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Chemical Synthesis of Long Chain 2-Alkynals, Alkynols, 2*c*- and 2*t*-Alkenals and 2*c*- and 2*t*-Alkenols

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Summary: The chemical synthesis of long chain alkynals and alkynols is described. Stereospecific reduction leads to 2*c*-alkenals and 2*c*-alkenols. 2*t*-Alkenals have been synthesized by adaption of the method of Corey *et al.* to long chain derivatives. The structures of all products have been ascertained by chemical and physical techniques. The intermediates connected with the high yields make these methods most suitable for radioactive labelling of these compounds. The 2*t*- and 2*c*-alkenals have served as substrates in the identification, isolation and characterization of the 2-alkenal reductase (2-alkenal: NADPH oxidoreductase, EC 1.3.1.?; not yet listed), which reduces the α,β-*trans* double bond of th 2*t*-hexadecenal to palmitaldehyde, a reaction operative in the metabolism of long chain sphingosine bases.

Chemische Synthese von langkettigen 2-Alkinalen und Alkinolen, 2c- und 2t-Alkenalen sowie 2c- und 2t-Alkenolen

Zusammenfassung: Die chemische Synthese von langkettigen Alkinalen und Alkinolen wird beschrieben. Stereospezifische Reduktion führt zu 2*c*-Alkenalen und 2*c*-Alkenolen. Langkettige 2*t*-Alkenale wurden mit Hilfe der von Corey et al. für kurzkettige Aldehyde beschriebenen Methode synthetisiert. Die chemische Struktur aller Produkte wurde durch chemische und physikalische Methoden bewiesen. Die Zwischenprodukte und die hohen Ausbeuten der Syntheseschritte schaffen sehr geeignete Voraussetzungen für die radioaktive Markierung dieser Verbindungen. Die 2*t*- und 2*c*-Alkenale dienten als Substrate bei der Identifizierung, Isolierung und Charakterisierung der 2-Alkenal-Reduktase (2-Alkenal: NADPH-Oxidoreduktase EC 1.3.1.?; noch nicht registriert). Dieses Enzym reduziert die α,β-ständige *trans*-Doppelbindung z. B. des 2*t*-Hexadecenals zu Palmitinaldehyd, eine Reaktion, die im Stoffwechsel der langkettigen Base Sphingosin von Bedeutung ist.

The biological degradation of the most abundant naturally occurring long chain base 4*t*-sphingenine (sphingosine), is initiated by the phosphorylation of the primary hydroxyl group by an ATP-dependent kinase, followed by an aldolase type lyase reaction, which cleaves the 2*S*,3*R*-2-amino-1,3-diol system between carbon atoms 2 and 3. 4*t*-Sphingenine then yields 2*t*-hexadecenal representing carbon atoms 3 to 18 and phosphorylethanolamine C-1 and C-2[1].

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Abbreviation: EGS = ethylene glycol succinate polyester.

In the subsequent paper[2] we describe the occurrence, isolation and characterization of a soluble NADPH-dependent 2-alkenal reductase which reduces α,β-*trans* double bonds, *e.g.* of 2*t*-hexadecenal to palmitaldehyde.

These and further studies on the properties of this enzyme were made possible after suitable procedures had been developed for the synthesis of labelled 2*t* and 2*c* long chain alkenals. This paper describes the chemical synthesis of long chain 2-alkynals and alkynols and their corresponding 2*c*-

[1] For Review see Stoffel, W. (1973) *Mol. Cell.* Biochem. **1**, 147–155.

[2] Stoffel, W. & W. Därr (1973) *this J.* **355**, in press.

and 2*t*-alkenals and 2*c*- and 2*t*-alkenols. The labelling technique is exemplified in the synthesis of 2*t*-hexadecenal.

