Title
Synthesis of High Specific Activity [1-{sup 3}H]Faresyl Pyrophosphate

Authors
Saljoughian, M.
Morimoto, H.
Williams, P.G.

Publication Date
1991-08-01

Lawrence Berkeley Laboratory
UNIVERSITY OF CALIFORNIA

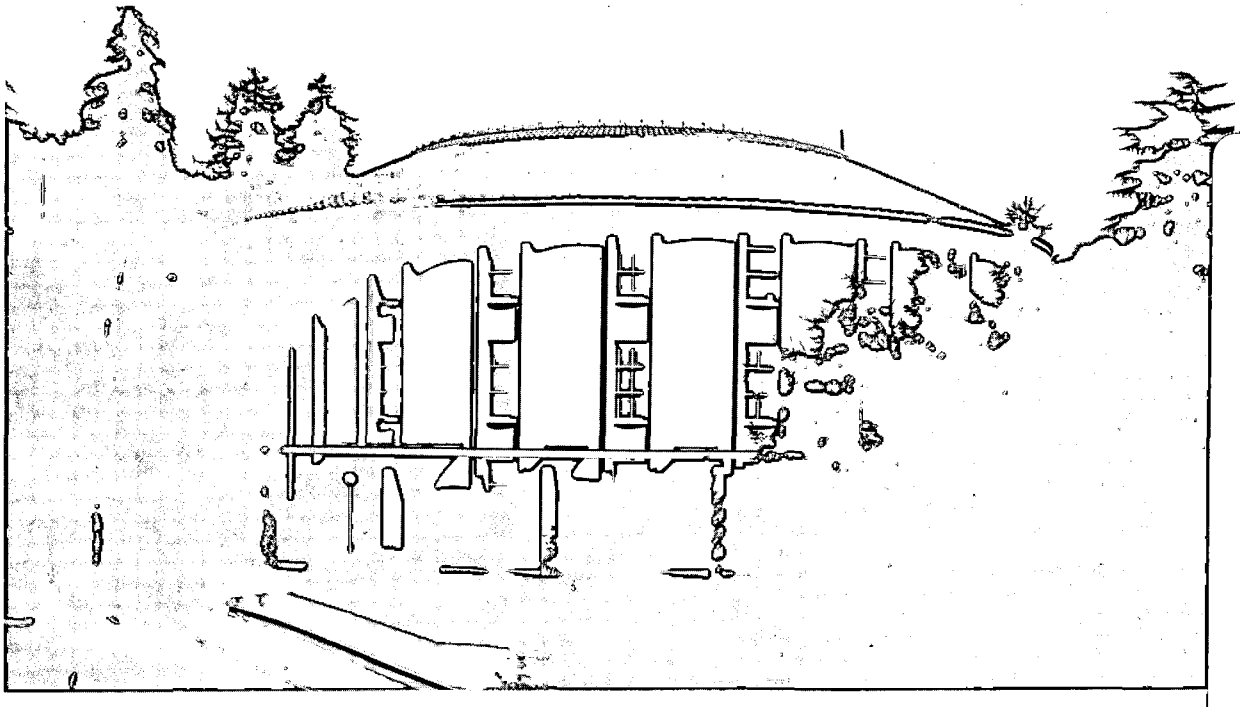
CHEMICAL BIODYNAMICS DIVISION

Presented at the Fourth Topical Meeting on Tritium Technology in Fission, Fusion, and Isotopic Applications, Albuquerque, NM, September 29–October 4, 1991, and to be published in the Proceedings

Synthesis of High Specific Activity [1-^{3}H]Farnesyl Pyrophosphate

M. Saljoughian, H. Morimoto, and P.G. Williams

August 1991



Prepared for the U.S. Department of Energy under Contract Number DE-AC03-76SF00098

DISCLAIMER

LBL-31140

SYNTHESIS OF HIGH SPECIFIC ACTIVITY [1-^{3}H]FARNESYL PYROPHOSPHATE

Manouchehr Saljoughian, Hiromi Morimoto and Philip G. Williams

National Tritium Labeling Facility
Chemical Biodynamics Division
Lawrence Berkeley Laboratory
University of California
Berkeley, California 94720.

This research was supported by the Biotechnology Resources Program, Division of Research Resources, National Institutes of Health under Grant P41 RR01237, through the Department of Energy under Contract DE-AC03-76SF00098.

ABSTRACT

The synthesis of tritiated farnesyl pyrophosphate with high specific activity is reported. trans-trans Farnesol was oxidized to the corresponding aldehyde followed by reduction with lithium aluminium tritide (5%-^{3}H) to give trans-trans [1-^{3}H]farnesol. The specific radioactivity of the alcohol was determined from its triphenylsilane derivative, prepared under very mild conditions. The tritiated alcohol was phosphorylated by initial conversion to an allylic halide, and subsequent treatment of the halide with tris-tetra-n-butylammonium hydrogen pyrophosphate. The hydride procedure followed in this work has advantages over existing methods for the synthesis of tritiated farnesyl pyrophosphate, with the possibility of higher specific activity and a much higher yield obtained.

