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Synthesis of heterocyclic platelet activating factor analogues† ☆

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Abstract

The synthesis of heterocyclic analogues of the platelet activating factor is described. The preparation starts with acylating *rac*-tetrahydro-1,3-thiazine-4-carboxylic acid ethyl ester, with palmitoyl chloride to form the amide linkage. Following ester reduction, the phosphocholine part is introduced via 2-chloro-2-oxo-1,3,2-dioxaphospholane and subsequent ring opening with trimethylamine under pressure. Furthermore, the related L-thiazolidine analogue is prepared using the same procedure. In addition the sulfinyl and sulfonyl derivatives of this compound are obtained by oxidation with 3-chloro-perbencoic acid. From one sulfinyl intermediate the diastereomeres are separated and their conformations are determinated by ^{13}C-NMR spectroscopy.

Keywords: Phospholipid synthesis; Cyclic platelet activating factor analogue; Cyclic thioether; Chiral sulphur compound

1. Introduction

Ether lipids represent a structural class of phospholipids that exhibit in vitro and in vivo activity against a variety of tumor cell lines [1]. However, the exact mechanism of the neoplastic cell growth inhibitory property is not fully understood at present. Considerable preclinical data suggest that the mechanism of action may be caused by generation of cytotoxic macrophages [2], direct cytotoxicity [3], diminished activity of alkyl cleavage enzymes in tumor cells [4], selective membrane interaction [5] and inhibition of protein kinase C [6].

In contrast to various conventional cytotoxic agents currently in use in the clinical treatment of malign tumors, alkyl lysophospholipids do not affect DNA [7] and cause only slight side effects, as shown in preliminary clinical phase I trials [8]. Therefore, synthetic ether lipids and related substances have been established as a new class of tumoricidal agents, and there seems to be various possibilities for their clinical application.

Well-known members of this class are ET-18-OCH_3 [9], Ilmofosine [10] and SRI-62-834 [11], a

† Dedicated to Prof. H.J. Roth on the occasion of his 65th birthday.

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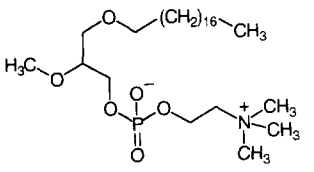
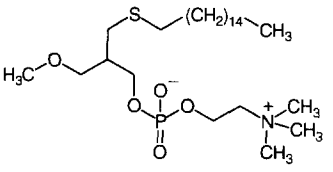
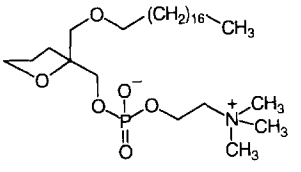


ET-18-OCH_3



Ilmofosine



SRI-62-834

cyclic analogue of ET-18-OCH_3. A common characteristic of these three substances is an ether or thioether function, which links the alkyl chain with the 'backbone'. Replacement of the ether linkage of ET-18-OCH_3 with an amide group leads to 1-*N*-alkyl amide analogues with activity against HL60 human leukemic cells which is comparable to that of ET-18-OCH_3 [12]. This indicates that the ether linkage at the position C(1) is not a necessary structural characteristic for anti-neoplastic cell growth inhibition by alkyl lysophospholipids.

Some time ago we described the synthesis of alkylamido phospholipids containing a methioninol backbone [13]. Inspired by the fact that cyclic oxygen analogues of alkyl lysophospholipids also exhibit strong cytotoxicity against a variety of tumor cell lines [14] and cause an enhancement of macrophage activity, we have developed a novel cyclic alkyl amide analogue containing a cyclical methioninol-related 'backbone'. Furthermore, the five-membered cyclic homologue and its sulfonyl and sulfinyl derivatives are described. These rigid analogues should exhibit a higher selectivity than non-cyclic compounds, since they show a decreased rotation around the 'backbone'. Contrary to SRI-62-834, the cycle of these lipids contains two heteroatoms, the framework has been incorporated into the ring, the 'backbone' consists only of two carbon atoms and the oxygen has been exchanged with sulphur. Furthermore, the distance between the 'backbone' and sulphur has been increased.

This work is a continuation of our previous efforts to synthesize ether lipid analogues, which should be useful for a better understanding of the mechanisms of cytotoxicity and for elucidating the structural features responsible for the observed anti-neoplastic property.

2. Materials and methods

2-Chloro-2-oxo-1,3,2-dioxaphospholane, 97% pure, trimethylamine anhydrous, 98% pure, L-thiazolidine-4-carboxylic acid, >97% pure, homocysteine thiolactone-HCl, >99% pure, triethylamine, > 98% pure, 3-chloro-perbencoic acid, 55% pure (containing 10% 3-chloro-benzoic acid and 35% water) and lithium borohydride, 95% pure, were purchased from Fluka AG (Neu-Ulm, Germany). Palmitoyl chloride, 98% pure, was purchased from Aldrich (Steinheim, Germany).

2.1. Analytical and preparative methods

^1H- and ^{13}C-NMR spectra: Brucker AC-80 spectrometer (80 MHz); internal standard: TMS; IR spectra: Perkin-Elmer FT-IR spectrometer 1750 (KBr); melting points: Gallenkamp Melting Point Apparatus, uncorrected; optical rotations were measured with a Perkin-Elmer 241.

