JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Synthesis of Deuterated γ-Lactones for Use in Stable Isotope Dilution Assays

JO-ANNA HISLOP, MARTIN B. HUNT, SIMON FIELDER, AND DARYL D. ROWAN*

The Horticulture and Food Research Institute of New Zealand Ltd., Private Bag 11030, Palmerston North, New Zealand

Two syntheses of deuterated γ-lactones for use as internal standards in stable isotope dilution assays (SIDA) were developed. [2,2,3,3-$^2H_4$]-γ-Octa-, -γ-deca-, and -γ-dodecalactones with >89% deuterium incorporation were prepared in 27, 17, and 19% overall yields, respectively, by the reduction of a doubly protected hydroxypropiolic acid with deuterium gas. [3,3,4-$^2H_3$]-γ-Octa- and -γ-dodecalactones were prepared in 6 and 23% yields with >92% deuterium incorporation by the free radical addition of 2-iodoacetamide to [1,1,2-$^2H_3$]-1-hexene and [1,1,2-$^2H_3$]-1-decene, respectively. Reaction yields were highly dependent upon the purity of the 1-alkene starting material. The deuterated γ-lactones were evaluated as internal standards for SIDA.

KEYWORDS: γ-Lactones; deuterium; synthesis; isotope labeling; SIDA; flavor compounds; γ-dodecalactone; γ-decalactone; γ-octalactone

INTRODUCTION

γ-Lactones (**Scheme 1**) are important flavor compounds that occur in a wide range of foods including stone fruit (*1*, *2*), wine (*3*), milk (*4*), and dairy products (*5*). γ-Lactones are formed by the cyclization of the corresponding γ-hydroxycarboxylic acids (*6*), with the even-numbered carbon chain lactones predominating in foodstuffs. The intense and characteristic odors of these lactones are described as being "fruity" and in some cases as "reminiscent of coconut" (γ-octalactone), "peach-like" (γ-deca- and γ-dodecalactones), and "fatty" (γ-dodecalactone) (*7*). The respective odor detection thresholds for γ-octa-, γ-deca-, and γ-dodecalactone in water are 7, 11, and 7 ppb (*2*). To enhance flavor, γ-lactones are added to many foodstuffs at concentrations often comparable to those occurring naturally and within a range of 0.01−60 ppm (*7*).

Accurate quantitation of flavor compounds is most readily achieved through the addition of a known quantity of a stable isotope labeled analogue to the sample as an internal standard (SIDA) (*8*). Due to the nearly identical physical and chemical properties of the labeled internal standard and the analyte, the labeled and unlabeled materials are recovered with equal efficiencies, and losses of the analyte during isolation and measurement are compensated for. To minimize interferences during GC-MS analysis, deuterium labeling should increase the molecular weight of a labeled internal standard by at least 2 mass units. For γ-lactones, under standard electron impact GC-MS conditions (70 eV), loss of the alkyl substituent at C4 is favored, leading to an intense base peak at *m*/*z* 85 in the mass spectrum (**Scheme 1**). For maximum sensitivity in SIDA, a deuterium label should therefore be placed in the lactone ring.

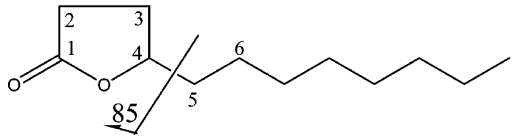
Although many syntheses of γ-lactone have been developed (*9*), few are suitable for the introduction of multiple deuterium atoms regioselectively into the lactone ring. Heiba et al. (*10*) have reported the synthesis of a range of γ-lactones via a one-step procedure involving the addition of substituted carboxylate salts to alkenes at elevated temperatures in the presence of manganese(III) salts. Curran and Ko (*11*) reported the one-step synthesis of multiply substituted γ-lactones by the free radical addition of 2-bromoacetic acid derivatives to butyl vinyl ether. Yorimitsu et al. (*12*) have used Curran's atom transfer process and reported the isolation of γ-lactones in good yields from the addition of a 2-iodocarbonyl compound to a 1-alken-ω-ol in water in the presence of a radical initiator. Thus, 2-iodoacetamide and 5-hexen-1-ol were reacted in water in the presence of a radical initiator to give 8-hydroxy-γ-octalactone in 95% yield. The reaction failed, however, to produce any lactone products when a water-insoluble 1-alkene was used.

Ring-deuterated γ-dodecalactone and γ-decalactone have been reported by Haffner and Tressl (*13*) as metabolic products of the yeast *Soridiobolus salmonicolo*. Ring-deuterated d_4-oak lactones have been prepared by Pollnitz et al. (*14*) by deuterium exchange and borodeuteride reduction of 3-methyl-4-oxo-octanoic acid. A similar strategy has been used to prepare d_5-γ- and δ-lactones with deuterium incorporated into both the ring positions (C3 and C4) and the side chain (C5) (*15*, *16*). This paper reports the synthesis of [3,3,4-$^2H_3$]- and [2,2,3,3-$^2H_4$]-ring-labeled γ-lactones using two general strategies: (1) hydrogenation of 4-hydroxypropargylic acids (*17*) with deuterium gas and (2) addition of carboxymethyl radicals to [1,1,2-

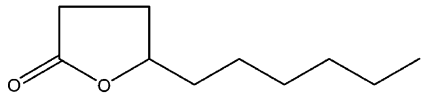
* Author to whom correspondence should be addressed (telephone +00-64-6-356-8080; fax +00-64-6-351-7011; e-mail drowan@hortresearch.co.nz).

10.1021/jf048885b CCC: $27.50
Published on Web 10/15/2004

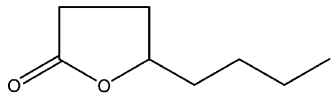
Scheme 1. Chemical Structures of γ-Dodeca- (**1**), γ-Deca- (**2**), and γ-Octalactones (**3**) and 6-Dodecyne-γ-lactone (**4**) Showing Origin of the Base Peak *m/z* 85 in the Mass Spectrum



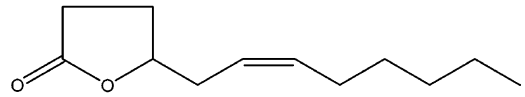
1 γ-dodecalactone



2 γ-decalactone



3 γ-octalactone



4 *Z*-6-dodeceno-γ-lactone

$^2H_3$]-1-alkenes (*10*−*12*) as well as a preliminary evaluation of use of these compounds for SIDAs.

MATERIALS AND METHODS

Chemicals. Reagents were obtained from the Aldrich Chemical Co. (Milwaukee, WI) and were used without further purification unless otherwise stated. Reactions were performed under an atmosphere of dry nitrogen in oven-dried glassware. Benzene, THF, and diethyl ether (BDH) were purified by distillation from sodium benzophenone ketyl. Reactions were monitored by thin-layer chromatography on aluminum-backed Kieselgel 60 F_{254} silica gel plates (Merck) with the specified solvents. Compounds were visualized under an ultraviolet lamp followed by treatment with alkaline potassium permanganate and strong heating. Flash column chromatography was carried out using silica gel (60−62 μm, Merck). Short path distillation was carried out using a GKR-51 Kugelrohr (Büchi).

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ on a JEOL HNM-GX270W NMR spectrometer. Chemical shifts (δ) are in parts per million relative to chloroform at 7.27 ppm for ^{1}H (270 MHz) and at 77.0 ppm for ^{13}C (67.8 MHz). The following abbreviations are used: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad. Infrared spectra were measured on a Paragon 1000 FT-IR spectrometer as thin films between NaCl plates. Capillary gas chromatography−mass spectrometry (GC-MS) was carried out on a VG70-250S magnetic sector instrument operating at a 70 eV ionization potential (spectral interface at 180 °C) coupled to a Hewlett-Packard 5890 series II gas chromatograph fitted with a 30 m × 0.25 mm i.d. DB1 column, film thickness = 0.25 μm; temperature was programmed from 40 °C (5 min) to 280° C at 5 °C/min (hold for 20 min), helium head pressure = 5 psi.

