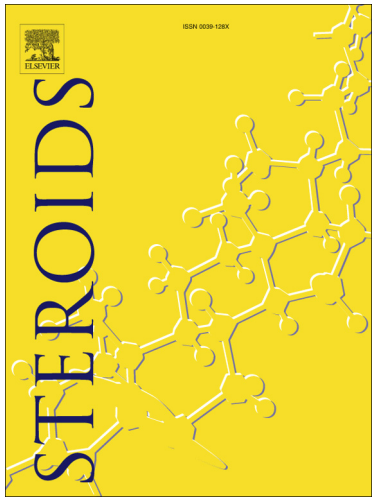

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PII: S0039-128X(18)30167-3
DOI: https://doi.org/10.1016/j.steroids.2018.09.004
Reference: STE 8310

To appear in: *Steroids*

Received Date: 27 April 2018
Revised Date: 17 August 2018
Accepted Date: 6 September 2018

Please cite this article as: Chanquia, S.N., Ripani, E., Baldessari, A., García Liñares, G., Bile acids: lipase-catalyzed synthesis of new hyodeoxycholic acid derivatives, *Steroids* (2018), doi: https://doi.org/10.1016/j.steroids.2018.09.004

Bile acids: lipase-catalyzed synthesis of new hyodeoxycholic acid derivatives

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Abstract

In this work we present an efficient, environmentally friendly approach to the synthesis of a series of hyodeoxycholic acid derivatives applying Biocatalysis. Fifteen acetyl and ester derivatives, twelve of them new, were obtained through an enzymatic strategy in a fully regioselective way and in very good to excellent yield. In order to find the optimal reaction conditions, the influence of several parameters such as enzyme source, alcohol or acylating agent:substrate ratio, enzyme:substrate ratio, temperature and reaction solvent was considered. The excellent results obtained made this procedure very efficient, particularly considering the low amount of enzyme required. In addition, this methodology uses mild reaction conditions and has reduced environmental impact, making biocatalysis a suitable way to obtaining these bile acids derivatives.

Keywords: Lipases; Bile acid; Biocatalysis; Hyodeoxycholic acid

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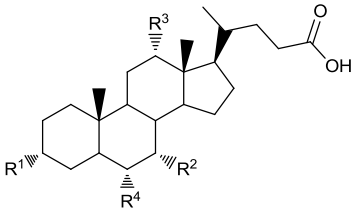
1. Introduction

Bile acids are found predominantly in the bile of mammals and other vertebrates as oxygenated metabolites of steroids [1, 2]. They are classified in two groups: primary bile acids (cholic, **CA**, and chenodeoxycholic, **CDCA**) which are synthesized by the liver, and secondary bile acids which are obtained through enterobacterial metabolism of primary bile acids, such as deoxycholic **(DCA)**, lithocholic **(LCA)**, and hyodeoxycholic **(HDCA)** acids (Scheme 1).



CA 1: R^1 = OH, R^2 = OH, R^3 = OH, R^4 = H
CDCA 2: R^1 = OH, R^2 = OH, R^3 = H, R^4 = H
DCA 3: R^1 = OH, R^2 = H, R^3 = OH, R^4 = H
LCA 4: R^1 = OH, R^2 = H, R^3 = H, R^4 = H
HDCA 5: R^1 = OH, R^2 = H, R^3 = H, R^4 =O H

Scheme 1. Structure of important bile acids

Bile acids are important metabolites that take part in many biological processes such as secretion, digestion and regulation of cholesterol metabolism. Therefore, they are the subject of several pharmacological studies [1, 3, 4]. Because of their amphiphilic properties they act as biological detergents that help in the absorption and dissolution of fatty acids and vitamins. Also, they show many pharmacological properties, including selective and potent farnesoid X receptor (FXR) antagonism [5], antimicrobial [6] and anticarcinogenic activity [7, 8]. In addition, they show promising activity for the treatment of various inflammation-related human diseases [9]. For these reasons, bile acid analogs are extensively used in drug formulations in order to improve absorption [10, 11], as excipients that can enhance transdermal penetration [12, 13], gastrointestinal solubility and stability of drugs [14, 15] and bring multiple benefits. Hyodeoxycholic acid (HDCA, **5**) is the main acid constituent of hog bile, and for many years it was used in the industry as a precursor in the synthesis of steroids. Moreover, HDCA has proved to inhibit atherosclerosis in mice by decreasing cholesterol levels [16].

Highly active and selective biocatalysts such as enzymes and whole cells are very powerful tools in organic synthesis, behaving in a chemo-, regio- and enantioselective way, simplifying the preparation of a wide variety complex organic molecules [17, 18]. Moreover, at the present time chemoenzymatic multi-step processes are a growing subject in organic synthesis [19], especially for the synthesis of novel pharmaceutical derivatives [20]. This approach has many advantages, namely it allows to avoid tedious protection and deprotection steps in chemical synthesis, which is especially useful when working with compounds with several functional groups.

Amid the wide variety of enzymes used in organic synthesis, lipases in non-aqueous media have an amazing promiscuity, being used for a broad range of reactions such as aminolysis, esterification, transesterification, polymerization, etc. [21-25]. In our laboratory several studies have been accomplished on the use of lipases, obtaining a wide variety of biologically active compounds, many of them new [26-35].

Usually, the potential application of bile acids depends on their amphiphilic properties, therefore we have recently studied and reported the synthesis of derivatives of many bile acids searching for an increase in its lipophilicity [28, 36, 37]. We described the application of lipases to the esterification, acetylation and alcoholysis of CDCA, DCA, LCA and CA. *Candida antarctica* B lipase gave the best results for esterification and acetylation reactions of CDCA, DCA and LCA and for alcoholysis reaction of the four bile acids. CA showed a different behavior in comparison with the other bile acids, probably due to its structure containing three hydroxyl groups. In the case of CA, *Rhizomucor miehei* lipase gave the best results for the esterification reaction, but it was not possible to obtain acetylation products with any of the lipases tested.

Continuing with this approach, we noticed that compared to the other bile acids, there are very few works about hyodeoxycholic acid (HDCA). Therefore, we thought it would be of great interest to analyze its reactivity and obtain different derivatives which might have useful applications. In the present work we present the synthesis of a series of derivatives of hyodeoxycholic acid, most of them new, obtained by lipase-catalyzed esterification, acetylation and alcoholysis reactions.

2. Experimental

2.1. General

Chemicals and solvents were purchased from Merck Argentina and Sigma–Aldrich de Argentina and used without further purification. Lipase from *Candida rugosa* (CRL) (905 U/mg solid) was purchased from Sigma Chemical Co.; *Candida antarctica* lipase B (CAL B): Novozym 435 (7400 PLU/g), Lipozyme from *Thermomyces lanuginosus* (TLIM) and Lipozyme from *Rhizomucor miehei* RM1M (LIP) (both 7800 U/g) were generous gifts of Novozymes Spain; *Carica papaya* lipase (CPL) is the remaining solid fraction of papaya latex, after wash off of proteases using distilled water. CPL is a naturally immobilized enzyme [38] and was a generous gift of Dr. Georgina Sandoval, CIATEJ, Mexico. All enzymes were used "straight from the bottle". Enzyme/substrate ratio (E/S): enzyme amount in mg/substrate amount in mg. Enzymatic reactions were carried out on MaxQ 4000 Thermo Scientific Co. digital incubator shaker at the corresponding temperature and 200 rpm. To monitor the reaction progress aliquots were withdrawn and analyzed by TLC performed on commercial 0.2 mm aluminum-coated silica gel plates (F254) and visualized by immersion in an aqueous solution of $(NH_4)_6Mo_7O_{24}.4H_2O$ (0.04 M), $Ce(SO_4)_2$ (0.003 M) in H_2SO_4 (10%). % Conversion was determined by analytical reverse phase HPLC employing a Phenomenex Phenogel column 5 μM 10E5A, 300 x 7.8 mm and eluting with MeOH:H_2O 80:20 at 1.00 mL/min. ^1H NMR and ^{13}C NMR spectra were recorded at room temperature in $CDCl_3$ as solvent using a Bruker AM-500 NMR instrument operating at 500.14 MHz and 125.76 MHz for ^1H and ^{13}C respectively. The ^1H NMR spectra are referenced to the residual $CHCl_3$ proton of the solvent $CDCl_3$ at δ = 7.26 ppm. Coupling constants are reported in Hertz (Hz). ^{13}C NMR spectra were fully decoupled and are referenced to the middle peak of the solvent $CDCl_3$ at δ = 77.0 ppm. Splitting patterns are designated as: s, singlet; d, doublet; t, triplet; q, quadruplet; dd, double doublet, etc. High Resolution Mass Spectrometry was recorded with Thermo Scientific EM/DSQ II – DIP. The results were within ±0.02% of the theoretical values.

