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^{1}H-, ^{13}C-, and ^{15}N-NMR and ESI-TOF^{+} MS studies of a supramolecular complex of silver(I) and a cholaphane

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Abstract

A novel application of the mixed anhydride procedure for synthesising lithocholic acid piperazine diamide, an important intermediate in designing bile acid-based supramolecular host molecules, is reported. The synthesis of a thiophene-containing cholaphane with transition metal complexation ability and ^{1}H-, ^{13}C-, and ^{15}N-NMR as well as ESI-TOF^{+} MS spectral characterisation of the ligand and its Ag(I) complex are included. The coordination of the Ag(I) ion as well as an ability of the cholaphane to recognise Ag(I) ion over alkali metal ions, especially potassium ion, is discussed. The possible medical applications are also presented.

Keywords: Cholaphane; Thiophene; Piperazine; Ag(I) complexation; Multinuclear magnetic resonance; ESI-TOF^{+} MS

1. Introduction

Cholaphanes (Fig. 1) are bile acid-based macrocycles consisting of bile acid units joined together by various spacer groups. Dimeric cholaphanes have less freedom and can be made more rigid and pre-organized for strong and selective binding compared to the linear bile acid-based structures [1]. Some of these cyclic systems have shown remarkable capability for diastereo- and enantioselective binding of carbohydrate derivatives in organic solvents [2]. Because the design of macrocyclic synthetic receptors with molecular cavities has been of considerable interest during recent years, the cholaphanes with their large, rigid, and curved steroidal skeletons, variable substituents at positions C7 and C12, and spacer groups with different chemical activities, represent an ideal group of compounds for serving as model compounds for complex biological systems and molecular recognition of substrates in enzymatic processes.

Bile acids and their derivatives are important compounds also from the pharmacological point of view. They have been used in the treatment of bile acid deficiency and liver diseases as well as in dissolution of cholesterol gallstones [3]. They have potential to act as carriers of liver-specific drugs, absorption enhancers, and as cholesterol level lowering agents [4]. In the body bile acids combine with glycine and taurine forming amides, which are hydrolysed by intestinal bacteria in the metabolic pathway. Therefore, it is expected, that analogous polymeric bile acid derivatives would also have a tendency of being biodegradable, or exhibit other biological activity [5]. Bile acid polyamine conjugates are potential agents in gene therapy [6] because of the Coulombic attraction between the polyamine moieties and the polyphosphate backbone of deoxyribonucleic acid [7]. Polymeric bile acid and insulin conjugate can be used for the production of a medicament for *Diabetes mellitus* [8].

Bile acid derivatives conjugated with metal ion chelated complexes have been used successfully as contrast agents in magnetic resonance imaging (MRI). They have been used for example in MRI assessment of microvascular hyperpermeability in a rat breast tumor as well as an anti-VEGF (vascular endothelial grown

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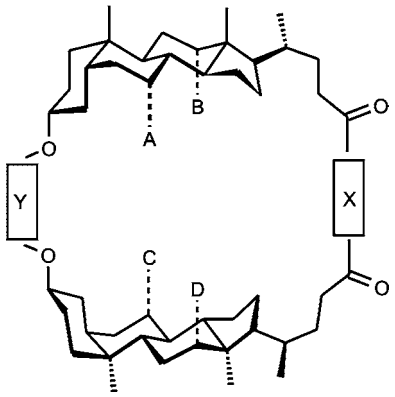


Fig. 1. General dimeric structure of a cholaphane (A–D = binding/catalytic function; X, Y = spacer).

factor) agents [9] and as blood pool agents for NMR diagnostics [10–12].

In our previous studies we have reported syntheses and spectral characterisations of some bile acid-based cleft-type compounds and cholaphanes [13–18]. We have also investigated their alkali metal [18] and Ag(I) cation [14,17] binding properties. In this study we have chosen 2,5-thiophene dicarboxylic acid as a structural fragment in order to tailor the cholaphane ring size thus modifying the coordination sphere suitable for various metal cation recognition. Piperazine has been maintained as the other bridging group. As a new detail a novel one-pot synthetic procedure for preparing the piperazine-containing dimer (3α,3′α-dihydroxy-5β-cholan-24-oic acid piperazine diamide) is presented. Piperazine has metal complexing capabilities and is a good hydrogen-bond acceptor [19], which makes its derivatives interesting for supramolecular complexation chemistry. In addition, it has been shown [20] that some *N*,*N*′-disubstituted piperazine compounds are high-affinity ligands for σ-1 and σ-2 type receptors, which have potential functional role in several important physiological and biochemical processes.

