One-Pot Synthesis of Cationic Amphiphiles from *n*-Alcohols and Allyl Alcohols

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Abstract: A novel, efficient one-pot method has been developed to synthesize amphiphiles such as N-alkylated/N-allylated triethylamines and pyridinium salts for the first time from *n*-alcohols and naturally occurring terpenes (allyl alcohols) in good yields. These amphiphiles have got industrial application as surfactants, DNA carriers, and other biological applications. The DNA delivery efficacy and cytotoxicity of N-alkylated and N-allylated triethylamine and pyridinium salts were studied.

Key words: one-pot synthesis, *n*-alcohols, allyl alcohols, amphiphiles, DNA delivering agents, surfactants, cytotoxic agents, trialkylamine salts, pyridinium salts

The concept of gene therapy involves the introduction of engineered or foreign genetic material into target cells or organs in order to induce protein expression. Various methods have been developed to deliver the engineered genetic material, mostly based upon either viral or nonviral carrier systems (vectors). At present, the most efficient methods for the transfer of genetic material (transfection) involve the use of viral vectors[1,2] (e.g., retrovirus, adenovirus), although there are strong arguments concerning the risks with regard to immunogenicity and propagation.[3,4] Synthetic cationic amphiphiles are considered to be promising carriers for gene delivery. They can transfect genes after complexation with DNA (lipoplex formation) and deliver genes into the cell, eventually leading to their expression.

Amphiphiles are chemical compounds possessing both hydrophilic (water-loving) and lipophilic (fat-loving) properties. Amphiphiles have very good surfactant property and wide industrial applications (Figure 1) such as in detergents,[5,6] cosmetics,[7] skin-care products,[8] shampoo preparations,[9–11] etc. They also show wide variety of biological activities, such as antifungal,[12–14] antibacterial,[14,15–18] antiparasitic,[19,20] cytotoxic,[14,21] etc.

General protocol for the synthesis of amphiphiles involves the alkylation of ring nitrogen of pyridine or trialkylamines using excess of alkyl halides such as bromides or iodides (route 1 in Scheme 1).[22,23] There are several disadvantages in the current protocols such as: (i) some of the alkyl halides used in N-alkylation/N-allylation are not available commercially in such cases they have to be prepared from their respective alcohols,[24–29] (ii) some of the commercially available alkyl halides/allyl halides are expensive than alcohols, (iii) prolonged reaction time (12 h to 3 weeks), and (iv) harsh reaction conditions[22,30] (Scheme 1). Our literature survey revealed that there is not a single method to prepare cationic amphiphiles from *n*-alcohols and allyl alcohols directly, which contains alkyl or allyl substitution on nitrogen of trialkylamines or pyridines.

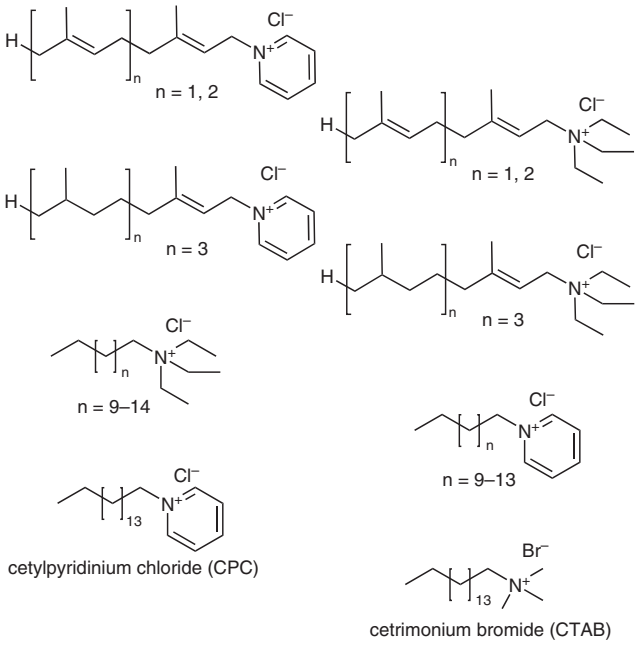
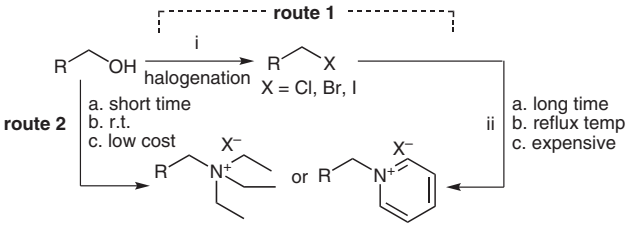


