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Influence of the Stereochemistry on the Sensory Properties of 4-Mercapto-2-heptanol and Its Acetyl-Derivatives

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ABSTRACT: 4-Mercapto-2-heptanol, previously described in cooked bell pepper, was used to determine the impact of the stereochemistry on the sensory properties of a thiol with a 1,3-oxygen−sulfur functionality. In addition, the acetyl-derivatives 4-acetylthio-2-heptanol, 4-mercapto-2-heptyl acetate and 4-acetylthio-2-heptyl acetate were investigated. The synthesized stereoisomers were separated via capillary gas chromatography (GC) using chiral stationary phases. The GC orders of elution were determined by assigning the absolute configurations via NMR analysis in combination with lipase-catalyzed kinetic resolutions. Odor thresholds and odor properties were determined by means of GC/Olfactometry. The data revealed that the sensory properties of the investigated compounds are not only significantly influenced by the acetylation but also by the configurations of the two asymmetric centers.

KEYWORDS: *4-mercapto-2-heptanol, 4-acetylthio-2-heptanol, 4-mercapto-2-heptyl acetate, 4-acetylthio-2-heptyl acetate, polyfunctional thiols, tropical olfactophore, absolute configuration, lipase, odor thresholds, sensory properties, GC/O, NMR*

INTRODUCTION

Sulfur-containing volatiles are known as outstanding contributors to the flavor of many foods.[1−3] In particular, polyfunctional thiols play important roles owing to their low odor thresholds and their pronounced odor qualities.[4−7] Their sensory properties not only depend on factors such as concentration, but also are determined by certain structural features.[1,8,9] For example, many compounds exhibiting tropical, fruity or vegetable odor notes share a 1,3-oxygen−sulfur functionality as common element.[10,11] 3-Mercapto-1-hexanol, first reported in yellow passion fruit and later in wines, is a long known representative fulfilling this structural requirement.[12,13] Another example is 3-mercapto-2-methyl-1-pentanol identified in raw onions after thermal processing.[14] Regarding the impact of the stereochemistry on the sensory properties of these chiral compounds, different odor qualities but no significant differences in odor thresholds have been reported for the enantiomers of 3-mercapto-1-hexanol.[15−17] In contrast, the odor thresholds determined for the stereoisomers of 3-mercapto-2-methyl-1-pentanol differed by factors up to 1000.[18]

Recently, 4-mercapto-2-heptanone and 4-mercapto-2-heptanol have been identified in cooked red bell pepper.[19] The sensory properties of this β-mercaptoalkanone and β-mercaptoalkanol have been assessed in NaCl and sugar solutions; however, racemic and diastereoisomeric mixtures, respectively, were employed, and thus a potential impact of the stereochemistry on the sensory properties of these chiral substances has not been considered. The significance of this phenomenon for these classes of substances has been indicated by a recent study demonstrating differences in odor thresholds between the enantiomers of 4-mercapto-2-alkanones and in odor qualities between the diastereoisomers of the corresponding 4-mercapto-2-alkanols.[20]

Therefore, the initial aim of this study was to use the naturally occurring 4-mercapto-2-heptanol as further representative to determine the sensory properties of the four stereoisomers of a compound exhibiting the 1,3-oxygen−sulfur functionality. Considering the suggestion that this "tropical olfactophore"-skeleton can be extended to the respective acetylthio-derivatives, the acetates 4-acetylthio-2-heptanol, 4-mercapto-2-heptyl acetate and 4-acetylthio-2-heptyl acetate were also included into the assessments.[21] The synthesized stereoisomers should be separated via capillary gas chromatography using chiral stationary phases, the GC orders of elution should be determined by assigning the absolute configurations, and the sensory properties of the stereoisomers should be evaluated by capillary gas chromatography/olfactometry.

MATERIALS AND METHODS

Chemicals. 3-Hepten-2-one was provided by TCI Europe, Belgium. Thioacetic acid, acetyl chloride, *trans*-2-decenal (≥95%), lithium aluminum hydride, sodium borohydride and porcine pancreas lipase type II (L 3126) were obtained from Sigma-Aldrich, Germany. Acetic anhydride was purchased from Fluka, Germany. 2-Propanol (HPLC grade) and *n*-hexane (HPLC grade) were from VWR Prolabo, Germany.

Syntheses. *4-Mercapto-2-heptanol* ***3***. A mixture of 3-hepten-2-one **1** (1.62 g, 14.5 mmol) and thioacetic acid (1.66 g, 21.8 mmol) was stirred for 1 h under ice cooling and for another 20 h at room temperature (25 °C). After removing the excess of thioacetic acid under reduced pressure using an aspirator at 40 °C, 4-acetylthio-2-heptanone **2** (2.71 g, 14.4 mmol, 99% yield) was obtained.

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Chromatographic, mass spectrometric and NMR data were in accordance with those previously reported.[22] 4-Acetylthio-2-heptanone **2** (2.0 g, 10.6 mmol) in 20 mL of dry tetrahydrofuran (THF) was added slowly to a suspension of lithium aluminum hydride (1.81 g, 47.8 mmol) in 50 mL of dry THF under argon atmosphere at 0 °C.[19,20] The reaction mixture was stirred at room temperature overnight. After careful quenching with 100 mL of distilled water under ice-cooling, the aqueous phase was adjusted to pH 2, using hydrochloric acid (5%) and extracted with dichloromethane (3 × 20 mL). The organic phase was dried with sodium sulfate, and the solvent was removed under reduced pressure to yield **3** as a pale yellow liquid (1.54 g, 10.4 mmol, 97% yield). GC analysis revealed a purity of 96% and the presence of a 39:61 mixture of diastereoisomers. The preparative separation of the diastereoisomers was carried out using a Dionex HPLC system (UltiMate 3000 series, Dionex, Germany) equipped with a wavelength detector-3100 adjusted to 210 nm. Samples (250 μL) were injected into a Nucleosil 50−5 column (250 × 8 mm i.d., CS-Chromatographie, Germany). The use of *n*-hexane containing 8% 2-propanol as eluent (flow rate 4.0 mL/min; 30 °C) resulted in the HPLC-separation of *anti*-**3** (4.94 min) and *syn*-**3** (5.43 min). Linear retention indices (LRI) determined by GC: 1751 (*anti*-**3**), 1768 (*syn*-**3**) on DB-Wax; 1109 (*anti*-**3**), 1118 (*syn*-**3**) on DB-1. GC−MS (*m*/*z*, rel. %) 55 [100], 45 [95], 71 [71], 43 [44], 114 [40], 41 [29], 97 [28], 87 [25], 61 [22], 70 [20], 148 (M^+, 4). ^{1}H NMR (500 MHz, $CDCl_3$) *δ anti*-**3**: *δ* 4.14 (ddt, *J* = 12.6, 6.3, 3.4 Hz, 1H, H-2), 3.08−2.99 (m, 1H, H-4), 1.79−1.71 (m, 1H, H-3a), 1.69−1.36 (m, 5H, H-3b, H-5, H-6), 1.19 (d, *J* = 6.2 Hz, 3H, H-1), 0.92 (t, *J* = 7.1 Hz, 3H, H-7). *syn*-**3**: 4.01 (dqd, *J* = 7.8, 6.2, 4.7 Hz, 1H, H-2), 2.90−2.81 (m, 1H, H-4), 1.79−1.71 (m, 1H, H-3a), 1.69−1.36 (m, 5H, H-3b, H-5, H-6), 1.19 (d, *J* = 6.6 Hz, 3H, H-1), 0.92 (t, *J* = 7.0 Hz, 3H, H-7). ^{13}C NMR (126 MHz, $CDCl_3$) *δ anti*-**3**: 67.2 (C-2), 48.4 (C-3), 41.9 (C-5), 38.8 (C-4), 23.7 (C-1), 20.1 (C-6), 13.9 (C-7). *syn*-**3**: 65.6 (C-2), 47.9 (C-3), 42.2 (C-5), 37.6 (C-4), 24.2 (C-1), 20.3 (C-6), 13.9 (C-7).

