CHARACTERIZATION AND INHIBITION OF A CLASS II DITERPENE CYCLASE FROM *MYCOBACTERIUM TUBERCULOSIS*: IMPLICATIONS FOR TUBERCULOSIS

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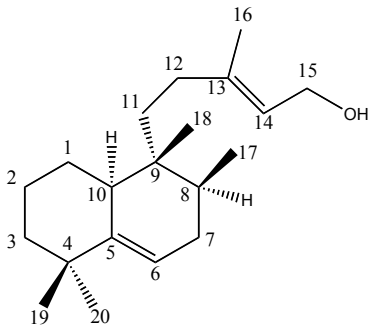
SUPPLEMENTAL INFORMATION

NMR Structural Analysis of Halimadien-15-ol (3b)

NMR spectra for the edaxadiene (3) product were recorded at 25 °C on a Bruker Avance 700 equipped with a probe with cryogenic detection for ^{1}H and ^{13}C. Structural analysis was undertaken using 1D ^{1}H, 1D ^{13}C, DQF-COSY, HSQC, multiplicity-edited HSQC, HMBC, HMQC-COSY and ROESY spectra acquired at 700 MHz using standard experimental protocols. Through this effort it was possible to verify the structural equivalence of **3b** to that previously reported.

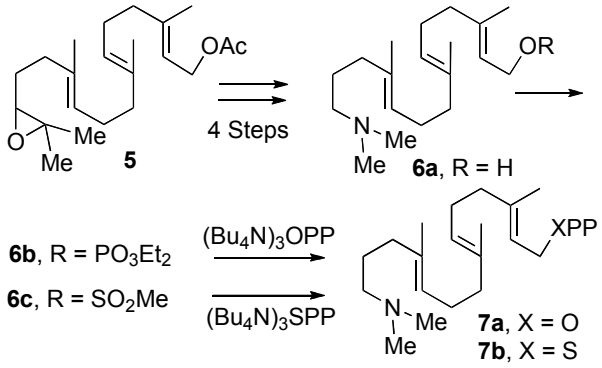


Halimadienol/Tuberculosinol (3b): Carbon numbering as indicated

NMR chemical shift data for 3b

C	δ_C (ppm)	δ_H (ppm)
1	28.17	1.079(m), 1.748(m)
2	22.99	1.558(2H, m)
3	41.63	1.269(m), 1.397(m)
4	36.67	
5	146.54	
6	117.12	5.547(bt)
7	32.35	1.829(2H, m)
8	34.07	1.512(m)
9	37.61	
10	40.66	2.259(bd, J=12.52)
11	35.70	1.380(m), 1.527(m)
12	33.41	1.873(m), 1.942(m)
13	139.56	
14	124.83	5.422(t)
15	59.80	3.988(d, J=6.45Hz)
16	16.78	1.511(3H, s)
17	15.66	0.807(3H, d, J=6.75)
18	30.43	1.149(3H, s)
19	29.55	1.100(3H, s)
20	16.83	0.694(3H, s)

General Aspects, Preparative Procedures, and Characterization Data for 3-AzaGGSPP



General Aspects

Solvents were distilled from the indicated drying agents prior to use. CH_3CN was distilled from P_2O_5 and stored over 3 Å molecular sieves. Et_3N and pyridine were distilled from CaH_2 and stored over KOH. Diethyl chlorophosphate and methanesulfonyl chloride were obtained from Aldrich Chemical Company and used without further purification. Dihydrogen disodium pyrophosphate was obtained from

Sigma Chemical Company and was converted to $HOPP(NBu_4)_3$ according to a literature procedure reported by Poulter and co-workers.[1] The progress of the reactions was followed by TLC unless stated otherwise. TLC analyses were performed on plastic-backed Merck Kieselgel 60 F254 plate with visualization by UV fluorescence at 254 nm, I_2 stain, or phosphomolybdic acid stain (PMA, 10 % in absolute ethanol) followed by development on a hot plate at 120 °C. Dowex AG 50W-X8 ion exchange resin (H^+ form) obtained from BioRad was converted to the ammonium form before each chromatography according to a published procedure.[1] Flash chromatography was performed according to the procedure of Still[2] using Merck 0.040-0.063 mm silica gel.

