JOC
The Journal of Organic Chemistry

VOLUME 66, NUMBER 17 AUGUST 24, 2001

© Copyright 2001 by the American Chemical Society

Articles

Biosynthesis of Acaterin: Coupling of C_5 Unit with Octanoate

Yasuyo Sekiyama,[‡] Yoshinori Fujimoto,*[,‡] Keiji Hasumi,[§] and Akira Endo[§]

Department of Chemistry and Materials Science, Tokyo Institute of Technology, Meguro, Tokyo 152-8551, Japan, and Department of Applied Biological Science, Tokyo Noko University, Fuchu, Tokyo 183-8509, Japan

fujimoto@cms.titech.ac.jp

Received March 6, 2001

Acaterin (**1**), produced by *Pseudomonas* sp. A 92, is a secondary metabolite having a 2-penten-4-olide structure. Feeding experiments with ^{2}H- and ^{13}C-labeled decanoic acid, their 3-oxygenated congeners, and octanoic acid have suggested that **1** is biosynthesized via coupling of a C_5 unit with octanoate, rather than via introduction of a C_3 unit at the α position of a decanoate derivative. Further feeding study of [2,3-$^{13}C_2$]decanoic acid concluded that the former route is operating in the biosynthesis of **1**.

Introduction

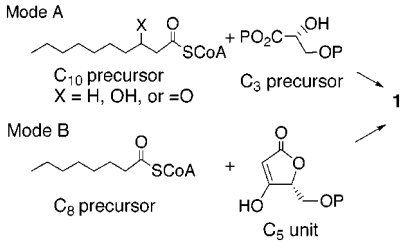
Natural compounds having a polyketide chain at the C-2 position of a 2-penten-4-olide structure have been found in a variety of organisms.[1−16] Acaterin (**1**), one of this group of compounds, was isolated from *Pseudomonas* sp. A 92 as an inhibitor of acyl-CoA:cholesterol acyltransferase,[17] and its absolute configuration was determined as (4*R*,1′*R*).[18] 4-Dehydroacaterin (**2**), an immediate precursor of **1**, was subsequently isolated from the same strain when cultured in a medium supplemented with a

* To whom correspondence should be addressed.
‡ Tokyo Institute of Technology.
§ Tokyo Noko University.

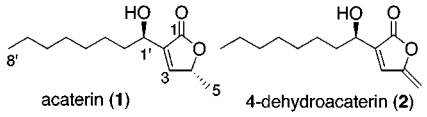
(1) (a) Pattenden, G. *Prog. Chem. Org. Nat. Prod.* **1978**, *35*, 133−198. (b) Zeng L.; Ye, Q.; Oberlies, N. H.; Shi, G.; Gu, Z.-M.; He, K.; McLaughlin, J. L. *Nat. Prod. Rep.* **1996**, *13*, 275−306.
(2) (a) Schmitz, F. J.; Kraus, K. W.; Ciereszko, L. S.; Sifford, D. H.; Weinheimer, A. J. *Tetrahedron Lett.* **1966**, 97−104. (b) Schmitz, F. J.; Lorance. E. D. *J. Org. Chem.* **1971**, *36*, 719−720.
(3) Kazlauskas, R.; Murphy, R. T.; Quinn, R. J.; Wells, R. J. *Tetrahedron Lett.* **1977**, 37−40.
(4) Ravi, B. N.; Wells, R. J. *Aust. J. Chem.* **1982**, *35*, 105−112.
(5) (a) Shoji, J.; Sakazaki, R.; Hattori, T.; Matsumoto, K.; Uotani, N.; Yoshida T. *J. Antibiot.* **1989**, *42*, 1729−1733. (b) Terui, Y.; Sakazaki, R.; Shoji, J. *J. Antibiot.* **1990**, *43*, 1245−1253.
(6) (a) Roggo, B. E.; Petersen, F.; Delmendo, R.; Jenny. H.-B.; Peter, H. H.; Roesel, J. *J. Antibiot.* **1994**,*47*, 136−142. (b) Roggo, B. E.; Hug, P.; Moss, S.; Raschdorf, F.; Peter, H. H. *J. Antibiot.* **1994**, *47*, 143−147. (c) Hamaguchi, T.; Sudo, T.; Osada, H. *FEBS Lett.* **1995**, *372*, 54−58.
(7) Yamada, A.; Yamaguchi, K.; Kitamura, H.; Yazawa, K.; Fukuzawa, S.; Wang, G.-Y. −S.; Kuramoto, M.; Uemura, D. *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu* **1995**, *37*, 224−229.
(8) Bohlmann, F.; Grenz, M. *Tetrahedron Lett.* **1971**, 3623−3626.
(9) Filho, R. B.; Diaz, P. P.; Gottlieb, O. R. *Phytochemistry* **1980**, *19*, 455−459.
(10) Vanwagenen, B. C.; Cardellina, J. H., II. *Tetrahedron* **1986**, *42*, 1117−1122.
(11) Hadacek, F.; Greger, H.; Grenz, M.; Bohlmann, F. *Phytochemistry* **1987**, *26*, 1527−1529.
(12) Takeda, K.; Sakurai, K.; Ishii, H. *Tetrahedron* **1972**, *28*, 3757−3766.
(13) Niwa, M.; Iguchi, M.; Yamamura, S. *Tetrahedron Lett.* **1975**, 1539−1542.
(14) Etse, J. T.; Waterman, P. G. *Phytochemistry* **1986**, *25*, 1903−1905.
(15) Reffstrup, T.; Boll, P. M. *Acta Chem. Scand.* **1980**, *B34*, 653−659.
(16) Keller-Juslén C.; King, H. D.; Kuhn, M.; Loosi, H.-R.; Pache, W.; Petcher, T. J.; Weber, H. P.; von Wartburg, A. *J. Antibiot.* **1982**, *35*, 142−150.
(17) Naganuma, S.; Sakai, K.; Hasumi, K.; Endo, A. *J. Antibiot.* **1992**, *45*, 1216−1221.
(18) Ishigami, K.; Kitahara, T. *Tetrahedron* **1995**, *51*, 6431−6442.

