Synthesis of (*E,E,E*)-(1,2,3,4-$^{13}C_4$)-Geranylgeraniol

Knut Eis, Hans-Günther Schmalz*

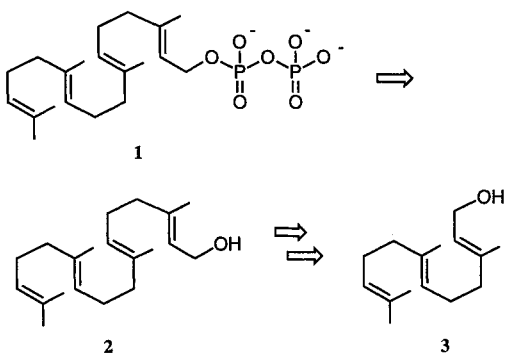
Institut für Organische Chemie, Technische Universität Berlin, Strasse des 17. Juni 135, D-10623 Berlin, Germany

Fax +49(30)31421105; E-mail Schmalz@WAP0109.chem.tu-berlin.de

Received 3 July 1996; revised 2 September 1996

A synthesis of (*E,E,E*)-(1,2,3,4-$^{13}C_4$)-geranylgeraniol (**2'**) (5 steps, ≥ 30% overall yield) is described starting from farnesol (**3**). The synthesis is based on the Sum/Weiler protocol, which was found to be superior after investigating two different routes in the non-labelled series.

The lipidation of proteins is of great importance for a variety of biological processes such as cell signaling.[1] An interesting example constitutes the Rab proteins, which are involved in the regulation of vesicular membrane traffic.[2] These small G proteins are subject to geranylgeranylation at two cysteine residues at or very near the CO_2H-terminus through a process involving a geranylgeranyltransferase (GGTase). In addition, this prenylation requires an accessory protein, the so-called Rab escort protein (REP).

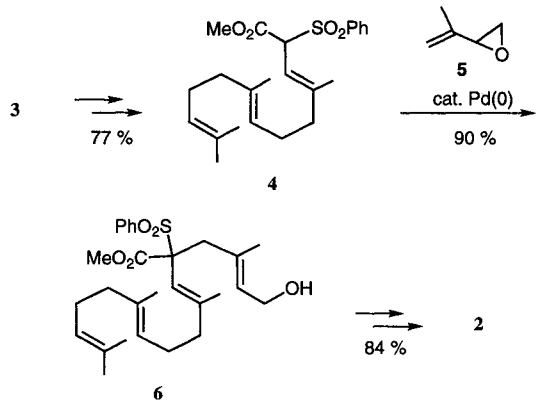


Scheme 1

In order to obtain more detailed structural information about the prenylated Rab proteins by means of NMR spectroscopy, the proteins with specifically ^{13}C-labelled geranylgeranyl sidechains are of high potential value. In an ideal case, several ^{13}C-substituted centers would be positioned close to the cysteine-bridge. Since the *in vitro* attachment of the geranylgeranyl chain to the purified Rab proteins is possible employing geranylgeranyl pyrophosphate (**1**) in the presence of RabGGTase and REP-1,[2] and since geranylgeraniol (**2**) can be converted into **1** in good yield using the two step protocol of Davisson, Woodside and Poulter,[3] the accessibility of ^{13}C-labelled **2** is the limiting prerequisite for the anticipated NMR spectroscopic investigation.

For the synthesis of ^{13}C-labelled **2**, farnesol (**3**) was projected as an ideal starting material (Scheme 1) because it is commercially available as the pure *E,E*-isomer and the ^{13}C-labels could be introduced in the course of the attachment of the lacking C_5-unit. Due to the high cost of ^{13}C-labelled starting materials, we had to find a highly efficient and reliable synthetic route for the conversion of **3** into **2** using a minimum number of steps. A literature search revealed that several synthetic routes to **2** had been described,[4-10] some of them[6,8-10] indeed starting from farnesol or derivatives thereof.

