Total Synthesis of Grassypeptolide

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General Methods

Unless otherwise stated, reagents were commercially available and used without further purification. All solvents were distilled prior to use: THF was distilled from Na/benzophenone, dichloromethane and DMF were distilled from CaH2, methanol was distilled under a N2 atmosphere from Mg / I2. Triethylamine, NMM, 2,6-lutidine, pyridine and diisopropylethylamine were distilled from CaH2. All non-aqueous reactions were performed under an atmosphere of nitrogen or argon using oven-dried glassware and standard syringe/septa techniques. NMR spectra were recorded on Bruker Avance AV-500 or DRX-300 instruments and calibrated using residual undeuterated chloroform (δH = 7.26 ppm) or CDCl3 (δC = 77.0 ppm) as an internal reference. Data are reported as follows: chemical shift as δ values (ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant in Hz, integration. High resolution mass spectra were measured on ABI Q-star Elite. Optical rotations were measured on a Perkin-Elmer 351 polarimeter at 589 nm with a 100 mm path length cell at 20°C (reported as follows: concentration (c in g/100mL), solvent). Thin-layer chromatography (TLC) was carried out using pre-coated sheets (Qingdao silica gel 60-F250, 0.2 mm) which, after development, were visualized directly under UV light at 254nm or I2, and/or staining in ninhydrin or phosphomolybdic acid solution followed by heating. Flash column chromatography was performed using the indicated solvents (with Rf = 0.2-0.3 for the desired component) on E. Qingdao silica gel 60 (230-400 mesh ASTM) or on E. Merck silica gel 60 (230-400 mesh ASTM). Yields refer to chromatographically purified compounds, unless otherwise stated.
To a solution of acid 11 (313 mg, 1.25 mmol) in CH₂Cl₂ (10 mL) at 0 °C, (COCl)₂ (255 μL, 3.0 mmol) was added, followed by DMF (10 μL, 10% in CH₂Cl₂, v/v). The reaction mixture was stirred for 4 h while slowly warming to room temperature and then concentrated in vacuo. The residue was dissolved in CH₂Cl₂ (10 mL) and concentrated again, these procedures were repeated three times to ensure complete removal of excess (COCl)₂. The residue, after being dried under high vacuum for 2h, was dissolved in CH₂Cl₂ (10 mL) and transformed via syringe to a solution of 10 (144 mg, 0.33 mmol) in CH₂Cl₂ (10 mL) at 0 °C. After DMAP (20 mg, 0.16 mmol) and NMM (330 μL, 3.0 mmol) were added, the reaction mixture was stirred overnight at room temperature. The reaction was quenched by addition of saturated aqueous solution of NaHCO₃ (20 mL). Layers were separated; the aqueous phase was further extracted with diethyl ether (20 mL x 2). The combined organics were washed sequentially with saturated aqueous solution of NaHCO₃ (20 mL x 2) and brine (20 mL), dried over anhydrous Na₂SO₄, filtered and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc : Hexanes = 1 : 2, v/v) on silica gel (Qing Dao) to give 4 (200 mg, 90%) as a colorless oil. 

