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Synthesis of cholic acid based calixpyrroles and porphyrins

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ABSTRACT

New cholic acid based calix[4]pyrroles and porphyrins were prepared and their properties were studied. It was confirmed by spectral measurements that the superassembly of 5,15-bis(3α,7α,12α-trihydroxy-5β-cholan-24-yl)-10,20-diphenylporphyrin, the best candidate for this study from the conjugates prepared, may be influenced not only by the solvent mixture composition (polar/non-polar component ratio) but by time as well.

1. Introduction

The steroid nucleus has been quite widely adopted as a building block for supramolecular chemistry. It is large, rigid, chiral, and thus suitable for creating extended architectures with well-defined conformations, capable in principle of enantiodiscrimination. Of the available steroidal starting materials, the most versatile bile acids are especially useful, providing inexpensive starting points with helpful substitution patterns, arising from both region- and stereo-chemical variation, and are thus well adapted to serve as a scaffold for functional group arrays [1] and functional devices and constructs [2–4]. Hence it is still very actual to search for new types of these compounds.

In this work we are further extending our studies [5–12] to pyrrole-bile acid conjugates, because calix[4]pyrroles and porphyrins represent a class of macrocycles, which are very important structural elements in host guest chemistry, since calix[4]pyrroles bind anions and neutral species and porphyrins bind cations in an efficient way [13] and some of them may be utilized as electrochemical sensing devices [14].

2. Experimental

2.1. Materials and methods

TLC was performed on either HF254 plates (Merck) with detection by UV light or on plates made from MP Biomedicals SilicaGel G with detection by spraying with a solution of 5 g of $Ce(SO_4)_2(H_2O)_4$ in 500 ml 10% H_2SO_4 and subsequent heating. Flash column chromatography was performed on silica gel (Merck, 100–160 μm) with solvents distilled prior to use.

As the source of microwave (MW) irradiation a microwave microprocessor controlled chemical reactor Plasmatronika M REOS with electromagnetic stirring, MW generator of 2.45G and temperature regulation was used.

FTIR spectrometer Nicolet iS10 (Thermo Scientific), automatic polarimeter Autopol VI (Rudolph Research Analytical), UV/VIS Varian Cary 50 UV–Vis, and Cary Eclipse fluorescence spectrophotometers were used for physicochemical characterisations. IR spectra (ν) are given in cm^{-1}.

All NMR experiments were run with Varian Gemini 300 HC, working at 299.97 M for protons, 75.44 M for carbon-13. ^1H and ^{13}C NMR chemical shifts were referenced to the signal of internal standard TMS, in chloroform-d solution, and are given in δ ppm, unless stated otherwise. Coupling constants *J* are given in Hz.

Mass spectrometric measurements were performed using Agilent LC/MSD SL/ESI with hyphenated HPLC and LCQ Fleet Ion Trap LC/MS^n mass spectrometer with electrospray resp. chemical ionization (ESI resp. APCI) or using Q TOF (Micromass) spectrometer with direct inlet (ESI), on a ZAB-EQ (VG Analytical) instrument (FAB) with Xe ionization, or Autospec Ultima (Micromass) by EI technique.

2.2. Chemical synthesis

2.2.1. Esterification of cholic acid **1** under microwave irradiation

A mixture of cholic acid (2.01 g, 4.9 mmol) and *p*-toluene sulphonyl acid monohydrate (500 mg, 2.63 mmol) in methanol

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(50 ml) was irradiated by MW for 6 min at 50% of maximum intensity (i.e. at 375 W). After evaporation of methanol, water was slowly added until the product starts to crystallize, and the crude product was filtered and washed with water. Recrystallization from methanol–water yielded 2.07 g (99%) of methyl cholate, m.p 107–109 °C. Literature [15] gives different value of m.p, 156–157 °C.

2.2.2. Methyl-7α,12α-diformyloxy-3α-hydroxy-5β-cholan-24-oate

A solution of methyl cholate (2.07 g, 4.9 mmol) in formic acid (5 ml) was standing at room temperature for three days. Then ice was added to the mixture, the white crystallized product was filtered and washed with water, yield 2.37 g (96%) of methyl tri-*O*-formylcholate.