INTRODUCTION

Allylic pyrophosphates and farnesyl pyrophosphate in particular are important substrates and key intermediates in several biosynthetic pathways. An example is the biosynthesis of pentalenene, the parent hydrocarbon of the pentalenolactone family of sesquiterpene antibiotics.[1] Labelled precursors were used to prove that the cyclization of farnesyl pyrophosphate is catalyzed by a single enzyme to give pentalenene.

It is also clear that certain eukaryotic proteins require post-translational prenylation to perform their membrane-associated cellular function. Recent studies[2] have suggested that the prenylation modification is essential to the ability of these proteins to associate with the membrane, and that the function of materials such as the oncogenic Ras protein may be suppressed by inhibition of the prenylation reaction. This work suggests that suppression of prenylation activity in some proteins may be an effective anti-cancer treatment.

In addition to these, many other mechanistic studies with enzymes of the isoprene pathway require radiolabeled forms of these pyrophosphates[3] and until recently none of these compounds were commercially available, usually being prepared from the corresponding labelled alcohols. trans-trans Farnesyl pyrophosphate and similar isoprenoid molecules have previously been labeled with tritium at the C-1,[4] C-8,[5] C-9,[1] and C-12[9] positions and used in biological studies. Difficulties in the preparation and purification of these compounds is partly due to the highly reactive 3,3-dialkylallylic moiety and to the fact that pyrophosphates are superb leaving groups when they are protonated.[3] Many of the problems associated with the preparation and purification of these compounds are resolved by introduction of the labile carbon-oxygen bond in the final step by a direct displacement with inorganic phosphate and by chromatographic purification on cellulose.[3] In the present work, we have modified the existing synthesis of farnesyl pyrophosphate tritiated at the C-1 position in order to achieve higher specific activity, and have prepared a new triphenylsilane derivative of [1-^{3}H]trans-trans farnesol to facilitate the determination of specific activity.

RESULTS AND DISCUSSION

The synthesis of high specific activity farnesyl pyrophosphate (Scheme) first involved the preparation of farnesal (2) from trans-trans farnesol (1, Figure 1A), using a chromium-pyridine complex. This procedure gave a mixture of isomeric aldehydes (83:17, Figure 1B), with the major isomer being the trans-trans form. Attempted reduction of (2) with freshly prepared supertritide[6] did not yield the corresponding [1-^{3}H] alcohol (3). Reduction with the same reagent at low temperature did not give satisfactory results either, and these problems may be due to the highly reactive nature of the supertritide over-reducing the conjugated system of farnesal. However, the reaction of farnesal (2) with lithium aluminium tritide at low temperature (-40^{o}C) gave a clean tritiated product (3), and the conditions were readily optimized. Analysis of the product by tritium

NMR spectroscopy (Figure 2A) revealed two tritium peaks in the ratio 87:13 arising from reduction of the two isomeric aldehydes, with the major compound being the [1-^{3}H]trans-trans farnesol.

For the purpose of specific activity measurement a new derivative was prepared. Initial tosylation reaction for the allylic alcohol was not successful and a triphenylsilyl derivative (4, Figure 2 B) was prepared under very mild conditions using triphenylsilyl chloride in pyridine at 0^{o}C in 30 min. Analysis of specific radioactivity of the derivative was achieved by radio-HPLC and the specific activity of the [1-^{3}H]farnesyl pyrophosphate was estimated to be 1 Ci/mmol. Since pyrophosphorylation of the tritiated farnesol needs a highly reactive form of this compound, [1-^{3}H]farnesyl chloride (5, Figure 2C) was prepared from [1-^{3}H]farnesol and N-chloro-succinimide in dichloromethane. Without purification, the halide was treated with tris-tetra-n-butylammonium pyrophosphate[10] and [1-^{3}H]farnesyl pyrophosphate (6, Figure 2D) was obtained as a white solid product and analyzed by radio-HPLC, and by ^{1}H, ^{3}H, ^{13}C and ^{31}P NMR spectroscopy (Figures 3A and 3B).

In conclusion, the experimental manipulations were simple and the desired product was the only phosphorylated radioactive species produced in this reaction. We prepared and used LAT with 5%-^{3}H in this work. The use of LAT at the maximum specific activity would yield the desired compound with 28.72 Ci/mmole. The overall radiochemical yield of [1-^{3}H]farnesyl pyrophosphate (83%) was also substantially higher than that of the existing procedures (21%).[4]

EXPERIMENTAL

1- Synthesis of *trans-trans* Farnesal (2)

Chromium trioxide (6.2 gm, 62 mmole) was added to pyridine (70 mL) in a chilled ice bath over a period of 10 min while stirring vigorously. *trans-trans* Farnesol (1, 2.9 mL, 11.6 mmole) in pyridine (10 mL) was added to the chromium-pyridine complex in one portion. The mixture was stirred for 30 min and then allowed to remain undisturbed for 2 hours at room temperature. The mixture was then poured into water (300 mL), and extracted with ether (100 mL, 3X). The combined ether extracts were washed successively with HCl (10%, 50 mL, 3X), Na_2CO_3 (10%, 25 mL, 2X) and water (25 mL). The ether layer was dried ($MgSO_4$) and the solvent removed to leave an oil residue (2.7 mL, 93%). ^1H NMR (C_6D_6) δ 1.47 (3H, s, methyl), 1.52 (3H, s, methyl), 1.56 (3H, s, methyl), 1.67 (3H, s, methyl), 1.74-2.14 (8H, m, C-4,C-5,C-8,C-9 methylenes), 5.00 (1H, t, C-6 vinyl), 5.18 (1H, t, C-10 vinyl), 5.82 (1H, d, C-2 vinyl).

