Preparation and Characterization of a Novel Series of Amphiphilic Phospholipids Compounds

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Received 4 July 2007; accepted 7 May 2008
DOI 10.1002/app.28694
Published online 7 August 2008 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: For application of phospholipids compounds in drug permeation field, a novel series of amphiphilic phospholipids compounds containing different aliphatic chains as the hydrophobic tail groups and phosphorylcholine as the hydrophilic head groups were synthesized in three steps: preparation of hydroxyalkyl methacrylate by the reaction of various aliphatic chain diols with α-methacryloyl chloride; preparation of 2-(2-oxo-1,3,2-dioxaphospholoyloxy)alkyl methacrylate by the reaction of hydroxyalkyl methacrylate with 2-chloro-2-oxo-1,3,2-dioxaphospholane; preparation of the amphiphilic phospholipids compounds by the ring-opening reaction of 2-(2-oxo-1,3,2-dioxaphospholoyloxy)alkyl methacrylate in the presence of trimethylamine. The chemical structure of the amphiphilic phospholipids compounds were supported by Fourier transform infrared spectroscopy and ^{1}H NMR. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 110: 2058–2062, 2008

Key words: phospholipids; synthesis; biomimetic; biomaterials; membranes

INTRODUCTION

Phospholipids compounds are composed of three essential features: a polar head group, one or more hydrophobic tails, and a backbone structure connecting them.[1] Tails contain saturated aliphatic chains or unsaturated aliphatic chains, and head groups consist of any number of charged polar moieties. A variety of mesophase structures, including monolayer, micelle, reverse micelle, bilayer, and hexagonal phase, are presented due to a strong tendency to self-assembly of phospholipids when dispersed in a solvent such as water. The aqueous phase behavior of phospholipids is dictated by intrinsic parameters such as phospholipids polarity, acyl chain length, location and extent of acyl chain unsaturation, acyl chain branching, head group size, head group polarity, and head group charge, as well as extrinsic parameters such as concentration (lyotropism) and temperature (thermotropism).[2–5]

Because of their amphiphilic structure and various morphologies in aqueous phase, phospholipids compounds are of great interest in drug delivery, tissue-engineering applications, improvement of the blood compatibility, and biomembranes.[6–12] Biomembranes play an important role in all essential biological phenomena. They organize a living matter in cell, create a fluid two-dimensional matrix, and allow for the controlled transport of solutes. The synthesis and property of polymers containing phospholipids are of increasing interest, because phospholipids are principal components of biomembranes.[13,14] The synthesis of phospholipids analogous compounds started in 1971 and developed rapidly in the last several decades.[15,16] Much work has focused on the synthesis of phospholipids polymers such as polymeric phosphatidylcholine and phosphatidylethanolamine analogues, polymers with cholesterol moieties and polymeric glycolipid analogues.[17–22] However, the synthesis routes of these polymers were complicated, and these phospholipid compounds were limited.

In this study, a novel series of phospholipids compounds were synthesized with a satisfactory yield by a relative simple method. First, the hydroxyalkyl methacrylates (HAMA) were prepared by the reaction of various aliphatic chains diols with methacryloyl chloride (MAC), then the HAMAs reacted with 2-chloro-2-oxo-1,3,2-dioxaphospholane (COP) to form 2-(2-oxo-1,3,2-dioxaphospholoyloxy)alkyl methacrylates (OPAMA), finally, the amphiphilic phospholipids

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Contract grant sponsor: National Natural Science Foundation of China; contract grant numbers: 30572271; 50673058.
Contract grant sponsor: Science and Technology Develop Foundation of Shanghai; contract grant number: 054119539.
Contract grant sponsor: Shanghai Foundation Research Key Project; contract grant number: 05JC14072.

compounds were obtained by the ring-opening reaction of OPAMAs in the presence of trimethylamine. The potential of using these phospholipids compounds in drug permeation field will be explored in our group.

EXPERIMENTAL

Materials and methods

2-Chloro-2-oxo-1,3,2-dioxaphospholane (COP) was purchased from Sigma-Aldrich Co. (Shanghai, China). 1,8-Octandiol, 1,10-decandiol and 1,12-dodecandiol were purchased from Beckman Co. (Shanghai, China) and purified by recrystallization before use. α-methacryloyl chloride (MAC) was synthesized using α-methylacrylic acid and thionyl chloride and purified by distillation, bp 95°C.[23] Tetrahydrofuran (THF) was distilled from sodium and stored over a sodium–potassium alloy in a drybox. Trimethylamine and triethylamine (Et_3N) were stirred overnight with KOH. Acetonitrile was stirred with K_2CO_3 for 24 h, then distilled from P_2O_5, and stored over 4-Å sieve. Hexane and acetoacetate (EtOAc) were purified by distillation. All other reagents were purchased from SCRC (Shanghai, China).

