Supporting information

APPLICATION OF REVERSIBLE DETECTION METHOD FOR N-TERMINUS AMINO GROUPS: SOLID PHASE SYNTHESIS OF STYLISSATIN B

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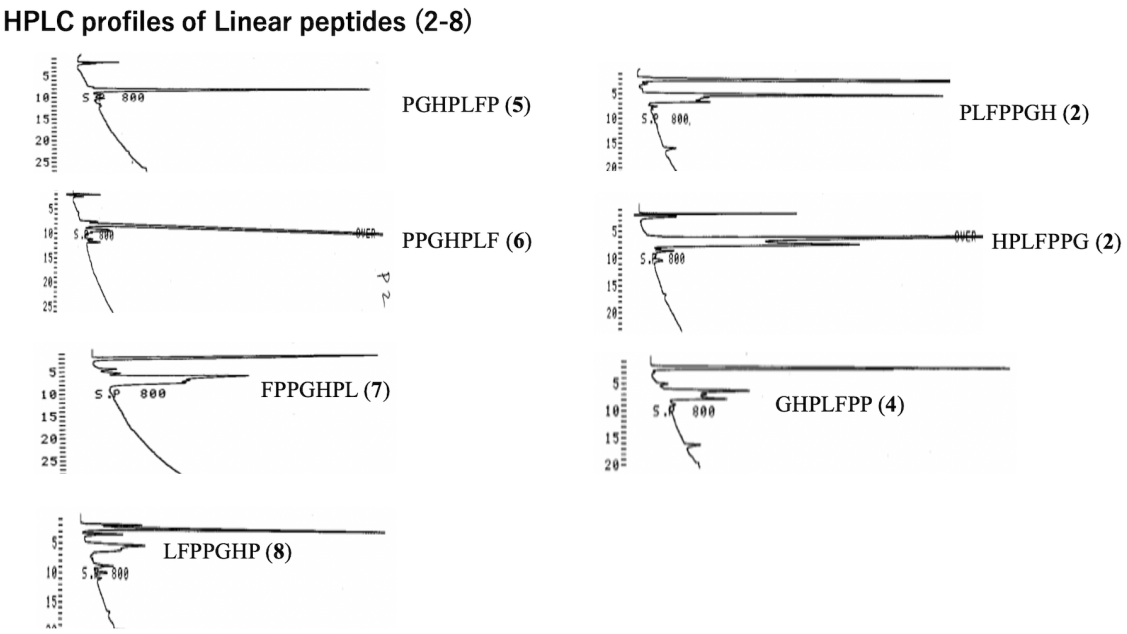


Figure S1. HPLC profiles of linear peptides (**2-8**). RP-HPLC conditions: flow rate of 1 mL/min, linear gradient from 10% to 90% MeCN over 30 min.

