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Synthesis and interfacial behavior of sulfur-containing analogs of lung surfactant dipalmitoyl phosphatidylcholine

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Abstract—Synthesis methods and initial surface property characterizations are reported for two sulfur-containing phosphonolipids related structurally to dipalmitoyl phosphatidylcholine (DPPC), the major lung surfactant glycerophospholipid. Sulfur linkages in these compounds affect molecular interactions relative to ester linkages, and are structurally resistant to cleavage by phospholipases. The SO_2-linked analog synthesized here had increased adsorption and improved film respreading compared to DPPC, while reaching very low surface tensions ($\leqslant$1 mN/m) in cycled interfacial films on both the Wilhelmy balance and the pulsating bubble surfactometer. This compound appears to have potential utility as a component in future phospholipase-resistant synthetic exogenous surfactants for treating clinical forms of inflammatory lung injury.

1. Introduction

Pulmonary surfactant, a complex mixture of glycerophospholipids and biophysically active proteins in the alveolar airsacs, is essential for normal respiratory function in air-breathing animals.[1,2] Lung surfactant is deficient in premature infants with the respiratory distress syndrome (RDS), and can become inactivated or dysfunctional during inflammatory acute lung injury (ALI) and the acute respiratory distress syndrome (ARDS). Life saving therapy with animal-derived exogenous surfactant drugs is now available for premature infants with RDS, and is being extended to patients with clinical lung injury syndromes.[1] However, significant interest remains in developing new, active synthetic lung surfactants with high inhibition resistance for therapeutic applications, particularly for adult and pediatric patients with severe ALI/ARDS.

During inflammatory lung injury, endogenous phospholipases are released that can degrade lung surfactant glycerophospholipids. One approach to designing novel synthetic surfactants involves the use of phospholipase-resistant structural analogs of dipalmitoyl phosphatidylcholine (DPPC), the major glycerophospholipid in mammalian pulmonary surfactant.[1,3–6] Analog compounds can be synthesized not only to be resistant to phospholipase activity, but also to have potentially improved surface active properties compared to DPPC. Our prior work has reported on a highly active diether phosphonolipid analog of DPPC (designated DEPN-8, compound **1**) and its use in synthetic surfactants.[3,6] The present study reports the synthesis of two sulfur-containing phosphonolipid analogs (compounds **2** and **3**, Fig. 1), along with preliminary assessments of their

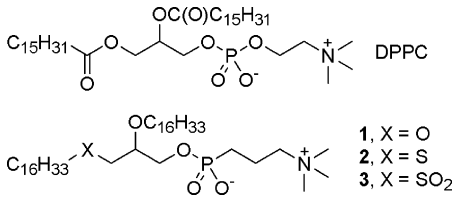

Figure 1. Structure of diether (**1**) and sulfur-containing phosphonolipid analogs (**2** and **3**) related to DPPC.

Keywords: Lung surfactant, Phospholipid; Surface tension; Sulfur; Respread.
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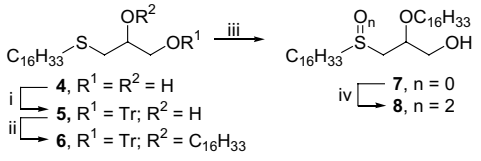
surface active properties in spread surface films at the air–water interface and in aqueous dispersions. Compared to the ester group, sulfur linkages have the potential to significantly alter molecular packing and interactions at the interface and in the aqueous phase in addition to providing resistance to endogenous phospholipases.

2. Results and discussion

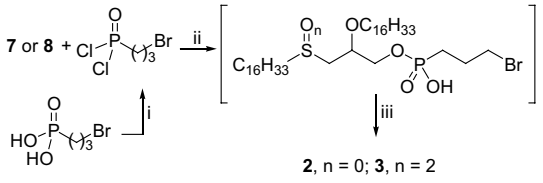
2.1. Synthetic chemistry

The sulfur-containing lipids were prepared as shown in Schemes 1 and 2. Commercially available 1-thioglycerol was converted to the 1-*S*-hexadecyl-*rac*-thioglycerol (**4**) by the alkylation with hexadecyl bromide in alcoholic KOH (95%). The primary hydroxyl group was selectively tritylated (TrCl, Et_3N), producing known[7] alcohol **5** in 93% yield. The remaining hydroxyl group of **5** was alkylated with hexadecyl bromide to yield **6** in 94% yield. The trityl group was cleaved with (pTSA) in 95% aq methanol to provide alcohol **7**, a known compound,[8] which could be smoothly oxidized to the corresponding sulfone **8** with MCPBA prior to installation of the phosphono head group.

