

Volatile Methyl Esters of Medium Chain Length from the Bacterium *Chitinophaga* Fx7914

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The analysis of the volatiles released by the novel bacterial isolate *Chitinophaga* Fx7914 revealed the presence of *ca.* 200 compounds including different methyl esters. These esters comprise monomethyl- and dimethyl-branched, saturated, and unsaturated fatty acid methyl esters that have not been described as bacterial volatiles before. More than 30 esters of medium C-chain length were identified, which belong to five main classes, methyl (*S*)-2-methylalkanoates (class *A*), methyl (*S*)-2,(ω – 1)-dimethylalkanoates (class *B*), methyl 2,(ω – 2)-dimethylalkanoates (class *C*), methyl (*E*)-2-methylalk-2-enoates (class *D*), and methyl (*E*)-2,(ω – 1)-dimethylalk-2-enoates (class *E*). The structures of the compounds were verified by GC/MS analysis and synthesis of the target compounds as methyl (*S*)-2-methyloctanoate (**28**), methyl (*S*)-2,7-dimethyloctanoate ((*S*)-**43**), methyl 2,6-dimethyloctanoate (**49**), methyl (*E*)-2-methylnon-2-enoate (**20a**), and methyl (*E*)-2,7-dimethyloct-2-enoate (**41a**). Furthermore, the natural saturated 2-methyl-branched methyl esters showed (*S*)-configuration as confirmed by GC/MS experiments using chiral phases. Additionally, the biosynthetic pathway leading to the methyl esters was investigated by feeding experiments with labeled precursors. The Me group at C(2) is introduced by propanoate incorporation, while the methyl ester is formed from the respective carboxylic acid by a methyltransferase using *S*-adenosylmethionine (SAM).

Introduction. – Fatty acids are one of the ubiquitous building blocks of biological systems. The typical chain length varies in bacteria between 14 and 20, with the most common fatty acids containing 16 or 18 C-atoms. In addition to these compounds, many bacteria also produce methyl-branched fatty acids, carrying an additional Me group often in the iso- or anteiso-position [1]. These acids are usually conjugated to other compounds forming the various lipid classes of living organisms. In bacteria, a second group of acids can be produced, short-chain, often amino-acid derived, volatile acids with up to six C-atoms [2]. Fatty acids with a chain length between these two classes are known, but are encountered relatively rarely in bacteria, often present only in low amounts compared to the more common acids with a longer C-chain.

While short-chain fatty acids are volatile and have a distinct odor, the long-chain fatty acids possess a much lower vapor pressure. Some bacteria increase the volatility by transformation of the fatty acids into the methyl ester, making the compound also unavailable for other biosynthetic transformations.

Methyl esters of intermediate chain length have only rarely been reported from bacteria. Methyl 5-methylhexanoate and methyl 9-methyldecanoate have been

detected in volatiles released by *Stigmatella aurantiaca* [3], while methyl 4-methylpentanoate is produced by different actinomycetes [4]. Furthermore, methyl octanoate was found in the odor of different Pecorino cheeses, most likely originating from bacteria [5].

Chitinophaga belongs to the sphingobacteria and is a gliding bacterium not well investigated for the production of secondary metabolites. The antibiotic elansolide is the only natural compound known to be produced by these bacteria [6]. We became interested in the volatiles released by *Chitinophaga* Fx7914 which proved to be very complex. Beside several other components like sulfur compounds, amino acid derivatives, and diterpenes, a complex set of saturated and unsaturated fatty acid methyl esters of medium chain length was identified, all of them carrying a Me branch at C(2). Many of these compounds have not been reported from bacteria or other natural sources, beside a few exceptions [7–10]. The identification of these esters by GC and GC/MS, the syntheses of representative examples as well as the determination of the absolute configuration by stereoselective synthesis, followed by GC/MS on chiral phases, will be presented here. Furthermore, some aspects of the biosynthesis of these compounds were probed by feeding experiments using deuterium ($^2\text{H}_1$)-labeled precursors. Finally, the bouquet of volatiles released by *Chitinophaga* Fx7914 is described.

Results and Discussion. – Liquid cultures of *Chitinophaga* Fx7914 were analyzed using the closed-loop-stripping methodology (CLSA) adopted to microbiological work as described in [11][12]. The volatiles emitted by the bacteria were trapped on activated charcoal. Elution with CH_2Cl_2 furnished an extract that was analyzed by GC/MS. *Chitinophaga* cultures release a large variety of compounds which were identified by comparison of mass spectra and GC retention indices with library data and with synthetic standards (Fig. 1 and Table 1).

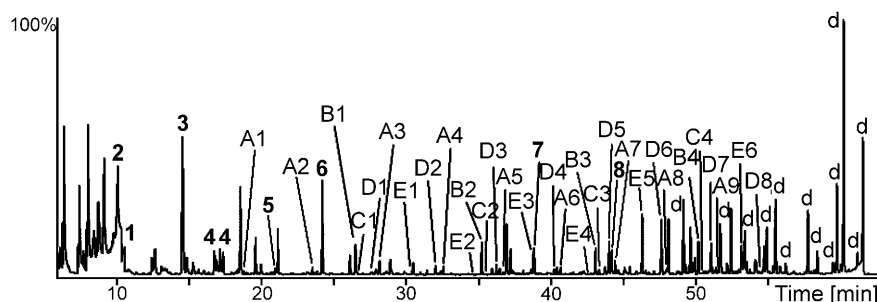


Fig. 1. Total ion chromatogram of a headspace extract of a liquid culture of *Chitinophaga* Fx7914

Characteristic compounds, repeatedly detected in large amounts in different experiments, were 2-methyl- and 3-methylbutanoic acid (**1** and **2**, resp.), 2-methyltetrahydrothiophenone (**3**), as well as the corresponding alcohol **4**, 2-phenylethanol (**5**), methyl phenylacetate (**6**), sesquiterpenes of the cadinane family such as γ -cadinene (**7**) and α -cadinol (**8**), several alcohols, ketones, and lactones, as well as diterpenoids of unknown structure. The biosynthesis of **3** has recently been investigated by us [13].

Table 1. *Volatiles Released by Chitinophaga Fx7914*

Entry	Compound	<i>I</i> ^{a)}	Ident. ^{b)}	Conc. ^{c)}
1	Methyl 2-methylpropionate		MS ^{d)}	xxx ^{e)}
2	4-Methylpentan-2-one		MS	xxx
3	Methyl 3-methylbutanoate		MS	xxx
4	3-Hydroxypentan-2-one	809	syn ^{f)}	xxx
5	2-Hydroxypentan-3-one	816	syn	xxx
6	5-Methylhexan-3-one	839	MS	xxx
7	4-Hydroxy-4-methylpentan-2-one	846	MS, <i>RI</i> ^{g)}	xxx
8	2-Hydroxy-4-methylpentan-3-one	853	MS	xxx
9	Ethylbenzene	860	MS, <i>RI</i>	xxx
10	Unknown 55, 73, 98 ^{h)}	862		xxx
11	<i>m</i> -Xylene	868	syn	xxx
12	Methyl 2-methylbut-2-enoate	870	MS, <i>RI</i>	xxx
13	2-Methoxy-1-methylethyl acetate	877	MS	xxx
14	<i>o</i> -Xylene	892	syn	xxx
15	2-Hydroxyhexan-3-one	898	MS	xxx
16	3-Methylbutanoic acid (2)	903	syn	xxx
17	2-Methylbutanoic acid (1)	908	syn	xxx
18	2-Butoxyethanol	910	MS	x ⁱ⁾
19	2,5-Dimethylpyrazine	913	syn	x
20	Butano-4-lactone	914	syn	x
21	But-2-eno-4-lactone	916	MS	x
22	Dimethylsulfone	923	syn	x
23	Hexane-2,5-dione	931	MS	x
24	Unknown 41, 73, 100	948		xxx
25	Pentano-4-lactone	953	syn	x
26	6-Methylheptan-2-one	957	MS, <i>RI</i>	xx ^{j)}
27	Benzaldehyde ^{k)}	958	syn	xx
28	Unknown 82, 132	962		xx
29	Dimethyl trisulfide	964	MS, <i>RI</i>	xx
30	Unknown 45, 88	968		x
31	Aniline	976	syn	x
32	2-Methyltetrahydrothiophen-3-one (3)	983	syn	xxx
33	2,4,6-Trimethylpyridine ^{k)}	990	MS	xxx
34	Methyl 4-methylhexanoate	999	MS, <i>RI</i>	xxx
35	3-Methylpenten-2-eno-4-lactone	1000	MS	xxx
36	2,4-Dimethylpent-2-eno-4-lactone	1011	MS	x
37	Unknown 43, 111, 126	1013		x
38	Unknown 43, 69, 113	1013		x
39	Mesitylene	1018	MS, <i>RI</i>	x
40	Unknown 43, 83, 98, 101	1020		x
41	Unknown 68, 100	1025		x
42	2-Methyltetrahydrothiophen-3-ol (4)	1027	syn	xxx
43	2-Ethylhexan-1-ol ^{k)}	1030	MS	x
44	Benzyl alcohol	1032	syn	xx
45	2-Methyltetrahydrothiophen-3-ol (4)	1035	syn	xxx
46	Unknown 43, 109	1039		xxx
47	Methyl 2-ethylhexanoate ^{k)}	1044	MS	x
48	7-Methyloctan-3-one	1050	MS, <i>RI</i>	x
49	Unknown 66, 132	1054		x

Table 1 (cont.)

Entry	Compound	<i>I</i> ^{a)}	Ident. ^{b)}	Conc. ^{c)}
50	Unknown 84, 129	1056		xx
51	1-Phenylethanol	1059	syn	xx
52	Acetophenone	1062	syn	xxx
53	<i>N</i> -Methylaniline	1062	syn	xxx
54	Methyl 2-methylheptanoate (A1)	1064	syn	xxx
55	2-Ethyl-4,6-dimethylpyridine ^{k)}	1067	MS	x
56	Methylthiobenzene	1079	MS	x
57	2-Phenylpropan-2-ol	1082	MS	xxx
58	<i>o</i> -Guaicol	1086	MS	x
59	Unknown 71, 95, 110, 128	1103		x
60	Unknown 43, 57, 83	1106		x
61	2-Phenylethanol (5)	1109	syn	xx
62	Heptano-4-lactone	1150	MS, <i>RI</i>	x
63	Methylundecene	1154	MS	x
64	8-Methylnonan-2-one	1156	MS, <i>RI</i>	x
65	Methyl 2-methyloctanoate (A2)	1161	syn	xx
66	Unknown 43, 77, 105	1164		x
67	Unknown 91, 119, 134	1168		x
68	Methyl 2-phenylacetate (6)	1175	syn	xxx
69	Decanal	1204	syn	x
70	Benzothiazole	1215	MS, <i>RI</i>	xxx
71	Methyl 2,7-dimethyloctanoate (B1)	1224	syn	xxx
72	Methyl 2,6-dimethyloctanoate (C1)	1226	syn	x
73	Methyl (<i>E</i>)-2-methyloct-2-enoate (D1)	1248	syn	x
74	9-Methyldecan-3-one	1251	MS, <i>RI</i>	x
75	Octano-4-lactone	1255	MS, <i>RI</i>	x
76	Benzyl propanoate	1257	MS	x
77	Methyl 2-methylnonanoate (A3)	1260	syn	x
78	Unknown 58, 87, 95	1269		x
79	Undecan-6-one	1271	MS, <i>RI</i>	x
80	Unknown 77, 106, 135, 159	1277		xxx
81	4-(Methylsulfanyl)thiophenol	1279	MS	xxx
82	Nonano-5-lactone	1281	MS, <i>RI</i>	x
83	Ethyl 4-methylbenzoate	1286	MS	x
84	Tridecane	1300	MS, <i>RI</i>	x
85	Methyl (<i>E</i>)-2,7-dimethyloct-2-enoate (E1)	1311	syn	xxx
86	Methyl geranate	1324	MS, <i>RI</i>	x
87	Methyl 3-methylalkanoate	1328	MS	x
88	Methyl (<i>E</i>)-2-methylnon-2-enoate (D2)	1348	syn	xx
89	Unknown 111, 184	1353		x
90	Methyl 2-methyldecenoate	1356	MS	x
91	Unknown 55, 70, 84, 124, 142	1358		x
92	Methyl 2-methyldecanoate (A4)	1360	syn	xx
93	2-Vinylnaphthalene ^{k)}	1373	MS	x
94	Dodecan-3-one	1389	MS, <i>RI</i>	x
95	Dodecan-2-one	1394	MS, <i>RI</i>	x
96	Tetradecane	1400	MS, <i>RI</i>	x
97	Methyl (<i>E</i>)-2,8-dimethylnon-2-enoate (E2)	1408	MS, <i>RI</i>	x
98	Methyl 2,9-dimethyldecenoate	1417	MS	x
99	Methyl 2,9-dimethyldecenoate (B2)	1423	MS, <i>RI</i>	xxx

Table 1 (cont.)

Entry	Compound	<i>I</i> ^{a)}	Ident. ^{b)}	Conc. ^{c)}
100	Methyl 2,8-dimethyldecanoate (C2)	1429	MS, <i>RI</i>	x
101	1,2-Dihydro-2,2,4-trimethylquinoline ^{k)}	1440	MS	x
102	Methyl (<i>E</i>)-2-methyldec-2-enoate (D3)	1448	syn	xx
103	Methyl 2-methylundecenoate	1453	MS	xx
104	Methyl 2-methylundecanoate (A5)	1460	syn	x
105	Dodecan-1-ol	1474	MS, <i>RI</i>	x
106	Decano-5-lactone	1493	MS, <i>RI</i>	x
107	Tridecan-2-ol	1499	MS, <i>RI</i>	x
108	Methyl (<i>E</i>)-2,9-dimethyldec-2-enoate (E3)	1510	MS, <i>RI</i>	xxx
109	γ -Cadinene (7)	1512	MS, <i>RI</i>	xxx
110	Sesquiterpene	1517		x
111	Sesquiterpene	1521		x
112	Sesquiterpene	1523		x
113	Methyl 3-methylalkanoate	1526	MS	x
114	(<i>E</i>)-Methyl 2-methylundec-2-enoate (D4)	1547	syn	x
115	Unknown 153, 237	1552		xx
116	Methyl 2-methyldodecanoate (A6)	1559	syn	xx
117	Nerolidol	1563	MS, <i>RI</i>	x
118	12-Methyltridecan-2-ol	1567	MS, <i>RI</i>	x
119	Undecano-4-lactone	1571	MS, <i>RI</i>	x
120	Sesquiterpene	1573		x
121	Tridecan-1-ol	1576	MS, <i>RI</i>	x
122	Unknown 41, 73, 88, 243	1597		x
123	Sesquiterpene	1600		xx
124	Hexadecane	1600	MS, <i>RI</i>	xx
125	Methyl (<i>E</i>)-2,10-dimethylundec-2-enoate (E4)	1609	MS, <i>RI</i>	x
126	Methyl 2,11-dimethyldodecanoate	1613	MS	x
127	Sesquiterpene	1618		x
128	Methyl 2,11-dimethyldodecanoate (B3)	1621	syn	xxx
129	1-Epicubenol	1626	MS, <i>RI</i>	x
130	Methyl 2,10-dimethyldodecanoate (C3)	1629	MS, <i>RI</i>	x
131	τ -Cadinol	1638	MS, <i>RI</i>	xx
132	Sesquiterpene	1640		xx
133	δ -Cadinol	1644	MS, <i>RI</i>	x
134	Methyl (<i>E</i>)-2-methyldodec-2-enoate (D5)	1647	syn	xxx
135	α -Cadinol (8)	1652	MS, <i>RI</i>	x
136	Methyl 2-methyltridecanoate (A7)	1659	syn	xx
137	Unknown 88, 101, 256 (ester)	1662		x
138	13-Methyltetradecan-3-ol	1664	MS, <i>RI</i>	xx
139	Tetradecan-1-ol	1677	MS, <i>RI</i>	xxx
140	Methyl alkanoate	1699	MS	x
141	2-Ethylhexyl benzoate ^{k)}	1707	MS	xxx
142	Methyl (<i>E</i>)-2,11-dimethyldodec-2-enoate (E5)	1710	syn	xxx
143	Methyl alkenoate	1717	MS	xx
144	Methyl 2-methyltetradecenoate	1733	MS	x
145	Methyl (<i>E</i>)-2-methyltridec-2-enoate (D6)	1747	syn	xxx
146	Methyl 2-methyltetradecenoate	1750	MS	xx
147	Unknown 105, 126	1753		x
148	Methyl 2-methyltetradecanoate (A8)	1759	syn	xx

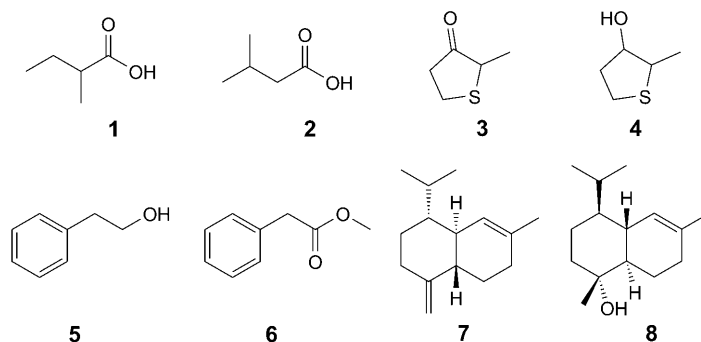
Table 1 (cont.)

