J Am Oil Chem Soc (2013) 90:437–448
DOI 10.1007/s11746-012-2177-9

ORIGINAL PAPER

Chemo-Enzymatic Synthesis and Antimicrobial Evaluation of Alkyloxy Propanol Amine-Based Cationic Ether Lipids

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Received: 26 June 2012 / Revised: 27 August 2012 / Accepted: 25 October 2012 / Published online: 23 November 2012

Abstract The present study involved the synthesis and antimicrobial evaluation of alkyloxy propanol amine-based cationic lipids *N*,*N*-dimethyl-1-octadecylamino-3-alkyloxy-2-propanol (series A, 7a–e) and *N*-methyl-*N*,*N*-di-(2-hydroxy-3-alkyloxy-2 propyl) octadecylamine (series B, 9a–e) and their acetylated derivatives (8a–e and 10a–e). A simple three-step chemo-enzymatic approach was employed for the synthesis of 7a–e and 9a–e in 71–80 and 67–88 % yields, respectively. The first step involved the synthesis of a series of glycidyl ethers from a series of alcohols (C_4, C_8, C_{10}, C_{12}, C_{14}) which were opened in the second step with octadecylamine to obtain 1-octadecylamino-3-alkyloxy-2 propanol (5a–e) and *N*,*N*-di-(2-hydroxy-3-alkyloxypropyl) octadecylamine (6a–e). In the third step, alkyloxy propanolamines (5a–e, 6a–e) were quaternized using methyl iodide to yield quaternized ammonium salts. The quaternized ammonium salts were enzymatically acetylated using *Candida antarctica* lipase-B based immobilized enzyme Novozym 435 to obtain their acetylated derivatives. The quaternized salts as well as their acetylated derivatives were evaluated for antibacterial and antifungal properties. The title compounds were found to possess moderate to good antibacterial activities against all the studied bacterial strains namely, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella aerogenes* compared to streptomycin and cetyl trimethyl ammonium bromide (CTAB). The title compounds exhibited relatively good antifungal activities against *Candida albicans* and no significant activities against other fungal strains namely, *Saccharomyces cerevisiae*, *Rhizopus oryzae* and *Aspergillus niger* when compared to amphotericin B and CTAB.

Keywords Antimicrobial activity · Cationic-ether lipids · Chemo-enzymatic synthesis · Quaternized salts · Alkyloxy-propanolamine

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Introduction

Glycidyl derivatives namely acyloxy or alkyloxy propanol amines have been widely investigated for a variety of applications [1–8]. The presence of several key functional groups on the glycerol backbone makes them attractive chemical intermediates for several applications. Lipids with ether bonds to long chain alkyl moieties in addition to ester bonds to fatty acids are in general called as ether lipids and are common in nature especially as membrane constituents. In general, the ether bond is located at the *sn*-1 position of a glycerol moiety, which may be part of a non-polar lipid or a phospholipid in animal tissues or in micro-organisms. Researchers are showing greater interest in these compounds as ether lipids were found in cancer tissues, followed by the discovery of distinct ether lipids such as the platelet-activating factor, with several important biological activities [7, 8]. Pure ether lipids are rarely easy to separate from the fully acylated forms, and often their presence in tissues is inferred from isolation of their hydrolysis products.

The existence of ether bonds which are resistant to hydrolysis and chemically stable makes it possible to apply this class of materials under drastic environmental

conditions. Furthermore, cationic lipids are attracting a lot of attention due to many unique applications in gene therapy, antimicrobial properties etc. [9–14]. These lipids are composed of three parts namely, a cationic head group, a lipophilic tail group, and a linker that tethers the hydrophilic head group and hydrophobic tail group. In general, cationic head groups include quaternary salt lipids, lipoamines and combinations of both. The tail groups consist of saturated or unsaturated alkyl chains or cholesteryl groups. The nature of head group as well as hydrophobic tail groups are important structural variables in a cationic lipid-based antimicrobial agent, which governs all major applications of cationic lipid.

Another unique class of ether lipids is alkanolamines and other derivatives. A few of such synthetic ether lipid analogues reported are terpene glyceryl ether lipids, isostearyl glyceryl ethers, 1-alkylamino-3-alkyloxy-2-propanols and *N*,*N*-di-(2-hydroxy-3-alkyloxypropyl) alkyl amines [6] with different active sites, which are responsible for various applications such as polyfunctional fuel additives [6]. In another study, a series of alkyloxy alkylamino propanols were synthesized from epichlorohydrin, straight or branched chain primary and secondary alcohols and tertiary alkyl primary amines as well as straight chain primary amine. In addition, their sedimentation inhibition characteristics in distillate fuels of various compositions were studied in comparison to tertiary alkyl primary amines as well as some known commercial additives of similar nature [15]. 1-Alkylamino-3-alkyloxy-2-propanols, and *N*,*N*-di-(2-hydroxy-3-alkyloxy propyl) alkyl amines were prepared with the same chain length both for alkylamino and alkyloxy groups by condensation of alkyl glycidyl ethers with long chain alkylamines by employing thermal and microwave-assisted methods [16]. However, the use of alkyloxy propanolamines as antimicrobial agents has not been studied by the researchers even though they can be potential molecules for biological applications.

Therefore, in the present study, homologous series (A and B) of alkyloxy propanol amine-based cationic ether-lipids and their acetylated derivatives, of varying alkyl chain lengths (C_4, C_8, C_{10}, C_{12}, C_{14}) in alkyloxy group were synthesized and evaluated for their antimicrobial activities.

Materials and Methods

Materials and Instruments

The raw materials needed for the synthesis, such as epichlorohydrin, butyl, octyl, decyl, dodecyl and tetradecyl alcohols, *n*-octadecylamine, potassium hydroxide, methyl iodide, vinyl acetate were purchased from Sd Fine Chemicals (Mumbai, India). Silica gel (60–120 mesh) for column chromatography was purchased from M/s Acme Synthetic Chemicals (Mumbai, India). All solvents, purchased from Sd Fine Chemicals, were of high purity grade and were used without further purification. The immobilized lipase from *Candida antarctica* (Novozym 435) was purchased from Novozyme A/S (Bangalore, India). The bacterial test organisms and the fungal strains were obtained from the Institute of Microbial Technology, Chandigarh, India. The media was procured from M/s Himedia Laboratories (Mumbai, India).

IR spectra were recorded on a Perkin Elmer (model: Spectrum BX) FT-IR Spectrometer using CH_2Cl_2 as solvent or as KBr pellets. All ^{1}H-NMR spectra were recorded on a Bruker (Wissembourg, France) ARX 400 Spectrometer (200 MHz) with $CDCl_3$ solvent. FAB mass spectra were recorded on a Micromass Autospec QFPD (Manchester, UK).

