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Analytical and sensory characterization of the stereoisomers of 2-mercapto-4-alkanols and related substituted 1,3-oxathianes

Anja Devenie Riegel

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Vorsitz: Prof. Dr. rer. nat. Michael Rychlik

Prüfer*innen der Dissertation:

1. Prof. Dr. rer. nat. Karl Heinz Engel
2. Prof. Dr. rer. nat. Wilfried Schwab

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“Words are pale shadows of forgotten names. As names have power, words have power. Words can light fires in the minds of men. Words can wring tears from the hardest hearts. There are seven words that will make a person love you. There are ten words that will break a strong man's will.” – NAME OF THE WIND BY PATRIK ROTHFUS

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ABBREVIATIONS

AcS	Acetylthio
AEDA	aroma extract dilution analysis
ANL	*Aspergillus niger* lipase
ATP	adenosine triphosphate
c	conversion rate
CAL-B	*Candida antarctica* Lipase B
cAMP	cyclic adenosine monophosphate
CCL	*Candida cylindracea* lipase
CD	Cyclodextrin
$CDCl_3$	Deuterochloroform
cf.	*Confer*
CH_2Cl_2	Dichloromethane
CH_3	methyl group
CoMFA	comparative molecular field analysis
CRL	*Candida rugosa* lipase
D	Diastereoselectivity
E	Enantioselectivity
ee_P	enantiomeric excess of the product
ee_S	enantiomeric excess of the substrate
EI	electron ionization
eq.	Equivalent
-Et	ethyl group
Et_2O	diethyl ether
EtOAc	ethyl acetate
eV	electron volt
FD	flavor dilution
FID	flame ionization detector
GC	gas chromatography
GC/O	gas chromatography/olfactometry
HCl	hydrochloric acid
HPLC	high-performance liquid chromatography
ID	inner diameter
k	reaction rate constant
kPa	kilo pascal
$LiAlH_4$	lithium aluminum hydride
LRI	linear retention index
-Me	methyl group
MeOH	Methanol

mm	millimetre
MS	mass spectrometry
n	straight-chain of carbon atoms
Na_2SO_4	anhydrous sodium sulfate
$NaBH_4$	sodium borohydride
-*n*-Bu	*n*-butyl group
NMR	nuclear magnetic resonance
-*n*-Pentyl	*n*-pentyl group
-*n*-Pr	*n*-propyl group
PLE	esterase from porcine liver
PPL	porcine pancreas lipase
ppm	parts per million
QSAR	Quantitative structure-activity relationship
R	Rest
R_f	response factor
RT	room temperature
SN	serial number
TBDMS	*tert*-butyldimethylsilyl
tert	Tertiary
THF	Tetrahydrofuran
TLC	thin-layer chromatography
WGL	wheat germ type I lipase

1. INTRODUCTION AND OBJECTIVES

Sulfur-containing volatiles are important for the flavor of various foods (Mussinan & Keelan, **1994**; Blank, **2002**; Vermeulen *et al.*, **2005**). Polyfunctional thiols are a subgroup of sulfur-containing volatiles (Boelens & van Gemert, **1993**; Vermeulen & Collin, **2003**). They play important roles due to their low odor thresholds and distinct odor qualities (Collin *et al.*, **2001**; Collin & Vermeulen, **2006**). Their sensory properties are associated with certain structural features, such as the so-called "tropical olfactophore", which is based on a 1,3-oxygen-sulfur relationship. This structural motif has been studied extensively as it occurs in many aroma compounds that exhibit strong tropical, fruity, or vegetal notes (Rowe, **2002**; Robert *et al.*, **2009**).

3-Mercaptohexanol is a well-known example of a polyfunctional thiol that meets these structural requirements. 3-Mercaptohexanol and its acetate are naturally occurring volatiles, identified for the first time in yellow passion fruits (Engel & Tressl, **1991**) and later shown to also occur in a variety of tropical fruits and wines (Cannon & Ho, **2018**). Since 3-mercaptohexanol is a chiral molecule, research has focused on assigning the configurations of the enantiomers (Heusinger & Mosandl, **1984**), determining their naturally occurring distribution (Weber *et al.*, **1994**), and evaluating their sensory properties (Steinhaus *et al.*, **2008**; Schoenauer *et al.*, **2015**).

As part of a programme aiming at the establishment of structure-odor relationships for other chiral polyfunctional thiols and the elucidation of the impact of the configurations of these molecules on their sensory properties, our research group has been focusing on homologous series of β-mercaptoalkanones and β-mercaptoalkanols. This work had been inspired by the fact that 4-mercapto-2-heptanone, its positional isomer 2-mercapto-4-heptanone, the corresponding *β*-mercaptoalkanols 4-mercapto-2-heptanol and 2-mercapto-4-heptanol, as well as the C9 homologue 4-mercapto-2-nonanol have been reported in cooked red bell peppers (*Capsicum annuum*) (Naef *et al.*, **2008**). The C5 homologue 4-mercapto-2-pentanone has been detected in cheddar cheese (Kleinhenz *et al.*, **2006**; Kleinhenz *et al.*, **2007**).

Analytical and sensory characterizations have been performed for homologous series (C5-C10) of 4-mercapto-2-alkanones (Wakabayashi *et al.*, **2011**; Wakabayashi *et al.*, **2015**) and the corresponding 4-mercapto-2-alkanols (Nörenberg *et al.*, **2017b**). Regarding the positional isomers, assignments of the configurations and assessments

of the sensory properties were available for the homologous series (C6-C10) of 2-mercapto-4-alkanones (Kiske *et al.*, **2019**). The first task in the course of the studies for this thesis was to fill the remaining gap and to elaborate analytical and sensory data for the homologous series (C6-C10) of the corresponding 2-mercapto-4-alkanols. The stereoisomers of these chiral β-mercaptoalkanols should be separated via capillary gas chromatography using chiral stationary phases, the configurations and the GC-order of elution of the stereoisomers should be assigned, and the sensory properties of the stereoisomers should be assessed via capillary gas chromatography/ olfactometry.

The second part of the thesis was devoted to derivatives of these β-mercaptoalkanols, i.e. alkyl-substituted 1,3-oxathianes resulting from their reaction with acetaldehyde. It is noteworthy that already several years before the identification of 3-mercaptohexanol in yellow passion fruits (Engel & Tressl, **1991**) *cis*- and *trans*-2-methyl-4-propyl-1,3-oxathiane, the reaction products from the condensation of 3-mercaptohexanol with acetaldehyde, have been described in a yellow passion fruit concentrate (Winter *et al.*, **1976**). The configurations as well as the sensory properties of the stereoisomers of 2-methyl-4-propyl-1,3-oxathiane were determined (Heusinger & Mosandl, **1984**; Mosandl & Heusinger, **1985**; Küntzel & Frater, **1989**), and the distribution of the stereoisomers was elucidated (Singer *et al.*, **1988**; Weber *et al.*, **1995**). More recently, the presence of *cis*-2-methyl-4-propyl-1,3-oxathiane has been detected in several wines (Chen *et al.*, **2018**; Wang *et al.*, **2021**). A new 1,3-oxathiane *cis*-2,4,4,6-tetramethyl-1,3-oxathiane has been described as a condensation product, resulting from the reaction of 4-mercapto-4-methylpentan-2-ol with acetaldehyde in wine (Wang *et al.*, **2021**).

In the light of these data, it seemed reasonable to devote research to structurally related 1,3-oxathianes resulting from the reaction of β-mercaptoalkanols with acetaldehyde. 2,4-Dimethyl-6-propyl-1,3-oxathiane and 2,6-dimethyl-4-propyl-1,3-oxathiane formed by the reaction of 2-mercapto-4-heptanol and its positional isomer 4-mercapto-2-heptanol, respectively, with acetaldehyde, were selected as examples. Both substances possess three asymmetric centers; the objectives of the study were (i) to separate for each substance the eight stereoisomers via capillary gas chromatography, (ii) to assign their configurations, and (iii) to assess their sensory properties.

The data sets on the sensory properties of the stereoisomers of the 2-mercapto-4-alkanol homologues and of the two alkyl-substituted 1,3-oxathianes should assist in extending the knowledge on the impact of the stereochemistry on the sensory properties of these sulfur-containing volatiles and to get more insight into the importance of stereochemistry for the olfactophore based on a 1,3-oxygen-sulfur structural motif.

2. BACKGROUND

2.1. Sulfur-Containing Volatiles

Polyfunctional thiols are a subgroup of sulfur-containing volatiles that includes a variety of chemical classes, such as mercaptoacids, -alcohols, -aldehydes, -ketones, -esters, -ethers, their corresponding *S*- and *O*-acetylated derivatives, aliphatic and aromatic mercaptans, terpenic mercaptans, acylic mercaptans containing another sulfur function, and heterocyclic mercaptans (Vermeulen *et al.*, **2005**). They have been identified in various natural sources like essential oils, oleoresins or absolutes (geranium, rose, *helichrysum*, pepper, patchouli, cassia, absinth, and hop), fruits (blackcurrant, passion fruit, melon, tomato, grape, pineapple, and grapefruit), vegetables (*Allium* species, *cruciform* families, radish, and truffle), beverages (coffee, beer), and foods (prepared meat, potato, bread). Several review articles regarding their occurrence are available (Boelens & van Gemert, **1993**; McGorrin, **2011**; Cannon & Ho, **2018**).

Volatile polyfunctional thiols are known to be outstanding contributors to the flavor of many foods, because of their low odor thresholds and their pronounced odor qualities. Although they are usually present at low concentrations in foods or beverages, they often contribute significantly to the overall characteristic flavor (Blank, **2002**). Their sensory properties are often strongly determined by their concentration. In most foods, sulfur-compounds contribute to a delicate pleasant flavor character when present at low concentrations (< 1µg/kg), while at higher concentrations, their flavors are perceived as sharp, irritating, unpleasant, and repulsive (Vermeulen *et al.*, **2005**; McGorrin, **2011**).

For example, 3-mercapto-2-methylpentanol exhibits a sulfurous, burnt gummy, and onion-like odor at 1 ppm in a 5% salt solution. At a concentration of 0.5 ppm in a 5% salt solution, the perceived odor changes to meat broth, sulfurous, onion, and leek-like. The corresponding 3-mercapto-2-methylpentanal showed a similar behavior; at 1 ppm the odor was described as sulfurous, pungent, meaty, pungent, oniony, roasty, and at 5 ppm as meaty broth, cooked meat, roasty, pungent (Widder *et al.*, **2000**).

Another example is the so-called “cat ketone” 4-mercapto-4-methyl-2-pentanone, which is repellent at high concentrations but is also key component in Sauvignon blanc wine (Darriet *et al.*, **1995**).

Sulfur-containing volatiles can also be responsible for off-flavor notes. Examples are 3-methyl-2-butene-1-thiol (stunky off-flavor in ale) (Goldstein *et al.*, **1993**) or 4-mercapto-4-methyl-2-pentanone and 3-mercapto-3-methylbutyl formate (cat ketone with "catty, ribes" smell) (McGorrin, **2011**). Table 1 gives examples of volatile polyfunctional thiols that have been found in foods.

Table 1: Polyfunctional volatile thiols, their odor qualities, and occurrence.

compound	odor quality	occurrence	literature
4-mercapto-4-methyl-2-pentanone	boxwood, catty, genista, black currant	wine (Scheurebe, Sauvignon Blanc), grapefruit, basil, sen-cha tea	(Darriet *et al.*, **1995**; Buettner & Schieberle, **2001**; Vermeulen *et al.*, **2006**)
1-*p*-menthene-8-thiol	juicy, grapefruit	grapefruit	(Demole *et al.*, **1982**; Buettner & Schieberle, **2001**)
2-methyl-4-propyl-1,3-oxathiane	tropical, green	passion fruit	(Winter *et al.*, **1976**)
3-mercaptohexanol	lime, rhubarb, citrus, tropical fruit	passion fruit, wine (Sauvignon blanc)	(Engel & Tressl, **1991**; Tominaga *et al.*, **2002**; Collin & Vermeulen, **2006**)
3-mercaptohexyl acetate	box-tree, passion fruit, black currant	wine (Riesling), pink guava	(Tominaga *et al.*, **1996**; Tominaga *et al.*, **1998a**; Steinhaus *et al.*, **2008**; Steinhaus *et al.*, **2009**)
4-mercapto-2-pentanone	black currant, green, potato	cheddar cheese, cocked red bell pepper	(Collin & Vermeulen, **2006**; Kleinhenz *et al.*, **2007**; Naef *et al.*, **2008**)
4-mercapto-2-pentanol	onion (raw), catty, black currant, genista	cheddar cheese, cooked red bell pepper	(Collin & Vermeulen, **2006**; Kleinhenz *et al.*, **2007**; Naef *et al.*, **2008**)

2.1.1. 3-Mercaptohexanol

3-Mercaptohexanol and the esters of 3-(methylthio)hexanol and 3-mercaptohexanol have been identified for the first time in yellow passion fruits (*Passiflora edulis* f. *flavicarpa*) (Engel & Tressl, **1991**). Their absolute configurations and sensory properties were determined; for example, (*R*)-3-mercaptohexyl acetate exhibited a tropical fruit flavor reminiscent of passion fruit, while the (*S*)-enantiomer smelled sulfury and herbal (Heusinger & Mosandl, **1984**; Weber *et al.*, **1992**). Weber *et al.* investigated the natural distribution of the enantiomers in yellow passion fruits and found predominantly the (*S*)-enantiomers to be present: 3-mercaptohexanol (51-81%), 3-(methylthio)hexanol (90-98%), 3-mercaptohexyl acetate (96%), 3-mercaptohexyl butanoate (> 96%) (Weber *et al.*, **1994**; Weber *et al.*, **1995**). Later, 3-mercaptohexanol, 3-mercaptohexanal, and their derivatives were detected in various tropical fruits, wine, and hop (Table 2).

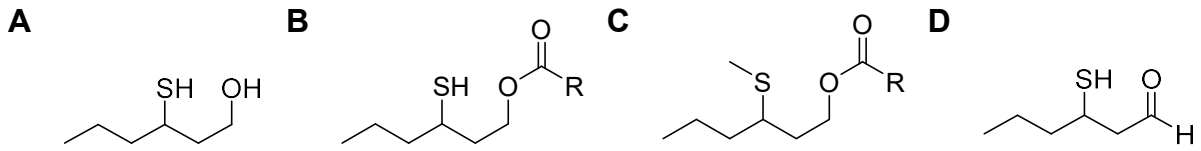


Figure 1: Structures of (A) 3-mercaptohexanol, (B) esters of 3-mercaptohexanol, (C) esters of 3-(methylthio)hexanol (Engel & Tressl, **1991**), and (D) 3-mercaptohexanal (Takoi *et al.*, **2009**), R = - CH_3, -C_3H_7, -C_5H_{11}.

Table 2: Examples of the natural occurrence of 3-mercaptohexanol, 3-mercaptohexanal, and their *S*-, *O*-, and *S*-methyl derivatives.

compound	food	literature
3-mercaptohexanol	hop (*Humulus luplus* L.) Cultivar Nelson Sauvin[1], star fruit (*Averrhoa carambola*)[2], grapefruit juice[3], *Vitis vinifera* (Var. Sauvignon Blanc and Semillon)[4], pink guava (*Psidium guajava* L.)[5], Chardonnay & Solaris[6], vietnamese coriander (*Persicaria odorata* (Lour.) Sojak))[7], *Humulus lupulus* L.[8]	[1](Takoi *et al.*, **2009**) [2](Mahattanatawee *et al.*, **2005**) [3](Lin *et al.*, **2002**) [4](Tominaga *et al.*, **1996**) [4](Tominaga *et al.*, **2006**) [5](Mahattanatawee *et al.*, **2005**) [5](Steinhaus *et al.*, **2009**) [6](Chenot *et al.*, **2020**) [7](Starkenmann *et al.*, **2006**) [8](Gros *et al.*, **2012**)
3-mercaptohexyl acetate	grapefruit juice[1], *Vitis vinifera* (Var. Sauvignon Blanc and Semillon)[2], pink guava (*Psidium guajava* L.)[3], *Humulus lupulus* L.[4]	[1](Lin *et al.*, **2002**) [2](Tominaga *et al.*, **1996**) [2](Tominaga *et al.*, **2006**) [3](Mahattanatawee *et al.*, **2005**) [3](Steinhaus *et al.*, **2009**) [4](Gros *et al.*, **2012**)
3-mercaptohexanal	Chardonnay & Solaris[1], vietnamese coriander (*Persicaria odorata* (Lour.) Sojak))[2], Ciflorette strawberries (*Fragaria* × *ananassa* 'Ciflorette')[3], persimmon (*Diospyros kaki* L.) var. Triumph[4]	[1](Chenot *et al.*, **2020**) [2](Starkenmann *et al.*, **2006**) [3](Cannon *et al.*, **2015**) [4](Wang *et al.*, **2012**)
3-(methylthio)hexanal	Ciflorette strawberries (*Fragaria* × *ananassa* 'Ciflorette')	Cannon *et al.*, 2015
3-acetylthiohexanal	Ciflorette strawberries (*Fragaria* × *ananassa* 'Ciflorette')	Cannon *et al.*, 2015

2.1.2. 2-Methyl-4-Propyl-1,3-Oxathiane

2-Methyl-4-propyl-1,3-oxathiane has been identified in passion fruits *Passiflora edulis* f. *flavicarpa* (Winter *et al.*, **1976**), and more recently also in wine (Chen *et al.*, **2018**). Weber *et al.* investigated the natural distribution of the enantiomers in yellow passion fruits and found predominantly the (*S*)-enantiomers of 2-methyl-4-propyl-1,3-oxathiane (100%) to be present (Weber *et al.*, **1995**). Mosandl & Heusinger described the odors of the four stereoisomers. The (2*S*,4*R*)-stereoisomer stands out in particular. It was described as "fatty, fruity-green, tropical fruits and grapefruit", while the other three stereoisomers were described as "sulfurous and green" (Pickenhagen & Broenner-Schindler, **1984**; Mosandl & Heusinger, **1985**). The *trans*-stereoisomers showed differences in their odor qualities as well; the (2*R*,4*R*)-stereoisomer had earthy notes, while the (2*S*,4*S*)-stereoisomer had more of a sweat character (Mosandl & Heusinger, **1985**). Also, a lower threshold in water (2 ppb) was determined for the (2*S*,4*R*)-stereoisomer than for the (2*R*,4*S*)-stereoisomer (4 ppb) (Pickenhagen & Broenner-Schindler, **1984**).

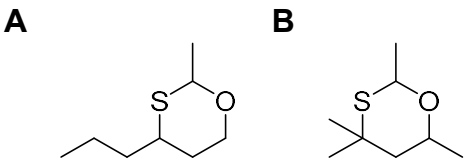


Figure 2: Structures of (A) 2-methyl-4-propyl-1,3-oxathiane (Winter *et al.*, **1976**) and (B) 2,4,4,6-tetramethyl-1,3-oxathiane (Wang *et al.*, **2021**).

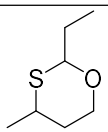
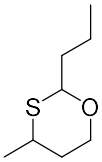
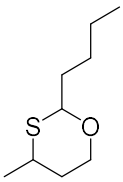
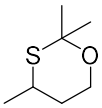
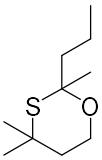
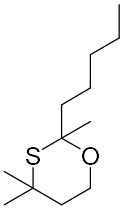
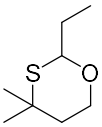
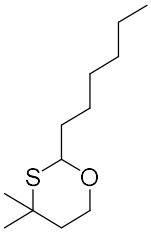
Several polyfunctional thiols have been found in Sauvignon blanc wine, including 3-mercaptohexanol, 3-mercaptohexyl acetate, 4-methyl-4-mercapto-2-pentanone, and 4-methyl-4-mercapto-2-pentanol (Darriet *et al.*, **1995**; Tominaga *et al.*, **1996**; Tominaga *et al.*, **1998a**). Among those compounds, 3-mercaptohexanol was described to react with acetaldehyde in wine to yield 2-methyl-4-propyl-1,3-oxathiane (Chen *et al.*, **2018**). Inspired by the identification of *cis*-2-methyl-4-propyl-1,3-oxathiane in wines, Wang *et al.* proposed that other polyfunctional thiols with structures similar to 3-mercaptohexanol could undergo the same reaction, resulting in the formation of the corresponding 1,3-oxathianes. Such candidates are 3-mercaptopentanol, 2-methyl-

3-mercaptobutanol, 3-mercaptoheptanol, and 4-methyl-4-mercapto-2-pentanol, all bearing a 1,3-sulfur-oxygen-functionality (Wang *et al.*, **2021**).

4-Methyl-4-mercapto-2-pentanol is not as abundant in wines as 3-mercaptohexanol and 3-mercaptohexyl acetate, but it has been reported in botrytized Semillon, Gewürztraminer, Muscat, Pinot Gris, and Riesling (Tominaga *et al.*, **2000**). The corresponding 1,3-oxathiane of 4-methyl-4-mercapto-2-pentanol, *cis*-2,4,4,6-tetramethyl-1,3-oxathiane, was identified in 10 out of 567 analyzed wines. The concentrations ranged from 5 ng/L to 28 ng/L. At lower concentrations, the aroma was described as citrus, green, sweet/caramel, and mango; at higher concentrations, mango/tropical, sweet/caramel, onion/sulfur/sweaty, and sulfurous notes were reported. Only one of the two possible *cis*-enantiomers was detected, but the configuration of this enantiomer has not been assigned yet (Wang *et al.*, **2021**).

The discovery of 2-methyl-4-propyl-1,3-oxathiane resulted in extensive research on modifications of the alkyl substituents at positions 2 and 4. Table 3 gives some examples of synthesized alkylated 1,3-oxathianes, focusing on the elongation of the alkyl chain in position 2. They exhibit a broad range of flavors. Regarding tropical and fruity notes, 2-ethyl-4-methyl-1,3-oxathiane stands out in particular; the odor profile has been described as “floral, fruity, apricot and dried papaya” (Winter & Mottu, **1977**; LeLandais, **2006**; Chen *et al.*, **2013**).

Table 3: Structures of synthesized 1,3-oxathianes and their odor profiles

structure	compound	odor profile
	2-ethyl-4-methyl-1,3-oxathiane	floral, fruity, apricot, dried papaya[1]
	2-propyl-4-methyl-1,3-oxathiane	onion, garlic, sweet[1]
	2-butyl-4-methyl-1,3-oxathiane	green, spicy, leafy, woody, pepper, oily[1]
	2,2,4-trimethyl-1,3-oxathiane	green, woody, terpenes[1]
	4,4-dimethyl-2-propyl-1,3-oxathiane	herbaceous, aromatic, parsley, thujone, lime, sulfurous, bucco leaf[2]
	2,4,4-trimethyl-2-pentyl-1,3-oxathiane	sweet, sugary, onion, basil leaves[2]
	2-ethyl-4,4-dimethyl-1,3-oxathiane	herbaceous, sage, marigold leaves, cassis, mint, sulfurous, onion, thyme[2]
	2-heptyl-4,4-dimethyl-1,3-oxathiane	Cassis, fruity, lemon, green[2]

[1] (Chen *et al.*, **2013**); [2] (LeLandais, **2006**)

2.2. Formation Pathways

Thiols are formed through different pathways from non-volatile precursors, like reducing sugars, thiamine (vitamin B_1), or sulfur-containing amino acids (cysteine and methionine) (Figure 3). Several biogenetic pathways for the formation of polyfunctional thiols are discussed in the literature (Starkenmann *et al.*, **2008b**; Cannon & Ho, **2018**; Muhl *et al.*, **2022**).

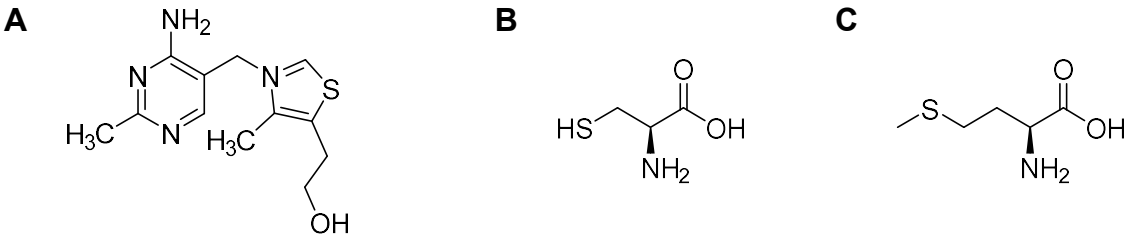


Figure 3: Structures of selected non-volatile precursors of thiols: (A) thiamine (vitamin B_1), (B) cysteine, and (C) methionine.

In thermal processes, one pathway to thiols leads through the MAILLARD reactions. In the first step, dicarbonyls are formed, which catalyze the STRECKER-degradation of cysteine and methionine. Compounds, which are formed in this process, like hydrogen sulfide or methyl mercaptan easily react with unsaturated and carbonyl compounds (Mussinan & Keelan, **1994**). For example, in cooked meat, fried or baked foods, thiols are formed by MAILLARD reactions from the thermal degradation of thiamine. These reactions are favored by acidic conditions (Vermeulen *et al.*, **2005**). A second pathway is the formation of dimethyl sulfide through enzyme-catalyzed reactions (Mussinan & Keelan, **1994**).

The formation of 3-mercaptohexanol (3MH) in wines has been studied by several research groups. Tominaga *et al.* first described the conversion of S-3-(hexanol)-L-cysteine (Cys-3MH) to 3MH by yeast alcoholic fermentation in wine (Tominaga *et al.*, **1998b**).

Later, a second pathway for the biosynthesis of Cys-3MH was discovered. The primary precursor is S-3-(hexanol)-glutathione (G-3MH), which can generate Cys-3MH through degradation by the enzymes γ-glutamyl transpeptidase and carboxypeptidase. This pathway was confirmed by the identification of the intermediate

S-3-(hexanol)-L-cysteinyl glycine (Cys-Gly-3MH) in Sauvignon blanc grape juice (Peyrot des Gachons *et al.*, **2002**; Capone *et al.*, **2011**).

A third proposed mechanism relates to the enzymatic oxidation of lipids to form (*E*)-2-hexenal. (*E*)-2-Hexenal can react with hydrogen sulfide or L-cysteine in the presence of enzymes. This mechanism was confirmed by using deuterated standards in wine. Later, (*E*)-2-hexenol was identified as another precursor of 3-mercaptohexanol in wine (Schneider *et al.*, **2006**; Harsch *et al.*, **2013**).

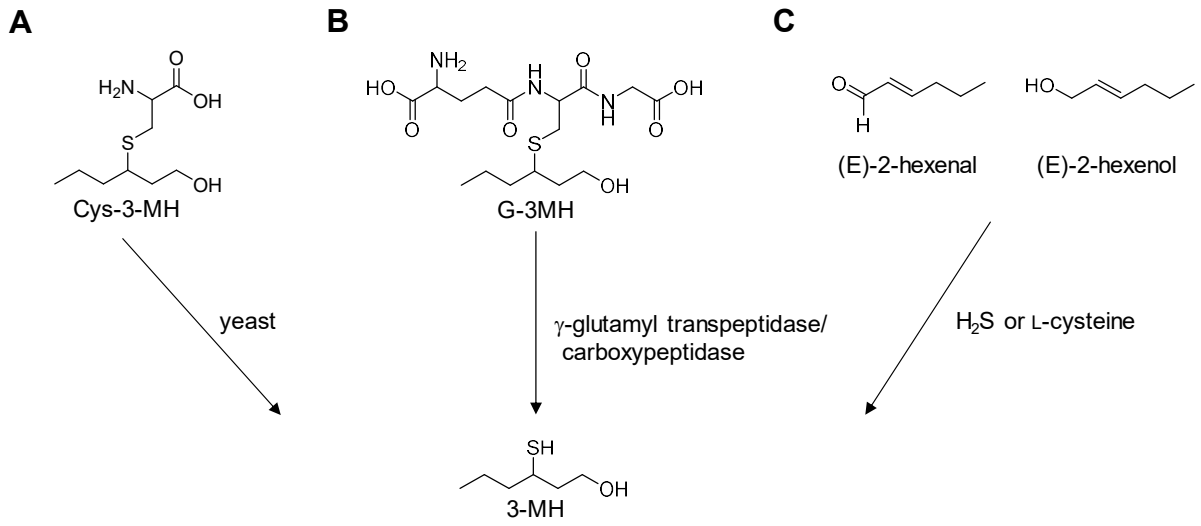


Figure 4: Biogenetic pathways for the formation of 3-mercaptohexanol (3MH) from (A) Cys-3MH, (B) G-3MH, and (C) (*E*)-2-hexenal or (*E*)-2-hexenol (Cannon & Ho, **2018**).

Clarification of the preferred mechanism in wine requires further investigation. Some studies concluded that instead of Cys-3MH and (*E*)-2-hexenal, G-3MH is the major precursor of 3MH. Other studies discovered a mechanism for 3MHA, involving yeast and acetyltransferase (Swiegers *et al.*, **2006**; Subileau *et al.*, **2008**; Roland *et al.*, **2010a**; Roland *et al.*, **2010b**). Roland *et al.* concluded that the formation of 3MH in wines has two distinct origins, one from naturally occurring precursors in grapes and the other from wine-making technology. The latter is associated with the pathway via (*E*)-2-hexenal (Roland *et al.*, **2010c**).

Cys-3MH was also discovered in passion fruits. An extract containing Cys-3MH was treated with β-lyase (from *Eubacterium limosum*). Subsequently, 3MH could be identified together with 3-mercapto-3-methylbutanol (Tominaga & Dubourdieu, **2000**). Fedrizzi *et al.* were able to identify, in addition to the two main precursors, *S*-glutathionylated- and *S*-cysteinylated-3MH, the three missing intermediates leading

to the S-cysteinylated precursor. This suggests that the plant can activate both metabolic pathways (Fedrizzi *et al.*, **2012**).

Biogenesis theories have been discussed for other polyfunctional sulfur-containing aroma compounds found in passion fruits (Tressl & Albrecht, **1986**). An enzyme capable of hydrolyzing thioesters to their respective thiols was found in the mesocarp of passion fruit, which may also be an explanation for the formation of 3MH, 3MHA, and the other mercaptohexyl esters in passion fruit (Tapp *et al.*, **2008**).

Another class of polyfunctional thiols are mercaptoketones. Their formation can be explained by the addition of H_2S to *α*,*β*-unsaturated ketones, which are in turn introduced by cysteine. Subsequent reductions would lead to unsaturated thiols, mercaptoalcohols, (methylthio)thiols, and dithiols. Starkenmann & Niclass confirmed the natural occurrence of cysteine-*S*-conjugates in bell pepper (*Capsicum annuum*) (Starkenmann & Niclass, **2011**).

Polyfunctional sulfur-containing volatile compounds were previously described to be present in cooked bell pepper, including 2-mercapto-4-heptanone and 4-mercapto-2-heptanone and the corresponding mercaptoalkanols 2-mercapto-4-heptanol and 4-mercapto-2-heptanol (Naef *et al.*, **2008**; Nörenberg *et al.*, **2017a**). The structures of the identified non-volatile cysteine-*S*-conjugates and the respective *β*-mercaptoalkanones and *β*-mercaptoalkanols are shown in Figure 5. Starkenmann & Niclass further showed that incubation of the purified precursors with a *β*-lyase (apotryptophanase from *Escherichia coli*) released the expected thiols (Starkenmann & Niclass, **2011**).

A biogenesis pathway could be excluded for the formation of 3MH and 3MHA in guava. Treatment of the isolated putative precursors with β-lyase did not lead to the formation of 3MH. Steinhaus *et al.* hypothesized that unsaturated carbonyls, such as (E)-2-hexenal, could be intermediates in the formation of 3MH (Steinhaus *et al.*, **2008**).

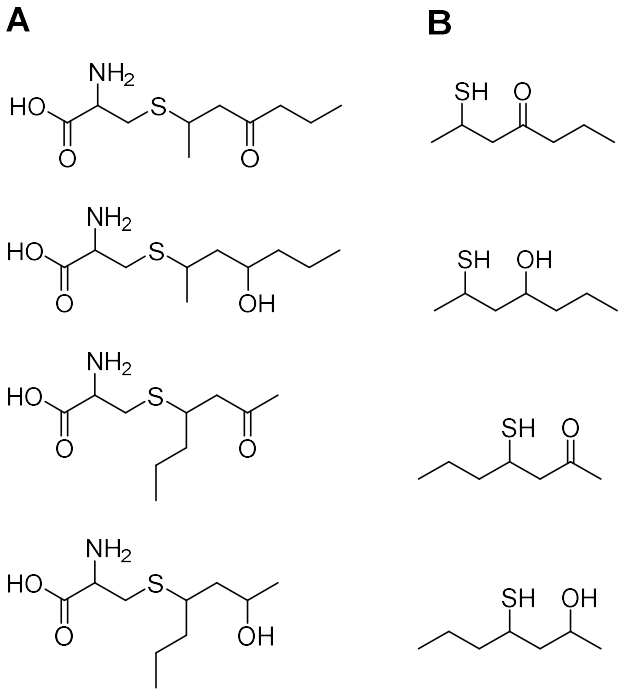


Figure 5: (A) Structures of the cysteine-*S*-conjugates *S*-(3-oxo-1-methylhexyl)-L-cysteine, *S*-(3-oxo-1-propylbutyl)-L-cysteine, *S*-(3-hydroxy-1-methylhexyl)-L-cysteine and *S*-(3-hydroxy-1-propylbutyl)-L-cysteine, and of the polyfunctional volatile thiols 2-mercapto-4-heptanone, 4-mercapto-2-heptanone, 2-mercapto-4-heptanol and 4-mercapto-2-heptanol, identified in bell peppers (*Capsicum annuum* L. cultivar).

Several volatile polyfunctional organo-sulfur compounds were found in noni fruit (*Morinda citrifolia* L.). Among those were 3-(methylthio)propanoic acid, methyl 3-(methylthio)propanate, and ethyl 3-(methylthio)propanate. A biosynthetic pathway from L-methionine was proposed. The first step is a transamination of L-methionine to a keto-acid. Subsequent decarboxylation of the keto-acid leads to the formation of a CoA-ester. Finally, through the reaction with water, methanol, or ethanol, 3-(methylthio)propanoic acid, methyl 3-(methylthio)propanoate, and ethyl 3-(methylthio)propanoate are formed (Wei *et al.*, **2011**).

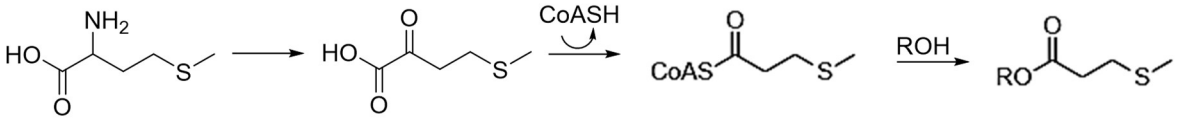


Figure 6: Proposed biosynthetic pathway of 3-(methylthio)propionic acid, methyl 3-(methylthio)-propionate, and ethyl 3-(methylthio)propionate from L-methionine (Wei *et al.*, **2011**)

The stereochemical course of the formation pathways of 3MH and 3MHA was investigated by Capone *et al.* (Table 4). Different grapes were examined and it was revealed that (*S*)-configured G-3MH (referring to the chiral center bearing the sulfur

atom) is present in the first step of the biosynthesis (Capone *et al.*, **2010**; Capone *et al.*, **2011**). This excess is slightly lower in the next step for Cys-3MH and an almost racemic ratio was observed for the free thiol 3MH. However, after the esterification of 3MH to 3MHA, again an excess of the (*S*)-enantiomer is observed (Tominaga *et al.*, **2006**; Chen *et al.*, **2018**).

Table 4: Enantiomeric ratios of G-3MH, Cys-3MH, 3MH, and 3MHA determined in Sauvignon blanc Grape Juice (Tominaga *et al.*, **2006**; Chen *et al.*, **2018**)

compound	enantiomeric ratio (*S*)	(*R*)
G-3MH	80%	20%
Cys-3MH	67%	33%
3MH	55%	45%
3MHA	72%	28%

The differences in the enantiomeric compositions observed in the steps described above could be explained by a stereoselective enzyme-catalyzed esterification of 3MH to 3MHA. The preferential conversion of the (*S)*-enantiomer of 3MHA would result in a racemic ratio of 3MH (Engel, **2020**). Another hypothesis for the almost racemic ratio of 3MH is the conversion of 3MH to other sulfur-containing polyfunctional volatiles, such as (4*S*)-configured 2-methyl-4-propyl-1,3-oxathiane which is the predominating stereoisomer in passion fruits. It is still unknown if the (4*S*)-stereoisomer of 2-methyl-4-propyl-1,3-oxathiane is also preferentially formed in wine (Singer *et al.*, **1988**; Chen *et al.*, **2018**; Engel, **2020**). Similarly, the previously described possibility of the addition of H_2S to (E)-2-hexenal may also influence the enantiomeric ratio of 3-mercaptohexanol (Schneider *et al.*, **2006**; Roland *et al.*, **2011**).

Starkenmann *et al.* found that naturally occurring, odorless cysteine-*S*-conjugates such as *S*-3-(1-hexanol)-L-cysteine in wine, *S*-(1-propyl)-L-cysteine in onions, and *S*-(2-heptyl)-L-cysteine in peppers are converted to volatile thiols in the mouth by microflora. After a time delay of 20-30 s, these volatile thiols were perceived, and the sustained perception of their odor lasted 3 min (Starkenmann *et al.*, **2008a**). In terms of odor perception in wine, this may be an intriguing factor.

2.3. Structure-Odor Correlations

2.3.1. Olfactophores Based on Oxygen-Sulfur-Relationships

For polyfunctional thiols, the arrangement of oxygen and sulfur in the molecules is of particular importance for their sensory properties (Rowe, **2002**). For example, a 1,2-oxygen-sulfur functionality, the so-called “savory olfactophore”, is found in meaty and savory compounds (Rowe, **2002**). Another important structural feature is the "tropical olfactophore" which is based on a 1,3-oxygen-sulfur functionality (Rowe, **2002**). Aroma compounds with this structural feature can exhibit tropical, fruity, and vegetable-like odor notes. The specific structural requirements “around” the basic structural skeleton are outlined in Figure 7. This model has later been extended to include also acetylated compounds (Robert *et al.*, **2009**).

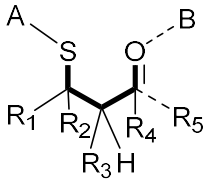
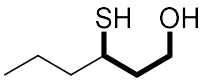
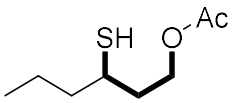
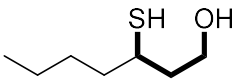
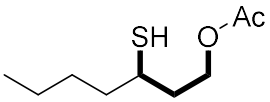
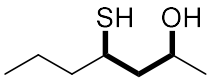
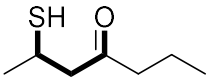
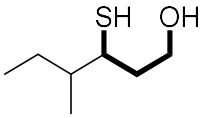
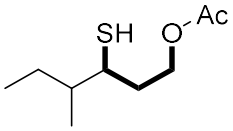


Figure 7: "Tropical olfactophor". A: H, SCH_3, ring; B: H, CH_3, acyl if carbonyl not present; R_1, R_2: H, acyl; R_3: H, acyl, ring; R_4: H, CH_3, ring, OR; R_5: H if carbonyl not present (Rowe, **2002**)

Tertiary thiols are often characterized by a “catty” smell, and their odor thresholds are lower by a factor of 300-3000 than the odor thresholds of primary and secondary thiols. Proton acceptors, such as carbonyl groups, influence the odor towards an intensified sweetness. Mercaptoaldehydes and thioesters are associated with a cheesy odor. Primary mercaptoalkanols (minimum 6 carbon atoms) exhibit delicate fragrances, which are described as greenish, carrot, or rhubarb. Smaller mercaptoalcohols with branching tend to have onion, pungent and plastic-like odors (Boelens & van Gemert, **1993**; Vermeulen & Collin, **2002**; Vermeulen *et al.*, **2005**; Polster & Schieberle, **2017**). Table 5 shows examples of compounds with the "tropical olfactophor" structural motif, with the respective key arrangement shown in bold.

Table 5: Examples of compounds with the "tropical olfactophor" structural motif.

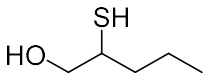
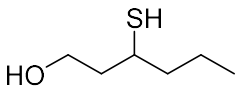
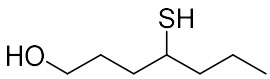
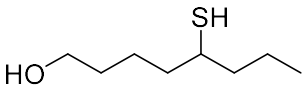
compound	structure	flavor description
3-mercaptohexanol		green, unpleasant[1], rhubarb, lime[2], sausage, meaty, tropical, alliaceous, green peel, guava[3]
3-mercaptohexyl acetate		tropical fruit, blackcurrant, box-tree, alliaceous, cooked, sweet, durian[3]
3-mercaptoheptanol		green, ink, rhubarb, grassy, vegetal, bitter grapefruit, guava, passion fruit, berry notes[3], citrus fruit, vinaigrette, carrot[2]
3-mercaptoheptyl acetate		green, 1-phenylethanol, cabbage, grapefruit, pear, peach[3]
4-mercapto-2-heptanol (isomer I and II)		fruity, tropical, guava, vegetable, watercress[4]
2-mercapto-4-heptanone		arugula, earthy, sesame, grapefruit[4]
3-mercapto-4-methylhexanol		green, ink, rhubarb, 1-phenyl ethanol, red fruit, sulfurous, rubbery, grapefruit, alliaceous[3]
3-mercapto-4-methylhexyl acetate		sage, sulfurous, garlic, buchu, catty[3]

[1](Ferreira *et al.*, **2002**), [2](Vermeulen *et al.*, **2003**), [3](Escher *et al.*, **2006**), [4](Naef *et al.*, **2008**)

The importance of the 1,3-oxygen-sulfur functionality becomes obvious when comparing 2-mercaptopentanol, 3-mercaptohexanol, 4-mercaptoheptanol, and 5-mercaptooctanol, with 1,2-, 1,3-, 1,4- and 1,5-oxygen-sulfur functionality, respectively (Table 6). All four mercaptoalkanols exhibit tropical fruity notes, like grapefruit or passion fruit. However, only 3-mercaptohexanol with a 1,3-oxygen-sulfur functionality shows a low odor threshold (0.053 ng/L air). An elongation of the alkyl chain between the functional groups leads to higher thresholds, as seen for 4-

mercaptoheptanol (31 ng/L in air) and 5-mercaptooctanol (58 ng/L in air). Also, a reduction of the length of the alkyl chain between the functional groups results in higher thresholds (Schellenberg, **2002**; Polster & Schieberle, **2015**).

Table 6: Structures, odor qualities, and odor thresholds of 2-mercaptopentanol, 3-mercaptohexanol, 4-mercaptoheptanol, and 5-mercaptooctanol.

structure	compound	odor	threshold (ng/L in air)
	2-mercaptopentanol	grapefruit, burnt	0.23[1]
	3-mercaptohexanol	grapefruit	0.053[1]
	4-mercaptoheptanol	green, tropical, passion fruit	31[2]
	5-mercaptooctanol	green, tropical, passion fruit	58[2]

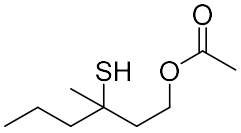
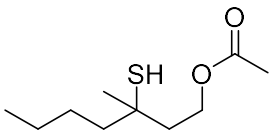
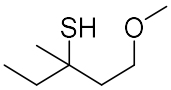
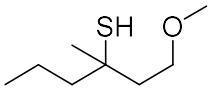
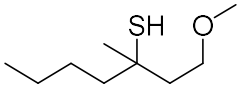
[1](Polster & Schieberle, **2015**), [2](Schellenberg, **2002**)

These structure-odor correlations have also been confirmed by more recent studies involving computer-assisted modulations. Odor thresholds and odor qualities of homologous series of alkane-1-thiols, alkane-2-thiols, alkane-3-thiols, 2-methylalkane-1-thiols, 2-methylalkane-3-thiols, 2-methylalkane-2-thiols and alkane-1,ω-dithiols were compared with those of the corresponding alcohols and methylthioalkanes to investigate the influence of the thiol group (Polster & Schieberle, **2015**). 3D-QSAR models were created using CoMFA to correlate the thresholds of the analyzed thiols with their molecular structures. The calculations showed that most of the differences in odor thresholds can be explained by steric effects. Electrostatic fields had only a minor effect on the odor thresholds. The odor thresholds simulated by the theoretical model agreed well with the experimentally determined data, but more data are needed to validate the model (Polster & Schieberle, **2015**). Similar structure-odor relationships

studies were employing 3D QASR calculations for homologous series of 1-mercapto-3-alkanols, 3-mercaptoalkanols, 2-mercaptoalkanols, 4-mercapto-2-alkanols, 3-mercapto-3-methylalkanols, 1-mercapto-2-methyl-3-alkanols, 3-mercapto-2-methylalkanols, and 2-ethyl-3-mercaptoalkanols (Polster & Schieberle, **2017**).

