Electronic Supplementary Material (ESI) for RSC Advances

Microwave-assisted solution phase peptide synthesis in neat water

Amit Mahindra, Karthik Nooney, Shrikant Uraon, Krishna K. Sharma, Rahul Jain*[a]

Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research, Sector 67, S. A. S. Nagar, Punjab 160062, India

E-mail: rahuljain@niper.ac.in

SUPPORTING INFORMATION

Table of Contents

1. General information

All starting materials were purchased from Sigma-Aldrich/Chem-Impex and used further without any additional purification. Analytical thin-layer chromatography (TLC) was performed using aluminum plates precoated with silica gel (0.25 mm, 60 Å pore-size) impregnated with a fluorescent indicator (254 nm). Visualization on TLC was achieved by the use of UV light (254 nm), treatment with 10% ninhydrin in ethanol or stained with Iodine. Flash column chromatography was undertaken on silica gel (400-630 mesh). Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on AVANCE III 400 Bruker (400 MHz). Proton chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to residual protium in the NMR solvent ($CDCl_3$, δ 7.26 and CD_3OD, δ 3.31). The following abbreviations were used to describe peak patterns when appropriate: br = broad, s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet. Coupling constants, *J*, were reported in Hertz unit (Hz). Carbon 13 nuclear magnetic resonance spectroscopy (^{13}C NMR) was recorded on AVANCE III 400 Bruker (100 MHz) and was fully decoupled by broad band decoupling. Chemical shifts were reported in ppm referenced to the centre line at a 77.0 and 49.0 ppm of $CDCl_3$ and CD_3OD. High resolution mass spectra were taken with MAXIS-Bruker using ESI-TOF method. HPLC analysis was performed on the SHIMADZU-prominence using Supelcosil^{TM} LC-18 column (25 cm × 4.6 mm, 5µm) run for 60 min with a flow of 1 mL/min, using a gradient of 95-5% where buffer A was 0.1% TFA in H_2O and buffer B was 0.1% TFA in CH_3CN and detection at 220 nm. All MW-irradiation reactions were carried out on a CEM Discover® microwave reactor.

2. Experimental procedure

2.1. General experimental procedure for dipeptides (2a-n)

In a 10 mL MW vial equipped with a magnetic stir bar, amino acid (AA_2) methyl ester·xHCl (1 mmol) was dissolved in H_2O (2.5 mL) followed by DIEA (2 mmol). Fmoc/Boc-AA_1-OH (1.2 mmol) was added followed by TBTU (1.2 mmol) and HOBt (1.2 mmol). Mixture was subjected to MW-irradiation (CEM Discover® microwave reactor) with gas cooling (pressure of 40 psi was maintained during irradiation) for 30 min at 40W with magnetic stirring, and a temperature limit of 60 ˚C (reaction time refers to the hold time at the desired set temperature). H_2O was evaporated and the reaction mixture was purified on automated flash column chromatography system (Biotage®) to give Fmoc/Boc-AA_1-AA_2-OMe.

2.2. General experimental procedure for *N*-α-Boc-protected peptides (3a-f, 4a-c)

In a 10 mL MW vial equipped with a magnetic stir bar, Boc-AA_1-AA_2-OMe (1 mmol) was subjected to 6N HCl (5 mL) at 25 ˚C for 30 min. Dihydrochloride salt of dipeptide was neutralized with DIEA (2 mmol). The resulting AA_1-AA_2-OMe (1 mmol) was dissolved in H_2O (2.5 mL). Boc-AA_3-OH (1.2 mmol) was added followed by TBTU (1.2 mmol) and HOBt (1.2 mmol). Mixture was subjected to MW-irradiation (CEM Discover® microwave reactor) with gas cooling (pressure of 40 psi was maintained during irradiation) for 30 min at 40W with magnetic stirring, and a temperature limit of 60 ˚C (reaction time refers to the hold time at the desired set temperature). H_2O was evaporated and the reaction mixture was purified on automated flash column chromatography system (Biotage®) to afford tripeptides (**3a-f**). The deprotection and coupling cycle described above was repeated to obtain tetrapeptides (**4a-c**).

2.3. General experimental procedure for *N*-α-Fmoc-protected peptides (3g-i, 4d)

In a 10 mL MW vial equipped with a magnetic stir bar, Fmoc-AA_1-AA_2-OMe (1 mmol) was subjected to 20% piperidine (2 mL) at 25 ˚C for 10 min followed by removal of excess piperidine. The resulting AA_1-AA_2-OMe (1 mmol) was dissolved in H_2O (2.5 mL) followed by addition of DIEA (2 mmol). Fmoc-AA_3-OH (1.2 mmol) was added followed by TBTU (1.2 mmol) and HOBt (1.2 mmol). Mixture was subjected to MW-irradiation (CEM Discover® microwave reactor) with gas cooling (pressure of 40 psi was maintained during irradiation) for 30 min at 40W with magnetic stirring, and a temperature limit of 60 ˚C (reaction time refers to the hold time at the desired set temperature). H_2O was evaporated and the reaction mixture was purified on automated flash column chromatography system (Biotage®) to afford tripeptides (**3g-i**). The deprotection and coupling cycle described above was repeated to obtain tetrapeptide (**4d**).

3. Product Characterization Data

Boc-Phe-Ile-OMe (2a)

^{1}H NMR (400MHz, CD_3OD): δ = 7.30-7.21 (m, 5H), 4.41-4.38 (m, 1H), 4.37-4.34 (m, 1H), 3.69 (s, 3H), 3.09 (dd, *J* = 5.8, 13.8 Hz, 1H), 2.82 (dd, *J* = 9.2, 13.7 Hz, 1H), 1.92-1.84 (m, 1H), 1.38 (s, 9H), 1.31-1.24 (m, 1H), 1.24-1.17 (m, 1H), 0.95-0.90 (m, 6H); ^{13}C NMR (100 MHz, CD_3OD): δ = 173.0, 171.9, 156.2, 137.1, 128.9, 127.9, 126.3, 79.3, 56.7, 55.8, 51.0, 37.6, 37.1, 27.2, 24.8, 14.5, 10.2; IR (neat): υ 3308, 2968, 2929, 2879, 1752, 1683, 1646, 1535, 1437, 1391, 1366, 1173, 1017, 989, 860, 716, 701; HRMS (ESI-TOF): calculated for [$M+H^+$] 393.2389; found 393.2384; HPLC: t_R= 47.80 min, 98%.

