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SYNTHESIS AND CONFORMATIONAL ANALYSIS OF DI-^{13}C-LABELED FARNESYL DIPHOSPHATE ANALOGS

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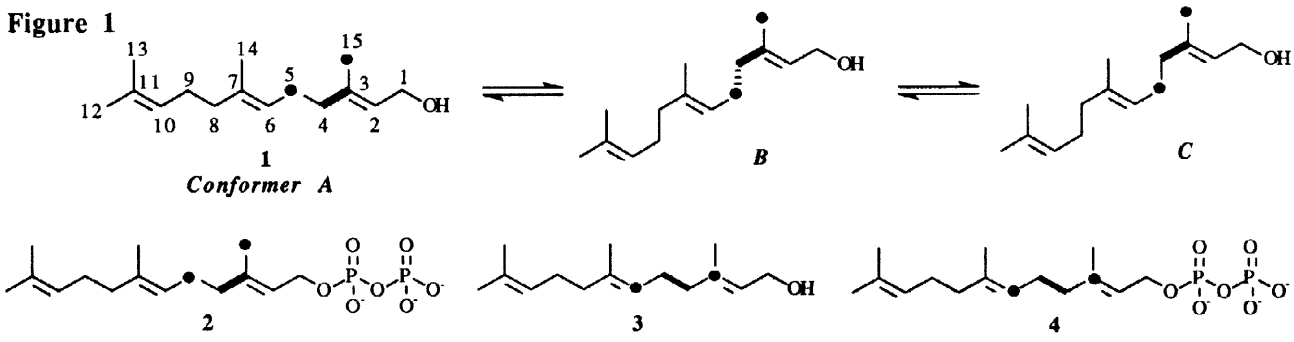
Abstract: Two di-^{13}C-labeled farnesyl diphosphates (**2** and **4**) have been prepared using modified versions of the isoprenoid triflate route previously developed in this laboratory. The $^{3}J_{CC}$ coupling constants for the precursor alcohols **1** and **3** are 1.6 Hz and 3.6 Hz, respectively, in $CDCl_3$, and very similar results were obtained for **2** and **4** in D_2O. This indicates a skew or gauche conformation about the C_3-C_4 bond and a trans conformation about the C_4-C_5 bond in both farnesol and FPP.

Isoprenoid diphosphates play a central role in cellular lipid metabolism, particularly the key intermediate farnesyl diphosphate (FPP), which serves as a precursor to cholesterol, dolichol, ubiquinone, and farnesylated proteins.[1] Surprisingly, little is known about the solution structure of isoprenoid diphosphates. Molecular mechanics and *ab initio* calculations[2,3] and solid state structural studies[4,5] have been employed to examine the conformations of prenyl derivatives and model systems. However, only Facke and Berger have examined the solution phase conformation of an isoprenoid, farnesol (**1**),[6] and no studies have been done on the solution phase conformation of any prenyl diphosphate. As shown below (Figure 1), the C_3-C_4 bond in farnesol could assume a trans (conformer **A**) or eclipsed (**C**) conformation, or an intermediate skew or gauche conformation (represented by **B**). Molecular mechanics and *ab initio* calculations indicate that the skew (**B**, where the angle between the labeled carbons is ~90°) and eclipsed conformations **C** are preferred.[2,6,7] Carbon-13 NMR of the C_5-C_{15} di-^{13}C-labeled analog **1** should be able to distinguish between **B** and the other two conformers, as a Karplus relationship is observed and the coupling constants between the labeled carbons in **A** and **C** are significantly larger (~4-5 Hz) than the coupling constant for the intermediate conformations represented by **B** (~0-2 Hz).[8-10] Therefore, as a part of efforts[11,12] to develop FPP-based inhibitors of protein-farnesyl transferase,[13] we have synthesized diphosphates **2** and **4** and obtained their ^{13}C-NMR spectra.[14,15] These compounds provide evidence concerning the preferred solution conformation about the C_3-C_4 and C_4-C_5 bonds of FPP, respectively.

