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Journal of Molecular Structure 693 (2004) 81–86
Journal of MOLECULAR STRUCTURE
www.elsevier.com/locate/molstruc

Synthesis and characterization of lithocholic acid derived dipyrromethanes: precursors for pyrrole-steroidal macrocycles

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Received 20 January 2004; revised 9 February 2004; accepted 11 February 2004

Abstract

Three steroidal dipyrromethanes, 3,3,24,24-tetrakis(pyrrol-2-yl)-5β-cholane **1**, 3,3-bis(pyrrol-2-yl)-5β-cholan-24-oic acid **2**, and methyl 3,3-bis(pyrrol-2-yl)-5β-cholan-24-oate **3**, have been prepared from 3α-hydroxy-5β-cholan-24-oic acid (lithocholic acid) **4** in good overall yields. The structures of **1**–**3** have been fully characterized by ^{1}H, ^{13}C, PFG DQF ^{1}H–^{1}H COSY, ^{1}H–^{1}H ROESY, ^{13}C DEPT-135, PFG ^{1}H–^{13}C HMQC, PFG ^{1}H–^{13}C HMBC, and PFG ^{1}H–^{15}N HMBC NMR spectra. Their molecular weights and compositions have been determined by ESI-TOF and EI mass spectra, and elemental analyses. The energetically optimised geometry and isotropic ^{13}C NMR chemical shifts of 3,3,24,24-tetrakis(pyrrol-2-yl)-5β-cholane **1** have been calculated by *ab initio* HF/6-31G* and DFT B3PW91/6-311G* methods.

Keywords: Pyrrole; Dipyrromethane; Lithocholic acid; Multinuclear magnetic resonance; Electrospray ionization and EI mass spectra

1. Introduction

Bile acids and their derivatives have been used in the treatment of bile acid deficiency and liver diseases, as well as in dissolution of cholesterol gallstones [1]. They can be used as cholesterol level lowering agents and as carriers for liver-specific drugs [2]. Previously, we have reported the synthesis and characterization of a thiophene and piperazine bearing cholaphane, which has cation (e.g. Ag^{+}) binding properties [3]. In this work we are extending our studies to pyrrole-bile acid conjugates, because pyrrole is a biochemically important structural fragment found in heme, chlorophyll and many alkaloid structures [4]. Additionally pyrrole derivatives are widely used in the pharmaceutical industry, for example as anti-inflammatory and anticancer drugs [5,6]. Porphines are used in photodynamic therapy (PDT) as photosensitizers, which tend to accumulate in diseased tissue. In visible light they react with molecular oxygen to generate radicals, which inactivate tumour cells [7]. A steroid-porhyrin conjugate has also been used for saccharide binding in protic media [8]. Dipyrromethanes are important precursors in syntheses of calix [4] pyrroles, porphyrins and other macrocyclic pyrrole derivatives [9]. Dipyrromethanes containing one or two hydrogen atoms at *meso* position can be oxidized to dipyrromethenes, which can chelate transition metal cations [9]. The aim of this study is to prepare steroidal oligopyrromethanes, which can be used further as precursors in the syntheses of pyrrole macrocycles. Molecules containing both steroid and pyrrole moieties are interesting and potential carriers for drug molecules. They also have potential to act as prodrugs or drugs by themselves [8].

2. Experimental

2.1. Spectroscopy

All NMR experiments were run with Bruker Avance DRX 500 FT NMR spectrometer equipped with a z-gradient accessory and a 5 mm diameter inverse detection probehead working at 500.13 MHz for proton, 125.77 MHz for carbon-13 and 50.70 MHz for nitrogen-15, respectively. ^{1}H and ^{13}C NMR chemical shifts were referenced to the trace signal of undeuteriated solvent, $\delta(^{1}H)$ $CHCl_3 = 7.26$ ppm, and to the signal of solvent, $\delta(^{13}C)$ $CDCl_3 = 77.00$ ppm, from internal

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doi:10.1016/j.molstruc.2004.02.014