2- Synthesis of [1-^3H]*trans-trans* Farnesol (3)

trans-trans Farnesal (128 µl, 0.5 mmole) was dissolved in dry THF (1 mL) and lithium aluminium tritide (5% ^3H, 0.25 mmole) was freshly made and added at -40°C. The yellowish color of the aldehyde disappeared after 10 min. The reaction was stirred for an additional 10 min and then quenched with methanolic HCl (10%, 100 µL) until no gas was evolved. The solvent was then carefully removed under vacuum, water (1 mL) was added and the mixture was stirred. The product was then extracted with EtOAc (2 mL), dried over magnesium sulfate (100 mg) and the radioactivity assessed by liquid scintillation counting (905 mCi). ^1H NMR (C_6D_6) δ 1.47 (3H, s, methyl), 1.57 (6H, s, 2 methyl groups), 1.68 (3H, s, methyl), 1.96-2.20 (8H, m, C-4, C-5, C-8, C-9 methylenes), 3.96 (2H, d, C-1 methylene), 5.22 (2H, t, C-6 , C-10 vinyls), 5.39 (1H, t, C-2 vinyl). ^3H NMR (proton-decoupled, C_6D_6) δ 3.95 (s, C_1-^3H, 87%) 3.97 (s, C_1-^3H,13%).

3- Synthesis of [1-^3H]Farnesyl Triphenylsilane (4)

[1-^3H]*trans-trans* Farnesol (25 µL, 0.1mmole, 93 mCi) was added to dry pyridine (100 µL) at -23°C (CCl_4/CO_2) and stirred for 5 min. Triphenylsilyl chloride (29 mg, 0.1 mmole) was added in one portion and the mixture was stirred -23°C for 30 min, then brought up to the room temperature over 30 min. $CHCl_3$ (2 mL) was added to give a clear solution. The solution was then washed with HCl (10%, 5 mL, 2X), $NaHCO_3$ (5%, 5 mL, 2X) and water (5 mL, 2X) and dried over anhydrous magnesium sulfate. Thin layer chromatography ($CHCl_3$:hexane-3:7) showed a new spot (R_f 0.4) as well as a base line spot which was separated by a small silica gel column. The residue remained as an oil (328 mg). ^1H NMR (C_6D_6) δ 1.36 (3H, s, methyl), 1.55 (6H, s, 2 methyl groups), 1.66 (3H, s, methyl), 1.95-2.17 (8H, m, C-4, C-5, C-8, C-9 methylenes), 4.48 (2H, d, C-1 methylene), 5.20 (2H, m, C-10, C-6 vinyls), 5.62 (1H, t, C-2

vinyl), 7.18 (9H, m, *p*- and *o*- aromatic), 7.81 (6H, m, *m*- aromatic). ^{3}H NMR (C_6D_6) δ 4.47(s, C_1-^{3}H, 87%), 4.50 (s, C_1-^{3}H, 13%).

4- Synthesis of [1-^{3}H]Farnesyl Chloride (5)

N-Chlorosuccinimide (58 mg, 1.65 mmole) was dissolved in dichloromethane (1.2 mL) and the mixture was cooled to -30°C (CH_3CN/CO_2). Dimethylsulfide (32 μL) was added dropwise from a syringe to the cold, well stirred, heterogeneous reaction flask. The contents of the flask were warmed to 0°C, and then cooled to -40°C. [1-^{3}H]Farnesol (94 μL, 0.37 mmole, 350 mCi) in dry dichloromethane (1 mL) was added by syringe over a 3 min interval to the milky white suspension. The reaction was allowed to warm to 0°C, during which time it became a clear, colorless solution, and was then stirred for 2 hours. The ice bath was then removed and the reaction was stirred at room temperature for 15 min, and then poured into a separatory funnel containing cold saturated sodium chloride (2.5 mL). The product was extracted with pentane (5 mL, 2X) and dried over anhydrous magnesium sulfate to give (69.2 mg, 0.29 mmol, approximate total activity 290 mCi). ^{1}H NMR (C_6D_6) δ 1.39 (3H, s, methyl), 1.46 (3H, s, methyl), 1.53 (3H, s, methyl), 1.56 (3H, s, methyl), 1.86-2.17 (8H, m, C-4, C-5, C-8, C-9 methylenes), 3.79 (2H, d, C-1 methylene), 5.149 (1H, t, C-6 vinyl), 5.23 (1H, t, C-10 vinyl), 5.33 (1H, t, C-2 vinyl). ^{3}H NMR (C_6D_6) δ 3.76 (s , C_1-^{3}H, 87%), 3.79 (s, C_1-^{3}H,13%).

