Synthesis of 1,2-Di-*O*-acyl-3-thioglycerols for Lipid Modification of Peptides and Proteins

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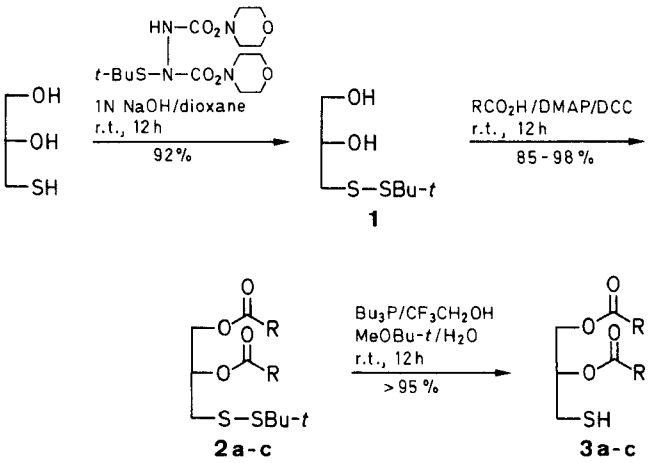
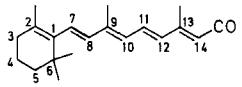
Thiol-functionalized lipids were synthesized to exploit thiol-specific reaction principles for the selective conjugation of lipid moieties to peptides and proteins. Thioglycerol (3-mercapto-1,2-propanediol) protected as *S-tert*-butylthio derivative served as starting product for the esterification of the two hydroxy groups with identical saturated or unsaturated fatty acids as well as for the preparation of mixed diacyl derivatives. Reductive cleavage of the thiol protecting group produced the (*RS*)-1,2-di-O-acyl-3-thioglycerols (*RS*)-2,3-diacyloxypropanethiols as suitable reagents for lipid modification of target molecules.

Post-translational processing of proteins and peptides by fatty acylation and their modification by phospholipids has been recognized to strongly influence the transmembrane pathways of these biomolecules and their distribution in cells.[1-4] Specific binding to membrane receptors or nonspecific incorporation of hydrophobic domains in membranes or the synergism of these two events provide mechanisms for protein trafficking across biomembranes. There is, therefore, much interest in developing efficient methods for the artificial attachment of lipid molecules to peptides and proteins in order to target exogeneous factors in living organisms.

In conjugate chemistry increasing attention has been paid to the thiol function. It allows selective crosslinking of molecules via disulfide or sulfide bonds exploiting mild thiol–disulfide interchange or thiol addition reactions. For latter reaction type, the maleinimide group as thiol acceptor[5] has found widespread application. In previous studies[6-8] we have shown that this group is sufficiently stable under certain conditions of peptide synthesis to enable its insertion at preselected peptide-chain positions



2, 3	RCO
a	$C_{15}H_{31}CO$ (palmitoyl)
b	$C_{13}H_{27}CO$ (myristoyl)
c	 (retinoyl)

Scheme A

and thus, to represent an ideal anchor for subsequent covalent linkage of thiol-functionalized molecules.

This observation compelled us to develop thiol-containing lipids for the selective attachment of lipid-

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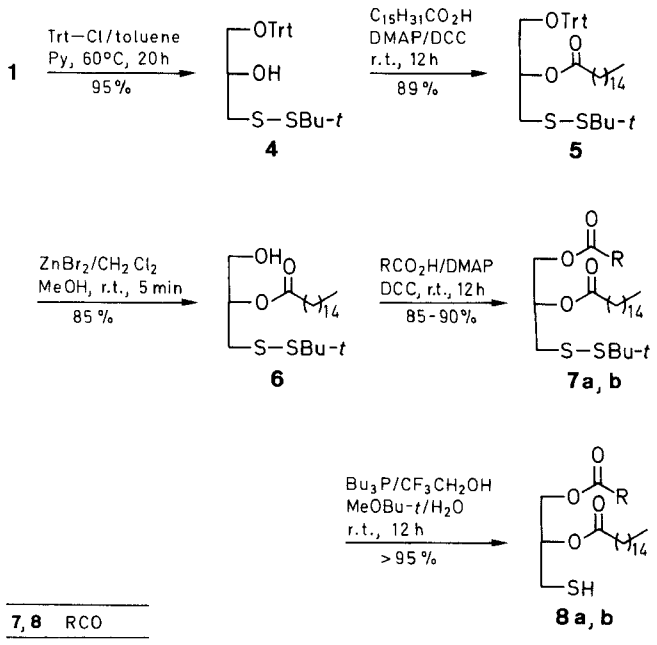
derived hydrophobic moieties to peptides and proteins. 2,3-Diacyloxypropanethiols containing identical or mixed fatty acyl residues were selected for this purpose to permit interdigitation of the acyl chains in membranes or enhance the tendency to liposome formation. Since the main scope of the present study is focused on effective methods for lipophylic modification of peptides and proteins, the work has been confined to the synthesis of racemic compounds.