Synthesis of [2,2,3,3-$^2H_4$]-γ-Lactones. *4-Hydroxydec-2-ynoic Acid Methyl Ester*. A solution of lithium diisopropylamide was prepared by stirring *n*-butyllithium (1.45 M [hexanes], 21.7 mL, 31.4 mmol) and diisopropylamine (4.12 mL, 31.4 mmol) in THF (20 mL) under argon at 0 °C. This solution was added to a solution of propiolic acid (1.0 g, 14.3 mmol) in THF (10 mL) at −78 °C. *N*,*N*,*N*′,*N*′-Tetramethylethylenediamine (5 mL) and heptanal (1.63 g, 14.3 mmol) were added, and the mixture was warmed to room temperature with stirring. After 1 h, water (10 mL), saturated NH_4Cl (20 mL), and aqueous HCl (10%, 10 mL) were added, and the aqueous layer was washed with ether (3 × 10 mL). The organic phase was evaporated, and the crude oil was dissolved in ether (40 mL) and extracted with 2% NaOH (2 × 10 mL). The aqueous phase was acidified with concentrated HCl (2 × 10 mL) and re-extracted with ether (3 × 10 mL). The combined organic phases were washed with water (2 × 10 mL) and brine (10 mL), dried over $MgSO_4$, and concentrated in vacuo to give 4-hydroxydec-2-ynoic acid as a white power (1.15 g, 44%). This was dissolved in ether (35 mL) and treated with excess diazomethane (prepared from *N*-methyl-*N*-nitrosotoluene-*p*-sulfonamide) at 0 °C for 5 min. Removal of volatiles in vacuo and purification by flash chromatography on silica gel eluting with hexane through to hexane/ethyl acetate (4:1) gave the title compound (0.80 g, 28%) as a pale yellow oil: R_f 0.59 (hexane/ethyl acetate 1:1); ν_{max} (film) 3422, 2956, 2931, 2860, 1720, 1458, 1437, 1254, 1049 cm^{-1}; ^{1}H NMR δ 0.86 (3H, t, J = 6.2 Hz, H10), 1.26 (6H, s, H7 to H 9), 1.43 (2H, m, H6), 1.73 (2H, m, H5), 3.75 (3H, s, OCH_3), 4.46 (1H, t, J = 6.6 Hz, H4); ^{13}C NMR δ 14.0, 22.5, 24.9, 28.8, 31.6, 36.8, 52.8, 61.9, 75.9, 88.6, 163.8; MS, *m/z* (rel int %) 197 (52), 181 (45), 113 (28), 69 (37), 55 (72), 43 (100). Found: $M^+ - H$ 197.1172, $C_{11}H_{17}O_3$ requires 197.1178.

*4-tert-Butyldimethylsiloxydec-2-ynoic Acid Methyl Ester (**5**).* To a stirred solution of 4-hydroxydec-2-ynoic acid methyl ester (0.80 g, 4.0 mmol) in DMF (2 mL) at 0 °C was added imidazole (0.33 g, 4.9 mmol) and *tert*-butyldimethylsilyl chloride (0.54 g, 5.0 mmol). The solution was brought to room temperature and stirred for 18 h. Water (2 mL) was added to the reaction, and the solution was partitioned against ether (3 × 25 mL). The combined organic extracts were washed with water (2 × 20 mL) and saturated brine (20 mL), dried over $MgSO_4$, concentrated in vacuo, and purified by flash chromatography on silica gel. Eluting with hexane through to hexane/ethyl acetate (5:1) gave **5** (0.84 g, 66%) as a colorless oil: R_f 0.59 (hexane/ethyl acetate 4:1); ν_{max} (film) 2957, 2859, 1720, 1464, 1436, 1362, 1341, 1249, 1096 cm^{-1}; ^{1}H NMR δ 0.11 (3H, s, SiCH_3), 0.14 (3H, s, SiCH_3), 0.89 (12H, s, H10 and C(CH_3)$_3$), 1.28 (6H, s, H7 to H9), 1.41 (2H, m, H6), 1.71 (2H, m, H5), 3.76 (3H, s, OCH_3), 4.44 (1H, t, J = 6.4 Hz, H4); ^{13}C NMR δ −5.1, −4.5, 14.1, 18.2, 22.6, 25.0, 25.7, 28.9, 31.7, 37.8, 52.6, 62.6, 75.6, 89.1, 153.8; MS, *m/z* (rel int %) 311 (2), 281 (7), 255 (52), 229 (24), 203 (34), 171(33), 149 (7), 89 (100), 73 (49), 55 (21), 41 (19). Found: $M^+ - H$ 311.2043, $C_{17}H_{31}O_3Si$ requires 311.2042.

*[2,2,3,3-$^2H_4$]-4-tert-Butyldimethylsiloxydecanoic Acid Methyl Ester **6**.* To **5** (0.83 g, 2.7 mmol) dissolved in freshly distilled benzene (25 mL) was added Wilkinson's catalyst (10 mol %, 0.125 g). The flask was fitted to a hydrogenation apparatus and degassed three times before stirring vigorously while the uptake of D_2 gas (99.8% atom D) was monitored. After consumption of 2.0 equiv of D_2 (60 h), the flask was isolated from the reservoir of deuterium gas. Benzene was removed under reduced pressure and the crude oil filtered through a small plug of Celite with hexane/ethyl acetate (20:1). The product was purified further by flash chromatography on silica gel eluting with hexane through to hexane/ethyl acetate (20:1) to give **6** (0.83 g, 97%) as a colorless oil: R_f 0.56 (hexane/ethyl acetate 4:1); ν_{max} (film) 2956, 2931, 2858, 1743, 1463, 1435, 1361, 1256, 1198, 1091 cm^{-1}; ^{1}H NMR δ 0.04 (6H, s, Si(CH_3)$_2$) 0.88 (12H, s, H10 and C(CH_3)$_3$), 1.27 (8H, br s, H6 to H9), 1.43 (2H, m, H5), 3.66 (3H, s, OCH_3); ^{13}C NMR δ −4.6, −4.4, 14.1, 18.1, 22.6, 25.2, 25.9, 29.5, 31.9, 37.0, 51.4, 71.0, 174.4; MS, *m/z* (rel int %) 320 (<1), 289 (10), 263 (100), 230 (76), 174 (13), 161 (7), 132 (7), 115 (15), 101 (9), 89 (76), 73 (89), 59 (44), 41 (27). Found: M^+ 320.2688, $C_{17}H_{32}D_4O_3Si$ requires 320.2688.

[2,2,3,3-$^2H_4$]-γ-Decalactone. To a stirred solution of **6** (0.73 g, 2.9 mmol) in THF (40 mL) was added concentrated HCl (1 mL). After 2 h of stirring, the reaction was diluted with ether (10 mL) and partitioned against saturated sodium bicarbonate (2 × 20 mL), water (2 × 20 mL), and saturated brine (20 mL). The solution was dried over $MgSO_4$ and concentrated in vacuo to give a pale oil. Flash chromatography on silica gel eluting with hexane/ethyl acetate (4:1) gave an oil that was further

purified by distillation (120 °C at 0.2 mmHg) to the title compound (0.365 g, 92%) as a colorless oil: R_f 0.29 (hexane/ethyl acetate 4:1); ν_{max} (film) 3524, 2931, 2859, 2361, 1770, 1467, 1379, 1348, 1202, 1112, 1062, 1112, 1062, 1012 cm^{-1}; ^{1}H NMR δ 0.88 (3H, t, J = 6.4 Hz, H10) 1.27 (8H, br s, H6 to H9), 1.60 (1H, m, H5), 1.71 (1H, m, H5), 4.47 (1H, t, J = 6.4 Hz, H4); ^{13}C NMR δ 14.1 (q), 22.6 (t), 27.9 (m, CD_2), 28.6 (m, CD_2) 25.2 (t), 29.0 (t), 31.7 (t), 35.6 (t), 80.9 (d), 177.2 (s); MS, *m*/*z* (rel int %) 174 (<1), 156 (1), 141 (4), 132 (8), 104 (4), 89 (100), 88 (51), 70 (7), 60 (7), 56 (8), 55 (9), 43 (15), 41 (17). Found: M^+ 174.1559, $C_{10}H_{14}D_4O_2$ requires 174.1557.

[2,2,3,3-$^2H_4$]-γ-Dodecalactone was prepared from nonanal as above. Flash chromatography on silica gel and distillation (140 ° C at 0.2 mmHg) gave the title compound (52 mg, 17% overall) as a colorless oil: R_f 0.29 (hexane/ethyl acetate 4:1); ν_{max} (film) 3519, 2927, 2856, 1770, 1467, 1378, 1348, 1203, 1161, 1113, 1060 cm^{-1}; ^{1}H NMR δ 0.88 (3H, t, J = 6.4 Hz, H10), 1.27 (12H, br s, H6 to H11), 1.60 (1H, m, H5), 1.71 (1H, m, H5), 4.47 (1H, t, J = 6.4 Hz, H4); ^{13}C NMR δ 14.2 (q), 22.7 (t), 25.3 (t), 27.5 (m, CD_2), 28.7 (m, CD_2), 29.2 (t), 29.4 (t), 29.5 (t), 31.9 (t), 35.6 (t), 80.9 (d), 177.1 (s); MS, *m*/*z* (rel int %) 141 (2), 132 (5), 104 (4), 89 (100), 88 (11), 70 (7), 69 (7), 60 (5), 56 (11), 55 (12), 43 (15), 41 (21). Found: M^+ 202.1868, $C_{12}H_{18}D_4O_2$ requires 202.1871.

[2,2,3,3-$^2H_4$]-γ-Octalactone was prepared from pentanal as above. Flash chromatography and distillation (110 °C at 2 mmHg) gave the title compound (0.36 g, 27% overall) as a colorless oil: R_f 0.29 (hexane/ethyl acetate 4:1); ν_{max} (film) 3520, 2958, 2934, 2863, 1772, 1467, 1380, 1348, 1202, 1108, 1061, 1009 cm^{-1}; ^{1}H NMR δ 0.88 (3H, t, J = 6.4 Hz, H8), 1.27 (4H, br s, H6, 7) 1.60 (1H, m, H5), 1.71 (1H, m, H5), 4.47 (1H, t, J = 6.4 Hz, H4); ^{13}C NMR δ 13.8 (q), 22.3 (t), 27.2 (t), 28.0 (m, CD_2), 28.8 (m, CD_2), 35.1 (t), 80.7 (d), 177.0 (s); MS, *m*/*z* (rel int %) 146 (<1), 125 (1), 104 (4), 90 (4), 89 (100), 88 (35), 60 (6), 41 (8). Found: M^+ 146.1246, $C_8H_{10}D_4O_2$ requires 146.1245.