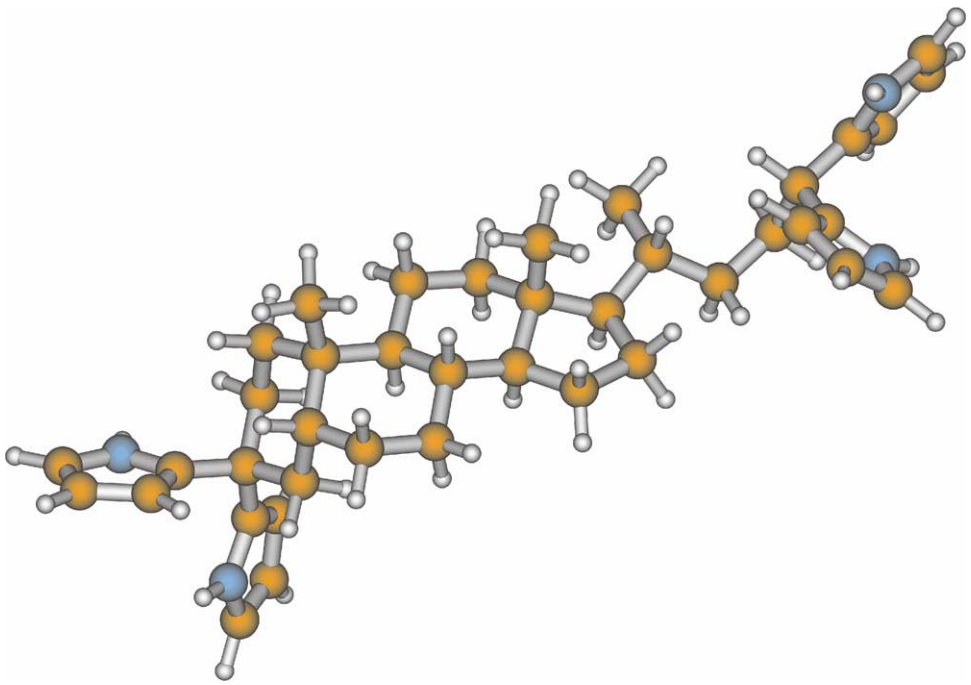


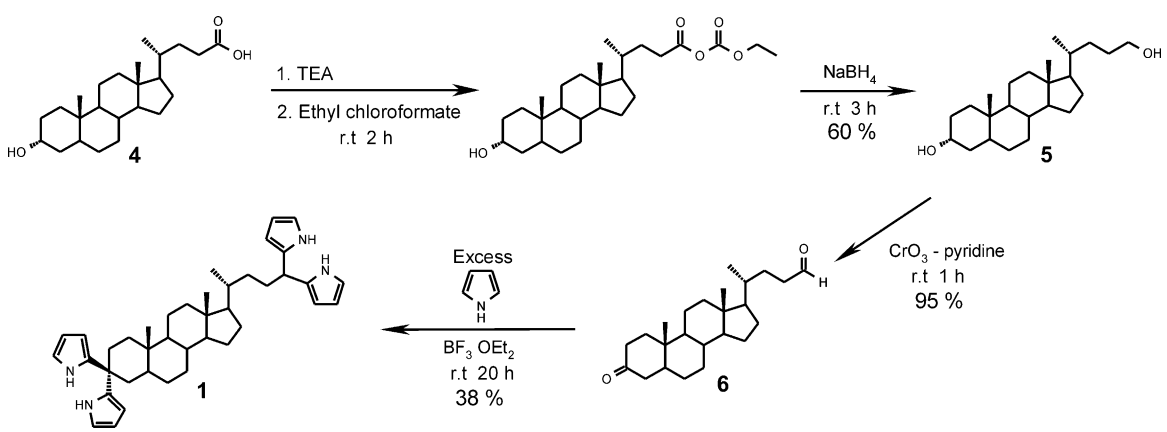
Fig. 1. *Ab initio* HF/6-31G* optimized structure of 3,3,24,24-tetrakis(pyrrol-2-yl)-5β-cholane.

