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Highly Efficient Preparation of Selectively Isotope Cluster-Labeled Long Chain Fatty Acids via Two Consecutive C_{sp^3}–C_{sp^3} Cross-Coupling Reactions

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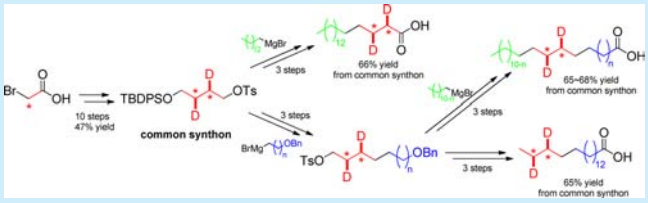
Supporting Information

ABSTRACT: An efficient synthesis involving two copper-catalyzed alkyl–alkyl coupling reactions has been designed to easily access doubly isotope-labeled fatty acids. Such NMR- and IR-active compounds were obtained in excellent overall yields and will be further used for determining the conformation of an alkyl chain of lipidic biomolecules upon interaction with proteins.



Conformation of a saturated alkyl chain is one of the fundamental concepts in organic chemistry, where three preferable states, anti (T), gauche+ (G+), and gauche− (G−), are adopted.[1]

The conformation of flexible molecules is strongly correlated with the macroscopic physical properties of hydrocarbon assemblages containing long alkyl chains such as polymers, detergent micelles, and biomembranes.[2] Therefore, experimental methods to explore alkyl chain conformation have been developed by means of vibration spectroscopy, neutron diffraction, and NMR, combined with isotopically labeled compounds.[3] In these methodologies, ^{13}C and/or ^{2}H are introduced at the methylene unit concerned to enable selective observation by giving separable signals from large background ones. Specifically, the $^{13}CH_2$ or CD_2 group provides information about the chain mobility at the labeled methylene or total information for two $^{13}CH_2$–C or two CD_2–C bonds respectively.

On the other hand, when investigating a dihedral angle of a particular C–C bond, introduction of a single $^{13}CH_2$ or CD_2 group is not sufficient. For obtaining the segmental dihedral angle in such dynamic molecules as fatty acids, it is a requisite to determine the relative orientation between two neighboring methylene groups by introducing multiple isotopes. The minimum structure for defining an alkane chain conformation is a butylene unit (C–CH_2–CH_2–C), where C2 and C3 should be CHDs with defined configurations, when aiming to discriminate the G+ and G− orientations.

Accordingly, a standardized cluster-labeling scheme for a saturated alkyl chain can be separated into two stages: a versatile preparation of the butylene common intermediate that can cover all combinations of ^{13}C and ^{2}H labeling patterns,[4] and the practical incorporation of the cluster-labeled common intermediate into an alkyl chain in high yield retaining the configuration of C2/C3.

In this study, by taking as an example [*n*, *n*+1-$^{13}C_2$, *n*, *n*+1-$^{2}H_2$] doubly labeled stearic acid (Figure 1), we aimed to establish a highly efficient route for the preparation of site-selectively cluster-labeled linear hydrocarbon chains involving two consecutive C_{sp^3}–C_{sp^3} cross-coupling reactions.

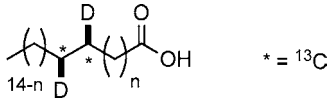


Figure 1. [*n*, *n*+1-$^{13}C_2$, *n*, *n*+1-$^{2}H_2$] stearic acids.

To establish the standardized labeling scheme, we designed two common intermediates **10** and **11**, which could be derived from bromoacetic acid.[5] In this study, we adopted [2-^{13}C]-bromoacetic acid twice to form a ^{13}C–^{13}C spin pair avoiding the use of expensive $^{13}C_2$ labeled precursors and constructed the *threo* configuration for C2/C3 of the common intermediate by the *syn*-selective deuteration of *E*-olefin (Scheme 1).

After activation as an acid chloride, the addition of ethanol afforded ester **1**, which was then immediately converted into the corresponding phosphonate **2** by Arbuzov reaction with triethylphosphite. Compound **2** was subsequently subjected to Horner–Wadsworth–Emmons (HWE) olefination with benzyloxyacetaldehyde, providing the monolabeled conjugated ester **3**, which was then reduced by diisobutylaluminium hydride (DIBAL-H) to generate alcohol **4**. Thus, a second protecting group was required that would be sufficiently robust for the conditions of our strategy while allowing an easy separation of the two aldehydes after degradation by ozonolysis. For this reason, we chose the *tert*-butyldiphenylsilyl (TBDPS) group that is stable under various conditions[6] and more lipophilic than benzyl. After protection of the hydroxyl group of

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Scheme 1. Synthesis of the Common Synthons 10 and 11

