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SYNTHESIS AND CHARACTERIZATION OF POLYMERIC PHOSPHOLIPID ANALOGUES CONTAINING BOTH ALKYL AND PHOSPHATIDYLCHOLINE GROUPS

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ABSTRACT

A series of new phospholipid analogous compounds, n-(methacryloyloxy)alkyl-2-(trimethylammonium)ethyl phosphates (**5a-c**), were prepared and characterized. The homopolymerizations and copolymerization of **5c** with methyl methacrylate (**MMA**) were carried out with α,α'-azobisisobutyronitrile(AIBN) as a radical initiator. The obtained monomers and polymers with high purity were confirmed by FT-NMR, IR spectral data, and elemental analyses method. Moreover, the viscosity behaviors of these new polymers which contain phosphatidylcholine groups in their side chains were also investigated.

INTRODUCTION

Recently, phospholipids and phospholipid analogues are widely studied in the fields of chemistry and biochemistry, because phospholipids are unquestionably important in various cellular membranes. Especially, considerable attention has been paid to polymeric phospholipid analogues containing phosphatidylcholine moieties, which exist on the extracellular surface of the phospholipid bilayer, concerning biocompatibility and other properties [1-6]. Since 2-(methacryloyloxy)ethyl-2-(trimethylammonium)ethyl phosphate

(**MTP**) was synthesized first by our research group in 1982 [7], a great amount of interest has been shown in the properties and applications of **MTP** [8-10]. We also reported the preparations and properties of methacrylic polymers containing both phosphatidylcholine group and long chain alkyl group in the side chains [11]. In order to obtain new biocompatible materials, it seemed very interesting to synthesize some novel polymerizable phospholipid analogous monomers, and to homo- and co-polymerize such monomers. Thus, we have prepared and polymerized 4-(methacryloyloxy)butyl-2-(trimethylammonium) ethyl phosphate (**MBTP**), 6-(methacryloyloxy)hexyl-2-(trimethylammonium)ethyl phosphate (**MHTP**) and 10-(methacryloyloxy)decyl-2-(trimethylammonium)ethyl phosphate (**MDTP**) in high yields. The synthesis and characterization of such monomers and polymers are mainly described in this paper.

RESULTS AND DISCUSSION

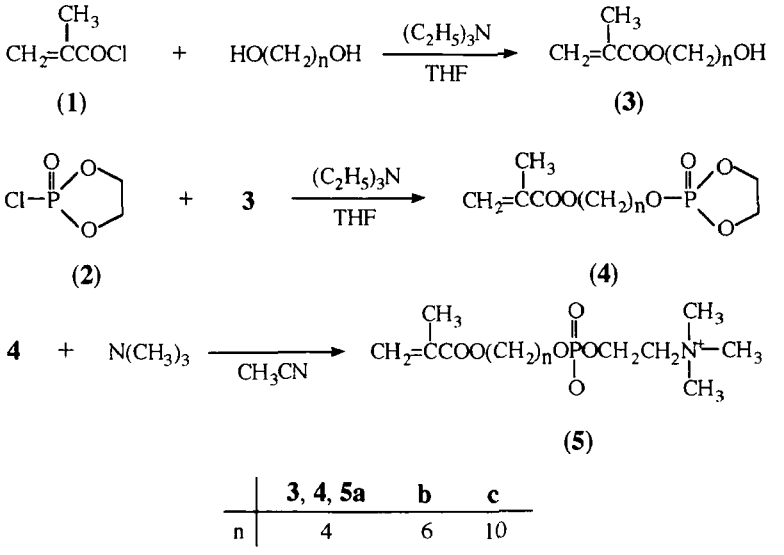
The synthetic procedure of the purposed monomers **5a-c**(**MBTP**, **MHTP** and **MDTP**) is outlined in Scheme 1.

n-Hydroxyalkylmethacrylates (**3a-c**) were obtained by the reaction of methacryloyl chloride (**1**) with 1,n-alkane glycols in the presence of triethylamine. n-(2-Oxo-1,3,2-dioxaphospholane-2-yloxy)alkyl methacrylates (**4a-c**) were synthesized by the reaction of **3a-c** with **2** in the presence of triethylamine. According to the method of Thoung and Chabrier[16], the reactions of **4a-c** with trimethylamine were carried out in anhydrous acetonitrile to give **5a-c** in good yields, respectively. These monomers were confirmed by their IR, FT-NMR and elemental analysis. They are white solids and soluble in methanol or chloroform but almost insoluble in acetone or diethylether. Their melting points could not be obtained because these monomers are very hygroscopic. As an example, the ^{1}H and ^{13}C FT-NMR spectra of compound **MDTP** are shown in Fig. 1.

