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Analysis and sensory evaluation of the stereoisomers of 4-mercapto-2-alkanols

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ABBREVIATIONS

AEDA	aroma extract dilution analysis
α	separation factor
BE-GC-LoADS	best estimated gas chromatography-lowest amounts detected sniffing
c	conversion rate
CAL-B	*Candida antarctica* lipase B
cAMP	adenosine 3,5-cyclic monophosphate
CD	cyclodextrin
Charme	combined hedonic response measurement
DCC	N,N'-dicyclohexylcarbodiimide
DMAP	4-dimethylaminopyridine
DMF	N,N-dimethylformamide
ee_p / ee_s	enantiomeric excess of product/substrate
EI	electron ionization
er	enantiomeric ratio
Et_2O	diethyl ether
FD factor	flavor dilution factor
FID	flame ionization detector
GC	gas chromatography
GC/O	gas chromatography/olfactometry
HPLC	high performance liquid chromatography
HTA	hydratropic acid
i.d.	internal diameter
LC	liquid chromatography
$LiAlH_4$	lithium aluminum hydride
LLE	liquid-liquid extraction
LOD	limit of detection
LOQ	limit of quantitation
LRI	linear retention index
MαNP	2-methoxy-2-(1-naphthyl)propionic acid
MCSS	moving column stream switching
MDGC	multidimensional gas chromatography
MPA	2-methoxy-2-phenylacetic acid
MS	mass spectrometry

$NaBH_4$	sodium borohydride
NaOH	sodium hydroxide
NMR	nuclear magnetic resonance spectroscopy
PPL	porcine pancreatic lipase
R_s	resolution
RT	room temperature
SD	absolute standard deviation
SDE	simultaneous distillation-extraction
SIM	single / selected ion monitoring
SPME	solid phase micro extraction
TBDMS	*tert*-butyl dimethylsilyl
THF	tetrahydrofuran
TIC	total ion chromatogram
TLC	thin layer chromatography
VCD	vibrational circular dichroism

1 INTRODUCTION AND OBJECTIVES

Polyfunctional thiols are known as outstanding contributors to the flavor of many foods because of their low odor thresholds and their pronounced odor qualities (Boelens and van Gemert, 1993; Mussinan and Keelan, 1994; Blank, 2002; Vermeulen *et al*., 2001, 2003 and 2005; Vermeulen and Collin, 2006). Their sensory properties not only depend on factors such as concentration, but also are determined by certain structural features. For instance, the "tropical olfactophore" based on a 1,3-oxygen-sulfur function relationship was emphasized as a descriptor for tropical, fruity, and vegetable odors (Rowe, 2002). Mercaptoalkanols and mercaptoketones such as 4-mercapto-2-pentanone and 4-mercapto-2-pentanol, tentatively identified in aged Cheddar cheese (Kleinhenz *et al*., 2006 and 2007), as well as 4-mercapto-2-heptanone, 4-mercapto-2-heptanol, their positional isomers, and 4-mercapto-2-nonanol, previously identified in cooked red bell pepper (Naef *et al*., 2008), are prominent examples.

The sensory assessment of racemic and diastereomeric mixtures of these *ß*-mercapto compounds in NaCl und sugar solutions resulted in fruity, green, vegetable, meaty, and synthetic notes (Naef *et al*., 2008). Comparable odor impressions were obtained for the diastereomers of 4-mercapto-2-alkanols (C5-C10) via capillary gas chromatography / olfactometry (GC/O) analysis. Odor thresholds determined at the sniffing port ranged from 0.072-12 ng/L in air; the lowest odor thresholds were obtained for the medium-chain homologs (Polster, 2012; Polster and Schieberle, 2017). Despite their interesting sensory properties, the impact of the configurations of these compounds has not been considered so far. The significance of this phenomenon for *β*-mercapto compounds has been indicated by a study demonstrating differences in the odor properties between the enantiomers of 4-mercapto-2-alkanones with carbon chain lengths of C5-C10 (Wakabayashi *et al*., 2011 and 2015).

Thus, the main objective of this thesis was to determine the odor qualities and odor intensities of the stereoisomers of 4-mercapto-2-alkanols. Homologs with carbon chain lengths of 5 to 10 (**1-6**) should be synthesized by Michael-type addition of thioacetic acid to the corresponding 3-alken-2-ones and subsequent reduction of the obtained 4-acetylthio-2-alkanones (**7-12**) (Naef *et al*., 2008; Wakabayashi *et al*., 2011 and 2015).

The four stereoisomers of each homolog should be separated via GC using chiral stationary phases, and GC orders of elution should be determined by assigning the absolute configurations.

In the first stage of research, the focus was based on the naturally occurring 4-mercapto-2-heptanol **3**. The C7 homolog should be used to determine the sensory properties of the four stereoisomers of a compound exhibiting the 1,3-oxygen-sulfur functionality. To this end, a chiral stationary phase suitable for the separation of the four stereoisomers of synthesized **3** had to be found and the absolute configurations of the stereoisomers of **3** had to be determined. Considering the suggestion that the "tropical olfactophore"-skeleton can be extended to the respective acetylthio-derivatives (Robert *et al*., 2004), the acetates 4-mercapto-2-heptyl acetate **13**, 4-acetylthio-2-heptyl acetate **14** and 4-acetylthio-2-heptanol **15** should be included into the assessment.
To determine the impact of the chain length and the stereochemistry on the odor thresholds and odor qualities of the stereoisomers of 4-mercapto-2-alkanols, the remaining homologs C5, C6, and C8 to C10 (**1**, **2**, **4-6**) should then also be investigated.

These analytical capabilities should finally be used to determine the naturally occurring distributions of the stereoisomers of 4-mercapto-2-heptanol **3** identified in cooked red bell peppers (*Capsicum annuum*) by Naef *et al*. (2008). The analysis was extended to the *β*-mercapto ketones 2-mercapto-4-heptanone **17** and 4-mercapto-2-heptanone **18** as well as to the stereoisomers of 2-mercapto-4-heptanol **19**.

2 BACKGROUND

2.1 Flavor Compounds

In addition to appearance and texture, flavor is one of the main sensory properties decisive in the selection and acceptance of foods by consumers (Fisher and Scott, 1997; Jeleń, 2011). According to DIN 10950-1:1999-04 (DIN, 1999), the term “flavor” refers to the overall sensation of smell, taste and tactile sensations evoked when consuming foods. Taste (sourness, sweetness, bitterness, saltiness and umami) and trigeminal sensations (pungency, astringency and cooling) are mainly caused by non-volatile, polar and water-soluble compounds upon contact of foods with the mouth (Fisher and Scott, 1997). For aroma sensations to occur, compounds have to fulfill certain molecular properties such as surface activity, low polarity, some water solubility, a high lipophilicity, a high vapor pressure and a molecular weight lower than 300 Dalton (Ohloff, 1994). They can reach the olfactory epithelium (Regio olfactoria) in the nasal cavity, either via the nasal (orthonasal perception) or the oral (retronasal perception) passage where they can activate olfactory receptors (Ohloff, 2004). Humans have ~ 400 intact receptors which were expressed by millions of sensory neurons (Hatt, 2004; Martin, 2013; Mainland *et al*., 2014). Thereby, each neuron expresses only one type of olfactory receptor. After binding, a G-protein-mediated stimulation of adenylyl cyclase induces the synthesis of adenosine 3,5-cyclic monophosphate (cAMP), this leads to an cAMP-mediated influx of Na^+ and Ca^{2+} ions through cyclic nucleotide-gated channels into the cell. Calcium-gated Cl^- channels are activated by the increased intracellular Ca^{2+} concentration, resulting in the depolarization of the cell membrane. Depending on the size, action potentials can be initiated and conducted along the axon of the olfactory cell into a region of the forebrain by passing the ethmoidal cribriform plate of bone. In the olfactory bulb, the axons of sensory neurons expressing the same type of odor receptors converge in so-called glomeruli. The olfactory signal is further transmitted via the mitral cells and the olfactory nerve tract to the primary olfactory cortex and from there to higher brain regions were the signaling process is decoded and olfactory interpretation and response occurs (Ohloff, 2004). It is estimated that humans are able to discrimate qualitatively about one trillion of different aroma impressions although having only about 400 different types of olfactory receptors (Niimura and Nei, 2003; Bushdid *et al*., 2014). Up to now, ligands for only about 10% of the human olfactory receptors have been identified (Mainland *et al*., 2014; Dunkel *et al*., 2014).

More data are necessary for a better understanding of the combinatorial receptor coding scheme which is used by the olfactory system for coding the identity of aroma compounds.

In foods, about 8,800 volatile compounds from different chemical classes (hydrocarbons, aldehydes, ketones, esters, acids, lactones, halogens, sulfur compounds, etc.) have been identified (Nijssen *et al*., 2017). It has been speculated that a total of 10,000 volatiles are to be expected (Rijkens and Boelens, 1975). However, according to a meta-analysis of Dunkel *et al*. (2014) less than 3% of the 10,000 volatiles are estimated to contribute to the overall aroma. This extrapolation was based on data obtained from 119 selected publications describing key food odorants of 227 different food products. The influence of each compound on the aroma of a food was estimated by calculating its odor activity value. According to Rothe and Thomas (1962), the odor activity value is the ratio of a compound's concentration to its odor threshold. Compounds with an odor activity value greater than or equal one are involved in the formation of the flavor, whereas compounds with an odor activity value lower than one are expected not to contribute to the overall aroma of the food.

Aroma compounds can be classified into so-called primary and secondary flavor substances. The first group comprises substances (mainly esters, alcohols, aldehydes and terpenes) which are generated via metabolic pathways (e.g. enzyme-catalyzed reactions) of living plants, fruits and vegetables. Secondary flavor compound arise from enzymatic, microbiologic and thermal conversion of respective non-volatile precursors during food production and processing (Matheis, 1991; Franzke, 1996).

Based on their abundance, odor-active volatiles can also be classified into generalists, intermediates and individualists. Generalists are widespread in food products whereas intermediates are less abundant. Representatives of individualists are known to impart characteristic aroma notes to selected foods. For example, the omission of the highly odor active thiols 4-mercapto-4-methylpentan-2-one and 1-*p*-menthene-8-thiol from a model mixture of a grapefruit juice containing 20 aroma-active compounds was recognized by the panelists. The model lacking 1-*p*-menthene-8-thiol was described as less fresh and grapefruit-like whereas an orange-like aroma was obtained for the model without 4-mercapto-4-methylpentan-2-one (Buettner and Schieberle, 2001).

In few cases, already one compound can mimic the smell of a whole food. Such compounds are called “character impact compounds” (Belitz *et al*., 2008). Examples are listed in Table 1.

Table 1. Character impact compounds in foods.

compound	odor description	occurrence
benzaldehyde	bitter almond	almond, cherry, plum
(*R*)-limonene	citrus	orange juice
1-(*p*-hydroxyphenyl)-3-butanone	raspberry	raspberry
(*R*)-(-)-1-octen-3-ol	mushroom	Camembert
(*E*,*Z*)-2,6-nonadienal	cucumber	cucumber

Belitz *et al*., 2008

2.1.1 Sulfur-Containing Volatiles

Sulfur-containing volatiles are characterized by low odor thresholds and strong penetrating odors which, depending on their concentrations, may contribute to the agreeable as well as to the disagreeable flavors of foods (Boelens and van Gemert, 1993; Franzke, 1996; Rowe *et al.*, 2004; McGorrin, 2011). Their important roles in flavor chemistry have been demonstrated in a variety of reviews (Maga, 1975a and 1975b; Maga, 1976; Shankaranarayana *et al*., 1982; Mussinan and Keelan, 1994; Blank, 2002; Vermeulen *et al*., 2005; McGorrin, 2011).

Already in the 1970´s, Maga summarized the roles of thiazoles, thiophenes and thiols as flavor compounds in foods (Maga, 1975a, 1975b and 1976). As reviewed by Rowe *et al*. (2004), the great interest in sulfur-containing compounds within the flavor and fragrance industry in the last decade was reflected by the increasing number, particularly of thiols, registered as ”generally regarded as safe” (GRAS) by the Flavor and Extract Manufacturers Association (FEMA). In 2002, Blank reviewed that the compilation of the Nutrition and Food Research Institute of the Netherlands (TNO) contains approximately 700 volatile organic sulfur compounds which have been found in edible products. This number corresponds to approximately 10% of all identified flavor substances (Blank, 2002).

Today, the number of sulfur-containing volatiles listed in the TNO publication website *Volatile Compounds in Foods* (VCF; www.vcf-online.nl) stands at over 900 (Nijssen *et al.*, 2017).

2.1.1.1 Thiols

Thiols belong to the most odorous aroma chemicals (Franzke, 1996; Rowe *et al.*, 2004). Their odor thresholds are often at the ppt level or even below. With thresholds in the range of pico- to femtogram levels, 4-methoxy-2-methyl-2-butanethiol, 1-*p*-menthen-8-thiol, 3-mercapto-3-methylbutyl formate and the (2*R*,3*S*)-configured stereoisomer of 3-mercapto-2-methylpentanol are typical examples among the most aroma-active odorants reported so far in literature (Table 2) (Demole *et al.*, 1982; Guth and Grosch, 1991; Blank *et al.*, 1992; Sabater Lüntzel *et al.*, 2000). Due to their high odor intensities, thiols play outstanding roles for the aroma of many foods even if they are only present at trace levels. So far, more than 100 volatile thiols have been identified in foods. Many of these thiols contain an additional functional group (e.g. alcohol, aldehyde, ketone or ester) and are therefore called polyfunctional thiols. Some representatives of this compound class are summarized in Table 2. A wide range of odor descriptions ranging from pleasant (e.g. fruity, vegetable-like, brothy, meaty) to unpleasant (e.g. sweaty, catty, rubber) odor notes have been determined for polyfunctional thiols (Vermeulen *et al.*, 2005; Vermeulen and Collin, 2006). Although most of them impart characteristic odor notes to foods, some polyfunctional thiols may be considered as off-flavors. For example, the roasted coffee note imparted by 2-furanmethanethiol contributes to the characteristic aroma of coffee. However, higher levels of this compound can cause an alteration of the typical coffee flavor, perceived as off-flavor (Tressl and Silwar, 1981). The skunky off-flavor of beer exposed to light is associated with 3-methyl-2-butene-1-thiol (Gunst and Verzele, 1978). Recently, this polyfunctional thiol has been detected in wine made from Prieto Picudo grapes in concentrations around its odor threshold (0.5-1 ng/L in wine). Its contribution to the overall aroma at this level was not yet clearly determinable as the thiol was described to impart herbal or fresh odor notes to the wine flavor on one hand and to reduce the fruity impression on the other hand when added in concentrations of 0.5 - 2 ng/L to wine with no analyte content (San-Juan *et al.*, 2012).

Table 2. Examples of polyfunctional thiols in foods.

compound	odor description	odor threshold	occurrence
4-methoxy-2-methyl-2-butanethiol	catty, blackcurrant-like[1], meaty[3]	0.00008-0.0003 ng/L in air[1]	olive oil[1], coffee[2], green tea[3], blackcurrant[4]
1-*p*-menthen-8-thiol	fresh grapefruit juice[5], grapefruit-like[6,7,30], sulfurous[7]	0.0001 µg/L in water (racemate)[5] 0.000034 ng/L in air (racemate)[30], 0.0000066 ng/L in air (*S*)[30], 0.000090 ng/L in air (*R*)[30]	grapefruit juice[5,6]
4-mercapto-4-methyl-2-pentanone	blackcurrant-like[6,7], catty[7], box tree[8], catty urine[8]	0.0001 µg/L in water (retronasal)[7], 0.066-0.165 ng/L in water[8]	grapefruit juice[6,7], Sauvignon wine[8]
3-mercapto-3-methylbutyl formate	sweaty, fruity, sweaty, catty, blackcurrant-like (~ 0.1 mg/L)[9], catty[11], roasty[11]	0.002-0.005 µg/L in water[9], 0.0002-0.0004 ng/L in air[11]	coffee[9,11], stored pale lager beer[10]
3-mercapto-2-methylpentanol	meat broth, onion, sweaty, leek (racemate at 0.5 ppb)[13], broth-like, sweaty, leek-like (enantiomers)[12]	0.15 µg/L in water (racemate)[13] 0.0007 µg/L in water (racemate)[14], > 12 µg/L in water (2*R*,3*R*)[12], > 30 µg/L in water (2*S*,3*S*)[12], 0.00007-0.0002 ng/L in air (2*R*,3*S*)[12], 0.003-0.007 ng/L in air (2*S*,3*R*)[12]	onion[13, 14], shallots[14], chives[14], scallions[14], leek[14]
4-mercapto-2-pentanone	grapefruit, sweet (*S*)[16], catty, fruity (*R*)[16]	0.99 ng/L in air (*S*)[15], 1.2 ng/L in air (*R*)[15]	aged Cheddar cheese[17]

Table 2 (continued).

4-mercapto-2-pentanol	broom, blackcurrant, catty (racemate)[18]	0.002 ng (BE-GC-LoADS)[18]	aged Cheddar cheese[17]
3-mercaptohexanol	grapefruit (rac., enantiomers)[19,20], grapefruit, citrus peel (*R*)[21], passion fruit (*S*)[21]	0.06 µg/L in water (racemate)[20], 0.07 ng/L in air (*S*)[19], 0.08 ng/L in air (*R*)[19]	guava[19,20], wine[21], yellow passion fruit[22]
2-furanmethanethiol	roasted coffee (0.01-0.5 ppb), staled coffee, sulfury (1-10 ppb)[23]	0.006 µg/L in water[24], 0.36 µg/L in water[25]	roasted coffee[23], popcorn[24,26], ham[27]
3-methyl-2-butene-1-thiol	skunky, coffee[28], marihuana, rubber, beer[29]	0.01 ng/L in water[29]	wine[29]

[1] Guth and Grosch, 1991; [2] Poisson *et al.*, 2005; [3] Kumazawa and Masuda, 1999; [4] Jung *et al.*, 2016; [5] Demole *et al.*, 1982; [6] Buettner and Schieberle, 1999; [7] Buettner and Schieberle, 2001; [8] Darriet *et al.*, 1995; [9] Holscher *et al.*, 1992; [10] Schieberle, 1991a; [11] Blank *et al.*, 1992; [12] Sabater Lüntzel *et al.* 2000; [13] Widder *et al.*, 2000; [14] Granvogl *et al.*, 2004; [15] Wakabayashi *et al.*, 2012; [16] Wakabayashi *et al.*, 2015; [17] Kleinhenz *et al.*, 2006; [18] Vermeulen *et al.*, 2003; [19] Steinhaus *et al.*, 2008; [20] Steinhaus *et al.*, 2009;[21] Tominaga *et al.*, 2006; [22] Engel and Tressl, 1991; [23] Tressl and Silwar, 1981; [24] Buttery *et al.*, 1997; [25] Czerny *et al.*, 2008; [26] Schieberle, 1991b; [27] Carrapiso *et al.*, 2002; [28] Gros and Collin, 2012; [29] San-Juan *et al.*, 2012; [30] Schoenauer and Schieberle, 2016

2.2 Correlation Between Structure and Sensory Properties of Thiols

Although thiols play outstanding roles for the aroma of many foods, the understanding of their extraordinary odor-activities is still in its infancy since systematic studies on their structure-odor relationships have scarcely been performed. An overview is given in the following chapter.

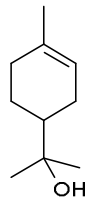
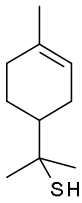
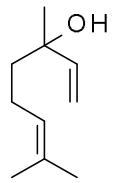
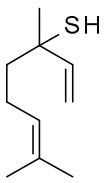
2.2.1 Influence of Thiol Exchange

One possibility to demonstrate the uniqueness of thiols is to compare their odor properties with those of structurally related oxygen-containing compounds. In many cases, the exchange of the hydroxyl group by a mercapto group results in remarkable changes of odor properties (Ohloff, 1994). Impressive examples such as the difference between α-terpineol and *p*-menthene-8-thiol have been described by Wannagat *et al*. (1987). As shown in Table 3a, the odor threshold of α-terpineol was by a factor of three million higher than that of *p*-menthene-8-thiol, respectively. This is in good agreement with the results of Schoenauer and Schieberle (2016) who showed that the odor thresholds of *p*-menthene-8-thiol and α-terpineol differed by factors of 51 and 86 million, respectively, for the (*R*)- and (*S*)-enantiomers. Apart from the thresholds, also the odor qualities changed. A floral-like odor reminiscent of lilac obtained for α-terpineol changed to a strong sulfur note for *p*-menthene-8-thiol which on dilution is characterized by a grapefruit-like aroma. A remarkable difference in the odor quality has also been reported when changing from linalool to thiolinalool.

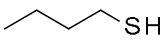
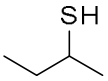
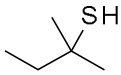
Sakoda and Hayashi (2002) subjected the results regarding odor qualities of the homologous series of 1- and 2-alkyl alcohols and thiols (C5–C11) to principal component analysis. The multivariate analysis resulted in a clear separation of alcohols and thiols, demonstrating that the panelists were able to distinguish between the two compounds classes. Moreover, within the compound classes, changes in the odor qualities between 1- and 2-alkyl compounds were detected. The analysis of 1-, 2- and 3-alkyl alcohols and thiols (C3/C4–C10) by GC/O revealed completely different threshold curves, whereas lower odor thresholds were obtained for the thiols with chain lengths up to C8 (Polster and Schieberle, 2011; 2015).

Table 3. Correlations between structures and sensory properties of thiols.

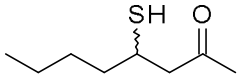
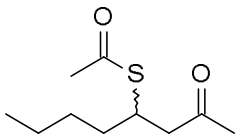
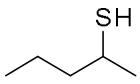
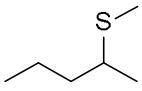
a) exchange of OH- by SH-group

α-terpineol		*p*-menthene-8-thiol	
	floral, sweet, lilac[1] 300 µg/L in water[2]		sulfury, nauseating (pure)[3], fresh grapefruit juice (1ppb)[4] 0.0001 µg/L in water[4]
linalool		thiolinalool	
	flowery, lavender, bergamot[3]		roasty, poultry (conc.)[5] fresh, fruity, bitter grapefruit (diluted)[5]

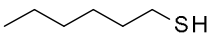
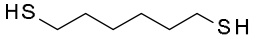
b) position of SH-group within the molecule

butane-1-thiol		0.7 µg/L in beer[6] 0.19 ng/L in air[7]	garlic, burned, sulfury[7]
butane-2-thiol		0.6 µg/L in beer[6] 0.035 ng/L in air[7]	garlic, burned, sulfury[7]
2-methylbutane-2-thiol		0.00007 µg/L in beer[6] 0.0023 ng/L in air[7]	burned, catty, sulfury[7]

c) blocking the SH-group

4-mercapto-2-octanone		4-acetylthio-2-octanone	
	0.09 ng/L in air (*R*)[8] 0.04 ng/L in air (*S*)[8]		24 ng/L in air (*R*)[8] 57 ng/L in air (*S*)[8]
pentane-2-thiol		2-(methylthio)pentane	
	0.0032 ng/L in air[7] burned, roasty, sulfury[7]		14 ng/L in air[7] garlic, sulfury, metallic[7]

d) introduction of an additional SH-group

hexane-1-thiol		hexane-1,6-dithiol	
	0.010 ng/L in air[7] burned, mushroom[7]		0.35 ng/L in air[7] cabbage, fatty[7]

[1] Bhatia *et al.*, 2008; [2] Buttery *et al.*, 1971; [3] Boelens, 1982; [4] Demole *et al.*, 1982; [5] Sigg-Grutter and Wild, 1974; [6] Meilgaard, 1975; [7] Polster and Schieberle, 2015; [8] Wakabayashi *et al.*, 2012

2.2.2 Influence of the Position of the Thiol Group Within Molecules

The odor properties of thiols also depend strongly on the position of the SH-group within the molecule. Already in 1975, Meilgaard showed that the tertiary thiol 2-methylbutane-2-thiol identified in beer has a much lower threshold than the corresponding secondary (butane-2-thiol) and primary (butane-1-thiol) homologs (Table 3b). This is in accordance with the results of Polster and Schieberle (2011 and 2015) who investigated homologous series (C3/C4–C10) of primary, secondary and tertiary thiols. They observed similar threshold pattern and threshold minima for medium-chain representatives of alkane-1-thiols, alkane-2-thiols, alkane-3-thiols and 2-methylalkane-2-thiols. However, at the minimum threshold levels (C5 and C6), the odor thresholds of the primary and secondary thiols were higher than those of the tertiary thiols. This might be explained by the hypothesis of Wang *et al*. (2003). They suggested that the Lewis basicity of the thiol group increases by a nearby substitution, and thus leads to a stronger binding effect of the volatile to the metal ions (copper (II) or zinc (II)) in the active center of the odor receptor. As a result, tertiary thiols often show lower odor thresholds than primary and secondary thiols.

The comparison of odor qualities of homologous series of primary, secondary and tertiary thiols revealed that similar odor notes were often detected for thiols with the same chain length. For example, garlic, burned and sulfury notes were detected for short chain thiols as shown in Table 3b using the example of 1- and 2-butanethiol. An additional catty odor, as it is characteristic for tertiary thiols, was perceived for 2-methyl-butane-2-thiol (Polak *et al*., 1988; Polster and Schieberle, 2011 and 2015).

The influence of the position of the SH-group in alkanols was investigated by Schellenberg (2002). GC/O analysis of 4- and 5-mercaptoalcohols (C5/C6 - C10/C11) revealed pleasant (e.g. green, tropical, melon-like) odor notes for compounds with the SH-group in ω4- and ω5-position and unpleasant (e.g. sulfury, burned) notes for compounds with the SH-group in ω2-, ω3-, ω6- and ω7-position. In both homologous series, the odor thresholds increased with increasing chain length, whereas lower thresholds were obtained for homologs having the SH-group in ω4-position. Recently, Schoenauer and Schieberle (2016) performed a structure-odor-activity study on monoterpenoid mercaptans. Their GC/O analyses of 1-*p*-menthene and *p*-menthane derivatives as well as of aromatic and open-chain mercapto monoterpenoids resulted in lower odor thresholds for derivatives having the SH-

group in the side chain compared to those having the SH-group attached at the ring, except for *p*-menthane-4-thiol having a tertiary thiol group.

2.2.3 Influence of Blocking the Free Thiol Group

Blocking the SH-group can lead to remarkable changes in odor properties. For example, the GC/O analysis of the homologous series (C5-C10) of 4-mercapto-2-alkanones and 4-acetylthio-2-alkanones revealed significantly higher odor thresholds for the acetylated compounds (Wakabayashi *et al*., 2012 and 2015). As illustrated for the C8 homolog, the odor thresholds of the enantiomers differed by factors of 260 for the (*R*)-enantiomer and by a factor of 1425 for the (*S*)-enantiomer (Table 3c).
The methylation of 1-, 2- and 3-alkane-thiols as well as of 2-methylalkane-2-thiols resulted in a clear decrease in odor intensities and changes in the odor qualities as exemplarily shown for pentane-2-thiol in Table 3c (Polster and Schieberle, 2015).

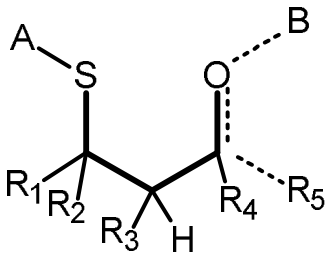
2.2.4 Influence of Additional Functional Groups

The presence of additional functional groups in the molecule can influence the sensory properties of thiols. This influence was recently investigated by Polster and Schieberle (2015) who compared odor thresholds of alkane-1-thiols (C3-C10) with those of the corresponding dithiols. The evaluation of the dithiols revealed the lowest odor thresholds for the short-chain representatives (C3-C5: 0.075-0.088 ng/L in air) and increasing values for the homologues with longer chain lengths. In contrast, the odor thresholds of the alkane-1-thiols decreased with increasing carbon number from C3 to C6 and increased after passing the minimum threshold level. At this minimum level, the greatest effect between the two compound classes has been detected. A 35 times higher odor intensity was perceived for hexane-1-thiol than for hexane-1,6-dithiol (Table 3d).
A decrease in odor strength was also mentioned by Wakabayashi *et al*. (2015) for 4-mercapto-2-alkanones (C5 and C6) after comparing the odor thresholds with those of corresponding alkane-thiols reported in literature.
In general, the existence of more than one functional group within molecules is often associated with increased odor thresholds (Ohloff, 1994).

2.2.5 Olfactophore Models

Further investigations on sulfur-containing compounds revealed the association of odor impressions with specific chemical structures (Dimoglo *et al.*, 1988; Rowe and Tangel, 1999; Rowe, 2002; Vermeulen *et al.*, 2003).

Fruity, tropical, and vegetable odor notes have been associated with numerous polyfunctional thiols possessing a 1,3-oxygen-sulfur function relationship (Rowe, 2002; Vermeulen *et al.*, 2003). Compounds fulfilling the essential structural feature of the so-called “tropical olfactophore” (Figure 1) are shown in Table 4.



A = H, SCH_3, ring
B = H, CH_3, acyl, absent if carbonyl
$\mathbf{R_1}$, $\mathbf{R_2}$ = H, alkyl
$\mathbf{R_3}$ = H, alkyl, ring
$\mathbf{R_4}$ = H, CH_3, ring, OR
$\mathbf{R_5}$ = H, absent if carbonyl

Figure 1. The “tropical olfactophore” according to Rowe, 2002.

Table 4. Examples of compounds showing the “tropical olfactophore”.

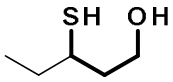
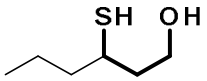
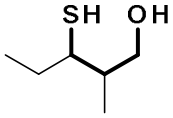
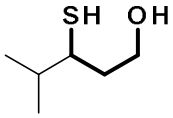
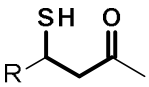
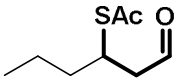
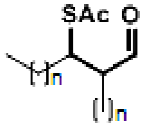
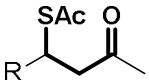
compound	structure	flavor description
3-mercapto-pentanol		citrus, sulfury, grapefruit[1]
3-mercapto-hexanol		grapefruit [2,3] (racemate and enantiomers)
3-mercapto-2-methyl-pentanol		meat broth, onion, sweaty, leek (racemate at 0.5 ppb)[4]
3-mercapto-4-methyl-pentanol		grapefruit, rhubarb (racemate)[5]
4-mercapto-2-alkanones (C5-C10)		fruity ((*S*)-enantiomers); catty vegetable, sulfury, peel oil ((*R*)-enantiomers)[6]

Table 4 (continued).

3-acetylthiohexanal		fruity, sweet, grapefruit ((*S*)-enantiomers); sulfury,citrus peel, roasted ((*R*)-enantiomers); sweet, grapefruit, citrus peel, (racemate)[7]
3-acetylthio-2-alkyl alkanals		fruity, tropical, grapefruit-like[8]
4-acetylthio-2-alkanones (C5-C10)		fruity, blackcurrant, citrus, bitter sweet (both enantiomers)[6]

[1] Sarrazin *et al.*, 2007; [2] Steinhaus *et al.*, 2008; [3] Steinhaus *et al.*, 2009; [4] Widder *et al.*, 2000; [5] Takoi *et al.*, 2009; [6] Wakabayashi *et al.*, 2015; [7] Wakabayashi *et al.*, 2003; [8] Robert *et al.*, 2004

The sensory evaluation of 3-acetylthiohexanal as well as of the series of 3-acetylthio-2-alkyl alkanals demonstrated for the first time that the “tropical olfactophore”-skeleton can be extended to acetylthio-compounds (Robert *et al.*, 2004; Wakabayashi, 2004). Moreover, the GC/O analysis of the enantiomers of 4-mercapto-2-alkanones and 4-acetylthio-2-alkanones (C5-C10) indicated that there might be specific stereochemical requirements that have to be taken into account in the establishment of olfactophore models (Wakabayashi *et al.*, 2015).

2.2.6 Impact of the Stereochemistry on Odor Properties

Chirality plays another important role in the olfactory perception of volatiles. To date, the sensory properties of more than 665 enantiomeric pairs have been described (Leffingwell, 2015). The sensory evaluations revealed that enantiomers can differ in their odor qualities and / or odor intensities. Great differences were also found for chiral sulfur-containing compounds. For example, the (*R*)-enantiomer of 1-*p*-menthene-8-thiol exhibited a strong grapefruit-like odor whereas a weak and non-specific odor was perceived for the (*S*)-enantiomer (Lehmann *et al.*, 1995). Different odor qualities have also been described for the esters of 3-(methylthio)hexanol which have been identified in yellow passion fruits (Engel and Tressl, 1991; Weber *et al.*, 1992). Fruity odor notes have been reported for the (*R*)-enantiomers, except for 3-(methylthio)hexyl hexanoate possessing a sulfury and cabbage-like odor note.

An intensive smell reminiscent of sulfur and vegetables (onion and cabbage) was perceived for the (*S*)-enantiomers. In contrast, similar odor qualities but different odor thresholds (up to a factor of 1000) were reported for the stereoisomers of 3-mercapto-2-methylpentanol as shown in Table 2 (Sabater Lüntzel *et al.*, 2000).

2.3 Analysis of Stereoisomers

2.3.1 Capillary Gas Chromatographic Analysis of Stereoisomers

Over the last decades there has been a substantial progress in the analysis of chiral compounds by capillary gas chromatography (GC). The first approaches have been based on the conversion of enantiomers into diastereomeric derivatives by reactions with chiral derivatization agents and the separation of the formed derivatives on achiral stationary phases (Bailey and Hass, 1941; Gil-Av and Nurok, 1962). Nowadays, this indirect method has been more and more replaced by the direct method since the use of chiral derivatization agents suffers from several drawbacks. Main disadvantages are the need for complete optical and chemical purity of the chiral derivatization agents, incomplete conversion reaction with the sample and longer sample preparation times (Souter, 1986; Schreier *et al*., 1995).

The use of chiral stationary phases is another possibility to provide an optically active environment needed for the separation of enantiomers. Based on the mode of selector-selectand interaction, chiral stationary phases can be divided into three main classes: 1) amino acid derivatives (hydrogen bonding), 2) metal complex phases (complexation) and 3) cyclodextrin phases (inclusion) (Schurig, 2001). The real breakthrough in enantioselective GC separation has been achieved with the introduction of cyclodextrin derivatives as chiral stationary phases (Smolkova *et al*., 1982; Kościelski *et al*., 1983; Juvancz *et al*., 1987; Schurig and Nowotny, 1988; König *et al*., 1989; Dietrich *et al*., 1992a and 1992b).

2.3.1.1 Cyclodextrin-Mediated Separations of Enantiomers

Cyclodextrins (CDs) are macrocyclic oligosaccharides with at least six and up to twelve α-D-glucopyranose units connected by α-1,4-glucoside bonds. The three smallest homologs, *α*- (n=6), *β*- (n=7) and *γ*-CD (n=8) are the major, industrially manufactured and applied CDs. The molecular structure of a *β*-CD is exemplarily shown in Figure 2A.

Based on the preferred chair conformation of the glucopyranose units, the free hydroxyl groups in position 2 and 3 (secondary OH-groups) are located on one rim of the cylinder and the hydroxyl groups in position 6 (primary OH-group) on the opposite rim (Figure 2B).

A **B**

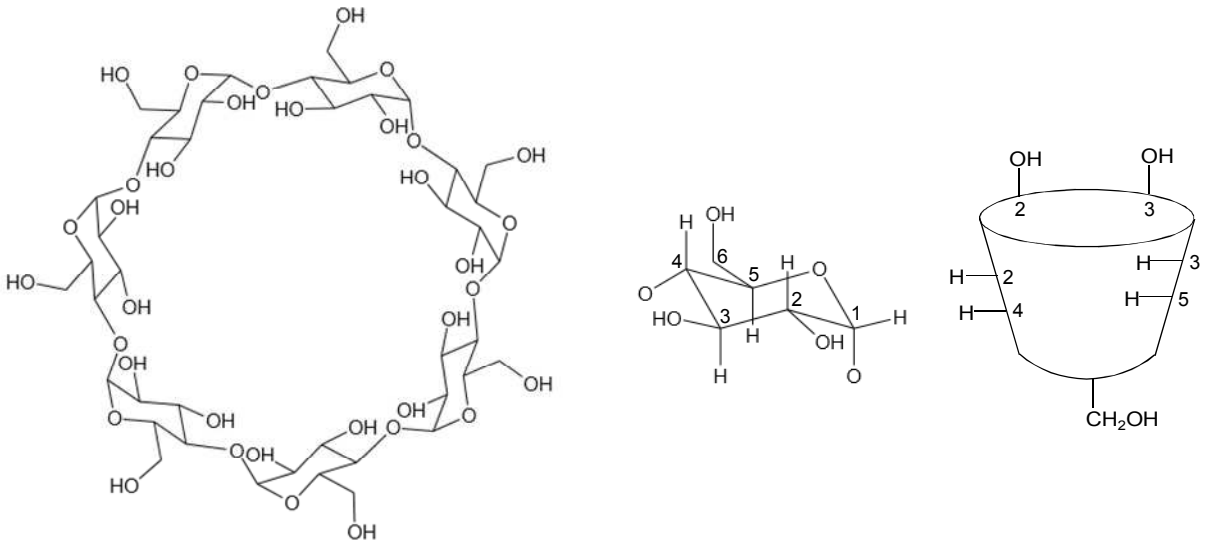


Figure 2. (**A**) Molecular structure of a *β*-cyclodextrin, (**B**) schematic view of the orientation of a glucopyranoside within a cavity model (modified after Dodziuk, 2006).