TMS. ^{15}N NMR chemical shifts were referenced to the signal of an external nitromethane in 1 mm diameter capillary inserted coaxially inside the 5 mm NMR tube, $\delta(CH_3^{15}NO_2) = 0.0$ ppm. ^{1}H, ^{13}C, and ^{15}N NMR chemical shift assignments were based on PFG DQF ^{1}H–^{1}H COSY [10], ^{1}H–^{1}H ROESY [11], ^{13}C DEPT-135, PFG ^{1}H–^{13}C HMQC [12], PFG ^{1}H–^{13}C HMBC [13], and PFG ^{1}H–^{15}N HMBC NMR measurements.

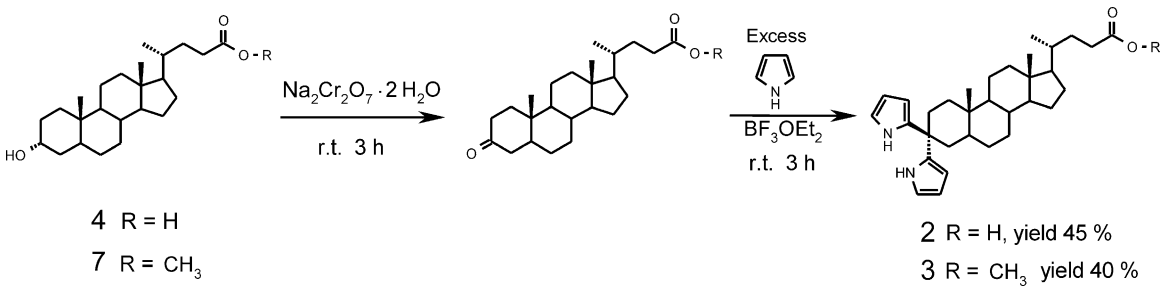
Mass spectrometric measurements were performed using VG AutoSpec EI mass spectrometer and Micromass LCT time of flight (TOF) mass spectrometer with electrospray ionization (ESI). Elemental analysis was performed using VarioEL III elemental analyzer.

2.2. Calculations

The geometry of **1** was optimized at the *ab initio* HF/6-31G*, and NMR shieldings were calculated at the B3PW91/6-311G* levels of theory using GAUSSIAN 98 [14]. The crystal structure of methyl 3-oxo-5β-cholan-24-oate was used as a starting structure for the steroidal part of molecule [15]. The optimized structure of **1** is shown in Fig. 1. The predicted NMR chemical shifts were derived from equation $\delta = \sigma_\nu - \sigma$, where δ is the chemical shift, σ is the absolute shielding, and σ_ν is the absolute shielding of the standard (TMS; 185.76 ppm).



Scheme 1. Synthesis of **1**.



Scheme 2. Syntheses of **2** and **3**.

2.3. Synthesis

3,3,24,24-tetrakis(pyrrol-2-yl)-5β-cholane **1**: 3α-hydroxy-5β-cholan-24-oic acid (lithocholic acid) **4** was used as a starting material in the synthesis of 3,3,24,24-tetrakis(pyrrol-2-yl)-5β-cholane **1**. In the first step LCA (3.28 mmol) was allowed to react with triethylamine (11.8 mmol) and ethyl chloroformate (10.8 mmol) resulting in a very reactive anhydride intermediate ($NaBH_4$ is not powerful enough to reduce acid directly), which was further reduced to 5β-cholane-3,24-diol **5** with $NaBH_4$ (36.1 mmol) [16]. The diol (1.97 mmol) was then oxidized to 3-oxo-5β-cholan-24-al **6** by using CrO_3-pyridine (CrO_3, 23.6 mmol; pyridine, 47.3 mmol) [17]. The desired product was obtained by allowing 3-oxo-5β-cholan-24-al (1.87 mmol) to react with a five-fold excess (20 equivalents) of pyrrole (37.4 mmol) using BF_3OEt_2 as a catalyst [18]. Excess pyrrole was the needed in reaction to prevent the formation of pyrrole macrocycles and other side products like tripyrranes. Reaction time was also crucial because the more reactive aldehyde reacted first with pyrrole forming dipyrromethane. Thus, when the reaction mixture was stirred for 3 h, the product was mainly 3-oxo-24,24-bis(pyrrol-2-yl)-5β-cholane. Increasing the reaction time to 20 h gave the product where both the aldehyde and the ketone were reacted. The crude product was purified by flash chromatography (silica gel, 0.040–0.063 mm; CH_2Cl_2). The overall yield of **1** was 22% (0.71 mmol) calculated from lithocholic acid. The reaction route to **1** is described in Scheme 1.

