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Communication

Practical highly enantioselective synthesis of (*R*)- and (*S*)-(*E*)-4-hydroxynon-2-enal

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Oxidative stress enhances lipid peroxidation (LPO) implicated in cancer promotion and progression. (*E*)-4-Hydroxynon-2-enal 1 (*trans*-4-hydroxy-2-nonenal, HNE) is one of the most abundant products of LPO. Reactions of HNE with DNA and proteins are responsible for its mutagenic and toxic effects. On the other hand, HNE is regarded as a key molecule in stress mediated cell cycle signaling. LPO generates racemic HNE (*rac*-1); however, it is expected that the individual enantiomers will behave differently in their interactions with cell components. The study of HNE stereochemistry in its chemical and biochemical interactions is hindered by the lack of expedient methods for preparation of pure enantiomers. This study presents one step synthesis of HNE in a cross-metathesis reaction between the commercially available oct-1-en-3-ol and acrolein in the presence of 2nd generation Grubbs catalyst. The use in the metathesis reaction of enantiomers of oct-1-en-3-ol obtained *via Candida antarctica* lipase resolution of the racemate allowed us to prepare of 4-(*R*)- and 4-(*S*)-enantiomers of HNE (*R*-1 and *S*-1, respectively) with excellent optical purity (97.5 and 98.4% ee, respectively) and good chemical yields (70%).

Keywords: (*E*)-4-hydroxynon-2-enal (4-HNE) synthesis, 4-HNE enantiomers, asymmetric synthesis, *Candida antarctica* lipase, lipid peroxidation

INTRODUCTION

Oxidative stress enhances lipid peroxidation (LPO) which is implicated in the promotion and progression of carcinogenesis. The oxidative stress driven LPO is a powerful source of endogenous reactive agents, including 2,3-unsaturated aldehydes (enals), such as acrolein, croton aldehyde and (*E*)-4-hydroxynon-2-enal **1** (*trans*-4-hydroxy-2-nonenal, HNE, Scheme 1) (Wu & Kin, 1995). Enals form exocyclic hydroxypropano adducts with DNA bases (Chung *et al.*, 1999), they can also form DNA interchain (Kozekov *et al.*, 2003) and DNA-protein cross-links (Kurtz & Lloyd, 2003) as well as protein adducts (Uchida *et al.*, 1998; Hashimoto *et al.*, 2003). The reactions of LPO products with DNA and proteins are responsible for their mutagenic and toxic effects. On the other hand, HNE, the major LPO-generated hydroxyalkenal, is regarded as a key molecule in stress mediated cell cycle signaling (Yang *et al.*, 2003). The present state of knowledge of HNE reactivity, metabolism, signaling, its modulatory effect in various human organs and contribution to the pathogenesis of major human chronic diseases has recently been reviewed by Poli *et al.* (2008).

HNE generated in rat liver microsomes, where LPO is induced by ADP/Fe^{++} treatment, is a racemic mixture consisting of the *R* and *S* enantiomers at a ratio 1:1 (Bringmann *et al.*, 1994a). This indicates that HNE is formed, at least in the liver, by a free radical mechanism without any enzymatic stereocontrol. It can be expected, however, that individual enantiomers will behave differently in their interactions.

The stereochemistry at C-4 of the chiral hydroxyalkenals is very often neglected even in studies

