Total Synthesis of Cytotoxic Anhydrophytosphingosine Pachastrissamine (Jaspine B)

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General experimental details:

All of the solvents and reagents used were obtained commercially and used as such, unless noted otherwise. Moisture or air sensitive reactions were conducted under argon atmosphere in oven dried (120°C) glass apparatus. Diethyl ether and THF were distilled from sodium benzophenone ketyl, while dichloromethane was distilled over calcium hydride, prior to use. Solvents were removed under reduced pressure using standard rotary evaporators. Flash column chromatography was carried out using Silica gel 60 (230-400 mesh), while thin layer chromatography (tlc) was carried out on Silica Gel HLF, pre-coated glass plates. All yields reported refer to isolated material judged to be homogeneous by tlc and NMR spectroscopy. Unless noted otherwise, NMR spectra were recorded with the chemical shifts (δ) reported in ppm relative to Me₄Si (for ¹H) and CDCl₃ (for ¹³C) as internal standards respectively. Melting points are uncorrected.

Experimental Procedures:

Synthesis of bicyclic lactone 2. To an ice-cold solution of the lactone **3** (1 g, 3.1 mmol) in CH_2Cl_2 (15 mL), formic acid (96%, 10 mL) was added with stirring. The reaction mixture was allowed to attain room temperature and stirring continued for another 3 h. Excess solvent was reduced under vacuum, the residual oil dissolved in ethyl acetate (25 mL) and slowly poured into 100 mL of saturated aqueous sodium bicarbonate solution. The resulting mixture was stirred overnight at room temperature, the organic layer separated, the aqueous layer extracted with ethyl acetate (2x50 mL), combined extract washed with brine, dried (anhydrous Na_2SO_4), concentrated and the residue purified by column chromatography (EtOAc/hexanes = 1:3) to yield the bicyclic lactone **3** as a white solid (682 mg, 79%); mp = 95–97 °C; $[\alpha]_D^{25}$ –90.0 (c 0.7, CHCl_3); IR (NaCl) 3300, 1784, 1713 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 2.71 (d, J = 19 Hz, 1H), 2.81 and 2.85 (dd, J = 6, 19 Hz, 1H), 3.52 (t, J = 9 Hz, 1H), 4.18 (t, J = 8 Hz, 1H), 4.53 (m, 1H), 4.85 (m, 1H), 4.98 (m, 1H), 5.14 (s, 2H), 5.25 (br s, 1H), 7.36 (m, 5H); ^{13}C NMR (125 MHz, CDCl_3) δ 36.5, 53.6, 67.1, 68.6, 77.2, 82.0, 128.0, 128.2, 128.4, 135.9, 155.8, 174.9; HRMS calcd. for $\text{C}_{14}\text{H}_{16}\text{NO}_5$ m/z ($M + H$)⁺, 278.1028; found, 278.1044.

Reduction of lactone 3 to the lactol 4. The lactone **3** (0.66 g, 2.38 mmol) was dissolved in anhydrous CH_2Cl_2 (8 mL) and cooled to –78 °C. To this stirring solution was added DIBAL-H (1 M in toluene, 3 mL, 3.0 mmol) dropwise. The reaction was stirred at –78 °C for 1.5 h and then quenched by careful addition of MeOH (1.0 mL). The reaction mixture was brought to room temperature, diluted with EtOAc (50 mL) and saturated aqueous sodium potassium tartrate (30 mL) was added to it. The resulting mixture was stirred until two clear layers were seen. The two layers were separated and the aqueous layer was extracted with EtOAc (3 × 25 mL). The combined extract dried (Na_2SO_4), concentrated, and the crude product purified by column chromatography (EtOAc/hexanes = 2:3 to 1:1) affording the lactol **4** as a white solid (551 mg, 83%); mp = 76–79 °C; IR (NaCl) 3407, 1701 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3 , mixture of anomers) δ 2.15 (m, 2H), 3.31 (m, 0.5H), 3.86–4.34 (m, 3.5H), 4.59–4.81 (m, 2H), 5.0 (br s, 2H), 5.52 – 5.89 (m, 2H), 7.34 (s, 5H); ^{13}C NMR (100 MHz, CDCl_3 , mixture of anomers) δ 41.5, 42.4, 54.0, 54.1, 67.4, 67.5, 69.4, 72.0, 80.7, 82.5, 83.3, 83.6, 100.2, 100.6, 128.6, 128.7, 128.9, 129.0, 136.5, 136.6, 156.6, 156.7; FAB+ calcd. for $\text{C}_{14}\text{H}_{18}\text{NO}_5$ m/z ($M + H$)⁺, 280.2; found, 280.2.

