Supporting Information

Chemical Synthesis

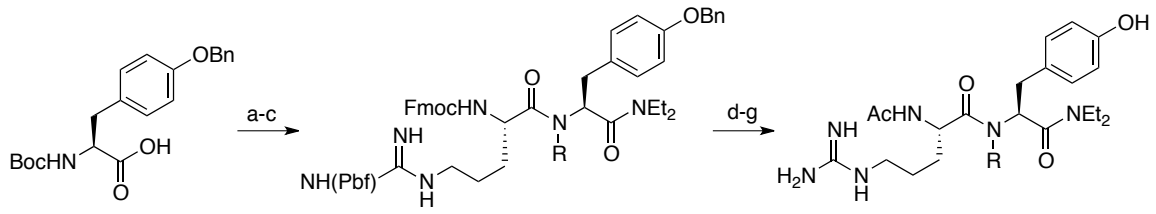
All reagents were obtained from Merck (Novabiochem), Sigma Aldrich or Fisher Scientific. Thin-layer chromatography was carried out on Merck silica gel aluminium-backed plates with a fluoresence indicator (254 nm). Visualisation of TLC plates was carried out by UV light, then by either a cerium sulphate/ammonium molybdate or $KMnO_4$ stain. Flash chromatography was preformed with 35–70 μm silica gel (Fisher Davisil). Infrared spectra were recorded using a FTIR spectrometer fitted with an ATR accessory. Absorption peaks were recorded in cm-1 and were described as strong (s), medium (m), weak (w) or broad (br). ^{1}H NMR and ^{13}C NMR were recorded using a Bruker DPX400 (400 and 100 MHz) in $(CD_3)_2SO$ with DMSO (2.50 ppm ^{1}H, 39.51 ppm ^{13}C) as an internal reference. Chemical shifts δ are given in ppm; multiplicities are indicated as s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), m (multiplet), and br (broad); coupling constants, J, are reported in Hz. ^{13}C signal arising from the quaternary carbon bearing the boronic acid group was not observed and therefore were not listed, this was accordance with work by Hall et al. in "Mothana, S.; Grassot, J.; Hall, D. G. *Angew. Chem. -Int. Edit.* (2010), 49, 2883-2887".

Cyclic peptide synthesis

The linear counterparts of the CRYTFNV alanine-scanning analogues were synthesized at the Hershey Macromolecular Core Facility of the Pennsylvania State University. The linear peptides were cyclized and purified as previously described for CRYFNV in "A. Tavassoli, S. J. Benkovic, *Angew. Chem. Int. Ed. Engl.* (2005), *44*, 2760-2763".

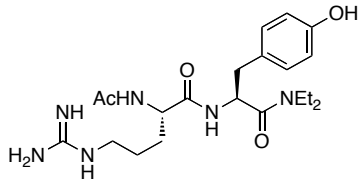
dipeptide synthesis

All capped dipeptides were synthesized on a similar scale as detailed below for compound 8, starting from the appropriate amino acids (from Merck Novabiochem or SigmaAldrich).



Scheme 1 Reagents and conditions: a) EDC, HOBt, Et_2NH, CH_2Cl_2; b) TFA/CH_2Cl_2; c) Fmoc-Arg(Pbf)-OH, EDC, HOBt, CH_2Cl_2; d) DBU, EtOAc; e) Ac_2O, Et_3N, CH_2Cl_2; f) 10% Pd/C, H_2, MeOH; g) TFA/TIS/H_2O.

Ac-Arg-Tyr-NEt_2



Chemical Formula: $C_{21}H_{34}N_6O_4$
Exact Mass: 434.2642
Molecular Weight: 434.5325

Synthesis of diethyl amide

To a stirred solution of N-α-t-Boc-O-benzyl-L-tyrosine (Novabiochem, Cat. No. 853065, 2.0 g, 5.38 mmol, 1 eq) dissolved in CH_2Cl_2 (30 ml) at room temperature, HOBt (730 mg, 5.38 mmol, 1 eq) and EDC.HCl (1.03 g, 5.38 mmol, 1 eq) were added, followed by diethyl amine (1.17 ml, 11.30 mmol, 2.1 eq). The reaction was stirred at room temperature for 4 hours, after which 10% citric acid (20 ml) was added. The organic layer was collected and the aqueous layer was re-extracted with CH_2Cl_2 (3 x 20 ml). The combined organic layers were washed with 10% citric acid (20 ml), saturated $NaHCO_3$ (20 ml) and brine (20 ml). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give a yellow oil (2.18 g, 5.12 mmol, 95 % crude yield) that was used without further purification.

Boc deprotection and peptide coupling

TFA (6 mL) was added to a stirred solution of Boc amine (1.0 g, 3.06 mmol) in CH_2Cl_2 (6 mL) at room temperature. The reaction was stirred at room temperature, and after 30 minutes the reaction was concentrated in vacuo to give a yellow oil, to which was added to a solution of N-α-Fmoc-N^G-(2,2,4,6,7-

pentamethyldihydrobenzofuran-5-sulfonyl)-L-arginine (NovaBiochem, Cat. No. 852067, 1.98 g, 3.06 mmol, 1 eq) in CH_2Cl_2 (25 ml), followed by HOBt (454 mg, 3.3 mmol, 1.1 eq) and EDC.HCl (632 mg, 3.3 mmol, 1.1 equiv). The reaction was stirred at room temperature, after 4 hours 10% citric acid (20 ml) was added. The organic layer was collected and the aqueous layer was re-extracted with CH_2Cl_2 (3 x 20 ml). The combined organic layers were washed with 10% citric acid (25 ml), saturated $NaHCO_3$ (25 ml) and brine (25 ml). The organic layer was dried ($MgSO_4$) and concentrated in vacuo to give a yellow oil that was purified by column chromatography (SiO_2, 75 % EtOAc-hexane) to give a white solid (1.36 g, 1.42 mmol, 46 % yield) that was characterized by mass spectroscopy.

Fmoc deprotection and acetylation

At room temperature, to a stirred solution of Fmoc protected amine (300 mg, 0.31 mmol) dissolved in EtOAc was added DBU (1.05 eq). The reaction was stirred at room temperature, after 30 minutes the reaction was concentrated in vacuo to give a yellow oil. Purification by SiO_2 (short- path) eluting with MeOH 1-10%:CH_2Cl_2 gave a yellow oil that was dissolved in CH_2Cl_2 (10 ml). To the stirred solution at room temperature, Ac_2O (47 µl, 0.34 mmol, 1.1 eq) and triethylamine (38 µl, 0.34 mmol, 1.05 eq) were added. After 15 hours the reaction was concentrated in vacuo and purified by column chromatography (SiO_2, 7% MeOH-CH_2Cl_2) to give a to give a yellow oil (209 mg, 0.27 mmol, 87 % yield).

Debenzylation

At room temperature under an atmosphere of H_2, a suspension of the protected dipeptide (100 mg, 0.13 mmol) and 10% Pd/C (18 mg, 0.012 mmol, 0.1 eq) in MeOH (10 ml) was was stirred for 15 hours. The reaction mixture was filtered through celite and concentrated in vacuo to give a yellow oil (77 mg) that was used in the next step without further purification.

Side chain deprotection

At room temperature, a solution containing TFA (4.75 ml), TIS (0.125 ml) and H2O 0.125 ml) at a % ratio of 95:2.5:2.5was added to the dipeptide (77mg, 0.11 mmol). The reaction was stirred at room temperature,

after 2 hours the reaction was concentrated in vacuo followed by diethyl ether precipitation to give a white solid. Purification by RP-HPLC using a Waters Alantis Prep T3 OBD 5µm 19 x 100 mm column at a 17 mL min-1 elution with a 95% A 5% B → 30% A 70% B gradient over 10 minutes (A = water with 1% TFA. B = acetonitrile with 1% TFA) gave the desired dipeptide after lyophilisation as a white solid (25 mg, 52%).

[α]26D -28.5 (MeOH, c 0.53).

IR νmax (neat) 3272 (m), 3192 (m), 1614 (s), 1541 (s), 1514 (s) cm-1.

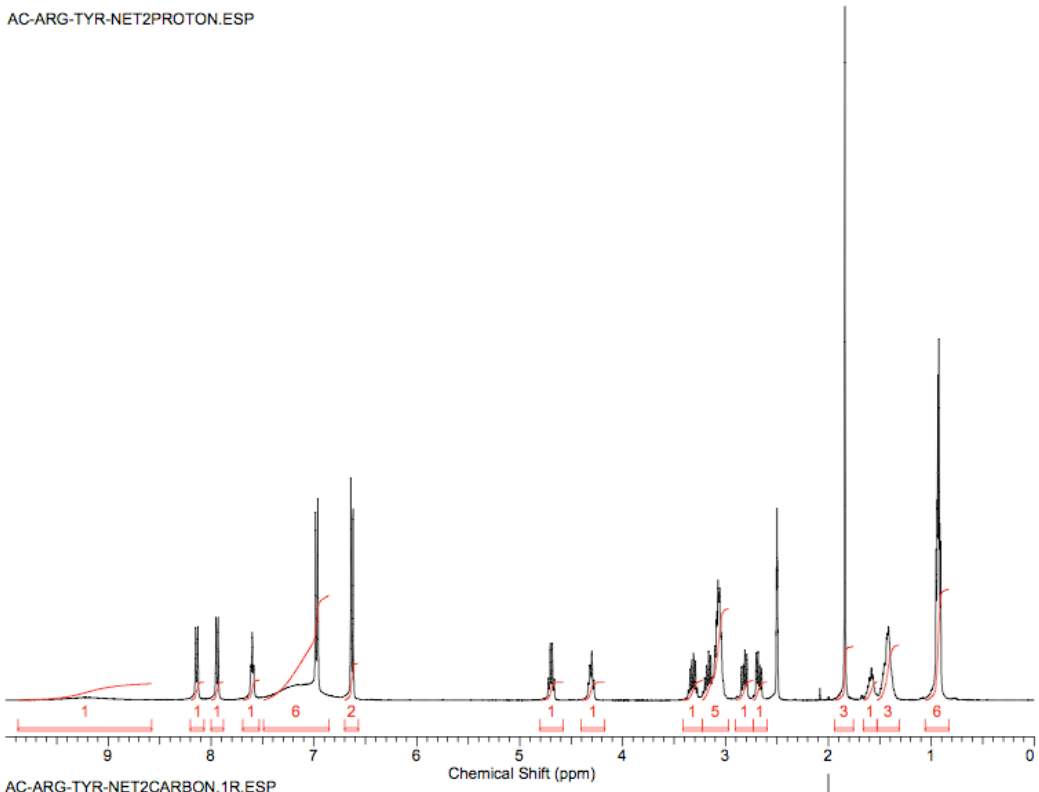
^{1}H NMR (400 MHz, DMSO-d6) δ 9.85 - 8.66 (br s, 1H), 8.14 (d, J = 7.5 Hz, 1H), 7.94 (d, J = 8.1 Hz, 1H), 7.60 (t, J = 5.6 Hz, 1H), 6.97 (d, J = 8.3 Hz, 2H), 7.54 - 6.86 (m, 3H), 6.63 (d, J = 8.3 Hz, 2H), 4.69 (dt, J = 6.8, 7.5 Hz, 1H), 4.31 (dt, J = 6.1, 8.1 Hz, 1H), 3.32 (dd, J = 7.0, 13.4 Hz, 1H), 3.23 - 2.99 (m, 5H), 2.82 (dd, J = 7.5, 13.2 Hz, 1H), 2.68 (dd, J = 6.8, 13.2 Hz, 1H), 1.84 (s, 3H), 1.65 - 1.51 (m, 1H), 1.51 - 1.33 (m, 3H), 0.94 (t, J = 7.0 Hz, 3H), 0.93 (t, J = 7.0 Hz, 3H).

^{13}C NMR (100 MHz, DMSO-d6) δ 170.9, 169.9, 169.1, 156.7, 156.0, 130.2, 127.2, 114.9, 51.6, 50.2, 41.0, 40.4, 39.7, 37.4, 29.3, 24.9, 22.5, 14.0, 12.7.

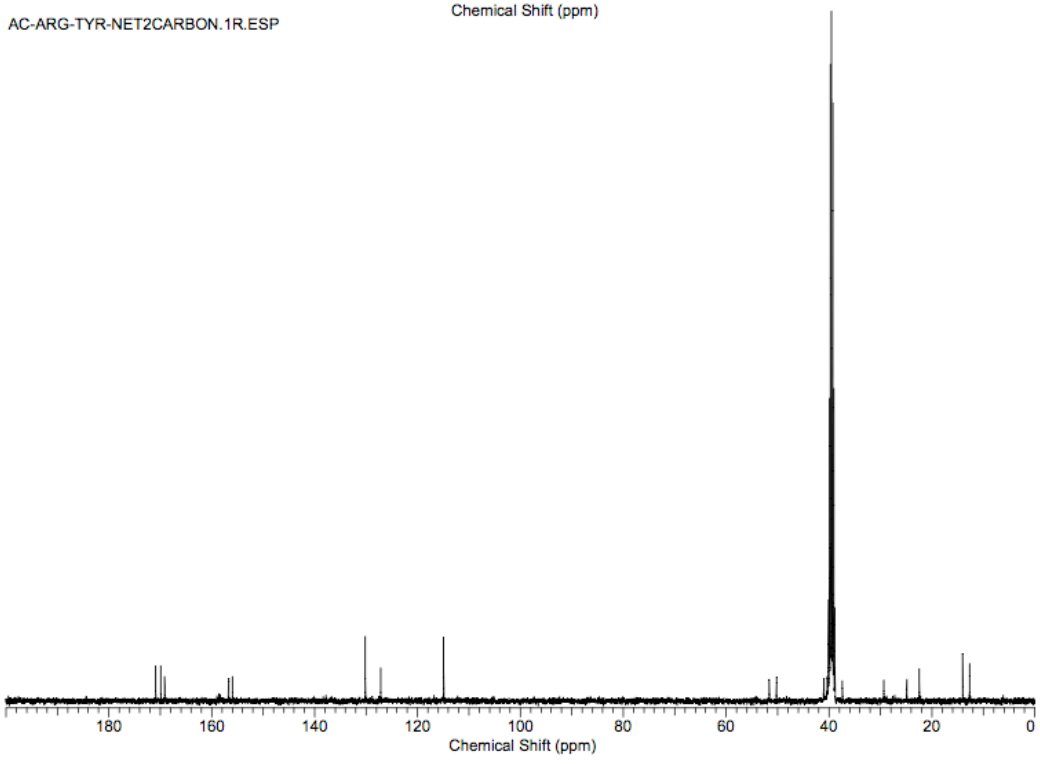
LRMS [M+H]+ 435 (100%).

HRMS ESI+ [M+H]+ calculated = 435.2714, [M+H]+ found = 435.2711.

AC-ARG-TYR-NET2PROTON.ESP

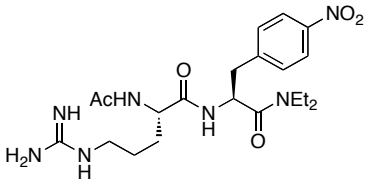


AC-ARG-TYR-NET2CARBON.1R.ESP



Ac-Arg-Phe(4-NO_2)-NEt_2

The protocol outlined above was repeated on a similar scale, with the appropriate amino acids, with an overall yield of 23%.



Chemical Formula: $C_{21}H_{33}N_7O_5$
Exact Mass: 463.2543
Molecular Weight: 463.5306

[α]27D -31.6 (MeOH, c 0.55).

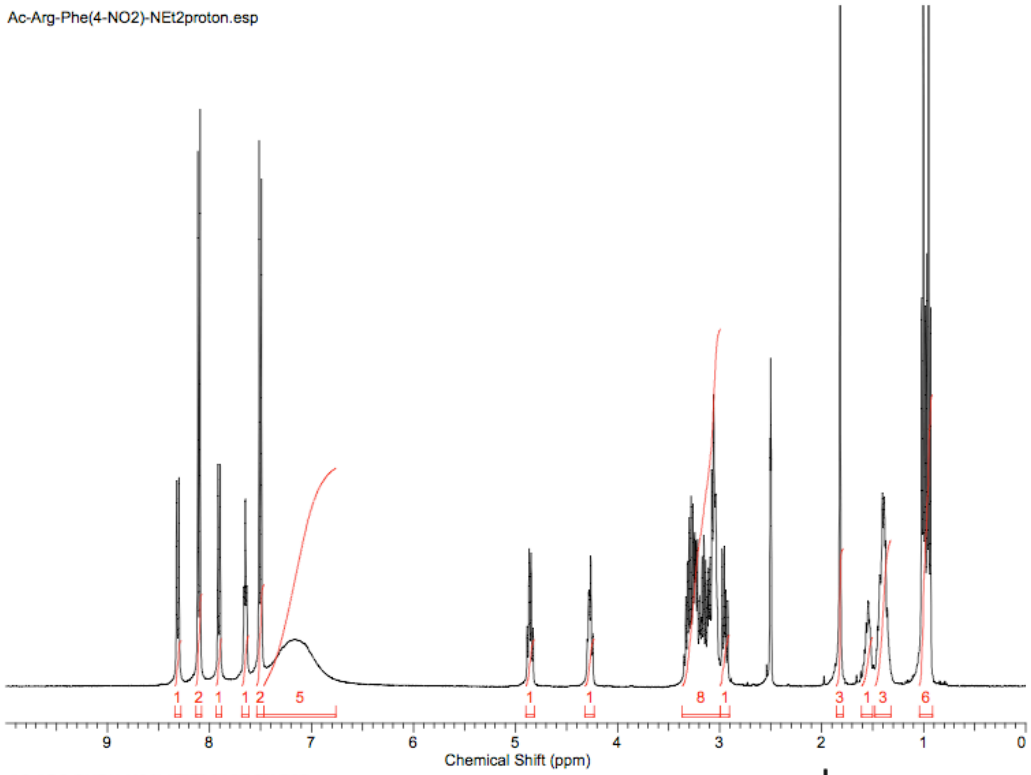
IR νmax (neat) 3278 (m), 3193 (m), 2979 (w), 2943 (w), 1621 (s), 1519 (s) cm-1.