The results of both investigations are in line with the olfactophor models proposed by Rowe and suggest that there are only a few possibilities, for yet unknown odor-intensive thiols contributing to food aroma. Table 7 lists newly synthesized thiols with low thresholds that have not yet been found in food (Rowe, **2002**; Schoenauer *et al.*, **2015**; Polster & Schieberle, **2017**).

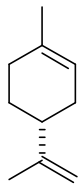
Table 7: Examples of polyfunctional volatile thiols that have not been identified in foods; their structures, odor descriptions, and odor thresholds (Polster & Schieberle, **2015**; Polster & Schieberle, **2017**).

compound	structure	flavor description	odor threshold (ng/L in air)
3-mercapto-3-methylhexyl acetate		black currant	0.0014
3-mercapto-3-methylheptyl acetate		black currant	0.0026
1-methoxy-3-methylpentane-3-thiol		black currant	0.0025
1-methoxy-3-methylhexane-3-thiol		black currant	0.0028
1-methoxy-3-methyl-heptane-3-thiol		black currant	0.0033

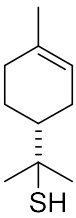
2.3.2. Impact of Chirality

Sensory differences between enantiomers have been first reported for the monoterpene alcohols citronellol (Rienäcker & Ohloff, **1961**) and linalool (Klein & Ohloff, **1962**) and for the monoterpene ketone carvone (Leitereg *et al.*, **1971a**; Leitereg *et al.*, **1971b**; Friedman & Miller, **1971**; Russell & Hills, **1971**). In the meantime, the impact of chirality on odor properties has been demonstrated for many volatiles and comprehensive reviews are available (Brenna *et al.*, **2003**; Sell, **2005**; Bentley, **2006**).

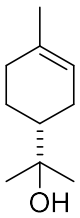
As examples for the impact of both, the exchange of the hydroxyl group by a thiol group and of the configurations, sensory properties of the two monoterpenoids *α*-terpineol and 1-*p*-menthene-8-thiol are summarized in Figure 8. Studies revealed that while the exchange of a carbon atom by a silicon atom did not influence the flavor, the exchange of oxygen by sulfur leads to a significant differences in the odor properties of the respective compounds (Wannagat *et al.*, **1987**).



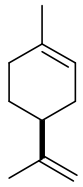
(*R*)-(+)-limonene
odor: orange[1]



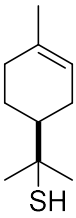
(*R*)-(+)-1-*p*-menthene-8-thiol
odor: fruity[1], grapefruit-like[2]
threshold: 0.000090 ng/L in air[2]



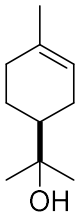
(*R*)-(+)- α-terpineol
odor: fruity, citrus-like[2], lilac[2]
threshold: 4600 ng/L in air[2]



(*S*)-(-)-limonene
odor: turpentine or lemon[1]



(*S*)-(-)-1-*p*-menthene-8-thiol
odor: nearly ordorless[1], grapefruit-like[2]
threshold: 0.0000066 ng/L in air[2]



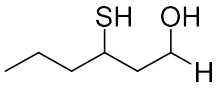
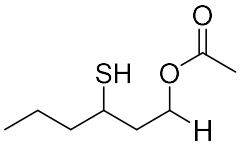
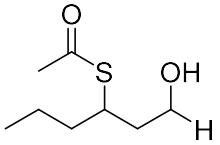
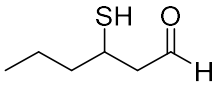
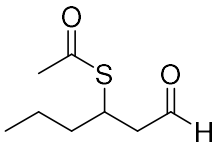
(*S*)-(+)- α-terpineol
odor: fruity, flowery[2], conifer-like[2]
threshold: 570 ng/L in air[2]

[1](Bentley, **2006**), [2](Schoenauer & Schieberle, **2016**)

Figure 8: Structures of the enantiomers of limonene, 1-*p*-menthene-8-thiol, and *α*-terpineol and their odor qualities and odor thresholds.

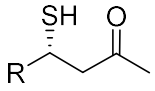
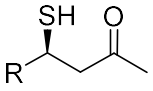
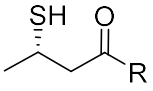
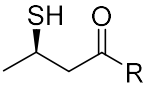
The sensory properties of the enantiomers of 3-mercaptohexanol, 3-mercaptohexanal and their acetyl-derivatives have been extensively studied (Heusinger & Mosandl, **1984**; Weber *et al.*, **1992**; Van De Waal *et al.*, **2002**; Wakabayashi *et al.*, **2003**; Tominaga *et al.*, **2006**; Steinhaus *et al.*, **2008**); they are summarized in Table 8.

Table 8: GC/O-determined odor qualities and thresholds reported for the enantiomers of 3-mercaptohexanol, 3-mercaptohexyl acetate (Steinhaus *et al.*, **2008**), 3-acetylthiohexanol, 3-mercaptohexanal and 3-acethylthiohexanal (Wakabayashi *et al.*, **2003**).

	(***R***)	(***S***)
3-mercaptohexanol 	odor (GC/O): grapefruit threshold: 0.08 ng/L in air	odor(GC/O): grapefruit threshold: 0.07 ng/L in air
3-mercaptohexyl acetate 	odor (GC/O): black currant threshold: 0.10 ng/L in air	odor (GC/O): blackcurrant threshold: 0.03 ng/L in air
3-acetylthiohexanol 	odor (GC/O): fruity, grapefruit, sulfury determined at: 0.3 µg	odor (GC/O): sulfury, roasted, rubber-like determined at: 0.3 µg
3-mercaptohexanal 	odor (GC/O): sulfurous, rubber-like determined at: 0.04 µg	odor (GC/O): green, citrus peel, fruity determined at: 0.04 µg
3-acetylthiohexanal 	odor (GC/O): sulfurous, roasted, citrus peel determined at: 0.1 µg	odor (GC/O): fruity, sweet, grapefruit determined at: 0.1 µg

The sensory properties of the enantiomers of homologous series of β-mercaptoalkanones have been investigated (Wakabayashi *et al.*, **2015**; Kiske *et al.*, **2019**). For the homologous series of 4-mercapto-2-alkanols (C6-C10) consistent differences in the odor qualities between the enantiomers were observed, the odor qualities of enantiomers of the homologous series of 2-mercapto-4-alkanols (C6-C10) showed no significant difference (Table 9).

Table 9: Odor qualities determined for the enantiomers of 4-mercapto-2-alkanones and 2-mercapto-4-alkanones (C6-C10) (Wakabayashi *et al.*, **2015**; Kiske *et al.*, **2019**).

	4-mercapto-2-alkanones		**2-mercapto-4-alkanones**	
				
	(4S)	**(4R)**	**(2S)**	**(2R)**
C6	sulfury, onion□like	citrus peel, blackcurrant	sweat, mustard pungent	urine, pungent
C7	slightly catty grapefruit peel	sweet, grapefruit	pungent, plastic, vegetable	plastic, pungent, vegetable
C8	sulfury, peel oil	blackcurrant, fruity	roasty, onion, vegetable broth	urine, sweet, vegetable broth
C9	sulfury, peel oil	grapefruit peel, fruity	vegetable, earthy, mushroom	vegetable, earthy, mushroom
C10	bell pepper, fruity	bell pepper, grassy	mushroom, dull, vegetables	mushroom, dull, earthy, musty

2.4. Capillary Gas Chromatographic Analysis of Stereoisomers

To enable the capillary gas chromatographic separation of enantiomers, chiral stationary phases are used. Three classes of chiral stationary phases can be distinguished: Amino acid derivatives, metal complexes, and cyclodextrins (Schurig & Nowotny, **1990**; Gey, **2015**).

Chiral stationary phases based on derivatized cyclodextrins (CD) have become of particular importance (Figure 9). Cyclodextrins are cyclic oligosaccharides that consist of at least six D-glucopyranose units, formed by the enzymatic degradation of starch. The glucose units are linked by *α*-1,4-glycosidic bonds. A distinction is made between *α*-, *β*-, and *γ*-cyclodextrins, which consist of six, seven, or eight glucose molecules, respectively (Szente & Szemán, **2013**).

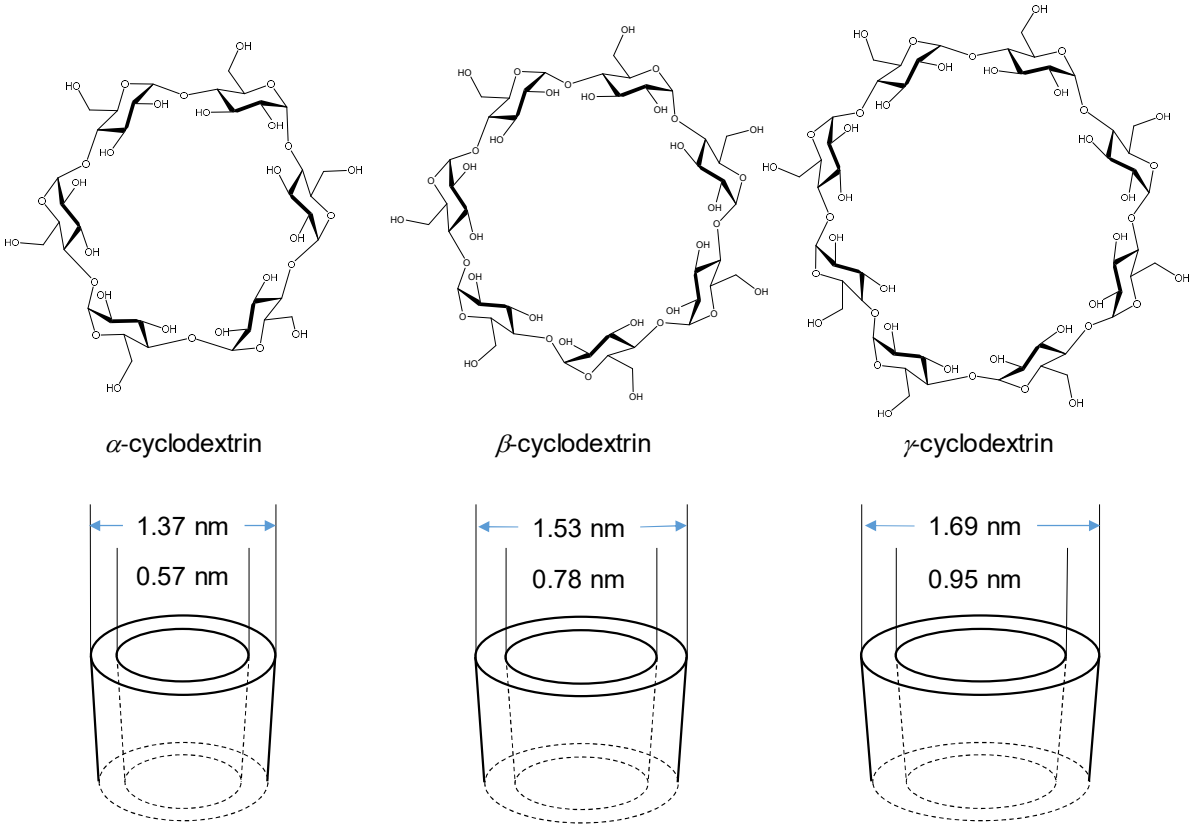


Figure 9: Structures of *α*-, *β*-, and *γ*-cyclodextrins and their chonical dimensions (Szente & Szemán, **2013**).

The glucose molecules of cyclodextrins are in an armchair conformation. The macrocyclic conformation has the shape of a hollow cone, with a hydrophobic interior (due to the carbon atoms) and a hydrophilic exterior (due to the primary and secondary hydroxyl groups). The primary hydroxyl groups (C6-OH) are located at the conically

narrowed opening of this cone and the secondary ones (C2-OH and C3-OH) at the wide opposite side. Due to the tendency of cyclodextrins to complex molecules, hydrophobic functional groups of an enantiomer can enter the cavity and form inclusion complexes (host-guest-complexes). Here, the chiral centers of the enantiomers interact with the hydrophilic centers at the cyclodextrin edge. This leads to chiral recognition and consequently to the separation of these molecules (Schurig & Nowotny, **1990**; Gey, **2015**).

Due to their physical properties, non-derivatized cyclodextrins are unsuitable for the coating of capillary columns. Modified cyclodextrins showed high enantioselectivity. The selectivity is influenced by the nature and position of the substituents. The two most commonly used cyclodextrin phases are heptakis 2,3-di-*O*-methyl-6-*O*-*tert*-butyl-dimethysilyl-*β*-cyclodextrin and 2,6-di-*O*-pentyl-3-*O*-trifluoroacetyl-*β*-cyclodextrin (Schurig & Nowotny, **1990**; Szente & Szemán, **2013**). Several others have been developed (König *et al.*, **1989**; Schurig & Nowotny, **1990**; Dietrich *et al.*, **1992**; Bicchi *et al.*, **1999**; Takahisa, **2005**; Takahisa & Engel, **2005**)

Schmarr developed a chiral stationary phase bearing butyryl-groups at positions 2 and 3 and a *tert*-butyl-dimethylsilyl-group at position 6 (Figure 10) and Nörenberg *et al.* demonstrated that this octakis(2,3-di-*O*-*n*-butyryl-6-*O*-*tert*-butyl-dimethylsilyl)-γ-cyclodextrin phase is suitable for the separation of the four stereoisomers of 2-mercapto-4-heptanol (Schmarr, **1992**; Nörenberg *et al.*, **2017a**). Takahisa & Engel developed a 2,3-di-*O*-methoxymethyl-6-*O*-*tert*-butyl-dimethylsilyl)-β-cyclodextrin phase, that was shown suitable for the separation of the stereoisomers of 4-mercapto-2-hexanol (Takahisa, **2005**; Takahisa & Engel, **2005**; Nörenberg *et al.*, **2017b**).

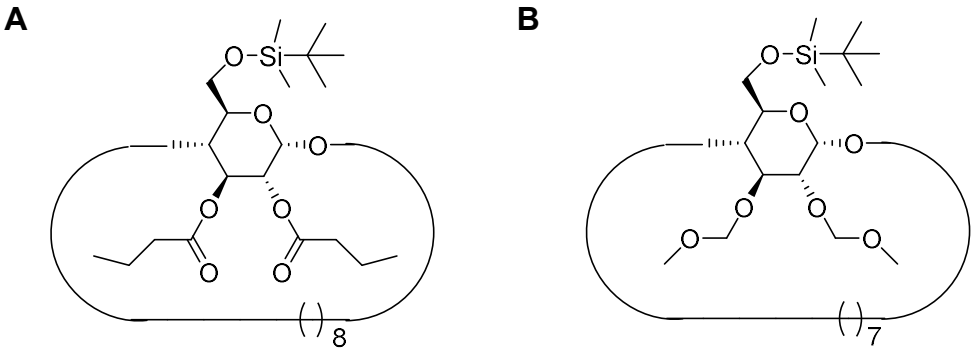


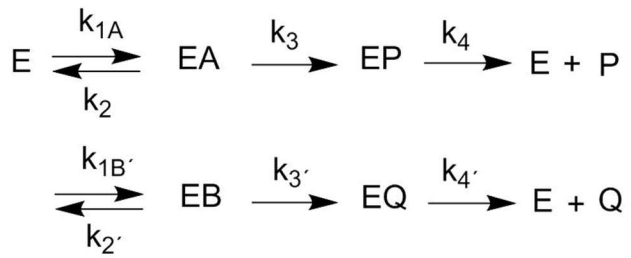
Figure 10: Schematic illustration of a modified glucose unit of (A) octakis-(2,3-di-*O*-*n*-butyryl-6-*O*-*tert*-butyl-dimethylsilyl)-γ-cyclodextrin, modified after (Schmarr, **1992**) and (B) 2,3-di-*O*-methoxymethyl-6-*O*-*tert*-butyl-dimethylsilyl)-β-cyclodextrin phase, modified after (Takahisa, **2005**; Takahisa & Engel, **2005**).

Knowledge on the mechanisms underlying the separations of enantiomers via GC is limited. Consequently, for an unknown compound, neither the order of elution of the enantiomers nor the usefulness of a given chiral stationary phase can be predicted (König *et al.*, **1988**).

2.5. Enzyme-Catalyzed Kinetic Resolutions

In an enzyme-catalyzed kinetic resolution, two enantiomers A and B are transformed at different rates by an enzyme. Ideally, only one of the enantiomers is exclusively accepted as substrate (Ghanem & Aboul-Enein, **2005**).

The sequence of an enzyme-catalyzed kinetic resolution is schematically shown in Figure 11. In this model, it is assumed that the reaction is irreversible and that no inhibitor is present. A and B are the fast- and slow-reacting enantiomers, respectively, competing for the same enzyme binding site. First, an enzyme-substrate-complex EA with the preferred enantiomer A is formed. Since only the formation of the enzyme-substrate-complex is reversible, the intermediate EA has two options: it can react back into enzyme E and substrate A with rate constant k_2, or it can be transformed into the product-enzyme-complex EP with rate constant k_3 and then into the product P and free enzyme E with rate constant k_4. Enzyme E may also react with the non-preferred enantiomer B, and the corresponding product Q is formed in the same manner. In this case, each of these steps proceeds more slowly than when the enzyme reacts only with enantiomer A (Chen et al., 1982).



E	Enzyme
A	Preferred enantiomer A
B	Non-preferred enantiomer B
EA	Enzyme-Substrate-Complex (A)
EB	Enzyme-Substrate-Complex (B)
EP	Enzyme-Product-Complex (A)
EQ	Enzyme-Product-Complex (B)
P	Product formed from Substrate A
Q	Product formed from Substrate B
k	rate constant

Figure 11: Schematic representation of an enzyme-catalyzed kinetic resolution, modified after (Chen *et al.*, **1982**).

The enantioselectivity E is used to measure the ability of an enzyme to distinguish between two enantiomers A and B (Bornscheuer & Kazlauskas, **2006**) and corresponds to the ratio of their specificity constants, as shown in equation 1 (Chen *et al.*, **1982**).

$$E = \frac{V_A/K_A}{V_B/K_B} \qquad \text{(equation 1)}$$

E	Enantioselectivity
V_A	Maximum Velocity for Enantiomer A
V_B	Maximum Velocity for Enantiomer B
K_A	Michaelis-Menten-Constant of Enantiomer A
K_B	Michaelis-Menten-Constant of Enantiomer B

When following kinetic resolutions via GC, the peak areas determined for the fast- and the slow-reacting enantiomers of the substrate (A and B) and of the corresponding products (P and Q) can be used to determine the enantiomeric excesses (ee) and the conversion rate (c), and to calculate the enantioselectivity (E), according to equations 2a and 2b.

$$E = \frac{ln\left[(1-c)(1-ee(S))\right]}{ln\left[(1-c)(1-ee(S))\right]} \qquad \text{(equation 2a)}$$

$$E = \frac{ln\left[1-c(1-ee(P))\right]}{ln\left[1-c(1-ee(P))\right]} \qquad \text{(equation 2b)}$$

E	Enantioselectivity
c	Conversion rate
V_A and V_B	Maximum Velocity for Enantiomer A and B
K_A and K_B	Michaelis-Menten-Constant of Enantiomer A and B
$ee(S)$ and $ee(P)$	Enantiomeric excess of the substrate S and product P

2.5.1. Lipase CAL-B

Lipases (EC 3.1.1.3) are a class of hydrolases, which are distributed in plants, higher animals, and microorganisms. They are responsible for the cleavage of fatty acids from emulsified triglycerides. The enzyme and the substrate have opposite polarities, hence the biotransformation occurs at an oil/water interface. Lipases show higher activities when the interface becomes larger (Schmid & Verger, **1998**). Lipases are applied, for example, to catalyze the hydrolysis of carboxylic acid esters or the reverse reaction in anhydrous organic solvents (Koeller & Wong, **2001**; Klibanov, **2001**). Lipases and esterases are used in manufacturing foods and leather, as well as in large-scale

applications in fine chemicals like pharmaceuticals and cosmetics (Schulze & Wubbolts, **1999**; Kovac *et al.*, **2000**)

The yeast *Candida antarctica* generates two lipases: Lipase A and B. Lipase A is calcium-dependent and thermostable, while lipase B is calcium-independent and less heat-stable, and more alkali-resistant than lipase A. Lipase A and B differ in their substrate specificities. Lipase A shows activity towards triglycerides, with a unique preference for an sn-2-bond (Rogalska *et al.*, **1993**), while lipase B shows activity toward esters, amides, and thiols. Lipase A has a molecular weight of 43 kD and an isoelectric point of 8.0 ± 0.2 (Michiyo, **1988**). Lipase B (CAL-B) consists of 317 amino acids with an approximate size of 30 Å x 40 Å x 50 Å and a molecular weight of 33 kDa (Uppenberg *et al.*, **1994a**). The pH optimum for CAL-B is 7. In aqueous solutions, the enzyme is stable in a pH range of 3.5-9.5. The denaturation temperature varies between 50 °C and 60 °C, depending on the pH value. Immobilization increases the stability of the enzyme and allows CAL-B to be used also in organic solvents (Anderson *et al.*, **1998**).

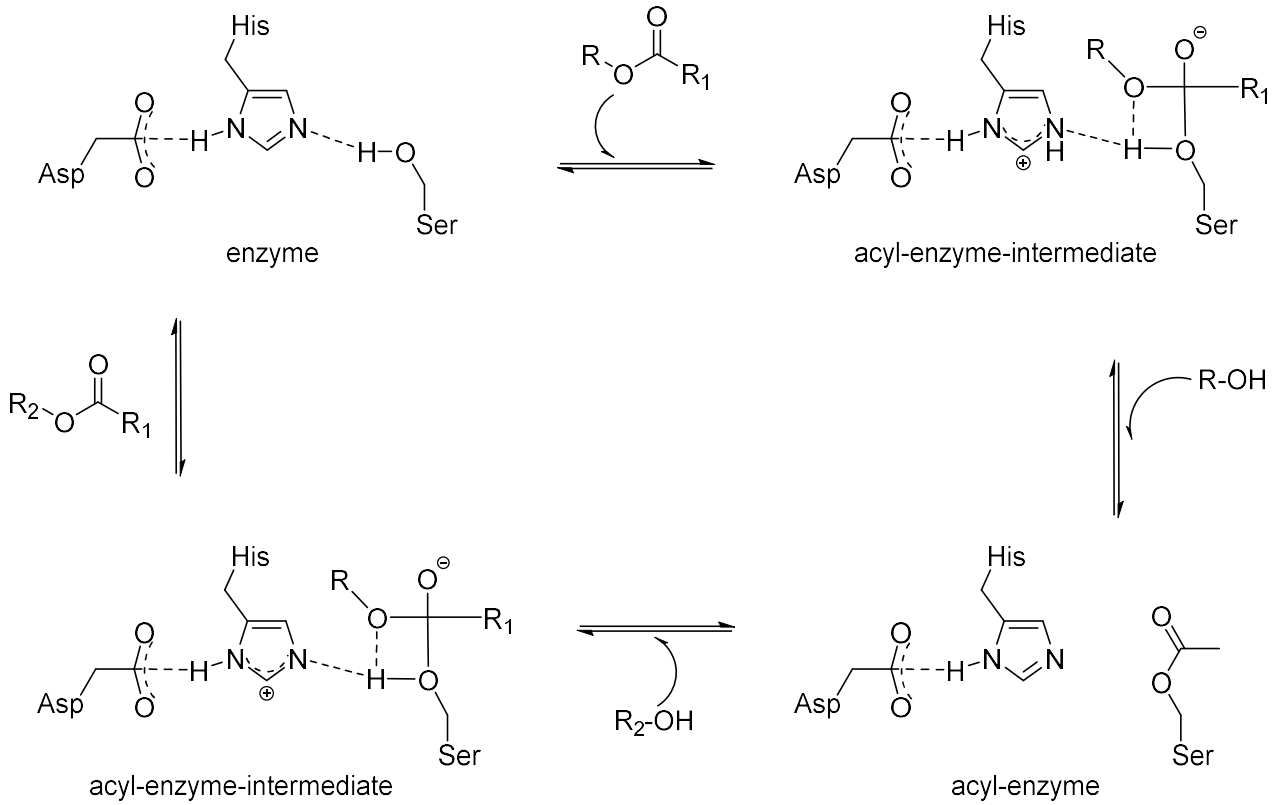


Figure 12: Catalytic mechanism of CAL-B, modified after (Anderson *et al.*, **1998**).

The central *β*-sheet is composed of seven strands (*β*1-*β*7). The last six of them are parallel (*β*2-*β*7). Ten *α*-helices were found in the enzyme (Uppenberg *et al.*, **1994a**).

The active site of the *α*/*β*-hydrolase-fold consists of three amino acid residues (Ser-His-Asp), the so-called catalytic triad, which is responsible for the enzyme-catalyzed reaction. CAL-B has the same mechanism of action as a serine protease (Figure 12) (Uppenberg *et al.*, **1994b**).

CAL-B prefers the (*R*)-enantiomer of secondary alcohols. This selectivity is used to obtain optically pure or enantiomerically enriched compounds via enzyme-catalyzed kinetic resolution (Anderson *et al.*, **1998**).

3. RESULTS

3.1. Publication I

Riegel, A. D.; Kiske, C., Dudko, V.; Poplacean, I.; Eisenreich, W.; Engel, K.-H. Assignment of the Absolute Configurations and Assessment of the Sensory properties of the Stereoisomers of a Homologous Series of 2-Mercapto-4-alkanols (C6-C10)
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A homologous series of 2-mercapto-4-alkanols (C6-C10) was synthesized, and their stereoisomers were separated by capillary gas chromatography (GC). The determination of the GC-order of elution of the *anti*- and *syn*-diastereoisomers was based on ^{1}H-NMR data. The configurations of the stereoisomers separated by GC on cyclodextrin derivatives as chiral stationary phases were assigned by obtaining enantiomerically enriched thiols via lipase-catalyzed kinetic resolutions. Odor thresholds and odor qualities were determined via GC/olfactometry, and structure-odor-relationships were elucidated.

The odor thresholds of the stereoisomers of 2-mercapto-4-alkanols were generally higher than those previously reported for a homologous series of 4-mercapto-2-alkanols. For the C7 and C8 homologues, the odor thresholds were impacted by the configuration of the asymmetric center bearing the hydroxyl group. The (2*S*,4*R*)-stereoisomers had significantly higher thresholds than the (2S,4*S*)-stereoisomers. For the other chain lengths, the odor thresholds of the stereoisomers were in the same order of magnitude.

Compared to the isomeric 4-mercapto-2-alkanols, fruity, tropical notes were lacking. The stereoisomers of the chain lengths C6-C8 were predominantly described as vegetable-like and pungent; for the chain lengths, C9 and C10 mushroom was the prevailing descriptor. These results were in agreement with those previously reported for the corresponding 2-mercapto-4-alkanones and 2-acethylthio-4-alkanones. No dependence on the configuration was observed for the odor qualities, indicating that for 2-mercapto-4-alkanols the chain length rather than the configuration is the main driver determining the odor properties.

Candidates Contribution

Syntheses and purifications of the homologous series of 2-mercapto-4-alkanols; development of the methods for the separation of the stereoisomers by capillary gas chromatography using chiral stationary phases; determination of the absolute configurations and the GC-order of elutions by interpretation of NMR-data and performance of enzyme-catalyzed kinetic resolutions; determination of the odor thresholds and the odor descriptions by GC/O; writing and revision of the manuscript, including Figures and Tables, and the Supporting Information.

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Absolute Configurations and Sensory Properties of the Stereoisomers of a Homologous Series (C6−C10) of 2-Mercapto-4-alkanols

Anja Devenie Riegel, Christiane Kiske, Viktoriia Dudko, Iulia Poplacean, Wolfgang Eisenreich, and Karl-Heinz Engel*

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ABSTRACT: A homologous series (C6−C10) of 2-mercapto-4-alkanols was obtained by the addition of thioacetic acid to the respective alkenones and subsequent reduction with $LiAlH_4$. Gas chromatographic separation of the stereoisomers was achieved using chiral stationary phases. Their absolute configurations were assigned by the correlation of ^1H NMR data and enzyme-catalyzed kinetic resolutions. Odor thresholds and odor qualities were determined by capillary gas chromatography/olfactometry. Compared to the odor qualities reported for the isomeric 4-mercapto-2-alkanols, the homologous series of 2-mercapto-4-alkanols lacked fruity, tropical notes. There was no consistent correlation between the configurations and the odor qualities. However, the observed odor thresholds indicated the importance of the configuration of the asymmetric center bearing the hydroxyl group and the alkyl substituent. The length of this alkyl chain is a main driver for the odor properties, ranging from pungent, vegetable to earthy, mushroom notes.

KEYWORDS: *2-mercapto-4-alkanols, absolute configuration, structure−odor relationships, odor threshold, odor quality, stereochemistry, chirality*

INTRODUCTION

Sulfur-containing volatiles are of importance to the flavor of various foods.[1−3] Polyfunctional thiols play particular roles, owing to their low odor thresholds and pronounced odor qualities.[4−6] Their sensory properties have been correlated with certain structural features, such as the so-called "tropical olfactophore", which is based on a 1,3-oxygen−sulfur function relationship.[7] Examples of compounds possessing this structural element are β-mercaptoalkanones and β-mercaptoalkanols. 4-Mercapto-2-heptanone, its positional isomer 2-mercapto-4-heptanone, and the respective β-mercaptoalkanols 4-mercapto-2-heptanol and 2-mercapto-4-heptanol, as well as the C9 homologue 4-mercapto-2-nonanol, have been reported in cooked red bell pepper (*Capsicum annuum*).[8] In cheddar cheese, the C5-homologue 4-mercapto-2-pentanone has been described.[9,10]

The aim of our studies was to establish structure−odor relationships for these compound classes, with particular focus on the impact of the configurations of these chiral molecules on their sensory properties. In previous investigations, (i) we implemented capillary gas chromatographic separations of the stereoisomers of homologous series of 4-mercapto-2-alkanones (C5−C10), 4-mercapto-2-alkanols (C5−C10), and 2-mercapto-4-alkanones (C6−C10) using various types of cyclodextrin derivatives as chiral stationary phases, (ii) we determined the absolute configurations and assigned the GC order of elution of the stereoisomers of members of these homologous series via combinations of lipase-catalyzed kinetic resolutions of the respective acetylthio-compounds and NMR analyses exploiting anisotropy effects in diastereoisomeric derivatives, and (iii) we finally assessed the sensory properties, i.e., odor thresholds and odor qualities of the stereoisomers, via capillary gas chromatography/olfactometry.[11−16]

The objective of this study was to complement the existing data on 4-mercapto-2-alkanones, 4-mercapto-2-alkanols, and 2-mercapto-4-alkanones by applying these concepts to the investigation of a homologous series (C6−C10) of 2-mercapto-4-alkanols. This should provide further insight into the influence of the spatial arrangements of the functional groups on the sensory properties of these molecules and thus create the basis for a comparative assessment of structure−odor relationships of chiral β-mercaptoalkanones and β-mercaptoalkanols.

MATERIALS AND METHODS

Chemicals. 4-Hexen-3-one, lipase from *Candida antarctica* (B lipase, adsorbed on a macroporous acrylic resin, CAL-B), lithium aluminum hydride ($LiAlH_4$), sodium borohydride ($NaBH_4$), (*E*)-2-decenal (≥95%), deuterochloroform ($CDCl_3$), tetrahydrofuran (THF), anhydrous sodium sulfate (Na_2SO_4), *n*-alkane standard solutions (C8−C40), dichloromethane (CH_2Cl_2), and ethyl acetate (EtOAc) were purchased from Sigma-Aldrich (Steinheim, Germany). Thioacetic acid was obtained from Merck Schuchardt OHG

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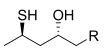
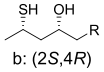
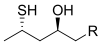
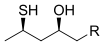
(Hohenbrunn, Germany). 2-Octen-4-one was purchased from TCI Europe (Zwijndrecht, Belgium). Silica gel (NormaSil60, 40−63 μm), *n*-hexane, methanol, and diethyl ether were from VWR Chemicals (Leuven, Belgium).

Syntheses. *2-Mercapto-4-alkanols.* According to previously described procedures, a homologous series of 2-acetylthio-4-alkanones (C6−C10) was synthesized by the Michael-type addition of thioacetic acid to the corresponding 2-alkene-4-ones.[14,16] 2-Mercapto-4-alkanols **1**−**5** (Table 1) were prepared by subsequent

Table 1. Structures of the Stereoisomers of 2-Mercapto-4-alkanols 1−5

2-mercapto-4-alkanols				
no.	chain length	R	structure	
			anti	*syn*
1	C6	methyl		
2	C7	ethyl	a: (2*R*,4*R*)	b: (2*S*,4*R*)
3	C8	*n*-propyl		
4	C9	*n*-butyl		
5	C10	*n*-pentyl	a': (2*S*,4*S*)	b': (2*R*,4*S*)

reduction using $LiAlH_4$.[8,13] The reaction was carried out under an argon atmosphere at 0 °C. The 2-acetylthio-4-alkanones (C6: 5.63 mmol, C7: 3.72 mmol, C8: 3.46 mmol, C9: 3.24 mmol, and C10: 3.05 mmol) were dissolved in 8 mL of dry THF. This solution was added dropwise to a suspension of $LiAlH_4$ (25.33 mmol for C6, 16.75 mmol for C7, 15.59 mmol for C8, 14.58 mmol for C9, and 13.72 mmol for C10) in 13 mL of dry THF. After 1 h, the cooling was removed and the mixture was stirred overnight at RT. The reaction was quenched carefully by adding distilled water under ice cooling. The aqueous phase was adjusted to pH 2 using hydrochloric acid (5%) and extracted three times with diethyl ether. The combined organic phases were dried with anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The crude products were purified via column chromatography. The fractions were checked by thin-layer chromatography (TLC, ALUGRAM SIL G/UV254, Macherey-Nagel, Germany) with 10% sulfuric acid as visualization reagent, using the same eluent as for the column chromatography. 2-Mercapto-4-hexanol **1**: 0.74 g (5.50 mmol; mol yield (corrected by taking the purity into account): 74%; purity (GC): 75%); purity (GC) after column chromatography on silica gel (*n*-hexane/EtOAc, 5 + 1, v/v): 95%; linear retention index (LRI):[17] 1632 (anti), 1655 (syn) on DB-Wax and 1010 (anti), 1021 (syn) on DB-1. 2-Mercapto-4-heptanol **2**: 0.57 g (3.83 mmol; mol yield: 83%; purity (GC): 81%); purity (GC) after column chromatography on silica gel (*n*-hexane/EtOAc, 9+2, v/v): 96%; LRI: 1719 (anti), 1742 (syn) on DB-Wax and 1106 (anti), 1117 (syn) on DB-1. 2-Mercapto-4-octanol **3**: 0.54 g (3.30 mmol; mol yield: 85%; purity (GC): 89%); purity (GC) after column chromatography on silica gel (*n*-hexane/EtOAc, 3+2, v/v): 92%; LRI: 1833 (anti), 1856 (syn) on DB-Wax and 1210 (anti), 1220 (syn) on DB-1. 2-Mercapto-4-nonanol **4**: 0.58 g (3.31 mmol; mol yield: 85%; purity (GC): 83%); purity (GC) after column chromatography on silica gel (*n*-hexane/EtOAc, 7 + 1, v/v): 94%; LRI: 1939 (anti), 1961 (syn) on DB-Wax and 1312 (anti), 1323 (syn) on DB-1. 2-Mercapto-4-decanol **5**: 0.61 g (3.20 mmol; mol yield: 87%; purity (GC): 83%); purity (GC) after column chromatography on silica gel (*n*-hexane/EtOAc, 5+2, v/v): 98%; LRI 2036 (anti), 2058 (syn) on DB-Wax and 1417 (anti), 1427 (syn) on DB-1. Chromatographic, mass spectrometric, and NMR data were in agreement with those previously reported.[8,18]

2-Acetylthio-4-hexanol. 2-Acetylthio-4-hexanol **6** was synthesized in analogy to the previously described procedure for the isomeric 4-acetylthio-2-alkanols.[13] 2-Acetylthio-2-hexanone (2.6 mmol) was dissolved in 20 mL of methanol and 80 mL of potassium phosphate buffer (pH 7.4). A solution of $NaBH_4$ (5.7 mmol) in 8 mL water was added dropwise under ice cooling. After 30 min, the reaction was stopped by adjusting the pH to 5, using hydrochloric acid (5%). The aqueous phase was extracted three times with CH_2Cl_2, and the combined organic phases were dried with anhydrous Na_2SO_4, filtered, and removed under reduced pressure, resulting in 0.54 g (3.05 mmol; yield (corrected by taking the purity into account): 83%; purity (GC): 78%; ratio of diastereoisomers: 49:51) of the crude product. The crude product (0.25 g) was subjected to column chromatography on silica gel (*n*-hexane/EtOAc, 3:1) to separate the diastereoisomers, yielding 0.12 g of *anti*-**6** (0.68 mmol, 47%, purity (GC): 56%) and 0.05 g of *syn*-**6** (0.35 mmol, 24%, purity (GC): 63%). *Anti*-**6** and *syn*-**6** were further purified by column chromatography on silica gel (*n*-hexane/EtOAc, 2:1), purity (GC): *anti*-**6**: 76% and *syn*-**6**: 89%; LRI: 1882 (*anti*-**6**), 1970 (*syn*-**6**) on DB-Wax; 1116 (*anti*-**6**), 1143 (*syn*-**6**) on DB-1. The fractions were checked by thin-layer chromatography (TLC, ALUGRAM SIL G/UV254, Macherey-Nagel, Germany) with 10% sulfuric acid as visualization reagent.

Mass spectrometric and NMR data of the synthesized compounds **1**−**6** are given in the Supporting Information.

Lipase-Catalyzed Kinetic Resolutions. Enantiomerically enriched 2-mercapto-4-alkanones with chain lengths C6−C10 were obtained following the previously described procedure.[14,16] Briefly, the respective racemic 2-acetylthio-4-alkanones were added to a 50 mM potassium phosphate buffer (pH 7.4) solution. CAL-B was added to the emulsion. After the mixture was stirred for a defined time at RT,[14,16] the reaction was stopped by filtration. The aqueous layer was extracted three times with diethyl ether, and the combined organic layers were dried with anhydrous Na_2SO_4, filtered, and removed under reduced pressure. The formed 2-(*R*)-mercapto-4-alkanones were separated from the unreacted 2-(*S*)-acetylthio-4-alkanones by column chromatography.

NMR Spectroscopy. The compounds were dissolved in $CDCl_3$. The spectra were recorded at 27 °C, with Avance 500 spectrometers (Bruker, Billerica, MA). ^1H NMR and ^{13}C NMR spectra were measured at 500 and 126 MHz, respectively. ^1H-detected experiments were done with an inverse $^1H/^{13}C$ probe head. 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. All signals were assigned by proton−proton and proton−carbon correlation experiments (H,H-COSY, HSQC, and HMBC). Data processing was done with the MestreNova software (Mestrelab Research, Santiago de Compostela, Spain).

GC Analyses. *Capillary Gas Chromatography (GC-FID).* A HP5890 gas chromatograph (Hewlett-Packard INC, Waldbronn, Germany) was equipped with a split/splitless injector (215 °C, split ratio 1:10) and an FID (300 °C). Hydrogen was used as the carrier gas at a constant pressure of 150 kPa. Column 1 was installed (Table 2); temperature program: from 40 °C (5 min hold) to 240 °C (30 min hold) at 4 °C/min.

A 6890N gas chromatograph (Agilent Technologies, Waldbronn, Germany) was equipped with a split/splitless injector (230 °C, split ratio 1:10) and an FID (300 °C). Hydrogen was used as the carrier gas at a constant flow of 1.8 mL/min. Column 2 was installed (Table 2); temperature program: from 60 °C (5 min hold) to 300 °C (5 min hold) at 5 °C/min.

Stereoselective Analysis. A 6890N chromatograph (Agilent Technologies) was equipped with a split/splitless injector (230 °C, a split ratio of 1:10) and an FID (300 °C). Hydrogen was used as the carrier gas at a constant flow of 1.6 mL/min. The enantioselective analysis of 2-acetylthio-4-alkanones and 2-mercapto-4-alkanones was carried out, as previously described.[14,16] For the stereoselective analysis of 2-mercapto-4-hexanol **1** columns 3[19] and 4, for the separation of the enantiomers of *syn*-**1** column 5,[20] and for analysis of 2-mercapto-4-alkanols (C7−C10) **2**−**5** column 6[20] were used (Table 2). Temperature programs are given in the Supporting Information.

Multidimensional Gas Chromatography (MDGC). The instrumentation consisted of two coupled GC 8000 (Carlo Erba Instruments). A 60 m × 0.32 mm i.d., 0.25 μm, DB-Wax column

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Table 2. Columns Employed for Capillary GC Separations

column no.	stationary phase; column diameters	compounds separated
1	DB-WAX; i.d.; *df*: 0.25 μm (Agilent Technologies, Waldbronn, Germany)	**1**−**5**
2	DB-1; 60 m × 0.32 mm i.d.; *df*: 0.25 μm (J&W Scientific, Waldbronn, Germany)	**1**−**5**
3	Octakis(2,3-di-*O*-[(*S*)-2-methylbutyryl]-6-*O*-*tert*-butyldimethylsilyl)-γ-cyclodextrin 25% in OV1701-vi; 30 m × 0.25 mm i.d., *df*: 0.25 μm[a]	**1**
4	Octakis(2,6-di-*O*-pentyl-3-*O*-butyryl)-γ-cyclodextrin(FS-Lipodex E); 25 m × 0.25 mm i.d (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany)	**1**
5	Octakis(2,3-di-*O*-*n*-butyryl-6-*O*-*tert*-butyldimethylsilyl)-γ-cyclodextrin 33% in SE 54; 30 m × 0.32 mm i.d.; *df*: 0.25 μm[a]	**1b**, **1b′**
6	Octakis(2,3-di-*O*-*n*-butyryl-6-*O*-*tert*-butyldimethylsilyl)-γ-cyclodextrin 50% in SE 54; 30 m × 0.32 mm i.d.; *df*: 0.25 μm[a]	**2**−**5**

[a]Synthesized as previously described.[19,20]

(J&W Scientific; Waldbronn, Germany) was installed as a precolumn into a GC oven 1 (GC 1). GC 1 was equipped with a split/splitless injector (215 °C, split ratio 1:5) and an FID (230 °C). Hydrogen was used as the carrier gas at a constant pressure of 165 kPa; temperature program: from 40 °C (5 min hold) to 240 °C (25 min hold) at 4 °C/min. Columns 3−6 (Table 2) were installed as the main columns into GC oven 2 (GC 2). GC 2 was equipped with an FID (230 °C). The outlet pressure was 85 kPa. A moving column stream switching device (MCSS) with a 1 m × 0.25 mm i.d. deactivated fused silica transfer capillary was used to transfer the diastereoisomers of **1**−**5** from GC 1 to GC 2. Data were processed via Chrom-Card software (Thermo Fisher Scientific, Dreieich, Germany). The temperature programs used for columns 3−6 in GC 2 are given in the Supporting Information.

Capillary Gas Chromatography/Olfactometry (GC/O). Sensory analyses were performed on a Trace GC Ultra (Thermo Fisher Scientific, Dreieich, Germany). The GC was equipped with a cold-on-column injector (35 °C), an FID (250 °C), and a heated sniffing port (200 °C). Hydrogen was used as the carrier gas at a constant pressure of 75 kPa. A deactivated fused silica precolumn was used (1 m × 0.53 mm i.d.; BGB Analytik AG, Rheinfelden, Germany). The effluent was split 1:1 via a press-fit Y-splitter among sniffing port and FID. A deactivated fused silica capillary column was used to connect between Y-splitter, the sniffing port and FID (30 cm × 0.25 mm i.d.; BGB Analytik AG, Rheinfelden, Germany). For the stereoisomers of 2−5, column 6[20] was utilized; for the stereoisomers of **1**, and **1b** and **1b′**, columns 4 and 5[20] were installed, respectively. The used temperature programs are given in the Supporting Information. The sensory analyses of the stereoisomers of **1**−**5** were performed by three panelists (females, 26−37 years old). Panelist 3 had no prior experience with GC/O assessments, whereas panelists 1 and 2 were experienced. Odor thresholds in air were determined according to the previously described procedure using (*E*)-2-decenal as the internal standard with a reported odor threshold of 2.7 ng/L in air.[21,22] Known amounts of 2-mercapto-4-alkanols **1**−**5** and of the internal standard were dissolved in diethyl ether and diluted stepwise (1 + 1 (v/v)). Each dilution was assessed three times by GC/O. A concentration level was considered as odor threshold if it was the lowest dilution step at which the odor was consistently perceived in three consecutive GC/O runs.[13] The odor qualities were determined at one dilution step above the odor threshold. Flavor dilution (FD) factors of the target compounds and of the internal standard were obtained by aroma extract dilution analysis (AEDA).[23]

Gas Chromatography−Mass Spectrometry (GC−MS). A Trace GC Ultra gas chromatograph directly coupled to an ISQ mass spectrometer (Thermo Fisher) was employed, using helium as the carrier gas (inlet pressure of 75 kPa; a split/splitless injector set to 220 °C with a split ratio of 1:10). The mass spectra recorded in the electron impact mode (EI) were at 70 eV in a scan range from *m*/*z* 30 to 250, at an ion source temperature of 250 °C and an interface temperature of 240 °C. A 30 m × 0.25 mm i.d., 0.5 μm, DB-WAXetr fused silica capillary column (J&W Scientific) was installed. The temperature was increased from 40 °C (5 min hold) to 240 °C (25 min hold) at 4 °C/min. Data acquisition was done via Xcalibur software, version 2.1 (Thermo Fisher Scientific, Waltham, MA).

