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Synthesis and Alkaline Metal Ion Binding Ability of New Steroid Dimers Derived from Cholic and Lithocholic Acids

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Abstract

The synthesis and NMR spectra of steroid dimers being derivatives of cholic and lithocholic acid methyl esters, in which the steroid residues are linked by the terephthaloyl spacer, are described. The complexing ability of the dimers towards alkaline metal cations has been studied by the electrospray mass spectrometry. Cholic acid derivatives show affinity toward metal cations and anions. Binding of metal cations by methyl lithocholate dimer has not been detected.

Introduction

Steroids are compounds of natural origin and play an important role in biological systems. Cholesterol is a component of cell membranes in *Eucaryotes*, and serves as substrate for production of bile acids and steroid hormones. In cells, steroids are recognised by antibodies and enzymes. Synthetic receptors [1] of steroids have been also reported. Some resorcin[4]arene hosts have shown capability of complexation of bile acids' derivatives [2], cholesterol [3a] and corticosteroids [3b], while cyclophanes have been tested for recognition of androsterone and estrogens [4]. Water-soluble cyclophane receptors are found effective for complexation of bile acid salts [5]. Steroids themselves have been used as building blocks for designing and construction of molecular receptors which serve for recognition of guest molecules of diverse chemical nature. Some excellent review papers have been published [6–9]. The first attempts to synthesize artificial receptors of steroid type have been reported by groups of Guthrie and Ueda [10] and McKenna *et al*. [11]. The major types of cyclic host structures derived from steroids are cyclocholates made of up to four bile acid units, cholaphanes which incorporate two or more bile acid residues joined together by spacer groups of diverse chemical character, and steroid cyclophanes. Other bile acid-based artificial receptors are molecular clefts and molecular tweezers [6]. The cleft-type steroid receptors are head-to-head dimers in which two rigid steroid frameworks are linked by spacer group of aliphatic or aromatic character. The spacer groups link C(3) and C(3′) [12, 13] or C(24)—C(24′) [14, 15] carbon atoms of cholic or lithocholic acid. Some head-to-tail steroid dimers have also been reported [16, 17]. Interestingly, steroid dimers have been found to be more efficient than monomers in complexing guest molecules [18, 19]. Steroid dimers are also attractive substrates for macrocyclisation. Steroid-based macrocyclic compounds prepared by this approach might function as artificial receptors. These macrocyclic artificial receptors are usually synthesised from appropriately functionalized monomers or dimers which themselves can serve as supramolecular hosts.

Electrospray ionisation mass spectrometry (ESI-MS) has only recently been used as a method of studying formation of complexes between steroids and metal ions [20, 21]. Metal complexing properties of monomeric bile acids, cyclocholates and cholaphanes of di-, tri- and tetramer structure have been investigated [20, 21].

In this paper we present a synthesis of new steroid dimers with aromatic spacer group of ester type, which are potential receptors for neutral molecules and metal cations. The alkaline metal ion binding ability of these compounds is investigated by the electrospray mass spectrometry.

Experimental

General experimental conditions

The IR spectra were taken on an FT-IR Bruker FS 113V spectrophotometer, in chloroform solutions or KBr discs. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 VT spectrometer operating in the Fourier transform mode, in deuteriochloroform

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solutions. The chemical shifts (δ) are expressed in ppm relative to tetramethylsilane. DEPT technique was used for the assignment of multiplicity of carbon signals in ^{13}C NMR spectra. The additivity rules and comparison with data reported [22] for compounds of similar structure were helpful for signal assignment. The progress of reactions and purity of compounds was monitored by TLC using a precoated aluminium-backed silica plates (E. Merck, no. 5554). Silica gel 60 (Merck 70–230 mesh, no. 7734) was used for flash chromatography.

Electrospray mass spectra

ESI MS experiments were performed using a Walters/Micromass ZQ ES mass spectrometer with methanol solvent system entering the chamber at a rate of 40 μL/min. The source temperature was 120 °C and the convoltage was set at 30 V. All the spectra were analysed as the sum of 10 scans, each of 0.6 s duration. Metal ions were added in the form of their perchlorate (Li), chloride (Na, K) or iodide (Rb, Cs) salts dissolved in MeOH (10^{-4} mol L^{-1}) to a dimer solution in MeOH (10^{-4} mol L^{-1}).