The truncated cone shape of the CDs is a result of the free rotation of the primary hydroxyl groups which reduces the diameter of the cavity (Schreier *et al*., 1995; Szejtli, 1998; Szente and Szemán, 2013). The presence of 18 (*α*-CD), 21 (*β*-CD) and 24 (*γ*-CD) substitutable hydroxyl groups was used to form more than fifty modified CD derivatives showing different chiral selectivities. The most frequently used CDs have been peralkylated, dialkylated or acetylated. Due to the fact that the mechanism of chiral recognition of CDs is still in its empirical stage, it is hard to predict which chiral stationary phase will separate a certain pair of enantiomers (Werkhoff *et al*., 1991; Juvancz and Petersson, 1996; Szejtli, 1998; Schurig 2001; Szente and Szemán, 2013). To overcome this problem, so-called binary or mixed CDs capable to resolve enantiomers of a wide variety of structurally diverse racemates have been investigated (Laub and Purnell, 1975; Nie *et al*., 2000; Kreidler *et al*., 2008).

As an example, Chiramix a column coated with a mixture of the two chiral stationary phases heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-*β*-cyclodextrin and octakis(2,6-di-*O*-methyl-3-*O*-trifluoroacetyl)-*γ*-cyclodextrin as shown in Figure 3 has been used by Tamogami *et al*. (2001).

A **B**

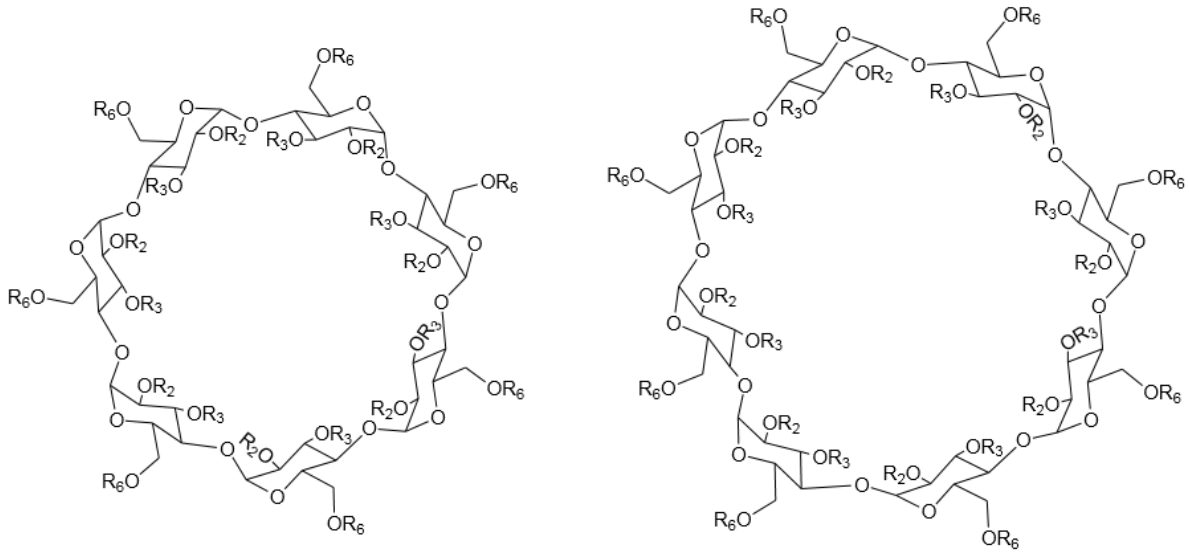


Figure 3. Molecular structures of the two chiral stationary phases used for the preparation of Chiramix. (**A**) Heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-*β*-cyclodextrin with R_2 = R_6= Me and R_3 = *n*-C_5H_{11}. (**B**) Octakis(2,6-di-*O*-methyl-3-*O*-trifluoroacetyl)-*γ*-CD with R_2 = R_6 = Me and R_3 = $COCF_3$. Modified after Akasaka *et al*., 2011.

The combination of the two chiral stationary phases enabled the simultaneous separation of enantiomers of different compound classes such as lactones and terpenes which could have been resolved on either heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-*β*-cyclodextrin or octakis(2,6-di-*O*-methyl-3-*O*-trifluoroacetyl)-*γ*-cyclodextrin (Bicchi *et al*., 1991; König *et al*., 1992; Bicchi *et al*., 1993; Tamogami *et al*., 2001 and 2004).

2.3.1.2 Enantioselective Multidimensional Gas Chromatography

Multidimensional gas chromatography (MDGC) is a suitable method for the isolation, identification and stereodifferentiation of volatile compounds present in complex mixtures. Separations in this system are realized by using two GC columns connected via a switching device as illustrated in Figure 4.

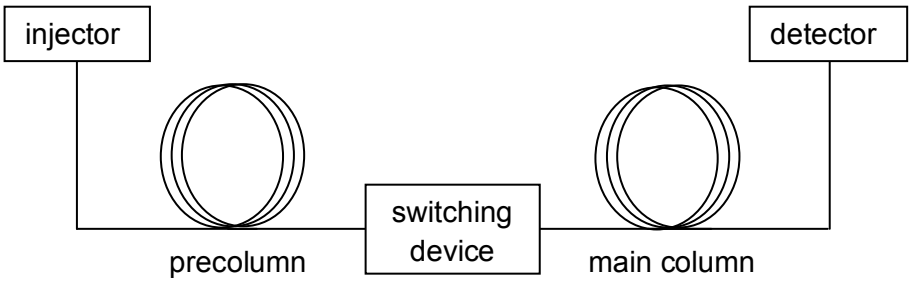


Figure 4. Schematic diagram of a MDGC system.

After a pre-separation of the compounds on the first column, selected compounds or fractions can be transferred via a so-called “heart-cut” using a switching device onto the main column where the compounds undergo further separation (Krammer *et al*., 1990). Employing a combination of an achiral precolumn and a chiral main column enables a rapid and simple stereodifferentiation of chiral compounds from complex mixtures without further sample clean-up, avoids the overlapping of peaks and the contamination of the main column (Werkhoff *et al*., 1991; Bicchi *et al*., 1999). Enantioselective MDGC is therefore widely used for determinations of naturally occurring enantiomeric compositions as well as for the authenticity control of samples from the food, flavor and fragrance industry (Hildenbrand *et al*., 1990; Werkhoff *et al*., 1991).

2.3.1.3 Sensory Analysis via Capillary Gas Chromatography/Olfactometry

Capillary gas chromatography/olfactometry (GC/O) describes a technique that uses the sensitivity of the human nose to determine the sensory relevance of volatile compounds based on parameters such as the odor quality, the odor quantity or the duration of the odor activity (Fuller *et al*. 1964; Blank, 1996; Delahunty *et al*., 2006).
It is carried out on a gas chromatograph equipped with a suitable capillary column and a column flow splitter to simultaneously perform sensory evaluation at the sniffing port and to record via a conventional detector (e.g. flame ionization detector (FID) or mass spectrometry (MS)) as shown in Figure 5.

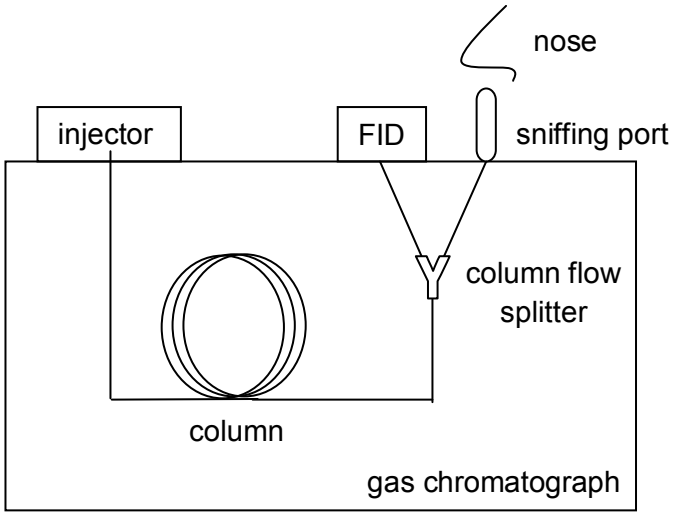


Figure 5. Schematic presentation of a GC/O system.

Several GC/O techniques are in practice. Depending on their principles, they can be classified into three categories: (i) detection frequency, (ii) dilution to threshold, and (iii) direct intensity (Delahunty *et al*., 2006). The detection frequency method is a simple technique where at least 10 panelists analyze the same extract by means of GC/O. The more often a compound at the same retention time is detected by panelists, the higher is its impact on the aroma profile (Linssen *et al*., 1993). The Charm-Analysis (combined hedonic response measurement) (Acree *et al*.,1984) as well as the aroma extract dilution analysis (AEDA) (Ullrich and Grosch, 1987) are common used dilution techniques. For both techniques a dilution series of an extract is prepared and analyzed by means of GC/O. In AEDA, the panelist sniffs one diluted extract after the other starting with the highest concentration until no odor is perceivable.

The odor activity of each compound is finally expressed as flavor dilution factor which represents its odor threshold at a given concentration. Using Charm-Analysis, the panelist evaluates the odor quality and the duration of each aroma-active compound in the diluted extracts which were presented in randomized order. The results are expressed as unitless "Charm values" and displayed as so-called Charm chromatograms. The chromatographic peaks are proportional to the amount of compound in the extract.
In comparison, the rarely used Osme technique (greek: *osme* meaning "smell") is not based on odor detection thresholds (McDaniel *et al*., 1990; Grosch, 2001). Assessors using this direct intensity method evaluate the odor quality as well as the intensity of aroma-active compounds at a defined concentration by using a computerized time-intensity device. Results are presented as so-called osmegrams in which the odor intensity is plotted versus the time (McDaniel *et al*.,1990; Miranda-Lopez *et al*., 1992).

In food aroma research, GC/O studies are used to determine aroma profiles of foods and beverages, to control odor changes in food due to processing techniques, to identify novel compounds with interesting odor characters from nature as well as to generate new flavors that “closely match natural products” (Delahunty *et al*., 2006; Brattoli *et al*., 2013). Another advantage of this technique is that it enables the detection and identification of aroma-active volatile compounds which are present at trace levels or even at concentrations lower than the detection limit of the conventional detector (Blank, 1996). For example, 1-*p*-menthene-8-thiol has been discovered by GC/O analysis as a new potent compound of grapefruit juice (Demole *et al*., 1982), and 2,3,6-trichloroanisole has been identified as off-flavor in green coffee (Spadone *et al*., 1990).
Furthermore, the use of chiral GC columns makes it possible to determine sensory properties of enantiomers and to give detailed information about their contribution to the overall aroma of investigated foods. For example, the determination of aroma-active compounds in cempedak and jack fruits revealed that the aroma difference between these two fruits might be caused by the occurrence of 2-(methylthio)butane and 2-(methylthio)pentane in cempedak fruits. Typical sulfury and cempedak-like odor notes and slight differences in the odor thresholds were determined for the enantiomers of 2-(methylthio)butane and 2-(methylthio)pentane by enantio GC/O analysis (Steinhaus and Grimm, 2015).

2.4 Peppers (*Capsicum*)

Peppers (*Capsicum* species) are among the first plants cultivated in the New World. Archeologists found remains of peppers in Mexico which were dated back to about 7,000 B.C. (Andrews, 1984). The introduction of "red pepper" (later classified as *Capsicum*) to Europe and subsequently to Africa and Asia is associated with the landing of Columbus in the Americas (Bosland and Votava, 1999). Today, pepper represents one of the most used condiments in the world (Kumar *et al*., 2006). Botanically, the pepper fruits are berries, but in general usage they are considered to be vegetables (Bosland *et al*., 1988). They are consumed as fresh and processed vegetables (e.g. cooked, pickled, sauces and beer) as well as in dried form (spice). They are known as an excellent source of vitamin A, C and E. In 1937, Albert Szente-Györgyi won the Nobel Prize i.a. for his discovery of ascorbic acid in Hungarian "paprika" peppers (Andrews, 1984; Teubner *et al*., 1993). Moreover, peppers are used as coloring and flavoring agents and as traditional and modern pharmaceuticals (for e.g. stimulating the flow of saliva and gastric juices, raising body temperature and reliving cramps) (Bosland and Votava, 1999; Kumar *et al*., 2006).

Peppers (*Capsicum* species) are classified among the large family *Solanaceae* that includes for example potato, tomato, eggplant, tobacco and petunia (Bosland *et al*., 1996). They are not related to black pepper (*Piper nigrum*) and Guinea pepper / grains of paradise (*Aframomum melegueta*) (Bosland, 1996; Bosland and Votava, 1999). The genus *Capsicum* (latin: *capsa* meaning "satchel" or greek: *kapto* meaning "to bite") is estimated to consist of twenty to thirty wild species, three to five semicultivated species and five domesticated species - *C. pubescens*, *C. baccatum*, *C. annuum*, *C. chinense* and *C. frutescens* (Andrews, 1984; Teubner *et al*., 1993; Bosland and Votava, 1999). The most widely spread and economically important domesticated species is *C. annuum* (Teubner *et al*., 1993; Kumar *et al*., 2006). Among the cultivars of this species, there is a tremendous phenotypic diversity in plant habit and fruit qualities such as pungency, size, color and shape (Bosland *et al.*, 1988; Kumar *et al*., 2006; Roth, 2014). Within the *Capsicum annuum* pod types, the majority of peppers of commerce are bell-type peppers (Bosland and Votava, 1999). Bell peppers, often so-called sweet peppers, are large, crisp and thick-walled fruits with a pleasant sweet taste and a slight pungency. A cross section of a bell-shaped pepper fruit is shown in Figure 6.

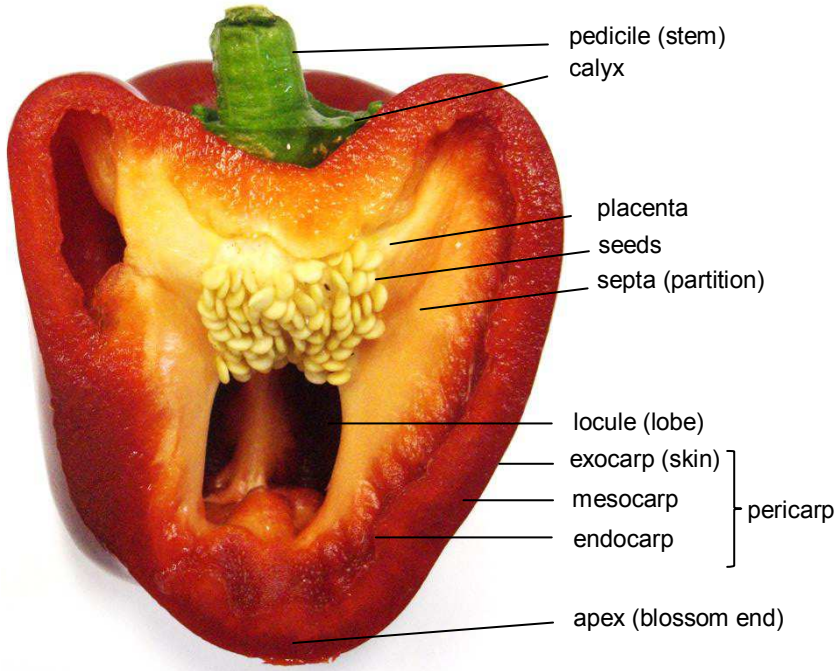


Figure 6. Cross section of a mature (red) fruit of the bell pepper cultivar California Wonder.

Depending on the level of ripeness, peppers (*Capsicum annuum*) are known to change their colors (Camara and Monéger, 1978; Bosland and Votava, 1999). The essential phenomenon taking place during maturation is the degradation of chlorophyll and the synthesis of carotenoids (Camara and Monéger, 1978; Gómez-Ladrón de Guevara *et al*., 2003). Up to 34 carotenoids have been identified in peppers of the species *Capsicum annuum* (Deli *et al*., 2001). The great variability in colors of the fruits is given by the combination of yellow and red carotenoids. Yellow pepper colors are primarily caused by *β*-carotene, lutein, zeaxanthin and violaxanthin. The color of red peppers is due to carotenoids such as capsanthin and capsorubin (Curl, 1962; Davies *et al*., 1970; Nagle *et al*., 1979; Deli *et al*., 2001). Figure 7 shows exemplarily three ripening stages – green, turning and red – of bell pepper fruits of the cultivar California Wonder. "California Wonder" or "Calwonder", introduced in the year 1928, is the oldest and most popular bell pepper cultivar (Andrews, 1984; Bosland and Votava, 1999).

A **B** **C**

Figure 7. Three ripening stages of bell pepper fruits of the cultivar California Wonder: (**A**) green, (**B**) yellow / orange, (**C**) red.

2.4.1 Volatile Compounds

Apart from color and pungency, aroma constitutes one of the most important quality parameter for *Capsicum* fruits (Luning *et al.*, 1994; Kollmannsberger *et al.*, 2007). According to Roth (2014), the majority of peppers are cultivated because of their outstanding flavors and not due to their sharpness. A great variability in flavor compositions depending on species, cultivar, maturation stage, tissues or usage (e.g. fresh, dried, homogenized and cooked) has been described in numerous publications. A comprehensive overview has been given by Lim (2013).

The analysis of 20 different cultivars of the five domesticated species revealed that cultivars of *Capsicum chinense* and *frutescens* have the highest contents of volatile compounds (Kollmannsberger, 2007). Their fruity/exotic flavor could be explained by a high contribution of esters and ionones and a lack of green, vegetable-like smelling volatiles (Rodríguez-Burruezo *et al.*, 2010). In contrast, powerful vegetable-like odors have been described for *Capsicum pubescens* fruits. The sensory analysis of the so-called rocotos revealed predominance of nitrogen and sulfur compounds (mainly pyrazines) as well as of aldehydes. A less intensive vegetable-like odor has been observed for *Capsicum baccatum* fruits due to a lower contribution of pyrazines and aldehydes and the presence of 2-heptanethiol and terpenoids (Kollmannsberger *et al.*, 2011).

Compared to other species, the total amount of volatiles of *Capsicum annuum* fruits was rather low (Kollmannsberger, 2007). However, the odor impressions of the fruits showed a great variability (Rodríguez-Burruezo *et al.*, 2010).

The sensory analysis of fruits of different cultivars of *Capsicum annuum* revealed fruity, vegetable-like, green, peasy, floral, and earthy notes (Chitwood *et al*., 1983; Kollmannsberger *et al*., 2007).

An overview of sensorially active compounds identified in different tissues of *Capsicum* fruits is given in Figure 8. An intensive smell of "green bell pepper" is associated with 2-methoxy-3-isobutylpyrazine. This pyrazine with an odor threshold of 0.002 ppb in water is considered as one of the most important constituents of *Capsicum* fruits (Buttery *et al*., 1969; Huffman *et al*., 1978; Rodríguez-Burruezo *et al*., 2010). During maturation and cooking, the so-called "bell pepper pyrazine" decreases or even disappears (Luning *et al*., 1994; Mazida *et al*., 2005; Pino *et al*., 2006; Elmore *et al*., 2010). The analysis of different tissues of fresh jalapeno peppers revealed an uneven distribution of 2-methoxy-3-isobutylpyrazine throughout the whole pod, the highest concentrations being determined in the outer wall (Huffman *et al*., 1978). Kollmannsberger (2007) also reported that the majority of 2-methoxy-3-isobutylpyrazine is located in the fruit flesh, especially near the apex (Figure 8).

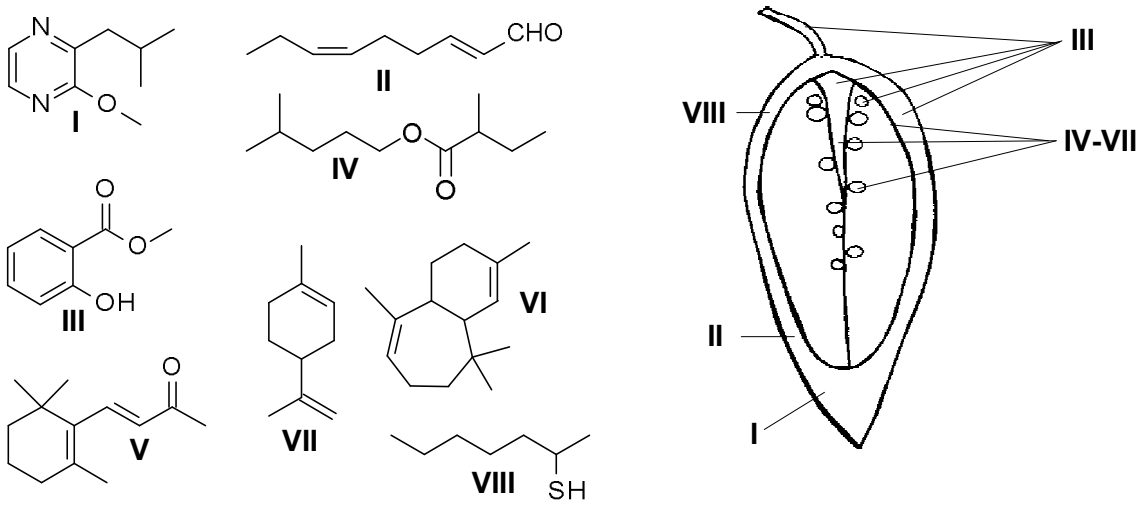


Figure 8. Sensorially active compounds identified in different tissues of *Capsicum* fruits (modified after Kollmannsberger, 2007).

I: 2-methoxy-3-isobutylpyrazine, II: (*E*,*Z*)-2,6-nonadienal, III: methylsalicylate, IV: 4-methylpentyl 2-methylbutanoate, V: β-ionone, VI: γ-himachalene, VII: limonene, VIII: 2-heptanethiol.

Lipoxygenase cleavage products such as hexanal as well as (*Z*)-, (*E*)-2-nonenal and (*E*,*Z*)-2,6-nonadienal with their green odor notes reminiscent of grass and cucumber were also shown to be located in the fruit flesh (Buttery *et al*., 1969; Kollmannsberger, 2007; Kollmannsberger *et al*., 2007).

The phenolic ester methyl salicylate with its potent sweet, green, and herbaceous odor (odor threshold: 40 ppb in water) was found as a major component in green bell peppers as well as in several cultivars of fully ripe red bell peppers (Buttery *et al*., 1969; Kollmannsberger *et al*., 2007). Its distribution within the fruit is not uniform. Differences among cultivars and tissues (stem, placenta/seeds, fruit flesh) have been reported (Kollmannsberger, 2007; Moreno *et al*., 2011). Esters, ionones and terpenoids possessing fruity/exotic and flowery odor notes were mainly found in placenta/seeds and septa (Kollmannsberger, 2007; Moreno *et al*., 2011). 4-Methylpentyl 2-methylbutanoate, β-ionone as well as the sequiterpene γ-himachalene and the monoterpene limonene are common representatives of these compound classes identified in *Capsicum* fruits (Kollmannsberger *et al*., 2007; Elmore *et al*., 2010; Rodríguez-Burruezo *et al*., 2010). 2-Heptanethiol, the first thiol identified in bell peppers, is mainly located in the fruit flesh, especially in the upper part near the stem (Simian *et al*., 2004; Kollmannsberger, 2007). Depending on the concentration, 2-heptanethiol is described as sulfury, onion, and mushroom-like in high concentrations (~ 1 mg/L) and as bell pepper, fruity, and vegetable-like when diluted (~ 0.1 mg/L). More pleasant attributes such as sweet, fruity, tropical, and floral were used by Sakoda and Hayashi (2002) to describe the odor of 2-heptanethiol. Orthonasal measurements resulted in the determination of an odor threshold of 10 µg/L in water (Simian *et al*., 2004).

2.4.1.1 Sulfur-Containing Compounds

The volatiles identified in *Capsicum* fruits belong to several chemical classes such as phenols, aldehydes, acids, ketones, alcohols, ethers, aromatic hydrocarbons, alkanes, esters and lactones. Nitrogen and sulfur-containing compounds are minor compound classes (Mateo *et al*., 1997; Kollmannsberger *et al*., 2007 and 2011; Rodríguez-Burruezo *et al*., 2010). A compilation of sulfur-containing compounds reported in *Capsicum* fruits is summarized in Table 5.

Table 5. Sulfur-containing compounds identified in *Capsicum* fruits.

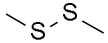
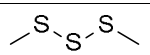
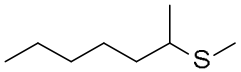
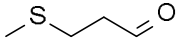
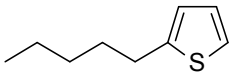
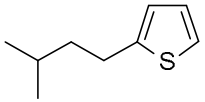
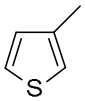
compound	structure	flavor description
sulfides		
dimethyl sulfide[1,2,7]		sulfury[10], tomato[9]
dimethyl disulfide[2,3]		cabbage-like[10]
dimethyl trisulfide[2,3,7]		sulfury[10], rotten, onion/leek[2,7], cooked cabbage[9]
2-heptyl methylsulfide[1]		no data found in literature
methional[4]		potato-like[4, 10], cooked potato[9]
thiophenes		
2-pentylthiophene[1,3]		moldy[1]
2-isopentylthiophene[7]		no data found in literature
3-methylthiophene[7]		fatty, winey[8]

Table 5 (continued).

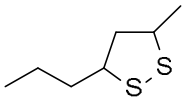
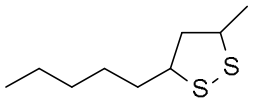
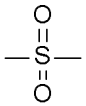
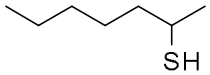
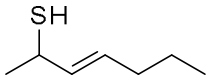
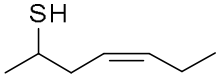
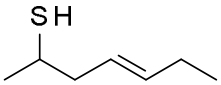
dithiolanes		
3-methyl-5-propyl-1,2-dithiolane[6]		very green, plastic, mustard, watercress, alliaceous (diastereomeric mixture, 2 ppm)[6]
3-methyl-5-pentyl-1,2-dithiolane[6]		rubbery, sulfury, green, rotten (2 ppm)[6]
sulfone		
dimethylsulfone[7]		sulfury, burned[11]
thiols		
2-heptanthiol[1,5,6]		bell pepper, fruity, vegetables (~ 0.1 mg/L); sulfury, onion, mushroom (~ 1.0 mg/L)[5]; sweet, fruity, tropical and floral[12]
(*E*)-3-heptene-2-thiol[6]		sesame, green, bell peppers, citrus, fresh (0.1 ppm)[6]
(*Z*)-4-heptene-2-thiol[6]		seanut, sesame, green coffee beans (10 ppm)[6]
(*E*)-4-heptene-2-thiol[6]		seanut, sesame, coffee, bitterness of peppers (2 ppm)[6]

Table 5 (continued).

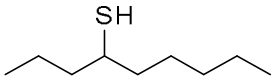
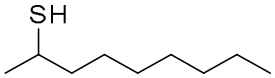
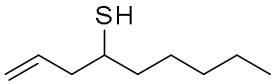
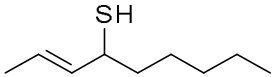
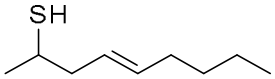
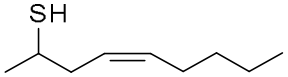
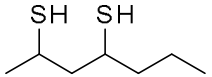
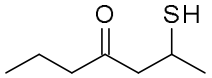
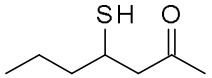
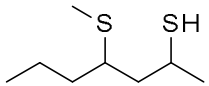
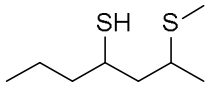
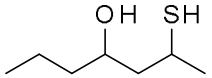
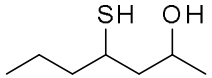
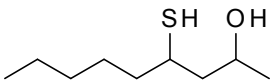
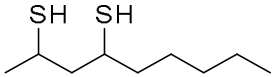
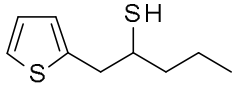
4-nonanethiol[6]		sulfury, fruity, sweaty, fatty (0.3 ppm)[6]
2-nonanethiol[6]		roasted, roasted chicken, sesame, lettuce (0.5 ppm)[6]
1-nonene-4-thiol[6]		berry, green, fruity, tropical fruit (0.2 ppm)[6]
(*E*)-2-nonene-4-thiol[6]		green, vegetal, weak (50 ppm)[6]
(*E*)-4-nonene-2-thiol[6]		green, mushroom, rubbery (1 ppm)[6]
(*Z*)-4-nonene-2-thiol[6]		fruity, green, vegetal, mushroom (2 ppm)[6]
2,4-heptane-dithiol[6]		spring onion, green, alliaceous (50 ppm)[6]
2-mercapto-4-heptanone[6]		grapefruit, sesame, earthy, rocket (0.5 ppm)[6]
4-mercapto-2-heptanone[6]		green, peely, vegetable (0.5 ppm)[6]; grapefruit ((*S*)-enantiomer), slightly catty, grapefruit peel ((*R*)-enantiomer)[14]

Table 5 (continued).

4-methylthio-2-heptanethiol[6]		fruity, minty, green, rhubarb, tropical fruit, alliaceous (mixture with 2-methylthio-4-heptanethiol, 1 ppm)[6]
2-methylthio-4-heptanethiol[6]		fruity, minty, green, rhubarb, tropical fruit, alliaceous (mixture with 4-methylthio-2-heptanethiol, 1 ppm)[6]
2-mercapto-4-heptanol[6]		fruity, tropical, guava, watercress, vegetal (diastereomeric mixture, 0.5 ppm)[6]
4-mercapto-2-heptanol[6]		onion, liver, meaty, sweaty, resinous (diastereomeric mixture, 50 ppm)[6]; grapefruit (each diastereomer)[15]
4-mercapto-2-nonanol[6]		synthetic, rubbery, unpleasant (isomer 1, 50 ppm)[6]; rhubarb, sweat (isomer 1), rhubarb, mushroom (isomer 2)[13]; grapefruit, fatty (each diastereomer)[15]
2,4-nonane-dithiol[6]		rotten, melon, chemical (0.5 ppm)[6]
1-(2-thienyl)-2-pentanethiol[6]		tropical, passion fruit, common, weak (1 ppm)[6]

[1] Kollmannsberger, 2007; [2] van Ruth and Roozen, 1994; [3] Luning *et al.*, 1994; [4] Zimmermann and Schieberle, 2000; [5] Simian *et al.*, 2004; [6] Naef *et al.*, 2008; [7] van Ruth *et al.*, 2003; [8] Flament, 1991; [9] Blank, 2002; [10] Rychlik *et al.*, 1998; [11] Tunick, 2014; [12] Sakoda and Hayashi, 2002; [13] Vermeulen and Collin, 2006; [14] Wakabayashi *et al.*, 2015; [15] Polster, 2012

Dimethyl disulfide, dimethyl trisulfide and 2-pentylthiophene were reported in fresh Mazurka bell peppers at three different ripening stages (green, turning and red) by Luning *et al*. (1994). Sulfides such as dimethyl sulfide, dimethyl disulfide and dimethyl trisulfide were identified in commercially dried red bell peppers (*Capsicum annuum*) from Chile, Hungary and Turkey after rehydration (van Ruth and Roozen, 1994). Methional with its potato-like odor was described as one of the most important odor-active volatile compound in Hungarian and Moroccan sweet bell pepper powders (Zimmermann and Schieberle, 2000). Two sulfides (dimethyl disulfide and dimethyl trisulfide), two thiophenes (3-methylthiophene and 2-isopentylthiophene) as well as dimethylsulphone were found by the GC-MS analysis of dried red bell peppers from Turkey (van Ruth *et al*., 2003). According to Simian *et al*. (2004) 2-heptanethiol was the first thiol which has been reported as flavor compound of bell peppers. The highest content was found in a cooked red bell pepper extract. The analysis of 20 different cultivars of the five domesticated species via solid phase micro extraction (SPME) yielded four different sulfur-containing volatiles: dimethyl sulfide, 2-pentylthiophene, 2-heptanethiol and 2-heptyl methylsulfide (Kollmannsberger, 2007). More recently, 19 new thiols (nine aliphatic (un)saturated thiols, two mercapto-ketones, three mercapto-alcohols, two dithiols, two methylthio-thiols) and two dithiolanes have been identified in red bell peppers (*Capsicum annuum*) after isolation via simultaneous distillation-extraction (SDE) and subsequent thiol-enrichment via Affi-Gel 501 (Naef *et al*., 2008). The sensorial evaluation of the identified heptane and nonane derivatives in NaCl (0.3%) and sugar (0.5%) solutions revealed a wide range of different odor descriptions which include fruity, green, vegetable-like, meaty, and in some cases rotten, synthetic, chemical, and rubbery notes (Table 5). The formation of some of these polyfunctional thiols such as the mercapto-ketones could be explained by the formal addition of H_2S, most probably introduced by cysteine, to *α*,*β*-unsaturated ketones. Additional reduction steps would finally lead to the synthesis of unsaturated thiols, mercapto-alcohols, methylthio-thiols and dithiols. The natural occurrence of cysteine-*S*-conjugates as non-volatile precursors of the two mercapto-ketones 2-mercapto-4-heptanone and 4-mercapto-2-heptanone as well as the corresponding mercapto-alcohols 2-mercapto-4-heptanol and 4-mercapto-2-heptanol was confirmed by Starkenmann and Niclass (2011).

The cysteine-*S*-conjugates identified included *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. Their structures are shown in Figure 9.

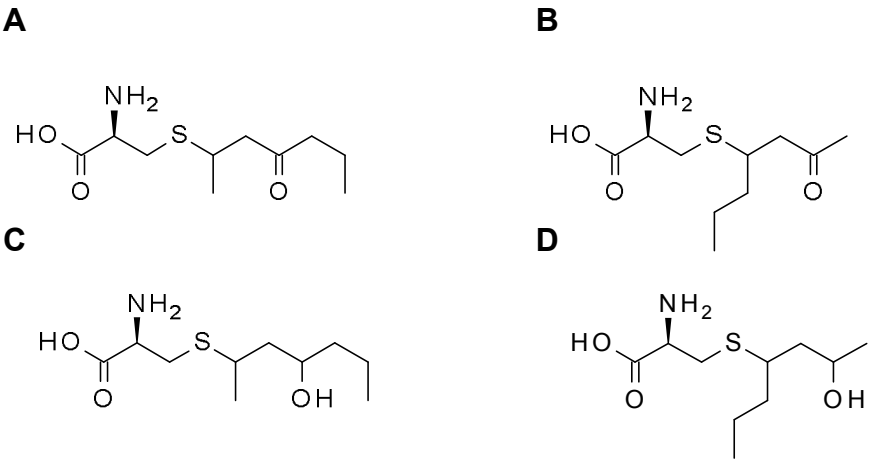


Figure 9. Structures of cysteine-*S*-conjugates identified in bell peppers (*Capsicum annuum* L. cultivar). (**A**) *S*-(3-oxo-1-methylhexyl)-*L*-cysteine, (**B**) *S*-(3-oxo-1-propylbutyl)-*L*-cysteine, (**C**) *S*-(3-hydroxy-1-methylhexyl)-*L*-cysteine, (**D**) *S*-(3-hydroxy-1-propylbutyl)-*L*-cysteine.

Quantitative estimations revealed concentrations in green and red bell peppers in a range of 3-108 µg/kg. The incubation of the purified cysteine-*S*-conjugates with a *β*-lyase (Apotryptophanase from *Escherichia coli*) enabled the liberation of the expected thiols (Starkenmann and Niclass, 2011).

