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Synthetic Receptors for Uronic Acid Salts Based on Bicyclic Guanidinium and Deoxycholic Acid Subunits

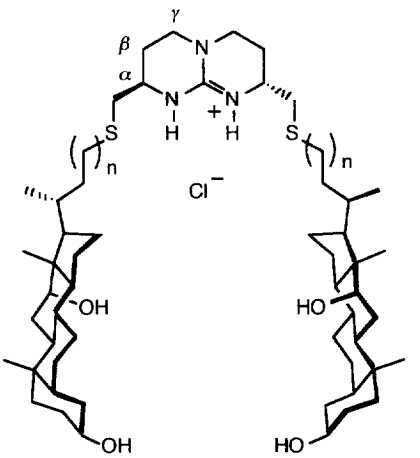
Margot Segura, Victoria Alcázar, Pilar Prados and Javier de Mendoza*

Departamento de Química Orgánica, Universidad Autónoma de Madrid
Cantoblanco, E- 28049, Madrid (Spain)

Abstract: Four receptors for uronic acid salts have been prepared using a chiral bicyclic guanidinium unit and deoxycholic acid derivatives as building blocks. Binding constants in $CD_3CN/CDCl_3$ ranged from 3200 to 7000 M^{-1} for glucuronate and galacturonate salts. Nordeoxycholic-containing receptors were found more efficient and moderately selective for glucuronic salts. © 1997 Elsevier Science Ltd.

Molecular recognition of bio-relevant substrates such as amino acids, peptides, or nucleotides has attained in recent years a considerable degree of success.[1] However, development of artificial receptors for the efficient and selective recognition of carbohydrates is still an ongoing endeavour, partly due to the complex three-dimensional structures of these substrates, but also to their highly solvated hydroxy functionalities, frequently involved in extensive hydrogen bond networks.[2]

Among carbohydrates, D-glucuronic acid deserves particular attention since it actively participates in biotransformation and detoxification processes of a number of endogenous compounds, through hepatic glucuronidation.[3] Many carboxy-containing drugs also undergo conjugation with glucuronic acid in humans, leading to the formation of acyl glucuronides, which are excreted into urine.[4] A precise knowledge of the binding behaviour of these metabolites would help understanding drug-glucuronide interactions. We describe herein receptors **1**-**4** as model systems designed to estimate the relative contributions of ionic and non-ionic hydrogen bonds in glucuronate binding.



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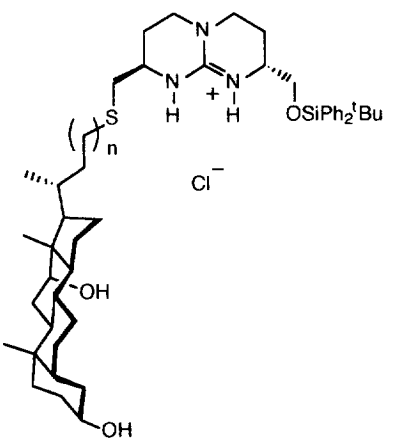
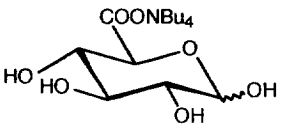
2 n = 2



3 n = 1
4 n = 2

Receptors **1** and **2** feature a chiral bicyclic guanidinium binding subunit attached through flexible spacers (differing in one carbon atom) to modified deoxycholic acid side arms. Guanidinium hosts are well known to form strong ion-paired complexes with oxoanions (carboxylates, phosphates or sulfates) even in competitive solvents.[5] Upon binding to uronic salts, the carboxylate-guanidinium ion-pair is expected to promote further contacts between hydroxy groups of the glycopyranosyl guest and the hydroxy groups converging at the inner concave sides of the curved profiles of deoxycholic acid derivatives, while the outer surface (steroid and bicyclic guanidinium) is essentially lipophilic and ensures good solubility in organic solvents.[6] In this study, complexation of tetrabutylammonium (TBA) D-glucuronate **5**, was compared with binding to tetraethylammonium (TEA) D-galacturonate salt **6**, differing in only the configuration at C-4.[7] Receptors **3** and **4**, having only one steroid side arm, as well as the model guanidinium salt **7**,[8] allow a more systematic study and the evaluation of the contribution of the various types of hydrogen bonds to the complexation, as well as to the extent of self- association in hosts **1-2**.



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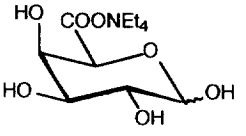

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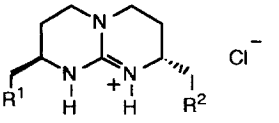