Results

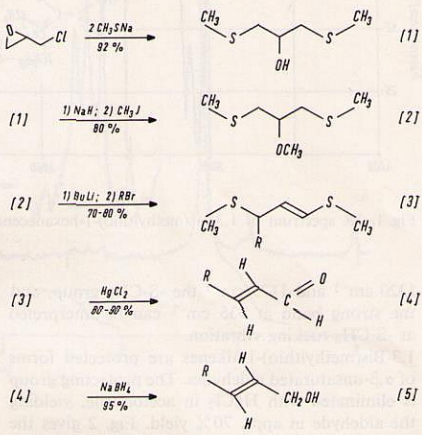
1. Synthesis of 2t-alkenals

Numerous methods for the synthesis of α,β-unsaturated aldehydes have been described in the literature. Acyl chlorides[3–5], acyl anilides[6] and acyl imidazols[7] have been reduced with complex metal hydrides to their corresponding aldheydes.

The BF_3-catalyzed condensation of 2 mol saturated aldehyde with 1 mol vinyl ether yields a cyclic acetal, which is cleaved by acid hydrolysis into the bis-homologous α,β-unsaturated aldehyde[8] Formylmethylene triphenylphosphorane reacts with benzaldehyde and oenanthaldehyde to cinnamaldehyde and 2-nonenal, respectively[9]. The Wittig reaction generally yields *cis*-olefins, but the ratio of *cis* to *trans* isomers depends on the reaction conditions[10–12].

A procedure used in the prostaglandin series by Corey *et al.*[13] appeared to be the most suitable method for the synthesis of long chain 2*t*-alkenals. Alkylhalides alkylate 1,3-bis(methylthio)allyllithium. The corresponding *trans*-alkenal is obtained in one additional step. We synthesized a number of homologous long chain 2*t*-alkenals by this procedure, which is outlined in the Table. The yields of the different intermediates and the products are also given.

Table: Scheme for the synthesis of 2*t*-alkenals.



R	Yield of [3] [%]	Yield of [4] [%]
n-C_5H_{11}-	80	74
n-C_7H_{15}-	81	70
n-$C_{11}H_{23}$-	81	62
n-$C_{13}H_{27}$-	80	65
n-$C_{15}H_{31}$-	73	59
[2,3-$^3H_4$]$C_{13}H_{27}$-	72	61

The reactivity of the alkyl bromides decreases with growing chain length, but the 1,3-bis(methylthio)-allyllithium is very reactive, and it is only necessary to be careful that the alkyl bromide remains in solution at the low temperature required for the alkylation reaction. Therefore when tridecyl- and pentadecyl bromide are used, the reaction temperature was kept at $-10\,^0$C.

The IR spectrum of 1,3-bis(methylthio)-1-hexadecene is given in Fig. 1.

The CH deformation at 955 cm^{-1} indicates the *trans* configuration of the double bond, the absorption bands at 1440 cm^{-1}, 1420 cm^{-1},

[3] Bayer, O. (1954) in Methoden d. Org. Chemie, Houben-Weyl-Müller, 4. Auflage, Bd. VII/1, S. 285, Verlag Georg Thieme, Stuttgart.

[4] Mosettig, E. & Mozingo, R. (1948) *Org. React.* **4**, 362–377.

[5] Brown, H. C. & Subba, B. C. (1958) *J. Amer. Chem. Soc.* **80**, 5377–5380.

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[7] Staab, H. A. & Bräunling, H. (1962) *Liebigs Ann. Chem.* **654**, 119–130.

[8] Hoagin, R. J. & Hirsch, O. H. (1950) D. B. P. Anm. U. 1390, Union Carbide Carbon Corp.

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[11] Bestmann, H. & Kratzer, O. (1962) *Ber. Deut. Chem. Ges.* **95**, 1894–1901.

[12] Schlosser, M. & Christmann, K. F. (1967) *Liebigs Ann. Chem.* **708**, 1–35.

[13] Corey, E. J., Erickson, B. W. & Noyon, R. (1971) *J. Amer. Chem. Soc.* **93**, 1724–1729.

