Pergamon

Tetrahedron Vol. 50, No. 34, pp. 10245-10252, 1994

0040-4020/94 $7.00+0.00

0040-4020(94)00612-1

4-Hydroxynon-2-enal, a Cytotoxic Lipid Peroxidation Product, and its C_5-Analog 4-Hydroxypent-2-enal: Enantioselective Synthesis and Stereoanalysis[1]

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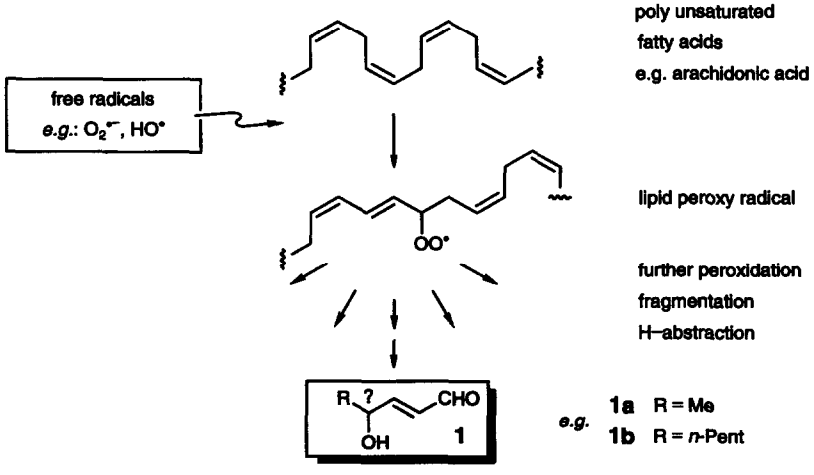
Abstract: A stereoselective synthesis of the lipid peroxidation products 4-hydroxypent-2-enal (**1a**) and 4-hydroxynon-2-enal (**1b**) in high optical purity is presented. The configuration of **1a** and **b** was established by Ru(III)-catalyzed oxidative degradation and subsequent stereoanalysis of the resulting α-hydroxy acids. It was demonstrated that **1a** is configuratively stable under physiological conditions.

INTRODUCTION

4-Hydroxyalk-2-enals **1**, which are formed *in vivo* as breakdown products of oxidized polyunsaturated lipids (Scheme 1), have recently received much attention in biological and biomedical science.[2-4] They result from a variety of physiological processes known as *oxidative stress*. The investigation of their biological activities revealed the ability to act as crosslinkers of enzymes and proteins such as low density lipoprotein[3] or microsomal glucose-6-phosphatase and cytochrome P_{450}.[4]



Scheme 1. *In vivo* formation of 4-hydroxyalkenals by lipid peroxidation.

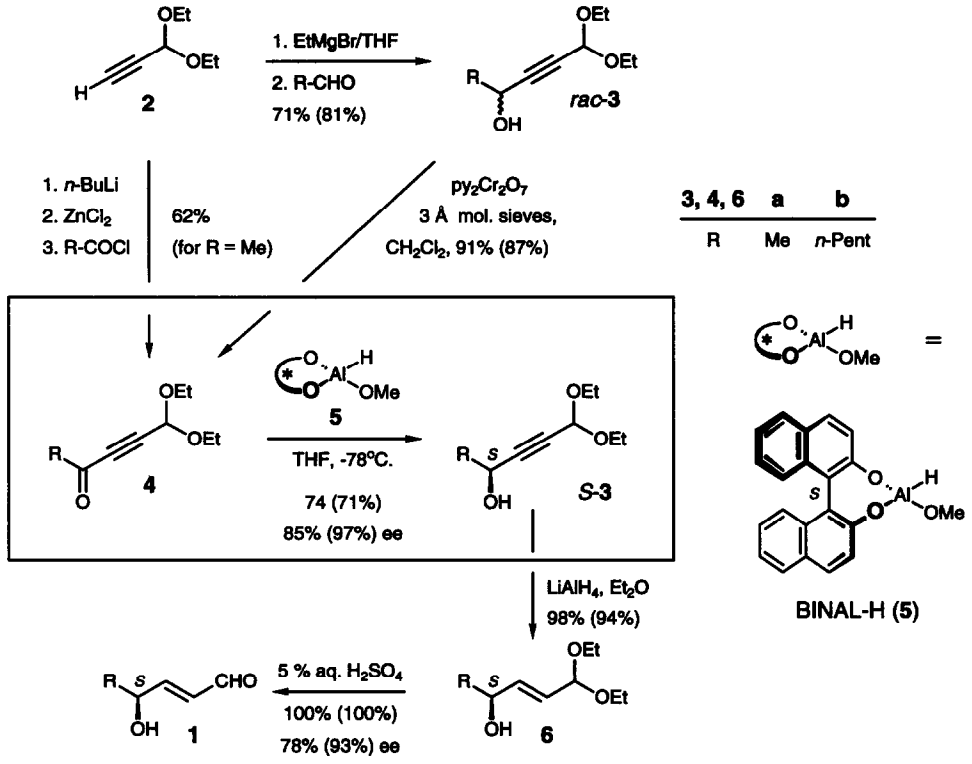
Compared with other aldehydic products of lipid peroxidation, 4-hydroxynon-2-enal (**1b**) shows the most pronounced biological effects and therefore has received the highest attention. Due to its simpler structure, 4-hydroxypent-2-enal (**1a**) is an ideal model compound for investigations on the reactivity of hydroxyalkenals.