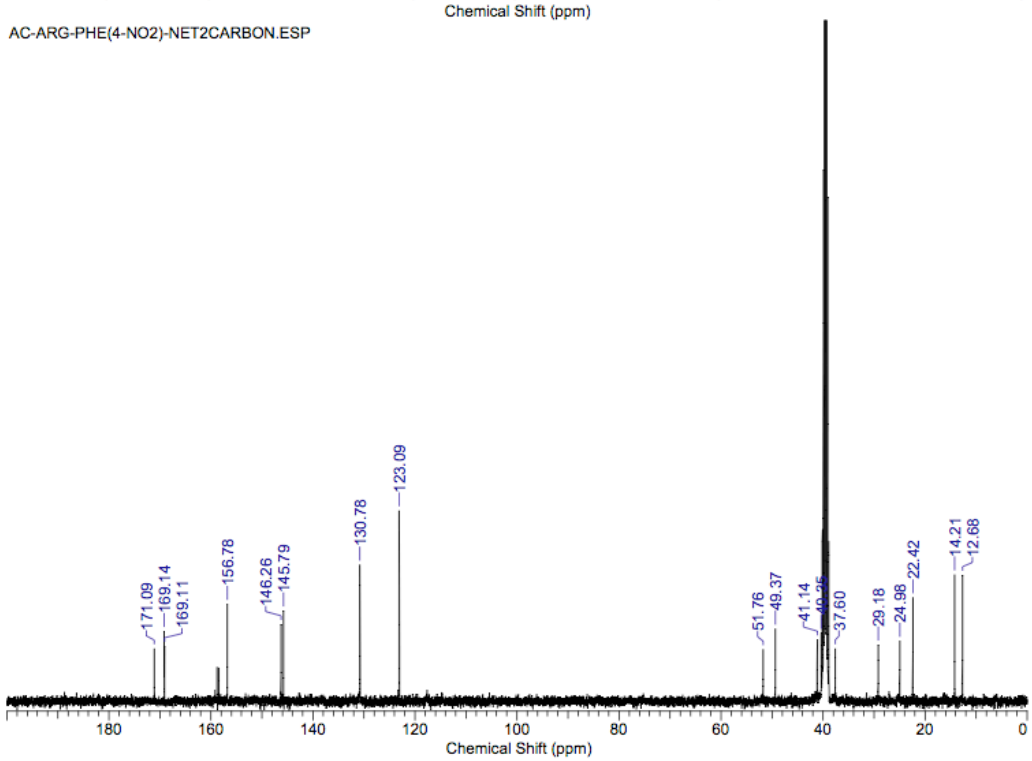
^{1}H NMR (400 MHz, DMSO-d6) δ 8.31 (d, J = 8.6 Hz, 1H), 8.10 (d, J = 8.6 Hz, 2H), 7.91 (d, J = 8.1 Hz, 1H), 7.65 (t, J = 5.6 Hz, 1H), 7.50 (d, J = 8.6 Hz, 2H), 7.17 (br. s., 3H), 4.90 - 4.82 (m, 1H), 4.32 - 4.23 (m, 1H), 3.37 - 3.00 (m, 7H), 2.95 (dd, J = 8.1, 13.6 Hz, 1H), 1.82 (s, 3H), 1.61 - 1.50 (m, 1H), 1.48 - 1.32 (m, 3H), 1.00 (t, J = 7.1 Hz, 3H), 0.95 (t, J = 7.1 Hz, 3H).

^{13}C NMR (100 MHz, DMSO-d6) δ 171.1, 169.1, 169.1, 156.8, 146.3, 145.8, 130.8, 123.1, 51.8, 49.4, 41.1, 40.4, 39.8, 37.6, 29.2, 25.0, 22.4, 14.2, 12.7.

LRMS [M+H]+ 464 (100%).

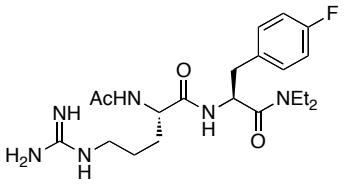
HRMS ESI+ [M+H]+ calculated = 464.2616, [M+H]+ found = 464.2610.


Ac-Arg-Phe(4-NO2)-NEt2proton.esp
1 2 1 1 2 5 1 1 8 1 3 1 3 6
9 8 7 6 5 4 3 2 1 0
Chemical Shift (ppm)


AC-ARG-PHE(4-NO2)-NET2CARBON.ESP
171.09
169.14
169.11
156.78
146.26
145.79
130.78
123.09
51.76
49.37
41.14
37.60
29.18
24.98
22.42
14.21
12.68
180 160 140 120 100 80 60 40 20 0
Chemical Shift (ppm)

Ac-Arg-Phe(4-F)-NEt_2

The above protocol was followed, on a similar scale with the appropriate amino acids. The overall yield of synthesis was 19%.



Chemical Formula: $C_{21}H_{33}FN_6O_3$
Exact Mass: 436.2598
Molecular Weight: 436.5235

[α]27D -29.1 (MeOH, c 0.54).

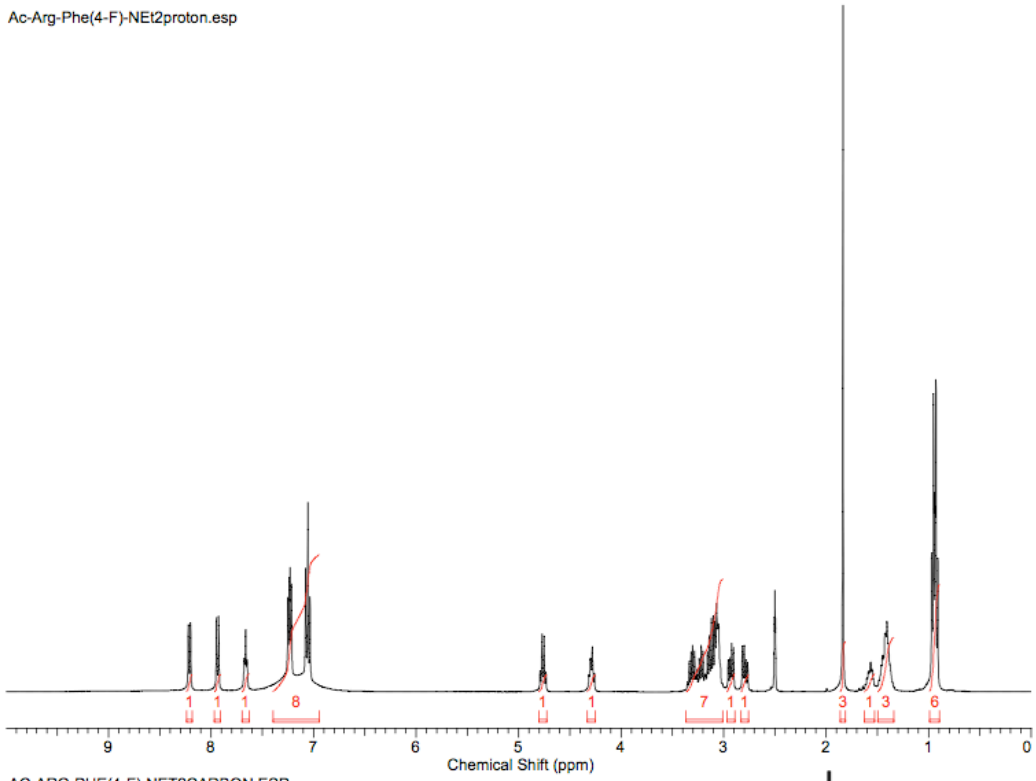
IR νmax (neat) 3274 (m), 1618 (s), 1590 (s), 1509 (s) cm-1.

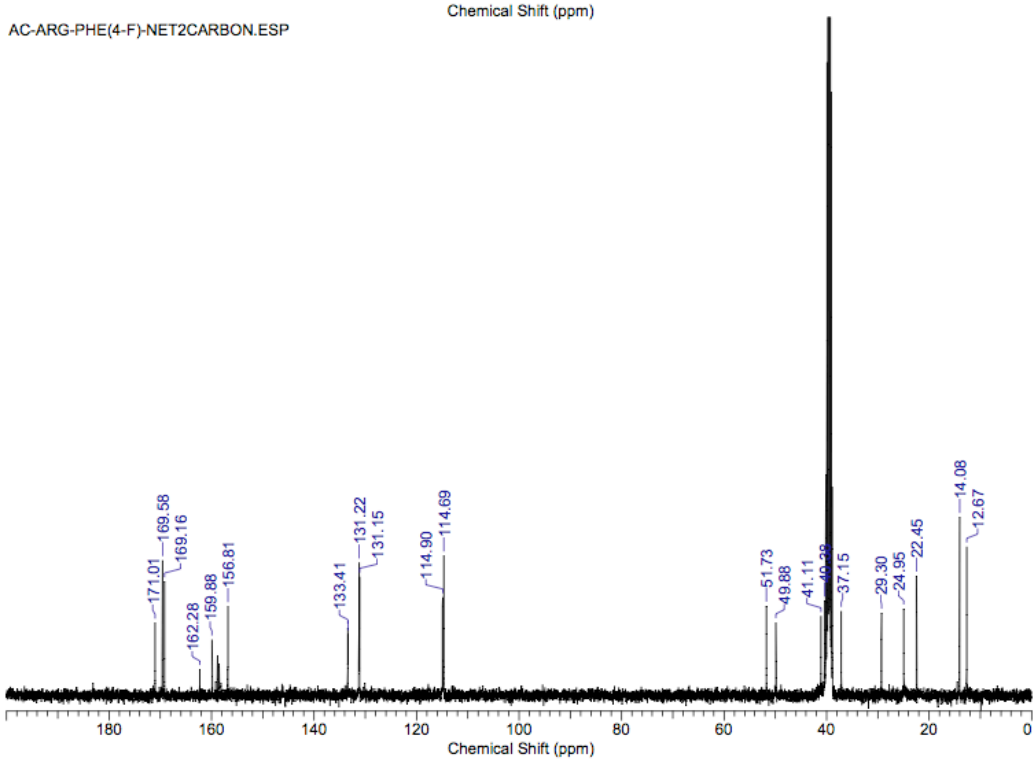
^{1}H NMR (400 MHz, DMSO-d6) δ 8.21 (d, J = 8.1 Hz, 1H), 7.94 (d, J = 8.1 Hz, 1H), 7.66 (t, J = 5.6 Hz, 1H), 7.23 (dd, J = 6.1, 8.6 Hz, 2H), 7.06 (t, J = 9.1 Hz, 2H), 7.40 - 6.94 (br s, 3H), 4.76 (ddd, J = 7.1, 7.3, 8.1 Hz, 1H), 4.33 - 4.25 (m, 1H), 3.37 - 3.01 (m, 6H), 2.93 (dd, J = 7.1, 13.3 Hz, 1H), 2.79 (dd, J = 7.3, 13.3 Hz, 1H), 1.84 (s, 3H), 1.63 - 1.52 (m, 1H), 1.49 - 1.34 (m, 3H), 0.95 (t, J = 7.1 Hz, 3H), 0.92 (t, J = 7.1 Hz, 3H).

^{13}C NMR (100 MHz, DMSO-d6) δ 171.0, 169.6, 169.2, 162.3, 159.9, 156.8, 133.4, 133.4, 131.2, 131.1, 114.9, 114.7, 51.7, 49.9, 41.1, 40.4, 39.8, 37.2, 29.3, 24.9, 22.4, 14.1, 12.7.

LRMS [M+H]+ 437 (100%).

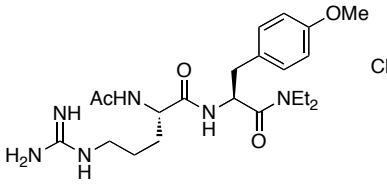
HRMS ESI+ [M+H]+ calculated = 437.2671, [M+H]+ found = 437.2666.


Ac-Arg-Phe(4-F)-NEt2proton.esp
1
1
1
8
1
1
7
1
1
3
1
3
6
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)


AC-ARG-PHE(4-F)-NET2CARBON.ESP
171.01
169.58
169.16
162.28
159.88
156.81
133.41
131.22
131.15
114.90
114.69
51.73
49.88
41.11
37.15
29.30
24.95
22.45
14.08
12.67
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

Ac-Arg-Tyr(Me)-NEt_2

The above procedure was repeated on a similar scale, with the appropriate amino acids, with a total yield of 22%.



Chemical Formula: $C_{22}H_{36}N_6O_4$
Exact Mass: 448.2798
Molecular Weight: 448.5590

[α]27D -22.5 (MeOH, c 0.54).

IR νmax (neat) 3273 (m), 1614 (s), 1539 (s), 1512 (s) cm-1.

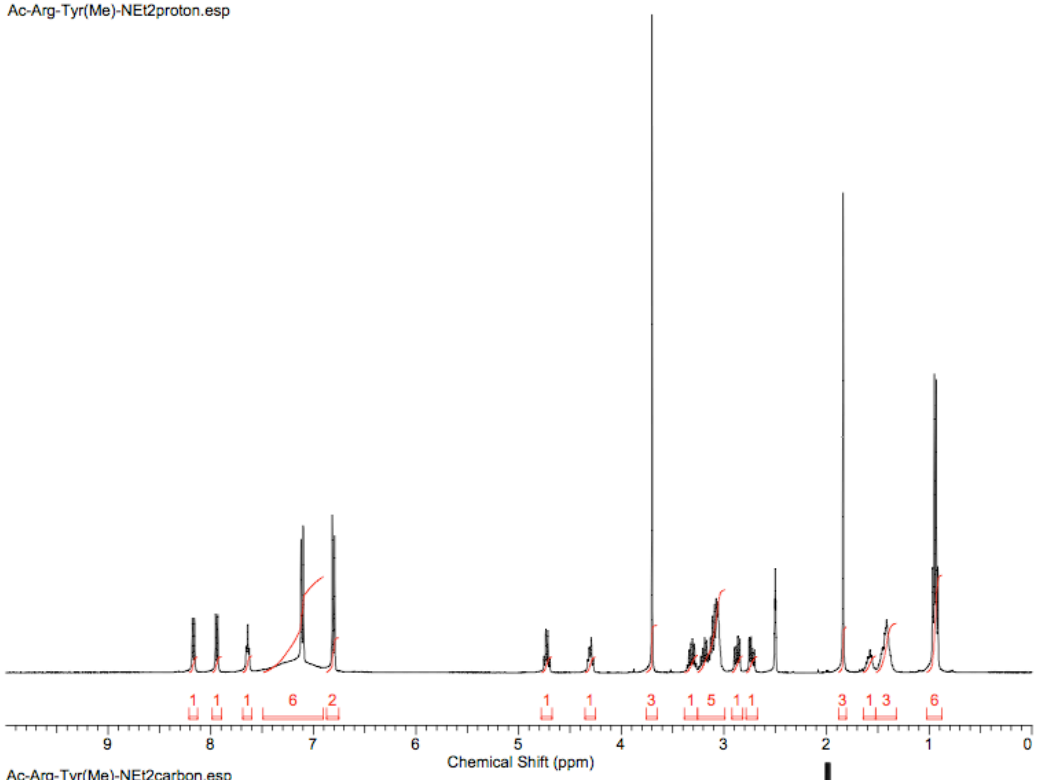
^{1}H NMR (400 MHz, DMSO-d6) δ 8.17 (d, J = 8.1 Hz, 1H), 7.94 (d, J = 8.6 Hz, 1H), 7.64 (t, J = 5.6 Hz, 1H), 7.11 (d, J = 8.6 Hz, 2H), 7.49 - 6.93 (m, 3H), 6.80 (d, J = 8.6 Hz, 2H), 4.73 (ddd, J = 7.1, 7.3, 8.1 Hz, 1H), 4.35 - 4.25 (m, 1H), 3.70 (s, 3H), 3.39 - 3.26 (m, 1H), 3.26 - 2.99 (m, 5H), 2.87 (dd, J = 7.3, 13.2 Hz, 1H), 2.73 (dd, J = 7.1, 13.2 Hz, 1H), 1.84 (s, 3 H), 1.64 - 1.53 (m, 1H), 1.51 - 1.34 (m, 3H), 0.99 - 0.89 (m, 6H).

^{13}C NMR (100 MHz, DMSO-d6) δ 170.9, 169.8, 169.1, 158.0, 156.8, 130.3, 129.1, 113.5, 55.0, 51.7, 50.1, 41.1, 40.4, 39.7, 37.3, 29.3, 24.9, 22.5, 14.0, 12.7.

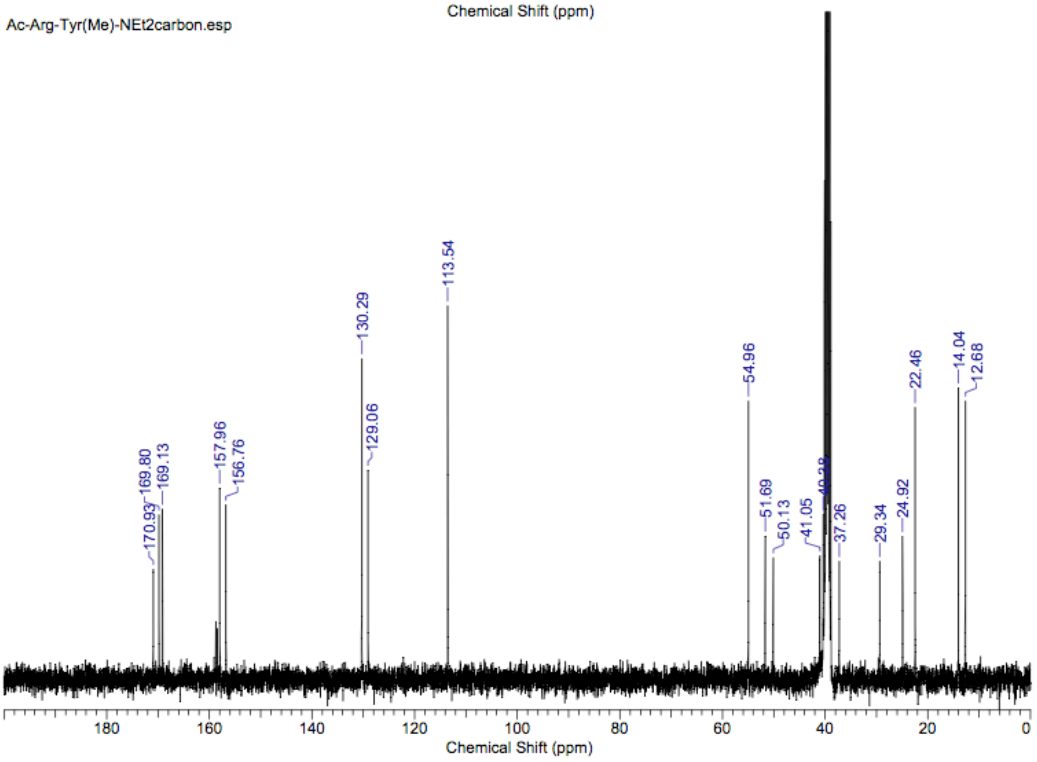
LRMS [M+H]+ 449 (100%).

HRMS ESI+ [M+H]+ calculated = 449.2871, [M+H]+ found = 449.2868.

Ac-Arg-Tyr(Me)-NEt2proton.esp

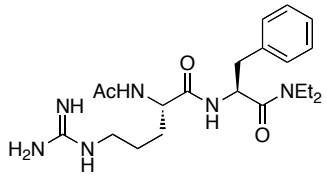


Ac-Arg-Tyr(Me)-NEt2carbon.esp



Ac-Arg-Phe-NEt_2

The above procedure was repeated on a similar scale, with the appropriate amino acids, with an overall yield of 19%.



