JOC The Journal of Organic Chemistry

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J. Org. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.joc.7b02169 • Publication Date (Web): 13 Nov 2017

Just Accepted

ACS Publications

The Journal of Organic Chemistry is published by the American Chemical Society.
1155 Sixteenth Street N.W., Washington, DC 20036

Site-specific deuteration of polyunsaturated alkenes

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Supporting Information Placeholder

ABSTRACT: Selective deuteration of drugs and biologically relevant molecules is increasingly becoming important in the pharmaceutical industry. Site-selective isotopic reinforcement of polyunsaturated fatty acids (PUFAs) at their bis-allylic sites has been identified as a unique approach in preventing oxidative damage in these molecules, which had been linked to neuronal and retinal diseases, atherosclerosis and aging. Typical methods for preparation of site-selectively deuterated PUFAs require rather long, laborious and expensive syntheses. In this report, we disclose a very efficient catalytic protocol for site-specific deuteration of PUFAs and analogous poly-alkenes under exceptional kinetic control. Deuterium oxide (D_2O) has been identified not only as a deuterium source but also as a crucial component in the overall reaction mechanism responsible for averting the formation of thermodynamically favored side-products.

INTRODUCTION.

The process of deuteration (or H/D exchange), which involves hydrogen (^{1}H) substitution with its heavier isotope deuterium (^{2}H or D), has found numerous applications in nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry, polymer science, etc.[1] Selective deuteration (H/D exchange at specific molecular sites) has been identified as one of the most promising tools in drug design to improve their pharmacokinetic and pharmacodynamic properties.[2] Recently, it has been shown that the lipid peroxidation process (LPO) of polyunsaturated fatty acids (PUFAs) that, for example, leads to impaired lipid membranes and protein/DNA damage,[3] can be inhibited by site-specific deuteration.[4] This is rather significant considering that the toxic effects of LPO are implicated in numerous pathologies including retinal and neuronal diseases, atherosclerosis and aging.[5] However, the preparation of PUFAs selectively deuterated at their bis-allylic sites has only been achieved through complex and multi-step synthetic procedures (full syntheses) limiting the accessibility of these materials.[4a,6] Therefore, the development of a one-step catalytic and selective H/D exchange process would be invaluable for further exploration and clinical applications of these biologically important molecules.

Catalytic deuteration of alkene-containing molecules (PUFAs are skipped poly-alkene species) has been exclusively performed by transition metal-based complexes.[7] However, to the best of our knowledge, none of reported catalytic systems is adequate for deuteration of PUFAs[6b] mainly due to the absence of kinetic control (producing only the thermodynamic products), which is required to keep the cis- and non-conjugated double bonds found in these molecules from forming more thermodynamically stable trans- and/or conjugated fragments. Herein, we wish to report a selective one-step H/D exchange process for various skipped poly-alkenes (including PUFAs) at their targeted bis-allylic sites (Table 1) under exceptional kinetic control using a Ru-based catalyst and deuterium oxide (D_2O). Detailed experimental and computational evidence suggested that D_2O not only acted as the deuterium source but it also prevented the formation of thermodynamic side products (trans-isomers and conjugated alkenes).

RESULTS AND DISCUSSION.

Our initial target was Grotjahn's catalyst **1** (Figure 1) as this complex was reported to deuterate allylic positions of several simple alkenes but in the absence of any kinetic control yielding only the most thermodynamically stable products.[7g,8] It was then not surprising to observe extensive alkene conjugation when a catalytic amount of **1** was introduced into an acetone-d_6 solution containing ethyl linoleate (**E-Lin**; entry 1, Table 1). However, as the imidazolyl moiety of **1** played a crucial role in the overall mechanism,[7g,8] we were hoping that a structural modification of this Ru-based complex would allow us to at least prevent alkene conjugation, which was considered as the most important kinetic impasse. Deconjugation would be quite difficult to achieve while *trans*-to-*cis* isomerization should be significantly

Figure 1. Various ruthenium-based complexes explored as deuteration catalysts.