So far biochemical studies have been carried out with racemic 4-hydroxyalk-2-enals, completely neglecting the stereocenter at C-4. A reason may be that until recently, nonracemic 4-hydroxyalk-2-enals were accessible only by a tedious racemate resolution *via* a chiral iron complex.[5] In 1993, Wang *et al.*[6] published a synthesis of optically active 4-hydroxyalk-2-enals, the stereocenter being generated by a Sharpless epoxidation. Yet, no experimental details and no optical yields were given for the most interesting representative 4-hydroxynon-2-enal (**1b**). Allevi *et al.*[7] prepared 4-hydroxyalk-2-enals with high optical activity by enzymatic resolution, unfortunately the yields with this procedure were low. This prompts us to present a straightforward and highly enantioselective four-step chemical synthesis of **1a** and **1b** from a joint achiral precursor **2**, and their degradative stereoanalysis.

RESULTS AND DISCUSSION

Key step of our synthesis (see Scheme 2) is the creation of the C-4 stereocenter by stereoselective reduction of the alkynones **4a** and **b** to give the acetylenic alcohols *S*-**3a,b**. Our synthesis starts from the achiral alkyne **2**, which is commercially available, but can also be obtained in three steps starting from acrolein.[8] From **2**, the ketones **4** can be prepared directly after lithiation, transmetallation with $ZnCl_2$ and addition to acetyl or *n*-hexanoic chloride. Although this procedure seems to be straightforward, it leads to the formation of several side products which are difficult to separate from the sensitive ketones **4**. Thus, it is more effective to prepare **4a** and **b** from the racemic alkohols *rac*-**3a,b**, which are obtained easily by deprotonation of **2** with ethylmagnesiumbromide and addition of the acetylide to acetic aldehyde or *n*-hexanal, respectively. This preparation of *rac*-**3a** and **b** has already been described by Esterbauer[9] as a part of his non-enantioselective hydroxyalkenal synthesis. For the oxidation, pyridinium dichromate with 3 Å molecular sieves[10] was found to be an excellent and mild reagent. The product **4** was separated from the much more polar by-products by filtration through silica and used without further purification.