	R^1	R^2
7	$OSiMe_2{}^tBu$	$OSiPh_2{}^tBu$
8	Br	Br
9	Br	$OSiPh_2{}^tBu$

RESULTS AND DISCUSSION

Synthesis of Receptors

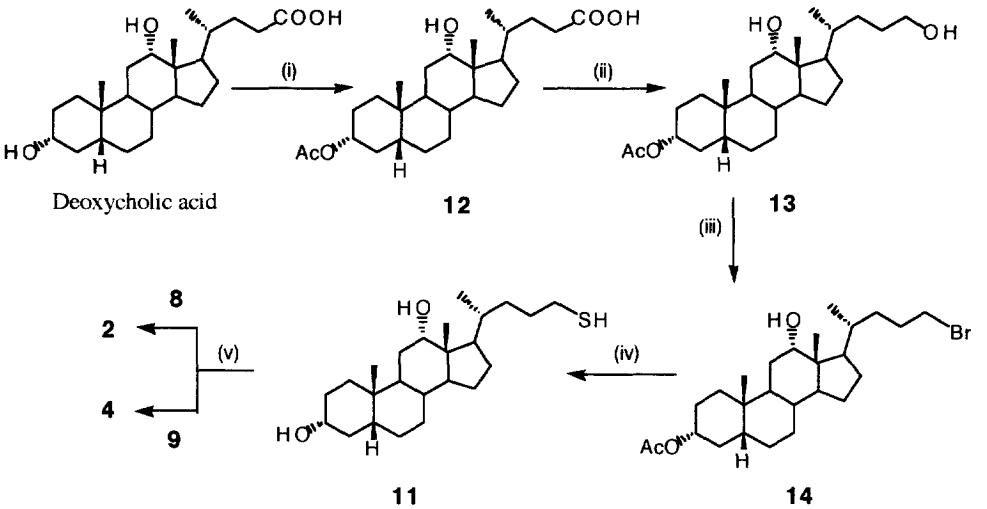
Compounds **1** and **2** were prepared by reaction of dibromoguanidinium chloride **8** with thiols **10** and **11**, respectively. Receptors **3** and **4** were obtained similarly from bromoguanidinium chloride **9**.[9] Both thiol precursors **10** and **11** were easily obtained from suitable protected forms of deoxycholic acid (Schemes 1 and 2). Thus, thiol **11** (Scheme 1) was assembled from mono-*O*-acetyl derivative **12**, which undergoes reduction to alcohol **13**, followed by bromination and reaction with thiourea. Although no protection of the hydroxyl groups was necessary in these reactions, acetylation of the group at C-3[10] resulted in an increased yield of 67 % for the bromination step.

For the preparation of thiol **10** (Scheme 2), the side chain of the steroid had to be shortened one carbon atom, through a modified Hundsdiecker reaction ($I_2/Pb(OAc)_4$).[11] Protection of both C-3 and C-12 hydroxy groups of deoxycholic acid was required. The choice of protective groups was made by solubility and ease of removal criteria. Although steroids are usually highly lipophilic materials, only a few protective groups are suitable in carbon tetrachloride, the solvent of choice for the Hundsdiecker reaction. Di-*O*-acetyl[10] and di-*O*-formyl[12] derivatives are easily available, but the latter was insoluble under the reaction conditions, while attempts to obtain the target thiol through alkaline hydrolysis of the di-*O*-acetyl thiouronium intermediate required longer

reaction times, leading to substantial amounts of undesired by-products. In contrast, simultaneous use of both protective groups, as in compound **15**, allowed an easy access to thiol **10**.

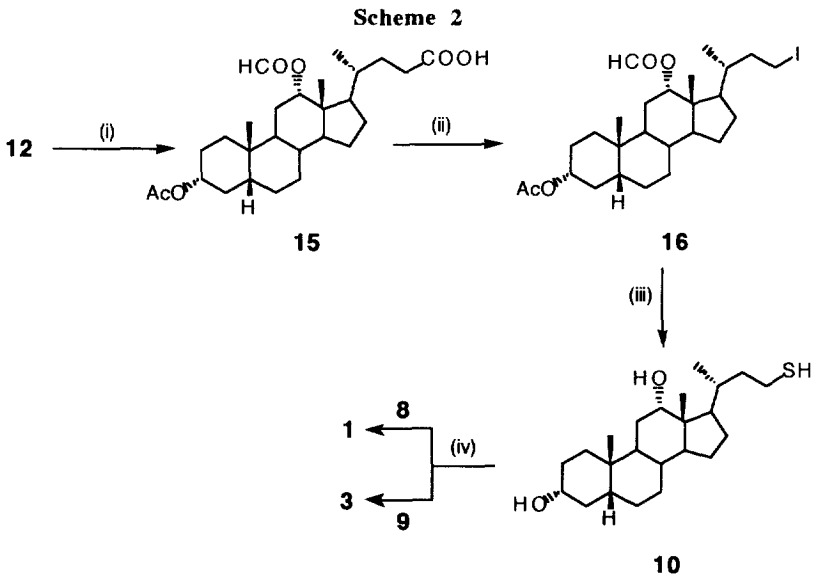
The thioether linkages were made by reaction of the thiolates (NaOMe, MeOH) with the corresponding bromoguanidinium salts.

Scheme 1



Conditions: (i) $(CH_3CO)_2O$, pyridine, 25 °C. (ii) BH_3.THF, THF, 40 °C. (iii) PPh_3, NBS, toluene, 111 °C. (iv) $(NH_2)_2CS$, EtOH, 78 °C, then NaOH aq., 78 °C. (v) NaOMe, MeOH, 25 °C.

Scheme 2



Conditions: (i) HCOOH, $HClO_4$, 50 °C, then $(CH_3CO)_2O$, 40 °C. (ii) $Pb(CH_3CO_2)_4$, I_2, CCl_4, hv, 77 °C. (iii) $(NH_2)_2CS$, EtOH, 78 °C, then NaOH aq., 78 °C. (iv) NaOMe, MeOH, 25 °C.