2.2. Lipase-catalyzed esterification. General procedure

LIP (10 mg) was added to a solution of hyodeoxycholic acid (20 mg) and the corresponding alcohol (1 eq.) in diisopropyl ether (DIPE) (5 mL). The suspension was shaken (200 rpm)

at 55 ºC and the reaction monitored by TLC. Once the reaction was finished, the enzyme was filtered off and the solvent evaporated under reduced pressure. The residue was purified by column chromatography on silica gel employing mixtures of hexane:ethyl acetate as eluent (1:0-1:1). Reuse experiments: the filtered and washed enzyme was used in the next enzymatic esterification under the same reaction conditions. LIP retained 80 % activity after three reaction cycles.

2.3. Lipase-catalyzed acetylation.

CAL B (10 mg) was added to a solution of hyodeoxycholic acid (20 mg) in ethyl acetate (5 mL). The suspension was shaken (200 rpm) at 55 ºC and the progress of the reaction monitored by TLC. Once the reaction finished, the enzyme was filtered off and the solvent evaporated under reduced pressure. Given that the reaction achieved full conversion no further purification was needed. Reuse experiments: the filtered and washed enzyme was used in the next enzymatic acetylation under the same reaction conditions. CAL B retained 95 % activity after five reaction cycles.

2.4. Lipase-catalyzed alcoholysis.

CAL B (1 g) was added to a solution of 3,6-diacetoxy hyodeoxycholic acid (50 mg) in 10 ml of hexane containing 1.2 mol equiv. of ethanol. The suspension was shaken (200 rpm) at 55 °C and the progress of the reaction was monitored by TLC. When the reaction finished, lipase was filtered off, the solvent was evaporated under reduced pressure, and the crude residue purified by column chromatography on silica gel employing mixtures hexane:ethyl acetate as eluent (4:1-1:1).

2.5. Chemical peracetylation

A mixture of hyodeoxycholic acid (100 mg), acetic anhydride (2 mL) and pyridine (2 mL) was stirred at room temperature for 16 hs. TLC was used to monitor the development of the reaction. After completion, the mixture was partitioned using ammonium chloride saturated solution (10 mL) and methylene chloride (10 mL). The aqueous phase was extracted with methylene chloride (3 x 10 mL). Afterwards, the combined organic layers were washed with sodium chloride saturated solution (5 x 10 mL), dried using anhydrous $MgSO_4$, and

the solvent was evaporated. The residue (125 mg) was purified by column chromatography (silica gel) employing mixtures of hexane/EtOAc as eluent (4:1–1:1) (**7**, m = 110 mg).

Ethyl hyodeoxycholate (6a): Yield 95%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.07 (2H, q, *J* = 6.8 Hz, -OC*H*$_2$CH$_3$), 3.96 (1H, dt, *J* = 12.2, 5.0 Hz, H-6), 3.53 (1H, m, H-3), 2.27 (1H, m, H-23b), 2.16 (1H, m, H-23a), 1.21 (3H, t, *J* = 6.8 Hz, -OCH$_2$C*H*$_3$), 0.86 (3H, d, *J* = 6.4 Hz, H-21), 0.85 (3H, s, H-19), 0.60 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 174.6 (C-24), 71.2 (C-6), 67.8 (C-3), 60.2 (-O*C*H$_2$CH$_3$), 56.0, 55.8, 48.2, 42.7, 39.8, 39.7, 35.8, 35.4, 35.2, 34.7, 31.2, 31.1, 30.9, 29.8, 29.6, 28.0, 24.1 (C-19), 23.4, 20.6, 18.1 (C-21), 14.1 (-OCH$_2$*C*H$_3$), 11.9 (C-18). HRMS: [M + Na]$^+$ Calcd. $C_{26}H_{44}NaO_4$ 443.3137. Found: $C_{26}H_{44}NaO_4$ 443.3139.

***n*-Propyl hyodeoxycholate (6b):** Yield 90%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.06 (1H, dt, *J* = 12.1, 5.3 Hz, H-6), 4.02 (2H, t, *J* = 6.65 Hz, -OC*H*$_2$CH$_2$CH$_3$), 3.62 (1H, m, H-3), 2.34 (1H, m, H-23b), 2.21 (1H, m, H-23a), 1.13 (3H, d, *J* = 6.2 Hz, H-21), 0.93 (3H, t, *J* = 7.1 Hz, -OCH$_2$CH$_2$C*H*$_3$), 0.89 (3H, s, H-19), 0.63 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 174.5 (C-24), 71.6 (C-6), 68.1 (C-3), 65.9 (-O*C*H$_2$CH$_2$CH$_3$), 56.1, 55.9, 48.3, 42.8, 39.9, 39.8, 35.9, 35.5, 35.3, 35.0, 31.3, 31.0, 30.2, 29.1, 28.1, 24.2 (C-19), 23.5, 22.9, 22.0, 20.7, 18.2 (C-21), 12.0 (-OCH$_2$CH$_2$*C*H$_3$), 10.4 (C-18). HRMS: [M + Na]$^+$ Calcd. $C_{27}H_{46}NaO_4$ 457.3294. Found: $C_{27}H_{46}NaO_4$ 457.3287.

***n*-Butyl hyodeoxycholate (6c):** Yield 82% after two purifications by column chromatography; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.10 (1H, q, *J* = 6.1 Hz, H-6), 4.04 (2H, t, *J* = 6.7 Hz, -OC*H*$_2$CH$_2$CH$_2$CH$_3$), 3.62 (1H, tt, *J* = 11.2, 6.3 Hz, H-3), 2.32 (1H, m, H-23b), 2.21 (1H, m, H-23a), 0.93 (3H, t, *J* = 6.8 Hz, -OCH$_2$CH$_2$CH$_2$C*H*$_3$), 0.91 (3H, d, *J* = 6.7 Hz, H-21), 0.90 (3H, s, H-19), 0.63 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 174.4 (C-24), 71.6 (C-6), 68.1 (C-3), 64.1 (-O*C*H$_2$CH$_2$CH$_2$CH$_3$), 56.1, 55.9, 48.4, 42.8, 40.0, 39.8, 35.9, 35.5, 35.3, 35.0, 34.8, 31.3, 31.0, 30.7, 30.2, 29.2, 28.1, 24.2 (C-19), 23.5, 20.8, 19.2, 18.2 (C-21), 13.7 (-OCH$_2$CH$_2$CH$_2$*C*H$_3$), 12.0 (C-18). HRMS: [M + Na]$^+$ Calcd. $C_{28}H_{48}NaO_4$ 471.3450. Found: $C_{28}H_{48}NaO_4$ 471.3453.

***n*-Pentyl hyodeoxycholate (6d):** Yield 80% after two purifications by column chromatography; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.06 (2H, t, *J* = 6.8 Hz, OC*H*$_2$CH$_2$CH$_2$CH$_2$CH$_3$), 4.04 (1H, dt, *J* = 11.7, 6.8 Hz, H-6), 3.62 (1H, tt, *J* = 11.1, 6.5 Hz, H-3), 2.32 (1H, m, H-23b), 2.22 (1H, m, H-23a), 0.94 (3H, t, *J* = 7.4 Hz, -OCH$_2$CH$_2$CH$_2$CH$_2$C*H*$_3$), 0.90 (3H, d, *J* = 7.3 Hz, H-21), 0.89 (3H, s, H-19), 0.64 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 174.4 (C-24), 71.6 (C-6), 68.1 (C-3), 64.4 (-O*C*H$_2$CH$_2$CH$_2$CH$_2$CH$_3$), 56.1, 55.9, 48.4, 42.8, 39.9, 39.8, 35.9, 35.5, 35.3, 35.1, 34.8, 31.3, 31.0, 30.2, 29.2, 29.2, 28.4, 28.1, 24.2 (C-19), 23.5, 22.3, 20.8, 18.2 (C-21), 14.0 (-OCH$_2$CH$_2$CH$_2$CH$_2$*C*H$_3$), 12.0 (C-18). HRMS: [M + Na]$^+$ Calcd. $C_{29}H_{50}NaO_4$ 485.3607. Found: $C_{29}H_{50}NaO_4$ 485.3612.

