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Analysis and Sensory Evaluation of the Stereoisomers of a Homologous Series (C5-C10) of 4-Mercapto-2-alkanols

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Abstract

A homologous series of 4-mercapto-2-alkanols (C5-C10) was used to investigate the impact of the stereochemistry on the sensory properties of a class of naturally occurring polyfunctional thiols having a 1,3-oxygen-sulfur functionality. Stereoisomers were obtained via syntheses of racemic mixtures and subsequent lipase-catalyzed kinetic resolutions. Analytical separations of the stereoisomers were achieved by capillary gas chromatography (GC) using chiral stationary phases. The absolute configurations were assigned via NMR analysis. Sensory evaluations by means of GC/Olfactometry revealed odor threshold minima for the medium-chain homologs (C7-C9) of the 4-mercapto-2-alkanol stereoisomers. Except for the C5 homolog, the lowest odor thresholds were determined for the (2*R*,4*R*)-configured stereoisomers. The variability in odor qualities was mainly determined by the chain length. None of the 4-mercapto-2-alkanol stereoisomers showed consistent odor qualities for all homologs.

Keywords

4-Mercapto-2-alkanols; polyfunctional thiols; absolute configuration; odor threshold; odor quality

Introduction

Polyfunctional thiols are known as one of the classes of sulfur-containing volatiles playing important roles for the aroma of various foods.[1-6] They are characterized by low odor thresholds and pronounced odor qualities.[4,7] Fruity, tropical and vegetable odor notes have been particularly associated with numerous polyfunctional thiols possessing a 1,3-oxygen-sulfur function.[7,8] Compounds fulfilling this essential structural feature of the so-called 'tropical olfactophore' are for example the fruity smelling 3-mercapto-1-pentanol, 3-mercapto-4-methyl-1-pentanol and its acetate identified in hop[9] and beer[10] as well as the broth-like, sweaty and leek-like smelling 3-mercapto-2-methyl-1-pentanol isolated from raw onions.[11,12] As known for other chiral aroma compounds, the stereochemistry also has been shown to play a role in the olfactory perception of polyfunctional sulfur-containing volatiles.[13] For example, different odor qualities have been described for the esters of 3-(methylthio)-1-hexanol identified in yellow passion fruits.[14,15] Enantiodifferentiation in terms of odor intensity has been recognized for 3-mercapto-2-methyl-1-pentanol; the odor thresholds of the stereoisomers differed by a factor up to 1000.[12]

The importance of the stereochemistry for the sensory properties has also been demonstrated for a homologous series (chain lengths C5-C10) of 4-mercapto-2-alkanones[16-18], a class of naturally occurring *β*-mercaptoketones.[19-21] For the corresponding 4-mercapto-2-alkanols, it was shown that the odor thresholds depend on the chain length and that there are differences in the sensory properties of the diastereomeric pairs.[22,23] However, data demonstrating differences between enantiomers have been limited to the respective C7-homolog[24,25] identified in cooked red bell pepper[21]. The analysis of 4-mercapto-2-heptanol and its acetyl derivatives via GC/O demonstrated that the odor properties of the stereoisomers were not only

affected by acetylation but also by the configurations of the two asymmetric centers.[24,25]

The aim of the present investigation was to evaluate the sensory properties of the stereoisomers of the complete homologous series (C5-C10) of 4-mercapto-2-alkanols. To this end, the first part of the study was devoted to the synthesis of the stereoisomers, their separation via GC using chiral stationary phases and the assignment of their absolute configurations. Subsequently, odor qualities and odor thresholds of the stereoisomers were determined via GC/O.

Materials and Methods

Chemicals. 3-Hepten-2-one and (*S*)-(+)-2-methoxy-2-(1-naphthyl)propionic acid ((*S*)-MαNP) were obtained from TCI Europe (Zwijndrecht, Belgium). 3-Octen-2-one was provided by Alfa Aesar (Karlsruhe, Germany) and 3-decen-2-one by SAFC (Buchs, Switzerland). 3-Penten-2-one, 3-nonen-2-one, (*E*)-2-decenal (≥ 95%), thioacetic acid, lithium aluminum hydride, lipases from porcine pancreas (Type II, PPL, lot numbers: 020M1589V and SLBL 2143V) and *Candida antarctica* (B lipase, adsorbed on a macroporous acrylic resin, CAL-B, lot number: SLBG4222V), deuterochloroform ($CDCl_3$), 4-(dimethylamino)pyridine (DMAP), *N*,*N'*-dicyclohexylcarbodiimide (DCC) and Celite® 503 were purchased from Sigma-Aldrich (Steinheim, Germany). 3-Hexen-2-one was synthesized by Knoevenagel reaction of 3-oxobutanoic acid and propanal as previously reported.[18]

Syntheses. A homologous series (chain lengths C5–C10) of 4-acetylthio-2-alkanones **7-12** were synthesized by Michael-type addition of thioacetic acid to the corresponding 3-alken-2-ones according to previously described procedures.[17,18] 4-Mercapto-2-alkanols **1-6** were prepared by adding the synthesized 4-acetylthio-2-alkanones (6.63 mmol for **1**, 5.74 mmol for **2**, 10.60 mmol for **3**, 3.11 mmol for **4**,

6.93 mmol for **5** and 12.93 mmol for **6**) dissolved in 20 mL of dry tetrahydrofuran (THF) slowly to a suspension of lithium aluminum hydride ($LiAlH_4$: 29.84 mmol for **1**, 25.83 mmol for **2**, 47.70 mmol for **3**, 14.01 mmol for **4**, 31.19 mmol for **5** and 58.19 mmol for **6**) in 50 mL of dry THF under argon atmosphere at 0 °C. The reaction mixture was stirred at RT overnight. After being cooled to 0 °C, distilled water was carefully added and the aqueous phase was adjusted to pH 2 using hydrochloric acid (5%) and extracted three times with dichloromethane. The organic phase was dried with anhydrous sodium sulfate and evaporated under reduced pressure to give the following crude products: 4-mercapto-2-pentanol, **1**: 0.41 g (3.41 mmol, mol yield: 51%, purity (GC): 76%, diastereomeric ratio, dr (%) = 37:63, purity after column chromatography on silica gel (*n*-hexane/Et_2O, 3:2, v/v): 96% (by GC)). 4-Mercapto-2-hexanol, **2**: 0.83 g (6.17 mmol, mol yield: 108%, purity: 84% by GC, dr (%) = 42:58). 4-Mercapto-2-heptanol, **3***:* 1.54 g (10.40 mmol, mol yield: 97%, purity: 96% by GC, dr (%) = 39:61). 4-Mercapto-2-octanol, **4**: 0.49 g (3.02 mmol, mol *y*ield: 97%, purity: 89% by GC, dr (%) = 47:53, purity after column chromatography on silica gel (*n*-hexane/Et_2O, 5:3, v/v): 96% (by GC)). 4-Mercapto-2-nonanol, **5**: 1.25 g (7.09 mmol, mol yield: 102%, purity: 92% by GC, dr (%) = 45:55). 4-Mercapto-2-decanol, **6**: 1.77 g (9.27 mmol, mol yield: 72%, purity: 91% by GC, dr (%) = 43:57). Chromatographic, mass spectrometric and NMR data were in agreement with those previously reported.[7,21,22,24,26]