Optimization Procedure for the Synthesis of γ-Decalactone 2. Typically, 1-octene (0.168 g, 1.50 mmol) was added to a glass scintillation vial containing a solution of 2-iodoacetamide or 2-iodoacetic acid (0.5 mmol) and radical initiator (1.0 mmol) in benzene (10 mL). The vial was flushed with argon, sealed (screw-cap lid and Parafilm), and marked to indicate the initial volume. A blank of benzene (10 mL) was also prepared for each run. Mole ratios were generally varied by factors of 2. The vials were placed in a vibrating water bath (200 rpm) at 75 °C. After 18 h, aqueous HCl (10%, 1 mL) and water (1 mL) were added to each vial. For GC analysis, 1 mL of the crude reaction mixture was diluted to 10 mL with ether. The blank (benzene only) was likewise diluted and spiked with γ-decalactone (1 μL). Product analysis was carried out on a Hewlett-Packard model 5890 series II gas chromatograph equipped with a flame ionization detector. Separations were achieved using a 30 m × 0.25 mm i.d. SE-30 Econo-Cap capillary column with a film thickness of 0.25 μm; the temperature was programmed from 50 °C (1 min) at 10 °C/min to 240 °C (5 min); the injector temperature was 200 °C, and the detector temperature was 250 °C. The column head pressure was 10 psi of hydrogen. The best results from each set of experiments were repeated and used as initial values in further optimization experiments.

Synthesis of γ-Decalactone 2. 1-Octene (0.168 g, 1.50 mmol) and water (0.90 mL, 50 mmol) were added to a stirred solution of 2-iodoacetamide (0.093 g, 0.503 mmol) and ACCN (0.242 g, 0.992 mmol) in benzene (10 mL). This mixture was refluxed under argon for 18 h, whereupon water (1 mL) and aqueous HCl (10%, 1 mL) were added. The reaction mixture was extracted with ether (3 × 5 mL), and the combined organic extracts were washed with water (2 × 5 mL) and saturated brine (5 mL), dried over $MgSO_4$, and concentrated in vacuo. Flash chromatography on silica gel, eluting with hexane through to hexane/ethyl acetate (4:1), gave the title compound as an oil, which was further purified by short path distillation (112−116 °C at 0.2 mmHg) (0.083 g, 32% based on 1-octene): R_f = 0.31 (hexane/ethyl acetate 4:1); ν_{max} (film) 2955, 2931, 2870, 1775, 1467, 1420, 1363, 1282, 1182, 1063, 1012 cm^{-1}; ^{1}H NMR δ 0.82 (3H, t, J = 6.4 Hz, H10), 1.23 (7H, br s, H5 and H7 to H9), 1.37 (1H, m, H6), 1.58 (1H, m, H6), 1.68 (1H, m, H5), 1.82 (1H, m, H3), 2.27 (1H, m, H3), 2.47 (2H, m, H2), 4.42 (1H, m, H4); ^{13}C NMR δ 14.1, 22.6, 25.2, 28.0, 28.9, 29.0, 31.7, 35.6, 81.0, 177.1; MS, *m*/*z* (rel int %) 170 (<1), 128 (9), 100 (5), 85 (100), 70 (4), 55 (10), 43 (9), 41 (13). Found: M^+ 170.1300, $C_{10}H_{18}O_2$ requires 170.1307.

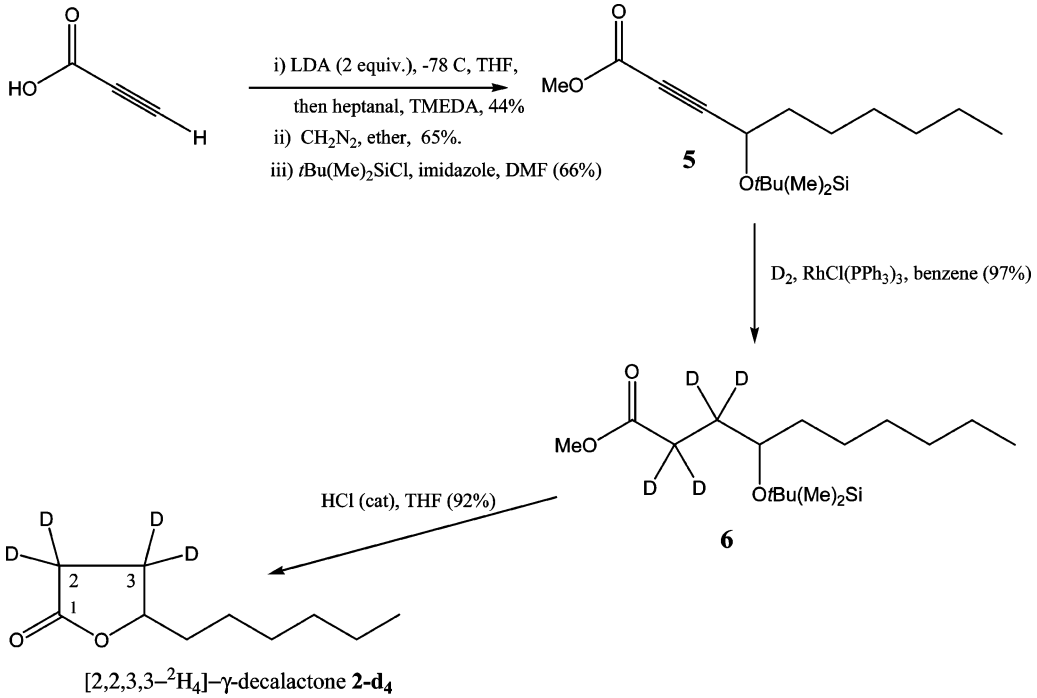
Synthesis of [3,3,4-$^2H_3$]-γ-Dodecalactone. 2-Iodoacetamide (0.215 g, 1.2 mmol) and ACCN (0.557 g, 2.3 mmol) were added to a stirred solution of [1,1,2-$^2H_3$]-1-decene (0.498 g, 3.5 mmol) in benzene (23 mL). Water (2.15 mL, 0.1 mol) was added, and the mixture was refluxed under nitrogen for 18 h. Aqueous HCl (10%, 2.5 mL) and water (2.5 mL) were added, and the mixture was extracted with ether (3 × 20 mL). The combined organic extracts were washed with water (2 × 20 mL) and saturated brine (20 mL), dried over $MgSO_4$, and concentrated in vacuo. The crude oil was purified by flash chromatography on silica gel, eluting with hexane through to hexane/ethyl acetate (4:1), to give recovered [1,1,2-$^2H_3$]-1-decene (17%) and ACCN (79%). Further elution gave a colorless oil, which was further purified by short path distillation (135−140 °C at 0.2 mmHg) to give the title compound (0.163 g, 23%) at 96% deuterium incorporation: R_f 0.45 (hexane/ethyl acetate 4:1); ν_{max} (film) 3525, 2927, 2856, 1778, 1467, 1424, 1378, 1259, 1228, 1187, 1124, 1010 cm^{-1}; ^{1}H NMR δ 0.88 (3H, t, J = 6.2 Hz, H12), 1.27 (11H, br s, H5 and H7 to H11), 1.46 (1H, m, H6), 1.58 (1H, m, H6), 1.73 (1H, m, H5), 2.50 (2H, s, H2), 4.47 (0.04H, m, residual H−); ^{13}C NMR δ 14.1 (q), 22.6, 25.2, 27.4 (m, C3), 28.7, 29.2, 29.3, 29.4, 31.8, 35.4, 80.4 (t, C4), 177.1; MS, *m*/*z* (rel int %) 201 (0.2), 183 (0.9), 141 (3), 131 (7), 103 (6), 98 (5), 88 (100), 87 (18), 84 (5), 70 (10), 69 (8), 60 (4), 56 (13), 55 (12), 43 (16), 41 (20). Found: M^+ 201.1804, $C_{12}H_{19}D_3O_2$ requires 201.1808.

Synthesis of [3,3,4-$^2H_3$]-γ-Dodecalactone (10). To a stirred solution of [1,1,2-$^2H_3$]-1-decene (0.113 g, 0.79 mmol, 93% ^{2}H) and acetic acid (10 mL) was added manganic acetate dihydrate (0.57 g, 2.1 mmol) and potassium acetate (3.56 g). The solution was refluxed for 1.5 h until the brown color had disappeared, and the reaction mixture was diluted with water (5 mL) and extracted with ether (3 × 15 mL). The organic extracts were combined, dried over $MgSO_4$, and concentrated in vacuo. The resulting crude material was passed through a short plug of silica gel with pentane (10 mL) and ethyl acetate (10 mL) to yield a pale yellow oil, which was further purified by short path distillation (136−140 °C at 0.2 mmHg) to give the title compound (0.11 g, 69%): ^{1}H NMR δ 4.46 (0.10H, m, residual H4) indicated 90% deuteration.

