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A PRACTICAL FORMAL SYNTHESIS OF A PHYSIOLOGICALLY ACTIVE ANALOGUE OF PLATELET ACTIVATING FACTOR

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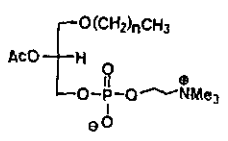
A synthesis of phosphocholine **9** was accomplished in 36% yield from isopropylidene glycerol in contrast to 2% yield as reported previously. Since **9** has been converted to platelet activating factor-analogue **2**, this report constitutes a more practical synthesis of **2**.

INTRODUCTION

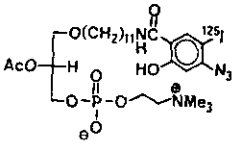
Platelet activating factor (PAF) (**1**) is a potent substance responsible for a wide spectrum of biological activities.[1,2,3] For example, PAF induces aggregation and secretion in rabbit platelets at an extremely low concentration level of 1×10^{-10} molar. Numerous studies addressing structure acitvity relationship have led to the proposal of a conformation of platelet PAF-specific binding site;[2] however, the putative receptor has not yet been isolated.

In order to facilitate the isolation of PAF-receptor, Bienvenue and coworkers[4] have designed a PAF-analogue **2** with a photoreactive azido group and radioactive iodine label present in the molecule. They found that this PAF-analogue displayed similar biological activity as the genuine PAF molecule. We have also engaged in the identification of PAF-receptor and were interested in the preparation of **2**.

In an effort to synthesize **2** according to the



1 n = 15, 17

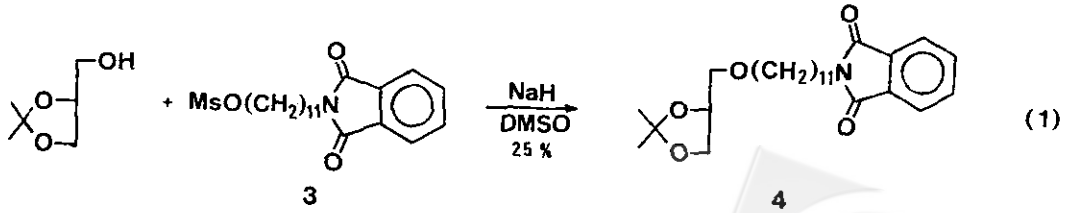


2

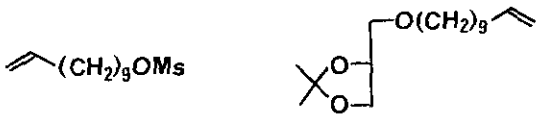
reported procedure[4] we found that there are at least two drawbacks involved in Bienvenue's process. Firstly, at the onset of the reported synthesis imide **4** was prepared in only 25% yield (eq. 1). Secondly, Bienvenue and coworkers employed an operationally complicated procedure reported by van Boom[5] in order to put on the phosphocholine moiety. Here we wish to report our modified high yield approach to the synthesis of the PAF-analogue **2**.

Results and Discussion

When we attempted the reaction as shown in equation (1) we were not able to obtain imide **4**.



