Total Synthesis of Grassypeptolide

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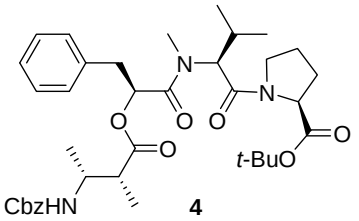
General Methods

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.



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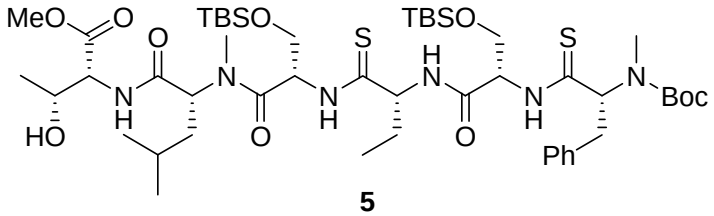
To a solution of acid **11** (313 mg, 1.25 mmol) in CH_2Cl_2 (10 mL) at 0 °C, $(COCl)_2$ (255 μL, 3.0 mmol) was added, followed by DMF (10 μL, 10% in CH_2Cl_2, v/v). The reaction mixture was stirred for 4 h while slowly warming to room temperature and then concentrated in *vacuo*. The residue was dissolved in CH_2Cl_2 (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 CH_2Cl_2 (10 mL) and transformed via syringe to a solution of **10** (144 mg, 0.33 mmol) in and CH_2Cl_2 (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 $NaHCO_3$ (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 $NaHCO_3$ (20 mL x 2) and brine (20 mL), dried over anhydrous Na_2SO_4, 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. $[\alpha]^{20}_D$ -29.9 (*c* 2.2, CH_2Cl_2), ^{1}H NMR (500 MHz, $CDCl_3$): 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, $CDCl_3$) δ 173.3, 171.0, 170.2, 168.6, 156.2, 137.0, 135.6, 129.1, 128.4, 128.3, 127.7, 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 $C_{37}H_{51}N_3O_8Na$ $(M+Na)^+$: 688.3574, found: 688.3568.



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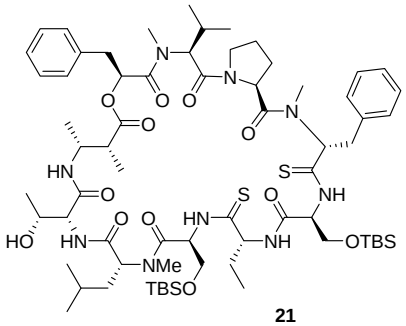
Compound **18** (1.04g, 1.20 mmol) was dissolved in CH_2Cl_2 (8 mL), 2, 6-lutidine (0.70 mL, 6.0 mmol) was added at room temperature, followed by dropwise addition of TMSOTf (0.87 mL, 4.81 mmol). The reaction mixture was stirred at room temperature for 10 min, then quenched by water (10 mL) and extracted with diethyl ether (50 mL). The organic phase was washed sequentially with saturated aqueous solution of $NaHCO_3$ and brine (20 mL for each), dried over anhydrous Na_2SO_4, 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 ^oC. 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 $NaHCO_3$ (20 mL x 2) and brine (20 mL), dried over anhydrous Na_2SO_4, 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. $[\alpha]^{20}_D$ +25.2 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) 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, 1H), 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. ^{13}C NMR (125 MHz, $CDCl_3$) 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, 67.9, 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 $C_{49}H_{88}N_6O_{10}SSi_2Na$ $(M+Na)^+$: 1063.5440, found: 1063.5421.

Compound **5** (539 mg, 0.52 mmol) in THF-^{t}BuOH (6 mL, 1:1) was treated with LiOH (1.1 mL, 1.1 mmol, 1.0 N in water) at 0 ^oC 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).

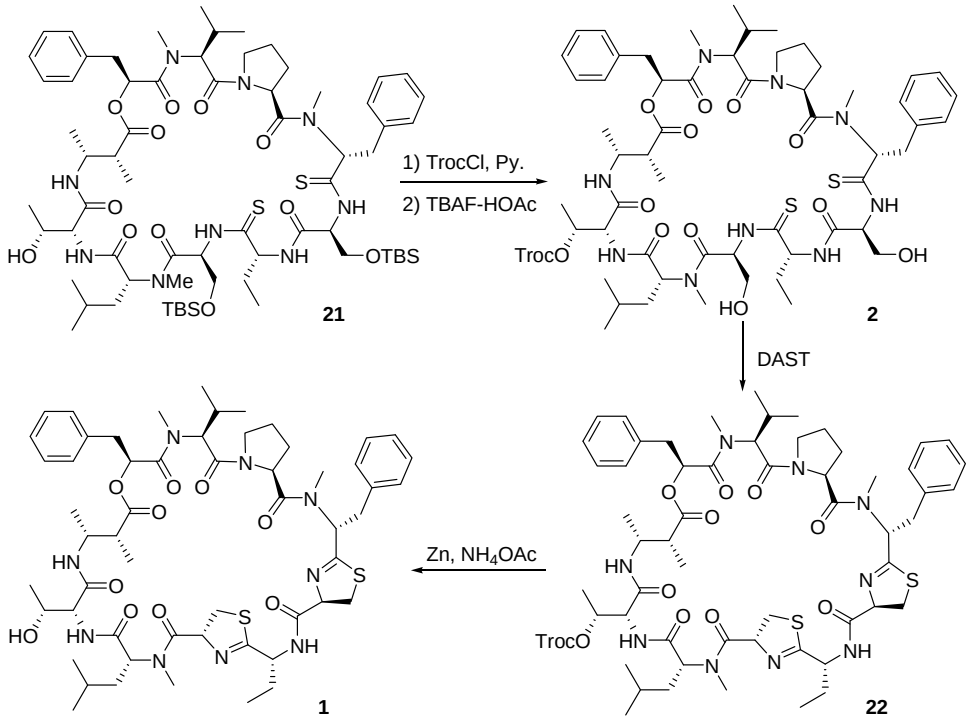
Extraction was performed with EtOAc (30 mL x 2). The combined organics were washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated in *vacuo* to provide the desired free acid **20** (510 mg, 95%).