***n*-Octyl hyodeoxycholate (6e):** Yield 78%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.07 (3H, m, H-6, -OC*H*$_2$(CH$_2$)$_6$CH$_3$), 3.64 (1H, tt, *J* = 11.1, 6.5 Hz, H-3), 2.34 (1H, m, H-23b), 2.22 (1H, m, H-23a), 0.92 (3H, d, *J* = 6.9 Hz, H-21), 0.91 (3H, s, H-19), 0.90 (3H, t, *J* = 6.9 Hz, -OCH$_2$(CH$_2$)$_6$C*H*$_3$), 0.65 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 174.4 (C-24), 71.6 (C-6), 68.1 (C-3), 64.4 (-O*C*H$_2$(CH$_2$)$_6$CH$_3$), 56.1, 55.9, 48.4, 42.8, 39.9, 39.8, 35.9, 35.5, 35.3, 35.1, 34.8, 31.8, 31.3, 31.0, 30.2, 29.2 (x3), 28.7, 28.1, 26.0, 24.2 (C-19), 23.5, 22.6, 20.8, 18.2 (C-21), 14.1 (-OCH$_2$(CH$_2$)$_6$*C*H$_3$), 12.0 (C-18). HRMS: [M + Na]$^+$ Calcd. $C_{32}H_{56}NaO_4$ 527.4076. Found: $C_{32}H_{56}NaO_4$ 527.4072.

***n*-Lauryl hyodeoxycholate (6f):** Yield 83%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.05 (3H, m, H-6, -OC*H*$_2$(CH$_2$)$_{10}$CH$_3$), 3.61 (1H, tt, *J* = 11.1, 6.5 Hz, H-3), 2.32 (1H, m, H-23b), 2.20 (1H, m, H-23a), 0.91 (3H, d, *J* = 6.8 Hz, H-21), 0.90 (3H, s, H-19), 0.87 (3H, t, *J* = 6.7 Hz, -OCH$_2$(CH$_2$)$_{10}$C*H*$_3$), 0.63 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 174.4 (C-24), 71.6 (C-6), 68.0 (C-3), 64.5 (-O*C*H$_2$(CH$_2$)$_{10}$CH$_3$), 56.1, 55.9, 48.4, 42.8, 39.9, 39.8, 35.9, 35.5, 35.3, 35.0, 34.9, 31.9, 31.3, 31.0, 30.2, 29.7 (x2), 29.6, 29.5, 29.4, 29.3, 29.1, 28.7, 28.1, 25.9, 24.2 (C-19), 23.5, 22.7, 20.8, 18.2 (C-21), 14.1 (-OCH$_2$(CH$_2$)$_{10}$*C*H$_3$), 12.0 (C-18). HRMS: [M + Na]$^+$ Calcd. $C_{36}H_{64}NaO_4$ 583.4702. Found: $C_{36}H_{64}NaO_4$ 583.4698.

***n*-Miristyl hyodeoxycholate (6g):** Yield 85%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.07 (3H, m, H-6, -OC*H*$_2$(CH$_2$)$_{12}$CH$_3$), 3.63 (1H, tt, *J* = 11.0, 6.2 Hz, H-3), 2.32 (1H, m, H-23b), 2.22 (1H, m, H-23a), 0.91 (3H, d, *J* = 6.7 Hz, H-21), 0.90 (3H, s, H-19), 0.87 (3H,

t, *J* = 6.5 Hz, -OCH$_2$(CH$_2$)$_{14}$C*H*$_3$), 0.63 (3H, s, H-18); ^{13}C NMR (CDCl$_3$, 500 MHz) δ 174.6 (C-24), 71.8 (C-6), 68.3 (C-3), 64.6 (-O*C*H$_2$(CH$_2$)$_{12}$CH$_3$), 56.3, 56.1, 48.5, 43.0, 40.1, 40.0, 36.1, 35.7, 35.5, 35.2, 35.0, 32.1, 31.5, 31.2, 30.4, 29.9, 29.8 (x2), 29.7 (x2), 29.6, 29.5, 29.4, 29.3, 28.8, 28.3, 26.1, 24.4 (C-19), 23.6, 22.9, 20.9, 18.4 (C-21), 14.3 (-OCH$_2$(CH$_2$)$_{12}$*C*H$_3$), 12.2 (C-18). HRMS: [M + Na]$^+$ Calcd. C$_{38}$H$_{68}$NaO$_4$ 611.5015. Found: C$_{38}$H$_{68}$NaO$_4$ 611.5011.

***n*-Palmityl hyodeoxycholate (6h):** Yield 82%; colorless oil; ^{1}H NMR (CDCl$_3$, 500 MHz) δ 4.07 (3H, m, H-6, -OC*H*$_2$(CH$_2$)$_{14}$CH$_3$), 3.64 (1H, tt, *J* = 11.1, 6.5 Hz, H-3), 2.34 (1H, m, H-23b), 2.21 (1H, m, H-23a), 0.94 (3H, d, *J* = 6.9 Hz, H-21), 0.92 (3H, s, H-19), 0.89 (3H, t, *J* = 6.7 Hz, -OCH$_2$(CH$_2$)$_{14}$C*H*$_3$), 0.65 (3H, s, H-18); ^{13}C NMR (CDCl$_3$, 500 MHz) δ 174.4 (C-24), 71.6 (C-6), 68.1 (C-3), 64.5 (-O*C*H$_2$(CH$_2$)$_{14}$CH$_3$), 56.1, 55.9, 48.4, 42.8, 39.9, 39.8, 36.0, 35.5, 35.3, 35.1, 34.8, 31.9, 31.3, 31.0, 30.3, 29.7 (x5), 29.6 (x2), 29.5, 29.4, 29.3, 29.2, 28.7, 28.1, 26.0, 24.2 (C-19), 23.5, 22.7, 20.7, 18.2 (C-21), 14.1 (-OCH$_2$(CH$_2$)$_{14}$*C*H$_3$), 12.0 (C-18). HRMS: [M + Na]$^+$ Calcd. C$_{40}$H$_{72}$NaO$_4$ 639.5328. Found: C$_{40}$H$_{72}$NaO$_4$ 639.5331.

***n*-Stearyl hyodeoxycholate (6i):** Yield 80%; colorless oil; ^{1}H NMR (CDCl$_3$, 500 MHz) δ 4.05 (3H, m, H-6, -OC*H*$_2$(CH$_2$)$_{16}$CH$_3$), 3.64 (1H, tt, *J* = 11.1, 6.5 Hz, H-3), 2.32 (1H, m, H-23b), 2.21 (1H, m, H-23a), 0.93 (3H, d, *J* = 6.9 Hz, H-21), 0.91 (3H, s, H-19), 0.87 (3H, t, *J* = 6.7 Hz, -OCH$_2$(CH$_2$)$_{16}$C*H*$_3$), 0.65 (3H, s, H-18); ^{13}C NMR (CDCl$_3$, 500 MHz) δ 174.4 (C-24), 71.6 (C-6), 68.1 (C-3), 64.5 (-O*C*H$_2$(CH$_2$)$_{16}$CH$_3$), 56.2, 56.0, 48.4, 42.9, 40.0, 39.9, 36.0, 35.6, 35.4, 35.1, 34.9, 32.0, 31.4, 31.1, 30.3, 29.8, 29.7 (x4), 29.6 (x4), 29.5, 29.4, 29.3, 29.2, 28.7, 28.2, 26.0, 24.2 (C-19), 23.5, 22.7, 20.8, 18.2 (C-21), 14.2 (-OCH$_2$(CH$_2$)$_{16}$*C*H$_3$), 12.1 (C-18). HRMS: [M + Na]$^+$ Calcd. C$_{42}$H$_{76}$NaO$_4$ 667.5641. Found: C$_{42}$H$_{76}$NaO$_4$ 667.5637.

