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Pheromone synthesis. Part 255: Synthesis and GC–MS analysis of pheromonal triacylglycerols of male *Drosophila* fruit flies☆

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ABSTRACT

Pheromonal triacylglycerols and their analogs, **1A**, **1B**, **2A**, **2B**, **3A**, **3B**, and **3C**, of male *Drosophila* fruit flies were synthesized and analyzed by GC–MS. Their GC retention times were found to be a reliable measure to analyze and identify these triacylglycerols with acetyl, oleoyl and tigloyl groups, although the stereo- and regioisomers of **1** (**1A** and **1B**), **2** (**2A** and **2B**), and **3** (**3A**, **3B**, and **3C**) could not be distinguished from each other by MS alone.

1. Introduction

Triacylglycerols (TAGs) are naturally occurring and abundant tricarboxylic esters of glycerol. Although triacylglycerol **I** (Fig. 1) with the same three acyl groups as well as another symmetrical one **II** are achiral, triacylglycerols such as **III** and **IV** are dissymmetric and chiral. Synthesis of enantiopure triacylglycerols is a long-standing challenge to chemists,[2] and several new chemical[3–6] and enzymatic[7] approaches have been reported recently together with development of reliable methods for determination of their enantiomeric purity.

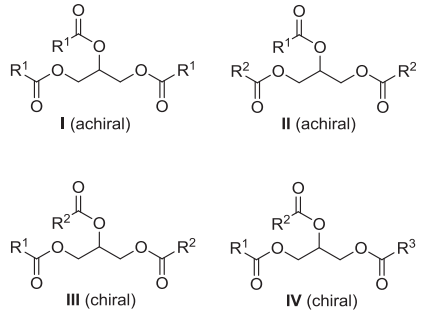

Fig. 1. Four structural types **I**–**IV** of triacylglycerols.

In 2011 Yew et al. demonstrated the presence of triacylglycerols in cuticles of two species of *Drosophila* fruit flies, *Drosophila arizonae* and *Drosophila mojavensis*.[8] The structures of some of the triacylglycerols were then proposed (Figs. 2 and 3), which were new and unusual to be comprised of zero or one acetic acid, two or one tiglic acid(s), and a fatty acid.[9] These triacylglycerols are secreted by male flies from the ejaculatory bulb, transferred to females during mating, and inhibit courtship from other males. Accordingly, *Drosophila* triacylglycerols are a novel class of pheromones.[9] Mori's synthetic triacylglycerols including (*R*)- and (*S*)-1,2-ditigloyl-3-oleoylglycerol (**1A**)[3] were bioassayed against *Drosophila* fruit flies, and (*R*)-**1A** was found to suppress copulation of the flies at a dosage of 75 ng per female, while (*S*)-**1A** was inactive.[9] This is another example to show the important role of chirality in chemical communications.[10,11]

However, there was an ambiguity with regard to the proposed structure **1A** of the natural triacylglycerol as pointed out by Francke in the course of the reviewing process of Ref. 9. The structure **1A** was proposed totally basing upon the mass spectroscopic (MS) analysis of the natural product. Is it possible to discriminate **1A** from **1B** on the basis of the MS data alone? Due to the scarcity of the natural triacylglycerol, none of its IR, ^{1}H and ^{13}C NMR data was available. Of course ^{13}C NMR analysis would be an appropriate tool to discriminate **1A** from **1B**, because the latter is a symmetrical

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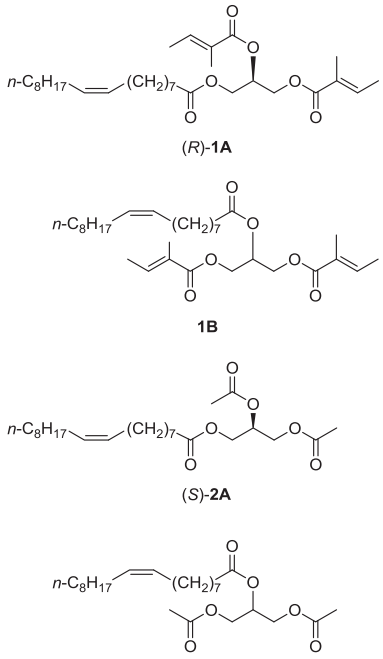


Fig. 2. Two candidate structures (*R*)-**1A** and **1B** of the triacylglycerol pheromone of male *Drosophila* fruit flies. Synthetic (*R*)-**1A**[3] suppresses the fly copulation at a dose of 75 ng per female, while (*S*)-**1A** is inactive.[9] Structures **2A** and **2B** of two non-natural analogs are also shown.

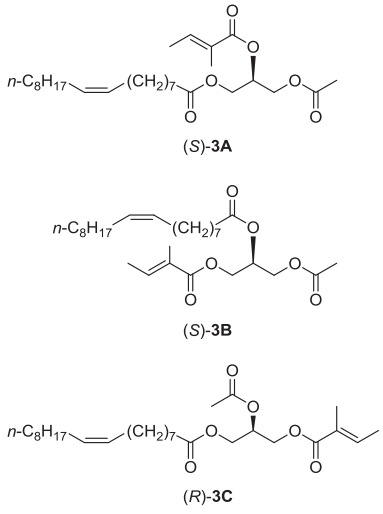


Fig. 3. Three candidate structures (*S*)-**3A**, (*S*)-**3B**, and (*R*)-**3C** for a *Drosophila* triacylglycerol with an acetyl, an oleoyl and a tigloyl groups. Their opposite enantiomers are also the candidates.

molecule. It therefore became necessary to synthesize **1B**, and find out a method to distinguish **1A** from **1B**. For the purpose of analyzing a similar case, non-natural diacetyloleoylglycerols, (*R*)- and (*S*)-**2A** and **2B**, were also chosen as the synthetic targets.

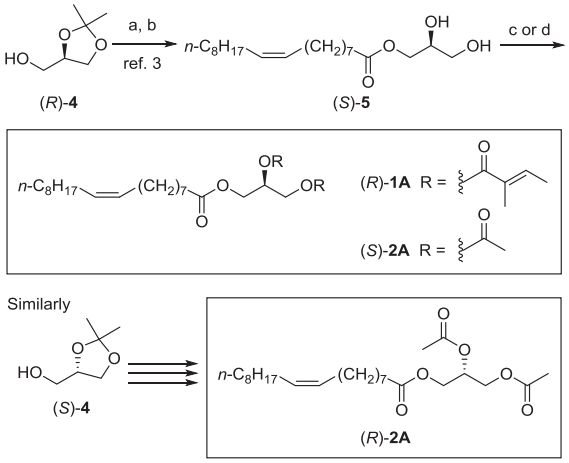
Another objective of the present work was to determine the substitution pattern of the *Drosophila* triacylglycerol(s) of type **IV** (Fig. 1) with an acetyl, an oleoyl and a tigloyl groups. Six isomers are possible for that triacylglycerol(s) of type **IV** as shown in Fig. 3: (*S*)-**3A**, (*S*)-**3B** and (*R*)-**3C** as well as their opposite enantiomers. It was therefore planned to synthesize and analyze all of them to find out a method for establishing (at least) the substitution pattern of the natural triacylglycerol(s) of type **IV**. Since the biological role of the type **IV** triacylglycerol is not yet known precisely, it is important to provide the enantiomers of **3A**, **3B**, and **3C** for bioassay.

The present paper describes in detail the synthesis and analysis of *Drosophila* triacylglycerols, and recommends the GC–MS analysis for their identification.[12]

2. Results and discussion

2.1. Synthesis of triacylglycerols 1A and 2A

Synthesis of the pheromonally active (*R*)-1,2-ditigloyl-3-oleoylglycerol (**1A**) was executed by the published method[3] as shown in Scheme 1. Acylation of (*R*)-2,3-acetoneglycerol (**4**) with oleoyl chloride was followed by deprotection with 80% acetic acid at 50 °C for 2 h to give (*S*)-1-oleoylglycerol (**5**), whose enantiomeric purity was determined as >98% ee by the ^{1}H NMR analysis of the corresponding bis-(*R*)-α-methoxy-α-trifluoromethylphenylacetic (MTPA) ester.[3]



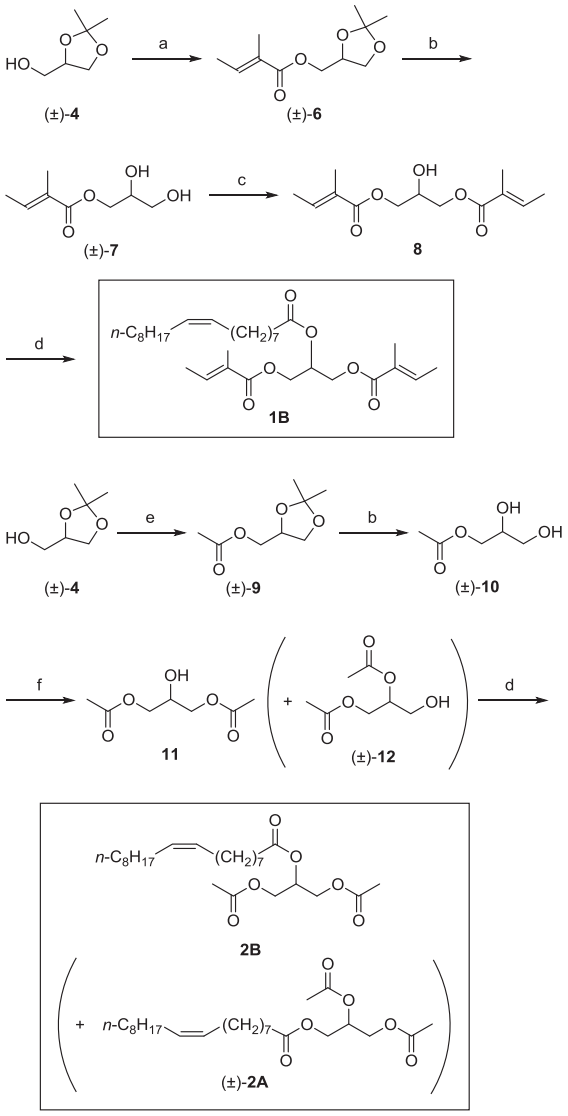
Scheme 1. Synthesis of triacylglycerols (*R*)-**1A**, (*R*)-**2A**, and (*S*)-**2A**. Reagents: (a) oleoyl chloride, DMAP, C_5H_5N, C_6H_6 [94% for (*R*)-isomer; 96% for (*S*)-isomer]; (b) 80% AcOH, 50 °C, 2 h [94% for (*R*)-**5**; 99% for (*S*)-**5**]; (c) tigloyl chloride, DMAP, C_5H_5N, C_6H_6 [76% for (*R*)-**1A**]; (d) acetyl chloride, C_5H_5N [80% for (*R*)-**2A**]; acetic anhydride, DMAP, C_5H_5N [83% for (*S*)-**2A**].

