Supporting Information

Farnesyl Diphosphate Synthase: The Art of Compromise between Stereoselectivity and Substrate Selectivity

Hirekodathakallu V. Thulasiram and C. Dale Poulter*

315 South 1400 East RM 2020; Department of Chemistry; University of Utah, Salt Lake City, UT, USA 84112

*Corresponding author: Phone: 801-581-6685; Fax: 801-581-4391; e-mail: poulter@chemistry.utah.edu

Supporting Information

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Materials and Methods

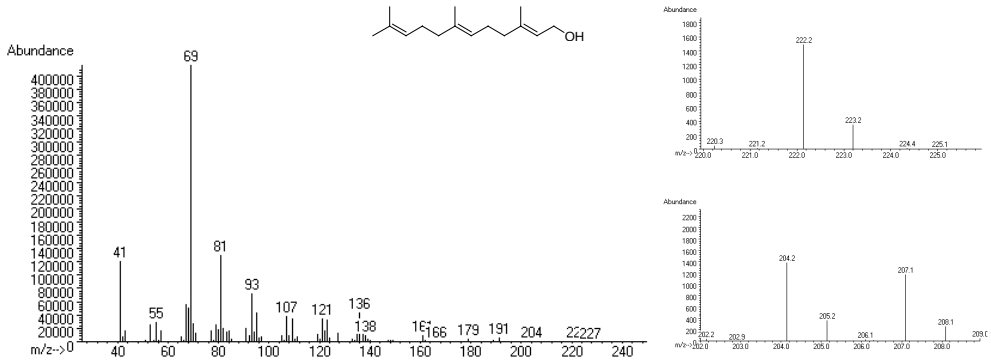
E. coli XA-90 were provided by Gregory Verdine (Harvard University, Boston, MA). Restriction enzymes were purchased from Invitrogen, Stratagene, or Amersham Biosciences. Unlabeled IPP, DMAPP, GPP, and NPP were synthesized as reported earlier[1] and concentrations were determined by phosphate analysis[2]. (*R*)-[2-^{2}H]IPP and (*S*)-[2-^{2}H]IPP were provided by Professor John Vederas. BglCV was purified as previously described.[3]

Synthesis of isomeric farnesols.

Ethyl (2Z/E,6E)-3,7,11-trimethyl-2,6,10-trienoate. A solution of triethyl phosphonoacetate (1.33 g, 5.95 mmol) was added dropwise to a suspension of sodium hydride (50% in oil, 285.6 mg, 5.95 mmol) in dry THF (5mL) at 0 °C under nitrogen. After 2 h, 1.05 g (5.41 mmol) of geranyl acetone was added dropwise and the solution was stirred for 3 h as the mixture warmed to room temperature. The reaction mixture was cooled to 0 °C and excess of hydride was quenched with 5 mL of water. The aqueous layer was extracted three times with diethyl ether. The combined ether layers were washed with water and saturated brine, and dried over anhydrous $NaSO_4$. Solvent was removed at reduced pressure, and the residue was chromatographed on silica gel (3% ethyl acetate in hexane) to give 1.05g (73%) of the α,β-unsaturated ester as a 4:1 of *E,E* and *Z,E* isomers. NMR (^{1}H and ^{13}C) spectra were consistent with those reported previously. [4]

(2Z,6E)-3,7,11-Trimethyldodeca-2,6,10-trien-1-ol. To a solution of ethyl (2Z/*E*,6E)-3,7,11-trimethyl-2,6,10-trienoate (0.75 mmol, 198 mg) in 7.5 mL of toluene at -78 °C under nitrogen was added 3.5 mL (1.0 M in toluene; 3.5 mol) of diisobutyl