The monomers (**MBTP**, **MHTP** and **MDTP**) were homopolymerized with AIBN (2% of monomer mol) as an initiator at 70°C for 16h to give the corresponding homopolymers (**6a-c**). The polymerization procedure of polymers is shown in Scheme 2.

The homopolymers were purified by precipitation and reprecipitation from anhydrous acetone, and they were investigated by IR, FT-NMR and melting points, respectively. The IR spectra showed that the absorption bands of C=C double bonds are absent, while the absorption bands of other groups appear as well as the corresponding monomers. In the FT-NMR spectra, only the peaks of C=C double bonds disappeared, and the others are similar to those of the monomers .

Using the same method, copolymerization of **MDTP** with **MMA** was also performed, and then the obtained copolymer **7** was purified by reprecipitation in anhydrous acetone as



	3, 4, 5a	b	c
n	4	6	10

Scheme 1. Synthesis for monomers **5a-c**.

well as the homopolymers. The pure copolymer **7** was determined by IR, FT-NMR and elemental analysis, respectively. Its ^{1}H-NMR spectrum did not show the peaks of C=C double bonds, while it showed the peak at δ=3.62ppm which attributes to the methyl of -COOCH$_3$ group.

The IR spectra of homopolymer **6c** and copolymer **7** are shown in Fig. 2. The obvious difference between the two polymers is that the characteristic absorption peaks of -COOCH$_3$ which exists in **MMA**, appear clearly at 1450 and 760cm^{-1} only in the IR spectrum of copolymer **7**.

All the obtained polymers are white solids and are soluble in methanol but almost insoluble in acetone and diethylether. In detailed, the homopolymers **6a** and **6b** are easily soluble, **6c** is soluble, and copolymer **7** is almost insoluble in water. No clear melting points were observed until the polymers were heated up to 250°C. Their yields are found about 65~70%.

In previous work[11], we have found that vinyl polymers having phosphatidylcholine in the side chains show the properties of polyelectrolytes in their viscosity behavior in aqueous solution. Therefore, in this study, viscosity measurements of these homopolymers **6a-c** were performed at 25°C in the presence of NaCl in water or in the mixture of methanol and

