Introduction

Phospholipids (PLs) are important constituents of most of the biological membranes. As the demand for bioactive molecules is increasing day by day, substantial interest is growing rapidly to design more and more structured phospholipid molecules useful for food, industrial and biological applications. The thesis entitled "**Chemo-enzymatic Synthesis of Novel Structured Phospholipids and their Biological Evaluation**" deals with the development of novel methodologies for the partial synthesis of structured phospholipids, their characterization and biological evaluation. The entire thesis is divided into five chapters.

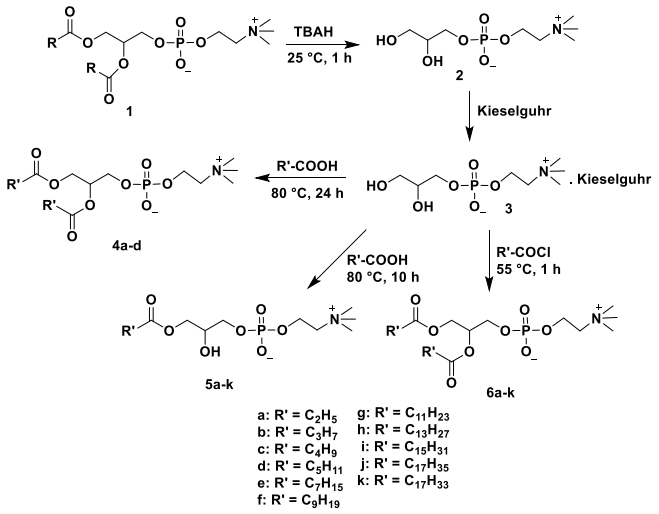
Chapter 1: Introduction to Phospholipids

This Chapter gives introduction about classification of phospholipids, nomenclature, structure and occurrence of phospholipids. Various methods used for qualitative and quantitative identification and analysis of phospholipids are briefly reviewed. The chapter also gives information on biological evaluation of various structured phospholipids synthesized either by partial or total synthesis. Salient features of various methods available in literature for the synthesis of mono acyl, diacyl and mixed chain phospholipids using chemical and enzymatic process are also briefly reviewed.

Chapter 2: A Novel Methodology for the Synthesis of 1-Acyl-*sn*-Glycero-3-Phosphocholine and 1,2-Diacyl-*sn*-Glycero-3-Phosphocholine

In addition to their normal biological role, phospholipids are being used in several areas like surfactants, pharmaceuticals, paint, dye, cosmetics and food industries. In this chapter, a simple, efficient and environmentally benign methodology was developed for the synthesis of saturated and unsaturated fatty acid containing phosphatidylcholines (1-acyl-*sn*-glycero-3-phosphocholine, 7 compounds and 1,2-diacyl-*sn*-glycero-3-phosphocholine, 11 compounds) were developed from GPC with fatty acids/fatty acid chlorides employing kieselguhr as catalyst in solvent free conditions and without using any additional acylating catalyst (**Scheme 1**). The yields of short chain fatty acid based PCs were very low when GPC-kieselguhr complex was reacted directly with C_3-C_6 fatty

acids. However, the yields were improved when shorter chain (C_3-C_6) fatty acid chlorides were used in place of fatty acids. 1-Acyl PCs were selectively obtained when GPC-kieselguhr complex was reacted with long chain (C_8-C_{18}) fatty acids. 1,2-Diacyl PCs were yielded when the reaction was carried out with long chain fatty acid chlorides. The yields of the products were in the range of 95-98% for 1-acyl PCs and 96-99% for 1,2-diacyl PCs.



