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AN NMR METHOD FOR ELUCIDATING THE STEREOCHEMISTRY OF PROTEIN FARNESYLATION

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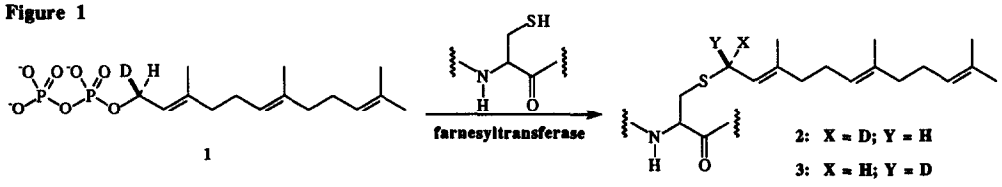
Abstract: The syntheses of the deuterium-labeled farnesylated peptides **11** and **12** are described. The 500 MHz NMR spectra of these two compounds exhibit clear differences in the signals of the two pro-chiral hydrogens on C-1 of the farnesyl moiety. This provides a method to determine the stereochemical course of the enzyme farnesyl:protein transferase.

Mutant ras proteins are believed to be involved in 20% of human cancers.[1] It has recently been discovered that, to be active, ras proteins must be modified with a farnesyl moiety at a cysteine four residues from the carboxy terminus.[2,3] This modification is carried out by an enzyme, farnesyl:protein transferase, which uses farnesyl pyrophosphate as the source of the prenyl moiety.[4-7] Inhibitors of this enzyme might block the action of the mutant ras proteins and thus act as potential anti-cancer agents.[8] The design of such inhibitors would be facilitated by a knowledge of the mechanism of this enzyme.[9a,b] We have embarked on a project to determine whether the alkyl group is transferred via a single displacement or a double displacement mechanism using stereospecifically labeled farnesyl pyrophosphate (**1**, Figure 1). A single displacement by an Sn2 mechanism would lead to inversion of configuration (**2**), whereas a double displacement mechanism would afford farnesylcysteine with retention of configuration (**3**). The stereochemical course of substitution reactions of allylic pyrophosphates with carbon nucleophiles has been extensively examined;[10] however, no stereochemical studies have been done on their enzymatic reaction with heteroatom nucleophiles. Herein it is shown that the pro-chiral protons on the C-1 allylic carbon of a farnesylcysteine can be distinguished by ^1H NMR.

