Synthesis of Deuterated Volatile Lipid Degradation Products To Be Used as Internal Standards in Isotope Dilution Assays. 1. Aldehydes

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The isotopically labeled compounds [5,6-$^2H_2$]hexanal (**d-I**), [2,3-$^2H_2$]-(*E*)-2-nonenal (**d-II**), [3,4-$^2H_2$]-(*E*,*E*)-2,4-nonadienal (**d-III**), and [3,4-$^2H_2$]-(*E*,*E*)-2,4-decadienal (**d-IV**) were prepared in good yields using new or improved synthesis procedures. Labeling position, chemical purity, and isotopic distribution of the compounds were characterized by various MS and NMR techniques. These molecules are used as internal standards in quantification experiments based on isotope dilution assay. Synthesis of **d-I**, **d-III**, and **d-IV** has not yet been reported in the literature.

Keywords: *Synthesis; isotope labeling; deuteration; flavor compounds; lipid degradation products; [5,6-$^2H_2$]hexanal; [2,3-$^2H_2$]-(E)-2-nonenal; [3,4-$^2H_2$]-(E,E)-2,4-nonadienal; [3,4-$^2H_2$]-(E,E)-2,4-decadienal; NMR; GC/MS; isotope dilution assay*

INTRODUCTION

Lipid-derived volatile compounds such as hexanal (**I**), (*E*)-2-nonenal (**II**), (*E*,*E*)-2,4-nonadienal (**III**), and (*E*,*E*)-2,4-decadienal (**IV**) play an important role in many food flavors (Figure 1). They are formed from linoleate (Grosch, 1987). These compounds contribute to the characteristic and desired note of a food but can also cause off-flavors depending on their concentration compared to other sensorially relevant odorants.

The green-smelling compound **I**, in combination with *trans*-4,5-epoxy-(*E*)-2-decenal, has been shown to be mainly responsible for the warmed-over flavor of cooked meat (Konopka et al., 1995). The fatty-smelling odorant **II** is known as an off-flavor compound of aged beer (Meilgaard and Peppard, 1986). The fatty-smelling compound **III** was identified as one of the key odorants in extruded oat meal (Guth and Grosch, 1993). Compound **IV**, also smelling fatty and deep-fried, was reported to significantly contribute to the typical note of cooked rice, chicken broth, and French fries (Buttery et al., 1988; Gasser and Grosch, 1990; Wagner and Grosch, 1997).

Sensorially relevant compounds such as **I**−**IV** often occur in low concentrations in foods. This makes their quantification rather difficult. Therefore, special efforts must be made to obtain reliable quantitative results. Isotope dilution assay (IDA) has been successfully applied to flavor research for several years [Schieberle and Grosch, 1987; review in Grosch (1994) and Schieberle (1995)]. This technique is based on the use of labeled internal standards added to the target product prior to sample preparation. In this way, losses can be accounted for because whatever changes occur in the natural substance occur in the labeled version as well. The labeled internal standard and the analyte are monitored by mass spectrometry (MS). Both the labeled and unlabeled flavor compounds of known purity must be available for establishing the calibration curves.

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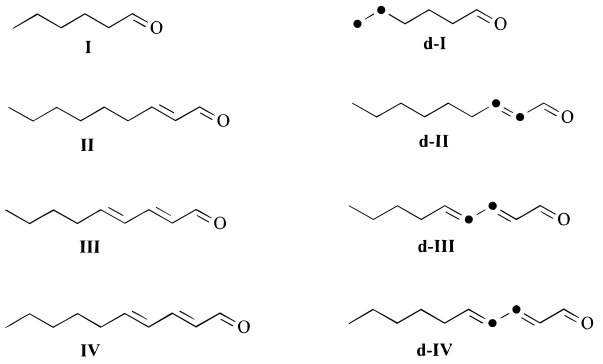

Figure 1. Chemical structures of hexanal (**I**), (*E*)-2-nonenal (**II**), (*E*,*E*)-2,4-nonadienal (**III**), (*E*,*E*)-2,4-decadienal (**IV**), and their corresponding deuterated analogues (● indicates the labeling position). The abbreviation "d" means that the compound is deuterated, without specification of the number or position of the deuterium atoms.

Availability of the labeled compounds is still the limiting factor in the development of IDA (Milo and Blank, 1998). Therefore, the crucial step is usually the synthesis of isotopically labeled internal standards and characterization of their chemical and isotopic purities. The aim of this work was to prepare deuterated analogues of **I**−**IV** based on the pioneering work of Guth and Grosch (1990). The following labeled compounds were synthesized and characterized (Figure 1): [5,6-$^2H_2$]-hexanal (**d-I**), [2,3-$^2H_2$]-(*E*)-2-nonenal (**d-II**), [3,4-$^2H_2$]-(*E*,*E*)-2,4-nonadienal (**d-III**), and [3,4-$^2H_2$]-(*E*,*E*)-2,4-decadienal (**d-IV**).

EXPERIMENTAL PROCEDURES

Materials and Reagents. The following unlabeled compounds were commercially available: hexanal (**I**, purity ≥98%, Fluka, Buchs, Switzerland); (*E*)-2-nonenal (**II**, ∼92% by NMR, Agipal, Paris, France); (*E*,*E*)-2,4-nonadienal (**III**, ∼90% by NMR, Aldrich, Buchs, Switzerland); and (*E*,*E*)-2,4-decadienal (**IV**, >95% by NMR, Fontarome, Cergy-Pontoise, France).

The starting materials for the syntheses were of highest purity: 1,4-dichloro-2-butyne (**IX**, >97%), pentanal (≥98%), hexanal (**I**, ≥98%), and 5-hexen-1-ol (**V**, ≥97%) were from

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Fluka. 2-Nonyn-1-ol (**VII**, 98%) was obtained from Lancaster (Morecambe, England).

The $^2H_2$-labeled compounds deuterium oxide (^2H_2O, >99.8% ^2H), lithium aluminum deuteride ($LiAl^2H_4$, >99% ^2H), and deuteroethanol (EtO^2H, >99.5% ^2H) were obtained from Fluka. Deuterium gas ($^2H_2$, >99.8% ^2H) was from Carbagas (Lausanne, Switzerland) and deuterated chloroform (C^2HCl_3, 99.8%) from Dr. Glaser AG (Basel, Switzerland).

Anhydrous dichloromethane (CH_2Cl_2), diethyl ether (Et_2O), pyridine, and tetrahydrofuran (THF) stored over molecular sieves (<0.005% H_2O) as well as ethanol (EtOH, >99.8%), methanol (MeOH, >99.5%), sulfuric acid (H_2SO_4, 98%), Celite 545, and Florisil adsorbent for chromatography (100−200 mesh) were purchased from Fluka; benzene (>99.7%), hexane, ethyl acetate, sodium hydrogencarbonate ($NaHCO_3$), sodium sulfate (Na_2SO_4), calcium chloride ($CaCl_2$), and sodium acetate (NaOAc) were from Merck (Darmstadt, Germany).

The following reagents were used: pyridinium chlorochromate (PCC, ≥98%), manganese dioxide (MnO_2), sodium methoxide (MeONa), and tris(triphenylphosphine)rhodium(I) chloride [$RhCl(PPh_3)_3$, Wilkinson's catalyst] from Fluka; ethylmagnesium bromide ($BrMgC_2H_5$, 1 M in THF) was from Aldrich.