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals

The following chemicals were used:

Acetic anhydride (98%)	Sigma-Aldrich, Steinheim, Germany
Acetone	AppliChem, Darmstadt, Germany
Acetyl chloride (p.a., ≥ 99.9% (T))	Sigma-Aldrich, Steinheim, Germany
Affi-Gel 10	Bio-Rad Laboratories, Munich, Germany
n-Alkane standard solutions (C8-C20 and C21-C40)	Sigma-Aldrich, Steinheim, Germany
p-Aminophenylmercuric acetate (≥ 90% titration)	Sigma-Aldrich, Steinheim, Germany
Ammonium chloride (p.a.)	VWR, Darmstadt, Germany
Ammonium sulfate (technical, ≥ 99%)	Sigma-Aldrich, Steinheim, Germany
Celite 503	Sigma-Aldrich, Steinheim, Germany
(*E*)-2-Decenal (≥ 95%)	Sigma-Aldrich, Steinheim, Germany
3-Decen-2-one (≥ 97%)	SAFC, Buchs, Switzerland
Deuterated chloroform (99.96 atom% D)	Sigma-Aldrich, Steinheim, Germany
Dichloromethane (p.a., ≥ 99.9% GC)	Sigma-Aldrich, Steinheim, Germany
N,*N'*-Dicyclohexylcarbodiimide	Sigma-Aldrich, Steinheim, Germany
Diethyl ether (p.a., distilled prior to use)	Merck, Darmstadt, Germany
4-Dimethylaminopyridine (99%)	Sigma-Aldrich, Steinheim, Germany
N,N-Dimethylformamide (≥ 99.8%, ACS reagent)	Sigma-Aldrich, Steinheim, Germany
DL-Dithiothreitol (≥ 99% titration)	Sigma-Aldrich, Steinheim, Germany
Ethanol (≥ 99.5%)	Merck, Darmstadt, Germany
Ethanolamine (p.a.)	Merck, Darmstadt, Germany
Ethyl 3-oxobutanoate	Frey & Lau GmbH, Henstedt-Ulzburg, Germany
3-Hepten-2-one (> 96%)	TCI Europe, Zwijndrecht, Belgium
n-Hexane (95%, AnalaR Normapur)	VWR, Darmstadt, Germany
n-Hexane (HPLC grade)	VWR, Darmstadt, Germany

Hydrochloric acid (min. 25%, puriss p.a.)	Sigma-Aldrich, Steinheim, Germany
Lithium aluminum hydride	Sigma-Aldrich, Steinheim, Germany
Methanol (HiPersolv Chromanorm)	VWR, Darmstadt, Germany
(*S*)-(+)-2-methoxy-2-(1-naphthyl)propionic acid	TCI Europe, Zwijndrecht, Belgium
3-Nonen-2-one (95%)	Sigma-Aldrich, Steinheim, Germany
3-Octen-2-one (97%)	Alfa Aesar, Karlsruhe, Germany
3-Oxobutanoic acid	Sigma-Aldrich, Steinheim, Germany
n-Pentane (AnalaR Normapur)	VWR, Darmstadt, Germany
3-Penten-2-one (≥70 %)	Sigma-Aldrich, Steinheim, Germany
Potassium dihydrogen phosphate	Riedel-de Haën, Seelze, Germany
di-Potassium hydrogen phosphate trihydrate	Merck, Darmstadt, Germany
Propanal (≥ 97%)	Sigma-Aldrich, Steinheim, Germany
2-Propanol (Ph. Eur.)	VWR, Darmstadt, Germany
2-Propanol (HPLC grade)	VWR, Darmstadt, Germany
Pyridine (p.a.)	Merck, Darmstadt, Germany
Tetrahydrofuran (anhydrous, ≥ 99.9%)	Sigma-Aldrich, Steinheim, Germany
Thioacetic acid (96%)	Sigma-Aldrich, Steinheim, Germany
Silica gel (NormaSil 60, 40-63 µm)	VWR, Darmstadt, Germany
Sodium borohydride (p.a., > 96% gas-volumetric)	Fluka, Buchs, Switzerland
Sodium hydrogen carbonate (purum p.a., ≥ 99.9%)	Fluka, Buchs, Switzerland
Sodium hydroxide (≥ 97.0%, pellets)	Sigma-Aldrich, Steinheim, Germany
Sodium sulfate (anhydrous)	VWR, Darmstadt, Germany
Sulfuric acid (95-98%, extra pure)	Merck, Darmstadt, Germany

3.1.2 Enzymes

The following lipases were purchased from Sigma-Aldrich (Steinheim, Germany):

(1) *Candida antarctica* lipase B
≥ 5.000 U/g, recombinant, expressed in *Aspergillus niger*, adsorbed on a macroporous acrylic resin (CAL-B), L4777

(2) Lipase from porcine pancreas
type II, 30-90 U/mg, (PPL), L3126

3.1.3 Bell Pepper (*Capsicum annuum*)

Red and green bell peppers (*Capsicum annuum*) of the cultivar California Wonder were purchased in a local store. Information on origin (according to the labeling), dates of purchase, color and the employed isolation method is presented in Table 6. The bell peppers were stored at 8 °C prior to analysis.

Table 6. Investigated batches of bell peppers.

batch	origin	date of purchase	color	method
1	Almeria, Spain	04/22/2014	red	SDE[a]
2	unknown, Spain	04/28/2014	red	SDE[a]
3a	Murcia, Spain	05/08/2014	red	SDE[a]
3b	Murcia, Spain	05/13/2014	green	SDE[a]
4a	Neufahrn, Germany	05/30/2014	red	SDE[a]
4b	Neufahrn, Germany	05/30/2014	red	LLE[b]
4c	Neufahrn, Germany	06/18/2014	red	LLE[b]
5	unknown, Netherlands	05/22/2014	green	SDE[a]

[a] Simultaneous distillation-extraction

[b] Liquid-liquid extraction

3.2 Syntheses

3.2.1 4-Acetylthio-2-alkanones

A homologous series (chain lengths C5–C10) of 4-acetylthio-2-alkanones **7-12** were synthesized by Michael-type addition of thioacetic acid to 3-alken-2-ones in analogy to the method described by Wakabayashi *et al*. (2011 and 2015).

A mixture of 3-alken-2-one and thioacetic acid was stirred for 1 h under ice cooling and subsequently overnight at room temperature (RT). The crude products were obtained after removing the excess of thioacetic acid under reduced pressure using an aspirator at 40 °C (Table 7).

Table 7. Syntheses of 4-acetylthio-2-alkanones **7-12**.

no.	3-alkene-2-one	thioacetic acid	yield			purity
	[mmol]	[mmol]	[g]	[mmol]	mol [%]	GC (%)
7	11.86	17.79	1.87	11.68	96	97
8	19.63	29.45	3.03	17.41	88	98[a]
9	19.79	26.69	4.01	21.32	107	92
10	11.92	17.88	2.63	13.01	109	83
11	11.86	17.79	2.53	11.69	98	96
12	25.05	37.58	6.19	26.90	108	88

[a] 4-Acetylthio-2-hexanone was purified by column chromatography on silica gel (silica gel 60, 0.063-0.200 mm, Merck, Germany) by elution with a mixture of *n*-hexane and diethyl ether (Et_2O) (4:1, v/v). The fraction was checked by thin layer chromatography (TLC, ALUGRAM SIL G/UV254, Macherey-Nagel, Germany) and visualization was achieved by spraying with 10% sulfuric acid and subsequent heating until dryness.

3.2.2 4-Mercapto-2-alkanols

4-Mercapto-2-alkanols with carbon chain lengths of C5-C10 (**1-6**) were prepared in analogy to the previously described procedure by Naef *et al*. (2008). The synthesized 4-acetylthio-2-alkanones (6.63 mmol for **1**, 5.74 mmol for **2**, 10.60 mmol for **3**, 3.11 mmol for **4**, 6.93 mmol for **5** and 12.93 mmol for **6**) dissolved in 20 mL of dry tetrahydrofuran (THF) were slowly added to a suspension of lithium aluminum hydride ($LiAlH_4$: 29.84 mmol for **1**, 25.83 mmol for **2**, 47.70 mmol for **3**, 14.01 mmol for **4**, 31.19 mmol for **5** and 58.19 mmol for **6**) in 50 mL of dry THF under argon atmosphere at 0 °C. The reaction mixture was stirred at RT overnight.

After being cooled to 0 °C, distilled water was carefully added and the aqueous phase was adjusted to pH 2 using hydrochloric acid (5%) and extracted three times with dichloromethane. The organic phase was dried with anhydrous sodium sulfate and evaporated under reduced pressure to give the following crude products:

4-Mercapto-2-pentanol, **1**: 0.41 g (3.41 mmol, mol yield: 51%, purity: 76% by GC (ratio of diastereomers, 37:63), purity after column chromatography on silica gel (*n*-hexane/Et_2O, 3:2, v/v): 96% (by GC). Linear retention indices (LRI) determined by GC: 1573 (*anti*), 1599 (*syn*) on DB-Wax; 915 (*anti*), 927 (*syn*) on DB-1. GC-MS (*m/z*, rel.%) (both isomers show the same fragmentation): 45 (100), 86 (44), 61 (38), 71 (37), 43 (32), 41 (30), 60 (27), 69 (23), 42 (20), 59 (13), 120 (M^+, 6). ^{1}H-NMR (500 MHz, $CDCl_3$): *δ* 4.02 (dqd, *J* = 9.3, 6.2, 3.0 Hz, 1H, H-2$_{anti}$), 3.89 (dqd, *J* = 7.6, 6.2, 5.0 Hz, 1H, H-2$_{syn}$), 3.16-3.06 (m, 1H, H-4$_{anti}$), 2.98 (hept, *J* = 7.1 Hz, 1H, H-4$_{syn}$), 1.67-1.61 (m, 2H, H-3$_{syn}$), 1.47-1.40 (m, 2H, H-3$_{anti}$), 1.33-1.30 (m, 6H,H-5$_{anti+syn}$), 1.16 (d, *J* = 6.2 Hz, 3H, H-1$_{anti}$), 1.14 (d, *J* = 7.2 Hz, 3H, H-1$_{syn}$). ^{13}C NMR (126 MHz, $CDCl_3$): *δ* 66.84 (C-2$_{syn}$), 65.77 (C-2$_{anti}$), 49.95 (C-3$_{syn}$), 49.47 (C-3$_{anti}$), 33.18 (C-4$_{syn}$), 32.49 (C-4$_{anti}$), 26.62 (C-5$_{anti}$), 26.24 (C-5$_{syn}$), 24.01 (C-1$_{anti}$), 23.79 (C-1$_{syn}$).

4-Mercapto-2-hexanol, **2**: 0.83 g (6.17 mmol, mol yield: 108%, purity: 84% by GC (ratio of diastereomers, 42:58), LRI: 1652 (*anti*), 1671 (*syn*) on DB-Wax; 1017 (*anti*), 1028 (*syn*) on DB-1. GC-MS (*m/z*, rel.%): 45 (100), 71 (58), 100 (48), 55 (47), 41 (47), 43 (31), 83 (28), 56 (28), 74 (27), 75 (21), 134 (M^+, 5). ^{1}H-NMR (500 MHz, $CDCl_3$): *δ* 4.08 (dqd, *J* = 9.2, 6.2, 2.8 Hz, 1H, H-2$_{anti}$), 3.94 (dqd, *J* = 7.8, 6.2, 4.5 Hz, 1H, H-2$_{syn}$), 2.94-2.86 (m, 1H, H-4$_{anti}$), 2.77-2.69 (m, 1H, H-4$_{syn}$), 1.73-1.39 (m, 8H, H-3$_{anti+syn}$, H-5$_{anti+syn}$), 1.16 (d, *J* = 6.2 Hz, 3H, H-1$_{anti}$), 1.14 (d, *J* = 6.2 Hz, 3H, H-1$_{syn}$), 0.95 (td, *J* = 7.3, 2.8 Hz, 6H, H-6$_{anti+syn}$). ^{13}C NMR (126 MHz, $CDCl_3$): *δ* 67.10 (C-2$_{syn}$), 66.50 (C-2$_{anti}$), 47.71 (C-3$_{syn}$), 47.31 (C-3$_{anti}$), 40.65 (C-4$_{syn}$), 39.48 (C-4$_{anti}$), 32.80 (C-5$_{anti}$), 32.46 (C-5$_{syn}$), 24.08 (C-1$_{anti}$), 23.61 (C-1$_{syn}$), 11.49 (C-6$_{anti}$), 11.24 (C-6$_{syn}$).

4-Mercapto-2-heptanol, **3***:* 1.54 g (10.40 mmol, mol yield: 97%, purity: 96% by GC (ratio of diastereomers, 39:61), LRI: 1751 (*anti*), 1768 (*syn*) on DB-Wax; 1109 (*anti*), 1118 (*syn*) on DB-1; 1279 (*anti*), 1305 (*syn*) on RTX-200. GC-MS (*m/z*, rel.%): 55 (100), 45 (95), 71 (71), 43 (44), 114 (40), 41 (29), 97 (28), 87 (25), 61 (22), 70 (20), 148 (M^+, 6). ^{1}H NMR (500 MHz, $CDCl_3$): *δ* 4.07 (dtd, *J* = 12.4, 6.2, 2.8 Hz, 1H, H-2$_{anti}$), 3.94 (dqd, *J* = 7.8, 6.2, 4.6 Hz, 1H, H-2$_{syn}$), 3.01-2.94 (m, 1H, H-4$_{anti}$), 2.79 (qt, *J* = 7.9, 5.2 Hz, 1H, H-4$_{syn}$), 1.73-1.29 (m, 12H, H-3$_{anti+syn}$, H-5$_{anti+syn}$, H-6$_{anti+syn}$), 1.16

(d, *J* = 6.2 Hz, 3H, H-1$_{anti}$), 1.13 (d, *J* = 6.2 Hz, 3H, H-1$_{syn}$), 0.85 (t, *J* = 7.1 Hz, 6H, H-7$_{anti+syn}$). ^{13}C NMR (126 MHz, $CDCl_3$): *δ* 67.43 (C-2$_{syn}$), 65.85 (C-2$_{anti}$), 48.65 (C-3$_{syn}$), 48.18 (C-3$_{anti}$), 42.43 (C-5$_{anti}$), 42.16 (C-5$_{syn}$), 39.07 (C-4$_{syn}$), 37.88 (C-4$_{anti}$), 24.46 (C-1$_{anti}$), 24.00 (C-1$_{syn}$), 20.54 (C-6$_{anti}$), 20.37 (C-6$_{syn}$), 14.15 (C-7$_{anti}$), 14.12 (C-7$_{syn}$).

4-Mercapto-2-octanol, **4**: 0.49 g (3.02 mmol, mol *y*ield: 97%, purity: 89% by GC (ratio of diastereomers, 47:53), purity after column chromatography on silica gel (*n*-hexane/Et_2O, 5:3, v/v): 96% by GC. LRI: 1847 (*anti*), 1862 (*syn*) on DB-Wax; 1209 (*anti*), 1218 (*syn*) on DB-1. GC-MS (*m/z*, rel.%): 45 (100), 69 (87), 71 (87), 55 (54), 43 (54), 41 (52), 128 (48), 60 (28), 74 (27), 102 (26), 162 (M^+, 5). ^{1}H NMR (500 MHz, $CDCl_3$): *δ* 4.07 (dqd, *J* = 9.1, 6.5, 3.2 Hz, 1H, H-2$_{anti}$), 3.97-3.90 (m, 1H, H-2$_{syn}$), 3.00-2.92 (m, 1H, H-4$_{anti}$), 2.82-2.74 (m, 1H, H-4$_{syn}$), 1.73-1.20 (m, 16H, H-3$_{anti+syn}$, H-5$_{anti+syn}$, H-6$_{anti+syn}$, H-7$_{anti+syn}$), 1.16 (d, *J* = 6.4 Hz, 3H, H-1$_{anti}$),1.13 (d, *J* = 6.0 Hz, 3H, H-1$_{syn}$), 0.84 (td, *J* = 7.2, 1.9 Hz, 6H, H-8$_{anti+syn}$).^{13}C NMR (126 MHz, $CDCl_3$): *δ* 66.96 (C-2$_{syn}$), 65.39 (C-2$_{anti}$), 48.22 (C-3$_{syn}$), 47.77 (C-3$_{anti}$), 39.60 (C-5$_{anti}$), 39.29 (C-5$_{syn}$), 38.92 (C-4$_{syn}$), 37.76 (C-4$_{anti}$), 29.17 (C-6$_{anti}$), 28.98 (C-6$_{syn}$), 24.06 (C-1$_{anti}$), 23.59 (C-1$_{syn}$), 22.42 (C-7$_{anti+syn}$), 14.02 (C-8$_{anti+syn}$).

4-Mercapto-2-nonanol, **5**: 1.25 g (7.09 mmol, mol yield: 102%, purity: 92% by GC (ratio of diastereomers, 45:55), LRI: 1946 (*anti*), 1961 (*syn*) on DB-Wax; 1311 (*anti*), 1319 (*syn*) on DB-1. GC-MS (*m/z*, rel.%): 45 (100), 71 (92), 55 (82), 69 (63), 41 (51), 43 (50), 142 (46), 83 (34), 58 (31), 57 (28), 176 (M^+, 4). ^{1}H NMR (500 MHz, $CDCl_3$): *δ* 4.08 (dqd, *J* = 9.1, 6.2, 2.8 Hz, 1H, H-2$_{anti}$), 4.01-3.89 (m, 1H, H-2$_{syn}$), 3.01-2.91 (m, 1H, H-4$_{anti}$), 2.78 (qt, *J* = 8.0, 5.1 Hz, 1H, H-4$_{syn}$), 1.73-1.17 (m, 20H, H-3$_{anti+syn}$, H-5$_{anti+syn}$, H-6$_{anti+syn}$, H-7$_{anti+syn}$,H-8$_{anti+syn}$), 1.16 (d, *J* = 6.2 Hz, 3H, H-1$_{anti}$), 1.13 (d, *J* = 6.2 Hz, 3H, H-1$_{syn}$), 0.83 (td, *J* = 7.0, 1.4 Hz 6H, H-9$_{anti+syn}$). ^{13}C NMR (126 MHz, $CDCl_3$): *δ* 67.05 (C-2$_{syn}$), 65.43 (C-2$_{anti}$), 48.20 (C-3$_{syn}$), 47.74 (C-3$_{anti}$), 39.88 (C-5$_{anti}$), 39.61 (C-5$_{syn}$), 39.01 (C-4$_{syn}$), 37.81 (C-4$_{anti}$), 31.73 (C-6$_{anti}$), 31.52 (C-6$_{syn}$), 26.69 (C-7$_{anti}$), 26.49 (C-7$_{syn}$), 24.07 (C-1$_{anti}$), 23.60 (C-1$_{syn}$), 22.60 (C-8$_{anti+syn}$), 14.08 (C-9$_{anti+syn}$).

4-Mercapto-2-decanol, **6**: 1.77 g (9.27 mmol, mol yield: 72%, purity: 91% by GC (ratio of diastereomers, 43:57), LRI: 2058 (*anti*), 2073 (*syn*) on DB-Wax; 1414 (*anti*), 1421 (*syn*) on DB-1. GC-MS (*m/z*, rel.%): 71 (100), 55 (98), 45 (93),43 (67), 41 (58), 69 (57), 156 (42), 83 (39), 58 (35), 102 (33), 190 (M^+, 4). ^{1}H NMR (500 MHz, $CDCl_3$): *δ* 4.06 (dqd, *J* = 9.1, 6.2, 2.9 Hz, 1H, H-2$_{anti}$), 3.98-3.88 (m, 1H, H-2$_{syn}$), 3.01-2.90 (m, 1H, H-4$_{anti}$), 2.83-2.72 (m, 1H, H-4$_{syn}$), 1.73-1.17 (m, 24H,H-3$_{anti+syn}$, H-5$_{anti+syn}$, H-

6$_{anti+syn}$, H-7$_{anti+syn}$,H-8$_{anti+syn}$, H-9$_{anti+syn}$), 1.15 (d, *J* = 6.3 Hz, 3H, H-1$_{anti}$), 1.13 (d, *J* = 6.2 Hz, 3H, H-1$_{syn}$), 0.81 (t, *J* = 6.7 Hz, 6H, H-10$_{anti+syn}$). ^{13}C NMR (126 MHz, $CDCl_3$): *δ* 66.85 (C-2$_{syn}$), 65.29 (C-2$_{anti}$), 48.20 (C-3$_{syn}$), 47.81 (C-3$_{anti}$), 39.90 (C-5$_{anti}$), 39.56 (C-5$_{syn}$), 38.87 (C-4$_{syn}$), 37.80 (C-4$_{anti}$), 31.76 (C-8$_{anti}$), 31.75 (C-8$_{syn}$), 29.03 (C-7$_{anti}$), 29.00 (C-7$_{syn}$), 26.96 (C-6$_{anti}$), 26.76 (C-6$_{syn}$), 24.03 (C-1$_{anti}$), 23.50 (C-1$_{syn}$), 22.61 (C-9$_{anti+syn}$), 14.10 (C-10$_{anti+syn}$).

Chromatographic, mass spectrometric and NMR data were in good agreement with those previously reported (Vermeulen *et al*., 2003; Ozeki *et al*., 2004; Naef *et al*., 2008; Polster, 2012; Polster and Schieberle, 2017).

3.2.3 4-Mercapto-2-heptyl acetate

A solution of 4-mercapto-2-heptanol (0.344 g, 2.32 mmol) in Et_2O (5 mL) was slowly treated with acetyl chloride (165 µL, 2.32 mmol) at 0 °C under argon atmosphere as previously reported (Vermeulen and Collin, 2003). The reaction mixture was stirred for 18 h at RT. The reaction was stopped by addition of 200 µL pyridine and the precipitate was filtered off. The organic phase was washed with water and brine, dried with sodium sulfate, and the solvent was evaporated. The crude product was purified by column chromatography on silica gel (silica gel 60, 0.063-0.200 mm, Merck, Germany) by elution with a mixture of *n*-hexane and Et_2O, 9:1, (v/v). The fractions were checked by thin layer chromatography (TLC, ALUGRAM® SIL G/UV$_{254}$, Macherey-Nagel, Germany), visualization was achieved by spraying with 10% sulfuric acid and subsequent heating until dryness.

4-Mercapto-2-heptyl acetate **13** was obtained as a colorless liquid, 122 mg (0.64 mmol, mol yield: 28%, purity: 97% by GC (ratio of diastereomers, 40:60), LRI: 1650 (*anti*), 1668 (*syn*) on DB-Wax; 1233 (*anti*), 1241 (*syn*) on DB-1. GC-MS (*m/z*, rel.%): 43 (100), 55 (81), 87 (47), 97 (46), 130 (45), 115 (22), 88 (19), 41 (18), 102 (17). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.24-5.16 (m, 1H, H-2$_{anti}$), 5.15-5.07 (m, 1H, H-2$_{syn}$), 2.86-2.74 (m, 2H, H-4$_{anti+syn}$), 2.03 (s, 6H, CH_3C=O-O-$_{anti+syn}$), 1.99-1.92 (m, 1H, H-5a$_{anti}$), 1.92-1.85 (m, 1H, H-5a$_{syn}$), 1.79-1.71 (m, 1H, H-5b$_{syn}$), 1.70-1.63 (m, 1H, H-3a$_{syn}$), 1.57-1.62 (m, 1H, H-3a$_{anti}$), 1.57-1.36 (m, 7H, H-5b$_{anti}$, H-3b$_{anti}$, H-3b$_{syn}$, H6$_{anti+syn}$), 1.26 (d, *J* = 6.4 Hz, H-1$_{anti}$), 1.22 (d, *J* = 6.2 Hz, 3H, H-1$_{syn}$), 0.94-0.87 (m, 6H, H7$_{anti+syn}$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.7 ($CH_3\underline{C}$=O-O-$_{anti}$), 170.6 ($CH_3\underline{C}$=O-O-$_{syn}$), 69.0 (C-2$_{syn}$), 68.91 (C-2$_{anti}$), 45.5 (C-5 $_{anti}$), 45.2 (C-5$_{syn}$), 41.3 (C-3$_{anti}$), 40.8

(C-3$_{syn}$), 37.0 (C-4$_{anti}$), 37.0 (C-4$_{syn}$), 21.4 (<u>C</u>H$_3$C=O-O-$_{anti}$), 21.4 (<u>C</u>H$_3$C=O-O-$_{syn}$), 20.6 (C-1/C-6), 20.1 (C-1/C-6), 20.0 (C-1/C-6), 19.9 (C-1/C-6), 13.8 (C-7$_{anti}$), 13.7 (C-7$_{syn}$).

3.2.4 4-Acetylthio-2-heptyl acetate

A solution of 4-mercapto-2-heptanol (0.538 g, 3.37 mmol) in 2.5 mL pyridine was treated with 4-dimethylaminopyridine (247 mg, 2.02 mmol) and acetic anhydride (1.60 mL, 16.9 mmol). The mixture was stirred for 24 h at RT. The solvent was removed under reduced pressure and the residue was dissolved in Et_2O. After washing with saturated aqueous solutions of sodium hydrogen carbonate (3x10 mL) and ammonium chloride (3x10 mL), the organic layer was dried over sodium sulfate and the solvent was removed under reduced pressure. The diastereomeric mixture (40:60 by GC, *anti*:*syn*) was separated by column chromatography on silica gel with *n*-hexane/Et_2O (5:1; v/v) and the obtained fractions were checked by TLC.

The separated diastereomers of 4-acetylthio-2-heptyl acetate **14** were isolated as *anti*-diastereomer (yield: 0.295 g, 1.27 mmol, 38%, purity: 94% by GC) and *syn*-diastereomer (yield: 0.442 g, 1.90 mmol, 56%, purity: 96% by GC) as colorless liquids. LRI: 1881 (*anti*), 1934 (*syn*) on DB-Wax; 1403 (*anti*), 1428 (*syn*) on DB-1. GC-MS (*m/z*, rel.%): 43 (100), 55 (38), 130 (32), 129 (30), 97 (24), 87 (22), 139 (18), 115 (13), 88 (11). ^{1}H NMR (500 MHz, $CDCl_3$): *anti*: δ 5.02-4.93 (m, 1H, H-2), 3.68-3.58 (m, 1H, H-4), 2.29 (s, 3H, CH_3C=O-S-), 2.03 (s, 3H, CH_3C=O-O-), 1.96-1.85 (m, 1H, H-3a), 1.65-1.48 (m, 3H, H-3b, H-5), 1.47-1.30 (m, 2H, H-6), 1.22 (d, *J* = 6.2 Hz, 3H, H-1), 0.90 (t, *J* = 7.3 Hz, 3H, H-7); *syn*: δ 5.08-4.90 (m, 1H, H-2), 3.60-3.47 (m, 1H, H-4), 2.31 (s, 3H, CH_3C=O-S-), 2.04 (s, 3H, CH_3C=O-O-), 1.95-1.82 (ddd, *J* = 14.1, 7.9, 7.1 Hz, 1H, H-3a), 1.78-1.70 (dt, *J* = 14.2, 6.4 Hz, 1H, H-3b), 1.65-1.56 (dddd, *J* = 13.8, 9.9, 6.0, 5.0 Hz, 1H, H-5a), 1.54-1.46 (m, 1H, H-5b), 1.45-1.29 (m, 2H, H-6), 1.22 (d, *J* = 6.2 Hz, 3H, H-1), 0.89 (t, *J* = 7.3 Hz, 3H, H-7). ^{13}C NMR (126 MHz, $CDCl_3$): *anti*: δ 195.5 (CH_3<u>C</u>=O-S-), 170.9 (CH_3<u>C</u>=O-O-), 68.5 (C-2), 41.3 (C-3), 40.8 (C-4), 37.9 (C-5), 30.8 (<u>C</u>H$_3$C=O-S-), 21.5 (<u>C</u>H$_3$C=O-O-), 20.5 (C-6), 20.0 (C-1), 14.0 (C-7); *syn*: δ 195.8 (CH_3<u>C</u>=O-S-), 170.7 (CH_3<u>C</u>=O-O-), 69.0 (C-2), 41.1 (C-3), 40.9 (C-4), 37.0 (C-5), 30.9 (<u>C</u>H$_3$C=O-S-), 21.5 (<u>C</u>H$_3$C=O-O-), 20.1 (C-6), 19.9 (C-1), 13.9 (C-7).

3.2.5 4-Acetylthio-2-heptanol

Sodium borohydride (201 mg, 5.31 mmol dissolved in 8 mL water) was added dropwise under ice-cooling to a solution of 4-acetylthio-2-heptanone **9** (0.50 g, 2.66 mmol) in 20 mL methanol and 80 mL potassium phosphate buffer (50 mM, pH 7.4). After 20 min, the pH was adjusted to 5 using hydrochloric acid (5%). The aqueous layer was washed with dichloromethane (3x20 mL), the organic phase was dried with sodium sulfate and the solvent was removed under reduced pressure. The diastereomers (55:45 by GC, *anti*:*syn*) were separated by column chromatography on silica gel (*n*-hexane/Et_2O, 2:1; v/v) to obtain *anti*-configured 4-acetylthio-2-heptanol *anti*-**15**: 153.5 mg (0.81 mmol, mol yield: 31%, purity: 92% by GC) and *syn*-configured 4-acetylthio-2-heptanol *syn*-**15**: 99.2 mg (0.52 mmol, mol yield: 20%, purity: 93% by GC) as yellow liquids. LRI: 1939 (*anti*), 2038 (*syn*) on DB-Wax; 1302 (*anti*), 1323 (*syn*) on DB-1. GC-MS (*m/z*, rel.%): 43 (100), 55 (41), 45 (32), 71 (23), 147 (18), 87 (17), 97 (16), 130 (15). ^{1}H NMR (500 MHz, $CDCl_3$): *anti*: δ 3.81-3.72 (m, 1H, H-2), 3.73-3.63 (m, 1H, H-4), 2.36 (s, 3H, CH_3C=O-S-), 1.75-1.68 (m, 1H, H-3a), 1.61-1.54 (m, 2H, H-5), 1.53-1.32 (m, 3H, H-3b, H-6), 1.18 (d, *J* = 6.3 Hz, 3H, H-1), 0.92-0.88 (m, 3H, H-7). ^{13}C NMR (125 MHz, $CDCl_3$): *anti*: δ 199.3 ($CH_3\underline{C}$=O-S-), 65.2 (C-2), 46.2 (C-3), 42.2 (C-4), 37.8 (C-5), 31.0 ($\underline{C}H_3C$=O-S-), 23.0 (C-1), 20.5 (C-6), 14.2 (C-7). NMR-data of *syn*-4-acetylthio-2-heptanol could not be obtained owing to the instability of the substance under the measurement conditions. GC analysis revealed *syn*-4-mercapto-2-heptyl acetate as rearrangement product.

3.2.6 2-Acetylthio-4-heptanone

2-Acetylthio-4-heptanone **16** was synthesized by Michael-type addition of thioacetic acid (1.1 equiv) to 2-hepten-4-one (1.0 equiv). As 2-hepten-4-one was not commercially available, it was synthesized according to Naef *et al*. (2008): mol yield: 45%, purity: 57% by GC. 2-Acetylthio-4-heptanone **16**: mol yield: 90%; purity: 96% by GC.

3.2.7 2-Mercapto-4-heptanone

2-Mercapto-4-heptanone **17** was synthesized by refluxing 2-acetylthio-4-heptanone **16** with methanol as described by Kiske *et al*. (2016). The crude product of **17** was purified by column chromatography on silica gel using a mixture of *n*-hexane/Et_2O 4:1 (v/v). The obtained fractions were checked by TLC. 2-Mercapto-4-heptanone **17**: mol yield: 79%, purity: 92% by GC. LRI: 1568 on DB-Wax; 1325 on RTX-200. Chromatographic and mass spectrometric data were in accordance with those previously reported (Naef *et al.*, 2008; Kiske *et al*., 2016).

3.2.8 4-Mercapto-2-heptanone

4-Mercapto-2-heptanone **18** was synthesized by refluxing 4-acetylthio-2-heptanone **9** with methanol as described by Kiske *et al*. (2016). The crude product of **18** was purified by column chromatography on silica gel using a mixture of *n*-hexane/ Et_2O 4:1 (v/v). The obtained fractions were checked by TLC. 4-Mercapto-2-heptanone **18**: mol yield: 63%, purity: 97% by GC. LRI: 1598 on DB-Wax; 1355 on RTX-200. Chromatographic and mass spectrometric data were in accordance with those previously reported (Naef *et al.*, 2008; Wakabayashi *et al*., 2011).

3.2.9 2-Mercapto-4-heptanol

To a suspension of $LiAlH_4$ (0.35 g, 9 mmol) dissolved in 20 mL THF under ice cooling, 2-mercapto-4-heptanone **17** (0.30 g, 2 mmol) in 3 mL THF was added dropwise. After stirring for 24 h at RT, the reaction mixture was quenched carefully with 20 mL of water, and then adjusted to pH 2 using hydrochloric acid (25%). The mixture was extracted with Et_2O (3x20 mL), the combined organic phases were dried over sodium sulfate and evaporated under reduced pressure. After column chromatography on silica gel (*n*-hexane/Et_2O, 5:3, v/v) 2-mercapto-4-heptanol **19** was obtained: yield: 20%, purity: 96% by GC (ratio of diastereomers, 45:55). LRI: 1742 (first eluting diastereomer), 1766 (second eluting diastereomer) on DB-Wax; 1276 (first eluting diastereomer), 1302 (second eluting diastereomer) on RTX-200. GC-MS (*m/z*, rel.%) (both isomers show the same fragmentation): 41 (47), 43 (66), 55 (100), 61 (85), 71 (62), 73 (43), 81 (16), 99 (26), 114 (36), 130 (13), 148 (M^+, 3). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 3.82 (m, 1H, H-$4_{1.Dia}$), 3.68 (m, 1H, H-$4_{2.Dia}$), 3.13 (ddq, J = 13.5, 6.8, 3.4 Hz, 1H, H-$2_{1.Dia}$), 3.03 (hept, J = 6.9, 1H, H-$2_{2.Dia}$), 1.63 (m, 4H, H-$3_{1.+2.Dia}$),

1.40 (m, 8H, H-$5_{1.+2.Dia}$, H-$6_{1.+2.Dia}$), 1.32 (d, *J* = 6.8, 3H, H-$1_{1.Dia}$), 1.31 (d, *J* = 6.7, 3H, H-$1_{2.Dia}$), 0.87 (m, 6H, H-$7_{1.+2.Dia}$). ^{13}C NMR (126 MHz, $CDCl_3$): *δ* 69.27 (C-$4_{2.Dia}$), 68.36 (C-$4_{1.Dia}$), 47.37 (C-$3_{2.Dia}$), 46.76 (C-$3_{1.Dia}$), 39.03 (C-$5_{1.Dia}$), 38.90 (C-$5_{2.Dia}$), 32.03 (C-$2_{2.Dia}$), 31.47 (C-$2_{1.Dia}$), 25.77 (C-$1_{1.Dia}$), 24.90 (C-$1_{2.Dia}$), 17.80 (C-$6_{1.Dia}$), 17.60 (C-$6_{2.Dia}$), 13.05 (C-$7_{1.Dia}$), 13.04 (C-$7_{2.Dia}$).

Chromatographic, mass spectrometric and NMR data were in accordance with those previously reported (Naef *et al*., 2008).

3.3 Lipase-Catalyzed Kinetic Resolutions

3.3.1 Preparation of Enantiomerically Enriched 4-Mercapto-2-alkanols

In analogy to the method described by Wakabayashi *et al*. (2011 and 2015), 5 mmol of synthesized 4-acetylthio-2-alkanone (**7-12**) were dissolved in 50 mL of potassium phosphate buffer (50 mM, pH 7.4). After adding 1.0 g of the enzyme preparation, the mixture was stirred at RT for a defined time (Table 8). The enzyme was filtered off and the aqueous phase was extracted four times with 50 mL Et_2O. The organic phase was dried with anhydrous sodium sulfate and the solvent was removed under reduced pressure using an aspirator at 40 °C. An aliquot of the reaction mixture (1 µL/mL in Et_2O) was subjected to GC analysis using heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyl dimethylsilyl)-*β*-cyclodextrin (CycloSil-B) as chiral stationary phase (GC-FID system IV). The order of elution of the enantiomers of the remaining acetylthioester substrate and of the obtained 4-mercapto-2-alkanone as product was assigned according to Kiske *et al*. (2016). The conversion rates of substrates as well as the enantiomeric excesses of products ee_P and of the remaining substrates ee_S were calculated according to equation [1] (Ghanem and Aboul-Enein (2005)).

$$c\ (\%) = \frac{ee_s}{ee_s + \mathrm{ee_p}} 100 \qquad [1]$$

The enantiomeric excess value is expressed using the GC integration areas P_1 and P_2 of the enantiomers (equation [2]).

$$ee\ (\%) = \frac{P_1 - P_2}{P_1 + P_2} 100 \qquad \text{for } P_1 > P_2 \qquad [2]$$

The separation of the reaction mixture was carried out by column chromatography on silica gel using a defined mixture of *n*-hexane and Et_2O. In Table 8, the amounts of racemic 4-acetylthio-2-alkanones, the employed enzymes, reaction times, conversion rates, enantiomeric excesses and conditions used for the separation of the reaction mixture are presented.