4-Mercapto-2-heptyl Acetate ***4***. A solution of **3** (0.344 g, 2.32 mmol) in diethyl ether (Et_2O, 5 mL) was slowly treated with acetyl chloride (165 μL, 2.32 mmol) at 0 °C under argon atmosphere as previously reported.[5] The reaction mixture was stirred for 18 h at room temperature. The precipitate was filtered off, the organic phase was washed with water and brine, dried with sodium sulfate, and the solvent was evaporated. The crude product was purified by column chromatography on silica gel (silica gel 60, 0.063−0.200 mm, Merck, Germany) by elution with a mixture of *n*-hexane and Et_2O, 9:1, (v/v). The fractions were checked by thin layer chromatography (TLC, ALUGRAM SIL G/UV_{254}, Macherey-Nagel, Germany), and visualization was achieved by spraying with 10% sulfuric acid and subsequent heating until dryness. Compound **4** was obtained as a colorless liquid (122.4 mg, 0.643 mmol, 28% yield). GC analysis revealed a purity of 97% and the presence of a 40:60 mixture of diastereoisomers. LRI: 1650 (*anti*-**4**), 1668 (*syn*-**4**) on DB-Wax; 1233 (*anti*-**4**), 1241 (*syn*-**4**) on DB-1. GC−MS (*m*/*z*, rel.%): 43 (100), 55 (81), 87 (47), 97 (46), 130 (45), 115 (22), 88 (19), 41 (18), 102 (17). ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 5.24−5.16 (m, 1H, H-2_{anti}), 5.15−5.07 (m, 1H, H-2_{syn}), 2.86−2.74 (m, 2H, H-$4_{anti+syn}$), 2.03 (s, 6H, $CH_3C(O)-O-_{anti+syn}$), 1.99−1.92 (m, 1H, H-$5a_{anti}$), 1.92−1.85 (m, 1H, H-$5a_{syn}$), 1.79−1.71 (m, 1H, H-$5b_{syn}$), 1.70−1.63 (m, 1H, H-$3a_{syn}$), 1.57−1.62 (m, 1H, H-$3a_{anti}$), 1.57−1.36 (m, 7H, H-$5b_{anti}$, H-$3b_{anti}$, H-$3b_{syn}$, H-$6_{anti+syn}$), 1.26 (d, *J* = 6.4 Hz, H-1_{anti}), 1.22 (d, *J* = 6.2 Hz, 3H, H-1_{syn}), 0.94−0.87 (m, 6H, H-$7_{anti+syn}$). ^{13}C NMR (126 MHz, $CDCl_3$) *δ* 170.7 ($CH_3\underline{C}(O)-O-_{anti}$), 170.6 ($CH_3\underline{C}(O)-O-_{syn}$), 69.0 (C-$2_{syn}$), 68.91 (C-$2_{anti}$), 45.5 (C-$5_{anti}$), 45.2 (C-$5_{syn}$), 41.3 (C-$3_{anti}$), 40.8 (C-$3_{syn}$), 37.0 (C-$4_{anti}$), 37.0 (C-$4_{syn}$), 21.4 ($\underline{C}H_3C(O)-O-_{anti}$), 21.4 ($\underline{C}H_3C(O)-O-_{syn}$), 20.6 (C-1/C-6), 20.1 (C-1/C-6), 20.0 (C-1/C-6), 19.9 (C-1/C-6), 13.8 (C-7_{anti}), 13.7 (C-7_{syn}).