Synthesis of the olefin 5. To a stirred solution of (dodecyl)triphenylphosphonium bromide (2.3 g, 4.5 mmol) in anhydrous THF (20 mL) at –78 °C was added n-butyl lithium (1 M soln. In hexanes, 4 mL, 4 mmol) dropwise and the resulting solution stirred for 45 min (solution turned yellow-orange). The lactol **4** (279 mg, 1 mmol) dissolved in anhydrous THF (6 mL) was then added dropwise to the reaction mixture. The solution was stirred at –78 °C for 2 h, followed by stirring at room temperature overnight. The reaction was quenched by addition of a saturated ammonium chloride solution (15 mL), organic layer was separated and the aqueous layer extracted with EtOAc (2 × 25 mL). The combined extract was dried (Na_2SO_4), concentrated, and the crude product purified by column chromatography (EtOAc/hexanes = 1:9 to 3:7) affording the mixture of olefins **5** as a viscous solid (262 mg, 81%); IR (teflon) 3278, 1758, 1735, 1710 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3 , mixture of *cis/trans* isomers) δ 0.89 (t, J = 7 Hz, 3H), 1.2 (br s, 18 H), 2.0 – 2.12 (2m, 2H), 2.48 – 2.61 (2m, 2H), 3.54 (m, 2H), 3.98 (d, J = 10 Hz, 1H), 4.40 (m, 1H), 4.98 (dd, J = 4, 8 Hz, 1H), 5.43–5.57 (m, 2H), 5.87 and 5.95 (2s, 1H); ^{13}C NMR (100

MHz, CDCl_3 , mixture of *cis/trans* isomers) δ 14.5, 23.1, 26.7, 27.8, 29.6, 29.7, 29.8, 29.9, 30.0, 30.1, 31.8, 32.3, 33.0, 57.3, 73.9, 77.6, 81.1, 83.3, 83.5, 123.9, 124.5, 133.9, 134.9, 159.4; HRMS calcd. for $\text{C}_{19}\text{H}_{34}\text{NO}_3$ m/z ($\text{M} + \text{H}$)⁺, 324.2539; found, 324.2520.

Hydrogenation of olefin 5 to form 6. To a solution of the olefin **5** (220 mg, 0.68 mmol) in ethyl acetate (10 mL), 10% Pd–C (70 mg) was added and the mixture stirred under hydrogen atmosphere for 6 h. The catalyst was filtered, washed thoroughly with ethyl acetate (3x30 mL) and the combined filtrate concentrated to yield the product **6** as an amorphous white solid (198 mg, 90%); mp = 115–118 °C; $[\alpha]_D^{25}$ 46.5 (c 0.4, CHCl_3); IR (teflon) 1749, 1718 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 0.89 (t, J = 7 Hz, 3H), 1.2–1.48 (br s, 24H), 1.78 (m, 2H), 3.55 (m, 2H), 3.96 (d, J = 10 Hz, 1H), 4.39 (m, 1H), 4.97 (m, 1H), 5.82 (s, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.5, 23.1, 26.4, 28.5, 29.7, 29.9, 30.0, 30.1, 32.3, 57.4, 73.7, 77.6, 81.3, 83.6, 159.4; HRMS calcd. for $\text{C}_{19}\text{H}_{36}\text{NO}_3$ m/z ($\text{M} + \text{H}$)⁺, 326.2695; found, 326.2691.

