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Advanced Synthesis & Catalysis

Synthesis of Perdeuterated Linoleic Acid-d_{31} and Chain Deuterated 1-Palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine-d_{62}

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Abstract: Herein, we report a gram-scale synthesis of perdeuterated linoleic acid-d_{31}. The starting materials for the synthesis are two saturated fatty acids, azelaic acid-d_{14} and pentanoic acid-d_9, which can be obtained by metal catalysed hydrothermal hydrogen-deuterium exchange. The synthesis utilises the fatty acids directly via decarboxylative coupling. Copper catalysed coupling of a terminal alkyne intermediate with a propargyl bromide derivative affords a skipped diyne, which can be reduced using P-2 nickel to obtain the desired *cis*,*cis*-diene geometry. The subsequent synthesis of the tail-deuterated phospholipid, 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine-d_{62} (PLPC-d_{62}) is also described. Optimised reaction conditions were developed to access this phospholipid and its regioisomeric purity was characterised by two complementary mass spectrometry techniques.

Keywords: Deuterium; Isotopic labelling; Synthetic methods; Fatty acids; Lipids

Introduction

Linoleic acid (9*Z*,12*Z*-octadecadienoic acid; LA; 18 : 2n−6), an omega-6 fatty acid, is one of two essential polyunsaturated fatty acids. Dietary requirement for omega-6 and omega-3 fatty acids is due to an absence of the desaturase enzymes necessary to insert a *cis* double bond at the requisite positions of saturated fatty acids. In humans, linoleic acid is catabolised to γ-linolenic acid and subsequently to longer chain dihomo-γ-linolenic acid and arachidonic acid, precursors to prostaglandins, thromboxanes and leukotrienes. Omega-6 fatty acids, including linoleic acid, are important structural components of cell membranes. Phospholipids derived from linoleic acid influence cell membrane properties including fluidity, flexibility, permeability and the activity of membrane-bound enzymes and cell-signalling pathways.[1] Recently, it was shown that polyunsaturated fatty acids bind to the novel coronavirus SARS-CoV-2 and effectively interfere with binding to its receptor, hACE2.[2] It was demonstrated that linoleic acid significantly blocks the entry of SARS-CoV-2 into cells. In addition, linoleoyl lipids have also been used as components in lipid nanoparticles to encapsulate mRNA for vaccine development and production.[3]

Deuterium-labelled compounds are important tools for investigating the structure and function of chemical and biological materials using a variety of characterisation techniques. The deuterium kinetic isotope effect

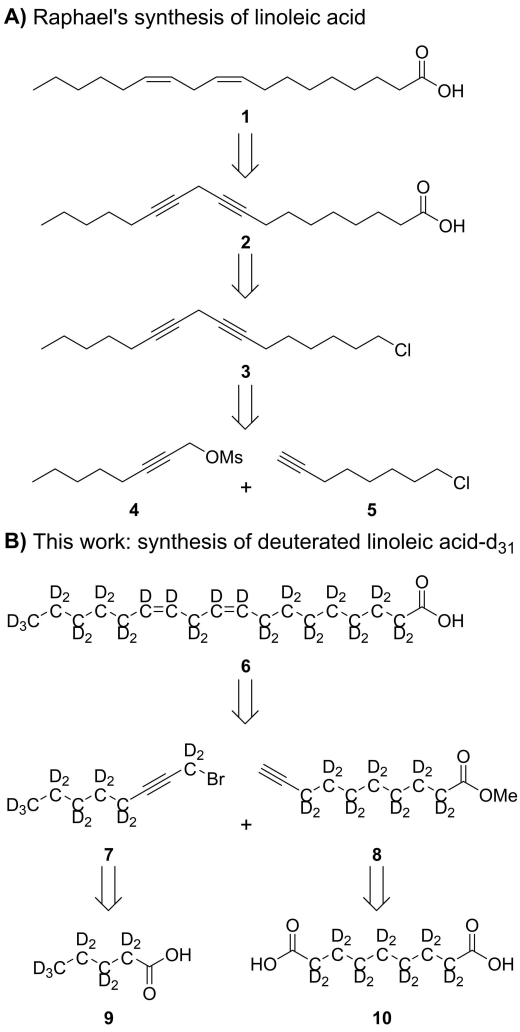
has also been effectively applied to prepare advanced materials with improved performance, most notably in the pharmaceutical industry, where selective hydrogen-deuterium exchange is a common strategy to improve the pharmacokinetic properties of drug leads.[4] Significant efforts have been made towards stabilising linoleic acid via specific isotopic labelling. The majority of these reports focus on linoleic acid with specific deuterium labelling at the allylic and *bis*-allylic positions. These analogues have been used to study the metabolism of linoleic acid and its phospholipids and for extracting active site geometries of the enzyme-substrate complexes in lipoxygenases.[5] An important finding has been that replacement of the *bis*-allylic hydrogens with deuterium arrests autoxidation due to the kinetic isotope effect.[6] This has resulted in numerous studies probing the potential of deuterated polyunsaturated fatty acids to diminish lipid peroxidation, which is a common feature of various neurological and age-related pathologies.[7]

In most cases, deuterated isotopologues have very similar physical and chemical properties to their naturally occurring protiated parent compounds. Deuterium labelling, however, does alter a number of properties that can be exploited by experimental techniques. For example, the increase in mass imparted by the additional neutrons can be exploited by mass spectrometry to differentiate isotope-labelled fatty acids (used as internal standards or tracers of metabolic flux) and endogenous fatty acids extracted from biological samples.[8] Moreover, deuteration results in vastly different NMR spectroscopic and neutron scattering signals. In neutron scattering experiments, deuteration changes the scattering length density (SLD) of selected constituents in multicomponent systems, allowing them to be visualised separately, thus simplifying data analysis and improving signal-to-noise.[9] While selective deuteration is useful for investigations related to biological kinetic isotopic effects, perdeuteration is generally required to investigate complex systems by neutron scattering studies. Model synthetic lipid bilayers have been used to investigate important biophysical and biochemical questions. This has resulted in a greater understanding of lipid membranes, including elucidating their structure, phase behaviour, water permeability and changes induced by membrane proteins.[10] Native-like lipid membranes, including those derived from unsaturated fatty acids, are required to accurately investigate membrane processes such as interactions with proteins and drug molecules.

Linoleic acid is an important precursor to many biologically relevant lipids. Although deuterated phospholipids with saturated acyl chains are widely available, there is a paucity of deuterated lipids with unsaturated (e. g. oleoyl and linoleoyl) chains available for investigations involving biomimetic cellular membranes. 1-Palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine (PC 16:0/18:2(9Z,12Z), or PLPC) is a glycerophospholipid, with substitution of palmitic acid and linoleic acid at the *sn*-1 and *sn*-2 positions of the glycerol backbone, respectively. It has found use in the generation of micelles, liposomes, and other types of artificial membranes, as well as the study of lipid peroxidation.[11] Deuteration of such biologically relevant lipids is of particular interest for small-angle neutron scattering (SANS) and neutron reflectometry studies, whereby differences in scattering length density is utilised to achieve contrast variation and elucidate structural and functional information about cellular membranes.[12] Of note, the use of deuterated lipids has been instrumental in determining how SARS-CoV-2 interacts with cell membranes.[13] In addition to neutron experiments,[14] deuterated phospholipids are required for particular NMR[15] and vibrational spectroscopy studies.[16] Monoglyceride-based liquid crystals have been increasingly used as extended-release drug delivery systems using glyceryl monooleate (GMO) and monolinoleate (GML). GML possesses superior properties in comparison to GMO, however, the majority of neutron scattering investigations requiring contrast matching by deuteration have been done with GMO only due to the availability of deuterated oleic acid.[17] Perdeuterated linoleic acid is therefore required to synthesise such linoleoyl lipids of interest.

