Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Peptide inhibitors of the Keap1-Nrf2 protein-protein interaction with improved binding and cellular activity

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Supplementary Information

Synthetic methods

All Fmoc α-amino acids had the L-configuration, reactive side chains were protected with *t*-Butyl. All chemicals were obtained from commercial suppliers and used without further purification. Diisopropylcarbodiimide (DIC), dichloromethane (DCM), trifluoroacetic acid (TFA), hydrazine, rhodamine isothiocyanate (RITC), stearic acid and methanol were purchased from Aldrich. Ninhydrin, piperidine, triisopropylsilane (TIS), diisopropylethylamine (DIPEA), and acetic anhydride were purchased from Fisher. Peptide synthesis grade dimethylformamide (DMF) was purchased from Rathburn Chemicals. All α-amino acids, resins and HATU were purchased from NovaBiochem (Merck).

Mass spectroscopy data were collected using a Waters Micromass ZQ instrument coupled to a Waters 2695 HPLC with a Waters 2996 PDA (LCMS instrument). Waters Micromass ZQ parameters used were: Capillary (kV), 3.50; Cone (V), 30; Extractor (V), 3.0; Source temperature (°C), 120; Desolvation temperature (°C), 350; Cone flow rate (L/h), 80; Desolvation flow rate (L/h), 650. LCMS analysis was used to monitor the progress of peptide synthesis and to characterise the final compound after HPLC purification. Solvents were removed by rotary evaporation at or below 40 °C and the compounds further dried using high vacuum pumps. The purification of the peptides was achieved using High Performance Liquid Chromatography. ^{1}H NMR and ^{13}C NMR were recorded on a Bruker Advance 400 Spectrophotometer at 400 MHz and 100 mHz, or Bruker Advance 500 Spectrophotometer at 500 MHz and 125 MHz respectively. Chemical shifts (δ H) are quoted in ppm (parts per million) and referenced to the d_6-DMSO residual dimethyl-sulphoxide signal ^{1}H δ = 2.50. Multiplicities in ^{1}H NMR spectra are quoted as: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. High resolution mass spectra (HRMS) were obtained on a Thermo Navigator mass spectrometer coupled to an HPLC instrument using electrospray (ES) ionisation and time-of-flight (TOF) mass spectrometry.

General procedure for peptide synthesis

When using unsubstituted Wang resin, the general procedure for peptide synthesis began with coupling the first amino acid. The peptide sequence was grown by sequential coupling of amino acids and deprotection of Fmoc protecting groups. The peptide was then capped using acetic anhydride or stearic acid, cleaved from the resin, isolated and purified. When using pre-loaded resins or Rink amide resin, synthesis began with Fmoc deprotection and was followed by sequential assembly of amino acids using the amino acid coupling procedure and deprotection of Fmoc protecting groups. The peptide was then capped using acetic anhydride, stearic acid or fluorescein isothiocyanate or rhodamine isothiocyanate, cleaved from the resin and isolated and purified.

Attachment of the first amino acid – Wang resin: The resin was allowed to swell in DMF at room temperature for 30 min in a solid phase synthesis tube. The Fmoc protected amino acid (10 eq. relative to resin loading) was allowed to dissolve in dry DCM (5 mL). A solution of DIC (5 eq.) in dry DCM was added to the amino acid solution. The mixture was allowed to stir for 20 min at 0 °C, the reaction mixture was kept free of moisture with a calcium chloride drying tube. The DCM was removed by evaporation under reduced pressure and the residue dissolved in DMF and added to the resin. DMAP (0.1 eq.) was dissolved in DMF and the solution added to the resin/amino acid mixture. The solution was allowed to mix at room temperature for 1 h. The procedure was repeated once.

Attachment of the first amino acid – Rink amide resin: The resin was allowed to swell in DMF at room temperature for 30 min in a solid phase synthesis tube. The Fmoc protecting group was removed using 20% piperidine in DMF and the first amino acid coupled using HATU (2.5 eq. relative to resin loading) and DIPEA (5 eq. relative to resin loading) in DMF.

Fmoc deprotection: A 5 mL solution of 20% piperidine in DMF was added to the resin and shaken for 10 min, this procedure was repeated twice. The resin was rinsed with DMF (3 x 5 mL).

Amino acid coupling: The Fmoc amino acid (2.5 eq. relative to resin loading), HATU (2.5 eq. relative to resin loading) and DIPEA (5 eq. relative to resin loading) were dissolved in DMF (5 mL). This solution was added to the resin and mixed for 1 h the resin was drained and washed with DMF (3 x 5 mL) and the procedure repeated once.

Capping with acetic anhydride: Acetic anhydride (5 eq. relative to resin loading) and DIPEA (10 eq. relative to resin loading) were dissolved in DMF. The solution was added to the resin and mixed for 1 h at room temperature. The resin was drained and washed with DMF (3 x 5 mL). The procedure was repeated once.

Capping with stearic acid: Stearic acid (2.5 eq. relative to resin loading), HATU (2.5 eq. relative to resin loading) and DIPEA (5 eq. relative to resin loading) were dissolved in DMF (5 mL). The solution was added to the resin and mixed for 2

h, the resin was drained and washed with DMF (3 x 5 mL). The procedure was repeated three times.

Removal of Dde protecting group: A 5 mL solution of 2% hydrazine in DMF was added to the resin and shaken for 3 min, repeated once. The resin was rinsed with DMF (3 x 5 mL).

Coupling rhodamine isothiocyanate to the ε-amino group of lysine: Rhodamine isothiocyanate (1.5 eq. relative to resin loading) and DIPEA (3 eq. relative to resin loading) were dissolved in DMF (5 mL). The solution was added to the resin and mixed for 2 h (the reaction tube was protected from light), the resin was drained and washed with DMF (3 x 5 mL). The procedure was repeated once.

Removal from the resin and side chain deprotection: The resin was washed with DMF (3 x 5 mL), DCM (3 x 5 mL) and methanol (3 x 5 mL) and dried overnight in a dessicater. The dry resin was transferred to a dry round bottom flask and a 10 mL solution of TFA/TIS/H_2O (95:2.5:2.5) added. The mixture was allowed to stir at room temperature for 2 h. The resin was filtered, washed twice with TFA and the filtrates combined. The TFA was removed initially by rotary evaporation and residual TFA removed using a high vacuum pump to leave a glassy film. The glassy film was washed with diethyl ether (10 mL) which allowed the crude peptide to precipitate. The crude peptide was triturated into the ether and the solution centrifuged at 10,000 RPM for 10 min to form a pellet of crude peptide. The ether was decanted and the crude peptide allowed to dry overnight.

