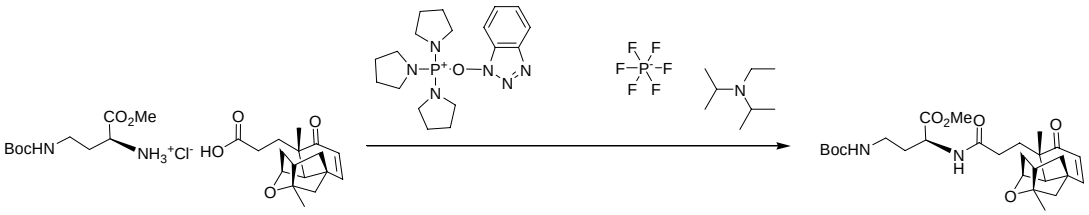
Structure and Semisynthesis of Platensimide A, Produced by Streptomyces platensis

Kithsiri B. Herath, Chaowei Zhang, Hiranthi Jayasuriya, John G. Ondeyka, Deborah L. Zink, Bruce Burgess, Jun Wang, Sheo B. Singh*

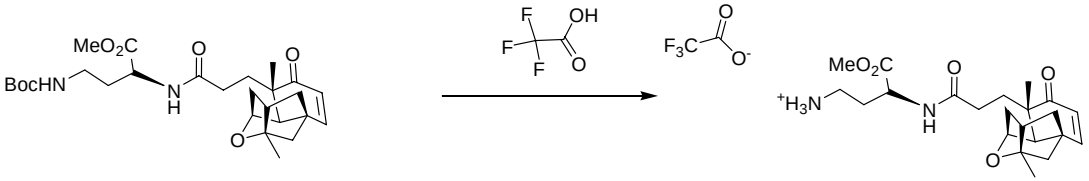
Merck Research Laboratories, Rahway, New Jersey 07065, USA

Experimental

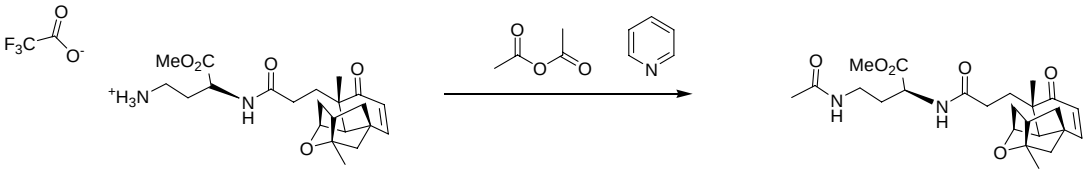
All NMR spectra is included as a separate file.



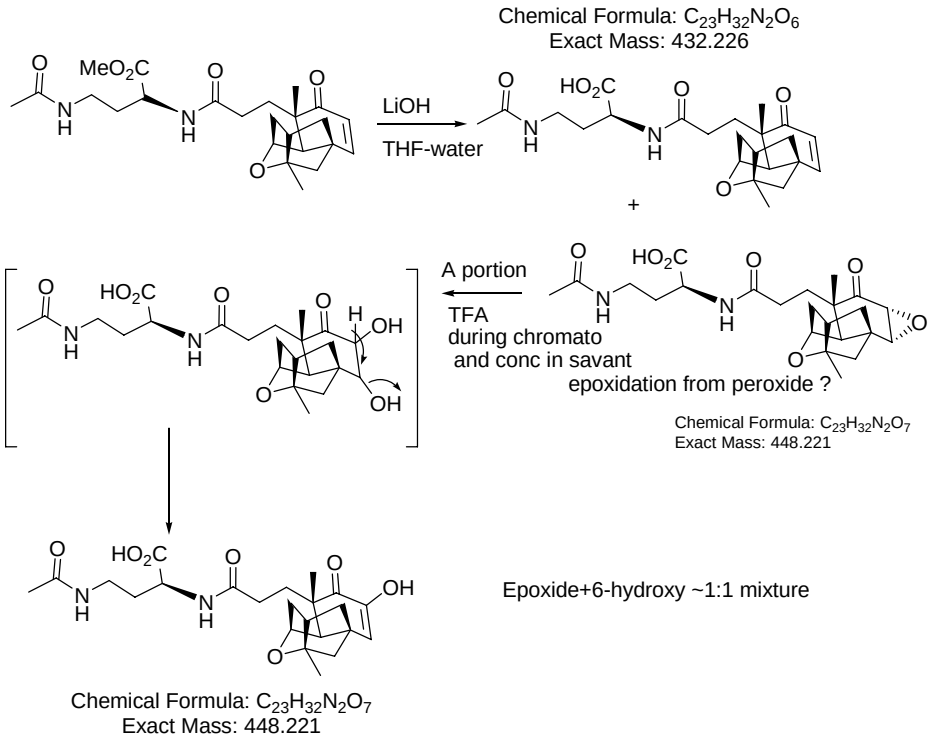
Synthesis of Compound 7: To a solution of platensic acid (**4**, 20.6 mg, 0.07 mmol) in DMF (1 mL) was added Boc-L-lysine methyl ester (22.9 mg, 0.085 mmol), PyBop reagent (44.3 mg, 0.085 mmol) followed by DIPEA (0.037 mL, 0.213 mmol). The reaction mixture was stirred at room temperature for 2 hrs and analyzed by HPLC [Zorbax RX C-8 (4.6 x 250 mm), 10-95% aqueous-MeCN +0.1% TFA gradient, 1 mL/min, product retention time 11.46 min]. The reaction mixture was diluted with one mL MeOH and directly prepped on RPHPLC (Zorbax RX C-8 (22 x 250 mm), 37 min 10-95% aq -MeCN +0.1% TFA gradient, 12 mL/min, 230 nm detection, two runs, product eluted at ret time 26-28 min, pooled from both run, lyophilized to give 30.5 mg (85% yield) of **7** as a colorless powder. LCMS *m/z* 505 (M+H). ^{1}H NMR (CD_3OD) δ 6.62 (1H, d, J = 10 Hz), 5.86 (1H, d, J = 10 Hz), 4.47 (1H, brs), 4.42 (1H, dd, J = 9, 5 Hz), 3.70 (3H, s), 3.15 (1H, m), 3.04 (1H, m), 2.42 (1H, t, J = 6 Hz), 2.37 (1H, brs), 2.25-2.15 (2H, m), 2.15-1.95 (4H, m), 1.85-1.75 (5H, m), 1.70 (1H, d, 11.5 Hz), 1.43 (9H, s), 1.42 (3H, s), 1.22 (3H, s).