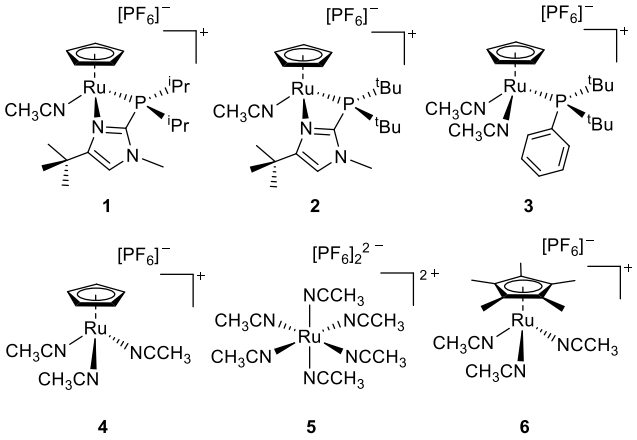
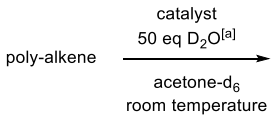
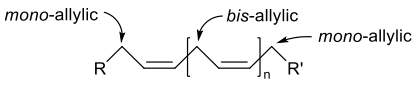
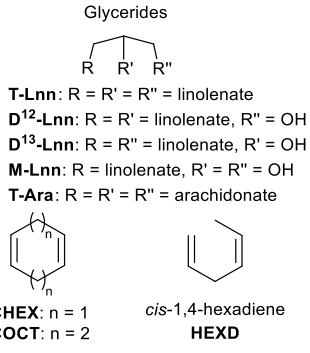


Table 1. General reaction conditions and examinable substrates for the target deuteration.





	n	R	R'
E-Lin	1	$(CH_2)_3CH_3$	$(CH_2)_6CO_2Et$
E-Lnn	2	CH_3	$(CH_2)_6CO_2Et$
A-Lnn	2	CH_3	$(CH_2)_6CO_2H$
O-Lnn	2	CH_3	$(CH_2)_6CH_2OH$
H-Lnn	2	CH_3	$(CH_2)_6CH_3$
E-Ara	3	$(CH_2)_3CH_3$	$(CH_2)_2CO_2Et$
E-DHA	5	CH_3	CH_2CO_2Et
A-DHA	5	CH_3	CH_2CO_2H
O-DHA	5	CH_3	CH_2CH_2OH
H-DHA	5	CH_3	CH_2CH_3



#	Complex (%)[b]	Substrate	Double bond conjugation	Time (h)	Yield (%)	Extent of deuteration (%) *Mono*-allylic	*Bis*-allylic
1	**1** (5%)	**E-Lin**	Yes[c] (82%)	170	n.d.	N/A[d]	N/A[d]
2	**2** (5%)	**E-Lin**	No	42	n.i.	82	0
3	**3** (5%)	**E-Lin**	No	16	n.i.	82	0
4	**4** (1%)	**E-Lin**	No	3	n.i.	95	0
5	**4** (1%)	**E-Lnn**	No	1	> 99	30 (34, 25)[e]	97
6	**4** (1%)	**E-Ara**	No	24	> 99	28 (32, 25)[e]	95
7	**4** (2%)	**E-DHA**	No	7	> 99	23 (34, 12)[e]	96
8[f]	**4** (1%)	**M-Lnn**	No	1	n.i.	10	94
9[f]	**4** (2%)	**D[12]-Lnn**	No	4	n.i.	23	95
10[f]	**4** (2%)	**D[13]-Lnn**	No	4	n.i.	15	94
11	**4** (1%)	**T-Lnn**	No	7	> 99	21	95
12	**4** (5%)	**T-Ara**	No	3	n.i.	19	96
13[g]	**4** (3%)	**E-Lnn** **E-Ara** **E-DHA**	No	1	n.i.	17	96
14	**4** (1 %)	**A-Lnn**	No	24	n.i.	10	76
15	**4** (1%)	**O-Lnn**	No	18	n.i.	22 (25, 19)[e]	96
16	**4** (1%)	**H-Lnn**	No	18	n.i.	31 (34, 28)[e]	97
17	**4** (2%)	**A-DHA**	No	24	n.i.	17 (26, 8)[e]	90
18	**4** (2%)	**O-DHA**	No	18	n.i.	47 (54, 39)[e]	98
19	**4** (2%)	**H-DHA**	No	18	n.i.	29 (34, 24)[e]	97
20	**5** (1%)	**E-Lnn**	No	24	n.d.	0	0
21	**6** (1%)	**E-Lnn**	No[h]	24	n.d.	N/A[d]	N/A[d]
22	**4** (1%)	**CHEX**	No	24	N/A	N/A	0
23	**4** (1%)	**COCT**	No	24	N/A	0	N/A
24[i]	**4** (1%)	**HEXD**	No	2	n.i.	95	0
25	**4** (1%)	***EE*-Lin**[j]	Yes[c] (75%)	72	n.d.	N/A[d]	N/A[d]
26[k]	**4** (1%)	**E-Lnn**	No	24	n.i.	n.d.	10