HPLC Methods

System A: Preparative reverse-phase high-performance liquid chromatography (HPLC) was carried out on a Zorbax Eclipse XDB C-18 column 250 x 9.4 mm. All HPLC experiments were performed with gradient conditions: initial fixed composition 5% B to 90% B over 20 min, held for 5 min at 90% B, then returned to 5% B in 10 min and held for 5 min. Total duration of gradient run was 40 min. Eluents used were solvent A (H_2O with 0.02% TFA) and solvent B (MeCN with 0.02% TFA). Flow rate: 5.00 mL/min.

System B: Preparative reverse-phase high-performance liquid chromatography (HPLC) was carried out on a Zorbax Eclipse XDB C-8 column 250 x 9.4 mm. All HPLC experiments were performed with gradient conditions: initial fixed composition 5% B to 90% B over 20 min, held for 5 min at 90% B, then returned to 5% B in 10 min and held for 5 min. Total duration of gradient run was 40 min. Eluents used were solvent A (H_2O with 0.02% TFA) and solvent B (MeCN with 0.02% TFA). Flow rate: 5.00 mL/min.

System C: Analytical reverse-phase high-performance liquid chromatography (HPLC) was carried out on a Zorbax Eclipse XDB C-18 column 150 x 4.6 mm. All HPLC experiments were performed with gradient conditions: initial fixed composition 5% B to 90% B over 20 min, held for 5 min at 90% B, then returned to 5% B in 10 min and held for 5 min. Total duration of gradient run was 40 min. Eluents used were solvent A (H_2O with 0.02% TFA) and solvent B (MeCN with 0.02% TFA). Flow rate: 1.20 mL/min.

System D: Analytical reverse-phase high-performance liquid chromatography (HPLC) was carried out on a Phenomenex Onyx Monolithic C-18 column 50 x 4.6 mm. All HPLC experiments were performed with gradient conditions: initial fixed composition 5% B to 50% B over 3 min, returned to 5% B in 2 min. Total duration of gradient run was 5 min. Eluents used were solvent A (H_2O with 0.1% formic acid) and solvent B (MeCN with 0.1% formic acid). Flow rate: 3.0 mL/min.

System E: Analytical reverse-phase high-performance liquid chromatography (HPLC) was carried out on a Zorbax Eclipse XDB C-8 column 150 x 4.6 mm. All HPLC experiments were performed with gradient conditions: initial fixed composition 5% B to 90% B over 20 min, held for 5 min at 90% B, then returned to 5% B in 10 min and held for 5 min. Total duration of gradient run was 40 min. Eluents used were solvent A (H_2O with 0.02% TFA) and solvent B (MeCN with 0.02% TFA). Flow rate: 1.20 mL/min.

System F: Analytical reverse-phase high-performance liquid chromatography (HPLC) was carried out on a Phenomenex Onyx Monolithic C-18 column 50 x 4.6 mm. All HPLC experiments were performed with gradient conditions: initial fixed composition 5% B, held for 2 min, 5% B to 50% B over 3 min, held at 50% B for 1 min, then 50% B to 95% B, returned to 5% B in 2.5 min and held at 5% B for 30 s. Total duration of gradient run was 10 min. Eluents used were solvent A (H_2O with 0.1% formic acid) and solvent B (MeCN with 0.1% formic acid). Flow rate: 3.0 mL/min.

Compound 4: Ac-Asp-Pro-Glu-Thr-Gly-Glu-Leu-NH_2

Rink amide MBHA resin (500 mg, 0.59 mmol/g) and the symmetrical anhydride of Fmoc-Leu-OH were used to prepare this compound according to the general methods. The crude material was subjected to preparative HPLC separation (system A) and lyophilisation to give a white powder. ^{1}H NMR (400 MHz, DMSO): δ 0.80-0.90 (6H, m), 1.05 (3H, d, *J* 6.34), 1.40-1.50 (2H, m), 1.50-1.65 (1H, m), 1.70-2.00 (6H, m), 1.80 (3H, s), 2.15-2.35 (4H, m), 2.60-2.70 (1H, m), 3.60-3.80 (6H, m), 4.00-4.90 (8H, m), 6.95 (1H, s), 7.15 (1H, s), 7.60-8.30 (6H, m). ^{13}C NMR (100 MHz, DMSO): δ 19.6, 21.5, 22.0, 22.9, 23.8, 27.6, 29.2, 30.4, 46.8, 47.7, 50.7, 51.3, 52.3, 58.5, 59.7, 66.4, 68.6, 72.9, 168.7, 169.0, 170.8, 171.4, 173.6, 173.9. HRMS (ESI): calculated for $C_{33}H_{45}N_7O_{16}$ [M+H$^+$] 799.3474, found 799.3514. MS (ESI): *m/z* = 801.22 [M+H$^+$, 100%], *m/z* = 799.21 [M-H$^+$, 100%]. Retention time (system C): t_R 6.27 min. Retention time (system D): t_R 2.08 min. Purity: > 95%. Melting point: > 250 °C (decomposes). Mass of crude peptide: 0.1460 g. Pure peptide obtained from 100 mg crude: 35.5 mg.