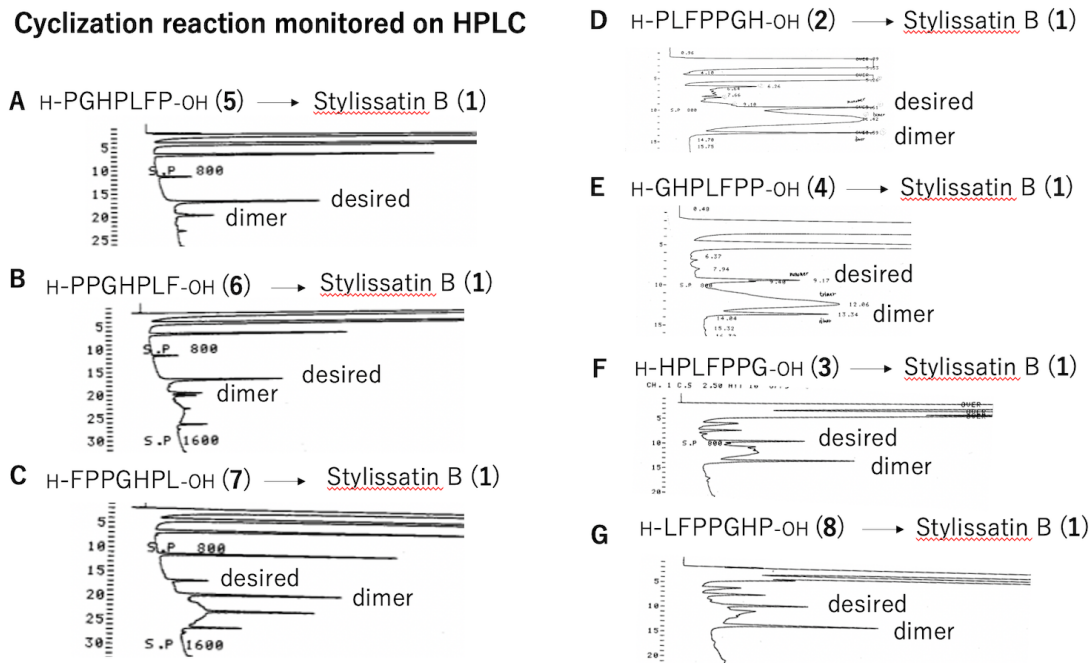


Figure S2. Cyclization of linear peptides (**2-8**) to yield the stylisattin B (**1**) and its dimer (After 24 h). RP-HPLC conditions: flow rate of 1 mL/min, linear gradient from 10% to 50% MeCN over 30 min (charts A-C) or 10% to 90% MeCN over 30 min (charts D-G).

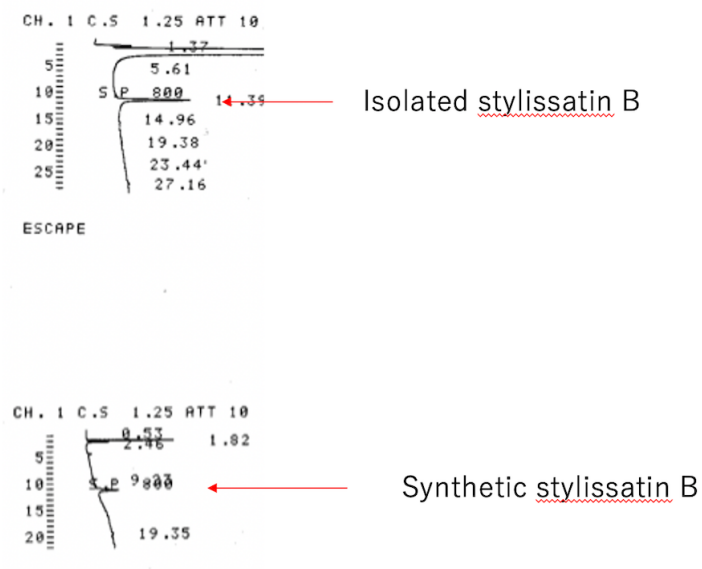


Figure S3. HPLC profiles of isolated stylissatin B and synthetic stylissatin B. RP-HPLC conditions: flow rate of 1 mL/min, linear gradient from 20% to 40% MeCN over 30 min.

EXPERIMENTAL

General All solvents were reagent grade. All commercial reagents were of the highest purity available. Optical rotations were determined with a JASCO P-2200 polarimeter at the sodium D line. IR spectra were recorded on a Spectrum Two (PerkinElmer, Waltham, MA, USA). ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) spectra were recorded on JNM-ECA600 spectrometer. Chemical shifts are reported in ppm with reference to solvent signals [^{1}H NMR: DMSO-d6 (2.50); ^{13}C NMR: DMSO-d6 (39.5)]. Mass spectra were obtained using JEOL AccuTOF JMS-T100LC (ESI-MS). Analytical HPLC was carried out using a COSMOSIL 5C_{18}-AR-II column (4.6ID×150 mm) eluting with a linear gradient of MeCN (0.1% TFA) in H_2O (0.1% TFA) over a run time of 30 min (flow rate of 1 ml/min), a HITACHI L-2400 as a UV-Vis detector, and a HITACHI L-2130 pump. Preparative HPLC was performed with a COSMOSIL 5C_{18}-AR-II column (10ID x 250 mm) eluting with a linear gradient of MeCN (0.1% TFA) in H_2O (0.1% TFA) at a run time of 30 min (flow rate of 2 mL/min), on a HITACHI L-2400 as a UV-Vis detector, HITACHI L-2130 Pump. UV measurements were recorded at a wavelength of 220 nm for the peptide analyses.