Analytical Methods. *Gas Chromatography (GC).* This was performed on a Hewlett-Packard HP-5890 gas chromatograph (Geneva, Switzerland) equipped with a splitless injector and an FID. Fused silica capillary columns were used: DB-5, DB-1701, DB-FFAP, and DB-Wax (J&W capillaries, Folsom, CA; 30 m × 0.32 mm, film thickness = 0.25 μm). Helium was used as carrier gas (100 kPa). The GC was operated at an injector temperature of 250 °C and at a detector temperature of 250 °C. The ovens were programmed as follows: 20 °C, 70 °C/min to 50 °C, 4 °C/min to 180 °C, 10 °C/min to 240 °C (10 min) for DB-5; 20 °C (2 min), 40 °C/min to 50 °C (2 min), 4 °C/min to 150 °C, 10 °C/min to 240 °C (15 min) for FFAP; 20 °C (1 min), 70 °C/min to 60 °C, 6 °C/min to 180 °C, 10 °C/min to 240 °C (15 min) for DB-1701 and DB-Wax. Linear retention indices (RI) were calculated according to the method of van den Dool and Kratz (1963).

GC/MS. This was performed on a Finnigan MAT 8430 mass spectrometer (Bremen, Germany). Electron impact (EI) mass spectra were generated at 70 eV and positive chemical ionization (PCI) at 150 eV with ammonia as the reagent gas. Volatile components were sampled via a Hewlett-Packard (Geneva, Switzerland) HP-5890 gas chromatograph equipped with an HP-7673 autosampler using the following conditions: cold on-column injector, fused silica capillary column DB-1701 or DB-Wax (30 m × 0.32 mm, film thickness = 0.25 μm), helium as carrier gas (90 kPa). The temperature program was 35 °C (2 min), 40 °C/min to 50 °C (1 min), 6 °C/min to 180 °C, 10 °C/min to 240 °C (10 min). Relative abundances of the ions are given in percent.

Alternatively, GC/MS was performed on a Finnigan SSQ 7000 (San Jose, CA) using PCI at 200 eV and isobutane as reagent gas. The samples were introduced by splitless injection (1 μL) using the DB-1701 capillary column described above. Helium was used as carrier gas (70 kPa). The temperature program was as follows: 20 °C (1 min), 70 °C/min to 60 °C, 6 °C/min to 180 °C, 10 °C/min to 240 °C (10 min).

Nuclear Magnetic Resonance (NMR) Spectroscopy. The samples for NMR spectroscopy were prepared in WILMAD 528-PP 5 mm Pyrex NMR tubes, using as solvent ~0.7 mL of C^2HCl_3 from a sealed vial. NMR spectra were acquired on a Bruker AM-360 spectrometer, equipped with a quadrinuclear 5 mm probe head, at 360.13 MHz for ^1H and at 90.56 MHz for ^{13}C under standard conditions. The probe temperature was 21 °C for the proton spectra and slightly higher for the carbon spectra, due to heteronuclear composite pulse decoupling. All shifts are cited in parts per million from the internal tetramethylsilane (TMS) standard.

(a) One-Dimensional NMR. Most **proton spectra** were acquired with a spectral width of 7575.758 Hz, 64K of analog/digital converted and Fourier transformed data points, resulting in a frequency resolution of 0.231 Hz/point. The pulse width was 8 μs, corresponding to a pulse angle of ~64°. The acquisition period was 4.325 s, and the relaxation delay (= waiting time between two pulse acquisition periods) usually 10 s, or longer when the slowly relaxing aldehyde protons or other protons surrounded by deuterium atoms needed to be quantified.

For compounds **II**−**IV**, homonuclear *Overhauser* enhancement (NOE difference) experiments were performed using a pulse width of 11 μs (90°). For **II** the interpulse delay was 12.83 s, of which 7.5 s was continuous wave preirradiation at a decoupler power level determined empirically. The number of pulses was 64. All other parameters were the same as for the normal one-dimensional proton spectra. For **III** and **IV**, the data files were again 64K, the preirradiation was 19 s after 1 s without decoupling, and the total number of scans was 512. To make assignments unequivocal, for example, for olefinic protons, selective homonuclear decoupling experiments were carried out for compound **II** at decoupler levels optimized empirically.

The composite pulse proton decoupled **carbon-13 spectra** at 90.56 MHz were usually measured with a spectral width of 21739.13 Hz, 64K data points for acquisition, a pulse angle of 4 μs (~64°), and a relaxation delay of 8.5 s. The resolution was 0.663 Hz/data point. DEPT spectra were measured using the same spectral width and sizes of the data fields, with a relaxation delay of 6 s and a variable flip angle proton pulse of 16 μs (~129°). ^{13}C spectra without proton decoupling during the free induction decay were also acquired to help with the assignments.

(b) Two-Dimensional NMR. Two basic two-dimensional NMR techniques were applied to obtain unequivocal signal assignments, that is, homonuclear ^1H correlation experiments (COSY; Aue et al., 1976; Nagayama et al., 1980), which reflect the scalar (through-bond) coupling network between the protons in the molecule, and heteronuclear one-bond $^1H/^{13}C$ correlation experiments (HETCOR; Bax and Morris, 1981), which identify the protons directly bound to a particular carbon atom.

COSY spectra were determined for compounds **II**−**IV**. The parameters were adjusted to the spectral properties of each molecule. Taking **II** as an example, the spectral width in both dimensions was 3289.5 Hz and the number of scans 16 with two dummy scans. The data field in the F_2 dimension was 2K, and in the F_1 dimension it was 1K with 512 executed experiments and 1-fold zero filling. Two 90° pulses (10.6 μs) and a relaxation delay of 5.7 s were used. The spectra were filtered with a sine function in both dimensions before Fourier transformation. They were plotted in the magnitude mode after symmetrization about the main diagonal.

HETCOR spectra were measured for compounds **I**−**IV**, **d-IV**, and (*Z*)-1-methoxy-1-buten-3-yne (**XI**). Again, the parameters were adjusted to the spectral widths for protons and carbons required for each molecule. As an example, the spectral width of molecule **II** in the carbon dimension F_2 was 16666.7 Hz for 8K data points, and in the proton dimension F_1 it was 3289.5 Hz for 512 data points (256 experiments, with 1-fold zero filling). The number of scans was 32 with two dummy scans. The polarization delay was 36 ms (optimal for one-bond proton-carbon couplings of 139 Hz) and the relaxation delay 5.5 s. The spectra were filtered with a shifted sine square function in both dimensions before Fourier transformation and plotted in the magnitude mode.

^{13}C NMR Spectra Calculation. The assignment of some of the ^{13}C NMR spectra (compounds **II** and **III**) was checked by predicting the chemical shifts from the structural formula, based on the chemical environment and bond topology of each carbon atom, with the help of the SPECINFO on-line program SPECAL (comprehensive spectral database of STN international, c/o Fachinformationszentrum Karlsruhe, Germany).

Determination of Chemical and Isotopic Purity. *Chemical purity* was determined on the basis of GC data. Purity was expressed as peak area in percent without using FID correction factors. GC peaks were generally composed of several isotopomers with the target compound as major isotopomer. NMR was applied to estimate the amount of solvent and nonvolatile impurities.

Table 1. Chemical and Isotopic Purities of the Labeled Compounds Synthesized

labeled compd	chemical purity in %[a] (byproducts in %)[b]	isotopic purity (%)[c] (byproducts in %)[c]
d−I	**94**	**93** ([$^2H_2$]-**I**)
	(6, hexanoic acid)	(5, **I**; 1, [^{2}H]-**I**; 1, [$^2H_3$]-**I**)
d-II	**99**	**97** ([$^2H_2$]-**II**)
	(1, *Z*-isomer)	(3, [^{2}H]-**II**)
d-III	**94**	**99** ([$^2H_2$]-**III**)
	(3, *E,Z*- isomer; 3, others)	(0.6, [^{2}H]-**III**, 0.4, [$^2H_3$]-**III**)
d-IV	**95**	**96** ([$^2H_2$]-**IV**)
	(3, *E,Z*- isomer; 2, others)	(4, [^{2}H]-**IV**)

[a] Estimation based on GC-FID analysis, see Experimental Procedures. [b] Presence of trace of solvents (determined by NMR). [c] Estimation based on GC/MS-PCI analysis, see Experimental Procedures.