n.d. = not determined; n.i. = not isolated.[13] [a]equivalents of D_2O with respect to a *bis*-allylic proton. [b]% of complex with respect to the substrate. [c]regardless of the presence or absence of D_2O. [d]conjugation and/or *cis-trans* isomerization prevented the estimation on the % deuteration. [e]Combination of ^1H and ^{13}C NMR spectroscopies allowed for estimation of the deuteration percentage at the aliphatic *mono*-allylic position on one end and acid/ester/alcohol/reduced-aliphatic *mono*-allylic position on the other end for selected substrates. [f]20 equivalents of D_2O. [g] 1:1:1 mol ratio of the substrates. [h]*cis-trans* isomerization observed with no conjugation. [i]10 equivalents of D_2O. [j]***EE*-Lin** is the *trans,trans*-isomer of **E-Lin**. [k]Methanol-d_4 (100 equivalents with respect to a *bis*-allylic proton) was used instead of D_2O.

more feasible.[9] Thus, complex **2** (Figure 1) was prepared by replacing the phosphine's isopropyl groups of **1** with *tert*-butyl substituents. After mixing complex **2**, **E-Lin** and D_2O in acetone-d_6 it was possible to observe H/D exchange solely at the PUFA's *mono*-allylic positions (~ 82%, entry 2, Table 1). More importantly, there were absolutely no signs of any alkene *cis*-to-*trans* isomerization or conjugation. This particular observation suggested that the imidazolyl moiety of the phosphine ligand found in **2** might not have been involved in the deuteration mechanism.

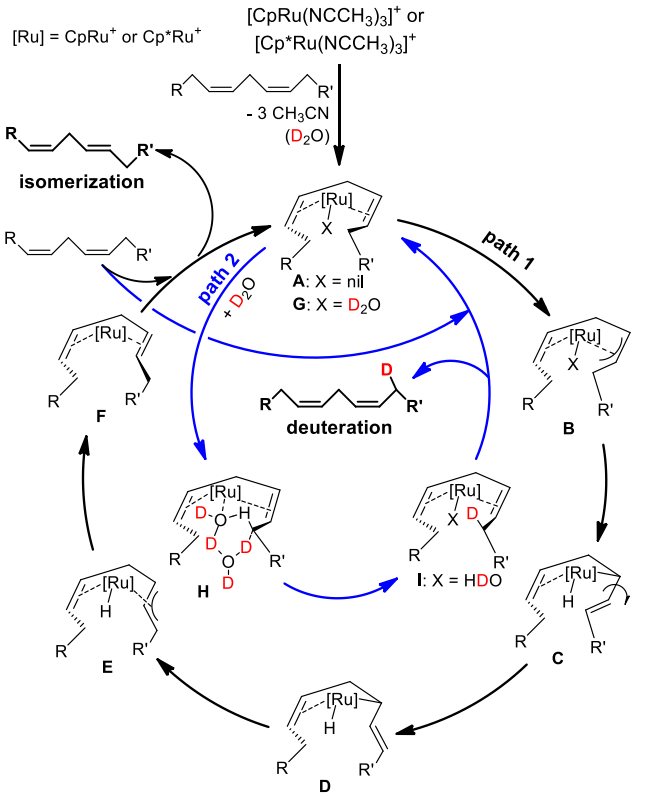
In order to probe this hypothesis, we prepared complex **3** (Figure 1) by replacing the imidazolyl fragment of **2** with a phenyl group. Indeed, this structural modification not only maintained the same deuteration selectivity and the kinetic control but it also resulted in a faster H/D exchange rate (entry 3, Table 1), further implying that the entire phosphine ligand was redundant. It was then not surprising that both **2** and **3** were outperformed by their common synthetic precursor i.e. phosphine-free complex **4** (Figure 1) with respect to the rate of deuteration of the *mono*-allylic positions of **E-Lin** (entry 4, Table 1). This collective evidence implied that the $[CpRu]^+$ fragment (Cp = cylopentadientyl) was the real catalyst and that complexes **2**, **3** and **4** could be viewed as pre-catalysts.