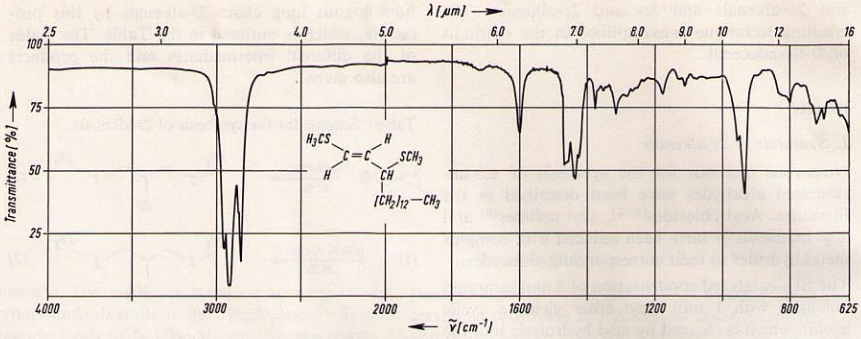
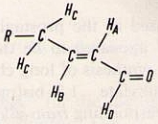


Fig. 1. IR spectrum of 1,3-bis(methylthio)-1-hexadecene.

1320 cm^{-1} and 1175 cm^{-1} the -S-CH_3 group, and the strong band at 935 cm^{-1} can be interpreted as -S-CH_3 rocking vibration.

1,3-Bis(methylthio)-1-alkenes are protected forms of α,β-unsaturated aldehydes. The protecting group is eliminated with $HgCl_2$ in acetonitrile, yielding the aldehyde in appr. 70% yield. Fig. 2 gives the IR spectrum of 2*t*-hexadecenal. The absorption of the carbonyl group appears at 1695 cm^{-1}, characteristic for α,β-unsaturated aldehydes, that of the *trans* double bond at 3020 cm^{-1} and 970 cm^{-1}. The AB system of the olefinic protons in the 60 MHz-NMR-spectrum, Fig. 3, also proves the correct structure. The aldehyde proton H_D splits the A part (H_A) into a doublet. The coupling constant J_{AD} is 8 Hz.



The proton couples with the two allylic hydrogen nuclei forming a triplet with J_{AC} = 1 Hz. The B part of the spectrum exhibits a doublet split into triplets with J_{AB} = 16 Hz and J_{BC} = 6 Hz. The coupling constant J_{AB} = 16 Hz proves the *trans*

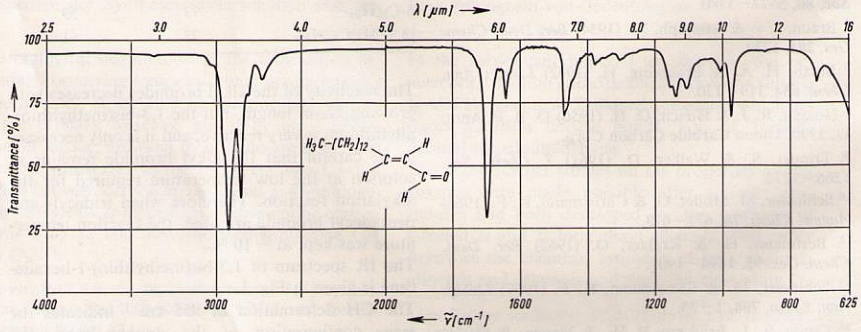


Fig. 2. IR spectrum of 2*t*-hexadecenal.

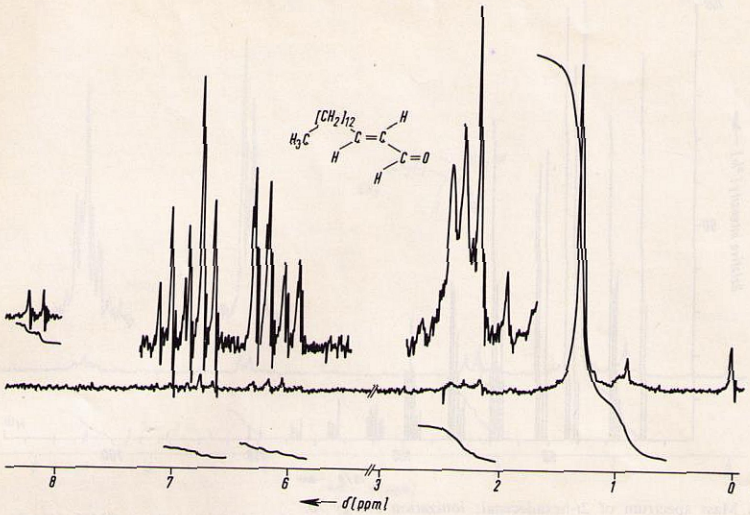


Fig. 3. 60 MHz-NMR spectrum of 2*t*-hexadecenal.