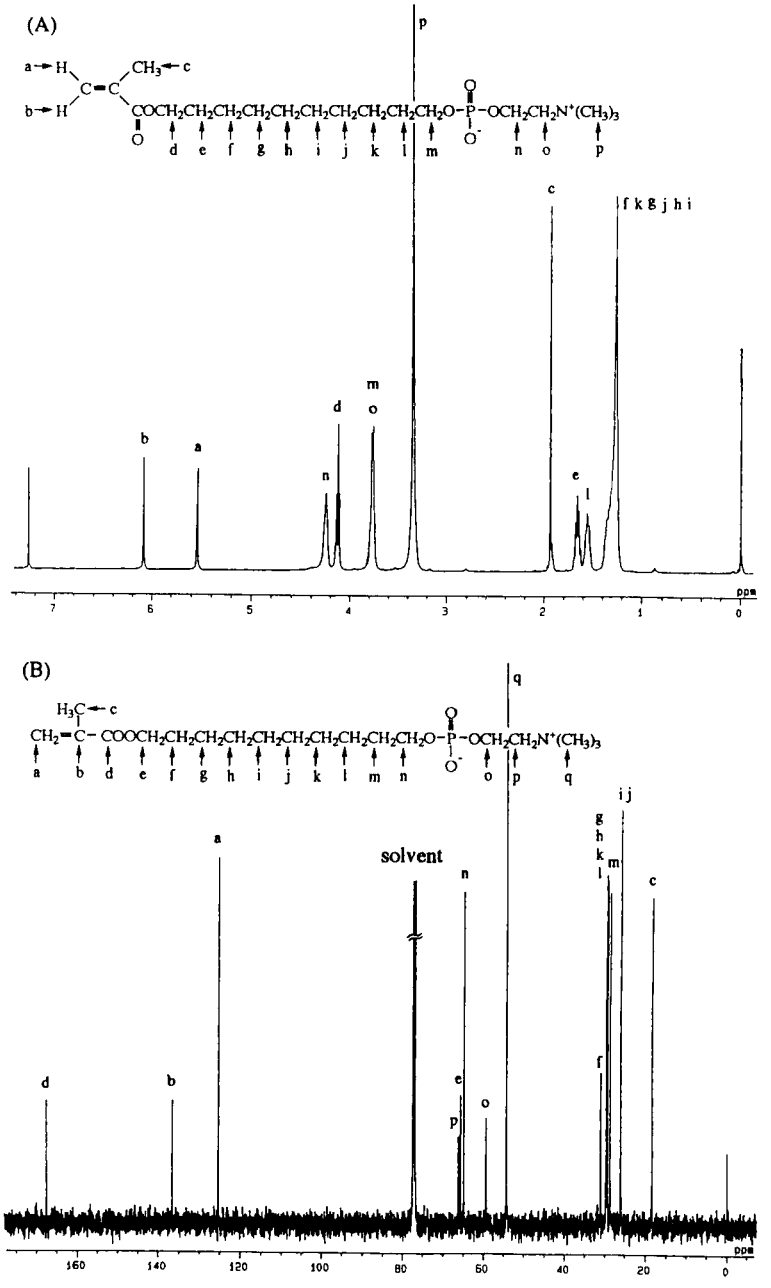
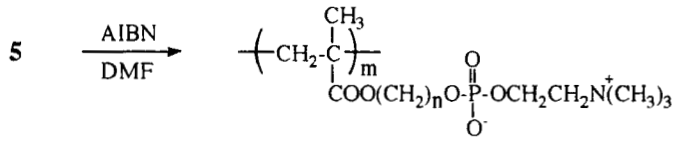
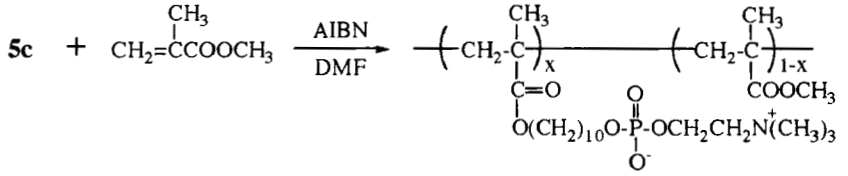


Fig. 1. ^{1}H FT-NMR(A) and proton noise decoupled ^{13}C FT-NMR(B) spectra of compound **MDTP** in $CDCl_3$, TMS as internal standard.



(6)



(7) (x=0.34)

Scheme 2. The homopolymerization of monomers **5a-c** and copolymerization process of monomer **5c** with **MMA**.

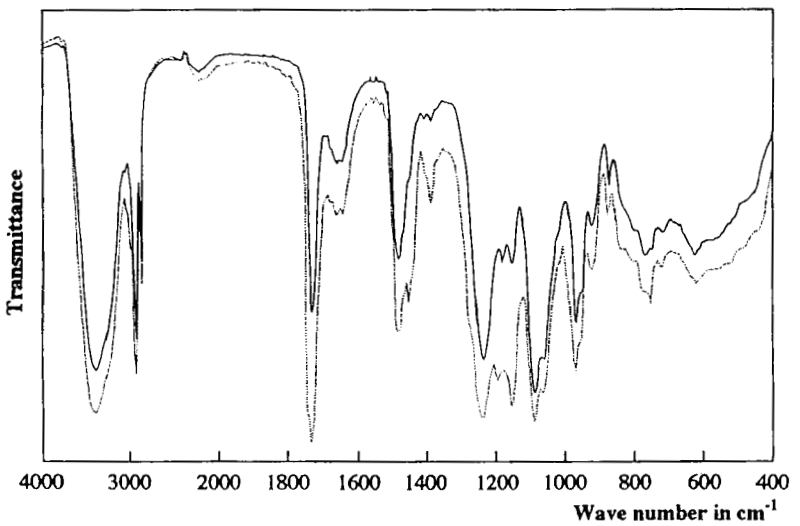


Fig. 2. IR spectra of homopolymer **6c** (——) and copolymer **7** (·····).

