PREPARATION OF METHYL 8c,11c-EICOSADIENOATE-17,17,18,18-d_4, METHYL 8c,11c,14c-EICOSATRIENOATE-17,17,18,18-d_4 AND METHYL 5c,8c,11c-EICOSATRIENOATE-17,17,18,18-d_4

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SUMMARY

Methyl 8c,11c-eicosadienoate, methyl 5c,8c,11c-eicosatrienoate and methyl 8c,11c,14c-eicosatrienoate (all 17,17,18,18-d_4) were prepared in multi-gram quantities for human metabolism studies. The deuterium atoms were incorporated by reduction of the appropriate acetylenic tetrahydropyranyl (THP) ethers with $(Ph_3P)_3RhCl$ and deuterium gas. The THP ethers were converted to the iodides with $H_3PO_4/P_2O_5/KI$. The deuterium-labelled fatty acid methyl esters were then synthesized from the alkyl iodide-d_4 via a series of acetylenic coupling reactions with the last double bond generated by the Wittig reaction. Acetylenic bonds were reduced to olefinic bonds by use of Lindlar's catalyst. Both 8,11-20:2 and 5,8,11-20:3 were synthesized from the same 8-carbon-d_4 starting fragment. The 8,11-20:2 isomer was prepared in a 10-step synthesis in 19% overall yield, the 5,8,11-20:3 in 12 steps (18% overall yield) and the 8,11,14-20:3 isomer in 12 steps with an overall yield of 10%. Trans isomers were removed by silver resin chromatography. The 8,11-20:2, 5,8,11-20:3 and 8,11,14-20:3 compounds had isotopic purities of 91-97%.

Key Words: Deuterium; fatty acids; Wittig; eicosadienoate; eicosatrienoate

INTRODUCTION

Methyl 5c,8c,11c-eicosatrienoate-17,17,18,18-d_4 (Me 5c,8c,11c-20:3-d_4), methyl 8c,11c-eicosadienoate-17,17,18,18-d_4 (Me 8c,11c-20:2-d_4) and methyl 8c,11c,14c-eicosatrienoate-17,17,18,18-d_4 (Me 8c,11c,14c-20:3-d_4) were synthesized as part of a study to investigate fatty acid metabolism in humans. Both 8,11-20:2 and 5,8,11-20:3 are produced in rats fed a fat-free diet, and the latter has been found to be selectively incorporated into rat liver lipids (1). The 5,8,11-20:3 fatty acid is produced at times of and used as an index for essential fatty acid deficiency (2,3). The 8,11,14-20:3 fatty acid is a

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recognized precursor to arachidonic acid (5,8,11,14-20:4) (4) and prostaglandin E_1 (5).

Small quantities (non-deuterated) of both the 5,8,11-20:3 (4,6,7) and 8,11,14-20:3 (5,8,9) have been prepared. Synthesis of the 8,11-20:2 fatty acid has not been reported.

To facilitate our studies of the metabolism of these fatty acids in humans, 5 to 6 g of each of the deuterated fatty acids was required per subject. Our objectives were to develop synthetic procedures for preparation of multi-gram quantities of these deuterated fats.

EXPERIMENTAL

Instruments. Isotopic purity and deuterium distribution were determined on a Finnigan 4500 Mass Spectrometer interfaced with a gas chromatograph containing a 30 m Supelcowax 10 capillary column (0.32 mm x .5 μ film thickness) (GC/MS) and programmed from 165 to 265°C at 5°C/min. Helium was utilized as the carrier gas. Samples were analyzed by chemical ionization (conditions: isobutane reagent gas, 70 eV). Cis and trans isomers were analyzed on a Packard Model 428 GC equipped with a 100 m SP2560 capillary column (He carrier gas). Purities of the intermediates were determined with a 10" x 3/8" packed 3% EGSS-X column. The preparation and utilization of the silver resin column for the separation of cis and trans isomers has been described elsewhere (10,11). The C18 reverse phase (C18 RP) chromatography column employed was 50 x 250 mm (5 μ particle size) and was purchased from Serva Feinbiochemica, Heidelberg, Germany. ^{13}C-NMR spectra were obtained with a Bruker WH300WB pulsed Fourier transform spectrometer operating at 75.5 MHz.