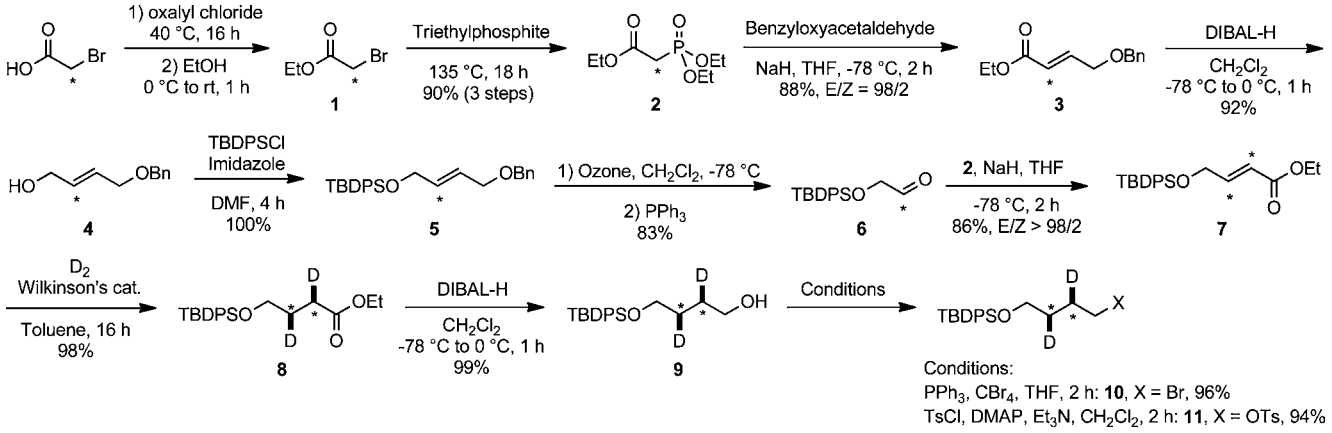
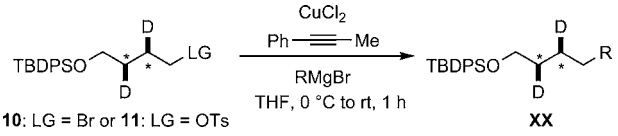


Table 1. Optimization of Alkyl–Alkyl Coupling



entry	starting material	$CuCl_2$ (mol %)	acetylene (mol %)	R	RMgBr (equiv)	yield[a] (%)	XX
1	**10**	2	10	C_6H_{13}	1.5	50	**12**
2	**10**	5	20	C_6H_{13}	2	80	**12**
3	**11**	5	20	C_6H_{13}	2	96	**12**
4	**11**	5	20	$C_{14}H_{29}$	2	98	**13**

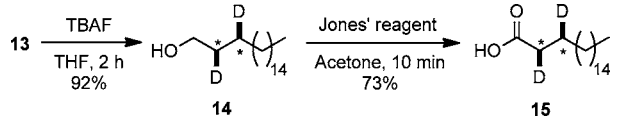
[a]Isolated yield.

4 as a corresponding TBDPS ether, the resulting olefin **5** was ozonolyzed to labeled aldehyde **6**. Using the previously synthesized phosphonate **2**, a second HWE reaction provided ester **7** in a separable mixture *E*/*Z* 98:2 with the two positions of the conjugated olefin labeled with ^{13}C. From olefin (***E***)-**7**, deuteration of the double bond was carried out with Wilkinson's catalyst to give labeled ester **8** in almost quantitative yield with full deuterium incorporation.[7,8] The diastereomeric purity of this reaction was found to be satisfactory in favor of the cis/threo compound.[8] A second reduction with DIBAL-H afforded hydroxyl **9**. Finally, two desired common synthons were obtained either as a brominated compound, **10**, or as a tosylated one, **11**, in overall yields, after 10 steps, of 48% and 47% respectively.

With those two labeled intermediates in hand, we envisaged that further extension of an acyl chain should be achieved through a direct cross-coupling between the alkyl electrophile **10** or **11** and an alkyl metal. Recently, remarkable advances have been brought about to accelerate applications of C_{sp^3}–C_{sp^3} cross-coupling reactions for organic synthesis. Alkyl electrophiles containing *β*-hydrogen atoms were originally thought to be unsuitable. Nevertheless, several groups have developed powerful C_{sp^3}–C_{sp^3} Suzuki–Miyaura, Negishi, or Kumada–Corriu couplings.[9] Among these, we adopted a method based on a copper-catalyzed Kumada–Corriu coupling developed by Kambe and co-workers,[10] to first link the tail of the stearic acid.