Boc-Val-Ile-OMe (2b)

^{1}H NMR (400MHz, CD_3OD): δ = 4.29 (d, *J* = 6.0 Hz, 1H), 3.80 (d, *J* = 7.3 Hz, 1H), 3.60 (s, 3H), 1.94-1.87 (m, 1H), 1.83-1.79 (m, 1H), 1.34 (s, 9H), 1.21-1.17 (m, 1H), 1.17-1.11 (m, 1H), 0.87-0.80 (m, 12H); ^{13}C NMR (100 MHz, CD_3OD): δ = 174.7, 173.4, 157.9, 80.9, 61.4, 58.2, 52.4, 38.4, 33.1, 28.4, 26.3, 19.8, 18.7, 15.9, 11.7; IR (neat): υ 3736, 3336, 2973, 2483, 1685, 1527, 1366, 1251, 1176, 1090, 1043, 1013, 880, 784; HRMS (ESI-TOF): calculated for [$M+H^+$] 345.2389; found 345.2382; HPLC: t_R= 46.23 min, 96.12%.

Boc-Phe-His-OMe (2c)

^{1}H NMR (400MHz, CD_3OD): δ = 7.72 (s, 1H), 7.27-7.19 (m, 5H), 6.92 (s, 1H), 4.70 (t, *J* = 6.1 Hz, 1H), 4.32 (dd, *J* = 5.4, 8.9 Hz, 1H), 3.67 (s, 3H), 3.19-3.11 (m, 1H), 3.10-3.07 (m, 1H), 3.07-3.01 (m, 1H), 2.82-2.74 (m, 1H), 1.35 (s, 9H); ^{13}C NMR (100 MHz, CD_3OD): δ = 172.9, 171.6, 156.3, 137.1, 134.6, 134.1, 128.9, 128.0, 126.3, 118.6, 79.4, 55.9, 52.6, 51.5, 37.7, 28.6, 27.3; IR (neat): υ 3406, 2956, 1757, 1661, 1601, 1448, 1391, 1365, 1275, 1206, 1176, 1094, 763; HRMS (ESI-TOF): calculated for [$M+H^+$] 417.2138; found 417.2138; HPLC: t_R= 30.87 min, 96.75%.

Boc-Trp-Ile-OMe (2d)

^{1}H NMR (400MHz, CD_3OD): δ = 7.48 (d, *J* = 7.5 Hz, 1H), 7.21 (d, *J* = 8.0 Hz, 1H), 7.01-6.93 (m, 2H), 6.93-6.86 (m, 1H), 4.31 (t, *J* = 7.0 Hz, 1H), 4.25 (d, *J* = 6.0 Hz, 1H), 3.51 (s, 3H), 3.11 (dd, *J* = 6.1, 14.4 Hz, 1H), 2.95 (dd, *J* = 7.4, 14.4 Hz, 1H), 1.73-1.64 (m, 1H), 1.32-1.22 (s, 9H), 1.11-1.05 (m, 1H), 1.05-0.97 (m, 1H), 0.79-0.72 (m, 6H); ^{13}C NMR (100 MHz, CD_3OD): δ = 173.3, 171.8, 156.2, 136.6, 127.5, 123.2, 120.9, 118.4, 118.0, 110.9, 109.5, 79.3, 56.7, 55.4, 51.1, 37.1, 27.6, 24.8, 14.4, 10.3; IR (neat): υ 3331, 2964, 2926, 2855, 1746, 1668, 1602, 1504, 1456,

1392, 1366, 1259, 1170, 1095, 763, 749; HRMS (ESI-TOF): calculated for [M+H$^+$] 432.2498, found 432.2494; HPLC: t_R= 46.93 min, 96.23%.

Boc-Met-Ile-OMe (2e)

^{1}H NMR (400MHz, CD_3OD): δ = 4.41 (d, *J* = 5.8 Hz, 1H), 4.23 (d, *J* = 5.8 Hz, 1H), 3.72 (s, 3H), 2.61-2.51 (m, 2H), 2.10 (s, 3H), 2.06-1.98 (m, 1H), 1.93-1.84 (m, 2H), 1.45 (s, 9H), 1.33-1.22 (m, 2H), 0.96-0.90 (m, 6H); ^{13}C NMR (100 MHz, CD_3OD): δ = 173.3, 171.9, 156.4, 79.3, 56.8, 53.4, 51.2, 37.0, 29.7, 27.3, 24.8, 15.9, 14.6, 13.9; IR (neat): υ 3336, 2967, 1744, 1658, 1523, 1456, 1366, 1275, 1260, 1170, 764; HRMS (ESI-TOF): calculated for [M+H$^+$] 377.2110; found 377.2107; HPLC: t_R= 42.73 min, 95.19%.

Boc-Ser(Bzl)-Ile-OMe (2f)

^{1}H NMR (400MHz, CD_3OD): δ = 7.37-7.22 (m, 5H), 4.53 (d, *J* = 5.0 Hz, 2H), 4.42 (d, *J* = 5.8 Hz, 1H), 4.32 (s, 1H), 3.75-3.69 (m, 2H), 3.68 (s, 3H), 1.90-1.83 (m, 1H), 1.44 (s, 9H), 1.31-1.27 (m, 1H), 1.24-1.13 (m, 1H), 0.92-0.85 (m, 6H); ^{13}C NMR (100 MHz, CD_3OD): δ = 171.9, 171.5, 156.3, 137.8, 127.9, 127.5, 127.4, 79.5, 72.9, 69.7, 56.8, 54.5, 51.1, 37.3, 27.3, 24.8, 14.5, 10.3; IR (neat): υ 3398, 3263, 2929, 2856, 1705, 1651, 1534, 1435, 1379, 1368, 1169, 1073, 967, 740, 698; HRMS (ESI-TOF): calculated for [M+H$^+$] 423.2495; found 423.2495; HPLC: t_R= 50.65 min, 99.34%.

Boc-Trp-His-OMe (2g)

^{1}H NMR (400MHz, CD_3OD): δ = 7.61-7.52 (m, 2H), 7.32 (d, *J* = 8.0 Hz, 1H), 7.11-7.05 (m, 2H), 7.04-6.97 (m, 1H), 6.79 (s, 1H), 4.68-4.62 (m, 1H), 4.40-4.32 (m, 1H), 3.63 (s, 3H), 3.21 (dd, *J* = 5.8, 14.6 Hz, 1H), 3.10-3.04 (m, 1H), 3.02 (d, *J* = 9.0 Hz, 1H), 3.00-2.94 (m, 1H), 1.36 (s, 9H); ^{13}C NMR (100 MHz, CD_3OD): δ = 173.3,171.5, 156.2, 136.6, 134.9, 127.5, 123.3, 120.9, 118.4, 117.9, 110.9, 109.5, 79.3, 55.5, 52.6, 51.4, 29.3, 28.6, 27.7; IR (neat): υ 3331, 3127, 2782, 1729, 1692, 1653, 1532, 1438, 1367, 1292, 1173, 972, 798, 781, 752; HRMS (ESI-TOF): calculated for [M+H$^+$] 456.2247, found 456.2247; HPLC: t_R= 30.95 min, 100%.