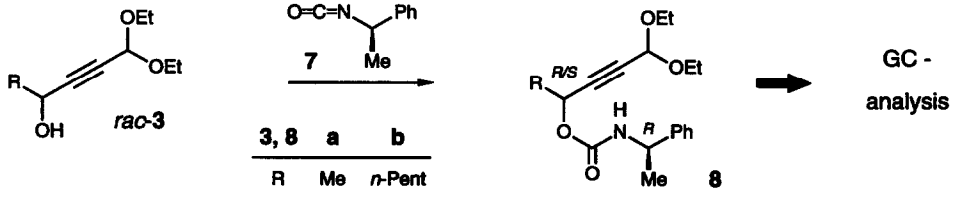
The stereoselective reduction of the ketones **4** was best brought about with Noyori's *S*-BINAL-H (**5**), a reagent that had already been used for the enantioselective preparation of other α,β-acetylenic alcohols.[11] With this efficient reagent, *S*-**3a** was obtained with 74 % yield and 85 % ee and the hydroxynonenal precursor *S*-**3b** with 71 % yield and 97 % ee. Careful preparation of *S*-**5** turned out to be of critical importance to avoid a decrease of optical yield. The ee values of **3** were determined by gas chromatography after derivatization with a chiral electrophile. Mosher's reagent[12] led to separable diastereomeric derivatives only for **3a**. By contrast, the diastereomeric carbamates **8a** and **b** obtained by treatment of **3a** and **b** with *R*-1-phenylethylisocyanate (**7**)[13] (see Scheme 3), displayed distinct peaks in the chromatogram. This reagent required more forcing conditions, which fortunately did not lead to racemization of the alkynols **3a,b**.



Scheme 2. Preparation of *S*-**1a** and **b**. Yields and optical purities refer to R = Me, those in brackets to R = *n*-Pent.

Subsequent *trans*-selective hydrogenation of the optically active alkynols **3a,b** to the alkenols **6a,b** was accomplished with good yields. The most critical step of the sequence was the final acid catalyzed hydrolysis of the acetal group of **6** leading to the desired aldehydes **1a** and **b**.

Such hydroxyalkenals show a strong tendency to racemize under acidic aqueous conditions, as was demonstrated examplarily for **1a**. We found that acetal cleavage by 1 h treatment with 2 % aqueous solution of citric acid as described by Esterbauer,[9] is far too harsh, leading to a considerable decrease of optical activity.



Scheme 3. Stereoanalysis of **3a** and **b** after derivatization with *R*-1-phenylethylisocyanate (**7**).

In order to minimize reaction time and thus exposure to critical acidic conditions, THF was added as a cosolvent to improve the solubility of the unpolar acetals **6**. Hydrolysis was complete within 1 min, leading only to a minor loss of stereochemical information and the products were sufficiently pure by NMR and GC. The ee of **1a** was found to be 77 % and of **1b** 93 %.

For the stereoanalysis of the hydroxyalkenals **1a** and **b** by GC, a new method had to be employed, because the sensitive compounds would most probably not survive the prolonged heating necessary for the derivatization with **7**. Optimum conditions for the enantiomer resolution of **1a** and **b** were found by separation on a chiral permethyl-β-cyclodextrin stationary phase after *O*-acetylation under mild conditions. The resolution of *R*- and *S*-**1a** was possible even without derivatization, therefore **1a** is a suitable model compound for biochemical experiments, where a rapid stereoanalysis is required.[14]

Optically active **1a** was subjected to physiological conditions (buffered aqueous solution at pH 7.1, 37 °C). Samples were analyzed by GC for *R*- and *S*-**1a**. An only 20 % decrease of optical activity was observed after 14 d, when the aldehyde had almost completely disappeared (Fig. 1). Thus the 4-hydroxyalkenals can be considered as configuratively stable *in vivo*, as their enzymatic metabolism, for which a biological halflife of < 30 min was reported,[15] is much more rapid than their racemization.

