Enantioselective Reduction of Vicinally Substituted Monocyclic Aldehydes with Horse Liver Alcohol Dehydrogenase; A New Approach to Chiral Alcohols and Aldehydes

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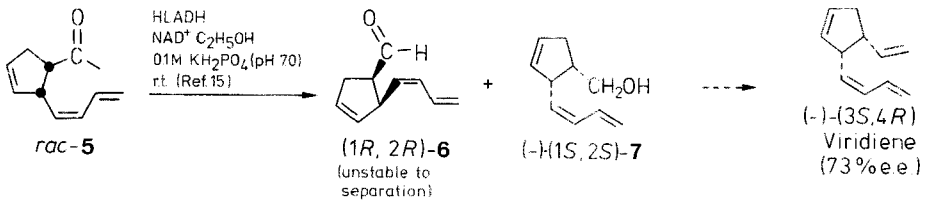
Optically active, vicinally substituted monocyclic aldehydes and alcohols can be conveniently prepared by enantioselective reduction of racemic aldehydes by means of horse liver alcohol dehydrogenase (HLADH) under kinetically controlled conditions.

Efficient and economic syntheses of optically active compounds are a major concern in modern organic chemistry. New concepts have been introduced such as standardized chromatographic separations of diastereomers,[1,2] alkylations of chiral enolates derived from camphor-[3,4] or proline-reagents,[5] asymmetric 4n+2 cycloadditions,[6] epoxidations with chiral complexes of transition metals[7] and enantioselective reductions with modified hydrides.[8,9] All these methods require chiral derivatization of either the substrate or the reagent prior to separation or chemical modification. With enzymes, however, as chiral host molecules such derivatization is unnecessary. Therefore enzymatic approaches to chiral synthons become more and more accepted also by synthetic chemists. Enzymes, that combine a high degree of enantioselectivity with a broad spectrum of substrates are particularly useful. Such enzymes are pig liver esterase[10] and several lipases[11] for enantioselective saponifications; aminoacid acylase for enantioselective deacetylation of natural and unnatural *N*-acetylamino acids;[12] and hydroxynitrile lyase for the enantiospecific formation of cyanohydrins.[13] Horse liver alcohol dehydrogenase (HLADH), has been used to catalyze enantiospecific oxidation of meso-[14] and non-meso diols[15] to lactones by the system nicotinamide-adenine dinucleotide/flavine mononucleotide (NAD^+/FMN); in its reductive mode the enzyme was utilized for the enantioselective synthesis of optically active alcohols from (poly)cyclic ketones.[16] In the same way the enantiospecific transfer of hydrogen to 1-^{2}H-aldehydes was the key step in several preparations of chirally labelled 1-^{2}H-alcohols.[17]

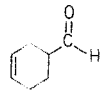
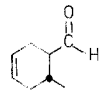
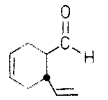
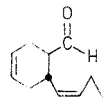
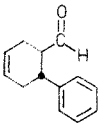
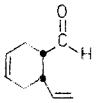
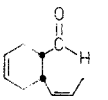
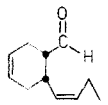
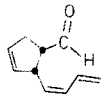
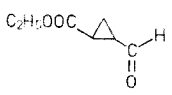
We found recently that vicinally substituted, monocyclic aldehydes are also good substrates of HLADH.[15] Under kinetically controlled conditions one enantiomer of these compounds is preferentially reduced, and the resulting alcohol is easily separated from the remaining antipodic aldehyde by chromatography on silica gel. (−)-(3*S*,4*R*)-3-[(1*Z*)-1,3-butadienyl]-4-vinylcyclopentene, the unnatural enantiomer of (+)-viridiene, a gamete-releasing and -attracting pheromone of several brown algae, was the first compound to be synthesized acccording to this method[15] (Scheme **A**).



