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Novel lithocholaphanes: Syntheses, NMR, MS, and molecular modeling studies

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Abstract

Novel head-to-head lithocholaphanes **6** and **11** have been synthesized via precursors **1**–**5** and **7**–**10** with overall good yields, and characterized by ^{1}H, ^{13}C, and ^{15}N NMR spectroscopy, ESI-TOF mass spectrometry, thermal analysis, and molecular modeling. In addition, the binding abilities of **6** and **11** towards alkali metal cations have been investigated via competitive complexation studies using equimolar mixtures of Li^+, Na^+, K^+, and Rb^+-cations, and cholaphanes **6** and **11**. The formation of cation–cholaphane adducts was detected by ESI-TOF mass spectrometry. The trends in these comparative binding studies are nicely reproduced theoretically with PM3 energetically optimized structures of **6** and **11** and their interaction energies with alkali metal cations calculated by molecular mechanics. Cholaphane **11** possessing a peptoid type structural fragment, $-(CH_2CONHCH_2CH_2)_2O-$, as a coordination sphere, shows binding tendency towards lithium and sodium cations, whereas **6** possessing an ester type, $-(CH_2OCOCH_2)_2O-$, moiety and a bigger cavity size than **11**, shows merely a tendency towards bigger alkali metal cations, potassium and rubidium.

Keywords: Lithocholaphane; Synthesis; NMR; ESI-TOF MS; Molecular modeling studies

1. Introduction

There exists a constantly growing need for new molecular frameworks with applications in the field of supramolecular chemistry, driving researchers to development of new host–guest structures. One approach is to design novel macrocyclic synthetic receptors with molecular cavities able to serve as model compounds for more complex biological systems. Typically macrocyclic host molecules bind substrates either in their defined cavity or above their plane [1]. Bile acids are widely used as building blocks for such supramolecular entities.

The most abundant naturally occurring bile acids in higher vertebrates are derivatives of cholanic acid, cyclopentanoperhydrophenanthrene ring-containing steroid consisting of 24 carbon atoms. Bile acids are end-products of cholesterol metabolism in the liver. The steroidal nucleus of cholesterol undergoes several hydroxylations followed by a loss of an isopropyl group from the side chain resulting in primary bile acids. Primary bile acids, among which cholic acid (3α,7α,12α-trihydroxy-5β-cholan-24-oic acid) and chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholan-24-oic acid) are the most important ones, are further transformed to secondary bile acids, such as deoxycholic (3α,12α-dihydroxy-5β-cholan-24-oic acid), hyodeoxycholic (3α,6α-dihydroxy-5β-cholan-24-oic acid), and lithocholic (3α-hydroxy-5β-cholan-24-oic acid) acids [2]. Synthesis of bile acids takes place in the liver, from where they are secreted into bile, stored in gallbladder,

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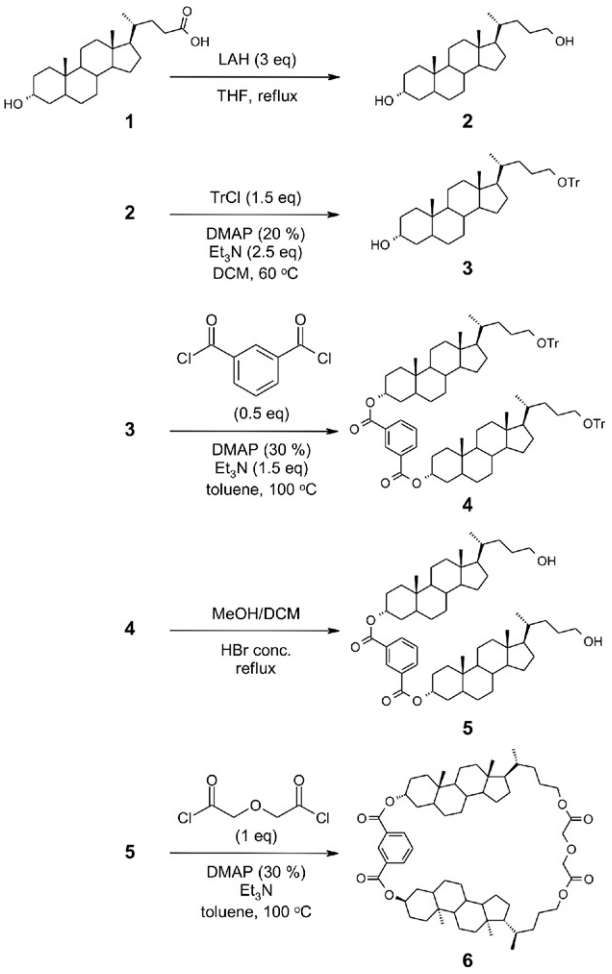
and emptied into the small intestine, where they are reabsorbed and carried to liver via portal vein. This cycle, called enterohepatic circulation, happens 6–15 times per day. Bile acids have an essential role in digestion and absorption of lipids and lipid-soluble vitamins. In addition, they prevent cholesterol from precipitating in the gall bladder [3]. Most prominent characteristics of bile acids include their large, rigid, and curved steroidal skeleton, chemically different hydroxyl groups, enantiomeric purity, and unique amphiphilicity. Furthermore, they are low-priced and easily available. Owing to these properties, bile acids are ideal building blocks in construction of novel molecular and supramolecular assemblies for molecular recognition.

Cholaphanes, bile acid-derived macrocycles, which consist of steroidal units joined together by various spacer groups, have been continuously in a focus of supramolecular chemistry owing to their versatile properties [4–16]. One way to tailor a bile acid derivative suitable for cation recognition is, to attach a crown ether type moiety either directly to a hydroxy group of a bile acid [17] or by bridging together two steroidal hydroxyls by a polyalkoxy chain [18]. Moreover, various crown ethers and bile acid derivatives are used as building blocks in synthetic ion channels and pores [19]. One future objective to synthesize ionophorous bile acid macrocycles could also be their suitability for preparation of ion-selective electrodes [20]. Recently, cholaphanes containing pyridine moieties are reported to have an ability to bind flavin analogues [21]. More generally, many bile acid derivatives are valuable compounds in supramolecular and pharmaceutical chemistry, as reviewed by us [22,23]. As a continuation of our previous studies on various cholaphanes [6,7,9,12,24] we now wish to report syntheses, spectral characterization (NMR and ESI-TOF MS), molecular modeling, and alkali metal cation binding properties of two novel cholaphanes, **6** and **11**, with aromatic and alkoxy/aminoalkoxy building blocks offering proper binding sites, for example, for alkali and earth alkaline metal cations (see Fig. 1). These structures resemble also, for example, cyclopeptides [25–27] and ionophorous antibiotics [28], which show interesting receptor properties.