The Ag(I) ion complexation experiments performed by modern multinuclear NMR experiments as well as by ESI-TOF$^+$ MS measurements are particularly interesting, because of a recent finding according to which Pt(II), Pd(II), and Au(III) complexes with bile acids, such as cholic, chenodeoxycholic, and ursodeoxycholic, and a halide or NH_3 may be useful cytostatic agents. One such complex actually has shown cytostatic activity against human colon adenocarcinoma cells [21].

2. Experimental

2.1. Compounds

Lithocholic acid (3α-hydroxy-5β-cholan-24-oic acid) was a 98% reagent and 2,5-thiophene dicarboxylic acid a 99% reagent from Aldrich Chemical Co. Piperazine was purchased from Fluka Chemie AG as >99% reagent. 3α,3′α-Dihydroxy-5β-cholan-24-oic acid piperazine diamide was synthesised by the mixed anhydride method following a previously reported procedure [22–25]. The ring was closed following a modification of the Yamaguchi macrolactonisation reaction [13,26] yielding 2,5-thiophene dicarboxylic acid diester of 3α,3′α-dihydroxy-5β-cholan-24-oic acid piperazine diamide (cholaphane).

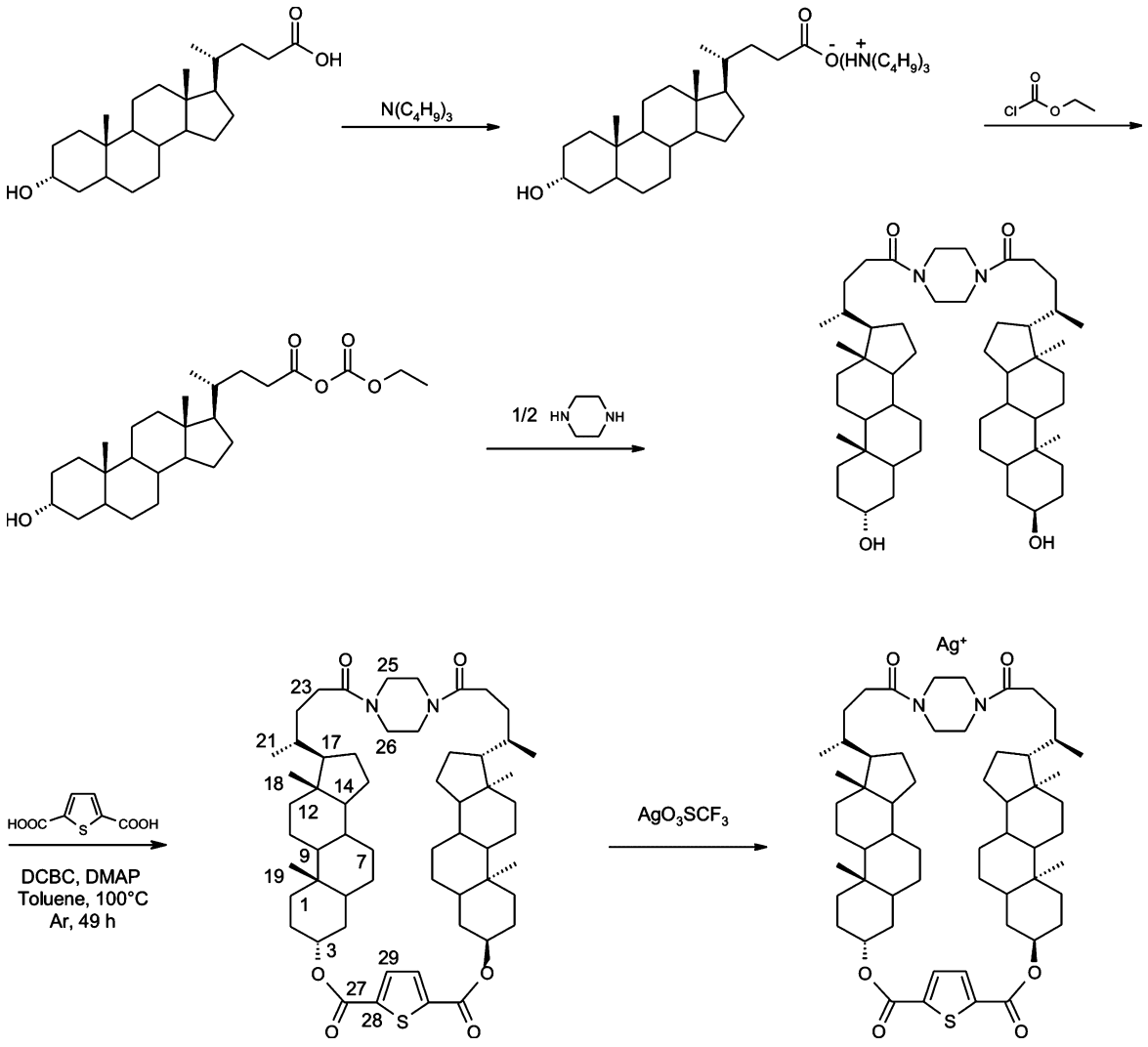
The synthetic route to the cholaphane is described in Scheme 1.