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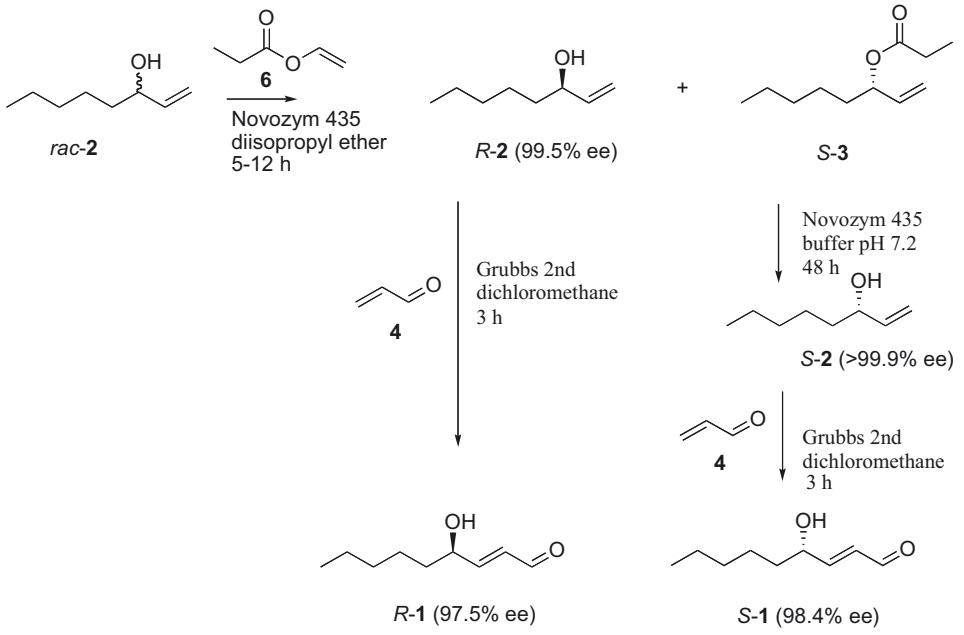
Abbreviations: ee, enantiomeric excess; HNE, (*E*)-4-hydroxynon-2-enal, *trans*-4-hydroxy-2-nonenal; HPLC, high-performance liquid chromatography; LPO, lipid peroxidation; TLC, thin-layer chromatography.



Sheme 1. Preparation of *R*- and *S*-(*E*)-4-hydroxynon-2–enal (*R*-1 and *S*-1).

concerning the biological activity of these toxic and mutagenic products; equally neglected is their metabolism. However, the obvious importance of the chirality of hydroxyalkenals in their chemical and biochemical reactions has been shown in some cases. For example, the stereoisomers of HNE adducts to guanine in DNA are markedly different in their chemical reactivity (Kurtz & Lloyd, 2003) as well as in their ability to cause mutations (Fernandes *et al*., 2003). 4*R*-HNE- and 4*S*-HNE-modified proteins are specifically recognized by different monoclonal antibodies (Hashimoto *et al*., 2003). It has also been shown in several recent studies that the metabolism of HNE, oxidation by aldehyde dehydrogenases and glutathione conjugation by glutathione *S*-transferases, is enantioselective (Brichac *et al*., 2007 and references therein).

The study of HNE stereochemistry in its chemical and biochemical interactions is hindered by the lack of expedient methods for preparation of pure enantiomers. The existing methods for preparation of racemic HNE are multistep and rather arduous. These include, among others: a classical Esterbauer method based on the reaction of 1,1-diethoxypropyne with n-hexanal (Esterbauer & Wegner, 1967), a method based on addition of pentylmagnesium bromide to bisfumaricaldehyde (Gree *et al*., 1986; Chandra & Srivastava, 1997) or on condensation of Wittig reagent bearing a hexanoyl group with glyoxal dimethyl acetal (Kurangi *et al*., 2006). Similarly, a multistep procedure employed for the synthesis of the *S* enantiomer of HNE was based on the Esterbauer method (Esterbauer & Wegner, 1967) with creation of the C-4 stereocenter by stereoselective reduction of 1,1-diethoxynon-2-yn-4-one (Bringmann *et al*., 1994b). Resolution of the HNE enantiomers was achieved by coupling of racemic HNE dimethylacetal with optically pure (–) sorbic acid iron tricarbonyl complex, subsequent chromatography and deacetalization (de Montarby *et al*., 1988). A more practical method, based on enzymatic enatioselective lipase-catalyzed esterification of the 4-hydroxy group, was used for separation of 4-(*R*)- and 4-(*S*)-hydroxyalk-2-enals, including enantiomers of HNE, to give products of enantiomeric purity > 95% ee (Allevi *et al*., 1993). This method was used recently by authors studying enantioselective metabolism of HNE (Brichac *et al*., 2007 and references therein).

