SYNTHESIS OF C-13 LABELED VITAMIN E, [4'a-^{13}C]all-rac-α-TOCOPHEROL[1)]

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Summary

Vitamin E with a ^{13}C-labeled isoprenoid side chain, [4'a-^{13}C]-all-rac-α-tocopherol (1), was synthesized by the coupling reaction of 6-methoxymethoxy-2-([4-methyl-^{13}C]5-bromo-4-methyl-pent-1-yl)chroman (8) with 3,7-dimethyl-1-(thiazolin-2-yl)thio-2,6-octadiene (9). Compound 8 was prepared using 2-(4,4-di-ethoxycarbonylbut-1-yl)-6-methoxymethoxy-2,5,7,8-tetramethyl-chroman (5) as a key intermediate and [^{13}C]methyl iodide as a ^{13}C source. The total yield of the labeled α-tocopherol based on [^{13}C]methyl iodide was 58.7%.

Key words: Vitamin E, [^{13}C]Labeled α-tocopherol, Synthesis, [4'a-^{13}C]all-rac-α-Tocopherol

Introduction

Vitamin E is considered not only to act as a biological anti-oxidant and radical scavenger[2)], but to stabilize biomembranes through a specific, physicochemical interaction between its isoprenoid side chain and fatty acyl, particularly arachidoyl, side chain of polyunsaturated phospholipids[3)]. However, it is

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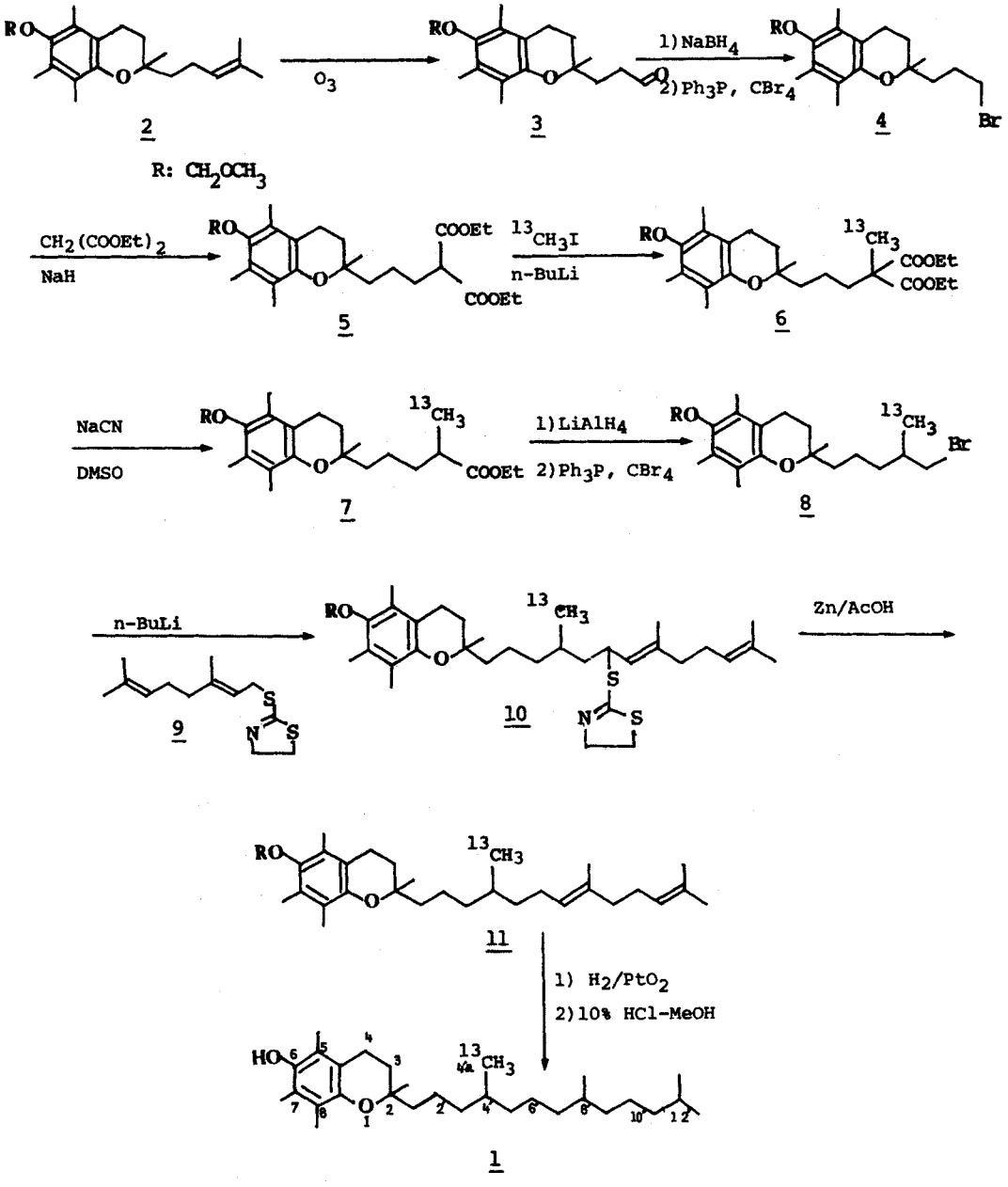
hardly clarified what interactions there are between vitamin E and lipids in biomembrane. For the investigation of them some new methods need to be developed. One of the hopeful ones is presumed to be the measurement of ^{13}C-relaxation time on vitamin E in biomembrane. Thus, α-tocopherol (vitamin E) with a labeled isoprenoid side chain must be very useful for the elucidation of its action in biomembrane. In order to obtain the labeled α-tocopherol, we have recently established a new route for the synthesis of α-tocopherol[4]. Several α-tocopherols which are labeled with ^{13}C at the different positions on isoprenoid side chain would be necessary to compare their relaxation times in biomembrane. We now wish to report the preparation of [4'a-^{13}C]-all-rac-α-tocopherol.