PLFPPGH (2) (20 mg, 27 μmol, 32.8%) ^{1}H-NMR (600 MHz, DMSO-d6) δ 8.81 (1H, s), 8.18 (1H, d, J = 9.0 Hz), 8.06 (2H, brs), 7.82 (1H, d, J = 9.6 Hz), 7.31 (1H, s), 7.15 (5H, m), 4.82 (1H, m), 4.63 (1H, m), 4.51 (1H, m), 4.25 (1H, m), 4.17 (1H, m), 4.13 (1H, m), 3.49 (8H, m), 3.02 (1H, m), 2.93 (1H, m), 2.91 (1H, m), 2.68 (1H, m), 2.06 (2H, m), 1.96 (1H, m), 1.86 (1H, m), 1.85 (8H, m), 1.47 (1H, m), 1.27 (2H, m), 0.77 (3H, d, J = 8.4 Hz), 0.70 (1H, d, J = 7.8 Hz). ^{13}C-NMR (150 MHz, DMSO-d6) δ 180.5, 173.8, 172.1, 170.4, 169.4, 168.3, 166.2, 134.4, 133.7, 129.7, 128.8, 128.4, 126.7, 121.1, 59.8, 58.9, 58.0, 52.0,

51.8, 47.2 (C2), 47.0 (C2), 44.0, 36.0, 29.6 (C2), 28.8, 27.6, 25.9, 24.9, 24.8, 24.6, 23.3, 21.8. IR (ATR) ν max cm^{-1}: 3279, 2959, 1666, 1633, 1544, 1440, 1200, 1178, 1130, 1024, 826, 800, 720. HRMS (ESI) *m/z*: calcd. for $C_{38}H_{54}N_9O_8$ $[M+H]^+$ 764.4088, found 764.4095. RP-HPLC (flow rate of 1 mL/min): t_R = 8.18 min (linear gradient from 10% to 90% MeCN over 30 min). $[\alpha]_D^{28}$ = -452.9 (c 0.295, MeOH).

HPLFPPG (3) (39 mg, 52 µmol, 65%) ^{1}H-NMR (600 MHz, DMSO-d6) δ 8.65 (1H, brs), 8.37 (1H, brs), 7.94 (1H, brs), 7.76 (1H, brs), 7.43 (1H, brs), 7.22 (1H, s), 7.00 (5H, m), 4.51 (1H, m), 4.34 (1H, m), 4.26 (1H, m), 4.16 (1H, m), 4.03 (1H, m), 4.20 (1H, m), 3.39 (8H, m), 3.06 (2H, m), 2.91 (1H, m), 2.79 (1H, m), 2.55 (1H, m), 2.01 (1H, m), 1.91 (1H, m), 1.66 (9H, m), 1.33 (1H, m), 1.20 (1H, m), 1.18 (1H, m), 0.62 (3H, m), 0.56 (3H, m). ^{13}C-NMR (150 MHz, DMSO-d6) δ 173.8, 172.6, 171.5, 171.3, 170.6, 169.2, 166.2, 137.5, 134.2, 129.5, 128.5, 119.2, 118.2, 115.9, 59.7, 59.4, 58.0, 52.0 (C2), 51.1, 47.2, 47.2, 47.0, 46.6, 44.0, 36.0, 33.0, 29.6, 28.8, 28.0, 24.8, 24.7, 24.3, 24.2, 22.8, 21.7. IR (ATR) ν max cm^{-1}: 3280, 2958, 1638, 1535, 1449, 1199, 1130, 1024, 827, 798, 719. $[\alpha]_D^{30}$ = -65.9 (c 1.3, MeOH).

GHPLFPP (4) (19 mg, 25 µmol, 31.8%) ^{1}H-NMR (600 MHz, DMSO-d6) δ 8.84 (1H, s), 8.60 (1H, d, J = 7.8 Hz), 8.43 (1H, d, J = 6.6 Hz), 8.14 (1H, d, J = 9.6 Hz), 8.06 (1H, d, J = 9.6 Hz), 7.25 (1H, brs), 7.18 (5H, m), 4.60 (1H, m), 4.50 (1H, m), 4.48 (1H, m), 4.25 (2H, m), 4.13 (1H, m), 3.72 (2H, m), 3.64 (1H, m), 3.48 (1H, m), 3.15 (5H, m), 3.00 (1H, m), 2.88 (1H, m), 2.69 (1H, m), 2.15 (1H, m), 2.10 (1H, m), 2.02 (1H, m), 1.90 (1H, m), 1.78 (8H, m), 1.47 (1H, m), 1.32 (2H, m), 0.81 (3H, d, J = 8.4 Hz), 0.77 (3H, d, J = 7.8 Hz).^{13}C-NMR (150 MHz, DMSO-d6) δ 172.8, 172.1, 171.6, 170.7, 169.5, 169.4, 168.3,

138.0, 134.1, 129.8, 129.6, 128.8, 128.5, 117.3, 60.2, 59.6, 59.1, 51.9, 51.7, 47.4, 46.1 (C2), 45.1, 42.5, 42.3, 36.0, 30.0, 29.5, 28.1, 26.6, 25.1, 25.0, 24.5, 23.5, 21.8. IR (ATR) ν max cm^{-1}: 3266, 2958, 1630, 1534, 1436, 1199, 1129, 1024, 827, 798, 719. $[\alpha]_D^{27}$ = -73.3 (c 1.36, MeOH).