Synthesis of pachastrissamine (1). To a solution of the carbamate derivative **6** (122 mg, 0.37 mmol) in ethanol (10 mL), aqueous potassium hydroxide (1 M solution in H_2O , 10 mL) was added and the mixture refluxed for 9 h. The reaction mixture was concentrated under vacuum and the residue purified by column chromatography ($\text{CHCl}_3/\text{MeOH}/\text{aq. NH}_4\text{OH}$ = 95 : 4 : 1) to afford pachastrissamine (**1**) as a white solid (86 mg, 77%); mp = 70–72 °C; $[\alpha]_D^{25}$ 9.6 (c 0.11, CHCl_3) [lit.² $[\alpha]_D^{25}$ 7.0 (c 0.1, CHCl_3)]; ^1H NMR (400 MHz, CDCl_3) δ 0.89 (t, J = 7 Hz, 3H), 1.32 (br s, 22H), 1.48–1.69 (m, 4H), 2.11 (br s, 3H, exchangeable with D_2O), 3.40–3.48 (2m, 2H), 3.63 (m, 1H), 4.14 (dd, J = 6.4, 8.6 Hz, 1H); ^{13}C NMR (125 MHz, CDCl_3) δ 14.0, 22.6, 25.7, 29.2, 29.4, 29.5, 29.6, 31.8, 33.6, 52.5, 73.1, 74.6, 85.1; HRMS calcd. for $\text{C}_{18}\text{H}_{38}\text{NO}_2$ m/z ($\text{M} + \text{H}$)⁺, 300.2903; found, 300.2902.

Current Data Parameters
NAME Pb-bicyclic-lac
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20030321
Time 17.04
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 16
DS 2
SWH 6009.615 Hz
FIDRES 0.183399 Hz
AQ 2.7263477 sec
RG 322.5
DM 83.200 usec
DE 6.00 usec
TE 300.0 K
D1 0.03000000 sec

***** CHANNEL f1 *****
NUC1 1H
P1 7.70 usec
PL1 -4.00 dB
SFO1 500.1320005 MHz

F2 - Processing parameters
SI 32768
SF 500.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

F1 - Processing parameters
SI 1024
MC2 OF
SF 500.1300000 MHz
WDW no
SSB 0
LB 0.30 Hz
GB 0

ID NMR plot parameters
CX 20.00 cm
CY 10.00 cm
FIP 10.008 ppm
F1 5005.33 Hz
F2P -2.008 ppm
F2 -1004.29 Hz
PPMCH 0.50081 ppm/cm
HZCH 300.48077 Hz/cm

Current Data Parameters
NAME Pb-bicyclic-lac
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20030321
Time 17.52
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 1024
DS 4
SWH 31446.548 Hz
FIDRES 0.479836 Hz
AQ 1.0420724 sec
RG 10321.3
DM 15.390 usec
DE 6.00 usec
TE 300.0 K
D1 0.10000000 sec
D11 0.03000000 sec
D12 0.00002000 sec

***** CHANNEL f1 *****
NUC1 13C
P1 6.50 usec
PL1 5.00 dB
SFO1 125.7719472 MHz

***** CHANNEL f2 *****
CPDPRG2 waltz16
NUC2 1H
PCPD2 95.00 usec
PL2 -4.00 dB
PL12 19.00 dB
PL13 30.00 dB
SFO2 500.1325000 MHz

F2 - Processing parameters
SI 65536
SF 125.7578008 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 1024
MC2 OF
SF 500.1300000 MHz
WDW no
SSB 0
LB 0.30 Hz
GB 0

ID NMR plot parameters
CX 20.00 cm
CY 5.00 cm
FIP 237.517 ppm
F1 20869.63 Hz
F2P -12.538 ppm
F2 -1576.91 Hz
PPMCH 12.50282 ppm/cm
HZCH 1572.32703 Hz/cm

Current Data Parameters
NAME PB-L-ser-lactol
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20030601
Time 16.04
INSTRUM drx400
PROBHD 5 mm Multinucl
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 16
DS 2
SWH 4789.272 Hz
FIDRES 0.146157 Hz
AQ 3.4210291 sec
RG 64
OW 104.400 usec
OE 4.50 usec
TE 300.0 K
D1 1.00000000 sec

***** CHANNEL f1 *****
NUC1 1H
P1 7.70 usec
PL1 -6.00 dB
SFO1 400.1320007 MHz

F2 - Processing parameters
SI 16384
SF 400.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

ID NMR plot parameters
CX 20.00 cm
CY 14.77 cm
FIP 10.985 ppm
F1 4395.28 Hz
F2P -0.985 ppm
F2 -393.99 Hz
PPMCH 0.59846 ppm/cm
HZCH 239.46362 Hz/cm

Current Data Parameters
NAME PB-L-ser-lac-13C
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20030601
Time 16.47
INSTRUM drx400
PROBHD 5 mm Multinucl
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 1024
DS 2
SWH 23148.148 Hz
FIDRES 0.353213 Hz
AQ 1.4156276 sec
RG 3251
OW 21.600 usec
OE 4.50 usec
TE 300.0 K
D1 0.05000000 sec
d11 0.63000000 sec
d12 0.00002000 sec