Thioglycerol (3-mercapto-1,2-propanediol) served as starting compound and was protected at the thiol function as an unsymmetrical disulfide using 1-(*tert*-butylthiohydrazine)-1,2-dicarboxmorpholide as a *tert*-butylthio donator.[9] The resulting *S*-protected derivative **1** was then esterified in straightforward manner with palmitic, myristic or retinoic acid using the DCC (1,3-dicyclohexylcarbodiimide) procedure in presence of 10 mol% *N,N*-dimethylaminopyridine (DMAP)[10] to produce in high yields the (*RS*)-1,2-diacyloxy-3-(*tert*-butyldithio) propanes **2** (Scheme **A**).

For the synthesis of the mixed diacyl derivatives the primary alcohol function of **1** (Scheme **B**) was converted to the corresponding trityl ether **4** by allowing **1** to react with triphenylchloromethane in toluene in the presence of pyridine.[11] Esterification of the secondary hydroxyl group with palmitic acid was again carried out by the DCC/DMAP method. Subsequent detritylation of the resulting compound **5** was attempted using various known acid-catalyzed cleavage procedures; but all were accompanied by substantial acyl migration. The best results in this context were obtained by silicic acid/boric acid column chromatography[12] or by cleavage with zinc bromide,[13] followed by silica gel chromatography. A residual 1–2% of the 1-acyl byproduct could not be removed. Compound **6** was then esterified with myristic or retinoic acid to produce the mixed diacyl derivatives **7**. The isomers resulting from the acyl migration byproduct present in **6** or possibly formed during the esterification process, were cleanly separated by silica gel chromatography as detected by comparative TLC analysis with crude compounds **7** obtained from a compound **6** with larger percentages of acyl migration byproduct.

In the synthetic steps involving retinoic acid, precautions were taken to avoid air contact, since an enhanced tendency to oxidation was observed for this unsaturated acid upon esterification. Thereby as main oxidation product 5,6-epoxy-5,6-dihydroretinoyl derivative is formed as determined by comparing the ^{1}H-NMR data of the isolated byproduct with the literature values.[14] Incorporation of unsaturated fatty acids in triacylglycerols is known to influence their relative oxidation rates;[15] our results with the retinoyl derivatives confirm these observations.

Quantitative removal of the thiol protecting group from **2** and **7** was achieved by reduction of the unsymmetrical disulfide with tributylphosphine in presence of water under conditions known to prevent desulfurization.[16–17] Concomitant O→S acyl migration was not observed to occur at noticeable extents, although such isomerization



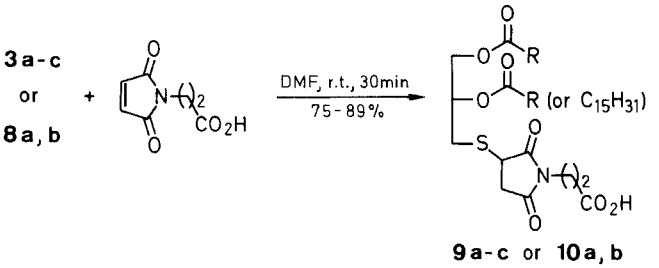
7, 8	RCO
a	myristoyl
b	retinoyl

Trt = CPh_3

Scheme **B**

at this synthetic step as well as during the addition reaction to maleoyl- peptides or proteins would not impair the homogeneity of the lipid moiety in the conjugate. In fact, the possible acyl migration byproduct (*RS*)-1-acyloxy-3-acylthio-2-propanol would be inert in the thiol-specific conjugation reactions.