HLADH = horse liver alcohol dehydrogenase NAD$^+$ = nicotinamide-adenine dinucleotide

Scheme A

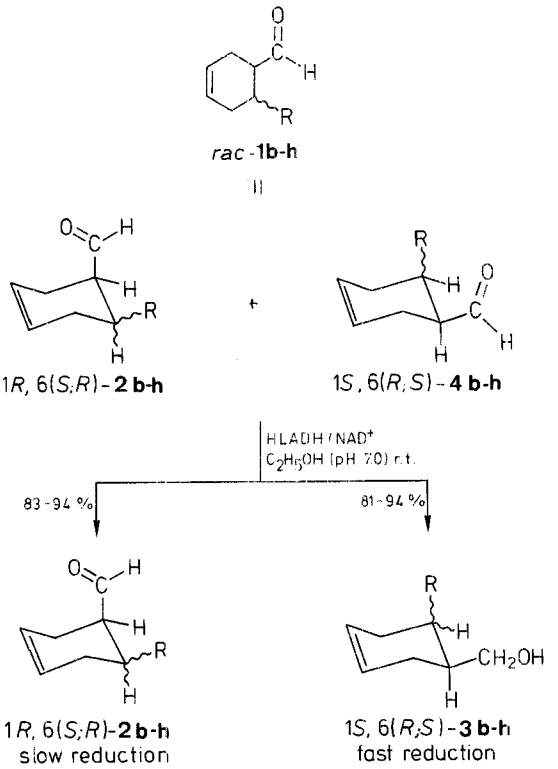
Table 1. Kinetically Controlled Reductions of Racemic Aldehydes **1a–h**, **5**, **9**

Racemic Aldehyde	Reduction[a] (%)	Rel.[b] Rate	Yield[c] (%)	$[\alpha]_{578}$			Refs.[e]
				Aldehyde	Alcohol	Aldehyde[d]	
1a 	50	100	82 (84)	**2a:** −1.08° (c = 1.86) CH_3OH	**3a:** +0.9° (c = 3.2) CH_3OH	**4a:** +1.12° (c = 2.90) CH_3OH	22
1b 	50	85	83 (81)	**2b:** −43.8° (c = 6.52) $CHCl_3$	**3b:** +39.2° (c = 8.24) $CHCl_3$	**4b:** +44.5° (c = 4.01) $CHCl_3$	23
1c 	47	46	90 (94)	**2c:** −80.4° (c = 3.11) CH_2Cl_2	**3c:** +73.9° (c 1.70) CH_2Cl_2	**4c:** +89.2° (c = 1.80) CH_2Cl_2	15
1d 	43	6	85 (87)	**2d:** −52.4° (c = 1.10) CH_2Cl_2	**3d:** +46.6° (c = 1.27) CH_2Cl_2	**4d:** +82.5° (c = 1.47) CH_2Cl_2	24
1e 	52	3	94 (92)	**2e:** −16.6° (c = 1.44) CH_2Cl_2	**3e:** +39.5° (c = 3.90) CH_2Cl_2	**4e:** +34.2° (c = 2.97) CH_2Cl_2	25
1f 	48	88	89 (92)	**2f:** −17.6° (c = 1.61) CH_2Cl_2	**3f:** −3.5° (c = 1.83) CH_2Cl_2	**4f:** +18.6° (c = 2.35) CH_2Cl_2	15
1g 	42	26	90 (92)	**2g:** −65.6° (c = 2.53) CH_2Cl_2	**3g:** −21.5° (c = 1.94) CH_2Cl_2	**4g:** +89.1° (c = 1.52) CH_2Cl_2	24
1h  E/Z: 87 : 13	42	6	92 (89)	**2h:** −30.8° (c = 1.72) CH_2Cl_2	**3h:** −9.4° (c = 1.59) CH_2Cl_2	**4h:** +46.5° (c = 0.75) CH_2Cl_2	24
5 	60		– (38)	**6:** –	**7:** −156.9° (c = 0.80) CH_2Cl_2	**8:** –	15
9 	55		87 (82)	**10:** −121.7° (c = 2.23) CH_2Cl_2	**11:** +30.5° (c = 2.33) CH_2Cl_2	**12:** +103.1° (c = 2.61) CH_2Cl_2	26

[a] %-Reduction was determined by GC of extracted aliquots.
[b] Relatives rates were calculated with respect to 3-cyclohexenecarbaldehyde **1a** (rel. rate = 100).
[c] Yields of isolated pure product. Yields in brackets refer to the alcohols **3a–h**, **7**, **11**.
[d] Optical rotations of aldehydes obtained by reoxidation of the alcohols **3a–h**, **11**.
[e] References providing synthetic- and analytical data.

