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MICROWAVE SYNTHESIS OF NOVEL CHENODEOXYCHOLIC ACID ESTERS AND COMPARATIVE STUDY OF CHROMATOGRAPHIC BEHAVIOR AND LIPOPHILICITY

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In this study, eco-friendly microwave-assisted esterification reactions of chenodeoxycholic acid with medium-chain diols (1,2-ethanediol, 1,4-butanediol, 1,6-hexanediol, 1,8-octanediol, and 1,10-decanediol) or tetraethylene glycol were accomplished. The synthesized bile acid esters were obtained with high yields (up to 81 %) and purity in a short reaction time (15 min). It is shown that the microwave technique is a suitable method for the preparation of chenodeoxycholic acid esters. In order to obtain detailed insight into the lipophilic behavior of the studied chenodeoxycholic acid esters, a comparative study of chromatographic behavior and lipophilicity was performed. Also, the present study deals with the estimation of pH – log*D* profiles in order to get an overview of the changes in lipophilicity related to the pH value changes. The obtained results indicate that studied compounds have log*D* values in the range acceptable for potential drug candidates. Chromatographic lipophilicity of synthesized chenodeoxycholic acid esters successfully correlate with *in silico* lipophilicity descriptors.

Keywords: chenodeoxycholic acid esters; diols; lipophilicity; liquid chromatography; microwaves

МИКРОБРАНОВА СИНТЕЗА НА НОВИ ЕСТЕРИ НА ХЕНОДЕОКСИХОЛНА КИСЕЛИНА И КОМПАРАТИВНА СТУДИЈА НА ХРОМАТОГРАФСКОТО ОДНЕСУВАЊЕ И ЛИПОФИЛНОСТА

Во оваа студија, со помош на микробранова печка се извршени еколошки реакции на естерификација на хенодеоксихолна киселина со диоли со средни низи (1,2-етандиол, 1,4-бутандиол, 1,6-хександиол, 1,8-октандиол и 1,10-деканедиол) или со тетраетилен гликол. Синтетизираните естери на жолчни киселини се добиени со високи приноси (до 81%) и чистота за кратко време на реакција (15 min). Се покажа дека техниката на микробранова печка е соодветен метод за подготовка на естери на хенодеоксихолна киселина. Со цел да се добие детален увид во липофилното однесување на испитуваните естери на хенодеоксихолната киселина, направено е компаративно проучување на хроматографското однесување и липофилноста. Исто така, во оваа студија е направена процена на профилите на pH – log*D* со цел да се добие преглед на промените во липофилноста поврзани со промените на вредноста на pH. Добиените резултати покажуваат дека проучуваните соединенија имаат вредности на log*D* во опсег прифатлив за потенцијалните корисници на лекови. Хроматографската липофилност на синтетизираните естери на хенодеоксихолната киселина успешно корелира со дескрипторите на липофилноста добиени со компјутерски симулации.

Клучни зборови: естери на хенодеоксихолна киселина; диоли; липофилност; течна хроматографија; микробранови

1. INTRODUCTION

Bile acids are widely used in pharmaceutical chemistry, and play a great role as intermediates in the synthesis of biologically active compounds or in the formation of a new potential drug.[1–5] Due to their amphiphilic character, they increase cell membrane permeability and enhance the absorption of hydrophobic drugs.[6] Furthermore, it has been reported that synthetic bile acid derivatives induced apoptosis in several human cancer cells.[7–10]

Somewhat surprisingly, there are only a few reports that have shown the synthesis and biologically active significance of bile acids esters.[11–15] Hu *et al*. described the synthesis of the ester of cholic acid with ethylene glycol by conventional method,[16] while the ester of chenodeoxycholic acid with the same diol was reported in 2007 by Gauthier.[17] Gauthier *et al*. revealed greater cytotoxicity in ethylene glycol esters of bile acids versus their free acid analogues owing to their greater hydrophobicity.[12] The ethylene glycol ester of lithocholic acid had unexpectedly low water–octanol partition coefficients (logK_{OW}) and high IC_{50} when compared with the other bile acid esters. The existence of a relationship between the nature of the group on position 24 and cytotoxicity has been previously shown in the literature.[13] Kuhajda *et al*.[14] reported the synthesis and cytotoxic activity of bile acid esters. Within these studies, the ethyl ester of 12-ketocholic acid showed very strong antiproliferative activity against particular tumor cell lines. Additionally, the importance of esters is reflected in their application as essential intermediates in organic synthesis yielding a great number of bile acid derivatives.[14,15]

During the past decade, microwave-assisted chemistry has emerged as a very efficient and powerful tool to heat reaction mixtures in dedicated sealed reaction vessels. In such reactors, remarkable rate enhancements have been observed along with cleaner reactions, with easier workup compared to conventional heating methods. Due to the unique nature of irradiation, microwaves (MW) significantly support organic synthesis and, at the same time, have found application in medicinal chemistry. Recently many laboratories, including our own, have described microwave-assisted reactions of bile acids.[15,18–20] Synthesis of bile acid derivatives proceeded in a highly-accelerated manner and the yields and purity of the final products were excellent. These results encouraged us to move forward in the synthesis of potentially biologically active bile acid esters with a slightly longer carbon chain, in contrast to common bile acid esters.