Reaction of the (*RS*)-2,3-diacyloxypropanethiols **3** and **8** with 3-maleinimidopropionic acid[7] as model compound (Scheme **C**) was found to proceed rapidly and in nearly quantitative manner to give **9** and **10**, respectively, as judged by TLC of the reaction mixture even at a thiol/maleinimide ratio of 1:1. In the case of maleoyl-peptides and proteins the use of an excess of the mercapto compounds should guarantee practically quantitative lipid modification with facile removal of the unreacted highly soluble lipid reagent.



9a-c or **10a, b**

3, 9	RCO	10	RCO
a	palmitoyl	a	myristoyl
b	myristoyl	b	retinoyl
c	retinoyl		

Scheme **C**

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Addition of the thiol group to the maleinimide function leads to a second chiral center, and thus with racemic glycerol derivatives as in the present case, to two diastereoisomeric racemic mixtures. Correspondingly, the related ^{1}H-NMR spectra are characterized by two sets of signals.

Besides the use of the efficient thiol/maleinimide method for the conjugation step, the (*RS*)-2,3-diacyloxypropanethiol **3** and **8** can be *S*-activated as sulfenohydrazides and then be used for selective unsymmetrical disulfide bridging with thiol-containing target molecules following procedures previously elaborated in our laboratory.[9,18]

Melting points were determined on a Büchi apparatus and are uncorrected. ^{1}H-NMR spectra (internal standard $CHCl_3$) were recorded on a Brucker AM 400 spectrometer. Elemental analyses were obtained with a Heraeus CHN-O-Rapid apparatus. TLC was carried out on silica gel 60 or Lichroprep RP-18 plates (Merck) using the solvent systems; 1. isopropyl ether; 2. cyclohexane/$CHCl_3$/AcOH (45:45:10); 3. hexane/*t*-BuOMe/AcOH (85:10:5); 4. hexane/*t*-BuOMe/pyridine (60:10:1); 5. EtOAc; 6. MeOH/H_2O (9:1); 7. hexane/*t*-BuOMe (2:1); 8. acetone/CH_3CN (1:1). Compounds were visualized by spraying with 0.5% $KMnO_4$ in 1 M NaOH followed by heating at 100 °C; the Ellman's reagent served for thiol compounds. 1-(*tert*-Butylthio)hydrazine 1,2-dicarboxmorpholide was prepared according to known procedures,[9] 3-maleinimidopropionic acid was prepared as previously descrived,[7] thioglycerol, palmitic acid, myristic acid and retinoic acid were purchased from Fluka AG; silica gel (230–400 mesh, ASTM) and Lichroprep RP-18 were from Merck AG. Reaction with retinoic acid and related workup procedures were performed in the dark or under red light and exclusion of air whenever possible, to avoid both photoisomerization[19] and oxidation. Elemental analyses are given wherever an analytically pure sample could be obtained. Otherwise the compounds are characterized by ^{1}H-NMR spectra.

(*RS*)-3-*tert*-Butyldithio-1,2-propanediol (1):
To a stirred ice-cold solution of thioglycerol (10.8 g, 0.1 mol) and 1-(*tert*-butylthio)hydrazine 1,2-dicarboxmorpholide (52.0 g, 0.15 mol) in Ar-saturated dioxane[11] is added dropwise 1 N NaOH (100 mL). After 12 h at r.t. the solvent is evaporated and the residue is partitioned between EtOAc and 2% aq $KHSO_4$. The organic phase is washed with water (3 × 100 mL), dried (Na_2SO_4), and concentrated to a small volume. The product crystallizes on addition of petroleum ether; yield: 18 g (92%); mp 48.5–49.5 °C; homogeneous on TLC (solvent systems: 1, 2).

