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Chemical synthesis of deuterium-labeled and unlabeled very long chain polyunsaturated fatty acids

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ABSTRACT

First syntheses of a deuterium-labeled very long C34-containing polyunsaturated fatty acid, 34:5*n*5, and three other unlabeled very long chain C30–32 containing polyunsaturated fatty acids are reported. These syntheses were achieved by coupling chemically modified C22- and C20-containing polyunsaturated fatty acids with carbanions derived from arylalkyl sulfones, followed by sodium amalgam-mediated desulfonylation.

Very long chain polyunsaturated fatty acids (VLC-PUFAs) are arbitrarily defined as fatty acids that have 24 or more carbon atoms in their molecules with cis-double bonds interrupted by methylene units. These fatty acids found in most living organisms from humans to autotrophic and heterotrophic lower organisms can be grouped into two molecular families: omega-6 (or *n*−6) and omega-3 (or *n*−3) families, where the number 6 or 3 represents the position of the first double bond proximal to the terminal methyl group.

Dietary intervention studies suggest that omega-3 PUFAs such as eicosapentaenoic acid (EPA; 20:5D5,8,11,14,17) and docosahexaenoic acid (DHA; 22:6D4,7,10,13,16,19) confer health benefits to humans.[1–3] PUFAs are also widely distributed in higher plants.[4] Humans, like other animals, can synthesize PUFAs from the essential α-linolenic acid[5] and the PUFAs are distributed throughout the body. However, the distribution of VLC-PUFAs appears to be more limited, being found in retina, brain,[1] testis, and spermatozoa.[6] The VLC-PUFAs with 3–9 double bonds are not only susceptible to oxidative damage but are also difficult to separate from mammalian tissues for characterization.[7] Therefore, not much is known about the metabolism and function of VLC-PUFAs.

Animal models of an early on set form for macular degeneration (autosomal dominant Stargardt-like macular dystrophy, STGD3) were used to show that this human disease arises due to a deficiency of VLC-PUFAs in the retina.[8] Mutation of the gene encoding elongation of very long chain fatty acid 4 (*ELOVL4*) leads to STGD3.[9] *ELOVL4* has been shown to elongate long fatty acids to form VLC-PUFAs.[10] Thus, the VLC-PUFAs present in retina have recently seen a renewed interest because of their important role in vision.

Even though aspects of metabolic engineering of some specific VLC-PUFA are reported,[16] to the best of our knowledge, their chemical laboratory synthesis has not been described. In order to evaluate the retinal role of VLC-PUFA in the vision process in our ongoing epidemiological studies, we have synthesized a variety of VLC-PUFAs with carbon chains varying between C-30 to C-34 and with three, four, and five *cis*-oriented C–C double bonds (Fig. 1). Additionally, we prepared a dideuterated version of a C-34 omega-3 VLC-PUFA, 34:5*n*3.d_2 to be used as a probe in our studies. For convenience, all VLC-PUFA materials were synthesized using C-20 and C-22-containing all-*cis* fatty acids available commercially (Nu-Chek Prep, Inc). Care was taken to select reagents that are not expected to invert geometrical integrity of the C–C double bonds. Additionally, all reactions were carried under argon in flasks covered with aluminium foil to prevent oxidation.

Our first priority was to prepare a deuterium-labeled omega-3 fatty acid 34:5*n*3 with a difference of at least 2 mass units. Cost considerations suggested incorporating two deuterium atoms at a location near the middle of the saturated part of the long carbon chain that was less likely to be prone to deuterium exchange under our bioassay conditions. To this end, 12-bromododecanol (**1**) (Scheme 1) was protected with a robust *t*-butyldiphenylsilyl (TBDPS) group to give the bromosilyl ether **2**. Next, the bromide function of **2** was displaced with a sulfinate anion in *N*,*N*-dimethylformamide (DMF) to generate the sulfone fragment **3** in 94% yield over two steps.[11]

In a separate pot, omega-3-all-*cis*-docosapentaenoic acid **4** was reduced either directly with lithium aluminium deuteride in tetrahydrofuran solvent, or via cyanuric fluoride-mediated acyl fluoride formation[12] followed by sodium borodeuteride reduction,[13] to give an alcohol that was immediately converted into the desired deuterated *p*-toluenesulfonate ester **5** in 71% yield over two steps.