configuration of the double bound. The singlet at 0.9 ppm represents the terminal CH_3 group and the singlet at 1.3 ppm, the integration of which yields 22 protons, represents the 11 CH_2 groups of the carbon chain. The asymmetric triplet around 2.3 ppm corresponds to the 2 allylic protons.

The purity of the 2*trans*-unsaturated aldehydes was checked by gas chromatography on 2m 2.5% EGS columns at 85 °C for 2*t*-octenal and 2*t*-decenal, 100 °C for 2*t*-tetradecenal, and 150 °C for 2*t*-hexadecenal and 2*t*-octadecenal. Thin-layer chromatography, solvent system hexane/ether/acetic acid 50:50:1, was also used.

$NaBH_4$ reduction in methanol of the 2*t*-alkenals leads to the corresponding 2*t*-alkenols. The IR spectrum of 2*t*-hexadecenol shows absorption bands characteristic for the OH-group at 3600 to 3100 cm^{-1}, *trans* double bonds at 1665 cm^{-1} and 960 cm^{-1}. The mass spectrum gives the final structural proof, Fig. 4. The molecular ion $M^{\oplus}$ appears at 238, *m/e* 220(M−18), *m/e* 209 (M−29) α-fission, *m/e* 194 (M−44) β-fission M−(29 + *n* × 28) *n* = 1−8 and M−(44 + *n* × 28) *n* = 1−8.

Synthesis of long chain 2-alkynols and 2-alkynals

2-Alkynols are easily available in good yields in a two-step synthesis by the condensation of the lithium salt of 2-propargyloxytetrahydropyrane[14] and long chain alkyl bromides, *e. g.* n-undecyl and n-tridecyl bromide. Acid hydrolysis of the tetrahydropyranyl ether yields the corresponding alcohol. The most suitable solvent for this condensation is dimethylsulfoxide, which reacts with lithiumamide to methylsulfinylmethyllithium. The latter base generates the acetylide. IR spectrum:

OH-stretching vibration at 3460 cm^{-1}.

C≡C-stretching vibration at 2290 cm^{-1}.

2-Alkynols were oxidized in high yield with dicyclohexylcarbodiimide in dimethylsulfoxide in the presence of catalytic amounts of crystalline phosphoric acid to 2*t*-alkynals. Dicyclohexylurea

[14] Franke, W., Ziegenbein, W. & Meister, H. (1960) *Angew. Chem.* **72**, 391−400.

[15] Agarin, C. (1965) *Bull. Soc. Chim. Fr.* 1021.

[16] Corey, E. J. & Chaykovsky, M. (1965) *J. Amer. Chem. Soc.* **87**, 1345−1353.

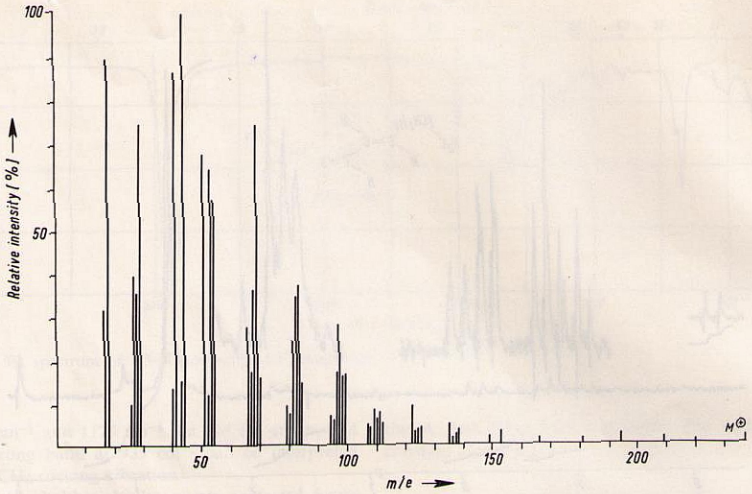


Fig. 4. Mass spectrum of 2*t*-hexadecenal; ionization energy 70.

was filtered off, the crude aldehyde chromatographed over Al_2O_3 with methylene chloride and the aldehyde distilled under high vacuum.