The plates for the thin-layer chromatography (TLC) were prepared in our group with a fluorescent indicator UV254. The silica gel for the silica gel column chromatography was zcx-2, 100–200 mesh, and purchased from Qingdao Ocean Chemical Co. (Qingdao, China).

^{1}H NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer (Swiss). FTIR spectra were measured using a Nicolet NEXUS-670 Fourier transform infrared spectrometer (America).

A novel series of phospholipids compounds containing different aliphatic chains were synthesized through a similar route. The synthesis of 2-methacryloyloxyoctyl phosphorylcholine is given as an example.

Synthesis of 8-hydroxyoctyl methacrylate

A solution of 1,8-octandiol (29.2 g, 0.2 mol) and anhydrous Et_3N (33.4 g, 46 mL, 0.33 mol) in dry THF (200 mL) was cooled to 0°C. α-MAC (20.9 g, 0.2 mol) was added dropwise over 1 h with stirring, keeping the temperature between 0 and 3°C. Then, the reaction mixture was warmed to 50°C and kept at this temperature for 2 h, followed by TLC. When the reaction was completed, the reaction mixture was cooled to room temperature and filtered off the precipitate. After the solvent was evaporated under vacuum, the residue was redissolved in EtOAc (150 mL). The organic phase was washed with 0.5*M* $NaHCO_3$ (2 × 100 mL) and 0.5*M* sodium citrate (2 × 100 mL) separately, dried over $MgSO_4$, filtered off the precipitate, and evaporated under vacuum. The residue was purified by flash chromatography on silica gel. The eluant initially used was EtOAc/hexanes in a ratio of 20/80 (v/v). Until the first product (TLC: $R_f = 0.9$) was entirely run-off, the ratio of the eluant was changed to EtOAc/hexanes 50/50 (v/v), and the next production, a colorless liquid 8-hydroxyoctyl methacrylate (TLC: $R_f = 0.6$, yield 24.3%) was collected. The 8-hydroxyoctyl methacrylate was supported by ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 1.34 ($-CH_2-CH_2-CH_2-CH_2-$, m, 8H), 1.55–1.57 ($-CH_2$, t, 2H), 1.66–1.69 ($-CH_2$, t, 2H), 1.94 ($-CH_3$, m, 3H), 2.9 (—OH, broad, 1H), 3.63 ($-OCH_2-$, t, 2H), 4.14 ($-CH_2O-$, t, 2H), 5.55 (=CH, t, 1H), 6.10 (=CH, t, 1H), and IR (cm^{-1}) 3420 (—OH), 2930 ($-CH_2$), 2857 ($-CH_3$), 1720 (C=O), 1640 ($C=CH_2$).

Synthesis of 8-(2-oxo-1,3,2-dioxaphospholoyloxy) octyl methacrylate

8-Hydroxyoctyl methacrylate (4.5 g, 0.021 mol) and anhydrous Et_3N (2.1 g, 0.021 mol) were dissolved in 100 mL dry THF. After the solution was cooled to −20°C, COP (3 g, 0.021 mol) in 50 mL dry THF was added dropwise with stirring over a period of 1 h, and then the reaction mixture was left at room temperature for 4 h until the reaction was completed. Subsequently, 3 mL anhydrous hexane was added into the mixture, then filtered off the precipitate of triethylammonium chloride, and evaporated under vacuum. For further purification, 50 mL of dry ethyl ether/THF (1 : 1) was added into the residue, and a small amount of triethylammonium chloride was precipitated and filtered off three times. After vacuum evaporation, a colorless liquid 8-(2-oxo-1, 3, 2-dioxaphospholoyloxy)octyl methacrylate was obtained without further characterization.

Preparation of 2-methacryloyloxyoctyl phosphorylcholine

A solution of 8-(2-oxo-1,3,2-dioxaphospholoyloxy)octyl methacrylate (3 g, 0.0087 mol) and 20 mL dry acetonitrile was added into a 100 mL dry glass pressure bottle (heated at 130°C overnight and cooled under nitrogen before use). After the bottle was cooled to −20°C, 1 mL anhydrous trimethylamine dissolved in 10 mL anhydrous acetonitrile was rapidly added into the solution. The pressure bottle was closed under nitrogen environment and allowed to warm up to room temperature. The reaction mixture was heated to 70°C for more than 48 h. When the reaction was completed, the reaction mixture was redissolved in water and the precipitate was filtered