RESULTS AND DISCUSSION

GC Separation of the Stereoisomers of 2-Mercapto-4-alkanols. 2-Mercapto-4-alkanols **1**−**5** (Table 1) were synthesized by the Michael-type addition of thioacetic acid to the respective alkenones, followed by reduction with $LiAlH_4$, as described for 4-mercapto-2-alkanols.[8,13,14] The GC separations of the obtained diastereoisomeric pairs of the homologous series of 2-mercapto-4-alkanols **1**−**5** on a DB-Wax column are shown in Figure 1A. For the homologues with chain lengths C7−C10, separations of the four stereoisomers were achieved using octakis(2,3-di-*O*-*n*-butyryl-6-*O*-*tert*-butylsilyl)-γ-cyclodextrin as the chiral stationary phase (Figure 1B); for the chain length C6, there was a coelution of the two anti-stereoisomers **1**. A complete separation of the four stereoisomers of this homologue was obtained by utilizing octakis-(2,3-di-*O*-[(*S*)-2-methylbutyryl]-6-*O*-*tert*-butyldimethylsilyl)-γ-cyclodextrin as the chiral stationary phase (Figure 1C).

Determination of the Absolute Configurations of the Stereoisomers of 2-Mercapto-4-alkanols. The assignment of the order of elution of the anti- and syn-diastereoisomers of **1**−**5** was based on the following sequence: (i) 2-acetylthio-4-hexanone was reduced with $NaBH_4$ to 2-acetylthio-4-hexanol **6**, following the previously described procedure for the isomeric 4-acetylthio-2-alkanols.[13] The resulting diastereoisomers D1-**6** and D2-**6** (first and second eluting on an achiral DB-Wax column) were separated via column chromatography; subsequent reduction with $LiAlH_4$ resulted in the corresponding diastereoisomers of 2-mercapto-4-hexanol (D1-**1** and D2-**1**) (Figure 2). (ii) The separated diastereoisomers D1-**1** and D2-**1** were subjected to NMR analysis. The ^{1}H NMR signals showed clearly distinguished chemical shifts for the protons in the positions 2 (H-2) and 4 (H-4) of the diastereoisomers D1-**1** and D2-**1** (Table 3). The NMR data of diastereoisomer D1-**1** were in agreement with those available for *anti*-(2*S*,4*S*)-2-mercapto-4-hexanol.[18] (iii) The pronounced differences in the chemical shifts for H-2 and H-4 were also reflected in the NMR spectrum of the mixture of stereoisomers **1** (Figure 3A). The calculated ratios between the pairs of NMR signals for H-2 and H-4 for **1** (36:64) and the ratios obtained for the diastereoisomeric pair on a DB-Wax column (35:65) were nearly the same (Table 4). This resulted in a GC order of elution for 2-mercapto-4-hexanol **1** as anti before syn on a DB-Wax column. (iv) The synthesized mixtures of stereoisomers for the homologues C7−C10 2−5 were also subjected to NMR analysis, and the ratios for the two pairs of signals for H-2 and H-4 were compared to those obtained for the diastereoisomers on a DB-Wax column (Table 4). Figure 3B,C exemplarily shows ^{1}H NMR data (H-4 and H-2) for the syn- and anti-diastereoisomers of 2-mercapto-4-heptanol **3** and 2-mercapto-4-heptanol **4**. As a result, the order of elution on a DB-Wax column for the homologues C7−C10 2−5 was consistently assigned as *anti* before syn (Figure 1A).

A procedure based on enzyme-catalyzed kinetic resolution[14,16] was applied to obtain enantiomerically enriched thiols to assign the order of elution of the stereoisomers of each homologue **1**−**5** on chiral stationary phases. As an example,

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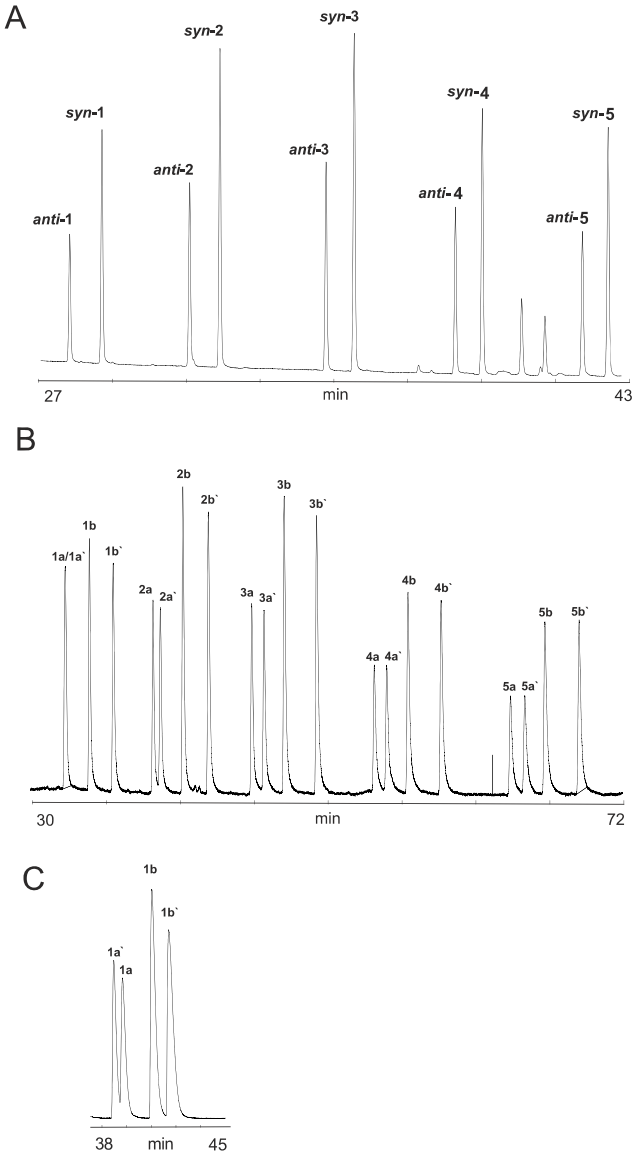

Figure 1. Capillary gas chromatographic separation of the diastereoisomers of 2-mercapto-4-alkanols **1**−**5** with chain lengths C6−C10 on (A) DB-Wax (column 1) and (B) octakis(2,3-di-*O*-*n*-butyryl-6-*O*-*tert*-butyldimethylsilyl)-γ-cyclodextrin (column 6); (C) separation of the stereoisomers of 2-mercapto-4-hexanol **1** on octakis(2,3-di-*O*-[(*S*)-2-methylbutyryl]-6-*O*-*tert*-butyldimethylsilyl)-γ-cyclodextrin (column 3). For conditions, see Materials and Methods.

the approach is shown for 2-mercapto-4-heptanol **2** in Figure 4. The first step was the hydrolysis of racemic 2-acetylthio-4-heptanone **7** with the lipase CAL-B from *Candida antarctica*. After 4 h, the preferentially formed 2-(*R*)-mercapto-4-heptanone **8** was separated from the unreacted substrate 2-(*S*)-acetylthio-4-heptanone **7** via column chromatography (Figure 4A).[14,16] In analogy to the isomeric 4-mercapto-2-alkanols,[13,15] the enantiomerically enriched 2-(*S*)-acetylthio-4-heptanone **7** was reduced with $LiAlH_4$ to 2-mercapto-4-heptanol **2**, exhibiting the corresponding excess of the (2*S*)-configured diastereoisomers (Figure 4B). These (2*S*)-configured 2-mercapto-4-heptanol **2** diastereoisomers were subjected to GC analysis on octakis(2,3-di-*O*-*n*-butyryl-6-*O*-*tert*-butyldimethylsilyl)-γ-cyclodextrin (column 6) as the chiral stationary phase. The comparison with the GC separation of the

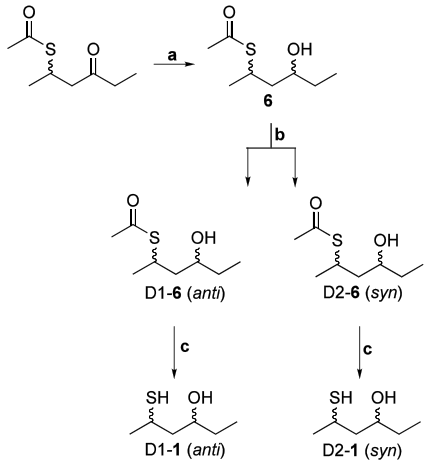

Figure 2. Approach applied to obtain the diastereoisomers of 2-mercapto-4-hexanol (*anti*-**1** and *syn*-**1**). a: $NaBH_4$, phosphate buffer; b: column chromatography on silica gel; and c: $LiAlH_4$.

synthesized mixture of 2-mercapto-4-heptanol stereoisomers **2** showed that the (2*S*)-diastereoisomers coeluted with the second and third eluting peaks of the mixture of stereoisomers of 2-mercapto-4-heptanol **2** (Figure 4C). MDGC was used to determine the syn- and anti-enantiomeric pairs, by separate transfers from the DB-Wax precolumn to the main column (column 6). As a result, the first and second eluting stereoisomers on column 6 were assigned as anti, and the third and fourth eluting stereoisomers as syn.

Analogous procedures were applied for the chain lengths C6 **1** and C8−C10 **3**−**5**, enabling the assignment of the order of elution on octakis(2,3-di-*O*-*n*-butyryl-6-*O*-*tert*-butyldimethylsilyl)-γ-cyclodextrin chiral stationary phase as (2*R*,4*R*)-**2a** before (2*S*,4*S*)-**2a**′ for the anti-stereoisomers and (2*S*,4*R*)-**2b** before (2*R*,4*S*)-**2b**′ for the syn-stereoisomers (Figure 1B). The coelution of the (2*R*,4*R*)-**1a** and (2*S*,4*S*)-**1a**′ stereoisomers was solved by using octakis(2,3-di-*O*-[(*S*)2-methylbutyryl]-6-*O*-*tert*-butyldimethylsilyl)-γ-cyclodextrin (column 3) as the chiral stationary phase, resulting in the same order of elution for the two *syn*-stereoisomers, but in a reversed order of elution of the two *anti*-stereoisomers (Figure 1C).

Determination of Odor Thresholds. Odor thresholds of the stereoisomers of 2-mercapto-4-alkanols **1**−**5** were determined by GC/O using the method described by Ullrich and Grosch.[21] The β-mercaptoalkanols were assessed by three panelists (Table 5). In some cases, the determined odor thresholds showed clear differences between the panelists, i.e., for (2*R*,4*R*)-2-mercapto-4-heptanol **2a** (factor 44 between panelists 1 and 2), for (2*R*,4*S*)-2-mercapto-4-octanol **3b**′ (factor 16 between panelists 1 and 2), and for stereoisomers of **5** (factor 7 between panelists 1 and 3). However, for the other stereoisomers, the odor thresholds were either the same or differed only by factors 2−6 between the panelists, corresponding to 1−3 dilution steps in the course of the AEDA; this indicates the reproducibility of the sensory assessments.

Figure 5 (solid lines) shows a graphical presentation of the geometric means of the odor thresholds of the stereoisomers of 2-mercapto-4-alkanols **1**−**5** depending on the chain lengths. Figure 5 (dotted lines) also depicts odor thresholds previously reported for the homologous series of the positional isomers 4-mercapto-2-alkanols.[15] The odor thresholds for the 2-mercapto-4-alkanols **1**−**5** were generally higher than those of

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Table 3. NMR Data of the Separated anti- and syn-Diastereoisomers of 2-Mercapto-4-hexanol D1-1 and D2-1 and of *anti*-(2*S*,4*S*)-2-Mercapto-4-hexanol[18]

	^{1}H (ppm)				^{13}C (ppm)		
	D1-**1** (anti)	D2-**1** (syn)	*anti*-(2*S*,4*S*)- 2-mercapto- 4-hexanol[18]		D1-**1** (anti)	D2-**1** (syn)	*anti*-(2*S*,4*S*)- 2-mercapto-4-hexanol[18]
H-1	1.37 (d, *J* = 6.8 Hz, 3H)	1.36 (d, *J* = 6.7 Hz, 3H)	1.38 (d, *J* = 6.8 Hz, 3H)	C-1	26.89	26.03	26.8
H-2	3.24−3.14 (m, 1H)	3.12−3.04 (m, 1H)	3.27−3.16 (m, 1H)	C-2	32.62	33.17	32.5
H-3 H-3′	1.68 (m, 1H) 1.52−1.44 (m, 1H)	1.72−1.63 (m, 2H)	1.67−1.45 (br, m, 1H)	C-3	47.52	48.07	47.3
H-4	3.82−3.75 (m, 1H)	3.67−3.62 (m, 1H)	3.80−3.78 (m, 1H)	C-4	71.04	71.95	71.0
H-5	1.51−1.44 (m, 2H)	1.54−1.38 (m,2H)	1.56−1.45 (m, 3H)	C-5	30.79	30.65	30.6
H-6	0.94 (t, *J* = 7.5 Hz, 3H)	0.93 (t, *J* = 7.5 Hz, 3H)	0.96 (t, *J* = 7.5 Hz, 3H)	C-6	10.04	9.89	9.9
SH	1.49 (d, *J* = 7.9 Hz, 1H)	1.59 (d, *J* = 7.1 Hz, 1H)	1.49 (d, *J* = 6.8 Hz, 1H)				
OH	1.84 (br, s, 1H)	1.94 (br, s, 1H)					

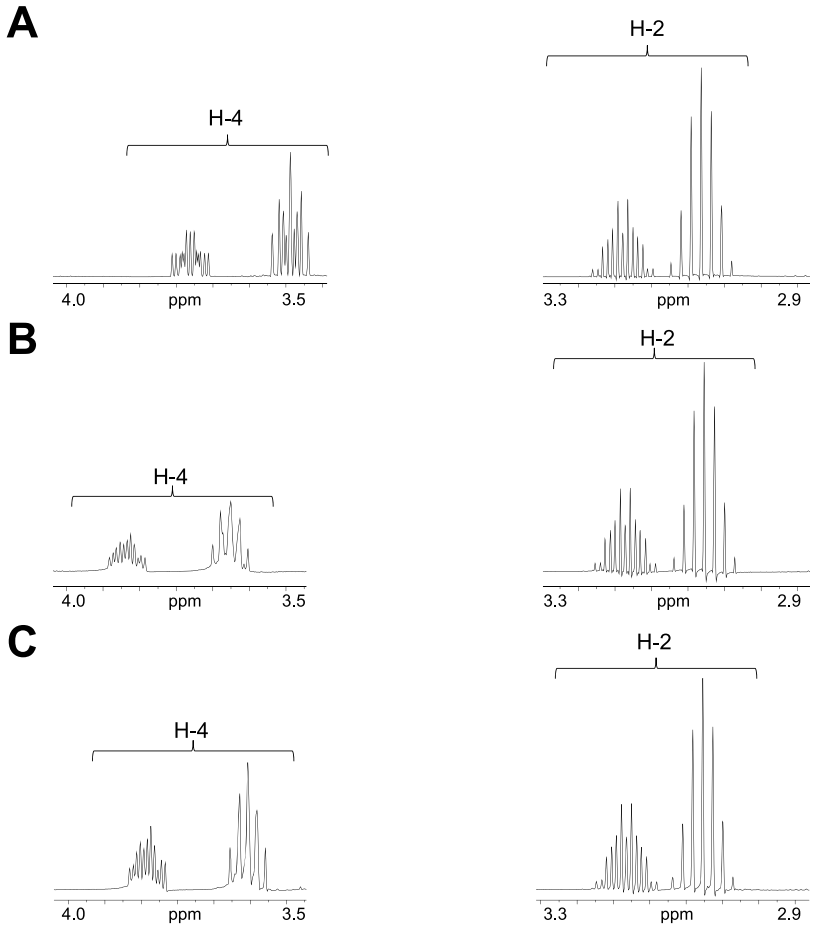

Figure 3. ^{1}H NMR data of the signals corresponding to the syn- and anti-diastereoisomers for H-4 and H-2 of (A) 2-mercapto-4-hexanol **1**, (B) 2-mercapto-4-heptanol **2**, and (C) 2-mercapto-4-octanol **3**.

the 4-mercapto-2-alkanols. Only the C7 (2*S*,4*S*)-stereoisomer **2a**′ exhibited a threshold in the same range as for the 4-mercapto-2-alkanols. For the 4-mercapto-2-alkanols, the (2*R*,4*R*)-stereoisomers consistently showed the lowest odor thresholds; the thresholds of the other stereoisomers were rather similar. This outstanding role of the (2*R*,4*R*)-stereoisomers was not observed for the 2-mercapto-4-alkanols **1**−**5**. For this homologous series, the configuration of the asymmetric center bearing the hydroxyl group seems to influence the odor thresholds, an effect particularly pronounced for the chain length C7, with 56 ng/L (air) for the (2*S*,4*R*)-stereoisomer **2b** and 0.2 ng/L (air) for the (2*S*,4*S*)-stereoisomer **2a**′, and for chain length C8 with a factor of 15 between **3b** and **3a**′ (Table 5).

Determination of Odor Qualities. The odor qualities of the stereoisomers of 2-mercapto-4-alkanols **1**−**5** were determined by GC/O at one dilution step above the odor threshold by three panelists; odor descriptions named by more than one panelist for one stereoisomer are marked in bold (Table 6). Compared to the odor qualities reported for the

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Table 4. Diastereoisomeric Ratios of the Synthesized Mixtures of Stereoisomers of 2-Mercapto-4-alkanols 1−5 Determined by GC and ^{1}H NMR Analyses

	diastereoisomeric ratios (%)		
	GC[a]	^{1}H NMR	
no.	anti/syn	H-2	H-4
1	35:65	36:64	36:64
2	37:63	38:62	38:62
3	40:60	40:60	40:60
4	38:62	40:60	40:60
5	36:64	38:62	36:64

[a]DB-WAX (column 1); see Figure 1A.

stereoisomers of the isomeric 4-mercapto-2-alkanols, the odor qualities of the 2-mercapto-4-alkanols **1−5** showed a lack of fruity and tropical notes.[15] The odor qualities resembled those of the respective 2-mercapto-4-alkanones and 2-acetylthio-4-alkanones.[16]

There was no consistent correlation between the configuration and the odor qualities. However, a chain-length-dependent change of odor qualities was observed. The stereoisomers of the chain lengths C6−C8 were predominantly described as vegetable-like or associated with cooked vegetable notes. The stereoisomers of the chain lengths C9 and C10 exhibited mainly mushroom notes. It is, however, striking that the (2*S*,4*R*)-stereoisomers of these chain lengths were not described as mushroom-like by any of the panelists; instead, the C8 homologue (2*S*,4*R*)-2-mercapto-4-octanol **3b** was perceived as vegetable- and mushroom-types by all three panelists.

Comparative Sensory Assessment of *β*-Mercaptoalkanone and *β*-Mercaptoalkanol Stereoisomers. Rowe[7] proposed structural requirements for a "tropical olfactophore", which were extended to the respective acetyl-compounds by Robert et al.[24] The comparison of the sensory data elaborated in this study for the 2-mercapto-4-alkanols **1−5** with those previously determined for the homologous series of 4-mercapto-2-alkanones,[12] 4-mercapto-2-alkanols,[15] and 2-mercapto-4-alkanones[16] demonstrates the key role of substituent R_4 in the "tropical olfactophore" model (Figure 6A). Two of the investigated compound classes, i.e., 4-mercapto-2-alkanones (Figure 6B) and the respective 4-mercapto-2-alkanols (Figure 6D), have a methyl group as substituent R_4 as required by the model, and both exhibited the expected fruity and sulfury notes. In contrast, the 2-mercapto-compounds (Figure 6C,E) showed a lack of fruity and tropical notes, owing to the presence of the longer alkyl group as substituent R_4. This phenomenon was also observed for 1-mercapto-3-alkanols and 3-mercapto-1-alkanols: Starting from chain length C5, for 1-mercapto-3-alkanols, mushroom and oily notes were reported. On the other hand, 3-mercapto-1-alkanols meet the requirement of the tropical olfactophore; hence, grapefruit flavors were observed up to chain length C8.[25]

In addition, the configuration had an impact on the sensory properties; the (*R*)-4-mercapto-2-alkanones (Figure 6B) and the (2*R*,4*R*)-stereoisomers of the 4-mercapto-2-alkanols (Figure 6D) showed the lowest thresholds.[12,15] The importance of the configuration of the asymmetric center

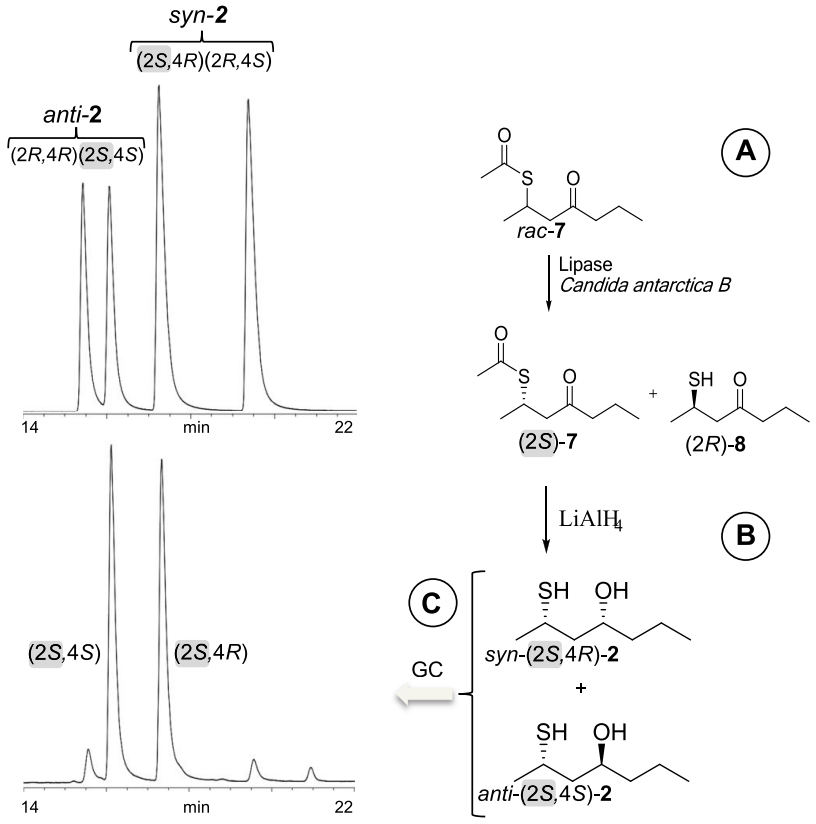

Figure 4. Approach applied to assign the GC order of elution of 2-mercapto-4-alkanols **1−5** shown for the C7 homologue 2-mercapto-4-heptanol **2**; A: lipase-catalyzed kinetic resolution of the 2-acetylthio-4-heptanone enantiomers; B: formation of (2*S*)-enriched diastereoisomers of 2-mercapto-4-heptanol; and C: GC separation on octakis(2,3-di-*O*-*n*-butyryl-6-*O*-*tert*-butyldimethylsilyl)-*γ*-cyclodextrin.

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Table 5. Odor Thresholds of the Stereoisomers of 2-Mercapto-4-alkanols 1−5 Determined by GC/O

	odor thresholds in air (ng/L)[a]											
	a (2*R*,4*R*)			**a′** (2*S*,4*S*)			**b** (2*S*,4*R*)			**b′** (2*R*,4*S*)		
	panelist			panelist			panelist			panelist		
no.	1	2	3	1	2	3	1	2	3	1	2	3
1 (C6)[c]	32	16	19	8.0	2.0	1.2	1.8[b]	11[b]	8.7[b]	1.8[b]	4.5[b]	1.6[b]
2 (C7)[d]	0.2	8.8	7.4	0.2	0.5	0.1	49	56	47	1.5	1.5	0.4
3 (C8)[d]	3.1	12	10	3.2	3.2	5.4	38	75	63	1.2	19	7.9
4 (C9)[d]	2.6	7.9	7.7	5.2	32	15	8.5	26	51	8.2	13	25
5 (C10)[d]	66	157	482	71	169	518	122	145	447	115	138	847

[a]Odor thresholds were determined by GC/O. [b]Arithmetic means; odor thresholds were determined twice by each panelist on two columns (**4** and **5**). [c]Separation of the stereoisomers of **1** on column 3 could not be applied for the sensory evaluation. Instead, columns **4** and **5** were applied. [d]Column 6.

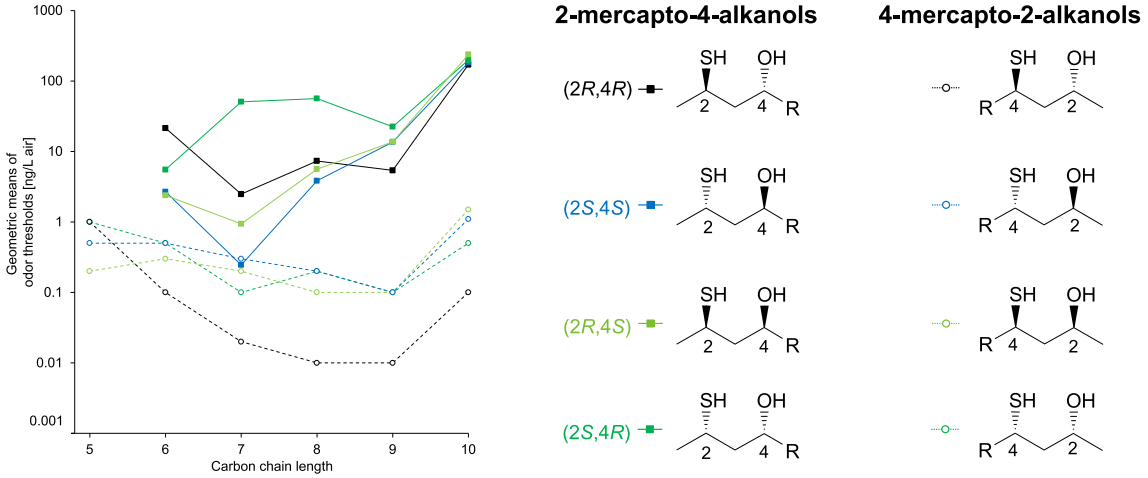

Figure 5. Geometric means of the odor thresholds of the stereoisomers of 2-mercapto-4-alkanols (solid lines): (2*R*,4*R*)-stereoisomer (black box solid), (2*S*,4*S*)-stereoisomer (blue box solid), (2*R*,4*S*)-stereoisomer (green box solid), (2*S*,4*R*)-stereoisomer (dark green box solid), and their positional isomers 4-mercapto-2-alkanols (dotted lines): (2*R*,4*R*)-stereoisomer (black circle open), (2*S*,4*S*)-stereoisomer (blue circle open), (2*R*,4*S*)-stereoisomer (green circle open), and (2*S*,4*R*)-stereoisomer (dark green circle open).

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

		odor descriptions[a]			
no.	panelist	**a** (2*R*,4*R*)	**a′** (2*S*,4*S*)	**b** (2*S*,4*R*)	**b′** (2*R*,4*S*)
1	1	cooked **vegetable**	green, cucumber	**sweat**, onion	roasty, pork
	2	**vegetable**, sour	nutty	**pungent**, burnt	sour
	3	burnt	sweet, sweat	**pungent**, **sweat**, sweet, green	sulfury
2	1	**pungent**	cooked onion	sweet, pungent	sweat
	2	**vegetable**, **pungent**	leek, sulfury	burnt, spicy, oily	**vegetable**, aromatic
	3	**vegetable**, watercress	sweet, vegetable-broth	vegetable-broth	**vegetable**-broth sweet
3	1	earthy	**vegetable**, coffee, moldy	**vegetable**-broth, **mushroom**	**vegetable**-broth, sweat, urine
	2	**vegetable**, pungent, fatty	leek, pungent	**vegetable**, **mushroom**	**vegetable**, pungent
	3	**vegetable**, watercress	**vegetable**-broth, spicy	**vegetable**, **mushroom**	**vegetable**, herbs, earthy
4	1	**mushroom**, **metallic**	**mushroom**, earthy	**earthy**	**mushroom**, moldy
	2	**mushroom**, **metallic**	**mushroom**, moldy	moldy, humid	**mushroom**, pungent
	3	**mushroom**	**mushroom**, vegetable-broth	**earthy**, vegetable, sweet	**mushroom**, earthy
5	1	**vegetable**, **pungent**	burnt	spicy	**vegetable**
	2	**pungent**, **woody**, mushroom, metallic	**mushroom**, **woody**, **metallic**	pungent, woody, moldy	**vegetable**, woody
	3	**vegetable**, **woody**, humid	**mushroom**, **woody**	vegetable-broth, sweet	**vegetable**

[a]GC/O descriptions were determined at a one dilution step above the odor threshold. Descriptions given by at least two panelists are marked in bold.

bearing the thiol group was also reflected by the observation that the (2*R*,4*R*)- and (2*S*,4*R*)-stereoisomers of 4-mercapto-2-heptyl acetate exhibited the lowest odor thresholds of the corresponding acetyl esters.[13] In contrast, for the positional isomers 2-mercapto-4-alkanones[16] and the corresponding 2-mercapto-4-alkanols (Figure 6C,E), the configuration of the asymmetric center bearing the thiol group did not play a significant role.[16] The odor thresholds of the 2-mercapto-4-

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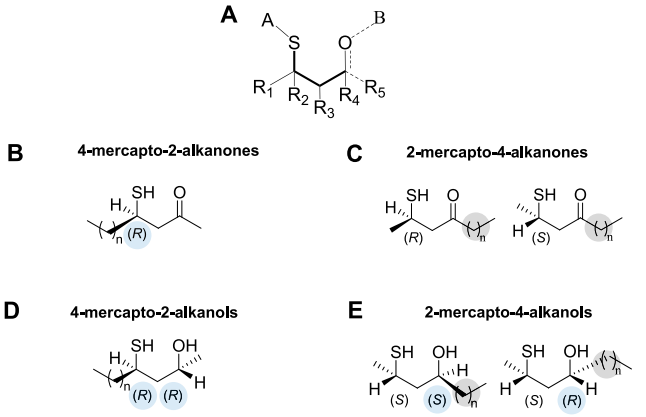

Figure 6. (A) Structural requirements for a "tropical olfactophore" as proposed by Rowe[7] and extended by Robert et al.[24] (A = H, SCH_3, ring; B = H, CH_3, acyl, absent if carbonyl; R_1, R_2 = H, alkyl; R_3 = H, alkyl, ring; R_4 = H, CH_3, ring, OR; R_5 = H absent if carbonyl); (B–E) spatial arrangements determining the sensory properties of β-mercaptoalkanones and β-mercaptoalkanols.

alkanols 1–5 were rather influenced by the configuration of the asymmetric center bearing the hydroxyl group and the substituent R_4. The length of this alkyl chain was the main driver for the odor properties, ranging from pungent, vegetable to earthy, mushroom notes. In analogy to 2-mercapto-4-alkanones,[16] the odor qualities of 2-mercapto-4-alkanols resemble those of the corresponding 1-alken-3-ols with one carbon atom less. This phenomenon was particularly pronounced for the chain length C9; except for the stereoisomer (2*S*,4*R*)-2-mercapto-4-nonanol **4b**, all stereoisomers exhibited mushroom odors, comparable to 1-octen-3-ol.[26−29] The odor properties determined for the other 2-mercapto-4-alkanols 1–3 and **5** are in agreement with odor properties described for the respective homologous series of racemic 1-alken-3-ols; e.g., pungent for 1-penten-3-ol and 1-hexen-3-ol, vegetable-like for 1-hepten-3-ol, and mushroom-like for 1-nonen-3-ol.[27] This supports the hypothesis that the β-mercapto-group plays the role of the vinyl group of the 1-alken-3-ols and 1-alken-3-ones, respectively.[16]

The sensory properties of the two diastereoisomeric mixtures of 2-mercapto-4-heptanol **2** tasted in NaCl (0.3%) and sugar (0.5%) have been described as fruity, tropical, guava, watercress, and vegetal.[8] In the GC/O assessment of the stereoisomers of 2-mercapto-4-heptanol **2**, vegetable was also the dominating odor description, and one panelist described the odor of (2*R*,4*R*)-2-mercapto-4-heptanol **2a** as watercress.

β-Mercaptoalkanones and β-Mercaptoalkanols in Cooked Bell Pepper (*Capsicum annuum*). 2-Mercapto-4-heptanone **7**, 2-mercapto-4-heptanol **2**, and the positional isomers 4-mercapto-2-heptanone and 4-mercapto-2-heptanol were among the sulfur-containing volatiles identified in bell peppers.[8] Distributions of the stereoisomers of these C7 homologues in cooked red bell pepper have previously been investigated.[30] The data elaborated in the present study allow complementing the missing assignments of the configurations of the 2-mercapto-4-heptanol stereoisomers. The (2*S*,4*S*)- and (2*S*,4*R*)-stereoisomers of this β-mercaptoalkanol are quantitatively dominating in cooked bell pepper. Biogenetically, this is in line with the reported high preponderance of the potential precursor (*S*)-2-mercapto-4-heptanone **8** in cooked bell pepper.[30] (2*S*,4*S*)-2-Mercapto-4-heptanol **2a**′ and (2*S*,4*R*)-2-mercapto-4-heptanol **2b** showed the lowest and highest thresholds, respectively, for the C7 homologues (Table 5). For both stereoisomers, the odor qualities showed a wide range, from sweet, vegetable to burnt, pungent; none of the panelists described a specific bell pepper flavor (Table 6). To identify the impact of these compounds on the aroma of bell peppers, it would be necessary to determine the odor thresholds in water and to calculate the respective odor activity values.

In conclusion, the assessment of the stereoisomers of 2-mercapto-4-alkanols confirms that the sensory properties of β-mercaptoalkanones and β-mercaptoalkanols are impacted by two main parameters: for representatives of substances with a 1,3-oxygen−sulfur function bearing an alkyl chain from ethyl to hexyl as substituent R_4 in the Rowe model for a "tropical olfactophor" (e.g., 2-mercapto-4-alkanols or 2-mercapto-4-alkanones),[7] the sensory properties are mainly determined by the chain length of this substituent, and tropical, fruity notes are lacking. For representatives possessing a methyl group as substituent R_4 (e.g., 4-mercapto-2-alkanols or 4-mercapto-2-alkanones), the configuration at the asymmetric center bearing the thiol group is the main parameter determining the sensory properties. In future studies, these observations might be corroborated by extending the number of panelists and by assessing the sensory properties of the neat compounds in aqueous solutions.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.0c00221.

MS and NMR data for 2-mercapto-4-alkanols (C6–C10) and 2-acetylthio-4-hexanol; temperature programs used for the separations of 2-mercapto-4-alkanols (C6–C10) with chain lengths; geometric means of the individual odor thresholds determined by the panelists via GC/O (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Karl-Heinz Engel − *Lehrstuhl für Allgemeine Lebensmitteltechnologie, Technische Universität München, D-85354 Freising, Weihenstephan, Germany;* orcid.org/0000-0001-8932-5175; Phone: +49-(0)8161-71-4259; Email: k.h.engel@wzw.tum.de; Fax: Tel: +49-(0)8161-71-4250

Authors

Anja Devenie Riegel − *Lehrstuhl für Allgemeine Lebensmitteltechnologie, Technische Universität München, D-85354 Freising, Weihenstephan, Germany*

Christiane Kiske − *Lehrstuhl für Allgemeine Lebensmitteltechnologie, Technische Universität München, D-85354 Freising, Weihenstephan, Germany*

Viktoriia Dudko − *Lehrstuhl für Allgemeine Lebensmitteltechnologie, Technische Universität München, D-85354 Freising, Weihenstephan, Germany*

Iulia Poplacean − *Lehrstuhl für Allgemeine Lebensmitteltechnologie, Technische Universität München, D-85354 Freising, Weihenstephan, Germany*

Wolfgang Eisenreich − *Lehrstuhl für Biochemie, Technische Universität München, D-85747 Garching, Germany*

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Notes

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3.2. Publication II

Riegel, A. D.; Wakabayashi, H.; Wakabayashi, M.; Rynešová, M.; V. Dudko, V.; Eisenreich W.; Engel, K.-H. Configurations and Sensory Properties of the Stereoisomers of 2,6-Dimethyl-4-propyl-1,3-oxathiane and 2,4-Dimethyl-6-propyl-1,3-oxathiane

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2,6-Dimethyl-4-propyl-1,3-oxathiane and 2,4-dimethyl-6-propyl-1,3-oxathiane were synthesized by reaction of acetaldehyde with 4-mercapto-2-heptanol and 2-mercapto-4-heptanol, respectively. For both heterocycles, the eight stereoisomers could be separated via capillary gas chromatography (GC), using a derivatized cyclodextrin as a chiral stationary phase. The configurations and the GC order of elution were determined by (i) syntheses of the 1,3-oxathianes starting from stereochemically defined stereoisomers of 4-mercapto-2-heptanol and 2-mercapto-4-heptanol obtained via lipase-catalyzed kinetic resolutions and HPLC separations, and (ii) by NMR analyses. The odor thresholds and odor qualities of the individual stereoisomers were determined via GC/olfactometry.

The mean odor thresholds of all stereoisomers of 2,6-dimethyl-4-propyl-1,3-oxathiane (1.5 – 12 ng/L in air) were lower than those determined for the stereoisomers of the positional isomer 2,4-dimethyl-6-propyl-1,3-oxathiane (1 – 1404 ng/L in air). The highest odor thresholds were observed for the stereoisomers (2*S*,4*R*,6*S* and 2*R*,4*S*,6*R*) with all alkyl-substituents in equatorial positions; this effect was particularly pronounced for 2,4-dimethyl-6-propyl-1,3-oxathiane. For both oxathianes, there were no differences between the odor thresholds of enantiomeric pairs of the stereoisomers. Regarding the odor qualities, six of the eight stereoisomers of 2,6-dimethyl-4-propyl-1,3-oxathiane exhibited pleasant flowery, fruity, or sweet nuances. In contrast, the stereoisomers of 2,4-dimethyl-6-propyl-1,3-oxathiane were mainly characterized as broth and mushroom-like, pungent and musty. The data demonstrate the importance of both the positions of substituents and the configurations on the sensory properties of these alkyl-substituted 1,3-oxathianes.

Candidates Contribution

Syntheses of 2,6-dimethyl-4-propyl-1,3-oxathiane and 2,4-dimethyl-6-propyl-1,3-oxathiane; development of the methods for the separation of the eight stereoisomers each by capillary gas chromatography on a chiral stationary phase; determination of their absolute configurations and the GC-order of elution by performing the respective enzyme-catalyzed kinetic resolutions and HPLC separations and by interpretation of the NMR data; determination of the odor thresholds and the odor descriptions; writing and revision of the manuscript, including Figures and Tables, and the Supporting Information.

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Configurations and Sensory Properties of the Stereoisomers of 2,6-Dimethyl-4-propyl-1,3-oxathiane and 2,4-Dimethyl-6-propyl-1,3-oxathiane

Anja Devenie Riegel, Hidehiko Wakabayashi, Motoko Wakabayashi, Markéta Rynešová, Viktoriia Dudko, Wolfgang Eisenreich, and Karl-Heinz Engel*

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ABSTRACT: The heterocyclic compounds 2,6-dimethyl-4-propyl-1,3-oxathiane **1** and 2,4-dimethyl-6-propyl-1,3-oxathiane **2** were obtained by condensing 4-mercapto-2-heptanol and 2-mercapto-4-heptanol, respectively, with acetaldehyde. For both, separation of the eight stereoisomers was achieved *via* capillary gas chromatography using heptakis(diethyl-*tert*-butyldimethylsilyl)-*β*-cyclodextrin as the chiral stationary phase. Their configurations were assigned by combinations of enzyme-catalyzed kinetic resolutions, HPLC separations, and assessments of NMR data. The odor thresholds and odor qualities of the stereoisomers were determined by capillary gas chromatography/olfactometry. The odor thresholds of the stereoisomers of **2** were generally higher than those of **1**. For both oxathianes, the stereoisomers in which all substituents are in equatorial positions showed the highest odor thresholds. Most of the stereoisomers of **1** exhibited pleasant flowery, fruity, or sweet nuances; the stereoisomers of **2** were mainly characterized by descriptors, such as broth, mushroom, or pungent. The data demonstrate the impact of the positions of substituents and their spatial orientations on the sensory properties of 1,3-oxathianes.

KEYWORDS: *1,3-oxathianes, structure−odor relationships, odor threshold, odor quality, stereochemistry, chirality*

INTRODUCTION

Polyfunctional thiols are an important subgroup of sulfur-containing volatiles exhibiting attractive sensory properties.[1−4] A 1,3-oxygen−sulfur relationship is seen in many compounds exhibiting powerful tropical, fruity, or vegetable notes.[5,6] A prominent example of a polyfunctional thiol fulfilling this structural requirement is 3-mercaptohexanol, reported for the first time as a naturally occurring volatile in yellow passion fruits.[7] This mercapto primary alcohol and its acetate were later shown to be present in other tropical fruits[8] and to play essential sensory roles as varietal thiols in wines.[9] 3-Mercaptohexanol is a chiral molecule; accordingly, research has focused on the assignment of the configurations of the enantiomers,[10] the assessment of their sensory properties,[11,12] and the determination of their naturally occurring distributions.[11,13−15]

Remarkably, several years before the detection of 3-mercaptohexanol in yellow passion fruits, *cis*- and *trans*-2-methyl-4-propyl-1,3-oxathiane, the reaction products resulting from the condensation of 3-mercaptohexanol with acetaldehyde, had been reported as volatile constituents in a yellow passion fruit concentrate.[16] Configurations and sensory properties of the stereoisomers of 2-methyl-4-propyl-1,3-oxathiane have been determined,[10,17−19] and their distribution in passion fruits has been elucidated.[13,20] More recently, the presence of *cis*-2-methyl-4-propyl-1,3-oxathiane has been reported in several wines,[21] and its enantiomeric distributions in wines have been described.[22] In addition, the occurrence of *cis*-2,4,4,6-tetramethyl-1,3-oxathiane, resulting from the reaction of 4-mercapto-4-methylpentan-2-ol with acetaldehyde, has been described in wine.[22]

In the context of studies on structure−odor relationships, in the past decade, several research activities have been devoted to the analytical and sensory characterization of homologous series of four other classes of polyfunctional thiols, that is, 4-mercapto-2-alkanones and 4-mercapto-2-alkanols[23−26] and their positional isomers 2-mercapto-4-alkanones and 2-mercapto-4-alkanols.[27,28] A particular focus was on the impact of the chirality of these flavor compounds on the perception of the individual stereoisomers.[29] For the C7 homologue 4-mercapto-2-heptanol, the influence of acetylations[30] and the change from an aliphatic to a cyclic structure[31] on the sensory properties of the stereoisomers has been investigated.