Compound 5: Ac-Asn-Pro-Glu-Thr-Gly-Glu-Leu-OH

Wang resin (500 mg, 0.9 mmol/g) and the symmetrical anhydride of Fmoc-Leu-OH were used to prepare this compound according to the general methods. The crude material was subjected to preparative HPLC separation (system A) and

lyophilisation to give a white powder. ^{1}H NMR (400 MHz, DMSO): δ 0.80-0.90 (6H, m), 1.00-1.10 (3H, d, *J* 6.25), 1.40-1.50 (2H, m), 1.60-1.70 (1H, m), 1.80 (3H, s), 1.70-2.25 (10H, m), 2.25-2.70 (4H, m), 3.60-4.30 (10H, m), 4.80 (1H, m), 7.80-8.20 (8H, m). ^{13}C NMR (100 MHz, DMSO): δ 19.3, 21.3, 22.1, 22.8, 24.1, 24.2, 26.1, 27.6, 29.1, 30.1, 30.3, 37.2, 42.1, 45.9, 47.5, 50.5, 51.6, 52.5, 58.3, 60.0, 66.0, 166.6, 168.9, 170.4, 170.6, 171.0, 171.4, 171.5, 172.1, 174.0. HRMS (ESI): calculated for $C_{33}H_{52}N_8O_{15}Na$ [M+Na$^+$] 822.6310, found 822.3618. MS (ESI): *m/z* = 801.28 [M+H$^+$, 100%], *m/z* = 799.25 [M-H$^-$, 100%]. Retention time (system C): t_R 4.64 min. Retention time (system D): t_R 2.18 min. Purity: > 95%. Melting point: > 250 °C (decomposes). Mass of crude product: 0.2414 g. Pure peptide obtained from 100 mg of crude: 31.1 mg.

Compound 6: Ac-Asn-Pro-Glu-Thr-Gly-Glu-Leu-NH$_2$

Rink amide resin (500 mg, 0.59 mmol/g) was used to prepare this compound according to the general methods. The crude material was subjected to preparative HPLC separation (system A) and lyophilisation to give a white powder. ^{1}H NMR (400 MHz, DMSO): δ 0.80-0.90 (6H, m), 1.00-1.10 (3H, d, *J* 6.25), 1.40-1.50 (2H, m), 1.60-1.70 (1H, m), 1.80 (3H, s), 1.70-2.10 (10H, m), 2.20-2.70 (4H, m), 3.60-4.30 (10H, m), 4.70-4.80 (1H, m), 4.80-5.10 (1H, s), 6.90-8.20 (10H, m). ^{13}C NMR (100 MHz, DMSO): δ 19.4, 21.5, 22.1, 22.9, 24.2, 26.0, 27.2, 29.1, 30.1, 30.3, 37.2, 40.6, 42.3, 46.9, 47.5, 50.9, 52.0, 52.6, 58.4, 60.0, 66.4, 168.9, 168.9, 170.5, 170.6, 170.8, 171.4, 171.6, 172.1, 173.9. HRMS (ESI): calculated for $C_{33}H_{53}N_9O_{14}Na$ [M+Na$^+$] 822.6310, found 822.3618. MS (ESI): *m/z* = 801.39 [M+H$^+$, 40%], *m/z* = 798.32 [M-H$^-$, 100%]. Retention time (system C): t_R 6.04 min. Retention time (system D): t_R 2.07 min. Purity: > 95%. Melting point: > 250 °C (decomposes). Mass of crude product: 0.1819 g. Pure peptide obtained from 100 mg of crude: 29.53 mg.

Compound 7: Ac-Asn-Pro-Glu-Thr-Gly-Gln-Leu-OH

Wang resin (500 mg, 0.9 mmol/g) and the symmetrical anhydride of Fmoc-Leu-OH were used to prepare this compound according to the general methods. The crude material was subjected to preparative HPLC separation (system A) and lyophilisation to give a white powder. ^{1}H NMR (400 MHz, DMSO): δ 0.80-0.90 (6H, m), 1.00-1.10 (3H, d, *J* 6.25), 1.50 (2H, m), 1.80 (3H, s), 1.60-2.00 (9H, m), 2.00-2.10 (2H, m), 2.20-2.70 (4H, m), 3.60-3.70 (2H, m), 3.70-3.80 (1H, m), 4.00-4.80 (8H, m), 6.80-7.80 (6H, m), 8.00-8.20 (4H, m). ^{13}C NMR (100 MHz, DMSO): δ 19.4, 21.4, 22.1, 22.8, 24.1, 24.2, 26.2, 28.2, 29.1, 30.5, 31.4, 37.2, 42.3, 46.9, 47.5, 50.8, 52.0, 52.7, 58.2, 60.0, 66.0, 168.5, 168.8, 170.5, 170.7, 171.5, 171.6, 172.7, 174.1. HRMS (ESI): calculated for $C_{33}H_{53}N_9O_{14}Na$ [M+Na$^+$] 822.6310, found 822.3618. MS (ESI): *m/z* = 800.24 [M+H$^+$, 100%], *m/z* = 798.19 [M-H$^+$, 100%]. Retention time (system C): t_R = 6.29 min. Retention time (system D): t_R = 2.12 min. Purity: > 95%. Melting point: > 250 °C (decomposes). Mass of crude product: 0.2371 g. Pure peptide obtained from 100 mg of crude: 15.3 mg.

Compound 8: Ac-Asn-Pro-Glu-Thr-Gly-Gln-Leu-NH$_2$

Rink amide resin (500 mg, 0.59 mmol/g) was used to prepare this compound according to the general methods. The crude material was subjected to preparative HPLC separation (system A) and lyophilisation to give a white powder. ^{1}H NMR (400 MHz, DMSO): δ 0.80-0.90 (6H, m), 1.00-1.10 (3H, d, *J* 6.30), 1.40-1.50 (2H, m), 1.50-1.60 (1H, m), 1.80 (3H, s), 1.70-2.10 (10H, m), 2.20-2.70 (4H, m), 3.60-4.30 (10H, m), 4.80 (1H, m), 6.80-7.50 (6H, m), 7.60-8.30 (6H, m). ^{13}C NMR (100 MHz, DMSO): δ 19.5, 21.4, 22.2, 22.9, 24.1, 27.8, 29.1, 31.4, 37.2, 42.4, 46.9, 47.6, 50.9, 52.4, 58.4, 60.0, 66.4, 168.9, 169.0, 170.7, 171.0, 171.7, 171.8, 172.1, 174.0, 174.1. HRMS (ESI): calculated for $C_{33}H_{54}H_{10}O_{13}$ [M+H$^+$] 799.3950, found 799.3947. MS (ESI): *m/z* = 799.05 [M+H$^+$, 100%], *m/z* = 797.00 [M-H$^+$, 100%]. Retention time (system C): t_R 4.09 min. Retention time (system D): t_R 2.00 min. Purity: > 95%. Melting point: > 250 °C (decomposes). Mass of crude product: 0.1816 g. Pure peptide obtained from 100 mg of crude: 16.87 mg.