Scheme 1. Preparation of cholaphane **6**.

2. Experimental

2.1. Syntheses and analytical data

The synthetic steps leading to cholaphanes **6** and **11** are illustrated in Schemes 1 and 2.

3α-Hydroxy-5β-cholan-24-oic acid (lithocholic acid) (***1***) (97% by titration). Compound **1** was purchased from Sigma

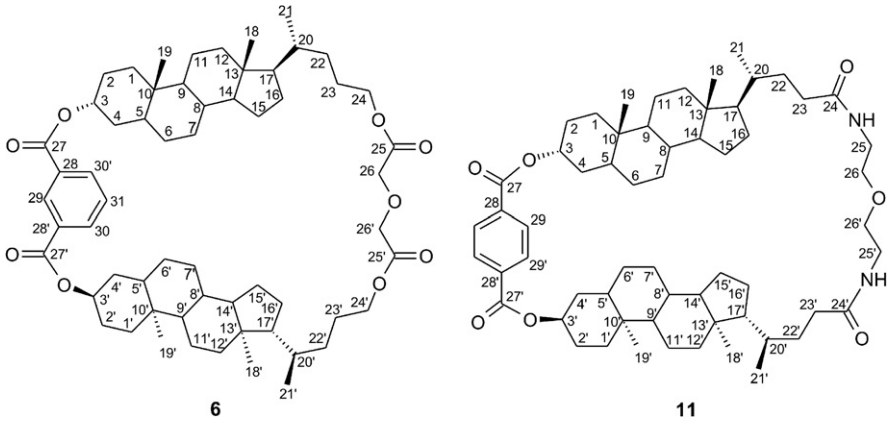
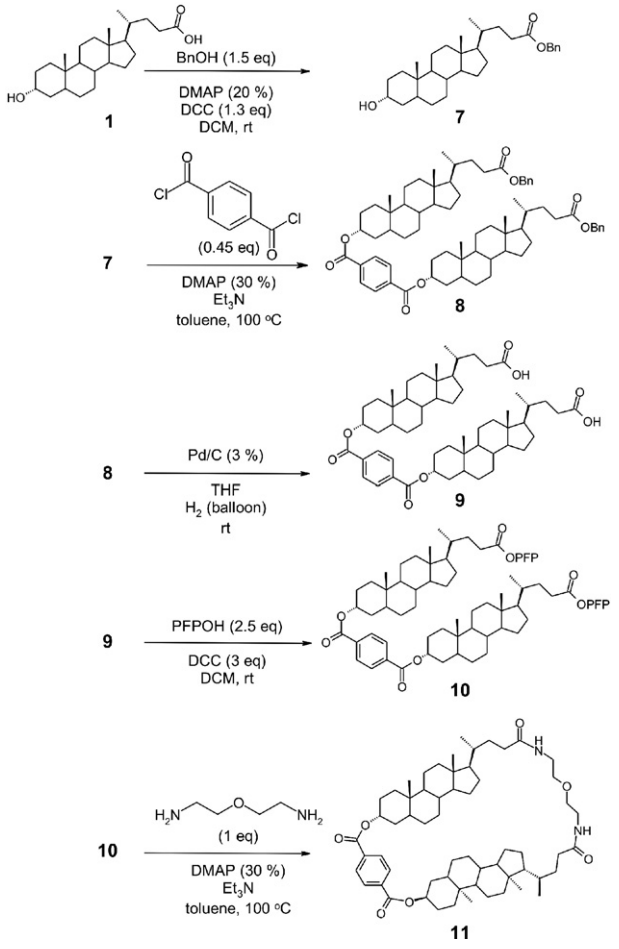


Fig. 1. Structures and numbering of cholaphanes **6** and **11.**



Scheme 2. Preparation of cholaphane **11**.

and used without further purification. Isophthalic and terephthalic acids and other reagents used in synthetic steps were analytical grade reagents and used as such. All solvents used in chromatography and recrystallization were also analytical grade reagents and used without further purification.

5β-Cholane-3α,24-diol (**2**). Compound **2** has been prepared as described before [29]. An argon-flushed two-necked flask equipped with a condenser, dropping funnel, and a magnetic stirrer was charged with the solution of lithocholic acid, **1**, (300 mg, 0.796 mmol) in anhyd. THF (8 mL) and cooled to 0 °C. Suspension of $LiAlH_4$ (LAH) (90 mg, 2.39 mmol) in 2 ml of THF was added. The mixture was stirred for 30 min at rt and then refluxed for 2 h. The cold (0 °C) reaction mixture was carefully quenched by dropwise addition of a 10% HCl solution (10 mL) and swirled at rt until the reaction mixture became transparent. The product was extracted with ether (3 × 30 mL), the ethereal solution was dried ($MgSO_4$), and evaporated to give **2** (289 mg, 100%) as a white crystalline solid, which was used without further purification since its mp corresponded to that reported in the literature: 172–173 °C from acetone. ^{1}H NMR ($CDCl_3$, 500 MHz, ppm): δ 3.65–3.55 (m, 3H, 3β-H and 24-CH_2), 0.92 (d, $J = 5.6$ Hz, 3H, 21-CH_3), 0.92 (s, 3H, 19-CH_3), 0.64 (s, 3H, 18-CH_3); ^{13}C NMR (126 MHz, $CDCl_3$, ppm): δ 71.87 (C-3), 63.57 (C-24), 56.54 (C-14), 56.22 (C-17), 42.72 (C-13), 42.13 (C-5), 40.48 (C-9), 40.22 (C-12), 36.48 (C-4), 35.88 (C-8), 35.58 (C-20), 35.37 (C-1), 34.58 (C-10), 31.85 (C-22), 30.56 (C-2), 29.45 (C-23), 28.29 (C-16), 27.21 (C-6), 26.44 (C-7), 24.22 (C-15), 23.36 (C-19), 20.84 (C-11), 18.64 (C-21), 12.04 (C-18).