Using the C7-homologues 4-mercapto-2-heptanol **3** and 2-mercapto-4-heptanol **4** as examples, the goal of this study was to investigate the impact of cyclization *via* reaction with acetaldehyde on the sensory properties of the resulting 2,6-dimethyl-4-propyl-1,3-oxathiane **1** and 2,4-dimethyl-6-propyl-1,3-oxathiane **2** (Figure 1). These alkyl-substituted 1,3-oxathianes have not been described in the literature so far; the presence of a second asymmetric center in the starting

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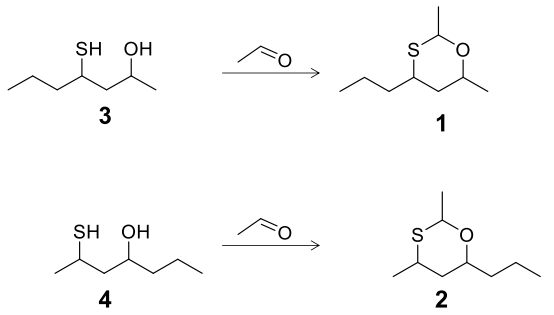

Figure 1. Formation of 2,6-dimethyl-4-propyl-1,3-oxathiane **1** and 2,4-dimethyl-6-propyl-1,3-oxathiane **2** by the reaction of 4-mercapto-2-heptanol **3** and 2-mercapto-4-heptanol **4** with acetaldehyde.

mercapto secondary alcohols compared to 3-mercaptohexanol and, thus in each case, the potential formation of eight 1,3-oxathiane stereoisomers (Figure 2), constituted a particular analytical challenge. Thus, the objectives of this investigation were (i) to establish a capillary gas chromatographic (GC) separation of the stereoisomers of 2,6-dimethyl-4-propyl-1,3-oxathiane **1** and 2,4-dimethyl-6-propyl-1,3-oxathiane **2**, (ii) to assign their configurations and to determine their GC-order of elution, and (iii) to assess their odor properties by means of capillary gas chromatography/olfactometry (GC/O).

MATERIALS AND METHODS

Chemicals. Lipase B from *Candida antarctica* (L4777, ≥5000 U/g, recombinant, expressed in *Aspergillus niger*, adsorbed on a macroporous acrylic resin, CAL-B), porcine pancreas lipase type II (L 3126, 100−500 U/mg, PPL), *p*-toluenesulfonic acid (*p*-TsOH), (*E*)-2-decenal (≥95%), deuterochloroform ($CDCl_3$), anhydrous sodium sulfate (Na_2SO_4), *n*-alkane standard solutions (C8−C40), *n*-hexane (HPLC grade), *i*-propanol (HPLC grade), and dichloromethane (DCM) were purchased from Sigma-Aldrich (Steinheim, Germany). Silica gel (NormaSil60, 40−63 μm), *n*-hexane, and diethyl ether were purchased from VWR Chemicals (Leuven, Belgium).

Syntheses. 4-Mercapto-2-heptanol **3** and 2-mercapto-4-heptanol **4** were obtained by Michael-type addition of thioacetic acid to the corresponding α,β-unsaturated carbonyls and subsequent reduction with $LiAlH_4$.[28,30] 2,6-Dimethyl-4-propyl-1,3-oxathiane **1** and 2,4-dimethyl-6-propyl-1,3-oxathiane **2** were synthesized by reacting these β-mercaptoalkanols with acetaldehyde.[21,32] To a solution of acetaldehyde in DCM (0.91 mmol in 1.5 mL for **3**; 2.99 mmol in 4 mL for **4**), *p*-TsOH (0.15 equiv) and 4 Å molecular sieves (0.2 g for **3**; 1 g for **4**) were added. Under an argon atmosphere and at 0 °C, solutions of **3** (0.13 mmol in 2 mL of DCM) and **4** (0.43 mmol in 2 mL of DCM), respectively, were added dropwise. The reaction mixture was stirred overnight at room temperature (RT). The organic layer was washed with 50 mM potassium phosphate buffer (pH 7.4) and brine, dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. 2,6-Dimethyl-4-propyl-1,3-oxathiane **1**: 82.5 mg [0.47 mmol; crude mol yield: 105%; purity (GC): 58%]; purity (GC) after column chromatography on silica gel (*n*-hexane/diethyl ether, 9 + 1, v/v): 92%; GC−MS (*m*/*z*, rel.%) for diastereoisomer **1**-I: 159 (31), 128 (10), 115 (45), 101 (17), 87 (48), 74 (20), 55 (100),

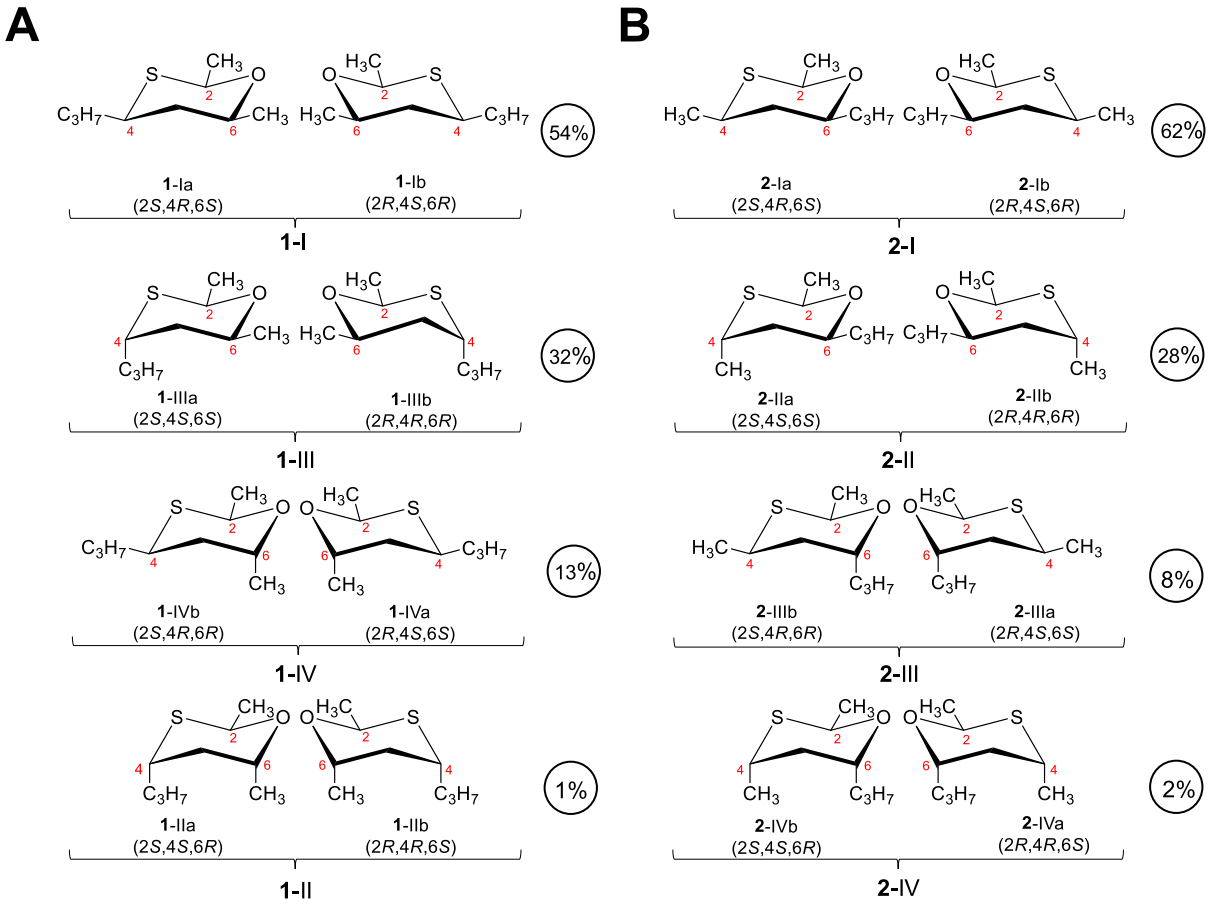

Figure 2. Structures of the stereoisomers of (A): 2,6-dimethyl-4-propyl-1,3-oxathiane and (B): 2,4-dimethyl-6-propyl-1,3-oxathiane. The encircled numbers correspond to the proportions of the stereoisomers in mixtures obtained upon the reaction of acetaldehyde with 4-mercapto-2-heptanol **3** and 2-mercapto-4-heptanol **4**, respectively.

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45 (38), 41 (34), 32 (32), 174 (M+, 31); apart from slight differences in intensities, **1**-III and **1**-IV showed nearly the same fragmentation patterns; the proportion of diastereoisomer **1**-II in the synthesized mixture was too low to obtain an appropriate mass spectrum under the employed conditions. 2,4-Dimethyl-6-propyl-1,3-oxathiane **2**: 40.9 mg [0.23 mmol; mol yield: 68%; purity (GC): 61%]; purity (GC) after column chromatography on silica gel (*n*-hexane/diethyl ether, 9 + 1, v/v): 92%; GC−MS (*m*/*z*, rel.%) for diastereoisomer **2**-I: 159 (11), 141 (3), 130 (23), 115 (31), 101 (65), 97 (24), 87 (33), 81 (37), 74 (28), 60 (100), 55 (82), 43 (27), 41 (48), 174 (M+, 39); apart from slight differences in the intensities, the other diastereoisomers (**2**-II, **2**-III, and **2**-IV) showed nearly the same fragmentation patterns. The diastereomeric ratios and linear retention indices (LRIs)[33] of DB-WAX and DB-1 are given in Table 1 and Figure 2.

Table 1. Ratios and Chromatographic Data of the Diastereoisomers Obtained upon Syntheses of 2,6-Dimethyl-4-propyl-1,3-oxathiane 1 and 2,4-Dimethyl-6-propyl-1,3-oxathiane 2

			linear retention index (LRI)	
no.	diastereoisomer[a]	diastereomeric ratio [%]	DB-WAX	DB-1
1	I	54	1475	1266
	II	1	1484	1275
	III	32	1495	1282
	IV	13	1480	1302
2	I	62	1437	1250
	II	28	1480	1278
	III	8	1508	1287
	IV	2	1528	1299

[a]The Roman numbering corresponds to the GC order of elution on a DB-WAX column (Figure 2).

Lipase-Catalyzed Kinetic Resolutions. As previously described,[25,30] 2-acetylthio-4-heptanone and 4-acetylthio-2-heptanone were subjected to kinetic resolutions using CAL-B and PPL, respectively, as the biocatalysts. The formed 2-(*R*)-mercapto-4-heptanone and 4-(*S*)-mercapto-2-heptanone were separated from the unreacted 2-(*S*)-acetylthio-4-heptanone and 4-(*R*)-acetylthio-2-heptanone by column chromatography. 4-(*S*)-Mercapto-2-heptanone (*ee*: 50%) and 2-(*S*)-acetylthio-4-heptanone (*ee*: 81%) were subsequently reduced with $LiAlH_4$ to (4*S*)-**3** and (2*S*)-**4**, respectively, which were used for further synthesis of enantiomerically enriched **1** and **2**.

NMR Spectroscopy. AVANCE 500 spectrometers (Bruker, Rheinstetten, Germany) were used to measure the compounds (dissolved in 0.5 mL of $CDCl_3$). The ^1H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra were recorded at 27 °C. ^1H-detection experiments were performed using an inverse $^1H/^{13}C$ probe head, and direct ^{13}C-measurements were performed using a QNP $^{13}C/^{31}P/^{29}Si/^{19}F/^1H$ cryoprobe. The ^{13}C NMR spectra were recorded in the proton-decoupled mode. The experiments were conducted in full automation using the standard parameter sets of the TOPSPIN 3.0 software package (Bruker). All signals were assigned by proton−proton and proton−carbon correlation experiments [H,H-correlation spectroscopy, H,H-nuclear Overhauser effect spectroscopy (NOESY), H,C-heteronuclear single quantum coherence spectroscopy, and H,C-heteronuclear multiple bond correlation]. MestreNova software (Mestrelab Research, Santiago de Compostela, Spain) was used for data processing.

High-Performance Liquid Chromatography. Semipreparative separations of the *anti*- and *syn*-diastereoisomers of 4-mercapto-2-heptanol **3** and 2-mercapto-4-heptanol **4** were carried out using a Dionex HPLC system (UltiMate 3000 series, Dionex, Germering, Germany) equipped with a 3100 wavelength detector adjusted to 210 nm using a Nucleosil 50−5 column (250 × 8 mm i.d.; CS chromatography, Langerwehe, Germany) for **3**[30] and a ChiraSper NT column (250 × 4 mm i.d.: LiChroCART, Merck, Darmstadt, Germany) for **4**. Isocratic elutions were performed at 30 °C. Separation of *anti*-**3** (17.9 min) and *syn*-**3** (20.0 min) using *n*-hexane containing 5% *i*-propanol as the eluent (flow rate 0.4 mL/min; 30 °C) resulted in enriched *anti*-**3** (97% *anti*-**3**: 3% *syn*-**3**) and enriched *syn*-**3** (97% *syn*-**3**: 3% *anti*-**3**). HPLC-separation of *anti*-**4** (15.0 min) and *syn*-**4** (15.7 min) using *n*-hexane containing 5% *i*-propanol as the eluent (flow rate 0.4 mL/min; 30 °C) yielded enriched *anti*-**4** (93% *anti*-**4**: 7% *syn*-**4**) and enriched *syn*-**4** (95% *syn*-**4**: 5% *anti*-**4**).

GC Analyses. *Capillary GC.* A DB-WAX (30 m × 0.25 mm i.d.; df: 0.25 μm; Agilent Technologies, Waldbronn, Germany) was installed in an HP5890 A gas chromatograph (Hewlett-Packard INC, Waldbronn, Germany), equipped with a split/splitless injector (215 °C, split ratio of 1:10) and an FID (300 °C); carrier gas: H_2 at a constant pressure of 150 kPa. The temperature program used was from 40 °C (5 min hold) to 240 °C (30 min hold) at 4 °C/min. A DB-1 column (60 m × 0.32 mm i.d.; df: 0.25 μm; J&W Scientific, Waldbronn, Germany) was installed in an HP5890 A gas chromatograph (Hewlett-Packard INC, Waldbronn, Germany), equipped with a split/splitless injector (215 °C, split ratio of 1:10) and an FID (300 °C); carrier gas: H_2 at a constant pressure of 150 kPa; temperature program: from 40 °C (5 min hold) to 240 °C (30 min hold) at 4 °C/min.

Stereoselective Analysis. A 6890N gas chromatograph (Agilent Technologies) was equipped with a split/splitless injector (230 °C, split ratio of 1:10) and an FID (300 °C). Hydrogen was used as carrier gas at a constant pressure of 75 KPa. The separation of the stereoisomers of **1** and **2** was achieved using a heptakis(diethyl-*tert*-butyldimethylsilyl)-β-cyclodextrin column (25 m × 0.25 mm i.d., 0.25 μm df; MEGA-DEX DET-Beta; Mega S.r.l., Legnano, Italy); temperature program: from 35 °C (0 min hold) to 125 °C (0 min hold) at 2 °C/min and from 125 to 180 °C (5 min hold) at 4 °C/min.

Multidimensional Gas Chromatography. The instrumentation consisted of two coupled GC 8000 (Carlo Erba Instruments). The diastereoisomers of **1** and **2** were transferred from GC oven 1 to GC oven 2 with a moving-column stream-switching device (MCSS) with a 1 m × 0.25 mm i.d. deactivated fused silica transfer capillary column. A DB-WAX column (60 m × 0.32 mm i.d., 0.25 μm; J&W Scientific; Waldbronn, Germany) was installed as a precolumn into GC oven 1, equipped with a split/splitless injector (215 °C, split ratio 1:5) and an FID (230 °C), carrier gas (H_2) at a constant pressure of 165 kPa, and temperature program from 40 °C (5 min hold) to 240 °C (25 min hold) at 4 °C/min. A heptakis(diethyl-*tert*-butyldimethylsilyl)-β-cyclodextrin column (25 m × 0.25 mm i.d., 0.25 μm df; MEGA-DEX DET-Beta; Mega S.r.l., Legnano, Italy) was installed as the main column into GC oven 2, equipped with an FID (230 °C). The outlet pressure was 85 kPa. The data were processed *via* Chrom-Card software (Thermo Fisher Scientific, Dreieich, Germany). Temperature program: from 38 °C (10 min hold) to 80 °C (0 min hold) at 2 °C/min, from 80 to 180 °C at 1 °C/min for **1** and from 38 °C (10 min hold) to 80 °C (20 min hold) at 2 °C/min, from 80 °C to 180 °C at 1 °C/min for **2**.

Capillary Gas Chromatography/Olfactometry. Sensory analyses were performed on a Trace GC Ultra (Thermo Fisher Scientific, Dreieich, Germany). The GC system was equipped with a cold on-column injector (35 °C), an FID (250 °C), and a heated sniffing port (200 °C). Hydrogen was used as the carrier gas at a constant pressure of 75 kPa; no makeup such as humidified air was used. A deactivated fused silica precolumn was used (1 m × 0.53 mm i.d.; BGB Analytik AG, Rheinfelden, Germany). The effluent was split 1:1 *via* a press-fit Y-splitter among the sniffing port and FID. A deactivated fused silica capillary column was used to connect between the Y-splitter and sniffing port and FID (30 cm × 0.25 mm i.d.; BGB Analytik AG, Rheinfelden, Germany). The temperature program used for the separation of the stereoisomers of **1** and **2** on the heptakis(diethyl-*tert*-butyldimethylsilyl)-β-cyclodextrin column was as follows: from 35 °C (0 min hold) to 80 °C (20 min hold) at 30 °C/min, from 80 to 115 °C (0 min hold) at 2 °C/min, and from 115 to 180 °C (10 min hold) at 4 °C/min. The odor thresholds were determined by three

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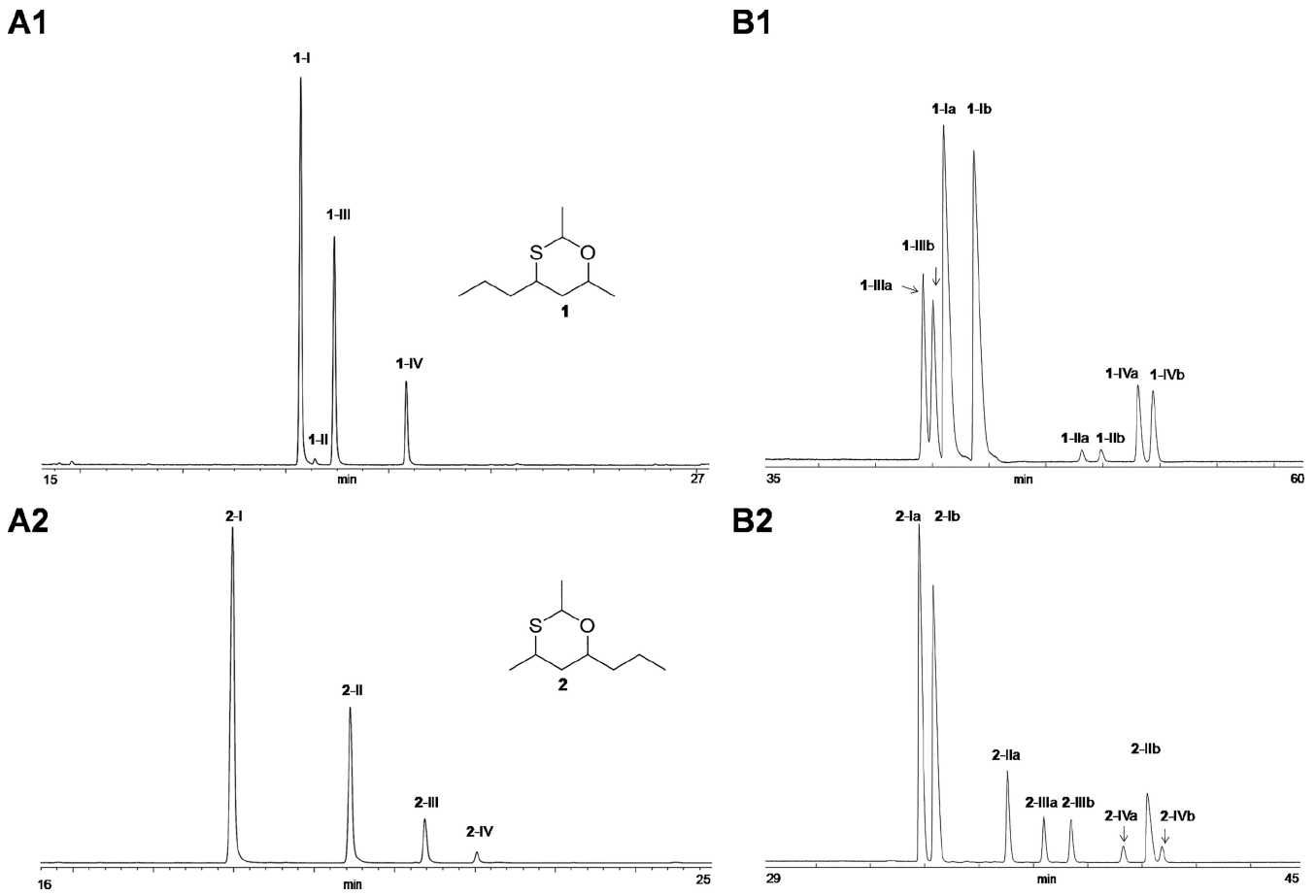

Figure 3. GC separation of the diastereoisomers of (A1) 2,6-dimethyl-4-propyl-1,3-oxathiane **1** and (A2) 2,4-dimethyl-6-propyl-1,3-oxathiane **2** on (DB-WAX); GC separation of the stereoisomers of (B1) **1** and (B2) **2** on heptakis(diethyl-*tert*-butylsilyl)-β-cyclodextrin.

female panelists (25, 26, and 33 years) according to the previously described procedure[34,35] using *trans*-2-decenal (odor threshold of 2.7 ng/L in air) as the internal standard. Solutions of **1**, **2**, and *trans*-2-decenal in diethyl ether were diluted stepwise (1 + 1, v/v), and each dilution was evaluated by GC/O three times by each panelist. The lowest dilution step at which a stereoisomer was consistently perceived in three consecutive GC/O-runs was considered as the odor threshold. The odor qualities of the stereoisomers were determined at one dilution step above the odor threshold.

Gas Chromatography–Mass Spectrometry. A DB-WAXetr fused silica capillary column (30 m × 0.25 mm i.d.; 0.5 μm; J&W Scientific) was installed into a Trace GC Ultra gas chromatograph coupled to an ISQ mass spectrometer (Thermo Fisher), with the carrier gas: He, an inlet pressure of 75 kPa, and a split/splitless injector set to 220 °C with a split ratio of 1:10. The mass spectra were recorded in the electron ionization mode (EI) at 70 eV in a scan range of m/z 30–250, an ion source temperature of 250 °C, and an interface temperature of 240 °C. The temperature program used was from 40 °C (5 min hold) to 240 °C (25 min hold) at 4 °C/min. Data acquisition was carried out *via* Xcalibur software, version 2.1 (Thermo Fisher Scientific, Waltham, MA, USA).

RESULTS AND DISCUSSION

GC Separation of the Stereoisomers of 1 and 2. Michael-type addition of thioacetic acid to 3-hepten-2-one and 2-hepten-4-one, respectively,[25,36] followed by the subsequent reduction of the β-acetylthioheptanones with $LiAlH_4$ resulted in 4-mercapto-2-heptanol **3** and 2-mercapto-4-heptanol **4**.[28,30] The heterocyclic compounds 2,6-dimethyl-4-propyl-1,3-oxathiane **1** and 2,4-dimethyl-6-propyl-1,3-oxathiane **2** were obtained by condensing these β-mercaptoalkanols **3** and **4** with acetaldehyde.[21] The GC separations of the resulting four diastereoisomeric pairs of **1** and **2** on a DB-WAX column are shown in Figure 3A-1, A-2. The ratios of the diastereoisomers are given in Table 1 and in Figure 2. For both substituted 1,3-oxathianes, separations of the eight stereoisomers were achieved by employing heptakis(diethyl-*tert*-butyldimethylsilyl)-β-cyclodextrin as the chiral stationary phase (Figure 3B-1, B-2).

Determination of the Configurations of the Stereoisomers of 1 and 2. The configurations of the stereoisomers of **1** and **2** were assigned based on an approach involving syntheses starting from stereochemically defined mixtures of stereoisomers of their precursors 4-mercapto-2-heptanol **3** and 2-mercapto-4-heptanol **4** and NMR analyses.

(i) Assignment of the stereoisomers possessing (4*S*)-configurations. Enantiomerically enriched 4-(*S*)-mercapto-2-heptanol **3**, obtained *via* $LiAlH_4$-reduction of 4-(*S*)-mercapto-2-heptanone (*ee*: 50%), resulting from PPL-catalyzed kinetic resolution of 4-acetylthio-2-heptanone,[25,30] was used as the starting compound to synthesize enantiomerically enriched (4*S*)-stereoisomers of 2,6-dimethyl-4-propyl-1,3-oxathiane **1** (Supporting Information). The comparison of the GC separation of these stereoisomers of **1** (Figure 4A-2) with the mixture of stereoisomers of **1** obtained upon synthesis starting from a diastereoisomeric mixture of **3** (Figure 4A-1) enabled assignment of the configuration of the stereo-

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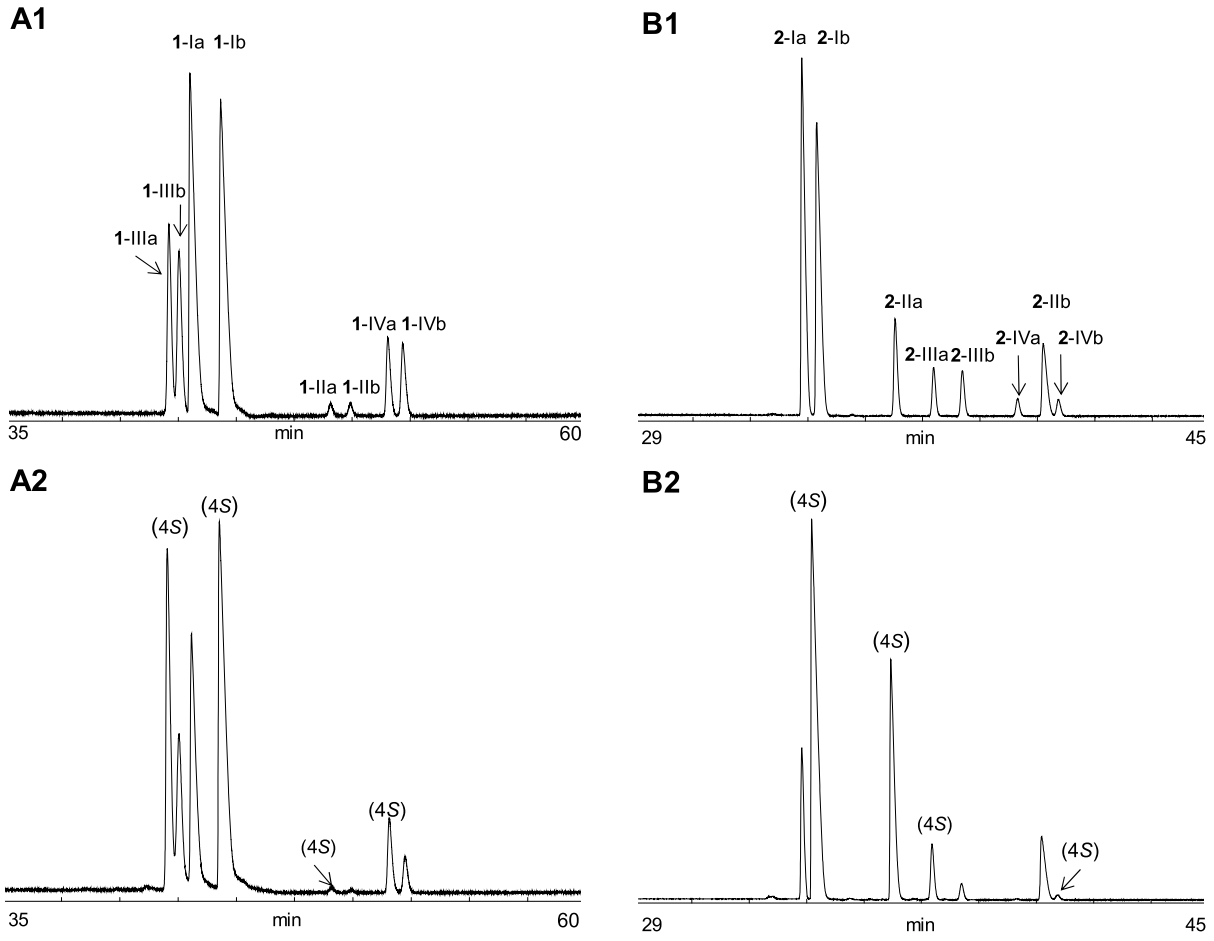

Figure 4. GC separation of the stereoisomers of (A1) synthesized 2,6-dimethyl-4-propyl-1,3-oxathiane **1**; (A2) (4*S*)-enriched 2,6-dimethyl-4-propyl-1,3-oxathiane **1**; (B1) synthesized 2,4-dimethyl-6-propyl-1,3-oxathiane **2**; (B2) (4*S*)-enriched 2,4-dimethyl-6-propyl-1,3-oxathiane **2**.

genic center at position 4. Stereoisomers **1**-IIIa, **1**-Ib, **1**-IIa, and **1**-IVa possess (4*S*)-configurations; consequentially, **1**-IIIb, **1**-Ia, **1**-IIb, and **1**-IVb are (4*R*)-configured. An analogous procedure was applied to assign the stereoisomers of 2,4-dimethyl-6-propyl-1,3-oxathiane **2** with (*S*)-configuration in position 4 (Figure 4B-2), using 2-(*S*)-mercapto-4-heptanol **4**, obtained *via* $LiAlH_4$-reduction of 2-(*S*)-acetylthio-4-heptanone (*ee*: 81%), resulting from the CAL-B-catalyzed kinetic resolution of 2-acetylthio-4-heptanone[25] as the starting compound to synthesize enantiomerically enriched (4*S*)-2,4-dimethyl-6-propyl-1,3-oxathiane **2** (Figure 4B-2; Supporting Information). The stereoisomers **2**-Ib, **2**-IIa, **2**-IIIa, and **2**-IVb possess the (4*S*)-configuration, and accordingly, the stereoisomers **2**-Ia, **2**-IIb, **2**-IIIb, and **2**-IVa possess the (4*R*)-configuration.

(ii) Assignment of the relative configurations in positions 4 and 6. To assign the stereoisomers of **1** and **2** that possess relative *trans*- and *cis*-configurations, respectively, regarding the substituents in positions 4 and 6, the 2,4-*anti*- and the 2,4-*syn*-diastereoisomers of **3** and **4** were separated *via* HPLC.[30] Condensation of 2,4-*anti*-**3** with acetaldehyde resulted in two pairs of stereoisomers of **1** with *trans*-configurations related to positions 4 and 6 (Figure 5A-2). The comparison with the GC separation of the mixture of stereoisomers obtained by the synthesis of **1** (Figure 5A-1) revealed that on the employed chiral stationary phase, the first (**1**-III) and the last (**1**-IV) eluting pairs of enantiomers possess relative 4,6-*trans*-configurations; accordingly, the second (**1**-I) and the third pair (**1**-II) possess relative 4,6-*cis*-configurations. In combination with the information gained in step (I), the following configurations were assigned at positions 4 and 6: **1**-Ia: (4*R*,6*S*), **1**-Ib: (4*S*,6*R*); **1**-IIa: (4*S*,6*R*), **1**-IIb: (4*R*,6*S*); **1**-IIIa: (4*S*,6*S*), **1**-IIIb: (4*R*,6*R*); **1**-IVa: (4*S*,6*S*), and **1**-IVb: (4*R*,6*R*).

Analogously, the HPLC-enriched 2,4-*syn*-diastereoisomer of **4** was used to synthesize the stereoisomers of 2,4-dimethyl-6-propyl-1,3-oxathiane **2** with *cis*-configurations related to positions 4 and 6 (Figure 5B-2). GC analysis (Figure 5B-2) revealed a main peak pair corresponding to the 4,6-*cis*-stereoisomers **2**-Ia and **2**-Ib (Figure 5B-1) and four minor peaks. Two of them corresponded to the 4,6-*trans*-stereoisomers formed as byproducts due to incomplete separation of the 2,4-*anti*- and 2,4-*syn*-diastereoisomers of 2-mercapto-4-heptanol **4** *via* HPLC (*syn*-**4**: 97% and *anti*-**4**: 3%); the comparison with the GC separation of the synthesized mixture of stereoisomers enabled assignment of the other pair as stereoisomers **2**-IVa and **2**-IVb possessing *cis*-configurations, related to the positions 4 and 6 (Figure 5B-1). Taking into account the information from step (i), the following configurations were assigned: **2**-Ia: (4*R*,6*S*), **2**-Ib: (4*S*,6*R*); **2**-IIa: (4*S*,6*S*), **2**-IIb: (4*R*,6*R*); **2**-IIIa: (4*S*,6*S*), **2**-IIIb: (4*R*,6*R*); **2**-IVa: (4*R*,6*S*), and **2**-IVb: (4*S*,6*R*)

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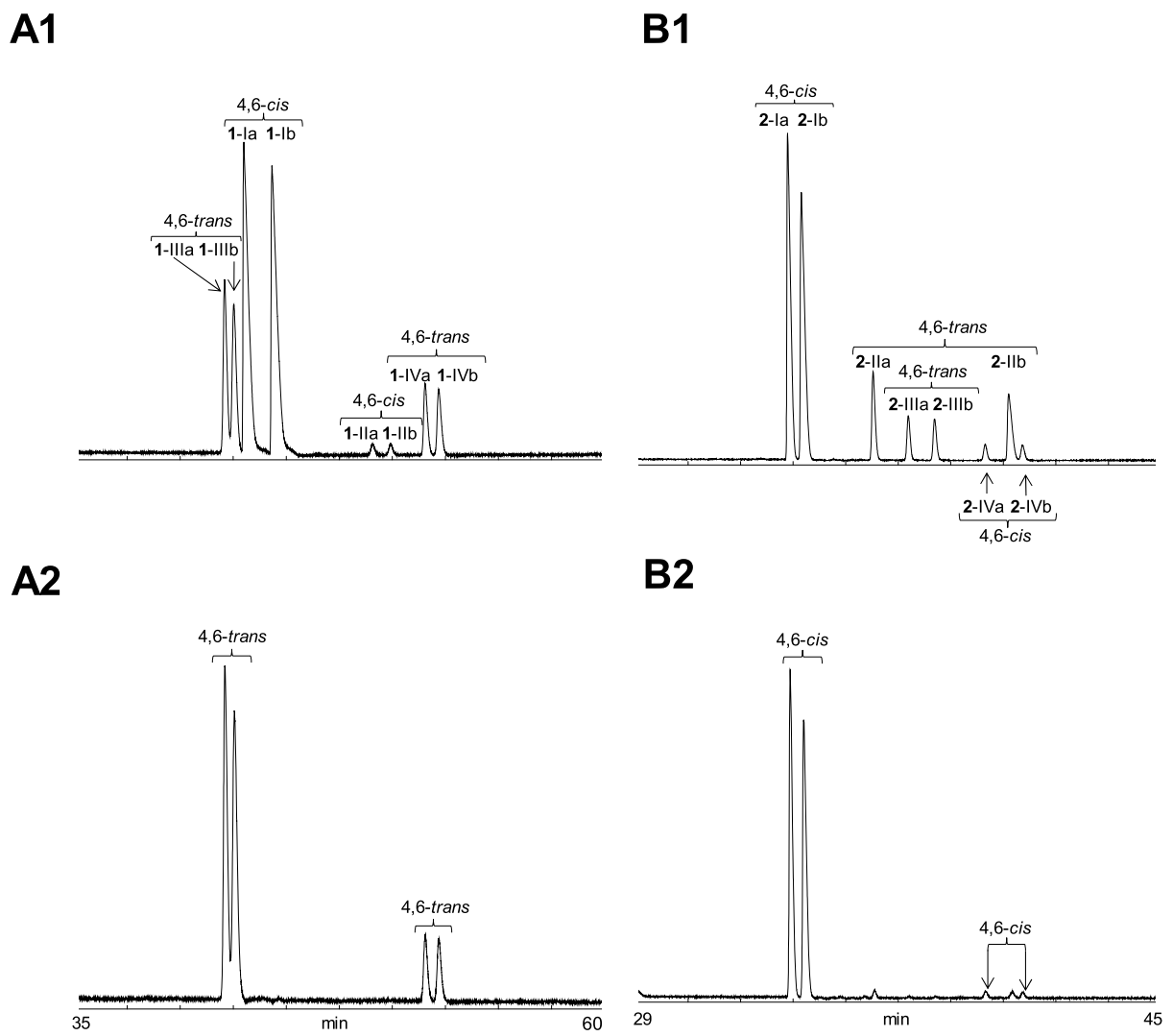

Figure 5. GC separation of stereoisomers of 2,4-dimethyl-6-propyl-1,3-oxathiane **1** and 2,4-dimethyl-6-propyl-1,3-oxathiane **2** on heptakis(diethyl-*tert*-butylsilyl)-*β*-cyclodextrin. (A1) mixture of stereoisomers of **1** obtained upon synthesis starting from a diastereoisomeric mixture of 4-mercapto-2-heptanol **3**; (A2) 4,6-*trans*-configured stereoisomers of **1** obtained upon synthesis starting from *anti*-4-mercapto-2-heptanol *anti*-**3**; (B1) Mixture of stereoisomers of **2** obtained upon synthesis starting from a diastereoisomeric mixture of 2-mercapto-4-heptanol **4**; (B2) 4,6-*cis*-configured stereoisomers of **2** obtained upon synthesis starting from *syn*-2-mercapto-4-heptanol *syn*-**4**.

(iii) NMR analyses. The ^{1}H and ^{13}C NMR data assigned to the stereoisomers of the synthesized mixtures of **1** and **2** are compiled in Tables 2 and 3. They were compared to the NMR data of 2-methyl-4-propyl-1,3-oxathiane,[16,17] as well as to data reported for other structurally related di- and trimethylated 1,3-oxathianes[22,37,38] (Supporting Information). The upfield shifts of H-2 (4.82 ppm) and H-6 (3.50 ppm) in **1**-I compared to H-2 (5.03 ppm) and H-6 (3.78 ppm) in **1**-III and the upfield shifts of H-2 (4.80 ppm) and H-6 (3.35 ppm) in **2**-I compared to H-2 (5.08 ppm) and H-6 (3.66 ppm) in **2**-II (Table 2) were in excellent agreement with the differences in chemical shifts reported for the structurally related trimethylated analogues *r*-2-*cis*-4-*cis*-6-1,3-oxathiane (H_a-2: 4.78 ppm; H_a-6: 3.45 ppm) and *r*-2-*trans*-4-*cis*-6-1,3-oxathiane (H_a-2: 5.02; H_a-6: 3.82 ppm).[38] Similar to the axial methyl substituent in position 4 of *r*-2-*cis*-4-*cis*-6-1,3-oxathiane, the axial propyl and methyl substituents in position 4 of **1**-III and **2**-II, respectively, have shielding effects on the protons H-2 and H-6. Similar differences in chemical shifts have been reported for *cis*- and *trans*-2,4,4,6-tetramethyl-1,3-oxathiane.[22] Regarding H-4, the ^{1}H NMR data of **1**-I (3.04 ppm), **1**-III (2.88 ppm), **2**-I (3.11−3.03 ppm), and **2**-II (3.15 ppm) fit to those of *cis*-2-methyl-4-propyl-1,3-oxathiane (H_a-4: 3.09−3.02 ppm), *trans*-2-methyl-4-propyl-1,3-oxathiane (H-4: 2.89 ppm), *r*-2-*cis*-4-*cis*-6-trimethyl-1,3-oxathiane (H_a-4: 3.02 ppm), and *r*-2-*trans*-4-*cis*-6-trimethyl-1,3-oxathiane (H_e-4: 3.14 ppm).[17,38] The chemical shifts of **1**-IV (H-2: 5.16 ppm, H-4: 3.25 ppm, and H-6: 4.36 ppm) were in agreement with those of *r*-2-*cis*-4-*trans*-6-trimethyl-1,3-oxathiane (H_a-2: 5.11 ppm, H_a-4: 3.23 ppm, and H_e-6: 4.31 ppm).[38]

The assignments of **1**-I, **1**-IV, and **2**-I as the stereoisomers with *cis*-configurations related to positions 2 and 4 and of **1**-III and **2**-II as those with *trans*-2,4-configurations were also supported by comparisons of the chemical shifts of the methyl groups and coupling constants of the respective structural analogues (Supporting Information).

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Table 2. ^{1}H NMR Data Assigned to Diastereoisomers Obtained upon Syntheses of 2,6-Dimethyl-4-propyl-1,3-oxathiane 1 and 2,4-Dimethyl-6-propyl-1,3-oxathiane 2 (for Structures and Positions, See Figure 2)

	2,6-dimethyl-4-propyl-1,3-oxathiane 1			2,4-dimethyl-6-propyl-oxathiane 2	
position	1-I (1-Ia and 1-Ib)	1-III (1-IIIa and 1-IIIb)	1-IV (1-IVa and 1-IVb)	2-I (2-Ia and 2-Ib)	2-II (2-IIa and 2-IIb)
H-2	4.82 (q, $^3J_{Ha\text{-}2/2\text{-}Me}$ = 6.2 Hz, 1H)	5.03 (q, $^3J_{Ha\text{-}2/2e\text{-}Me}$ = 6.2 Hz, 1H)	5.16 (q, $^3J_{Ha\text{-}2/2e\text{-}Me}$ = 6.2 Hz, 1H)	4.80 (q, $^3J_{Ha\text{-}2/2e\text{-}Me}$ = 6.2 Hz, 1H)	5.08 (t, $^3J_{Ha\text{-}2/2e\text{-}Me}$ = 6.1 Hz, 1H)
H-6	3.50 (m, $^3J_{Ha\text{-}6/Ha\text{-}5}$ = 11.1 Hz, $J_{H\text{-}6/6\text{-}Me}$ = 6.0 Hz, $J_{Ha\text{-}6/He\text{-}5}$ = 2.1 Hz, 1H)	3.78 (dqd, $^3J_{Ha\text{-}6/Ha\text{-}5}$ = 12.4 Hz, $^3J_{H\text{-}6/6\text{-}Me}$ = 6.2 Hz, $^3J_{Ha\text{-}6/He\text{-}5}$ = 2.0 Hz, 1H)	4.36 (qdd, $^3J_{He\text{-}6/6a\text{-}Me}$ = 7.1 Hz, $^3J_{He\text{-}6/Ha\text{-}5}$ = 5.2 Hz, $^3J_{He\text{-}6/He\text{-}5}$ = 2.2 Hz, 1H)	3.35 (dddd, $^3J_{Ha\text{-}6/Ha\text{-}5}$ = 11.2 Hz, $J_{H\text{-}6/Ha\text{-}7}$ = 7.5 Hz, $J_{H\text{-}6/Hb\text{-}7}$ = 4.6 Hz, $J_{H\,a\text{-}6/H\,e\text{-}5}$ = 1.9 Hz, 1H)	3.66 (dddd, $^3J_{Ha\text{-}6/Ha\text{-}5}$ = 11.9 Hz, $J_{H\text{-}6/Ha\text{-}7}$ = 7.8 Hz, $J_{H\text{-}6/Hb\text{-}7}$ = 4.7 Hz, $J_{Ha\text{-}6/He\text{-}5}$ = 1.9 Hz, 1H)
H-4	3.04 (dddd, $^3J_{Ha\text{-}4/Ha\text{-}5}$ = 11.6, $^3J_{Ha\text{-}4/H\text{-}7}$ = 7.3, $^3J_{Ha\text{-}4/H\text{-}7}$ = 5.7, $^3J_{Ha\text{-}4/He\text{-}5}$ = 2.7 Hz, 1H)	2.88 (dddd, $^3J_{He\text{-}4/H\text{-}7}$ = 10.7, $^3J_{He\text{-}4/H\text{-}7}$ = 7.6 Hz, $^3J_{He\text{-}4/Ha\text{-}5}$ = 4.9 Hz, 1H), $^3J_{He\text{-}4/He\text{-}5}$ = 2.8 Hz, 1H)	3.25 (dtd, $^3J_{He\text{-}4/Ha\text{-}5}$ = 12.0 Hz, $^3J_{Ha\text{-}4/H\text{-}7}$ = 6.3 Hz, $^3J_{Ha\text{-}4/H\text{-}7}$ = 5.5 Hz, $^3J_{He\text{-}4/He\text{-}5}$ = 2.7 Hz, 1H)	3.11–3.03 (m, 1H)	3.15 (qdd, $^3J_{He\text{-}4/4\text{-}Me}$ = 7.3, $J_{He\text{-}4/Ha\text{-}5}$ = 4.8, $^3J_{He\text{-}4/He\text{-}5}$ = 2.8 Hz, 1H)
H_e-5	1.75 (ddd, $^2J_{He\text{-}5/Ha\text{-}5}$ = 13.6 Hz, $^3J_{He\text{-}5/Ha\text{-}4}$ = 2.6 Hz, $^3J_{He\text{-}5/Ha\text{-}6}$ = 2.0 Hz, 1H)	1.60 (ddd, $^2J_{He\text{-}5/Ha\text{-}5}$ = 13.9 Hz, $^3J_{He\text{-}5/He\text{-}4}$ = 2.8 Hz, $^3J_{He\text{-}5/Ha\text{-}6}$ = 1.9 Hz, 1H)	1.73–1.61 (m, 1H)	1.74 (ddd, $^3J_{He\text{-}5/Ha\text{-}5}$ = 13.6 Hz, $^3J_{He\text{-}5/Ha\text{-}4}$ = 2.7 Hz, $^3J_{He\text{-}5/Ha\text{-}6}$ = 1.9 Hz, 1H),	1.49 (ddd, $^2J_{He\text{-}5/Ha\text{-}5}$ = 13.5 Hz, $^3J_{He\text{-}5/Ha\text{-}4}$ = 2.7 Hz, $^3J_{He\text{-}5/Ha\text{-}6}$ = 1.8 Hz, 1H)
H_a-5	1.24–1.19 (m, 1H)	1.82 (ddd, $^2J_{Ha\text{-}5/He\text{-}5}$ = 13.9 Hz, $^3J_{Ha\text{-}5/Ha\text{-}6}$ = 11.5, $^3J_{Ha\text{-}5/He\text{-}4}$ = 4.8 Hz, 1H)	[a]	1.21–1.12 (m, 1H)	1.81 (ddd, $^2J_{Ha\text{-}5/He\text{-}5}$ = 13.8 Hz, $^3J_{Ha\text{-}5/Ha\text{-}6}$ = 11.7 Hz, $^3J_{Ha\text{-}5/He\text{-}4}$ = 4.9 Hz, 1H)
4-Me/6-Me	1.22 (d, $^3J_{6e\text{-}Me/Ha\text{-}6}$ = 6.3 Hz, 3H)	1.17 (d, $^3J_{6e\text{-}Me/Ha\text{-}6}$ = 6.2 Hz, 3H)	1.20 (d, $^3J_{6a\text{-}Me/He\text{-}6}$ = 7.0 Hz, 1H)	1.20 (d, $^3J_{4e\text{-}Me/Ha\text{-}4}$ = 6.7 Hz, 3H)	1.54 (d, $^3J_{4e\text{-}Me/He\text{-}4}$ = 7.3 Hz, 3H)
2-Me	1.48 (d, $^3J_{2e\text{-}Me/Ha\text{-}2}$ = 6.3 Hz, 3H)	1.43 (d, $^3J_{2e\text{-}Me/Ha\text{-}2}$ = 6.2 Hz, 3H)	1.41 (d, $^3J_{2e\text{-}Me/Ha\text{-}2}$ = 6.1 Hz, 1H)	1.47 (d, $^3J_{2e\text{-}Me/Ha\text{-}2}$ = 6.2 Hz, 3H)	1.43 (d, $^3J_{2e\text{-}Me/Ha\text{-}2}$ = 6.2 Hz, 3H)

[a] Data could not be assigned.