4-Acetylthio-2-heptyl Acetate ***5***. A solution of **3** (0.538 g, 3.37 mmol) in pyridine (2.5 mL) was treated with 4-dimethylaminopyridine (247 mg, 2.02 mmol) and acetic anhydride (1.60 mL, 16.9 mmol), and the mixture was stirred for 24 h at room temperature. The solvent was removed under reduced pressure, and the residue was dissolved in diethyl ether. After washing with saturated aqueous solutions of sodium hydrogen carbonate (3 × 10 mL) and ammonium chloride (3 × 10 mL), the organic layer was dried over sodium sulfate, and the solvent was removed under reduced pressure. The diastereoisomeric mixture (40:60 by GC analysis, *anti*:*syn*) was separated by column chromatography on silica gel with *n*-hexane/Et_2O (5:1), and the obtained fractions were checked by TLC. The separated diastereoisomers were isolated as *anti*-**5** (0.295 g, 1.27 mmol, 38% yield, purity: 94% by GC) and *syn*-**5** (0.442 g, 1.90 mmol, 56% yield, purity: 96% by GC) as colorless liquids. LRI: 1881 (*anti*-**5**), 1934 (*syn*-**5**) on DB-Wax; 1403 (*anti*-**5**), 1428 (*syn*-**5**) on DB-1. GC−MS (*m*/*z*, rel.%): 43 (100), 55 (38), 130 (32), 129 (30), 97 (24), 87 (22), 139 (18), 115 (13), 88 (11). ^{1}H NMR (500 MHz, $CDCl_3$): *anti*-**5**: *δ* 5.02−4.93 (m, 1H, H-2), 3.68−3.58 (m, 1H, H-4), 2.29 (s, 3H, $CH_3C(O)-S-$), 2.03 (s, 3H, $CH_3C(O)-O-$), 1.96−1.85 (m, 1H, H-3a), 1.65−1.48 (m, 3H, H-3b, H-5), 1.47−1.30 (m, 2H, H-6), 1.22 (d, *J* = 6.2 Hz, 3H, H-1), 0.90 (t, *J* = 7.3 Hz, 3H, H-7). *syn*-**5**: *δ* 5.08−4.90 (m, 1H, H-2), 3.60−3.47 (m, 1H, H-4), 2.31 (s, 3H, $CH_3C(O)-S-$), 2.04 (s, 3H, $CH_3C(O)-O-$), 1.95−1.82 (ddd, *J* = 14.1, 7.9, 7.1 Hz, 1H, H-3a), 1.78−1.70 (dt, *J* = 14.2, 6.4 Hz, 1H, H-3b), 1.65−1.56 (dddd, *J* = 13.8, 9.9, 6.0, 5.0 Hz, 1H, H-5a), 1.54−1.46 (m, 1H, H-5b), 1.45−1.29 (m, 2H, H-6), 1.22 (d, *J* = 6.2 Hz, 3H, H-1), 0.89 (t, *J* = 7.3 Hz, 3H, H-7). ^{13}C NMR (126 MHz, $CDCl_3$) *anti*-**5**: *δ* 195.5 ($CH_3\underline{C}(O)-S-$), 170.9 ($CH_3\underline{C}(O)-O-$), 68.5 (C-2), 41.3 (C-3), 40.8 (C-4), 37.9 (C-5), 30.8 ($\underline{C}H_3C(O)-S-$), 21.5 ($\underline{C}H_3C(O)-O-$), 20.5 (C-6), 20.0 (C-1), 14.0 (C-7). *syn*-**5**: *δ* 195.8 ($CH_3\underline{C}(O)-S-$), 170.7 ($CH_3\underline{C}(O)-O-$), 69.0 (C-2), 41.1 (C-3), 40.9 (C-4), 37.0 (C-5), 30.9 ($\underline{C}H_3C(O)-S-$), 21.5 ($\underline{C}H_3C(O)-O-$), 20.1 (C-6), 19.9 (C-1), 13.9 (C-7).

4-Acetylthio-2-heptanol ***6***. Sodium borohydride (201 mg, 5.31 mmol), dissolved in 8 mL water, was added dropwise under ice-cooling to a solution of 4-acetylthio-2-heptanone **2** (0.50 g, 2.66 mmol) in 20 mL of methanol and 80 mL of potassium phosphate buffer (50 mM, pH 7.4). After 20 min, the pH was adjusted to 5 using hydrochloric acid (5%). The aqueous layer was washed with dichloromethane (3 × 20 mL), the organic phase was dried with sodium sulfate, and the solvent was removed under reduced pressure. The diastereoisomers (55:45 by GC, *anti*:*syn*) were separated by column chromatography on silica gel (*n*-hexane/Et_2O, 2:1) to yield *anti*-**6** (153.5 mg, 0.81 mmol, 31%, purity: 92% by GC) and *syn*-**6** (99.2 mg, 0.52 mmol, 20%, purity: 93% by GC) as yellow liquids. LRI: 1939 (*anti*-**6**), 2038 (*syn*-**6**) on DB-Wax; 1302 (*anti*-**6**), 1323 (*syn*-**6**) on DB-1. GC−MS (*m*/*z*, rel.%): 43 (100), 55 (41), 45 (32), 71 (23), 147 (18), 87 (17), 97 (16), 130 (15). ^{1}H NMR (500 MHz, $CDCl_3$) *anti*-**6**: *δ* 3.81−3.72 (m, 1H, H-2), 3.73−3.63 (m, 1H, H-4), 2.36 (s, 3H, $CH_3C{=}O{-}S-$), 1.75−1.68 (m, 1H, H-3a), 1.61−1.54 (m, 2H, H-5), 1.53−1.32 (m, 3H, H-3b, H-6), 1.18 (d, *J* = 6.3 Hz, 3H, H-1), 0.92−0.88 (m, 3H, H-7). ^{13}C NMR (125 MHz, $CDCl_3$) *anti*-**6**: *δ* 199.3 ($CH_3\underline{C}(O)-S-$), 65.2 (C-2), 46.2 (C-3), 42.2 (C-4), 37.8 (C-5), 31.0 ($\underline{C}H_3C(O)-S-$), 23.0 (C-1), 20.5 (C-6), 14.2 (C-7). NMR data of *syn*-**6** could not be obtained because of the instability of the substance under the measurement conditions; GC-analysis revealed *syn*-**4** as rearrangement product.

Kinetic Resolution of Racemic 4-Acetylthio-2-heptanone. In accordance with the procedure described by Wakabayashi et al.,[22] **2** (1.08 g, 5.74 mmol) was dissolved in 50 mL of potassium phosphate buffer (50 mM, pH 7.4).[22] 1.0 g of porcine pancreas lipase (PPL) was added, and the solution was stirred at room temperature for 7.5 h. The enzyme was filtered off, and the aqueous layer was extracted with dichloromethane (4 × 50 mL). The organic phase was dried with sodium sulfate and the solvent was evaporated; GC analysis revealed a conversion rate of 79%. Column chromatography on silica gel (*n*-hexane/Et_2O, 4:1) yielded 4-mercapto-2-heptanone **7** (430.2 mg, 2.94 mmol, 51% yield) and the remaining substrate (*S*)-**2** (116.3 mg, 0.618 mmol, 11% yield). The enantiomeric purity of 4-acetylthio-2-heptanone **2** was determined using heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-*β*-cyclodextrin as chiral stationary phase (98% (*S*): 2% (*R*)).

The obtained (*S*)-**2** was reduced to the (4*S*)-configured diastereoisomers of 4-mercapto-2-heptanol **3**. This was used as starting substance to synthesize the (4*S*)-configured diastereoisomers

of the acetates **4**, **5** and **6**, employing the same procedures as described above (see also Figure 1).

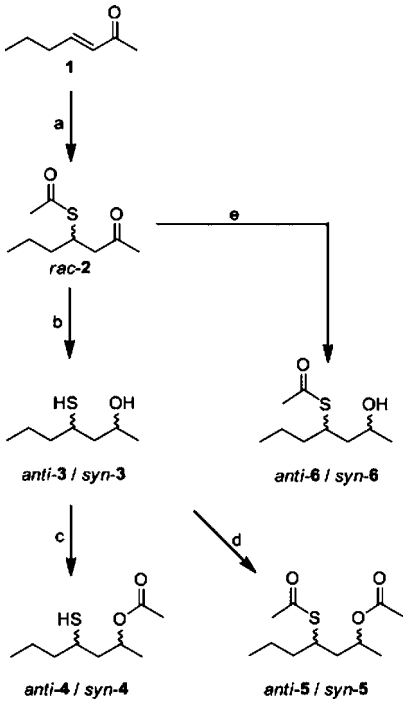


Figure 1. Syntheses of the stereoisomers of 4-mercapto-2-heptanol **3**, 4-mercapto-2-heptyl acetate **4**, 4-acetylthio-2-heptyl acetate **5**, and 4-acetylthio-2-heptanol **6**. (a) Thioacetic acid; (b) $LiAlH_4$, THF; (c) acetyl chloride, Et_2O, pyridine; (d) acetic anhydride, 4-(dimethylamino)pyridine, pyridine; (e) $NaBH_4$, phosphate buffer.