Results and Discussion

A starting material, 6-methoxymethoxy-2,5,7,8-tetramethyl-2-(4-methyl-3-penten-1-yl)chroman (2), which was prepared as discrived previously[4], was ozonized in a dry ice-acetone bath to give 6-methoxymethoxy-2,5,7,8-tetramethyl-2-(3-oxoprop-1-yl)chroman (3) in 73.2% yield. Reduction of 3 with $NaBH_4$, followed by bromination using carbon tetrabromide and triphenylphosphine, led to 2-(3-bromoprop-1-yl)-6-methoxymethoxy-2,5,7,8-tetramethylchroman (4) in 63.8% yield from 3. Compound 4 was reacted with ethyl malonate in the presence of sodium hydride to afford 2-(4,4-diethoxycarbonylbut-1-yl)-6-methoxymethoxy-2,5,7,8-tetramethylchroman (5) in 80.5% yield. Treatment of 5 with [^{13}C]-methyl iodide and n-butyllithium gave 2-([5-^{13}C]4,4-diethoxycarbonylpent-1-yl)-6-methoxymethoxy-2,5,7,8-tetra-



methylchroman (6) in 88.8% yield. The labeled position in 6 was confirmed spectroscopically: in the ^{1}H-NMR spectrum ($CDCl_3$) a signal at 1.42 ppm is split with a coupling constant of 126.0 Hz (J_{C-H}) and in the ^{13}C-NMR spectrum ($CDCl_3$) the intensity of a signal at 19.9 ppm is extremely enhanced and a signal at 53.7

ppm is split with a coupling constant of 35.4 Hz (J_{C-C}). With sodium cyanide 6 was refluxed in dimethylsulfoxide for 24 hours to give 2-([5-^{13}C]4-ethoxycarbonyl-1-yl)-6-methoxymethoxy-2,5,7,8-tetramethylchroman (7) in 92.3% yield. After reduction of 7 with $LiAlH_4$ in dry ether at 0°C, the resultant alcohol obtained was converted into 2-([4-methyl-^{13}C]5-bromo-4-methylpent-1-yl)-6-methoxymethoxy-2,5,7,8-tetramethylchroman (8) in 86.3% yield.