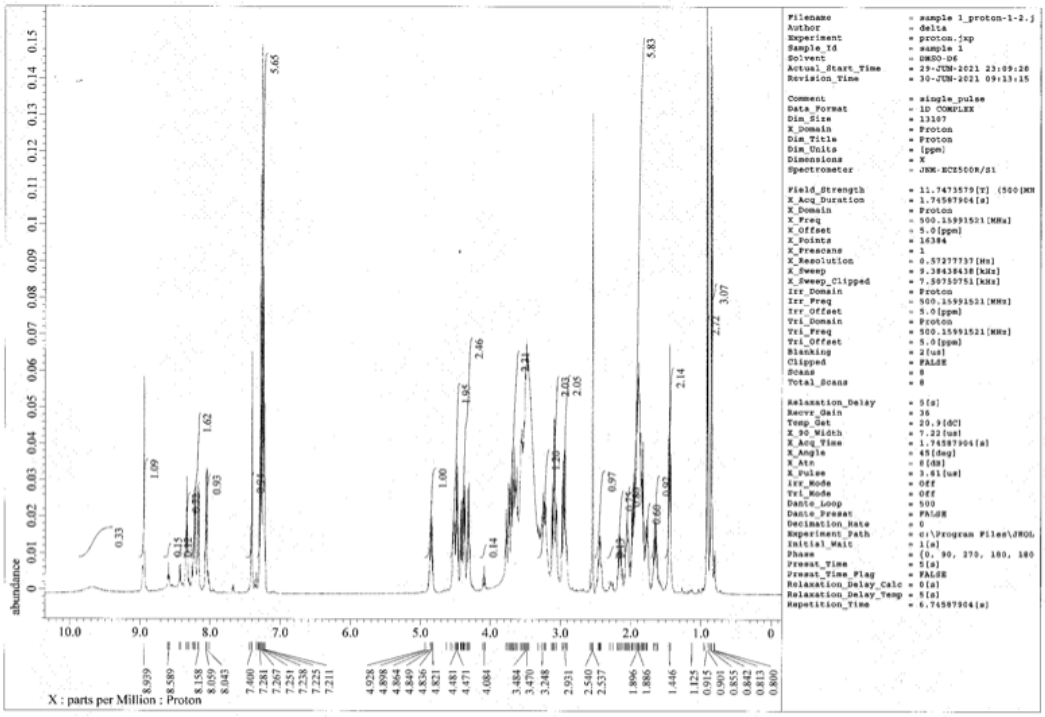
PGHPLFP (5) (32 mg, 42 μmol, 53.0%) ^{1}H-NMR (600 MHz, DMSO-d6) δ 8.94 (1H, s), 8.33 (1H, t, *J* = 6.6 Hz), 8.22 (1H, d, *J* = 9.6 Hz), 8.17 (1H, d, *J* = 9.0 Hz), 8.04 (1H, d, *J* = 9.6 Hz), 7.40 (1H, s), 7.30 (2H, m), 7.24 (1H, m), 7.23 (2H, m), 4.86 (1H, q, *J* = 9.0 Hz), 4.45 (1H, t, *J* = 6.0 Hz), 4.40 (1H, dt, *J* = 9.6, 6.6 Hz), 4.36 (1H, dd, *J* = 9.6, 6.0 Hz), 4.29 (1H, dd, *J* = 10.2, 5.4 Hz), 4.23 (1H, q, *J* = 8.4 Hz), 3.76 (2H, m), 3.75 (2H, m), 3.55 (2H, m), 3.55 (2H, m), 3.10 (1H, dd, *J* = 10.2, 6.0 Hz), 3.07 (1H, dd, *J* = 10.2, 6.0 Hz), 2.97 (1H, dd, *J* = 17.4, 9.0 Hz), 2.91 (1H, dd, *J* = 17.4, 9.0 Hz), 2.45 (1H, m), 2.15 (1H, m), 2.05 (1H, m), 1.93 (1H, m), 1.85 (2H, m), 1.80 (3H, m), 1.80 (2H, m), 1.75 (1H, m), 1.66 (1H, m), 1.45 (2H, m), 0.91 (3H, d, *J* = 8.4 Hz), 0.85 (3H, d, *J* = 7.8 Hz). ^{13}C-NMR (150 MHz, DMSO-d6) δ 173.6, 172.1, 172.0, 169.6, 169.0, 168.7, 168.6, 137.4, 133.8, 129.7, 129.0, 128.3, 126.6, 117.9, 60.0, 59.3, 59.1, 51.9, 51.7, 50.1, 47.3, 46.8, 46.0, 42.0, 41.1, 37.0, 30.2, 30.2, 29.8, 28.9, 24.8, 24.7, 24.4, 23.8, 23.1, 22.6. IR (ATR) ν max cm^{-1}: 3283, 2959, 1665, 1633, 1539, 1438, 1198, 1179, 1129, 1024, 827, 790, 719. $[\alpha]_D^{26}$ = -75.3 (c 2.0, MeOH).