Figure 1 Prototype amphiphiles prepared and commercially available surfactants CPC and CTAB.



Scheme 1 Comparison between previously reported method (route 1) and our new method (route 2) for the synthesis of amphiphiles.

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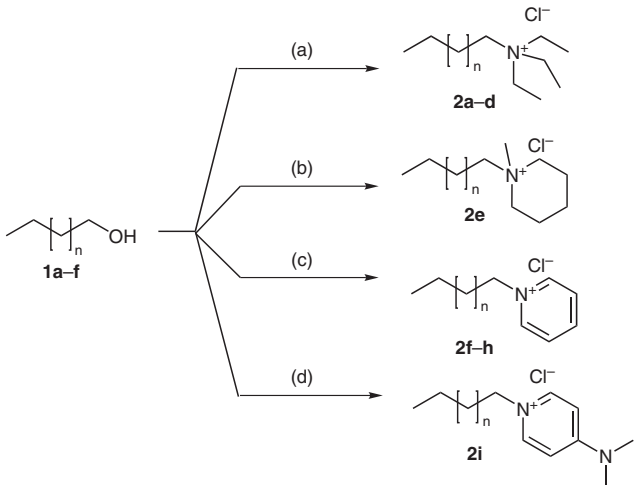
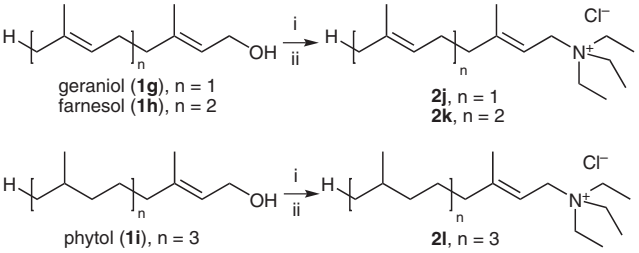
We therefore wanted to develop a one-pot method for the synthesis of amphiphiles with an *n*-alkyl/allyl substitution on the nitrogen of pyridine or trialkylamine using *n*-alcohols and naturally occurring allyl alcohols (terpenols) such as geraniol (monoterpene),[31–33] farnesol (sesquiterpene),[34,35] and phytol (diterpene)[36–38] and to evaluate the resulting amphiphile's efficacy in DNA/gene delivery and also their biological activity.

Large numbers of cationic amphiphiles and surfactants have been reported in the literature, which possess straight-chain lipophilic moieties. We therefore carried out a reaction with *n*-alcohol and triethylamine to generate the N-alkylated triethylamine salts. The mixture of *n*-alcohol **1a** and triethylamine in dichloromethane (CH_2Cl_2) was added dropwise to the stirred solution of phosphorus oxychloride ($POCl_3$) in CH_2Cl_2 at 0 °C to give alkylated phosphorodichloridate. After stirring for 1 hour, a solution of *N*,*N*-diethylethanolamine in triethylamine was then added at 0 °C with the anticipation that the remaining chlorides of $ROPOCl_2$ would react with *N*,*N*-diethylethanolamine to provide a better leaving group. The resulting solution was stirred for 1 hour at 0 °C and then for an additional 2 hours at room temperature. The workup of the reaction mixture and subsequent silica gel column chromatography provided the desired N-alkylated triethylamine salt **2a** (Scheme 2) in good yields.[39] To demonstrate the generality and application of our procedure for the synthesis of amphiphiles and surfactants, we carried out a reaction with *n*-alcohols **1a–f** and obtained their respective N-alkylated triethylamine salts **2a–d**, *N*-methylpiperidine salt **2e**, pyridine salts **2f–h**, and *N*,*N*-dimethylaminopyridine salt **2i** (Scheme 2, Table 1).[40]



