Biosynthesis of Volatiles by the Myxobacterium *Myxococcus xanthus*

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The volatiles emitted from cell cultures of myxobacterium Myxococcus xanthus *were collected by use of a closed-loop stripping apparatus (CLSA) and analyzed by GC-MS. Two new natural products, (S)-9-methyldecan-3-ol ((S)-**1**) and 9-methyldecan-3-one (**2**), were identified and synthesized, together with other aliphatic ketones and alcohols, and terpenes. Biosynthesis of the two main components (S)-**1** and **2** was examined in feeding experiments carried out with the wild-type strain DK1622 and two mutant strains JD300 and DK11017, which are impaired in the degradation pathway from leucine to isovaleryl-SCoA. Isovaleryl-SCoA is used as a starter, followed by chain elongation with two malonate units. Subsequent use of methyl malonate and decarboxylation leads to (S)-**1** and **2**. Furthermore, 3,3-dimethylacrylic acid (DMAA) can be used by the mutant strain to form isovaleryl-SCoA, which corroborates recent data on the detection of a novel variety of the mevalonate pathway giving rise to isovaleryl-SCoA from HMGCoA.*

Introduction

Myxobacteria are well known for their capability to produce a diverse array of natural products.[1,2] Several important compounds with promising pharmacological activities, among them the epothilones,[3,4] have been found. Recently, the biosynthetic genes for several polyketides formed by Myxobacteria have been identified.[5–9] Nevertheless, more genes encoding typical secondary metabolite biosynthetic pathways than known secondary metabolites have been found in two *Stigmatella aurantiaca* strains.[10,11] Preliminary analysis of the genome sequence of *Sorangium cellulosum* So ce56[1,12] and a statistical evaluation of gene fragments encoding polyketide synthases from strain So ce90 suggest that this is also the case in the genus *Sorangium*.[13] In addition, the genome sequence of *M. xanthus* DK1622 is currently being determined at the Institute for Genomic Research (TIGR). Although no structure of any natural product from this strain has been published to date, sequence analysis again indicates the presence of a variety of typical secondary metabolite biosynthetic gene clusters.[14] In order to correlate the genetic potential of *M. xanthus* DK1622 with its production of natural products, we recently initiated a program aimed at the identification of secondary metabolites from this strain. Because identification of secondary metabolites is usually performed by isolating pure compounds in considerable amounts, to enable extensive NMR studies, minor compounds may not be found by the procedure typically used. Furthermore, volatile compounds are usually lost during the purification process. We therefore reasoned that analysis of volatiles released by *M. xanthus* might give new insight into the biosynthetic capabilities of this species. In addition, the high sensitivity of GC-MS analyses would enable direct sampling of volatiles from agar plate cultures. We have shown recently that this method is useful for analysis of volatiles in other Myxobacteria.[15]

Results and Discussion

The volatiles emitted into the headspace by cell cultures of *M. xanthus* DK1622, DK11017 and JD300 (Myxobacteriales) grown on agar plates were collected on activated charcoal by use of a modified closed-loop stripping apparatus (CLSA) and extracted with dichloromethane.[16] The following GC-MS analysis of the extracts (for total results see Table 1, for TIC see Figure 1) showed the presence of two 3-ketones, each with a molecular mass of 170 amu, corresponding to, for example, a molecular formula of $C_{11}H_{22}O$. Other main components were the corresponding alcohols, with slightly higher retention indices, and geosmin (**5**), which is responsible for the characteristic odour of *M. xanthus* and was the first volatile identified from Myxobacteria.[17] Furthermore, several other interesting compounds, including the terpenes (−)-germacrene D (**6**) and (1(10)*E*,5*E*)-germacradien-11-ol (**7**), are also produced by this species. The absolute configuration of **6** was elucidated by GC on a chiral stationary phase and comparison with a synthetic sample, while the absolute configuration of **7** remains unknown. Other volatiles are 2-aminoacetophenone (**8**) and 4-methylquinoline (**9**), which has the same carbon backbone as similar quinoline derivatives isolated from different Myxobacteria such as *Archangium gephyra* (4-quinolylaldoxime, 4-(hydroxymethyl)quinoline, 4-quinolinecarbaldehyde and 4-quinolinecarboxylic acid), *Myxococcus fulvus* (4-(hydroxymethyl)quinoline),

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Table 1. *Compounds identified in headspace analysis of* Myxococcus xanthus. *Compounds mentioned in the text are in bold type; artefacts originating from the medium are in italic.*[a]

GC	Compound	*I*	Identification	1	2	3	4	5	B
	5-methylhex-4-en-3-one		ms			x			
a	5-methylhexan-3-one		inc	x	x	x		x	
	5-methylhexan-3-ol		inc			x			
b	butyl acetate		ms	x					
	6-methyl-5-hepten-2-one		ms	x					
	butyl propionate	918	ms		x				
	2-acetylfuran	929	ms		x	x			
c	*2,5-dimethylpyrazine*	930	syn	x					x
d	dimethyltrisulfide	983	ms	x					
	octan-3-one	998	inc		x				
e	*2-ethylhexan-1-ol*	1037	ms	x	x	x			x
f	*benzyl alcohol*	1051	syn	x	x	x			x
	7-methyloctan-3-one	1057	inc, syn		x				
	acetophenone	1080	syn		x				x
	nonan-3-one	1091	inc		x				
	nonanal	1114	inc			x			x
g	2-phenylethanol	1125	syn	x	x	x			
	unsaturated 3-ketone	1136		x					
	benzyl cyanide	1161	syn		x				
h	**decan-3-one (4)**	1195	inc, syn	x	x	x			
i	**(*S*)-decan-3-ol ((*S*)-3)**	1206	inc, syn, chgc	x	x				
j	*decanal*	1216	syn	x		x			x
k	dimethyltetrasulfide	1234	ms	x					
	benzothiazole	1246	ms			x			
L	**9-methyldecan-3-one (2)**	1260	inc, syn	x	x	x	x	x	
m	**(*S*)-9-methyldecan-3-ol ((*S*)-1)**	1273	inc, syn, chgc	x	x	x	x	x	
	undecan-3-one	1298	inc, syn		x	x			
	tridecane	1300	syn			x			
	undecan-3-ol	1308	inc, syn		x				
	2-aminoacetophenone (8)	1322	ms		x				
	4-methylquinoline (9)	1399	syn		x	x	x	x	
n	**geosmin (5)**	1430	syn	x	x	x	x	x	
o	geranylacetone	1457	ms	x		x			
	cyanoisoquinoline	1475	ms		x	x		x	
p	**(−)-germacrene D (6)**	1490	syn, chgc	x	x				
q	*pentadecane*	1500	syn	x		x			x
r	*diethyl phthalate*	1605	ms	x	x	x			x
s	**(1(10)*E*,5*E*)-germacradien-11-ol (7)**	1655	syn	x	x		x	x	
t	*heptadecane*	1700	syn	x					x
u	*diisobutyl phthalate*	1876	ms	x	x	x			x
v	*nonadecane*	1900	syn	x					x
w	*dibutyl phthalate*	1973	ms	x	x	x			x

[a] GC: marker in TIC in Figure 1, *I*: retention index, 1.–5.: different samples of *M. xanthus*, B: blank analysis of medium without bacteria, ms: mass spectrum, inc: retention index increment system, syn: synthetic sample, chgc: GC on chiral stationary phase.