Finally, (*S*)-4-acetylthio-2-pentanone, (*S*)-4-mercapto-2-hexanone, (*R*)-4-acetylthio-2-heptanone, (*R*)-4-acetylthio-2-octanone, (*R*)-4-acetylthio-2-nonanone and (*S*)-4-mercapto-2-decanone obtained after lipase-mediated hydrolysis (Table 8) were subjected to reduction using $LiAlH_4$ in analogy to the method described in chapter 3.2.2 in order to obtain the (4*S*)-configured diastereomers of 4-mercapto-2-pentanol (4*S*)-**1**, 4-mercapto-2-hexanol (4*S*)-**2** and 4-mercapto-2-decanol (4*S*)-**6** as well as the (4*R*)-configured diastereomers of 4-mercapto-2-heptanol (4*R*)-**3**, 4-mercapto-2-octanol (4*R*)-**4** and 4-mercapto-2-nonanol (4*R*)-**5**, respectively.

Table 8. Preparation of enantiomerically enriched mercaptoalkanones and 4-acetylthio-2-alkanones via lipase-catalyzed hydrolysis of racemic 4-acetylthio-2-alkanones **7-12**.

racemic 4-acetylthio-2-alkanone		lipase[a]	reaction time (h)	conversion[b] (%)	ee_p[c] (%)	ee_s[c] (%)	er (%)[d] = [(*S*):(*R*)][e]		conditions for the separation of the reaction mixture by column chromatography
chain length	[g]						hydrolysis product	remaining substrate	
C5	0.80	CAL-B	4	56	76.0	> 99	12:88	> 99:n.d.[f]	*n*-hexane:Et_2O, 3:1, v/v
C6	0.87	PPL	6	75	31.4	93.2	66:34	3:97	*n*-hexane:Et_2O, 4:1, v/v
C7	0.94	PPL	7.5	79	24.0	95.9	62:38	2:98	*n*-hexane:Et_2O, 4:1, v/v
C8	1.01	PPL	1	43	63.7	47.6	82:18	26:74	*n*-hexane:Et_2O, 5:1, v/v
C9	1.08	PPL	1	38	71.6	43.2	86:14	28:72	*n*-hexane:Et_2O, 5:1, v/v
C10	1.15	PPL	2	63	43.1	n.d.[f]	72:28	-[g]	*n*-hexane:Et_2O, 6:1, v/v

[a] 1 g

[b] Calculated according to equation 1

[c] Calculated according to equation 2

[d] Enantiomeric ratio determined by capillary gas chromatography

[e] Configurations were assigned according to Kiske *et al.* (2016)

[f] Not determined under used conditions

[g] No data available

3.3.2 Preparation of Enantio-Enriched Acetates of 4-Mercapto-2-heptanol

3.3.2.1 (4*R*)-Configured Diastereomers of 4-Mercapto-2-heptyl acetate

The (4*R*)-configured diastereomers of 4-mercapto-2-heptyl acetate (4*R*)-**13** were prepared by selective *O*-acetylation of (4*R*)-4-mercapto-2-heptanol (4*R*)-**3** obtained via PPL-catalyzed kinetic resolution of racemic 4-acetylthio-2-heptanone **9** and subsequent reduction with $LiAlH_4$. The acetylation was performed in analogy to the method described in chapter 3.2.3.

3.3.2.2 (4*R*)-Configured Diastereomers of 4-Acetylthio-2-heptyl acetate

The (4*R*)-configured diastereomers of 4-acetylthio-2-heptyl acetate (4*R*)-**14** were prepared by acetylation of (4*R*)-4-mercapto-2-heptanol (4*R*)-**3** obtained via PPL-catalyzed kinetic resolution of racemic 4-acetylthio-2-heptanone **9** and subsequent reduction with $LiAlH_4$. The acetylation was performed in analogy to the method described in chapter 3.2.4.

3.3.2.3 (4*R*)-Configured Diastereomers of 4-Acetylthio-2-heptanol

The (4*R*)-configured diastereomers of 4-acetylthio-2-heptanol (4*R*)-**15** were prepared by reduction of (4*R*)-4-acetylthio-2-heptanone (4*R*)-**9** obtained via PPL-catalyzed kinetic resolution of racemic 4-acetylthio-2-heptanone **9** with sodium borohydride, employing the same procedure as described in chapter 3.2.5.

3.3.3 Preparation of Enantio-Enriched 2-Mercapto-4-heptanone

Enantiomerically enriched 2-mercapto-4-heptanone was obtained by adding 20 mg of CAL-B resin to 85 µmol of synthesized 2-acetylthio-4-heptanone **16**, dissolved in 1 mL of potassium phosphate buffer (50 mM, pH 7.4). After stirring the mixture with a Teflon stir bar at RT for 30 minutes, an aliquot of 100 µL was extracted with 1 mL of dichloromethane using a vortex shaker for 1 minute, dried over anhydrous sodium sulfate, filtered, and finally refilled by adding 1 mL of dichloromethane. GC analyses of the reaction mixtures using a CycloSil-B column (GC-FID system IV) resulted in (*R*)-**17** (er (%) = 97:3) according to the absolute configuration assigned by Kiske *et al*. (2016).

3.3.4 Preparation of Enantio-Enriched 4-Mercapto-2-heptanone

(*S*)-Configured 4-mercapto-2-heptanone (*S*)-**18** was obtained by adding 20 mg of PPL to 85 µmol of synthesized 4-acetylthio-2-heptanone **9**, dissolved in 1 mL of potassium phosphate buffer (50 mM, pH 7.4). After stirring the mixture with a Teflon stir bar at RT for 30 minutes, an aliquot of 100 µL was extracted with 1 mL of dichloromethane using a vortex shaker for 1 minute, dried over anhydrous sodium sulfate, filtered, and finally refilled by adding 1 mL of dichloromethane. GC analyses of the reaction mixtures using a CycloSil-B column (GC-FID system IV) resulted in (*S*)-**18** (er (%) = 71:29) according to the absolute configuration assigned by Kiske *et al*. (2016).

3.4 Preparation of (*S*)-MαNP Thioesters of (*R*)- and (*S*)-4-Mercapto-2-octanone

(*R*)-4-Mercapto-2-octanone, (*R*)-**21**. In accordance with the previously described enzyme-catalyzed kinetic resolution (Wakabayashi *et al*., 2011), 37.5 mmol of racemic 4-acetylthio-2-octanone **10** were dissolved in potassium phosphate buffer and 7.5 g of PPL were added. After stirring for 3 h at RT, the enzyme was filtered off using Celite and the aqueous phase was extracted with Et_2O (4x25 mL). The organic phase was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. (*R*)-4-Acetylthio-2-octanone (*R*)-**10** was obtained after column chromatography on silica gel using a mixture of *n*-hexane and Et_2O (7:1, v/v): mol yield: 1.7%, purity (GC): 97.8%, er (%) = 91:9. (*R*)-4-Mercapto-2-octanone (*R*)-**21** was obtained via transesterification (Kiske *et al*., 2016) starting with 0.5 mmol of (*R*)-**10** followed by purification (column chromatography on silica gel using a mixture of *n*-hexane and Et_2O (7:1, v/v)): mol yield: 0.7%, purity (GC): 97.5%, er (%) = 76:14.
(*S*)-4-Mercapto-2-octanone, (*S*)-**21**. For (*S*)-**21**, 12.5 mmol of racemic 4-acetylthio-2-octanone **10** were dissolved in potassium phosphate buffer and 5 g of PPL were added. After stirring for 1 h at RT, the enzyme was filtered off using Celite and the aqueous phase was extracted with Et_2O (4x25 mL). The organic phase was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. (*S*)-**21** was obtained after column chromatography on silica gel using a mixture of *n*-hexane and Et_2O (7:1, v/v): mol yield: 6.4%, purity (GC): 97.1%, er (%) = 88:12.

(*S*)-MαNP thioesters of (*R*)- and (*S*)-**21**. The diastereomers were prepared according to Kiske *et al*. (2016) and separated by semi-preparative HPLC using a Dionex HPLC system (UltiMate 3000 series, Dionex, Germering, Germany) equipped with a 3100 wavelength detector set at 254 nm using a 250 x 8 mm i.d. Nucleosil 50-5 column (CS Chromatography, Langerwehe, Germany). Isocratic elution was performed at 30 °C with a mixture of *n*-hexane/isopropanol 96:4 (v/v) as the eluent and a flow rate of 2 mL/min. (*S*)-M*α*NP thioesters of (*R*)-**21**: 10.0 mg, 31.0%; (*S*)-MαNP thioesters of (*S*)-**21**: 5.5 mg, 17.2%.

3.5 Analyses

3.5.1 Capillary Gas Chromatography (GC)

3.5.1.1 GC-Flame Ionization Detector (GC-FID)

Achiral GC analyses were performed on the following GC systems:

GC-FID I:

Instrument:	HP5890 A (Hewlett-Packard, Heilbronn, Germany)
Column:	DB-Wax column (J&W Scientific, Agilent Technologies, Waldbronn, Germany); 30 m x 0.25 mm i.d.; 0.5 µm film thickness
Temperature program:	40 °C/5 min//4 °C/min//240 °C/30 min
Carrier gas:	Hydrogen (135 kPa), constant pressure
Injector:	Split injection (ratio of 1:7), 215 °C
Detector:	350 °C

GC-FID II:

Instrument:	CE 5160 instrument (Carlo Erba Instruments, Hofheim, Germany)
Column:	DB-1 column (J&W Scientific, Agilent Technologies); 30 m x 0.25 mm i.d.; 1.0 µm film thickness
Temperature program:	60 °C/5 min//5 °C/min//250 °C/5 min
Carrier gas:	Hydrogen (74 kPa), constant pressure
Injector:	Split injection (ratio of 1:10), 200 °C
Detector:	260 °C

GC-FID III:

Instrument:	CE 5160 instrument (Carlo Erba Instruments)
Column:	RTX-200 column (Restek, Bad Homburg, Germany); 30 m x 0.32 mm i.d.; 1.0 µm film thickness
Temperature program:	40 °C/5 min//2 °C/min//240 °C/30 min
Carrier gas:	Hydrogen (75 kPa), constant pressure
Injector:	Split injection (ratio of 1:4), 230 °C
Detector:	350 °C

Linear retention indices (LRI) were calculated using the retention time R_t of the unknown compound, the retention time Rt_n of the *n*-alkane eluting earlier than the unknown compound, the retention time Rt_{n+1} of the *n*-alkane eluting after the unknown compound, and the number C of carbon atoms of the earlier eluting *n*-alkane (equation [3]). *n*-Alkane standard solutions were used as references (van den Dool and Kratz, 1963).

$$\mathrm{LRI} = \left[C + \frac{Rt - Rt_n}{Rt_{n+1} - Rt_n}\right] 100 \qquad [3]$$

Enantioselective analyses of 4-acetylthio-2-alkanones **7-12**, 4-mercapto-2-alkanones, 4-mercapto-2-heptyl acetate **13**, 4-acetylthio-2-heptyl acetate **14**, 4-acetylthio-2-heptanol **15** and 2-mercapto-4-heptanone **17** were performed on the following GC system:

GC-FID IV:

Instrument:	HP5890 Series II (Hewlett-Packard)
Column:	CycloSil-B (J&W Scientific, Agilent Technologies); 30 m x 0.25 mm i.d.; 0.25 µm film thickness
Temperature program:	
for **7-13**, **17** and	
4-mercapto-2-alkanones:	75 °C/0 min//1 °C/min//180 °C/5 min
for **14**:	75 °C/10 min//0.5 °C/min//100 °C/45 min// 2 °C/ min//180 °C/5 min
for **15**:	75 °C/0 min//2 °C/min//180 °C/5 min
Carrier gas:	Hydrogen (160 kPa for **15** and 176 kPa for **7-14**, **16** and 4-mercapto-2-alkanones), constant pressure
Injector:	Split injection (ratio of 1:5), 200 °C
Detector:	350 °C

Enantioselective analyses of the stereoisomers of 4-mercapto-2-alkanols (**1** and **3-6**) were performed on the following GC system:

GC-FID V:

Instrument:	CE 5160 instrument (Carlo Erba Instruments)
Column:	50% Heptakis(2,3-di-*O*-acetyl-6-*O*-TBDMS)-*β*-CD in OV1701-vi; 30 m x 0.25 mm i.d., in-house prepared according to Dietrich *et al*., 1992a
Temperature program:	85 °C/0 min//0.5 °C/min//110 °C/50 min// 2 °C/min//180 °C/5 min
Carrier gas:	Hydrogen (75 kPa), constant pressure
Injector:	Split injection (ratio of 1:10), 200 °C
Detector:	260 °C

The separation of the *syn*-configured isomers of 4-mercapto-2-heptanol *syn*-**3** was performed on GC system VI:

GC-FID VI:

Instrument:	CE 5160 instrument (Carlo Erba Instruments)
Column:	50% Octakis(2,3-di-*O*-*n*-butyryl-6-*O*-TBDMS)-*γ*-CD in SE 54; 30 m x 0.25 mm i.d., in-house prepared according to Schmarr, 1992
Temperature program:	75 °C/0 min//0.5 °C/min//93 °C/0 min// 1 °C/min//180 °C/5 min
Carrier gas:	Hydrogen (75 kPa), constant pressure
Injector:	Split injection (ratio of 1:10), 200 °C
Detector:	260 °C

Enantioselective analysis of the stereoisomers of 4-mercapto-2-hexanol **2** was performed on GC system VII:

GC-FID VII:

Instrument:	CE 5160 instrument (Carlo Erba Instruments)
Column:	28% Heptakis(2,3-di-*O*-methoxymethyl-6-*O*-TBDMS)-*β*-CD in OV1701-vi; 30 m x 0.25 mm i.d., in-house prepared according to Takahisa and Engel, 2005
Temperature program:	55 °C/20 min//0.5 °C/min//85 °C/15 min// 2 °C/min//180 °C/5 min
Carrier gas:	Hydrogen (110 kPa), constant pressure
Injector:	Split injection (ratio of 1:10), 200 °C
Detector:	260 °C

Enantioselective analyses of the stereoisomers of 4-mercapto-2-alkanols (**1-6**) were performed on the following GC system:

GC-FID VIII:

Instrument:	HP5890 Series II (Hewlett-Packard)
Column:	Inert Cap^{TM} Chiramix (GL Science, Tokyo, Japan); 30 m x 0.25 mm i.d.; 0.25 µm film thickness
Temperature program:	60 °C/0 min//0.7 °C/min//180 °C/30 min
Carrier gas:	Hydrogen (110 kPa), constant pressure
Injector:	Split injection (ratio of 1:30), 230 °C
Detector:	250 °C

In general, deactivated fused silica capillaries (0.25 mm i.d.; BGB Analytik AG, Rheinfelden, Germany) were installed in the injector and detector to protect the chiral stationary phases.

The quality of the enantiomeric separations was judged by calculating the separation factor (α) and the resolution (R_s). The separation factor α was calculated according to equation [4].

$$\alpha = \frac{k_2}{k_1} \qquad [4]$$

The retention factor k measures the retention of a compound on the chromatographic column by determining the retention time (min) of a non-retained compound t_0 and of the first t_1 or second t_2 eluting enantiomer (equation [5]. A separation between

$$k_1 = \frac{t_1 - t_0}{t_0} \quad \text{or} \quad k_2 = \frac{t_2 - t_0}{t_0} \qquad [5]$$

enantiomers occurs if the separation factor α is greater than 1 (Rood, 1991).

The resolution R_s is calculated by the retention time (min) of the first t_1 and second t_2 eluting enantiomer, and the width of the peak at half-height of the first w_{b1} and second w_{b2} eluting enantiomer (equation [6]). A baseline separation is achieved with $R_s \geq 1.5$ (Mosandl, 1992).

$$R_s = 1.177 \frac{t_2 - t_1}{w_{b1} + w_{b2}} \qquad [6]$$

3.5.1.2 Multidimensional GC (MDGC)

The instrumentation consisted of two coupled GC 8000 (Carlo Erba Instruments). A Moving Column Stream Switching device (MCSS) and a 1 m x 0.25 mm i.d. deactivated fused silica transfer capillary (BGB Analytik AG) were used to transfer the compounds from the precolumn (GC 1) onto the main column (GC 2).

Enantioselective MGDC Analysis of 4-Mercapto-2-alkanols

The orders of elution of the stereoisomers of the 4-mercapto-2-alkanols **1-6** were determined via enantioselective MDGC:

Precolumn:	DB-Wax (J&W Scientific, Agilent Technologies);
	60 m x 0.32 mm i.d.; 0.25 µm film thickness
Temperature program:	40 °C/5 min//4 °C/min//240 °C/25 min
Carrier gas:	Hydrogen (165 kPa), constant pressure
Injector:	Split injection (ratio of 1:5), 215 °C
Detector:	230 °C (FID)

The cut intervals were as follows: 27.88 - 28.99 min for *anti*-**1**, 29.18 - 30.00 min for *syn*-**1**, 31.15 - 31.85 min for *anti*-**2**, 32.05 - 32.80 min for *syn*-**2**, 33.75 - 34.22 min for *anti*-**3**, 34.52 - 35.04 min for *syn*-**3**, 36.50 - 37.16 min for *anti*-**4**, 37.32 - 37.83 min for *syn*-**4**, 39.42 - 39.92 min for *anti*-**5**, 40.11 - 40.57 min for *syn*-**5**, 42.05 - 42.69 min for *anti*-**6**, 42.85 - 43.36 min for *syn*-**6**.

The following chiral stationary phases were installed into GC 2 (FID: 230 °C, outlet pressure: 98 kPa) as main columns:

Main column (a):	50% Heptakis(2,3-di-*O*-acetyl-6-*O*-TBDMS)-*β*-CD
	in OV1701-vi; 30 m x 0.25 mm i.d.
Temperature program:	40 °C/0 min//2 °C/min//85 °C/5 min//
	0.5 °C/min//110 °C/50 min//2 °/min//180 °C/15 min
Main column (b):	28% Heptakis(2,3-di-*O*-methoxymethyl-6-*O*-TBDMS)-*β*-CD
	in OV1701-vi; 30 m x 0.25 mm i.d.
Temperature program:	40 °C/16 min//1 °C/min//55 °C/20 min//
	0.5 °C/min//85 °C/10 min//2 °C/min//180 °C/15 min
Main column (c):	Inert Cap^{TM} Chiramix (GL Science);
	30 m x 0.25 mm i.d.; 0.25 µm film thickness
Temperature program:	37 °C/0 min//1 °C/min//60 °C/5 min//
	0.7 °C/min//180 °C/15 min

Data were processed via Chrom-Card software (Thermo Fisher Scientific, Dreieich, Germany).

Enantioselective MGDC Analysis of ß-Mercapto Compounds in Bell Pepper Extracts

To determine the naturally occurring distributions of the stereoisomers of 4-mercapto-2-heptanol **3**, 2-mercapto-4-heptanone **17**, 4-mercapto-2-heptanone **18** and 2-mercapto-4-heptanol **19** in bell pepper, enantioselective MDGC was performed using two different precolumns in combination with two different chiral stationary phases as main columns.

Precolumn (a):	DB-Wax (J&W Scientific, Agilent Technologies);
	60 m x 0.32 mm i.d.; 0.25 µm film thickness
Temperature program:	40 °C/5 min//4 °C/min//240 °C/25 min
Carrier gas:	Hydrogen (165 kPa), constant pressure
Injector:	Split injection (ratio of 1:5), 215 °C
Detector:	230 °C (FID)

The cut intervals using DB-Wax as precolumn were as follows:
27.48 - 27.68 min for **17**, 28.68 - 28.92 min for **18**, 33.49 - 33.52 min for the first eluting diastereomer of **19**, 33.72 - 33.93 min for *anti*-**3**, 33.86 - 34.19 min for the second eluting diastereomer of **19** in combination with *anti*-**3** and 34.29 - 34.56 min for *syn*-**3**.

Precolumn (b):	RTX-200 column (Restek)
	30 m x 0.32 mm i.d.; 1.0 µm film thickness
Temperature program:	40 °C/5 min//2°C/min//120 °C/0 min//4 °C/min//
	240 °C/15 min
	Hydrogen (165 kPa), constant pressure
Injector:	Split injection (ratio of 1:5), 215 °C
Detector:	230 °C (FID)

The cut intervals using RTX-200 as precolumn were as follows:
27.94 - 28.63 min for the first eluting diastereomer of **19** in combination with *anti*-**3**, 28.35 - 28.65 min for *anti*-**3**, 29.66 - 29.84 min for the second eluting diastereomer of **19**, 30.05 - 30.13 min for *syn*-**3**, 30.89 - 31.58 min for **17**, 32.94 - 33.29 min for **18**.

The following chiral stationary phases were installed into GC 2 (FID: 230 °C, outlet pressure: 98 kPa) as main columns:

Main column (a):	Inert Cap^{TM} Chiramix (GL Science);
	30 m x 0.25 mm i.d.; 0.25 µm film thickness
Temperature program:	37 °C/0 min//1 °C/min//60 °C/5 min//
	0.7 °C/min//180 °C/10 min
Main column (b):	50% Octakis(2,3-di-*O*-*n*-butyryl-6-*O*-TBDMS)-*γ*-CD
	in SE 54; 30 m x 0.25 mm i.d.
Temperature program:	40 °C/0 min//2°C/min//85 °C/7 min//0.5°C/min//
	110 °C/50 min//2°C/min//180 °C/10 min

Data were processed via Chrom-Card software (Thermo Fisher Scientific).

3.5.1.3 GC/Olfactometry (GC/O)

Sensory evaluations were performed by three panelists (females, 20-30 years old). Panelist 1 had extensive training for more than three years, whereas panelist 2 and 3 had no prior experience with GC/O assessments.
Odor thresholds in air were determined following the procedure described by Ullrich and Grosch (1987). Stock solutions containing the internal standard (*E*)-2-decenal and the respective target compounds in known concentrations (0.1 mg/mL-2.0 mg/mL in Et_2O) were diluted stepwise by a factor of 1:2 (v/v) using Et_2O as solvent. They were freshly prepared for each panelist. The aliquots were analyzed by GC/O until no odor was perceivable. The panelists considered a concentration level only as odor threshold if it was the lowest dilution step at which the odor was consistently perceived in three consecutive GC/O-runs.

The result of the aroma extract dilution analysis (AEDA) was expressed as flavor dilution (FD) factor according to equation [7], with n as the number of 1+1 dilutions (Grosch, 1993).

$$FD = 2^n \qquad [7]$$

The odor threshold (ng/L in air) of the investigated substance is calculated using the odor threshold O_s of the internal standard (*E*)-2-decenal of 2.7 ng/L in air (Boelens and van Gemert, 1986), the concentration of substance C_x, the concentration C_s of the internal standard, the flavor dilution factor FD_x of the substance, and the flavor dilution factor FD_s of the internal standard (equation [8]).

$$O_x = \frac{O_s C_x FD_s}{C_s FD_x} \qquad [8]$$

The odor qualities obtained during the AEDA were collected and those with injection volumes corresponding to approximately 1.5 ng for each stereoisomer at the sniffing port were used as descriptors of the odor qualities.

Instrument:	HP5890 A Series II (Hewlett-Packard)
Injector:	Cold on-column injection (40 °C), samples were applied onto a deactivated precolumn 30 cm x 0.32 mm i.d. (BGB Analytik AG))
Detector:	250 °C (FID)
Sniffing port:	200 °C

Instrument:	Fractovap 4200 (Carlo Erba Instruments)
Injector:	Split injection (ratio of 1:10), 220 °C
Detector:	230 °C (FID)
Sniffing port:	230 °C
Make-up gas:	Nitrogen (50 kPa)

Effluent between FID and sniffing port was split 1:1 via a press-fit Y-splitter and deactivated fused silica capillaries (50 cm x 0.25 mm i.d.; BGB Analytik AG).

GC/O analyses of the diastereomers of 4-mercapto-2-heptanol **3** and its acetyl derivatives **13-15** were performed by panelist 1. Conditions were as follows:

GC/O I:

Instrument:	HP5890 A Series II (Hewlett-Packard)
Column:	DB-Wax column (J&W Scientific, Agilent Technologies); 30 m x 0.25 mm i.d.; 0.5 µm film thickness
Temperature program:	40 °C/5 min//4 °C/min//240 °C/30 min
Carrier gas:	Hydrogen (70 kPa for **3**, **14** and **15**; 75 kPa for **13**), constant pressure

The sensory analyses of the stereoisomers of the 4-mercapto-2-alkanols (**1**, **3**, **4**, *anti*-**5** and **6**) by panelist 2 were accomplished using the following chiral stationary phase installed into the HP5890 A Series II gas chromatograph.

GC/O II:

Instrument:	HP5890 A Series II (Hewlett-Packard)
Column:	50% Heptakis(2,3-di-*O*-acetyl-6-*O*-TBDMS)-*β*-CD in OV1701-vi; 30 m x 0.25 mm i.d.
Temperature program:	40 °C/0 min//30 °C/min//85 °C/0 min// 0.5 °C/min//110 °C/50 min//2 °/min//180 °C/20 min
Carrier gas:	Hydrogen (75 kPa), constant pressure

Sensory evaluation of the stereoisomers of 4-mercapto-2-heptyl acetate **13**, 4-acetylthio-2-heptyl acetate **14** and 4-acetylthio-2-heptanol **15** was performed by panelist 1 using the following conditions:

GC/O III:

Instrument:	HP5890 A Series II (Hewlett-Packard)
Column:	25% Heptakis(2,3-di-*O*-methyl-6-*O*-TBDMS)-*β*-CD in SE 54; 30 m x 0.25 mm i.d.
Temperature program:	for **13**: 40 °C/0 min // 30 °C /min//80 °C/35 min//4 °C/min// 180 °C/5 min
	for **14**: 40 °C/0 min//30 °C/min//75 °C/10 min//0.5 °C /min// 100 °C/10 min//2 °C/min//180 °C/5 min
	for **15**: 40 °C/0 min//30 °C/min//75 °C/0 min//2 °C/min// 180 °C/5 min
Carrier gas:	Hydrogen (75 kPa)

The *syn*-configured isomers of 4-mercapto-2-heptanol *syn*-**3** were analyzed by panelist 1 and 2 using the following conditions:

GC/O IV:

Instrument:	HP5890 A Series II (Hewlett-Packard)
Column:	50% Octakis(2,3-di-*O*-*n*-butyryl-6-*O*-TBDMS)-*γ*-CD in SE 54; 30 m x 0.25 mm i.d.
Temperature program:	40 °C/0 min//30 °C/min//75 °C/0 min// 0.5 °C/min//93 °C/0 min//1 °C/min//180 °C/5 min
Carrier gas:	Hydrogen (75 kPa), constant pressure

The separation of the stereoisomers of 4-mercapto-2-hexanol **2** was performed under the following conditions. Sensory analysis was performed by panelist 1.

GC/O V:

Instrument:	HP5890 A Series II
Column:	28% Heptakis(2,3-di-*O*-methoxymethyl-6-*O*-TBDMS)-*β*-CD in OV1701-vi; 30 m x 0.25 mm i.d.
Temperature program:	40 °C/0 min//30 °C/min//55 °C/20 min// 0.5 °C/min//85 °C/10 min//4 °C/min//180 °C/20 min
Carrier gas:	Hydrogen (75 kPa), constant pressure

Sensory evaluation of the stereoisomers of 4-mercapto-2-alkanols **1**, **2** and **4-6** was performed by panelist 3 on the following GC/O system:

GC/O VI:

Instrument:	Fractovap 4200 (Carlo Erba Instruments)
Column:	Inert Cap^{TM} Chiramix (GL Science);
	30 m x 0.25 mm i.d.; 0.25 µm film thickness
Temperature program:	60 °C/0 min//0.7 °C/min//180 °C/30 min
Carrier gas:	Hydrogen (98 kPa), constant pressure

3.5.1.4 GC-Mass Spectrometry (GC-MS)

GC-MS conditions were as follows:

Instrument:	GC 8000^{TOP} Voyager
	(CE Instruments, Hindley Green, United Kingdom)
Detector:	Fisons MD8000^{TOP}
	(Fisons Instruments, Manchester, United Kingdom)
Column:	DB-Waxetr (J&W Scientific, Agilent Technologies)
	30 m x 0.25 mm i.d.; 0.5 µm film thickness
Temperature program:	40 °C/5 min//4 °C/min//240 °C/25 min
Carrier gas:	Helium (75 kPa), constant inlet pressure
Injector:	Split injection (ratio of 1:50), 220 °C
Ionization mode:	Electron Ionization (EI), ionization energy: 70 eV
Scan mode:	m/z 30-250
MS temperature:	200 °C (ion source), 240 °C (interface)

Data acquisition was done via Xcalibur (version 1.4, Thermo Fisher Scientific).

Compound identifications were performed in total ion chromatogram (TIC) mode. The quantifications of the investigated *β*-mercapto compounds were done in single/selected (SIM) mode using the following ions (m/z): 45, 55, 102 and 148 for both diastereomers of **3**; 43, 71, 75 and 146 for **17**; 43, 55, 113 and 146 for **18**; and 55, 61, 101 and 148 for both diastereomers of **19**.

3.5.2 High Performance Liquid Chromatography (HPLC)

The semi-preparative separation of the diastereomers of 4-mercapto-2-heptanol **3** and 4-mercapto-2-nonanol **5** was carried out under the following conditions:

Instrument:	UltiMate 3000 series (Dionex, Germering, Germany)
Column:	Nucleosil 50–5 column, 250 x 8 mm i.d.
	(CS-Chromatographie, Langerwehe, Germany)
Mobil phase:	for **3**: *n*-hexane / 2-propanol (97:3, v/v)
	for **5**: *n*-hexane / 2-propanol (96:4, v/v)
Flow rate:	4.0 mL/min (30 °C)
Detector:	Wavelength detector-3100
	for **3**: adjusted to 220 nm
	for **5**: adjusted to 200 nm
Injection volume:	250 µL
Sample concentration:	for **3**: 1 µL/mL in *n*-hexane
	for **5**: 10 mg/mL in *n*-hexane

The purification of *(S)-MαNP thioesters of (R)- and (S)*-configured 4-mercapto-2-octanone **21** was obtained by repetitive HPLC fractionations using following conditions:

Instrument:	UltiMate 3000 series (Dionex)
Column:	Nucleosil 50–5 column, 250 x 8 mm i.d.
	(CS-Chromatographie)
Mobil phase:	*n*-hexane / 2-propanol (96:4, v/v)
Flow rate:	2.0 mL/min (30 °C)
Detector:	Wavelength detector-3100, adjusted to 254 nm
Injection volume:	350 µL
Sample concentration:	for (*S*)-**21**: 34.1 mg/mL in *n*-hexane /2-propanol (96:4, v/v)
	for (*R*)-**21**: 23.3 mg/mL in *n*-hexane /2-propanol (96:4, v/v)

3.5.3 Nuclear Magnetic Resonance Spectroscopy (NMR)

^{1}H NMR and ^{13}C NMR spectra were recorded at 500 MHz and 126 MHz, respectively with Avance500 spectrometers (Bruker, Billerica, MA, USA).

^{1}H-detected experiments were done with an inverse ^{1}H/^{13}C probehead, direct ^{13}C-measurements were performed with a QNP ^{13}C/^{31}P/^{29}Si/^{19}F/^{1}H cryoprobe. The experiments were done in full automation using standard parameter sets of the TOPSPIN 3.0 software package (Bruker).

^{13}C NMR spectra were recorded in proton-decoupled mode. The compounds were dissolved in deuterated chloroform. The spectra were recorded at 27 °C. All signals were assigned by proton-proton and proton-carbon correlation experiments (COSY, HSQC and HMBC). Data processing was typically done with the MestreNova software (Mestrelab Research, Santiago de Compostela, Spain).

3.5.4 Determination of Optical Rotations

Optical rotations were measured on a Polartronic-E polarimeter (Schmidt & Haensch, Berlin, Germany) fitted with a measuring cell (path length 1 dm) and a sodium lamp (wavelength 589 nm). (*S*)- and (*R*)-configured 4-mercapto-2-octanone **21** as well as (*R*)-configured 4-acetylthio-2-octanone (*R*)-**10** were diluted in ethanol and the measurements were performed at a temperature of 21 °C. (*S*)-**21**: $[\alpha]_D$ +17.0, concentration: 2.07 g/100 mL, purity: 97% by GC, enantiomeric excess (ee): 75.9%; (*R*)-**10**: $[\alpha]_D$ +15.6, concentration: 0.96 g/mL, purity: 98% by GC, ee: 82.8%; (*R*)-**21**: $[\alpha]_D$ -16.9, concentration: 1.16 g/mL, purity: 97.5% by GC, ee: 71.5%.

3.6 Investigation of *β*-Mercapto Compounds in Bell Pepper

3.6.1 Preparation of Bell Pepper Extracts

Simultaneous Distillation-Extraction (SDE)

500 g of sliced pericarp was homogenized (Moulinex Turbo blender, Alençon, France) with 350 mL of distilled water. The slurry was transferred into a 2 L round-bottom flask, rinsing the blender with 150 mL of distilled water. The homogenate was continuously extracted for 90 min with 150 mL of a Et_2O/*n*-pentane mixture (1:1, v/v) in a modified Likens-Nickerson apparatus (Schultz *et al*., 1977). The obtained organic extract was dried over sodium sulfate and concentrated at ~ 40 °C to a volume of 1 mL using a 30 cm x 2 cm i.d. Vigreux-column. The sample was stored at -18 °C prior to the thiol-selective enrichment.

Liquid-Liquid Extraction (LLE)

500 g of pericarp were sliced and homogenized (Moulinex Turbo blender) without adding water. The purée was centrifugated at 2500 rpm (himacCT6EL, Tokyo, Japan) for 5 min and pressed through a sieve to obtain the juice. After adding 250 mL of distilled water to the remaining purée, the mixture was again centrifuged (2500 rpm, 5 min) and filtered. The juice was transferred into a Kutscher-Steudel liquid-liquid extractor (Wieland and Sucrow, 1982) and distilled water was filled up to 1 L. Extraction was performed for 24 h using 150 mL of an Et_2O/*n*-pentane mixture (1:1, v/v) as solvent. The obtained organic extract was dried with sodium sulfate and concentrated to a volume of 1 mL using a 30 cm x 2 cm i.d. Vigreux-column. The sample was stored at - 18 °C prior to the thiol-selective enrichment.

3.6.2 Thiol Enrichment via Affinity Chromatography

3.6.2.1 Preparation of Mercurated Affi-Gel 10

The derivatization of Affi-Gel 10 was performed as previously described (Steinhaus *et al*., 2007). 25 mL of Affi-Gel 10 was transferred into a Buchner funnel and washed with 75 mL of 2-propanol. After washing, 0.38 g of *p*-aminophenylmercury acetate dissolved in 8 mL of N,N-dimethylformamide (DMF) was added and the mixture was stirred at RT. After four hours, 0.25 mL of ethanolamine was added to the gel slurry and the mixture was stirred for another hour.

Finally, the mercurated gel slurry was transferred into a Buchner funnel and washed with 63 mL of DMF followed by 175 mL of 2-propanol. After the final wash, the mercurated Affi-Gel 10 was suspended in 75 mL of 2-propanol and stored at 4 °C.

3.6.2.2 Thiol Enrichment via Mercurated Affi-Gel 10

A Pasteur pipet (0.5 cm i.d.) was loaded with mercurated Affi-Gel 10 (~ 2.0-2.5 cm, cotton wool at the bottom). In analogy to Schneider *et al.* (2003), the gel was conditioned with 5 mL of 2-propanol and 5 mL of an *n*-pentane/dichloromethane mixture (2:1, v/v). The extracts obtained from 4 kg of bell peppers (8 x 1 mL; after isolation via SDE or LLE) were pooled. The pooled extract of cooked red bell peppers was directly applied onto the gel whereas the pooled extract of raw bell peppers was first divided into two aliquots (2 x 4 mL of extract diluted in 4 mL of an *n*-pentane/Et_2O mixture (1:1, v/v)). The non-thiol compounds were removed by washing the gel with 25 mL of an *n*-pentane/dichloromethane mixture (2:1, v/v). The thiols were liberated by elution with 4 mL of a 10 mM solution of DL-dithiothreitol in an *n*-pentane/dichloromethane mixture (2:1, v/v). DL-Dithiothreitol was removed by washing the thiol extract with 1 mL of distilled water. After drying over sodium sulfate, the extract was concentrated to a volume of 1 mL under a nitrogen flow for GC-MS and GC-FID analysis, and subsequently to ~ 200 µL for the analysis of cooked red bell pepper extracts and to ~ 100 µL for the analysis of green cooked and raw red bell pepper extracts via enantioselective MDGC.

3.6.3 Quantitative Estimations of *β*-Mercapto Compounds in Cooked Bell Pepper Extracts

Quantitations were performed in a two-step procedure: (i) The concentrations of **17** and **18** as well as of the diastereomeric pairs of **3** and **19** were estimated in extracts of cooked bell pepper via GC-MS in SIM mode based on external calibration curves with synthesized reference substances. Recovery rates of the thiols determined for the enrichment-step using mercurated Affi-Gel 10 were taken into account. (ii) Based on the estimated concentrations of **17**, **18** and the diastereomeric pairs of **3** and **19**, the concentrations of the respective stereoisomers were calculated using the ratios determined via enantio-MDGC.