Entry	Compound	<i>I</i> ^{a)}	Ident. ^{b)}	Conc. ^{c)}
149	Alcohol	1779		x
150	Methyl farnesoate	1784	MS	x
151	Diterpene	1791		xxx
152	Methyl alkenoate	1795	MS	xx
153	Octadecane	1800	MS, <i>RI</i>	x
154	Diterpene	1805		xxx
155	Methyl 2,13-dimethyltetradecenoate	1811	MS	xxx
156	Diterpene	1814		xxx
157	Methyl 2,13-dimethyltetradecanoate (B4)	1822	MS, <i>RI</i>	xxx
158	Methyl 2,12-dimethyltetradecanoate (C4)	1829	MS, <i>RI</i>	x
159	Methyl alkanolate	1833	MS	x
160	Hexahydrofarnesylacetone	1845	syn	xx
161	Methyl (<i>E</i>)-2-methyltetradec-2-enoate (D7)	1848	syn	xxx
162	Diterpene	1855		x
163	Diterpene	1858		x
164	Methyl 2-methylpentadecanoate (A8)	1859	syn	x
165	Diterpene	1863		x
166	Diterpene	1869		xxx
167	Diterpene	1881		xxx
168	Diterpene	1889		xxx
169	Diterpene	1894		x
170	Nonadecane	1900		x
171	Diterpene	1905		xx
172	Diterpene	1906		xx
173	Methyl (<i>E</i>)-2,13-dimethyltetradec-2-enoate (E6)	1911	MS, <i>RI</i>	xxx
174	Diterpene	1914		xx
175	Diterpene	1918		xxx
176	Diterpene	1922		xxx
177	Diterpene	1929		xx
178	Diterpene	1935		x
179	Methyl ester	1935		
180	Diterpene	1941		xxx
181	Diterpene	1944		xxx
182	Methyl (<i>E</i>)-2-methylpentadec-2-enoate (D8)	1949	syn	x
183	Diterpene	1951		x
184	Diterpene	1957		x
185	Diterpene	1966		xxx
186	Diterpene	1971		xx
187	Diterpene	1980		xx
188	Diterpene	1995		xx
189	Diterpene	2003		xx
190	Diterpene	2058		xxx
191	Diterpene	2066		xx
192	Diterpene	2071		xx
193	Diterpene	2079		xxx
194	Diterpene	2115		xxx
195	Diterpene	2125		xxx
196	Diterpene	2133		xxx

Table 1 (cont.)

Entry	Compound	<i>I</i> ^{a)}	Ident. ^{b)}	Conc. ^{c)}
197	Diterpene	2141		xxx
198	Diterpene	2153		xx
199	Diterpene	2173		xxx
200	Diterpene	2186		xxx

^{a)} *I* = GC Retention index. ^{b)} Ident. = Identification method. ^{c)} Conc. = concentration. ^{d)} MS = Mass spectrum. ^{e)} xxx = Main compound (> 0.1%). ^{f)} syn: Synthetic compound. ^{g)} *RI* = Retention index. ^{h)} Numbers indicate characteristic ions in the mass spectra of unknown compounds. ⁱ⁾ x = Trace compound (< 0.04% of total area of the TIC). ^{j)} xx = Minor compound (0.04–0.1%). ^{k)} Medium constituent.



In addition to these compounds, a series of esters was detected featuring characteristic ions at *m/z* 88 and 101 (Fig. 2). The ion corresponding to *m/z* 88 is generated by a *McLafferty* rearrangement of the ester function, while the ion ascribed to *m/z* 101 is caused by a primary H shift from C(6) to the CO group, followed by H-transfer and cleavage of the C(3)–C(4) bond [14]. Characteristic for ethyl esters, usually associated with the ions corresponding to *m/z* 88 and 101, are those at *m/z* 73 and $[M-45]^+$ that arise by cleavage next to the CO group. The latter ions were missing; instead, *m/z* 59 and $[M-31]^+$ were present, indicating the presence of a methyl ester functional group. In this case, peaks at *m/z* 88 and 101 indicate the presence of a Me branch at C(2), as shown in Scheme 1. These methyl 2-methylalkanoates were accompanied by compounds which exhibit a similar mass spectrum but a molecular-ion 2 amu lower than the parent compound, indicating the presence of a C=C bond equivalent. A derivatization of the extract with dimethyl disulfide (MeSSMe) to locate the position of the C=C bond in the chain furnished no derivatized esters. This behavior strongly suggested the presence of a C=C bond at C(2), because a CO-conjugated C=C bond does not react with MeSSMe due to its lower reactivity [15–17]. The occurrence of ions with peaks at *m/z* 88 and 101 in the unsaturated methyl esters, at first hand surprising when a C=C bond is located at C(2), might be explained by the migration of the C=C bond out of conjugation before fragmentation [18]. A diagnostic ion peak of considerable intensity in the spectra of some unsaturated esters is at *m/z* 127. Its formation can be explained by the mechanism

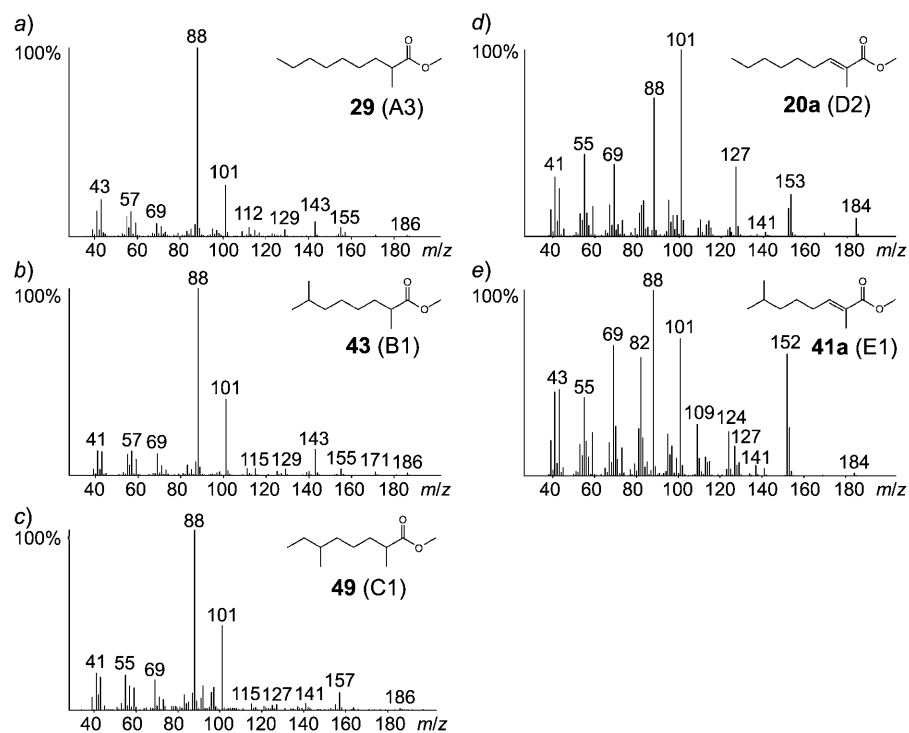
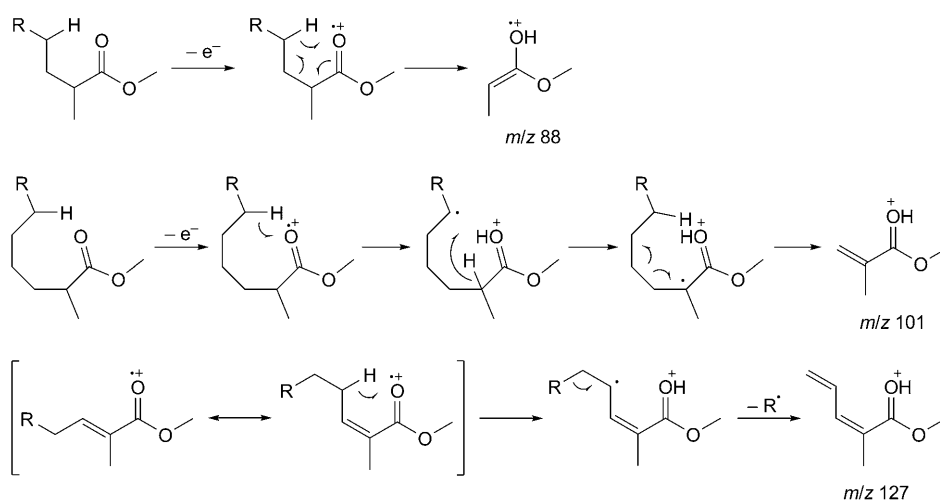


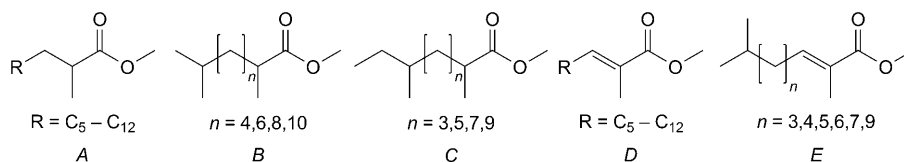
Fig. 2. Mass spectra of a) methyl 2-methylnonanoate (**29**; A3); b) methyl 2,7-dimethyloctanoate (**43**; B1); c) methyl 2,7-dimethylnonanoate (**49**; C1); d) methyl 2-methylnon-2-enoate (**20a**; D2); and e) methyl 2,7-dimethyloct-2-enoate (**41a**; E1)

Scheme 1. Characteristic Mass-Spectral Fragmentations of Saturated and Unsaturated Methyl Esters



shown in *Scheme 1* [19]. Transfer of a H-atom of C(6) to the CO group is followed by loss of the γ -alkyl group leading to an extended conjugated C=C bond system. This reaction can only occur in α,β -unsaturated methyl esters, pointing again to a C=C bond at C(2).

More than 30 different methyl esters containing between 9 to 17 C-atoms were detected in the natural samples (*Tables 1* and *2*). The determination of the GC retention indices (*RI*s) revealed the presence of five different subtypes of these esters [20]. The saturated methyl esters had *RI*s ending around 60, 22, and 28 (classes *A*, *B*, and *C*), while the unsaturated ones exhibited values around 48 and 10 (class *D* and *E*).



The *RI*s of aliphatic compounds with a terminal functional group (FG) can be calculated using an empirical *RI* model developed by us and used successfully in several projects [3][21–24]. The functional group (FG) increment for methyl esters is 331, calculated from the retention index of 1331 of methyl decanoate. The FG of a Me group in α -position to a CO group was determined to be 30 [25]. The compound class *A* can thus be identified to constitute methyl 2-methylalkanoates, exemplified by identification of compound A3, the most prominent member of this class. The molecular-ion peak at m/z 186 in the mass spectrum (*Fig. 2, a*) indicates a methyl ester with ten C-atoms in the acid part. The fragmentation pattern establishes the position of a Me group at C(2). The calculated retention index RI_{calc} for methyl 2-methylnonanoate (A3) is therefore $900 + 331 + 30 = 1261$, in good agreement with the observed value of 1259 (*Table 2*) of the synthetic material (see below). The class-*B* compound B1 (mass spectrum *Fig. 2, b*) has the same molecular mass as A3, but is eluted earlier in GC. The *RI* of 1224 indicates an additional Me branch in the chain. By use of the known increments for a Me branch at various positions along the chain, a $\omega - 1$ Me group seems to be most plausible [21]. The value $RI_{\text{calc}} = 800 + 331 + 30 + 60 = 1221$ is very close to the observed value. The second best position would be $\omega - 3$ with $RI_{\text{calc}} = 800 + 331 + 30 + 56 = 1217$. The mass spectra of the class-*B* compounds were very similar to those of the class *A* compounds, but showed a small intensity increase in the $[M - 43]^+$ ion, typical for iso-esters [26]. Methyl esters branched at $\omega - 3$ also show a $[M - 43]^+$ ion, but with higher intensity [26]. In addition to the classes *A* and *B*, the class *C* esters proved to have a very similar mass spectrum (*Fig. 2, c*), also with a molecular-ion peak at m/z 186 in case of C1. The class *C* esters are eluted directly after the corresponding class *B* esters, pointing to a second Me branch in a different position than $\omega - 1$. Only the anteiso-esters show a higher *RI* value compared to the iso-esters [21]. Therefore, the second Me branch in the class-*C* compounds is located at the $\omega - 2$ position. The corresponding calculated value for C1 is $RI_{\text{calc}} = 800 + 331 + 30 + 73 = 1234$ which is close to the observed value of 1226. The mass spectra of the class-*C* esters (*Fig. 2, c*) also showed an enhanced formation of $[M - 29]^+$ which can be explained the loss of the Et group at the end of the chain. The unsaturated esters of class *D* and *E* showed a *RI*

Table 2. *Different Methyl Esters from Chitinophaga Fx7914*

Compound	Peak ^{a)}	RI_{nat} ^{b)}	RI_{calc} ^{c)}	RI_{exp} ^{d)}
Methyl 2-methylheptanoate (27)	A1	1064	1061	1060
Methyl 2-methyloctanoate (28)	A2	1161	1161	1161
Methyl 2-methylnonanoate (29)	A3	1259	1261	1258
Methyl 2-methyldecanoate (30)	A4	1360	1361	1359
Methyl 2-methylundecanoate (31)	A5	1460	1461	1459
Methyl 2-methyldodecanoate (32)	A6	1559	1561	1559
Methyl 2-methyltridecanoate (33)	A7	1659	1661	1660
Methyl 2-methyltetradecanoate (34)	A8	1759	1761	1759
Methyl 2-methylpentadecanoate (35)	A9	1859	1861	1859
Methyl 2,7-dimethyloctanoate (43)	B1	1224	1221	1223
Methyl 2,9-dimethyldecanoate	B2	1423	1421	
Methyl 2,11-dimethyldodecanoate (44)	B3	1621	1621	1620
Methyl 2,13-dimethyltetradecanoate	B4	1822	1821	
Methyl 2,6-dimethyloctanoate (49)	C1	1226	1234	1229
Methyl 2,8-dimethyldecanoate	C2	1429	1434	
Methyl 2,10-dimethyldodecanoate	C3	1629	1634	
Methyl 2,12-dimethyltetradecanoate	C4	1829	1834	
Methyl (<i>E</i>)-2-methyloct-2-enoate (19a)	D1	1248	1251	1245
Methyl (<i>E</i>)-2-methylnon-2-enoate (20a)	D2	1348	1351	1346
Methyl (<i>E</i>)-2-methyldec-2-enoate (21a)	D3	1448	1451	1446
Methyl (<i>E</i>)-2-methylundec-2-enoate (22a)	D4	1547	1551	1547
Methyl (<i>E</i>)-2-methyldodec-2-enoate (23a)	D5	1647	1651	1648
Methyl (<i>E</i>)-2-methyltridec-2-enoate (24a)	D6	1747	1751	1746
Methyl (<i>E</i>)-2-methyltetradec-2-enoate (25a)	D7	1848	1851	1848
Methyl (<i>E</i>)-2-methylpentadec-2-enoate (26a)	D8	1949	1951	1949
Methyl (<i>E</i>)-2,7-dimethyloct-2-enoate (41a)	E1	1311	1311	1313
Methyl (<i>E</i>)-2,8-dimethylnon-2-enoate	E2	1408	1411	
Methyl (<i>E</i>)-2,9-dimethyldec-2-enoate	E3	1510	1511	
Methyl (<i>E</i>)-2,10-dimethylundec-2-enoate	E4	1609	1611	
Methyl (<i>E</i>)-2,11-dimethyldodec-2-enoate (42a)	E5	1710	1711	1710
Methyl (<i>E</i>)-2,13-dimethyltetradec-2-enoate	E6	1912	1911	

^{a)} Peak: see Fig. 1. ^{b)} RI_{nat} : Retention index of the naturally occurring ester. ^{c)} RI_{calc} : Calculated retention index. ^{d)} RI_{exp} : Retention index of the synthetic standard.