In this communication we present one step synthesis of HNE, in a cross-metathesis reaction between the commercially available oct-1-en-3-ol *rac*-**2** and acrolein **4** in the presence of 2nd generation Grubbs catalyst. The use in the metathesis reaction of enantiomers of oct-1-en-3-ol obtained *via Candida antarctica* lipase resolution of racemate, allowed us to prepare the 4-(*R*)- and 4-(*S*)-enantiomers of HNE with excellent optical purity (97.5 and 98.4% ee, respectively).

MATERIALS AND METHODS

Caution. HNE and acrolein are potentially carcinogenic and should be handled with protective clothing in a well-ventilated fume hood.

Chemicals. Racemic oct-1-en-3-ol, vinyl propionate, vinyl acetate, vinyl crotonate and Grubbs catalyst 2nd generation (benzylidene[1,3-bis(2,4,6-tri-

methylphenyl)-2-imidazolidinylidene]dichloro(tricyclohexylphosphine)ruthenium) were purchased from Sigma Aldrich Chemicals. Acrolein (containing 10% water) was from Fluka. Novozym 435 (immobilized *Candida antarctica* lipase B, >2 units/mg) was provided by Novozyme.

Methods. TLC was performed using silica gel 60 F_{254} aluminum sheets (Merck). Detection was carried out by charring of silica gel previously sprayed with a solution of 5% anisaldehyde in ethanol containing 10% H_2SO_4. For column chromatography silica gel 60 (Merck) was used. Enantiomeric composition of oct-1-en-3-ol was determined on a Thermo-Trace-GC Ultrachromatograph by using a Supelco B-dex 120 chiral column (30 m×0.25 mm, d_f 0.25 μm) at 65°C after derivatization by 1-(trimethylsilyl)-imidazole. Separations were performed at 100 kPa. Enantiomeric composition of HNE was determined by HPLC with a Chiralpak AS-H chiral column (250 mm×4.6 mm). Separations were performed in isocratic 5% isopropanol in hexane (v/v) as a mobile phase, flow 1 mL/min, UV detection at 225 nm. NMR measurements were performed on a Varian Inova 400 MHz Spectrometer. Optical rotation was measured on a Jasco P-2000 polarimeter.

Lipase-mediated kinetic resolution of racemic oct-1-en-3-ol. General Procedure. Vinyl propionate **6** (32.5 g (40 mL), 325 mmol, 6.5 equiv.) and Novozym 435 lipase (1.78 g) were added to a solution of ***rac*-2** (6.4 g (5 mL), 50 mmol, 1 equiv.) in 100 mL of diisopropyl ether and the whole mixture was stirred at room temperature for 6 h (or for other time periods indicated in Table 2). The reaction was stopped by filtering off the enzyme beads. The solvent was removed in vacuum to give a mixture of unreacted starting alcohol ***R*-2** (yield 3.07 g, 48% after purification) and propionate of ***S*-3** (yield 4.59 g, 50% after purification) which are easily separable by silica gel column chromatography (hexane, followed by 10% ethyl acetate in hexane, v/v). The reaction and chromatographic separation were followed by TLC in 10% ethyl acetate/hexane (v/v) (alcohol is more polar than propionate). The propionate ester ***S*-3** (1.84 g, 10 mmol) was then hydrolyzed to alcohol ***S*-2** in 10 mL of 0.1 M sodium phosphate buffer (pH 7) in the presence of 1.1 g of Novozym 435 lipase; 2.5 mL of acetone was used as a co-solvent and reaction was stirred at room temp. for 48 h. The reaction was stopped by filtering off the enzyme beads. Acetone was removed in vacuum, reaction mixture was extracted with ethyl ether (three times), the combined ether extracts were dried over $MgSO_4$ and then evaporated to give 1.15 g of ***S*-2** (9 mmol, 95% yield). The product was used for next reaction without further purification because only a trace of unreacted ester was detected by TLC.

Cross-metathesis synthesis of (*E*)-4-hydroxynon-2-enal. General Procedure. Reaction between oct-1-en-3-ol ***rac*-2** (640 mg, 5 mmol, 1 equiv.) and acrolein **4** (835 mg, 15 mmol, 3 equiv.) was carried out under argon in 25 mL of dry dichloromethane for 3 h in the presence of 2nd generation Grubbs catalyst (42.5 mg, 0.05 mmol, 0.01 equiv.) at room temp. The solvent was evaporated and reaction mixture was purified by silica gel chromatography (hexane/ethyl acetate, 4:1, v/v) to give HNE (545 mg, 70% isolation yield). The purified compound moved as a single spot on TLC in 30% ethyl acetate-hexane (v/v).