3.6.3.1 External Calibration Curves

Four stock solutions containing synthesized **17**, **18**, **3** or **19** (each 1 mg/mL in Et_2O) were prepared and stepwise diluted. Four concentrations in the following ranges were analyzed by GC-MS in the SIM mode in triplicate analyses: 24-9460 ng/mL for **17**; 60-2547 ng/mL for **18**; 20-1999 ng/mL for *anti*-**3**; 30-2992 ng/mL for *syn*-**3**; 170-2712 ng/mL for **19** (first eluting diastereomer) and 175-2806 ng/mL for **19** (second eluting diastereomer). Plotting the integrated peak areas against the concentrations (ng/mL) resulted in following linear regressions: y = 157.21x + 40868 (R^2 = 0.982) for **17**; y = 295.19x - 7918 (R^2 = 0.947) for **18**; y = 120.97x + 7708 (R^2 = 0.998) for *anti*-**3**; y = 126.57x + 4725 (R^2 = 0.996) for *syn*-**3**; y = 194.66x - 42596 (R^2 = 0.937) for **19** (diastereomer I) and y = 193.23x - 45284 (R^2 = 0.943) for **19** (diastereomer II).

3.6.3.2 Recovery Rates

Recovery rates of **3**, **17-19** after affinity chromatography were determined in triplicate from model experiments. Stock solutions of **3**, **17-19** with concentrations of 5 µg/mL in *n*-pentane/Et_2O (1:1, v/v) were prepared; 1 mL of each stock solution was added to 7 mL of an *n*-pentane/Et_2O (1:1, v/v) mixture. After stirring, the mixture was applied onto the mercurated Affi-Gel 10.

The recovery rate is calculated by the integrated peak area A_{x_i} of the investigated substance x_i, which is determined in the SDE extract, and the peak area A_{x_0} of substance x_i in the stock solution (equation [9]).

$$\text{Recovery } (\%) = \frac{A_{x_i}}{A_{x_0}} \, 100 \qquad [9]$$

The following recovery rates were determined: 2-mercapto-4-heptanone **17** (65 ± 11%), 4-mercapto-2-heptanone **18** (64 ± 6%), 4-mercapto-2-heptanol **3** (*anti*: 64 ± 10%, *syn*: 66 ± 9%) and 2-mercapto-4-heptanol **19** (diastereomer I: 73 ± 17%, diastereomer II: 67 ± 15%).

3.6.3.3 Determination of Limits of Detection and Limits of Quantitations

The instrumental limits of detection (LOD) and limits of quantitations (LOQ) of 2-mercapto-4-heptanone **17**, 4-mercapto-2-heptanone **18** as well as of the diastereomers of 4-mercapto-2-heptanol **3** and 2-mercapto-4-heptanol **19** were determined according to the method described by Vogelgesang and Hädrich (1998).
To this end, a series of six dilutions of each reference compound was analyzed in triplicate ranging from 7-1141 ng/mL by GC-MS in SIM mode. The integrated peak areas were plotted against the used concentrations. The relationship is described by the equation y = a + bx.

The residual standard deviation is calculated by the calibration line using the number of measured values n, the index of calibration analyses i, the peak area y_i of the substance x, the y-intercept a, the fortification concentration x_i of sample i, and the slope of the calibration line b (equation [10]).

$$s_y = \sqrt{\frac{\sum_{i=1}^{n}\left(y_i - (a + bx_i)\right)^2}{n-2}} \qquad [10]$$

The limit of detection (equation [11]) and the limit of quantitation (equation [12]) are obtained from the linear regression analysis with the quantil $t_{f;a}$ of t-distribution for $f = n\text{-}2$ degrees of freedom and a probability of 95%, the mean value $\bar{x}$ of all concentrations, the mean value $\bar{y}$ of the signal value of all calibration analyses, and the identification limit $ID = 2\ LOD$.

$$LOD = \frac{s_y}{b} t_{f;a} \sqrt{1 + \frac{1}{n} + \frac{\bar{x}^2}{\sum_{i=1}^{n}(x_i - \bar{x})^2}} \qquad [11]$$

$$LOQ = \frac{\left(\left(\bar{y} + b(ID - \bar{x}) + s_y t_{f;a} \sqrt{1 + \frac{1}{n} + \frac{(ID - \bar{x})^2}{\sum_{i=1}^{n}(x_i - \bar{x})^2}}\right) - a\right)}{b} \qquad [12]$$

The calculated limits of detection and limits of quantitation of the investigated compounds (LOD; LOQ) are as follows: *anti*-configured 4-mercapto-2-heptanol *anti*-**3** (32 ng/mL; 93 ng/mL), *syn*-configured 4-mercapto-2-heptanol *syn*-**3** (52 ng/mL; 152 ng/mL), 2-mercapto-4-heptanone **17** (90 ng/mL; 267 ng/mL), 4-mercapto-2-heptanone **18** (68 ng/mL; 202 ng/mL), first eluting diastereomer of 2-mercapto-4-heptanol **19** (116 ng/mL; 346 ng/mL) and for the second eluting diastereomer of 2-mercapto-4-heptanol **19** (110 ng/mL; 326 ng/mL).

4 RESULTS AND DISCUSSION

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

4.1.1 Syntheses

4-Mercapto-2-heptanol **3** and its acetyl derivatives **13-15** were prepared as outlined in Figure 10. 4-Mercapto-2-heptanol **3** was synthesized by Michael-type addition of thioacetic acid to 3-hepten-2-one and subsequent reduction of the formed 4-acetylthio-2-heptanone **9** with lithium aluminum hydride.

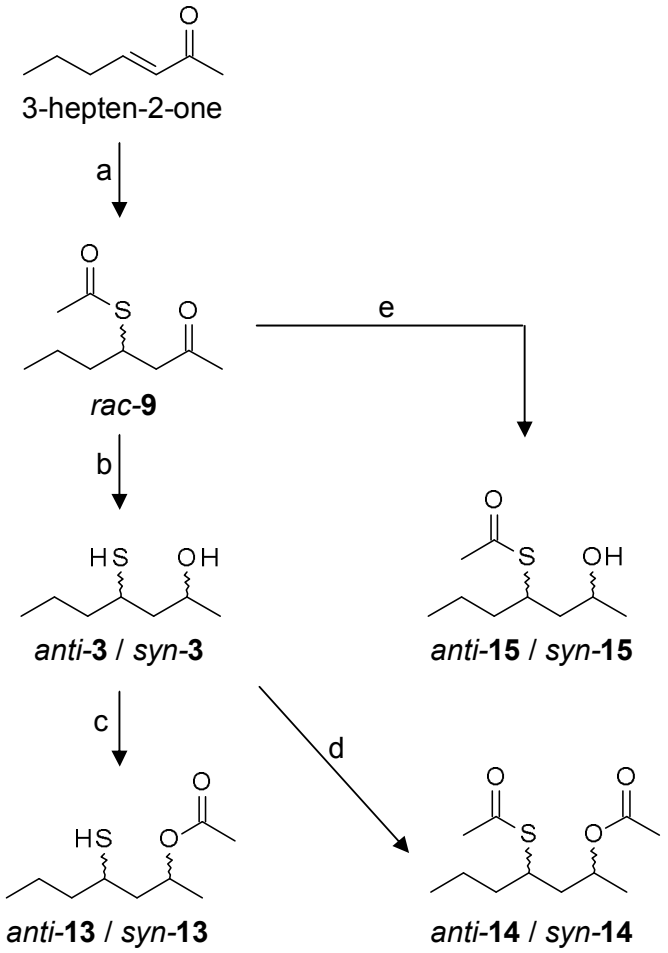


Figure 10. Syntheses of the stereoisomers of 4-mercapto-2-heptanol **3**, 4-mercapto-2-heptyl acetate **13**, 4-acetylthio-2-heptyl acetate **14**, and 4-acetylthio-2-heptanol **15**.

(a) thioacetic acid; (b) lithium aluminum hydride, tetrahydrofuran; (c) acetyl chloride, Et_2O, pyridine; (d) acetic anhydride, 4-(dimethylamino)pyridine, pyridine; (e) sodium borohydride, phosphate buffer

The selective *O*-acetylation of **3** resulting in 4-mercapto-2-heptyl acetate **13** was achieved by using acetyl chloride. 4-Acetylthio-2-heptyl acetate **14** was obtained by treatment of **3** with acetic anhydride. The reduction of 4-acetylthio-2-heptanone **9** with sodium borohydride yielded 4-acetylthiol-2-heptanol **15**.

4.1.2 GC Separations

The capillary gas chromatographic separations of the diastereomers and enantiomeric pairs of 4-mercapto-2-heptanol **3** and the corresponding acetyl derivatives **13-15** are shown in Figure 11.

In case of **3**, the reaction sequence resulted in a 39:61 mixture of diastereomers (Figure 11A). The chromatographic and mass spectrometric data were in agreement with those previously reported (Naef *et al*., 2008; Polster, 2012; Polster and Schieberle, 2017). GC analysis of **3** using heptakis(2,3-di-*O*-acetyl-6-*O*-TBDMS)-*β*-CD as chiral stationary phase enabled the separation of the four stereoisomers.

4-Mercapto-2-heptyl acetate **13**, 4-acetylthio-2-heptyl acetate **14** and 4-acetylthio-2-heptanol **15** are described here for the first time. A comparison of the DB-Wax chromatogram shown in Figure 11D to those depicted in Figures 11A and 11B demonstrates that under the employed experimental conditions the reported intramolecular acetyl transfer (resulting in **13**) and deacetylation (resulting in **3**) upon reduction with sodium borohydride occurred only to a minor extent (Trost *et al*., 1971; Rowe and Tangel, 1999). The use of heptakis(2,3-di-*O*-methyl-6-*O*-TBDMS)-*β*-CD as chiral stationary phase enabled the separation of the four stereoisomers of each acetyl ester **13**-**15**.

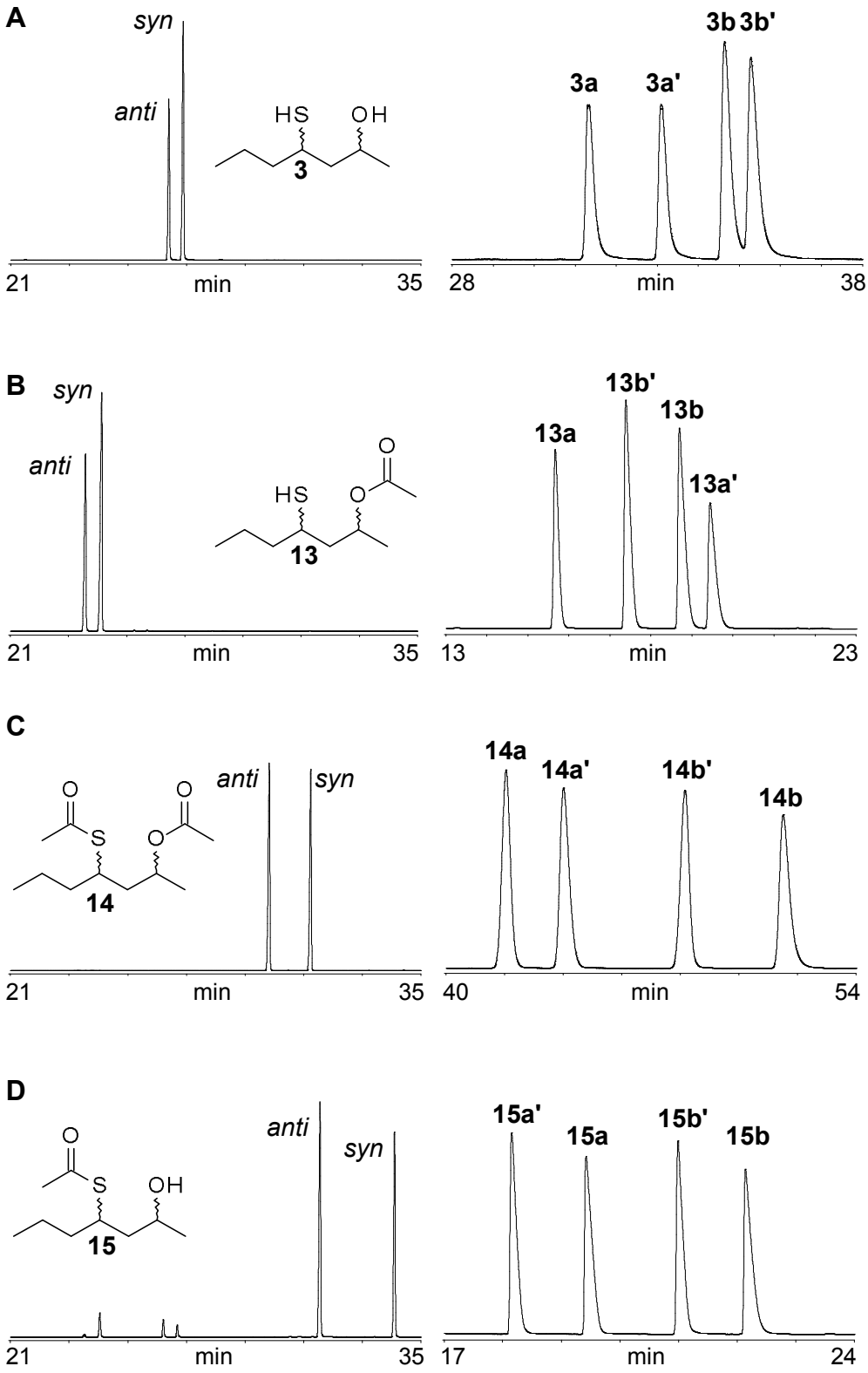


Figure 11. GC separation of the diastereomers and the enantiomeric pairs of (**A**) 4-mercapto-2-heptanol **3**, (**B**) 4-mercapto-2-heptyl acetate **13**, (**C**) 4-acetylthio-2-heptyl acetate **14**, and (**D**) 4-acetylthio-2-heptanol **15**. For conditions, see Materials and Methods (GC-FID systems I, IV and V).

4.1.3 Determination of the Absolute Configurations

4.1.3.1 Assignment of the Order of Elution of the Stereoisomers of 4-Mercapto-2-heptanol

The GC order of elution of the stereoisomers of 4-mercapto-2-heptanol **3** was determined by assigning the absolute configurations via NMR analysis in combination with lipase-catalyzed kinetic resolution. The sequence employed to determine the absolute configurations of the stereoisomers of **3** is shown in Figure 12.

Step (1) HPLC-analysis of **3** on a normal phase silica column resulted in a separation sufficient for preparative isolation of the diastereomers.

Step (2) As shown in the table, the NMR data of the first eluted diastereomer were in excellent agreement with the data reported for *anti*-configured (2*S*,4*S*)-**3**, the product obtained from 3-hepten-2-one utilizing tandem Michael addition-Meerwein-Ponndorf-Verley reduction (Ozeki *et al.*, 2004). Therefore, the diastereomer eluted first from the HPLC column constitutes the pair of *anti*-configured (2*S*,4*S*)- and (2*R*,4*R*)-enantiomers.

Step (3) Reinvestigation by GC showed that this HPLC fraction corresponded to the first eluted peak in the capillary gas chromatogram and to the first pair of stereoisomers separated on the chiral stationary phase.

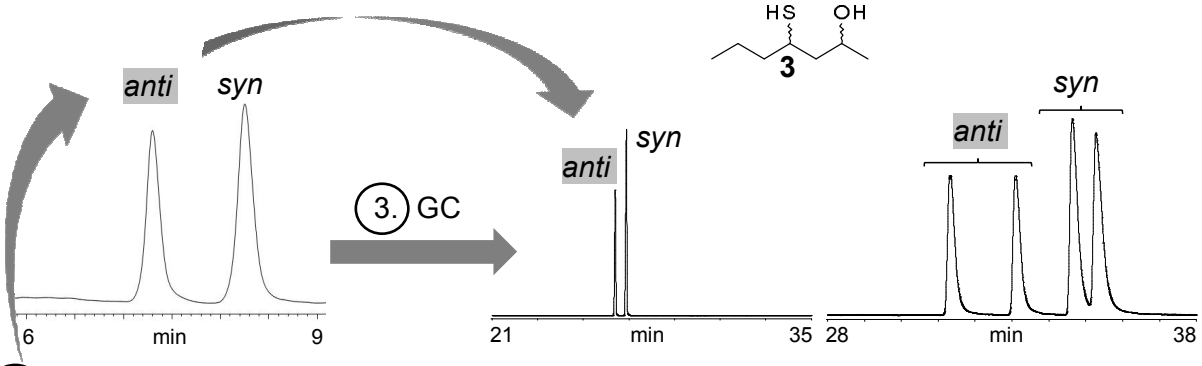
Step (4) Racemic 4-acetylthio-2-heptanone *rac*-**9** was subjected to kinetic resolution via lipase-catalyzed hydrolysis. In accordance with the stereochemical course described for this reaction, porcine pancreas lipase (PPL)-mediated hydrolysis of *rac*-**9** resulted in the liberation of (*R*)-configured 4-mercapto-2-heptanone (*R*)-**18** (enantiomeric ratio, er (%) = 62:38) as product and (*S*)-configured 4-acetylthio-2-heptanone (*S*)-**9** with high optical purity (er (%) = 98:2) as remaining substrate (Wakabayashi *et al*., 2011).

The enantiomeric excesses were determined by capillary gas chromatography using CycloSil-B as chiral stationary phase (for conditions, see Materials and Method, GC-FID system IV).

After separation of the reaction mixture by silica gel column chromatography, the isolated remaining substrate (*S*)-**9** was subjected to reduction using lithium aluminum hydride.

Step (5) Enantioselective GC analysis demonstrated that the resulting mixture of (4*S*)-configured diastereomers co-eluted with the second peaks of the pairs of stereoisomers obtained for **3**. Taking into account the assignment of the *anti*- and *syn*-diastereomers achieved in the first steps, the absolute configurations and the order of elution of the four stereoisomers of **3** could be assigned as shown in Figure 11A as (2*R*,4*R*) **3a** before (2*S*,4*S*) **3a'** and (2*S*,4*R*) **3b** before (2*R*,4*S*) **3b'** using heptakis(2,3-di-*O*-acetyl-6-*O*-TBDMS)-*β*-CD as chiral stationary phase.

1. HPLC separation of the diastereomers



2. NMR analysis of the separated isomers

position	δ, ^{1}H, chemical shifts, ppm		
	HPLC		Ozeki *et al*. (2004)
	isomer **I**	isomer **II**	*anti*-(2*S*,4*S*)-4-mercapto-2-heptanol
H-2	4.14, (ddt), 1H	4.01, (dqd), 1H	4.15, (ddq), 1H
H-4	3.08-2.99, (m), 1H	2.90-2.81, (m), 1H	3.09-2.99, (m), 1H
H-3a	1.79-1.71, (m), 1H	1.79-1.71, (m), 1H	1.76, (ddd), 1H
H-3b, H5, H-6	1.69-1.36, (m), 5H	1.69-1.36, (m), 5H	1.65-1.40, (m), 4H + 1.37, (d), 1H
H-1	1.23, (d), 3H	1.19, (d), 3H	1.23, (d), 3H
H-7	0.92, (t), 3H	0.92, (t), 3H	0.92, (t), 3H

4. Lipase-catalyzed kinetic resolution

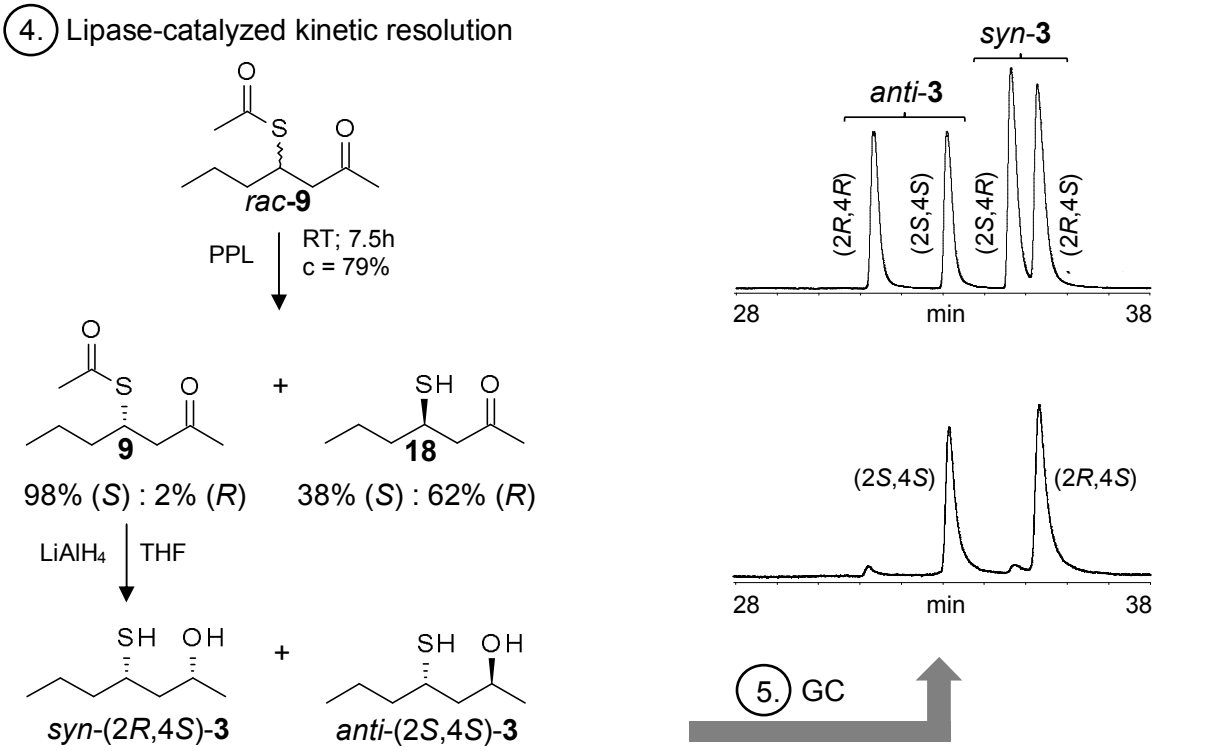


Figure 12. Approach to assign the GC elution order of the stereoisomers of 4-mercapto-2-heptanol **3**.

4.1.3.2 Assignment of the Order of Elution of the Stereoisomers of the Acetates of 4-Mercapto-2-heptanol

4-Mercapto-2-heptyl acetate

The assignment of the order of elution of 4-mercapto-2-heptyl acetate **13** was based on the following procedure:

Step (1) Separation of the diastereomers of 4-acetylthio-2-heptanol **15** via column chromatography on silica gel. A direct LC-separation of the diastereomers of **13** could not be achieved.

Step (2) Deacetylation of the separated *syn*-diastereomer of **15** by reduction with lithium aluminum hydride resulted in the formation of *syn*-**3**.

Step (3) *O*-acetylation of *syn*-**3** using acetyl chloride and assignment of the obtained *syn*-**13** by comparison of the GC retention time of the resulting diastereomer to those of the reference sample (Figure 11B, chapter 4.1.2).

Step (4) Preparation of the (4*S*)-configured diastereomers (4*S*)-**13** by selective *O*-acetylation of the (4*S*)-configured diastereomers of 4-mercapto-2-heptanol (4*S*)-**3** obtained via PPL-mediated hydrolysis of racemic 4-acetylthio-2-heptanone *rac*-**9** and subsequent reduction with lithium aluminum hydride.

Step (5) The comparison of the GC chromatograms of *syn*-**13** and (4*S*)-**13** resulted in the following order of elution of the stereoisomers using CycloSil-B as chiral stationary phase: (2*R*,4*R*) **13a** before (2*R*,4*S*) **13b'** and (2*S*,4*R*) **13b** before (2*S*,4*S*) **13a'**.

4-Acetylthio-2-heptyl acetate

The assignment of the order of elution of the stereoisomers of 4-acetylthio-2-heptyl acetate **14** was based on the following procedure in analogy to the strategy employed for 4-mercapto-2-heptanol **3**.

Step (1) Separation of the diastereomers of **14** via column chromatography on silica gel.

Step (2) Deacetylation of the separated diastereomers of **14** by alkaline cleavage and assignment of the *anti*-/*syn*-configurations by comparison of the GC retention times of the resulting stereoisomers to those of 4-mercapto-2-heptanol **3** (Figure 11A, chapter 4.1.2).

Step (3) Preparation of the (4*S*)-configured diastereomers of 4-acetylthio-2-heptyl acetate (4*S*)-**14** by acetylation of (4*S*)-4-mercapto-2-heptanol (4*S*)-**3** obtained via lipase-catalyzed kinetic resolution of racemic 4-acetylthio-2-heptanone *rac*-**9** and subsequent reduction with $LiAlH_4$.

Step (4) GC analysis was performed using CycloSil-B as chiral stationary phase. Taking into account the assignment of the *anti*- and *syn*-diastereomers achieved in the first steps, the absolute configurations and the order of elution of the four stereoisomers of **14** could be assigned as (2*R*,4*R*) **14a** before (2*S*,4*S*) **14a'** and (2*R*,4*S*) **14b'** before (2*S*,4*R*) **14b** (Figure 11C, chapter 4.1.2).

4-Acetylthio-2-heptanol

The same procedure as described for **14** was applied to assign the order of elution of the stereoisomers of 4-acetylthio-2-heptanol **15**, except that in step (3) the (4*S*)-configured diastereomers were prepared by reduction of (*S*)-configured 4-acetylthio-2-heptanone (*S*)-**9** obtained via lipase-catalyzed kinetic resolution of racemic 4-acetylthio-2-heptanone *rac*-**9** and subsequent reduction with sodium borohydride. As shown in Figure 11D, the order of elution of the stereoisomers of **15** could be assigned as (2*S*,4*S*) **15a'** before (2*R*,4*R*) **15a** and (2*R*,4*S*) **15b'** before (2*S*,4*R*) **15b** using CycloSil-B as chiral stationary phase.

4.1.4 Corrigendum of the Assignment of the Absolute Configurations of 4-Mercapto-2-heptanol and Its Acetyl Derivatives

In 2016, Kiske *et al*. reinvestigated the configurations of 4-mercapto-2-pentanone **20**, 2-mercapto-4-heptanone **17** and 4-mercapto-2-heptanone **18** by vibrational circular dichroism (VCD) and ^{1}H NMR analyses of (*R*)-hydratropic acid thioesters (HTA) and 2-methoxy-2-phenylacetic acid (MPA) thioesters. The determined absolute configurations of the enantiomers of the investigated *β*-mercaptoalkanones were not in agreement with those determined via the ^{1}H NMR anisotropy method using (*S*)-2-methoxy-2-(1-naphthyl)propionic acid (MαNP) as chiral reagent (Wakabayashi *et al*., 2011 and 2015). As a consequence, the previous assignments of the absolute configurations of 4-mercapto-2-heptanol **3** and its derivatives **13-15** had to be revised since their assignments were based on the absolute configuration of 4-mercapto-2-heptanone **18** determined by Wakabayashi *et al*. (2011).

4.1.4.1 Reinvestigation of the Absolute Configurations of ß-Mercaptoalkanones

The absolute configurations of the enantiomers of 4-mercapto-2-alkanones (C5-C10) have been assigned for the first time by Wakabayashi *et al*. (2011, 2012 and 2015).
To this end, synthesized 4-acetylthio-2-alkanones were subjected to enzyme-catalyzed kinetic resolution to obtain enantiomerically enriched 4-mercapto-2-alkanones which were derivatized with (*S*)-MαNP. The resulting thioesters were separated via HPLC and analyzed by ^{1}H NMR analysis. The absolute configurations of the synthesized diastereomeric (*S*)-MαNP thioesters were assigned by the sector rule as previously established for MαNP esters of secondary alcohols (Taji *et al*., 2002). According to this method, the order of elution of the (*S*)-MαNP thioesters was (*S*,*R*) before (*S*,*S*) by HPLC. GC analysis of the 4-mercapto-2-alkanones revealed an order of elution of (*R*) before (*S*) using heptakis(2,3-di-*O*-methyl-6-*O*-TBDMS)-*β*-CD as chiral stationary phase.

Recently, *ß*-mercaptoalkanones such as 4-mercapto-2-pentanone **20** and 4-mercapto-2-heptanone **18** were analyzed by VCD, a powerful tool for the determination of the absolute configuration of chiral compounds (Kiske *et al*., 2016). The configurations determined for **18** and **20** by means of VCD were not in agreement with those previously reported by Wakabayashi *et al*. (2011, 2012 and 2015). To substantiate this result, the enantiomers of **18** and **20**, analyzed via VCD, were also derivatized with (*S*)-MαNP in the presence of N,N'-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) and analyzed via HPLC, as previously described (Wakabayshi *et al*., 2011 and 2015). The HPLC analyses of the (*S*)-MαNP thioesters of **18** and **20** resulted in reversed orders of elution compared to those determined by Wakabayashi *et al*. (2011 and 2015).

Next, Kiske *et al*. (2016) decided to examine the NMR behavior of (*R*)-HTA and (*S*)- or (*R*)-MPA thioesters of the enantiomers of **18** and **20**. The determined absolute configurations were in agreement with those obtained via VCD and were therefore opposite to the assignments of the respective MαNP thioesters (Wakabayashi *et al*., 2011, 2012 and 2015). The order of elution of the enantiomers of **18** and **20** using heptakis(2,3-di-*O*-methyl-6-*O*-TBDMS)-*ß*-CD as chiral stationary phase was (*S*) before (*R*).

Considering these conflicting results, Kiske *et al*. (2016) tried to elucidate why the model applied by Wakabayashi *et al*. (2011, 2012 and 2015) is not suitable for the determination of the absolute configuration of *ß*-mercaptoalkanones. To this end, (*R*)-2-mercapto-4-heptanone (*R*)-**17** and (*R*)-2-heptanethiol were derivatized with (*R*)- and (*S*)-MPA and analyzed via ^{1}H NMR. The evaluation based on the model developed by Porto *et al*. (2007), suitable for the assignment of the absolute configuration of chiral secondary thiols, resulted in the (*R*)-configuration for both thiols showing that the presence of an additional carbonyl group has no influence on the assignment of the absolute configuration. Moreover, (*R*)-configured 2-mercapto-4-heptanone (*R*)-**17**, 2-heptanethiol and 2-heptanol were derivatized with (*S*)- and (*R*)-MαNP, analyzed via ^{1}H NMR and subjected to the model of Porto *et al*. (2007). The consideration of the ^{1}H chemical shifts caused by the naphthyl group of MαNP resulted in the (*R*)-configuration for the investigated thiols and in the (*S*)-configuration for 2-heptanol. This phenomenon may result from the different predominating conformers. Conformational analysis showed that the *syn* periplanar conformation is the preferred conformation of MαNP and MPA esters.

In contrast, the *anti* periplanar conformation is dominating for MPA thioesters (Kasai *et al*., 2007; Porto *et al*., 2007). This indicates that MαNP thioesters also prefer an *anti* periplanar conformation which would cause different anisotropy effects compared to the respective MαNP esters. Based on these results, Kiske *et al*. (2016) adapted the sector rule of Taji *et al*. (2002) for the assignment of the absolute configurations of chiral *β*-mercaptoalkanones. The formula as well as an example of use are shown in Figure 21 (chapter 4.2.2).

4.1.4.2 Revised Assignment of the Orders of Elution of the Stereoisomers of 4-Mercapto-2-heptanol and Its Acetyl Derivatives

The assignments of the absolute configurations of the stereoisomers of 4-mercapto-2-heptanol **3** and its derivatives **13-15** as reported in chapter 4.1.3 were corrected taking into account the determined absolute configuration of 4-mercapto-2-heptanone **18** by Kiske *et al*. (2016).

In case of 4-mercapto-2-heptanol **3**, the determination of the absolute configuration is still based on the procedure described in chapter 4.1.3.1 using the combination of ^{1}H NMR analysis and enzyme-catalyzed kinetic resolution. Considering the reversed order of elution of the enantiomers of 4-mercapto-2-heptanone **18** determined by Kiske *et al*. (2016), the PPL-mediated hydrolysis of racemic 4-acetylthio-2-heptanone *rac*-**9** results in the formation of the (*S*)-configured thiol (*S*)-**18** as product and the (*R*)-configured 4-acetylthio-2-heptanone (*R*)-**9** as remaining substrate with high optical purity (Figure 13). Subjecting isolated 4-acetylthio-2-heptanone (*R*)-**9** to reduction with $LiAlH_4$ results in the formation of the (4*R*)-configured diastereomers of 4-mercapto-2-heptanol **3**, respectively. Taking into account the assignment of the *anti*- and *syn*-diastereomers as described in chapter 4.1.3.1, the absolute configurations and the order of elution of the four stereoisomers of 4-mercapto-2-heptanol **3** could be assigned as (2*S*,4*S*) **3a** before (2*R*,4*R*) **3a'** and (2*R*,4*S*) **3b** before (2*S*,4*R*) **3b'** using heptakis(2,3-di-*O*-acetyl-6-*O*-TBDMS)-*ß*-cyclodextrin as stationary phase (Figure 14A).

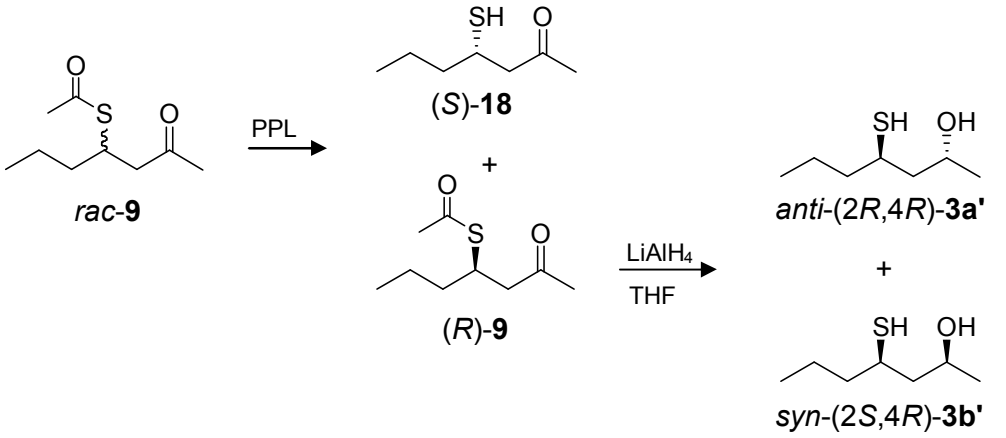


Figure 13. Revised stereochemical course of the lipase-catalyzed resolution of racemic 4-acetylthio-2-heptanone *rac*-**9** resulting in the formation of (4*R*)-configured diastereomers of 4-mercapto-2-heptanol **3a'** and **3b'** after reduction of (*R*)-**9** with $LiAlH_4$.

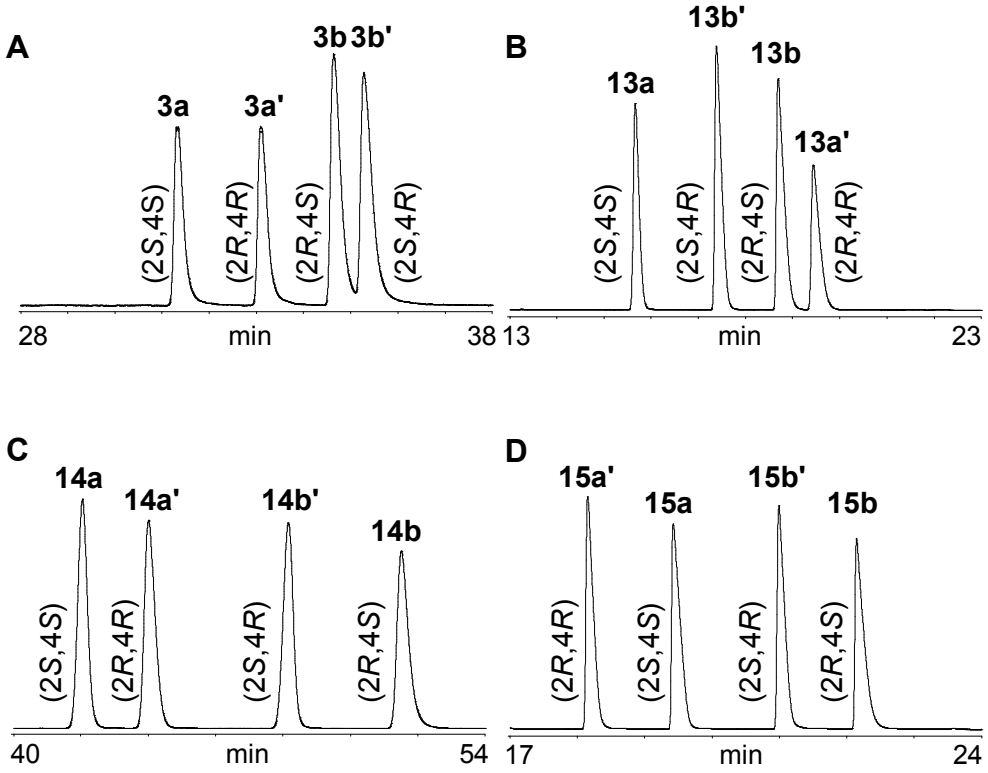


Figure 14. Revised GC order of elution of the enantiomeric pairs of (**A**) 4-mercapto-2-heptanol **3**, (**B**) 4-mercapto-2-heptyl acetate **13**, (**C**) 4-acetylthio-2-heptyl acetate **14**, (**D**) 4-acetylthio-2-heptanol **15** taking into account the reinvestigation of the absolute configuration of 4-mercapto-2-heptanone **18** by Kiske *et al*. (2016). For conditions, see Materials and Methods (GC-FID systems IV and V).