Analytical Methods. *Capillary Gas Chromatography (GC-FID).* A DB-Wax column (30 m × 0.25 mm i.d.; 0.5 μm film thickness; J&W Scientific; column 1) installed into an HP5890 A gas chromatograph (Hewlett-Packard INC; GC-system 1) equipped with a split/splitless injector (215 °C, split ratio of 1:7) and FID (350 °C) was used (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 DB-1 column (30 m × 0.25 mm i.d.; 1.0 μm film thickness; J&W Scientific; column 2) installed into a Carlo Erba GC, model 5160 Mega Series (GC-system 2), equipped with a split/splitless injector (200 °C, split ratio of 1:10), FID (260 °C) was used (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 (1963), using C_8−C_{40} *n*-alkane standard solutions (Fluka).[23]

Enantioselective Analysis. For enantioseparation of **3** fused silica capillary columns (30 m × 0.25 mm i.d.) coated with heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyl dimethylsilyl)-β-cyclodextrin in OV1701-vi (column 3) and oktakis(2,3-di-*O*-*n*-butyryl-6-*O*-*tert*-butyl dimethylsilyl)-γ-cyclodextrin in SE 54 (column 4) were installed into GC-system 2. The syntheses of the cyclodextrin derivatives were performed according to previously described procedures.[24,25] The following temperature programs were used: from 85 °C (0 min hold) to 110 °C (50 min hold) at 0.5 °C/min and from 110 to 180 °C (5 min hold) at 2 °C/min for column 3, and from 75 °C (0 min hold) to 93 °C (0 min hold) at 0.5 °C/min and from 93 to 180 °C (5 min hold) at 1 °C/min for column 4. Hydrogen was used as carrier gas at a constant pressure of 75 kPa.

For enantioseparation of **2** and **4**−**7** a fused silica capillary column (30 m × 0.25 mm i.d.) coated with heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyl dimethylsilyl)-β-cyclodextrin in SE 54 (column 5) was installed into an HP5890 A gas chromatograph (GC-system 3) equipped with a split/splitless injector (200 °C, split ratio of 1:5) and a FID (350 °C). The cyclodextrin derivative was synthesized according to a previously described procedure.[26] The following temperature programs were used: for **2**, **6** and **7** from 75 °C (0 min hold) to 180 °C (5 min hold) at 2 °C/min, for **4** from 75 °C (0 min hold) to 180 °C (5 min hold) at 1 °C/min, and for **5** from 75 °C (10 min hold) to 100 °C (45 min hold) at 0.5 °C/min and from 100 to 180 °C (5 min hold) at 2 °C/min. Carrier gas used was hydrogen at a constant pressure of 176 kPa for **2**, **4**, **5** and **7** and 160 kPa for **6**.

Capillary Gas Chromatography/Olfactometry (GC/O). Sensory analyses of **3**−**6** were performed on an HP5890 A Series II gas chromatograph (Hewlett-Packard INC; GC-system 4). Samples were applied onto a deactivated precolumn (30 cm × 0.32 mm i.d.; BGB Analytik AG) using a cold-on-column injector (40 °C). The analyses were accomplished using columns 1 and 3−5. The effluent was split 1:1 via a press-fit Y-splitter and deactivated fused silica capillaries (50 cm × 0.25 mm i.d.; BGB Analytik AG) to a heated sniffing port (200 °C) and FID (250 °C). The following temperature programs were used: (I) for **3**−**6** (column 1) from 40 °C (5 min hold) to 240 °C (30 min hold) at 4 °C/min; (II) for **3** (column 3) from 40 °C (0 min hold) to 85 °C (0 min hold) at 30 °C/min and from 85 to 110 °C (0 min hold) at 0.5 °C/min and from 110 to 180 °C (20 min hold) at 2 °C/min; (III) for **3** (column 4) from 40 °C (0 min hold) to 75 °C (0 min hold) at 30 °C/min and from 75 to 93 °C (0 min hold) at 0.5 °C/min and from 93 to 180 °C (5 min hold) at 1 °C/min; (IV) for **4** (column 5) from 40 °C (0 min hold) to 80 °C (35 min hold) at 30 °C/min and from 80 to 180 °C (5 min hold) at 4 °C/min; (V) for **5** (column 5) from 40 °C (0 min hold) to 75 °C (10 min hold) at 30 °C/min and from 75 to 100 °C (10 min hold) at 0.5 °C/min and from 100 to 180 °C (5 min hold) at 2 °C/min; (VI) for **6** (column 5) from 40 °C (0 min hold) to 75 °C (0 min hold) at 30 °C/min and from 75 to 180 °C (5 min hold) at 2 °C/min. Hydrogen was used as carrier gas for column 1 at 70 kPa for **3**, **5** and **6**, at 75 kPa for **4**, for column 3 at 85 kPa and for columns 4 and 5 at 75 kPa.

Odor thresholds in air were determined following the procedure described by Ullrich and Grosch (1987).[27] Known amounts of the internal standard *trans*-2-decenal, and the respective target compounds were dissolved in Et_2O and diluted stepwise by a factor of 1:1 (v/v). The aliquots were analyzed by GC/O until no odor was perceivable. Flavor dilution (FD) factors were calculated according to Grosch (1993).[28]

Capillary Gas Chromatography−Mass Spectrometry (GC−MS). MS data of compounds **2**−**7** were obtained on a GC 8000^{TOP} with a Voyager GC−MS (Thermo Fischer Scientific) equipped with a split/splitless injector (220 °C, split ratio 1:50). The installed column was a 30 m × 0.25 mm (i.d.) fused silica capillary column coated with DB-Wax (0.5 μm film thickness; J&W Scientific). The temperature was programmed from 40 °C (5 min hold) to 240 °C (25 min hold) at 4 °C/min. Carrier gas used was hydrogen at a constant inlet pressure of 75 kPa. The mass spectrometer was operated on scan mode at 25−445 amu, and the ionization energy was set at 70 eV. The source temperature was 200 °C and the interface temperature was 240 °C. Data acquisition was done via Xcalibur (version 1.4; Thermo Scientific).