[α]$_{D}^{20}$ -29.9 (c 2.2, CH₂Cl₂), $^1$H NMR (500 MHz, CDCl₃): δ 7.38-7.28 (m, 5H), 7.22-7.15 (m, 5H), 6.00 (d, $J = 10.0$ Hz, 1H), 5.38 (dd, $J = 6.3$, 7.5 Hz, 1H), 5.13 (br, s, 2H), 5.05 (d, $J = 13.0$ Hz, 1H), 4.29 (dd, $J = 4.3$, 8.6 Hz, 1H), 3.95-3.91 (m, 1H), 3.72-3.68 (m, 2H), 3.06-3.03 (m, 2H), 3.01 (s, 3H), 2.62-2.59 (m, 1H), 2.30-2.21 (m, 1H), 2.18-2.11 (m, 1H), 2.02-1.83 (m, 3H), 1.45 (s, 9H), 1.18 (d, $J = 7.2$ Hz, 3H), 1.07 (d, $J = 6.9$ Hz, 3H), 0.99 (d, $J = 6.3$ Hz, 3H), 0.84 (d, $J = 6.6$ Hz, 3H) ppm; $^{13}$C NMR (125 MHz, CDCl₃) δ 173.3, 171.0, 170.2, 168.6, 156.2, 137.0, 135.6, 129.1, 128.4, 128.3, 127.7, 127.1, 81.1, 71.5, 66.2, 59.7, 59.2, 49.2, 47.3, 44.7, 37.2, 29.8, 29.1, 27.9, 27.3, 24.6, 19.4, 18.9, 18.3, 14.0 ppm; HRMS (ESI) m/z calculated for C$_{37}$H$_{51}$N$_{3}$O$_{8}$Na (M+Na)$^+$: 688.3574, found: 688.3568.
Compound 18 (1.04g, 1.20 mmol) was dissolved in CH₂Cl₂ (8 mL), 2, 6-lutidine (0.70 mL, 6.0 mmol) was added at room temperature, followed by dropwise addition of TMSOTf (0.87 mL, 4.81 mmol). The reaction mixture was stirred at room temperature for 10 min, then quenched by water (10 mL) and extracted with diethyl ether (50 mL). The organic phase was washed sequentially with saturated aqueous solution of NaHCO₃ and brine (20 mL for each), dried over anhydrous Na₂SO₄, filtered and concentrated in vacuo. The residue was dissolved in THF (20 mL), 19 (636 mg, 1.44 mmol) in THF (10 mL) was added dropwise at 0 °C. Upon the completion of addition, the reaction mixture was concentrated in vacuo, the residue was dissolved in diethyl ether (50 mL). This organic solution was washed sequentially with aqueous HCl solution (0.5 N, 20 mL x 2), saturated aqueous solution of NaHCO₃ (20 mL x 2) and brine (20 mL), dried over anhydrous Na₂SO₄, filtered and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc : Hexanes = 1 : 3, v/v) on silica gel (Qing Dao) to give hexapeptide 5 (1.02 g, 82%) as a white foam. [α]₂⁰ D +25.2 (c 1.0, CHCl₃); ¹H NMR (500 MHz, CDCl₃) exists as rotational conformers: δ 8.82 & 8.51 (both br, s, 1H), 8.65 (br, s, 1H), 7.26-7.18 (m, 6H), 7.13 (d, J = 7.9 Hz, 1H), 5.54-5.51 (m, 1H), 5.22 (dd, J = 6.3, 8.9 Hz, 1H), 5.04 (dd, J = 5.0, 10.0 Hz, 1H), 4.97-4.93 (m, 1H), 4.55 (br, s, 1H), 4.53 (dd, J = 3.7, 8.1 Hz, 1H), 4.13-4.10 (m, 1H), 4.09-4.07 (m, 1H), 3.94-3.87 (m, 3H), 3.74 (s, 3H), 3.73-3.69 (m, 2H), 3.16-3.13 (m, 3H), 3.03 (s, 3H), 2.74 & 2.72 (both br, s, 3H), 1.91-1.86 (m, 1H), 1.78-1.73 (m, 2H), 1.67-1.63 (m, 1H), 1.53-1.50 (m, 1H), 1.36 & 1.26 (both br, s, 9H), 1.19 (d, J = 6.3 Hz, 3H), 0.94 (d, J = 6.7 Hz, 3H), 0.91 (d, J = 6.5 Hz, 3H), 0.88-0.87 (m, 3H), 0.88 (s, 9H), 0.87 (s, 9H), 0.07-0.05 (m, 12H) ppm. ¹³C NMR (125 MHz, CDCl₃) exists as rotational conformers: δ 203.9, 203.8, 203.3, 171.0, 170.8, 170.8, 170.7, 168.6, 168.4, 156.7, 137.6, 128.9, 128.3, 126.4, 80.7, 67.9, 679.6, 66.9, 62.1, 62.0, 61.7, 61.6, 59.9, 59.6, 57.6, 57.6, 56.8, 56.7, 55.0, 52.2, 37.3, 36.8, 32.2, 31.2, 29.6, 29.3, 28.2, 28.1, 25.8, 25.7, 24.7, 23.1, 21.9, 19.3, 18.2, 18.1, 10.1, 10.0, -5.3, -5.5, -5.5, -5.6 ppm; HRMS (ESI) m/z calculated for C₄₉H₇₃N₄O₁₀S₂Si₂Na (M+Na)+: 1063.5440, found: 1063.5421.