Methods

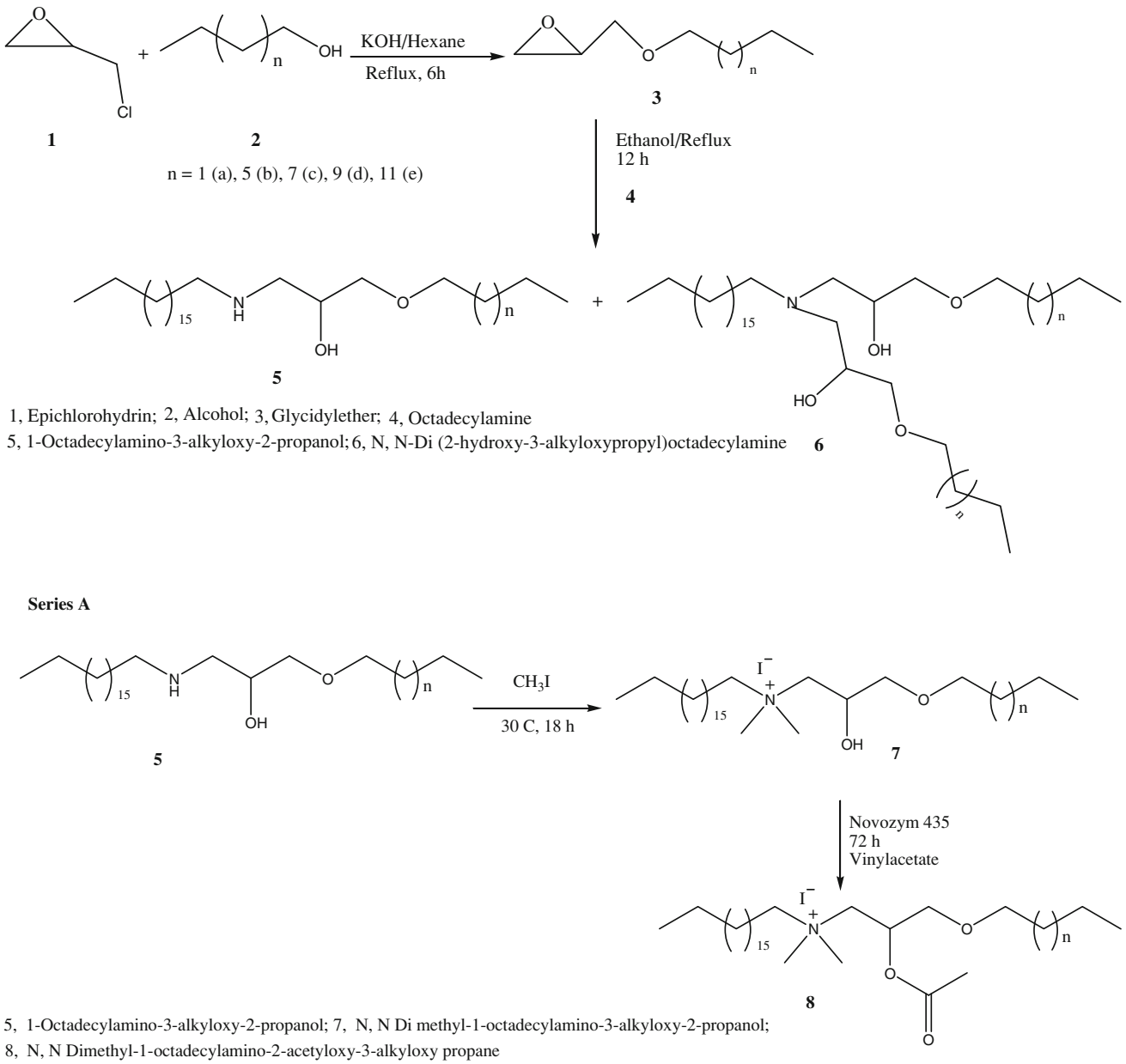
Synthesis of Alkyloxy Propanol Amine-Based Cationic Ether Lipids

A Schematic diagram illustrating the synthesis of the two types (series A and B) of alkyloxy propanol amine-based cationic lipids and their acetylated derivatives is presented in Scheme 1.

A Typical Procedure for the Synthesis of n-Tetradecyl Glycidyl Ether (3e)

The synthesis of glycidyl ethers were carried by following the method reported by Karuna et al. [16]. Tetradecyl glycidyl ether (3e) was prepared by stirring a suspension of epichlorohydrin (1, 40 g, 0.44 mol), *n*-tetradecanol (2e, 94.2 g, 0.44 mol), potassium hydroxide pellets (56 g, 1 mol) in hexane (500 mL) at reflux temperature for 6 h (Fig. 1). The reaction was monitored by TLC using the solvent system hexane/ethyl acetate (90:10, v/v). At the end of the reaction time, the reaction mixture was cooled, filtered to remove excess potassium hydroxide. The filtrate was further washed with water till the washings were free from alkali. The hexane layer was passed through anhydrous sodium sulfate to remove traces of moisture. The solvent was evaporated under reduced pressure. The title product was purified using silica gel column chromatography eluted with hexane/ethyl acetate (98:2, v/v). The compound obtained was 64.6 g, a yield of 54.4 %.

^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.87 (3H, H_a, t, –C$\underline{H}_3$), 1.2–1.4 [22H, H_b, m, –(C$\underline{H}_2$)$_{11}$], 1.4–1.6 (2H, H_c, m, –CH$_2$C$\underline{H}_2$), 2.5, 2.7 (2H, H_d, H_e, 2 m, 2 epoxy protons), 3.05–3.1 (1H, H_f, m, 1 epoxy proton), 3.24, 3.36 (3H, H_g, H_h, 2dd, –C$\underline{H}$–O–C$\underline{H}_2$–), 3.5–3.6 (1H, H_i, d, –C$\underline{H}$–O–CH$_2$); IR (KBr, cm^{-1}): 1112 (C–O–C–str), 3048, 1253, 912.2 (epoxy ring).



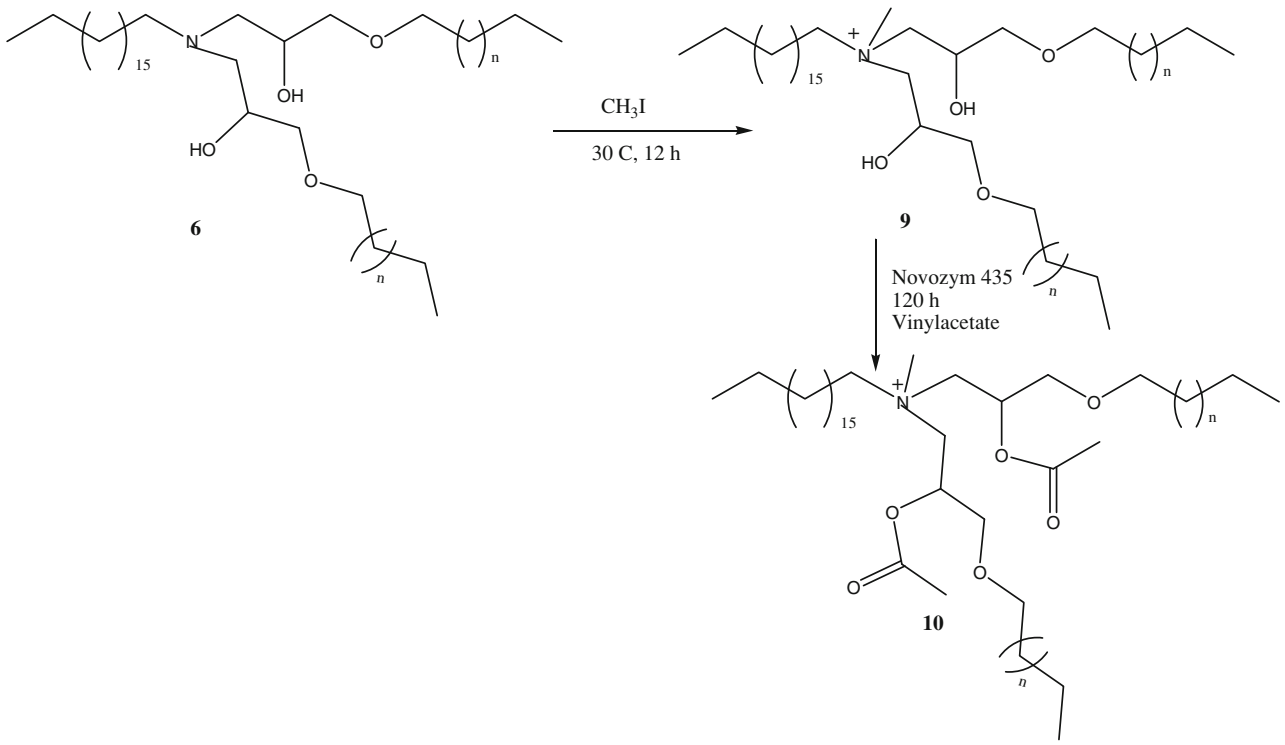
Scheme 1 Synthetic route of *N,N*-dimethyl-1-octadecylamino-3-alkyloxy-2-propanol (7a–e) and *N*-methyl-*N,N*-di-(2-hydroxy-3-alkyloxy-2 propyl) octadecylamine (9a–e), and their acetylated derivatives (8a–e, 10a–e)

