SYNTHESIS OF BILE ACID AMINES VIA MICROWAVE IRRADIATION

Vasiljević Bojana[1], Jovana Prekodravac[1], Dragana Jovanović[1]

[1] *Vinča Institute of Nuclear Science-National Institute of Republic of Serbia, University of Belgrade, Belgrade, Serbia*
e-mail: bojana.vasiljevic@vin.bg.ac.rs

Abstract
Herein, we present microwave-assisted reductive amination of oxo derivative of deoxycholic acid with morpholine in the presence of sodium-cyanoborohydride. These chemical transformation produces a majority of the 3β-amino isomer **5** as a new compound after five minutes of irradiation. In addition, formylated bile acid have been proved as excellent starting material for the synthesis of bile acid`s *N*-morpholino amine. Microwave-assisted reactions of formylation in the absence of catalyst, selective deformylation, as well as further oxidation with N-bromosuccinimide gained 3-oxo derivatives of deoxycholic acid acid in high yield. Compared to the conventional protocol a remarkable reduction in overall processing time from hours to a few minutes was achieved.

Introduction
Microwave-assisted organic synthesis has revolutionized organic chemistry [1]. These new technique is considered as an important approach toward green chemistry, medicinal chemistry and drug development, since small molecules can be built in a fraction of time required by conventional heating methods [2].
In the chemistry of bile acids there are significant advantages in the replacement of hydroxyl group by amino functionality [3]. Till date various aminosterols have been discovered, but their synthesis usually need longer heating time and tedious apparatus setup, which resulted in the higher cost of the process and the excessive use of solvents [4-6]. Only few reports on the use of microwave irradiation in chemistry of bile acids confirmed its efficiency in synthesis of various bile acids derivatives [7]. Furtheremore, synthesis of bile acids oxo derivatives as well as insertion of protecting groups presents one of the time consuming steps in organic synthesis. The acetyl protecting group has generally been more widely used than any other function-protecting group due to its stability in various reaction conditions and its ease of removal. However, reactions of acetylation are usually accompanied with unpure products and demand for further purification. Neverthelles, formylated bile acid have been proved as excellent starting material for the synthesis of different bile acid derivatives [8,9]. Taking that under consideration our goal is in investigating and expanding microwave technology in the chemistry of bile acids.
Herein we reported the synthesis of new bile acid amine, 3β-(*N*-morpholino)-12α-hydroxy-5β-cholanoic acid, via fast and efficient microwave irradiation. In-core microwave heating lead to pure formylated and partially deformylated 3α,12α-dihydroxy-5β-cholanoic acid (deoxycholic acid, DCA).

Experimental
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. Reaction temperatures were monitored by an external infrared (IR) sensor. Reaction cooling is performed by compressed air automatically after the heating period has elapsed. Reactions were monitored by thin layer chromatography (TLC) on

silica gel plates (Silica gel 60 F_{254}). Purification of products was carried out by flash column chromatography using Kieselgel 60 (0.040-0.063, Merck). NMR spectra were recorded on a Bruker AC 250 E spectrometer operating at 250 MHz (^{1}H) and 62.5 MHz (^{13}C). Chemical shifts are expressed as ppm downfield from TMS using $CDCl_3$ as solvent. 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.

3α,12α-diformyloxy-5β-cholanoic acid 1

Starting compound 3α,12α-dihydroxy-5β-cholanic acid (500 mg, 1.27 mmol) and metanoic acid (2 mL, 52 mmol) were placed into a 10 mL microwave process vial equipped with a magnetic stir bar. The reaction mixture was heated in a microwave reactor at 60 °C for 30 min. After the reaction time elapsed, reaction mixture was cooled by gas jet cooling. Acetic anhydride was then added dropwise until a large quantity of bubbles appeared. The solution was then poured into 50 mL of cold water with stirring and the reaction product was extracted with chloroform (3 x 20 mL). After removing the solvent under *vacuo*, the TLC chromatography (chloroform : acetone = 6 : 4) confirmed high purity of compound **1**, as white crystals (562 mg, 98 %).
1H NMR ($CDCl_3$, δ, ppm): 0.75 (s, 3H, CH_3-18); 0.83 (d, 3H, CH_3-21); 0.93 (s, 3H, CH_3-19); 1.09-2.40 (m, 27H, CH, CH_2); 4.83 (m, 1H, H-3); 5.25 (s, 1H, H-12); 8.04 (s, 1H, CHO-3); 8.14 (s, 1H, CHO-12). 13C NMR ($CDCl_3$, δ, ppm): 12.33 (CH_3-18); 17.43 (CH_3-21); 22.92 (CH_3-19); 23.43, 25.74, 25.89, 26.47, 26.77, 27.34, 30.47, 30.87, 32.09, 34.02, 34.20, 34.66, 34.77, 35.60, 41.73, 45.01, 47.35 and 49.25 (C-1, C-2, C-4, C-5, C-6, C-7, C-8, C-9, C-10, C-11, C-13, C-14, C-15, C-16, C-17, C-20, C-22, C-23); 74.10 (C-3); 76.49 (C-12); 160.56 (CHO-3); 160.69 (CHO-12); 179.78 (CO_2H).

