Pergamon

Tetrahedron Letters 41 (2000) 8495–8498

TETRAHEDRON LETTERS

Simplified syntheses of polymerizable bis-substituted phosphatidylcholines with various chain lengths

Soo K. Bae,[a] Se-H. Kim,[a] Jong-D. Kim,[a] Kyo I. Koo,[b] Tai K. Ryeom,[b] Kon Ryeom,[b] Xiaolin Fu[c] and Young H. Chang[c,*]

[a]*Department of Chemical Engineering, KAIST, Taejon 305-701, South Korea*
[b]*Department of Microbiology, Dan Kook University, Chonan 330-714, South Korea*
[c]*Department of Chemistry, KAIST, Taejon 305-701, South Korea*

Received 14 July 2000; revised 4 September 2000; accepted 8 September 2000

Abstract

Syntheses of new compounds, 1,2-bis[15-(2′,4′-hexadienoyloxy)pentadecanoyl]- and 1,2-bis[15-(2′,4′-hexadienoyloxy)dodecanoyl]-sn-glycero-3-phosphatidylcholines, via a simplified procedure are reported. The new approach utilizes the conversion of commercially available lactones into polymerizable bis-substituted phosphatidylcholines after two steps of reaction.

Polymerizable phospholipids have been utilized in the preparation of vesicles that are more stable to destructive physical disturbances, such as protein insertion into the membrane,[1] or attack of surfactants[2] and blood components,[3] that may possibly occur during in vivo drug delivery. Furthermore, polymerizable phospholipids have recently found use in the gating of vesicles, such that partial polymerization of membrane lipids in situ cause formation of membrane openings, through which drug molecules can be effectively released to the environment.[4,5] A number of polymerizable lipids have been synthesized,[6–8] but those multi-step, low-yield preparation methods have impeded active utilization of the products. Therefore, we have developed a simplified synthesis approach, in which commercially available lactones (or ω-hydroxylalkanoic acids) can be easily converted to polymerizable bis-substituted (**1a**, **1b**, **1c**) phosphatidylcholines after two steps of reaction (Fig. 1).

* Corresponding author. E-mail: yhchang@sorak.kaist.ac.kr

0040-4039/00/$ - see front matter
PII: S0040-4039(00)01537-9

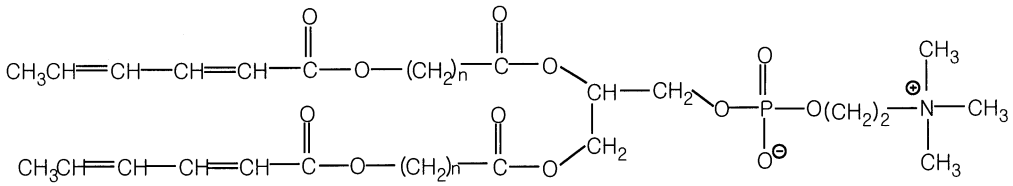
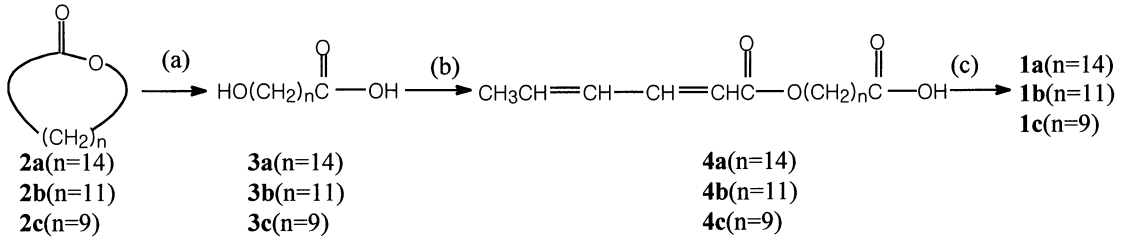


Figure 1. Bis-substituted polymerizable phosphatidylcholines; **1a**($n=14$), **1b**($n=11$) and **1c**($n=9$)

We have confirmed the polymerizability of the synthesized lipids by preparing liposomes loaded with actinomycin D, an anticancer drug, from the lipids and polymerizing them under 254 nm UV light.[9] The liposomes were prepared by extrusion through 200 nm membranes, yielding a unimodal population with an average size of 230 nm, and the drug was encapsulated in the liposomes via the pH-gradient method. A further study on the possibility of photosensitive gating of drug-loaded vesicles is underway. The synthesis procedure for the lipids is outlined in Scheme 1.