A Typical Procedure for the Synthesis of 1-Octadecylamino-3-tetradecyloxy-2 Propanol (5e) and N,N-Di-(2-hydroxy-3-tetradecyloxypropyl) Octadecylamine (6e)

The synthesis of the title compounds was carried out by following the method reported by Karuna et al. [16]. Tetradecyl glycidyl ether (3e, 25.5 g, 0.1 mol) and octadecylamine (4, 26.9, 0.1 mol) were taken in ethanol (100 mL) and the contents were refluxed over a period of 12 h. The course of the reaction was monitored by TLC using a solvent system of chloroform/methanol (90:10 v/v). TLC clearly indicated the presence of two products due to the formation of monomer (5e) and dimer (6e). After 12 h, the reaction mixture was concentrated in a rotary evaporator to get the crude product. The two pure title products 5e (17.1 g, a yield of 31.8 %) and 6e (36.9 g, a yield of 44.8 %) were purified using silica gel column chromatography eluted with chloroform/methanol (99:1, 98:2, v/v).

Spectral data of 5e: ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.9 (6H, H_a, t, –$(CH_2)_{15}$–$\underline{CH_3}$,–$(CH_2)_{11}$–$\underline{CH_3}$, 1.2–1.4 [52H, H_b, m, –$(\underline{CH_2})_{15}$–CH_3), –$(\underline{CH_2})_{11}$–CH_3], 1.5–1.6 (4H, H_c, m, –HN–CH_2–$\underline{CH_2}$; –O–CH_2–$\underline{CH_2}$), 2.7–2.85 (4H, H_d, m, $\underline{CH_2}$–NH–$\underline{CH_2}$), 3.3–3.45 (4H, H_e, m, –$\underline{CH_2}$–O–$\underline{CH_2}$–), 3.85–3.95 (1H,

Series B



6, N, N-Di (2-hydroxy-3-alkyloxypropyl)octadecylamine; 9, N methyl, N, N-Di (2-hydroxy-3-alkyloxypropyl)octadecylamine; 10, N Methyl, N, N-Di (2-acetyloxy-3-alkyloxypropyl)octadecylamine

Scheme 1 continued

H_f, m, –CH–(OH); IR (KBr, cm^{-1}): 3451 (–OH, NH str); 1216, 1117 (–C–O–C–str); FABMS (*m/z*, rel. int.): $C_{35}H_{73}O_2N$, *m/z* 540 ($[M + H]^+$, 100); *m/z* 282 ($C_{19}H_{39}O^+$, 25.8).

Spectral data of 6e: ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.9 (9H, H_a, t, $–(CH_2)_{15}–CH_3$, $[–(CH_2)_{11}–CH_3]_2$, 1.2–1.4 (74 H, H_b, m,$–(CH_2)_{15}–CH_3$, $[–(CH_2)_{11}–CH_3]_2$, 1.5–1.65 (6H, H_c, m, 2 X $–CH_2–CH_2–O–$, $–CH_2–CH_2–N–$), 2.5–2.6 [6H, H_d, m,$–(CH_2)–N(CH_2)–CH_2$], 3.4–3.5 [8H, H_e, m, 2 X $CH_2–O–CH_2–$ CH–(OH)], 3.7–3.85 (2H, H_f, m, 2 X $–CH_2–CH–(OH)–CH_2–$); FABMS (*m/z*, rel. int.): $C_{52}H_{107}O_4N$, *m/z* 811 ([M + 2], 100), *m/z* 553 ($C_{36}H_{74}O_2N^+$, 47.5), *m/z* 282 ($C_{19}H_{39}O^+$, 25.8); IR (KBr, cm^{-1}): 3408 (C–OH str), 1112 (C–O–C–str).

A Typical Procedure for the Synthesis of N,N-Di-methyl-1-octadecylamino-3-tetradecyloxy-2-propanol (7e)

1-Octadecylamino-3-tetradecyloxy-2-propanol (5e, 5.39 g, 0.01 mol) was taken in excess methyl iodide and stirred magnetically at 25–30 °C for 18 h. The progress of the reaction was monitored by TLC using a solvent system of chloroform/methanol (90:10 v/v). The reaction was quenched at 18 h and then the reaction mixture was concentrated in a rotary evaporator to remove unreacted methyl iodide. The title product was separated using silica gel column chromatography eluted with chloroform/methanol (98:2, v/v). As the product (more polar) and unreacted compound have a sufficient polarity difference to differentiate on the TLC and separation using silica gel column chromatography the unreacted compounds and product were easily separated from the crude product by eluting chloroform/methanol (98:2, 99.5:0.5, v/v) respectively. The compound obtained was 4.6 g, a yield of 80.3 %.

^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.9 (6H, H_a, t, $–(CH_2)_{15}–CH_3$,$–(CH_2)_{11}–CH_3$, 1.2–1.4 [52H, H_b, m, $–(CH_2)_{15}–CH_3$), $–(CH_2)_{11}–CH_3$], 1.5–1.8 (4H, H_c, m,–HN–$CH_2–CH_2$; –O–$CH_2–CH_2$), 3.1–3.24 (2H, H_d, t,–$^+$N–$CH_2–CH_2$-), 3.4–3.6 (6H, H_e, m, $^+$N–CH_2–CH(OH)–CH_2–O–CH_2–), 3.7 (6H, H_f, s, $–CH_2–N^+(CH_3)_2–CH_2–CH(OH)$, 4.1–4.3 (1H, H_g, m, $^+$N–$CH_2–CH(OH)–CH_2–O–CH_2–$), Fig. 1a; FABMS (*m/z*, rel. int.): $C_{37}H_{78}O_2N$, *m/z* 569 ($[M + H]^+$, 50), Fig. 1b; IR (KBr, cm^{-1}): 3391 (C–OH str), 1117 (C–O–C str), Fig. 1c.

A Typical Procedure for the Synthesis of N-Methyl-N,N-Di-(2-hydroxy-3-tetradecyloxy-2-propyl) Octadecylamine (9e)

N,N-di-(2-hydroxy-3-tetradecyloxypropyl) octadecylamine (6e, 8.1 g, 0.01 mol) was taken in excess methyl iodide and stirred magnetically at 25–30 C over a period of 12 h. The