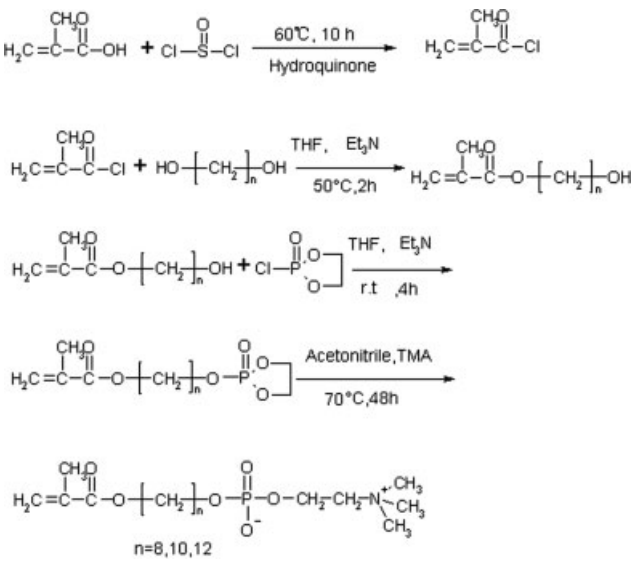

Figure 1 The synthesis outline of phospholipids compounds.

off. After 20 mL dry diethyl ether was added into the solution, a viscous precipitate was formed, washed three times with dry THF and dry acetone, respectively, recrystallized from dry acetonitrile, filtrated under nitrogen protection, and a white hydroscopic power was obtained (total yield of last two step: 72%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 1.34 (—CH_2—CH_2—CH_2—CH_2—, m, 8H), 1.55–1.57 (—CH_2, t, 2H), 1.66–1.69 (—CH_2, t, 2H), 1.94 (—CH_3, m, 3H), 2.85 (—$N(CH_3)_3$, m, 9H), 3.15 (—CH_2N—, t, 2H), 3.70 (—OCH_2—, t, 2H), 4.06 (—$POCH_2$—, t, 2H), 4.29 (—CH_2OP—, t, 2H), 5.56 (=CH, t, 1H), 6.10 (=CH, t, 1H) and IR(cm^{-1}) 2930 (C—H), 2500–2700 (N—C), 1720 (C=O), 1640 (C=C), 1300, 1240, 1160, and 1080 (—$POCH_2$—).

RESULTS AND DISCUSSION

In the present study, a novel family of phospholipids analogous compounds was synthesized in three steps. The synthesis outline is shown in Figure 1. Briefly, various aliphatic chain diols first react with MAC to form HAMA; OPAMA was subsequently obtained by HAMA reaction with COP; finally, the ring-opening step was carried out with trimethylamine, which rapidly added into dry acetonitrile solution.

Because of the low yield of HAMA, Yamasaki et al. reported another synthetic route of HAMA. In brief, one hydroxy group of diol was first protected by chlorotrimethylsilane and then another hydroxy group reacted with MAC, and finally the protective group was removed to product HAMA.[10] The advantage of our approach was that the reaction was carried out in one step and the yield of HAMA was relatively satisfactory. Figure 2 shows that the

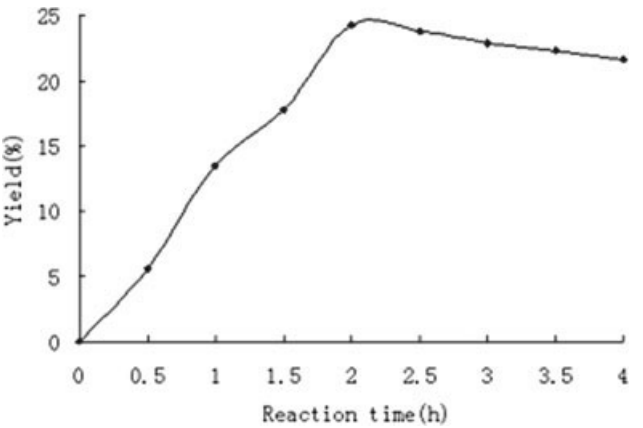

Figure 2 The relationship between reaction time and the yield of HAMA. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

yield of HAMA increases by extending the reaction time at the beginning; however, when the reaction time exceeds a certain time, further increase in the reaction time will decrease the yield of HAMA. In this reaction, the MAC not only reacted with one hydroxy group of diols, but also considerable by-products from the reaction of MAC with the total hydroxy groups of diols were also produced. Because of excess amount of diols and slow dropwise addition of MAC, a relatively high yield of HAMA would be obtained. However, on increasing the reaction time, HAMA will react with MAC and cut down the yield of HAMA consequently. The chemical structure of the obtained 8-hydroxyoctyl methacrylate was supported by ^{1}H NMR as the existence of the peak of 1.34 ppm (—CH_2—CH_2—CH_2—CH_2—), 1.55–1.57 ppm (—CH_2), 1.66–1.69 ppm (—CH_2), and 2.9 ppm (—OH) in Figure 3 and the absorption of 3420 (—OH), 2930 (—CH_2) in Figure 5(a).