The IR spectrum of 2-hexadecynal is given in Fig. 5. Absorptions at: C≡C-stretching at 2200 cm^{-1}; C=O-stretching at 1675 cm^{-1} (conj. with the C≡C-bond).

The 2-alkynals and 2-alkynols can be stereospecifically reduced to 2*c*-alkenals and 2*c*-alkenols with Lindlar catalyst[17]. The IR spectrum of 2*c*-hexadecenol shows a broad absorption at 3600 to 3150 cm^{-1}: O−H-stretching; at 3010 cm^{-1} C=C-H-stretching; 1660 cm^{-1} C=C-stretching; 710 cm^{-1} C−H-deformation vibration.

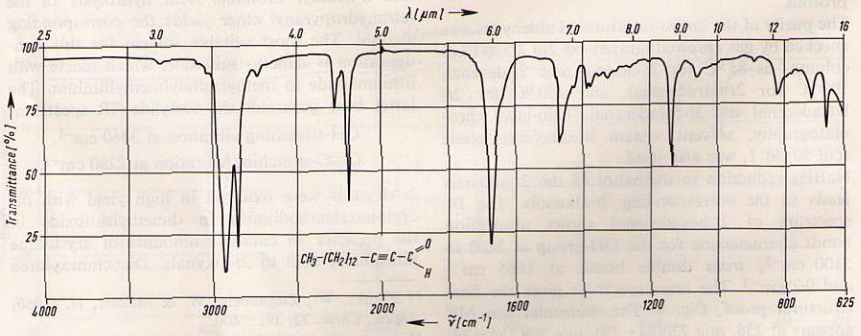


Fig. 5. IR spectrum of 2-hexadecynal.

[17] Lindlar, H. (1952) *Helv. Chim. Acta* **35**, 446–450.

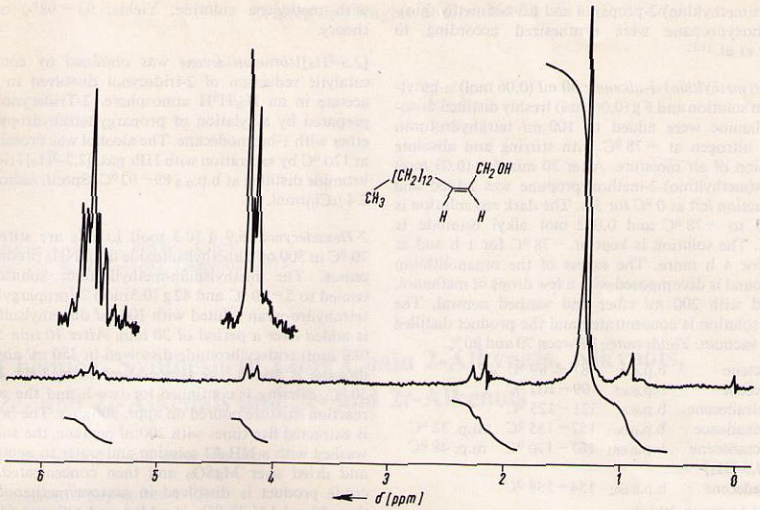
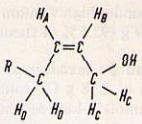


Fig. 6. 60 MHz NMR spectrum of 2*c*-hexadecenol.

The IR spectrum of 2*c*-hexadecenal indicates the following structure: 1690 cm^{-1} α,β-unsaturated carbonyl absorption and C=C-stretching; at 1630 cm^{-1}, conjugation with the carbonyl group; *cis* configuration of double bond; C−H-deformation vibration at 695 cm^{-1}.

The NMR spectrum of 2*c*-hexadecenol (Fig. 6) shows six groups of signals, the intensities of which are 3:22:1:2:2:2.