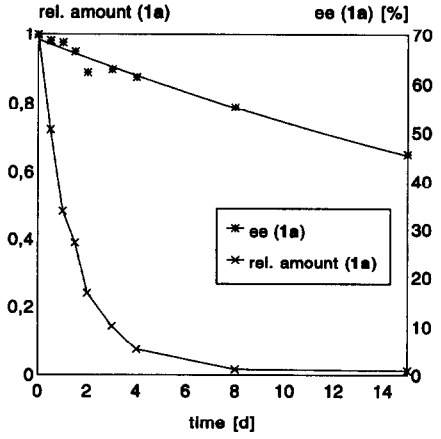
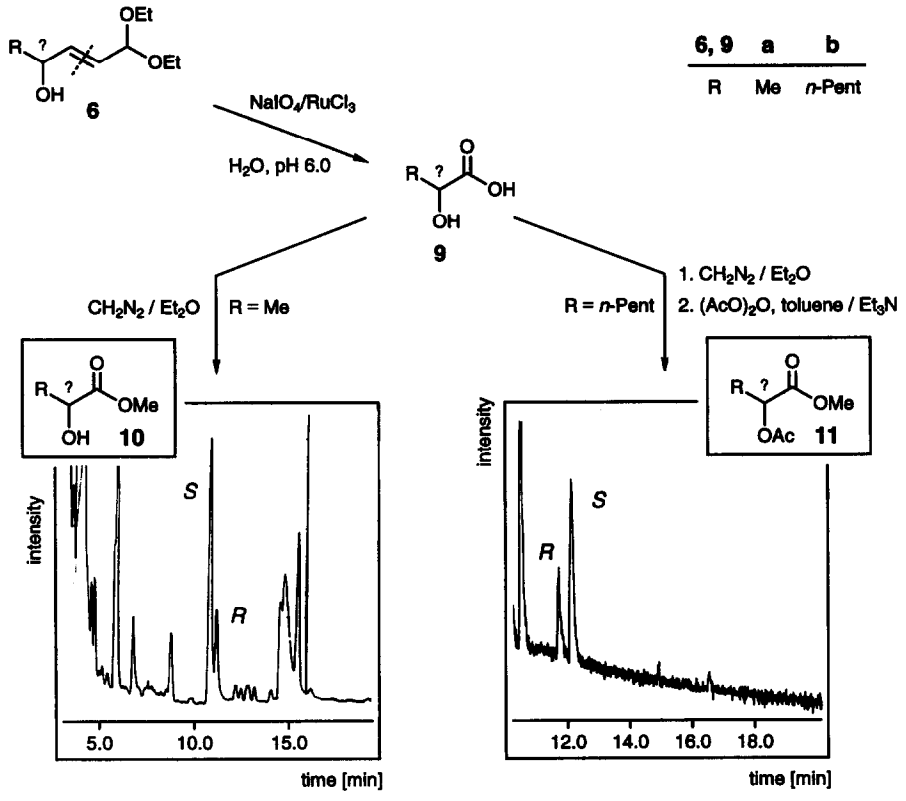


Fig. 1. Stability of **1a** under physiological conditions.

According to Noyori,[11] the absolute configuration of BINAL-H (**5**) reduction products can be predicted quite reliably to be *S* if *S*-**5** is employed and *vice versa*. Still, as there are some exceptions to this rule,[11] we decided to unambiguously assign the stereochemistry by a first application of a Ru(III) catalyzed oxidative degradation[16] to alkenols like **6a,b** (Scheme 4). The α-hydroxy acid **9a** resulting after double bond cleavage of **6a** was transferred into the methyl ester **10**, whereas **9b** was methylated and additionally acetylated at 4-OH to yield **11**. The derivatives were subjected to gas chromatography on chiral stationary phases. The enantiomers were identified by comparison with 2*S*-lactic acid (**9a**) (commercially available) and *R*-2-hydroxyheptanoic acid (**9b**) (prepared according to a literature procedure[17]). Although partial racemization occurred during the oxidative degradation, we still could establish the absolute configurations of both **6a** and **6b** to be *S* if *S*-BINAL-H (**5**) had been used for the reduction of the ketones **4a,b**.



Scheme 4. Oxidative Degradation of **6a** and **b** and identification of the products.

Combined with the enantiomer analysis, the enantioselective synthesis of hydroxyalkenals now opens up the possibility to investigate biologically important questions such as the stereochemistry of the formation and metabolism and the enantiospecific biological activities of these important natural products.