To outline the ease and broad scope of this procedure as well as the principal stereochemical requirements for an enantioselective reduction, a number of systematically altered racemic 6-alkenyl- or 6-alkyl-3-cyclohexencarboxaldehydes **1a–h** and other ring systems **5** and **9** with similar substitution patterns (Table 1) were synthesized and reduced with HLADH under kinetically controlled conditions.

The method simply involves addition of HLADH to a slowly stirred suspension of aldehyde and NAD^+ in a 0.1 molar phosphate buffer at pH 7 using excess ethanol as the coupled substrate to recycle the nicotinamide coenzyme[18] and as cosolvent for poorly water soluble aldehydes. The reductions were followed by GC (on SE 30; 50 m × 0.31 mm) and were stopped after 50% conversion of the aldehyde by extraction with ether. The remaining antipodic aldehydes **2a–h, 6** and **10** as well as the produced alcohols **3a–h, 7** and **11** (Scheme **B**) were obtained chemically pure after chromatography on silica gel with a petrolether/ether gradient.



HLADH = horse liver alcohol dehydrogenase
NAD^+ = nicotinamide-adenine dinucleotide

Scheme **B**. The stereochemical course of the kinetically controlled enzymatic reduction of **1b < h** is also representative for **1a**, **5** and **9**, respectively.

Table 2. Spectral Data of Compounds **1a–h**, **3a–h**, **7**, **9** and **11**

Compound No.	^{1}H-NMR (CCl_4) δ(ppm)	IR (Neat) ν(cm^{-1})	MS *m/e* (M^+)
1a	1.50–3.00 (m, 7H); 5.50–5.90 (m, 2H); 9.72 (s, 1H)	3005, 2905, 2820, 2695, 1715, 1640, 1430, 920, 650	110
3a	1.50–2.35 (m, 7H); 3.18 (s, 1H); 3.40 (d, 2H, J = 6.5 Hz); 5.65 (br. s, 2H)	3450, 3030, 2920, 2940, 1650, 1435, 1095, 1030, 655	112
1b	0.95 (d, 3H, J = 6.5 Hz); 1.50–2.40 (m, 6H); 5.55 (br. s, 2H); 9.52 (d, 1H, J = 1.5 Hz)	3005, 2945, 2890, 2820, 2790, 1715, 1645, 1425, 750, 655	124
3b	0.88 (d, 3H, J = 6.7 Hz); 1.15–2.35 (m, 6H); 3.35–4.75 (m, 3H); 5.57 (br. s, 2H)	3330, 3010, 2940, 2870, 2820, 1650, 1430, 1065, 995, 655	122
1c	1.45–2.95 (m, 6H); 4.80–5.25 (m, 2H); 4.75 (br. s, 2H); 9.65 (d, 1H, J = 1.5 Hz)	3090, 3040, 2990, 2925, 2850, 2725, 1720, 1670, 1000, 925	136
3c	1.30–2.75 (m, 6H); 3.52 (m, 2H); 2.75 (s, 1H); 4.85–6.05 (m, 3H); 5.67 (m, 2H)	3340, 3080, 3025, 2900, 2840, 1660, 1045, 995, 915	138
1d	1.00 (t, 3H, J = 7.0 Hz); 1.60–2.55 (m, 5H); 2.80 (m, 1H); 5.05–5.60 (m, 2H); 5.75 (br. s, 2H)	3020, 3000, 2960, 2910, 2830, 2700, 1720, 1650, 1430, 660	164
3d	1.05 (t, 3H, J = 8.5 Hz); 1.40 (s, 1H); 1.35–2.70 (m, 6H); 3.25–3.50 (m, 2H); 5.05–5.55 (m, 2H); 5.62 (br. s, 2H)	3340, 3060, 3015, 2960, 2900, 2820, 1650, 1430, 1040, 660	166
1e	1.50–3.20 (m, 6H); 5.80 (br. s, 2H); 7.23 (br. s, 5H); 9.48 (d, 1H, J = 2.0 Hz)	3080, 3060, 3020, 2900, 2830, 2710, 1720, 1600, 1490, 1430, 760, 700, 660	186
3e	1.40–2.85 (m, 6H); 3.30 (m, 2H); 5.75 (br. s, 2H); 7.23 (br. s, 5H)	3380, 2960, 2920, 2900, 2830, 1650, 1600, 945, 760, 660	188
1f	1.80–3.10 (m, 6H); 4.85–6.20 (m, 3H); 5.70 (br. s, 2H); 9.68 (d, (d, 1H, J = 1.0 Hz)	3090, 3040, 2990, 2925, 2855, 2730, 1725, 1650, 1000, 920	124
3f	1.50–2.65 (m, 6H); 2.90 (m, 1H); 3.15–3.60 (m, 2H); 4.85–6.00 (m, 3H); 5.58 (s, 2H)	3350, 3080, 3030, 2960, 2910, 2840, 1640, 1440, 1030, 920	126
1g	1.68 (d, 3H, J = 5.5 Hz); 1.80–2.90 (m, 5H); 3.18 (m, 1H); 5.25–5.60 (m, 2H); 5.68 (br. s, 2H); 9.65 (s, 1H)	3020, 2910, 2840, 2710, 1725, 1650, 1435, 1245, 1035, 720	150
3g	1.68 (d, 3H, J = 5.7 Hz); 1.70–2.65 (m, 6H); 2.85 (m, 1H); 3.33 (m, 2H); 5.20–5.80 (m, 2H)	3330, 3020, 2905, 2840, 1650, 1435, 1020, 920, 660	152
1h	0.97 (t, 3H, J = 6.6 Hz); 1.55–3.55 (m, 6H); 5.20–5.60 (m, 2H); 5.68 (br. s, 2H); 9.68 (s, 1H)	3060, 3025, 2960, 2910, 2830, 2710, 1720, 1645, 1460, 1430, 730, 660	164
3h	0.97 (t, 3H, J = 6.6 Hz); 2.67 (s, 1H); 1.50–2.65 (m, 7H); 2.90 (m, 1H); 3.40 (m, 2H); 5.15–5.60 (m, 2H); 5.65 (br. s, 2H)	3340, 3060, 3020, 2960, 2910, 2840, 1650, 1460, 1435, 1025, 735, 660	166
7	1.20–3.10 (m, 4H); 3.20–4.00 (m, 3H); 4.80–6.95 (m, 7H)	3350, 3080, 3050, 3000, 2920, 2840, 1640, 1600, 1005, 965, 900, 720	150
9	1.28 (t, 3H, J = 7.5 Hz); 1.30–1.65 (m, 2H); 1.95–2.50 (m, 2H); 4.10 (q, 2H, J = 7.0 Hz); 9.38 (d, 1H, J = 3.5 Hz)	3100, 3060, 2980, 2840, 2730, 1715 (broad), 1380, 1315, 1180, 980, 880, 815, 750, 665	142
11	1.65–1.80 (m, 4H); 1.22 (t, 3H); 3.44 (m, 2H); 3.78 (s, 1H); 4.03 (q, 2H, J = 7.5 Hz)	3430, 2980, 2940, 2870, 1720, 1450, 1410, 1315, 1180, 1095, 1030, 885, 860, 795, 740	144