5- Synthesis of tetra-n-Butylammonium Hydrogen Pyrophosphate

A 2 x 30 cm column was slurry packed with Dowex AG 50W-8X cation exchange resin (hydrogen form) in deionized water. After washing the column with two column volumes of deionized water, disodium dihydrogen pyrophosphate (3.13 gm,14 mmole) in deionized water (30 mL) was applied to the resin. The pH of the eluent was monitored, and was collected when the pH became acidic. Collection was stopped when the pH returned to that of deionized water. The solution of pyrophosphoric acid was immediately titrated to pH 7.3 with tetra-n- butylammonium hydroxide. The salt was dried by lyophylization and gave a hydroscopic white solid: 12.53 gm (98%).

6- Synthesis of [1-^{3}H]Farnesyl Pyrophosphate (6)

The reaction was run with stirring, under nitrogen, in a flame-dried round bottom flask. Tris (tetra-n-butyl) ammonium hydrgen pyrophosphate (500 mg) was dissolved in dry acetonitrile (1.5 mL) and [1-^{3}H]*trans-trans* farnesyl chloride (69.2 mg, 290 mCi) in dry acetonitrile (0.5 mL) was added and stirred for 2 hours at room temperature. The solvent was removed and the resulting residue was dissolved in isopropanol: 25 mmole ammonium bicarbonate-1:49 (2 mL). The resulting cloudy solution was centrifuged before it was passed through a column containing

Dowex AG 50W-8X (14 mL, 24 meq.) cation exchange resin. This column was equilibrated with two column volumes of ion exchange buffer and then eluted with the same buffer (10 mL) before it was used. The ammonium form of the resin was made by the treatment of the resin with concentrated ammonium hydroxide, after washing two times with deionized water. About 30 mL of the yellowish eluent was collected (total activity 253 mCi), and a second fraction (50 mL) was also collected (40 mCi). Both fractions were lyophilized and yielded yellowish solids which were independently analyzed by tritium and phosphorus NMR spectroscopy. ^{1}H NMR (D_2O/ND_4OD) δ 1.55 (3H, s, methyl), 1.65 (3H, s, methyl), 1.69 (3H, s, methyl), 1.73 (3H, s, methyl), 2.06 (8H, m, C-4, C-5, C-8, C-9 methylenes), 4.48 (2H, t, C-1 methylene), 5.17 (2H , m, C-6, C-10 vinyls), 5.48 (1H, t, C-2 vinyl). ^{3}H NMR (320MHz, D_2O/ND_4OD) δ 4.49 (d, C_1-^{3}H). ^{13}C NMR (75MHz, D_2O/ND_4OD, DEPT experiment) δ 18.04, 18.43, 19.80, 27.76 (C-12, C-13, C-14, C-15) δ 28.41, 28.53, 41.67 (2 carbons) (C-4, C-5, C-8, C-9), 65.015 (C-1: d, $J_{^{13}C,^{31}P}$ = 4.36 Hz), δ 122.868 (C-2: d, $J_{^{13}C,^{31}P}$ = 8.71 Hz), δ 127.010, 127.167, (C-6, C-10), C-3,7,11: not observed. ^{31}P NMR (32MHz, D_2O/ND_4OD) δ –6.04 (P_2, $J_{P,P}$ = 21.97 Hz), -9.99 (P_1, $J_{P,P}$ = 21.98 Hz, $J_{P,H}$ = 6.0 Hz).

ACKNOWLEDGEMENTS

This research was supported by the Biotechnology Resources Program, Division of Research Resources, National Institutes of Health under Grant P41 RR01237, through the Department of Energy under Contract DE-AC03-76SF00098.

REFERENCES

1. D.E. CANE, C. ABELL, and A.M. TILLMAN, "Pentalenene Biosynthesis and the Enzymatic Cyclization of Farnesyl Pyrophosphate: Proof that the Cyclization is Catalyzed by a Single Enzyme", *Bioorg. Chem.*, 12, 312-328 (1984).

2. W.R. SCHAFER, C.E. TRUEBLOOD, C.-C. YANG, M.P. MAYER, S. ROSENBERG, C.D. POULTER, S.-H. KIM, and J. RINE, "Enzymatic Coupling of Cholesterol Intermediates to a Mating Pheromone Precursor and to Ras protein", *Science*, 249, 1133-1139 (1990).

3. V.J. DAVISSON, A.B. WOODSIDE, and C.D. POULTER, "Synthesis of Allylic and Homoallylic Isoprenoid Pyrophosphates", in *Methods in Enzymology* (Law, J.H., and Rilling, H.C., Eds.), Academic Press, New York, 110, 130-144 (1985).

4. V.J. DAVISSON, T.M. ZABRISKIE, and C.D. POULTER, "Radiolabeled Allylic Isoprenoid Pyrophosphates: Synthesis, Purification, and Determination of Specific Activity", *Bioorg. Chem.*, 14, 46-54 (1986).