Elemental analysis of **1**: C: 81.70, H: 9.59, N: 9.06%. Calculated: C: 81.31, H: 9.21, N: 9.48%.

3,3-Bis(pyrrol-2-yl)-5β-cholan-24-oic acid **2** was prepared by oxidizing **4** (5.43 mmol) with $Na_2Cr_2O_7$. 2 H_2O (5.43 mmol) [19], and formed 3-oxo-5β-cholan-24-oic acid (5.38 mmol) was stirred for 3 h at r.t. with 10 equivalents of pyrrole (53.8 mmol) using BF_3OEt_2 as a catalyst. The crude product was purified by flash

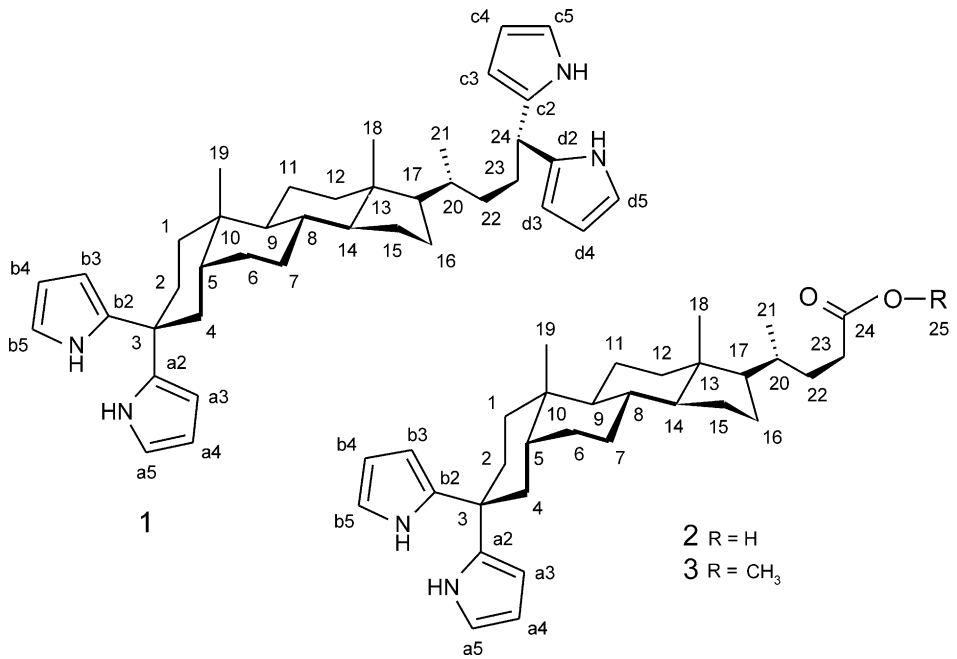


Fig. 2. Structures and numbering of **1**–**3**.

chromatography (silica gel, 0.040–0.063 mm; CH_2Cl_2: MeOH, 99:1). The overall yield of **2** was 45% (2.42 mmol) calculated from **4**.

Elemental analysis of **2**: C: 76.01, H: 9.44, N: 4.97, O: 9.58%. Calculated: C: 75.55, H: 9.51, N: 5.50, O: 9.43%, contains 2 + H_2O.

Methyl 3,3-Bis(pyrrol-2-yl)-5β-cholan-24-oate **3** was prepared by the same procedure as **2**, except that methyl lithocholate **7** (1.46 mmol) was used as starting compound [20]. The overall yield of **3** was 40% (0.59 mmol) calculated from **7**. The synthetic route to **2** and **3** is described in Scheme 2.