Synthesis of [3,3,4-$^2H_3$]-γ-Octalactone. 2-Iodoacetamide (0.13 g, 0.7 mmol) and ACCN (0.339 g, 1.4 mmol) were added to a stirred solution of [1,1,2-$^2H_3$]-1-hexene (0.183 g, containing some [1-$^2H_1$]-1-hexyne) in benzene (14 mL). Water (1.26 g, 70 mmol) was added, and the mixture was refluxed under nitrogen for 18 h. Aqueous HCl (10%, 2.5 mL) and water (2.5 mL) were added, and the mixture was extracted with ether (3 × 10 mL). The combined organic extracts were washed with water (2 × 10 mL) and brine (10 mL), dried over $MgSO_4$, and concentrated in vacuo. The resulting material was purified by flash chromatography on silica gel, eluting with hexane through to hexane/ethyl acetate (4:1) to give the title compound (0.017 g, 6%) as a pale oil with 92% deuterium incorporation: R_f 0.21 (hexane/ethyl acetate 6:1); ^{1}H NMR δ 0.92 (3H, t, J = 6.4 Hz, H8), 1.39 (3H, m, H7 and H5), 1.43 (1H, m, H-6), 1.74 (1H, m, H5), 2.52 (2H, s, H2), 4.46 (0.08H, m, residual H4); ^{13}C NMR δ 13.9, 22.5, 27.3 (m, C3), 28.7, 28.8, 35.1, 80.5 (t, C4), 177.0; MS, *m*/*z* (rel int %) 145 (0.8), 144 (0.6), 127 (1), 126 (2), 103 (7), 102 (4), 88 (100), 87 (79), 86 (15), 85 (5), 71 (5), 57 (7), 41 (7). Found: M^+ 145.1178, $C_8H_{11}D_3O_2$ requires 145.1167.

Synthesis of [1,1,2-$^2H_3$]-1-Alkenes. *[1-$^2H_1$]-1-Decyne.* To a stirred solution of 1-decyne (1.53 g, 11.1 mmol) in THF (40 mL) at −78 °C was added *n*-butyllithium (1.2 M [hexanes], 10.8 mL, 13.3 mmol). After 10 min of stirring, D_2O (0.24 mL, 13 mmol, 99.9% atom D) was added. The reaction mixture was warmed to room temperature, stirred (2 h), and washed with ether (3 × 20 mL) and aqueous HCl (10%, 10 mL). The organic phase was washed with water (3 × 10 mL) and saturated brine (10 mL), and the aqueous fractions were back-extracted with ether (2 × 10 mL). The combined organic fractions were dried over $MgSO_4$ and concentrated in vacuo to give the title compound (1.50 g, 97%) with 98% deuterium incorporation as a pale oil: ν_{max} (film) 2927, 2857, 2598, 1467, 1378, 1327, 1116 cm^{-1}; ^{1}H NMR δ 0.88 (3H, t, J = 6.2 Hz, H10) 1.28 (8H, br s, H6 to H9), 1.40 (2H, m, H5), 1.51 (2H, quintet, J = 6.8 Hz, H4), 1.92 (0.02H, t, residual H1), 2.18 (2H, t, J 6.8 Hz, H3); ^{13}C NMR δ 14.2, 18.4, 22.7, 28.6, 28.8, 29.1, 29.2, 31.9, 67.9 (t,

Scheme 2. Synthesis of [2,2,3,3-$^2H_4$]-γ-Decalactone (**2**-d_4) by Alkylation of Propiolic Acid with Heptanal



[2,2,3,3–$^2H_4$]–γ-decalactone **2-d$_4$**

Cl), 84.2 (t, C2); MS, *m*/*z* (rel int %) 139 (<1), 110 (6), 96 (31), 82 (90), 68 (51), 57 (49), 55 (100), 43 (66), 41 (99). Found: M^+ 139.1475, $C_{10}H_{17}D$ requires 139.1471.

[1,1,2-$^2H_3$]-1-Decene. Lindlar's catalyst (0.33 g) and quinoline (0.25 mL) were added to a stirred solution of [1-$^2H_1$]-1-decyne (1.50 g, 108 mmol) in pentane (40 mL). The reaction flask was flushed three times with deuterium gas (~10 mL, 99.8% atom D) and attached to a hydrogenation apparatus, where the solution was stirred vigorously while D_2 uptake was monitored. After 18 h, deuterium uptake was complete, and the mixture was filtered through a short plug of Celite and rinsed with ether (2 × 20 mL), then washed with aqueous HCl (10%, 20 mL), saturated sodium bicarbonate (2 × 10 mL), water (2 × 10 mL), and saturated brine (10 mL). The combined organic phases were dried over $MgSO_4$ and concentrated in vacuo to give a yellow oil. Short path distillation (76−80 °C at 15 mmHg) gave the title compound (0.944 g, 61%) as a colorless oil with 93% deuterium incorporation: ν_{max} (film) 2958, 2926, 2855, 1584, 1467, 1378, 1118 cm^{-1}; ^1H NMR 0.92 (3H, t, J = 6.2 Hz, H10) 1.31 (12H, br s, H4 to H9), 2.07 (2H, t, J = 6.2 Hz, H3), 4.90−4.95 (0.07H, m, residual H1), 5.42 (0.035H, m, residual H2); ^{13}C NMR 14.2, 22.8, 29.0, 29.3, 29.4, 29.6, 32.0, 33.7, 113.6 (m, C1), 138.9 (t, C2); MS, *m*/*z* (rel int %) 143 (15), 114 (7), 100 (14), 98 (12), 86 (22), 83 (20), 76 (75), 56 (100), 43 (91), 41 (59). Found: M^+ 143.1753, $C_{10}H_{17}D_3$ requires 143.1753.

Synthesis of [1-$^2H_1$]-1-Octyne. To a stirred solution of 1-octyne (3.75 g, 34 mmol) in THF (100 mL) at −78 °C was added *n*-butyllithium (2.0 M [hexanes], 20 mL, 40 mmol). After 10 min of stirring, D_2O (0.83 mL, 40 mmol, 99.9% atom D) was added. The reaction mixture was warmed to room temperature and stirred (2 h) with ether (40 mL) and aqueous HCl (10%, 20 mL). The organic phase was washed with water (3 × 20 mL) and saturated brine (20 mL), and the aqueous fractions were back-extracted with ether (2 × 10 mL). The combined organic fractions were dried over $MgSO_4$ and concentrated in vacuo to give the title compound (3.28 g, 87%). Attempted purification by distillation gave a mixture (bp 81−86 °C at 16 mmHg) of [1-$^2H_1$]-1-octyne (92% deuterium incorporation) and 1-butanol (ca. 1:1). *For [1-$^2H_1$]-1-octyne:* ^1H NMR δ 0.88 (3H, t, J = 6.2 Hz, H8), 1.29 (6H, br s, H5, 6, and 7), 1.51 (2H, quintet, H4), 1.93 (0.08H, t, J = 6.8 Hz, residual H1), 2.16 (2H, t, J = 7.0 Hz, H3); ^{13}C NMR δ 14.0, 18.4, 22.6, 28.5, 31.3, 67.8, 84.2 (t, C-1); MS, *m*/*z* (rel int %) 111 (2), 96 (16), 82 (99), 68 (41), 55 (60), 43 (100), 41 (77). Found: M^+ 111.1147, $C_8H_{13}D$ requires 139.1158.

Synthesis of [1-$^2H_1$]-1-Octyne (Sodium Hydride). 1-Octyne (3.75 g, 34.0 mmol) was added to ether-washed NaH (2.64 g, 0.11 mol) in ether (100 mL) under argon and sonicated for 30 min. The mixture was quenched at 0 °C with D_2O (3.41 g, 0.17 mol) in the presence of d_4-acetic acid (1.95 mL, 34 mmol). The reaction mixture was washed sequentially with 10% HCl (20 mL), water (2 × 20 mL), and brine (20 mL). The combined aqueous fractions were back-extracted with diethyl ether (2 × 20 mL). The organic fractions were combined, dried ($MgSO_4$), and evaporated to give [1-$^2H_1$]-1-octyne (87% deuteration).

Synthesis of [1,1,2-$^2H_3$]-1-Hexene. 1-Hexyne (3.60 g, 44 mmol) in tetradecane (50 mL) was stirred with prewashed NaH (1.10 g, 46 mmol) for 2 h at 0 °C. The reaction was quenched with D_2O (0.95 mL, 48 mmol) and stirred for 2 h. Lindlar's catalyst (1.32 g) and quinoline (1.0 mL) were added, and the flask was swept with deuterium gas (2 × ~15 mL) prior to reduction with deuterium gas. Additional catalyst (150 mg) was added after 1 week. Deuterium uptake ceased after 2 weeks when the required volume was consumed. The reaction mixture was filtered through a short column of $MgSO_4$ and distilled. A fraction (bp 72−80 °C, 42% yield) was shown by ^1H NMR to consist of a mixture of [1,1,2-$^2H_3$]-1-hexene and [1-$^2H_1$]-1-hexyne in a ratio of 1.0:0.8. *For [1,1,2-$^2H_3$]-1-hexene:* ^1H NMR δ 0.90 (3H, t, J 7.2 Hz, H6), 1.36 (4H, m, H4, 5), 2.03 (2H, m, H3), 4.92−4.96 (0.06H, m, residual H1), 5.42 (0.03H, m, residual H2); ^{13}C NMR δ 13.9, 22.2, 31.1, 33.3, 113.5 (m, C1), 131.2 (t, C2); GC-MS, *m*/*z* (rel int %) 87 (27), 74 (13), 70 (14), 57 (88), 43 (91), 41 (100). M^+ 87.1127 $C_6H_9D_3$ requires 87.1127. *For [1-$^2H_1$]-1-hexyne:* ^1H NMR δ 1.56 (1.6H, m, H3), other signals obscured; ^{13}C NMR δ 13.8, 19.0, 22.3, 31.3, 68.2 (m, C2), 84.3 (m, C1).