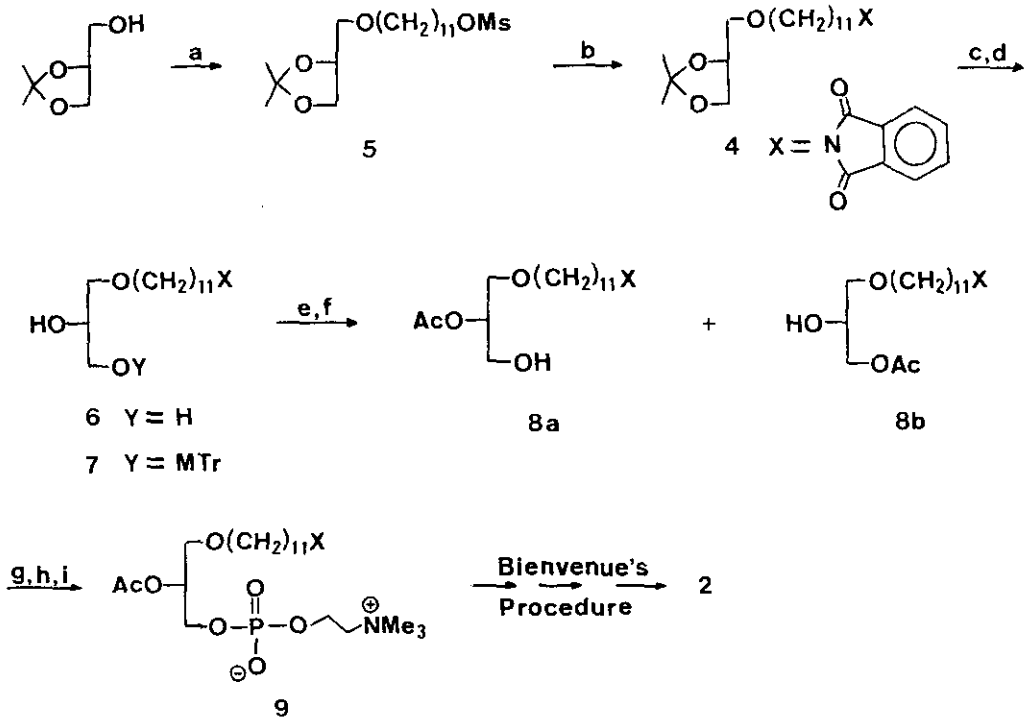
3 **4** (1)



10 11 12

Instead we found that the alkoxide preferentially attacked the imide carbonyl. To solve this problem we prepared 11-bromoundecyl mesylate by treating 11-bromoundecanol with mesyl chloride (1.3 equiv) and triethylamine (1.5 equiv) in dichloromethane at 0°C. When the crude bromo mesylate (1 equiv) was reacted with isopropylidene glyceroxide generated from isopropylidene glycerol and sodium hydride (1.5 equiv) in a mixture of anhydrous dimethyl sulfoxide and tetrahydrofuran at 0°C for 1.5 h, we obtained mesylate **5** (Scheme I) in 56% yield. In addition, we isolated 26% of mesylate **10** and 5% of olefin **11** resulting most likely from E2 process of the corresponding bromide and mesylate, respectively. We have also isolated 6% of an ether type compound **12** whose origin was not certain. With a slight modification, when we mixed isopropylidene glycerol with 1.5 equivalents of 11-bromoundecyl mesylate in

Scheme I



(a) $Br(CH_2)_{11}OMs$, NaH, DMSO, THF, 0°C, 2h (b) potassium phthalimide, DMSO, 95°C, 2h (c) TsOH (cat), MeOH, RT (d) 4-methoxyphenydiphenylmethyl chloride (MTrCl), Et_3N, CH_2Cl_2, 0°C, 30min (e) Ac_2O, Et_3N, 4-DMAP (cat), CH_2Cl_2, RT, 3h (f) H_3BO_3/SiO_2 (g) $Cl_2PO_2CH_2CH_2Br$, Et_3N, $ClCH{=}CCl_2$, 0°C, 35 min (h) NaOAc, EDTA, H_2O, THF, RT, 1h (i) Me_3N (45%), 2-PrOH, CH_3CN, $CHCl_3$, 60°C, 4h.

a mixture of anhydrous dimethyl sulfoxide and tetrahydrofuran at 0°C followed by the addition of 3 equivalents of sodium hydride, a delightful 91% yield of mesylate 5 was obtained.