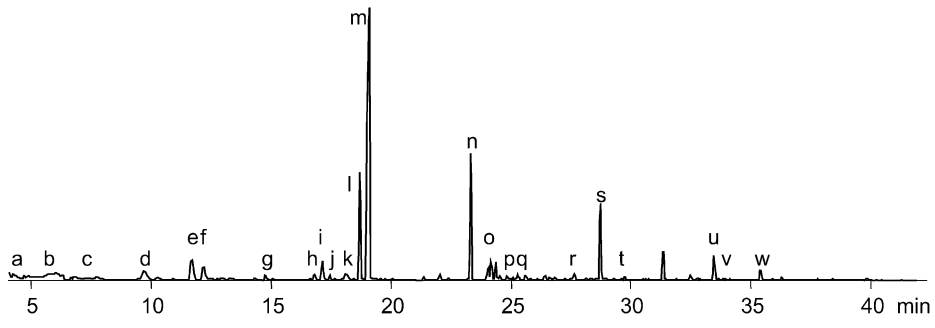
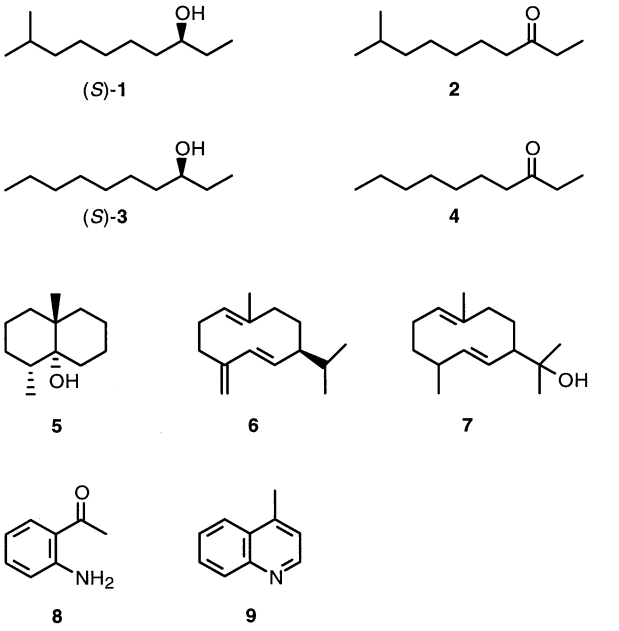


Figure 1. *Total ion chromatogram of an extract of* Myxococcus xanthus *(sample 1). Letters refer to compounds in Table 1.*

and *Myxococcus virescens* (4-quinolylaldoxime and 4-(hydroxymethyl)quinoline).[18] The analyses were repeated five times to check the reproducibility of the results. Some variation occurred in that not all compounds were always detected; the produced amounts were variable (see Table 1). For example, experiment four yielded only small amounts of material, so that only the major compounds were detected.

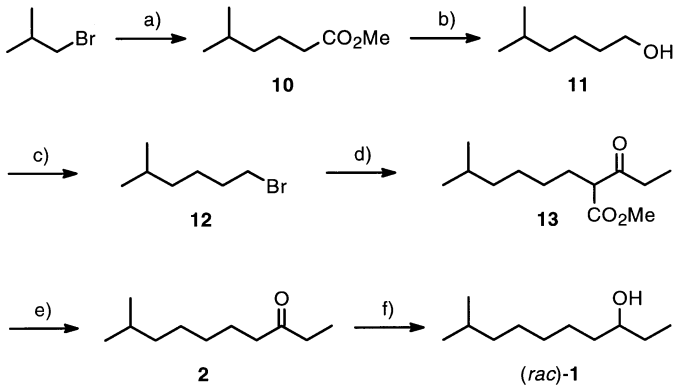


The two ketones had very similar mass spectra, and the later eluting one was readily identified as undecan-3-one by comparison with a synthetic sample. Further characterisation of the other ketone was based upon gas chromatographic retention indices,[19] because it was assumed that it was a branched compound. The relative position of a methyl group in a saturated carbon chain has a strong influence on the retention index. We have developed an empirical model for the calculation of the retention index, which follows Equation (1):[20]

$$I_C = N + FG + \sum Me_i - \sum S \qquad (1)$$

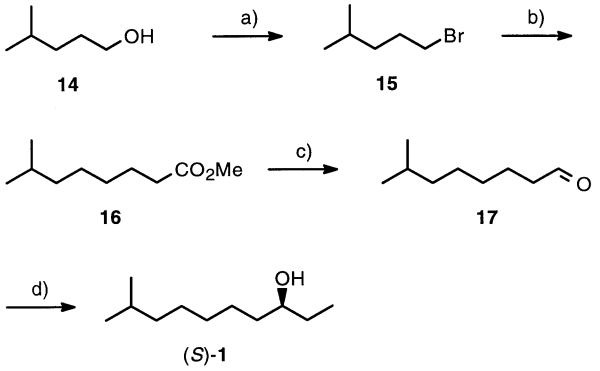
N is the number of carbon atoms in the longest alkyl chain times one hundred, *FG* is an increment for the functional group, previously determined to be *FG*(3-one) = 195 ± 3 for 3-ketones and *FG*(3-ol) = 205 ± 2 in the case of 3-alcohols, Me_i is an increment corresponding to the position of methyl branches in the chain (summarized in ref. [20]), and *S* is a steric increment to be considered if there are two or more methyl branches in one molecule. Normally, the observed and the calculated retention indices do not fall more than five to ten units apart. With this empirical knowledge, only a few structural options remained: the unknown alcohol (I = 1273) of the molecular formula $C_{11}H_{24}O$ could be branched in the positions ω-1 (**1**, I_C = 1265) or ω-2 (8-methyldecan-3-ol, I_C = 1278), and the corresponding ketone (I = 1260) in the positions ω-1 (**2**, I_C = 1255), ω-2 (8-methyldecan-3-one, I_C = 1268) or ω-3 (7-methyldecan-3-one, I_C = 1251). Methyl branches originating from amino acids such as leucine and valine (leading to an ω-1-methyl branch) or isoleucine (corresponding to a methyl branch in the ω-2 position) are often found in polyketides. The best match of measured and calculated retention indices was found for the ω-1 position.

The synthesis of 9-methyldecan-3-one (**2**) and 9-methyldecan-3-ol ((*rac*)-**1**) was performed to confirm the proposed



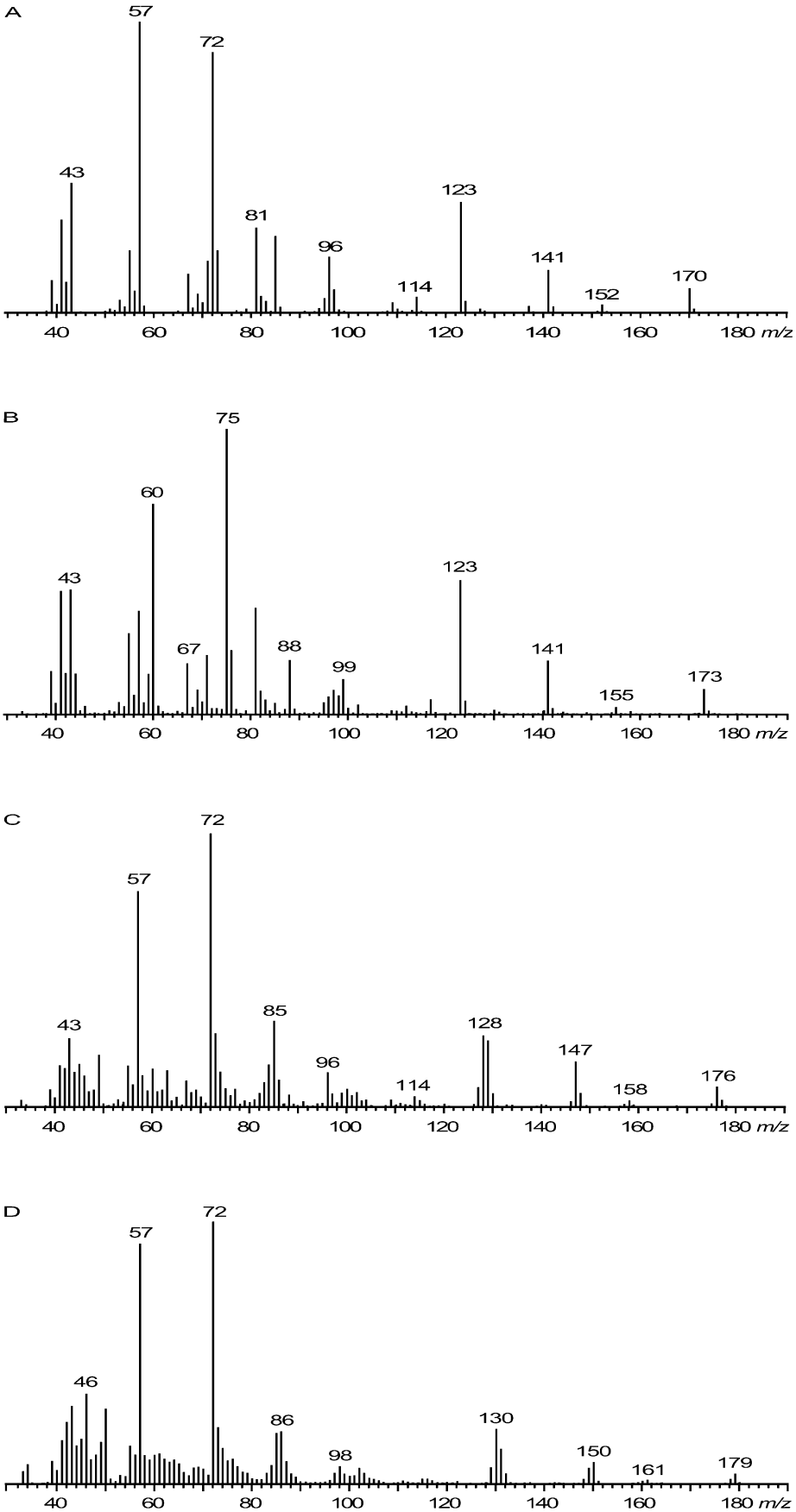
Scheme 1. *Synthesis of (rac)-**1**. a) 1. Mg, 2. CuI, Me_2S, DMAP, methyl acrylate, TMSCl, 78%; b) $LiAlH_4$, 90%; c) Br_2, PPh_3, 87%; d) NaH, 3-oxopentanoic acid methyl ester, 60%; e) NaCl, H_2O, DMSO, 88%; f) $LiAlH_4$, 95%.*