Compound 9: Bz-Asp-Pro-Glu-Thr-Gly-Glu-Leu-OH

Wang resin (500 mg, 0.9 mmol/g) and the symmetrical anhydride of Fmoc-Leu-OH were used to prepare this compound according to the general methods. The crude material was subjected to preparative HPLC separation (system A) and lyophilisation to give a white powder. ^{1}H NMR (400 MHz, DMSO): δ 0.70-0.90 (6H, m), 1.05 (3H, d, *J* 6.35), 1.45-1.55 (1H, m,), 1.55-1.80 (2H, m), 1.82-2.35 (8H, m), 2.72 (2H, m), 3.60-3.80 (6H, m), 3.80-5.10 (9H, m), 7.40-7.85 (5H, m), 7.65-8.70 (6H, m), 12.00 (4H, s). ^{13}C NMR (100 MHz, DMSO): δ 19.4, 21.3, 22.8, 24.1, 24.2, 26.2, 27.4, 29.2, 30.4, 42.4, 48.4, 50.5, 51.7, 58.9, 60.0, 66.8, 127.2, 128.1, 131.3, 133.7, 166.0, 168.5, 170.6, 171.1, 171.6, 174.0. HRMS (ESI): calculated for $C_{33}H_{45}N_7O_{16}$ [M+H$^+$] 864.3627, found 864.3640. MS (ESI): *m/z* = 864.35 [M+H$^+$, 100%], *m/z* = 862.30 [M-H$^+$, 100%]. Retention time (system C): t_R 8.69 min. Retention time (system D): t_R 2.58 min. Purity: > 95%. Melting point: > 250 °C (decomposes). Mass of crude peptide: 0.2373 g. Pure peptide obtained from 100 mg crude: 26.1 mg.

Compound 10: St-Asp-Pro-Glu-Thr-Gly-Glu-Leu-OH

Wang resin (500 mg, 0.9 mmol/g) and the symmetrical anhydride of Fmoc-Leu-OH was used to prepare this compound according to the general methods. The crude material was subjected to preparative HPLC separation (system B) and lyophilisation to give a white powder. ^{1}H NMR (400 MHz, DMSO): δ 0.80-0.90 (9H, m), 1.04 (3H, d, *J* 6.34), 1.25 (28H, s), 1.40-1.50 (3H, m), 1.50-1.60 (2H, m), 1.60-1.80 (2H, m), 1.80-2.10 (6H, m), 2.20-2.40 (4H, m), 2.60-2.80 (1H, m), 3.60-4.90 (14H, m), 7.50-8.20 (6H, m). ^{13}C NMR (100 MHz, DMSO): δ 13.9, 19.3, 21.2, 22.0, 22.8, 24.2, 25.0, 26.3, 27.5, 28.5, 28.6, 28.7, 28.8, 28.9, 29.0, 29.9, 30.2, 31.2, 34.7, 46.6, 47.3, 50.2, 51.6, 53.7, 168.4, 171.1, 171.5, 171.8, 173.7, 173.8, 174.0. HRMS (ESI): calculated for $C_{49}H_{83}N_7O_{16}Na$ [M+Na$^+$] 1048.5793, found 1048.5767. MS (ESI): *m/z* = 1026.42

[M+H$^+$, 100%], *m/z* = 1024.42 [M-H$^+$, 100%]. Retention time (system E): t_R 19.84 min. Retention time (system F): t_R 8.10 min. Purity: > 95%. Melting point: > 250 °C (decomposes). Mass of crude peptide: 0.2326 g. Pure peptide obtained from 100 mg crude: 21.5 mg.

Compound 11: St-Asn-Pro-Glu-Thr-Gly-Glu-Leu-OH
Wang resin (500 mg, 0.9 mmol/g) and the symmetrical anhydride of Fmoc-Leu-OH were used to prepare this compound according to the general methods. The crude material was subjected to preparative HPLC separation (system B) and lyophilisation to give a white powder. HRMS (ESI): calculated for $C_{49}H_{84}N_8O_{15}$ [M+H$^+$] 1025.6135, found 1025.6180. MS (ESI): *m/z* = 1025.45 [M+H$^+$, 100%], *m/z* = 1023.53 [M-H$^-$, 100%]. Retention time (system E): t_R 19.41 min. Retention time (system F): t_R 8.02 min. Purity: > 95%. Melting point: > 250 °C (decomposes). Mass of crude product: 0.2861 g. Pure peptide obtained from 100 mg of crude: 26.01 mg.

Compound 12: St-Asn-Pro-Glu-Thr-Gly-Glu-Leu-NH$_2$
Rink amide resin (500 mg, 0.59 mmol/g) was used to prepare this compound according to the general methods. The crude material was subjected to preparative HPLC separation (system B) and lyophilisation to give a white powder. HRMS (ESI): calculated for $C_{49}H_{85}N_9O_{14}$ [M+H$^+$] 1024.6294, found 1024.6311. MS (ESI): *m/z* = 1024.49 [M+H$^+$, 10%], *m/z* = 1023.46 [M-H$^+$, 30%]. Retention time (system E): t_R 19.39 min. Retention time (system F): t_R 8.00 min. Purity: > 95%. Melting point: > 250 °C (decomposes). Mass of crude product: 0.1101 g. Pure peptide obtained from 100 mg of crude: 16.5 mg.

Compound 13: St-Asp-Glu-Glu-Thr-Gly-Glu-Phe-OH
Fmoc-Phe-wang resin (500 mg, 0.65 mmol/g) was used to prepare this compound according to the general methods. The crude material was subjected to preparative HPLC separation (system B) and lyophilisation to give a white powder. ^{1}H NMR (400 MHz, DMSO): δ 0.80-0.90 (3H, m), 1.04 (3H, s), 1.65-2.00 (6H, m), 1.25 (28H, s), 1.50-1.60 (2H, m), 1.60-1.80 (2H, m), 1.85-2.15 (6H, m), 2.20-2.40 (2H, m), 3.50-3.65 (2H, m), 3.65-4.80 (9H, m), 7.20-7.32 (5H, m), 7.65-8.30 (7H, m). ^{13}C NMR (100 MHz, DMSO): δ 14.3, 18.7, 22.5, 24.8, 26.3, 28.5, 28.8, 29.1, 29.4, 29.9, 31.3, 35.6, 36.2, 42.8, 53.7, 56.4, 56.5, 58.9, 61.8, 67.9, 126.3, 127.5, 128.9, 135.9, 170.1, 170.3, 171.1, 173.5, 173.9, 174.8, 178.6. HRMS (ESI): calculated for $C_{52}H_{81}N_7O_{18}Na$ [M+Na$^+$] 1114.5638, found 1114.5679. MS (ESI): *m/z* = 1092.19 [M+H$^+$, 100%], *m/z* = 1090.12 [M-H$^+$, 100%]. Retention time (system E): t_R 18.31 min. Retention time (system F): t_R 7.85 min. Purity: > 95%. Melting point: > 250 °C (decomposes). Mass of crude peptide: 0.2165 g. Pure peptide obtained from 100 mg crude: 18.3 mg.