High-performance liquid chromatography (HPLC) is often used to determine the lipophilicity of newly-synthesized biologically active and drug-like compounds.[21–25] On the other hand, different *in silico* lipophilicity descriptors are widely used for the prediction of chromatographic behavior and biological and physicochemical characteristics of various molecules with biomedical importance.[26–29] In order to facilitate the discovery of new potential drugs, many drug-likeness filters were developed involving lipophilicity compounds.[30] The passive transport through biological membranes is driven by lipophilicity, so it represents a crucial characteristic when it comes to pharmacological behavior and drug activity. When considering *in silico* lipophilicity descriptors, they are often used as elimination factors for the design and synthesis of new drug-like compounds. According to *in silico* lipophilicity descriptors, molecules of interest can be distinguished and chosen for further *in vitro* and *in vivo* trials.

In the present paper, we want to report the microwave-assisted esterification reactions of chenodeoxycholic acid, using a catalytic quantity of hydrochloric acid in medium-chain diols (1,2-ethanediol (ethylene glycol), 1,4-butanediol, 1,6-hexanediol, 1,8-octanediol, 1,10-decanediol, or tetraethylene glycol). To the best of our knowledge, this type of bile acid ester has not been synthesized so far, except compound **3** (Scheme 1), which we synthesized earlier in a preliminary experiment,[20] and here we optimized synthesis to get a higher yield. An ethylene glycol-based derivative, synthesized earlier under conventional conditions,[17] was the compound which we used for optimizing the reaction conditions for MW-assisted syntheses. Based on the biomedical importance of the selected novel chenodeoxycholic acid esters, the chromatographic lipophilicity of six compounds was examined under different chromatographic conditions. Their pH–log*D* profiles were also studied with the aim to reveal the changes in lipophilicity related to the pH value changes.

2. EXPERIMENTAL

2.1. *Chromatographic instrumentation and chemicals*

For the chromatographic analysis Agilent Technologies 1200 Series HPLC system (Santa Clara, California, USA) with evaporative light scattering detector (ELSD) was used. HPLC system contained binary pump, degasser and automatic injector connected to a computer for data pro-

cessing with AgilentChemStation program. Chromatographic column ZORBAX SB C-18, (3 × 250 mm, 5 μm) (Santa Clara, California, USA) was used as the stationary phase. 2-Propanol used for dissolving, and methanol and acetonitrile for the mobile phase were all HPLC grade, purchased from J. T. Baker (Deventer, The Netherlands).

2.2. *Chromatographic procedure*

All studied compounds were dissolved in 2-propanol at a concentration of 1 mg/ml and filtered throughout Captiva Econofilter (PTFE syringe filters, 25 mm diameter, 0.45 μm pore size) (Santa Clara, California, USA). Binary mixtures of methanol/acetonitrile and 2-propanol were used as a mobile phase, with methanol/acetonitrile volume fractions in the range of 70 – 95 v/v. Isocratic elutions were performed at the flow rate and injection of 0.8 ml/min and 10 μl, respectively. The column temperature was kept at 30 °C. ELSD was kept at 30 °C and 3.6 bar. Retention data were expressed as the logarithm of retention factor (log*k*) values, defined by the following equation, and were used for chromatographic lipophilicity modeling:

$$\log k = \log\left(\frac{t_r - t_0}{t_0}\right) \quad (1)$$

where t_r is the retention time of a compound and t_0 the dead time (the retention time of the first peak on the chromatogram). All analyses were done in triplicate.

2.3. *In silico lipophilicity and distribution coefficient modeling*

Prior to *in silico* lipophilicity descriptor calculation, molecular structure design was done using 2D structure MarvinSketch 17.2.3 (Chem Axon) and for 3D structure ChemBio3D Ultra 12.0.[31] For the calculation of different log*P* and log*D* values, four programs were used: ChemBioDraw Ultra 12.0,[31] ChemBio3D Ultra 12.0,[31] ALOGPS 2.1,[32] and MarvinSketch 17.2.3.[31] All *in silico* lipophilicity descriptors were calculated on the basis of 2D structures, therefore the structural optimization and energy minimization were not required.

The calculation of distribution coefficients of the studied chenodeoxycholic acid esters were carried out on the basis of 2D molecular structures using MarvinSketch 17.2.3[33] and the weighted method. The tautomerization and resonance in a molecule were considered. The electrolyte (Na^+, K^+ and Cl^-) concentrations were set at 0.1 mol/l.