The chemical synthesis of linoleic acid **1** was first reported by Raphael and Sondheimer in *Nature* in 1950 (Scheme 1, A).[18] The synthesis, like all subsequent approaches, encompasses a key skipped diyne or β-diacetylenic acid intermediate, **2**, which can be partially hydrogenated to afford the requisite *cis*, *cis* alkene geometry of linoleic acid. The skipped diyne **2** was synthesised by treatment of the Grignard complex of acetylenic chloride **5** with an excess of acetylenic methanesulfonate **4**, followed by a malonic ester two carbon homologation. Mesylate **4** can be derived from the corresponding alcohol, while acetylenic chloride **5** was synthesised from 1,6-dichlorohexane. In the following year, Walborsky and co-workers reported an alternative six-step synthesis starting from 1,9-decadiyne.[19] Coupling to form the desired skipped diyne intermediate was achieved using a catalytic amount of copper bromide to prevent side-reactions resulting from the high reactivity of the central methylene group of the product. As in the previous report, semihydrogenation was challenging, this time achieved in low yield as a mixture of products using Raney nickel.

In more recent times, synthetic efforts to produce isotopically labelled derivatives have given rise to improved methods for key steps in the production of linoleic acid, namely the coupling reaction to form β-diacetylenic acid intermediate **2** and its selective



Scheme 1. Retrosynthetic approaches to the synthesis of linoleic acid.

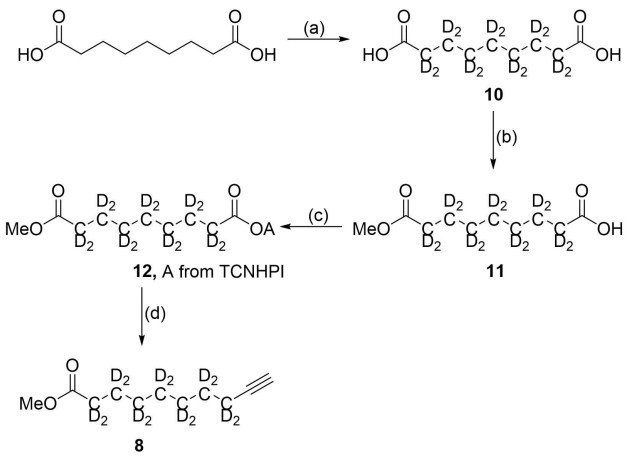
reduction.[5,20] The development of stereoselective methods for the partial reduction of alkynes to *cis*-alkenes, including, but not limited to, hydrogenation in the presence of Lindlar's[21] or P2-nickel catalyst[22] and hydrometallation using boron species[23] has allowed for the reliable semihydrogenation of skipped diynes.

Herein, we report our approach to access gram quantities of deuterated (ca. 98%D) linoleic acid-d_{31} **6** (Scheme 1, B) and its chain deuterated phospholipid, 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine-d_{62} (PLPC-d_{62}). In designing the synthesis, careful consideration was taken to consider the availability and high costs associated with the use of deuterated reagents. In addition, issues pertaining to the synthesis of deuterated molecules such as the avoidance of back-exchange, consideration of deuterium kinetic isotope effects and the structural characterisation of isotopologue mixtures were also contemplated. While saturated fatty acids undergo reliable hydrogen-deuterium exchange under hydrothermal conditions,[24] unsaturated fatty acids are not suitable substrates for such harsh conditions. Instead, deuterated saturated fatty acids can be used as precursors, which after functional group interconversion, can be stitched together to form the desired polyunsaturated product. A synthesis utilising a series of Wittig olefinations could be envisioned, analogous to our synthesis of deuterated oleic acid.[25] However, to achieve this, a large number of functional group interconversion steps as well as a complex protecting group strategy would need to be employed. Rather, the key skipped diyne was synthesised by copper(I)-mediated coupling of propargylic halide **7** with terminal alkyne **8** (Scheme 1, B).[26] It was envisioned that key intermediates **7** and **8** could be accessed from saturated fatty acids **9** and **10** via step-efficient decarboxylative coupling. With the skipped diyne in hand, established semihydrogenation methodologies could be employed to produce **6**. The synthesis of mixed acyl glycerophospholipids is deceptively challenging. Optimised reaction conditions were developed to synthesise the tail deuterated linoleoyl lipid PLPC (**20**) using a stoichiometric amount of the precious labelled fatty acid **6**.

There is an evident need for a convenient and scalable synthesis of deuterated linoleic acid to produce deuterated linoleoyl lipids. The large-scale production of deuterated lipids is generally more suited to chemical synthesis over biosynthetic methods,[27] from which it is difficult to purify well defined molecular species in sufficient quantities.

Results and Discussion

Metal catalysed hydrothermal hydrogen-deuterium exchange in heavy water is a reliable method to produce deuterated saturated fatty acids. Highly deuterated pentanoic acid **9** (96.8%D) and azelaic acid **10** (98.5% D) could be obtained after two cycles at 220 °C in alkaline D_2O, in the presence of a platinum on carbon catalyst (Scheme 2). It was envisioned that the carboxylic acids could be used directly as functional handles to obtain the desired coupling partners **7** and **8**, rather than undergoing a series of functional group interconversions. In the case of azelaic acid, this required protection of one of the carboxylic acids as the methyl ester. Using methods we have previously reported, methyl ester **11** could be synthesised in a two-step procedure via barium hydroxide-mediated selective hydrolysis of the corresponding diester.[25,28] From here, the desired terminal alkyne **8** was installed via decarboxylative alkynylation. Decarboxylative cross couplings have emerged in recent years as a powerful strategy to form carbon-carbon or carbon-heteroatom bonds from widely available, low cost and chemically stable carboxylic acids.[29] In particular, there have been a number of recent reports for the decarboxylative

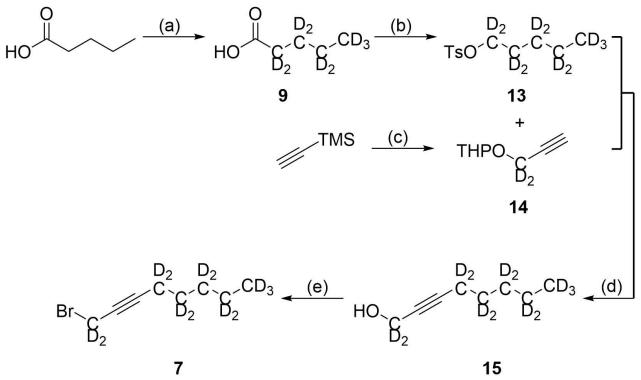


Scheme 2. Synthesis of terminal alkyne **8**. *Reagents and conditions*: (a) Pt/C, NaOD, D_2O, 220 °C, 3 days, 2 cycles, 98% D; (b) i) MeOH, H_2SO_4, reflux, 48 h, quant.; ii) $Ba(OH)_2.8H_2O$, MeOH, rt, 24 h, 70%; (c) TCNHPI, DCC, DMAP, CH_2Cl_2, rt, 8 h, 86%; (d) Ethynyl zinc chloride LiCl, $NiCl_2.6H_2O$, 4-4′-dimethoxy-2-2′-bipyridine, DMF, THF, rt, 16 h, 61%.