As shown in Table 1, the initial attempt gave a promising 50% yield (entry 1). To improve the yield, we optimized the reaction conditions by increasing the amount of the catalyst, additive, and organomagnesium reagent. After such adjustments, the yield rose to a good 80% (entry 2) with brominated **10**. Nevertheless, in contrast to the report by Kambe,[10] tosylated **11** proved to be a more suitable precursor than **10** as the coupling product was obtained under the same conditions in almost quantitative yield with both short (C_6H_{13}) and long ($C_{14}H_{29}$) alkyl chains (entries 3 and 4). As depicted in Scheme 2, two more steps from **13**, i.e. desilylation by tetrabutylalu-

Scheme 2. Final Steps for 2,3-Labeled Stearic Acid 15



minium fluoride (TBAF) and Jones' oxidation, led to the preparation of **15**, one of the labeled stearic acids, with a 66% overall yield from the common synthon **11**.

While the tail part was successfully installed, we found that the coupling of the fatty acyl head was more challenging. Because of the propensity of carboxylic acids to bind metals,[11] direct coupling is delicate[12] and the acid moiety is often protected as an ester. Various methodologies involving alkylmetal bearing functional groups, such as Suzuki–Miyaura[9,13] and Negishi[9,14] couplings, have been elaborated. However, no reaction occurred between **10** (or **11**) and the appropriate ester as a coupling partner.[13b,c,14a] This forced us to adopt an alternative strategy where a protected hydroxyl is to be converted to the expected fatty acids by a routine deprotection and oxidation sequence. In order to accomplish such a coupling reaction, the previously used methodology was employed using freshly prepared Grignard reagents bearing a protected hydroxyl group. To our knowledge, this coupling reaction involving Grignard reagents with heteroatoms has not been

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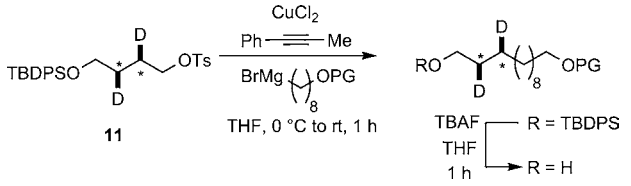
described to date. A survey over several possible protecting groups[15] revealed three candidates to be promising: tetrahydropyran (THP), *p*-methoxybenzyl (PMB), and benzyl (Bn) as shown in Table 2. After two steps, THP afforded the desired compound **16** in only 43% yield, much lower than our expectations. The more robust PMB and Bn groups revealed excellent yields in the two-step sequence. Because Bn exhibited a better result, we chose it as the protecting group for the rest of our study.

Table 2. Survey of Different Protecting Groups for C_{sp^3}–C_{sp^3} Coupling[a]

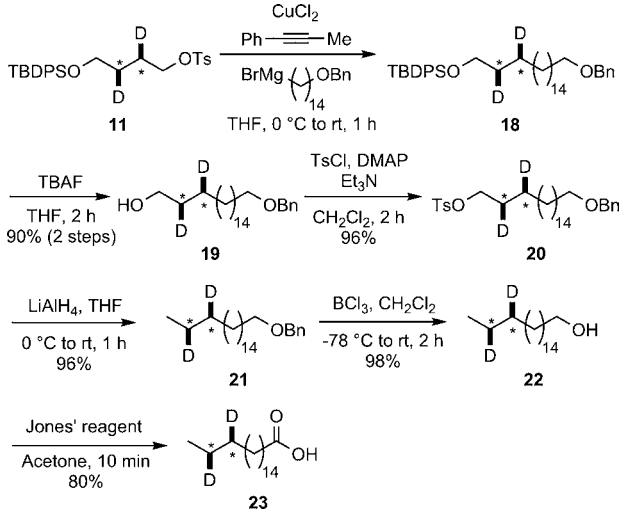


entry	PG	compound	yield[b] (% after 2 steps)
1	THP	**16**	43
2	PMB	**17**	93
3	Bn	**25b**	96

[a]Reaction conditions: **11** (0.5 mmol), $CuCl_2$ (5 mol %), methylphenylacetylene (20 mol %), Grignard reagent (1 mmol), THF (0.5 mL), 0 °C to rt, 1 h. [b]Isolated yield.

The synthesis of 16,17-labeled stearic acid **23** was realized by the same strategy with the appropriate chain length (Scheme 3). As expected, the C_{sp^3}–C_{sp^3} coupling and subsequent desilylation afforded the labeled monoprotected diol **19** in 90% yield after two steps. Activation as tosylate **20** and subsequent nucleophilic reduction afforded the protected alcohol **21**. Deprotection of the Bn group to give 16,17-labeled stearyl alcohol **22** was found to be more efficient and much faster with a Lewis acid such as boron trichloride than with an oxidant such as dichlorodicyanoquinone (DDQ) or by classical catalytic hydrogenation with Pd/C. Finally, Jones' oxidation led to the desired stearic acid **23** in 65% overall yield after six steps from **11**.