***n*-Benzyl hyodeoxycholate (6j):** Yield 92%; colorless oil; ^{1}H NMR (CDCl$_3$, 500 MHz) δ 7.35 (5H, s, - CH$_2$*Ph*), 5.11 (2H, s, -C*H*$_2$Ph), 4.05 (1H, dt, *J* = 12.0, 4.8 Hz, H-6), 3.62 (1H, tt, *J* = 11.4, 6.7 Hz, H-3), 2.38 (1H, m, H-23b), 2.27 (1H, m, H-23a), 0.90 (6H, t, *J* = 6.9 Hz, H-19, H-21), 0.61 (3H, s, H-18); ^{13}C NMR (CDCl$_3$, 500 MHz) δ 174.2 (C-24), 136.3 (Ph-C1), 128.7 (Ph, C2,C2'), 128.4 (Ph, C3,C3'), 128.3 (Ph, C4), 71.8 (C-6), 68.3

(C-3), 66.3 (-O*C*H$_2$Ph), 56.3, 56.1, 48.6, 43.0, 40.1, 40.0, 36.1, 35.7, 35.5, 35.2, 35.0, 31.4, 31.1, 30.4, 29.4, 28.3, 24.4 (C-19), 23.6, 20.9, 18.4 (C-21), 12.2 (C-18). HRMS: $[M + Na]^+$ Calcd. $C_{31}H_{46}NaO_4$ 505.3294. Found: $C_{31}H_{46}NaO_4$ 505.3289.

3α-Acetoxy hyodeoxycholic acid (7): Yield 91% after two purifications by column chromatography; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.71 (1H, tt, *J* = 11.2, 6.6 Hz, H-3), 4.07 (1H, dt, *J* = 11.6, 4.7 Hz, H-6), 2.37 (1H, m, H-23b), 2.25 (1H, m, H-23a), 2.02 (3H, s, -COC*H*$_3$), 0.93 (3H, d, *J* = 6.3 Hz, H-21), 0.91 (3H, s, H-19), 0.64 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 179.3 (C-24), 170.6 (-*C*OCH$_3$), 74.1 (C-6), 67.9 (C-3), 56.1, 55.9, 48.2, 42.8, 39.9, 39.8, 35.9, 35.3, 35.2, 34.7 (x2), 30.9, 30.8, 28.1, 26.5, 25.3, 24.1 (C-19), 23.4, 21.4 (-CO*C*H$_3$), 20.8, 18.2 (C-21), 12.0 (C-18). HRMS: $[M + Na]^+$ Calcd. $C_{26}H_{42}NaO_5$ 457.2930. Found: $C_{26}H_{42}NaO_5$ 457.2925.

Ethyl 3α-Acetoxy hyodeoxycholate (8): Yield 88% after two purifications by column chromatography; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.73 (1H, tt, *J* = 11.2, 6.5 Hz, H-3), 4.14 (2H, q, *J* = 6.7 Hz, -OC*H*$_2$CH$_3$), 4.07 (1H, dt, *J* = 11.3, 4.7 Hz, H-6), 2.34 (1H, m, H-23b), 2.21 (1H, m, H-23a), 2.04 (3H, s, -COC*H*$_3$), 1.27 (3H, t, *J* = 6.7 Hz, -OCH$_2$C*H*$_3$), 0.93 (3H, d, *J* = 6.3 Hz, H-21), 0.93 (3H, s, H-19), 0.65 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 174.3 (C-24), 170.5 (-*C*OCH$_3$), 74.1 (C-6), 67.9 (C-3), 60.2 (-O*C*H$_2$CH$_3$), 56.1, 56.0, 48.3, 42.8, 39.9, 39.8, 36.0, 35.3, 35.2, 34.8, 34.7, 31.3, 31.0, 28.1, 26.5, 25.3, 24.2 (C-19), 23.4, 21.4 (-CO*C*H$_3$), 20.7, 18.3 (C-21), 14.3 (-OCH$_2$*C*H$_3$), 12.0 (C-18). HRMS: $[M + Na]^+$ Calcd. $C_{28}H_{46}NaO_5$ 485.3243. Found: $C_{28}H_{46}NaO_5$ 485.3238.

3α,6α-Diacetoxy hyodeoxycholic acid (9a): Yield 89%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.14 (1H, dt, *J* = 11.6, 4.7 Hz, H-6), 4.70 (1H, tt, *J* = 11.3, 6.4 Hz, H-3), 2.38 (1H, m, H-23b), 2.25 (1H, m, H-23a), 2.03 (3H, s, -COC*H*$_3$), 2.01 (3H, s, -COC*H*$_3$), 0.97 (3H, s, H-19), 0.92 (3H, d, *J* = 6.6 Hz, H-21), 0.64 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 179.5 (C-24), 170.5 (-*C*OCH$_3$), 170.5 (-*C*OCH$_3$), 73.7 (C-6), 71.0 (C-3), 56.1, 55.9, 45.3, 42.9, 39.8 (x2), 36.0, 35.2, 35.0, 34.6, 31.3, 30.9, 30.7, 28.1, 26.4, 26.2, 24.1 (C-19), 23.3, 21.4 (-CO*C*H$_3$), 21.4 (-CO*C*H$_3$), 20.7, 18.2 (C-21), 12.0 (C-18). HRMS: $[M + Na]^+$ Calcd. $C_{28}H_{44}NaO_6$ 499.3036. Found: $C_{28}H_{44}NaO_6$ 499.3039.

Ethyl 3α,6α-Diacetoxy hyodeoxycholate (9b): Yield 85%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.15 (1H, dt, *J* = 12.2, 4.8 Hz, H-6), 4.71 (1H, tt, *J* = 11.4, 6.6 Hz, H-3), 4.13 (2H, q, *J* = 6.7 Hz, -OC*H*$_2$CH$_3$), 2.34 (1H, m, H-23b), 2.23 (1H, m, H-23a), 2.05 (3H, s, -COC*H*$_3$), 2.03 (3H, s, -COC*H*$_3$), 1.27 (3H, t, *J* = 7.0 Hz, -OCH$_2$C*H*$_3$), 0.98 (3H, s, H-19), 0.93 (3H, d, *J* = 6.5 Hz, H-21), 0.65 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 174.3 (C-24), 170.5 (-*C*OCH$_3$), 170.5 (-*C*OCH$_3$), 73.7 (C-6), 71.0 (C-3), 60.2 (-O*C*H$_2$CH$_3$), 56.2, 56.0, 45.4, 42.9, 39.9, 36.1, 35.3, 35.0, 34.6, 31.3 (x2), 30.9, 29.7, 28.1, 26.4, 26.2, 24.1 (C-19), 23.3, 21.4 (-CO*C*H$_3$), 21.4 (-CO*C*H$_3$), 20.7, 18.3 (C-21), 14.3 (-OCH$_2$*C*H$_3$), 12.0 (C-18). HRMS: $[M + Na]^+$ Calcd. $C_{30}H_{48}NaO_6$ 527.3349. Found: $C_{30}H_{48}NaO_6$ 527.3352.

Ethyl 6α-Acetoxy hyodeoxycholate (10): Yield 83% after two purifications by column chromatography; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.13 (1H, dt, *J* = 11.6, 4.8 Hz, H-6), 4.12 (2H, q, *J* = 6.8 Hz, -OC*H*$_2$CH$_3$), 3.60 (1H, tt, *J* = 11.2, 6.5 Hz, H-3), 2.32 (1H, m, H-23b), 2.21 (1H, m, H-23a), 2.02 (3H, s, -COC*H*$_3$), 1.26 (3H, t, *J* = 6.8 Hz, -OCH$_2$C*H*$_3$),), 0.96 (3H, s, H-19), 0.92 (3H, d, *J* = 6.5 Hz, H-21), 0.64 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 174.3 (C-24), 170.5 (-*C*OCH$_3$), 71.3 (C-3, C-6), 60.2 (-O*C*H$_2$CH$_3$), 56.1, 55.9, 45.3, 42.9, 39.9 (x2), 36.1, 35.3 (x2), 34.7, 31.3 (x2), 30.9, 30.3, 30.1, 28.1, 24.1 (C-19), 23.3, 21.4 (-CO*C*H$_3$), 20.7, 18.3 (C-21), 14.3 (-OCH$_2$*C*H$_3$), 12.0 (C-18). HRMS: $[M + Na]^+$ Calcd. $C_{28}H_{46}NaO_5$ 485.3243. Found: $C_{28}H_{46}NaO_5$ 485.3246.