Enzyme-catalyzed kinetic resolution of racemic 4-acetylthio-2-alkanones. In analogy to the method described by Wakabayashi et al.[17,18], 5 mmol of synthesized 4-acetylthio-2-alkanone (0.80 g of **7**, 0.87 g of **8**, 0.94 g of **9**, 1.01 g of **10**, 1.08 g of **11** and 1.15 g of **12**, respectively) were dissolved in 50 mL of potassium phosphate buffer (50 mM, pH 7.4). After adding 1.0 g of the enzyme preparation (CAL-B resin for **7** and PPL for **8-12**), the mixture was stirred at RT for a defined time (4 h for **7**, 6 h

for **8**, 7.5 h for **9**, 1 h for **10** and **11**, and 2 h for **12**). The enzyme was filtered off and the aqueous phase was extracted four times with 50 mL Et_2O. The organic phase was dried with anhydrous sodium sulfate and the solvent was removed under reduced pressure using an aspirator at 40 °C. An aliquot of the reaction mixture (1 µL/mL in Et_2O) was subjected to GC analysis using heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyl dimethylsilyl)-*β*-cyclodextrin as chiral stationary phase. The following conversion rates of substrates (56% for **7**, 75% for **8**, 79% for **9**, 43% for **10**, 38% for **11** and 63% for **12**) as well as the enantiomeric ratios (er) of products (4-mercapto-2-alkanones (C5-C10, **13-18**), er (%) = 12:88 for **13**, 66:34 for **14**, 38:62 for **15**, 82:18 for **16**, 86:14 for **17** and 72:28 for **18**) and of the remaining substrates (4-acetylthio-2-alkanones, er (%) = 99.6:0.4 for **7**, 97:3 for **8**, 98:2 for **9**, 74:26 for **10** and 72:28 for **11**) were calculated.[27]

The separation of the reaction mixture was carried out by column chromatography on silica gel using a mixture of *n*-hexane and Et_2O (3:1 for **7** and **13**, 4:1 for **8** and **14** as well as for **9** and **15**, 5:1 for **10** and **16** as well as for **11** and **17**, and 6:1 for **12** and **18**, v/v). In analogy to the method described for the C7 homolog[24,25], the separated 4-acetylthio-2-alkanones of **10** and **11** were used as starting substances to synthesize the (4*R*)-configured diastereomers of 4-mercapto-2-octanol **4** and 4-mercapto-2-nonanol **5** employing the same procedure as described above for the synthesis of 4-mercapto-2-alkanols. The reduction of the remaining substrate of the C5 homolog **7** resulted in the formation of the (4*S*)-configured diastereomers of 4-mercapto-2-pentanol **1** since CAL-B resin was used for the enzyme-catalyzed hydrolysis instead of PPL.[28] In case of the C6 and C10 homolog, the obtained products 4-mercapto-2-hexanone **14** and 4-mercapto-2-decanone **18** were reduced to the (4*S*)-configured diastereomers of the corresponding mercaptoalkanols **2** and **6** using $LiAlH_4$.

Preparation of (*S*)-MαNP thioesters of (*R*)- and (*S*)-4-mercapto-2-octanone. *(R)-4-mercapto-2-octanone, (R)-**16***. In accordance with the previously described enzyme-catalyzed kinetic resolution[17], 37.5 mmol of racemic 4-acetylthio-2-octanone **10** were dissolved in potassium phosphate buffer and 7.5 g of PPL were added. After stirring for 3 h at RT, the enzyme was filtered off using Celite® and the aqueous phase was extracted with Et_2O (4x25 mL). The organic phase was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. (*R*)-4-acetylthio-2-octanone **10** was obtained after column chromatography on silica gel using a mixture of *n*-hexane and Et_2O (7:1, v/v): mol yield: 1.7%, purity (GC): 97.8%, er (%) = 91:9. (*R*)-4-mercapto-2-octanone (*R*)-**16** was obtained via transesterification[28] starting with 0.5 mmol of (*R*)-**10** followed by purification (column chromatography on silica gel using a mixture of *n*-hexane and Et_2O (7:1, v/v)): mol yield: 0.7%, purity (GC): 97.5%, er (%) = 76:14.

*(S)-4-mercapto-2-octanone, (S)-**16***. For (*S*)-**16**, 12.5 mmol of racemic 4-acetylthio-2-octanone **10** were dissolved in potassium phosphate buffer and 5 g of PPL were added. After stirring for 1 h at RT, the enzyme was filtered off using Celite® and the aqueous phase was extracted with Et_2O (4x25 mL). The organic phase was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. (*S*)-**16** was obtained after column chromatography on silica gel using a mixture of *n*-hexane and Et_2O (7:1, v/v): mol yield: 6.4%, purity (GC): 97.1%, er (%) = 88:12.

*(S)-MαNP thioesters of (R)- and (S)-**16***. The diastereomers were prepared according to Kiske et al.[28] and separated by semi-preparative HPLC using a Dionex HPLC system (UltiMate 3000 series, Dionex, Germering, Germany) equipped with a 3100 wavelength detector set at 254 nm using a 250 x 8 mm i.d. Nucleosil 50-5 column (CS Chromatography, Langerwehe, Germany). Isocratic elution was performed at

30 °C with a mixture of *n*-hexane/isopropanol 96:4 (v/v) as the eluent and a flow rate of 2 mL/min. (*S*)-M*α*NP thioesters of (*R*)-**16**: 10.0 mg, 31.0%; (*S*)-MαNP thioesters of (*S*)-**16**: 5.5 mg, 17.2%.

Capillary Gas Chromatography (GC-FID). The column used was a 30 m x 0.25 mm i.d., 0.5 µm; DB-Wax column (J&W Scientific, Agilent Technologies, Waldbronn, Germany, column 1) installed into an HP5890 A gas chromatograph (Hewlett-Packard, Heilbronn, Germany) equipped with a split/splitless injector (215 °C, split ratio of 1:7) and an FID (350 °C); temperature program: from 40 °C (5 min hold) to 240 °C (30 min hold) at 4 °C/min; carrier gas: hydrogen at a constant pressure of 135 kPa.

A 30 m x 0.25 mm i.d.,1.0 µm; DB-1 column (J&W Scientific, Agilent Technologies, column 2) was installed into a CE 5160 instrument (Carlo Erba Instruments, Hofheim, Germany) equipped with a split/splitless injector (200 °C, split ratio of 1:10) and an FID (260 °C); temperature program: from 60 °C (5 min hold) to 250 °C (5 min hold) at 5 °C/min; carrier gas: hydrogen at a constant pressure of 74 kPa. Linear retention indices (LRI) were determined according to van den Dool and Kratz[29], using C_8-C_{40} *n*-alkane standard solutions (Sigma-Aldrich).

Enantioselective analyses of 4-acetylthio-2-alkanones **7-12** and 4-mercapto-2-alkanones **13-18** were performed on a 30 m x 0.25 mm i.d., 0.25 µm; CycloSil-B column (J&W Scientific, Agilent Technologies, column 3) installed into an HP5890 Series II gas chromatograph (Hewlett-Packard) equipped with a split/splitless injector (200 °C, split ratio of 1:5) and an FID (350 °C); temperature program: from 75 °C (0 min hold) to 180 °C (5 min hold) at 1 °C/min; carrier gas: hydrogen at a constant pressure of 176 kPa.

