N-Amino-Imidazolidin-2-one Peptidomimetics

Ngoc-Duc Doan, Robert Hopewell and William D. Lubell

Address: Département de Chimie, Université de Montréal, C.P. 6128, Succursale Centre-Ville, Montréal, Québec, Canada H3C 3J7

Email Address: william.lubell@umontreal.ca

Table of Contents

I. Experimental section

1. General

Unless specified, all non-aqueous reactions were run under an argon atmosphere. Analytical thin-layer chromatography (TLC) was performed on glass-backed silica gel plates (Merck 60 F254). Visualization of the developed chromatogram was performed by UV absorbance or staining with ceric ammonium molybdate. Silica gel chromatography was performed using 230-400 mesh silica gel (Silicycle). Melting points were obtained on a Buchi melting point apparatus and are uncorrected. Nuclear magnetic resonance spectra (^{1}H, ^{13}C, COSY) were recorded either on a Bruker AV 400, AMX 400, or AV 500 spectrometer. Optical rotations were determined with a Perkin-Elmer 341 polarimeter at 589 or 546 nm. Data are reported as follows: [α]λtemp, concentration (*c* in g/100 mL), and solvent. High resolution mass analyses were obtained by the Centre régional de spectroscopie de masse de l'Université de Montréal. Analytical supercritical fluid chromatography (SFC) was performed at the Laboratoire d'analyse et de séparation chirale par SFC de l'Université de Montréal and data are reported as follows: column type, eluent, flow rate, temperature, backpressure, wavelength and retention times (R*t*).

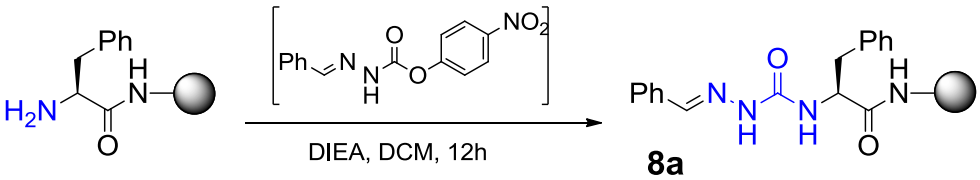
Polystyrene Rink Amide resin (0.63 mmol/g, 75-100 mesh) was purchased from Advanced Chemtech™ and the loading of the resin was determined by a standard Fmoc loading test.[1] Reagents including benzaldehyde, hydrazine hydrate, *p*-nitrophenyl chloroformate, *tert*-butylimino-tri(pyrrolidino)phosphorane (BTPP), tetraethylammonium hydroxide (TEAH), tetrabutylammonium hydroxide (TBAH), hydroxylamine hydrochloride, pyridine, formic acid (FA), and *N,N*-diisopropylethylamine (DIEA), all were purchased from Aldrich and used without further purification. 1,2-Dibromoethane was purchased from Aldrich and purified by filtration through a plug of silica gel prior to use. N-protected amino acids such as Boc-Gly, Boc-Met, Fmoc-Cys(Trt), Boc-Ala, Fmoc-Ala, Fmoc-Asn(Trt), Fmoc-Asp(Boc), Fmoc-Arg(Pbf), Boc-Tyr(OtBu), Fmoc-Pro and Boc-Pro, Fmoc-Lys(Boc), Fmoc-D-Phe, Fmoc-Trp(Boc), Fmoc-D-Trp(Boc), and Boc-His(Boc) were purchased from Novabiochem (EMD Bioscience Inc., San Diego, California) or GL Biochem Ltd. (Shangai, China). All solvents were obtained from VWR international. Anhydrous solvents

(THF, MeCN, DCM and DMF) were obtained by passage through solvent filtration systems (Glass-Contour, Irvine, CA). Analytical LCMS and HPLC analyses were performed on 5 µM, 150 or 50 mm × 4.6 mm C18 Phenomenex Gemini columns™ with a flow rate of 0.5 mL/min using a distilled water containing 0.1% formic acid (FA) /acetonitrile with 0.1% FA gradient. Peptide analogues were purified on a semi-preparative column (5 µM, 250 mm × 21.2 mm, C18 Gemini column™) using various gradients of distilled water containing 0.1% FA /acetonitrile with 0.1% FA gradient at a flow rate of 10.0 mL/min.

2. Fmoc-based SPPS: Fmoc deprotection and HBTU couplings

Peptide syntheses were performed under standard conditions[1] on an automated shaker using polystyrene Rink amide resin (0.63 mmol/g, 75-100 mesh). Couplings of amino acids (3 equiv) were performed in DMF using HBTU (3 equiv) as coupling reagent and DIEA (6 equiv). Fmoc group removal was performed by treating the resin with 20% piperidine in DMF for 30 min. The Resin was washed after each coupling and Fmoc-group removal step sequentially with DMF (3 × 10 mL), MeOH (3 × 10 mL), THF (3 × 10 mL), and DCM (3 × 10 mL). The purity of peptide fragments was ascertained by LCMS analysis after cleavage and deprotection of a small aliquot of resin as described below.

3. Representative protocol for preparation of semicarbazone-protected aza-Gly-peptide resin: synthesis of bezylidene-aza-glycinyl-phenylalaninamide resin 8a

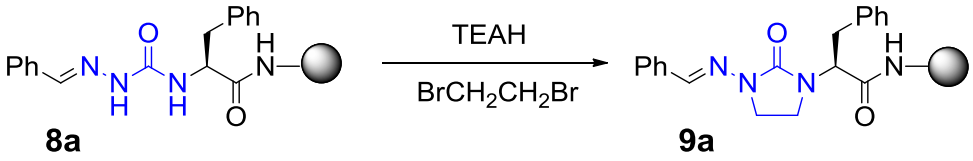


8a

To a stirred solution of hydrazine hydrate (150 µL, 4.5 mmol) in EtOH (3 mL) at 0°C, benzaldehyde (150 µL, 1.5 mmol) was added drop-wise. Complete formation of benzaldehyde hydrazone was monitored by TLC (R_f = 0.6, 2:1 hexane:EtOAc). After stirring for 2h, the mixture was poured directly into H_2O (20 mL) and extracted with DCM (3 x 30 mL). The organic phase was separated, dried with $MgSO_4$ and concentrated *in-vacuo* to 3 mL of a benzaldehyde hydrazone solution in DCM. Without further purification,

the hydrazone solution was added drop-wise over 20 min to a solution of *p*-nitrophenylchloroformate (0.3 g, 1.5 mmol) in DCM (5 mL) at 0°C. The reaction mixture was allowed to warm to room temperature and stirred under argon for an additional 2 h, cooled to 0°C, and treated with DIEA (0.52 mL, 3.0 mmol). The resulting suspension was quickly transferred to a plastic syringe tube equipped with Teflon™ filter, stopper and stopcock containing 0.5 g of pheylalaninamide linked to Rink resin (0.63 mmol/g). After agitation on an automated shaker for 16 h at room temperature, the resin was filtered and washed with DMF (3 x 5 mL), MeOH (3 x 5 mL), THF (3 x 5 mL), MeOH (3 x 5 mL), and DCM (3 x 5 mL). The reaction was shown to have quantitative conversion by monitoring, using LCMS [5-80% MeCN (0.1% FA) in water (0.1% FA), 20 min, R*t* = 8.3 min], of the residue obtained from cleavage of a resin aliquot (3 mg) with 1 mL of TFA/TES/H_2O (95:2.5:2.5, v/v/v), after resin filtration and evaporation of the volatiles.

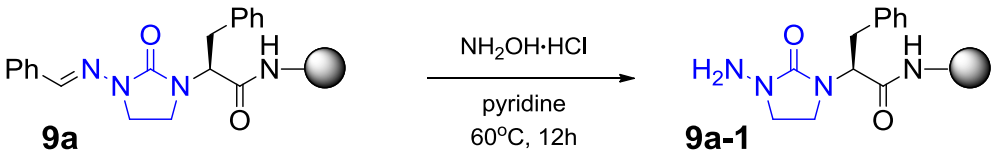
4. Representative protocol for preparation of semicarbazone-protected Aid-containing peptide-resin: synthesis of *N*-benzylidene-amino-imidazolidin-2-one-phenylalaninamide 9a



8a **9a**

A solution of 40% tetra-ethylammonium hydroxide (TEAH) in water (232 µL, 0.8 mmol, 5 equiv) was added to a plastic syringe tube equipped with Teflon™ filter, stopper and stopcock containing a suspension of semicarbazone-protected aza-Gly-Phe linked to Rink amide resin **8a** (200 mg, 0.63 mmol/g) swollen in THF (3 mL) and agitated for 30 min at room temperature on an automated shaker. 1,2-Dibromoethane was filtered through a pad of silica gel, and a portion of the filtered dihalide (27.2 µL, 0.32 mmol, 2.5 equiv) was subsequently added to the resin mixture, which was agitated for an additional 16 h. The reaction was shown to have a quantitative conversion by monitoring, using LCMS [5-80% MeCN (0.1% FA) in water (0.1% FA), 20 min, R*t* = 8.8 min], of the residue obtained from cleavage of a resin aliquot (3 mg) with 1 mL of TFA/TES/H_2O (95:2.5:2.5, v/v/v), resin filtration and evaporation of the volatiles.

5. Representative protocol for deprotection of semicarbazone on solid support: synthesis of amino-indolizidin-2-one-phenylalaninamide 9a-1.



Semicarbazone-protected Aid-Phe linked to Rink amide resin **9a** (200 mg, 0.126 mmol) in a plastic syringe tube equipped with Teflon™ filter, stopper and stopcock was treated with a pre-made stock solution of 1.5 M NH_2OH•HCl in pyridine[2] (5 mL) and heated with sonication in a water bath at 60 °C for 12 h. The resin was filtered and washed with 10% DIEA in DMF (3 × 10 mL), then DMF (3 × 10 mL), MeOH (3 × 10 mL), THF (3 × 10 mL), and DCM (3 × 10 mL). The reaction was shown to have 87% conversion to semicarbazide **9a-1** by monitoring using LCMS [5-80% MeCN (0.1% FA) in water (0.1% FA), 20 min, R*t* = 4.7 min] of the residue obtained from cleavage of a resin aliquot (3 mg) with 1 mL of TFA/TES/H_2O (95:2.5:2.5, v/v/v), resin filtration and evaporation of the volatiles. In cases in which the LCMS analysis revealed incomplete semicarbazone deprotection, the procedure was repeated.

6. Representative protocol for coupling of protected-amino acid to semicarbazide moieties on solid support: synthesis of *N*-Boc-Gly-Aid-Phe resin 11c.

N-(Boc)Glycine (110 mg, 0.32 mmol, 5 equiv) and *N*,*N*'-diisopropylcarbodiimide (DIC, 44 µL, 0.32 mmol, 2.5 equiv) were reacted in dry DCM (5 mL) and stirred at room temperature for 30 min. The resulting suspension was concentrated *in-vacuo* to a residue, which was dissolved in DMF (3 mL), and added to a plastic syringe tube equipped with Teflon™ filter, stopper and stopcock containing unprotected semicarbazide Aid-Phe linked to Rink resin **9a-1** (200 mg, 0.126 mmol). The suspension was agitated for 16 h at room temperature by an automatic shaker. The reaction was shown to have > 90% conversion to

the semicarbazide by monitoring, using LCMS [5-80% MeCN (0.1% FA) in water (0.1% FA), 20 min, R*t* = 9.1 min] of the residue obtained from cleavage of a resin aliquot (3 mg) with 1 mL of TFA/TES/H_2O (95:2.5:2.5, v/v/v), resin filtration and evaporation of the volatiles. Subsequent Fmoc removal and couplings to complete the target sequences were performed according to conventional Fmoc-based SPPS protocols [1].

7. Deprotection and cleavage of Aid-peptides from the resin.

The Rink resin-bound Aid-peptide was deprotected and cleaved from the support using a freshly made solution of TFA/H_2O/TES (95:2.5:2.5, v/v/v, 20 mL/g of peptide resin) at room temperature for 2 h. The resin was filtered and rinsed with TFA. The filtrate and rinses were concentrated until a crude oil persisted, from which a precipitate was obtained by addition of cold ether (10-15 mL). After centrifugation, the supernatant was removed and the crude Aid-peptide was taken up in aqueous acetonitrile (10% v/v) and freeze-dried to a white solid, which was analyzed by HPLC to assess purity.

8. Analysis and purification of Aid-peptides

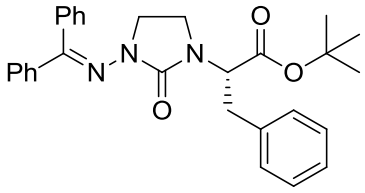
Analyses and characterization of Aid-peptides were performed on either an Agilent™ Technologies 1100 Series LCMS instrument with ESI ion-source, single quadrupole mass detection and positive mode ionization or a ThermoFinnigan™ LCQ Advantage MS, with ESI ion-source, ion-trap mass detection, and positive mode ionization, equipped with a Gilson™ LC 322 pump containing auto-sampler and injector. Prior to injection, samples were dissolved in 10% H_2O in MeOH. The LCMS analyses were performed on a Gemini™ C18 reverse-phase column (150 × 4.60 mm, 5 µm), using a binary solvent system consisting of 0.1% FA in H_2O, and 0.1% FA in MeOH at a flow rate of 0.5 mL/min and UV detection at 254 nm. Linear gradients of the mobile phase [2-80% methanol (0.1% FA) in water (0.1% FA), over 15 min] were used for analyses of crude peptides.

Purification of Aid-peptides was conducted on a Waters™ PrepLC instrument equipped with a reverse-phase Gemini™ C18 column (250 × 21.2 mm, 5 µm), using binary solvent systems consisting of MeCN

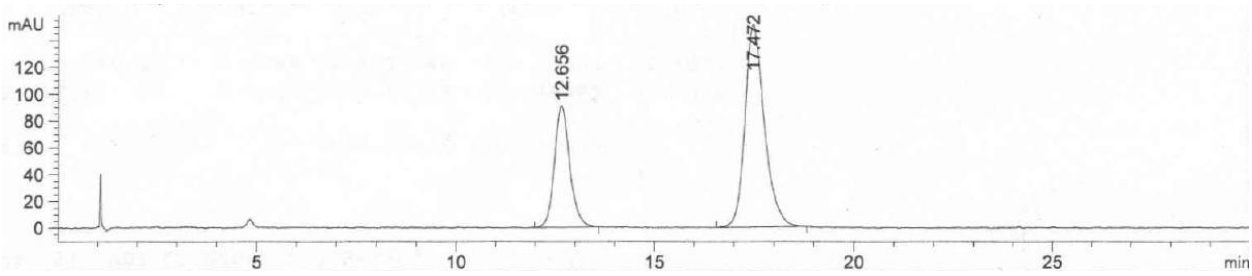
(0.1% FA) in H_2O (0.1% FA) at a flow rate of 10.0 mL/min and UV detection at 214 nm. Purest fractions were combined and freeze-dried to a white powder. Each purified sample was analyzed for purity by LCMS with a Gemini™ C18 reverse-phase column (150 × 4.60 mm, 5 µm) at a flow rate of 0.5 mL/min and UV detection at 214 nm. The purity of each Aid-peptide was analysed in two binary solvent systems respectively consisting of gradients of 2-80% MeOH (0.1% FA) in H_2O (0.1% FA), and 2-40% acetonitrile (0.1% FA) in H_2O (0.1% FA).

9. Synthetic Experimental Procedures and Characterization Data

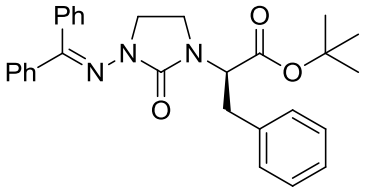
(2'*S*)-1-((Diphenylmethylene)amino)-3-(*tert*-butyl-3'-phenylpropanoate)-imidazolidin-2-one [(*S*)-2]



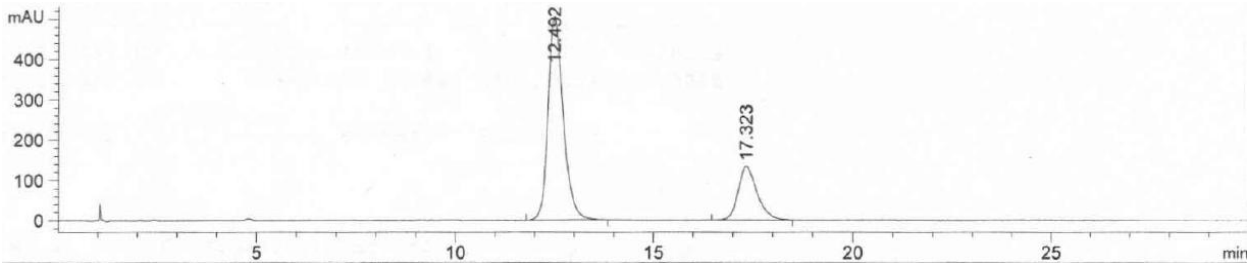
Benzhydrylidene aza-Gly-Phe-O*t*Bu [(*S*)-**1**, 220 mg, 0.5 mmol, synthesized as previously reported[3]] was dissolved in 4 mL of anhydrous THF, cooled to 0°C, treated with 5 equiv. of 40% tetraethylammonium hydroxide (TEAH) in water (0.92 mL, 2.5 mmol), stirred for 30 min, and treated with 2.5 equiv of 1,2-dibromo-ethane (108 µL, 1.25 mmol). The ice bath was removed. The reaction was allowed to warm to room temperature and stirred overnight. After evaporation of the volatiles under reduced pressure, the residue was purified by chromatography on silica gel using 15% EtOAc in hexanes as eluent. Evaporation of the collected fractions gave imidazolidin-2-one (*S*)-**2** as yellow oil (123 mg, 53%): R_f 0.41 (3:7 EtOAc : hexanes). $[\alpha]_D^{20}$ –3.4 (*c* 1.04, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (9H, s), 2.85-2.90 (1H, ddd, *J* = 7.0, 9.0, 15.9 Hz), 2.92-3.01 (2H, m), 3.13-3.17 (1H, ddd, *J* = 5.4, 8.8, 14.1 Hz), 3.27-3.30 (1H, dd, *J* = 6.1, 14.7 Hz), 3.38-3.43 (1H, ddd, *J* = 7.1, 8.8, 15.2 Hz), 4.92-4.95 (1H, q, *J* = 6.2, 10.2 Hz), 7.22-7.24 (3H, d, *J* = 7.2 Hz), 7.26-7.33 (7H, m), 7.41-7.43 (3H, m), 7.56-7.58 (2H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1, 159.3, 157.6, 138.5, 136.8, 136.3, 129.6, 129.1, 129.0, 128.7, 128.5, 128.4, 128.4, 127.9, 126.7, 82.0, 56.4, 45.9, 38.8, 35.1, 28.0. HRMS m/z 470.2398, $(M+H)^+$ calcd for $[C_{29}H_{32}N_3O_3]^+$: 470.2399. SFC analysis detected a 32:68 enantiomeric ratio of *R*- and *S*-**2** on a chiral stationary phase [Chiralcel AD-H 25 cm, 5 µm, 10% *i*-PrOH, 3mL/min, 35°C, 150 bar, R*t* (minor) 12.65 min, R*t* (major) 17.47 min].



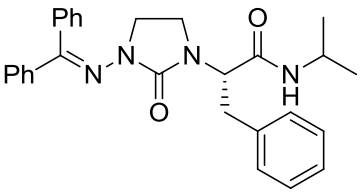
(2'*R*)-1-((Diphenylmethylene)amino)-3-(*tert*-butyl-3'-phenylpropanoate)-imidazolidin-2-one [(*R*)-2]



Imidazolidin-2-one (*R*)-**2** was synthesized from benzhydrylidene aza-Gly-D-Phe-O*t*Bu ((*R*)-**1**, 110 mg, 0.25 mmol) using the same protocol as (*S*)-**2**, and isolated as yellow oil (72 mg, 0.15 mmol, 62% yield): R_f 0.40 (3:7 EtOAc : hexanes). $[\alpha]_D^{20}$ 5.2 (*c* 1.04, $CHCl_3$). SFC analysis detected a 63:37 enantiomeric ratio of *R*- and *S*-**2** on a chiral stationary phase [Chiralcel AD-H 25 cm, 5 µm, 10% i-PrOH, 3mL/min, 35°C, 150 bar, R*t* (major) 12.49 min, R*t* (minor) 17.32 min].

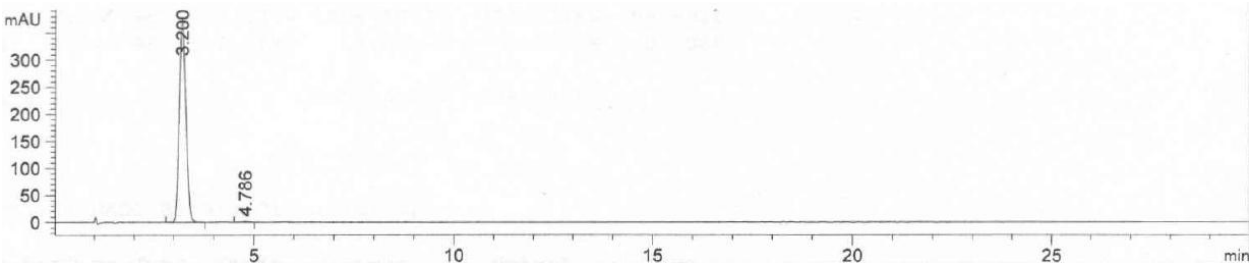


(*S*)-2-(3-((Diphenylmethylene)amino)-2-oxoimidazolidin-1-yl)-*N*-isopropyl-3-phenylpropanamide [(*S*)-4]

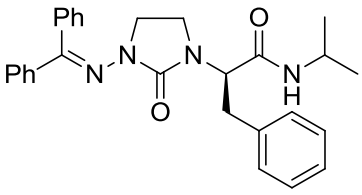


Imidazolidin-2-one (*S*)-**4** was synthesized from benzhydrylidene aza-glycinyl-phenylalanine isopropyl amide ((*S*)-**3**, 108 mg, 0.25 mmol, synthesized as previously reported[4]) using the same protocol described for (*S*)-**2**. Purification by chromatography on silica gel using a gradient of 20-30% EtOAc in hexanes gave a white powder (63 mg, 0.14 mmol, 55% yield): R_f 0.33 (4:6 EtOAc :

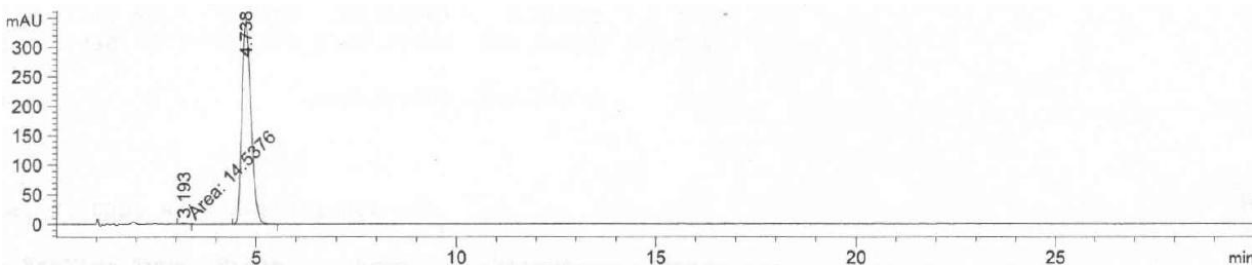
hexanes). $[\alpha]_D^{20}$ –22.8 (*c* 1.04, $CHCl_3$), mp: 70-72°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.02 (3H, d, *J* = 6.5 Hz), 1.10 (3H, d, *J* = 6.5 Hz), 2.90-3.02 (3H, m), 3.24-3.30 (3H, m), 3.94-4.01 (1H, m), 4.61-4.64 (1H, t, *J* = 8.0 Hz), 6.16-6.18 (1H, d, *J* = 7.9 Hz), 7.22-7.23 (3H, m), 7.28-7.32 (6H, m), 7.36-7.39 (1H, m), 7.42-7.45 (3H, m), 7.56-7.57 (2H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.7, 159.6, 159.0, 138.3, 137.0, 136.0, 129.8, 129.2, 129.1, 129.0, 128.6, 128.5, 128.5, 128.0, 126.7, 58.0, 45.9, 41.4, 39.3, 34.4, 22.6, 22.4. HRMS 455.2440, $(M+H)^+$ calcd for $[C_{28}H_{31}N_4O_2]^+$: 455.2442. An enantiomeric ratio of >99:1 of *S*:*R*-isomer was ascertained by SFC analysis on a chiral stationary phase [Chiralcel AD-H 25 cm, 5 µm, 20% i-PrOH, 3mL/min, 35°C, 150 bar, R*t* (major) 3.20 min, R*t* (trace) 4.79 min].



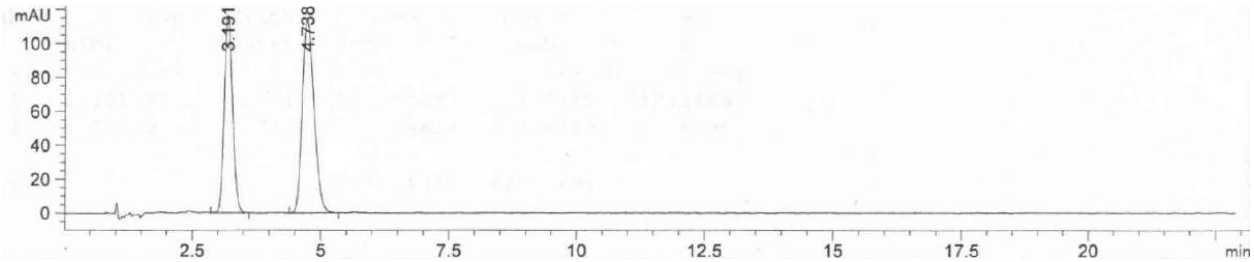
(*R*)-2-(3-((Diphenylmethylene)amino)-2-oxoimidazolidin-1-yl)-N-isopropyl-3-phenylpropanamide [(*R*)-4]



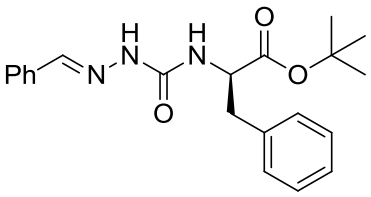
Imidazolidin-2-one (*R*)-**4** was synthesized from benzhydrylidene aza-glycinyl-D-phenylalanine isopropyl amide ((*R*)-**3**, 108 mg, 0.25 mmol, synthesized as previously reported[4]) using the protocol described for (*S*)-**2** and isolated by chromatography on silica gel using 20-30% EtOAc in hexanes, as a white powder (58 mg, 0.13 mmol, 51% yield): R_f 0.33 (4:6 EtOAc : hexanes). $[\alpha]_D^{20}$ 22.5 (*c* 1.04, $CHCl_3$), mp: 70-72°C. An enantiomeric ratio of >99:1 *R*:*S*-isomer was ascertained by SFC analysis on a chiral stationary phase [Chiralcel AD-H 25 cm, 5 µm, 20% i-PrOH, 3mL/min, 35°C, 150 bar, R*t* (trace) 3.20 min, R*t* (major) 4.74 min].



Co-injection of (*S*)-**4** and (*R*)-**4** gave two peaks at 3.10 min and 4.79 min.