For coupling the sulfone fragment **3** with the fatty acid tosylate fragment **5**, the sulfone **3** was deprotonated to a lithocarbanion

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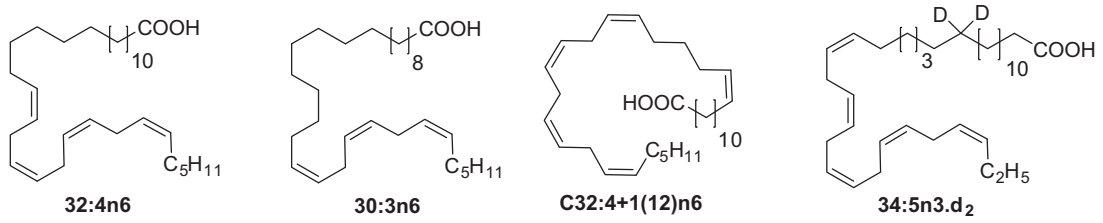
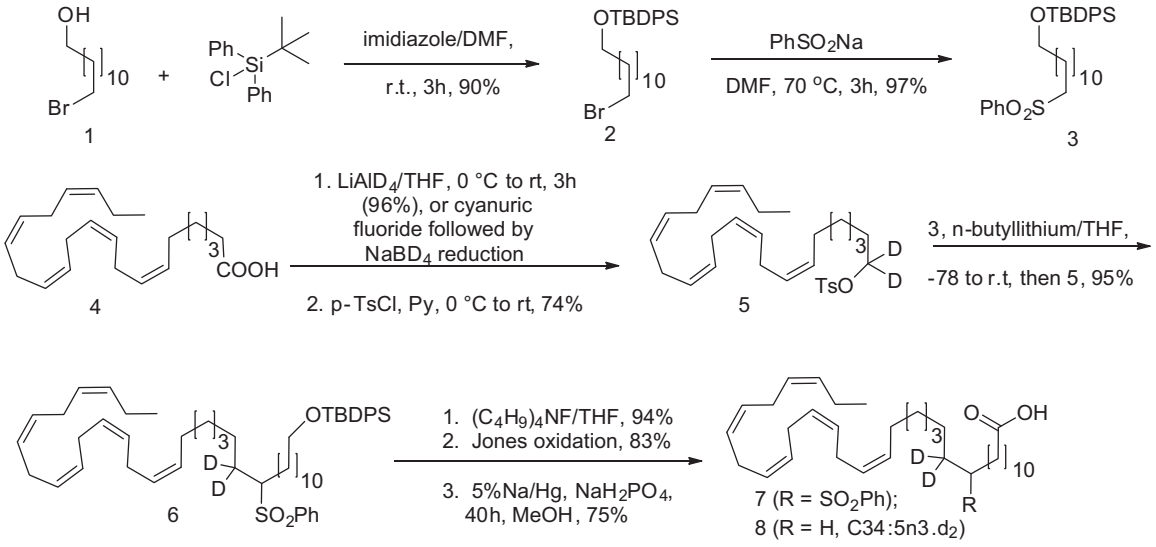
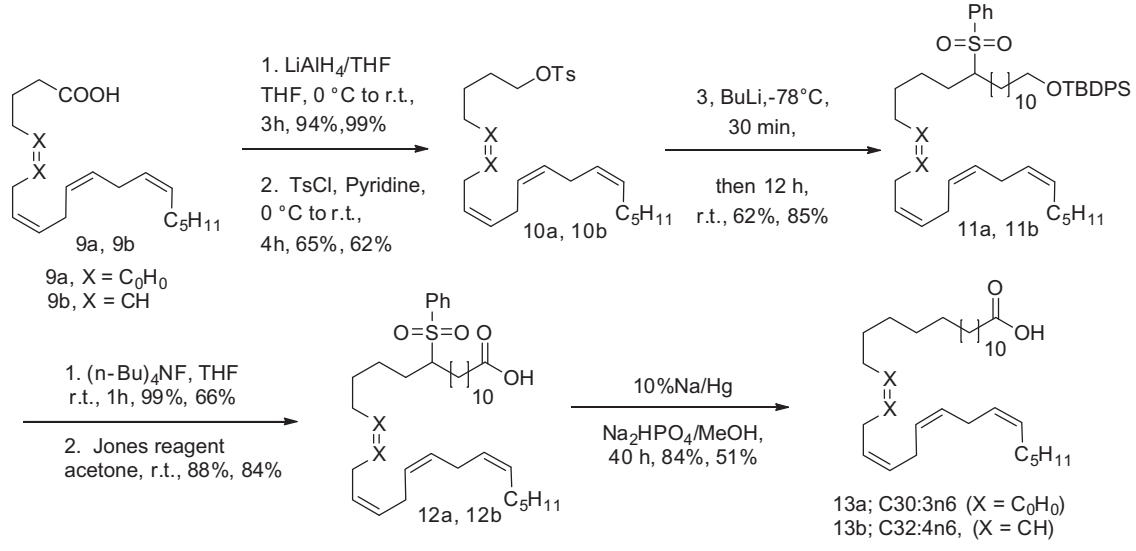


Figure 1. Structures of synthesized VLC-PUFAs.