2.1.1. 3α,3′α-Dihydroxy-5β-cholan-24-oic acid piperazine diamide

Lithocholic acid (7.54 g, 20 mmol) was dissolved in 120 ml of Na dried 1,4-dioxane. Then 3.70 g (20 mmol) of tri-*n*-butylamine was added. The solution was cooled to +10 °C and 2.18 g (20 mmol) of ethyl chloroformate in 10 ml of dioxane was added dropwise during 15 min. The temperature was kept constant while stirring the reaction mixture for additional 30 min. A solution of 0.86 g (10 mmol) of piperazine in 10 ml of water was added dropwise at the same temperature, and the resulting solution was stirred for 2 h. During the stirring the temperature was allowed to reach room temperature spontaneously. The reaction mixture was dissolved to $CHCl_3$ (75 ml), washed with sat. $NaHCO_3$ (2 × 60 ml) and water (2 × 60 ml), dried ($MgSO_4$), and evaporated under vacuum. The crude product was purified by sequential column chromatography: (i) silica gel (0.040–0.063 mm), $CHCl_3$–C_3H_6O (80:20); (ii) silica gel (0.040–0.063 mm), $CHCl_3$–C_3H_6O (70:30); (iii) silica gel (0.040–0.063 mm), $CHCl_3$–C_3H_6O–MeOH (70:30:1). The pure product was obtained in 51% yield. The purity and molecular structure of 3α,3′α-dihydroxy-5β-cholan-24-oic acid piperazine diamide were ascertained by comparing the ^{1}H- and ^{13}C-NMR spectra to the corresponding spectra of the previously [17] synthesised product.

2.1.2. 2,5-Thiophene dicarboxylic acid diester of 3α,3′α-dihydroxy-5β-cholan-24-oic acid piperazine diamide (cholaphane)

To a suspension of 1.20 g (1.49 mmol) of 3α,3′α-dihydroxy-5β-cholan-24-oic acid piperazine diamide and 0.26 g (1.49 mmol) of 2,5-thiophene dicarboxylic acid in sodium dried $C_6H_5CH_3$ (150 ml) 1.50 g (4.0 equivalents) of 4-(*N*,*N*-dimethyl)aminopyridine (DMAP) was added and the reaction mixture heated to 100 °C. After that 0.70 g (1.1 equivalents) of 2,6-dichlorobenzoyl chloride (DCBC) was added and the mixture kept under Ar-atmosphere at 100 °C for 49 h. The crude product was dissolved in $CHCl_3$ (75 ml) and washed with sat. $NaHCO_3$ (2 × 60 ml) and water (2 × 60 ml), dried ($MgSO_4$), and evaporated to dryness under vacuum. The product was separated by sequential



Scheme 1.

column chromatography: (i) silica gel (0.063–0.200 mm), $CHCl_3$–C_3H_6O–MeOH (95:4:1); (ii) silica gel (0.040–0.063 mm), $CHCl_3$–C_3H_6O–MeOH (70:20:10); yield 0.39 g (28%).

2.1.3. Ag(I) complex of 2,5-thiophene dicarboxylic acid diester of 3α,3′α-dihydroxy-5β-cholan-24-oic acid piperazine diamide

The Ag(I) binding experiments were done by dissolving 13.67 mg (0.05 mmol) of AgO_3SCF_3 in 1 ml of $CDCl_3$ containing 50 mg (0.05 mmol) of the cholaphane so that the cation–ligand molar ratio was 1:1. For the MS measurements the sample solutions were diluted to concentrations of 0.01 mg ml^{-1} by using MeOH (p.a. grade).