FPPGHPL (7) (22 mg, 29 μmol, 36.8%) ^{1}H-NMR (600 MHz, DMSO-d6) δ 8.82 (1H, s), 8.30 (1H, d, *J* = 11.4 Hz), 8.26 (1H, d, J = 9.6 Hz), 8.24 (1H, t, *J* = 6.6 Hz), 8.09 (1H, d, *J* = 10.2 Hz), 7.30 (6H, m), 4.79 (1H, q, *J* = 8.4 Hz), 4.60 (1H, m), 4.38 (1H, m), 4.33 (1H, m), 4.32 (1H, m), 4.21 (1H, m), 3.72 (2H, m), 3.68 (2H, m), 3.61 (2H, m), 3.55 (2H,

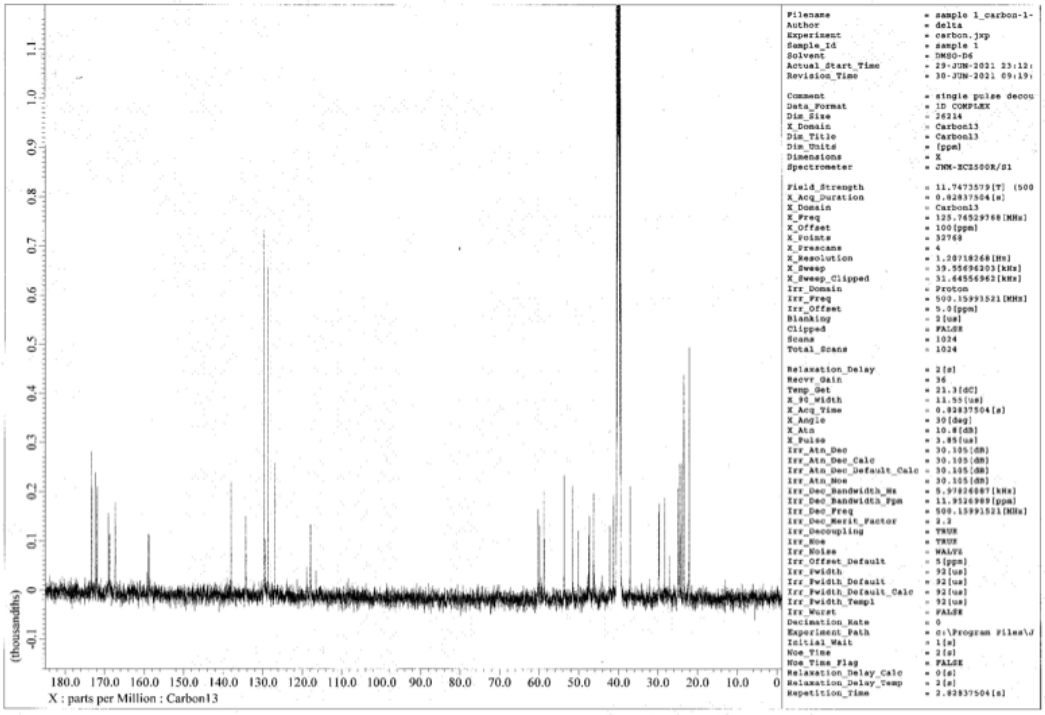
m), 3.06 (1H, m), 3.03 (1H, m), 2.91 (1H, m), 2.89 (1H, m), 2.20 (1H, m), 2.08 (1H, m), 2.05 (1H, m), 1.95 (1H, m), 1.85 (6H, m), 1.80 (2H, m), 1.67 (1H, m), 1.50 (2H, m), 0.89 (3H m), 0.82 (3H, m). ^{13}C-NMR (150 MHz, DMSO-d6) δ 174.5, 173.9, 172.4, 170.2, 169.1, 168.4, 166.8, 134.7, 130.4, 129.0, 128.8, 127.7, 116.3, 60.2, 59.8, 58.3, 52.8, 52.6, 50.8, 47.5(C2), 47.3, 47.1, 42.2, 36.0, 29.8(C2), 29.7, 28.5, 25.3, 25.0, 24.8, 24.7, 23.4, 21.8. IR (ATR) ν max cm^{-1}: 2958, 1628, 1532, 1447, 1182, 1130, 833, 798, 720. $[\alpha]_D^{28}$ = -50.0 (c 1.80, MeOH).