Solvents were removed under vacuum at 40°C. Ethanol and diethyl ether were dried with sodium and used immediately after distillation. Benzene, acetonitrile and tetrahydrofuran were distilled from calcium hydride directly into the dry reaction pots. Triethylamine was dried over potassium hydroxide and then distilled. Palmitoyl chloride was distilled before use (158–160°C, 0.2 mbar). *rac*-Tetrahydro-1,3-thiazine-4-carboxylic ethyl

ester-HCl and L-thiazolidine-4-carboxylic acid ethyl ester-HCl were prepared by passing a stream of HCl gas through a suspension of the relevant compound in dry ethanol as described elsewhere [15].

Phospholipids were purified by MPLC (Labomat MD 80/100, Labomatic) on silica gel (Labogel NP 30–60, 30m/60A from Labomatic); column: vol. 410 ml, diameter 37 mm. Pressure: 10–12 bar. Flow: 10–12 ml/min. Solvent: chloroform/methanol/NH_3 25% (65:35:6 by vol.).

Column chromatography of intermediates: silical gel (Silicagel 60, particle size: 0.063–0.2 mm, Merck, Germany).

The purity of the lipids and intermediates was checked by thin-layer chromatography (TLC) (Polygram SIL G/UV 254, Macherey-Nagel, layer: 0.25 mm silica gel, 40 × 80 mm), without saturation.

Detection: alkalised aqueous solution of $KMnO_4$. Lipids were detected by spraying with Molybdenum Blue (Sigma)/0.1 mol H_2SO_4 (3:1).

2.2. *rac-(N-hexadecanoyl)-tetrahydro-1,3-thiazine-4-carboxylic acid ethyl ester* (1)

4.0 g (0.0189 mol) of *rac*-tetrahydro-1,3-thiazine-4-carboxylic acid ethyl ester-HCl was stirred in 10.0 g (0.099 mol) of dry triethylamine containing 5 ml of dry tetrahydrofuran. After 30 min the mixture was diluted with 100 ml of dry tetrahydrofuran, and 5.0 g (0.0181 mol) of palmitoyl chloride was added dropwise at 0°C. The reaction mixture was allowed to warm slowly to room temperature, and stirring was continued for 3 h. Then 250 ml of water was added and the emulsion was extracted with diethyl ether (250 ml). The organic layer was washed with water, dried with anhydrous Na_2CO_3, filtered and extracted with 1 N HCl (4 × 10 ml). The organic layer was then extracted with a saturated NaCl solution, dried with Na_2SO_4, filtered and evaporated to dryness to give the product **1** as a white wax. TLC: R_f ~ 0.8 (ethyl acetate : petroleum ether (60/80); 3:2; v/v). Yield: 7.0 g (93.9%). $C_{23}H_{43}NO_3S$ (413.66). m.p.: 42–43°C. Calculated: C 66.78 H 10.48 N 3.39. Found: C 67.26 H 10.82 N 3.31.

^{1}H-NMR (chloroform-d_1): δ (ppm) = 0.84 (t, 3H, CH_3); 1.15–1.72 (m, 26H, CH_2); 1.22 (t, 3H, CH_3-ester); 1.79–3.09 (m, 6H, CH_2CON, CH_2CH_2S); 3.84–4.35 (q + additional peaks 2H, CH_2OOC); 4.57 (s, 2H, NCH_2S); 5.49 (t, 1H, CH). ^{13}C-NMR (chloroform-d_1): δ (ppm) = 13.53, 13.66 ($2CH_3$); 22.13 (CH_2CH_3); 23.92, 24.33, 24.64, 26.97, 27.89, 28.82, 28.92, 29.15 $12CH_2$ + additional peaks; 31.41 (CH_2S); 32.35, 32.57 (CH_2CON + additional peak); 39.90, 44.33 (NCH_2S + additional peak); 51.30, 55.44 (CH + additional peak); 60.68, 61.12 (CH_2OOC + additional peak); 169.20, 169.78 (C=O-ester + additional peak); 170.69, 171.38 (C=O-amid +additional peak). IR: 2952, 2918, 2871, 2850 (CH_2); 1733 (C=O-amid); 1642 (C=O-ester).

2.3. *rac-(N-hexadecanoyl)-4-hydroxymethyl-tetrahydro-1,3-thiazine* (2)

6.0 g (0.0145 mol) of the intermediate **1** was dissolved in 100 ml of dry diethyl ether. 0.20 g (0.0092 mol) of lithium borohydride was added, and the mixture was heated to reflux for 4 h. The mixture was quenched with water (5 ml), and extracted twice with 10 ml of water and once with 10 ml of a saturated NaCl solution. The organic layer was dried with Na_2SO_4, filtered and evaporated to dryness. The residue was purified by column chromatography on silica gel, using diethyl ether as a solvent. TLC: R_f ~ 0.7 (diethyl ether : petroleum ether (60/80); 3:2; v/v). Yield: 3.2 g (59.4%) $C_{21}H_{41}NO_2S$ (371.62); m.p.: 41–43°C. Calculated: C 67.87 H 11.12 N 3.77. Found: C 67.87 H 11.15 N 3.83.