NMR Spectroscopy. ^1H NMR and ^{13}C NMR spectra were recorded at 500 and 126 MHz, respectively, with Avance500 spectrometers. ^1H-detected experiments were done with an inverse $^1H/^{13}C$ probehead, and direct ^{13}C-measurements were performed with a QNP $^{13}C/^{31}P/^{29}Si/^{19}F/^1H$ 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.

RESULTS AND DISCUSSION

GC-Separation and Assignment of the Order of Elution of the Stereoisomers of 4-Mercapto-2-heptanol. 4-Mercapto-2-heptanol **3** was synthesized by Michael-type addition of thioacetic acid to 3-hepten-2-one **1** and subsequent reduction of the formed 4-acetylthio-2-heptanone **2** with lithium aluminum hydride, according to previously described procedures (Figure 1).[22,29] The reaction sequence resulted in a 39:61 mixture of diastereoisomers (Figure 2A). The chromatographic and mass spectrometric data were in agreement with those previously reported.[19] Capillary gas chromatographic analysis of **3** using heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyl dimethylsilyl)-β-cyclodextrin as chiral stationary phase enabled the separation of the four stereoisomers (Figure 2B). The assignment of the order of elution was based on a two-step procedure: (i) separation of the two diastereoisomers via HPLC and comparison of the NMR data to those reported for *anti*-(2*S*,4*S*)-4-mercapto-2-heptanol, the product obtained from 3-hepten-2-one utilizing tandem Michael addition-Meerwein-Ponndorf-Verley reduction, and (ii) preparation of the (4*S*)-configured stereoisomer via lipase-catalyzed resolution of 4-acetylthio-2-heptanone **2** and subsequent reduction of the remaining substrate with lithium aluminum hydride.[22,30]

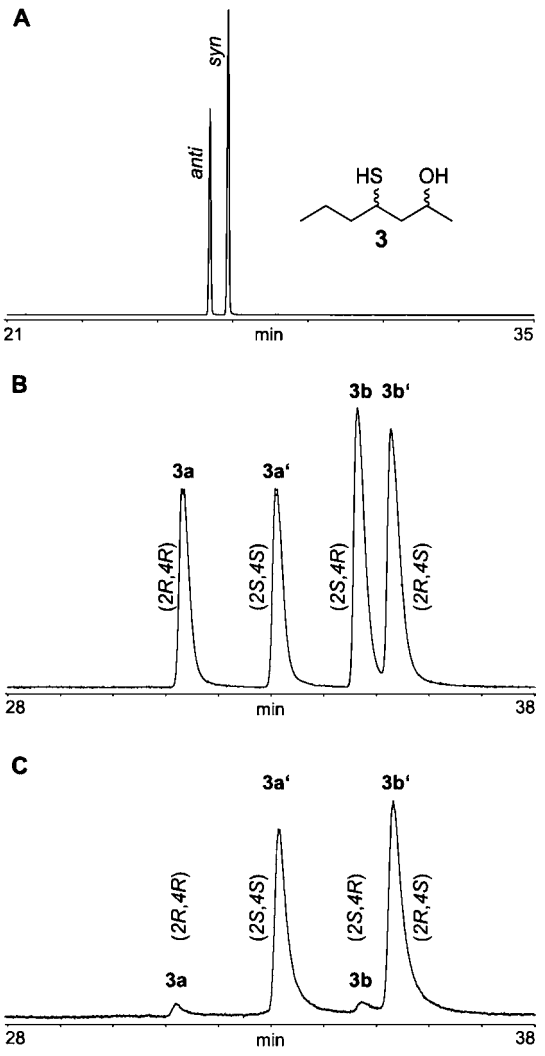


Figure 2. Capillary gas chromatographic separation of (A) the diastereoisomers, (B) the enantiomeric pairs, and (C) the (4*S*)-configured diastereoisomers of 4-mercapto-2-heptanol **3**. For conditions, see Material and Methods.

Step (i). HPLC of **3** on a normal phase silica column resulted in a separation sufficient for preparative isolation of the diastereoisomers. As shown in Table 1, the NMR data of the first eluted diastereoisomer were in excellent agreement with the data reported for *anti*-configured (2*S*,4*S*)-4-mercapto-2-heptanol.[30] Therefore, the diastereoisomer eluted first from the HPLC column constitutes the pair of *anti*-configured (2*S*,4*S*)- and (2*R*,4*R*)-enantiomers. Reinvestigation by GC showed that this HPLC fraction corresponded to the first eluted peak in the capillary gas chromatogram (Figure 2A) and to the first pair of stereoisomers separated on the chiral stationary phase (Figure 2B).

Step (ii). Racemic 4-acetylthio-2-heptanone *rac*-**2** was subjected to kinetic resolution via lipase-catalyzed hydrolysis (Figure 3). In accordance with the stereochemical course described for this reaction, porcine pancreas lipase-mediated hydrolysis of *rac*-**2** resulted in the liberation of the (*R*)-configured thiol (*R*)-**7** (24% ee) as product and (4*S*)-acetylthioheptan-2-one (*S*)-**2** with high optical purity (96% ee) as remaining substrate.[22] The enantiomeric excesses were determined by capillary gas chromatography using 2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl-β-cyclodextrin as chiral stationary phase. After separation of the reaction mixture by silica gel column chromatography, the isolated remaining substrate was subjected to reduction using lithium aluminum hydride. GC analysis demonstrated that the resulting mixture of (4*S*)-configured diastereoisomers coeluted with the second peaks of the pairs of stereoisomers obtained for **3** (Figure 2B,C). Taking into account the assignment of the *anti*- and *syn*-diastereoisomers achieved in the first step, the absolute configurations and the order of elution of the four stereoisomers of **3** could be assigned as shown in Figure 2B.

GC-Separation and Assignment of the Order of Elution of the Stereoisomers of the Acetates of 4-Mercapto-2-heptanol. Acetyl-derivatives of 4-mercapto-2-heptanol were prepared as outlined in Figure 1. 4-Acetylthio-2-heptyl acetate **5** was obtained by treatment of **3** with acetic anhydride. The selective *O*-acetylation of **3** resulting in 4-mercapto-2-heptyl acetate **4** was achieved by using acetyl chloride.[5] The reduction of 4-acetylthio-2-heptanone **2** with sodium borohydride yielded the *S*-acetylated alkanol **6**. The GC-separations of the obtained mixtures of diastereoisomers are shown in Figure 4. A comparison of the chromatogram shown in Figure 4C to those depicted in Figures 4A and 2A demonstrates that under the employed experimental conditions, the previously reported intramolecular acetyl transfer (resulting in **4**) and deacetylation (resulting in **3**) upon reduction with sodium borohydride occurred only to a minor extent.[11,31] As to the authors' knowledge, 4-acetylthio-2-heptanol **6**, 4-acetylthio-2-heptyl acetate **5** and 4-mercapto-2-heptyl acetate **4** have not been described so far.