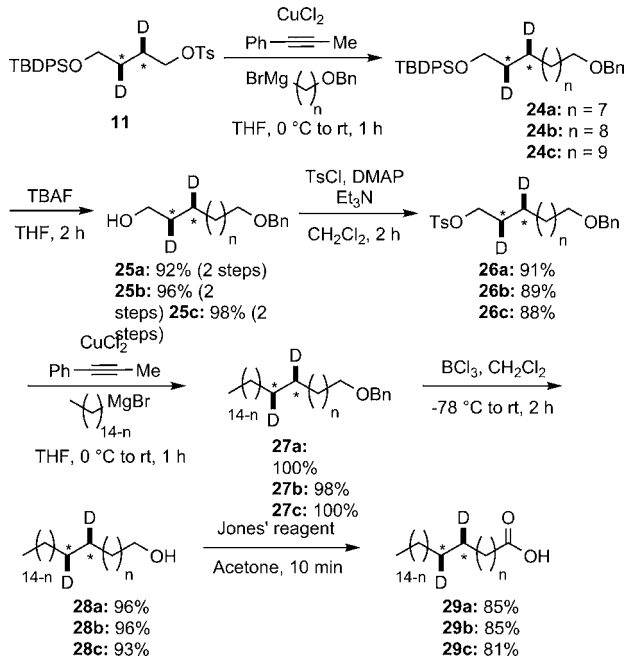
Scheme 3. Synthesis of 16,17-Labeled Stearic Acid 23



After synthesizing the two stearic acids **15** and **23** containing the isotope labels near both of the acyl termini, we had all the tools in hand to produce fatty acids where the labeled moiety would be located in the central portion. However, the order of the two couplings had to be reexamined. Due to the volatility of the intermediates if the tail of the fatty acid was coupled first, we had to carry out the troublesome separation of the desired compound from the byproducts after the second coupling with protected-hydroxyl Grignard reagents. Thus, it quickly came to light that the head of the fatty acid had to be coupled first.

On this basis, pure products **25a–c** were obtained in satisfactory yield after coupling the protected-hydroxyl chain and deprotection of TBDPS (Scheme 4). Tosylation and the subsequent second coupling afforded the desired protected stearyl alcohols **27a–c** in an efficient way. Finally, debenzylation furnished alcohols **28a–c**, which were oxidized to complete the synthesis of the fatty acids **29a–c** in overall yields of, respectively, 68%, 68%, and 65% from **11**.

Scheme 4. Synthesis of "Middle"-Labeled Stearic Acids 29a–c



In summary, from [2-^{13}C]-bromoacetic acid and via a versatile common precursor, we established a simple and efficient route for accessing a range of stearic acids bearing variable labeled positions with a ^{13}CHD-^{13}CHD moiety in their aliphatic chain. This methodology involves two powerful C_{sp^3}–C_{sp^3} couplings that could also be applied for the synthesis of other labeled fatty acids or more complex molecules possessing an alkyl chain linker. Their application in the investigation on protein–lipid interactions is now in progress by means of solid NMR and will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, full spectroscopic data, and copies of ^{1}H and ^{13}C NMR spectra for labeled stearic acids and their intermediates are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(4) For example, conformation of labeled units, such as C-CHD-CHD-C, ^{13}C-CH$_2$-CH$_2$-^{13}C, and C-^{13}CHD-^{13}CHD-C can be site-selectively investigated by vibrational circular dichroism (VCD), NMR *J*-coupling analysis, and/or NMR distance measurement.

(5) Commercial sources of bromoacetic acid cover all patterns of ^{13}C labeling: [1-^{13}C], [2-^{13}C], and [1,2-^{13}C$_2$].

(6) For a comparative study of silyl ether protecting groups, see: Davies, J. S.; Higginbotham, C. L.; Treemer, E. J.; Brown, C.; Treatgold, R. C. *J. Chem. Soc., Perkin Trans. 1* **1992**, 3043.

(7) Determined by NMR.

(8) See Supporting Information.

(9) For representative reviews, see: (a) Cárdenas, D. J. *Angew. Chem., Int. Ed.* **2003**, *42*, 384. (b) Netherton, M. R.; Fu, G. C. *Adv. Synth. Catal.* **2004**, *346*, 1525. (c) Terao, J.; Kambe, N. *Acc. Chem. Res.* **2008**, *41*, 1545. (d) Jana, R.; Pathak, T. P.; Sigman, M. S. *Chem. Rev.* **2011**, *111*, 1417.

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