24-Triphenylmethoxy-5β-cholan-3α-ol ***(3)***. In a vial with a screw cap the mixture of **2** (90 mg, 0.25 mmol), triphenylmethylchloride (tritylchloride = TrCl) (104 mg, 0.37 mmol), 4-(dimethylamino)pyridine (DMAP) (5 mg, 0.04 mmol), and triethylamine (Et_3N) (84 μL, 0.6 mmol) in dichloromethane (DCM) (1.5 mL) was heated on an oil bath (60 °C) for 15 h. The solvent was removed under reduced pressure and the residue was subjected to column chromatography (DCM, $R_f = 0.30$) to give **3** as a white crystalline solid (119 mg, 83%); mp 93–94 °C; Anal. found: C, 85.61; H, 9.44. Calcd for $C_{43}H_{56}O_2$: C, 85.38; H, 9.33%; ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.44–7.19 (m, 15H, OTr), 3.61 (m, 1H, 3β-H), 3.00 (m, 2H, 24-CH_2), 0.90 (s, 3H, 19-CH_3), 0.88 (d, 3H, 21-CH_3), 0.60 (s, 3H, 18-CH_3).

Bis(24-triphenylmethoxy)-3α,3′α-(isophthaloyloxy)-bis(5β-cholane) ***(4)***. An argon-flushed two-necked flask equipped with a condenser, dropping funnel, and a magnetic stirrer was charged with **3** (120 mg, 0.2 mmol), DMAP (7 mg, 0.06 mmol), Et_3N (44 μL, 0.3 mmol), and isophthaloyl dichloride (20 mg, 0.1 mmol) in anhyd. toluene (2 mL) in the order given at rt. The mixture was stirred at 100 °C for 15 h. After cooling to rt the mixture was diluted with DCM and washed twice with sat. aq. $NaHCO_3$ solution. The organic layer was dried ($MgSO_4$) and the solvent evaporated under reduced pressure. The product was purified by column chromatography (petroleum ether/EtOAc, 10:1, $R_f = 0.50$) to give **4** as a white crystalline solid (112 mg, 85%); mp 150–152 °C; Anal. found: C, 84.18; H, 8.85. Calcd for $C_{94}H_{114}O_6$: C, 84.26; H, 8.58%; ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 8.69 (m, 1H, 29-H), 8.19 (m, 2H, 30-H, 30′-H), 7.51–7.18 (m, 16H, 31-H, OTr), 4.98 (m, 2H, 3β-H, 3′β-H), 3.00 (m, 4H, 24-CH_2, 24′-CH_2), 0.95 (s, 6H, 19-CH_3, 19′-CH_3), 0.89 (d, 6H, 21-CH_3, 21′-CH_3), 0.62 (s, 6H, 18-CH_3, 18′-CH_3).

3α,3′α-(Isophthaloyloxy)-bis(5β-cholan-24-ol) ***(5)***. Compound **4** (106 mg, 0.08 mmol) was dissolved in the mixture of MeOH (6 mL) and DCM (2 mL). To this solution aqueous 48% HBr (200 μL) was added and the mixture was refluxed for 3 h. The product was extracted with DCM (2 × 20 mL), the organic layer was washed with water, dried ($MgSO_4$), and concentrated. The resulting oil was subjected to column chromatography (DCM/MeOH, 20:1, $R_f = 0.20$) to give **5** as a white crystalline solid (50 mg, 74%); mp 188–190 °C; Anal. found: C, 78.64; H, 10.35. Calcd for $C_{56}H_{86}O_6$: C, 78.64; H, 10.14%; ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 8.68 (m, 1H, 29-H), 8.19 (m, 2H, 30-H, 30′-H), 7.49 (m, 1H, 31-H), 4.98 (m, 2H, 3β-H, 3′β-H), 3.60 (m, 4H, 24-CH_2, 24′-CH_2), 0.95 (s, 6H, 19-CH_3, 19′-CH_3), 0.92 (d, 6H, 21-CH_3, 21′-CH_3), 0.65 (s, 6H, 18-CH_3, 18′-CH_3).

Cyclic 3α,3′α-(isophthaloyloxy)-bis(5β-cholan-24-yloxy)-3-oxaglutarate ***(6)***. In a vial with a screw cap the mixture of **5** (68 mg, 0.08 mmol), DMAP (3 mg, 0.02 mmol), 2,2′-oxydiacetyl

chloride (14 mg, 0.08 mmol), and Et_3N (0.5 mL) in toluene (8 mL) was heated on an oil bath (100 °C) until TLC analysis revealed the reaction was no longer proceeding (15–20 h). The solvent was removed under reduced pressure and the residue was subjected to column chromatography (DCM, $R_f \sim 0.2$–0.25) to give **6** as a white crystalline solid (14 mg, 15%); $T_m = 314.6$ °C; ^{1}H NMR ($CDCl_3$, 500 MHz, ppm): δ 8.45 (t, $J = 1.6$ Hz, 1H, 29-H), 8.26 (dd, 2H, $J = 7.8$, 1.7 Hz, 30-H, 30′-H), 7.54 (t, 1H, $J = 7.8$ Hz, 31-H), 4.95 (m, 2H, $J = 7.3$, 4.3 Hz, 3β-H, 3′β-H), 4.25-4.12 (m, 4H, 26-CH_2, 26′-CH_2), 4.20 and 4.24 (ABq, 4H, $J^{AB} = 16.5$ Hz, 24-CH_{ab}, 24′-CH_{ab}), 0.99 (s, 6H, 19-CH_3, 19′-CH_3), 0.93 (d, 6H, $J = 6.5$ Hz, 21-CH_3, 21′-CH_3), 0.68 (s, 6H, 18-CH_3, 18′-CH_3); ^{13}C NMR ($CDCl_3$, 126 MHz, ppm): δ 169.9 (C-25, C-25′), 165.8 (C-27, C-27′), 134.3 (C-30, C-30′), 131.3 (C-28, C-28′), 128.8 (C-29), 128.6 (C-31), 76.4 (C-3, C-3′), 68.5 (C-26, C-26′), 65.3 (C-24, C-24′), 56.5 (C-14, C-14′), 56.4 (C-17, C-17′), 42.8 (C-13, C-13′), 42.3 (C-5, C-5′), 40.6 (C-9, C-9′), 40.2 (C-12, C-12′), 35.9 (C-8, C-8′), 35.2 (C-1, C-1′), 35.1 (C-20, C-20′), 34.8 (C-10, C-10′), 32.4 (C-4, C-4′), 32.1 (C-22, C-22′), 28.3 (C-16, C-16′), 27.2 (C-6, C-6′), 26.8 (C-2, C-2′), 26.5 (C-7, C-7′), 25.3 (C-23, C-23′), 24.3 (C-15, C-15′), 23.4 (C-19, C-19′), 20.9 (C-11, C-11′), 18.6 (C-21, C-21′), 12.0 (C-18, C-18′); MS *m/z* ESI-TOF$^+$ found 975.8177 $[M+Na]^+$ (100%) and 991.7653 $[M+K]^+$(42.5%), $C_{60}H_{88}O_9Na$ requires 975.6326 and $C_{60}H_{88}O_9K$ 991.6066, respectively.