The broad triplet at 0.87 ppm corresponds to the terminal CH_3-group, the singlet at 1.3 ppm to 12 CH_2-groups, the sharp signal at 2.15 ppm to the OH-proton. Two allylic protons give rise to the signal at 2.3 ppm. The second allylic CH_2 group adsorbs downfield at 4.15 ppm as a doublet due to the inductive effect of the neighboring OH-group. The coupling constant is $J_{BC} = 5$ Hz.



$R = -[CH_2]_{11}-CH_3$

The multiplet due to the olefinic protons centers around 5.6 ppm. The coupling constants J_{AB}, J_{AD} and J_{BC} are almost equal. Each olefinic proton possesses three neighboring nuclei, which should yield a quartet. The chemical shift of the A and B part of the AB system is small, however, with superposition of the quartets. The coupling constant J_{AB} with 7 Hz proves the *cis* configuration of the double bond.

We gratefully acknowledge the support by the *Deutsche Forschungsgemeinschaft*.

Experimental

Melting and boiling points are uncorrected.

IR spectra were recorded with the spectrophotometer Perkin Elmer, model 257, between NaCl plates.

NMR spectra were recorded with a NMR spectrometer Varian A-60-D in deuterochloroform with tetramethylsilane as international standard.

Gas liquid chromatographic analyses were performed with a Perkin Elmer gas chromatograph, model F-20.

Mass spectrometry was carried out with a Varian-MAT mass spectrometer, model CH 5.

1,3-Bis(methylthio)-2-propanol and 1,3-bis(methylthio)-2-methoxypropane were synthesized according to Corey *et al.* [13].

1,3-Bis(methylthio)-1-alkenes: 30 ml (0.06 mol) n-butyllithium solution and 6 g (0.06 mol) freshly distilled diisopropylamine were added to 100 ml tetrahydrofuran under nitrogen at −78 °C with stirring and absolute exclusion of air moisture. After 30 min 5 g (0.03 mol) 1,3-bis(methylthio)-2-methoxypropane was added and the reaction left at 0 °C for 5 h. The dark red solution is cooled to −78 °C and 0.032 mol alkyl bromide is added. The solution is kept at −78 °C for 1 h and at 0 °C for 4 h more. The excess of the organolithium compound is decomposed with a few drops of methanol, diluted with 200 ml ether and washed neutral. The dried solution is concentrated and the product distilled under vacuum. Yields range between 70 and 80%.

R-1-octene	$b.p._{0.4}$	87 − 89 °C	
R-1-decene	$b.p._{0.07}$	99 − 102 °C	
R-1-tetradecene	$b.p._{0.1}$	121 − 125 °C	
R-1-hexadence	$b.p._{0.001}$	152 − 155 °C	m.p. 32 °C
R-1-octadecene	$b.p._{0.001}$	167 − 170 °C	m.p. 48 °C
R-1-[4,5-$^3H_4$]-hexadecene	$b.p._{0.001}$	154 − 158 °C	

R = 1,3-bis(methylthio)-

2t-Alkenals: A solution of 0.05 mol 1,3-bis(methylthio)-1-alkene in 50 ml tetrahydrofuran is added dropwise within 10 min to a vigorously stirred acetonitrile/water 8:1 solution of 0.2 mol $HgCl_2$. A white precipitate forms on slight warming. The suspension is stirred for another 12 h at 30 °C and filtered. The residue is extracted with five 50 ml portions of ether. The combined ether phases are washed twice with 100 ml H_2O, dried over $MgSO_4$ and the solvent evaporated at 40 °C under vacuum. The crude aldehyde is chromatographed over 400 g Al_2O_3 with methylene chloride. The product elutes with the solvent front. It is distilled under vacuum.