LFPPGHP (8) (21 mg, 28 μmol, 35.0%) ^{1}H-NMR (600 MHz, DMSO-d6) δ 8.60 (1H, s), 8.22 (1H, brs), 7.98 (2H, brs), 7.87 (1H, brs), 7.03 (6H, m), 4.61 (2H, m), 4.35 (3H, m), 4.02 (1H, m), 3.50 (6H, m), 3.28 (2H, m), 2.86 (2H, m), 2.71 (1H, m), 2.64 (1H, m), 1.93 (1H, m), 1.83 (2H, m), 1.71 (2H, m), 1.63 (7H, m), 1.40 (1H, m), 1.31 (2H, m), 0.63 (6H, m). ^{13}C-NMR (150 MHz, DMSO-d6) δ 173.6, 172.9, 172.0, 170.7, 169.4, 169.1, 168.2, 137.5, 133.7, 129.4, 129.0, 126.7, 128.5, 117.5, 60.2, 59.4, 58.1, 52.3, 51.5, 50.5, 47.2, 47.1 (C2), 36.0, 29.3, 24.6, 47.2, 42.0, 28.8, 28.3, 27.6, 26.0, 24.8, 23.6, 22.9, 21.6. IR (ATR) ν max cm^{-1}: 3280, 2960, 1633, 1536, 1445, 1199, 1179, 1130, 1024, 826, 800, 719. $[\alpha]_D^{28}$ = -20.9 (c 1.78, MeOH).

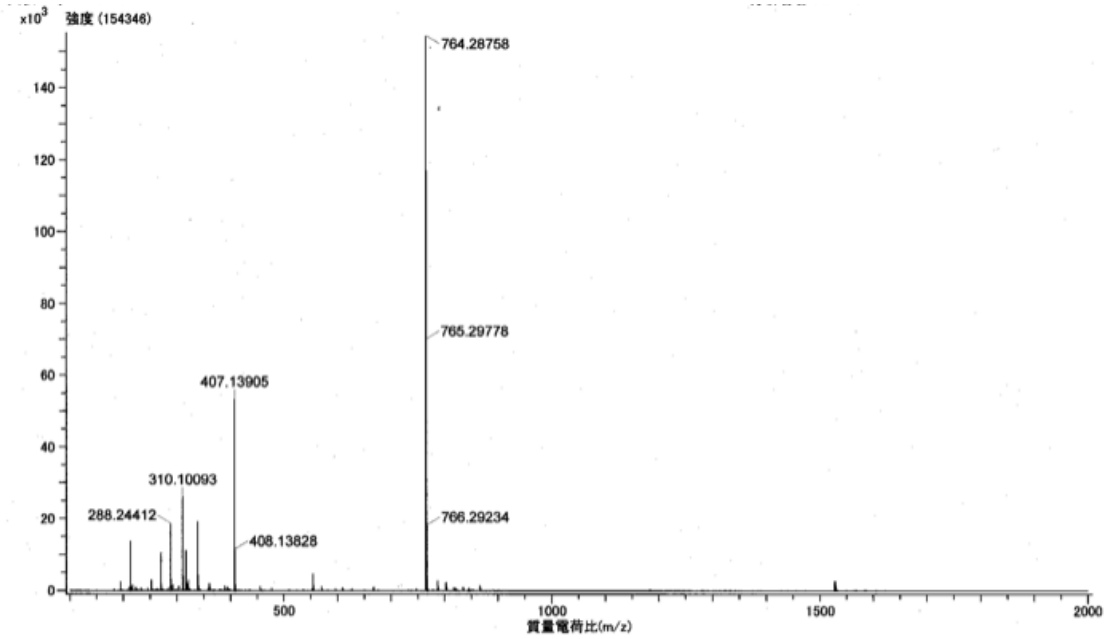
^{1}H-NMR of PPGHPLF (**6**)