21

To a solution of compound **3** (155 mg, 0.10 mmol) in CH_2Cl_2 (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×3). The combined organics were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated in *vacuo*. The residue was dissolved in CH_2Cl_2 (100 mL) and cooled to 0 ^oC, 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), $NaHCO_3$ (20 mL, 5% aqueous solution) and brine (20 mL), dried over anhydrous Na_2SO_4, 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. $[\alpha]^{20}_D$ -22.6 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) 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, $CDCl_3$) 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, 128.4, 127.0, 126.3, 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, 25.8, 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 $C_{68}H_{112}N_9O_{12}S_2Si_2$ $(M+H)^+$: 1366.7410, found: 1366.7417.



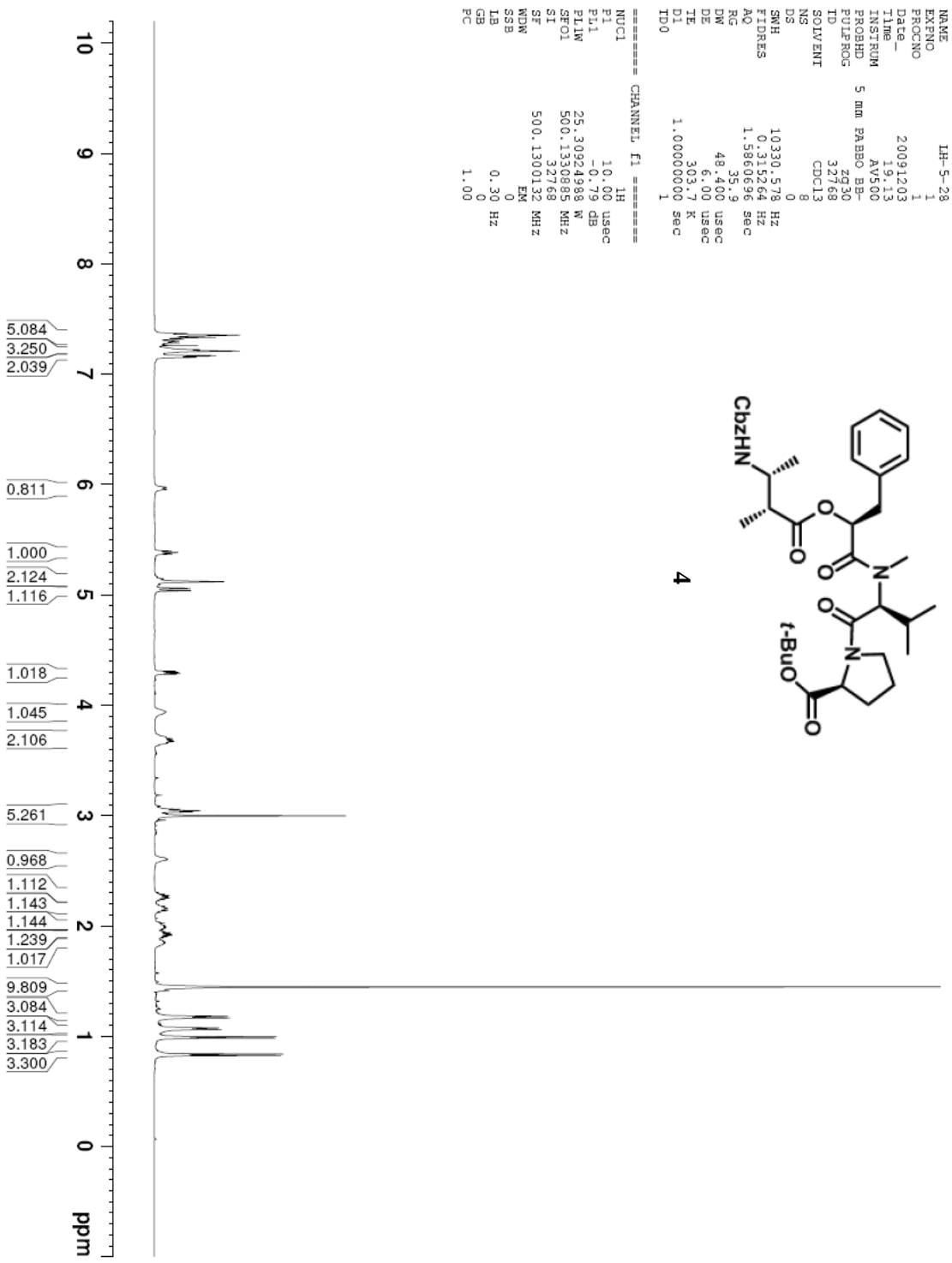
Macrocycle **21** (23 mg, 0.014mmol) in CH_2Cl_2 (4 mL) was treated with TrocCl (7.0 μL, 0.050 mmol) and pyridine (0.136 mL, 1.52 mmol) at 0 ^oC for 1 h. After being quenched by water (10 mL), the reaction mixture was extracted with EtOAc (10 mL x 2). The combined organics were washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated in *vacuo*. The residue was dissolved in THF (3 mL) at 0 ^oC, 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_2O 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_2SO_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 $C_{59}H_{84}Cl_3N_9O_{14}S_2Na$ $(M+Na)^+$: 1334.4542, found: 1334.4463.