Binding Studies

Guanidinium complexes with oxoanions in apolar solvents are too strong to be evaluated by ^{1}H NMR titrations. Therefore, a more polar solvent that could compete to some extent for the H-bonding centers of the binding partners was required. Acetonitrile (dielectric constant $\varepsilon = 37.5$) has been succesfully employed to this respect.[13] For our study, a 92:8 acetonitrile-chloroform mixture was employed, with tetraalkylammonium salts of glucuronic and galacturonic acids **5** and **6** as soluble guests. Dilution experiments in this mixture of solvents showed very weak self-association for receptors and substrates above 10^{-3} M. In titrations, host concentration was kept constant while the concentration of the guest was gradually increased. The receptor guanidinium NH-signals were monitored as a function of the guest concentration and the complexation-induced change in chemical shift, $\Delta\delta$, was plotted against the guest concentration. Job plots were performed on a sufficient number of complexes so that 1:1 stoichiometry could be assumed for all cases under study[14]. Association constants and binding free energies were obtained by using a nonlinear least-squares curve-fitting program[15] and are collected in Table 1.

Table 1. Evaluated Host Protons, Association Constants K_a, and Binding Free Energies ΔG° for 1:1 Complexes of Receptors **1-4** and **7** with Carboxylate Salts in $CD_3CN/CDCl_3$ (92:8) at 298 K

Entry	Host	Guest	Evaluated protons ($\Delta\delta_{sat}$[a]; $\Delta\delta_{obs}$[b])	K_a ($l\ mol^{-1}$)	ΔG° (kcal mol^{-1})
1	**1**	**5**	NH (2.23; 2.05)	7000	-5.28
2	**1**	**6**	NH (2.44; 2.07)	5800	-5.16
3	**2**	**5**	NH (2.20; 1.84)	2800	-4.73
4	**2**	**6**	NH (2.76; 2.11)	3200	-4.81
5	**3**	**5**	NH (1.95; 1.75)	5900	-5.17
6	**3**	**6**	NH (2.41; 2.02)	5500	-5.13
7	**4**	**5**	NH (2.02; 1.74)	3300	-4.83
8	**4**	**6**	NH (2.56; 2.10)	3500	-4.86
9	**7**	**5**	NH (2.05; 1.53)	2800	-4.73
10	**7**	**6**	NH (2.73; 2.10)	2200	-4.59

[a] Estimated change in chemical shift at saturating binding. [b] Largest change in chemical shift observed during titration.

Association constants for receptors **1** and **3** with shorter spacers are higher than for deoxycholic hosts **2** and **4**. With glucuronate, values for constants were at least double with **1** and **3** (compare entries 1 and 3, or 5 and 7). This could be attributed to the increased loss of entropy upon complex formation in these more flexible receptors with longer side arms. In all cases, however, values ranged around $10^3\ M^{-1}$, only slightly higher than the constants with the simple model guanidinium salt **7** (entries 1 and 9). Thus, steroid units only influence binding weakly. For compounds **1** and **2**, with two side arms, extensive intramolecular hydrogen bonding before complexation could also account for the weak association observed. Indeed, IR studies in chloroform in the range 10^{-3} - 10^{-4} *M* revealed strongly chelated species, but this was also observed for the simple guanidinium model **7**

and for single armed receptors **3** and **4**, indicating that association is always present between the guanidinium moieties and their counterions.

Only a slight selectivity for glucuronate over galacturonate was observed for receptors **1** and **3**. Since the only difference between both carbohydrates is the configuration at C-4, the different counterions playing no significant role,[16] the lack of selectivity is not surprising.

CONCLUSIONS

Carbohydrate recognition is complicated by the formation of intramolecular hydrogen bonds and by solvation. Use of a framework with two steroids[17] as in **1** or **2**, should allow a single molecule of the receptor to fully wrap around the substrate, promoting the formation of a stable 1:1 complex of defined structure, as it has been previously shown for related systems.[18] However, bile acid derivatives connected through too flexible spacers do not efficiently contribute to binding. Shortening the side chain by just one carbon atom results in improved complexation. It could be predicted that use of more rigid receptors, i.e. in a macrocyclic framework, would result in higher stabilities and selectivities.

EXPERIMENTAL

Melting points are uncorrected. ^{1}H NMR spectra were recorded on a AMX 300 (300 MHz) spectrometer. ^{13}C NMR spectra were recorded using AMX 300 (75 MHz) or AC 200 (50 MHz) spectrometers. Chemical shifts are reported in parts per million (δ, ppm) relative to the solvent residual signal (assignments labelled with an asterisk are only tentative). Fast atom bombardment (FAB, NBA matrix) and electron impact (EI) mass spectra were recorded on a VG Autospec instrument. Optical rotations (1 dm cells, 20 °C) were measured in a Perkin-Elmer 24.1 MC polarimeter. Elemental analyses were recorded using Perkin-Elmer 2400 CHN or Perkin-Elmer 2400 CHNS/O instruments. Flash column chromatography separations were performed using SDS 60 (230-400 mesh) silica gel. Commercial grade reagents and solvents were used without further purification, unless otherwise specified.