2.4. *Synthetic procedures*

All reagents and solvents were obtained from commercial suppliers and used without further purification. Microwave-assisted reactions were carried out in a CEM Discover BenchMate single-mode microwave reactor (300 W max magnetron power output) in 10 ml sealed process Pyrex vials with magnetic stirring. The microwave-assisted reaction time was the hold time at the designated temperature. Reaction temperatures were monitored by an external infrared (IR) sensor. Reaction cooling was performed by compressed air automatically after the heating period had elapsed. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (Silica gel 60 F254). Purification of products was carried out by flash column chromatography using Kieselgel 60 (0.040–0.063 mm, Merck). NMR Spectra were recorded on a Bruker AC 250 E (250 MHz ^{1}H, 62.9 MHz ^{13}C) instrument and chemical shifts are expressed as ppm downfield from TMS using $CDCl_3$ as solvent. The letters s, d, t, q and m are used to indicate singlet, doublet, triplet, quadruplet and multiples. High resolution mass spectroscopy (HRMS) spectra were recorded on a 6210 Time-of-Flight LC/MS Agilent Technologies (ESI+) instrument. All organic extracts were dried with anhydrous Na_2SO_4. Organic solutions were concentrated in a rotary evaporator under reduced pressure at a bath temperature above 30 °C.

2.4.1. *2'-Hydroxyethyl 3α,7α-dihydroxy-5β-cholan-24-oate* (**1**)

Method A. Starting compound 3α,7α-dihydroxy-5β-cholanic acid (128 mg, 0.326 mmol) was dissolved in ethylene glycol (2 ml) and then cc HCl (0.01 ml) was added. The reaction mixture was heated at 80 °C for 2 hours. The resulting reaction mixture was poured into water and extracted with ethyl acetate (3 × 15 ml). Combined organic extracts were washed with 5 % Na_2CO_3 (2 × 10 ml), then water (2 × 10 ml), and dried. The resulting crude product was purified by flash column chromatography (7 g silica gel, toluene ethyl acetate = 1:5), affording pure compound **1** as white crystals (35 mg, 4 2 %).

Method B. 3α,7α-Dihydroxy-5β-cholanic acid (132 mg, 0.336 mmol), ethylene glycol (2 ml) and cc HCl (0.01 ml) were placed into a 10 ml microwave process vial equipped with a magnetic stir bar. The reaction mixture was heated in a micro-

wave reactor at 100 °C for 15 min. After the reaction time elapsed, the mixture was cooled by gas jet cooling, poured into water, and extracted with ethyl acetate (3 × 15 ml). Combined organic extracts were washed with 5 % Na_2CO_3 (2 × 10 ml), then water (2 × 10 ml), and dried. After removing the solvent *in vacuo*, the residue was purified by flash column chromatography (7 g silica gel, chloroform : acetone = 2:3), affording pure compound **1** as white crystals (32 mg, 72 %); mp: 140–141 °C; ^{1}H NMR ($CDCl_3$, δ, ppm): 0.65 (s, 3H, 18-CH_3), 0.90 (s, 3H, 19-CH_3), 0.93 (d, 3H, 21-CH_3), 3.44 (m, 1H, 3β-CH), 3.83 (m, 3H, 7β-CH, $COOCH_2C\underline{H}_2OH$), 4.20 (t, 2H, $COOC\underline{H}_2CH_2OH$); ^{13}C NMR ($CDCl_3$, δ, ppm) 11.74 (C-18), 18.30 (C-19), 60.93 ($C\underline{H}_2OH$), 65.89 (-$COOC\underline{H}_2$), 68.46 (C-7), 71.9 (C-3), 174.80 (C-24); HRMS: calculated for $C_{26}H_{44}O_5$ $[M+Na]^+$: 459.30810; found: 459.30811.

2.4.2. *General procedure for preparation of compounds* **2**, **3**, **4**, **5** *and* **6**

3α,7α-Dihydroxy-5β-cholanic acid (1 eq) and the appropriate diol (1,4-butanediol, 1,6-hexanediol, 1,8-octanediol, 1,10-decanediol or tetraethylene glycol) (100 eq) and cc HCl (0.03 ml) were added to a 10 ml microwave process vial equipped with a magnetic stir bar. The reaction mixture was irradiated in a microwave reactor for 15 min at 100 °C. Compressed air was used to quickly cool down the sample after the heating was completed. The reaction mixture was poured into water and extracted with ethyl acetate (3 × 15 ml). Combined organic extracts were washed with 5% Na_2CO_3 (2 × 10 ml), then water (2 × 10 ml), and dried. The resulting crude products were purified by flash column chromatography (8 g silica gel, petroleum ether : acetone = 7:3) to obtain pure compounds **2**, **3**, **4**, **5,** or **6,** respectively.

4'-Hydroxybutyl 3α,7α-dihydroxy-5β-cholan-24-oate (**2**)

Compound **2** was obtained as white crystals (81%); mp: 106 – 107 °C; ^{1}H NMR ($CDCl_3$, δ, ppm): 0.69 (s, 3H, 18-CH_3), 0.80 (s, 3H, 19-CH_3), 0.94 (d, *J*=6.4 Hz, 3H, 21-CH_3), 3.48 (m, 1H, 3β-CH), 3.69 (t, 2H, $COOCH_2(CH_2)_2C\underline{H}_2OH$), 3.87 (s, 1H, 7β-CH), 4.12 (t, 2H, $COOC\underline{H}_2CH_2CH_2CH_2OH$). ^{13}C NMR ($CDCl_3$, δ, ppm): 11.77 (C-18), 18.28 (C-19), 62.37 ($C\underline{H}_2OH$), 64.10 (-$COOC\underline{H}_2$), 68.56 (C-7), 72.04 (C-3), 174.44 (C-24). HRMS: calculated for $C_{28}H_{48}O_5$ $[M+Na]^+$: 487.33940; found: 487.33928.