Isotopic purity was calculated from GC/MS data (Rohwedder, 1985; Rakoff and Rohwedder, 1992). Clusters of ions representing the species from $[M + 3]^+$ to $[M - 2]^+$ of both the deuterated and nonlabeled reference compounds were measured in the PCI mode on the SSQ 7000 using selected ion monitoring and isobutane as reactant gas. The nondeuterated substances were analyzed for isotope correction of the labeled compounds.

Synthesis of [5,6-$^2H_2$]Hexanal (d-I). *[5,6-$^2H_2$]Hexan-1-ol (**d-VI**).* 5-Hexen-1-ol (**V**, 8.0 g, 80 mmol) was placed into a reaction flask containing 250 mL of benzene. Wilkinson's catalyst was added at 15 wt % (1.2 g) of the substrate to be deuterated. The flask was filled (3×) with deuterium gas by alternating evacuation of the system and flushing with $^2H_2$. Pressure was maintained at slightly above atmospheric pressure. The process of deuteration was periodically monitored by withdrawing a small sample of the solution with a syringe and analyzing by GC. When deuteration was complete, the solvent was evaporated. Et_2O was then added to dissolve the deuterated compound and to precipitate the catalyst. After removal of Et_2O, 7.0 g (67.3 mmol, 84% yield) of **d-VI** was obtained by distillation under vacuum (37−38 °C, 4 mbar) with a purity of 97% (GC): GC RI(DB-5) = 875, RI(DB-1701) = 966, RI(FFAP) = 1350, RI(DB-Wax) = 1358; MS-EI 86 (25, $[M - H_2O]^+$), 85 (4), 71 (28), 70 (24), 59 (6), 58 (100), 57 (42), 56 (25), 55 (25), 45 (25), 44 (30), 43 (28), 42 (27), 41 (10); MS-CI (NH_3) 139 (15, $[M + NH_4 + NH_3]^+$), 122 (100, $[M + NH_4]^+$).

*[5,6-$^2H_2$]Hexanal (**d-I**).* In a 250 mL three-neck flask fitted with a thermometer and a reflux condenser were suspended 14.5 g (67.5 mmol) of PCC and 1.1 g (13.5 mmol) of anhydrous NaOAc in 130 mL of anhydrous CH_2Cl_2. The system was kept under nitrogen to avoid moisture. **d-VI** (6.0 g, 57.7 mmol) in 5 mL of anhydrous CH_2Cl_2 was added in one portion to the magnetically stirred solution cooled at ∼0 °C. The temperature was then allowed to increase to 15 °C. The reaction was monitored by GC. When it was finished, 100 mL of anhydrous Et_2O was added and the supernatant was separated from the black gum. The insoluble residue was washed with anhydrous Et_2O (3 × 20 mL). The combined organic solutions were passed through a short pad of Florisil, and the solvent was removed by evaporation. Distillation of the oily residue through a short Vigreux column (30 × 1 cm) under vacuum (21−22 °C, 10 mbar) resulted in ∼2.2 g of **d-I** (21.6 mmol, 37% yield): GC RI(DB-5) = 801, RI(DB-1701) = 877, RI(FFAP) = 1063, RI-(DB-Wax) = 1080; MS-CI 137 (25, $[M + NH_4 + NH_3]^+$), 120 (100, $[M + NH_4]^+$), 102 (5, M^+ or $[M + NH_4 - H_2O]^+$). Chemical and isotopic purities are shown in Table 1.

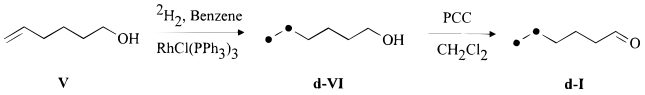
Synthesis of [2,3-$^2H_2$]-(*E*)-2-Nonenal (d-II). *[2,3-$^2H_2$]-(E)-2-Nonen-1-ol (**d-VIII**).* In a 200 mL three-neck reactor fitted with a reflux condenser and a thermometer was suspended 3.0 g (80 mmol) of $LiAl^2H_4$ in anhydrous THF. 2-Nonyn-1-ol (**VII**, 8.0 g, 57 mmol) in anhydrous THF (10 mL) was slowly added to the magnetically stirred solution. The mixture was refluxed for 1 h under nitrogen and then stored overnight at room temperature. GC analysis indicated complete reduction of **VII** to **d-VIII**. After cooling with an ice bath, 20 mL of ^2H_2O was added drop by drop, followed by 80 mL of aqueous H_2SO_4 (4 N) to dissolve any insoluble residues. The organic phase was separated from the water phase, and the aqueous solution was then extracted with Et_2O (3 × 50 mL). The combined organic solutions were washed successively with saturated solutions of $NaHCO_3$ (2 × 10 mL) and NaCl (2 × 10 mL) and then dried over anhydrous Na_2SO_4. After removal of the solvent by evaporation and distillation under vacuum (64−66 °C, 10 mbar, Büchi GKR-50, Flawil, Switzerland), 6.84 g (47.5 mmol, 83% yield) of a colorless oil of **d-VIII** with a purity of 97% (GC) was obtained: GC RI(DB-5) = 1170, RI(DB-1701) = 1283, RI(FFAP) = 1718, RI(DB-Wax) = 1719; MS-EI 144 (2, M^+), 126 (4), 99 (3), 98 (9), 97 (27), 96 (10), 95 (3), 85 (4), 84 (26), 83 (25), 82 (20), 81 (7), 72 (7), 71 (16), 70 (37), 69 (32), 68 (18), 67 (7), 60 (8), 59 (100), 58 (23), 57 (29), 56 (46), 55 (38), 45 (17), 44 (10), 43 (55), 42 (32), 41 (33), 40 (9), 39 (11); MS-CI (NH_3) 162 (8, $[M + NH_4]^+$), 126 (3, $[M - H_2O]^+$), 144 (100, M^+ or $[M + NH_4 - H_2O]^+$).

*[2,3-$^2H_2$]-(E)-2-Nonenal (**d-II**).* This could be obtained by oxidation of **d-VIII** (6.5 g, 45.1 mmol) with PCC using the same procedure as described for the oxidation of **d-VI**. Distillation of the oily residue through a short Vigreux column under vacuum (45−46 °C, 2 mbar) resulted in 2.72 g (19.2 mmol, 43% yield) of a colorless oil of **d-II**. The oil smelled intensely fatty. Alternatively, **d-II** was prepared with better yield from **d-VIII** by stirring **d-VIII** (200 mg, 1.4 mmol) with MnO_2 (2.0 g, 23 mmol) in hexane (10 mL) overnight at room temperature. The suspension was filtered through Celite. The solvent was evaporated, yielding 150 mg of **d-II** (1.1 mmol, 76% yield): GC RI(DB-5) = 1158, RI(DB-1701) = 1277, RI(FFAP) = 1529, RI-(DB-Wax) = 1536; MS-CI (NH_3) 160 (100, $[M + NH_4]^+$), 142 (2, M^+ or $[M + NH_4 - H_2O]^+$). Chemical and isotopic purities are shown in Table 1.