Compound 5 (539 mg, 0.52 mmol) in THF-²BuOH (6 mL, 1:1) was treated with LiOH (1.1 mL, 1.1 mmol, 1.0 N in water) at 0 °C for 1 h. Volatiles were removed by concentration, the aqueous residue was made up to 5 mL with water and adjusted to pH 4 with citric acid (10% aqueous solution).
Extraction was performed with EtOAc (30 mL x 2). The combined organics were washed with brine (10 mL), dried over anhydrous Na₂SO₄, filtered and concentrated in *vacuo* to provide the desired free acid 20 (510 mg, 95%).

To a solution of compound 3 (155 mg, 0.10 mmol) in CH₂Cl₂ (4 mL), 2,6-lutidine (176 μL, 1.52 mmol) and TMSOTf (183 μL, 1.01 mmol) were added at room temperature. The reaction mixture was stirred for 4 h before it was quenched by pH 5.0 buffer (10 mL) and extracted with EtOAc (20 mL x 3). The combined organics were washed with brine (20 mL), dried over anhydrous Na₂SO₄, filtered and concentrated in *vacuo*. The residue was dissolved in CH₂Cl₂ (100 mL) and cooled to 0 °C, 2,6-lutidine (117 μL, 1.01 mmol) was added, followed by portionwise addition of BOPCl (77 mg, 0.30 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 3 days before it was quenched by addition of water (20 mL). Volatiles were removed by concentration. The aqueous residue was extracted with EtOAc (30 mL x 3). The combined organics were washed sequentially with HCl (20 mL x 2, 0.5 N aqueous solution), NaHCO₃ (20 mL, 5% aqueous solution) and brine (20 mL), dried over anhydrous Na₂SO₄, filtered and concentrated in *vacuo*. The residue was purified by flash chromatography (EtOAc : Hexanes = 1 : 1, v/v) on silica gel (Merck) to give 21 (98 mg, 72%) as a white foam. [α]$_{D}^{20}$ -22.6 (c 1.0, CHCl₃); $^1$H NMR (500 MHz, CDCl₃) exists as rotational conformers: δ 9.40 & 9.07 (br, s, 1H), 8.69 & 8.25 (br, s, 1H), 7.33-7.15 (m, 10H), 5.62 (br, 1H), 5.54 (br, s, 1H), 5.48-5.41 (m, 1H), 5.06-5.02 (m, 1H), 4.94 (br, s, 1H), 4.78-4.75 (m, 1H), 4.65-4.60 (m, 2H), 4.21-4.10 (m, 3H), 4.04 (br, s, 1H), 3.97-3.75 (m, 5H), 3.69 (br, s, 1H), 3.34-3.30 (m, 2H), 3.17-3.02 (m, 5H), 2.94 (s, 3H), 2.87-2.83 (m, 3H), 2.48 (qd, J = 3.2, 7.0 Hz, 1H), 2.31-2.21 (m, 2H), 1.94 (br, 2H), 1.83-1.78 (m, 2H), 1.67-1.60 (m, 3H), 1.52-1.45 (m, 1H), 1.25 (br, 3H), 1.19 (d, J = 6.9 Hz, 3H), 1.08 (d, J = 5.1 Hz, 3H), 1.04-0.71 (m, 33H), 0.31-0.04 (m, 12H) ppm. $^{13}$C NMR (125 MHz, CDCl₃) exists as rotational conformers: δ 204.0, 203.5, 172.3, 172.3, 170.4, 170.3, 170.0, 168.8, 168.8, 168.5,
138.5, 136.1, 131.0, 129.4, 129.3, 128.8, 128.6, 128.5, 127.0, 72.3, 68.0, 61.7, 61.4, 59.8, 59.0, 57.0, 56.9, 55.0, 52.5, 48.1, 47.8, 45.0, 38.2, 37.2, 36.3, 31.3, 30.8, 29.7, 28.4, 27.5, 25.8, 25.7, 25.1, 24.5, 23.3, 21.6, 20.1, 20.0, 19.0, 18.6, 18.2, 18.1, 14.9, 10.1, -5.1, -5.2, -5.4, -5.5 ppm; HRMS (ESI) m/z calculated for C_{68}H_{112}N_{9}O_{12}S_{2}Si_{2} (M+H)^{+}: 1366.7410, found: 1366.7417.