Synthesis of Compound 11: To a solution of 24 mg (0.048 mmol) of platensyl-Boc-(S)-Lys-OMe (**7**) in $CHCl_3$ (1 mL) was added TFA (0.366 mL) at 0 ^oC. The reaction mixture was stirred for 2 hr. RPHPLC analysis (same condition as above) indicated completion of the reaction. Starting material t_R = 11.46 min, product t_R 6.84 min. The reaction mixture was concentrated to dryness under a stream of N_2, re-dissolved in 1 mL $CHCl_3$, 200 uL was taken out and 800 uL was used for the next step. 200 uL was diluted with 400 uL MeOH and prepped on RPHPLC (Zorbax RX C-8 (22 x 250 mm), 37 min 10-95% aqueous -MeCN +0.1% TFA gradient, 12 mL/min, 230 nm detection. Fractions eluting at 17-19 min were pooled and lyophilized to yield 3.9 mg (80%) of **11** as a powder. LCMS *m/z* 405 (M+H), ^1H NMR (C_5D_5N) δ 9.44 (1H, d, J = 7.5 Hz), 6.37 (1H, d, J = 10 Hz), 5.94 (1H, d, J = 10 Hz), 5.19 (1H, dt, J = 5, 8.5 Hz), 4.48 (1H, brs), 3.64 (1H, m), 3.61(3H, s), 3.57 (1H, m), 2.79 (1H, m), 2.61-2.45 (4H, m), 2.43 (1H, brs), 2.19 (1H, t, J = 6.5 Hz), 2.03 (1H, m), 1.89 (1H, m), 1.81 (1H, dd, J = 9.5, 3 Hz), 1.80 (1H, d, J = 11.5 Hz), 1.73 (1H, dd, J = 11, 3.5 Hz), 1.56 (1H, m), 1.48 (1H, d, J = 11 Hz), 1.39 (3H, s), 1.13 (3H, s).