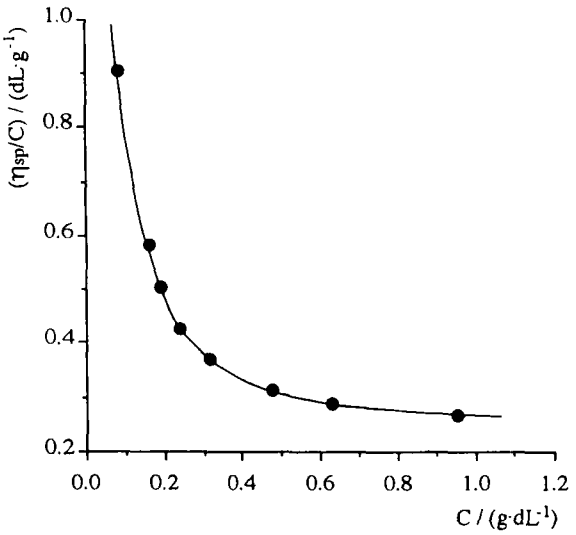


Fig. 3. Reduced viscosity of copolymer **7** in the mixed solvent of chlorobenzene and methanol (7:4, v/v) at 25°C.

water. Their η_{sp}/C were found to increase with the dilution of concentration in the absence of NaCl, and the inherent viscosity $[\eta]$ were found as 0.42, 0.39 and 0.32 in existence of 0.1M NaCl, respectively. In the case of copolymer **7**, the viscosity was measured in the mixture of chlorobezene and methanol (7:4; v/v) because the copolymer is insoluble in water. Fig. 3 shows the plot of reduced viscosity η_{sp}/C vs. C for copolymer **7**, its η_{sp}/C was found to increase rapidly with the reduce of concentration. It is revealed that copolymer **7** shows the viscosity behavior of usual polyelectrolytes in the presence of polar solvent due to containing cations and anions in the side chains.

The present synthesized monomers and polymers may be useful for designing and constructing synthetic phospholipid membrane. To prepare amphiphilic phospholipid analogous polymers, the copolymerizations of these monomers with long chain methacrylate or acrylamide monomers, and the adsorption of protein on the basis of copolymer **7** which contains both **MMA** and **MDTP** groups in the side chains are currently under investigation in our laboratory.

EXPERIMENTAL

Materials and measurements

Chloroform, benzene, chlorobenzene, tetrahydrofuran (THF), methanol, acetone,

acetonitrile, *N*, *N*-dimethylformamide (DMF) were commercially obtained and purified by distillation. All chemicals are of the highest purity available and used without further purification. Methacryloyl chloride (**1**) was prepared by the reaction of methacrylic acid with benzoyl chloride in the presence of a small amount of copper (II) chloride, according to the method of Stempel *et al.*[12]. 2-Chloro-2-oxo-1,3,2-dioxaphospholane (**2**), b.p. 102.5-105.0°C (1.0 mbar), was prepared according to the procedure described by Lucas and Edmundson[13,14]. Trimethylamine was prepared by the reaction of trimethylamine hydrochloride with 40% sodium hydroxide[15].

Proton(^{1}H) FT-NMR(400MHz) and carbon(^{13}C) FT-NMR(100.40MHz) spectra were recorded on a JEOL α-400. Their chemical shifts were referenced to tetramethylsilane directly as an internal standard. Infrared(IR) spectra (KBr discs) were obtained using a Jasco Model A 202 spectrometer and reported in wave numbers(cm^{-1}). The melting points of monomers and polymers were measured by a Micro Melting Point Apparatus (Yanaco MP-J3). Column chromatography was carried out on silica gel (Wakogel C-200). Viscometric measurements were performed with a Ubbelohde type viscometer at 25°C.

Synthesis

4-Hydroxybutylmethacrylate (3a)

Into a thoroughly dried 500mL three-necked round-bottomed flask equipped with a mechanical stirrer, a drying tube, and a dropping funnel were placed 25.00g (0.28mol) of 1,4-butanediol and 28.08g (0.28mol) of triethylamine in 250mL of dry THF. After cooling with ice-water bath (0°C), 29.02g (0.28mol) of **1** was slowly added to the stirred solution by dropwise over a period of 1h. During the dropping, the mixture was maintained at 0°C, and triethylamine hydrochloride as a white solid was precipitated. After being dropped, the reaction mixture was then allowed to warm up to 20°C and stirred for further 1h. The precipitate was filtered off and washed with 30mL THF. The filtrate was concentrated on rotary evaporator under reduced pressure to give crude product as a pale yellow liquid. The crude product was purified by column chromatography eluting with chloroform, and the pure compound **3a** as a colorless liquid was obtained. Yield: 22.21g (50.5%).