Benzyl 3α-hydroxy-5β-cholan-24-oate ***(7)***. To a stirred solution of lithocholic acid, **1**, (500 mg, 1.33 mmol) in anhydrous DCM (3 mL), DMAP (32 mg, 0.27 mmol) and benzyl alcohol (216 mg, 2 mmol) were added. Then dicyclohexyl carbodiimide, DCC (357 mg, 1.73 mmol), was added to the mixture. After stirring overnight in a closed flask the mixture was diluted with DCM and filtered. The filtrate was washed with 5% aq. HCl, sat. $NaHCO_3$, and H_2O, then dried ($MgSO_4$), and evaporated to dryness. The crude product was purified by column chromatography (petroleum ether/EtOAc, 4:1, $R_f = 0.28$) and recrystallized from hexane/EtOAc. The yield of **7** was 432 mg (74%); ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.33 (m, 5H, Ph), 5.09 (m, 2H, $PhCH_2$), 3.58 (m, 1H, 3β-H), 2.42-2.21 (m, 2H, 23-CH_2), 0.88 (s and d, 6H, 19-CH_3, 21-CH_3), 0.59 (s, 3H, 18-CH_3). These correspond to those reported previously [30].

Dibenzyl 3α,3′α-(terephthaloyloxy)-bis(5β-cholan-24-oate) ***(8)***. To the terephthaloyl dichloride (90 mg, 0.443 mmol) in anhyd. toluene (3 mL), DMAP (36 mg, 0.29 mmol), Et_3N (0.5 mL), and **7** (430 mg, 0.98 mmol) were added at rt. The mixture was stirred in argon atmosphere at rt for 1 h and then at 100 °C for 12 h. After cooling to rt the mixture was diluted with DCM and washed twice with sat. $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$ and the solvent evaporated under reduced pressure. The product was purified by column chromatography (DCM, $R_f = 0.55$) to give **8** as a white crystalline solid (401 mg, 86%, based on terephthaloyl dichloride); mp 132–134 °C; Anal. found: C, 78.99; H, 9.05. Calcd for $C_{70}H_{94}O_8$: C, 79.06; H, 8.91%; ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 8.07 (s, 4H, 29-H, 29′-H), 7.34 (m, 10H, Ph), 5.09 (m, 4H, $PhCH_2$), 4.97 (m, 2H, 3β-H, 3′β-H), 2.43–2.22 (m, 4H, 23-CH_2, 23′-CH_2), 0.95 (s, 6H, 19-CH_3, 19′-CH_3), 0.90 (d, 6H, 21-CH_3, 21′-CH_3), 0.59 (s, 6H, 18-CH_3,18′-CH_3).

3α,3′α-(Terephthaloyloxy)-bis(5β-cholan-24-oic acid) ***(9)***. To the mixture of **8** (2.045 g, 1.92 mmol) and 10% Pd/C (61 mg, 3%) in argon atmosphere, 20 mL of anhydrous THF was added. Then the flask was purged with H_2 for 1–2 s, connected to a balloon of H_2, and stirred for 3 h at rt. The mixture was filtered through a short plug of silica gel to remove the catalyst. The filtrate was evaporated to dryness to give **9** as a white crystalline solid (1.637 g, 96%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 11.56 (br s, 2H, 24-COOH, 24′-COOH), 7.98 (s, 4H, 29-H, 29′-H), 4.86 (m, 2H, 3β-H, 3′β-H), 2.24-2.02 (m, 4H, 23-CH_2, 23′-CH_2), 0.88 (s, 6H, 19-CH_3,19′-CH_3), 0.84 (d, 6H, 21-CH_3, 21′-CH_3), 0.57 (s, 6H, 18-CH_3,18′-CH_3).

Bis(pentafluorophenyl) 3α,3′α-(terephthaloyloxy)-bis(5β-cholan-24-oate) ***(10)***. To a stirred solution of **9** (1.637 g, 1.84 mmol) in anhydrous DCM (50 mL) pentafluorophenol (PFPOH) (846 mg, 4.60 mmol) and *N*,*N*′-dicyclohexyl carbodiimide (DCC) (1.140 g, 5.53 mmol) was added in the order given. After being stirred overnight the mixture was diluted with DCM and filtered. The filtrate was washed with sat. $NaHCO_3$, and H_2O, then dried (Na_2SO_4), and evaporated to dryness. The crude product was purified by column chromatography (petroleum ether/DCM, 1:1, $R_f = 0.30$). The yield of **10** was 1.888 g (84%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 8.07 (s, 4H, 29-H, 29′-H), 4.98 (m, 2H, 3β-H, 3′β-H), 2.73-2.53 (m, 4H, 23-CH_2, 23′-CH_2), 0.97 (s, d, s and d, 12H, 19-CH_3, 21-CH_3, 19′-CH_3, 21′-CH_3), 0.67 (s, 6H, 18-CH_3, 18′-CH_3); MS *m/z* ESI-TOF$^+$ found 1237.7355 $[M+MeOH+Na]^+$ (100%) and 1253.7007 $[M+MeOH+K]^+$ (46.3%), $C_{68}H_{80}F_{10}O_6CH_3OHNa$ requires 1237.5955 and $C_{68}H_{80}F_{10}O_6CH_3OHK$ 1253.5695, respectively.

