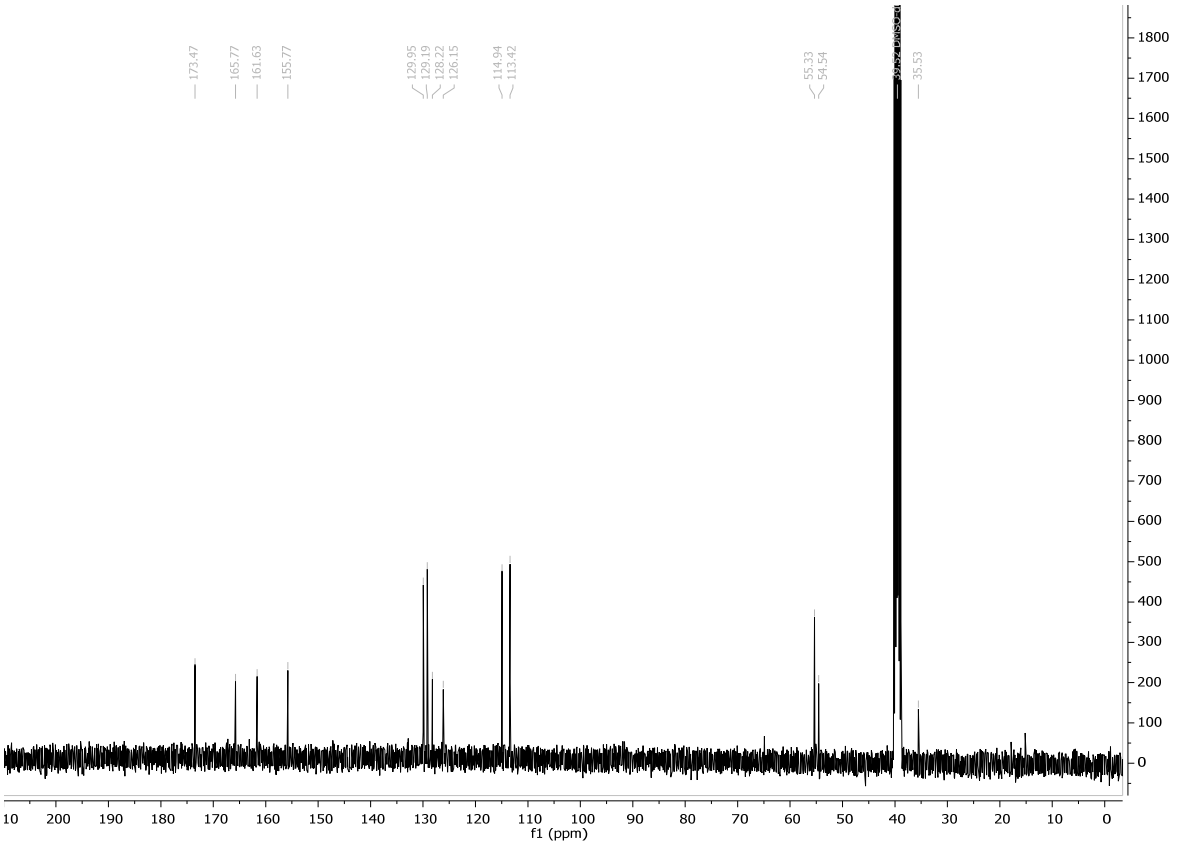

12.61
7.89
7.89
7.88
7.88
7.87
7.86
7.86
7.05
7.04
7.03
7.02
7.02
7.01
4.43
4.42
4.40
4.40
4.22
4.21
4.20
4.19
4.19
4.18
4.17
4.16
3.82
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
1.14
1.13
O
H
N
O
O
OH
OH
C12H15NO5
MW: 253.3
0.65
2.80
1.94
1.00
1.10
3.10
2.81
f1 (ppm)


172.26
166.13
161.77
129.20
126.17
113.57
66.60
58.63
55.39
40.15 DMSO
39.94 DMSO
39.73 DMSO
39.52 DMSO
39.31 DMSO
39.10 DMSO
38.89 DMSO
20.55
f1 (ppm)