GC separation of the stereoisomers of **1-6** was performed on two in-house prepared 30 m x 0.25 mm i.d. fused silica capillary columns coated with 50% heptakis(2,3-di-

O-acetyl-6-*O*-*tert*-butyl dimethylsilyl)-*β*-cyclodextrin (column 4) and 28% heptakis(2,3-di-*O*-methoxymethyl-6-*O*-*tert*-butyl dimethylsilyl)-*β*-cyclodextrin in OV1701-vi (column 5), respectively. The syntheses of the cyclodextrins and the column preparations were carried out as previously described.[30,31] Columns 4 and 5 were installed into a CE 5160 instrument (Carlo Erba Instruments) equipped with a split/splitless injector (200 °C, split ratio of 1:10) and an FID (260 °C); hydrogen at constant pressure of 75 kPa and 110 kPa, respectively, was used as carrier gas. A 30 m x 0.25 mm i.d., 0.25 µm; Inert Cap™ Chiramix column (GL Science, Tokyo, Japan, column 6) installed into an HP5890 Series II gas chromatograph (Hewlett-Packard) equipped with a split/splitless injector (230 °C, split ratio of 1:30) and an FID (250 °C); carrier gas: hydrogen at a constant pressure of 110 kPa was also used to separate the stereoisomers of **1-6**.

Multidimensional Gas Chromatography (MDGC). The instrumentation consisted of two coupled GC 8000 (Carlo Erba Instruments). A Moving Column Stream Switching device (MCSS) and a 1 m x 0.25 mm i.d. deactivated fused silica transfer capillary were used to transfer the diastereomers of **1-6** from the pre-column (GC 1) onto the main column (GC 2). A 60 m x 0.32 mm i.d., 0.25 µm; DB-Wax column (J&W Scientific, Agilent Technologies) was installed into GC 1, equipped with a split/splitless injector (215 °C, split ratio of 1:5) and an FID (230 °C); temperature program: from 40 °C (5 min hold) to 240 °C (25 min hold) at 4 °C/min; carrier gas: hydrogen at a constant pressure of 165 kPa. The columns 4-6 were installed as main columns in GC 2 equipped with an FID (230 °C); outlet pressure was 98 kPa. Data were processed via Chrom-Card software (Thermo Fisher Scientific, Dreieich, Germany).

Capillary Gas Chromatography-Olfactometry (GC/O). Sensory analyses of the stereoisomers of **1-6** were performed on columns 4-6. Columns 4 and 5 were

installed into an HP5890 A Series II gas chromatograph (Hewlett-Packard) equipped with a cold-on-column injector (40 °C), a heated sniffing port (200 °C) and an FID (250 °C); carrier gas: hydrogen at a constant pressure of 75 kPa. Column 6 was installed into a Fractovap 4200 (Carlo Erba Instruments), equipped with a split/splitless injector (220 °C, split ratio of 1:10), a sniffing port (230 °C) and an FID (230 °C); carrier gas: hydrogen at a constant pressure of 98 kPa; make-up gas: nitrogen at 50 kPa. For both GC/O systems, the effluent was split 1:1 via a press-fit Y-splitter and 30 cm x 0.25 mm i.d. deactivated fused silica capillaries (BGB Analytik AG, Rheinfelden, Germany) among sniffing port and FID.

The sensory analyses of the stereoisomers of **1-6** were performed by three panelists (females; 20-30 years old). Panelist 1 and 2 had no prior experience with GC/O assessments, whereas panelist 3 had extensive training for more than three years. Panelist 1 determined the odor properties of the stereoisomers of **1**, **3a/a'**, **4**, **5a/a'** and **6** using column 4. The sensory evaluation of the *syn*-enantiomeric pair of **3b/b'** was performed using octakis(2,3-di-*O*-*n*-butyryl-6-O-*tert*-butyl dimethylsilyl)-*γ*-CD as chiral stationary phase.[24,25] Panelist 2 used column 6 for the determination of odor thresholds and odor qualities of the stereoisomers of **1**, **2**, and **4-6**. Panelist 3 determined the odor properties of the stereoisomers of **2** and **3** using column 4 and 5.[24,25] Odor thresholds in air were determined following the procedure described by Ullrich and Grosch using (*E*)-2-decenal with the reported odor threshold of 2.7 ng/L in air as internal standard.[32,33] Known amounts of the internal standard and of the 4-mercapto-2-alkanols were dissolved in Et_2O and diluted stepwise by a factor of 1:2 (v/v). Stock solutions were freshly prepared for each panelist. The aliquots were analyzed by GC/O until no odor was perceivable. The panelists considered a concentration level only as odor threshold if it was the lowest dilution step at which the odor was consistently perceived in three consecutive GC/O-runs.[24] Flavor dilution

factors (FD) of the internal standard and of the target compounds were obtained by aroma extract dilution analysis (AEDA).[34] The odor impressions obtained during the AEDA were collected and those with injection volumes corresponding to approximately 1.5 ng for each stereoisomer at the sniffing port were used as descriptors of the odor qualities.

Capillary Gas Chromatography - Mass Spectrometry (GC-MS).

A 30 m x 0.25 mm i.d., 0.5 µm; DB-Waxetr fused silica capillary column (J&W Scientific, Agilent Technologies) installed into a GC 8000^{TOP} gas chromatograph (CE Instruments, Hindley Green, United Kingdom) directly coupled to a Fisons MD8000^{TOP} mass spectrometer (Fisons Instruments, Manchester, UK) was used for compound identifications. The temperature was programmed from 40 °C (5 min hold) to 240 °C (25 min hold) at 4 °C/min. A split/splitless injector (220 °C, split ratio 1:50) was used and the carrier gas was helium at a constant inlet pressure of 75 kPa. The mass spectra in the electron impact mode (EI) were measured at 70 eV in a scan range from *m/z* 30 - 250. The source temperature was 200 °C and the interface temperature 240 °C. Data acquisition was done via Xcalibur software, version 1.4 (Thermo Fisher Scientific).

NMR Spectroscopy. ^{1}H NMR and ^{13}C NMR spectra were recorded at 500 MHz and 126 MHz, respectively with Avance 500 spectrometers (Bruker, Billerica, MA, USA). ^{1}H-detected experiments were done with an inverse ^{1}H/^{13}C probehead, direct ^{13}C-measurements were performed with a QNP ^{13}C/^{31}P/^{29}Si/^{19}F/^{1}H cryoprobe. The experiments were done in full automation using standard parameter sets of the TOPSPIN 3.0 software package (Bruker). ^{13}C NMR spectra were recorded in proton-decoupled mode. The compounds were dissolved in deuterated chloroform.

The spectra were recorded at 27 °C. All signals were assigned by proton-proton and proton-carbon correlation experiments (COSY, HSQC and HMBC). Data processing

was typically done with the MestreNova software (Mestrelab Research, Santiago de Compostela, Spain).

Optical Rotations. Optical rotations were measured on a Polartronic-E polarimeter (Schmidt & Haensch, Berlin, Germany) fitted with a measuring cell (path length 1 dm) and a sodium lamp (wavelength 589 nm). Samples were diluted in ethanol and the measurements were performed at a temperature of 21 °C. **(*S*)-16**: $[\alpha]_D$ +17.0, concentration (c): 2.07 g/100 mL, GC purity (p): 97.1%, enantiomeric excess (ee): 75.9%; **(*R*)-10**: $[\alpha]_D$ +15.6, c: 0.96, p: 97.8, ee: 82.8; **(*R*)-16**: $[\alpha]_D$ -16.9, c: 1.16, p: 97.5, ee: 71.5.

