Synthetic Scheme for the Preparation of ^{13}C-Labeled 2,7-Dimethylocta-2,4,6-triene-1,8-dial, the Central Part of Carotenoids

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Starting from acetic acid and methyl iodide an efficient modular synthetic scheme has been developed for the synthesis of ^{13}C-labeled 2,7-dimethylocta-2,4,6-triene-1,8-dial, which is a suitable building block in the synthesis of carotenoids with tenfold ^{13}C-enrichment in the central part.

Introduction

Fundamental information about the amount of the various carotenoids in the human body and the various organs down to the cellular level is essential when studying the health effects of carotenoids.[1−4] It is possible to use Resonance Raman spectroscopy in a noninvasive way to obtain information about the amount of carotenoids in living human tissue, such as skin and retina.[5] When ^{13}C-labeled carotenoids are added to the food of individuals, Resonance Raman spectroscopy can be used to distinguish between different carotenoids and determine their presence and amount in humans in vivo in a noninvasive way.[6]

Together with two other groups we recently published a method to quantify, without perturbation, the nutritional status of β-carotene at the physiological level in individuals, by addition of ^{13}C-labeled β-carotene to their food.[7] Using mass spectrometry we could monitor the levels of the isotopically ^{13}C-labeled β-carotene and its metabolites.

Extending the use of mass spectrometry and Resonance Raman spectroscopic methods to other carotenoids that are present in our diet is now of utmost importance if we are to study the presence, quantity, bioconversion, and bioavailablity of carotenoids in individuals. Therefore, there is a great need for ^{13}C-labeled carotenoids, and for an efficient synthetic strategy applicable to a range of carotenoids. Recently, we described the synthesis of uniformly ^{13}C-labeled retinal via a modular synthetic strategy.[8] Using a reductive McMurry dimerization, [U-^{13}C] *all-trans*-retinal was converted into [U-^{13}C] *all-trans*-β-carotene.[9]

However, the McMurry dimerization is only applicable for symmetrical carotenoids without functionalities, which makes this method unsuitable for most carotenoids. It is well known that almost all carotenoids can be prepared from 2,7-dimethylocta-2,4,6-triene-1,8-dial (C_{10}-dialdehyde) and the appropriate end groups.[10] The synthesis of ^{13}C-labeled C_{10}-dialdehyde described in earlier work has the severe drawback that it is inconvenient for multifold ^{13}C-labeling, and different schemes are necessary to introduce ^{13}C labels at different positions.[11]

This paper describes an efficient synthetic scheme for ^{13}C-enriched C_{10}-dialdehyde up to the $^{13}C_{10}$ level. Using a modular total organic synthetic strategy we can now specifically introduce ^{13}C labels at any position or combination of positions. By varying the end groups, we can easily obtain a library of multifold ^{13}C-labeled carotenoids.

Results

We describe the scheme in which the sp^2-atoms of C_{10}-dialdehyde are derived from acetic acid and the methyl groups from methyl iodide. Both starting materials are commercially available in 99% ^{13}C-enriched form at reasonable price. Previously, we have described the high-yield conversion of $^{13}C_2$ acetic acid into $^{13}C_4$ diethyl fumarate (**1**)[12] and $^{13}C_2$ acetic acid and ^{13}C methyl iodide into [1-(ethoxycarbonyl)ethyl]triphenylphosphonium iodide (**3**).[13] Scheme 1 indicates that **1** can be reduced with Dibal-H into but-2-ene-1,4-diol (**2**). This diol can be converted in a double MnO_2 oxidation and double Wittig coupling, in a one-pot reaction, to diethyl 2,7-dimethylocta-2,4,6-trien-1,8-oate (**4**) in excellent yield.

Treatment with Dibal-H followed by oxidation with MnO_2 gives the required *all-trans*-2,7-dimethylocta-2,4,6-triene-1,8-dial (**5**).[14] The final product **5** proved to be identical in all analytical aspects to the authentic material.