Cyclic N,N′-(3-oxa-1,5-pentanediyl) 3α,3′α-(terephthaloyloxy)-bis(5β-cholan-24-amide) ***(11)***. To a stirred solution of **10** (73 mg, 0.06 mmol), DMAP (22 mg, 0.18 mmol) and Et_3N (25 μL, 0.18 mmol) in anhydrous DCM (6 mL), and 2,2′-oxybis(ethylamine) (7 mg, 0.07 mmol) was added at once. After being stirred in a stoppered flask for 12–15 h the mixture was diluted with DCM and washed with 10% aq. KOH and with water. The organic layer was dried ($MgSO_4$) and concentrated. The crude product was purified by column chromatography (DCM/MeOH, 30:1, $R_f \sim 0.2$–0.3). The yield of **11** was 21 mg (37%); $T_m = 352.8$ °C (decomposition during melting); ^{1}H NMR ($CDCl_3$, 500 MHz, ppm): δ 8.10 (s, 4H, 29-H, 29′-H), 5.74 (t, 2H, NH), 5.06 (m, 2H, $J = 5.8$, 5.3, 4.9 Hz, 3β-H, 3′β-H), 3.62-3.34 (m, 8H, 26-H, 26′-H, 25-H, 25′-H), 0.96 (s, 6H, 19-CH_3, 19′-CH_3), 0.95 (d, 6H, $J = 6.7$ Hz, 21-CH_3, 21′-CH_3), 0.68 (s, 6H, 18-CH_3, 18′-CH_3); ^{13}C NMR ($CDCl_3$, 126 MHz, ppm): δ 173.8 (C-24, C-24′), 165.1 (C-27, C-27′), 134.6 (C-28, C-28′), 129.4 (C-29, C-29′), 74.7 (C-3, C-3′), 69.7 (C-26, C-26′), 57.1 (C-14, C-14′), 55.2 (C-17, C-17′), 42.7 (C-13, C-13′), 41.7 (C-5, C-5′), 40.7 (C-9, C-9′), 40.5 (C-12, C-12′), 39.0 (C-25, C-25′), 35.6 (C-8, C-8′), 35.1 (C-20, C-20′), 34.9 (C-1, C-1′), 34.4 (C-10, C-10′),

32.2 (C-23, C-23′), 32.1 (C-4, C-4′), 31.8 (C-22, C-22′), 28.3 (C-16, C-16′), 26.8 (C-6, C-6′), 26.6 (C-2, C-2′), 26.2 (C-7, C-7′), 24.0 (C-15, C-15′), 23.2 (C-19, C-19′), 21.0 (C-11, C-11′), 18.4 (C-21, C-21′), 12.0 (C-18, C-18′); MS *m/z* ESI-TOF$^+$ found 973.8069 $[M+Na]^+$ (100%) and 989.7914 $[M+K]^+$ (5%), $C_{60}H_{90}N_2O_7Na$ requires 973.6646 and $C_{60}H_{90}N_2O_7K$ 989.6385, respectively.

2.2. Differential scanning calorimetry

Melting transitions (T_m) of **6** and **11** were determined on power compensation type Perkin-Elmer PYRIS DIAMOND DSC. The measurements were carried out under nitrogen atmosphere (flow rate 50 mL min^{-1}) using 50 μL sealed aluminum sample pans with pin holes. The sealing was made by using a 30 μL aluminum pan with pinholes as cover-pan to ascertain good thermal contact between the sample and pan, and to minimize the free volume inside the pan, as the sample volume is gently squeezed by the cover-pan. The temperature calibration was carried out using two standard materials (*n*-decane, In) and energy calibration by an indium standard (28.45 J g^{-1}). As small sample weights were used, the heating rates of 20 and/or 40 °C were used to enhance sensitivity of the measurements. Samples were heated from 25 to 400 °C and the sample weights used were about 0.15–1 mg. The sample weights were checked afterwards to see potential changes in the weights that may have occurred during heating. Uncertainty for temperature is less than 0.8 °C.

2.3. Mass spectrometry

In order to ascertain the molecular weights of the compounds, ESI-TOF MS measurements were performed. For the MS measurement the NMR sample of **6** (in $CDCl_3$) was diluted to a concentration of ~0.5 mmol/L by using acetonitrile (HPLC grade)–formic acid (0.1%) mixture. The NMR sample of **11** was diluted to a concentration of ~0.05 mmol/L by using methanol (HPLC grade).

The adduct formation experiments with alkali metal chlorides were performed by preparing 0.05 mM solutions of LiCl, NaCl, KCl, and RbCl (first dissolved in H_2O and then diluted with acetonitrile or methanol, respectively). The ligands were first dissolved in $CHCl_3$ and then diluted to a concentration of 0.05 mM by using acetonitrile (**6**) or methanol–formic acid (0.1%) mixture (**11**). An equal volume of each individual alkali metal solution was taken, mixed thoroughly, and the same volume of the ligand solution added resulting in a molar ratio of 1:1 (cation/ligand). After 15 min the measurement for the equimolar mixture of the alkali metal chlorides and the ligand was performed. For **11** adduct formation with earth alkaline metal chlorides was tested as well. $MgCl_2$, $CaCl_2$, and $SrCl_2$ were first dissolved in H_2O and then diluted to a concentration of 0.05 mM by using MeOH. The experimental procedure was analogous to that described for adduct formation experiment with alkali metal chlorides.

Adduct formation between the ligands and selected tetraalkyl- or dialkyldiarylammonium salts (tetramethyl-, dimethyldipropyl-, diethyldihexyl-, dimethyldibenzyl-, and dimethyldioctylammonium bromides, respectively) was tested without success. Finally, adduct formation experiment between the ligands and pyridinium cation was performed. 0.5 mM solutions of pyridine in MeCN/HCOOH (for experiment with **6**) as well as in MeOH (for experiment with **11**) were prepared and equal volumes of pyridine solution and ligand solution mixed and stirred thoroughly. For **11** no adduct with pyridinium cation was observed to form. For **6**, however, a signal with a rather weak intensity (~3%) of $[M+Py+H]^+$ ion was detected. $[M+Na]^+$ ion gave the most intense signal in the spectrum, even though no alkali metal salts were added to the sample solution.

Electrospray mass spectrometric measurements were performed by using LCT time of flight (TOF) mass spectrometer with electrospray ionization (ESI; Micromass LCT). Controlling the LCT as well as acquiring and processing the data were performed with a MassLynx NT software system. In the experiments aimed at molecular weight determinations a flow rate of 80 μL/min was used for the sample solution and the sample droplets were dried with nitrogen gas. Positive ions were chosen for detection. For **6** the potentials of 150 and 6 V for the sample and extraction cones were applied. RF lens was set at a potential of 1100 V and the potential in the capillary at 4500 V. For **11** the potentials for sample and extraction cones were set at 100 and 6 V, respectively. Potentials of 1000 and 4000 V for RF lens and capillary were applied. For **10** potentials of 195 and 6 V for sample cone and extraction cone, respectively, were applied. The potential at capillary was set at 4200 V and that of RF lens at 1200 V. For all of the three compounds the desolvation temperature was set at 120 °C and the source temperature at 80 °C.