The relative rates of HLADH-catalyzed reductions of the aldehydes **1a–h** are compiled in Table 1 and are standardized against 3-cyclohexenecarboxaldehyde (**1a**). The unsubstituted aldehyde **1a** and compounds with small substituents (**1b**; R = CH_3, **1c**, **1f**; $CH{=}CH_2$, **1g**; $CH{=}CH{-}CH_3$) are rapidly reduced under standard conditions.

From *cis*-disubstituted aldehydes **1f–h** less homogeneous products are obtained. GC-analysis indicated small amounts (3–7%) of epimerized *trans*-disubstituted isomers. In addition, an *E*-contaminant of the side chain of **1h** (*E*/*Z* = 87:13) is preferentially reduced, leaving a stereochemically homogeneous aldehyde and an alcohol showing an *E*/*Z*-ratio of ca 75:25. In all other cases the geometrical isomers of the side chain were reduced with approximately the same ease.

The optical purity of the aldehydes was determined by one of the following methods (cf. Table 3):

- correlation of the optical rotations with literature data;
- GC-analysis (on SE 30) of the diastereomeric excess (d.e.) after derivatization of the aldehydes with (−)-(2*R*,3*R*)-butanediol;[19]
- analysis of suitable ^{13}C-NMR resonances of these acetals.

Particularly the acetal carbons showed useful $\Delta\delta$ shifts of 0.2–0.25 ppm. The acetals of racemic aldehydes were used as references, and their d.e. was determined with ± 15% accuracy. Alcohols **3a–h** and **11** were oxidized to the aldehydes **4a–h** and **12** with pyridiniumchlorochromate (PCC) according to standard procedures.