Synthesis of [3,4-$^2H_2$]-(*E,E*)-2,4-Nonadienal (d-III) and [3,4-$^2H_2$]-(*E,E*)-2,4-Decadienal (d-IV). *(Z)-1-Methoxy-1-buten-3-yne (**XI**).* An aqueous NaOH solution (150 g, 40%) was added dropwise to an ethanolic solution (80 mL) of **IX** (30 g, 0.24 mol) and pyridine (1.5 g) to form **X**. The gas was then purged from the reaction mixture into a container cooled with dry ice while passing through two traps, the first trap containing an aqueous NaOH solution (1 M) and the second trap solid $CaCl_2$. This step was almost quantitative. A mixture composed of **X** (∼11 g, 0.22 mol) and MeONa (2.7 g) in methanol (100 mL) was heated in an autoclave at 75 °C for 5 h. After cooling, water (200 mL) was added and the organic phase was separated from the aqueous layer. The water phase was treated with an aqueous H_2SO_4 solution (25 mL, 20%) and then extracted with Et_2O (3 × 100 mL). The combined organic layers were washed with $NaHCO_3$ (1 M, 2 × 10 mL) and dried over Na_2SO_4. After removal of the solvent, the residue was distilled under vacuum, yielding 8.9 g (0.11 mol, 50% yield) of a yellow oil of **XI**, which polymerized at room temperature: [MS-EI was identical with that found in the MS library for (*Z*)-1-methoxy-1-buten-3-yne] MS-EI 82 (100), 81 (18), 39 (75), 38 (21), 37 (12), 50 (30), 53 (28), 51 (20),49 (10), 52 (9); ^{1}H NMR δ 6.354 (dd, J = 6.5, 0.85 Hz, 1H, 1-CH), 4.519 (dd, J = 6.5, 2.3 Hz, 1H, 2-CH), 3.800 (s, 3H, OCH_3), 3.086 (dd, J = 2.4, 0.9 Hz, 1H, 4-CH) [The proton NMR data are in good agreement with those reported by Corey and Albright (1983). The extremely long spin−lattice relaxation time of the acetylenic proton demanded a very long relaxation delay (15 min) between the pulses to permit quantitative integration. The 6.5 Hz coupling constant between 1-CH and 2-CH indicates *Z*-configuration for the double bond]; ^{13}C NMR δ 158.08 (d, 1-CH), 84.31 (d, 2-CH), 80.68 (d, 4-CH), 78.25 (s, 3-C), 60.84 (q, OCH_3) [These assignments were corroborated by a ^{13}C spectrum without proton decoupling (characteristic acetylenic $^2J_{CH}$ of ∼50 Hz) and for the protonated carbons by a HETCOR experiment].

*[3,4-$^2H_2$]-(E,E)-2,4-Nonadienal (**d-III**).* **XI** (0.75 g, 9.2 mmol), dissolved in anhydrous THF (12 mL), was added dropwise to a solution of $BrMgC_2H_5$ (8 mL, 1.0 M in THF) while the mixture was maintained at ∼40 °C. After an additional hour of stirring at room temperature, the reaction flask was cooled in an ice−water bath, and a solution of pentanal (0.6 g, 7 mmol) dissolved in anhydrous THF (5 mL) was added dropwise. The mixture was stirred at room temperature until the

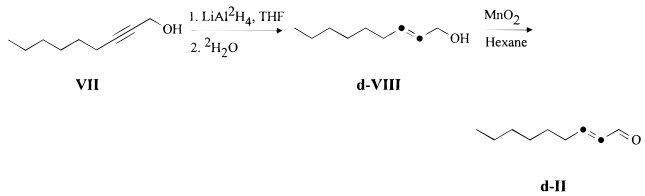
Scheme 1. Synthesis of [5,6-$^2H_2$]Hexanal (d-I)[a]



V d-VI d-I

[a] ● indicates the labeling position.

Scheme 2. Synthesis of [2,3-$^2H_2$]-(*E*)-2-Nonenal (d-II)[a]



VII d-VIII d-II

[a] ● indicates the labeling position.

reaction was completed (monitoring by GC), then cooled, and treated with EtO^2H (0.35 g, 7 mmol). After 20 min, solid $LiAl^2H_4$ (0.3 g, 7 mmol) was added in small portions over a period of 10 min. The mixture was stirred for 2 h at room temperature and stored overnight. It was then treated successively with ethyl acetate (0.4 mL), ^2H_2O (2 mL), and H_2SO_4/H_2O (4 N, 10 mL). The organic layer was separated and the aqueous phase was extracted with Et_2O. The organic layer and the ether extracts were combined, washed with water (3 × 10 mL), and dried over Na_2SO_4. Most of the solvent was distilled off on a Vigreux column (50 × 1 cm) at atmospheric pressure. Finally, the residual solvent was evaporated under reduced pressure. The residue was purified by flash chromatography using a mixture of pentane and Et_2O (9 + 1, v/v). A yellow oil of **d-III** was obtained (0.5 g, 3.6 mmol, 51% yield based on pentanal). It had an intense fatty note. GC RI(DB-5) = 1213, RI(DB-1701) = 1353, RI(FFAP) = 1692, RI(DB-Wax) = 1696; MS-CI (NH_3) 158 (100, $[M + NH_4]^+$), 141 (37, $[M + 1]^+$). Chemical and isotopic purities are shown in Table 1.

*[3,4-$^2H_2$]-(E,E)-2,4-Decadienal (**d-IV**).* This compound was prepared following the same procedure as described above for **d-III**. Starting with **XI** (2.0 g, 24.4 mmol) and hexanal (2.0 g, 20 mmol), **d-IV** was obtained (1.55 g, 10.1 mmol, 50% yield). It also had an intense fatty note. *GC* RI(DB-5) = 1317, RI(DB-1701) = 1461, RI(FFAP) = 1803, RI(DB-Wax) = 1806; MS-CI (NH_3) 172 (100, $[M + NH_4]^+$), 155 (50, $[M + 1]^+$). Chemical and isotopic purities are shown in Table 1.

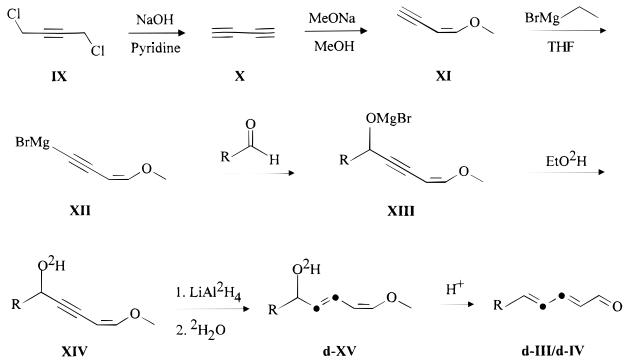
RESULTS AND DISCUSSION

Preparation of isotopically labeled analogues of compounds **I**−**IV** was first described by Guth and Grosch (1990, 1993). In most cases, the authors obtained mixtures of several isotopomers that were mainly characterized by GC/MS. Time-consuming purification methods (HPLC) were used, for example, for the separation of trans and cis isomers. In this work, the synthesis procedures were modified to improve yields and to favor formation of stereochemically defined isomers. Furthermore, rapid cleanup procedures were used, and analytical characterization was based on both GC/MS and NMR data.

In our work, the target compounds **d-I**−**d-IV** were synthesized using new or improved methods (Schemes 1−3). Starting materials for the synthesis of **d-I** and **d-II** were commercially available, that is, 5-hexen-1-ol (**V**) and 2-nonyn-1-ol (**VII**), respectively. Carbon−carbon connection required for the preparation of **d-III** and **d-IV** was achieved by combining Grignard reagents with aldehydes, resulting in satisfactory yields (∼50−60%).