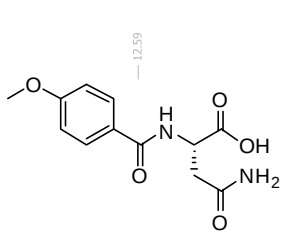
Compound **4e**



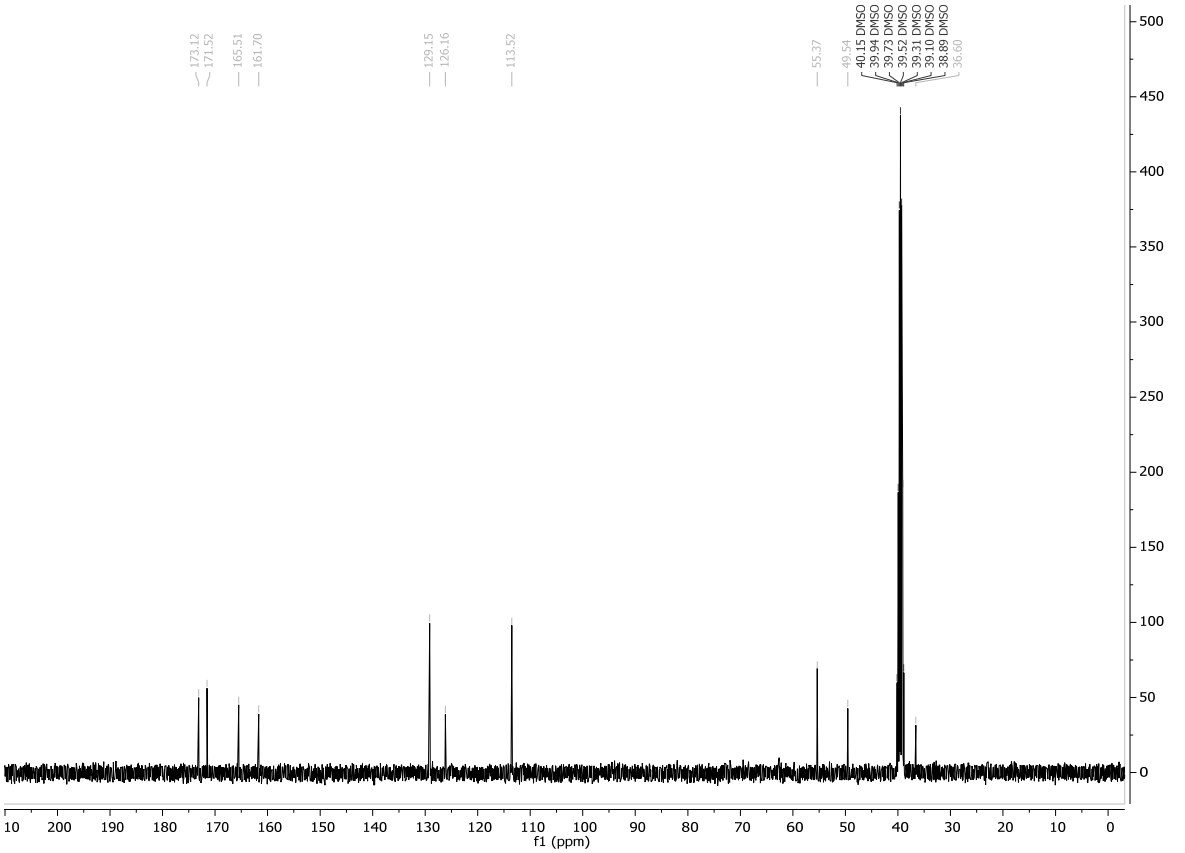
$C_{17}H_{17}NO_5$
MW: 315.3



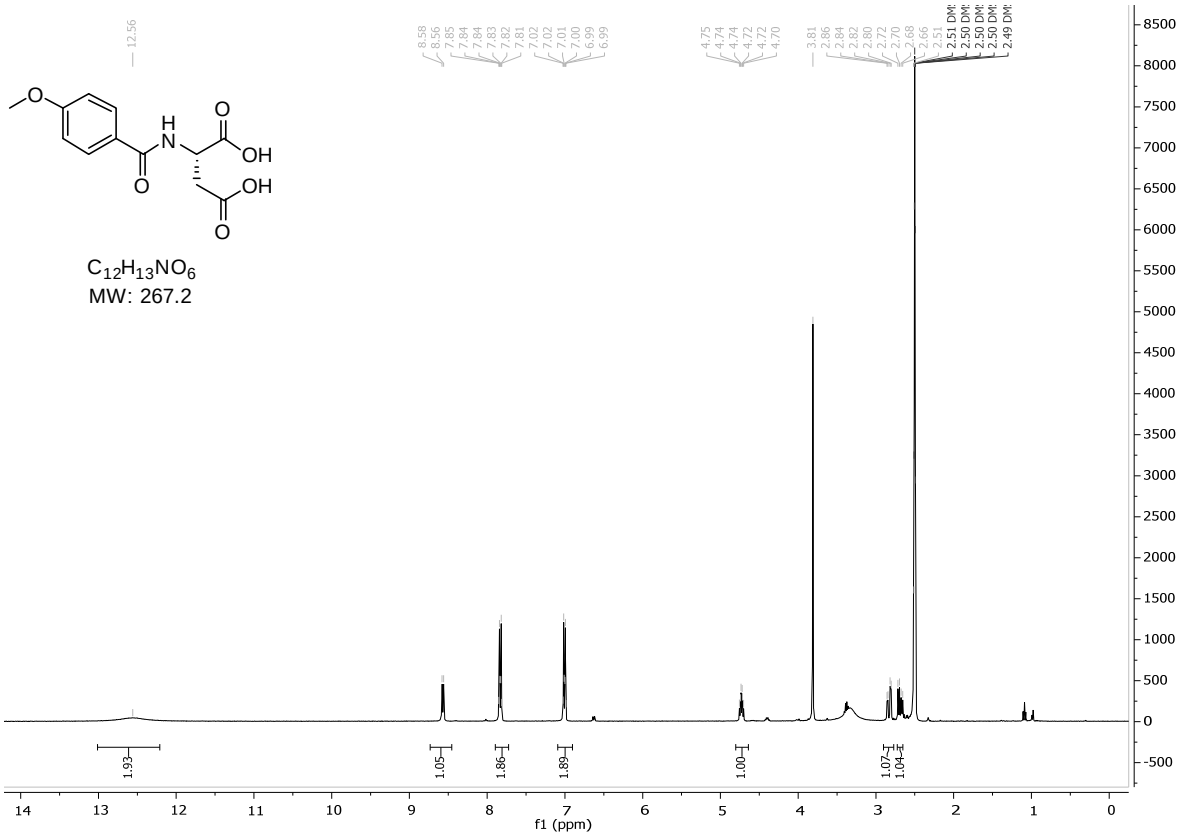
Compound **4f**



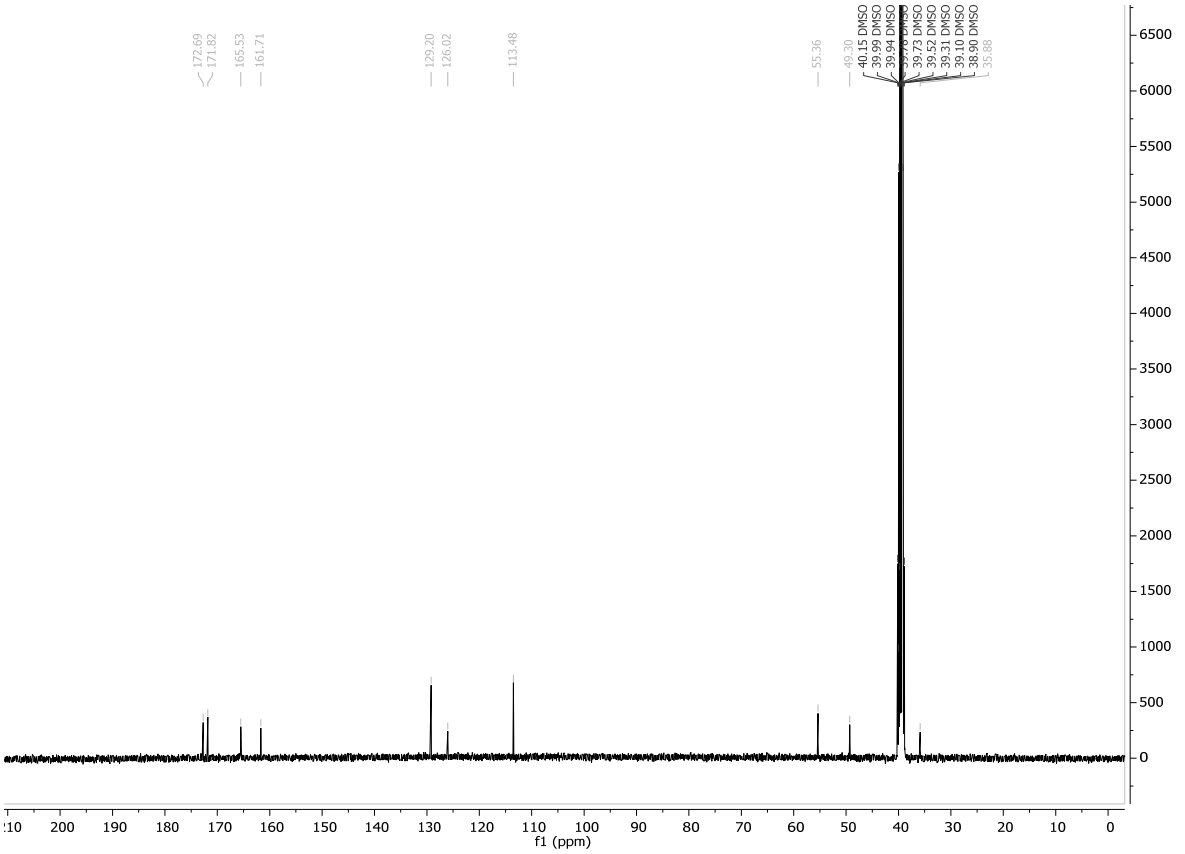
$C_{12}H_{14}N_2O_5$
MW: 266.3



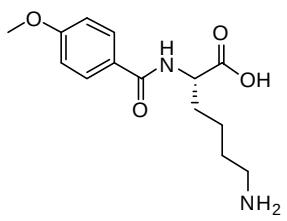
Compound **4g**



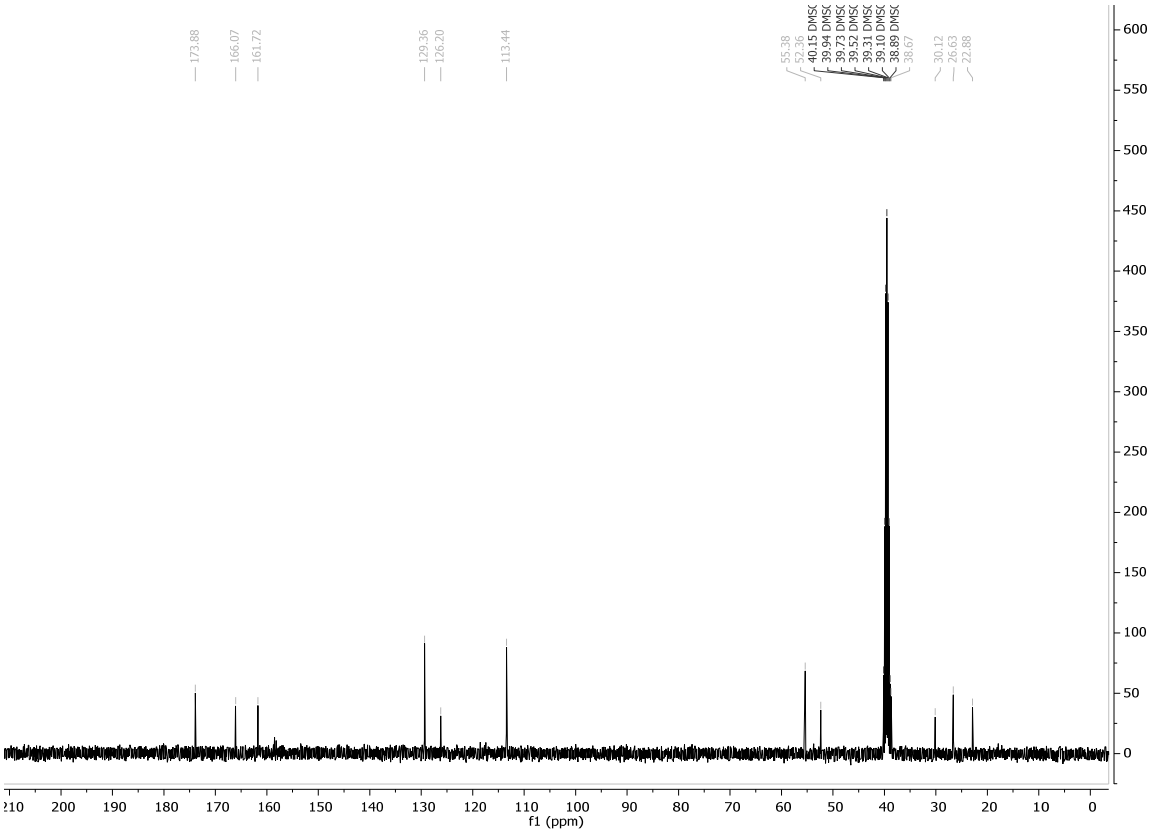
$C_{12}H_{13}NO_6$
MW: 267.2



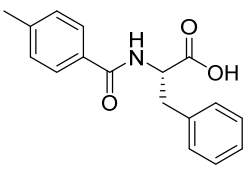
Compound **4h**



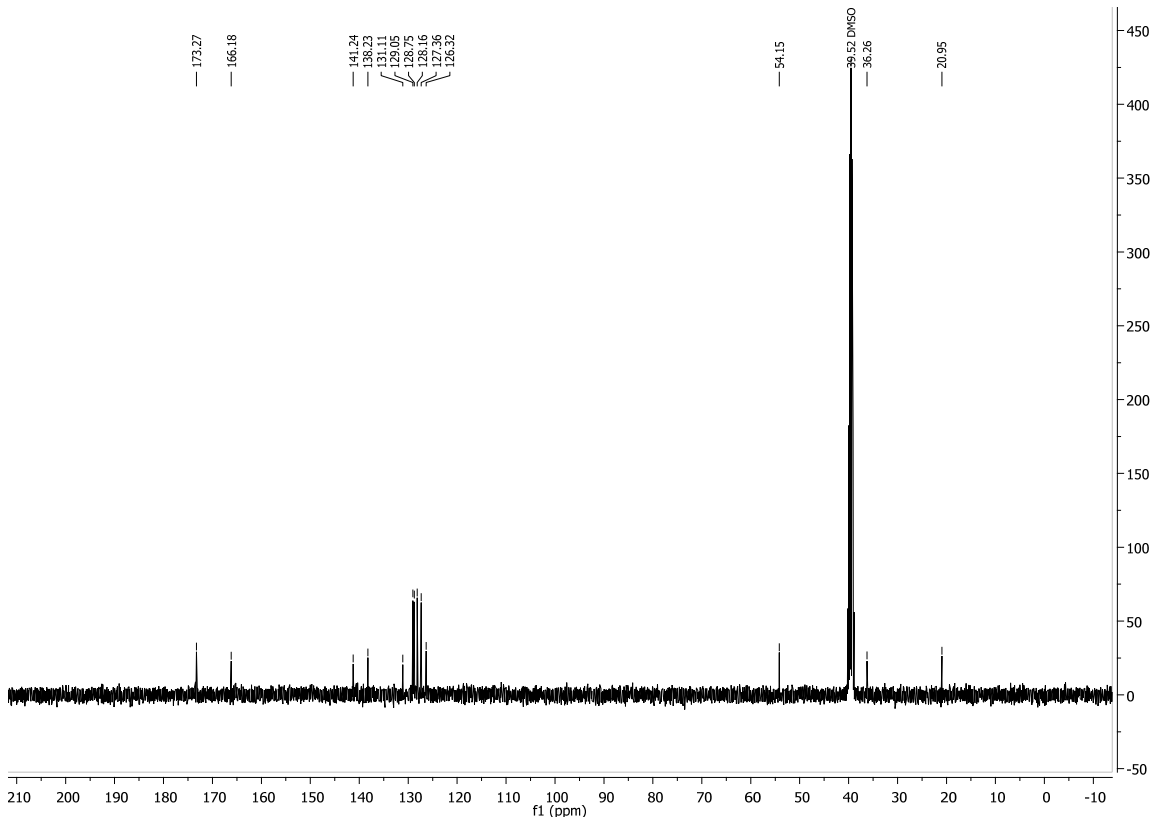
$C_{14}H_{20}N_2O_4$
MW: 280.3



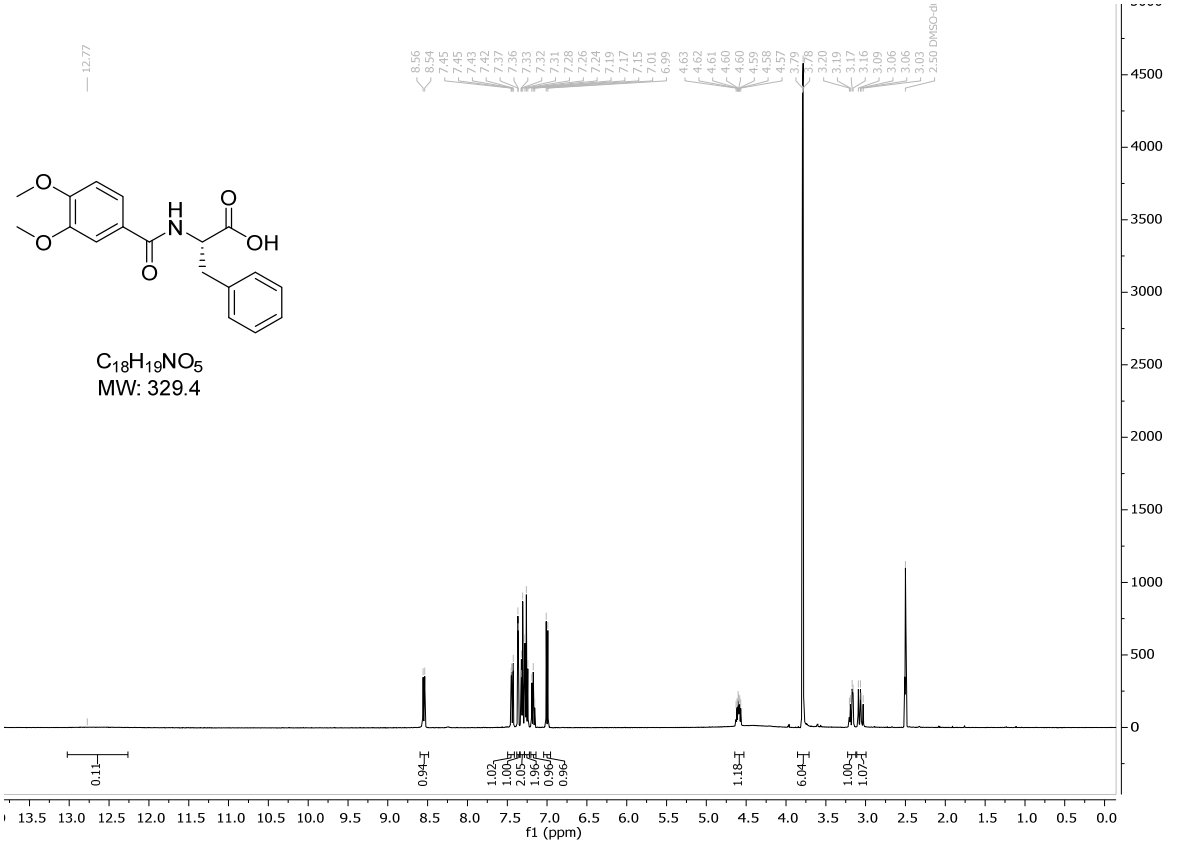
Compound **5a**

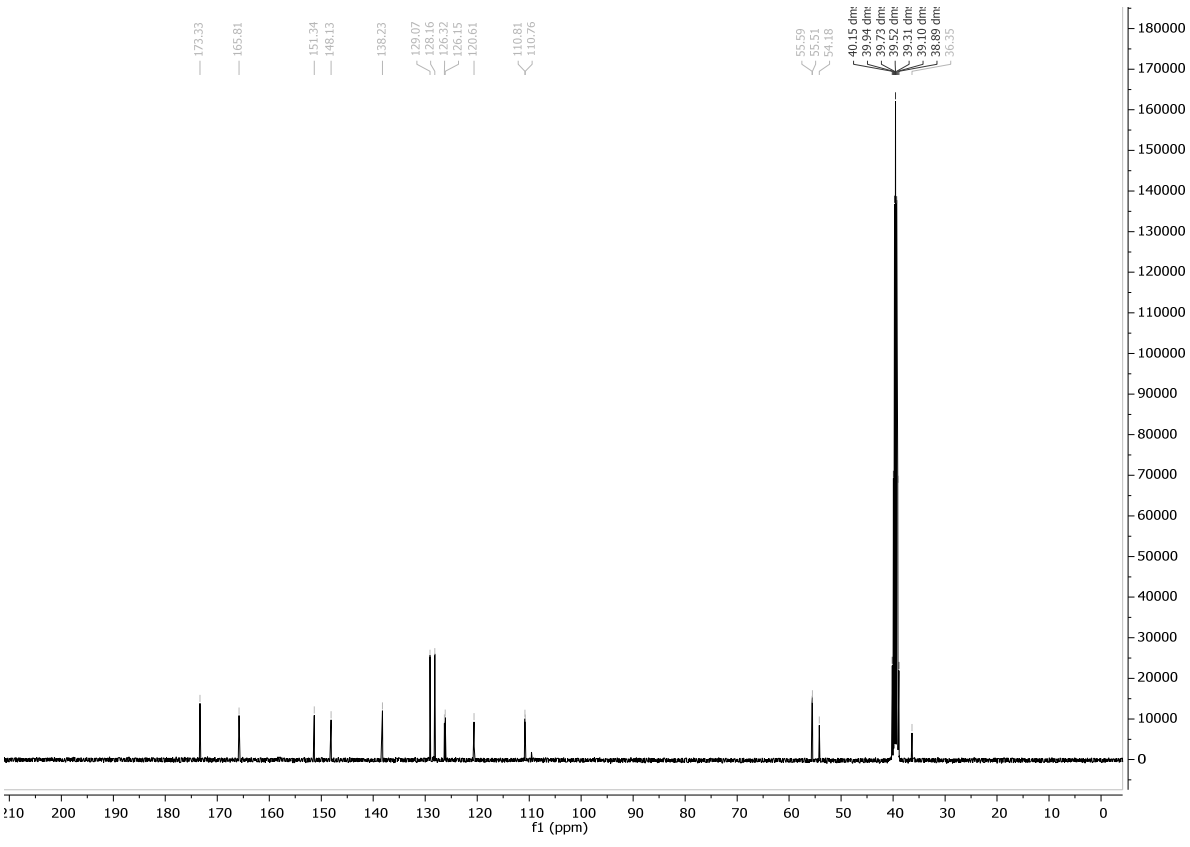


$C_{17}H_{17}NO_3$
MW: 283.3

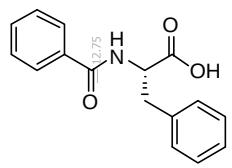


Compound **5b**

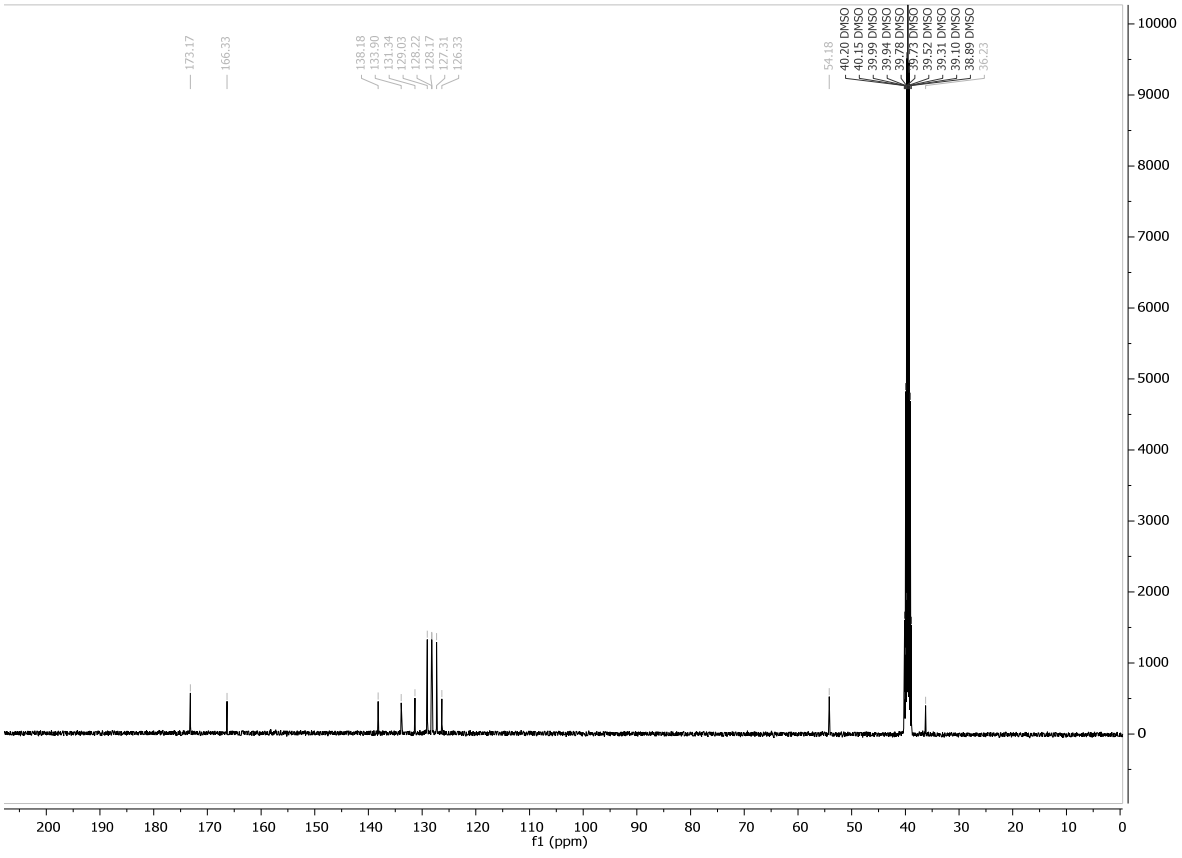

12.77
8.56
8.54
7.45
7.43
7.42
7.37
7.36
7.33
7.32
7.31
7.28
7.26
7.24
7.19
7.17
7.15
7.01
6.99
4.63
4.62
4.61
4.60
4.60
4.59
4.58
4.57
3.78
3.20
3.19
3.17
3.16
3.09
3.06
3.06
3.03
2.50 DMSO-d
O
O
H
N
O
O
OH
$C_{18}H_{19}NO_5$
MW: 329.4
0.11
0.94
1.02
1.00
2.05
1.96
0.96
0.96
1.18
6.04
1.00
1.07
f1 (ppm)


173.33
165.81
151.34
148.13
138.23
129.07
128.16
126.32
126.15
120.61
110.81
110.76
55.59
55.51
54.18
40.15 dmt
39.94 dmt
39.73 dmt
39.52 dmt
39.31 dmt
39.10 dmt
38.89 dmt
36.35
f1 (ppm)

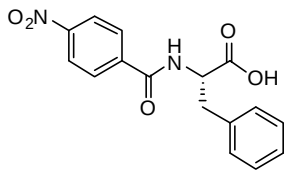
Compound **5c**



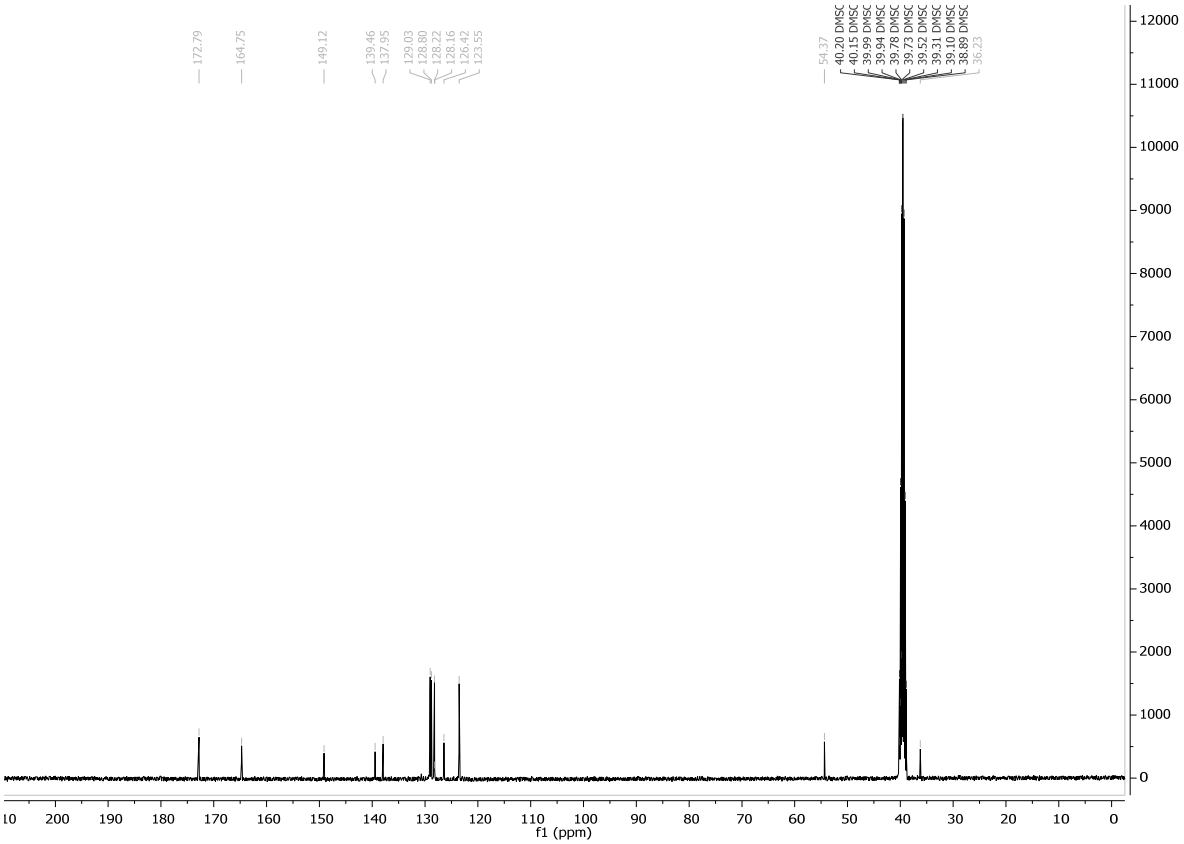
$C_{16}H_{15}NO_3$
MW: 269.3



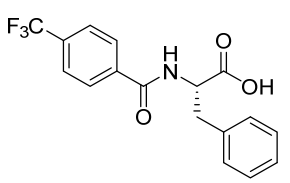
Compound **5d**



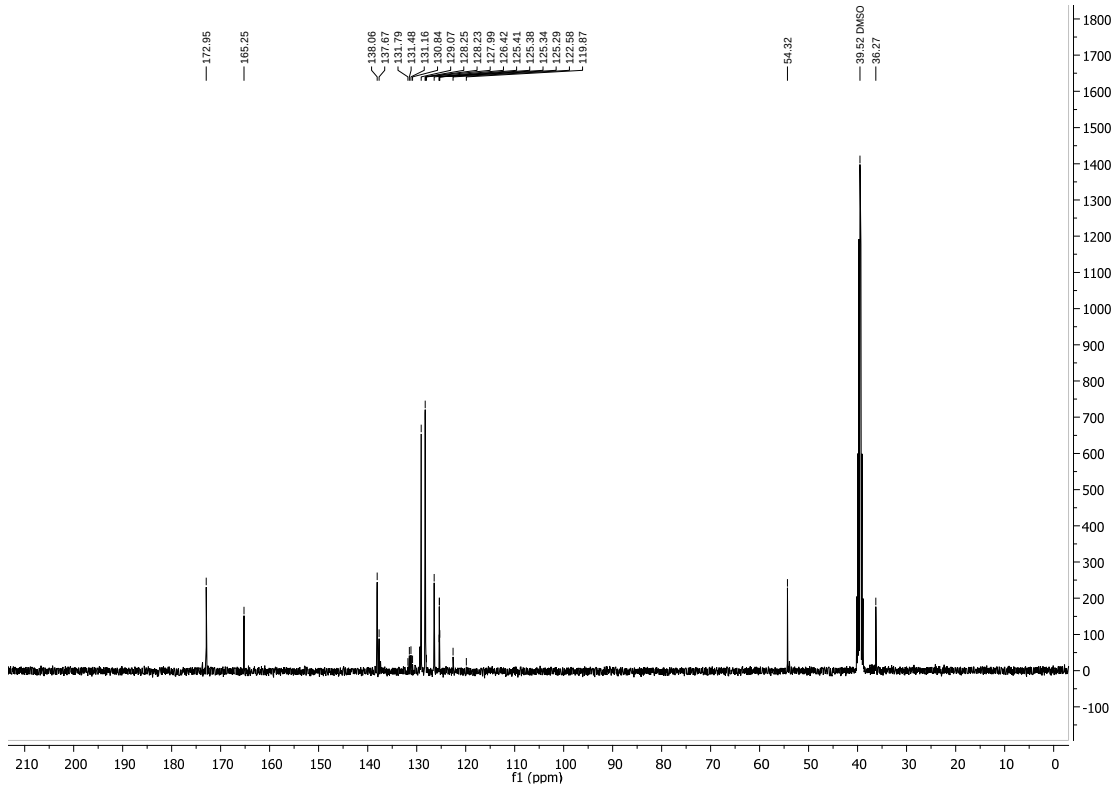
$C_{16}H_{14}N_2O_5$
MW: 314.3



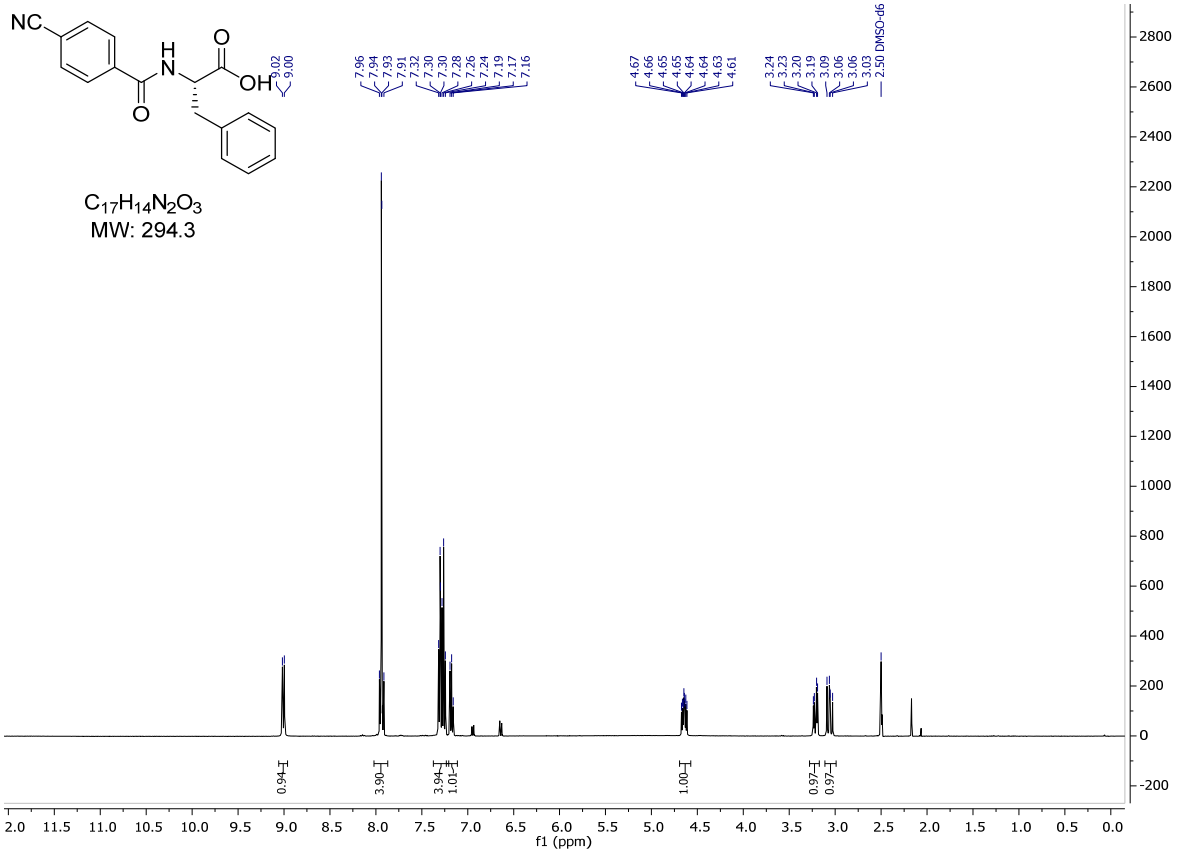
Compound **5e**

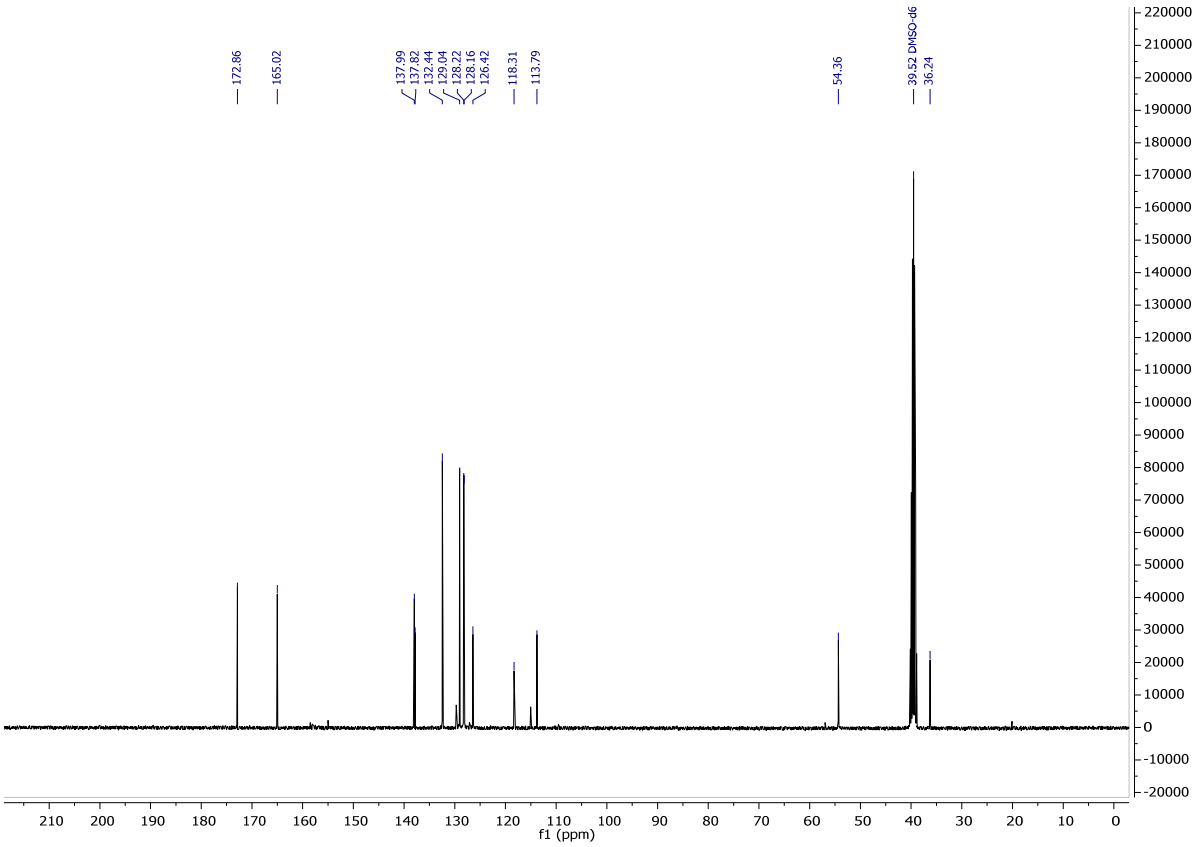


$C_{17}H_{14}F_3NO_3$
MW: 337.3

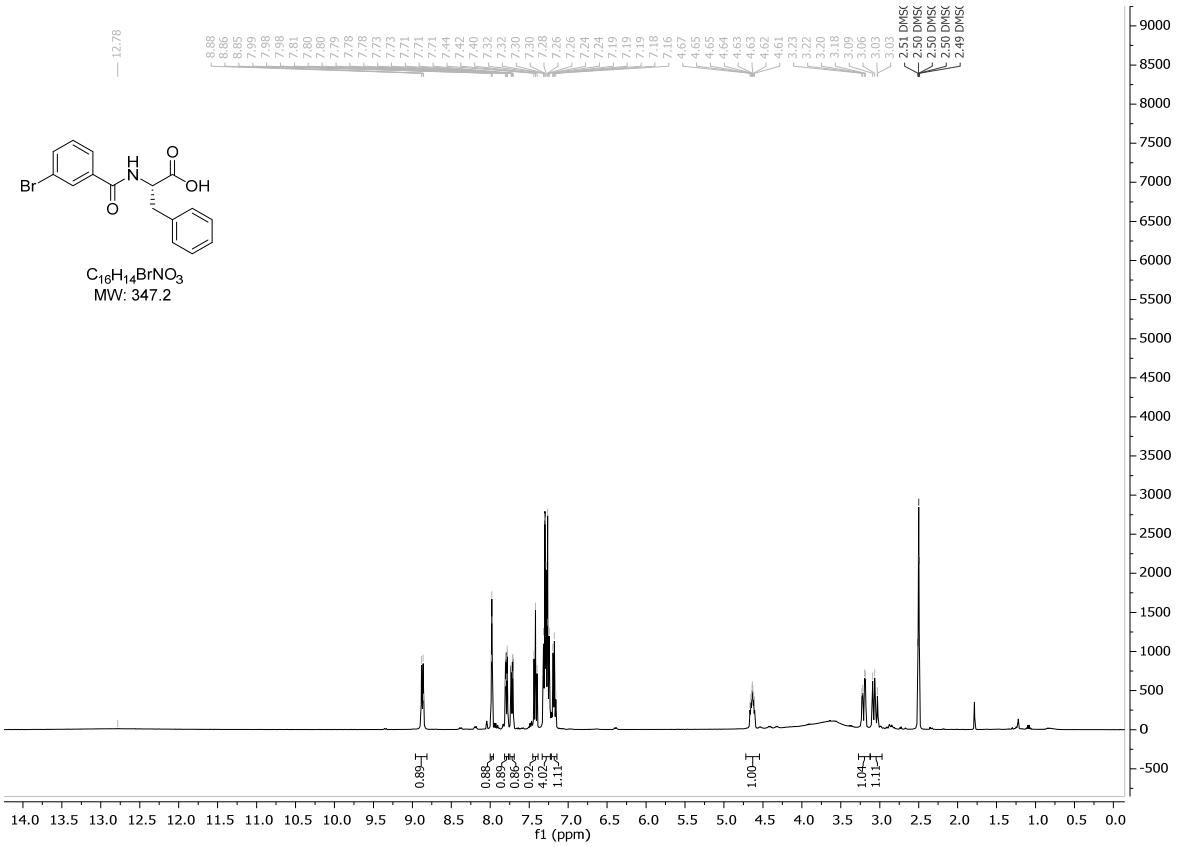


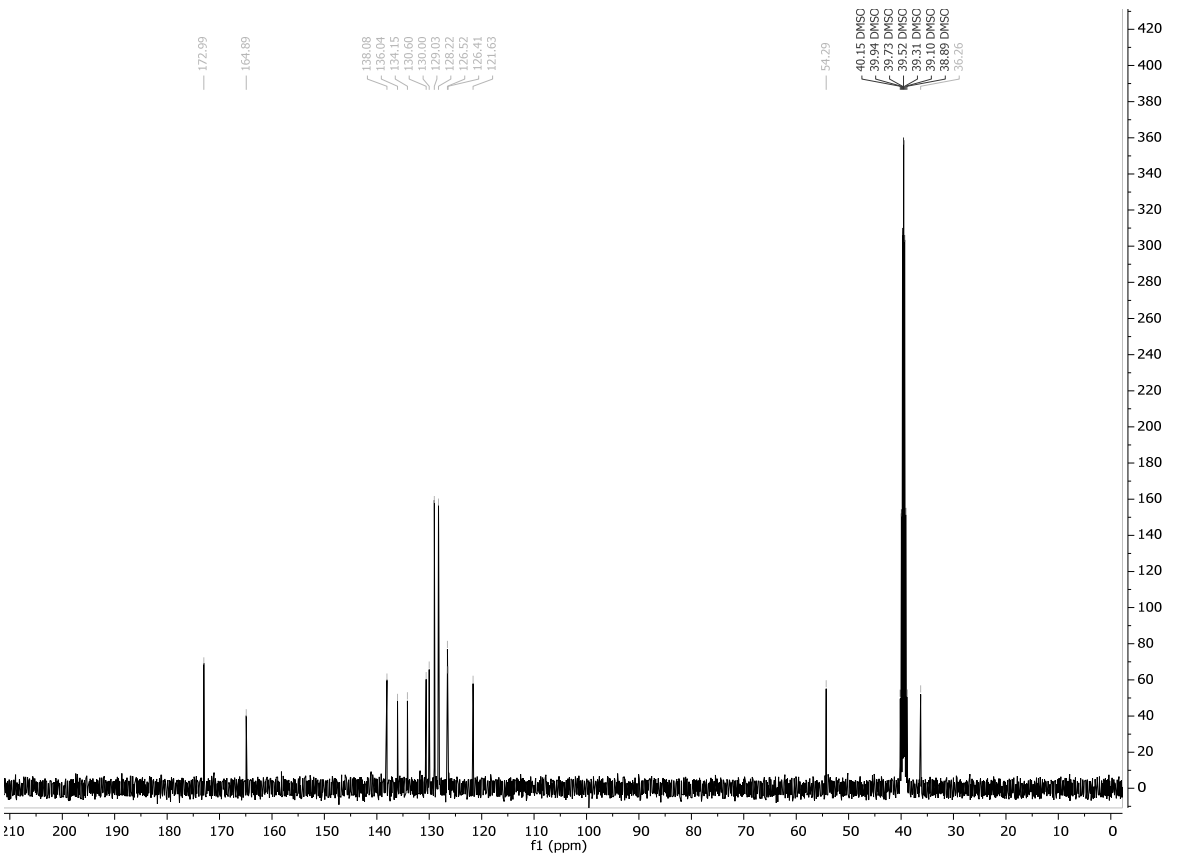
Compound **5f**


NC
H
N
O
OH
O
$C_{17}H_{14}N_2O_3$
MW: 294.3
9.02
9.00
7.96
7.94
7.93
7.91
7.32
7.30
7.30
7.28
7.26
7.24
7.19
7.17
7.16
4.67
4.66
4.65
4.65
4.64
4.64
4.63
4.61
3.24
3.23
3.20
3.19
3.09
3.06
3.06
3.03
2.50 DMSO-d6
0.94
3.90
3.94
1.01
1.00
0.97
0.97
f1 (ppm)


172.86
165.02
137.99
137.82
132.44
129.04
128.22
128.16
126.42
118.31
113.79
54.36
39.52 DMSO-d6
36.24
f1 (ppm)