Results and Discussion. *GC-separation of the stereoisomers of 4-mercapto-2-alkanols*. 4-Mercapto-2-alkanols **1-6** (Table 1) were synthesized by Michael-type addition of thioacetic acid to the corresponding 3-alken-2-ones and subsequent reduction of the obtained 4-acetylthio-2-alkanones **7-12** with lithium aluminum hydride.[16-18,21,24] The GC separations of the diastereomeric pairs of the homologous series (chain lengths C5-C10) are shown in Figure 1A. For separation of the four stereoisomers of each homolog several chiral stationary phases were tested. Figure 1B shows the separation obtained by using heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyl dimethylsilyl)-*β*-cyclodextrin as chiral stationary phase. The use of this cyclodextrin derivative was suitable for the separation of the four stereoisomers, except for chain length C6. For this homolog **2**, only an incomplete separation could be achieved (Figure 1C), and there were coelutions with stereoisomers of **1**. Therefore, heptakis(2,3-di-*O*-methoxymethyl-6-*O*-*tert*-butyl dimethylsilyl)-*β*-cyclodextrin was employed; the use of this chiral stationary phase enabled the separation of all four stereoisomers of **2** (Figure 1D).

Determination of the absolute configurations and order of elution of the stereoisomers of 4-mercapto-2-alkanols. The absolute configurations and the order of elution of the stereoisomers of 4-mercapto-2-alkanols with chain lengths C5 **1**, C6 **2**, and C8-C10 **4-6** were determined according to the principles previously reported for the assignment of the GC-elution order of the stereoisomers of the C7 homolog 4-mercapto-2-heptanol **3**.[24,25] This involved (i) a comparison of the NMR data of the LC-separated diastereomers of **3** with those reported for *anti*-(2*S*,4*S*)-4-mercapto-2-heptanol and (ii) the formation of enantio-enriched diastereomers of **3** via lipase-catalyzed kinetic resolution of 4-acetylthio-2-heptanone **9** and subsequent reduction of the remaining substrate.[24-26] Recently, the configurations of 4-mercapto-2-pentanone **13** and 4-mercapto-2-heptanone **15** have been re-investigated by vibrational circular dichroism (VCD) and ^{1}H NMR analyses of (*R*)-hydratropic acid thioesters and 2-methoxy-2-phenylacetic acid thioesters.[28] The absolute configurations of the enantiomers of **13** and **15** determined with these approaches were not in agreement with those deduced via the ^{1}H NMR anisotropy method using (*S*)-2-methoxy-2-(1-naphthyl)propionic acid (MαNP) as chiral reagent.[16-18] As a consequence, the published assignments of the absolute configurations of the stereoisomers of **3** had to be revised since their assignments were based on the absolute configuration of 4-mercapto-2-heptanone **15** determined by Wakabayashi et al.[17,24,25] As a result, the order of elution of the diastereomers of **3** were assigned as *anti*- before *syn*- on a DB-Wax column (Figure 1A) and as (2*S*,4*S*)-**3a** before (2*R*,4*R*)-**3a'** and (2*R*,4*S*)-**3b** before (2*S*,4*R*)-**3b'** for the stereoisomers using heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyl dimethylsilyl)-*β*-cyclodextrin as chiral stationary phase (Figure 1B).

The analysis of the diastereomeric mixture of **3** via ^{1}H NMR spectroscopy enabled the determination of the diastereomeric ratios (H-2 and H-4) at the stereogenic

centers (Figure 2A). As outlined in Table 2, the ratios measured via integral analysis of the appropriate pairs of protons (*syn*/*anti*) were nearly identical to the ratio of *anti*- and *syn*-diastereomers determined by GC analysis (Figure 1A). Based on this result, the comparison of diastereomeric ratios of synthesized 4-mercapto-2-alkanols obtained via capillary gas chromatography and ^{1}H NMR spectroscopy was used to assign the *anti*-/*syn*-configurations for the complete homologous series. For the synthesized mercaptoalkanols (chain lengths C5 **1**, C6 **2**, and C8-C10 **4-6**) there were also good agreements between the GC and the NMR data, as shown in Table 2. The diastereomeric ratios determined at the H-2 and H-4 positions of the mercaptoalkanols with the shortest (C5, **1**) and the longest chain lengths (C10, **6**) are exemplarily shown in Figure 2B and 2C. As a result, the order of elution of the diastereomers of the investigated 4-mercapto-2-alkanols was consistently assigned as *anti* before *syn* (Figure 1A).

The final step was the assignment of the order of elution of the two *anti*-configured [(2*S*,4*S*) and (2*R*,4*R*)] and the two *syn*-configured [(2*S*,4*R*) and (2*R*,4*S*)] enantiomers. To this end, a procedure based on enzyme-catalyzed kinetic resolution was used to obtain enantiomerically enriched thiols. The used approach is exemplarily shown for 4-mercapto-2-pentanol **1** in Figure 3. The first step was the CAL-B-mediated hydrolysis of the thioester bond of racemic 4-acetylthio-2-pentanone **7** which resulted in the formation of the (*R*)-configured thiol **13** as product and (4*S*)-configured **7** as remaining substrate (Figure 3, step A). The absolute configurations were assigned according to Kiske et al.[28] GC analysis of the enantiomers of **7** and **13** was performed using heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyl dimethylsilyl)-*β*-cyclodextrin as chiral stationary phase (Figure 3, step A). In the next step, the reaction mixture was separated by column chromatography and the nearly enantiomerically pure 4-acetylthio-2-pentanone (*S*)-**7** was subjected to reduction with

$LiAlH_4$ to form the stereoisomers of 4-mercapto-2-pentanol **1** with the corresponding excess of the (4*S*)-configured diastereomers (Figure 3, step B). GC analysis using heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyl dimethylsilyl)-*β*-cyclodextrin as chiral stationary phase demonstrated that the (4*S*)-configured diastereomers coeluted with the first peaks of the pairs of stereoisomers obtained for the synthesized 4-mercapto-2-pentanol **1**. The assignment of the *anti*- and *syn*-diastereomers achieved in the first step in combination with the assignment of the enantiomeric pairs to their corresponding diastereomers via MDGC enabled the determination of the absolute configurations and GC orders of elution of the four stereoisomers of 4-mercapto-2-pentanol (Figure 3; step C) as (2*S*,4*S*)-**1a** before (2*R*,4*R*)-**1a'** and (2*R*,4*S*)-**1b** before (2*S*,4*R*)-**1b'**.

An analogous procedure was applied to assign the order of elution of the stereoisomers of 4-mercapto-2-octanol **4.** To this end, the (4*S*)- and (4*R*)-configured enantiomers of **16** were prepared via kinetic resolution using PPL in accordance with the previously described procedure.[28] The obtained enantiomerically enriched mercaptoalkanones were reacted with (*S*)-MαNP (Figure 4), purified with semi-preparative HPLC, and the diastereomers were analyzed by NMR spectroscopy (Table 3). Previous studies had demonstrated consistent ^{1}H NMR anisotropy effects for the complete homologous series of (*S*)-MαNP thioesters of 4-mercapto-2-alkanones of chain lengths C5 to C10.[17,18] Therefore, it was assumed that the recently revised sector rule for secondary thiols, verified via VCD and ^{1}H NMR analysis of diastereomeric thioesters formed with other auxiliary reagents for 4-mercapto-2-alkanones of chain lengths C5 and C7,[28] can be applied. The Δ*δ* values of H-1 and H-3 are positive (0.06 and 0.05, respectively) and are placed on the left side whereas the Δ*δ* values for H-5 – H-8 are negative (-0.02, -0.03, -0.03 and -0.04, respectively) and are placed on the right side. This results in (*R*)-configuration at the

C-4 position of the first eluting diastereomer and thus corresponds to the (*S*,*R*) diastereomeric MαNP thioester of **16** which elutes before the (*S*,*S*) diastereomer. Based on this result, PPL-mediated hydrolysis of racemic 4-acetylthio-2-octanone *rac*-**10** resulted in the formation of the (*S*)-configured thiol **16** as product and (4*R*)-configured **10** as remaining substrate. After column chromatography, (4*R*)-configured **10** was subjected to reduction with $LiAlH_4$ to form the (4*R*)-configured diastereomers of **4**. Comparing the order of elution of the (4*R*)-configured diastereomers of **4** to those of the enantiomeric pairs of *anti*- and *syn*-configured **4** via MDGC resulted in an order of elution of (2*S*,4*S*)-**4a** before (2*R*,4*R*)-**4a'** and (2*R*,4*S*)-**4b** before (2*S*,4*R*)-**4b'** using heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyl dimethylsilyl)-*β*-cyclodextrin as chiral stationary phase (Figure 1B).