Boc-His-His-OMe (2h)

^{1}H NMR (400 MHz, CD_3OD): δ = 8.21 (br.s., 1H), 8.18-8.09 (m, 1H), 7.12 (s, 2H), 4.74 (dd, *J* = 5.1, 7.9 Hz, 1H), 4.36 (t, *J* = 6.7 Hz, 1H), 3.74 (s, 3H), 3.28-3.20 (m, 1H), 3.15 (d, *J* = 8.0 Hz, 1H), 3.12-3.07 (m, 1H), 2.95 (dd, *J* = 8.5, 14.6 Hz, 1H), 1.42 (s, 9H); ^{13}C NMR (100 MHz, CD_3OD): δ = 173.7, 172.6, 157.6, 135.8, 132.9, 131.3, 124.7, 117.9, 118.4, 81.0, 55.7, 54.9, 53.7, 29.6, 28.9, 28.7, 23.6; IR (neat): υ 3524, 2976, 1734, 1541, 1275, 1260, 764, 750. HRMS (ESI-TOF): calculated for [M+H$^+$] 407.2043, found 407.2040; HPLC: t_R= 13.10 min, 100%.

Boc-His(Bom)-Arg-OMe (2i)

^{1}H NMR (400MHz, CD_3OD): δ = 7.74 (s, 1H), 7.33 (s, 5H), 6.87 (s, 1H), 5.49 (s, 2H), 4.52 (s, 2H), 4.44 (dd, *J* = 5.0, 8.8 Hz, 1H), 4.39 (dd, *J* = 5.9, 8.7 Hz, 1H), 3.69 (s, 3H), 3.21-3.16 (m, 3H), 2.98 (dd, *J* = 8.8, 15.6 Hz, 1H), 1.92-1.84 (m, 1H), 1.62 (d, *J* = 7.3 Hz, 3H), 1.40 (s, 9H); ^{13}C NMR (100 MHz, CD_3OD): δ = 172.6, 171.9, 157.2, 156.2, 136.8, 128.2, 127.7, 79.5, 73.7, 70.1, 53.8, 51.7, 51.6, 40.5, 29.3, 28.3, 27.3, 25.9, 24.7; IR (neat): υ 3336, 2928, 1671, 1537, 1454, 1366, 1283, 1252, 1170, 1085, 1026, 742; HRMS (ESI-TOF): calculated for [M+H$^+$] 546.3040, found 546.3048; HPLC: t_R= 27.98 min, 99.18%.

Boc-Arg-Arg-OMe (2j)

^{1}H NMR (400 MHz, CD_3OD): δ = 4.46 (s, 1H), 4.21 (s, 1H), 3.74 (s, 3H), 3.28-3.20 (m, 4H), 2.07-1.90 (m, 1H), 1.90-1.77 (m, 2H), 1.76-1.64 (m, 5H), 1.46 (s, 9H); ^{13}C NMR (100 MHz, CD_3OD): δ = 173.8, 172.2, 157.3, 157.2, 156.4, 79.4, 54.1, 51.9, 40.6, 40.5, 29.8, 28.1, 27.4, 24.9; IR (neat): υ 3163, 2479, 1662, 1554, 1456, 1368, 1252, 1169, 1018, 871, 685; HRMS (ESI-TOF): calculated for [M+H$^+$] 445.2887, found 445.2887; HPLC: t_R= 17.49 min, 97%.

Boc-His(Bzl)-His-OMe (2k)

^{1}H NMR (400MHz, CD_3OD): δ= 7.64 (s, 1H), 7.57 (s, 1H), 7.37-7.28 (m, 3H), 7.22 (d, *J* = 6.8 Hz, 2H), 6.86 (d, *J* = 6.3 Hz, 2H), 5.13 (s, 2H), 4.64 (t, *J* = 6.3 Hz, 1H), 4.29 (dd, *J* = 5.3, 8.5 Hz, 1H), 3.65 (s, 3H), 3.11-3.06 (m, 1H), 3.03 (d, *J* = 7.5 Hz, 1H), 2.99-2.89 (m, 1H), 2.77 (dd, *J* = 8.9, 14.7 Hz, 1H), 1.37 (s, 9H); ^{13}C NMR (100 MHz, CD_3OD): δ = 172.9, 171.6, 156.2, 137.3, 136.9, 136.8, 134.9, 128.5, 127.7, 127.2, 117.4, 79.3, 52.5, 51.4, 50.3, 30.1, 28.6, 27.3; IR (neat): υ 3292, 2924, 1674, 1501, 1391, 1366, 1259, 1168, 1048, 750; HRMS (ESI-TOF): calculated for [M+H$^+$] 497.2512; found 497.2514; HPLC: t_R= 26.43 min, 98.42%.

Fmoc-Phe-Ile-OMe (2l)

^{1}H NMR (400MHz, CD_3OD): δ = 7.78 (d, *J* = 7.5 Hz, 2H), 7.60 - 7.54 (m, 2H), 7.38 (t, *J* = 7.5 Hz, 2H), 7.31-7.23 (m, 6H), 7.22-7.16 (m, 1H), 4.46 (dd, *J* = 5.5, 9.3 Hz, 1H), 4.38 (d, *J* = 6.3 Hz, 1H), 4.32-4.18 (m, 2H), 4.17-4.10 (m, 1H), 3.67 (s, 3H), 3.10 (dd, *J* = 5.6, 13.7 Hz, 1H), 2.85 (dd, *J* = 9.3, 13.8 Hz, 1H), 1.91-1.80 (m, 1H), 1.26-1.15 (m, 2H), 0.90-0.86 (m, 6H); ^{13}C NMR (100 MHz, CD_3OD): δ = 172.7, 171.8, 156.7, 143.8, 143.7, 141.1, 137.0, 129.0, 128.0, 127.3, 126.7, 126.3, 124.8, 124.7, 119.5, 66.6, 56.8, 56.1, 51.1, 37.6, 37.0, 24.8, 14.5, 10.3; IR (neat): υ 3307, 3029, 2964, 2876, 2468, 1739, 1690, 1651, 1535, 1433, 1354, 1256, 1144, 1031, 847, 739; HRMS (ESI-TOF): calculated for [M+H$^+$] 515.2546, found 515.2537; HPLC: t_R= 58.68 min, 98%.

Fmoc-Ser(*t*-butyl)-Ile-OMe (2m)

^{1}H NMR (400MHz, CD_3OD): δ = 7.69 (d, *J* = 7.5 Hz, 2H), 7.61-7.52 (m, 2H), 7.29 (t, *J* = 7.5 Hz, 2H), 7.21 (t, *J* = 7.4 Hz, 2H), 4.34 (d, *J* = 6.0 Hz, 1H), 4.28 (d, *J* = 6.8 Hz, 2H), 4.19 (br.s., 1H), 4.13 (t, *J* = 6.7 Hz, 1H), 3.60 (s, 3H), 3.56-3.52 (m, 1H), 3.48 (d, *J* = 5.8 Hz, 1H), 1.78 (td, *J* = 6.5, 13.1 Hz, 1H), 1.18 (s, 2H), 1.09 (s, 9H), 0.83-0.78 (m, 6H); ^{13}C NMR (100 MHz, CD_3OD): δ = 171.9, 171.4, 156.9, 143.9, 141.2, 127.4, 126.8, 124.8, 119.6, 73.5, 66.7, 61.7, 56.7, 55.3, 51.1, 37.3, 28.1, 24.2, 14.5, 10.3; IR (neat): υ 3273, 2966, 2923, 2872, 2528, 2442, 1722, 1662, 1520, 1450, 1414, 1250, 1084, 1018, 870, 585, 540; HRMS (ESI-TOF): calculated for [M+H$^+$] 511.2808; found 511.2810; HPLC: t_R= 56.79 min, 98.78%.