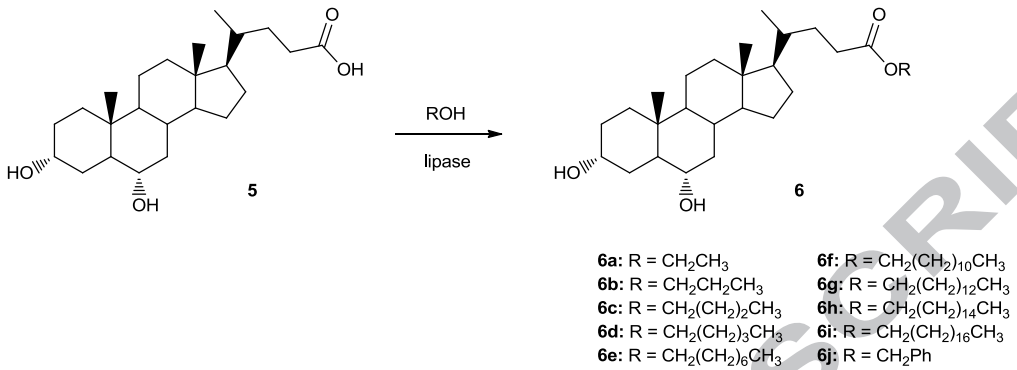
3. Results and Discussions

3.1. Enzymatic Esterification

According to literature, only methyl and ethyl esters of hyodeoxycholic acid have been described so far, which were prepared using methanol or ethanol and sulphuric acid [39, 40].

Searching for a mild alternative procedure for obtaining different esters of HDCA, we performed the lipase-catalyzed esterification of this acid, using different alcohols (Scheme 2). In order to optimize the reaction conditions we made several experiments, including a lipase screening and variation of other reaction parameters such as temperature, solvent,

enzyme:substrate ratio (E/S) and alcohol:substrate ratio (Nu/S) using ethanol as model nucleophile.



Scheme 2. Enzymatic synthesis of hyodeoxycholic esters.

In the beginning, we studied the behavior of lipases from several sources in the reaction of **5** with ethanol: from the yeasts *Candida rugosa* (CRL) and *Candida antarctica B* (CAL B), Lipozyme from the fungus *Rhizomucor miehei* (LIP) and *Thermomyces lanuginosus* (TLIM). Also from plants: the naturally immobilized *Carica papaya* lipase (CPL), which is the remaining solid fraction of papaya latex after washing off proteases.

In lipase-catalyzed reactions it is complicated to predict the effect of solvent because it depends on the substrate used [21, 24]. Therefore to choose an appropriate solvent, screening experiments are made. The solvents evaluated were hexane, diisopropyl ether (DIPE), *t*-butanol and acetone. In addition, the reaction using ethanol both as nucleophile and solvent was also performed. Taking into account the optimal reaction conditions for similar transformations, the reactions were carried out at 55 °C using E/S: 10 and nucleophile:substrate ratio Nu/S: 10 [36, 37].

Among the enzymes evaluated, LIP and CAL B were the best lipases toward the esterification of HDCA. With CRL, CPL, TLIM or in the absence of biocatalyst no product was obtained. In terms of conversion, both LIP and CAL B proved to be more efficient than the other evaluated lipases, being equally efficacious affording **6a** (100% conversion) at 72 h of reaction. But taking into account that LIP is significantly cheaper than CAL B, LIP was the enzyme of choice. Both lipases were more active in DIPE than in the other solvents tested, as shown in the Figure 1.

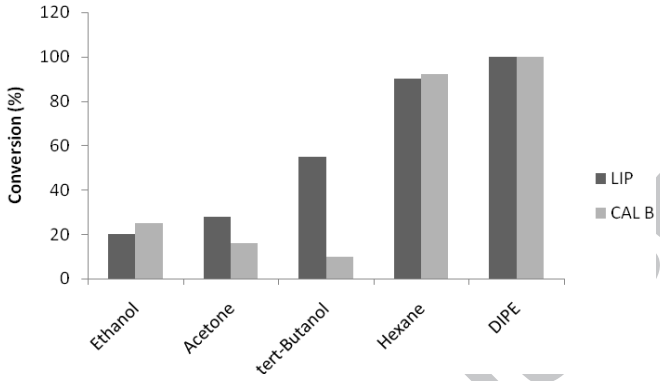


Figure 1. Effect of solvent for esterification of HDCA (**5**) with the most effective lipases evaluated.

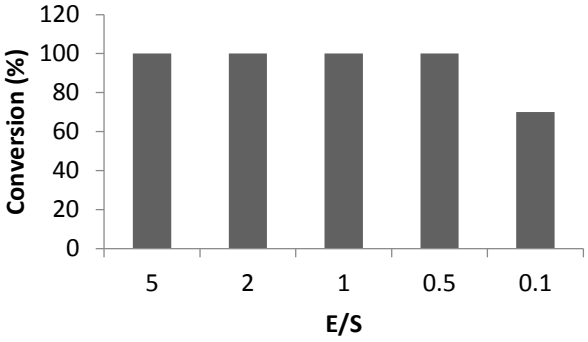


Figure 2. Result from the E/S screening experiments showing optimal ratio for LIP.

Then, the influence of E/S in the enzymatic esterification was evaluated using Nu/S: 10, DIPE as solvent at 55 °C and variable amounts of LIP (Figure 2). From the obtained results, it can be seen that 100% conversion was achieved even at E/S = 0.5 after 72 hs of reaction. LIP showed a superb efficiency for this reaction, since this value of E/S is very low in comparison with previous work [36, 37]. Considering the results schematized in the figure 2, E/S = 0.5 was the ratio of choice.

The influence of Nu/S on esterification yield was also evaluated at 72 h in DIPE using LIP (E/S 0.5) at 55 °C. We have observed that an equimolar ratio was enough to achieve the

best results. Lastly, in order to analyze the influence of temperature, the reaction was carried out at 25 °C, 40 °C and 55 ºC, keeping the rest of the reaction parameters at their optimal values (LIP, DIPE, E/S: 0.5 and Nu/S: 1). Maximum conversion was reached at 55 °C after 72 h. At lower temperatures a decrease in conversion percentages was observed at the same reaction time. In addition, methyl *t*-butyl ether (MTBE) was used as reaction solvent at 25 °C and 40 °C. However, yields were lower than those with DIPE at 55 °C. Therefore, 55 °C was selected as the optimum temperature.

Based on these results, the optimal conditions for the enzymatic esterification of hyodeoxycholic acid are: LIP as biocatalyst, DIPE as solvent, temperature: 55 °C, E/S: 0.5 and Nu/S: 1.0.

It is interesting to note that towards biocatalytic esterification, HDCA behaves differently in comparison to other dihydroxylated bile acids previously studied, whereas its reactivity is similar to cholic acid (CA) [36, 37]. In the case of chenodeoxycholic acid (CDCA) and deoxycholic acid (DCA), CAL B was the best biocatalyst and the conditions involved E/S: 10, Nu/S: 5, hexane as solvent and a temperature of 55 °C. On the other hand, the optimal conditions for esterification of CA were LIP as biocatalyst, temperature: 40 °C, E/S: 10, Nu/S: 20 and DIPE as solvent.

Table 1. Enzymatic synthesis of hyodeoxycholic esters.

Entry	Nucleophile	Product	Yield (%)
1	CH_3CH_2OH	**6a**	95
2	$CH_3CH_2CH_2OH$	**6b**	90
3	$CH_3(CH_2)_2CH_2OH$	**6c**	82
4	$CH_3(CH_2)_3CH_2OH$	**6d**	80
5	$CH_3(CH_2)_6CH_2OH$	**6e**	78
6	$CH_3(CH_2)_{10}CH_2OH$	**6f**	83
7	$CH_3(CH_2)_{12}CH_2OH$	**6g**	85
8	$CH_3(CH_2)_{14}CH_2OH$	**6h**	82
9	$CH_3(CH_2)_{16}CH_2OH$	**6i**	80
10	$PhCH_2OH$	**6j**	92

Reaction conditions: Enzyme: LIP; Solvent: DIPE; Temperature: 55 °C; E/S: 0.5 Nu/S: 1

Once the experimental conditions were optimized, we applied them to the esterification of **5** with a variety of alcohols to afford products **6a-j**. The results, expressed as yield of isolated product, are summarized in Table 1.