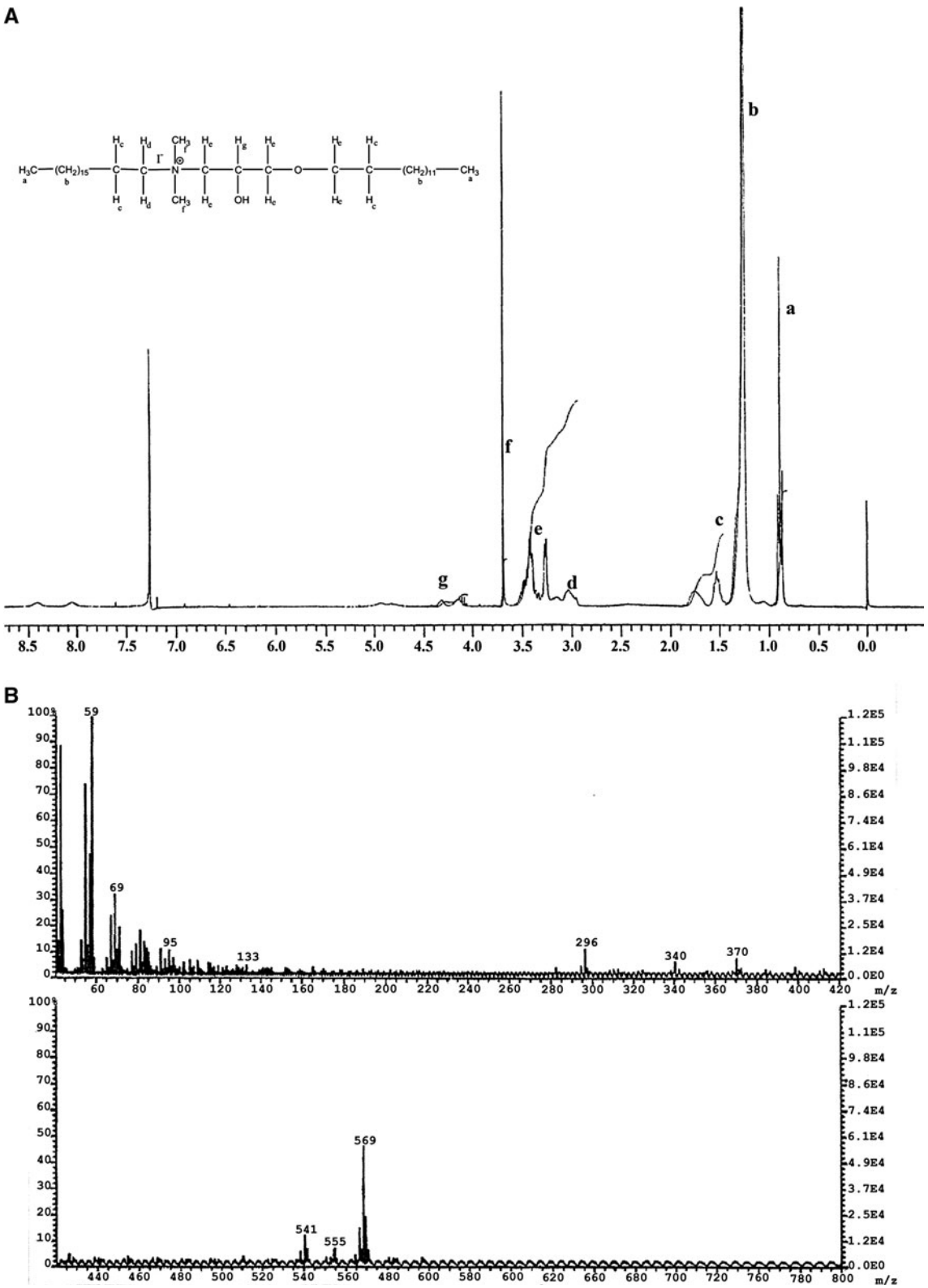


Fig. 1 **a** ^{1}H-NMR spectrum, **b** FAB mass spectrum, **c** FT-IR spectrum of *N*,*N*-di-methyl-1-octadecylamino-3-tetradecyloxy-2-propanol (7e)

progress of the reaction was monitored by TLC using a solvent system of chloroform/methanol (90:10 v/v). The reaction was stopped at 12 h and the reaction mixture was concentrated in a rotary evaporator to remove excess methyl iodide. The title product, 9e was purified using silica gel column chromatography eluted with chloroform/

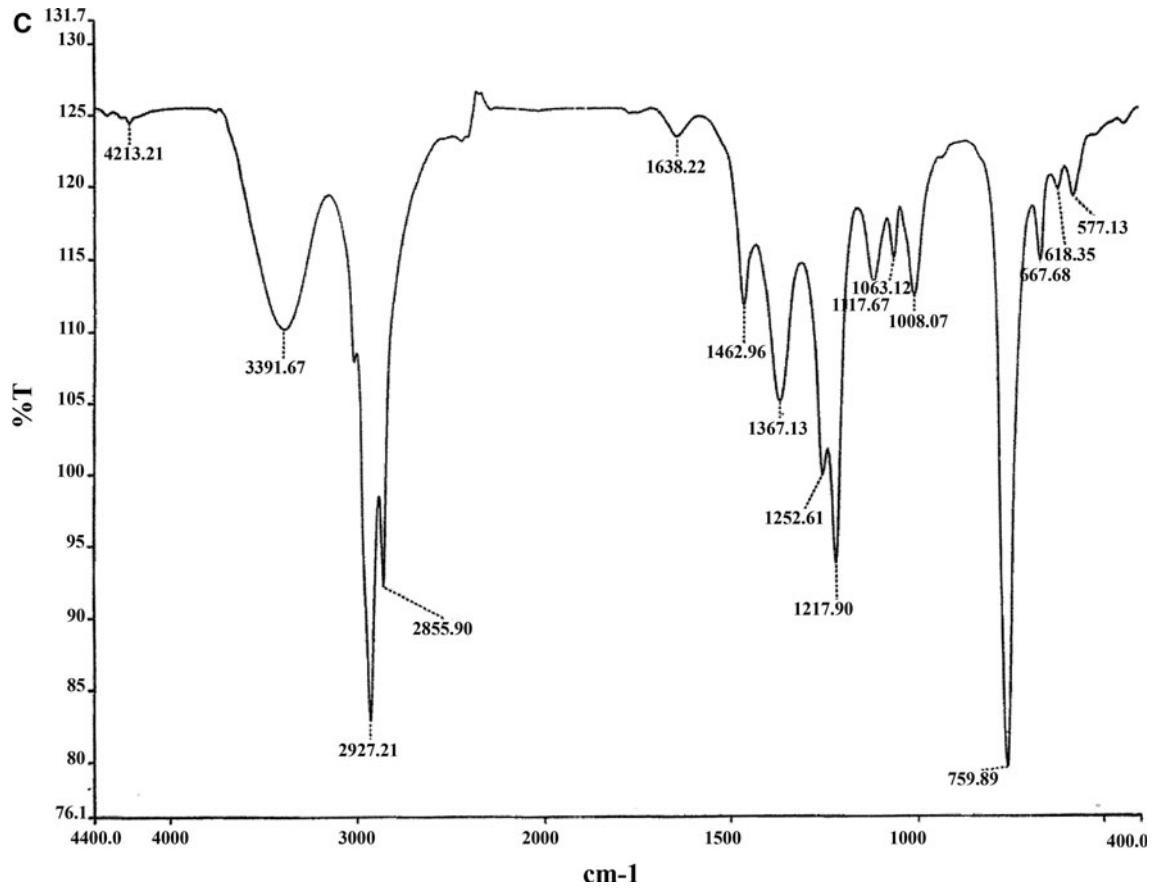


Fig. 1 continued

methanol (98:2, v/v) and the unreacted compound was eluted with the pure chloroform. The compound obtained was 7.4 g, a yield of 88.5 %.

^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.9 (9H, H_a, t, $-(CH_2)_{15}-\underline{C}H_3$, $[-(CH_2)_{11}-\underline{C}H_3]_2$, 1.2–1.4 (74 H, H_b, m, $-(\underline{C}H_2)_{15}-CH_3$, $[-(\underline{C}H_2)_{11}-CH_3]_2$, 1.5–1.75 (6H, H_c, m, 2 X $-\underline{C}H_2-CH_2-O-$, $-\underline{C}H_2-CH_2-N^+-$), 3.3–3.6 (14H, H_d, m,$-^+N-\underline{C}H_2-CH_2-$, $^+N-[\underline{C}H_2-CH(OH)-\underline{C}H_2-O-\underline{C}H_2-]_2$, 3.7 (3H, H_e, s,$-CH_2-N^+(\underline{C}H_3)-CH_2-CH(OH)$, 4.4 (2H, H_f, m, $^+N-[CH_2-\underline{C}H(OH)-CH_2-O-CH_2-]_2$, Fig. 2a; FABMS (*m/z*, rel. int.): $C_{53}H_{110}O_4N$, *m/z* 825 ($[M + H]^+$, 91.2), *m/z* 296 ($C_{21}H_{44}^+$, 46), Fig. 2b; IR (KBr, cm^{-1}): 3330 (C–OH str), 1117 (C–O–C str), Fig. 2c.