^{1}H-NMR (chloroform-d_1): δ (ppm) = 0.85 (t, 3H, CH_3); 1.23–1.71 (m, 26H, CH_2); 1.87–3.08 (m, 6H, CH_2CON, CH_2CH_2S); 3.65–4.63 (m, 6H, CH, CH_2OH, NCH_2S). ^{13}C-NMR (chloroform-d_1): δ (ppm) = 13.67 (CH_3); 22.24 (CH_2CH_3); 22.69, 23.11, 24.55, 24.91, 25.38, 28.68, 28.95, 29.06, 29.27 31.50 ($12CH_2$ + additional peaks); 32.94, 33.43 (CH_2S + additional peak); 37.09 (CH_2CON); 42.31 (NCH_2S); 51.19, 54.28 (CH + additional peak); 59.75, 60.84 (CH_2O + additional peak); 172.42, 173.02 (C=O + additional peak). IR: 3393 (OH); 2956, 2922, 2851 (CH_2); 1624 (C=O) cm^{-1}.

2.4. rac-[(N-hexadecanoyl)-tetrahydro-1,3-thiazine-4-oxymethyl]-2-oxo-1,3,2-dioxaphospholane (**3**)

Compound **2** (2.5 g, 0.00672 mol) was solved together with 1.0 g (0.0071 mol) of 2-chloro-2-oxo-1,3,2-dioxaphospholane in 20 ml of dry benzene. At 5°C 0.72 g (0.00713 mol) of dry triethylamine was added in one portion. The mixture was stirred at room temperature for 24 h, the precipitated salt was filtered and the solvent was removed under reduced pressure. The residue obtained was used for the next step without further purification. $C_{23}H_{44}NO_5PS$ (477.6).

2.5. rac-(N-hexadecanoyl)-4-methyl-tetrahydro-1,3-thiazine-phosphocholine (**4**)

The obtained amount of compound **3** was dissolved in 60 ml of dry acetonitrile and transferred to a pressure bottle. 10 ml of dry trimethylamine was added, and the solution was stirred at 65°C for 40 h. The precipitate formed was collected by suction and purified by mean pressure liquid chromatography, using chloroform/methanol/NH_3 25% (65:35:6 by vol.) as a solvent. Yield: 0.8 g (22.2%) of a white hygroscopic solid. TLC: R_f ~ 0.3 (chloroform : methanol : NH_3 25%; 65:35:6; v/v/v). $C_{26}H_{53}N_2O_5PS$ (536.8). m.p.: 180–181°C. Calculated: C 58.18 H 09.95 N 5.22. Found: C 56.23 H 10.37 N 5.01.

^{1}H-NMR (methanol-d_4): δ (ppm) = 0.89 (t, 3H, CH_3); 1.28–1.58 (m, 26H, CH_2); 1.82–3.04 (2m, 6H, CH_2NOC, CH_2CH_2S); 2.91–3.28 (m, 2H, CCH_2S); 3.22 (s, 9H, $^+N(CH_3)_3$) 3.52–5.15 (m, 9H, CH, $2CH_2O$, CH_2N^+, NCH_2S). ^{13}C-NMR (methanol-d_4): δ (ppm) = 14.51 (CH_3); 23.67 (CH_2CH_3); 23.91, 26.09, 28.52, 27.01, 28.35, 30.41, 30.74, 33.01 ($12CH_2$ + additional peaks); 34.17, 34.56 (CH_2S + additional peak); 38.31 (CH_2CON); 43.38 (NCH_2S); 50.85 (CH); 54.65 (3 $^+NCH_3$); 60.35 (CH_2O-choline); 64.55 (CH_2O), 67.34 (CH_2N^+); 174.42, 174.72 (C=O + additional peak). IR: 2920, 2851 (CH_2); 1641 (C=O) cm^{-1}.

2.6. L-(N-hexadecanoyl)-thiazolidine-4-carboxylic acid ethyl ester (**5**)

The preparation was carried out as described for compound **1**, using L-thiazolidine-4-carboxylic acid ethyl ester-HCl as starting material. TLC: R_f ~ 0.5 (diethyl ether : petroleum ether (60/80); 1:1; v/v). Yield: 84.9% of a white solid. $C_{22}H_{41}NO_3S$ (399.6) m.p.: 39–40°C. Calculated: C 66.12 H 10.34 N 3.50. Found: C 66.15 H 10.06 N 3.52 $[\alpha]_{578}^{20} = -80.7°$ (c = 1.25, $CHCl_3$).

^{1}H-NMR (chloroform-d_1): δ (ppm) = 0.85 (t, 3H, CH_3); 1.16–1.71 (m, 26H, CH_2); 1.22 (t, 3H, CH_3-ester); 2.25 (m, 2H, CH_2CON); 3.24 (m, CH_2S); 4.22 (q, 2H, CH_2OOC + additional peaks); 4.56 (d, 2H, NCH_2S); 5.08 (t + additional peak at 4.75, 1H, CH). ^{13}C-NMR (chloroform-d_1): δ (ppm) = 13.24 ($2CH_3$); 21.84 (CH_2CH_3); 23.85 (CH_2CH_2CON); 28.53, 28.72, 28.87, 31.11 ($11CH_2$); 31.84 (CH_2S); 33.68 (CH_2CON); 47.49 (NCH_2S); 60.38 (CH_2OOC); 60.75, 60.97 (CH + additional peak); 168.85 (C=O-ester); 170.12, 170.53 (C=O-amid + additional peak). IR: 2921, 2850 (CH_2); 1741 (C=O-amid); 1649 (C=O-ester) cm^{-1}.