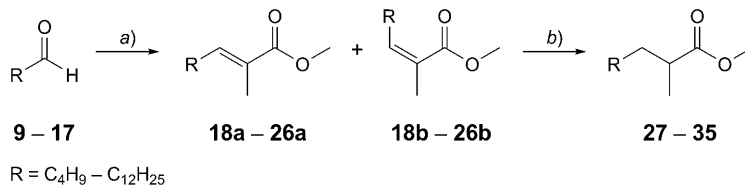
value 90 units higher than that of the respective saturated compounds of class *A* and *B*. This retention behavior is typical for α,β -unsaturated CO compounds and point together with the MS data to a C=C bond at C(2) (Fig. 2, *d* and *e*). In conclusion, class-*D* and -*E* compounds were suggested to be analogs of the class *A* and *B* compounds with an additional C=C bond at C(2). Besides these esters, several other methyl esters of low abundance occurred in the headspace extract, but their concentration was too low to confirm their structure (Table 1).

Several of the methyl esters of classes *A*–*E* were then synthesized to verify the proposed structures and to clarify the configuration of the C=C bond in the unsaturated esters.

Synthesis of Methyl Esters. Different aldehydes **9**–**17**, ranging from pentanal (**9**) to tridecanal (**17**), were transformed by a *Horner–Wadsworth–Emmons* reaction with

methyl 2-(dimethoxyphosphoryl)propanoate to the unsaturated methyl esters **18a/18b–26a/26b**, respectively, as diastereoisomeric mixtures in varying ratios (*Scheme 2*) [27]. NMR Analysis verified the C=C bond configuration of the product diastereoisomers, and GC/MS comparison with the natural esters established that the class-*D* esters are constituted of methyl (*E*)-2-methylalk-2-enoates.

Scheme 2. Synthesis of Unsaturated and Saturated Methyl 2-Methylalkanoates

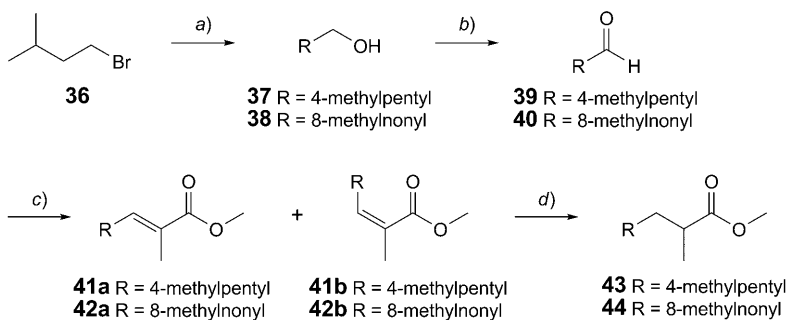


a) NaH, methyl 2-(dimethoxyphosphoryl)propanoate; 30–78%. b) H₂, PtO₂; 40–100%.

In a final reaction step, the diastereoisomer mixtures of the unsaturated esters were hydrogenated in MeOH with PtO₂ as catalyst, giving racemic mixtures of the corresponding saturated mono-Me-branched methyl esters **27–35** (*Scheme 2*) [28]. Comparison of the synthetic material with the natural extract proved that the class-A esters were indeed methyl 2-methylalkanoates.

The iso-esters of classes *B* and *E* were synthesized as shown in *Scheme 3*. Isobutyl bromide (**36**) was transformed into 5-methylhexan-1-ol (**37**) or 9-methyldecan-1-ol (**38**) by a Li₂CuCl₄-mediated chain elongation with 3-bromopropan-1-ol or 6-bromohexan-1-ol, respectively [29]. The alcohols **37** and **38** were then oxidized with PCC to furnish 5-methylhexanal (**39**) and 9-methyldecanal (**40**) [30]. The transformation to the unsaturated esters as just described afforded methyl 2,7-dimethyloct-2-enoate (**41a** and **41b**) and 2,11-dimethyldodec-2-enoate (**42a** and **42b**), respectively, as mixtures of diastereoisomers. After separation of the isomers, the (*E*)-isomers showed the same mass spectrum and *R*_Is as the corresponding volatiles from *Chitinophaga* Fx7914, verifying the suggested structures for the class-*E* compounds.

Scheme 3. Synthesis of Iso-Branched Unsaturated and Saturated Methyl 2-Methylalkanoates

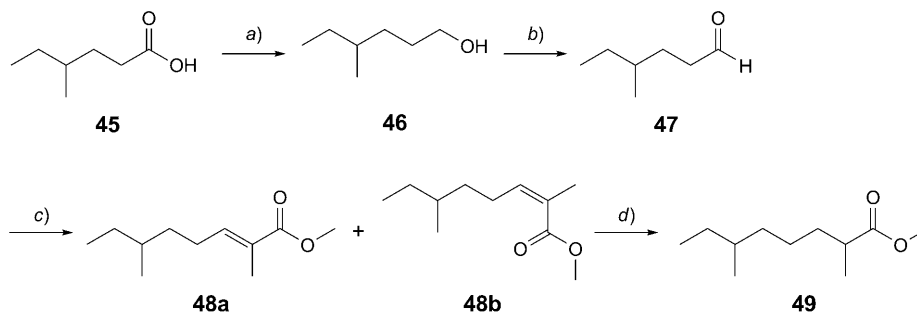


a) Mg, bromoalcohol, Li₂CuCl₄; 85% (**37**), 68% (**38**). b) Pyridinium chlorochromate (PCC); 69% (**39**), 86% (**40**). c) NaH, methyl 2-(dimethoxyphosphoryl)propanoate; 32% (**41**), 44% (**42**). d) H₂, PtO₂; 66% (**43**), 61% (**44**).

Finally, hydrogenation of **41** or **42** provided racemic methyl 2,7-dimethyloctanoate (**43**) and methyl 2,11-dimethyldodecanoate (**44**), respectively (*Scheme 3*), confirming the proposed structure for the class-*B* compounds of *Chitinophaga* Fx7914.

The synthesis of the anteiso-esters of class *C* started from 4-methylhexanoic acid (**45**) that was reduced with LiAlH_4 to give 4-methylhexan-1-ol (**46**; *Scheme 4*) [22]. Following oxidation with PCC afforded the corresponding aldehyde **47** that was transformed as described into methyl (*E*)- and (*Z*)-2,6-dimethyloct-2-enoate (**48**) [27][30]. In a final reaction, **48** was hydrogenated in MeOH using PtO_2 as catalyst [28] to yield methyl 2,6-dimethyloctanoate (**49**) which proved identical to the natural compound C1.

Scheme 4. Synthesis of Anteiso-Branched Saturated Methyl 2-Methylalkanoates



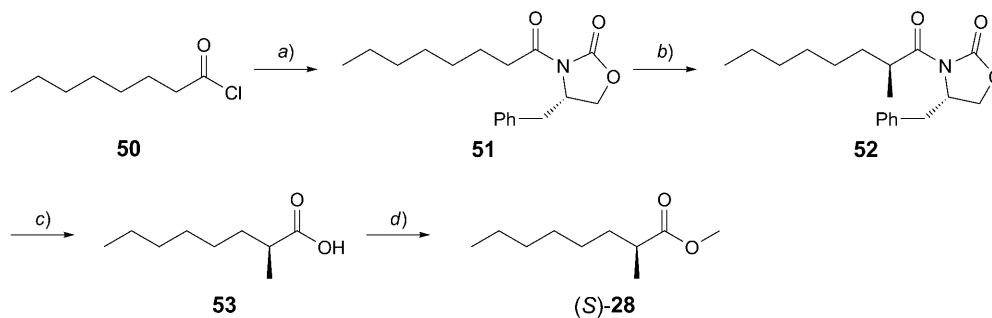
a) LiAlH_4 ; 75% (**46**). b) PCC (**47**). c) NaH, methyl 2-(dimethoxyphosphoryl)propanoate; 38% (over two steps) (**48**). d) H_2 , PtO_2 ; quant. (**49**).

Although not all esters listed in *Table 2*, i.e., A1–E6, were synthesized, the structures of B2, B4, C2, C3, C4, E2, E3, E4, and E6 were confirmed by their MS data and *R*_Is, showing the expected class values.

Determination of the Absolute Configuration. The mono- and dimethyl-branched saturated methyl esters both contained a stereogenic center at C(2). Therefore, a representative of class-*A* and -*B* esters was synthesized stereoselectively for the determination of their configuration by GC/MS on chiral phases. Methyl (*S*)-2-methyloctanoate ((*S*)-**28**) was synthesized as depicted in *Scheme 5*.

Octanoyl chloride (**50**) was transformed with the *Evans* chiral auxiliary (4*S*)-4-benzyl-1,3-oxazolidin-2-one to the corresponding derivative **51** [31]. The following stereoselective methylation with MeI under basic conditions furnished compound **52** in a high diastereoisomeric ratio of 98:2, which was purified by column chromatography to give only one diastereoisomer [32]. The chiral auxiliary was cleaved off with H_2O_2 and LiOH, providing (*S*)-2-methyloctanoic acid (**53**) [32]. Finally, an acid-catalyzed esterification with methanol afforded enantiomerically pure methyl (*S*)-2-methyloctanoate ((*S*)-**28**) [33].

The methyl ester (*S*)-**28** and *rac*-**28** were used for GC analyses using a chiral β -Dex 225 GC phase (*Fig. 3, a*). The analyses confirmed the ee value of synthetic (*S*)-**28** to be 100%, and proved that the natural compound also shows this configuration.

Scheme 5. Stereoselective Synthesis of Methyl (*S*)-2-Methyloctanoate ((*S*)-**28**)

a) BuLi, (4*S*)-4-benzyl-1,3-oxazolidin-2-one; 84%. b) Sodium hexamethyldisilazane (NaHMDS), MeI; 66%. c) H₂O₂, LiOH; 100%. d) MeOH, HCl; 92%.

Furthermore, natural **28** is produced as single enantiomer. This results suggest that all esters of class A possess an (*S*)-configuration.

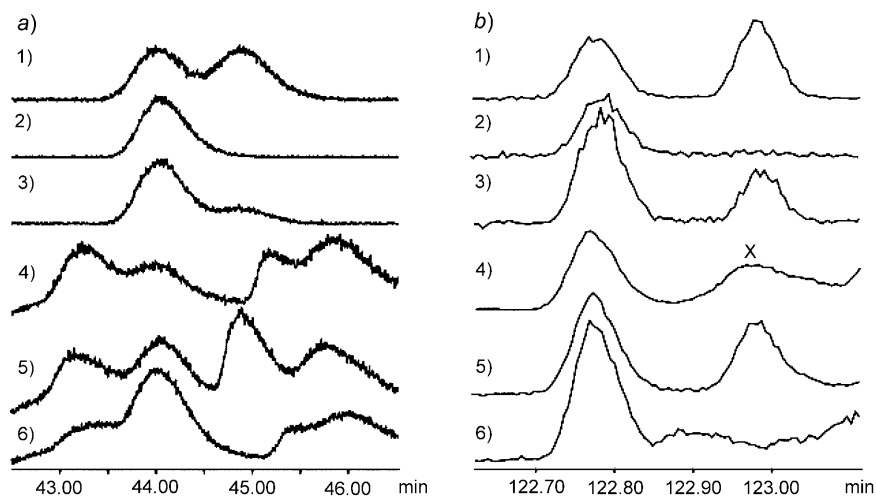
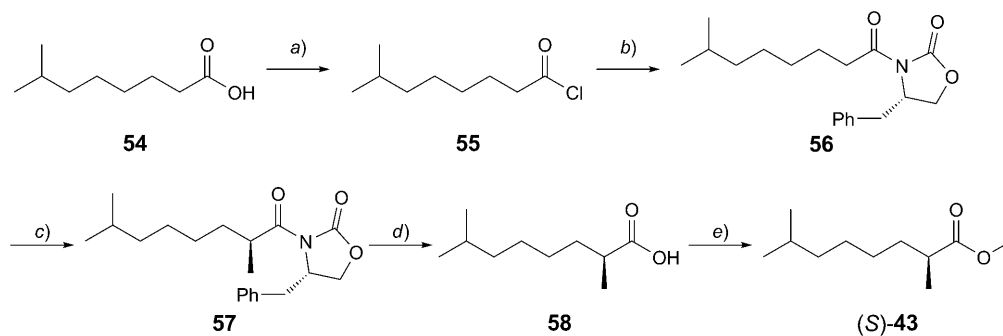


Fig. 3. Determination of the absolute configuration of a) methyl (*S*)-2-methyloctanoate ((*S*)-**28**) and b) methyl 2,7-dimethyloctanoate ((*S*)-**43**) on a chiral β-Dex 225 phase. a) 1) Synthetic *rac*-**28**. 2) Synthetic (*S*)-**28**. 3) Coinjection of *rac*- and (*S*)-**28**. 4) Natural extract of *Chitinophaga* Fx7914. 5) Coinjection of *rac*-**28** and natural extract. 6) Coinjection of (*S*)-**28** and natural sample. b) 1) Synthetic *rac*-**43**. 2) Synthetic (*S*)-**43**. 3) Coinjection of *rac*- and (*S*)-**43**. 4) Natural extract of *Chitinophaga* Fx7914. 5) Coinjection of *rac*-**43** and natural extract. 6) Coinjection of (*S*)-**43** and natural sample.

To extend these investigations to the dimethyl-branched saturated methyl esters of class B, methyl (*S*)-2,7-dimethyloctanoate ((*S*)-**43**) was prepared (Scheme 6). Previously prepared 7-methyloctanoic acid (**54**) was converted to the corresponding acid chloride **55** that was transformed to the Evans oxazolidinone **56** [17][31][34]. Methylation led to compound **57** with a diastereoisomeric ratio of 96:4 that gave

Scheme 6. Stereoselective Synthesis of Methyl (*S*)-2,7-Dimethyloctanoate ((*S*)-**43**)

a) Oxalyl chloride; 80%. b) BuLi, (4*S*)-4-benzyl-1,3-oxazolidin-2-one; 48%. c) NaHMDS, MeI; 46%. d) H₂O₂, LiOH; 89%. e) MeOH, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC), 4-(dimethylamino)pyridine (DMAP); 66%.

enantiomerically pure **57** after purification [32]. The following cleavage of the oxazolidinone produced acid **58** [32]. Finally, EDC-assisted esterification with MeOH furnished the target compound (*S*)-**43** that was used for GC/MS analyses on a chiral β -Dex 225 column (Fig. 3, b) [35].

The natural methyl 2,7-dimethyloctanoate ((*S*)-**43**) is also produced exclusively as pure (*S*)-enantiomer. Most likely, all class-*B* compounds possess (*S*)-configuration as the class-*A* esters do. The configuration of the two stereogenic centers in the class-*C* compounds was not determined because of their low natural abundance.

Biosynthesis of the Methyl Esters. It is well known that a ($\omega - 1$)-Me group in fatty acids containing an even number of C-atoms in the chain originates from 3-methylbutyryl-CoA, derived from the amino acid leucine, while, in case of an odd number of C-atoms, isobutyryl-CoA, derived from valine, serves as starter compound. Similarly, the ($\omega - 2$)-Me group occurs only in fatty acids with an even number of C-atoms in the chain and is formed from 2-methylbutyryl-CoA derived from isoleucine [24][36][37]. The Me groups in the esters of *Chitinophaga* located at the end of the chain are most likely biosynthetically formed *via* these pathways. In contrast, the biosynthetic origin of the Me group at C(2) was unknown. Possibilities are either the addition of the Me group to a C=C bond *via* (*S*)-adenosylmethionine (SAM) or incorporation of methylmalonate in the carboxylic acid chain extension by fatty acid synthases [27][38][39].