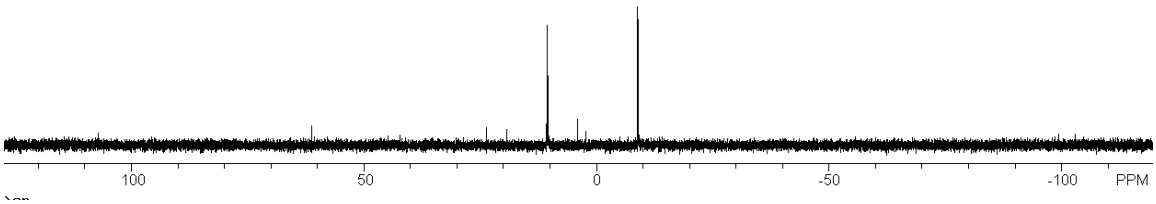
^1H NMR spectra were acquired on Unity 400 or Unity 500 spectrometers at 400 MHz or 500 MHz in $CDCl_3$ with $CHCl_3$ as the internal reference ($CHCl_3$ = 7.26 ppm) or C_6D_6 with C_6D_5H as the internal reference (C_6D_5H = 7.16 ppm), or CD_3OD with CD_3OH as the internal reference (CD_3OH = 4.78 ppm). ^{31}P NMR spectra were recorded with the Unity 400 at 162 MHz in CD_3OD with 85 % H_3PO_4 as the external reference (H_3PO_4 = 0.00 ppm), unless indicated otherwise.

(*E*, *E*, *E*)-15-Aza-14,15-dihydrogeranylgeraniol (6a). The amino alcohol (200 mg) was prepared from (*E*, *E*, *E*)-geranylgeraniol (2.04 g)[3] via 14,15-epoxygeranylgeranyl acetate (**5**). Terminal hypobromination of GGOAc and conversion to the 14,15-epoxy acetate (790 mg) followed by acid-catalyzed hydrolysis provided the 14,15-diol acetate intermediate (150 mg).[4,5] $NaIO_4$ cleavage of the 14,15-diol, reductive amination of the trisnor aldehyde acetate with dimethylamine, and acetate hydrolysis as previously described gave 15-azaGGOH (**6a**).[4] The physical properties and NMR spectra agreed with the literature values.[4]

(*E*, *E*, *E*)-Geranylgeranyl Thiolodiphosphate, Ammonium Salt.

The procedure was modeled after a literature description for preparation of the same compound,[6] except that the meslyate intermediate generated in dry acetonitrile, was used instead of geranylgeranyl bromide. A solution of (*E*, *E*, *E*)-geranylgeraniol[3] (30.0 mg , 0.103 mmol) and triethylamine (28.0 μL, 20.3 mg, 0.201 mmol) in 1.5 mL of dry acetonitrile was stirred and cooled at -30 °C under N_2 as neat methanesulfonyl chloride (12.0 μL, 17.7 mg, 0.154 mmol) was added slowly. After 30 min at -30 °C, solid tetrabutylammonium thiopyrophosphate salt (230 mg, 0.198 mmol)[6] and 150 mg of 3Å molecular sieves were added, and the suspension was stirred for another 30 min at same temp. The mixture was warmed up to 0 °C and stirred for 1 h. The solids were removed by filtration, and the filter cake was washed with 30 mL of MeCN. The filtrate was concentrated at room temperature under reduced pressure.

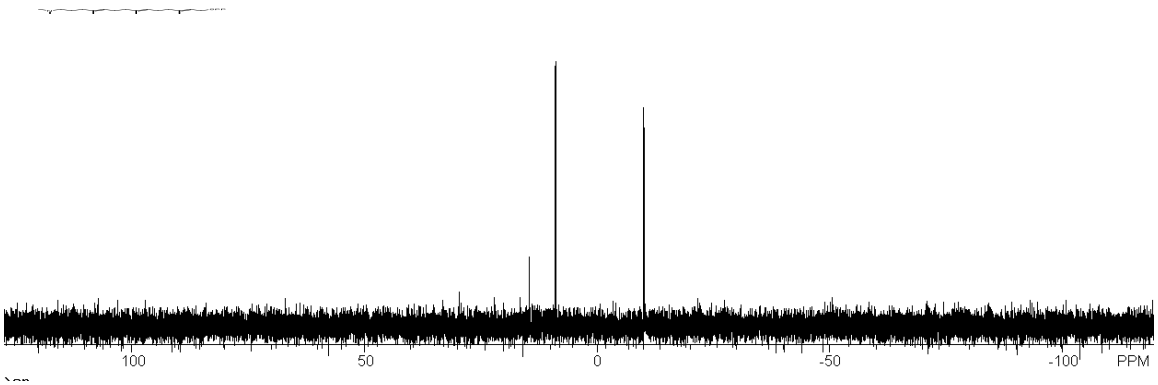
Ion exchange to the ammonium salt was accomplished by dissolving the residue in aqueous buffer composed of 2% v/v 25 mM aqueous NH_4HCO_3 and i-PrOH, and passage of the solution through a Dowex column (2 × 25 cm) in the ammonium form and elution with 50 mL of buffer solution.[5] The eluate was lyophilized, and the remaining salt was extracted with MeOH (3 x 8 mL). The combined MeOH extracts were evaporated to give 87 mg of the crude product as a white solid. Purification by flash column chromatography on cellulose[1] using MeCN : i-PrOH : 50 mM aqueous NH_4HCO_3 = 2 : 1 :1 afforded 44.0 mg (0.0854 mmol, 83%) of the known thiolodiphosphate[6b] as a white solid: ^{31}P NMR (CD_3OD, 400 MHz, unreferenced) δ 10.60 (d, J = 27.2 Hz, 1P), -8.94 (d, J = 27.2 Hz, 1P). The ^{31}P NMR spectrum also showed a small singlet (7%) attributed to a PPi contaminant.