EXPERIMENTAL

Racemic 1,1-diethoxynon-2-yn-4-ol was prepared according to Esterbauer,[9] *S*-binaphthol was obtained by resolution of racemic material as described in the literature.[18] All other reagents were used as commercially available. GC analyses were performed with a HP 5890 series II instrument, equipped with a HP 5971 A MSD (Hewlett Packard) and with a Carlo Erba GC 2000 Vega Series 2. ^{1}H NMR spectra were recorded on a Bruker AC 200 spectrometer in $CDCl_3$. The chemical shifts δ are given in parts per million (ppm), with reference to the proton signal of $CHCl_3$ in the deuterated solvent. Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter. Mass spectra were determined on a Finnigan MAT 8200 mass spectrometer. IR spectra were obtained from a Perkin-Elmer 1420 infrared spectrometer and are given in cm^{-1}. The intensities are denoted by: strong (s) and weak (w).

1,1-Diethoxypent-2-yn-4-one (**4a**): To a solution of *rac*-**3a** (172 mg, 1.00 mmol) in CH_2Cl_2 (5 mL), pyridinium dichromate (1.06 g, 2.82 mmol) and powdered 3 Å molecular sieves (1.50 g) were consecutively

added. The slowly darkening mixture was stirred at r.t. for 1 h. Et_2O (10 mL) was added and the brownish slurry was filtered through a 0.5 cm layer of silica gel. The silica was washed (CH_2Cl_2 / Et_2O 1:2), filtrate and washing solutions were combined and the solvents were evaporated *in vacuo* to yield 155 mg (91 %) of the ketone **4a** as a colorless liquid, which rapidly turned yellow at r.t. IR (Film): ν = 2980, 2920, 2880 (s, C-H), 2220 (w, C C), 1670 (s, C=O), 1210; ^{1}H NMR: δ = 1.19 (t, 6 H, *J* = 7.1, OCH_2CH_3), 2.31 (s, 3 H, 5-H), 3.48-3.75 (m, 4 H, OCH_2CH_3), 5.32 (s, 1H, 1-H); MS (EI, 70 eV): *m/z* (%) = 170 (0.1) [M^+], 169 (1.3) [M^+ - 1], 125 (95) [M^+ - OC_2H_5], 97 (100) [M^+ - C_4H_9O]; Calcd. for $C_9H_{14}O_3$ (170.2): C, 63.50; H, 8.25. Anal. found: C, 63.19; H, 8.19.

1,1-Diethoxynon-2-yn-4-one (**4b**): *rac*-**3b** (228 mg, 1.00 mmol) was analogously oxidized to yield 197 mg (87 %) of the ketone **4b**. IR (Film): ν = 2980, 2920, 2880 (s, C-H), 2220 (w, C H), 1670 (s, C=O), 1210; ^{1}H NMR: δ = 0.90 (t, 3 H, *J* = 6.6, 9-H), 1.27 (t, 6 H, *J* = 7.1, OCH_2CH_3), 1.29-1.75 (m, 6 H, 6-H, 7-H, 8-H), 2.59 (t, *J* = 6.9, 2 H, 5-H), 3.62-3.76 (m, 4 H, OCH_2CH_3), 5.39 (s, 1 H, 1-H); MS (EI, 70 eV): *m/z* (%) = 226 (0.2) [M^+], 225 (2.1) [M^+ - 1], 181 (81) [226 - OC_2H_5], 111 (92) [$C_7H_{11}O^+$]; Calcd. for $C_{13}H_{22}O_3$ (226.3): C, 69.00; H, 9.80. Anal. found: C, 68.99; H, 9.89.

The products were used for the following reduction without further purification.

(S)-1,1-Diethoxypent-2-yn-4-ol (**3a**): A solution of *S*-BINAL-H (**5**) (1.00 mmol) was freshly prepared from $LiAlH_4$ (1.00 mmol, 1.45 mL of a 0.69 M solution), methanol (32.0 mg, 1.00 mmol, 40.5 µL) and *S*-binaphthol (289 mg, 1.01 mmol) as published by Noyori.[11] Ketone **4a** (56.7 mg, 333 µmol) dissolved in THF (1 mL) was added at -100 °C over a period of 15 min. Subsequently, the mixture was allowed to warm to -78 °C and stirring was continued until the reaction was complete (monitored by TLC on SiO_2, CH_2Cl_2 / MeOH 98:2). Reaction time varied between 10 min and 1 h. Excessive **5** was destroyed with methanol. The solution was allowed to warm to r.t. and saturated aqueous NH_4Cl solution (10 mL) was added. After addition of Et_2O (20 mL), the organic layer was separated, washed with H_2O and dried (Na_2SO_4). The crude mixture as obtained after evaporation of the solvent *in vacuo*, was subjected to column chromatography (SiO_2, 63 - 200 mesh, CH_2Cl_2 / MeOH 98:2) to yield *S*-**3a** (42.4 mg, 74 %) as a colorless oil. $[\alpha]_D^{22}$ = -8.75 ° (*c* = 0.37 in CH_2Cl_2); ee = 85 %.