Liquid cultures of *Chitinophaga* Fx7914 were supplemented with either [1'-²H₃]methionine as precursor of SAM or with sodium [1-¹³C]propanoate as precursor of methylmalonate to investigate whether one of the proposed biosynthetic pathways is operating. The emitted headspace volatiles were then analyzed by GC/MS [11][12].

The labeled precursors were incorporated in both experiments. Upon feeding of [1'-²H₃]methionine the molecular ion of methyl 2,7-dimethyloctanoate (**43**) shifted from *m/z* 186 to 189, while the characteristic ions at *m/z* 88 and 101 were replaced by the corresponding values at *m/z* 91 and 104 respectively (Fig. 4, a), indicating the formation of [²H₃]methyl 2,7-dimethyloctanoate ([²H₃]-**43**) (incorporation rate

>95%). Furthermore, the $[M-31]^+$ ion was not present, but instead a $[M-34]^+$ fragment appeared. This shift indicated incorporation of the ^2H label only into the MeO group of the ester, but not into the Me group at C(2).

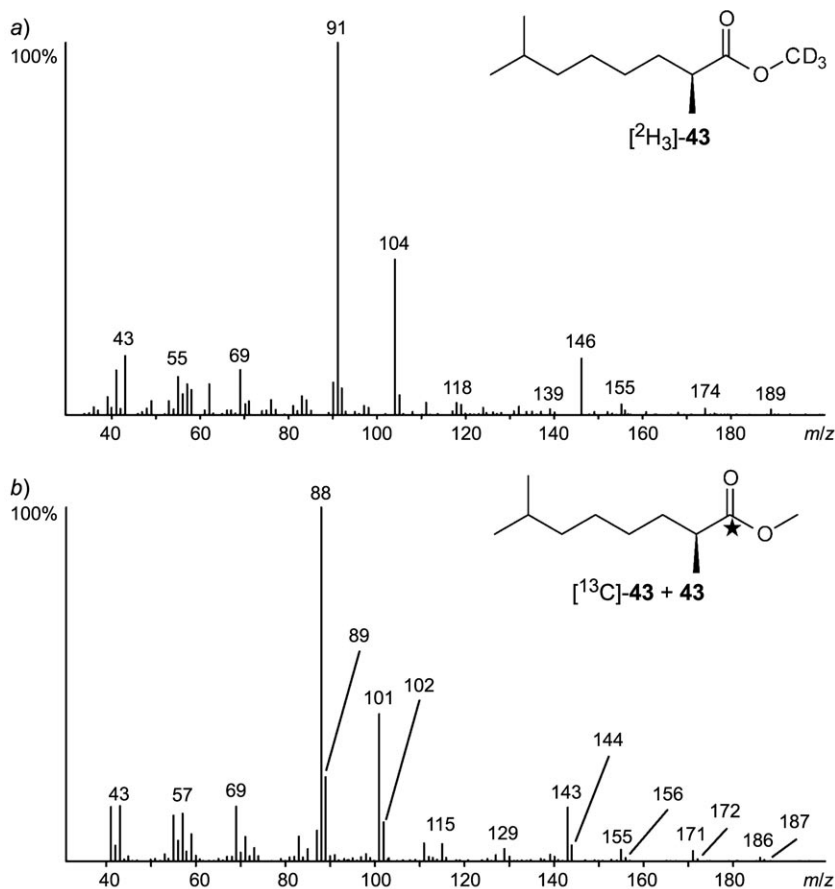


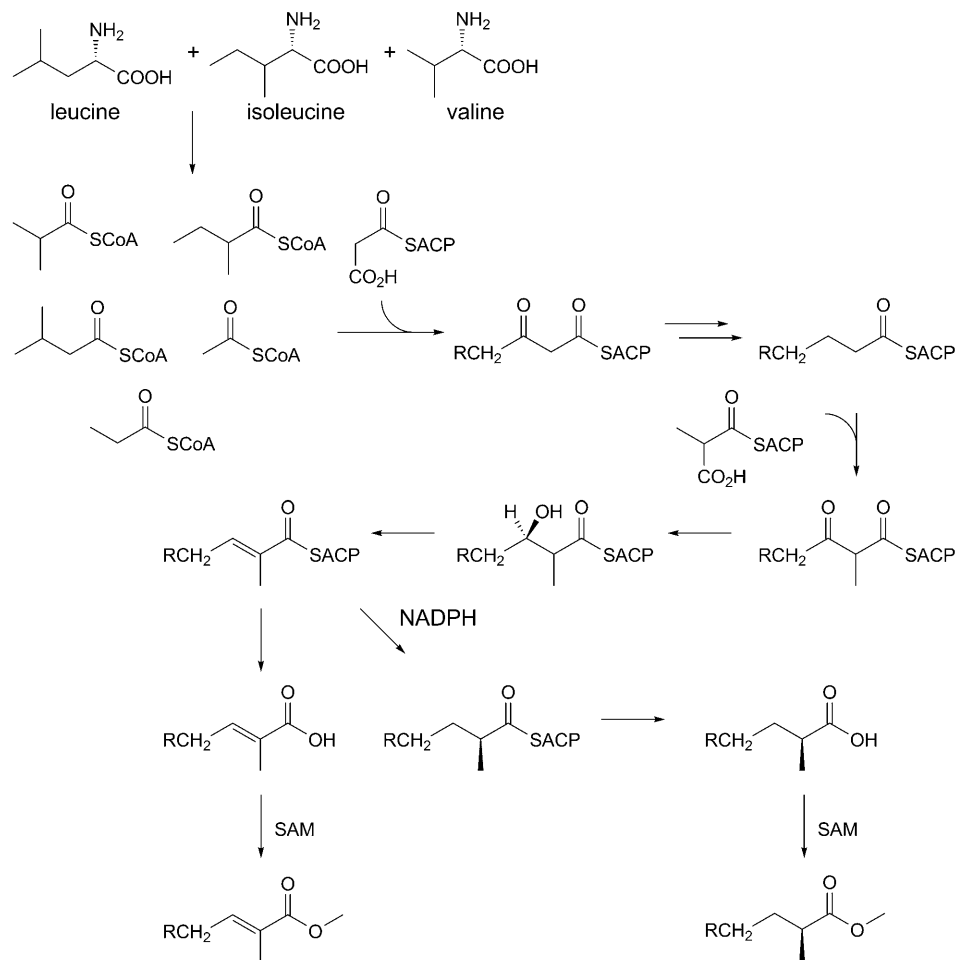
Fig. 4. Mass spectra of methyl 2,7-dimethyloctanoate (**43**) from *Chitinophaga Fx7914* after feeding of a) $[1,1',1'-^2\text{H}_3]$ methionine and b) sodium $[1-^{13}\text{C}]$ propanoate

The feeding of sodium $[1-^{13}\text{C}]$ propanoate furnished labeled esters (incorporation rate 20%). Contrary to ^2H isotopomers that have shorter GC retention times compared to their parent compounds [3][22][24], ^{13}C -labelled compounds show no retention-time shift. In the mass spectrum of labeled methyl 2,7-dimethyloctanoate (**43**) an intensity increase of the ion peaks at m/z 89, 102, 144, 156, 172, and 187, compared to the spectrum of unlabelled **43**, was observed, indicating the incorporation of labeled propanoate into **43** (Fig. 4, b). The mass spectrum showed the position of the label at C(1), thus proving that the Me branch at C(2) originates from methylmalonate and not from SAM.

On the basis of the known fatty acid biosynthesis and the labeling experiments, a biosynthetic pathway for the formation of the different methyl-ester classes can be

postulated (*Scheme 7*). Starting either with acetyl-CoA, propionyl-CoA (class *A* and *D*), 3-methylbutyryl-CoA derived from leucine (class *B* and *E*), 2-methylbutyryl-CoA derived from isoleucine (class *C*), or isobutyryl-CoA derived from valine (class *E*), methyl-branched saturated and unsaturated methyl esters are synthesized.

Scheme 7. Biosynthetic Pathway for the Formation of the Different Methyl Ester Classes



The chain is extended from the starter molecules *via* malonyl-SCoA [2][22][40]. The odd or even chain length of the esters can be explained by use of the various starters, but an α -oxidation process, leading to a loss of one C-atom during or after chain extension, cannot be ruled out [24][37]. The feeding experiments with [¹³C]propanoate did not clarify whether a propionyl starter is really used, because the few ions which contain the alkyl end of the esters are of low abundance in the mass spectra. A definite proof for a propionyl-CoA starter would be the enhanced intensity

of the $[M+2]^+$ ion, requiring incorporation of two propanoate units. The abundance of such ions was too low for a definite conclusion.

In the final chain-extension step methylmalonyl-CoA derived from propanoate is added to the chain, leading to the Me branch at C(2). Hydrolysis from the acyl carrier protein (ACP) before reduction and final methylation with SAM leads to the unsaturated esters, while reduction of the C=C bond with NADPH, followed by hydrolysis from ACP and SAM mediated methylation, affords the saturated esters. The methylation of free fatty acids with SAM, established here by the labeling experiments, has been described before by the action of a methyltransferase in *Mycobacterium phlei* and the myxobacterium *S. aurantiaca*, and also in the rat hypothalamus [41–43].

As discussed in the introduction, methyl esters of medium chain length have only occasionally been reported from bacteria. Most of the compounds described in Table 2 have not been reported before from nature. The related unsaturated fatty acids are also rare. In mycobacteria, 2-methylalk-2-enoates of longer chain length have been found as constituents of complex lipids [44][45]. In contrast, simple 2-methylalkanoic acids are found in bacteria, plants, and animals. The parent acids of the 2,X-dimethyl-branched esters reported here are also not known from nature, except for 2,7-dimethyloctanoic acid which occurs bound to glycerol in epidermal glands of the reptile, *Sphenodon punctatus* [46].

In summary, the identification, biosynthesis, and synthesis of novel Me-branched aliphatic methyl alkanoates and methyl alkenoates of medium chain length is described. Similar compounds have not been reported before as a major volatile class released by bacteria. Why these esters are formed is unknown, but they might function as signals or spacing compounds, inhibiting microbial growth next to *Chitinophaga* cells, thus contributing to the success of these gliding bacteria.

We thank the *Deutsche Forschungsgemeinschaft* for financial support (DFG Schu 984-6).

Experimental Part

General. Chemicals were purchased from *Sigma–Aldrich* (Germany), *Fluka* (Switzerland), *Acros* (Belgium), *Merck* (Germany), *Cambridge Isotope Laboratories* (USA), and *Deutero* (Germany). All solvents were purified by distillation and dried according to standard methods. TLC: 0.2-mm pre-coated plastic sheets (*Polygram Sil G/UV₂₅₄*; *Macherey-Nagel*). Column chromatography (CC): *Merck* silica gel 60 (SiO₂; 70–200 mesh); solvent mixtures as used for the determination of the R_f values in TLC; compounds were detected using a molybdatophosphoric acid soln. (5% in EtOH) or a KMnO₄ soln., followed by heat-gun treatment. NMR Spectra: *Bruker AVII-300*, *DRX-400*, or *AVIII-400* spectrometers; chemical shifts δ in ppm relative to Me₄Si as internal standard and coupling constants J in Hz.

Media and Growth Conditions. T2 Medium of the following composition was used to study the production of metabolites and to perform feeding experiments under defined conditions: glutamine, 0.2% (w/w); MgSO₄ × 7 H₂O, 0.02% (w/w); Fe-EDTA, 8 mg/l; MnSO₄, 5 mg/l; ZnCl₂, 0.25 mg/l; HEPES, 12 g/l; *XAD 16* adsorber resin (*Rohm and Haas*), 2%; pH adjusted to 7.0. 5 ml of a 20% (w/w) autoclaved sucrose soln. and 1 ml of a 7% (w/w) KH₂PO₄ autoclaved soln. were added to 100 ml of the above medium. Liquid cultures were started in 106 ml T2 medium in 250-ml *Erlenmeyer* flasks inoculated with 2% of a preculture and incubated on a rotary shaker at 30° for 4 d. The feeding experiments were carried out by the addition of [³H₃]methionine (50 mg, 5.5 mM final concentration) or sodium [1-¹³C]propanoate (200 mg, 31.2 mM final concentration) to well grown cultures of *Chitinophaga* Fx7914.

Sampling of Volatiles. Volatile org. compounds emitted by bacterial cultures were collected by the closed-loop stripping analysis (CLSA) using the mentioned 250-ml *Erlenmeyer* flasks [11][12]. The

volatiles were adsorbed on charcoal (*Chromtech*; Precision Charcoal Filter, 5 mg) for 24 h and extracted with 30 μ l of CH_2Cl_2 . These extracts were immediately analyzed by GC/MS and then stored at -30° .

GC/MS Analysis. GC/MS Analyses were carried out on a *HP-6890* GC system connected to a *HP-5973* mass-selective detector fitted with a *BPX5* fused-silica cap. column (25 m, 0.22 mm i.d., 0.25 μ m film; *SGE*, Australia) or on an *Agilent 7890A* GC system connected to an *Agilent 5975C* inert mass detector fitted with a *HP-5MS* fused silica cap. column (30 m, 0.25 mm i.d., 0.25 μ m film; *J&W Scientific*, USA). Conditions for the *HP-6890/HP-5973* system were: inlet pressure: 77.1 kPa, 23.3 ml He min^{-1} ; injection volume: 1 μ l; transfer line: 300° ; electron energy: 70 eV. GC Program: 5 min at 50° , increasing with $10^\circ \text{ min}^{-1}$ to 320° , operated in either split or splitless mode (60 s valve time); for the *Agilent 7890A/Agilent 5975C* system, the following conditions were used: inlet pressure: 77.1 kPa, 23.3 ml He min^{-1} ; injection volume: 2 μ l; transfer line: 300° ; electron energy: 70 eV. GC Programm: 5 min at 40° , increasing with 3° min^{-1} to 320° , operated in either split or splitless mode (60 s valve time); He carrier gas at 1 ml min^{-1} (*HP-6890*) or 1.2 ml min^{-1} (*Agilent 7890A*).

RI Values were determined from a homologous series of *n*-alkanes ($\text{C}_8\text{--C}_{35}$) [20]. Identification of compounds was performed by comparison of mass spectra to the *Wiley-6 Library* and the *Essential Oils Library* (*Massfinder*) and by comparison of *RI* data from the literature or with synthetic standards.

GC/MS on Chiral Stationary Phase. Enantiomer separations were carried out on the *Agilent 7890A/5975C* system equipped with a β -*Dex* 225 fused-silica cap. column (30 m, 0.32 mm i.d., 0.25 μ m film; *Supelco/Sigma–Aldrich*, Germany). The carrier gas was He at 1.6 ml min^{-1} (valve time: 60 s, spitless injection), injection volume: 1 μ l. The mono-methyl esters were separated with the following temp. program: 50 min at 60° , then with $10^\circ \text{ min}^{-1}$ to 210° . The dimethyl esters were separated with the following temp. program: 120 min at 50° , then with $20^\circ \text{ min}^{-1}$ to 210° .

Derivatization with Dimethyl Disulfide (DMS). According to the method of *Leonhardt and DeVilbiss* [16], a soln. of the natural extract (10 μ l), DMS (50 μ l), and an I_2 soln. (5 μ l, 5% in Et_2O) was heated to 60° for 8 h. The soln. was diluted with pentane, and excess I_2 was removed by washing with sat. aq. $\text{Na}_2\text{S}_2\text{O}_3$ soln. The org. layer was separated, dried (MgSO_4), concentrated, and analyzed by GC/MS.

