SYNTHESIS OF METHYL *cis*-9-OCTADECENOATE-14,14,15,15,17,18-d_6

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Received July 5, 1977
Revised October 2, 1977

SUMMARY

A six-step synthesis is described which gives a 45-55% overall yield of methyl *cis*-9-octadecenoate-d_6. All reactions except the last give over 90% yields. Incorporation of deuterium is simple, efficient, and produces isotope purities of over 84%. The 2-alkoxytetrahydropyran is a key intermediate in this reaction sequence. The discovery that this intermediate can be directly converted to the alkyl iodide in >90% yields resulted in a significant increase in overall yields and simplified the total synthesis. The general synthetic scheme can be used for preparation of most positional monoenoic fatty acid isomers by varying the chain length of the deutero alkyl iodide which is coupled to an appropriate aldehydic ester using the Wittig reaction.

Key words: deuterium, catalytic reduction, tetrahydropyranyl, methyl 9-octadecenoate.

INTRODUCTION

The preparation of large (100 g) quantities of methyl *cis*-9-octadecenoate-d_6 (oleate-d_6) was required for use as a standard in a series of triple labelled human metabolism studies (1). The synthesis of a similar oleate-d_6 has been described by DeJarlais and Emken (2). Their method, however, requires the aldehydic ester to be refluxed several times with D_2O/pyridine (3) in order to incorporate two deuteriums into the aldehydic ester for final coupling by the Wittig reaction to the appropriate iodoalkane-d_4. The exchange of two deuterium atoms for hydrogen by this method inefficiently utilizes D_2O, is very tedious, and results in low yields (68%) of the deuterated aldehydic ester. The synthesis

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0362-4803/78/0015-0097$01.00

described in this paper efficiently incorporates six deuteriums into the iodoalkane fragment by catalytic deuteration of an "ene-yne" intermediate prepared by coupling of an acetylene and an olefin. Overall yields of 45-55% methyl *cis*-9-octadecenoate-d_6 are readily achieved by the synthetic sequence shown in Figure 1, while both the time and expense of preparation are minimized.

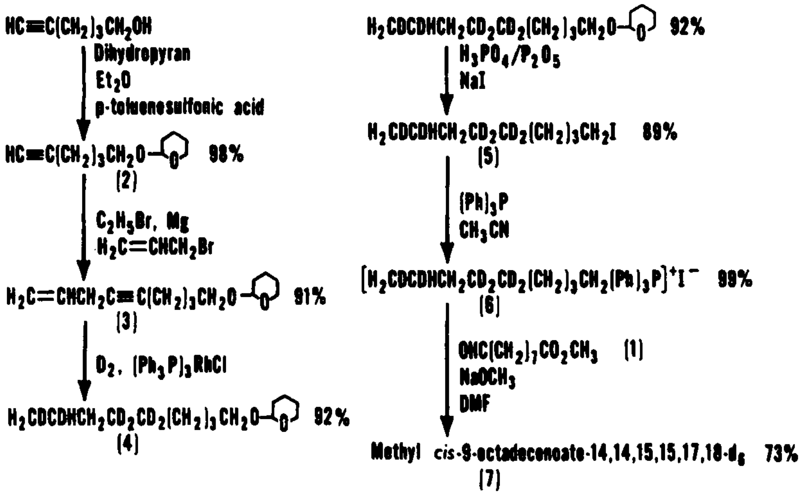

Figure 1. Synthetic Scheme for Methyl *cis*-9-Octadecenoate-14,14,15,15,17,18-d_6.

EXPERIMENTAL

Instruments--Isotopic purities were determined on a Nuclide 12-906 mass spectrometer (MS) with 70 eV impact ionization inlet at 150°C for tetrahydropyranyl ether and 200°C for the methyl *cis*-9-octadecenoate-d_6 (4). *cis/trans* Ratios in methyl 9-octadecenoate-14,14,15,15,17,18-d_6 were determined on a Packard Model 7400 gas-liquid chromatograph (GC) equipped with a 4 mm x 20 ft, 10% Silar 10C glass column. Runs were made at 205°C using helium as the carrier gas and a flame-ionization detector (1). Purity and completeness of reactions were determined by analyzing the products on an Aerograph 600-B GC furnished with a flame-ionization detector and N_2 as the carrier gas. A 12 ft x 1/8 in. stainless-steel column packed with 3% EGSS-X on Gas Chrom Q, 100/120 mesh was employed. Infrared spectra (IR) were obtained with a Perkin-Elmer Model 621 using 1 mm KBr cells and CS_2 as the solvent.

Reagents--The following compounds and reagents were used as received: 5-Hexyn-1-ol (Farchan), Dihydropyran (Quaker Oats Co.), Allyl Bromide and Bromoethane (98%)

(Aldrich Chemical Co.), *Tris*(triphenylphosphine)chlororhodium (Strem Chemicals), Methyl Oleate (Emery Industries, Inc.), Molecular Sieve 3A (Alfa Products), Silica Gel 60-200 mesh (Baker), Sodium Methoxide (Harshaw Chemical Co).