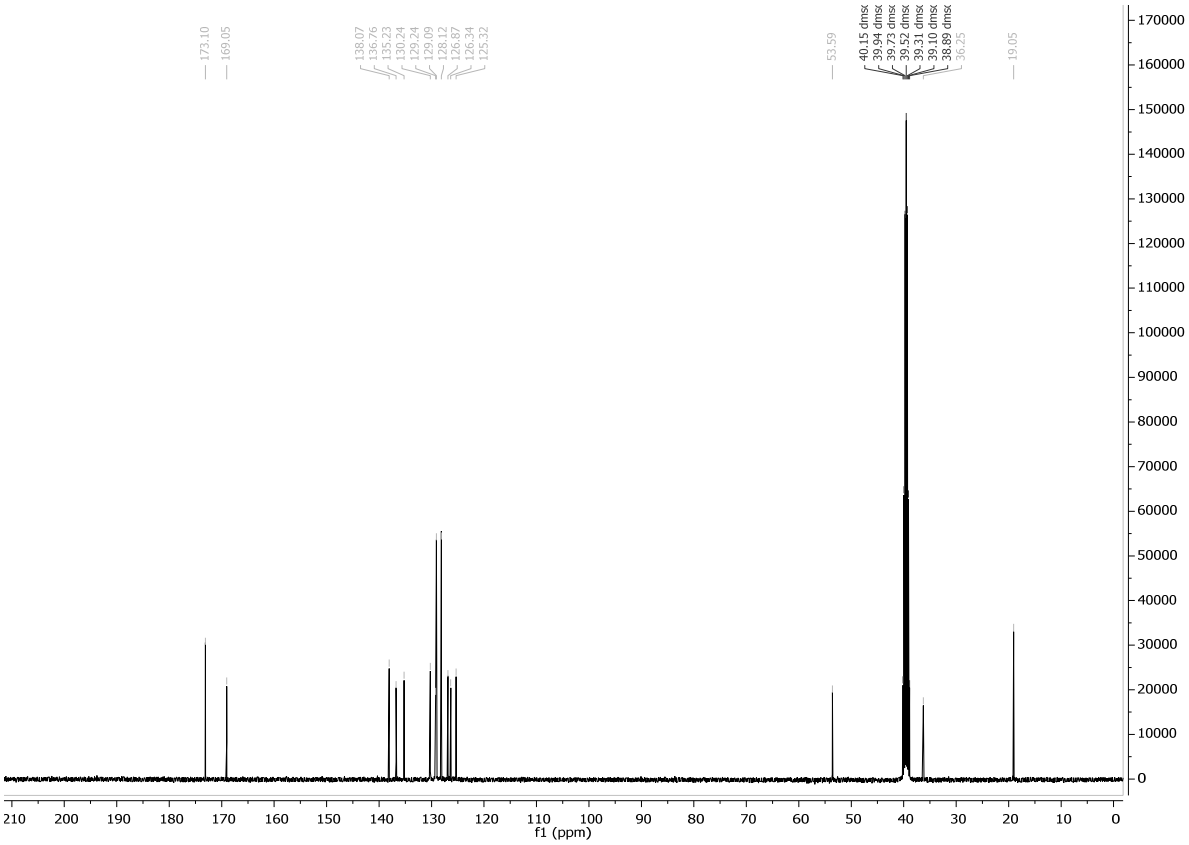
Compound **5g**


12.78
8.88
8.86
8.85
7.99
7.98
7.98
7.81
7.80
7.80
7.79
7.78
7.78
7.73
7.73
7.71
7.71
7.71
7.44
7.42
7.40
7.32
7.32
7.30
7.30
7.28
7.26
7.26
7.24
7.24
7.19
7.19
7.18
7.16
4.67
4.65
4.65
4.64
4.63
4.63
4.62
4.61
3.23
3.22
3.20
3.18
3.09
3.06
3.03
3.03
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
Br
H
N
O
O
OH
C16H14BrNO3
MW: 347.2
0.89
0.88
0.88
0.86
0.92
4.02
1.11
1.00
1.04
1.11
f1 (ppm)

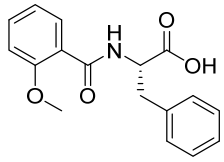

172.99
164.89
138.08
136.04
134.15
130.60
130.00
129.03
128.22
126.52
126.41
121.63
54.29
40.15 DMSO
39.94 DMSO
39.73 DMSO
39.52 DMSO
39.31 DMSO
39.10 DMSO
38.89 DMSO
36.26
f1 (ppm)