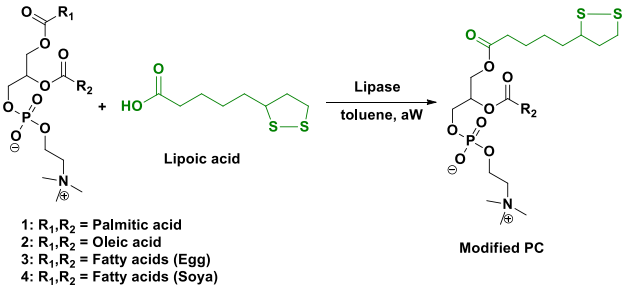
Scheme 1. **Synthesis of 1-Acyl-s*n*-Glycero-3-Phosphocholines and 1,2-Diacyl-*sn*-Glycero-3-Phosphocholines**

The novelty of this methodology is employing kieselguhr as a heterogeneous catalyst for the first time and the kieselguhr was separated from reaction mixture and washed with methanol and reused for 5 times without loss of any activity. All the products were characterized by NMR and MS analysis and also further confirmed by chromatographic (HPLC and GC) techniques. This method is quite attractive for the preparation of symmetrical phosphocholines. As all the reaction steps are very simple, the method can be upscaled very easily for commercial exploitation for the preparation of both lyso PC and symmetrical 1,2-acyl PCs.

Chapter 3: Enzymatic Synthesis of 1-Lipoyl-2-Acyl Phosphatidylcholines

Phospholipids especially structured phospholipids are known to have applications in studies related to functions of biological membranes and in studies of function and folding of membrane-bound proteins. Literature reports are abundantly available for the synthesis and studies of phospholipids with a variety of fatty acids, but same is not the

case with fatty acids with different functionalities like hetero atom containing fatty acids. Very few reports are available on the synthesis of PLs involving active functional groups of fatty acids like sulphur based, amines, carboxylic acids, carbonyl compounds etc which suggests that there is scope for further research in this direction. Sulfur containing lipoic acid (LA) has been gaining importance as drug of the future due to its numerous biological activities. LA exhibits antioxidant activity in both oxidized and reduced forms. LA has potential as therapeutic agent in ischemia-reperfusion injury, heavy metal poisoning, radiation damage, neuro degeneration, HIV infection and the treatment of diseases such as atherosclerosis, thrombosis, diabetes, inflammatory. This chapter deals with the bioorganic synthesis of four novel phospholipids, 1-lipoyl-2-acyl phosphatidylcholine from 1, 2-diacylphosphatidylcholine (DPPC, DOPC, soya/egg PC) and lipoic acid as substrates *via* lipase catalyzed (Lipozyme *RMIM*, *TL IM*, *Candida antarctica* and *R. oryzae* lipases) transesterification reaction at controlled water activity (**Scheme 2**) and only Lipozyme *RM IM* exhibited the maximum incorporation of lipoic acid into phosphatidylcholines. The optimum conditions for the formation of desired products with *RM IM* lipase as catalyst were found to be 1:30 molar equivalents of PC to lipoic acid with a lipase concentration of 75 mg/mL of substrate mixture at room temperature with various reaction time periods (96-120 h) at a_W of 0.11. A low water activity, high lipoic acid concentration and prolonged reaction times were necessary for the product formation (70-87%) and maximum conversion (80-97%) of the starting material.



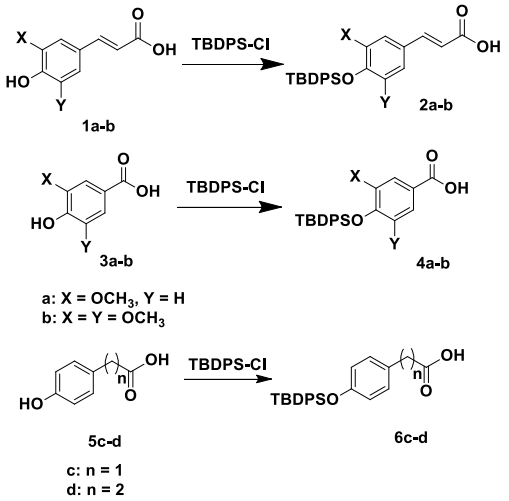
Scheme 2. Synthesis of 1-Lipoyl-2-Acyl Phosphatidylcholines