The use of 2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl-β-cyclodextrin as chiral stationary phase enabled the separation of the four stereoisomers of each acetyl ester **4**-**6** (Figure 4).

The assignment of the order of elution of the stereoisomers of 4-acetylthio-2-heptyl acetate **5** was based on a three-step procedure analogous to the strategy employed for 4-mercapto-2-heptanol **3**: (i) separation of the diastereoisomers via column chromatography on silica gel, (ii) deacetylation of the separated diastereoisomers by alkaline cleavage and assignment of the

Table 1. ^{1}H NMR Data of the Diastereoisomers of 4-Mercapto-2-heptanol 3 Separated via HPLC and the Enantioselectively Synthesized (2*S*,4*S*)-4-Mercapto-2-heptanol[28]

	δ, ^{1}H, chemical shifts, ppm		
	HPLC		Ozeki et al. (2004)[28]
position	LC-peak I (*anti*-**3**)	LC-peak II (*syn*-**3**)	*anti*-(2*S*,4*S*)-**3**
H-2	4.14 (ddt), 1H	4.01 (dqd), 1H	4.15 (ddq), 1H
H-4	3.08−2.99 (m), 1H	2.90−2.81 (m), 1H	3.09−2.99 (m), 1H
H-3a	1.79−1.71 (m), 1H	1.79−1.71 (m), 1H	1.76 (ddd), 1H
H-3b, H-5, H-6	1.69−1.36 (m), 5H	1.69−1.36 (m), 5H	1.65−1.40 (m), 4H + 1.37, (d), 1H
H-1	1.23 (d), 3H	1.19 (d), 3H	1.23 (d), 3H
H-7	0.92 (t), 3H	0.92 (t), 3H	0.92 (t), 3H

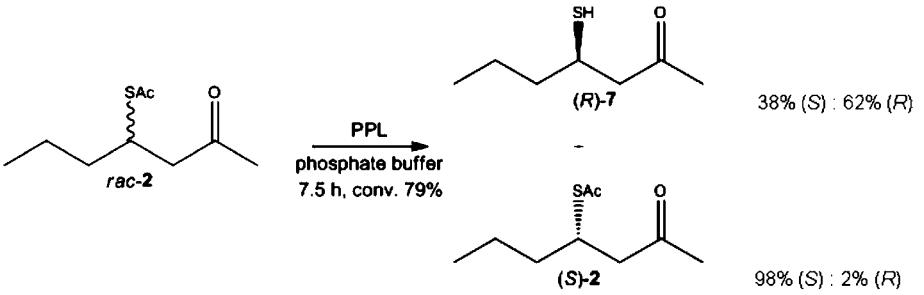


Figure 3. Lipase-catalyzed resolution of racemic 4-acetylthio-2-heptanone **2** resulting in the formation of the enantioenriched product 4-mercapto-2-heptanone (*R*)-**7** and the remaining substrate 4-acetylthio-2-heptanone (*S*)-**2** after 7.5 h.

syn-/*anti*-configurations by comparison of the GC retention times of the resulting stereoisomers of **3** to those in Figure 2A, and (iii) preparation of the (4*S*)-configured diastereoisomers by acetylation of (4*S*)-4-mercapto-2-heptanol obtained via lipase-catalyzed kinetic resolution of 4-acetylthio-2-heptanone **2** and subsequent reduction with lithium aluminum hydride.

The same procedure was applied to assign the order of elution of the stereoisomers of 4-acetylthio-2-heptanol **6**, except that in step (iii) the (4*S*)-configured diastereoisomers were prepared by reduction of (4*S*)-4-acetylthio-2-heptanone obtained via lipase-catalyzed kinetic resolution of racemic 4-acetylthio-2-heptanone with sodium borohydride.

The assignment of the order of elution of 4-mercapto-2-heptyl acetate **4** was impaired by the fact that the separation of the diastereoisomers of **4** via column chromatography was not possible. Therefore, the procedure was based on (i) separation of the diastereoisomers of **6** via column chromatography on silica gel, (ii) deacetylation of the separated *syn*-diastereoisomer of **6** by reduction with lithium aluminum hydride, (iii) *O*-acetylation using acetyl chloride and assignment of *syn*-**4** by comparison of the GC retention time of the resulting diastereoisomer to those in Figure 4A, and (iv) preparation of the (4*S*)-configured diastereoisomers by selective *O*-acetylation of (4*S*)-4-mercapto-2-heptanol obtained via lipase-catalyzed kinetic resolution of 4-acetylthio-2-heptanone **2** and subsequent reduction with lithium aluminum hydride.

Determination of Odor Thresholds. The odor thresholds of the stereoisomers of 4-mercapto-2-heptanol **3** and the corresponding esters **4**−**6** were determined by GC/O using the method described by Ullrich and Grosch (1987).[27] Table 2 shows the data obtained for the *anti*- and *syn*-diastereoisomers via GC/O on an achiral stationary phase and for the enantiomeric pairs employing chiral stationary phases.

The GC/O-assessments underlying the data compiled in Table 2 were performed by one person. To reduce the potential uncertainties associated with this approach, the following measures were taken: (i) the panelist 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. (ii) For the *anti*- and *syn*-diastereoisomers, the sensory evaluations were performed in duplicate starting from two separately prepared stock solutions. Except for the *syn*-diastereoisomers of **5** and **6**, the results obtained in these independently performed experiments were very consistent (Table 2). In order to rule out that the results obtained for **6** might be affected by the previously reported rearrangement to **4**, the stability of this substance in the solvent diethyl ether was checked by periodic GC analysis of the stored stock solution; no formation of **4** was observed.[11,31] (iii) For the pair of enantiomers showing the lowest resolution ((2*S*,4*R*) and (2*R*,4*S*)-4-mercapto-2-heptanol; peaks **3b** and **3b**′ in Figure 2B), the sensory assessment was performed by a second panelist. In addition, another chiral stationary phase was employed to rule out that the determination of the odor thresholds might be hampered by odor adaptation or saturation effects as indicated for long-lasting odors.[32] As shown in Figure 5, the use of 2,3-di-*O*-*n*-butyryl-6-*O*-*tert*-butyl dimethylsilyl-γ-cyclodextrin as stationary phase resulted in a coelution of the *anti*-enantiomers and a reversed order of elution of the *syn*-enantiomers. The odor thresholds shown in Figure 5 demonstrate the excellent agreement between the two panelists. A comparison with the thresholds determined on the 2,3-di-*O*-acetyl-6-*O*-*tert*-butyl dimethylsilyl-β-cyclodextrin phase (Table 2) confirmed that the order of elution of the enantiomers did not influence the odor thresholds.