Scheme 1. (a) 95% ethanol and KOH, (b) sorbyl chloride ($CH_3CH{=}CH{-}CH{=}CH{-}COCl$)[5] with pyridine, (c) 4′-dimethylamine pyridine (DMAP) and l-glycerophosphorylcholine–cadmium chloride complex

General methods

All reactions were run under an atmosphere of highly pure nitrogen. All reagents (Aldrich Chemical Co.) were used as received, and all solvents (Aldrich) were purified and dried according to conventional methods. Analytical TLC employed 2.5 cm×10 cm plates coated with a 250 μm layer of silica gel GF (Analtech) I_2 and a 254 nm UV lamp was used for component visualization. Silica gel (70–200 mesh) from J. T. Baker Chemical Co. was used for the chromatography columns. NMR spectra were obtained on a Bruker AMX 300 spectrometer. ^1H and ^{13}C NMR chemical shifts (ppm) were referenced to TMS ($CDCl_3$) or TSP (D_2O) (Table 1). Mass spectra were recorded with VG Autospec Ultima Spectrometer at 70 eV ionizing energy.

*15-Hydroxylpentadecanoic acid (**3a**).* ω-Pentadecalactone, **2a**, (8.173 g, 34.0 mmol) was hydrolyzed under reflux temperature for 2 h in 100 mL of 95% ethanol and potassium hydroxide (2.862 g, 51.0 mmol). Two-thirds of the ethanol was removed, and the residue was diluted with 150 mL of water and was acidified with 5N sulphuric acid. **3a** was obtained as a precipitate, which was filtered, washed with water (3×20 mL), and dried in vacuo [yield: 8.625 g (98.0%)]. 12-Hyroxydodecanoic acid (**3b**) and 10-hydroxydecanoic acid (**3c**) were synthesized similarly with 95.9 and 96.2% yields, respectively.

*15-(2′,4′-Hexadienoyloxy)pentadecanoic acid (**4a**).* Freshly distilled sorbyl chloride[5] (2.99 g, 23.3 mmol) in $CHCl_3$ (10 mL) was added dropwise to a mixture of **3a** (4.00 g, 15.5 mmol) with pyridine (9.2 mL, 93.0 mmol) in $CHCl_3$ (50 mL) over 30 min, and was stirred for 2 days at room temperature. The reaction mixture was washed with 2N sodium hydroxide (4×20 mL) and

