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Newly synthesized bolaamphiphiles from castor oil and their aggregated morphologies for potential use in drug delivery

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ABSTRACT

The present study focused on synthesizing bolaamphiphiles from the readily available and inexpensive castor oil, a vegetable oil, which contains about 90% of ricinoleic acid. Two classes of symmetric and asymmetric bolaamphiphiles with acetylcholine head groups were synthesized and characterized by spectroscopic analysis. These novel bolaamphiphilic compounds self-assemble in aqueous media to form stable cationic spherical nano-sized vesicles that are potential drug delivery systems.

1. Introduction

A new trend in the design of drug delivery systems is to combine the principles of supramolecular chemistry and green chemistry by exploiting renewable plant-derived resources as a starting material for the fabrication of nanoscale structures. Vegetable oils are generally considered to be the most important class of renewable resources, because of their ready availability, competitive cost and built-in functionality that make them attractive for numerous applications in the chemical industry.[1,2]

Lipid-based nanoparticles are generally the least toxic nanostructures compared to other nanomaterials (dendrimers, nanotubes, nanocrystals, inorganic nanoparticles), and therefore, they helped to achieve significant progress in drug delivery.[3–6]

New compounds—bolaamphiphiles—have recently emerged as excellent candidates for making vesicles (nanostructures) with unique properties for drug delivery. These novel amphiphiles that have attracted much interest in the recent years are composed of two charged head groups connected by a long linear polymethylene chain.[7–9] Several cationic bolas with acetylcholine (ACh) head groups, synthesized from vernonia oil, have been shown to form cationic nano-size vesicles by self-assembly.[10–12] The choline esters surface groups of these vesicles were hydrolysed by acetylcholine esterase (AChE) and it resulted in the disruption of the vesicular structure and the release of encapsulated compounds at the hydrolysis site.[13,14] AChE is an enzyme, which is highly active in several tissues throughout the body (e.g., in the skeletal muscle, heart and the central nervous system). The primary function of this enzyme is to hydrolyse ACh and thus, terminate cholinergic neurotransmission.[15–17] But this enzyme can also be used to achieve selective drug release at sites where the enzyme is highly active. The hydrolysis of choline esters head groups by AChE was described as a means to achieve active targeting, based on increased enzyme activity in the diseased tissues.[18–20] However, since acetylcholine esterase is highly active in several tissues throughout the body, release from vesicles with acetylcholine head groups will occur in all of these tissues and not only in one specific tissue. Yet, it was demonstrated that by treating animals with pyridostigmine, an inhibitor of acetylcholine esterase that does not penetrate via the BBB into the brain, the release of encapsulated fluorescent marker was primarily in the brain.[13]

However, the synthesis of bola compounds from vernonia oil was not easy because of the formation of various structural isomers that are difficult to separate and also, this vegetable oil is not

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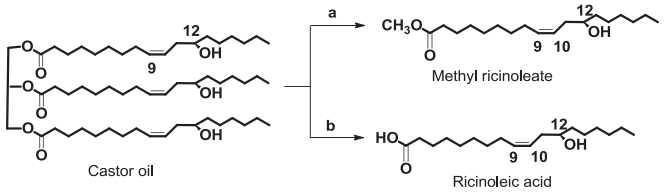
commercially available and may pose a problem for a scale-up synthesis. Therefore, alternatives to vernonia oil are being sought and, in the present study, we investigated the possibility of using castor oil as the starting material for the synthesis of such bolaamphiphiles.

Castor oil is a readily available and inexpensive vegetable oil obtained from the seeds of *Ricinus communis* (Euphorbiaceae), which contains about 90% of ricinoleic acid, a monounsaturated 18-carbon fatty acid with a hydroxyl functional group on the 12th carbon. The high content of ricinoleic acid is the reason for the high value of castor oil and its versatile application possibilities in the chemical industry. The high availability of castor oil and the hydroxyl group in ricinoleic acid make this oil as a good candidate for the synthesis of bolaamphiphiles.

In the present study based on castor oil, the newly synthesized bolaamphiphiles were designed with the molecular parameters required to form, by self-assembly, stable nanostructures with properties needed for drug carriers. According to the nature and targeting properties of acetylcholine, series of symmetric and asymmetric bolaamphiphiles with acetylcholine head groups bound to the bolaskeleton either via nitrogen atom or through oxygen atom of the acetylcholine (ACh) head groups were prepared. Upon self-assembly in aqueous media, the novel bolaamphiphilic compounds formed stable spherical cationic nano-sized vesicles.

2. Results and discussion

Based on their design, the novel bolaamphiphiles were synthesized following a strategy similar to the one we previously developed.[10,21] The first synthetic step is the formation of the bolaamphiphile hydrophobic skeleton by the reaction of the carboxylic group of the fatty acid or fatty acid ester with aliphatic diols, and the second synthetic step is the incorporation of the ACh head groups by esterification of the secondary hydroxyl groups located on C12 of the ricinoleic acid moiety with a halo carboxylic or a dicarboxylic acid. To simplify the separation and purification of the intermediate and the final products, methyl ricinoleate and ricinoleic acid (Scheme 1) were used rather than castor oil itself.[22–25]



Scheme 1. Synthesis of methyl ricinoleate and ricinoleic acid from castor oil. a) MeOH/CH_3ONa, 80 °C, 2.5 h; b) KOH/EtOH, reflux, 1 h.