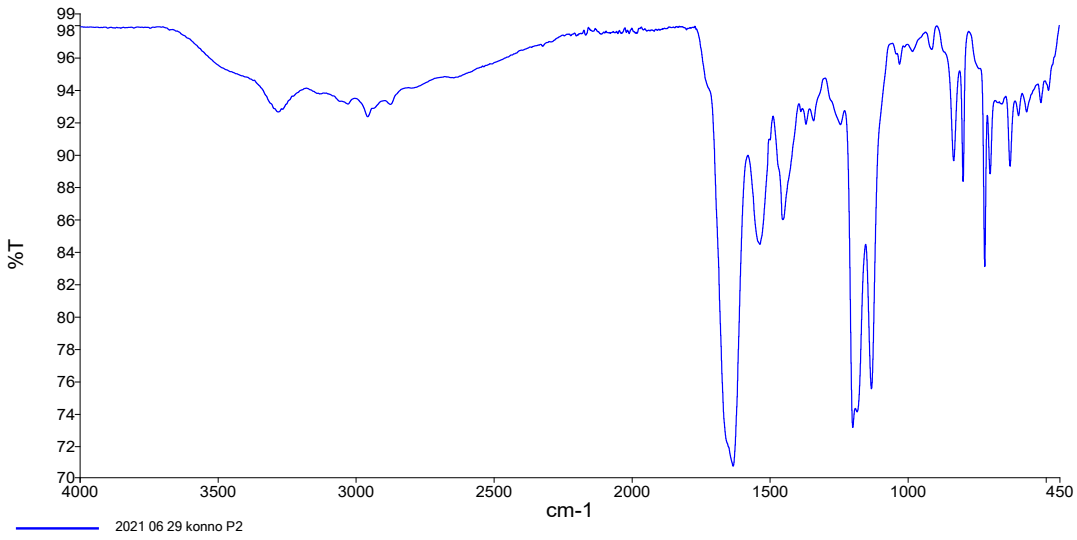
^{13}C-NMR of PPGHPLF (**6**)



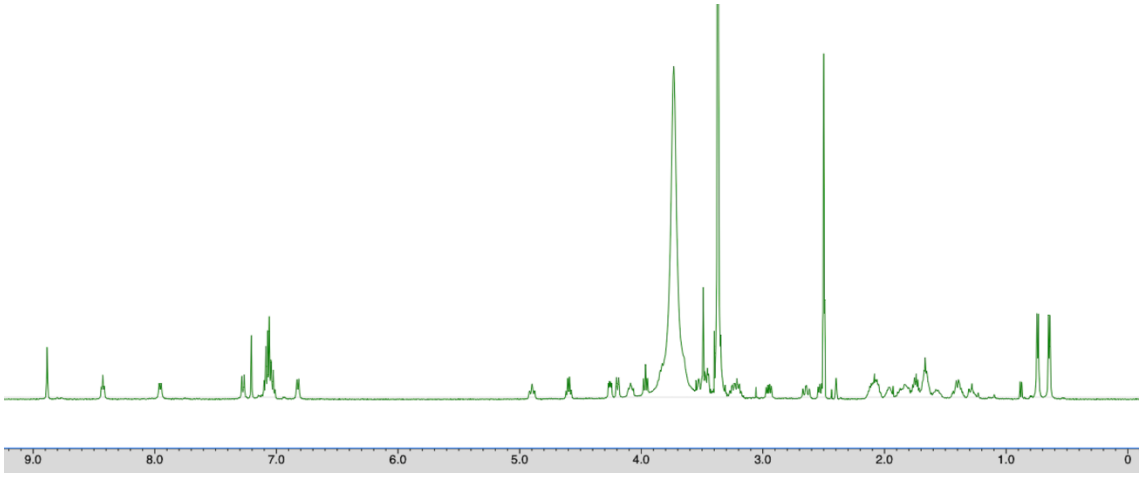
ESI-MS of PPGHPLF (**6**)