All the products were characterized by NMR and MS analysis. All the products were evaluated using three *in vitro* methods such as 2,2-diphenyl-1-picryl-hydrazyl radical scavenging activity, superoxide radical scavenging activity and inhibition of lipid peroxidation assay compared with BHT and α-tocopherols as standard antioxidants. All

the products exhibited lower antioxidant activity as compared to the reference compounds. The synthesized lipoic acid containing phosphatidylcholine derivatives have potential in drug delivery research as the lipoic acid is known to be effective in various disease conditions, in addition to being an effective antioxidant and biological activities.

Chapter 4: Chemo-enzymatic Synthesis and *in vitro* Antioxidant and Antimicrobial Studies of Phenolic Acid Based-Structured Phosphatidylcholines

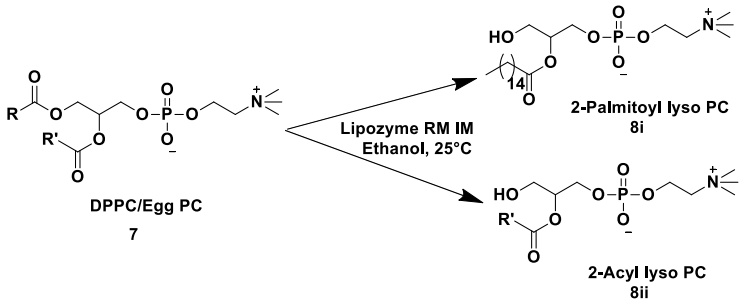
Recent literature reveals that several phospholipids are being synthesized by incorporating polyunsaturated fatty acids and unusual fatty acids for specific applications. Literature reveals that phenolic acids containing phospholipids are scarce even though they may be promising for some novel applications. Phenolic acids are ubiquitous natural antioxidants accounting for approximately one third of the phenolic compounds in our daily diet. In addition to antioxidant activity, it is reported that phenolic acid derivatives exhibit several biological activities. In this chapter, chemo-enzymatic synthesis of twelve novel phosphatidylcholines containing phenolic acids (4-hydroxy phenyl acetic, 4-hydroxy phenyl propanoic, ferulic, sinapic, vanillic and syringic acids) in *sn*-1 and fatty acids in *sn*-2 position is described for the first time (**Scheme 3-7**). Initially, hydroxyl group of phenolic acids was protected with *tert*-butyl diphenyl silyl chloride in presence of imidazole to obtain 4-(*tert*-butyl diphenyl silyloxy) phenolic acids in 82-90% yields.



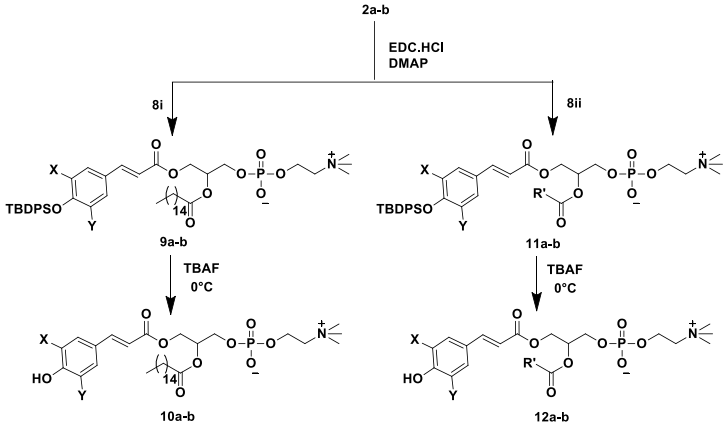
Scheme 3. Synthesis of Protected Phenolic Acids