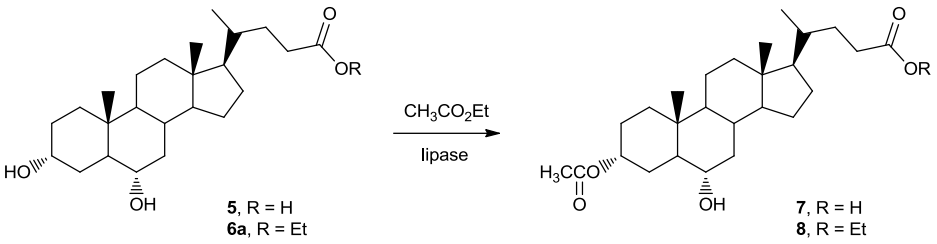
Alkyl esters containing linear alkyl chains from two to eighteen carbon atoms were synthesized in very good to excellent yield (78-95 %). A relation was not observed between yield and alkyl chain length as in the case of cholic acid. Every product was completely characterized by spectroscopic methods and seven of them (**6b-6h**) were not previously described in literature. The use of an equivalent amount of alcohol makes this procedure even more advantageous, especially in the case of longer-chain derivatives in terms of product isolation, process simplicity and economy.

3.2. Enzymatic Acetylation

To the best of our knowledge, monoacetyl derivatives of hyodeoxycholic acid have not been described so far. Similarly to other bile acids, bisacetylation of HDCA was carried out at room temperature in acetic anhydride, DMAP and pyridine.

Recently, we have reported the synthesis of 3α-acetyl derivatives of chenodeoxycholic and deoxycholic acid using CAL B as biocatalyst. Under the same reaction conditions, with lithocholic acid as substrate, the lipase catalyzed both the acetylation and esterification reactions affording ethyl lithocholate 3α-acetate as the only product in one pot-two steps [36]. In addition, it was not possible to acetylate cholic acid in a regioselective way, chemically or enzymatically. However, the ethyl ester of cholic acid was enzymatically acetylated on a regioselective way affording 3α-acetyl derivative [37].

Therefore, we sought a regioselective enzymatic acetylation of hyodeoxycholic acid (Scheme 3). We began by applying the optimal conditions determined for the acetylation of CDCA and DCA: CAL B as biocatalyst, ethyl acetate as acylating agent, E/S: 5, A/S: 5 in hexane at 55 °C. In addition, we carried out the reaction using ethyl acetate both as solvent and acylating agent. The 3α-acetyl derivative (**7**) was obtained in both cases in excellent yield after 1 h of reaction. The lipase showed a fully regioselective behavior, reacting only with the hydroxyl moiety on carbon 3, leaving the one on carbon 6 unscathed under the evaluated conditions.



Scheme 3. Enzymatic synthesis of acetyl derivatives of HDCA (**5**) and ethyl ester (**6a**).

Taking into account that 100% of conversion was observed for the HDCA under the conditions of acetylation for the CDCA, we carried out the reaction with lower E/S ratios: the acetylation finished after 3 h using a E/S ratio of 1, and finished after 24 h with a E/S ratio of 0.5. The low amount of enzyme necessary for catalyzing the acetylation reaction confers an additional advantage to this enzymatic methodology.

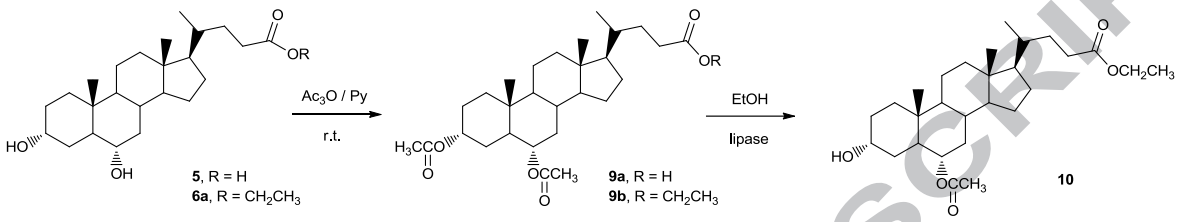
The same conditions (CAL B, ethyl acetate, hexane, E/S: 0.5, A/S: 5, 55 °C) were used to carry out acetylation of ethyl ester **6a**. Again, we observed acetylation of hydroxyl group on 3-position, obtaining the corresponding ethyl ester 3-acetate (**8**) after 8 h of reaction. The acetylation of HDCA was also tested in the presence of LIP as biocatalyst but no conversion was observed after 24 h of reaction.

It is also important to note the significant difference in the reaction time of acetylation of HDCA compared to that for CDCA and DCA. While the compound **7** was obtained after 1 h of reaction, the 3-acetate derivatives of the other bile acids were obtained in 48 h. If the reaction mixture is left stirring five days, esterification also occurs, obtaining ethyl 3-acetyl hyodeoxycholate (**8**). These results suggest that it is not possible to acetylate the 6-position applying this methodology and, on the other hand, the ethanol generated in the reaction was capable to esterify the carboxylic acid moiety given enough time. We had already observed the same behavior in the case of CAL B catalyzed acetylation of lithocholic acid (LCA) [36].

On the other hand, it is important to remark the considerable difference observed in the reaction times for acetylation (1 h) compared to esterification (72 h) of HDCA.

3.3. Enzymatic Alcoholysis

In order to obtain the 6-acetyl derivative of HDCA we carried out bisacetylation of both HDCA and its ethyl ester, followed by enzymatic alcoholysis. Products of peracetylacion, **9a** and **9b**, were obtained respectively in 89 % and 85% yield, treating **5** and **6a** with acetic anhydride and pyridine at room temperature during 16 h (Scheme 4).



Scheme 4. Chemical synthesis and enzymatic alcoholysis of peracetylated derivatives of HDCA (**5**) and the ethyl ester (**6a**).

We carried out the enzymatic alcoholysis of **9a** and **9b** applying the optimal conditions determined for alcoholysis of peracetylated chenodeoxycholic acid: CAL B as biocatalyst (E/S: 5) ethanol as nucleophile (Nu/S: 1.2), hexane as solvent, temperature: 55 °C [36]. For both substrates the used lipase was regioselective, reacting only at 3-position. Using these conditions, we obtained ethyl 6-acetyl hyodeoxycholate (**10**) from **9b** in excellent yield (89%) after 2 h of reaction (Scheme 3). As observed in previous work [36, 37] , starting from **9a**, the alcoholysis reaction also afforded **10** (92% yield), using ethanol as both alcoholysis and esterifying agent. In this case, at shorter reaction times it was observed the formation of a compound in very low proportion, which quickly becomes the reaction product **10**. Probably this intermediate compound corresponds to the 6-acetyl hyodeoxycholic acid, which is immediately esterified. In both cases CAL B behaved regioselectively, as in acetylation reactions, providing an efficient alternative to obtain the derivative without altering the 6-position, agreeing with previous work of our laboratory [26, 36, 41, 42].

4. Conclusions

This work describes the synthesis of hyodeoxycholic acid derivatives, most of them new compounds in a regioselective way, using enzymes as catalysts. By this methodology, we were able to accurately control the reaction, obtaining very good conversions rapidly and, in most cases, only one product. Furthermore, the catalyst is removed by a mere filtration step and can be reused, retaining excellent activity after five reaction cycles.

Amongst the various reaction parameters analyzed for each reaction, we tested different lipases, obtaining the best results with both *Candida antarctica* B and Lipozyme from *Rhizomucor miehei* for esterification reactions. Given that LIP is cheaper than CAL B, and thus considering the overall reaction applicability we bent towards the use of LIP to esterify this bile acid.

For both acetylation and alcoholysis the enzyme of choice was CAL B, since LIP showed no activity for those reactions.

To sum up, not only we present the synthesis of several new compounds from a substrate that has been avoided for many years, but also we make it in a simple, environmentally friendly way and the reactions are efficiently performed at mild temperature and are purified easily.

Supporting Information. Spectral data for compounds **6-10** associated with this article are supplied.

Acknowledgments

We thank UBA (UBACYT 20020130100212) and CONICET (PIP 11220110100653) for partial financial support. G.G.L. and A.B. are Research Members of CONICET (Argentina).

References

[1] K.D. Setchell, D. Kritchevsky, P.P. Nair, The Bile Acids: Chemistry, Physiology, and Metabolism: Volume 4: Methods and Applications, Springer Science & Business Media2012.