6'-Hydroxyhexyl 3α,7α-dihydroxy-5β-cholan-24-oate (**3**)

Compound **3** was obtained as a yellow oil (60 %); ^{1}H NMR ($CDCl_3$, δ, ppm): 0.68 (s, 3H, 18-CH_3), 0.95 (s, 3H, 19-CH_3), 0.97 (d, J = 6.4 Hz, 3H, 21-CH_3), 3.47 (m, 1H, 3β-CH), 3.66 (t, 2H, $COOCH_2(CH_2)_4C\underline{H}_2OH$), 3.86 (s, 1H, 7β-CH), 4.08 (t, 2H, $COOC\underline{H}_2(CH_2)_4CH_2OH$); ^{13}C NMR ($CDCl_3$, δ, ppm): 11.78 (C-18), 18.28 (C-19), 62.81 ($C\underline{H}_2OH$), 64.27 (-$COOC\underline{H}_2$), 68.53 (C-7), 72.01 (C-3), 174.46 (C-24); HRMS: calculated for $C_{30}H_{52}O_5$ $[M+Na]^+$: 515.37070; found: 515.37106.

8'-Hydroxyoctyl 3α,7α-dihydroxy-5β-cholan-24-oate (**4**)

Compound **4** was obtained as a yellow oil (44 %). ^{1}H NMR ($CDCl_3$, δ, ppm): 0.67 (s, 3H, 18-CH_3), 0.92 (s, 3H, 19-CH_3), 0.94 (d, *J* = 6.4 Hz, 3H, 21-CH_3), 3.48 (m, 1H, 3β-CH), 3.65 (t, *J* = 6.4 Hz, 2H, $COOCH_2(CH_2)_6C\underline{H}_2OH$), 3.86 (s, 1H, 7β-CH), 4.07 (t, *J* = 6.8 Hz, 2H, $COOC\underline{H}_2(CH_2)_6CH_2OH$). ^{13}C NMR ($CDCl_3$, δ, ppm): 11.78 (C-18), 18.27 (C-19), 63.00 ($C\underline{H}_2OH$), 64.40 (-$COOC\underline{H}_2$), 68.54 (C-7), 72.03 (C-3), 174.44 (C-24). HRMS: calculated for $C_{32}H_{56}O_5$ $[M+Na]^+$: 543.40200; found: 543.40072.

10'-Hydroxydecyl 3α,7α-dihydroxy-5β-cholan-24-oate (**5**)

Compound **5** was obtained as a yellow oil (37 %). ^{1}H NMR ($CDCl_3$, δ, ppm): 0.67 (s, 3H, 18-CH_3), 0.92 (s, 3H, 19-CH_3), 0.94 (d, *J* = 6.4 Hz, 3H, 21-CH_3), 3.48 (m, 1H, 3β-CH), 3.65 (t, *J* = 6.8 Hz, 2H, $COOCH_2(CH_2)_8C\underline{H}_2OH$), 3.86 (s, 1H, 7β-CH), 4.07 (t, *J* = 6.8 Hz, 2H, $COOC\underline{H}_2(CH_2)_8CH_2OH$). ^{13}C NMR ($CDCl_3$, δ, ppm): 11.77 (C-18), 18.27 (C-19), 63.03 ($C\underline{H}_2OH$), 64.43 (-$COOC\underline{H}_2$), 68.53 (C-7), 72.02 (C-3), 174.44 (C-24). HRMS: calculated for $C_{34}H_{60}O_5$ $[M+Na]^+$: 571.43330; found: 571.43449.

12'-Hydroxy-1,4,7,10-tetraoxadodecyl 3α, 7α-dihydroxy-5β-cholan-24-oate (**6**)