RESULTS AND DISCUSSION

Two approaches to the synthesis of ring-deuterated γ-lactones were evaluated: first, the synthesis of [2,2,3,3-$^2H_4$] ring-deuterated γ-lactones by the reduction of a 4-hydroxypropargylic acid (*17*) with deuterium gas and, second, the synthesis of [3,3,4-$^2H_3$] ring-deuterated compounds by the addition of a carboxymethyl radical to a [1,1,2-$^2H_3$]-1-alkene (*10*−*12*).

[2,2,3,3-$^2H_4$]-γ-Lactones by Reduction of 4-Hydroxypropargylic Acids with Deuterium Gas. The [2,2,3,3-$^2H_4$]-γ-octa-, deca-, and dodecalactones were synthesized by adaptation of the reaction schemes reported by Midland and Tramontano (*17*) and Herrman et al. (*18*) (**Scheme 2**). Thus, alkylation of the dianion of propiolic acid with heptanal gave 4-hydroxydec-2-ynoic acid in 44% yield. A homogeneous hydrogenation

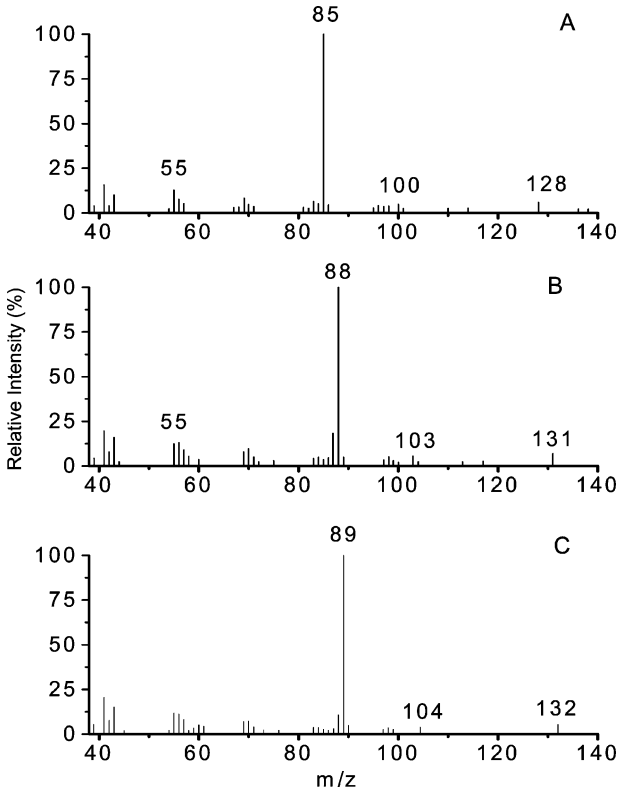


Figure 1. Mass spectra (GC-MS, 70 eV) of (**A**) γ-dodecalactone, (**B**) [3,3,4-$^2H_3$]-γ-dodecalactone, and (**C**) [2,2,3,3-$^2H_4$]-γ-dodecalactone.

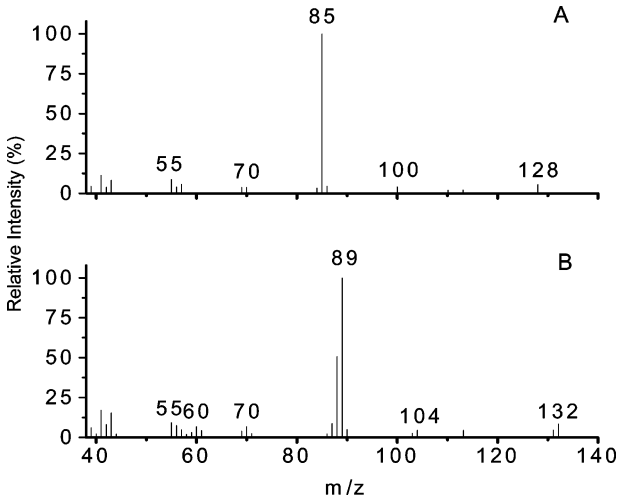


Figure 2. Mass spectra (GC-MS, 70 eV) of (**A**) γ-decalactone and (**B**) [2,2,3,3-$^2H_4$]-γ-decalactone.

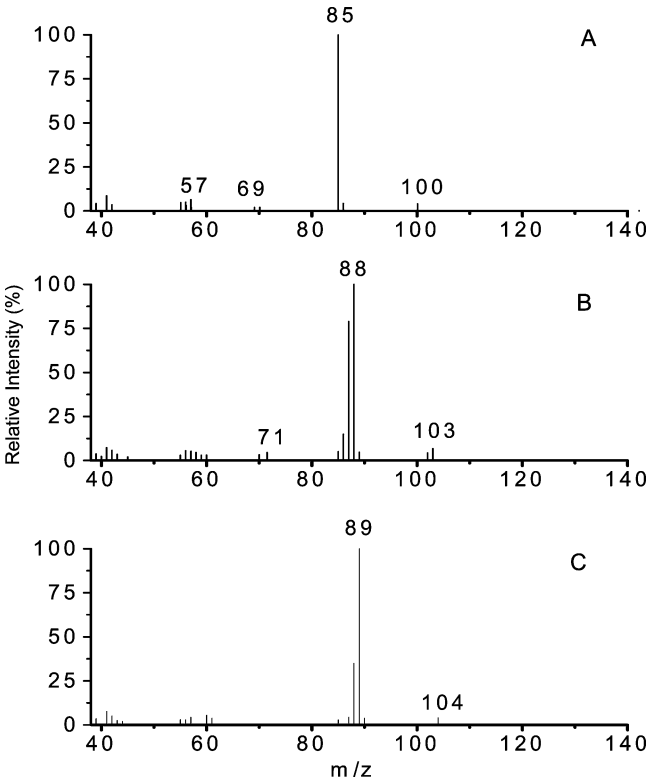


Figure 3. Mass spectra (GC-MS, 70 eV) of (**A**) γ-octalactone, (**B**) [3,3,4-$^2H_3$]-γ-octalactone, and (**C**) [2,2,3,3-$^2H_4$]-γ-octalactone.

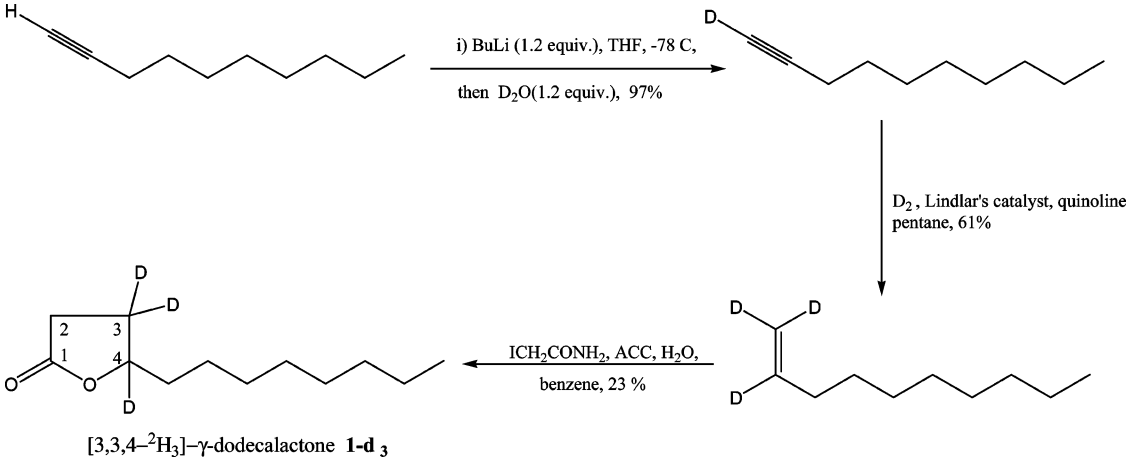
catalyst [Wilkinson's, $RhCl(PPh_3)_3$] was chosen to prevent scrambling of the deuterium label (*19*); however, a trial reaction involving the reduction with hydrogen of 4-hydroxydodec-2-ynoic acid proceeded very slowly and in poor yield (6 weeks and 24% yield, respectively). 4-Hydroxydec-2-ynoic acid was therefore protected as the methyl ester *tert*-butyldimethsilyl ether 5 (43% yield over two steps). Reduction with deuterium gas and Wilkinson's catalyst in benzene then gave the deuterated derivative **6** in 97% yield after 60 h. Deprotection with catalytic HCl in THF gave [2,2,3,3-$^2H_4$]-γ-decalactone (**2**-d_4) in 92% yield after distillation (17% overall yield). Incorporation of deuterium at C2 and C3 was shown by the nearly complete absence of signals at δ 2.34 and 2.18 (H2 and H3, respectively) and the collapse of a multiplet (δ 4.40, H4) into a triplet in the ^1H NMR. Integration of residual signals indicated 89% incorporation of deuterium.