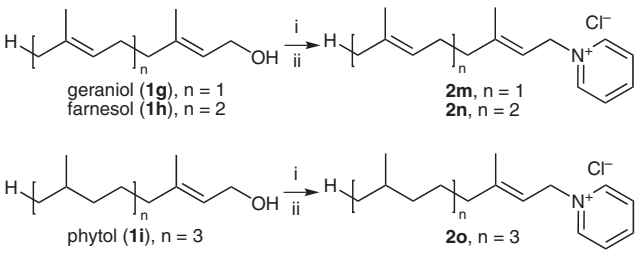
Scheme 2 Synthesis of N-alkylated triethylamines and pyridinium salts (DNA carriers and surfactants). *Reagents and conditions*: (a) i. $POCl_3$, Et_3N, CH_2Cl_2, 0 °C; ii. *N*,*N*-diethylethanolamine, Et_3N, 0 °C; (b) i. $POCl_3$, *N*-methylpiperidine, CH_2Cl_2, 0 °C; ii. *N*,*N*-diethylethanolamine, *N*-methylpiperidine, 0 °C; (c) i. $POCl_3$, pyridine, CH_2Cl_2, 0 °C; ii. *N*,*N*-diethylethanolamine, pyridine, 0 °C; (d) i. $POCl_3$, *N*,*N*-dimethylaminopyridine, CH_2Cl_2, 0 °C; ii. *N*,*N*-diethylethanolamine, *N*,*N*-dimethylaminopyridine, 0 °C.

We then focused on the synthesis of cationic amphiphiles, which contain terpenyl groups on nitrogen of pyridine or trialkylamine using naturally occurring terpenols (allyl alcohols). Terpenoids are plant secondary metabolites, which play an important role in plant–insect, plant–pathogen, and plant–plant interactions. The majority of volatile compounds released from plants are monoterpenes(C_{10}) and sesquiterpenes (C_{15}). Plants utilize these terpenes in the self-defense against herbivores and microbial pathogens. Various monoterpenes (C_{10}) are toxic to insects,[41] fungi,[42] and bacteria[43] and serve as feeding deterrents to mollusks,[44] insects,[45] and mammals.[46] We therefore chose few naturally occurring terpenols such as geraniol (monoterpene: C_{10}), farnesol (sesquiterpene: C_{15}), phytol (diterpene: C_{20}) to synthesize amphiphiles and to evaluate the DNA delivery capacity and biological importance. To our knowledge there is no synthetic methodology available for the synthesis of this class of compounds. We carried out a reaction with geraniol (**1g**) and triethylamine under similar reaction conditions,[39] which provided the desired geranylated triethylamine salt **2j** (Scheme 3) in good yields. The chemical structure of **2j** was confirmed by extensive 2D NMR spectral data such as COSY, HSQC, HMBC, etc. Similar results were obtained with other allyl alcohols such as farnesol (**1h**) and phytol (**1i**) to provide respective salts **2k** and **2l** (Table 1).