Conversion of 5 to acetate 8a with optimized reaction conditions was shown in Scheme I. Thus mesylate 5 was coupled with potassium phthalimide to give 4 in 94% yield. Removal of the isopropylidene group was accomplished by stirring imide 4 in methanol with a catalytic amount of *p*-toluenesulfonic acid to give 89% of the diol 6 with 11% recovery of 4. The primary hydroxyl group was then protected by reacting diol 6 with *p*-methoxyphenyldiphenylmethyl chloride (MTrCl) to give 86% of alcohol 7. Conversion of 7 to the corresponding acetate was accomplished with acetic anhydride and triethylamine catalyzed by 4-*N*,*N*-dimethylaminopyridine (4-DMAP). Trityl group of the crude acetate was removed according to Buchnea's procedure[6] using a boric acid-impragnated silica gel column. Straight silica gel or the presence of catalytic amount of triethylamine would cause 1,2-migration of the acetyl group and should be avoided. Thus, the acetate alcohol 8 was obtained from 7 in 84% yield. ^{1}H NMR analysis indicated that this material was a 93 : 7 mixture of the desired alcohol 8a and its regioisomer 8b.

As mentioned at the beginning, Bienvenue[4] converted 8a to 9 with a complicated procedure using at least three very polar organic reagents which made isolation of the polar phosphocholine very difficult. In addition, the phosphocholine obtained this way was a 75 : 25 mixture of 9 and its regioisomer. Apparently, the reaction condition employed caused isomerization of 8a to 8b to some extent. We found that Eibl's procedure[7] was better because simpler reagents were used and no isomerization occurred. Thus, when the mixture of 8 was treated with crude 2-bromoethylphosphoric acid chloride[7] followed by hydrolysis and amination gave phosphocholine 9 in 66% yield as a 95 : 5 mixture of 9 and its regioisomer as determined by HPLC analysis.[8] The undesired isomer of 9 was removed by preparative HPLC. With pure 9 in hand the rest of the synthesis of 2 was carried out according to the reported procedure.[4]

In conclusion, we have accomplished the synthesis of phosphocholine 9 in a total of 36% yield from isopropylidene glycerol compared with 2% as reported previously.[4] Since 9 has been converted to PAF-analogue 2 by Bienvenue *et al.*,[4] thus we have accomplished a more practical formal total synthesis of 2. Although racemic isopropylidene glycerol was used throughout this report, our approach could also be applied to the synthesis of homochiral 2 if one starts from (*R*)-isopropylidene glycerol.

Experimental Section

All melting points were taken with a Yanaco micro melting point apparatus and are uncorrected. ^{1}H magnetic resonance spectra were recorded on Varian Associates EM-390 or Bruker AM-300WB spectrometers and are reported in parts per million from internal tetramethylsilane on the δ scale. Infrared spectra were taken with a Perkin-Elmer 1310 or 983G instrument. Mass spectra were recorded on a Finnigan TSQ-46C instrument operating at an ionization voltage of 70 eV. Elemental analyses were carried out on a Perkin-Elmer 240C instrument.

The following solvents and reagents were dried and purified prior to use: tetrahydrofuran (distilled from sodium benzophenone ketyl); dimethyl sulfoxide, triethylamine, and methanesulfonyl chloride (distilled from calcium hydride). Reactions requiring an inert atmosphere were run under a blanket of nitrogen. Analytical thin layer chromatography was performed using EM Laboratories 0.25 mm thick precoated silica gel 60 F-254 plates. Column chromatography was performed over EM Laboratories silica gel (70 – 230 mesh). High pressure luquid chromatography was carried out on a Waters Associates instrument equipped with ultraviolet and refractive index detectors.