5. D.E. CANE, and A.M. TILLMAN, "Pentalenene Biosynthesis and the Enzymatic Cyclization of Farnesyl Pyrophosphate", *J. Am. Chem. Soc.*, 105, 122-124 (1983).

6. H. ANDRES, H. MORIMOTO, and P.G. WILLIAMS, "Preparation and Use of $LiEt_3BT$ and $LiAlT_4$ at Maximum Specific Activity", *J. Chem. Soc., Chem. Commun.*, 627-628 (1990).

7. J.R. HOLUM, "Study of the Chromium (VI) Oxide-Pyridine Complex", *J. Am. Chem. Soc.*, 26, 4814-4816 (1961).

8. H. NAKAI, N. HAMANAKA, H. MIYAKE, and M. HAYASHI, "Synthesis of 5-Fluoro-Prostaglandins", *Chem. Lett.*, 1499-1502 (1979).

9. W.-S. ENG, and G.D. PRESTWICH, "A Short Radiosynthesis of Natural Juvenile Hormone III, Methyl [12-^{3}H]-(10R)-10,11-Epoxyfarnesoate", *J. Labelled Compd. Radiopharm.*, 25, 627-633 (1987).

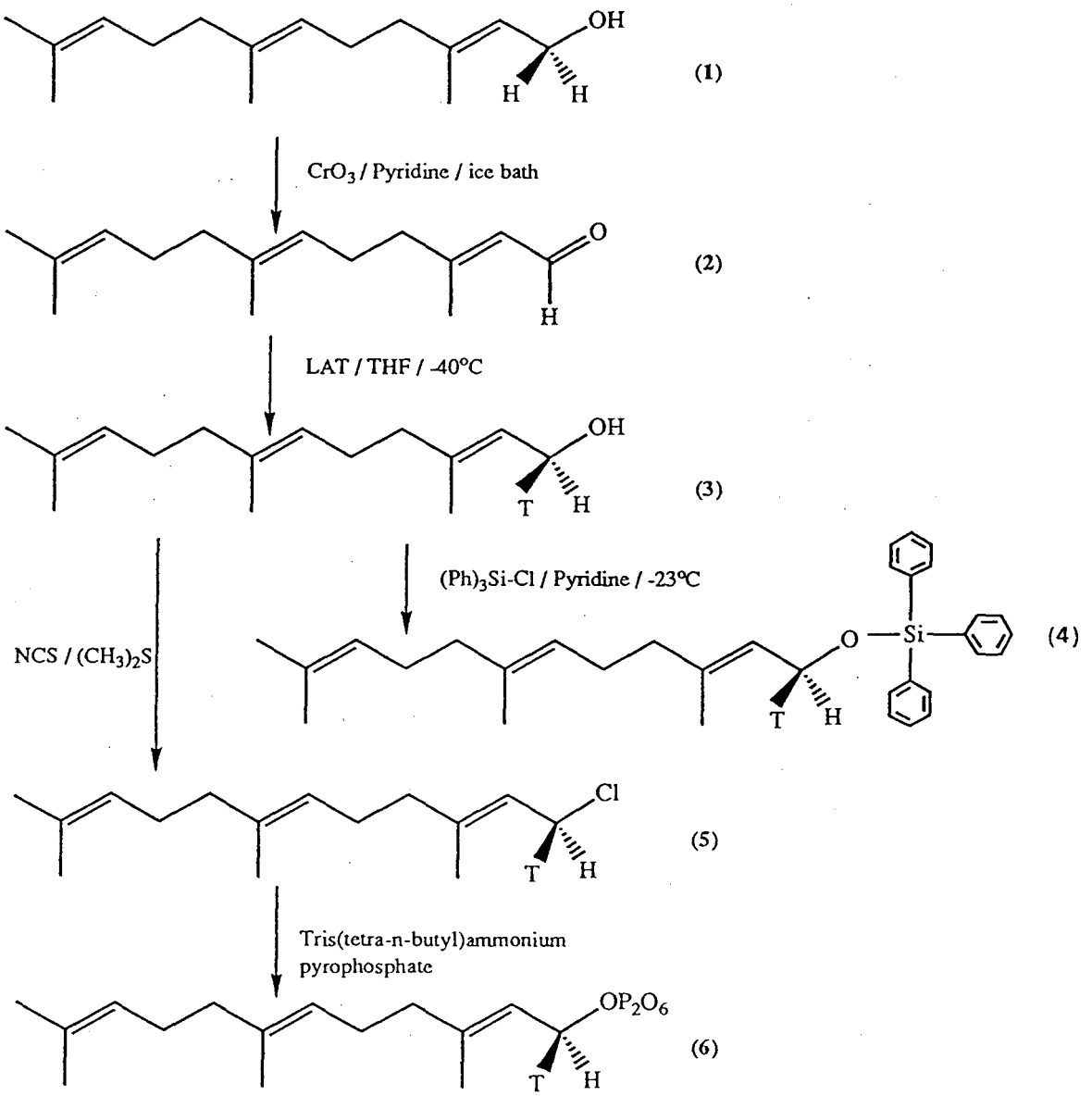
10. V.J. DAVISSON, A.B. WOODSIDE, T.R. NEAL, K.E. STREMLER, M. MUEHLBACHER, and C.D. POULTER, "Phosphorylation of Isoprenoid Alcohols", *J. Org. Chem.*, 51, 4768-4779 (1986).