In analogy, the absolute configurations of the stereoisomers of the remaining homologs **2**, **5** and **6** were assigned via PPL-mediated hydrolyses of 4-acetylthio-2-hexanone **8**, 4-acetylthio-2-nonanone **11** and 4-acetylthio-2-decanone **12**. The formed (*S*)-configured thiols (**14**, **17** and **18**) and the remaining (*R*)-configured substrates (**8**, **11** and **12**) were analyzed using heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyl dimethylsilyl)-*β*-cyclodextrin as chiral stationary phase.[16-18,28] After column chromatography, (4*R*)-configured **11** as well as (4*S*)-configured **14** and **18** were subjected to reduction with $LiAlH_4$ to form the corresponding enantiomerically enriched diastereomers of **2**, **5** and **6**. GC analyses of (4*R*)-configured **5**, (4*S*)-configured **6** and the respective racemic reference substances resulted in orders of elution of (2*S*,4*S*)-**a** before (2*R*,4*R*)-**a'** and (2*R*,4*S*)-**b** before (2*S*,4*R*)-**b'** using heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyl dimethylsilyl)-*β*-cyclodextrin as chiral stationary phase (Figure 1B). The same order of elution was assigned for the stereoisomers of the C6-homolog **2** separated on heptakis(2,3-di-*O*-methoxymethyl-6-*O*-*tert*-butyl dimethylsilyl)-*β*-cyclodextrin as stationary phase (Figure 1D).

An interesting phenomenon was observed when screening Chiramix®, a column coated with a mixture of the two chiral stationary phases heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-*β*-cyclodextrin and octakis(2,6-di-*O*-methyl-3-*O*-trifluoroacetyl)-*γ*-cyclodextrin as alternative chiral stationary phase (Figure 5).[35] There were changes in the order of elution of the stereoisomers of **1-6** depending on the chain lengths. For the long-chain homologs **4-6** the *anti*-configured (2*S*,4*S*)-**a** and (2*R*,4*R*)-**a'** stereoisomers consistently eluted before the corresponding *syn*-configured (2*S*,4*R*)-**b'** and (2*R*,4*S*)-**b** stereoisomers. The changed order of elution of the four stereoisomers of the short-chain homologs **1** and **2** as well as the coelution of the (4*R*)-configured diastereomers of the C7 homolog **3a'** and **3b'** appears to be due to an increasing shift of the (2*R*,4*R*)-configured stereoisomers **1-3a'** to later retention times with decreasing chain lengths.

Determination of odor thresholds. The odor thresholds of the stereoisomers of **1-6** were determined via GC/O (Table 4). In total three panelists participated; except for **5b** and **5b'**, each stereoisomer was evaluated by two assessors. Regarding the variability of the panelists, there were a few cases in which high differences between odor thresholds were observed, i.e. for **4a'** (factor: 60) and **5a'** (factor: 30) between panelists 1 and 2 as well as for **2b'** (factor: 30), **2a** (factor: 22) and **2b** (factor: 7) between panelists 2 and 3. However, for most of the stereoisomers the individual odor thresholds were either the same or differed up to a maximum of factors 3 to 5, corresponding to approximately two dilution steps in the course of the AEDA. Figure 6 illustrates the odor threshold curves for the four stereoisomers of 4-mercapto-2-alkanols **1-6** based on the geometric means calculated from the assessments by the respective panelists (except for **5b** and **5b',** for which only single sensory evaluations were available).

405 The data set revealed two effects: (i) For all stereoisomers odor threshold minima
406 were observed for the medium-chain homologs (C7-C9). This is in good agreement
407 with the recently reported odor thresholds for the racemic mixtures of a homologous
408 series of 4-mercapto-2-alkanols.[22] The individual curves for the diastereomers
409 [(2R,4S), (2S,4R)] and [(2S,4S), (2R,4R)] also fit very well to those reported for the
410 respective diastereomeric mixtures.[23] This good agreement with sensory data
411 generated in a different laboratory and by trained panelists[22,23] supports the reliability
412 of the data obtained in the present study, despite the limited number of (trained)
413 panelists and the observed individual differences. Threshold minima for medium-
414 chain representatives have been observed within homologous series of various
415 mercaptoalkanols[22] and for the enantiomers of the homologous series of 4-mercapto-
416 2-alkanones (minima at carbon chain lengths C8) and 4-acetylthio-2-alkanones
417 (minima at carbon chain lengths C7/C8).[18] (ii) Except for C5, the lowest odor
418 thresholds were determined for the (2*R*,4*R*)-configured stereoisomers. The visual
419 differences seen in Figure 6 were verified by calculating the ratios of the geometrical
420 means of the thresholds determined for the (2*R*,4*R*)-configured stereoisomers and
421 those of the second most intensive smelling stereoisomers. Particularly for the
422 homologs with chain lengths C8 and C9 these ratios were pronounced
423 (approximately 18).
424 Using the example of 4-mercapto-2-heptanol and its acetyl derivatives, it has been
425 shown that the odor thresholds were nearly independent from *S*- and/or *O*-acetylation
426 if the structural prerequisite of (2*R*)-configuration was fulfilled. [24,28] A high sensory
427 potency was also observed for the (4*R*)-configured isomers of short- and medium-
428 chain homologs (C5-C8) of 4-mercapto-2-alkanones (C5-C10) taking into account the
429 re-investigated absolute configurations.[18,28] These data indicate that the (*R*)-
430 configurations might play key roles for the thresholds of these ß-mercaptoalcohols

and *ß*-mercaptoketones. However, *S*-acetylation of 4-mercapto-2-alkanones (C5-C10) reduced the odor intensity, and the odor thresholds of the enantiomers were highly impacted by the chain length.

Determination of odor qualities. Odor qualities of the stereoisomers of **1-6** were also determined by panelists 1-3 via GC/O (Table 5). It is known that odor qualities may show pronounced concentration-dependent changes. Nevertheless, for the purpose of comparison the sensory assessments were performed at constant amounts of 1.5 ng at the sniffing port for each stereoisomer. Considering the variability of the dominant odor impressions (printed in bold type), it is obvious that none of the stereoisomers showed consistent odor qualities for all homologs. For example, the odor qualities of the (2*R*,4*R*)-configured stereoisomers (**1-6a'**) ranged from onion to plastic-solvent-like odor notes. The comparison of the odor descriptions for the different chain lengths showed that for the C5 homolog **1** similar odor qualities such as onion (**1a** and **1a'**) or sweat (**1b** and **1b'**) were detected for the enantiomeric pairs. Different odor notes such as fruity (**2a** and **3a**), onion (**2a'**, **3a'** and **3b**), savory (**2b** and **3b'**), and green/herb-like (**2b'**) were obtained for the isomers of the chain lengths C6 and C7. The odor descriptions of the stereoisomers of the chain lengths C8 to C10 changed towards unpleasant chemical notes such as burnt, plastic, solvent, or pungent. These data demonstrate that the chain length is the main factor determining the variability in odor qualities of stereoisomers of 4-mercapto-2-alkanols. A similar effect was reported for the odor properties of the diastereomers of 4-mercapto-2-alkanols (C5-C10).[22,23] GC/O analysis resulted in onion and meaty notes for the C5 homologs, a fruity-like odor reminiscent of grapefruit dominated the odor impressions of the C6-C9 homologs, and further elongation led to fatty and burnt odor notes. No significant differences in the odor qualities have been described for the diastereomeric pairs of the 4-mercapto-2-alkanols (C5-C10), except for the C6