[2] H.E. Karpen, S.J. Karpen, Bile Acid Metabolism During Development, Fetal and Neonatal Physiology (Fifth Edition), Elsevier2017, pp. 913-929. e4.
[3] A.F. Hofmann, L.R. Hagey, Key discoveries in bile acid chemistry and biology and their clinical applications: history of the last eight decades, Journal of lipid research 55(8) (2014) 1553-1595.
[4] J.J. Feher, Quantitative human physiology: an introduction, Academic press2017.
[5] D. Yu, D.L. Mattern, B.M. Forman, An improved synthesis of 6α-ethylchenodeoxycholic acid (6ECDCA), a potent and selective agonist for the Farnesoid X Receptor (FXR), Steroids 77(13) (2012) 1335-1338.
[6] S. Bansal, M. Singh, S. Kidwai, P. Bhargava, A. Singh, V. Sreekanth, R. Singh, A. Bajaj, Bile acid amphiphiles with tunable head groups as highly selective antitubercular agents, MedChemComm 5(11) (2014) 1761-1768.
[7] D. Brossard, L. El Kihel, M. Clément, W. Sebbahi, M. Khalid, C. Roussakis, S. Rault, Synthesis of bile acid derivatives and in vitro cytotoxic activity with pro-apoptotic process on multiple myeloma (KMS-11), glioblastoma multiforme (GBM), and colonic carcinoma (HCT-116) human cell lines, European journal of medicinal chemistry 45(7) (2010) 2912-2918.
[8] R.M. Gadaleta, O. Garcia-Irigoyen, A. Moschetta, Bile acids and colon cancer: Is FXR the solution of the conundrum?, Molecular Aspects of Medicine 56(Supplement C) (2017) 66-74.
[9] T. Li, J.Y. Chiang, Bile acids as metabolic regulators, Current opinion in gastroenterology 31(2) (2015) 159.
[10] N. Hassan, A. Ahad, M. Ali, J. Ali, Chemical permeation enhancers for transbuccal drug delivery, Expert opinion on drug delivery 7(1) (2010) 97-112.
[11] M. Stojančević, N. Pavlović, S. Goločorbin-Kon, M. Mikov, Application of bile acids in drug formulation and delivery, Frontiers in Life Science 7(3-4) (2013) 112-122.
[12] J. Jampilek, K. Brychtova, Azone analogues: classification, design, and transdermal penetration principles, Medicinal research reviews 32(5) (2012) 907-947.
[13] M. Cirri, F. Maestrelli, N. Mennini, P. Mura, Combined use of bile acids and aminoacids to improve permeation properties of acyclovir, International Journal of Pharmaceutics 490(1) (2015) 351-359.

[14] L. Yang, J.P. Fawcett, J. Østergaard, H. Zhang, I.G. Tucker, Mechanistic studies of the effect of bile salts on rhodamine 123 uptake into RBE4 cells, Molecular pharmaceutics 9(1) (2011) 29-36.

[15] L. Yang, H. Zhang, J.P. Fawcett, M. Mikov, I.G. Tucker, Effect of bile salts on the transport of morphine-6-glucuronide in rat brain endothelial cells, Journal of pharmaceutical sciences 100(4) (2011) 1516-1524.

[16] D.M. Shih, Z. Shaposhnik, Y. Meng, M. Rosales, X. Wang, J. Wu, B. Ratiner, F. Zadini, G. Zadini, A.J. Lusis, Hyodeoxycholic acid improves HDL function and inhibits atherosclerotic lesion formation in LDLR-knockout mice, The FASEB Journal 27(9) (2013) 3805-3817.

[17] K. Buchholz, V. Kasche, U.T. Bornscheuer, Biocatalysts and enzyme technology, John Wiley & Sons2012.

[18] K. Faber, Biotransformations in organic chemistry: a textbook, Springer Science & Business Media2011.

[19] J. Whitthall, P. Sutton, Practical Methods for Biocatalysis and Biotransformations 2, John Wiley & Sons Ltd., New York, 2012.

[20] J.A. Tao, G.-Q. Lin, A. Liese, Biocatalysis for the pharmaceutical industry: discovery, development, and manufacturing, John Wiley & Sons2009.

[21] G. Carrea, S. Riva, Organic synthesis with enzymes in non-aqueous media, John Wiley & Sons2008.

[22] A. Baldessari, L.E. Iglesias, Lipases in green chemistry: acylation and alcoholysis on steroids and nucleosides, Lipases and Phospholipases: Methods and Protocols (2012) 457-469.

[23] M. Hall, W. Kroutil, K. Faber, The evolving role of biocatalysis in asymmetric synthesis, Asymmetric Synthesis II: More Methods and Applications (2013) 221-231.

[24] V. Gotor, I. Alfonso, E. García-Urdiales, Asymmetric organic synthesis with enzymes, John Wiley & Sons2008.

[25] L.N. Monsalve, G. Petroselli, R. Erra-Ballsells, A. Vázquez, A. Baldessari, Chemoenzymatic synthesis of novel N-(2-hydroxyethyl)-β-peptoid oligomer derivatives and application to porous polycaprolactone films, Polymer International 63(8) (2014) 1523-1530.

[26] L.N. Monsalve, M.Y. Machado Rada, A.A. Ghini, A. Baldessari, An efficient enzymatic preparation of 20-pregnane succinates: chemoenzymatic synthesis of 20β-hemisuccinyloxy-5αH-pregnan-3-one, Tetrahedron 64(8) (2008) 1721-1730.
[27] P.G. Quintana, A. Baldessari, Lipase-catalyzed regioselective preparation of fatty acid esters of hydrocortisone, Steroids 74(13) (2009) 1007-1014.
[28] P.G. Quintana, A. Canet, M. Marciello, F. Valero, J.M. Palomo, A. Baldessari, Enzyme-catalyzed preparation of chenodeoxycholic esters by an immobilized heterologous Rhizopus oryzae lipase, Journal of Molecular Catalysis B: Enzymatic 118 (2015) 36-42.
[29] P.G. Quintana, G. García Liñares, S.N. Chanquia, R.M. Gorojod, M.L. Kotler, A. Baldessari, Improved Enzymatic Procedure for the Synthesis of Anandamide and N-Fatty Acylalkanolamine Analogues: A Combination Strategy to Antitumor Activity, European Journal of Organic Chemistry 2016(3) (2016) 518-528.
[30] P.G. Quintana, M. Guillén, M. Marciello, F. Valero, J.M. Palomo, A. Baldessari, Immobilized heterologous Rhizopus oryzae lipase as an efficient catalyst in the acetylation of cortexolone, European Journal of Organic Chemistry 2012(23) (2012) 4306-4312.
[31] E.M. Rustoy, A. Baldessari, An efficient chemoenzymatic synthesis of the bactericide lapyrium chloride, European journal of organic chemistry 2005(21) (2005) 4628-4632.
[32] E.M. Rustoy, Y. Sato, H. Nonami, R. Erra-Balsells, A. Baldessari, Lipase-catalyzed synthesis and characterization of copolymers from ethyl acrylate as the only monomer starting material, Polymer 48(6) (2007) 1517-1525.
[33] G. García Liñares, P. Arroyo Mañez, A. Baldessari, Lipase-Catalyzed Synthesis of Substituted Phenylacetamides: Hammett Analysis and Computational Study of the Enzymatic Aminolysis, European Journal of Organic Chemistry 2014(29) (2014) 6439-6450.
[34] G. García Liñares, G. Parraud, C. Labriola, A. Baldessari, Chemoenzymatic synthesis and biological evaluation of 2- and 3-hydroxypyridine derivatives against Leishmania mexicana, Bioorganic & Medicinal Chemistry 20(15) (2012) 4614-4624.
[35] S.N. Chanquia, N. Boscaro, L. Alche, A. Baldessari, G.G. Liñares, An Efficient Lipase-Catalyzed Synthesis of Fatty Acid Derivatives of Vanillylamine with Antiherpetic Activity in Acyclovir-Resistant Strains, ChemistrySelect 2(4) (2017) 1537-1543.