The reaction was first tried with two equivalents of phosphorane **3**, which gave 61% of the desired product (**4**) and

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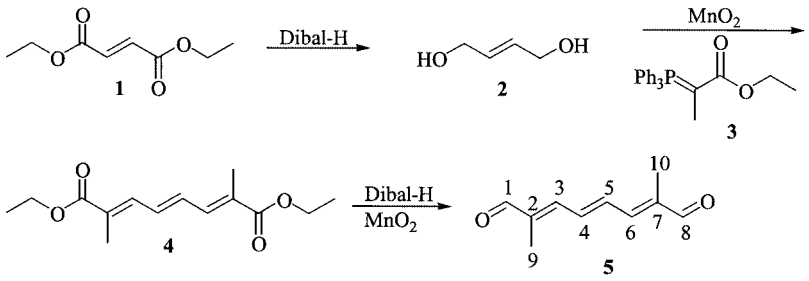


Scheme 1. Synthesis of 2,7-dimethylocta-2,4,6-triene-1,8-dial (**5**)

19% of ethyl 2-methyl-6-oxo-2,4-hexadienoate (**8**; see Scheme 2). After optimization, best results for this reaction were obtained with 2.4 equivalents of the phosphorane. This is a drawback when labeled materials are used, but the good yield [91% based on (**2**)], short route and easy workup and purification make this reaction very useful.

The overall yield for the synthesis, based on methyl iodide and acetic acid, is 61% for ^{13}C-labels at positions 1−2 and 7−10, and 45% for the central positions 3−6.

Discussion

The main feature in this scheme is the double oxidation and double Wittig coupling of but-2-ene-1,4-diol (**2**) to give the C_{10}-diester **4** in a one-pot reaction.[15] But-2-ene-1,4-diol (**2**) is oxidized with MnO_2, in the presence of phosphorane **3**. When following the reaction closely by TLC, we found that ethyl 2-methyl-6-oxo-2,4-hexadienoate (**8**) is formed as an intermediate, which reacts with the phosphorane **3** in a Wittig coupling to give the diester **4**.

Probably the reactions proceed by oxidation of one hydroxy group of **2** to an aldehyde, which reacts with the phosphorane **3** to give ethyl 6-hydroxy-2-methyl-2,4-hexadienoate. Subsequently, the second hydroxy group is oxidized to an aldehyde, which reacts in a second Wittig coupling with **3** to give the desired product **4**.

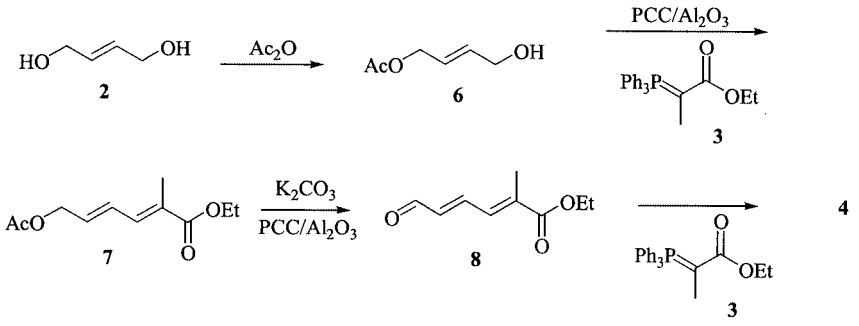
Before we developed the method described above, a protection and deprotection scheme was developed to synthesize C_{10}-diester **4**, as shown in Scheme 2.

The diol (**2**) reacted with one equivalent of acetic anhydride to give a mixture consisting of 50% monoacetate, 25% unchanged diol, and 25% diacetate.[16] These products can easily be separated by washing the organic solution containing the mixture with water, which removes the diol. The diester can be separated by silica-gel column chromatography. The diester is reconverted into the diol with KOH, and, together with the unchanged diol from the acetylation reaction, acetylated again as described above. The monoacetate **6** is oxidized with pyridinium chlorochromate on basic alumina (PCC/Al) to give 4-acetoxycrotonaldehyde, which is allowed to react under Wittig conditions with **3** to give ethyl 6-acetoxy-2-methylhexa-2,4-dienoate (**7**).