10.1021/jo015614g CCC: $20.00 © 2001 American Chemical Society
Published on Web 07/28/2001

Scheme 1. Two Possible Modes in the Biosynthesis of 1



long chain fatty acid such as dodecanoic acid, tetradecanoic acid, and oleic acid.[19] Although ^{13}C-acetate was found to be hardly incorporated into **1**, feeding [1-^{13}C]-dodecanoic acid gave rise to **1** labeled at the C-1, -1′, -3′, -5′, and -7′ positions.[20] Feeding experiments of ^{13}C- and ^{2}H-labeled glycerols revealed that the branched C_3 moiety (C-3, -4, and -5 positions) of **1** and **2** originates from a glycerol metabolite having a carboxylic group, and this implies that a 3-oxo intermediate would be involved in acaterin biosynthesis.[20] Furthermore, it was suggested that the glycerol metabolite (C_3 precursor) is neither pyruvate nor lactate, but 1,3-bisphosphoglyceric acid or its biological equivalent, based on the findings that feeding of *sn*-(3*R*)- and *sn*-(3*S*)-[3-^{2}H]glycerols furnished compound **2** stereospecifically labeled at (*E*)-H and (*Z*)-H on C-5, respectively.[21]



Concerning the mode of the formation of acaterin framework, two distinct modes can be considered: mode A, introduction of the C_3 precursor at the α position of a decanoate derivative, and mode B, coupling of octanoate with a C_5 unit (hereafter tentatively represented as a C_5-lactone) which is made up of glycerate and acetate (or malonate) (Scheme 1). It has been proposed that more than a dozen of secondary metabolites possessing such a lactone moiety are biosynthesized via mode A.[8−16] Furthermore, virginiae butanolide A, a butylolactone autoregulator isolated from *Streptomyces antibioticus*, is known to be biosynthesized via introduction of a branched C_3 unit (dihydroxyacetone) at the α position of a C_9-polyketide precursor.[22] It was also reported that tenuazonic acid, a tetramic acid derivative, was biosynthesized via introduction of a branched C_6 unit (L-isoleucine) at the α position of 3-oxobutanoate in *Alternaria tenuis*.[23]

In this paper, we report the results of labeling studies which led to the conclusion that **1** was biosynthesized via mode B rather than mode A.

Results and Discussion

Feeding Experiments with ^{2}H-Labeled Fatty Acids. We initiated feeding studies of C_{10} fatty acid derivatives in view of the hypothesis that related compounds are biosynthesized via mode A. Potential ^{2}H-labeled C_{10} precursors, (4*RS*)-[4-^{2}H]decanoic acid (**3**), (3*RS*,4*RS*)-[4-^{2}H]-3-hydroxydecanoic acid (**4**), and ethyl (4*RS*)-[4-^{2}H]-3-oxodecanoate (**5**) were synthesized and fed to *P.* sp. A 92, individually. Compounds **3**, **4**, and **5** (carrying 80% ^{2}H at C-4, estimated by ^{1}H NMR) were readily prepared by two carbon extension of ethyl (2*RS*)-[2-^{2}H]octanoate which was obtained by exchange of α proton of the nonlabeled material with D_2O. The ^{2}H NMR spectra of **1** obtained by feeding **3**−**5** exhibited a signal at δ 1.71 due to 2′-^{2}H (Figure 1a−c). These results suggested that the substrates **3**−**5** were indifferently incorporated into **1** after conversion to a common precursor. To obtain the information on the C-3 oxidation level of the postulated C_{10} precursor, the metabolic fate of the C-3 hydrogens was investigated by feeding (3*RS*,4*RS*)-[3,4-$^{2}H_2$]-3-hydroxydecanoic acid (**6**) (carrying 80% ^{2}H at C-4 and >98% ^{2}H at C-3, estimated by ^{1}H NMR) and (3*RS*)-[3-^{2}H]-decanoic acid (**7**) (carrying 90% ^{2}H at C-3, estimated by ^{1}H NMR). Compound **6** was prepared by reduction of **5** with $NaBD_4$ and **7** was prepared by one carbon extension of ethyl (2*RS*)-[2-^{2}H]nonanoate. The ^{2}H NMR spectrum of **1** obtained by feeding **6** showed a signal due to 2′-^{2}H of **1**, but not due to 1′-^{2}H (Figure 1d). Furthermore, the ^{2}H NMR spectrum of **1** obtained by feeding **7** exhibited no deuterium signals. Since decanoic acid is known to be incorporated into **1** (vide supra), this observation implies that two hydrogens at the C-3 of decanoate were completely lost during the transformation. These results suggested that decanoate and 3-hydroxydecanoate were not incorporated as such, but incorporated into **1** after conversion to 3-oxodecanoate. Alternatively, 3-oxodecanoate was further degraded to octanoate, which was then incorporated into the C_8 side chain of **1** via mode B. Feeding studies of (2*RS*)-[2-^{2}H]octanoic acid (**8**) (carrying 97% ^{2}H at C-2, estimated by ^{1}H NMR) revealed that octanoate was incorporated into **1** as shown in the ^{2}H NMR spectrum (Figure 1e) of the resulting **1**. Thus, the candidates of the polyketide precursor of **1** were narrowed down to 3-oxodecanoate or octanoate (Scheme 2).

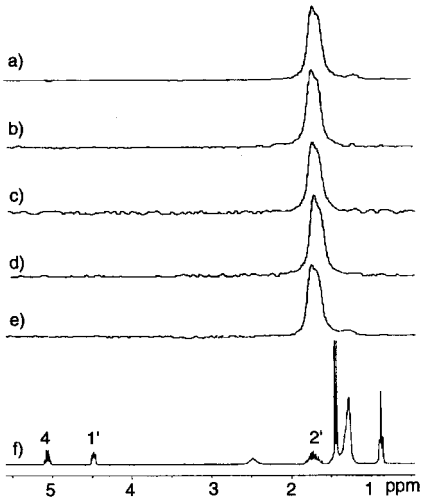


Figure 1. ^{2}H NMR spectra (61 MHz) of **1**. (a) Derived from (4*RS*)-[4-^{2}H]decanoic acid (**3**); (b) derived from (3*RS*,4*RS*)-[4-^{2}H]-3-hydroxydecanoic acid (**4**); (c) derived from ethyl (4*RS*)-[4-^{2}H]-3-oxodecanoate (**5**); (d) derived from (3*RS*,4*RS*)-[3,4-$^{2}H_2$]-3-hydroxydecanoic acid (**6**); (e) derived from (2*RS*)-[2-^{2}H]octanoic acid (**8**); (f) ^{1}H NMR spectrum (300 MHz) of nonlabeled **1**.

(19) Sekiyama, Y.; Araya, H.; Hasumi. K.; Endo, A.; Fujimoto, Y. *Nat. Prod. Lett.* **1997**, *11*, 61−66.
(20) Sekiyama, Y.; Araya, H.; Hasumi. K.; Endo, A.; Fujimoto, Y. *Tetrahedron Lett.* **1998**, *39*, 6233−6236.
(21) Sekiyama, Y.; Fujimoto, Y.; Hasumi. K.; Endo, A. *Tetrahedron Lett.* **1999**, *40*, 4223−4226.
(22) (a) Sakuda, S.; Higashi, A.; Tanaka, S.; Nihira, T.; Yamada, Y. *J. Am. Chem. Soc.* **1992**, *114*, 663−668. (b) Sakuda, S.; Tanaka, S.; Mizuno, K.; Sukcharoen, O.; Nihira, T.; Yamada, Y. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2309−2315.
(23) Gatenbeck, S.; Sierankiewicz, J. *Acta Chem. Scand.* **1973**, *27*, 1825−1827.