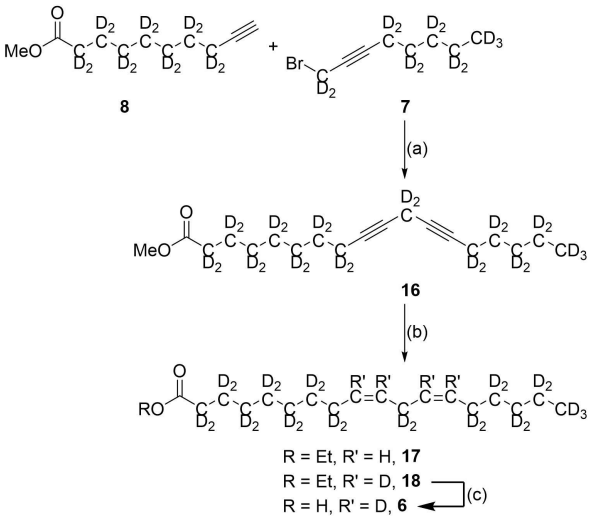
alkynylation of aliphatic carboxylic acids.[30] Decarboxylative alkynylation reduces the number of synthetic steps to access such key intermediates as terminal alkyne **8** or propargylic halide **7** from azelaic acid and hexanoic acid, respectively. This approach avoids traditional selective reduction of carboxylic acid derivatives followed by functional group interconversions and substitution with organometallic alkyne derivatives. In our previous report for the synthesis of oleic acid, selective reduction of the carboxylic acid in **11** was achieved using protiated boron dimethyl sulfide complex, which eventually leads to one proton in the final structure.[25] The decarboxylative alkynylation method reported by Baran and co-workers[30e] employs an inexpensive, commercially available alkyne source to reliably synthesise terminal alkynes. Activation of the carboxylic acid as the *N*-hydroxytetrachlorophthalimide (TCNHPI) ester **12** followed by treatment with ethynyl zinc chloride lithium chloride afforded **8** via nickel catalysed decarboxylative coupling in moderate yield. Baran and co-workers have reported this method on large scale (1 mole), and in our hands no issues were observed when increasing the scale.

In a similar manner as above, it was anticipated that decarboxylative alkynylation could be employed to synthesise a terminal alkyne or the desired propargylic halide derivative, directly, from deuterated hexanoic acid. The volatility of 1-heptyne-d_{11} precludes its synthesis in high yield using the methods above. Decarboxylative coupling of an activated hexanoic acid derivative, with protected propargyl alcohol was attempted using conditions reported by Baran and co-workers[30e] and Weix and co-workers (See Supporting Information).[30a] However, the desired compound (**15**) was obtained only in low yield. Rather, a more traditional approach was taken, starting from deuterated pentanoic acid **9** (Scheme 3). The acid **9** was reduced with lithium aluminium deuteride and immediately activated as the tosylate (**13**). Protected propargyl alcohol **14** was synthesised in a three-step procedure reported by Dodo and co-workers.[31] Deprotonation of **14** and substitution of tosylate **13** furnished alkylated propargyl alcohol **15** after removal of the tetrahydropyran protecting group. An Appel reaction afforded propargyl bromide coupling partner **7** in high yield. To reduce the number of steps to synthesise **7**, commercially available tetrahydropyran protected propargyl alcohol could be used in this synthesis to provide linoleic acid-d_{29}, without the *bis*-allylic position labelled. The site-specific deuteration methodology reported by Smarun et al. could then be applied to synthesise linoleic acid-d_{31}.[32] This experiment is undergoing further investigation, subject to accessibility of the requisite catalyst.

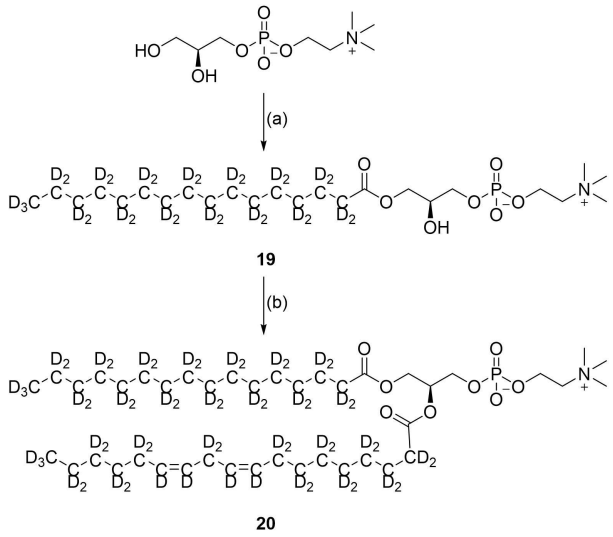
A copper-mediated coupling of terminal alkyne **8** and propargyl bromide derivative **7** afforded skipped diyne **16** (Scheme 4).[26] Stereoselective reduction of **16** to the desired *cis*,*cis*-1,4-diene was initially attempted via semi-hydrogenation over Lindlar's catalyst (See Supporting Information, Table S1 for reaction optimisation details). Optimisation of the reaction conditions (10 mol% Lindlar's catalyst, 5% w/w quinoline with respect to Lindlar's catalyst, in ethyl acetate/hexane 1 : 1 at room temperature for 2 hours) afforded the desired product as a mixture, contaminated with over-reduced and isomerised compounds. Although separation of saturated impurities was



Scheme 3. Synthesis of propargyl bromide derivative **7**. *Reagents and conditions:* (a) Pt/C, NaOD, D_2O, 220 °C, 3 days, 2 cycles, 97%D; (b) i) $LiAlD_4$, Et_2O, 0 °C-rt, 16 h; ii) TsCl, DMAP, Et_3N, CH_2Cl_2, 0 °C-rt, 24 h, 78%; (c) i) *n*-BuLi, $(CD_2O)_n$, THF, −40-0 °C, 16 h; ii) DHP, $TsOH.H_2O$, CH_2Cl_2, rt, 16 h; iii) K_2CO_3, MeOH, rt, 4 h, 59%; (d) i) *n*-BuLi, NMP, THF, 0 °C-rt, 16 h; ii) $TsOH.H_2O$, MeOH, rt, 2 h, 57%; (e) Br_2, PPh_3, CH_2Cl_2, 0 °C, 30 min, 92%.



Scheme 4. Synthesis of linoleic acid-d_{31} **6**. *Reagents and conditions:* (a) CuI, NaI, K_2CO_3, DMF, rt, 16 h, 86%; (b) $Ni(OAc)_2.4H_2O$, $NaBH_4$, ethylenediamine, H_2 or D_2, EtOH, rt, 16 h, 86%; (c) $LiOH.H_2O$, MeOH, rt, 24 h, 96%.



Scheme 5. Synthesis of 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphocholine-d_{62} (PLPC-d_{62}) **20**. *Reagents and conditions*: (a) (i) Bu_2SnO, iPrOH, reflux 1 h, (ii) palmitoyl chloride-d_{31} (from **21**), Et_3N, rt, 30 mins, 47%; (b) **6**, 4-PyOH, DCC, $CHCl_3$, rt, 40 h, 74%.

possible with silver ion chromatography, alternative conditions were pursued to achieve improved selectivity. Reduction with nickel P2 catalyst under an atmosphere of hydrogen yielded **17** with high selectivity. It is of note that in the ethanolic reaction medium, transesterification occurred, quantitatively. The ^{1}H NMR spectrum of **17** provides evidence for the required *cis*,*cis* alkene geometry. As the allylic and *bis*-allylic positions are deuterated, the alkene region of the spectrum is simplified and coupling constants of 10.5–11 Hz can be measured for the alkene vicinal coupling (See Supporting Information, Figure S1). Ethyl linoleate-d_{31} **18** was obtained using the same conditions, under a deuterium atmosphere. Hydrolysis of the ester afforded linoleic acid-d_{31} **6** in gram-scale quantities, from readily available amounts of the deuterated fatty acid precursors, azelaic acid and pentanoic acid (10 g and 5 g, respectively). Comparison of the NMR and IR spectroscopy data with commercially sourced protiated linoleic acid (See Supporting Information S44, S45 and S47) confirms the structure of **6** and mass spectrometry supports the high overall deuteration level (97.6%D, See Supporting Information).