Boc-Arg(Mtr)-Ile-OMe (2n)

^{1}H NMR (400MHz, $CDCl_3$): δ = 6.63 (s, 1H), 4.32-4.13 (m, 2H), 3.78 (s, 3H), 3.67 (s, 3H), 3.52 (d, *J* = 3.3 Hz, 2H), 3.25 (br.s., 2H), 2.61 (s, 3H), 2.52 (s, 3H), 2.06 (s, 3H), 1.80 (dd, *J* = 7.9, 13.9 Hz, 2H), 1.63 (d, *J* = 7.5 Hz, 1H), 1.38 (s, 9H), 1.04 (d, *J* = 6.3 Hz, 2H), 0.88-0.78 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 180.0, 179.4, 162.9, 160.7, 158.6, 142.2, 141.0, 136.1, 128.5, 115.4, 83.2, 58.6, 56.2, 46.8, 45.2, 32.7, 31.2, 28.7, 26.3, 23.8, 21.3, 17.0, 14.6; IR (neat): υ 3163, 2479, 1662, 1554,1534, 1435, 1379, 1368, 1169, 1073, 967, 740, 698; HRMS (ESI-TOF): calculated for [M+H$^+$] 613.3145; found 613.3147; HPLC: t_R= 36.23 min, 93.89%.

Boc-Val-Phe-Ile-OMe (3a)

^{1}H NMR (400MHz, CD_3OD): δ = 7.27-7.15 (m, 5H), 4.76 (t, *J* = 7.0 Hz, 1H), 4.35 (d, *J* = 6.3 Hz, 1H), 3.89 (d, *J* = 6.5 Hz, 1H), 3.65 (s, 3H), 3.06 (dd, *J* = 6.5, 13.6 Hz, 1H), 2.90 (dd, *J* = 8.2, 13.7 Hz, 1H), 1.97-1.89 (m, 1H), 1.86-1.78 (m, 1H), 1.41 (s, 9H), 1.30-1.26 (m, 1H), 1.23-1.14 (m, 1H), 0.89-0.83 (m, 12H); ^{13}C NMR (100 MHz, CD_3OD): δ =172.7, 171.8, 170.3, 156.4, 136.7, 129.1, 128.0, 126.3, 79.1, 60.0, 56.8, 56.7, 54.1, 37.7, 29.3, 27.4, 24.8, 18.4, 17.2, 14.5, 10.3; IR (neat): υ 3277, 2966, 2414, 1648, 1527, 1458, 1366, 1246, 1175, 1017, 928, 878, 741, 697; HRMS (ESI-TOF): calculated for [M+H$^+$] 492.3073; found 492.3065; HPLC: t_R= 44.27 min, 93.48%.

Boc-Ile-Phe-Ile-OMe (3b)

^{1}H NMR (400MHz, CD_3OD): δ = 7.30-7.18 (m, 5H), 4.63 (s, 1H), 4.38-4.33 (m, 1H), 4.14-4.07 (m, 1H), 3.66 (s, 3H), 3.07 (s, 1H), 2.95-2.87 (m, 1H), 1.88-1.80 (m, 1H), 1.74-1.64 (m, 1H), 1.44 (s, 9H), 1.31-1.22 (m, 2H), 1.22-1.14 (m, 1H), 1.09 (d, *J* = 6.0 Hz, 1H), 0.91-0.80 (m, 12H); ^{13}C NMR (100 MHz, CD_3OD): δ = 172.7, 171.8, 171.7, 156.4, 136.7, 129.0, 128.0, 126.3, 79.2, 60.2, 59.2, 56.7, 51.1, 37.6, 37.0, 27.4, 24.8, 24.3, 14.5, 10.3, 10.1; IR (neat): υ 3328, 2964, 2925, 1750, 1674, 1601, 1455, 1260, 1174, 1092, 748; HRMS (ESI-TOF): calculated for [M+H$^+$] 506.323; found 506.325; HPLC: t_R= 48.93 min, 95.32%.

Boc Abu-Phe-Ile-OMe (3c)

^{1}H NMR (400MHz, CD_3OD): δ = 7.29-7.19 (m, 5H), 4.74-4.67 (m, 1H), 4.34 (d, *J* = 6.3 Hz, 1H), 3.88 (d, *J* = 5.5 Hz, 1H), 3.68 (s, 3H), 3.11 (dd, *J* = 6.3, 13.8 Hz, 1H), 2.96 (d, *J* = 8.0 Hz, 1H), 1.89 - 1.80 (m, 1H), 1.67 (d, *J* = 6.5 Hz, 1H), 1.57-1.49 (m, 1H), 1.43 (s, 9H), 1.30 (s, 1H), 1.26-1.17 (m, 1H), 0.92-0.86 (m, 9H); ^{13}C NMR (100 MHz, CD_3OD): δ = 173.3, 171.8, 171.7, 156.4, 136.7, 129.7, 129.1, 128.0, 126.3, 79.2, 56.7, 56.1, 54.0, 51.1, 37.6, 37.1, 27.4, 25.3, 24.8, 14.5, 10.3, 9.3; IR (neat): υ 3329, 3306, 2965, 2931, 2877, 2476, 2421, 1738, 1685, 1644, 1532, 1499, 1461, 1366, 1302, 1261, 1176, 1043, 1022, 847, 747, 701; HRMS (ESI-TOF): calculated for [M+H$^+$] 478.2917; found 478.2919; HPLC: t_R= 46.29 min, 97.78%.

Boc-Phg-Trp-Ile-OMe (3d)

^{1}H NMR (400MHz, CD_3OD): δ = 7.59 (d, *J* = 7.8 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.27 (s, 5H), 7.12 - 6.99 (m, 3H), 5.15 (s, 1H), 4.76 (t, *J* = 6.4 Hz, 1H), 4.27 (d, *J* = 6.0 Hz, 1H), 3.61 (s, 3H), 3.30-3.21 (m, 1H), 3.21-3.14 (m, 1H), 1.77-1.67 (m, 1H), 1.43 (s, 9H), 1.33-1.25 (m, 2H), 0.84-0.75 (m, 6H); ^{13}C NMR (100 MHz, CD_3OD): δ = 172.1, 172.0, 171.6, 171.3, 137.5, 136.6, 128.3, 127.7, 127.4,123.3, 121.0, 118.5, 117.9, 110.8, 109.2, 79.6, 60.2, 58.7, 56.7, 53.9, 36.9, 27.3, 24.9, 24.7, 14.4, 10.3; IR (neat): υ 3297, 2969, 2935, 2425, 1747, 1683, 1656, 1541, 1455, 1366, 1248, 1171, 1087, 1013, 866, 745, 696; HRMS (ESI-TOF): calculated for [M+H$^+$] 565.3026; found 565.3029; HPLC: t_R= 50.43 min, 95.34%.