The carbonyl functions were introduced either by oxidation of primary alcohols to aldehydes or by hydrolysis of semiacetals to aldehydes. Oxidation of alcohols was performed with pyridinium chlorochromate

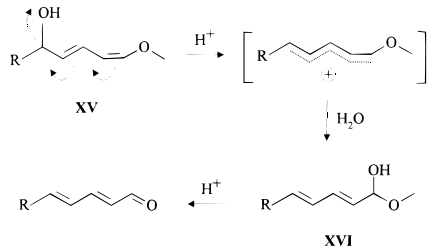
Scheme 3. Synthesis of [3,4-$^2H_2$]-(*E*,*E*)-2,4-Nonadienal (R = C_4H_9, d-III) and [3,4-$^2H_2$]-(*E*,*E*)-2,4-Decadienal (R = C_5H_{11}, d-IV)[a]



IX X XI XII XIII XIV d-XV d-III/d-IV

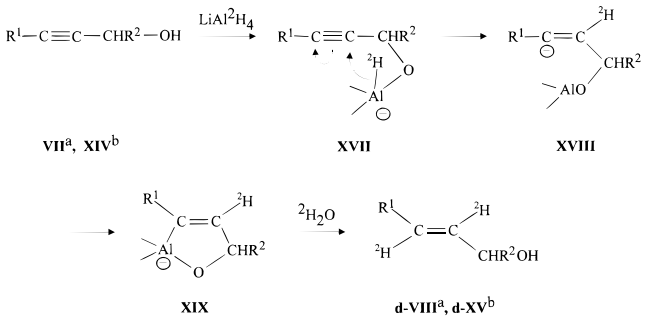
[a] ● indicates the labeling position.

Scheme 4. Formation of (*E*,*E*)-2,4-Dienals from Enol Vinyl Ethers (XV) via Semiacetals (XVI)



XV XVI

Scheme 5. Reduction of α-Alkynols to *trans*-Olefinic Alcohols with Lithium Aluminum Deuteride ($LiAl^2H_4$) and Subsequent Deuterolysis (Adapted from Snyder, 1967)[a]



VII[a], XIV[b] XVII XVIII XIX d-VIII[a], d-XV[b]

[a] Symbols R^1 and R^2 represent (for a) $R^1 = C_6H_{13}$, $R^2 = H$; and (for b) $R^1 = CH{=}CHOCH_3$, $R^2 = C_4H_9$ or C_5H_{11}.

(PCC) and manganese dioxide (MnO_2) following the general procedures of Corey and Suggs (1975) and Attenburrow et al. (1952), respectively. In general, the yields obtained with PCC were moderate (∼40%). Significantly better yield was obtained for **d-II** (76%) when using MnO_2 for oxidation of the primary allylic alcohol **d-VIII**.

In the preparation of 2,4-dienals (**d-III**, **d-IV**), the aldehyde function was introduced by acidic hydrolysis of semiacetals according to Pippen and Nonaka (1958). As shown in Scheme 4, the formation of (*E*,*E*)-2,4-dienals can be explained by spontaneous dehydration of the enol vinyl ether (**XV**) and concomitant rearrangement of the 1,3-cis/trans-configured double bonds to 2,4-trans/trans-configured semiacetals (**XVI**) under acidic conditions. The yields obtained were ∼50%.

Deuterium atoms were introduced either by catalytic deuteration using homogeneous (Wilkinson's) catalysts or by reduction with $LiAl^2H_4$. In general, the yields were good (80−90%). Homogeneous catalytic deuteration

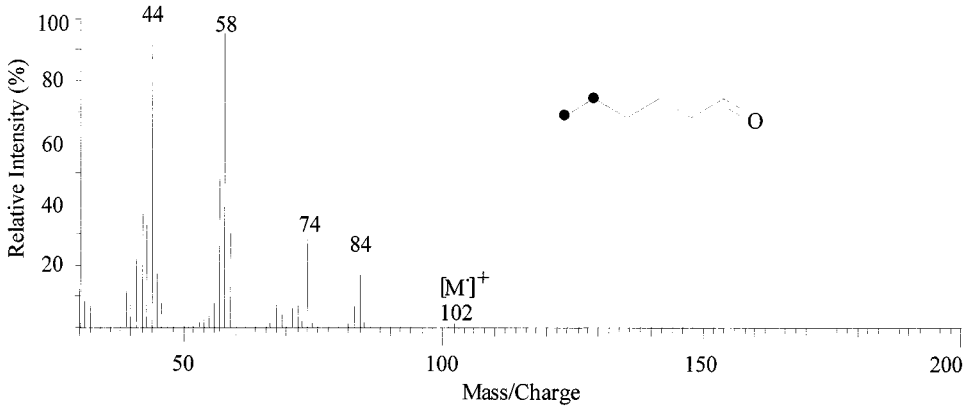


Figure 2. Mass spectrum (EI) of [5,6-$^2H_2$]hexanal (**d-I**).

allows preparation of well-defined deuterated compounds with less "scrambling" of deuterium atoms (Morandi and Jensen, 1969; Birch and Walker, 1966). Therefore, compound **d-I** was obtained with Wilkinson's catalyst by adapting the procedure of Young et al. (1965). This method has already been applied to unsaturated aldehydes (Jardine and Wilkinson, 1967), but not to hexenals.

In the case of compounds **d-II**−**d-IV**, deuterium was introduced by reduction of acetylenic alcohols with $LiAl^2H_4$ and subsequent deuterolysis of the intermediate metal alkenyl. In general, reduction of α-alkynols to *trans*-olefinic alcohols with $LiAlH_4$ (Bates et al., 1954) proceeds nonstereospecifically as shown by Snyder (1967). The latter author explained the preferred formation of trans-configured olefinic alcohols (Scheme 5) by geometrical constraints of the cyclic intermediate (**XIX**). The transfer of hydride (or deuteride) from aluminum to carbon (**XVII**) is facilitated by coordination of Al with a Lewis base, for example, THF.

Characterization of the compounds synthesized was performed using chromatographic techniques (GC-FID, GC/MS) to determine the chemical and isotopic purity. NMR methods were applied for unequivocal identification of the labeling position.

[5,6-$^2H_2$]Hexanal (d-I). Guth and Grosch (1990, 1993) reported the synthesis of [2,3-$^2H_2$]- and [3,4-$^2H_2$]-hexanal by heterogeneous catalytic deuteration of the corresponding hexenals with $Pd/CaCO_3$ or PtO_2. [2,3-$^2H_2$]Hexanal proved to be unstable toward $^2H/H$ exchange during sample preparation and even under cold storage conditions (Prof. W. Grosch, Garching, Germany, personal communication). [3,4-$^2H_2$]Hexanal was shown to be more stable with respect to $^2H/H$ exchange. In this work (Scheme 1), the target compound was prepared in two steps by deuteration of 5-hexen-1-ol (**V**) using Wilkinson's catalyst (Morandi and Jensen, 1969) followed by oxidation of the resulting [5,6-$^2H_2$]hexan-1-ol (**d-VI**) to **d-I** with PCC according to the method of Corey and Suggs (1975). **d-I** is the first well-defined and characterized deuterated hexanal reported in the literature so far.

The mass spectrum of **d-I** is shown in Figure 2. The molecular ion at *m/z* 102 and the fragments at *m/z* 84, 74, and 58 indicated the incorporation of two deuterium atoms into the molecule, which is in good agreement with the CI data showing the ion $[M + NH_4]^+$ at *m/z* 120. The fragment at *m/z* 44, obtained by McLafferty rearrangement, ruled out 1-C and 2-C as labeling position. The exact labeling positions were determined by NMR spectroscopy (Table 2).