Even though only the *mono*-allylic positions of **E-Lin** were deuterated, we hypothesized that, based on the identity of a key intermediate (see below), it would be possible to perform H/D exchange at the *bis*-allylic positions of substrates that contain three or more double bonds. Indeed, when **E-Lin** was replaced with ethyl linolenate (**E-Lnn**), 97% of *bis*-allylic and 30% of *mono*-allylic protons underwent H/D exchange in just 1 hour at room temperature using a catalytic amount (1%) of **4**, signifying our targeted deuteration selectivity (entry 5, Table 1). Additionally, ethyl arachidonate (**E-Ara**, entry 6, Table 1), ethyl docosahexaenoate (**E-DHA**, entry 7, Table 1), mono- (**M-Lnn**, entry 8, Table 1), 1,2-di- (**D^{12}-Lnn**, entry 9, Table 1), 1,3-di- (**D^{13}-Lnn**, entry 10, Table 1) and tri-glycerides of linolenic (**T-Lnn**; entry 11, Table 1) and arachidonic (**T-Ara**; entry 12, Table 1) acids were successfully and selectively deuterated at their *bis*-allylic positions with complex **4**. More importantly, the selective H/D exchange using a mixture of **E-Lnn**, **E-Ara** and **E-DHA** (mol ratio of 1:1:1, entry 13, Table 1) was also achieved, demonstrating an immense potential of this method to avoid costly PUFA separations. Furthermore, the alcohol (**O-Lnn** and **O-DHA**; entries 15 and 18) and hydrocarbon (**H-Lnn** and **H-DHA**; entries 16 and 19) analogues of **E-Lnn** and **E-DHA** were also adequate substrates for the target deuteration. On the other hand, deuteration of the free acid analogues of these substrates (i.e. **A-Lnn** and **A-DHA**, entries 14 and 17) were performed at slower reaction rates. This is because 0.3 to 1% of these acids were expected to dissociate into the corresponding carboxylates (pK_a values for fatty acids were reported to be between 4 and 5)[10] which would then compete with the alkene fragments for the coordination to the ruthenium centre.[11] It is also noteworthy that the average deuteration percentage at the *bis*-allylic positions was around 95% while the *mono*-allylic positions were deuterated at ~ 30% or less for selected substrates. A higher degree of deuteration (above 97%) at the *bis*-allylic positions was achievable but with loss of selectivity with respect to the *mono*-allylic positions (see, for example, entries 16 and 18, Table 1). In fact, when reaction mixtures were left for extended periods of time (~ 168 h) the deuteration percentage of *mono*-allylic site never exceeded 50% and we never observed any decrease in deuteration at both *mono*- and *bis*-allylic sites during these experiments. It is also worth noting that using ^{13}C NMR spectroscopy[12] it was possible to estimate the relative percentage of deuteration at different (the aliphatic vs ester/acid/alcohol/reduced-aliphatic ends of substrate molecules) *mono*-allylic positions. In all cases, the *mono*-allylic sites with a longer chain or presence of acid/ester/alcohol groups were deuterated to a lesser extent presumably due to a higher steric influence (e.g. entry 7, Table 1).

Furthermore, a series of control experiments has been performed by using H_2O instead of D_2O in order to examine whether any *cis-trans* isomerization and/or conjugation occurred in the course of deuteration. Detrimental side effects could be easily detected by ^1H and ^{13}C NMR spectroscopies. For example, δ_C signals assigned to allylic positions of PUFAs would be downfield shifted by about 5 ppm for each adjacent double bond that was isomerized from *cis* to *trans*.[14] Additionally, if double bond migration and consequent conjugation were to occur during these experiments it would be highlighted by disappearance of the δ_H and δ_C resonances associated with the *bis*-allylic positions followed by noticeable shifts of vinylic δ_H and δ_C resonances.[15] Using this information, we repeated our experiments described for **E-Lnn**, **E-Ara** and **E-DHA** by replacing D_2O with H_2O. According to ^1H and ^{13}C NMR spectroscopies there was absolutely no evidence for the formation of either *trans*- or positional isomers for any of the attempted substrates even after leaving these reaction mixtures for more than 48 hours, which is up to 48 times longer than the completion time of the deuteration process for **E-Lnn**. In fact, even after exposing a mixture of **E-Lnn**, **4** (1 mol%) and H_2O (50 equivalents per *bis*-allylic proton) for 6 days at 40 °C there were no detectable signs of any deleterious processes in the reaction medium.[12] It is also worth mentioning that no observable de-deuteration of acetone-d_6 was observed under standard reaction conditions.