With gram-scale quantities of linoleic acid-d_{31} in hand, we set out to synthesise more complex lipids from this key deuterated building block. 1-Palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine (PLPC) is a lipid of interest, in particular, for research investigating the influence of lipid unsaturation on cellular structure and function. Chain deuterated PLPC-d_{62} was synthesised in a two-step procedure from commercially available L-α-glycerophosphorylcholine (Scheme 5). Regioselective monoacylation at the *sn*-1 position was achieved via acylation of an activated tin ketal, prepared by treatment of L-α-glycerophosphorylcholine with dibutyltin oxide.[33] Purification of the 2-lyso phospholipid **19** by recrystallisation was necessary to obtain high regioisomeric purity.[34] Purification via column chromatography results in 1,2-acyl migration, whereby a small amount (approximately 10%) of the 1-lyso phospholipid is formed. In the ^{1}H NMR spectrum, the characteristic *sn*-2 methine proton residue is shifted based on whether the adjacent hydroxyl group is esterified, and hence the regiopurity of the lyso lipid can be readily determined.[35] A more challenging feat is to determine the extent of 1,2-acyl migration after the second esterification to form the diacylglycerol phospholipid has occurred. In biological systems, most phospholipids have a saturated fatty acid ester at the *sn*-1 position and an unsaturated fatty acid ester at the *sn*-2 position. However, the distribution of fatty acids within phospholipids is in continual flux, due to phospholipid degradation and continuous phospholipid remodelling within cell membranes. Isomeric mixtures of common mixed acyl glycerophospholipids have been identified at high abundance in various samples.[36] In most cases, commercially available lipids are comprised of regioisomeric mixtures. For the construction of model membranes, it is unclear whether a single regioisomer is biologically relevant in many cases. Recently, an elegant chemoenzymatic procedure for producing chain deuterated 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) with high regiopurity was reported.[37] While

the method does reduce the amount of 1-oleoyl-2-palmitoyl-*sn*-glycero-3-phosphocholine (OPPC) normally produced in a purely chemical synthesis, the multistep procedure generates a small amount (3.6%) of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) as an inseparable by-product. In our approach to synthesise chain deuterated PLPC, we endeavoured to optimise the esterification of 2-lyso lipid **19** to minimise the amount of regioisomeric product, 1-linoleoyl-2-palmitoyl-*sn*-glycero-3-phosphocholine (LPPC) produced. 2-Lyso lipid **19** is known to readily undergo 1,2-acyl migration in aqueous solutions, and the process is catalysed by either acid or base.[38] Lysophosphatidylcholine lipids rapidly equilibrate to a 90:10 mixture of the more stable 2-lysophosphatidylcholine and 1-lysophosphatidylcholine, respectively. However, Kielbowicz et al. reported minimal 1,2-acyl migration of 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine in organic solvents, by high performance liquid chromatography (HPLC) analysis (~1% in 96 h).[39] Under standard 4-dimethylaminopyridine catalysed esterification conditions, Gupta et al. utilised ^{14}C labelling to estimate the degree of migration.[40] After 30 hours, a small amount (5%) of the migrated product was observed. In a similar manner, Roberts and co-workers did not observe a measurable amount of OPPC by NMR measurements after the Steglich esterification of 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine using ^{13}C labelled oleic acid.[41]

Standard conditions for the esterification of 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine generally call for an excess of the carboxylic acid to ensure sufficient conversion of the poorly nucleophilic secondary hydroxyl group to the desired ester. Under Steglich esterification conditions, in the absence of a strong nucleophile, the carboxylic acid is consumed by irreversible formation of an *N*-acyl urea by-product. A nucleophilic catalyst, in most cases 4-dimethylaminopyridine, is necessary, in stoichiometric or superstoichiometric amounts, to achieve satisfactory yield.[40] The use of a large excess of **6** is obviously not ideal, and hence efforts were made to optimise the esterification reaction using stoichiometric amounts of the fatty acid. Under Steglich esterification conditions using a slight excess of **6** (1.05 eq.) and either no or catalytic 4-dimethylaminopyridine (10 mol%), very low yield of **20** was obtained (< 10%). In addition, the carbonyl region of the ^{13}C NMR spectrum of the product indicated the presence of significant amounts of the regioisomeric product LPPC. It is thought that the small amount of 1-lysophosphatidylcholine present reacts preferentially, due to the superior nucleophilicity of its primary hydroxyl group. Increasing the amount of nucleophilic catalyst (1 equivalent) improved the yield of the reaction (25%), but also resulted in the formation of numerous byproducts, and again a significant amount LPPC as estimated by ^{13}C NMR. A method reported by Vodovozova and Molotkovsky for the acylation of 2-lysophosphatidylcholine without the need for an excess of fatty acid was adopted.[42] The method utilises an alternative nucleophilic catalyst, 4-hydroxypyridine. In the presence of *N*,*N*′-dicyclohexylcarbodiimide and an excess of 4-hydroxypyridine, *O*-acylation occurs via formation of a stable, activated 4-hyroxypyridine ester. The 4-hydroxypyridine also acts as a mild base to catalyse transesterification, while resulting in minimal by-product formation. The esterification of **19** with 1.05 equivalents of **6**, 1.5 equivalents of *N*,*N*′-dicyclohexylcarbodiimide and 5 equivalents of 4-hydroxypyridine afforded **20** in significantly improved yield (74%).

Differentiation and quantification of regioisomeric diacylglycerol phospholipids is immensely challenging. For instance, comparison of the NMR spectra of commercially sourced POPC and OPPC shows no difference in the ^{1}H, ^{31}P NMR spectra and only marginal differences in the ^{13}C spectra. The two carbonyl carbons of POPC and OPPC are separable, and can be used to estimate the ratio of the two species. The carbonyl region of the ^{13}C NMR spectrum of **20** indicated the formation of one predominant regioisomer. However, due to the low abundance of the ^{13}C isotope, accurate quantification is not possible. Combinations of collision-induced dissociation (CID) and ozone-induced dissociation (OzID) mass spectrometry yielded diagnostic fragment ions for assigning regiochemistry, which indicated the presence of (86 ± 2)% PLPC.[43] For confirmation, a complementary MS technique was performed, via online enzymatic digestion with phospholipase A1 (PLA1).[44] High resolution mass spectrometric measurements of the relative abundance of the resulting 1-lysophosphatidylcholine products products is indicative of the initial PLPC : LPPC ratio. The value obtained from this method was (89 ± 2)% PLPC. Detailed analysis is provided in the Supporting Information. Both mass spectrometry-based methods yield consistent results, demonstrating the success of the synthetic strategy for minimising acyl migration and the formation of LPPC.

Conclusion

A route to synthesise perdeuterated linoleic acid **6** on a gram-scale has been developed. Decarboxylative alkynylation has been employed along with optimised methods to utilise easily accessible deuterated saturated fatty acid building blocks. The modular nature of the synthesis can be exploited to access linoleic acid derivatives with selective deuteration in different portions of the molecule. The use of linoleic acid-d_{31} to produce deuterated materials was demonstrated by the synthesis of chain deuterated PLPC-d_{62} **20**. High yields (74%) of the phospholipid were obtained using only a stoichiometric amount of the precious labelled unsatu-

rated fatty acid. Perdeuterated linoleic acid-d_{31} and its chain deuterated phospholipid, PLPC-d_{62} were fully characterised by NMR and IR spectroscopy and mass spectrometry. The regiopurity of the mixed acyl glycerophospholipid was accurately determined by two complementary mass spectrometry techniques. The highly deuterated products will expedite chemical or biomolecular studies using neutron scattering techniques, infrared spectroscopy or NMR to answer important questions about membrane structure and function and lipid metabolism. The methods reported can be applied to the synthesis of other deuterated polyunsaturated lipids of interest such as 1,2-dilinoleoyl-*sn*-glycero-3-phosphocholine, glyceryl monolinoleate and linolein.