An intermediate, 3,7-dimethyl-1-(thiazolin-2-yl)thio-2,6-octadiene (9), was prepared by a reaction of geranyl bromide with 2-mercaptothiazoline. Condensation of 9 with the bromide 8 in a mixture of dry tetrahydrofuran (THF) and hexamethylphosphoramide (HMPA) in the presence of n-butyllithium gave 6-methoxymethoxy-2,5,7,8-tetramethyl-2-([4-methyl-^{13}C](7E,11E)4,8,12-trimethyl-6-(thiazolin-2-yl)thio-7,11-tridecadien-1-yl)chroman (10) in 98.2% yield. With zinc powder 10 was desulfurized in acetic acid to give 6-methoxymethoxy-2,5,7,8-tetramethyl-2-([4-methyl-^{13}C](7E,11E)-4,8,12-trimethyl-7,11-tridecadien-1-yl)chroman (11) in 92.3% yield. Finally, 11 was reduced under 50 atm of hydrogen in the presence of platinum oxide at room temperature and then the methoxymethoxy protecting group was removed by methanolic hydrogen chloride. The desired [4'a-^{13}C]all-rac-α-tocopherol (1) was obtained in nearly quantitative yield. The ^{13}C-labeling was confirmed on the basis of the marked enhancement of a signal at 19.7 ppm (C-4'a) and coupling between C-4'a and C-4' (J_{C-C}=35.4 Hz) in ^{13}C-NMR spectrum. The overall yield of 1 based on [^{13}C]methyl iodide was 58.7%.

Experimental

^{1}H- and ^{13}C-NMR spectra were recorded on a Varian XL-200 Spectrometer employing $CDCl_3$ as a solvent and tetramethylsilane

as an internal standard. Mass, UV and IR spectra were taken with spectrometers as follows: Shimadzu-LKB 9000, Cary 118C and Jasco IRA-2, respectively. Silica gel C-200 (Wako, Osaka, Japan) was used for column chromatography and silica gel PF_{254} (Merck, Darmstadt, BRD) for thin layer chromatography. [^{13}C]Methyl iodide (^{13}C 90 atom %) was purchased from Merck Sharp Dohme Canada Limited (Montreal, Canada).

6-Methoxymethoxy-2,5,7,8-tetramethyl-2-(3-oxoprop-1-yl)chroman (3). Ozone gas was introduced into a solution of 2 (4.0g, 12 mmol) in dry methylene chloride (20 ml) at -78°C in a dry ice-acetone bath for 3 hours. After being warmed to room temperature, the reaction mixture was washed with water and dried over sodium sulfate. The solvent was evaporated. The residue was purified by a silica gel chromatography using benzene as an eluent to give 3 (2.7 g, 73.2%). Mass (M^+) 306; IR (neat) 1680 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ, 9.81 (bt, 1H, -CHO), 2.61 (m, 2H, $-CH_2-CHO$); ^{13}C-NMR ($CDCl_3$) δ, 202.2 (d, -CHO).

2-(3-Bromoprop-1-yl)-6-methoxymethoxy-2,5,7,8-tetramethylchroman (4). Sodium borohydride (0.25 g, 7.0 mmol) was added with stirring at 0°C to a solution of 3 (2.7 g, 8.8 mmol) in a mixture of THF and methanol (1:1, 15 ml). The stirring was continued for 3 hours. The reaction mixture was poured into ice water (150 ml) and then extracted with ether. The ether extract was washed with brine, 10% acetic acid and water, in turn. The extract was dried and evaporated. The residue was chromatographed on a silica gel column and eluted with a mixture of benzene and ether (5:1) to give a corresponding alcohol (2.0 g, 73.6%). Mass (M^+) 308; IR (neat) 3425 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ, 3.70 (t, 2H, J=7.0 Hz, $-CH_2OH$); ^{13}C-NMR ($CDCl_3$) δ, 62.9 (t, $-CH_2OH$). The mixture of the alcohol

obtained (2.9 g, 9.0 mmol), carbon tetrabromide (11.0 mmol) and triphenylphosphine (2.96 g, 11.0 mmol) was dissolved in dry benzene (30 ml). The solution was refluxed with stirring for one hour. To this solution hexane (100 ml) was added and cooled to room temperature. The reaction mixture was filtered. The filtrate was evaporated. The residue was chromatographed on a silica gel column using a mixture of hexane and ether (5:1) as an eluent to obtain 4 (3.0 g, 86.7%). Mass (M^+) 371; ^{1}H-NMR ($CDCl_3$) δ, 3.45 (m, 2H, $-C\underline{H}_2-Br$); ^{13}C-NMR ($CDCl_3$) δ, 38.5 (t, $-\underline{C}H_2-Br$).