2.7. L-(N-hexadecanoyl)-4-hydroxymethyl-thiazolidine (**6**)

The ester **5** was treated with lithium borohydride in the same manner as described for compound **2**. The product was purified by column chromatography, using diethyl ether/petroleum ether (60/80) (3:2) as a solvent. TLC: R_f ~ 0.7 (diethyl ether : petroleum ether (60/80); 1:1: v/v). Yield: 54.4% of a white solid. $C_{20}H_{39}NO_2S$ (357.6) m.p.: 41–43°C. Calculated: C 67.17 H 10.99 N 3.92. Found: C 67.00 H 11.21 N 3.83 $[\alpha]_{578}^{20} = -33.3°$ (c = 1.77, $CHCl_3$).

^{1}H-NMR (chloroform-d_1): δ (ppm) = 0.84 (3H, CH_3); 1.25–1.71 (m, 26H, CH_2); 2.34 (m, 2H, CH_2CON); 2.77–3.25 (m, CH_2S); 3.64 (broad peak, 3H, CH_2OH); 3.47–4.71 (m, 3H, CH, NCH_2S). ^{13}C-NMR (chloroform-d_1): δ (ppm) = 13.58 (CH_3); 22.17 (CH_2CH_3); 24.29, 24.79 (CH_2CH_2NCO + additional peak); 28.88–29.19 ($11CH_2$ + additional peaks); 31.42, 32.12 (CH_2S + additional peak); 34.49 (CH_2CON); 46.55, 48.32 (NCH_2S + additional peak); 60.81 (CH); 61.45 (CH_2OH); 171.53, 171.78 (C=O + additional peak). IR: 3235 (OH); 2922, 2850 (CH_2); 1644 (C=O) cm^{-1}.

2.8. L-[*(N-hexadecanoyl)-thiazolidine-4-oxymethyl*] *2-oxo-1,3,2-dioxaphospholane (7)*

The cyclic triester was prepared by treatment of **6** with 2-chloro-2-oxo-1,3,2-dioxaphospholane as described above. $C_{22}H_{41}NO_5PS$ (477.6).

2.9. L-*(N-hexadecanoyl)-4-methyl-thiazolidine-phosphocholine* (**8**)

The cyclic phosphoric acid ester **7** was treated as described for compound **4**. Yield: 59% of a white hygroscopic solid. $C_{25}H_{51}N_2O_5PS$ (522.7). TLC: R_f ~ 0.3 (chloroform:methanol:NH_3 25%; 65:35:6; v/v/v); m.p.: 237–238°C. Calculated: C 57.44 H 09.83 N 5.36. Found: C 54.04 H 10.18 N 5.20 $[\alpha]_{578}^{20} = -22.4°$ (c = 1.67, $CHCl_3$).

^{1}H-NMR (methanol-d_4): δ (ppm) = 0.89 (t, 3H, CH_3); 1.28–1.59 (m, 26H, CH_2); 2.30–2.52 (m, 2H, CH_2CNO); 2.91–3.34 (m, 2H, CH_2S); 3.25 (s, 9H, $^+N(CH_3)_3$); 3.54–4.78 (br.m, 9H, CH, $2CH_2O$, CH_2N^+, NCH_2S). ^{13}C-NMR (methanol-d_4): δ (ppm) = 14.44 (CH_3); 23.67 (CH_2CH_3); 25.95, 26.42 (CH_2CH_2CON + additional peak); 30.39, 30.73 ($11CH_2$); 32.62, 33.01 (CH_2S + additional peak); 35.72 (CH_2CON); 50.04 (NCH_2S); 54.71 ($^+NCH_3$); 60.31 (CH_2O-choline); 60.99 (CH); 64.45, 65.52 (CH_2O + additional peak); 67.47 (CH_2N^+); 173.64, 174.49 (C=O + additional peak). IR: 2921, 2851 (CH_2); 1646 (C=O) cm^{-1}.