structures (see Scheme 1). A Michael addition of 2-methylpropylmagnesium bromide to methyl acrylate introduced the required methyl branch and afforded methyl 5-methylhexanoate (**10**).[21] Reduction to the alcohol **11** and bromination with triphenylphosphane and bromine led to 1-bromo-5-methylhexane (**12**).[22] The compound **12** was used to alkylate methyl 3-oxopentanoate, furnishing the required methyl ester **13** in a moderate yield.[23] Krapcho deethoxycarbonylation[24] afforded **2**, which was subsequently reduced with $LiAlH_4$ to form (*rac*)-**1**. Comparison of the retention indices and mass spectra of the synthetic compounds with those of the natural products confirmed our structural proposals.



Scheme 2. *Synthesis of (S)-**1**. a) Br_2, PPh_3, 85%; b) 1. Mg, 2. CuI, Me_2S, DMAP, methyl acrylate, TMSCl, 62%; c) DIBAH, 85%, d) Ti(OiPr)$_4$, (1*R*,2*R*)-N,N′-bis-(trifluoromethylsulfonyl)-1,2-diaminocyclohexane, $ZnEt_2$, 76%, 98% ee.*

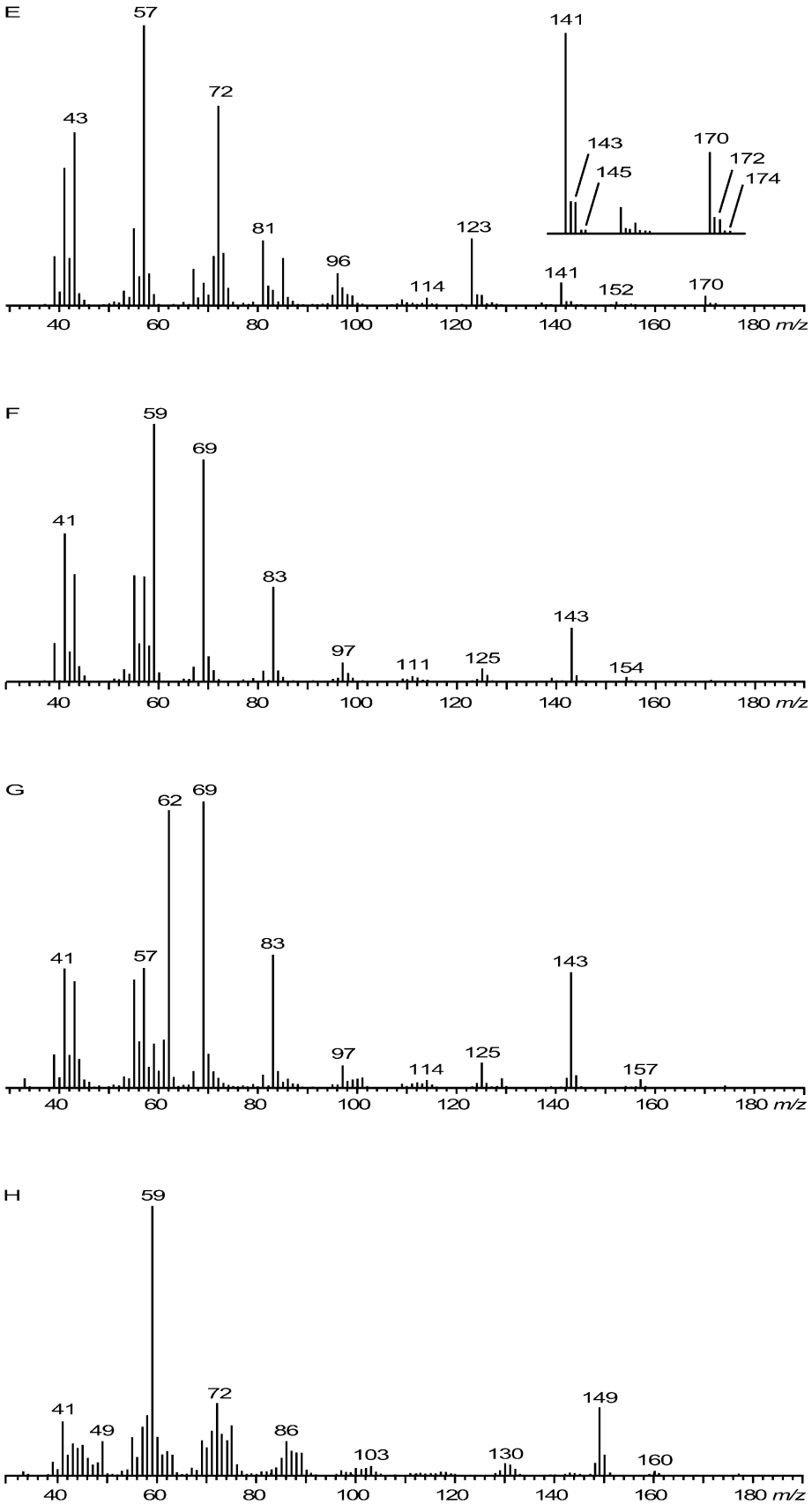
An enantioselective synthesis of **1** for the determination of the absolute configuration was then carried out according to Scheme 2. The alcohol 4-methyl-1-pentanol (**14**) was transformed into the corresponding alkyl bromide **15**. Its Grignard reagent was then added to methyl acrylate, forming methyl 7-methyloctanoate (**16**), which was reduced with DIBAH to yield the aldehyde **17**.[25] We then used enantioselective diethylzinc addition to form the corresponding carbinol.[26] With aliphatic aldehydes, (1*R*,2*R*)-*N*,*N*′-bis-(trifluoromethylsulfonyl)-1,2-diaminocyclohexane as chiral ligand in combination with Ti(O*i*Pr)$_4$ is



known to give particularly high *ee* values in the products and to form the *S* enantiomers preferentially. This method afforded (*S*)-**1** in good yield and high enantiomeric purity (76%, 98% *ee*). Analysis by GC with a chiral stationary phase showed the natural product to be pure (*S*)-**1**. In the same manner, the absolute configuration of a minor component emitted by *M. xanthus*, decan-3-ol (**3**), was also shown to be *S*.

The biosynthetic pathways to 3-alcohols or 3-ketones are not known. Therefore we undertook feeding experiments with labelled precursors to clarify this point. We suggest that **2** is formed from isovaleryl-SCoA, which originates from leucine degradation by transamination and oxidative decarboxylation.[27] Compound **2** might be generated by extension with two malonate units and one final methylmalonate building block by a fatty acid synthase or polyketide synthase.[27,28] The sequence would be completed by reductive loss of the carboxylic acid group. Final reduction should then lead from the ketone **2** to the alcohol (*S*)-**1**.