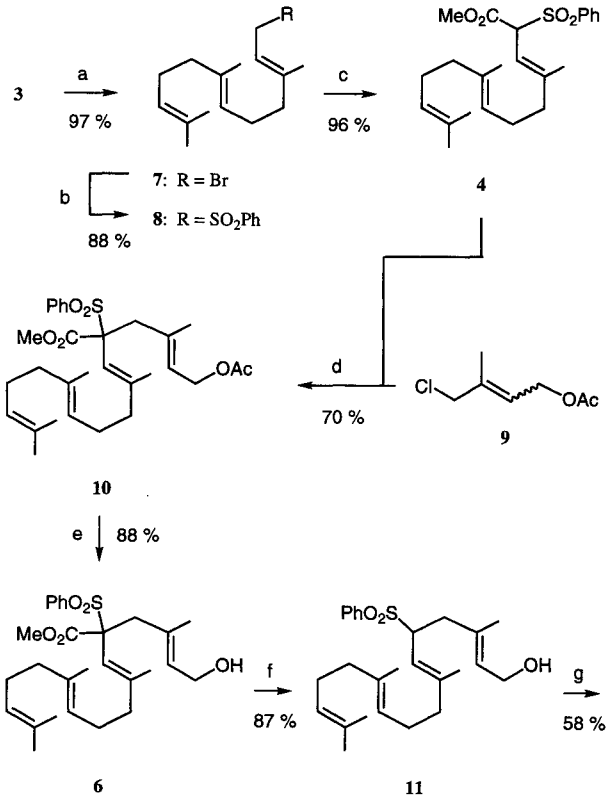
The synthesis which initially looked most appealing to us was the one of Bouzbouz and Kirschleger.[10] In the key step of their synthesis (Scheme 2), these authors couple the farnesol-derived sulfonyl ester **4** with the epoxide **5** to the geranylgeraniol derivative **6**, which is then converted to pure (*E,E,E*)-geranylgeraniol (**2**) in high overall yield (57%).



Scheme 2

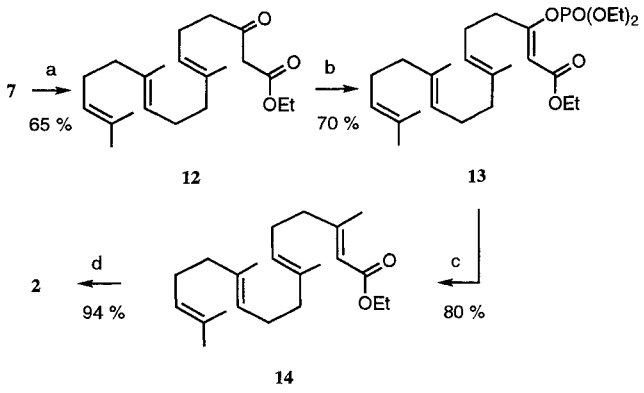
While we had no problems to prepare the α-sulfonyl ester **4** (via **7** and **8**) following the literature procedures[10] (Scheme 3), we were not able to obtain the epoxide **5** in a satisfying manner.[10] In addition, the starting materials which would be needed for the preparation of ^{13}C-labelled **5** are not easily available. We therefore decided to modify the synthesis and to employ **9**[11] as an alternative C_5-building block. According to Bäckvall,[11] this compound was obtained from isoprene as a 4.5:1-mixture of the *E*- and *Z*-isomers. Coupling[12] of **4** and **9** employing $(PPh_3)_4Pd$ as a catalyst and sodium hydride as a base gave the acetate **10** in 70% yield.[13] Treatment of **10** with potassium carbonate in methanol resulted in the selective cleavage of the acetate function to afford **6** in 88% yield.[13] This material was finally demethoxylated (→ **11**) and desulfonated (→ **2**) again using literature procedures.[10] After chromatography of the crude product (*E/Z*-mixture)[13] pure (*E,E,E*)-geranylgeraniol (**2**) was isolated in 58% yield (Scheme 3).

The synthetic route thus elaborated provides a reliable method for the conversion of **3** to **2** (seven steps; 25% overall yield). Compared to the original Bouzbouz/Kirschleger synthesis, the advantage of our modification



a) PBr_3, Et_2O, RT, 3 h; b) $NaSO_2Ph$, DMF, RT, 22.5 h; c) Me_2CO_3, KO^tBu, DMF, -40° C for 7 h, then RT for 14 h; d) NaH, cat. $Pd(PPh_3)_4$, THF, RT, 2h, E/Z-selectivity ≥ 9:1; e) K_2CO_3, CH_3OH, RT, 1.25 h; f) C_6H_5SH, cat. Cs_2CO_3, DMF, 90° C, 4.25h; g) $LiBHEt_3$, $PdCl_2(dppp)$, THF, RT, 2 h, then chromatography (SiO_2, hexanes/EtOAc = 4:1; removal of ca. 10 % of the (2Z)-diastereomer).

Scheme 3



a) dianion of ethyl acetoacetate, THF, 0°C, 1 h; c) NaH, $Cl(O)P(OEt)_2$, Et_2O, 0°C, 2.3 h; d) Me_2CuLi, Et_2O, -78° C for 3 h, then -50 °C for 1 h; e) DIBAH, toluene, 0°C, 1 h.