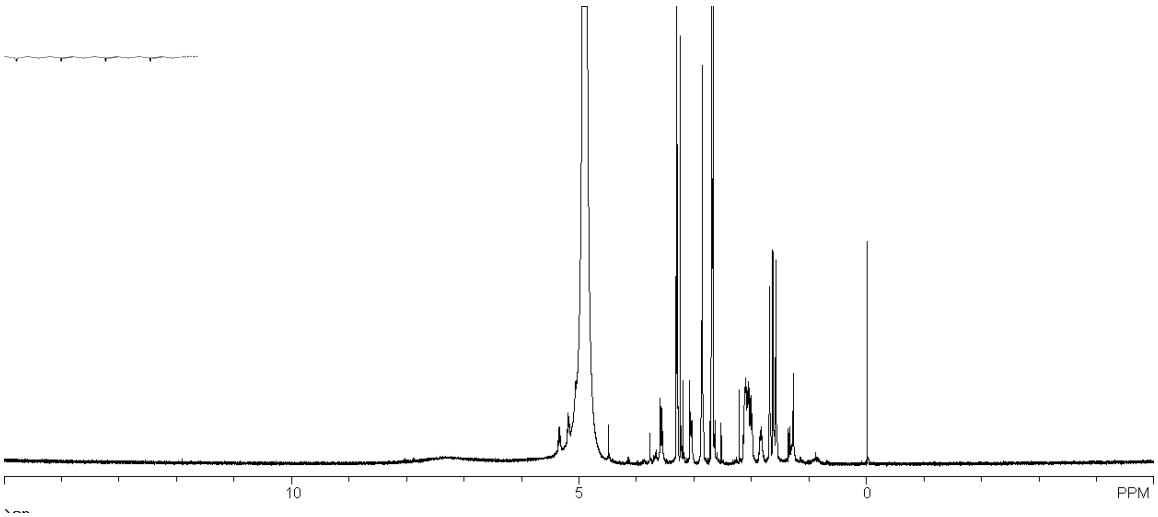
^{31}P NMR Spectrum (CD_3OD, 162 MHz)

(*E*, *E*, *E*)-15-Aza-14, 15-dihydrogeranylgeranyl Thiolodiphosphate (7b).

Reaction of 15-azaGGOH (**6a**, 30.5 mg, 0.104 mmol) (ref 4) with triethylamine, mesyl chloride, and tetrabutylammonium thiopyrophosphate in dry acetonitrile, ion exchange, MeOH extractions, and purification by cellulose chromatography as described above for geranylgeranyl SPP afforded the aza analogue as a white solid: yield 46.0 mg (0.0887 mmol, 85%): ^{31}P NMR (CD_3OD, 162 MHz) δ 8.97 (d, J = 27.7 Hz, 1P), -10.03 (d, J = 27.5 Hz, 1P). ^{1}H NMR (CD_3OD, 400 MHz) δ 4.6 – 6.0 (m, OH), 3.54 – 3.62 (m, 2H), 3.04 – 3.12 (m, 2H), 2.87 (s, 6H), 1.79 – 2.17 (m, 12H), 1.69 (s, 3H), 1.64 (s, 3H), 1.58 (s, 3H).



^{31}P NMR spectrum (CD_3OD, 162 MHz)



^{1}H NMR Spectrum (CD_3OD, 400 MHz)

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6. (a). Phan, R. M. and Poulter, C. D. (2000) *Org. Lett.* **2**, 2287 – 2289. (b) Phan, R. M. and Poulter, C. D. (2001) *J. Org. Chem.* **66**, 6705 – 6710.