Experimental Section

General Experimental

All reactions were performed under an atmosphere of nitrogen unless otherwise specified. Chemicals and reagents of the highest grade were purchased from Sigma-Aldrich (Sydney, Australia) and were used without further purification. Solvents were purchased from Sigma-Aldrich and Merck. NMR solvents were purchased from Cambridge Isotope Laboratories Inc. (MA, USA) and Sigma-Aldrich and were used without further purification. D_2O (99.8%) was supplied by AECL, Canada. Anhydrous dichloromethane, tetrahydrofuran and diethyl ether were obtained from a LC Technology Solutions Inc. SP-1 Stand Alone Solvent Purification System. Analytical thin-layer chromatography (TLC) was performed using Merck aluminium backed silica gel 60 F_{254} (0.2 mm) plates, which were visualised with shortwave (254 nm) ultraviolet light or with potassium permanganate, vanillin, Hanessian's or bromocresol green stains. Flash column chromatography was performed using Merck Kieselgel 60 (230-400 mesh) silica gel, with the eluent mixture reported as the volume:volume ratio. Hydrothermal reactions were performed in D_2O at high temperatures by mixing the appropriate fatty acid with NaOD and Pt/C (10% w/w) in a Mini Benchtop 4560 Parr reactor (600 mL vessel capacity, 3000 psi maximum pressure, 350 °C maximum temperature) (Moline, USA). Infrared absorption spectra were recorded on a Thermo Scientific NicoletTM iSTM10 FTIR spectrometer as a solid or a thin film from ethanol, and the data are reported as vibrational frequencies (cm^{-1}). Nuclear magnetic resonance spectra were recorded at 300 K using either a Bruker AVANCE DRX400 (400 MHz) spectrometer. ^1H chemical shifts are expressed as parts per million (ppm) with residual chloroform (δ 7.26), and dimethyl sulfoxide (δ 2.50) as reference and are reported as chemical shift (δ); relative integral; multiplicity; coupling constants (*J*) reported in Hz. ^{13}C chemical shifts are expressed as parts per million (ppm) with residual chloroform (δ 77.16) and dimethyl sulfoxide (δ 39.52) as reference and reported as chemical shift (δ); multiplicity. ^2H chemical shifts are reported as parts per million (ppm) and are reported as chemical shift (δ); multiplicity. Low-resolution mass spectrometry (LRMS) was recorded using electrospray ionisation (ESI) recorded on a 4000 QTrap mass spectrometer (AB Sciex). The overall percentage deuteration of the molecules were calculated by MS using the isotope distribution analysis of the different isotopologues. This was calculated taking into consideration the ^{13}C natural abundance, whose contribution was subtracted from the peak area of each M + 1 isotopologue to allow for accurate estimation of the percentage deuteration of each isotopologue. High resolution mass spectrometry was performed on an Orbitrap Elite high-resolution MS with robotic nanoESI source (Advion Triversa NanoMate). Regiopurity analysis was performed on an Orbitrap Elite high-resolution MS with robotic nanoESI source (Advion Triversa NanoMate). Optical rotation was measured using a Rudolph Research Analytical Autopol III automatic polarimeter. Specific rotations based on the equation $[\alpha] = (100\alpha)/(lc)$ are reported as unitless numbers and are in the form: $[\alpha]_D^T \pm xx$ (c, solvent), where c is the concentration in g/l00 mL the path length, l, is in decimetres and T is temperature.

Azelaic Acid-d_{14} (10)

Azelaic acid-d_{14} was prepared using previously reported methods,[25] **^1H NMR** (400 MHz, MeOD) δ 2.24 (bs, residual), 1.55 (bs, residual), 1.29 (bs, residual) ppm; **^2H NMR** (61.4 MHz, MeOD) δ 2.23 (4D, s), 1.54 (4D, s), 1.28 (6D s) ppm; **$^{13}C\{^1H, ^2H\}$ NMR** (100.6 MHz, $CDCl_3$) δ 177.8, 34.1, 28.7, 24.9 ppm; **ESI-MS**: $[M–H]^-$ 201.2 (major isotopologue), 98.5%D.

9-Methoxy-9-oxononanoic Acid-d_{14} (11)

The monoester of azelaic acid-d_{14} was prepared using previously reported methods,[25] **^1H NMR** (400 MHz, $CDCl_3$) δ 3.65 (3 H, s), 2.30 (bs, residual), 2.26 (b s, residual), 1.56 (bs, residual), 1.25 (bs, residual) ppm; **^2H NMR** (61.4 MHz, $CDCl_3$) δ 2.46–2.10 (4D, bs), 1.56 (4D, s), 1.26 (6D, s) ppm; **$^{13}C\{^1H, ^2H\}$ NMR** (100.6 MHz, $CDCl_3$) δ 180.1, 174.5, 51.6, 33.4, 33.3, 27.7, 27.6, 23.9, 23.6 ppm.

1-Methyl 9-(4,5,6,7-tetrachloro-1,3-dioxoisoindolin-2-yl) nonanedioate-d_{14} (12)

To a solution of monoester **11** (5 g, 23.1 mmol) and *N*-hydroxytetrachlorophthalimide (7.0 g, 23.1 mmol) in anhydrous dichloromethane (100 mL) at 0 °C was added 4-dimethylaminopyridine (280 mg, 2.3 mmol) followed by a solution of *N*,*N*′-dicyclohexylcarbodiimide (5.25 g, 25.4 mmol) in anhydrous dichloromethane (20 mL), dropwise. The mixture was warmed to room temperature and stirred for 8 hours. The suspension was filtered through a pad of silica, and eluted with a mixture of dichloromethane, hexane (4 : 1). The filtrate was concentrated under reduced pressure to obtain the activated ester **12** (9.9 g, 86%) as an off-white solid, **^1H NMR** (400 MHz, $CDCl_3$) δ 3.66 (3 H, s), 2.62 (bs, residual), 2.27 (bs, residual), 1.80-1.70 (m, residual), 1.66–1.55 (m, residual), 1.48-1.21 (m, residual) ppm; **^2H NMR** (61.4 MHz, $CDCl_3$) δ 2.62 (2D, bs), 2.28 (2D, bs), 1.73 (2D, bs), 1.59 (2H, bs), 1.48-1.20 (6D, m) ppm; **$^{13}C\{^1H, ^2H\}$ NMR** (100.6 MHz, $CDCl_3$) δ 174.4, 169.3, 157.7, 141.1, 130.6, 124.9, 51.6, 33.3, 30.2, 27.6, 27.5, 27.4, 23.8, 23.7 ppm.

Methyl dec-9-ynoate-d_{14} (8)

A mixture of dried lithium chloride (1.05 g, 24.8 mmol) and zinc chloride (3.4 g, 24.9 mmol) was dissolved in anhydrous tetrahydrofuran (25 mL, 1 M) and treated with ethynyl magnesium bromide (0.37 M in THF, 66.9 mL, 24.8 mmol) and stirred at room temperature for 30 minutes. An aliquot was titrated with iodine[45] (0.26 M).

In a separate flask a solution of nickel chloride hexahydrate (560 mg, 2.34 mmol) and 4,4'-dimethoxy-2,2'-bipyridine (510 mg, 2.34 mmol) in anhydrous *N*,*N*-dimethylformamide (59 mL) was stirred for 15 minutes at room temperature (green solution).

A separate flask was charged with TCNHPI ester **12** (5.8 g, 11.7 mmol) and cooled to 0 °C. The flask was evacuated and backfilled with nitrogen three times. The premixed nickel solution and ethynyl zinc chloride (90 mL) solution were added in quick succession (dark brown solution). The reaction mixture was stirred at room temperature for 16 hours. The mixture was quenched with dilute hydrochloric acid (1 M, 100 mL) and extracted with diethyl ether (3 × 100 mL). The combined organic extracts were washed with aqueous lithium chloride (1 M, 100 mL) and brine (100 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude residue was purified by flash column chromatography using dichloromethane, hexane (0 : 1 to 1 : 1) as an eluent to obtain the terminal alkyne **8** (1.4 g, 61%) as a yellow liquid, **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.65 (3 H, s), 2.26 (bs, residual), 2.13 (bs, residual), 1.92 (1H, s), 1.56 (bs, residual), 1.46 (bs, residual), 1.36-1.21 (m, residual) ppm; **^{2}H NMR** (61.4 MHz, $CDCl_3$) δ 2.26 (2D, bs), 2.13 (2D, bs), 1.57 (2D, bs), 1.46 (2D, bs), 1.40–1.15 (6D, m) ppm; **^{13}C{^{1}H, ^{2}H} NMR** (100.6 MHz, $CDCl_3$) δ 174.5, 84.8, 68.2, 51.6, 33.4, 27.8, 27.5, 27.3, 27.3, 23.9, 17.7 ppm; **ESI-MS**: $[M+Na]^+$ 219.4 (major isotopologue).