(S)-1,1-Diethoxynon-2-yn-4-ol (**3b**): **4b** (75.4 mg, 333 µmol) was analogously reduced to yield 54.0 mg (71 %) of the secondary alcohol **3b**. $[\alpha]_D^{22}$ = -4.50 ° (*c* = 0.24 in CH_2Cl_2); ee = 97 %. Other physical data of **3a** and **b** were identical to those published[9] for the racemic compounds.

Enantiomer analysis of **3a** *and* **b**: **3a** or **b** (1.00 mg) and 3.41 mg (23.2 µmol, 3.32 µl) of *(R)*-phenylethylisocyanate (**7**), were dissolved in toluene (300 µl) and heated to 90 °C for 48 h, until derivatization was complete (TLC). The reaction mixture was analyzed by GC on a DB-1 (J&W Scientific) fused silica capillary column [30 m x 0.3 mm ID, film thickness 0.3 µm, temperature: 150 °C (3 min), 5 °C / min up to 190 °C (5 min), 1 °C / min up to 210 °C (10 min), 5 °C / min up to 280 °C (5 min)] producing two peaks with identical mass spectra.
R,S-**8a**: t_R = 22.83; *R,R*-**8a**: t_R = 23.65 min; GC/MS (EI, 70 eV): m/z (%) = 318 (0.6) [M^+ - 1], 274 (40) [M^+ - OC_2H_5], 170 (32) [$C_9H_{15}O_3^+$ - 1], 128 (100) [$C_7H_{12}O_2^+$], 105 (91) [Ph-$C_2H_4^+$].
R,S-**8b**: t_R = 31.61; *R,R*-**8b**: t_R = 31.96 min; GC/MS (EI, 70 eV): m/z (%) = 374 (0.16) [M^+], 330 (50) [M^+ - OC_2H_5], 226 (7.1) [$C_{13}H_{23}O_3^+$ - 1], 182 (27) [$C_{11}H_{18}O_2^+$], 127 (49) [$C_8H_{15}O^+$], 105 (100) [Ph-$C_2H_4^+$].

(S)-1,1-Diethoxypent-2-en-4-ol (**6a**): $LiAlH_4$ (16.4 mg, 432 µmol) was added to a stirred solution of **3a** (36.2 mg, 210 µmol) in Et_2O (10 mL) at -25 °C. Temperature and stirring were maintained for 30 min. Excessive reagent was quenched with semi-saturated aqueous NH_4Cl solution (1 mL). After warming to r.t.,

the organic layer was separated and the aqueous phase was extracted twice with Et_2O. The combined extracts were dried (Na_2SO_4) and the solvent was removed *in vacuo* leaving 35.9 mg (98 %) of the product **6a** as a colorless oil. $[\alpha]_D^{22}$ = -8.25 ° (*c* = 0.61 in CH_2Cl_2).

(S)-1,1-Diethoxynon-2-en-4-ol (**6b**): Analogous *trans*-hydrogenation of **3b** (47.9 mg, 210 µmol) yielded 45.5 mg (94 %) of **6b**. $[\alpha]_D^{22}$ = +11.3 ° (*c* = 0.36 in CH_2Cl_2). Other physical data of **6a** and **b** were identical to those published[9] for the racemic compounds.