2.2. Spectroscopy

All ^1H, ^{13}C, ^{13}C DEPT-135, PFG ^1H,^{13}C HMQC [27,28], PFG ^1H,^{13}C HMBC and PFG ^1H,^{15}N HMBC [29], and ^1H,^1H COSY [30,31] NMR measurements were performed in 0.05–0.1 M $CDCl_3$ solutions with a Bruker Avance DRX 500 spectrometer equipped with a 5 mm diameter broad band inverse observation (proton) probehead with a z-gradient accessory [32] working at 500.13 MHz in ^1H, 125.77 MHz in ^{13}C, and 50.70 MHz in ^{15}N experiments, respectively. The ^1H- and ^{13}C-NMR chemical shifts were referenced to the residual signal of partly deuteriated solvent: $\delta(C^1HD_2) = 7.26$ ppm from the internal Me_4Si and to the signal of solvent $\delta(^{13}CD_3) = 77.00$ ppm from the internal Me_4Si, respectively. The ^{15}N-NMR chemical shifts were referenced to the signal of external nitromethane, $\delta(CH_3^{15}NO_2) = 0.0$ ppm, in a 1 mm diameter capillary inserted coaxially inside the 5 mm NMR tube. The complete lists of the acquisition and processing parameters are available from E.K. on request.

In the PFG ^1H,^{15}N HMBC experiment the pulse program, inv4gslplrnd, was selected from the manufacturer's library and a 50 ms evolution delay (correspond-

ing to 10 Hz proton–nitrogen-15 spin–spin coupling) was incorporated in the pulse program. The PFG $^{1}H,^{15}N$ HMBC (heteronuclear multiple bond correlation) pulse program was selected because both in the reference and the studied compound the nitrogens are unprotonated. However, in the HMBC experiment all proton bearing nitrogens are also visible as doublets split by proton–nitrogen spin–spin couplings when the low-pass filter set at the start of the pulse program does not exactly match the evolution delay of $^{1}J(N,H)$. By this arrangement the information given by both HMQC and HMBC is obtained by a single heteronuclear chemical shift correlation experiment.

Electrospray mass spectrometric measurements were performed using an LCT time of flight (TOF) mass spectrometer with electrospray ionisation (ESI; Micromass LCT). The spectrometer utilised two hexapole RF lenses to transfer ions from the source to the orthogonal acceleration TOF mass analyser. Ions emerging from the analyser were detected by a dual microchannel plate detector and ion counting system. Controlling the LCT as well as acquiring and processing the data were performed with a MassLynx NT software system. In each experiment a flow rate of 10 μl min^{-1} was used for the sample solution and the sample droplets were dried with nitrogen gas. The potentials of 118 and 3 V for the sample and extraction cones were applied. The RF lens was set at a potential of 500 V and the potential in the capillary at 3187 V. The desolvation temperature was set at 200 °C and the source temperature at 100 °C. The metal complexation experiment was done equally. For the sample and extraction cones potentials of 125 and 3 V were applied. The RF lens was set at a potential of 500 V and the potential in the capillary at 3200 V. The temperature of the desolvation was decreased for 20 °C.

For the accurate mass measurement the calibration of the instrument was made with NaI. The nominally measured mass and the centroided spectrum were subjected to a mass scale correction obtained from the variation of the observed value of the reference mass from its theoretical monoisotopic mass. The necessary mass scale correction was applied to the spectrum relative to the fifth order calibration curve used for the acquisition of the raw data. An additional mass correction was applied from an algorithm, which accounts for the observed statistical shift in the centroided mass with signal intensity resulting from the detector dead time. The reference was introduced by flow injection (10 μl min^{-1}) and the sample by loop injection. The potentials of 60 and 3 V for the sample and extraction cones were applied. The RF lens was set at a potential of 500 V and the potential in the capillary at 3187 V. The desolvation temperature was set to 200 °C and the source temperature to 100 °C. Leucin-enkephalin (1 ng μl^{-1}) was used as a reference ion.