Pentanoic Acid-d_9 (9)

Pentanoic acid (13 g, 127 mmol), sodium hydroxide (6.1 g, 153 mmol) and platinum on activated carbon (10% w/w, 0.6 g) were added to a 600 mL Parr reactor vessel and suspended in deuterium oxide (100 mL). The vessel was flushed with nitrogen and heated at 220 °C for 3 days. The mixture was cooled to room temperature, acidified with hydrochloric acid (5 M), diluted with ethyl acetate (100 mL) and stirred for 10 minutes. The biphasic mixture was filtered through a pad of Celite®. The filtrate was transferred to a separatory funnel and the layers separated. The aqueous phase was extracted with ethyl acetate (3 × 50 mL), the organic phases combined, dried over anhydrous magnesium sulfate and concentrated under reduced pressure, to obtain pentanoic acid-d_9 (87%D by MS). The material was subjected to another cycle using the same conditions as above to obtain **9** (96.8%D, quant.) as a colourless liquid, **^{1}H NMR** (400 MHz, $CDCl_3$) δ 2.31 (bs, residual), 1.57 (bs, residual), 1.31 (bs, residual), 0.86 (bs, residual) ppm; **^{2}H NMR** (61.4 MHz, $CDCl_3$) δ 2.32 (2D, bs), 1.57 (2D, bs), 1.32 (2D, bs), 0.87 (3D, s) ppm; **^{13}C{^{1}H, ^{2}H} NMR** (100.6 MHz, $CDCl_3$) δ 180.6, 33.1, 25.7, 21.0, 12.6 ppm; **ESI-MS**: $[M-H]^-$ 110.2, overall deuteration level 96.8%D, isotopologue distribution 71% d_9, 29% d_8.

Pentyl 4-methylbenzenesulfonate-d_9 (13)

Lithium aluminium deuteride (3.4 g, 80.9 mmol) was suspended in anhydrous diethyl ether (60 mL) and cooled to 0 °C. A solution of pentanoic acid-d_9 (**9**) (5 g, 45 mmol) in diethyl ether (20 mL) was added dropwise. The mixture was warmed to room temperature and stirred for 16 hours. The reaction was cooled to 0 °C and quenched with water (3.4 mL), aqueous sodium hydroxide (15% w/w. 3.4 mL) and water (10.2 mL). The suspension was warmed to room temperature and stirred for 15 minutes. Anhydrous magnesium sulfate was added and the mixture stirred for a further 10 minutes before the solids were removed by filtration through a pad of Celite®. The filtrate was concentrated under reduced pressure until approximately 30 mL of diethyl ether remained.

To the solution was added triethylamine (9.4 mL, 67.5 mmol) and 4-dimethylaminopyridine (550 mg, 4.45 mmol) and the mixture cooled to 0 °C. A solution of 4-toluenesulfonyl chloride (10.3 g, 54 mmol) in anhydrous dichloromethane (50 mL) was added dropwise. The reaction mixture was warmed to room temperature and stirred for 24 hours. The suspension was poured into dilute hydrochloric acid (1 M, 50 mL), extracted with dichloromethane (3 × 50 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:1 to 1:9) as an eluent to obtain tosylate **13** (8.9 g, 78%) as a colourless oil, **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.78 (2H, d, J = 8.3 Hz), 7.34 (2H, d, J = 8.0 Hz), 2.44 (3H, s), 1.58 (bs, residual), 1.23–1.17 (m, residual), 0.79 (bs, residual) ppm; **^{2}H NMR** (61.4 MHz, $CDCl_3$) δ 4.00 (2D, bs), 1.59 (2D, bs), 1.32-1.12 (4D, m), 0.80 (3D, s) ppm; **^{13}C{^{1}H, ^{2}H} NMR** (100.6 MHz, $CDCl_3$) δ 144.7, 133.4, 129.9, 128.0, 70.1, 27.5, 26.2, 21.7, 20.8, 12.8 ppm.

2-(Prop-2-yn-1-yloxy)Tetrahydro-2H-pyran-d_2 (14)

The protected propargyl alcohol **14** was prepared using previously reported methods,[31] **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.81 (^{1}H, t, J = 3.4 Hz), 3.87-3.78 (1H, m), 3.57-3.49 (1H, m), 2.39 (1H, s), 1.89-1.46 (6H, m) ppm; **^{2}H NMR** (61.4 MHz, $CDCl_3$) δ 4.24 (2D, d, J = 2.7 Hz) ppm; **^{13}C{^{1}H, ^{2}H} NMR** (100.6 MHz, $CDCl_3$) δ 96.9, 79.8, 74.1, 62.1, 53.6, 30.3, 25.5, 19.1 ppm.

Oct-2-yn-1-ol-d_{13} (15)

The protected propargyl alcohol **14** (4.8 g, 33.8 mmol) was dissolved in anhydrous tetrahydrofuran (10 mL) and cooled to 0 °C. *n*-Butyl lithium (1.45 M in cyclohexane, 25 mL, 36.4 mmol) was added dropwise and the mixture stirred for 30 minutes before *N*-methyl-2-pyrrolidone (10 mL) was added. A solution of the tosylate **13** (6.6 g, 26.0 mmol) in *N*-methyl-2-pyrrolidone (10 mL) was added dropwise and the mixture was slowly brought to room temperature and stirred for 16 hours. The reaction mixture was cooled again to 0 °C and quenched with saturated aqueous ammonium chloride (5 mL), diluted with water (20 mL) and extracted with diethyl ether (3 × 30 mL). The combined organic extracts were washed with saturated aqueous lithium chloride (20 mL), dried over anhydrous magnesium sulfate and concentrated. The residue was

filtered through a plug of silica eluting with ethyl acetate, hexane (1:4). The filtrate was concentrated under reduced pressure and the crude protected alcohol used immediately without further purification or characterisation.

The crude product was taken up in methanol (60 mL) and treated with 4-toluenesulfonic acid monohydrate (980 mg, 5.6 mmol) and the mixture stirred at room temperature for 2 hours. The reaction was quenched with saturated aqueous sodium hydrogen carbonate (20 mL) and the methanol removed under reduced pressure. The aqueous residue was extracted with ethyl acetate (3×20 mL), dried over anhydrous magnesium sulfate and concentrated. The crude product was purified by flash column chromatography using ethyl acetate, hexane (0:1 to 1:9) as an eluent to obtain the title compound **15** (2.08 g, 57%) as a light yellow liquid, **^{1}H NMR** (400 MHz, $CDCl_3$) δ 2.16 (bs, residual), 1.63 (1H, s), 1.45 (bs, residual), 1.29 (bs, residual), 1.26 (bs, residual), 0.83 (bs, residual) ppm; **^{2}H NMR** (61.4 MHz, $CDCl_3$) δ 4.22 (2D, s), 2.16 (2D, bs), 1.46 (2D, bs), 1.37-1.18 (4D, m), 0.85 (3D, s) ppm; **^{13}C{^{1}H, ^{2}H} NMR** (100.6 MHz, $CDCl_3$) δ 86.7, 78.3, 51.0, 29.8, 27.2, 21.0, 18.0, 12.9 ppm.