Chemical Formula: $C_{21}H_{34}N_6O_3$
Exact Mass: 418.2692
Molecular Weight: 418.5331

[α]27D -25.6 (MeOH, c 0.57).

IR νmax (neat) 3273 (m), 1777 (w), 1618 (s), 1538 (s) cm-1.

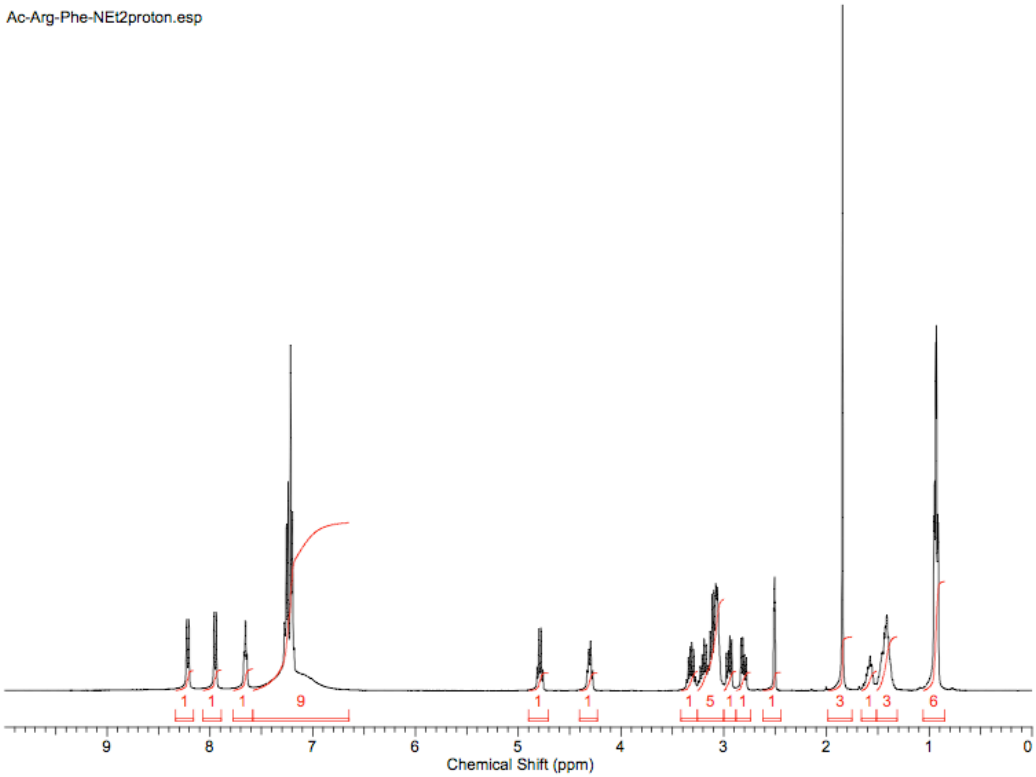
^{1}H NMR (400 MHz, DMSO-d6) δ 8.22 (d, J = 8.1 Hz, 1H), 7.95 (d, J = 8.1 Hz, 1H), 7.65 (t, J = 5.3 Hz, 1H), 7.46 - 7.02 (m, 8H), 4.79 (ddd, J = 8.1, 7.3, 7.1 Hz, 1H), 4.35 - 4.26 (m, 1H), 3.42 - 3.26 (m, 1H), 3.26 - 3.00 (m, 5H), 2.95 (dd, J = 7.3, 13.5 Hz, 1H), 2.81 (dd, J = 7.1, 13.5 Hz, 1H), 1.84 (s, 3H), 1.66 - 1.51 (m, 1H), 1.51 - 1.33 (m, 3H), 0.94 (t, J = 7.1 Hz, 3H), 0.93 (t, J = 7.1 Hz, 3H).

^{13}C NMR (100 MHz, DMSO-d6) δ 171.1, 169.1, 169.1, 156.8, 146.3, 145.8, 130.8, 123.1, 51.8, 49.4, 41.1, 40.4, 39.8, 37.6, 29.2, 25.0, 22.4, 14.2, 12.7.

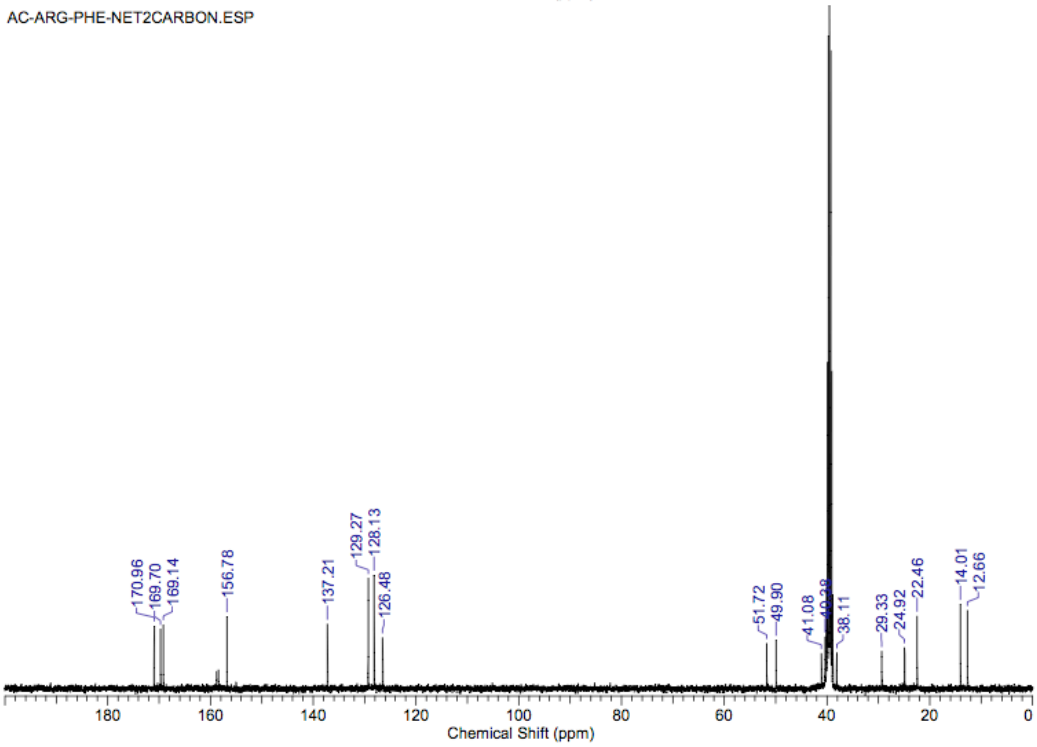
LRMS [M+H]+ 419 (100%).

HRMS ESI+ [M+H]+ calculated = 419.2765, [M+H]+ found = 419.2760.

Ac-Arg-Phe-NEt2proton.esp

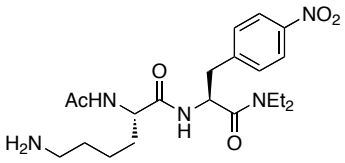


AC-ARG-PHE-NET2CARBON.ESP



Ac-Lys-Phe(4-NO_2)-NEt_2

The above protocol was repeated with the appropriate amino acids, on a similar scale, with an overall yield of 25%.



Chemical Formula: $C_{21}H_{33}N_5O_5$
Exact Mass: 435.2482
Molecular Weight: 435.5172

[α]26D -46.9 (MeOH, c 0.48).

IR νmax (neat) 3272 (m), 3077 (w), 2941 (m), 1776 (w), 1621 (s), 1519 (s) cm-1.

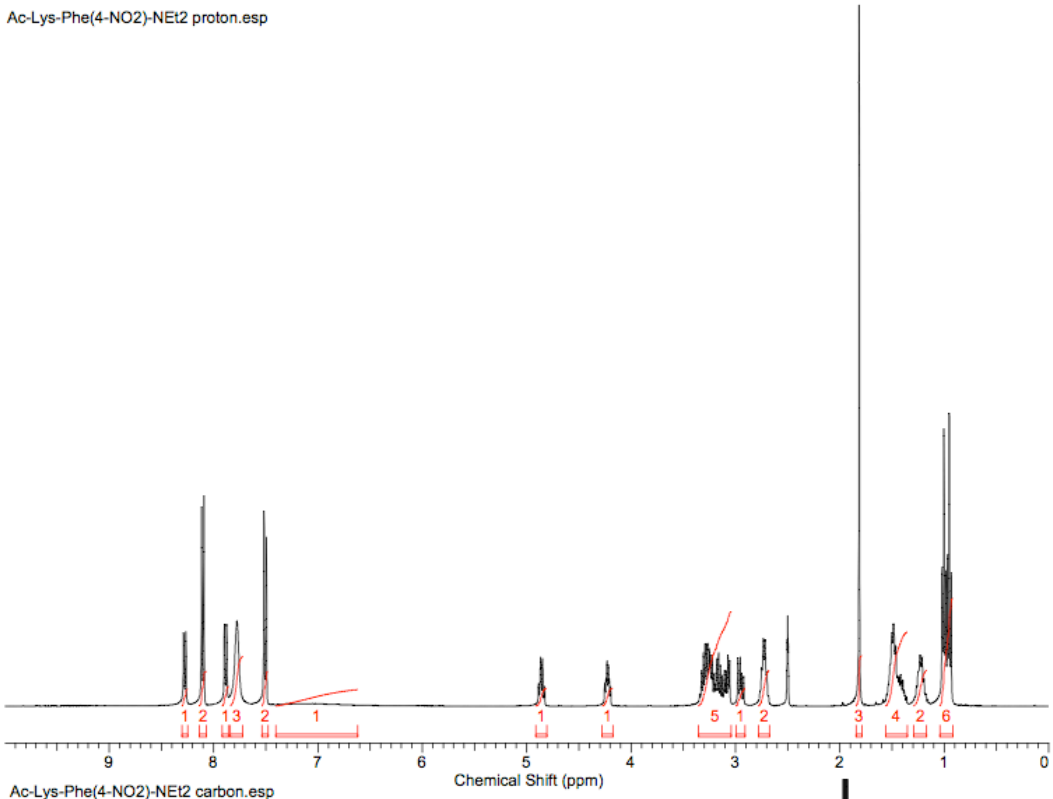
^{1}H NMR (400 MHz, DMSO-d6) δ 8.28 (d, J = 8.6 Hz, 1H), 8.10 (d, J = 8.6 Hz, 2H), 7.88 (d, J = 8.6 Hz, 1H), 7.78 (br. s., 2H), 7.51 (d, J = 8.6 Hz, 2H), 7.01 (br. s, 1H), 4.86 (ddd, J = 6.3, 8.1, 8.6 Hz, 1H), 4.22 (dt, J = 5.1, 8.6 Hz, 1H), 3.35 - 3.12 (m, 4H), 3.08 (dd, J = 6.3, 13.4 Hz, 1H), 2.95 (dd, J = 8.1, 13.4 Hz, 1H), 2.78 - 2.67 (m, 2H), 1.81 (s, 3H), 1.56 - 1.35 (m, 4H), 1.29 - 1.17 (m, 2H), 1.00 (t, J = 7.1 Hz, 3H), 0.95 (t, J = 7.0 Hz, 3H).

^{13}C NMR (100 MHz, DMSO-d6) δ 171.3, 169.2, 169.1, 146.3, 145.8, 130.8, 123.1, 52.0, 49.3, 41.1, 39.0, 38.7, 37.6, 31.4, 26.6, 22.5, 22.2, 14.2, 12.7.

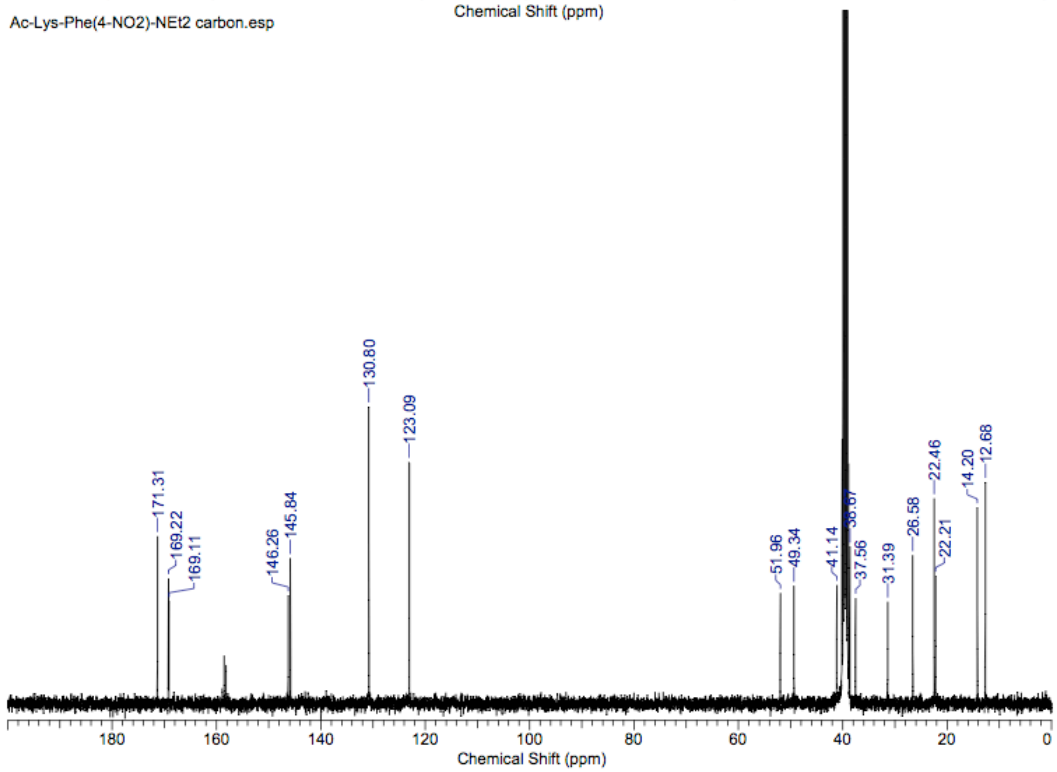
LRMS [M+H]+ 436 (100%).

HRMS ESI+ [M+H]+ calculated = 435.2482, [M+H]+ found = 436.2559.

Ac-Lys-Phe(4-NO2)-NEt2 proton.esp

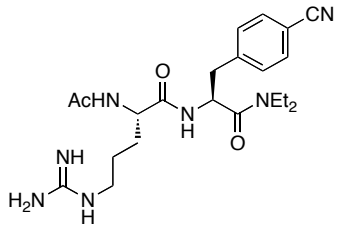


Ac-Lys-Phe(4-NO2)-NEt2 carbon.esp



Ac-Arg-Phe(4-CN)-NEt_2

The above procedure was followed at a similar concentration, with the appropriate amino acids,, with an overall yield of 22%.



Chemical Formula: $C_{22}H_{33}N_7O_3$
Exact Mass: 443.2645
Molecular Weight: 443.5425

[α]26D -31.6 (MeOH, c 0.51).

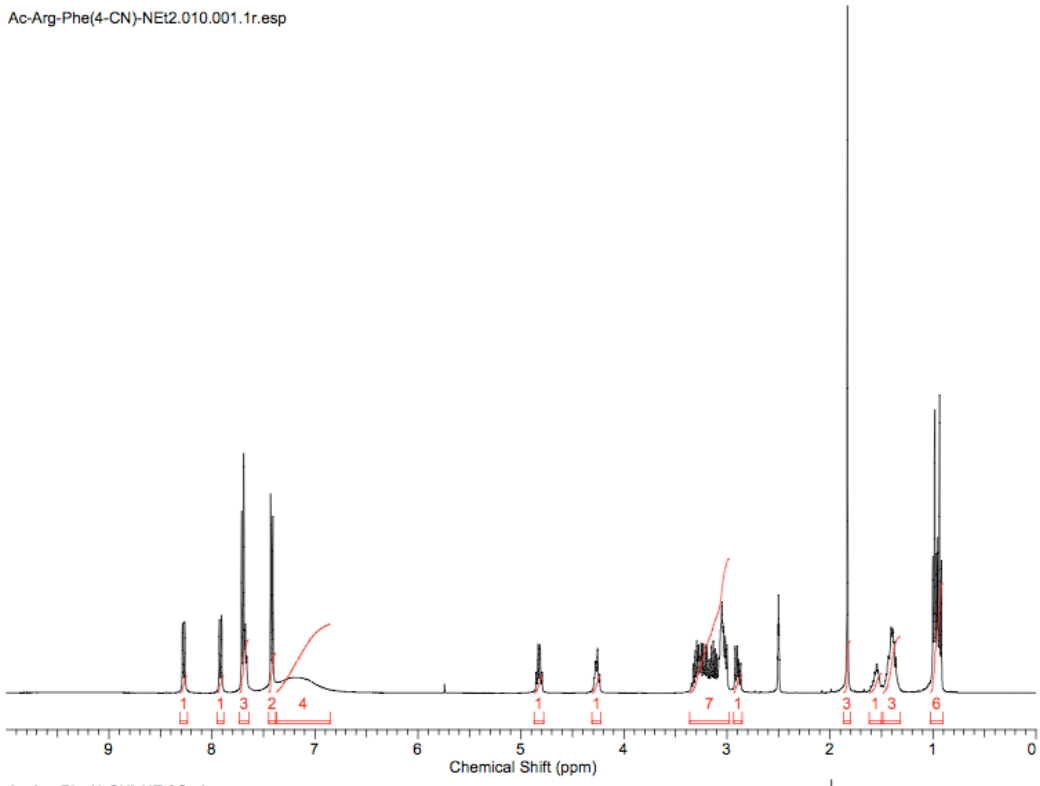
IR νmax (neat) 3278 (m), 2230 (w), 1777 (w), 1620 (s), 1538 (s) cm-1.