Preparation of Methyl 2-(Dimethoxyphosphoryl)propanoate. A mixture of methyl 2-bromopropanoate (18.37 g, 0.11 mol) and $(\text{MeO})_3\text{P}$ (12.40 g, 0.1 mol) was stirred and heated to $105\text{--}110^\circ$ for 2 h [47]. The MeBr formed during the reaction was removed continuously, finally furnishing the crude product. This preparation was purified on SiO_2 using Et_2O as eluent. The purified product still contained small amounts of irremovable side product. R_f (Et_2O) 0.27. GC (*BPX5*): *I* 1293. $^1\text{H-NMR}$ (400 MHz, CDCl_3): 1.42–1.50 (*m*, 3 H); 3.02–3.14 (*m*, 1 H); 3.76–3.77 (*m*, 3 H); 3.78–3.80 (*m*, 3 H); 3.81–3.83 (*m*, 3 H). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): 11.6 (Me); 38.6 (*d*, $J=134.5$, CH); 52.6 (Me); 53.3 (Me); 53.4 (Me); 170.0 (C). EI-MS (70 eV): 196 (1, M^+), 181 (21), 165 (40), 151 (4), 141 (15), 137 (31), 127 (15), 109 (100), 93 (12), 79 (27), 55 (53).

Preparation of Unsaturated Methyl Esters. According to *Dickschat et al.* [27], NaH (1.3 equiv., 60% in mineral oil) was suspended in THF (1 ml/mmol) and cooled to 0° . Methyl 2-(dimethoxyphosphoryl)propanoate (1.3 equiv.) dissolved in THF (0.5 ml/mmol) was added dropwise to this suspension. The resulting mixture was stirred for 1 h at 40° . After cooling to 0° the respective aldehyde (1–1.3 equiv.) in THF (0.5 ml/mmol) was added dropwise. The mixture was stirred under reflux for 24 h, and the reaction was quenched by the addition of 2M HCl . The aq. layer was extracted three times with Et_2O , the combined org. phases were dried with MgSO_4 and finally concentrated under vacuum. Column chromatography on SiO_2 using a gradient of pentane/ Et_2O (200 : 1 to 100 : 1) gave the respective ester as a diastereoisomeric mixture.

Methyl 2-Methylhept-2-enoate (18). Diastereoisomeric ratio (crude product): (*E*)/(*Z*) 2 : 1. Yield: 42% (328 mg, 2.10 mmol). R_f (pentane/ Et_2O 10 : 1): (*Z*): 0.61; (*E*): 0.49. GC (*HP-5MS*): (*Z*): *RI* 1088; (*E*): *RI* 1145. $^1\text{H-NMR}$ (400 MHz, CDCl_3): (*E*): 0.85–0.93 (*m*, 3 H); 1.24–1.46 (*m*, 4 H); 1.83–1.84 (*m*, 3 H); 2.17 (*q*, $J=7.1$, 2 H); 3.73 (*s*, 3 H); 6.77 (*td*, $J=1.5, 7.5$, 1 H). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): (*E*): 12.3 (Me); 13.8 (Me); 22.4 (CH_2); 28.3 (CH_2); 30.7 (CH_2); 51.6 (Me); 127.4 (C); 142.7 (CH); 168.7 (C). EI-MS (70 eV): (*Z*): 156 (46, M^+), 127 (100), 101 (33), 95 (47), 88 (27), 81 (10), 67 (27), 55 (55), 41 (29); (*E*): 156 (35, M^+), 127 (63), 114 (7), 101 (92), 88 (65), 81 (17), 69 (29), 55 (100), 54 (11), 41 (40).

Methyl 2-Methyloct-2-enoate (19). Diastereoisomeric ratio (crude product): (*E*)/(*Z*) 3 : 1. Yield: 30% (257 mg, 1.48 mmol). R_f (pentane/ Et_2O 10 : 1): (*Z*): 0.64; (*E*): 0.46. GC (*HP-5MS*): (*Z*): *RI* 1189;

(*E*): *R*_f 1245. ¹H-NMR (400 MHz, CDCl₃): (*E*): 0.89 (*t*, *J* = 6.9, 3 H); 1.27–1.37 (*m*, 4 H); 1.40–1.49 (*m*, 2 H); 1.83–1.84 (*m*, 3 H); 2.17 (*dq*, *J* = 0.8, 7.4, 2 H); 3.73 (*s*, 3 H); 6.77 (*tq*, *J* = 1.4, 7.5, 1 H). ¹³C-NMR (100 MHz, CDCl₃): (*E*): 12.3 (Me); 14.0 (Me); 22.5 (CH₂); 28.2 (CH₂); 28.6 (CH₂); 31.5 (CH₂); 51.7 (Me); 127.4 (C); 142.8 (CH); 168.8 (C). EI-MS (70 eV): (*Z*): 170 (42, *M*⁺), 139 (19), 127 (100), 101 (58), 95 (42), 88 (45), 83 (21), 81 (14), 67 (25), 55 (18), 53 (20), 41 (63); (*E*): 170 (19, *M*⁺), 139 (27), 127 (45), 101 (100), 95 (21), 88 (69), 82 (21), 69 (53), 59 (20), 55 (56), 41 (38).

Methyl 2-Methylnon-2-enoate (20). Diastereoisomeric ratio (crude product): (*E*)/(*Z*) 1.3:1. Yield: 78% (714 mg, 3.88 mmol). *R*_f (pentane/Et₂O 10:1): (*Z*): 0.65; (*E*): 0.50. GC (*HP-5MS*): (*Z*): *R*_f 1282; (*E*): *R*_f 1346. ¹H-NMR (400 MHz, CDCl₃): (*E*): 0.89 (*t*, *J* = 6.9, 3 H); 1.21–1.36 (*m*, 6 H); 1.40–1.47 (*m*, 2 H); 1.82–1.83 (*m*, 3 H); 2.17 (*dq*, *J* = 0.9, 7.4, 2 H); 3.73 (*s*, 3 H); 6.74–6.79 (*m*, 1 H). ¹³C-NMR (100 MHz, CDCl₃): (*E*): 12.3 (Me); 14.1 (Me); 22.6 (CH₂); 28.5 (CH₂); 28.7 (CH₂); 29.0 (CH₂); 31.7 (CH₂); 51.6 (Me); 127.4 (C); 142.8 (CH); 168.8 (C). EI-MS (70 eV): (*Z*): 184 (34, *M*⁺), 153 (20), 127 (100), 101 (55), 95 (37), 88 (43), 83 (14), 69 (27), 55 (34), 41 (28); (*E*): 184 (10, *M*⁺), 153 (23), 127 (37), 101 (100), 88 (74), 82 (17), 69 (39), 55 (45), 41 (32).

Methyl 2-Methyldec-2-enoate (21). Diastereoisomeric ratio (crude product): (*E*)/(*Z*) 2:1. Yield: 52% (514 mg, 2.60 mmol). *R*_f (pentane/Et₂O 10:1): (*Z*): 0.64; (*E*): 0.51. GC (*HP-5MS*): (*Z*): *R*_f 1381; (*E*): *R*_f 1446. ¹H-NMR (400 MHz, CDCl₃): (*E*): 0.88 (*t*, *J* = 6.9, 3 H); 1.23–1.48 (*m*, 9 H); 1.60–1.67 (*m*, 1 H); 1.82–1.83 (*m*, 3 H); 2.13–2.19 (*m*, 2 H); 3.73 (*s*, 3 H); 6.77 (*tq*, *J* = 1.4, 7.5, 1 H). ¹³C-NMR (100 MHz, CDCl₃): (*E*): 12.3 (Me); 14.1 (Me); 22.6 (CH₂); 28.6 (CH₂); 29.0 (CH₂); 29.1 (CH₂); 29.3 (CH₂); 31.8 (CH₂); 51.6 (Me); 127.4 (C); 142.9 (CH); 168.8 (C). EI-MS (70 eV): (*Z*): 198 (29, *M*⁺), 167 (18), 127 (100), 114 (12), 101 (67), 95 (33), 88 (52), 83 (13), 69 (24), 55 (32), 41 (29); (*E*): 198 (7, *M*⁺), 167 (19), 127 (29), 101 (100), 95 (17), 88 (71), 83 (17), 69 (29), 55 (36), 41 (29).

Methyl 2-Methylundec-2-enoate (22). Diastereoisomeric ratio (crude product): (*E*)/(*Z*) 2:1. Yield: 74% (601 mg, 2.84 mmol). *R*_f (pentane/Et₂O 10:1): (*Z*): 0.64; (*E*): 0.50. GC (*HP-5MS*): (*Z*): *R*_f 1480; (*E*): *R*_f 1547. ¹H-NMR (400 MHz, CDCl₃): (*E*): 0.88 (*t*, *J* = 6.8, 3 H); 1.20–1.52 (*m*, 11 H); 1.56–1.68 (*m*, 1 H); 1.82–1.83 (*m*, 3 H); 2.16 (*dq*, *J* = 0.8, 7.4, 2 H); 3.73 (*s*, 3 H); 6.77 (*tq*, *J* = 1.4, 7.5, 1 H). ¹³C-NMR (100 MHz, CDCl₃): (*E*): 12.3 (Me); 14.1 (Me); 22.6 (CH₂); 28.7 (CH₂); 29.2 (CH₂); 29.3 (CH₂); 29.4 (CH₂); 29.6 (CH₂); 31.8 (CH₂); 51.6 (Me); 127.4 (C); 142.8 (CH); 168.8 (C). EI-MS (70 eV): (*Z*): 212 (22, *M*⁺), 181 (17), 127 (100), 114 (13), 101 (77), 95 (32), 88 (57), 83 (13), 81 (13), 69 (25), 55 (31), 41 (30); (*E*): 212 (6, *M*⁺), 181 (16), 127 (27), 101 (100), 95 (17), 88 (73), 83 (15), 69 (28), 55 (33), 41 (28).

Methyl 2-Methyldodec-2-enoate (23). Diastereoisomeric ratio (crude product): (*E*)/(*Z*) 2:1. Yield: 76% (665 mg, 2.94 mmol). *R*_f (pentane/Et₂O 10:1): (*Z*): 0.61; (*E*): 0.48. GC (*HP-5MS*): (*Z*): *R*_f 1580; (*E*): *R*_f 1648. ¹H-NMR (400 MHz, CDCl₃): (*E*): 0.88 (*t*, *J* = 6.8, 3 H); 1.20–1.52 (*m*, 13 H); 1.55–1.69 (*m*, 1 H); 1.82–1.83 (*m*, 3 H); 2.13–2.19 (*m*, 2 H); 3.73 (*s*, 3 H); 6.77 (*tq*, *J* = 1.6, 7.5, 1 H). ¹³C-NMR (100 MHz, CD₃OD): (*E*): 12.3 (Me), 14.1 (Me), 22.7 (CH₂), 28.7 (CH₂), 29.3 (CH₂), 29.4 (2 CH₂), 29.5 (CH₂), 29.6 (CH₂), 31.9 (CH₂), 51.6 (Me), 128.6 (C), 142.8 (CH), 168.7 (C). EI-MS (70 eV): (*Z*): 226 (23, *M*⁺), 195 (16), 127 (100), 115 (17), 101 (66), 95 (32), 88 (59), 69 (23), 55 (29), 41 (30); (*E*): 226 (5, *M*⁺), 195 (16), 127 (25), 101 (100), 95 (17), 88 (77), 83 (17), 69 (26), 55 (31), 41 (27).

Methyl 2-Methyltridec-2-enoate (24). Diastereoisomeric ratio (crude product): (*E*)/(*Z*) 1:1. Yield: 60% (554 mg, 2.31 mmol). *R*_f (pentane/Et₂O 10:1): (*Z*): 0.61; (*E*): 0.48. GC (*HP-5MS*): (*Z*): *R*_f 1677; (*E*): *R*_f 1746. ¹H-NMR (400 MHz, CDCl₃): (*E*): 0.88 (*t*, *J* = 6.8, 3 H); 1.23–1.34 (*m*, 14 H); 1.36–1.47 (*m*, 2 H); 1.82–1.83 (*m*, 3 H); 2.13–2.20 (*m*, 2 H); 3.73 (*s*, 3 H); 6.77 (*dq*, *J* = 1.4, 7.5, 1 H). ¹³C-NMR (100 MHz, CDCl₃): (*E*): 12.3 (Me); 14.1 (Me); 22.6 (CH₂); 28.6 (CH₂); 29.3 (2 CH₂); 29.4 (2 CH₂); 29.6 (2 CH₂); 31.9 (CH₂); 51.6 (Me); 127.3 (C); 142.8 (CH); 168.7 (C). EI-MS (70 eV): (*Z*): 240 (22, *M*⁺), 209 (15), 143 (9), 127 (100), 114 (14), 101 (69), 95 (31), 88 (63), 83 (15), 81 (15), 69 (23), 55 (29), 41 (31); (*E*): 240 (5, *M*⁺), 209 (15), 127 (25), 114 (9), 101 (100), 95 (17), 88 (82), 83 (16), 81 (14), 69 (24), 55 (30), 41 (28).

Methyl 2-Methyltetradec-2-enoate (25). Diastereoisomeric ratio (crude product): (*E*)/(*Z*) 1:1. Yield: 73% (715 mg, 2.81 mmol). *R*_f (pentane/Et₂O 10:1): (*Z*): 0.59; (*E*): 0.48. GC (*HP-5MS*): (*Z*): *R*_f 1778; (*E*): *R*_f 1848. ¹H-NMR (400 MHz, CDCl₃): (*E*): 0.88 (*t*, *J* = 6.9, 3 H); 1.23–1.33 (*m*, 16 H); 1.36–1.45 (*m*, 2 H); 1.82–1.83 (*m*, 3 H); 2.13–2.20 (*m*, 2 H); 3.73 (*s*, 3 H); 6.77 (*dq*, *J* = 1.4, 7.5, 1 H). ¹³C-NMR (100 MHz, CDCl₃): (*E*): 12.3 (Me); 14.1 (Me); 22.7 (CH₂); 28.6 (CH₂); 29.3 (2 CH₂); 29.4 (2 CH₂); 29.6 (3 CH₂); 31.9 (CH₂); 51.6 (Me); 127.4 (C); 142.8 (CH); 168.8 (C). EI-MS (70 eV): (*Z*): 254 (22, *M*⁺), 223

(14), 157 (9), 143 (21), 127 (100), 114 (14), 101 (69), 95 (30), 88 (65), 83 (14), 81 (15), 69 (23), 55 (29), 41 (31); (*E*): 254 (4, M^+), 223 (12), 143 (5), 127 (24), 114 (8), 101 (100), 95 (17), 88 (91), 83 (16), 81 (14), 69 (26), 55 (31), 41 (28).

Methyl 2-Methylpentadec-2-enoate (26). Diastereoisomeric ratio (crude product): (*E*)/(*Z*) 1:1. Yield: 65% (667 mg, 2.49 mmol). R_f (pentane/Et₂O 10:1): (*Z*): 0.60; (*E*): 0.48. GC (*HP-5MS*): (*Z*): R_I 1877; (*E*): R_I 1949. ¹H-NMR (400 MHz, CDCl₃): (*E*): 0.88 (*t*, $J=6.8$, 3 H); 1.23–1.33 (*m*, 18 H); 1.35–1.45 (*m*, 2 H); 1.82–1.83 (*m*, 3 H); 2.13–2.19 (*m*, 2 H); 3.73 (*s*, 3 H); 6.77 (*dq*, $J=1.4$, 7.5, 1 H). ¹³C-NMR (100 MHz, CDCl₃): (*E*): 12.3 (Me); 14.1 (Me); 22.7 (CH₂); 28.6 (CH₂); 29.3 (2 CH₂); 29.4 (2 CH₂); 29.6 (4 CH₂); 31.9 (CH₂); 51.6 (Me); 127.3 (C); 142.8 (CH); 168.7 (C). EI-MS (70 eV): (*Z*): 268 (22, M^+), 237 (14), 171 (9), 157 (23), 127 (100), 114 (14), 101 (73), 95 (30), 88 (69), 83 (15), 81 (15), 69 (24), 55 (30), 41 (32); (*E*): 268 (5, M^+), 237 (12), 157 (5), 127 (23), 114 (8), 101 (100), 99 (6), 98 (12), 95 (16), 88 (83), 83 (15), 81 (14), 69 (23), 55 (29), 41 (25).