1-Bromooct-2-yne-d_{13} (7)

Triphenylphosphine (4 g, 15.5 mmol) was dissolved in anhydrous dichloromethane (70 mL) and cooled to 0 °C. To this was added bromine (0.8 mL, 15.5 mmol) dropwise until a yellow colour persisted. A small amount of triphenylphosphine was added to return the solution to colourless. A solution of the alcohol **15** (2 g, 14.4 mmol) in dichloromethane (10 mL) was added dropwise and the solution stirred for 30 minutes. Hexane (100 mL) was added and the resulting suspension filtered through a pad of silica, eluting with ethyl acetate, hexane (1:19). The filtrate was concentrated under reduced pressure to obtain the alkyl bromide **7** (2.66 g, 92%) as a colourless liquid, **^{1}H NMR** (400 MHz, $CDCl_3$) δ 2.19 (bs, residual), 1.46 (bs, residual), 1.30 (bs, residual), 0.84 (bs, residual) ppm; **^{2}H NMR** (61.4 MHz, $CDCl_3$) δ 3.92 (2D, s), 2.19 (2D, bs), 1.46 (2D, bs), 1.39–1.18 (4D, m), 0.85 (3D, s) ppm; **^{13}C{^{1}H, ^{2}H} NMR** (100.6 MHz, $CDCl_3$) δ 88.5, 75.3, 29.8, 27.0, 21.0, 18.3, 15.6, 12.9 ppm.

Methyl octadeca-9,12-diynoate-d_{27} (16)

A solution of terminal alkyne **8** (1.4 g, 7.13 mmol) and propargyl bromide derivative **7** (1.9 g, 9.24 mmol) in degassed, anhydrous *N,N*-dimethylformamide (12 mL) was added to a flask charged with copper(I) iodide (2.85 g, 15.0 mmol), sodium iodide (2.2 g, 15.0 mmol) and potassium carbonate (1.58 g, 11.4 mmol). The suspension was stirred at room temperature for 16 hours. The reaction was quenched with saturated aqueous ammonium chloride (10 mL), extracted with diethyl ether (3×10 mL), washed with saturated aqueous lithium chloride (20 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude residue was purified by flash column chromatography using diethyl ether, hexane (0:1 to 3:97) as an eluent to obtain the skipped diyne **16** (1.79 g, 79%) as a light yellow oil, **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.65 (3H, s), 2.26 (bs, residual), 2.10 (bs, residual), 1.43 (bs, residual), 1.30-1.21 (m, residual), 0.84 (bs, residual) ppm; **^{2}H NMR** (61.4 MHz, $CDCl_3$) δ 3.08 (2D, bs), 2.26 (2D, bs), 2.10 (4D, bs), 1.56 (2D, bs), 1.43 (4D, bs), 1.37-1.08 (10D, m), 0.84 (3D, s) ppm; **^{13}C{^{1}H, ^{2}H} NMR** (100.6 MHz, $CDCl_3$) δ 174.5, 80.6, 80.5, 74.6, 74.5, 51.5, 33.4, 29.8, 27.8, 27.6, 27.6, 27.5, 27.4, 23.9, 21.0, 18.0, 18.0, 12.9, 9.4 ppm; **ESI-MS**: $[M+Na]^+$ 340.6 (major isotopologue).

Ethyl Linoleate-d_{27} (17)

Nickel acetate tetrahydrate (63 mg, 0.252 mmol) was dissolved in ethanol (0.7 mL) with gentle heating. The system was evacuated and backfilled with hydrogen gas three times. A solution of sodium borohydride (1 M, 0.38 mL) in ethanol was added dropwise with liberation of hydrogen gas. The system was evacuated and backfilled with hydrogen gas three times and stirred for 30 minutes. Ethylenediamine (67 μL, 1.0 mmol) was added in one portion and the system was evacuated and backfilled with hydrogen gas three times and stirred for 5 minutes before a solution of the skipped diyne **16** (80 mg, 0.252 mmol) in ethanol (0.5 mL) was added. The system was evacuated and backfilled with hydrogen gas three times and the reaction stirred under a hydrogen atmosphere for 16 hours. The reaction mixture was filtered through a pad of silica, eluting with diethyl ethyl, hexane (1:9) and the filtrate concentrated under reduced pressure to obtain the ethyl ester **17** (68 mg, 80%) as a colourless oil, **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.41–5.26 (4H, m), 4.11 (2H, q, $J=7.2$ Hz), 1.24 (3H, t, $J=7.2$ Hz) ppm; **^{2}H NMR** (61.4 MHz, $CDCl_3$) δ 2.74 (2D, bs), 2.24 (2D, bs), 2.00 (4D, bs), 1.56 (2D, bs), 1.41-1.05 (14D, m), 0.83 (3D, s) ppm; **^{13}C{^{1}H, ^{2}H} NMR** (100.6 MHz, $CDCl_3$) δ 174.1, 130.3, 130.1, 128.1, 128.0, 60.2, 33.7, 30.3, 28.5, 28.3, 28.0, 27.9, 27.9, 26.4, 26.4, 25.1, 24.0, 21.4, 14.4, 13.0 ppm; **ESI-MS**: $[M+Na]^+$ 358.7 (major isotopologue).

Ethyl Linoleate-d_{31} (18)

Nickel acetate tetrahydrate (1.25 g, 5.04 mmol) was dissolved in ethanol (5 mL) with gentle heating. The system was evacuated and backfilled with deuterium gas three times. A solution of sodium borohydride (1 M, 7.56 mL) in ethanol was added dropwise with liberation of hydrogen gas. The system was evacuated and backfilled with deuterium gas three times and stirred for 30 minutes. Ethylenediamine (1.35 mL, 20.2 mmol) was added in one portion and the system was evacuated and backfilled with deuterium gas three times and stirred for 5 minutes before a solution of the skipped diyne **16** (1.6 g, 5.04 mmol) in ethanol (3 mL) was added. The system was evacuated and backfilled with deuterium gas three times and the reaction stirred under a deuterium atmosphere for 16 hours. The reaction mixture was filtered through a pad of silica, eluting with diethyl ethyl, hexane (1:9) and the filtrate concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silver nitrate impregnated silica using diethyl ether, hexane (0:1 to 1:9) as an eluent to obtain the ethyl ester **18** (1.47 g, 86%) as a light yellow oil, **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.35 (residual), 4.11 (2H, q, $J=7.2$ Hz), 2.73 (residual), 2.26 (residual), 1.99 (residual), 1.57 (residual), 1.24 (3H, t, $J=7.2$ Hz), 0.83 (residual) ppm; **^{2}H NMR** (61.4 MHz, $CDCl_3$) δ 5.52-5.21 (4D, m), 2.74 (2D, bs), 2.25 (2D, bs), 2.00 (4D, bs), 1.57 (2D, bs),

1.41–1.05 (14D, m), 0.84 (3D, s) ppm; **^{13}C{^{1}H, ^{2}H} NMR** (100.6 MHz, $CDCl_3$) δ 174.1, 129.8, 129.6, 127.6, 127.5, 60.3, 33.7, 30.3, 28.5, 28.3, 28.0, 27.9, 27.9, 26.2, 26.2, 24.9, 24.0, 21.4, 14.4, 13.0 ppm; **ESI-MS**: $[M+Na]^+$ 362.7 (major isotopologue).