A mixture of methyl tri-*O*-formylcholate (2.37 g, 4.68 mmol) and *p*-toluene sulphonyl acid monohydrate (890 mg, 4.68 mmol) in propan-2-ol (30 ml) was irradiated by MW for 8 min at 50% of maximum intensity (i.e. at 375 W). The residue after evaporation of propan-2-ol was dissolved in ether (25 ml) and Na_2CO_3 was added. Solids were filtered off, the filtrate was evaporated and the crude product was purified by chromatography on a column of silica gel (45 g) in cyclohexane: diethyl ether 1:1 and then diethyl ether, yield 1.46 g (65%) of methyl-7α,12α-diformyloxy-3α-hydroxy-5β-cholan-24-oate, m.p 59–62 °C. The product was characterized by $[\alpha]_D^{20}$ −43.5 ($c = 8.78 \times 10^{-3}$ g/ml in $CHCl_3$). ^{1}H NMR: 0.76 (s, 3 H, 19-CH_3), 0.84 (d, J = 6.4, 3 H, 21-CH_3), 0.93 (s, 3 H, 18-CH_3), 3.51 (quin, J = 4.3, 1 H, 3β-H), 3.66 (s, 3 H, 24-$COOCH_3$), 5.07 (d, J = 2.6, 1 H, 7β-H), 5.27 (br. s, 1 H, 12β-H), 8.15 (s, 1 H, 7α-OOCH) 8.11 (s, 1 H, 12α-OOCH). IR ($BaCl_2$) 3500 (OH), 2952, 2928, 2872 (CH_2, CH_3), 1720 (C=O), 1177 (C–O). MS (ES-API): for $C_{27}H_{42}O_7$ (478.62), calculated monoisotopic m/z: 478.29 (496.33) Da, found: M+NH_4 496.2. It may be noted that biological data of methyl-7α,12α-diformyloxy-3α-hydroxy-5β-cholan-24-oate in inflammation were described elsewhere [16].

2.2.3. Methyl 7α,12α-diformyloxy-3-oxo-5β-cholan-24-oate ***2***

Fresh distilled pyridine (4.7 ml, 59 mmol) was added to the mixture of dry chromium (VI) oxide (1.96 g, 19.6 mmol) and dichloromethane (10 ml) at 0 °C, and the mixture was stirred until a suspension was formed. Then methyl 7α,12α-diformyloxy-3-hydroxy-5β-cholan-24-oate (1.56 g, 3.2 mmol) dissolved in dichloromethane (15 ml) was added. The mixture was irradiated by MW for 6 min at 50% of maximum intensity (i.e. at 375 W). Dichloromethane was distilled off and the residue was diluted by diethyl ether (20 ml), filtered through a layer of alumina (30 g) and the filtrate was evaporated and co-evaporated with toluene (3 × 10 ml). Recrystallization from diethyl ether yielded 1.48 g (95%) of methyl 7α,12α-diformyloxy-3-oxo-5β-cholan-24-oate **2**, m.p 123–124.5 °C. Product **2** was characterized by $[\alpha]_D^{20}$ −51.1 ($c = 7.10 \times 10^{-3}$ g/ml in $CHCl_3$). ^{1}H NMR: 0.78 (s, 3 H, 18-CH_3), 0.84 (d, J = 6.4, 3 H, 21-CH_3), 1.03 (s, 3 H, 19-CH_3), 3.65 (s, 3 H, 24-$COOCH_3$), 5.14 (d, J = 2.6, 1 H, 7β-H), 5.30 (br. s, 1 H, 12β-H), 8.08 (s, 1 H, 7α-OOCH), 8.15 (s, 1 H, 12α-OOCH). IR ($BaCl_2$) 2952, 2928, 2872 (CH_2, CH_3), 1720 (C=O), 1177 (C–O). MS (ES-API): for $C_{27}H_{40}O_7$ (476.60), calculated monoisotopic m/z: 476.27 (494.31) Da, found: M+NH_4 494.0.

2.2.4. Calix[4]pyrrole-cholic acid conjugates

Calix[4]pyrrole-cholic acid conjugate ***3****:* A mixture of methyl 7α, 12α-diformyloxy-3-oxo-5β-cholan-24-oate (220 mg, 0.46 mmol), 5,5-dimethyldipyrromethane (350 mg, 2,0 mmol) and trifluoroacetic acid (16 μl, 0.2 mmol) in dichloromethane (30 ml) under argon was strongly stirred in room temperature for 4 h. Then dried acetone (20 ml) was added, and the mixture was stirred for further 1 h, and the reaction was ended by adding Na_2CO_3. The mixture was filtered through a layer of silica gel, and the filtrate was evaporated. The crude product was purified by chromatography (silica gel, 32–63 μM, elution by cyclohexane – ether 9:1) to yield 198 mg calix[4]pyrrole **3** (51%) as amorphous solid. $[\alpha]_D^{20}$ −7.6 ($c = 1.05 \times 10^{-3}$ g/ml in $CHCl_3$). ^{1}H NMR: 0.73 (s, 3 H, 18-CH_3), 0.84 (d, 3 H, 21-CH_3), 0.86 (s, 3 H, 19-CH_3), 1.49 (bs, 6 CH_3 calix[4]pyrrole), 3.66 (s, 3 H, 24-$COOCH_3$), 5.04 (bs, 1 H, 7β-H), 5.26 (bs, 1 H, 12β-H), 5.89 (m, calix[4]pyrrole), 6.86 (bs, 1 H, NH), 6.91 (bs, 1 H, NH), 7.04 bs, 2 H, 2 NH), 8.11 (s, 1 H, 7α-OOCH), 8.16 (s, 1 H, 12α-OOCH). IR ($BaCl_2$) 3416, 3107 (N–H), 2952 $_{as}$(CH_3), 2928 $_{as}$(CH_2), 2872 $_s$(CH_3), 1720 (C=O), 1177 (C–O). MS (ES-API): $C_{52}H_{70}N_4O_6$ (847.14), calculated monoisotopic m/z: 846.52 Da, found: 864.2.