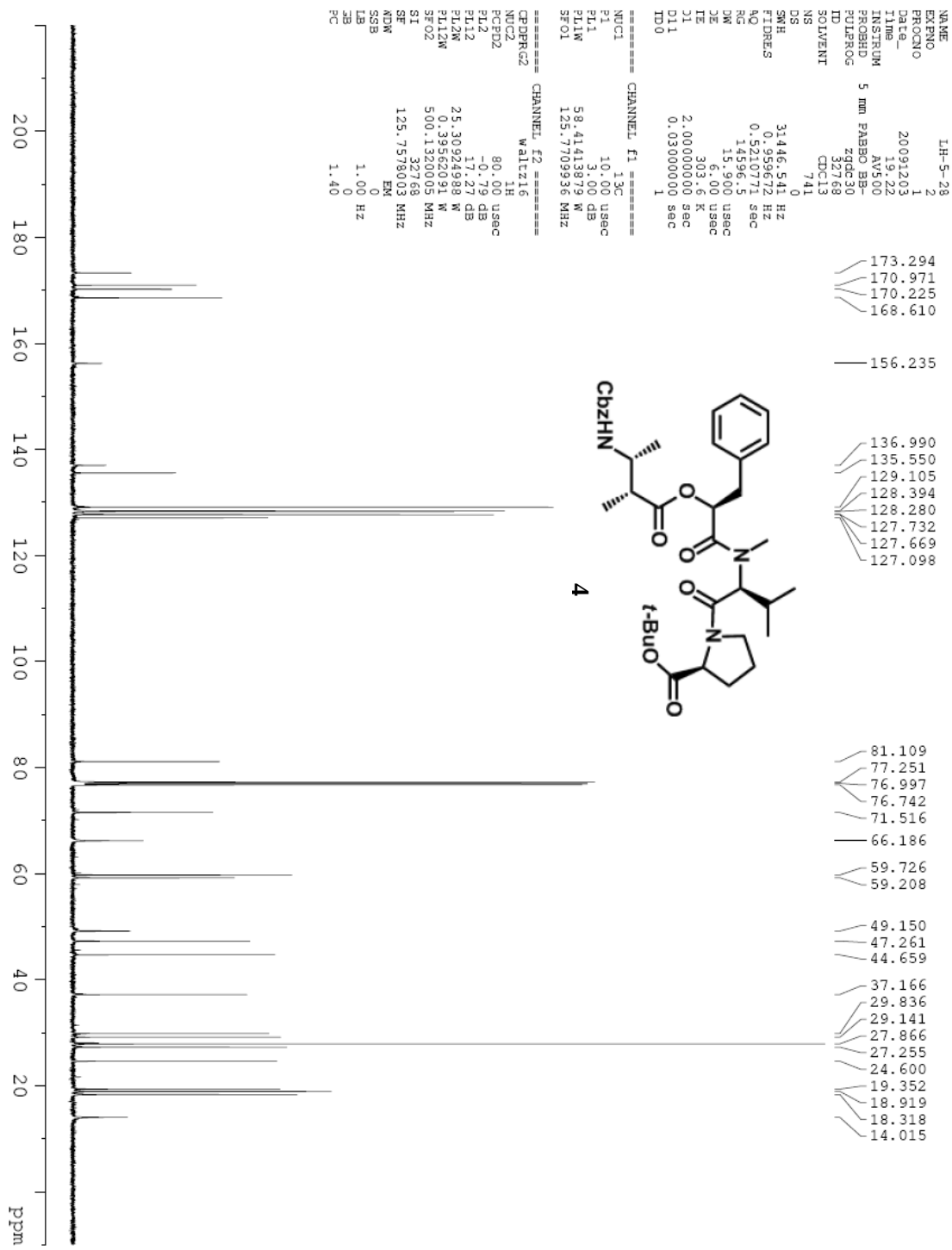
To a solution of **2** (20 mg) in CH_2Cl_2 (3 mL), DAST (11 μL, 0.084 mmol) was dropwise added at -78

^{o}C. The reaction mixture was slowly warmed to -50 ^{o}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_2SO_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 ^{o}C under a protective flow of Ar, followed by dropwise addition of aqueous solution of NH_4OAc (0.7 mL, 1.0 N) at 0 ^{o}C. The reaction mixture was stirred at 0 ^{o}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_2SO_4, filtered and concentrated in *vacuo*. The residue was purified by flash chromatography (CH_2Cl_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_2Cl_2), lit.[1] $[\alpha]^{20}_{D}$ +76 (*c* 0.1, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 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 (dqd, *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 (dqq, *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, $CDCl_3$): δ 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 $C_{56}H_{79}N_9O_{10}S_2Na$ $(M+Na)^+$: 1124.5284, found: 1124.5241; HRMS (ESI) *m/z* calculated for $C_{56}H_{80}N_9O_{10}S_2$ $(M+H)^+$: 1102.5464, found: 1102.5442, lit.[3] 1102.5438

[1] Kwan, J. C.; Rocca, J. R.; Abboud, K. A.; Paul, V. J.; Luesch, H. *Org. Lett.* **2008**, *10*, 789-792.