6-Hydroxyhexylmethacrylate (3b)

Using the same method for preparing **3a**, crude compound **3b** was also obtained as a pale yellow liquid from the reaction of hexamethylene glycol [25.0g (0.21mol)] with **1** [22.07g (0.21mol)]. The reaction was performed at 0°C as dropping temperature and 25°C~30°C as maintaining temperature. Then, it was purified by column chromatography with chloroform as a solvent to give pure **3b** as a colorless liquid. Yield: 20.59g (53.8%).

10-Hydroxydecylmethacrylate (3c)

With the same procedure for preparing **3a**, the reaction of 1,10-decanediol [25.0g (0.14mol)] with **1** [16.32g (0.15mol)] was carried out at 0°C as dropping temperature and

40~50°C as maintaining temperature. Then the obtained crude product was purified by column chromatography eluting with chloroform/acetone(50:1; v/v), and the pure compound **3c** was obtained as a colorless liquid. Yield: 20.52g (60.5%).

IR(KBr): 3400(s: -OH, ν_{O-H}); 2910 and 2850(vs: -$(CH_2)_n$-, ν_{C-H}); 1715(vs: C=O, $\nu_{C=O}$); 1630cm^{-1}(s: C=C, $\nu_{C=C}$).

^{1}H FT-NMR(400MHz, $CDCl_3$): Compound **3a**: δ=1.66(m: 2H, -C<u>H</u>$_2$CH_2OH); 1.78(m: 2H, -COOCH_2C<u>H</u>$_2$-); 1.94(s: 3H, -CH_3); 2.57(bs: 1H, -OH); 3.68(t: 2H, -C<u>H</u>$_2$OH); 4.19(t: 2H, -COOC<u>H</u>$_2$-); 5.56 (t: 1H, C<u>H</u>=C(CH_3)COO-, trans); and 6.10ppm (s: 1H, C<u>H</u>=C(CH_3)COO-, cis). Compound **3b**: δ=1.40(m: 4H, -COOCH_2CH_2(C<u>H</u>$_2$)$_2$-); 1.58(m: 2H, -C<u>H</u>$_2$CH_2OH); 1.69(m: 2H, -COOCH_2C<u>H</u>$_2$-); 1.94(s: 3H, -CH_3); 2.17(bs: 1H, -OH); 3.63(t: 2H, -C<u>H</u>$_2$OH); 4.15(t: 2H, -COOC<u>H</u>$_2$-); 5.56 (t: 1H, C<u>H</u>=C(CH_3)COO-, trans); and 6.10ppm (s: 1H, C<u>H</u>=C(CH_3)COO-, cis). Compound **3c**: δ=1.30(m: 12H, -COOCH_2CH_2(C<u>H</u>$_2$)$_6$-); 1.56(m: 2H, -C<u>H</u>$_2$CH_2OH); 1.67(m: 2H, -COOCH_2C<u>H</u>$_2$-); 1.94(s: 3H, -CH_3); 2.17(bs: 1H, -OH); 3.62(t: 2H, -C<u>H</u>$_2$OH); 4.14(t: 2H, -COOC<u>H</u>$_2$-); 5.55 (t: 1H, C<u>H</u>=C(CH_3)COO-, trans); and 6.10ppm (s: 1H, C<u>H</u>=C(CH_3)COO-, cis).

4-(2-Oxo-1,3,2-dioxaphospholane-2-yloxy)butyl methacrylate (4a)

Into a thoroughly dried 500mL three-necked round-bottomed flask equipped with a mechanical stirrer, a drying tube, and a dropping funnel were placed 22.21g (0.14mol) of **3a** and 15.15g (0.15mol) of triethylamine in 300mL of dry THF. After cooling with dry-ice/methanol bath (-20°C), 21.30g (0.15mol) of **2** were slowly added to the stirred solution by dropwise over a period of 1.5h. During the dropping, the mixture was maintained at -20°C ~ -15°C, and triethylamine hydrochloride as a white solid was precipitated. After being dropped, the reaction mixture was allowed to warm up to 0°C and stirred for further 2h. The precipitate was filtered off and washed with 30mL THF. The filtrate was concentrated on rotary evaporator under reduced pressure to obtain 18.11g (0.9mol) of **4a** as a pale yellow liquid. Yield: 36.13 (98.2%).