Analogous procedures were used to synthesize [2,2,3,3-$^2H_4$]-γ-dodecalactone (19% overall yield, 97% deuterium incorporation by ^1H NMR) and [2,2,3,3-$^2H_4$]-γ-octalactone (27% overall yield, 90% deuterium incorporation).

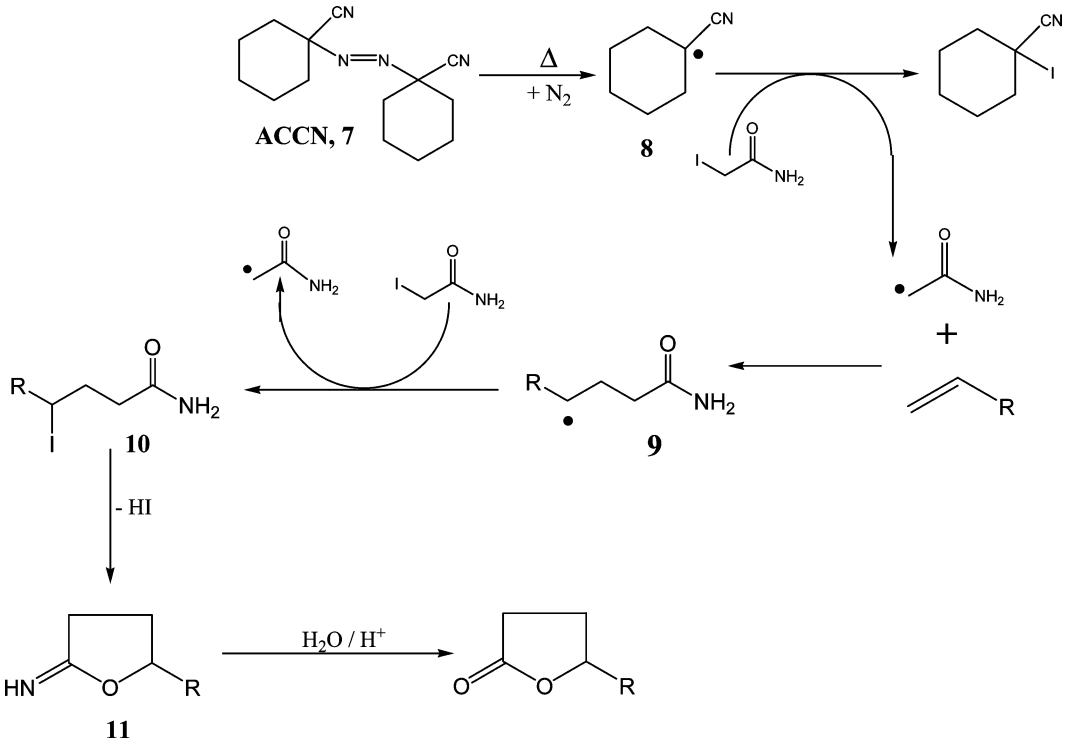
GC-MS analysis gave mass spectra (**Figures 1**−**3**) with a base peak at *m*/*z* 89, 4 mass units higher than in the unlabeled compound and corresponding to the incorporation of four deuterium atoms. The mass spectra of some samples (e.g., **Figures 2** and **3**) also included a major peak at *m*/*z* 88, indicating the presence of significant amounts of a trideuterated species, inconsistent with the results of ^1H NMR analysis. The molecular ion (<1%) appeared as a cluster (201:202:203:204 = 39:68:100:70) centered at *m*/*z* 203, confirming some loss of label; however, similar multiple ions were observed for both deuterated and nondeuterated γ-lactones. Attempted CI-GC-MS of deuterated and nondeuterated samples gave similar molecular ion clusters. On the basis of varying ion ratios (*m*/*z* 89:*m*/*z* 88) between samples, it was concluded that partial loss of deuterium from C2 was occurring in the GC injector port through enolization of the deuterium α to the lactone carbonyl, possibly with residual water in the samples. Although the extent of this loss was reproducible throughout a series of GC-MS samples, and could be corrected for in SIDA, it provided the impetus to develop an improved synthesis of labeled γ-lactones.

[3,3,4-$^2H_3$]-γ-Lactones by Carboxymethyl Radical Addition to [1,1,2-$^2H_3$]-1-Alkenes. The method of Yorimitsu et al. (*12*) for the synthesis, in water, of ω-hydroxyalkyl-γ-lactones was adapted (**Scheme 3**) and optimized for the synthesis of water-insoluble γ-lactones. The introduction of deuterium into the ring of the γ-lactone requires the availability of the corresponding [1,1,2-$^2H_3$]-1-alkenes. Sirokman et al. (*20*) have reported the synthesis of [1-$^2H_1$]-1-alkynes with high isotopic purity by the quenching of the corresponding sodium acetylide

Scheme 3. Synthesis of [3,3,4-$^2H_3$]-γ-Dodecalactone (**1**-d_3) from 1-Decyne



Scheme 4. Mechanism Proposed (*12*) for the Free Radical Addition of 2-Iodoacetamide to 1-Alkenes To Give γ-Lactones



with deuterium oxide. Reduction of the deuterated acetylene with gaseous deuterium in the presence of Lindlar's catalyst would then afford the required [1,1,2-$^2H_3$]-1-alkenes (**Scheme 3**).

The formation of γ-lactones by free radical addition to 1-alkenes is the result of a complex series of reactions (**Scheme 4**) initiated by the thermal decomposition of the radical initiator 1,1′-azo-bis(cyclohexanecarbonitrile) (**7**, ACCN) to generate two equivalent cyclohexyl radicals **8**. In a second step, the cyclohexyl radical **8** abstracts an iodine atom from a 2-iodocarbonyl species to give an acetamide radical. The acetamide radical adds to the 1-alkene to produce the secondary carbon-centered radical **9**, to which iodine is transferred either from a second molecule of 2-iodoacetamide or from iodocyclohexylnitrile formed initially in the iodine abstraction step. Cyclization of **10** under the reaction conditions, with loss of HI, gives the imino-ether **11** (*12*), which is hydrolyzed to give the γ-lactone product. Initially this reaction, when run in benzene, produced no γ-lactone products. Optimization of the reaction condition was carried out with 1-octene using either 2-iodoacetamide or 2-iodoacetic acid, initially in small glass vials and later in conventional glassware. The reaction conditions of Yorimitsu et al. (*12*) with olefin, 2-iodocarbonyl, and radical initiator in a 1.5:1.0:0.5 mole ratio were chosen as a starting point. Initial results indicated ACCN **7** gave marginally higher yields (~3%) than either 4,4′-azobis(4-cyanopentanoic acid) or 2,2′-azobis(isobutyronitrile) (0−3%), and further optimization used only this compound. Two cycles of optimization identified the combination of 1-octene (1.5 mmol), 2-iodoacetamide (0.5 mmol), and ACCN (1.0 mmol) as giving a 24% GC yield of γ-decalactone **2**. The proposed mechanism (*12*) requires water to hydrolyze the imino-ether intermediate **11**. The significance of extraneous water was realized when a 22% isolated (24% GC yield) yield of γ-decalactone obtained above from a reaction in a scintillation vial gave no γ-lactone in a round-bottom flask under similar "anhydrous" conditions. A final optimization was carried out under nitrogen in conventional round-bottom flasks (20 mL) (**Table 1**). A 28% GC yield was obtained with 50 equiv of water

Table 1. Effect of Concentration of Water on GC Yield of γ-Decalactone (**2**) Produced from Reaction of 2-Iodoacetamide with 1-Octene

1-octene (mmol)	2-iodoacetamide (mmol)	ACCN (mmol)	water (mmol)	yield of γ-decalactone (**2**)[a] (%)
1.5	0.5	1.0	5.0	3
1.5	0.5	1.0	10.0	12
1.5	0.5	1.0	50.0	28
1.5	0.5	1.0	100.0	14

[a] Based on 1-octene.

Table 2. Isolated Yields (Based on 1-Alkene) of Deuterated and Nondeuterated γ-Lactones Obtained by Addition of Iodoacetamide and $Mn(OAc)_3$-Derived Acetoxy Radicals to Appropriate 1-Alkene Precursors

product lactone	reagents	yield % of γ-lactone	yield % of [3,3,4-$^2H_3$]-γ-lactone
γ-octa-	2-iodoacetamide/ACCN	32	6
γ-deca-	2-iodoacetamide/ACCN	33	0[a]
	$Mn(OAc)_3$/HOAc	38	
γ-dodeca-	2-iodoacetamide/ACCN	32	23
	$Mn(OAc)_3$/HOAc		69
6-dodecyno-γ-	2-iodoacetamide/ACCN	5.2[b]	
	$Mn(OAc)_3$/HOAc	40	

[a] Olefin precursor, [1,1,2-$^2H_3$]-1-octene, contaminated with *n*-butanol. [b] Olefin precursor, 1-decen-4-yne. Yield excludes recovered starting material (40%).

under a nitrogen atmosphere. When repeated preparatively, γ-decalactone was isolated in 28% yield after distillation. Comparable yields of γ-octa- and γ-dodecalactone (32%) were also obtained under these conditions (**Table 2**).