A Typical Procedure for the Synthesis of N,N-Di-methyl-1-octadecylamino-3-tetradecyloxy-2-acetyloxy Propane (8e)

N,N-di-methyl-1-octadecylamino-3-tetradecyloxy-2-propanol (7e, 5.68 g, 0.01 mol) was taken in vinyl acetate (8.6 g, 0.1 mol) and to this commercial lipase Novozym 435 (20 wt% of 7e) was added. The contents were stirred magnetically at 25–30 °C for 72 h. The reaction progress was monitored by TLC using a solvent system of chloroform/methanol (90:10 v/v). After 72 h, the reaction mixture was filtered to separate enzyme and concentrated in a rotary evaporator to remove excess vinyl acetate. The title product and un-acetylated compound were separated using silica gel column chromatography eluted with chloroform/methanol (98:2, 97:3, v/v) respectively. The compound obtained was 5 g, a yield of 80.4 %.

^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.9 (6H, H_a, t, $-(CH_2)_{15}-\underline{C}H_3$,$-(CH_2)_{11}-\underline{C}H_3$, 1.2–1.4 [52H, H_b, m, $-(\underline{C}H_2)_{15}-CH_3$), $-(\underline{C}H_2)_{11}-CH_3$], 1.5–1.8 (4H, H_c, m,–HN–$CH_2-\underline{C}H_2$; $-O-CH_2-\underline{C}H_2$), 2.0 (3H, H_d, s, $-O-CO-CH_3$), 3.1–3.2 (2H, H_e, t,$-^+N-\underline{C}H_2-CH_2-$), 3.4–3.6 (6H, H_f, m, $^+N-\underline{C}H_2-CH(OH)-\underline{C}H_2-O-\underline{C}H_2-$), 3.7 (6H, H_g, s,$-CH_2-N^+(\underline{C}H_3)_2-CH_2-CH(OH)$, 4.9 (1H, H_h, m, $^+N-CH_2-\underline{C}H(OCOCH_3)$; IR (KBr, cm^{-1}): 1738 (C = O str), 1217, 1116 (–C–O–C–str).

A Typical Procedure for the Synthesis of N-methyl-N,N-di-(2-acetyloxy-3-tetradecyloxy-2-propyl)octadecylamine (10e)

N-methyl-*N*,*N*-di-(2-hydroxy-3-tetradecyloxypropyl) *n*-octadecylamine (8e, 8.24 g, 0.01 mol) was taken in vinyl acetate (8.6 g, 0.1 mol) and to this Novozym 435 (20 wt% of 8e) was added and stirred magnetically at 25–30 °C for

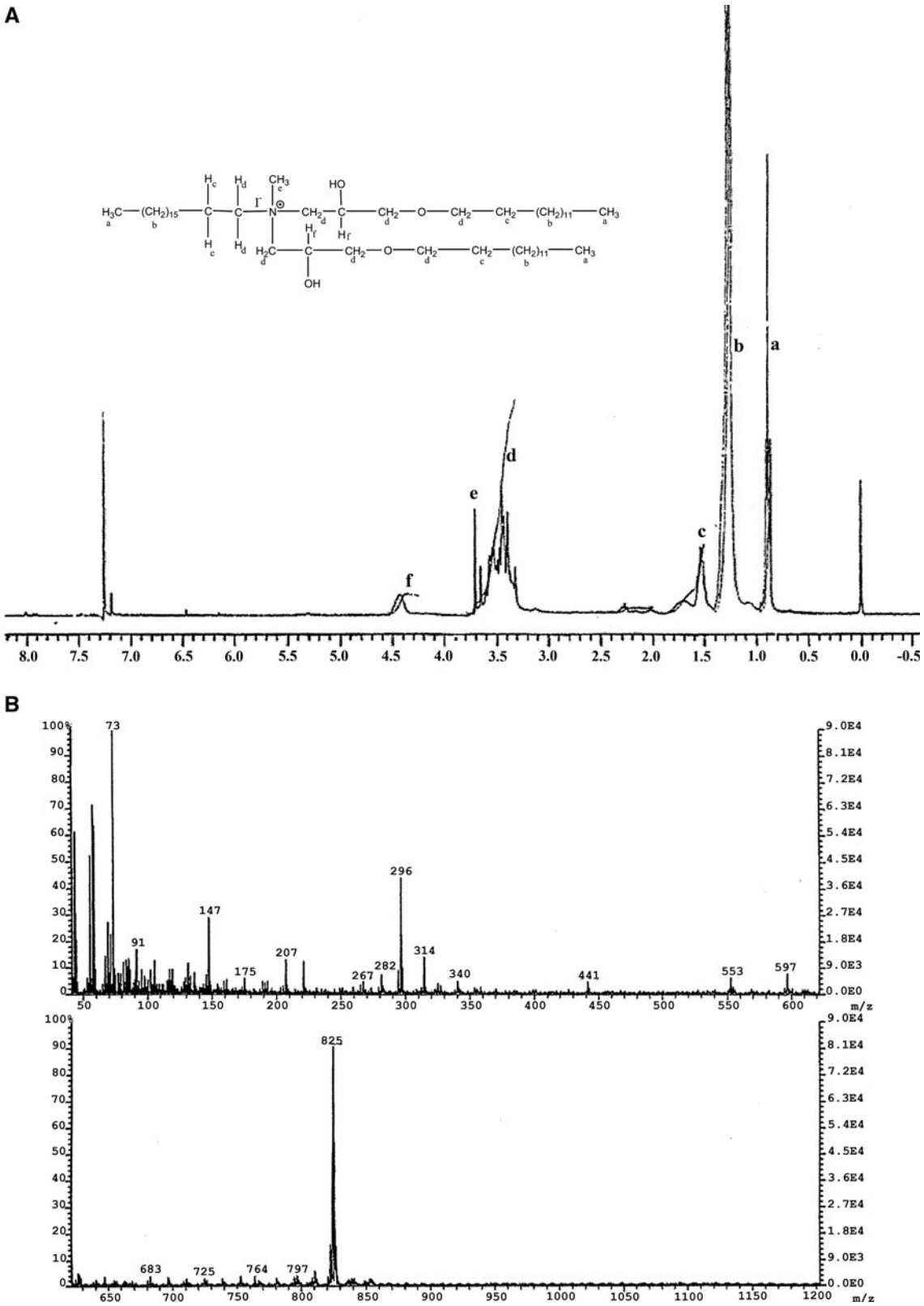


Fig. 2 **a** ^{1}H-NMR spectrum, **b** FAB mass spectrum, **c** FT-IR spectrum of *N*-methyl-*N*,*N*-di-(2-hydroxy-3-tetradecyloxy-2-propyl) octadecylamine (9e)