11-(3,4-Isopropylideneglyceroxy)undecyl me-

thanesulfonate (5). To a solution of 1.53 g (6.1 mmol) of 11-bromo-1-undecanol and 1.3 mL (9.1 mmol) of triethylamine in 6 mL of dichloromethane cooled at 0°C under nitrogen was added dropwise a solution of 0.62 mL (7.9 mmol) of methanesulfonyl chloride in 6 mL of dichloromethane dropwise over a period of 50 min. The resulting mixture was stirred at 0°C for 30 min and then partitioned between 100 mL of ether and 50 mL of water. The ether layer was washed with 50 mL of water, 50 mL of saturated sodium chloride solution, dried ($MgSO_4$) and concentrated in vacuo to give 2.24 g of a yellow liquid. ^{1}H NMR analysis of this crude 11-bromo-1-undecyl mesylate indicated that it was quite pure and was used directly in the preparation of **5**. However, if necessary, the mesylate can be purified via column chromatography over silica gel. IR (neat) 2960, 2900, 1480, 1360, 1180, 960 cm^{-1}; ^{1}H NMR (CCl_4, 90 MHz) δ 1.02–2.01 (*m*, 18H, methlene manifold), 2.87 (*s*, 3H, CH_3), 3.31 (*t*, J = 6 Hz, 2H, $BrCH_2$), 4.10 (*t*, J = 6Hz, 2H, OCH_2). Anal. Calcd for $C_{12}H_{25}BrO_3S$: C, 43.77; H, 7.65. Found: C, 43.89; H, 7.77.

To a solution of 1.67 g (5.1 mmol) of the crude bromo mesylate in 5.5 mL of dry dimethyl sulfoxide and 4.5 mL of dry tetrahydrofuran cooled in an ice-water bath under nitrogen was added 0.42 mL (3.4 mmol) of isopropylideneglycerol followed by 386 mg (10 mmol) of sodium hydride (60% dispersion in mineral oil). The reaction mixture was stirred at 0°C for 2 h and partitioned between 100 mL of ether and 50 mL of water. The ether layer was washed with 50 mL of water, 50 mL of saturated sodium chloride solution, dried ($MgSO_4$) and concentrated in vacuo to give 2.13 g of a pale yellow liquid. This liquid was chromatographed over 60 g of silica gel (eluted with ethyl acetate/hexane, 1:9, followed by ethyl acetate/hexane, 25:75) to give 1.17 g (91%) of **5** as a pale yellow liquid: IR (neat) 3000, 2940, 2860, 1460, 1360, 1260, 1220, 1180, 1120, 1060, 960, 950 cm^{-1}; ^{1}H NMR (CCl_4, 90 MHz) δ 1.10–1.90 (*m*, 24H, methylene manifold and CH_3), 2.85 (*s*, 3H, OSO_2CH_3), 3.11–4.22 (*m* with *t*, J = 7 Hz, at 4.12, 9H, OCH and OCH_2); mass spectrum, *m/e* (relative intensity) 381 (0.5, M^+), 365 (6), 153 (6), 111 (12), 101 (100), 97 (51), 83 (47), 79 (22), 73 (33), 69 (39). Anal. Calcd for $C_{18}H_{36}O_6S$: C, 56.81; H, 9.54. Found: C, 56.80; H, 9.67.

1,2-Isopropylidene-3-*O*-(11-phthalimidoundecyl) glycerol (4). A mixture of 1.41 g (3.7 mmol) of the mesylate **5** and 786 mg (4.2 mmol) of potassium phthalimide in 7 mL of dry dimethyl sulfoxide was heated under nitrogen at 95°C for 2h. The resulting mixture was partitioned between 100 mL of ether and 50 mL of water. The organic layer was washed with 50 mL of water, 50 mL of saturated sodium chloride solution, dried ($MgSO_4$) and concentrated in vacuo to give 1.77 g of a yellow oil. The oil was chromatographed over 50 g of silica gel (eluted with ethyl acetate/hexane, 15:85) to give 1.5 g (94%) of imide **4** as a pale yellow oil: IR (neat) 2980, 2940, 2860, 1770, 1710, 1460, 1385, and 1360 cm^{-1}; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.10–1.85 (*m* with two *s* at 1.35 and 1.40, 24H, methylene manifold and CH_3), 3.30–3.80 (*m* with *t*, J = 7 Hz, at 3.65, 7H, OCH_2 and NCH_2), 3.93–4.33 (a six line multiplet, 2H, OCH and OCH_2), 7.60–7.93 (*m*, 4H, Ar-H); mass spectrum, *m/e* (relative intensity) 416 (8, $M^+ - CH_4$), 300 (23), 174 (10), 160 (100), 148 (28), 130 (22), 104 (12), 83 (19). Anal. Calcd for $C_{25}H_{37}NO_5$: C, 69.58; H, 8.64; N, 3.25. Found: C, 69.43; H, 8.67; N, 3.28.