(S)-4-Hydroxypent-2-enal (**1a**): To a well stirred solution of acetal **6a** (3.48 mg, 20 µmol) in THF (300 µL), 5% H_2SO_4 (5 µL)[19] was added. The reaction was complete within 1 min. Saturated aqueous NH_4Cl solution (5 mL) was added and the mixture was extracted with Et_2O. The extracts were combined, washed with H_2O, dried (Na_2SO_4) and the solvent was evaporated *in vacuo* to yield 2.00 mg (100 %) of the free aldehyde *S*-**1a** as a colorless oil. $[\alpha]_D^{22}$ = +15.3 ° (*c* = 0.90 in CH_2Cl_2); ee = 78 %.

(S)-4-Hydroxynon-2-enal (**1b**): Analogous cleavage of the acetal **6b** (4.61 mg, 20.0 µmol) yielded 3.12 mg (100 %) of the free aldehyde. $[\alpha]_D^{22}$ = +49.3 ° (CH_2Cl_2, *c* = 0.46); ee = 93 %. Other physical data of **1a** and **b** were identical to those published[9] for the racemic compounds.

Enantiomer analysis of **1a** *and* **b**: 1 mg **1a** or **1b** was dissolved in toluene / 0.1 M Et_3N (300 µL) and acetic anhydride (100 µL) was added. The sample was heated to 60 °C for 1.5 h, washed with aqueous phosphate buffer (pH 7.0, 300 µL) and analyzed by GC on a C-DEX B (*J&W*) fused silica capillary column [stationary phase: permethyl-β-cyclodextrin, 30 m x 0.2 mm ID, film thickness 0.25 µm, temperature: 100 °C (3 min), 2 °C / min up to 170 °C (5 min)]. Two peaks with identical mass spectra were obtained.
O-acetylated *R*-**1a**: t_R = 10.01 min; *O*-acetylated *S*-**1a**: t_R = 10.67 min; GC/MS: m/z (%) = 100 (90) [M^+ - OAc], 81 (C_5H_5O, 100), 71 (87) [100 - C_2H_5].
O-acetylated *R*-**1b**: t_R = 28.31 min; *O*-acetylated *S*-**1b**: t_R = 28.45 min; GC/MS: m/z (%) = 156 (93) [M^+ - OAc], 127 (85) [156 - C_2H_5], 81 (100) [C_5H_5O].

Oxidative cleavage of **6a** *and* **b**: The acetals **6a** (1.00 mg, 5.74 µmol) or **6b** (1.00 mg, 4.34 µmol) were dissolved in 1 mL of a buffered (pH 6.0) solution of $RuCl_3$ (40.0 µg, $1.93 \cdot 10^{-7}$ mol) and $NaIO_4$ (400 mg, 1.87 mmol) and stirred for 2 h at r.t. The solution was freeze dried and the solid residue was extracted with MeOH. The extracts were passed through a 4 µm filter cartridge (*Millipore Co.*) and the solvent was evaporated *in vacuo*.

Derivatization of lactic acid (**9a**) *and resolution of R- and S-methyl lactate* (**10**) *by GC*: A solution of CH_2N_2 in Et_2O was added to the samples until the yellow color of the reagent persisted. The solution was directly subjected to gas chromatography on a Chirasil-Val (*Machery & Nagel*) fused silica capillary column (25 m x 0.32 mm ID, film thickness 0.14 µm), temperature: 80 °C (2 min), 1 °C / min up to 95 °C (2 min), 5 °C / min up to 150 °C (5 min).
S-**10**: t_R = 10.56 min; *R*-**10**: t_R = 10.84.

Derivatization of R- and S-2-hydroxyheptanoic acid (**9b**) *and separation of the derivatives R- and S*-**11**: Samples containing 2-hydroxyheptanoic acid (**9b**) were treated with CH_2N_2 as described for **9a**. The solvent was removed *in vacuo* and the 2-hydroxycarboxylic esters were acetylated and analyzed by GC as described for **1b**.
R-**11**: t_R = 11.72 min; *S*-**11**: t_R = 12.01.

ACKNOWLEDGEMENT

Generous financial support of this project by the Fonds der Chemischen Industrie is gratefully acknowledged. R.L. thanks the Erasmus Program and the Région Rhône-Alpes for granting a scholarship.

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(*Received in Germany* 24 *June* 1994; *accepted* 6 *July* 1994)