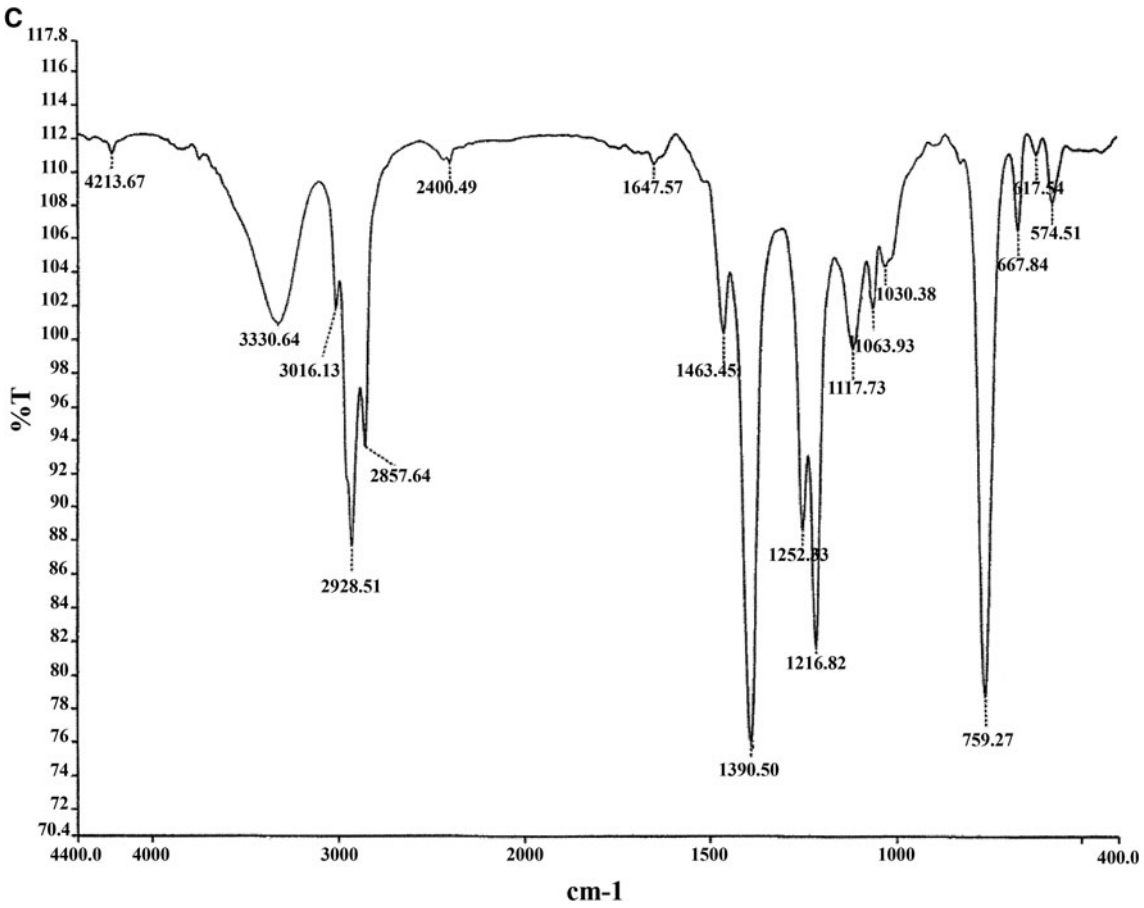


Fig. 2 continued

120 h. The reaction was monitored by TLC using solvent system of chloroform/methanol (90:10, v/v). After 120 h, reaction mixture was filtered to separate enzyme and concentrated in a rotary evaporator. The title product and un-acetylated compound were separated using silica gel column chromatography eluted with chloroform/methanol (99.5:0.5, 97.5:2.5, v/v) respectively. The compound obtained was 6.7 g,a yield of 70.3 %.

^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.9 (9H, H_a, t,–$(CH_2)_{15}$–$C\underline{H}_3$, [–$(CH_2)_{11}$–$C\underline{H}_3]_2$, 1.2–1.4 (74 H, H_b, m, –$(C\underline{H}_2)_{15}$–CH_3, [–$(C\underline{H}_2)_{11}$–$CH_3]_2$, 1.5–1.75 (6H, H_c, m, 2 X –$C\underline{H}_2$–CH_2–O–, –$C\underline{H}_2$–CH_2–N^+–), 2.2 (6H, H_d, s, 2 X –O–CO–$C\underline{H}_3$), 3.3–3.6 (14H, H_e, m,–$^+$N–$C\underline{H}_2$–CH_2-, $^+$N–[$C\underline{H}_2$–CH($OCOCH_3$)–$C\underline{H}_2$–O–$C\underline{H}_2$–$]_2$,3.7(3H, H_f, s,–CH_2–N^+($C\underline{H}_3$)–CH_2–CH($OCOCH_3$), 4.95 (2H, H_g, m,$^+$N–[CH_2–$C\underline{H}$($OCOCH_3$)–CH_2–O–CH_2–$]_2$; IR (KBr, cm^{-1}): 1744 (C = O str); 1230, 1121 (–C–O–C–str).

Biological Procedures

Antibacterial Activity

The minimum inhibitory concentration (MIC) of the various evaluated compounds were tested against two representative gram-positive organisms, viz. *Bacillus subtilis* (MTCC-441), *Staphylococcus aureus* (MTCC-96), and gram-negative organisms, viz. *Escherichia coli* (MTCC-443), *Pseudomonas aeruginosa* (MTCC- 741) and *Klebsiella aerogenes* (MTCC-39), by the well diffusion method recommended by the National Committee for Clinical Laboratory (NCCL) standards [17]. Streptomycin and cetyl trimethyl ammonium bromide (CTAB) were used as positive controls and chloroform as a negative control for antibacterial activity.

Antifungal Activity

The antifungal activities were assayed against four fungal strains, namely *Candida albicans* (MTCC-227), *Saccharomyces cerevisiae* (MTCC-36), *Rhizopus oryzae* (MTCC-262) and *Aspergillus niger* (MTCC-282), by the agar-well diffusion method [18]. Cultures of test organisms were maintained on potato dextrose agar (PDA) slants and subcultured on petri dishes before examining for antifungal activity employing the agar cup bioassay method. A commercially prepared PDA medium (composition: potato infusion, 200 g; dextrose, 20 g; agar, 15 g) was used in this evaluation study. The PDA medium (39.0 g) was suspended

in distilled water (1000 mL) and heated to boiling until it dissolved completely (pH 5.6). The medium and the Petri dishes were autoclaved at 120 °C at a pressure of 15 psi, for 20 min. The medium was poured into sterile petri dishes under aseptic conditions in a laminar flow chamber. When the medium in the plates had solidified, 0.5 mL of the test culture was inoculated and uniformly spread over the agar surface. Solutions were prepared at two concentrations (100 and 150 μg/mL) by dissolving the test compounds in chloroform. After inoculation, cups were scooped out with a 6-mm sterile cork borer and the lids of the dishes were replaced. The different concentrations (100 and 150 μg/mL) of test solutions were added separately to each cup. The treated and the controlled cups were kept at 27 °C for 48 h. The diameters (mm) of the inhibition zones were then measured. Amphotericin B and CTAB were taken as positive controls and chloroform as negative control. Three replicates were maintained for each treatment.