1-*O*-(11-Phthalimidoundecyl)glycerol (6). To a solution of 1.5 g (3.5 mmol) of imide **4** in 20 mL of methanol was added 20 mg (0.1 mmol) of *p*-toluenesulfonic acid monohydrate. The resulting solution was stirred at room temperature overnight followed by the addition of 20 μL (0.14 mmol) of triethylamine and then concentrated in vacuo. The resulting solid residue was chromatographed over 25 g of silica gel (eluted with ethyl acetate/hexane, 4:6, followed by ethyl acetate/hexane, 8:2) to give 173 mg (11%) of unreacted **4** as a pale yellow oil and 1.21 g (89%) of diol **6** as a white solid: mp 57°C;

IR (CCl_4) 3500 (br), 2955, 2875, 1770, 1720, 1445, 1435, 1395, 1365 cm^{-1}; ^1H NMR (CCl_4, 90 MHz) δ 1.19–1.80 (*m*, 18H, methylene manifold), 2,19–2.60 (br *s*, 1H, OH), 2.60–2.95 (br *s*, 1H, OH), 3.31–3.82 (*m*, 9H, OCH, OCH_2 and NCH_2), 7.72–7.95 (*m*, 4H, Ar-H); mass spectrum, *m/e* (relative intensity) 391 (0.5, M^+), 360 (2), 331 (6), 316 (12), 300 (13), 160 (100), 148 (13), 130 (11). Anal. Calcd for $C_{22}H_{33}NO_5$: C, 67.99; H, 8.50; N, 3.58. Found: C, 67.82; H, 8.74; N, 3.49.

1-*O*-(4-methoxyphenyldiphenyl)methyl-3-*O*-(11-phthalimidoundecyl)-glycerol (7). To a solution of 1.221 g (3.1 mmol) of diol **6** and 0.86 mL (6.2 mmol) of dry triethylamine in 5 mL of dichloromethane cooled at 0°C under nitrogen was added dropwise over a period of 30 min a solution of 1.53 g (4.9 mmol) of (4-methoxyphenyl)diphenylmethyl chloride in 5 mL of dichloromethane. After stirring at 0°C for another 30 min the resulting mixture was partitioned between 100 mL of ether and 50 mL of water. The organic layer was washed with 50 mL of saturated sodium chloride solution, dried ($MgSO_4$) and concentrated in vacuo to give a yellow oil. The oil was chromatographed over 60 g of silica gel (eluted with ethyl acetate/hexane, 2:8, followed by ethyl acetate/hexane, 1:1) to give 1.76 g (86%) of alcohol **7** as a yellow oil: IR (neat) 3500 (br), 3400, 2960, 2880, 1770, 1710, 1610, 1515, 1470, 1440, 1395, 1360, 1240, 1180, 1140 cm^{-1}; ^1H NMR (CCl_4, 90 MHz) δ 1.10–1.80 (*m*, 18H, methylene manifold), 3.09 (*d*, *J* = 6 Hz, 1H, OH), 3.23–3.90 (*m* with *s* at 3.71, 10H, OCH, OCH_2, OCH_3 and NCH_2), 6.70 (br *d*, *J* = 9Hz, 2H, Ar-H), 7.05–7.47 (*m*, 12H, Ar-H), 7.47–7.85 (*m*, 4H, phthalimidyl-Ar-H). Anal. Calcd for $C_{42}H_{49}NO_6$: C, 75.99; H, 7.44: N, 2.11. Found: C, 76.53: H, 7.82: N, 1.75.