Compound **5h**

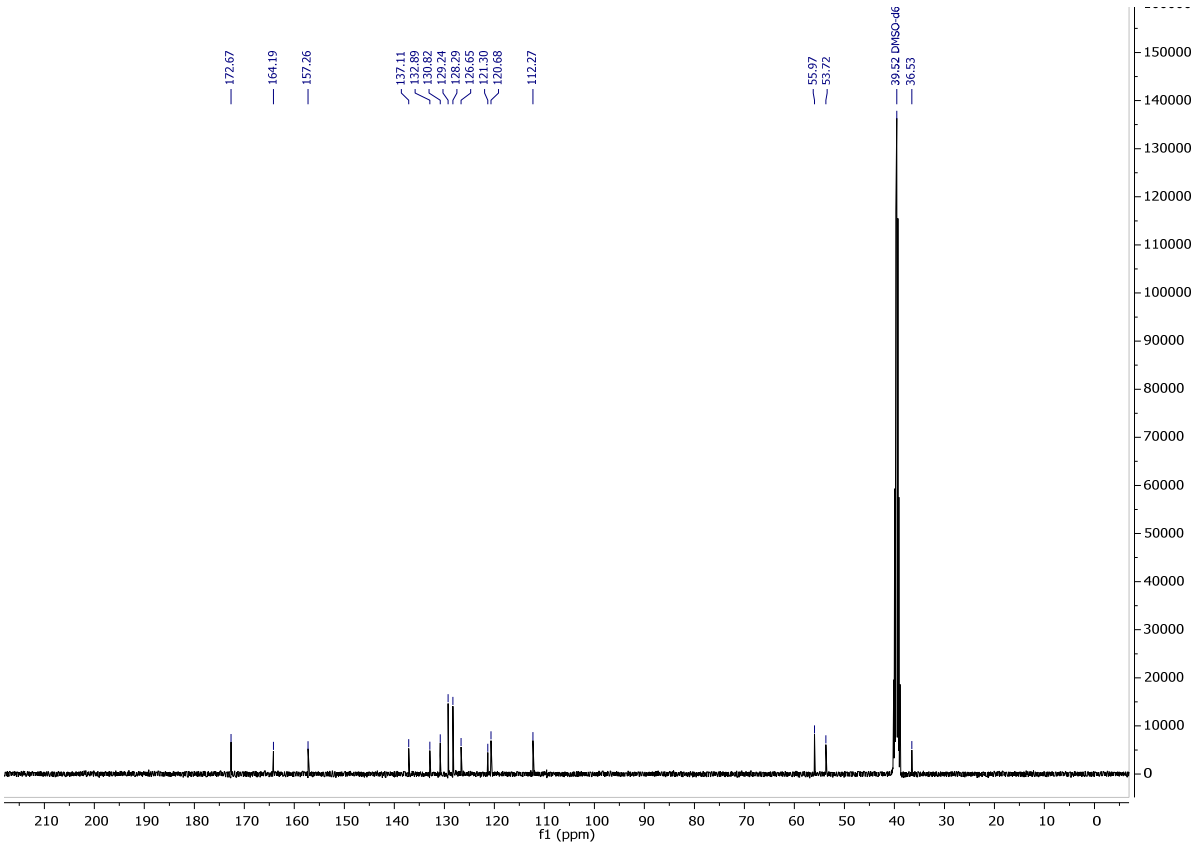
$C_{17}H_{17}NO_3$
MW: 283.3



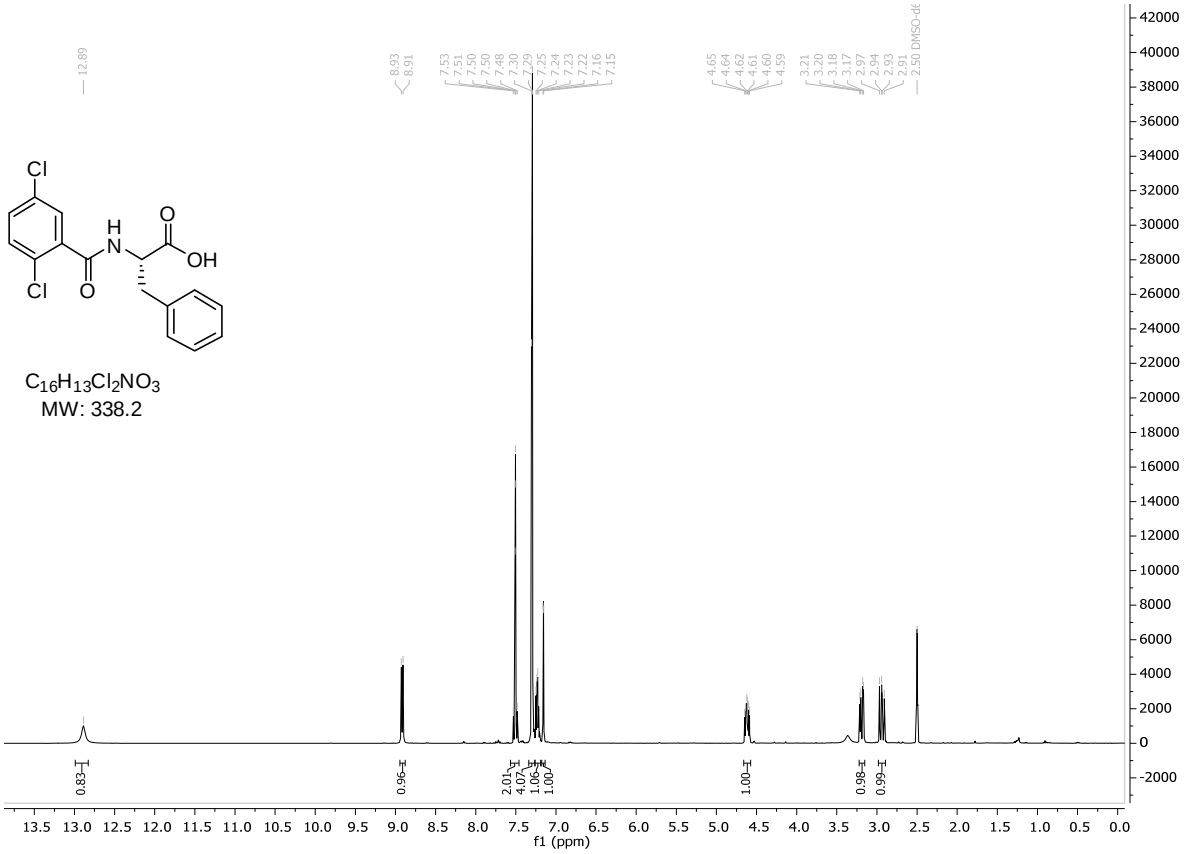
Compound **5i**

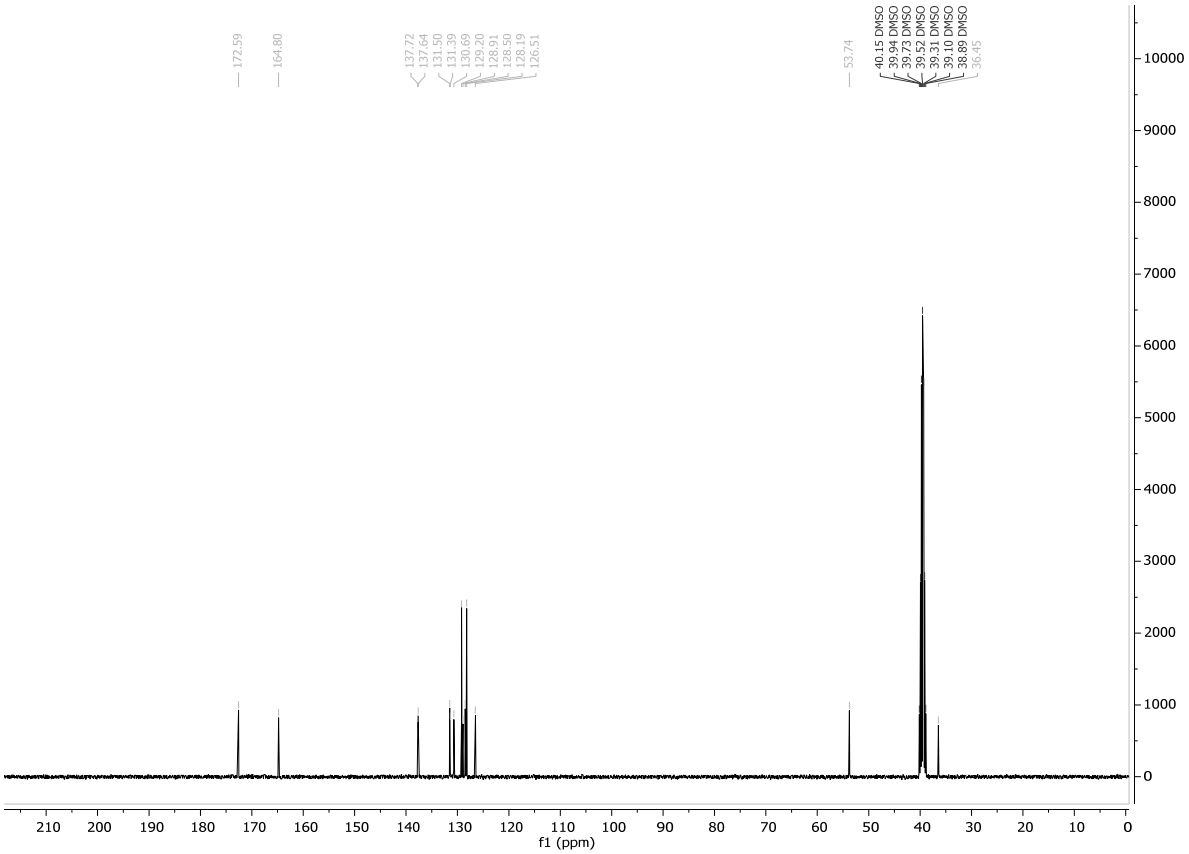


$C_{17}H_{17}NO_4$
MW: 299.3

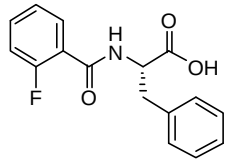


Compound **5j**

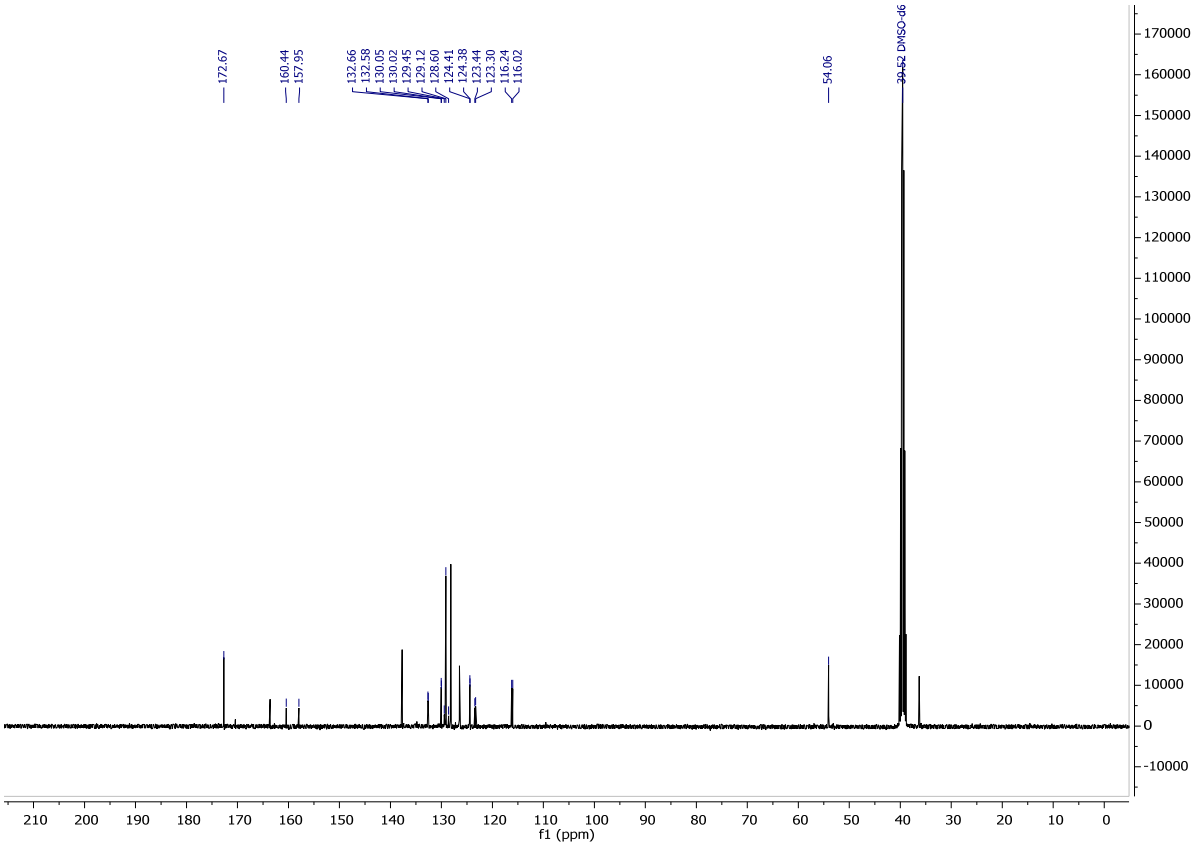




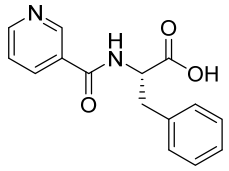
Compound **5k**



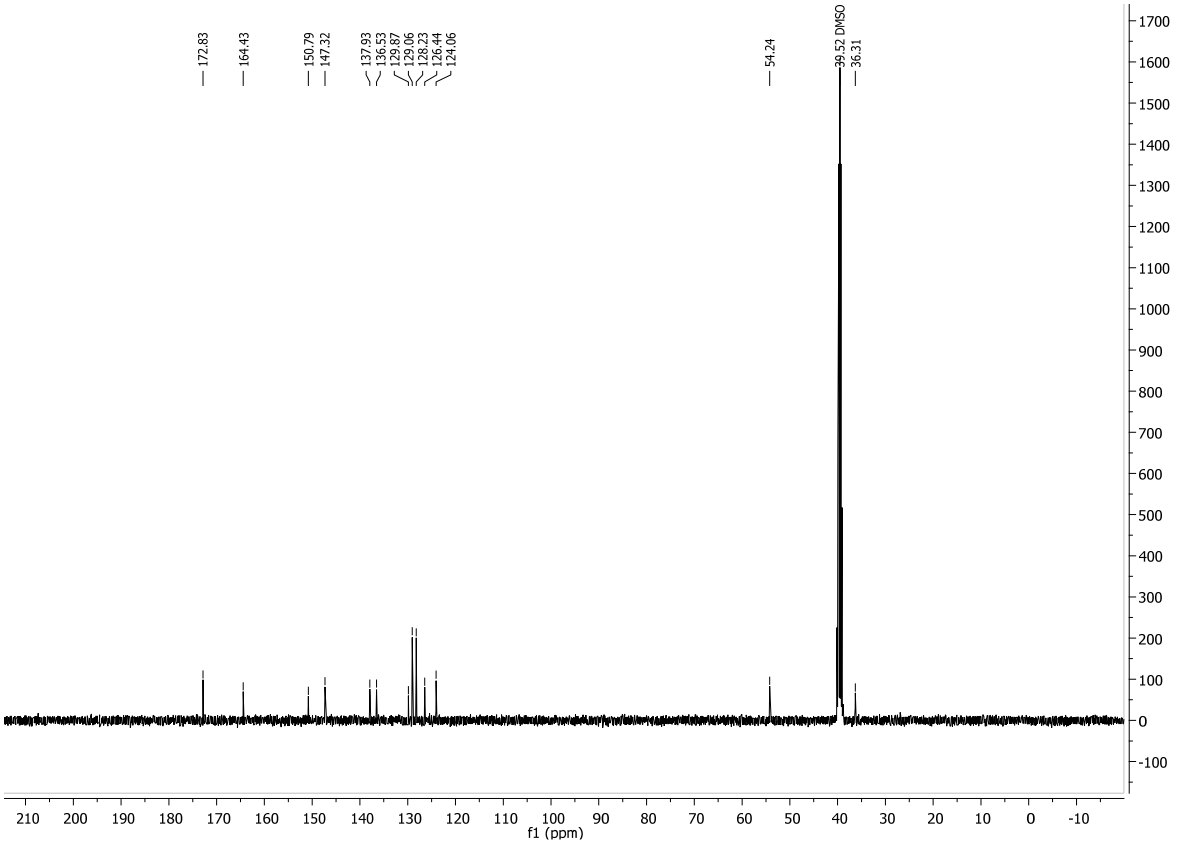
$C_{16}H_{14}FNO_3$
MW: 287.3



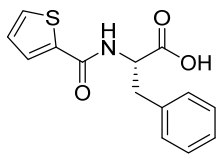
Compound **5l**



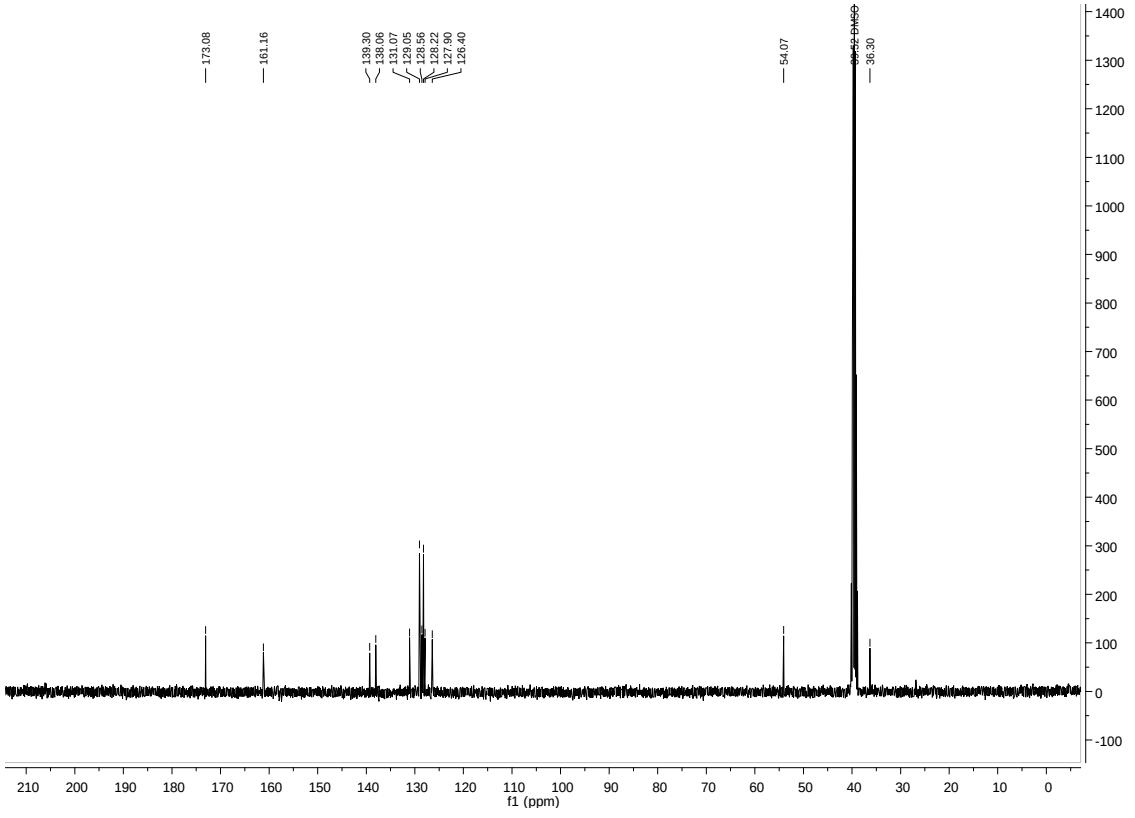
$C_{15}H_{14}N_2O_3$
MW: 270.3



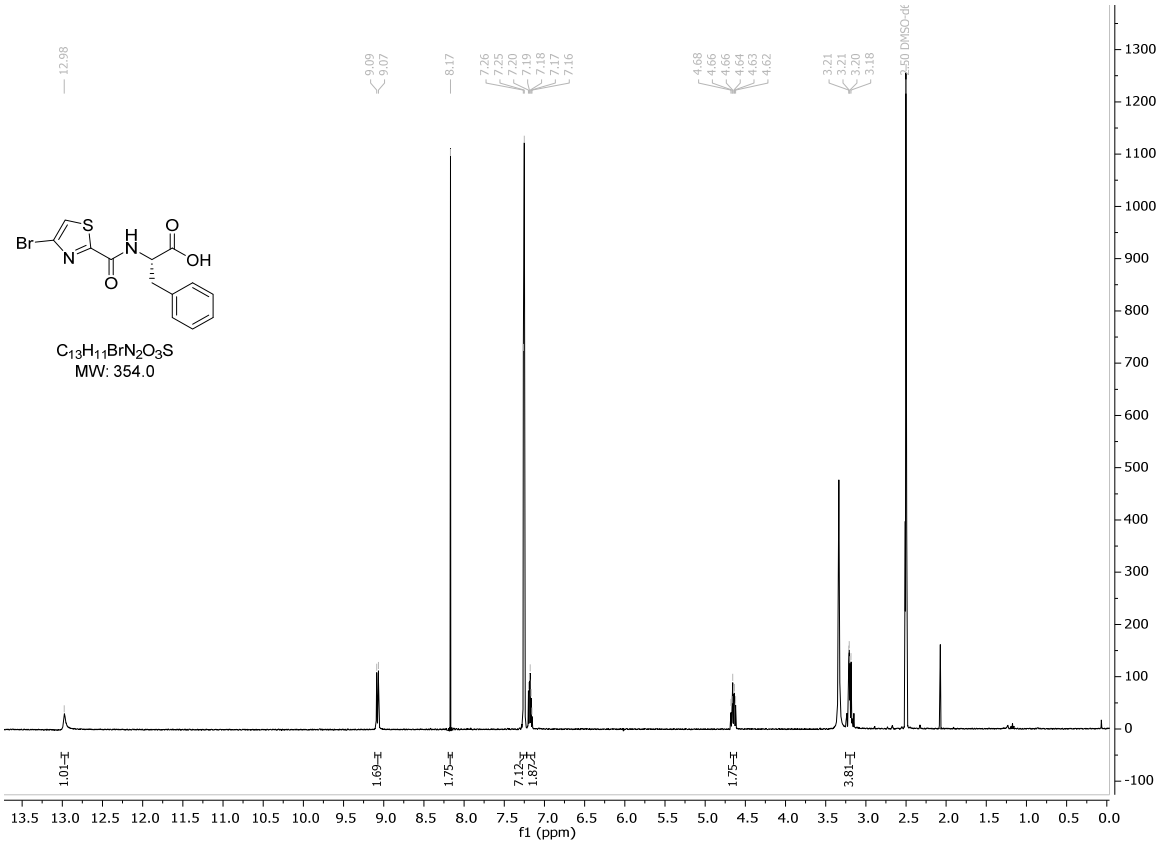
Compound **5m**

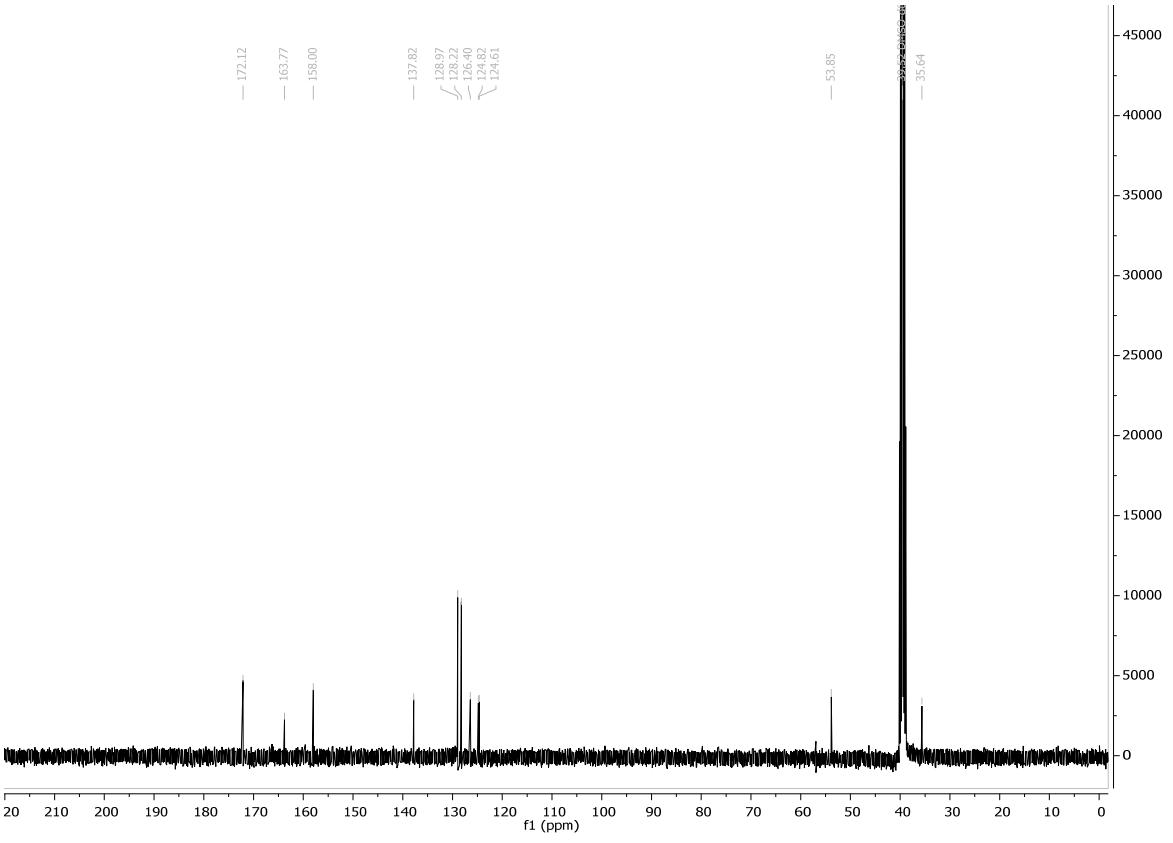


$C_{14}H_{13}NO_3S$
MW: 275.3

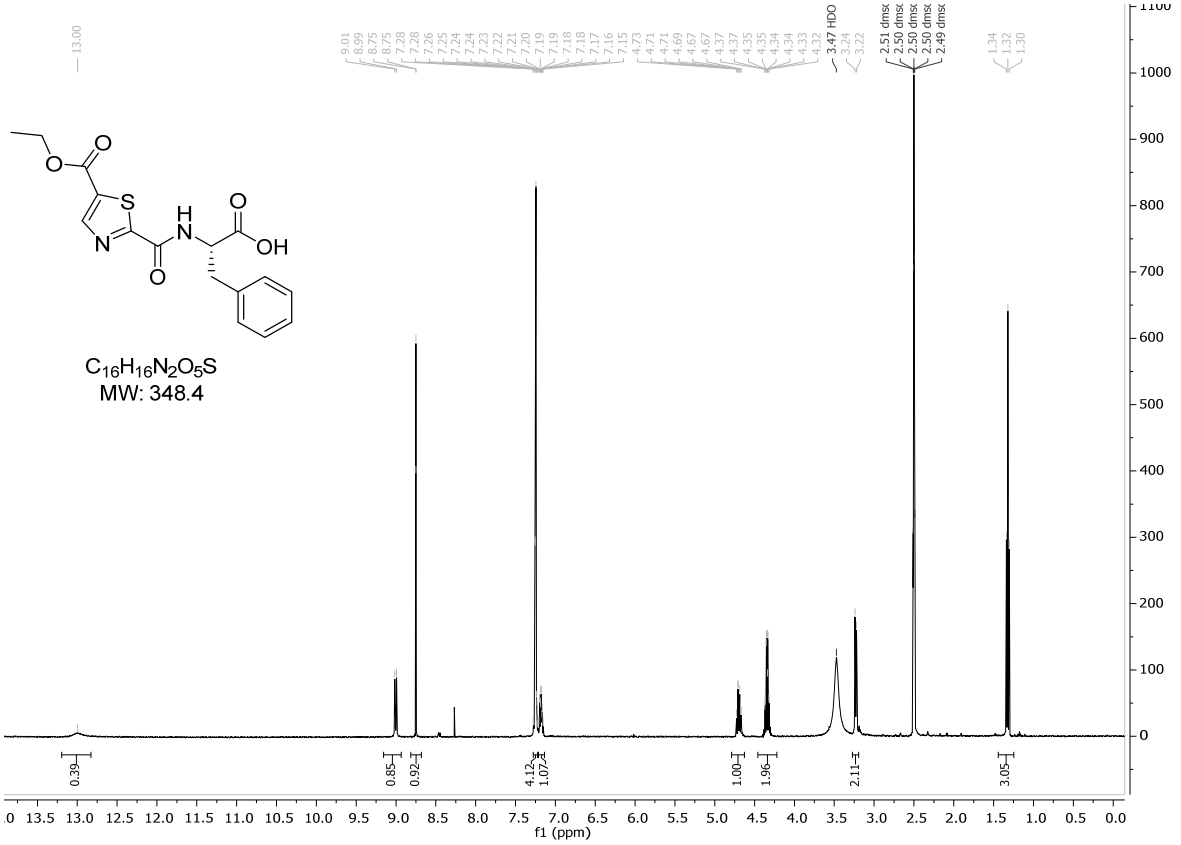


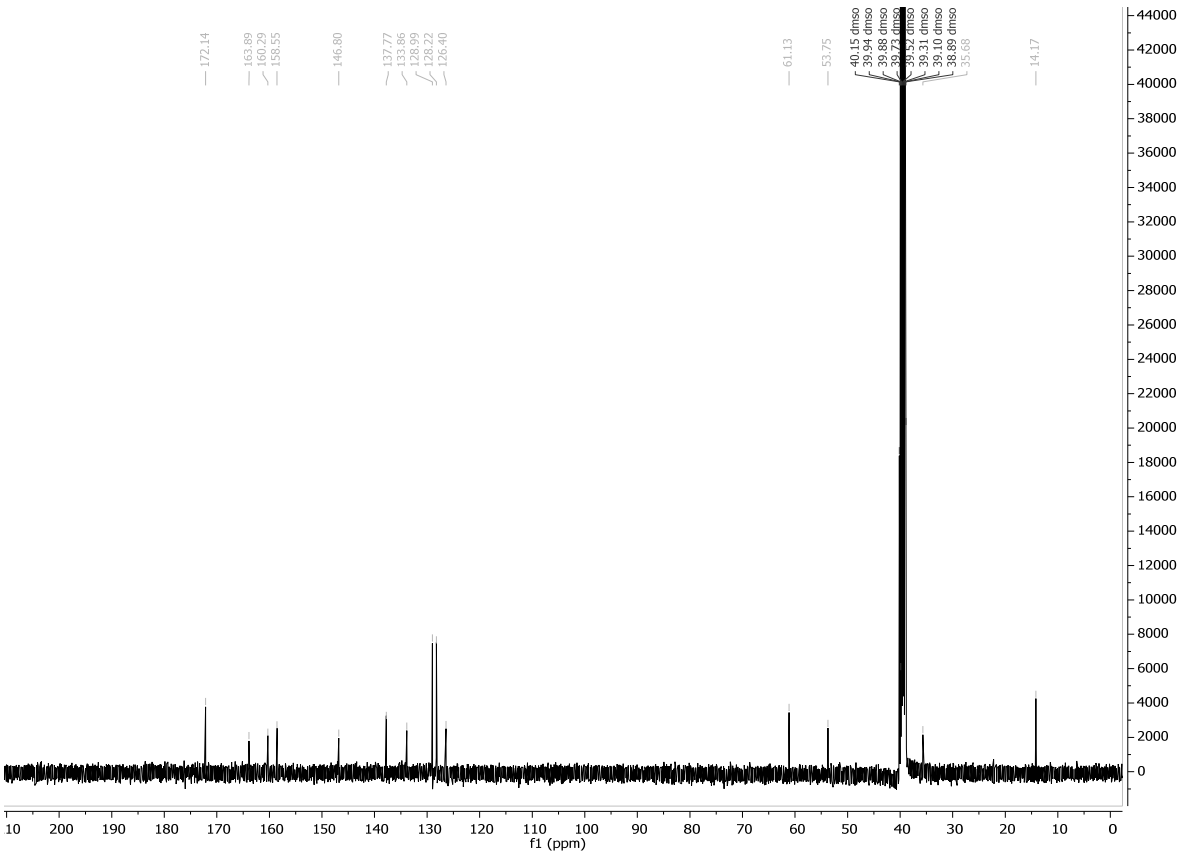
Compound **5n**


12.98
9.09
9.07
8.17
7.26
7.25
7.20
7.19
7.18
7.17
7.16
4.68
4.66
4.66
4.64
4.63
4.62
3.21
3.21
3.20
3.18
2.50 DMSO-d6
Br
S
N
H
N
O
O
OH
C13H11BrN2O3S
MW: 354.0
1.01
1.69
1.75
7.12
1.87
1.75
3.81
f1 (ppm)

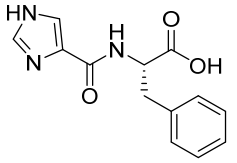

172.12
163.77
158.00
137.82
128.97
128.22
126.40
124.82
124.61
53.85
35.64
f1 (ppm)

Compound **5o**

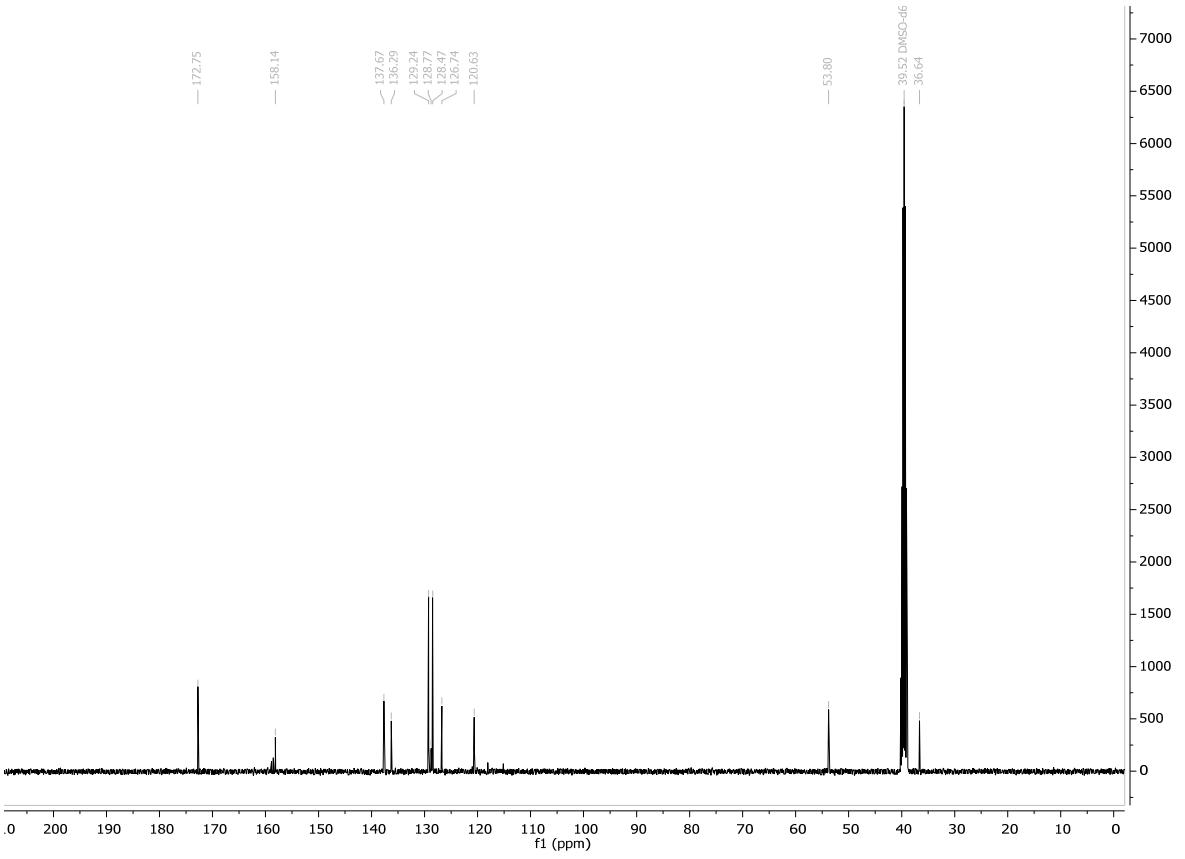




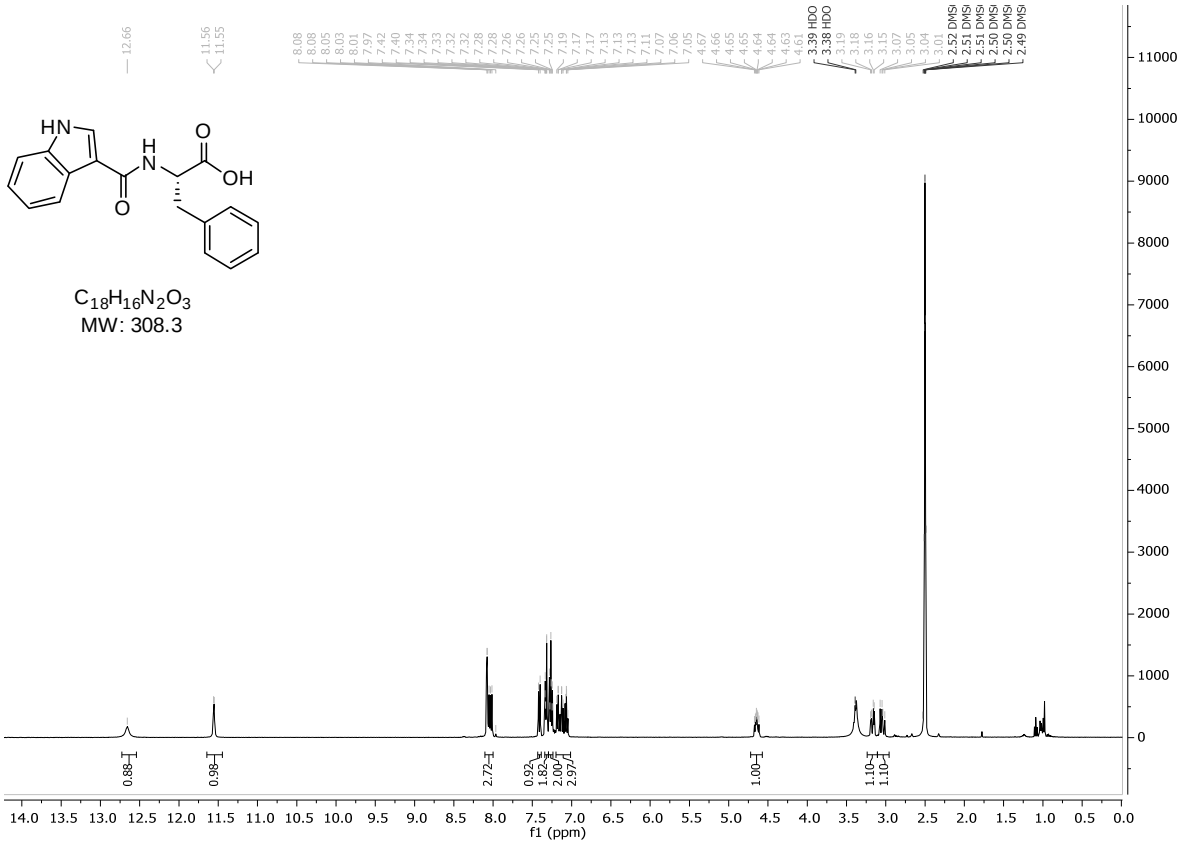
Compound **5p**

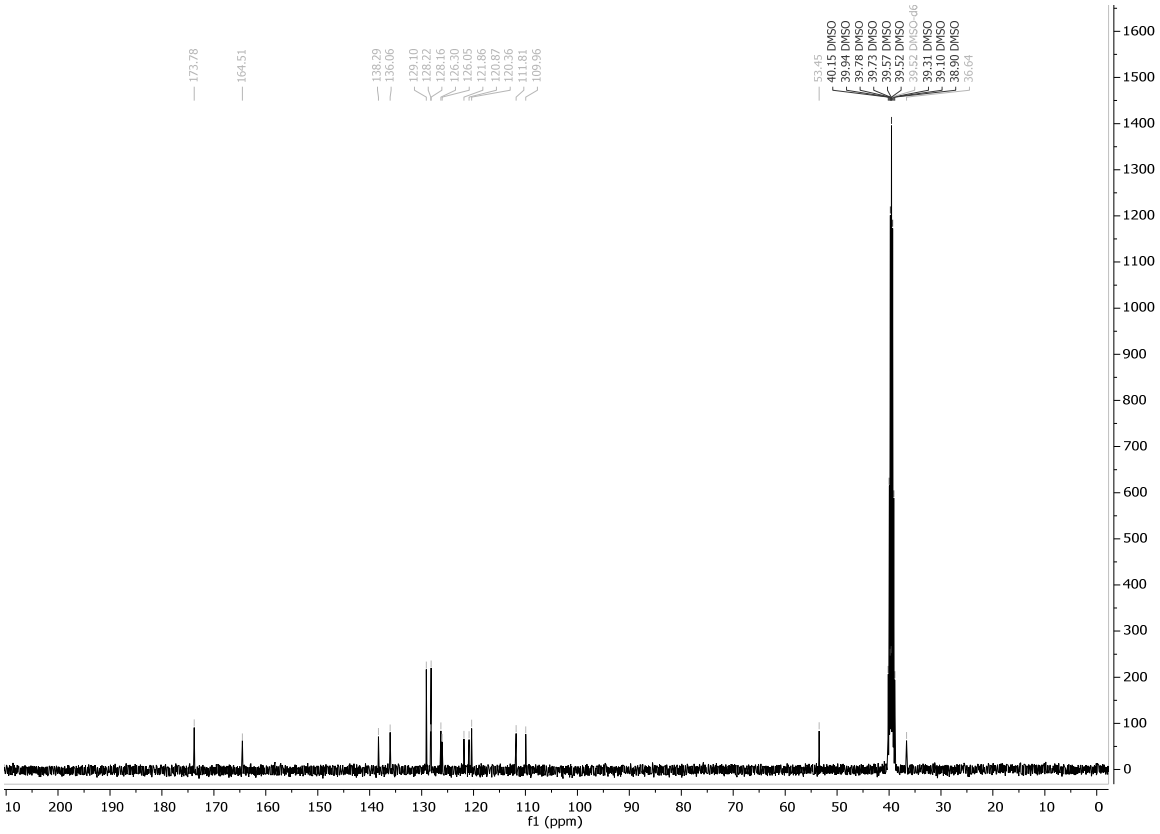


$C_{13}H_{13}N_3O_3$
MW: 259.3

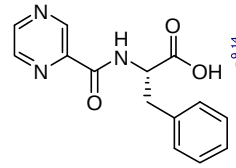


Compound **5q**

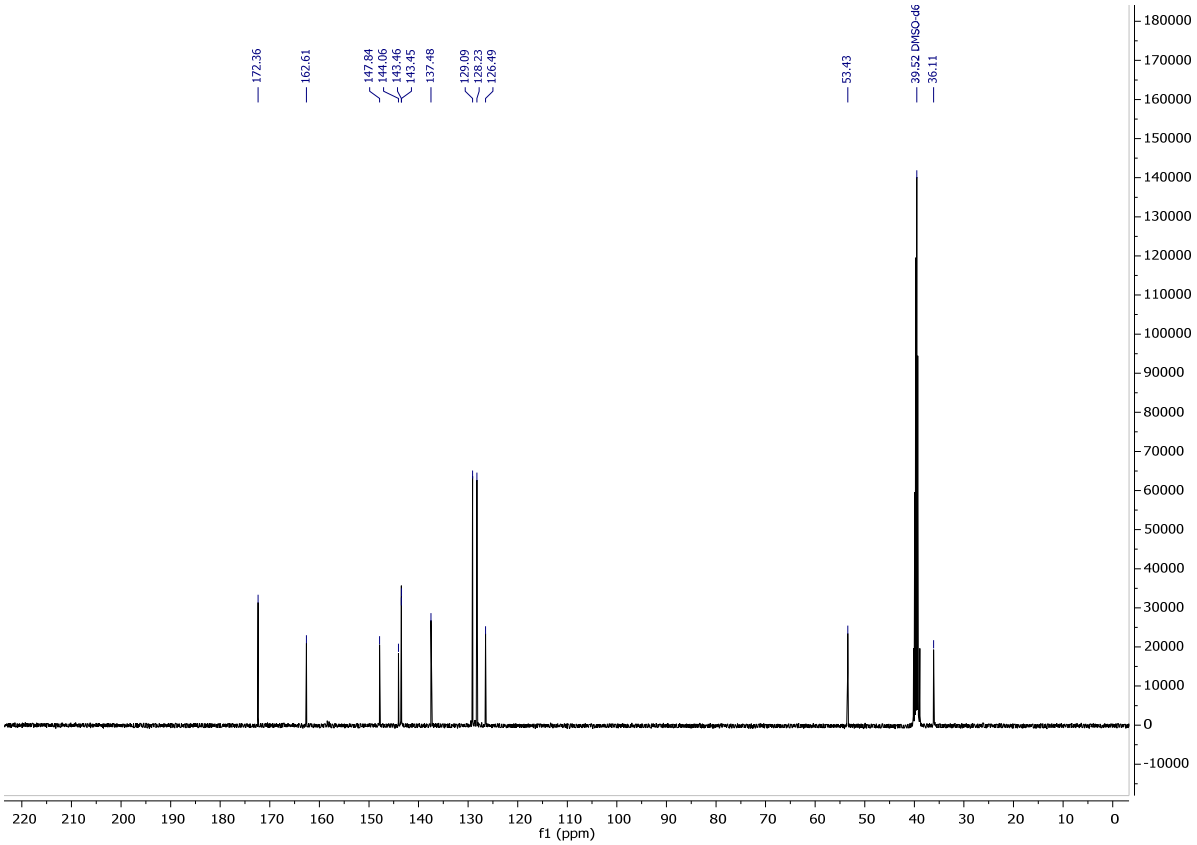




Compound **5r**



$C_{14}H_{13}N_3O_3$
MW: 271.3



Experimental procedures for peptides **6a**, [^{13}C]-**6a*** and **6b**

Procedures used in Scheme 2:

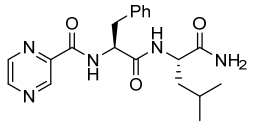
General procedure for Fmoc-SPPS: The peptides were synthesized on Biotage® Initiator+ Alstra™ Automated Microwave Peptide Synthesizer (Biotage AB, Uppsala, Sweden) using standard Fmoc-SPPS from Rink Amide PS resin (0.68 mmol/g) on a 0.1 mmol scale. The side chain protections for the Fmoc amino acids were Gln(Trt), Ser(OtBu), and Lys(Alloc), respectively. Fmoc deprotection was performed using 20% Pip in DMF for 1 × 3 min and 1× 10 min, followed by repeated washing with DMF. Each Fmoc protected amino acid was coupled sequentially to the Rink Amide PS resin in a five-fold excess (5 equiv, 0.5 M) using HBTU (5 equiv, 0.6 M) and DIPEA (10 equiv, 2.0 M) in DMF for 40 min at r.t. The first coupling to the resin was repeated twice. At the end of the synthesis the partially protected peptide resin was washed with DCM and dried under vacuum to ensure complete removal of solvent.

General procedure for Alloc deprotection: A 8 mL vial was charged with the alloc protected peptide resin and $Pd(PPh_3)_4$ (5mol%, 0.005 mmol, 6 mg). The vial was sealed with a screw cap fitted with a Teflon® seal and evacuated, and backfilled with nitrogen gas. Thereafter, dry DCM (3 mL) and $PhSiH_3$ (10 equiv, 10 mmol, 123 µL) were added through the self-sealing septa by a syringe using a 21G needle. The needle was removed and the reaction was allowed to agitate for 2 h at r.t. Next, excess pressure was released using a needle and thereafter the resin was separated by filtration and washed with DCM (3 × 5 mL) and MeOH (3 × 5 mL). The procedure was repeated if necessary.