Reagents. The following reagents were used as received: Bromoethane, 2-propyn-1-ol, Lindlar catalyst, triphenylphosphine, δ-valerolactone, cyclooctene, dihydropyran, ethylmagnesium bromide (2.0 M in hexane), n-butyl lithium (2.6 M in hexane) (all Aldrich Chemical Co., Milwaukee, WI), lithium metal (Alfa Products, Danvers, MA), 3-butyn-1-ol, 5-hexyn-1-ol (Farchan Laboratories, Gainesville, FL), tris-triphenylphosphine chlororhodium (I) (Strem Chemicals, Newburyport, MA) and deuterium gas, 98% (Matheson Gas Products, Secaucus, NJ).

The normalities of the ethylmagnesium bromide and the n-butyl-lithium were determined before use (12). Other reagents were synthesized according to Figures 1 and 2 as follows:

methyl 4-formylbutanoate, 1. Compound 1 (bp 64-69°C/5.0 mm Hg) was prepared by the hydrolysis of 0.1 mol of δ-valerolactone and subsequent oxidation by pyridinium chlorochromate (13) in 40% overall yield.

methyl 7-formylheptanoate, 2. Compound 2 (bp 86-90°C/0.4 mm Hg) was prepared in 70% yield by the ozonolysis of cyclooctene (14) and esterification of the resultant aldehydic acid (bp 140-146/0.20 mm Hg) by methanol/HCl (15).

Methyl 8c,11c-eicosadienoate-17,17,18,18-d_4 (See Figure 1)

5-octyn-1-ol, 3. Liquid ammonia (NH_3; 1.3 L) was added to a 3-necked, 2 L round-bottomed flask equipped with N_2 and NH_3 inlets, thermometer, mechanical stirrer, and a dry ice/isopropanol-cooled reflux condenser. The temperature was maintained at -35°C by a dry ice/isopropanol bath. Ferric nitrate (0.4 g) was added, the slurry was stirred for 15 minutes and lithium metal (7.74 g; 1.12 mol) was added in 0.3-0.4 g pieces over a 30-minute period. The blue-black slurry was stirred until the color had changed to gray (30 minutes) and then for another 30 minutes. 5-hexyn-1-ol (50.0 g; 0.51 mol) in 250 ml of tetrahydrofuran (THF) was added dropwise over 30 minutes at -35 to -40°C. The slurry was stirred for 1 hour at -35°C and then bromoethane (50.0 g; 0.46 mol) in 250 ml THF was added dropwise over 30 minutes. Stirring was continued for another 30 minutes and the slurry was allowed to gradually warm to room temperature overnight. The thick slurry was warmed to 40°C by water bath and flushed with nitrogen to remove most of the remaining NH_3. Approximately 500 ml of water and 5 g of ammonium chloride were added, the reaction mixture was transferred with 250 ml diethyl ether (Et_2O) to a separatory funnel, and the layers were separated. The water layer was acidified to pH 2 with 5 N sulfuric acid (H_2SO_4) and extracted twice with 250 ml portions of Et_2O. The Et_2O extractions were combined and washed successively with saturated ammonium chloride, 5% H_2SO_4 and saturated sodium chloride. The Et_2O layer was dried

over sodium sulfate (Na_2SO_4) for 2 hours. The Na_2SO_4 was removed by filtration and the Et_2O by rotary evaporation. The residue was distilled through a 6 in. Vigreux column to yield 57.8 g of 3 (94% pure; 94% yield; bp = 85-90°C/3.5 mm Hg).

2-(5-octynyloxy)tetrahydropyran, 4. A 115.5 g (92% pure; 93% yield; bp 84-86°C/0.15 mm Hg) sample of 4 was prepared as described previously for the 6-carbon analogue (16).

2-octyloxy-5,5,6,6,-d_4)tetrahydropyran, 5. Compound 4 (92 g; 0.44 mol) was deuterated by means of $(Ph_3P)_3RhCl$ catalyst and D_2 gas to 5 (96% yield; bp 88-92/0.05 mm Hg) in a manner analogous to the 5-carbon analogue described previously (17).

1-iodooctane-5,5,6,6-d_4, 6. The iodide (93.8 g; 83% yield; bp 74-78°C/ 0.2 mm Hg) was prepared from the tetrahydropyranyl (THP) precursor as described previously for the 5-carbon analogue (17).

3-dodecyn-1-ol-9,9,10,10-d_4, 7. Compound 6 and 3-butyn-1-ol were coupled (Li/NH_3) to produce 7 (61.4 g; 91% pure; 84% yield; bp 88-90°C/0.10 mm Hg) as described in the preparation of 3.