Selective conversion of the 6-acetoxy function in **7** to the alcohol without saponification of the ethyl ester is effected by hydrolysis with K_2CO_3 in ethanol to give ethyl 6-hydroxy-2-methyl-2,4-hexadienoate. Subsequent oxidation of the alcohol gives ethyl 2-methyl-6-oxo-2,4-hexadienoate (**8**), which is coupled with **3** via another Wittig reaction to give diethyl 2,7-dimethylocta-2,4,6-trien-1,8-oate (**4**). This is converted into the C_{10}-dialdehyde **5** as described above.

In this scheme both Wittig couplings are carried out with one equivalent of phosphorane, which makes this scheme useful for the efficient introduction of ^{13}C-labels at positions 1, 2, and 9 of the final C_{10}-dialdehyde **5**.

To reduce the number of steps we tried to convert **3** into ethyl pyruvate according to a literature procedure,[17] and couple this to either 1,4-bis(diethoxyphosphoryl)-2-butene or 1,4-bis(triphenylphosphonium)-2-butene dibromide, which are both made in two steps from but-2-ene-1,4-diol (**2**). Both the Wittig coupling and the phosphonate coupling of ethyl pyruvate only gave degradation products and start-



Scheme 2. Alternative synthesis of diethyl 2,7-dimethylocta-2,4,6-trien-1,8-oate (**4**)

ing material, even though several different conditions were tried.

Conclusion

The synthetic scheme described in this paper allows the quick and easy preparation of isotopically ^{13}C-labeled 2,7-dimethylocta-2,4,6-triene-1,8-dial. The key step in this synthesis is the double MnO_2 oxidation of but-2-ene-1,4-diol (**2**) in the presence of the C_3-phosphorane **3**, to give the C_{10}-diester **4** in a one-pot reaction. All carotenoids can now be prepared with selective or uniform ^{13}C-enrichment in the central part via this synthetic scheme for the preparation of 2,7-dimethylocta-2,4,6-triene-1,8-dial.

Experimental Section

General Remarks: Dry reactions were carried out in a dry nitrogen atmosphere, and reaction vessels were flame-dried prior to use. Dry solvents were dried by distillation [low-boiling petroleum ether (PE) 40−60 °C from P_2O_5, toluene and dichloromethane (DCM) from CaH_2] and kept dry by storing over sodium wire. Solutions of NaCl refer to saturated solutions of salt in water. SiO_2/H_2O is a homogeneous mixture of 400 g of SiO_2 and 120 mL of water.

Reactions were monitored by thin-layer chromatography (TLC), on Merck F_{254} silica gel 60 aluminum sheets, 0.2 mm; spots were visualized with UV light (254 nm) or treated with an oxidizing spray ($KMnO_4$ (2 g) and K_2CO_3 (4 g) in water (100 mL). Column chromatography was performed on Merck silica gel 60 (0.040−0.063 nm, 230−400 mesh).

^1H NMR and ^{13}C NMR spectra were recorded on a Bruker WM-300 apparatus with tetramethylsilane (TMS; δ = 0.00 ppm) or $CDCl_3/CH_3OD$ (δ = 77.00/49.00 ppm) as internal standard.

All chemicals were purchased from Aldrich, Fluka or Acros Chimica.

But-2-ene-1,4-diol (2): Under a nitrogen atmosphere, a solution of diethyl fumarate (**1**; 2.0 g, 11.6 mmol) in dry PE (bp, 40−60 °C) was cooled to −60 °C, and diisobutylaluminum hydride (Dibal-H; 4.4 equiv., 51 mL 1 M solution in hexanes, 51 mmol) was added with a syringe. The solution was allowed to warm to −20 °C in 1 hour, after which TLC (diethyl ether) showed complete conversion of the starting material. At −20 °C, SiO_2/H_2O (83.9 g) was added, and the mixture was stirred for 1 hour at 0 °C. K_2CO_3 and $MgSO_4$ were added, and the solids were removed by filtration and washed thoroughly with diethyl ether. The solution was concentrated in vacuo, to give but-2-ene-1,4-diol (**2**; 0.82 g, 9.3 mmol, 80%). ^1H NMR (300 MHz, CD_3OD): δ = 4.11 (d, *J* = 3.8 Hz, 4 H, 1-H/4-H), 4.91 (s, 2 H, OH), 5.87 (t, *J* = 3.8 Hz, 2 H, 2-H/3-H) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 63.0 (C-1/C-4), 131.1 (C-2/C-3) ppm.