Macrocycle 21 (23 mg, 0.014 mmol) in CH_{2}Cl_{2} (4 mL) was treated with TrocCl (7.0 μL, 0.050 mmol) and pyridine (0.136 mL, 1.52 mmol) at 0 °C for 1 h. After being quenched by water (10 mL), the reaction mixture was extracted with EtOAc (10 mL x 2). The combined organics were washed with brine (10 mL), dried over anhydrous Na_{2}SO_{4}, filtered and concentrated in vacuo. The residue was dissolved in THF (3 mL) at 0 °C, HOAc (9.6 μL, 0.17 mmol) was added, followed by dropwise addition of TBAF (0.17 mL, 0.17 mmol, 1 M in THF). The mixture was stirred at room temperature overnight before it was diluted with EtOAc (50 mL) and washed with H_{2}O and brine (10 mL for each). The combined aqueous phase was back-extracted with EtOAc (20 mL x 2). The combined organics were washed with brine (20 mL), dried over anhydrous Na_{2}SO_{4}, filtered and concentrated in vacuo. The residue was purified by a short flash chromatography (EtOAc : MeOH = 9 : 1, v/v) on silica gel (Merck) to give 2 (~20 mg) as a viscous oil, which was used immediately in the following reaction.

HRMS (ESI) m/z calculated for C_{59}H_{90}Cl_{3}N_{9}O_{14}S_{2}Na (M+Na)^{+}: 1334.4542, found: 1334.4463.