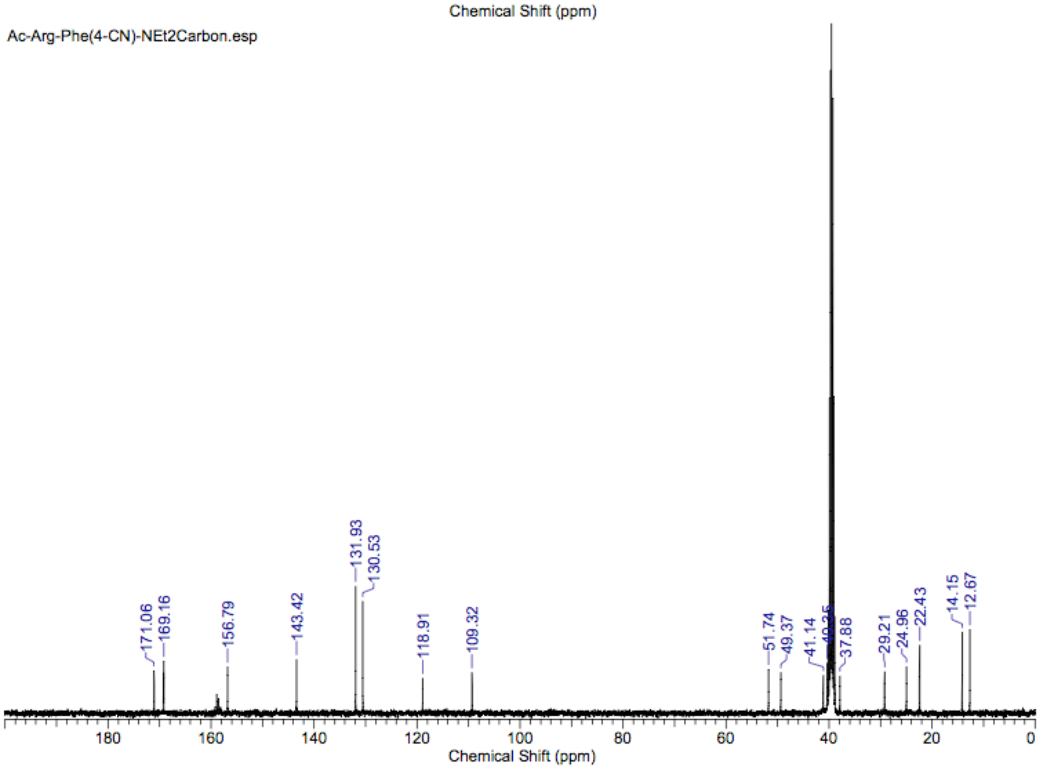
^{1}H NMR (400 MHz, DMSO-d6) δ 8.28 (d, J = 8.6 Hz, 1H), 7.92 (d, J = 8.6 Hz, 1H), 7.74 - 7.64 (m, 3H), 7.42 (d, J = 8.1 Hz, 2H), 7.23 (br. s., 3H), 4.87 - 4.77 (m, 1H), 4.31 - 4.22 (m, 1H), 3.37 - 2.98 (m, 7H), 2.89 (dd, J = 7.8, 13.4 Hz, 1H), 1.83 (s, 3H), 1.63 - 1.50 (m, 1H), 1.49 - 1.31 (m, 3H), 0.98 (t, J = 7.1 Hz, 3H), 0.94 (t, J = 7.1 Hz, 3H).

^{13}C NMR (100 MHz, DMSO-d6) δ 171.1, 169.2, 169.2, 156.8, 143.4, 131.9, 130.5, 118.9, 109.3, 51.7, 49.4, 41.1, 40.4, 39.8, 37.9, 29.2, 25.0, 22.4, 14.2, 12.7.

LRMS [M+H]+ 444 (100%).

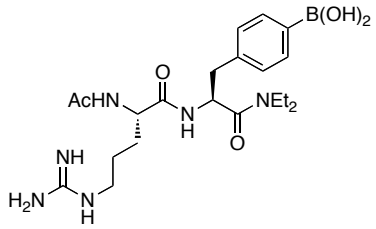
HRMS ESI+ [M+H]+ calculated = 444.2718, [M+H]+ found = 444.2723.


Ac-Arg-Phe(4-CN)-NEt2.010.001.1r.esp
1
1
3
2
4
1
1
7
1
3
1
3
6
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)


Ac-Arg-Phe(4-CN)-NEt2Carbon.esp
171.06
169.16
156.79
143.42
131.93
130.53
118.91
109.32
51.74
49.37
41.14
37.88
29.21
24.96
22.43
14.15
12.67
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

Ac-Arg-Phe(4-B(OH)$_2$)-NEt$_2$

The above procedure was repeated with the appropriate amino acids, on a similar scale, to give an overall yield of 13%.



Chemical Formula: $C_{21}H_{35}BN_6O_5$
Exact Mass: 462.2762
Molecular Weight: 462.3508

[α]26D -21.8 (MeOH, c 0.50).

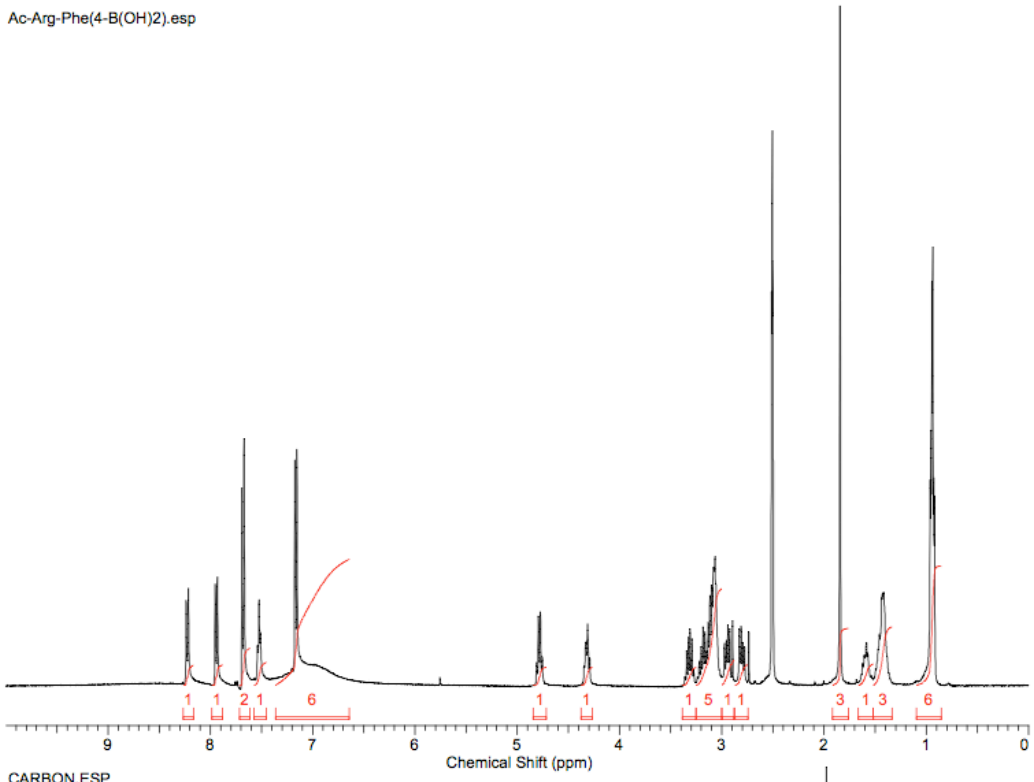
IR νmax (neat) 3289 (m), 2981 (w), 1777 (w), 1614 (s), 1539 (s) cm-1.

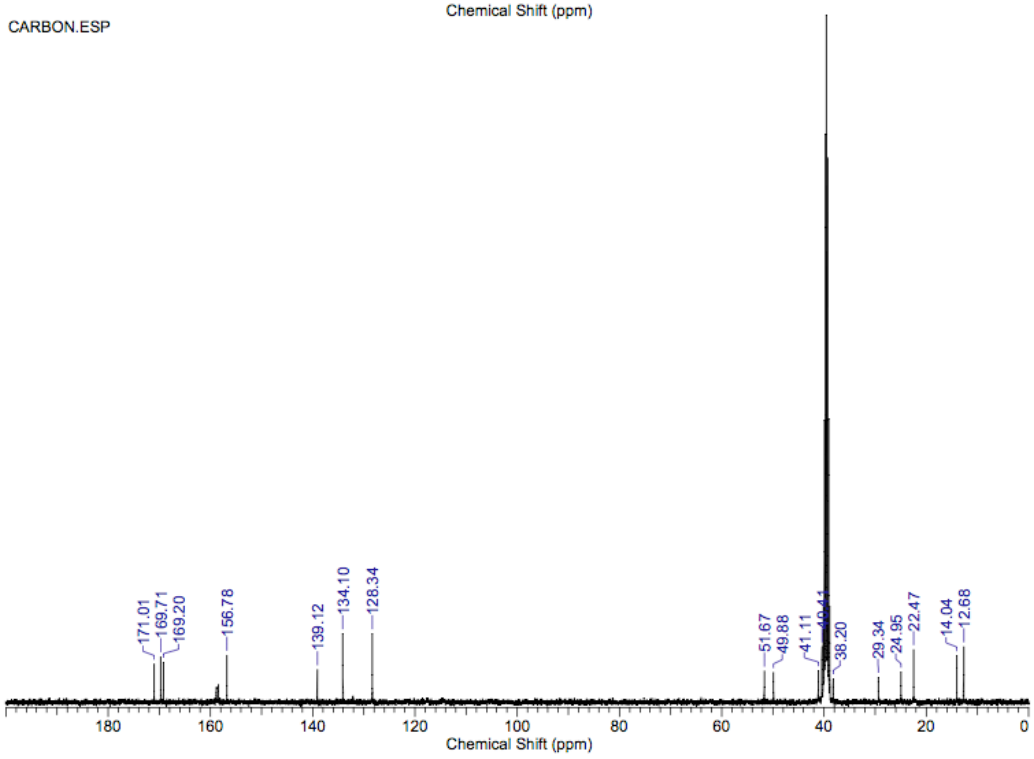
1H NMR (400 MHz, DMSO-d6) δ 8.23 (d, J = 8.1 Hz, 1H), 7.94 (d, J = 8.6 Hz, 1H), 7.68 (d, J = 8.1 Hz, 2H), 7.56 - 7.48 (m, 1H), 7.16 (d, J = 8.1 Hz, 2H), 4.78 (q, J = 7.2 Hz, 1H), 4.36 - 4.27 (m, 1H), 3.37 - 3.26 (m, ^{1}H), 3.25 - 3.01 (m, 5H), 2.95 (dd, J = 7.2, 13.2 Hz, 1H), 2.80 (dd, J = 7.2, 13.2 Hz, 1H), 1.84 (s, 3H), 1.66 - 1.52 (m, 1H), 1.52 - 1.34 (m, 3H), 0.96 - 0.90 (m, 6H).

^{13}C NMR (100 MHz, DMSO-d6) δ 171.0, 169.7, 169.2, 156.8, 139.1, 134.1, 128.3, 51.7, 49.9, 41.1, 40.4, 39.0, 38.2, 29.3, 24.9, 22.5, 14.0, 12.7.

LRMS [M+H]+ 463 (100%).

HRMS ESI+ $[M+H]^+$ calculated = 463.2835, $[M+H]^+$ found = 463.2844.


Ac-Arg-Phe(4-B(OH)2).esp
1 1 2 1 6 1 1 1 5 1 1 3 1 3 6
9 8 7 6 5 4 3 2 1 0
Chemical Shift (ppm)


CARBON.ESP
171.01
169.71
169.20
156.78
139.12
134.10
128.34
51.67
49.88
41.11
40.41
38.20
29.34
24.95
22.47
14.04
12.68
180 160 140 120 100 80 60 40 20 0
Chemical Shift (ppm)

Synthesis of N-methylated dipeptides

The above procedure was followed, on a similar scale, starting with the appropriate amino acids, with the following deviation. After the first reaction with diethylamine, the boc-protected amino acid was N-methylated as outlined below. HATU/DIPEA were used in place of PyBOP for coupling to arginine, as outlined below.

Methylation of Boc-Protected Amino acids

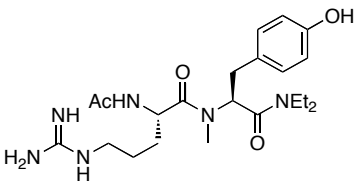
To a stirred solution of Boc-protected amino acid (1.54 mmol) dissolved in THF/DMF 20:1 (8ml) at 0°, 60% NaH mineral oil (136 mg, 3.39 mmol, 2.2 eq) was added. The reaction was stirred at 0°, and after 30 minutes, methyl iodide (0.714 ml, 7.70 mmol, 5 eq) was added. The reaction was stirred at 0° for a further 30 minutes, then at room temperature for 15 hours. The reaction was quenched with water (added slowly dropwise until no effervescence was observed). The organic layer was separated. The aqueous layer was re-extracted with EtOAc (3 x 10 ml). The combined organic phases were dried ($MgSO_4$) and concentrated in vacuo to give yellow/orange oil. Purification on SiO_2 eluting with EtOAc/hexane gave the methyl amine as a yellow oil (42% yield for tyrosine, 38% for p-nitrotyrosine).

Peptide coupling with N-Methyl amine

At room temperature, to a stirred solution of Fmoc-protected arginine (441 mg, 0.68 mmol) dissolved in CH_2Cl_2 (5 ml) was added HATU (260 mg, 0.68 mmol, 1 eq) and DIPEA (0.240 mg, 1.37 mmol, 2 eq). The N-methylated amino acid (0.68 mmol, 1 eq) in CH_2Cl_2 (5 ml) was added and the reaction was stirred for 15 hours. The reaction was diluted with 10% citric acid (10 ml). The organic layer was collected and the aqueous layer was re-extracted with CH_2Cl_2 (3 x 10 ml). The combined organic layers were washed with 10% citric acid (10 ml), saturated $NaHCO_3$ (10 ml) and brine (10 ml). The organic layer was dried ($MgSO_4$) and concentrated in vacuo to give a yellow oil (crude yield ~70%) that was used directly in the next step.

Ac-Arg-NMe-Tyr-NEt_2

The above procedure for the synthesis of N-methylated dipeptides was followed. The overall yield for the synthesis was 11%.



Chemical Formula: $C_{22}H_{36}N_6O_4$
Exact Mass: 448.2798
Molecular Weight: 448.5590

[α]26D -139.6 (MeOH, c 0.6).

IR νmax (neat) 3273 (m), 3183 (m), 2978 (m), 1614 (s), 1515 (s) cm-1.

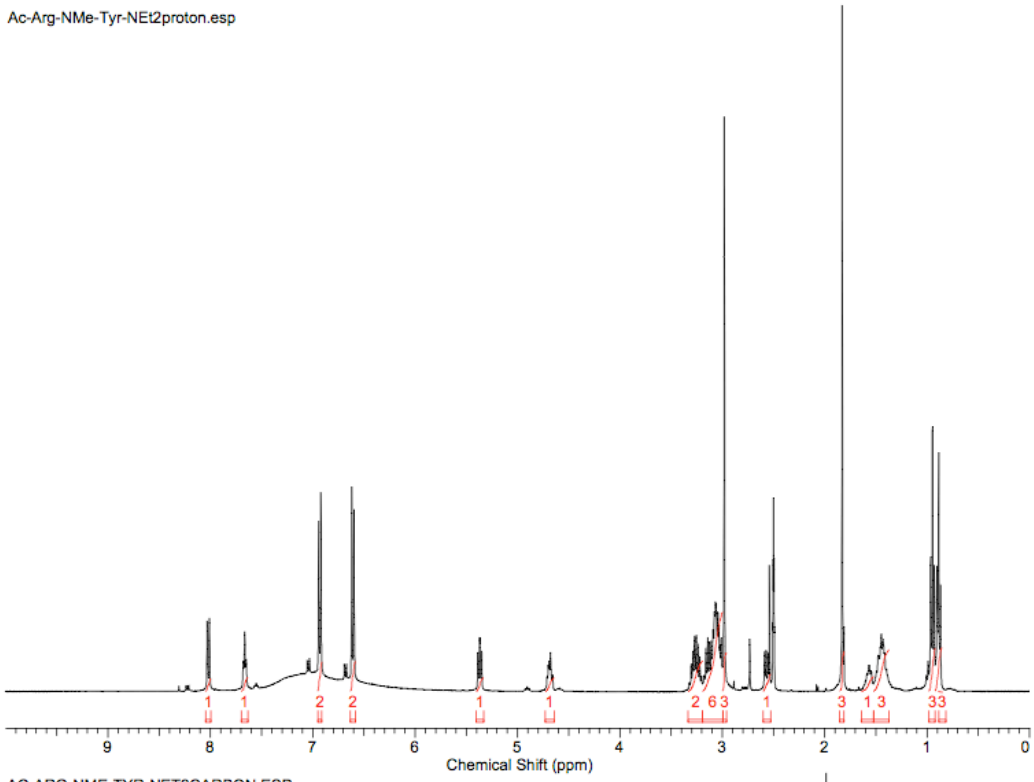
^{1}H NMR (400 MHz, DMSO-d6) δ 8.02 (d, J = 8.6 Hz, 1H), 7.67 (t, J = 5.6 Hz, 1H), 6.93 (d, J = 8.6 Hz, 2H), 6.61 (d, J = 8.6 Hz, 2H), 5.37 (dd, J = 6.5, 8.1 Hz, 1H), 4.72 - 4.65 (m, 1H), 3.33 - 3.19 (m, 2H), 3.19 - 3.00 (m, 6H), 2.98 (s, 3H), 2.56 (dd, J = 6.5, 13.6 Hz, 1H), 1.83 (s, 3H), 1.63 - 1.52 (m, 1H), 1.52 - 1.35 (m, 3H), 0.95 (t, J = 6.8 Hz, 3H), 0.89 (t, J = 6.8 Hz, 3H).

^{13}C NMR (100 MHz, DMSO-d6) δ 171.3, 169.0, 168.1, 156.7, 155.7, 130.0, 127.6, 115.0, 53.7, 48.1, 40.7, 40.4, 39.6, 34.0, 30.2, 28.7, 24.8, 22.3, 14.2, 12.7.

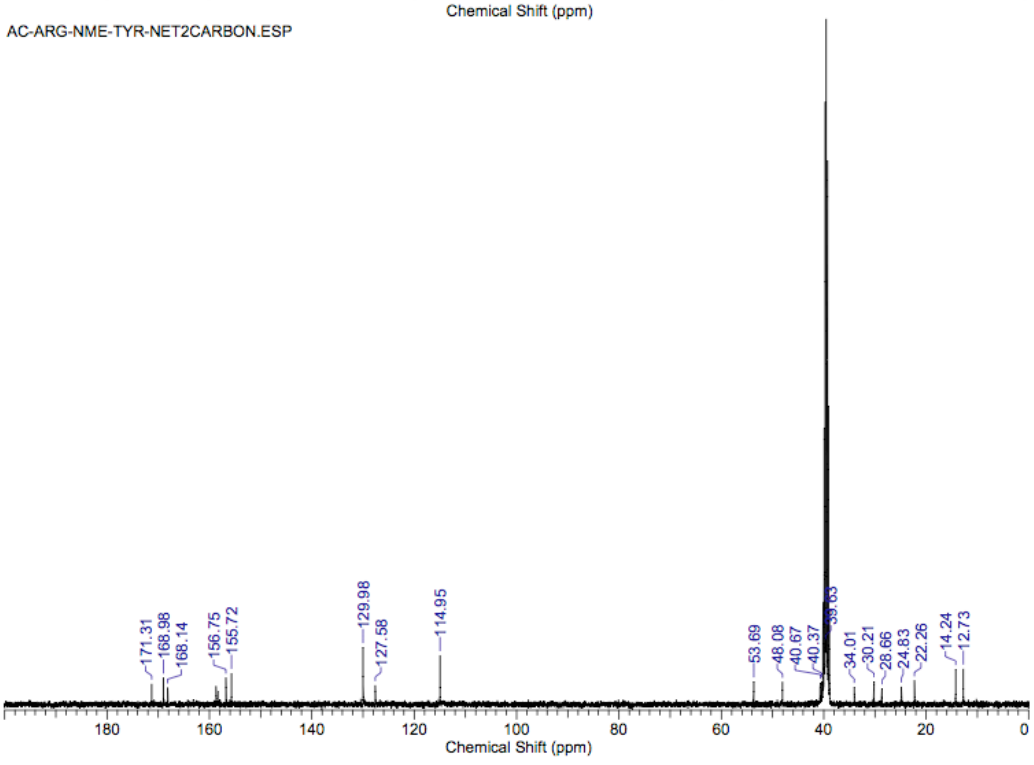
LRMS [M+H]+ 449 (100%).