Acylation of (*S*)-**5** with excess tigloyl chloride afforded the known (*R*)-**1A**, $[\alpha]_D^{21}$ +4.11 (*c* 4.13, hexane), in 67% overall yield based on (*R*)-**4** (three steps). Acylation of (*S*)-**5** with excess acetyl chloride furnished (*S*)-1,2-diacetyl-3-oleoylglycerol (**2A**), $[\alpha]_D^{23}$ −0.88 (*c* 4.19, hexane). Similarly, (*S*)-**4** was converted to (*R*)-**2A**, $[\alpha]_D^{24}$ +1.33 (*c* 4.02, hexane). The overall yield of (*R*)-**2A** and that of (*S*)-**2A** were 71% and 78%, respectively, based on (*S*)-**4** and (*R*)-**4** (three steps).

2.2. Synthesis of symmetrical triacylglycerols 1B and 2B

Scheme 2 summarizes the synthesis of two symmetrical triacylglycerols, 1,3-ditigloyl-2-oleoylglycerol (**1B**) and 1,3-diacetyl-2-oleoylglycerol (**2B**). Although pure **1B** could be obtained by the depicted synthetic scheme, **2B** could not be secured in pure state.

Synthesis of **1B** started from (±)-2,3-acetoneglycerol (**4**). Acylation of (±)-**4** with tigloyl chloride gave (±)-**6**, which was stirred with 80% acetic acid and heated at 130 °C for 10 min. The mixture was concentrated in vacuo, and the residue was diluted with toluene. The solution of (±)-**7** in toluene was again concentrated in vacuo to give (±)-**7** as a viscous oil. Selective mono-acylation of (±)-**7** to give 1,3-ditigloylglycerol (**8**) was executed by treatment with an equivalent amount of tigloyl chloride in the presence of di(*n*-butyl)tin oxide and triethylamine in CH_2Cl_2 under the



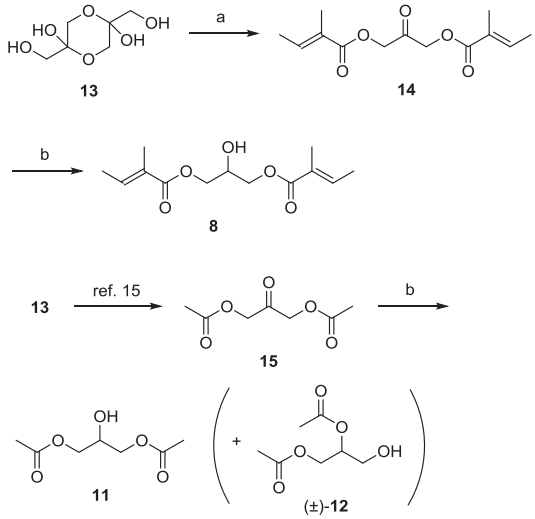
Scheme 2. Synthesis of triacylglycerols **1B** and **2B**. Reagents: (a) tigloyl chloride, DMAP, C_5H_5N, C_6H_6 (quant.); (b) 80% AcOH, 130 °C, 10 min [quant. for (±)-**7**; 97% for (±)-**10**]; (c) $(n\text{-Bu})_2SnO$, Et_3N, tigloyl chloride, CH_2Cl_2 (70%); (d) oleoyl chloride, DMAP, C_5H_5N, C_6H_6 [77% for **1B**; 87% for **2B**+(±)-**2A**]; (e) acetyl chloride, DMAP, C_5H_5N, C_6H_6 (quant.); (f) $(n\text{-Bu})_2SnO$, Et_3N, acetyl chloride, CH_2Cl_2 (86%).

conditions reported by Martinelli et al. for the selective monotosylation of 1,2-diols.[13] Regioselective alkylation and esterification of a specific hydroxyl group via organotin intermediates are well established methods as reviewed by David and Hanessian.[14] In the present case, (±)-**7** afforded **8** (98.8% purity by GC–MS analysis) in 70% yield. Acylation of **8** with oleoyl chloride afforded **1B** in 77% yield. The structure **1B** assigned to the product was consistent with its ^{13}C NMR spectrum, in which only two signals (δ 167.4 and 172.9) for *C*=O carbons and four signals (δ 128.0, 129.7, 130.0, 138.2) for *C*=*C* carbons could be observed owing to its molecular symmetry. The overall yield of **1B** was 54% based on (±)-**4** (four steps).

Similarly, acetylation of (±)-**4** gave (±)-**9**, which was deprotected with hot 80% acetic acid to furnish (±)-1-acetylglycerol (**10**). Its di(*n*-butyl)tin oxide-catalyzed monoacetylation, however, gave a 65:35 mixture of the desired **11** and unwanted (±)-**12** instead of pure **11**. It must be due to the extremely facile acetyl migration from C-1 to C-2. Since **11** could not be separated from (±)-**12** by conventional silica gel chromatography, the mixture was acylated with oleoyl chloride to give a 2:1 mixture of the desired **2B** and undesired (±)-**2A**. The minor component was identified as (±)-**2A** by GC–MS comparison with (*R*)-**2A**. It is to be noted that tigloyl migration in the case of 1,3-ditigloylglycerol (**8**) is a far less facile process.

2.3. Alternative synthesis of 1,3-diacylglycerols 8 and 11

An alternative synthetic route for 1,3-diacylglycerols **8** and **11** was also explored as shown in Scheme 3. Commercially available 1,3-dihydroxyacetone dimer (**13**) in pyridine was treated with excess tigloyl chloride to give crystalline 1,3-ditigloyloxyacetone (**14**), mp 46.0–46.5 °C, in 91% yield after chromatographic purification. Reduction of **14** with zinc borohydride in THF furnished 1,3-ditigloylglycerol (**8**) in quantitative yield. The above preparative method is superior to the previous one [(±)-**4**→**8**] in view of the better yield, shorter steps, and less complicated experimental procedures.



Scheme 3. Alternative synthesis of **8** and **11**. Reagents: (a) tigloyl chloride, C_5H_5N, C_6H_6 (91%); (b) $Zn(BH_4)_2$, THF [quant. for **8**; 61% for a 2:1 mixture of **11** and (±)-**12**].

When the above synthetic method was used for the preparation of **11**, it resulted in failure, although a successful case was recorded in 1986.[15] Acetylation of **13** with acetic anhydride and pyridine gave crude **15** as a colorless solid, which was recrystallized from EtOAc/hexane to give **15** as needles in 75% yield.[15,16] Reduction of **15** with zinc borohydride [prepared from sodium borohydride (washed with EtOAc to remove alkaline impurities)[16] and zinc chloride in THF] unfortunately afforded a 2:1 mixture of **11** and (±)-**12** in 61% yield. Extremely facile acetyl migration in the course of the reduction could not be avoided in my hands. In the successful case reported in 1986, zinc borohydride in Et_2O was employed as the reducing agent.[15] Acylation of the resulting mixture of **11** and (±)-**12** with oleoyl chloride furnished a mixture of **2B** and (±)-**2A**. Unfortunately, therefore, pure **2B** could not be secured.

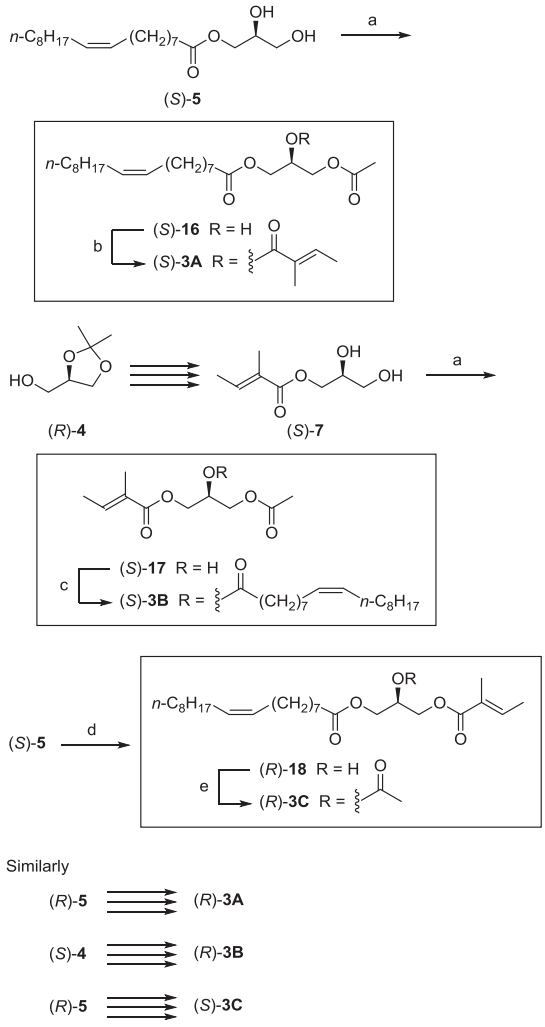
2.4. Synthesis of the enantiomers of chiral triacylglycerols 3A, 3B, and 3C

The synthetic plan for these chiral triacylglycerols **3A**, **3B**, and **3C** was to employ the di(*n*-butyl)tin oxide-catalyzed terminal acylation of monoacylglycerols **5** and **7**. However, the facile acyl migration in the course of the final acylation at C-2 might cause severe decrease in the purity of the desired products **3A**, **3B**, and **3C**.

Notwithstanding the above problem, the synthesis of (*S*)-**3A** started from (*S*)-**5**. Treatment of (*S*)-**5** with acetyl chloride in the presence of di(*n*-butyl)tin oxide and triethylamine in CH_2Cl_2[13] was followed by chromatographic purification to give (*S*)-**16** (90.1% pure

by GC–MS analysis) in 82% yield. Acylation of (*S*)-**16** with tigloyl chloride in the presence of 4-dimethylaminopyridine (DMAP) and pyridine unfortunately caused extensive acyl migration. The product was a mixture of (*S*)-**3A** (62.3%), (*R*)-**3C** (15.6%; generated by 1→2 acetyl migration), and (*S*)-**3B** (11.1%; generated by 1→2 oleoyl migration). Fortunately, the desired (*S*)-**3A** was the major component, and the product could be used successfully as a reference sample for the subsequent analytical works.

(*R*)-1,2-Acetoneglycerol (**4**) was the starting material for the synthesis of (*S*)-**3B**. As described in Section 2.2 for the conversion of (±)-**4** to (±)-**7**, (*R*)-**4** was converted to (*S*)-1-tigloylglycerol (**7**) via (*S*)-**6**. The enantiomeric purity of (*S*)-**7**, $[\alpha]_D^{22}$ −2.28 (*c* 4.50, $CHCl_3$), was 90% ee as determined by Mosher's MTPA/NMR method.[3] Acetylation of (*S*)-**7** with acetyl chloride in the presence of di(*n*-butyl)tin oxide and triethylamine in CH_2Cl_2 afforded (*S*)-**17**, which was 99.5% pure by its GC–MS analysis. Treatment of (*S*)-**17** with oleoyl chloride in the presence of DMAP and pyridine gave a mixture of triacylglycerols, whose major component was desired (*S*)-**3B** (62.6%) contaminated with 26.6% of (*S*)-**3C**, which was generated by 1→2 acetyl migration prior to the oleoylation (Scheme 4).