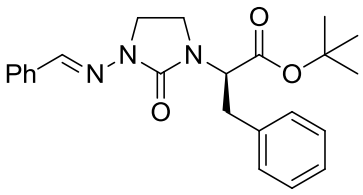
Benzylidene aza-glycinyl-D-phenylalanine *tert*-butyl ester [5]



To a stirred solution of hydrazine hydrate (1.5 mL, 45 mmol) in EtOH (30 mL) at 0°C, benzaldehyde (1.5 mL, 15 mmol) was added dropwise. Formation of benzaldehyde hydrazone was monitored by TLC (Rf = 0.6: 2:1 hexane:EtOAc). After 2h, the mixture was poured into H_2O (200 mL) and extracted with DCM (3 x 200 mL). The organic phase was separated, dried with $MgSO_4$ and concentrated in-vacuo to a 30 mL solution of benzaldehyde hydrazone in DCM. Without further purification, the hydrazone solution was added drop-wise over 30 min to a 0°C solution of p-nitrophenylchloroformate (3 g, 15 mmol) in DCM (50 mL). The reaction mixture was allowed to warm to room temperature with stirring for 2 h, cooled to 0°C, and treated with DIEA (5.2 mL, 30 mmol). The resulting suspension was quickly transferred to a mixture of D-phenylalanine tert-butyl ester hydrochloride (2.57 g, 10 mmol) and DIEA (2.5 mL, 15 mmol) in 100 mL of DCM. The reaction was stirred overnight at room temperature, and concentrated under vacuum to a residue, which was purified by chromatography using 10-30% EtOAc in hexanes. Evaporation of the collected fractions gave a yellow oil (3.1 g, 57%): Rf 0.26 (4:6 EtOAc : hexanes); $[\alpha]_D^{20}$ –12.4 (c 1.04, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (9H, s), 3.18-

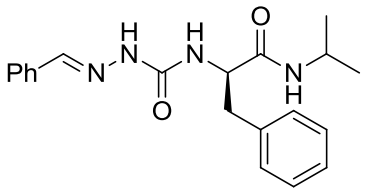
3.22 (1H, dd, J = 6.3, 13.7 Hz), 3.25-3.29 (1H, dd, J = 5.7, 13.7 Hz), 4.81-4.85 (1H, ddd, J = 8.4, 5.8, 12.1 Hz), 6.75-6.76 (1H, d, J = 8.3 Hz), 7.28-7.43 (8H, m), 7.61-7.63 (2H, m), 7.83 (1H, s), 10.43 (1H, s). ^{13}C NMR (100 MHz, CDCl3) δ 170.9, 156.4, 141.6, 136.5, 134.3, 129.7, 129.6, 128.6, 128.4, 127.0, 126.9, 82.0, 54.1, 38.9, 28.0. HRMS m/z 368.1966, $(M+H)^+$ calcd for $[C_{21}H_{26}N_3O_3]$+: 368.1969

(2'*R*)-1-((phenylmethylene)amino)-3-(*tert*-butyl-3'-phenylpropanoate)-imidazolidin-2-one [5c]



Benzylidene aza-Gly-Phe-O*t*Bu (**5**, 98 mg, 0.25 mmol), was dissolved in 2 mL of dry THF, cooled to 0°C, treated with 5 equiv. of BTPP base (382 µL, 1.25 mmol), stirred for 30 min, and treated with 2.5 equiv of 1,2-dibromo-ethane (54 µL, 0.63 mmol). The ice bath was removed. The reaction was allowed to warm to room temperature and stirred overnight. After evaporation of the volatiles under reduced pressure, the residue was purified by chromatography on silica gel using 15% EtOAc in hexanes to give the imidazolidin-2-one **5c** as a yellow oil (54 mg, 52%): R_f 0.32 (3:7 EtOAc : hexanes). $[\alpha]_D^{20}$ –9.4 (c 1.04, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (9H, s), 3.00-3.06 (1H, dd, *J* = 10.5, 14.8 Hz), 3.34-3.40 (1H, dd, *J* = 6.0, 14.8 Hz), 3.48-3.53 (1H, m), 3.57-3.62 (1H, m), 3.73-3.84 (2H, m), 4.99-5.04 (1H, dd, *J* = 5.9, 10.5 Hz), 7.22-7.54 (8H, m), 7.54 (1H, s), 7.71-7.73 (2H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.8, 155.9, 139.8, 136.3, 134.2, 128.9, 128.3, 128.2, 128.1, 126.7, 126.4, 81.9, 55.8, 40.8, 37.9, 34.6, 27.6. HRMS m/z 394.2083, $(M+H)^+$ calcd for $[C_{23}H_{28}N_3O_3]^+$: 394.2086

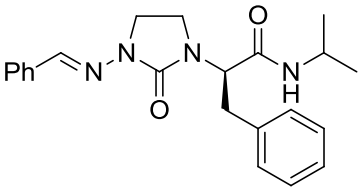
Benzylidene aza-glycinyl-D-phenylalanine isopropyl amide [6]



tert-Butyl ester **5** (1.84 g, 5 mmol, 1 equiv) was dissolved in 12 mL of a 1:1 DCM:TFA mixture, stirred for 2 h, and evaporated under reduced pressure. The resulting acid was dissolved in 10 mL of a DCM:DMF (95:5 v/v) mixture, treated with isopropylamine (1.23 mL, 15 mmol, 3 equiv), *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(1H-benzotriazol-1-yl)uronium hexafluoro-phosphate (HBTU, 1.89 g, 5 mmol, 1 equiv) and hydroxybenzotriazole (HOBt, 0.68 g, 5 mmol, 1 equiv), and stirred overnight at room temperature. Evaporation of the volatiles under vacuum gave a residue, which was purified by

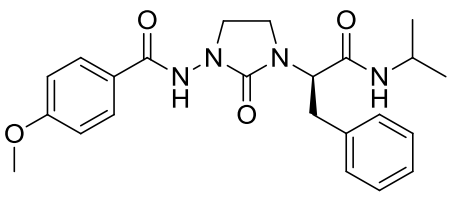
chromatography eluting with 5% MeOH in DCM. Evaporation of the collected fractions gave a white powder (1.48 g, 84%): R*f* 0.49 (1:9 MeOH : DCM); $[\alpha]_D^{20}$ –31.4 (*c* 1.04, $CHCl_3$), mp: 159-161°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.03-1.04 (3H, d, *J* = 6.6 Hz), 1.09-1.10 (3H, d, *J* = 6.6 Hz), 3.05-3.11 (1H, dd, *J* = 7.5, 13.5 Hz), 3.32-3.37 (1H, dd, *J* = 5.7, 13.6 Hz), 4.01-4.10 (1H, m), 4.60-4.65 (1H, ddd, *J* = 5.8, 7.7, 13.6 Hz), 5.82-5.84 (1H, d, *J* = 7.7 Hz), 6.74-6.76 (1H, d, *J* = 8.0 Hz), 7.28-7.37 (5H, m), 7.42-7.44 (3H, m), 7.61-7.63 (2H, m), 7.78 (1H, s), 9.63 (1H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 156.2, 142.0, 136.8, 133.9, 129.9, 129.6, 128.7, 127.1, 127.0, 126.9, 55.1, 41.5, 38.8, 22.6, 22.5. HRMS m/z 353.1965, $(M+H)^+$ calcd for $[C_{20}H_{25}N_4O_2]$: 353.1972.

(*R*)-2-(3-((Phenylmethylene)amino)-2-oxoimidazolidin-1-yl)-*N*-isopropyl-3-phenylpropanamide [6c]



Imidazolidin-2-one **6c** was synthesized from benzylidene aza-glycinyl-D-phenylalanine isopropyl amide (**6**, 1 g, 2.84 mmol) using the protocol described for the synthesis of (*S*)-**2c**. Purification by chromatography on silica gel using 40-50% EtOAc in hexanes gave 971 mg (2.56 mmol, 87% yield) of a white powder: R_f 0.27 (6:4 EtOAc : hexanes). $[\alpha]_D^{20}$ 88.6 (*c* 1.04, $CHCl_3$), mp: 72-74°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.04-1.06 (3H, d, *J* = 6.6 Hz), 1.11-1.13 (3H, d, *J* = 6.6 Hz), 3.02-3.09 (1H, dd, *J* = 8.5, 14.1 Hz), 3.31-3.37 (1H, dd, *J* = 7.56, 14.15 Hz), 3.55-3.66 (3H, m), 3.70-3.73 (1H, m), 3.97-4.05 (1H, m), 4.76-4.80 (1H, t, *J* = 8.1 Hz), 6.51-6.53 (1H, d, *J* = 7.8 Hz), 7.21-7.23 (1H, m), 7.27-7.29 (4H, m), 7.33-7.39 (3H, m), 7.43 (1H, s), 7.68 (1H, d, *J* = 1.2 Hz), 7.70 (1H, d, *J* = 1.7 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.7, 156.2, 140.0, 136.9, 134.6, 129.4, 129.0, 128.6, 128.6, 127.0, 126.8, 57.3, 41.5, 41.0, 38.6, 34.6, 22.5, 22.4. HRMS m/z 379.2086, $(M+H)^+$ calcd for $[C_{22}H_{27}N_4O_2]$+: 379.2089

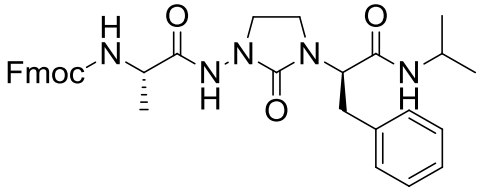
(2'*R*)-1-*p*-Methoxybenzamido-3-(3'-phenyl-*N*'-isopropyl-2'-propionamide)-imidazolidin-2-one [7a]



Imidazolidin-2-one **6c** (189 mg, 0.5 mmol) was treated with a 1:1 mixture of 1 N HCl (5 mL) and THF (5 mL) at 40°C overnight. After evaporation of the volatiles under reduced pressure, 5 mL of

water was added to the residue, which was freeze-dried to give a yellow oil that was dissolved in 10 mL of DCM, treated with DIEA (250 µL, 15 mmol, 3 equiv) and 4-methoxybenzoyl chloride (127.5 mg, 0.75 mmol, 1.5 equiv), and stirred overnight at room temperature. Evaporation of the volatiles under vacuum gave a residue, which was purified by chromatography eluting with 40-60% EtOAc in hexanes to give amide **7a** (189 mg, 0.44 mmol, 89%) as white powder: R_f 0.39 (7:3 EtOAc : hexanes). $[\alpha]_D^{20}$ 91.0 (*c* 1.04, $CHCl_3$), mp: 86-88°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.13-1.15 (3H, d, *J* = 6.63 Hz), 1.16-1.17 (3H, d, *J* = 6.63 Hz), 2.97-3.01 (1H, dd, *J* = 9.83, 14.35 Hz), 3.37-3.40 (2H, m), 3.47-3.55 (3H, m), 3.76 (3H, s), 4.04-4.12 (1H, m), 4.52-4.56 (1H, q, *J* = 6.26, 9.75 Hz), 6.69-6.70 (2H, d, *J* = 8.90 Hz), 6.91-6.93 (1H, d, *J* = 7.93 Hz), 7.17-7.22 (5H, m), 7.70-7.71 (2H, d, *J* = 8.89 Hz), 10.10 (1H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.4, 165.8, 162.6, 161.7, 137.7, 129.4, 128.8, 128.6, 126.6, 123.5, 113.6, 58.4, 55.3, 45.7, 41.7, 40.0, 34.1, 22.6, 22.4. HRMS m/z 425.2178, $(M+H)^+$ calcd for $[C_{23}H_{29}N_4O_4]^+$: 425.2183.

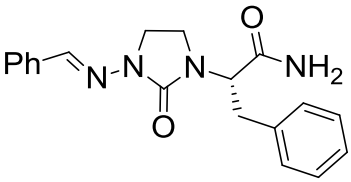
***N*-(Fmoc)Alaninyl-aminoimidazolidin-2-one-D-phenylalanine isopropyl amide [7b]**



Imidazolidin-2-one **6c** (189 mg, 0.5 mmol) was converted to its semicarbazide counterpart using the HCl in THF protocol described above for the synthesis of **7a**. The resulting residue was dissolved in 10 mL of DCM, treated with DIEA (83 µL, 5 mmol, 1 equiv) and the symmetric anhydride of Fmoc-Ala, which was prepared by mixing *N*-(Fmoc)alanine (467 mg, 1.5 mmol, 3 equiv) and DIC (116 µL, 0.75 mmol, 1.5 equiv) in DCM. Evaporation of the volatiles under vacuum gave a residue, which was purified by chromatography eluting with 3% MeOH in DCM. Evaporation of the collected fractions gave a white powder (198 mg, 68%): R*f* 0.32 (1:19 MeOH : DCM) ^{1}H NMR (400 MHz, CDCl3) δ 1.04 (3H, d, *J* = 6.51 Hz), 1.12 (3H, d, *J* = 6.58 Hz), 1.38 (3H, d, *J* = 6.81 Hz), 2.96-3.01 (1H, dd, *J* = 8.88, 14.23 Hz), 3.41-3.48 (3H, m), 3.50-3.56 (1H, m), 4.00-4.05 (1H, m), 4.20-4.23 (1H, dd, *J* = 6.84, 6.96 Hz), 4.33-4.40 (3H, m), 4.53-4.57 (1H, dd, *J* = 6.63, 7.96 Hz), 5.53 (1H, d, *J* = 7.55 Hz), 6.33 (1H, d, *J* = 7.40 Hz), 7.20-7.33 (8H, m), 7.40-7.43 (2H, dd, *J* = 6.80, 7.53 Hz), 7.58-7.60 (2H, m), 7.77 (1H, s), 7.79 (1H, s), 8.44 (1H, s). ^{13}C NMR (100 MHz, CDCl3) δ 171.8, 168.4, 160.1, 156.1, 143.7, 141.3, 137.2, 128.9, 128.6, 127.8, 127.1, 126.7, 125.0, 120.1, 67.3, 58.1,

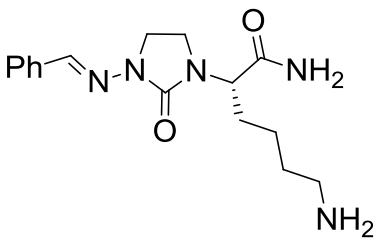
48.9, 47.2, 45.6, 41.6, 39.4, 34.2, 22.6, 22.3, 17.8. HRMS m/z 584.2878, (M+H)+ calcd for $[C_{33}H_{38}N_5O_5]$+: 584.2879

Benzylidene-Aid-Phe-NH_2 (10a).



Aid-peptide **10a** was prepared from benzylidene-aza-Gly-Phe-NH-resin **8a** (100 mg, 0.9 mmol/g) following the protocol described above for **9a**. The reaction was shown to have a quantitative conversion by monitoring with LCMS analysis of the crude mixture obtained from cleavage of resin **9a** with 6 mL of TFA/H_2O (95: 5, v/ v), resin filtration and evaporation. Purification of **10a** was conducted on a Waters™ PrepLC instrument equipped with a reverse-phase Gemini™ C18 column (250 × 21.2 mm, 5 µm), using a binary solvent system [20-60% of MeCN (0.1% FA) in water (0.1% FA), 40 min] at a flow rate of 10.0 mL/min. Purest fractions of **9a** were combined and freeze-dried to a white powder (12.4 mg, 41% overall). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.04-3.08 (1H, dd, *J* = 9.59, 14.6 Hz), 3.36-3.40 (1H, dd, *J* = 6.37, 14.7 Hz), 3.52-3.59 (4H, m), 4.88-4.91 (1H, dd, *J* = 6.4, 9.6 Hz), 5.60 (1H, s), 7.21-7.22 (1H, m), 7.26-7.31 (6H, m), 7.35-7.40 (3H, m), 7.62 (1H, d, *J* = 1.46 Hz), 7.64 (1H, d, *J* = 1.97 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.7, 156.5, 140.1, 136.9, 134.4, 129.6, 128.8, 128.7, 128.6, 126.9, 126.8, 56.6, 40.8, 38.5, 33.9. HRMS m/z 337.1660, $(M+H)^+$ calcd for $[C_{19}H_{21}N_4O_2]^+$: 337.1659

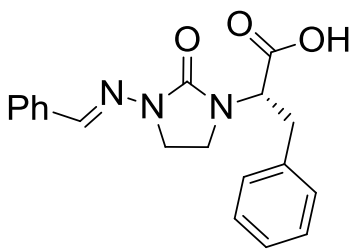
Benzylidene-Aid-Lys-NH_2 (10b).



Aid-Peptide **10b** was prepared from benzylidene-azaGly-Lys(Boc)-NH-resin **8b** (100 mg, 0.9 mmol/g) following the protocols described for the synthesis of **9a**. The reaction was shown to have a quantitative conversion by monitoring with LCMS analysis of crude mixture obtained from cleavage of resin with 6 mL of TFA/TES/H_2O (95: 2.5:2.5, v/v/v). Purification of **9b** was conducted using a reverse-phase Gemini™ C18 column [0-50% MeCN (0.1% FA) in water (0.1% FA), 45 min] at a flow rate of 10.0 mL/min. Purest fractions of **9b** were combined and freeze-dried to a white powder (13.5 mg, 47% overall). ^{1}H NMR (500 MHz, CD_3OD) δ 1.40-

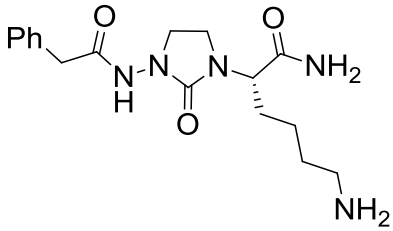
1.51 (2H, m), 1.71-1.80 (2H, m), 1.83-1.91 (1H, m), 1.98-2.03 (1H, m), 2.94-2.98 (2H, m), 3.63-3.68 (1H, m), 3.75-3.80 (1H, m), 3.86-3.90 (2H, m), 4.54-4.57 (1H, dd, *J* = 5.3, 10.4 Hz), 7.36-7.43 (3H, m), 7.72 (1H, s), 7.78-7.81 (2H, m), 8.56 (1H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.1, 159.0, 142.2, 136.4, 130.6, 129.7, 128.2, 56.4, 42.1, 40.5, 39.4, 28.8, 28.0, 24.1. HRMS m/z 318.1925, $(M+H)^+$ calcd for $[C_{16}H_{24}N_5O_2]^+$: 318.1936.

Benzylidene-Aid-Phe-OH (10j).



Aid-Peptide **10j** was prepared from benzylidene-azaGly-Phe ester linked to Wang resin **8j** (100 mg, 0.67 mmol/g) following the protocols described for the synthesis of **9a**, exceptionally, BTPP (104 mg, 0.34 mmol) was used as base. The reaction was shown to have 82% conversion by monitoring with LCMS analysis of crude mixture obtained from cleavage of resin with 5 mL of TFA/TES/H_2O (95: 2.5:2.5, v/v/v). Purification of **10j** was conducted using a reverse-phase Gemini™ C18 column [20-80% MeCN (0.1% FA) in water (0.1% FA), 30 min, at a flow rate of 10.0 mL/min]. Purest fractions of **10j** were combined, and freeze-dried to a white powder (4.8 mg, 21% overall). ^{1}H NMR (500 MHz, CD_3OD) δ 3.14 (1H, dd, *J* = 11.7, 14.8 Hz), 3.45 (1H, dd, *J* = 4.8, 14.8 Hz), 3.61-3.73 (3H, m), 3.80-3.85 (1H, m), 4.89-4.93 (1H, dd, *J* = 4.8, 11.4 Hz), 7.20-7.24 (1H, m), 7.29-7.40 (7H, m), 7.62 (1H, s), 7.75-7.77 (2H, m). ^{13}C NMR (100 MHz, CD_3OD) δ 172.4, 157.5, 140.5, 137.3, 135.0, 129.0, 128.3, 128.2, 128.2, 126.8, 126.4, 56.5, 40.5, 38.6, 34.2. HRMS m/z 338.1460, $(M+H)^+$ calcd for $[C_{19}H_{20}N_3O_3]^+$: 338.1457

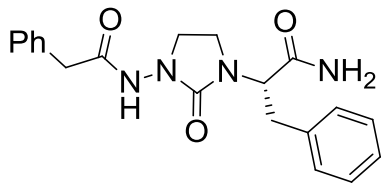
Phenylacetyl-Aid-Lys-NH_2 (12a):



Aid-peptide **12a** was prepared from benzylidene-Aid-Lys(Boc) linked to Rink resin **9b** (100 mg, 0.9 mmol/g). The benzylidene protecting group was first removed using a solution of hydroxyamine (1.5 M) in pyridine following the protocol described for the synthesis of **9a-1**. The acylation step was performed using phenylacetyl chloride (41.6 mg, 0.27 mmol, 3 equiv) and DIEA (47 μL, 0.27 mmol, 3 equiv) in DCM (4 mL) on an automated shaker overnight. After filtration, the resin was treated with 6 mL of a TFA/TES/H_2O (95: 2.5:2.5, v/v/v) cocktail for 2h. After resin filtration and

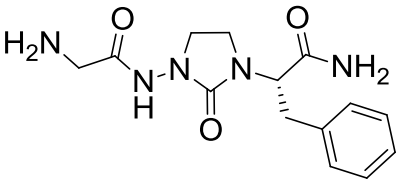
evaporation, the crude residue was purified using preparative HPLC on a Gemini™ C18 column [10-50% MeCN (0.1% FA) in water (0.1% FA), 40 min]. Purest fractions of **12a** were combined and freeze-dried to white powder (10.6 mg, 34% overall) ^{1}H NMR (500 MHz, CD_3OD) δ 1.37-1.43 (1H, m), 1.45-1.52 (1H, m), 1.64-1.69 (1H, m), 1.71-1.76 (1H, m), 1.81-1.86 (1H, m), 1.91-1.97 (1H, m), 2.91-2.97 (2H, m), 3.44-3.50 (1H, m), 3.56-3.59 (5H, m), 4.39-4.42 (1H, q, *J* = 5.40, 10.36 Hz), 7.25-7.29 (1H, m), 7.33-7-34 (4H, m), 8.19 (1H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.1, 173.3, 162.2, 135.9, 130.3, 129.6, 128.1, 56.4, 46.6, 41.5, 40.7, 39.5, 28.6, 27.8, 23.8. HRMS m/z 348.2029, $(M+H)^+$ calcd for $[C_{17}H_{26}N_5O_3]^+$: 348.2030

Phenylacetyl-Aid-Phe-NH_2 (12b):



Aid-peptide **12b** was prepared from benzylidene-Aid-Phe linked to Rink resin **9a** (100 mg, 0.9 mmol/g) following a similar protocol as described for the synthesis of **12a**. After purification by preparative HPLC on a Gemini™ C18 column [10-50% MeCN (0.1% FA) in water (0.1% FA), 40 min], purest fractions of **10b** were combined and freeze-dried to a white powder (10.2 mg, 31% overall). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.97-3.03 (1H, dd, *J* = 10.2, 15.7 Hz), 3.35-3.39 (2H, dd, *J* = 7.2, 8.3 Hz), 3.44-3.53 (3H, m), 3.56 (2H, s), 4.66-4.69 (1H, q, *J* = 5.8, 10.1 Hz), 5.74 (1H, s), 6.79 (1H, s), 7.21-7.23 (3H, m), 7.26-7.30 (5H, m), 7.33-7.36 (2H, m), 8.18 (1H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2, 170.8, 160.6, 137.1, 133.6, 130.1, 129.3, 129.0, 128.7, 127.5, 126.8, 57.4, 45.5, 41.2, 39.4, 33.7. HRMS m/z 367.1757, $(M+H)^+$ calcd for $[C_{20}H_{23}N_4O_3]^+$: 367.1765

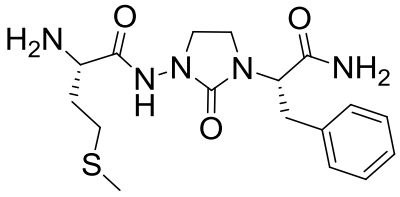
Gly-Aid-Phe-NH_2 (12c):



Aid-peptide **12c** was prepared from benzylidene-Aid-Phe linked to Rink resin **9a** (100 mg, 0.9 mmol/g) as previously described. Liberation of the semicarbazide was accomplished employing $NH_2OH{\cdot}HCl$ in pyridine at 60°C for 12h to give resin **9a-1**. Acylation of **9a-1** was subsequently performed by coupling *N*-(Boc)glycine (79 mg, 0.45 mmol, 5 equiv) activated by way of its symmetric anhydride which was freshly prepared using DIC (32 µL, 0.23 mmol, 2.5 equiv) to give resin **11c**. After resin cleavage [TFA/TES/H_2O (95: 2.5:2.5, v/v/v)], filtration, washing, and

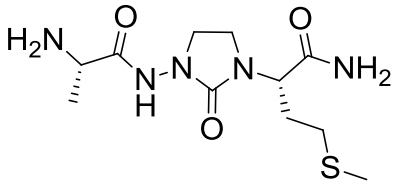
evaporation of the filtrate and washings, the crude residue was purified using preparative HPLC on a Gemini™ C18 column [0-30% MeCN(0.1% FA) in water (0.1% FA), 40 min]. Purest fractions of **12c** were combined and freeze-dried to white powder (9.9 mg, 36% overall). ^{1}H NMR (400 MHz, CD_3OD) δ 3.02-3.07 (1H, dd, *J* = 10.0, 14.9 Hz), 3.30-3.31 (1H, dd, *J* = 3.3, 8.8 Hz), 3.34 (1H, overlay with solvent peak), 3.48-3.61 (4H, m), 3.73 (2H, s), 4.69-4.72 (1H, dd, *J* = 6.1, 9.9 Hz), 7.21-7.25 (1H, m), 7.30 (2H, d, *J* = 1.9 Hz), 7.03 (2H, s), 7.32-7.34 (2H, m), 8.11 (1H, brd). ^{13}C NMR (100 MHz, CD_3OD) δ 174.6, 167.4, 161.6, 138.5, 130.0, 129.6, 127.8, 58.5, 46.6, 40.4, 40.3, 35.6. HRMS m/z 306.1571, $(M+H)^+$ calcd for $[C_{14}H_{20}N_5O_3]^+$: 306.1561**.**

Met-Aid-Phe-NH_2 (12d):



Similar to the synthesis of **12c**, Aid-peptide **12d** was prepared from benzylidene-Aid-Phe linked to Rink resin **9a** (100 mg, 0.9 mmol/g). Acylation was performed using *N*-(Boc)methionine (112 mg, 0.45 mmol, 5 equiv), which was activated with DIC (32 µL, 0.23 mmol, 2.5 equiv) in DCM (5 mL). After resin cleavage [TFA/TES/H_2O (95: 2.5:2.5, v/v/v)], filtration, washing, and evaporation of the filtrate and washings, the crude residue was purified using preparative HPLC on a Gemini™ C18 column [10-40% MeCN (0.1% FA) in water (0.1% FA), 40 min]. Purest fractions of **12d** were combined and freeze-dried to white powder (8.9 mg, 33% overall). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.08-2.20 (2H, m), 2.13 (3H, s), 2.64-2.67 (2H, t, *J* = 7.5 Hz), 3.02-3.07 (1H, dd, *J* = 9.9, 14.4 Hz), 3.31-3.35 (1H, dd, *J* = 6.2, 14.3 Hz), 3.51-3.61 (4H, m), 3.91-3.94 (1H, t, *J* = 6.6 Hz), 4.68-4.71 (1H, dd, *J* = 6.2, 9.9 Hz), 7.21-7.24 (1H, m), 7.29-7.33 (5H, m), 8.43 (1H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.6, 170.6, 168.6 (formic acid) 161.6, 138.5, 130.0, 129.6, 127.8, 58.5, 52.5, 46.5, 40.5, 35.5, 32.2, 29.8, 15.1. HRMS m/z 380.1750, $(M+H)^+$ calcd for $[C_{17}H_{26}N_5O_3S]^+$: 380.1751**.**

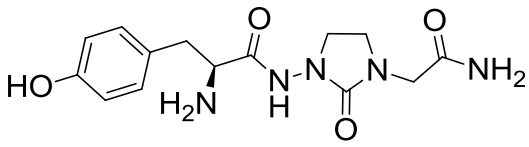
Ala-Aid-Met-NH_2 (12e):



Aid-peptide **12e** was prepared from benzylidene-Aid-Met resin **9i** (100 mg, 0.9 mmol/g), which was prepared according to the protocols described for the synthesis of **9a**. Acylation was performed using *N*-(Boc)alanine (85 mg, 0.45 mmol, 5 equiv) activated with DIC (32 uL,

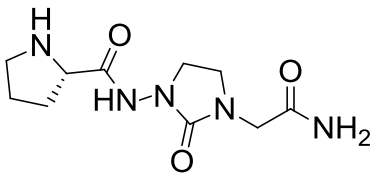
0.23 mmol, 2.5 equiv) in DCM (5 mL). After resin cleavage (TFA/TES/H_2O (95: 2.5:2.5, v/v/v)), filtration, washing, and evaporation of the filtrate and washings, the crude residue was purified using preparative HPLC on a RP-Polar column (150 × 21.2 mm, 5 µm), using a gradient of 0-20% MeCN (0.1% FA) in water (0.1% FA) over 40 min. Purest fractions of **12e** were combined and freeze-dried to white powder (4.6 mg, 17% overall). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.55 (3H, d, *J* = 7.1 Hz), 2.03-2.10 (1H, m), 2.12 (3H, s), 2.18-2.25 (1H, m), 2.47-2.53 (1H, m), 2.57-2.62 (1H, m), 3.51-3.55 (1H, m), 3.62-3.66 (3H, m), 3.93-3.97 (1H, dd, *J* = 7.6, 15.1 Hz), 4.54-4.57 (1H, dd, *J* = 5.1, 10.2 Hz), 8.49 (1H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.8, 171.5, 161.8, 56.3, 46.5, 40.0, 31.4, 29.4, 29.1, 17.5, 15.3. HRMS m/z 304.1447, $(M+H)^+$ calcd for $[C_{11}H_{22}N_5O_3S]^+$: 304.1440

Tyr-Aid-Gly-NH_2 (12f):



Aid-peptide **12f** was prepared from benzylidene-Aid-Gly resin **9h** (100 mg, 0.9 mmol/g) that was prepared according to the protocols to make **9a**. Acylation was performed using Fmoc-Tyr(OtBu) (207 mg, 0.45 mmol, 5 equiv) and DIC (32 µL, 0.23 mmol, 2.5 equiv) in DCM (5 mL). The Fmoc-protecting group was removed using 20% piperidine in DMF (5 mL) for 30 min. After resin cleavage [TFA/TES/H_2O (95: 2.5:2.5, v/v/v)], filtration, washing, and evaporation of the filtrate and washings, the crude residue was purified using preparative HPLC equipped with a Gemini™ C18 column (250 × 21.2 mm, 5 µm) using a gradient of 0-5% MeCN (0.1% FA) in water (0.1% FA) over 40 min. Purest fractions of **12f** were combined and freeze-dried to white powder (10.1 mg, 35% overall). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.88-2.92 (1H, dd, *J* = 7.1, 13.8 Hz), 2.97-3.01 (1H, dd, *J* = 7.4, 13.8 Hz), 3.29-3.32 (1H, m), 3.38-3.47 (3H, m), 3.78 (2H, d, *J* = 2.1 Hz), 3.81-3.84 (1H, m), 6.67-6.69 (2H, d, *J* = 8.5 Hz), 7.00-7.02 (2H, d, *J* = 8.4 Hz), 8.28 (1H, s, brd). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2, 170.4, 162.1, 158.3, 131.7, 126.0, 116.8, 54.8, 47.6, 46.1, 43.6, 38.0. HRMS m/z 322.1507, $(M+H)^+$ calcd for $[C_{14}H_{19}N_5O_4]^+$: 322.1510