HRMS ESI+ [M+H]+ calculated = 449.2871, [M+H]+ found = 449.2866.

Ac-Arg-NMe-Tyr-NEt2proton.esp

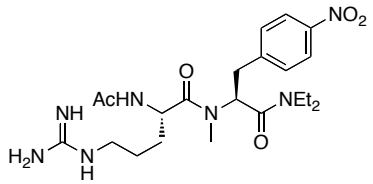


AC-ARG-NME-TYR-NET2CARBON.ESP



Ac-Arg-NMe-Phe(4-NO_2)-NEt_2

The above procedure for the synthesis of N-methylated dipeptides was followed. The overall yield for the synthesis was 15%.



Chemical Formula: $C_{22}H_{35}N_7O_5$
Exact Mass: 477.2700
Molecular Weight: 477.5572

[α]26D -206.6 (MeOH, c 0.51).

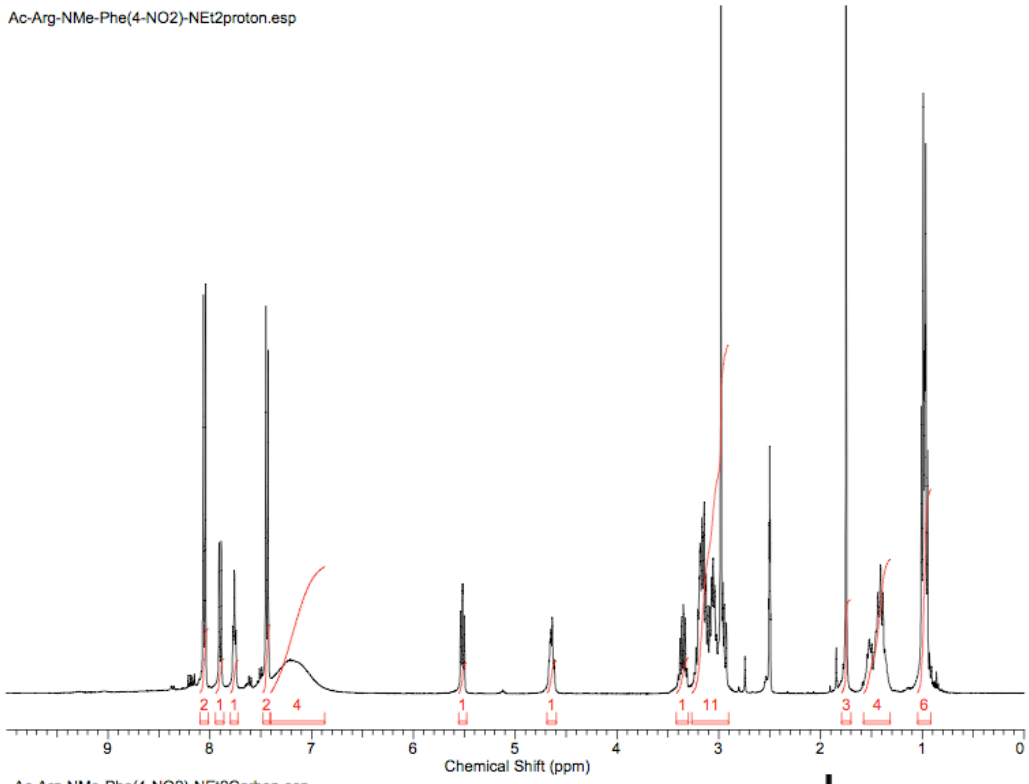
IR νmax (neat) 3294 (m), 3190 (w), 1776 (w), 1620 (s), 1518 (s) cm-1.

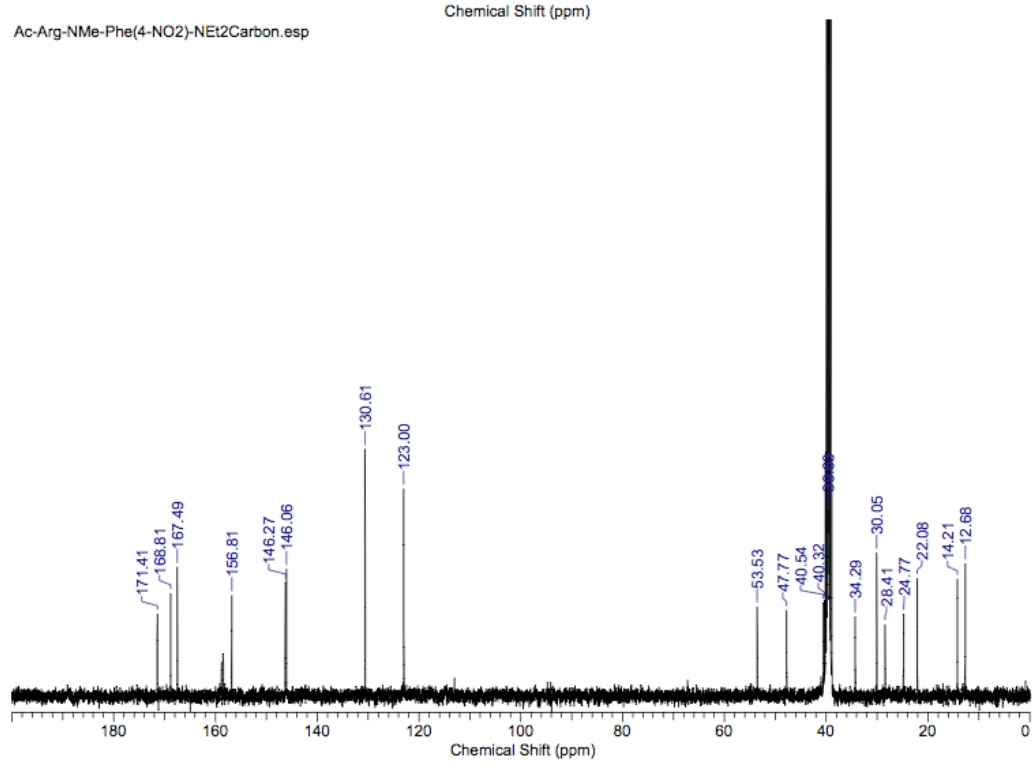
^{1}H NMR (400 MHz, DMSO-d6) δ 8.05 (d, J = 8.6 Hz, 2H), 7.90 (d, J = 8.6 Hz, 1H), 7.76 (t, J = 5.6 Hz, 1H), 7.44 (d, J = 8.6 Hz, 2H), 7.19 (br. s., 3H), 5.56 - 5.46 (m, 1H), 4.69 - 4.60 (m, 1H), 3.36 (qd, J = 6.9, 13.7 Hz, 1H), 3.26 - 2.90 (m, 10H), 1.75 (s, 3H), 1.58 - 1.31 (m, 4H), 0.99 (t, J = 7.1 Hz, 3H), 0.98 (t, J = 7.1 Hz, 3H).

^{13}C NMR (100 MHz, DMSO-d6) δ 171.4, 168.8, 167.5, 156.8, 146.3, 146.1, 130.6, 123.0, 53.5, 47.8, 40.5, 40.3, 39.6, 34.3, 30.1, 28.4, 24.8, 22.1, 14.2, 12.7.

LRMS [M+H]+ 478 (100%).

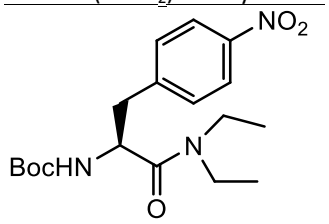
HRMS ESI+ [M+H]+ calculated = 478.2772, [M+H]+ found = 478.2768.


Ac-Arg-NMe-Phe(4-NO2)-NEt2proton.esp
2 1 1 2 4
1 1
1 11
3 4 6
9 8 7 6 5 4 3 2 1 0
Chemical Shift (ppm)

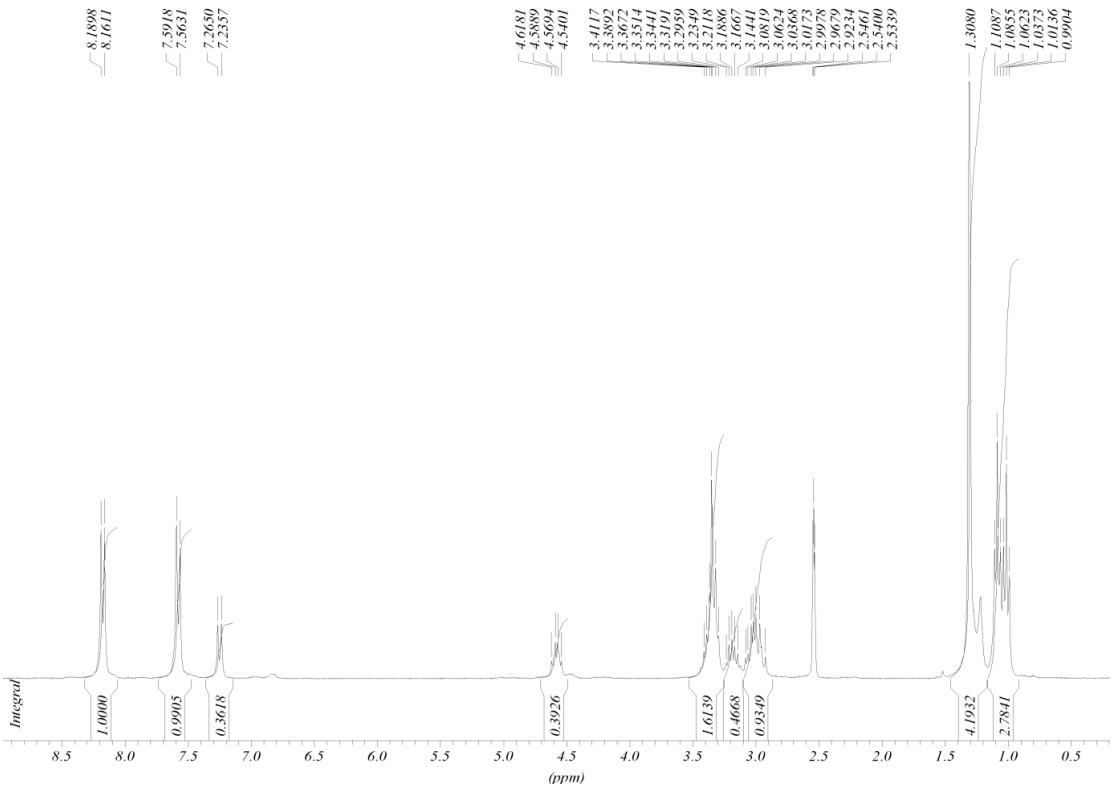

Ac-Arg-NMe-Phe(4-NO2)-NEt2Carbon.esp
171.41
168.81
167.49
156.81
146.27
146.06
130.61
123.00
53.53
47.77
40.54
40.32
39.68
34.29
30.05
28.41
24.77
22.08
14.21
12.68
180 160 140 120 100 80 60 40 20 0
Chemical Shift (ppm)

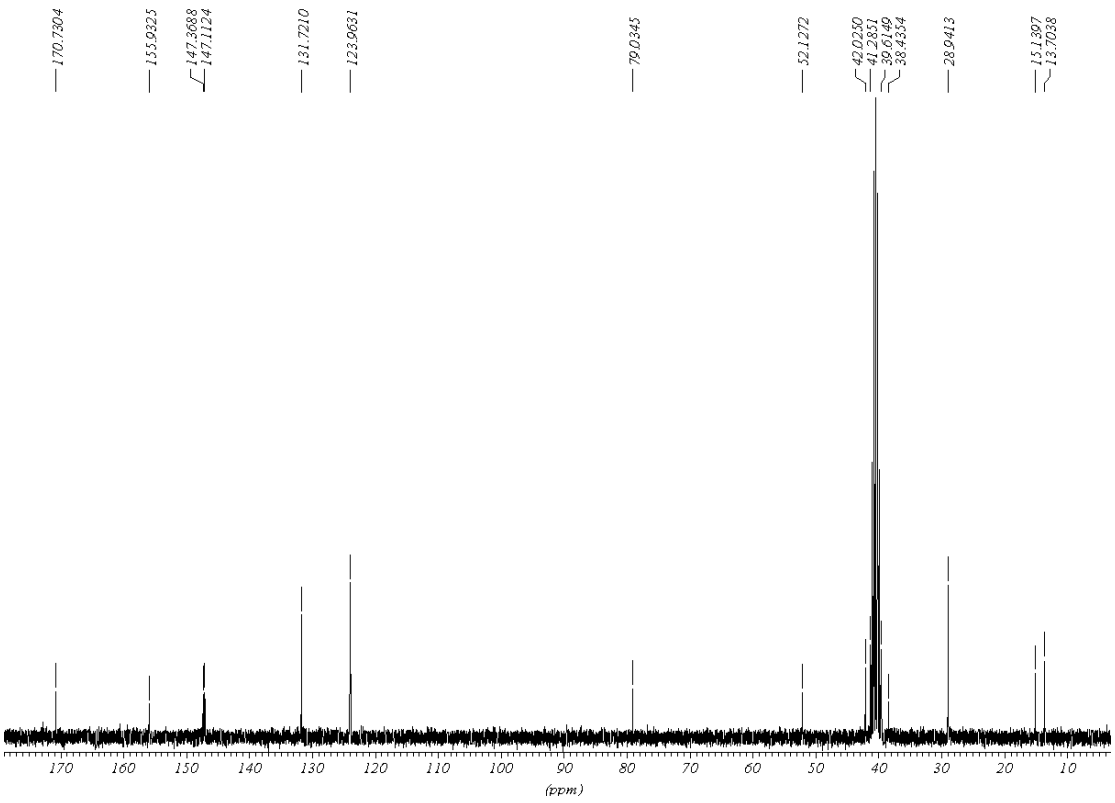
Spectra for the intermediates in the synthesis of compound 14

Boc-Phe(4-NO_2)-diethylamide

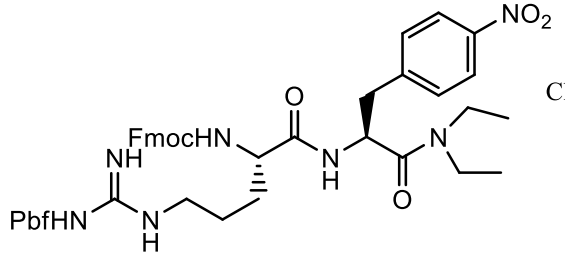


Chemical Formula: $C_{18}H_{27}N_3O_5$
Exact Mass: 365.20
Molecular Weight: 365.42

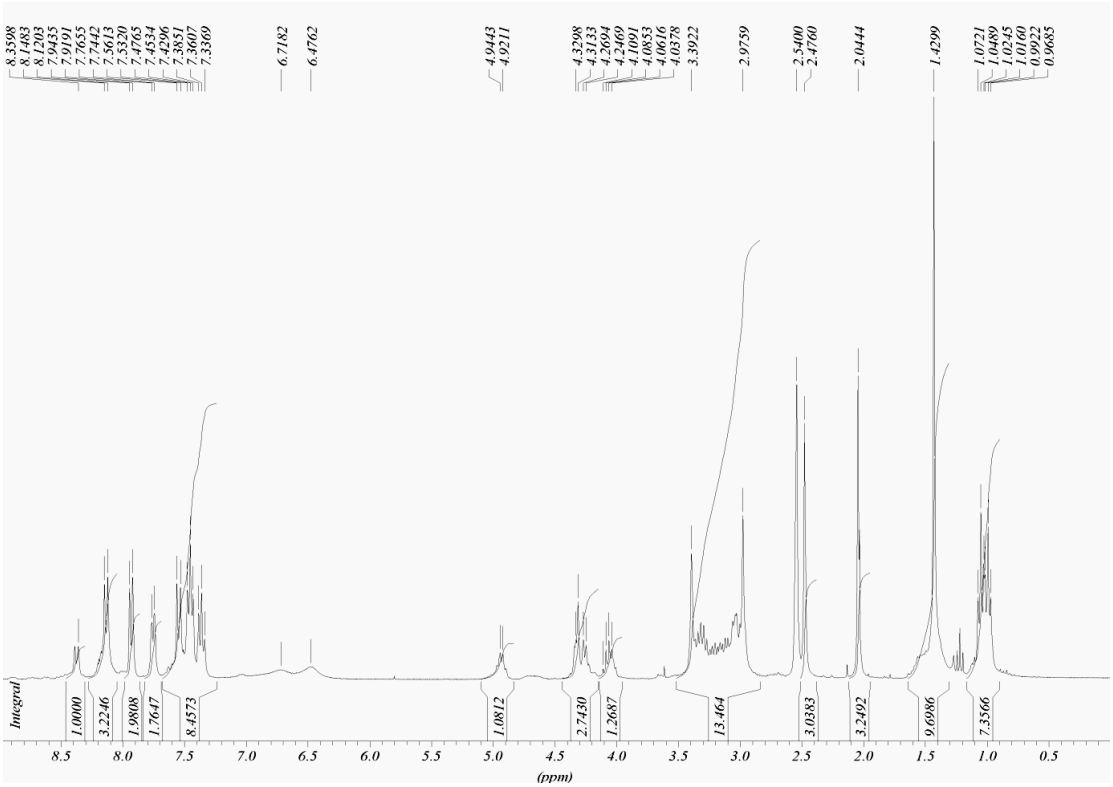


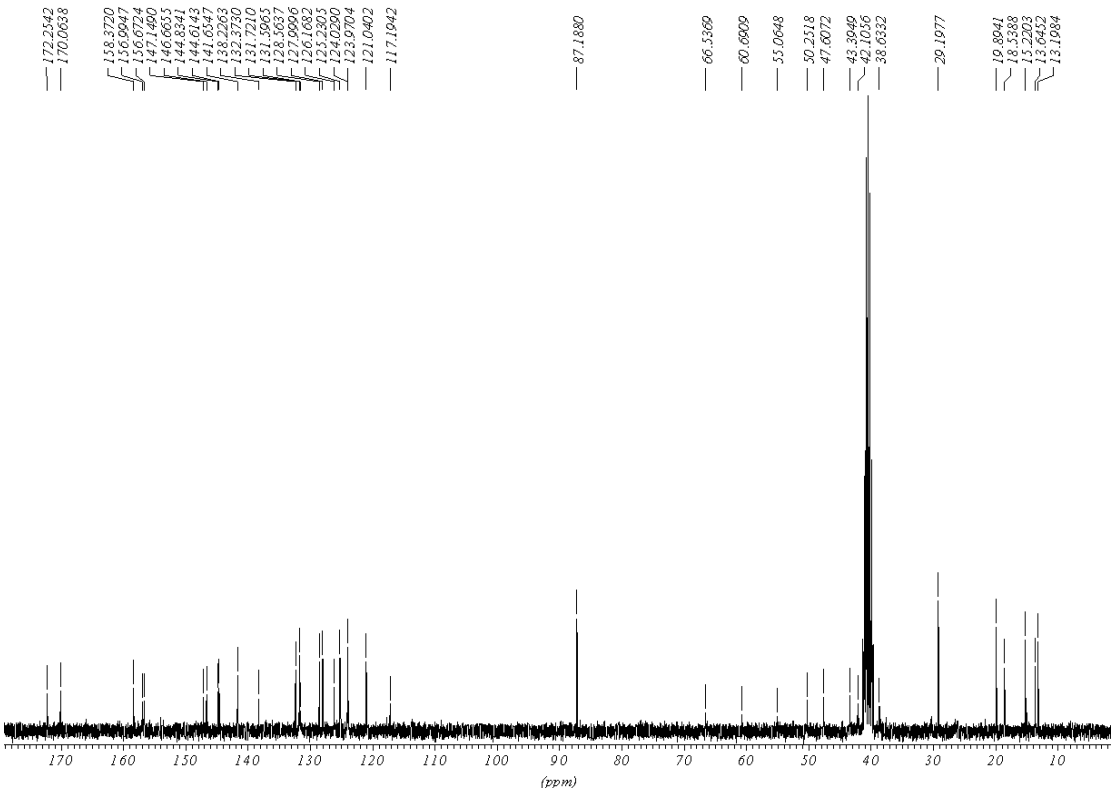

170.7304
155.9325
147.3688
147.1124
131.7210
123.9631
79.0345
52.1272
42.0250
41.2851
39.6149
38.4534
28.9413
15.1397
13.7038
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