For the assignment of the configurations at position 2 of the diastereoisomers of **1** and **2**, NOE data were used. Figure 6 shows a part of the NOESY-spectrum of **1**. For diastereoisomer **1**-I, NOE correlations were observed between H-2/H-4 and H-2/H-6, indicating that all three protons at the stereogenic centers in positions 2, 4, and 6 are axial to the ring plane. For diastereoisomer **1**-III, one NOE signal was observed between H-2/H-6, indicating axial positions for H-2 and H-6. For **1**-IV, one NOE signal was observed between H-2/H-4, indicating axial positions for H-2 and H-4. The weakness of the signal was probably due to the fact that the stereoisomers of **1**-IV only constitute 13% of the synthesized mixture (Table 1) subjected to NMR analysis. Taking into account the results from steps (i) and (ii), for the diastereoisomers of **1**, the configurations and spatial orientations as summarized in Table 4 were assigned. For **1**-II, no NOE data could be obtained because its proportion (1%) in the synthesized mixture of diastereoisomers of **1** was too low. Considering the configurations assigned at position 4 in step (i) and the relative configurations related to positions 4 and 6 assigned in step (ii), the spatial orientations of the substituents in **1**-IIa and **1**-IIb were deduced, assuming the stable equatorial orientation of the methyl substituent in position 2 (Figure 2). Figure 7 shows a part of the NOESY spectrum of 2,4-dimethyl-6-propyl-1,3-oxathiane **2**. NOE signals were observed for diastereoisomer **2**-I between H-4/H-6, H-2/H-6, and H-2/H-4; for diastereoisomer **2**-II, a NOE signal was observed between H-2/H-6. These results are also in agreement with the previous assignments from steps (i) and (ii) (Table 4). For the quantitatively minor stereoisomers **2**-III and **2**-IV, no NMR data were available. Comparable to the approach taken for **1**-II, the spatial orientations of **2**-III and **2**-IV were deduced based on the configurations determined in position 4, the relative configurations regarding positions 4 and 6, and the assumption that the methyl substituent in position 2 is in the preferred equatorial position (Figure 2).

In the mixtures obtained upon the reaction of acetaldehyde with the *β*-mercaptoalkanols **3** and **4**, the stereoisomers possessing all substituents in equatorial positions (**1**-I and **2**-I, respectively) were quantitatively predominating. There was also a prevalence of the stereoisomers with *cis*-configurations of the substituents in positions 2 and 6 of the 1,3-oxathiane rings. For **1**, the ratio of the sum of the *cis*-2,6-configured **1**-I and **1**-II and the sum of the *trans*-2,6-configured **1**-II and **1**-IV was 86%:14%; for **2**, the corresponding ratio of the sum of *cis*-2,6-configured **2**-Ia and **2**-IIa and the sum of *trans*-2,6-configured **2**-IIIa and **2**-IVa was 90%:10%. This is in agreement with the ratios of *cis*- and *trans*-configured stereoisomers reported for other substituted 1,3-oxathianes, such as 2-methyl-4-propyl-1,3-oxathiane[10,16] or *cis*-2,4,4,6-tetramethyl-1,3-oxathiane.[22]

The GC separation of the enantiomers of **2**-II on the employed chiral stationary phase was much better than those of the enantiomers of the other stereoisomers of **1** and **2**. The known mechanisms underlying the GC separation of enantiomers on modified cyclodextrins did not provide an obvious explanation for this notable phenomenon.

Sensory Properties of the Stereoisomers of 1 and 2. *Evaluation via GC/O.* The odor thresholds of the stereoisomers of **1** and **2** were determined *via* GC/O by three panelists using the method described by Ullrich and Grosch[34] (Table 5). Only for **1**-IIa and **2**-IIb were there clear differences (factors 18 and 22, respectively) between the odor thresholds determined by two panelists. For all other stereoisomers, the

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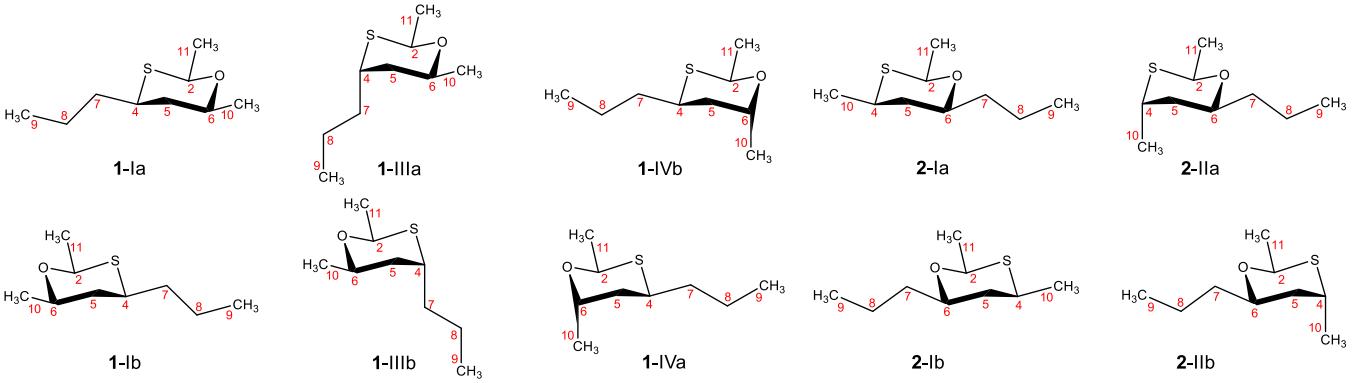
Table 3. ^{13}C NMR Data Assigned to Diastereoisomers Obtained upon Syntheses of 2,6-Dimethyl-4-propyl-1,3-oxathiane **1** and 2,4-Dimethyl-6-propyl-1,3-oxathiane **2**

	2,6-dimethyl-4-propyl-1,3-oxathiane **1**			2,4-dimethyl-6-propyl-1,3-oxathiane **2**	
position	**1**-I (**1**-Ia and **1**-Ib)	**1**-III (**1**-IIIa and **1**-IIIb)	**1**-IV (**1**-IVa and **1**-IVb)	**2**-I (**2**-Ia and **2**-Ib)	**2**-II (**2**-IIa and **2**-IIb)
2	78.90	73.97	69.35	79.04	73.92
4	42.47	39.48	35.80	37.30	33.98
5	40.19	37.01	38.72	40.11	36.75
6	75.74	70.23	70.26	79.54	73.56
7	38.38	36.14	36.87	38.56	38.79
8	19.51	21.42	[a]	18.63	18.62
9	14.03	13.90	14.18	14.14	14.25
10	22.09	22.09	22.09	21.92	21.25
11	22.29	22.58	[a]	21.73	22.06



[a]Data could not be assigned.

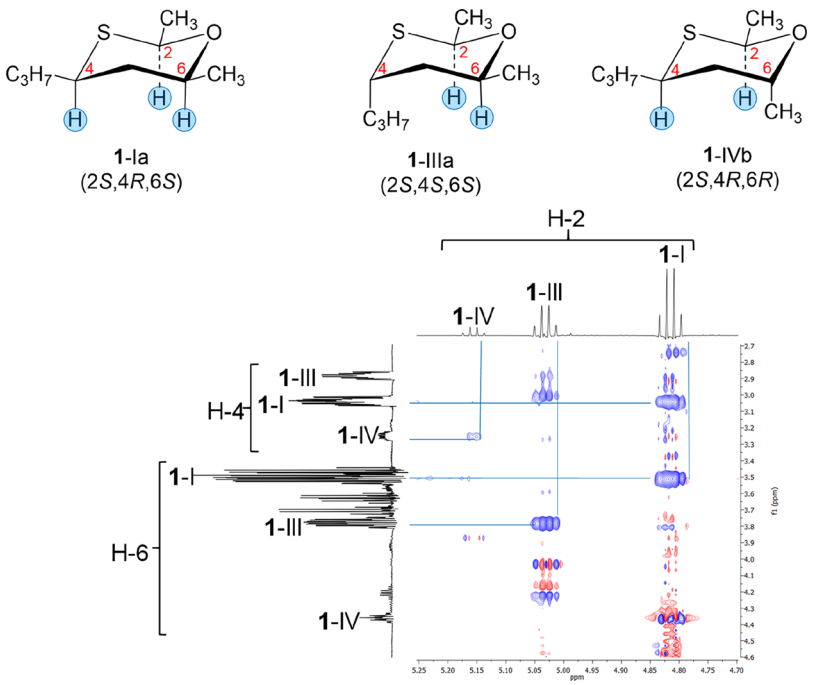


Figure 6. Part of the NOESY spectrum of 2,6-dimethyl-4-propyl-1,3-oxathiane **1**. The protons showing NOE correlations are marked in blue in the structures exemplarily shown for **1**-Ia, **1**-IIIa, and **1**-IVb.

odor thresholds determined by the individual panelists were either the same or differed by factors of 2−5, corresponding to only one or slightly more than two dilution steps, as applied in the course of the sensory evaluation procedure. This indicates the reproducibility of the sensory assessments performed by the panelists.

The odor thresholds of the stereoisomers of **2** were generally higher than those of the corresponding stereoisomers of **1**. This difference (factor of 178 between the geometric means) was particularly pronounced for the (2*S*,4*R*,6*S*)-stereoisomers **1**-Ia and **2**-Ia; for the other stereoisomers of **1** and **2**, the geometric means of the odor thresholds differed by factors ranging from 6 to 18.

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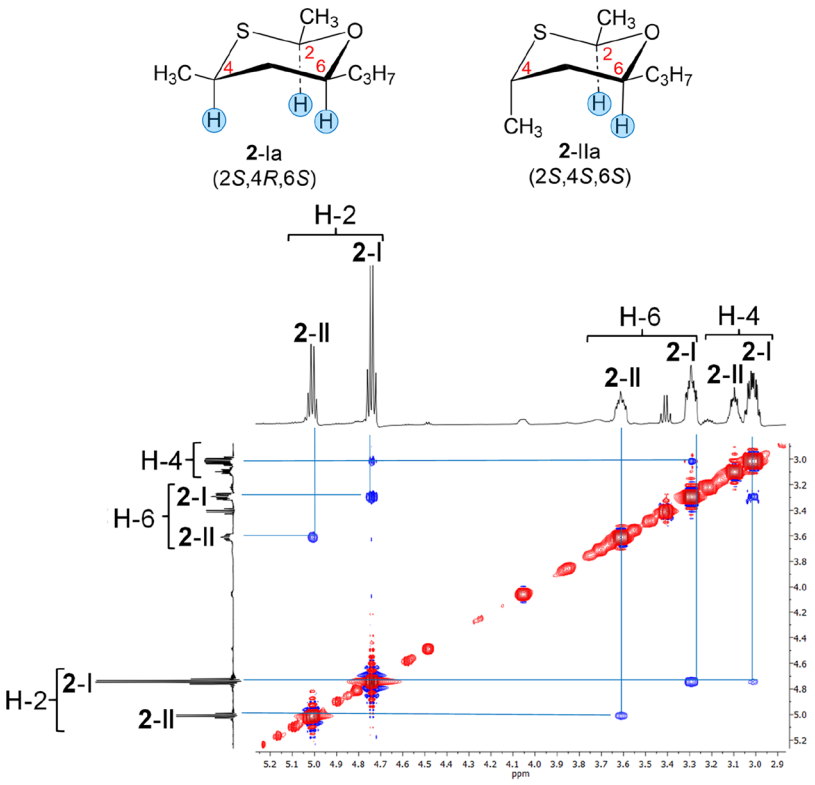

Figure 7. Part of the NOESY spectrum of 2,4-dimethyl-6-propyl-1,3-oxathiane **2**. The protons showing NOE correlations are marked in blue in the structures exemplarily shown for **2**-Ia and **2**-IIa.

Table 4. Overview on Configurations and Spatial Arrangements Assigned for the Stereoisomers of 2,6-Dimethyl-4-propyl-1,3-oxathiane 1 and 2,4-Dimethyl-6-propyl-1,3-oxathiane 2

		relative configuration			spatial arrangements[a]			absolute configuration		
stereoisomer		4,6	2,4	2,6	H-2	H-4	H-6	2	4	6
1-Ia	*r*-2-*cis*-4-*cis*-6	*cis*	*cis*	*cis*	ax.	ax.	ax.	*S*	*R*	S
1-Ib	*r*-2-*cis*-4-*cis*-6	*cis*	*cis*	*cis*	ax.	ax.	ax.	*R*	*S*	R
1-IIa	*r*-2-*trans*-4-*trans*-6	*cis*	*trans*	*trans*	ax.	eq.	eq.	*S*	*S*	R
1-IIb	*r*-2-*trans*-4-*trans*-6	*cis*	*trans*	*trans*	ax.	eq.	eq.	*R*	*R*	S
1-IIIa	*r*-2-*trans*-4-*cis*-6	*trans*	*trans*	*cis*	ax.	eq.	ax.	*S*	*S*	S
1-IIIb	*r*-2-*trans*-4-*cis*-6	*trans*	*trans*	*cis*	ax.	eq.	ax.	*R*	*R*	R
1-IVa	*r*-2-*cis*-4-*trans*-6	*trans*	*cis*	*trans*	ax.	ax.	eq.	*R*	*S*	*S*
1-IVb	*r*-2-*cis*-4-*trans*-6	*trans*	*cis*	*trans*	ax.	ax.	eq.	*S*	*R*	R
2-Ia	*r*-2-*cis*-4-*cis*-6	*cis*	*cis*	*cis*	ax.	ax.	ax.	*S*	*R*	*S*
2-Ib	*r*-2-*cis*-4-*cis*-6	*cis*	*cis*	*cis*	ax.	ax.	ax.	*R*	*S*	*R*
2-IIa	*r*-2-*trans*-4-*cis*-6	*trans*	*trans*	*cis*	ax.	eq.	ax.	*S*	*S*	*S*
2-IIb	*r*-2-*trans*-4-*cis*-6	*trans*	*trans*	*cis*	ax.	eq.	ax.	*R*	*R*	*R*
2-IIIa	*r*-2-*cis*-4-*trans*-6	*trans*	*cis*	*trans*	ax.	ax.	eq.	*R*	*S*	*S*
2-IIIb	*r*-2-*cis*-4-*trans*-6	*trans*	*cis*	*trans*	ax.	ax.	eq.	*S*	*R*	*R*
2-IVa	*r*-2-*trans*-4-*trans*-6	*cis*	*trans*	*trans*	ax.	eq.	eq.	*R*	*R*	*S*
2-IVb	*r*-2-*trans*-4-*trans*-6	*cis*	*trans*	*trans*	ax.	eq.	eq.	*S*	*S*	*R*

[a]For **1**-IIa and **1**-IIb, no NOESY data and for **2**-IIIa, **2**-IIIb, **2**-IVa, and **2**-IVb no NMR data were available; for these stereoisomers, the spatial arrangements were deduced by taking into account the absolute configurations determined at position 4 in step (ii) and the relative configurations in positions 4 and 6 determined in step (ii) and assuming the stable equatorial orientation of the methyl substituent in position 2.

For both oxathianes, the enantiomeric pairs of (2*S*,4*R*,6*S*)- and (2*R*,4*S*,6*R*)-stereoisomers (**1**-Ia, **1**-Ib and **2**-Ia, **2**-Ib, respectively) showed the highest odor thresholds. These are the stereoisomers in which all substituents are in equatorial positions and which were quantitatively dominating in the synthesized mixtures (Figure 2). A comparison of the highest and lowest geometric mean odor thresholds of the stereoisomers of **1** and **2**, respectively, shows that this impact of the spatial arrangements of the substituents in positions 2, 4, and 6 was more pronounced for oxathiane **2**. The mean odor threshold determined for **2**-Ia (1404 ng/L) was approximately 100 times higher than the mean threshold of **2**-IIIb (14 ng/L), whereas for oxathiane **1**, the mean thresholds of **1**-Ib (12 ng/L) and **1**-IIb (1.5 ng/L) differ only by a factor of 8. Within the group of stereoisomers of **1** and **2**, respectively, having at least one of the substituents in axial position, that is, **1**-II, **1**-III, and

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Table 5. Odor Thresholds of the Stereoisomers of 2,6-Dimethyl-4-propyl-1,3-oxathiane 1 and 2,4-Dimethyl-6-propyl-1,3-oxathiane 2 Determined by GC/O

	odor threshold in air (ng/L)									
	2,6-dimethyl-4-propyl-oxathiane **1**					2,4-dimethyl-6-propyl-oxathiane **2**				
		panelist					panelist			
stereoisomer	no.	1	2	3	mean[a]	no.	1		2	3	mean[a]
(2*S*,4*R*,6*S*)	**1**-Ia	9.3	11	4.8	7.9	**2**-Ia	1884	3747	844	652	1404
(2*R*,4*S*,6*R*)	**1**-Ib	9.2	10	19	12	**2**-Ib	123	233	419	168	212
(2*S*,4*S*,6*R*)	**1**-IIa	1.0	18	-[b]	4.2	**2**-IVb	53	15	27	19	25
(2*R*,4*R*,6*S*)	**1**-IIb	1.0	2.2	-[b]	1.5	**2**-IVa	16	22	80	18	27
(2*S*,4*S*,6*S*)	**1**-IIIa	2.7	1.5	5.6	2.8	**2**-IIa	53	15	27	91	37
(2*R*,4*R*,6*R*)	**1**-IIIb	2.9	1.6	2.8	2.4	**2**-IIb	8	11	20	177	24
(2*R*,4*S*,6*S*)	**1**-IVa	1.8	8.1	1.6	2.9	**2**-IIIa	36	14	26	27	24
(2*S*,4*R*,6*R*)	**1**-IVb	1.0	2.2	3.2	1.9	**2**-IIIb	16	14	13	13	14

[a]Geometric mean. [b]Not determined.

1-IV and **2**-II, **2**-III, and **2**-IV, there were only subtle differences in the odor thresholds. For both oxathianes, there were also no differences between the odor thresholds of enantiomeric stereoisomers.

The odor qualities of the stereoisomers of **1** and **2** were recorded at the GC/O-dilution step above the odor threshold level (Table 6); odor descriptions given by at least two of the panelists are marked in bold. For 2,6-dimethyl-4-propyl-1,3-oxathiane **1**, six of the eight stereoisomers exhibited pleasant flowery, fruity, or sweet nuances. Only the stereoisomers **1**-IIa and **1**-IVa showed more unpleasant moldy, earthy, or rancid notes. For the stereoisomers of 2,4-dimethyl-6-propyl-1,3-oxathiane **2**, fruity, sweet, and flowery descriptions were rare; except for **2**-IVb, the other stereoisomers were mainly characterized by descriptors, such as broth, mushroom, pungent, and musty.

*Comparison of the Sensory Properties of the Stereoisomers of **1** and **2** to Those of the Corresponding β-Mercaptoheptanols **3** and **4**.* The odor thresholds of the stereoisomers of 2,6-dimethyl-4-propyl-1,3-oxathiane **1** (1.0−9.3 ng/L) were higher than those reported for the stereoisomers of 4-mercapto-2-heptanol **3** (0.05−0.3 ng/L).[39] Similarly, the odor thresholds of 2,4-dimethyl-6-propyl-1,3-oxathiane **2** (14−1404 ng/L) were higher than those of the stereoisomers of 2-mercapto-4-heptanol **4** (0.2−51 ng/L).[28] For both β-mercaptoalkanols, bridging the 1,3-oxygen sulfur functionality *via* reaction with acetaldehyde resulted in increases of the odor thresholds. Pronounced increases were particularly observed if the cyclization of a *syn*-mercaptoalkanol resulted in a 1,3-oxathiane possessing all substituents in equatorial positions; examples are the clear differences in the odor thresholds between *syn*-(2*S*,4*R*)-**3** (0.2 ng/L)[39] and (2*R*,4*S*,6*R*)-**1**-Ib (12 ng/L) and between *syn*-(2*R*,4*S*)-**3** (0.9 ng/L)[28] and (2*S*,4*R*,6*S*)-**2**-Ia (1404 ng/L). These differences in the odor thresholds in air determined by GC/O for the free β-mercaptoalkanols **3** and **4** and the corresponding 1,3-oxathianes **1** and **2** are in agreement with the clear differences between the odor thresholds in alcoholic/aqueous solutions reported for 3-mercaptohexanol (60 ng/L)[15] and 2-methyl-4-propyl-1,3-oxathiane (7.1 μg/L)[21] and for 4-mercapto-4-methylpentan-2-ol (55 ng/L)[40] and *cis*-2,4,4,6-tetramethyl-1,3-oxathiane (14.9 μg/L).[22]

Regarding the odor qualities, the onion and sulfury notes reported for three of the four stereoisomers of **3**[39] were not perceived for the stereoisomers of **1** (Table 6). The conversion of **3** to the 1,3-oxathiane **1** resulted mostly in fruity, flowery, and sweet odor notes. The impact of the configuration of the newly formed asymmetric center in position 2 becomes obvious when comparing the odor descriptions for the stereoisomers of **1** resulting from the reaction of (2*S*,4*S*)-4-mercapto-2-heptanol **3** with acetaldehyde: (2*S*,4*S*,6*S*)-configured **1**-IIIa exhibited flowery, sweet, herbal, and lemon peel odor notes, whereas the diastereoisomeric (2*R*,4*S*,6*S*)-configured **1**-IVa was described as moldy, musty, vegetable, earthy, and metallic (Table 6). There was, however, no consistent pattern regarding the influence of the configurations of the three substituents at positions 2, 4, and 6 of the stereoisomers of 1,3-oxathiane **1** on their odor qualities.

For the stereoisomers of 2-mercapto-4-heptanol **4**, vegetable broth-type notes were reported as the common descriptors. This difference from the odor qualities of the stereoisomers of 4-mercapto-2-heptanol **3** was also reflected in the odor descriptions of the stereoisomers of oxathiane **2** (Table 6); in contrast to the stereoisomers of **1**, fruity and flowery notes were rare, whereas mushroom, earthy, moldy, and pungent nuances were prevailing.

*Comparison of the Sensory Properties of the Stereoisomers of **1** and **2** to Those of 2-Methyl-4-propyl-1,3-oxathiane.* 2,6-Dimethyl-4-propyl-1,3-oxathiane **1** differs from 2-methyl-4-propyl-1,3-oxathiane by the presence of a methyl substituent at position 6 of the oxathiane ring and consequentially by an additional asymmetric center. For *cis*-(2*S*,4*R*)-2-methyl-4-propyl-1,3-oxathiane, the odor from a smelling strip has been described as typically sulfurous with a rubbery onion note and a fruitiness reminiscent of grapefruit peel, mango, and passion fruit, whereas the odor of the *cis*-(2*R*,4*S*)-enantiomer was much weaker, without pronounced sulfur character and with a fresh iris-type note.[18] These differences in odor qualities between the two enantiomers have later been confirmed,[19] and the sensory evaluation of all stereoisomers of 2-methyl-4-propyl-1,3-oxathiane revealed that only the *cis*-(2*S*,4*R*)-stereoisomer exhibits the fatty, fruity green, tropical fruits, and grapefruit odor notes.[17] The odor descriptions recorded for the two stereoisomers of **1** possessing *cis*-(2*S*,4*R*)-configurations, that is, **1**-Ia and **1**-IVb, revealed that with the additional methyl substituent in position 6 compared to *cis*-(2*S*,4*R*)-2-methyl-4-propyl-1,3-oxathiane, the molecules still exhibited fresh, fruity, sweet, and flowery notes; however, there were also cooked vegetable-type and herbal nuances, and a pronounced passion fruit odor was lacking. The unique role

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Table 6. GC/O-Odor Descriptions of the Stereoisomers of 2,6-Dimethyl-4-propyl-1,3-oxathiane 1 and 2,4-Dimethyl-6-propyl-1,3-oxathiane 2[a]

		odor description	
stereoisomer	panelist	oxathiane **1**	oxathiane **2**
(2*S*,4*R*,6*S*)		**1**-Ia	**2**-Ia
	1	**fresh**, **sweet**, green tea, cooked vegetable	**broth**, roasted meat, vegetable
	2	**fresh**, broth, fruity, herbs, eucalyptus	**broth**, **mushroom**, rancid fatty, cheesy
	3	**sweet**, lemon peel	**mushroom**, metallic
(2*R*,4*S*,6*R*)		**1**-Ib	**2**-Ib
	1	**flowery**, **sweet**, fresh, fruity, herbs	**sour**, **fruity**, herbs, citrus
	2	**flowery**, **sweet**, broth, citrus, lemon	pickled **mushroom**, bay leaf, vinegar, dried apple, broth
	3	**flowery**, **sweet**, moldy	**mushroom**, **sour**, **fruity**, metallic, moldy
(2*S*,4*S*,6*R*)		**1**-IIa	**2**-IVb
	1	sweet, herbs, earthy	**fruity**, **sweet**, soapy, fresh
	2	fatty, rancid, oily	**fruity**, **sweet**, **herbal**, **flowery**, earthy, potage
	3	-[b]	**fruity**, **sweet**, **herbal**, **flowery**, pine
(2*R*,4*R*,6*S*)		**1**-IIb	**2**-IVa
	1	**flowery**, sweet, fresh	**herbs**, moldy, musty, earthy, cooked vegetable
	2	**flowery**, fruity	**herbs**, flowery
	3	-[b]	sweet, pungent, gasoline, sweat
(2*S*,4*S*,6*S*)		**1**-IIIa	**2**-IIa
	1	**flowery**, **sweet**, **herbal**	**sweat**, pungent, cheesy, **sweet**, fruity
	2	**flowery**	**sweat**, pungent, meat broth, rancid
	3	**sweet**, **herbal**, lemon peel	**sweet**, pungent, gasoline, sour, burnt
(2*R*,4*R*,6*R*)		**1**-IIIb	**2**-IIb
	1	**citrus**, **fruity**, **sweet**, fresh, orange peel	**earthy**, **roasted**, **sour**, broth, fruity
	2	**citrus**, **fruity**, cooked apple	**earthy**, **roasted** meat, woody, mushroom
	3	**sweet**, **fruity**, lemon peel, herbal	**sour**, pungent, gasoline, bitter, burnt
(2*R*,4*S*,6*S*)		**1**-IVa	**2**-IIIa
	1	**moldy**, musty, vegetable, earthy, clay	**sour**, **sweet**, **vinegar**, fruity
	2	**moldy**, herbs, black tea, woody	**sour**, **sweet**, cooked **mushroom** in **vinegar**, herbs
	3	**moldy**, metallic, fruity	**mushroom**, **sweet**, moldy, pungent, gasoline, metallic
(2*S*,4*R*,6*R*)		**1**-IVb	**2**-IIIb
	1	**sweet**, fruity, flowery	**earthy**, **wood**, **sweet**, musty, wet, cheesy
	2	**sweet**, cooked vegetable	**earthy**, **wood**, **sweet**, sour, herbs, cooked
	3	**sweet**, sour bitter	**sweet**, mushroom, gasoline

[a]Descriptions given by at least two of the panelists are marked in bold [b]Not determined.

of *cis*-(2*S*,4*R*)-2-methyl-4-propyl-1,3-oxathiane as the only one of the four stereoisomers exhibiting tropical fruit notes[17] is lost in the series of homologous stereoisomers of **1** possessing an additional methyl substituent in position 6. The importance of the spatial orientation of this substituent is demonstrated by the two stereoisomers of **1** possessing *cis*-(2*R*,4*S*)-configurations: Stereoisomer **1**-Ib was consistently described by the panelists as flowery and sweet, whereas stereoisomer **1**-IVa was described as moldy, musty, and earthy.

In the stereoisomers of 2,4-dimethyl-6-propyl-1,3-oxathiane **2**, there is a reversal of the substituents in positions 4 and 6 compared to **1**, and the stereoisomers of **2** possess an additional propyl substituent in position 6 compared to 2-methyl-4-propyl-1,3-oxathiane. The observation that mushroom, earthy, and pungent rather than fruity and flowery nuances were predominating supports the hypothesis that, in analogy to β-mercaptoalkanones and β-mercaptoalkanols, for alkyl-substituted 1,3-oxathianes, the length of the substituent in position 6, corresponding to the substituent R4 in the model for a "tropical olfactophor",[5,6] plays an essential role for the sensory properties.

The odor properties determined for the stereoisomers of **1** and **2** are in line with the available data, indicating that modifications of the type and the position of substituents at the 1,3-oxathiane backbone result in molecules with interesting sensory characteristics.[17,41] So far, most of these structural modifications have focused on the substituents in positions 2 and 4; the present study indicates that variations of the substituents in position 6 (including variations of the stereochemical configurations) also offer the potential for "tuning" the sensory properties of 1,3-oxathianes in quite a broad way.

The recent publications on wine demonstrate that foods containing the appropriate precursor molecules, that is, β-mercaptoalkanols, are promising sources for also identifying the corresponding 1,3-oxathianes to be anticipated, for example, from the reaction with acetaldehyde. 2,6-Dimethyl-4-propyl-1,3-oxathiane **1** and 2,4-dimethyl-6-propyl-1,3-oxathiane **2** have not been identified in nature so far. The precursors 2-mercapto-4-heptanol **4** and 4-mercapto-2-heptanol **3** have been reported as volatile constituents of cooked bell pepper,[36] and their naturally occurring enantiomeric distributions have been investigated.[26] The analytical and sensory data provided in this study might assist in searching, for example, for the presence of stereoisomers of **1** and **2** in this natural source. Sensory assessments of the individual stereoisomers of **1** and **2** not only by GC/O but also in aqueous solutions or ideally in food matrices would be another valuable approach to extend the knowledge on the contributions of this class of sulfur-containing volatiles to odor and taste and on the impact of their configurations on sensory perceptions.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.2c00509.

NMR data of stereoisomers of **1** and **2** and of structurally related supporting substances and reaction schemes to obtain (4*S*)-enriched stereoisomers of **1** and **2** (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Karl-Heinz Engel − *Lehrstuhl für Allgemeine Lebensmitteltechnologie, Technische Universität München, Freising-Weihenstephan D-85354, Germany;* orcid.org/0000-0001-8932-5175; Email: k.h.engel@tum.de

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Authors

Anja Devenie Riegel − *Lehrstuhl für Allgemeine Lebensmitteltechnologie, Technische Universität München, Freising-Weihenstephan D-85354, Germany*

Hidehiko Wakabayashi − *Lehrstuhl für Allgemeine Lebensmitteltechnologie, Technische Universität München, Freising-Weihenstephan D-85354, Germany*

Motoko Wakabayashi − *College of Bioresource Sciences, Nihon University, Fujisawa 252-0880, Japan*

Markéta Rynešová − *Lehrstuhl für Allgemeine Lebensmitteltechnologie, Technische Universität München, Freising-Weihenstephan D-85354, Germany*

Viktoriia Dudko − *Lehrstuhl für Allgemeine Lebensmitteltechnologie, Technische Universität München, Freising-Weihenstephan D-85354, Germany*

Wolfgang Eisenreich − *Bayerisches NMR Zentrum, Strukturelle Membranbiochemie, Technische Universität München, Garching D-85747, Germany*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jafc.2c00509

Notes

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4. DISCUSSION

The numbering of the compounds in this chapter was adopted from Riegel *et al.*, **2020**; Publication I and Riegel *et al.*, **2022;** Publication II.

4.1. Separation of Stereoisomers via Capillary Gas Chromatography

As outlined in chapter 2.4, the development of derivatized cyclodextrins constituted a major breakthrough for the analysis of enantiomers by capillary gas chromatography. This type of chiral stationary phases had been successfully employed in previous studies related to the separation of enantiomers of β-mercaptoalkanones (Wakabayashi *et al.*, **2011**; Wakabayashi *et al.*, **2015**; Kiske *et al.*, **2019**) and β-mercaptoalkanols (Nörenberg *et al.*, **2017b**). Therefore, they were also tested in this thesis for the GC-separation of the stereoisomers of the homologous series of 2-mercapto-4-alkanols (C6-C10) and 2,4-dimethyl-6-propyl-1,3-oxathiane and 2,6-dimethyl-4-propyl-1,3-oxathiane.

To this day, no logical relationship could be found between molecular size, structure, or functional groups of chiral compounds and the recognition of their stereoisomers on cyclodextrin derivatives. It is assumed that the process involves a complexity of different interactions, such as hydrogen bonds, dipole-dipole interactions, and others (Volker, **2001**). Although many cyclodextrin stationary phases with different derivatization patterns are commercially available, it is still a challenge to find a suitable chiral cyclodextrin phase for the separation of stereoisomers. This is reflected in the fact that for the separation of the enantiomers of β-mercaptoalkanones, β-mercaptoalkanols, and β-mercaptocycloalkanols various cyclodextrin derivatives differing in ring size as well as in the type of derivatization have been applied (Table 10).

Table 10: Examples of cyclodextrin derivatives applied as stationary phases for the separation of the enantiomers of sulfur-containing polyfunctional flavor compounds

Compounds	**chiral stationary phase**	**literature**
2-acetylthio-4-alkanones (C6-C10) 2-mercapto-4-alkanones (C6-C10)	diethyl-*tert*-butylsilyl-*β*-cyclodextrin (MEGA-DEX DET-Beta; Mega s.n.c., Legnano, Italy)	(Kiske *et al.*, **2019**)
4-acetylthio-2-alkanones (C5-C10) 4-mercapto-2-alkanones (C5-C10)	30% heptakis (2,3-di-*O*-methyl-6-*O*-*tert*-butyl-dimethylsilyl)-*β*-cyclodextrin in OV1701 (CycloSil-B; Agilent technology INC, Santa Clara, California, USA)	(Wakabayashi *et al.*, **2011**; Wakabayashi *et al.*, **2015**)
3-mercaptocycloalkanols (C5, C6)	octakis(2,3-di-*O*-*n*-butyryl-6-*O*-*tert*-butyldimethylsilyl)-*γ*-cyclodextrin	(Wakabayashi *et al.*, **2020**)
4-mercapto-2-alkanols (C5, C7-C10)	heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-*β*-cyclodextrin	(Nörenberg *et al.*, **2017b**)
4-mercapto-2-hexanol	heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-*β*-cyclodextrin heptakis(2,3-di-*O*-methoxymethyl-6-*O*-*tert*-butyldimethylsilyl)-*β*-cyclodextrin	(Nörenberg *et al.*, **2017b**)
3-mercaptocycloheptanol	heptakis(2,3-di-*O*-ethyl-6-*tert*-butylsilyl)-*β*-cyclodextrin	(Wakabayashi *et al.*, **2020**)

4.1.1. GC-Separation of the Stereoisomers of 2-Mercapto-4-alkanols

For 2-mercapto-4-alkanols with chain lengths C7-C10 the four stereoisomers could be separated using a commercially available column coated with octakis(2,3-di-*O*-*n*-butyryl-6-*O*-*tert*-butyldimethylsilyl)-γ-cyclodextrin phase as chiral stationary phase (Figure 13). There was a consistent order of elution of the diastereoisomeric pairs, i.e. *anti* before *syn*, as well as of the individual enantiomers, i.e. (2*R*,4*R*) before (2*S*,4*S*) and (2*S*,4*R*) before (2*R*,4*S*), respectively. However, for 2-mercapto-4-hexanol there was a co-elution of the first eluted *anti*-configured enantiomers. Their separation could be achieved using octakis(2,3-di-*O*-[(*S*)-2-methylbutryl]-6-*O*-*tert*-butyldimethyl)-γ-cyclodextrin. This previously synthesized chiral stationary phase (Schmarr, **1992**; Takahisa & Engel, **2005**) is also an acylated γ-cyclodextrin derivative; however, the hydroxyl groups in positions 2 and 3 of the glucose units in the cyclodextrin backbone are esterified with (*S*)-2-methylbutyric acid rather than with butyric acid. It is noteworthy that the order of elution of the *anti*- and *syn*-configured stereoisomers of 2-mercapto-4-hexanol were the same as those of the 2-mercapto-4-alkanol homologues with chain lengths C7-C10; however, the order of elution of the *anti*-configured enantiomers was reversed. This confirms that for stereoisomers separated via GC using chiral stationary phases, individual assignments of the configurations are always required, and “read across” has to be performed with the greatest precaution.

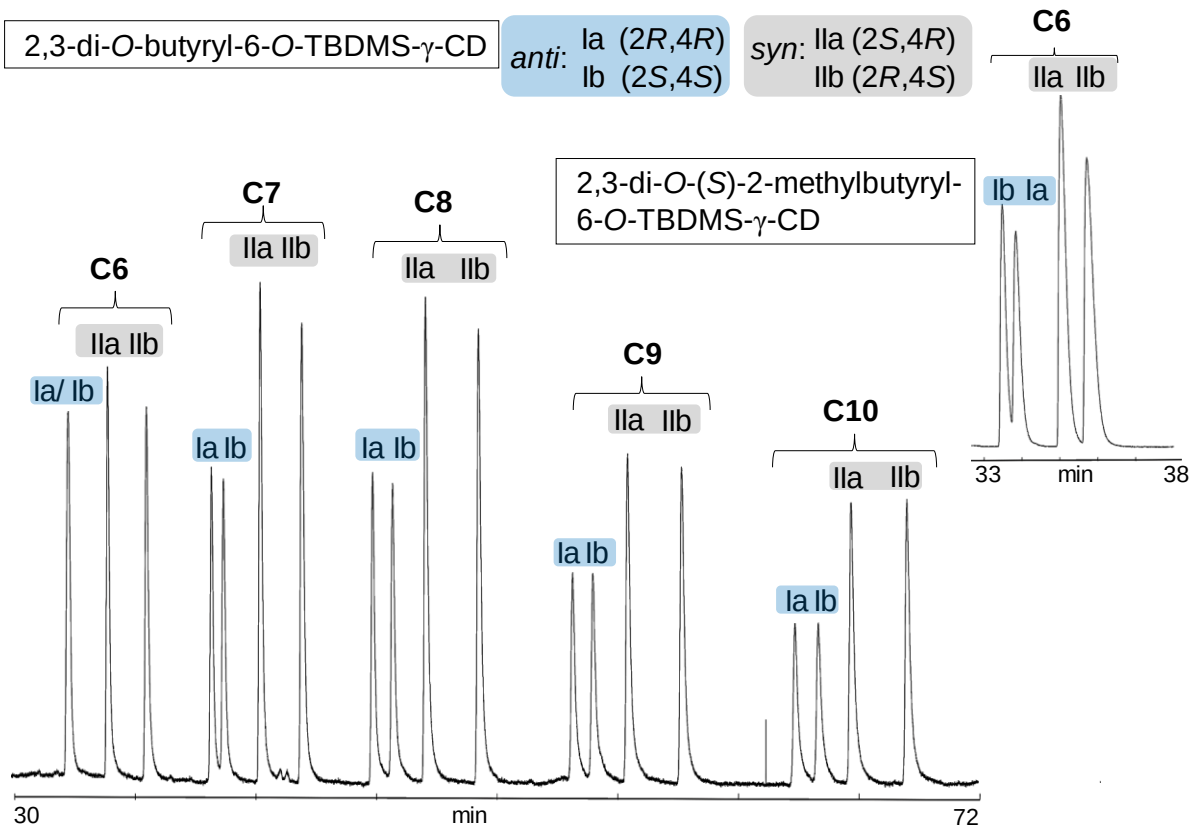


Figure 13: GC separation of the stereoisomers of the homologous series of 2-mercapto-4-alkanols using octakis(2,3-di-*O*-*n*-butyryl-6-*O*-*tert*-butyldimethylsilyl)-γ-cyclodextrin (C7-C10) and octakis(2,3-di-*O*-[(*S*)-2-methylbutyryl]-6-*O*-*tert*-butyldimethyl)-γ-cyclodextrin (C6) as chiral stationary phases (Riegel *et al.*, **2020;** Publication I).

If one compares the separation of the stereoisomers achieved for 2-mercapto-4-alkanols with those for a homologous series (C5-C10) of the isomeric 4-mercapto-2-alkanols (Nörenberg *et al.*, **2017b**), similar phenomena were observed. Capillary gas chromatographic separations of the stereoisomers of 4-mercapto-2-alkanols with chain lengths C5 and C7-C10 were achieved on heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-*β*-cyclodextrin. That means, an acylated (in this case acetylated) cyclodextrin was used similar to the separation of the 2-mercapto-4-alkanols; however, the cyclodextrin backbone was *β*-cyclodextrin (7 glucose units) rather than γ-cyclodextrin (8 glucose units). Interestingly, again the homologue with chain length C6 could not be separated on this chiral stationary phase. For the separation of stereoisomers of this homologue, heptakis(2,3-di-*O*-methoxymethyl-6-*O*-*tert*-butyldimethylsilyl)-*β*-cyclodextrin was used as a stationary phase. This is one of the examples of a cyclodextrin derivative in which the hydroxyl

groups in positions 2 and 3 of the glucose units were not converted into ethers or esters, respectively, but into acetals (Takahisa & Engel, **2005**; Engel, **2020**).

These results obtained for the homologous series of the positional isomers 2-mercapto-4-alkanols and 4-mercapto-2-alkanols demonstrate that „trial-and-error“ is still a basic principle when searching for chiral stationary phases enabling the separation of stereoisomers. In the meantime, a broad spectrum of cyclodextrin derivatives is commercially available; fortunately, for the studies underlying this thesis, also various in-house synthesized stationary phases were available for screening.

4.1.2. GC-Separation of the Stereoisomers of 1,3-Oxathianes

The GC-analysis of 2,4-dimethyl-6-propyl-1,3-oxathiane and 2,6-dimethyl-4-propyl-1,3-oxathiane presented a challenge because they possess three chiral centers at positions 2, 4, and 6, and for both eight stereoisomers are possible.

For both alkyl-substituted 1,3-oxathianes, the four diastereoisomers could be well separated using a non-chiral DB-WAX column. Figure 14 shows the chromatograms obtained for the mixtures of stereoisomers obtained by the reaction of acetaldehyde with 4-mercapto-2-heptanol and 2-mercapto-4-heptanol, respectively.