Synthesis

Methyl 3α-hydroxy-5β-cholan-24-oate **1**, methyl 3α,7α,12α-triacetoxy-5β-cholan-24-oate [23] and methyl 3α-hydroxy-7α,12α-diacetoxy-5β-cholan-24-oate **3** [24] were prepared according to literature procedures.

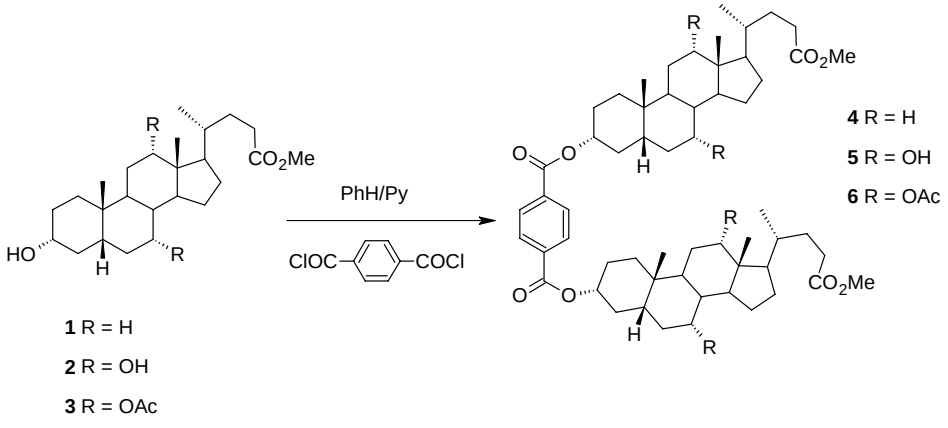
Bis(methyl 5β-cholan-24-oate-3α-yl)terephthalate **4**
To the solution of methyl lithocholate **1** (200 mg, 0.52 mmol) in anhydrous benzene (5 mL) and anhydrous pyridine (2 mL) terephthaloyl chloride (68 mg, 1.2 equiv., 0.33 mmol) was added. The solution was stirred at room temperature for 24 h (TLC control). When the substrate disappeared, a few mL of benzene-diethyl ether (1:1) mixture was added and the formed precipitate was filtered off and dissolved in $CHCl_3$. This solution was washed with H_2O, HCl (1 N), $NaHCO_3$ (5%) solution, dried with magnesium sulphate and evaporated to give 188 mg of the pure solid product **4.** The additional crop of the product was isolated from the benzene–Et_2O–Py solution. It was chromatographed on a silica gel column to give dimer **4** (15 mg, 87% total yield); ν_{max} (cm^{-1})(KBr): 1740, 1717, 1272, 1252 and 730.

Bis(methyl 7α,12α-diacetoxy-5β-cholan-24-oate-3α-yl) terephthalate **6**
Compound **6** was prepared from **3** according to the procedure described above. After the work-up, the crude product was chromatographed on an SiO_2 column to give dimer **6** as an oil in 85% yield; ν_{max} (cm^{-1})(KBr): 1736, 1713, 1271, 1250 and 734.

Bis(methyl 7α,12α-dihydroxy-5β-cholan-24-oate-3α-yl) terephthalate **5**
To a solution of methyl cholate **2** [25] (500 mg, 1.18 mmol) in anhydrous benzene (10 mL) and pyridine (0.5 mL) terephthaloyl chloride (180 mg, 0.72 mmol) was added. The mixture was stirred for 12 h at room temperature (TLC control). The mixture was diluted with $CHCl_3$ (10 mL), the solution was filtered off and washed with HCl (1 N), Na_2CO_3 solution (10%) and brine. The solvents were evaporated and the residue was chromatographed on an SiO_2 column with $CHCl_3$/AcOEt(3:1) and $CHCl_3/Me_2CO$ (20:1) as eluents to give compound **5** (200 mg, 35% yield) as the solidifying oil; ν_{max} (cm^{-1})($CHCl_3$): 3614, 3530, 1740, 1711, 1274 and 1251.