General procedure D: The partially protected peptide resin (1 equiv, 0.1 mmol, 0.68 mmol/g) was transferred to a 8 mL vial charged with (hetero)aryl iodide (4 equiv, 0.4 mmol), $MePh_2SiCOOH$ **3** (3 equiv, 0.3 mmol, 73 mg) or $MePh_2Si^{13}COOH$ [^{13}C]-**3*** (3 equiv, 0.3 mmol, 73 mg, to label peptide [^{13}C]-**6a***), KF (3 equiv, 0.3 mmol, 17 mg), palladacycle precatalyst **2** (5 mol%, 0,005 mmol, 5 mg) and TEA (4 equiv, 0.4 mmol, 56 µL). The vial was sealed with a screw cap fitted with a Teflon® seal and evacuated, and backfilled with argon/nitrogen gas. Thereafter, dry DMF (3 mL) was added through the self-sealing septa by a syringe using a 21G needle. The needle was removed and the reaction was allowed to agitate for 15 h at r.t. using ultrasonication. Next, excess pressure was released using a needle and thereafter the resin was separated by filtration and washed with DMF (3 × 5 mL), DCM (3 × 5 mL) and MeOH (3 × 5 mL). The resin was dried under vacuum and the final product was thereafter cleaved from the resin by treatment with 95% aqueous TFA (2 mL) and TES (100 µL) followed by agitation for 2 h at r.t. The resin was filtered off and washed with TFA (3 × 300µL). The filtrate was collected in a centrifuge tube and concentrated in a stream of argon/nitrogen to a volume of < 2 mL. The product was precipitated by the addition of cold diethylether (12 mL), collected by centrifugation, washed with diethylether (3 × 10 mL) and dried in a stream of argon/nitrogen and in vacuum over night. The crude peptide was purified by preparative RP-HPLC.

Characterization data, NMR spectra and RP-HPLC Chromatograms for peptides **6a** and [^{13}C]-**6a***

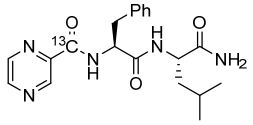
N-((*S*)-1-(((*S*)-1-Amino-4-methyl-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)pyrazine-2-carboxamide (Peptide 6a)



$C_{20}H_{25}N_5O_3$
MW: 383.2

General procedure for Fmoc-SPPS was followed for synthesizing the dipeptide and general procedure D was followed for the aminocarbonylation using 2-iodopyrazine (4 equiv, 0.4 mmol, 40 µL). The title peptide **6a** (30 mg, 78%) was obtained as a white solid after RP-HPLC purification. RP-HPLC purity: Restek Alure biphenyl > 99%, Thermo Hypersil Fluophase RP > 99%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.12 (d, J = 1.6 Hz, 1H), 8.87 (d, J = 2.3 Hz, 1H), 8.73 (dd, J = 2.4, 1.6 Hz, 1H), 8.69 (d, J = 8.5 Hz, 1H), 8.27 (d, J = 8.4 Hz, 1H), 7.34 (s, 1H), 7.26-7.10 (m, 5H), 7.02 (s, 1H), 4.83 (dd, J = 8.5, 4.7 Hz, 1H), 4.30 (d, J = 8.5, 6.5 Hz, 1H), 3.17 (dd, J = 13.8, 4.7 Hz, 1H), 3.08 (dd, J = 13.8, 8.5 Hz, 1H), 1.65-1.53 (m, 1H), 1.53-1.43 (m, 2H), 0.88 (d, J = 6.5 Hz, 3H), 0.84 (d, J = 6.5 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.8, 170.0, 162.3, 147.8, 144.1, 143.4, 137.3, 129.3, 128.1, 126.4, 53.9, 50.9, 41.1, 37.5, 24.3, 23.0, 21.7. HRMS (ES) m/z calcd for [M+H+]: $C_{20}H_{26}N_5O_3$ 384.2036 found 384.2035.

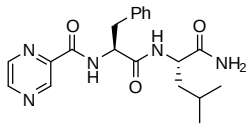
N-((*S*)-1-(((*S*)-1-Amino-4-methyl-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)pyrazine-2-carboxamide-^{13}C (Peptide [^{13}C]-6a*)



$C_{19}{}^{13}CH_{25}N_5O_3$
MW: 384.2

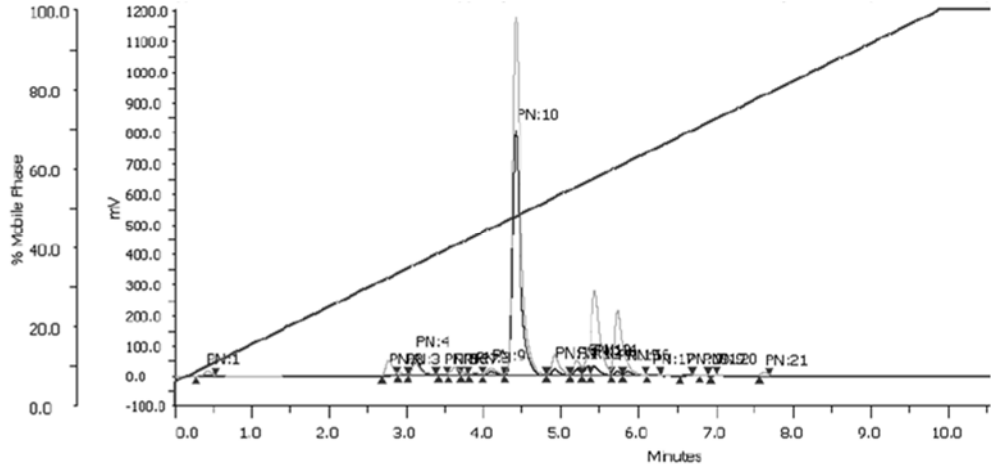
General procedure for Fmoc-SPPS was followed for synthesizing the dipeptide and general procedure D was followed for the aminocarbonylation using 2-iodopyrazine (4 equiv, 0.4 mmol, 40 µL) and $MePh_2Si^{13}COOH$ [^{13}C]-**3*** (3 equiv, 0.3 mmol, 73 mg). The title peptide [^{13}C]-**6a*** (28 mg, 73%) was obtained as a white solid after RP-HPLC purification. RP-HPLC purity: Restek Alure biphenyl > 98%, Thermo Hypersil Fluophase RP > 98%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.12 (s, 1H), 8.88 (dd, J = 2.3, 1.6 Hz, 1H), 8.74 (dd, J = 2.4, 1.6 Hz, 1H), 8.69 (dd, J = 8.5, 3.2 Hz, 1H), 8.26 (d, J = 8.5 Hz, 1H), 7.33 (s, 1H), 7.26-7.12 (m, 5H), 7.01 (s, 1H), 4.82 (dd, J = 7.3, 4.3 Hz, 1H), 4.34-4.25 (m, 1H), 3.17 (dd, J = 13.8, 4.7 Hz, 1H), 3.08 (dd, J = 13.8, 8.5 Hz, 1H), 1.65-1.53 (m, 1H), 1.48 (ddd, J = 8.5, 5.6, 2.3 Hz, 2H), 0.88 (d, J = 6.5 Hz, 2H), 0.84 (d, J = 6.5 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.8, 169.9, 162.3 (^{13}C-enriched), 147.8, 143.42, 143.38, 137.3, 129.3, 128.0, 126.3, 53.8, 50.9, 41.1, 37.5, 24.2, 23.0, 21.7. HRMS (ES) m/z calcd for [M+H+]: $C_{19}{}^{13}CH_{26}N_5O_3$ 385.2069 found 385.2067.

Peptide 6a

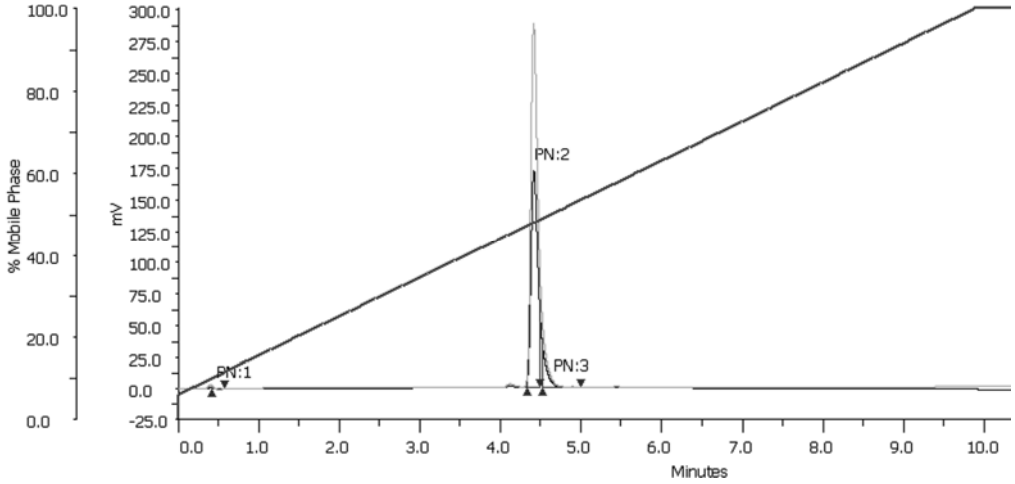


$C_{20}H_{25}N_5O_3$
MW: 383.2

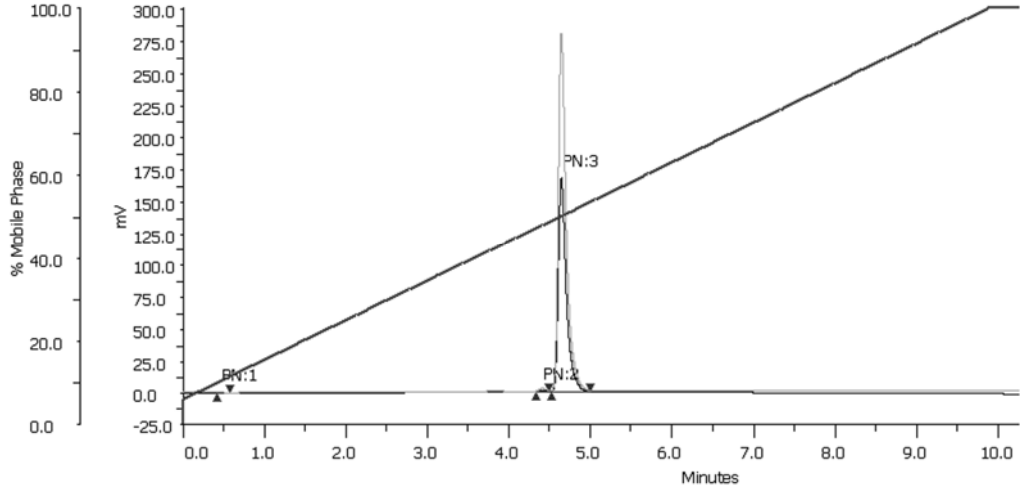
RP-HPLC Crude peptide: PN 10 is the desired peptide.