Scheme 2. Incorporation of ^{2}H-Labeled Fatty Acids into 1

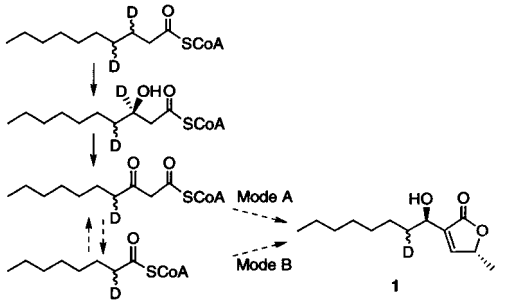


Table 1. ^{13}C-Enrichments in 1 Obtained by Feeding [1-^{13}C]-Labeled Fatty Acids

carbon (δ, ppm)	relative ^{13}C−enrichment[a] from [1-^{13}C]-decanoic acid (**9**)	[1-^{13}C]-octanoic acid (**10**)	[1-^{13}C]-dodecanoic acid
C-1 (172.6)	13.1	25.2	8.6
C-2 (136.2)	0.8	1.0	0.7
C-3 (149.3)	1.3	0.9	0.9
C-4 (77.9)	1.2	1.0	0.9
C-5 (18.9)	1.0	1.0	1.0
C-1′ (67.0)	3.1	43.8	2.7
C-2′ (35.4)	1.0	1.0	1.0
C-3′ (25.2)	3.0	4.7	4.0
C-4′ (29.3)	0.8	0.9	0.9
C-5′ (29.1)	2.5	5.2	3.6
C-6′ (31.7)	0.7	1.0	0.8
C-7′ (22.6)	2.2	3.6	3.6
C-8′ (14.0)	1.0	1.0	0.9

[a] Relative to the abundance at C-5 = 1.0.

Feeding Experiments with ^{13}C-Labeled Fatty Acids. To shed light on which fatty acid, C_{10} or C_8, is the obligatory precursor, feeding studies of [1-^{13}C]decanoic acid (**9**) and [1-^{13}C]octanoic acid (**10**) were carried out. ^{13}C-Enrichments in the resulting **1** are listed in Table 1. The ^{13}C NMR spectrum of **1** obtained by feeding **9** showed an enrichment of the C-1 signal (13.1-fold of natural abundance) accompanied by lower level enrichments of the signals at C-1′, -3′, -5′, and -7′ (2.7-fold, average of the 4 carbons). These lower level enrichments were due to the incorporation of [1-^{13}C]acetate arising from the labeled fatty acid via β-oxidation. On the other hand, the ^{13}C NMR spectrum of **1** obtained by feeding **10** displayed a remarkable enrichment of the C-1′ signal (43.8-fold). The specific uptake of **10** indicated that octanoate was more efficiently utilized in the biosynthesis of **1** than decanoate, supporting mode B. The spectrum also showed that the C-3′, -5′, -7′ signals were enriched to some extent (4.5-fold, average of the three carbons). Interestingly, the C-1 signal was much more intense (25.2-fold) than the three signals. These results are in agreement with our earlier observation that feeding [1-^{13}C]dodecanoic acid gave rise to **1** which was labeled at C-1 (8.6-fold) and C-1′, -3′, -5′, and -7′ (3.5-fold, average of the four carbons).[20] It is likely that acetyl CoA produced by β-oxidation of fatty acids was more efficiently used for the C_5-unit formation, probably via a different pool of acetate, than the C_8 appendage. Therefore, the aforementioned enrichment of the C-1 signal of **1** obtained by feeding **9** can be rationalized by assuming such acetate incorporation. Under these experimental conditions, no enrichment was observed at the C-3, -4, and -5 positions. These studies suggested that octanoate is an obligatory precursor, rather than 3-oxodecanoate.

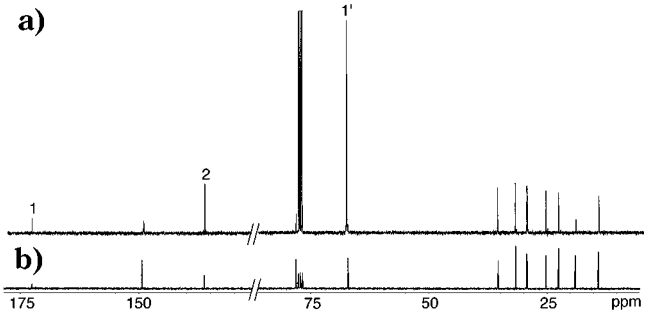
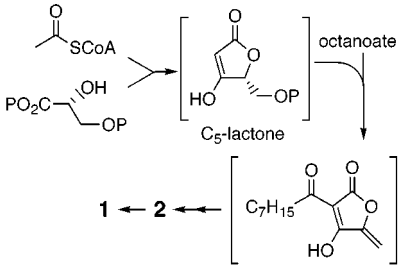


Figure 2. ^{13}C NMR spectra (100 MHz, $CDCl_3$) of **1**. (a) Derived from [2,3-$^{13}C_2$]decanoic acid (**11**); (b) nonlabeled **1**. The weak flanking doublet signals for C-1′ and C-2 may be attributed to recombination of [1-^{13}C]octanoate and [2-^{13}C]acetate arising from **11**.

Scheme 3. Plausible Pathway for the Biosynthesis of 1



This was rigorously established by feeding studies of [2,3-$^{13}C_2$]decanoic acid (**11**), which was synthesized from [1-^{13}C]octanoic acid in 10 steps with K^{13}CN as the label source. The ^{13}C NMR spectrum of **1** (Figure 2) biosynthesized from **11** showed that the intensity of the C-1′ signal (19.8-fold, normalized as C-5 = 1.0) is considerably larger than that of C-2 (10.9-fold). These data clearly indicated that the doubly labeled substrate was cleaved between C-2 and C-3, and then the resulting [1-^{13}C]-octanoate was incorporated into the C_8 side chain of **1**. As expected, the C-1 (9.2-fold) and C-2 signals of **1** in this feeding experiment were considerably enriched,[24] due to the contribution of [1-^{13}C]- and [2-^{13}C]acetate arising from **11**.

In conclusion, we have demonstrated that **1** is biosynthesized via mode B, i.e., coupling of octanoate and a C_5 unit. The result is in contrast with the biosynthesis of structurally related natural compounds. We would like to propose a pathway for acaterin biosynthesis as shown in Scheme 3, on the basis of our previous and present data. Coupling of a hypothesized C_5-lactone with octanoate, elimination of phosphoric acid followed by reduction at C-3 and C-1′ would produce **2**, which is finally reduced to furnish **1**. Investigation of the exact structure of the C_5 unit and the order of the above events is now in progress in our laboratory.