3α-Acetoxy-12α-hydroxy-5β-cholan-24-oic acid (12). A mixture of deoxycholic acid (5.0 g, 12.74 mmol), acetic anhydride (50 ml) and pyridine (50 ml) was stirred at room temperature for 6 h. The solution was poured into ice and extracted with ethyl acetate. The organic layer was washed with 5 % HCl and brine, dried (Na_2SO_4), concentrated *in vacuo* and purified by column chromatography (dichloromethane-ethyl acetate, 7:3, 0.25 % AcOH) to yield **12** as a white solid (4.22 g, 76 %), m.p. 94-96 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ_H 4.69 (tt, 1H, J = 11.2, 4.7 Hz, CH-3), 3.99 (t, 1H, J = 2.6 Hz, CH-12), 2.40 (m, 1H, CH_2-23), 2.25 (m, 1H, CH_2-23), 2.00 (s, 3H, CH_3CO), 0.97 (d, 3H, J = 6.1 Hz, CH_3-21), 0.90 (s, 3H, CH_3-19), 0.67 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$, 50 MHz) δ_C 179.4 (COOH), 170.8 (*C*OCH_3), 74.3 (C-3), 73.2 (C-12), 48.0 (C-14), 47.1 (C-17), 46.3 (C-13), 41.7 (C-5), 35.8 (C-8), 35.0 (C-20), 34.7 (C-1), 34.0 (C-10), 33.4 (C-9), 32.0 (C-4), 30.9 (C-22), 30.6 (C-23), 28.5 (C-11), 27.4 (C-16), 26.8 (C-6), 26.3 (C-2), 25.9 (C-7), 23.5 (C-15), 23.0 (C-19), 21.4 (*C*H_3CO), 17.1 (C-21), 12.6 (C-18); FABMS *m/z* 435.4 (MH^+, 2 %), 417.4 (MH^+-H_2O, 2 %), 357.3 (MH^+-H_2O-AcOH, 73 %); $[\alpha]_D$ + 52.7 ° (c = 1.04, $CHCl_3$); Anal. Calcd. for $C_{26}H_{42}O_5$: C, 71.85; H, 9.74. Found: C, 71.51; H, 9.55.

3α-Acetoxy-12α,24-dihydroxy-5β-cholane (13). Borane-tetrahydrofuran complex (1M, 30.0 mmol) was added under argon to an ice-cooled stirred solution of **12** (2.2 g, 5.06 mmol) in dry tetrahydrofuran (30 ml), and the reaction mixture was heated at 40 °C for 18 h. Water was then added, the solvent was removed and the resulting residue was redissolved in dichloromethane, washed with 5 % HCl and brine, dried (Na_2SO_4) and concentrated *in vacuo*. The crude compound was purified by column chromatography (dichlorometane-ethyl acetate, 7:3, 0.25 % AcOH) to afford **13** as a white solid (1.63 g, 78 %), m.p. 130-132 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ_H 4.70 (tt, 1H, J = 11.3, 4.8 Hz, CH-3), 3.99 (t, 1H, J = 2.9 Hz, CH-12), 3.60 (t, 2H, J = 6.2 Hz, CH_2O), 2.01 (s, 3H, CH_3CO), 0.98 (d, 3H, J = 6.5 Hz, CH_3-21), 0.90 (s, 3H, CH_3-19), 0.68 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$, 50 MHz) δ_C 170.7 (*C*OCH_3), 74.3 (C-3), 73.0 (C-12), 63.2 (C-24), 48.1 (C-14), 47.4 (C-17), 46.3 (C-13), 41.7 (C-5), 35.8 (C-8), 35.2 (C-20), 34.7 (C-1), 34.0 (C-10), 33.5 (C-9), 32.0 (C-4), 31.7 (C-22), 29.3 (C-23), 28.5 (C-11), 27.5 (C-16), 26.8 (C-6), 26.3 (C-2), 25.9 (C-7), 23.5 (C-15), 23.0 (C-19), 21.4 (*C*H_3CO), 17.5 (C-21), 12.6 (C-18); FABMS *m/z* 421.4 (MH^+, 2 %), 403.4 (MH^+-H_2O, 3 %), 343.3 (MH^+-H_2O-AcOH, 100 %); $[\alpha]_D$ + 57.5 ° (*c* = 1.06, $CHCl_3$); Anal. Calcd. for $C_{26}H_{44}O_4$: C, 74.24; H, 10.54. Found: C, 73.92; H, 10.60.