In order to gather more insights into the deuteration mechanism we examined the role of the Cp ligand and the fact that the *bis*-allylic position of **E-Lin** has not been deuterated. Hexa(acetonitrile) complex **5** (Figure 1) showed no deuteration ability towards **E-Lnn** signifying the importance of the cyclic substituent (entry 20, Table 1). On the other hand, if the permethylated analogue of **4** (i.e. **6**) was used only *cis*-to-*trans* isomerization was detected (entry 21, Table 1).[14c] We attributed this observation to the steric factors that influence the bonding interaction between the central Ru and the substrate.[14c] Then, deuteration of 1,4-cyclohexadiene (**CHEX**) and 1,5-cyclooctadiene (**COCT**) were attempted under standard reaction conditions yielding no observable signs of isotope scrambling within 24 h (entries 22 and 23, Table 1). This suggested that the allylic positions of these two substrates and the *bis*-allylic protons of **E-Lin** are not in correct stereoelectronical orientation with respect to the central Ru atom preventing them from the H/D exchange. This was supported by performing deuteration using *cis*-1,4-hexadiene (**HEXD**; it could be viewed as an acyclic analogue of **CHEX**) during which only the allylic methyl group was isotopically enriched with no signs of H/D exchange occurring at the *bis*-allylic position (entry 24, Table 1). Therefore, this collective evidence indicated that a *cis,cis-bis*-alkene coordinated Ru complex (**A**, Scheme 1) was most likely one of the key intermediates in the overall deuteration mechanism. This idea was reinforced by observing alkene conjugation when *trans,trans*-ethyl linoleate (***EE*-Lin**) was subjected to the normal deuteration reaction conditions (entry 25, Table 1) presumably due to the difference in coordination abilities between *cis*- and *trans*- double bonds to a $RuCp^{(*)}$-containing moiety.[16]

In order to propose the most likely mechanism for the observed deuteration it is also important to mention the most prominent mechanistic steps for the alkene-assisted *cis-trans* isomerization of *cis,cis*-dienes (pathway 1, Scheme 1) involving permethylated complex **6**.[14c] In fact, apart from alkene assistance, which prevents alkene migration and consequently the formation of positional isomers,[14c] this mechanism is regularly used to describe *cis-trans* isomerization of various alkenes catalyzed by transition metal complexes that do not contain a hydride ligand.[17] Accordingly, initial *cis,cis*-diene coordination (**A**, Scheme 1) is subjected to oxidative addition to form η^3-allyl intermediate **B**, which is then followed by conversion to η^1-intermediate **C** and rotation around the C-C bond to generate intermediate **D**. Reverting back to another η^3-allyl



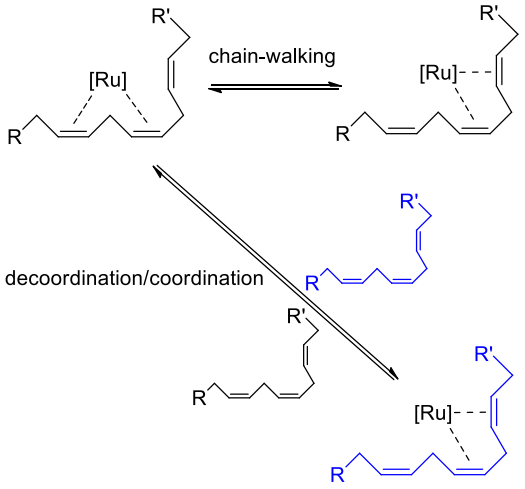
Scheme 1. Proposed *cis-trans* isomerization (path 1) and deuteration (path 2) mechanisms.

intermediate (**E**) allows for reductive elimination and formation of *cis,trans*-diene intermediate **F**. Further evidence for this mechanism was gathered through computational studies that determined the activation energy of around 30 kcal/mol for the allyl rotation (**B** → **E**) - the rate determining step of this reaction.[14c] It is reasonable to assume that the existence of the allyl intermediate **B** is the key step in the overall mechanism because, according to the numerous literature reports,[7g,8b] its formation is essential for consequent *cis-trans* isomerization and/or alkene migration. Thus, the lack of any alkene isomerization or migration products in the described deuteration experiments indicated that either the η^3-allyl intermediate did form but it was prevented from the usual reaction progression (i.e. through **C**, **D**, **E**, etc.) or this intermediate was absent from the overall deuteration mechanism.

Even though initial computational studies (see the Supporting Information for full details) could not adequately describe the proper intermediate/transition state, it is strongly believed that the formation of the allylic intermediate is absent in the overall deuteration mechanism. It is then proposed that two (**H**, path 2, Scheme1) or more water molecules are actively involved in the overall mechanism.[18] This concept was further supported by using methanol-d_4 (CD_3OD) as a deuterating reagent for deuteration of **E-Lnn** (entry 26, Table 1), which is not expected to form as extensive hydrogen bonding network as water, resulting in significantly reduced rate for the H/D exchange process.