On the basis of this hypothesis, [D_{10}]leucine was fed to *M. xanthus* wild-type strain DK1622 and to the mutant strain JD300, which is impaired in the degradation of branched-chain keto acids due to a mutation in the *bkd* gene (*b*ranched-chain *k*eto acid *d*ehydrogenase; *bkd* is also called *esg* in *M. xanthus*).[29,30] We have recently shown that the mutant is "leaky" and is still able to generate minute amounts of isovaleryl-SCoA from leucine.[31] In earlier investigations a newly generated *bkd*$^-$ mutant strain of *S. aurantiaca* was found to be a producer of the polyketide myxothiazol even though the biosynthesis of this compound is initiated by isovaleryl-SCoA.[32] Because the



mutant reported in that study is not "leaky", a *bkd*-independent pathway to isovaleryl-SCoA must exist. Feeding experiments employing different ^{13}C-labelled acetates pointed to the formation of this starter unit via hydroxymethylglutaryl-SCoA and 3,3-dimethylacryloyl-SCoA.[32]

Similar results were found during analysis of ω-1-methyl-branched fatty acids of both *M. xanthus* strain DK1622 and *S. aurantiaca* strain DW4/3–1.[32] We therefore reasoned that the biosynthesis of (*S*)-**1** and **2** could also be dependent on 3,3-dimethylacrylic acid, and so we fed this compound in deuterated form to wild-type and *bkd*$^-$ mutant strains of *M. xanthus* to see whether it would be accepted as precursor.

Labelled [D_{10}]leucine offered to *M. xanthus* DK1622 was incorporated into **2** (25%), as discussed below (see Scheme 3 and Figure 2A and D). The effective mass, μ, of a C–D group ($\mu = {}^{24}/_{14} \approx 1.71$) is higher than that of a C–H group ($\mu = {}^{12}/_{13} \approx 0.92$). For this reason the vibrational ground state of a C–D group is at lower energy than that of a C–H group. The anharmonic Morse potential thus makes a C–D bond shorter than a C–H bond,[33] and this is the reason for a decrease in the GC retention times of deuterated compounds. Because of their high deuterium content, labelled compounds identified in this study elute earlier in the GC (ca. 5 to 10 s) than their unlabelled counterparts. Therefore, pure mass spectra of labelled compounds could be obtained. The peaks in the mass spectrum of **2** at $m/z = 123$, 141 and 152 were shifted to $m/z = 132$, 150 and 161 and the molecular ion from $m/z = 170$ to $m/z = 179$, all demonstrating the incorporation of nine deuterium atoms. One deuterium was lost during the transamination from [D_{10}]leucine to

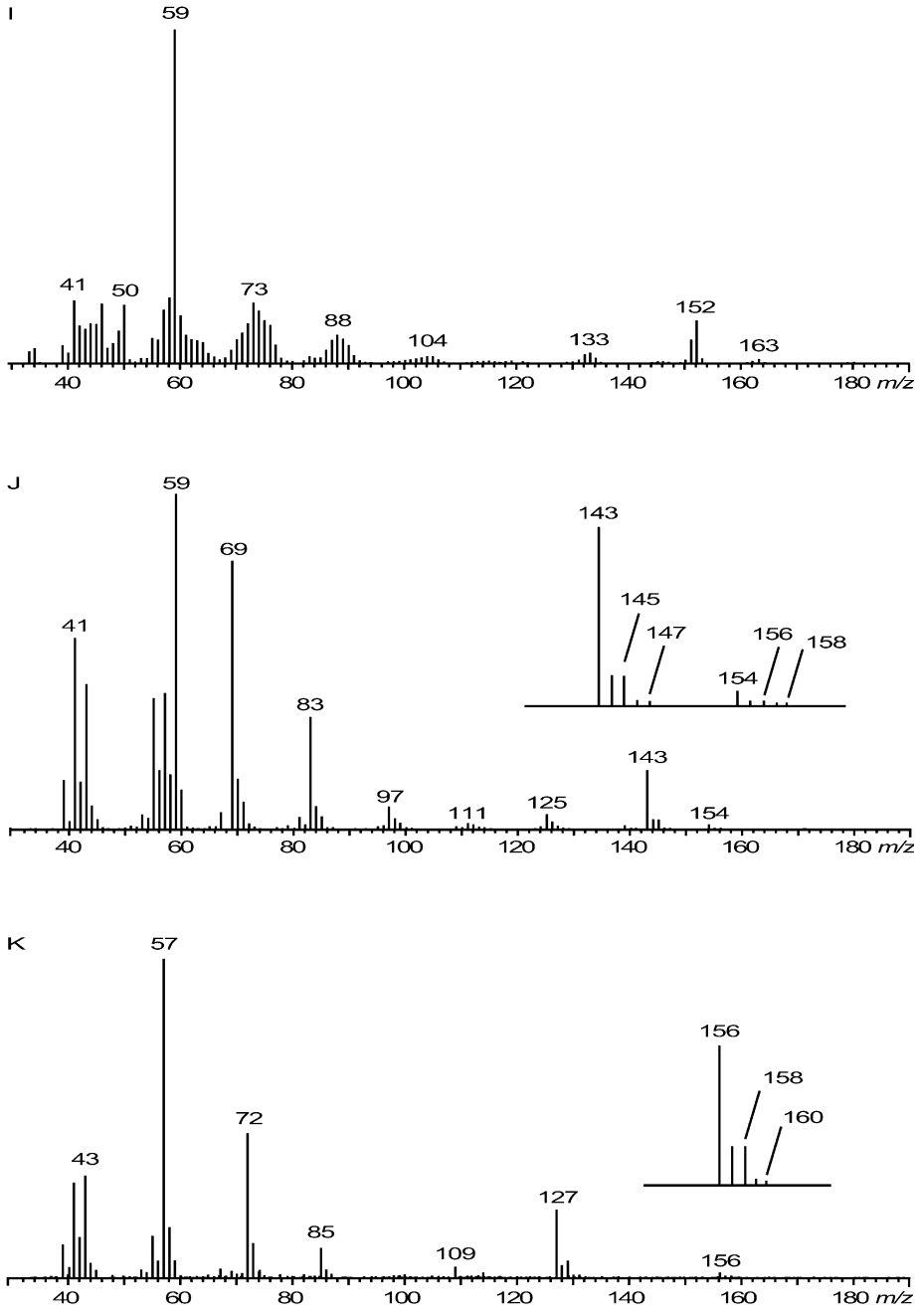
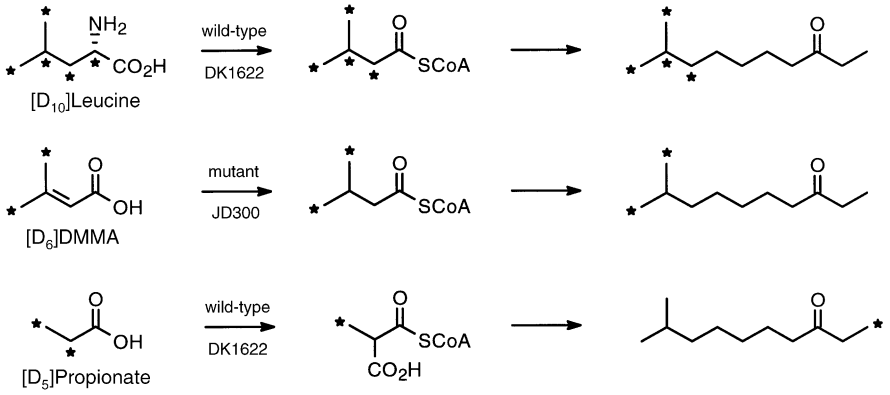


Figure 2. *Mass spectra of labelled and unlabelled 9-methyldecan-3-one (**2**) and (S)-9-methyldecan-3-ol (S)-**1**: Compound **2** (A), [D_3]-**2** after feeding of strain DK1622 with [D_5]propionate (B), [D_6]-**2** after feeding of mutant strain JD300 with [D_6]DMAA (C), [D_9]-**2** after feeding of DK1622 with [D_{10}]leucine (D), [$^{13}C_2$]-**2** after feeding of DK1622 with [$^{13}C_2$]acetate (E), Compound (S)-**1** (F), [D_3]-(S)-**1** after feeding of DK1622 with [D_5]propionate (G), [D_6]-(S)-**1** after feeding of JD300 with [D_6]DMAA (H), [D_9]-(S)-**1** after feeding of DK1622 with [D_{10}]leucine (I), [$^{13}C_2$]-(S)-**1** after feeding of DK1622 with [$^{13}C_2$]acetate (J), [$^{13}C_2$]-**4** after feeding of DK1622 with [$^{13}C_2$]acetate (K).*