The phosophonocholine head group was then installed through a previously established protocol as shown in Scheme 2.[6] Final isolation and recrystallization afforded lipids **2** and **3** in 48% and 59% yields, respectively. Selected synthetic procedures and characterization data are given in the References and notes section.[9] Although a number of phosphatidylcholines with sulfur-linked fatty alkyl chains are known,[8,10–21] including some compounds with thioether linkages,[8,20,21] lipids **2** and **3** represent the first examples of thioether- or sulfone-containing *phosphonocholine* derivatives.



Scheme 1. Reagents and conditions: (i) TrCl, Et_3N, CH_3CN/THF, 93%; (ii) KOH, DMSO, $nC_{16}H_{33}Br$, 94%; (iii) *p*-TSA, 95% aq MeOH, 85%; (iv) 2.3 equiv MCPBA, CH_2Cl_2, 76%.



Scheme 2. Reagents and conditions: (i) PCl_5, $CHCl_3$; (ii) Et_3N, $CHCl_3$; (iii) Me_3N, $CHCl_3$/CH_3CN/*i*PrOH, 2 days, 60 °C. Yields over all steps: **2**: 48%; **3**: 59%.

2.2. Biophysical properties of sulfur-containing lipids 2 and 3

The biophysical properties of the S-lipid **2** and the SO_2-lipid **3** were examined in comparison to DPPC and to **1** (DEPN-8) by several methods. Surface films spread to a uniform high initial concentration of 15 Å^2/molecule were studied on a Wilhelmy balance to highlight respreading behavior,[1,22,23] and adsorption to the air–water interface was measured for surfactant dispersions in a dish with a stirred subphase to minimize diffusion resistance.[24] Surface pressure–area isotherms on the Wilhelmy balance showed that both sulfur-containing analogs **2** and **3** had improved film respreading compared to DPPC and diether **1** on cycle 2/1 (Table 1). SO_2-lipid **3** also had improved film respreading compared to DPPC and **1** on cycle 7/1 (respreading for **2** could not accurately be defined for cycle 7/1 because of substantial isotherm shape changes that occurred after cycle 3). If cycle number was not considered, spread films of all four compounds (**1**–**3** and DPPC) had maximum surface pressures of 72 mN/m on the Wilhelmy balance at 23 °C. In studies on dispersed lipids, compounds **2** and **3** had increased adsorption surface pressures compared to **1** and DPPC at 0.25 and 5 min (Table 1).

The dynamic surface activity of the sulfur lipids was examined further at environmental conditions relevant for the lungs in vivo on a pulsating bubble surfactometer (37 °C, 20 cycles/min, 50% area compression).[25] Measurements of minimum surface tension on this instrument have been shown in multiple studies to provide a physiologically relevant assessment of overall lung surfactant activity that combines effects from both adsorption and dynamic film compression.[1] Pulsating bubble measurements indicated that lipid **3** had overall dynamic surface activity approaching that of **1** at the surfactant concentration studied (2.5 mg/mL) (Fig. 2). The dynamic surface activity of **2** on the pulsating bubble was significantly less than that of **3** (Fig. 2). This reduced activity on the bubble apparatus for **2** is presumably related to the lower maximum surface pressures found in isotherms of spread surface excess films of this compound during cycles 1–3 on the Wilhelmy balance (Table 1 legend). Finally, as reported in a number of prior studies (see Ref. 1 for review), the surface tension lowering ability of DPPC was substantially worse on the pulsating bubble apparatus (Fig. 2) than on the Wilhelmy balance (Table 1). This reduced activity for DPPC in bubble experiments is due to its extremely poor adsorption when dispersed in the aqueous phase.