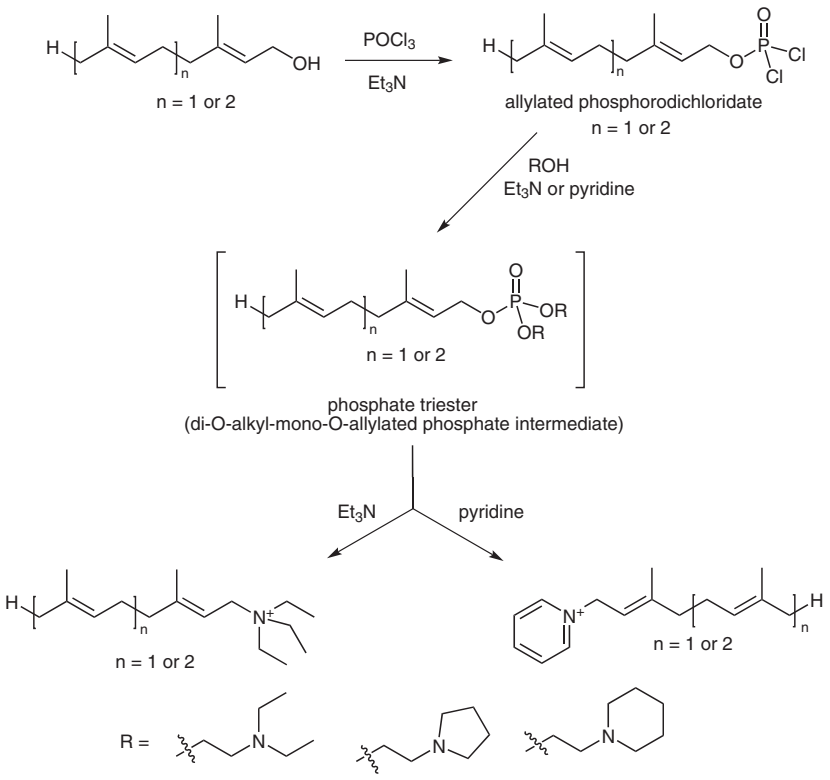


Scheme 3 Synthesis of geranylated and farnesylated triethylamine salts. *Reagents and conditions*: i. $POCl_3$, Et_3N, CH_2Cl_2, 0 °C; ii. *N*,*N*-diethylethanolamine, Et_3N, 0 °C.

Encouraged by these results, we synthesized the allylated pyridinium salts of **1g–i** (Scheme 4) by replacing triethylamine with pyridine, which provided the required pyrdinium salts **2m–o**, respectively[40] (Table 1).



Scheme 4 Synthesis of geranylated, farnesylated, and phytylated pyridinium salts. *Reagents and conditions*: i. $POCl_3$, pyridine, CH_2Cl_2, 0 °C; ii. *N*,*N*-diethylethanolamine, pyridine, 0 °C.



Scheme 5 Possible reaction mechanism in the formation of N-alkylated and N-allylated triethylamines or pyridinium salts