It is noteworthy that the order of elution of the four diastereoisomers differed for the two 1,3-oxathianes. For 2,6-dimethyl-4-propyl-1,3-oxathiane, the diastereoisomer **2**-I, in which all alkyl substituents in positions 2, 4, and 6 are equatorial, exhibited the shortest retention time, whereas diastereoisomer **2**-IV, in which the methyl and propyl substituents in positions 4 and 6, respectively, are axial, showed the longest retention time (Figure 14B).

For 2,4-dimethyl-6-propyl-1,3-oxathiane, the diastereoisomer **1**-I, in which all alkyl substituents are equatorial, also showed the shortest retention time. However, the axial orientation of the propyl group in position 6 resulted in a significant shift in the retention time and a much earlier elution of this diastereoisomer **1**-II on the DB-WAX column (Figure 15A).

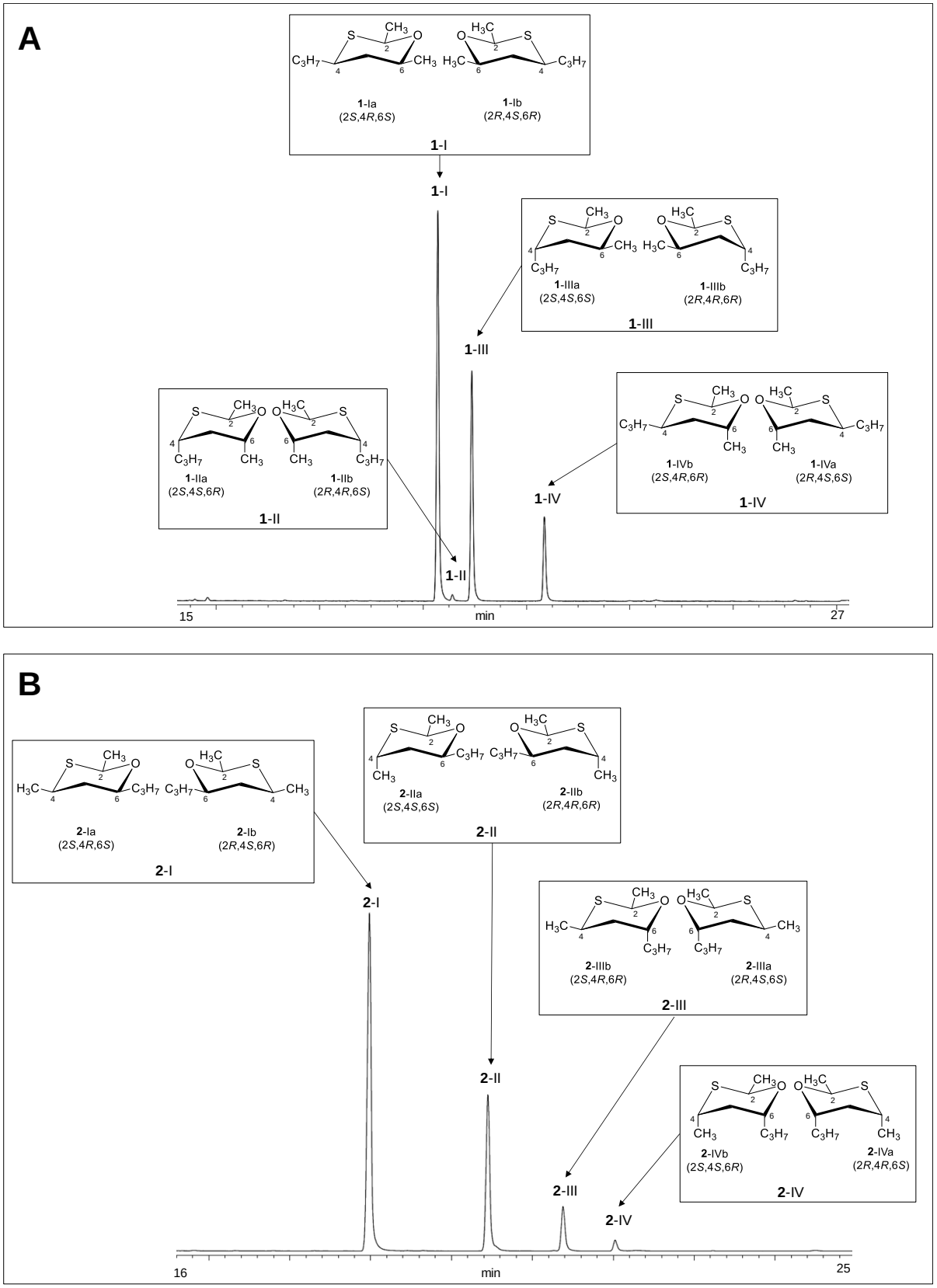


Figure 14: GC separation of the diastereoisomers of (A) 2,6-dimethyl-4-propyl-1,3-oxathiane and (B) 2,4-dimethyl-6-propyl-1,3-oxathiane on DB-WAX (Riegel *et al.*, **2022;** Publication II).

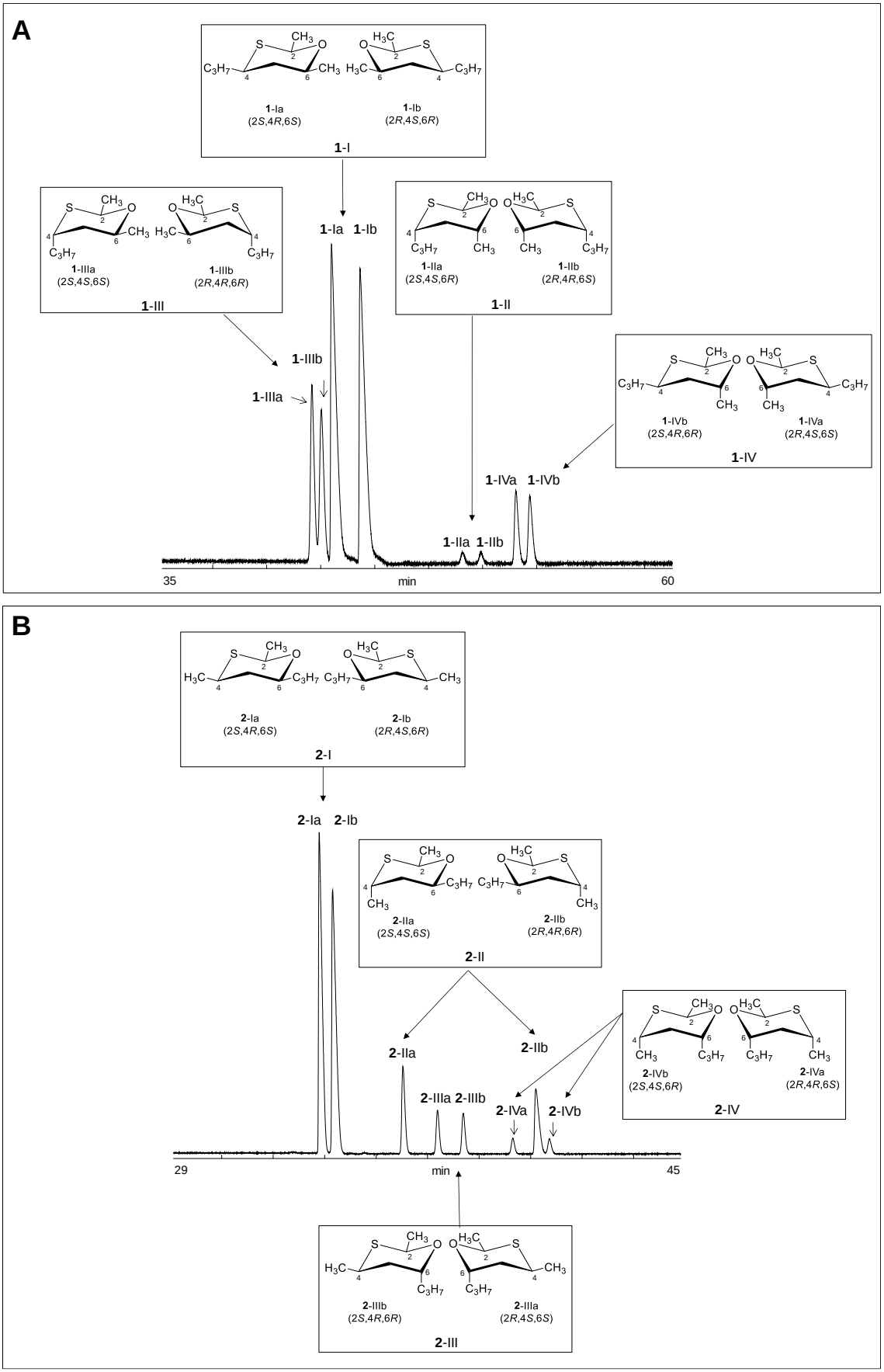


Figure 15: GC separation of the stereoisomers of (A) 2,6-dimethyl-6-propyl-1,3-oxathiane and (B) 2,4-dimethyl-4-propyl-1,3-oxathiane on heptakis(diethyl-*tert*-butyldimethylsilyl)-β-cyclodextrin (Riegel *et al.*, **2022;** Publication II) .

For each 1,3-oxathiane, baseline separations of the eight stereoisomers were achieved on heptakis(diethyl-*tert*-butyldimethylsilyl)-β-cyclodextrin phase as chiral stationary phase (Figure 15). There was no obvious correlation between the order of elutions of the diastereoisomers on the DB-WAX column and the order of elution of the enantiomeric pairs on the chiral stationary phase. The separation of the enantiomers of **2**-II on heptakis(diethyl-*tert*-butyldimethylsilyl)-β-cyclodextrin was outstandingly high compared to those observed for the other stereoisomers (Figure 15B). The present knowledge of mechanisms underlying the separation of enantiomers on cyclodextrin derivatives does not allow to explain this result.

The separation of the four stereoisomers of 2-methyl-4-propyl-1,3-oxathiane on Ni(II)-[bis(3-heptafluorobutyryl-(1*R*)-camphorate] as chiral stationary phase has been an impressive example for the application of complexation gas chromatography (Mosandl *et al.*, **1984**; Singer *et al.*, **1988**). Recently, the separation of the enantiomers of *cis*-2-methyl-4-propyl-1,3-oxathiane and *cis*-2,4,4,6-tetramethyl-1,3-oxathiane and their determination in wine has been reported (Wang *et al.*, **2021**).

Another example of the separation of all 8 stereoisomers of a chiral compound on a cyclodextrin derivative as a chiral stationary phase is the GC-analysis of 3-butyl(hexahydro)phthalide (Bartschat *et al.*, **1997**).

4.2. Assignment of the Absolute Configurations

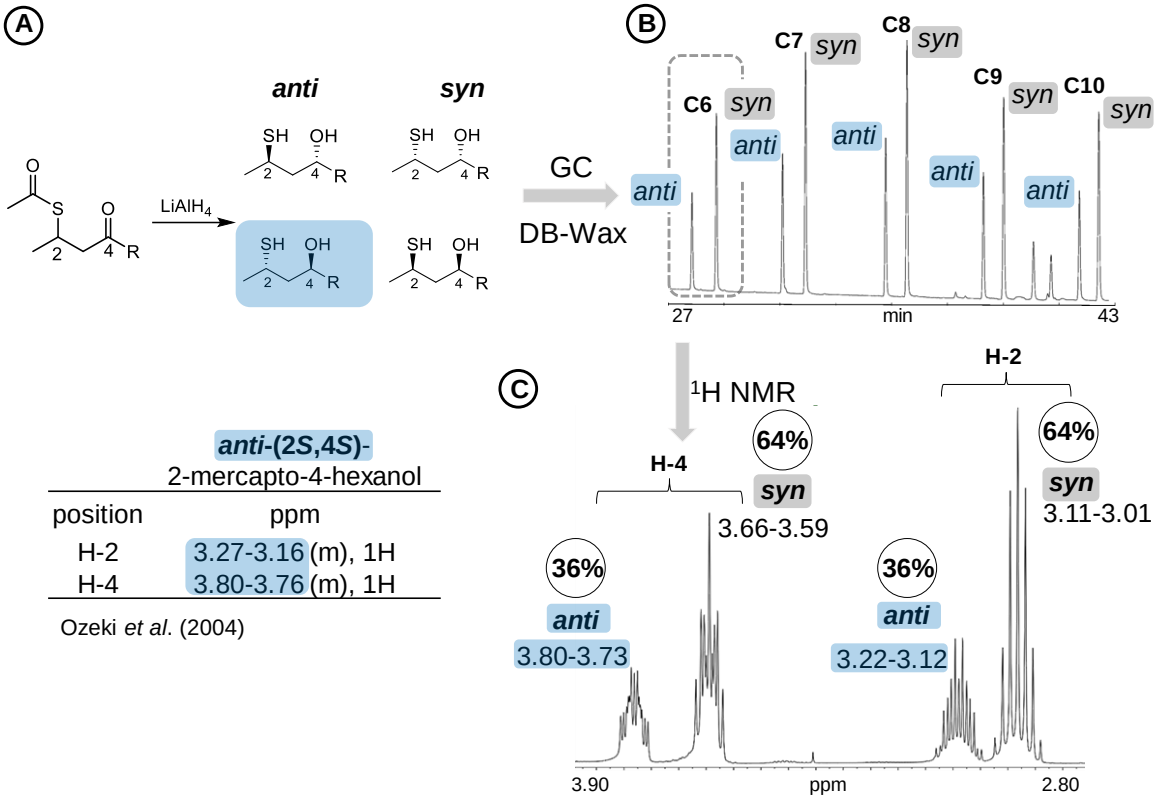
4.2.1. Configurations of the Stereoisomers of 2-Mercapto-4-alkanols (C6-C10)

The assignment of the absolute configurations and the GC-order of elution of the stereoisomers of the homologous series (C6-C10) of 2-mercapto-4-alkanols was based on a two-step procedure:

(i) In the first step, the order of elution of the *cis*- and *trans*-configured diastereoisomers separated via GC on a DB-WAX column was determined.

- 2-Acetylthio-4-hexanone, obtained by addition of thioacetic acid to 4-hexene-3-one, was reduced with $NaBH_4$ to 2-acetylthio-4-hexanol. The resulting *syn*- and *anti*-configured diastereoisomers were separated by column chromatography and subsequently reduced with $LiAlH_4$ to yield *syn*- and *anti*-2-mercapto-4-hexanol, respectively.
- The obtained diastereoisomers were subjected to NMR analysis, and the chemical shifts observed for the protons in positions 2 and 4 were compared to those available for *anti*-(2*S*,4*S*)-2-mercapto-4-hexanol (Ozeki *et al.*, **2004**). *Anti*-(2*S*,4*S*)-2-mercapto-4-hexanol has been synthesized via stereoselective synthesis and after derivatization, the structure was confirmed by X-ray crystal structure analysis (Ozeki *et al.*, **2004**).
- The ratios between the pairs of NMR signals for H-2 and H-4 calculated for the synthesized mixture of 2-mercapto-4-hexanol (36:64) were nearly the same as the ratio determined for the peak areas in the GC-chromatogram on DB-WAX (35:65), thus allowing to determine the GC order of elution for 2-mercapto-4-hexanol as *anti* before *syn*.
- This comparison of ratios of the NMR signals and the peak areas in the GC chromatogram was also performed for the homologous 2-mercapto-4-alkanols with chain lengths C6 and C8-C10.

The employed procedure is depicted in Figure 16.



***anti*-(2*S*,4*S*)-2-mercapto-4-hexanol**

position	ppm
H-2	3.27-3.16 (m), 1H
H-4	3.80-3.76 (m), 1H

Ozeki *et al*. (2004)

Figure 16: (A) Syntheses of 2-mercapto-4-alkanols via reduction with $LiAlH_4$ of the respective *β*-acetylthioalkanones; (B) separation of the *syn*- and *anti*-pairs of 2-mercapto-4-alkanols (C6-C10) via GC on a DB-WAX column; (C) ^{1}H-NMR spectrum (2.90 – 2.80 ppm) showing the signals corresponding to H-4 and H-2 at the chiral centers (Riegel *et al.*, **2020;** Publication I).

(ii) In the second step, the order of elution of the stereoisomers separated via GC on the employed chiral stationary phases was determined.

- Enantiomerically enriched 2-mercapto-4-alkanols were obtained by enzyme-catalyzed kinetic resolutions of racemic 2-acetylthio-4-alkanones and subsequent reduction of the remaining substrates with $LiAlH_4$. The procedure is outlined exemplarily for 2-mercapto-4-heptanol in Figure 17. The lipase CAL-B from *Candida antarctica* preferentially hydrolyzes (*R*)-configured 2-acetylthio-4-heptanone. The remaining non-hydrolyzed (2S)-configured substrate is reduced with LiAlH4, and the enantiomerically enriched mixture of stereoisomers is used to assign the GC order of elution.

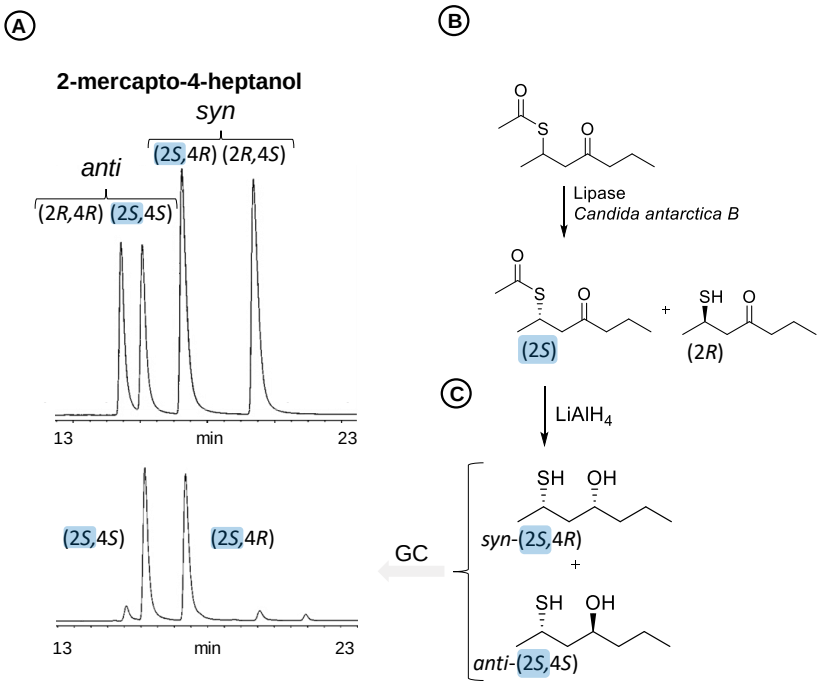


Figure 17: (A) GC separation of the stereoisomers of 2-mercapto-4-heptanol; (B) enzyme-catalyzed kinetic resolution of 2-acetylthio-4-heptanone with CAL-B and (C) reduction of 2-(*S*)-mercapto-4-heptanone to the corresponding 2-(*S*)-mercapto-4-heptanol diastereoisomers and comparison of the GC-chromatograms (Riegel *et al.*, **2020;** Publication I).

A similar approach has been applied to the assignment of the absolute configurations of the stereoisomers of a homologous series of 4-mercapto-2-alkanols (Nörenberg *et al.*, **2017b**). In this case, the NMR data of the separated diastereoisomers were compared with the NMR data of *anti*-(2*S*,4*S*)-4-mercapto-2-heptanol synthesized by Ozeki *et al.*, **2004**. Another difference is that the diastereoisomers of 4-mercapto-2-alkanols could be directly separated via semi-preparative HPLC. For 2-mercapto-4-alkanols such a semipreparative HPLC was not possible, because they are more susceptible to a formation of disulfides during the HPLC separation step.

4.2.2. Configurations of the Stereoisomers of 1,3-Oxathianes

The determination of the absolute configurations of the stereoisomers of 2,4-dimethyl-6-propyl-1,3-oxathiane and 2,6-dimethyl-4-propyl-1,3-oxathiane was based on a three-steps procedure:

(i) Assignment of the stereoisomers possessing (4*S*)-configurations:

- Enzyme-catalyzed kinetic resolution of 4-acetylthio-2-heptanone with porcine pancreas lipase (PPL) was used to obtain enantiomerically enriched 4-(*S*)-mercapto-2-heptanone (Figure 18A). This hydrolysis product was separated from the non-hydrolyzed remaining substrate 4-(*R*)-acetylthio-2-heptanone by column chromatography (Figure 18B).
- 4-(*S*)-Mercapto-2-heptanone was reduced via $LiAlH_4$ to the corresponding 4-(*S*)-mercapto-2-heptanol diastereoisomers (Figure 18C) which were subsequently treated with acetaldehyde to form the corresponding (4*S*)-2,4-dimethyl-6-propyl-1,3-oxathianes (Figure 18D).
- The comparison with the GC chromatogram of the racemic mixture allowed the assignment of those stereoisomers possessing an (*S*)-configuration in position 4. Consequently, the remaining stereoisomers had a (4*R*)-configuration (Figure 18D).
- A similar approach was used to obtain (4*S*)-2,6-dimethyl-4-propyl-1,3-oxathianes; in this case, lipase B from *Candida antarctica* (CAL-B) was employed to obtain 2-(*S*)-mercapto-4-heptanol as a precursor.

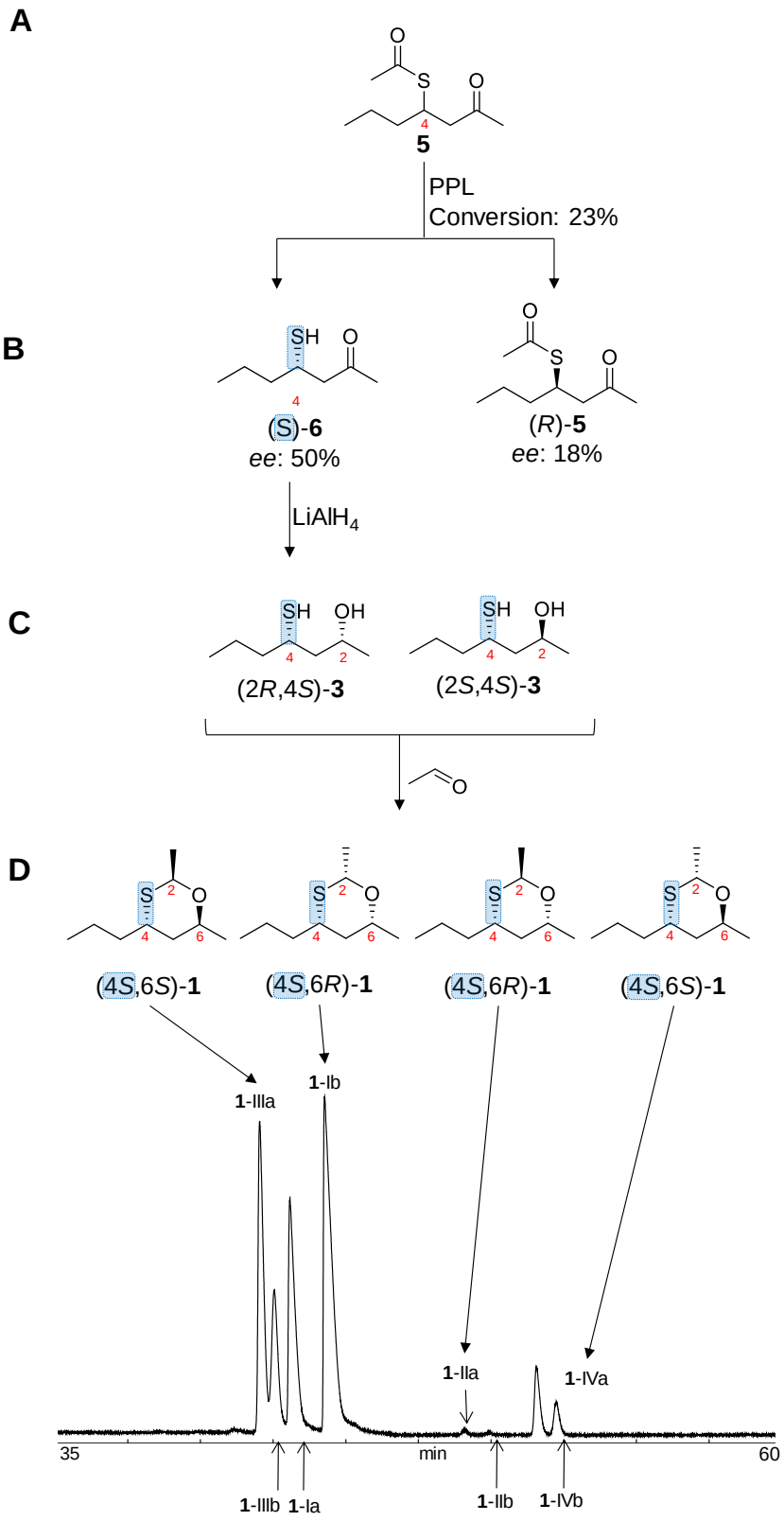


Figure 18: Assignment of the absolute configuration in position 4 of 2,6-dimethyl-4-propyl-1,3-oxathiane: (A) enzyme-catalyzed kinetic resolution of 4-acetylthio-2-heptanone with PPL, (B) separation of 4-(*S*)-mercapto-2-heptanone and 4-(*R*)-acetylthio-4-heptanone via column chromatography, (C) reduction of 4-(*S*)-mercapto-2-heptanone with LiAlH4 to the corresponding 4-(*S*)-mercapto-2-heptanol and (D) synthesis of (4*S*)-2,6-dimethyl-4-propyl-1,3-oxathiane and the separation of the stereoisomers via GC on a chiral stationary phase.

(ii) Assignment of the relative configurations in positions 4 and 6:

- The *anti*- and *syn*-diastereoisomers of 4-mercapto-2-heptanol were separated via semi-preparative HPLC (Figure 19A). Their assignment was based on the previously reported assignments of the *syn*- and *anti*-diastereoisomers of β-mercaptoalkanols (Nörenberg *et al.*, **2017b**).
- The separated pair of *anti*-diastereoisomers of 4-mercapto-2-heptanol was subsequently condensed with acetaldehyde to form the corresponding 2,6-dimethyl-4-propyl-1,3-oxathianes with *trans*-configuration of the substituents in positions 4 and 6 (Figure 19B).
- The comparison of the GC chromatograms allowed the assignment of the stereoisomers with a relative 4,6-*trans*-configuration. The remaining pairs of diastereoisomers consequently had a relative 4,6-*cis*-configuration (Figure 19C). By combining this information with that gained in step (i), the absolute configurations for position 6 could be assigned (Table 11).

The assignments of the relative configuration of positions 4 and 6 for 2,4-dimethyl-6-propyl-1,3-oxathiane followed the same steps, starting from 2-mercapto-4-heptanol.

Table 11: Assignments of the absolute and relative configurations of 2,6-dimethyl-4-propyl-1,3-oxathiane based on steps (i) and (ii)

stereoisomer	relative configuration	absolute configuration	
	4,6	4	6
1-Ia	*cis*	*R*	*S*
1-Ia'	*cis*	*S*	*R*
1-IIb	*cis*	*S*	*R*
1-IIb'	*cis*	*R*	*S*
1-IIIc	*trans*	*S*	*S*
1-IIIc'	*trans*	*R*	*R*
1-IVd	*trans*	*S*	*S*
1-IVd'	*trans*	*R*	*R*

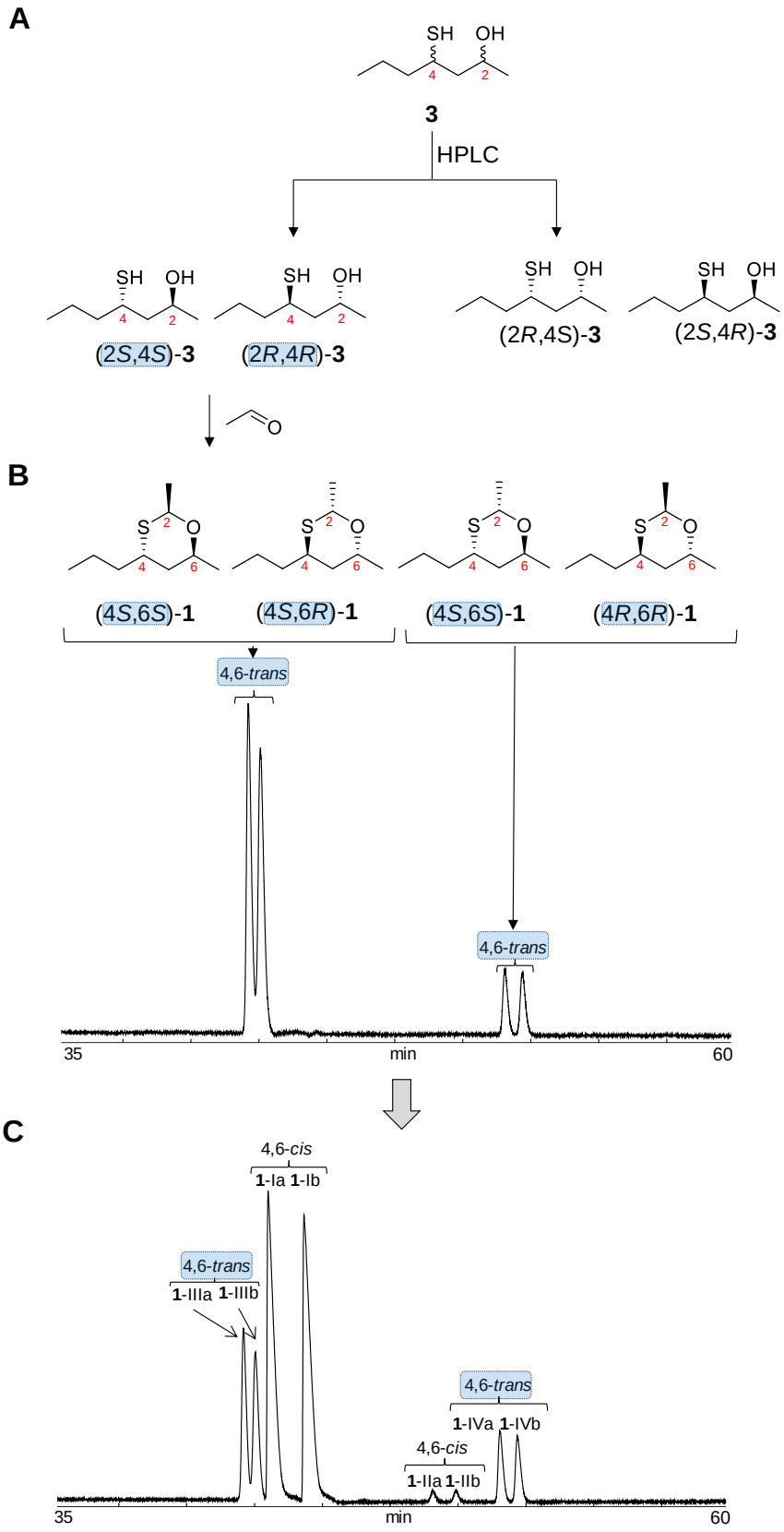


Figure 19: Assignment of the 4,6-*cis*- and 4,6-*trans*-stereoisomers of 2,6-dimethyl-4-propyl-1,3-oxathiane: (A) HPLC separation of *anti*- and *syn*-4-mercapto-2-heptanol, (B) condensation of *anti*-4-mercapto-2-heptanol with acetaldehyde to the corresponding 4,6-*trans*-2,6-dimethyl-4-propyl-1,3-oxathiane stereoisomers and (C) GC separation of the formed 2,6-dimethyl-4-propyl-1,3-oxathiane stereoisomers on a chiral stationary phase and comparison to the racemic mixture.

(iii) NMR Analysis:

- The third stereogenic center in position 2, introduced by the reaction of 4-mercapto-2-heptanol and 2-mercapto-4-heptanol, respectively, with acetaldehyde, was assigned by NMR analysis. The chemical shifts and the coupling constants observed for the diastereoisomers were compared with those of structurally related 1,3-oxathianes such as 2-methyl-4-propyl-1,3-oxathiane and di- and trimethylated 1,3-oxathianes (Pasanen, **1972**; Mosandl & Heusinger, **1985**).
- Configurations at position 2 were assigned based on NOE correlations. Figure 20A shows as an example the relevant part of the NOESY spectrum of 2,6-dimethyl-4-propyl-1,3-oxathiane. The NOE signals observed for diastereoisomers **1**-I indicate that all protons in positions 2, 4, and 6 are in an axial conformation (Figure 20B).
- Due to the low percentage in the synthesized mixture of oxathianes (1%), no NOE signals could be assigned for the pair of diastereoisomers **1**-IIa. To determine the absolute configurations for the stereoisomers **1**-IIb and **1**-IIb', the spatial orientations of the substituents in positions 4 and 6 were deduced, taking into account the results from steps (i) and (ii). For the methyl substituent in position 2, the stable equatorial orientation was postulated.
- The diastereomeric pairs **2**-III and **2**-IV were also present in the synthesized mixture at very low concentrations, hence NMR data were not available. In analogy to the determination of the absolute configuration for **1**-II, the spatial orientations of **2**-III and **2**-IV were assigned based (i) on the configurations determined in position 4, (ii) the relative configurations assigned for positions 4 and 6, and the assumption that the methyl substituent in position 2 is preferentially in an equatorial position.

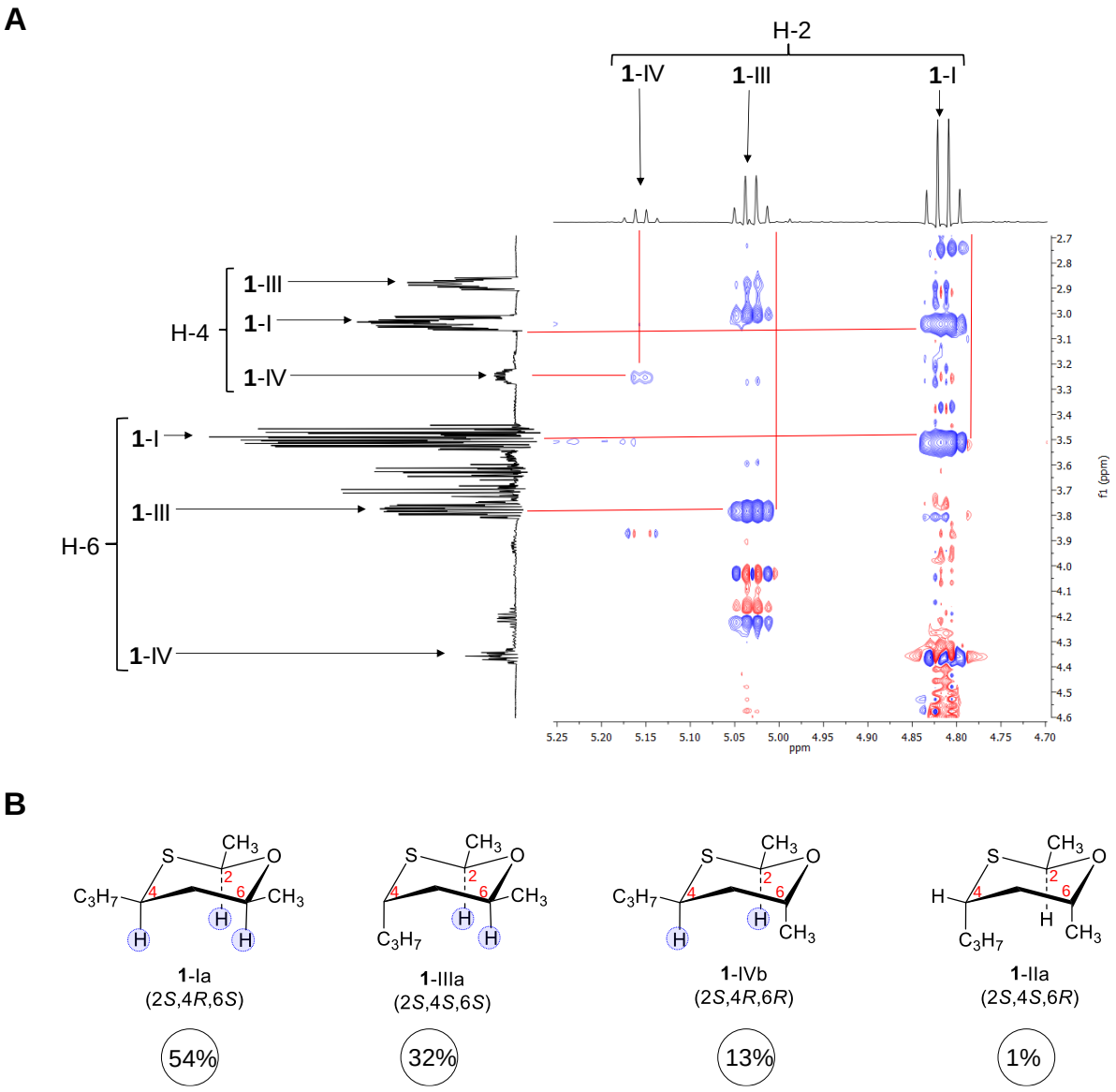


Figure 20: (A) NOESY spectrum of 2,6-dimethyl-4-propyl-1,3-oxathiane and (B) the structures of one of the pairs of diastereoisomers.

The NOE method has also been shown to be suitable to determine the absolute configurations of β-mercaptocycloalkanones and β-mercaptocycloalkanols and their respective *S*-acetyl-derivatives, other cyclic sulfur-containing compound classes with relatively rigid spatial conformations (Wakabayashi *et al.*, **2020**).

4.3. Determination of Odor Thresholds and Odor Qualities via Gas Chromatography/ Olfactometry (GC/O)

The odor thresholds of the stereoisomers of the 2-mercapto-4-alkanol homologues (C6-C10) and of 2,4-dimethyl-6-propyl-1,3-oxathiane and 2,6-dimethyl-4-propyl-1,3-oxathiane were determined via GC/O according to the procedure developed by (Ullrich & Grosch, **1987**). The method is based on the use of (*E*)-2-decenal as an internal standard, for which an odor threshold of 2.7 ng/L in air has been reported (Boelens & van Gemert, **1986**). The coupling of the GC-sniffing port with capillary columns coated with cyclodextrin derivatives-based chiral stationary phases enabled to assess the sensory properties of the individual stereoisomers.

The assessments were performed by three panelists. To counteract the subjectivity inherent to evaluations by human assessors and to reduce the potential uncertainties, each dilution was evaluated three times. A concentration level was considered as an odor threshold if it was the lowest dilution step at which the odor was consistently perceived in three consecutive GC runs.

Apart from a few outliers, for both the 2-mercapto-4-alkanol homologues and the two 1,3-oxathianes the odor thresholds determined by the individual panelists were either the same or differed only by factors 2-6. This corresponds to 1-3 dilution steps as performed in the course of the employed procedure; it is in line with the variance expected for this type of approach and confirms the reproducibility of the sensory assessments by the panelists.

Regarding odor qualities, the panelists were free to use any type of descriptor and to report as many odor impressions as needed. In the course of the interpretation of the data, attention was particularly paid to descriptions given by at least two panelists. In agreement with the procedure applied in previous studies on the sensory evaluations of the stereoisomers of 4-mercapto-2-alkanones and 4-acetylthio-2-alkanones (Wakabayashi *et al.*, **2015**), of 4-mercapto-2-heptanol and its acetyl-derivatives (Nörenberg *et al.*, **2013**) and of 2-mercapto-4-alkanones (Kiske *et al.*, **2019**), the odor qualities were determined at one dilution level above the odor threshold. For the stereoisomers of the homologous series of 4-mercapto-2-alkanols, for each stereoisomer, the odor impressions recorded for the injection volume corresponding to approximately 1.5 ng at the sniffing port have been used as descriptors (Nörenberg *et al.*, **2017b**).

4.3.1. Sensory Properties of the Stereoisomers of 2-Mercapto-4-Alkanols

Odor Thresholds.

The geometric means of the odor thresholds determined by the panelists for the stereoisomers of the 2-mercapto-4-alkanol homologues are depicted in Figure 21. To facilitate a direct comparison, the geometric means of the odor thresholds previously reported for the respective 4-mercapto-2-alkanol homologues (Nörenberg *et al.*, **2017b**) are also shown in Figure 21. The following phenomena were observed:

- Except for the C7 (2*S*,4*S*)-stereoisomer, the odor thresholds determined for the stereoisomers of the 2-mercapto-4-alkanols were consistently higher than those of the homologous series of 4-mercapto-2-alkanols.
- For the 4-mercapto-2-alkanol homologues (C6-C10), the odor thresholds of the (2*R*,4*R*)-stereoisomers were significantly lower than those of the other stereoisomers.
- The consistent triggering of lower odor thresholds by this configuration was not observed for the 2-mercapto-4-alkanols. Only for the C7 and C8 homologues, the configuration of the asymmetric center bearing the hydroxyl group (position 4) was clearly impacted the odor thresholds. The geometric mean of the odor threshold of (2*S*,4*R*)-2-mercapto-4-heptanol (51 ng/L in air) was 255 times higher than that of (2*S*,4*S*)-2-mercapto-4-heptanol (0.2 ng/L in air), and the mean odor threshold of (2*S*,4*R*)-2-mercapto-4-octanol (56 ng/L in air) was 15 times higher than that of the (2*S*,4*S*)-stereoisomer (3.8 ng/L in air). For the other chain lengths, the odor thresholds of the stereoisomers were in the same order of magnitude.

The outstanding role of the (2*R*,4*R*)-configuration for the stereoisomers of 4-mercapto-2-alkanols has been also been observed for acetyl-derivatives of 4-mercapto-2-heptanol. For 4-mercapto-2-heptyl acetate, 4-acetylthio-2-heptyl acetate, and 4-acetylthio-2-heptanol possessing this configuration, the odor thresholds were consistently lower than those of the three other stereoisomers (Nörenberg *et al.*, **2013**).

The odor thresholds of the stereoisomers of 4-mercapto-2-alkanols were in the same range as the odor thresholds of the homologous series of 3-mercaptoalkanals (C6-C9), 3-mercaptoalkanols (C4-C10), 3-mercaptoalkyl acetates (C4-C10) and

1-mercapto-3-alkanols (C3-C10) (Vermeulen & Collin, **2002**; Polster & Schieberle, **2017**). No differences between the odor thresholds of the enantiomers of 3-mercaptoalkanols were found. In contrast, for the homologous series of 3-acethylthioalkanols, the odor thresholds of the stereoisomers differed; this was particularly pronounced for the chain length C6 (Schoenauer *et al.*, **2015**)

The investigation of the stereoisomers of 3-mercaptocycloalkanols (C5-C7) revealed that their odor thresholds were in the same range as those determined for the stereoisomers of 2-mercapto-4-alkanols. Comparable results were determined for the stereoisomers of 3-acetylthiocycloalkanols (C5-C7), 3-mercaptocycloalkanones (C5-C7), and 3-acetylthiocycloalkanones (C5-C7). For all four homologous series, the configuration did not play a decisive role for the odor thresholds (Wakabayashi *et al.*, **2020**).

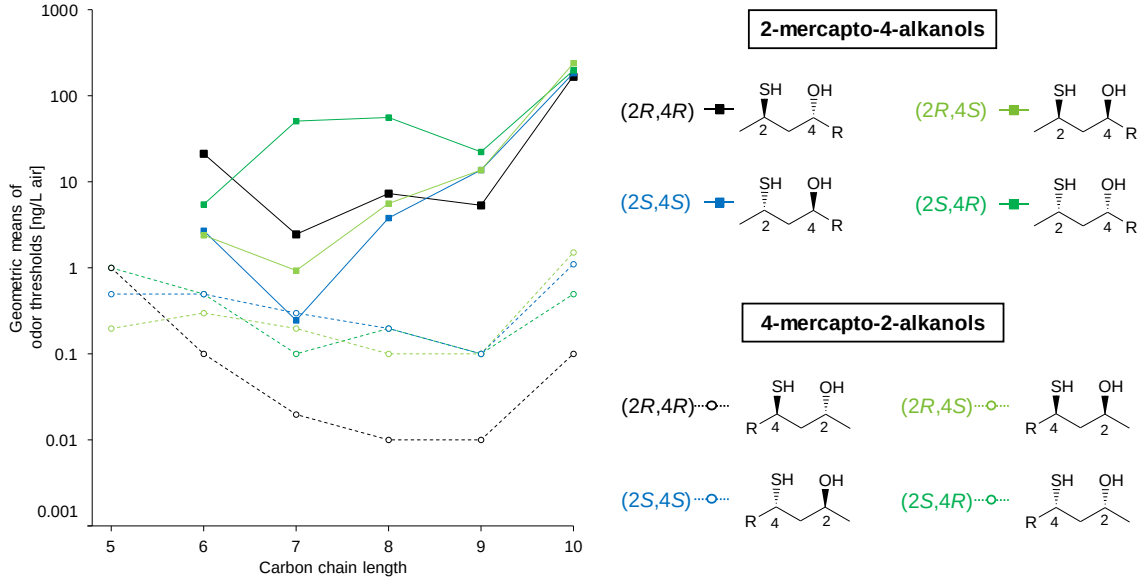


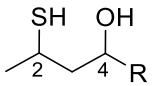
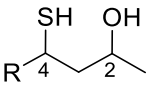
Figure 21: Odor thresholds determined for the stereoisomers of 2-mercapto-4-alkanols (C6-C10) (Riegel *et al.*, **2020;** Publication I) and 4-mercapto-2-alkanols (C5-C10) (Nörenberg *et al.*, **2017b**).