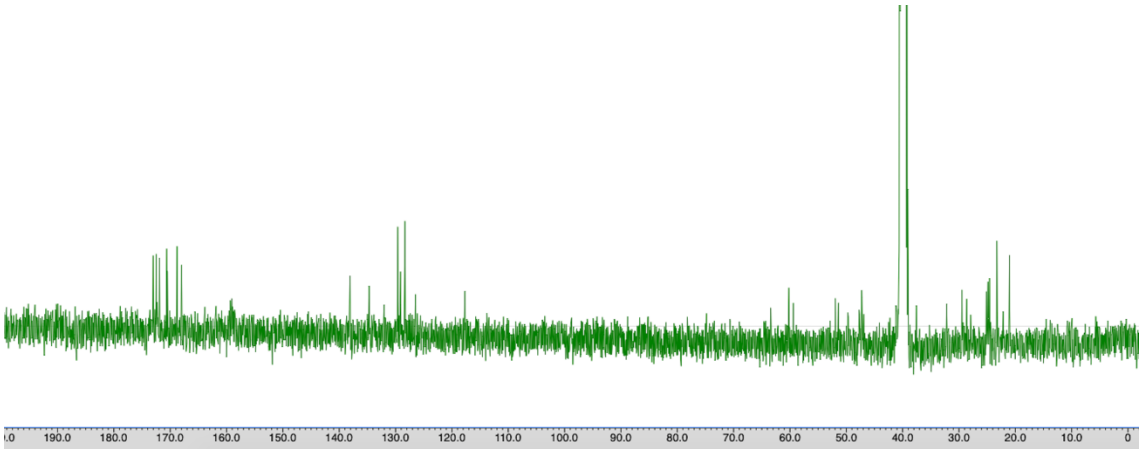
IR (ATR) of PPGHPLF (**6**)



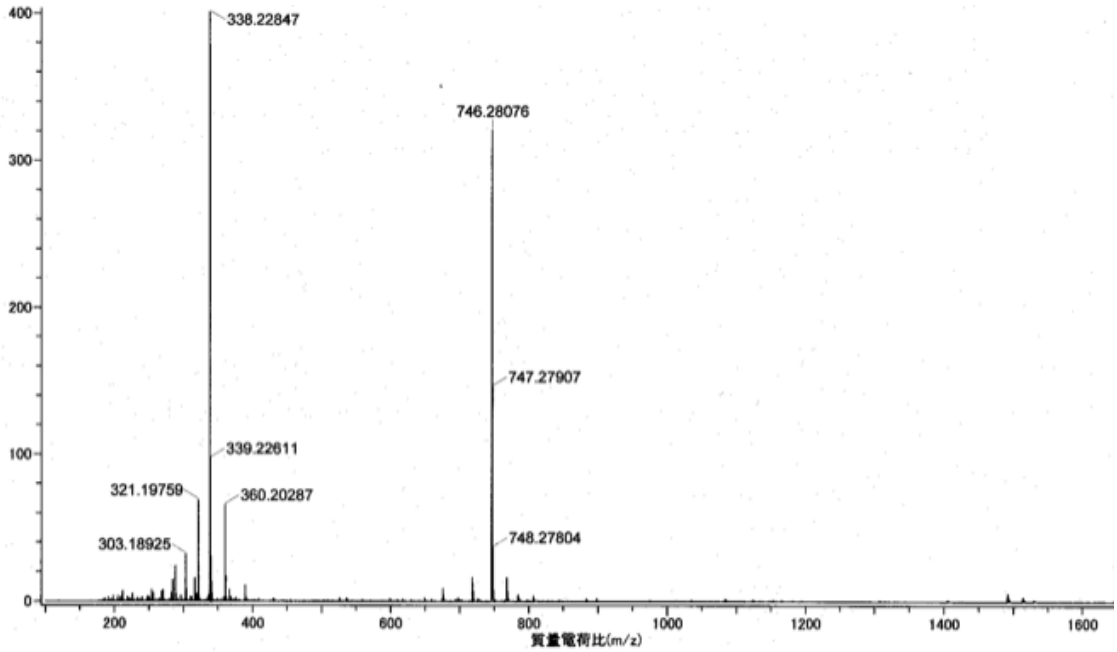
^{1}H-NMR of Stylisattin B (**1**)



^{13}C-NMR of Stylisattin B (**1**)



ESI-MS of Stylisattin B (**1**)



IR (ATR) of Stylisattin B (**1**)