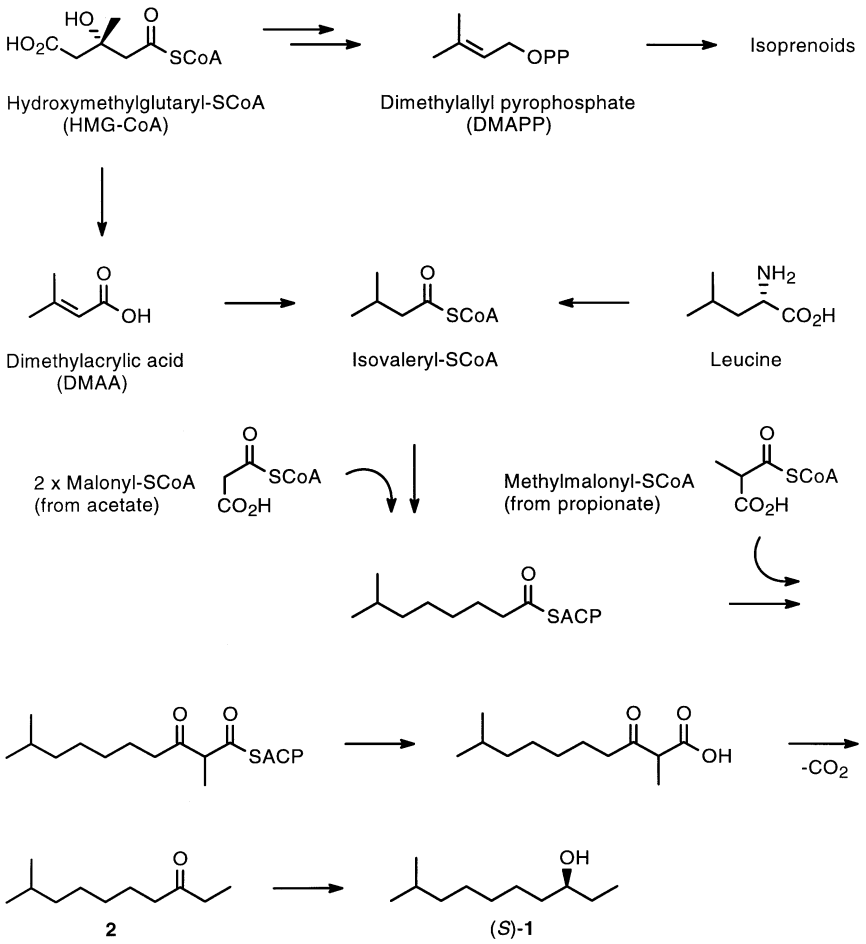
In further experiments, [D_6]-3,3-dimethylacrylic acid ([D_6]DMAA) was fed both to the wild-type and to one mutant strain. Strain DK1622 formed neither labelled (*S*)-**1** nor labelled **2**. In contrast, mutant JD300 was able to produce [D_6]-(*S*)-**1** and [D_6]-**2** after feeding with [D_6]DMAA (see Scheme 3). Thus, there must be another pathway to the isovaleryl-SCoA starter unit, and the incorporation rates of [D_6]DMAA into (*S*)-**1** (73%) and **2** (60%) indicate the participation of the mevalonate pathway in the formation of isovaleryl-SCoA via hydroxymethylglutaryl-SCoA (HMG-CoA), as also shown in previous experiments.[32] To the best of our knowledge, this is only the second example of the use of this novel biosynthetic branch of the mevalonate pathway. Comparison of the mass spectra of (*S*)-**1** and [D_6]-(*S*)-**1** (see Figure 2F and H) shows characteristic shifts of 6 amu in the fragment ions at $m/z = 97$, 125, 143 and 154 to $m/z = 103$, 131, 149 and 160, respectively. Similar results were found for the ketone **2** (see Figure 2A and C).

To investigate the biosynthesis of (*S*)-**1** and **2** further, [D_5]propionate was offered to wild-type *M. xanthus*. Incorporation of three deuterium atoms was indicated by shifts in the fragment ions ($[M-H_2O]^+$) from $m/z = 154$ to 157 (Figure 2F and G, (*S*)-**1**) and $m/z = 170$ to 173 (see Figure 2A and B, **2**). In the same manner, fragments of α-cleavage increased from $m/z = 59$ to 62 and $m/z = 57$ to 60. The ion originating from the McLafferty rearrangement in the case of **2** moves from $m/z = 72$ to 75. The α-fragments originating from cleavage next to the other side of the functional groups ($m/z = 141$ and $m/z = 143$), however, do not increase. All these data indicate incorporation of one propionate unit at C-1 and C-2 of the compounds. The two deuterium atoms at C-2 of propionate are lost during the formation of methylmalonyl-SCoA and by hydrogen exchange on this acidic position.

[D_9]α-ketoisovaleric acid. Incorporation of [D_{10}]leucine was also found in the case of (*S*)-**1** (26%). Characteristic fragment ions (compare Figure 2F and I) were shifted from $m/z = 143$, 154 and 171 to 152, 163 and 180, respectively, while the molecular ions were hard to detect because of their low abundance. The mutant strain JD300 also turned out to be a producer of [D_9]-**2** when fed with [D_{10}]leucine. The smaller incorporation rate (3%) indicated that the mutant is "leaky". Incorporation into (*S*)-**1** could not be observed.



Scheme 3. *Feeding experiments with* Myxococcus xanthus.



Scheme 4. *Biosynthetic pathways to 9-methyldecan-3-one (**2**) and (S)-9-methyldecan-3-ol ((S)-**1**).*

Finally, [$^{13}C_2$]acetate was fed to *Myxococcus xanthus*. In this case the GC retention times of labelled and unlabelled compounds do not differ, as the effective mass of a ^{13}C–H group ($\mu = {}^{13}/_{14} \approx 0.929$) is almost the same as that of a ^{12}C–H group ($\mu = {}^{12}/_{13} \approx 0.923$) so there are no significant differences in bond lengths. The effect on a C–C bond is even smaller. In consequence, no pure mass spectra of labelled compounds could be obtained, but incorporation of [$^{13}C_2$]acetate is indicated by stronger peaks at $m/z = 172$ (**2**, see Figure 2E) and $m/z = 156$ ($[M-H_2O]^+$, (*S*)-**1**, see Figure 2J). Twofold incorporation of [$^{13}C_2$]acetate can also be observed through the occurrence of small peaks at $m/z = 174$ (**2**) and $m/z = 158$ ($[M-H_2O]^+$, (*S*)-**1**). As expected, incorporation of [$^{13}C_2$]acetate into decan-3-one **4** also occurred (see Figure 2K).

Thus, biosynthesis of (*S*)-**1** and **2** is shown to start with isovaleryl-SCoA, followed by two chain-elongation steps with malonyl-SCoA, and completed by reaction with propionate-derived methylmalonyl-SCoA. Final removal of the carboxylic group results in the formation of **2**, which is reduced to (*S*)-**1**. In *M. xanthus* two pathways to isovaleryl-SCoA exist: the well known transformation of leucine and a new pathway from acetate via HMG-CoA and DMAA, respectively. The results of these biosynthetic investigations are summarized in Scheme 4.

Whether the biosynthetic machinery for the production of the 3-ketones is encoded in unassigned polyketide genes is currently being investigated in our laboratories. Another possibility is the formation of (*S*)-**1** and **2** by fatty acid biosynthesis.

The results presented here show that the small quantities of volatiles produced by cultures on agar plates are sufficient for investigation of the biosynthetic pathways of volatiles. To the best of our knowledge, this is the first work to make use of this approach, most studies in this area being performed with liquid cultures of microorganisms.