Scheme 4. Synthesis of triacylglycerols **3A**, **3B**, and **3C**. Reagents: (a) $(n\text{-Bu})_2SnO$, Et_3N, acetyl chloride, CH_2Cl_2 [73% for (*S*)-**16**; 71% for (*R*)-**16**; 70% for (*S*)-**17**; 71% for (*R*)-**17**]; (b) tigloyl chloride, DMAP, C_5H_5N, C_6H_6 [71% for (*S*)-**3A**; 78% for (*R*)-**3A**]; (c) oleoyl chloride, DMAP, C_5H_5N, C_6H_6 [91% for (*S*)-**3B**, 82% for (*R*)-**3B**]; (d) $(n\text{-Bu})_2SnO$, Et_3N, tigloyl chloride, CH_2Cl_2 [99% for (*R*)-**18**; quant. for (*S*)-**18**]; (e) acetyl chloride, DMAP, C_5H_5N, C_6H_6 [83% for (*R*)-**3C**; 73% for (*S*)-**3C**].

For the preparation of (*R*)-**3C**, (*S*)-**5** was treated with tigloyl chloride in the presence of di(*n*-butyl)tin oxide and triethylamine in CH_2Cl_2 to give (*R*)-**18** (99.1% pure as determined by GC–MS analysis). Acetylation of (*R*)-**18** with acetyl chloride and DMAP in pyridine afforded (*R*)-**3C** (71.5% pure). The by-products were (*S*)-**3A** (13.7%; generated by 1→2 tigloyl migration) and (*R*)-**3B** (4.9%; generated by 1→2 oleoyl migration).

Similarly, (*R*)-**5** was converted to (*R*)-**3A** [79.6% pure by GC–MS; contaminated with (*R*)-**3B** (3.2%) and (*S*)-**3C** (7.7%)]. (*S*)-1-Oleoylglycerol (**5**) was converted to (*R*)-**3B** [64.3% pure by GC–MS; contaminated with (*R*)-**3C** (28.8%)], and (*R*)-**5** was converted to (*S*)-**3C** [74.9% pure by GC–MS; contaminated with (*R*)-**3A** (5.7%) and (*S*)-**3B** (2.9%).

2.5. Comments on the acylation of 1-acyl- and 1,3-diacylglycerols

2.5.1. Selective acylation is possible at C-3 of 1-acylglycerol. 1-Acylglycerols could be acylated selectively at C-3 by treatment with acyl chloride in the presence of di(*n*-butyl)tin oxide and triethylamine to give 1,3-diacylglycerols. As described in 2.4, 1,3-diacylglycerols (*S*)-**16**, (*S*)-**17** and (*R*)-**18** could be secured in 90.1%, 99.5% and 99.1% purity, respectively, by the selective acylation at C-3. Martinelli's di(*n*-butyl)tin oxide-catalyzed acylation[13] is an excellent method for the preparation of 1,3-diacylglycerols.

2.5.2. 1→2 Acyl migration prevents the synthesis of pure triacylglycerols. Acylation at C-2 of 1,3-diacylglycerols **16**, **17** and **18** ought to give triacylglycerols **3A**, **3B**, and **3C**, respectively, with newly introduced acyl group at C-2, if there is no acyl migration prior to the acylation reaction. When the acylation was actually carried out, the results were surprising and disappointing after GC–MS analysis of the products. As shown in Table 1, the generated **3A**, **3B**, and **3C** were not pure.

Fortunately, the expected products were the major components in each case. But cross-contamination with other triacylglycerols took place. Prior to the acylation with the respective acyl chloride, the starting 1,3-diacylglycerol suffered from 1→2 (or 3→2) acyl migration to give 1,2-diacylglycerols, whose subsequent acylation yielded undesired triacylglycerol(s).

The results listed in Table 1 indicate that the ease of migration is acetyl>tigloyl>oleoyl. The acetyl group was so easily transferred to C-2 that in the case of the oleoylation of 1-acetyl-3-tigloylglycerol (**17**), no tigloyl-migrated product **3A** could be detected as can be seen from (B). It is also to be noted that the tigloyl group is more apt to migrate than the oleoyl group as seen from the results in (C).

Through the acylation experiments, an important lesson was learned that the acyl migration prevents the synthesis of pure triacylglycerols from 1,3-diacylglycerols. Fig. 4 shows a possible mechanism for the acyl migration, giving a mixture of **3a**, **3b**, and **3c** instead of pure **3a**.

2.6. Spectroscopic comparison of the synthetic samples of *Drosophila* triacyglycerols

It was previously reported that (*R*)-**1A** was pheromonally active against male *Drosophila* fruit flies.[9] However, there was no strong reason to exclude **1B** as the correct structure of the natural pheromone. Various spectra of (*R*)-**1A** and**1B** were therefore compared whether the two isomers exhibit different spectral properties or not.

The IR spectrum of (*R*)-**1A** was almost identical with and indistinguishable from that of **1B**. The ^1H NMR spectrum of (*R*)-**1A** was slightly different from that of **1B**, because the molecular symmetry of **1B** made both the $CH_3C{=}C$ and OCH_2 signals simpler than the corresponding signals of (*R*)-**1A**. Differences were more evident in the ^{13}C NMR spectra of (*R*)-**1A** and **1B**. Two signals (δ 62.2 and 62.3) were observed for the CH_2O carbons of (*R*)-**1A**, while only

Table 1
Migration of the acyl group at position 1 or 3 in the course of the acylation at position 2 of 1,3-diacylglycerols **16**, **17** and **18** to give triacylglycerols **3A**, **3B**, and **3C**, respectively. A=CH_3CO–; OL=(*Z*)-*n*-$C_8H_{17}CH{=}CH(CH_2)_7CO$–; T=(*E*)-$CH_3CH{=}C(CH_3)CO$–

	Starting 1,3-diacylglycerol	Acylating agent	Desired product	Ratio (%) of the generated triacylglycerols* **3A** t_R = 21.30 min	**3B** t_R = 21.41 min	**3C** t_R = 21.54 min
(A)	(*R*)-**16**	TCl	(*R*)-**3A**	88.0	3.5	8.5
	(*S*)-**16**	TCl	(*S*)-**3A**	77.0	4.9	18.1
(B)	(*R*)-**17**	OLCl	(*R*)-**3B**	-	69.1	30.9
	(*S*)-**17**	OLCl	(*S*)-**3B**	-	70.2	29.8
(C)	(*R*)-**18**	ACl	(*R*)-**3C**	15.2	5.4	79.4
	(*S*)-**18**	ACl	(*S*)-**3C**	6.8	3.5	89.7

*listed in the increasing order of GC-MS retention times (t_R).

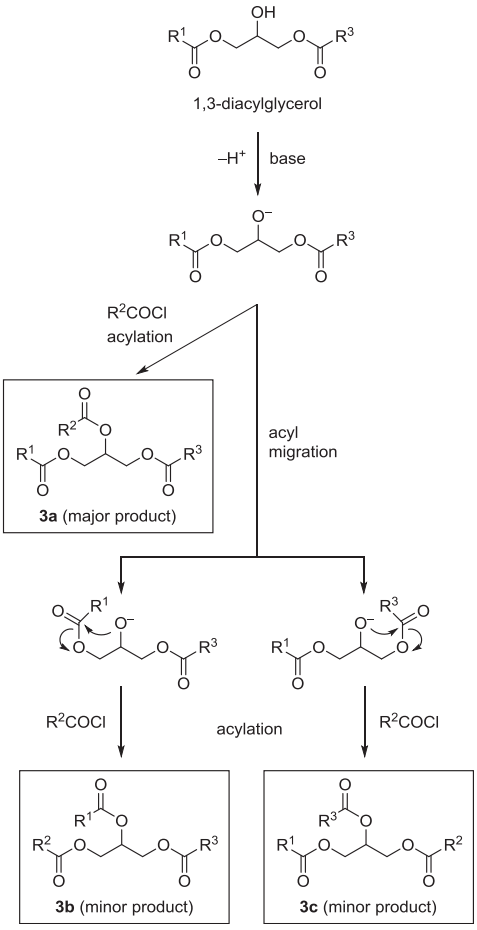


Fig. 4. Acyl migration in the course of the acylation of a 1,3-diacylglycerol prevents the formation of the pure triacylglycerol **3a** and gives a mixture of **3a**, **3b**, and **3c**.

a single signal (δ 62.5) was observed for the CH_2O carbons of **1B** due to its symmetrical structure. Similarly, six *C*=*C* signals and three *C*=O signals were observed for (*R*)-**1A**, while **1B** exhibited only four *C*=*C* signals and two *C*=O signals due to its molecular symmetry. However, these NMR differences were of no use in the present case, because the *Drosophila* triacylglycerols were detected and analyzed by MS alone, and their pure samples were unavailable in an amount sufficient for ^{13}C NMR measurements.

It was therefore of utmost importance to compare the mass spectrum of (*R*)-**1A** with that of **1B**. As shown in Fig. 5, the mass spectrum of (*R*)-**1A** was almost identical with and indistinguishable from that of **1B**. This result annoyed me, because it meant that all of the spectroscopic methods were inappropriate for the identification of the nanogram amounts of the *Drosophila* triacylglycerols.

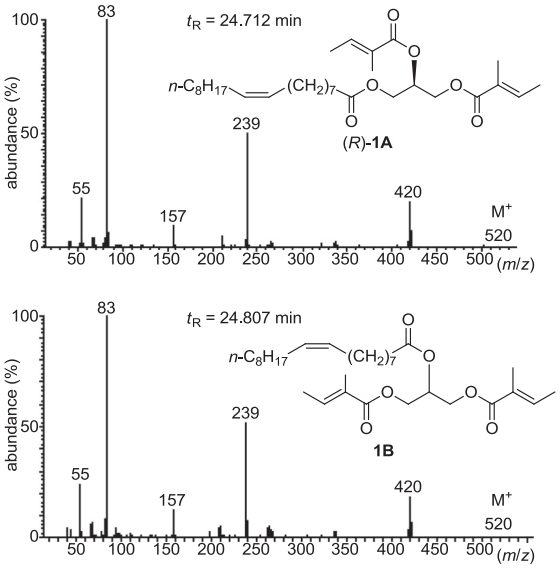


Fig. 5. Mass spectra (EI, 70 eV) of (*R*)-**1A** and **1B**. They are almost the same.