In analogy to the procedure described for the correction of the absolute configuration of the stereoisomers of 4-mercapto-2-heptanol **3**, the revised GC orders of elution of the stereoisomers of 4-mercapto-2-heptyl acetate **13**, 4-acetylthio-2-heptyl acetate **14** and 4-acetylthio-2-heptanol **15** separated on heptakis(2,3-di-*O*-methyl-6-*O*-TBDMS)-*ß*-cyclodextrin are shown in Figure 14 (B-D).

4.1.5 Sensory Evaluation

4.1.5.1 Experimental Set-Up

The odor properties of the stereoisomers of 4-mercapto-2-heptanol **3** and the corresponding esters **13-15** were determined by GC/O using the method described by Ullrich and Grosch (1987). The following measures were taken to reduce the potential uncertainties associated with this approach:

(i) The panelist considered a concentration level only as odor threshold if it was the lowest dilution step at which the odor was consistently perceived in three consecutive GC/O runs.

(ii) For the *anti*- and *syn*-diastereomers, the sensory evaluations were performed in duplicate starting from two separately prepared stock solutions.

(iii) In order to rule out that the sensory evaluation of 4-acetylthio-2-heptanol **15** might be affected by the previously reported re-arrangement to 4-mercapto-2-heptyl acetate **13** (see chapter 4.1.2), the stability of this substance in the solvent Et_2O was checked by periodic GC analysis of the stored stock solution.

(iv) For the pair of enantiomers showing the lowest resolution ((2*R*,4*S*) and (2*S*,4*R*)-4-mercapto-2-heptanol; peaks **3b** and **3b'** in Figure 14A), the sensory assessment was performed by a second panelist (panelist 2).

In addition, another chiral stationary phase was employed to rule out that the determination of the odor thresholds might be hampered by odor adaptation or saturation effects as indicated for long-lasting odors (Begnaud *et al*., 2006).
It is known that odor qualities may show pronounced concentration-dependent changes. For the purpose of comparison, the sensory assessments were performed at amounts of approximately 6 ng at the sniffing port for each stereoisomer.

4.1.5.2 Determination of Odor Thresholds

The odor thresholds obtained for the *anti*- and *syn*-diastereomers via GC/O on an achiral stationary phase and for the enantiomeric pairs of 4-mercapto-2-heptanol **3** and its acetates **13-15** employing chiral stationary phases are summarized in Table 9.

Table 9. Odor thresholds of the stereoisomers of 4-mercapto-2-heptanol **3** and the corresponding acetyl esters **13-15** determined by GC/O.

no.	compound	odor thresholds of the stereoisomers in air (ng/L)							
		anti[a]		*syn*[a]		a (*2S,4S*)	a' (*2R,4R*)	b (*2R,4S*)	b' (*2S,4R*)
3	4-mercapto-2-heptanol	0.01	0.01	0.08	0.08	0.1[b]	0.05[b]	0.2[b]	0.3[b]
13	4-mercapto-2-heptyl acetate	0.07	0.07	0.2	0.2	2.1[c]	0.03[c]	0.2[c]	6.1[c]
14	4-acetylthio-2-heptyl acetate	0.01	0.01	0.3	1.0	5.5[c]	0.09[c]	0.3[c]	1.3[c]
15	4-acetylthio-2-heptanol	0.09	0.14	4.6	1.2	17.2[c]	0.03[c]	0.2[c]	4.9[c]

Odor thresholds were determined by GC/O using fused silica capillary columns coated with: [a] DB-Wax; sensory evaluations of the diastereomers were done in duplicate; [b] heptakis(2,3-di-*O*-acetyl-6-*O*-TBDMS)-*β*-CD; [c] heptakis(2,3-di-*O*-methyl-6-*O*-TBDMS)-*β*-CD; for further conditions, see Materials and Methods (GC/O systems I-III).

The sensory evaluations of the diastereomers of 4-mercapto-2-heptanol **3** and 4-mercapto-2-heptyl acetate **13,** independently performed by two panelists, resulted in consistent odor thresholds. In contrast, slight differences in the odor thresholds were obtained for the *syn*-diastereomers of 4-acetylthio-2-heptyl acetate **14** and 4-acetylthio-2-heptanol **15**. In case of **15**, a rearrangement to **13** was excluded since the stability of 4-acetylthio-2-heptanol **15** was proven by periodical GC analysis of the stored stock solution.

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

This is in agreement with the significantly increased thresholds of the enantiomers of a homologous series of 4-acetylthio-2-alkanones compared with the respective 4-mercapto-2-alkanones (Wakabayashi *et al*., 2012 and 2015).

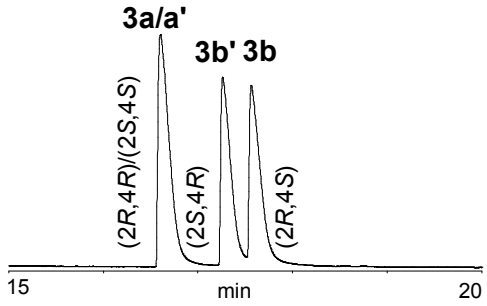
For the *anti*-diastereomer of **3** the impact of the *O*-acetylation on the odor threshold was in the same order of magnitude as that of the *S*-acetylation.

With an odor threshold of 0.2 ng/L in air, the increase in odor threshold of *syn*-**13** was less pronounced compared to *syn*-**3**. Previous sensory evaluations of a spectrum of mercapto alkyl-acetates also revealed mostly higher odor thresholds compared to the corresponding mercapto alcohols (Vermeulen and Collin, 2003; Vermeulen *et al*., 2003). Interestingly, the odor threshold of *anti*-**14** was the same as that of *anti*-**3**. For the *syn*-diastereomer diacetylation resulted in a loss in odor intensity.

In general, the *anti*-configuration of the diastereomers of **3** and **13-15** was found to be more sensorially active (0.01-0.14 ng/L in air) than the *syn*-configuration (0.08-4.6 ng/L in air). A similar influence of the stereochemistry on odor intensities has been reported for the stereoisomers of 3-mercapto-2-methyl-1-pentanol (Sabater Lüntzel *et al.*, 2000). Low odor threshold values in water (0.03 and 0.04 µg/L) have been determined for the isomers having the thiol and the methyl group in *anti*-position compared to the odor impressions of the *syn*-isomers which were estimated to be higher by a factor of 300 and 1000.

The assessment of the odor intensities of the four stereoisomers of **3** and **13-15** revealed that for each pair of enantiomers the (2*R*)-configured isomers showed the lowest odor thresholds. If this structural prerequisite of (2*R*)-configuration is fulfilled, the odor thresholds were nearly independent from the degree of acetylation. The consistently lowest odor thresholds (0.03–0.09 ng/L in air) were observed for the (2*R*,4*R*)-configured isomers. The odor thresholds of the (2*S*)-configured isomers were highly impacted by the degree of acetylation. For example, the odor threshold of (2*S*,4*S*)-configured 4-acetylthio-2-heptanol **15a** was 570 times higher than that of **15a'**. As shown in Figure 15, the use of octakis(2,3-di-*O*-*n*-butyryl-6-*O*-TBDMS)-*γ*-CD (*n*-butyryl-*γ*-CD) as stationary phase resulted in a co-elution of the *anti*-enantiomers and a reversed order of elution of the *syn*-enantiomers. The odor thresholds shown in Figure 15 demonstrate the excellent agreement between the two panelists.

A comparison with the thresholds determined on heptakis(2,3-di-*O*-acetyl-6-*O*-TBDMS)-*β*-CD (Table 9) confirmed that the order of elution of the enantiomers did not influence the odor thresholds.



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

Figure 15. GC separation of the stereoisomers of 4-mercapto-2-heptanol **3** using *n*-butyryl-*γ*-CD and determination of the odor thresholds of *syn*-enantiomeric pair of **3** by GC/O. For conditions, see Materials and Methods (GC-FID system VI; GC/O system IV).

4.1.5.3 Determination of Odor Qualities

Odor descriptions for the stereoisomers of 4-mercapto-2-heptanol **3** and the corresponding acetates **13-15** are summarized in Table 10.

In general, the *anti*-configured isomers of **3** and **13-15** were found to possess sulfuric and onion-type odors, whereas the *syn*-configured isomers exhibited sulfuric, green and fruity notes, except for *syn*-**3** possessing an additional savory odor note.

Concerning the stereoisomers, the (2*R*,4*R*)-configuration (**3**, **13-15a'**) appears to be a prerequisite for the intensive smell of raw onion. The corresponding enantiomers were described as being sweet, fruity (**3**, **13** and **14a**) or savory-like (**15a**). The assessment of the *syn*-enantiomeric pairs of **3** and **13-15** revealed a strong influence of the acetylation on the odor property.

Sulfuric, green, and tropical (grapefruit and passion fruit) odor notes were obtained for the *syn*-enantiomeric pairs of the acetylated derivatives (**13-15b** and **b'**). In contrast, the *syn*-isomers of 4-mercapto-2-heptanol exhibited savory, meaty (**3b**), and green aroma notes reminiscent of dill (**3b'**).

Table 10. Odor qualities of the stereoisomers of 4-mercapto-2-heptanol **3** and the corresponding acetyl esters **13-15** determined by GC/O.

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

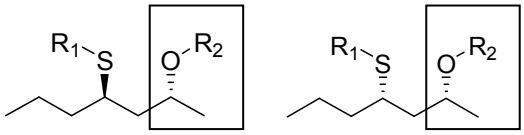
[a] GC/O descriptions were made with injection volumes corresponding to ~ 6 ng for each stereoisomer at the sniffing port; odor qualities were determined by GC/O using fused silica capillary columns coated with: [b] DB-Wax; [c] heptakis(2,3-di-*O*-acetyl-6-*O*-TBDMS)-*β*-CD; [d] heptakis(2,3-di-*O*-methyl-6-*O*-TBDMS)-*β*-CD; for further conditions, see Materials and Methods (GC/O systems I-III).

Comparable odor descriptions such as onion, liver, meaty, sweaty, and resinous have been reported for the diastereomeric mixture of **3** tasted in 50 ppm NaCl and sugar solutions (Naef *et al*., 2008). GC/O analysis of the diastereomers of **3** revealed a grapefruit-like odor at threshold level (Polster and Schieberle, 2017). Moreover, the odor qualities of the stereoisomers of 4-mercapto-2-heptanol **3** and 4-mercapto-2-heptyl acetate **13** are in good agreement with the tropical, fruity, and vegetable odor notes described for the "1,3-oxygen-sulfur olfactophore" (Rowe and Tangel, 1999; Rowe, 2002).

The sensory evaluation of the structure-related 4-acetylthio-2-heptyl acetate **14** and 4-acetylthio-2-heptanol **15** would support the extension of the "tropical olfactophore"' to *S*-acetylated compounds as suggested by Robert *et al.* (2004) and Wakabayashi (2004).

Moreover, a significant impact not only of the acetylation but also of the configurations of the two asymmetric centers in these molecules on the sensory properties was observed.

The results indicate that the (*R*)-configuration at position 3 of the 1,3-oxygen-sulfur functionality plays a decisive role as trigger for low odor thresholds and specific odor descriptions as demonstrated in Figure 16.



(2*R*,4*R*) (2*R*,4*S*) $R_{1,2}$ = H, acetyl

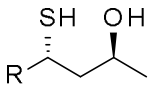
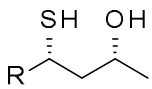
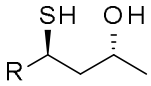
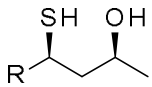
Figure 16. Configurations found as triggers for low odor thresholds [(2*R*,4*R*) << (2*R*,4*S*)] and specific odor descriptions [(2*R*,4*R*): onion; (2*R*,4*S*): tropical].

4.2 Analysis and Sensory Evaluation of the Stereoisomers of the Homologous Series of 4-Mercapto-2-alkanols

4.2.1 Syntheses and GC Separations

4-Mercapto-2-alkanols **1**, **2** and **4-6** were synthesized by Michael-type addition of thioacetic acid to the corresponding 3-alken-2-ones and subsequent reduction of the obtained 4-acetylthio-2-alkanones **7**, **8** and **10-12** with $LiAlH_4$, in analogy to the procedure described for 4-mercapto-2-heptanol **3** (chapter 4.1.1). The structures of the investigated mercaptoalkanols are shown in Table 11.

Table 11. Structures of the stereoisomers of 4-mercapto-2-alkanols **1-6** with carbon chain lengths from C5 to C10.

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

The GC separations of the diastereomeric pairs of the homologous series (chain lengths C5-C10) are shown in Figure 17A. For separation of the four stereoisomers of each homolog several chiral stationary phases were tested. Figure 17B shows the separation obtained by using heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyl dimethylsilyl)-*β*-cyclodextrin as chiral stationary phase. The use of this cyclodextrin derivative was suitable for the separation of the four stereoisomers, except for chain length C6. For this homolog **2**, only an incomplete separation could be achieved (Figure 17C), and there were coelutions with stereoisomers of **1**. Therefore, heptakis(2,3-di-*O*-methoxymethyl-6-*O*-*tert*-butyl dimethylsilyl)-*β*-cyclodextrin was employed; the use of this chiral stationary phase enabled the separation of all four stereoisomers of **2** (Figure 17D). Separation factors (α) and resolutions (R_s) are summarized in Table 12.

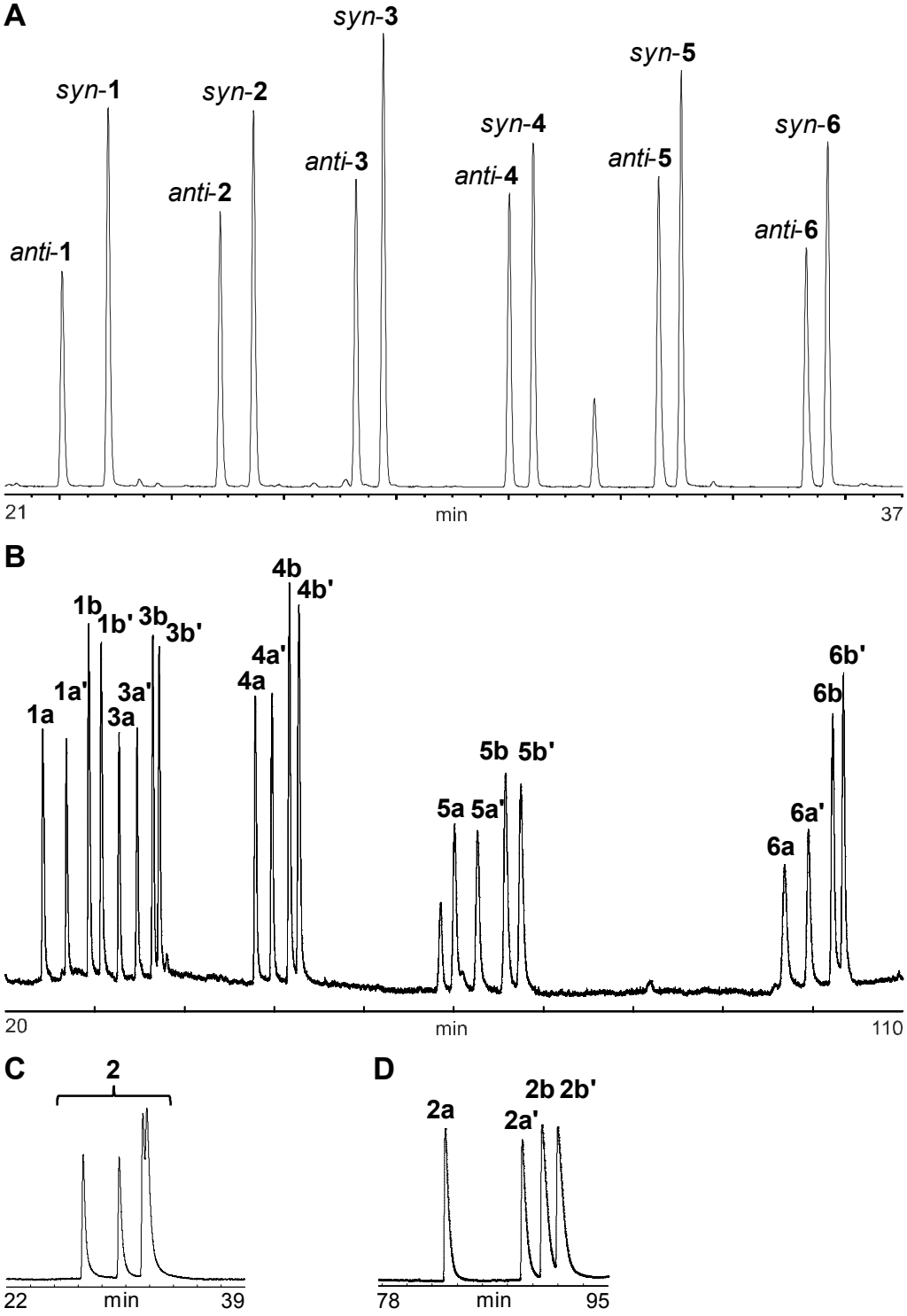


Figure 17. (**A**) GC separation of the diastereomers of 4-mercapto-2-alkanols **1-6** on a DB-Wax column (GC-FID system I), (**B**) separation of the stereoisomers of 4-mercapto-2-alkanols **1**, **3-6** on heptakis(2,3-di-*O*-acetyl-6-*O*-TBDMS)-*β*-CD (GC-FID system V), (**C**) separation of the stereoisomers of 4-mercapto-2-hexanol **2** on heptakis(2,3-di-*O*-acetyl-6-*O*-TBDMS)-*β*-CD (GC-FID system V), (**D**) separation of the stereoisomers of **2** on heptakis(2,3-di-*O*-methoxymethyl-6-*O*-TBDMS)-*β*-CD (GC-FID system VII). a (2*S*,4*S*), a' (2*R*,4*R*), b (2*R*,4*S*), b' (2*S*,4*R*).

Table 12. Comparison of the separations of the stereoisomers of 4-mercapto-2-alkanols **1-6**.

no.	**1**[c]		**2**[d]		**3**[c]		**4**[c]		**5**[c]		**6**[c]	
	anti	*syn*	*anti*	*syn*	*anti*	*syn*	*anti*	*syn*	*anti*	*syn*	*anti*	*syn*
α[a]	1.10	1.04	1.08	1.01	1.06	1.02	1.04	1.02	1.04	1.02	1.02	1.01
R_s[b]	6.56	3.47	10.57	1.50	5.67	1.83	5.14	2.31	3.66	2.19	3.39	2.11

[a] Separation factor α was calculated according to equation 4
[b] Resolution R_s was calculated according to equation 6
[c] Stereoisomers separated on heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyl dimethylsilyl)-*β*-cyclodextrin
[d] Stereoisomers separated on heptakis(2,3-di-*O*-methoxymethyl-6-*O*-*tert*-butyl dimethylsilyl)-*β*-cyclodextrin

4.2.2 Determination of the Absolute Configurations and the Order of Elution of the Stereoisomers

The determination of the absolute configurations and the order of elution of the stereoisomers of 4-mercapto-2-heptanol **3** have been described in detail in the chapters 4.1.3 and 4.1.4. The reinvestigation of the absolute configurations of the stereoisomers of 4-mercapto-2-heptanol (chapter 4.1.4.2) based on the published data of Kiske *et al*. (2016) resulted in an order of elution of *anti*- before *syn*- on a DB-Wax column for the diastereomers (Figure 17A) and of (2*S*,4*S*) **3a** before (2*R*,4*R*) **3a'** and (2*R*,4*S*) **3b** before (2*S*,4*R*) **3b'** for the stereoisomers using heptakis(2,3-di-*O*-acetyl-6-*O*-TBDMS)-*β*-CD as chiral stationary phase (Figure 17B).

The analysis of the diastereomeric mixture of 4-mercapto-2-heptanol **3** via ^{1}H NMR spectroscopy enabled the determination of the diastereomeric ratios (H-2 and H-4) at the stereogenic centers (Figure 18A). As outlined in Table 13, the ratio measured via integral analysis of the appropriate pairs of protons (*syn*/*anti*) was nearly identical to the ratio of *anti*- and *syn*-diastereomers determined by GC analysis (Figure 17A).

Table 13. Diastereomeric ratios of synthesized 4-mercapto-2-alkanols **1-6** (C5-C10) determined by GC and ^{1}H NMR analysis.

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

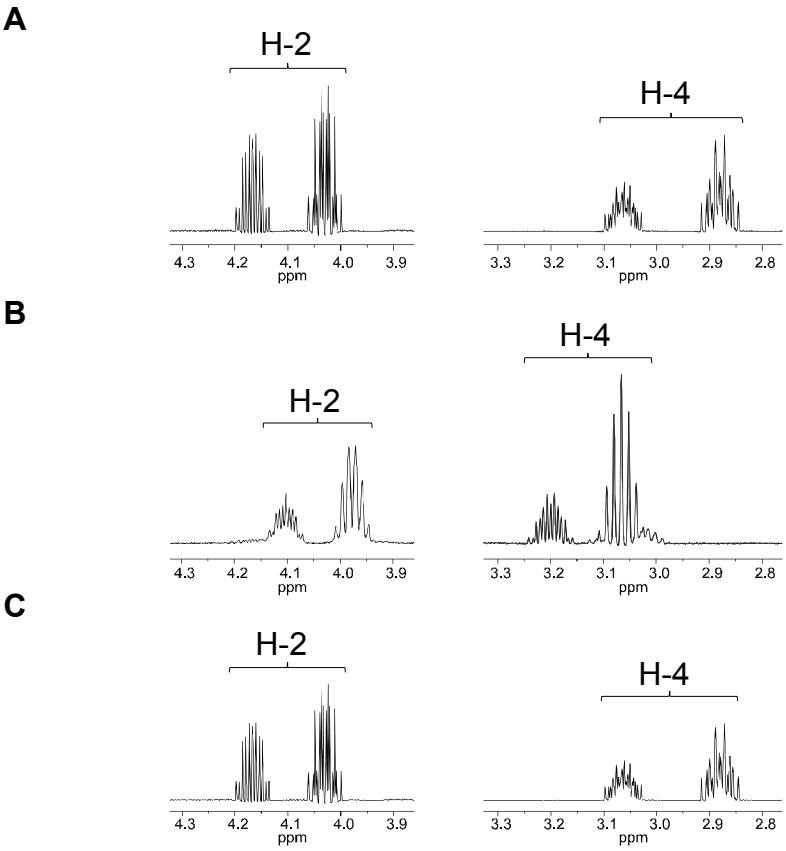


Figure 18. ^{1}H NMR data of synthesized (**A**) 4-mercapto-2-heptanol **3**, (**B**) 4-mercapto-2-pentanol **1** and (**C**) 4-mercapto-2-decanol **6**.

Based on this result, the comparison of diastereomeric ratios of synthesized 4-mercapto-2-alkanols **1-6** obtained via GC and ^{1}H NMR spectroscopy was used to assign the *anti*-/*syn*-configurations for the complete homologous series. For the synthesized mercaptoalkanols **1**, **2** and **4-6** there were also good agreements between the GC and the NMR data, as shown in Table 13. The diastereomeric ratios determined at the H-2 and H-4 positions of the mercaptoalkanols with the shortest (C5, **1**) and the longest chain lengths (C10, **6**) are exemplarily shown in Figure 18B and 18C. As a result, the order of elution of the diastereomers of the investigated 4-mercapto-2-alkanols **1-6** was consistently assigned as *anti* before *syn* (Figure 17A).

The final step was the assignment of the order of elution of the two *anti*-configured [(2*S*,4*S*) and (2*R*,4*R*)] and the two *syn*-configured [(2*S*,4*R*) and (2*R*,4*S*)] enantiomers. To this end, a procedure based on enzyme-catalyzed kinetic resolution was used to obtain enantiomerically enriched thiols. The used approach is exemplarily shown for 4-mercapto-2-pentanol **1** in Figure 19. The first step was the *Candida antarctica* lipase B (CAL-B) mediated hydrolysis of the thioester bond of racemic 4-acetylthio-2-pentanone **7** which resulted in the formation of the (*R*)-configured thiol **20** as product and (4*S*)-configured **7** as remaining substrate (Figure 19, step 1). The absolute configurations were assigned according to Kiske *et al*. (2016). GC analysis of the enantiomers of **7** and **20** was performed using heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyl dimethylsilyl)-*β*-cyclodextrin as chiral stationary phase (Figure 19, step 1).

In the next step, the reaction mixture was separated by column chromatography and the nearly enantiomerically pure 4-acetylthio-2-pentanone (*S*)-**7** was subjected to reduction with $LiAlH_4$ to form the stereoisomers of 4-mercapto-2-pentanol **1** with the corresponding excess of the (4*S*)-configured diastereomers (Figure 19, step 2). GC analysis using heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyl dimethylsilyl)-*β*-cyclodextrin as chiral stationary phase demonstrated that the (4*S*)-configured diastereomers coeluted with the first peaks of the pairs of stereoisomers obtained for the synthesized 4-mercapto-2-pentanol **1**. The assignment of the *anti*- and *syn*-diastereomers achieved in the first step in combination with the assignment of the enantiomeric pairs to their corresponding diastereomers via MDGC enabled the determination of the absolute configurations and GC orders of elution of the four stereoisomers of 4-mercapto-2-pentanol (Figure 19; step 3) as (2*S*,4*S*) **1a** before (2*R*,4*R*) **1a'** and (2*R*,4*S*) **1b** before (2*S*,4*R*) **1b'**.

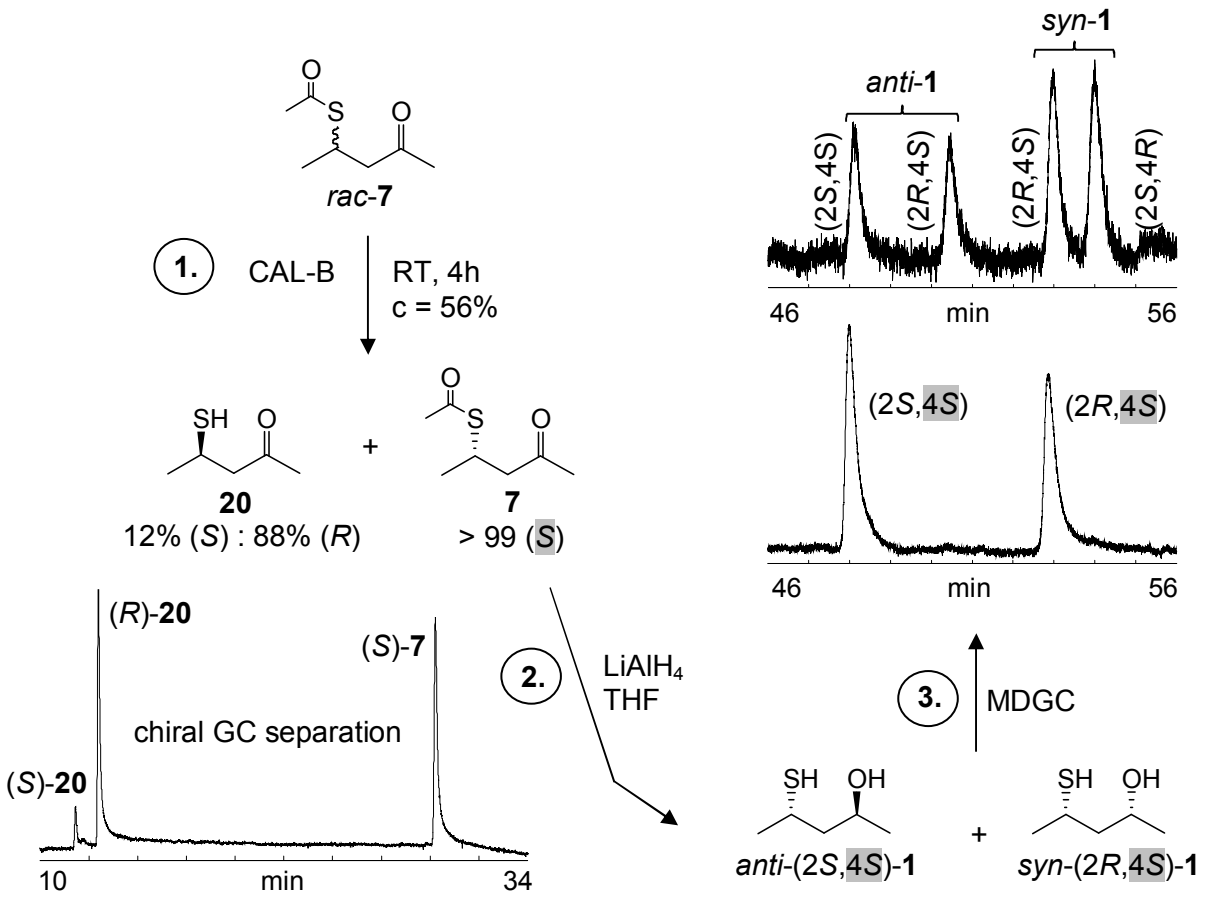


Figure 19. Approach employed to assign the GC order of elution of the stereoisomers of 4-mercapto-2-alkanols, shown for 4-mercapto-2-pentanol **1**.

An analogous procedure was applied to assign the order of elution of the stereoisomers of 4-mercapto-2-octanol **4**. To this end, the (4*S*)- and (4*R*)-configured enantiomers of 4-mercapto-2-octanone **21** were prepared via kinetic resolution using PPL in accordance with the previously described procedure (Kiske *et al*., 2016). The obtained enantiomerically enriched mercaptoalkanones were reacted with (*S*)-MαNP (Figure 20), purified with semi-preparative HPLC, and the diastereomers were analyzed by NMR spectroscopy (Table 14). Previous studies had demonstrated consistent ^{1}H NMR anisotropy effects for the homologous series of (*S*)-MαNP thioesters of 4-mercapto-2-alkanones of chain lengths C5 to C10 (Wakabayashi *et al*., 2011 and 2015). Therefore, it was assumed that the recently revised sector rule for secondary thiols (Figure 21) can be applied (Kiske *et al*., 2016). The Δδ values of H-1 and H-3 are positive (0.06 and 0.05, respectively) and are placed on the left side whereas the Δδ values for H-5 – H-8 are negative (-0.02, -0.03, -0.03 and -0.04, respectively) and are placed on the right side.

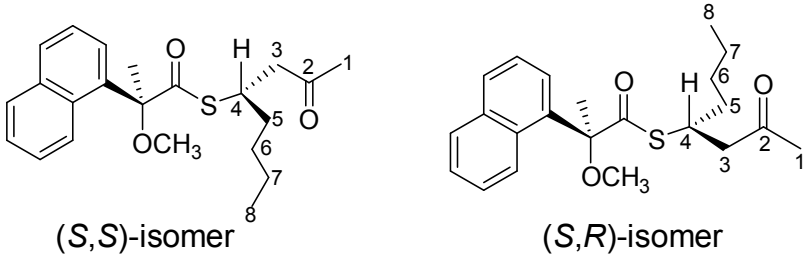


Figure 20. Structures of synthesized diastereomeric (*S*)-MαNP thioesters of 4-mercapto-2-octanone **21**.

Table 14. ^{1}H NMR data and Δ*δ* values of (*S*)-MαNP thioesters of both enantiomers of 4-mercapto-2-octanone **21**.

(*S*)-MαNP thioester of 4-mercapto-2-octanone **21**			
H	(*S*,*R*)-**21**	(*S*,*S*)-**21**	Δ*δ*
1	1.98 (s)	2.04 (s)	0.06
3	2.62 (d, 2.1)	2.67 (dd, 16.5, 6.4)	0.05
3'	2.60 (d, 3.1)	2.60 (dd, 18.8, 7.0)	0
4	3.74 (m)	3.75 (m)	0
5	1.52 (m)	1.50 (m)	-0.02
6	1.21 (m)	1.18 (m)	-0.03
7	1.21 (m)	1.18 (m)	-0.03
8	0.79 (t, 7.3)	0.75 (t, 7.3)	-0.04

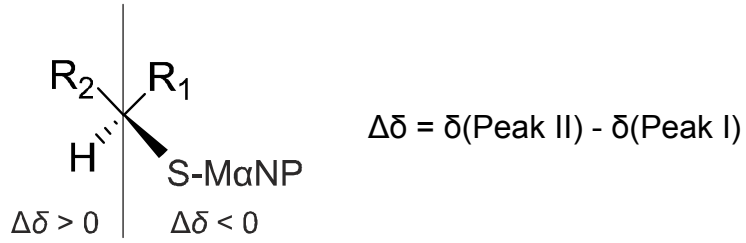


Figure 21. Sector rule for secondary thiols according to Kiske *et al*. (2016).

This results in (*R*)-configuration at the C-4 position of the first eluting diastereomer and thus corresponds to the (*S*,*R*) diastereomeric MαNP thioester of **21** which elutes before the (*S*,*S*) diastereomer. Based on this result, PPL-mediated hydrolysis of racemic 4-acetylthio-2-octanone *rac*-**10** resulted in the formation of the (*S*)-configured thiol **21** as product and (4*R*)-configured **10** as remaining substrate. After column chromatography, (4*R*)-configured **10** was subjected to reduction with $LiAlH_4$ to form the (4*R*)-configured diastereomers of **4**. Comparing the order of elution of the (4*R*)-configured diastereomers of **4** to those of the enantiomeric pairs of *anti*- and *syn*-configured **4** via MDGC resulted in an order of elution of (2*S*,4*S*) **4a** before (2*R*,4*R*) **4a'** and (2*R*,4*S*) **4b** before (2*S*,4*R*) **4b'** using heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyl dimethylsilyl)-*β*-cyclodextrin as chiral stationary phase (Figure 17B).

In analogy, the absolute configurations of the stereoisomers of the remaining homologs **2**, **5** and **6** were assigned via PPL-mediated hydrolyses of 4-acetylthio-2-hexanone **8**, 4-acetylthio-2-nonanone **11** and 4-acetylthio-2-decanone **12**. The formed (*S*)-configured thiols (4-mercapto-2-hexanone, 4-mercapto-2-nonanone and 4-mercapto-2-decanone) and the remaining (*R*)-configured substrates (**8**, **11** and **12**) were analyzed using heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyl dimethylsilyl)-*β*-cyclodextrin as chiral stationary phase (Wakabayashi *et al*., 2011, 2012 and 2015; Kiske *et al*., 2016). After column chromatography, (4*R*)-configured 4-acetylthio-2-nonanone (*R*)-**11** as well as (4*S*)-configured 4-mercapto-2-hexanone and (4*S*)-configured 4-mercapto-2-decanone were subjected to reduction with $LiAlH_4$ to form the corresponding enantiomerically enriched diastereomers of **2**, **5** and **6**. GC analyses of (4*R*)-configured **5**, (4*S*)-configured **6** and the respective racemic reference substances resulted in orders of elution of (2*S*,4*S*) **a** before (2*R*,4*R*) **a'** and (2*R*,4*S*) **b** before (2*S*,4*R*) **b'** using heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyl dimethylsilyl)-*β*-cyclodextrin as chiral stationary phase (Figure 17B). The same order of elution was assigned for the stereoisomers of the C6-homolog **2** separated on heptakis(2,3-di-*O*-methoxymethyl-6-*O*-*tert*-butyl dimethylsilyl)-*β*-cyclodextrin as stationary phase (Figure 17D).

An interesting phenomenon was observed when screening Chiramix, a column coated with a mixture of the two chiral stationary phases heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-*β*-CD and octakis(2,6-di-*O*-methyl-3-*O*-trifluoroacetyl)-*γ*-CD (Tamogami *et al*., 2001) as alternative chiral stationary phase (Figure 22). There were changes in the order of elution of the stereoisomers of **1-6** depending on the chain lengths.

For the long-chain homologs **4-6** the *anti*-configured (2*S*,4*S*) **a** and (2*R*,4*R*) **a'** stereoisomers consistently eluted before the corresponding *syn*-configured (2*S*,4*R*) **b'** and (2*R*,4*S*) **b** stereoisomers. The changed order of elution of the four stereoisomers of the short-chain homologs **1** and **2** as well as the coelution of the (4*R*)-configured diastereomers of the C7 homolog **3a'** and **3b'** appears to be due to an increasing shift of the (2*R*,4*R*)-configured stereoisomers **1-3a'** to later retention times with decreasing chain lengths. Separation factors (α) and resolutions (R_s) are summarized in Table 15.