For 4-mercapto-2-heptanol **3** odor thresholds of 0.01 and 0.08 ng/L air were determined for the *anti*- and *syn*-diastereoisomers, respectively. Blocking the SH-group of **3** by acetylation resulted in a significant loss in odor intensity. The odor thresholds of the resulting stereoisomers of 4-acetylthio-2-heptanol **6** were increased by a factor of at least 9 for the *anti*-diastereoisomer and at least 15 for the *syn*-diastereoisomer.

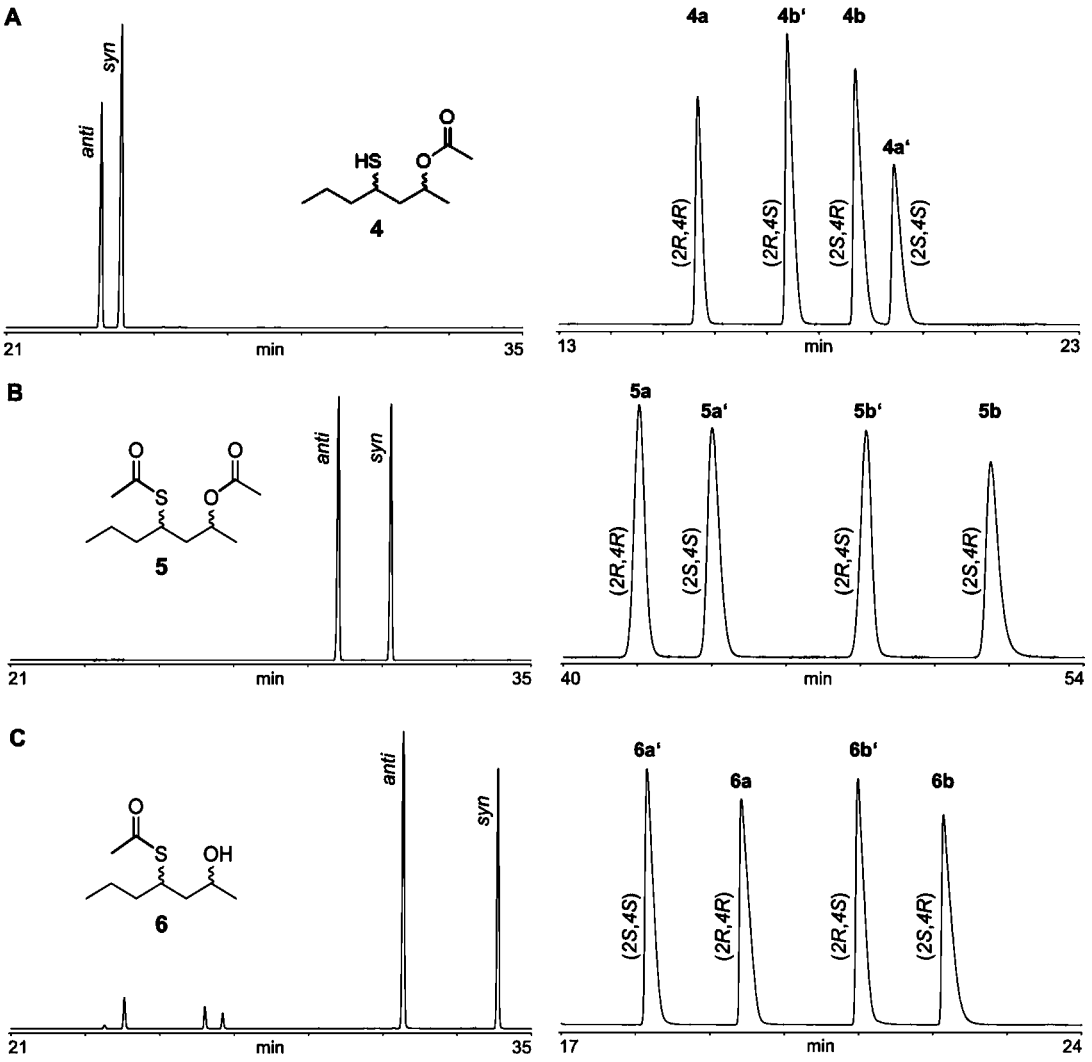


Figure 4. Capillary gas chromatographic separation of the diastereoisomers and the enantiomeric pairs of (A) 4-mercapto-2-heptyl acetate **4**, (B) 4-acetylthio-2-heptyl acetate **5**, and (C) 4-acetylthio-2-heptanol **6**. For conditions, see Material and Methods.

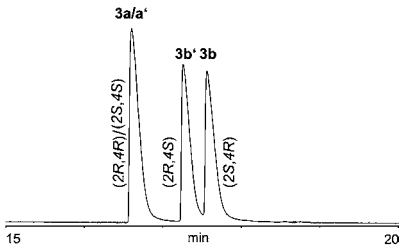
Table 2. Odor Thresholds of the Stereoisomers of 4-Mercapto-2-heptanol 3 and the Corresponding Acetyl Esters 4−6 Determined by GC/O

		odor thresholds of the stereoisomers in air (ng/L)							
no.	compound	*anti*[a]		*syn*[a]		a (2*R*,4*R*)	a′ (2*S*,4*S*)	b (2*S*,4*R*)	b′ (2*R*,4*S*)
3	4-mercapto-2-heptanol	0.01	0.01	0.08	0.08	0.1[b]	0.05[b]	0.2[b]	0.3[b]
4	4-mercapto-2-heptyl acetate	0.07	0.07	0.2	0.2	2.1[c]	0.03[c]	0.2[c]	6.1[c]
5	4-acetylthio-2-heptyl acetate	0.01	0.01	0.3	1.0	5.5[c]	0.09[c]	0.3[c]	1.3[c]
6	4-acetylthio-2-heptanol	0.09	0.14	4.6	1.2	17.2[c]	0.03[c]	0.2[c]	4.9[c]

[a]Odor thresholds were determined by GC/O using fused silica capillary columns coated with a DB-Wax, sensory evaluations of the diastereoisomers were done in duplicate. [b]Heptakis(2,3-di-*O*-acetyl-6-*O*-TBDMS)-β-CD. [c]Heptakis(2,3-di-*O*-methyl-6-*O*-TBDMS)-β-CD. For further conditions, see Material and Methods.