aluminum hydride. The mixture was stirred for 1 h at -78 °C and then quenched with 25 mL of saturated aqueous potassium sodium tartrate. The organic phase was separated, and the aqueous phase was extracted with ethyl acetate. The combined organic layers were washed with water and brine (20 mL) and dried over $MgSO_4$. Solvent was removed at reduced pressure and part of the residue was chromatographed on a preparative1% silver nitrate silica TLC plate developed with hexane:ethylacetate:methanol:acetone (7:1:1:1). NMR (^{1}H and ^{13}C) spectra for *Z*,*E*-farnesol [5,6] (R_f = 0.40) and *E*,*E*-farnesol [6,7] (R_f =0.30) were consistent with those reported previously.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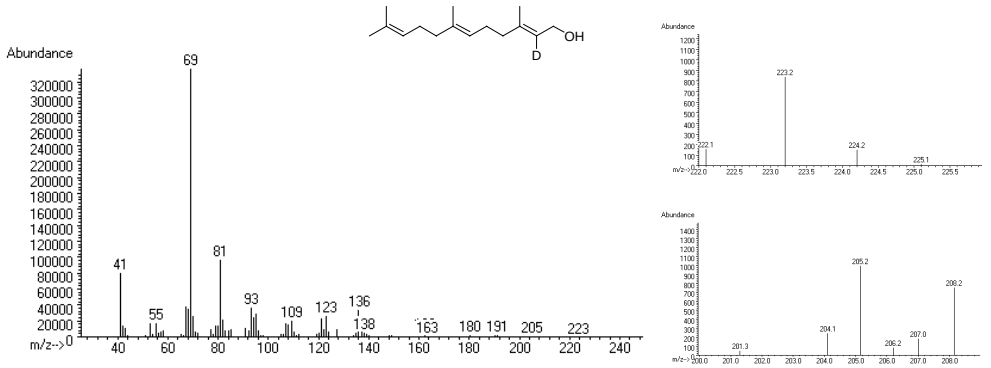
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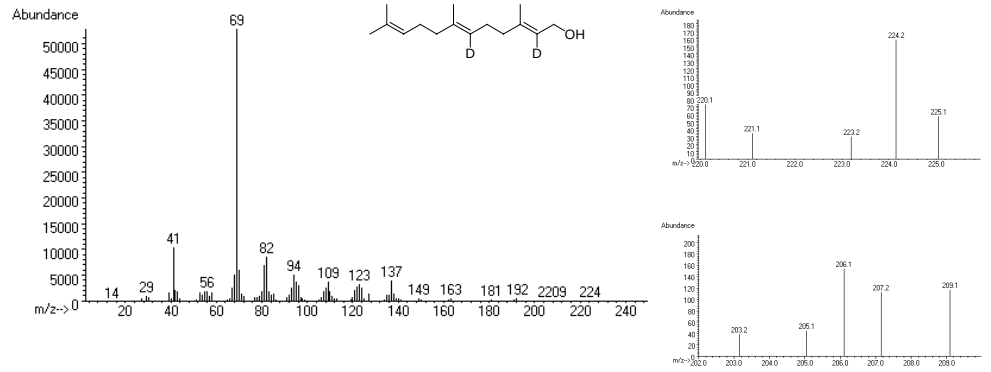
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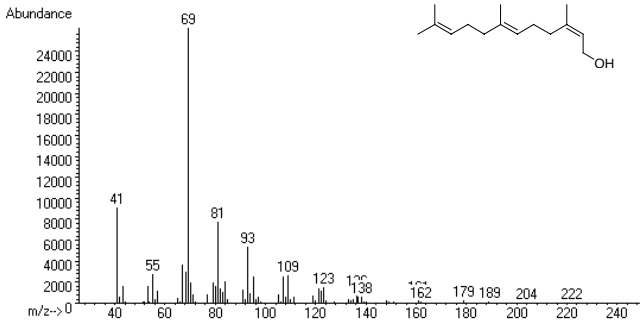