Experimental Section

Strains, culture conditions, feeding experiments: The strains *Myxococcus xanthus* DK1622 and the corresponding kanamycin-resistant esg mutants JD300 and DK11017 were cultured in TS medium composed of tryptone (Difco, 0.1%), $MgSO_4 \cdot 7H_2O$ (Merck,0.02%), starch (Merck, 0.04%), HEPES (Roth, 0.119%), pH 7.2. All feeding ex-

periments were conducted in glass petri dishes containing 50 mL of tryptone starch agar (10 g L^{-1} tryptone, 2 g L^{-1} $MgSO_4 \cdot 7H_2O$, 4 g L^{-1} soluble starch, 11.9 g L^{-1} HEPES buffer; pH adjusted to 7.2 with KOH; 15 g L^{-1} agar containing 0.2% Difco agar) containing the labelled precursors (1 mM final concentration). For racemic [D_{10}]leucine only, the final concentration was 8 mM. About 500 μL liquid culture (10^8 cells) were placed on the prepared agar plates and dried. The plates were incubated at 30 °C for 1 to 3 d and then analysed.

Racemic [D_{10}]leucine (98% deuterium) and [$^{13}C_2$]acetate (99% ^{13}C) were obtained from Campro Scientific, [D_6]propionic acid (98.5% deuterium) from Acros Organics (Geel, Belgium), and [D_6]-3,3-dimethylacrylic acid by synthesis.

Sampling: Volatile organic compounds emitted by cell cultures of *Myxococcus xanthus* were collected by the CLSA technique.[16] The volatiles were adsorbed on charcoal (Chromtech Gesellschaft für analytische Meßtechnik mbH, Idstein, Germany, Precision Charcoal Filter, 5 mg) for 24 hours, and then eluted with 30 μL of dichloromethane. The obtained solutions were immediately analysed by GC-MS and stored at −70 °C.

GC-MS: GC-MS analyses were carried out on a HP 6890 Series GC System connected to a HP 5973 Mass Selective Detector (Hewlett–Packard Company, Wilmington, USA) fitted with a BPX5 fused silica capillary column (25 m × 0.22 mm i.d., 0.25 μm film, SGE Inc., Melbourne, Australia). Conditions were as follows: inlet pressure: 77.1 kPa, He 23.3 mL min^{-1}; injection volume: 1 μL; transfer line: 300 °C; electron energy: 70 eV. The GC was programmed as follows: 5 min at 50 °C, increasing at 5 °C min^{-1} to 300 °C, and operated in splitless mode (60 s valve time). The carrier gas was He at 1 mL min^{-1}. Retention indices *I* were determined from a homologous series of *n*-alkanes (C_8–C_{25}). Identification of compounds was performed by comparison of mass spectra with the Wiley 6 Library and the Essential Oils Library (Massfinder) and with synthetic standards (see Table 1 for details).

Chiral GC: Separation of the enantiomers of **1** and **3** was carried out on a 8000Top GC (ThermoQuest, Toronto, Canada) instrument fitted with a heptakis-(6-*O*-TBDMS-2,3-di-*O*-acetyl)-β-cyclodextrin fused silica column (15 m × 0.25 mm i.d.) and a FID. Conditions were as follows: inlet pressure: 30 kPa, H_2 20 mL min^{-1}; injection volume: 1 μL. The GC was programmed as follows: 3 min at 70 °C, increasing at 0.5 °C min^{-1} to 130 °C (9-methyldecan-3-ol (**1**)) and 100 °C isotherm (decan-3-ol (**3**)), and operated in splitless mode (60 s valve time). The carrier gas was H_2 at 1 mL min^{-1}.

Synthesis (general methods): Chemicals: Chemicals were purchased from Fluka Chemie GmbH (Buchs, Switzerland) or Sigma–Aldrich Chemie GmbH (Steinheim, Germany). ^1H NMR: spectra were obtained on a Bruker AC 200 (200 MHz) or AMX 400 (400 MHz) spectrometer with TMS as an internal standard. ^{13}C NMR: spectra were recorded on a Bruker AC 200 (50 MHz) or AMX 400 (100 MHz) instrument with TMS as an internal standard. FTIR: spectra were obtained on a HP 6890 Series GC system connected to a HP 5965A Infrared Detector (Hewlett–Packard Company, Wilmington, USA). Optical rotation: optical rotation was determined on a Dr. Kernchen Propol Digital Automatic Polarimeter. Column chromatography: column chromatography was carried out on Merck Kieselgel 60. Thin layer chromatography: TLC was carried out on 0.2 mm precoated plastic sheets Polygram Sil G/UV_{254} (Macherey–Nagel, Düren, Germany). Solvents: solvents were purified by distillation and dried according to standard methods.

Methyl esters 10 and 16: A mixture of methyl acrylate (0.86 g, 10 mmol) and Me_3SiCl (2.18 g, 20 mmol) in THF (10 mL) was added dropwise over 30 min to a cooled (−78 °C) THF (40 mL) solution of alkylmagnesium bromide (prepared from Mg (10 mmol) and the alkyl bromides isobutyl bromide or **12** (10 mmol)), DMAP (2.44 g, 20 mmol), CuBr (144 mg, 1 mmol) and dimethyl sulfide (62 mg, 1 mmol), as reported by Nakamura et al.[21] After the mixture had been stirred for 3 h at −78 °C, diethyl ether (100 mL) and HCl (2 N, 50 mL) were added. The aqueous phase was separated and extracted three times with diethyl ether. The combined organic layers were dried with $MgSO_4$. The pure 1,4-adduct was obtained as a colourless liquid after solvent evaporation and column chromatography.

Compound 10: 78%; TLC: $R_F = 0.80$ (pentane/diethyl ether 5:1); GC: $I = 993$; ^1H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 0.88$ (d, $J =$ 6.6 Hz, 6H; $2 \times CH_3$), 1.13–1.26 (m, 2H; CH_2), 1.42–1.70 (m, 3H; CH, CH_2), 2.29 (t, $J = 7.6$ Hz, 2H; CH_2), 3.67 (s, 3H; CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 22.5$ ($2 \times CH_3$), 22.9 (CH_2), 27.8 (CH), 34.3 (CH_2), 38.4 (CH_2), 51.4 (CH_3), 174.3 (CO) ppm; FTIR (gas phase): $\tilde{\nu} = 2961$ (C–H), 1759 (C=O), 1589, 1444, 1357, 1236, 1173, 1025, 855 cm^{-1}; EI-MS (70 eV): *m/z* (%): 129 (1) $[M-CH_3]^+$, 113 (10), 101 (30), 87 (27), 74 (100) $[C_3H_6O_2]^+$, 69 (27), 59 (23), 55 (15), 43 (36), 41 (28).

Compound 16: 62%; TLC: $R_F = 0.65$ (pentane/diethyl ether 10:1); GC: $I = 1194$; ^1H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 0.89$ (d, $J =$ 6.6 Hz, 6H; $2 \times CH_3$), 1.13–1.22 (m, 2H; CH_2), 1.25–1.34 (m, 4H; $2 \times CH_2$), 1.52 (non, $J = 6.6$ Hz, 1H; CH), 1.59–1.66 (m, 2H; CH_2), 2.33 (t, $J = 7.5$ Hz, 2H; CH_2), 3.69 (s, 3H; CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 22.5$ ($2 \times CH_3$), 24.9 (CH_2), 26.9 (CH_2), 27.8 (CH), 29.3 (CH_2), 34.0 (CH_2), 38.7 (CH_2), 51.3 (CH_3), 174.2 (CO) ppm; FTIR (gas phase): $\tilde{\nu} = 2954$ (C–H), 1759 (C=O), 1446, 1357, 1214, 1170, 1047 cm^{-1}; EI-MS (70 eV): *m/z* (%): 172 (3) $[M]^+$, 157 (7) $[M-CH_3]^+$, 141 (8), 129 (27), 123 (9), 101 (10), 97 (16), 87 (79), 74 (100) $[C_3H_6O_2]^+$, 69 (17), 59 (16), 55 (35), 43 (32), 41 (30).

Alcohols 11 and (*rac*)-1: According to standard procedures, a solution of the appropriate methyl ester (15.0 mmol) in dry diethyl ether (20 mL) was added dropwise to a suspension of $LiAlH_4$ (1.14 g, 30.0 mmol) in dry diethyl ether (50 mL). After the system had been heated at reflux for 3 h, the reaction mixture was cooled with ice, diluted with water (30 mL) and HCl (2 N, 30 mL), and extracted with diethyl ether (3 × 50 mL). The combined extracts were dried with $MgSO_4$. The residue obtained after concentration was separated by chromatography on silica gel. After evaporation of the eluate the pure alcohol was obtained as a colourless liquid.