Experimental Section

General Procedures and Materials. The ^{1}H and ^{13}C NMR spectra were recorded on JEOL LA-400 or LA-300 or GSX-270 spectrometers in $CDCl_3$ at 298 K. ^{2}H NMR spectra

(24) Relative ^{13}C-enrichments in **1** obtained by feeding [2,3-$^{13}C_2$]-decanoic acid are 9.2 (C-1), 10.9 (C-2), 1.4 (C-3), 1.3 (C-4), 1.0 (C-5), 19.8 (C-1′), 4.5 (C-2′), 3.3 (C-3′), 3.7 (C-4′), 4.7 (C-5′), 3.4 (C-6′), 2.7 (C-7′), and 2.9 (C-8′).

were recorded on a JEOL LA-400 spectrometer in $CHCl_3$ at 298 K. EI-MS (70 eV), FAB-MS (3-nitrobenzyl alcohol as matrix), and GC-MS [EI-MS mode, capillary column HEWLETT PACKARD Ultra 1 (25 m × 0.20 mm i.d., film thickness 0.33 mm), oven temp programmed from 100 °C to 120 °C at the increasing rate 2 °C/min, injection temp. 200 °C, separator temp. 200 °C, ion source temp 300 °C] spectra were taken on a JEOL JMS-AX505HA spectrometer. Melting points were measured by a Yazawa BY-1 hot-stage microscope and are uncorrected. Analytical TLC was carried out on Merck silica gel 60F-254 plates (0.25 mm precoated). Column chromatography was performed on 70−230 mesh silica gel from Merck. Reaction solvents were purified by distillation from appropriate drying agents: THF, ether ($LiAlH_4$), diisopropylamine, DMSO (NaH), CH_2Cl_2 (P_2O_5), and benzene (CaH_2). Except for hydrogenolysis, all reactions were performed under nitrogen atmosphere. $K^{13}CN$ (99% ^{13}C), [1-^{13}C]octanoic acid (**10**) (>99% ^{13}C), [1-^{13}C]decanoic acid (**9**) (>99% ^{13}C) and [1-^{13}C]dodecanoic acid (>99% ^{13}C) were purchased from Isotec Inc. D_2O (>99.8% D) was purchased from Merck. $NaBD_4$ (98% D) was purchased from Aldrich.

Fermentation. *Pseudomonas.* sp. A 92 was maintained at 4 °C on Oxoid Brain Heart Infusion agar slants. The production medium contained glucose (0.5%), soybean meal (1.0%), peptone (0.5%), and $CaCO_3$ (0.2%). The pH of the medium was adjusted to 7.0 with 1.0 M KOH. The medium was sterilized for 20 min at 120 °C in a steam autoclave. A loopful of the organism from a slant culture was inoculated into 100 mL of the production medium in a 500 mL baffled Erlenmeyer flask. The inoculated cultures were incubated at 25 °C in the dark for 48 h on a rotary shaker at 200 rpm.

Feeding Experiments with Labeled Fatty Acids. In general, 100 mg each of labeled fatty acid was individually added to 100 mL of the production medium before sterilization. In the feeding experiment of [2,3-$^{13}C_2$]decanoic acid, a mixture of 30 mg of the labeled acid and 70 mg of unlabeled acid was used. The cultures were worked up, as previously described,[19] to give **1** in the amount indicated in parentheses: (4*RS*)-[4-^{2}H]decanoic acid (**3**) (10 mg), (3*RS*,4*RS*)-[4-^{2}H]-3-hydroxydecanoic acid (**4**) (12 mg), ethyl (4*RS*)-[4-^{2}H]-3-oxodecanoate (**5**) (4 mg), (3*RS*,4*RS*)-[3,4-$^2H_2$]-3-hydroxydecanoic acid (**6**) (12 mg), (3*RS*)-[3-^{2}H]decanoic acid (**7**) (10 mg) [2-^{2}H]octanoic acid (**8**) (10 mg), [1-^{13}C]decanoic acid (**9**) (6 mg), [1-^{13}C]octanoic acid (**10**) (6 mg), [2,3-$^{13}C_2$]decanoic acid (**11**) (17 mg).

(2*RS*)-[2-^{2}H]Octanoic Acid (8). A solution of ethyl octanoate (5.0 g, 29 mmol) in THF (15 mL) was added dropwise to a freshly prepared solution of LDA [n-BuLi (23 mL, 1.5 M solution in hexane, 35 mmol) was added to diisopropylamine (4.9 mL, 35 mmol) in THF (60 mL) at −78 °C, and the mixture was stirred at the same temperature for 10 min. The acetone bath was removed, and the reaction mixture was stirred at ambient temperature for 10 min and then recooled to −78 °C] in THF at −78 °C. After the mixture was stirred at this temperature for 1 h, D_2O (1.6 mL, 88 mmol) in THF (13 mL) was added dropwise to the solution. The resulting mixture was allowed to warm to room temperature and diluted with ether and saturated aqueous NH_4Cl. The layers were separated, and the aqueous layer was repeatedly extracted with ether. The combined organic layer was washed with 2 N HCl, saturated aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated under reduced pressure. Column chromatography of the resulting crude product with hexane/AcOEt (10:1**)** afforded ethyl (2*RS*)-[2-^{2}H]octanoate (**12**) (2.0 g, 76%) as a colorless oil: ^{1}H NMR (400 MHz) δ 0.88 (3H, t, J = 6.8 Hz), 1.26−1.36 (11H, m), 1.57−1.66 (2H, m), 2.32−2.37 (1.03H, m, H-2), 4.12 (2H, q, J = 7.1 Hz); ^{13}C NMR (100 MHz) δ 14.05, 14.24, 22.58, 24.84 (C-3 for doubly labeled **12**), 24.90 (C-3 for singly labeled **12**), 24.96 (C-3 for unlabeled **12**), 28.91, 29.02 (C-4 for doubly labeled **12**), 29.05 (C-4 for singly labeled **12**), 29.08 (C-4 for unlabeled **12**), 31.64, 34.08 (t, $^1J_{C-D}$ = 19.3 Hz, C-2 for singly labeled **12**), 34.38 (C-2 for unlabeled **12**), 60.13, 173.95; GC-MS *m*/*z* 174 (1.14), 173 (2.98), 172 (M^+ for unlabeled **12**, 0.57), 129 (14.9), 128 (321), 127 (5.75), 90 (21.1), 89 (100), 88 (21.2).

Lithium hydroxide monohydrate (604 mg, 14.4 mmol) was added to the solution of **12** (250 mg, 1.44 mmol) in DME (12.5 mL). The mixture was stirred at room temperature for 10 h and then diluted with ether (20 mL) and H_2O (50 mL). The separated organic layer was repeatedly extracted with 15% aqueous NaOH. The combined aqueous layer was acidified with 2 N HCl and extracted with ether. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Column chromatography of the resulting crude product with hexane/AcOEt (10:1) afforded **8** (160 mg, 77%) as a colorless oil: ^{1}H NMR (400 MHz) δ 0.88 (3H, t, J = 6.8 Hz), 1.22−1.39 (8H, m), 1.60−1.65 (2H, m), 2.31−2.37 (1.03H, m, H-2); ^{13}C NMR (100 MHz) δ 14.05, 22.59, 24.54 (C-3 for doubly labeled **8**), 24.60 (C-3 for singly labeled **8**), 24.67 (C-3 for unlabeled **8**), 28.90, 28.95 (C-4 for doubly labeled **8**), 28.97 (C-4 for singly labeled **8**), 29.00 (C-4 for unlabeled **8**), 31.60, 33.72 (t, $^1J_{C-D}$ = 19.4 Hz, C-2 for singly labeled **8**), 34.01 (C-2 for unlabeled 8), 179.95; ^{2}H NMR δ 2.33 (D-2); EI-MS *m*/*z* 146 (1.51), 145 (3.23), 144 (M^+ for unlabeled **8**, 1.14), 62 (34.9) 61 (100), 60 (42.5).