The mass spectra of (*S*)-**3A**, (*R*)-**3B**, and (*R*)-**3C** were also almost identical to each other as shown in Fig. 6, and could not be used for the identification of each of them. It must be added that their IR and NMR data were also identical, and useless for the purpose of identification.

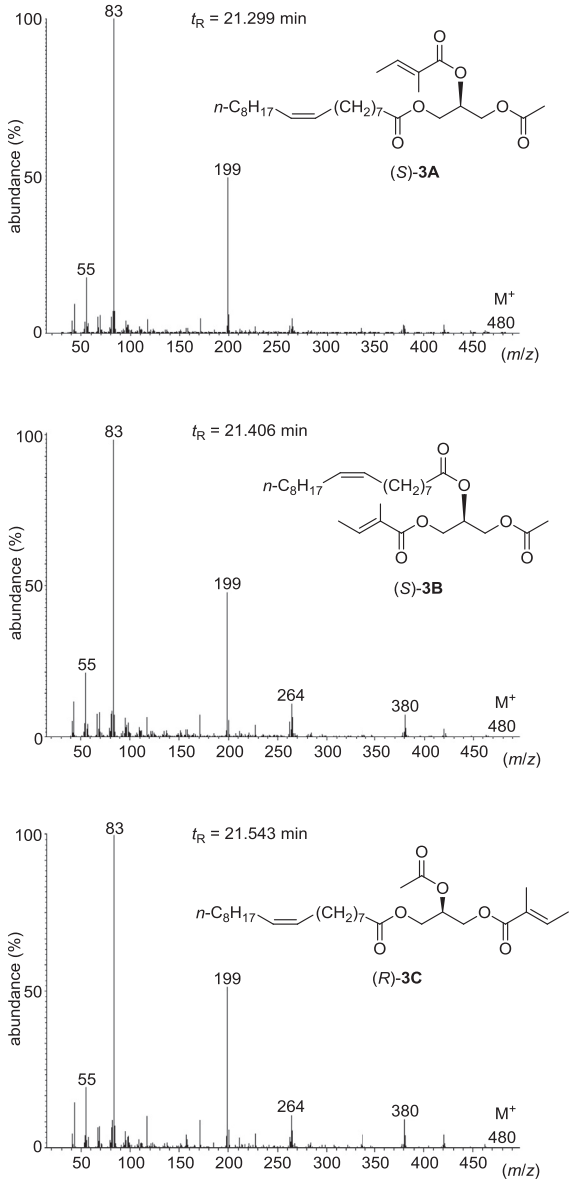


Fig. 6. Mass spectra (EI, 70 eV) of (*S*)-**3A**, (*S*)-**3B**, and (*R*)-**3C**. They are very similar to each other.

2.7. Chromatographic analysis of the *Drosophila* triacylglycerols

At this stage it seemed hopeless to discriminate (*R*)-**1A** from **1B**, because their mass spectra were almost the same. Their GC retention times were not so much different either [t_R=24.7 min for (*R*)-**1A** and t_R=24.8 min for **1B**; see Fig. 5], when they were analyzed separately. Even with these very small difference in their retention times, GC co-injection experiment of a 1:2 mixture of (*R*)-**1A** and **1B** was attempted, because in the case of GC–MS analysis of a 1:2 mixture of **2A** and **2B**, a very small difference (0.035 min) in their retention times could be detected as two partially resolved peaks.

Fig. 7(a) shows the chromatogram obtained by the co-injection experiment for (*R*)-**1A** and **1B**. They were cleanly separable: t_R=24.6 min for (*R*)-**1A** and 24.8 min for **1B**. Accordingly, identification of the *Drosophila* pheromone must be possible by carrying out GC (or LC)-MS co-injection experiments with the synthetic samples (*R*)-**1A** and **1B**.

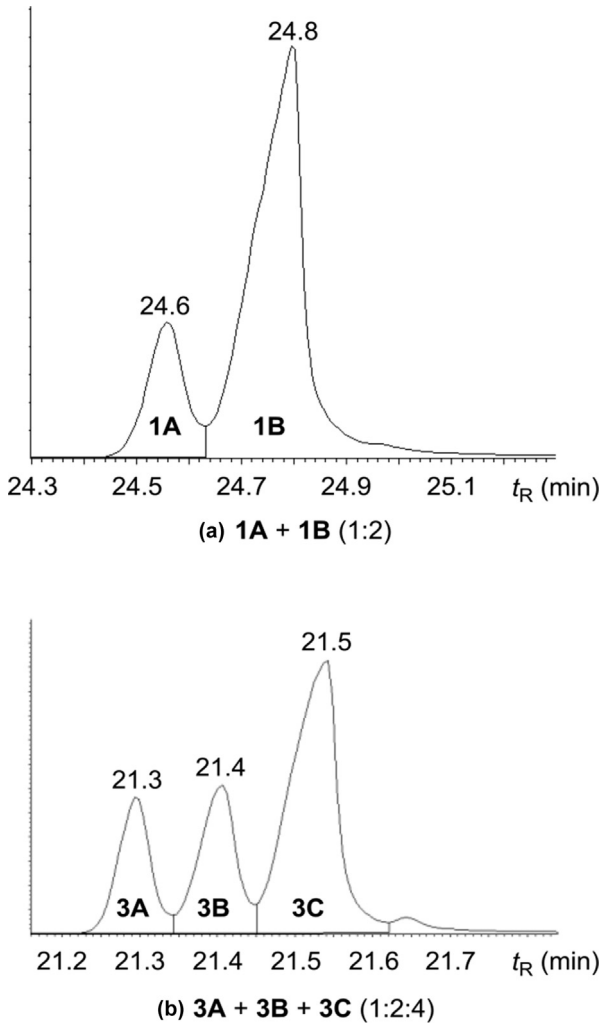


Fig. 7. GC separation of (a) a 1:2 mixture of (*R*)-**1A** and **1B** and (b) a 1:2:4 mixture of (*S*)-**3A**, (*S*)-**3B**, and (*R*)-**3C**. Instrument: Agilent 5975 inert XL; Column: HP-5MS, 5% phenylmethylsiloxane, 30 m×0.25 mm i.d.

As shown in Fig. 7 (b), a 1:2:4 mixture of the synthetic (*S*)-**3A**, (*S*)-**3B**, and (*R*)-**3C** was also separable by GC–MS:t_R=21.3 min for (*S*)-**3A**, 21.4 min for (*S*)-**3B** and 21.6 min for (*R*)-**3C**. The *Drosophila* triacylglycerols with an acetyl, an oleoyl and a tigloyl group can therefore be identified by GC (or LC)–MS co-injection experiments, although their absolute configuration remains unknown. A suitable chiral stationary phase for the enantiomer identification must be found out.

HPLC–MS methods are known to be useful in determining the ratio of positional isomers of triacylglycerols in vegetable oils.[17–19] These HPLC–MS methods were also examined for the analysis of *Drosophila* triacylglycerols. Separation was possible only in the case of (*R*)-**1A** and **1B** by employing the method of Nagai et al.[19] Neither a mixture of **2A** and **2B** nor a mixture of **3A**, **3B**, and **3C** could be separated. Therefore, in the case of the *Drosophila* triacylglycerols, analysis by GC–MS is preferable to HPLC–MS analysis.

3. Conclusion

Synthesis of 1,3-diacylglycerols was achieved by selective acylation of 1-acylglycerols with acyl chlorides in the presence of di(*n*-butyl)tin oxide and triethylamine. Acylation of 1,3-diacylglycerols with different acyl chlorides in the presence of DMAP and pyridine was found to be accompanied with acyl migration of the pre-existing acyl group(s), and therefore the resulting triacylglycerols were mixtures of positional isomers, whose major component was the desired one.

Preliminary bioassay by Yew et al. of (*R*)-**1A** and **1B** showed the former to be far more active than the latter as the *Drosophila* pheromone. The final identification of the natural triacylglycerols as well as the bioassay of all of the synthetic samples will be reported by Yew and her co-workers in due course.

4. Experimental

4.1. General

Melting points are uncorrected values. Refractive indices (n_D) were measured on an Atago DMT-1 refractometer. Optical rotations were measured on a Jasco P-1020 polarimeter. IR spectra were measured on a Jasco FT/IR-410 spectrometer. ^{1}H NMR spectra (400 MHz, TMS at δ=0.00 as internal standard) and ^{13}C NMR spectra (100 MHz, $CDCl_3$ at δ=77.0 as internal standard) were recorded on a Jeol JNM-AL 400 spectrometer. GC–MS were measured on Agilent Technologies 5975 inert XL. HRMS were recorded on Jeol JMS-SX 102A or Varian 901-MS. Column chromatography was carried out on Merck Kieselgel 60 Art 1.07734.

4.2. (*R*)-1,2-Ditigloyl-3-oleoylglycerol (1A)

This was prepared from enantiomerically pure (*S*)-**5** as previously reported,[3] and obtained as an oil, n_D^{17}=1.4750; $[\alpha]_D^{21}$ +4.11 (*c* 4.13, hexane); GC–MS [column: HP-5MS, 5% phenylmethylsiloxane, 30 m×0.25 mm i.d.; carrier gas, He; press: 52.8 kPa, temp 50 °C (2 min), then +15 °C/min, 300 °C (60 min)]: t_R 24.98 min (92.1%). Its IR, ^{1}H and ^{13}C NMR, and MS spectral data were identical to those reported previously.[3]

4.3. 1,2-Diacetyl-3-oleoylglycerol (2A)

4.3.1. (R)-Isomer. Acetyl chloride (785 mg, 10 mmol) was added dropwise to a stirred and ice-cooled solution of (*R*)-**5** {$[\alpha]_D^{24}$ −2.94 (*c* 3.57, pyridine), enantiomerically pure as determined by MTPA-NMR analysis;[3] 703 mg, 1.97 mmol} in dry pyridine (3 mL). The mixture was left to stand for 3 d in a refrigerator, diluted with ice and water, and extracted with Et_2O. The extract was washed successively with dil HCl, $NaHCO_3$ solution and brine, dried ($MgSO_4$) and concentrated in vacuo. The residue (0.84 g) was chromatographed over SiO_2 (5 g). Elution with hexane/EtOAc (15:1) gave (*R*)-**2A** (691 mg, 80%) as a colorless oil, n_D^{20}=1.4547; $[\alpha]_D^{24}$ +1.33 (*c* 4.02, hexane); ν_{max} (film): 3005 (w), 2926 (s), 2854 (m), 1749 (vs), 1653 (w), 1458 (m), 1371 (m), 1223 (s), 1169 (m), 1098 (w), 1051 (m), 1017 (w); δ_H ($CDCl_3$): 0.88 (3H, t, *J* 7.2), 1.22–1.40 (20H, br, peaks at 1.27 and 1.30), 1.57–1.65 (2H, m), 1.98–2.05 (4H, m), 2.08 (3H, s), 2.09 (3H, s), 2.32 (2H, t, *J* 7.6), 4.13–4.18 (2H, m), 4.27–4.33 (2H, m), 5.23–5.26 (1H, m), 5.32–5.40 (2H, m); δ_C ($CDCl_3$) 14.1, 20.7, 20.9, 22.7, 24.9, 27.18, 27.23, 29.08, 29.11, 29.17, 29.3, 29.5, 29.7, 31.4, 34.0, 62.0, 62.3 69.1, 129.7, 130.0, 170.1, 170.5, 173.3; GC–MS [same conditions as those for **1A**]: t_R 19.38 min (83.1%); MS (70 eV, EI): *m/z*: 440 (<1)[M^+], 380 (6) [M^+−AcOH], 265 (22), 264 (28), 159 (100), 43 (45); HRMS calcd for $C_{25}H_{44}O_6Na^+$: 463.3030, found: 463.3030.