fmoc-Arg(Pbf)-CONH-Phe(4-NO_2)-L-Diethylamide

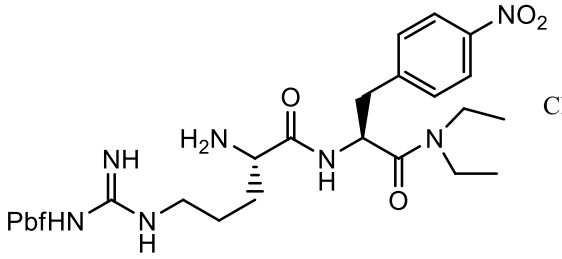


Chemical Formula: $C_{47}H_{57}N_7O_9S$
Exact Mass: 895.39
Molecular Weight: 896.06

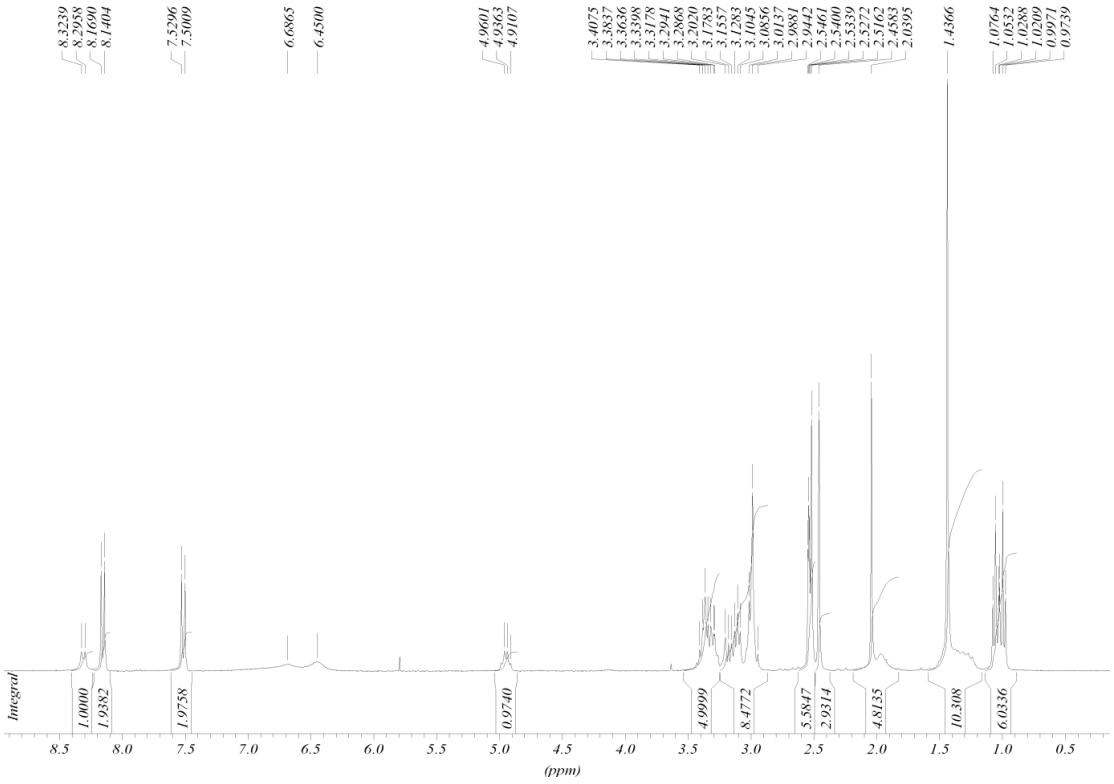


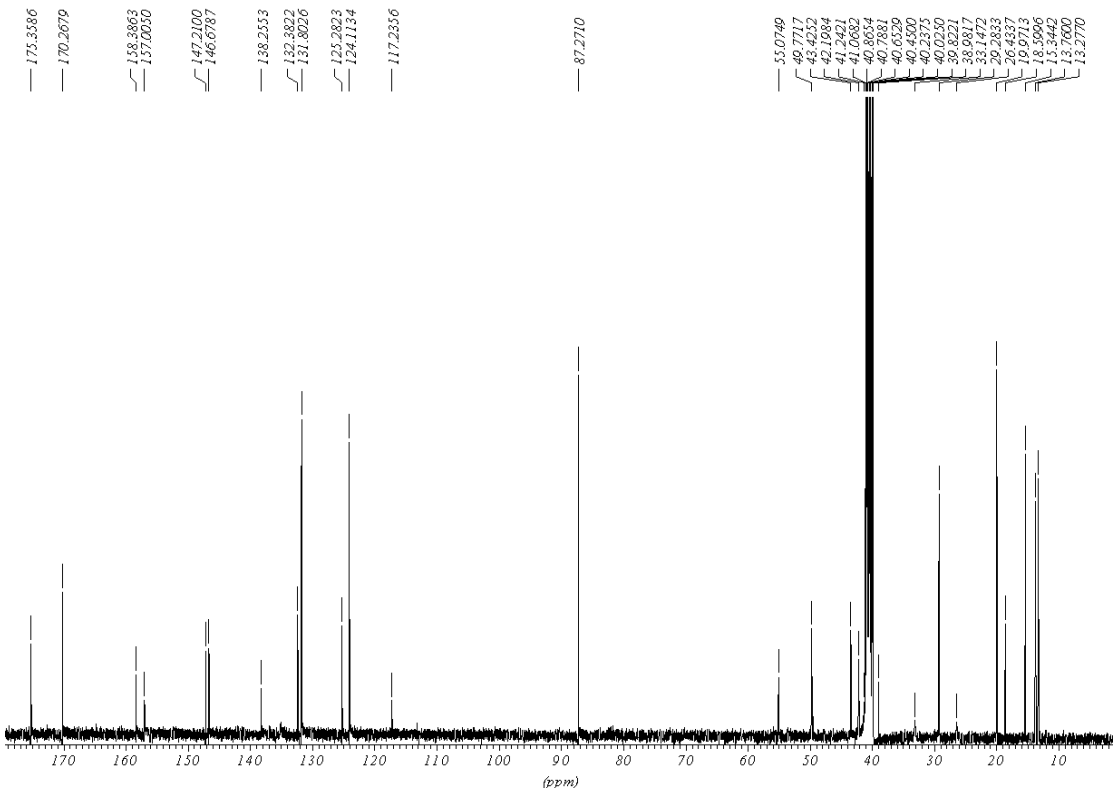

172.2542
170.0638
158.3720
156.9947
156.6724
147.1490
146.6655
144.8341
144.6143
141.6547
138.2263
132.3730
131.7210
131.5965
128.5637
127.9996
126.1682
125.2305
124.0290
123.9704
121.0402
117.1942
87.1880
66.5369
60.6909
55.0648
50.2518
47.6072
43.3949
42.1056
38.6332
29.1977
19.8941
18.3388
15.2203
13.6452
13.1984
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

Amino-Arg(Pbf)-CONH-Phe(4-NO_2)-L-Diethylamide

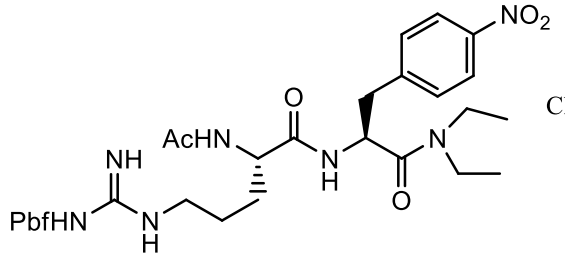


Chemical Formula: $C_{32}H_{47}N_7O_7S$
Exact Mass: 673.33
Molecular Weight: 673.82

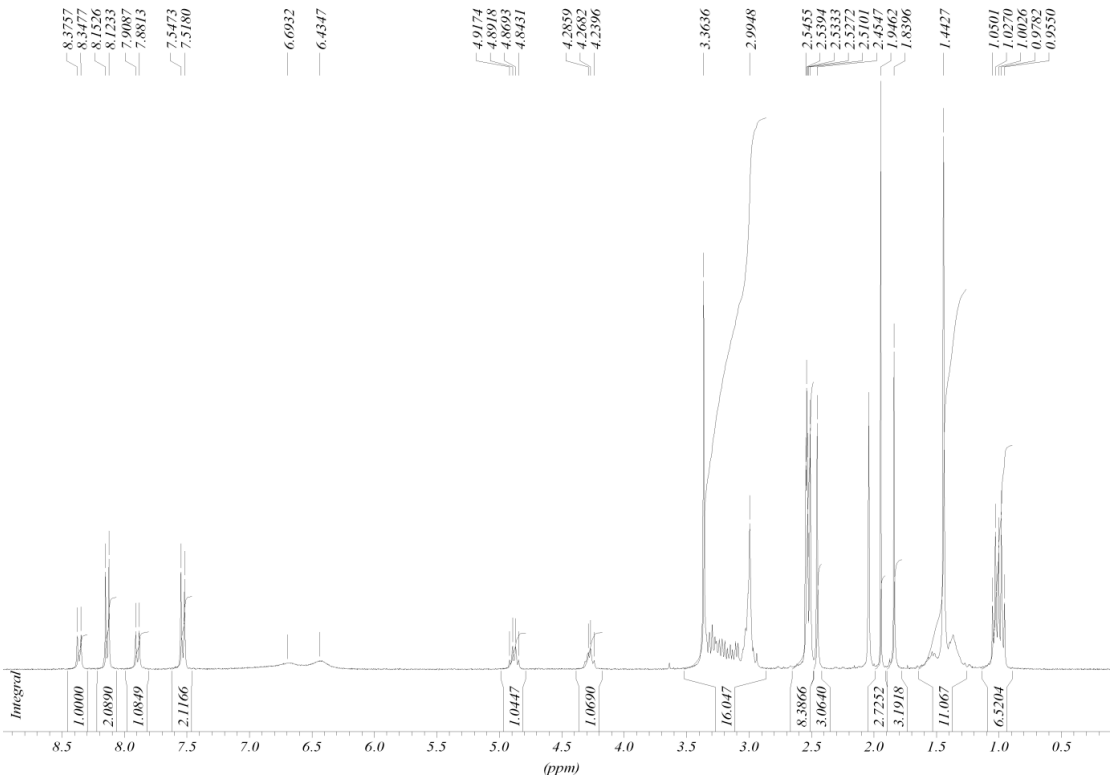


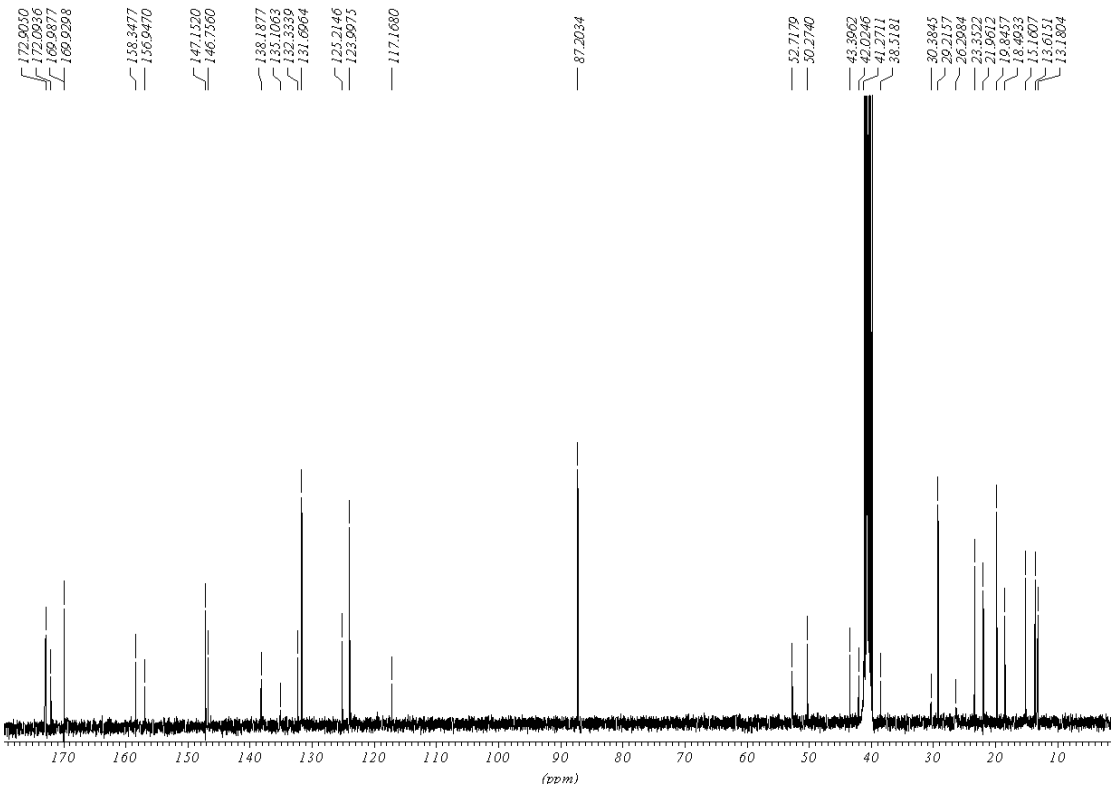

175.3586
170.2679
158.3863
157.0050
147.2100
146.6787
138.2553
132.3822
131.8026
125.2823
124.1134
117.2356
87.2710
55.0749
49.7717
43.4252
42.1984
41.1421
41.0682
40.8654
40.7881
40.6529
40.4500
40.2375
40.0250
39.8221
38.0817
33.1472
29.2833
26.4437
20.9713
18.5906
13.3442
13.1760
13.2770
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

Acetyl-Arg(Pbf)-CONH-Phe(4-NO_2)-L-Diethylamide



Chemical Formula: $C_{34}H_{49}N_7O_8S$
Exact Mass: 715.34
Molecular Weight: 715.86




172.9050
172.0936
169.9877
169.9298
158.3477
156.9470
147.1520
146.7560
138.1877
135.1063
132.3339
131.6964
125.2146
123.9975
117.1680
87.2034
52.7179
50.2740
43.3962
42.0246
41.2711
38.5181
30.3845
29.2157
26.2984
23.3522
21.9612
19.8457
18.4933
15.1607
13.6151
13.1804
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

Protein expression and enzyme kinetics

Avian and human ATIC fused to an *N*-terminal 6X histidine tag were expressed from pET28 vectors in BL21(DE3) strains of *E. coli*, and purified by standard protocols. The proteins were purified on an AKTAprime FPLC (GE Healthcare) by nickel affinity. AICAR Tfase assays were conducted with avian ATIC and carried out in 1 cm path length quartz cuvettes at 25 °C as previously described in "A. Tavassoli, S. J. Benkovic, *Angew Chem Int Ed Engl* (2005), *44*, 2760-2763". Each inhibitor was dissolved in DMSO to give a 100 mM stock solution that was further diluted for use in each assay. Each data point was repeated in triplicate. The data was analyzed using KaleidaGraph (Synergy Software) and Microsoft Excel, by assuming competitive inhibition with 10-f-thf as previously described in "A. Tavassoli, S. J. Benkovic, *Angew Chem Int Ed Engl* (2005), *44*, 2760-2763". Errors in the K_i were calculated by linear regretion. Compounds 1, 8, and 14 were also assayed with human ATIC, with the same K_i (within error) being observed for each compound as for the avian enzyme.