(4*RS*)-[4-^{2}H]Decanoic Acid (3). $LiAlH_4$ (356 mg, 9.38 mmol) was added to a solution of **12** (3.25 g, 18.8 mmol) in ether (33.0 mL), and the mixture was stirred at room temperature for 30 min. The reaction mixture was diluted with ether and 2 N HCl. The separated organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated under reduced pressure. Column chromatography of the resulting crude product with hexane/AcOEt (7:1) gave (2*RS*)-[2-^{2}H]octanol (**13**) (1.60 g, 65%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$ containing a small amount of D_2O), 0.88 (3H, t, J = 6.6 Hz), 1.18−1.40 (10H, m), 1.44−1.66 (1.1H, m, H-2), 3.60−3.68 (2H, m).

PDC (5.73 g, 15.2 mmol), NaOAc (500 mg, 6.10 mmol), and molecular sieves 4A powder (5.73 g) were added to the solution of **13** (1.00 g, 7.62 mmol) in CH_2Cl_2 (20.0 mL). After being stirred at room temperature for 2 h, the reaction mixture was diluted with ether and filtered through a Florisil column. The filtrate was washed with 2 N HCl, saturated aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated under reduced pressure to give a crude (2*RS*)-[2-^{2}H]octanal (**14**) (624 mg) as a yellow oil which was used for the next step without further purification.

Ethyl (triphenylphosphoranylidene)acetate (1.23 g, 3.53 mmol) was added to the solution of crude **14** (230 mg, 1.78 mmol) in benzene (2.30 mL), and the mixture was stirred at room temperature for 1 h. The reaction mixture was worked up as described for **13** to afford ethyl (4*RS*)-[4-^{2}H]-2-decenoate (**15**) (215 mg, 38% from **13**) as a colorless oil: ^{1}H NMR (300 MHz) δ 0.88 (3H, t, J = 6.7 Hz), 1.16−1.38 (11H, m), 1.38−1.52 (2H, m) 2.14−2.21 (1.1 H, m, H-4), 4.18 (2H, q, J = 7.1 Hz) 5.81 (1H, d, J = 15 Hz), 6.92−7.10 (1H, m).

Pd-C (10%, 109 mg) was added to a solution of **15** (543 mg, 2.72 mmol) in MeOH (54.3 mL), and the mixture was stirred under hydrogen at room temperature for 12 h. The reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure. Column chromatography of the resulting crude product with hexane/AcOEt (10:1) afforded ethyl (4*RS*)-[4-^{2}H]decanoate (**16**) (450 mg, 82%) as a colorless oil: ^{1}H NMR (270 MHz) δ 0.89 (3H, t, J = 6.8 Hz), 1.16−1.36 (14.2H, m), 1.50−1.68 (2H, m) 2.28 (2 H, t, J = 6.1 Hz), 4.12 (2H, q, J = 7.2 Hz).

Ester **16** (447 mg, 2.22 mmol) was hydrolyzed to **3** (256 mg, 67%) in the same manner as described for **8**. **3**: white plates; mp 27−29 °C; ^{1}H NMR (400 MHz) δ 0.88 (3H, t, J = 6.8 Hz), 1.20−1.38 (11.2H, m), 1.60−1.65 (2H, m), 2.35 (2H, t, J = 7.6 Hz); ^{13}C NMR (100 MHz) δ: 14.10, 22.65, 24.45 (C-3 for doubly labeled **3**), 24.55 (C-3 for singly labeled **3**), 24.65 (C-3 for unlabeled **3**), 28.63 (t, $^1J_{C-D}$ = 19.0 Hz, C-4 for singly labeled **3**), 29.04 (C-4 for unlabeled **3**), 29.13 (C-5 for singly labeled **3**), 29.24 (C-5 for unlabeled **3** and C-7), 29.32 (C-6 for doubly labeled **3**) 29.35 (C-6 for singly labeled **3**), 29.37 (C-6 for unlabeled **3**), 31.84, 34.06 (C-2 for doubly labeled **3**) 34.09 (C-2 for singly labeled **3**), 34.11 (C-2 for unlabeled **3**), 180.57; ^{2}H NMR δ 1.31 (1D, D-4), 1.62 (0.03D, D-3), 2.32 (0.09D, D-2); EI-MS *m*/*z* 173 (M^+ for singly labeled **3**, 4.67), 144 (5.62), 143 (3.98), 117 (1.57), 116 (8.19), 115 (12.4), 131 (12.2), 130 (19.3), 128 (28.1), 73 (100), 60 (78.2).

(3*RS*,4*RS*)-[4-^{2}H]-3-Hydroxydecanoic Acid (4). Ethyl bromoacetate (1.28 mL, 11.6 mmol) and zinc powder (760 mg, 11.6 mmol) were added to the solution of crude **14** (300 mg, 2.32 mmol) in benzene (3.00 mL), and the mixture was heated to 85 °C for 15 min, and then cooled to room temperature. The reaction mixture was worked up as described for **13** to afford ethyl (3*RS*,4*RS*)-[4-^{2}H]-3-hydroxydecanoate (**17**) (412 mg, 82%) as a colorless oil: ^{1}H NMR (300 MHz) δ 0.88 (3H, t, J = 6.7 Hz), 1.16−1.58 (14.2H, m), 2.39 (1H, dd, J = 16.4, 8.8 Hz); 2.51 (1H, dd, J = 16.4, 3.2 Hz), 3.01 (1H, bs), 3.97−4.01 (1H, m), 4.17 (2H, q, J = 7.1 Hz).

Ester **17** (350 mg, 1.61 mmol) was hydrolyzed to **4** (160 mg, 53%) in the same manner as described for **8**. **4**: white plates; mp 52−53 °C; ^{1}H NMR (400 MHz) δ 0.88 (3H, t, J = 6.8 Hz), 1.19−1.65 (11.2H, m), 2.48 (1H, dd, J = 16.6, 9.0 Hz), 2.58 (1H, dd, J = 16.6, 3.4 Hz) 4.00−4.07 (1H, m); ^{13}C NMR (100 MHz) δ 14.06, 22.61, 25.20 (C-5 for doubly labeled **4**), 25.31 (C-5 for singly labeled **4**), 25.41 (C-5 for unlabeled **4**), 29.18, 29.34 (C-6 for doubly labeled **4**), 29.37 (C-6 for singly labeled **4**), 29.39 (C-6 for unlabeled **4**), 31.74, 35.85 (t, $^1J_{C-D}$ = 19.0 Hz, C-4 for singly labeled **4**), 36.42 (C-4 for unlabeled **4**), 40.97, 67.87 (C-3 for doubly labeled **4**), 67.93 (C-3 for singly labeled **4**), 177.82; ^{2}H NMR δ 1.45 and 1.52 (D-4); FAB MS m/z 191 (27.4), 190 (62.3), 189 (MH^+ for unlabeled **4**, 41.0); EI MS m/z 171 (1.82), 170 (1.02), 89 (100).