4.3.2. (S)-Isomer. Similarly, (*S*)-**5** {$[\alpha]_D^{23}$ +3.22 (*c* 4.57, pyridine), enantiomerically pure as determined by MTPA-NMR analysis;[3] 724 mg, 2.04 mmol} in pyridine (5 mL) was treated with Ac_2O (1 mL, excess) and DMAP (50 mg) for 3 d in a refrigerator to give 746 mg (83%) of (*S*)-**2A** as a colorless oil, n_D^{19}=1.4569; $[\alpha]_D^{23}$ −0.88 (*c* 4.19, hexane); GC–MS [same conditions as those for (*R*)-**1A**]: t_R 19.39 min (88.3%). Its IR, ^{1}H and ^{13}C NMR, and MS spectral data were identical to those reported for (*R*)-**2A**. HRMS calcd for $C_{25}H_{44}O_6Na^+$: 463.3030, found: 463.3030.

4.4. 1-Tigloyl-2,3-acetoneglycerol (6)

4.4.1. Racemate. A solution of tigloyl chloride (1.18 g, 10 mmol) in dry C_6H_6 (5 mL) was added dropwise to a stirred and ice-cooled solution of (±)-**4** (1.00 g, 7.6 mmol) and DMAP (50 mg, 0.4 mmol) in dry C_5H_5N (5 mL) at 5–10 °C. Stirring was continued for 30 min at 0–5 °C and for 1 h at room temperature. The mixture was then diluted with ice and water, and extracted with Et_2O. The extract was washed successively with dil HCl, $NaHCO_3$ solution and brine, dried ($MgSO_4$), and concentrated in vacuo to give an oil (3.0 g). This was chromatographed over SiO_2 (20 g). Elution with hexane/EtOAc (10:1) gave (±)-**6** (1.62 g, quant.) as a colorless oil, n_D^{18}=1.4575; ν_{max} (film): 2987 (m), 1714 (s), 1652 (m), 1257 (s), 1218 (m), 1153 (m), 1078 (m), 845 (m), 735 (m); δ_H ($CDCl_3$): 1.38 (3H, s), 1.44 (3H, s), 1.80 (3H, d, *J* 6.8), 1.84 (3H, s), 3.79 (1H, dd, *J* 7.2, 8.0), 4.09 (1H, dd, *J* 6.4, 8.0), 4.19 (2H, d, *J* 5.2), 4.36 (1H, dt, *J* 11.2, 5.6), 6.90 (1H, q, *J* 7.2); GC–MS [column: HP-5MS, 5% phenylmethylsiloxane, 30 m×0.25 mm i.d.; carrier gas, He; press: 52.7 kPa, temp 50–160 °C (+10 °C/min), then 160–220 °C (+4 °C/min)]: t_R 11.20 min (95.0%). MS (70 eV, EI): *m/z*: 200 (12) [M^+−CH_2], 199 (100) [M^+−CH_3], 114 (5), 101 (35), 83 (69), 73 (8), 55 (22), 43 (27). HRMS calcd for $C_{10}H_{15}O_4$ [M^+−CH_3]: 199.0970, found: 199.0978.

4.4.2. (R)-Isomer. Similarly, (*S*)-**4** (1.00 g, 7.6 mmol) gave (*R*)-**6** (1.50 g, 98%) as a colorless oil, n_D^{17}=1.4592; $[\alpha]_D^{20}$ +17.7 (*c* 5.12, hexane); Its IR, ^{1}H NMR and MS spectra were identical to those of (±)-**6**. GC–MS [same conditions as those for (±)-**6**]: t_R 11.22 min (93.6%). HRMS calcd for $C_{10}H_{15}O_4$ [M^+−CH_3]: 199.0970, found: 199.0976.

4.4.3. (S)-Isomer. Similarly, (*R*)-**4** (1.00 g, 7.6 mmol) gave (*S*)-**6** (1.53 g, quant.) as a colorless oil, n_D^{22}=1.4570; $[\alpha]_D^{20}$ −17.9 (*c* 5.15, hexane). Its IR, NMR and MS spectra were identical to those of (±)-**6**. GC–MS [same conditions as those for (±)-**6**]: t_R 11.21 min (95.7%). HRMS calcd for $C_{10}H_{15}O_4$ [M^+−CH_3]: 199.0970, found: 199.0973.

4.5. 1-Tigloylglycerol (7)

4.5.1. Racemate. 80% Acetic acid [AcOH/H_2O=80:20 (v/v), 8 mL] was added to (±)-**6** (1.60 g, 7.6 mmol), and the mixture was stirred and heated at 130 °C (bath temperature) for 10 min. The solution was concentrated in vacuo, and the residue was diluted with toluene. The solution was concentrated again in vacuo to remove toluene, acetic acid and water to give 1.40 g (quant.) of (±)-**7** as a colorless and viscous oil, n_D^{19}=1.4742; ν_{max} (film): 3414 (m, br), 2948 (m), 1712 (s), 1651 (m), 1384 (m), 1269 (s), 1146 (m), 1079 (m), 735 (m), δ_H ($CDCl_3$): 1.80 (3H, d, *J* 7.2), 1.84 (3H, s), 3.61 (1H, dd, *J* 6.0, 7.6), 3.71 (1H, dd, *J* 3.6, 7.2), 3.94–3.99 (1H, m), 4.10–4.30 (2H, m), 4.22 (2H, d, *J* 6.4), 6.90 (1H, q, *J* 7.2). HRMS calcd for $C_8H_{14}O_4$: 174.0892, found: 174.0898.

4.5.2. (R)-Isomer. Similarly, (*R*)-**6** (1.48 g, 7.3 mmol) and 80% acetic acid (17 mL) was stirred and heated at 50 °C for 1 h. The resulting

homogeneous solution was concentrated in vacuo at <60 °C. The residue was dissolved in C_6H_6 and concentrated in vacuo. This operation was repeated three times to remove acetic acid and water completely, yielding (*R*)-**7** (1.30 g, quant.) as a colorless oil, n_D^{17}=1.4764; $[\alpha]_D^{20}$+2.73 (*c* 3.64, $CHCl_3$); Its IR and ^1H NMR spectra were identical to those of (±)-**7**. (*R*)-**7** was enantiomerically pure as analyzed by MTPA/NMR method.[3] HRMS calcd for $C_8H_{14}O_4$: 174.0892, found: 174.0899.

4.5.3. (S)-Isomer. Similarly, (*S*)-**6** (1.43 g, 7.1 mmol) yielded (*S*)-**7** (1.29 g, quant.) as a colorless oil, n_D^{22}=1.4710; $[\alpha]_D^{22}$ −2.28 (*c* 4.50, $CHCl_3$); Its IR and ^1H NMR spectra were identical with those of (±)-**7**. (*S*)-**7** was of 90% ee as analyzed by MTPA/NMR method [signal area of *δ* 4.60–4.65 (dd)/*δ* 4.70–4.75 (dd)=5:95]. HRMS calcd for $C_8H_{14}O_4$: 174.0892, found: 174.0897.

4.6. 1,3-Ditigloylglycerol (8)

Triethylamine (950 mg, 9.4 mmol) and $(n\text{-Bu})_2SnO$ (2.01 g, 8.07 mmol) were added to a solution of (±)-**7** (1.40 g, 8.07 mmol) in dry CH_2Cl_2 (70 mL) under argon. The suspension was stirred for 15 min at room temperature. Subsequently, a solution of tigloyl chloride (971 mg, 8.2 mmol) in dry CH_2Cl_2 (4 mL) was added dropwise to the ice-cooled and stirred suspension at 10–15 °C. The milky white mixture was stirred for 4.5 h at room temperature, and filtered through Celite. The Celite layer was washed with EtOAc, and the combined filtrate and washings were concentrated in vacuo. The residue was chromatographed over SiO_2 (20 g). Elution with hexane/EtOAc (20:1) gave 1,2,3-tritigloylglycerol (228 mg). Further elution with hexane/EtOAc (20:1 to 10:1) gave **8** (1.44 g, 70%) as a colorless oil, n_D^{18}=1.4858; ν_{max} (film): 3480 (m, br), 2954 (m), 1713 (s), 1651 (m), 1442 (m), 1382 (m), 1344 (m), 1261 (s), 1143 (s), 1079 (s), 735 (s); δ_H ($CDCl_3$): 1.80 (6H, d, *J* 7.2), 1.84 (6H, s), 2.74 (1H, s, br), 4.14–4.20 (1H, m), 4.24 (4H, dd, *J* 6.4, 7.0), 6.90 (2H, q, *J* 7.2); δ_C ($CDCl_3$): 12.0, 14.4, 65.3, 68.5, 128.0, 138.3, 168.1; GC–MS [same conditions as those used for **6**]: t_R 16.34 min (98.8%); MS (70 eV, EI): *m*/*z*: 238 (7) [M^+-H_2O], 157 (12), 156 (12), 143 (11), 83 (100), 55 (31). HRMS calcd for $C_{13}H_{20}O_5$: 256.1311, found: 256.1310.