Although specific molecular mechanisms were not studied here, the fact that the newly synthesized SO_2-lipid **3** had interfacial activity approaching or exceeding that of compound **1** is significant. A synthetic exogenous surfactant containing **1** plus 1.5% by weight of mixed bovine lung surfactant proteins (SP)-B and C has recently been shown by our group to have extremely high surface activity that is fully maintained in the presence of phospholipase A_2.[6] This synthetic surfactant (**1**+1.5% SP-B/C) also had high resistance to biophysical inhibition by plasma proteins and cellular lipids, which can be present

Table 1. Respreading ratios, maximum film surface pressures and adsorption surface pressures for sulfur-containing lipids **2** and **3** relative to DPPC and diether lipid **1**

Compound	Wilhelmy balance measurements			Adsorption measurements	
	Film respreading cycle 2/1	Film respreading cycle 7/1	Maximum film surface pressure (mN/m)	Surface pressure at 0.25 min (mN/m)	Surface pressure at 5 min (mN/m)
DPPC	28.3 ± 0.3	48 ± 0.3	72	0	1.2 ± 0.2
DEPN-8, **1**	19.5 ± 1.0	33.1 ± 1.5	72	0	7.2 ± 0.5
SO_2-Lipid, **3**	14.3 ± 0.7	21.6 ± 0.4	72	20.0 ± 0.0	30.3 ± 1.3
S-Lipid, **2**	0.2 ± 0	—	72 (cycles 4–7)	8.7 ± 1.3	30.7 ± 3.2

Data are mean ± standard error for $n = 3$–5 experiments. Films were spread to 15 Å^2/molecule on a Wilhelmy balance (compression ratio 4.35:1, rate 5 min/cycle, 23 °C). Maximum surface pressure for **2** was significantly less (~51–56 mN/m) for cycles ⩽ 3. Respreading is based on the area (arbitrary units) between compression curves 1, 2 or 1, 7.[22,23] An area of 0 between compressions indicates complete respreading, and larger areas indicate less respreading. For adsorption, surfactants were added at time 0 to a dish with a stirred subphase, and surface pressure was measured with a hanging Wilhelmy slide (2.5 mg surfactant phospholipid/40 mL of subphase, 37 °C).[1,24]

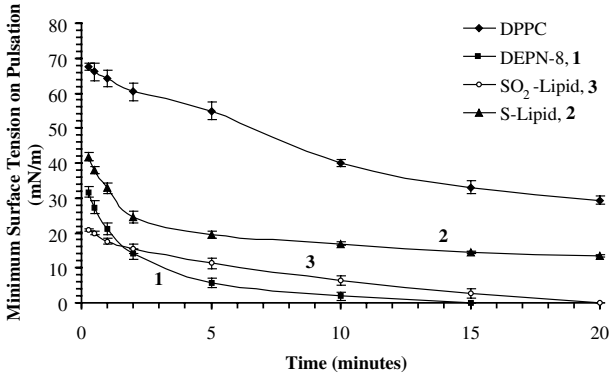


Figure 2. Minimum surface tension as a function of time for sulfur-containing lipids compared to DPPC and **1**. Surface tension at minimum bubble radius was measured as a function of time of pulsation on a bubble surfactometer (37 °C, 20 cycles/min, 50% area compression, 2.5 mg phospholipid/mL) for surfactant dispersions in 0.15 M NaCl. Data are mean ± standard error for $n = 3$–5 experiments. Data for DPPC are adapted from Ref. 6.

in the alveoli during inflammatory lung injury (ALI/ARDS).[6] Initial biophysical assessments of sulfur-containing analogs in the present paper did not address their properties and interactions with lung surfactant proteins or related synthetic peptides. Studies are currently in progress to assess the surface active properties of the SO_2-lipid **3** in combination with purified bovine SP-B, SP-C, and mixed SP-B/C to more fully define its potential utility as a component in synthetic exogenous lung surfactants for possible use in ALI/ARDS.