Compound 14: St-Asp-Pro-Gly-Glu-Glu-Thr-Leu-OH
Wang resin (500 mg, 0.9 mmol/g) and the symmetrical anhydride of Fmoc-Leu-OH were used to prepare this compound according to the general methods. The crude material was subjected to preparative HPLC separation (system B) and lyophilisation to give a white powder. ^{1}H NMR (400 MHz, DMSO): δ 0.80-0.90 (9H, m), 1.04 (3H, d, *J* 6.31), 1.25 (28H, s), 1.40-1.55 (3H, m), 1.60-1.70 (2H, m), 1.70-1.80 (2H, m), 1.80-2.10 (8H, m), 2.20-2.40 (2H, m), 2.65-2.75 (1H, m), 3.60-3.90 (6H, m), 3.90-4.90 (8H, m), 7.70-8.20 (6H, m). ^{13}C NMR (100 MHz, DMSO): δ 12.0, 13.9, 17.8, 19.6, 21.3, 22.0, 22.8, 24.1, 25.1, 27.1, 27.3, 28.5, 28.6, 29.0, 31.2, 34.8, 45.7, 47.2, 47.7, 50.3, 52.0, 58.2, 59.9, 66.5, 70.6, 74.2, 168.7, 169.7, 170.9, 171.8, 172.3, 173.8, 173.9, 174.0. HRMS (ESI): calculated for $C_{33}H_{45}N_7O_{16}$ [M+H$^+$] 1026.5974, found 1026.6011. MS (ESI): *m/z* = 1026.42 [M+H$^+$, 100%], *m/z* = 1024.42 [M-H$^+$, 100%]. Retention time (system E): t_R 17.45 min. Retention time (system F): t_R 7.60 min. Purity: > 95%. Melting point: > 250 °C (decomposes). Mass of crude peptide: 0.2736 g. Pure peptide obtained from 100 mg crude: 22.6 mg.

Compound 15: St-Asp-Glu-Gly-Glu-Glu-Thr-Phe-OH
Fmoc-Phe-wang resin (500 mg, 0.65 mmol/g) was used to prepare this compound according to the general methods. The crude material was subjected to preparative HPLC separation (system B) and lyophilisation to give a white powder. ^{1}H NMR (400 MHz, DMSO): δ 0.80-0.90 (3H, m), 1.02 (3H, s), 1.65-2.20 (6H, m), 1.25 (28H, m), 1.53-1.64 (2H, m), 1.80-2.30 (8H, m), 2.30-2.50 (2H, m), 3.45-3.65 (2H, m), 3.65-4.85 (9H, m), 7.20-7.35 (5H, m), 7.60-8.35 (7H, m). ^{13}C NMR (100 MHz, DMSO): δ 14.1, 18.5, 22.3, 25.4, 26.7, 28.9, 29.0, 29.2, 29.5, 30.1, 31.5, 35.8, 36.4, 41.9, 53.6, 56.5, 56.6, 59.1, 61.4, 67.3, 125.9, 127.6, 128.7, 135.7, 170.0, 170.3, 171.4, 173.7, 174.2, 174.6, 178.9. HRMS (ESI): calculated for $C_{52}H_{81}N_7O_{18}Na$ [M+Na$^+$] 1114.5619, found 1114.5693. MS (ESI): *m/z* = 1093.64 [M+H$^+$, 100%], *m/z* = 1091.53 [M-H$^+$, 100%]. Retention time (system E): t_R 18.34 min. Retention time (system F): t_R 7.87 min. Purity: > 95%. Melting point: > 250 °C (decomposes). Mass of crude peptide: 0.1937 g. Pure peptide obtained from 100 mg crude: 15.1 mg.

Compound 16: Ac-Asp-Pro-Gly-Glu-Glu-Thr-Leu-OH
Wang resin (500 mg, 0.9 mmol/g) and the symmetrical anhydride of Fmoc-Leu-OH were used to prepare this compound according to the general methods. The crude material was subjected to preparative HPLC separation (system A) and lyophilisation to give a white powder. ^{1}H NMR (400 MHz, DMSO): δ 0.80-0.90 (6H, m), 1.05 (3H, d, *J* 6.31), 1.40-1.60 (2H, m), 1.60-1.70 (1H, m), 1.70-2.10 (6H, m), 1.80 (3H, s), 2.15-2.35 (4H, m), 3.60-3.80 (6H, m), 3.90-4.90 (8H, m), 7.70-8.20 (6H, m). ^{13}C NMR (100 MHz, DMSO): δ 19.6, 21.4, 22.2, 22.8, 24.1, 27.1, 27.3, 29.1, 30.1, 36.6, 42.1, 46.8, 47.3, 50.3, 51.7, 52.0, 58.1, 60.0, 66.5, 168.7, 168.8, 169.7, 170.0, 170.9, 171.8, 172.4, 173.8, 173.9, 174.0. HRMS (ESI): calculated for $C_{33}H_{45}N_7O_{16}$ [M+H$^+$] 802.3470, found 802.3485. MS (ESI): *m/z* = 802.11 [M+H$^+$, 70%], *m/z* = 800.09 [M-H$^+$, 100%].

Retention time (system D): t_R 2.15 min. Purity: > 95%. Melting point: > 250 °C (decomposes). Mass of crude peptide: 0.2571 g. Pure peptide obtained from 100 mg crude: 37.0 mg.