NMR spectra for compound 4, 5, 21 and 1

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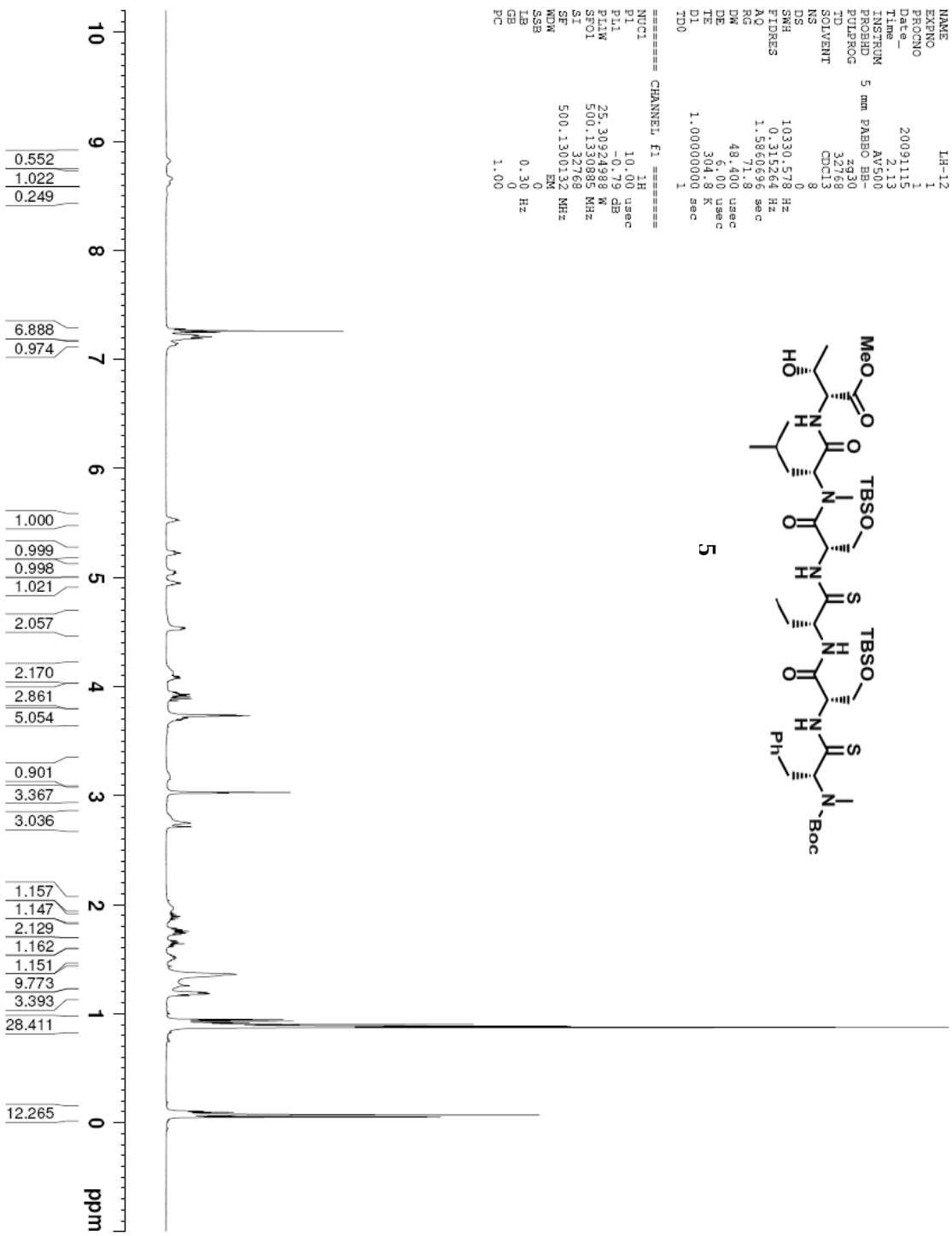
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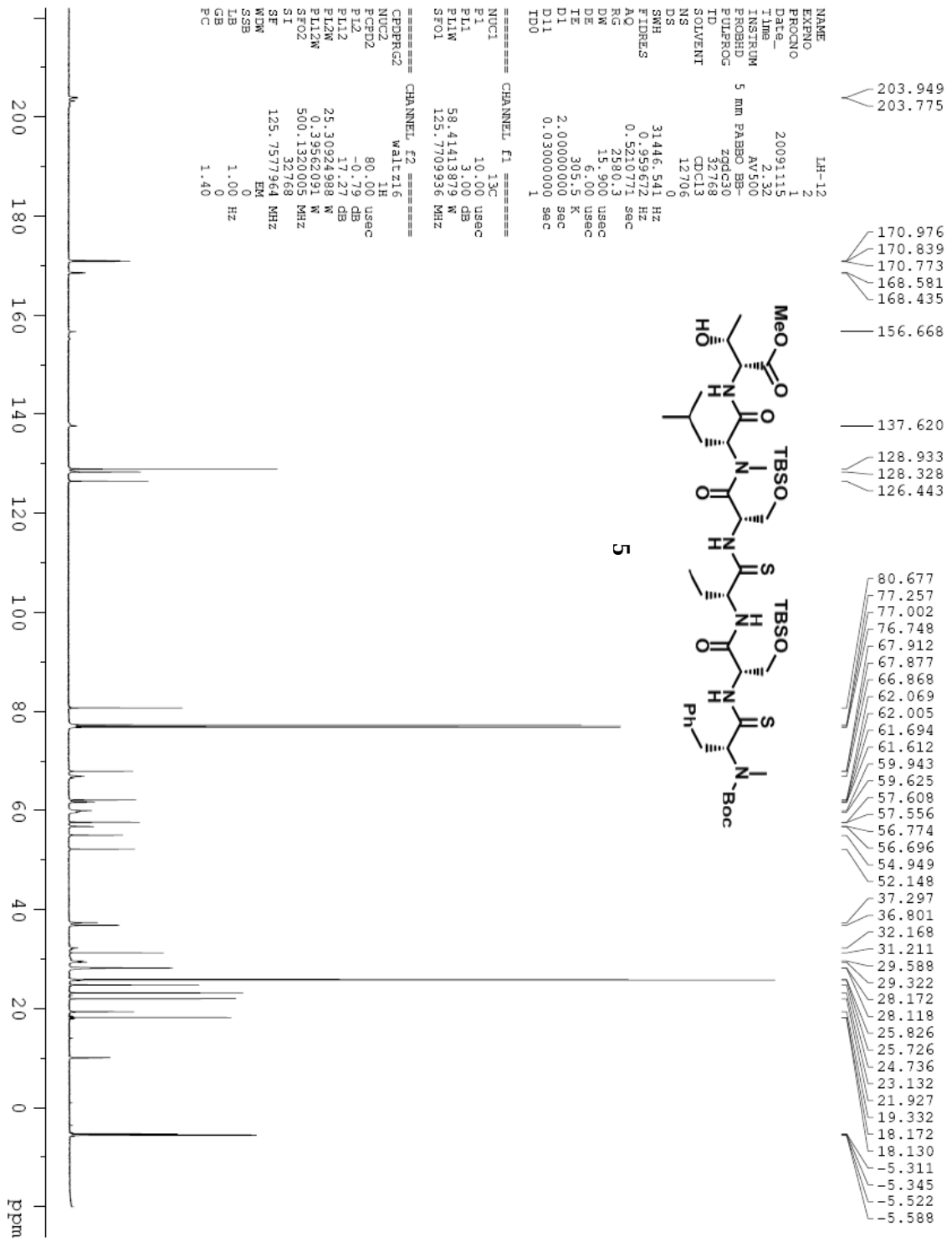
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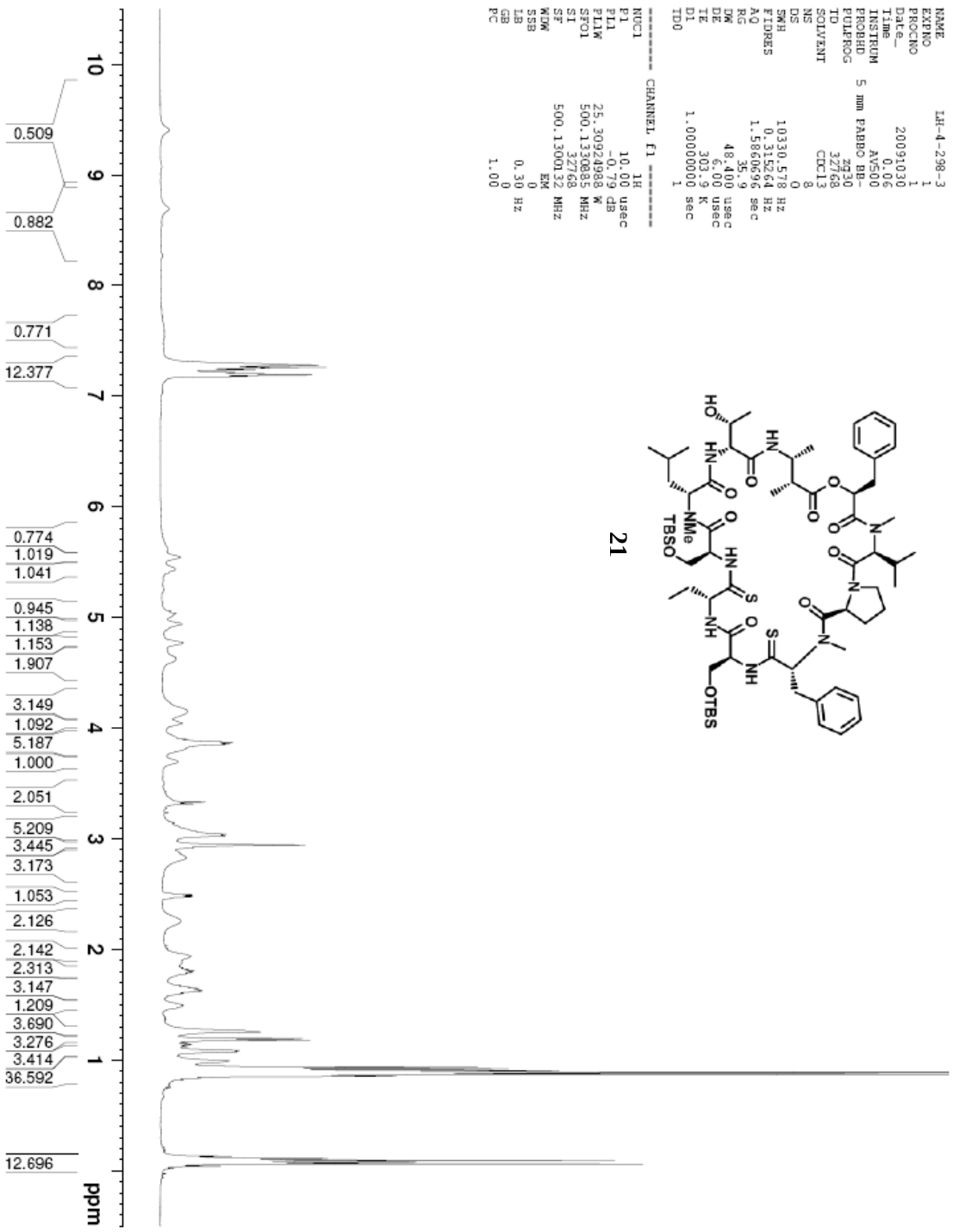
SFO1 125.7709936 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -0.79 dB
PL12 17.27 dB
PL2W 25.30924988 W
PL12W 0.39562091 W
SFO2 500.1320005 MHz
SI 32768
SF 125.7577964 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
203.949
203.775
170.976
170.839
170.773
168.581
168.435
156.668
137.620
128.933
128.328
126.443
80.677
77.257
77.002
76.748
67.912
67.877
66.868
62.069
62.005
61.694
61.612
59.943
59.625
57.608
57.556
56.774
56.696
54.949
52.148
37.297
36.801
32.168
31.211
29.588
29.322
28.172
28.118
25.826
25.726
24.736