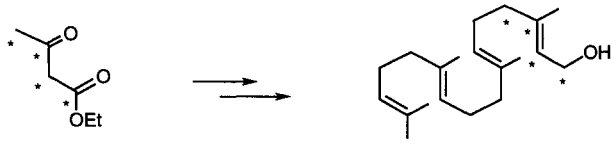
Scheme 4

is that we can employ the much more accessible C_5-building block **9**, which can also be (even more stereoselectively) prepared by an alternative method starting from chloroacetone and vinylmagnesium bromide.[14] The synthesis described above should therefore be suited for the preparation of ^{13}C-labelled **2** starting from (1,2,3-$^{13}C_3$)-acetone. On a small scale, however, problems could arise due to the volatility of the early intermediates.

At this time, we became aware of a paper of Sum and Weiler[15] describing an elegant method for the C_5-chain elongation of terpenoids. We immediately decided to check this protocol and to apply it to the synthesis of geranylgeraniol **(2)** (Scheme 4).

The dianion of ethyl acetoacetate (obtained from ethyl acetoacetate by treatment with sodium hydride and butyllithium) was reacted with farnesyl bromide **(7)** at 0 °C to give the β-keto ester **12** (65 % yield). On deprotonation with sodium hydride and trapping of the resulting enolate with diethyl chlorophosphate at 0 °C, **12** was converted into the enol phosphate **13**, which was obtained as a pure diastereomer. Treatment of **13** with lithium dimethylcuprate at −78 °C then provided ethyl geranylgeranoate **(14)** which was finally reduced with DIBAH at 0 °C to give pure (*E,E,E*)-geranylgeraniol **(2)** in high yield (Scheme 4).[16] This second synthesis has several advantages compared to the first one: It is shorter, the overall yield is higher and no *E*/*Z*-mixtures were obtained.[17] In addition, the C_5-building block (ethyl acetoacetate) is commercially available[18] in several ^{13}C-substituted forms (with respect to the ^{13}C-labelled positions) and can be directly employed.

Thus, starting from (1,2,3,4-$^{13}C_4$)-ethyl acetoacetate **(15)**, the synthesis of (*E,E,E*)-(1,2,3,4-$^{13}C_4$)-geranylgeraniol **(2′)** was carried out (see experimental section for details). The desired product **2′** was obtained with 38 % overall yield (Scheme 5) and can now be employed for the projected NMR spectroscopic investigation of geranylgeranylated proteins. We believe that the work described herein (in full detail) should be of value for the future investigation of biological phenomena related to prenylated proteins.



15 2′

Scheme 5

All reactions were routinely carried out under anhydrous conditions in an argon atmosphere. Et_2O and THF were distilled from sodium/benzophenone in an argon atmosphere prior to use. DMF was dried over molecular sieves 4 Å. Toluene was dried by passing through ICN alumina B (activity I) prior to use. MeOH was refluxed with Mg, distilled and stored over molecular sieves 3 Å. All other chemicals were used as received. The (1,2,3,4-$^{13}C_4$)-substituted ethyl acetoacetate was purchased from Cambridge Isotope Laboratories, Inc. The Pd catalysts used were prepared using literature procedures $(Ph_3P)_4Pd$,[19] and $PdCl_2dppp$.[20] NMR spectra were recorded in $CDCl_3$ on a Bruker AM 270 or AM 400 spectrometer. All NMR recordings were referenced to $CHCl_3$ resonances (7.25 and

77.0 ppm). ^{1}H NMR splitting patterns abbreviations are: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad; ψ, pseudo. ^{13}C NMR multiplicity was determined by DEPT sequences, abbreviations are: s, CH_3; d, CH_2; t, CH; q, quaternary carbons. IR spectra were recorded on a Nicolet Magna FT-IR spectrometer in CCl_4. Wavenumbers (ν) are quoted on cm^{-1}, abbreviations are: s, strong; m, medium; w, weak and br, broad. Mass spectra (MS) and high resolution mass spectra (HRMS) were carried out on a Varian MAT 711 spectrometer (70 eV); the calculated mass of the molecular peak was always confirmed by the peak matching method (accuracy ± 1 ppm). Analytical thin layer chromatography (TLC) was performed using Merck Silica 60 F 254 glass plates; the chromatograms were visualized under ultraviolet light and/or by staining with a cerium reagent [prepared by dissolving phosphomolybdic acid (2 g), $CeSO_4$ (1 g) and conc. H_2SO_4 (10 mL) in H_2O (90 mL)] followed by heating. Flash chromatography[21] was performed using Merck silica gel 60 (230–400 mesh). Preparative TLC was carried out on a Chromatotron (Harrison Research, model no. 7924 T) employing glass rotors coated with 1 to 4 mm layers of Merck silica gel PF 60 F254 containing gypsum.