4.7. 1,3-Ditigloyl-2-oleoylglycerol (1B)

A solution of oleoyl chloride (2.00 g, 6.6 mmol) in dry C_6H_6 (5 mL) was added dropwise to a stirred and ice-cooled solution of **8** (1.41 g, 5.5 mmol) and DMAP (20 mg) in dry C_5H_5N (6 mL) at 5–10 °C. The mixture was stirred for 15 min at 5–10 °C, and for 1 h at room temperature. It was then diluted with ice and water, and extracted with Et_2O. The extract was washed successively with dil HCl, $NaHCO_3$ solution and brine, dried ($MgSO_4$), and concentrated in vacuo to give an oily residue (3.62 g). This was chromatographed over SiO_2 (30 g). Elution with hexane/EtOAc (20:1) gave 2.20 g (77%) of **1B** as a slightly yellowish oil, n_D^{19}=1.4770; ν_{max} (film): 2925 (s), 2855 (s), 1744 (s), 1718 (vs), 1653 (m), 1461 (m), 1380 (m), 1343 (w), 1254 (s), 1135 (m), 1079 (m), 733 (m); δ_H ($CDCl_3$): 0.88 (3H, t, *J* 6.8), 1.20–1.40 (20H, peaks at 1.27 and 1.29), 1.55–1.65 (2H, m), 1.79 (6H, d, *J* 7.2), 1.82 (6H, s), 1.95–2.08 (4H, m), 2.32 (2H, t, *J* 7.6), 4.22–4.37 (4H, m), 5.30–5.40 (3H, m), 6.82–6.90 (2H, m); δ_C ($CDCl_3$): 12.0, 14.1, 14.4, 22.7, 24.9, 25.6, 27.13, 27.18, 29.0, 29.1, 29.2, 29.3, 29.5, 29.68, 29.73, 31.9, 34.2, 62.5, 68.9, 128.0, 129.7, 130.0, 138.2, 167.4, 172.9; GC–MS [same conditions as those used for **1A**]: t_R 24.80 min (88.2%); MS (70 eV, EI): *m*/*z*: 520 (<1) [M^+], 420 (16) [M^+–tiglic acid (TA)], 239 (47) [M^+-oleic acid (OA)], 157 (12), 83 (100), 55 (22). HRMS calcd for $C_{31}H_{52}O_6$: 520.3764, found: 520.3754.

4.8. (±)-1-Acetyl-2,3-acetoneglycerol (9)

A solution of acetyl chloride (1.30 g, 17 mmol) in dry C_6H_6 (5 mL) was added to a stirred and ice-cooled solution of (±)-**4** (1.53 g, 11.6 mmol) and DMAP (25 mg) in dry C_5H_5N (5 mL). The mixture was stirred for 1 h at room temperature, and worked up as described for (±)-**6** to give 2.34 g (quant.) of crude (±)-**9** as a colorless oil, n_D^{19}=1.4332; ν_{max} (film): 2988 (m), 1746 (s), 1372 (m), 1233 (s), 1159 (w), 1055 (m), 984 (w), 683 (m); δ_H ($CDCl_3$): 1.37 (3H, s), 1.44 (3H, s), 2.09 (3H, s), 3.70–3.57 (1H, m), 4.04–4.10 (2H, m), 4.15–4.19 (1H, m), 4.30–4.34 (1H, m). These IR and ^1H NMR data are in good accord with those of (*S*)-**9**.[14] GC–MS [same conditions as those used for (±)-**6**]: t_R 6.69 min (99.7%); MS (70 eV,EI): *m*/*z*: 159 (100) [M^+-CH_3], 101 (29), 72 (14), 57 (9), 43 (97).

4.9. (±)-1-Acetylglycerol (10)

A mixture of (±)-**9** (1.80 g, 10.3 mmol) and 80% acetic acid (10 mL) was stirred and heated at 130 °C (bath temperature) for 10 min. The resulting homogeneous solution was concentrated in vacuo. The residue was mixed with toluene, and concentrated again in vacuo to remove toluene, acetic acid and water. The remaining oil (±)-**10** (1.35 g, 97%) was used in the next step without further purification, n_D^{15}=1.4502; ν_{max} (film): 3394 (m, br), 2952 (w), 1737 (s), 1376 (m), 1247 (s), 1119 (w), 1046 (s), 981 (w), 933 (w), 848 (w); δ_H ($CDCl_3$): 2.11 (3H, s), 3.57–3.72 (2H, m), 3.50–3.70 (2H, br), 3.90–3.98 (1H, m), 4.10–4.20 (2H, m). These IR and ^1H NMR data are in good accord with those of (*S*)-**10**.[15]

4.10. 1,3-Diacetylglycerol (11) contaminated with (±)-1,2-diacetylglycerol [(±)-12]

Triethylamine (1.01 g, 10 mmol) and $(n\text{-Bu})_2SnO$(2.42 g, 9.7 mmol) were added to a solution of (±)-**10** (1.32 g, 9.7 mmol) in dry CH_2Cl_2(70 mL) under argon. The suspension was stirred for 15 min at room temperature. A solution of acetyl chloride (790 mg, 10.1 mmol) in dry CH_2Cl_2 (4 mL) was added dropwise to the stirred and ice-cooled suspension. Stirring was continued for 30 min at 5–10 °C and for 2 h at room temperature. The mixture was filtered through Celite. The Celite layer was washed with EtOAc, and the combined filtrate and washings were concentrated in vacuo. The residue (1.8 g) was chromatographed over SiO_2 (25 g). Elution with hexane/EtOAc (2:1) gave 1.48 g (86%) of a 65:35 mixture of **11** and (±)-**12** as a colorless oil, n_D^{20}=1.4382; ν_{max} (film): 3463 (m, br), 2959 (m), 1741 (s), 1439 (m), 1374 (s), 1233 (s), 1048 (s), 607 (m); δ_H ($CDCl_3$): 2.11 (6H, s), 2.94 (1H, br), 3.74 (0.7H, d, *J* 4.8), 4.08–4.25 (4H, m), 5.06–5.09 (0.3H, m); GC–MS [same conditions as those used for **6**]: t_R 8.74 min (64.9%, **11**), 8.78 min [35.1%, (±)-**12**]; MS (70 eV, EI): *m*/*z*: 103 (59), 43 (100) for **11**; 103 (41), 43 (100) for (±)-**12**. HRMS calcd for $C_7H_{11}O_4$ [M^+–OH]: 159.0657, found: 159.0666.

4.11. 1,3-Diacetyl-2-oleoylglycerol (2B) contaminated with 1,2-diacetyl-3-oleoylglycerol [(±)-2A]

A solution of oleoyl chloride (0.85 g, 2.8 mmol) in dry C_6H_6 (3 mL) was added dropwise to a stirred and ice-cooled solution of the above mixture of **11** and (±)-**12** (440 mg, 2.5 mmol) and DMAP (10 mg) in dry C_5H_5N (3 mL) at 5–10 °C. The mixture was stirred at 5–10 °C for 15 min and then for 1 h at room temperature. It was then diluted with ice and water, and extracted with Et_2O. The extract was washed successively with dil HCl, $NaHCO_3$ solution and brine, dried ($MgSO_4$), and concentrated in vacuo to give a residual oil (1.58 g). This was chromatographed over SiO_2 (20 g). Elution with hexane/EtOAc (20:1) gave 0.95 g (87%) of a 2:1 mixture of **2B** and (±)-**2A** as an oil, n_D^{20}=1.4558; ν_{max} (film): 3005 (w), 2925 (s),

2854 (s), 1749 (s), 1459 (m), 1369 (m), 1224 (s), 1167 (m), 1100 (w), 1051 (m); Its ^{1}H NMR spectrum was almost identical to that of **2A**. The ^{13}C NMR spectrum of **2B** was different from that of **2A** reflecting its symmetrical structure: (1) Only two signals for RCOOCH_2– could be observed at δ 62.3 and 68.8 and (2) Only two signals for *C*=O could be observed at δ 170.5 and 173.0. GC–MS [same conditions as those used for **1A**]: t_R 19.35 min (61.4%, **2B**), 19.39 min [28.8%, (±)-**2A**]; MS of **2B** (70 eV, EI): *m*/*z*: 440 (<1)[M^+], 380 (7) [M^+–AcOH], 265 (67), 264 (57), 159 (100), 43 (54); MS of (±)-**2A** (70 eV, EI); *m*/*z*: 440 (<1)[M^+], 380 (7) [M^+–AcOH], 265 (26), 264 (31), 159 (100), 43 (44). HRMS calcd for $C_{25}H_{44}O_6Na^+$ [M+Na]$^+$: 463.3030, found: 463.3030.

4.12. 1,3-Ditigloyloxyacetone (14)

Tigloyl chloride (1.25 g, 10.5 mmol) in dry C_6H_6 (2 mL) was added to a stirred and ice-cooled solution of **13** (0.45 g, 2.5 mmol) in dry C_5H_5N (2 mL) at 0–5 °C. The mixture was stirred for 2 h at room temperature to precipitate solid $C_5H_5N\cdot HCl$. It was then diluted with ice-water, and extracted with Et_2O. The extract was washed successively with dil HCl, $NaHCO_3$ solution and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue (1.073 g) was chromatographed over SiO_2 (15 g). Elution with hexane/EtOAc (20:1–10:1) gave 580 mg (91%) of **14** as a colorless solid. Recrystallization from hexane/EtOAc gave rhombs, mp 46.0–46.5 °C; ν_{max} (Nujol): 1745 (s), 1712 (s), 1650 (s), 1269 (s), 1149 (s), 733 (s), δ_H ($CDCl_3$): 1.83 (6H, d, *J* 6.8), 1.87 (6H, s), 3.91 (4H, s), 6.98 (1H, q-like, *J* 6.8); δ_C ($CDCl_3$): 12.0, 14.6, 66.6, 127.6, 139.3, 167.1, 199.0; GC–MS [same conditions as those used for **6**]: t_R 16.29 min (97.6%); MS (70 eV, EI): *m*/*z*: 254 (<1)[M^+], 154 (13) [M^+–tiglic acid], 141 (51), 83 (100), 55 (42). HRMS calcd for $C_{13}H_{18}O_5$: 254.1154, found: 254.1151.

4.13. 1,3-Ditigloylglycerol (8) by reduction of 14

A solution of **14** (374 mg, 1.5 mmol) in dry THF (3 mL) was added dropwise to a solution of $Zn(BH_4)_2$ in dry THF [prepared by the addition of a solution of $ZnCl_2$ (68 mg, 0.5 mmol) in dry THF (2 mL) to stirred $NaBH_4$ (38 mg, 1.0 mmol)] under ice-cooling and stirring. After stirring for 20 min at 5–10 °C, the reaction was quenched by the addition of acetic acid (0.1 mL). The mixture was concentrated in vacuo. The residue was diluted with hexane/EtOAc (10:1), and chromatographed over SiO_2 (5 g). Elution with hexane/EtOAc (10:1) gave 377 mg (quant.) of **14** as a colorless oil, n_D^{20}=1.4858. Its IR, ^{1}H NMR and MS spectra were identical with those of **8** described in 4.6 (vide supra). GC–MS [same conditions as those used for **6**]: t_R 16.61 min (94.6%).

4.14. 1,3-Diacetoxyacetone (15)

This was prepared according to Suemune et al.[15] as needles, mp 46 °C, in 77% yield based on **13**.