23.132
21.927
19.332
18.172
18.130
-5.311
-5.345
-5.522
-5.588
MeO
HO
TBSO
TBSO
Ph
Boc
5
200
180
160
140
120
100
80
60
40
20
0
ppm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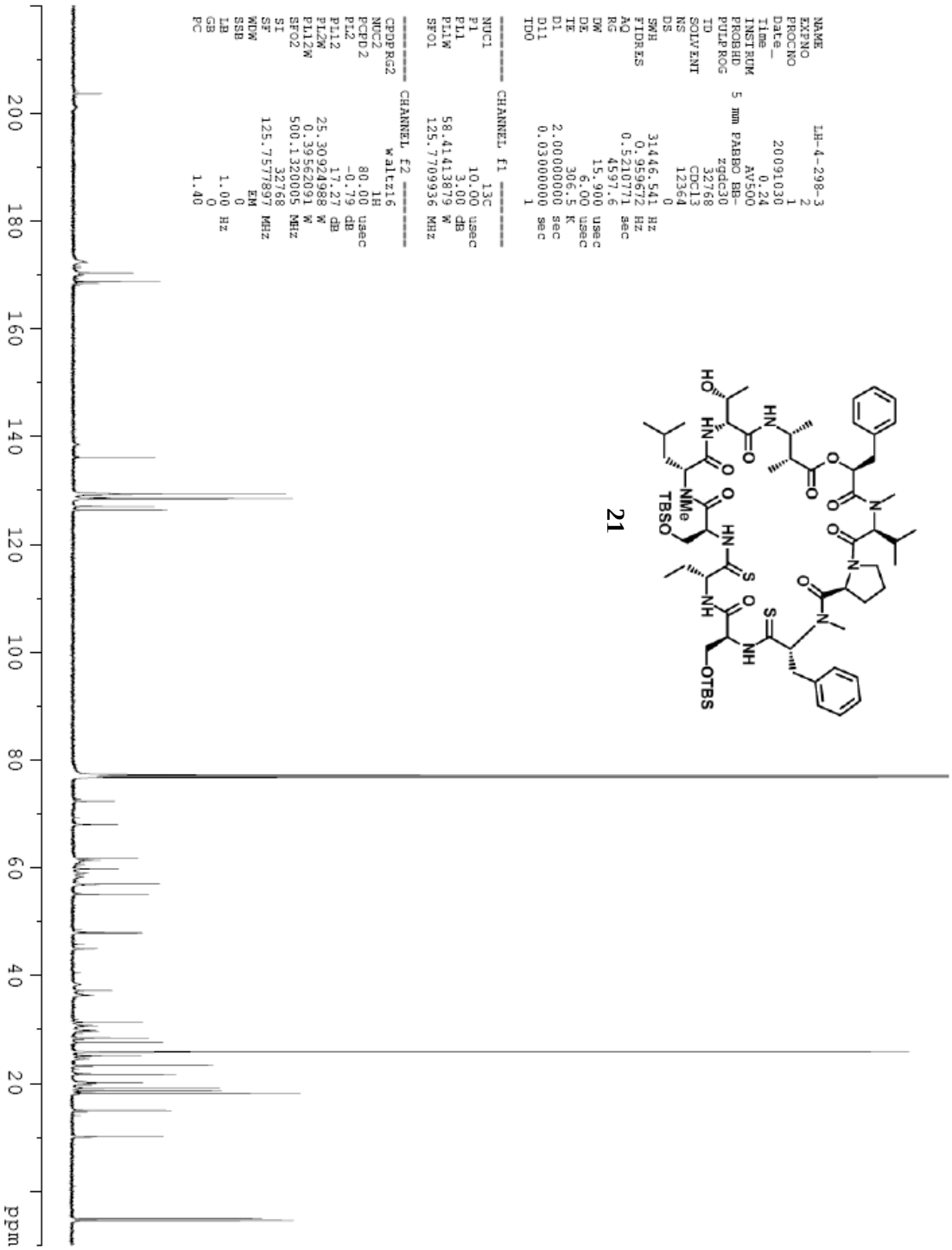
EXPNO 1
PROCNO 1
Date_ 20091030
Time 0.06
INSTRUM AV500
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 8
DS 0
SWH 10330.578 Hz
FIDRES 0.315264 Hz
AQ 1.5860696 sec
RG 35.9
DW 48.400 usec
DE 6.00 usec
TE 303.9 K
D1 1.00000000 sec
TD0 1
========== CHANNEL f1 ==========
NUC1 1H
P1 10.00 usec
PL1 -0.79 dB
PLW1 25.30924988 W
SFO1 500.13308885 MHz
SI 32768
SF 500.13000132 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
0.509
0.882
0.771
12.377
0.774
1.019
1.041
0.945
1.138
1.153
1.907
3.149
1.092
5.187
1.000
2.051
5.209
3.445
3.173
1.053
2.126
2.142
2.313
3.147
1.209
3.690
3.276
3.414
36.592
12.696
ppm
21
HO
HN
O
NMe