Table 1 One-Pot Synthesis of Amphiphiles

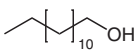
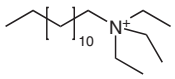
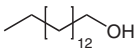
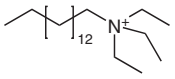
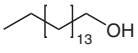
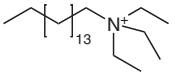
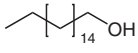
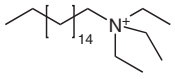
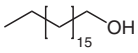
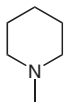
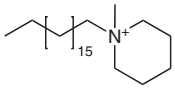
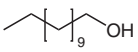
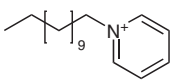
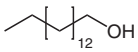
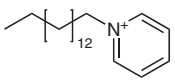
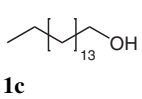
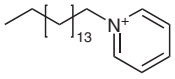
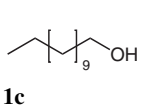
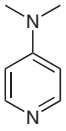
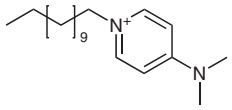
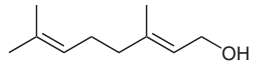
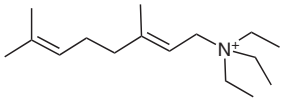
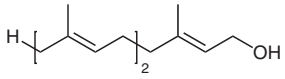
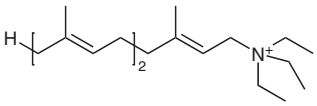
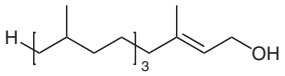
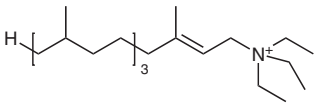
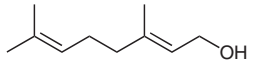
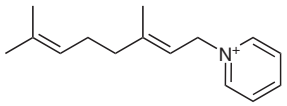
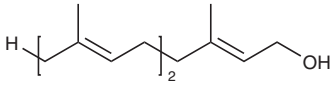
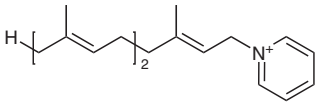
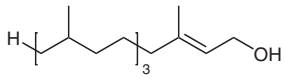
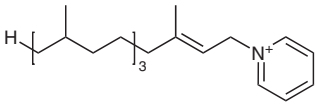
Entry	Alcohol	Amine/pyridine	N-Alkylated chloride salts	Isolated yields (%)
1	 **1a**		 **2a**	60
2	 **1b**		 **2b**	62
3	 **1c**		 **2c**	55
4	 **1d**		 **2d**	51
5	 **1e**		 **2e**	58
6	 **1f**		 **2f**	55
7	 **1b**		 **2g**	57

Table 1 One-Pot Synthesis of Amphiphiles (continued)

Entry	Alcohol	Amine/pyridine	N-Alkylated chloride salts	Isolated yields (%)
8	 **1c**		 **2h**	59
9	 **1c**		 **2i**	63
10	 **1g**		 **2j**	59
11	 **1h**		 **2k**	64
12	 **1i**		 **2l**	63
13	 **1g**		 **2m**	65
14	 **1h**		 **2n**	67
15	 **1i**		 **2o**	69

The reaction mechanism in the formation of N-alkylated/N-allylated triethylamine and pyridinium salts appears to be initial phosphorylation of the *n*-alcohol or allyl alcohol in the presence of triethylamine to provide alkylated/allylated phosphorodichloridate. Phosphates and its esters are known as leaving groups similar to triflates, tosylates, and mesylates.[47–49] Addition of *N*,*N*-diethylethanolamine in the presence of triethylamine replaces other two chlorides of alkylated/allylated phosphorodichloridate to give phosphate triester (tri-O-alkylated/di-*O*-alkyl-mono-*O*-allylphosphate intermediate) which might be behaving as better leaving group. Attack of triethylamine or pyridine on carbon (C) rather than phosphorus (P) of phosphate triester might be resulting in cleavage of C–O bond and formation of C–N bond to produce alkylated/allylated triethylamine or pyridinium salts (Scheme 5). It is noteworthy to mention here that in absence of *N*,*N*-diethylethanolamine, pyrrolidineethanolamine, or piperidineethanolamine (Scheme 5) the formation of alkylated triethylamine or pyridinium salts are not observed. These findings supports our proposed reaction mechanism, however, further studies are required to confirm the exact reaction mechanism.

In summary, we developed a novel and simple one-pot method for the synthesis of alkylated and allylated triethylamines and pyridinium salts (amphiphiles/industrial surfactants) in reasonably good yields from *n*-alcohols and allyl alcohols such as geraniol, farnesol, and phytol. This new method overcomes the disadvantages associated with the previous methods such as prolonged reaction time, harsh reaction conditions (reflux temperature), and preparation of alkyl halides (bromides or iodides) from their respective alcohols in case commercial nonavailability. This improved method is useful for the industries, which are involved in the synthesis of surfactants and DNA carriers. This method can also be used in the synthe-

sis of terpenoid salts which may improve the oral bioavailability when compared to natural terpenols. The results of DNA transfection will be communicated elsewhere in due course.