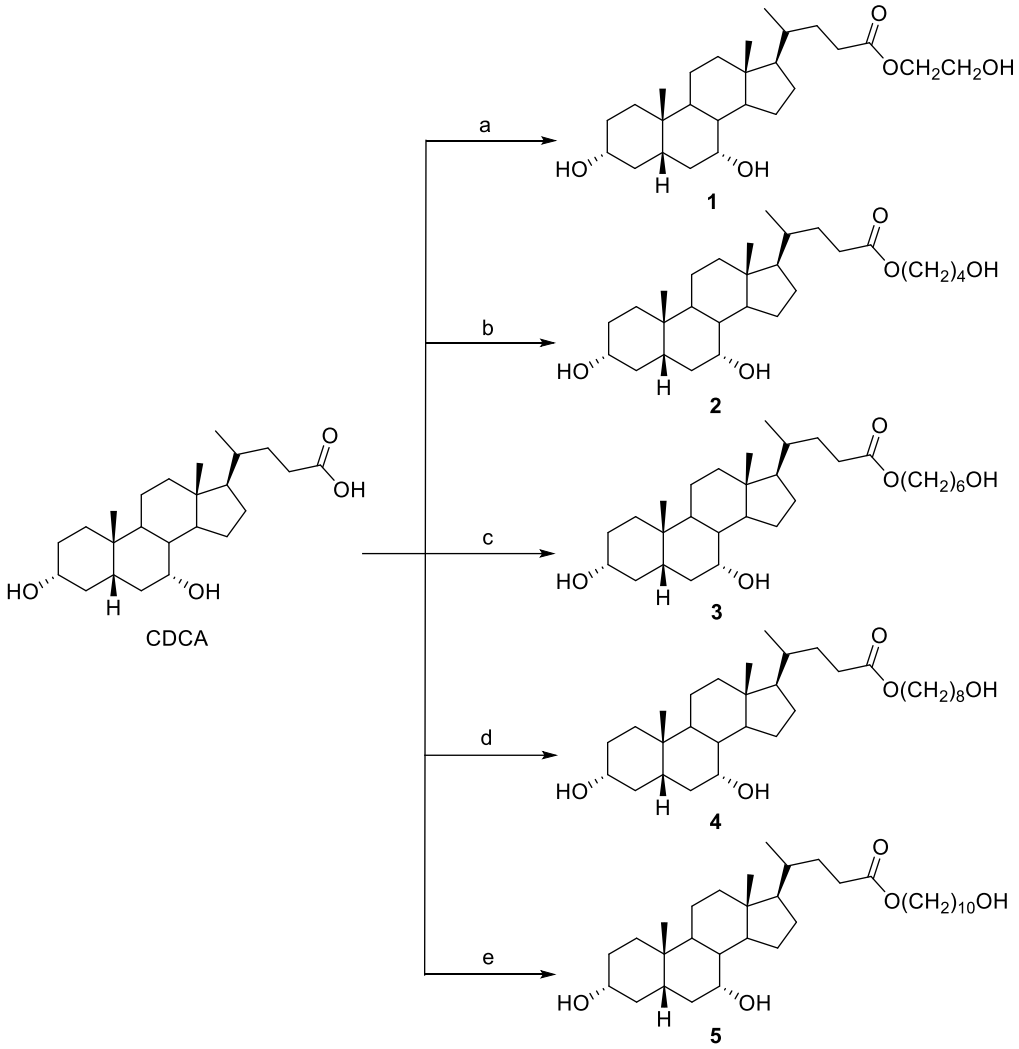
Compound **6** was obtained as a yellow oil (31 %). ^{1}H NMR ($CDCl_3$, δ, ppm): 0.67 (s, 3H, 18-CH_3), 0.92 (s, 3H, 19-CH_3), 0.94 (d, *J* = 6.4 Hz, 3H, 21-CH_3), 3.47 (m, 1H, 3β-CH), 3.63 (t, *J* = 6.8 Hz, 2H, $COO(CH_2CH_2O)_3CH_2C\underline{H}_2OH$), 3.68-3.74 (group of signals, 12H, CH_2), 3.87 (d, *J* = 2.4 Hz, 1H, 7β-CH), 4.25 (q, 2H, $COOC\underline{H}_2CH_2(OCH_2CH_2)_3OH$). ^{13}C NMR ($CDCl_3$, δ, ppm): 11.78 (C-18), 18.28 (C-19), 61.76 ($C\underline{H}_2OH$), 63.39 (-$COOC\underline{H}_2$), 68.53 (C-7), 72.01 (C-3), 174.30 (C-24). HRMS: calcu-

lated for $C_{34}H_{60}O_5$ $[M+Na]^+$: 591.38674; found: 591.38666.

3. RESULTS AND DISCUSSION

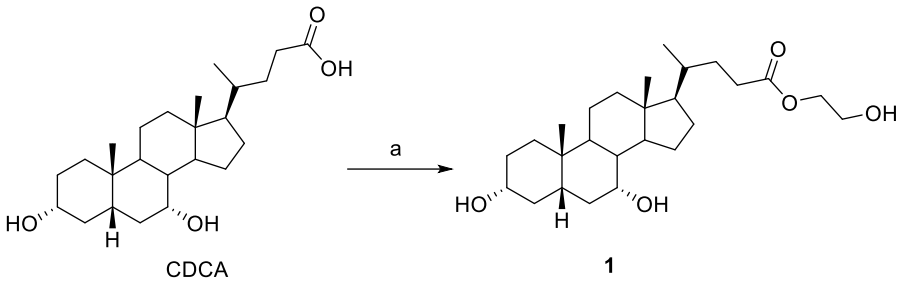
3.1. *Chemical synthesis*

Chenodeoxycholic acid (CDCA), one of the primary bile acids, with two hydroxyl groups at positions 3α and 7α, was used as a compound to study the efficiency of MW-assisted esterification with different diols. To introduce green chemistry concepts and decrease production of hazardous waste, esterification of chenodeoxycholic acid was carried out applying a catalytic quantity of hydrochloric acid in medium-chain diols (1,2-ethanediol (ethylene glycol), 1,4-butanediol, 1,6-hexanediol, 1,8-octanediol, or 1,10-decanediol). In temperature-controlled mode of the MW reactor, chenodeoxycholic acid esters (**1**, **2**, **3**, **4** and **5**) were synthesized with high yields and purity in very short reaction times at 100 °C (Scheme 1).