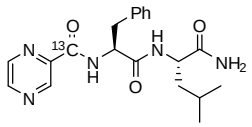
RP-HPLC purity: Restek Alure biphenyl



RP-HPLC purity: Thermo Hypersil Fluophase RP

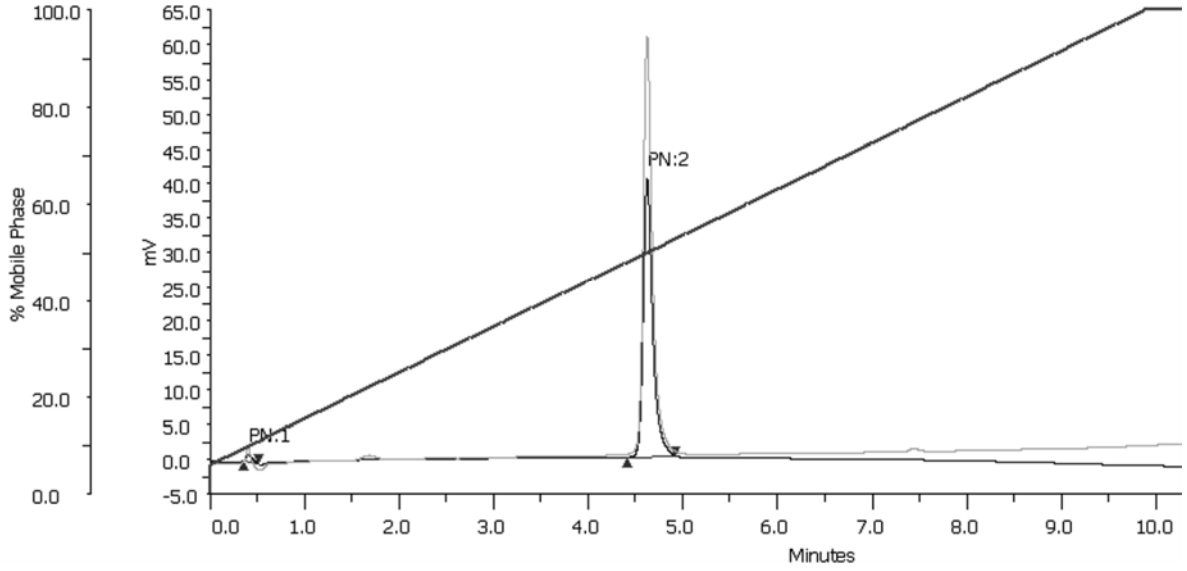


Peptide [^{13}C]-6a*

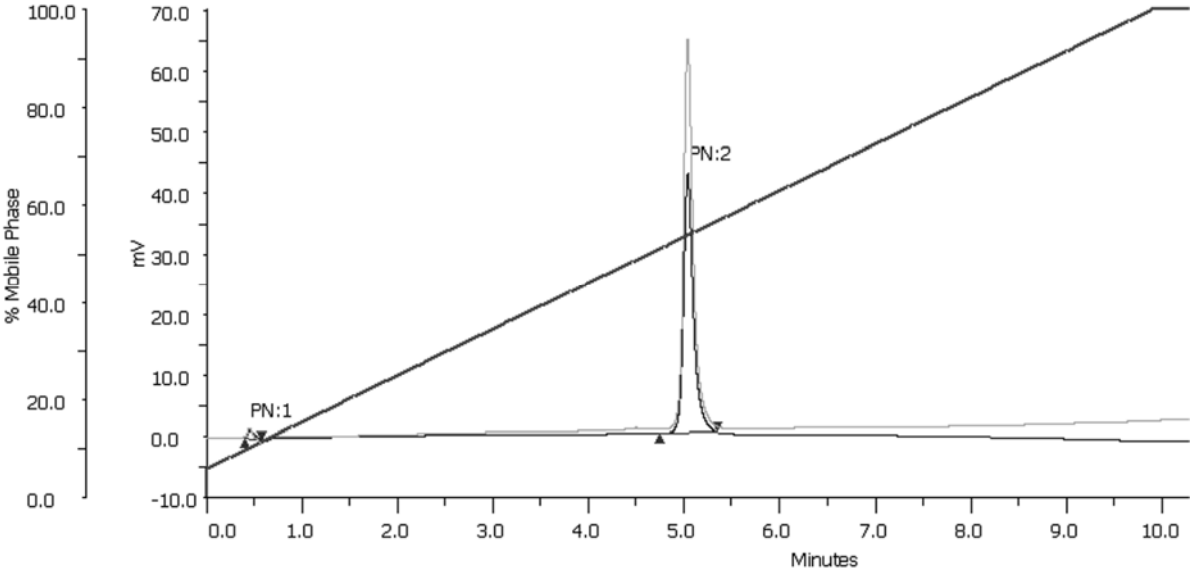


$C_{19}{}^{13}CH_{25}N_5O_3$
MW: 384.2

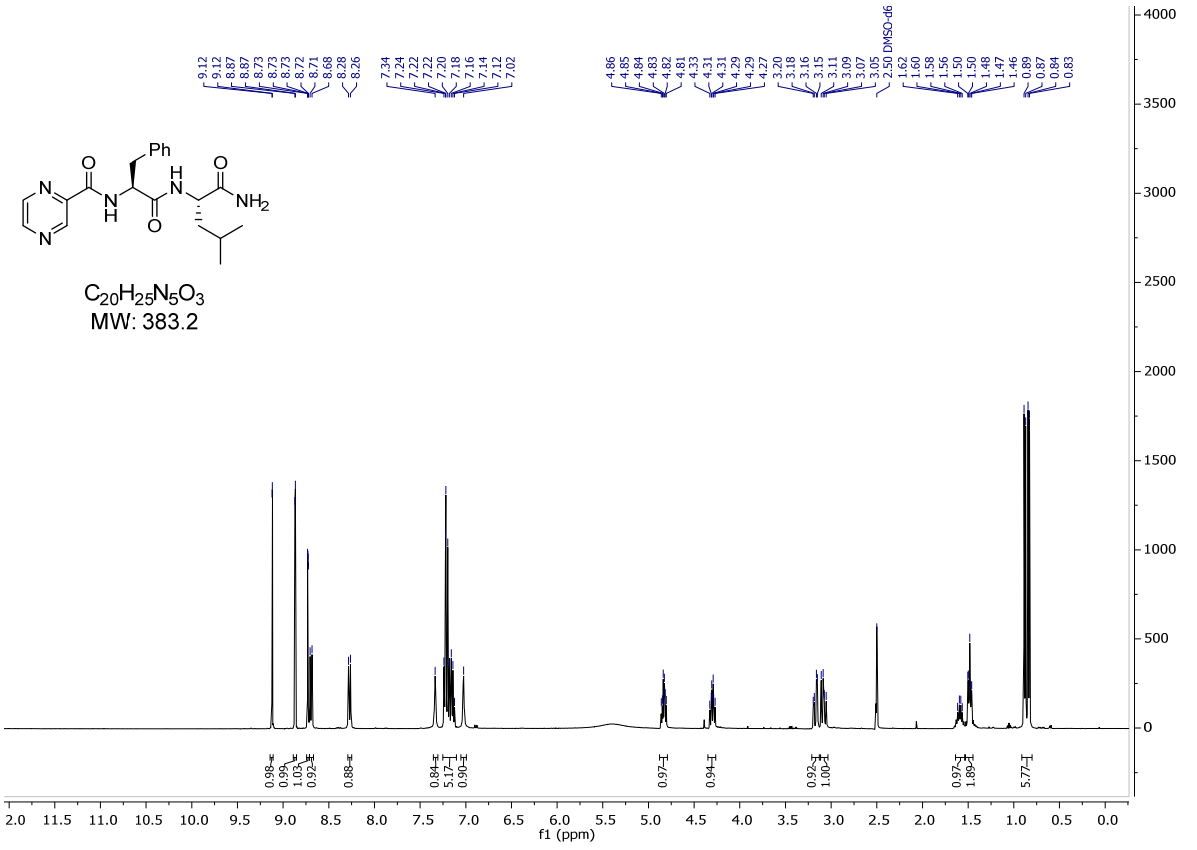
RP-HPLC purity: Restek Alure biphenyl

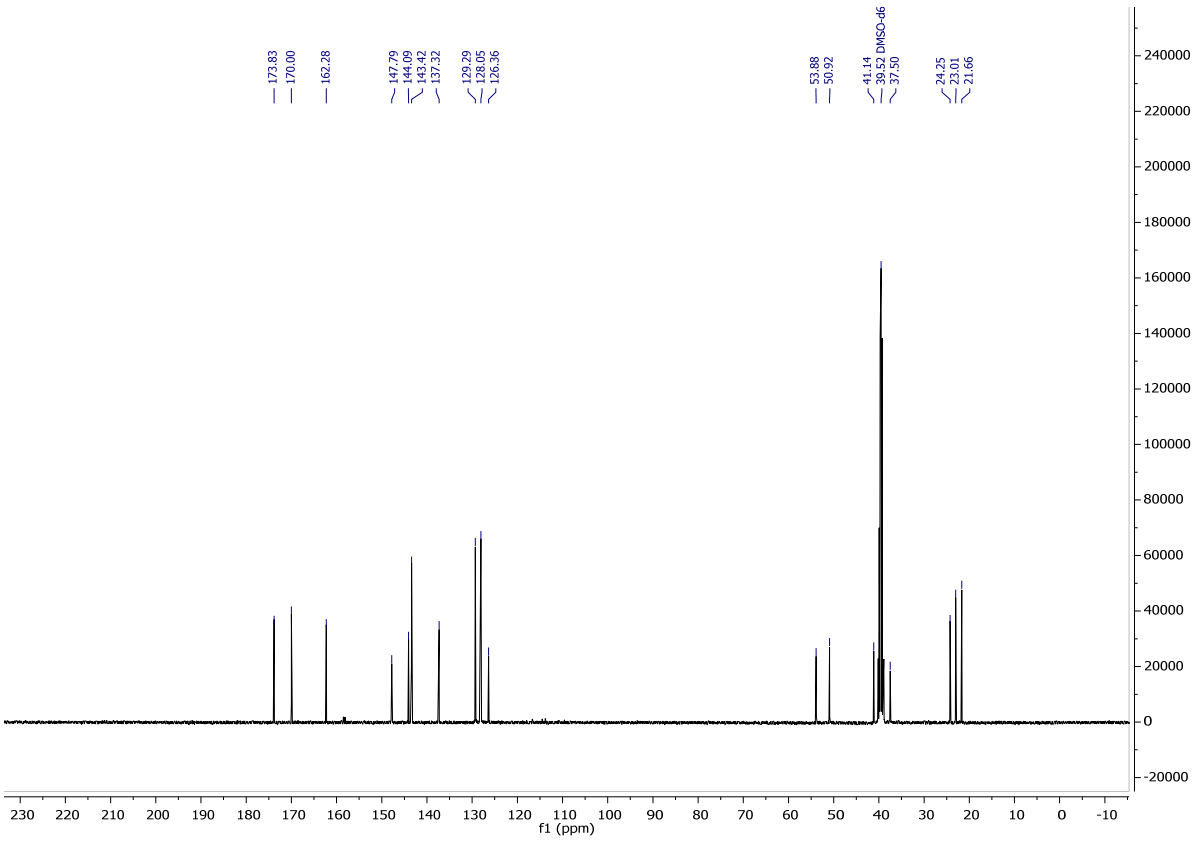


RP-HPLC purity: Thermo Hypersil Fluophase RP

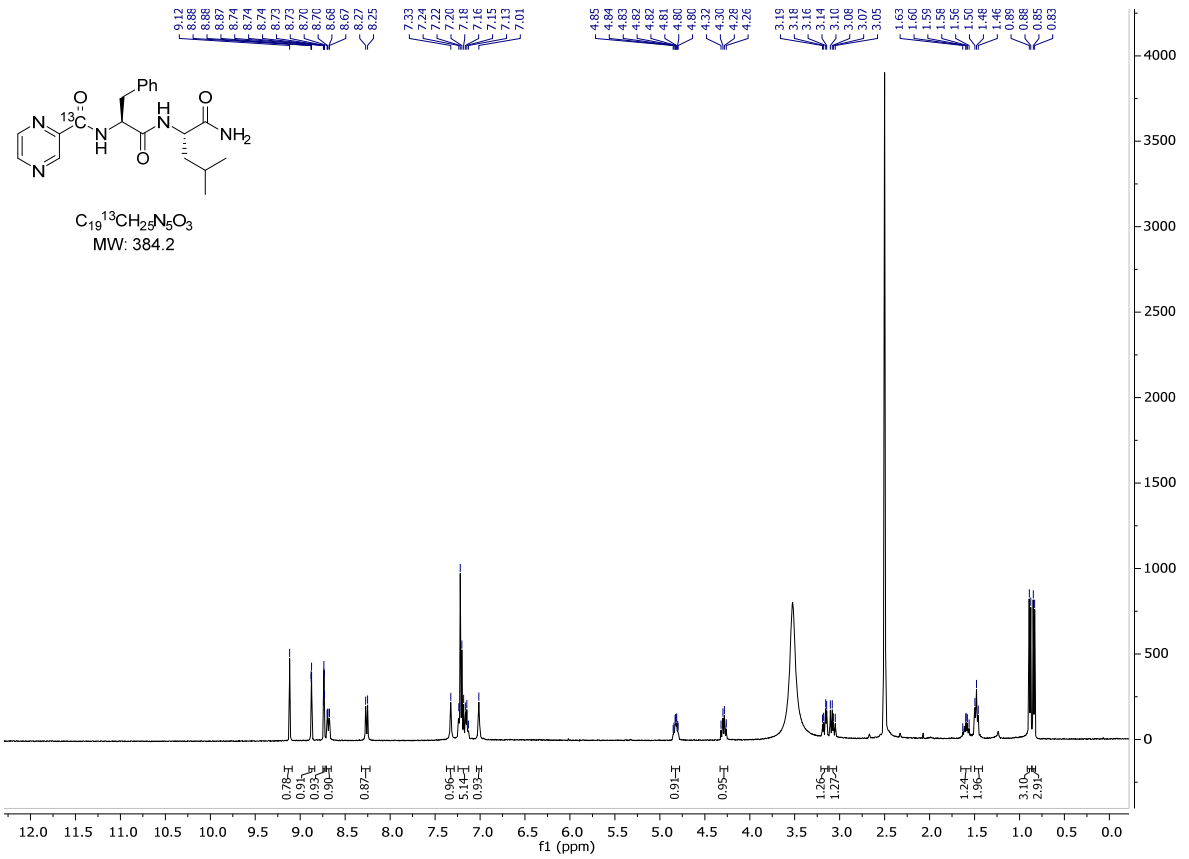


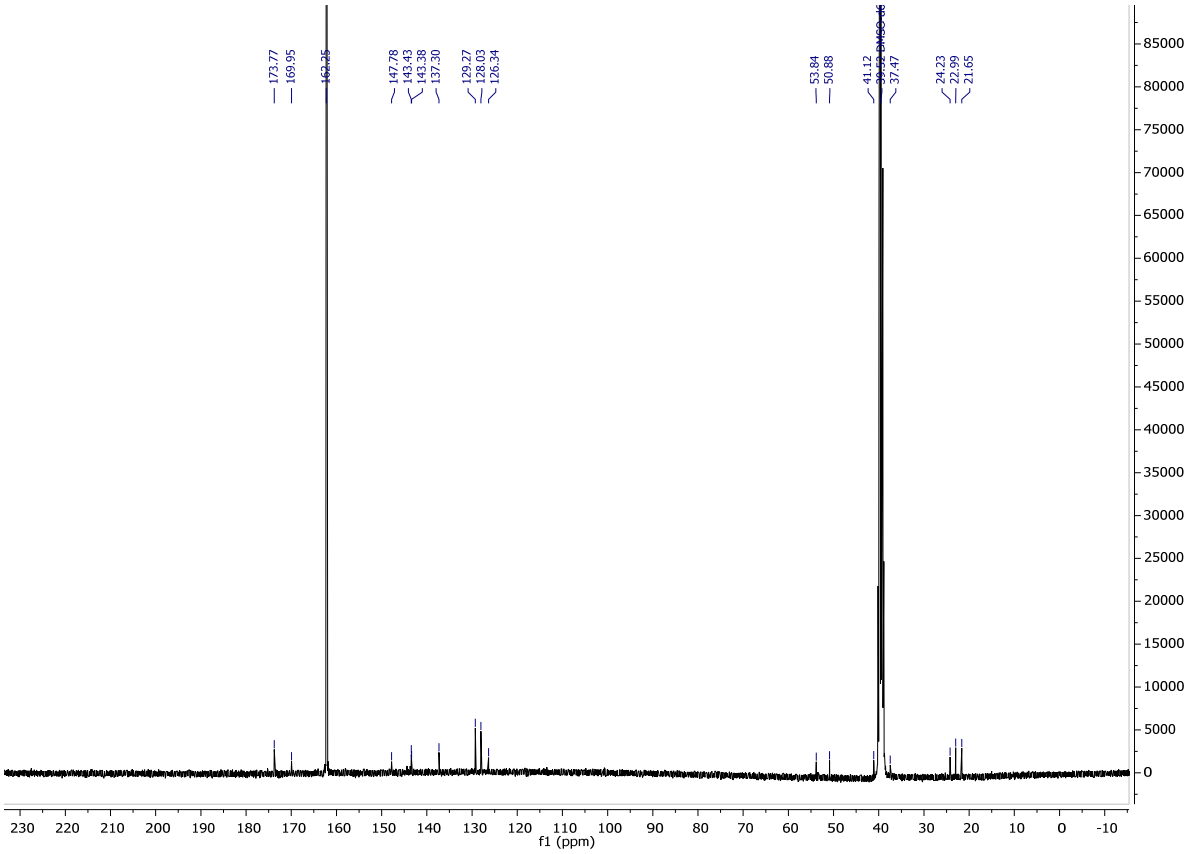
Peptide 6a





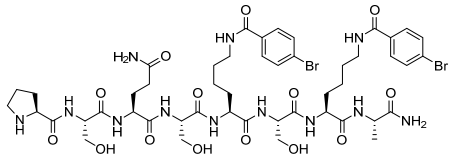
Peptide [^{13}C]-6a*





Characterization data, RP-HPLC Chromatograms and NMR spectra for peptide **6b**

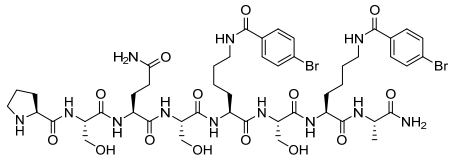
(*S*)-*N*[1]-((7*S*,10*S*,13*S*,16*S*)-7-(((*S*)-1-Amino-1-oxopropan-2-yl)carbamoyl)-13-(4-(4-bromobenzamido)butyl)-1-(4-bromophenyl)-17-hydroxy-10-(hydroxymethyl)-1,9,12,15-tetraoxo-2,8,11,14-tetraazaheptadecan-16-yl)-2-((S)-3-hydroxy-2-((S)-pyrrolidine-2-carboxamido)propanamido)pentanediamide (Peptide 6b)[11]



$C_{48}H_{68}Br_2N_{12}O_{14}$
MW: 1194.3

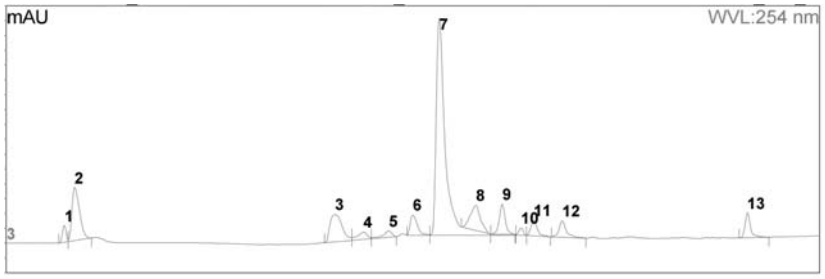
General procedure for Fmoc-SPPS was followed for synthesizing the peptide and the Fmoc protecting group in the N-terminal was removed after the aminocarbonylation. General procedure for alloc deprotection was performed and thereafter general procedure D was followed for the aminocarbonylation using 1-bromo-3-iodobenzene (8 equiv, 0.8 mmol, 227 mg), $MePh_2SiCOOH$ **3** (4 equiv, 0.4 mmol, 97 mg), KF (4 equiv, 0.4 mmol, 24 mg). The title peptide **6b** (73 mg, 61%) was obtained as a white solid after RP-HPLC purification. RP-HPLC purity: Restek Alure biphenyl > 98.3%, Thermo Hypersil Fluophase RP > 99.3%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.15 (s, 1H), 8.68 (d, J = 7.6 Hz, 1H), 8.55-8.45 (m, 3H), 8.15 (d, J = 7.7 Hz, 1H), 8.09-7.94 (m, 3H), 7.84 (d, J = 7.6 Hz, 1H), 7.79-7.75 (m, , J = 8.9Hz, 4H), 7.65 (d, J = 8.9Hz, 4H), 7.24 (s, 1H), 7.12 (s, 1H), 7.00 (s, 1H), 6.79 (s, 1H), 5.17- 5.05 (m, 2H), 5.04-4.97 (m, 1H), 4.44-4.35 (m, 1H), 4.34-4.22 (m, 4H), 4.18-4.11 (m, 2H), 3.66-3.50 (m, 6H), 3.26 – 3.18 (m, 4H), 2.14-2.08 (m, 2H), 1.93-1.81 (m, 4H), 1.80-1.65 (m, 4H), 1.60-1.44 (m, 6H), 1.38-1.27 (m, 4H), 1.18 (d, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.1, 174.0, 171.7, 171.1, 171.0, 170.3, 170.0, 169.3, 168.3, 168.1, 165.2, 133.7, 131.2, 129.3, 124.7, 61.7, 61.5, 58.9, 48.1, 45.9, 34.2, 31.3, 31.2, 29.6, 28.8, 28.8, 28.6, 28.0, 23.5, 22.8, 18.0. HRMS (ES) m/z calcd for [M+H+]: $C_{48}H_{69}Br_2N_{12}O_{14}$ 1195.3417 found 1195.3429.

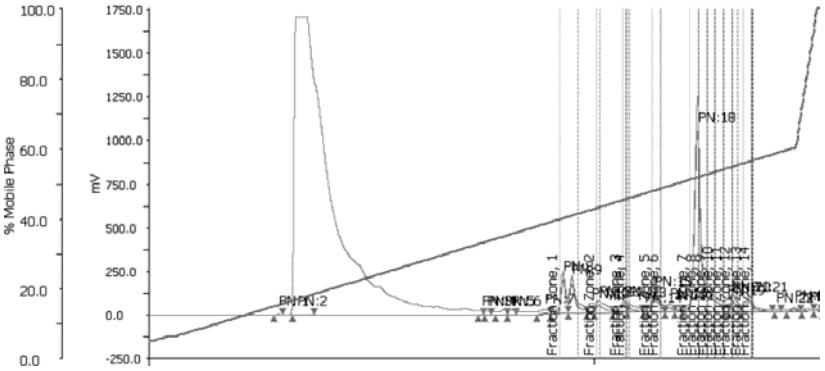
Peptide 6b



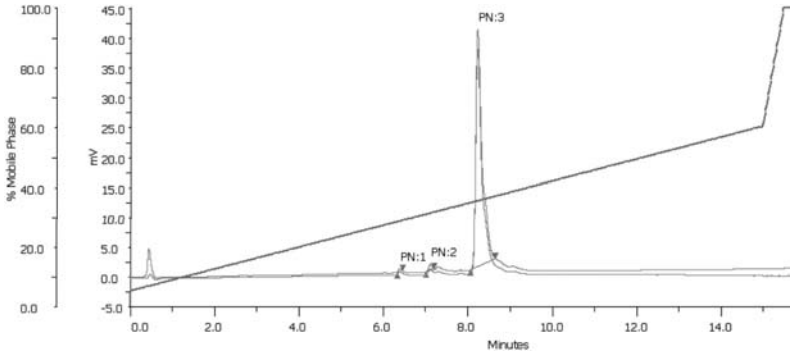
$C_{48}H_{68}Br_2N_{12}O_{14}$
MW: 1194.3

RP-HPLC Crude peptide: peak 7 (PN18 below) is the desired peptide.

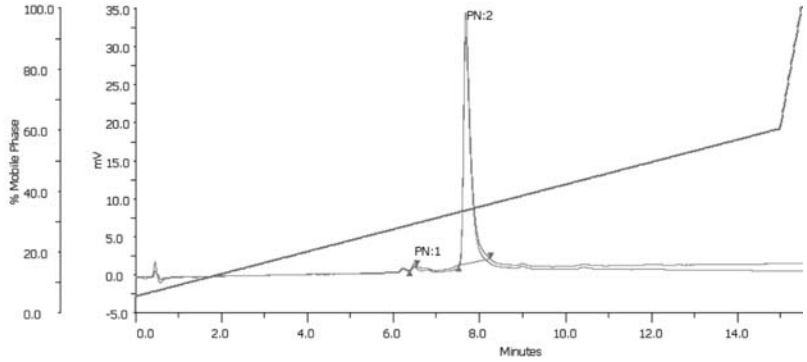




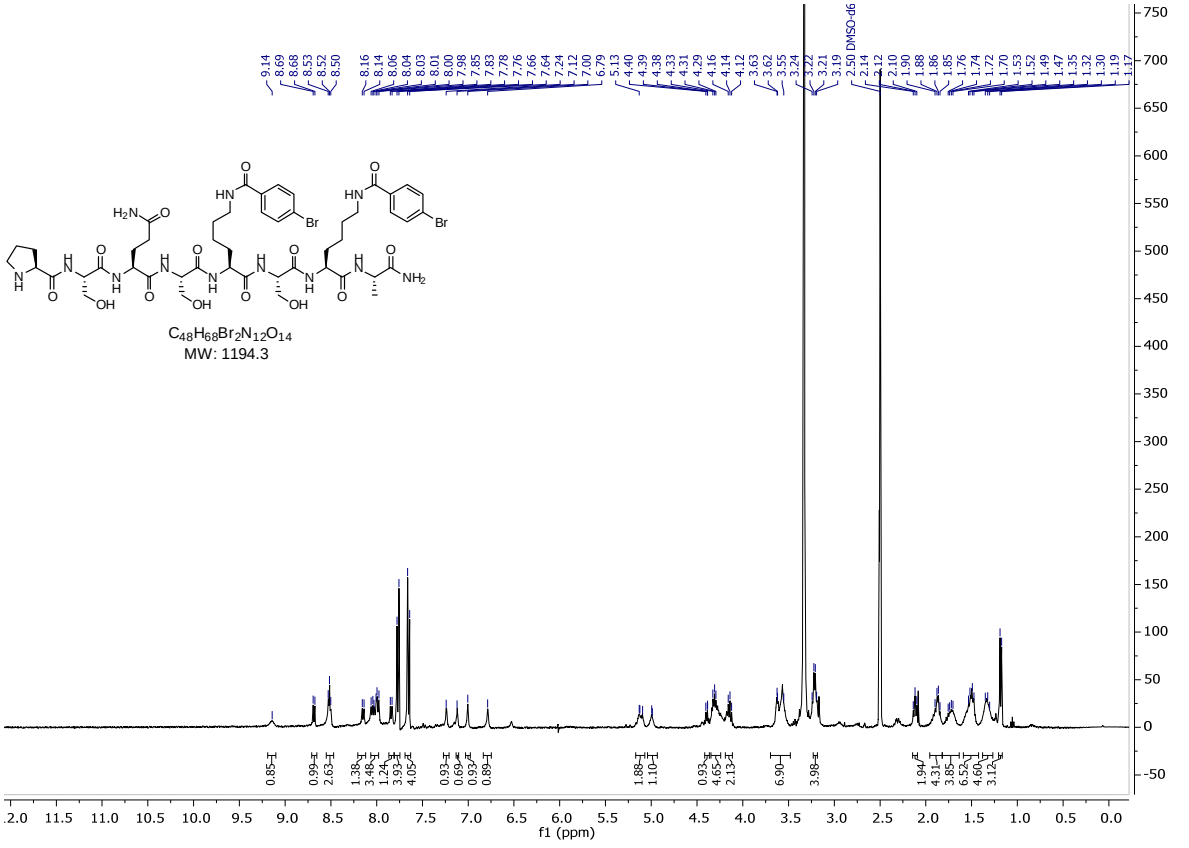
RP-HPLC purity: Restek Alure biphenyl

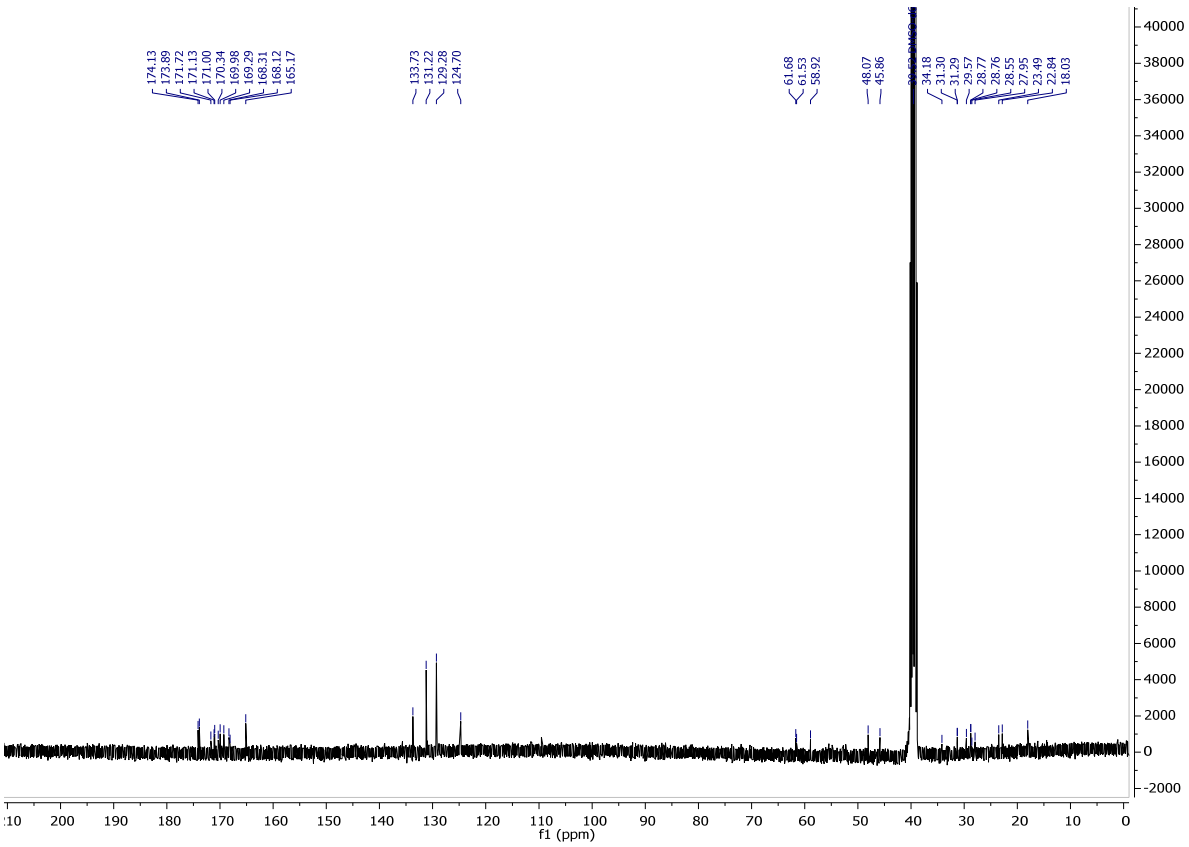


RP-HPLC purity: Thermo Hypersil Fluophase RP



Peptide 6b





Experimental procedures for peptides **6c** and [^{13}C]-**6c***

Procedures used in Scheme 3:

General procedure for Fmoc-SPPS: The precursor peptides were synthesized on Biotage® Initiator+ Alstra™ Automated Microwave Peptide Synthesizer (Biotage AB, Uppsala, Sweden) using standard Fmoc-SPPS from Rink Amide PS resin (0.68 mmol/g) on a 0.1 mmol scale. The side chain protections for the Fmoc amino acids were Asp(OtBu) and Lys(Mtt), respectively. Fmoc deprotection was performed using 20% Pip in DMF for 1 × 3 min and 1× 10 min, followed by repeated washing with DMF. Each Fmoc protected amino acid was coupled sequentially to the Rink Amide PS resin in a five-fold excess (5 equiv, 0.5 M) using HBTU (5 equiv, 0.6 M) and DIPEA (10 equiv, 2.0 M) in DMF for 40 min at r.t. The first coupling to the resin was repeated twice. Boc-Phe-OH was incorporated in the final step in the synthesis. At the end of the synthesis the partially protected peptide resin was washed with DCM and dried under vacuum to ensure complete removal of solvent.

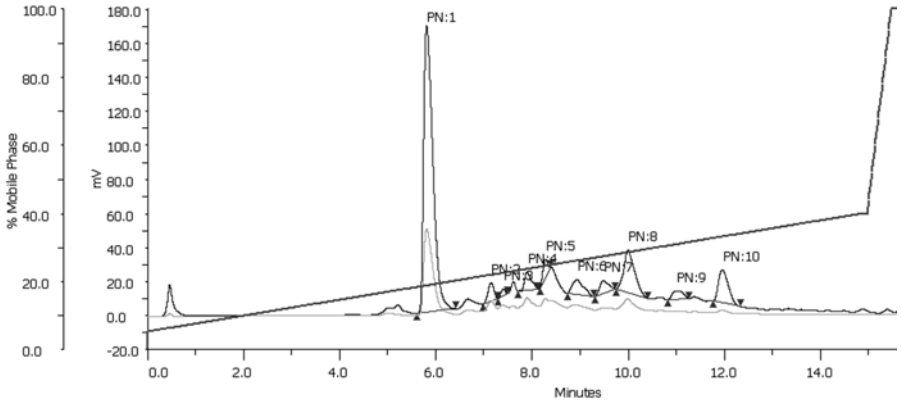
General procedure for Mtt deprotection:[10] The Mtt protected peptide resin was transferred to a 6 mL disposable syringe fitted with porous polyethylene filter and washed with 1.8% TFA in DCM. Total 10 washes (2 mL/was) and 3 min/wash. LC-MS was monitoring the Mtt-removal. Traces of peptide were observed in the final washes.

General procedure D: The partially protected peptide resin (1 equiv, 0.1 mmol, 0.68 mmol/g) was transferred to a 8 mL vial charged with $MePh_2SiCOOH$ **3** (3 equiv, 0.3 mmol, 73 mg) or $MePh_2Si^{13}COOH$ [^{13}C]-**3*** (3 equiv, 0.3 mmol, 73 mg, to label peptide [^{13}C]-**6c***), KF (3 equiv, 0.3 mmol, 17 mg), palladacycle precatalyst **2** (5 mol%, 0,005 mmol, 5 mg) and TEA (4 equiv, 0.4 mmol, 56 µL). The vial was sealed with a screw cap fitted with a Teflon® seal and evacuated, and backfilled with argon/nitrogen gas. Thereafter, dry DMF (3 mL) was added through the self-sealing septa by a syringe using a 21G needle. The needle was removed and the reaction was allowed to agitate for 15 h at at 45 °C using ultrasonication. (Increasing the temperature even further, to 65 °C only increased the side-product formed from intermolecular cyclization, see p S63). Next, excess pressure was released using a needle and thereafter the resin was separated by filtration and washed with DMF (3 × 5 mL), DCM (3 × 5 mL) and MeOH (3 × 5 mL). The resin was dried under vacuum and the final product was thereafter cleaved from the resin by treatment with 95% aqueous TFA (2 mL) and TES (100 µL) followed by agitation for 2 h at r.t. The resin was filtered off and washed with TFA (3 × 300µL). The filtrate was collected in a centrifuge tube and concentrated in a stream of argon/nitrogen to a volume of < 2 mL. The product was precipitated by the addition of cold diethylether (12 mL), collected by centrifugation, washed with diethylether (3 × 10 mL) and dried in a stream of argon/nitrogen and in vacuum over night. The crude peptide was purified by preparative RP-HPLC.

Analysis of the side-product formed during synthesis of peptide [^{13}C]-**6c***

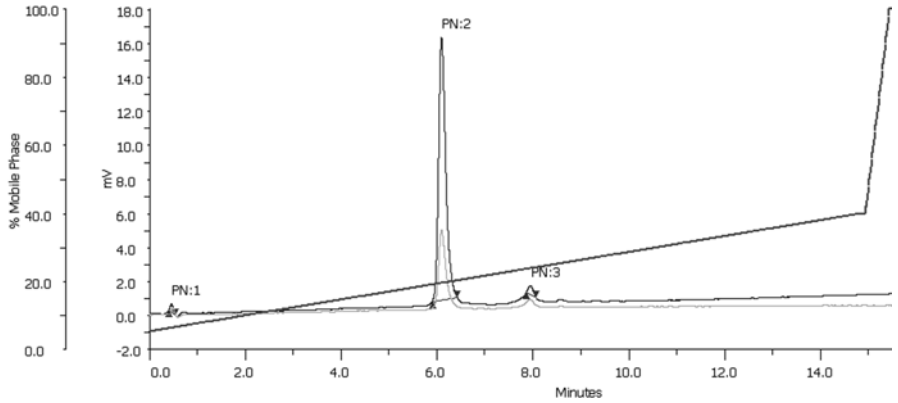
In the first attempt to synthesize the peptide [^{13}C]-**6c*** at 45 °C using ultrasonication, an isolated yield of 15% was obtained (16 mg) after RP-HPLC purification.

RP-HPLC Crude peptide: PN1 containes the cis/trans rotamers and the side-product formed due to intermolecular cyclization (see identification procedure as follows). All the fractions containing product (PN1) were pooled and lyophilized.

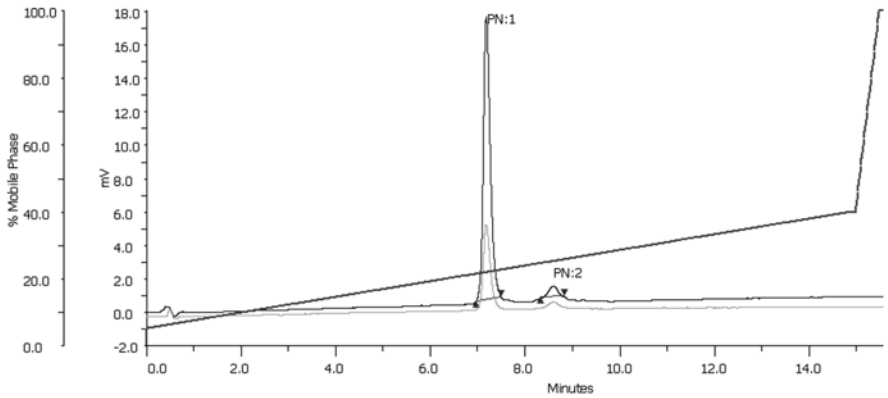


Purity check revealed a purity of Restek Alure biphenyl > 96.7%, Thermo Hypersil Fluophase RP > 96.0%, respectively (see below) which means that the product contained approx. 4% of the side-product.