3α-Acetoxy-24-bromo-12α-hydroxy-5β-cholane (14). *N*-bromosuccinimide (245 mg, 1.38 mmol) was added slowly to a cooled solution of triphenylphosphine (362 mg, 1.38 mmol) in toluene (30 ml). A vigorous reaction occurred and a brownish syrup developed. After 1 h at room temperature, **13** (385 mg, 0.91 mmol) was added and the mixture was refluxed for 38 h. The solvent was removed *in vacuo* and the residue was redissolved in dichloromethane, washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. The crude was purified by column chromatography (dichloromethane-ethyl acetate, 15:1) to yield **14** as a white solid (297 mg, 67 %), m.p. 126-128 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ_H 4.70 (tt, 1H, J = 11.3, 4.7 Hz, CH-3), 3.99 (m, 1H, CH-12), 3.41 (dt, 1H, J = 9.7, 6.7 Hz, CH_2Br), 3.36 (dt, 1H, J = 9.7, 6.9 Hz, CH_2Br), 2.01 (s, 3H, CH_3CO), 0.98 (d, 3H, J = 6.5 Hz, CH_3-21), 0.91 (s, 3H, CH_3-19), 0.68 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$, 50 MHz) δ_C 170.3 (*C*OCH_3), 74.1 (C-3), 72.9 (C-12), 48.1 (C-14), 47.2 (C-17), 46.3 (C-13), 41.7 (C-5), 35.8 (C-8), 34.8 (C-20), 34.7 (C-1), 34.3 (C-22), 34.2 (C-24), 34.0 (C-10), 33.4 (C-9), 32.0 (C-4), 29.4 (C-23), 28.5 (C-11), 27.4 (C-16), 26.8 (C-6), 26.3 (C-2), 25.9 (C-7), 23.5 (C-15), 23.0 (C-19), 21.3 (*C*H_3CO), 17.5 (C-21), 12.6 (C-18); EIMS *m/z* 466.3/464.3 (M^+-H_2O, 6 %), 424.3/422.3 (M^+-AcOH, 12 %), 406.3/404.3 (M^+-H_2O-AcOH, 44 %); $[\alpha]_D$ + 55.8 ° (*c* = 1.04, $CHCl_3$); Anal. Calcd. for $C_{26}H_{43}O_3Br$: C, 64.58; H, 8.96. Found: C, 64.69; H, 8.88.

3α,12α-Dihydroxy-24-mercapto-5β-cholane (11). A mixture of **14** (118 mg, 0.24 mmol), thiourea (21 mg, 0.27 mmol) and 95 % ethanol (5 ml) was refluxed for 24 h. The solution was cooled to room temperature and aqueous sodium hydroxide (1*N*, 0.50 mmol) was then added; the mixture was refluxed under argon for 3.5 h. The reaction mixture was cooled to room temperature, acidified with 5 % HCl and concentrated *in vacuo*. The residue was redissolved in ethyl acetate, washed with brine, dried (Na_2SO_4) and evaporated. The crude was purified by column chromatography (dichloromethane-ethyl acetate, 1:1) to yield thiol **11** as a white solid (68 mg, 70 %), m.p. 74-76 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ_H 3.98 (t, 1H, J = 2.9 Hz, H-12), 3.59 (tt, 1H, J = 11.0, 4.6 Hz, CH-3), 2.46 (m, 2H, CH_2S), 0.96 (d, 3H, J = 6.5 Hz, CH_3-21), 0.90 (s, 3H, CH_3-19), 0.67 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$, 50 MHz) δ_C 73.0 (C-12), 71.5 (C-3), 48.0 (C-14), 47.3 (C-17), 46.3 (C-13), 41.9 (C-5), 36.2 (C-4), 35.9 (C-8), 35.2 (C-20), 34.9 (C-1), 34.2 (C-22), 34.2 (C-10), 33.4 (C-9), 30.7 (C-23), 30.2 (C-2), 28.4 (C-11), 27.5 (C-16), 27.0 (C-6), 26.0 (C-7), 24.9 (C-24), 23.6 (C-15), 23.0 (C-19), 17.5 (C-21), 12.6 (C-18); FABMS *m/z* 377.3 (MH^+-H_2O, 19 %), 359.3 (MH^+-$2H_2O$, 60 %); $[\alpha]_D$ + 41.0 ° (*c*

= 0.5, $CHCl_3$); Anal. Calcd. for $C_{24}H_{42}O_2S$: C, 73.04; H, 10.73; S, 8.12. Found: C, 72.65; H, 10.44; S, 8.31.

3α-Acetoxy-12α-formyloxy-5β-cholan-24-oic acid (15). A suspension of **12** (3.68 g, 8.47 mmol) in 15 ml of 90 % formic acid containing 0.03 ml of 70 % perchloric acid was stirred at 50 °C for 1.5 h. The reaction mixture was then cooled to 40 °C, acetic anhydride (12 ml) was carefully added and the mixture was further stirred for 10 minutes. The solution was cooled to room temperature and poured into 185 ml of water with vigorous stirring. The precipitate was filtered, washed with water, redissolved in diethyl ether, and the organic layer was washed with brine, dried (Na_2SO_4) and evaporated to afford **15** as a white solid (3.59 g, 92 %), m.p. 190-192 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ_H 8.13 (s, 1H, HCOO), 5.23 (bs, 1H, CH-12), 4.69 (tt, 1H, J = 11.2, 4.7 Hz, CH-3), 2.38 (m, 1H, CH_2-23), 2.23 (m, 1H, CH_2-23), 2.02 (s, 3H, CH_3CO), 0.90 (s, 3H, CH_3-19), 0.83 (d, 3H, J = 6.2 Hz, CH_3-21), 0.74 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$, 50 MHz) δ_C 180.1 (COOH), 170.6 ($COCH_3$), 160.5 (HCOO), 75.9 (C-12), 74.1 (C-3), 49.2 (C-14), 47.3 (C-17), 44.9 (C-13), 41.6 (C-5), 35.5 (C-8), 34.7 (C-20), 34.6 (C-1), 34.1 (C-9), 34.0 (C-10), 32.0 (C-4), 30.9 (C-22), 30.4 (C-23), 27.2 (C-16), 26.7 (C-6), 26.4 (C-2), 25.8 (C-11), 25.7 (C-7), 23.4 (C-15), 22.9 (C-19), 21.4 (CH_3CO), 17.3 (C-21), 12.1 (C-18); FABMS m/z 463.5 (MH^+, 1 %), 417.5 (MH^+-H_2CO_2, 13 %), 357.4 (MH^+-H_2CO_2-AcOH, 100 %); $[\alpha]_D$ + 87.5 ° (c = 1.0, $CHCl_3$); Anal. Calcd. for $C_{27}H_{42}O_6$: C, 70.10; H, 9.15. Found: C, 69.78; H, 9.14.