Scheme 1. Reagents and conditions: MW, 100 °C, 15 min, cc HCl, (a) $HO(CH_2)_2OH$, yield 72 %; (b) $OH(CH_2)_4OH$, yield 81 %; (c) $OH(CH_2)_6OH$, yield 60 %; (d) $OH(CH_2)_8OH$, yield 44 %; (e) $OH(CH_2)_{10}OH$, yield 37%

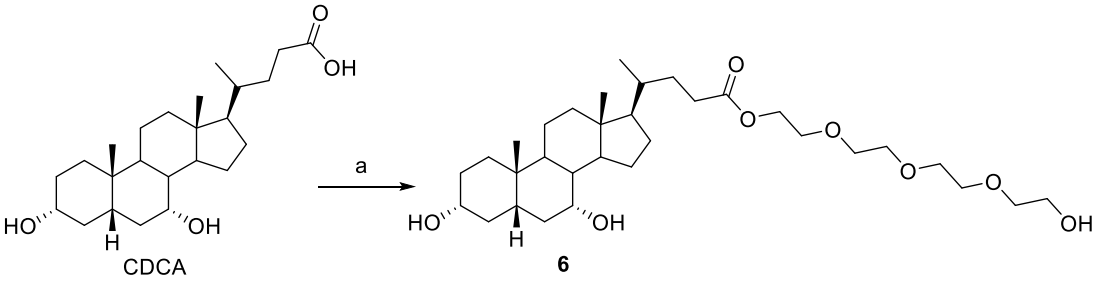
In order to obtain reference data for the microwave-assisted experiments, synthesis of 2-hydroxyethyl 3α,7α-dihydroxy-5β-cholan-24-oate **1** was carried out by acid-catalyzed esterification and conventional heating. Scheme 1 shows that synthesis of compound **1** in a closed vessel system of a MW reactor was significantly shorter compared to results obtained by conventional methods (Scheme 2).



Scheme 2. Reagents and conditions: (a) $HOCH_2CH_2OH$, cc HCl, 80 °C, 2h, 42 %

Microwave versus traditional heating reactions of chenodeoxycholic acid esterification with ethylene glycol revealed a significant jump in the yield of the desired products. It should be emphasized that extended reaction time does not change the final composition of the products. Furthermore, higher reaction temperatures appeared inappropriate since they led to the formation of byproducts and caused further decomposition. The closed vessel system of microwave synthesis was chosen, mainly with the aim of reaching the reaction temperatures in a very short time and to drive the equilibrium towards ester formation.

Satisfactory yields were also obtained in the microwave-assisted synthesis of compound **6** (Scheme 3). These results have indicated that faster derivatization of the bile acid under microwave irradiation is a consequence of the reaction temperatures that can be rapidly attained in a sealed vessel and to the more efficient heat transfer to the reaction mixture by applying direct core microwave dielectric heating.



Scheme 3. Reagents and conditions: (a) MW, $HO(CH_2CH_2O)_4H$, cc HCl, 100 °C, 15 min, yield 31 %

3.2. *Chromatographic retention of studied compounds*

One of the most used bonded phases is octadecyl (C18), which represents silica gel that is modified with long hydrocarbon chains with 18 carbon atoms. This kind of structure makes it possible to have a stationary phase that is less polar than the mobile phase. It is well known that strong interactions occur between the polar mobile phase and polar molecules. Since polar molecules travel faster through the column, they have shorten retention time than non-polar molecules. Non-polar molecules form attractions with hydrocarbon groups on the basis of Van der Waals dispersion forces and they are being retained longer on the column (higher retention). Generally, as methanol is a more polar solvent than acetonitrile, it will travel faster through the column and have lower retention times in the methanol-2-propanol system. Since chenodeoxycholic acid esters are not very polar, it could be assumed that the interactions between them and an acetonitrile-2-propanol mobile phase would be stronger than the interactions between them and a mobile phase containing methanol-2-propanol. Therefore, the retention of the studied chenodeoxycholic acid esters in the system with methanol-2-propanol was lower than the retention in the system with acetonitrile-2-propanol. Retention data for both chromatographic systems were expressed as the logarithm of the retention factor (log*k*) and results are shown in Table 1 and Table 2, as well as graphically presented in Supplementary Data Figure S1. It can be noticed that for both systems log*k* values are higher in chromatographic systems with higher volume fraction of methanol/acetonitrile. Hence, the highest log*k* values for the methanol-2-propanol system were obtained when methanol 95 v/v was used; similar to the acetonitrile-2-propanol system when acetoni-

trile 95 v/v was used. Generally speaking, it can be noticed that as the methanol/acetonitrile volume fraction decreases the retention time also decreases. With respect to the polarity, all investigated compounds behaved in accordance with their functional groups, considering that compounds **1** and **2** have the least polar functional groups, while compound **6** has the most polar functional group in its structure. All of this is in accordance with chromatographic theory.