4-Acetoxy-2-buten-1-ol (6): Acetic anhydride (2.55 g, 25 mmol) was slowly added to a solution of but-2-ene-1,4-diol (**2**; 2.20 g, 25 mmol) and pyridine (2.37 g, 30 mmol) in dry toluene. The mixture was stirred at room temperature for 1 hour; after which time TLC (diethyl ether) showed a mixture of starting compound, monoacetate and diacetate. H_2O was added, and the mixture was extracted three times with diethyl ether. The combined organic layers were dried with $MgSO_4$ and concentrated in vacuo. After purification on a silica column (50% diethyl ether/PE) the monoacetate (**6**; 1.36 g, 10.5 mmol, 42%) was collected. Unchanged diol was collected by extracting the water layer thoroughly with dichloromethane; the organic layers were combined, dried with $MgSO_4$ and the solvents were removed in vacuo. Diacetate, collected after column chromatography, was treated with KOH to obtain another batch of diol. ^1H NMR (300 MHz, $CDCl_3$): δ = 2.06 (s, 3 H, CH_3 acetoxy), 4.24 (d, *J* = 6.2 Hz, 2 H, 4-H), 4.57 (br. s, 1 H, OH), 4.66 (d, *J* = 6.5 Hz, 2 H, 1-H), 5.62 (dt, *J* = 6.2 Hz/12.3 Hz, 1 H, 3-H), 5.80 (dt, *J* = 6.5, 12.3 Hz, 1 H, 2-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 20.7 (CH_3 acetoxy), 57.9 (C-4), 59.9 (C-1), 125.0 (C-2), 133.1 (C-3), 171.1 (C=O acetoxy) ppm.

Ethyl 6-Acetoxy-2-methylhexa-2,4-dienoate (7): At 0 °C, 4-acetoxy-2-buten-1-ol (**6**; 1.36 g, 10.5 mmol) was added to a suspension of pyridinium chlorochromate (14.8 g) on basic alumina (PCC/Al 20 wt%) (13.7 mmol) in DCM. The mixture was stirred at room temp. until TLC analysis (diethyl ether) showed complete conversion of the starting compound. PCC/Al was removed by flash chromatography, the solids were washed with diethyl ether and the organic solvents were removed in vacuo, to give 4-acetoxy-crotonaldehyde (1.0 g, 7.8 mmol, 74%). ^1H NMR (300 MHz, $CDCl_3$): δ = 2.14 (s, 3 H, CH_3 acetoxy), 4.87 (d, *J* = 4.4 Hz, 2 H, 4-H), 6.28 (dd, *J* = 15.9 Hz/7.9 Hz, 1 H, 2-H), 6.88 (dt, *J* = 15.9 Hz/4.3 Hz, 1 H, 3-H), 9.59 (d, *J* = 7.9 Hz, 1 H, 1-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 20.2 (CH_3 acetoxy), 62.0 (C-4), 131.6 (C-2), 149.5 (C-3), 169.8 (C=O acetoxy), 192.5 (C-1) ppm.