Compound 17: Ac-Asp-Glu-Gly-Glu-Glu-Thr-Phe-OH

Fmoc-Phe-Wang resin (500 mg, 0.65 mmol/g) was used to prepare this compound according to the general methods. The crude material was subjected to preparative HPLC separation (system A) and lyophilisation to give a white powder. ^{1}H NMR (400 MHz, DMSO): δ 1.04 (3H, d, *J* 6.33), 1.68-2.01 (6H, m), 1.81 (3H, s), 2.19-2.32 (6H, m), 2.60-2.91 (2H, m), 3.03 (1H, dd, *J* 13.64, 6.11), 3.55-3.71 (2H, m), 3.88-3.97 (1H, m), 4.25-4.68 (6H, m), 7.19-7.31 (5H, m), 7.68-8.21 (7H, m). ^{13}C NMR (100 MHz, DMSO): δ 24.2, 27.9, 33.1, 35.0, 35.4, 41.2, 41.5, 54.6, 56.9, 57.0, 58.5, 64.0, 132.1, 133.7, 142.9, 174.1, 174.7, 175.3, 176.1, 176.2, 176.3, 177.2, 178.1, 179.3, 179.5. HRMS (ESI): calculated for $C_{36}H_{49}N_7O_{18}$ [M+H$^+$] 868.3212, found 868.3215. MS (ESI): *m/z* = 869.35 [M+H$^+$, 100%], *m/z* = 867.19 [M-H$^+$, 100%]. Retention time (system C): t_R 5.95 min. Retention time (system D): t_R 2.27 min. Purity: > 95%. Melting point: > 250 °C (decomposes). Mass of crude peptide: 0.2385 g. Pure peptide obtained from 100 mg crude: 26.0 mg

Compound 18: St-Lys(RITC)-Asn-Pro-Glu-Thr-Gly-Glu-Leu-OH

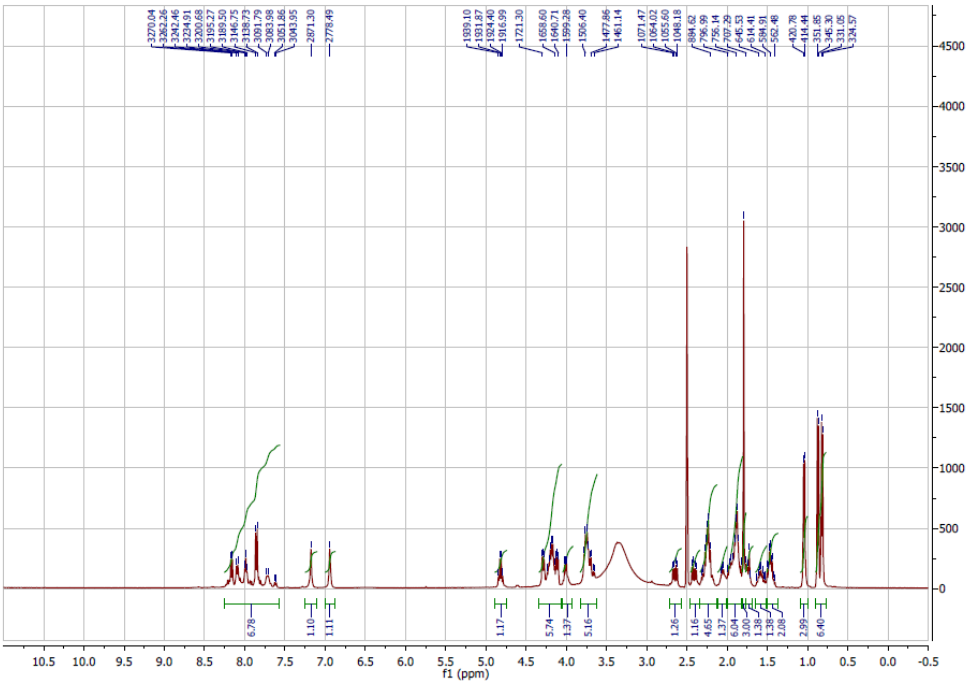
Wang resin (500 mg, 0.9 mmol/g) and the symmetrical anhydride of Fmoc-Leu-OH were used to prepare this compound according to the general methods. The crude material was subjected to preparative HPLC separation (system B) and lyophilisation to give a pink powder. HRMS (ESI): calculated for $C_{84}H_{126}N_{13}O_{19}S$ [M+H$^+$]^2 826.9546, found 826.9565. MS (ESI): *m/z* = 827.3 [M-826.7, 100%], *m/z* = 1653.3 [M-H$^+$, 10%], *m/z* = 825.45 [M-825.55, 100%]. Retention time (system E): t_R 18.51 min. Retention time (system F): t_R 7.83 min. Purity: > 95%. Melting point: > 250 °C (decomposes). Mass of crude product: 0.4578 g. Pure peptide obtained from 100 mg of crude: 12.3 mg.

Compound 19: St-Lys(RITC)-Asn-Pro-Glu-Thr-Gly-Glu-Leu-NH_2

Rink amide resin (500 mg, 0.59 mmol/g) was used to prepare this compound according to the general methods. The crude material was subjected to preparative HPLC separation (system B) and lyophilisation to give a pink powder. HRMS (ESI): calculated for $C_{84}H_{127}N_{14}O_{18}S$ [M+H$^+$] 1651.9174, found 1651.9098. MS (ESI): *m/z* = 817.97 [M-835.13, 100%], *m/z* = 1652.70 [M-H$^+$, 10%], *m/z* = 824.41 [M-828.69, 100%]. Retention time (system E): t_R 18.74 min. Retention time (system F): t_R 7.92 min. Purity: > 95%. Melting point: > 250 °C (decomposes). Mass of crude product: 0.1827 g. Pure peptide obtained from 100 mg of crude: 12.8 mg.