***** CHANNEL f1 *****
NUC1 13C
P1 12.30 usec
PL1 2.00 dB
SFO1 100.6232933 MHz

***** CHANNEL f2 *****
CPDPRG2 waltz16
NUC2 1H
PCPD2 100.00 usec
PL2 0.00 dB
PL12 18.00 dB
PL13 18.00 dB
SFO2 400.1316005 MHz

F2 - Processing parameters
SI 32768
SF 100.6127290 MHz
WDW EN
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

ID NMR plot parameters
CX 20.00 cm
CY 5.67 cm
FIP 220.036 ppm
F1 221.38.41 Hz
F2P -10.036 ppm
F2 -1009.74 Hz
PPMCH 11.50359 ppm/cm
HZCH 1157.40747 Hz/cm

Current Data Parameters
 NAME KM 1-28 2
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20041203
 Time 18.33
 INSTRUM drx400
 PROBHD 5 mm QNP 1H/13
 PULPROG zg30
 TO 32768
 SOLVENT CDCl3
 NS 16
 DS 2
 SWH 4789.272 Hz
 FIDRES 0.146157 Hz
 AQ 3.4210291 sec
 RG 287.4
 OW 104.400 usec
 OE 5.50 usec
 FE 293.2 K
 O1 1.00000000 sec
 MCREST 0.00000000 sec
 MCWRR 0.01500000 sec

***** CHANNEL f1 *****
 NUC1 1H
 P1 9.35 usec
 PL1 -4.00 dB
 SF01 400.1320007 MHz

F2 - Processing parameters
 SI 32768
 SF 400.1300000 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00

1D NMR plot parameters
 CX 20.00 cm
 CY 12.50 cm
 F1P 10.985 ppm
 F1 4395.28 Hz
 F2P -0.985 ppm
 F2 -393.99 Hz
 PPMCH 0.59846 ppm/cm
 HZCH 239.46362 Hz/cm

Current Data Parameters
 NAME KM 1-28 2
 EXPNO 2
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20041203
 Time 20.14
 INSTRUM drx400
 PROBHD 5 mm QNP 1H/13
 PULPROG zgpg30
 TO 65536
 SOLVENT CDCl3
 NS 2307
 DS 4
 SWH 23148.148 Hz
 FIDRES 0.353213 Hz
 AQ 1.4156276 sec
 RG 4096
 DW 21.600 usec
 DE 5.50 usec
 TE 293.2 K
 O1 0.15000001 sec
 d11 0.03000000 sec
 DELTA 0.05000000 sec
 MCREST 0.00000000 sec
 MCWRR 0.01500000 sec

***** CHANNEL f1 *****
 NUC1 13C
 P1 11.25 usec
 PL1 2.00 dB
 SF01 100.6232933 MHz

***** CHANNEL f2 *****
 CPDPRG2 waltz16
 NUC2 1H
 PCPD2 100.00 usec
 PL2 -4.00 dB
 PL12 16.58 dB
 PL13 17.00 dB
 SF02 400.1316005 MHz

F2 - Processing parameters
 SI 65536
 SF 100.6127290 MHz
 WDW EM
 SSB 0
 LB 1.00 Hz
 GB 0
 PC 1.40

1D NMR plot parameters
 CX 20.00 cm
 CY 12.50 cm
 F1P 220.036 ppm
 F1 22138.41 Hz
 F2P -10.036 ppm
 F2 -1009.74 Hz
 PPMCH 11.50359 ppm/cm
 HZCH 1157.40747 Hz/cm

Current Data Parameters
 NAME 1_29
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20030724
 Time 13.06
 INSTRUM drx400
 PROBHD 5 mm Multinuc1
 PULPROG zg30
 TO 32768
 SOLVENT CDCl3
 NS 16
 DS 2
 SWH 4789.272 Hz
 FIDRES 0.146157 Hz
 AQ 3.4210291 sec
 RG 161.3
 OW 104.400 usec
 DE 4.50 usec
 TE 300.0 K
 D1 1.0000000 sec

***** CHANNEL f1 *****
 NUC1 1H
 P1 7.70 usec
 PL1 -6.00 dB
 SFO1 400.1320007 MHz

F2 - Processing parameters
 SI 16384
 SF 400.1300000 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00