The absolute configurations of **2a–c, 2f, 7** and **10** are known (cf. references of Table 3). Compounds **3d–e** and **3g–h** belong to homologous series and exhibit no deviations in their sign of rotation or the ^{13}C-NMR of their diastereomeric acetals. Therefore, the assumption of a general absolute configuration within these series *seems* to be justified. The cyclopropane **10** was converted into the known diacetate and its e.e. and configuration obtained from published data.[20]

The active center of HLADH forms a specifically shaped cavern with hydrophobic areas of limited accessibility.[21] Additional substituents in juxtaposition to the carbonyl group of the alicyclic aldehydes **1a–h**, **5** and **9** should therefore strongly reduce the number of possible orientations of the substrate at the active center leading to enantioselective reductions. Comparing the data of Table 3, the low enantiomeric excess of the alcohol **3a** obtained by reduction of the unsubstituted 3-cyclohexenecarbaldehyde **1a** (0.9% e.e) clearly substantiates the need for additional substituents as directing elements. In general the *trans* series **1b–e** gives higher optical yields than the corresponding *cis* derivatives **1f–h**. Increasing chain length of *R* at first significantly enhances the e.e. of the products, but further enlargement of substituents has little or no effect (Table 3).

According to Table 3 and Scheme **B** aldehydes having the carbonyl group next to a center with *S*-configuration are preferentially reduced and lead to alcohols of the same configuration. This stereochemical course is not altered by *cis* or *trans* geometry or ring size, respectively (Table 3). This may be compared to the well documented oxidation of monocyclic meso-diols to lactones which also involves an enzymatic attack at $-CH_2OH$ groups attached to centers with *S*-configuration.[12] Thus, a major advantage of this enantiotopic constancy of HLADH-catalyzed reductions of aldehydes is the predictability of the absolute configurations of the products. Whether this predictability is general has to await further systematic investigations with other systems, substituents or substitutions patterns.

The ease of the procedure, the unambiguous stereochemical course and the simple chromatographic separation of the products makes this enantioselective reduction a useful and versatile alternative to current chemical approaches. Scaling up these reductions to a several 100 mmol range presents no problem (see experimental), but the e.e. of the obtained products proved to be lower than for the small scale reductions (51% and 52% e.e. *versus* 26% and 38% e.e. for **2b** and **3b**, respectively). Furthermore, immobilized HLADH[27,28] may be repeatedly used without loss of optical purity of the products and lets this method favourably compete with others in economy and efficiency.

Kinetically Controlled Reduction of Racemic Aldehydes 1a–h, 5 and 9 with HLADH; General Procedure:

To a slowly stirred solution of NAD^+ (100 mg, free acid, grade III; Boehringer, Mannheim) in 0.1 normal phosphate buffer (100 ml, pH 7) and ethanol (40 ml) are added HLADH (300 µl, 2.7 U/mg; 10 mg/ml, Boehringer, Mannheim) followed by the aldehyde (2.0 g) in ethanol (10 ml). The progress of the reaction is followed by GC (on SE 30, 50 m × 0.31 mm). In the case of slow reacting substrates additional portions of the enzyme are added, and the reductions are generally terminated after 50% conversion by extraction with ether (3 × 75 ml).

Table 3. Absolute Configuration and e.e. of Aldehydes **2a–h**, **6**, **10** and Alcohols **3a–h**, **7** and **11**

Aldehyde				Alcohol				
	Configuration	e.e. (%)	Method[a]		Configuration	e.e. (%)	Method[a]	Refs.
2a	(−)-(1*R*)	0.90	A	**3a**	(+)-(1*S*)	0.93	A	22
2b	(−)-(1*R*, 6*R*)	51	A	**3b**	(+)-(1*S*, 6*S*)	52	A	23
2c	(−)-(1*R*, 6*S*)	61	A	**3c**	(+)-(1*S*, 6*R*)	67	A	15
2d	(−)-(1*R*, 6*S*)*	52	B	**3d**	(+)-(1*S*, 6*R*)*	82	B	–
2e	(−)-(1*R*, 6*R*)*	42	B	**3e**	(+)-(1*S*, 6*S*)*	87	B	–
2f	(−)-(1*R*, 6*R*)	22	A	**3f**	(−)-(1*S*, 6*S*)	21	A	15
2g	(−)-(1*R*, 6*R*)*	62	B	**3g**	(+)-(1*S*, 6*S*)*	84	B	–
2h	(−)-(1*R*, 6*R*)*	52	C	**3h**	(+)-(1*S*, 6*S*)*	79	C	–
6	–[b]	–	–	**7**	(−)-(1*S*, 2*S*)	73	A	15
10	(−)-(1*R*, 2*R*)	48	A	**11**	(+)-(1*S*, 2*S*)	41	A	20