Because the ring-opening reagent triethylamine is added in the second step, the obtained 8-(2-oxo-1,3,2-dioxaphospholoyloxy)octyl methacrylate that possesses a five-membered ring was purified immediately and used in the next reaction without further characterization.

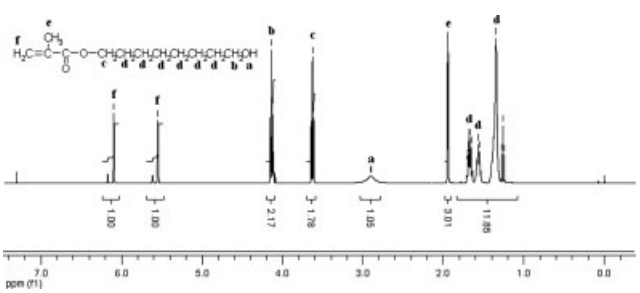

Figure 3 ^{1}H NMR spectrum of 8-hydroxyoctyl methacrylate in $CDCl_3$.

In the last reaction, utilization of different ring-opening regents (such as trimethylamine, dimethylamine analogues or its derivatives incorporating vinyl group) in polar aprotic solvents such as acetonitrile, DMF, and DMSO will provide various phospholipids analogues. Compared to trimethylamine, dimethylamine analogues or its derivatives such as 2-dimethylaminoethyl methacrylate and 6-dimethylaminohexyl methacrylate as ring-opening reagents, a different type of vinyl phospholipids monomers were obtained, in which the phosphatidycholine analogous polar head groups are located in the middle of the molecule, and hydrophobic portions and vinyl groups are located at both ends. However, using trimethylamine as a ring-opening reagent, phosphatidylcholine analogous vinyl monomers have been synthesized, and their polar head group is located at one side of the molecule.[6] The chemical structure of phospholipid analogous compounds that used trimethylamine as ring-opening reagent were confirmed by ^{1}H NMR in Figure 4. The existence of the peaks at 2.85 ppm (—$N(CH_3)_3$), 3.15 ppm (—CH_2N—), 4.29 ppm (—CH_2OP—) powerfully verified the completion of the ring-opening reaction, which further confirmed in IR by the disappearance of absorption peaks of 3420 (—OH) and the existence of absorption peaks of 2500–2700 (N—C), 1300 cm^{-1}, 1240 cm^{-1}, 1160 cm^{-1}, 1080 cm^{-1} (—$POCH_2$—) in Figure 5(b).

CONCLUSIONS

A novel series of amphiphilic phospholipids compounds containing different aliphatic chains as the hydrophobic tails and phosphorylcholine as the hydrophilic head groups were successfully synthesized in three steps: preparation of HAMA by the reaction of various aliphatic chain diols with MAC; preparation of OPAMA by the reaction of HAMA with COP; preparation of the amphiphilic phospholipids analogous compounds by the ring-opening reaction of OPAMA with trimethylamine as a ring-opening reagent. The structure of the amphiphilic phospholipids compounds and their precursors were confirmed by ^{1}H NMR and FTIR. Compared to other synthetic methods, our approach provided phospholipids compounds with satisfactory yield. Their potential application in drug permeation field is under study in our group.

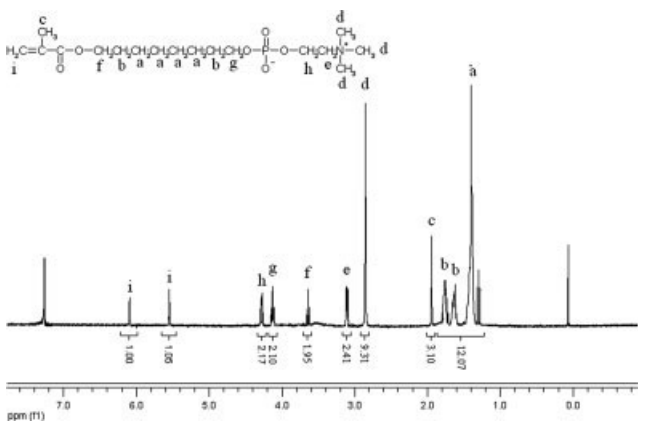

Figure 4 ^{1}H NMR spectrum of 2-methacryloyloxyoctyl phosphorylcholine in $CDCl_3$.