In the adduct formation experiment of **6** with alkali metal chlorides a flow rate of 80 μL/min was used for the sample solution. The potentials for sample and extraction cones were set at 75 and 5 V and those of 750 and 3800 V for RF lens and capillary were applied, respectively. In the experiments aimed at investigation of adduct formation of **11** with alkali and earth alkaline metal chlorides, the values of 100 and 80 and 5 V for sample and extraction cones were applied. The potential at the capillary was set at 3800 V and that of RF lens at 2000 V. A flow rate of 40 μL/min of the sample solution for both of the experiments was used. In each of the adduct formation experiments the desolvation temperature was set at 120 °C and the source temperature at 80 °C, respectively.

2.4. NMR spectroscopy

^1H, ^{13}C, ^{13}C DEPT-135, PFG (pulsed field gradient) DQF (double quantum filtered) ^1H,^1H COSY (correlation spectroscopy) [31,32], PFG ^1H,^{13}C HMQC (heteronuclear

multiple quantum coherence) [33,34], and PFG ^{1}H,X HMBC (heteronuclear multiple quantum correlation, X = ^{13}C or ^{15}N) [35] spectra of **2**, **6**, and **11** were recorded in dilute $CDCl_3$-solutions at 30 °C with Bruker Avance DRX 500 FT NMR spectrometer equipped with an inverse detection dual probehead (BBI) and z-gradient accessory working at 500.13 MHz for proton, at 125.77 MHz for carbon-13, and at 50.69 MHz for nitrogen-15, respectively. ^{1}H NMR spectra of the precursors **1**, **3**–**5**, and **7**–**10** have been measured with Bruker Avance DPX 400 FT NMR spectrometer. The ^{1}H and ^{13}C chemical shifts are referenced to the signal of internal TMS ($\delta = 0.0$ ppm) and the ^{15}N shift to the N-15 signal of an external CH_3NO_2 ($\delta = 0.0$ ppm) capillary inserted coaxially inside the 5 mm diameter NMR tube. The complete lists of the Bruker Avance DRX 500 acquisition and processing parameters are available from E.K. on request.

2.5. Calculations

The molecular frameworks were drawn in HyperChem molecular modeling software [36], after which optimization of the geometries was performed using molecular mechanics MM+ force field [37]. In order to locate the possible global energy minimum on the potential surface, 10 simulated annealing runs in MM+ force field were performed for both cholaphanes. Each run started from 0 K, followed by 5 ps heating to 300 K in 25 K temperature steps, 10 ps simulation at 300 K, and 5 ps cooling close to 0 K, all in 0.0001 ps time steps. After each run the obtained structure was further optimized using molecular mechanics and then subjected to semiempirical PM3 calculations [38]. The calculations were performed on Dell Optiplex GX 280 PC (3 GHz Intel® Pentium® 4 CPU, 2 GB). In order to locate the most probable interaction site of the alkali metal, the cation was introduced to three different locations inside the cavity as well as to the close vicinity of both spacer groups outside the cavity in relation to the PM3-optimized cholaphane conformation lowest in energy, given a charge of +1, and the resulting complex optimized using molecular mechanics.

3. Results and discussion

3.1. NMR

The ^{13}C NMR chemical shift assignments of **6** and **11** and their precursors **1**–**5** and **7**–**10** are based on the large reference data collected in our review on several bile acid derivatives [39], related terephthalate containing cholaphanes [12], and ^{13}C DEPT-135, PFG DQF ^{1}H,^{1}H COSY, PFG ^{1}H,^{13}C HMQC, as well as PFG ^{1}H,^{13}C HMBC experiments of **6** and **11**. In Figs. 2 and 3 are depicted the PFG ^{1}H,^{13}C HMQC spectra of **6** and **11**, respectively. The ^{13}C NMR chemical shifts are referenced to the center peak of the solvent $CDCl_3$, 77.0 ppm from the signal of an internal $(CH_3)_4Si$ ($\delta = 0.0$ ppm). ^{15}N NMR chemical shift of **11** is determined by PFG ^{1}H,^{15}N HMBC experiment using a 100 ms evolution delay and referenced to the signal of an external CH_3NO_2 sample in a 1 mm diameter capillary tube inserted coaxially inside the 5 mm NMR-tube. The value of −272.0 ppm of **11** is very typical for carboxylic acid amides [40]. The complete ^{1}H NMR spectral assignment of the steroidal skeleton is not possible due to seriously overlapping signals. The ^{1}H NMR chemical shifts are referenced to the resonance of the residual $CHCl_3$, 7.26 ppm from an internal $(CH_3)_4Si$ ($\delta = 0.0$ ppm).

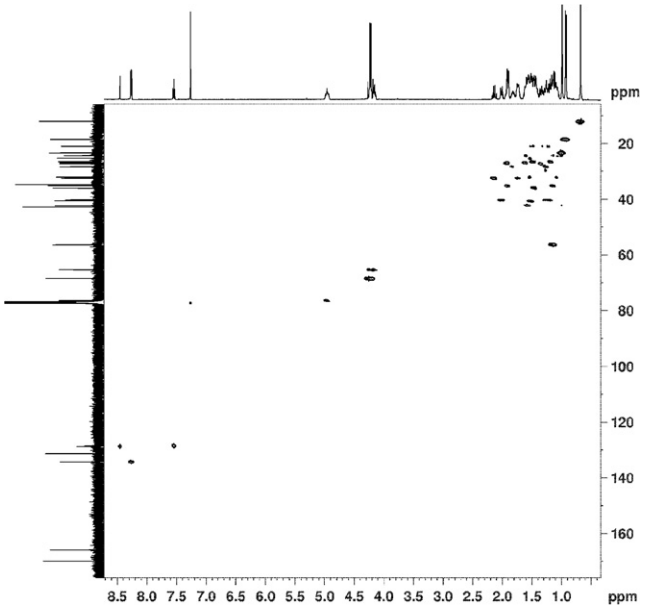


Fig. 2. PFG ^{1}H,^{13}C HMQC spectrum of **6**.