Synthesis of [3,3,4-$^2H_3$]-γ-Dodecalactone. Deprotonation of 1-decyne with *n*-BuLi in THF at −78 °C and quenching of the reaction mixture with deuterium oxide gave [1-$^2H_1$]-1-decyne in 97% isolated yield after distillation. Integration of the residual signal in the ^1H NMR at 1.94 ppm (alkynic proton, 0.02 H) indicated 98% deuterium incorporation. In addition, the two-proton triplet of doublets for H3 had collapsed to a simple triplet at 2.18 ppm. The ^{13}C NMR spectra showed the terminal alkynic signals (C1 and C2) much reduced and replaced by a pair of 1:1:1 triplets. GC-MS confirmed the deuteration with a molecular ion of *m*/*z* 139, 1 mass unit higher than for 1-decyne.

Hydrogenation of **12** with deuterium gas in the presence of Lindlar's catalyst poisoned with quinoline gave **13** in 61% yield after distillation. The ^1H NMR showed the nearly complete absence of the alkenic protons, corresponding to 93% deuterium incorporation. The two-proton multiplet (H3) of 1-decene had collapsed into a triplet at 2.07 ppm. ^{13}C NMR data showed a reduced intensity for the deuterated signals with splitting of the two alkenic signals into a multiplet at 113.6 ppm (C1) and a 1:1:1 triplet at 138.9 ppm (C2). Mass spectral analysis showed a molecular ion of *m*/*z* 143 (15%), 3 mass units above that of 1-decene.

Using the optimized conditions described above, [1,1,2-$^2H_3$]-1-decene (3.5 mmol) was refluxed with 2-iodoacetamide (1.2 mmol), ACCN (2.3 mmol), and water (0.1 mol) in benzene (23 mL) for 18 h under an atmosphere of nitrogen. [3,3,4-$^2H_3$]-γ-Dodecalactone was obtained in 23% yield after chromatography and distillation with 96% deuterium incorporation as judged by ^1H NMR. The ^1H NMR spectra showed the expected simplification of splitting patterns with the disappearance of the one-proton quintet at 4.31 ppm (H4) and two methylene multiplets at 1.82 and 2.26 ppm (H3). The two-proton multiplet (H2) at 2.52 ppm had collapsed into a broadened singlet of comparable chemical shift (2.52 ppm). The ^{13}C NMR signals for the deuterated carbons were easily distinguishable, with C4 appearing as a 1:1:1 triplet at 80.8 ppm and C3 as a multiplet centered around 27.4 ppm. A molecular ion of *m*/*z* 201 (<1%) was obtained by GC-MS analysis of the distilled material, 3 mass units above that of γ-dodecalactone. The expected base peak of *m*/*z* 88 was observed (**Figure 1B**).

Attempted Synthesis of [3,3,4-$^2H_3$]-γ-Decalactone. Treatment of 1-octyne with 1.2 equiv each of *n*-butyllithium and then D_2O gave an 87% yield of crude [1-$^2H_1$]-1-octyne contaminated with residual hexanes. Attempts to purify this product resulted in further significant losses (80−90%), so the crude product was hydrogenated directly with deuterium gas using Lindlar's catalyst and quinoline in pentane to give a 61% yield after distillation of [1,1,2-$^2H_3$]-1-octene with 88% deuterium incorporation by ^1H NMR. This product was contaminated with significant quantities of 1-butanol (~40%), presumably from reaction of the *n*-butyllithium reagent with atmospheric oxygen. All attempts to use this product in the preparation of [3,3,4-$^2H_3$]-γ-decalactone were unsuccessful. Alternatively, [1-$^2H_1$]-1-octyne was prepared by reaction of 1-octyne with sodium hydride and quenching with D_2O/acetic acid-d_4, but at best an 87% incorporation of deuterium was achieved. Although disappointing, unlabeled γ-decalactone was isolated in 33% yield by this procedure, suggesting that the target compound would be accessible by this reaction given access to pure [1,1,2-$^2H_3$]-1-octene.

Synthesis of [3,3,4-$^2H_3$]-γ-Octalactone. Due to the volatility of reagents and products, the synthesis of [1,1,2-$^2H_3$]-1-hexene required a different approach, and the direct transformation from 1-hexyne (bp 64 °C) with recovery by distillation from high boiling solvents was investigated. 1-Hexyne in tetradecane was treated sequentially with 1.05 equiv each of sodium hydride and D_2O followed by hydrogenation in situ with Lindlar's catalyst and deuterium gas. Distillation gave a 42% yield of a mixture (1:0.8) of [1,1,2-$^2H_3$]-1-hexene (with 94% deuterium incorporation) and [1-$^2H_1$]-1-hexyne. To test if residual 1-hexyne would interfere with γ-lactone synthesis, 1-hexyne was substituted for the 1-alkene under otherwise identical reaction conditions. The only product isolated from the reaction was identified by NMR as a 6:1 cis/trans mixture of 4-iodo-3-octenamide (14%). Significant in the ^1H NMR was the appearance of two doublets H2 (3.00 and 3.12 ppm) in a ratio of 6:1 and a single proton triplet H3 (5.79 ppm). The ^{13}C NMR showed amide (172.3 and 174.5 ppm) and alkenic carbons [94.6 and 98.7 (C4), and 114.6 and 116.4 (C3) ppm]. This result suggested that the lactonization reaction should proceed in the presence of residual 1-hexyne.

Reaction of [1,1,2-$^2H_3$]-1-hexene (contaminated with [1-$^2H_1$]-1-hexyne) with 2-iodoacetamide under optimized conditions gave [3,3,4-$^2H_3$]-γ-octalactone in a disappointing 6% yield after purification by flash chromatography with 92% deuterium incorporation as judged by ^1H NMR. Comparison of ^1H NMR spectra with that of unlabeled γ-octalactone showed the disappearance of the one-proton multiplet at 4.46 ppm (H4) and the two methylene multiplets at 1.88 and 2.31 ppm (H3). ^{13}C NMR showed splitting of the deuterated carbons with C4 appearing as a 1:1:1 triplet at 80.5 ppm and C3 as a multiplet centered at 27.3 ppm. A molecular ion of *m*/*z* 145 (<1%) was obtained by GC-MS, corresponding to the presence of three deuterium atoms. Similarly, a base peak of *m*/*z* 88 (100%), increased by 3 mass units, was also observed (**Figure 3B**).

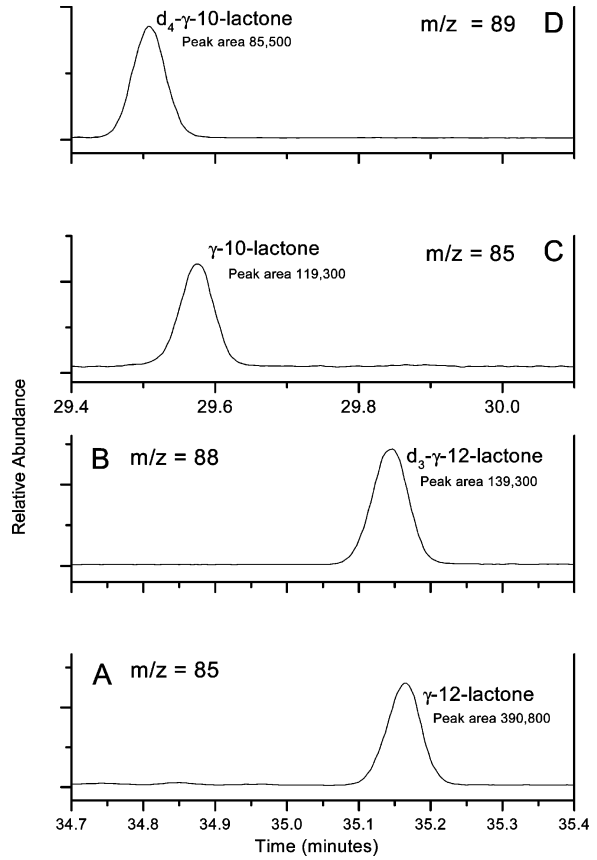


Figure 4. GC-MS-SIM mass chromatograms of a butter flavor extract spiked with [2,2,3,3-$^2H_4$]-γ-decalactone and [3,3,4-$^2H_3$]-γ-dodecalactone at 36 and 90 ng g^{-1} butter, respectively. Unlabeled γ-decalactone (**C**) and γ-dodecalactone (**A**) were measured at *m/z* 85, [2,2,3,3-$^2H_4$]-γ-decalactone at *m/z* 89 (**D**), and [3,3,4-$^2H_3$]-γ-dodecalactone at *m/z* 88 (**B**).

With freshly distilled 1-hexene, this reaction resulted in the successful isolation of 32% γ-octalactone after distillation and suggests that with access to sufficient quantities of pure [1,1,2-$^2H_3$]-1-hexene, the corresponding deuterated γ-octalactone would also be accessible.