TBSO
HN
O
HN
S
O
NH
S
NH
OTBS
O
N
O
O
N
O
N
O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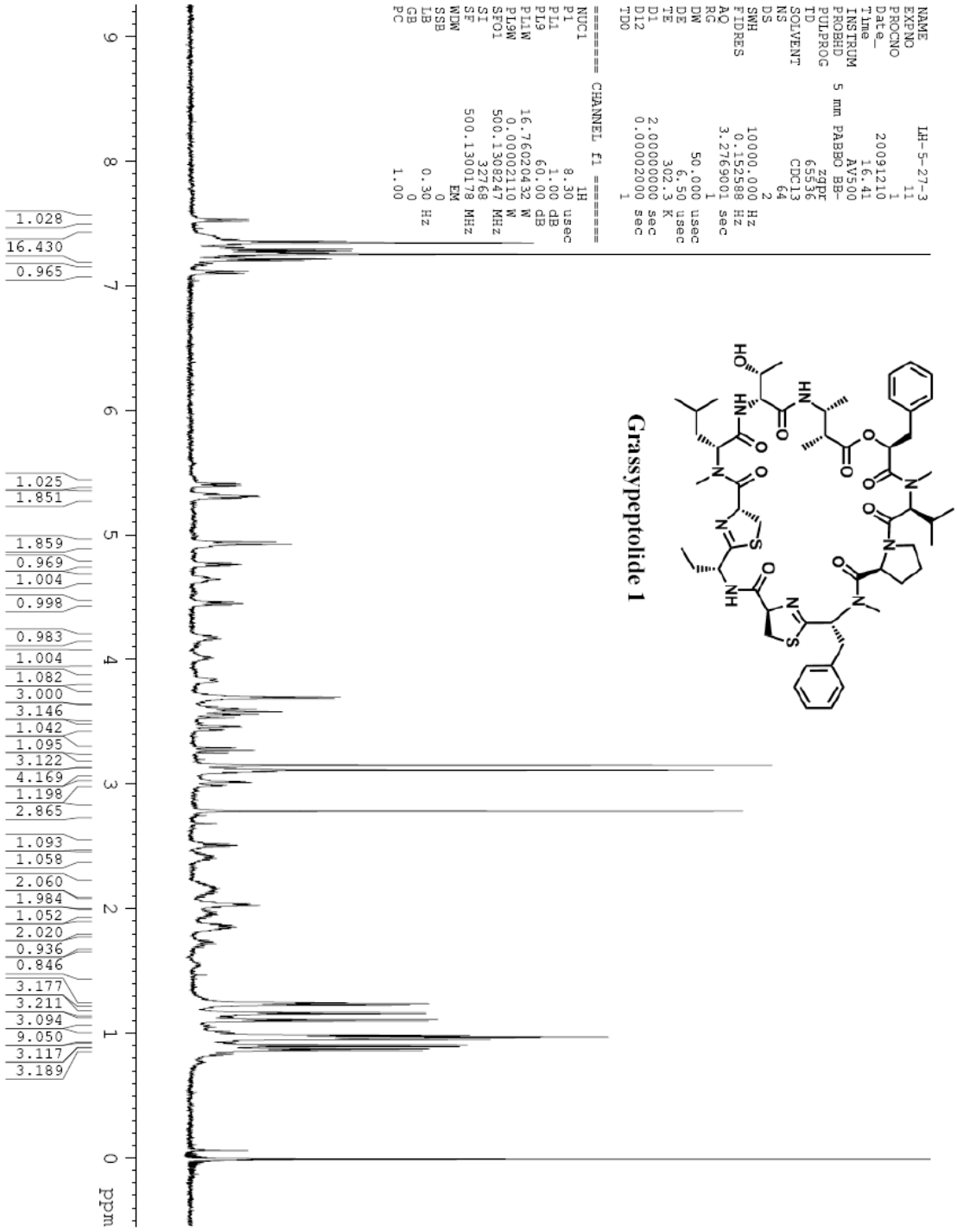
EXPNO 2
PROCNO 1
Date_ 20091030
Time 0.24
INSTRUM AV500
PROBHD 5 mm PABBO BB-
PULPROG zgdc30
TD 32768
SOLVENT CDCl3
NS 12364
DS 0
SWH 31446.541 Hz
FIDRES 0.959672 Hz
AQ 0.5210771 sec
RG 4597.6
DW 15.900 usec
DE 6.00 usec
TE 306.5 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
CHANNEL f1
NUC1 13C
P1 10.00 usec
PL1 3.00 dB
PL1W 58.41413879 W
SFO1 125.7709936 MHz
CHANNEL f2
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -0.79 dB
PL12 17.27 dB
PL2W 25.30924988 W
PL12W 0.39562091 W
SFO2 500.1320005 MHz
SI 32768
SF 125.7577897 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
HO
HN
O
NMe
TBSO
HN
S
O
NH
OTBS
21
200
180
160
140
120
100
80
60
40
20
ppm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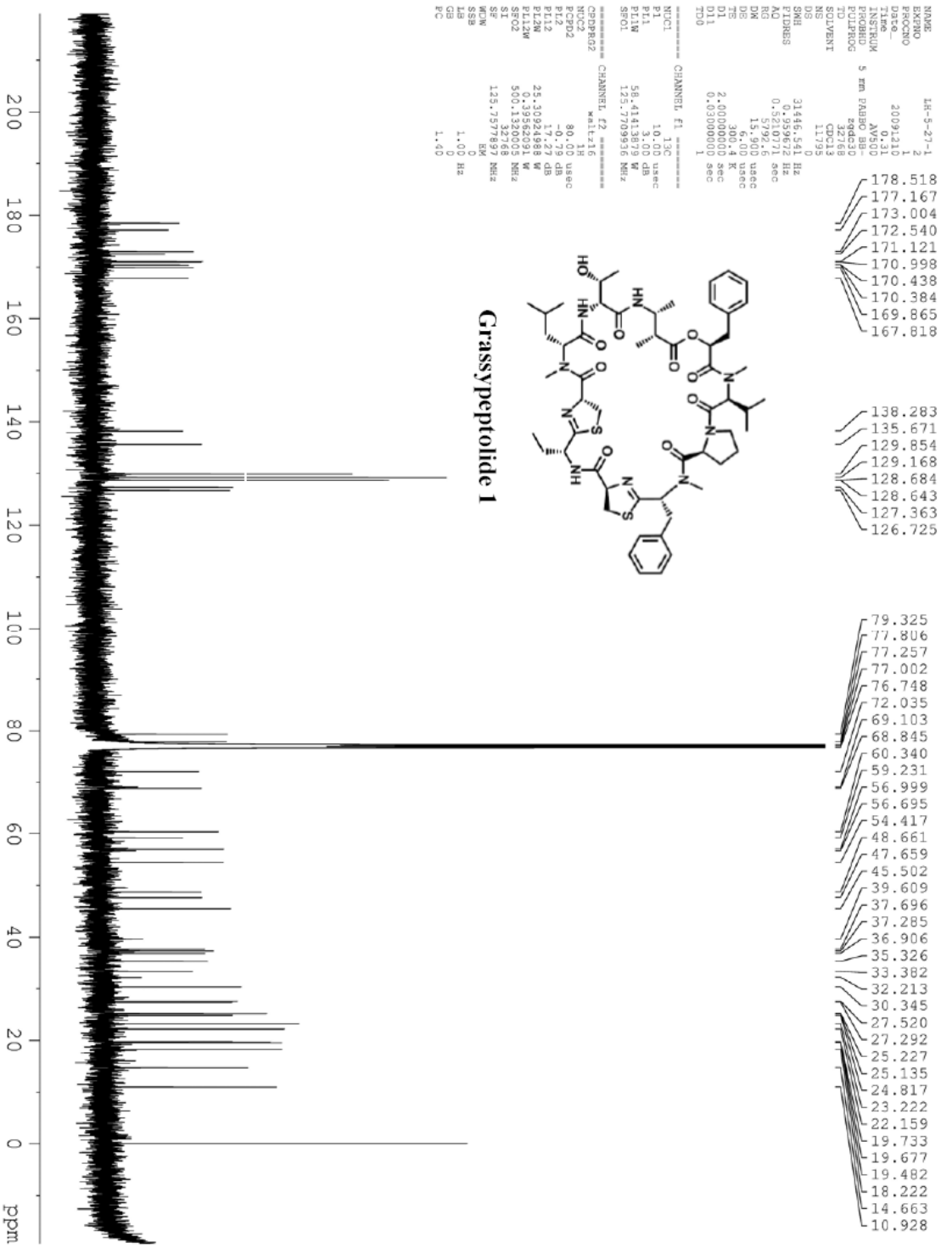