Compounds **7** and **8** were prepared according to literature.[10]

(3*E*,7*E*)-Methyl 2-Benzenesulfonyl-4,8,12-trimethyltrideca-3,7,11-trienoate (4):

To a cold solution (−40 °C) of **8** (4.06 g; 11 mmol) and dimethyl carbonate (6.5 mL, 77 mmol) in DMF (45 mL) was added *t*-BuOK (3.284 g, 29.2 mmol) at −40 °C . The resulting orange mixture was stirred for 7 h at 0 °C and for 7 h at r.t. and worked up as described in the literature.[10] According to NMR and TLC (hexane/EtOAc, 6:1) the crude product (brown oil) was very pure and was directly used for the preparation of **10**; yield: 4.56 g (96%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.52 (s, 3H), 1.60 (s, 3H), 1.62 (s, 3H), 1.68 (s, 3H), 1.94–2.12 (m, 8H), 3.76 (s, 3H), 4.82 (d, 2H, J = 10.5 Hz), 5.07 (m, 2H), 5.29 (br d, 1H, J = 10.5 Hz), 7.54 (br t, 2H, J = 7.5 Hz), 7.66 (tt, 1H, J = 7.5, 1.5 Hz), 7.86 (br dd, 2H, J = 7.5, 1.5 Hz).

^{13}C NMR (270 MHz, $CDCl_3$): δ = 14.2 (q), 16.0 (q), 16.9 (q), 25.7 (q), 26.0 (t), 26.6 (t), 39.6 (t), 39.7 (t), 53.0 (q), 70.2 (d), 111.8 (d), 123.0 (d), 124.2 (d), 128.8 (2d), 129.6 (2d), 131.4 (s), 134.1 (d), 135.8 (s), 137.2 (s), 147.8 (s), 165.6 (s).

IR (CCl_4): ν = 3070 (w), 2928 (m), 2917 (m), 2856 (m), 1746 (s), 1448 (m), 1329 (s), 1311 (m), 1155 (m), 1083 cm^{-1} (m).

HRMS: 404.2021 as calc. for $C_{23}H_{32}O_4S$.

(2′*E*,3*E*,7*E*)-Methyl- 2-(4-Acetoxy-2-methylbut-2-enyl)-2-benzenesulfonyl-4,8,12-trimethyltrideca-3,7,11-trienoate (10):

A solution of **4** (201 mg, 0.5 mmol) in THF (5 mL) was added slowly to a stirred suspension of NaH (15 mg, 0.63 mmol) in THF (1 mL) to give an orange solution. Separately, a solution of **9** (121 mg, 0.74 mmol; E/Z = 4.5:1)[11] in THF (5 mL) was added to a stirred suspension of $(Ph_3P)_4Pd$ (60 mg, 0.05 mmol) in THF (1 mL) resulting in a dark brown solution. This solution was transferred to the orange solution of the anion of **4** by means of a transfer needle (vigorous reaction) and the dark reaction mixture was stirred for 2 h at r.t. After addition of satd. aq NH_4Cl (10 mL), the aqueous layer was extracted with hexane/EtOAc, (4:1, 3 × 10 mL). The combined organic layers were washed with H_2O (2 × 10 mL), brine (10 mL), dried ($MgSO_4$) and the solvents were removed in vacuo. The crude product was dissolved in hexane/EtOAc (4:1) and (after filtering off a precipitate) purified by flash chromatography (hexane/EtOAc, 4:1) to give 185 mg (70%) of **10** as a pale yellow oil.[13]

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.40 (s, 3H), 1.58 (br s, 6H), 1.87 (s, 3H), 1.89 (s, 3H), 2.02 (s, 3H), 2.00–2.12 (m, 8H), 2.90 (d, 1H, J = 14 Hz), 3.20 (d, 1H, J = 14 Hz), 3.67 (s, 3H), 4.52 (d, 2H, J = 7 Hz), 5.02–5.13 (m, 2H), 5.27–5.50 (m, 2H), 7.49–7.52 (br t, 2H, J = 7.5 Hz), 7.60–7.66 (br t, 1H, J = 7.5 Hz), 7.82–7.92 (m, 2H).

^{13}C NMR (270 MHz, $CDCl_3$): δ = 15.9 (q), 17.3 (q), 18.3 (q), 20.8 (q), 25.58 (t), 25.62 (t), 26.2 (t), 26.6 (q), 39.6 (t), 41.7 (t), 42.1 (t), 52.5 (q), 60.8 (d), 77.2 (s), 117.1 (d), 123.2 (d), 123.7 (d), 124.1 (d), 128.2 (2d), 131.9 (2d), 131.3 (s), 133.8 (d), 135.6 (s), 135.8 (s), 136.9 (s), 146.9 (s), 168.1 (s), 170.7 (s).