Methyl (2*E*)-2,7-Dimethyloct-2-enoate (41a). Yield: 13% (91 mg, 0.49 mmol). R_f (pentane/Et₂O 10:1) 0.40. GC (*HP-5MS*): R_I 1313. ¹H-NMR (400 MHz, CDCl₃): 0.88 (*d*, $J=6.5$, 6 H); 1.17–1.24 (*m*, 2 H); 1.44 (*dt*, $J=7.6$, 15.6, 2 H); 1.49–1.58 (*m*, 1 H); 1.83–1.84 (*m*, 3 H); 2.12–2.18 (*m*, 2 H); 3.74 (*s*, 3 H); 6.74–6.79 (*m*, 1 H). ¹³C-NMR (100 MHz, CDCl₃): 12.3 (Me); 22.5 (2 Me); 26.4 (CH₂); 27.9 (CH); 28.9 (CH₂); 38.3 (CH₂); 51.6 (Me); 127.4 (C); 142.8 (CH); 168.8 (C). EI-MS (70 eV): 184 (1, M^+), 152 (68), 141 (4), 124 (25), 113 (10), 101 (75), 95 (23), 88 (100), 83 (21), 82 (65), 81 (26), 69 (71), 55 (42), 41 (45).

Methyl (2*Z*)-2,7-Dimethyloct-2-enoate (41b). Yield: 19% (138 mg, 0.75 mmol). R_f (pentane/Et₂O 10:1) 0.53. GC (*HP-5MS*): R_I 1246. EI-MS (70 eV): 184 (9, M^+), 153 (14), 152 (32), 141 (6), 127 (67), 114 (11), 101 (55), 95 (48), 88 (67), 83 (22), 82 (59), 81 (33), 69 (66), 55 (60), 41 (100), 39 (50).

Methyl (2*E*)-2,11-Dimethyldodec-2-enoate (42a). Yield: 25% (228 mg, 0.95 mmol). R_f (pentane/Et₂O 10:1) 0.58. GC (*HP-5MS*): R_I 1710. ¹H-NMR (400 MHz, CDCl₃): 0.86 (*d*, $J=6.8$, 6 H); 1.12–1.17 (*m*, 2 H); 1.25–1.35 (*m*, 8 H); 1.40–1.46 (*m*, 2 H); 1.48–1.55 (*m*, 1 H); 1.82–1.83 (*m*, 3 H); 2.13–2.19 (*m*, 2 H); 3.73 (*s*, 3 H); 6.77 (*td*, $J=1.4$, 7.5, 1 H). ¹³C-NMR (100 MHz, CDCl₃): 12.3 (Me); 22.6 (2 Me); 27.3 (CH₂); 27.9 (CH); 28.6 (CH₂); 28.7 (CH₂); 29.4 (CH₂); 29.5 (CH₂); 29.8 (CH₂); 39.0 (CH₂); 51.6 (Me); 127.4 (C); 142.8 (CH); 168.7 (C). EI-MS (70 eV): 240 (5, M^+), 209 (11), 208 (10), 127 (24), 114 (8), 101 (100), 95 (22), 88 (72), 83 (17), 81 (17), 69 (32), 55 (31), 41 (35).

Methyl (2*Z*)-2,11-Dimethyldodec-2-enoate (42b). Yield: 19% (174 mg, 0.73 mmol). R_f (pentane/Et₂O 10:1) 0.69. GC (*HP-5MS*): R_I 1643. EI-MS (70 eV): 240 (26, M^+), 209 (15), 127 (100), 114 (14), 101 (67), 95 (36), 88 (59), 83 (15), 81 (17), 69 (27), 55 (30), 43 (33), 41 (36).

Methyl 2,6-Dimethyloct-2-enoate (48). Diastereoisomeric ratio (crude product): (*E*)/(*Z*) 6:1. Yield: 38% (203 mg, 1.10 mmol, over two steps). R_f (pentane/Et₂O 10:1): (*Z*): 0.74; (*E*): 0.62. GC (*BPX5*): (*Z*): R_I 1247; (*E*): R_I 1317. ¹H-NMR (400 MHz, CDCl₃): (*E*): 0.75–0.84 (*m*, 6 H); 1.04–1.42 (*m*, 4 H); 1.50 (*m*, 1 H); 1.75–1.78 (*m*, 3 H); 2.04–2.14 (*m*, 2 H); 3.66 (*s*, 3 H); 7.19 (*s*, 1 H). ¹³C-NMR (100 MHz, CDCl₃): (*E*): 11.3 (Me); 12.3 (Me); 19.0 (Me); 26.3 (CH₂); 29.3 (CH₂); 34.1 (CH); 35.3 (CH₂); 51.7 (Me); 127.2 (C); 143.0 (CH); 168.8 (C). EI-MS (70 eV): (*Z*): 184 (19, M^+), 127 (100), 101 (21), 95 (28), 88 (7), 67 (15), 55 (1541 (17)); (*E*): 184 (4, M^+), 153 (14), 127 (23), 114 (10), 101 (100), 95 (20), 88 (20), 84 (33), 69 (37), 55 (30), 41 (31).

Preparation of Saturated Methyl Esters. According to Suzukamo *et al.* [28], the respective unsaturated ester (1 mmol) was dissolved in 10 ml of dry MeOH, and 1 mol-% PtO₂ was added. The resulting suspension was stirred, while H₂ was passed through it, and the progress of the reaction was checked with GC. After the reaction was completed, column chromatography on SiO₂ with pentane/Et₂O 10:1 as eluent afforded the pure product.

Methyl 2-Methylheptanoate (27). Yield: quant. (332 mg, 2.10 mmol). R_f (pentane/Et₂O 10:1) 0.59. GC (*HP-5MS*): R_I 1060. ¹H-NMR (400 MHz, CDCl₃): 0.88 (*t*, $J=6.9$, 3 H); 1.14 (*d*, $J=7.0$, 3 H); 1.25–1.46 (*m*, 7 H); 1.59–1.70 (*m*, 1 H); 2.44 (*sext.*, $J=7.0$, 1 H); 3.67 (*s*, 3 H). ¹³C-NMR (100 MHz, CDCl₃): 14.0 (Me); 17.0 (Me); 22.5 (CH₂); 26.9 (CH₂); 31.7 (CH₂); 33.8 (CH₂); 39.4 (CH); 51.4 (Me); 177.4 (C). EI-MS (70 eV): 143 (2, [$M-15$]⁺), 127 (6), 115 (8), 101 (25), 88 (100), 69 (5), 57 (28), 41 (12).

Methyl 2-Methyloctanoate (28). Yield: 57% (138 mg, 0.80 mmol). R_f (pentane/Et₂O 10:1) 0.53. GC (*HP-5MS*): R_I 1161. ¹H-NMR (400 MHz, CDCl₃): 0.88 (*t*, $J=6.9$, 3 H); 1.14 (*d*, $J=7.0$, 3 H); 1.22–1.33 (*m*, 8 H); 1.36–1.47 (*m*, 1 H); 1.61–1.69 (*m*, 1 H); 2.43 (*sext.*, $J=7.0$, 1 H); 3.67 (*s*, 3 H). ¹³C-NMR

(100 MHz, CDCl_3): 14.0 (Me); 17.1 (Me); 22.6 (CH_2); 27.2 (CH_2); 29.2 (CH_2); 31.7 (CH_2); 33.8 (CH_2); 39.5 (CH); 51.4 (Me); 177.4 (C). EI-MS (70 eV): 172 (1, M^+), 143 (3), 141 (4), 129 (4), 115 (6), 101 (24), 88 (100), 71 (7), 57 (16), 41 (11).

Methyl 2-Methylnonanoate (29). Yield: 40% (176 mg, 0.95 mmol). R_f (pentane/ Et_2O 10:1) 0.53. GC (HP-5MS): RI 1258. $^1\text{H-NMR}$ (400 MHz, CDCl_3): 0.88 (t , $J=6.9$, 3 H); 1.14 (d , $J=7.0$, 3 H); 1.21–1.33 (m , 10 H); 1.36–1.45 (m , 1 H); 1.60–1.69 (m , 1 H); 2.43 (sext. , $J=7.0$, 1 H); 3.67 (s , 3 H). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): 14.0 (Me); 17.0 (Me); 22.6 (CH_2); 27.2 (CH_2); 29.1 (CH_2); 29.5 (CH_2); 31.8 (CH_2); 33.8 (CH_2); 39.4 (CH); 51.4 (Me); 177.4 (C). EI-MS (70 eV): 186 (1, M^+), 171 (1), 157 (3), 143 (8), 129 (4), 115 (3), 101 (27), 88 (100), 69 (5), 55 (9), 41 (11).

Methyl 2-Methyldecanoate (30). Yield: 80% (285 mg, 1.43 mmol). R_f (pentane/ Et_2O 10:1) 0.52. GC (HP-5MS): RI 1359. $^1\text{H-NMR}$ (400 MHz, CDCl_3): 0.88 (t , $J=7.1$, 3 H); 1.14 (d , $J=6.8$, 3 H); 1.19–1.46 (m , 13 H); 1.60–1.69 (m , 1 H); 2.43 (sext. , $J=7.0$, 1 H); 3.67 (s , 3 H). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): 14.1 (Me); 17.1 (Me); 22.7 (CH_2); 27.3 (CH_2); 29.3 (CH_2); 29.5 (2 CH_2); 31.9 (CH_2); 33.8 (CH_2); 39.5 (CH); 51.4 (Me); 177.4 (C). EI-MS (70 eV): 200 (2, M^+), 169 (3), 157 (7), 143 (9), 115 (3), 101 (30), 88 (100), 69 (6), 57 (11), 41 (10).

Methyl 2-Methylundecanoate (31). Yield: 76% (237 mg, 1.11 mmol). R_f (pentane/ Et_2O 10:1) 0.53. GC (HP-5MS): RI 1459. $^1\text{H-NMR}$ (400 MHz, CDCl_3): 0.88 (t , $J=6.9$, 3 H); 1.14 (d , $J=7.0$, 3 H); 1.20–1.34 (m , 14 H); 1.35–1.45 (m , 1 H); 1.60–1.69 (m , 1 H); 2.43 (sext. , $J=7.0$, 1 H); 3.67 (s , 3 H). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): 14.1 (Me); 17.1 (Me); 22.7 (CH_2); 27.2 (CH_2); 29.3 (CH_2); 29.5 (3 CH_2); 31.9 (CH_2); 33.8 (CH_2); 39.5 (CH); 51.4 (Me); 177.4 (C). EI-MS (70 eV): 214 (2, M^+), 183 (3), 171 (5), 157 (9), 143 (7), 115 (4), 101 (32), 88 (100), 69 (6), 57 (11), 41 (10).

Methyl 2-Methyldodecanoate (32). Yield: 71% (332 mg, 1.46 mmol). R_f (pentane/ Et_2O 10:1) 0.52. GC (HP-5MS): RI 1559. $^1\text{H-NMR}$ (400 MHz, CDCl_3): 0.88 (t , $J=6.9$, 3 H); 1.14 (d , $J=7.0$, 3 H); 1.22–1.33 (m , 16 H); 1.35–1.44 (m , 1 H); 1.60–1.70 (m , 1 H); 2.43 (sext. , $J=7.0$, 1 H); 3.67 (s , 3 H). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): 14.1 (Me); 17.0 (Me); 22.7 (CH_2); 27.2 (CH_2); 29.3 (CH_2); 29.5 (2 CH_2); 29.6 (2 CH_2); 31.9 (CH_2); 33.8 (CH_2); 39.5 (CH); 51.4 (Me); 177.4 (C). EI-MS (70 eV): 228 (3, M^+), 197 (2), 185 (5), 171 (6), 157 (6), 143 (7), 115 (3), 101 (34), 98 (2), 88 (100), 69 (6), 57 (10), 41 (10).

Methyl 2-Methyltridecanoate (33). Yield: 76% (226 mg, 0.93 mmol). R_f (pentane/ Et_2O 10:1) 0.53. GC (HP-5MS): RI 1660. $^1\text{H-NMR}$ (400 MHz, CDCl_3): 0.88 (t , $J=6.8$, 3 H); 1.14 (d , $J=6.8$, 3 H); 1.21–1.47 (m , 19 H); 1.57–1.70 (m , 1 H); 2.43 (sext. , $J=7.0$, 1 H); 3.67 (s , 3 H). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): 14.1 (Me); 17.0 (Me); 22.7 (CH_2); 27.2 (CH_2); 29.3 (CH_2); 29.5 (2 CH_2); 29.6 (3 CH_2); 31.9 (CH_2); 33.8 (CH_2); 39.5 (CH); 51.4 (Me); 177.4 (C). EI-MS (70 eV): 242 (4, M^+), 211 (2), 199 (6), 185 (6), 157 (7), 143 (9), 115 (3), 101 (36), 88 (100), 69 (7), 55 (10), 41 (10).

Methyl 2-Methyltetradecanoate (34). Yield: 73% (336 mg, 1.31 mmol). R_f (pentane/ Et_2O 10:1) 0.57. GC (HP-5MS): RI 1759. $^1\text{H-NMR}$ (400 MHz, CDCl_3): 0.88 (t , $J=6.9$, 3 H); 1.14 (d , $J=7.0$, 3 H); 1.21–1.46 (m , 21 H); 1.59–1.70 (m , 2 H); 2.43 (sext. , $J=7.0$, 1 H); 3.67 (s , 3 H). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): 14.1 (Me); 17.1 (Me); 22.7 (CH_2); 27.2 (CH_2); 29.4 (CH_2); 29.5 (2 CH_2); 29.6 (3 CH_2); 29.7 (CH_2); 31.9 (CH_2); 33.8 (CH_2); 39.5 (CH); 51.4 (Me); 177.4 (C). EI-MS (70 eV): 256 (5, M^+), 225 (2), 213 (6), 199 (8), 157 (8), 143 (9), 115 (3), 101 (38), 88 (100), 69 (7), 55 (10), 41 (10).

Methyl 2-Methylpentadecanoate (35). Yield: 83% (250 mg, 0.93 mmol). R_f (pentane/ Et_2O 10:1) 0.56. GC (HP-5MS): RI 1859. $^1\text{H-NMR}$ (400 MHz, CDCl_3): 0.88 (t , $J=6.9$, 3 H); 1.14 (d , $J=7.0$, 3 H); 1.21–1.46 (m , 23 H); 1.59–1.70 (m , 2 H); 2.43 (sext. , $J=7.0$, 1 H); 3.67 (s , 3 H). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): 14.1 (Me); 17.1 (Me); 22.7 (CH_2); 27.2 (CH_2); 29.4 (CH_2); 29.5 (2 CH_2); 29.6 (3 CH_2); 29.7 (2 CH_2); 31.9 (CH_2); 33.8 (CH_2); 39.5 (CH); 51.4 (Me); 177.4 (C). EI-MS (70 eV): 270 (7, M^+), 239 (2), 227 (5), 213 (8), 199 (2), 171 (2), 157 (9), 143 (8), 101 (38), 88 (100), 69 (8), 55 (11), 41 (10).

Methyl 2,7-Dimethyloctanoate (43). Yield: 66% (153 mg, 0.82 mmol). R_f (pentane/ Et_2O 10:1) 0.58. GC (HP-5MS): RI 1223. $^1\text{H-NMR}$ (400 MHz, CDCl_3): 0.86 (d , $J=6.6$, 6 H); 1.12–1.18 (m , 5 H); 1.24–1.35 (m , 4 H); 1.36–1.56 (m , 2 H); 1.60–1.70 (m , 1 H); 2.43 (sext. , $J=7.0$, 1 H); 3.67 (s , 3 H). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): 17.1 (Me); 22.6 (2 Me); 27.3 (CH_2); 27.5 (CH_2); 27.9 (CH); 33.9 (CH_2); 38.8 (CH_2); 39.5 (CH); 51.4 (Me); 177.4 (C). EI-MS (70 eV): 186 (1, M^+), 171 (2), 155 (3), 143 (13), 129 (3), 115 (4), 101 (40), 88 (100), 69 (13), 57 (14), 55 (13), 43 (15), 41 (18).