6-(2-Oxo-1,3,2-dioxaphospholane-2-yloxy)hexyl methacrylate (4b)

Using the same method for preparing **4a**, **4b** was obtained as a pale yellow liquid from the reaction of **3b** [20.59g (0.11mol)] with **2** [17.18g (0.12mol)]. The reaction was performed at -15°C as dropping temperature and 0°C~10°C as maintaining temperature. Yield: 31.07g (97.8%).

10-(2-Oxo-1,3,2-dioxaphospholane-2-yloxy)decyl methacrylate (4c)

The similar reaction for preparing **4a**, was carried out between 20.52g (0.08mol) of **3c** and 12.78g (0.09mol) of **2** to give **4c** as a pale yellow liquid(0°C as dropping temperature

and 15~20°C as maintaining temperature). Yield: 22.27g (98.0%).

IR(KBr): 2910 and 2850(vs: -$(CH_2)_n$-, ν_{C-H}); 1715(vs:C=O, $\nu_{C=O}$); 1630cm^{-1}(s: C=C, $\nu_{C=C}$); 1275(s: O-P=O, ν_{P-O}) and 1050cm^{-1}(vs: P-O-C, ν_{C-O}).

4-(Methacryloyloxy)butyl-2-(trimethylammonium)ethyl phosphate (MBTP) (5a)

After 36.13g (0.14mol) of **4a** and 120mL dry acetonitrile were placed into a 300mL glass pressure bottle, 16.55g (0.28mol) of trimethylamine was quickly added into the same bottle. The pressure bottle was closed and then shaken in a thermostat at 50°C for 20h. After the reaction, it was cooled to room temperature. The pressure bottle was opened, then the solution was concentrated to give crude product as a yellow liquid. The crude product was dissolved in methanol, and then reprecipitated from dry acetone. This operation was repeated for three times to afford pure product **5a** as a white solid. Yield: 30.11g (66.5%).

^{1}H FT-NMR(400MHz, $CDCl_3$): δ=1.75(m: 2H, -$COOCH_2C\underline{H}_2$-), 1.66(m: 2H, -$C\underline{H}_2CH_2OPO$-), 1.92(s, 3H, -CH_3), 3.38(s, 9H, -$N^+(CH_3)_3$), 3.82(m: 2H, -$C\underline{H}_2N^+$-), 3.85(m: 2H, -$C\underline{H}_2OPO$-), 4.15(t: 2H, -$COOCH_2$-), 4.27(bs: 2H, -$OPOC\underline{H}_2$-), 5.55(t: 1H, $C\underline{H}$=C(CH_3)-, trans), 6.08ppm(s: 1H, $C\underline{H}$=C(CH_3)-, cis).

^{13}C FT-NMR(100.40MHz, $CDCl_3$): δ=18.36(-CH_3), 25.29(-$C\underline{H}_2CH_2OPO$-), 27.28(-$COOCH_2C\underline{H}_2$-), 54.22(-$N^+(CH_3)_3$), 59.25(-$OPOC\underline{H}_2$-), 64.40(-$C\underline{H}_2OPO$-), 64.81(-$COOC\underline{H}_2$-), 66.20(-CH_2N^+-), 125.50($C\underline{H}_2$=C(CH_3)-), 136.32(CH_2=$\underline{C}$(CH_3)-), 167.50ppm(-COO-).

6-(Methacryloyloxy)hexyl-2-(trimethylammonium)ethyl phosphate (MHTP) (5b)

Monomer **5b** was prepared by the reaction of **4b** [31.01g (0.11mol)] and trimethylamine [13.00g (0.22mol)] at 50°C for shaking 24h. Then it was refined with precipitation and reprecipitation in dry acetone, and the pure compound **5b** as a white solid was obtained. Yield: 25.40g (65.7%).