2.10. L-*(N-hexadecanoyl)-thiazolidine-S-dioxide-4-carboxylic acid ethyl ester* (**9**)

To a solution of 11.1 g (0.0278 mol) **1** in dichloromethane (150 ml), 13.5 g (0.0782 mol) of 3-chloro-perbencoic acid was added in several portions. After stirring for 10 h at room temperature the formed precipitate was removed by suction and the mixture was alkalised with an aqueous Na_2CO_3 solution (500 ml). The dichloromethane was distilled off under reduced pressure and the precipitate formed was collected. The crude product was purified by column chromatography, using petroleum ether (60/80)/diethyl ether (2:3) as a solvent. TLC: R_f ~ 0.6 (petroleum ether (60/80) : diethyl ether; 2:3; v/v). Yield: 8.2 g (68.3%) of a white solid. $C_{22}H_{41}NO_5S$ (431.6), m.p.: 69–71°C. Calculated: C 61.22 H 9.57 N 3.25. Found: C 61.24 H 9.66 N 3.21 $[\alpha]_{578}^{20} = -48.5°$ (c = 1.64, $CHCl_3$). ^{1}H-NMR (chloroform-d_1): δ (ppm) = 0.54 (t, 3H, CH_3), 1.16–1.82 (m, 26H, CH_2); 1.28 (t, 3H, CH_3-ester); 2.31(m, 2H, CH_2CON); 3.48 (d + additional peaks, 2H, CH_2SO_2); 4.22 (q, 2H, CH_2OOC); 4.48 (d + additional peak, 2H, NCH_2SO_2); 5.41 (t + additional peaks at 4.99, 1H, CH). ^{13}C-NMR (chloroform-d_1): δ (ppm) = 13.87 ($2CH_3$); 22.48 (CH_2CH_3); 24.28 (CH_2CH_2CON); 29.17, 29.26, 29.47, 31.73 ($11CH_2$); 34.32 (CH_2CON); 51.28, 52.50 (CH_2SO_2 + additional peak); 53.86, 56.06 (CH + additional peak); 62.40 (CH_2OOC); 61.75, 63.16 (NCH_2SO_2 + additional peak); 167.96 (C=O-ester); 171.80 (C=O-amid). IR: 2952, 2918, 2850 (CH_2); 1755, 1736 (C=O-ester); 1682, 1659 (C=O-amid) cm^{-1}; (CCl_4) 1752 (C=O-ester); 1684 (C=O-amid) cm^{-1}.

2.11. L-*(N-hexadecanoyl)-4-hydroxymethyl-thiazolidine-S-dioxide* (**10**)

To a solution of 6.4 g (0.0148 mol) **9** in dry diethyl ether (100 ml), 0.18 g (0.00826 mol) of lithium borohydride was added. The reaction mixture was stirred for 1 h at room temperature and then refluxed for another hour. After cooling, 100 ml of water was added carefully and the mixture was extracted with diethyl ether/ethyl acetate (4:1, 200 ml). The organic layer was separated, dried with Na_2SO_4 and evaporated to dryness. The residue was purified by column chromatography, using petroleum ether (60/80)/diethyl ether (2:3) as a solvent. TLC: R_f ~ 0.7 (petroleum ether (60/80):diethyl ether; 2:3, v/v). Yield: 3.6 g (62.5%). $C_{20}H_{39}NO_4S$ (389.6). m.p.: 110–120°C. Calculated: C 61.66 H 10.09 N 3.60. Found: C 61.38 H 10.31 N 3.46 $[\alpha]_{578}^{20} = -46.9°$ (c = 1.50, $CHCl_3$).

^{1}H-NMR (chloroform-d_1): δ (ppm) = 0.85 (t, 3H, CH_3); 1.20–1.80 (m, 26H, CH_2); 2.32 (m, 2H, CH_2CON); 3.38 (d, 2H, CH_2SO_2); 3.82 (m, 2H, CH_2O); 4.12–5.37 (br.m, 3H, CH, NCH_2SO_2). ^{13}C-NMR (chloroform-d_1): δ (ppm) = 13.83 (CH_3); 22.42 (CH_2CH_3); 24.51 (CH_2CH_2CON); 29.10, 29.21, 29.45, 31.67 ($11CH_2$); 32.54, 34.65

(CH_2CON + additional peak); 49.54, 51.09 (CH_2SO_2 + additional peak); 54.47, 56.29 (CH + additional peak); 61.48, 62.19, 63.72 (NCH_2SO_2, CH_2O, + additional peak); 172.74 (C=O). IR: 3468 (OH); 2922, 2851 (CH_2); 1651, 1630 (C=O, peak doubling) cm^{-1}.

2.12. L-[*(N-hexadecanoyl)-thiazolidine-S-dioxide-4-oxymethyl]-2-oxo-1,3,2-dioxaphospholane* (**11**)

$C_{22}H_{42}N_2O_5PS$ (463.6) and

2.13. L-*(N-hexadecanoyl)-4-methyl-thiazolidine-S-dioxide-phosphocholine* (**12**)

Yield: 46.5% of a white hygroscopic solid. $C_{25}H_{51}N_2O_5PS$ (522.7) was prepared as described for compounds **3** and **4**. TLC: R_f ~ 0.25 (chloroform:methanol:NH_3 25%; 65:35:6; v/v/v); m.p.: 240°C (decomposition). Calculated: C54.13 H 9.27 N 5.05. Found: C 51.80 H 9.46 N 4.82 $[\alpha]_{578}^{20} = -49.7°$ (c = 1.74, $CHCl_3$). ^{1}H-NMR (methanol-d_4): δ (ppm) = 0.89 (t, 3H, CH_3); 1.12–1.87 (m, 26H, CH_2); 2.40 (m, 2H, CH_2CON); 3.23(s, 9H, $^+N(CH_3)_3$); 3.58 (m, 4H, CH_2SO_2, CH_2N^+); 3.90–5.31 (broad m, 7H, CH, $2CH_2O$, NCH_2SO_2). ^{13}C-NMR (methanol-d_4): δ (ppm) = 14.46 (CH_3); 23.66 (CH_2CH_3); 25.84 (CH_2CH_2CON); 30.40, 30.71, 32.98 ($11CH_2$); 35.52 (CH_2CON); 50.36 (CH_2SO_2); 54.69, 56.62 ($^+N(CH_3)_3$, CH + additional peak); 60.42 (CH_2O-choline); 62.42, 64.60 (NCH_2SO_2 + additional peak); 65.82 (CH_2O); 67.60 (CH_2N^+). IR: 2920, 2851 (CH_2); 1660 (C=O) cm^{-1}.