3. Results and discussion

3.1. Synthesis

The traditional method for preparing 3α,3′α-dihydroxy-5β-cholan-24-oic acid piperazine diamide, an important intermediate in designing bile acid-based supramolecular host molecules [17], starts with the protection of the 3α-OH group of lithocholic acid by trifluoroacetic acid anhydride. The resulting 3α-trifluoroacetoxy derivative is then refluxed with thionyl chloride in order to convert it to the activated acid chloride. The protected acid chloride and piperazine are allowed to react for 4 days followed by a deprotection of the 3α-trifluoroacetoxy groups. The successive reaction steps of this four-step synthesis are extremely time-consuming. Additionally, at every reaction step some of the product is lost leading to moderate or even low yields. In this work a time-saving one-pot synthesis with a reasonably good yield for the preparation of 3α,3′α-dihydroxy-5β-cholan-24-oic acid piperazine diamide is described. The product is synthesised by the mixed anhydride method following a previously reported procedure [22–25] and also applied by us in the synthesis of *N*-deoxycholyl-L-tryptophan [33]. The synthetic procedure starts by allowing tri-*n*-butylamine and lithocholic acid to react forming a salt, which is further converted to a very reactive anhydride derivative by ethyl chloroformate. Addition of piperazine in the molar ratio of 2:1 results to the formation of the desired product with a 51% yield. The most significant advantage of this method is that the product can be obtained in a few days (including the column chromatographic purifications) compared to duration for over a week of the former method. Also the yield (51%) is slightly better compared to the previous case (46%). The reported application of the mixed anhydride method provides a simple way of synthesising not only mono- but also diamides of bile acids. The diamide obtained was then allowed to react with 2,5-thiophene dicarboxylic acid in the presence of DMAP and DCBC. The resulting cholaphane was purified by sequential column chromatography and exposed to a reaction with AgO_3SCF_3.

3.2. Spectroscopy

The ^{1}H- and ^{13}C-NMR chemical shifts of 2,5-thiophene dicarboxylic acid diester of 3α,3′α-dihydroxy-5β-cholan-24-oic acid piperazine diamide and its Ag(I) complex are collected in Tables 1 and 2, respectively.

For the lithocholyl fragment only some proton chemical shifts are given in Table 1 because at 500 MHz unambiguous assignments of all protons are not possible. The ^{1}H- and ^{13}C-NMR chemical shift assignments are based on the literature spectra of previously synthesised cholaphanes [13,17] and 2,5-thiophene di-

Table 1
Selected ^{1}H-NMR chemical shifts (ppm from $CDCl_3$, $\delta = 7.26$) of the cholaphane and its Ag(I) complex

Proton	Ligand	Ligand + Ag^+
3β-H	4.90	4.91
18-CH_3	0.64	0.66
19-CH_3	0.94	0.95 (overl.)
21-CH_3	0.95	0.95 (overl.)
23-CH_α	2.14	2.26
23-CH_β	2.34	2.40
pipe 1-H	3.53	3.61
pipe 2-H	3.62	3.67
thio CH (ar.)	7.78	7.80

Table 2
^{13}C- and ^{15}N-NMR chemical shifts (ppm from $CDCl_3$, $\delta = 77.00$ and from ext. CH_3NO_2, $\delta = 0.0$) of the cholaphane and its Ag(I) complex

Assignment	Ligand	Ligand + Ag^+
1	34.95	35.02
2	26.71	26.78
3	76.14	76.21
4	32.34	32.41
5	41.98	42.05
6	27.02	27.10
7	26.42	26.49
8	35.78	35.87
9	40.51	40.55
10	34.64	34.71
11	20.75	20.82
12	40.12	40.15
13	42.65	42.73
14	56.48	56.47
15	24.18	24.26
16	28.14	28.14
17	54.68	54.62
18	11.83	11.88
19	23.32	23.38
20	34.37	34.44
21	18.61	18.60
22	31.02	31.16
23	28.23	28.41
24	172.24	174.07
25	45.70	45.48
26	41.24	41.39
27	160.94	161.06
28	138.17	138.25
29	133.56	133.63
pipe N	−267.6	−264.4

carboxylic acid as well as on the present ^{13}C DEPT-135, PFG ^{1}H,^{13}C HMQC, and PFG ^{1}H,^{13}C HMBC experiments.

At room temperature the ^{1}H-NMR chemical shift pattern of piperazine appears asymmetric. This is believed to be due to the steric strain of the cholaphane ring observed also in the ^{13}C-NMR chemical shifts as described later in this paper and reported by us previously [17]. In order to investigate the effect of temperature to the ^{1}H-NMR chemical shift pattern of piperazine variable temperature ^{1}H-NMR experiments between 323 and 223 K in 10 K temperature steps were performed. At 323 K the signal of piperazine 2-H (3.62 ppm) appears as a broad singlet in contrast to the doubly split signal of 1-H (3.54 ppm). As the temperature is lowered first the fine structure of the pattern becomes more visible until the signals of 1-H and 2-H start to coalesce. At 273 K the chemical shift pattern of piperazine is almost symmetrical. Finally, at 223 K the piperazine signal appears as a singlet (3.59 ppm). This behaviour is believed to be due to the conformational equilibrium between the chair, half-chair, twist-boat, and boat conformations of the piperazine moiety.