4-Acetoxy-crotonaldehyde (1.0 g, 7.8 mmol) was dissolved in 5 mL DCM and C_3-phosphorane **3** (2.83 g, 7.8 mmol) in DCM was added. The solution was stirred for 30 min at room temp. and followed by TLC analysis (15% diethyl ether/PE). When TLC showed complete conversion of the aldehyde, the solvent was removed in vacuo. The product was purified on a flash-column (10% diethyl ether/PE), to yield (ethyl 6-acetoxy-2-methylhexa-2,4-dienoate (**7**; 1.66 g, 7.8 mmol, 100%). ^1H NMR (300 MHz, $CDCl_3$): δ = 1.31 (t, *J* = 7.1 Hz, 3 H, OCH_2CH_3), 1.96 (s, 3 H, 2-CH_3), 2.10 (s, 3 H, CH_3 acetoxy), 4.21 (q, *J* = 7.1 Hz, 2 H, OCH_2CH_3), 4.69 (d, *J* = 6.0 Hz, 2 H, 6-H), 6.09 (dt, *J* = 6.0 Hz/15.2 Hz, 1 H, 5-H), 6.59 (dd, *J* = 15.2 Hz/11.4, 1 H, 4-H), 7.17 (d, *J* = 11.4 Hz, 1 H, 3-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.2 (2-CH_3), 13.8 (OCH_2CH_3), 20.3 (CH_3 acetoxy), 60.1 (OCH_2CH_3), 63.7 (C-6), 128.1 (C-2), 131.5 (C-5), 133.2 (C-3), 136.0 (C-4), 167.4 (C-1), 169.8 (C=O acetoxy) ppm.

Ethyl 6-Oxo-2-methyl-2,4-hexadienoate (8): K_2CO_3 (2 g) was added to a solution of (**7**; 1.66 g, 7.8 mmol) in ethanol. After 2 hours at room temp., TLC (diethyl ether) showed complete conversion of the starting material. The solution was concentrated to a volume of 20 mL, and NaCl solution (50 mL, sat.) was added. The water layer was extracted three times with diethyl ether, and the combined organic layers were dried with $MgSO_4$, which was removed by filtration. After evaporation of the solvent, the desired ethyl 6-hydroxy-2-methyl-2,4-hexadienoate (1.28 g, 7.5 mmol, 96%) was obtained. No further purification was necessary. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.31 (t, *J* = 7.1 Hz, 3 H, OCH_2CH_3), 1.94 (s, 3 H, 2-CH_3), 4.21 (q, *J* = 7.1 Hz, 2 H, OCH_2CH_3), 4.28 (d, *J* = 5.1 Hz, 2 H, 6-H), 6.17 (dt, *J* = 15.2 Hz/5.1 Hz, 1 H, 5-H), 6.59 (dd, *J* = 15.2 Hz/11.4 Hz, 1 H, 4-H), 7.18 (d, *J* = 11.4 Hz, 1 H, 3-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.4 (2-CH_3), 14.1 (OCH_2CH_3), 60.7 (OCH_2CH_3), 62.7 (C-6), 125.2 (C-4), 127.1 (C-2), 137.3 (C-5), 139.7 (C-3), 168.5 (C-1) ppm.

Ethyl 6-hydroxy-2-methylhexa-2,4-dienoate (1.28 g, 7.5 mmol) was added at 0 °C to a solution of PCC/Al (20 wt%, 10.5 g , 9.8 mmol) in dry DCM. The mixture was stirred for 16 hours, until TLC analysis (50% diethyl ether/PE) showed complete conversion of the starting material. PCC was removed by filtration through silica and washed with diethyl ether. The organic solvents were removed in vacuo to yield ethyl 6-oxo-2-methyl-2,4-hexadienoate (**8**; 1.0 g, 6.0 mmol, 80%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.34 (t, *J* = 7.2 Hz, 3 H, OCH_2CH_3), 2.13 (s, 3 H, 2-CH_3), 4.27 (q, *J* = 7.2 Hz, 2 H, OCH_2CH_3), 6.39 (dd, *J* = 7.9 Hz/14.4 Hz, 1 H, 5-H), 7.33 (d, *J* = 12.0 Hz, 1 H, H3), 7.48 (dd, *J* = 12.0 Hz/14.4 Hz, 1 H, 4-H), 9.61 (d, *J* = 7.9 Hz, 1 H, 6-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.3 (2-CH_3), 14.0 (OCH_2CH_3), 61.1 (OCH_2CH_3), 133.8 (C-2), 135.8 (C-5), 136.9 (C-3), 144.8 (C-4), 166.8 (C-1), 192.9 (C-6) ppm.