Acknowledgements

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References and notes

1. Notter, R. H. *Lung Surfactants: Basic Science and Clinical Applications*; Marcel Dekker: New York, 2000.
2. Notter, R. H.; Wang, Z. *Rev. Chem. Eng.* **1997**, *13*, 1–118.
3. Turcotte, J. G.; Lin, W. H.; Pivarnik, P. E.; Sacco, A. M.; Bermel, M. S.; Lu, Z.; Notter, R. H. *Biochim. Biophys. Acta* **1991**, *1084*, 1–12.
4. Turcotte, J. G.; Sacco, A. M.; Steim, J. M.; Tabak, S. A.; Notter, R. H. *Biochim. Biophys. Acta* **1977**, *488*, 235–248.
5. Turcotte, J. G.; Lin, W. H.; Pivarnik, P. E.; Motola, N. C.; Bhongle, N. N.; Heyman, H. R.; Notter, R. H. *Chem. Phys. Lipids* **1991**, *58*, 81–95.
6. Wang, Z.; Schwan, A. L.; Lairson, L. L.; O'Donnell, J. S.; Byrne, G. F.; Foye, A.; Holm, B. A.; Notter, R. H. *Am. J. Physiol.* **2003**, *285*, L550–L559.
7. Hong, C. I.; Kirisits, A. J.; Nechaev, A.; Buchheit, D. J.; West, C. R. *J. Med. Chem.* **1990**, *33*, 1380–1386.
8. Nali, M.; Rindone, B.; Bosone, E.; Farina, P.; Innocenti, S.; Valcavi, U. *Gazz. Chim. Ital.* **1986**, *116*, 25–27.
9. Selected experimental procedures: *Synthesis of 1-S-hexadecyl-2-O-hexadecyl-rac-sulfonylglycerol* (**8**). 1-*S*-hexadecyl-2-*O*-hexadecyl-*rac*-thioglycerol (**7**)[8] (2.0 g, 3.7 mmol) was dissolved in dry CH_2Cl_2 (35 mL), and the MCPBA (1.93 g, 8.6 mmol) in CH_2Cl_2 (60 mL) was added to the sulfide, the reaction mixture was stirred at rt overnight. Satd Na_2CO_3 (40 mL) was added and the mixture was extracted with CH_2Cl_2, washed with water, brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (eluent: hexane/EtOAc = 5:1) to give alcohol **8**, 76% yield, mp: 60–61 °C. Data for **8**: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.96 (m, 1H), 3.88 (dt, $J = 3.6$ and 11.6 Hz, 1H), 3.62–3.51 (m, 3H), 3.37 (ddd, $J = 1.2$, 8.4, and 14.8 Hz, 1H), 3.09–3.05 (m, 3H), 1.84 (m, 3H), 1.58 (m, 2H), 1.41 (m, 2H), 1.34–1.26 (m, 50H), 0.88 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 74.79, 70.20, 62.38, 54.94, 54.69, 31.89, 29.92, 29.67 (several C's), 29.52, 29.46, 29.33, 29.30, 29.08, 28.49, 26.16, 22.66, 21.84, 14.09. IR (CH_2Cl_2): ν, 3443, 2925, 2853, 1466, 1264 cm^{-1}. Anal. Calcd for $C_{35}H_{72}O_4S$: C, 75.47; H, 13.03. Found: C, 75.58; H, 12.72.
General procedure for installation of phosphonocholine head group. Synthesis of Lipids **2** *and* **3**. Using 800–1100 mg of alcohols **7** or **8**, the procedure followed a previously published method,[6] with the exception that after flash chromatography of the lipid, crystallization was from $CHCl_3$/acetone = 2:1. Yield of **2**: 48% yield, mp: 203–204 °C (decomp.). ^{1}H NMR ($CDCl_3/CD_3OD$ = 1:1, 400 MHz): δ 4.02 (t, $J = 5.6$ Hz), 3.64–3.52 (m, 3H), 3.44 (m, 2H), 3.15 (s, 9H), 2.72 (ABX pattern, $J_{AX} = 5.6$ Hz; $J_{BX} = 6.0$ Hz; $J_{AB} = 13.6$ Hz, 2H), 2.59 (t, $J = 7.4$ Hz, 2H), 2.04 (m, 2H), 1.70 (dt, $J = 7.2$ and 17.2 Hz, 2H), 1.58 (m,