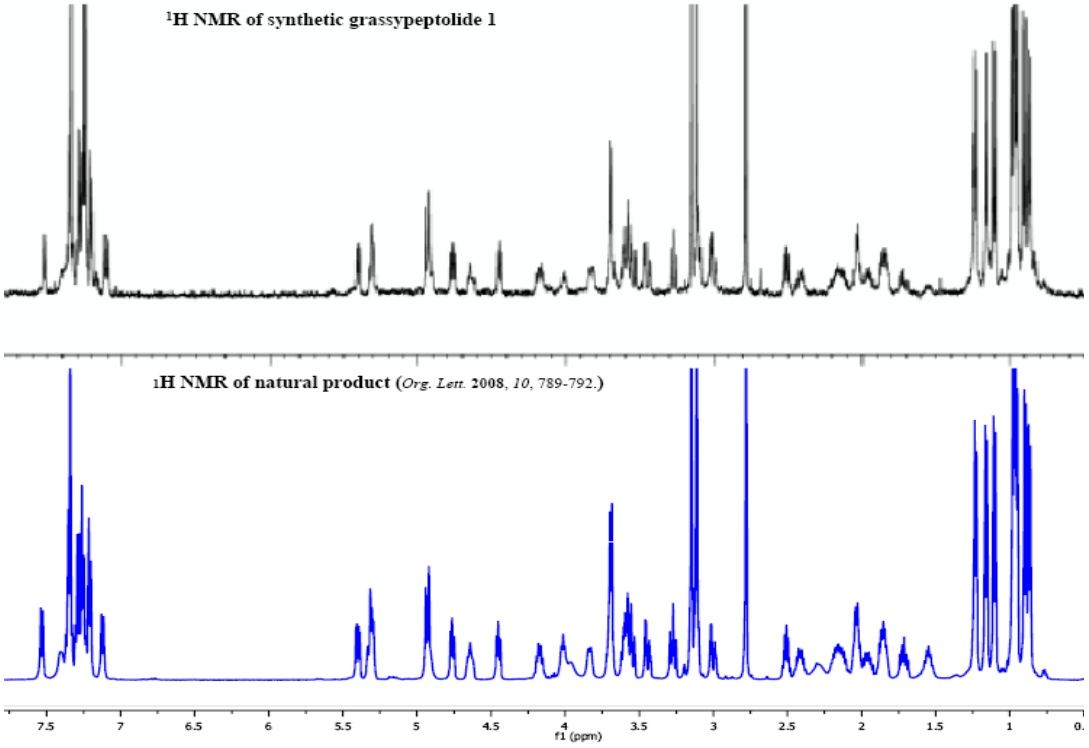
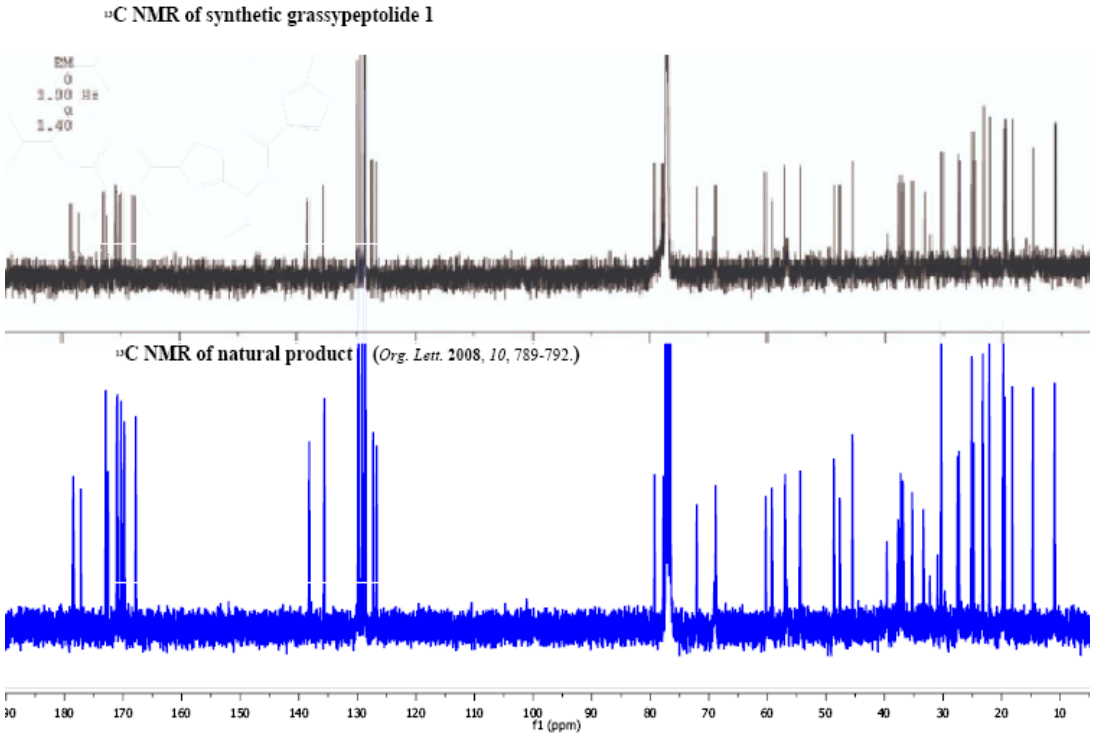
EXPNO 11
PROCNO 1
Date_ 20091210
Time 16.41
INSTRUM AV500
PROBHD 5 mm PABBO BB-
PULPROG zgpr
TD 65536
SOLVENT CDCl3
NS 64
DS 2
SWH 10000.000 Hz
FIDRES 0.152588 Hz
AQ 3.2769001 sec
RG 1
DW 50.000 usec
DE 6.50 usec
TE 302.3 K
D1 2.00000000 sec
D12 0.00002000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 8.30 usec
PL1 1.00 dB
PL9 60.00 dB
PL1W 16.76020432 W
PL9W 0.00002110 W
SFO1 500.1308247 MHz
SI 32768
SF 500.1300178 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
Grassypeptolide 1
1.028
16.430
0.965
1.025
1.851
1.859
0.969
1.004
0.998
0.983
1.004
1.082
3.000
3.146
1.042
1.095
3.122
4.169
1.198
2.865
1.093
1.058
2.060
1.984
1.052
2.020
0.936
0.846
3.177
3.211
3.094
9.050
3.117
3.189
9
8
7
6
5
4
3
2
1
0
ppm