Linoleic Acid-d_{31} (6)

The ethyl ester **18** (1.45 g, 4.27 mmol) was dissolved in methanol (60 mL) and treated with an aqueous solution of lithium hydroxide monohydrate (2.4 M, 4.1 mL) and the mixture was stirred at room temperature overnight. The reaction mixture was acidified with dilute aqueous hydrochloric acid and the volatiles removed under reduced pressure. The aqueous residue was extracted with diethyl ether (3 × 50 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate, dichloromethane (0 : 1 to 1 : 9) as an eluent to obtain the title compound (1.28 g, 96%) as a very faint yellow oil, **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.34 (residual), 2.31 (residual), 2.00 (residual), 1.59 (residual), 1.35-1.20 (residual), 0.83 (residual) ppm; **^{2}H NMR** (61.4 MHz, $CDCl_3$) δ 5.56–5.24 (4D, m), 2.74 (2D, bs), 2.32 (2D, bs), 2.00 (4D, bs), 1.59 (2D, bs), 1.47–1.04 (14D, m), 0.84 (3D, s) ppm; **^{13}C{^{1}H, ^{2}H} NMR** (100.6 MHz, $CDCl_3$) δ 180.2, 129.8, 129.6, 127.7, 127.5, 33.4, 30.3, 28.5, 28.3, 28.0, 27.9, 27.9, 26.3, 26.2, 24.9, 23.7, 21.4, 13.0 ppm; **ESI-MS**: $[M-H]^-$ 310.5, overall deuteration level 97.6%D, isotopologue distribution 47.5% d_{31}; 35.9% d_{30}, 12.6% d_{29}, 3.2% d_{28}, 0.9% d_{27}.

Palmitic Acid-d_{31} (21)

Palmitic acid-d_{31} was prepared using previously reported methods,[46] **^{1}H NMR** (400 MHz, $CDCl_3$) δ 2.31 (bs, residual), 1.58 (bs, residual), 1.31-1.12 (m, residual), 0.82 (bs, residual) ppm; **^{2}H NMR** (61.4 MHz, $CDCl_3$) δ 2.31 (2D, s), 1.58 (2D, s), 1.40–1.02 (24D, m), 0.82 (3D, s) ppm; **^{13}C{^{1}H, ^{2}H} NMR** (100.6 MHz, $CDCl_3$) δ 179.9, 33.4, 30.6, 28.4, 23.8, 21.5, 13.0, ppm; **ESI-MS**: $[M-H]^-$ 286.5 (major isotopologue), 97.8%D.

*1-Palmitoyl-*sn*-glycero-3-phosphocholine-d_{31} (19)*

A suspension of L-α-glycerophosphorylcholine (3.2 g, 12.44 mmol) and dibutyltin oxide (3.41 g, 13.68 mmol) in anhydrous isopropanol (130 mL) was heated at reflux for 1 hour.

Palmitoyl chloride-d_{31} was prepared from **21** (4.65 g, 16.17 mmol) by treatment with thionyl chloride (11.7 mL, 161.7 mmol) and *N*,*N*-dimethylformamide (2 drops). The mixture was stirred at reflux for 3 hours before excess thionyl chloride was removed *in vacuo*.

The solution of L-α-glycerophosphorylcholine and dibutyltin oxide was cooled to room temperature and treated with triethylamine (2.1 mL, 14.93 mL) followed by the freshly prepared palmitoyl chloride-d_{31}. The white suspension was stirred at room temperature for 30 minutes. The reaction was quenched with water (130 mL) and washed with heptane (130 mL). The water-alcohol solution was further washed with heptane (3 × 65 mL). The aqueous phase was freeze dried to obtain a crude residue (8.1 g), which was taken up in ethanol (30 mL) and precipitated with acetone (130 mL) at −20 °C. The resulting solid was suspended in anhydrous ethanol (30 mL) and dissolved with very gentle heating. The solution was allowed to age at 4 °C for 16 hours. The resulting solid was collected by filtration and dried *in vacuo*, to obtain the title compound (3.1 g, 47%) as a white powder, **^{1}H NMR** (400 MHz, $CDCl_3$, MeOD) δ 4.33–4.22 (2H, m), 4.08 (2H, d, J = 5.6 Hz), 4.00-3.89 (2H, m), 3.89-3.79 (1H, m), 3.69-3.62 (2H, m), 2.25 (residual), 1.51 (residual), 1.15 (residual), 0.78 (residual) ppm; **^{2}H NMR** (61.4 MHz, $CDCl_3$, MeOD) δ 2.23 (2D, bs), 1.50 (2D, bs), 1.32-1.00 (24D, m), 0.77 (3D, s) ppm; **^{31}P NMR** (161.9 MHz, $CDCl_3$, MeOD) δ 0.045 ppm; **^{13}C{^{1}H} NMR** (100.6 MHz, $CDCl_3$, MeOD) δ 174.3, 68.9 (d, J = 5.4 Hz), 67.1 (d, J = 5.7 Hz), 66.5 (m), 64.9, 59.2 (d, J = 5.3 Hz), 53.4 (t, J = 3.4 Hz), 28.2 (m) ppm; **ESI-MS**: $[M+Na]^+$ 549.9 *m/z*, isotopologue distribution 45.3% d_{31}; 36.0% d_{30}, 13.8% d_{29}, 3.8% d_{28}, 0.8% d_{27}, 0.3% d_{26}.

*1-Palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine-d_{62} (20)*

A solution of **6** (100 mg, 0.32 mmol) and **19** (161 mg, 0.31 mol) in anhydrous chloroform (4 mL) was cooled to 0 °C and treated with 4-hydroxypyridine (145 mg, 1.53 mmol) followed by a solution of *N*,*N*′-dicyclohexylcarbodiimide (95 mg, 0.46 mmol) in chloroform (2 mL), dropwise. The mixture was warmed to room temperature and stirred for 40 hours in the absence of light. The volatiles were removed under a stream of nitrogen and the residue purified by flash column chromatography using methanol with 0.4% water, dichloromethane (0:1 to 3:7) as an eluent to obtain the title compound (185 mg, 74%) as a colourless solid, R_f = 0.15, 3 : 7 MeOH with 0.4% H_2O, CH_2Cl_2); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.37-5.28 (residual), 5.21–5.12 (1H, m), 4.37 (1H, dd, J = 2.5, 12.2 Hz), 4.33–4.22 (2H, m), 4.10 (1H, dd, J = 7.4, 12.1 Hz), 4.10-3.83 (2H, m), 3.80–3.70 (2H, m), 3.33 (9H, s), 2.27–2.20 (residual), 2.00–1.96 (residual), 1.55–1.48 (residual), 1.33–1.14 (residual), 0.90–0.77 (residual) ppm; **^{2}H NMR** (61.4 MHz, $CDCl_3$) δ 5.55-5.22 (4D, m), 2.72 (2D, bs), 2.25 (4D, bs), 1.99 (4D, bs), 1.51 (4D, bs), 1.41–0.96 (38D, bs), 0.82 (6D, s) ppm; ^{31}P NMR (161.9 MHz, $CDCl_3$) δ −1.037 ppm; **^{13}C{^{1}H} NMR** (100.6 MHz, $CDCl_3$) δ 173.8, 173.4, 70.6 (d, J = 8.0 Hz), 66.4 (m), 63.5 (d, J = 4.5 Hz), 63.1, 59.5 (d, J = 5.6 Hz), 54.5, 28.3 (m) ppm; **^{13}C{^{1}H, ^{2}H} NMR** (100.6 MHz, $CDCl_3$) δ 173.8, 173.4, 129.8, 129.5, 127.6, 127.5, 70.6 (d, J = 8.0 Hz), 66.4 (m), 63.5 (d, J = 3.7 Hz), 63.1, 59.5 (d, J = 5.6 Hz), 54.5, 33.6, 33.5, 30.6, 30.2, 28.6, 28.5, 28.5, 28.5, 28.4, 28.2, 28.1, 28.1, 28.0, 28.0, 28.0, 26.3, 26.2, 24.9, 24.0, 23.9, 21.5, 21.4, 13.1, 13.0 ppm; **ESI-MS**: $[M+Na]^+$ 843 *m/z*, isotopologue distribution 16.7% d_{62}; 31.2% d_{61}, 26.4% d_{60}, 14.8% d_{59}, 6.3% d_{58}, 2.8% d_{57}, 1.1% d_{56}, 0.7% d_{55}; **HRMS**: Cald for $C_{42}H_{18}D_{42}NO_8PNa$: 842.9410, found 842.9401; $[\alpha]_D^{23}$: +6.13 (0.508, $CHCl_3$).

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