3-dodecen-1-ol-9,9,10,10-d_4, 8. Compound 8 (28 g; 94% purity; 94% yield) was prepared by the Lindlar catalyzed reduction of 7 as described previously (18) for the 10-carbon analogue. The final product contained 3% of the saturated alcohol and 3% under-reduced material.

1-bromo-3-dodecene-9,9,10,10-d_4, 9. Compound 9 (37.8 g; 92% pure; 82% yield; bp 78-80°C/0.2 mm Hg) was prepared from compound 8 by reaction with Ph_3PBr_2 as described previously (18) for the 10-carbon analogue.

1-iodo-3-dodecene-9,9,10,10-d_4, 10. Compound 10 (37.1 g) was prepared from bromide, 9 (see compound 20, ref. 18). The final material contained 82% iodide 10 and 11% bromide 9. Since both halides could be used in the preparation of 11, no further purification was attempted.

1-dodec-3-enyl-9,9,10,10-d_4-triphenylphosphonium iodide, 11. The triphenylphosphonium salt (69.2 g) was prepared as decribed previously (18) for the 10-carbon analogue. Compound 11 was glassy in nature and had a melting point of 65-68°C.

methyl 8c,11c-eicosadienoate-17,17,18,18-d_4, 12. Compound 12 (16 g; 99% pure) was prepared by the Wittig coupling of 2 and 11 by means of n-BuLi in THF (see compound 22, ref. 18 for procedure). However, the theoretical amount of n-BuLi was added only after enough n-BuLi had been added initially to maintain a reddish tinge in the slurry. Trans isomers were removed by silver resin chromatography [2.5% acetonitrile (ACN) in methanol]. A methyl 8c,11c-18:2-d_4 impurity was removed by chromatographic separation on a C18 reverse phase column (ACN as solvent). The overall yield of compounds 10 to 12 was 41%.

Methyl 5c,8c,11c-eicosatrienoate-17,17,18,18-d_4 (See Figure 1)

2-undecyn-1-ol-8,8,9,9-d_4, 13. See synthesis of 3. Compound 6 was coupled to 2-propyn-1-ol (Li/NH_3) to prepare 13 (48.6 g; 97% pure; 98% yield; bp 78-80°C/0.05 mm Hg).

1-bromo-2-undecyne-8,8,9,9-d_4, 14. See synthesis of compound 7, ref. 18. The bromide 14 (61.7 g; 92% pure; 85% yield; bp 95-102°C/0.5 mm Hg) was prepared via Ph_3PBr_2.

3,6-pentadecadiyn-1-ol-12,12,13,13-d_4, 15. See synthesis of compound 17, ref. 18. Compound 15 (49.0 g; 87% pure; 95% yield; bp 137-141°C/0.05 mm Hg) was prepared by the Grignard coupling of 3-butyn-1-ol and 14.

3,6-pentadecadien-1-ol-12,12,13,13-d_4, 16. See preparation of compound 8. Compound 16 (19.3 g; 95% yield) was prepared by the Lindlar reduction of 15. The final product was composed of 95% 16, 2% 15 and 3% over-reduced (mono-unsaturated) alcohol-d_4.

1-bromo-3,6-pentadecadiene-12,12,13,13-d_4, 17. See synthesis of compound 7, ref. 18. The bromo-compound (18.8 g; 92% pure; 83% yield; bp 108-110°C/0.10 mm Hg) was prepared by the reaction of Ph_3PBr_2 and compound 16.

1-iodo-3,6-pentadecadiene-12,12,13,13-d_4, 18. See preparation of compound 20, ref. 18. Exchange with sodium iodide was not complete (11% bromide, 81% iodide) but final product (21.3 g) was used without additional purification.

1-pentadeca-3,6-dienyl-12,12,13,13-d_4-triphenylphosphonium iodide, 19. See synthesis of compound 8, ref. 18. Refluxed 18 with ACN and Ph_3P for 21

hours. Work-up yielded 35.8 g of sticky white solid (compound 19).

methyl 5c,8c,11c-eicosatrienoate-17,17,18,18-d_4, 20. Preparation was similar to that of compound 22, ref. 18. Silver resin chromatography (5% ACN in methanol) was used to remove trans isomers formed during the preparation of 20. Small amounts (6-7%) of C-18 impurities were removed by C18 reverse phase chromatography (ACN as solvent) to yield 6.0 g of 20 (98% pure; 40% yield from compound 17).