Additionally, our investigations have shown that, irrespective of the nature of the substrate, water itself played a pivotal role in preventing the formation of more thermodynamically favored side-products. Specifically, repeating standard deuteration protocol while using various amounts of H_2O and monitoring mixtures by means of ^1H and ^{13}C NMR spectroscopies revealed that by using no less than 4000 equivalents of water per molecule of the catalyst was necessary to prevent the formation of *trans*- and positional isomers in a span of at least 8 days. Meanwhile, as it was expected, repeating same experiment with 4000 equivalents of CH_3OH per molecule of the catalyst resulted in slow *cis-trans* isomerization and conjugation of **E-Lnn** (about 6% of double bonds were *trans*-isomerized and signs of conjugation were observed after keeping the mixture for 3 days). Furthermore, computational analysis confirmed that D_2O coordination to (CpRu(*bis*-alkene)$^+$ fragment is not thermodynamically favored but it could be made possible by adding a large excess of D_2O i.e. the formation constant for this process was calculated to be around $3.4x10^{-5}$ ($\Delta G = +6.1$ kcal/mol). These collective observations not only indicated the importance of a large excess of D_2O in avoiding the formation of deleterious products but also suggested that the presence of more than two molecules of D_2O was quite possibly involved in the formation of proposed transition state **L** in the solution state (*44*).[18] Therefore, based on the obtained experimental and computational evidence, the most probable H/D mechanism involves a concerted step (path 3) that avoids the formation of an allyl intermediate.

Lastly, the fact that observed deuteration process is quite efficient under the set catalytic conditions is most likely due to "decoordination/coordination" mechanism among various PUFAs (Scheme 2) because the "chain-walk" mechanism would probably be more efficient under stoichiometric conditions.



Scheme 2. De-coordination/coordination vs chain-walking mechanisms.

In conclusion, we have succeeded in selective deuteration of various poly-alkenes including several examples of biologically important polyunsaturated fatty acids at room temperature using the least expensive deuterium source (i.e. D_2O). A very simple and commercially available Ru-based catalyst has been identified to perform the target H/D exchange with unprecedented kinetic control. Even though all the substrates examined contained thermodynamically unfavourable alkene fragments there was no evidence for either alkene conjugation or *cis-trans* isomerization under the tested reaction conditions. In fact, apart from acting as the deuterium source, the presence of D_2O also prevented the formation of any deleterious products. Experimental and computational evidence strongly suggest that the most likely mechanistic pathway involves a concerted transition state in which the observed C-H/D and O-D/H bond breaking and forming processes occur concurrently.

EXPERIMENTAL SECTION.

General Methods: Even though all the reagents used in the study are only oxygen sensitive all synthetic and reactivity studies have been performed using standard dry-box and Schlenk techniques. Acetone and acetone-d_6 were distilled over B_2O_3 and stored

under inert atmosphere. D_2O, H_2O, CD_3OD and CH_3OH were deoxygenated by bubbling an inert gas and kept in storage Schlenk flasks (CD_3OD and CH_3OH were stored over 4 Å molecular sieves). Complexes **1**, **2**, **4**, **5** and **6** were synthesized according to the published reports.[8a,19] The ester forms of polyunsaturated fatty acids, the mono-, di- and triglycerides, cyclooctadiene, cyclohexadiene, *cis*-1,4-hexadiene and *trans,trans*-isomer of ethyl linoleate (ethyl linolelaidate) were purchased from commercial sources and used without further purification. The acid, alcohol and hydrocarbon forms of selected fatty acids were synthesized according to the published reports.[4d,20]

Synthesis of complex 3

Complex **3** was synthesized using modified method for synthesis of complex [CpRu(^{t}Bu$_2$P-Im)(NCCH$_3$)][PF$_6$] (i.e. complex **2**).[19a] Stock solution of ^{t}Bu$_2$PPh in hexane (8.0 mL, 18.51 mM, 1.48 mmol) was transferred into a 50 mL Schlenk flask and all volatiles were removed *in vacuo* after which phosphine was re-dissolved in 5 mL of acetone. This solution was then added to the solution of complex [CpRu(NCCH$_3$)$_3$][PF$_6$] (i.e. complex **4**, 64 mg, 1.47 mmol) in 5 mL of acetone and reaction mixture was stirred for 1 hour. The solution was concentrated *in vacuo* until a precipitate started to form, after which an excess of hexane (20 mL) was added with vigorous stirring. The mixture was stirred overnight, solid was then isolated by filtration, washed three times with hexane (10 mL) and dried under vacuum for several hours. The obtained solid was then recrystallized from a 1:1 acetone/ether mixture, filtered, washed twice with ether (10 mL) and hexane (10 mL) and dried under vacuum to afford the desired product. Yield: 71 mg (78%, yellow-brown powder). ^{1}H NMR (500 MHz, acetone-d$_6$) δ 8.06-8.10 (m, 2H), 7.48-7.50 (m, 3H), 4.60 (s, 5H), 2.58 (br s, 6H), 1.39 (d, 18H, $^3J_{PH}$ = 12.7 Hz) ppm. ^{13}C{^{1}H} NMR (125.7 MHz, acetone-d$_6$) δ 135.97 (d, $^2J_{CP}$ = 10.3 Hz), 134.36 (d, $^1J_{CP}$ = 25.7 Hz), 129.71 (s), 127.31 (d, $^3J_{CP}$ = 8.8 Hz), 81.60 (s), 75.97 (s), 39.11 (d, $^1J_{CP}$ = 11.9 Hz), 30.66 (d, $^2J_{CP}$ = 4.6 Hz) ppm. ^{31}P{^{1}H} NMR (202 MHz, acetone-d$_6$) δ 72 (s), -144 (sept, $^1J_{PF}$ = 706 Hz) ppm. Anal.Calcd for $C_{23}H_{34}F_6N_2P_2Ru$: C, 44.88; H, 5.57; N, 4.55. Found: C, 44.76; H, 5.59; N, 4.56. HRMS: m/z (M-CH$_3$CN)$^+$ = (Calculated for $RuC_{21}H_{31}NP$, 430.1238) found 430.1241, (M-2CH$_3$CN)+ = (Calculated for $RuC_{19}H_{28}P$, 389.0972) found 389.0973.