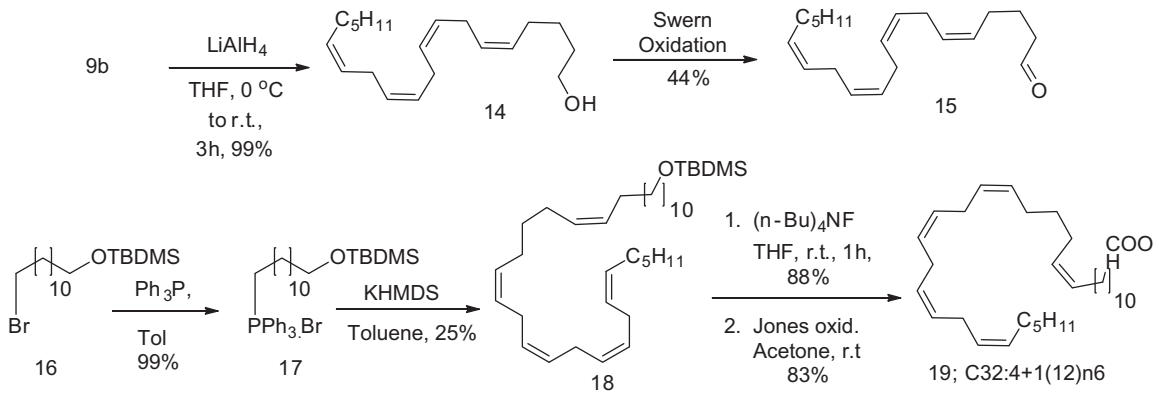
Scheme 1. Synthesis of 34:5*n*3.d2.



Scheme 2. Synthesis of 32:4*n*6 and 30:3 *n*6. (Yields reported in series a, X = C_0H_0; in series b, X = CH.)

with *n*-butyllithium in tetrahydrofuran at −78 °C and reacted with the tosylate **5** to furnish the deuterated sulfone **6** in 95% yield. The TBDPS group was removed with tetra-*n*-butylammonium fluoride (94%) and the resulting alcohol was carefully oxidized with the Jones reagent[14] in acetone (83%) to give the polyunsaturated fatty acid **7**. Finally, the phenylsulfone moiety of **7** was reductively removed by treating it with sodium amalgam in methanol under phosphate buffer conditions[15] furnishing the VLC-PUFA 34:5*n*3 **8** after purification to homogeneity by flash silica gel chromatography. The overall yield of this eight-step convergent synthetic sequence leading to the VLC-PUFA **8** was 38%.

For some of our ongoing projects, two unlabeled omega-6 VLC-PUFAs, namely, 30:3*n*6 and 32:4*n*6 (Scheme 2) were also synthesized using the methodology described above. These syntheses employed omega-6-all-*cis*-octadecatrienoic acid (18:3*n*6, **9a**) and omega-6-all-*cis*-icosatetraenoic acid (20:4*n*6, **9b**) as the starting polyunsaturated fatty acid starting materials. Starting from the sulfone **3**, the entire synthetic sequence proceeded without incident



Scheme 3. Synthesis of 32:4+1(12)*n*6.

and furnished the final VLC-PUFAs 30:3*n*6 (**13a**) and 32:4*n*6 (**13b**) with overall yields similar to **8**.

As a negative control, we also synthesized 32:4+1(12)*n*6, an unusual VLC-PUFA with a *cis* C–C double bond at the C-12 position, separated from the nearest C–C double bond by three methylene groups rather than the usual one. As graphically represented in Scheme 3, the omega-6-all-*cis*-icostetraenoic acid 20:4*n*6, **9b**, was reduced with lithium aluminium hydride in THF to the primary alcohol **14**, which was then partially oxidized to the aldehyde **15**. In a Wittig reaction, the aldehyde **15** was coupled with the phosphorane derived from phosphonate **17**, itself derived from the protected bromoacohol **16**, to give a silyl-protected alcohol **18**, from which the alcohol was recovered by desilylation and oxidized under Jones oxidation to the VLC-PUFA 32:4+1(12)*n*6 **19**. In this case, the overall synthetic sequence starting from **9b** and 12-bromododecanol (**2**) proceeded in 8.1% combined yield.

The VLC-PUFAs as well as all the synthetic intermediates were purified by flash silica gel chromatography and their structures were confirmed by ^{1}H NMR and ESI/APCI-mass spectroscopy. Furthermore, in view of their expected rapid oxidation, all synthesized VCL-PUFAs were stored away from light at −80 °C under argon. They were found to be stable at least for several weeks as judged by their unchanged spectral properties.

In conclusion, in order to elucidate their biological role in the vision process and related areas, the first practical and general chemical synthetic method for several very long chain polyunsaturated fatty acids is described. The overall eight-step synthesis methodology reported herein proceeded in reasonable yield and can be employed for most VLC-PUFAs required for activity and role elucidation in any biological system.

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Supplementary data

Supplementary data (chemical synthesis procedures and spectral data of the final VLC-PUFAs and their synthetic intermediates) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.09.139.

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