12α-formyloxy-3α-hydroxy-5β-cholanoic acid 2

To a stirred solution of 3α,12α-diformyloxy-5β-cholanoic acid **1** (100 mg, 0.22 mmol) in acetone (1.12 mL) was added 0.2 M NaOH (0.48 mmol, 2.4 mL) dropwise. The reaction mixture was heated in a microwave reactor at 60 °C for 5 min. After the reaction time elapsed, the solution was cooled by gas jet cooling and acidified with dilute acetic acid (13 µL glacial acetic acid in 102 µL of water). The reaction product was extracted with chloroform (3 x 20 mL). After removing the solvent under *vacuo*, the TLC chromatography (chloroform : acetone = 6 : 4) confirmed high purity of compound **2**, as white crystals (89 mg, 95 %).
1H NMR ($CDCl_3$, δ, ppm): 0.75 (s, 3H, CH_3-18); 0.83 (d, 3H, CH_3-21); 0.91 (s, 3H, CH_3-19); 1.12-2.39 (m, 28H, CH, CH_2); 3.59 (m, 1H, H-3); 5.24 (s, 1H, H-12); 8.12 (s, 1H, CHO-12).

12α-formyloxy-3-oxo-5β-cholanoic acid 3

Solution of 12α-formyloxy-3α-hydroxy-5β-cholanoic acid **2** (0.25 mmol, 103 mg) in *t*-butanole (2 mL) and solution of *N*-bromosuccinimide (0.46 mmol, 82 mg) in water (1 mL) were placed into a 10 mL microwave process vial equipped with a magnetic stir bar. The reaction mixture was heated in a microwave reactor at 80 °C for 1 min. When the reaction time elapsed, reaction mixture was cooled by gas jet cooling. After removing the most of *t*-butanole under *vacuo*, the reaction product was extracted with chloroform (3 x 20 mL). TLC chromatography (chloroform : acetone = 6 : 4) confirmed high purity of compound **3**, as white crystals (95 mg, 92 %).
1H NMR ($CDCl_3$, δ, ppm): 0.79 (s, 3H, CH_3-18); 0.84 (d, 3H, CH_3-21); 1.01 (s, 3H, CH_3-19); 1.17-2.17 (m, 27H, CH, CH_2); 5.29 (s, 1H, H-12); 8.13 (s, 1H, CHO-12). 13C NMR ($CDCl_3$, δ, ppm): 12.38 (CH_3-18); 17.44 (CH_3-21); 22.27 (CH_3-19); 23.41, 25.36, 26.08, 26.39, 27.31, 30.45, 30.87, 34.27, 34.54, 34. 77, 35.33, 36.58, 36.89, 42,17, 43.95, 45.07, 47.37 and 49.21 (C-1, C-2, C-4, C-5, C-6, C-7, C-8, C-9, C-10, C-11, C-13, C-14, C-15, C-16, C-17, C-20, C-22, C-23); 160.47 (CHO-12); 179.65 (CO_2H); 212.93 (C-3).

12α-hydroxy-3-oxo-5β-cholanoic acid 4

Starting compound 12α-formyloxy-3-oxo-5β-cholanoic acid **3** (0,38 mmol, 150 mg) and 0.2 M NaOH (0.77 mmol, 3.84 mL) were placed into a 10 mL microwave process vial equipped with a magnetic stir bar. The reaction mixture was heated in a microwave reactor at 60 °C for 2 min. After the reaction time elapsed, reaction mixture was cooled by gas jet cooling and poured into dilute acetic acid (13 μL glacial acetic acid in 102 μL of water). The reaction product was extracted with chloroform (3 x 20 mL). TLC chromatography (chloroform : acetone = 6 : 4) confirmed high purity of compound **4**, as white crystals (104 mg, 74 %).