Lyso PCs were prepared by enzymatic hydrolysis of DPPC and egg PC in 90-93% yields. Lyso PCs were esterified employing N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride in presence of 4-dimethylaminopyridine with protected

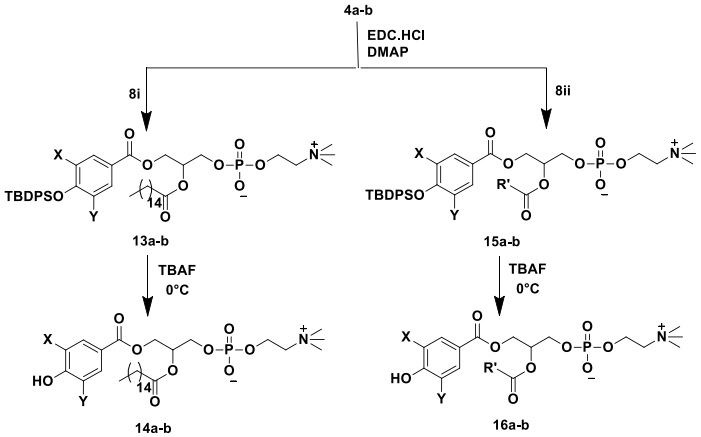
phenolic acids to obtain 1-(4-*tert*-butyl diphenyl silyloxy) phenoyl-2-acyl-*sn*-glycero-3-phosphocholines in 48-56% yields. The final step, *tert*-butyl diphenyl silyl group was removed from 1-(4-*tert*-butyl diphenyl silyloxy) phenoyl-2-acyl-*sn*-glycero-3-phosphocholines with tetrabutylammonium fluoride to obtain 1-phenoyl-2-acyl phosphatidylcholines in 87-94% yields. The overall yields of the products are in the range of 30.8-44.0% from PC.



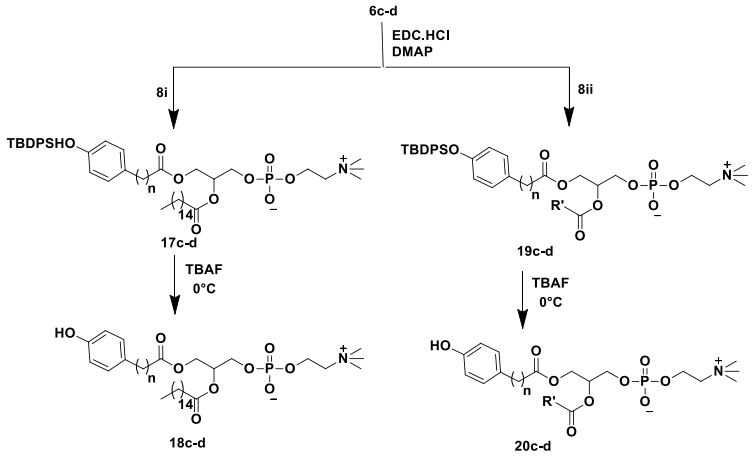
Scheme 4. Synthesis of 2-Palmitoyl and 2-Acyl Lyso Phosphatidylcholine