Odor Qualities.

The odor qualities determined for the stereoisomers of the homologous series (C6-C10) of 2-mercapto-4-alkanols are summarized in Table 12. For comparison, the odor qualities previously reported for 4-mercaptoalkanols (Nörenberg *et al.*, **2017b**) are also listed.

For the chain lengths C6-C8, the stereoisomers of the 2-mercapto-4-alkanols were predominantly described as vegetable-like and pungent; for the chain lengths C9 and C10, mushroom-like descriptors prevailed (Table 12). In contrast, 4-mercapto-2-alkanols exhibited a sulfury and fruity smell for the chain lengths C6 and C7 and pungent, plastic-like notes for the chain lengths C8-C10. For 4-mercapto-2-alkanols, the odor qualities revealed a dependence on the configuration for chain lengths C6 and C7. (2*S*,4*S*)-4-mercapto-2-heptanol and (2*S*,4*S*)-4-mercapto-2-hexanol exhibited fruity notes, while the three remaining stereoisomers were predominantly described as sulfury (Nörenberg *et al*., **2017b**). For both homologous series of 2-mercapto-4-alkanols and 4-mercapto-2-alkanols, the chain length was the main driver regarding the odor qualities.

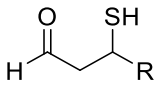
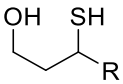
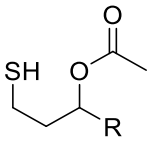
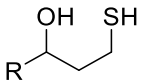
Table 12: GC/O-odor descriptions for the stereoisomers of homologous series (C6-C10) of 4-mercapto-2-alkanols (Riegel *et al.*, **2020;** Publication I**)** and 2-mercapto-4-alkanols (Nörenberg *et al.*, **2017b**).

	2-mercapto-4-alkanols 				**4-mercapto-2-alkanols** 			
	(2*S*,4*S*)	**(2*R*,4*R*)**	**(2R,4S)**	**(2S,4R)**	**(2*S*,4*S*)**	**(2*R*,4*R*)**	**(2*R*,4*S*)**	**(2*S*,4*R*)**
C6	green cucumber	vegetable sour	sour meaty	pungent onion	fruity sour	onion pungent	savory onion	green herbs
C7	pungent onion	vegetable pungent	sweat vegetable	sweet rotten	fruity sulfury	onion sulfury	onion sulfury	savory meaty
C8	vegetable moldy	vegetable earthy	vegetable fatty	green oily	plastic pungent	plastic pungent	burnt pungent	burnt onion
C9	earthy mushroom	metallic mushroom	moldy mushroom	vegetable mushroom	rubber burnt	plastic pungent	pungent onion	solvent onion
C10	metallic mushroom	vegetable mushroom	vegetable woody	spicy woody	burnt plastic	plastic fruity	plastic pungent	plastic solvent

These results are in line with the odor qualities determined for the corresponding β-mercaptoalkanones. The stereoisomers of 4-mercapto-2-alkanones revealed a dependence on the configuration: the (4*S*)-enantiomers exhibited mainly sulfury notes, while the (4*R*)-enantiomers smelled predominantly fruity (Wakabayashi *et al.*, **2015**). In contrast, the enantiomers of the positional isomers 2-mercapto-4-alkanones showed no dependence on the configuration (chapter 1.1.1). The chain length was the main driver of the odor qualities. 2-Mercapto-4-alkanones with chain lengths C6-C8 smelled vegetable-like, those with chain lengths C9 and C10 were reminiscent of mushrooms (Kiske *et al.*, **2019**), similar to 2-mercapto-4-alkanols. The respective acetylthio-alkanones exhibited comparable odor profiles.

These results are also in agreement with sensory data reported for other structurally related compounds like 3-mercaptoalkanals (C6-C9), 3-mercaptoalkanols (C4-C10), 3-mercaptoalkyl acetates (C4-C10) and 1-mercapto-3-alkanols (C3-C10) (Vermeulen & Collin, **2002**; Polster & Schieberle, **2017**). For 3-mercaptoalkanals, 3-mercaptoalkanols, and 3-mercaptoalkyl acetates, especially the mid-range chain lengths C5-C8 exhibited fruity notes (Table 13). In contrast, 1-mercapto-3-alkanols (C3-C10) did not exhibit fruity notes, similar to 2-mercapto-4-alkanols The investigation of the enantiomers of 3-mercaptoalkanols revealed that the odor qualities were the same, similar to those determined for the racemic mixtures (Schoenauer *et al.*, **2015**).

Table 13: GC/O-odor descriptions for 3-mercaptoalkanals (C5-C8), 3-mercaptoalkanols (C5-C8), and 3-mercaptoalkyl acetates (C5-C8) and 1-mercapto-3-alkanols (C5-C8) (Vermeulen & Collin, **2002**; Polster & Schieberle, **2017**).

	3-mercaptoalkanals	**3-mercaptoalkanols**	**3-mercaptoalkyl acetates**	**1-mercapto-3-alkanols**
				
C5	broth, onion flowery	grapefruit	black currant	burnt
C6	citrus, fruit peel fresh	grapefruit	black currant	burnt
C7	flowery, citrus fruit peel	grapefruit	black currant	burnt
C8	citrus grapefruit	grapefruit burnt	black currant	burnt mushroom

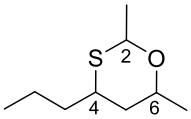
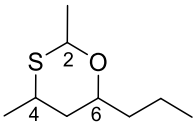
The odor qualities of the stereoisomers of 3-acetylthiocycloalkanols (C5-C7), 3-mercaptocycloalkanones (C5-C7), and 3-acetylthiocycloalkanones (C5-C7) (roasted, onion, bitter, burnt, rubbery, potato, and meaty) were comparable to those determined for the homologous series of 2-mercapto-4-alkanols (C6-C10). Fruity notes were not observed, and the odor qualities were similar to those of 3-mercaptocycloalkanols. The configurations were not essential for the odor qualities (Wakabayashi *et al.*, **2020**).

4.3.2. Sensory Properties of the Stereoisomers of 1,3-Oxathianes

Odor Thresholds.

The geometric means of the odor thresholds determined for the stereoisomers of 2,6-dimethyl-4-propyl-1,3-oxathiane and 2,4-dimethyl-6-propyl-1,3-oxathiane are listed in Table 14.

Table 14: Mean odor thresholds of the stereoisomers of 2,6-dimethyl-4-propyl-1,3-oxathiane and 2,4-dimethyl-6-propyl-1,3-oxathiane (Riegel *et al.*, **2022;** Publication II)

	2,6-dimethyl-4-propyl-1,3-oxathiane		**2,4-dimethyl-6-propyl-1,3-oxathiane**	
				
stereoisomer[a]	no.	mean[b]	no.	mean[b]
(2*S*,4*R*,6*S*)	**1**-Ia	7.9	**2**-Ia	1404
(2*R*,4*S*,6*R*)	**1**-Ia'	12	**2**-Ia'	212
(2*S*,4*S*,6*R*)	**1**-IIb	4.2	**2**-IVd'	25
(2*R*,4*R*,6*S*)	**1**-IIb'	1.5	**2**-IVd	27
(2*S*,4*S*,6*S*)	**1**-IIIc	2.8	**2**-IIb	37
(2*R*,4*R*,6*R*)	**1**-IIIc'	2.4	**2**-IIb'	24
(2*R*,4*S*,6*S*)	**1**-IVd	2.9	**2**-IIIc	24
(2*S*,4*R*,6*R*)	**1**-IVd'	1.9	**2**-IIIc'	14

[a]regarding the structures, see Figures 15 and 16
[b]geometric mean

The mean odor thresholds of all stereoisomers of 2,6-dimethyl-4-propyl-1,3-oxathiane (1.5 – 12 ng/L in air) were lower than those determined for the stereoisomers of the positional isomer 2,4-dimethyl-6-propyl-1,3-oxathiane (14 – 1404 ng/L in air). The highest odor thresholds were observed for those stereoisomers (2*S*,4*R*,6*S* and 2*R*,4*S*,6*R*) in which all alkyl-substituents are in equatorial positions; this effect was particularly pronounced for 2,4-dimethyl-6-propyl-1,3-oxathiane. For both 1,3-oxathianes, the configuration at the chiral centers did not play an important role.

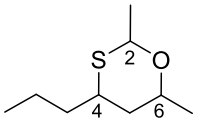
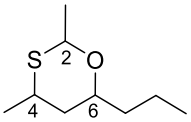
Odor Qualities.

Table 15 shows a comparison of the main odor qualities of the stereoisomers of 2,6-dimethyl-4-propyl-1,3-oxathiane and 2,4-dimethyl-6-propyl-1,3-oxathiane. Fruity, sulfury, herbal, and flowery notes were mainly observed for the stereoisomers of 2,6-dimethyl-4-propyl-1,3-oxathiane. In contrast, broth, mushroom, earthy, and wood notes were dominating odor descriptions for the eight stereoisomers of 2,4-dimethyl-6-propyl-1,3-oxathiane.

Surprisingly, sweet, fruity, herbal, and flowery odor notes were observed for some stereoisomers of 2,4-dimethyl-6-propyl-1,3-oxathianes. This was particularly pronounced for the (2*S*,4*S*,6*R*)-stereoisomer (25 ng/L (air)). The (2*S*,4*S*,6*R*)- and (2*R*,4*R*,6*S*)-stereoisomers, with axial orientations of the substituents in positions 4 and 6, were among the few stereoisomers with fruity and herbal flavor notes (Riegel *et al.*, **2022;** Publication II). These descriptions are in line with those described for the homologous series of 2-mercapto-4-alkanols.

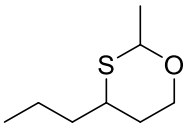
In contrast, the odor descriptions for stereoisomers of 2,6-dimethyl-4-propyl-1,3-oxathiane changed compared to the corresponding stereoisomers of 4-mercapto-2-heptanol (Riegel *et al.*, **2022;** Publication II).

Table 15: GC/O-Odor Descriptions of the Stereoisomers of 2,6-Dimethyl-4-propyl-1,3-oxathiane and 2,4-Dimethyl-6-propyl-1,3-oxathiane (Riegel *et al.*, **2022;** Publication II).

	odor description	
stereoisomer	2,6-dimethyl-4-propyl-1,3-oxathiane	2,4-dimethyl-6-propyl-1,3-oxathiane
		
(2*S*,4*R*,6*S*)	**1**-Ia fresh, sweet, broth, herbs	**2**-Ia broth, roasted meat, oily, mushroom
(2*R*,4*S*,6*R*)	**1**-Ia' flowery, sweet, citrus, herbs	**2**-Ia' mushroom, sour, fruity, citrus
(2*S*,4*S*,6*R*)	**1**-IIb sweet, herbs, earthy, oily	**2**-IVd' fruity, sweet, herbal, flowery
(2*R*,4*R*,6*S*)	**1**-IIb' flowery, sweet, flowery, fruity	**2**-IVd herbs, musty, earthy, flowery
(2S,4*S*,6*S*)	**1**-IIIc flowery, sweet, herbal, lemon peel	**2**-IIb sweat, pungent, sour, sweet
(2*R*,4*R*,6*R*)	**1**-IIIc' citrus, fruity, sweet, citrus peel	**2**-IIb' earthy, roasted, sour, mushroom
(2*R*,4*S*,6*S*)	**1**-IVd moldy, musty, vegetable, earthy	**2**-IIIc sour, sweet, mushroom, vinegar
(2*S*,4*R*,6*R*)	**1**-IVd' sweet, fruity, flowery vegetable	**2**-IIIc' earthy, wood, sweet, mushroom

The odor qualities of the stereoisomers of 2,6-dimethyl-4-propyl-1,3-oxathiane were similar to those determined for the stereoisomers of 2-methyl-4-propyl-1,3-oxathiane found in passion fruit *Passiflora edulis* f. *flavicarpa* (Winter *et al.*, **1976**), and wine (Chen *et al.*, **2018**). (2*S*,4*R*)-2-Methyl-4-propyl-1,3-oxathiane stands out in particular (Table 16); it was described as "fatty, fruity-green, tropical fruits and grapefruit", while the other three stereoisomers were described as "sulfurous and green" (Mosandl & Heusinger, **1985**).

Table 16: Odor qualities (in water) of the stereoisomers of 2-methyl-4-propyl-1,3-oxathiane (Pickenhagen & Broenner-Schindler, **1984**; Mosandl & Heusinger, **1985**).

	2-methyl-4-propyl-1,3-oxathiane
	
	odor descriptions
(2*S*,4*R*)	fruity-green, tropical, grapefruit
(2*R*,4*S*)	sulfury, herbal, green, linseed oil-like
(2*S*,4*S*)	green-grass, root, earthy, red radish
(2*R*,4*R*)	sulfury, bloomy, sweet

4.4. Impact of Structure and Stereochemistry on the Olfactophore Based on a 1,3-Oxygen-Sulfur Functionality

4.4.1. *β*-Mercaptoalkanones and *β*-Mercaptoalkanols

The results obtained in this thesis regarding the sensory properties of the stereoisomers of a homologous series of 2-mercapto-4-alkanols complement the data previously reported for homologous series of the corresponding 2-mercapto-4-alkanones (Kiske *et al.*, **2019**) and of the positional isomers, i.e. 4-mercapto-2-alkanones (Wakabayashi *et al.*, **2015**) and 4-mercapto-2-alkanols (Nörenberg *et al.*, **2017b**). A comparison of the data sets elaborated for these substance classes possessing a 1,3-oxygen-sulfur functionality and thus meeting one of the basic requirements for a so-called "tropical olfactophore" (Rowe, **2002**; Robert *et al.*, **2004**) is outlined in Figure 22 and can be summarized as follows:

(i) For 4-mercapto-2-alkanones, the configuration of the asymmetric center bearing the thiol group impacted both, the odor thresholds and the odor properties. In particular, (4*R*)-mercapto-2-hexanone showed a significantly lower odor threshold than the (4S)-enantiomer; with increasing chain lengths this effect diminished. Fruity notes were more often reported for the (*R*)-enantiomers (Wakabayashi *et al.*, **2015**).

(ii) This importance of the configuration of the asymmetric center bearing the thiol group for the odor thresholds was very pronounced for the corresponding 4-mercapto-2-alkanols. Independent from the chain length, the (2*R*,4*R*)-stereoisomers consistently showed the lowest odor thresholds. Regarding the odor properties, fruity, sulfury notes prevailed for the short-chain, and plastic, pungent notes for the long-chain homologues (Nörenberg *et al.*, **2017b**).

This picture regarding the influence of the configuration of the asymmetric center bearing the thiol group on the odor thresholds and the predominating odor properties changed completely for the positional isomers.

(iii) For the homologous series of 2-mercapto-4-alkanones, there were no significant differences between the odor thresholds of the (2*R*)- and (2*S*)-enantiomers. Regarding the odor properties, fruity notes as observed for

4-mercapto-2-alkanones were lacking. The chain length of the alkyl substituent at the carbonyl group turned out to be the main driver for the odor properties. The homologues C6-C8 showed pungent, vegetable notes, whereas the higher homologues exhibited mushroom, earthy properties (Kiske *et al.*, **2019**).

(iv) These phenomena were confirmed for the 2-mercapto-4-alkanols. The odor thresholds were not impacted by the configuration of the asymmetric center bearing the thiol group but by the asymmetric center bearing the hydroxyl group. For chain lengths C7 and C8, the (2*S*,4*S*)-stereoisomers showed significantly lower thresholds than the (2*S*,4*R*)-stereoisomers. Regarding the odor properties, again pungent, vegetable notes prevailed for the shorter and mushroom, earthy properties for the higher homologues (Riegel *et al.*, **2020;** Publication I).

These results are in agreement with other structurally related polyfunctional thiols like 3-mercaptoalkanols. While 3-mercaptoalkanols exhibit mainly fruity notes, the positional isomers of 1-mercapto-3-alkanols did not reveal fruity notes. 3-mercaptoalkanols are in accordance with the “tropical olfactophor” and 1-mercapto-3-alkanols do not meet the criteria (Polster & Schieberle, **2017**).

Similarly, for 3-mercaptocycloalkanols, 3-mercaptocycloalkanones, and their acetyl-derivatives there were no fruity notes (Wakabayashi *et al.*, **2020**). These results are also in agreement with the “tropical olfactophor” model postulated by Rowe; as the ring closure occurred at position 3 and not at position 2 as intended in the model (Rowe, **2002**).

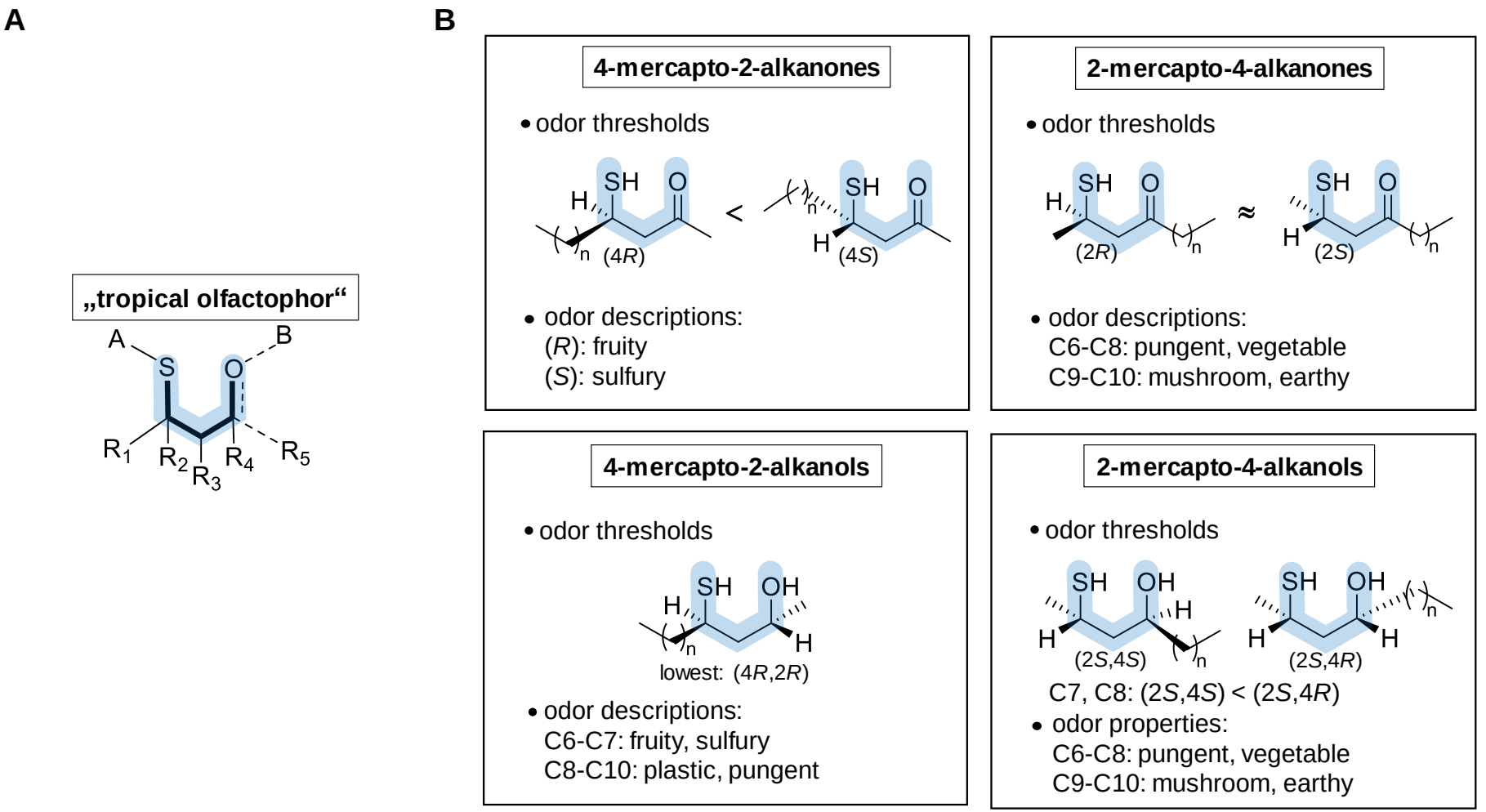


Figure 22: (A) Structural requirements for the “tropical olfactophor”: A: H, SCH_3, ring; B: H, CH_3, acyl if carbonyl not present; R_1, R_2: H, acyl; R_3: H, acyl, ring; R_4: H, CH_3, ring, OR; R_5: H if carbonyl not present (Rowe, **2002**); (B) Comparison of the sensory properties of 4-mercapto-2-alkanones (Wakabayashi *et al.*, **2015**), 2-mercapto-2-alkanones (Kiske *et al.*, **2019**), 4-mercapto-2-alkanols (Nörenberg *et al.*, **2017b**) and 2-mercapto-4-alkanols (Riegel *et al.*, **2020;** Publication I); adapted from (Engel, **2020**).

4.4.2. Alkyl-Substituted 1,3-Oxathianes

A comparison of the sensory data previously reported for the stereoisomers of 4-mercapto-2-heptanol (Nörenberg *et al.*, **2017b**) and those elaborated in this thesis for the stereoisomers of 2-mercapto-4-heptanol (Riegel *et al.*, **2020;** Publication I) and of 2,6-dimethyl-4-propyl-1,3-oxathiane and 2,4-dimethyl-6-propyl-1,3-oxathiane (Riegel *et al.*, **2022;** Publication II) allows to assess the influence of a cyclization resulting in a blocking of the free thiol and hydroxyl groups on the sensory properties of molecules possessing the 1,3-oxygen-sulfur functionality. The data are outlined in Figure 23 and Figure 24 and can be summarized as follows:

(i) For nearly all *β*-mercaptoalkanol stereoisomers, the blocking of the functional groups, as a result of the reaction with acetaldehyde, leads to increases of the odor thresholds of the formed 1,3-oxathiane stereoisomers. The only exceptions are the thresholds of *syn*-(2*S*)-mercapto-(4*R*)-heptanol and the respective 2,4-dimethyl-6-propyl-1,3-oxathiane stereoisomers which are in the same order of magnitude.

(ii) The configuration of the newly formed asymmetric center in position 2 of the 1,3-oxathiane rings plays an essential role. Among the 1,3-oxathiane stereoisomers resulting from the cyclization step, the enantiomeric (2*S*,4*R*,6*S*)- and the (2*R*,4*S*,6*R*)-stereoisomers, i.e. those possessing all alkyl substituents in the same spatial orientations, have the highest odor thresholds.

(iii) Regarding the odor properties, the important role of the substituent R4 in the model for a “tropical olfactophore” was also confirmed for 1,3-oxathianes. Only stereoisomers of 2,6-dimethyl-4-propyl-1,3-oxathiane possessing a methyl group as substituent R4 showed fruity, flowery notes; in contrast, for 2,4-dimethyl-6-propyl-1,3-oxathiane possessing a propyl group as substituent R4, broth, mushroom or pungent notes were predominating.

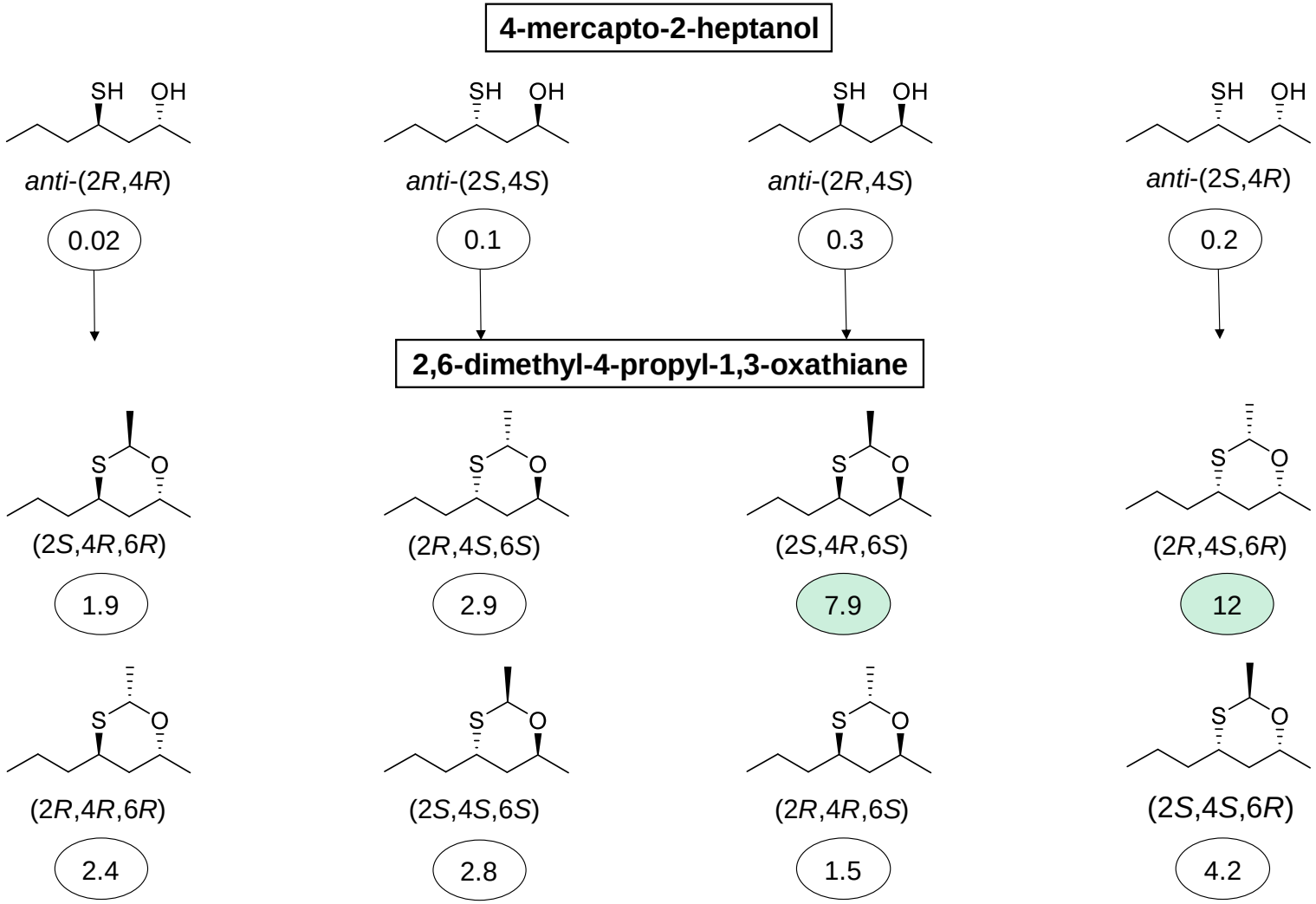


Figure 23: Comparison of the mean odor thresholds (ng/L in air; values presented in the circles) of the stereoisomers of 4-mercapto-2-heptanol (Nörenberg et al., 2017b) and of the 2,6-dimethyl-4-propyl-1,3-oxathiane stereoisomers formed upon reaction with acetaldehyde (Riegel et al. 2022, Publication II).

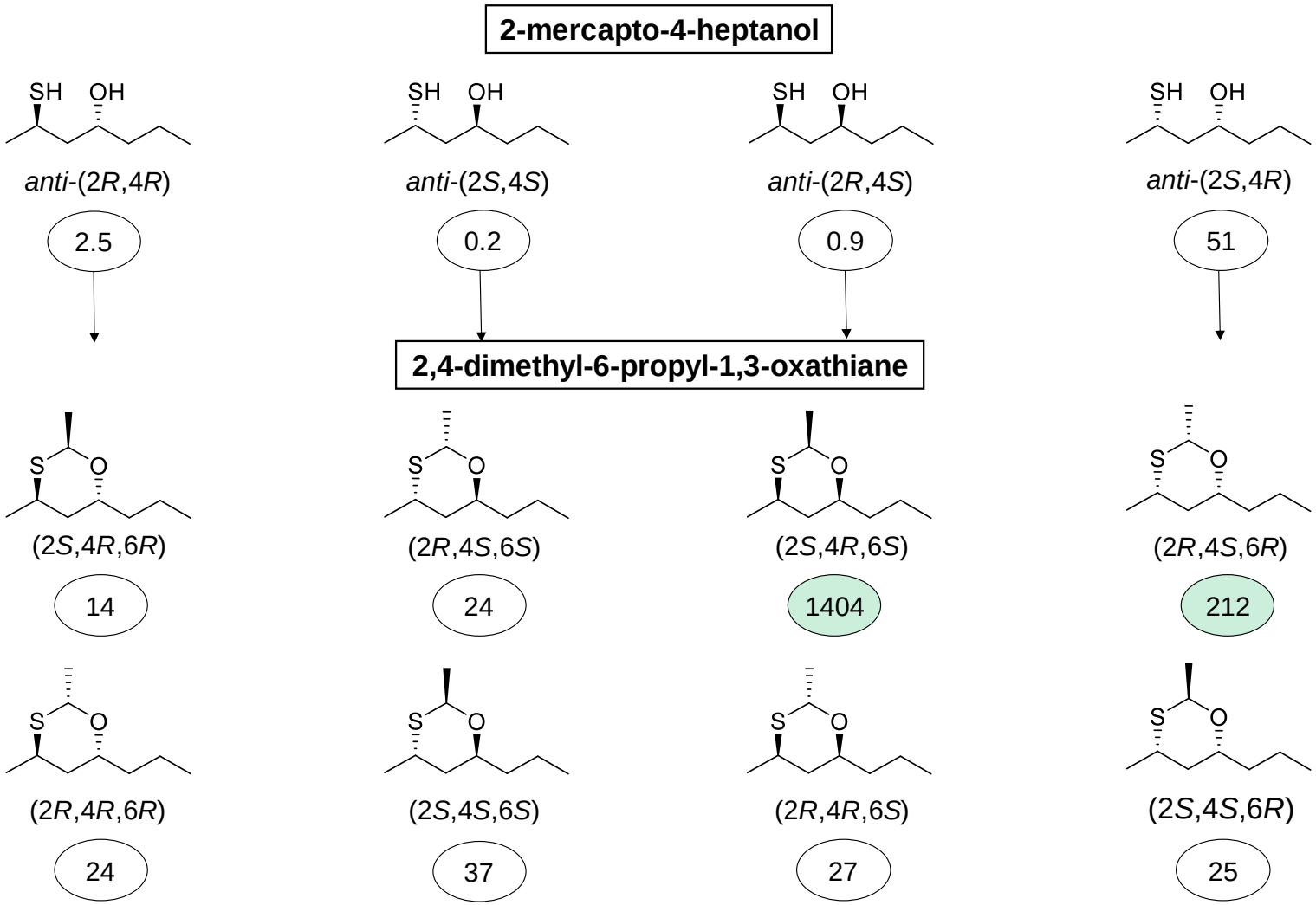


Figure 24: Comparison of the mean odor thresholds (ng/L in air; values presented in the circles) of the stereoisomers of 2-mercapto-4-heptanol (Riegel *et al*., **2020**; Publication I) and of the 2,4-dimethyl-6-propyl-1,3-oxathiane stereoisomers formed upon reaction with acetaldehyde (Riegel *et al*., **2022**; Publication II).

4.5. Perspectives

The set of analytical data acquired for the stereoisomers of the homologous series of β-mercaptoalkanols and of the alkyl-substituted 1,3-oxathianes should provide a valuable basis for further investigations.

The chromatographic data regarding separations of the individual stereoisomers on non-chiral and chiral stationary phases as well as the provided mass spectral and NMR data should assist in their identifications and quantifications and support the search for further foods in which these sulfur-containing volatiles are naturally occurring. The studies in bell pepper (Naef *et al.*, **2008**) have shown that for β-mercaptoalkanones and β-mercaptoalkanols natural systems containing the respective precursors, e.g. the structurally related α,β-unsaturated carbonyls, should be promising candidates. The studies on wine (Chen *et al.*, **2018**; Wang *et al.*, **2021**) indicate that the identification of the respective β-mercaptoalkanol precursors is a promising indicator that the related alkyl-substituted 1,3-oxathianes might also be present.

The set of analytical tools could also be used to support further studies on the biogenesis of these compounds. The first investigations in bell peppers (Nörenberg *et al.*, **2017a**) revealed exciting results regarding the distributions of the stereoisomers of β-mercaptoheptanones and β-mercaptoheptanols depending on the state of maturation. They indicated that stereochemistry may play an essential role in the steps involved in the biotransformation of the non-volatile precursors to the sulfur-containing volatiles.

In the studies for this thesis, the GC/O-based sensory evaluations of stereoisomers have been performed with a limited number of panelists. In the future, these results could be further corroborated by the use of additional panelists. Furthermore, sensory evaluations might not only be performed via GC/O but also in aqueous media or even in the respective food matrices. In combination with quantitative determinations of naturally occurring distributions of stereoisomers, this would allow the calculations of aroma activity values and thus assessments of the actual contributions of the stereoisomers to the food flavor. However, it has to be kept in mind that the assessment of individual stereoisomers in aqueous matrices would require extensive purification steps.

To this day the understanding of the interactions between odor receptors and flavor molecules is a major challenge; as the human genome contains ~ 800 odor receptor (OR) genes and many odorants activate a combination of odorant receptors (Dunkel *et al.*, **2014**; Mainland *et al.*, **2014**). Several studies indicate that olfactory receptors were formed around naturally occurring compounds and that they are very narrowly tuned (Dunkel *et al.*, **2014**). For example, only one of 391 tested human olfactory receptors, OR2M3, responded to 3-mercapto-2-methyl-pentanol (Noe *et al.*, **2017**). Regarding chirality, OR1A1 was found to be a candidate for the formation of a carvone-enantioselective phenotype; a discovery that may help to explain the mechanisms underlying (*R*)-(-)-carvone-specific anosmia in humans (Geithe *et al.*, **2017**). One potential application would be to screen the enantiomers of the homologues series of β-mercaptoalkanones against ORs, to determine if OR2M3 is activated, or if the enantiomers trigger further ORs since these enantiomers exhibit a broad range of odor qualities.

Lastly, potential industrial and commercial applications may arise from the acquired analytical and sensory data on alkyl-substituted 1,3-oxathianes, as they indicate that the flavor properties of these molecules may be tailored not only by the type and the positions of the alkyl substituents but also by the configurations of these molecules.

5. SUMMARY

Polyfunctional thiols are examples of sulfur-containing volatiles that play important roles as flavor compounds. In particular, substances with a 1,3-oxygen-sulfur arrangement as a structural feature often show low odor thresholds and pronounced odor properties. The studies underlying this thesis were embedded in a research project with the objectives to investigate structure-odor correlations for chiral *β*-mercaptoalkanones and the corresponding *β*-mercaptoalkanols and to elucidate the impact of the configurations of these molecules on their sensory properties.

In the first study, a homologous series of 2-mercapto-4-alkanols (C6-C10) was synthesized and their stereoisomers were separated by capillary gas chromatography (GC). The GC-order of elution of the *anti*- and *syn*-diastereoisomers was determined based on correlations of ^{1}H-NMR data. The configurations of the stereoisomers separated by GC on cyclodextrin derivatives as chiral stationary phases were assigned by using enantiomerically enriched *β*-mercaptoalkanols obtained via lipase-catalyzed kinetic resolutions as references.

The odor thresholds and the odor qualities were determined via GC/olfactometry. The odor thresholds of the stereoisomers of 2-mercapto-4-alkanols were generally higher than those previously reported for a homologous series of 4-mercapto-2-alkanols. For the C7 and C8 homologues, the odor thresholds were influenced by the configuration of the asymmetric center bearing the hydroxyl group. The (2*S*,4*R*)-stereoisomers had significantly higher thresholds than the (2*S*,4*S*)-stereoisomers. For the other chain lengths, the odor thresholds of the stereoisomers were in the same order of magnitude.

In contrast to the isomeric 4-mercapto-2-alkanols, fruity or tropical notes were lacking as odor descriptors for 2-mercapto-4-alkanols. The stereoisomers of the chain lengths C6-C8 were predominantly described as vegetable-like and pungent; for the chain lengths C9 and C10, mushroom was the prevailing descriptor. These results were in agreement with those previously reported for 2-mercapto-4-alkanones and the corresponding 2-acethylthio-4-alkanones. No dependence on the configuration was observed for the odor qualities, indicating that for 2-mercapto-4-alkanols the chain length rather than the configuration was the main driver determining the odor properties.

The second study dealt with derivatives of *β*-mercaptoalkanols, i.e. alkyl-substituted 1,3-oxathianes resulting from the reaction with acetaldehyde. As examples, 2,6-dimethyl-4-propyl-1,3-oxathiane and 2,4-dimethyl-6-propyl-1,3-oxathiane were synthesized by reaction of acetaldehyde with 4-mercapto-2-heptanol and 2-mercapto-4-heptanol, respectively. For both heterocycles, the eight possible stereoisomers could be separated via capillary gas chromatography (GC), using a derivatized cyclodextrin as a chiral stationary phase. The configurations and the GC order of elution were determined by (i) syntheses of the 1,3-oxathianes starting from stereochemically defined stereoisomers of 4-mercapto-2-heptanol and 2-mercapto-4-heptanol obtained via lipase-catalyzed kinetic resolutions and HPLC separations, and (ii) by NMR analyses.

The odor thresholds and odor qualities of the individual stereoisomers were determined via GC/olfactometry. The mean odor thresholds of all stereoisomers of 2,6-dimethyl-4-propyl-1,3-oxathiane (1.5 - 12 ng/L in air) were lower than those determined for the stereoisomers of the positional isomer 2,4-dimethyl-6-propyl-1,3-oxathiane (14 - 1404 ng/L in air). The highest odor thresholds were observed for the stereoisomers (2*S*,4*R*,6*S* and 2*R*,4*S*,6*R*) with all alkyl-substituents in equatorial positions; this effect was particularly pronounced for 2,4-dimethyl-6-propyl-1,3-oxathiane. For both oxathianes, there were no differences between the odor thresholds of enantiomeric pairs of the stereoisomers. Regarding the odor qualities, six of the eight stereoisomers of 2,6-dimethyl-4-propyl-1,3-oxathiane exhibited pleasant flowery, fruity, or sweet nuances. In contrast, the stereoisomers of 2,4-dimethyl-6-propyl-1,3-oxathiane were mainly characterized as broth and mushroom-like, pungent and musty. The data demonstrate the importance of both the positions of substituents and the configurations on the sensory properties of these alkyl-substituted 1,3-oxathianes.

The acquired data on individual stereoisomers of the 2-mercapto-4-alkanol homologues and of the investigated 1,3-oxathianes show that the sensory properties are not only triggered by the 1,3-oxygen-sulfur structural motif but also by the configurations of these sulfur-containing volatiles.

6. ZUSAMMENFASSUNG

Polyfunktionelle Thiole sind eine Untergruppe schwefelhaltiger flüchtiger Verbindungen, die wichtige Rollen als Aromastoffe spielen. Insbesondere Substanzen mit einer 1,3-Sauerstoff-Schwefel-Anordnung als Strukturmerkmal weisen häufig niedrige Geruchsschwellen und markante Geruchseigenschaften auf. Die dieser Arbeit zugrundeliegenden Untersuchungen waren eingebettet in ein Forschungsprojekt mit dem Ziel, Struktur-Geruchs-Korrelationen für chirale *β*-Mercaptoalkanone und die entsprechenden *β*-Mercaptoalkanole zu untersuchen und dabei insbesondere den Einfluss der Konfigurationen dieser Moleküle auf ihre sensorischen Eigenschaften zu beleuchten.

In der ersten Studie wurde eine homologe Reihe von 2-Mercapto-4-alkanolen (C6-C10) synthetisiert. Ihre Stereoisomere wurden durch Kapillar-gaschromatographie (GC) getrennt. Die GC-Elutionsreihenfolge der *anti*- und *syn*-Diastereoisomere wurde mittels Korrelationen von ^{1}H-NMR-Daten bestimmt. Die Stereoisomere wurden durch GC auf Cyclodextrin-Derivaten als chiralen stationären Phasen getrennt, und ihre Konfigurationen wurden unter Verwendung von enantiomeren-angereicherten *β*-Mercaptoalkanolen, die durch Lipase-katalysierte kinetische Racematspaltungen erhalten wurden, als Referenzen zugeordnet.
Die Geruchsschwellen und die Geruchsqualitäten wurden mittels GC/Olfaktometrie bestimmt. Die Geruchsschwellen der Stereoisomere von 2-Mercapto-4-alkanolen waren im Allgemeinen höher als die, welche zuvor für eine homologe Reihe der positionsisomeren 4-Mercapto-2-alkanole bestimmt worden waren. Bei den C7- und C8-Homologen wurden die Geruchsschwellen durch die Konfiguration des asymmetrischen Zentrums, welches die Hydroxylgruppe trägt, beeinflusst. Die (2*S*,4*R*)-Stereoisomere hatten deutlich höhere Schwellenwerte als die (2*S*,4*S*)-Stereoisomere. Für die anderen Kettenlängen lagen die Geruchsschwellen der Stereoisomere in der gleichen Größenordnung.
Im Gegensatz zu den isomeren 4-Mercapto-2-alkanolen fehlten fruchtige oder tropische Noten als Geruchsdeskriptoren für 2-Mercapto-4-alkanole. Die Stereoisomere der Kettenlängen C6 bis C8 wurden überwiegend als gemüseartig und stechend beschrieben; für die Kettenlängen C9 und C10 war Pilz der vorherrschende

Deskriptor. Diese Ergebnisse stimmen mit denen überein, welche zuvor für 2-Mercapto-4-alkanone und die entsprechenden 2-Acethylthio-4-alkanone berichtet worden waren. Für die Geruchseigenschaften wurde keine Abhängigkeit von der Konfiguration festgestellt, was darauf hindeutet, dass bei 2-Mercapto-4-alkanolen die Kettenlänge und nicht die Konfiguration der Hauptfaktor ist, welcher die Geruchseigenschaften bestimmt.

Die zweite Studie befasste sich mit Derivaten von *β*-Mercaptoalkanolen, d.h. mit alkylsubstituierten 1,3-Oxathianen, die aus der Reaktion mit Acetaldehyd hervorgehen. Als Beispiele wurden 2,6-Dimethyl-4-propyl-1,3-oxathian und 2,4-Dimethyl-6-propyl-1,3-oxathian durch Reaktion von Acetaldehyd mit 4-Mercapto-2-heptanol bzw. 2-Mercapto-4-heptanol synthetisiert. Für beide Heterocyclen konnten die acht möglichen Stereoisomere mittels Kapillargaschromatographie unter Verwendung eines derivatisierten Cyclodextrins als chirale stationäre Phase getrennt werden. Die Konfigurationen und die GC-Elutionsreihenfolge wurden durch (i) Synthesen der 1,3-Oxathiane ausgehend von stereochemisch definierten Stereoisomeren von 4-Mercapto-2-heptanol und 2-Mercapto-4-heptanol, welche durch Lipase-katalysierte kinetische Racematspaltung und HPLC-Trennungen erhalten wurden, und (ii) durch NMR-Analysen bestimmt.

Die Geruchsschwellen und Geruchsqualitäten der einzelnen Stereoisomere wurden mittels GC/Olfaktometrie bestimmt. Die ermittelten mittleren Geruchsschwellen aller 2,6-Dimethyl-4-propyl-1,3-oxathian Stereoisomere (1,5 – 12 ng/L in Luft) waren niedriger als die der Stereoisomere des Positionsisomers 2,4-Dimethyl-6-propyl-1,3-Oxathian (14 – 1404 ng/L in Luft). Die höchsten Geruchsschwellen wurden für die (2*S*,4*R*,6*S*)- und (2*R*,4*S*,6*R*)-Stereoisomere, bei denen alle Alkylsubstituenten äquatorial angeordnet sind, beobachtet. Dieser Effekt war für 2,4-Dimethyl-6-propyl-1,3-oxathian besonders ausgeprägt. Bei beiden Oxathianen gab es keine Unterschiede zwischen den Geruchsschwellen der enantiomeren Paare der Stereoisomere. Hinsichtlich der Geruchsqualitäten wiesen sechs der acht Stereoisomere von 2,6-Dimethyl-4-propyl-1,3-oxathian angenehme blumige, fruchtige oder süße Nuancen auf. Im Gegensatz dazu wurden die Stereoisomere von 2,4-Dimethyl-6-propyl-1,3-oxathian hauptsächlich als brühe- und pilzartig, stechend und moschusartig charakterisiert. Die Daten zeigen, dass für die sensorischen

Eigenschaften dieser alkylsubstituierten 1,3-Oxathiane sowohl die Positionen der Substituenten als auch die Konfigurationen wichtig sind.