Compound 11: 90%; TLC: $R_F = 0.43$ (pentane/diethyl ether 1:1); GC: $I = 941$; ^1H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 0.88$ (d, $J =$ 6.5 Hz, 6H; $2 \times CH_3$), 1.13–1.64 (m, 7H; CH, $3 \times CH_2$), 1.81 (br s, 1H; OH), 3.63 (t, $J = 6.5$ Hz, 2H; CH_2) ppm; ^{13}C NMR (50 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 22.6$ ($2 \times CH_3$), 23.6 (CH_2), 28.0 (CH_2), 33.1 (CH_2), 38.8 (CH), 63.0 (CH_2) ppm; FTIR (gas phase): $\tilde{\nu} = 3667$ (O–H), 2949 (C–H), 1466, 1382, 1044 cm^{-1}; EI-MS (70 eV): *m/z* (%): 98 (2) $[M-H_2O]^+$, 83 (28), 73 (17), 70 (50), 69 (48), 56 (92), 55 (100), 43 (57), 41 (65), 39 (22).

Compound (*rac*)-1: 95%; TLC: $R_F = 0.23$ (pentane/diethyl ether 5:1); GC: $I = 1273$; ^1H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 0.86$ (d, $J = 6.6$ Hz, 6H; $2 \times CH_3$), 0.94 (t, $J = 7.5$ Hz, 3H; CH_3), 1.14–1.19 (m, 2H; CH_2), 1.28–1.35 (m, 5H; $2 \times CH_2$, CH), 1.38–1.55 (m, 6H; $3 \times CH_2$), 1.63 (br s; OH), 3.49–3.55 (m, 1H; CH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 10.0$ (CH_3), 22.8 ($2 \times CH_3$), 25.8 (CH_2), 27.5 (CH_2), 28.1 (CH), 30.1 (CH_2), 30.3 (CH_2), 37.1 (CH_2), 39.1 (CH_2), 73.5 (CH) ppm; FTIR (gas phase): $\tilde{\nu} = 3656$ (O–H), 2937 (C–H),

1463, 1379, 1230, 1110, 975 cm^{-1}; EI-MS (70 eV): *m/z* (%): 154 (2) $[M-H_2O]^+$, 143 (28), 125 (6), 111 (3), 97 (9), 83 (44), 69 (100), 59 (96), 57 (44), 55 (44), 43 (33), 41 (44).

Alkyl bromides 12 and 15: Bromine (3.20 g, 20.0 mmol) was added dropwise to a solution of triphenylphosphane (5.24 g, 20.0 mmol) at 0 °C until the yellow colour persisted, as described by Sonnet.[22] The alcohol **11** or **14** (15.0 mmol), diluted with dichloromethane (5 mL), was added at one time. After stirring for 2 h at 0 °C, the reaction mixture was diluted with diethyl ether (150 mL) and washed with saturated $NaHSO_3$ solution to remove excess bromine. The organic layer was separated, dried with $MgSO_4$ and concentrated in vacuo. The residue was separated by column chromatography on silica gel with pentane/diethyl ether (5:1) to provide the pure bromide as a colourless liquid.

Compound 12: 87%; TLC: $R_F = 0.95$ (pentane/diethyl ether 5:1); GC: $I = 1002$; ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 0.88$ (d, $J =$ 6.6 Hz, 6H; 2×CH_3), 1.12–1.64 (m, 5H; CH, CH_2, CH_2), 1.77–1.96 (m, 2H; CH_2), 3.41 (t, $J = 6.8$ Hz, 2H; CH_2) ppm; ^{13}C NMR (50 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 22.5$ (2×CH_3), 26.0 (CH_2), 27.9 (CH), 33.1 (CH_2), 34.0 (CH_2), 38.1 (CH_2) ppm; FTIR (gas phase): $\tilde{\nu} = 2960$ (C–H), 1460, 1380, 1252 cm^{-1}; EI-MS (70 eV): *m/z* (%): 137 (100) $[C_4H_8{}^{81}Br]^+$, 135 (100) $[C_4H_8{}^{79}Br]^+$, 123 (2), 121 (2), 109 (3), 107 (3), 83 (17), 69 (4), 57 (19), 55 (61), 43 (56), 41 (51), 39 (21).

Compound 15: 85%; TLC: $R_F = 0.95$ (pentane/diethyl ether 5:1); GC: $I = 904$; ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 0.90$ (d, $J =$ 6.4 Hz, 6H; 2×CH_3), 1.28–1.34 (m, 2H; CH_2), 1.58 (non, $J = 6.6$ Hz, 1H; CH), 1.82–1.90 (m, 2H; CH_2), 3.40 (t, $J = 6.9$ Hz, 2H; CH_2) ppm; ^{13}C NMR (50 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 22.5$ (2×CH_3), 27.5 (CH), 30.9 (CH_2), 34.3 (CH_2), 37.4 (CH_2) ppm; FTIR (gas phase): $\tilde{\nu} = 2961$ (C–H), 2417, 1461, 1381, 1252, 649 cm^{-1}; EI-MS (70 eV): *m/z* (%): 166 (7) $[C_6H_{13}{}^{81}Br]^+$, 164 (7) $[C_6H_{13}{}^{79}Br]^+$ $[M]^+$, 151 (5), 149 (5), 123 (1), 121 (1), 109 (5), 107 (5), 85 (100), 69 (46), 43 (87), 41 (71), 39 (27).

Methyl 7-methyl-2-propionyloctanoate (13): A procedure similar to that described by Geremia et al.[23] was used. NaH (1.56 g, 39.0 mmol, 60% in mineral oil) was washed three times with pentane and then suspended in dry DME (40 mL). The suspension was cooled down to 0 °C, and methyl 3-oxovalerate (5.07 g, 39.0 mmol) in DME (5 mL) was added dropwise. After the system had been stirred for half an hour, tetra-*n*-butylammonium iodide (554 mg, 1.95 mmol) was added, followed by 1-bromo-5-methylhexane (**12**; 3.49 g, 19.5 mmol). The reaction mixture was heated at reflux for 20 h, quenched with 2 N HCl and extracted with diethyl ether (3×). The combined organic layers were dried with $MgSO_4$. Pure methyl 7-methyl-2-propionyloctanoate (**13**; 2.66 g, 11.7 mmol, 60%) was obtained as a colourless liquid after evaporation of the solvent and column chromatography on silica gel with pentane/diethyl ether (5:1). TLC: $R_F = 0.58$ (pentane/diethyl ether 5:1); GC: $I = 1523$; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 0.84$ (d, $J = 6.6$ Hz, 6H; 2×CH_3), 1.06 (t, $J = 7.2$ Hz, 3H; CH_3), 1.12–1.34 (m, 6H; 3×CH_2), 1.51 (non, $J = 6.6$ Hz, 1H; CH), 1.80–1.89 (m, 2H; CH_2), 2.49 (dq, ^2J(H,H) = 18.1 Hz, $J = 7.2$ Hz, 1H; CH_2), 2.59 (dq, ^2J(H,H) = 18.1 Hz, $J = 7.3$ Hz, 1H; CH_2), 3.45 (t, $J = 7.4$ Hz, 1H; CH), 3.72 (s, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.5$ (2×CH_3), 22.5 (CH_3), 27.0 (CH_2), 27.7 (CH_2), 27.8 (CH), 28.3 (CH_2), 35.1 (CH_2), 38.6 (CH_2), 52.3 (CH_3), 58.7 (CH), 170.4 (CO), 205.8 (CO) ppm; FTIR (gas phase): $\tilde{\nu} = 2958$ (C–H), 1736 (C=O), 1451, 1340, 1246, 1166 cm^{-1}; EI-MS (70 eV): *m/z* (%): 199 (1) $[M-C_2H_5]^+$, 172 (9), 130 (29), 101 (14), 87 (26), 57 (100), 41 (19); elemental analysis calcd (%) for $C_{13}H_{24}O_3$ (228.33): C 68.38, H 10.59; found C 68.58, H 10.78.