Figure 1: A. 300 MHz Proton NMR spectrum of *trans-trans* farnesol in benzene-d_6.
B. 300 MHz Proton NMR spectrum of *trans-trans* farnesal in benzene-d_6.

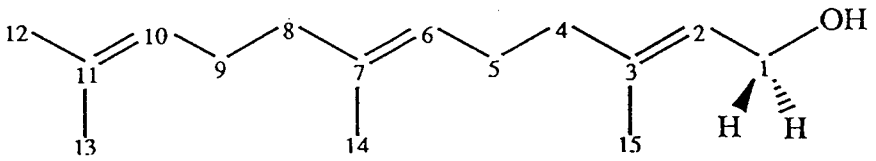
Figure 2: 320 MHz Tritium NMR spectra (proton decoupled) showing the region from 3.5—5 ppm. All the spectra were aquired with 8K points, and the FID was Gaussian multiplied (LB = -1, GB = 0.15) and zero-filled to 16K points before Fourier transformation. (A) [1-^{3}H]*trans-trans* farnesol in benzene-d_6. (B) [1-^{3}H]*trans-trans* farnesyl triphenylsilane in benzene-d_6. (C) [1-^{3}H]*trans-trans* farnesyl chloride in benzene-d_6. (D) [1-^{3}H] *trans-trans* farnesyl pyrophosphate in D_2O/ND_4OD at pH 8.

Figure 3: 121 MHz ^{31}P NMR spectrum of [1-^{3}H]*trans-trans* farnesyl pyrophosphate in D_2O/ND_4OD. A. Proton Decoupled. B. Proton Coupled.

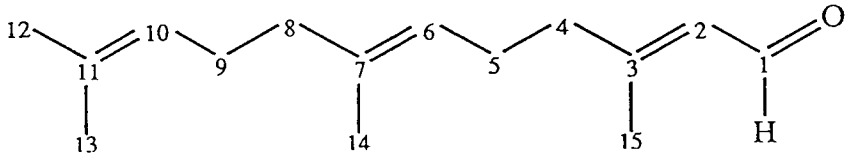
SCHEME


OH
H
H
(1)
CrO_3 / Pyridine / ice bath
O
H
(2)
LAT / THF / -40°C
OH
T
H
(3)
$(Ph)_3Si$-Cl / Pyridine / -23°C
O
Si
T
H
(4)
NCS / $(CH_3)_2S$
Cl
T
H
(5)
Tris(tetra-n-butyl)ammonium
pyrophosphate
OP_2O_6
T
H
(6)