Results and discussion

The effective coupling of the two steroidal residues was achieved in the reaction of monomeric bile acid methyl esters with acylating reagent (Scheme 1). Thus, the



Scheme 1. Input file for the *SPACE* program.

Table 1. The characteristic ^{1}H NMR chemical shifts (δ) of the bile acid dimers **4–6**

^{1}H-NMR Proton	**4**	**5**	**6**
H-3	4.98 (m, 2H)	4.85 (m, 2H)	4.82 (m, 2H)
H-7		3.88 (bs, 2H)	4.93 (bd, J = 2.5 Hz, 2H)
H-12		4.02 (bs, 2H)	5.11 (bs, 2H)
H-18	0.66 (s, 6H)	0.72 (s, 6H)	0.75 (s, 6H)
H-19	0.97 (s, 6H)	0.946 (s, 6H)	0.96 (s, 6H)
H-21	0.91–0.93	0.98–1.00	0.81–0.83
	(d, J = 6.3 Hz, 6H)	(d, J = 6 Hz, 6H)	(d, J = 6.3 Hz, 6H)
tere-phtalate	8.08 (s, 4H)	8.07 (s, 4H)	8.09 (s, 4H)
—COOC**H**$_3$	3.66 (s, 6H)	3.67 (s, 6H)	3.67 (s, 6H)
—C**H**$_2$—COOMe	2.17–2.41 (two m, 4H)		
C**H**$_3$COO-(7)			2.07 (s, 6H)
C**H**$_3$COO-(12)			2.15 (s, 6H)

Table 2. ^{13}C NMR chemical shifts of bile acid dimers **4–6**

Carbon	**4**	**5**	**6**
C-1	34.97	34.84	34.67
C-2	28.12	26.69	27.03
C-3	75.51	75.39	75.37
C-4	32.23	35.13	34.81
C-5	41.89	42.06	41.04
C-6	26.65[a]	34.37	31.32
C-7	26.27[a]	68.25	70.71
C-8	35.73	39.49	37.84
C-9	40.41	26.77	29.02
C-10	34.57	34.69	34.43
C-11	20.81	28.37	25.67
C-12	40.04	72.92	75.37
C-13	42.66	46.54	45.12
C-14	56.39	41.20	43.46
C-15	24.11	23.11	22.89
C-16	26.97[a]	27.42	27.25
C-17	55.90	47.22	47.43
C-18	11.98	12.55	12.32
C-19	23.29	22.53	22.66
C-20	35.29	35.13	34.67
C-21	18.21	17.34	17.59
C-22	30.93[b]	31.01[b]	30.96[b]
C-23	30.96[b]	30.82[b]	30.85[b]
C-24	174.73	174.72	174.28
—O**C**H$_3$	51.44	51.52	51.52
tere-**C**=O	165.32	165.45	165.11
tere C-1/4	134.41	134.42	134.41
tere C-2/3	129.32	129.35	129.26
CH$_3$**C**OO-(7)			170.02
CH$_3$**C**OO-(12)			170.18
CH$_3$COO-(7)			21.44
CH$_3$COO-(12)			21.64

[a,b] These signals may be interchanged.

steroid dimers **4** and **6** were prepared from lithocholic and 7,12-diacetylcholic acid methyl esters **1** and **3,** in acylation of the free 3α-hydroxy group with terephthaloyl chloride in 87 and 85% yield, respectively. The enhanced reactivity of the equatorial 3-OH group in cholic acid enabled preparation of dimer **5** in 35% yield from methyl cholate [25]. TLC analysis of the crude reaction product showed the presence of other products, possibly 3,7′- and 3,12′-dimers. This was confirmed by ^{1}H NMR spectrum, which showed signals at δ 5.22 and 5.40. The dimers **4–6** were fully characterised by NMR spectra, which were in agreement with the symmetric structure of the dimers. ^{1}H NMR spectra showed all characteristic signals in the low frequency region (Table 1).