Figure 1

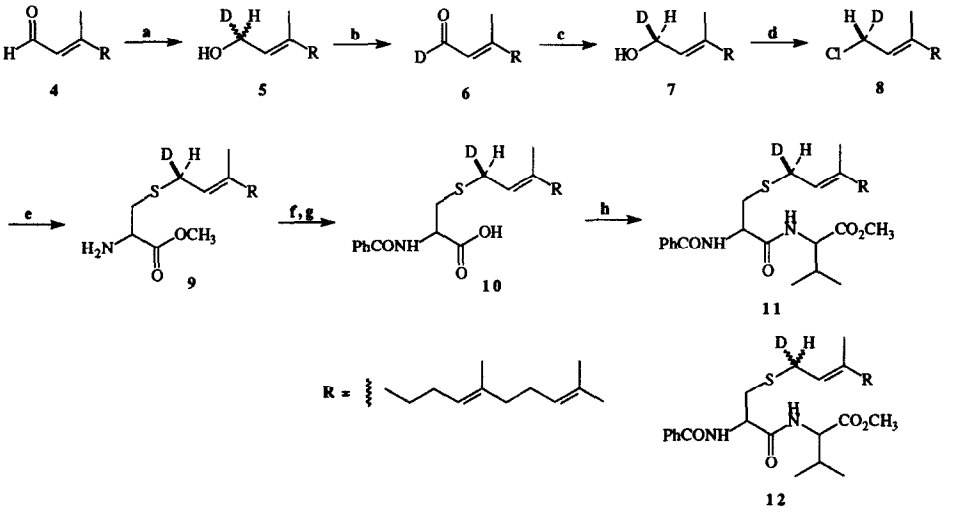


1

The dipeptide N-benzoyl-S-farnesylcysteinylvaline methyl ester was chosen as a model system for our NMR investigations, since the Cys(S-farnesyl)^{186}-Val^{187} sequence is present in human p21 H-ras. The synthesis began with farnesal (**4**, Figure 2) which was converted to [1-^{2}H]-farnesal as previously described by Cane *et al.*[11] Reduction of **4** with $NaBD_4$ afforded the racemic monodeuterated alcohol **5**, which was oxidized to afford **6** (93% D). The high level of deuterium incorporation is due to the very large deuterium isotope effect on the MnO_2 oxidation of alcohols.[12] The deuterated aldehyde was then reduced with R-Alpine Borane

to afford **7**.[13-15] The stereochemistry of the alcohol was assigned based on the well-established stereoselectivity of the Alpine Borane reagent. Alcohol **7** has been prepared previously by reduction with binapthol-modified $LiAlH_4$ and by enzymatic reduction.[11,16,17]

Figure 2



Reagents: a) $NaBD_4$, MeOH (92%); b) MnO_2, hexanes (93%); c) R-Alpine Borane, THF; H_2O_2, NaOH (98%); d) N-chlorosuccinimide,Me_2S, CH_2Cl_2; e) CysOMe, NH_3/MeOH (78% for two steps); f) $(PhCO)_2O$, Et_3N, CH_2Cl_2 (94%); g) NaOH, CH_3CN (98%); h) ValOMe, EDC, Et_3N, THF (80%)

The enantiomeric deuterated alcohol was then converted into the chloride **8** using the method of Corey and coworkers.[18] It has been demonstrated that, using Corey's method, chiral deuterated prenyl alcohols are converted into chlorides with inversion of configuration.[19] The resulting chloride was then coupled with cysteine methyl ester to afford **9**.[20] Acylation of the farnesylcysteine methyl ester with benzoic anhydride followed by hydrolysis of the ester afforded **10**.[21] Finally, coupling of the carboxyl group of the farnesylcysteine residue with valine methyl ester gave the desired dipeptide model for the ras protein carboxyl terminus, **11**. The non-stereospecifically deuterated dipeptide was prepared by converting **5** to racemic [1-^{2}H]-farnesyl chloride, and transforming it to **12** using the same procedure described above.[22]

The ^{1}H-NMR spectra of farnesylcysteine derivatives have previously been assigned.[23,24] The peak at 3.3 ppm assigned to the protons on C-1 of the farnesyl moiety, changes from a multiplet (integrating to 2H) in the unlabeled compound to an apparent quartet (1H) in the non-stereospecifically deuterium labeled compound (**12**). The signal at 5.3 ppm assigned to the C-2 vinylic proton also changes from a triplet to a doublet in **11** and **12**. Inspection of the NMR spectra of **11** and **12** (Figure 3) indicates that the apparent quartet seen for **12** is instead two doublets resulting from the 1R and 1S protons. If the alkylation of cys-OMe by farnesyl chloride proceeds (as expected) by a Sn2 mechanism, then the signal for the *pro*-1R proton appears downfield of that for the *pro*-1S proton. Thus NMR spectroscopy provides a simple method to determine the stereochemical course of the farnesyltransferase reaction.