^{1}H NMR (CD_3OD) δ 0.97 (t, J=6.7, 3H), 1.37–1.44 (m, 4H), 1.47–1.70 (m, 4H), 4.28 (d, J=5.3, 1H), 4.38–4.46 (m, 1H), 6.30 (ddd, J=1.7, 8.0 and 15.6, 1H), 7.05 (dd, J=4.3, 15.6, 1H), 9.6 (d, J=8.0, 1H); ^{13}C NMR (CD_3OD) δ 14.4, 23.6, 26.1, 32.9, 37.3, 71.6, 131.3, 162.5, 195.8.

Essentially the same procedures were applied for the synthesis of ***R*-1** (α_D^{19} = –48.5°, CH_2Cl_2, c = 0.50, 97.5% ee) and ***S*-1** (α_D^{19} = +48.9°, CH_2Cl_2, c = 0.50, 98.4% ee) obtained in similar yields. The measured optical rotations are in conformity to those reported (***R*-1**: α_D^{25} = –46°, $CHCl_3$, c = 0.45; ***S*-1**: α_D^{25}= +48°, $CHCl_3$, c = 0.69 (de Montarby *et al.*, 1988)). NMR spectra were identical with the spectra of ***rac*-1**.

RESULTS AND DISCUSSION

The cross-metathesis reaction between oct-1-en-3-ol ***rac*-2** and acrolein **4** in the presence of 2nd generation Grubbs catalyst appears to be a very simple and efficient method for preparation of (*E*)-4-hydroxynon-2-enal (HNE, ***rac*-1**). In contrast to the previously used multistep procedures requiring strictly anhydrous conditions (Esterbauer & Wegner, 1967; Gree *et al.*, 1986; Bringmann *et al.*, 1994b; Chandra & Srivastava, 1997; Kurangi *et al.*, 2006), the one step procedure presented here does not need anhydrous conditions (the preparation of acrolein used here contains 10% water) and gives HNE with 70% yield after simple silica gel column chromatography. The method was applied for the synthesis of racemic HNE and without any changes also for the synthesis of its both enantiomers (***R*-1** and ***S*-1**), using as a starting material enantiomers of 1-octen-3-ol, ***R*-2** and ***S*-2**, respectively. The HPLC profiles of synthesized ***R*-1** and ***S*-1** are presented in Fig. 1. During completion of this study a paper appeared describing the synthesis of HNE and related compounds *via* a cross-metathesis reaction, but the authors limited their studies to the synthesis of racemic compounds (Soulere *et al.*, 2007). The conditions were essentially the same as in our procedure with the exception that a different catalyst, 2nd

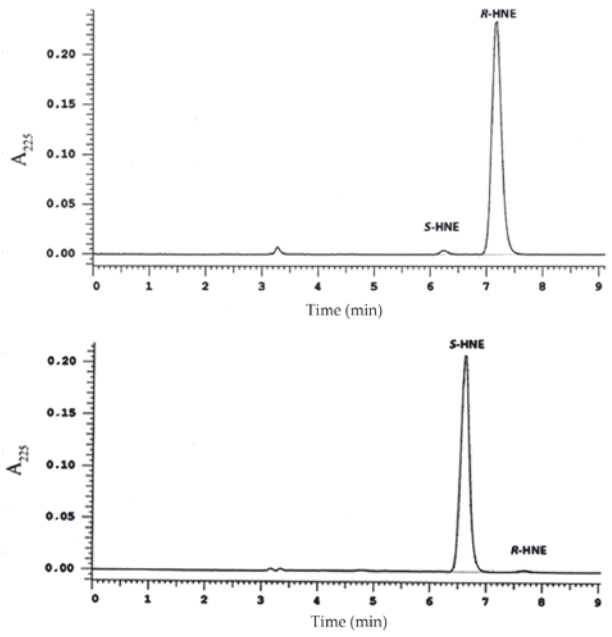


Figure 1. HPLC analysis of synthesized HNE enantiomers.
Upper panel: *R*-HNE, lower panel: *S*-HNE.

generation Hoveyda-Grubbs, was used. The yields of cross-metathesis reactions products (50–85%) were comparable to the yields obtained with our method (70%).