We synthesized two classes of synthetic bolaamphiphiles with ACh head groups from castor oil: (a) cationic bolaamphiphiles (symmetric and asymmetric) with acetylcholine head groups bound to the hydrophobic skeleton via its nitrogen atom (Schemes 2 and 3); (b) cationic bolaamphiphile (symmetric) with ACh head groups attached to the bola skeleton through its oxygen atom (Scheme 4).

2.1. Synthesis of bolaamphiphiles

2.1.1. Symmetric bolaamphiphiles with ACh head groups attached through nitrogen atom

2.1.1.1. Building the hydrophobic skeletons. The hydrophobic skeletons of the symmetric bolaamphiphiles **Bola-1** are the diesters **4a**, **4b** and **4c** synthesized by the extension of the ricinoleic chain in a chemoenzymatic esterification of ricinoleic acid (**1**, R=H) or transesterification of methyl ricinoleate (**1**, R=CH_3) with aliphatic diols **2a**, **2b** or **2c** and a lipase as the catalyst.[21,26,27] Lipases are known as superior catalysts that accept a wide array of complex molecules as substrates and catalyse reactions with high enantio- and regio-selectivities.[28–30] In the present study, in order to prepare diesters with better yield and purity, lipase catalyst was preferred to classical esterification techniques, which could induce the formation of by-products or the loss of materials due to the multi-step reactions occurring in the course of the protection and deprotection of the secondary hydroxyl group of ricinoleic moiety.

The reaction was carried out by reacting ricinoleic acid (**1**, R=H)) with 1, 10-decanediol **2a** (molar ratio acid/diol: 2/1) in toluene under vacuum azeotropic distillation in the presence of the immobilized *Candida antarctica* lipase.[10,31,32] This catalyst exhibited high regioselectivity by acting exclusively at the primary alcoholic positions of the molecules. The diester **4a** (with n=10 in the midsection) was obtained in high yield (72%) and purity (91%, HPLC analysis). Because the diols with a shorter aliphatic chain e.g., 1,4-butanediol **2b** or ethylene glycol **2c** are insoluble in toluene, the synthesis of their corresponding diesters **4b** and **4c** was performed by transesterification of methyl ricinoleate (**1**, R=CH_3) with these diols (**2b** or **2c**) again in a chemoenzymatic reaction in the presence of *C. antarctica* lipase, in *tert*-BuOH at room temperature for 24 h. The esterification reaction is a consecutive nucleophilic substitution reaction and a mixture of monoester **3** and diester **4** was obtained (Scheme 2). The products were separated by flash column chromatography.

The FTIR spectrum of the diester **4a** showed the disappearance of the absorption band of the carboxylic carbonyl group at 1710 cm^{-1}, and the appearance of the absorption band, characteristic of the new ester group at 1736 cm^{-1}.

^1H and ^{13}C NMR spectra of diester **4a** showed the appearance of a new signal of the alkoxy groups (CH_2—O) as a triplet at 4.08 ppm in ^1H spectrum and at 64.41 ppm in ^{13}C spectrum. For the diesters **4c**, an intense singlet at 4.26 ppm was observed for the new alkoxy group (CH_2—O) in ^1H NMR spectrum. MS (ESI) spectra confirmed the formation of the diesters **4**.

2.1.1.2. Head group attachment of the acetylcholine through the nitrogen atom. The attachment of the acetylcholine (ACh) head groups to the symmetric hydrophobic skeleton, through the nitrogen atom of the acetylcholine, was performed in a two-step reaction: firstly the chloroacetylation of the hydroxyl groups of the diesters **4** to obtain the dichloroacetate intermediates **6** that will be used in the second step as the alkylating agent to quaternize the *N*,*N*-dimethylaminoethyl acetate **7** to form the symmetric bola compounds **Bola-1** with acetylcholine head groups.

*2.1.1.2.1. Chloroacetylation of the diesters **4**.* The dichloroacetate intermediates **6** were prepared in a high yield (≈70%) by acylation of the secondary alcohols of the diesters **4** using an excess of chloroacetyl chloride **5** ($ClCH_2COCl$) in dry diethyl ether at room temperature for 24 h. In FTIR spectra, we noticed the absence of the absorption band of hydroxyl group (3383 cm^{-1}), the appearance of the new chloroacetate ester group at 1759 cm^{-1} in addition to the original carbonyl ester peak at 1736 cm^{-1} and the presence of the characteristic band of C—H bending of the terminal alkyl halide CH_2—Cl at 1291 cm^{-1} and C—Cl stretch at 719 cm^{-1} (data from **6a**, with n=10 in the midsection).

In the ^1H NMR spectrum of the symmetric dichloroacetate intermediate **6a**, we distinguished the overlapping of the triplet of methylenic protons of CH_2—O—CO groups located in the midsection at 4.05 ppm, with the two doublets belonging to the two diastereotopic protons of the terminal methylene chloride (CH_2Cl) at 4.04 ppm and 4.02 ppm. These diastereotopic protons possess a geminal coupling (J_{gem}=14.5 Hz) and undergo a 'roof' effect due to their very close chemical shifts (Fig. 1).