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(39) **Representative Procedure for the Preparation of *N*,*N*,*N*-Triethyltridecan-1-aminium Chloride (2a)**
A solution of **1a** (5.0 g, 25.0 mmol) in CH_2Cl_2 (10 mL) and Et_3N (10 mL) was added dropwise to a stirred solution of $POCl_3$ (7.6 g, 50.0 mmol) in dichloromethane (10 mL) at 0 °C. After stirring the solution for 1 h, a solution of *N*,*N*-diethylethanolamine (4.4 g, 37.5 mmol) in Et_3N (10 mL) was added. The whole reaction mixture was stirred for 1 h at 0 °C followed by 2 h at r.t. Water (5 mL) was added dropwise to the reaction mixture and stirred for additional 30 min. Then, 10 mL of 10% solution of citric acid and MeOH–H_2O (1:1) was added to the reaction mixture and extracted into $CHCl_3$ (3 × 100 mL). The combined organic layer was washed with MeOH–H_2O (1:1) and dried over anhyd Na_2SO_4. The solvent was evaporated under reduced pressure, and the crude product was chromatographed on silica gel as stationary phase and $CHCl_3$–MeOH–H_2O (91.5:8:0.5) as mobile phase to afford the compound **2a** (4.26 g, 60%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.60–3.03 (m, 8 H), 1.43 (m, 2 H), 1.18–1.04 (m, 29 H), 0.70 (t, *J* = 6.2 Hz, 3 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 57.6, 53.6 (3 C), 32.2, 30.0 (6 C), 29.8, 29.7, 29.5, 26.8, 26.1, 23.0, 22.2, 14.4. ESI-MS: *m/z* = 284.4.
(40) **Representative Procedure for the Preparation of 1-Nonylpyridinium Chloride (2f)**
A solution of **1f** (5 g, 26.9 mmol) in CH_2Cl_2 (10 mL) and pyridine (10 mL) was added dropwise to the stirred solution of $POCl_3$ (8.25 g, 53.7 mmol) in CH_2Cl_2 (10 mL) at 0 °C. After stirring for 1 h, a solution of *N*,*N*-diethylethanolamine (4.7 g, 40.3 mmol) in pyridine (10 mL) was then added at 0 °C. The whole reaction mixture was stirred for 1 h at 0 °C followed by 2 h at r.t. Water (5 mL) was added dropwise to the reaction mixture and stirred for additional 30 min. Then, 10 mL of 10% solution of citric acid and MeOH–H_2O (1:1) was added in the reaction mixture and extracted into $CHCl_3$ (2 × 100 mL). The combined organic layer was washed with MeOH–H_2O (1:1) and dried over anhyd Na_2SO_4. The

solvent was evaporated under reduced pressure, and the crude product was chromatographed on silica gel as stationary phase and $CHCl_3$–MeOH–H_2O (89.5:10:0.5) as mobile phase to afford the compound **2f** (3.67 g, 55%). ^1H NMR (200 MHz, CD_3OD): δ = 9.04 (d, *J* = 5.8 Hz, 2 H), 8.61 (t, *J* = 7.7 Hz, 1 H), 8.13 (t, *J* = 6.9 Hz, 2 H), 4.66 (t, *J* = 7.6 Hz, 2 H), 2.03 (t, *J* = 6.9 Hz, 2 H), 1.38–1.28 (m, 18 H), 0.89 (t, *J* = 6.7 Hz, 3 H). ^{13}C NMR (50 MHz, CD_3OD): δ = 145.9, 145.0 (2 C), 128.6 (2 C), 62.2, 32.1, 31.5, 29.7 (2 C), 29.6, 29.5, 29.1, 26.2, 22.7, 13.5. ESI-MS: *m/z* = 248.3.

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