Ethyl (4*RS*)-[4-^{2}H]-3-Oxodecanoate (5). Ester **17** (752 mg, 3.46 mmol) was oxidized to **5** (374 mg, 50%) in the same manner as described for **14**. **5**: pale yellow oil; ^{1}H NMR (400 MHz) δ 0.88 (3H, t, J = 6.8 Hz), 1.23−1.34 (11H, m), 1.52−1.67 (2H, m), 2.14−2.22 (0.1H, m, H-4 for the enol form), 2.47−2.56 (1.1H, m, H-4 for the keto form), 3.43 (1.9H, s, H-2 for the keto form), 4.20 (2H, q, J = 7.2 Hz), 4.97 (0.1H, s, H-2 for the enol form), 12.1 (0.1H, OH for the enol form); ^{13}C NMR (100 MHz) δ 14.05, 14.07, 14.25, 22.56, 22.59, 23.37 (C-5 for singly labeled **5**, the keto form), 25.42 (C-5 for unlabeled **5**, the keto form), 26.15 (C-5 for singly labeled **5**, the enol form), 26.21 (C-5 for unlabeled **5**, the enol form), 28.91 (C-6 for singly labeled **5**), 28.93 (C-6 for unlabeled **5**) 29.99, 31.60, 31.65, 34.65 (C-4 for singly labeled **5**, the enol form), 35.03 (C-4 for unlabeled **5**, the enol form), 42.67 (t, $^1J_{C-D}$ = 19.4 Hz, C-4 for singly labeled **5**, the keto form), 42.03 (C-4 for unlabeled **5**, the keto form), 42.29, 59.88, 61.32, 88.94, 167.28, 172.77, 179.02, 203.07, 203.15; ^{2}H NMR δ 2.16 (0.2D, D-4 for enol form), 2.51 (1D, D-4 for keto form); EI MS m/z 215 (M^+ for singly labeled **5**, 2.56), 197 (1.66), 196 (2.41), 131 (84.7), 130 (76.3), 128 (51.4), 115 (24.9), 88 (67.2), 85 (32.6), 57 (100).

(3*RS*,4*RS*)-[3,4-$^2H_2$]-3-Hydroxydecanoic Acid (6). $NaBD_4$ (111 mg, 2.65 mmol) was added to a solution of **5** (380 mg, 1.77 mmol) in EtOH (3.80 mL), and the mixture was stirred at room temperature for 5 min. The reaction mixture was worked up as described for **13** to afford ethyl (3*RS*,4*RS*)-[3,4-$^2H_2$]-3-hydroxydecanoate (**18**) (286 mg, 74%) as a colorless oil: ^{1}H NMR δ 0.88 (3H, t, J = 6.8 Hz), 1.16−1.56 (14.2H, m), 2.39 (1H, d, J = 16.4 Hz), 2.50 (1H, d, J = 16.4 Hz), 4.17 (2H, q, J = 7.1 Hz).

Ester **18** (286 mg, 1.31 mmol) was hydrolyzed to **6** (210 mg, 84%) in the same manner as described for **8**. **6**: white plates; mp 53−54 °C; ^{1}H NMR (400 MHz) δ 0.88 (3H, t, J = 6.6 Hz), 1.20−1.59 (11.2H, m), 2.47 (1H, d, J = 16.4 Hz), 2.57 (1H, d, J = 16.4 Hz); ^{13}C NMR δ (100 MHz) 14.06, 22.63, 25.20 (C-5 for **6** doubly labeled at C-4), 25.30 (C-5 for **6** singly labeled at C-4), 25.40 (C-5 for **6** unlabeled at C-4), 29.20 29.37 (C-6 for **6** doubly labeled at C-4), 29.39 (C-6 for **6** singly labeled at C-4), 29.41 (C-6 for **6** unlabeled at C-4), 31.77, 35.93 (t, $^1J_{C-D}$ = 18.5 Hz, C-4 for **6** singly labeled at C-4), 36.33 (C-4 for 6 unlabeled at C-4), 40.90, 67.53 (m, C-3), 177.85; ^{2}H NMR δ 1.45 and 1.52 (0.8D, D-4), 3.99 (1D, 3-D); FAB MS m/z 192 (16.1), 191 (52.09), 190 (20.43), 189 (MH^+ for unlabeled 6, 4.82); EI MS m/z 173 (0.37), 172 (1.39), 171 (1.02), 90 (100).

(3*RS*)-[3-^{2}H]Decanoic Acid (7). Ethyl nonanoate (5.0 g, 27 mmol) was converted to ethyl (2*RS*)-[2-^{2}H]nonanoate (**19**) (5.0 g, 99%) in the same manner as described for **12**. **19**: colorless oil; ^{1}H NMR (400 MHz) δ 0.88 (3H, t, J = 6.8 Hz), 1.21−1.35 (13H, m), 1.58−1.64 (2H, m), 2.24−2.31 (1.02 H, m. H-2), 4.13 (2H, q, J = 7.2 Hz); ^{13}C NMR (100 MHz) δ 14.05, 14.19, 22.60, 24.40 (C-3 for doubly labeled **19**), 24.87 (C-3 for singly labeled **19**), 24.94 (C-3 for unlabeled **19**), 29.09 (C-4 for singly labeled **19** and C-6), 29.10 (C-4 for unlabeled **19**), 29.19, 31.76, 34.04 (t, $^1J_{C-D}$ = 19.7 Hz, C-2 for singly labeled **19**), 34.33 (C-2 for unlabeled **19**), 60.09, 173.89; GC-MS m/z 188 (1.41), 187 (3.24), 186 (M^+ for unlabeled **19**, 0.34), 158 (8.96), 143 (16.4), 142 (24.22), 141 (3.17), 90 (40.69), 89 (100), 88 (13.5).

Ester **19** (5.0 g, 27 mmol) was converted to (2*RS*)-[2-^{2}H]-nonanol (**20**) (2.9 g, 73%) in the same manner as described for **13**. **20**: colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$ containing a small amount of D_2O) δ 0.88 (3H, t, J = 6.7 Hz), 1.18−1.40 (12H, m), 1.46−1.62 (1.02H, m), 3.51−3.66 (2H, m).

p-TsCl (5.7 g, 30 mmol) was added to a solution of alcohol **20** (2.9 g, 20 mmol) in pyridine (15 mL) at 0 °C, and the mixture was allowed to stand at 4 °C for 10 h. Ice chips and water were added, and the whole was stirred for 10 min. The mixture was worked up as described for **13** to afford (2*RS*)-[2-^{2}H]nonyl *p*-toluenesulfonate (**21**) (5.9 g, 99%) as a colorless oil: ^{1}H NMR (300 MHz) δ 0.87 (3H, t, J = 6.7 Hz), 1.14−1.37 (12H, m), 1.55−1.71 (1.1H, m), 2.45 (3H, s), 3.96−4.05 (2H, m), 7.35 and 7.79 (each 2H, d, J = 8.3 Hz).