Methyl 8-Formyloctanoate *1*: This compound was prepared by ozonolysis of methyl oleate and subsequent zinc/acetic acid reduction as described previously (5).

2-(5-hexynyloxy) tetrahydropyran *2* (6): The tetrahydropyranyl ether of 5-hexyn-1-ol was prepared by dissolving 5-hexyn-1-ol (50 g; 0.51 mol), dihydropyran (63 g; 0.75 mol), and *p*-toluenesulfonic acid (2.2 g) in 500 ml diethyl ether. A water bath was used to maintain the temperature at 25°C. The mixture was allowed to react for 18 hr and then was analyzed for completeness by GC (127°C; 50 ml/min N_2). Sodium carbonate (3 g) and 200 ml of saturated sodium bicarbonate were then added to the reaction. After stirring for 1/2 hr, the mixture was washed 4 times with 250-ml portions of H_2O and the Et_2O layer was dried over sodium sulfate. The Na_2SO_4 was removed by vacuum filtration and the Et_2O was evaporated with a rotary evaporator. Distillation of the residue through a 3 in. Vigreaux column (b.p. 76-80°C/0.35 mm Hg) resulted in 92.8 g of *2* (98% pure by GC) and a yield of 98%.

2-(non-8-en-5-ynyloxy)tetrahydropyran *3*: Magnesium metal turnings (18.0 g; 0.75 mol) and 155 ml tetrahydrofuran were combined in a 2-liter round-bottomed flask equipped with a thermometer, N_2 inlet, addition funnel, and electric stirrer. Bromoethane (120.0 g; 1.10 mol) in 85 ml THF was added dropwise while the temperature was maintained below 20°C. Compound *2* (94.0 g; 0.51 mol) in 30 ml THF was added over 45 min while the temperature was kept at 10°C. The mixture was then heated to 42°C and excess bromoethane was removed by vigorously flushing with N_2. The reaction mixture was cooled to 18°C, 0.9 g of cuprous chloride was added, and the mixture was further cooled to 10°C after 15 min. Allyl bromide (90.0 g; 0.75 mol) was added dropwise over a 2-hr period and the reaction was stirred at room temperature overnight. Water (1.1 liter) was then added and the mixture was acidified with 1 N H_2SO_4 until pH ~2. After two extractions with 350-ml portions of Et_2O, the Et_2O extracts were combined, washed with 300 ml saturated ammonium chloride, 300 ml saturated sodium bicarbonate, 2 x 350 ml H_2O and placed over Na_2SO_4 to dry. Filtration and evaporation of the Et_2O resulted in 119.6 g of dark green-gold oil. Since a previous distillation had resulted in decomposition (see Results and Discussion), the product was dissolved in 1 liter of petroleum ether (PE) and vacuum filtered through a 4 in. Buchner funnel containing 1/2 in. celite and 3/4 in. silica gel. Removal of the PE by rotary evaporator resulted in 110.8 g (93% pure by GC; 91% yield) of *3*.

2-(nonyloxy-5,5,6,6,8,9-d_6)tetrahydropyran *4* (2,7): *Tris*(triphenylphosphine)chlororhodium (8.0 g) was added to 1 liter of pre-dried (calcium sulfate) and degassed (argon) benzene, and the mixture was evacuated and flushed several times with D_2. Compound *3* (107.0 g; 0.43 mol) was added, the reaction was flushed with D_2 and then magnetically stirred. Deuterium uptake ceased after 10.5 hr. The benzene was evaporated by rotary evaporator and the residue was chromatographed on a 45 x 600 mm column containing 200 g of silica gel. The product was eluted with hexane, and the hexane was removed by rotary evaporator. This gave 94.5 g of *4* (97+% pure by GC; 92% yield).

1-iodononane-5,5,6,6,8,9-d_6 *5*: The iodide *5* was synthesized directly from the tetrahydropyranyl ether *4*. Phosphorous pentoxide (42.7 g; 0.30 mol), 85% phosphoric acid (139.2 g; 1.21 mol), and sodium iodide (195.7 g; 1.31 mol) were mixed in a 500-ml round-bottomed flask under N_2. The spontaneously heating mixture was cooled to 50°C and *4* (50.0 g; 0.207 mol) was added dropwise. After heating at 112-113°C for 18 hr (overnight), the viscous purplish-black solution was cooled and dissolved in ∿2 liters Et_2O. The Et_2O solution was washed with 400 ml of saturated sodium thiosulfate, 3 x 400 ml H_2O, and placed over Na_2SO_4 to dry. Removal of the Na_2SO_4 by filtration and the Et_2O by rotary evaporator yielded 79.2 g of tar which was distilled through a short-path column (52-53°C/ 0.07 mm Hg) to give 48.9 g of *5* (97+% pure by GC; 91% yield). Use of a slightly smaller molar excess of KI produced yields of ∿85%.