3α-Acetoxy-12α-formyloxy-23-iodo-24-nor-5β-cholane (16). To a 5 % w/v suspension of lead tetraacetate (4.80 g, 10.83 mmol) in refluxing carbon tetrachloride (tungsten lamp), was added **15** (5.00 g, 10.81 mmol) and then iodine (2.75 g, 10.83 mmol) in the same solvent until the iodine colour persisted. The reaction mixture was cooled to room temperature, washed with 10 % $Na_2S_2O_3$ and brine, dried (Na_2SO_4) and evaporated. The residue was purified by column chromatography (hexane-ether, 8:2) to yield **16** as a white solid (3.7 g, 63 %), m.p. 168-170 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ_H 8.13 (s, 1H, HCOO), 5.25 (bs, 1H, CH-12), 4.70 (tt, 1H, J = 11.4, 4.6 Hz, CH-3), 3.29 (ddd, 1H, J = 9.2, 9.2, 3.8 Hz, CH_2-23), 3.05 (ddd, 1H, J = 9.5, 9.5, 8.0 Hz, CH_2-23), 2.03 (s, 3H, CH_3CO), 0.92 (s, 3H, CH_3-19), 0.83 (d, 3H, J = 6.2 Hz, CH_3-21), 0.76 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 170.4 ($COCH_3$), 160.5 (HCOO), 76.0 (C-12), 74.1 (C-3), 49.2 (C-14), 47.2 (C-17), 45.1 (C-13), 41.7 (C-5), 39.9 (C-22), 36.6 (C-20), 35.8 (C-8), 34.7 (C-1), 34.2 (C-9), 34.0 (C-10), 32.1 (C-4), 27.3 (C-16), 26.8 (C-6), 26.5 (C-2), 25.9 (C-11), 25.7 (C-7), 23.4 (C-15), 22.9 (C-19), 21.4 (CH_3CO), 17.0 (C-21), 12.3 (C-18), 4.8 (C-23); EIMS m/z 498.3 (M^+-H_2CO_2, 8 %), 438.2 (M^+-H_2CO_2-AcOH, 100 %), 311.3 (M^+-H_2CO_2-AcOH-I, 53 %); $[\alpha]_D$ + 99.4 ° (c = 0.5, $CHCl_3$); Anal. Calcd. for $C_{26}H_{42}O_4I$: C, 57.35; H, 7.59. Found: C, 56.92; H, 7.56.

3α,12α-Dihydroxy-23-mercapto-24-nor-5β-cholane (10). A mixture of **16** (1.78 g, 3.27 mmol), thiourea (300 mg, 3.94 mmol) and 95 % ethanol (90 ml) was refluxed for 48 h. The solvent was removed *in vacuo* and the resulting residue was purified by chromatography (dichloromethane-methanol, 11:1) to afford the intermediate iodide salt (1.36 g, 67 %) as a yellow solid. To a stirred solution of this salt (1.27 g, 2.05 mmol) in 35 ml 95 % ethanol, aqueous sodium hydroxide (2N, 6.80 mmol) was then added and the mixture was refluxed under argon for 2.5 h. The solution was cooled to room temperature, acidified with 5 % HCl and concentrated *in vacuo*. The residue was redissolved in ethyl acetate, washed with brine, dried (Na_2SO_4) and evaporated. The crude was purified by column chromatography (dichloromethane-ethyl acetate, 5:2) to yield thiol **10** as a white solid (520 mg, 67 %), m.p. 84-86 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ_H 3.98 (t, 1H, J = 2.9 Hz, CH-12), 3.61 (tt, 1H, J = 11.0, 4.6 Hz, CH-3), 2.62 (dddd, 1H, J = 12.6, 9.5, 7.6, 4.7 Hz, CH_2S), 2.43 (dddd, 1H, J =

12.8, 8.9, 7.4, 7.4 Hz, CH_2S), 0.97 (d, 3H, J = 6.3 Hz, CH_3-21), 0.90 (s, 3H, CH_3-19), 0.68 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 73.1 (C-12), 71.8 (C-3), 48.2 (C-14), 47.4 (C-17), 46.5 (C-13), 42.0 (C-5), 40.4 (C-22), 36.4 (C-4), 36.0 (C-8), 35.1 (C-1), 34.8 (C-20), 34.1 (C-10), 33.6 (C-9), 30.4 (C-2), 28.6 (C-11), 27.5 (C-16), 27.1 (C-6), 26.1 (C-7), 23.6 (C-15), 23.1 (C-19), 21.9 (C-23), 17.3 (C-21), 12.7 (C-18); EIMS m/z 362.3 (M^+-H_2O, 51 %), 344.3 (M^+-$2H_2O$, 29 %); $[\alpha]_D$ + 50.8 ° (c = 0.5, $CHCl_3$); Anal. Calcd. for $C_{23}H_{40}O_2S$: C, 72.58; H, 10.59; S, 8.42. Found: C, 72.45; H, 10.67; S, 8.53.