^{1}H-NMR Spectra

Compound **4**



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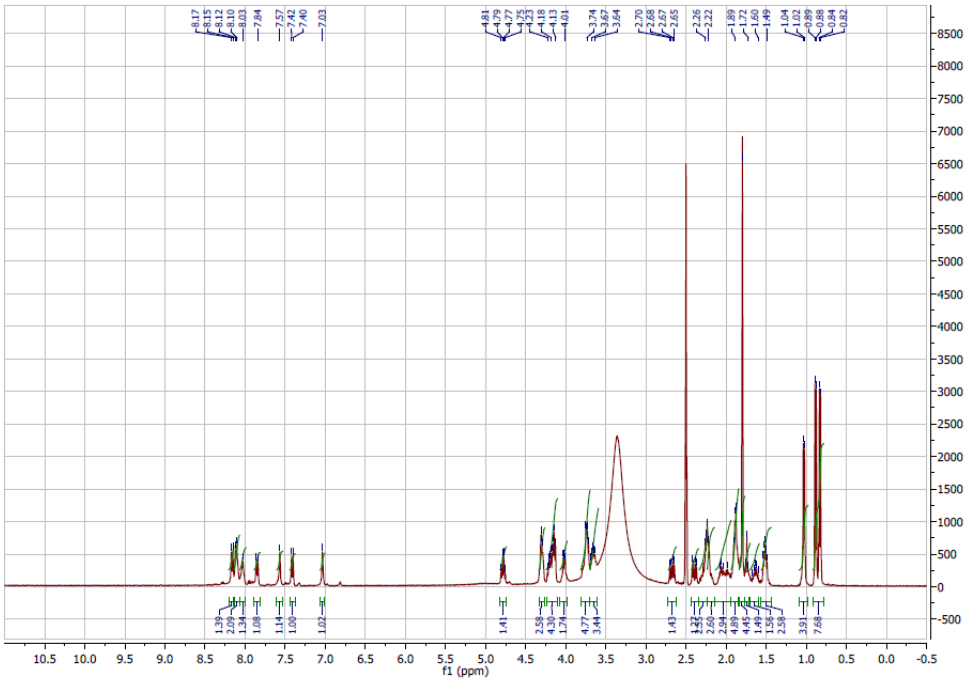

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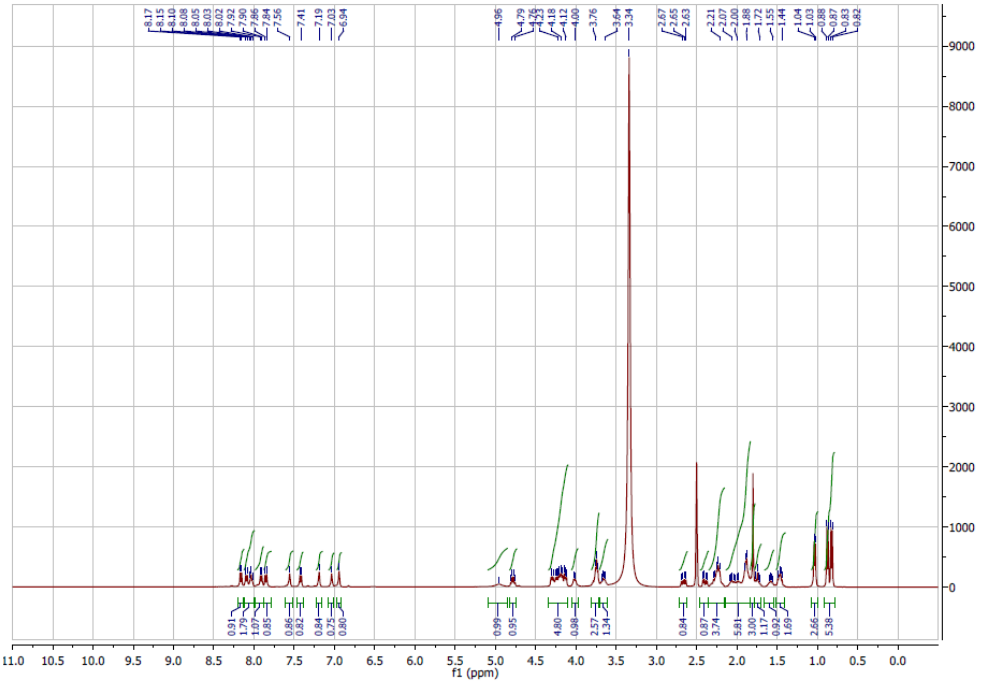

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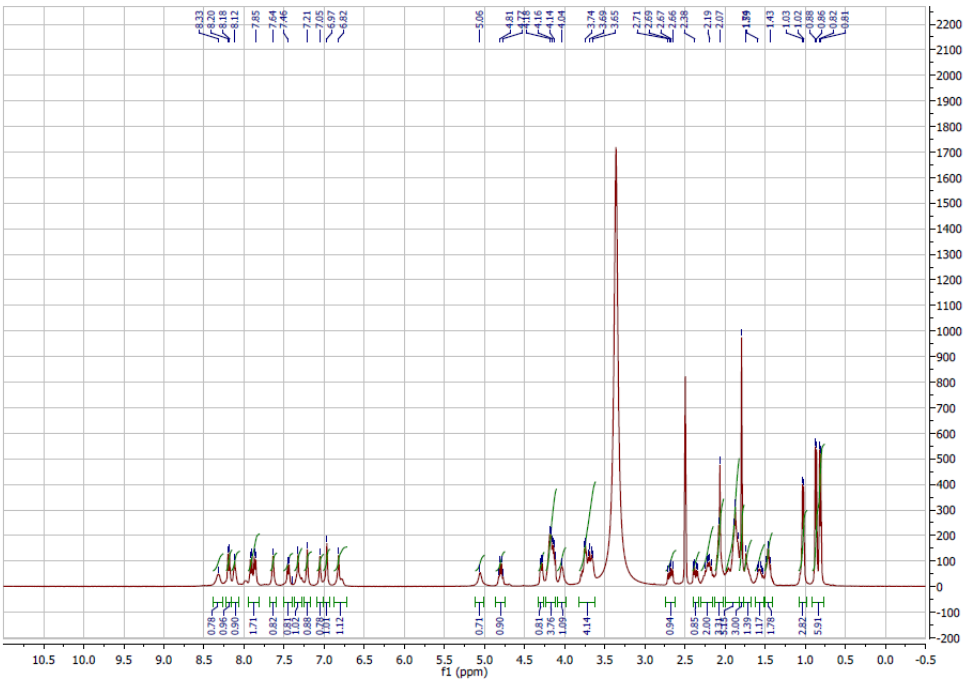