As can be seen from Table 1, the most significant ^{1}H-NMR chemical shift changes associated with AgO_3SCF_3 addition occur in the geminal protons at C23 (23-H_α and 23-H_β). The form of the piperazine chemical shift pattern at room temperature also differs clearly from the corresponding pattern of the free ligand. When Ag(I) cation is added to the solution, the pattern changes from unsymmetric to symmetrical, that is in the complex the piperazine chemical shift pattern appears as two singlets. Variable temperature ^{1}H-NMR experiments at 303, 273, 243, and 223 K show the same kind of behaviour as detected for the free ligand, that is the signals start to coalesce for conformational reasons. At low temperatures the overall resolution of the proton spectrum is decreased.

Previously we have reported significant differences in the ^{13}C-NMR chemical shifts of carbons C17 and C20 between an open and a terephthalic acid-closed lithocholic acid-based dimers [17]. The ^{13}C-NMR chemical shifts of C17 and C20 in an open dimer are typically ca. 56 and 36 ppm, respectively. In a closed cholaphane the ^{13}C-NMR chemical shifts of those particular carbons are shifted upfield more than 2 ppm [17], indicating a change in the conformational preferences of the flexible tail as the cycle is formed. The same trend denoting steric strain of the ring is observed also in the present cholaphane.

The formation of the Ag(I) complex of the cholaphane in solution can unambiguously be detected by ^{13}C-NMR chemical shift changes and ESI-TOF$^+$ MS results. The most significant changes in ^{13}C-NMR chemical shifts are observed for carbons C22 (0.14 ppm), C23 (0.18 ppm), C24 (1.83 ppm), C25 (0.22 ppm; piperazine-C1), and C26 (0.15 ppm; piperazine-C2) as can be seen both in Fig. 2 and Table 2.

The ^{13}C-NMR chemical shifts of carbons C22, C23, C24, and C26 are shifted downfield. The downfield ^{15}N-NMR chemical shift difference of the piperazine nitrogen between the free and complexed ligands is surprisingly small, only 3.2 ppm (see Table 2). This together with the carbon shift changes suggests that the strongest coordination occurs between the Ag(I) cation and the

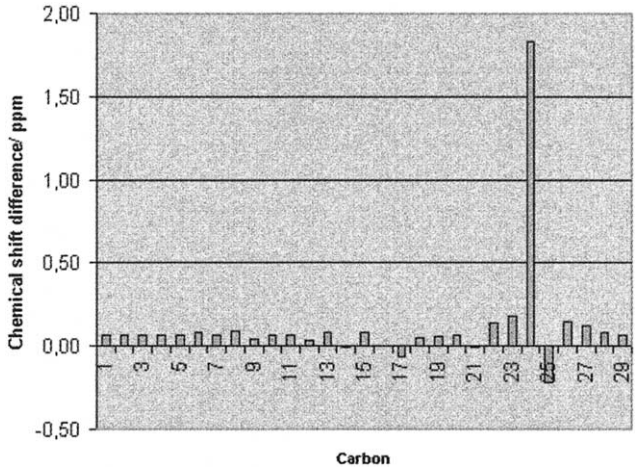


Fig. 2. Changes in ^{13}C-NMR chemical shifts of the cholaphane due to the complexation with Ag(I) ion. A downfield shift is indicated with a +-sign and an upfield shift with a −-sign.

carbonyl oxygens of the bile acid residues even though some interaction between the metal and the amide nitrogens can also be detected. The positively charged Ag(I) ion withdraws the lone electron pairs of the carbonyl oxygens and the amide nitrogens causing a deshielding effect to the nuclei of C24 and piperazine-N. The same effect causes similar but weaker deshielding of the nuclei of carbons C22, C23, and C26. Interestingly, the chemical shift of C25 is shifted upfield meaning that the nuclei are experiencing a shielding effect. The piperazine moiety probably possesses a slightly twisted chair conformation like in the terephthalic acid-closed cholaphane [17] causing an increased electron density around the C25 nuclei mediated by the withdrawal of the lone electron pairs by the cation. This also suggests that the cation is complexed outside the cholaphane ring.