Table 1
^{1}H, ^{13}C NMR and mass data of synthesized compounds

3a	^{1}H NMR ($CDCl_3$, Me_4Si) δ 3.64–3.61 (t, $J=6$ Hz, 2H, CH_2OH), 2.35–2.31 (t, $J=8$ Hz, 2H, CH_2CO_2H), 1.63–1.51 (m, 4H, CH_2CH_2R), 1.29–1.24 (br s, 20H, CH_2); ^{13}C NMR (CD_3COCD_3, Me_4Si) δ 174.1, 61.9, 33.6, 33.2, 29.9, 29.8, 29.7, 29.6, 29.5, 29.3, 29.1, 28.9, 28.7, 26.2, 25.1
3b	^{1}H NMR ($CDCl_3$, Me_4Si) δ 3.64–3.61 (t, $J=6$ Hz, 2H, CH_2OH), 2.35–2.31 (t, $J=8$ Hz, 2H, CH_2CO_2H), 1.63–1.51 (m, 4H, CH_2CH_2R), 1.29–1.26 (br s, 14H, CH_2) [Ref. 10]; ^{13}C NMR (CD_3COCD_3, Me_4Si) δ 174.3, 62.0, 33.8, 33.3, 29.8, 29.6, 29.4, 29.2, 29.0, 28.8, 26.2, 25.2
3c	^{1}H NMR ($CDCl_3$, Me_4Si) δ 3.64–3.61 (t, $J=6$ Hz, 2H, CH_2OH), 2.35–2.31 (t, $J=8$ Hz, 2H, CH_2CO_2H), 1.63–1.52 (m, 4H, CH_2CH_2R), 1.28–1.26 (br s, 10H, CH_2) [Ref. 10]; ^{13}C NMR (CD_3COCD_3, Me_4Si) δ 174.1, 62.0, 33.3, 33.2, 29.5, 29.4, 29.2, 28.9, 26.3, 25.1
4a	^{1}H NMR ($CDCl_3$, Me_4Si) δ 7.26–7.20 (m, 1H, CH=$CHCO_2$), 6.20–6.08 (m, 2H, CH_3CH=CH–), 5.77–5.73 (d, $J=15.2$ Hz, 1H, C=$CHCO_2$), 4.12–4.09 (t, $J=6$ Hz, 2H, CO_2CH_2), 2.35–2.31 (t, $J=8$ Hz, 2H, CH_2CO_2H), 1.84–1.82 (d, $J=5.6$ Hz, 3H, C=$CHCH_3$), 1.65–1.59 (m, 4H, CH_2CH_2R), 1.28–1.24 (br s, 20H, CH_2); ^{13}C NMR ($CDCl_3$, Me_4Si) δ 179.9, 167.5, 144.9, 139.1, 129.8, 119.0, 64.4, 34.4, 34.0, 29.6, 29.5, 29.4, 29.2, 29.2, 29.1, 29.0, 28.7, 28.6, 25.9, 24.6, 14.0
4b	^{1}H NMR ($CDCl_3$, Me_4Si) δ 7.25–7.19 (m, 1H, CH=$CHCO_2$), 6.16–6.12 (m, 2H, CH_3CH=CH–), 5.77–5.73 (d, $J=16$ Hz, 1H, C=$CHCO_2$), 4.12–4.09 (t, $J=6$ Hz, 2H, CO_2CH_2), 2.34–2.30 (t, $J=8$ Hz, 2H, CH_2CO_2H), 1.84–1.82 (d, $J=6$ Hz, 3H, C=$CHCH_3$), 1.64–1.57 (m, 4H, CH_2CH_2R), 1.28–1.23 (br s, 14H, CH_2); ^{13}C NMR ($CDCl_3$, Me_4Si) δ 179.9, 167.5, 144.9, 139.2, 119.0, 64.4, 34.4, 33.9, 29.5, 29.4, 29.2, 29.0, 28.7, 28.6, 25.9, 24.7, 18.6
4c	^{1}H NMR ($CDCl_3$, Me_4Si) δ 7.25–7.19 (m, 1H, CH=$CHCO_2$), 6.20–6.08 (m, 2H, CH_3CH=CH–), 5.77–5.73 (d, $J=15.6$ Hz, 1H, C=$CHCO_2$), 4.12–4.08 (t, $J=6.8$ Hz, 2H, CO_2CH_2), 2.34–2.30 (t, $J=7.4$ Hz, 2H, CH_2CO_2H), 1.83–1.82 (d, $J=6$ Hz, 3H, C=$CHCH_3$), 1.66–1.57 (m, 4H, CH_2CH_2R), 1.33–1.23 (br s, 10H, CH_2) [Ref. 5]; ^{13}C NMR ($CDCl_3$, Me_4Si) δ 179.6, 167.5, 144.9, 139.2, 129.8, 119.0, 64.4, 34.1, 34.0, 29.2, 29.1, 29.0, 28.7, 25.9, 24.6, 18.6