IR (CCl_4): ν = 3070 (w), 2968 (m), 2927 (m), 2856 (m), 1743 (s), 1447 (m), 1324 (s), 1231 (s), 1147 cm^{-1} (s).

MS: m/z (%) = 531 (1.3), 389 (64), 329 (28), 251 (35), 193 (37), 81 (38), 69 (100).

HRMS: 530.2702 as calc. for $C_{30}H_{42}O_6S$.

(2′*E*,3*E*,7*E*)-Methyl 2-Benzenesulfonyl-2-(4-hydroxy-2-methylbut-2-enyl)-4,8,12-trimethyltrideca-3,7,11-trienoate (6):

To a suspension of K_2CO_3 (373 mg, 1.46 mmol) in MeOH (10 mL) was added a solution of **10** (517 mg, 0.97 mmol) in MeOH (30 mL). After stirring for 1.25 h, the pale green mixture was poured into H_2O and the resulting milky suspension was extracted with a 1:1 mixture of hexane/EtOAc (3 × 30 mL). The combined organic layers were washed with H_2O (3 × 30 mL), brine (30 mL) dried (Na_2SO_4) and evaporated. The crude product was purified by preparative TLC (hexane/EtOAc, 1:1) to yield 419 mg (88%) of **6** as a pale yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.38 (s, 3H), 1.59 (br s, 6H), 1.67 (br s, 6H), 2.00–2.10 (m, 8H), 2.99 (d, 1H, J = 14 Hz), 3.18 (d, 1H, J = 14 Hz), 3.70 (s, 3H), 4.10 (d, 2H, J = 7 Hz), 5.03–5.12 (m, 2H), 5.34 (s, 1H), 5.47 (br t, 1H, J = 7 Hz), 7.50 (br t, 2H, J = 8.5 Hz), 7.61 (br t, 1H, J = 7.5 Hz), 7.86 (m, 2H).

^{13}C NMR (270 MHz, $CDCl_3$): δ = 16 (q), 17.3 (q), 17.6 (q), 18.3 (q), 25.6 (q), 26.2 (t), 26.6 (t), 39.6 (t), 41.6 (t), 42.8 (t), 52.6 (q), 59.0 (t), 77.1 (s), 117.6 (d), 123.1 (d), 124.1 (d), 128.3 (2d), 129.0 (d), 130.9 (s + 2d), 133.2 (s), 133.7 (d), 135.8 (s), 136.9 (s), 146.8 (s), 168.3 (s).

IR (CCl_4): ν = 3622 (w), 3545 (w, br), 3070 (w), 2968 (m), 2950 (m), 2927 (m), 2857 (m), 1743 (s), 1447 (m), 1324 (s), 1214 (m), 1147 (s), 1083 cm^{-1} (m).

MS: m/z (%) = 488 (1.3), 347 (97), 209 (38), 165 (32), 81 (38), 69 (100).

HRMS: 488.2596 as calc. for $C_{28}H_{40}O_5S$.

The spectral data are in reasonable accordance with those reported in the literature[10] for the same compound obtained by a different procedure.

(2*E*,6*E*,10*E*)-5-Benzenesulfonyl-2,6,10,14-tetramethylhexadeca-tetra-2,6,10,14-en-1-ol (11):

According to the literature procedure,[10] a mixture of Cs_2CO_3 (103 mg, 0.32 mmol), **6** (706 mg, 1.44 mmol) and thiophenol (0.385 mL, 3.77 mmol) in DMF (15 mL) was heated to 90 °C for 4.25 h. After workup, the crude product was purified by preparative TLC (hexane/EtOAc, 3:2) to give 539 mg (87%) of **11** as a pale yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.16 (d, 3H, J = 1 Hz), 1.57 (s, 3H), 1.61 (s, 3H), 1.62 (s, 3H), 1.68 (s, 3H), 1.88–2.10 (m, 8H), 2.34 (dd, 1H, J = 13.5, 11 Hz), 2.94 (dd, 1H, J = 13.5, 2.5 Hz), 3.92 (ddd, J = 11, 10, 2.5 Hz), 4.03–4.17 (m, 2H), 4.93 (d, 1H, J = 10 Hz), 4.99–5.11 (m, 2H), 5.42 (br t, 1H, J = 7 Hz), 7.52 (br t, 2H, J = 8 Hz), 7.64 (tt, 1H, J = 8, 1.5 Hz), 7.86 (dd, 2H, J = 8, 1.5 Hz).