Methyl 8c,11c,14c-eicosatrienoate-17,17,18,18-d_4 (See Figure 2)

2-pentyn-1-ol, 21. Compound 21 (64.0 g; 98% pure; 97% yield; bp 68-70°C/20 mm Hg) was prepared by the coupling (Li/NH_3) of bromoethane and 2-propyn-1-ol (see compound 3).

2-(2-pentynyloxy)tetrahydropyran, 22. Compound 22 (128.1 g; 98% pure; 99% yield; bp 98-102/4.0 mm Hg) was prepared as described previously (17).

2-(pentyloxy-2,2,3,3-d_4)tetrahydropyran, 23. See synthesis of compound 3 and ref. 17. Compound 20 (129.7 g; 98% pure) was prepared in 92% yield, but was chromatographed on silica gel (PE as solvent) instead of distilled.

1-iodopentane-2,2,3,3-d_4, 24. The iodide 24 (108.3 g; 98% pure; 88% yield; bp 100-120/760 mm Hg) was prepared as described previously (17).

2-octyn-1-ol-5,5,6,6-d_4, 25. See preparation of compound 7. Compound 25 (57.9 g; 97% pure; 88% yield; bp 86-88°C/3.3 mm Hg) was prepared by the coupling of 24 and 2-propyn-1-ol by means of Li/NH_3.

1-bromo-2-octyne-5,5,6,6-d_4, 26. See synthesis of compound 9. Compound 20 (38.3 g; 94% pure; 87% yield; 88-92°C/4.5 mm Hg).

3,6-dodecadiyn-1-ol-9,9,10,10-d_4, 27. See preparation of compound 17, ref. 18, for synthesis of the 10-carbon analogue. Compound 27 (30.6 g; 91% pure; 85% yield; bp 102-106/0.1 mm Hg).

3,6-dodecadien-1-ol-9,9,10,10-d_4, 28. See preparation of compound 16. Compound 28 was prepared by the Lindlar catalysed reduction of 27. Work-up afforded 30.7 g of 28 (90% pure; 90% yield) which contained 5% over-reduced (monoenoic) and 4% under-reduced (ene-ynoic) alcohols.

1-bromo-3,6-dodecadiene-9,9,10,10-d_4, 29. See synthesis of compound 19,

ref. 18, for preparation of the 10-carbon analogue. The bromo-compound 29 (33.7 g; 92% pure; 84% yield; bp 84-86°C/0.2 mm Hg) was prepared from 28 by Ph_3PBr_2.

1-iodo-3,6-dodecadiene-9,9,10,10-d_4, 30. See synthesis of compound 20, ref. 18, for preparation of the 10-carbon analogue. Compound 20 (42.1 g; 92% pure) was prepared from 29 by exchange with sodium iodide in acetone.

1-dodeca-3,6-dienyl-9,9,10,10-d_4-triphenylphosphonium iodide, 31. See synthesis of compound 8, ref. 18. Compound 31 (22.6 g) was a white, sticky solid which was difficult to isolate and transfer.

methyl 8c,11c,14c-eicosatrienoate-17,17,18,18-d_4, 32. Compound 32 was prepared by the Wittig coupling (n-BuLi/THF) of compounds 1 and 31 in a manner similar to the preparation of compound 22, ref. 18. Final purification by silver resin chromatography (5% ACN in methanol) resulted in 6.5 g (99% pure; 27% yield) of compound 32.

RESULTS AND DISCUSSION

Many syntheses of both labelled (19,20) and unlabelled (20,21) polyunsaturated fats have been reported. Our most reproducible results and highest yields were obtained when a variation of Bergelson's (23) procedure was utilized. The double bond closest to the carboxylic acid was incorporated by the Wittig reaction while the other double bonds were introduced by acetylenic coupling followed by reduction with Lindlar's catalyst (17). The deuterium atoms were located in the 17 and 18 positions to minimize potential isotope effects.

The synthetic sequences for methyl 8c,11c-eicosadienoate-17,17,18,18-d_4 and 5c,8c,11c-eicosatrienoate-17,17,18,18-d_4 are given in Figure 1; the sequence for methyl 8c,11c,14c-eicosatrienoate-17,17,18,18-d_4 in Figure 2. Individual yields are given for each step except the final 3 (9→12; 17→20; 29→32). In these instances, a combined yield for these 3 steps is presented after final purification. Overall yields were 19% for 8,11-20:2, 18% for 5,8,11-20:3 and 10% for 8,11,14-20:3.

Regarding these syntheses, several points of interest deserve comment.