The ^{13}C-NMR chemical shift changes after addition of silver triflate for the thiophene moiety of the present cholaphane remain negligible supporting the assumption according to which the complexation of the cation occurs at the piperazine end. This observation differs from the previously reported Ag(I) complexation studies of isomeric 3α,3′α-bis(pyridine-*n*-carboxy)-5β-cholan-24-oic acid piperazine diamides ($n = 2$–4) in which the complexation of the metal occurred unambiguously at the pyridine moieties and no changes in the piperazine ^{13}C-NMR chemical shifts were detected [17]. The isomeric 3α,3′α-bis(pyridine-*n*-carboxy)-5β-cholan-24-oic acid piperazine diamides are sterically less restricted than the dimeric cholaphanes. This property gives the cation more freedom in selecting its coordination environment. The two aromatic ring systems capable of simultaneous π-cation interaction of these molecular clefts offer an attractive complexation surroundings for Ag(I) cation. The aromatic thiophene part of the present cholaphane contains not only one aromatic ring but an almost 'electroneutral' heteroatom. Additionally, if the sulphur atom is oriented inside the cholaphane cavity the steric reasons support complexation of the cation to the other part of the cholaphane. So the coordination environment containing two oxygens and two nitrogens of the piperazine end of the molecule appears as a logic alternative.

The Hg^+ and Cd^{2+} complexes were also tried to form by saturating the $CDCl_3$ solution of the cholaphane with Hg_2Cl_2 and $Cd(ClO_4)_2 \cdot 6H_2O$ but any changes due to possible complexation were not observed in ^{1}H-NMR spectra. Because of the previously mentioned finding according to which Pt(II), Pd(II), and Au(III) complexes with bile acids and a halide or NH_3 may be useful cytostatic agents [21] also Au^{3+} complexation was tried by adding $HAuCl_4$ to the NMR sample solution so that the cation–ligand ratio was 1:1. A change in the colour of the solution was observed simultaneously with a formation of a yellow solid. An overall decrease of resolution in ^{1}H-NMR spectrum after addition of Au(III) was detected. Unfortunately, the solution was so dilute that a more detailed analysis of the spectrum was impossible.

In order to ensure the molecular weight of the ligand, an accurate mass measurement using leucin-enkephalin as a lock mass reference was performed. A centroided spectrum for the ligand resulted in MS *m*/*z*, ESI-TOF$^+$ found 939.6285 $[M+H]^+$, $C_{58}H_{86}N_2O_6S$ requires $[M+H]^+ = 939.6304$. The measured value deviated 2.0 ppm from the theoretical one.

In the spectrum measured without the lock mass reference singly charged $[M+H]^+$, $[M+Na]^+$, $[M+K]^+$, $[2M+H]^+$, $[2M+Na]^+$, and $[2M+K]^+$ ions were detected, even though alkali metal salts were not added to the sample solution. The intensities of the $[M+H]^+$ and $[M+K]^+$ ions were almost equal $[M+Na]^+$ ion appearing somewhat lower in intensity. The monomeric adducts prevailed over the dimeric ions. After addition of AgO_3SCF_3 the intensities of the alkali metal adducts, especially the potassium adducts, of the ligand were significantly decreased and an $[M+Ag]^+$ ion with an isotope pattern consistent with the theoretical one was observed (see Fig. 3). Also in the dimeric ion region of the spectrum a low-intensity signal of $[2M+Ag]^+$ ion was clearly detected in addition to the signals of $[2M+H]^+$ and $[2M+Na]^+$ ions. $[2M+K]^+$ ion was not observed at all. The similar ionic radii of Ag^+ and K^+ ions probably induced a competition between the metal ions of the binding site of the cholaphane leading to displacement of potassium by silver. This is likely to be due to the polarisable $4d^{10}$ outer electron configuration of Ag^+ ion that can be involved in coordination bonding.