$C_7H_{16}O_2S_2$	calc.	C 42.82	H 8.21	S 32.66
(196.3)	found	42.86	8.27	32.38

(*RS*)-3-(*tert*-Butyldithio-1,2-dipalmitoyloxypropane (2a); Typical Procedure:
Compound **1** (0.2 g, 1 mmol) and palmitic acid (0.77 g, 3 mmol) in dry THF (10 mL) are reacted with DCC (0.62 g, 3 mmol) and DMAP (37 mg, 0.3 mmol) at r.t. for 12 h. The dicyclohexylurea is removed by filtration, the solvent evaporated and the residue is chromatographed on a silica gel column (3 × 21 cm) using hexane/*t*-BuOMe/AcOH (88:10:2) as eluent. Pure fractions are collected and upon removal of the solvents *in vacuo*, a low-melting solid is obtained; yield: 0.64 g (95%); mp 39–41 °C; homogeneous on TLC (solvent system: 3).

$C_{39}H_{76}O_4S_2$	calc.	C 69.59	H 11.38	S 9.53
(673.2)	found	69.62	11.35	9.32

^{1}H-NMR ($CDCl_3$): δ = 0.81 (t, 6H, J = 7.0 Hz, 2 × CH_3), 1.16–1.25 [m, 48H, 2 × $(CH_2)_{12}$], 1.26 (s, 9H, *t*-C_4H_9), 1.52–1.57 (m, 4H, 2 × $COCH_2CH_2$), 2.24 (t, 2H, J = 7.6 Hz), $COCH_2$), 2.24 (t, 2H, J = 7.6 Hz, $COCH_2$), 2.85 (d, 2H, J = 6.5 Hz, CH_2S), 4.12, (dd, 1H, J = 12.0, 5.6 Hz, CH_2O), 4.29 (dd, 1H, J = 12.0, 3.6 Hz, CH_2O), 5.18–5.23 (m, 1H, CHO).

2b; yield: 85%, wax-like material, homogeneous on TLC (solvent system: 3). The ^{1}H-NMR spectrum exhibited the identical set of signals as for **2a** and was consistent with the assigned structure.

2c; yield: 98%, wax-like material, eluent for chromatographic purification, hexane/*t*-BuOMe/pyridine (60:10:1), homogeneous on TLC (solvent systems: 1, 4, 5).

^{1}H-NMR ($CDCl_3$): δ = 0.96 (s, 12H, 2 × 16, 17-CH_3), 1.26 [s, 9H, $SC(CH_3)_3$], 1.38–1.41 (m, 4H, 2 × 2-CH_2), 1.52–1.58 (m, 4H, 2 × 3-CH_2), 1.64 (s, 6H, 2 × 18-CH_3), 1.93 (s, 6H, 2 × 19-CH_3), 1.95 (t, 4H, 2 × 4-CH_2), 2.27 (s, 3H, 20-CH_3), 2.28 (s, 3H, 20-CH_3), 2.93 (d, 2H, CH_2S), 4.24–4.34 (m, 2H, CH_2O), 5.25–5.31 (m, 1H, CHO), 5.71 (s, 2H, 2 × 14-CH), 6.07 (d, 2H, 2 × 8-CH), 6.07 (d, 2H, 2 × 10-CH), 6.21 (d, 2H, 2 × 7-CH), 6.21 (d, 2H, 2 × 12-CH), 6.94 (dd, 2H, 2 × 11-CH).

(*RS*)-2,3-Dipalmitoyloxypropanethiol (3a); Typical Procedure:
To an Ar-saturated solution of **2a** (0.18 g, 0.27 mmol) in CF_3CH_2OH (5 mL) *t*-BuOMe (5 mL) and water (0.1 mL) is added Bu_3P (92 µL, 0.37 mmol). The mixture is stirred at r.t. for 12 h, then the solvent is evaporated and the residue taken up in cyclohexane. The solution is washed with CF_3CH_2OH (3 × 10 mL) and evaporated to afford the product **3a** as a wax-like solid; yield: 0.16 g (quantitative); homogeneous on TLC (solvent system: 3). The product is used directly for the next step.

Product **3b** and **3c** are also obtained similarly in quantitative yield and used directly for the next step.

(*RS*)-3-*tert*-Butyldithio-1-trityloxy-2-propanol (4):
Compound **1** (1.7 g, 8.66 mmol) is heated in dry toluene (50 mL) with triphenylchloromethane (2.9 g, 10.39 mmol) and pyridine (2.5 mL) at 60 °C for 20 h. The mixture is concentrated to small volume and then diluted with hexane/*t*-BuOMe (100 mL, 9:1). Insoluble material is filtered the filtrate is evaporated and the residue is purified by reversed phase chromatography on a Lichroprep RP-18 column (3 × 20 cm) with MeOH/H_2O (9:1) as eluent followed by column chromatography on silica gel using the solvent system hexane/*t*-BuOMe (6:1). Evaporation of the solvents produced the trityl ether **5** as an oil; yield: 3.6 g (95%); homogeneous on TLC (solvent system: 3) and RP-18 TLC (solvent system: 6).