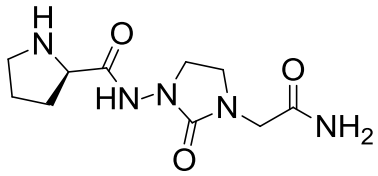
Pro-Aid-Gly-NH_2 (12g):



As described for the synthesis of Aid-peptide **12f**, **12g** was prepared from benzylidene-Aid-Gly resin **9h** (100 mg, 0.9 mmol/g). Acylation was

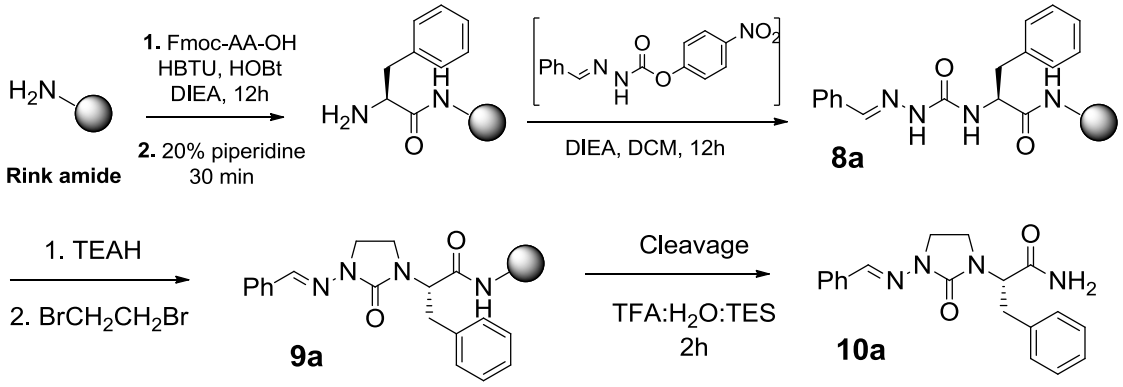
performed using *N*-(Fmoc)proline (151 mg, 0.45 mmol, 5 equiv) activated with DIC (32 uL, 0.23 mmol, 2.5 equiv) in DCM (5 mL). The Fmoc-protection was removed using 20% piperidine in DMF (5 mL) for 30 min. After the resin cleavage [TFA/H_2O (95:5, v/v)] protocol, the crude residue was purified using preparative HPLC on a Gemini™ C18 column (250 × 21.2 mm, 5 µm), using a gradient of 0-5% MeCN (0.1% FA) in water (0.1% FA) over 40 min. Purest fractions of **12g** were combined and freeze-dried to white powder (5.5 mg, 26% overall). ^{1}H NMR (500 MHz, CD_3OD) δ 2.02-2.13 (3H, m), 2.38-2.42 (1H, m), 2.68 (3H, m), 3.27-3.35 (2H, m), 3.54-3.57 (2H, m), 3.63-3.67 (2H, m), 3.91 (2H, s), 4.19-4.22 (1H, m), 8.52 (1H, s). ^{13}C NMR (100 MHz, CDCl3) δ 171.8, 170.1 (formic acid), 168.4, 160.6, 58.6, 46.2, 44.9, 42.2, 39.0, 29.5, 24.1. HRMS m/z 256.1404, $(M+H)^+$ calcd for $[C_{10}H_{18}N_5O_3]^+$: 256.1404

D-Pro-Aid-Gly-NH_2 (12h):

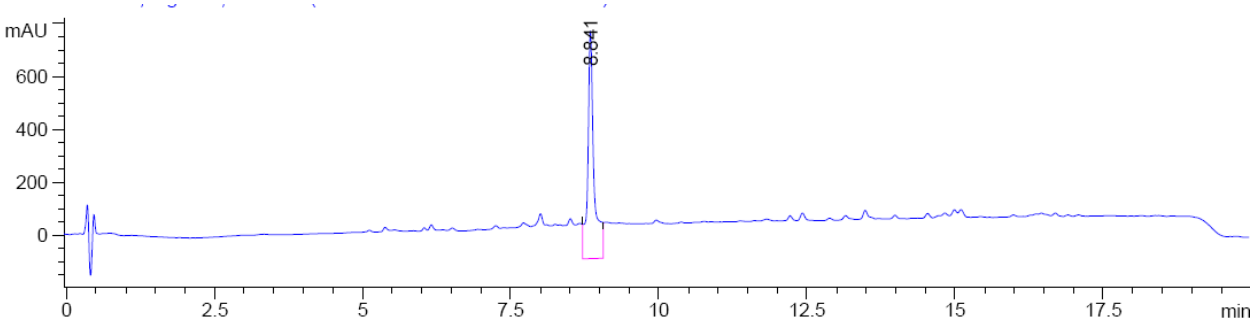


Similar to **12g**, tripeptide **12h** was prepared from benzylidene-Aid-Gly resin **9h** (100 mg, 0.9 mmol/g). After the resin cleavage, the crude residue was purified using preparative HPLC and, freeze-dried to give **12g** as a white powder (7.1 mg, 31% overall). HRMS m/z 256.1404, $(M+H)^+$ calcd for $[C_{10}H_{18}N_5O_3]^+$: 256.1404.

10. LC-MS profiles of crude benzylidene-Amino-imidazolidin-2-one dipeptides 10a-j



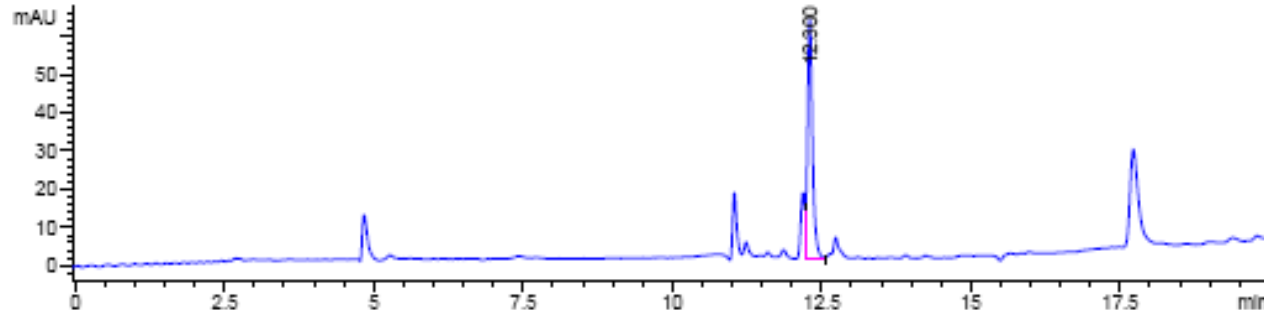
Benzylidene-Aid-Phe-NH_2 (10a).



LCMS (05-80% MeCN, 20 min) R.T. = 8.8 min;

LCMS (ESI) found m/e 337.2, $(M+H)^+$ calcd for $[C_{17}H_{19}N_4O_2]^+$: 337.2.

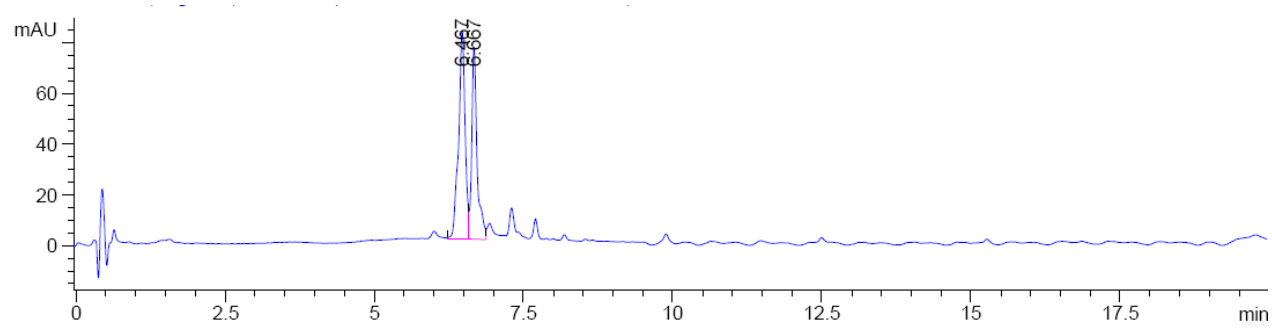
Benzylidene-Aid-Lys-NH_2 (10b).



LCMS (05-80% MeCN, 20 min) R.T. = 12.3 min;

LCMS (ESI) found m/e 318.2, $(M+H)^+$ calcd for $[C_{16}H_{24}N_5O_2]^+$: 318.2,

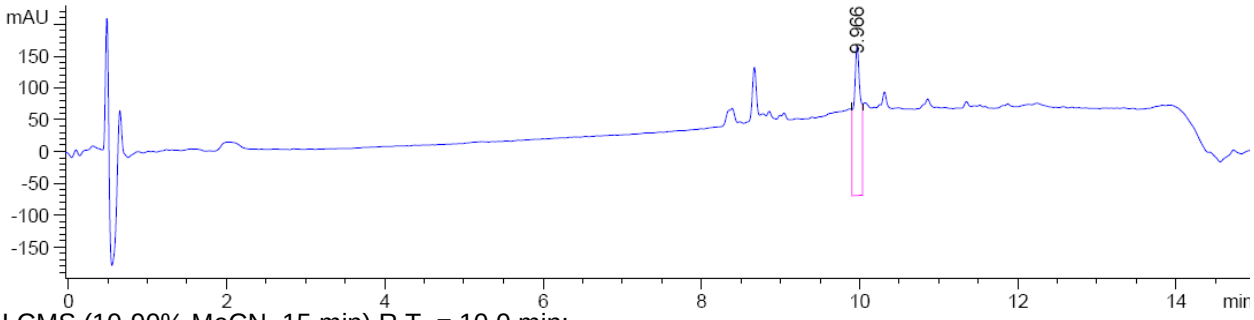
Benzylidene-Aid-Asp-NH_2 (9d).



LCMS (05-80% MeCN, 20 min) R.T. = 6.5 and 6.7 min;

LCMS (ESI) found m/e 305.1, $(M+H)^+$ calcd for $[C_{14}H_{17}N_4O_4]^+$: 305.1.

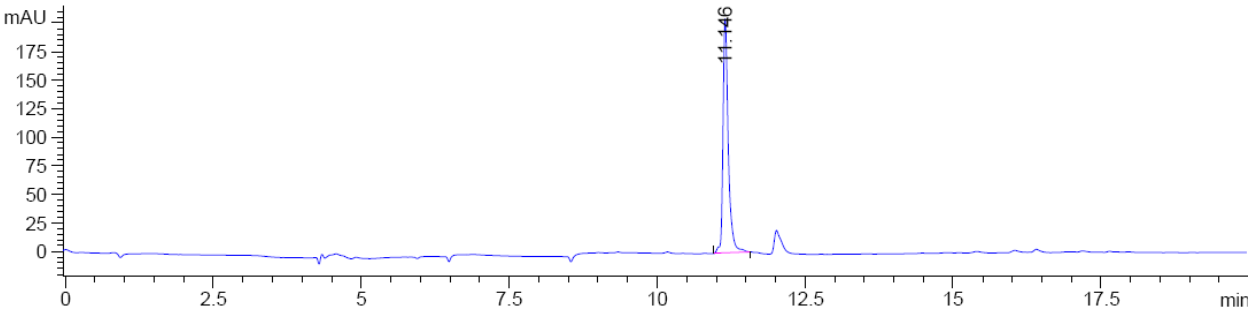
Benzylidene-Aid-Asp(OBn)-NH_2 (10e).



LCMS (10-90% MeCN, 15 min) R.T. = 10.0 min;

LCMS (ESI) found m/e 395.2, $(M+H)^+$ calcd for $[C_{21}H_{23}N_4O_4]^+$: 395.2,

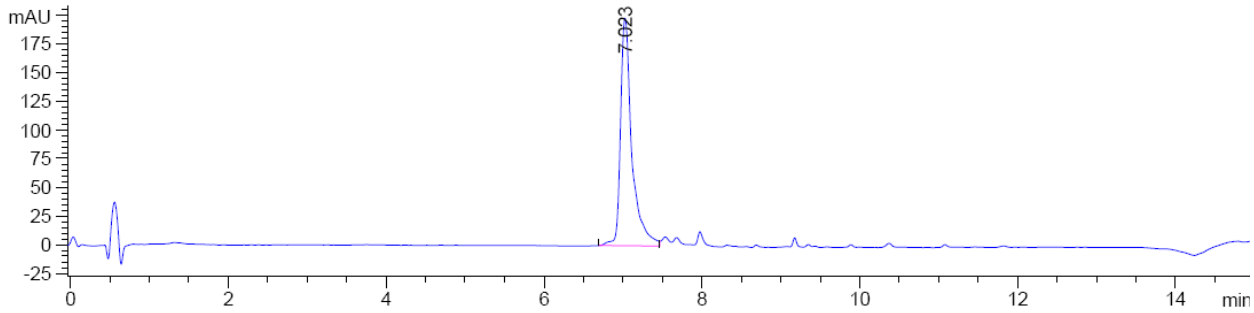
Benzylidene-Aid-Asn-NH_2 (10f).



LCMS (05-80% MeCN, 20 min) R.T. = 11.2 min;

LCMS (ESI) found m/e 304.1, $(M+H)^+$ calcd for $[C_{14}H_{18}N_5O_3]^+$: 304.1.

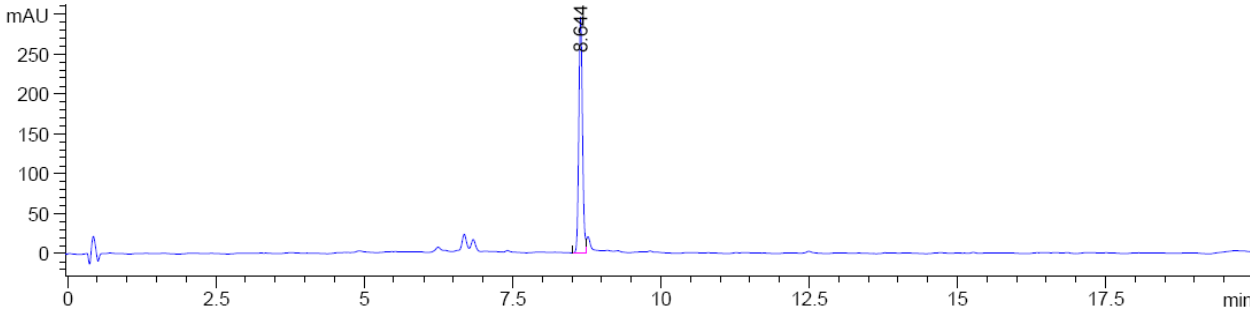
Benzylidene-Aid-Gly-NH_2 (10g).



LCMS (10-90% MeCN, 15 min) R.T. = 7.0 min;

LCMS (ESI) found m/e 247.1, $(M+H)^+$ calcd for $[C_{12}H_{15}N_4O_4]^+$: 247.12.

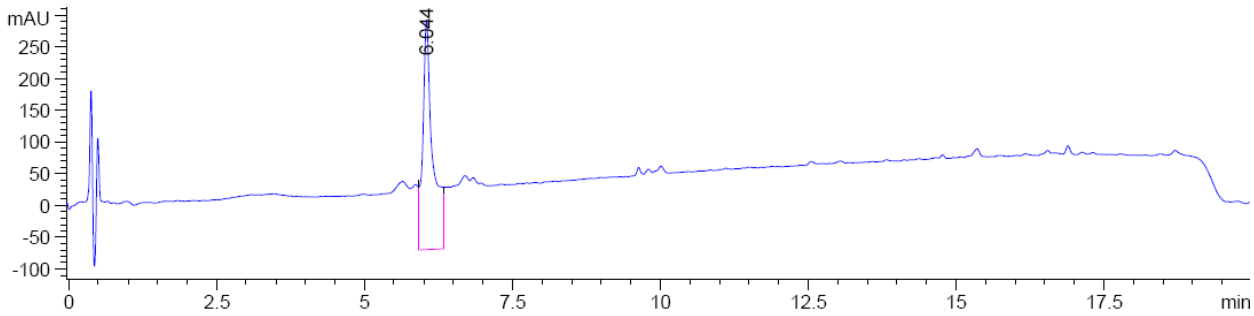
Benzylidene-Aid-Met-NH_2 (10h).



LCMS (05-80% MeCN, 20 min) R.T. = 8.6 min;

LCMS (ESI) found m/e 321.1, $(M+H)^+$ calcd for $[C_{15}H_{20}N_4O_2S]^+$: 321.1.

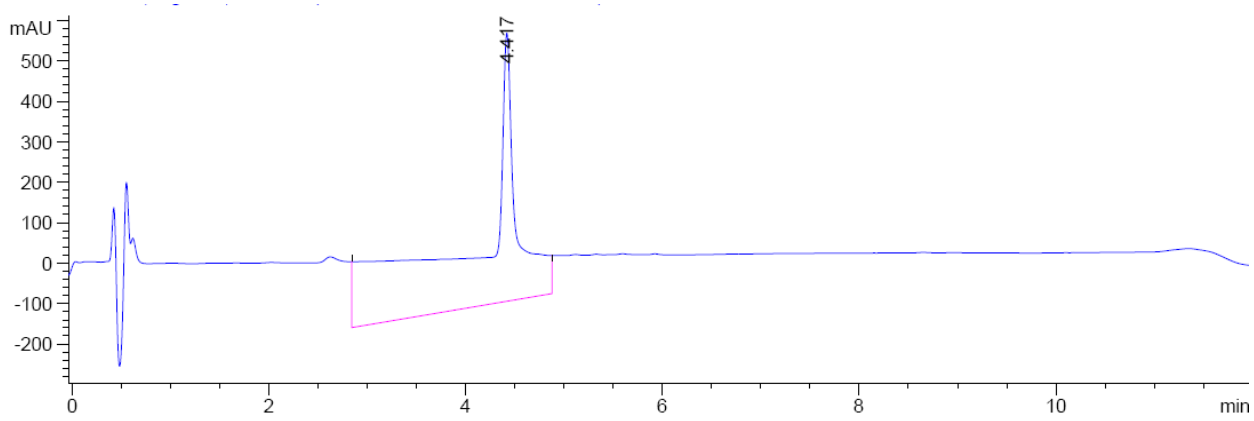
Benzylidene-Aid-Ser-NH2 (10i).



LCMS (05-80% MeCN, 20 min) R.T. = 6.0 min;

LCMS (ESI) found m/e 277.1, $(M+H)^+$ calcd for $[C_{13}H_{17}N_4O_3]^+$: 277.1.

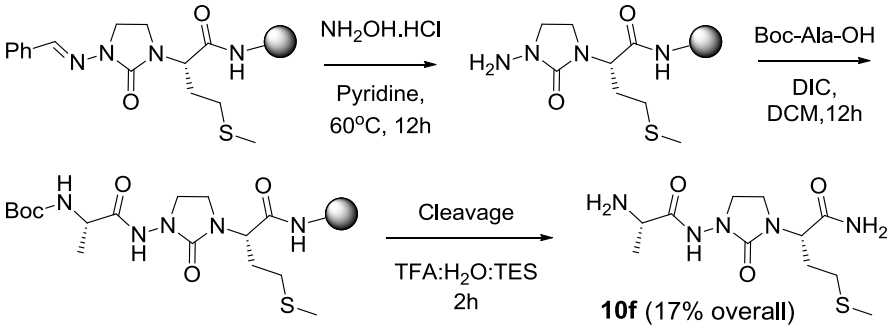
Benzylidene-Aid-Phe-OH (10j).



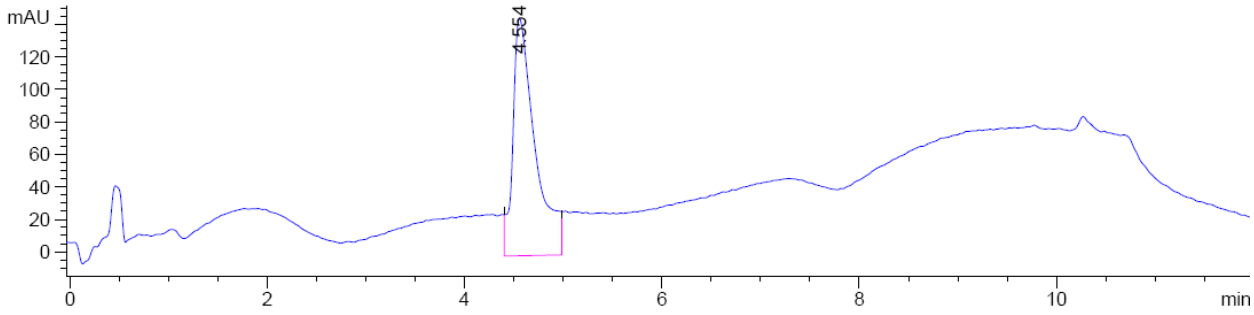
LCMS (30-90% MeCN, 12 min) R.T. = 4.4 min;

LCMS (ESI) found m/e 338.1, $(M+H)^+$ calcd for $[C_{19}H_{20}N_3O_3]^+$: 338.1

11. LC-MS profiles of crude N-Amino-imidazolidin-2-one peptidomimetics 12a-h



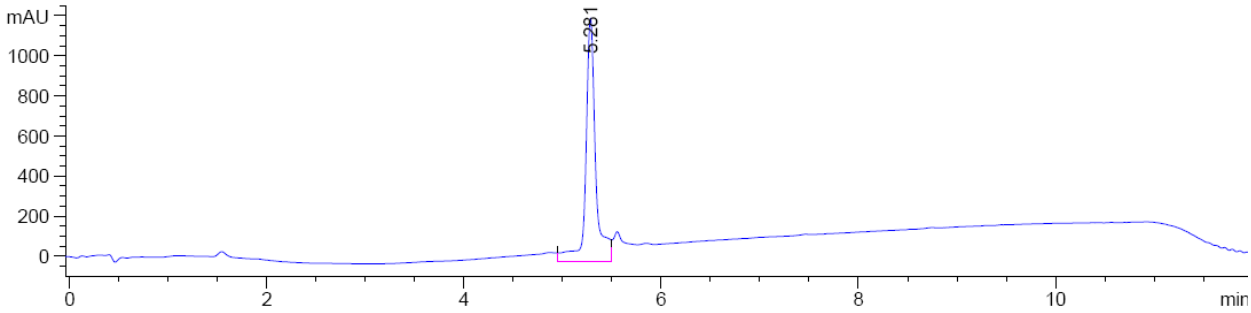
Phenylacetyl-Aid-Lys-NH_2 (12a):



LCMS (00-50% MeCN, 12 min) R.T. = 4.6 min;

LCMS (ESI) found m/e 348.2, $(M+H)^+$ calcd for $[C_{17}H_{26}N_5O_3]^+$: 348.2

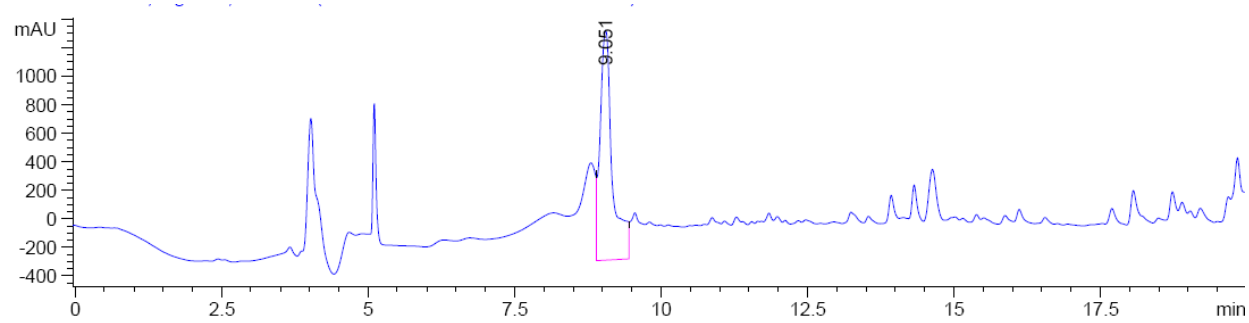
Phenylacetyl-Aid-Phe-NH_2 (12b):



LCMS (30-90% MeCN, 12 min) R.T. = 5.3 min;

LCMS (ESI) found m/e 367.2, $(M+H)^+$ calcd for $[C_{20}H_{23}N_4O_3]^+$: 367.2

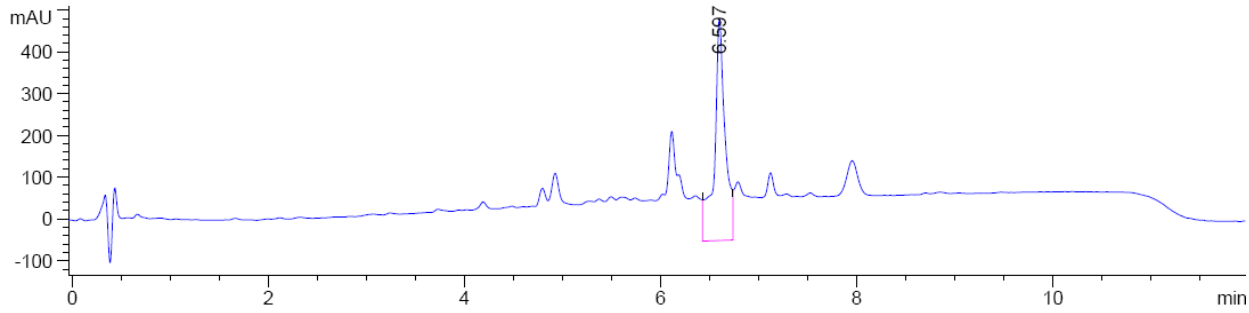
Gly-Aid-Phe-NH_2 (12c):



LCMS (05-80% MeCN, 20 min) R.T. = 9.1 min;

LCMS (ESI) found m/e 306.2, $(M+H)^+$ calcd for $[C_{14}H_{20}N_5O_3]^+$: 306.2

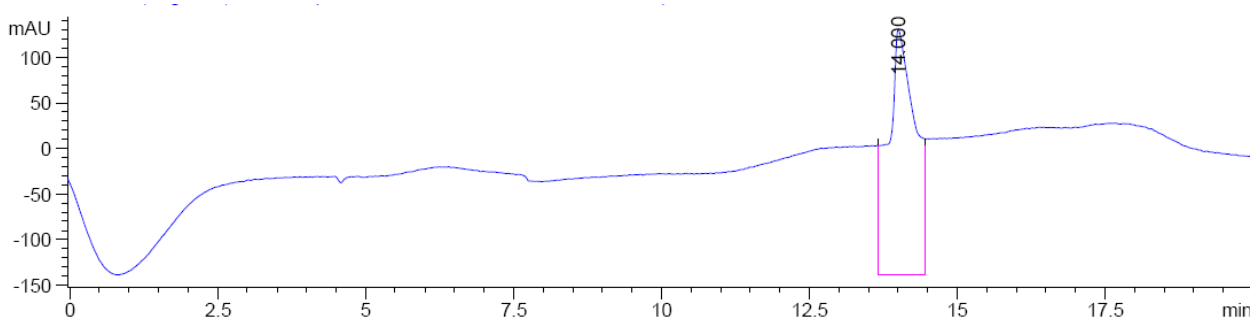
Met-Aid-Phe-NH_2 (12d):



LCMS (20-80% MeCN, 12 min) R.T. = 6.6 min;

LCMS (ESI) found m/e 380.2, $(M+H)^+$ calcd for $[C_{17}H_{26}N_5O_3S]^+$: 380.2

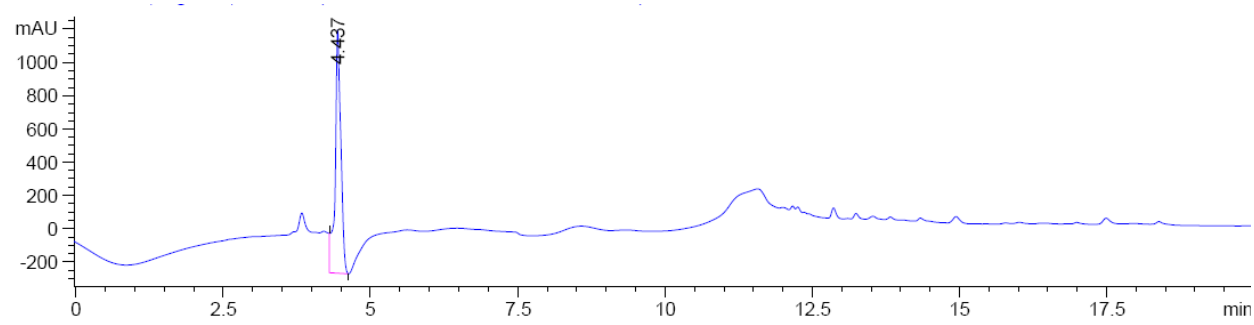
Ala-Aid-Met-NH_2 (12e):



LCMS (00-30% MeCN, 20 min) R.T. = 14.0 min;