(4*R*, 8*R*)-4,8-Bis[(3α,12α-dihydroxy-24-nor-5β-cholan-23-thiomethyl)]-1,5,7-triazabicycle [4.4.0]-5-decene, hydrochloride (1). To a solution of **10** (480 mg, 1.26 mmol) in dry methanol (10 ml), a freshly prepared 1*M* NaOMe solution in MeOH (1.77 mmol) was added and the mixture was stirred at room temperature for 1.5 h. A solution of **8** (264 mg, 0.69 mmol) in dry methanol (5 ml) was added in portions, the reaction mixture was stirred at room temperature for 15 h, and then acidified with 5 % HCl. The solvent was removed *in vacuo*, and the residue was redissolved in ethyl acetate, washed with 2*M* KOH, 5 % HCl, and brine, dried (Na_2SO_4), concentrated *in vacuo* and purified by column chromatography (dichloromethane-methanol, 14:1) to afford **1** as a white solid (402 mg, 66 %), m.p. 130-132 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ_H 8.84 (s, 2H, NH), 3.96 (bs, 2H, H-12), 3.67-3.51 (m, 4H, CH-α, CH-3), 3.33 (t, 4H, J = 5.6 Hz, CH_2-γ), 2.88 (dd, 2H, J = 13.5, 3.8 Hz, CH_2S), 2.74-2.45 (m, 6H, CH_2S, CH_2-23), 1.00 (d, 6H, J = 6.0 Hz, CH_3-21), 0.89 (s, 6H, CH_3-19), 0.66 (s, 6H, CH_3-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 150.9 (C guan.), 72.9 (C-12), 71.6 (C-3), 48.5 (C-α), 48.1 (C-14), 46.7 (C-17), 46.5 (C-13), 45.2 (C-γ), 42.0 (C-5), 36.7 (CH_2S), 36.4 (C-4), 35.9 (C-8), 35.7 (C-22*), 35.3 (C-1), 35.2 (C-20), 34.0 (C-10), 33.5 (C-9), 30.4 (C-2), 30.3 (C-23*), 28.5 (C-11), 27.7 (C-16), 27.1 (C-6), 26.1 (C-7), 25.0 (C-β), 23.7 (C-15), 23.1 (C-19), 17.4 (C-21), 12.6 (C-18); FABMS m/z 924.5 (M^+-Cl, 79 %); $[\alpha]_D$ + 40.0 ° (c = 0.5, $CHCl_3$); Anal. Calcd. for $C_{55}H_{94}O_4N_3S_2Cl$: C, 68.75; H, 9.86; N, 4.37; S, 6.67. Found: C, 69.04; H, 10.22; N, 4.28; S, 6.98.

(4*R*, 8*R*)-4,8-Bis[(3α,12α-dihydroxy-5β-cholan-24-thiomethyl)]-1,5,7-triazabicycle[4.4.0]-5-decene, hydrochloride (2). Prepared as **1**, from **11** (100 mg, 0.25 mmol) in dry methanol (2 ml), 1 *M* NaOMe in methanol (0.303 mmol), and **8** (48 mg, 0.13 mmol) in dry methanol (1 ml). Compound **2** was isolated as a white solid (0.06 g, 46 %), ^1H NMR ($CDCl_3$, 300 MHz) δ_H 8.86 (s, 2H, NH), 3.97 (bs, 2H, CH-12), 3.67-3.48 (m, 4H, CH-α, CH-3), 3.31 (t, 4H, J = 5.9 Hz, CH_2-γ), 2.90 (dd, 2H, J = 13.5, 3.8 Hz, CH_2S), 2.43 (dd, 2H, J = 13.7, 9.3 Hz, CH_2S), 2.64-2.42 (m, 4H, CH_2-23), 0.97 (d, 6H, J = 6.4 Hz, CH_3-21), 0.90 (s, 6H, CH_3-19), 0.67 (s, 6H, CH_3-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 150.9 (C guan.), 73.2 (C-12), 71.8 (C-3), 48.4 (C-α), 48.2 (C-14), 47.0 (C-17), 46.5 (C-13), 45.3 (C-γ), 42.1 (C-5), 36.4 (CH_2S, C-4), 36.0 (C-8), 35.3 (C-20), 34.8 (C-1), 34.1 (C-10), 33.6 (C-9), 33.5 (C-22*), 30.5 (C-2), 30.3 (C-23*), 28.5 (C-11), 27.6 (C-16), 27.1 (C-6), 26.3 (C-24*), 26.1 (C-7), 25.0 (C-β), 23.7 (C-15), 23.1 (C-19), 17.8 (C-21), 12.7 (C-18); FABMS m/z 952.9 (M^+-Cl, 100 %); $[\alpha]_D$ + 39.2 ° (c = 0.5, $CHCl_3$); Anal. Calcd. for $C_{57}H_{98}O_4N_3S_2Cl$: C, 69.22; H, 9.99; N, 4.25; S, 6.48. Found: C, 69.15; H, 9.60; N, 4.08; S, 6.23.