1-nonyl-5,5,6,6,8,9-d_6-triphenylphosphonium iodide *6*: Triphenylphosphine (53.9 g; 0.21 mol), acetonitrile (350 ml) and *5* (48.1 g; 0.19 mol) were combined and refluxed for 18 hr under a N_2 atmosphere. The solution was then cooled, the acetonitrile was removed by rotary evaporator, and the viscous residue was triturated with Et_2O until crystallization occurred. The crystals were separated by filtration and dried in a vacuum dessicator. This resulted in 94.4 g (99% yield) of *6* having a melting point of 68-72°C.

Methyl *cis*-9-octadecenoate-14,14,15,15,17,18-d_6 *7*: This compound was prepared by the Wittig coupling of the aldehydic ester *1* and the triphenylphosphonium nonyl-5,5,6,6,8,9-d_6 *6*. Compound *6* (96.4 g; 0.185 mol) was dissolved in 190 ml of N,N dimethylformamide (predried over Molecular Sieve 3A) and added to a 1-liter round-bottomed flask containing sodium methoxide (10.0 g; 0.185 mol) and equipped with a thermometer N_2 ebullator, electric stirrer, and addition funnel. The temperature was maintained at 15°C. The deep orange solution which formed was stirred at room temperature for 45 min, then cooled to 16°C and *1* (95% pure by GC; 39.3 g; 0.201 mol) in 100 ml DMF was added dropwise. The addition of *1* was stopped when an aldehydic ester peak appeared during analysis of the reaction mixture by GC. The reaction mixture was transferred to 1 liter of H_2O and extracted 3 times with 170-ml portions of petroleum ether (PE). A white, flocculant precipitate formed upon the addition of water, which was removed by filtration. The PE extracts were combined and washed with 2 x 400 ml of H_2O, then dried over Na_2SO_4. The Na_2SO_4 was removed by filtration and the PE by rotary evaporation. The residue was then distilled through a 3 in. Vigreux column (136-138°C/0.3 mm Hg) which resulted in 40.8 g (73% yield) of *7*. GC analysis indicated the product to be 94% *cis*/6% *trans*. The isomers were later separated by silver-resin chromatography using the procedure of Emken et al. (8).

RESULTS AND DISCUSSION

Several aspects of this synthesis of methyl 9-octadecenoate-d_6 require comment. The distillation of compound *3* through a 3 in. Vigreaux (110°C/1.2 mm Hg) was accompanied by vigorous gas evolution. GC analysis of the distillate indicated two components which were deuterated and separated by column chromatography using silica gel and distilled hexane. IR showed the primary component to be the alcohol (>80%). Due to this problem, purification was accomplished by silica gel/Celite instead.

The use of the tetrahydropyranyl derivatives (THP's) has several advantages. They are easily prepared in high yields, are excellent protecting groups for the hydroxide during the Grignard coupling, and do not react nor exchange hydrogen during deuteration. Unlike iodides, mesylates and methyl ethers, the THP's could be quickly analyzed by MS to determine isotopic purity. These results agreed quite well with the isotopic purity of the final methyl 9-octadecenoate-d_6 (see Table I).

TABLE I: Mass Spectral Analysis for Deuterium

	Deuterium Distribution (%)	
Number of Deuterium Atoms/Molecule	2-(nonyloxy-5,5,6,6,8,9-d_6) tetrahydropyran *4*	Methyl 9-octadecenoate-14,14,15,15,17,18-d_6 *7*
4	0.6	3.2
5	7.2	7.5
6	82.2	84.4
7	8.8	4.3
8	1.0	0.6
9	0.2	0.0
Average Deuterium Atoms/Molecule:	6.03	5.92
Isotopic Purity (%):	100.5	98.7

The THP's can be directly converted to the iodide (yield ∿90%). The procedure is easier and offers higher yields than hydrolysis and subsequent conversion to the iodide by reaction with $(PhO)_3P$ and CH_3I (9), I_2 and P_4 (10), or *via* the chloride intermediate.

The final Wittig coupling affords good yields if certain precautions are taken. The sodium methoxide had to be very dry. Lower yields resulted if $NaOCH_3$ prepared by the Na/CH_3OH method was used, perhaps due to incomplete removal of the adsorbed methanol. The aldehydic ester *1* forms trimers during storage. The trimers are unreactive toward the base-catalyzed Wittig and need to be removed by distillation or preparation of the acetal and hydrolysis just before use (11). These precautions increased the yield of the Wittig reaction from 45 to 75%.

The synthesis described in this paper could be extended to the preparation of other deuterium labelled monoenoic fatty acids by simply varying the number of carbon atoms in the compounds used in the final Wittig reaction. In this manner, double bond position as well as the chain length of the fatty acid could be varied.

Incorporation of deuteriums two or more carbons away from the double bond prevents scrambling of the deuterium label during the final Wittig coupling step (12). Biological isotope effects are also minimized by this procedure. In less than 3 weeks, large batches of distilled methyl *cis*-9-octadecenoate (>50% yield) can be prepared by this synthetic scheme.

ACKNOWLEDGMENTS

W. K. Rohwedder for MS analyses.

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