Boc-Trp-Phe-Ile-OMe (3e)

^{1}H NMR (400MHz, CD_3OD): δ = 7.46 (d, *J* = 7.8 Hz, 1H), 7.22 (d, *J* = 8.0 Hz, 1H), 7.13-7.05 (m, 3H), 7.02-6.94 (m, 4H), 6.93 - 6.88 (m, 1H), 4.55 (t, *J* = 6.0 Hz, 1H), 4.25-4.19 (m, 2H), 3.56 (s, 3H), 3.21 (td, *J* = 1.5, 3.2 Hz, 1H), 3.05 (dd, *J* = 5.0, 14.6 Hz, 1H), 2.90 (dd, *J* = 7.9, 14.7 Hz, 1H), 2.78 (d, *J* = 7.3 Hz, 1H), 1.71 (br.s., 1H), 1.27 (s, 9H), 1.08-1.02 (m, 2H), 0.77 (t, *J* = 7.8 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD): δ = 173.0, 171.7, 156.2, 136.6, 136.4, 129.1, 128.0, 127.4, 126.4, 123.2, 121.0, 118.4, 118.0, 110.9, 109.5, 79.4, 56.9, 56.8, 55.5, 51.1, 37.3,

36.9, 29.3, 27.2, 24.9, 14.5, 10.3; IR (neat): υ 3313, 3062, 2964, 2926, 2876, 2854, 2475, 1741, 1689, 1646, 1524, 1456, 1366, 1249, 1170, 1015, 857, 741; HRMS (ESI-TOF): calculated for [M+H$^+$] 579.3182; found 579.3181; HPLC: t_R= 43.67 min, 96.12%.

Boc-Ile-Ser(Bzl)-Ile-OMe (3f)

^{1}H NMR (400MHz, CD_3OD): δ = 7.24-7.13 (m, 5H), 4.58 (s, 1H), 4.43 (s, 2H), 4.32 (d, *J* = 5.8 Hz, 1H), 3.88 (d, *J* = 6.5 Hz, 1H), 3.69 - 3.60 (m, 2H), 3.57 (s, 3H), 1.81-1.67 (m, 2H), 1.32 (s, 9H), 1.28-0.98 (m, 4H), 0.83-0.75 (m, 12H); ^{13}C NMR (100 MHz, CD_3OD): δ = 173.0, 171.7, 170.5, 170.4, 156.8, 137.8, 127.9, 127.5, 127.3, 79.3, 72.8, 69.4, 59.4, 56.9, 56.8, 51.1, 37.1, 36.8, 27.3, 24.8, 24.4, 14.6, 10.3; IR (neat): υ 3317, 2966, 2474, 1740, 1687, 1640, 1527, 1454, 1366, 1263, 1171, 1023, 849, 777, 670, 617; HRMS (ESI-TOF): calculated for [M+H$^+$] 536.3336; found 536.3332; HPLC: t_R= 54.72 min, 93.96%.

Fmoc-Abu-Phe-Ile-OMe (3g)

^{1}H NMR (400MHz, $CDCl_3$): δ = 7.78 (d, *J* = 7.5 Hz, 2H), 7.60 (d, *J* = 7.0 Hz, 2H), 7.42 (t, *J* = 7.5 Hz, 2H), 7.33 (t, *J* = 7.5 Hz, 2H), 7.27-7.17 (m, 5H), 4.72 (d, *J* = 7.3 Hz, 1H), 4.51-4.41 (m, 2H), 4.39-4.30 (m, 1H), 4.25-4.18 (m, 1H), 4.13 (d, *J* = 6.0 Hz, 1H), 3.69 (s, 3H), 3.08 (d, *J* = 6.8 Hz, 2H), 1.82 (s, 2H), 1.68-1.59 (m, 1H), 1.40-1.25 (m, 1H), 1.13-1.01 (m, 1H), 0.92-0.79 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.6, 171.5, 170.3, 143.7, 141.3, 136.2, 129.2, 128.6, 127.7, 127.1, 127.0, 125.0, 120.0, 67.1, 56.6, 56.2, 54.4, 52.0, 47.1, 38.1, 37.7, 25.0, 15.3, 11.4, 9.7; IR (neat): υ 3382, 2965, 2931, 2877, 2477, 1746, 1691, 1641, 1524, 1454, 1366, 1250, 1175, 1044, 1019, 867, 698; HRMS (ESI-TOF): calculated for [M+H$^+$] 600.3073; found 600.306; HPLC: t_R= 55.63 min, 95.17%.

Fmoc-Leu-Phe-Ile-OMe (3h)

^{1}H NMR (400MHz, $CDCl_3$): δ = 7.78 (d, *J* = 7.5 Hz, 2H), 7.59 (d, *J* = 7.5 Hz, 2H), 7.42 (t, *J* = 7.5 Hz, 2H), 7.32 (t, *J* = 7.4 Hz, 2H), 7.24 - 7.12 (m, 5H), 4.70 (d, *J* = 6.8 Hz, 1H), 4.50-4.42 (m, 2H), 4.38-4.29 (m, 1H), 4.20 (t, *J* = 6.8 Hz, 2H), 3.69 (s, 3H), 3.07 (d, *J* = 7.0 Hz, 2H), 1.81 (s, 1H), 1.60 (d, *J* = 5.8 Hz, 2H), 1.39 - 1.23 (m, 2H), 1.12-0.99 (m, 1H), 0.95-0.80 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.1, 171.5, 170.3, 143.7, 141.3, 136.3, 129.2, 128.6, 127.7, 127.1, 126.9, 125.0, 124.9, 120.0, 119.9, 67.0, 56.6, 54.4, 52.0, 47.2, 41.3, 25.1, 24.6, 21.9, 15.3, 11.5; IR (neat): υ 3306, 2964, 2931, 2877, 2476, 1737, 1686, 1644, 1533, 1452, 1366, 1302, 1260, 1177, 1044, 740, 700, 620; HRMS (ESI-TOF): calculated for [M+H$^+$] 628.3386; found 628.3389; HPLC: t_R= 56.73 min, 98.51%.