T a b l e 1

Logarithm of the retention factor (logk) for studied chenodeoxycholic acid esters (methanol-2-propanol ratio)

Compound	log*k* (± SD)			
	70:30	80:20	90:10	95:5
1	–2.695 (± 0.001)	–1.553 (± 0.001)	–1.260 (± 0.000)	–1.380 (± 0.001)
2	–2.131 (± 0.001)	–1.486 (± 0.001)	–1.342 (± 0.002)	–1.456 (± 0.001)
3	–1.539 (± 0.004)	–1.247 (± 0.000)	–1.082 (± 0.005)	–1.043 (± 0.000)
4	–1.214 (± 0.003)	–0.972 (± 0.001)	–0.768 (± 0.002)	–0.758 (± 0.003)
5	–0.955 (± 0.002)	–0.760 (± 0.005)	–0.536 (± 0.001)	–0.502 (± 0.006)
6	–0.751 (± 0.001)	–0.573 (± 0.003)	–0.338 (± 0.001)	–0.290 (± 0.002)

T a b l e 2

Logarithm of the retention factor (logk) for studied chenodeoxycholic acid esters (acetonitrile-2-propanol ratio)

Compound	log*k* (± SD)			
	70:30	80:20	90:10	95:5
1	–0.687 (± 0.001)	–0.553 (± 0.001)	–0.331 (± 0.000)	–0.182 (± 0.001)
2	–0.630 (± 0.001)	–0.556 (± 0.001)	–0.401 (± 0.000)	–0.339 (± 0.001)
3	–0.494 (± 0.004)	–0.397 (± 0.000)	–0.233 (± 0.005)	–0.149 (± 0.004)
4	–0.382 (± 0.000)	–0.269 (± 0.001)	–0.098 (± 0.001)	–0.012 (± 0.003)
5	–0.250 (± 0.002)	–0.121 (± 0.003)	0.060 (± 0.001)	0.148 (± 0.003)
6	–0.113 (± 0.001)	0.037 (± 0.002)	0.231 (± 0.003)	0.327 0.002)

3.3. *Distribution coefficients of studied compounds*

Since the log*D* values can significantly vary in a certain pH range, it is necessary to emphasize at what pH value the log*D* parameter was calculated. The purpose of pH – log*D* profiles is to evaluate how stable the log*D* values in the pH range between 0 and 14 are, and how the changes in pH can affect the distribution of ionizable compounds between the water and organic phase. In the case of the chenodeoxycholic acid esters, it can be concluded that these compounds have similar pH – log*D* profiles (regarding trends) and log*D* does not significantly change within the pH range (Fig. 1). Presented profiles indicate that compounds **1** and **2** have almost the same pH–log*D* profiles.

If observed from the domain of oral administration, the compound has to pass through different gastrointestinal tract regions: saliva (pH = 6.4), stomach (1 < pH < 3), duodenum and jejunum (4.4 < pH < 6.6), and ileum (6.8 < pH < 8).[34] According to the presented pH–log*D* profiles, since the log*D* values do not vary significantly, it follows that the absorption in the gastrointestinal tract will not vary in different regions.

The intravenous route of administration brings the compound into the domain of a pH around 7.4. Values of log$D_{7.4}$ of the examined chenodeoxycholic acid esters fell in the range between 3 and 5 (excluding compounds **5** and **6**), which indicated good permeability, but lower absorption due to lower solubility.[34] Compounds **5** and **6** have log$D_{7.4}$ values higher than 5, which imply that these compounds tend to have low bioavailability and absorption due to low solubility, as well as high metabolic clearance.[34]