Scheme 5. Synthesis of Cinnamic Acid Derivatives of Phosphatidylcholine



Scheme 6. Synthesis of Benzoic Acid Derivatives of Phosphatidylcholine



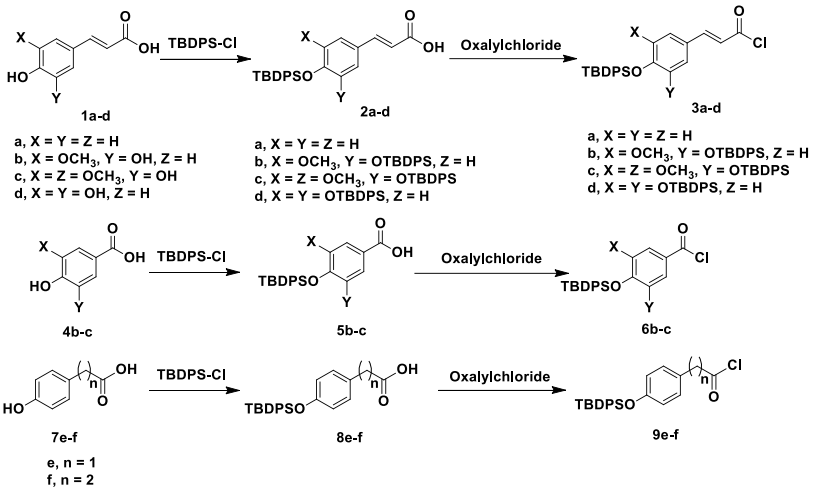
Scheme 7. Synthesis of Phenolic Acid Derivatives of Phosphatidylcholine

All the products were characterized by FT-IR, NMR and MS analysis. All the compounds were tested for *in vitro* antioxidant and anti-microbial activities. Among the active derivatives, compound 1-(4-hydroxy-3,5-dimethoxy) cinnamoyl-2-acyl-*sn*-glycero-3-phosphocholine exhibited excellent antioxidant activity which was higher than the standard BHT. Preliminary investigation of antimicrobial evaluation of the prepared phenol phospholipids exhibited moderate to good antimicrobial activity on some strains. Phenolic phosphatidylcholines may have potential antioxidant activity in variety of food matrices.

Chapter 5: Synthesis of Novel Phosphatidylethanolamine-N-phenolic Acid Derivatives and their evaluation for *in vitro* Antioxidant activity

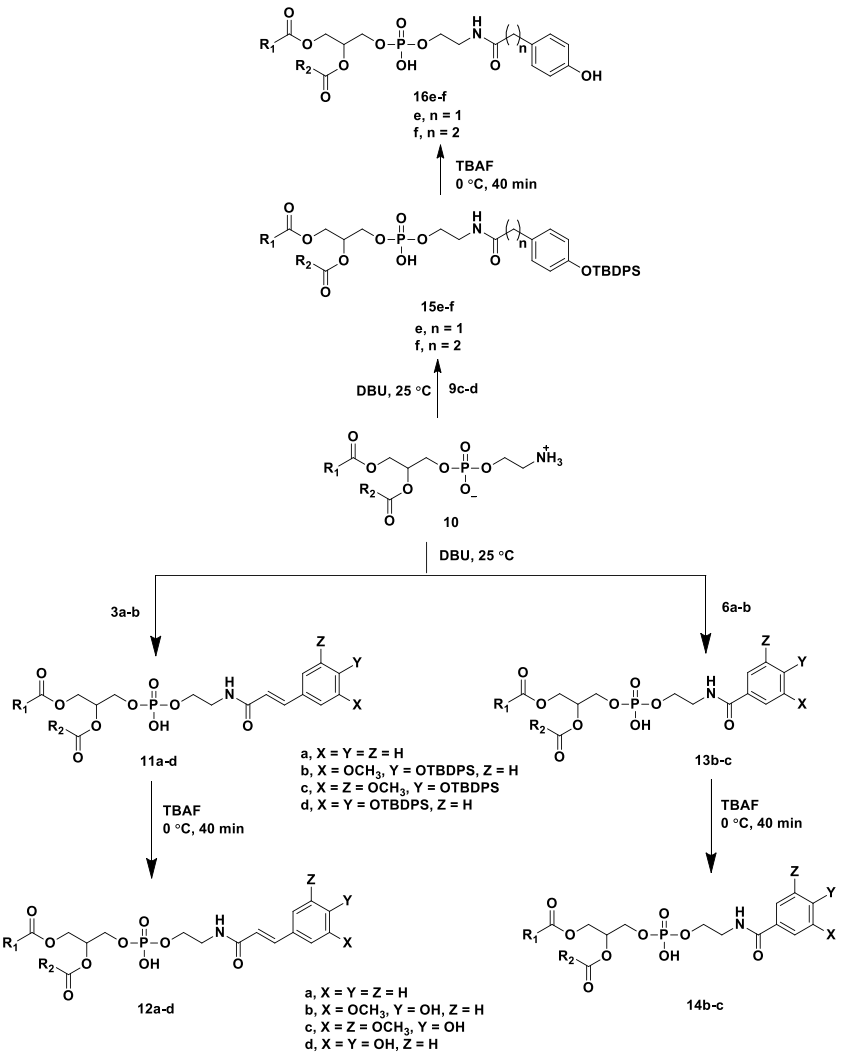
Several biologically active fatty acids including unusual fatty acids and their derivatives were incorporated into several phospholipids at *sn*-1 and *sn*-2 positions and evaluated for some specific properties. Similarly, a number of studies were reported by modifying the head group of PE at amino-functionality. N-acylethanolamines are known to exhibit variety of biological activities depending on its acyl chain. N-Palmitoylethanolamine and N-oleoylethanolamine have been documented as anti-inflammatory, analgesic and appetite-suppressing substances. *N*-Acyl phospholipids represent an unusual class of phospholipids that contain head groups modified with fatty acids. NAPEs play a vital role during injury or stress and also some NAPEs function as signaling molecules. Head group modification of phosphatidylethanolamine with fatty acids was extensively studied but similar modification with phenolics acids has not