2.14. L-*(N-hexadecanoyl)-thiazolidine-S-oxide-4-carboxylic acid ethyl ester* (**13**) (mixture of diastereomeres)

6.6 g (0.0382 mol) of 3-chloro-perbencoic acid, dissolved in 150 ml methylene chloride, was added dropwise to a cooled solution (0°C) of 14.0 g (0.0350 mol) **12** in dichloromethane (200 ml). After stirring at room temperature for 12 h, the dichloromethane was extracted once with water (50 ml) and ten times with a saturated Na_2CO_3 solution. After drying the organic solvent with Na_2SO_4, the dichloromethane was removed and the residue was purified by column chromatography, using petroleum ether (60/80)/diethyl ether (2:3) to elute the more lipophilic impurities. Then the elution was continued with dichloromethane/methanol (9:1), yielding 11.5 g (79%) of the desired product. TLC: R_f ~ 0.7 (chloroform : methanol; 9:1; v/v). $C_{22}H_{41}NO_4S$ (415.6) m.p.: 40–41°C. Calculated: C 63.58 H 09.94 N 3.37. Found: C 62.80 H 10.19 N 3.24 $[\alpha]_{578}^{20} = -97.4°$ (c = 2.02, $CHCl_3$). ^{1}H-NMR (chloroform-d_1): δ (ppm) = 0.81 (t, 3H, CH_3); 1.14–1.82 (m, 26H, CH_2); 1.22 (t, 3H, CH_3-ester); 2.29 (m, 2H, CH_2CON); 2.75–3.54 (m, 2H, CH_2SO); 4.03–5.41 (m, 5H, CH, CH_2OOC, NCH_2SO). ^{13}C-NMR (chloroform-d_1): δ (ppm) = 13.25 (CH_3); 21.64 (CH_2CH_3); 23.98 (CH_2CH_2CON); 28.53, 28.88, 31.12 ($11CH_2$); 32.73, 33.61 (CH_2CON + additional peak); 52.09, 53.56 (CH_2SO + additional peak); 57.30, 58.06 (CH + additional peak); 60.90, 61.40 (CH_2OOC + additional peak); 67.06, 68.25, 68.82 (NCH_2SO + additional peak); 168.29, 169.43 (C=O-ester + additional peak); 170.81, 171.86 (C=O-amid + additional peak). IR: 2919, 2851 (CH_2); 1742 (C=O-ester); 1657 (C=O-amid) cm^{-1}.

2.15. L-*(N-hexadecanoyl)-4-hydroxymethyl-thiazolidine-S-oxide* (**14**) (mixture of diastereomeres)

A solution of 9.0 g (0.0216 mol) **13** and of 0.24 g (0.0170 mol) lithium borohydride in 100 ml of benzene/diethyl ether (1:1) was heated to reflux for 5 h. After cooling, the reaction mixture was quenched with water (10 ml) and evaporated to dryness. Then 100 ml of water was added to the residue and the mixture was extracted with ethyl acetate/diethyl ether (1:1). The organic solvent was separated, dried with Na_2SO_4 and evaporated to dryness. The crude product was chromatographied at silica gel, using diethyl ether for eluting the more lipophilic impurities and then with diethyl ether/methanol (45:5) to obtain 4.5 g (55.7%) of pure **14** as a white solid. TLC: R_f ~ 0.4 and 0.45 (diethyl ether : methanol; 95:5; v/v) $C_{20}H_{39}NO_3S$ (373.6) m.p.: 103–104°C. Calculated: C 64.30 H 10.52 N 3.75. Found: C 63.69 H 10.69 N 3.64 $[\alpha]_{578}^{20} = -69.2°$ (c = 1.90, $CHCl_3$). ^{1}H-NMR (chloroform-d_1): δ (ppm) = 0.79 (t, 3H, CH_3);

1.08–1.77 (m, 26H, CH_2); 2.28 (m, 2H, CH_2CON); 3.10 (m, 2H, CH_2SO); 3.37–5.25 (m, 6H, CH, CH_2OH, NCH_2SO). ^{13}C-NMR (chloroform-d_1): δ (ppm) = 13.93 (CH_3); 22.48 (CH_2CH_3); 24.60, 24.80 (CH_2CH_2CON); 29.17, 29.28, 29.50, 31.74 ($11CH_2$); 33.47, 34.81 (CH_2CON + additional peak); 52.47, 54.20 (CH_2SO + additional peak); 57.66, 60.43 (CH); 62.59, 62.68 (CH_2O); 67.55, 69.38, 69.56 (NCH_2SO + additional peak); 172.14, 173.52 (C=O + additional peak). IR: 3401 (OH); 2955, 2920, 2850 (CH_2); 1650 (C=O) cm^{-1}.

2.16. L-*[(N-hexadecanoyl)-thiazolidine-S-oxide-4-oxymethyl]-2-oxo-1,3,2-dioxaphospholane* **(15)** *(mixture of diastereomeres)*

14 was phosphorylated as described for **3**. $C_{22}H_{42}NO_6PS$ (479.6).