LCMS (ESI) found m/e 304.1, $(M+H)^+$ calcd for $[C_{11}H_{22}N_5O_3S]^+$: 304.1

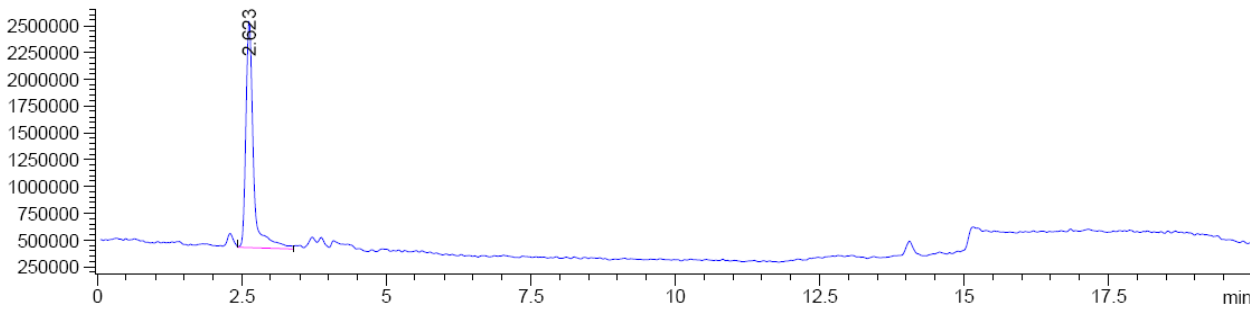
Tyr-Aid-Gly-NH_2 (12f):



LCMS (00-30% MeCN, 20 min) R.T. = 4.4 min;

LCMS (ESI) found m/e 322.2, $(M+H)^+$ calcd for $[C_{14}H_{20}N_5O_4]^+$: 322.2

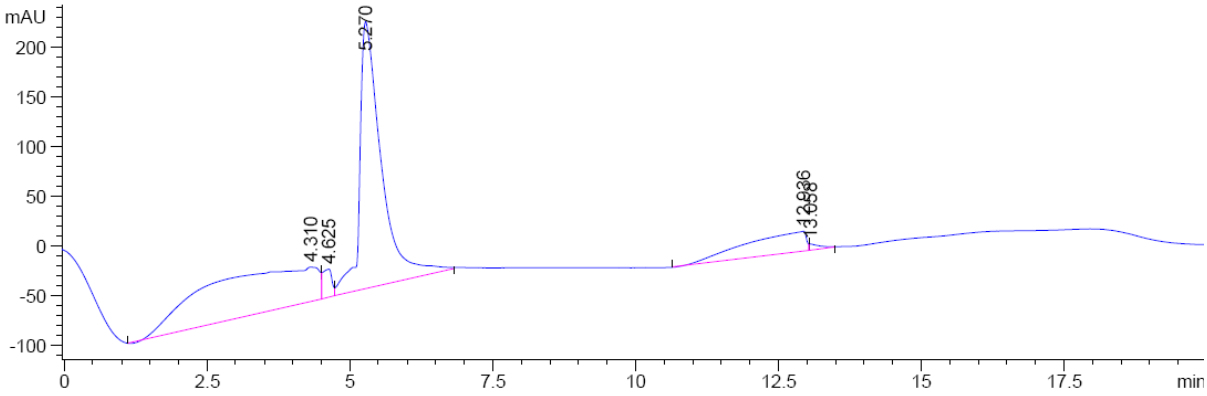
Pro-Aid-Gly-NH_2 (12g):



LCMS (00-30% MeCN, 20 min) R.T. = 2.6 min;

LCMS (ESI) found m/e 256.1, $(M+H)^+$ calcd for $[C_{10}H_{18}N_5O_3]^+$: 256.1

D-Pro-Aid-Gly-NH_2 (**12h**):

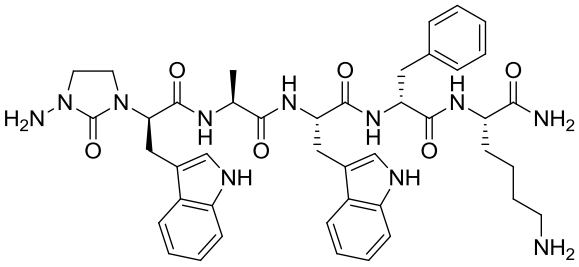


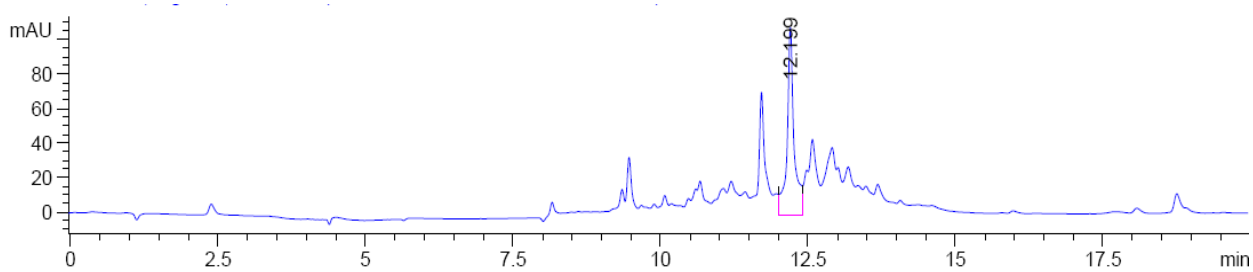
LCMS (00-20% MeCN, 20 min,) on a RP-polar column (150 × 4.60 mm, 5 µm), R.T. = 5.3 min;

LCMS (ESI) found m/e 256.1, $(M+H)^+$ calcd for $[C_{10}H_{18}N_5O_3]^+$: 256.1

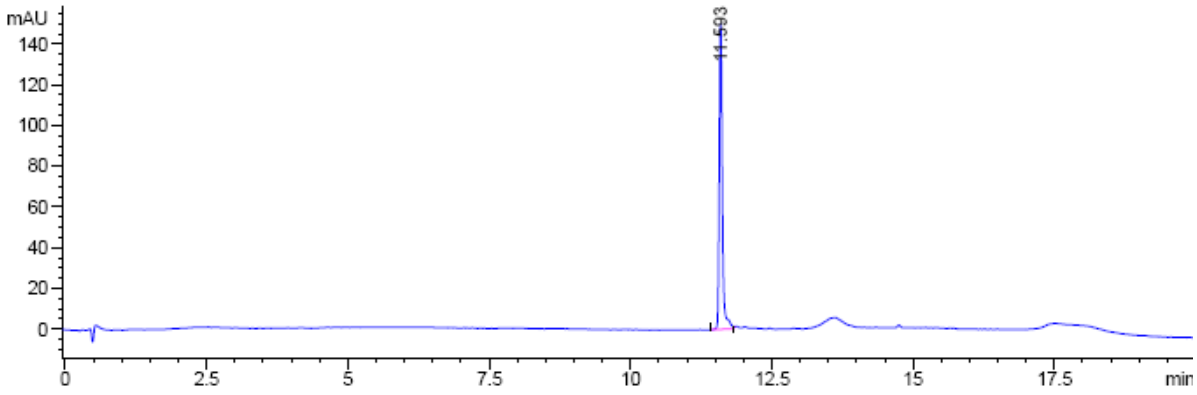
12. LC-MS profiles of GHRP-6 peptides

Aid-D-Trp-Ala-Trp-D-Phe-Lys-NH_2 ([Aid^1]GHRP-6)

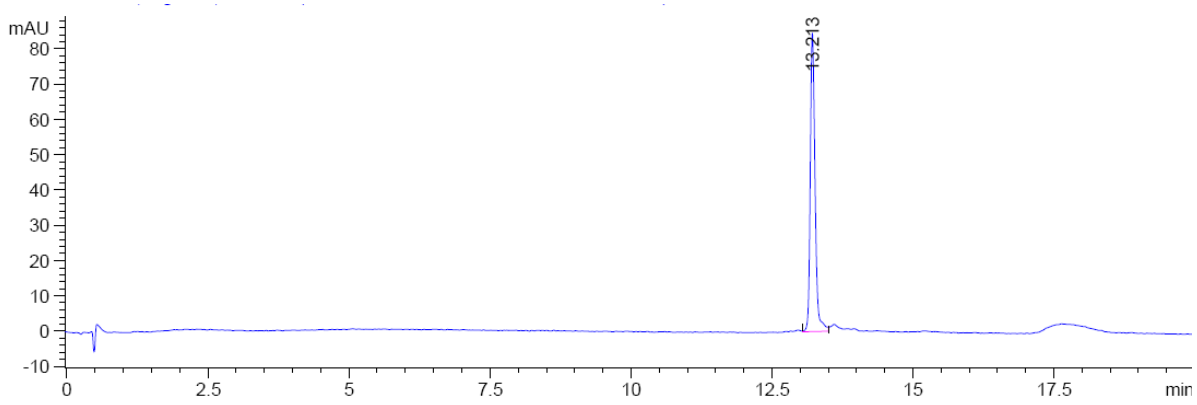




LCMS chromatogram (05-80% MeCN, 20 min, R.T. = 12.2 min) of crude [Aid[1]]GHRP-6 (**Aid**-D-Trp-Ala-Trp-D-Phe-Lys-NH_2). LCMS (ESI) found m/e 820.4, $(M+H)^+$ calcd for $[C_{38}H_{51}N_{12}O_6]^+$: 820.4

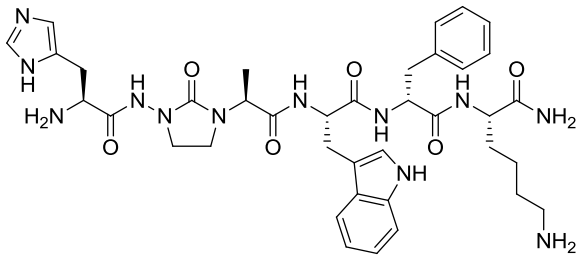


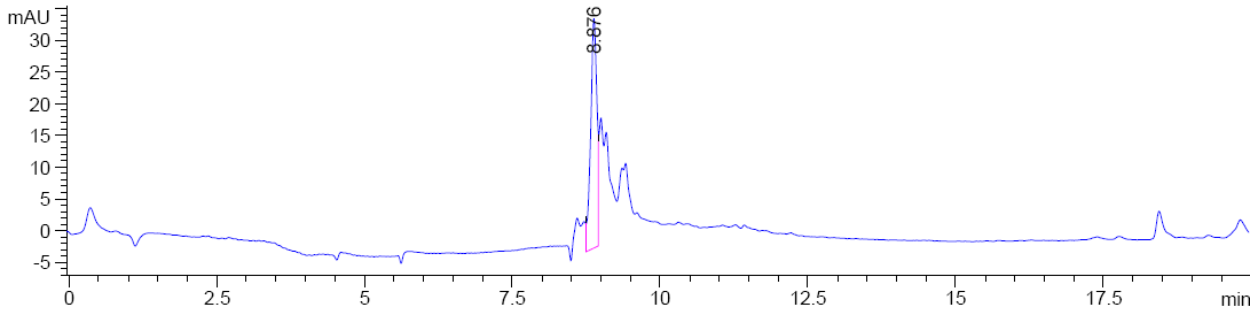
LCMS chromatogram (00-50% MeCN, 20 min, R.T. = 11.6 min) of purified [Aid[1]]GHRP-6.



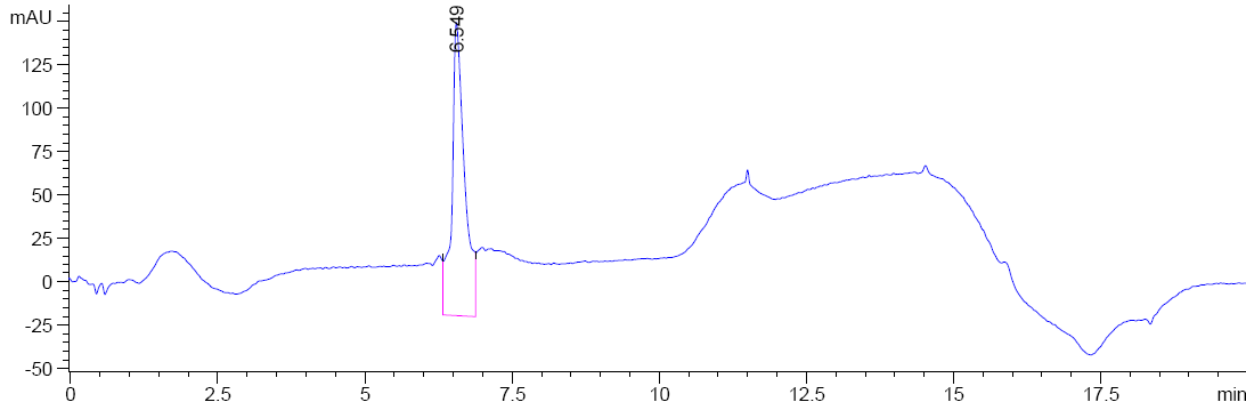
LCMS chromatogram (00-50% MeOH, 20 min, R.T. = 13.2 min) of purified [Aid[1]]GHRP-6.

His-Aid-Ala-Trp-D-Phe-Lys-NH_2 ([Aid[2]]GHRP-6)

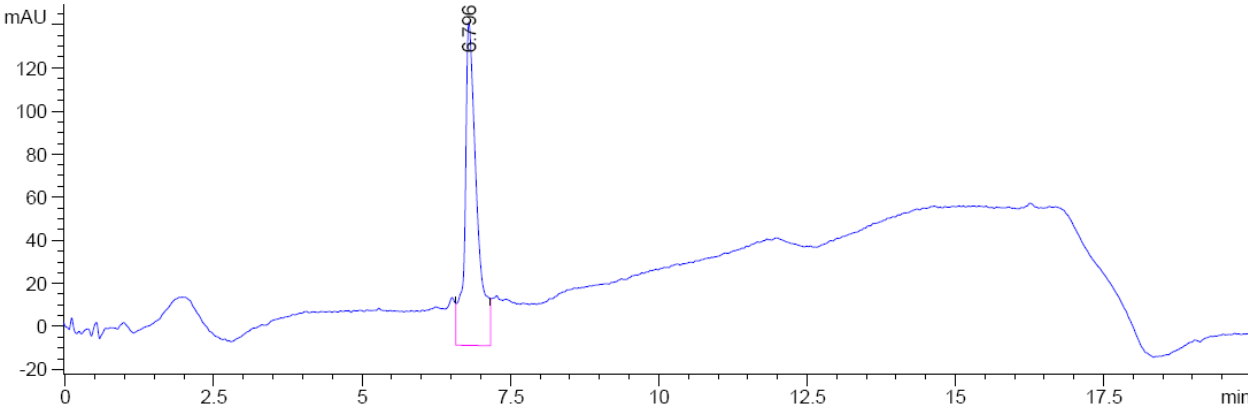




LCMS chromatogram (05-80% MeCN, 20 min, R.T. = 8.9 min) of crude [Aid^2]GHRP-6 (His-**Aid**-Ala-Trp-D-Phe-Lys-NH_2). LCMS (ESI) found m/e 771.4, (M+H)+ calcd for $[C_{43}H_{54}N_{11}O_6]^+$: 771.4.

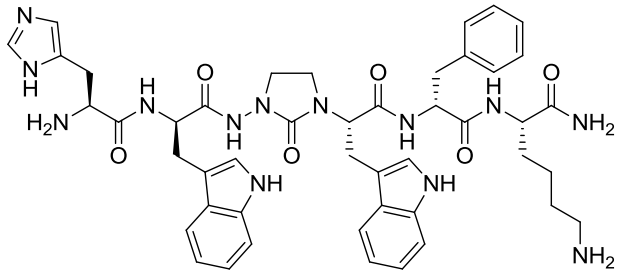


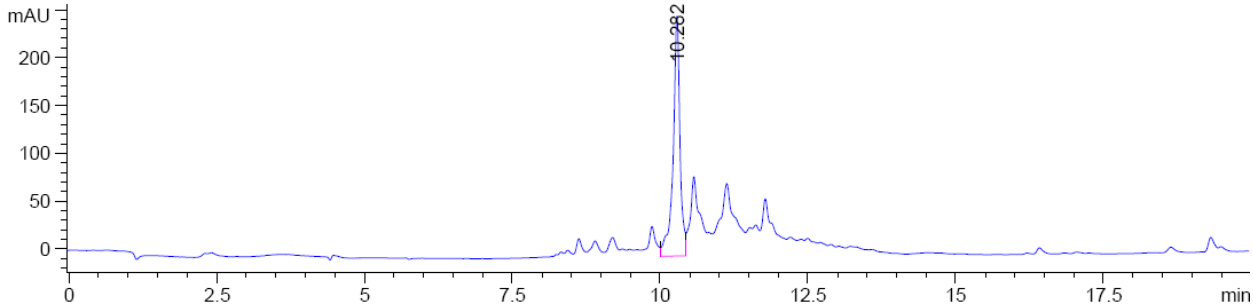
LCMS chromatogram (00-50% MeCN, 20 min, R.T. = 6.5 min) of purified [Aid^2]GHRP-6.



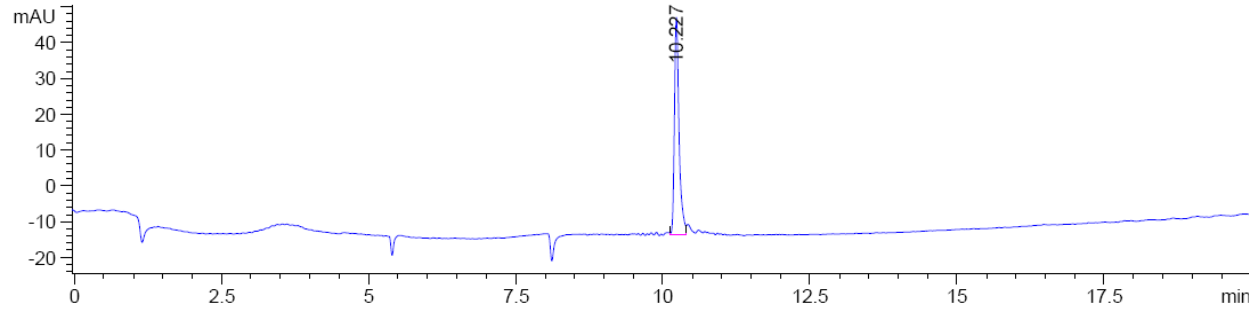
LCMS chromatogram (00-50% MeOH, 20 min, R.T. = 6.8 min) of purified [Aid^2]GHRP-6.

His-D-Trp-Aid-Trp-D-Phe-Lys-NH_2 ([Aid^3]GHRP-6)

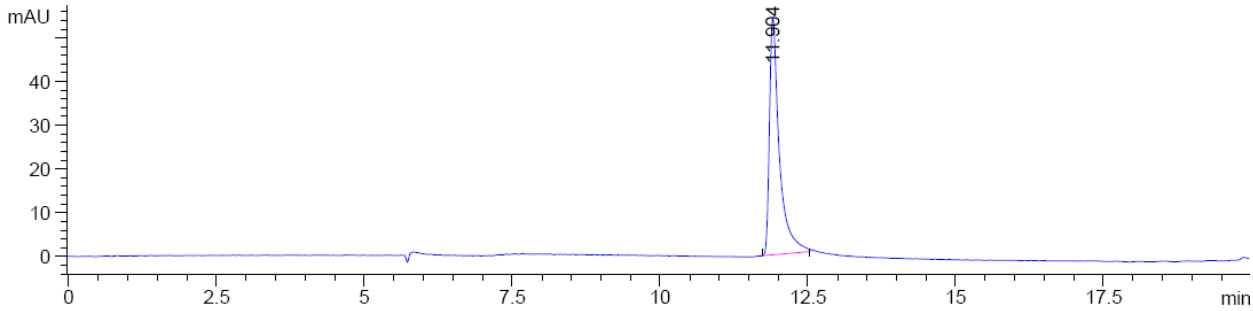




LCMS chromatogram (0-50% MeCN, 20 min, R.T. = 10.3 min) of crude [Aid^3]GHRP-6 (His-D-Trp-**Aid**-Trp-D-Phe-Lys-NH_2). LCMS (ESI) found m/e 886.5, $(M+H)^+$ calcd for $[C_{46}H_{56}N_{13}O_6]^+$: 886.4.

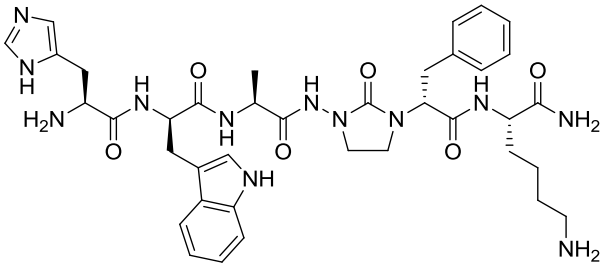


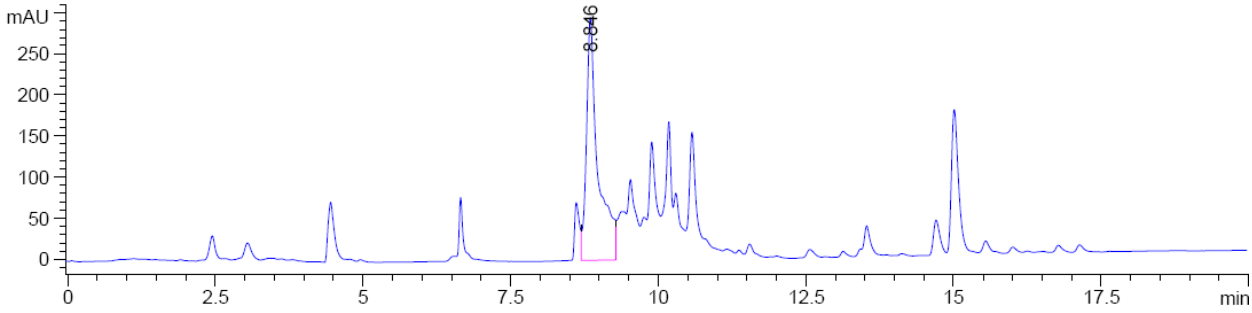
LCMS chromatogram (0-50% MeCN, 20 min, R.T. = 10.2 min) of purified [Aid^3]GHRP-6.



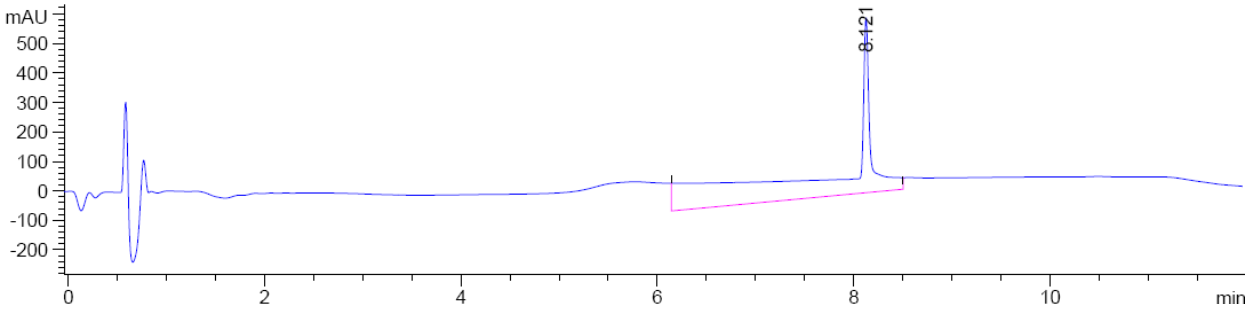
LCMS chromatogram (0-50% MeOH, 20 min, R.T. = 11.9 min) of purified [Aid^3]GHRP-6.

His-D-Trp-Ala-Aid-D-Phe-Lys-NH_2 ([Aid^4]GHRP-6)

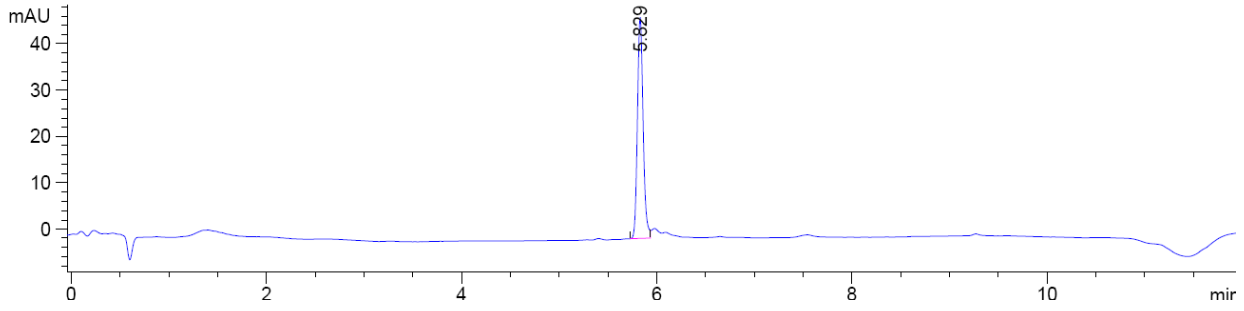




LCMS chromatogram (0-50% MeCN, 20 min, R.T. = 8.8 min) of crude [Aid^4]GHRP-6 (His-D-Trp-Ala-**Aid**-D-Phe-Lys-NH_2). LCMS (ESI) found m/e 771.4, $(M+H)^+$ calcd for $[C_{43}H_{54}N_{11}O_6]^+$: 771.4.

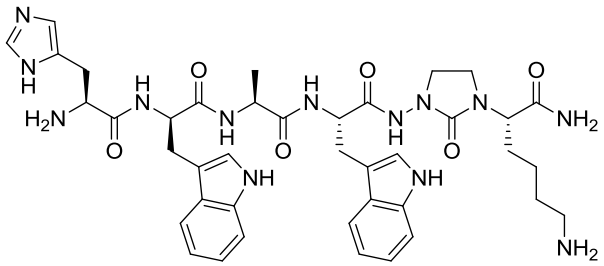


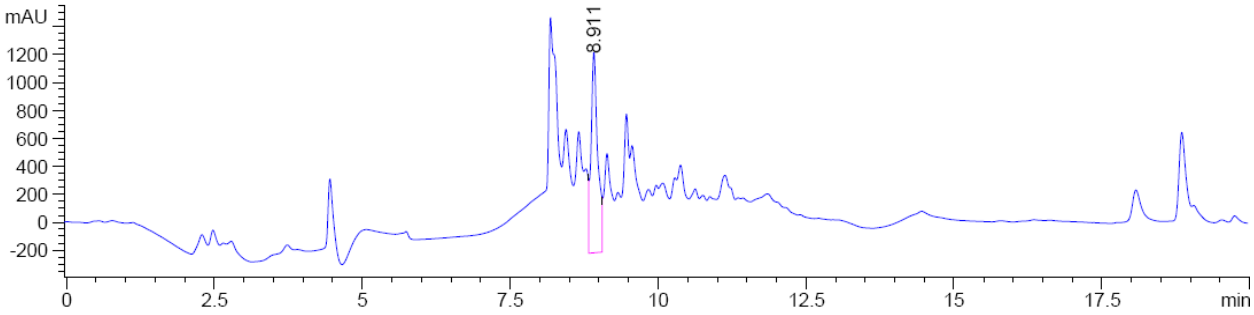
LCMS chromatogram (10-50% MeCN, 12 min, R.T. = 8.1 min) of purified [Aid^4]GHRP-6.



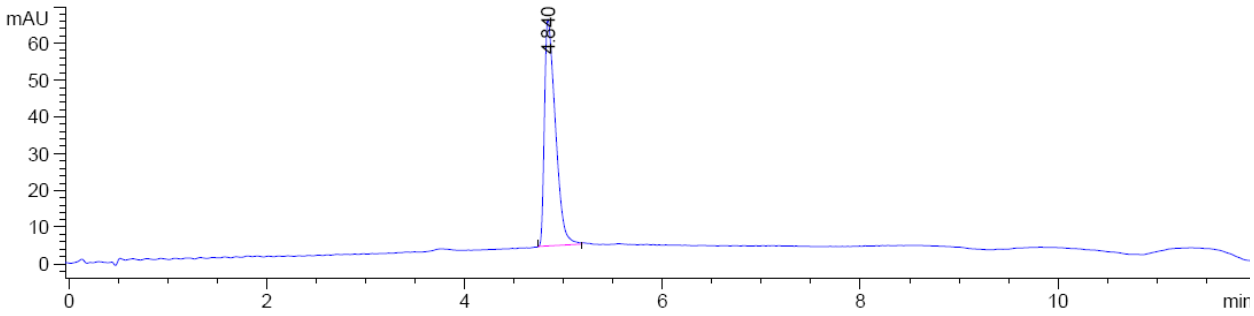
LCMS chromatogram (5-80% MeOH, 12 min, R.T. = 5.8 min) of purified [Aid^4]GHRP-6.

His-D-Trp-Ala-Trp-Aid-Lys-NH_2 ([Aid^5]GHRP-6)

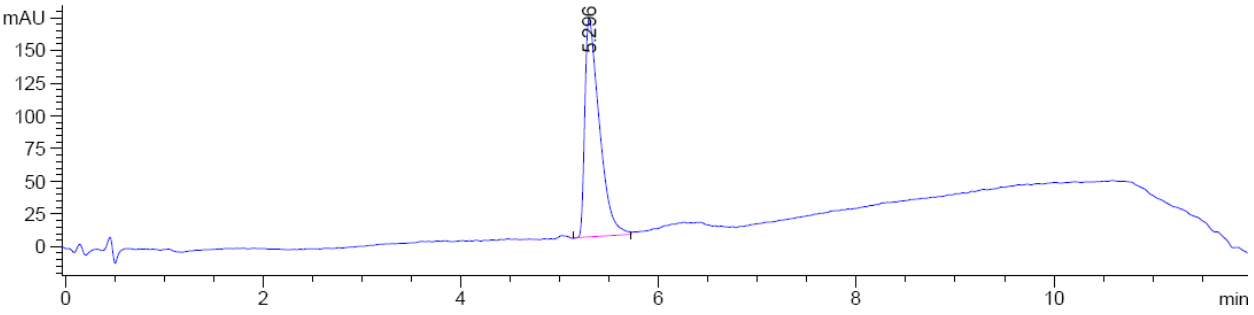




LCMS chromatogram (0-50% MeCN, 20 min, R.T. = 8.8 min) of crude [Aid[4]]GHRP-6 (His-D-Trp-Ala-**Aid**-D-Phe-Lys-NH_2). LCMS (ESI) found m/e 810.4, $(M+H)^+$ calcd for $[C_{40}H_{52}N_{13}O_6]^+$: 810.4.