Spectral data

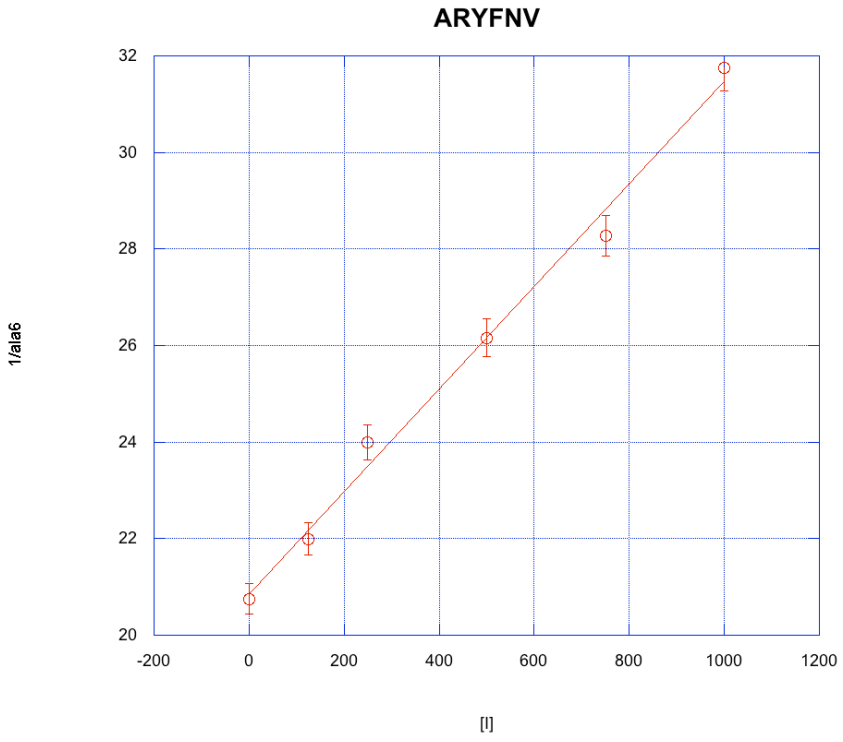


Figure 1. ARYFNV

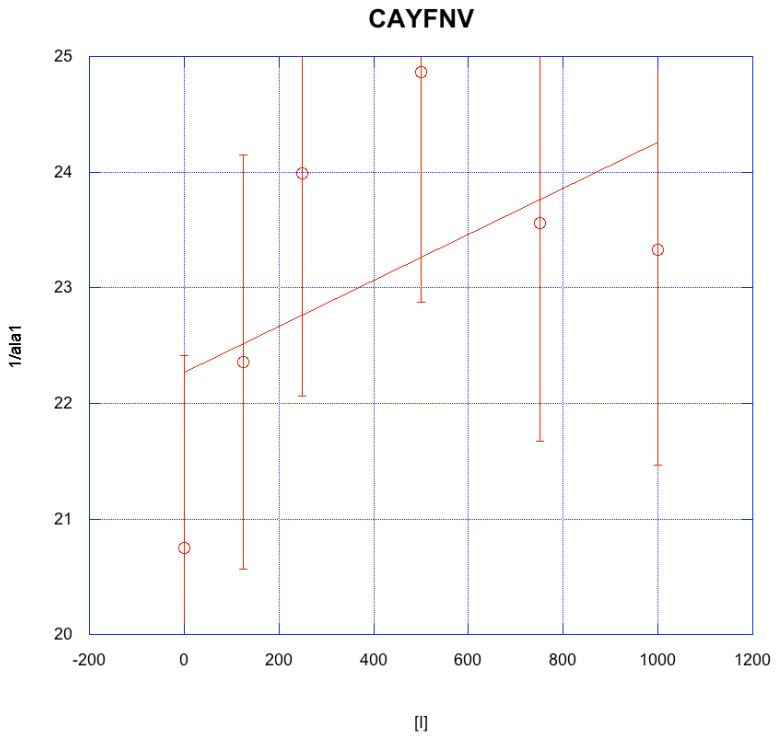


Figure 2. CAYFNV

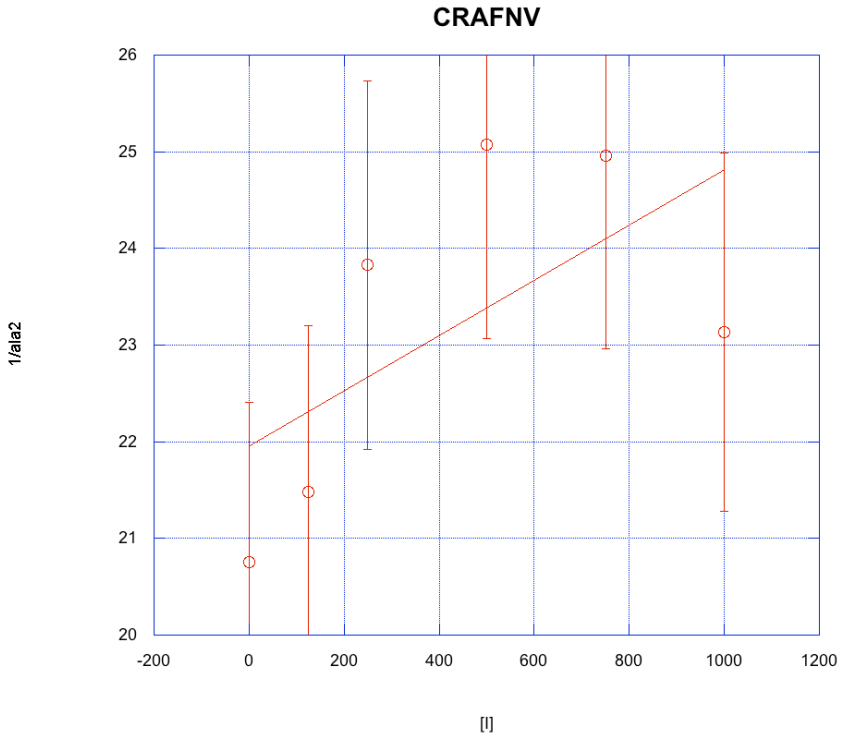


Figure 3. CRAFNV

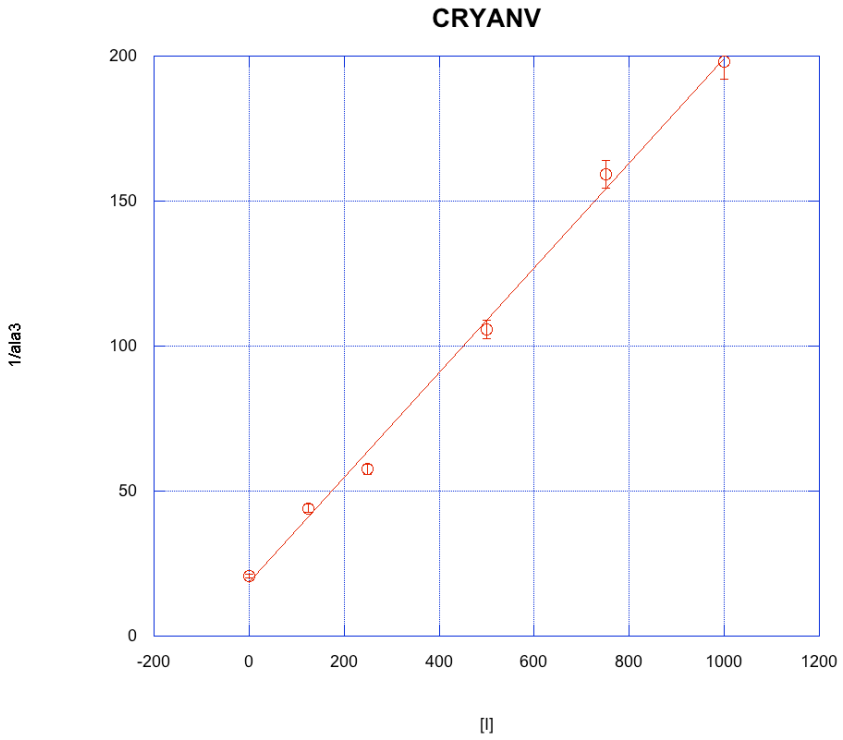


Figure 4. CRYANV

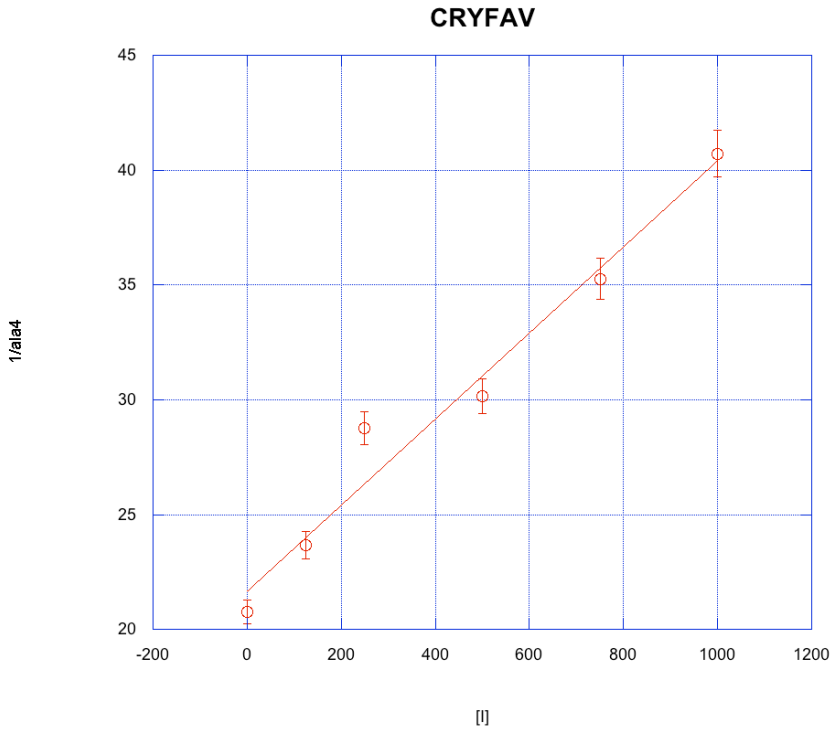


Figure 5. CRYFAV

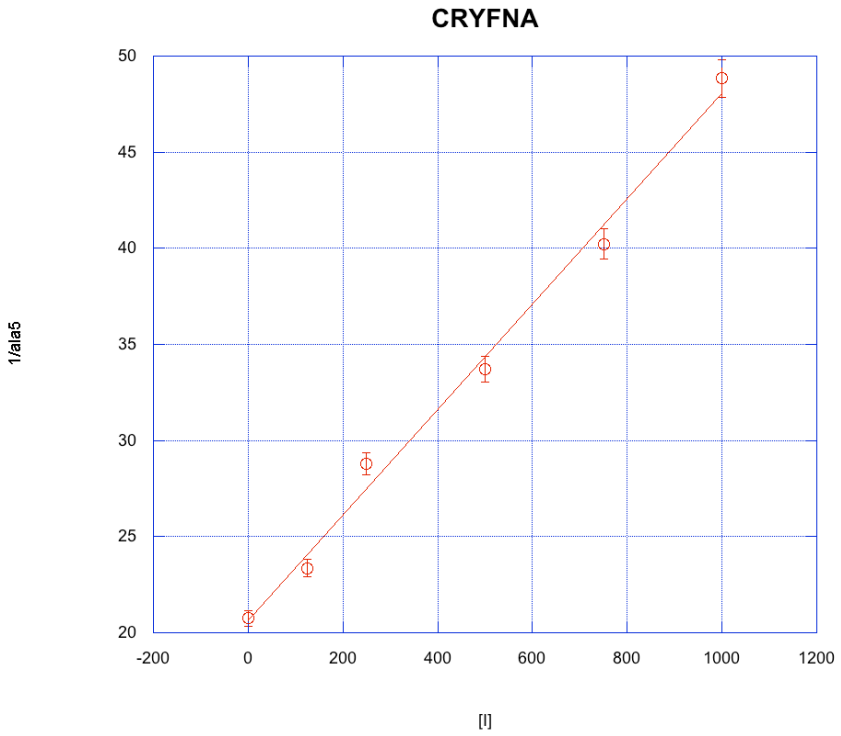


Figure 6. CRYFNA

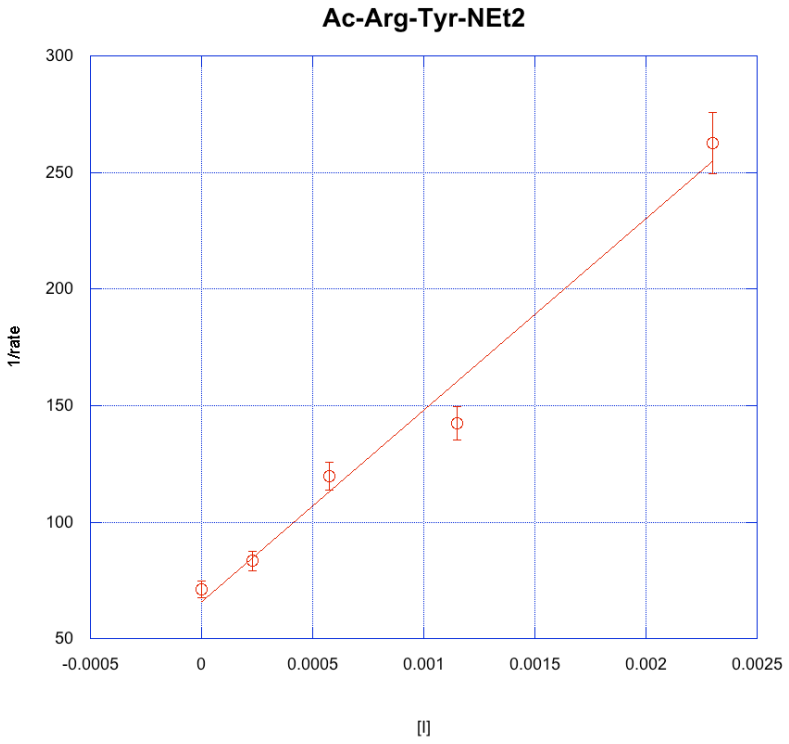


Figure 7. Ac-Arg-Tyr-NEt_2

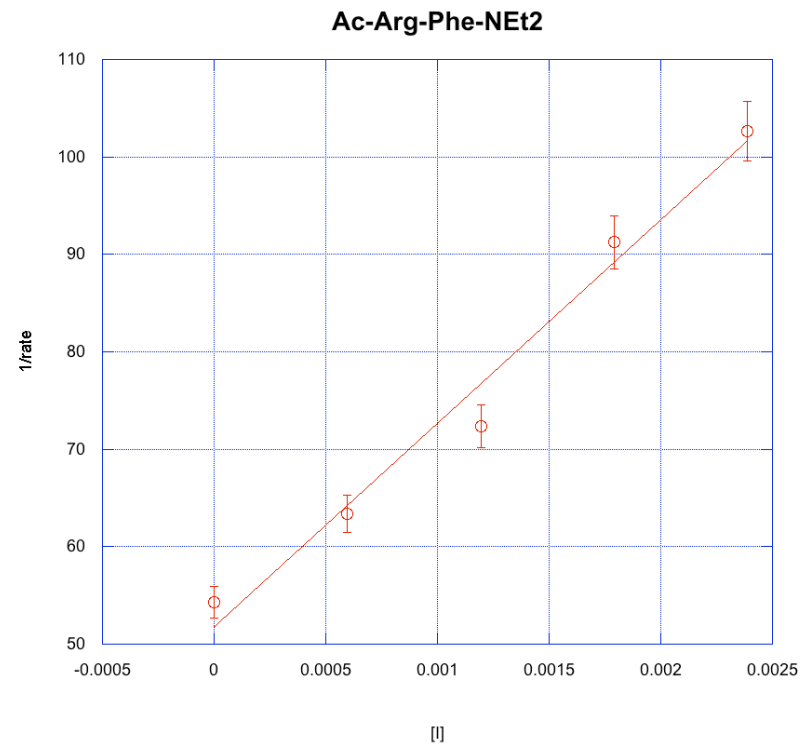


Figure 8. Ac-Arg-Phe-NEt_2

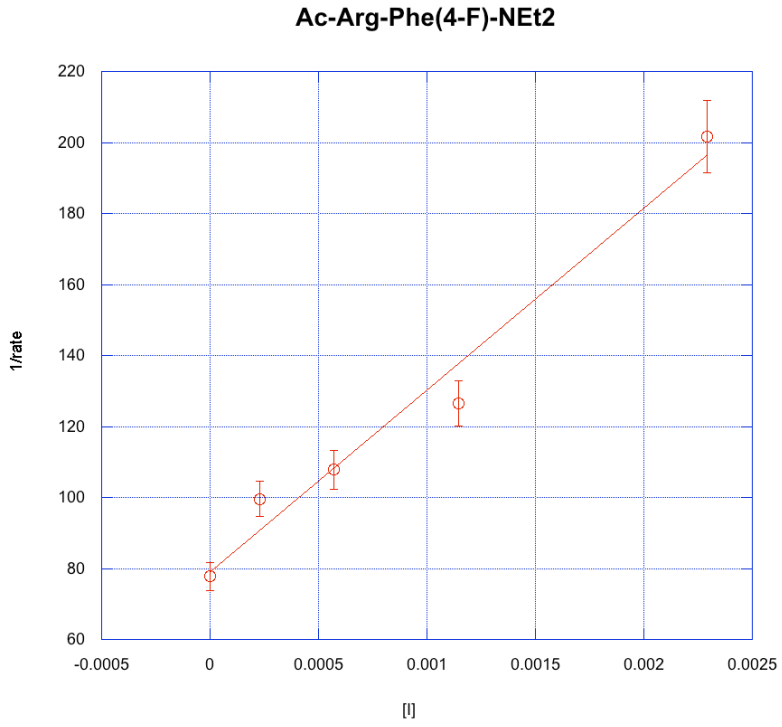


Figure 9. Ac-Arg-Phe(4-F)-NEt_2

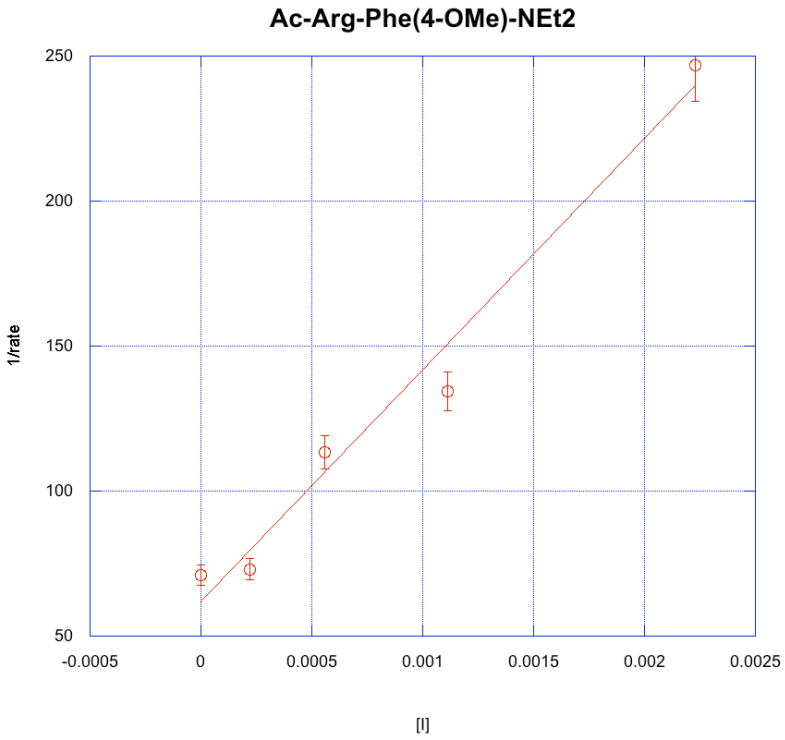


Figure 10. Ac-Arg-Phe (4-OMe)-NEt_2

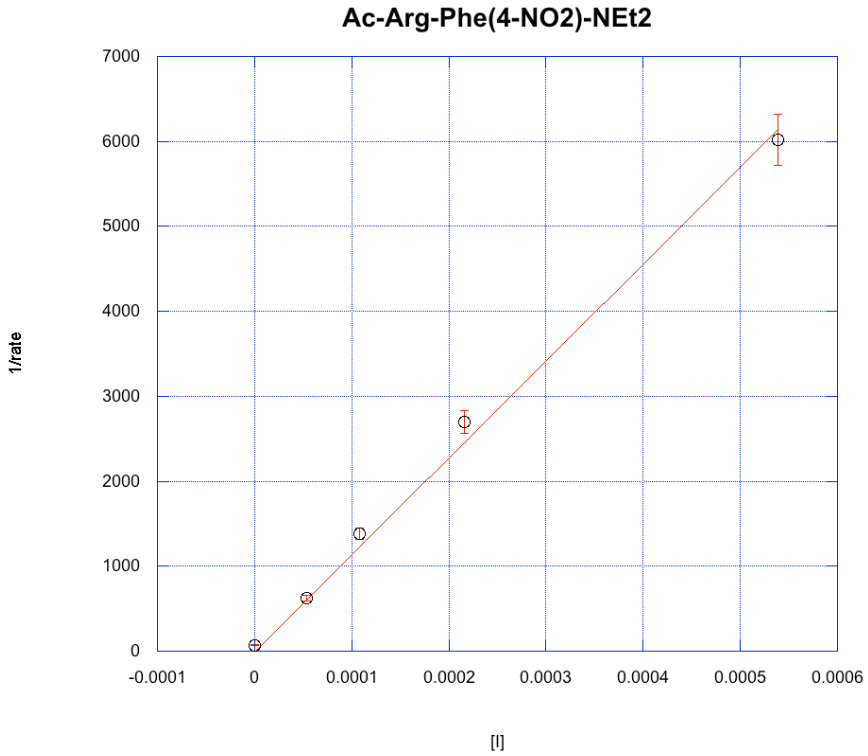


Figure 11. Ac-Arg-Phe(4-NO_2)-NEt_2

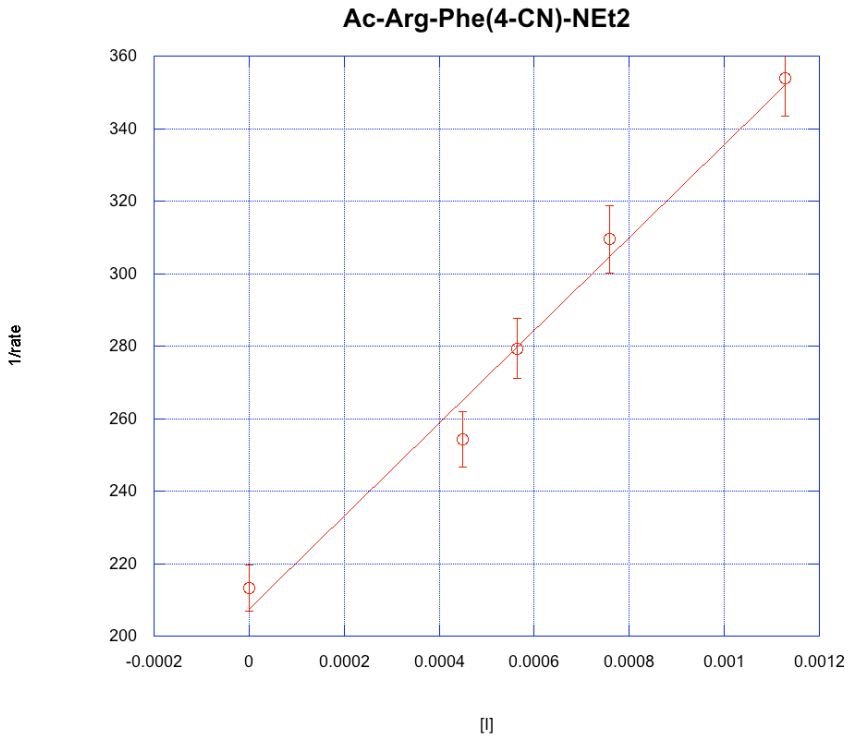


Figure 12. Ac-Arg-Phe(4-CN)-NEt_2

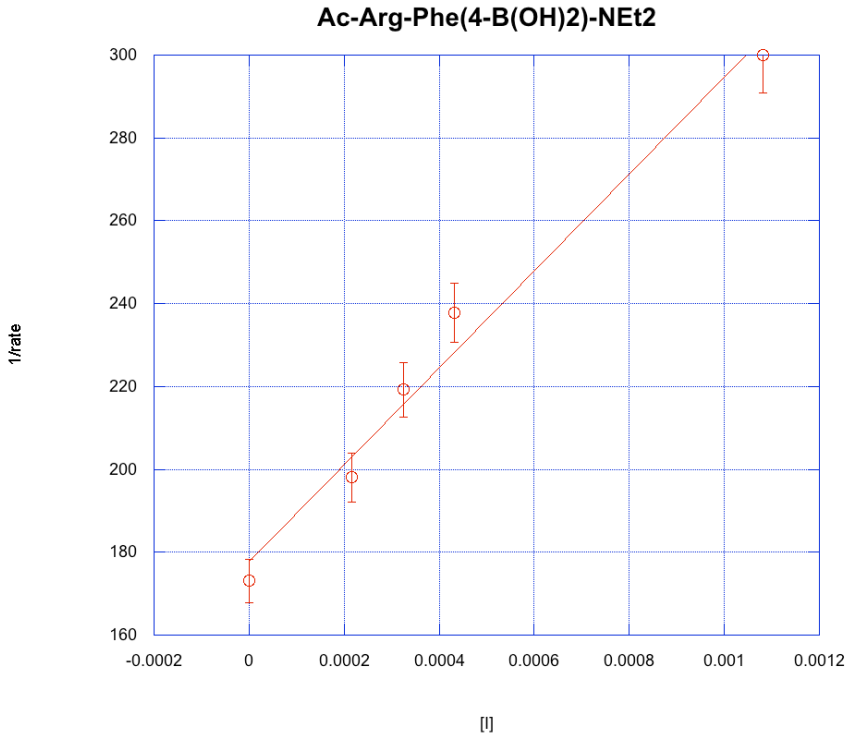


Figure 13. Ac-Arg-Phe(4-$B(OH)_2$)-NEt_2

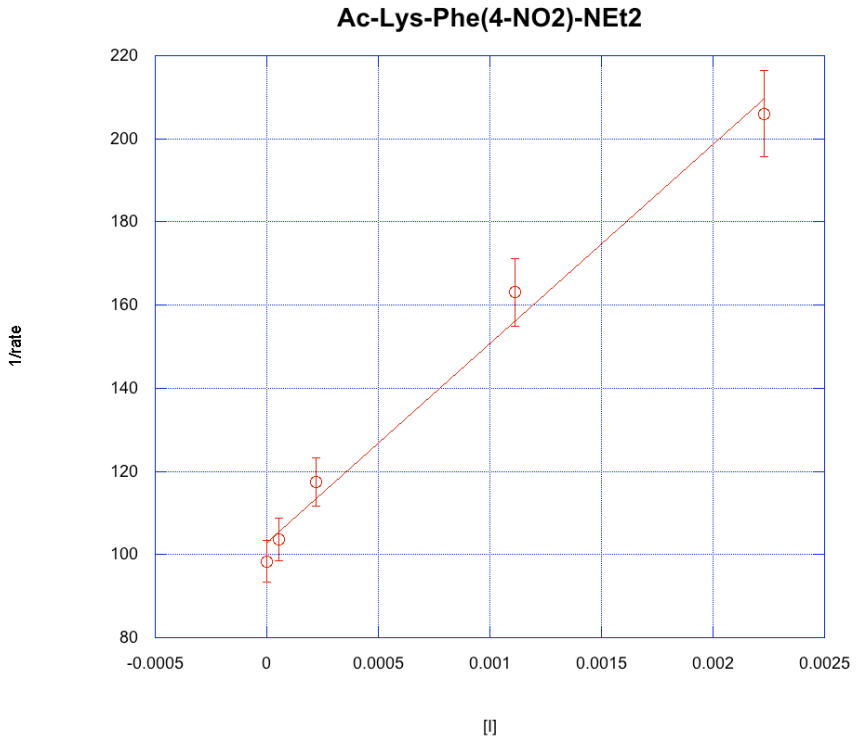


Figure 14. Ac-Lys-Phe(4-NO_2)-NEt_2

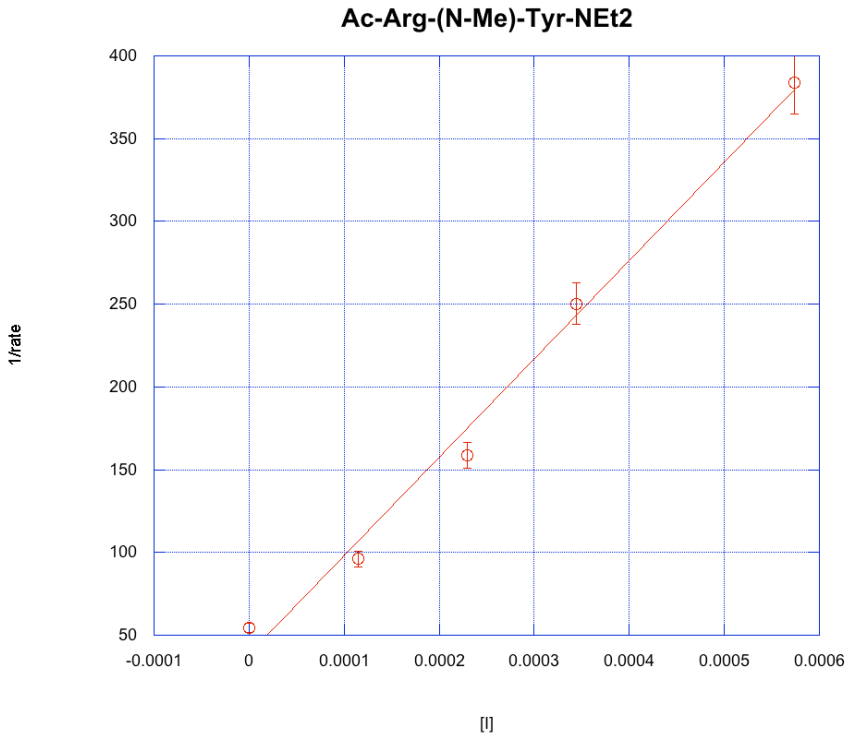


Figure 15. Ac-Arg-(N-Me)-Tyr-NEt_2

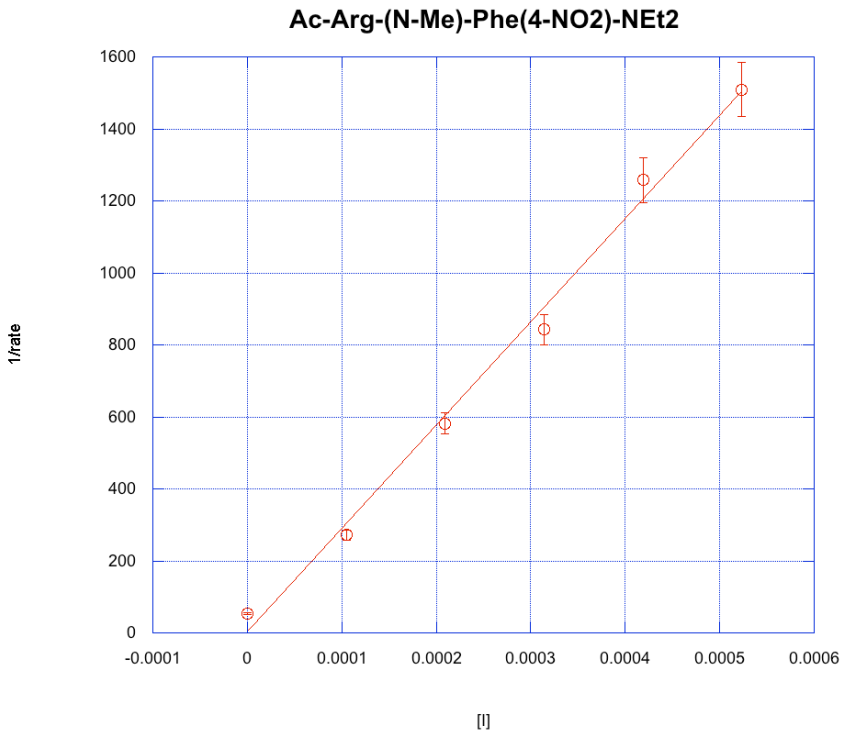