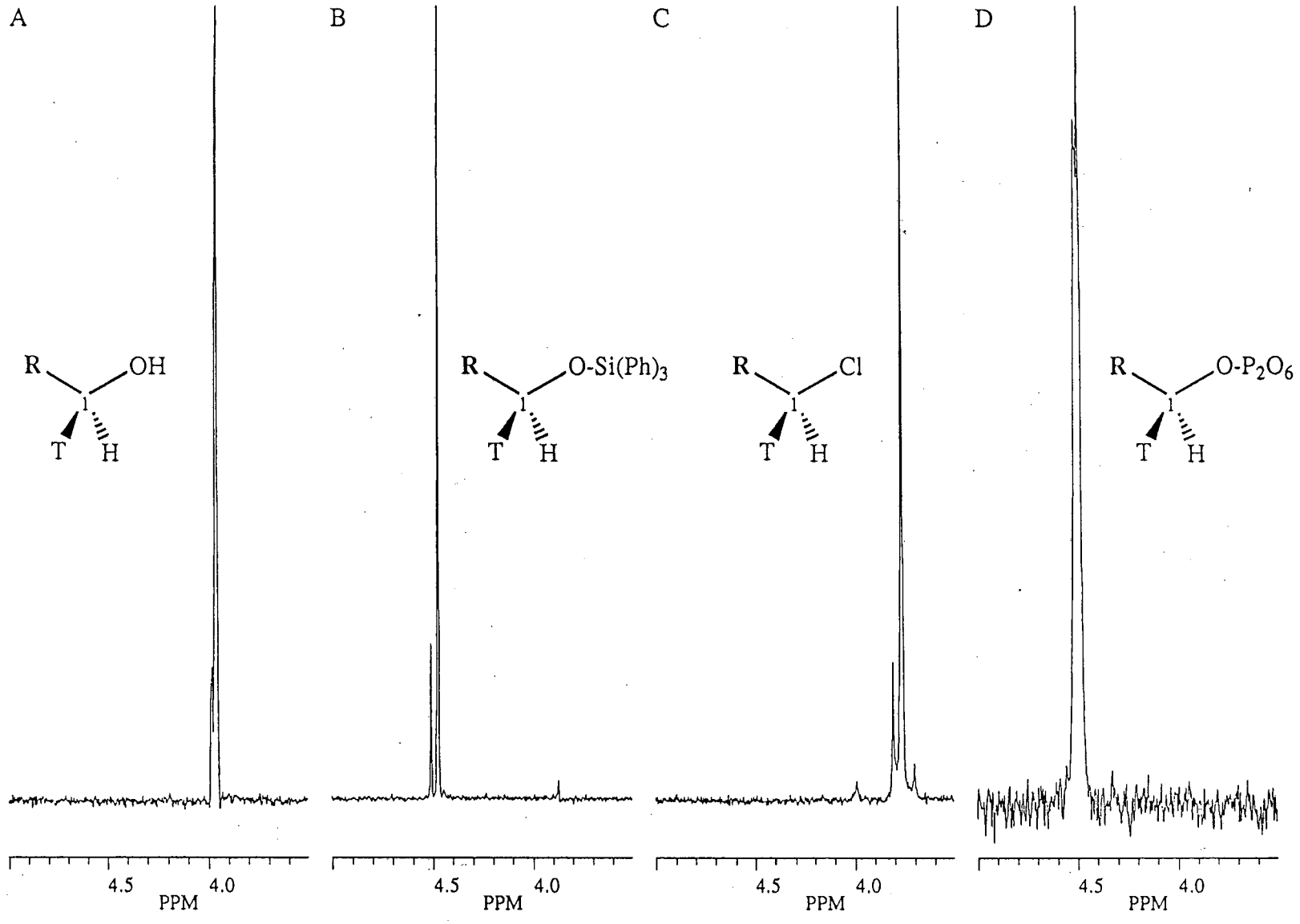
A

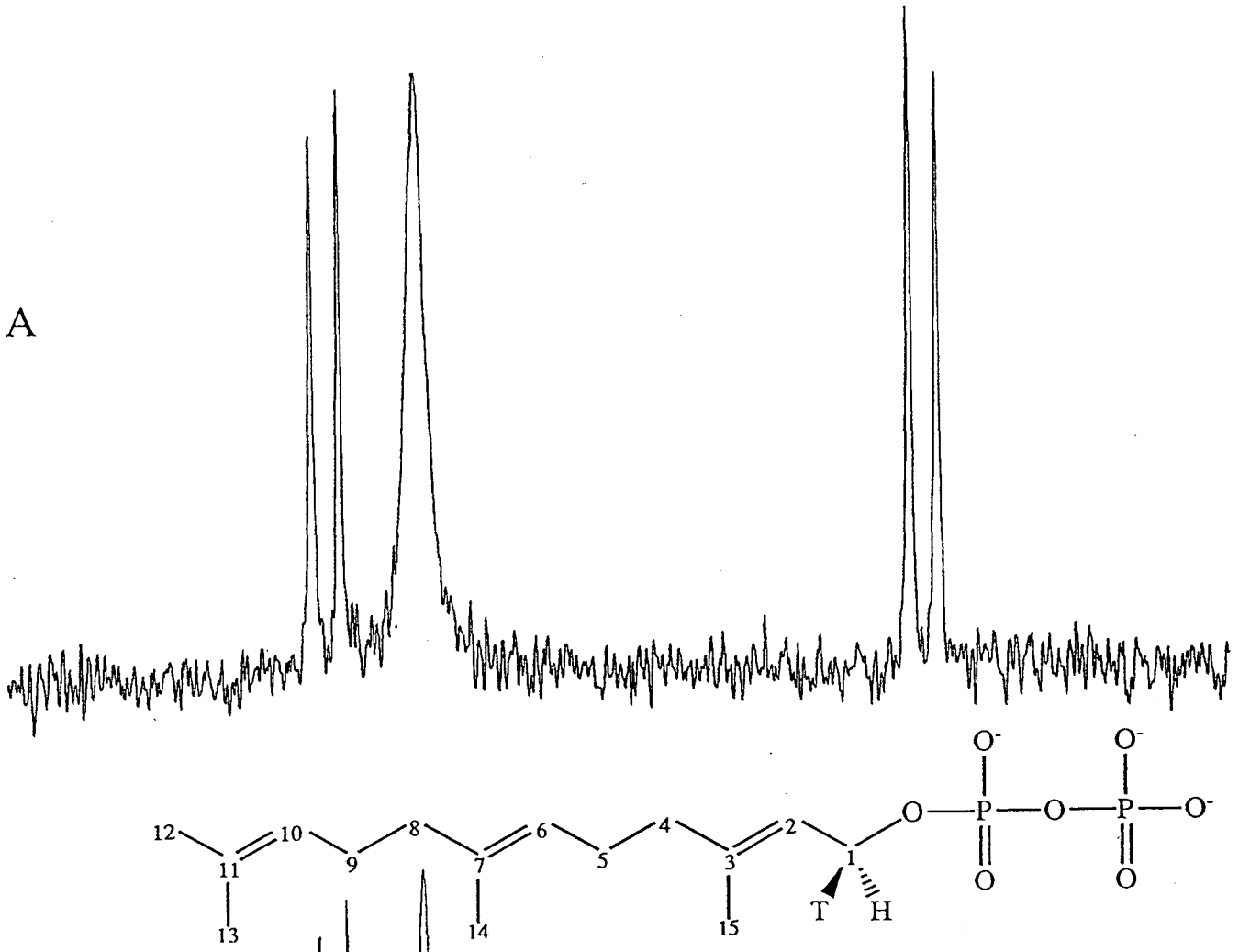

12
11
10
9
8
7
6
5
4
3
2
1
OH
H
H
13
14
15

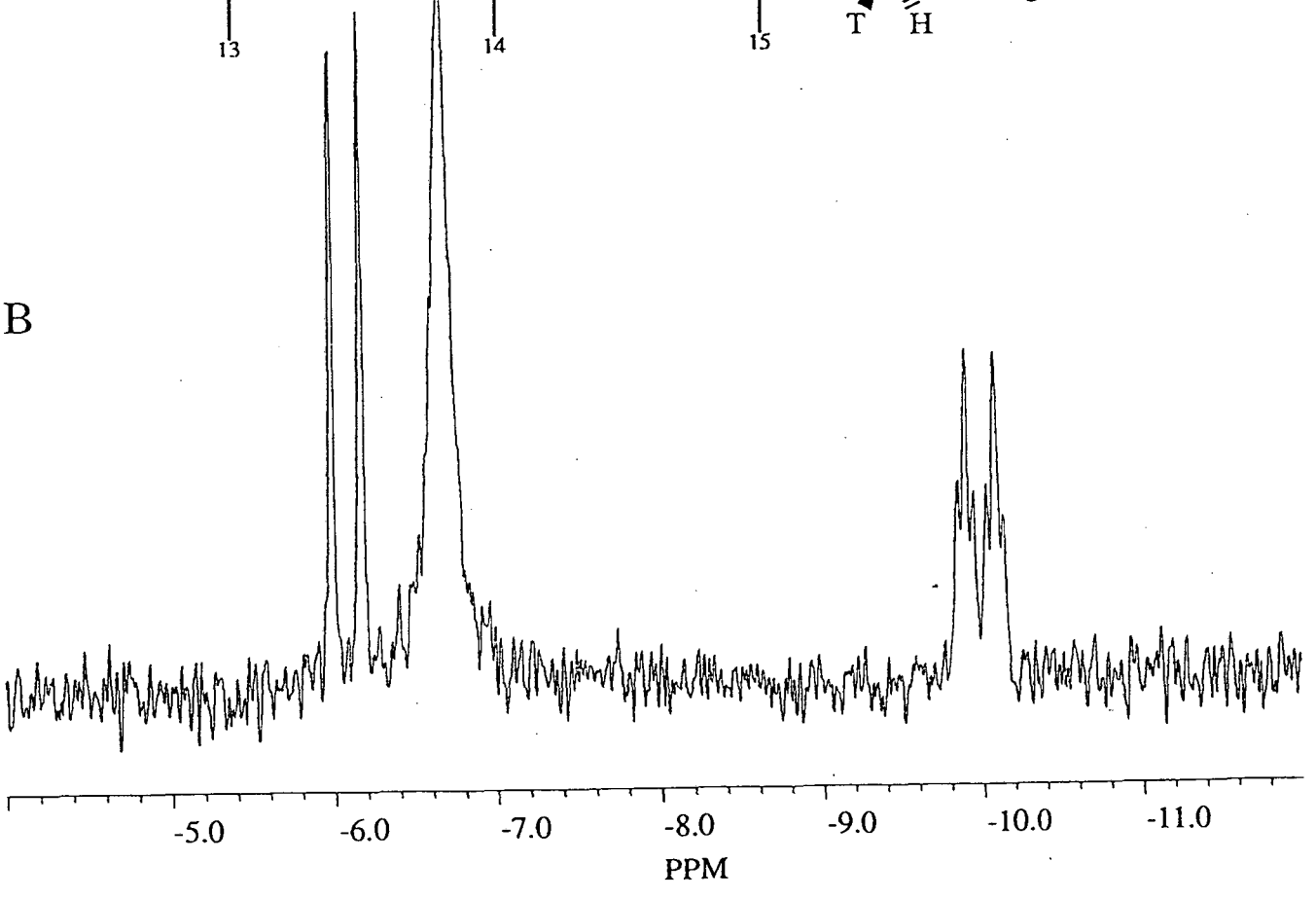
B


12
11
10
9
8
7
6
5
4
3
2
1
O
H
13
14
15

10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
PPM


A
B
C
D
R
OH
O-Si(Ph)3
Cl
O-P2O6
T
H
4.5
4.0
PPM


A
O⁻
O⁻
O
P
O
P
O⁻
O
O
12
11
10
9
8
7
6
5
4
3
2
1
13
14
15
T
H


B
-5.0
-6.0
-7.0
-8.0
-9.0
-10.0
-11.0
PPM

LAWRENCE BERKELEY LABORATORY
TECHNICAL INFORMATION DEPARTMENT
1 CYCLOTRON ROAD
BERKELEY, CALIFORNIA 94720