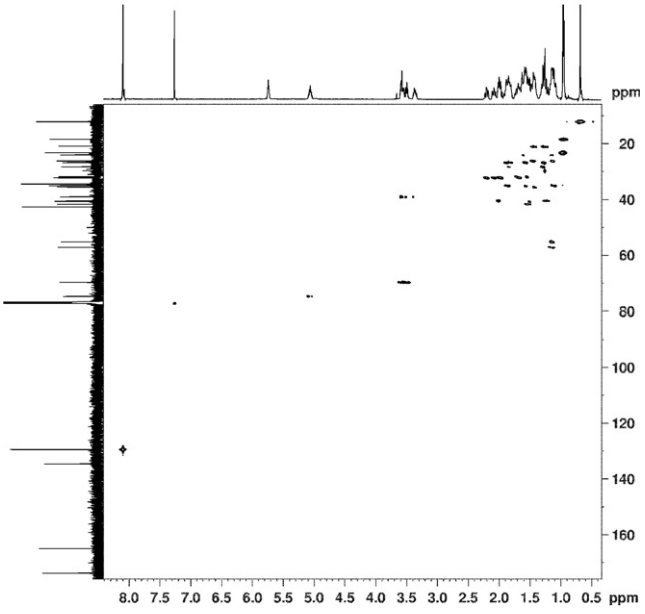


Fig. 3. PFG ^{1}H,^{13}C HMQC spectrum of **11**.

3.2. MS

In order to ascertain the molecular weights of the ligands **6** and **11** ESI-TOF mass spectrometric measurements were

performed. An experiment for **6** resulted in MS *m/z* ESI-TOF$^+$ found 975.8177 $[M+Na]^+$ (100%) and 991.7653 $[M+K]^+$ (42.5%), $C_{60}H_{88}O_9Na$ requires 975.6326 and $C_{60}H_{88}O_9K$ 991.6066. The corresponding results for **11** were MS *m/z* ESI-TOF$^+$ found 973.8069 $[M+Na]^+$ (100%) and 989.7914 $[M+K]^+$ (5%), $C_{60}H_{90}N_2O_7Na$ requires 973.6646 and $C_{60}H_{90}N_2O_7K$ 989.6385.

The ability of the ligands to form adducts with alkali metal cations was investigated by mixing equimolar amounts of LiCl, NaCl, KCl, and RbCl, respectively, with the ligand. The resulting solution was thoroughly mixed and the measurement performed after 15 min. Similarly, as reported recently for ionophorous lacalosid-derived antibiotic [28] as well as maleonitrile thiacrown ethers [41], alkali metal adducts with **6** were also detected in competitive ESI-TOF$^+$ MS binding studies. $[\mathbf{6}+M]^+$-adducts appeared at *m/z* values of 959.6570 $[M+Li]^+$, 975.6213 $[M+Na]^+$, 991.6106 $[M+K]^+$, and 1037.5194 $[M+Rb]^+$, respectively. The most intense ion was the adduct ion with potassium (100%). The second most intense ion was the adduct ion with sodium (61.3%), the third most intense ion the adduct ion with rubidium (42.5%), and the least intense ion that with lithium (18.1%). Thus it seems as **6** does not show very significant selectivity towards any of the alkali metal cations investigated. On the other hand, for **11**, the most intense ion in the spectrum was the adduct ion formed with lithium (100%; MS *m/z* ESI-TOF$^+$ found 957.6642). The other observed adduct ions were those with sodium (46.9%; MS *m/z* ESI-TOF$^+$ found 973.6648), with potassium (6.9%; MS *m/z* ESI-TOF$^+$ found 989.6382), and with rubidium (0.9%, MS *m/z* ESI-TOF$^+$ found 1035.6111), respectively.

Even though adduct ions with all alkali metal cations with cholaphane **11** were detected, the ligand, however, seemed to possess some selectivity towards cations with smaller ionic radii. In order to ensure this observation, adduct formation tendency with some earth alkaline metal cations of the ligand was conducted as well. Equimolar amounts of $MgCl_2$, $CaCl_2$, and $SrCl_2$ were thoroughly mixed, the ligand **11** added into the mixture, and the measurement performed after 15 min. Ions at *m/z* values of 951.6459 (29.4%), 973.6121 (100%), 989.5849 (6.3%), and 1037.5742 (2.5%) were observed. Those were interpreted to correspond the adduct ions $[M+H]^+$, $[M-H+Mg]^+$, $[M-H+Ca]^+$, and $[M-H+Sr]^+$, respectively. The most intense ion in this series was the adduct ion $[M-H+Mg]^+$. The ionic radius of Mg^{2+} is ca. 65 pm being close to that of Li^+, which is approximately 60 pm. The second most intense ion was $[M+H]^+$, and the third most intense one the adduct ion $[M-H+Ca]^+$. The intensity of the adduct ion formed with strontium was practically negligible, as was the one formed with rubidium in the alkali metal cation series. These results thus suggest that the cavity of **11** is smaller than that of **6** thus preventing the binding of cations with larger ionic radii and, on the other hand, favoring the binding of cations with relatively small ionic radius (60–65 pm). Pyridinium, tetraalkyl-, and dialkyldiarylammonium cations did not show significant adduct formation with **6** and **11** in ESI-TOF$^+$ MS experiments.

3.3. Molecular modeling

For **11** the simulated annealing runs led to three conformations, which were further optimized semiempirically. For **6** only one conformation was found using molecular dynamics simulations. The PM3-optimized conformations, lowest in energy for both cholaphanes **6** and **11**, are presented in Fig. 4.

As can be seen, the diameter of the cavity of **11** is smaller compared to the cavity of **6**. The width of the cavities is more or less the same order of magnitude, whereas the cavity of **6** is almost 2.5 Å longer than that of **11**. The geometries for the alkali metal ion adducts, lowest in energy for

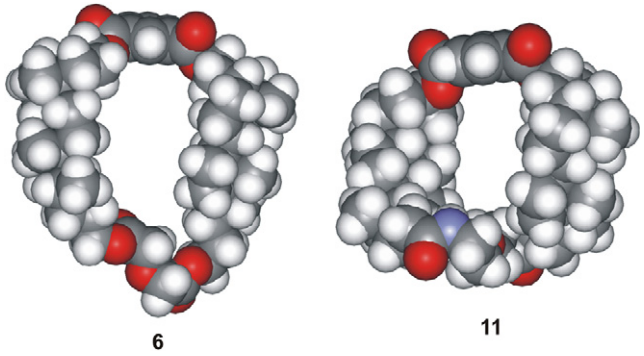


Fig. 4. The conformations lowest in energy for **6** (on the left) and for **11** (on the right), respectively, optimized at the semiempirical PM3 level of theory.