1a	^{1}H NMR ($CDCl_3$, Me_4Si) δ 7.25–7.18 (m, 2H, CH=$CHCO_2$), 6.16–6.08 (m, 4H, CH_3CH=CH–), 5.77–5.73 (d, $J=15.6$ Hz, 2H, C=$CHCO_2$), 5.20–5.19 (br s, 1H, $POCH_2CH$), 4.37–4.32 (br m, 4H, $POCH_2CH$ and $CHCH_2O_2C$), 4.14–4.10 (t, $J=7.4$ Hz, 4H, =$CHCO_2CH_2$), 3.96–3.94 (br s, 2H, NCH_2CH_2), 3.79 (br s, 2H, NCH_2CH_2), 3.46 (s, 9H, NCH_3), 2.29–2.23 (m, 4H, CH_2CO_2), 1.84–1.82 (d, $J=5.6$ Hz, 6H, =$CHCH_3$), 1.65–1.55 (m, 8H, CH_2CH_2R), 1.23 (br s, 40H, CH_2); ^{13}C NMR ($CDCl_3$, Me_4Si) δ 173.5, 173.2, 167.4, 144.8, 139.1, 129.8, 118.9, 70.2, 64.3, 62.7, 59.4, 54.6, 50.8, 34.3, 34.2, 29.7, 29.6, 29.5, 29.3, 29.2, 29.1, 28.7, 26.0, 24.9, 18.6; m/z 925 $(M+H)^+$ (calcd: 925.6044)
1b	^{1}H NMR ($CDCl_3$, Me_4Si) δ 7.22–7.18 (m, 2H, CH=$CHCO_2$), 6.19–6.06 (m, 4H, CH_3CH=CH–), 5.76–5.72 (d, $J=15.2$ Hz, 2H, C=$CHCO_2$), 5.16 (br s, 1H, $POCH_2CH$), 4.37–4.30 (m, 4H, $POCH_2CH$ and $CHCH_2O_2C$), 4.11–4.08 (t, $J=6$ Hz, 4H, =$CHCO_2CH_2$), 3.91 (br s, 2H, NCH_2CH_2), 3.77 (br s, 2H, NCH_2CH_2), 3.45 (s, 9H, NCH_3), 2.29–2.23 (m, 4H, CH_2CO_2), 1.83–1.82 (d, $J=5.6$ Hz, 6H, =$CHCH_3$), 1.64–1.56 (m, 8H, CH_2CH_2R), 1.24–1.19 (br s, 28H, CH_2); ^{13}C NMR ($CDCl_3$, Me_4Si) δ 174.0, 173.6, 167.4, 144.9, 139.2, 129.8, 119.0, 70.3, 64.4, 62.8, 58.4, 54.6, 50.7, 34.4, 34.3, 29.6, 29.5, 29.4, 29.3, 28.7, 28.6, 26.0, 25.0, 18.6; m/z 841 $(M+H)^+$ (calcd: 841.5105)
1c	^{1}H NMR ($CDCl_3$, Me_4Si) δ 7.24–7.17 (m, 2H, CH=$CHCO_2$), 6.19–6.07 (m, 4H, CH_3CH=CH–), 5.75–5.71 (d, $J=15.6$ Hz, 2H, C=$CHCO_2$), 5.16 (br s, 1H, $POCH_2CH$), 4.34–4.32 (br m, 4H, $POCH_2CH$ and $CHCH_2O_2C$), 4.10–4.06 (t, $J=6.8$ Hz, 4H, =$CHCO_2CH_2$), 3.95–3.92 (br s, 2H, NCH_2CH_2), 3.72 (br s, 2H, NCH_2CH_2), 3.43 (s, 9H, NCH_3), 2.28–2.24 (m, 4H, CH_2CO_2), 1.82–1.81 (d, $J=5.6$ Hz, 6H, =$CHCH_3$), 1.63–1.54 (m, 8H, CH_2CH_2R), 1.25–1.21 (br s, 20H, CH_2). [Ref. 5]; ^{13}C NMR ($CDCl_3$, Me_4Si) δ 173.5, 173.2, 167.4, 144.9, 139.3, 129.7, 118.9, 70.1, 64.3, 62.7, 59.4, 54.5, 50.5, 34.2, 34.0, 29.6, 29.3, 29.0, 28.6, 25.9, 24.8, 18.6; m/z 785 $(M+H)^+$ (calcd: 785.4479)