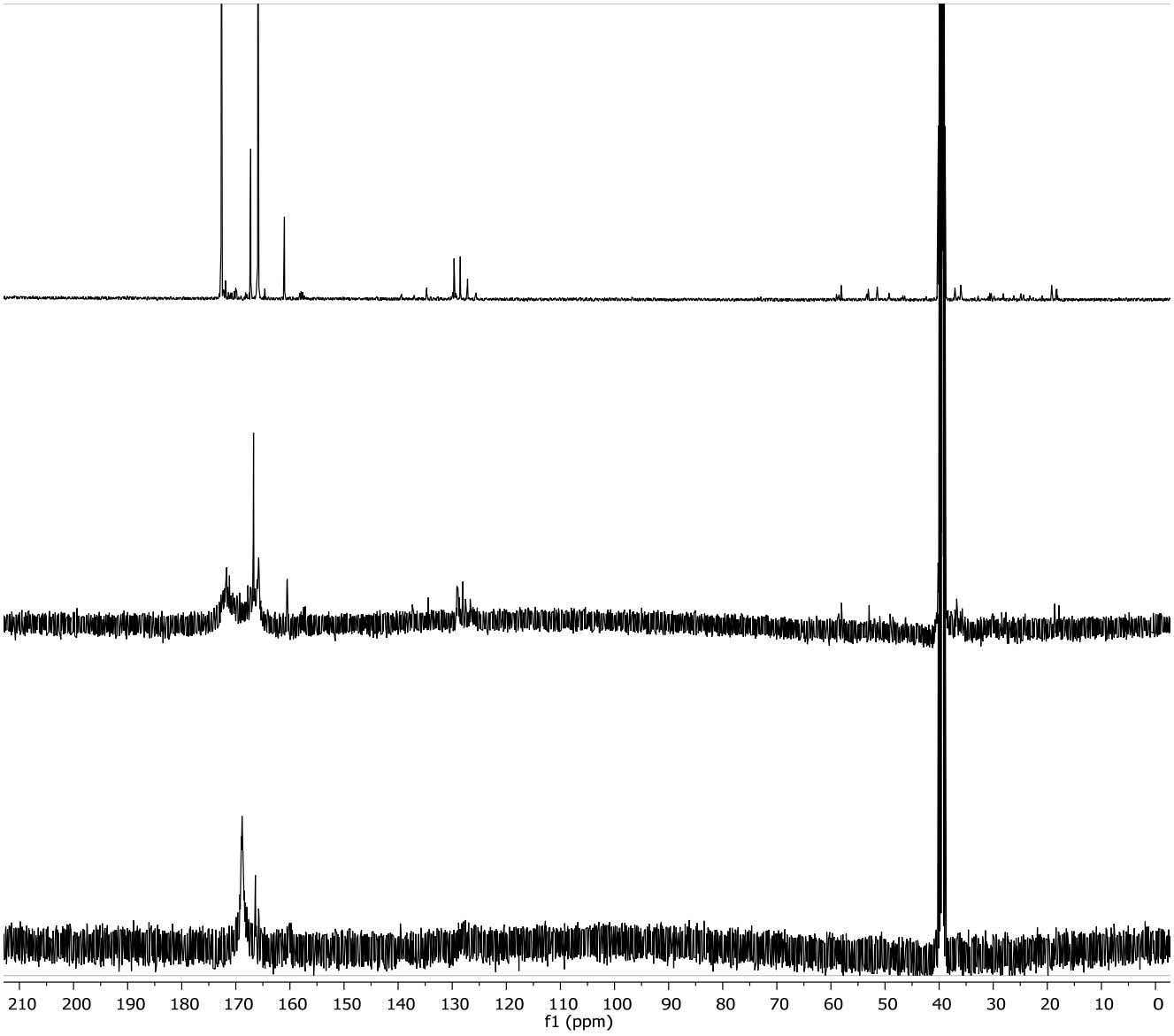
RP-HPLC purity: Restek Alure biphenyl. PN2 is the desired product and PN3 the side-product.



RP-HPLC purity: Thermo Hypersil Fluophase. PN1 is the desired product and PN2 the side-product.



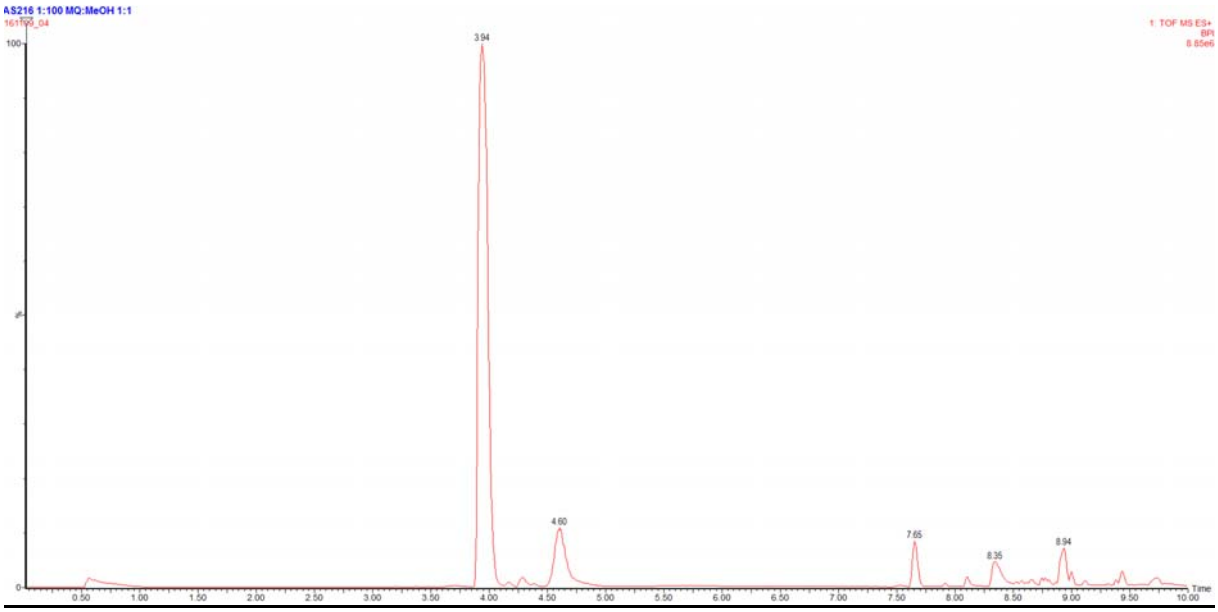
In order to identify the side-product formed using this reaction setup, ^{13}C-NMR analysis for peptide [^{13}C]-**6c*** at different temperatures was performed:
Topp, 25 °C; Middle, 80 °C; Bottom, 130 °C.



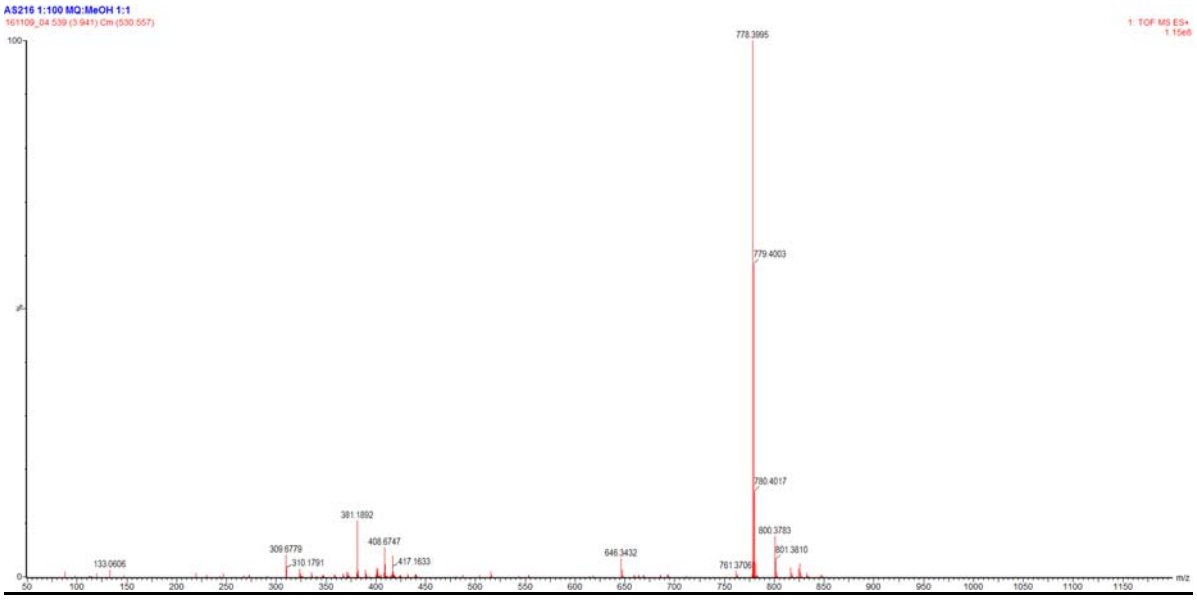
The ^{13}C NMR analysis shows that two of the ^{13}C-enriched peaks (at 172.7 and 165.9 ppm) converge to a single broad signal (at 168.8 ppm) at elevated temperature. These likely correspond to the cis/trans rotamers of the amide bond of the desired product formed after intramolecular cyclization,.

The other two ^{13}C-enriched peaks (at 167.3 and 161.0 ppm) are not affected by the elevated temperature and likely corresponds to the side-product formed from intermolecular cyclization which could be confirmed through exact mass analysis (see below).

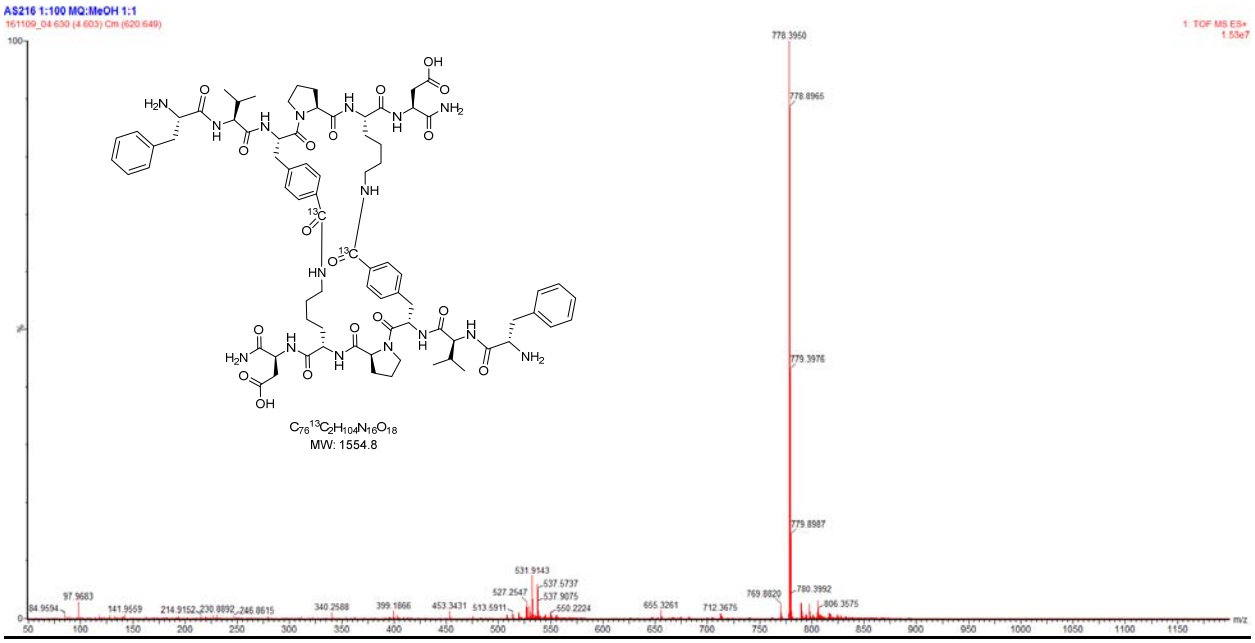
Furthermore, the exact mass for the compounds were determinate with a Q-ToF instrument (Synapt G2-S, from Waters Corporation) equipped with an electrospray ion source used in positive ionization mode. For chromatographic separation, an Acquity C18 BEH column (50 × 2.1 mm, particle size1.7 µm; Waters Corp.) was used, with a H_2O/MeCN gradient with 0.1% formic acid as mobile phase:



Peak at 3.94 min: desired product $[M+H]^+ = 778.3995$



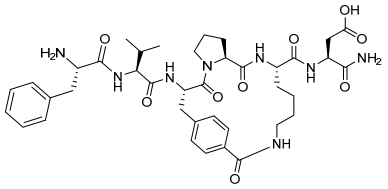
Peak at 4.60 min: side-product $[M+H]^{2+}$ = 778.3950 → 778. 3950 × 2 = 1556.7900 (double charged) desired molecular weight = 1556.7900 – 2 = 1554.7900



Here, the isotope pattern reveals a side-product that has a molecular weight, which is twice as high as that of the desired product and thus a result from intermolecular cyclization.

Characterization data, RP-HPLC Chromatograms and NMR spectra for peptide **6c**

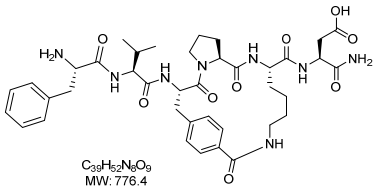
(*S*)-4-amino-3-((12*S*,3*S*,12*S*)-3-((*S*)-2-((*S*)-2-amino-3-phenylpropanamido)-3-methylbutanamido)-2,6,14-trioxo-7,13-diaza-1(1,2)-pyrrolidina-5(1,4)-benzenacyclotetradecaphane-12-carboxamido)-4-oxobutanoic acid (Peptide 6c)



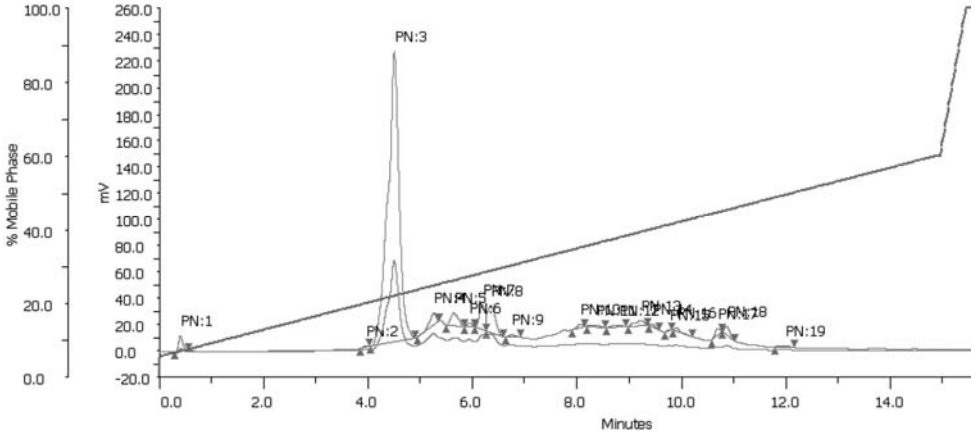
$C_{39}H_{52}N_8O_9$
MW: 776.4

General procedure for Fmoc-SPPS was followed for synthesizing the peptide besides that Boc-Phe-OH was incorporated as the last amino acid in the N-terminal. General procedure for Mtt deprotection was performed and thereafter general procedure D was followed for the aminocarbonylation at 65 °C using ultrasonication. The title peptide **6c** (8.8 mg, 11%) was obtained as a white solid after RP-HPLC purification and isolated as a 1:1 mixture of cis/trans rotamers. RP-HPLC purity: Restek Alure biphenyl > 99.9%, Thermo Hypersil Fluophase RP > 99.9%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.36 (s, 1H), 8.71-8.58 (m, 1H), 8.25 (s, 1H), 8.21-8.06 (m, 4H), 7.97-7.85 (m, 1H), 7.71 (d, J = 7.9 Hz, 1H), 7.64 (dd, J = 9.0, 4.7 Hz, 1H), 7.38-7.07 (m, 10H), 5.01-4.88 (m, 1H), 4.50-4.39 (m, 1H), 4.35 (t, J = 7.8 Hz, 1H), 4.19 (d, J = 7.4 Hz, 1H), 4.05 (s, 1H), 3.77 (d, J = 10.1 Hz, 1H), 3.71-3.61 (m, 1H), 3.20-2.92 (m, 5H), 2.65 (dd, J = 16.5, 5.7 Hz, 2H), 2.17-0.96 (m, 12H), 0.91 (dt, J = 14.1, 6.9 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.68, 172.30, 172.19, 171.88, 171.37, 170.99, 170.74, 170.25, 169.92, 169.85, 168.19, 167.99, 167.53, 167.14, 165.91, 158.31, 158.00, 157.69, 157.39, 139.37, 137.01, 134.74, 134.27, 132.31, 129.90, 129.66, 129.33, 128.52, 127.18, 125.61, 58.99, 58.50, 58.09, 53.40, 53.06, 51.45, 51.34, 49.29, 49.19, 46.73, 46.37, 42.40, 37.06, 36.46, 36.04, 35.82, 32.78, 30.73, 30.65, 30.36, 29.85, 28.23, 28.14, 26.20, 24.90, 24.40, 23.22, 20.95, 19.21, 19.14, 18.40, 18.23. HRMS (ES) m/z calcd for [M+H+]: $C_{39}H_{53}N_8O_9$ 777.3930 found 777.3931.

Peptide 6c



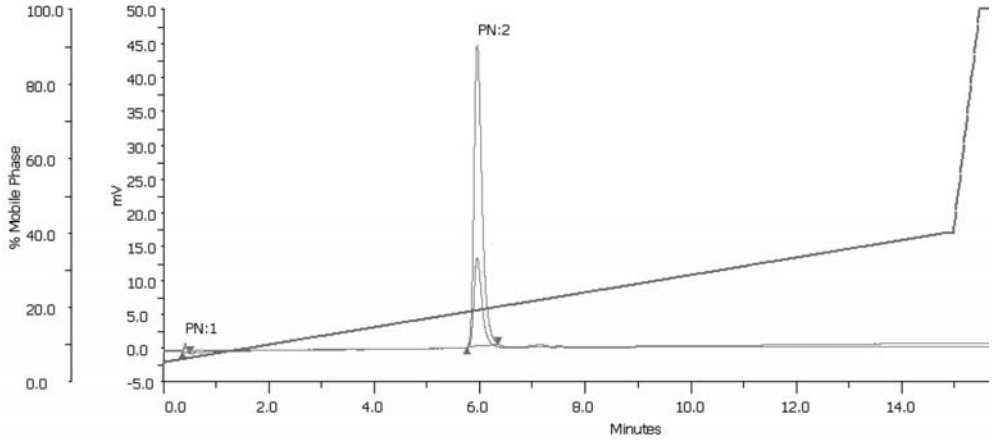
RP-HPLC Crude peptide: PN3 is the desired peptide.



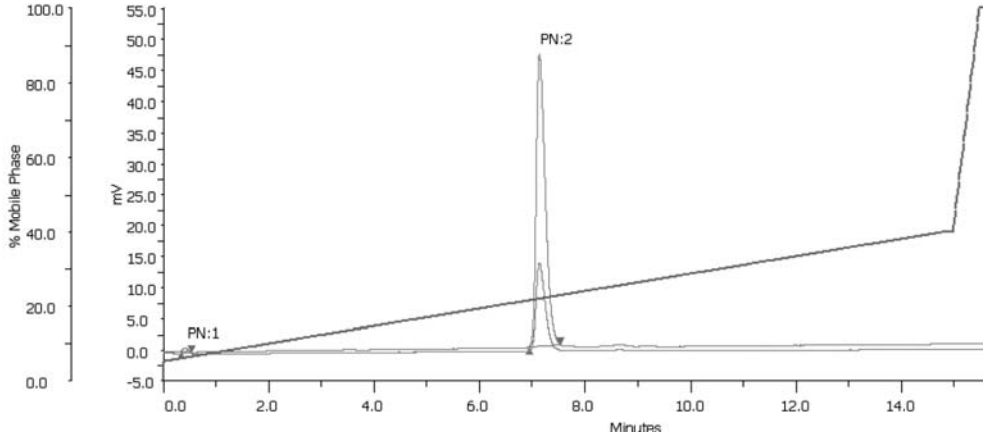
PN3 containes both the cis/trans rotamers derived from intramolecular cyclization and the side-product derived from intermolecular cyclization. In order to separate the desired product from the side-product the fractions containing the front part of the peak (PN3) was pooled and lyophilized. These pooled fraction was called **batch 1** and contained 8.8 mg product. Both purity check and NMR was run, see below.

Also, the back part of the peak (PN3) was pooled and lyophilized. This batch (**batch 2**) contained both the product and the side-product and had a total weight of 1.7 mg, see purity check below. Again, the aqurate mass for the compounds in **Batch 2** were determined with a Q-ToF instrument in order to comfirm the side-product formed due to intermolecular cyclization. One observation that was made was that the formation of the side-product was favored when increasing the reaction temperature even further, to 65 °C using ultrasonication.

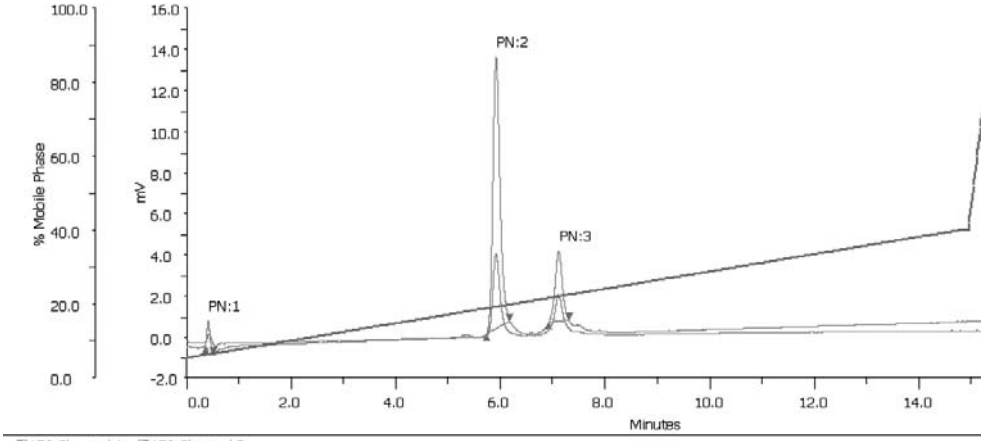
RP-HPLC purity Restek Alure biphenyl (**batch 1**)



RP-HPLC purity: Thermo Hypersil Fluophase (**batch 1**)



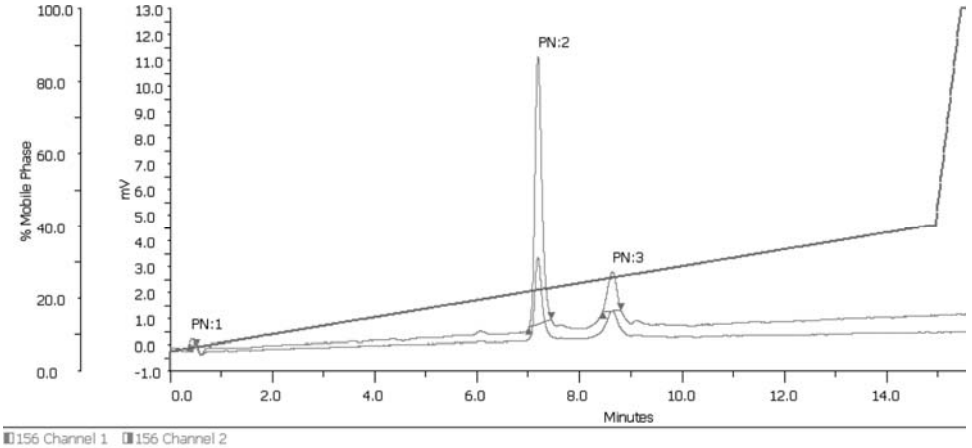
RP-HPLC purity Restek Alure biphenyl (**batch 2**) PN2 is the product and PN3 is the side-product.



Sample Table

Injection Number	Peak Name	Retention Time (min)	Area (uVmin x100)	Area %	Sample Name	Sample Location
8	1	0.417	9071.6667	3.344	AS218_no2_PC2	Sample Zone->2
8	2	5.923	208175.8333	76.741	AS218_no2_PC2	Sample Zone->2
8	3	7.121	54024.1667	19.915	AS218_no2_PC2	Sample Zone->2

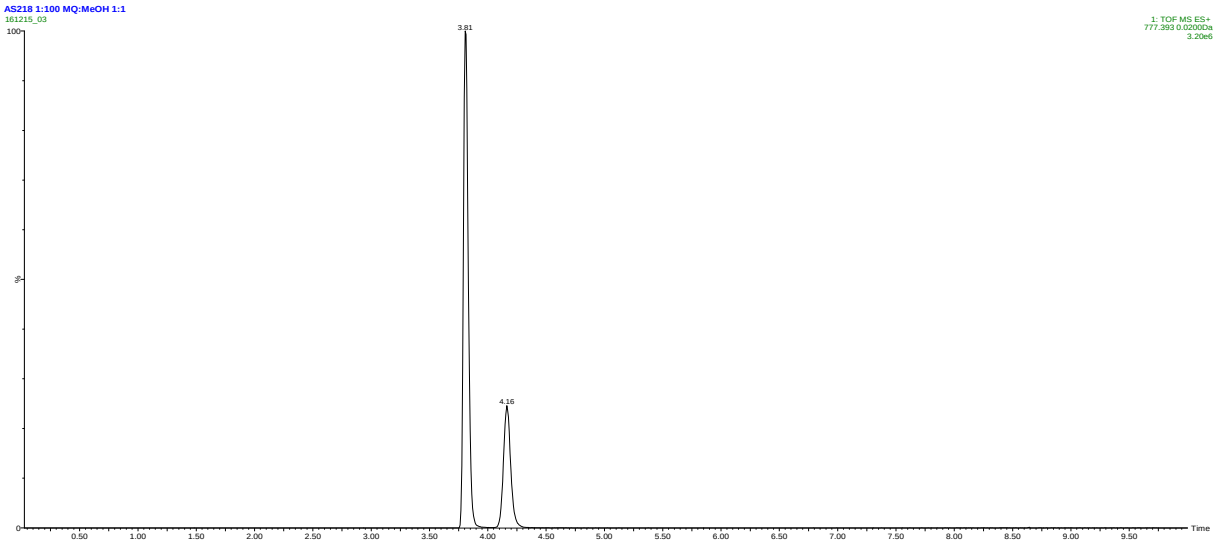
RP-HPLC purity: Thermo Hypersil Fluophase (**batch 2**) PN2 is the product and PN3 is the side-product.