Table 2. ^1H NMR and ^{13}C NMR Data of [5,6-$^2H_2$]Hexanal (d-I, ∼20 mg)[a]

proton/carbon	^1H NMR	^{13}C NMR
1-CHO	9.77, t, J = 1.9 Hz, ∼0.8 H[b]	203.0, d
2-CH_2	2.43, d t, J = 7.4, 1.9 Hz, 2 H	43.9, t
3-CH_2	1.64, m ("quintet"), J_{av} = 7.5 Hz, 2 H	21.8, t
4-CH_2, 5-CH^2H	∼1.3, complex m, ∼3 H	31.2 (C-4), t 22.0 (C-5)[c], d (1:1:1), $^1J_{C^2H}$ = 19.2 Hz
6-$CH_2{}^2H$	∼0.9, complex m, ∼2 H	13.5[c], t (1:1:1), $^1J_{C^2H}$ = 19.2 Hz

[a] Chemical shift δ in ppm from internal TMS. The multiplicity abbreviations used to describe NMR signals are s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet. For the proton spectra, d t means doublet of triplets, with decreasing values of the absolute coupling constants (here: $|J|_{doublet} > |J|_{triplet}$). Quotes ("...") mean approximate description of the multiplet. For ^{13}C NMR spectra, s, d, t, and q, denominate quaternary, CH, CH_2, and CH_3 carbons, respectively. [b] Aldehyde protons have very long T_1 relaxation times and appear in the spectrum with a reduced integral value. An integral value of one proton was obtained when the interpulse delay was made longer (220 s). [c] Accompanied by signals most likely due to traces of nondeuterated hexanal: 22.7 (5-CH_2), t and 14.1 (6-CH_3), q.

In nondeuterated hexanal (**I**), the proton NMR signals of 4-CH_2 and 5-CH_2 could not be individually resolved at 360 MHz, and a multiplet equivalent to four protons was found. For **d-I**, the corresponding integral was equivalent to three protons (the unequivocal location of the deuterium on 5-C had to be deduced from ^{13}C NMR). The methyl proton signal was well resolved, and a single deuterium substitution of the methyl group could be clearly demonstrated by the integral value, the change in line pattern from a triplet to a doublet of 1:1:1 signals, and the small isotopic upfield shift effect. The assignment of the carbons was deduced from the usual shift rules and made unambiguous by comparison with a HETCOR spectrum of nondeuterated hexanal (data not shown). The deuterium atoms were identified by the typical 1:1:1 pattern of the signals of 5-C and 6-C. Compared to the carbon signals of **I**, the normal upfield isotope shift effect (∼0.4−0.5 ppm) was found for the directly deuterium-labeled carbon atoms, and a smaller influence on the shift of the more remote carbons was noted.

[2,3-$^2H_2$]-(*E*)-2-Nonenal (d-II). Synthesis of **d-II** by catalytic deuteration of 2-nonyn-1-ol and oxidation of the resulting labeled 2-nonen-1-ol with Cr(VI) oxide was reported by Guth and Grosch (1990). In this work, the synthesis procedure was modified to favor formation of

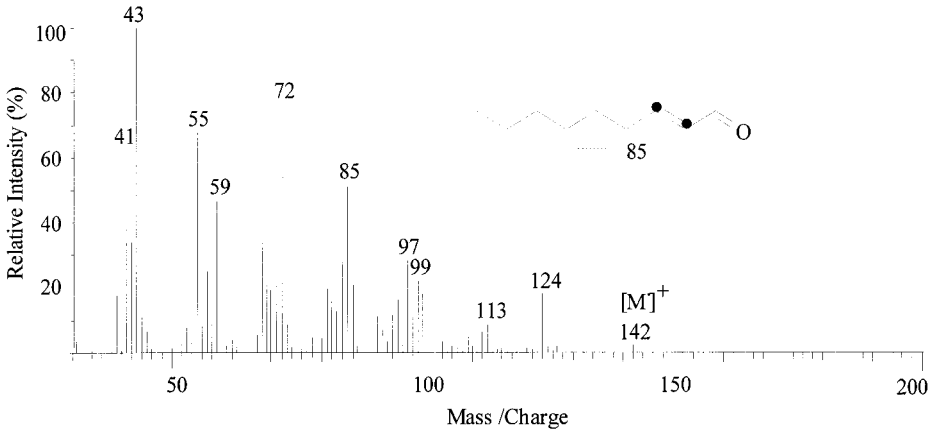


Figure 3. Mass spectrum (EI) of [2,3-$^2H_2$]-(*E*)-2-nonenal (**d-II**).

Table 3. ^{1}H NMR Data of (*E*)-2-Nonenal (II) and [2,3-$^2H_2$]-(*E*)-2-Nonenal (d-II)[a]

proton	(*E*)-2-nonenal (**II**)	[2,3-$^2H_2$]-(*E*)-2-nonenal (**d-II**)[b]
1-CHO	9.51, d, J = 7.9 Hz, ~0.8 H[c]	9.51, ~1:1:1 signal, $^3J_{H^2H}$ = 1.1 Hz, ~0.93 H[d]
2-CH	6.12, d d t, J = 15.6, 7.9, 1.5 Hz, ~0.7 H[c]	(6.11)[e]
3-CH	6.87, d t, J = 15.6, 6.8 Hz, ~0.9 H[c]	(6.86)[f]
4-CH_2	2.34, "q" d[g], J = 7.2, 1.5 Hz, 2 H	2.34, "t" (slightly broad), J = 7.4 Hz, 2 H
5-CH_2	1.51, m ("quintet"), J_{av} = 7.5 Hz, 2 H	1.51, m ("quintet"), J_{av} = 7.5 Hz, 2.1 H[h]
6,7,8-CH_2	1.4−1.2, complex multiplet, 6 H	1.4−1.2, m (complex), 6.3 H[h]
9-CH_3	0.89, "t", "J" = 6.9 Hz, 3H	0.89, "t", "J" = 6.9 Hz, 3.2 H[h]

[a] Chemical shift δ in ppm from internal TMS. The multiplicity abbreviations used to describe NMR signals are s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet. For the proton spectra, d t means doublet of triplets, with decreasing values of the absolute coupling constants (here: $|J|_{doublet} > |J|_{triplet}$). Quotes ("...") mean approximate description of the multiplet. [b] Spectrum acquired with a very long relaxation delay (220 s) to obtain quantitative integrals for slowly relaxing protons. [c] Integral value reduced because of long spin−lattice relaxation time. [d] Integral cannot be precisely determined because of overlap with doublet signal of [3-^{2}H]-(*E*)-2-nonenal. [e] Residual signal due to traces of [3-^{2}H]-(*E*)-2-nonenal: d (complex), J = 7.9 Hz, ~0.05 H. [f] Residual signal due to traces of [2-^{2}H]-(*E*)-2-nonenal: t (1:1:1), J = 6.9 Hz, $J_{2-C^2H,3-CH}$ = 2.3 Hz, ~0.02 H. [g] Probably an incompletely resolved d t d with J = 7.9, 6.8, 1.5 Hz. [h] Integrals larger than expected because of impurities.

Table 4. ^{13}C NMR Data of (*E*)-2-Nonenal (II) and [2,3-$^2H_2$]-(*E*)-2-Nonenal (d-II)[a]

carbon	(*E*)-2-nonenal (**II**)	[2,3-$^2H_2$]-(*E*)-2-nonenal (**d-II**)
1-CHO	194.2, d	194.2, d
2-CH	133.0, d	(132.9)[b]
2-C^{2}H		132.6, s (1:1:1), $^1J_{C^2H}$ = 24.6 Hz
3-CH	159.2, d	(159.1)[b]
3-C^{2}H		158.7, s (1:1:1), $^1J_{C^2H}$ = 23.1 Hz
4-CH_2	32.8, t	32.6, t
5-CH_2	27.8, t	27.8, t
6-CH_2	28.8, t	28.8, t,
7-CH_2	31.6, t	31.6, t,
8-CH_2	22.6, t	22.6, t
9-CH_3	14.1, q	14.1, q

[a] Chemical shift δ in ppm from internal TMS. The multiplicity abbreviations used to describe ^{13}C NMR spectra are s, d, t, and q, denominating quaternary, CH, CH_2, and CH_3 carbons, respectively. [b] Small signals due to undeuterated carbons.

the trans isomer. This was achieved by preparing [2,3-$^2H_2$]-(*E*)-2-nonen-1-ol (**d-VIII**) through stereospecific trans reduction (Snyder, 1967) of 2-nonyn-1-ol (**VII**) with $LiAl^2H_4$ (Scheme 2). **d-II** was obtained by oxidation of **d-VIII** with PCC (Corey and Suggs, 1975) or MnO_2 (Attenburrow et al., 1952) in good yields and with high stereoselectivity employing a simplified cleanup procedure.