Two procedures for preparation of methyl 4-formylbutanoate (1) were investigated. Ozonolysis of cyclopentene (in a manner similar to the preparation of 2) resulted in <10% of the desired product. Hydrolysis of δ-valerolactone with

Figure 1. Synthetic schemes for methyl 8c,11c-eicosadienoate-17,17,18,18-d_4 (Scheme A) and methyl 5c,8c,11c-eicosatrienoate-17,17,18,18-d_4 (Scheme B). Yields are provided in brackets. 1-iodooctabe-5,5,6,6-d_4 (6) = C_5d_4I.

subsequent oxidation by pyridinium chlorochromate consistently produced yields of 40%. Phosphonium salts 11, 19 and 31 were prepared from the appropriate unsaturated iodide rather than bromide precursors, because the phosphonium

iodide was formed more rapidly, was easier to isolate and resulted in higher yields in the final Wittig coupling reaction (18). While conversion of the bromides to the iodides was not complete, all of the advantages just mentioned were still realized. Also, care should be exercised when coupling alkyl

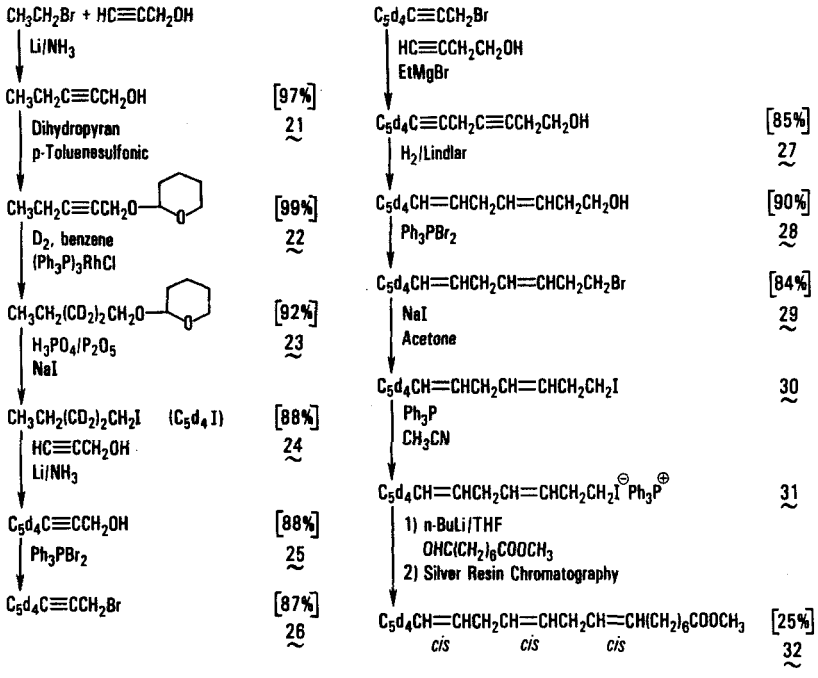

Figure 2. Synthetic scheme for methyl 8c,11c,14c-eicosatrienoate-17,17,18,18-d_4. Yields are provided in brackets. 1-iodopentane-2,2,3,3,-d_4 (24) = C_5d_4I.

halides to acetylenic alcohols with lithium metal in liquid ammonia. Reaction temperatures should be maintained at -35 to -40°C. When the temperature was maintained below -50°C during the alkyl halide and acetylenic alcohol addition, a vigorous exotherm suddenly occurred as the slurry was slowly warmed. The temperature surge resulted in a loss of liquid NH_3 and product through the reflux condenser. This problem did not occur when reaction temperatures were maintained at -35 to -40°C.

During the synthesis of the 8,11,14-20:3 isomer, addition of the theoretical amount of n-BuLi during the final Wittig coupling step resulted in lower (20-25%) combined yields than expected. Higher combined yields (40-41%) were obtained when the theoretical amount of n-BuLi was added after enough n-BuLi

had already been added to maintain a faint reddish tinge (due to formation of a reactive intermediate) of the slurry.

The synthesis of the 5,8,11-20:3 isomer was also attempted by coupling of a diacetylenic (rather than diolefinic) phosphonium salt to the appropriate aldehydic ester. The use of a mono-acetylenic phosphonium salt in the preparation of methyl crepenynate (9c,12a-18:2) has been accomplished with no trans in the 9-position (Wittig coupling with n-BuLi usually produces 15-20% trans) (24). Our attempts to couple the diacetylenic phosphonium salt resulted in no yield of product when n-BuLi in Et_2O (24) or n-BuLi in THF was used. Lack of product may have been due to formation of a very stable complex or abstraction of the highly acidic hydrogens on the carbon atom between acetylenic bonds in the phosphonium salt.