(4*R*, 8*R*)-4-(*tert*-Butyldiphenylsilyloxymethyl)-8-(3α,12α-dihydroxy-24-nor-5β-cholan-23-thiomethyl)-1,5,7-triazabicycle[4.4.0]-5-decene, hydrochloride (3). Prepared as **1**, from **10** (103 mg, 0.21 mmol) in dry methanol (1 ml), 1 *M* NaOMe in methanol (0.303 mmol), and **9** (137 mg, 0.38 mmol) in dry methanol (1 ml). Compound **3** was isolated as a white solid (0.10 g, 55 %), m.p. 130-132 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ_H 9.10 (s, 1H, NH), 8.70 (s, 1H, NH), 7.70-7.55 (m, 4H, Ar), 7.50-7.30 (m, 6H, Ar), 3.94 (bs, 1H, CH-12), 3.81 (d, 1H, J = 6.0 Hz, CH_2O), 3.65-3.40 (m, 4H, CH-α, CH_2O, CH-3), 3.30-3.10 (m, 4H, CH_2-γ), 2.88 (d, 1H, J = 11.9 Hz, CH_2S), 2.70-2.40 (m, 3H, CH_2S, CH_2-23), 1.04 (s, 9H, ^tBu),

0.97 (d, 3H, J = 6.2 Hz, CH_3-21), 0.88 (s, 3H, CH_3-19), 0.64 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 151.1 (C guan.), 135.6, 135.5, 132.7, 132.6, 129.9, 127.9, 127.8 (Ar), 73.0 (C-12), 71.6 (C-3), 65.2 (CH_2O), 49.0, 48.4 (C-α), 48.1 (C-14), 47.1 (C-17), 46.5 (C-13), 45.3, 44.6 (C-γ), 42.0 (C-5), 36.7 (CH_2S), 36.4 (C-4), 36.0 (C-8), 35.7 (C-22*), 35.2 (C-1), 35.1 (C-20), 34.1 (C-10), 33.5 (C-9), 30.4 (C-2), 28.5 (C-11), 27.6 (C-16), 27.1 (C-6), 26.8 [C(CH_3)$_3$], 26.1 (C-7), 24.9 (C-β), 23.6 (C-15), 23.1 (C-19), 22.8 (C-23*), 19.1 [C(CH_3)$_3$], 17.4 (C-21), 12.7 (C-18); FABMS m/z 800.7 (M^+-Cl, 100 %); $[\alpha]_D$ + 12.8 ° (c = 0.5, $CHCl_3$); Anal. Calcd. for $C_{48}H_{74}O_3N_3SSiCl$: C, 68.90; H, 8.91; N, 5.02; S, 3.83. Found: C, 68.81; H, 8.47; N, 4.67; S, 3.72.

(4*R*, 8*R*)-4-(*tert*-Butyldiphenylsilyloxymethyl)-8-(3α,12α-dihydroxy-5β-cholan-24-thiomethyl)-1,5,7-triazabicycle[4.4.0]-5-decene, hydrochloride (4). Prepared as **1**, from **11** (243 mg, 0.62 mmol) in dry methanol (2 ml), 1 *M* NaOMe in methanol (0.83 mmol), and **9** (315 mg, 0.59 mmol) in dry methanol (1 ml). Compound **4** was isolated as a white solid (230 mg, 44 %), m.p. 92-94 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ_H 9.17 (s, 1H, NH), 8.72 (s, 1H, NH), 7.70-7.55 (m, 4H, Ar), 7.50-7.30 (m, 6H, Ar), 3.96 (bs, 1H, CH-12), 3.82 (d, 1H, J = 6.1 Hz, CH_2O), 3.65-3.40 (m, 4H, CH-α, CH_2O, CH-3), 3.30-3.10 (m, 4H, CH_2-γ), 2.89 (dd, 1H, J = 13.3, 4.0 Hz, CH_2S), 2.56 (dd, 1H, J = 13.3, 9.7 Hz, CH_2S), 2.55-2.45 (m, 2H, CH_2-23), 1.06 (s, 9H, ^{t}Bu), 0.96 (d, 3H, J = 6.1 Hz, CH_3-21), 0.90 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 151.0 (C guan.), 135.5, 135.4, 132.6, 129.9, 127.8 (Ar), 73.1 (C-12), 71.6 (C-3), 65.1 (CH_2O), 49.0, 48.4 (C-α), 48.1 (C-14), 47.2 (C-17), 46.4 (C-13), 45.2, 44.5 (C-γ), 42.0 (C-5), 36.5 (CH_2S), 36.2 (C-4), 35.9 (C-8), 33.5 (C-22*), 35.2 (C-1, C-20), 34.0 (C-10), 33.5 (C-9), 30.3 (C-2), 28.4 (C-11), 27.6 (C-16), 27.0 (C-6), 26.8 [C(CH_3)$_3$], 26.4 (C-23*), 26.0 (C-7), 24.7 (C-β), 23.6 (C-15), 23.0 (C-19), 22.7 (C-24*), 19.1 [C(CH_3)$_3$], 17.6 (C-21), 12.6 (C-18); FABMS m/z 814.4 (M^+-Cl, 100 %); $[\alpha]_D$ + 32.3 ° (c = 1.0, $CHCl_3$); Anal. Calcd. for $C_{49}H_{76}O_3N_3SSiCl$: C, 69.18; H, 9.00; N, 4.94; S, 3.77. Found: C, 68.85; H, 8.70; N, 4.53; S, 3.63.

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