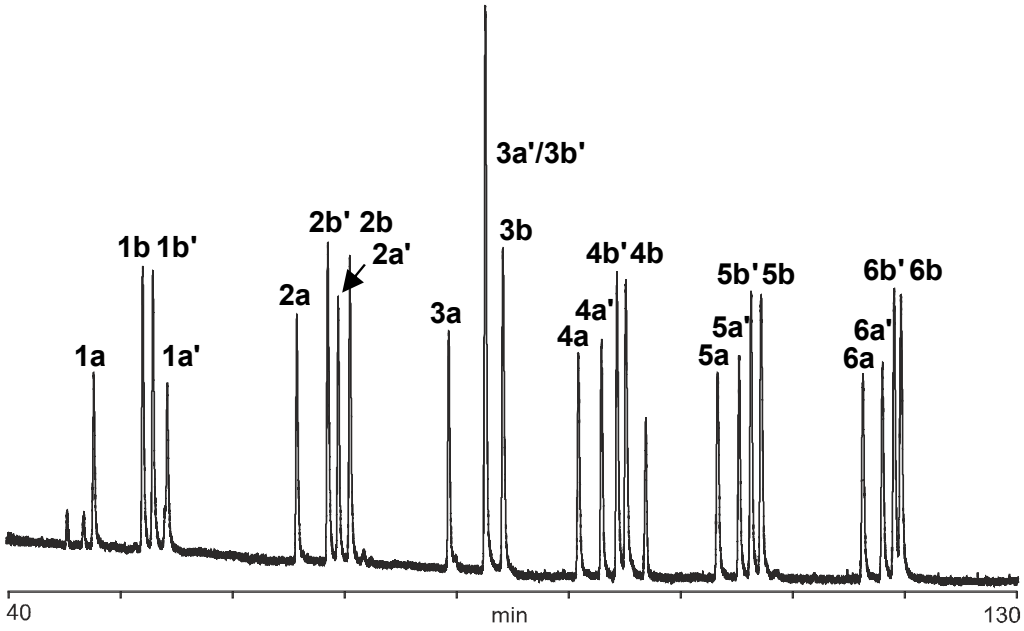


Figure 22. GC separation of the stereoisomers of 4-mercapto-2-alkanols **1-6** on Chiramix (GC-FID system VIII). a (2*S*,4*S*), a' (2*R*,4*R*), b (2*R*,4*S*), b' (2*S*,4*R*)

Table 15. Comparison of the separations of the stereoisomers of 4-mercapto-2-alkanols **1**, **2**, and **4-6** on Chiramix.

no.	**1**		**2**		**4**		**5**		**6**	
	anti	*syn*	*anti*	*syn*	*anti*	*syn*	*anti*	*syn*	*anti*	*syn*
α[a]	1.14	1.02	1.06	1.03	1.02	1.01	1.02	1.01	1.02	1.00
R_s[b]	16.06	2.30	9.78	4.96	4.81	1.74	4.44	1.99	3.81	1.29

[a] Separation factor α was calculated according to equation 4
[b] Resolution R_s was calculated according to equation 6

4.2.3 Sensory Evaluation of the Stereoisomers of 4-Mercapto-2-alkanols

Odor thresholds and odor qualities of the stereoisomers of 4-mercapto-2-alkanols **1-6** were determined via GC/O. In total three panelists participated; except for **5b** and **5b'**, each stereoisomer was evaluated by two assessors.

Panelist 1 determined the odor properties of the stereoisomers of 4-mercapto-2-hexanol **2** and 4-mercapto-2-heptanol **3** using heptakis(2,3-di-*O*-methoxymethyl-6-*O*-TBDMS)-*β*-CD and heptakis(2,3-di-*O*-acetly-6-*O*-TBDMS)-*β*-CD as chiral stationary phases. The last-mentioned column was used by panelist 2 to perform the sensory evaluation of the stereoisomers of 4-mercapto-2-alkanols **1-6**. No data were available for the stereoisomers of 4-mercapto-2-hexanol **2** due to insufficient separation (Figure 17C) and for the *syn*-configured isomers of 4-mercapto-2-nonanol because of a coelution with the internal standard (*E*)-2-decenal. Sensory properties of the *anti*-configured isomers of 4-mercapto-2-nonanol *anti*-**5** were obtained after HPLC-separation of the diastereomers. GC/O analysis using Chiramix as chiral stationary phase was performed by panelist 3. The use of this column enabled the determination of odor impressions of the stereoisomers of 4-mercapto-2-alkanols with chain lengths from C5-C10, except for C7 (Figure 22).

4.2.3.1 Determination of Odor Thresholds

The odor thresholds of the stereoisomers of **1-6** were determined via GC/O using the method described by Ullrich and Grosch (1987). Table 16 summarizes the individual odor thresholds of the investigated stereoisomers of 4-mercapto-2-alkanols **1-6**.

Regarding the variability of the three panelists, there were a few cases in which high differences between odor thresholds were observed, i.e. for **4a'** (factor: 60) and **5a'** (factor: 30) between panelists 2 and 3 as well as for **2b'** (factor: 30), **2a** (factor: 22) and **2b** (factor: 7) between panelists 1 and 3. However, for most of the stereoisomers the individual odor thresholds were either the same or differed up to a maximum of factors 3 to 5, corresponding to approximately two dilution steps in the course of the AEDA. Figure 23 illustrates the odor threshold curves for the four stereoisomers of 4-mercapto-2-alkanols **1-6** based on the geometric means (Table 17) calculated from the assessments by the respective panelists (except for **5b** and **5b',** for which only single sensory evaluations were available).

Table 16. Odor thresholds of the stereoisomers of 4-mercapto-2-alkanols **1-6** determined by GC/O.

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

[a] Odor thresholds were determined by GC/O
[b] Not determined by this panelist
[c] Not determined due to insufficient separation on heptakis(2,3-di-*O*-acetyl-6-*O*-TBDMS)-*β*-CD
[d] Not determined due to insufficient separation on Chiramix
[e] Not determined due to coelution with the internal standard (*E*)-2-decenal under used conditions

Table 17. Geometric means of the individual odor thresholds determined by the panelists via GC/O.

	geometric means of odor thresholds (ng/L air)[a]				
no.	a (2*S*,4*S*)	a' (2*R*,4*R*)	b (2*R*,4*S*)	b' (2*S*,4*R*)	ratio of thresholds[b]
1	0.9899	0.9899	0.2236	0.5000	[c]
2	0.4690	0.1140	0.2646	0.5477	2.3
3	0.1000	0.0224	0.2000	0.3000	4.5
4	0.2236	0.0077	0.1414	0.1732	18.4
5	0.1414	0.0055	0.1000[d]	0.1000[d]	18.2
6	0.5477	0.1000	1.4731	1.0954	5.5

[a] Geometric mean values calculated based on the individual odor thresholds determined by the assessors via GC/O shown in Table 16; [b] geometric mean of second most intensive smelling stereoisomer / geometric mean of (2*R*,4*R*)-configured stereoisomer; [c] no ratio was determined as the (2*R*,4*R*)-configured stereoisomer is not the most intensive smelling stereoisomer; [d] only one sensory evaluation available.

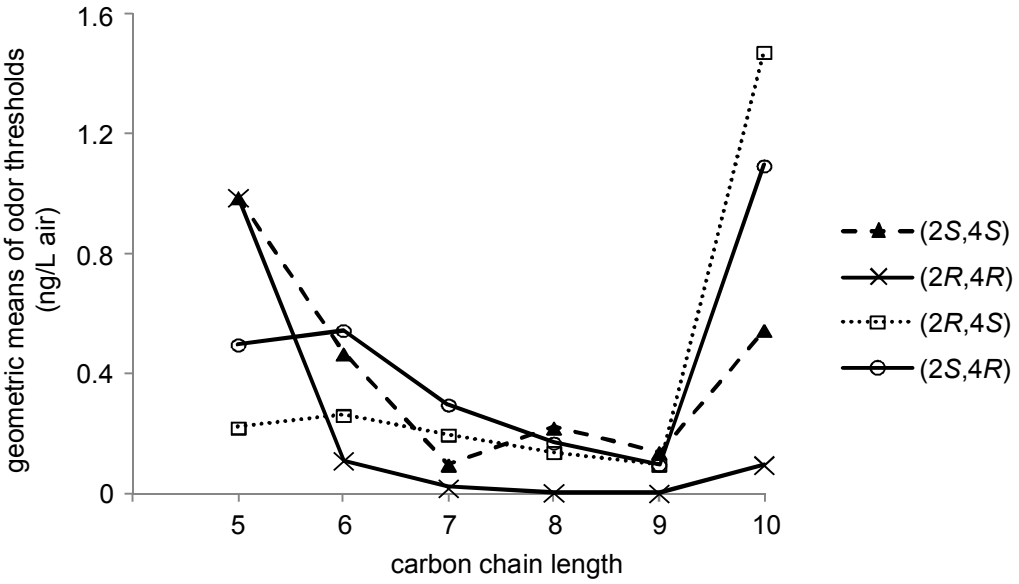


Figure 23. Geometric means of the odor thresholds of the stereoisomers of 4-mercapto-2-alkanols (except for (2*R*,4*S*) and (2*S*,4*R*) of C9, for which only single sensory evaluations were available).

The data set revealed two effects: (i) For all stereoisomers odor threshold minima were observed for the medium-chain homologs (C7-C9). This is in good agreement with the recently reported odor thresholds for the racemic mixtures of a homologous series of 4-mercapto-2-alkanols (Polster and Schieberle, 2017). The individual curves for the diastereomers [(2*R*,4*S*), (2*S*,4*R*)] and [(2*S*,4*S*), (2*R*,4*R*)] also fit very well to those reported for the respective diastereomeric mixtures (Polster, 2012). This good agreement with sensory data generated in a different laboratory and by trained panelists support the reliability of the data obtained in the present study, despite the limited number of (trained) panelists and the observed individual differences (Polster, 2012; Polster and Schieberle, 2017). Threshold minima for medium-chain representatives have been observed within homologous series of various mercaptoalkanols (Polster and Schieberle, 2017) and for the enantiomers of the homologous series of 4-mercapto-2-alkanones (minima at carbon chain lengths C8) and 4-acetylthio-2-alkanones (minima at carbon chain lengths C7/C8) (Wakabayashi *et al.*, 2015). (ii) Except for C5, the lowest odor thresholds were determined for the (2*R*,4*R*)-configured stereoisomers.

The visual differences seen in Figure 23 were verified by calculating the ratios of the geometrical means of the thresholds determined for the (2*R*,4*R*)-configured stereoisomers and those of the second most intensive smelling stereoisomers. Particularly for the homologs with chain lengths C8 and C9 these ratios were pronounced (approximately 18).

Regarding the odor thresholds determined for 4-mercapto-2-heptanol and its acetyl derivatives (Table 9, chapter 4.1.5.2), it has been shown that the odor thresholds were nearly independent from *S*- and/or *O*-acetylation if the structural prerequisite of (2*R*)-configuration was fulfilled. In contrast, the odor intensities of the (2*S*)-configured stereoisomers of 4-mercapto-2-heptanol significantly decreased by a factor of 20 after the acetylation of the OH-group. Blocking the SH-group resulted in a loss of odor intensity by a factor of 16 for (2*S*,4*R*)-configured 4-mercapto-2-heptanol and by a factor of 172 for (2*S*,4*S*)-configured 4-mercapto-2-heptanol (Table 9, chapter 4.1.5.2). A high sensory potency was also observed for the (4*R*)-configured isomers of short- and medium-chain homologs (C5-C8) of 4-mercapto-2-alkanones (C5-C10) taking into account the re-investigated absolute configurations (Wakabayashi *et al*., 2015; Kiske *et al*., 2016). *S*-Acetylation reduced the odor intensities of 4-mercapto-2-alkanones (C5-C10) whereas the (4*R*)-configured enantiomers were more affected than the corresponding (4*S*)-configured enantiomers with exception of the C5 and the C10 homolog. The greatest effects of acetylation were observed for the (4*R*)-configured enantiomers of the C6 and the C8 homolog with factors of 1500 (obtained by panelist 1) and 4000 (obtained by panelist 2). These data indicate that the (*R*)-configurations might play key roles for the thresholds of these *ß*-mercaptoalcohols and *ß*-mercaptoketones.

4.2.3.2 Determination of Odor Qualities

Odor qualities of the stereoisomers of **1-6** were also determined by panelists 1-3 via GC/O (Table 18). It is known that odor qualities may show pronounced concentration-dependent changes. Nevertheless, for the purpose of comparison the sensory assessments were performed at constant amounts of 1.5 ng at the sniffing port for each stereoisomer.

Considering the variability of the dominant odor impressions (printed in bold type), it is obvious that none of the stereoisomers showed consistent odor qualities for all homologs. For example, the odor qualities of the (2*R*,4*R*)-configured stereoisomers (**1-6a'**) ranged from onion to plastic-solvent-like odor notes. The comparison of the odor descriptions for the different chain lengths showed that for the C5 homolog **1** similar odor qualities such as onion (**1a** and **1a'**) or sweat (**1b** and **1b'**) were detected for the enantiomeric pairs. Different odor notes such as fruity (**2a** and **3a**), onion (**2a'**, **3a'** and **3b**), savory (**2b** and **3b'**), and green/herb-like (**2b'**) were obtained for the isomers of the chain lengths C6 and C7. The odor descriptions of the stereoisomers of the chain lengths C8 to C10 changed towards unpleasant chemical notes such as burned, plastic, solvent or pungent. These data demonstrate that the chain length is the main factor determining the variability in odor qualities of stereoisomers of 4-mercapto-2-alkanols. A similar effect was reported for the odor properties of the diastereomers of 4-mercapto-2-alkanols (C5-C10) (Polster, 2012; Polster and Schieberle, 2017). GC/O analysis resulted in onion and meaty notes for the C5 homologs, a fruity-like odor reminiscent of grapefruit dominated the odor impressions of the C6-C9 homologs, and further elongation led to fatty and burned odor notes. No significant differences in the odor qualities have been described for the diastereomeric pairs of the 4-mercapto-2-alkanols (C5-C10), except for the C6 homolog, having either a more meaty or fruity odor note.

The comparison of odor qualities of the enantiomers of the corresponding 4-mercapto-2-alkanones resulted in fruity notes for the (*R*)-configured enantiomers and in more unpleasant notes such as sulfury and catty-like for the (*S*)-configured enantiomers of the C5-C9 homologs taking into account the results of the reinvestigation of the absolute configurations of *β*-mercaptoalkanones. In case of 4-mercapto-2-decanone, both enantiomers smell like bell pepper (Wakabayashi *et al*., 2012 and 2015; Kiske *et al*., 2016).

Table 18. Odor descriptions of the stereoisomers of 4-mercapto-2-alkanols **1-6** determined by GC/O.

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

[a] GC/O descriptions were made for injection volumes corresponding to ~1.5 ng for each stereoisomer at the sniffing port; dominant odor impressions are bold typed
[b] Not determined due to coelution with the internal standard (*E*)-2-decenal under used conditions (GC/O system II)

These data demonstrate that the stereochemistry and not the chain length as in case of the 4-mercapto-2-alkanols (C5-C10) is the main factor determining the variability in odor qualities of the enantiomers of 4-mercapto-2-alkanones (C5-C10).

As expected for compounds possessing a 1,3-oxygen-sulfur functionality, tropical, fruity, and vegetable odor notes were obtained for stereoisomers of the investigated 4-mercapto-2-alkanols (Table 18). However, specific notes such as meaty, savory, sweaty as well as chemical notes were additionally perceived by the panelists (Table 18) and have also been reported in literature to describe the odor qualities of 4-mercapto-2-alkanols (Vermeulen *et al*., 2003; Naef *et al*., 2008).

In conclusion, GC/O analyses revealed that the odor thresholds of the stereoisomers of 4-mercapto-2-alkanols (C5-C10) were highly impacted by the stereochemistry as the lowest odor thresholds were determined for the (2*R*,4*R*)-configured stereoisomers, except for C5. In contrast, the odor qualities were mainly influenced by the chain length.

4.3 Distributions of the Stereoisomers of *β*-Mercaptoheptanones and *β*-Mercaptoheptanols in Cooked Bell Pepper (*Capsicum annuum*)

In bell peppers (*Capsicum annuum*), the detection of 2-heptanethiol as new aroma compound has been followed by the identification of a broad spectrum of sulfur-containing volatiles in an extract obtained by simultaneous distillation-extraction (SDE) and subsequent thiol-enrichment using Affi-Gel 501 (Simian *et al*., 2004; Naef *et al*., 2008). Among them, 2-mercapto-4-heptanone **17**, 4-mercapto-2-heptanone **18**, 4-mercapto-2-heptanol **3** and 2-mercapto-4-heptanol **19** which have been reported as examples of *β*-mercaptoalkanones and *β*-mercaptoalkanols naturally occurring in cooked red bell pepper (Naef *et al*., 2008). The respective nonvolatile precursors, that is cysteine-*S*-conjugates, have also been identified in bell peppers (Starkenmann and Niclass, 2011).

In former studies, GC separations of the stereoisomers of 4-mercapto-2-alkanones with carbon chain lengths from C5 to C10 using chiral stationary phases have been reported, and differences in odor thresholds and odor qualities between the stereoisomers have been determined by means of GC/O (Wakabayashi *et al*., 2011 and 2015). The absolute configurations of the stereoisomers of **17** and **18** have previously been assigned (Kiske *et al*., 2016). Analytical and sensorial data of the stereoisomers of **3** are given in the chapters 4.1 and 4.2.

The objective of the present study was to apply these analytical capabilities to determine the naturally occurring distributions of the stereoisomers of *β*-mercaptoheptanones and *β*-mercaptoheptanols in cooked red bell peppers. The investigated polyfunctional thiols were isolated via SDE, enriched by affinity chromatography, and subjected to enantioselective multidimensional gas chromatography (MDGC). Preliminary analyses of the stereoisomers of **3** and **17-19** in cooked green as well as in raw, unheated red bell peppers were also performed to investigate a potential influence of the maturation as well as to elucidate the interplay of biogenetic and process-induced formation of these sensorially interesting polyfunctional thiols.

4.3.1 Isolation and Enrichment of Thiols from Cooked Red Bell Pepper

The ß-mercapto compounds 2-mercapto-4-heptanone **17**, 4-mercapto-2-heptanone **18**, 4-mercapto-2-heptanol **3** and 2-mercapto-4-heptanol **19** have previously been reported in extracts obtained from cooked red bell pepper (Naef *et al*., 2008). 2-Heptanethiol has also been found in higher amounts in cooked bell pepper than in raw bell pepper samples (Simian *et al.*, 2004). Therefore, analogous to the procedures applied in these investigations, volatiles were isolated from bell peppers by means of SDE. The distillation step of the SDE is of course not a common household way of preparing food; however, it resulted in cooking of the bell peppers, and simultaneous extraction allowed easy isolation of volatiles. The SDE extracts obtained from a total of 4 kg of red bell peppers were pooled and subjected to a selective thiol-enrichment via mercurated Affi-Gel 10, according to the previously described principle (Full and Schreier, 1994). Thus, the isolation of the target compounds 2-mercapto-4-heptanone **17**, 4-mercapto-2-heptanone **18**, 4-mercapto-2-heptanol **3** and 2-mercapto-4-heptanol **19** was achieved as shown exemplarily in Figure 24 (batch 1). The investigated batches of bell peppers are summarized in Table 6. The identities of **3** and **17-19** were confirmed by comparing GC retention times and MS fragmentation patterns with those of synthesized racemic reference compounds.

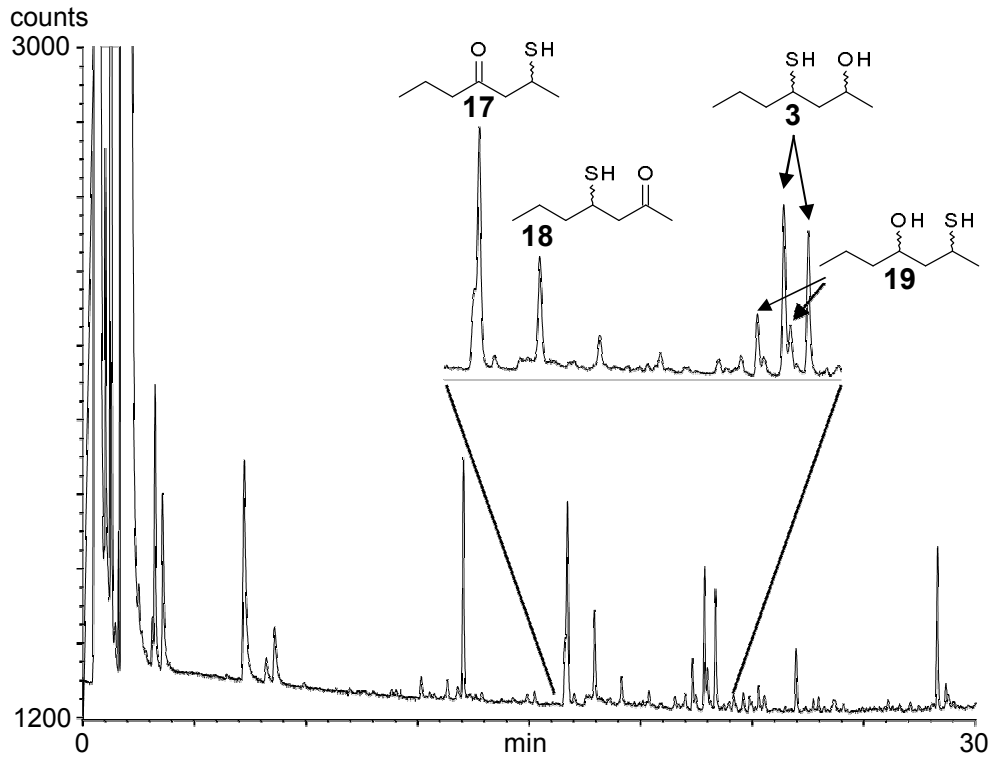


Figure 24. Capillary gas chromatogram (DB-Wax) of a red bell pepper extract (batch 1) obtained after isolation via SDE and thiol-selective enrichment using mercurated Affi-Gel 10. 2-Mercapto-4-heptanone **17**, 4-mercapto-2-heptanone **18**, 4-mercapto-2-heptanol **3** and 2-mercapto-4-heptanol **19**. For conditions see Materials and Methods (GC-FID system I).

4.3.2 Enantioselective Analysis Using MDGC

To determine the distributions of the stereoisomers of **3** and **17-19**, enantioselective MDGC was performed. In a preliminary study, the suitability of several chiral stationary phases was tested.

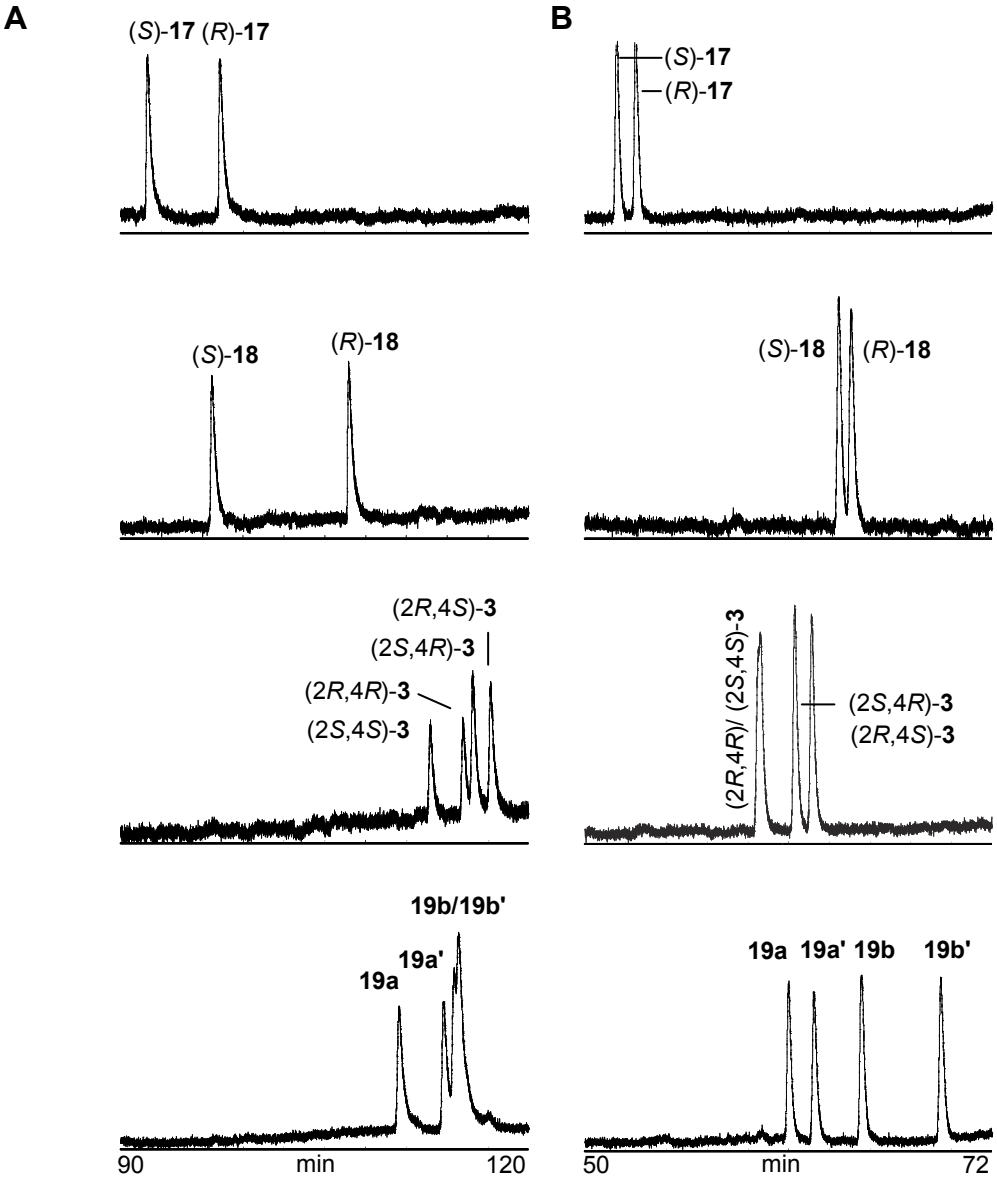


Figure 25. Stereodifferentiation of synthesized 2-mercapto-4-heptanone **17**, 4-mercapto-2-heptanone **18**, 4-mercapto-2-heptanol **3** and 2-mercapto-4-heptanol **19** obtained on (**A**) Chiramix and (**B**) octakis(2,3-di-*O*-*n*-butyryl-6-*O*-TBDMS)-γ-CD after transfer from a DB-Wax column. a/a': isomers of the first eluting diastereomer, b/b': isomers of the second eluting diastereomer

The separations obtained for the stereoisomers of synthesized **3**, **17-19** on (A) Chiramix and (B) octakis(2,3-di-*O*-*n*-butyryl-6-*O*-TBDMS)-γ-CD (*n*-butyryl-γ-CD) after the transfer from a DB-Wax column are shown in Figure 25. The order of elution of the enantiomers of **17** and **18** was determined by preparing enantiomerically enriched **17** and **18** via lipase-catalyzed kinetic resolution of racemic 2-acetylthio-4-heptanone **16** and 4-acetylthio-2-heptanone **9**, and assigning the order of elution of the enantiomers according to Kiske *et al*. (2016).

As shown in Figure 22, direct injection of **3** onto a column coated with Chiramix as chiral stationary phase resulted in coelutions of the (4*R*)-configured diastereomers. However, under the conditions of the MDGC analysis the separation of all four stereoisomers of **3** could be achieved (Figure 25, A). The use of *n*-butyryl-*γ*-CD as chiral stationary phase enabled only a separation of the *syn*-configured isomers of **3** (Figure 25, B). The order of elution was assigned by comparing the GC chromatogram of the (4*R*)-configured isomers of **3** obtained via kinetic resolution of racemic 4-acetylthio-2-heptanone **9** using PPL and subsequent reduction of the remaining substrate **9** using $LiAlH_4$ with those of the enantiomeric pairs of synthesized **3** separately transferred via MDGC. For **19**, only a partial separation of the four stereoisomers was obtained on Chiramix (Figure 25, A); therefore, as alternative approach *n*-butyryl-*γ*-CD was employed as chiral stationary phase enabling baseline separations of the stereoisomers of **19** (Figure 25, B). The determination of the absolute configurations of the stereoisomers of **19** is still in progress; separate transfers of the diastereomers of **19** from a DB-Wax column onto the chiral columns via MDGC were used to assign the enantiomeric pairs (**19a**/**a'** and **19b**/**b'**).

The distribution of the stereoisomers of **3**, **17-19** determined in a cooked red bell pepper extract (batch 2, Table 6) using Chiramix as chiral stationary phase is exemplarily shown in Figure 26 (chromatogram A1). The determined ratios of stereoisomers are presented in Table 19. 2-Mercapto-4-heptanone **17** was found to be present with a high excess of the (*S*)-enantiomer. In contrast, only a moderate excess of the (*R*)-enantiomer was obtained for the positional isomer 4-mercapto-2-heptanone **18**. A similar ratio, with a higher preponderance of the (4*R*)-configured stereoisomer was determined for the *syn*-enantiomeric pair of the corresponding mercaptoalcohol **3**. A clear enantiomeric excess, however, in favor of the (4*S*)-configured stereoisomer, was found for the *anti*-configured enantiomeric pair of **3**.

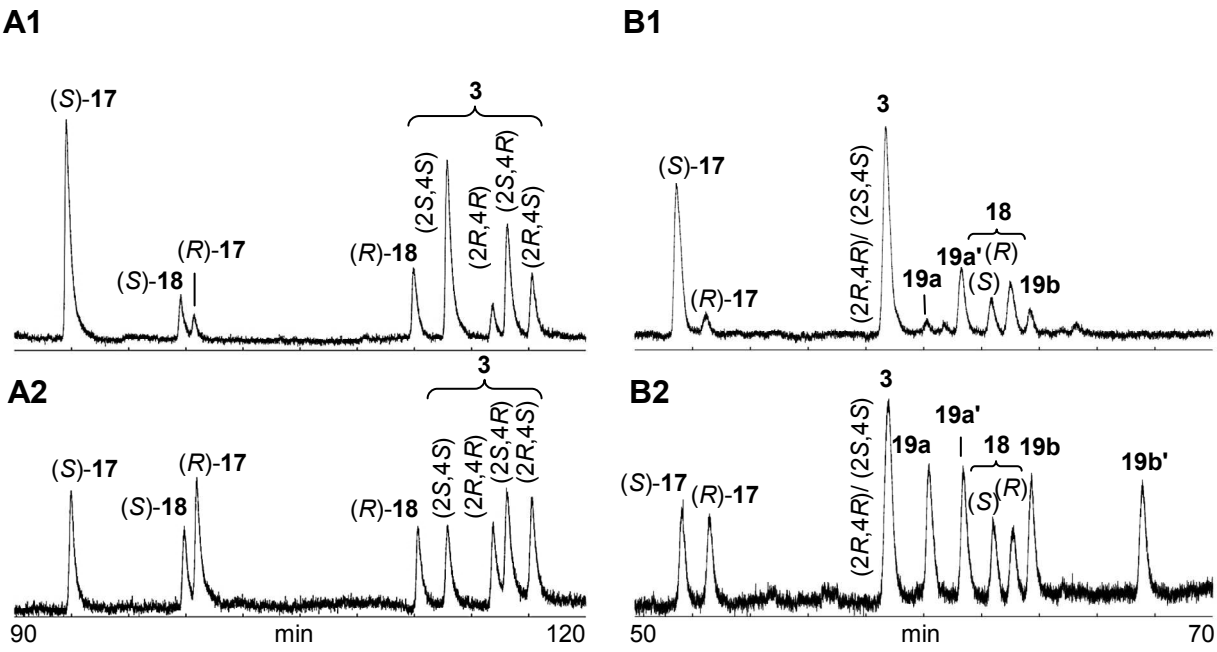


Figure 26. Stereodifferentiation of 2-mercapto-4-heptanone **17**, 4-mercapto-2-heptanone **18**, 4-mercapto-2-heptanol **3** and 2-mercapto-4-heptanol **19**: (**A1** and **B1**) red bell pepper extract (batch 2) obtained after isolation via SDE and thiol-selective enrichment using mercurated Affi-Gel 10 and (**A2** and **B2**) racemic reference compounds. Separations were performed on (**A**) Chiramix and (**B**) *n*-butyryl-*γ*-CD after transfer from a DB-Wax column.

In case of **19**, a clear excess of one of the enantiomers for both diastereomeric pairs was determined in the extract from the cooked red bell pepper (batch 2) using *n*-butyryl-*γ*-CD as chiral stationary phase (Figure 26, chromatogram B1). For the enantiomeric pair (**19a**/**a'**) of the first eluting diastereomer, the second eluting isomer (**19a'**) was predominating. For the second eluting diastereomer only one stereoisomer (**19b**) was found under the employed conditions. The enantioseparations on *n*-butyryl-*γ*-CD were also used to verify the enantiomeric ratios obtained for **17** and **18** on Chiramix. 2-Mercapto-4-heptanone **17** was again found to be nearly enantiomerically pure. The results obtained for 4-mercapto-2-heptanone **18** confirmed that the mercaptoketone was formed in a ratio of approximately 40% to 60% in favor of the (*R*)-enantiomer. No separation was obtained for the *anti*-configured isomers of 4-mercapto-2-heptanol **3**. The *syn*-configured diastereomer of **3** was not transferred onto the chiral column in order to avoid a coelution with the isomers of the first eluting diastereomer of **19** (Figure 25).

Table 19. Distributions of the stereoisomers of **3**, **17-19** from cooked red bell pepper extracts of batches 2 and 3a after SDE and thiol-selective enrichment.

				enantiomeric ratios (%)											
batch	MDGC			**17**		**18**		**3**				**19**			
	column 1	column 2	run	(*S*)	(*R*)	(*S*)	(*R*)	(2*S*,4*S*)	(2*R*,4*R*)	(2*S*,4*R*)	(2*R*,4*S*)	a	a'	b	b'
2	DB-Wax	Chiramix	1	87.5	12.5	40.1	59.9	82.8	17.2	67.9	32.1	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]
			2	-[a]	-[a]	35.4	64.6	85.8	14.2	67.1	32.9	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]
			3	92.0	8.0	35.1	65.0	87.6	12.4	64.5	35.5	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]
		n-butyryl-*y*-CD	4	90.6	9.4	38.7	61.3	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]	13.4	86.6	> 99	n.d.[c]
			5	89.3	10.7	38.4	61.6	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]	15.3	84.7	> 99	n.d.[c]
			mean	**89.9**	**10.1**	**37.5**	**62.5**	**85.4**	**14.6**	**66.5**	**33.5**	**14.4**	**85.6**	**> 99**	**n.d.**[c]
			± SD	**1.9**		**2.2**		**2.1**		**1.8**		**-**		**-**	
3a	RTX-200	Chiramix	1	-[a]	-[a]	-[a]	-[a]	-[a]	-[a]	61.4	38.6	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]
			2	95.2	4.8	44.0	56.0	92.4	7.6	57.8	42.2	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]
		n-butyryl-*y*-CD	3	92.8	7.2	-[a]	-[a]	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]	27.3	72.7	> 99	n.d.[c]
			4	93.1	6.9	39.6	60.4	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]	23.3	76.7	> 99	n.d.[c]
			5	92.6	7.4	44.2	55.8	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]	28.4	71.6	> 99	n.d.[c]
	DB-Wax	*n*-butyryl-*y*-CD	6	91.2	8.8	44.0	56.0	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]	30.1	69.9	> 99	n.d.[c]
			mean	**93.0**	**7.0**	**43.0**	**57.0**	**92.4**[d]	**7.6**[d]	**59.6**	**40.4**	**27.3**	**72.7**	**> 99**	**n.d.**[c]
			± SD	**1.4**		**2.2**		**-**		**-**		**2.9**		**-**	

[a] Compound was not analyzed during this MDGC run

[b] Not analyzed on this stationary phase

[c] Not determined under used conditions

[d] Single analysis

SD: absolute standard deviation

The reproducibility of the transfer step was checked for each investigated compound by determining the enantiomeric ratios in duplicate or triplicate analyses on each column used for the separation. A comparison of the single MDGC runs showed absolute differences of the enantiomeric ratios up to a maximum of 5% (Table 19).

To rule out the transfer of coeluting compounds, MDGC analysis of another red bell pepper extract (batch 3a) was performed using not only a DB-Wax but also a RTX-200, a medium-polar stationary phase, as precolumn. A comparison of the enantiomeric ratios of the investigated compounds obtained after transfer onto *n*-butyryl-*γ*-CD showed that the use of both stationary phases as precolumn delivered similar results for **17-19** (batch 3a, Table 19).