457 homolog, having either a more meaty or fruity odor note.[22,23] The odor qualities of the
458 enantiomers of the corresponding 4-mercapto-2-alkanones (C5-C10) are highly
459 impacted by the stereochemistry.[16,18] GC/O analyses revealed fruity odor notes for
460 the (*R*)-enantiomers and more unpleasant notes such as catty and sulfury for the (*S*)-
461 enantiomers, taking into account the results of the reinvestigation of the absolute
462 configurations of *β*-mercaptoalkanones.[16,18,28] As expected for compounds
463 possessing a 1,3-oxygen-sulfur functionality, tropical, fruity, and vegetable odor notes
464 were obtained for stereoisomers of the investigated 4-mercapto-2-alkanols (Table 5).
465 However, specific notes such as meaty, savory, sweaty as well as chemical notes
466 were additionally perceived by the panelists (Table 5) and have also been reported in
467 literature to describe the odor qualities of 4-mercapto-2-alkanols.[7,21]
468 In conclusion, the GC separation and the assignment of the absolute configurations
469 of the stereoisomers for the homologous series of 4-mercapto-2-alkanols with chain
470 lengths from C5 to C10 have been achieved. GC/O analyses revealed that the odor
471 thresholds of the stereoisomers of 4-mercapto-2-alkanols were highly impacted by
472 the stereochemistry as the lowest odor thresholds were determined for the (2*R*,4*R*)-
473 configured stereoisomers, except for C5. In contrast, the odor qualities were mainly
474 influenced by the chain length. The data provide another example for the impact of
475 both chain length and stereochemistry on the sensory properties of members of
476 homologous series of aroma compounds.
477

478 **ASSOCIATED CONTENT**

479 Supporting information: Experimental details of the syntheses of 4-acetylthio-2-
480 alkanones (C5-C10). MS and NMR data of the synthesized 4-mercapto-2-alkanols
481 (C5-C10). Temperature programs used for the GC separations of the 4-mercapto-2-
482 alkanol stereoisomers. GC linear retention indices of the diastereomers of 4-

mercapto-2-alkanols on DB-Wax and DB-1. Separation factors α and resolutions R_s of the enantiomeric pairs of 4-mercapto-2-alkanols. Geometric means of the individual odor thresholds of the stereoisomers of 4-mercapto-2-alkanols determined by the panelists via GC/O.

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Notes

The authors declare no competing financial interest.

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Figure Captions

Figure 1.

(A) Capillary gas chromatographic separation of the diastereomers of 4-mercapto-2-alkanols **1-6** on a DB-Wax column; (B) separation of the stereoisomers of 4-mercapto-2-alkanols **1**, **3-6** on heptakis(2,3-di-*O*-acetyl-6-*O*-TBDMS)-*β*-CD; (C) separation of the stereoisomers of 4-mercapto-2-hexanol **2** on heptakis(2,3-di-*O*-acetyl-6-*O*-TBDMS)-*β*-CD; (D) separation of the stereoisomers of 4-mercapto-2-hexanol **2** on heptakis(2,3-di-*O*-methoxymethyl-6-*O*-TBDMS)-*β*-CD.

Figure 2.

^{1}H NMR data of synthesized (A) 4-mercapto-2-heptanol **3**, (B) 4-mercapto-2-pentanol **1**, and (C) 4-mercapto-2-decanol **6**.

Figure 3.

Approach employed to assign the GC order of elution of the stereoisomers of 4-mercapto-2-alkanols, shown for 4-mercapto-2-pentanol **1** as example.

Figure 4.

Structures of synthesized diastereomeric (*S*)-MαNP thioesters of (*R*)- and (*S*)-4-mercapto-2-octanone **16**.

Figure 5.

GC separation of the stereoisomers of 4-mercapto-2-alkanols **1-6** on Chiramix®.

642 **Figure 6.**

643 Geometric means of the odor thresholds of the stereoisomers of 4-mercapto-2-
644 alkanols (except for (2*R*,4*S*) and (2*S*,4*R*) of C9, for which only single sensory
645 evaluations were available).

Table 1. Structures of the Stereoisomers of 4-Mercapto-2-alkanols **1-6** with Chain Lengths from C5 to C10

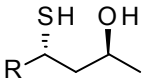
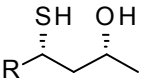
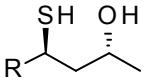
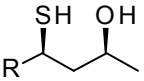
4-mercapto-2-alkanols				
no.	chain length	R	structure	
1	C5	$-CH_3$	*anti*	*syn*
2	C6	$-CH_2-CH_3$		
3	C7	$-(CH_2)_2-CH_3$	a: (2*S*,4*S*)	b: (2*R*,4*S*)
4	C8	$-(CH_2)_3-CH_3$		
5	C9	$-(CH_2)_4-CH_3$		
6	C10	$-(CH_2)_5-CH_3$	a': (2*R*,4*R*)	b': (2*S*,4*R*)

Table 2. Diastereomeric Ratios of Synthesized 4-Mercapto-2-alkanols **1-6** Determined by GC and ^{1}H NMR Analysis

	diastereomeric ratios (%)		
	GC	^{1}H NMR	
no.	*anti : syn*	H-2	H-4
1	37 : 63	34 : 66	34 : 66
2	42 : 58	42 : 58	41 : 59
3	39 : 61	40 : 60	40 : 60
4	47 : 53	47 : 53	45 : 55
5	45 : 55	47 : 53	44 : 56
6	43 : 57	43 : 57	44 : 56

Table 3. ^{1}H NMR Data and Δδ Values of (*S*)-MαNP Thioesters of Both Enantiomers of 4-Mercapto-2-octanone **16**

(S)-MαNP thioesters of 4-mercapto-2-octanone **16**			
H	(*S*,*R*)-**16**	(*S*,*S*)-**16**	Δδ
1	1.98 (s)	2.04 (s)	0.06
3	2.62 (d, 2.1)	2.67 (dd, 16.5, 6.4)	0.05
3'	2.60 (d, 3.1)	2.60 (dd, 18.8, 7.0)	0
4	3.74 (m)	3.75 (m)	0
5	1.52 (m)	1.50 (m)	-0.02
6	1.21 (m)	1.18 (m)	-0.03
7	1.21 (m)	1.18 (m)	-0.03
8	0.79 (t, 7.3)	0.75 (t, 7.3)	-0.04

Table 4. Odor Thresholds of the Stereoisomers of 4-Mercapto-2-alkanols **1-6** Determined by GC/O

	odor thresholds in air (ng/L)[a]											
	a (2*S*,4*S*)			a' (2*R*,4*R*)			b (2*R*,4*S*)			b' (2*S*,4*R*)		
	panelist			panelist			panelist			panelist		
no.	1	2	3	1	2	3	1	2	3	1	2	3
1	1.4	0.7	[b]	0.7	1.4	[b]	0.5	0.1	[b]	0.5	0.5	[b]
2	[b]	0.1	2.2	[b]	0.1	0.13	[b]	0.1	0.7	[b]	0.1	3.0
3	0.1	[b]	0.1	0.01	[b]	0.05	0.2	[b]	0.2	0.3	[b]	0.3
4	0.5	0.1	[b]	0.06	0.001	[b]	0.1	0.2	[b]	0.3	0.1	[b]
5	0.2	0.1	[b]	0.03	0.001	[b]	[b]	0.1	[b]	[b]	0.1	[b]
6	1.0	0.3	[b]	0.1	0.1	[b]	0.7	3.1	[b]	1.5	0.8	[b]

[a] Odor thresholds were determined by GC/O.