1H NMR ($CDCl_3$, δ, ppm): 0.67 (s, 3H, CH_3-18); 0.96 (d, 3H, CH_3-21); 1.21 (s, 3H, CH_3-19); 1.26-2.75 (m, 27H, CH, CH_2); 4.01 (s, 1H, H-12); 5.97 (br s, 2H, OH i CO_2H). 13C NMR ($CDCl_3$, δ, ppm): 12.59 (CH_3-18); 17.11 (CH_3-21); 23.49 (CH_3-19); 26.40, 27.36, 28.66, 29.17, 29.54, 30.60, 31.61, 32.70, 33.60, 35.53, 36.70, 42.13, 44.14, 46.40, 48.18, 53.68, 36.91 and 54.81 (C-1, C-2, C-4, C-5, C-6, C-7, C-8, C-9, C-10, C-11, C-13, C-14, C-15, C-16, C-17, C-20, C-22, C-23); 73.22 (C-12); 178.77 (CO_2H); 213.97 (C-3).

3β-(*N*-morpholino)-12α-hydroxy-5β-cholanoic acid 5

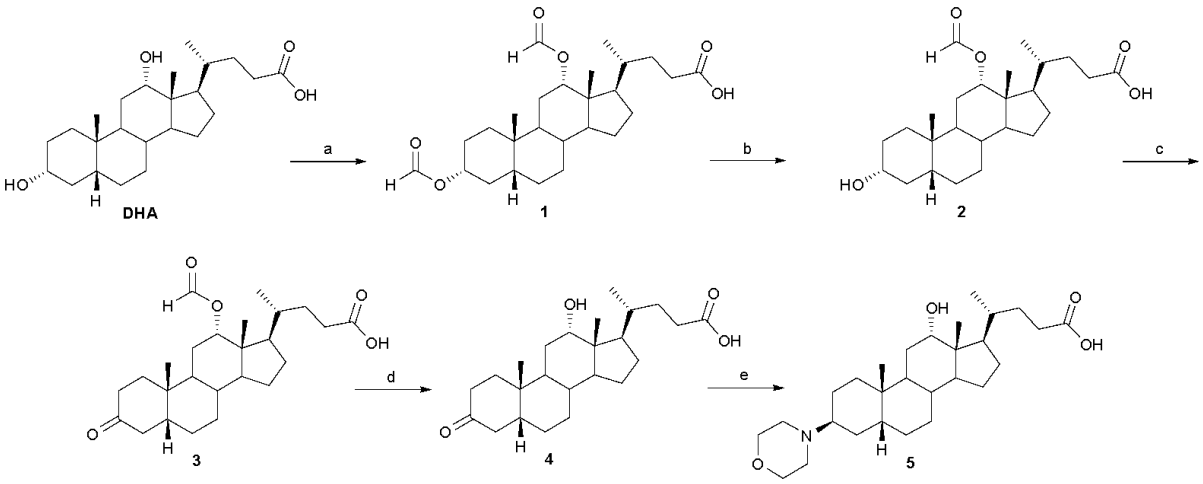
Starting compound 12α-hydroxy-3-oxo-5β-cholanoic acid **4** (0.97 mmol, 380 mg), MeOH (2 mL), morpholine (9.74 mmol, 0.843 mL), sodium-cyanoborohydride (5 mmol, 0.314 g) and glacial acetic acid (100 μL) were placed in a 10 mL microwave process vial equipped with a magnetic stir bar. The reaction mixture was heated in a microwave reactor at 100 °C for 5 min. After the reaction time elapsed, reaction mixture was cooled by gas jet cooling. The reaction mixture was added saturated solution of $NaHCO_3$ and then extracted with methylene chloride (3 x 20 mL). The residue was purified by flash column chromatography affording pure compound **5**, as white crystals (140 mg, 31 %).

1H NMR ($CDCl_3$, δ, ppm): 0.68 (s, 3H, CH_3-18); 0.97 (s, 3H, CH_3-19); 0.99 (d, 3H, CH_3-21); 1.01-1.80 (m, 24H, CH, CH_2); 2.0-2.3 (1H, H-3 i 2H, CH_2-23), 2.4-2.5 (m, 4H, CH_2NCH_2), 3.60-3.80 (m, 4H, CH_2OCH_2); 3.99 (s, 1H, H-12); 6.30 (br s, 2H, OH and CO_2H).

13C NMR ($CDCl_3$, δ, ppm): 12.77 (CH_3-18); 17.48 (CH_3-19); 21.21 (CH_3-21); 22.61, 23.66, 23.68, 23.78, 26.19, 27.32, 27.51, 28.69, 28.91, 30.38, 31.82, 33.58, 34.07, 34.67, 35.38, 36.05, 36.74 (C-1, C-2, C-4, C-5, C-6, C-7, C-8, C-9, C-10, C-11, C-13, C-14, C-15, C-16, C-17, C-20, C-22, C-23); 50.63 (CH_2NCH_2); 59. 89 (C-3); 67.29 (CH_2OCH_2); 73.36 (C-12); 180.34 (CO_2H).