Grassypeptolide 1
178.518
177.167
173.004
172.540
171.121
170.998
170.438
170.384
169.865
167.818
138.283
135.671
129.854
129.168
128.684
128.643
127.363
126.725
79.325
77.806
77.257
77.002
76.748
72.035
69.103
68.845
60.340
59.231
56.999
56.695
54.417
48.661
47.659
45.502
39.609
37.696
37.285
36.906
35.326
33.382
32.213
30.345
27.520
27.292
25.227
25.135
24.817
23.222
22.159
19.733
19.677
19.482
18.222
14.663
10.928
200
180
160
140
120
100
80
60
40
20
0
ppm

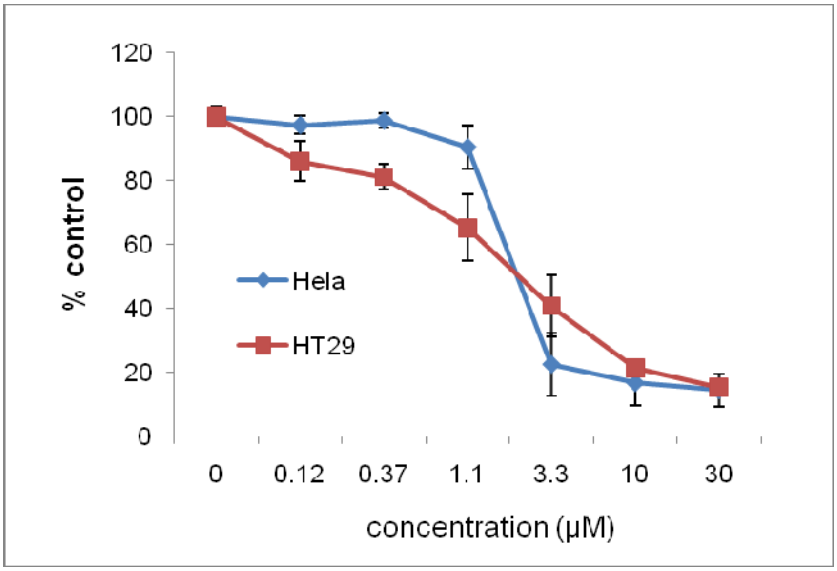
Comparison of NMR spectra of synthetic grassypeptolide 1 with that of natural product

Comparison of ^{1}H NMR and ^{13}C NMR





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 (**1**) on cell proliferation in Hela and HT29 cell lines. Cells were cultured for 48 h in the presence of various concentrations of grassypeptolide (**1**). Proliferation was measured by MTS assay. Each point represents the mean±SE from four determinations.