Methyl 2,11-Dimethyldodecanoate (44). Yield: 61% (156 mg, 0.64 mmol). R_f (pentane/ Et_2O 10:1) 0.59. GC (HP-5MS): RI 1620. $^1\text{H-NMR}$ (300 MHz, CDCl_3): 0.86 (d , $J=6.6$, 6 H); 1.12–1.46 (m , 10 H);

1.47–1.56 (*m*, 1 H); 1.58–1.71 (*m*, 1 H); 2.43 (*s*_{ext.}, *J* = 7.0, 1 H); 3.67 (*s*, 3 H). ¹³C-NMR (75 MHz, CDCl₃): 17.1 (Me); 22.6 (2 Me); 27.2 (CH₂); 27.4 (CH₂); 28.0 (CH); 29.5 (2 CH₂); 29.6 (CH₂); 29.9 (CH₂); 33.8 (CH₂); 39.0 (CH₂); 39.5 (CH); 51.4 (Me); 177.4 (C). EI-MS (70 eV): 242 (4, *M*⁺), 211 (3), 199 (11), 185 (7), 157 (8), 143 (10), 101 (41), 88 (100), 69 (9), 55 (13), 43 (15), 41 (13).

Methyl 2,6-Dimethyloctanoate (49). Yield: quant. (183 mg, 0.99 mmol). *R*_f (pentane/Et₂O 10:1) 0.55. GC (*HP-5MS*): *RI* 1229. ¹H-NMR (400 MHz, CDCl₃): 0.83–0.94 (*m*, 6 H); 1.07–1.15 (*m*, 5 H); 1.20–1.44 (*m*, 6 H); 1.54–1.70 (*m*, 1 H); 2.39–2.49 (*m*, 1 H); 3.67 (*s*, 3 H). ¹³C-NMR (100 MHz, CDCl₃): 11.4 (Me); 17.1 (Me); 19.1 (Me); 24.7 (CH₂); 29.4 (CH₂); 34.1 (CH₂); 34.2 (CH); 36.4 (CH₂); 39.5 (CH); 51.4 (Me); 177.4 (C). EI-MS (70 eV): 186 (2, *M*⁺), 157 (14), 129 (9), 115 (3), 101 (46), 88 (100), 69 (15), 55 (28), 41 (24).

Preparation of (ω-1)-Me-Branched Alcohols. According to the procedure of Fürstner *et al.* [29], a soln. of *i*-BuMgBr (40 mmol, 2.5 equiv.) in THF (12.5 ml), freshly prepared from Mg (40 mmol, 2.5 equiv.) and *i*-BuBr (40 mmol, 2.5 equiv.), was added to an ice-cooled soln. of the respective 1-bromoalcohol (16 mmol, 1 equiv.) and Li₂CuCl₄ (0.1M soln. in THF, 3 mmol, 0.19 mmol). The mixture was stirred for 1.5 h at 0° and was then allowed to warm up to r.t. Then, conc. HCl (10–15 ml) was added. The aq. layer was separated and extracted three times with Et₂O. The combined org. extracts were successively washed with sat. NaHCO₃ and brine, dried (MgSO₄), and then concentrated under vacuum. The crude product was purified by CC on SiO₂ with pentane/Et₂O (5:1) as eluent to yield the pure alcohol.

5-Methylhexan-1-ol (37). Yield: 85% (1.571 g, 13.55 mmol). *R*_f (pentane/Et₂O 5:1) 0.07. GC (*BPX5*): *RI* 942. ¹H-NMR (400 MHz, CDCl₃): 0.88 (*d*, *J* = 6.5, 6 H); 1.16–1.22 (*m*, 2 H); 1.31–1.38 (*m*, 2 H); 1.51–1.59 (*m*, 3 H); 3.61–3.69 (*m*, 2 H). ¹³C-NMR (100 MHz, CDCl₃): 22.5 (2 Me); 23.5 (CH₂); 27.9 (CH); 32.9 (CH₂); 38.7 (CH₂); 62.9 (CH₂). EI-MS (70 eV): 98 (1, [*M* – 18]⁺), 83 (21), 70 (40), 69 (42), 56 (90), 55 (100), 43 (73), 42 (30), 41 (85).

9-Methyldecan-1-ol (38). Yield: 68% (1.161 g, 6.75 mmol). *R*_f (pentane/Et₂O 5:1) 0.07. GC (*BPX5*): *RI* 1338. ¹H-NMR (400 MHz, CDCl₃): 0.86 (*d*, *J* = 6.8, 6 H); 1.12–1.17 (*m*, 2 H); 1.24–1.38 (*m*, 9 H); 1.46–1.60 (*m*, 4 H); 3.63 (*t*, *J* = 6.7, 2 H). ¹³C-NMR (100 MHz, CDCl₃): 22.6 (2 Me); 25.7 (CH₂); 27.4 (CH₂); 27.9 (CH); 29.4 (CH₂); 29.6 (CH₂); 29.8 (CH₂); 32.8 (CH₂); 39.0 (CH₂); 63.0 (CH₂). EI-MS (70 eV): 139 (2, [*M* – 43]⁺), 126 (5), 111 (12), 98 (13), 97 (12), 83 (34), 69 (78), 57 (63), 56 (100), 55 (84), 43 (86), 41 (91).

Preparation of 4-Methylhexan-1-ol (46). The alcohol was prepared according to known methods by LiAlH₄ reduction of 4-methylhexanoic acid (0.5 g, 3.85 mmol) [22]. Aqueous workup and removal of the solvents furnished pure **46**. Yield: 75% (0.335 g, 2.89 mmol). *R*_f (pentane/Et₂O 5:1) 0.09. GC (*BPX5*): *RI* 954. ¹H-NMR (300 MHz, CDCl₃): 0.84–0.89 (*m*, 6 H); 1.08–1.23 (*m*, 2 H); 1.28–1.42 (*m*, 3 H); 1.45–1.67 (*m*, 2 H); 2.37 (*s*, 1 H); 3.60 (*t*, *J* = 6.7, 2 H). ¹³C-NMR (75 MHz, CDCl₃): 11.7 (Me); 19.5 (Me); 29.8 (CH₂); 30.6 (CH₂); 32.9 (CH₂); 34.6 (CH); 63.6 (CH). EI-MS (70 eV): 98 (4, *M*⁺), 83 (8), 70 (100), 69 (88), 57 (27), 56 (32), 55 (40), 43 (18), 42 (19), 41 (74), 39 (17).

Preparation of (ω-1)- and (ω-2)-Me-Branched Aldehydes. The aldehydes were synthesized from the corresponding alcohols with PCC according to the standard procedure of Corey and Suggs [30]. Purification was performed by CC on SiO₂ using pentane/Et₂O in the ratio mentioned for the respective aldehyde.

5-Methylhexanal (39). Yield: 69% (1.017 g, 8.92 mmol). *R*_f (pentane/Et₂O 3:1) 0.61. GC (*BPX5*): *RI* 869. ¹H-NMR (400 MHz, CDCl₃): 0.89 (*d*, *J* = 6.6, 6 H); 1.18–1.24 (*m*, 2 H); 1.50–1.68 (*m*, 3 H); 2.31–2.43 (*m*, 2 H); 9.77 (*t*, *J* = 1.9, 1 H). ¹³C-NMR (100 MHz, CDCl₃): 19.9 (CH₂); 22.4 (2 Me); 27.8 (CH); 38.3 (CH₂); 44.1 (CH₂); 203.0 (CH). EI-MS (70 eV): (33, [*M* – 18]⁺), 86 (8), 81 (38), 71 (49), 55 (81), 43 (100), 41 (90), 39 (48).

9-Methyldecanal (40). Yield: 86% (0.958 g, 5.64 mmol). *R*_f (pentane/Et₂O 2:1) 0.75. GC (*BPX5*): *RI* 1267. ¹H-NMR (400 MHz, CDCl₃): 0.86 (*d*, *J* = 6.8, 6 H); 1.12–1.17 (*m*, 2 H); 1.24–1.35 (*m*, 8 H); 1.44–1.57 (*m*, 1 H); 1.63 (*quint.*, *J* = 7.3, 2 H); 2.42 (*dt*, *J* = 1.9, 7.3, 2 H); 9.77 (*t*, *J* = 1.9, 1 H). ¹³C-NMR (100 MHz, CDCl₃): 22.1 (CH₂); 22.6 (2 Me); 27.3 (CH₂); 27.9 (CH); 29.2 (CH₂); 29.4 (CH₂); 29.7 (CH₂); 39.0 (CH₂); 43.9 (CH₂); 202.9 (CH). EI-MS (70 eV): 152 (1, [*M* – 18]⁺), 142 (3), 137 (6), 124 (9), 109 (25), 96 (35), 95 (39), 82 (49), 81 (41), 69 (60), 57 (89), 43 (94), 41 (100).

4-Methylhexanal (47). R_f (pentane/Et₂O 3:1) 0.60. ¹H-NMR (200 MHz, CDCl₃): 0.84–0.92 (*m*, 6 H); 1.09–1.55 (*m*, 4 H); 1.57–1.76 (*m*, 1 H); 2.30–2.49 (*m*, 2 H); 9.78 (*t*, *J* = 1.9, 1 H). ¹³C-NMR (50 MHz, CDCl₃): 11.9 (Me); 19.5 (Me); 29.2 (CH₂); 29.8 (CH₂); 34.7 (CH); 42.4 (CH₂); 203.6 (CH). EI-MS (70 eV): 96 (13, *M*⁺), 81 (17), 71 (29), 70 (100), 57 (63), 55 (63), 43 (31), 42 (19), 41 (78), 39 (29).

Preparation of 7-Methyloctanoyl Chloride (55). According to the procedure of Spessard *et al.* [34], 7-methyloctanoic acid (**54**) (140 mg, 0.89 mmol) was dissolved in 5 ml of dry Et₂O and cooled to 0°. Oxalyl chloride (225 mg, 1.77 mmol) was added dropwise, and the mixture was stirred for 24 h at r.t. The solvent and excess oxalyl chloride were removed under vacuum to produce pure **55** (125 mg, 0.71 mmol, 80%). R_f (pentane/Et₂O 2:1) 0.93. GC (BPX5): *RI* 1202. ¹H-NMR (400 MHz, CDCl₃): 0.87 (*d*, *J* = 6.5, 6 H); 1.14–1.19 (*m*, 2 H); 1.26–1.38 (*m*, 4 H); 1.52 (*non.*, *J* = 6.6, 1 H); 1.63 (*quint.*, *J* = 7.3, 2 H); 2.88 (*t*, *J* = 7.3, 2 H). ¹³C-NMR (100 MHz, CDCl₃): 22.6 (2 Me); 25.1 (CH₂); 26.8 (CH₂); 27.9 (CH); 28.7 (CH₂); 38.6 (CH₂); 47.1 (CH₂); 173.9 (C). EI-MS (70 eV): 141 (15, [*M* – 35/37]⁺), 125 (18), 123 (17), 107 (16), 98 (33), 97 (42), 96 (30), 84 (55), 69 (48), 55 (100), 43 (93), 41 (73).

Preparation of Evans Chiral Auxiliary Acid Derivatives. As described by Gage and Evans [31], a soln. of (4*S*)-4-benzyl-1,3-oxazolidin-2-one (1 equiv.) in THF (3 ml/mmol) was cooled to –78°. Then, BuLi (1.6M in hexane, 1 equiv.) was added, and the resulting mixture was stirred for 30 min under these conditions. The respective octanoyl chloride (1.1 equiv.) was added dropwise, and the stirring was continued for 4 h at –78°. The mixture was allowed to warm up to r.t., and the reaction was quenched by the addition of sat. NH₄Cl soln. Then, the org. solvents were removed under vacuum, and the resulting aq. layer was extracted three times with CH₂Cl₂. The combined org. layers were successively washed with 1M NaOH soln. and brine, dried (MgSO₄), and concentrated under vacuum. Purification of the crude product by CC on SiO₂ with pentane/Et₂O (5:1) furnished the pure product.

(4*S*)-4-Benzyl-3-octanoyl-1,3-oxazolidin-2-one (51). Yield: 84% (2.54 g, 8.38 mmol). R_f (pentane/Et₂O 5:1) 0.14. GC (BPX5): *RI* 2459. ¹H-NMR (400 MHz, CDCl₃): 0.89 (*t*, *J* = 6.9, 3 H); 1.25–1.42 (*m*, 8 H); 1.63–1.77 (*m*, 2 H); 2.77 (*dd*, *J* = 9.7, *J* = 13.4, 1 H); 2.86–3.01 (*m*, 2 H); 3.30 (*dd*, *J* = 3.4, 13.4, 1 H); 4.14–4.22 (*m*, 2 H); 4.64–4.70 (*m*, 1 H); 7.20–7.35 (*m*, 5 H). ¹³C-NMR (100 MHz, CDCl₃): 14.1 (Me); 22.6 (CH₂); 24.2 (CH₂); 29.0 (CH₂); 29.1 (CH₂); 31.7 (CH₂); 35.5 (CH₂); 37.9 (CH₂); 55.1 (CH); 66.1 (CH₂); 127.3 (CH); 128.9 (2 CH); 129.4 (2 CH); 135.3 (C); 153.4 (C); 173.4 (C). EI-MS (70 eV): 303 (6, *M*⁺), 232 (3), 219 (8), 212 (18), 178 (4), 127 (100), 117 (10), 109 (4), 91 (17), 57 (32), 41 (9).

(4*S*)-4-Benzyl-3-(7-methyloctanoyl)-1,3-oxazolidin-2-one (56). Yield: 48% (96 mg, 0.31 mmol). R_f (pentane/Et₂O 5:1) 0.12. GC (BPX5): *RI* 2521. ¹H-NMR (400 MHz, CDCl₃): 0.87 (*d*, *J* = 6.8, 6 H); 1.15–1.21 (*m*, 2 H); 1.29–1.41 (*m*, 4 H); 1.48–1.58 (*m*, 1 H); 1.66–1.74 (*m*, 2 H); 2.77 (*dd*, *J* = 9.6, 13.1, 1 H); 2.85–3.01 (*m*, 2 H); 3.30 (*dd*, *J* = 3.3, 13.4, 1 H); 4.15–4.22 (*m*, 2 H); 4.64–4.69 (*m*, 1 H); 7.20–7.36 (*m*, 5 H). ¹³C-NMR (100 MHz, CDCl₃): 22.6 (2 Me); 24.3 (CH₂); 27.2 (CH₂); 27.9 (CH); 29.4 (CH₂); 35.6 (CH₂); 38.0 (CH₂); 38.8 (CH₂); 55.2 (CH); 66.1 (CH₂); 127.3 (CH); 129.0 (2 CH); 129.4 (2 CH); 135.3 (C); 153.5 (C); 173.5 (C). EI-MS (70 eV): 317 (13, *M*⁺), 302 (2), 226 (33), 219 (19), 178 (18), 141 (100), 123 (78), 117 (27), 91 (37), 81 (28), 71 (15), 67 (14), 57 (20), 55 (23), 43 (28), 41 (20).

Preparation of Methylated Evans Chiral Auxiliary Acid Derivatives. According to the procedure of Siebum *et al.* [32], a soln. of the acylated Evans oxazolidinone (1 equiv.) in dry THF (3 ml/mmol) was added dropwise to a cold (–78°) soln. of NaHMDS (1.0M soln. in THF, 2.5 equiv.) in THF (3 ml/mmol). The resulting mixture was stirred for 1 h at –78°, and then MeI (2 equiv.) was added. The mixture was stirred for 4 h at –78° and then slowly allowed to warm up to r.t. Workup was carried out by the addition of sat. NH₄Cl soln., H₂O, and 1M H₂SO₄ to adjust the pH to 1–2. The aq. layer was first separated and then extracted three times with Et₂O. The combined org. extracts were successively washed with sat. NaHCO₃, sat. Na₂S₂O₃, and brine, dried (MgSO₄), and then concentrated applying vacuum. The crude product (diastereoisomeric ratio 98:2 (**52**) and 96:4 (**57**), resp.) was purified by CC on SiO₂ with pentane/Et₂O (gradient from 10:1 to 7:1) as eluent. The obtained products were diastereoisomerically almost pure.