2-(4,4-Diethoxycarbonylbut-1-yl)-6-methoxymethoxy-2,5,7,8-tetramethylchroman (5). Diethyl malonate (0.29 g, 3.0 mmol) was added to a dispersion of sodium hydride (0.16 g, 4.0 mmol) in dry benzene (10 ml) with stirring at 0°C for one hour. To a clear solution was added dropwise a solution of 4 in dry benzene (5 ml). The mixture was heated under reflux with stirring for 3 hours. The reaction mixture was poured into ice water and extracted with ether. The ether extract was washed with brine and then dried over sodium sulfate. The solvent was evaporated. The residue was purified by a silica gel chromatography using a mixture of hexane and ether (15:1) to give 5 (0.98 g, 80.5%). Mass (M^+) 450; IR (neat) 1730 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ, 4.18 (q, 4H, J=8.0 Hz, $-O-C\underline{H}_2-CH_3$ x 2), 3.38 (t, 1H, J=7.5 Hz, $CO-C\underline{H}-CO$), 1.27 (t, 6H, J=8.0 Hz, $-O-CH_2-C\underline{H}_3$ x 2); ^{13}C-NMR ($CDCl_3$) δ, 169.4 (s, $-\underline{C}O_2Et$), 61.2 (t, $O-\underline{C}H_2$), 52.8 (d, $-\underline{C}H(CO_2Et)_2$).

2-([5-^{13}C]4,4-Diethoxycarbonylpent-1-yl)-6-methoxymethoxy-2,5,7,8-tetramethylchroman (6). A hexane solution of n-butyllithium (1.6 mol), 2 ml) was added to a solution of 5 (0.49 g, 1.1 mmol) in dry THF (10 ml), which was stirred under nitrogen in a dry ice-acetone bath. The stirring was continued for 30 min. A solution of [^{13}C]methyl iodide (0.23 g, 1.6 mmol) in dry THF

(3 ml) was added to the well-stirred reaction mixture. After one hour, the reaction mixture was warmed to 0°C very slowly, poured into ice water and then extracted with ethyl acetate. The organic layer was washed with brine and dried over sodium sulfate. The ethyl acetate was evaporated. The residue was chromatographed on a silica gel column using benzene as an eluent to give 6 (0.45 g, 88.8%). Mass (M^+) 465; IR (neat) 1730 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ, 1.42 (d, 3H, J_{C-H}=126.0 Hz, $-^{13}\underline{C}H_3$); ^{13}C-NMR ($CDCl_3$) δ, 53.7 (d, J_{C-C}=35.4 Hz, $^{13}CH_3-\underline{C}(CO_2Et)_2$), 19.9 (q, $-^{13}\underline{C}H_3$, ^{13}C-enriched).

2-([5-^{13}C]4-Ethoxycarbonylpent-1-yl)-6-methoxymethoxy-2,5,7,8-tetramethylchroman (7). A mixture of 6 (0.44 g, 0.9 mmol), sodium cyanide (0.06 g, 1.2 mmol) and dimethylsulfoxide (20 ml) was heated at 160°C for 6 hours. The cooled reaction mixture was poured into cold water (100 ml) and extracted with ether. The extract was washed with water and then dried over sodium sulfate. The residue was chromatographed on a silica gel column using benzene as an eluent to obtain 7 (0.34 g, 92.3%). Mass (M^+) 393; IR (neat) 1730 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ, 2.42 (m, 1H, $-\underline{C}H(^{13}CH_3)-CO_2Et$); 1.16 (dd, 3H, J_{C-H}=34.2 Hz, J_{H-H}=8.0 Hz, $^{13}\underline{C}H_3$) ^{13}C-NMR ($CDCl_3$) δ, 39.5 (dd, J_{C-C}=34.2 Hz, $-\underline{C}H-^{13}CH_3$), 17.1 (q, $-^{13}\underline{C}H_3$, ^{13}C-enriched).