To a solution of 2 (20 mg) in CH_{2}Cl_{2} (3 mL), DAST (11 μL, 0.084 mmol) was dropwise added at -78
The reaction mixture was slowly warmed to -50 °C within 1 h, then poured to ice-water (10 mL) and extracted with EtOAc (20 mL x 2). The combined organics were washed with brine (10 mL), dried over anhydrous Na$_2$SO$_4$, filtered and concentrated in vacuo to give a residue as crude 22. Compound 22 was dissolved in THF (2 mL), excess Zn powder (200mg) was added at 0 °C under a protective flow of Ar, followed by dropwise addition of aqueous solution of NH$_4$OAc (0.7 mL, 1.0 N) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, and then filtered through a pad of Celite and eluted with EtOAc (20 mL). The combined organics were washed with brine (10 mL), dried over anhydrous Na$_2$SO$_4$, filtered and concentrated in vacuo. The residue was purified by flash chromatography (CH$_2$Cl$_2$ : MeOH = 20 : 1, v/v) on silica gel (Merck) to give grassypeptolide 1 (6.8 mg, 37% over 4 steps from 21) as a white powder. $[\alpha]^{20}_{D} +87$ (c 0.12, CH$_2$Cl$_2$), lit. $[\alpha]^{20}_{D} +76$ (c 0.1, CH$_2$Cl$_2$); $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.52 (d, $J = 8.0$ Hz, 1H), 7.39 (br, d, $J = 6.7$ Hz, 1H), 7.35-7.21 (m, 10H), 7.11 (d, $J = 8.0$ Hz, 1H), 5.41 (dd, $J = 3.5$, 9.9 Hz, 1H), 5.31 (ddd, $J = 1.5$, 7.2, 9.9 Hz, 1H), 5.28-2.30 (m, 1H), 4.93 (d, $J = 10.8$ Hz, 1H), 4.92 (br, 1H), 4.76 (dd, $J = 5.8$, 7.1 Hz, 1H), 4.62-4.66 (m, 1H), 4.45 (dd, $J = 6.6$, 7.4 Hz, 1H), 4.17 (dq, $J = 6.4$, 6.5, 6.7 Hz, 1H), 4.01 (dq, $J = 6.3$, 6.4 Hz, 1H), 3.83 (dd, $J = 2.8$, 9.9 Hz, 1H), 3.69-3.72 (m ,2H), 3.68-3.70 (m, 1H), 3.59-3.63 (m, 1H), 3.58 (dd, $J = 9.9$, 10.6 Hz, 1H), 3.56 (dd, $J = 10.4$, 13.7 Hz, 1H), 3.45 (dd, $J = 3.7$, 13.9 Hz, 1H), 3.27 (dd, $J = 10.1$, 10.7 Hz, 1H), 3.15 (s, 3H), 3.11 (dd, $J = 5.4$, 14.0 Hz, 1H), 3.10 (dd, $J = 3.4$, 14.5 Hz, 1H), 3.10 (s, 3H), 2.78 (s, 3H), 2.51 (qd, $J = 6.0$, 6.8 Hz, 1H), 2.41 (dq, $J = 6.5$, 6.6, 10.9 Hz, 1H), 2.15-2.19 (m, 1H), 2.10-2.14 (m, 1H), 2.01-2.05 (m, 2H), 1.95-1.98 (m, 1H), 1.85-1.89 (m, 1H), 1.83-1.85 (m, 1H), 1.72 (ddd, $J = 6.1$, 8.3, 15.0 Hz, 1H), 1.53-1.57 (m, 1H), 1.23 (d, $J = 7.0$ Hz, 3H), 1.16 (d, $J = 6.7$ Hz, 3H), 1.11 (d, $J = 7.0$ Hz, 3H), 0.98 (d, $J = 5.5$ Hz, 3H), 0.97 (t, $J = 7.0$ Hz, 3H), 0.96 (d, $J = 6.7$ Hz, 3H), 0.90 (d, $J = 6.5$ Hz, 3H), 0.87 (d, $J = 6.7$ Hz, 3H) ppm; $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 178.5, 177.2, 173.0, 172.5, 171.1, 171.0, 170.4, 170.4, 169.9, 167.8, 138.3, 135.7, 129.9, 129.2, 128.7, 128.6, 127.4, 126.7, 79.3, 77.8, 72.0, 69.1, 68.8, 60.3, 59.2, 57.0, 56.7, 54.4, 48.7, 47.7, 45.5, 39.6, 37.7, 37.3, 36.9, 35.3, 33.4, 32.2, 30.3, 27.5, 27.3, 25.2, 25.1, 24.8, 23.2, 22.2, 19.7, 19.7, 19.5, 18.2, 14.7, 10.9 ppm; HRMS (ESI) m/z calculated for C$_{56}$H$_{79}$N$_9$O$_{10}$S$_2$Na (M+Na)$^+$: 1124.5284, found: 1124.5248; HRMS (ESI) m/z calculated for C$_{56}$H$_{80}$N$_9$O$_{10}$S$_2$ (M+H)$^+$: 1102.5464, found: 1102.5442, lit. 1 1102.5438

NMR spectra for compound 4, 5, 21 and 1
Comparison of NMR spectra of synthetic grassypeptolide 1 with that of natural product
Comparison of $^1$H NMR and $^{13}$C NMR

$^1$H NMR of synthetic grassypeptolide 1

$^1$H NMR of natural product (Org. Lett. 2008, 10, 785-792)

$^{13}$C NMR of synthetic grassypeptolide 1

$^{13}$C NMR of natural product (Org. Lett. 2008, 10, 785-792)
Effects of synthetic grassypeptolide on cancer cell line proliferation. Cells were seeded into 96-well plates and incubated overnight. Grassypeptolide and its analogues were added in serial dilutions in the medium containing 1% FCS and the plates were incubated for another 48 hours. Cell proliferation was performed using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay by adding CellTiter96 Aqueous solution (Promega Co., Madison, WI) according to the manufacturer’s protocol.

Effect of grassypeptolide (I) on cell proliferation in Hela and HT29 cell lines. Cells were cultured for 48 h in the presence of various concentrations of grassypeptolide (I). Proliferation was measured by MTS assay. Each point represents the mean±SE from four determinations.