Introduction

Lipids are a major class of biomolecules responsible for critical biological roles in regulating many vital biological pathways and pathophysiological events. Moreover, lipids act as potent signaling agents since many diseases are associated with disruptions in lipid metabolism and function including neurological disorders, autoimmune diseases, and cancer. \cite{1,2}

A considerable amount of lipid research relies on the use of chemical tools or probes. Regarding live cell experiments, fluorescence-based techniques are the least invasive, allowing the real-time observation of biological membranes and their characteristic physical properties. Therefore, fluorescent labelling is used as the preferred tool for the investigation of biological functions involving lipids, namely for clarifying metabolic pathways and molecular mechanisms of diseases where these molecules are of crucial importance \cite{3}. Additionally, the lack of functionalyzed lipid probes with the biological and physicochemical properties suitable for these types of studies is still a major limitation concerning this research area. Hence, the development of fluorescent lipid analogues has received increasing interest in recent years.

Aim

➢ Development of a bioconjugation method based on N-hydroxysuccinimide (NHS) chemistry to easily obtain fluorescent lipid probes.
➢ Implement an automated flow procedure based on multi-syringe flow injection to allow the synthesis and preparation of these lipid bioconjugate probes with minimal consumption of expensive reagents.

Methodology

A two-step classic organic synthesis procedure was established concerning the functionalization of phospholipid analogues with coumarin fluorescent probes through NHS coupling (Scheme 1 and 2).

Results

The synthesis of 2-oleyl-1-palmitoyl-sn-glycero-3-phosphoethanolamine (POPE) fluorescent analogue 5 was achieved through a classic organic synthesis method in a two-step reaction. In the first step, the coumarin-3-carboxylic acid 1 was activated to the corresponding succinimidy ester 3 followed by the direct conjugation to the POPE analogue 4 via amide bond formation to obtain the desired fluorescent lipid probe 5 with an overall yield of 36% as a preliminary result.

Concluding remarks

Lipids and cellular membranes are very challenging to study, and their investigation is strongly dependent on the use of fluorescence probes. In this work, a straightforward method to obtain fluorescent lipid derivatives by classic methods was applied. The implementation of a continuous flow procedure will allow a significant improvement regarding the versatility and easy access to lipid-fluorescent probes in an automated and efficient reagent’s consumption method.

References


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