The low standard deviations (1.4 - 2.9%) of the diastereomeric ratios observed in the course of the investigation of the two batches (2 and 3a) of cooked red bell peppers demonstrated the reproducibility of the MDGC-transfer step and the absence of coeluting compounds.

To obtain further insight into the potential variability of the enantiomeric ratios of **3**, **17-19**, two additional batches (1 and 4a) of red bell pepper of the cultivar California Wonder grown at different locations in Spain and Germany, respectively, and purchased at different dates (Table 6) were investigated (Table 20). In both extracts of cooked red bell peppers, the distributions of the stereoisomers of **3**, **17-19** were comparable to those of the first investigated batches of cooked red bell peppers from Spain. This indicates that the ratios of stereoisomers of the *β*-mercaptoketones **17** and **18** and the *β*-mercaptoalkanols **3** and **19** in cooked red bell pepper in this cultivar seem to be rather stable independent of origin and date of purchase.

Table 20. Distributions of the stereoisomers of **3**, **17-19** from cooked red bell pepper extracts of batches 1 and 4a after SDE and thiol-selective enrichment.

				enantiomeric ratios (%)											
batch	MDGC			**17**		**18**		**3**				**19**			
	column 1	column 2	run	(*S*)	(*R*)	(*S*)	(*R*)	(2*S*,4*S*)	(2*R*,4*R*)	(2*S*,4*R*)	(2*R*,4*S*)	a	a'	b	b'
1	DB-Wax	Chiramix	1	91.3	8.7	40.0	60.0	-[a]	-[a]	66.8	33.2	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]
		n-butyryl-*y*-CD	2	93.8	6.2	36.8	63.2	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]	12.5	87.5	> 99	n.d.[c]
			3	94.0	6.0	-[a]	-[a]	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]	17.0	83.0	> 99	n.d.[c]
			4	92.1	7.9	-[a]	-[a]	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]	14.4	85.6	> 99	n.d.[c]
			mean	**92.8**	**7.2**	**38.4**	**61.6**	**-**	**-**	**66.8**[d]	**33.2**[d]	**14.6**	**85.4**	**> 99**	**n.d.**[c]
			± SD	**1.3**		**-**		**-**		**-**		**2.3**		**-**	
4a	DB-Wax	Chiramix	1	92.2	7.8	39.5	60.5	91.2	8.8	56.1	43.9	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]
			2	-[a]	-[a]	-[a]	-[a]	93.0	7.0	58.0	42.0	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]
		n-butyryl-*y*-CD	3	-[a]	-[a]	43.7	56.3	n.a.[b]	n.a.[b]	60.1	39.9	-[a]	-[a]	> 99	n.d.[c]
			4	94.6	5.4	39.1	60.9	n.a.[b]	n.a.[b]	-[a]	-[a]	-[a]	-[a]	> 99	n.d.[c]
			5	-[a]	-[a]	39.9	60.1	n.a.[b]	n.a.[b]	58.1	41.9	-[a]	-[a]	> 99	n.d.[c]
			6	92.6	7.4	40.2	59.8	n.a.[b]	n.a.[b]	60.0	40.0	-[a]	-[a]	> 99	n.d.[c]
			7	92.6	7.4	39.1	60.9	n.a.[b]	n.a.[b]	-[a]	-[a]	9.7	90.3	> 99	n.d.[c]
			mean	**92.2**	**7.8**	**40.2**	**59.8**	**92.1**	**7.9**	**58.5**	**41.5**	**9.7**[d]	**90.3**[d]	**> 99**	**n.d.**[c]
			± SD	**1.1**		**1.8**		**-**		**1.7**		**-**		**-**	

[a] Compound was not analyzed during this MDGC run
[b] Not analyzed on this stationary phase
[c] Not determined under used conditions
[d] Single analysis
SD: absolute standard deviation

4.3.3 Quantitative Estimations

The concentrations of **3**, **17-19** were estimated based on external calibration curves with authentic reference substances and taking into consideration recovery rates for the thiol enrichment-step using mercurated Affi-Gel 10. The concentrations of the respective stereoisomers were then calculated using the ratios determined via enantioselective GC. As shown in Table 21, the concentrations of the stereoisomers of **3**, **17-19** in the bell pepper batches 1,2 and 3a originating from Spain were in comparable orders of magnitude. In contrast, the concentrations in the batch 4a from Germany were on average 5 times (from 13 to 2) higher. Similar concentrations in the lower µg/kg-range have been reported for the C5-homologs 4-mercapto-2-pentanol **1** (3.3 µg/kg) and 4-mercapto-2-pentanone **20** (1.5-9.9 µg/kg) tentatively identified in aged Cheddar cheese (Kleinhenz, 2006 and 2007). Regarding the respective cysteine-*S*-conjugates, the concentration of the precursor of **18** (*S*-(3-oxo-1-propylbutyl)-L-cysteine) has been reported to be lower than the sum of the concentrations of the precursors of **3** (*S*-(3-hydroxy-1-propylbutyl)-L-cysteine) and of **19** (*S*-(3-hydroxy-1-methylhexyl)-L-cysteine) (Starkenmann and Niclass, 2011). The estimated concentrations of **18** and **3** listed in Table 22 and the chromatogram shown in Figure 24 demonstrate that these differences in concentrations are similar for the liberated thiols. Treatment of (S-(3-hydroxy-1-propylbutyl)-L-cysteine) with a *β*-lyase has been shown to release both diastereomers of **3** (Starkenmann and Niclass, 2011).

Table 21. Estimated concentrations of the stereoisomers of **3**, **17-19** from cooked red bell pepper extracts after SDE and thiol-selective enrichment.

	estimated concentrations (µg/kg)[a]											
	17		**18**		**3**				**19**			
batch	(*S*)	(*R*)	(*S*)	(*R*)	(2*S*,4*S*)	(2*R*,4*R*)	(2*S*,4*R*)	(2*R*,4*S*)	a	a'	b	b'
1	1.6	0.1	0.2	0.3	n.a.[b]	n.a.[b]	0.5	0.2	0.04	0.3	0.2	n.q.[c]
2	3.1	0.3	0.3	0.5	1.7	0.3	1.0	0.5	0.1	0.4	0.3	n.q.[c]
3a	1.6	0.1	0.3	0.5	2.3	0.2	1.0	0.6	0.1	0.2	0.3	n.q.[c]
4a	10.2	0.9	1.6	2.3	9.1	0.8	3.6	2.6	0.2	1.4	0.9	n.q.[c]

[a] Single analysis
[b] Not analyzed
[c] No quantitation

Table 22. Distributions and estimated concentrations of the stereoisomers of **3**, **17-19** in cooked green and red bell pepper extracts after SDE and thiol-selective enrichment.

	batch	color	**17**		**18**		**3**			
			(*S*)	(*R*)	(*S*)	(*R*)	(2*S*,4*S*)	(2*R*,4*R*)	(2*S*,4*R*)	(2*R*,4*S*)
enantiomeric ratios (%)	5	green	72.3[a]	27.7[a]	22.1[b]	77.9[b]	89.2[c]	10.8[c]	83.8[c]	16.2[c]
	3b	green	71.6[c]	28.4[c]	20.9[c]	79.1[c]	79.1[c]	20.9[c]	80.4[c]	19.6[c]
	3a	red	93.0[d]	7.0[d]	43.0[e]	57.0[e]	92.4[c]	7.6[c]	59.6[a]	40.4[a]
estimated concentrations (µg/kg)[c]	5	green	0.22	0.08	0.04	0.16	0.80	0.10	0.50	0.10
	3b	green	0.07	0.03	0.02	0.08	0.16	0.04	0.08	0.02
	3a	red	1.58	0.12	0.34	0.46	2.31	0.19	0.95	0.65

[a] Duplicate analyses (mean value)
[b] Triplicate analyses (absolute standard deviation = ± 2.0)
[c] Single analysis
[d] Quintuple analyses (standard deviations are shown in Table 19)
[e] Quadruplicate analyses (standard deviations are shown in Table 19)

4.3.4 Influence of the State of Maturation

To investigate the potential impact of the state of maturation on the enantiomeric ratios and concentrations of the investigated compounds, green bell peppers were also analyzed. The GC separation of the stereoisomers of **3**, **17** and **18** in an SDE extract obtained from green bell peppers originating from The Netherlands (batch 5) is shown in Figure 27.

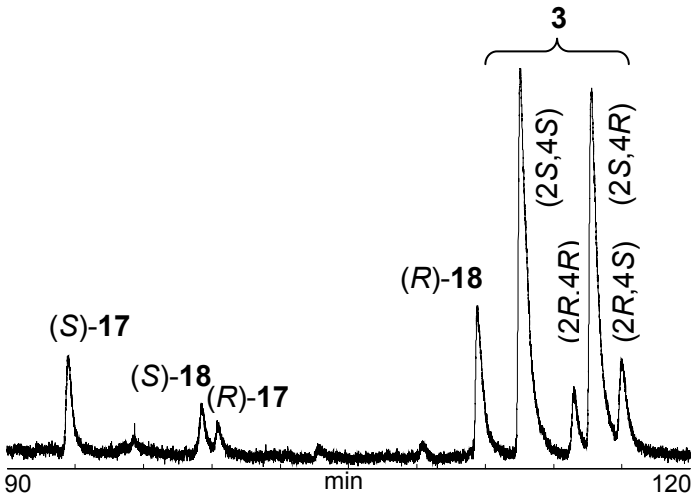


Figure 27. Stereodifferentiations of 2-mercapto-4-heptanone **17**, 4-mercapto-2-heptanone **18** and 4-mercapto-2-heptanol **3** determined in a green bell pepper extract (batch 5) obtained by isolation via SDE and thiol-selective enrichment using mercurated Affi-Gel 10. Separations were performed on Chiramix after transfer from a DB-Wax column.

The stereoisomeric ratios determined in this batch are given in Table 22. In addition, a batch of green bell peppers originating from Spain (batch 3b) was analyzed. Considering the difference in provenience, the stereoisomeric ratios determined in these two green bell pepper batches were quite comparable. The batch of green bell peppers from Spain (batch 3b) originated from the same location (Murcia) as one of the investigated batches of red bell peppers (batch 3a). Therefore, the data obtained for these two batches were compared to get a first picture of the influence of the state of maturation on the stereoisomeric compositions (Table 22). The data suggest that the enantiomeric ratios determined for the mercaptoketones **17** and **18** change during ripening in favor of the (*S*)-enantiomers.

For the stereoisomers of 4-mercapto-2-heptanol **3**, increasing ratios of the (4*S*)-configured stereoisomers were observed. The comparison also suggested that the estimated concentrations of the stereoisomers of **3**, **17** and **18** increased from green to red (Table 22). The greatest effect of maturation was observed for the (2*R*,4*S*)-configured **3** with a 32-fold difference between green and red bell peppers. Owing to the low number of samples analyzed and the fact that the maturation process was not followed for the same plant, the data are too limited to draw final conclusions. However, they suggest an impact of the state of maturation on both the concentrations and the distributions of the stereoisomers. This is supported by the fact that the concentrations of the cysteine-*S*-conjugate precursors of **18** and the sum of the concentrations of the respective precursors of **3** and **19** have been reported to be higher in red than in green bell peppers (Starkenmann and Niclass, 2011).

4.3.5 Biogenetical Aspects

The combination of stereodifferentiations and quantitative estimations allowed first hints regarding the potential stereochemical courses of reactions involved in the formation of 4-mercapto-2-heptanol **3**. As shown in Table 22, in all batches 4-mercapto-2-heptanone **18** showed a preponderance of the (4*R*)-enantiomer. However, among the stereoisomers of the corresponding 4-mercapto-2-heptanol **3** the (2*S*,4*S*)-configured stereoisomer was quantitatively predominating. This suggests that an enzyme-catalyzed reduction of the keto group of 4-mercapto-2-heptanone **18** might proceed with a preference of the (4*S*)-stereoisomer as substrate and the preferred formation of the (2*S*)-configured 4-mercapto-2-alkanol **3** as product. The configuration of 4-mercapto-2-heptanone **18** might thus not only be due to a stereoselective addition of cysteine to the double bond of the respective alkenone but also to a depletion of the (4*S*)-enantiomer in the course of the subsequent reduction of the keto group resulting in the preferred formation of (2*S*,4*S*)-4-mercapto-2-heptanol **3**. Owing to the limited data available, this is of course only a preliminary working hypothesis. Further research should focus on the influence of this interplay between the addition of cysteine to the alkenone and the subsequent reduction on the stereoisomeric composition of the resulting mercaptoalcohol. In addition, it should consider the impact of the maturation on the stereochemical course of the biosynthesis and the degradation of the nonvolatile precursors identified in bell peppers (Starkenmann and Niclass, 2011).

4.3.6 Influence of the Heat Treatment on the Stereoisomeric Compositions of *β*-Mercaptoheptanones and *β*-Mercaptoheptanols in Red Bell Pepper (*Capsicum annuum*)

To investigate the potential impact of the heat treatment on the stereoisomeric compositions of 2-mercapto-4-heptanone **17**, 4-mercapto-2-heptanone **18**, 4-mercapto-2-heptanol **3** and 2-mercapto-4-heptanol **19** which takes place during the isolation of volatiles via SDE, red bell peppers were also analyzed by liquid-liquid extraction (LLE).

The investigated *β*-mercapto compounds were isolated from 4 kg of red bell peppers originating from Germany (batch 4b, Table 6) by means of LLE. Enantioselective MDGC was performed to determine the distributions of the stereoisomers of the *β*-mercapto compounds after loading the LLE extract onto mercurated Affi-Gel 10.

The obtained enantiomeric ratios are summarized in Table 23. 2-Mercapto-4-heptanone **17** was found to be almost enantiomerically pure. In comparison, the enantiomeric excess of the positional isomer **18** in favor of the (*R*)-enantiomer was not so pronounced. For 4-mercapto-2-heptanol **3**, an excess of one of the isomers of the enantiomeric pairs of **3** was obtained. For both, the isomer with the (4*S*)-configuration was predominating. No enantiomeric ratios were determined for **19** as the concentration of the first eluting diastereomer (96 ng/mL) was below its limit of detection (116 ng/mL) and that of the second eluting diastereomer (354 ng/mL) only slightly above its limit of quantification (326 ng/mL).

In order to confirm that the ratios of stereoisomers obtained in this LLE extract (batch 4b) would not only reflect a unique assessment of one raw red bell pepper extract, a second batch of red bell pepper (batch 4c) originating from the same growing location (Neufahrn, Germany) was analyzed. As shown in Table 23, the stereoisomeric ratios determined in these two bell pepper batches were comparable.

Table 23. Distributions of the stereoisomers of 2-mercapto-4-heptanone **17**, 4-mercapto-2-heptanone **18** and 4-mercapto-2-heptanol **3** in raw and cooked red bell pepper extracts after thiol-selective enrichment using mercurated Affi-Gel 10.

batch	method	enantiomeric ratios (%)							
		17		**18**		**3**			
		(*S*)	(*R*)	(*S*)	(*R*)	(2*S*,4*S*)	(2*R*,4*R*)	(2*S*,4*R*)	(2*R*,4*S*)
4b	LLE[a]	92.9 ±1.8[c]	7.1 ±1.8[c]	34.7 ±2.1[c]	65.3 ±2.1[c]	95.5 ±1.0[d]	4.5 ±1.0[d]	40.8 ±0.5[c]	59.2 ±0.5[c]
4c	LLE[a]	86.3 ±2.4[d]	13.7 ±2.4[d]	40.7 ±3.1[d]	59.3 ±3.1[d]	> 99[e]	n.d.[e,f]	37.0 ±4.0[d]	63.0 ±4.0[d]
4a	SDE[b]	92.2 ±1.1[c]	7.8 ±1.1[c]	39.5 ±1.8[g]	60.5 ±1.8[g]	92.1[h]	7.9[h]	58.5 ±1.7[i]	41.5 ±1.7[i]

[a] LLE: liquid-liquid extraction
[b] SDE: simultaneous distillation-extraction
[c] Quadruplicate analyses (mean value ± standard deviation)
[d] Triplicate analyses (mean value ± standard deviation)
[e] Single analysis
[f] Not detectable under the used conditions
[g] Sextuple analyses (mean value ± standard deviation)
[h] Duplicate analyses (mean value)
[i] Quintuple analyses (mean value ± standard deviation)

Batch 4b belongs to the same lot of red bell peppers as one of the investigated batches (batch 4a) which has previously been analyzed via SDE and thiol-selective enrichment. Therefore, the data obtained for these two batches were compared to get a first picture of the influence of heat treatment on the stereoisomeric compositions (Table 23). The data indicate that the enantiomeric ratios determined for **17**, **18** and *anti*-**3** were rather stable. In contrast, differences were detected for the enantiomers of *syn*-**3**. In the raw red bell pepper extract (Figure 28A) *syn*-**3** was formed in a ratio of about 40% to 60% in favor of the (2*R,*4*S*)-enantiomer. A similar ratio, however in favor of the (2*S,*4*R*)-enantiomer was detected for *syn*-**3** in the cooked red bell pepper extract (Figure 28B).

Owing to the low number of samples analyzed and the fact that the preparation process was not followed for the same plant, the data are too limited to draw final conclusions. However, it is suggested that the enzymatic cleavage of the nonvolatile precursor of 4-mercapto-2-heptanol **3** (*S*-(3-hydroxy-1-propylbutyl)-L-cysteine) is ongoing during the isolation via LLE. Taking into account the increasing ratios of the (4*S*)-configured stereoisomers, in particular of the (2*R*,4*S*)-configured stereoisomer of **3** during ripening, this might result in the obtained ratio of *syn*-**3** in the extracts of unheated bell peppers.

Further studies will be needed to understand the stereospecific formation of these chiral compounds, in particular concerning the ratios of head-induced versus enzyme-catalyzed formation. This could be achieved, for example, by the inhibition of enzymes and the isolation of cysteine-*S*-conjugates and the assignment of their configurations. In addition, the data set on natural variability of the stereoisomers has to be extended.

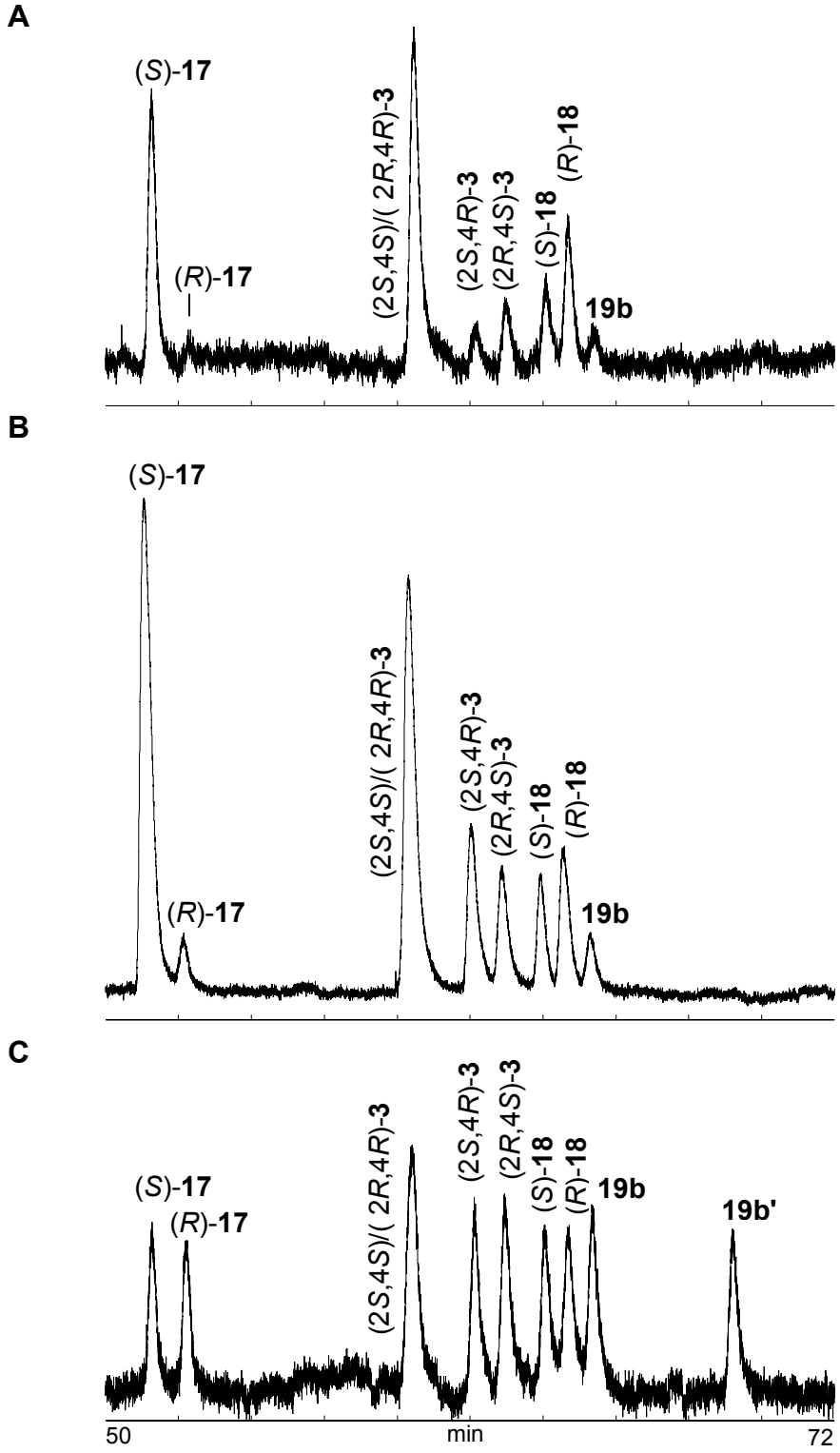


Figure 28. Stereodifferentiation of 2-mercapto-4-heptanone **17**, 4-mercapto-2-heptanone **18**, 4-mercapto-2-heptanol **3** and 2-mercapto-4-heptanol **19** of (**A**) a raw, (**B**) a cooked red bell pepper extract after isolation and thiol-selective enrichment and of (**C**) authentic reference samples obtained on *n*-butyryl-*γ*-CD after transfer from a DB-Wax column.

4.3.7 Sensory Aspects

The sensory evaluations of racemic **17** and **18** and of the diastereomeric mixtures of **3** and **19** assessed in NaCl and sugar solutions as well as the GC/O analyses of the stereoisomers of **18** and **3** resulted in tropical, fruity, and vegetable-like odor notes as expected for compounds possessing a 1,3-oxygen-sulfur function (Rowe, 2002; Naef *et al*., 2008; Wakabayashi *et al*., 2015). Sensory data of the stereoisomers of **3** are summarized in the Tables 16-18 (chapter 4.2.3.1). Considering the quantitative estimations of 4-mercapto-2-heptanone **18** (Table 21), it is interesting to note that (*R*)-**18**, which has been reported to have a low odor threshold (0.2 ng/L air) and a fresh, fruity, and grapefruit-like odor, was present in cooked red bell pepper extracts in slightly higher concentrations than the corresponding (*S*)-enantiomer having a slightly catty and grapefruit peel-like odor as well as a 3 times higher odor threshold (0.6 ng/L in air) (Wakabayashi *et al*., 2015; Kiske *et al*., 2016). In case of 4-mercapto-2-heptanol **3**, the (2*R*,4*R*)-configured stereoisomer, which was found to have the lowest odor threshold (0.02 ng/L air) and to be reminiscent of onion, was present at the lowest concentration in cooked red bell peppers (Tables 16-18 and 21). In contrast, a fruity odor note and an odor threshold of 0.1 ng/L in air has been perceived for the most abundant (2*S*,4*S*)-configured stereoisomer (Tables 16-18 and 21). At this point, a conclusion on the potential impact of the stereoisomers of the investigated mercaptoalkanones and mercaptoalcohols on the overall aroma of bell peppers is not possible. This would require determinations of the odor thresholds in water and calculations of the respective odor activity values.

5 SUMMARY

4-Mercapto-2-heptanol recently identified in cooked red bell peppers was used as naturally occurring representative to determine the sensory properties of the four stereoisomers of a polyfunctional compound possessing a 1,3-oxygen-sulfur functionality. Compounds fulfilling this structural requirement of the so-called "tropical olfactophore" are known to exhibit tropical, fruity, and vegetable-like odor notes.

4-Mercapto-2-heptanol was synthesized and the four stereoisomers were separated using heptakis(2,3-di-*O*-acetyl-6-*O*-TBDMS)-*β*-CD as chiral stationary phase. The determination of the absolute configuration was based on the combination of NMR analysis and lipase-catalyzed kinetic resolution. The sensory assessment via capillary gas chromatography/olfactometry (GC/O) resulted in differences in odor properties between the diastereomers and the enantiomeric pairs.

Including the respective acetyl-derivatives, 4-mercapto-2-heptyl acetate, 4-acetylthio-2-heptyl acetate and 4-acetylthio-2-heptanol into the analysis demonstrated that the sensory properties of the investigated compounds were impacted not only by the acetylation but also by the configurations of the two asymmetric centers. It seems that the configuration at position 3 of the 1,3-oxygen-sulfur functionality plays a decisive role as trigger for low odor thresholds and specific odor descriptions. Furthermore, the suggestion to extend the "tropical olfactophore"-skeleton to *S*-acetylated compounds was supported by the fact that the structurally related acetylthio-compounds were also described as fruity, green, and vegetable-like.

The impact of the stereochemistry on the odor properties as determined for 4-mercapto-2-heptanol and its acetyl derivatives was further investigated by analyzing other homologs of this series of *β*-mercaptoalkanols. 4-Mercapto-2-alkanols with chain lengths of C5, C6 and C8 to C10 were synthesized and their stereoisomers were separated via GC using heptakis(2,3-di-*O*-acetyl-6-*O*-TBDMS)-*β*-CD as chiral stationary phase. No separation of all four stereoisomers was obtained for 4-mercapto-2-hexanol; for this homolog, heptakis(2,3-di-*O*-methoxymethyl-6-*O*-TBDMS)-*β*-CD was employed as chiral stationary phase. The absolute configurations were assigned in analogy to the procedure described for 4-mercapto-2-heptanol. However, the formerly assigned configurations of 4-mercapto-2-heptanol and its acetates based on ^{1}H NMR anisotropy effects of derivatives of (*S*)-2-methoxy-2-(1-naphthyl) propionic acid had to be revised due to recent findings based on vibrational

circular dichroism and ^{1}H NMR analyses of (*R*)-hydratropic acid and 2-methoxy-2-phenylacetic acid derivatives. The reinvestigation of the absolute configurations of the stereoisomers of 4-mercapto-2-alkanols (C5-C10) resulted in an order of elution of *anti*- before *syn*- on a DB-Wax column for the diastereomers and of (2*S*,4*S*) before (2*R*,4*R*) and (2*R*,4*S*) before (2*S*,4*R*) for the stereoisomers separated on heptakis(2,3-di-*O*-acetyl-6-*O*-TBDMS)-*β*-CD. The same order of elution was obtained for the stereoisomers of 4-mercapto-2-hexanol using heptakis(2,3-di-*O*-methoxymethyl-6-*O*-TBDMS)-*β*-CD as chiral stationary phase. Changes in the order of elution depending on the chain length have been determined when using Chiramix, a column coated with a mixture of the two chiral stationary phases heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-*β*-CD and octakis(2,6-di-*O*-methyl-3-*O*-trifluoroacetyl)-*γ*-CD as alternative chiral stationary phase.
GC/O analyses revealed that the odor thresholds of the stereoisomers of 4-mercapto-2-alkanols (C5-C10) were highly impacted by the stereochemistry as the lowest odor thresholds were determined for the (2*R*,4*R*)-configured stereoisomers, except for C5. In contrast, the odor qualities were mainly influenced by the chain length. The data provide another example for the impact of both chain length and stereochemistry on the sensory properties of members of homologous series of aroma compounds.

2-Mercapto-4-heptanone, 4-mercapto-2-heptanone and the corresponding mercaptoalcohols, previously identified in cooked red bell pepper (*Capsicum annuum*), were used as examples to determine the distributions of stereoisomers of naturally occurring polyfunctional thiols. The thiols were isolated using simultaneous distillation-extraction and enriched by affinity chromatography. Enantioselective analysis was performed via multidimensional gas chromatography. For the studied cultivar California Wonder, the investigation of different batches revealed consistent ratios of the stereoisomers independent of origin and date of purchase. Quantitative estimations showed that the stereoisomers were present in cooked red bell pepper at concentrations in the range of 0.04-10 µg/kg. Lower concentrations were observed in cooked green bell pepper. The change from green to red bell pepper was also accompanied by shifts in the proportions of stereoisomers in favor of the (*S*)-enantiomers of the mercaptoheptanones and of the (4*S*)-configured stereoisomers of 4-mercapto-2-heptanol. Finally, a first insight into the distribution of the stereoisomers of the investigated compounds in raw red bell pepper has been given.

6 ZUSAMMENFASSUNG

4-Mercapto-2-heptanol ist ein in gekochter roter Paprika vorkommender Aromastoff. An dessen vier Stereoisomeren wurden beispielhaft die sensorischen Eigenschaften eines natürlichen Aromastoffes mit einer 1,3-Sauerstoff-Schwefel-Verbindung untersucht. Tropisch, fruchtige und gemüseartige Noten sind charakteristisch für Verbindungen, die diese strukturelle Anforderung des sogenannten "tropischen Olfaktophors" erfüllen. 4-Mercapto-2-heptanol wurde synthetisiert, und die vier Stereoisomere wurden auf einer Heptakis(2,3-di-*O*-acetyl-6-*O*-TBDMS)-*β*-Cyclodextrinphase (CD) getrennt. Die Bestimmung der absoluten Konfiguration basierte auf einer Kombination aus NMR Analytik und Lipase-katalysierter kinetischer Racematspaltung. Mittels Gaschromatographie-Olfaktometrie (GC/O) wurden sensorische Unterschiede zwischen den Diastereomeren und den Enantiomerenpaaren festgestellt. Unter Einbezug der korrespondierenden Acetylderivate 4-Mercapto-2-heptylacetat, 4-Acetylthio-2-heptylacetat und 4-Acetylthio-2-heptanol konnte gezeigt werden, dass die sensorischen Eigenschaften der untersuchten Verbindungen nicht nur durch eine Acetylierung, sondern auch durch die vorliegenden Konfigurationen an den beiden Stereozentren beeinflusst werden. Dabei scheint besonders die Konfiguration in Position 3 der 1,3-Sauerstoff-Schwefel-Verbindung eine entscheidende Rolle für niedrige Geruchsschwellenwerte und spezifische Geruchseindrücke zu spielen. Des Weiteren konnte der Vorschlag, das "tropische Olfaktophor" für *S*-acetylierte Verbindungen zu erweitern, durch den Erhalt von fruchtig, grünen und gemüseartigen Noten für die strukturrelevanten Acetylverbindungen bestärkt werden.

Der Einfluss der Stereochemie auf Geruchseigenschaften wurde nicht nur für 4-Mercapto-2-heptanol und dessen Acetylderivate, sondern auch für weitere Homologe aus der Reihe dieser *β*-Mercaptoalkanole untersucht. Hierfür wurden die 4-Mercapto-2-alkanole mit den Kettenlängen C5, C6 und C8-C10 synthetisiert und die Stereoisomere mittels Gaschromatographie unter Verwendung einer Heptakis(2,3-di-*O*-acetyl-6-*O*-TBDMS)-*β*-CD getrennt. Lediglich die vier Stereoisomere von 4-Mercapto-2-hexanol konnten nicht getrennt werden. Ihre Trennung erfolgte auf einer Heptakis(2,3-di-*O*-methoxymethyl-6-*O*-TBDMS)-*β*-CD Cyclodextrinphase. Die absoluten Konfigurationsbestimmungen erfolgten in Analogie zu der für 4-Mercapto-2-heptanol angewandten Methode.

Allerdings mussten die basierend auf Anisotropie-Effekten von (*S*)-2-Methoxy-2-(1-naphthyl)propionsäure-Derivaten ermittelten absoluten Konfigurationen von 4-Mercapto-2-heptanol und dessen Acetaten aufgrund neuer Erkenntnisse aus Untersuchungen mittels VCD-Spektroskopie und aus der ^{1}H NMR Analyse von (*R*)-2-Phenylpropansäure und 2-Methoxy-2-phenylessigsäure-Derivaten revidiert werden.
Daraus ergab sich eine Elutionsreihenfolge von *anti*- vor *syn*- für die Diastereomere auf einer DB-Wax Säule, sowie von (2*S*,4*S*) vor (2*R*,4*R*) und (2*R*,4*S*) vor (2*S*,4*R*) für die Stereoisomere der 4-Mercapto-2-alkanole auf einer Heptakis(2,3-di-*O*-acetyl-6-*O*-TBDMS)-*β*-CD. Die auf einer Heptakis(2,3-di-*O*-methoxymethyl-6-*O*-TBDMS)-*β*-Cyclodextrinphase getrennten Stereoisomere von 4-Mercapto-2-hexanol eluieren in derselben Reihenfolge. Abhängig von der Kettenlänge wurden Änderungen in der Elutionsreihenfolge der Stereoisomere unter Verwendung einer Chiramix Säule festgestellt. Diese Säule setzt sich aus den beiden chiralen stationären Phasen Heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-*β*-CD und Octakis(2,6-di-*O*-methyl-3-*O*-trifluoroacetyl)-*γ*-CD zusammen.
Bei GC/O Analysen der homologen Reihe der 4-Mercapto-2-alkanole (C5-C10) wurden, mit Ausnahme für das C5 Homolog, die niedrigsten Schwellenwerte für die (2*R*,4*R*)-konfigurierten Stereoisomere erhalten. Darüber hinaus zeigten die sensorischen Daten der homologen Reihe, dass die Geruchsqualitäten hauptsächlich von der Kettenlänge beeinflusst werden. Die Daten stellen somit ein weiteres Beispiel für den Einfluss der Kettenlänge und der Stereochemie auf die Geruchseigenschaften von Aromastoffen einer homologen Reihe dar.

Am Beispiel von 4-Mercapto-2-heptanon, 4-Mercapto-2-heptanol sowie den dazugehörigen Mercaptoalkoholen, die in gekochter roter Paprika nachgewiesen wurden, wurden Stereoisomerenverhältnisse von natürlich vorkommenden polyfunktionalen Thiolen bestimmt. Die Thiole wurden via simultaner Destillation/Extraktion isoliert und mit Hilfe von Affinitätschromatographie angereichert. Anschließend erfolgte die enantioselektive Analyse mittels multidimensionaler Gaschromatographie. Bei der Aufarbeitung von unterschiedlichen Chargen gekochter roter Paprika der Sorte California Wonder wurden stabile Enantiomerenverhältnisse für die polyfunktionalen Thiole, unabhängig von Anbauort und Einkaufsdatum erhalten. Quantitativen Abschätzungen zur Folge liegen die Stereoisomere in Konzentrationen von 0.04 - 10 µg/kg vor.

Geringere Konzentrationen wurden in gekochter grüner Paprika nachgewiesen. Zudem wurde eine Verschiebung der Enantiomerenverhältnisse zu Gunsten der (*S*)-konfigurierten Isomere der Mercaptoheptanone sowie der (4*S*)-konfigurierten Isomere von 4-Mercapto-2-heptanol während der Reifung beobachtet. Abschließend wurden erste Daten zu den Stereoisomerenverhältnissen der untersuchten Verbindungen in roher roter Paprika ermittelt.

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APPENDIX

Publications and Presentations

Publications (Peer-Reviewed)

Nörenberg, S.; Kiske, C.; Burmann, A.; Poplacean, I.; Engel, K.-H. Distributions of the stereoisomers of *β*-mercaptoheptanones and *β*-mercaptoheptanols in cooked bell pepper (*Capsicum annuum*). *J. Agric. Food Chem*. ***2017****, 65, 10250-10257.*

Nörenberg, S.; Kiske, C.; Reichardt, B.; Andelfinger, V.; Pfeiffer, A.; Schmidts, F.; Eisenreich, W.; Engel, K.-H. Analysis and sensory evaluation of the stereoisomers of a homologous series (C5-C10) of 4-mercapto-2-alkanols. *J. Agric. Food Chem*. ***2017****, 65, 8913-8922.*

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Frank, T.; Nörenberg, S.; Engel, K.-H. Metabolite profiling of two novel low phytic acid (*lpa*) soybean mutants. *J. Agric Food Chem*. **2009**, *57*, 6408-6416.

Oral Presentations

14th Weurman Symposium, Cambridge (UK) **2014**
Distributions of the stereoisomers of *β*-mercaptoheptanone and *β*-mercaptoheptanol in cooked red bell pepper (*Capsicum annuum*).

248th American Chemical Society National Meeting, Chemistry and Global Stewardship, San Francisco (US) **2014**
Stereoisomers of 4-mercapto-2-alkanols: analysis, sensory evaluations and natural occurrence.

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