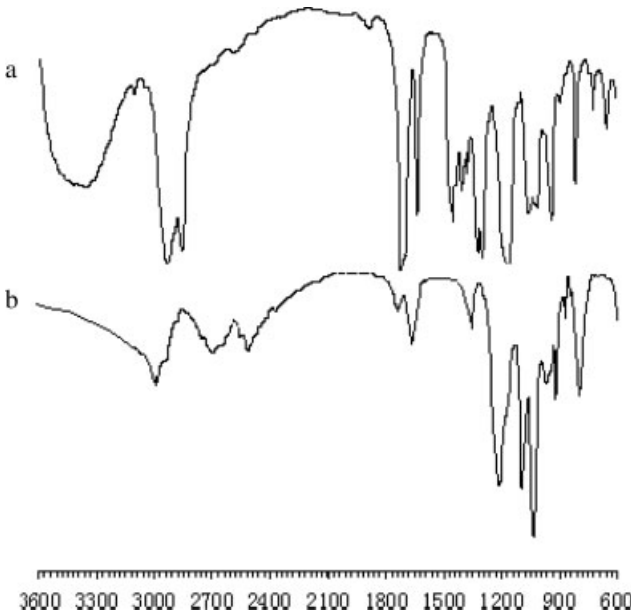

Figure 5 IR spectrum of phospholipids compound. (a) 8-Hydroxyoctyl methacrylate; (b) 2-methacryloyloxyoctyl phosphorylcholine.

The author is grateful to the analysis center of Donghua University.

References

1. Joel, H. C.; Phillip, B. M. Annu Rev Mater Res 2001, 31, 237.
2. Jones, M. N.; Chapman, D.; Micelles, Monolayers, and Biomembranes; Wiley-Liss: New York, 1995.
3. Epand, R. M.; Lipid Polymorphism and Membrane Properties; Academic Press: San Diego, 1997.
4. Israelachvili, J. Intermolecular and Surface Forces; Academic Press: London, 1992.
5. Seddon, J. M.; Cevc, G. In Phospholipids Handbook; Cevc, G., Ed.; Dekker: New York, 1993; pp 403–454.
6. Nakaya, T.; Li, Y. J Prog Polym Sci 1999, 24, 143.
7. Matthew, G. M.; John, M. L.; Matthew, P. C.; Timothy, E. L. Science 2006, 311, 353.
8. Kheirolomoom, A. J Controlled Release 2007, 118, 275.
9. Caseli, L. M.; Douglas, C.; Furriel, R. P. M. L.; Francisco, A. Z.; Maria, E. D. Thin Solid Films 2007, 515, 4801.
10. Akira, Y.; Yousuke, I.; Kimio, K.; Yasuhiko, I.; Nobuo, N.; Kazuhiko, I. Colloid Surf B Biointerfaces 2003, 28, 53.
11. Kazuhiko, I.; Tomoko, U.; Nobuo, N. Polym J 1990, 22, 355.
12. Kazuhiko, I.; Kikuko, F.; Yasuhiko, I.; Nobuo, N. Biomaterials 1999, 20, 1545.

13. Singer, S. J.; Nicolson, G. L. Science 1972, 175, 720.
14. Fendler, J. H. Acc Chem Res 1980, 13, 7.
15. Doiuchi, T.; Nakaya, T.; Imoto, M. Makromol Chem 1974, 175, 43.
16. Yamada M.; Li Y. J.; Nakaya T Macromolecules 1995, 28, 2590.
17. Susan, L.; Natschke, M.; Marasco, J. F. G. C. J.; Karen, L. M.; Michael, M.; Piantadosi, C.; Matthew, D. L.; Edward, J. M. J Med Chem 2018 1993, 36.
18. Suresh, K. B.; Joseph, H. J Org Chem 1988, 53, 5034.
19. Fredric, M. M.; Andrey, V. P. A. J Am Chem Soc 2001, 123, 5614.
20. Colin, B.; Stephen, C.; Nigel, P. G.; Catherine, H.; Clive, G. J.; William, U. P.; Gordon, C. K. R.; Robinson, J.; David, H.; Pritpal, K. S. J Med Chem 1992, 35, 2939.
21. Nakaya, T.; Nishio, K.; Memita, M.; Imoto, M. Makromol Chem Rapid Commun 1993, 14, 77.
22. Nakaya, T.; Memita, M.; Kang, M.; Macromol Reports 1993, A30, 349.
23. Hosoya, K. Anal Chem 1998, 70, 943.