Elemental analysis of **3**: C: 77.74, H: 9.66, N: 5.06, O: 7.55%. Calculated: C: 77.14, H: 9.61, N: 5.45, O: 7.78%, contains 2* **3** + H_2O

3. Results and discussion

The *ab initio* HF/6-31G* optimised minimum energy structure of **1** and numbering of **1**–**3** are shown in Figs. 1 and 2. The optimised structure of **1** was necessary for determination of proton–proton distances, which were crucial in estimating intramolecular nuclear Overhauser effects (NOE) and assigning four pyrrole moieties. Unfortunately, we were not able to grow single crystals suitable for X-ray structural analyses.

^{1}H NMR chemical shifts of the pyrrole moieties of **1**–**3** are presented in Table 1, ^{13}C NMR chemical shifts of **1**–**3** in Table 2, and ^{15}N NMR chemical shifts of **1** in Table 3. In Table 1 the assignments of the ^{1}H chemical shifts of pyrrole rings were based on ^{1}H–^{1}H Rotating Frame NOESY (ROESY with spin lock time 50 ms) measurement of **1**.

Table 1
^{1}H NMR chemical shifts of pyrrole rings

Proton	**1** δ (ppm)	**2** δ (ppm)	**3** δ (ppm)
a-3	6.04 (m)	6.04 (m)	6.04 (m)
a-4	6.11 (ddd, $J \approx 2.8$ Hz each)	6.12 (ddd, $J \approx 2.7$ Hz each)	6.11 (ddd, $J \approx 2.8$ Hz each)
a-5	6.53 (d, $J = 1.35$ Hz)	6.53 (d, $J = 1.51$ Hz)	6.53 (d, $J = 1.49$ Hz)
a-N	7.58 (s)	7.59 (s)	7.59 (s)
b-3	6.19 (m)	6.19 (m)	6.19 (m)
b-4	6.17 (ddd, $J \approx 2.8$ Hz each)	6.17 (ddd, $J \approx 2.7$ Hz each)	6.17 (ddd, $J \approx 2.7$ Hz each)
b-5	6.67 (d, $J = 1.18$ Hz)	6.67 (d, $J = 1.48$ Hz)	6.66 (d, $J = 1.06$ Hz)
b-N	7.80 (s)	7.82 (s)	7.83 (s)
c-3	6.10 (m)	–	–
c-4	6.15 (ddd, $J \approx 2.8$ Hz each)	–	–
c-5	6.63 (d, $J = 1.1.33$ Hz)	–	–
c-N	7.78 (s)	–	–
d-3	6.10 (m)	–	–
d-4	6.15 (ddd, $J \approx 2.8$ Hz each)	–	–
d-5	6.64 (d, $J = 1.37$ Hz)	–	–
d-N	7.78 (s)	–	–

Table 2
^{13}C NMR chemical shifts

Carbon	**1** Measured δ (ppm)	**1** Calculated δ (ppm)	**2** Measured δ (ppm)	**3** Measured δ (ppm)
1	33.0	31.2	33.0	33.0
2	31.3[a]	28.9	31.3	31.3
3	40.6	43.5	40.6	40.5
4	37.9	39.1	37.9	37.9
5	39.3	40.7	39.3	39.3
6	27.2	26.8	27.1	27.1
7	26.4	26.1	26.3	26.3
8	35.8	36.5	35.8	35.8
9	40.4	41.2	40.4	40.4
10	35.0	38.2	35.0	34.9
11	20.9	21.3	20.9	20.9
12	40.2	40.2	40.2	40.2
13	42.7	46.3	42.8	42.8
14	56.5	56.8	56.5	56.5
15	24.2	24.2	24.2	24.2
16	28.2	27.4	28.2	28.2
17	56.0	58.4	56.0	56.0
18	12.0	9.7	12.1	12.0
19	23.8	21.3	23.8	23.8
20	35.7	33.4	35.3	35.4
21	18.7	13.9	18.3	18.3
22	33.8	30.9	31.0[a]	31.1[a]
23	31.2[a]	27.5	30.8[a]	31.0[a]
24	38.1	35.6	179.9	174.7
25	–	–	–	51.4
a2	141.3	139.9	141.3	141.3
a3	101.6	101.5	101.6	101.6
a4	107.8	107.7	107.8	107.8
a5	116.3	114.7	116.3	116.3
b2	134.9	134.3	134.9	134.9
b3	106.4[b]	106.8	106.4[b]	106.4[b]
b4	108.0[b]	107.9	108.0[b]	107.9[b]
b5	117.2	115.6	117.2	117.1
c2	134.0	133.0	–	–
c3	105.1	103.6	–	–
c4	108.1[c]	107.9	–	–
c5	116.8[d]	115.2	–	–
d2	133.5	131.7	–	–
d3	105.5	108.9	–	–
d4	108.1[c]	108.1	–	–
d5	116.9[d]	116.1	–	–

Superscripts denote pairs of exchangeable assignments.