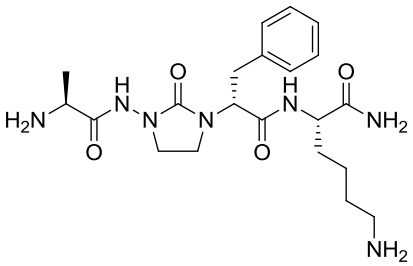


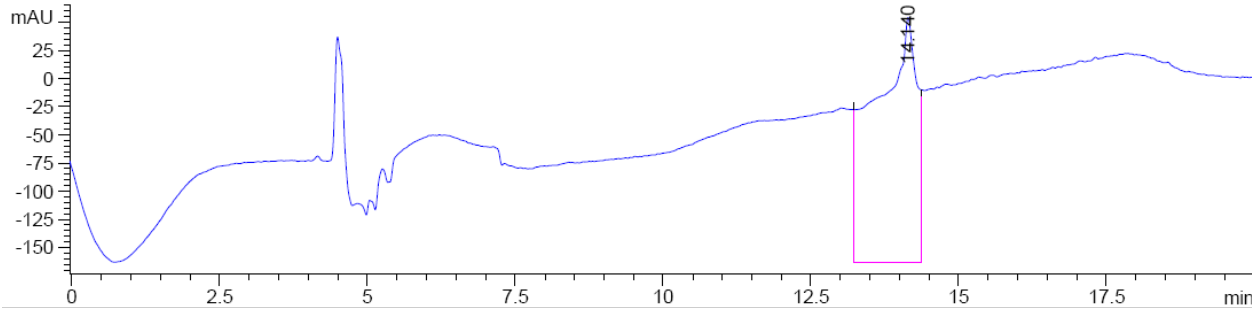
LCMS chromatogram (10-40% MeCN, 12 min, R.T. = 4.8 min) of purified [Aid[5]]GHRP-6.



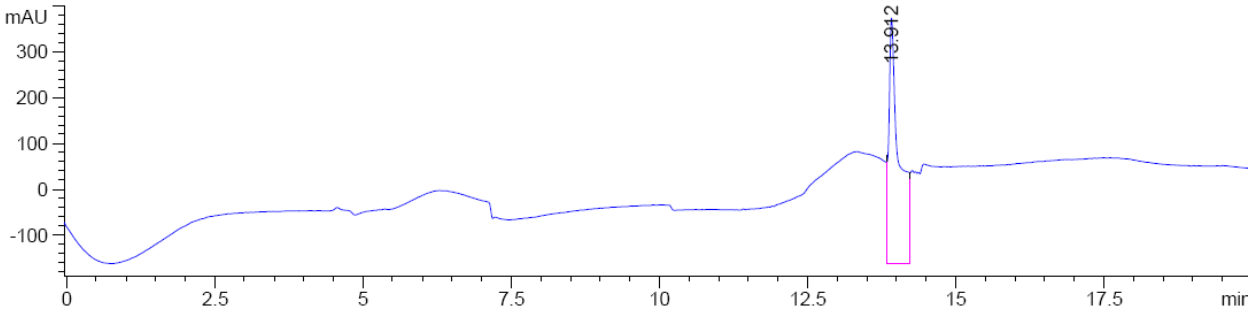
LCMS chromatogram (10-40% MeOH, 12 min, R.T. = 5.3 min) of purified [Aid[5]]GHRP-6.

Ala-Aid-D-Phe-Lys-NH_2 ([Aid^4]GHRP-6(3-6))

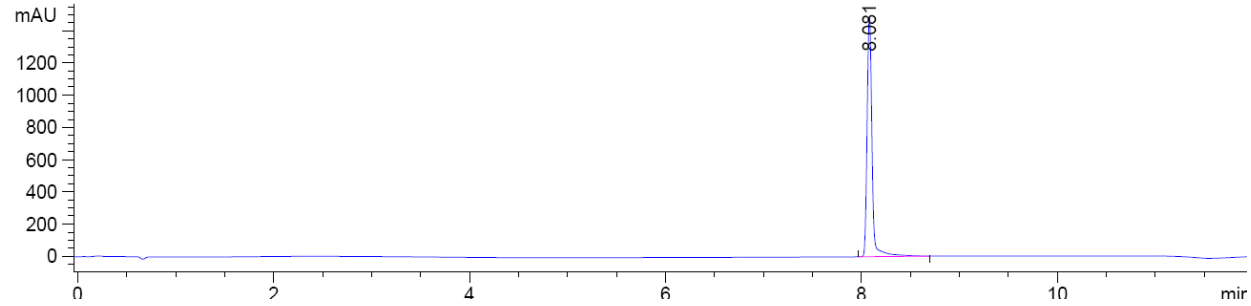




LCMS chromatogram (0-30% MeCN, 20 min, R.T. = 14.1 min) of crude [Aid^4]GHRP-6(3-6) (Ala-Aid-D-Phe-Lys-NH_2) on a RP-polar column (150 × 4.60 mm, 5 µm). LCMS (ESI) found m/e 448.3, $(M+H)^+$ calcd for $[C_{21}H_{34}N_7O_4]^+$: 448.3.

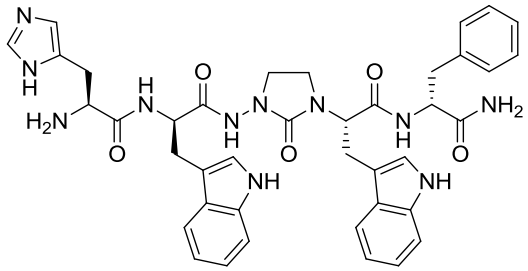


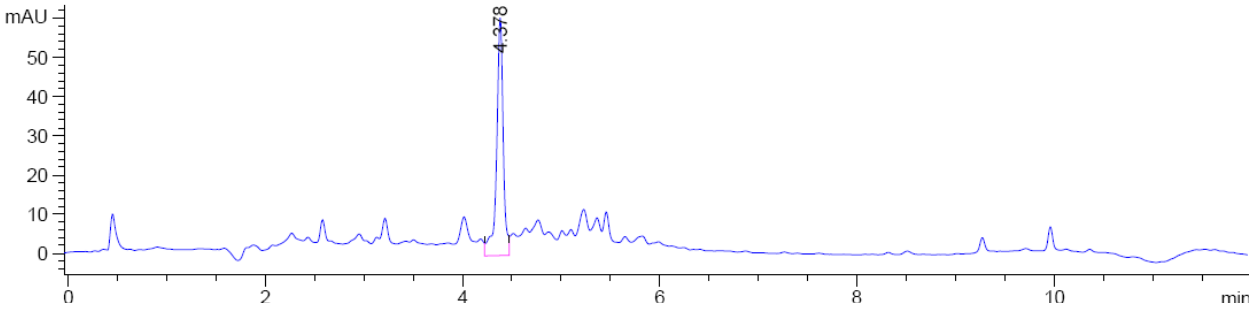
LCMS chromatogram (0-30% MeCN, 20 min, R.T. = 14.1 min) of purified [Aid^4]GHRP-6(3-6) on a RP-polar column (150 × 4.60 mm, 5 µm).



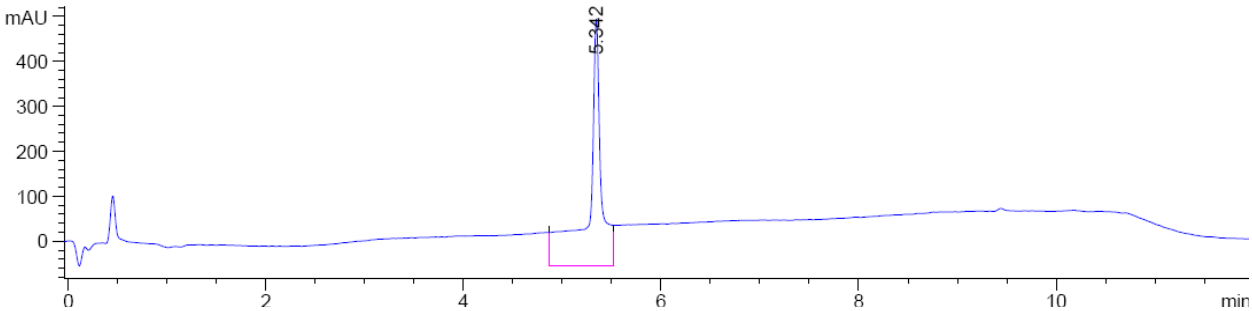
LCMS chromatogram (0-30% MeOH, 12 min, R.T. = 8.1 min) of purified [Aid^4]GHRP-6(3-6).

His-D-Trp-Aid-Trp-D-Phe-NH_2 ([Aid^3]GHRP-6(1-5))

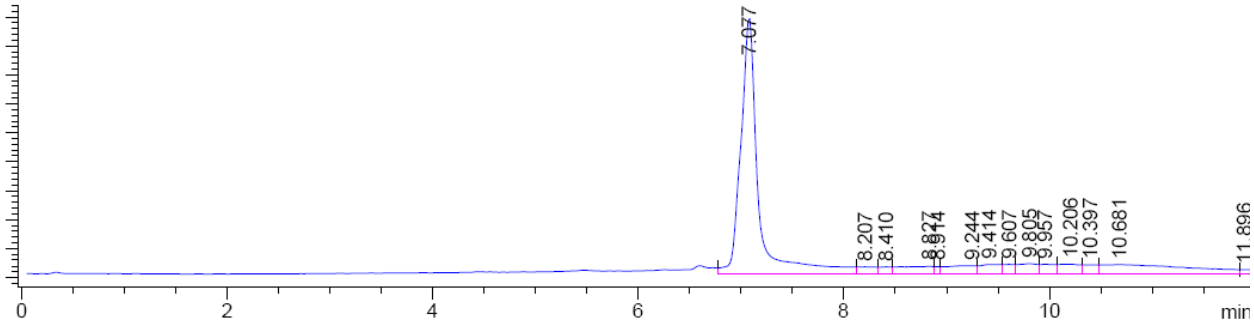




LCMS chromatogram (20-60% MeCN, 12 min, R.T. = 4.4 min) of crude [Aid^3]GHRP-6(1-5) (His-D-Trp-Aid-Trp-D-Phe- NH_2). LCMS (ESI) found m/e 758.4, $(M+H)^+$ calcd for $[C_{40}H_{44}N_{11}O_5]^+$: 758.3.

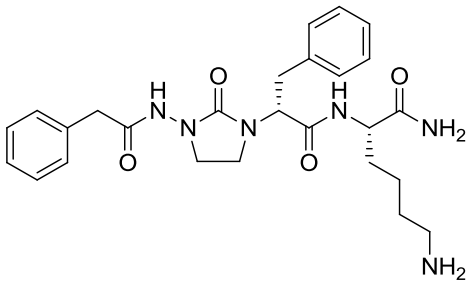


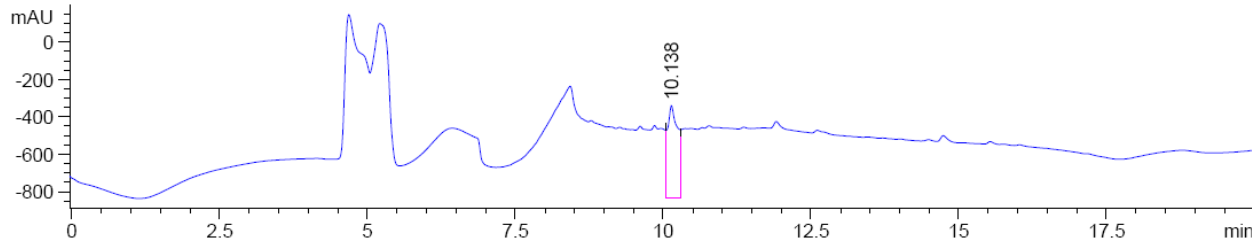
LCMS chromatogram (10-60% MeCN, 12 min, R.T. = 5.3 min) of purified [Aid^3]GHRP-6(1-5).



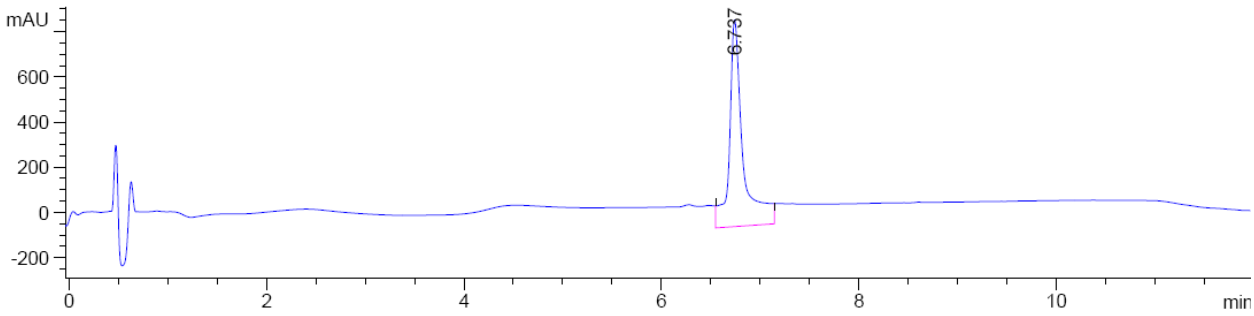
LCMS chromatogram (10-60% MeOH, 12 min, R.T. = 7.1 min) of purified [Aid^3]GHRP-6(1-5).

PhAc-Aid-D-Phe-Lys-NH$_2$ (PhAc-[Aid^4]GHRP-6(4-6))

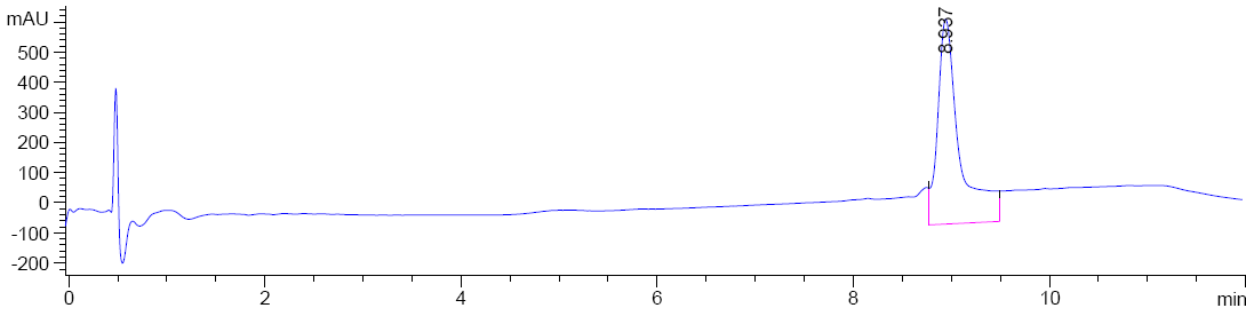




LCMS chromatogram (0-90% MeCN, 20 min, R.T. = 10.1 min) of crude PhAc-[Aid^4]GHRP-6(4-6) (PhAc-**Aid**-D-Phe-Lys-NH$_2$). LCMS (ESI) found m/e 495.3, $(M+H)^+$ calcd for $[C_{26}H_{35}N_6O_4]^+$: 495.3.



LCMS chromatogram (0-50% MeCN, 12 min, R.T. = 6.7 min) of purified PhAc-[Aid^{4}]GHRP-6(4-6).



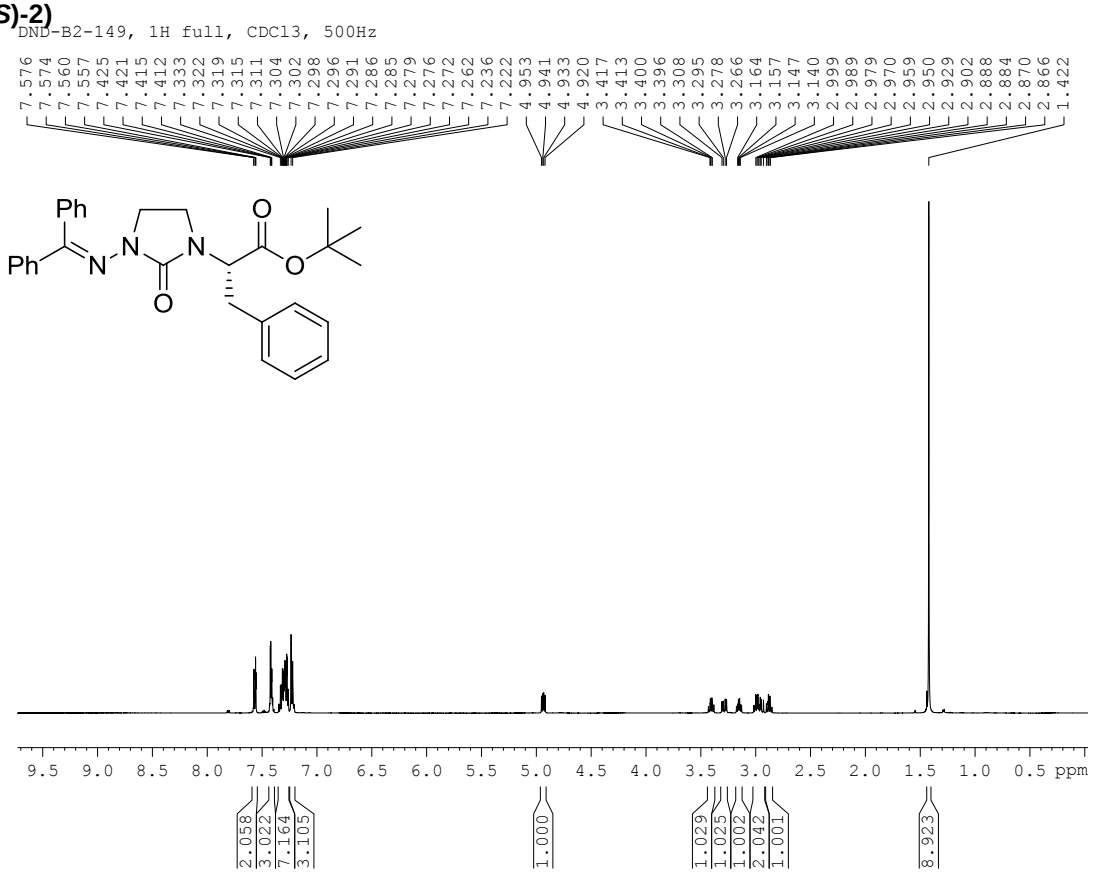
LCMS chromatogram (0-50% MeCN, 12 min, R.T. = 8.9 min) of purified PhAc-[Aid^{4}]GHRP-6(4-6).

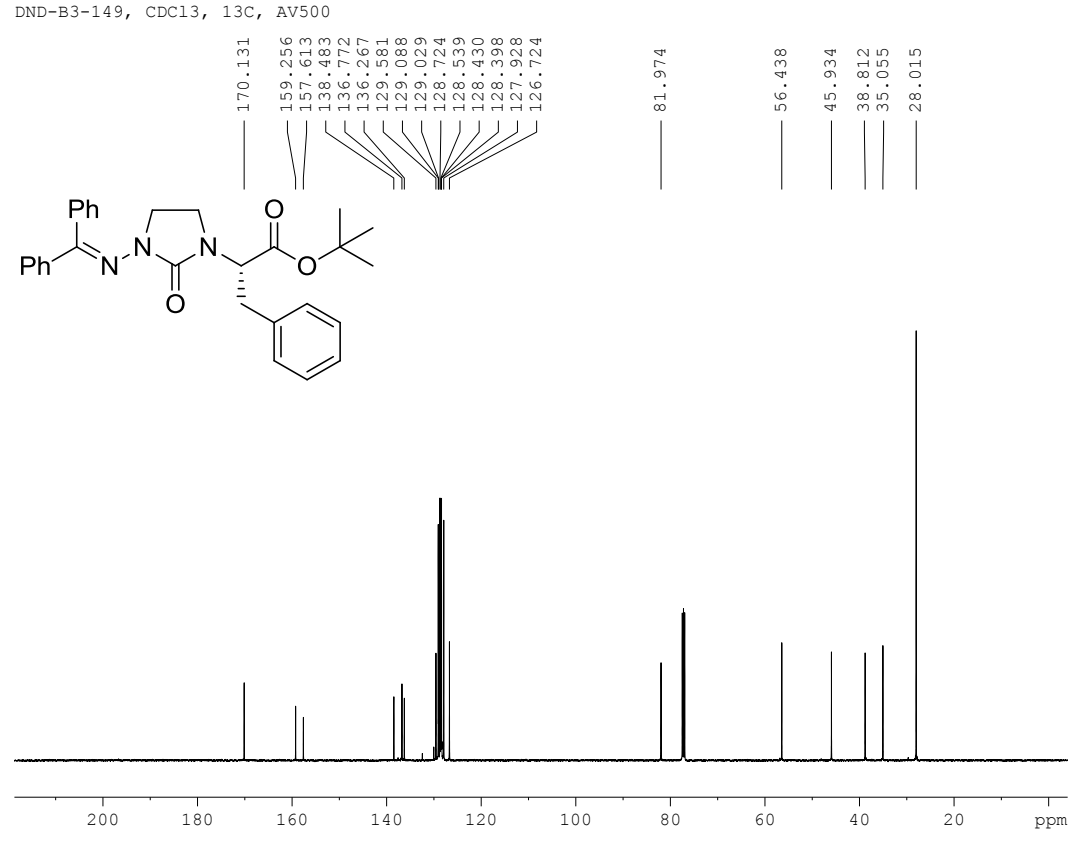
References

(1) Lubell, W. D.; Blankenship, J. W.; Fridkin, G.; Kaul, R. In *Science of Synthesis 21.11, Chemistry of Amides.*; Thieme: Stuttgart, Germany, 2005, p 713-809.
(2) Zhang, J.; Proulx, C.; Tomberg, A.; Lubell, W. D. *Org Lett* **2014**, *16*, 298-301.
(3) Garcia-Ramos, Y.; Lubell, W. D. *J Pept Sci* **2013**, *19*, 725-9.
(4) Proulx, C.; Lubell, W. D. *Org Lett* **2012**, *14*, 4552-5.

II. NMR spectra

(2'S)-1-((Diphenylmethylene)amino)-3-(tert-butyl-3'-phenylpropanoate)-Imidazolidin-2-one ((*S*)-2)

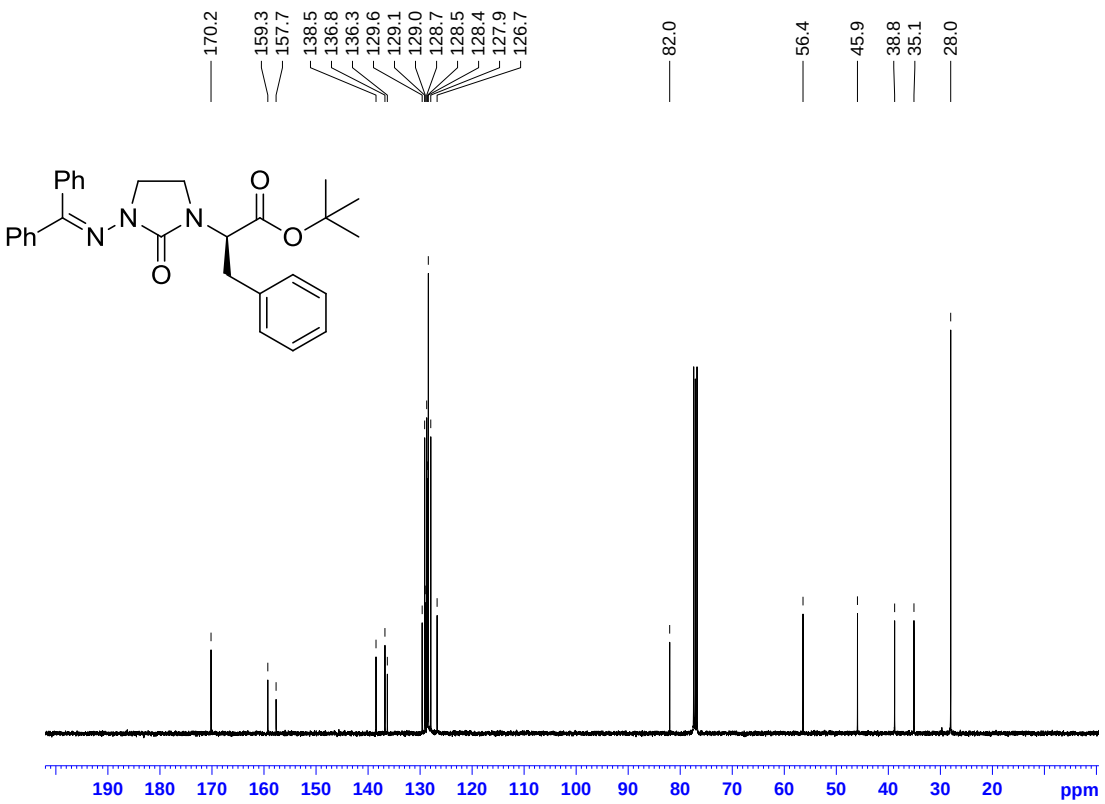




(2'R)-1-((Diphenylmethylene)amino)-3-(tert-butyl-3'-phenylpropanoate)-Imidazolidin-2-one ((*R*)-2)

DND-B3-67, 1H full, CDCl3, 400Hz

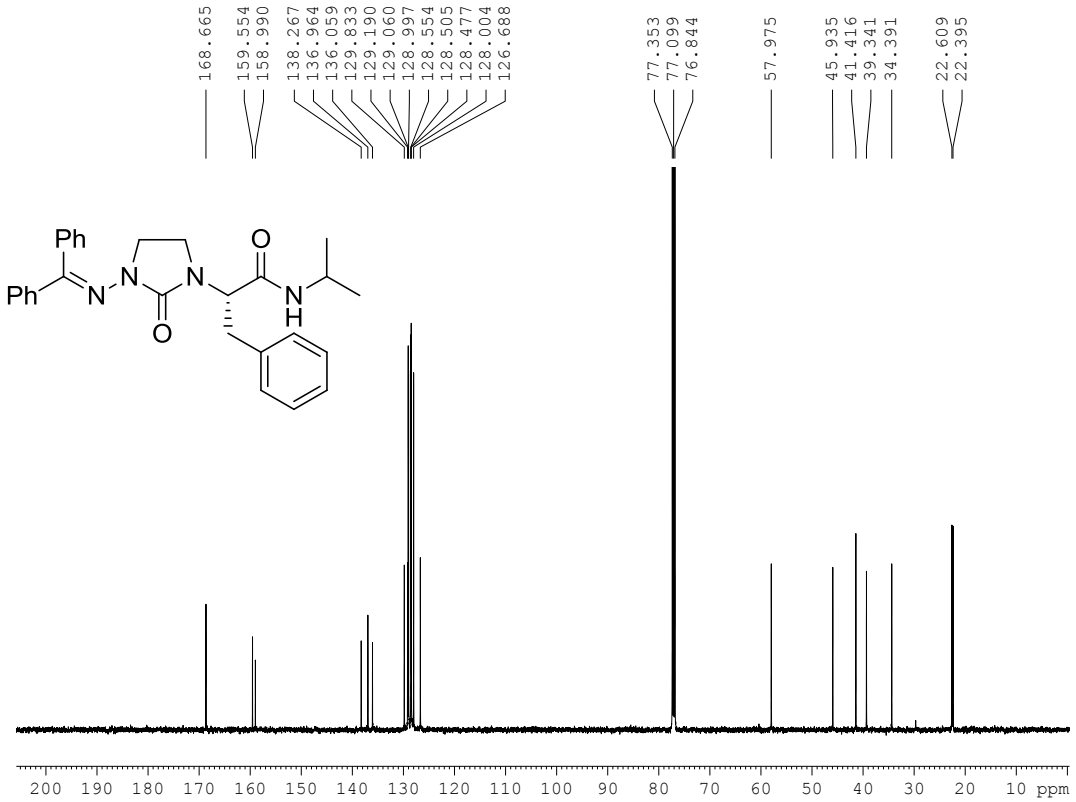
DND-B3-67, 13C full, CDCl3, AV400



(S)-2-(3-((diphenylmethylene)amino)-2-oxoimidazolidin-1-yl)-N-isopropyl-3-phenylpropanamide ((*S*)-4)