subsequently with water (2×10 mL). The solution was dried with Na_2SO_4, and the solvent was removed by rotary evaporation. The residue was purified by silica gel chromatography (ethyl acetate in hexane, 25→100%) to give **4a** [yield: 3.556 g (65.0%); TLC: $R_f = 0.55–0.60$, $CHCl_3$/$CH_3OH = 3:1$]. 12-(2′,4′-hexadienoyloxy)dodecanoic acid [**4b**; yield: 68.7%; TLC: $R_f = 0.50–0.60$] and 10-(2′,4′-hexadienoyloxy)decanoic acid [**4c**; yield: 62.4%; TLC: $R_f = 0.50–0.60$] were similarly prepared.

*1,2-Bis[15-(2′,4′-hexadienoyloxy)pentadecanoyl]-sn]glycerol-3-phosphatidylcholine(bis-sorb PC) (**1a**).* l-Glycerophosphorylcholine–cadmuim chloride complex (444.9 mg, 1.01 mmol), dried by repeated evaporation from a benzene suspension (8 mL), was mixed with **4a** (1.555 g, 4.41 mmol) in DMAP (304.7 mg, 2.47 mmol) and was suspended in 8 mL $CHCl_3$. 1,3-Dicyclohexylcarbodiimide (843.8 mg, 4.09 mmol) was added, and the mixture was stirred at room temperature for 1 week in the dark under nitrogen. The white suspension was filtered, washed with $CHCl_3$ (2×15 mL), and concentrated on the rotary evaporator. The residue was washed with methanol (2×15 mL) and was purified by silica gel chromatography (methanol in CH_2Cl_2, 0→100%) to give the final product, **1a** [yield: 853 mg (97.1%); TLC. $R_f = 0.4–0.48$, $CHCl_3$/CH_3OH/H_2O (65:25:4)]. 1,2-Bis[15-(2′,4′-hexadienoyloxy)dodecanoyl]-sn]glycerol-3-phosphatidylcholine [**1b**; yield: 94.1%; TLC: $R_f = 0.40–0.48$] and 1,2-bis[15-(2′,4′-hexadienoyloxy)-decanoyl]-sn]glycerol-3-phosphatidylcholine [**1c**; yield: 92.5%; TLC: $R_f = 0.42–0.48$] were also obtained in a similar way.

Acknowledgements

This work was supported by the MOHW (Ministry of Health and Welfare) of Korea.

References

1. Juliano, R. L.; Hsu, M. J.; Peterson, D.; Regen, S. L.; Singh, A. *Exp. Cell. Res.* **1983**, *146*, 422–427.
2. Hupfer, B.; Ringsdorf, H.; Schupp, H. *Chem. Phys. Lipids.* **1983**, *33*, 355–374.
3. Bonte, F.; Hsu, M. J.; Papp, A.; Wu, K.; Regen, S. L.; Juliano, R. L. *Biochim. Biophys. Acta* **1987**, *900*, 1–9.
4. Siegel, D. P. *Chem. Phys. Lipids* **1987**, *42*, 279–301.
5. Lamparski, H.; Liman, U.; Frankel, D. A.; Barry, J. A.; Ramaswami, V.; Brown, M. F.; O'Brien, D. F. *Biochemistry* **1992**, *31*, 685–694.
6. Gupta, C. M.; Radhakrishnan, R.; Khorana, H. G. *Proc. Natl. Acad. Sci. USA* **1977**, *74*, 4315–4319.
7. Eibl, H. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 257–271.
8. Srisiri, W.; Lee, Y. S.; O'Brien, D. F. *Tetrahedron Lett.* **1995**, *36*, 8945–8948.
9. Bae, S. K.; Kim, S. H.; Kim, J. D.; Koo, K. I.; Ryeom, K.; Fu, X.; Chang, Y. H., manuscript in preparation.
10. Pouchert, C. J.; Behnke, J. *The Aldrich Library of ^{13}C and ^{1}H FT NMR Spectra*; Aldrich Chemical Company, Inc., 1993; Vol. 1, p. 810.