KCN (262 mg, 4.02 mmol) was added to a solution of **21** (1.00 g, 3.35 mmol) in DMSO (12.5 mL), and the mixture was stirred at 90 °C for 3 h. The reaction mixture was diluted with saturated aqueous Na_2CO_3 and ether. The separated aqueous layer was repeatedly extracted with ether. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. Column chromatography of the resulting crude product with hexane/AcOEt (8:1) gave (3*RS*)-[3-^{2}H]decanenitrile (**22**) (480 mg, 94%) as a colorless oil: ^{1}H NMR (270 MHz) δ 0.88 (3H, t, J = 6.6 Hz), 1.16−1.38 (12H, m), 1.38−1.53 (2H, m), 1.56−1.72 (1.1 H, m), 2.28−2.37 (2H, m).

The mixture of **22** (480 mg, 3.13 mmol), EtOH (24.0 mL), KOH (1.76 g, 31.4 mmol), and H_2O (4.10 mL) was heated for reflux and stirred for 20 h. The reaction mixture was worked up as described for **8** to afford **7** (416 mg, 77%) as white plates: mp 26−28 °C; ^{1}H NMR (400 MHz) δ 0.88 (3H, t, J = 6.8 Hz), 1.20−1.38 (12H, m), 1.57−1.68 (1.1H, m), 2.27−2.39 (2H, m); ^{13}C NMR (100 MHz) δ 14.07, 22.65, 24.28 (t, $^1J_{C-D}$ = 19.4 Hz, C-3 for singly labeled **7**), 24.64 (C-3 for unlabeled **7**), 28.84 (C-4 for doubly labeled **7**), 28.94 (C-4 for singly labeled **7**), 29.04 (C-4 for unlabeled **7**), 29.21 (C-5 for single labeled **7**), 29.24 (C-5 for unlabeled **7** and C-7), 29.38, 31.84, 33.96 (C-2 for doubly labeled **7**), 34.05 (C-2 for singly labeled **7**), 34.13 (C-2 for unlabeled **7**), 180.74; ^{2}H NMR δ 1.62 (D-3); EI-MS m/z 174 (2.49), 173 (8.26), 172 (M^+ for unlabeled **7**, 2.63), 75 (13.5), 74 (66.0), 73 (24.9), 60 (100).

[2,3-$^{13}C_2$]Decanoic Acid (11). [1-^{13}C]Octanoic acid (500 mg, 3.44 mmol) was treated with ethereal diazomethane, and the mixture was concentrated under reduced pressure. Column chromatography of the resulting crude product with hexane/AcOEt (10:1) gave methyl [1-^{13}C]octanoate (**23**) (540 mg, 99%) as a pale yellow oil. ^{1}H NMR (300 MHz) δ 0.88 (3H, t, J = 6.6 Hz), 1.18−1.36 (8H, m), 1.50−1.68 (2H, m), 2.30 (2H, q, $^2J_{C-H}$ = 7.3, J_{H-H} = 7.3 Hz), 3.67 (3H, d, $^3J_{C-H}$ = 3.9 Hz); ^{13}C NMR (75 MHz) δ 14.04, 22.57, 24.93 (d, $^2J_{C-C}$ = 1.9 Hz, C-3), 28.89, 29.08 (d, $^3J_{C-C}$ = 3.8 Hz, C-4), 31.62, 34.07 (d, $^1J_{C-C}$ = 56.8 Hz, C-2), 51.41, (d, $^2J_{C-C}$ = 2.5 Hz), 174.33 (enhanced signal, C-1).

Ester **23** (540 mg, 3.39 mmol) was converted to [1-^{13}C]-octanol (**24**) (440 mg, 99%) in the same manner as described for **13**. **24**: colorless oil; ^{1}H NMR(300 MHz) δ 0.88 (3H, t, J = 6.8 H), 1.14−1.38 (10H, m), 1.44−1.64 (2H, m), 3.64 (2H, dt, $^1J_{C-H}$ = 140.6, J_{H-H} = 6.6 Hz); ^{13}C NMR (75 MHz), δ 14.09, 22.64, 25.72, 29.26, 29.37 (d, $^3J_{C-C}$ = 4.4 Hz, C-4), 31.80, 32.78 (d, $^1J_{C-C}$ = 37.1 Hz, C-2), 63.1 (enhanced signal, C-1).

Alcohol **24** (472 mg, 3.60 mmol) was converted to [1-^{13}C]-octyl *p*-toluenesulfonate (**25**) (896 mg, 87%) in the same manner as described for **21**. **25**: colorless oil; ^{1}H NMR (300 MHz) δ 0.87 (3H, t, J = 7.3 Hz), 1.12−1.34 (10H, m), 1.50−1.68 (2H, m), 2.45 (3H, s), 4.02 (2H, dt, $^1J_{C-H}$ = 148.4, $^3J_{H-H}$ = 6.5 Hz), 7.35 and 7.79 (each 2H, d, J = 8.3 Hz); ^{13}C NMR (75 MHz) δ 14.05, 21.61, 22.57, 25.29, 28.76 (d, $^1J_{C-C}$ = 27.8

Hz, C-2), 28.85 (d, $^3J_{C-C}$ = 4.3 Hz, C-4), 29.02, 31.66, 70.69 (enhanced signal, C-1), 127.87, 129.77, 133.18, 144.58.

Tosylate **25** (896 mg, 3.14 mmol) was converted to [1,2-$^{13}C_2$]-nonanenitrile (**26**) (410 mg, 92%) in the same manner as described for **22**, using K^{13}CN. **26**: colorless oil; ^{1}H NMR (300 MHz) δ 0.89 (3H, t, J = 6.7 Hz), 1.21−1.37 (8H, m), 1.37−1.51 (2H, m), 1.60−1.73 (2H, m), 2.34 (2H, ddt, $^1J_{C-H}$ = 134.0, $^2J_{C-H}$ = 9.6, J_{H-H} =7.2 Hz); ^{13}C NMR (75 MHz) δ 14.05, 17.10 (d, $^1J_{C-C}$ = 55.5 Hz, enhanced signal, C-2), 22.58, 25.33, (dd, $^1J_{C-C}$ = 33.0, $^2J_{C-C}$ = 2.8 Hz, C-3), 28.64 (d, $^3J_{C-C}$ = 4.3 Hz), 28.70 (d, $^3J_{C-C}$ = 4.7 Hz), 28.94, 31.67, 119.88 (d, $^1J_{C-C}$ = 55.5 Hz, C-1).