4.15. 1,3-Diacetylglycerol (11) contaminated with (±)-12 by reduction of 15

$NaBH_4$ used in this experiment was washed thoroughly by stirring with EtOAc for 3 h at room temperature, collected on a glass filter, washed with Et_2O, and dried to remove alkaline impurities.[16] A solution of $ZnCl_2$ (612 mg, 4.5 mmol) in dry THF (8 mL) was added to a stirred and ice-cooled suspension of $NaBH_4$ (342 mg, 9 mmol) in dry THF (12 mL). A solution of **15** (3.00 g, 17 mmol) in dry THF (10 mL) was added dropwise to the stirred and ice-cooled solution of $Zn(BH_4)_2$. Stirring was continued for 20 min at 0–5 °C. The reaction was quenched by the addition of acetic acid (1 mL), and the mixture was concentrated in vacuo. The residue was triturated with hexane/EtOAc (3:2), and transferred on a SiO_2 (25 g) column. Elution with hexane/EtOAc (3:2) gave 2.46 g (61%) of a mixture of **11** and (±)-**12**, ν_{max} (film):3462 (m), 2961 (m), 1740 (s), 1436 (m), 1374 (s), 1236 (s), 959 (m), 607 (m); GC–MS [same conditions as those for **6**]. t_R 8.86 min (63.4%, **11**), 8.91 min [28.1%, (±)-**12**]. MS of **11** (70 eV, EI): *m*/*z*: 158 (<1) [M^+–H_2O], 103 (52), 86 (6), 74 (8), 43 (100); MS of (±)-**12** (70 eV, EI): *m*/*z*: 158 (<1) [M^+–H_2O], 145 (5), 103 (38), 86 (12), 74 (5), 43 (100).

4.16. 1-Acetyl-3-oleoylglycerol (16)

4.16.1. (S)-Isomer. Triethylamine (505 mg, 5 mmol) and (n-Bu)$_2$SnO (1.230 g, 5 mmol) were added to a solution of (*S*)-**5** (1.78 g, 5 mmol) in dry CH_2Cl_2 (70 mL) under argon. The suspension was stirred for 10 min at room temperature. Then a solution of acetyl chloride (393 mg, 5 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise over 10 min to the stirred and ice-cooled suspension at 10–15 °C. Subsequently, the milky white mixture was stirred for 1.5 h at room temperature. The mixture was filtered through Celite. The Celite layer was washed with EtOAc, and the combined filtrate and washings were concentrated in vacuo. The residue (2.2 g) was chromatographed over SiO_2 (30 g). Elution with hexane/EtOAc (20:1–10:1) gave 387 mg of triacylglycerol. Further elution with hexane/EtOAc (5:1) furnished (*S*)-**16** (1.44 g, 73%) as a colorless oil, n_D^{18}=1.4612. $[\alpha]_D^{19}$–0.81 (*c* 5.26, hexane); ν_{max} (film): 3471 (m, br), 3005 (m), 2925 (s), 2854 (4), 1743 (s), 1654 (w), 1459 (m), 1377 (m), 1235 (s), 1177 (m), 1119 (w), 1047 (m), 944 (w), 723 (w); δ_H ($CDCl_3$): 0.88 (3H, t, *J* 6.8), 1.20–1.40 (20H, br, peaks at 1.27 and 1.30), 1.60–1.68 (2H, m), 1.98–2.08 (4H, m), 2.11 (3H, s), 2.35 (2H, t, *J* 7.6), 2.47–2.50 (1H, br), 4.05–4.25 (5H, m), 5.30–5.40 (2H, m); GC–MS [same conditions as those used for **1A**]: t_R 19.26 min (86.7%); MS (70 eV, EI): *m*/*z*: 380 (7) [M^+–H_2O], 265 (32), 207 (12), 129 (13), 117 (100), 98 (28), 81 (27), 69 (28), 67 (27), 55 (38), 43 (69). HRMS calcd for $C_{23}H_{42}O_5Na^+$:421.2924, found: 421.2926.

4.16.2. (R)-Isomer. Similarly, 1.78 g (5 mmol) of (*R*)-**5** gave 1.41 g (71%) of (*R*)-**16**, n_D^{18}=1.4628; $[\alpha]_D^{15}$+0.64 (*c* 5.06, hexane). Its IR, ^{1}H NMR and MS spectra were identical to those of (*S*)-**16**. GC–MS [same conditions as those used for **1A**]: t_R 19.09 min (77.4%). HRMS calcd for $C_{23}H_{42}O_5Na^+$: 421.2924, found: 421.2926.

4.17. 1-Acetyl-2-tigloyl-3-oleoylglycerol (3A)

4.17.1. (S)-Isomer. A solution of tigloyl chloride (534 mg, 4.5 mmol) in dry CH_2Cl_2 (3 mL) was added dropwise to a stirred and ice-cooled solution of (*S*)-**16** (1.36 g, 3.4 mmol) and DMAP (50 mg) in dry C_5H_5N (3 mL). The mixture was stirred for 30 min at 5–15 °C and for 2 h at room temperature until white $C_5H_5N\cdot HCl$ separated out. It was then diluted with ice and water, and extracted with Et_2O. The extract was washed successively with dil HCl, $NaHCO_3$ solution and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue (1.61 g) was chromatographed over SiO_2 (15 g). Elution with hexane/EtOAc (20:1) gave (*S*)-**3A** (1.17 g, 71%) as a slightly yellowish oil, n_D^{20}=1.4660; $[\alpha]_D^{23}$+0.85 (*c* 5.04, hexane); ν_{max} (film): 3004 (m), 2920 (s), 2855 (s), 1748 (s), 1717 (s), 1652 (m), 1458 (m), 1380 (m), 1368 (m), 1229 (s), 1153 (m), 1134 (m), 1075 (m), 1049 (m), 1020 (m), 733 (m); δ_H ($CDCl_3$): 0.88 (3H, t, *J* 6.8), 1.22–1.41 (20H, br, peaks at 1.27 and 1.30), 1.60 (2H, *t*-like, *J* 7.2), 1.80 (3H, d, *J* 6.8), 1.83 (3H, s), 1.98–2.10 (4H, m), 2.07 (3H, s), 2.31 (2H, t, *J* 7.6), 4.15–4.25 (2H, m), 4.28–4.38 (2H, m), 5.30–5.40 (3H, m), 6.80–7.00 (1H, m); δ_C ($CDCl_3$): 11.9, 14.0, 14.4, 14.8, 20.6, 22.6, 24.8, 25.5, 27.09, 27.13, 28.99, 29.02, 29.2, 29.5, 29.62, 29.69, 31.8, 33.9, 62.0, 62.2, 69.0, 128.0, 129.6, 129.9, 138.3, 166.9, 170.5, 173.2; GC–MS [same conditions as those used for **1A**]: t_R 21.62 min [69.8%, (*S*)-**3A**], 21.68 min [4.4%, (*S*)-**3B**], 21.78 min [16.4%, (*R*)-**3C**]; MS of (*S*)-**3A** (70 eV, EI): *m*/

z 480 (<1) [M^+], 420 (4) [M^+−AcOH], 380 (10) [M^+−tiglic acid], 264 (14), 199 (52) [M^+−oleic acid]. 83 (100), 55 (23), 43 (12). HRMS calcd for $C_{28}H_{48}O_6Na^+$: 503.3343, found: 503.3343.

4.17.2. (R)-Isomer. Similarly, (*R*)-**16** (1.19 g, 3.3 mmol) gave (*R*)-**3A** (1.11 g, 78%) as an oil, n_D^{20}=1.4674; $[\alpha]_D^{23}$ −0.60 (*c* 5.06, hexane). Its IR, NMR and MS spectra were identical with those of (*S*)-**3A**. GC−MS [same conditions as those used for **1A**]: t_R 21.55 min [79.6%, (*R*)-**3A**], 21.63 min [3.2%, (*R*)-**3B**], 21.73 min [7.7%, (*S*)-**3C**]. HRMS calcd for $C_{28}H_{48}O_6Na^+$: 503.3343, found: 503.3344.

4.18. 1-Acetyl-3-tigloylglycerol (17)

4.18.1. (S)-Isomer. Triethylamine (404 mg, 4 mmol) and (*n*-Bu)$_2$SnO (996 mg, 4 mmol) was added to a solution of (*S*)-**7** (702 mg, 4 mmol) in dry CH_2Cl_2 (40 mL) under argon. The suspension was stirred for 15 min at room temperature. A solution of acetyl chloride (315 mg, 4 mmol) in dry CH_2Cl_2 (3 mL) was added dropwise to the stirred and ice-cooled suspension at 10−15 °C. The milky white mixture was stirred for 3 h at room temperature, and filtered through Celite. The Celite layer was washed with EtOAc, and the combined filtrate and washings were concentrated in vacuo. The residue (1.1 g) was chromatographed over SiO_2 (20 g). Elution with hexane/EtOAc (20:1) gave 80 mg of triacylglycerol. Further solution with hexane/EtOAc (4:1) afforded 609 mg (70%) of (*S*)-**17** as a colorless oil, n_D^{25}=1.4650; $[\alpha]_D^{26}$−2.20 (*c* 4.49, Et_2O); ν_{max} (film): 3472 (m, br), 2957 (m), 1742 (s), 1714 (s), 1651 (m), 1442 (m), 1375 (m), 1254 (s), 1146 (m), 1080 (m), 1047 (m), 946 (w), 736 (m); δ_H ($CDCl_3$): 1.81 (3H, d, *J* 6.8), 1.85 (3H, s), 2.11 (3H, s), 2.75 (1H, br, s), 4.10−4.20 (3H, m), 4.24 (2H, *t*-like *J* 5.6), 6.90 (1H, q, *J* 6.8); GC−MS [same conditions as those used for **6**]; t_R 13.04 min (88.1%); MS (70 eV, EI): *m*/*z*: 216 (<1) [M^+], 198 (9), [M^+−H_2O], 156 (8) [M^+−AcOH], 143 (7), 117 (10) [M^+−tiglic acid], 99 (13), 83 (100), 55 (36), 43 (32). HRMS calcd for $C_{10}H_{16}O_5$: 216.0998, found 216.0992.

4.18.2. (R)-Isomer. Similarly, (*R*)-**7** (1.40 g, 8 mmol) gave (*R*)-**17** (1.24 g, 71%) as a colorless oil, n_D^{21}=1.4638; $[\alpha]_D^{23}$ +2.33 (*c* 4.18, Et_2O); GC−MS [same conditions as those used for **6**]; t_R 13.02 min (98.0%). Its IR, ^{1}H NMR and MS spectra were identical with those of (*S*)-**17**, HRMS calcd for $C_{10}H_{16}O_5$: 216.0998, found: 216.1008.