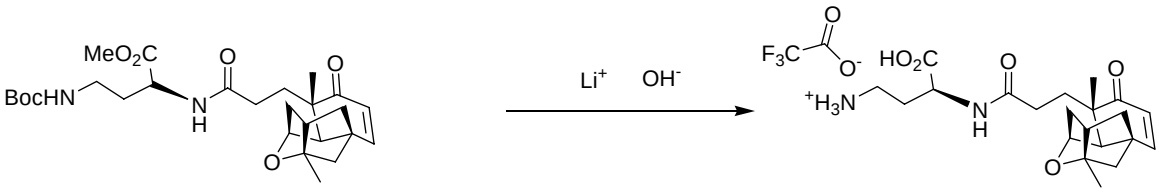


Synthesis of Compound 12: 80% of the product from the above reaction in 800 uL $CHCl_3$ was cooled to 0 ^oC and Ac_2O (0.38 mL) was added first followed by pyridine (0.31 mL). Reaction was complete in less than 10 min. Starting material (**11**) eluted at t_R 6.84 min and the product (**12**) eluted at t_R 7.95 min (HPLC condition; Zorbax Rx C-8 (4.6 x 250 mm), 10-95% Aq MeCN+0.1% TFA in 15 min,1 mL/min). The reaction mixture was quenched with methanol, concentrated to dryness (N_2), and prepped by RPHPLC, (Zorbax RX C-8 (22 x 250 mm), 37 min 10-95% aqueous -MeCN +0.1% TFA gradient, 12 mL/min, 230 nm detection. Fractions eluting at 20-23 min were pooled and lyophilized to give **12** (11.2 mg, 65.9% two step yield). LCMS *m/z* 447 (M+H), ^1H NMR (C_5D_5N) δ 9.13 (1H, d, J = 8 Hz), 8.52 (1H, brs), 6.36 (1H, d, J = 10 Hz), 5.93 (1H, d, J = 10 Hz), 5.14 (1H, dt, J = 5, 8.5 Hz), 4.46 (1H, brs), 3.75 (1H, m), 3.62 (3H, s), 3.53 (1H, m), 2.62 (1H, ddd, J = 13.5, 11, 4.5 Hz), 2.54 (1H, ddd, J = 13.5, 11, 5 Hz), 2.46 (1H, ddd, J = 13.5, 10.5, 4.5 Hz), 2.41 (1H, brs), 2.37 (1H, m), 2.18 (1H, t, J = 7 Hz), 2.13 (1H, m), 2.05 (1H, m), 2.03 (3H, s), 1.86 (1H, m), 1.80 (1H, dd, J = 9.5, 3 Hz), 1.78 (1H, d, J = 11.5 Hz), 1.74 (1H, dd, J = 10.5, 3.5 Hz), 1.56 (1H, m), 1.47 (1H, d, J = 11 Hz), 1.39 (3H, s), 1.13 (3H, s).

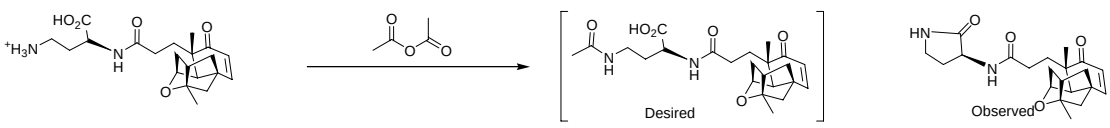


Synthesis of Compound 5, 13 and 14: To a solution of 7 mg of **12** in 1 mL THF-water (1:1) was added 25 mg of LiOH and stirred for 2 hrs. HPLC analysis indicated the presence of 50% of starting material. Additional 20 mg of LiOH was added and stirred for additional 2 hrs. The starting material was used up and three products were formed. The reaction mixture was cooled to 0 ^{o}C and quenched with TFA and directly purified on RPHPLC, (Zorbax RX C-8 (22 x 250 mm), 37 min 10-95% aq -MeCN +0.1% TFA gradient, 12 mL/min, 230 nm detection. Fractions eluting at 19-20 were pooled and lyophilized to give 5 mg of a mixture of products. A 4 mg aliquot was re-purified using a 22% MeCN +0.1% TFA isocratic system on a Zorbax RX C-8 (4.6 x 250 mm), 1 mL/min, 4 injections. Lyophilization of pooled fractions 10-12 and 13-15 afforded colorless powders of inseparable mixtures of **13**+**14** (LCMS of both *m/z* 449 (M+H) and **5** (LCMS *m/z* 433 (M+H), respectively. The epoxide in fractions 10-12 hydrolyzed and dehydrated to produce a ~1:1 mixture of epoxide and 6-hydroxy analog which were difficult to separate and were tested together. The formation of the oxidized products **13** and **14** was prevented when 1 mg of **12** was hydrolyzed by using LiOH (2 mg) in fresh THF-water mixture (0.3 mL, 1:1) for 2 hrs and analyzed by HPLC and LCMS. Under this condition product **5** was formed exclusively. No oxidized products were formed.), Approxinmately 1:1 ratio of 13+14, ^{1}H NMR (C_5D_5N) δ 9.13, 8.95 (1H, d, J = 8 Hz), 8.53, 8.50 (1H, brs), 5.86 (0.5 H, s), 5.25 (1H, m), 4.42, 4.30 (1H, brs), 3.86 (1H, m), 3.68, 3.59 (3H, s), 3.58 (1H, m), 3.44 (0.5H, d, J = 4.0 Hz), 3.15 (0.5 H, d, 4 Hz), 2.71 (0.5H, ddd, J = 13.5, 11, 4.5 Hz), 2.61-2.38 (5H, m), 2.3-2.05 (m), 2.00 (3H, s), 1.95 (1H,

dd, J = 11, 3.5 Hz), 1.85-1.78 (m), 1.56 (1H, m), 1.48, 1.44 (1H, d, J = 11 Hz), 1.35 (3H, s), 1.26 (1H, dd, J = J = 11.5, 3.5 Hz), 1.17 , 1.1 (3H, s).