H/D exchange studies

In most cases (entries 1-4, 8-10 and 12-25, Table 1) the reaction was prepared and monitored using a J. Young NMR tube according to the following general procedure: A J. Young NMR tube was charged with 20 mg of a substrate followed by D_2O (10, 20 or 50 equivalents per *bis*-allylic proton) and acetone-d$_6$ (~ 1 mL) after which first ^{1}H NMR spectrum was acquired. Inside a glove box a ruthenium complex was weighed in a scintillation vial to which then a D_2O-substrate mixture was added. Resulting mixture was thoroughly mixed, transferred back into the NMR tube and heated using an oil bath, if necessary. Reaction progress was monitored by hourly ^{1}H NMR scans during first 12 hours followed by daily scans. For entry 26, the general procedure was followed and CD_3OD was used instead of D_2O.

For selected runs (entries 5-7, 11, Table 1) the reaction was performed using 100 mg of substrates to emphasize that virtually quantitative yields of these reactions could be obtained: Inside a glove box two scintillation vials were charged with a substrate (**E-Lnn**, **E-Ara**, **E-DHA** or **T-Lnn**) and complex **4**, respectively. Both were transferred into two separate Schlenk flasks using three 0.5 mL acetone portions each. D_2O was added to the flask containing PUFA followed by the amount of acetone necessary to form a homogenous solution. Then a solution of complex **4** in acetone was transferred to the substrate/D_2O-containing solution and reaction was left stirring at room temperature. Upon completion of the reaction, excess of 2 N HCl (not less than 5 times the volume of reaction mixture) was added and the mixture was allowed to stir vigorously for 15 minutes. The product was extracted with 100 mL of hexane and the solution was then washed with saturated $NaHCO_3$ and NaCl solutions and dried over anhydrous Na_2SO_4. The solution was filtered and activated carbon was added. Stirring for another 15 minutes, filtration and removal of volatiles *in vacuo* afforded desired products.

Synthesis of deuterated ethyl linolenate

100 mg of **E-Lnn** (0.326 mmol), 1.18 mL of D_2O (65.40 mmol) and 1.42 mg of complex **4** (1%, 3.26 μmol) in 10 mL of acetone and stirring for 1 hour to afford desired deuterated product (101.06 mg, 99.6% yield). Starting material ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.31-5.45 (m, 5.67H), 4.14 (q, *J* = 7.2 Hz, 1.94H), 2.80-2.86 (m, 3.97H), 2.32 (t, *J* = 7.5 Hz, 2.00H), 2.06-2.13 (m, 3.90H), 1.61-1.68 (m, 2.02H), 1.25-1.40 (m, 11.18H), 1.00 (t, *J* = 7.5 Hz, 2.78H) ppm. Product ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.32-5.46 (m, 5.54H), 4.12-4.16 (m, 1.93H), 2.80 (br s, 0.12H), 2.29-2.32 (m, 2.00H), 2.02-2.13 (m, 2.81H), 1.60-1.68 (m, 2.03H), 1.24-1.41 (m, 11.24H), 0.98-1.01 (m, 2.74H) ppm.