Table 3
^{15}N NMR chemical shifts of 1

Pyrrole	δ (ppm)
a	−234,8
b	−236,8
c	−234,8
d	−234,8

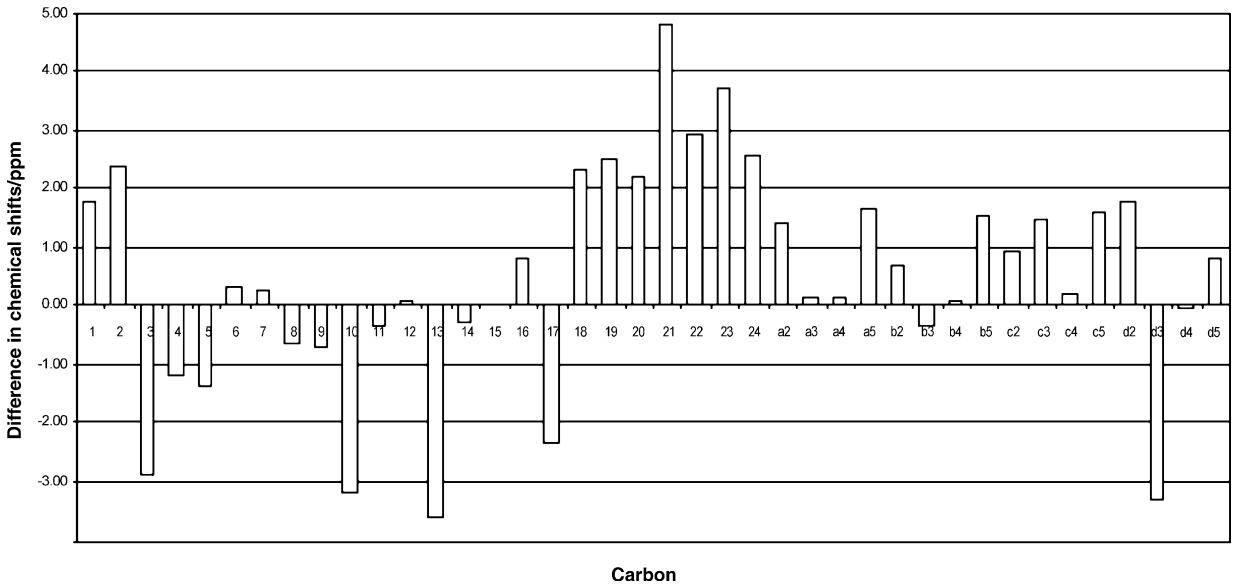


Fig. 3. Differences in calculated ^{13}C NMR chemical shifts of **1** compared to measured shifts.

NOE connectivities were observed between H-b3 in the axial pyrrole b and H-5_{ax} (distance H-b3–H-5_{ax} = 2.35 Å) and the H-4_{eq} (distance H-b3–H-4_{eq} = 2.42 Å) of the cyclohexane ring A of the cholane moiety, while H-a3 in the equatorial pyrrole a showed NOEs with H-2_{ax} (distance H-a3–H-2_{ax} = 2.28 Å) and H-4_{ax} (distance H-a3–H-4_{ax} = 3.58 Å) in cyclohexane ring A, respectively (the distances are measured from minimum energy structure of **1**, so they are not the shortest possible distances). The complete ^{1}H NMR shift assignment of the steroidal part of **1** was not carried out owing to seriously overlapping resonance lines.