[36] G. García Liñares, M. Antonela Zígolo, L. Simonetti, S.A. Longhi, A. Baldessari, Enzymatic synthesis of bile acid derivatives and biological evaluation against Trypanosoma cruzi, Bioorganic & Medicinal Chemistry 23(15) (2015) 4804-4814.
[37] M.A. Zígolo, G. García Liñares, A. Baldessari, New cholic acid derivatives: Biocatalytic synthesis and molecular docking study, Steroids 107 (2016) 10-19.
[38] C. Tecelão, I. Rivera, G. Sandoval, S. Ferreira-Dias, Carica papaya latex: A low-cost biocatalyst for human milk fat substitutes production, European journal of lipid science and technology 114(3) (2012) 266-276.
[39] G. Haslewood, Comparative studies ofbile salts'. 7. Bile acids of the coypu, Myocastor coypus, Biochemical Journal 56(4) (1954) 581.
[40] G. Haslewood, Comparative studies ofbile salts'. 9. The isolation and chemistry of hyocholic acid, Biochemical Journal 62(4) (1956) 637.
[41] A. Baldessari, Lipases as catalysts in synthesis of fine chemicals, Lipases and Phospholipases: Methods and Protocols (2012) 445-456.
[42] A. Baldessari, A.C. Bruttomesso, E.G. Gros, Lipase-Catalysed Regioselective Deacetylation of androstane derivatives, Helvetica chimica acta 79(4) (1996) 999-1004.

Highlights

- Hyodeoxycholic acid derivatives were prepared by an enzymatic approach in a regioselective way.
- The influence of several parameters was considered.
- The lipase catalysis was a very efficient procedure, especially considering the low amount used.

Figures and schemes captions

Scheme 1. Structure of important bile acids.

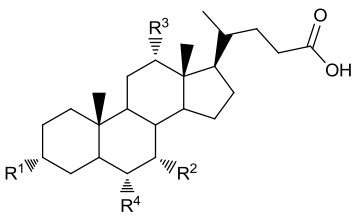
Scheme 2. Enzymatic synthesis of hyodeoxycholic alkyl esters.

Figure 1. Effect of solvent for esterification of HDCA (**1**) with the most effective lipases evaluated.

Figure 2. Results from the E/S screening experiments, showing optimal relation for LIP.

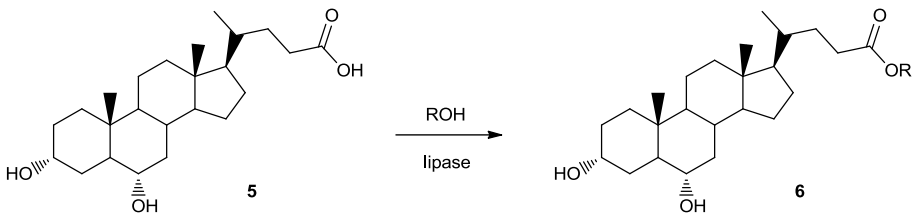
Scheme 3. Enzymatic synthesis of acetyl derivatives of HDCA (**1**) and ethyl ester (**2a**).

Scheme 4. Chemical synthesis and enzymatic alcoholysis of peracetylated derivatives of HDCA (**1**) and the ethyl ester (**2a**).



CA 1: R^1 = OH, R^2 = OH, R^3 = OH, R^4 = H
CDCA 2: R^1 = OH, R^2 = OH, R^3 = H, R^4 = H
DCA 3: R^1 = OH, R^2 = H, R^3 = OH, R^4 = H
LCA 4: R^1 = OH, R^2 = H, R^3 = H, R^4 = H
HDCA 5: R^1 = OH, R^2 = H, R^3 = H, R^4 =O H

Scheme 1



6a: R = CH_2CH_3
6b: R = $CH_2CH_2CH_3$
6c: R = $CH_2(CH_2)_2CH_3$
6d: R = $CH_2(CH_2)_3CH_3$
6e: R = $CH_2(CH_2)_6CH_3$
6f: R = $CH_2(CH_2)_{10}CH_3$
6g: R = $CH_2(CH_2)_{12}CH_3$
6h: R = $CH_2(CH_2)_{14}CH_3$
6i: R = $CH_2(CH_2)_{16}CH_3$
6j: R = CH_2Ph

Scheme 2

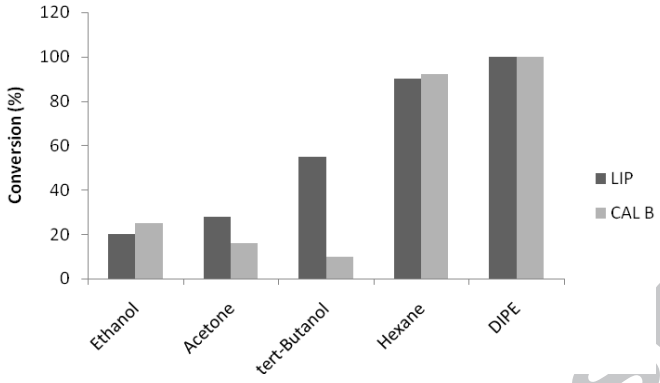


Figure 1

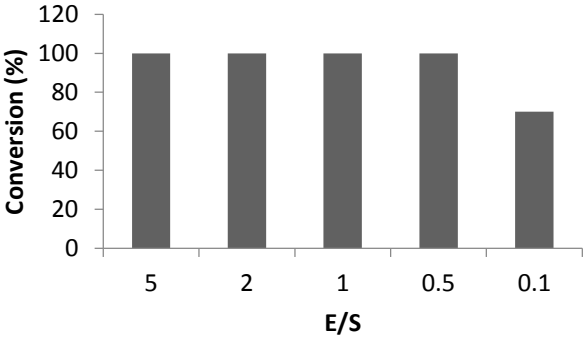
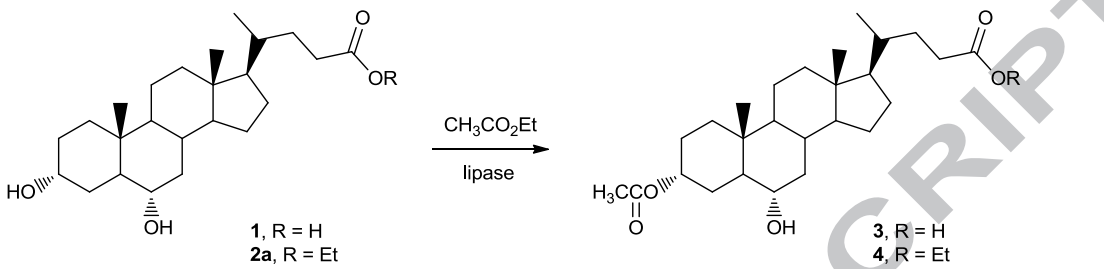
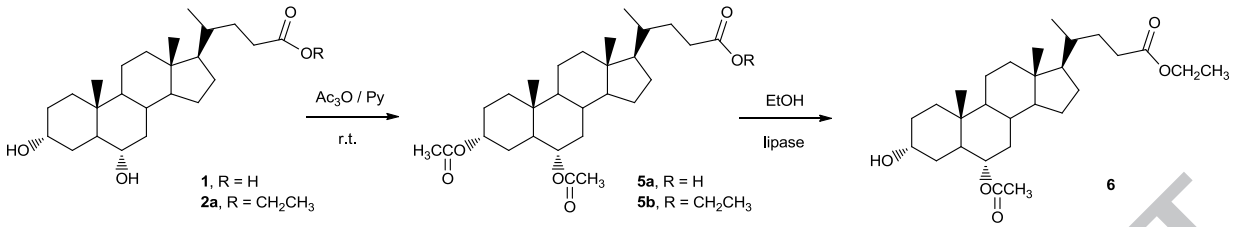


Figure 2



Scheme 2



Scheme 3

Table 1. Enzymatic synthesis of hyodeoxycholic esters.

Entry	Nucleophile	Product	Yield (%)
1	CH_3CH_2OH	**6a**	95
2	$CH_3CH_2CH_2OH$	**6b**	90
3	$CH_3(CH_2)_2CH_2OH$	**6c**	82
4	$CH_3(CH_2)_3CH_2OH$	**6d**	80
5	$CH_3(CH_2)_6CH_2OH$	**6e**	78
6	$CH_3(CH_2)_{10}CH_2OH$	**6f**	83

7	$CH_3(CH_2)_{12}CH_2OH$	**6g**	85
8	$CH_3(CH_2)_{14}CH_2OH$	**6h**	82
9	$CH_3(CH_2)_{16}CH_2OH$	**6i**	80
10	$PhCH_2OH$	**6j**	92

Reaction conditions: Enzyme: LIP; Solvent: DIPE; Temperature: 55 °C; E/S: 0.5 Nu/S: 1