Fmoc-Cys(*t*- butyl)-Ser(*t*-butyl)-Ile-OMe (3i)

^{1}H NMR (400MHz, $CDCl_3$): δ = 7.78 (d, *J* = 7.5 Hz, 2H), 7.61 (d, *J* = 7.5 Hz, 2H), 7.45-7.39 (m, 2 H), 7.35-7.31 (m, 2H), 4.60 (dd, *J* = 5.3, 8.8 Hz, 1H), 4.50-4.45 (m, 1H), 4.43-4.36 (m, 2H), 4.28 - 4.21 (m, 1H), 3.73 (s, 3H), 3.42 (t, *J* = 7.5 Hz, 1H), 3.14-3.05 (m, 1H), 2.89 (dd, *J* = 8.0, 13.3 Hz, 1H), 2.06 (s, 1H), 1.91 (s, 1H), 1.41 (s, 9H), 1.30-1.25 (m, 2H), 1.23 (s, 9H), 0.95-0.89 (m, 6H); ^{13}C NMR (101MHz, $CDCl_3$): δ =171.9, 169.8, 169.7, 143.8, 143.6, 141.3, 127.7, 127.0, 125.1, 119.9, 74.2, 67.3, 61.1, 56.7, 55.0, 53.6, 51.9, 47.1, 43.6, 37.8, 31.0, 27.4, 25.1, 15.5, 11.5; IR (neat): υ 3286, 2967, 1743, 1708, 1678, 1644, 1523, 1450, 1397, 1363, 1256, 1224, 1143, 1095, 1049, 877, 761, 662; HRMS (ESI-TOF): calculated for [M+H$^+$] 670.3526; found 670.3543; HPLC: t_R= 51.43 min, 97.33%.

Boc-Phe-Ile-Phe-Ile-OMe (4a)

^{1}H NMR (400MHz, CD_3OD): δ = 7.28-7.17 (m, 10H), 4.65 (dt, *J* = 5.4, 8.6 Hz, 1H), 4.21 (t, *J* = 7.4 Hz, 2H), 4.17-4.11 (m, 1H), 3.59 (s, 3H), 2.97 (dd, *J* = 4.9, 14.2 Hz, 1H), 2.87 (dd, *J* = 3.6, 13.9 Hz, 1H), 2.78 (dd, *J* = 9.0, 13.8 Hz, 1H), 2.69 (dd, *J* = 10.7, 13.7 Hz, 1H), 1.81-1.72 (m, 1H), 1.67 (d, *J* = 6.3 Hz, 1H), 1.43-1.33 (m, 2H), 1.28 (s, 9H), 1.18-1.11 (m, 1H), 1.08-0.97 (m, 1H), 0.84-0.74 (m, 12H); ^{13}C NMR (100 MHz, CD_3OD): δ = 172.1, 171.7, 171.5, 171.1, 155.7, 138.7, 137.9, 129.6, 129.5, 128.4, 126.6, 126.5, 78.5, 56.9, 56.7, 56.1, 53.7, 52.1, 37.9, 37.5, 36.7, 28.5, 28.2, 25.0, 24.3, 15.7, 11.4; IR (neat): υ 3410, 2950, 2843, 1641, 1454, 1399, 1053, 1032, 1017, 696; HRMS (ESI-TOF): calculated for [M+H$^+$] 653.3914; found 653.3925; HPLC: t_R= 46.89 min, 98.96%.

Boc-Glu(OBzl)-Phg-Trp-Ile-OMe (4b)

^{1}H NMR (400MHz, CD_3OD): δ = 7.58 (d, *J* = 8.0 Hz, 1H), 7.37-7.35 (m, 3H), 7.34-7.25 (m, 8H), 7.08 (t, *J* = 7.4 Hz, 1H), 7.06-6.98 (m, 2H), 5.43 (br.s., 1H), 5.13 (s, 2H), 4.78-4.75 (m, 1H), 4.62 (br.s., 1H), 4.27 (d, *J* = 5.8 Hz, 1H), 3.61 (s, 3H), 3.29 (d, *J* = 6.8 Hz, 1H), 3.15 (dd, *J* = 7.4, 14.4 Hz, 1H), 2.48 (t, *J* = 7.4 Hz, 2H), 2.05 (d, *J* = 7.3 Hz, 1H), 1.95-1.85 (m, 1H), 1.73 (br.s., 1H), 1.41 (s, 2H), 1.33 (s, 9H), 0.83-0.73 (m, 6H); ^{13}C NMR (100 MHz, CD_3OD): δ = 173.1,173.0, 171.7, 171.5, 156.2, 136.6, 128.4, 128.1, 127.8, 127.4, 127.2, 120.9, 118.4, 117.9, 110.8, 79.5, 66.0, 56.8, 53.9, 50.9, 36.8, 29.9, 27.3, 27.1, 24.7, 14.4, 10.2; IR (neat): υ 3749, 3315, 2930, 2471, 1716, 1636, 1541, 1524, 1456, 1340, 1252, 1160, 905, 820, 740, 695, 609, 574, 541; HRMS (ESI-TOF): calculated for [M+H$^+$] 784.3921; found 784.3932; HPLC: t_R= 49.86 min, 96.42%.

Boc-Ala(2-naphthyl)-Tyr(Bzl)-Phe-Ile-OMe (4c)

^{1}H NMR (400MHz, $CDCl_3$): δ = 7.82-7.77 (m, 2H), 7.62 (s, 1H), 7.50-7.44 (m, 2H), 7.41-7.36 (m, 4H), 7.33 (d, *J* = 6.5 Hz, 1H), 7.29-7.19 (m, 4H), 7.12 (d, *J* = 7.3 Hz, 2H), 6.86 (d, *J* = 7.8 Hz, 1H), 6.74 (d, *J* = 8.0 Hz, 2H), 6.58 (br.s., 1H), 6.51 (d, *J* = 6.3 Hz, 1H), 4.96 (s, 2H), 4.70 (d, *J* = 7.0 Hz, 1H), 4.56 (d, *J* = 6.5 Hz, 1H), 4.52-4.46 (m, 1H), 4.37 (d, *J* = 6.3 Hz, 1H), 3.71 (s, 3H), 3.20-3.12 (m, 2H), 2.88 (t, *J* = 6.0 Hz, 2H), 1.81 (br.s., 1H), 1.34 (s, 9H), 1.28 (br.s., 1H), 1.15 (d, *J* = 7.8 Hz, 1H), 0.94-0.85 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.7, 171.4, 170.4, 170.3, 157.8, 136.8, 136.7, 133.7, 133.5, 132.5, 130.1, 129.1, 128.5, 128.0, 127.9, 127.7, 127.5, 127.4, 127.1, 126.8, 126.4, 125.9, 115.0, 80.6, 69.9, 56.8, 54.5, 54.3, 52.0, 37.7, 36.5, 29.7, 28.2, 15.3, 11.5; IR (neat): υ 3288, 3062, 2925, 1742, 1691, 1643, 1527, 1365, 1248, 1174, 1019, 851, 807, 745, 696; HRMS (ESI-TOF): calculated for [M+H$^+$] 843.4333, found 843.4322; HPLC: t_R= 49.73 min, 96.86%.