The ^{13}C NMR chemical shift assignments of **1**–**3** were based on ^{13}C DEPT-135, PFG ^{1}H,^{13}C HMQC and PFG ^{1}H,^{13}C HMBC (with 3.45 ms low pass filter to remove the effect of direct couplings and 50 ms evolution time for geminal and vicinal couplings) measurements, and the data were compared with our previously published data of similar compounds [3,15]. The ^{13}C NMR chemical shift ranges of two pyrroles at C-3 in **1** are clearly larger than those attached to C-24. This is mainly due to the effect two β-carbons (C-2 and C-4) to C-a2 and C-b2 while C-c2 and C-d2 in the pyrroles at C-24 have only one β-carbon (C-23). Another reason can be that pyrroles at C-24 have a greater motional freedom than those at C-3.

The B3PW91/6-311G*/HF/6-31G* calculated ^{13}C NMR chemical shifts of **1** are also presented in Table 2. The greatest differences between the calculated and experimental data are −3.63 (C-13) and 4.83 ppm (C-21). In general, it can be mentioned that the calculated ^{13}C NMR chemical shifts of the acyclic carbons (such as the methyls 18, 19 and 21 and the side chain) in cholane moiety are more deshielded than the measured ones, whereas carbons 10, 13 and 17, which are members of the cyclic system, and attached to an acyclic carbon, are more shielded. The calculated ^{13}C NMR chemical shifts of the carbons in pyrroles are generally slightly deshielded, but an exception is C-3 in pyrrole d, which is shielded in comparison with the experimental value. The differences between calculated and measured ^{13}C NMR chemical shifts are presented in a graphical form in Fig. 3.

^{15}N NMR chemical shifts of **1** have been determined by PFG ^{1}H–^{15}N HMBC measurement (evolution delay 100 ms) They are close to that of pyrrole itself measured in $CDCl_3$, 145.2 ppm from $\delta(^{15}NH_3) = 0.0$ ppm [21], which corresponds to −235.0 ppm from $\delta(CH_3^{15}NO_2) = 0.0$ ppm.

The ESI-MS measurement of **1** showed the ion $m/z = 625.48$ $[M + Cl]^-$ ($M[C_{40}H_{54}N_4] = 590.90$). EI mass measurement showed ions $m/z = 590$ $[M]^+$, 523 $[M-\text{pyrrole}]^+$, 456 $[M-2\ \text{pyrrole}]^+$, 320 $[M-4\ \text{pyrrole}]^+$ and lower fragments from the steroidal moiety. As EI mass measurement shows, the first two pyrroles are cleaved one at the time, and the last two pyrroles are cleaved at the same time. Probably the pyrroles attached to carbon 3 in steroidal skeleton are the ones, which are first cleaved one at the time due to the more sterically hindered structure. The pyrrole rings attached to the carbon 24 are equal, and so it can be assumed that they are cleaved at the same time.

The ESI-MS measurement showed following ions for **2**: $m/z = 489.40$ $[M\text{-}H]^-$, 625.48 $[M + Cl]^-$, ($M[C_{32}H_{46}N_2O_2] = 490.74$) and for 3: $m/z = 503.38$ $[M-H]^-$, 539.37 $[M + Cl]^-$, ($M[C_{33}H_{48}N_2O_2] = 504.76$).

EI mass measurement showed following ions of interest for **2**: $m/z = 490$ $[M]^+$, 423 $[M-\text{pyrrole}]^+$, and for **3** $m/z = 504$ $[M]^+$, 437 $[M-\text{pyrrole}]^+$, and also the lower fragments from the steroidal moiety. In the cases of **2** and **3** only the cleavage of one pyrrole ring can be observed in EI mass spectra.

4. Conclusions

We describe the syntheses and characterization of three lithocholic acid based dipyrromethanes. Because the syntheses take place with good overall yields, these compounds can be used further in syntheses of other oligopyrroles and complexation studies with transition metal cations.

Acknowledgements

The authors acknowledge Spec. Lab. Tech. Reijo Kauppinen for his help in running the NMR spectra, Spec. Lab. Tech. Mirja Lahtiperä for mass spectra, Lab. Tech. Elina Hautakangas for elemental analysis, and M.Sc. Heikki Tuononen for his assistance in molecular modeling.

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