4H), 1.27–1.41 (m, 52H), 0.89 (t, $J = 6.8$ Hz, 6H); ^{13}C NMR ($CDCl_3/CD_3OD$ = 1:1, 100 MHz): δ 78.16 (d, $J = 7.1$ Hz), 69.83, 66.09 (d, $J = 13.7$ Hz), 63.88 (d, $J = 5.6$ Hz), 52.18, 32.78, 31.31, 29.42, 29.07 (several C's), 28.97, 28.90, 28.74, 28.67, 28.28, 25.48, 22.61, 16.72, 13.17; ^{31}P NMR ($CDCl_3/CD_3OD$ = 1:1, 162 MHz): δ 26.1; IR (Nujol): ν, 2963, 2906, 1412, 1261, 1096 cm^{-1}. HRMS, ESI (+ve), *m/z*: calcd for $C_{41}H_{87}NO_4PS$ $[M+H]^+$: 720.6093; found: 720.6126.
Yield of **3**: 59% yield, mp: 234–235 °C (decomp.). ^1H NMR ($CDCl_3/CD_3OD$ = 1:1, 400 MHz): δ 4.07–3.95 (m, 2H), 3.83 (m, 1H), 3.70 (dt, $J = 6.4$ and 9.2 Hz, 1H), 3.49 (dt, $J = 6.4$ and 8.8 Hz, 1H), 3.41–3.25 (m, 4H), 3.17–3.08 (m, 2H), 3.11 (s, 9H), 1.98 (m, 2H), 1.82 (m, 2H), 1.62–1.53 (m, 4H), 1.41 (m, 2H), 1.26 (m, 50H), 0.86 (t, $J = 6.8$ Hz, 6H); ^{13}C NMR ($CDCl_3/CD_3OD$ = 1:1, 100 MHz): δ 73.77 (d, $J = 7.1$ Hz), 69.73, 66.29 (d, $J = 13.5$ Hz), 61.84 (d, $J = 5.5$ Hz), 54.48, 54.20, 52.12, 31.27, 29.45, 29.04 (several C's), 29.00, 28.93, 28.77, 28.70, 28.50, 27.89, 25.65, 23.11, 21.99, 21.75, 20.98, 17.06, 13.15; ^{31}P NMR ($CDCl_3/CD_3OD$ = 1:1, 162 MHz): δ 23.2; IR (Nujol): ν, 2963, 2907, 1412, 1262, 1085 cm^{-1}; HRMS, ESI TOF (+ve), *m/z*: calcd for $C_{41}H_{87}NO_6PS$ $[M+H]^+$: 752.5992; found: 752.5974.

10. Cox, J. W.; Horrocks, L. A. *J. Lipid Res.* **1981**, *22*, 496–505.
11. Hendrickson, E. K.; Hendrickson, H. S. *Chem. Phys. Lipids* **2001**, *109*, 203–207.
12. Yu, L.; Dennis, E. A. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 9325–9329.
13. Bhatia, S. K.; Hajdu, J. *Tetrahedron Lett.* **1987**, *28*, 3767–3770.
14. Sutton, L. D.; Froelich, S.; Hendrickson, S. H.; Quinn, D. M. *Biochemistry* **1991**, *30*, 5888–5893.
15. Hendrickson, H. S.; Hendrickson, E. K. *Chem. Phys. Lipids* **1990**, *53*, 115–120.
16. Murata, M.; Ikoma, S.; Achiwa, K. *Chem. Pharm. Bull.* **1991**, *39*, 1335–1336.
17. Murata, M.; Uchida, H.; Achiwa, K. *Chem. Pharm. Bull.* **1992**, *40*, 2849–2851.
18. Hajdu, J.; Sturtevant, J. M. *Chem. Phys. Lipids* **1990**, *55*, 323–330.
19. Kan, C. C.; Bittman, R.; Hajdu, J. *Biochim. Biophys. Acta* **1991**, *1066*, 95–101.
20. Morris-Natschke, S.; Surles, J. R.; Daniel, L. W.; Berens, M. E.; Modest, E. J.; Piantadosi, C. *J. Med. Chem.* **1986**, *29*, 2114–2117.
21. Letourneux, Y.; Bourass, J.; Boucrot, P.; Elkihel, L.; Petit, J. Y. *Pharmacol. Res.* **1997**, *35*, 73–78.
22. Wang, Z.; Gurel, O.; Baatz, J. E.; Notter, R. H. *J. Lipid Res.* **1996**, *37*, 1749–1760.
23. Wang, Z.; Hall, S. B.; Notter, R. H. *J. Lipid Res.* **1995**, *36*, 1283–1293.
24. Notter, R.; Taubold, R.; Finkelstein, J. *Chem. Phys. Lipids* **1983**, *33*, 67–80.
25. Enhorning, G. *J. Appl. Physiol.* **1977**, *43*, 198–203.