4.19. 1-Acetyl-2-oleoyl-3-tigloylglycerol (3B)

4.19.1. (S)-Isomer. A solution of oleoyl chloride (900 mg, 3 mmol) in dry C_6H_6 (3 mL) was added dropwise to a stirred and ice-cooled solution of (*S*)-**17** (450 mg, 2.1 mmol) and DMAP (10 mg) in dry C_5H_5N (3 mL) at 5−10 °C. The mixture was stirred at 5−10 °C for 30 min and 1 h at room temperature. It was then diluted with ice and water, and extracted with Et_2O. The extract was washed successively with dil HCl, $NaHCO_3$ solution and brine, dried ($MgSO_4$), and concentrated in vacuo to give an oily residue (1.28 g). This was chromatographed over SiO_2 (7 g). Elution with hexane/EtOAc gave 907 mg (91%) of (*S*)-**3B** as a colorless to slightly yellowish oil, n_D^{23}=1.4665; $[\alpha]_D^{24}$−4.52 (*c* 5.80, hexane) ν_{max} (film): 3005 (w), 2926 (s), 2855 (s), 1748 (s), 1718 (s), 1652 (w), 1458 (m), 1368 (m), 1231 (s), 1155 (s), 1080 (m), 1051 (m), 733 (m); δ_H ($CDCl_3$): 0.88 (3H, t, *J* 7.2), 1.22−1.41 (20H, br, peaks at 1.27 and 1.30), 1.58−1.68 (2H, m), 1.80 (3H, d, *J* 6.8), 1.82 (3H, s), 2.00−2.10 (4H, m), 2.07 (3H, s), 2.33 (2H, t, *J* 7.2), 4.15−4.25 (2H, m), 4.29−4.35 (2H, m), 5.30−5.42 (3H, m), 6.86 (1H, q, *J* 6.8); δ_C ($CDCl_3$): 12.0, 14.1, 14.4, 20.7, 22.6, 24.9, 25.6, 27.12, 27.18, 29.0, 29.06, 29.14, 29.28, 29.48, 29.66, 29.72, 31.9, 34.2, 62.3, 62.5, 68.8, 128.0, 129.7, 130.0, 138.2, 167.4, 170.5, 172.9; GC−MS [same conditions as those used for **1A**]; t_R 21.72 min [62.6% (*S*)-**3B**], 21.80 min [26.6%, (*S*)-**3C**]; MS (70 eV, EI): *m*/*z*: 480 (<1) [M^+], 420 (4) [M^+−AcOH], 380 (10)[M^+−tiglic acid], 264 (14), 199 (52) [M^+-oleic aid], 83 (100), 55 (23), 43 (12). HRMS calcd for $C_{28}H_{48}O_6Na^+$: 503.3343, found 503.3343.

4.19.2. (R)-Isomer. Similarly, (*R*)-**17** (1.05 g, 4.9 mmol) afforded (*R*)-**3B** (1.91 g, 82%) as a slightly yellowish oil, n_D^{17}=1.4674; $[\alpha]_D^{20}$+4.44 (*c* 5.20, hexane). Its IR, ^{1}H NMR and MS spectra were identical with those of (*S*)-**17**. GC−MS [same conditions as those used for **1A**]; t_R 21.68 min [64.0%, (*R*)-**3B**], 21.77 min [28.6%, (*R*)-**3C**]. HRMS calcd for $C_{28}H_{48}O_6Na^+$: 503.3343, found: 503.3343.

4.20. 1-Oleoyl-3-tigloylglycerol (18)

4.20.1. (R)-Isomer. Triethylamine (364 mg, 3.6 mmol) and (*n*-Bu)$_2$SnO (871 mg, 3.5 mmol) were added to a solution of (*S*)-**5** (1.26 g, 3.5 mmol) in dry CH_2Cl_2 (50 mL) under argon. The suspension was stirred for 10 min at room temperature. A solution of tigloyl chloride (427 mg, 3.6 mmol) in dry CH_2Cl_2 (4 mL) was added dropwise to the stirred and ice-cooled suspension at 10−15 °C. The milky white mixture was stirred for 30 min at 5−10 °C, and then for 3 h at room temperature. The mixture was then filtered through Celite. The Celite layer was washed with EtOAc, and the combined filtrate and washings were concentrated in vacuo to give an oily residue (2.52 g). This was chromatographed over SiO_2 (20 g). Elution with hexane/EtOAc (50:1−20:1) gave 157 mg of impurities. Further elution with hexane/EtOAc (10:1) gave 1.54 g (99%) of (*R*)-**18** as a colorless oil, n_D^{19}=1.4722; $[\alpha]_D^{20}$ +1.14 (*c* 4.24, hexane); ν_{max} (film): 3852 (m, br), 3004 (m), 2925 (s), 2854 (s), 1741 (s), 1715 (s), 1652 (m), 1458 (m), 1380 (m), 1266 (s), 1145 (s), 1080 (m), 734 (m); δ_H ($CDCl_3$): 0.88 (3H, t, *J* 6.8), 1.20−1.40 (20H, br, peaks at 1.27 and 1.36), 1.60−1.70 (2H, m), 1.80 (3H, d, *J* 6.8), 1.84 (3H, s), 1.96−2.10 (4H, m), 2.35 (2H, t, *J* 7.6), 2.60 (1H, br), 4.10−4.30 (5H, m), 5.32−5.41 (2H, m), 6.86 (1H, q, *J* 6.8); GC−MS [same conditions as those used for **1A**]; t_R 21.10 min (99.1%); GC−MS (70 eV, EI): *m*/*z* 438 (<1) [M^+], 420 (4) [M^+−H_2O], 338 (3) [M^+−tiglic acid], 207 (5), 157 (36), 83 (100), 55 (23), HRMS calcd for $C_{26}H_{46}O_5Na^+$: 461.3237, found: 461.3237.

4.20.2. (S)-Isomer. Similarly, (*R*)-**6** (1.20 g, 3.4 mmol) gave (*S*)-**18** (1.49 g, quant.) as a colorless oil, n_D^{17}=1.4733; $[\alpha]_D^{18}$ −1.24 (*c* 5.07, hexane); GC−MS [same conditions as those used for **1A**]; t_R 21.11 min (98.3%); Its IR, ^{1}H NMR and MS spectra were identical with those of (*R*)-**18**. HRMS calcd for $C_{26}H_{46}O_5Na^+$: 461.3237, found: 461.3237.

4.21. 1-Oleoyl-2-acetyl-3-tigloylglycerol (3C)

4.21.1. (R)-Isomer. A solution of acetyl chloride (471 mg, 6 mmol) in dry C_6H_6 (2 mL) was added dropwise to a stirred and ice-cooled solution of (*R*)-**18** (1.38 g, 3.1 mmol) and DMAP (50 mg) in dry C_5H_5N (2 mL) and dry C_6H_6 (2 mL) at 10−15 °C. The mixture was stirred for 30 min at 10−15 °C, and then for 3 h at room temperature. It was diluted with ice and water, and extracted with Et_2O. The extract was washed successively with dil HCl, $NaHCO_3$ solution and brine, dried ($MgSO_4$), and concentrated in vacuo. The residual oil (2.00 g) was chromatographed over SiO_2 (5 g). Elution with hexane/EtOAc (20:1) gave 1.26 g (83%) of (*R*)-**3C** as a colorless oil, n_D^{22}=1.4653; $[\alpha]_D^{24}$ +3.93 (*c* 4.14, hexane); ν_{max} (film): 3004 (m), 2925 (s), 2855 (s), 1748 (s), 1718 (s), 1653 (m), 1459 (m), 1372 (m), 1258 (s), 1230 (s), 1153 (m), 1136 (m), 1079 (m), 1018 (w), 959 (w), 733 (m); δ_H ($CDCl_3$): 0.88 (3H, t, *J* 7.2), 1.20−1.40 (20H, br, peaks at 1.27 and 1.30), 1.61 (2H, t-like, *J* 7.2), 1.80 (3H, d, *J* 6.8), 1.83 (3H, s), 1.96−2.08 (4H, m), 2.08 (3H, s), 2.32 (2H, t, *J* 7.6), 4.15−4.25 (2H, m), 4.30−4.36 (2H, m), 5.28−5.40 (3H, m), 6.86 (1H, q, *J* 6.8); δ_C ($CDCl_3$): 11.9, 14.0, 14.1, 14.4, 20.8, 22.6, 24.8, 25.6, 27.09, 27.14, 29.00, 29.04, 29.3, 29.5,

29.6, 29.7, 31.8, 34.0, 62.3, 62.4, 69.1, 128.0, 129.9, 130.1, 138.2, 167.3, 170.0, 173.3; GC–MS [same conditions as those used for **1A**]; t_R 21.28 min [13.7%, (*S*)-**3A**], 21.37 min [4.9%, (*R*)-**3B**], 21.53 min [71.5%, (*R*)-**3C**]; MS (70 eV, EI): *m*/*z*: 480 (<1) [M^+], 420 (5) [M^+–AcOH], 380 (9) [M^+–tiglic acid], 264 (11), 199 (50), 83 (100), 55 (18). HRMS calcd for $C_{28}H_{48}O_6Na^+$503.3343, found: 503.3343.

4.21.2. (S)-Isomer. Similarly, (*S*)-**18** (1.33 g, 30 mmol) afforded (*S*)-**3C** (1.06 g, 73%) as a colorless oil, n_D^{20}=1.4668; $[\alpha]_D^{22}$ −3.79 (*c* 4.63, hexane); GC–MS [same conditions as those used for **1A**]; t_R 21.51 min [5.7%, (*R*)-**3A**], 21.61 min [2.9% (*S*)-**3B**], 21.77 min [74.9%, (*S*)-**3C**]. Its IR, ^{1}H NMR and MS spectra were identical with those of (*R*)-**3C**. HRMS calcd for $C_{28}H_{48}O_6Na^+$: 503.3343, found: 503.3343.

4.22. GC–MS co-injection experiments

*4.22.1. Co-injection of (R)-**1A** with **1B**.* A 1:2 mixture of (*R*)-**1A** and **1B** in Et_2O was analyzed by GC–MS under the conditions used for the analysis of (*R*)-**1A**: t_R 24.56 min for (*R*)-**1A** and 24.80 min for **1B** [see Fig. 6a]. They showed almost the same MS spectra.

*4.22.2. Co-injection of (S)-**3A**. (S)-**3B**, and (R)-**3C**.* A 1:2:4 mixture of (*S*)-**3A**, (*S*)-**3B**, and (*R*)-**3C** in Et_2O was analyzed by GC–MS under the conditions used for the analysis of (*R*)-**1A**: t_R 21.30 min for (*S*)-**3A**, 21.41 min for (*S*)-**3B**, and 21.54 min for (*R*)-**3C** [see Fig. 6b].

4.23. HPLC analysis of (*R*)-1A, and 1B

The synthetic triacylglycerols **1A**, **1B**, **3A**, **3B**, and **3C** were subjected to HPLC analysis. Only the separation of (*R*)-**1A** from **1B** was possible under the following conditions. Apparatus: Shimadzu prominence; column: Sunrise C_{28} 5μ, 250 mm×4.6 mm i.d. (ChromaNik Technology); column temperature: 10 °C; eluent: MeCN/THF=100:0 (0 min)–75:25 (50 min); flow rate: 1.0 mL/min; sample: (*R*)-**1A**+**1B** in MeCN; t_R 20.797 min [(*R*)-**1A**], 21.591 min (**1B**).

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