The full assignment of signals in ^{13}C NMR spectra of the dimers is presented in Table 2. These compounds can exist in conformation 'syn' and 'anti' as shown in Figure 1. In solution, the equilibrium between the two conformers is expected to depend on the polarity of the solvent. The dimers **4–6** are potential receptors for neutral and ionic guest molecules.

It has been shown that electrospray mass spectrometry experiments involving competition between a range of metal ions and a single host can yield useful information on relative binding affinities [20, 26]. Thus, the alkaline metal ion binding ability of the synthesised dimers has been investigated.

The positive ion electrospray mass spectrum of solution of **5** in MeOH showed peaks at m/z 997 and 1013 (Figure 2(a)), assigned to sodium and potassium complexes, respectively, formed with traces of both ions in the mobile phase. The spectrum of **5** with added KCl showed the expected peak $[M + K]^+$ and a low intensity peak of $[M + Na]^+$ resulting from sodium impurities. When a mixture of 10^{-4} M solutions of NaCl and KCl with 10^{-4} M solution of compound **5** was injected into the spectrometer, the intensity of the peaks $[M + K]^+$ and $[M + Na]^+$ was 100 and 40%, respectively, indicating a selectivity towards potassium (Figure 2b). Furthermore, peaks of $[M + Rb]^+$ and $[M + Cs]^+$ were identified in the spectra of **5** in MeOH with added solution of RbI and CsI. However, in these spectra no peak of the background sodium was detected, suggesting a strong preference for binding larger cations of rubidium and cesium, which might be explained by the π-cation interaction [20, 27]. In the presence of cations of small size, like Li^+ and Na^+, the 2:1 complexes with **5** were detected by the signals of ions

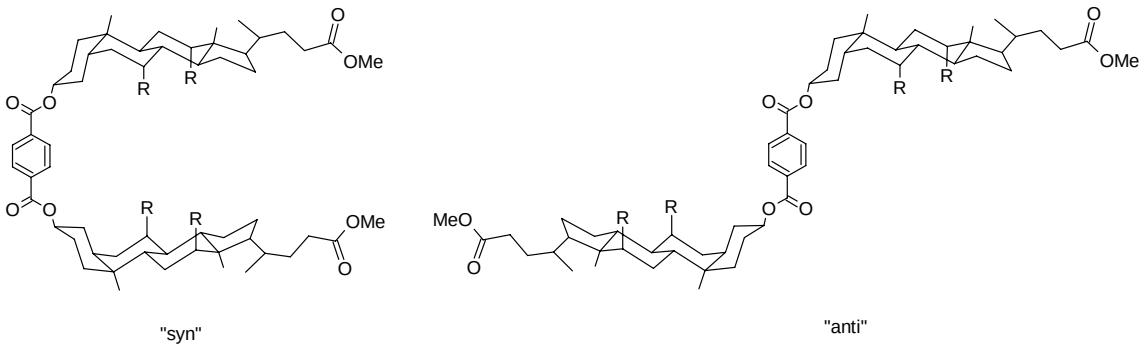


Figure 1. The two-low energy conformations of steroid dimers **4**–**6**.

$[M + Li + LiClO_4]^+$ at *m/z* 1087 and $[M + 2Na]^{2+}$ at *m/z* 511.5 in the spectra. In general, only singly charged peaks were observed for larger cations in the conditions applied. The reduced affinity for complexation of another metal ion in the case of K^+, Rb^+ and Cs^+ complexes suggests that in these adducts ligand **5** assumes the conformation 'syn' and the cation is placed in the cavity of the dimer. ESI mass spectrum of **5** in the presence of a mixture of equimolar amounts of Li^+, Na^+, K^+, Rb^+ and Cs^+ was also measured (Figure 3a).

The spectrum revealed peaks corresponding to complexes with all cations studied, the ones assigned to caesium $[M + Cs]^+$ at *m/z* 1107 and sodium $[M + Na]^+$ at *m/z* 997 being the most intense. The spectra shown in Figure 3 reflect the complicated equilibrium existing in solution when all metal cations compete for binding with the steroidal dimer ligand.