^{1}H FT-NMR(400MHz, $CDCl_3$): δ=1.37(m: 4H, -$COOCH_2CH_2(C\underline{H}_2)_2$-), 1.58(m: 2H, -$COOCH_2C\underline{H}_2$-), 1.66(m: 2H, -$C\underline{H}_2CH_2OPO$-), 1.93(s, 3H, -$CH_3$), 3.37(s, 9H, -$N^+(CH_3)_3$), 3.80(m: 4H, -$C\underline{H}_2OPO$- and -$C\underline{H}_2N^+$-), 4.12(t: 2H, -$COOCH_2$-), 4.26(bs: 2H, -$OPOC\underline{H}_2$-), 5.55(t: 1H, $C\underline{H}$=C(CH_3)COO-, trans), 6.08ppm(s: 1H, $C\underline{H}$=C(CH_3)COO-, cis)

^{13}C FT-NMR(100.40MHz, $CDCl_3$): δ=18.36(-CH_3), 25.48(-$COO(CH_2)_3C\underline{H}_2$-), 25.69(-$COO(CH_2)_2C\underline{H}_2$-), 28.57(-$C\underline{H}_2CH_2OPO$-), 30.81(-$COOCH_2C\underline{H}_2$-), 54.22(-$N^+(CH_3)_3$), 59.24(-$OPOC\underline{H}_2$-), 64.60(-$C\underline{H}_2OPO$-), 65.36(-$COOC\underline{H}_2$-), 66.16(-$CH_2N^+$-), 125.37($C\underline{H}_2$=C($CH_3$)-), 136.41($CH_2$=$\underline{C}$($CH_3$)-), 167.54ppm(-COO-).

10-(Methacryloyloxy)decyl-2-(trimethylammonium)ethyl phosphate (MDTP) (5c)

The similar process was performed as preparing **5a**, **5c** as a white solid was prepared from **4c** [22.27g (0.08mol)] and trimethylamine [14.19g (0.24mol)] (shaking 30h at 50°C), and then it was refined by reprecipitation from dry acetone. Yield: 22.27g (68.1%).

IR(KBr): 2910 and 2850(vs: $-(CH_2)_n-$, ν_{C-H}); 1715(vs: C=O, $\nu_{C=O}$); 1630(vs: C=C, $\nu_{C=C}$); 1230(vs: O-P=O, ν_{P-O}) and 1050-1080cm^{-1}(vs: P-O-C, ν_{C-O}).

^1H FT-NMR(400MHz, $CDCl_3$) and ^{13}C FT-NMR(100.40MHz, $CDCl_3$) spectra of compound **5c** are shown in Fig. 1.

Elemental analysis for compounds **5a-c**:

5a : $C_{13}H_{26}O_6NP$	Calc.(%)	C 48.28	H 8.12	N 4.33
(323.38)	Found(%)	C 48.19	H 8.20	N 4.27
5b : $C_{15}H_{30}O_6NP$	Calc.(%)	C 51.26	H 8.62	N 3.99
(351.44)	Found(%)	C 51.32	H 8.73	N 3.83
5c : $C_{19}H_{38}O_6NP$	Calc.(%)	C 55.99	H 9.42	N 3.44
(407.56)	Found(%)	C 55.85	H 9.56	N 3.36

Polymerization procedure

In a sealed ampoule, the homopolymerizations of monomers **5a-c** were carried out in dry DMF with α,α'-azobisisobutyronitrile (AIBN) (2% of monomer mol ratio) as an initiator by shaking at 70°C for 16h. After opening the ampoules, the solutions were poured into anhydrous acetone to precipitate the corresponding homopolymers. Then, the crude products were purified by reprecipitation with acetone for three times. The pure homopolymers **6a-c** were obtained as white solids. The structures of homopolymers **6a-c** were confirmed by IR(KBr) and FT-NMR(CD_3OD) spectra. Yields: About 65~70%. Melting points: >250°C.

Using the same procedure, copolymerization of monomer **5c** with methyl methacrylate (**MMA**) was carried out. The copolymer **7** as a white solid was obtained. The structure of copolymer **7** was investigated by IR(KBr) and FT-NMR(CD_3OD) spectra, while the composition was determined by elemental analysis. Yield: 72%. m.p.: >250°C. Found(%): C: 57.56; H: 8.93; N: 2.35.

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