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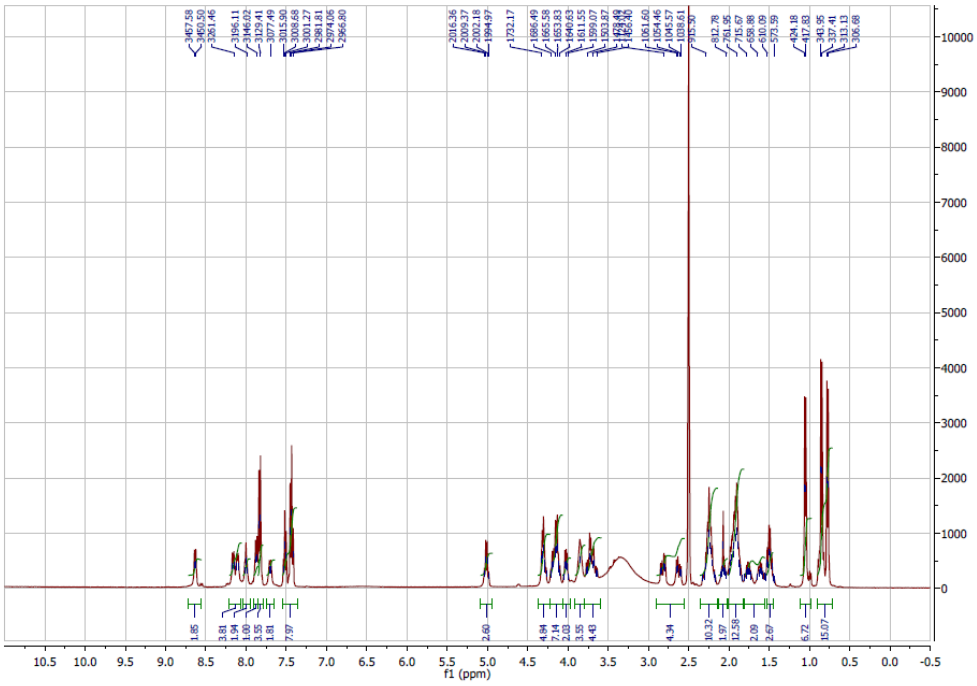

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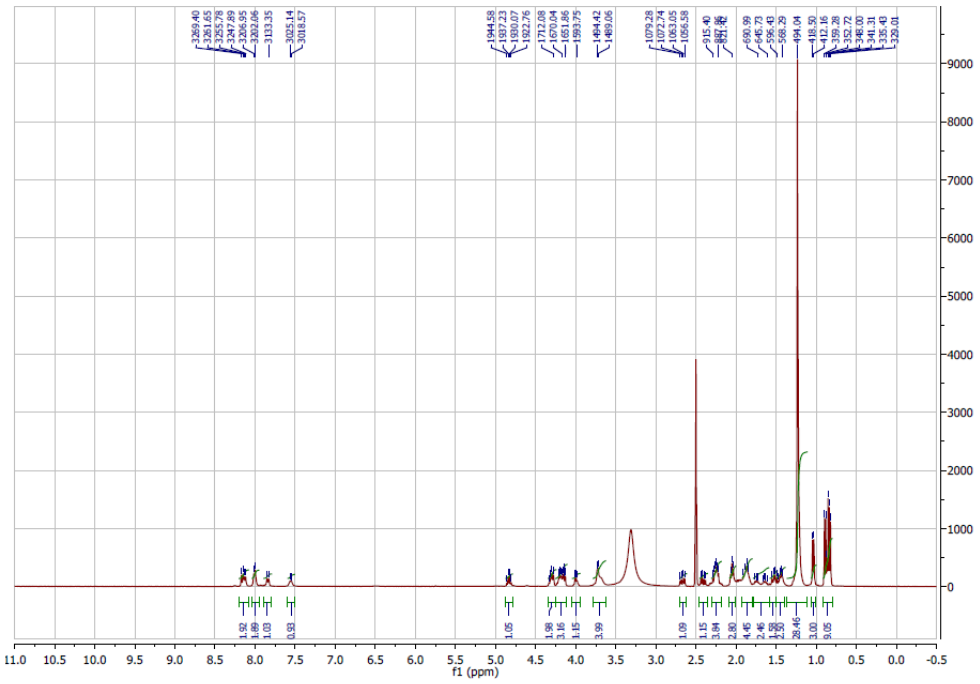

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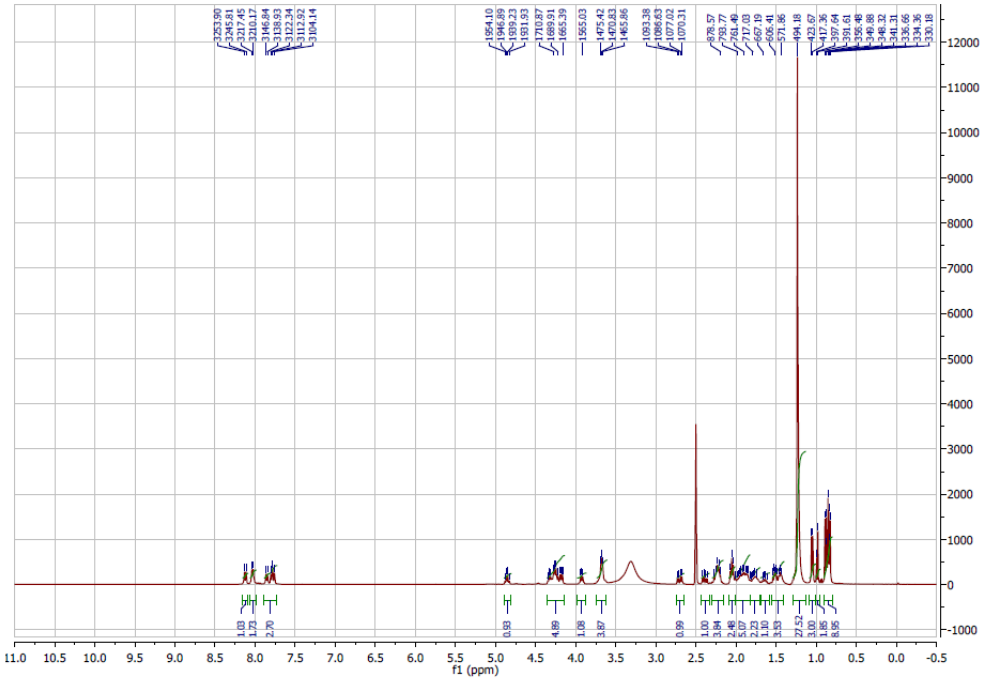

Compound **16**