Figure 1



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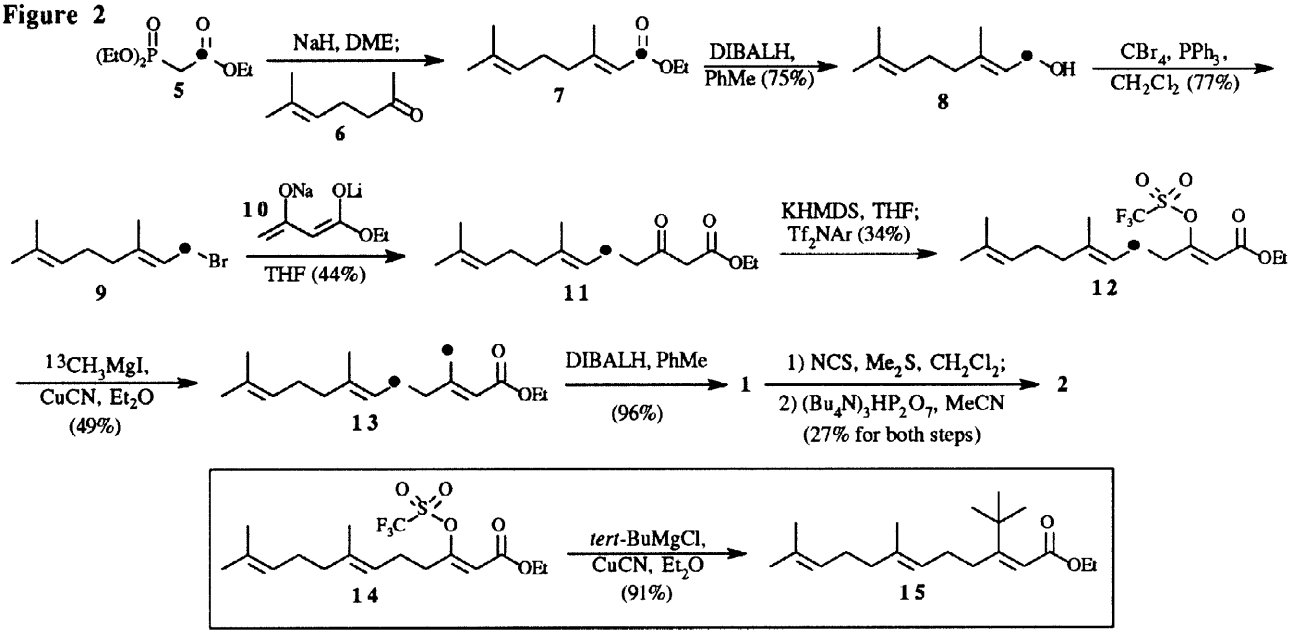
Work in this laboratory has demonstrated that vinyl triflates are very useful, versatile intermediates for the preparation of isoprenoid analogs.[11,12,16,17] Thus, the synthetic scheme shown in Figure 2 was used to prepare **1** and **2**. Horner-Emmons coupling of the commercially available ^{13}C-labeled phosphonate **5** with ketone **6**, followed by reduction and bromination, led to 1-^{13}C-geranyl bromide **9** in essentially the same manner as previously described.[18] Coupling of **9** and the acetoacetate dianion **10**,[11,16,19] afforded the β-ketoester **11**. Intermediate **11** was then transformed in a highly stereoselective fashion to the vinyl triflate **12**, again in the same fashion as described previously for the unlabeled triflate. The key new step in the synthesis was the attachment of a ^{13}C-labeled methyl group to the 3-position of the isoprenoid moiety. This was achieved with a copper cyanide mediated coupling of ^{13}C-methylmagnesium iodide[20] with vinyl triflate **12** to yield the desired di-^{13}C ester **13**. The utility of this new coupling method was demonstrated via the copper-mediated reaction of *tert*-BuMgCl with the unlabeled vinyl triflate **14** to give **15**, the key intermediate in the synthesis of a potent protein-farnesyl transferase inhibitor.[12] Note that the unoptimized yield in this reaction was higher than that achieved in the coupling of either *tert*-BuCu(CN)Li or *tert*-$Bu_2Cu(CN)Li_2$ with **14**.[12] Thus the use of Grignard reagents enhances the versatility of our vinyl triflate route to isoprenoids. Reduction of ester **13** to the alcohol **1**, followed by the two-step diphosphorylation procedure of Poulter and coworkers,[21] led to the desired di-labeled FPP **2**. The incorporation of the appropriate ^{13}C labels in **1** was confirmed by the intense, coupled (*vide infra*) ^{13}C-NMR signals at 16.3 and 26.3 ppm, along with the observation of ^{13}C coupling with the C_{15}-CH_3 and C_5-CH_2 signals in the proton NMR spectrum.[22]