Synthesis of Compounds 8 and 9: To a stirred solution of 11 mg (0.022 mmol) of platensyl-Boc-(S)-Lys-OMe (**7**) in THF-water (1:1, 0.5 mL) was added 40 mg (1.67 mmol) of solid LiOH. Hydrolysis of methyl ester was complete within 30 min. Starting material t_R 11.75 min (Zorbx RX C-8, 4.6 x 250 mm, 20-95% MECN-H20+0.1% TFA in 15 min, 1 mL/min) hydrolyzed acid (**8**) t_R 10.06 min. LCMS (m/z) 491 (M+H); This mixture was cooled to 0 ^oC and acidified with TFA (600 uL) and allowed to stir at RT overnight. Boc group was cleaved eluting ay t_R = 6.01 min (Zorbax RX C8, 4.6 x 250 mm, 20% aq MeCN+0.1% TFA, isocratic). The mixture was concentrated under a stream of N2 and chromatographed on Zorbax RX C8 (21.2 x 250 mm), gradient 10-95% in 37 min, 12 mL/min. Fractions eluting at 16-19 min were pooled and lyophilized to give 13 mg of the TFA salt (**9**). This material was contaminated with an unrelated material and needed further purification. A 5 mg aliquot of this impure material was used for acetylation reaction and remaining 8 mg was purified using 20% aqueous MeCN +0.1% TFA, isocratic, Zorbax RX C8 (9.4x 250 mm), 4 mL/min, 3 runs, fractions 7-9 pooled and lyophilized to give 4 mg (47%, adjusted yield for the sample split 75%) of the clean product **9** as a TFA salt. LCMS *m/z* 392 (M+H), ^1H NMR (C_5D_5N) δ 9.00 (1H, d, J = 7 Hz), 8.72 (1H, brs), 6.36 (1H, d, J = 10 Hz), 5.94 (1H, d, J = 10 Hz), 4.96 (1H, td, J = 8.5, 9.5 Hz), 4.46 (1H, brs), 3.20 (2H, m), 2.64 (1H, ddd, J = 13.5, 11, 4.5 Hz), 2.57 (1H, m), 2.53 (1H, ddd, 13.5, 11, 5 Hz), 2.46 (1H, ddd, J = 13.5, 10.5, 4.5 Hz), 2.43 (1H, brs), 2.18 (1H, t, J = 6.5 Hz), 2.04 (2H, m), 1.88 (1H, m), 1.80 (1H, dd, J = 9.5, 3 Hz), 1.79 (1H, d, J = 11.5 Hz), 1.74 (1H, dd, J = 10.5, 3.5 Hz), 1.56 (1H, m), 1.46 (1H, d, J = 11 Hz), 1.39 (3H, s), 1.13 (3H, s).



Synthesis of Compound 10: 5 mg of the TFA salt of the impure material of **9** from the above experiment was dissolved in 250 uL pyridine and 25 uL of Ac_2O was added. Reaction was complete within 20 min (HPLC, t_R = 19.8 min, 20% aq MeCN+0.1% TFA, isocratic, Zorbax RX

C-8, 4.6 x 250 mm, 1 mL/min). Volatile materials were removed by blowing a stream of N2 and the product was purified by RPHPLC on Zorbax RX C8 (9.4 x 250 mm), 20% Aq MeCN+0.1% TFA, 4 mL/min, 3 rums of 75 uL injection. The product eluted in fractions 13-16, they were pooled and lyophilized to afford the lactam **10** instead of N-acetylated acid. LCMS *m/z* 373 (M+H), ^{1}H NMR (C_5D_5N) δ 9.10 (1H, d, J = 6.5 Hz), 6.38 (1H, d, J = 10 Hz), 5.95 (1H, d, J = 10 Hz), 5.27 (1H, brm), 4.48 (1H, brs), 3.69 (1H, m), 3.60 (1H, m), 2.92 (1H, m), 2.61-2.46 (4H, m), 2.43 (1H, brs), 2.19 (1H, t, J = 6.5 Hz), 2.01 (1H, m), 1.88 (1H, m), 1.81 (1H, dd, J = 9.5, 3 Hz), 1.79 (1H, d, J = 11 Hz), 1.76 (1H, dd, J = 10.5, 3.5 Hz), 1.56 (1H, m), 1.48 (1H, d, J = 10.5 Hz), 1.38 (3H, s), 1.13 (3H, s).