Results and Discussion

Synthesis of Alkyloxy Propanol Amine-based Cationic Ether Lipids

Glycidyl ethers are versatile intermediates in organic chemistry and their reactions with different nucleophiles have been of extensive interest. In the present study, the reaction of epichlorohydrin with short, medium and long chain alcohols for 6 h (of varying C_4, C_8, C_{10}, C_{12} and C_{14} alkyl chain lengths) in the presence of potassium hydroxide was carried out in a hexane medium which yielded the glycidyl ethers (3a–e). However, further prolongation of reaction up to 10 h did not increase the yield of the products. The different glycidyl ethers prepared were *n*-butyl (3a), *n*-octyl (3b), *n*-decyl (3c), *n*-dodecyl (3d) and *n*-tetradecyl glycidyl ethers (3e) with yields in the range of 54–57 %. The epoxy of glycidyl ethers was opened with octadecylamine (4) in ethanol to produce 1- octadecylamino-3-alkyloxy-2-propanol (5a–e) and *N*,*N*-di-(2-hydroxy-3-alkyloxy propyl) octadecylamine (6a–e) with a yield of 28–41 and 41–46 %, respectively. The structures of 5a–e, 6a–e were confirmed by ^{1}H NMR which showed the characteristic peaks at δ 2.7–2.85 due to the presence of NH $(CH_2)_2$ and at 2.5 due to the presence of N $(CH_2)_3$, respectively. These compounds were then quaternized for 18 h using methyl iodide (Scheme 1) at 30 °C to yield the corresponding quaternary compounds (7a–e, 71–80 %; 9a–e, 67–88 % yields). However, the reasons are not clear for longer reaction times for quaternization of 5a–e (18 h) compared to 6a–e (12 h). It should be pointed out that further prolongation of the quaternization reactions in both the cases did not increase their respective yields. These synthesized compounds were unambiguously established from the analysis of their spectral data (IR, ^{1}H NMR and FABMS). The presence of peaks at δ 3.3–3.7 in ^{1}H NMR indicating $–N^+(CH_3)_2$ and $–N^+(CH_2)_2$ groups confirmed the quaternization of monomer (5a–e) and dimer (6a–e), respectively. The quaternary compounds 7a–e, 9a–e were further acetylated using vinyl acetate in the presence of Novozym 435 to produce the corresponding acetylated quaternary compounds 8a–e (a yield of 80–89 %) and 10a–e (a yield of 70–85 %). The presence of peak at δ 2.2 in both 8a–e, 10a–e further confirmed the acetylation of the quaternized salts. Interestingly, there was a clear difference in the reaction periods of 7a–e (72 h) and 9a–e (120 h); this could be due to steric hindrance in dimer.

Antibacterial Properties of Alkyloxy Propanol Amine-Based Cationic Ether Lipids

Antimicrobial activities of alkyloxy propanol amine-based cationic ether lipids with varied alkyl chains and head group are reported for the first time. All the synthesized cationic lipids exhibited good antibacterial activities at 100 μg/mL concentration against the studied strains (Fig. 3). However, tetradecyl chain length based compounds exhibited slightly poorer activity. In general, the quaternary ammonium salts are being used as antibacterial agents that disrupt the cell membrane through the binding of their ammonium cations to anionic sites in the outer layer tissue of bacteria [19]. However, in our study the nature of the quaternary head group (dimethyl type or monomethyl and long chain type), free secondary –OH group, or the presence of a short chain ester group (acetyl moiety) in place of the hydroxy group did not have a significant influence on antimicrobial activity. This reveals that there is no influence of acetylation of secondary –OH on the activity. This may be due to the presence of common long chain (octadecylamine moiety) on the amine side in all the synthesized compounds, further it is noteworthy that the presence of a common long chain on the amine side in all the synthesized cationic lipids made them behave similarly towards all the studied strains.

As a result, the present study did not reveal any preferential absorption at the bacterial cell wall to disrupt the bacterial cell membrane and further no clear trend was observed with both the types (series A and series B) of compounds with respect to variation in the head group and alkyloxy moiety. Incidentally, all the studied cationic lipids showed better activity (10–17 mm) than CTAB (7.5–9 mm), another well known cationic lipid. However, they exhibited inferior activity compared to streptomycin (20–29 mm).

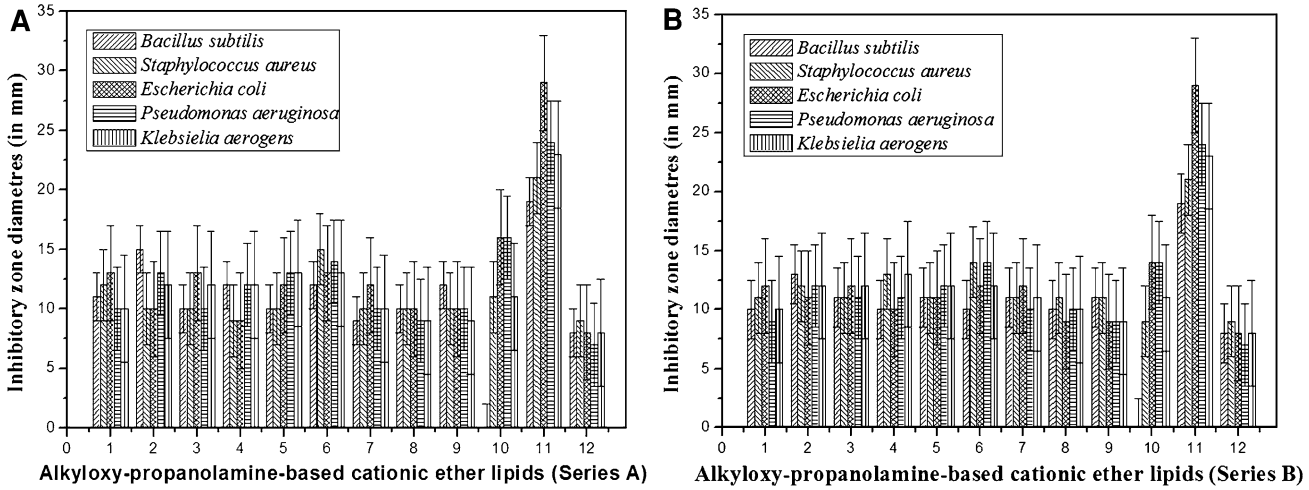


Fig. 3 **a** Antibacterial activity of alkyloxy propanol amine-based cationic ether lipids (Series A: 7a–e, 1–5; 8a–e, 6–10): **1** *N*,*N*-dimethyl-1-octadecylamino-3-butyloxy-2-propanol, **2** *N*,*N*-dimethyl-1-octadecylamino-3-octyloxy-2-propanol, **3** *N*,*N*-dimethyl-1-octadecylamino-3-decyloxy-2-propanol, **4** *N*,*N*-dimethyl-1-octadecylamino-3-dodecyloxy-2-propanol, **5** *N*,*N*-dimethyl-1-octadecylamino-3-tetradecyloxy-2-propanol, **6** *N*,*N*-dimethyl-1 octadecylamino-2-acetyloxy-3-butyloxy-2-propane, **7** *N*,*N*-dimethyl-1-octadecylamino-2-acetyloxy-3-octyloxy-2-propane, **8** *N*,*N*-dimethyl-1-octadecylamino-2 acetyloxy-3-decyloxy-2-propane, **9** *N*,*N*-dimethyl-1-octadecylamino-2-acetyloxy-3-dodecyloxy-2-propane, **10** *N*,*N*-dimethyl-1-octadecylamino-2-acetyloxy-3-tetradecyloxy-2-propane, **11** Streptomycin (standard), **12** Cetyl trimethyl ammonium bromide (CTAB, standard). **b** Antibacterial activity of alkyloxy propanol amine-based cationic ether lipids of (Series B: 9a–e, 1–5; 10a–e, 6–10): **1** *N*-methyl *N*,*N*-di-(2-hydroxy-3-butyloxypropyl) octadecylamine, **2** *N*-methyl *N*,*N*-di-(2-hydroxy-3-octyloxy propyl) octadecylamine, **3** *N*-methyl *N*,*N*-di-(2-hydroxy-3-decyloxy propyl) octadecylamine, **4** *N*-methyl *N*,*N*-di-(2-hydroxy-3-dodecyloxy propyl) octadecylamine, **5** *N*-methyl *N*,*N*-di-(2-hydroxy-3-tetradecyloxypropyl) octadecylamine, **6** *N*-methyl *N*,*N*-di-(2-acetyloxy-3 butyloxy propyl) octadecylamine, **7** *N*-methyl *N*,*N*-di-(2acetyloxy-3-octyloxypropyl) octadecylamine, **8** *N*-methyl*N*,*N*-di-(2-acetyloxy-3decyloxypropyl)octa decylamine, **9** *N*-methyl *N*,*N*-di-(2acetyloxy-3–dodecyloxypropyl)octa-decylamine, **10** *N*-methyl *N*,*N*-di-(2acetyloxy-3 tetradecyl oxypropyl) octadecylamine, **11** Streptomycin (standard), **12** Cetyl trimethyl ammonium bromide (CTAB, standard)