1D NMR plot parameters
 CX 20.00 cm
 CY 12.50 cm
 F1P 10.985 ppm
 F1 4395.28 Hz
 F2P -0.985 ppm
 F2 -393.99 Hz
 PPHCH 0.59846 ppm/cm
 HZCH 239.46362 Hz/cm

Current Data Parameters
 NAME 1_29
 EXPNO 2
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20030725
 Time 10.16
 INSTRUM drx400
 PROBHD 5 mm Multinuc1
 PULPROG zgpg30
 TO 65536
 SOLVENT CDCl3
 NS 956
 DS 2
 SWH 23148.148 Hz
 FIDRES 0.353213 Hz
 AQ 1.4156276 sec
 RG 3649.1
 OW 21.600 usec
 DE 4.50 usec
 TE 300.0 K
 D1 0.05000000 sec
 D11 0.03000000 sec
 D12 0.00020000 sec

***** CHANNEL f1 *****
 NUC1 13C
 P1 12.30 usec
 PL1 2.00 dB
 SFO1 100.6232933 MHz

***** CHANNEL f2 *****
 CPDPRG2 waltz16
 NUC2 1H
 PCPD2 100.00 usec
 PL2 0.00 dB
 PL12 18.00 dB
 PL13 18.00 dB
 SFO2 400.1316005 MHz

F2 - Processing parameters
 SI 32768
 SF 100.6127290 MHz
 WDW EN
 SSB 0
 LB 1.00 Hz
 GB 0
 PC 1.40

1D NMR plot parameters
 CX 20.00 cm
 CY 12.50 cm
 F1P 215.000 ppm
 F1 21631.74 Hz
 F2P -50.00 ppm
 F2 -503.06 Hz
 PPHCH 11.00000 ppm/cm
 HZCH 1106.73999 Hz/cm

Current Data Parameters
 NAME PB-1asp
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20030430
 Time 9.20
 INSTRUM dr400
 PROBHD 5 mm Multinucl
 PULPROG zg30
 TO 32768
 SOLVENT COC13
 NS 16
 DS 2
 SWH 4789.272 Hz
 FIDRES 0.146157 Hz
 AQ 3.4210291 sec
 RG 125
 DM 104.400 usec
 DE 4.50 usec
 TE 300.0 K
 D1 1.00000000 sec

===== CHANNEL f1 =====
 NUC1 1H
 P1 7.70 usec
 PL1 -6.00 dB
 SF01 400.1320007 MHz

F2 - Processing parameters
 SI 16384
 SF 400.1300000 MHz
 MDW EN
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00

1D NMR plot parameters
 CX 20.00 cm
 CY 12.50 cm
 F1P 10.985 ppm
 F1 4395.28 Hz
 F2P -0.985 ppm
 F2 -393.99 Hz
 PPHCH 0.59846 ppm/cm
 HZCH 239.46362 Hz/cm

Current Data Parameters
 NAME PB-1asp
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20030430
 Time 18.27
 INSTRUM spect
 PROBHD 5 mm BBO BB-1H
 PULPROG zgpg30
 TO 65536
 SOLVENT COC13
 NS 1024
 DS 4
 SWH 31446.541 Hz
 FIDRES 0.479836 Hz
 AQ 1.0420724 sec
 RG 11985.2
 DM 15.900 usec
 DE 6.00 usec
 TE 300.0 K
 D1 0.10000000 sec
 d11 0.03000000 sec
 d12 0.00002000 sec

===== CHANNEL f1 =====
 NUC1 13C
 P1 6.50 usec
 PL1 5.00 dB
 SF01 125.7719472 MHz

===== CHANNEL f2 =====
 CPDPRG2 waltz16
 NUC2 1H
 P2 95.00 usec
 PL2 -4.00 dB
 PL12 19.00 dB
 PL13 30.00 dB
 SF02 500.1325000 MHz

F2 - Processing parameters
 SI 65536
 SF 125.7578008 MHz
 MDW EN
 SSB 0
 LB 1.00 Hz
 GB 0
 PC 1.40

1D NMR plot parameters
 CX 20.00 cm
 CY 18.00 cm
 F1P 136.908 ppm
 F1 17217.31 Hz
 F2P -8.876 ppm
 F2 -1118.27 Hz
 PPHCH 7.28924 ppm/cm
 HZCH 916.87869 Hz/cm