[a] Absolute configurations and e.e. were determined according to the methods given: A = optical rotation; B = GC of diastereomeric acetals after derivatization with (−)-(2*R*, 3*R*)-butanediol; C = ^{13}C-NMR of diastereomeric acetals. Absolute configurations marked with an asterisk (*) are tentatively assigned. The references provide optical rotations and enantiomeric purities of the compounds.

[b] (+)-(1*R*, 2*R*) **6** obtained as the remaining antipodic aldehyde from the HLADH reduction of racemic **5** is unstable and could not be isolated by chromatography.[15]

The combined organic layers were dried with magnesium sulfate, concentrated *in vacuo* and chromatographed on silica gel. Aldehydes eluted first with hexane/ether (9:1) followed by the alcohols with hexane/ether in the ratio of 7:3.

Reductions with Immobilized HLADH; General Procedure:
Reductions with the immobilized enzyme[27,28] (immobilized on CNBr-activated Sepharose and stabilized by adenosine 5'-monophosphate; specific activity (ethanol) ΔE_{340}/min mg = 8.8) followes in all respects the above procedure, except that twice the amount of enzyme is used to compensate for its somewhat lower activity (ca. 50% the activity of the soluble enzyme under standard assay conditions[27]). Slow mechanical stirring is maintained to avoid damage of the polymeric support and to keep the enzyme suspended. After 50% reduction of the substrate the enzyme is removed from the reaction mixture by filtration (sintered glass filter G3) and washed with phosphate buffer (50 ml; pH 7). The products are extracted as described.

Preparative Scale Reductions of Racemic Aldehydes:
NAD^+ (0.8 g) and HLADH (1.0 ml) are placed in a mixture of phosphate buffer (1000 ml; pH 7.0) and ethanol (400 ml). Racemic aldehhyde **1b** (30.0 g) in ethanol (100 ml) is added, and the suspension is slowly stirred until GC indicated ca. 50% conversion. The reaction is terminated by extraction with ether (4 × 200 ml), and the combined organic layer is thoroughly washed with saturated brine (4 × 100 ml) followed by evaporation of the solvent *in vacuo*. Distillation of the crude mixture over a 20 cm column filled with stainless steel spirals gives the aldehyde **2b**; yield: 7.2 g (48%); b.p. 72°C/14 torr, an aldehyde/alcohol mixture; yield: 8.8 g; b.p. 75–90°C/14 torr and alcohol **3b** yield: 8.5 g (57%); b.p. 92°C/14 torr. Final purification of aliquots over silica gel gave **2b**; $[\alpha]_{578} = +28{,}4°$ ($c = 4.422$, $CHCl_3$) and **3b**; $[\alpha]_{578} = +22.1°$ ($c = 11.12$, $CHCl_3$). This corresponds to an e.e. of 25% for **2b** and 38% for **3b**.

Acetalization of Aldehydes with (−)-(2*R*,3*R*)-2,3-Butanediol; General Procedure:
Aldehyde (2.0 mmol), (−)-(2*R*,3*R*)-2,3-butanediol (4.0 mmol) and pyridinium-*p*-toluenesulfonate (50 mg) in tetrachloromethane (5 ml) are stirred at 40° for 4 h. The organic layer is washed with water (3 × 5 ml), dried with magnesium sulfate and evaporated *in vacuo*. The acetals are purified by chromatography on silica gel prior to spectroscopic analysis. Their d.e. was determined either by GC (on SE 30, 50 m × 0.31 mm) at properly adjusted isothermal temperature levels or by ^{13}C-NMR. Particularly the acetalcarbons showed useful $\Delta\delta$ shifts of 0.1–0.2 ppm (accuracy: ± 15%).

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