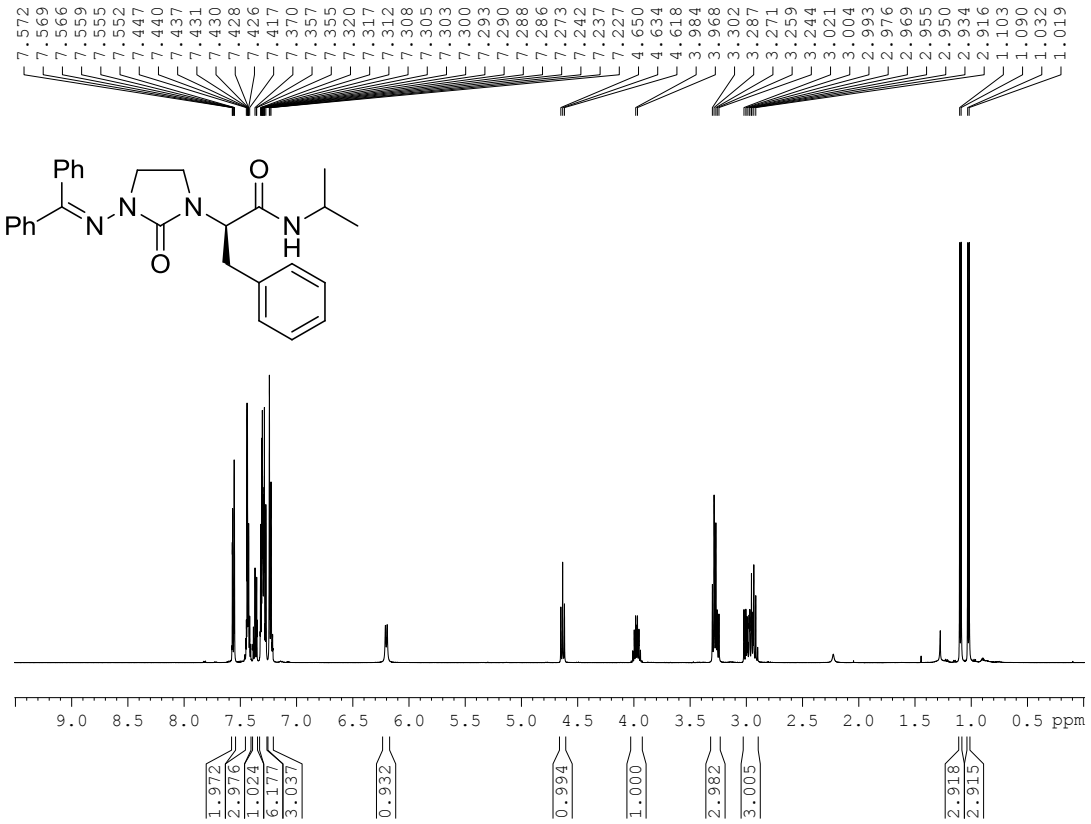
DND-B3-152, 1H full, CDCl3, 500Hz

DND-B3-151, 13C, CDCl3, AV500

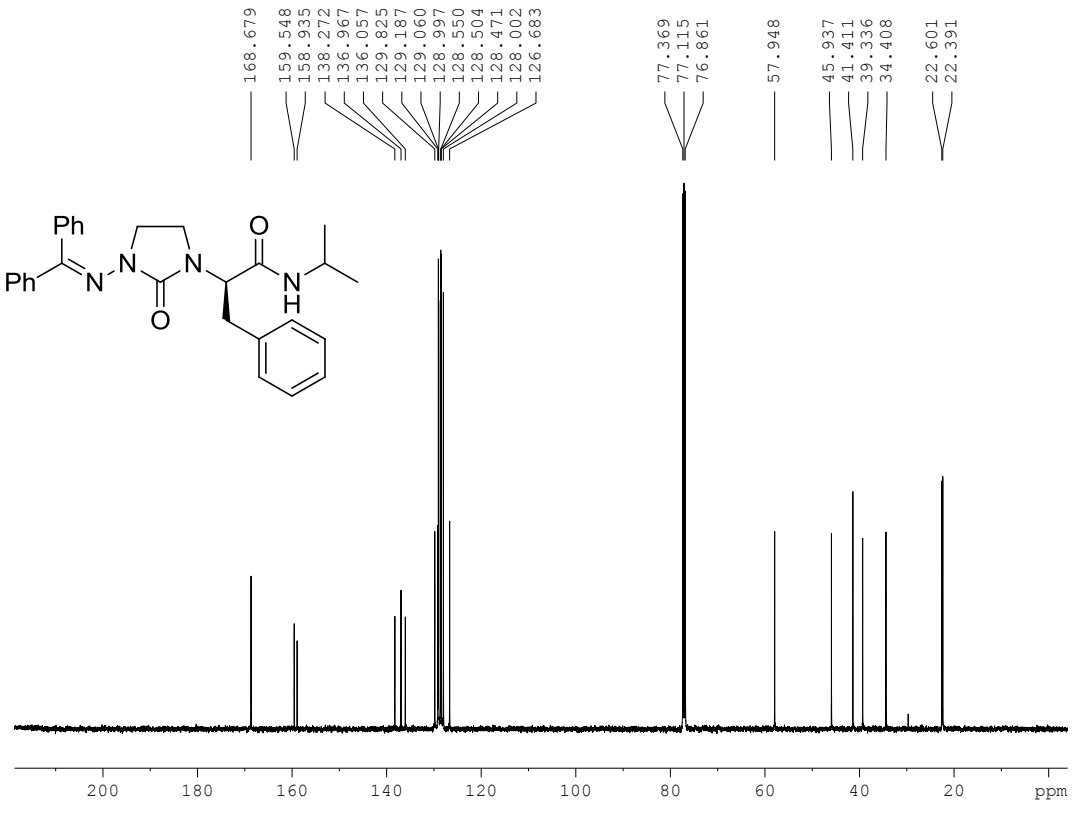


(R)-2-(3-((diphenylmethylene)amino)-2-oxoimidazolidin-1-yl)-N-isopropyl-3-phenylpropanamide ((*R*)-4)

DND-B3-97, 1H full, CDCl3, 500Hz

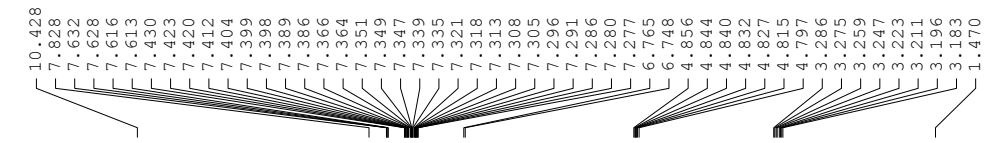


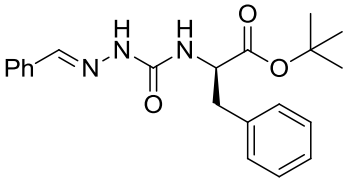
DND-B3-97, 13C, CDCl3, AV500



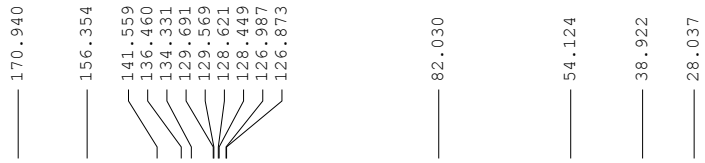
Benzylidene aza-glycinyl-D-phenylalanine tert-butyl ester (5)

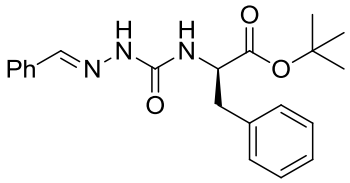
DND-B3-23, 1H full, CDCl3, 500Hz

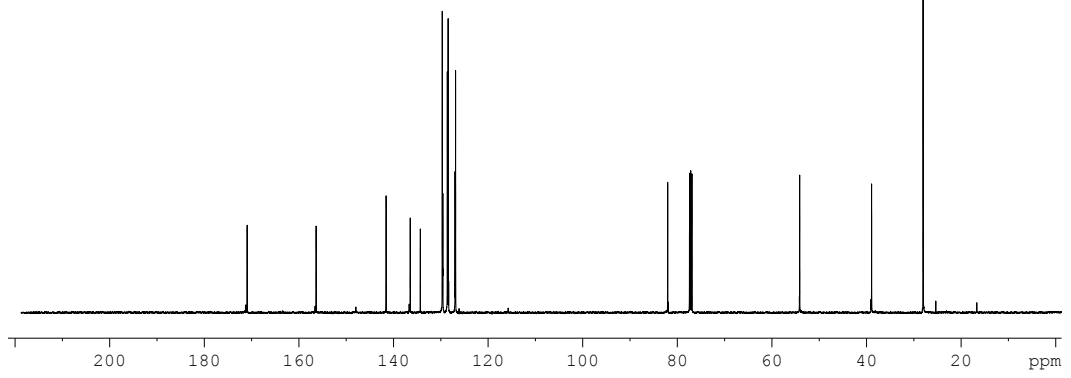




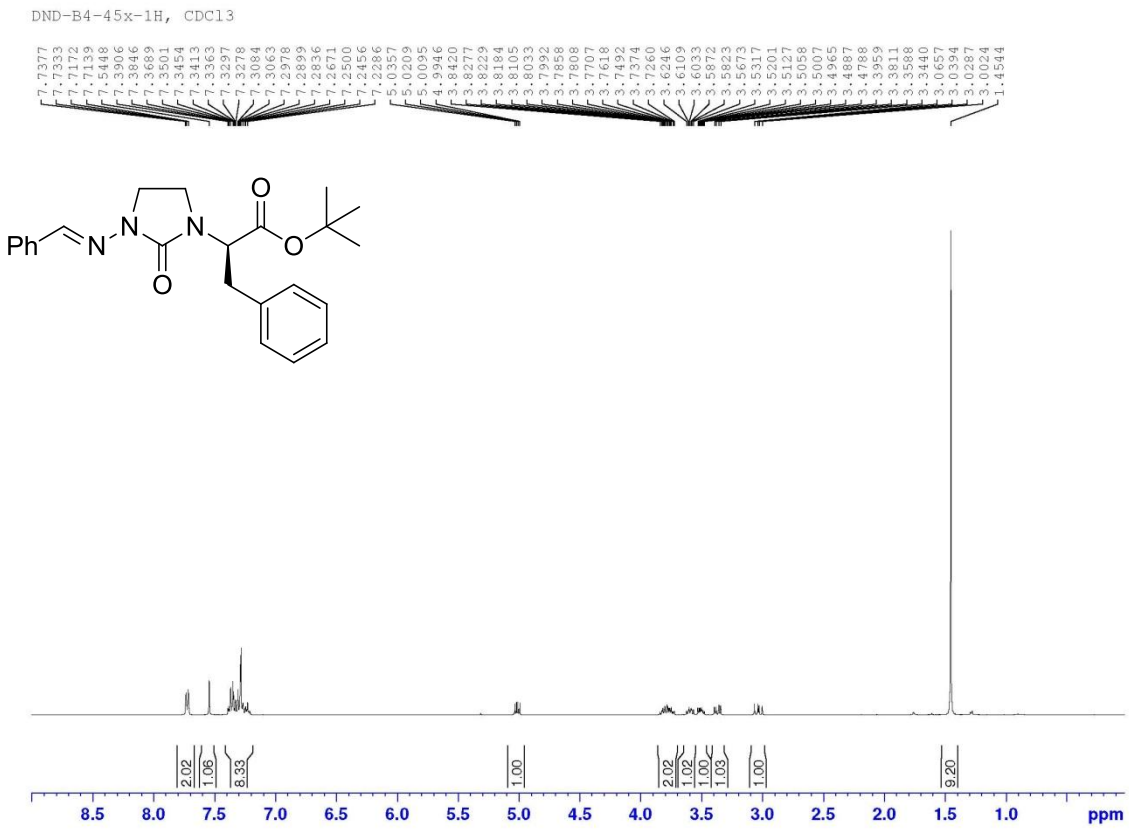
DND-B3-23, 13C, CDCl3, AV500

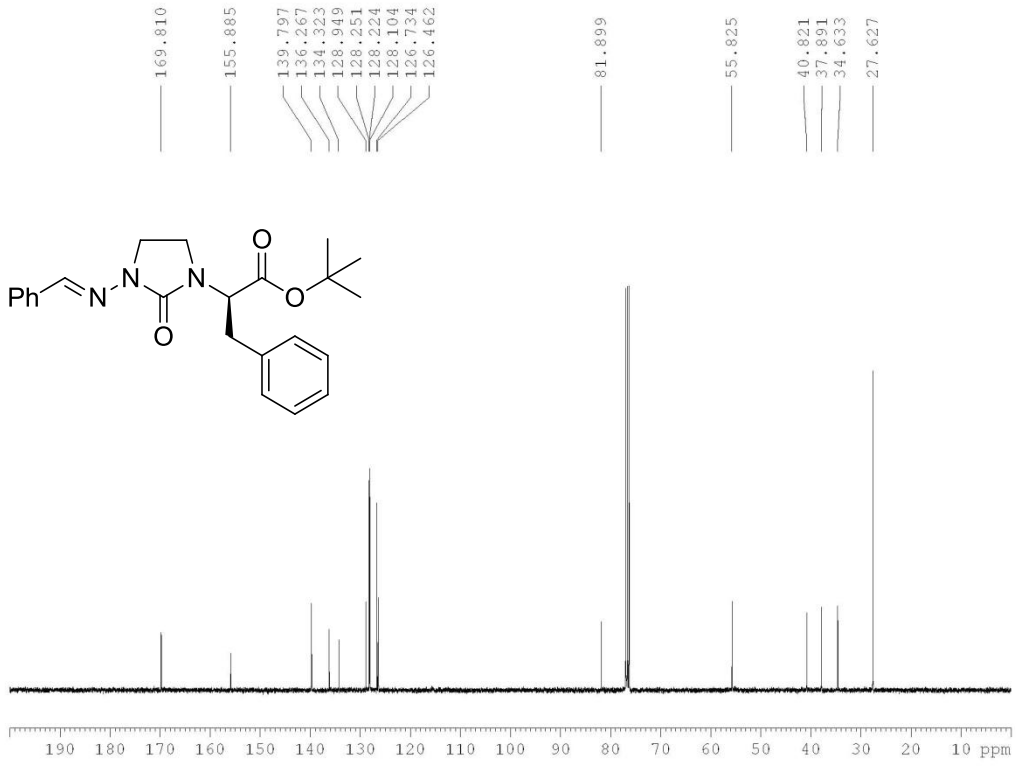




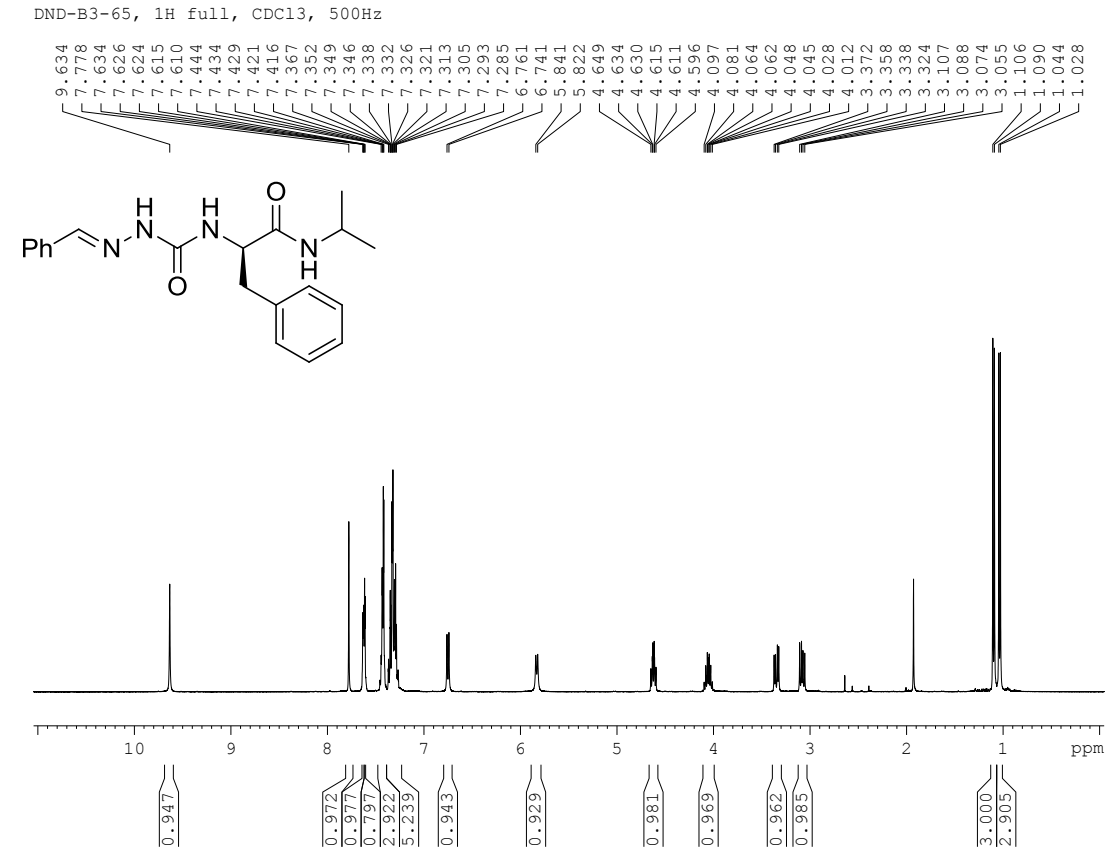


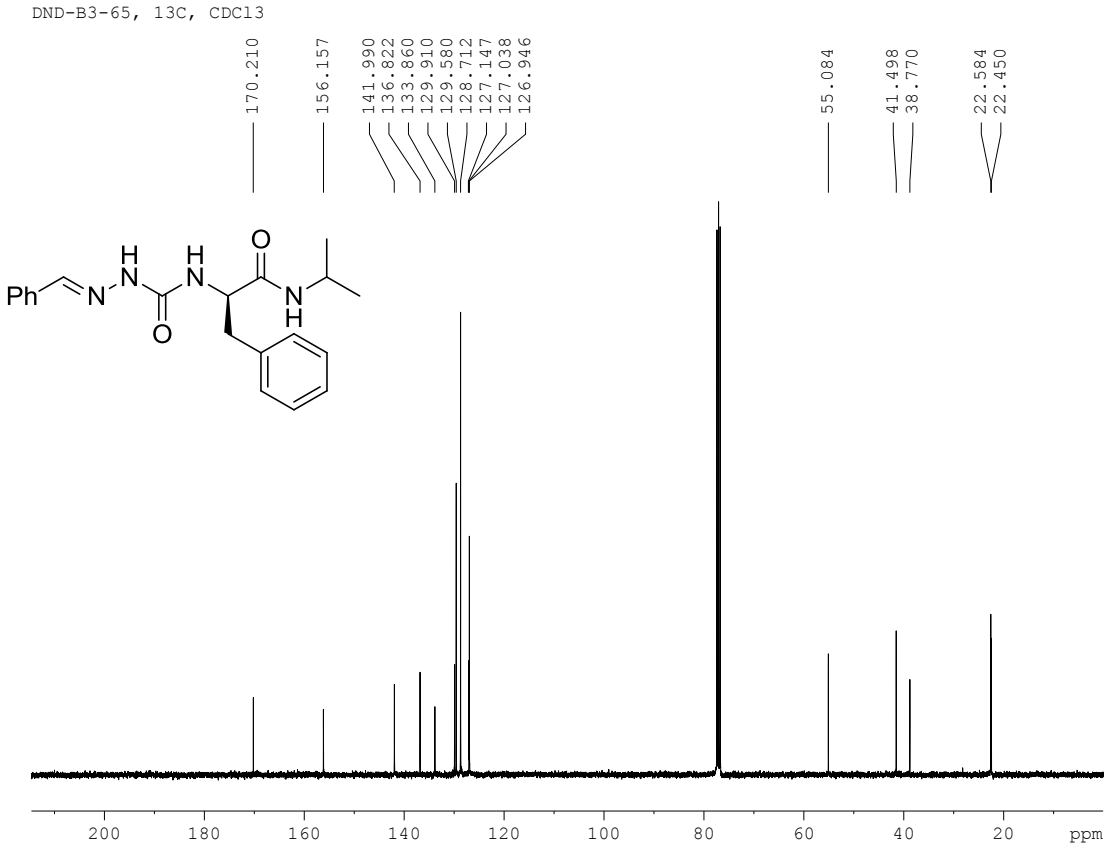
(2'*R*)-1-((phenylmethylene)amino)-3-(*tert*-butyl-3'-phenylpropanoate)-imidazolidin-2-one [5c]





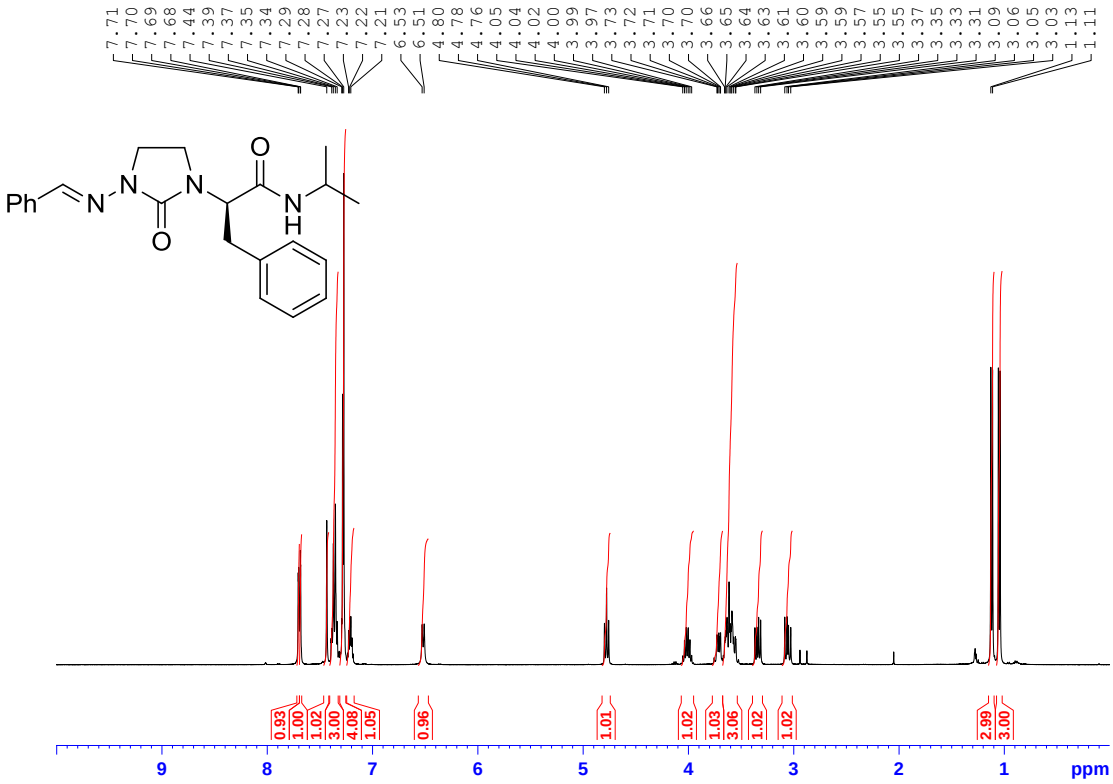
Benzylidene aza-glycinyl-D-phenylalanine isopropyl amide (6)



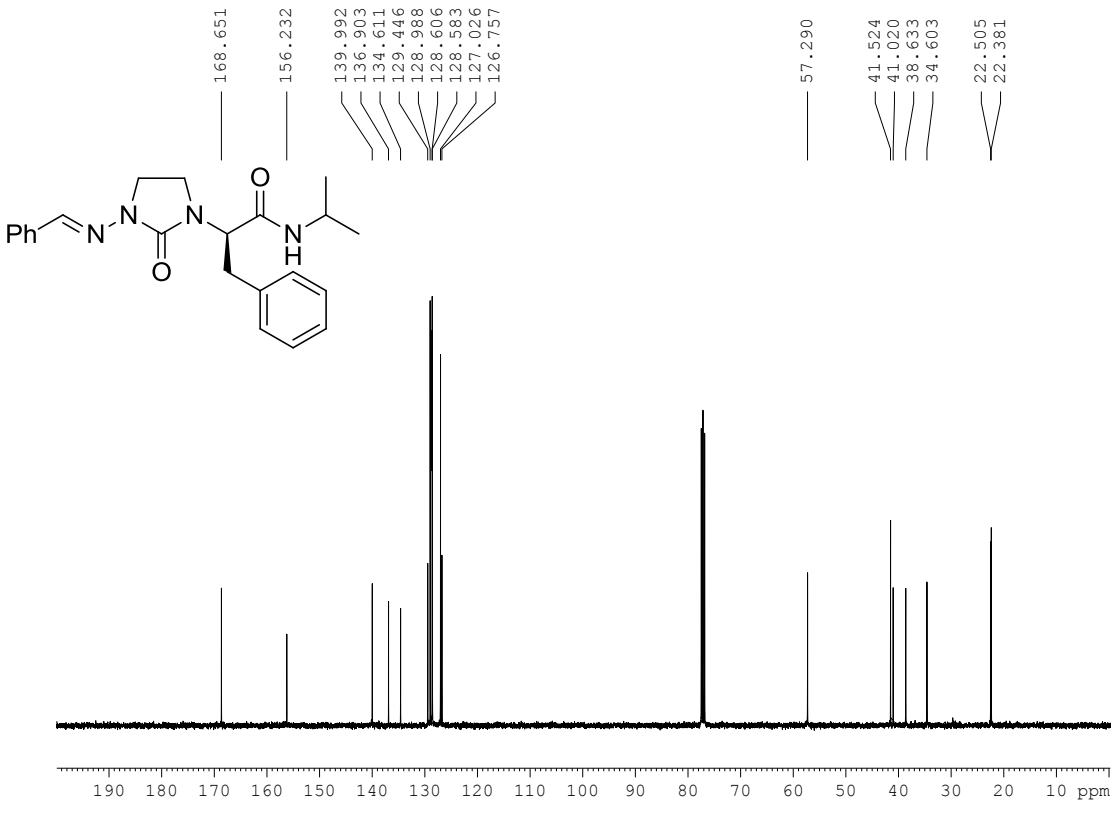


(R)-2-(3-((phenylmethylene)amino)-2-oxoimidazolidin-1-yl)-N-isopropyl-3-phenylpropanamide (6c)