Calix[4]pyrrole-cholic acid conjugate ***4****:* A mixture of **3** (0.092 g, 0.109 mmol) and NaOH (0.024 g, 0.600 mmol) in methanol (10 ml) was stirred in room temperature over night, then NH_4Cl was added, methanol was evaporated, and the crude product was purified by flash chromatography, elution by diethyl ether, yield 0.035 g (42%) as amorphous solid. $[\alpha]_D^{20}$ −8.1 ($c = 2.35 \times 10^{-3}$ g/ml in $CHCl_3$). ^{1}H NMR: 0.73 (s, 3 H, 18-CH_3), 0.84 (d, 3 H, 21-CH_3), 0.86 (d, 3 H, 19-CH_3), 1.67 (bs, 6 CH_3 calix[4]pyrrole), 3.62 (s, 3 H, 24-$COOCH_3$), 4.05 (bs, 1 H, 7β-H), 4.22 (bs, 1 H, 12β-H), 5.15 (m, calix[4]pyrrole), 5.25 (m, calix[4]pyrrole), 6.05 (m, calix[4]pyrrole), 8.00 (bs, 1 H, NH), 8.45 (bs, 1 H, NH), 9.39 (bd, 2 H, 2 NH). IR ($BaCl_2$) 3390(OH, NH), 2952 $_{as}$(CH_3), 2928 $_{as}$(CH_2), 2872 $_s$(CH_3), 1720 (C=O). MS (ES-API): $C_{50}H_{70}N_4O_4$ (791.12) calculated monoisotopic m/z: 790.53 (808.57) Da, found: M+NH_4 808.4.

2.2.5. Methyl 3α,12α-diformyloxy-7-oxo-5β-cholan-24-oate ***5***

Fresh distilled pyridine (10 ml, 124 mmol) was added to the mixture of dry chromium (VI) oxide (2.84 g, 28.4 mmol) and dichloromethane (30 ml) at 0 °C, and the mixture was stirred until a suspension was formed. Then methyl cholate (1.96 g, 4.64 mmol) dissolved in dichloromethane (15 ml) was added. The reaction mixture was stirred 40 min at 0 °C, then the mixture was filtered through a layer of alumina (30 g) and the filtrate was evaporated and co-evaporated with toluene (3 × 10 ml).

The crude product was dissolved in 5 ml of formic acid, the reaction mixture was standing at room temperature for three days. Then ice was added to the mixture, organic product was extracted to ether, organic solution was washed with concentrated solution of Na_2CO_3, water and concentrated solution of NaCl, and dried with $MgSO_4$ over night. Next day the solution was filtered, ether was distilled off. Crystallization from ether yielded 1.1 g (49%) of **5**, m.p 127–128 °C. $[\alpha]_D^{20}$ −44.0 ($c = 9.50$ g/ml in $CHCl_3$). ^{1}H NMR: 0.75 (s, 3 H, 18-CH_3), 0.84 (d, J = 6.1, 3 H, 21-CH_3), 1.21 (s, 3 H, 19-CH_3), 3.66 (s, 3 H, 24-$COOCH_3$), 4.81 (tt, J = 10.1, 5.2, 1 H, 3β-H), 5.27 (br. s, 1 H, 12β-H), 7.98 (s, 1 H, 3α-OOCH), 8.11 (s, 1 H, 12α-OOCH). IR ($BaCl_2$) 2951, 2928, 2875 (CH_2, CH_3), 1720 (C=O), 1177 (C–O). MS (ES-API): $C_{27}H_{40}O_7$ (476.60) calculated monoisotopic m/z: 476.27 (494.31) Da, found M+NH_4 494.4.

2.2.6. Porphyrin-cholic acid conjugates

3α,7α,12α-Trihydroxy-24,24-di(pyrrol-2-yl)-5β-chol-23-ene ***7****:* To a solution of pyrrolyl- magnesium bromide (851 mg, 5 mmol) in dichloromethane (55 ml) was added methyl cholate (2.00 g, 4.74 mmol) and the reaction mixture was refluxed in an oil bath in 75 °C with stirring for 60 h. After cooling, the complex was decomposed by pouring its solution into a mixture of about 100 g of ice and 200 ml of 10% NH_4Cl solution. After thorough shaking, the layers are separated, and the aqueous layer was extracted twice with ether. The combined ether solution was washed with water, 5% Na_2CO_3 solution, finally with concentrated NaCl solution, and organic solution was dried with $MgSO_4$ over night. The solvent was evaporated, the residue of crude product was purified by chromatography (silica gel, 32–63 μM, toluene and ether), yield 1.11 g **7** (46%) as amorphous solid. $[\alpha]_D^{20}$ −12.7 ($c = 2.13 \times 10^{-3}$ g/ml in