9-Methyldecan-3-one (2): Water (0.36 g, 20 mmol) and NaCl (1.17 g, 20 mmol) were added to a solution of methyl 7-methyl-2-propionyloctanoate (**13**; 2.25 g, 9.87 mmol) in DMSO (10 mL), as in Krapcho and Lovey's method.[24] The reaction mixture was heated at reflux for 24 h, diluted with water (50 mL) and finally extracted three times with diethyl ether. The combined extracts were dried with $MgSO_4$ and concentrated. Purification of the crude product by column chromatography on silica gel with pentane/diethyl ether (10:1) afforded 9-methyldecan-3-one (**2**; 1.47 g, 8.65 mmol, 88%) as a colourless liquid. TLC: $R_F = 0.59$ (pentane/diethyl ether (10:1); GC: $I = 1260$; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 0.86$ (d, $J =$ 6.6 Hz, 6H; 2×CH_3), 1.05 (t, $J = 7.3$ Hz, 3H; CH_3), 1.12–1.29 (m, 2H; CH_2), 1.21–1.32 (m, 4H; 2×CH_2), 1.51 (non, $J =$ 6.6 Hz, 1H; CH), 1.54–1.61 (m, 2H; CH_2), 2.37–2.45 (m, 4H; 2×CH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 8.1$ (CH_3), 22.9 (CH_3), 24.3 (CH_2), 27.4 (CH_2), 28.2 (CH), 29.8 (CH_2), 36.1 (CH_2), 39.1 (CH_2), 42.7 (CH_2), 212.1 (CO) ppm; FTIR (gas phase): $\tilde{\nu} = 2944$ (C–H), 1727 (C=O), 1590, 1461, 1356, 1149, 953 cm^{-1}; EI-MS (70 eV): *m/z* (%): 170 (4) $[M]^+$, 152 (1) $[M-H_2O]^+$, 141 (10) $[M-C_2H_5]^+$, 123 (29), 81 (25), 72 (77), 57 (100), 43 (56); elemental analysis calcd (%) for $C_{11}H_{22}O$ (170.29): C 77.58, H 13.02; found C 77.43, H 13.22.

7-Methyloctanal 15: Zakharkin and Sorokina's method was used.[25] The ester **16** (860 mg, 5.00 mmol) was dissolved in dry dichloromethane (50 mL). The solution was cooled down to −78 °C and then DIBAH (5.0 mL, 1 M in cyclohexane, 5.0 mmol) was added dropwise. The reaction mixture was stirred for 30 min, quenched with saturated Na tartrate solution and allowed to warm up to room temperature. The aqueous layer was separated and extracted three times with diethyl ether. The combined organic layers were dried with $MgSO_4$ and concentrated in vacuo. The pure product 7-methyloctanal (**17**; 607 mg, 4.27 mmol, 85%) was obtained by column chromatography on silica gel (pentane/diethyl ether 10:1) as a colourless liquid. TLC: $R_F = 0.47$ (pentane/diethyl ether 10/1); GC: $I = 1077$; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 0.86$ (d, $J =$ 6.6 Hz, 6H; 2×CH_3), 1.16–1.21 (m, 2H; CH_2), 1.28–1.35 (m, 4H; 2×CH_2), 1.52 (non, $J = 6.6$ Hz, 1H; CH), 1.60–1.67 (m, 2H; CH_2), 2.42 (dt, $J = 1.9$, 7.4 Hz, 2H; CH_2), 9.76 (t, $J = 1.9$ Hz, 1H; CHO) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 22.1$ (CH_2), 22.6 (2×CH_3), 27.1 (CH_2), 27.9 (CH), 29.4 (CH_2), 38.7 (CH_2), 43.9 (CH_2), 202.8 (CO) ppm; FTIR (gas phase): $\tilde{\nu} = 2943$ (C–H), 2813 (C–H), 2712, 1742 (C=O), 1462, 1381, 1168 cm^{-1}; EI-MS (70 eV): *m/z* (%): 124 (4) $[M-H_2O]^+$, 109 (20), 81 (26), 67 (19), 57 (100), 43 (76), 41 (81), 39 (30).

(*S*)-3-Alkanols ((*S*)-1 and (*S*)-3: The method reported by Kobayashi et al.[26] was used. $Ti(OiPr)_4$ (483 mg, 1.7 mmol, 1.2 equiv) was added to a solution of (1*R*,2*R*)-*N*,*N*′-bis-(trifluoromethylsulfonyl)-1,2-diaminocyclohexane (11 mg, 0.03 mmol) in dry toluene (1 mL). The solution was stirred for one hour and cooled to −78 °C. A solution of Et_2Zn in hexane (1.7 mL, 1 mol L^{-1}, 1.7 mmol, 1.2 equiv) was added, and the colour of the solution turned to orange. The aldehyde **17** (1.41 mmol, 1.0 equiv) was diluted with dry toluene (1 mL) and added dropwise. The reaction mixture was allowed to warm up to −20 °C, stirred for 2 h and quenched with HCl (2 N). The aqueous layer was separated and extracted three times with diethyl ether. The combined organic layers were dried with $MgSO_4$ and concentrated. The residue was separated by chromatography on silica gel with pentane/diethyl ether (5:1) to provide the pure (*S*)-3-alkanol as a colourless liquid.

Compound (*S*)-1: 76%; 98% *ee* determined by chiral GC, $t_R(R) =$ 61.1 min, $t_R(S) = 62.6$ min; TLC: $R_F = 0.23$ (pentane/diethyl ether 5:1); GC: $I = 1273$; $[\alpha]_D^{20.5}$ +5.01 ($c = 3.25$ in pentane); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 0.86$ (d, $J = 6.6$ Hz, 6H; 2×CH_3),

0.94 (t, $J=7.5$ Hz, 3H; CH_3), 1.14–1.19 (m, 2H; CH_2), 1.28–1.35 (m, 5H; $2\times CH_2$, CH), 1.38–1.55 (m, 6H; $3\times CH_2$), 1.63 (brs, OH), 3.49–3.55 (m, 1H; CH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): $\delta=$ 10.0 (CH_3), 22.8 ($2\times CH_3$), 25.8 (CH_2), 27.5 (CH_2), 28.1 (CH), 30.1 (CH_2), 30.3 (CH_2), 37.1 (CH_2), 39.1 (CH_2), 73.5 (CH) ppm; FTIR (gas phase): $\tilde{\nu}=3656$ (O–H), 2937 (C–H), 1463, 1379, 1230, 1110, 975 cm^{-1}; EI-MS (70 eV): *m/z* (%): 154 (2) $[M-H_2O]^+$, 143 (28) $[M-C_2H_5]^+$, 125 (6), 111 (3), 97 (9), 83 (44), 69 (100), 59 (96), 57 (44), 55 (44), 43 (33), 41 (44); elemental analysis calcd (%) for $C_{11}H_{22}O$ (172.30): C 76.68, H 14.04; found C 76.30, H 14.06.

Compound (*S*)-3: 89%; 100% *ee* determined by chiral GC, $t_R(R)=$ 16.9 min, $t_R(S)=17.3$ min; TLC: $R_F=0.20$ (pentane/diethyl ether 5:1); GC: $I=1206$; $[\alpha]_D^{21.2}$ +9.95 ($c=3.96$ in pentane); Naoshima et al.[34] reported an optical rotary power of $[\alpha]_D^{20}$ −9.07 ($c=3.76$ in pentane) for the *R* isomer; ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): $\delta=0.88$ (t, 3H; $J=6.7$ Hz, CH_3), 0.94 (t, 3H; $J=7.4$ Hz, CH_3), 1.17–1.63 (m, 14H; $7\times CH_2$), 1.89 (brs, 1H; OH), 3.47–3.54 (m, 1H, CH) ppm; ^{13}C NMR (50 MHz, $CDCl_3$, 25 °C, TMS): $\delta=9.9$ (CH_3), 14.1 (CH_3), 22.7 (CH_2), 25.7 (CH_2), 29.3 (CH_2), 29.7 (CH_2), 30.1 (CH_2), 31.9 (CH_2), 37.0 (CH_2), 73.3 (CH) ppm; FTIR (gas phase): $\tilde{\nu}=3654$ (O–H), 2936 (C–H), 2874 (C–H), 1460, 1379, 1231, 1117, 974 cm^{-1}; EI-MS (70 eV): *m/z* (%) = 140 (5) $[M-H_2O]^+$, 129 (30) $[M-C_2H_5]^+$, 111 (30), 97 (3), 83 (8), 69 (100), 59 (100), 55 (39), 41 (38).

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