^{1}H-NMR ($CDCl_3$): δ = 1.30 (s, 9H, *t*-C_4H_9), 2.78, (dd, 1H, J = 13.4, 7.5 Hz, CH_2O), 2.88 (dd, 1H, J = 13.4, 4.9 Hz, CH_2O), 3.21 (d, 2H, J = 5.2 Hz, CH_2S), 3.98–4.03 (m, 1H, CHO), 7.19–7.48 (m, 15 H_{arom}).

(*RS*)-3-(*tert*-Butyldithio-2-palmitoyloxy-1-trityloxypropane (5):
Compound **4** (2.1 g, 4.8 mmol) and palmitic acid (2.5 g, 9.6 mmol) are reacted in anhydrous CH_2Cl_2 (50 mL) with DCC (2.0 g, 9.6 mmol) and DMAP (0.12 g, 0.96 mmol) at r.t. for 20 h. The precipitate is filtered and the filtrate evaporated to an oily residue which is purified on a silica gel column (3 × 22 cm) with hexane/*t*-BuOMe (6:1) as eluent. The pure fractions are combined and the solvents removed *in vacuo* to give an oil; yield: 2.9 g (89%); homogeneous on TLC (solvent system: 3).

^{1}H-NMR ($CDCl_3$): δ = 0.84 (t, 3H, J = 6.6 Hz, CH_3), 1.16–1.24 [m, 24H, $(CH_2)_{12}$], 1.27 (s, 9H, *t*-C_4H_9), 1.57–1.65 (m, 2H, $COCH_2CH_2$), 2.28–2.33 (m, 2H, $COCH_2$), 2.93, (dd, 1H, J = 13.5, 7.1 Hz, CH_2S), 2.99, (dd, 1H, J = 13.5, 5.7 Hz, CH_2S), 3.20 (dd, 1H, J = 10.0, 5.1 Hz, CH_2O), 3.25 (dd, 1H, J = 10.0, 4.2 Hz, CH_2O), 5.19–5.24 (m, 1H, CHO), 7.175–7.14 (m, 15 H_{arom}).

(*RS*)-3-*tert*-Butyldithio-2-palmitoyloxy-2-propanol (6):
To an ice-cold solution of **5** (0.72 g, 1.06 mmol) in CH_2Cl_2 (7.5 mL) and MeOH (0.75 mL) is added anhydrous $ZnBr_2$ (2.39 g, 10.6 mmol). After vigorous stirring for 5 min at r.t. the reaction is quenched by the addition of 5% aq NH_4OAc (50 mL). The aqueous layer is extracted with *t*-BuOMe and the organic phase is washed with water, and dried (Na_2SO_4). The solvent is removed *in vacuo* and the residue containing about 10% of the 1-palmitoyl isomer was chromatographed on a silica gel column (3 × 22 cm) with hexane/*t*-BuOMe (2:1). Fractions containing low amounts of the acyl migration byproduct are combined and evaporated *in vacuo* to afford a wax-like solid. According to TLC (solvent

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systems: 3, 7) and ^{1}H-NMR, the product is contaminated by 1–2% of the 1-palmitoyl isomer and is used without further purification directly for the next reaction step; yield: 0.39 g (85%).

^{1}H-NMR ($CDCl_3$): $\delta = 0.81$ (t, 3H, $J = 7.0$ Hz, CH_3), 1.18–1.26 [m, 24H, $(CH)_{12}$], 1.27 (s, 9H, t-C_4H_9), 1.53–1.60 (m, 2H, $COCH_2CH_2$), 2.28 (t, 2H, $J = 7.4$ Hz, $COCH_2$), 2.90 (d, 2H, $J = 6.5$ Hz, CH_2S), 3.74–3.78 (m, 2H, CH_2O), 5.01–5.06 (m, 1H, CHO).