[b] Not determined by this panelist.

Table 5. Odor Descriptions of the Stereoisomers of 4-Mercapto-2-alkanols **1-6** Determined by GC/O

		odor descriptions[a]			
no.	panelist	a (2*S*,4*S*)	a' (2*R*,4*R*)	b (2*R*,4*S*)	b' (2*S*,4*R*)
1	1	**onion**, sweaty, meaty	**onion**, savory	**sweaty**, onion	**sweaty**, meaty
	2	**onion**, sweaty	**onion**, sweet	**sweaty**, onion	**sweaty**, pungent
2	2	**fruity**, sour, onion	**onion**, pungent	**savory**, onion, sweaty	**green**, onion, sour
	3	**fruity**, tropical, sulfury	**onion**, rhubarb	**savory**, onion	**herbs**, savory
3	1	**fruity**, sulfury, onion	**onion**, fermented	**onion**, sulfury	**savory**, meaty
	3	**fruity**, sulfury	**onion**, sulfury	**onion**, savory, meaty	**savory**, green, herbs
4	1	**plastic**, sulfury, green	**plastic**, green	**burnt**, tomato plant	**burnt**, green
	2	**pungent**, onion	**pungent**, onion	**pungent**, onion	**onion**, fruity, sour
5	1	**rubber**, **burnt**, sulfury	**plastic**, onion	-[b]	-[b]
	2	**garage**, onion	**pungent**, sweaty	**pungent**, onion	**solvent**, onion
6	1	**burnt**, **plastic**, sulfury	**plastic**, **rhubarb**	**plastic**, sulfury	**plastic**, fruity, citrus
	2	**onion**, sweaty	**solvent**, **fruity**, onion	**pungent**, onion	**solvent**, sour, onion

[a] GC/O descriptions were made for injection volumes corresponding to ~1.5 ng for each stereoisomer at the sniffing port. Dominant odor impressions are bold typed. [b] Not determined by this panelist.

Figures

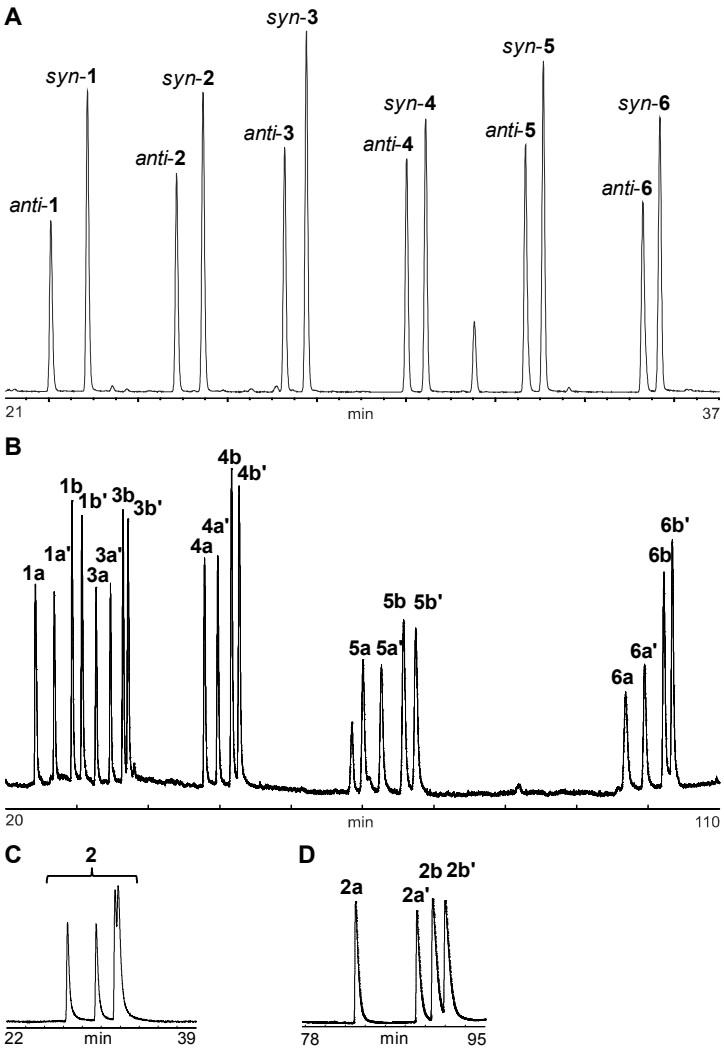


Figure 1

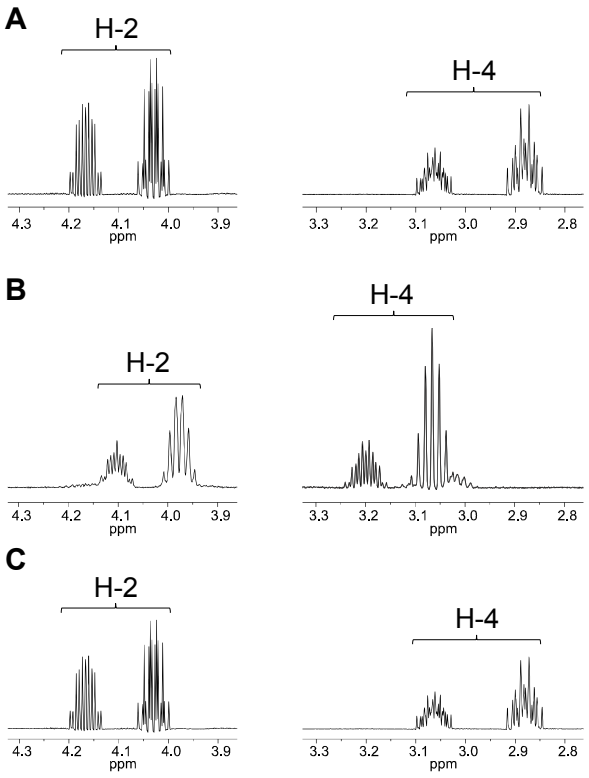

A
H-2
H-4
4.3 4.2 4.1 4.0 3.9
3.3 3.2 3.1 3.0 2.9 2.8
ppm
B
H-4
H-2
4.3 4.2 4.1 4.0 3.9
3.3 3.2 3.1 3.0 2.9 2.8
ppm
C
H-2
H-4
4.3 4.2 4.1 4.0 3.9
3.3 3.2 3.1 3.0 2.9 2.8
ppm