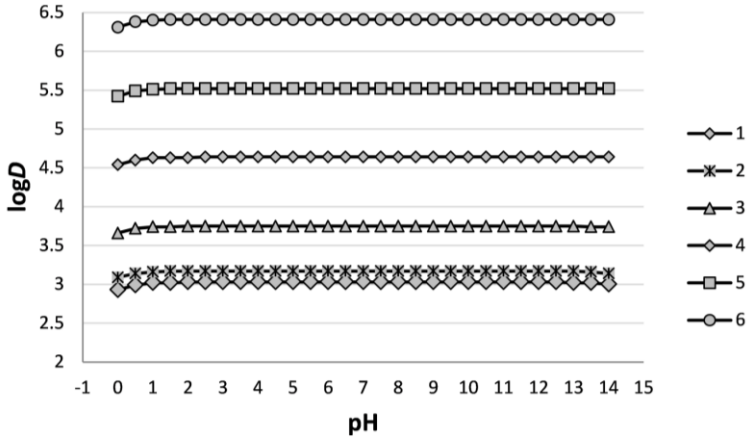


Fig. 1. pH–log*D* profiles of the studied chenodeoxycholic acid esters

3.4. *Lipophilicity of studied compounds*

Computationally calculated lipophilicity descriptors, eight of them (Supplementary data Table S1), have been considered regarding correlation with experimentally obtained retention data. All studied chenodeoxycholic acid esters can be considered as lipophilic, according to the obtained log*P* values (log*P* > 1). The values of log*k* parameters calculated for different chromatographic systems were correlated with computationally calculated lipophilicity descriptors. The best linear dependence was found for the relationships between log*k* versus logP^1 (obtained from program MarvinSketch 17.2.3) and logP^1 (obtained from program ChemBio Draw Ultra 12.0.) values (Fig. 2). There are significant correlations between logk_{70} determined in the mobile phase methanol-2-propanol and logP^1 ($R^2 \geq 0.9358$; $y = 0.4290x - 3.1589$) and logP^2 ($R^2 \geq 0.9403$; $y = 0.4091x - 3.4905$), as well as between logk_{95} determined in the mobile phase acetonitrile-2-propanol and logP^1 ($R^2 \geq 0.8898$; $y = 0.1771x - 0.7437$) and logP^2 ($R^2 \geq 0.8974$; $y = 0.1692x - 0.8821$). These significant correlations between log*k* values with lipophilicity descriptors indicate that chromatographic retention (log*k*) can be used as a chromatographic lipophilicity parameter.

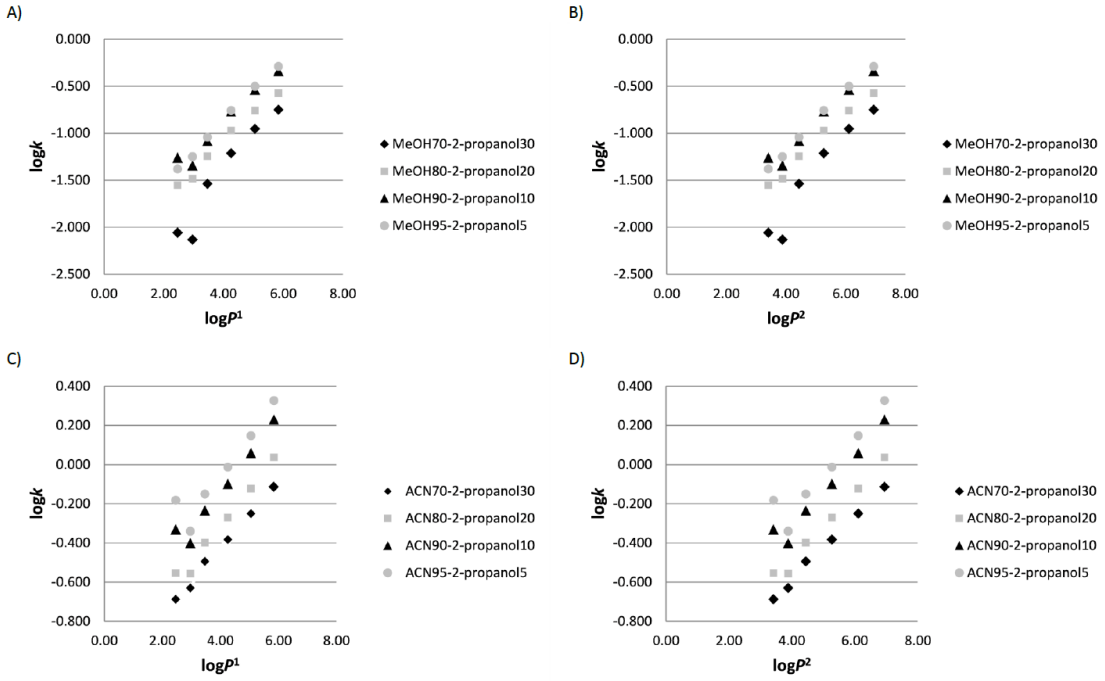


Fig. 2. The correlation between retention parameter (log*k*) determined in the mobile phase methanol-2-propanol and logP^1 lipophilicity parameter (A); the correlation between log*k* determined in the mobile phase methanol-2-propanol and logP^2 lipophilicity parameter (B); the correlation between log*k* determined in the mobile phase acetonitrile-2-propanol and logP^1 lipophilicity parameter (C); the correlation between log*k* determined in the mobile phase acetonitrile-2-propanol and logP^2 lipophilicity parameter (D); logP^1 – MarvinSketch 17.2.3; logP^2 – ChemBioDraw Ultra 12.0

4. CONCLUSION

In conclusion, we have reported the synthesis and comparative study of the chromatographic behavior and lipophilicity of chenodeoxycholic acid esters **1**–**6**. The experimental results presented herein demonstrated that reactions of bile acid esterification can be conveniently performed within 15 minutes by microwave irradiation. Microwave chemistry has again drawn considerable attention with excellent synthetic results of the synthesized chenodeoxycholic acid esters. Furthermore, the presented results indicate that the studied chenodeoxycholic acid esters have log*D* values in the range acceptable for potential drug candidates. Chromatographic lipophilicity of the studied compounds successfully correlates with *in silico* lipophilicity descriptors, which shows them to be potentially biologically active compounds.

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Supplementary data. Supplementary data associated with this article can be found in the online version.

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