The ESI mass spectra of the complexed dimer **6** were more complicated. The spectra obtained for solutions of **6** containing alkaline metal cations exhibited peaks indicating formation of singly charged adducts for each cation. In the spectrum of **6** with equimolar amounts of K^+ and Na^+ the peak $[M + K]^+$ was slightly more intense (Figure 2c), while the mixture of **6** with added mixture of K^+ and Rb^+ gave similar intensity peaks $[M + metal]^+$ in the spectrum. Despite the mild ionisation conditions, in all spectra of **6** with alkaline metal cations, peaks indicating fragmentation of the molecular complex were observed. For example, solution of **6** and KCl gave intense peak at *m/z* 587 besides the low intensity peak corresponding to $[M + K]^+$ at *m/z* 1181. The spectrum of the mixture of **6** and KCl + NaCl, revealed fragments *m/z* 571 and 587, while that of the mixture of **6** with Li^+, Na^+, K^+, Rb^+ and Cs^+, showed

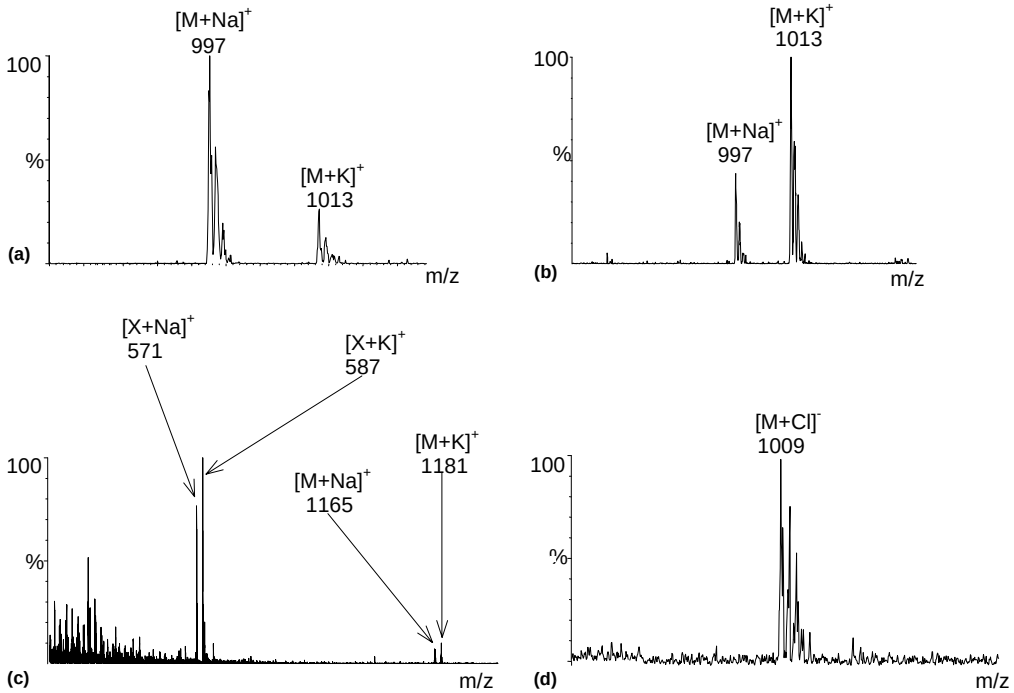


Figure 2. ESI-MS of compounds **5** (a,b,d) and **6** (c).