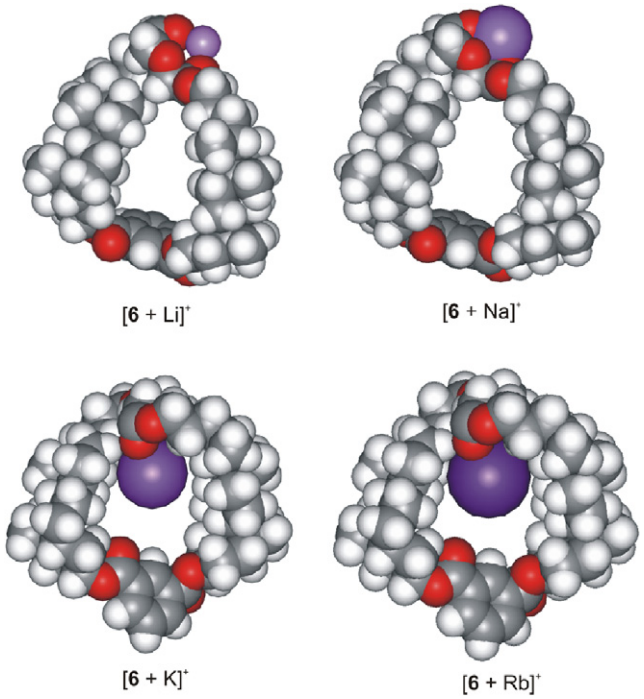


Fig. 5. The optimized geometries of the adducts formed by **6** with the alkali metal ions Li^+, Na^+, K^+, and Rb^+, respectively. The optimizations were performed using molecular mechanics and PM3-optimized conformation of **6**.

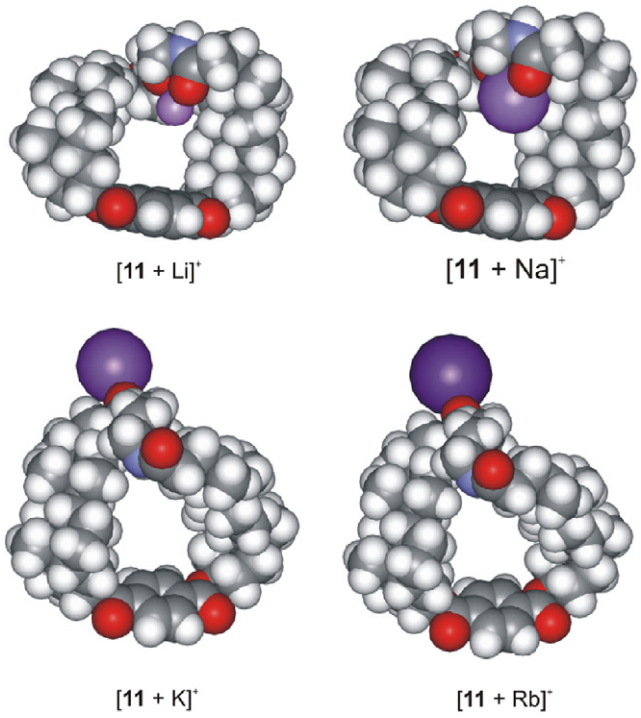


Fig. 6. The optimized geometries of the adducts formed by **11** with the alkali metal ions Li^+, Na^+, K^+, and Rb^+, respectively. The optimizations were performed using molecular mechanics and PM3-optimized conformation of **11**.

both cholaphanes **6** and **11**, are shown in Figs. 5 and 6, respectively.

In the case of **6** the alkali metal cations with smaller ionic radius (Li^+ and Na^+, respectively) resettled outside the cholaphane cavity. The carbonyl oxygens of the carboxymethoxyacetyl spacer seem to form a "tweezer-like" arrangement, which pinches the cations between the partially negatively charged oxygen atoms. On the other hand, K^+ and Rb^+ thrive inside the cholaphane cavity. The carbonyl oxygens of the carboxymethoxyacetyl spacer seem to be responsible for the electrostatic attraction of the positively charged ions in these cases as well, except that they are oriented towards the cavity instead of pointing outwards from it.

In the case of **11**, Li^+ and Na^+ ions exhibiting smaller ionic radii nicely fit in a partially negatively charged "pocket" formed by the carbonyl and ether oxygens of the (2-amidoethoxy)ethylamido spacer. The alkali metal cations with larger ionic radius (K^+ and Rb^+, respectively), for one, move outside the cholaphane cavity resettling to the close vicinity of the carbonyl oxygen of the (2-amidoethoxy)ethylamido spacer.

The molecular modeling results support the mass spectrometric ones, according to which cholaphane **6** formed adducts with all of the four alkali metal cations willingly, whereas cholaphane **11** excluded the ions with larger ionic radii. Based on molecular modeling results, in the case of **6** K^+ and Rb^+ are located inside the cavity, whereas Li^+ and Na^+ are sited outside the cavity. Cholaphane **11** behaves oppositely; the cations with smaller ionic radii are inside the cavity, whereas K^+ and Rb^+ are excluded outside. Most probably the arrangement with the cation inside the cavity corresponds to an adduct detected by mass spectrometric measurements. The tendency of **6** to form adducts with cations located outside the cavity according to the present molecular modeling calculations could be explained by the favorable coordination environment of two carbonyl and ether oxygen of cholaphane **6**. Cholaphane **11** provides only one carbonyl oxygen for electrostatic interaction for cations sited outside the cavity, resulting in a remarkably more instable adduct, respectively.

4. Conclusions

Two novel head-to-head cholaphanes with different cavity sizes have been designed and synthesized via several steps with overall good yields. These cholaphanes show different alkali metal cation binding properties, one favoring the lighter ones, and the other one the heavier ones, which possess larger ionic radii, respectively These findings can be further utilized in designing other bile acid-derived cholaphanes for molecular recognition, antibiotic drug substances, and other medicinal as well as analytical uses.

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