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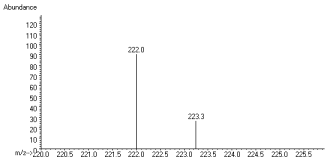
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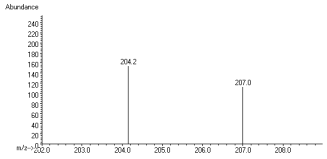
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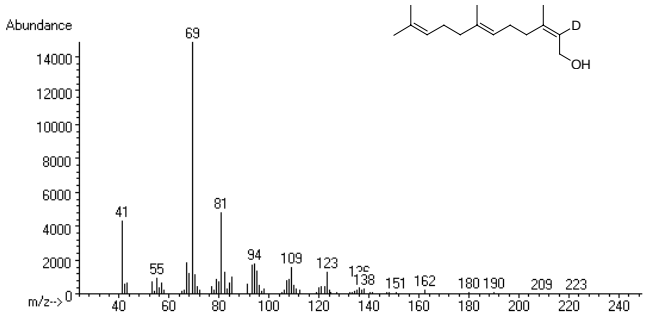
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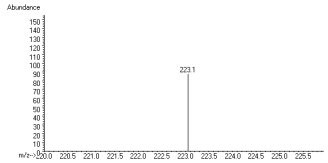
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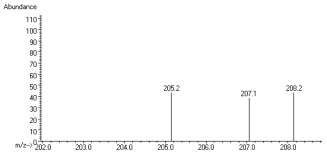
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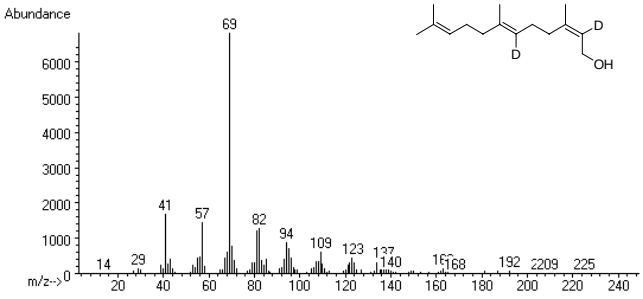
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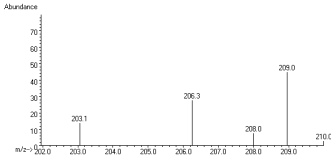
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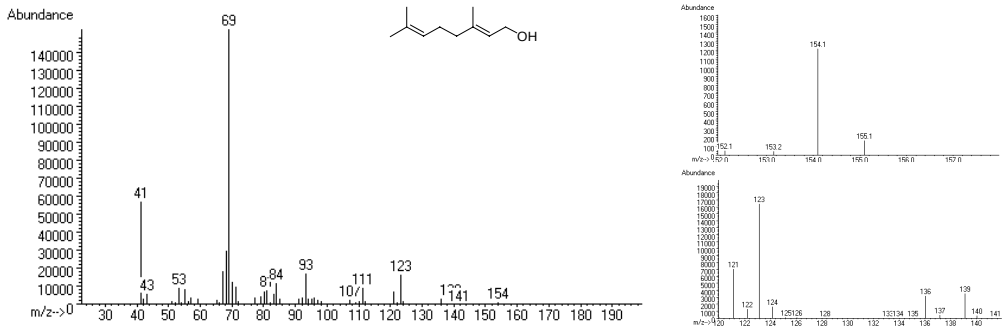
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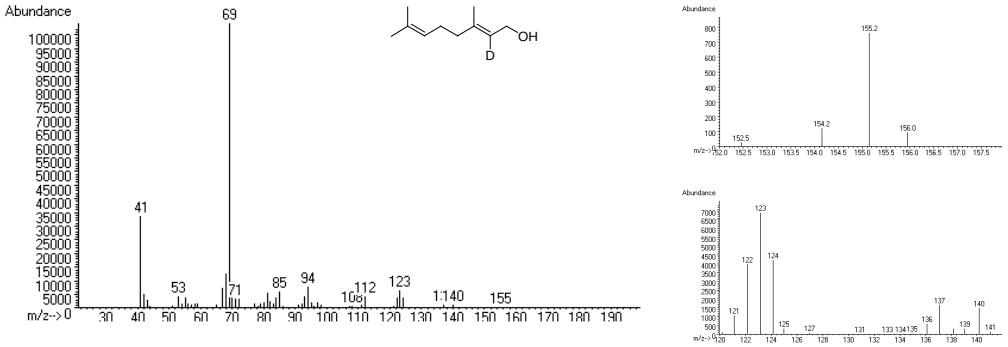