2-([4-Methyl-^{13}C]5-bromo-4-methylpent-1-yl)-6-methoxymethoxy-2,5,7,8-tetramethylchroman (8). Lithium aluminium hydride (0.05 g, 1.2 mmol) was added with stirring at 0°C to a solution of 7 0.34 g, 0.9 mmol) in dry ether (10 ml). The stirring was continued for 4 hours. The reaction mixture was poured into ice water and then extracted with ether. The extract was washed with brine 10% acetic acid and water, in turn. The ether was dried and evaporated. The residue was chromatographed on a silica gel column and eluted

with a mixture of benzene and ether (8:1) to give an alcohol (0.28 g, 92.2%). Mass (M^+) 351; IR (neat) 3420 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ, 3.41 (m, 2H, -C$\underline{H}_2$OH), 0.90 (dd, 3H, J_{C-H}=126.0 Hz, J_{H-H}=8.0 Hz, -CH-^{13}C$\underline{H}_3$); ^{13}C-NMR ($CDCl_3$) δ, 35.7 (d, J_{C-C}=35.2 Hz, -$\underline{C}$H-^{13}CH$_3$), 16.5 (q, $^{13}\underline{C}H_3$, ^{13}C-enriched). This alcohol obtained was dissolved in dry benzene (12 ml), and carbon tetrabromide (0.33 g, 1.0 mmol) and triphenylphosphine (0.27 g, 1.0 mmol) were added. The mixture was refluxed with stirring for one hour. To this solution hexane was added and cooled to room temperature. The reaction mixture was filtered. The filtrate was evaporated and residue was chromatographed on a silica gel column using the mixture of hexane and benzene (1:1) as an eluent to obtain 8 (0.28 g, 86.3%). Mass (M^+) 414; ^{1}H-NMR ($CDCl_3$) δ, 3.38 (m, 2H, -C$\underline{H}_2$-Br), 1.01 (dd, 3H, J_{C-H}=126.0 Hz, J_{H-H}=8.0 Hz, -^{13}C$\underline{H}_3$); ^{13}C-NMR ($CDCl_3$) δ, 34.9 (dd, J_{C-C}=31.2 Hz, -$\underline{C}$H-^{13}CH$_3$), 18.8 (q, -$^{13}\underline{C}H_3$, ^{13}C-enriched).

3,7-Dimethyl-1-(thiazolin-2-yl)thio-2,6-octadiene (9). To a solution of geraniol (2.0 g, 13.0 mmol) in dry ether (20 ml) was added a solution of PBr_3 (3.5 g, 13.0 mmol) in dry ether (5 ml) with stirring at 0°C for 20 min. The reaction mixture was poured into ice water and extracted with ether. The ether extract was washed with water and dried over sodium sulfate. The ether was evaporated at 40°C to give crude geranyl bromide, which was used in the next step without purification. Sodium hydride (0.3 g, 13.0 mmol) was added to a solution of 2-mercaptothiazoline (1.5 g, 13.0 mmol) in dry THF (8 ml) with stirring at 0°C. To this mixture was added a solution of the crude geranyl bromide in dry THF (10 ml) with stirring for 30 min. The reaction mixture was poured into ice water and extracted with ether. The ether extract was washed with saturated sodium chloride solution and dried over sodium

sulfate. After evaporation of ether the residue was chromatographed on a silica gel column using benzene as an eluent to yield 9 (2.7 g, 81.5%). Mass (M^+) 255; IR (neat) 1570 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ, 4.23 (t, 2H, J=8.0 Hz, N-C$\underline{H}_2$), 3.80 (d, 2H, J=8.0 Hz, S-C$\underline{H}_2$), 3.38 (t, 2H, J=8.0 Hz, S-C$\underline{H}_2$), 1.68 (s, 6H, -C$\underline{H}_3$ x 2), 1.59 (s, 3H, -C$\underline{H}_3$); ^{13}C-NMR ($CDCl_3$) δ, 165.7 (s, N=$\underline{C}$-S_2), 64.3 (t, N-$\underline{C}H_2$), 39.5 (t, S-$\underline{C}H_2$), 35.5 (t, S-$\underline{C}H_2$).