Several impurities were generated during our syntheses. Over- or under-reduction with Lindlar catalyst of the mono- or di-acetylenic alcohols (7, 15 and 27) resulted in either less double bonds than required or the presence of acetylenic bonds in the final product. The Wittig coupling also produced trans isomers (15-20%). All of these by-products were easily removed by silver resin chromatography. During the syntheses of the 8,11-20:2 and 5,8,11-20:3 fatty esters, an 18-carbon analogue appeared. This analogue resulted from incomplete coupling in the preparation of compound 3, so that some 5-hexyn-1-ol was carried along for the remainder of the syntheses. The undesired 18:2 and 18:3 analogues were easily removed by chromatography on a C18 reverse-phase column. Thus, the intermediates do not have to be 99% pure to be utilized in these syntheses. Only the formation of C-20 positional isomers could cause problems, since they cannot be separated easily. However, the procedures described do not generate positional isomers.

The ^{13}C-NMR chemical shifts for 8,11-20:2-d_4, 5,8,11-20:3-d_4 and 8,11,14-20:3-d_4 fatty esters are listed in Table I. The 8,11-20:2 and 5,8,11-20:3 isomers had not been studied previously by ^{13}C-NMR, but the chemical shifts of the olefinic carbons and the carbons alpha to them are indicative of the double-bond position in these polyunsaturated fatty acid methyl esters. No chemical shifts are listed in Table I for carbons 17 and 18, since these are the carbons on which the deuterium atoms are located.

Table 1. ^{13}C-NMR chemical shifts for methyl 8c,11c-eicosadienoate, 8c,11c,14c- and 5c,8c,11c-eicosatrienoates-17,17,18,18-d_4*

	8c,11c	8c,11c,14c		5c,8c,11c
Carbon	Obs	Lit (25)	Obs	Obs
1	174.12	174.13	173.97	173.75
2	34.09	34.12	34.03	33.37
3	24.98	24.99	24.94	24.83
4	28.98	28.96	28.93	27.30
5	29.13	29.11	29.08	128.95
6	29.37	29.47	29.46	128.45
7	27.21	27.25	27.01	26.58
8	129.88	130.16	130.01	127.89
9	128.17	127.93	127.84	128.78
10	25.70	25.71	25.66	25.64
11	127.91	128.27	128.13	127.56
12	130.17	128.36	128.26	130.34
13	27.32	25.71	25.66	27.30
14	29.53†	127.73	127.63	29.72
15	29.13†	130.41	130.27	29.33
16	29.77†	27.25	27.17	29.33
17	--	29.47	--	--
18	--	31.59	--	--
19	22.49	22.62	22.35	22.47
20	14.08	14.06	14.01	14.05

* ppm downfield from $(CH_3)_4Si$

† Tentative Assignments

The deuterium distribution for the intermediates and final products are listed in Table II. A sample of the 8,11,14-20:3-d_4 isomer was reduced to 20:0-d_4 [$(Ph_3P)_3RhCl$, benzene, H_2 gas] for the MS deuterium distribution analysis. The 8,11-20:2-d_4 and 5,8,11-20:3-d_4 were compared to 11,14-20:2-d_0 and 8,11,14-20:3-d_0, respectively, on our mass spectrometer to obtain the deuterium distribution. There is good agreement between the THP intermediates and the final fatty ester products.

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Table 2. Mass analysis for deuterium

Compound (number)*	Number of deuterium atoms (%)							Average number of deuterium atoms per molecule
	1	2	3	4	5	6	7	
2-(Octyloxy-5,5,6,6-d_4)-tetrahydropyran (5)	0.0	0.0	1.1	97.0	1.8	0.0	0.0	4.00
Methyl 8c,11c-eicosadienoate-d_4 (12)	0.1	0.0	1.6	96.9	1.3	0.0	0.0	3.99
Methyl 5c,8c,11c-eicosatrienoate-d_4 (20)	0.0	0.2	1.2	96.7	0.9	0.5	0.2	4.01
2-(Pentyloxy-2,2,3,3-d_4)-tetrahydropyran (23)	0.1	0.5	4.4	90.8	4.0	0.2	0.0	3.99
Methyl 8c,11c,14c-eicosatrienoate-d_4 (32)	0.7	0.0	4.5	90.7	3.4	0.5	0.0	3.97

*See Figures 1 and 2.

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