2.17. L-*(N-hexadecanoyl)-4-methyl-thiazolidine-S-oxide-phosphocholine* **(16)** *(mixture of diastereomeres)*

The synthesis was carried out as described for **4**. TLC: R_f ~ 0.2 (chloroform : methanol : NH_3 25%; 65:35:6; v/v/v). $C_{25}H_{51}N_2O_6PS$ (538.7). m.p.: 223–225°C decomposition. Calculated: C 55.74 H 09.54 N 5.20. Found: C 53.61 H 10.04 N 6.34 $[\alpha]_{578}^{20}$ = −60.6° (c = 1.59, $CHCl_3$).

^{1}H-NMR (methanol-d_4): δ (ppm) = 0.90 (t, 3H, CH_3); 1.15–1.87 (m, 26H, CH_2); 2.49 (m, 2H, CH_2CON); 3.24 (s, 9H, $^+N(CH_3)_3$); 3.15–3.35 (2H, CH_2SO), 3.59 (m, 2H, CH_2N^+); 4.19 (m, 6H, $2CH_2O$, NCH_2SO); 5.10 (m, 1H, CH). ^{13}C-NMR (methanol-d_4): δ (ppm) = 14.97 (CH_3); 24.17 (CH_2CH_3); 26.70 (CH_2CH_2CON); 30.90, 31.25, 33.51 ($11CH_2$); 36.16 (CH_2CON); 52.67 (CH_2SO); 55.20 ($^+N(CH_3)_3$); 55.00, 55.20 (CH); 60.95 (CH_2O-choline); 66.51 (CH_2O); 67.94 (CH_2N^+); 70.68, 71.16 (NCH_2SO) 176.14 (C=O). IR: 2919, 2850 (CH_2); 1651 (C=O) cm^{-1}.

2.18. Separation of the diastereomeres **14a** *and* **14b**

To separate the diastereomeres **14a** and **14b**, 2 g of the mixture **14** dissolved in diethyl ether was applied to a column of silica gel (100 g) equilibrated with diethyl ether. The column was first eluted with diethyl ether (2 l) and then developed with diethyl ether/methanol (9:1) to give two fractions of the accumulated diastereomeres. The separated diastereomeres were chromatographed once more, using first diethyl ether (2 l) and then diethyl ether/methanol (95:5) as solvents. Using the described procedure, analytical samples of both diastereomeres were obtained.

2.19. (1R,4R)-(N-hexadecanoyl)-4-hydroxymethyl-thiazolidine-S-oxide **(14a)**

TLC: R_f ~ 0.45 (diethyl ether: methanol; 95:5; v/v). m.p.: 101–102°C $[\alpha]_{578}^{20}$ = −10.8° (c = 1.11, $CHCl_3$). ^{13}C-NMR (chloroform-d_1): δ (ppm) = 13.85 (CH_3); 22.41 (CH_2CH_3); 24.52 (CH_2CH_2CON); 29.09, 29.27, 29.41, 31.65 ($11CH_2$); 34.61 (CH_2CON); 52.69 (CH_2SO); 60.32 (CH); 62.58 (CH_2O); 69.48 (NCH_2SO); 172.03 (C=O). IR: 3370 (OH); 2957, 2919, 2851 (CH_2); 1655 (C=O) cm^{-1}.

2.20. (1S,4R)-(N-hexadecanoyl)-4-hydroxymethyl-thiazolidine-S-oxide **(14b)**

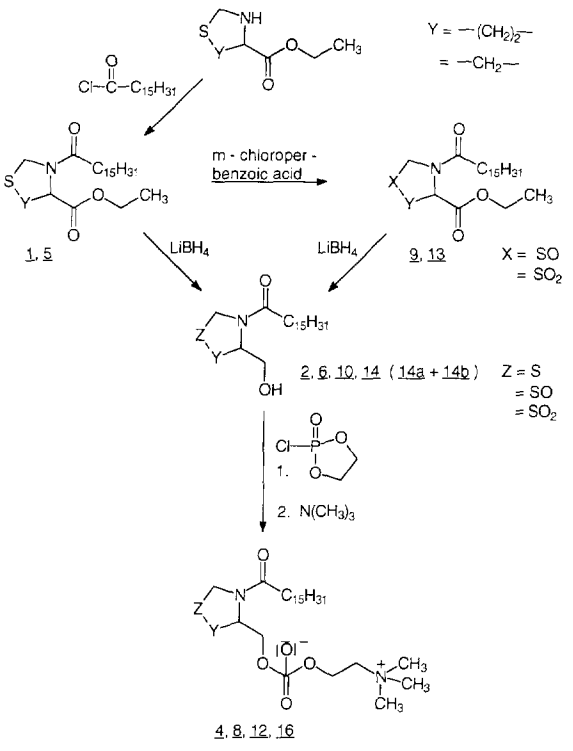
TLC: R_f ~ 0.4 (diethyl ether : methanol; 95:5; v/v). m.p.: 104–105°C $[\alpha]_{578}^{20}$ = −96.2° (c = 1.98, $CHCl_3$). ^{13}C-NMR (chloroform-d_1): δ (ppm) = 13.92 (CH_3); 22.47 (CH_2CH_3); 24.81 (CH_2CH_2CON); 29.17, 29.27, 29.50, 31.72 ($11CH_2$); 34.81 (CH_2CON); 52.41, 54.16 (CH_2SO); 57.61 (CH); 62.47, 63.11 (CH_2O); 67.48, 69.37 (NCH_2SO); 173.58 (C=O). IR: 3469 (OH); 2917, 2850 (CH_2); 1637 (C=O) cm^{-1}.