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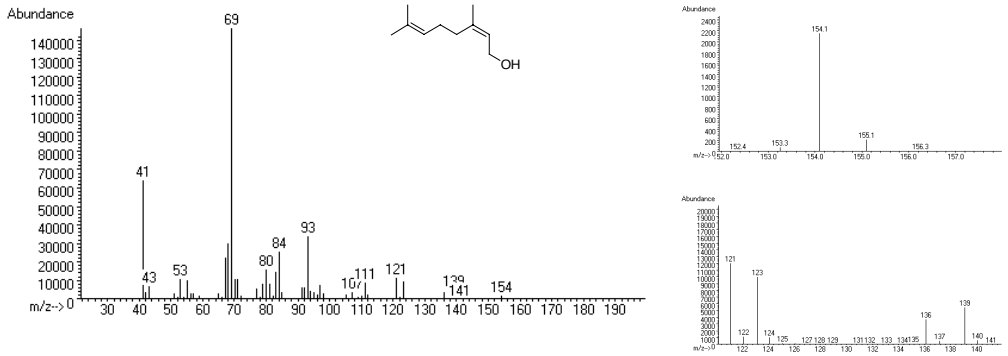
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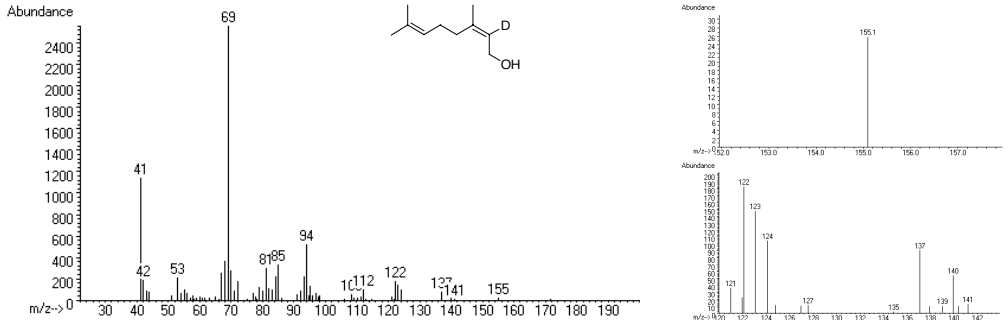
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REFERENCES

1. Davisson, V. J.; Woodside, A. B.; Neal, T. R.; Stremler, K. E.; Muehlbacher, M.; Poulter, C. D. *J. Org. Chem.* **1986**, *51*, 4768–4779.

2. Reed, B. C.; Rilling, H. C. *Biochemistry* **1976**, *15*, 3739–3745.

3. Erickson, H. K.; Poulter, C. D. *J. Am. Chem. Soc.* **2003**, *125*, 6886-6888.

4. Gibbs, R. A.; Krishnan, U.; Dolence, J. M.; Poulter, C. D. *J. Org. Chem.* **1995**, *60*, 7821-7829.

5. Xie, H.; Shao, Y.; Becker, J. M.; Naider, F.; Gibbs, R. A. J. Org. Chem. **2000**, *65*, 8552-8563.

6. Yu, J. S.; Kleckley, T. S.; Weimer, D. F. *Org. Lett*, **2005**, *7*, 4803-4806.

7. Burrell, J. W. K.; Garwood, R. F.; Jackman, L. M.; Oskay, E.; Weedon, B. C. L. *J. Chem. Soc. C* **1966**, 2144-2154.