(*RS*)-3-(*tert*-Butyldithio-1-myristoyloxy-2-palmitoyloxypropane (7a); Typical Procedure:

Compound **6** (0.24 g, 0.55 mmol) and myristic acid (0.38 g, 1.65 mmol) are reacted in dry CH_2Cl_2 (15 mL) with DCC (0.34 g, 1.65 mmol) and DMAP (20 mg, 0.165 mmol) at r.t. for 20 h. The precipitated urea is removed by filtration, the solvent is evaporated and the crude residue was purified on a silica gel column (3 × 22 cm) using hexane/t-BuOMe (8:1) as eluent. Evaporation of the solvents furnishes a low-melting solid; yield: 0.32 g (90%); mp 38–40°C; homogeneous on TLC (solvent systems: 1, 3).

$C_{37}H_{72}O_4S_2$	calc.	C 68.89	H 11.25	S 9.94
(645.1)	found	68.98	11.36	9.89

^{1}H-NMR ($CDCl_3$): $\delta = 0.84$ (t, 6H, $J = 7.0$ Hz, 2xCH_3), 1.21–1.28 [m, 44H, $(CH_2)_{12}$ + $(CH_2)_{10}$], 1.29 [s, 9H, t-C_4H_9), 1.53–1.68 (m, 4H, 2 × $COCH_2CH_2$), 2.27 (t, 2H, $J = 7.5$ Hz, $COCH_2$), 2.28 (t, 2H, $J = 7.5$ Hz, $COCH_2$), 2.88 (d, 2H, $J = 6.5$ Hz, CH_2S), 4.15, (dd, 1H, $J = 12.0$, 5.6 Hz, CH_2O), 4.32 (dd, 1H, $J = 12.0$, 3.6 Hz, CH_2O), 5.225–5.265 (m, 1H, CHO).

7b; yield: 87%; oil; eluent for chromatographic purification, hexane/t-BuOMe/pyridine (60:10:1); homogeneous on TLC (solvent system: 4) and RP-18 TLC (solvent system: 8).

^{1}H-NMR ($CDCl_3$): $\delta = 0.81$ (t, 3H, ω-CH_3), 0.96 (s, 6H, 16,17-CH_3), 1.11–1.25 [m, 24H, $(CH_2)_{12}$], 1.26 [s, 9H, $SC(CH_3)_3$], 1.40 (dd, 2H, 2-CH_3), 1.52–1.58 [overlapping m, 4H, 3-CH_2 and $COCH_2CH_2$), 1.64 (s, 3H, 18-CH_3), 1.93 (s, 3H, 19-CH_3), 1.95 (t, 2H, 4-CH_2), 2.255 (t, 2H, $COCH_2$), 2.28 (s, 3H, 20-CH_3), 2.88 (d, 2H, CH_2S), 4.20 and 4.30 (dd, respectively, 2H, CH_2O), 5.23–5.26 (m, 1H, CHO), 5.70 (s, 1H, 14-CH), 6.07 (d, 1H, 8-CH), 6.07 (d, 1H, 10-CH), 6.21 (d, 1H, 12-CH), 6.215 (d, 1H, 7-CH), 6.94 (dd, 1H, 11-CH).

(*RS*)-3-Myristoyloxy-2-palmitoyloxypropanethiol (8a) and (*RS*)-2-Palmitoyloxy-3-retinoyloxypropanethiol (8b):

These compounds are prepared by reduction of the unsymmetrical disulfides **7a,b** with Bu_3P as described for **3a–c**. Yields are > 95% and the products are used directly for the next step.

(*RS*)-3-[(3ξ*N*-Carboxyethyl-2,5-dioxo-3-pyrrolidyl)thio]-1,2-dipalmitoyloxypropane (9a); Typical Procedure:

To an Ar-saturated solution of the mercapto compound **3a** (165 mg, 0.28 mmol) in DMF (2 mL) is added 3-maleinimidopropionic acid (47 mg, 0.28 mmol) in DMF (1 mL). After stirring at r.t. for 30 min, the solvent is evaporated and the residue is partitioned between t-BuOMe and 5% AcOH. The organic phase is washed with water, dried (Na_2SO_4), and evaporated to dryness. The solid is recrystallized from hexane; yield: 0.18 g (85%); mp 55–57°C; homogeneous on TLC (solvent systems: 2, 3).