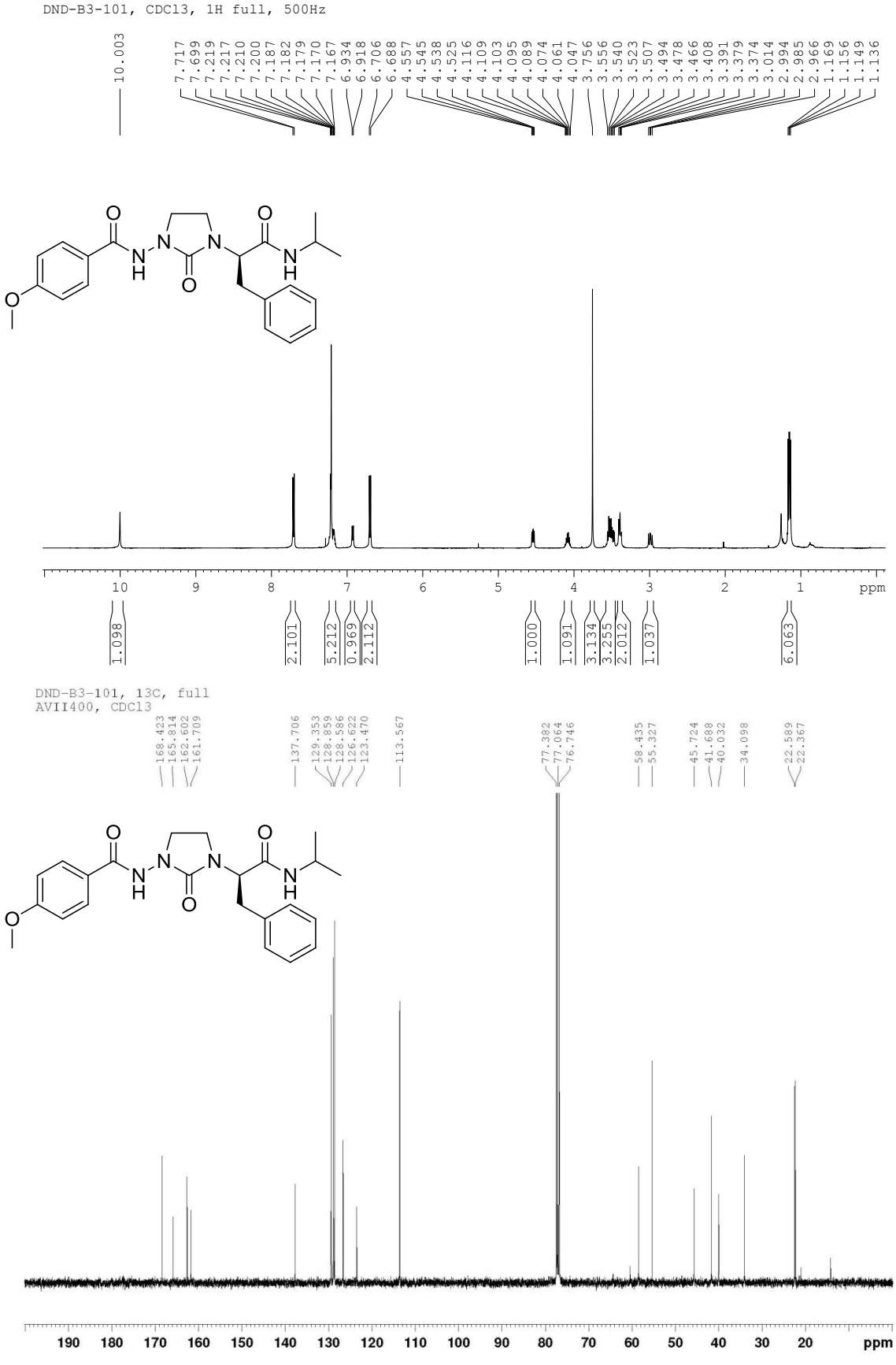
DND-B4-13, 1H full, CDCl3, 400Hz



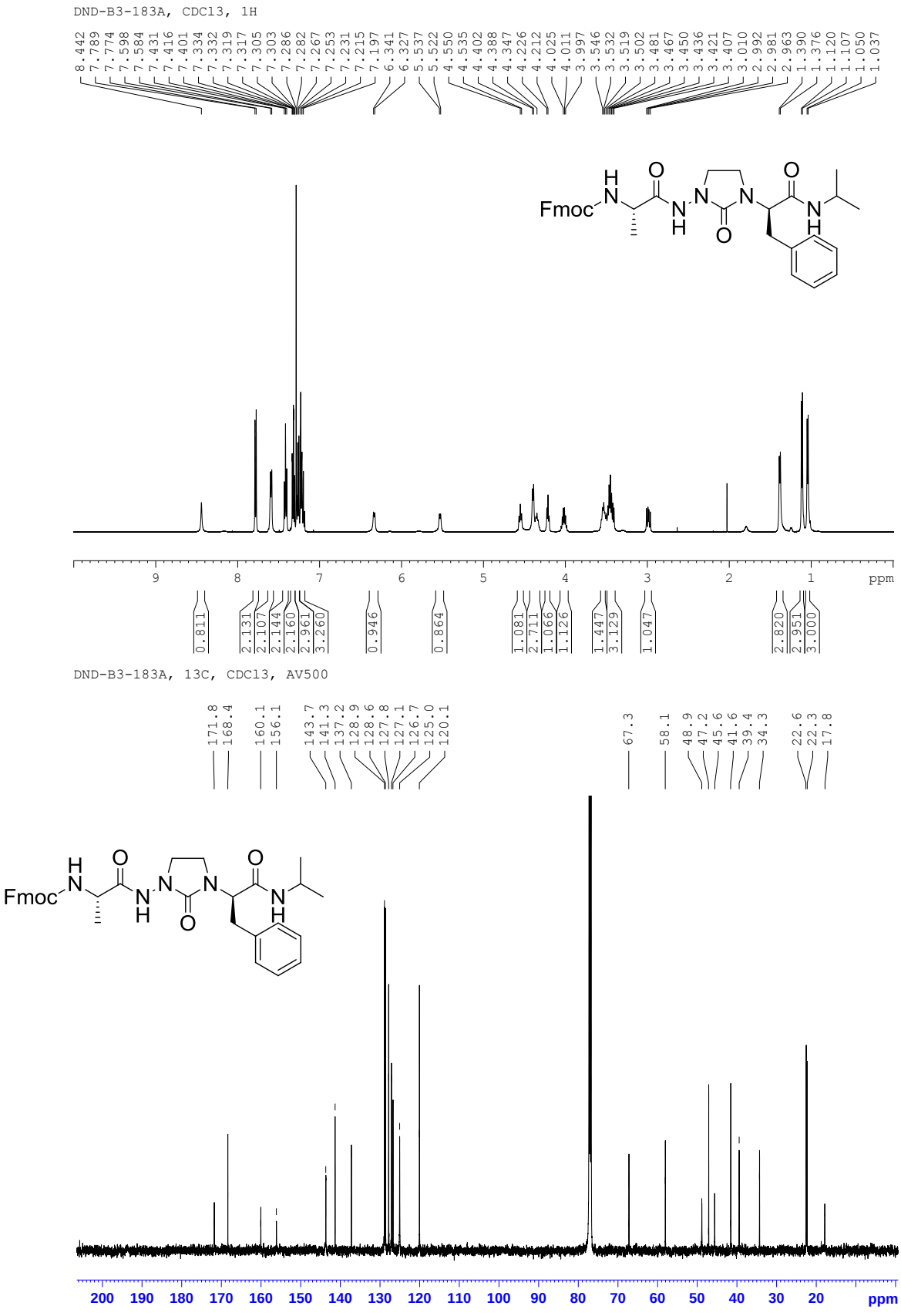
DND-B4-13, 13C, CDCl3, AV400



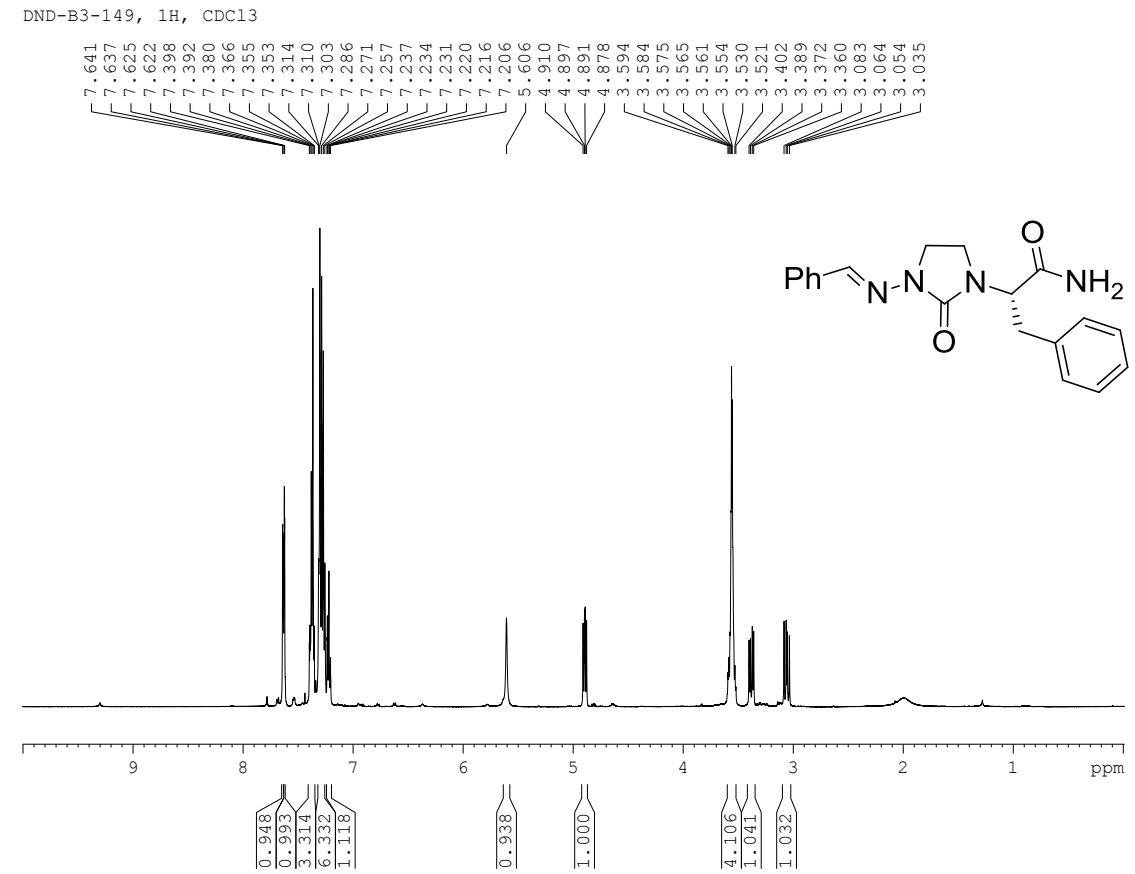
(2'*R*)-1-*p*-Methoxybenzamido-3-(3'-phenyl-*N*'-isopropyl-2'-propionamide)-imidazolidin-2-one (7a)

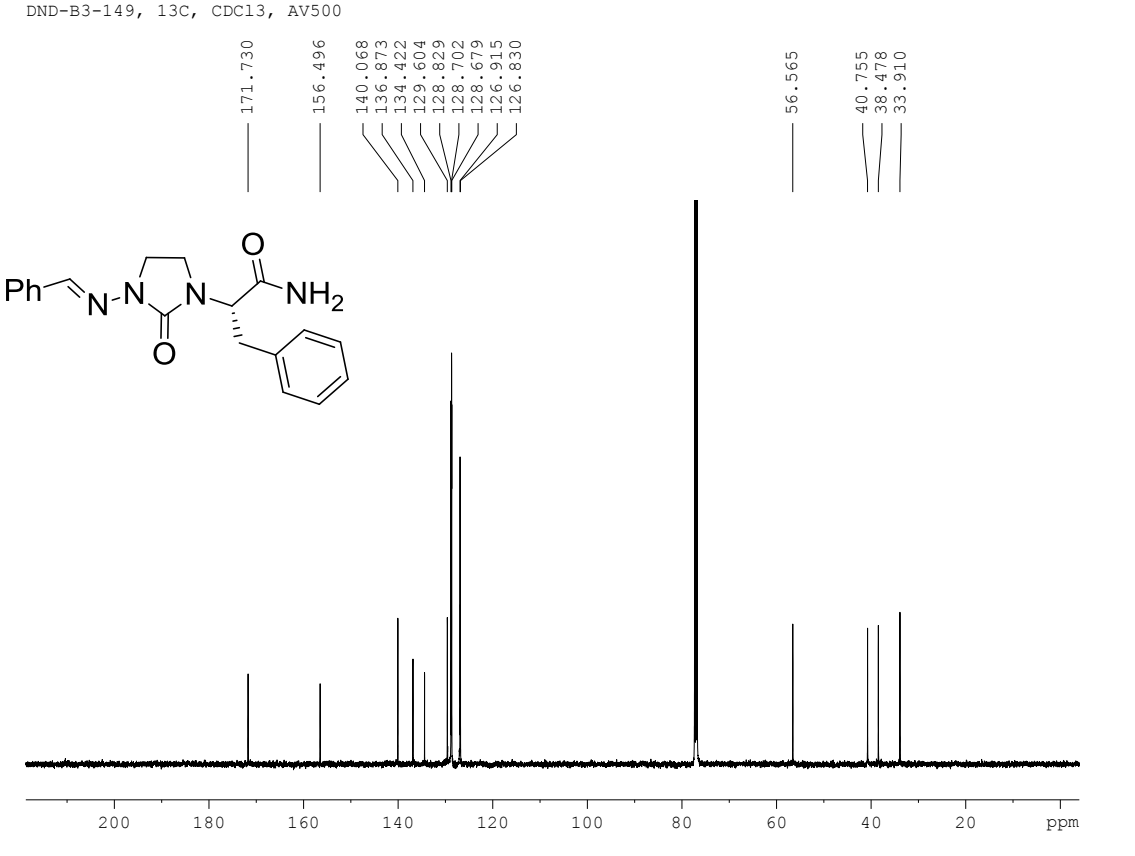


N-Fmoc-Alanine-1-amino-imidazolidin-2-one-D-phenylalanine isopropyl amide (7b)

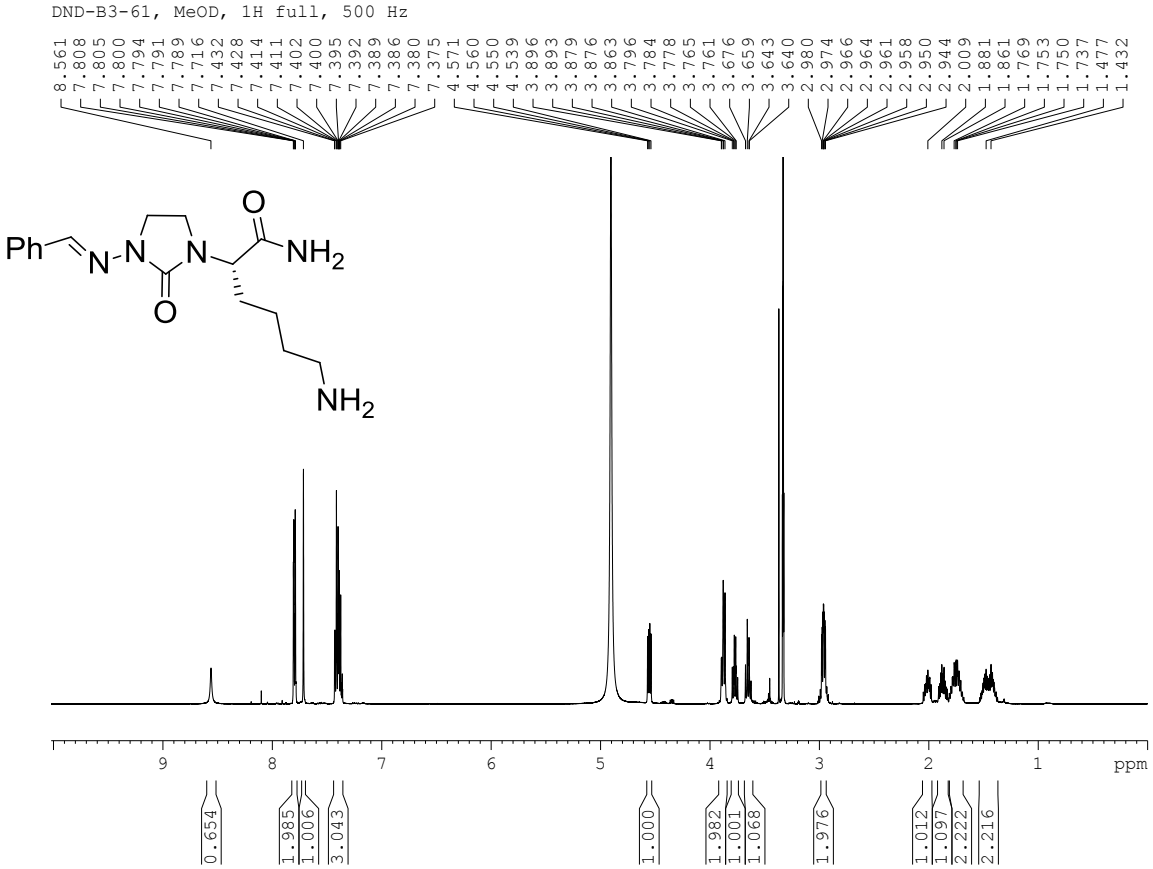


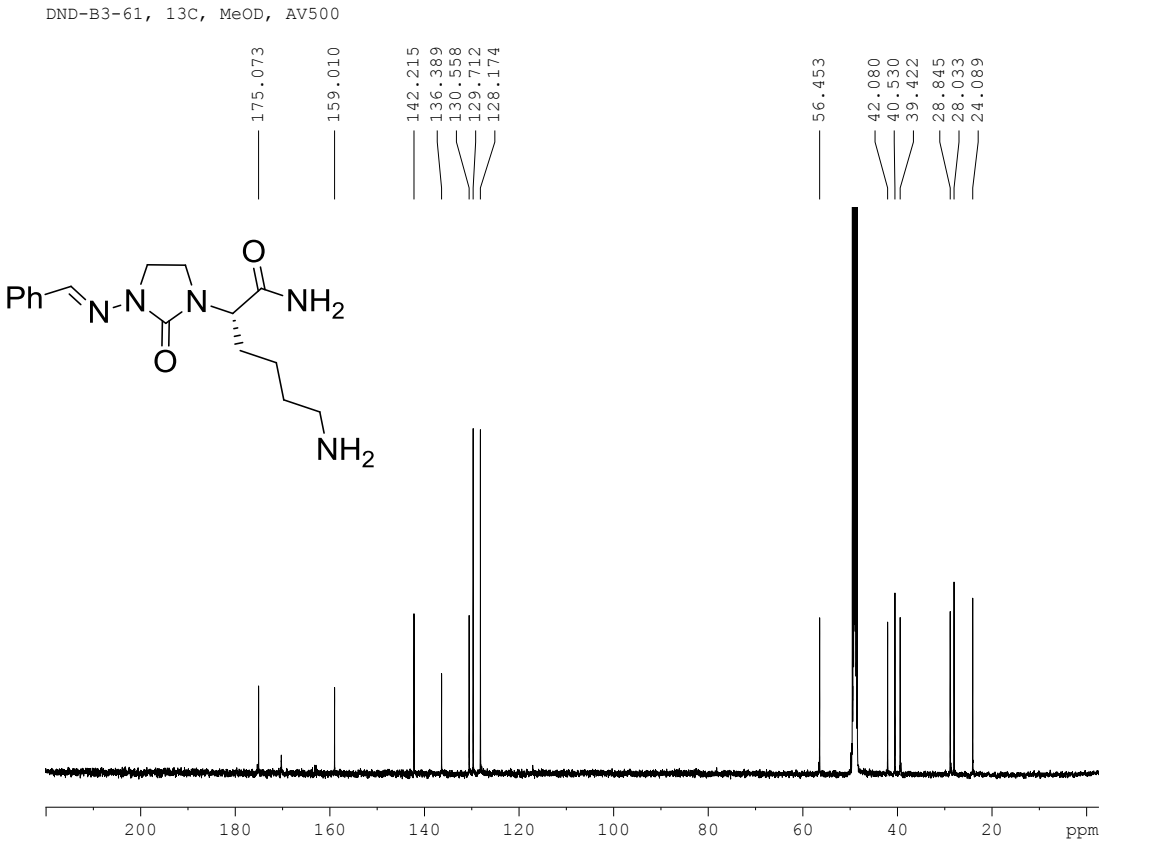
Benzylidene-Aid-Phe-NH2 (10a)



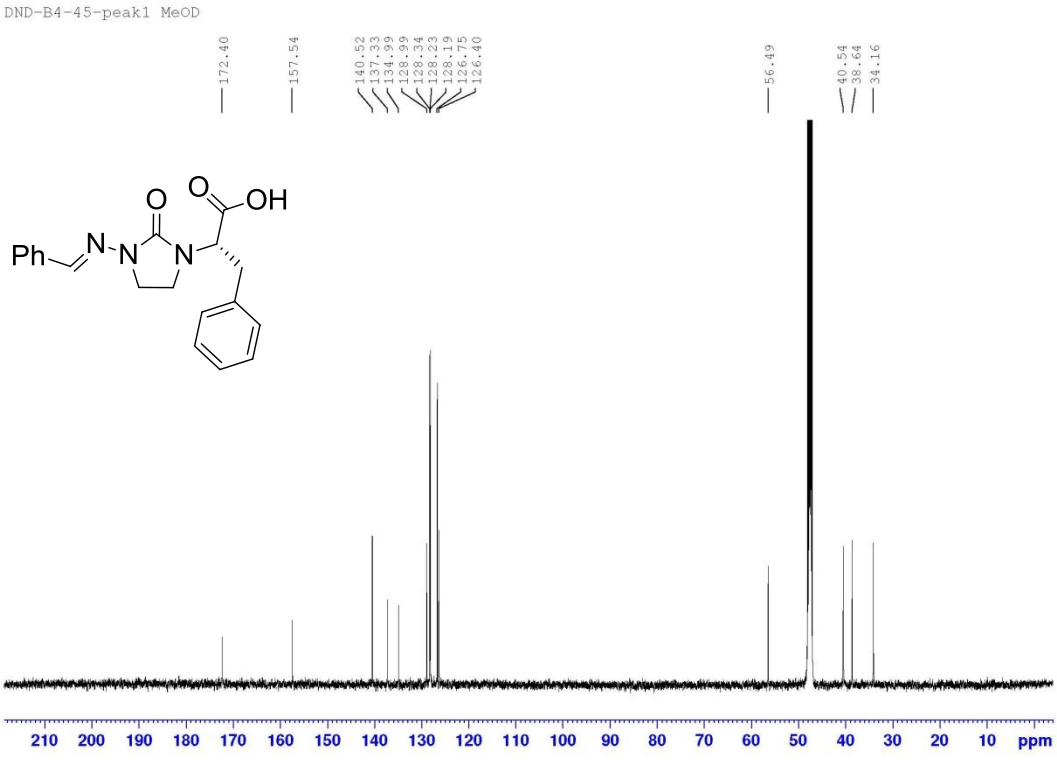


Benzylidene-Aid-Lys-NH2 (10b)

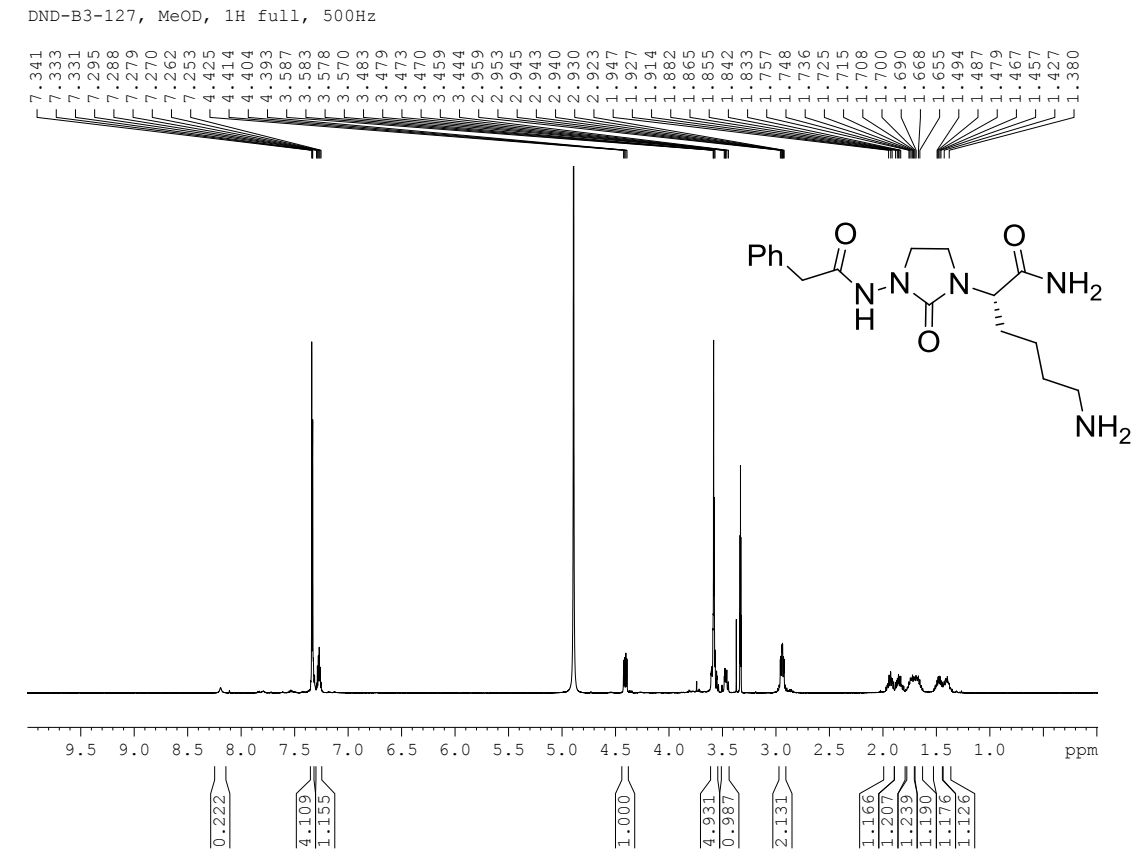


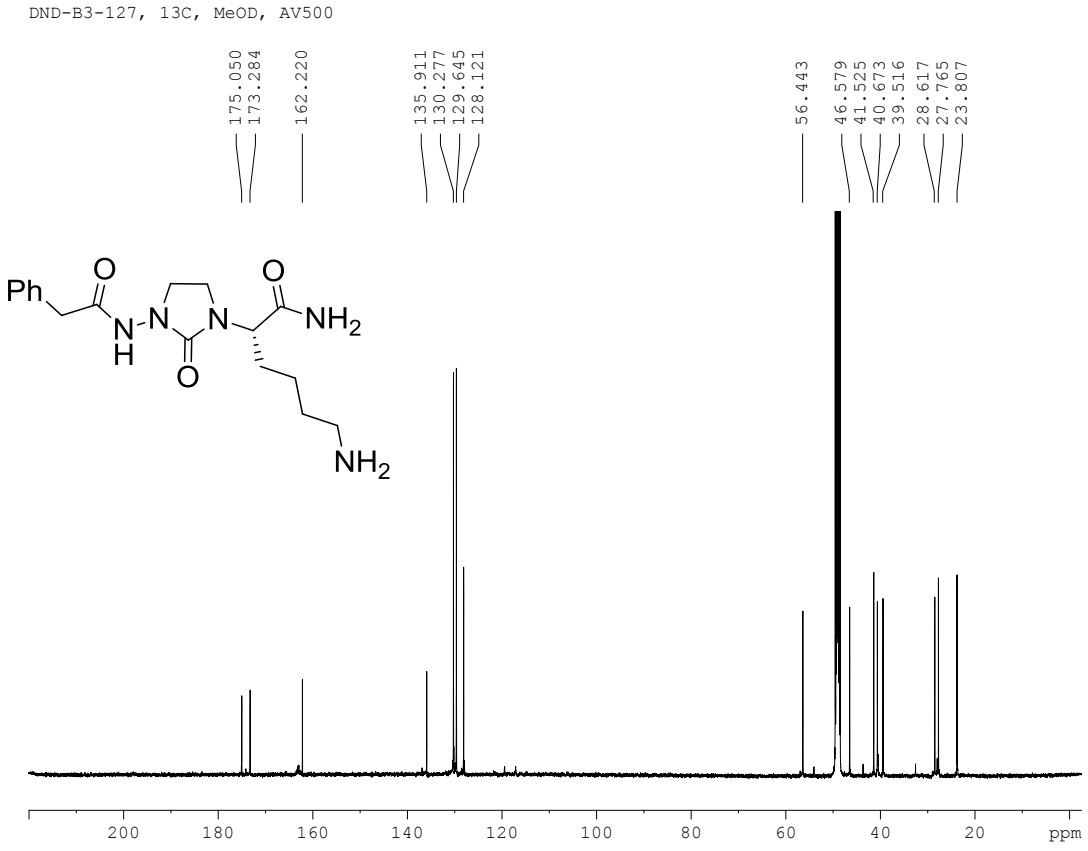


Benzylidene-Aid-Phe-OH (10d)

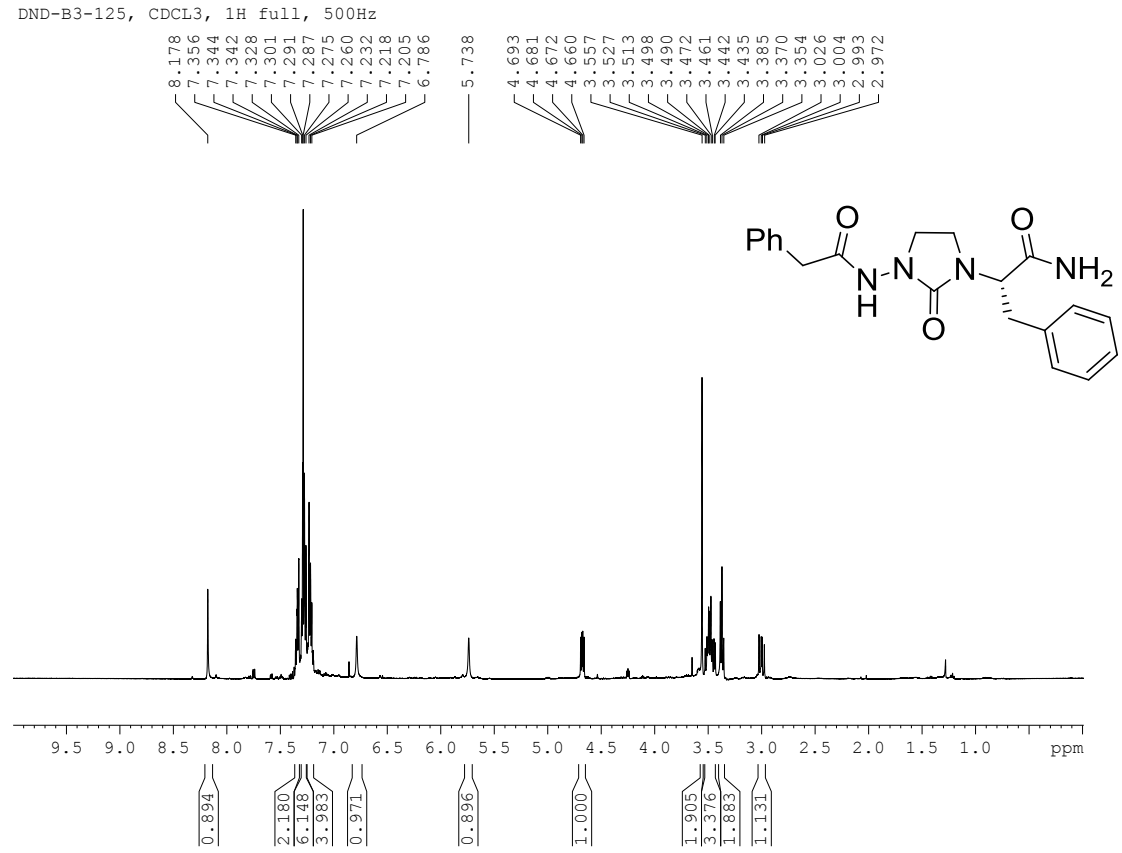


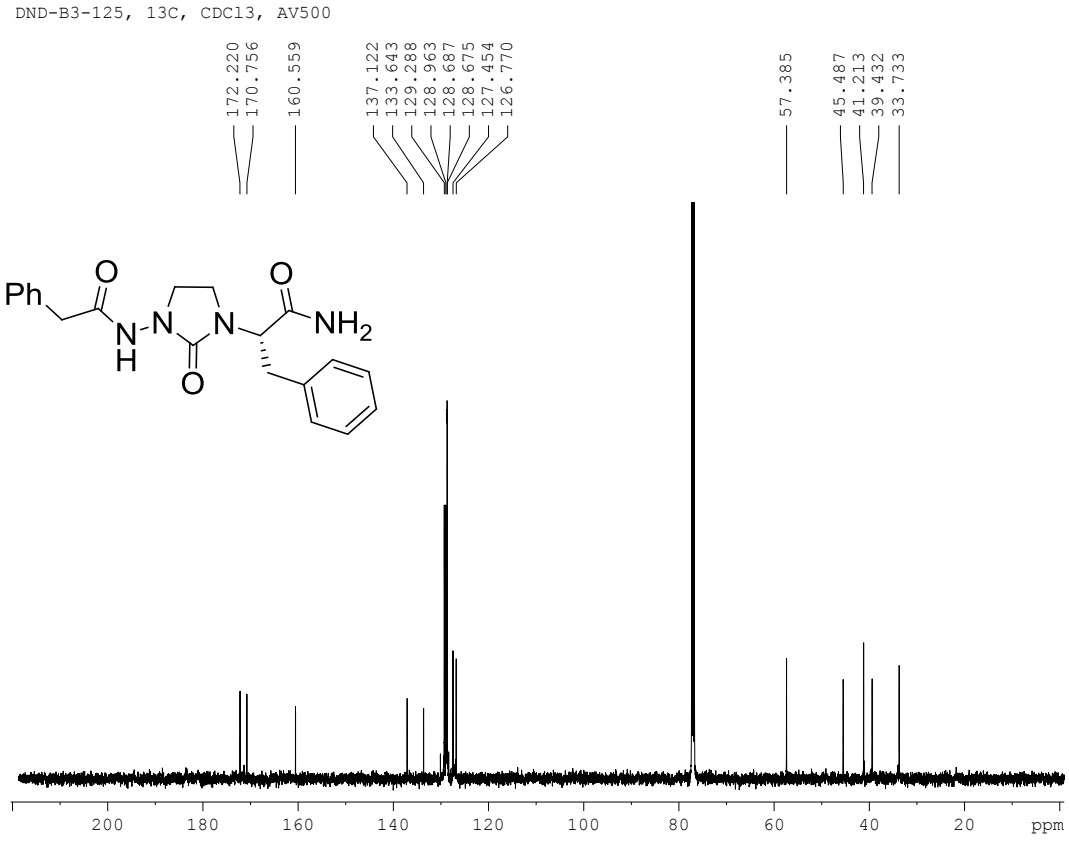
PhAc-Aid-Lys-NH2 (12a)