An essential step in the synthesis of enantiomers of HNE was the preparation of starting compounds, pure enantiomers of oct-1-en-3-ol, ***R*-2** and ***S*-2**. For this purpose we used lipase-catalyzed enantioselective acylation of ***rac*-2**. We decided to accomplish the enzymatic resolution of enantiomers of octenol rather than of enantiomers of HNE because of the reported instability of 4-hydroxyalkenals under conditions of lipase-catalyzed deacylation (Bringmann *et al.*, 1994b). Lipase-catalyzed resolution of racemate is well recognized as a useful procedure for obtaining chiral blocks for organic synthesis. A *Pseudomonas fluorescens* lipase was successfully used for kinetic resolution of several 4-(*R*)- and 4-(*S*)-hydroxyalk-2-enals, including enantiomers of HNE (Allevi *et al.*, 1993). The influence of lipase source, solvent and acyl donor nature, and time of reaction on enantioselective

Table 1. Novozym 435-catalyzed transesterification of oct-1-en-3-ol (*rac*-2) with various acyl donors

Acyl donor	% ee of remaining alcohol (reaction time)
Vinyl propionate	95 (6 h)
Vinyl acetate	92 (8 h)
Vinyl crotonate	50 (48 h)

acylation of several secondary allylic alcohols was studied recently (Fujii *et al.*, 2006; Chojnacka *et al.*, 2007). Based on the results of those studies we decided to use for kinetic resolution of racemic oct-1-en-3-ol the Novozym 435 preparation of lipase B from *Candida antarctica* and diisopropyl ether as solvent. Since Novozym 435 is a polymer-supported lipase, the enzyme could be easily recovered and recycled. The use of vinyl crotonate, an acyl donor reportedly giving a high enantioselectivity in acylation of oct-1-en-3-ol (Fujii *et al.*, 2006), surprisingly gave very low and unacceptable enantioselectivity (50% ee of remaining alcohol). Therefore, we examined other donors, vinyl propionate and vinyl acetate, and found that the former one assures satisfactory enantioselectivity of acylation (Table 1). Similar results with vinyl propionate as the donor in *Candida antarctica* lipase-mediated acylation of hept-1-en-3-ol were reported (Chojnacka *et al.*, 2007).

The impact of the time of reaction on its enantioselectivity and the yield of products in Novozym 435-mediated propionylation of ***rac*-2** is presented in Table 2. According to expectation shorter reaction favors higher optical purity of esterified octenol ***S*-3** (>99.9% ee after 5 h) whereas longer time favors higher optical purity of remaining octenol ***R*-2** (99.5% ee after 12 h). Novozym 435-catalyzed hydrolysis of octenol propionate ***S*-3** in buffered water solution gives octenol ***S*-2**. Under conditions of cross-metathesis some racemization occurs which is manifested by lowering of the ee value by 1.5–2%.

The chemoenzymatic synthesis of pure ***R*** and ***S*** enantiomers of (*E*)-4-hydroxynon-2-enal reported here is simple, efficient and easy to carry out even in laboratories not well equipped for advanced organic syntheses. The ready access to enantiomers of HNE

Table 2. Optical and chemical yields of lipase mediated kinetic resolution of oct-1-en-3-ol (*rac*-2) using vinyl propionate as the acyl donor

Reaction time (h)	% ee of remaining *R*-octenol[a] (yield)[b]	% ee of *S*-octenol propionate[c] (yield)[b]
5	90 (52%)	>99.9 (40%)
6	95 (48%)	98 (45 %)
12	99.5 (42%)	96 (46%)

[a]determined by GC analysis on chiral column; retention times for trimethylsilylated ***S*-2** and ***R*-2** are 20.6 min and 21.1 min, respectively; [b]determined after silica gel column purification; [c]determined by GC analysis on chiral column after Novozym 435-catalyzed hydrolysis to *S*-octenol

will facilitate studies of a variety of their chemical and biological interactions.

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