1-*O*-(4-Methoxyphenyldiphenyl)methyl-3-*O*-(11-phthalimidoundecyl)-glycerol acetate (13). To a solution of 2.15 g (3.2 mmol) of alcohol **7**, 0.91 mL (6.5 mmol) of dry triethylamine and 8 mg (0.06 mmol) of 4-*N*,*N*-dimethylaminopyridine in 10 mL of dichloromethane under nitrogen was added 0.49 mL (5.2 mmol) of acetic anhydride in one portion. The resulting solution was stirred at room temperature for 3 h and partitioned between 100 mL of ether and 50 mL of water. The organic layer was washed with 50 mL of saturated sodium chloride solution, dried ($MgSO_4$) and concentrated in vacuo to give 2.32 g of acetate **13** as a yellow oil. This oil was used directly in the next step without further purification. However, for full characterization, acetate **13** could be purified via silica gel column chromatography (eluted with ethyl acetate/hexane, 2:8, followed by ethyl acetate/hexane, 3:7): IR (neat) 3080, 2960, 2880, 1840, 1780, 1740, 1520, 1380, 1280 cm^{-1}; ^1H NMR ($CDCl_3$, 90 MHz) δ 1.05–1.89 (*m*, 18H, methylene manifold), 2.07 (*s*, 3H, $COCH_3$), 3.17–3.88 (*m* with *s* at 3.79, 11H, OCH_2, OCH_3 and NCH_2), 5.19 (*qu*, *J* = 6 Hz, 1H, OCH), 6.83 (br *d*, *J* = 9 Hz, 2H, Ar-H), 7.13–7.53 (*m*, 12H, Ar-H), 7.63–7.94 (*m*, 4H, phthalimidyl-Ar-H). Anal. Calcd for $C_{44}H_{51}NO_7$: C, 74.87; H, 7.28; N, 1.98. Found: C, 74.80; H, 7.53; N, 1.83.

2-*O*-Acetyl-3-*O*-(11-phthalimidoundecyl)glycerol (8a). Boric acid- impregnated silica gel was prepared according to Buchnea's procedure.[6] Thus, 10 g of silica gel was thoroughly mixed with a hot solution of 6.3 g of boric acid in 34 mL of water. The resulting mixture was filtered by suction and the filter cake was dried at 100°C for 24 h. The crude acetate **13** (76 mg) was dry-loaded on silica gel and then eluted by gravity over the boric acid coated silica gel (2g) with ethyl acetate/hexane (15:85). Fractions containing unreacted **13** were combined, concentrated and reloaded on the column and eluted with the same solvent system. The eluent was then changed to ethyl acetate-hexane (3:7) and finally with ethyl acetate/hexane (1:1). Fractions that contained alcohol **8** were combined and concentrated in vacuo to give an orange solid. The solid residue was rechromatographed over 1 g of the boric acid coated silica gel (eluted first with ethyl acetate/hexane, 3:7, followed by ethyl acetate/hexane, 1:1, and finally with ethyl acetate/hexane, 4:6). The desired