6-Methoxymethoxy-2,5,7,8-tetramethyl-2-([4-methyl-^{13}C](7E,11E)4,8,12-trimethyl-6-(thiazolin-2-yl)thio-7,11-tridecadien-1-yl)chroman (10). To a solution of 9 (0.21 g, 0.8 mmol) in 25 ml of a mixture of THF and HMPA (24:1) with stirring under nitrogen gas in a dry ice-acetone bath was added 2 ml of 1.6 mol n-butyllithium in hexane. After 30 min, 8 (0.84 g, 0.7 mmol) in 5 ml of THF was added dropwise. The stirring was continued for additional one hour at -78°C. The reaction mixture was warmed to 0°C very slowly, poured into ice water, and extracted with ethyl acetate. The organic layer was washed with water, the residue was chromatographed on a silica gel column using a mixture of benzene and ether (10:1) as an eluent to yield 10 (0.40 g, 98.2%). Mass (M^+) 588; IR (neat) 1575 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ, 0.95 (dd, 3H, J_{C-H}=126.0 Hz, J_{H-H}= 7.5 Hz, -^{13}C$\underline{H}_3$), 3.36 (t, 2H, J=8.0 Hz, S-C$\underline{H}_2$), 4.21 (t, 2H, J= 8.0 Hz, N-C$\underline{H}_2$), 4.54 (m, 1H, S-C$\underline{H}_2$), 5.08 (bt, 2H, =C-$\underline{H}$); ^{13}C-NMR ($CDCl_3$) δ, 124.2, 123.5 (each d, C=$\underline{C}$-H), 30.4 (dd, J_{C-C}=36.0 Hz, $\underline{C}$H-^{13}CH_3), 18.7 (q, -^{13}$\underline{C}H_3$, ^{13}C-enriched).

6-Methoxymethoxy-2,5,7,8-tetramethyl-2-([4-methyl-^{13}C](7E,11E)-4,8,12-trimethyl-7,11-tridecadien-1-yl)chroman (11). To a solution of 10 (0.4 g, 0.67 mmol) in THF (5 ml) was added several drops of acetic acid and zinc powder (0.2 g). The mixture was stirred for 2 hours at room temperature, then poured into ice water and extracted with ether. The extract was washed with

saturated sodium chloride solution, saturated sodium bicarbonate solution and water, in turn. The ether was evaporated. The residue was purified by silica gel column chromatography using hexane as an eluent to give 11 (0.293 g, 92.3%). Mass (M^+) 471; ^{1}H-NMR ($CDCl_3$) δ, 0.87 (dd, 3H, J_{C-H}=126.0 Hz, J_{H-H}=8.0 Hz, -$^{13}C\underline{H}_3$) 1.60 (s, 6H, -$C\underline{H}_3$ x 2), 1.70 (s, 3H, -$C\underline{H}_3$); ^{13}C-NMR ($CDCl_3$) δ, 33.1 (dd, J_{C-C}=36.0 Hz, $\underline{C}H$-$^{13}CH_3$), 19.4 (q, -$^{13}\underline{C}H_3$, ^{13}C-enriched)

[4'a-^{13}C]all-rac-α-Tocopherol (1). A mixture of a solution of 11 (0.293 g, 0.6 mmol) in ethyl acetate (10 ml) and platinum oxide (0.1 g) was shaken under 50 atm of hydrogen at room temperature. After the calculated amount of hydrogen had been absorbed, the catalyst was filtered off. The filtrate was concentrated. The residue was purified by silica gel column chromatography using hexane as an eluent to yield the hydrogenated compound, 6-methoxymethoxy-2,5,7,8-tetramethyl-2-([4-methyl-^{13}C]4,8,12-tridec-1-yl)chroman (0.295 g, 99.8%). Mass (M^+) 475; ^{1}H-NMR ($CDCl_3$) δ, 0.86 (dd, 3H, J_{C-H}=126.0 Hz, J_{H-H}=8.0 Hz, -$^{13}C\underline{H}_3$); ^{13}C-NMR ($CDCl_3$) δ, 32.7 (dd, J_{C-C}=35.5 Hz, $\underline{C}H_2$-$^{13}CH_3$), 19.6 (q, -$^{13}\underline{C}H_3$, ^{13}C-enriched). The compound obtained (0.295 g, 0.6 mmol) was dissolved in a methanol solution of hydrogen chloride (10%, 5 ml) and stirred at room temperature for 30 min. The solution was evaporated to give 1 (0.265 g, 99.0%). Mass (M^+) 431; ^{1}H-NMR ($CDCl_3$) δ, 0.87 (dd, 3H, J_{C-H}=126.0 Hz, J_{H-H}=8.0 Hz, -$^{13}C\underline{H}_3$); ^{13}C-NMR ($CDCl_3$) δ, 32.8 (dd, J_{C-C}=35.4 Hz, $\underline{C}H$-$^{13}CH_3$), 19.7 (q, -$^{13}\underline{C}H_3$, ^{13}C-enriched).

References and Notes

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