Die erarbeiteten Daten zu den einzelnen Stereoisomeren der 2-Mercapto-4-alkanol-Homologen und der untersuchten 1,3-Oxathiane zeigen, dass die sensorischen Eigenschaften nicht nur durch das 1,3-Sauerstoff-Schwefel-Strukturmotiv, sondern auch durch die Konfigurationen dieser schwefelhaltigen Aromastoffe bestimmt werden.

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8. APPENDIX

8.1. Supporting Information Publication I

MS and NMR Data of 2-Mercapto-4-alkanols (C6-C10).

2-Mercapto-4-hexanol (mixture of stereoisomers) (**1**): ^{1}H-NMR (500 MHz, $CDCl_3$): *δ* 3.73 (dddd, *J* = 9.7. 6.9, 5.4, 2.7 Hz, 1H, H-4$_{anti}$), 3.59 (tt, *J* = 7.5, 4.8 Hz, 1H, H-4$_{syn}$), 3.14 (dtq, *J* = 13.7, 6.8, 3.4 Hz, 1H, H-2$_{anti}$), 3.03 (hept, *J* = 6.9 Hz, 1H, H-2$_{syn}$), 1.70-1.56 (m, 4H, H-3$_{syn+anti}$), 1.50-1.34 (m, 4H, H-5$_{syn+anti}$), 1.32 (d, *J* = 6.8 Hz, 3H, H-1$_{anti}$), 1.31 (d, *J* = 6.7 Hz, 3H, H-1$_{syn}$), 0.89 (td, 7.5,3.4,6H, H-6$_{syn+anti}$). ^{13}C-NMR (126 MHz, $CDCl_3$): δ 71.5 (C-4$_{syn}$), 70.8 (C-4$_{anti}$), 47.8 (C-3$_{syn}$), 47.3 (C-3$_{anti}$), 32.8 (C-2$_{syn}$), 32.4 (C-2$_{anti}$), 30.5 (C-5$_{anti}$), 30.4 (C-5$_{syn}$), 26.7 (C-1$_{anti}$), 25.7 (C-1$_{syn}$), 9.9 (C-6$_{anti}$), 9.7 (C6$_{syn}$).

anti-2-Mercapto-4-hexanol (D1-**1**): ^{1}H-NMR (500 MHz, $CDCl_3$): δ [ppm] 3.82-3.75 (m, 1H, H-4), 3.24-3.14 (m, 1H, H-2), 1.84 (br, s, 1H, O-H), 1.68 (m, 1H, H-3), 1.52-1.44 (m, 1H, H-3`), 1.51-1.44 (m, 2H, H-5), 1.49 (d, *J* = 7.9 Hz, 1H, S-H), 1.37 (d, J = 6.8 Hz, 3H, H-1), 0.94 (t, *J*= 7.5 Hz, 3H, H-6). ^{13}C-NMR (126 MHz, $CDCl_3$): δ [ppm] 71.04 (C-4), 47.52 (C-3), 32.62 (C-2), 30.79 (C-5), 26.89 (C-1), 10.04 (C-6).

syn-2-Mercapto-4-hexanol (D2-**2**): ^{1}H-NMR (500 MHz, $CDCl_3$): δ [ppm] 3.67-3.62 (m, 1H, H-4), 3.12-3.04 (m, 1H, H-2), 1.94 (br, s, 1H, O-H), 1.72-1.63 (m, 2H, H-3), 1.59 (d, *J* = 7.1 Hz, 1H, S-H), 1.54-1.38 (m, 2H, H-5), 1.36 (d, 6.7 H7, 3H, H-1), 0.93 (t, *J* = 7.5 Hz, 3H, H-6). ^{13}C-NMR (126 MHz, $CDCl_3$): δ [ppm] 71.95 (C-4), 48.07 (C-3), 33.17 (C-2), 30.65 (C-5), 26.03 (C-1), 9.89 (C-6).

GC-MS (*m/z*, rel.%) (both isomers show the same fragmentation): 59 (100), 61 (98), 41 (35), 100 (31), 43 (30), 71 (30), 85 (20), 116 (18), 134 (M^+,4).

2-Mercapto-4-heptanol (**2**): GC-MS (*m/z*, rel.%) (both isomers show the same fragmentation): 59 (100), 61 (94), 41 (38), 43 (32), 71 (31), 100 (31), 85 (23), 116 (20), 134 (4), 147 (M^+,1). ^{1}H-NMR (500 MHz, CDCl3): δ 3.82 (dddd, *J* = 9.8, 7.2, 4.7, 2.6 Hz 1H, H-4$_{anti}$), 3.71-3.64 (m, 1H, H-4$_{syn}$), 3.14 (dtd, *J* = 10.4, 6.8, 3.7 Hz, 1H, H-2$_{anti}$), 3.03 (hept, *J* = 7.8, 6.9 Hz, 1H, H-2$_{syn}$), 1.67-1.58 (m, 4H, H-3$_{syn+anti}$), 1.49-1.35 (m, 8H, H-5$_{syn+anti}$, H-6$_{syn+anti}$), 1.32 (d, *J* = 6.8 Hz, 3H, H-1$_{anti}$), 1.31 (d, *J* = 6.7 Hz, 3H, H-1$_{syn}$), 0.87 (td, 6H, H-7$_{syn+anti}$). ^{13}C-NMR (126 MHz, $CDCl_3$): δ 70.2 (C-4$_{syn}$), 69.3 (C-4$_{anti}$), 48.8 (C-3$_{syn}$), 47.8 (C-3$_{anti}$), 40.0 (C-5$_{anti}$), 39.9 (C-5$_{syn}$), 33.0 (C-2$_{syn}$), 32.5 (C-2$_{anti}$), 26.8 (C-1$_{anti}$), 25.8 (C-1$_{syn}$), 18.8 (C-6$_{anti}$), 18.6 (C-6$_{syn}$), 14.0 (C-7$_{anti+syn}$).

2-Mercapto-4-octanol (**3**): GC-MS (*m/z*, rel.%) (both isomers show the same fragmentation): 101 (100), 69 (83), 87 (58), 61 (56), 111 (56), 41 (55), 128 (47), 74 (31), 43 (30), 115 (30), 144 (25), 162 (M^+,4). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.84 (dddd, *J* =9.9, 7.3, 4.9, 2.6 Hz, 1H, H-4$_{anti}$), 3.73-3.67 (m, 1H, H-4$_{syn}$), 3.18 (dpt, *J* = 10.5, 6.8, 3.6 Hz, 1H, H-2$_{anti}$), 3.07 (hept, *J* = 6.9 Hz, 1H, H-2$_{syn}$), 1.72-1.62 (m, 4H, H-3$_{syn+anti}$), 1.45-1.26 (m, 12H, H-5$_{syn+anti}$, H-6$_{syn+anti}$, H7$_{syn+anti}$), 1.29 (d, *J* = 6.8 Hz, 3H, H-1$_{anti}$), 1.28 (d, *J* = 6.7 Hz, 3H, H-1$_{syn}$), 0.91-0.87 (m, 6H, H-8$_{syn+anti}$). ^{13}C-NMR (126 MHz, $CDCl_3$): δ 70.6 (C-4$_{syn}$), 69.8 (C-4$_{anti}$), 48.6 (C-3$_{syn}$), 48.0 (C-3$_{anti}$), 37.8 (C-5$_{anti}$), 37.6

(C-5$_{syn}$), 33.2 (C-2$_{syn}$), 32.7 (C-2$_{anti}$), 28.0 (C-6$_{anti}$), 27.8 (C-6$_{syn}$), 27.0 (C-1$_{anti}$), 26.0 (C-1$_{syn}$), 22.9 (C-7$_{syn+anti}$), 14.3 (C-8$_{anti+syn}$).

2-Mercapto-4-nonanol (**4**): GC-MS (*m/z*, rel.%) (both isomers show the same fragmentation): 55 (100), 61 (95), 101 (59), 41 (50), 43 (50), 83 (45), 71 (41), 115 (30), 142 (12), 158 (12), 176 (M^+,1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.80 (dqd, *J* = 12.2, 7.1, 6.0, 2.8 Hz, 1H, H-4$_{anti}$), 3.66 (qd, *J* = 7.1, 4.8 Hz, 1H, H-4$_{syn}$), 3.14 (dpd, *J* = 10.5, 6.8, 3.7 Hz, 1H, H-2$_{anti}$), 3.03 (hept, *J* = 6.9 Hz, 1H, H-2$_{syn}$), 1.64-1.61 (m, 4H, H-3$_{syn+anti}$), 1.27-1.22 (m, 16H, H-5$_{syn+anti}$, H-6$_{syn+anti}$, H7$_{syn+anti}$, H-8$_{syn+anti}$), 1.32 (d, *J* = 6.9 Hz, 3H, H-1$_{anti}$), 1.31 (d, *J* = 6.7 Hz, 3H, H-1$_{syn}$), 0.85-0.80 (m, 6H, H-9$_{syn+anti}$). ^{13}C-NMR (126 MHz, $CDCl_3$): δ 70.5 (C-4$_{syn}$), 69.6 (C-4$_{anti}$), 48.4 (C-3$_{syn}$), 47.8 (C-3$_{anti}$), 37.9 (C-5$_{anti}$), 37.7 (C-5$_{syn}$), 33.0 (C-2$_{syn}$), 32.5 (C-2$_{anti}$), 31.8 (C-7$_{syn+anti}$), 26.8 (C-1$_{anti}$), 25.9 (C-1$_{syn}$), 25.3 (C-6$_{anti}$), 25.1 (C-6$_{syn}$), 22.6 (C-8$_{syn+anti}$), 14.0 (C-9$_{anti+syn}$).

2-Mercapto-4-decanol (**5**): GC-MS (*m/z*, rel.%) (both isomers show the same fragmentation): 55 (100), 61 (86), 43 (67), 41 (46), 101 (43), 74 (36), 97 (34), 115 (31), 129 (22), 143 (10), 172 (7), 190 (M^+,1). ^{1}HNMR (500 MHz, $CDCl_3$): δ 3.80 (tdd, *J* = 7.2, 5.7, 3.8 Hz, 1H, H-4$_{anti}$), 3.70-3.63 (m, 1H, H-4$_{syn}$), 3.13 (dtq, *J* = 13.6, 6.8, 3.4 Hz, 1H, H-2$_{anti}$), 3.03 (hept, *J* = 6.9 Hz, 1H, H-2$_{syn}$), 1.81-1.20 (m, 8H, H-3$_{syn+anti}$, H-5$_{syn+anti}$), 1.28-1.18 (m, 16H, H-6$_{syn+anti}$, H7$_{syn+anti}$, H-8$_{syn+anti}$, H-9$_{syn+anti}$), 1.31 (d, *J* = 6.8 Hz, 3H, H-1$_{anti}$), 1.31 (d, *J* = 6.7 Hz, 3H, H-1$_{syn}$), 0.84-0.79 (m, 6H, H-10$_{syn+anti}$). ^{13}C-NMR (126 MHz, $CDCl_3$): δ 70.5 (C-4$_{syn}$), 69.6 (C-4$_{anti}$), 48.4 (C-3$_{syn}$), 47.8 (C-3$_{anti}$), 37.9 (C-5$_{anti}$), 37.6 (C-5$_{syn}$), 33.0 (C-2$_{syn}$), 32.5 (C-2$_{anti}$), 31.8 (C-8$_{syn+anti}$), 29.3 (C-7$_{syn+anti}$), 26.7 (C-1$_{anti}$), 25.8 (C-1$_{syn}$), 25.6 (C-6$_{syn}$), 25.4 (C-6$_{anti}$), 22.6 (C-9$_{syn+anti}$), 14.1 (C-10$_{anti+syn}$).

2-Acetylthio-4-hexanol (**6**): GC-MS (*m/z*, rel.%) (both isomers show the same fragmentation): 43 (100), 59 (45), 133 (30), 74 (29), 116 (25), 101 (25), 87 (25), 146 (2), 158 (1), 176 (M^+,1).

anti-2-Acetylthio-4-hexanol (D1-**6**): ^{1}H-NMR (500 MHz, Chloroform-d) δ [ppm] 3.74 (dtd, *J* = 14.3, 7.2, 3.4 Hz, 1H, H-2), 3.44 (dddd, *J* = 9.9, 7.3, 5.1, 2.3 Hz, 1H, H-4), 2.32 (s, 3H, H-8), 1.87 (ddd, *J* = 14.6, 9.0, 5.8 Hz, 1H, H-3), 1.72 – 1.33 (m, 6H, H1,H-3`, H-5), 1.31 (d, *J* = 6.7 Hz, 3H, H-1), 0.90 (t, *J* = 7.4 Hz, 3H, H-6). ^{13}C-NMR (126 MHz, CDCl3): δ 198.78 (C-7), 70.27 (C-4), 45.49 (C-3), 36.76 (C-2), 27.65 (C-5), 30.79 (C-8), 22.09 (C-1), 10.04 (C-6).

syn-2-acetylthio-4-hexanol (D2-**6**): NMR data could not be obtained because of the instability of the substance under the measurement conditions. NMR-analysis revealed *syn*-2-mercapto-4-hexyl acetate as a rearrangement product.

syn-2-Mercapto-4-hexyl acetate: GC-MS (*m/z*, rel.%): 43 (100), 74 (66), 61 (52), 116 (45), 55 (40), 87 (43), 101 (25), 177 (M+,1). ^{1}H-NMR (500 MHz,$CDCl_3$) *δ* [ppm] 5.07 (dtd, *J* = 9.4, 6.1, 3.1 Hz, 1H, H-4$_{anti}$), 2.94 (dtq, *J* = 13.5, 6.7, 4.0, 3.3 Hz, 1H, H-2$_{anti}$), 2.06 (s, 3H, H-8$_{anti}$), 1.88 (ddd, *J* = 14.4, 9.7, 4.0 Hz, 1H, H-3a$_{anti}$), 1.73 (d, *J* = 6.3 Hz, 1H$_{anti}$), 1.66 – 1.58 (m, 1H, H-3b$_{anti}$), 1.63 – 1.56 (m, 2H, H-5$_{anti}$), 1.36 (d, J = 6.8 Hz, 3H, H-1$_{anti}$), 0.91 (t, *J* = 7.5 Hz, 3H, H-6$_{anti}$). ^{13}C-NMR (126 MHz, $CDCl_3$) *δ* [ppm] 171.01 (C-7), 73.63 (C-4), 45.24 (C-3), 32.10 (C-2), 27.65 (C-5), 25.83 (C-1), 21.30 (C-8), 9.60 (C-6).

Table S1. Temperature programs used for the separation of the stereoisomers of 2-mercapto-4-alkanols **2**-**5** with chain lengths C7-C10 using octakis(2,3-di-*O*-*n*-butyryl-6-*tert*-butyl-silyl)-*γ*-cyclodextrin 50% in SE-54; I: 30 m, I.D: 0.3 mm, df: 2,5 µm (column 6)[a]

method	**temperature program**
GC-FID	C7 and C8: from 85 °C (0 min hold) to 120 °C (0 min hold) at 1 °C/min and from 120 °C to 180 °C (5 min hold) at 4 °C/min C9 and C10: from 95 °C (0 min hold) to 125 °C (0 min hold) at 0.5 °C/min and from 125 °C to 180 °C (5 min hold) at 4 °C/min
GC/O	from 35 °C (0 min hold) to 95 °C (0 min hold) at 30 °C/min, from 95 °C to 145 °C (0 min hold) at 1 °C/min and from 145 °C to 180 °C (10 min hold) at 5 °C/min
MDGC	from 40 °C (0 min hold) to 85 °C (0 min hold) at 2 °C/min, from 85 °C to 120°C (0 min hold) at 1°C/min, from 120 °C to 180°C (10 min hold) at 4°C/min

[a] Synthesized according to (1)

Table S2. Temperature programs used for the separation of the stereoisomers 2-mercapto-4-hexanol **1** using octakis(2,3-(*S*)-methylbutyryl-6-*O*-*tert*-butyl-dimethylsilyl-γ-cyclodextrin 25% in OV1701-vi; 30 m x 0.32 mm i.d.; 0.25 µm (column 3)[a]

method	temperature program
GC-FID	from 85 °C (0 min hold) to 120 °C (0 min hold) at 1 °C/min and from 120 °C to 180 °C (5 min hold) at 4 °C/min
MDGC	from 40 °C (0 min hold) to 60 °C (0 min hold) at 2 °C/min, from 60 °C to 85°C (10 min hold) at 1°C/min, from 85 °C to 180°C (10 min hold) at 4°C/min

[a] Synthesized according to (2)

Table S3. Temperature programs used for the separation of the stereoisomers of 2-mercapto-4-hexanol **1** using octakis(2,6-di-*O*-*n*-pentyl-3-*O*-butyryl)-*γ*-cyclodextrin; I: 25 m, I.D: 0.25 mm; FS-Lipodex® E (column 4)

method	temperature program
GC-FID	from 85 °C (0 min hold) to 120 °C (0 min hold) at 1 °C/min and from 120 °C to 180 °C (10 min hold) at 4 °C/min
GC/O	from 35 °C (0 min hold) to 85 °C (0 min hold) at 30 °C/min, from 85 °C to 110 °C (0 min hold) at 1 °C/min and from 110 °C to 180 °C (4 min hold) at 5 °C/min

Table S4. Temperature programs used for the separation of the (2*S*,4*R*)-- and (2*R*,4*S*)-2-mercapto-4-hexanol stereoisomers **1b** and **1b'** using octakis(2,3-di-*O*-*n*-butyryl-6-*O*-*tert*-butyl-dimethylsilyl)-γ-cyclodextrin 33% in SE 54; 30 m x 0.32 mm i.d.; 0.25 µm (column 5)[a]

method	temperature program
GC/FID	from 85 °C (0 min hold) to 120 °C (0 min hold) at 1 °C/min and from 120 °C to 180 °C (10 min hold) at 4 °C/min
GC/O	from 35 °C (0 min hold) to 85 °C (0 min hold) at 30 °C/min, from 85 °C to 120 °C (0 min hold) at 1 °C/min, from 120 °C to 180 °C (5 min hold) at 4 °C/min

[a] Synthesized according to (1)

Table S5. Geometric Means of the Individual Odor Thresholds Determined by the Panelists via GC/O

		geometric means of odor thresholds (ng/L air)[a]				
no.	compound	a (2*R*,4*R*)	a' (2*S*,4*S*)	b (2*S*,4*R*)	b' (2*R*,4*S*)	ratio [b]
1	2-mercapto-4-hexanol	21	2.7	5.5	2.4	9
2	2-mercapto-4-heptanol	2.5	0.2	51	0.9	255
3	2-mercapto-4-octanol	7.3	3.8	56	5.6	15
4	2-mercapto-4-nonanol	5.4	14	22	14	4
5	2-mercapto-4-decanol	171	183	199	238	1

[a] Geometric mean values calculated based on the individual odor thresholds determined by the assessors via GC/O (n=3)

[b] ratio between the highest and lowest odor threshold for each chain length

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8.2. Supporting Information Publication II

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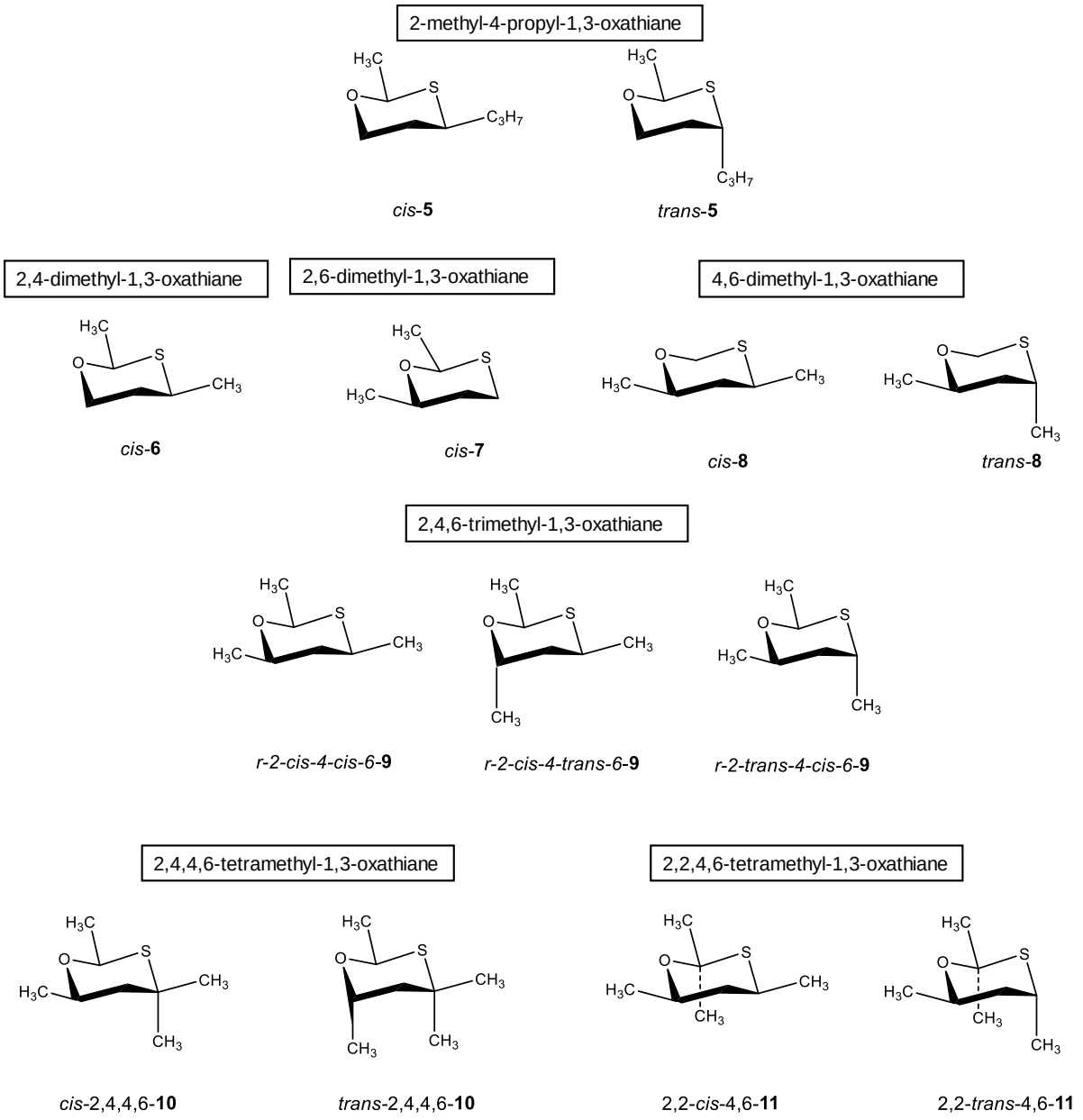

Figure S1. Stereoisomers of substituted 1,3-oxathianes used as supporting substances to assign the configurations of **1** and **2**:
2-methyl-4-propyl-1,3-oxathiane (*cis*-**5** and *trans*-**5**);[1] 2,4-dimethyl-1,3-oxathiane (*cis*-**6**), 2,6-dimethyl-1,3-oxathiane (*cis*-**7**), 4,6-dimethyl-1,3-oxathiane (*cis*-**8** and *trans*-**8**), 2,4,6-trimethyl-1,3-oxathiane (*r*-2-*cis*-4-*cis*-6-**9**, *r*-2-*trans*-4-*cis*-6-**9** and *r*-2-*cis*-4-*trans*-6-**9**) and 2,4,4,6-tetramethyl-1,3-oxathiane (*cis*-2,4,4,6-**10** and *trans*-2,4,4,6-**10**);[2] and 2,2,4,6-tetramethyl-1,3-oxathiane (2,2-*cis*-4,6-**11** and 2,2-*trans*-4,6-**11**).[3]

Table S1: ^{1}H and ^{13}C NMR data of 2-methyl-4-propyl-oxathiane **5**.[a]

position	^{1}H [ppm] *cis*-**5**	^{1}H [ppm] *trans*-**5**	position	^{13}C [ppm] *cis*-**5**	^{13}C [ppm] *trans*-**5**
H-2	4.80 (q, $^{3}J_{H\text{-}2/2\text{-}Me}$ = 6.2 Hz, 1H)	5.02 (q, $^{3}J_{H\text{-}2/2\text{-}Me}$ = 6.2 Hz, 1H)	2	79.25	73.89
2-Me	1.47 (d, $^{3}J_{2\text{-}Me/H\text{-}2}$ = 6.2 Hz, 3H)	1.43 (d, $^{3}J_{2\text{-}Me/H\text{-}2}$ = 6.3 Hz, 3H)	4	42.38	38.50
H-4	3.09 – 3.02 (m, 1H)	2.89 (dq, ^{3}J = 9.3, $^{3}J_{H\text{-}5a/H\text{-}4a}$ = 4.3 Hz, 1H)	5	38.46	35.66
H_e-5	1.72 (dq, $^{2}J_{Ha\text{-}5/He\text{-}5}$ = 13.9 Hz, $^{5}J_{He\text{-}5/Ha\text{-}4}$ = 2.5 Hz, 1H)	1.58 – 1.49 (m, 1H)	6	70.08	64.66
H_a-5	1.66 – 1.57 (m, 1H)	2.27 – 2.18 (m, 1H)	7	33.37	30.33
H_e-6	4.18 (ddd, $^{2}J_{He\text{-}6/H\text{-}6a}$ = 12.0 Hz, $^{3}J_{H\text{-}6/Ha\text{-}5}$ = 4.0 Hz, $^{3}J_{He\text{-}6/He\text{-}5}$ = 2.3 Hz, 1H)	3.98 – 3.92 (m, 1H)	8	19.41	21.17
H_a-6	3.56 (td, $^{2}J_{Ha\text{-}6/He\text{-}6}$ = 12.2 Hz, $^{3}J_{Ha\text{-}6/He\text{-}5}$ = 2.2 Hz, 1H),	3.83 (td, $^{2}J_{H\text{-}6a/H\text{-}6e}$ = 12.2 Hz, $J_{H\text{-}6a/H\text{-}5e}$ = 2.3 Hz, 1H),	9	14.04	13.94
			10	22.10	22.04

[a] The obtained NMR are in agreement with those previously reported.[4]

Table S2: Chemical shifts (^{1}H) determined for 2,6-dimethyl-4-propyl-1,3-oxathiane (**1**-I, **1**-III and **1**-VI) and 2,4-dimethyl-6-propyl-1,3-oxathiane (**2**-I and **2**-II) and reported for structurally related alkyl-substituted 1,3-oxathianes (structures see Figure S1).

	ppm											
	H_a-2	H_e-2	H_a-4	H_e-4	H_a-6	H_e-6	2_a-Me	2_e-Me	4_a-Me	4_e-Me	6_a-Me	6_e-Me
1-I	4.82	-	3.04	-	3.50	-	-	1.48	-	-	-	1.22
1-III	5.03	-	-	2.88	3.78	-	-	1.43	-	-	-	1.17
1-IV	5.16	-	3.25	-	-	4.36	-	1.41	-	-	1.20	-
2-I	4.80	-	3.11 – 3.03	-	3.35	-	-	1.47	-	1.20	-	-
2-II	5.08	-	-	3.15	3.66	-	-	1.43	1.54	-	-	-
trans-**5**	5.02	-	2.89		3.83	3.98 – 3.92	-	1.43	-	-	-	-
cis-**5**	-	4.80	3.09 – 3.02	-	3.56	4.18	-	1.47	-	-	-	-
cis-**6**	4.76	-	3.02	-	3.45	4.1	-	1.39	-	1.19	-	-
cis-**7**	4.79	-	[a]	[a]	3.44	-	-	1.40	-	-	-	1.17
cis-**8**	4.80	4.80	3.01	-	3.46	-	-	-	-	1.22	-	1.18
trans-**8**	4.94	4.64	-	3.10	3.09	-	-	-	1.52	-	-	1.17
r-2-*cis*-4-*cis*-6-**9**	4.78	-	3.02		3.45	-	-	1.40	-	1.17	-	1.17
r-2-*trans*-4-*cis*-6-**9**	5.02	-	-	3.14	3.82	-	-	1.38	1.55	-	-	1.15
r-2-*cis*-4-*trans*-6-**9**	5.11	-	3.23		-	4.31	-	1.37	-	1.19	1.31	
cis-2,4,4,6-**10**	4.84	-	-	-	3.67 - 3.62	-	-	1.35 - 1.38	[a]	[a]	-	1.12
trans-2,4,4,6-**10**	-	5.12	-	-		4.14 - 4.08	-	[a]	[a]	[a]	[a]	[a]
2,2-*cis*-4,6-**11**	-	-	3.11	-	3.80	-	1.61	1.45	-	1.16	-	1.14
2,2-*trans*-4,6-**11**	-	-	-	3.13	4.03	-	1.54	1.52	1.34	-	-	1.16

[a] not determined

Table S4: Vicinal coupling constants determined for 2,6-dimethyl-4-propyl-1,3-oxathiane (**1**-I, **1**-III and **1**-VI) and 2,4-dimethyl-6-propyl-1,3-oxathiane (**2**-I and **2**-II) and reported for structurally related alkyl-substituted 1,3-oxathianes (structures see Figure S1).

	J (Hz)							
compound	H_a-	H_e-4/H_e-5	H_a-4/H_e-5	H_e-4/H_a-5	H_a-6/H_a-5	H_e-6/H_e-5	H_a-6/H_e-5	H_e-6/H_a-5
1-I	11.6	-	2.7	-	11.1	-	2.1	-
1-III	-	2.8	-	5.3	12.4	-	2.0	-
1-IV	12.0	-	2.7	-	-	2.2	-	5.2
2-I	11.4	-	2.8	-	11.2	-	1.9	-
2-II	-	2.8	-	4.8	11.9	-	1.9	-
trans-**5**	4.5	-	5.0	-	12.0	4.5	2.2	4.0
cis-**5**	12.0	-	2.5	-	12.0	2.5	2.5	4.0
cis-**6**	10.6	-	3.7	-	10.5	3.3	3.0	3.4
cis-**7**	12.75	3.5	2.82	3.69	11.22	-	2.04	-
cis-**8**	11.45	-	2.75	-	10.5	-	2.2	-
trans-**8**	-	4.0	-	4.5	9.65	-	2.95	-
r-2-*cis*-4-*cis*-6-**9**	11.35	-	2.75	-	10.85	-	2.10	-
r-2-*trans*-4-*cis*-6-**9**	-	3.15	-	4.95	10.85	-	2.40	-
r-2-*cis*-4-*trans*-6-**9**	9.8	-	4.2	-	-	3.0	-	4.45
cis-2,4,4,6-**10**	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*
trans-2,4,4,6-**10**	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*
2,2-*cis*-4,6-**11**	11.9	-	2.85	-	11.2	-	2.15	-
2,2-*trans*-4,6-**11**	-	7.8	-	5.8	11.2	-	4.45	-

[a] no data available

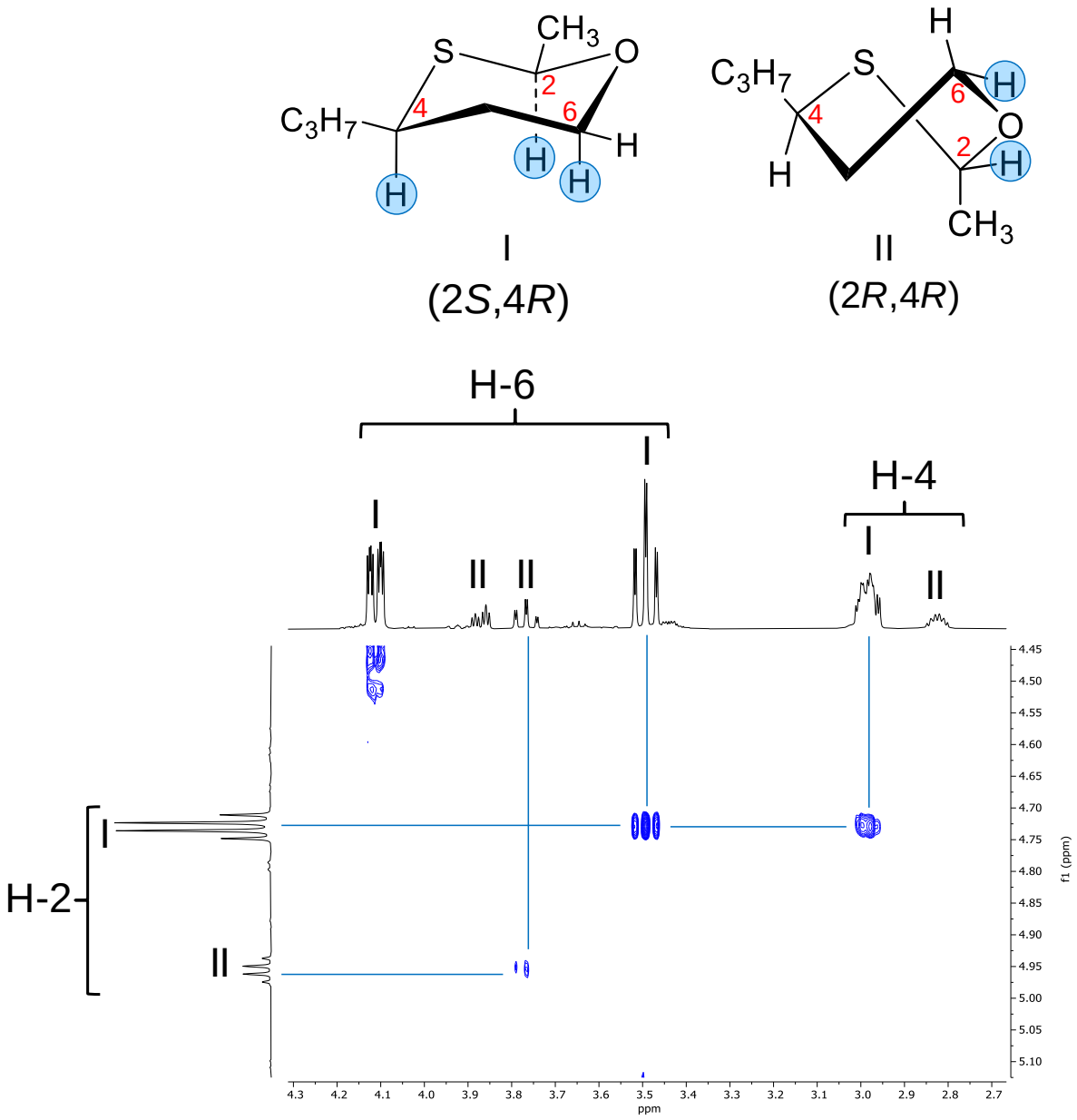

Figure S2. Part of the NOESY-spectra of 2-methyl-4-propyl-1,3-oxathiane. Chair and twist conformation according to Mosandl & Heusinger[4]

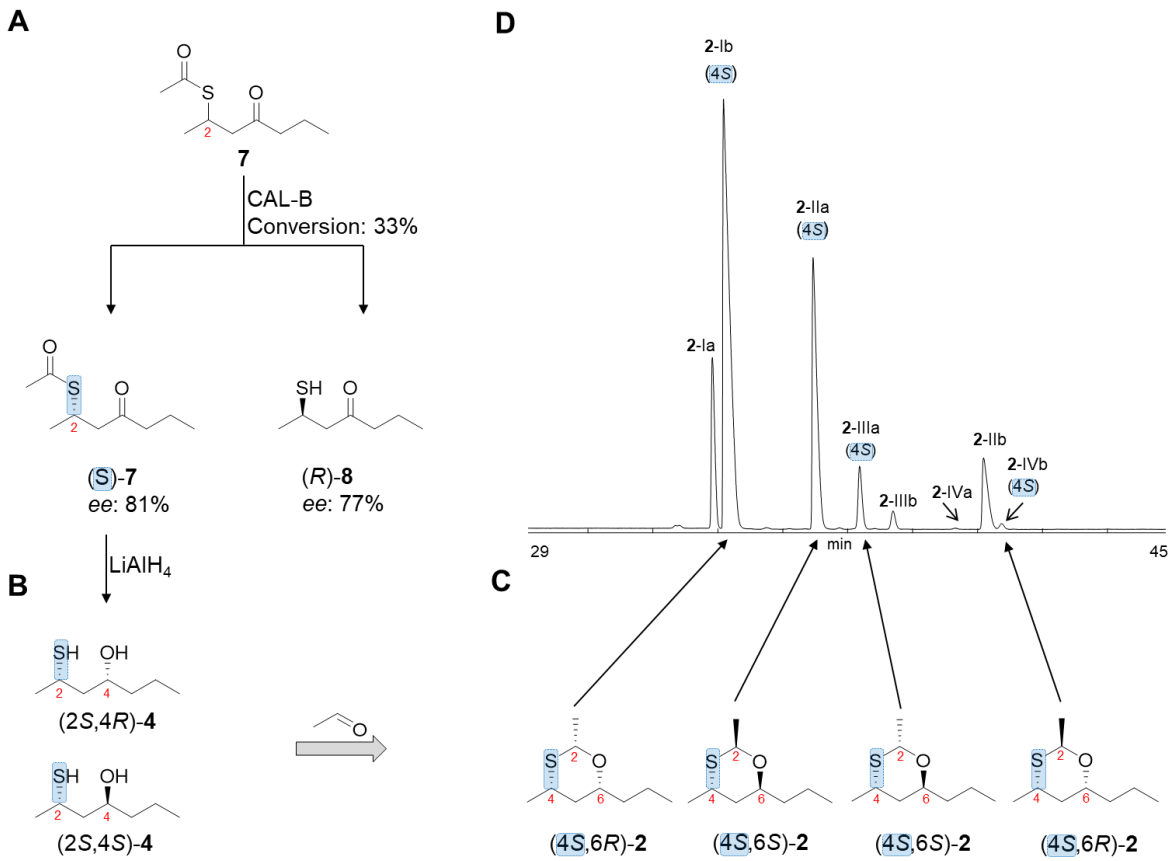

Figure S3: (A) CAL-B-catalyzed kinetic resolution of 2-acetylthio-4-heptanone; (B) reduction of the remaining (2*S*)-enriched substrate with $LiAlH_4$; (C) reaction of the formed stereoisomers of 2-mercapto-4-heptanol with acetaldehyde; (D) assignment of the GC-order of elution of the (4*S*)-configured stereoisomers of **2** on heptakis(diethyl-*tert*-butyldimethylsilyl)-*β*-cyclodextrin.

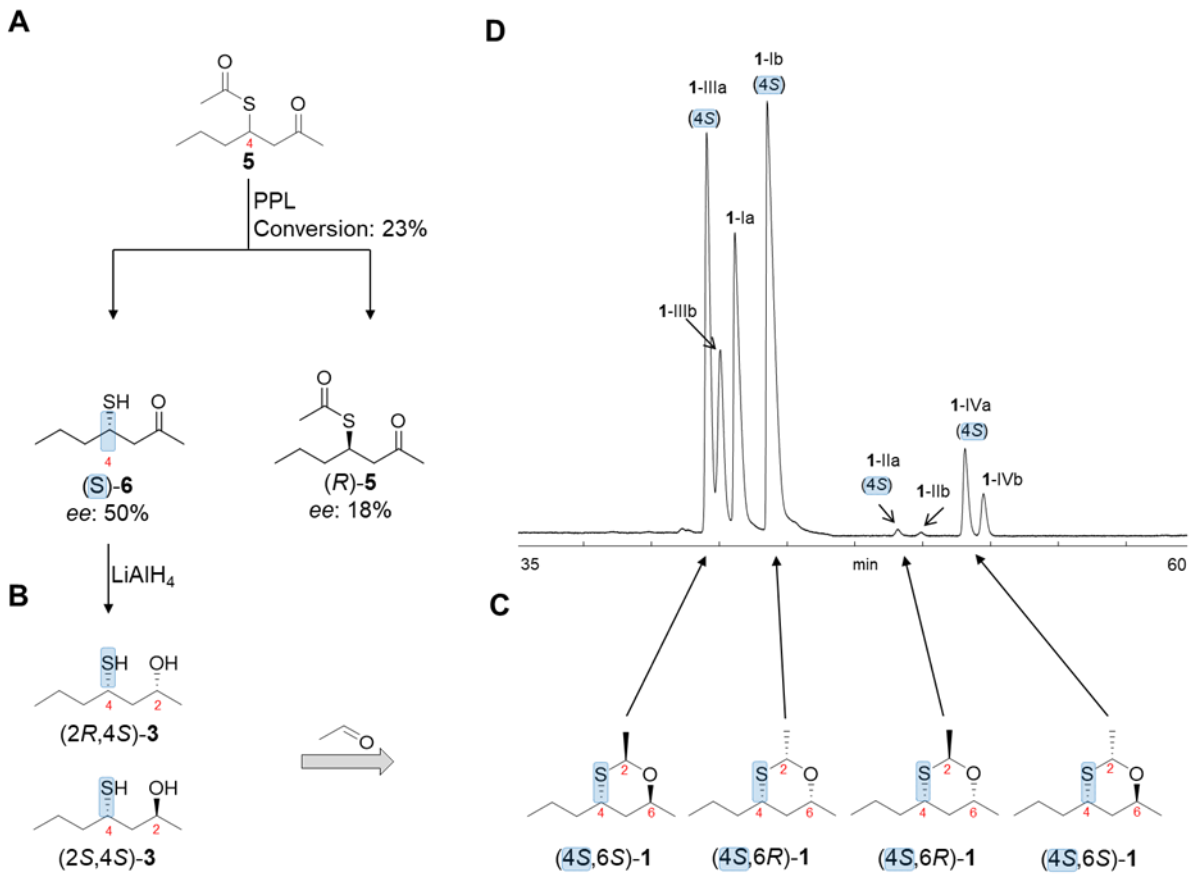

Figure S4: (A) PPL-catalyzed kinetic resolution of 4-acetylthio-2-heptanone; (B) reduction of the (4*S*)-enriched product with $LiAlH_4$; (C) reaction of the formed stereoisomers of 4-mercapto-2-heptanol with acetaldehyde; (D) assignment of the GC-order of elution of the (4*S*)-configured stereoisomers of **1** on heptakis(diethyl-*tert*-butyldimethylsilyl)-*β*-cyclodextrin.

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PUBLICATIONS (PEER-REVIEWED)

Riegel, A. D.; Wakabayashi, H.; Wakabayashi, M.; Rynešová, M.; V. Dudko, V.; Eisenreich W.; Engel, K.-H. Configurations and Sensory Properties of the Stereoisomers of 2,6-Dimethyl-4-propyl-1,3-oxathiane and 2,4-Dimethyl-6-propyl-1,3-oxathiane.
J. Agric. Food Chem. **2022**, *70*, 4712–4724

Riegel A. D.; Kiske C.; Dudko V.; Poplacean I.; Eisenreich W.; Engel K.-H.; Absolute Configurations and Sensory Properties of the Stereoisomers of a Homologous Series (C6-C10) of 2-Mercapto-4-alkanols.
J. Agric. Food Chem. **2020**, *68*, 2738–2746

Wakabayashi M.; Wakabayashi H.; **Riegel A. D.**; Eisenreich Wolfgang; and Engel K.-H. Analytical and Sensory Characterization of the Stereoisomers of 3-Mercaptocycloalkanones and 3-Mercaptocycloalkanols.
J. Agric. Food Chem. **2020**, *68*, 7184–7193

Kiske C.; **Riegel A. D**.; Hopf R.; Kvindt A.; Poplacean I.; Taniguchi T.; Swamy M. M. M.; Monde K.; Eisenreich W.; Engel K.-H.; Determination of the Absolute Configurations and Sensory Properties of the Enantiomers of a Homologous Series (C6-C10) of 2-Mercapto-4-alkanones.
J. Agric. *Food Chem*. **2019**, *67*, 1187-1196.

ORAL PRESENTATIONS

257th American Chemical Society National Meeting, Chemistry of new Frontiers, Orlando, Florida, (US) **2019**
Analytical and sensory characterization of chiral β-mercaptoalkanones and β-mercaptoalkanols.

12th Wartburg Symposium on Flavour Chemistry & Biology (Eisenach, GER) **2019**
Structure-odor relationships of chiral β-mercaptoalkanones and β-mercaptoalkanols.