alcohol fractions were combined, washed with water to remove boric acid, dried ($MgSO_4$) and concentrated *in vacuo* to give 38.5 mg (84% from 7) of alcohol 8 as a pale yellow oil. ^{1}H NMR analysis of this material showed that it was a mixture of the desired alcohol **8a** and its regioisomer **8b** in a ratio of 93 : 7. **8a**: IR (*neat*) 3460 (br), 2920, 2840, 1760, 1720, 1680, 1460, 1390, 1380, 1250, 1100, 1050 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.00–1.37 (*m*, 14H, methylene manifold), 1.50 (br *qu*, *J* = 7 Hz, 2H, N-C-CH_2-), 1.62 (br *qu*, *J* = 7 Hz, 2H, O-C-CH_2-), 1.80 (br *s*, 1H, OH), 2.06 (*s*, 3H, $COCH_3$), 3.30–3.48 (*m*, 2H, -OCH_2CH_2), 3.59 (*dd*, *J* = 5,3 Hz, 2H, -CH_2OH), 3.69 (*t*, *J* = 7 Hz, 2H, NCH_2), 3.78 (*d*, *J* = 5 Hz, 2H, -CH_2OR), 5.01 (*qu*, *J* = 5 Hz, 1H, RCOOCH), 7.60–7.71 (*m*, 2H, Ar-H), 7.74–7.83 (*m*, 2H, Ar-H); characteristic signals for the minor isomer **8b**: δ 2.04 (*s*, 3H, $COCH_3$), 4.00 (*q*, *J* = 5 Hz, 1H, HO-C<u>H</u>-), 4.07 (*dd*, *J* = 11, 6Hz, 1H, RCOOCH_2), 4.15 (*dd*, *J* = 11, 4 Hz, 1H, RCOOCH_2). Anal. Calcd for $C_{24}H_{35}NO_6$: C, 66.49; H, 8.14; N, 3.23. Found: C, 66.52; H, 8.23; N, 3.15.

2-*O*-Acetyl-3-*O*-(11-phthalimidoundecyl)glycerophosphocholine (9). To a solution of 27 μL (0.2 mmol) of 2-bromoethylphosphoric acid dichloride[7] in 0.2 mL of trichloroethylene cooled in an ice-water bath under nitrogen was added 25 μL (0.18 mmol) of dry triethylamine in one portion. To the resulting solution was added dropwise a solution of 34.5 mg (0.08 mmol) of the mixture of alcohol **8a** and **8b** over a period of 15 min. The resulting mixture was stirred at 0°C for another 35 min, filtered and concentrated in vacuo. The residue was then dissolved in 0.67 mL of tetrahydrofuran followed by the addition of 0.67 mL of a 0.5 M sodium acetate solution (pH 8.5) and 0.04 mL of a 0.5 M ethylenediaminetetraacetate solution (pH 10.5). The resulting turbid solution turned clear after stirring at room temperature for 5 min. After 1h, the reaction mixture was extracted with 25 mL of a 9:1 mixture of chloroform and methanol. The organic layer was dried ($MgSO_4$) and concentrated in vacuo to give 60.9 mg of a residue. This residue was mixed with 0.26 mL of chloroform, 0.45 mL of 2-propanol and 0.45 mL of acetonitrile followed by the addition of 0.62 mL of a 45% aqueous trimethylamine solution. The resulting mixture was stirred at 60°C for 4 h and then extracted with 25 mL of a 9:1 mixture of chloroform and methanol. The organic layer was dired ($MgSO_4$) and concentrated in vacuo to give 57.3 mg of a solid residue. This material was chromatographed over 0.6 g of silica gel (eluted with dichloromethane/methanol/water, 65:15:1, followed by dichloromethane/methanol/water, 65:35:5) to give 31.4 mg (66%) of **9** as a colorless oil. HPLC analysis[8] (nucleosil 50-5 column, 4.7 mm x 25 cm, preequilibrated with a solvent system of acetonitrile/methanol/85% phosphoric acid, 130:5:1.5, flow rate = 1 mL/min) of this material indicated the presence of 5% of the regioisomer (R_t = 21 min). The desired phosphocholine **9** (R_t = 22 min) was freed from its regioisomer by preparative HPLC and was identical to the material synthesized by Bienvenue[4] by comparison with the reported ^{1}H NMR spectrum.

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