The ability of the cholaphane to recognise Ag(I) ion over alkali metal ions is an important feature concerning its potential applications in medicine. In designing

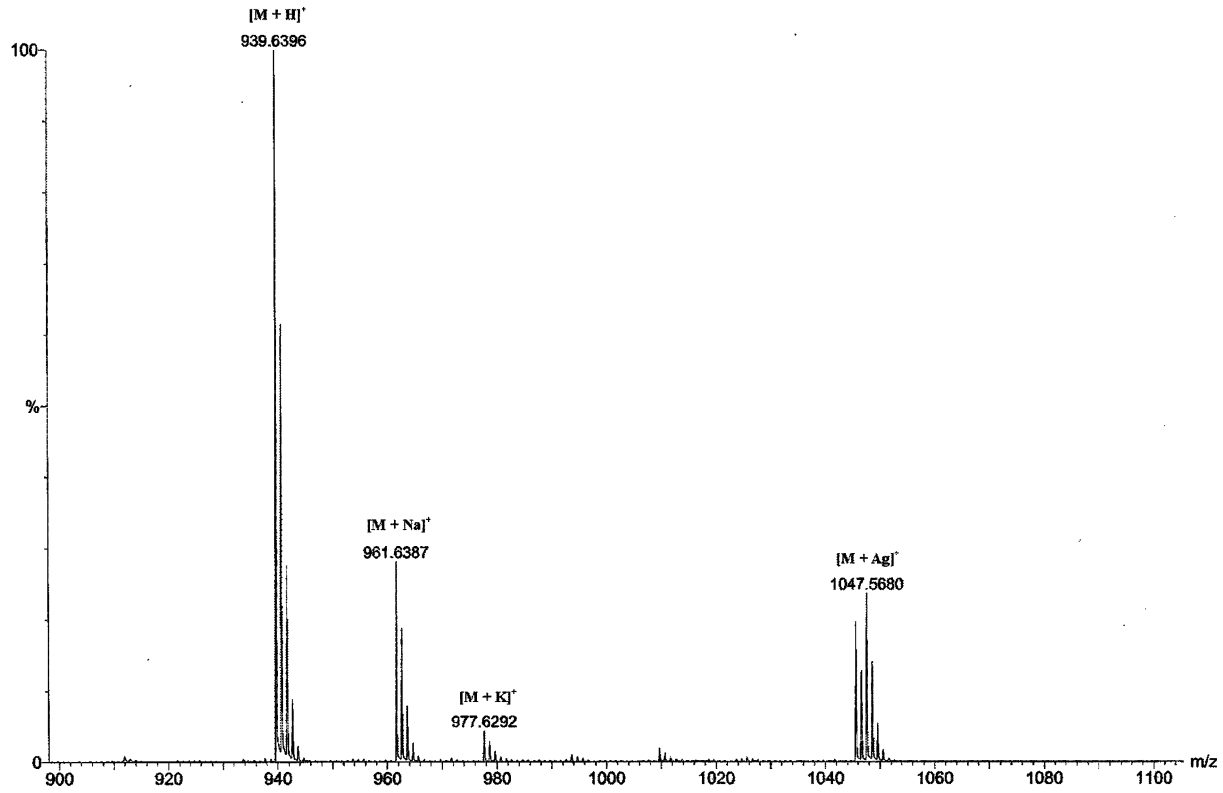


Fig. 3. Partial ESI-TOF$^+$ MS spectrum of Ag(I) complex of the cholaphane showing the proton, sodium, potassium, and Ag(I) adducts, respectively.

potential cytostatic agents [21], in which Pd(II), Pt(II), or Au(III) forms complexes with bile acid-based hosts, it is extremely important that the ligand has a stronger affinity to noble metal ions than to the alkali and earth-alkaline metals naturally existing in the mammalian body.

4. Conclusions

It has been shown that an application of the mixed anhydride procedure provides a fast and efficient method for synthesising not only mono- but also diamides of bile acids. A full spectral characterisation of a cholaphane consisting of lithocholic acid piperazine diamide and 2,5-thiophene dicarboxylic acid with a transition metal complexation ability is included. The NMR spectroscopic measurements suggest that Ag(I) ion forms a complex with the cholaphane. The coordination occurs between the Ag(I) cation and the carbonyl oxygens of the bile acid residues. Some interaction between the metal and the amide nitrogens of the piperazine moiety is also detected. The results also propose that the cation is complexed outside the cholaphane ring. The ESI-TOF$^+$ MS results for the present cholaphane show a tendency to form adducts with both alkali and transition metal ions. An ability of the cholaphane to recognise Ag(I) ion over alkali metal ions, especially potassium ion, is also observed. The transition metal complexing bile acid-based host molecules, such as synthesised and characterised in this work, capable of acting as liver-specific carrier molecules, absorption enhancers, and cholesterol-level lowering agents are potential building blocks in designing interesting chemical and pharmacological applications, especially because of recent findings according to which metal complexes of bile acid conjugates are potential contrast agents in MRI and useful cytostatic agents.

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