Figure 2

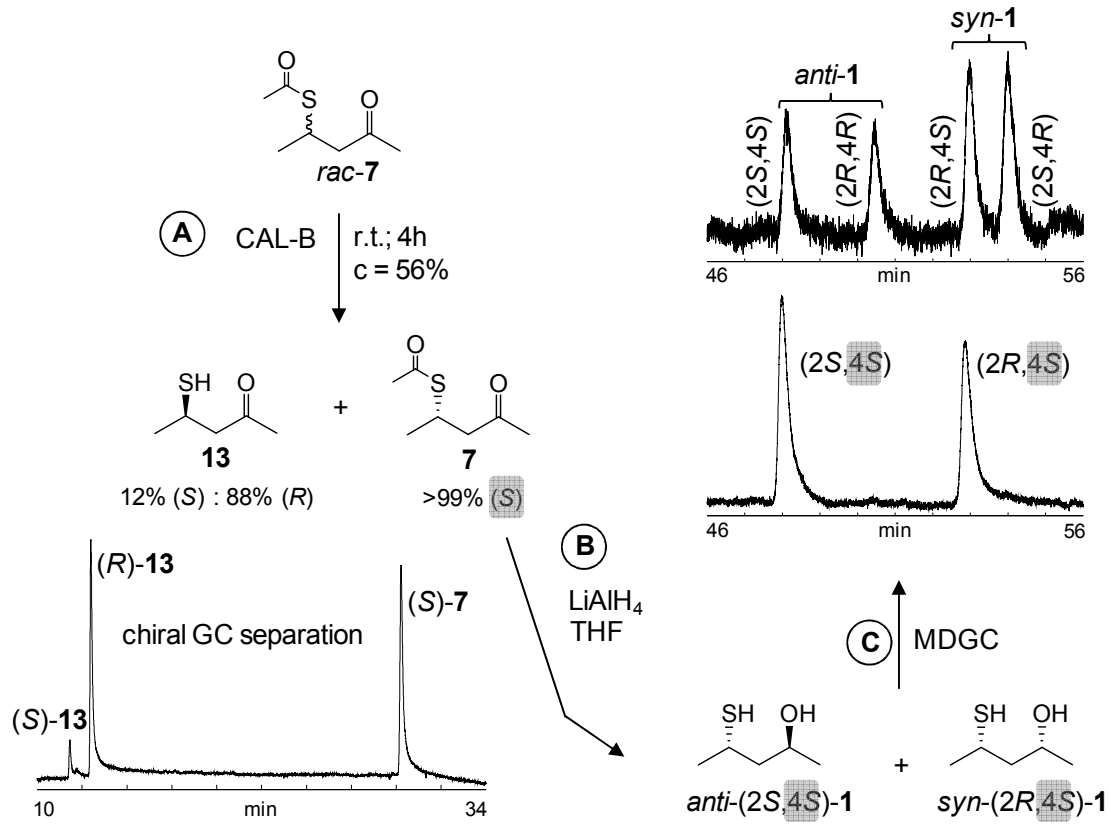
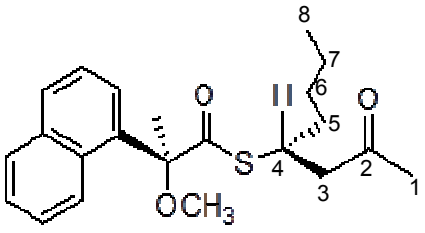
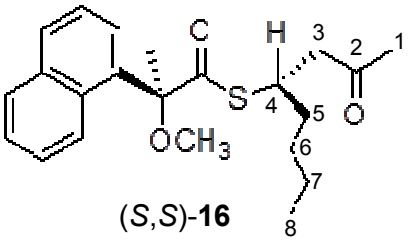

rac-7
A
CAL-B
r.t.; 4h
c = 56%
13
12% (S) : 88% (R)
7
>99% (S)
(R)-13
(S)-7
chiral GC separation
(S)-13
10
min
34
B
LiAlH4
THF
C
MDGC
anti-(2S,4S)-1
syn-(2R,4S)-1
anti-1
syn-1
(2S,4S)
(2R,4R)
(2R,4S)
(2S,4R)
46
min
56
(2S,4S)
(2R,4S)
46
min
56

Figure 3



(*S*,*R*)-**16**



(*S*,*S*)-**16**

Figure 4

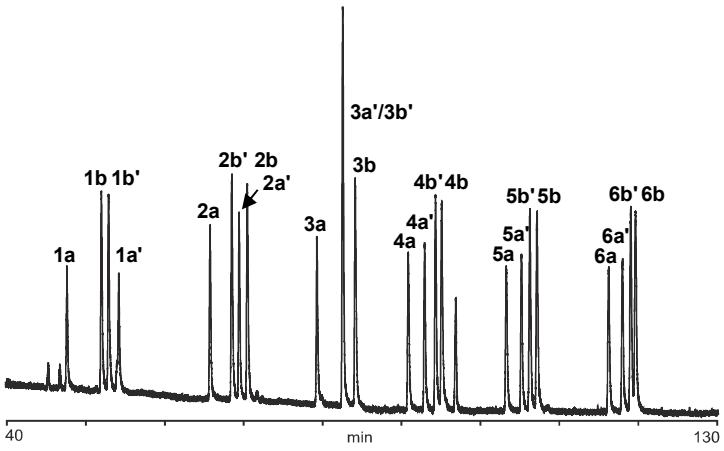

1a
1b 1b'
1a'
2a
2b' 2b
2a'
3a
3a'/3b'
3b
4a
4a'
4b' 4b
5a
5a'
5b' 5b
6a
6a'
6b' 6b
40
min
130

Figure 5

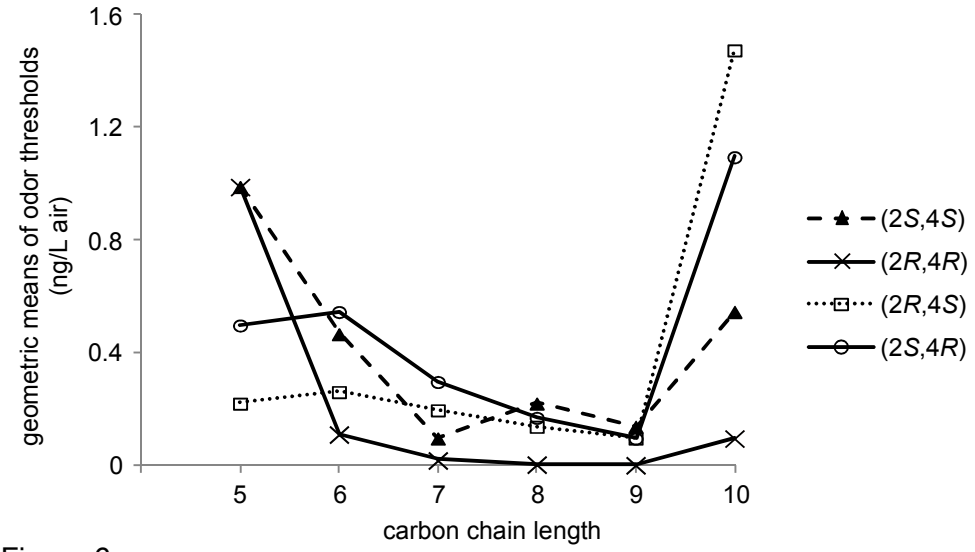
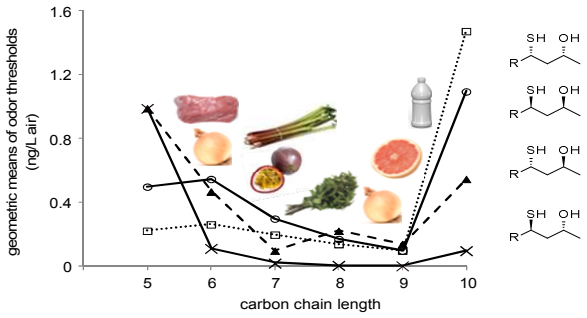

1.6
1.2
0.8
0.4
0
geometric means of odor thresholds (ng/L air)
5
6
7
8
9
10
carbon chain length
(2S,4S)
(2R,4R)
(2R,4S)
(2S,4R)

Figure 6

TOC graphic


1.6
1.2
0.8
0.4
0
geometric means of odor thresholds (ng/L air)
5
6
7
8
9
10
carbon chain length
SH OH
R