First evidence for an *E*-configuration in **d-II** was obtained by retention indices (RI) on different stationary phases. About 1% of the deuterated (*Z*)-2-nonenal was detected (Table 1), which was confirmed by GC/MS analysis. The molecular ion at *m*/*z* 142 and the fragments at *m*/*z* 124, 85, 72, and 59 indicated the incorporation of two deuterium atoms into the molecule (Figure 3). The intense $[M + NH_4]^+$ ion at *m*/*z* 160 obtained by MS-CI confirmed a shift of two units. The even-numbered fragment at *m*/*z* 72 in MS-EI of **d-II** derived from a McLafferty rearrangement of the molecular ion at *m*/*z* 142.

The assignments of ^{1}H NMR and ^{13}C NMR signals are summarized in Table 3 and Table 4, respectively. Compound **II** was used as reference for its deuterated analogue (**d-II**). Proton assignments of **II** were obtained from a COSY spectrum and by homonuclear decoupling. One-dimensional NOE difference spectra proved the all trans arrangement of the carbonyl and the double bond. Carbon assignments were confirmed by a HETCOR experiment and with the help of SPECAL ^{13}C NMR spectra prediction.

The proton spectrum of **d-II** showed only traces of its undeuterated and monodeuterated analogues. The position of monodeuteration could be proved from the carbon spectrum by the typical 1:1:1 line shapes for the 2-C^{2}H and 3-C^{2}H signals. They were each accompanied by a doublet signal, which stood for the monodeuterated nonenals. Note that these carbon signals are not quantitative, because they depend on the parameters of the acquisition and strongly on the relaxation properties of the different species.

[3,4-$^2H_2$]-(*E*,*E*)-2,4-Nonadienal (d-III) and [3,4-$^2H_2$]-(*E*,*E*)-2,4-Decadienal (d-IV). The first synthesis of deuterated **V** and **VI** (Guth and Grosch, 1990, 1993) was based on the procedure described by Pippen and Nonaka (1958) for the synthesis of the unlabeled analogues. Reaction of an aldehyde with the Grignard reagent **XII**, obtained by reacting $BrMgC_2H_5$ with (*Z*)-1-methoxy-1-buten-3-yne (**XI**), resulted in intermediate **XIII** (Scheme 3). Under acidic conditions, the labeled enol vinyl ether **d-XV** (labeling in R) spontaneously

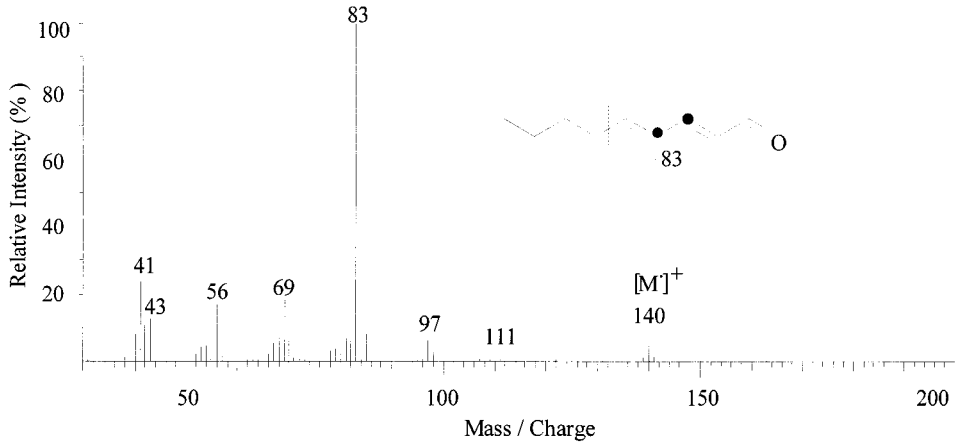


Figure 4. Mass spectrum (EI) of [3,4-$^2H_2$]-(*E*,*E*)-2,4-nonadienal (**d-III**).

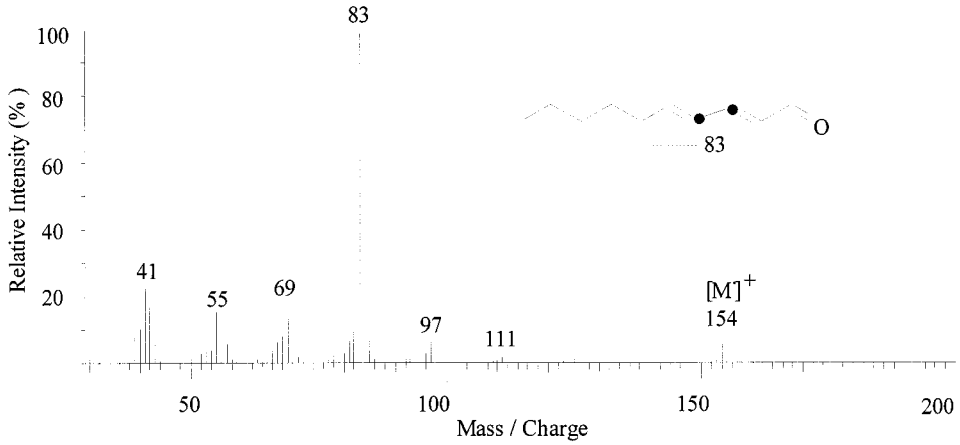


Figure 5. Mass spectrum (EI) of [3,4-$^2H_2$]-(*E*,*E*)-2,4-decadienal (**d-IV**).

Table 5. ^{1}H NMR Data of (*E*,*E*)-2,4-Nonadienal (III), [3,4-$^2H_2$]-(*E*,*E*)-2,4-Nonadienal (d-III), (*E*,*E*)-2,4-Decadienal (IV), and [3,4-$^2H_2$]-(*E*,*E*)-2,4-Decadienal (d-IV)[a]