Comparative Procedure: Mn(III) Acetate Chemistry. The relative efficiency of the free radical reaction above was compared with that of the one-step γ-lactone synthesis of Heiba et al. (*10*), which involves refluxing the appropriate 1-alkene in acetic acid in the presence of manganese(III) acetate. Removal of acetic acid from the reaction mixture proved to be difficult; nonetheless, γ-decalactone was obtained in 38% yield after distillation (**Table 2**). The harsh experimental conditions raised concerns as to the integrity of a deuterium label. Nevertheless, treatment of [1,1,2-$^2H_3$]-1-decene (93% deuterium incorporation) under Heiba's conditions gave [3,3,4-$^2H_3$]-γ-dodecalactone in 69% yield after distillation with 90% deuterium incorporation as judged by ^{1}H NMR. For the reactions tested, the manganese-(III) acetate procedure produced better yields of γ-lactones.

The extension of this reaction scheme to γ-lactones bearing unsaturated side chains, such as 6-(*Z*)-dodeceno-γ-lactone **4**, was explored. The olefin precursor, 1-decen-4-yne, was prepared in 80% yield by copper-catalyzed alkylation of 1-lithioheptyne with allyl bromide (*21*). Reaction with 2-iodoacetaminde under standard conditions gave 6-dodecyno-γ-lactone in only 5% yield. Reaction under the conditions of Heiba et al. (*10*) gave a 40% isolated yield (**Table 2**). This reaction was not pursued further; however, the product was subsequently converted to [6,7-$^2H_2$]-6-(*Z*)-dodeceno-γ-lactone in 75% yield after reduction with deuterium gas and Lindlar's catalyst (*16*).

SIDA Evaluation. The use of the deuterated lactones in SIDA of butter flavor was investigated. A sample of [2,2,3,3-$^2H_4$]-γ-decalactone and [3,3,4-$^2H_3$]-γ-dodecalactone in heptane (10 μL) were added to a stirred slurry of butter (28 g) at 40 °C so as to give concentrations of internal standards of 36 and 90 ng g^{-1} of butter, respectively. A flavor extract was prepared by adsorption of the butter onto Celite and extraction with acetonitrile (*22*) and analyzed by GC-MS with selected ion monitoring. The deuterated internal standards (*m/z* 88 and 89) were fully resolved from their unlabeled analogues (*m/z* 85) (**Figure 4**). Using appropriate response factors, the concentrations of γ-decalactone and γ-dodecalactone in this sample were calculated as 19 and 185 ng g^{-1}, respectively.

This result demonstrates the suitability of both the [3,3,4-$^2H_3$]- and [2,2,3,3-$^2H_4$]-γ-lactones for use in SIDAs, although care must be taken to correct for any deuterium exchange that may occur with the [2,2,3,3-$^2H_4$]-labeled compounds. The [3,3,4-$^2H_3$]-γ-lactones are not subject to deuterium exchange, and a good source of [1,1,2-$^2H_3$]-1-alkenes and further optimization of yields (*23*) would make these compounds more readily available for use in flavor analysis.

ACKNOWLEDGMENT

We thank a reviewer for drawing our attention to further research by Yorimitsu et al. (*23*), which reports the synthesis of γ-decalactone in 83% yield by the free radical cyclization of 1-octene and 2-iodoacetamide using aqueous ethanol as solvent and a novel radical initiator. We also thank John Allen for the HRMS measurements

LITERATURE CITED

(1) Derail, C.; Hofmann, T.; Schieberle, P. Differences in key odorants of handmade juice of yellow-flesh peaches (*Prunus persica* L.) induced by the workup procedure. *J. Agric. Food Chem.* **1999**, *47*, 4742−4745.

(2) Engle, K.-H.; Flath, R. A.; Buttery, R. G.; Mon, T. R.; Ramming, D. W.; Teranishi, R. Investigation of volatile constituents in nectarines. 1. Analytical and sensory characterization of aroma components in some nectarine cultivars. *J. Agric. Food Chem.* **1988**, *36*, 549−553.

(3) Aznar, M.; López, R.; Cacho, J. F.; Ferreira, V. Identification and quantification of impact odorants of aged red wines from Rioja. GC-olfactometry, quantitative GC-MS, and odor evaluation of HPLC fractions. *J. Agric. Food Chem.* **2001**, *49*, 2924−2929.

(4) Bendall, J. G. Aroma compounds of fresh milk from New Zealand cows fed different diets. *J. Agric. Food Chem.* **2001**, *49*, 4825−4832.

(5) Lehmann, D.; Maas, B.; Mosandl, A. Stereoisomeric flavour compounds LXIX: stereodifferentiation of $\delta(\gamma)$-lactones C_8−C_{18} in dairy products, margarine and coconut. *Lebensm.-Unters. -Forschung* **1995**, *201*, 55−61.

(6) Haffner, T.; Tressl, R. Biosynthesis of (*R*)-γ-decanolactone in the yeast *Sporobolomyces odorus*. *J. Agric. Food Chem.* **1996**, *44*, 1218−1223.

(7) Fenaroli, *G. Fenaroli's Handbook of Flavour Ingredients*; Furia, T. E., Bellanca, N., Eds.; CRC Handbook; The Chemical Rubber Co.: Cleveland, OH, 1971.

(8) Grosch, W. Detection of potent odorants in foods by aroma extract dilution analysis. *Trends Food Sci. Technol.* **1993**, *4*, 68−73.

(9) Collins, I. Saturated and unsaturated lactones. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1377−1395.

(10) Heiba, E. I.; Dessau, R. M.; Rodewald, P. G. Oxidation by metal salts. X. One-step synthesis of γ-lactones from olefins. *J. Am. Chem. Soc*. **1974**, *96*, 7977−7981.
(11) Curran, D. P.; Ko, S. B. Additions of electrophilic radicals to electron rich alkenes by the atom transfer method, surmounting potentially reversible radical atom transfer reactions by irreversible ionic reactions. *Tetrahedron Lett.* **1998**, *39*, 6629−6632.
(12) Yorimitsu, H.; Wakabayashi, K.; Shinokubo, H.; Oshima, K. Radical addition of 2-iodoalkanamide or 2-iodoalkanoic acid to alkenols using a water-soluble radical initiator in water. A facile synthesis of γ-lactones. *Tetrahedron Lett.* **1999**, *40*, 519−522.
(13) Haffner, T.; Tressl, R. Stereospecific metabolism of isomeric epoxyoctadecanoic acids in the lactone-producing yeast *Sporidiobolus salmonicolor*. *Lipids* **1998**, *33*, 47−58.
(14) Pollnitz, A. P.; Jones, G. P.; Sefton, M. A. Determination of oak lactones in barrel-aged wines and in oak extracts by stable isotope dilution analysis. *J. Chromatogr. A* **1999**, *857*, 239−246.
(15) Milo, C.; Reineccius, G. A. Identification and quantification of potent odorants in regular-fat and low-fat mild cheddar cheese. *J. Agric. Food Chem*. **1997**, *45*, 3590−3594.
(16) Schieberle, P.; Gassenmeier, K.; Guth, H.; Sen, A.; Grosch, W. Character impact odour compounds of different kinds of butter. *Lebensm.-Wiss. -Technol*. **1993**, *26*, 3247−356.
(17) Midland, M. M.; Tramontan, A. The synthesis of naturally occurring 4-alkyl and 4-alkenyl-γ-lactones using the asymmetric reducing agent β-3-pinanyl-9-borabicyclo[3.3.1]nonane. *Tetrahedron Lett*. **1980**, *21*, 3549−3552.
(18) Herrmann, J. L.; Berger, M. H.; Schlessinger, R. H. A total synthesis of racemic Avenaciolide. *J. Am. Chem. Soc*. **1979**, *101*, 1544−1549.
(19) Mohrig, J. R.; Dabora, S. L.; Foster, T. F.; Schultz, S. C. Stereospecific synthesis of 2-deuterio-3-hydroxybutanoate esters. Regiochemistry and stereochemistry of homogeneous hydrogenation with Wilkinson's catalyst. *J. Org. Chem.* **1984**, *49*, 5179−5182.
(20) Sirokman, G.; Molner, A.; Bartok, M. Synthesis of deuterium-labeled alkenes. *J. Labelled Compd. Radiopharm.* **1998**, *27*, 1−11.
(21) Burns, D. H.; Miller, J. D.; Chan, H.-K.; Delaney, M. O. Scope and utility of a new soluble copper catalyst [CuBr−LiSPh−LiBr−THF]: A comparison with other copper catalysts in their ability to couple one equivalent of a Grignard reagent with an alkyl sulfonate. *J. Am. Chem. Soc.* **1997**, *119*, 2125−2133.
(22) Wong, N. P.; Parks, O. W. Simple technique for extracting flavor compounds from fatty foods. *J. Dairy Sci.* **1994**, *51*, 1768−1769.
(23) Yorimitsu, H.; Wakabayashi, K.; Shinokubo, H.; Oshima, K. Radical addition of 2-iodoalkanamide or 2-iodoalkanoic acid to alkenes with a water-soluble radical initiator in aqueous media: facile synthesis of γ-lactones. *Bull. Chem. Soc. Jpn.* **2001**, *74*, 1963−1970.

Received for review July 6, 2004. Revised manuscript received September 7, 2004. Accepted September 8, 2004.

JF048885B