Figure 16. Ac-Arg-(N-Me)-Phe(4-NO_2)-NEt_2

Size exclusion chromatography

Human ATIC was prepared as detailed above, and divided into 100 nM solutions in 1 mL of buffer (20 mM Tris-Cl, pH 7.5, 150 mM NaCl, 50 mM KCl, 5 mM EDTA, 5 mM dithiothreitol). Inhibitor was added to each solution to give mixtures containing 0, 1, and 10µM of compound 14. Each solution was filtered through a Millex GP 0.22 µm filter (Millipore), and loaded onto a HiLoad 16/60 Superdex 200 gel filtration column (GE Healthcare) pre-equilibrated with the above buffer. The flow rate was 1 mL/min and the flow monitored by UV on an AKTAprime FPLC (GE Healthcare). The column was calibrated using a HMW gel filtration calibration kit (GE Healthcare).

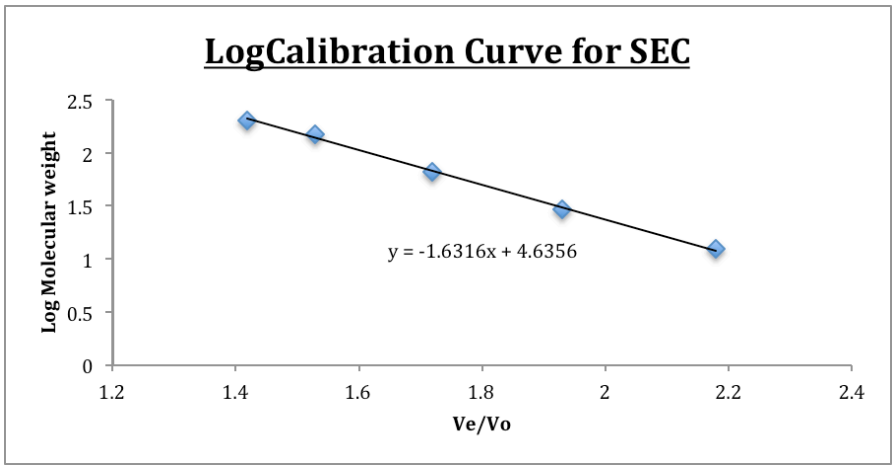


Figure 17: SEC calibration curves.

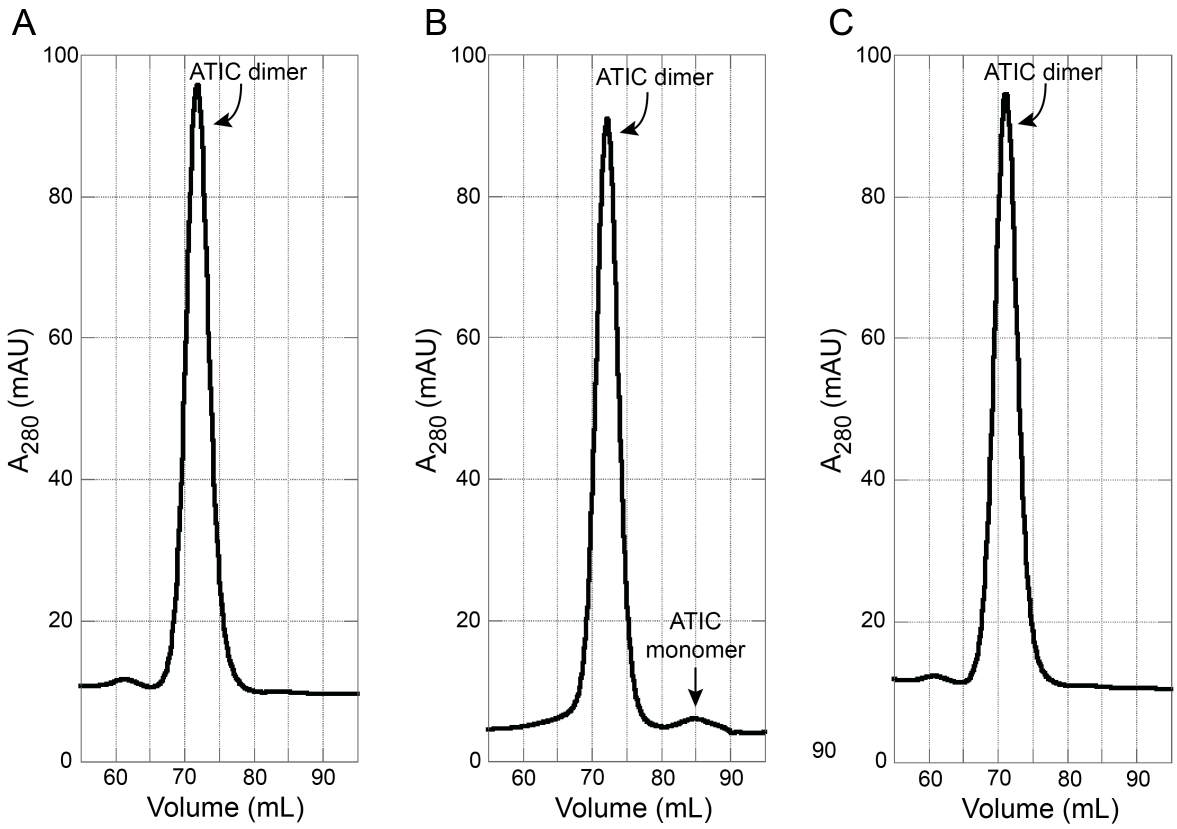


Figure 18: Size exclusion chromatography. (A) The ratio of monomer to dimer is shown in a 100 nM solution of ATIC (B) The same mixture with 10 µM of compound 14 shows the presence of monomeric ATIC at 85 mL (C) ATIC incubated with compound 9 (negative control) shows no monomer species.