Fmoc-Phe-Abu-Phe-Ile-OMe (4d)

^{1}H NMR (400MHz, $CDCl_3$): δ = 7.77-7.73 (m, 2H), 7.59 (d, *J* = 7.5 Hz, 1H), 7.50 (d, *J* = 7.5 Hz, 1H), 7.41-7.37 (m, 2H), 7.30-7.25 (m, 3H), 7.21-7.14 (m, 5H), 7.04 (br.s., 4H), 5.06-4.90 (m, 2H), 4.81 (d, *J* = 6.5 Hz, 1H), 4.51 (dd, *J* = 6.1, 8.7 Hz, 1H), 4.28 (d, *J* = 7.3 Hz, 1H), 4.17-4.09 (m, 2H), 3.45 (s, 3H), 3.10-2.97 (m, 4H), 1.81 (s, 2H), 1.75 (br.s., 1H), 1.63 (td, *J* = 6.9, 13.8 Hz, 1H), 1.27 (s, 3H), 1.12-1.02 (m, 1H), 0.79-0.74 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.7, 171.5, 171.0, 170.3, 153.9, 143.6, 142.3, 136.9, 130.3, 127.9, 126.9, 126.7, 126.2, 125.9, 125.2, 120.5, 63.6, 59.6, 58.7, 56.3, 51.4, 47.9, 37.3, 25.7, 15.0, 11.2, 9.1; IR (neat): υ 3286, 2967, 1743, 1708, 1678, 1644, 1523, 1450, 1250, 1175, 1044, 1019, 867, 698; HRMS (ESI-TOF): calculated for [M+H$^+$] 746.3679, found 746.3682; HPLC: t_R= 56.93 min, 98.41%.

Boc-β-Ala-Trp-Met-Asp-Phe-NH_2 (Pentagastrin, 5a)

^{1}H NMR (400MHz, CD_3OD): δ = 7.58 (d, *J* = 8.0 Hz, 1H), 7.35 (d, *J* = 8.3 Hz, 1H), 7.31-7.26 (m, 2H), 7.24-7.17 (m, 4H), 7.13-7.07 (m, 1H), 7.04-6.99 (m, 1H), 4.69 (dd, *J* = 5.9, 7.9 Hz, 2H), 4.57 (t, *J* = 6.5 Hz, 1H), 4.29 (dd, *J* = 4.9, 8.4 Hz, 1H), 3.65 (s, 3 H), 3.26-3.21 (m, 2H), 3.19-3.12 (m, 1H), 3.10-3.01 (m, 1H), 2.81 (dd, *J* = 5.8, 16.6 Hz, 1H), 2.69 (dd, *J* = 7.8, 16.6 Hz, 1H), 2.40 (t, *J* = 6.8 Hz, 2H), 2.30-2.14 (m, 2H), 1.99 (s, 2H), 1.91-1.78 (m, 2H), 1.38 (s, 9H); ^{13}C NMR (100 MHz, CD_3OD): δ = 174.9, 173.4, 173.2, 172.2, 156.9, 136.8, 135.4, 128.6, 128.3, 127.7, 125.6, 121.7, 119.8, 118.1, 109.5, 79.4, 59.3, 57.2, 53.3, 37.2, 35.4, 31.2, 29.3, 28.4, 15.4; IR (neat): υ 3288, 2969, 1742, 1698, 1640, 1525, 1395, 1050, 1034, 1017, 699; HRMS (ESI-TOF): calculated for [M+H$^+$] 767.3312, found 767.3319; HPLC: t_R= 50.85 min, 99.19%.