(4*S*)-4-Benzyl-3-[(2*S*)-2-methyloctanoyl]-1,3-oxazolidin-2-one (52). Diastereoisomeric ratio: 99:1. Yield: 66% (859 mg, 8.38 mmol). R_f (pentane/Et₂O 10:1) 0.13. GC (BPX5): *RI* 2442. ¹H-NMR (400 MHz, CDCl₃): 0.87 (*t*, *J* = 6.8, 3 H); 1.22 (*d*, *J* = 6.8, 3 H); 1.24–1.35 (*m*, 6 H); 1.36–1.46 (*m*, 2 H); 2.77 (*dd*, *J* = 9.6, 13.4, 1 H); 3.27 (*dd*, *J* = 3.3, 13.1, 1 H); 3.71 (*sext.*, *J* = 6.8, 1 H); 4.14–4.22 (*m*, 2 H); 4.64–4.70 (*m*, 1 H); 7.20–7.35 (*m*, 5 H). ¹³C-NMR (100 MHz, CDCl₃): 14.0 (Me); 17.3 (Me); 22.6 (CH₂); 27.2 (CH₂); 29.3 (CH₂); 31.7 (CH₂); 33.4 (CH₂); 37.7 (CH); 37.9 (CH₂); 55.3 (CH); 66.0 (CH₂); 127.3

(CH); 128.9 (2 CH); 129.4 (2 CH); 135.3 (C); 153.0 (C); 177.3 (C). EI-MS (70 eV): 317 (5, M^+), 233 (18), 226 (20), 178 (14), 141 (100), 134 (7), 117 (21), 113 (22), 91 (39), 86 (21), 71 (65), 57 (57), 43 (27), 41 (32).

(4*S*)-4-Benzyl-3-[(2*S*)-2,7-dimethyloctanoyl]-1,3-oxazolidin-2-one (**57**). Diastereoisomeric ratio: 100:0. Yield: 46% (70 mg, 0.22 mmol). R_f (pentane/Et₂O 5:1) 0.12. GC (BPX5): *RI* 2494. ¹H-NMR (400 MHz, CDCl₃): 0.86 (*d*, *J*=6.8, 6 H); 1.12–1.19 (*m*, 2 H); 1.22 (*d*, *J*=6.8, 3 H); 1.25–1.33 (*m*, 4 H); 1.37–1.56 (*m*, 2 H); 1.70–1.79 (*m*, 1 H); 2.77 (*dd*, *J*=9.7, 13.4, 1 H); 3.27 (*dd*, *J*=3.1, 13.4, 1 H); 3.67–3.75 (*m*, 1 H); 4.15–4.22 (*m*, 2 H); 4.65–4.70 (*m*, 1 H); 7.21–7.35 (*m*, 5 H). ¹³C-NMR (100 MHz, CDCl₃): 17.4 (Me); 22.6 (2 Me); 27.4 (CH₂); 27.5 (CH₂); 27.9 (CH); 33.5 (CH₂); 37.7 (CH); 37.9 (CH₂); 38.8 (CH₂); 55.4 (CH); 66.0 (CH₂); 127.3 (CH); 128.9 (2 CH); 129.4 (2 CH); 135.3 (C); 153.1 (C); 177.4 (C). EI-MS (70 eV): 331 (5, M^+), 316 (2), 240 (19), 233 (23), 178 (22), 155 (100), 134 (8), 133 (8), 127 (22), 117 (22), 91 (30), 85 (34), 71 (50), 57 (30), 43 (29), 41 (23).

Preparation of Carboxylic Acids from Acylated Evans Oxazolidinones. As reported by Siebum *et al.* [32], a H₂O₂ soln. (30% in H₂O, 5 equiv.) and LiOH (2.5 equiv.), dissolved in H₂O (0.5 ml/mmol), were added at 0° to a soln. of the respective oxazolidinone (1 equiv.) in THF (10 ml/mmol) and H₂O (5 ml/mmol). The mixture was stirred for 2 h under these conditions. Sodium sulfite in H₂O (1.9 equiv., 4 ml/mmol) was added, and the mixture was stirred for 15 min at 0°. The soln. was adjusted to pH 9–10 by the addition of sat. NaHCO₃ soln. THF was evaporated, and the residual aq. layer was extracted two times with CH₂Cl₂. The combined org. phases were dried (MgSO₄) and then concentrated under vacuum to give the recovered auxiliary (4*S*)-4-benzyl-1,3-oxazolidin-2-one. The aq. soln. was acidified to pH 1–2 with 1*M* H₂SO₄ soln. and extracted three times with Et₂O. The combined org. extracts were dried (MgSO₄), and the solvents were evaporated to furnish the respective pure carboxylic acid.

(2*S*)-2-Methyloctanoic Acid (**53**). Yield: quant. (300 mg, 1.90 mmol). GC (BPX5): *RI* 1269. ¹H-NMR (400 MHz, CDCl₃): 0.88 (*t*, *J*=6.9, 3 H); 1.18 (*d*, *J*=6.8, 3 H); 1.24–1.37 (*m*, 8 H); 1.39–1.47 (*m*, 1 H); 1.64–1.73 (*m*, 1 H); 2.46 (*sext.*, *J*=6.9, 1 H); 10.52 (*br. s*, 1 H). ¹³C-NMR (100 MHz, CDCl₃): 14.0 (Me); 16.8 (Me); 22.6 (CH₂); 27.1 (CH₂); 29.2 (CH₂); 31.7 (CH₂); 33.5 (CH₂); 39.4 (CH); 183.4 (C). EI-MS (70 eV): 158 (1, M^+), 129 (5), 115 (6), 101 (8), 87 (30), 74 (100), 69 (5), 55 (11), 43 (14), 41 (17).

(2*S*)-2,7-Dimethyloctanoic Acid (**58**). Yield: 89% (32 mg, 0.19 mmol). GC (BPX5): *RI* 1337. ¹H-NMR (400 MHz, CDCl₃): 0.86 (*d*, *J*=6.6, 6 H); 1.13–1.23 (*m*, 5 H); 1.24–1.35 (*m*, 4 H); 1.39–1.56 (*m*, 2 H); 1.64–1.73 (*m*, 1 H); 2.46 (*sext.*, *J*=7.0, 1 H). ¹³C-NMR (100 MHz, CDCl₃): 16.8 (Me); 22.6 (2 Me); 27.3 (CH₂); 27.4 (CH₂); 27.9 (CH); 33.6 (CH₂); 38.8 (CH₂); 39.3 (CH); 183.0 (C). EI-MS (70 eV): 172 (1, M^+), 157 (4), 129 (21), 115 (6), 101 (4), 87 (56), 74 (100), 69 (20), 57 (16), 55 (21), 43 (25), 41 (28).

*Preparation of Methyl (2*S*)-2-Methyloctanoate ((*S*)-**28**).* Similar to the procedure of Wipf *et al.* [33], the acid **53** (258 mg, 1.63 mmol) was dissolved in an excess of dry MeOH (16 ml), and a few drops of conc. HCl were added. The mixture was stirred under reflux for 4 h, concentrated, and then filtered through SiO₂ with Et₂O as eluent. After drying (MgSO₄), all solvents were removed under vacuum to give the pure product (257 mg, 1.49 mmol, 92%). R_f (pentane/Et₂O 10:1) 0.53. GC (HP-5MS): *RI* 1161. ¹H-NMR (400 MHz, CDCl₃): 0.88 (*t*, *J*=6.9, 3 H); 1.14 (*d*, *J*=7.0, 3 H); 1.22–1.33 (*m*, 8 H); 1.36–1.45 (*m*, 1 H); 1.61–1.69 (*m*, 1 H); 2.43 (*sext.*, *J*=7.0, 1 H); 3.67 (*s*, 3 H). ¹³C-NMR (100 MHz, CDCl₃): 14.0 (Me); 17.0 (Me); 22.6 (CH₂); 27.2 (CH₂); 29.1 (CH₂); 31.7 (CH₂); 33.8 (CH₂); 39.4 (CH); 51.4 (Me); 177.4 (C). EI-MS (70 eV): EI-MS (70 eV): 172 (1, M^+), 143 (5), 141 (4), 129 (4), 115 (6), 101 (25), 88 (100), 71 (6), 57 (17), 41 (12).

*Preparation of Methyl (2*S*)-2,7-Dimethyloctanoate ((*S*)-**43**).* According to Patel *et al.* [35], compound **58** (16 mg, 0.09 mmol) was dissolved in 3.5 ml of dry CH₂Cl₂. A few drops of MeOH were added to the soln. before it was cooled to 0°. DMAP (15 mg, 0.01 mmol) and EDC were added. The mixture was stirred for 1 h under these conditions and for 1.5 h at r.t. Then, Et₂O was added, and the org. layer was successively washed with H₂O and sat. NaHCO₃ soln. The combined org. layers were dried (MgSO₄) and concentrated under vacuum. Purification on SiO₂ with pentane/Et₂O 2:1 as eluent afforded the pure product (11 mg, 0.57 mmol, 61%). R_f (pentane/Et₂O 10:1) 0.58. GC (HP-5MS): *RI* 1223. ¹H-NMR (400 MHz, CDCl₃): 0.86 (*d*, *J*=6.6, 6 H); 1.12–1.18 (*m*, 5 H); 1.24–1.35 (*m*, 4 H); 1.36–1.56 (*m*, 2 H); 1.60–1.70 (*m*, 1 H); 2.43 (*sext.*, *J*=7.0, 1 H); 3.67 (*s*, 3 H). ¹³C-NMR (100 MHz, CDCl₃): 17.1 (Me); 22.6 (2 Me); 27.3 (CH₂); 27.5 (CH₂); 27.9 (CH); 33.9 (CH₂); 38.8 (CH₂); 39.5 (CH); 51.4 (Me);

177.4 (C). EI-MS (70 eV): 186 (1, M^+), 171 (2), 155 (3), 143 (14), 129 (3), 115 (4), 101 (41), 88 (100), 69 (13), 57 (14), 55 (13), 43 (14), 41 (17).

REFERENCES

- [1] T. Kaneda, *Microbiol. Rev.* **1991**, 55, 288.
- [2] S. Schulz, J. S. Dickschat, *Nat. Prod. Rep.* **2007**, 24, 814.
- [3] J. S. Dickschat, H. B. Bode, S. C. Wenzel, R. Müller, S. Schulz, *ChemBioChem* **2005**, 6, 2023.
- [4] C. E. G. Schöller, H. Gürtler, R. Pedersen, S. Molin, K. Wilkins, *J. Agric. Food Chem.* **2002**, 50, 2615.
- [5] R. Coda, E. Brechany, M. De Angelis, S. De Candia, R. Di Cagno, M. Gobbetti, *J. Dairy Sci.* **2006**, 89, 4126.
- [6] K. Gerth, H. Steinmetz, G. Höfle, to Helmholtz-Zentrum für Infektionsforschung, European Patent Application (09/103534), 2009.
- [7] B. Maudinas, J. Villoutreix, *Phytochemistry* **1977**, 16, 1299.
- [8] A. Sawabe, Y. Matsuda, H. Toyoda, K. Maeda, T. Nonomura, N. Shimizu, N. Fujita, S. Ouchi, *J. Oleo Sci.* **2003**, 52, 171.
- [9] M. D. Forero, C. E. Quijano, J. A. Pino, *Flavour Fragr. J.* **2009**, 24, 25.
- [10] H. Tsuji, A. Mizuno, *J. Food Sci.* **2010**, 75, C79.
- [11] W. Boland, P. Ney, L. Jaenicke, G. Gassmann, in 'Analysis of Volatiles', Ed. P. Schreier, de Gruyter, Berlin, 1984, p. 371.
- [12] J. S. Dickschat, T. Martens, T. Brinkhoff, M. Simon, S. Schulz, *Chem. Biodiversity* **2005**, 2, 837.
- [13] T. Nawrath, K. Gerth, R. Müller, S. Schulz, *ChemBioChem* **2010**, in press, doi: 10.1002/cbic.201000296.
- [14] H. Budzikiewicz, C. Djerassi, D. H. Williams, 'Mass spectrometry of organic compounds', Holden-Day, San Francisco, 1967.
- [15] N. M. Carballreira, F. Shalabi, C. Cruz, *Tetrahedron Lett.* **1994**, 35, 5575.
- [16] B. A. Leonhardt, E. D. DeVilbiss, *J. Chromatogr., A* **1985**, 322, 484.
- [17] V. Thiel, B. Kunze, P. Verma, I. Wagner-Döbler, S. Schulz, *ChemBioChem* **2009**, 10, 1861.
- [18] F. W. McLafferty, F. Tureček, 'Interpretation of mass spectra', University Science Books, Sausalito, 1993.
- [19] C. C. van de Sande, C. DeMeyer, A. Maquestiau, *Bull. Soc. Chim. Belges* **1976**, 85, 79.
- [20] H. van den Dool, P. D. Kraatz, *J. Chromatogr., A* **1963**, 11, 463.
- [21] S. Schulz, *Lipids* **2001**, 36, 637.
- [22] J. S. Dickschat, S. C. Wenzel, H. B. Bode, R. Müller, S. Schulz, *ChemBioChem* **2004**, 5, 778.
- [23] J. S. Dickschat, E. Helmke, S. Schulz, *Chem. Biodiversity* **2005**, 2, 318.
- [24] J. S. Dickschat, H. B. Bode, R. M. Kroppenstedt, R. Müller, S. Schulz, *Org. Biomol. Chem.* **2005**, 3, 2824.
- [25] S. Schulz, unpublished results.
- [26] R. Ryhage, E. Stenhagen, *Ark. Kemi* **1960**, 15, 291.
- [27] J. S. Dickschat, T. Nawrath, V. Thiel, B. Kunze, R. Müller, S. Schulz, *Angew. Chem., Int. Ed.* **2007**, 46, 8287.
- [28] G. Suzukamo, M. Fukao, M. Tamura, *Tetrahedron Lett.* **1984**, 25, 1595.
- [29] A. Fürstner, J. Ruiz-Caro, H. Prinz, H. Waldmann, *J. Org. Chem.* **2004**, 69, 459.
- [30] E. J. Corey, J. W. Suggs, *Tetrahedron Lett.* **1975**, 16, 2647.
- [31] J. R. Gage, D. A. Evans, *Org. Synth.* **1990**, 68, 83.
- [32] A. H. G. Siebum, W. S. Woo, J. Lugtenburg, *Eur. J. Org. Chem.* **2003**, 4664.
- [33] P. Wipf, Y. Uto, S. Yoshimura, *Chem.-Eur. J.* **2002**, 8, 1670.
- [34] G. O. Spessard, W. K. Chan, S. Masamune, *Org. Synth.* **1983**, 61, 134.
- [35] J. Patel, J. C. Hoyt, R. J. Parry, *Tetrahedron* **1998**, 54, 15927.
- [36] T. Kaneda, *Bacteriol. Rev.* **1977**, 41, 391.
- [37] H. B. Bode, J. S. Dickschat, R. M. Kroppenstedt, S. Schulz, R. Müller, *J. Am. Chem. Soc.* **2005**, 127, 532.

- [38] T. Ramakrishnan, P. S. Murthy, K. P. Gopinathan, *Bacteriol. Rev.* **1972**, 36, 65.
- [39] D. L. Rainwater, P. E. Kolattukudy, *J. Biol. Chem.* **1985**, 260, 616.
- [40] P. M. Dewick, 'Medicinal Natural Products: A Biosynthetic Approach', 3rd edn., John Wiley & Sons, Chichester, 2009.
- [41] Y. Akamatsu, J. H. Law, *J. Biol. Chem.* **1970**, 245, 709.
- [42] I. Müller, S. Weinig, H. Steinmetz, B. Kunze, S. Veluthoor, T. Mahmud, R. Müller, *ChemBioChem* **2006**, 7, 1197.
- [43] L. E. Eiden, R. T. Borchart, C. O. Rutledge, *J. Neurochem.* **1982**, 38, 631.
- [44] J. Fourche, M. Capdepuy, J. Texier-Maugein, *J. Chromatogr., B.* **1989**, 487, 142.
- [45] V. S. Dubey, T. D. Sirakova, M. H. Cynamon, P. E. Kolattukudy, *J. Bacteriol.* **2003**, 185, 4620.
- [46] B. Flachsbarth, M. Fritzsche, P. J. Weldon, S. Schulz, *Chem. Biodiversity* **2009**, 6, 1.
- [47] A. Friese, K. Hell-Momeni, I. Zündorf, T. Winckler, T. Dingermann, G. Dannhardt, *J. Med. Chem.* **2002**, 45, 1535.

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