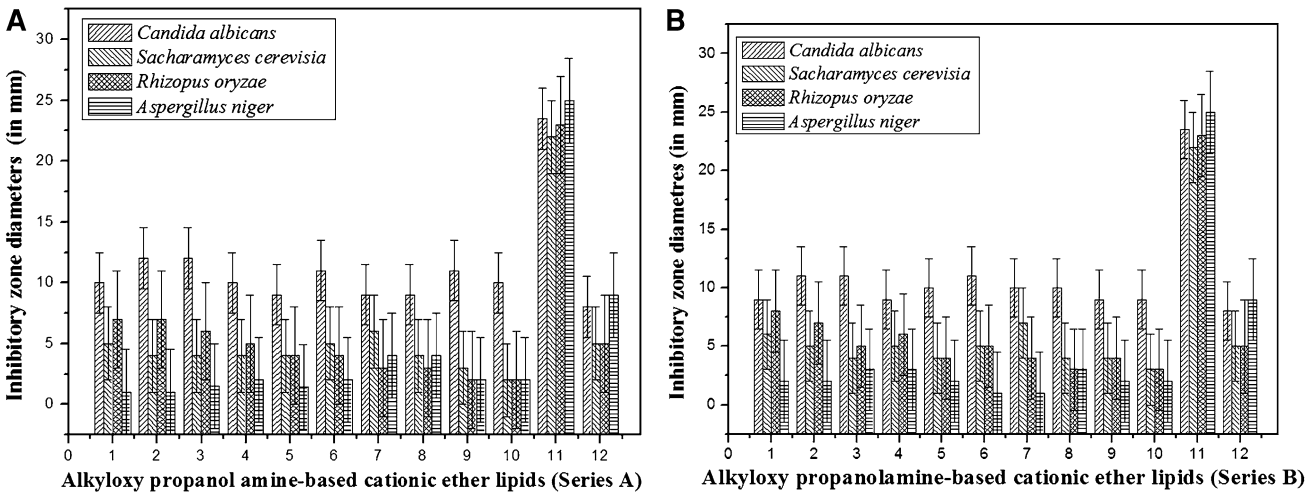


Fig. 4 **a** Antifungal activity of alkyloxy propanol amine-based cationic ether lipids (Series A: 7a–e, 1–5; 8a–e, 6–10): **1** *N*,*N*-dimethyl-1-octadecylamino-3-butyloxy-2-propanol, **2** *N*,*N*-dimethyl-1-octadecylamino-3-octyloxy-2-propanol, **3** *N*,*N*-dimethyl-1-octadecylamino-3-decyloxy-2-propanol, **4** *N*,*N*-dimethyl-1-octadecylamino-3-dodecyloxy-2-propanol, **5** *N*,*N*-dimethyl-1-octadecylamino-3-tetradecyloxy-2-propanol, **6** *N*,*N*-dimethyl-1 octadecylamino-2-acetyloxy-3-butyloxy-2-propane, **7** *N*,*N*-dimethyl-1-octadecylamino-2-acetyloxy-3-octyloxy-2-propane, **8** *N*,*N*-dimethyl-1-octadecylamino-2 acetyloxy-3-decyloxy-2-propane, **9** *N*,*N*-dimethyl-1-octadecylamino-2-acetyloxy-3-dodecyloxy-2-propane, **10** *N*,*N*-dimethyl-1-octadecylamino-2-acetyloxy-3-tetradecyloxy-2propane, **11** Amphotericin B (standard), **12** Cetyl trimethyl ammonium bromide (CTAB, standard). **b** Antifungal activity of alkyloxy propanol amine-based cationic ether lipids of (Series B: 9a–e, 1–5; 10a–e, 6–10): **1** *N*-methyl *N*,*N*-di-(2-hydroxy-3-butyloxypropyl) octadecylamine, **2** *N*-methyl *N*,*N*-di-(2-hydroxy-3-octyloxy propyl) octadecylamine, **3** *N*-methyl *N*,*N*-di-(2-hydroxy-3-decyloxy propyl) octadecylamine, **4** *N*-methyl *N*,*N*-di-(2-hydroxy-3-dodecyloxy propyl) octadecylamine, **5** *N*-methyl *N*,*N*-di-(2-hydroxy-3-tetradecyloxypropyl)octadecylamine, **6** *N*-methyl *N*,*N*-di-(2-acetyloxy-3-butyloxy propyl) octadecylamine, **7** *N*-methyl *N*,*N*-di-(2acetyloxy-3-octyloxypropyl)octadecylamine, **8** *N*-methyl *N*,*N*-di-(2-acetyloxy-3decyloxypropyl)octadecylamine, **9** *N*-methyl *N*,*N*-di-(2acetyloxy-3–dodecyloxypropyl)octadecylamine, **10** *N*-methyl *N*,*N*-di-(2acetyloxy-3 tetradecyl oxypropyl) octadecylamine, **11** Amphotericin B (standard), **12** Cetyl trimethyl ammonium bromide (CTAB, standard)

Antifungal Properties of Alkyloxy Propanol Amine-Based Cationic Ether Lipids

The comparison of antifungal activities of all the synthesized compounds with standards is depicted in Fig. 4. All the synthesized compounds with the alkyl chain lengths starting from C_4 to C_{14} exhibited moderate activity against *C. albicans* (10–12.5 mm); indeed, the activities in this case are comparable to CTAB (7–8 mm) and milder than amphotericin (24–26 mm). However, once again the same phenomena was observed as incase of antibacterial activity, i.e. no significant difference in antifungal activity was found even after acetylation of both A and B series compounds. Further, irrespective of variation in alkyl chain lengths, milder activities against *S. cerevisiae*, *R. oryzae* strains were found with all the synthesized cationic lipids.

On the whole, the synthesized cationic lipids exhibited lower antifungal activities than corresponding antibacterial activities. These results are in good agreement with previous results that quaternary ammonium salt-type antibacterial agents are in general less active against yeasts and fungi than against bacteria since the structure of the former is more complex than that of the latter [19–22]. The mode of action of cationic lipids and surfactants for their antifungal activity was reported by Viera and Carmona [14]. According to them, the action of the cationic detergent (CTAB) or the bilayer-forming cationic synthetic lipid DODAB does not involve fungus cell lysis but rather the change of cell surface charge from negative to positive [14]. This indicates that the compounds (series A and B) are more specific towards bacteria in influencing membrane physiology compared to fungi. The mode of action of these lipids is not clear in the present study. However, further studies are required with similar types of compounds in order to achieve complete antimicrobial evaluation.

Conclusion

In the present work, synthesis of a series of alkyloxy propanol amine-based cationic lipids, *N*,*N*-dimethyl-1-octadecylamino-3-alkyloxy-2-propanol, series A (7a–e), and, *N*-methyl-*N*,*N*-di-(2-hydroxy-3-alkyloxy-2 propyl) octadecylamine, series B (9a–e), and their acetylated derivatives (8a–e and 10a–e) of varying alkyloxy chain lengths (C_4, C_8, C_{10}, C_{12}, C_{14}), in 71–80, 67–88 % yields was carried out. The title compounds as well as their acetylated derivatives were evaluated for antimicrobial activity against two pathogenic strains of gram-positive bacteria, namely *B. subtilis*, *S. aureus* and gram-negative bacteria, namely *E. coli*, *P. aeruginosa*, and *K. aerogenes*. Their activities were compared with the standards Streptomycin and CTAB. All the synthesized compounds were found to possess moderate to good antibacterial activities. Furthermore, both the series A and B and their acetylated derivatives exhibited good antifungal activity against *C. albicans* compared to their activity against other fungal strains. No difference in activity was observed with respect to acetylation; however, an increase in the alkyl chain to tetradecyl, lowers both the antibacterial and antifungal activities.

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