examined so far. In this chapter, eight novel phosphatidylethanolamine-N-phenolic acid derivatives were synthesized by modifying the head group of egg PE at amine functionality with phenolic acids (4-hydroxy phenyl acetic, 4-hydroxy phenyl propanoic, cinnamic, ferulic, sinapic, caffeic, vanillic and syringic acids) for the first time (**Schemes 8 & 9**). Initially, hydroxyl group of phenolic acids was protected with *tert*-butyl diphenyl silyl chloride in presence of imidazole to obtain 4-(*tert*-butyl diphenyl silyloxy) phenolic acids in 82-90% yields.



Scheme 8. Synthesis of Protected Phenolic Acid Chlorides

The resulting 4-(*tert*-butyl diphenyl silyloxy) phenolic acids were converted into 4-(*tert*-butyl diphenyl silyloxy) phenolic acid chlorides which were reacted with phosphatidylethanolamine (PE) isolated from egg yolk lecithin in presence of 1,8-diazabicyclo[5.4.0] undec-7-ene to obtain PE-N-4-(*tert*-butyl diphenyl silyloxy) phenolic acids in 60-79% yields. In the final step, *tert*-butyl diphenyl silyl group was removed from PE-N-4-(*tert*-butyl diphenyl silyloxy) phenolic acids to obtain PE-N-phenolic acid derivatives in 70-85% yields. The overall yields of the products are in the range of 34.4-60.4% from PE. All the products were characterized by FT-IR, NMR and MS analysis. The compounds PE-N-4-hydroxy-3-methoxy cinnamic acid, PE-N-4-hydroxy-3,5-dimethoxy cinnamic acid and PE-N-3,4-dihydroxy cinnamic acid exhibited excellent radical scavenging activity and the EC_{50} values were found to be lower than the standard BHT and α-tocopherols. The compounds PE-N-4-hydroxy-3-methoxy benzoic acid and PE-N-4-hydroxy-3,5-dimethoxy benzoic acid also exhibited good antioxidant activities.



Scheme 9. Synthesis of Phosphatidylethanolamine-N-Phenolic Acid Derivatives

These are novel PE-N-phenolic acid derivatives which may have potential applications in the interface of chemistry and biology as existing PE-N-acylated long chain derivatives are reported to posses several biological activities. The presence of phenolics has to be examined and compared for biological activities that are reported for the PE-N-acyl derivatives in order to know the potential of these novel compounds.