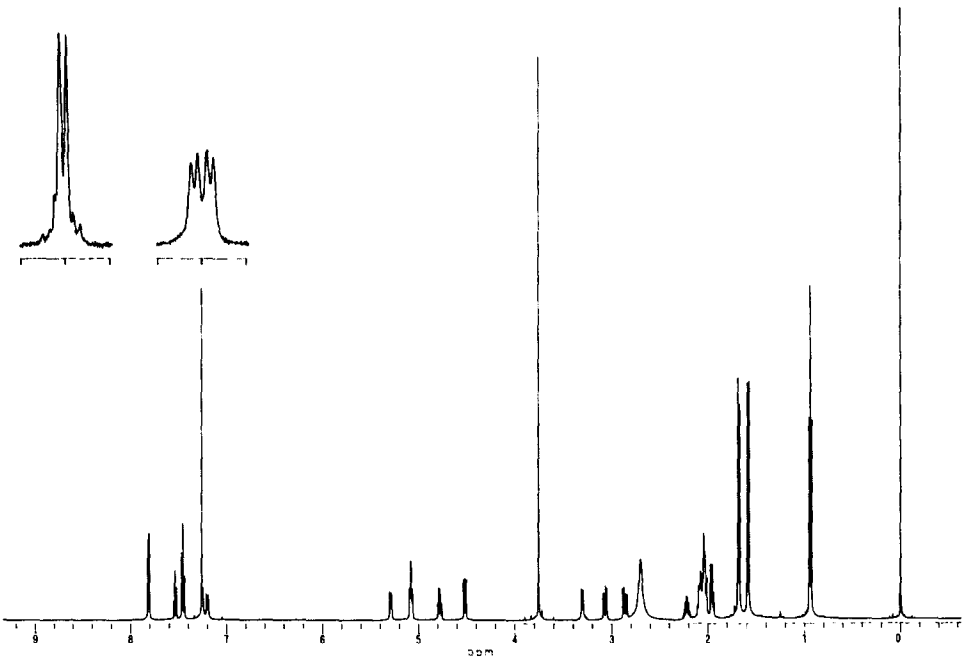


Figure 3. The 500 MHz ^{1}H NMR spectrum of **11**. The insets show an expansion of the region from 3.2 to 3.4 ppm showing the peaks due to the C-1 hydrogen for **11** (left) and **12** (right).

Farnesyl pyrophosphate chirally labeled at C-1 can be prepared from **7**.[11,19] The farnesyl moiety of this labeled substrate should then be readily transferred to the tetrapeptide BzCysValIleMet by rat brain farnesyl:protein transferase.[25,26] The stereochemistry of the resulting deuterium labeled peptide BzCys(S-farnesyl)ValIleMet should then be easily determined using ^{1}H-NMR as described above. The stereospecifically deuterated farnesylated peptide **11** could also be a valuable tool for investigating the mechanisms of chemical transformations of farnesylcysteine residues.[27-29] Experiments in these two areas are currently underway.

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(21) ^{13}C-NMR spectrum of **10** (75.4 MHz): 16.0, 16.2, 17.7, 25.7, 26.4, 26.7, 29.8 (t, $J_{C\text{-}D}$ 22 Hz), 32.8, 39.6, 39.7, 52.2, 119.3, 123.7, 124.3, 127.2, 128.7, 132.2, 133.2, 135.4, 140.4, 167.9, 174.1 ppm. Insufficient material was obtained for a ^{13}C-NMR of **11**.

(22) Compounds **11** and **12** were purified to homogeneity by reversed phase HPLC using a linear gradient of 35% A/65% B to 100% B over 30 min (A: 0.1%TFA/H_2O; B: 0.1%TFA/CH_3CN; column: Waters Nova-Pak C_{18} 8mm x 100mm Radial-Pak cartridge). The C-1 epimer of **11** was also synthesized as outlined in Figure 2, with the exception that S-Alpine Borane was used to reduce **6** to (1R)-[1-^{3}H]-farnesol. Unfortunately, this sample was contaminated by a minor impurity which could not be separated by HPLC and which exhibited a minor signal at ≈3.3 ppm which complicated the analysis of the NMR spectrum. However, the major peak seen in this region is a doublet which appears just upfield of 3.3 ppm, which is consistent with our analysis of the NMR spectra of **11** and **12** (Figure 3).

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