^{13}C NMR (270 MHz, $CDCl_3$): δ = 15.9 (q), 16.2 (q), 16.3 (q), 17.6 (q), 25.6 (q), 26.2 (t), 26.6 (t), 37.2 (t), 39.6 (2t), 59.1 (t), 63.1 (d), 117.9 (d), 123.3 (d), 124.1 (d), 127.2 (d), 128.7 (2x d), 129.2 (d), 131.4 (s), 133.4 (d), 134.1 (s), 135.6 (s), 137.7 (s), 145.5 (s).

IR (CCl_4): ν = 3620 (w), 3545 (w, br), 3070 (w), 2969 (m), 2917 (m), 2857 (m), 1447 (m), 1319 (m), 1307 (m), 1147 (s), 1086 cm^{-1} (m).

MS: m/z (%) = 430 (2.5), 289 (11), 271 (12), 203 (13), 137 (18), 81 (51), 69 (100).

HRMS: 430.2542 as calc. for $C_{26}H_{38}O_3S$.

The spectral data are in reasonable accordance with those reported in the literature.[10]

(2*E*,6*E*,10*E*)-2,6,10,14-Tetramethylhexadecatetra-2,6,10,14-en-1-ol (2):

To a cold (0 °C) suspension of **11** (480 mg, 1.12 mmol) and $PdCl_2$dppp (34 mg, 0.058 mmol) in THF (20 mL) was added a 1M solution of $LiBHEt_3$ in THF (2.4 mL, 2.4 mmol) over a period of 1 h. After workup[10] the crude product was purified by preparative

TLC (hexane/EtOAc, 4:1) to yield 188 mg (58%) of **2** as a colorless oil. In addition, 48 mg (15%) of a 1:1 mixture of (2*E*,6*E*,10*E*)- and (2*Z*,6*E*,10*E*)-geranylgeraniol was obtained.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.60 (br s, 9H), 1.68 (br s, 6H), 1.93–2.16 (m, 12H), 4.16 (d, 2H, J = 7 Hz), 5.07–5.15 (m, 3H), 5.42 (br t, 1H, J = 7 Hz).

^{13}C NMR (270 MHz, $CDCl_3$): δ = 16.0 (2q), 16.2 (q), 17.6 (q), 25.7 (q), 26.3 (t), 26.6 (t), 26.7 (t), 39.5 (t), 39.6 (t), 39.7 (t), 59.3 (t), 123.3 (d), 123.7 (d), 124.1 (d), 124.3 (d), 131.2 (s), 134.9 (s), 135.3 (s), 139.7 (s).

IR (CCl_4): ν = 3622 (w), 3499 (w, br), 3050 (w), 2968 (m), 2927 (m), 2856 (m), 1666 (w), 1449 (m), 1383 (m), 996 cm^{-1} (m).

MS: m/z (%) = 272 (3.9), 93 (35), 81 (39), 69 (100).

HRMS: 272.2504 as calc. for $C_{20}H_{32}$ ($M^+ - H_2O$).

The spectral data (IR, ^{1}H NMR) are in accordance with those given in the literature.[6]

(6*E*,10*E*)-(1,2,3,4-$^{13}C_4$)-7,11,15-Trimethyl-3-oxohexadeca-6,10,14-trienoic Acid Ethyl Ester (12′):

The procedure of Sum and Weiler[15] was slightly modified; all operations were carried out in an ice bath. To a suspension of NaH (297 mg, 7.43 mmol, 60% in mineral oil) in THF (25 mL) was added dropwise 1,2,3,4-$^{13}C_4$ ethyl acetoacetate (500 mg, 3.73 mmol). After 10 min, BuLi (3.1 mL of a 1.49 M solution in hexane, 5.8 mmol) was slowly added and stirring was continued for 10 min before a solution of farnesyl bromide (1.012 g, 3.55 mmol) in THF (4 mL) was added in one portion. After 1.5 h, the reaction was quenched by adding 2 N HCl (15 mL) and the ice bath was removed. The layers were separated and the aqueous layer was extracted with methyl *tert*-butyl ether. The combined organic layers were washed with brine (3 × 20 mL), dried ($MgSO_4$) and evaporated. The crude product was purified by flash chromatography (hexane/EtOAc, 4:1) to yield 784 mg (65%) of **12′** as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.27 (t, 3H, J = 7 Hz), 1.58 (s, 3H), 1.59 (s, 3H), 1.61 (s, 3H), 1.68 (s, 3H), 1.93–2.11 (m, 10H), 2.23–2.33 (m, 2H), 2.56 (dq, 2H, $^1J_{C,H}$ = 126 Hz, J_q = 6 Hz), 3.42 (dt, 2H, $^1J_{C,H}$ = 130 Hz, J_t = 7 Hz), 4.19 (qd, 2H, J_q = 7 Hz, J_d = 3 Hz), 5.09 (m, 3H).