2*t*-octenal	$b.p._{12}$	73 − 75 °C	
2*t*-decenal	$b.p._{0.25}$	45 − 48 °C	
2*t*-tetradecenal	$b.p._{0.4}$	95 − 98 °C	m.p. 35 °C
2*t*-hexadecenal	$b.p._{0.3}$	116 − 118 °C	
2*t*-octadecenal	$b.p._{0.001}$	123 − 125 °C	
2*t*-[4,5-$^3H_4$]-hexadecenal	$b.p._{0.2}$	114 − 115 °C	

2t-Alkenols: 3 mmol 2*t*-alkenal, dissolved in 20 ml methanol is added dropwise to a stirred solution of 1 g $NaBH_4$ in 50 ml methanol. The solution is stirred at room temperature for 12 h, excess $NaBH_4$ is destroyed with a few drops of 10% H_2SO_4, and the solvent is evaporated largely under vacuum. The residue is dissolved in 50 ml ether, and the solution is washed with a 5% $NaHCO_3$ solution and water to neutrality, then dried over $MgSO_4$. The ether is evaporated and the residual alcohol chromatographed over 100 g Al_2O_3 with methylene chloride. Yields: 93 − 98% of the theory.

[2,3-$^3H_4$]1-*bromotridecane* was obtained by complete catalytic reduction of 2-tridecynol dissolved in ethyl acetate in an H_2/H^3H atmosphere. 2-Tridecynol was prepared by alkylation of propargyltetrahydropyranyl ether with 1-bromodecane. The alcohol was brominated at 120 °C by saturation with HBr gas. [2,3-$^3H_4$]Tridecylbromide distilled at $b.p._{0.2}$ 89 − 92 °C. Specif. radioactiv. 2.4 μCi/μmol.

2-Hexadecynol: 6.9 g (0.3 mol) $LiNH_2$ are stirred at 70 °C in 300 ml dimethylsulfoxide until NH_3 production ceases. The methylsulfin-methyllithium solution is cooled to 5 − 10 °C and 42 g (0.3 mol) 2-(propargyloxy)-tetrahydropyran diluted with 100 ml dimethylsulfoxide is added over a period of 30 min. After 10 min 52.6 g (0.2 mol) tridecylbromide dissolved in 150 ml absolute dioxane is added, the temperature being kept below 50 °C. Stirring is continued for two h and the cooled reaction mixture poured on appr. 500 g ice. The product is extracted five times with 200 ml pentane, the solution washed with a NH_4Cl solution and water to neutrality, and dried over $MgSO_4$ and then concentrated. The crude product is dissolved in acetone/methanol 1:2, then 50 ml 1% H_2SO_4 is added and refluxed for 2 h under vacuum. The cooled reaction mixture is concentrated at the rotary still, 150 ml water is added and the product extracted with ether. The dried solution is concentrated and the residue distilled under vacuum.

$b.p._{0.15}$ 120 °C; 34.7 g (70% of theory).

2-Tridecynol can be synthesized by this procedure in a 72% yield, $b.p._{0.6}$ = 114 − 118 °C.

2-Hexadecynal: A solution of 25 g (0.105 mol) 2-hexadecynol in 50 ml absolute benzene is added dropwise with stirring to a mixture of 50 g *N,N*-dicyclohexylcarbodiimide, 1 g crystalline H_3PO_4 and 250 ml dimethylsulfoxide at 50 °C. The alcohol is added at a rate such that a temperature of 50 − 60 °C is sustained, and the stirring is continued for 1 h. *N,N*-Dicyclohexylurea is filtered off, the filtrate is diluted with 500 ml water and 50 ml 10% HCl and stirred for 10 min. Residual dicyclohexylurea is filtered, washed with pentane and the filtrate extracted five times with 150 ml portions of pentane. The combined pentane extracts are dried over $MgSO_4$, the solvent evaporated under vacuum and the residual aldehyde chromatographed over 200 g Al_2O_3 with methylene chloride. The aldehyde was distilled under high vacuum at $b.p._{0.02}$ 108 bis 110 °C. Yield: 22.9 g (92.5% of theory).

2c-Hexadecenol and *2c-hexadecenal* are obtained by catalytic reduction of 2.38 g (10 mmol) 2-hexadecynol and 2.36 g (10 mmol) 2-hexadecynal in 80 ml ethyl acetate with 0.5 g Lindlar catalyst. The yield was quantitative.