3. Results and discussion

The synthesis described here appears to provide an attractive route for obtaining heterocyclic platelet activating factor analogues. As described by Lewis et al. [15], *rac*-tetrahydro-1,3-thiazine-4-carboxylic acid-HCl is available by the reaction of *rac*-homocysteine thiolactone with 40% formaldehyde in the presence of dilute hydrochloric acid. Passing a stream of dry HCl gas through a suspension of the cyclic amino acid hydrochloride in absolute ethanol leads to the related ethyl ester-HCl

in good yield, as described for other amino acids [16]. This ester presents the starting material for the synthesis of lipid **4** by a short, four-step sequence. In the first step the amino group is acylated with palmitoyl chloride, yielding 93% of the desired compound **1**. For this the free base of the ester is prepared in situ by pre-treatment with a large excess of triethylamine. The following selective reduction of the ester group gives the amphiphilic alcohol **2** and is achieved by reaction with lithium borohydride in dry diethyl ether. Although using these mild conditions for ester reduction, many side products formed, perhaps by decomposition of the thiazine cycle. To introduce the phosphocholine part the alcohol **2** is allowed to react with 2-chloro-2-oxo-1,3,2-dioxaphospholane in the presence of triethylamine [17]. The extremely water-sensitive intermediate **3** is used for the next step without further treatment. Then the crude cyclic phosphate triester is cleaved by excess trimethylamine under pressure to give directly the inner salt of the desired phospholipid **4**. Using the described procedure, the five-membered cyclic thiazolidine analogue **8** is obtained from L-thiazolidine-4-carboxylic acid as a highly hygroscopic compound. Using the chirality of the commercially available precursor, it is possible to synthesize optically active heterocyclic phosphatidylcholine derivatives. We have prepared only the L-enantiomer, but the D-form should be obtainable by starting with D-thiazolidine-4-carboxylic acid in the same way. In comparison with the cyclic oxygen function of SRI-62-834, the thioether linkage is more lipophilic and unable to form hydrogen-bridges. Therefore, it seems possible that this molecule part plays an important role in the affinity to the binding sites of tumor cells or to exhibit anti-neoplastic activity. To increase the polarity of the cyclically short side-chain we have prepared the related sulfonyl and sulfinyl derivatives of the thiazolidine lipid **8**. Oxidation of the thioether group with *m*-chloroperbenzoic acid affords the sulfon **9** and the sulfin **13** in 63% and 79% yields, respectively. The corresponding lipids **12** and **16** are obtained from these intermediates, as outlined in scheme 1.

The preparation of the sulfinyl derivative **13** causes the generation of a new chiral centre, and



Scheme. 1.
Reaction scheme of lipid synthesis.

consequently a mixture of two diastereomeres originates. For instance, the existence of two isomers is demonstrable by measuring carbon-13 nuclear magnetic resonance spectra.

In fact the ^{13}C-NMR spectrum (broad-band decoupling of protons) of **13** shows a remarkable signal doubling for the carbons of the thiazolidine cycle. Measurements of ^{13}C-gated and 135°-dept-NMR spectra indicate that the double signals represent carbon atoms of the same kind. The large difference in the chemical shifts for the C4 carbon of the cycle (60.3 and 57.6 ppm) is suspicious. This spectroscopic difference may also be due to an anisotropic effect of the sulfoxide function. Depending on the conformation of the sulfoxide oxygen, the carbon resonances of the heterocyclic ring could be shifted upfield or downfield relative to the resonances of the comparable isomeric carbon atoms. To prove this hypothesis, the

diastereomeres of **14** were separated by column chromatography. As expected, the ^{13}C-NMR spectra of the pure diastereomeres **14a** and **14b** show no more signal doubling, and the superposition of these two spectra is identical with the spectrum obtained from the mixture. in contrast with this, the IR and ^{1}H-NMR spectra of the separated diastereomeres exhibit no significant differences. Lambert et al. [18] described a similar difference in chemical shifts in ^{13}C-NMR for the β-carbons of tetrahydrothiapyrane-*S*-oxide (23.3 and 15.5 ppm) at −93°C. They found that the β-carbon of the heterocycle with an equatorial orientation of the oxygen is resonating at a lower field. Accordingly, it seems to be reasonable to assume that the diastereomere **14a** exhibits an axial and **14b** an equatorial orientation of the oxygen. Because of the rigidity of the thiazolidine cycle, the steric arrangement of the sulfoxide oxygen is fixed, and the diastereomeres are obtainable in their optical pure forms by column chromatography.

These new heterocyclic phospholipids are of interest for testing cytotoxic effects and PAF antagonism. They also contribute to the clarification of the structure-activity relationship of biologically active phospholipids.

Preliminary in vitro tests show that these new compounds exhibit cytotoxic activity against various tumor cell lines. The further pharmacological properties of these lipids are under investigation.

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