$C_{42}H_{75}NO_8S$	calc.	C 66.89	H 10.02	N 1.86
(754.1)	found	66.20	10.04	1.85

^{1}H-NMR ($CDCl_3$): *racemic mixture* 1, $\delta = 0.81$ (t, 6H, $J = 7.0$ Hz, 2 × CH_3), 1.19–1.26 [m, 48H, 2 × $(CH_2)_{12}$], 1.52–1.57 (m, 4H, 2 × $COCH_2CH_2$), 2.25 (t, 4H, $J = 7.5$ Hz, 2 × $COCH_2CH_2$), 2.60–2.66 (overlapping m, 2H, CH_2CO_2H), 2.39 (dd, 1H, $J = 18.6$, 3.6 Hz, CH_2CO), 3.05 (dd, 1H, $J = 18.7$, 9.1 Hz, CH_2CO), 2.76 (dd, 1H, $J = 14.3$, 7.5 Hz, CH_2S), 3.22 (dd, 1H, $J = 14.3$, 5.0 Hz, CH_2S), 3.69–3.82 (overlapping m, 3H, $NCH_2CH_2CO_2H$ + SCHCO), 4.11 (dd, 1H, $J = 12.2$, 5.9 Hz, CH_2O), 4.31 (dd, 1H, $J = 12.0$, 3.6 Hz, CH_2O), 5.28 (m, 1H, CHO).

racemic mixture 2; $\delta = 0.81$ (t, 6H, $J = 7.0$ Hz, 2 × CH_3), 1.19–1.26 [m, 48H, 2 × $(CH_2)_{12}$], 1.52–1.57 (m, 4H, 2 × $COCH_2CH_2$), 2.25 (t, 4H, $J = 7.5$ Hz, 2 × $COCH_2CH_2$), 2.60–2.66 (overlapping m, 2H, CH_2CO_2H), 2.39 (dd, 1H, $J = 18.5$, 3.8 Hz, $SCHCH_2CO$), 3.05 (dd, 1H, $J = 18.7$, 9.1 Hz, $SCHCH_2CO$), 2.91 (dd, 1H, $J = 14.1$, 6.7 Hz, CH_2S), 3.11 (dd, 1H, $J = 14.1$, 6.5 Hz, CH_2S), 3.69–3.83 (overlapping m, 3H, NCH_2 + SCH), 4.14 (dd, 1H, $J = 12.3$, 6.1 Hz, CH_2O), 4.27 (dd, 1H, $J = 12.0$, 3.4 Hz, CH_2O), 5.13 (m, 1H, CHO).

9b; yield: 87%; mp 43–45°C.

$C_{38}H_{67}NO_8S$	calc.	C 65.39	H 9.68	N 2.01	S 4.59
(698.0)	found	65.45	9.72	1.99	4.34

The ^{1}H-NMR spectrum is consistent with the assigned structure.

9c: In this case, the reaction mixture is worked up as follows. The residue obtained after removal of the solvent is dissolved in cyclohexane and washed with 20% aq CF_3CH_2OH. The cyclohexane layer is dried (Na_2SO_4), evaporated and the residue is triturated with hexane; yield: 78%; wax-like product homogeneous on TLC (solvent systems: 2, 3). The ^{1}H-NMR spectrum is consistent with the assigned structure.

(*RS*)-3-[(3ξ*N*-Carboxyethyl-2,5-dioxo-3-pyrrolidyl)thio]-1-myristoyloxy-2-palmitoyloxypropane (10a); Typical Procedure:

The title compound is prepared from **8a** and 3-maleinimidopropionic acid and worked up as described for **9a**; yield: 79%; mp 51–52°C. The ^{1}H-NMR spectrum is consistent with the assigned structure.

$C_{40}H_{71}NO_8S$	calc.	C 66.17	H 9.86	N 1.93	S 4.42
(726.1)	found	66.25	9.90	1.90	4.37

10b; The mercapto compound **8b** was reacted with 3-maleinimidopropionic acid as described for **9a** and the mixture is worked up as reported for **9c**; oil; yield: 75%; homogeneous on TLC (solvent systems: 2, 3) and RP-18 TLC (solvent system: 8).

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