Synthesis of deuterated ethyl arachidonate

100 mg of **E-Ara** (0.301 mmol), 1.63 mL of D_2O (90.22 mmol) and 1.31 mg of complex **4** (1%, 3.01 μmol) in 12.5 mL of acetone and stirring for 24 hours to afford desired deuterated product (101.36 mg, 99.5% yield). Starting material ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.34-5.45 (m, 7.70H), 4.15 (q, *J* = 7.1 Hz, 1.94H), 2.82-2.87 (m, 5.99H), 2.33 (t, *J* = 7.6 Hz, 2.00H), 2.06-2.16 (m, 4.01H), 1.73 (quint, *J* = 7.4 Hz, 2.02H), 1.26-1.41 (m, 8.94H), 0.91 (t, *J* = 7.1 Hz, 2.91H) ppm. Product ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.34-5.45 (m, 7.64H), 4.15 (q, *J* = 7.1 Hz, 1.98H), 2.80-2.85 (m, 0.30H), 2.33 (t, *J* = 7.6 Hz, 2.00H), 2.03-2.16 (m, 2.87H), 1.73 (quint, *J* = 7.4 Hz, 1.99H), 1.26-1.41 (m, 9.08H), 0.91 (t, *J* = 7.1 Hz, 2.96H) ppm.

Synthesis of deuterated ethyl docosahexaenoate

100 mg of **E-DHA** (0.280 mmol), 2.53 mL of D_2O (0.14 mol) and 2.44 mg of complex **4** (2%, 5.61 μmol) in 15 mL of acetone and stirring for 18 hours to afford desired deuterated product (101.96 mg, 99.8% yield). Starting material ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.31-5.45 (m, 11.54H), 4.15 (q, *J* = 7.2 Hz, 1.94H), 2.83-2.90 (m, 10.00H), 2.36-2.44 (m, 4.03H), 2.10 (quint, *J* = 8.1 Hz, 1.88H), 1.28 (t, *J* = 7.2 Hz, 3.00H), 1.00 (t, *J* = 7.6 Hz, 2.79H) ppm. Product ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.30-5.43 (m, 10.70H), 4.14 (q, *J* = 7.2 Hz, 1.83H), 2.80-2.86 (m, 0.36H), 2.35-2.42 (m, 3.73H), 2.05-2.12 (m, 1.35H), 1.26 (t, *J* = 7.2 Hz, 3.00H), 0.99 (t, *J* = 7.6 Hz, 2.67H) ppm.

Synthesis of deuterated trilinolenin

100 mg of **T-Lnn** (0.115 mmol), 1.24 mL of D_2O (68.70 mmol) and 0.50 mg of complex **4** (1%, 1.15 mmol) in 20 mL of acetone and stirring for 7 hours to afford desired deuterated product (101.34 mg, 99.7% yield). Starting material ^{1}H NMR (500 MHz, acetone-d$_6$) δ 5.27-5.43 (m, 17.55H), 4.34 (dd, *J* = 4.0, 11.9 Hz, 2.16H), 4.19 (dd, *J* = 6.3, 11.9 Hz, 2.19H), 2.81-2.87 (m, 11.54H), 2.34 (t, *J* = 7.4 Hz, 6.00H), 2.05-2.13 (m, 11.64H), 1.57-1.66 (m, 6.06H), 1.31-1.42 (m, 24.18H), 0.97 (t, *J* = 7.6 Hz, 7.98H) ppm. Product ^{1}H NMR (500 MHz, acetone-d$_6$) δ 5.26-5.43 (m, 16.67H), 4.35 (dd, *J* = 4.0, 11.9 Hz, 2.16H), 4.19 (dd, *J* = 6.3, 11.9 Hz, 2.17H), 2.79-2.84 (m, 0.66H), 2.34 (t, *J* = 7.4 Hz, 6.00H), 2.03-2.13 (m, 9.08H), 1.57-1.66 (m, 6.14H), 1.30-1.42 (m, 24.44H), 0.97 (t, *J* = 7.6 Hz, 7.93H) ppm.

ASSOCIATED CONTENT

Supporting Information

Copies of multinuclear NMR spectra as well as detailed computational information. The Supporting Information is available free of charge on the ACS Publications website.

Conflicts of Interest

M. S. S. owns stocks in Retrotope. All other authors declare no competing financial interests.

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ACKNOWLEDGMENT

We thank NTU (SUG M4080534), Retrotope (www.retrotope.com) and the Ministry of Education, Science, and Technological Development of the Republic of Serbia within the framework of the project (MP, 172040) for the financial support and chemicals. Computational analyses were performed on the PARADOX cluster at the Scientific Computing Laboratory of the Institute of Physics Belgrade, supported in part by the Ministry of Education, Science, and Technological Development of the Republic of Serbia under project No. ON171017 (MP).

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(12) See the Supporting Information.

(13) Purification and isolation was not very practical at the NMR scale reactions. However, as we have shown on several examples (entries 5, 6, 7 and 11; Table 1) performed at larger scales (~ 100 mg) the isolate yields of these reactions are quantitative.

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