Diethyl 2,7-Dimethylocta-2,4,6-triene-1,8-oate (4). Method A: But-2-ene-1,4-diol (**2**; 0.56 g, 6.3 mmol) in DCM (10 mL) was added to a suspension of activated MnO_2 (10 g) in DCM (25 mL)at 0 °C. C_3-phosphorane **3** (5.5 g, 15.2 mmol) in DCM (15 mL)was added and the mixture was followed by TLC (50% diethyl ether/PE). The mixture was stirred overnight and MnO_2 was removed by filtration through Hyflo™. The filtrate was concentrated in vacuo and triphenylphosphonium oxide was removed by flash chromatography over silica gel (50% diethyl ether/PE). After a second purification on a short silica-gel column, diethyl 2,7-dimethylocta-2,4,6-triene-1,8-oate (**4**; 1.5 g, 5.8 mmol, 92%) was obtained.

Method B: Ethyl 2-methyl-6-oxo-2,4-hexadienoate (**8**; 1.0 g, 6.0 mmol) was dissolved in DCM (10 mL), and C_3-phosphorane **3** (2.2 g, 6.0 mmol) in DCM was added. The reaction was stirred at room temp. for 5 hours, until TLC (diethyl ether) showed that the reaction was complete. The solvent was evaporated in vacuo and the triphenylphosphane oxide formed was removed on a short flash column (50% diethyl ether/PE) to yield the C_{10}-diester (**4**; 1.5 g, 5.9 mmol, 99%) as a pure, white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.32 (t, *J* = 7.1 Hz, 6 H, 2 × OCH_2CH_3), 2.02 (s, 6 H, 2-CH_3/7-CH_3), 4.23 (q, *J* = 7.1 Hz, 4 H, 2 × OCH_2CH_3), 6.81 (dd, *J* = 7.8 Hz/3.1 Hz, 2 H, 3-H/6-H), 7.29 (m, 2 H, 4-H/5-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.8 (2-CH_3/7-CH_3), 14.1 (2 × OCH_2CH_3), 60.6 (2 × OCH_2CH_3), 129.9 (C-2/C-7), 133.2 (C-4/C-5), 136.8 (C-3/C-6), 167.5 (C-1/C-8) ppm.

2,7-Dimethylocta-2,4,6-triene-1,8-dial (5): A solution of **4** (1.5 g, 5.8 mmol) in dry PE was cooled to −80 °C, and 4.4 equiv. Dibal-H (25.9 mL of a 1 M solution in hexanes, 25.9 mmol) was added with a syringe. The yellow-orange mixture was allowed to warm to −20 °C in 1 hour. When TLC showed complete conversion of the diester, SiO_2/H_2O (45.4 g) was added, and the mixture was stirred at 0 °C for 1 hour. K_2CO_3(s) and $MgSO_4$(s) were added, and the solids were removed by filtration and washed with DCM. The product was concentrated in vacuo, and the resulting diol was oxidized, without further purification.

2,7-Dimethylocta-2,4,6-triene-1,8-diol was dissolved in acetone (10 mL), and MnO_2 (10 g) was added at 0 °C. The reaction was stirred overnight, and followed by TLC (diethyl ether). When the reaction was complete, the Mn salts were removed by filtration through Hyflo™, washed with DCM and the product was concentrated in vacuo. After purification on a silica column, pure *all-trans*-C_{10}-dialdehyde **5** (0.77 g, 4.7 mmol, 80%) was obtained. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.95 (s, 6 H, 2-CH_3/7-CH_3), 7.06 (m, 4 H, 3-H/4-H/5-H/6-H), 9.55 (s, 2 H, 1-H/8-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 9.7 (2-CH_3/7-CH_3), 134.2 (C-4/C-5), 141.0 (C-2/C-7), 146.0 (C-3/C-6), 194.3 (C-1/C-8) ppm.

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[15] When we submitted the first version of this paper, the referee suggested converting but-2-ene-1,4-diol in one step to the corresponding dialdehyde. We had tried this before with PCC/Al_2O_3, but the referee suggested using the milder oxidants TPAP/NMO or Dess−Martin. These oxidations did not give the dialdehyde, but we found that MnO_2 oxidation in the presence of the phosphorane circumvents the problem and results in double oxidation and double Wittig coupling in a one-pot reaction, which is a great improvement on the earlier work.

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