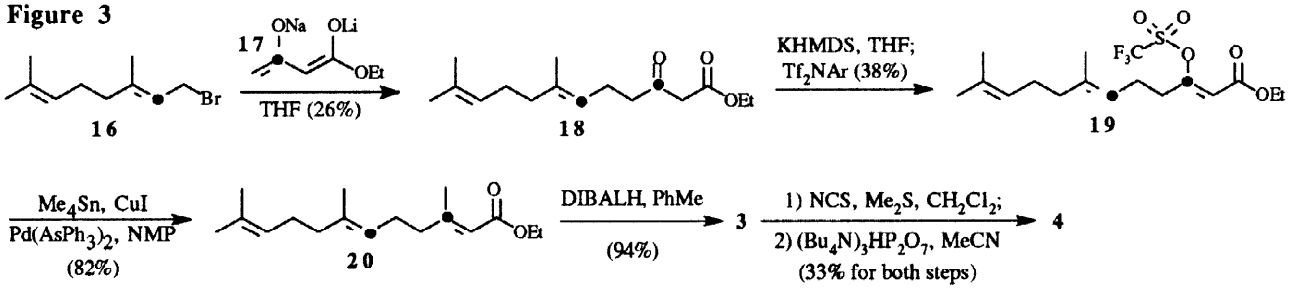
Figure 2



The 3,6-di-labeled derivatives **3** and **4** were prepared using a very similar synthetic route, as illustrated in Figure 3. The appropriately labeled geranyl bromide **16** was prepared in the same manner as **9**, with the exception that 2-^{13}C-triethylphosphonoacetate was used as starting material. It was then coupled with **17**, the dianion of 3-^{13}C-ethyl acetoacetate,[23] to give the di-^{13}C-labeled β-ketoester **18**. Since triflate **19** already possesses the two appropriate ^{13}C labels, it was transformed to ester **20** in high yield using the previously

developed Pd/CuI-mediated coupling with organostannanes.[11,16,24] Reduction of **18** afforded **3**, which was then converted to 3,6-^{13}C-FPP **4** in the same manner as described for **2**. The incorporation of the appropriate ^{13}C labels in **2** was confirmed by the intense, coupled (*vide infra*) ^{13}C-NMR signals at 123.8 and 139.8 ppm, along with the observation of ^{13}C coupling with the C_6 vinylic signal in the proton NMR spectrum.[22]

Figure 3



With the target compounds synthesized, the coupling constants of interest for the two alcohols and two diphosphates were determined in a straightforward fashion using ^{13}C-NMR in $CDCl_3$. The 1.6 Hz coupling constant for **1** is consistent with a skew (~90°) conformation about the C_3-C_4 bond of farnesol, while the 3.6 Hz coupling observed for **3** indicates that the C_4-C_5 bond primarily exists in a trans conformation. These values are identical to those determined by Facke and Berger for unlabeled farnesol using a pulse-transfer sequence for the determination of J_{CC} values,[6] and this confirms the validity of their method. The ^{13}C-NMR spectra of diphosphates **2** and **4** were determined in D_2O, as FPP is water soluble and water is the biologically relevant solvent. It might be supposed that the hydrophobic farnesyl moiety of FPP would exist in a more folded, globular form in water, and that gauche conformations in the hydrocarbon chain would predominate. Such folded conformations correspond to those required for the cyclization of FPP to various sesquiterpene structures.[25] However, the coupling constants observed for **2** (1.6 Hz) and **4** (3.5 Hz) in D_2O are *virtually identical* to those observed for **1** and **3** (respectively) in $CDCl_3$. Thus the conformational stability of the trans arrangement about the C_4-C_5 bond in the farnesyl chain outweighs any unfavorable interactions incurred by the hydrophobic group in D_2O. This surprising observation is consistent with the findings of Menger and D'Angelo,[26] who determined by $^3J_{CC}$ measurements that the trans/gauche ratio of the C_3-C_4 bond in *n*-undecane does not vary as the solvent is changed from chloroform to a water/ethanol mixture.[27] Therefore, the solution structure of FPP does not resemble the folded conformation found in the active sites of sesquiterpene cyclases,[28,29] but it closely approximates the extended conformation proposed to exist in the high-affinity FPP binding site in mammalian protein-farnesyl transferase.[30] It is anticipated that various prenylated peptides and proteins derived from **1-4** will prove to be valuable tools to investigate, in conjunction with solution phase or solid-state NMR,[31] the conformation of the farnesyl group in a variety of different environments.

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