Results and discussion

The significance of hydroxyl-protected groups in the chemistry of bile acids turned our attention to finding more appropriate and greener reactions conditions for synthesis of bile acid formates. Herein, we developed a simple method of formylation, that involves heating of bile acid in methanoic acid under microwave irradiation (Scheme 1).



Scheme 1 Reagents and conditions: MW (a) HCOOH, , 60 °C, 30 min, 98 % (b) 0.2 N NaOH, $(CH_3)_2CO$, 60 °C, 5 min, 95 % (c) NBS, $CH_3(CH_2)_3OH$, 80 °C, 1 min, 92 % (d) 0.2 N NaOH, 60 °C, 2 min, 74 % (e) $O(CH_2CH_2)_2N$, MeOH, CH_3COOH, $NaBH_3CN$, 100 °C, 5 min, 31 %

Pure product of 3α,12α-diformyloxy-5β-cholanoic acid **1** was obtained in high yield from DCA after 30 minutes of microwave iradiation. The TLC chromatography confirmed only trace amounts of starting material. Pure performilated bile acids are isolated simply by diluting the reaction mixture with water. Compared to the conventional heated reacton of DCA formilation, the presence of an acidic catalyst was unnecessary [8]. In the next phase, a partial deformylation of 3α,12α-diformyloxy-5β-cholanoic acid **1** with sodium-hydroxide in acetone was achieved at 60 ºC during 5 minutes of MW exposure.
Microwave-assisted selective oxidation with *N*-bromosuccinimide in *t*-butanol (80 °C, 1 min) and further deformylation (60 °C, 2 min) gave 12α-hydroxy-3-oxo-5β-cholanoic acid **4** in high yield and purity. Compared to the conventional protocol a remarkable reduction in overall processing time from hours to a few minutes was achieved. Structures of synthesized bile acid derivatives have been confirmed by ^{1}H- and ^{13}C- NMR spectroscopic data.

Finally, we have report efficient synthesis of new compound, 3β-(*N*-morpholino)-12α-hydroxy-5β-cholanoic acid **5** under high intensity of microwave irradiation. Target compound **5** was obtained by reductive amination of 3-oxo-12α-hydroxy-5β-cholanoic acid **4** with morpholine in the presence of $NaBH_3CN$ (Scheme 1), after 5 min of MW heating.
The resulting 3-hydroxy formyl bile acid proved to be the best starting materials for the synthesis of bile acids derivatives with specific modification at 3-hydroxyl group, such as the synthesis of bile acid`s *N*-morpholino amine. The structure of synthesized bile acid`s *N*-morpholino amine was confirmed by the presence of a signal at 2.54 ppm in the 1H NMR spectrum derived from hydrogen at C-3. Coupling constants are not observed on the given signal, indicating the equatorial position of the mentioned hydrogen.

Conclusion

In summary, we have shown that the use of bile acid formates, obtained in high yield and purity by a new formylation procedure, resulted in a much cleaner product and hence in higher yields and simplified procedures. Microwave irradiation has once again confirmed its efficiency within synthesis of new bile acid derivative, 3β-(*N*-morpholino)-12α-hydroxy-5β-cholanoic acid **5** in very short reaction time.

References

[1] J.M. Kremsner, A. Stadler, A Chemist's Guide to Microwave Synthesis, Anton Paar GmbH, Austria, 2018.
[2] C.O. Kappe, D. Dallinger, S.S. Murphree, Practical Microwave synthesis for Organic Chemists, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 2009.
[3] M. Blanchet, J. M. Brunel, Curr. Org. Chem., 25 (2018) 3613.
[4] B. Brycki, H. Koenig, T. Pospieszny, Molecules 20 (2015) 20887.
[5] S. Jones, W. Kinney, X. Zhang, L. Jones, B. Selinsky, Steroids 61 (1996) 565.
[6] M. Zasloff, W. Kinney, S. Jones, United States Patent number 5,856,535, 1999.
[7] Lj.M. Grbović, K.J. Pavlović, S.S. Jovanović-Šanta, B.R. Vasiljević, Curr. Org. Chem. 23 (2019) 256.
[8] K.-Y. Tserng, P. D. Klein, Steroids 25 (1977) 635.
[9] W. Kozanecka-Okupnik, B. Jasiewicz, T. Pospieszny, M. Matuszak, L. Mrówczyńska, Steroids 126 (2017) 50.