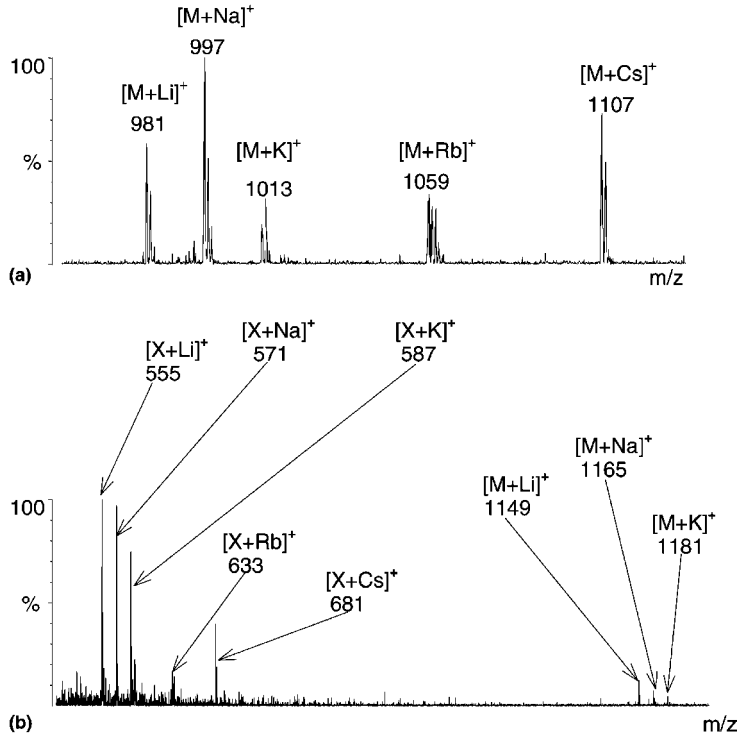
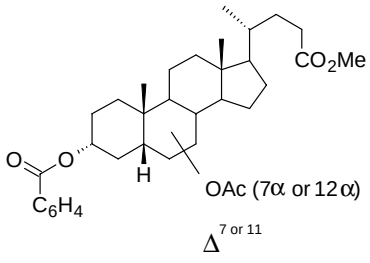


Figure 3. ESI-MS of equimolar mixture of compounds **5** (a) or **6** (b) with Li^+, Na^+, K^+, Rb^+ and Cs^+.

all relevant peaks at m/z 555, 571, 587, 633 and 681, respectively (Figure 3b). These peaks correspond to $[Z + metal]^+$ fragmentation ions, with Z most probably having the structure shown in formula **7**. The fragment Z might arise from elimination of one steroid unit from the molecular complex $[M + metal]^+$ according to the equation:

$$[M + metal]^+ \rightarrow [Z + metal]^+ + steroid + AcOH.$$



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In the spectrum of **6** with $LiClO_4$, ions $[M + Li]^+$ at m/z 1149, $[M + LiClO_4 + Li]^+$ at m/z 1255 and $[M + LiClO_4 + 2Li]^{2+}$ at m/z 631 were present, thus indicating a more complicated coordination pattern of a hard cation of a small radius [28].

It is interesting to note that in the negative ion electrospray mass spectra of the dimers, coordination of anions was also observed. The complexation of Cl^- was proved by the presence of the peak m/z 1009 in the spectrum of **5** with NaCl (Figure 2d), while that of I^- was detected in the spectrum of **6** with iodide salts. This type of encapsulation of anions by electroneutral hosts has been reported earlier [29].

The ESI mass spectra of methyl lithocholate dimer **4** with all alkaline metal salts gave no indication of a strong binding of cations to this ligand. This result is in contrast to the reported affinity of lithocholic acid based cholaphanes towards sodium or potassium [21].

Conclusion

The synthesis of new bile acid derived dimers of cleft type was described. In contrast to the behaviour of lithocholic acid methyl ester dimer **4**, high affinity of cholic acid methyl ester dimer **5** and its 7,12-diacetyl derivative **6** towards alkaline metal cations was demonstrated. It is assumed that due to conformational flexibility of the dimers no selectivity of binding a specific cation was clearly observed. The preference of binding K^+ more effectively than Na^+ was shown for the dimer **5**. The stable ions indicating fragmentation of dimer complexes $[M + metal]^+$ resulting in formation of $[Z + metal]^+$ ions were observed in all spectra of **6** in solution with alkaline metal salts studied. The preference for singly charged ions $[M + metal]^+$ suggests formation of complexes in which the ligand **5** or **6** takes conformation 'syn'. The coordination ability of **5** and **6** towards Cl^- and I^- anions was also evidenced.

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