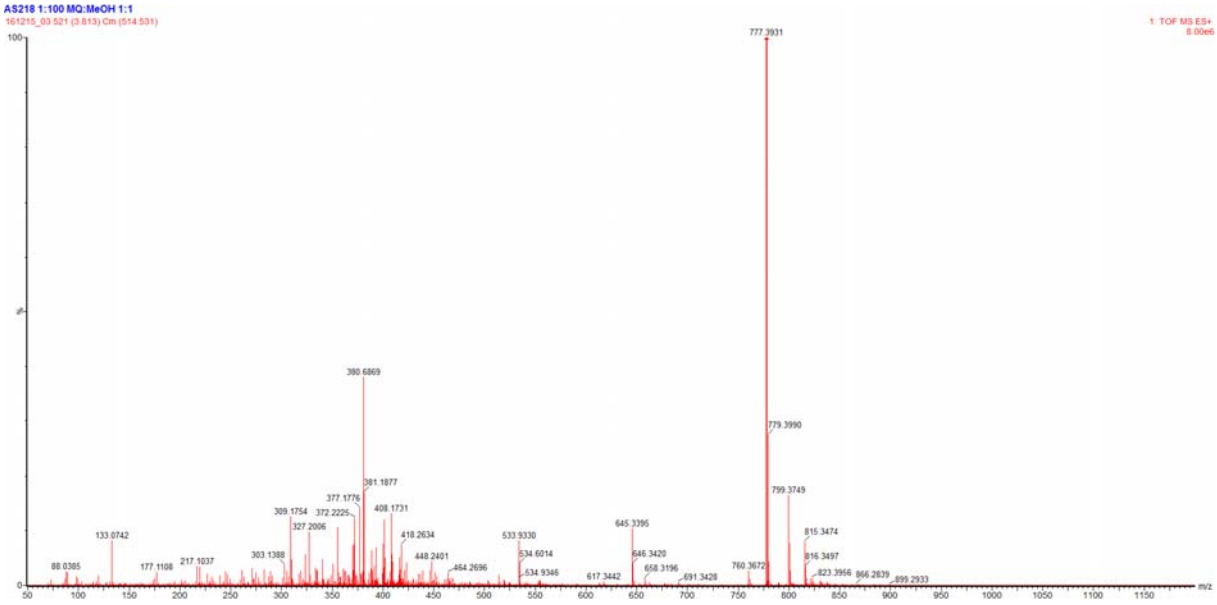
Sample Table

Injection Number	Peak Name	Retention Time (min)	Area (uVmin x100)	Area %	Sample Name	Sample Location
10	1	0.472	2702.5	1.379	AS218_no2_PC3	Sample Zone->2
10	2	7.193	163732.5	83.538	AS218_no2_PC3	Sample Zone->2
10	3	8.645	29562.5	15.083	AS218_no2_PC3	Sample Zone->2

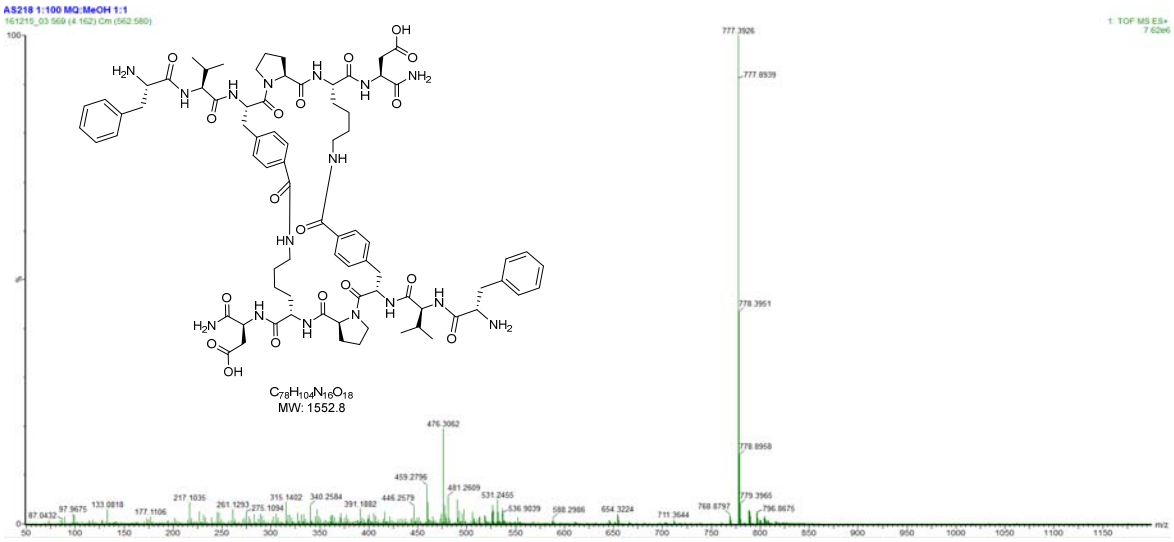
Exact mass for the compounds in **Batch 2** were determinate with a Q-ToF instrument (Synapt G2-S, from Waters Corporation) equipped with an electrospray ion source used in positive ionization mode. For chromatographic separation, an Acquity C18 BEH column (50 × 2.1 mm, particle size1.7 µm; Waters Corp.) was used, with a H_2O/MeCN gradient with 0.1% formic acid as mobile phase.



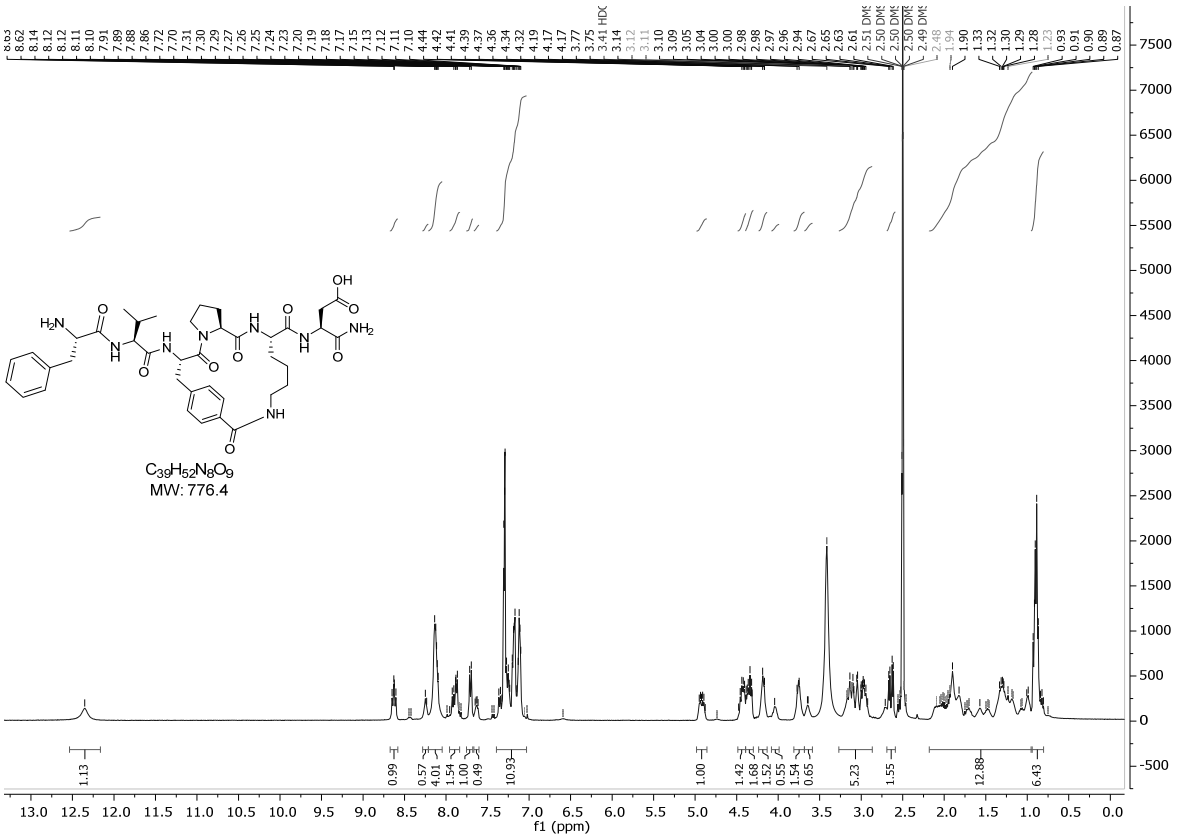
Peak at 3.81 min: desired product $[M+H]^+ = 777.3931$

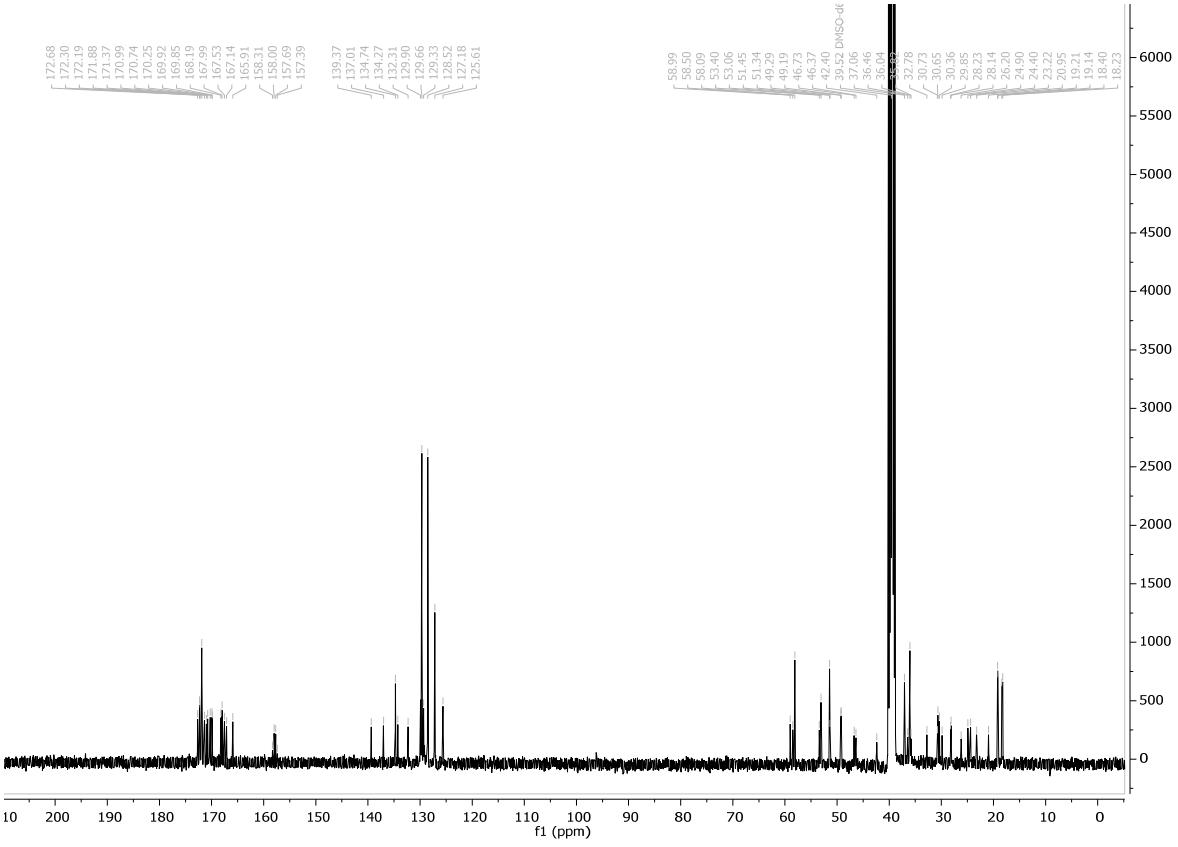


Peak at 4.16 min: side-product $[M+H]^{2+}$ = 777.3926 → 777. 3926 × 2 = 1554.7852 (double charged) desired molecular weight = 1554.7852 – 2 = 1552.7852



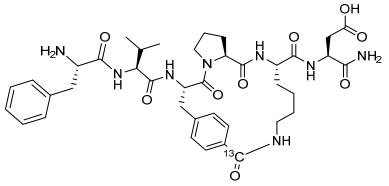
Peptide 6c (batch 1)





Characterization data, RP-HPLC Chromatograms and NMR spectra for peptide [^{13}C]-**6c***

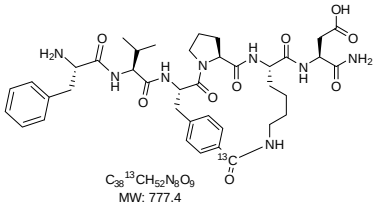
(*S*)-4-amino-3-((12*S*,3*S*,12*S*)-3-((*S*)-2-((*S*)-2-amino-3-phenylpropanamido)-3-methylbutanamido)-2,6,14-trioxo-7,13-diaza-1(1,2)-pyrrolidina-5(1,4)-benzenacyclotetradecaphane-12-carboxamido-6-^{13}C)-4-oxobutanoic acid (Peptide [^{13}C]-6c*)



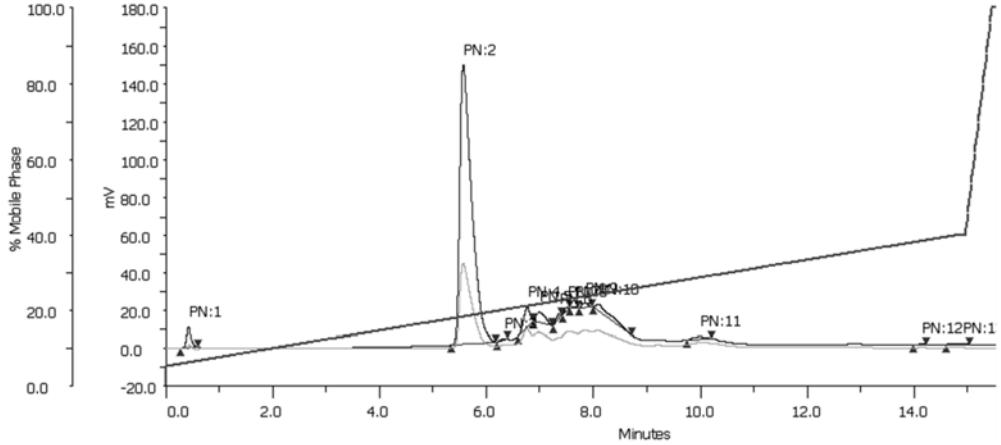
$C_{38}{}^{13}CH_{52}N_8O_9$
MW: 777.4

General procedure for Fmoc-SPPS was followed for synthesizing the peptide besides that Boc-Phe-OH was incorporated as the last amino acid in the N-terminal. General procedure for Mtt deprotection was performed and thereafter general procedure D was followed for the aminocarbonylation at 45 °C using ultrasonication. The title peptide **[^{13}C]-6c*** (4.6 mg, 6%) was obtained after two runs as a white solid after RP-HPLC purification and isolated as a 1:1 mixture of cis/trans rotamers. RP-HPLC purity: Restek Alure biphenyl > 99.9%, Thermo Hypersil Fluophase RP > 99.9%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.36 (s, 1H), 8.71-8.58 (m, 1H), 8.25 (s, 1H), 8.21-8.06 (m, 4H), 7.97-7.85 (m, 1H), 7.71 (d, J = 7.9 Hz, 1H), 7.64 (dd, J = 9.0, 4.7 Hz, 1H), 7.38-7.07 (m, 10H), 5.01-4.88 (m, 1H), 4.50-4.39 (m, 1H), 4.35 (t, J = 7.8 Hz, 1H), 4.19 (d, J = 7.4 Hz, 1H), 4.05 (s, 1H), 3.77 (d, J = 10.1 Hz, 1H), 3.71-3.61 (m, 1H), 3.20-2.92 (m, 5H), 2.65 (dd, J = 16.5, 5.7 Hz, 2H), 2.17-0.96 (m, 12H), 0.91 (dt, J = 14.1, 6.9 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.68 (^{13}C-enriched), 172.30, 172.19, 171.88, 171.37, 170.99, 170.74, 170.25, 169.92, 169.85, 168.19, 167.99, 167.53, 167.14, 165.91 (^{13}C-enriched), 158.31, 158.00, 157.69, 157.39, 139.37, 137.01, 134.74, 134.27, 132.31, 129.90, 129.66, 129.33, 128.52, 127.18, 125.61, 58.99, 58.50, 58.09, 53.40, 53.06, 51.45, 51.34, 49.29, 49.19, 46.73, 46.37, 42.40, 37.06, 36.46, 36.04, 35.82, 32.78, 30.73, 30.65, 30.36, 29.85, 28.23, 28.14, 26.20, 24.90, 24.40, 23.22, 20.95, 19.21, 19.14, 18.40, 18.23. HRMS (ES) m/z calcd for [M+H+]: $C_{38}{}^{13}CH_{53}N_8O_9$ 778.3964 found 778.3976.

Peptide [^{13}C]-6c*

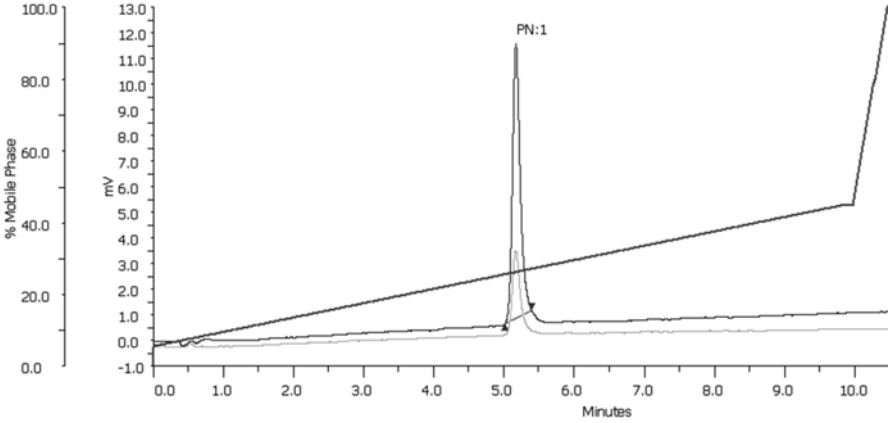


RP-HPLC Crude peptide: PN2 is the desired peptide.

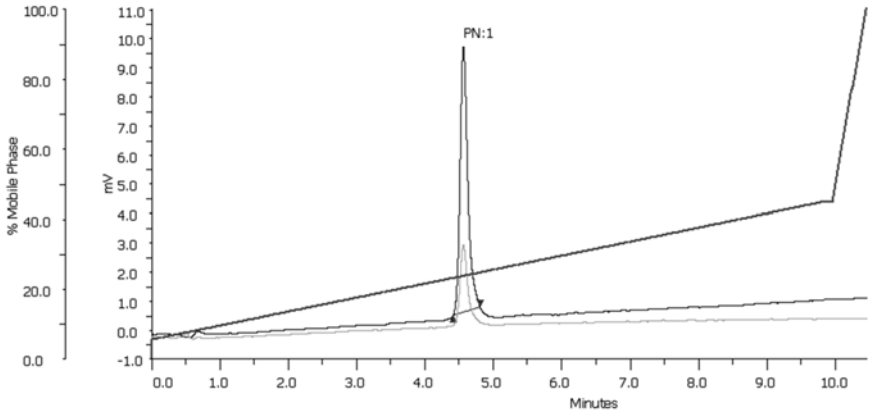


The peptide was purified in two runs using RP-HPLC. The fractions containing the front part of the peak (PN2) was pooled and lyophilized. Purity check and NMR was run.

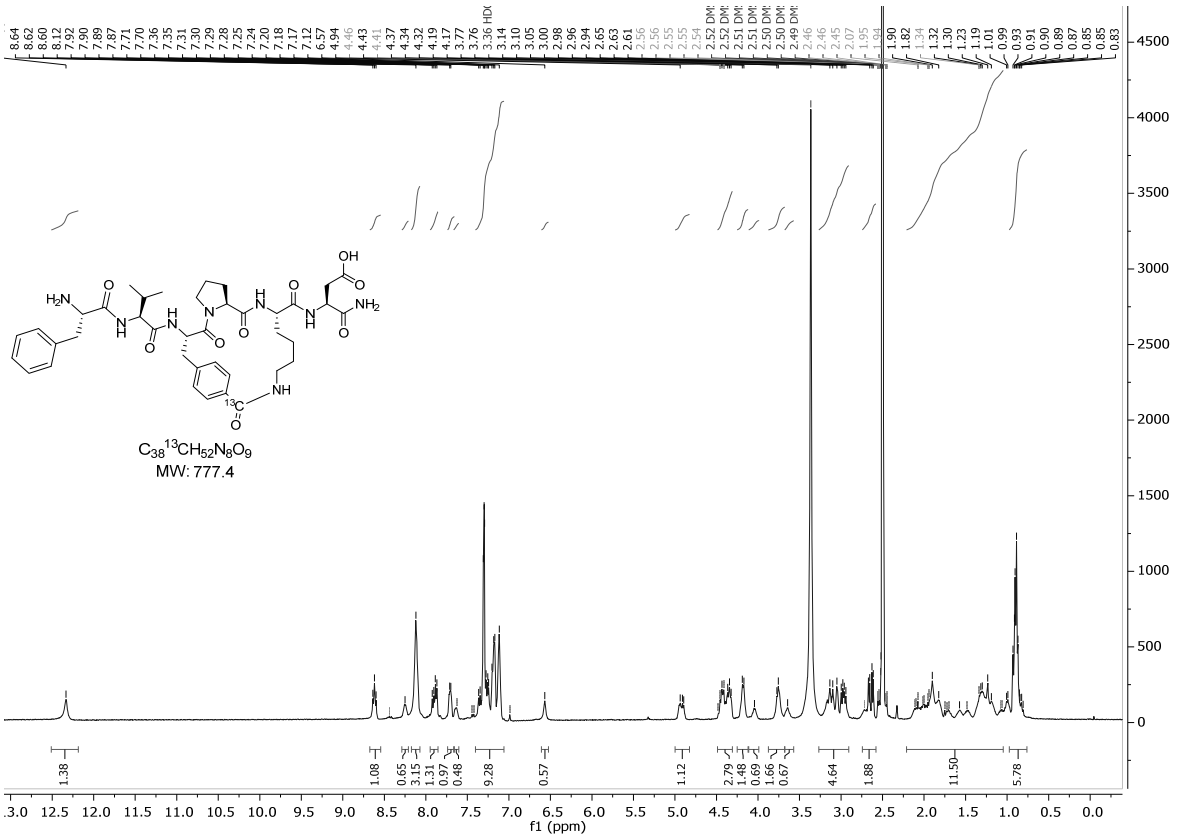
RP-HPLC purity: Restek Alure biphenyl



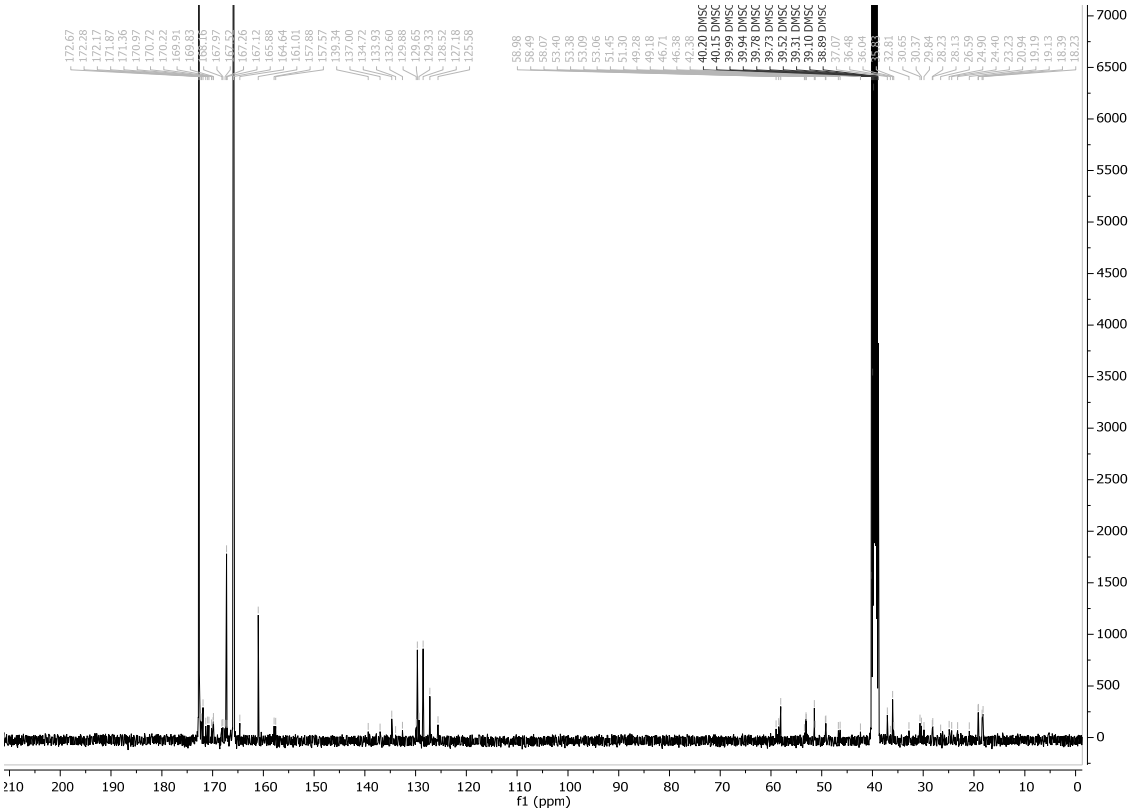
RP-HPLC purity: Thermo Hypersil Fluophase



Peptide [^{13}C]-6c*



^{13}C-enriched signals at 167.3 and 161.0 ppm, respectively, is trace amounts of the side-product formed due to intermolecular cyclization.



References

(1) Perrin, D.; Armarego, W. *Purification of Laboratory Chemicals 3rd Ed*; Pergamon Press, 1988.
(2) Friis, S. D.; Skrydstrup, T.; Buchwald, S. L. *Org. Lett.* **2014**, *16*, 4296.
(3) Bruno, N. C.; Niljianskul, N.; Buchwald, S. L. *J. Org. Chem.* **2014**, *79*, 4161.
(4) Friis, S. D.; Taaning, R. H.; Lindhardt, A. T.; Skrydstrup, T. *J. Am. Chem. Soc.* **2011**, *133*, 18114.
(5) Benvenuti, S.; Severi, F.; Costantino, L.; Vampa, G.; Melegari, M. *Farm.* **1998**, *53*, 439.
(6) Kawae, M.; Miyamae, H.; Narita, M.; Kurihara, T. *Tetrahedron Lett.* **1993**, *34*, 859.
(7) Luo, J.-M.; Dai, C.-F.; Lin, S.-Y.; Huang, P.-Q. *Chem. Asian J.* **2009**, *4*, 328.
(8) Pace, R. D.; Kabalka, G. W. *J. Org. Chem.* **1995**, *1600*, 4838.
(9) Fu, Z.; He, J.; Tong, A.; Xie, Y.; Wei, Y. *Synthesis (Stuttg).* **2013**, *45*, 2843.
(10) Li, D.; Elbert, D. L. *J. Pept. Res.* **2002**, *60*, 300.
(11) Patra, C. R.; Rupasinghe, C. N.; Dutta, S. K.; Bhattacharya, S.; Wang, E.; Spaller, M. R.; Mukhopadhyay, D. *ACS Chem. Biol.* **2012**, *7*, 770.