(2*E*,6*E*,10*E*)-(1,2,3,4-$^{13}C_4$)-3-Diethoxyposphoryloxy-7,11,15-trimethylhexadecatetra-2,6,10,14-enic Acid Ethyl Ester (13′):

A suspension of 70 mg (2.9 mmol) NaH in Et_2O (6 mL) was cooled in an ice bath and a solution of **12′** (784 mg, 2.32 mmol) in Et_2O (15 mL) was added dropwise. After stirring for 1.5 h, diethyl chlorophosphate (380 μL, 2.6 mmol) was added and stirring was continued for 2.3 h before NH_4Cl (1.3 g) was added. The ice bath was removed and after 0.5 h the mixture was filtered through a pad of Celite and the solvent was evaporated. Preparative TLC (hexane/EtOAc, 4:1) of the crude product yielded 867 mg (78%) of **13′** as a pale yellow oil.

^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.27 (t, 3H, J = 7 Hz), 1.37 (br t, 6H, J = 7 Hz), 1.60 (s, 6H), 1.62 (s, 3H), 1.68 (s, 3H), 1.93–2.13 (m, 8H), 2.17–2.34 (m, 3H), 2.43–2.55 (m), 4.16 (qd, 2H, J_q = 7 Hz, J_d = 4 Hz), 4.27 (ψ-quint, 4H, J = 8 Hz), 5.02–5.15 (m, 3.5H), 5.66 (m, 0.5H).

(2*Z*,6*E*,10*E*)-(1,2,3,4-$^{13}C_4$)-3,7,11,15-Tetramethylhexadecatetra-2,6,10,14-enoic Acid Ethyl Ester (14′):

A stirred suspension of CuI (806 mg, 2 mmol) in Et_2O (26 mL) was cooled to 0 °C and a 1.45 M solution of MeLi (5.5 mL, 8 mmol) in Et_2O was introduced via a syringe. After 1.5 h at 0 °C, the resulting solution was cooled to −78 °C and a solution of the enol phosphate **13′** (867 mg, 1.83 mmol) in Et_2O (4 mL) was added. The resulting orange solution was then allowed to warm to −40 °C over a period of 3 h. At this point, a 1:1 mixture of satd aq NH_4Cl and satd aq K_2CO_3 (25 mL) was added and stirring was continued for 0.5 h. The aqueous layer was extracted with methyl *tert*-butyl ether (15 mL), the combined organic layers were washed with a 1:1 mixture of satd. aq NH_4Cl and satd. aq K_2CO_3 (2 × 20 mL), satd. aq $Na_2S_2O_3$ (20 mL), dried ($MgSO_4$) and evaporated. Purification of the crude product by preparative TLC (hexane/EtOAc, 4:1) yielded 589 mg (96%) of **14′** as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.28 (t, 3H, 6 Hz), 1.55–1.71 (m, 12H), 1.94–2.21 (m, 14H), 2.31 (1H), 4.15 (qd, 2H, J_q = 7 Hz, J_d = 3 Hz), 5.08–5.12 (m, 3H), 5.66 (dd, 1H, $^1J_{CH}$ = 160 Hz, J_d = 6 Hz).

HRMS: 336.2849 as calc. for $C_{16}{}^{13}C_4H_{36}O_2$.

The spectral data of unlabelled **14** were in accordance to those given in the literature.[17]

(2*E*,6*E*,10*E*)-(1,2,3,4-$^{13}C_4$)-3,7,11,15-Tetramethylhexadecatetra-2,6,10,14-en-1-ol (2′):

To a cooled (0 °C) solution of **14′** (586 mg, 1.74 mmol) in toluene (20 mL) was slowly added DIBAH (3.75 mL of a 1.5 M solution in toluene, 5.6 mmol). After 0.5 h, the stirred reaction was quenched with H_2O (20 mL) and the resulting mixture was poured into a separating funnel containing 2 N HCl (25 mL). The aqueous layer was extracted with *tert*-butyl methyl ether (3 × 20 mL). The combined organic layers were washed with satd. aq $NaHCO_3$ (40 mL), H_2O (40 mL), brine (40 mL), dried ($MgSO_4$) and evaporated. Preparative TLC (hexane/EtOAc, 4:1) yielded 399 mg (78%) of **2′** as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.59–1.72 (m, 15H), 1.84–2.44 (m, 12H), 4.15 (br d, 2H, $^1J_{C,H}$ = 140 Hz), 5.08–5.15 (m, 3H), 5.52 (dq, 1H, $^1J_{C,H}$ = 154 Hz, J_q = 7 Hz).