This is in agreement with the significantly increased thresholds of the enantiomers of a homologous series of 4-acetylthio-2-alkanones compared to the respective 4-mercapto-2-alkanones.[20] For the *anti*-diastereoisomer of **3** the impact of the *O*-acetylation on the odor threshold was in the same order of magnitude as that of the *S*-acetylation. With an odor threshold of 0.2 ng/L air the increase in odor threshold of *syn*-**4** was less pronounced compared to *syn*-**3**. Previous sensory evaluations of a spectrum of mercapto alkyl-acetates also revealed mostly higher odor thresholds compared to the corresponding mercapto alcohols.[5,6] Interestingly, the odor threshold of *anti*-4-acetylthio-2-heptyl acetate was the same as that of *anti*-**3**. For the *syn*-diastereoisomer diacetylation resulted in a loss in odor intensity.

In general, the *anti*-configuration of the diastereoisomers of **3**−**6** was found to be more sensorially active (0.01−0.14 ng/L of air) than the *syn*-configuration (0.08−4.6 ng/L of air). A similar influence of the stereochemistry on odor intensities has been reported for the stereoisomers of 3-mercapto-2-methyl-1-pentanol.[18] Low odor threshold values in water (0.03 and 0.04



		2,3-di-*O*-*n*-butyryl-6-*O*-TBDMS-γ-CD	
		3b' (2*R*,4*S*)	3b (2*S*,4*R*)
odor threshold in air (ng/L)	panelist 1	0.32	0.15
	panelist 2	0.32	0.15

Figure 5. Capillary gas chromatographic separation of the stereoisomers of 4-mercapto-2-heptanol **3** using oktakis(2,3-di-*O*-*n*-butyryl-6-*O*-TBDMS)-γ-CD and determination of the odor thresholds of the *syn*-enantiomeric pair of **3** by GC/O. For conditions, see Material and Methods.

μg/L) have been determined for the isomers having the thiol and the methyl group in *anti*-position compared to the odor impressions of the *syn*-isomers, which were estimated to be higher by a factor of 300 and 1000.

The assessment of the odor intensities of the four stereoisomers of **3**−**6** revealed that for each pair of enantiomers the (2*S*)-configured isomers showed the lowest odor thresholds. If this structural prerequisite of (2*S*)-configuration is fulfilled, the odor thresholds were nearly independent from the degree of acetylation. The consistently lowest thresholds (0.03−0.09 ng/L air) were observed for the (2*S*,4*S*)-configured isomers. The odor thresholds of the (2*R*)-configured isomers were highly impacted by the degree of acetylation. For example, the odor threshold of (2*R*,4*R*)-configured 4-acetylthio-2-heptanol **6a** was 570 times higher than that of **6a**′.

Determination of Odor Properties. Odor descriptions for the stereoisomers of 4-mercapto-2-heptanol **3** and the corresponding acetates **4**−**6** are summarized in Table 3. There were concentration-dependent changes of the odor properties; for the purpose of comparison, all assessments were performed with injection volumes corresponding to approximately 6 ng for each stereoisomer at the sniffing port. In general, the *anti*-configured isomers of **3**−**6** were found to possess sulfuric and onion-type odors, whereas the *syn*-configured isomers exhibited sulfuric, green and fruity notes, except for *syn*-**3** possessing an additional savory odor note. Concerning the stereoisomers, the (2*S*,4*S*)-configuration (**3**−**6a**′) appears to be a prerequisite for the intensive smell of raw onion. The corresponding enantiomers were described as being sweet, fruity (**3**−**5a**) or savory-like (**6a**). The assessment of the *syn*-enantiomeric pairs of **3**−**6** revealed a strong influence of the acetylation on the odor property. Sulfuric, green and tropical (grapefruit, passion fruit) odor notes were obtained for the *syn*-enantiomeric pairs of the acetylated derivatives (**4**−**6b** and **b**′). In contrast, the *syn*-isomers of 4-mercapto-2-heptanol exhibited savory, meaty (**3b**) and green aroma notes reminiscent of dill (**3b**′). Comparable odor descriptions such as onion, liver, meaty, sweaty and resinous have been reported for the diastereoisomeric mixture of **3** tasted in 50 ppm NaCl and sugar solutions.[19] Moreover, the odor qualities of the stereoisomers of 4-mercapto-2-heptanol **3** and 4-mercapto-2-heptyl acetate **4** are in good agreement with the tropical, fruity and vegetable odor notes described for the "1,3-oxygen−sulfur olfactophore".[10,11] The sensory evaluation of the structure-related 4-acetylthio-2-heptanol **6** and 4-acetylthio-2-heptyl acetate **5** would support the extension of the "tropical olfactophore" to *S*-acetylated compounds as suggested by Robert et al. (2004).[21]

In conclusion, the obtained GC/O-data reveal a significant impact not only of the acetylation but also of the configurations of the two asymmetric centers on the sensory properties of the investigated compounds. The results indicate that there might be specific stereochemical requirements that have to be taken into account in the establishment of "olfactophore-models". However, further studies involving, for example, additional homologues would be needed to substantiate the general validity of the results elaborated in this study. The conclusions might also be strengthened by increasing the number of panelists and by determining odor thresholds in water and other matrices.

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Notes

The authors declare no competing financial interest.

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Table 3. Odor Properties of the Stereoisomers of 4-Mercapto-2-heptanol 3 and the Corresponding Acetyl Esters 4−6 Determined by GC/O

	odor description of the stereoisomers[a]					
no.[b]	*anti*[c]	*syn*[c]	a (2*R*,4*R*)	a′ (2*S*,4*S*)	b (2*S*,4*R*)	b′ (2*R*,4*S*)
3	sulfury, onion, green	sulfury, green, savory	sulfury, fruity, flowery[d]	sulfury, onion, sweet[d]	sulfury, savory, meaty[d]	sulfury, green, dill[d]
4	sulfury, onion, sweet	sulfury, green, fruity, fig	sulfury, sweet[e]	sulfury, onion[e]	sulfury, passion fruit[e]	sulfury, grapefruit[e]
5	sulfury, onion, sweet	sulfury, fruity, green tea	sulfury, fruity, fresh[e]	sulfury, onion, fruity[e]	sulfury, grapefruit[e]	sulfury, green[e]
6	sulfury, onion	sulfury, fruity	savory, sweet[e]	sulfury, onion, sweet[e]	grapefruit, refreshing[e]	grapefruit, fruity, sweet[e]

[a]GC/O descriptions were made with injection volumes corresponding to ∼6 ng for each stereoisomer at the sniffing port. [b]Arabic numerals correspond to the investigated compounds, see Figure 1; odor properties were determined by GC/O using fused silica capillary columns coated with the following. [c]DB-Wax. [d]Heptakis(2,3-di-*O*-acetyl-6-*O*-TBDMS)-β-CD. [e]Heptakis(2,3-di-*O*-methyl-6-*O*-TBDMS)-β-CD. For further conditions, see Material and Methods.

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