proton	III	d-III[b]	IV	d-IV[b]
1-CHO	9.54, d, J = 8.1 Hz, ~0.9 H[c]	9.54, d, J = 8.1 Hz, ~0.9 H[c]	9.54, d, J = 8.1 Hz, ~1 H	9.54, d, J = 8.1 Hz, ~0.9 H[c]
2-CH	6.08, d d, J = 15.3, 8.1 Hz, ~0.9 H[c]	6.07, d (1:1:1), sl br[d], $^3J_{\mathbf{HC-CH}}$ = 8.1, $^4J_{\mathbf{HC=C^2H}}$ ~ 2.1 Hz (trans!), ~0.9 H[c]	6.08, d d, J = 15.3, 8.1 Hz, ~1 H	6.07, d (1:1:1), sl br[d], $^3J_{\mathbf{HC-CH}}$ = 8.1, $^4J_{\mathbf{HC=C^2H}}$ ~ 2.1 Hz (trans!), ~0.9 H[c]
3-CH	7.09, m, 1 H	(7.09)[e]	7.09, m, 1 H	(7.09)[e]
4-CH and 5-CH	6.4−6.2, m, strong coupling, 2 H	6.29 (5-CH), t (1:1:1), sl br[d], $^3J_{\mathbf{HC-CH}}$ = 7.1, $^4J_{\mathbf{HC=C^2H}}$ ~ 2.1 Hz (trans!), ~1 H	6.4−6.2, m, strong coupling, 2 H	6.28 (5-CH), t (1:1:1), sl br[d], $^3J_{\mathbf{HC-CH}}$ = 7.1, $^4J_{\mathbf{HC=C^2H}}$ ~ 2.1 Hz (trans!), ~1 H
6-CH_2	2.23, m, 2 H	2.23, "q", "J" = 7.2 Hz, 2 H	2.22, m, 2 H	2.22, "q", "J" = 7.2 Hz, 2 H
7-CH_2	1.5−1.4, m, 2 H	1.5−1.4, m, 2 H	1.47, m ("quintet"), 2 H	1.46, m ("quintet"), "J" = 7.4 Hz, 2 H
8-CH_2	1.4−1.3, m, 2 H	1.4−1.3, m, 2 H		
9-CH_3	0.92 ppm, "t", "J" = 7.1 Hz, 3 H	0.92, "t", "J" = 7.2 Hz, 3 H		
8,9-CH_2			1.4−1.3, m, 4 H	1.4−1.3, m, 4 H
10-CH_3			0.90, "t", "J" = 7.1 Hz, 3 H	0.90, "t", "J" = 7.0 Hz, 3 H

[a] Chemical shift δ in ppm from internal TMS. The multiplicity abbreviations used to describe NMR signals are s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet. For the proton spectra, d t means doublet of triplets, with decreasing values of the absolute coupling constants (here: $|J|_{\text{doublet}} > |J|_{\text{triplet}}$). Quotes ("...") mean approximate description of the multiplet. [b] 84.3 s between pulses. [c] Reduced intensity because of long T_1 relaxation time. [d] sl br = slightly broadened due to long-range couplings with deuterium. [e] Residual signals due to traces of 4-monodeuterated (*E*,*E*)-2,4-dienals.

rearranged to the target 2,4-dienals. The labeling position was mainly at 6-C and 7-C, introduced via the deuterated aldehydes, which had to be prepared, however.

In this work, labeling was introduced at a later stage of the synthesis by stereospecific trans reduction of the acetylenic group in **XIV** with $LiAl^2H_4$ and subsequent deuterolysis (Scheme 3). Unfortunately, the starting material (*Z*)-1-methoxy-1-buten-3-yne (**XI**) was no longer commercially available and had, therefore, to be synthesized by heating diacetylene (**X**) in MeOH containing MeONa as catalyst (Herbertz, 1952). Diacetylene gas

Table 6. ^{13}C NMR Data of (*E*,*E*)-2,4-Nonadienal (III), [3,4-$^2H_2$]-(*E*,*E*)-2,4-Nonadienal (d-III), (*E*,*E*)-2,4-Decadienal (IV), and [3,4-$^2H_2$]-(*E*,*E*)-2,4-Decadienal (d-IV)[a]

carbon	III	d-III[b]	IV	d-IV[b]
1-CHO	194.0, d	194.0, d	194.0, d	194.0, d
2-CH	130.0, d	129.9, d	130.0, d	129.9, d
3-CH	153.0, d	(152.9)[c]	153.0, d	(152.9)[c]
3- C^2H		152.6, s (1:1:1), $^1J_{C^2H} = 23.2$ Hz		152.6, s (1:1:1), $^1J_{C^2H} = 23.2$ Hz
4-CH	128.7, d		128.6, d	
4-C^2H		128.3, s (1:1:1), $^1J_{C^2H} = 23.8$ Hz		128.3, s (1:1:1), $^1J_{C^2H} = 23.8$ Hz
5-CH	147.5, d	147.3, d	147.5, d	147.4, d
6-CH_2	32.9, t	32.9, t	33.2, t	33.2, t
7-CH_2	30.7, t	30.7, t	28.2, t	28.2, t
8-CH_2	22.3, t	22.3, t	31.4, t	31.4, t
9-CH_3	13.9, q	13.9, q		
9-CH_2			22.5, t	22.5, t
10-CH_3			14.0, q	14.0, q

[a] Chemical shift δ in ppm from internal TMS. The multiplicity abbreviations used to describe ^{13}C NMR spectra are s, d, t, and q, denominating quaternary, CH, CH_2, and CH_3 carbons, respectively. [b] 21.5 s between pulses. [c] Residual signals with "d" multiplicity due to traces of 4-monodeuterated (*E*,*E*)-2,4-dienals.

was obtained by dehydrochlorination of 1,4-dichloro-2-butyne (**IX**). This is a convenient method for synthesizing various 3,4-labeled (*E*,*E*)-2,4-dienals: it is a one-vessel reaction, labeling with $LiAl^2H_4$ is quantitative, the target products can easily be purified by distillation or column chromatography, and yields are fairly good.

The EI mass spectra of **d-III** (Figure 4) and **d-IV** (Figure 5) show similar fragmentation, so that they can be distinguished only by their molecular ions, that is, at *m*/*z* 140 and 154, respectively. Both **d-III** and **d-IV** have the same fragmentation patterns as the unlabeled compounds, except that the *m*/*z* values of some of the ions are shifted higher by two units, for example, at *m*/*z* 83 and 69. Incorporation of two deuterium atoms was confirmed by the MS-CI data.

Proton-NMR data of **d-III** and **d-IV** are summarized in Table 5, together with those of the unlabeled reference compounds **III** and **IV**. To account for the long T_1 relaxation times, especially of isolated protons in the deuterated compounds, sufficient time was allowed between the radio frequency pulses. Assignment of the signals was achieved via COSY spectra of the unlabeled analogues. The 4- and 5-protons formed a strongly coupled system, so that they could not be individually assigned at spectral frequency of 360 MHz. In **IV** and **d-IV** the signals of the 8-CH_2 and 9-CH_2 protons resulted in an overlapping complex multiplet, which also precluded individual assignment. The data for **III** and **IV** were in good agreement with those reported by Boosfeld and Vitzthum (1995); that is, their shift values, obtained at 250 MHz, were ~0.03−0.05 ppm lower than those reported in Table 5. Because the proton signals of 4-CH_2 and 5-CH_2 could not be distinguished, only the deuterium in 3-C position was confirmed by proton NMR. The position of the other deuterium could be deduced from the integral values, the coupling constants, and the correlations of the COSY spectrum. Traces of (*E*,*Z*) isomers were also identified in our samples, both chromatographically and by NMR.

Assignment of most of the ^{13}C NMR signals (Table 6) was straightforward, based on heteronuclear correlation spectra. Where strong coupling between protons led to ambiguities (e.g., 4-C and 5-C in both nonadienals and decadienals, 8-C and 9-C in **IV** and **d-IV**), the shift differences of 10 ppm or more predicted by the SPECAL program permitted clear differentiation of the corresponding carbons. Deuteration in positions 3 and 4 of **d-III** and **d-IV** was confirmed. Except for a 20 MHz ^{13}C NMR spectrum of **III** in the SPECINFO data bank (origin not specified, but well compatible with our results), no previous ^{13}C NMR data for 2,4-nonadienals or 2,4-decadienals were found in the literature.

Conclusions. Four labeled compounds were prepared by applying new or improved synthesis procedures. Their yields as well as chemical and isotopic purities were characterized using MS and NMR techniques. The synthetic routes described in this work are fully documented in terms of yield for each step, purity of the final product, and analytical characterization including NMR. In view of the likely future demand for such labeled reference compounds, more work on optimization of synthesis procedures from a yield and cost perspective may be justified.

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