HRMS: 294.2744 as calc. for $C_{16}{}^{13}C_4H_{34}O$.

This work was financially supported by the Fonds der Chemischen Industrie. We thank Dr. H. Oschkinat and Dr. M. Zerail (both EMBL, Heidelberg) for an inspiring discussion initiating this work and, in addition, for a sample of (1,2,3,4-$^{13}C_4$)-substituted ethyl acetoacetate. We are indebted to Dr. P. Pritschow and Dr. C. Zdero for performing the NMR spectra, Dr. G. Höhne for performing the MS spectra and Ms. C. Klose for performing the IR spectra. We also thank the Chemetall GmbH for generous gifts of chemicals.

(1) Casey, P.J. *Science* **1995**, *268*, 221.
(2) Zerial, M.; Stenmark, H. *Curr. Opin. Cell. Biol.* **1993**, *5*, 613.
Simons, K.; Zerial, M. *Neuron* **1993**, *11*, 789.
Alexandrov, K.; Horiuchi, H., Steele-Mortimer, O.; Seabra, M.C.; Zerial, M. *EMBO J.* **1994**, *13*, 5262.
(3) Davisson, V.J.; Woodside, A.B.; Poulter, C.D. *Methods Enzymol.* **1985**, *110*, 130.
Davisson, V.J.; Woodside, A.B.; Neal, T.R.; Stremler, K.E.; Muehlbacher, M.; Poulter, C.D. *J. Org. Chem.* **1986**, *51*, 4768.
Woodside, A.B.; Huang, Z.; Poulter, C.D. *Org. Synth.* **1988**, *66*, 211.
(4) Ruzicka, L.; Firmenich, G. *Helv. Chim. Acta* **1939**, *22*, 392.
(5) Corey, E.J.; Shieh, W.C. *Tetrahedron Lett.* **1992**, *33*, 6435.
(6) Coates, R.M.; Ley, D.A.; Cavender, P.L. *J. Org. Chem.* **1978**, *43*, 4915.
(7) Altman, L.J.; Ash, L.; Marson, S. *Synthesis* **1974**, 129.
(8) Trost, B.M.; Weber, L. *J. Org. Chem.* **1975**, *40*, 3617.
(9) Klinge, S.; Demuth, M. *Synlett* **1993**, 783.
(10) Bouzbouz, S.; Kirschleger, B. *Synlett* **1994**, 763,
Bouzbouz, S.; Kirschleger, B. *Synthesis* **1994**, 714.
(11) Nyström, J.-E.; Rein, T.; Bäckvall, J.-E. *Org. Synth.* **1989**, *67*, 105.
(12) For a review on Pd-catalysed allylic substitutions, see:
Trost, B.M. *Acc. Chem. Res.* **1980**, *13*, 385.
(13) Compounds **10**, **6** and **11** exhibited a clean NMR spectrum but contained ca. 10% of the (2*Z*)-diastereomer as judged by the fact that at the stage of compound **2** the (2*Z*)-diastereomer (ca. 10%) was detected in the crude product by means of ^{1}H NMR spectroscopy. Separation was achieved at this stage via preparative TLC.
(14) Tietze, L.-F.; Eicher, T. *Reaktionen und Synthese im organisch chemischen Praktikum*, 2nd ed, Georg Thieme: Stuttgart, 1991; p 112.
(15) Sum, F.-W.; Weiler, L. *Can. J. Chem.* **1979**, *57*, 1431.
Huckin, S.N.; Weiler, L. *J. Am. Chem. Soc.* **1974**, *96*, 1082.

(16) An attempt to reduce **14** with $LiAlH_4$ afforded a 2:1 mixture of **2** and its 2,3-dihydro derivative.
(17) While our work was in progress, Mu and Gibbs (Mu, Y.-Q.; Gibbs, R.A. *Tetrahedron Lett.* **1995**, *36*, 5669) communicated a somewhat related method for the conversion of **3** to **2** which also follows the Sum/Weiler strategy (27% overall yield). Instead of the enol phosphate **13**, these authors used the corresponding enol triflate which was converted to **14** via Suzuki coupling.
(18) This material is commercially available from Cambridge Isotope Laboratories, Inc..
(19) Colquhoun, H.M.; Holton, J.; Thompson, D.J.; Twigg, M.V. *New Pathways for Organic Syntheses*; Plenum Press: New York, 1984, p 383.
(20) Steffen, W.L.; Palenik, G.J. *Inorg. Chem.* **1976**, *15*, 2432.
(21) Still, W.C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.