Nitrile **26** (410 mg, 2.90 mmol) was converted to [1,2-$^{13}C_2$]-nonanoic acid (**27**) (385 mg, 83%) in the same manner as described for **7**. **27**: colorless oil; ^{1}H NMR (400 MHz) δ 0.89 (3H, t, J = 6.8 Hz), 1.18−1.41 (10H, m), 1.53−1.72 (2H, m), 2.35 (2H, dq, $^1J_{C-H}$ = 128.0, $^2J_{C-H}$ = 7.3, J_{H-H} = 7.3 Hz); ^{13}C NMR (100 MHz) δ 14.07, 22.62, 24.66 (dd, $^1J_{C-C}$ = 33.7, $^2J_{C-C}$ = 1.7 Hz, C-3), 29.05 (d, $^3J_{C-C}$ = 4.9 Hz), 29.08, 29.18 (d, $^3J_{C-C}$ = 4.1 Hz), 31.78, 33.94 (d, $^1J_{C-C}$ = 54.7 Hz, enhanced signal, C-2), 179.61 (d, $^1J_{C-C}$ = 54.7 Hz, enhanced signal, C-1).

Carboxylic acid **27** (385 mg, 2.40 mmol) was converted to methyl [1,2-$^{13}C_2$]nonanoate (**28**) (418 mg, 83%) in the same manner as described for **23**. **28**: colorless oil; ^{1}H NMR (400 MHz) δ 0.87 (3H, t, J = 7.0 Hz), 1.16−1.37 (10H, m), 1.52−1.67 (2H, m), 2.30 (2H, dq, $^1J_{C-H}$ = 127.6, $^2J_{C-H}$ = 7.5, J_{H-H} = 7.5 Hz), 3.67 (3H, d, $^3J_{C-H}$ = 4.0 Hz); ^{13}C NMR (100 MHz) δ 14.08, 22.63, 24.93 (dd, $^1J_{C-C}$ = 34.6, $^2J_{C-C}$ = 1.7 Hz, C-3), 29.10, 29.14 (d, $^3J_{C-C}$ =4.1 Hz), 29.20 (d, $^3J_{C-C}$ = 4.1 Hz), 31.79, 34.09 (d, $^1J_{C-C}$ = 56.7 Hz, enhanced signal, C-2), 51.41 (d, $^2J_{C-C}$ = 1.7 Hz), 174.35 (d, $^1J_{C-C}$ = 56.7 Hz, enhanced signal, C-1).

Ester **28** (418 mg, 2.34 mmol) was converted to [1,2-$^{13}C_2$]-nonanol (**29**) (340 mg, 99%) in the same manner as described for **13**. **29**: colorless oil; ^{1}H NMR (300 MHz) δ 0.88 (3H, t, J = 6.7 Hz), 1.14−1.42 and 1.68−1.84 (16H, m, H-3 to H-8 and H-2), 3.63 (2H, brd, $^1J_{C-H}$ = 140.6 Hz); ^{13}C NMR (75 MHz) δ 14.09, 22.65, 25.70 (d, $^1J_{C-C}$ = 34.6 Hz, C-3), 29.24, 29.42 (d, $^3J_{C-C}$ = 5.0 Hz), 29.55 (d, $^3J_{C-C}$ = 3.7 Hz), 31.86, 32.78 (d, $^1J_{C-C}$ = 37.1 Hz, enhanced signal, C-2), 63.06 (d, $^1J_{C-C}$ = 37.1 Hz, enhanced signal, C-1).

Alcohol **29** (340 mg, 2.32 mmol) was converted to [1,2-$^{13}C_2$]-nonyl *p*-toluenesulfonate (**30**) (639 mg, 92%) in the same manner as described for **21**. **30**: colorless oil; ^{1}H NMR (300 MHz) δ: 0.87 (3H, t, J = 6.8 Hz), 1.13−1.37 (12H, m), 1.63 (2H, dm, $^1J_{C-H}$ = 123.0 Hz), 2.45 (3H, s), 4.01 (2H, dtd, $^1J_{C-H}$ = 148.2, J_{H-H} = 6.4, $^2J_{C-H}$ = 2.6 Hz), 7.34 and 7.79 (each 2H, d, J = 8.2 Hz); ^{13}C NMR (75 MHz) δ 14.06, 21.59, 22.60, 25.25 (d, $^1J_{C-C}$ = 33.9 Hz, C-3), 28.75 (d, $^1J_{C-C}$ = 37.7 Hz, enhanced signal, C-2), 28.88 (d, $^3J_{C-C}$ = 4.3 Hz), 29.30 (d, $^3J_{C-C}$ = 3.8 Hz), 31.76, 70.68 (d, $^1J_{C-C}$ = 37.7 Hz, enhanced signal, C-1), 127.85, 129.75, 133.19, 144.58.

Tosylate **30** (639 mg, 2.13 mmol) was converted to [2,3-$^{13}C_2$]-decanenitrile (**31**) (301 mg, 92%) in the same manner as described for **22**. **31**: colorless oil; ^{1}H NMR (300 MHz) δ 0.88 (3H, t, J = 6.7 Hz), 1.20−1.41 (10H, m), 1.41−1.51 and 1.81−1.93 (4H, m, H-4 and H-3), 2.32 (2H, dm, $^1J_{C-H}$ = 143.8 Hz); ^{13}C NMR (75 MHz) δ 14.04, 17.08 (d, $^1J_{C-C}$ = 32.7 Hz, enhanced signal, C-2), 22.59, 25.32 (d, $^1J_{C-C}$ = 32.7 Hz, enhanced signal, C-3), 28.63 (dd, $^1J_{C-C}$ = 34.6 Hz, $^2J_{C-C}$ = 2.5 Hz), 28.72 (d, $^3J_{C-C}$ = 4.4 Hz), 29.12, 29.22 (d, $^3J_{C-C}$ = 4.3 Hz), 31.76, 118.09 (d, $^1J_{C-C}$ = 41.9 Hz, C-1).

Nitrile **31** (301 mg, 1.95 mmol) was converted to **11** (232 mg, 68%) in the same manner as described for **7**. **11**: white plates; mp 27−28 °C; ^{1}H NMR (400 MHz) δ 0.88 (3H, t, J = 6.8 Hz), 1.20−1.39 (12H, m), 1.64 (2H, dm, $^1J_{C-H}$ = 128.0 Hz), 2.35 (2H, dtd, $^1J_{C-H}$ = 127.4 Hz, J_{H-H} = 7.5 Hz, $^2J_{C-H}$ = 4.5 Hz); ^{13}C NMR (100 MHz) δ 14.08, 22.65, 24.63 (d, $^1J_{C-C}$ = 33.7 Hz, enhanced signal, C-3), 29.04 (d, $^1J_{C-C}$ = 34.6 Hz, C-4), 29.24, 29.38 (d, $^3J_{C-C}$ = 4.1 Hz), 31.84, 34.12 (d, $^1J_{C-C}$ = 33.7 Hz, enhanced signal, C-2), 180.72 (dd, $^1J_{C-C}$ = 54.3 Hz, $^2J_{C-C}$ = 1.6, C-2); Anal. Calcd for $C_8{}^{13}C_2H_{20}O_2$: C+^{13}C, 68.93; H, 11.57. Found: C+^{13}C, 69.23; H, 11.85; EI-MS *m*/*z* 174 (M^+ 12.2), 75 (91.94), 61 (100).

JO015614G