4. Representative HPLC Chromatograms of 2a, 2g, 3a, 3c and 4a

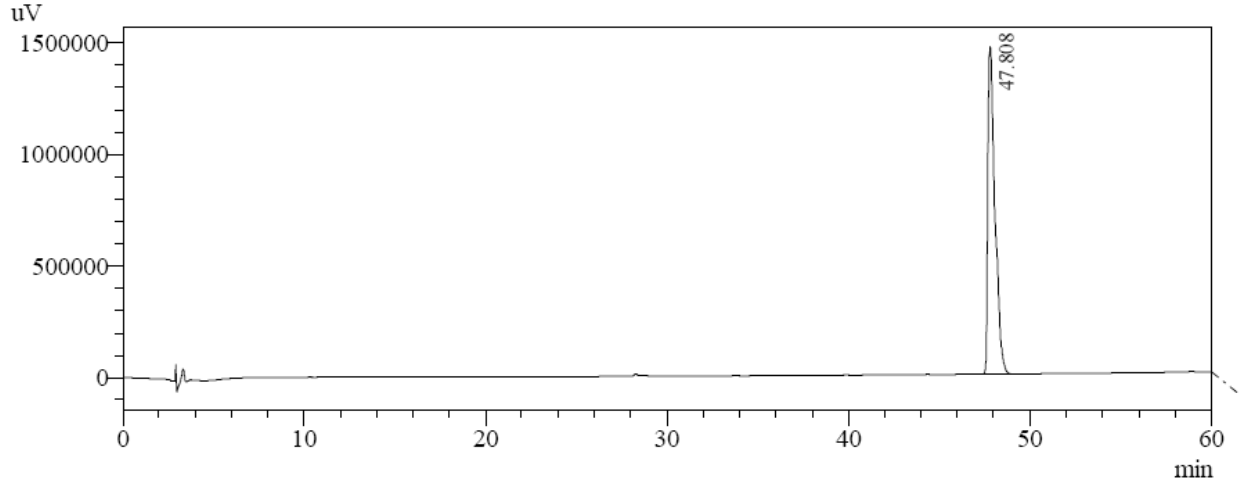

Figure 1 HPLC Chromatograms of 2a

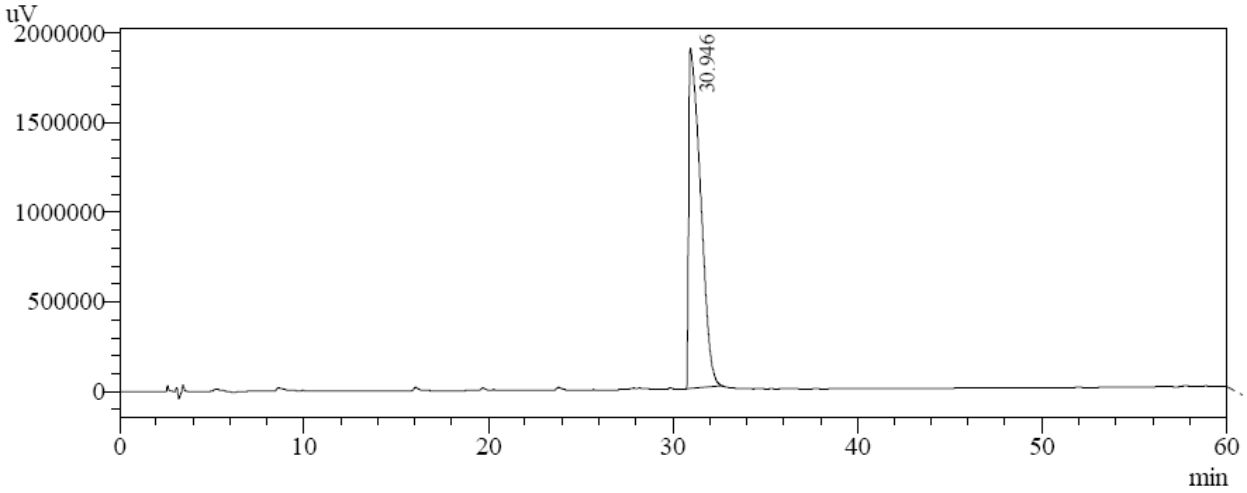

Figure 2 HPLC Chromatograms of 2g

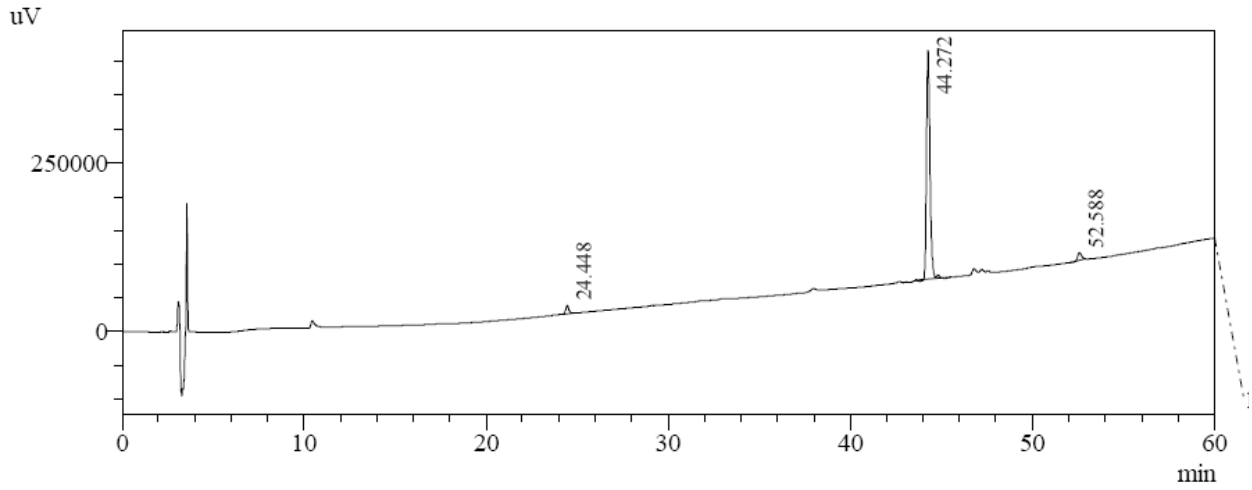

Figure 3 HPLC Chromatograms of 3a

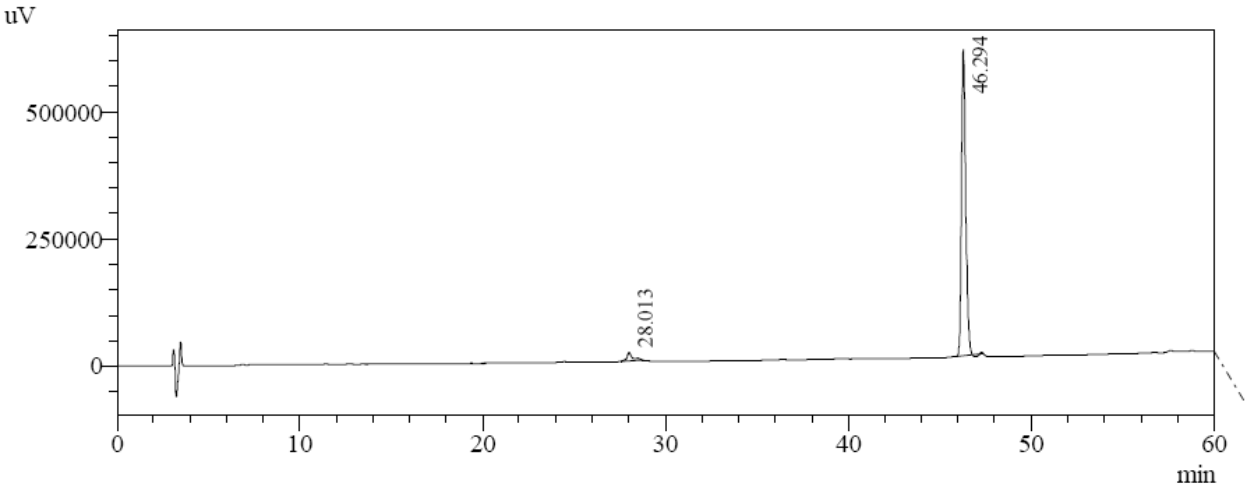

Figure 4 HPLC Chromatograms of 3c

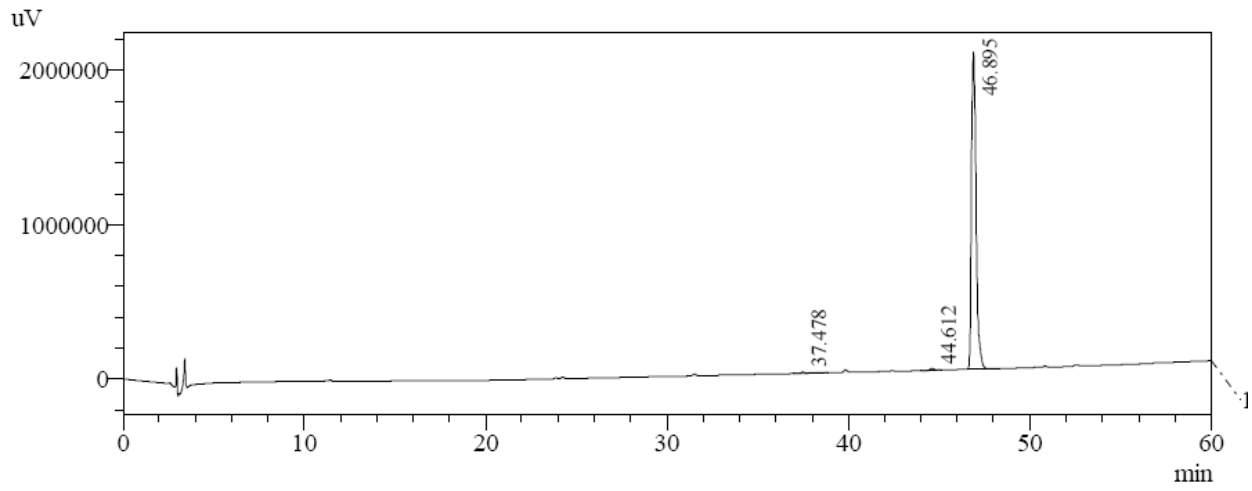

Figure 5 HPLC Chromatograms of 4a