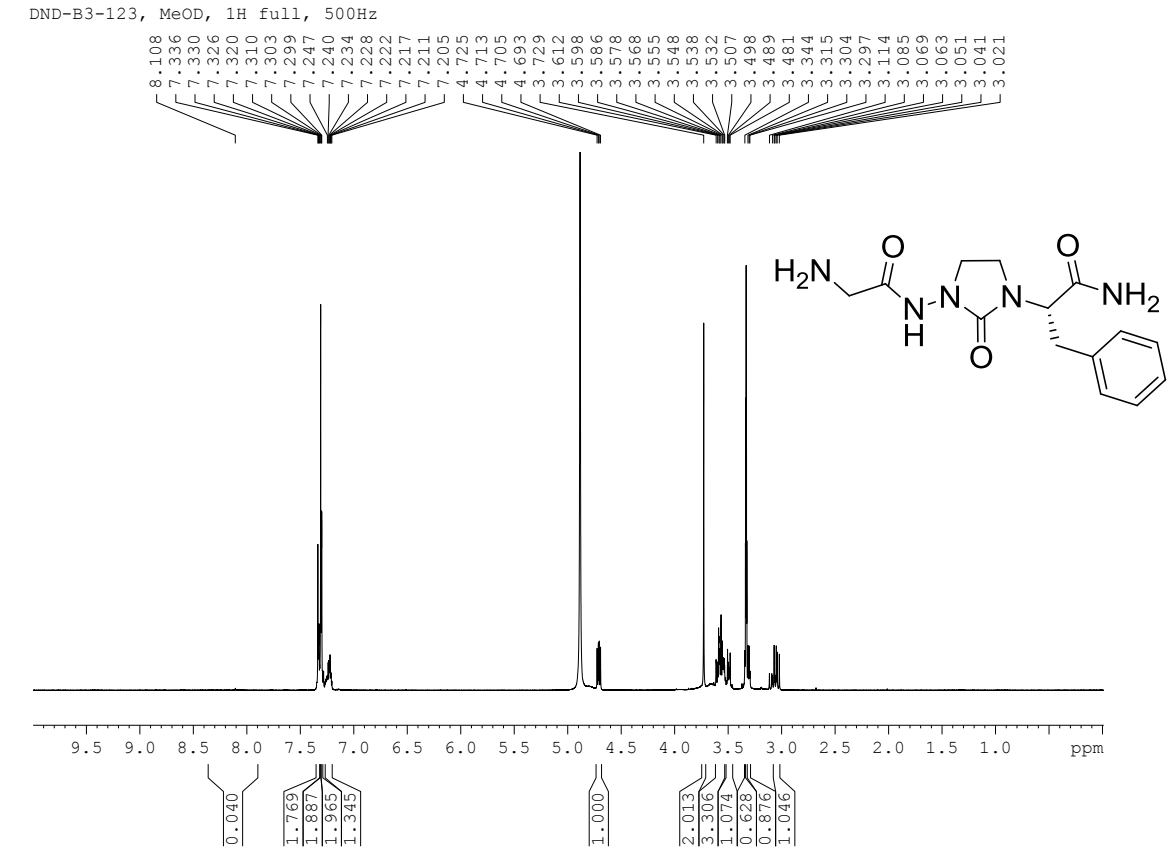


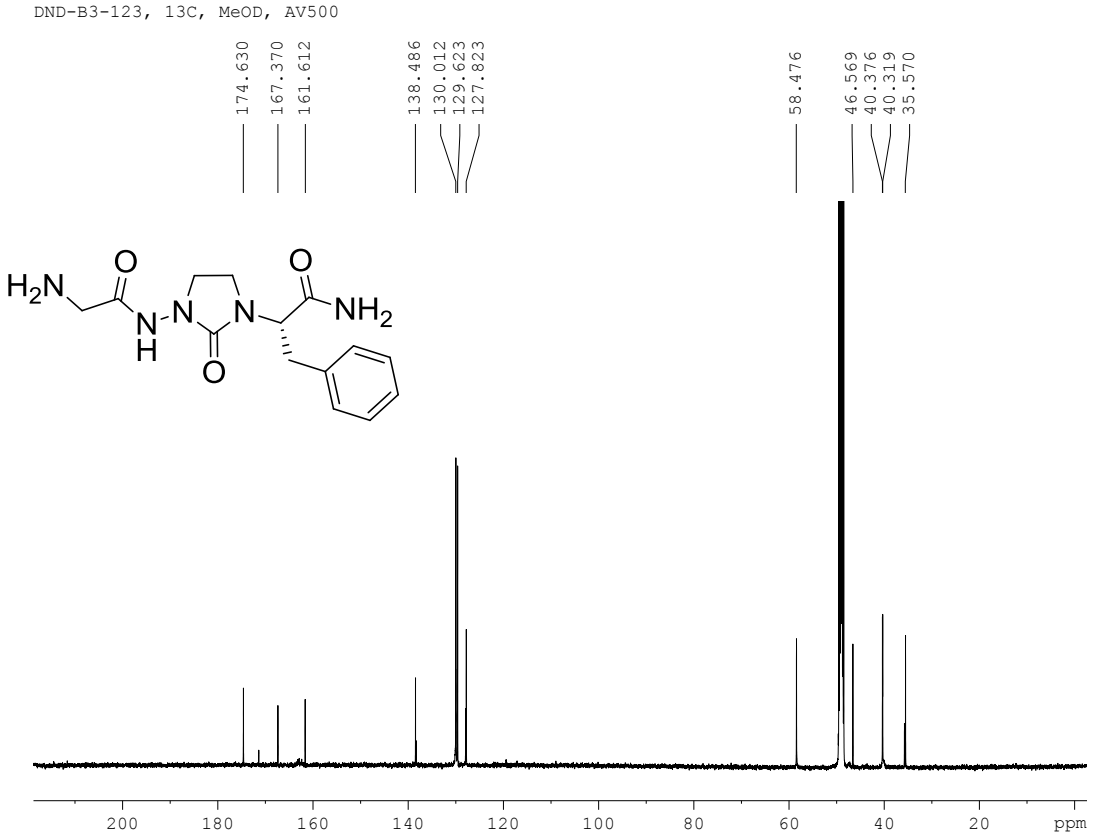
PhAc-Aid-Phe-NH2 (12b)



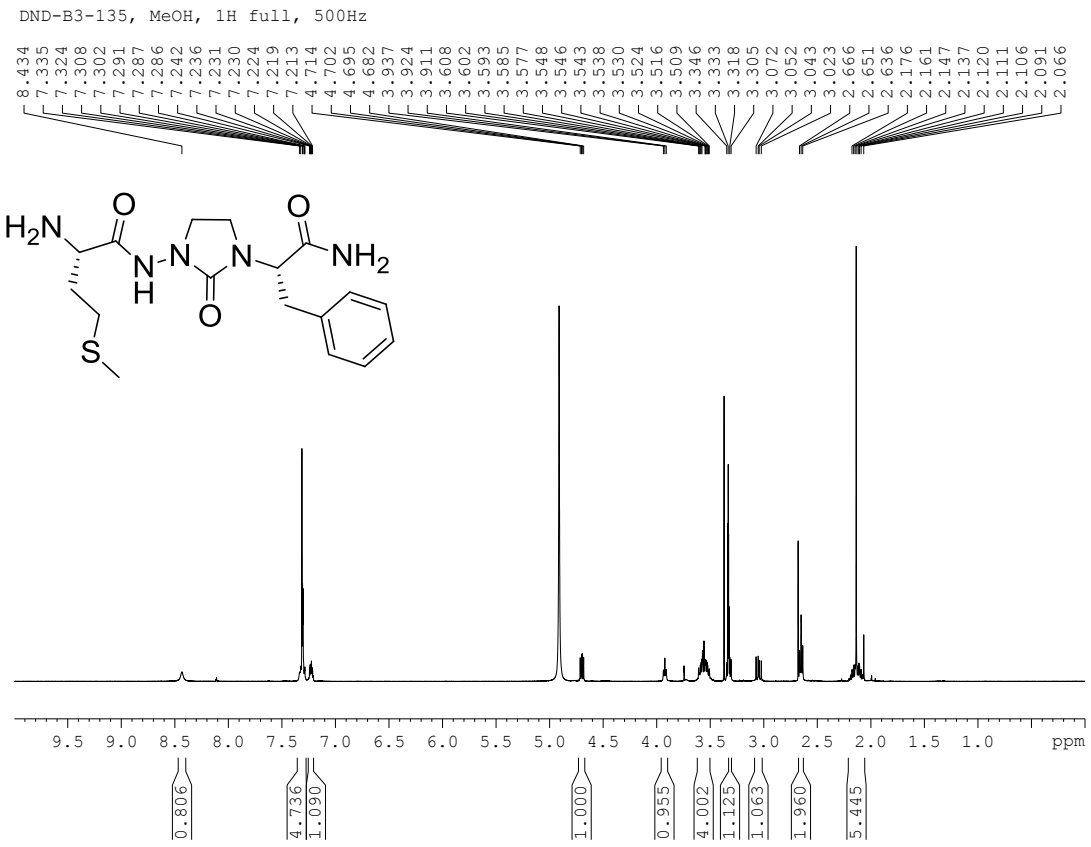


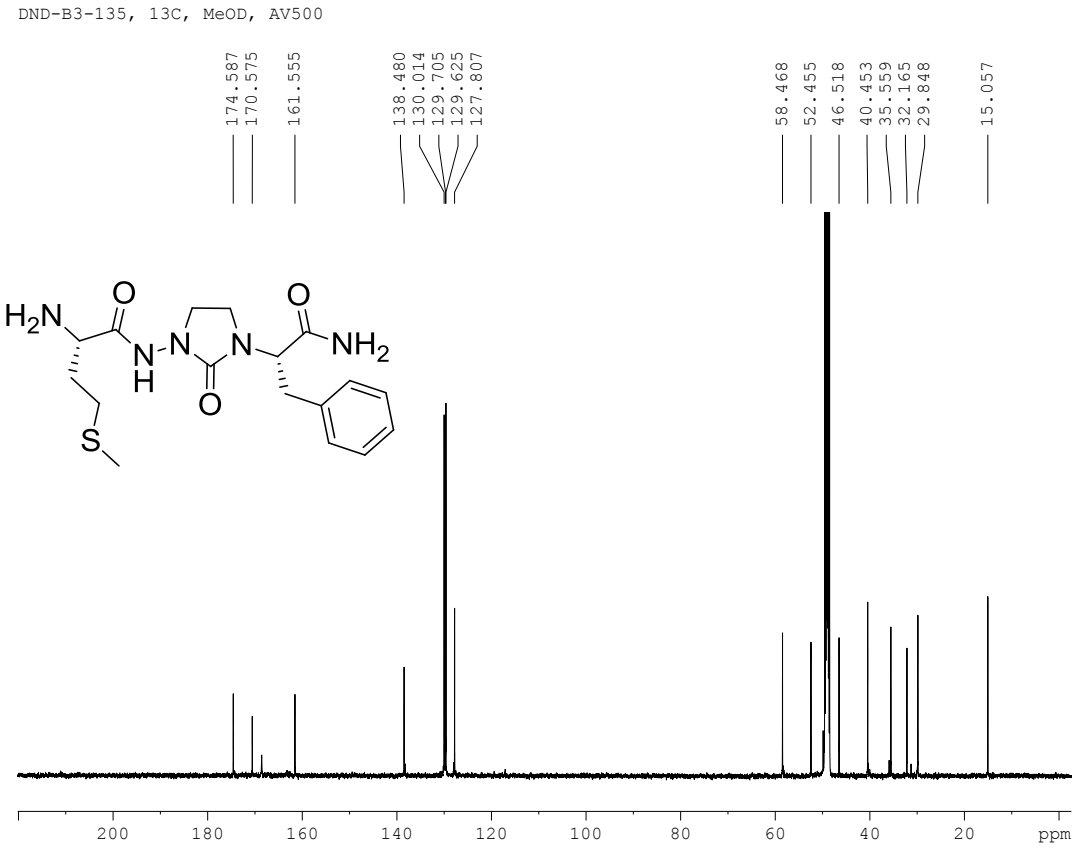
Gly-Aid-Phe-NH2 (12c)



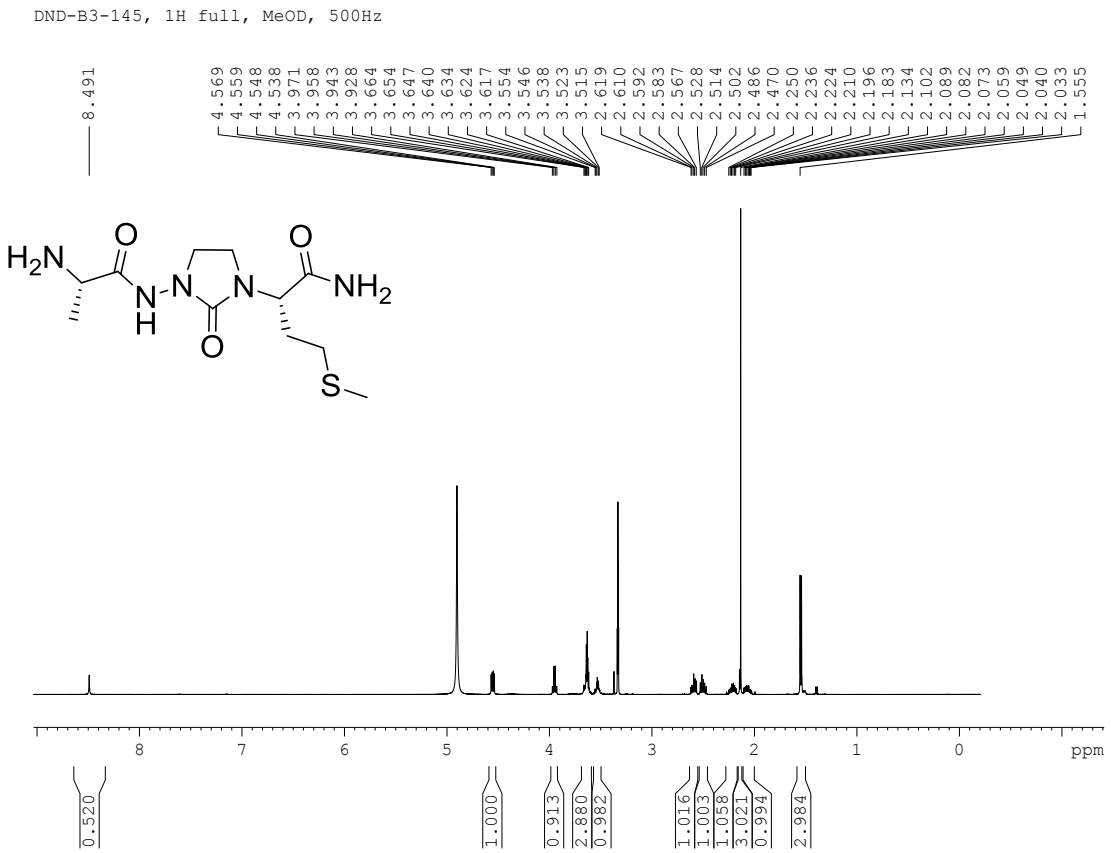


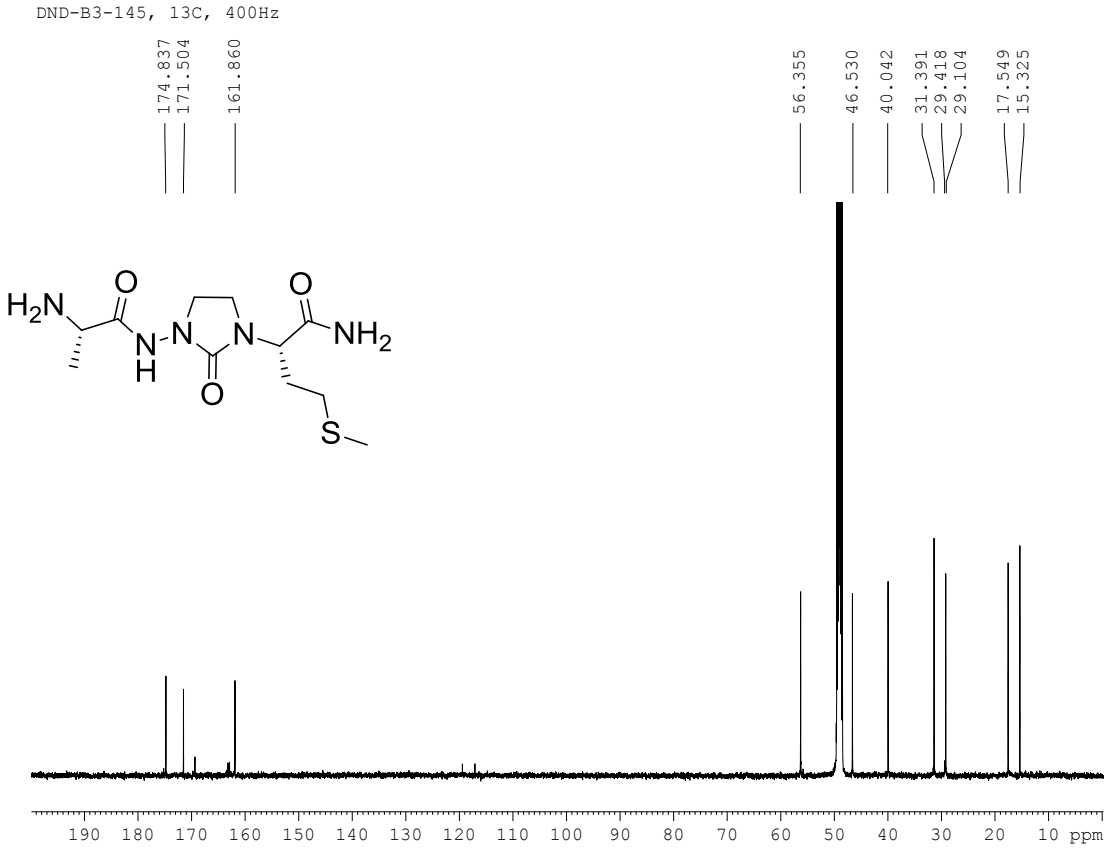
Met-Aid-Phe-NH2 (12d)



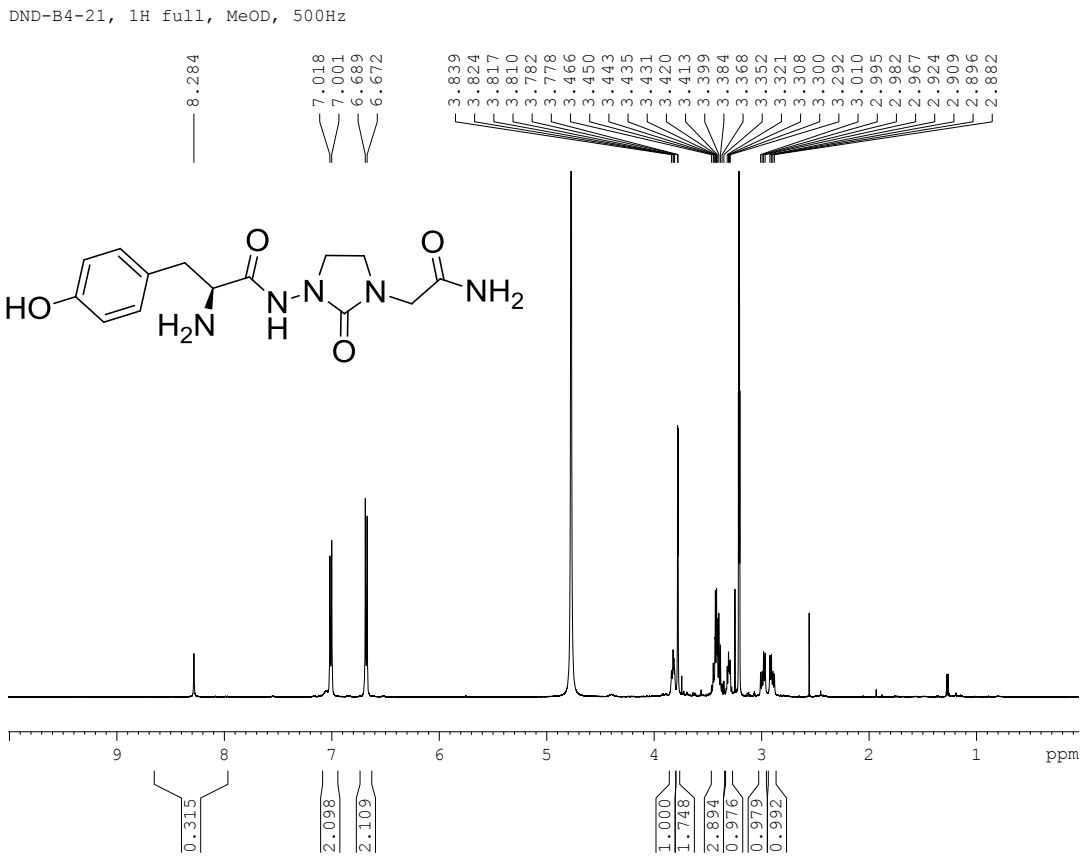


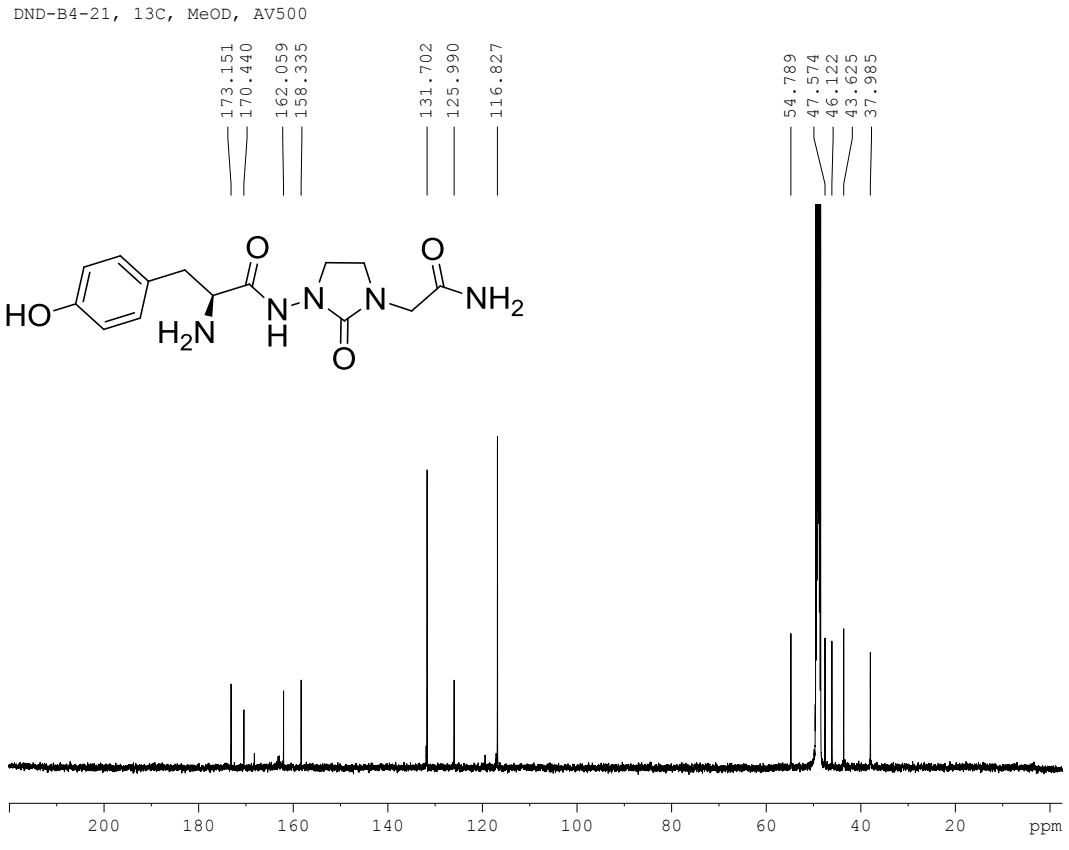
Ala-Aid-Met-NH2 (10f)





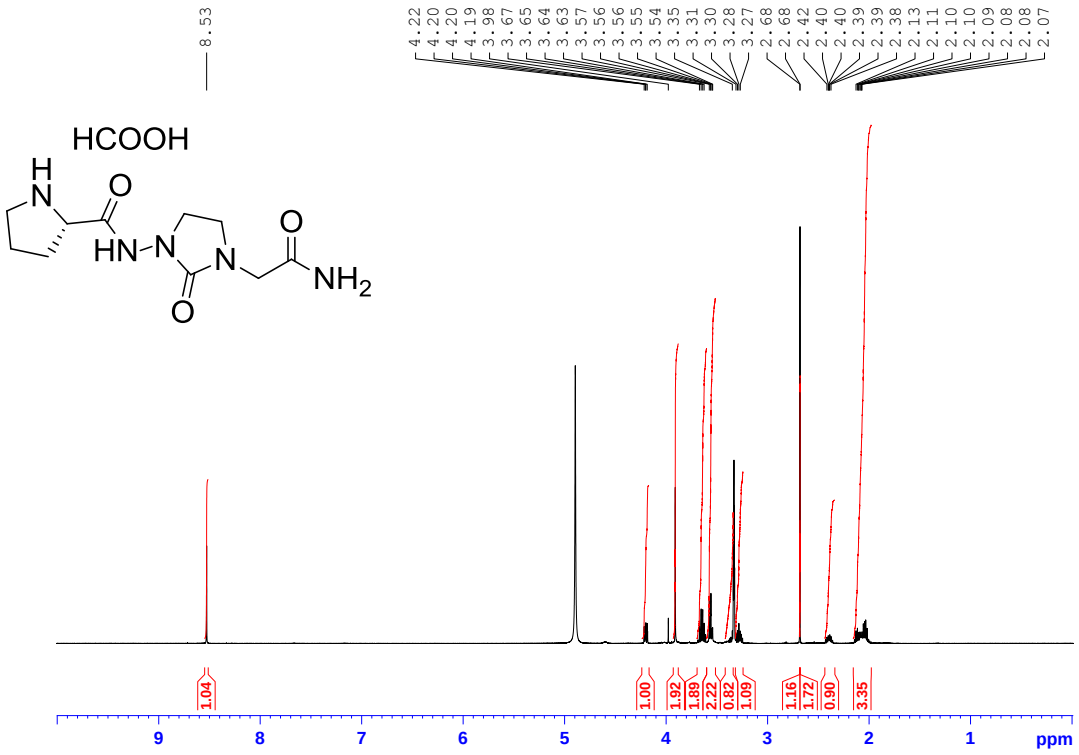
Tyr-Aid-Gly-NH2 (12e)





Pro-Aid-Gly-NH2 (12g)

DND-B3-179L, 1H, MeOH, AV500



DND-B3-179L, 13C, MeOD, AV500