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Synthesis of 7-deoxycholic amides or cholanes containing distinctive ion-recognizing groups at C3 and C12 and evaluation for ion-selective ionophores

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ABSTRACT

Tweezer-type ionophores containing C3-bipyridyl and C12-dithiocarbamoyl groups, or C3-bithiophenyl with C12-dithiocarbamoyl groups on a 7-deoxycholic amide or cholane derivatives were designed and synthesized. Potentiometric evaluation of the PVC membranes containing those deoxycholic amides/cholanes linked with unsymmetrically substituted tweezer-type bipyridyl or bithiophenyl with dithiocarbamoyl moieties proved their good affinity and selectivity for silver(I) ion. Especially, ionophores with bithiophenyl moiety on the 3α-position and diphenylaminothioxomethylthioacetyloxy group on 12α-position of cholan-24-amide or cholane reveal the most excellent result with a theoretical slope value of 59 mV/decade.

1. Introduction

Ion-selective electrodes (ISEs) are a distinctive example of chemical sensors that use the principle of molecular recognition chemistry. For this reason, new molecular tweezer-type neutral carriers based on a cholic acid backbone (CAB) have been designed, synthesized, and successfully utilized to yield highly selective ionophores for carbonate, chloride, silver, and calcium by us in recent years.[1–6] The CAB-based carriers are relatively easy to design and straightforward to synthesize. Moreover, previous examples show that the CAB frame is as versatile as calix[*n*]arenes for the development of selective ionophores. The selective ion-tweezing ability of a CAB-based neutral carrier can be controlled by changing the several factors; (1) variation in cavity size between two binding sites by changing the length of the linking chain between the substituent and the hydroxyl linker; (2) differentiation of the steric hindrance between C3 and C12 carbons of the backbone, and modification of ion-recognizing functional groups. Thus, many different CAB-based neutral carriers can be designed by substituting various types of ion-recognizing groups to the two hydroxyl linkers, which are approximately parallel at the C3 and C12 carbons of the frame and about 6 Å apart that is a reasonable distance for tweezing the various ions.[7]

Our initial success in several CAB-based neutral carriers has encouraged us to develop ion-selective ionophores for calcium, magnesium, anions, and transition metal ions, including silver ion with the same frame.[5] Most known transition metal-selective ionophores, such as silver ion-selective ionophores, possess sulfur and/or nitrogen atoms in their proposed binding sites.[8] Therefore, bipyridyl moieties containing nitrogen atoms[4] or dithiocarbamoyl moieties containing sulfur atoms[6] as a binding site for the silver ion-selective ionophores were adopted to 7-deoxycholic amide previously and showed reasonable affinity and selectivity to silver(I) ions over other metal cations.

Up to the present, all the CAB-based neutral carriers we have reported have the same ion-recognizing groups at C3 and C12 because introducing the different functional groups at C3 and C12 was hardly possible. Fortunately, we recently found a synthetic method for introducing different functional groups in a step-by-step fashion at C3 and C12 on CAB, which could be a cornerstone for developing diverse types of CAB-based neutral carriers with different ion-recognizing groups in the same molecule. Consequently, unsymmetrically substituted tweezer-type CAB-based ionophores, that is, the nitrogen atom-binding site containing moiety at C3 and sulfur atom-binding site containing moiety at C12, or sulfur atom-binding site containing moieties with different functional groups, such as C3-bithiophenyl and C12-dithiocarbamoyl moieties were designed and synthesized. Newly synthesized unsymmetrically substituted tweezer-type CAB-based ionophores showed excellent affinity and selectivity to silver(I) ions over alkali, alkaline earth, and other transition metal ions. Herein, we report on the synthesis and application to ionophores of CAB-based ionophores that have substituted with different ion-recognizing functional groups at C3 and C12 of 7-deoxycholic amide or cholane.

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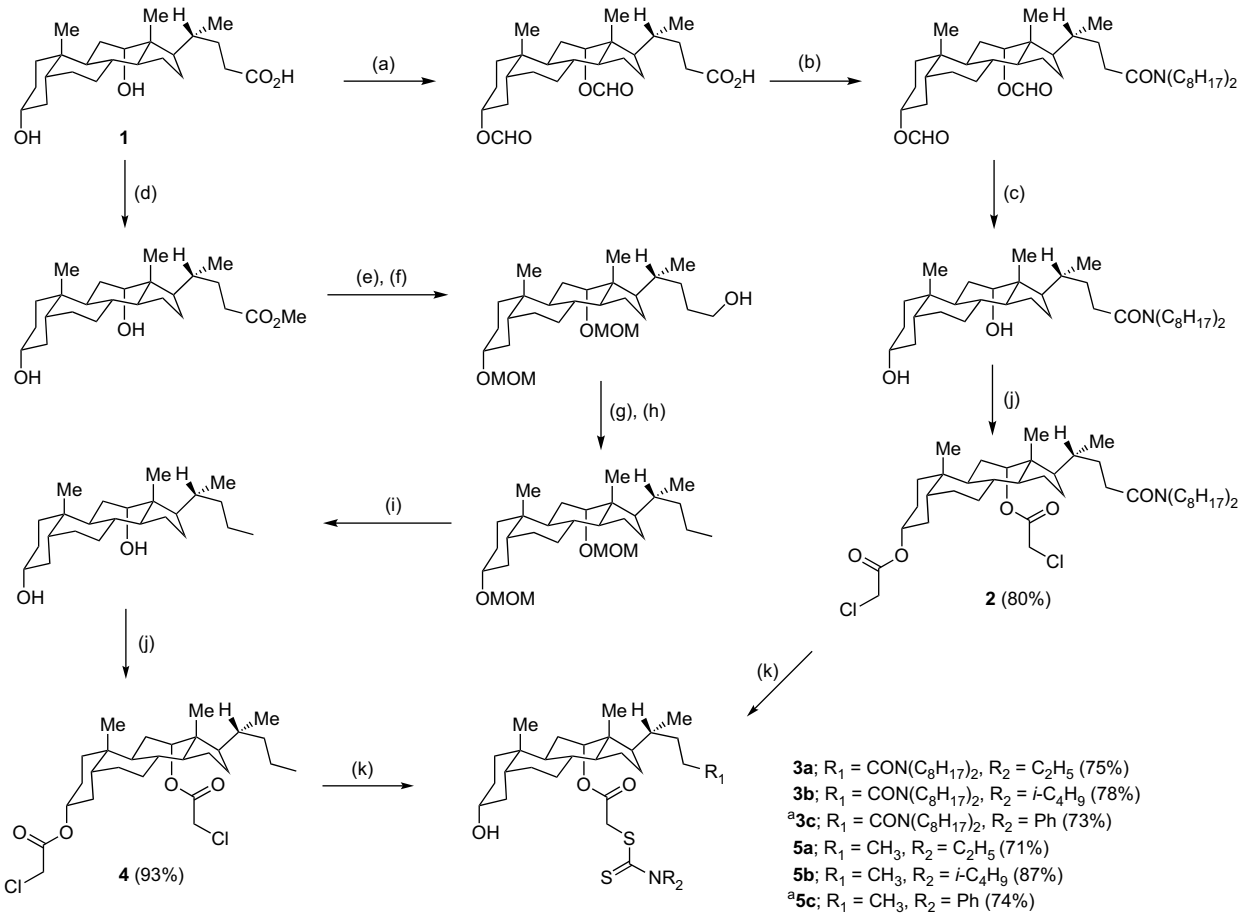


Scheme 1. (a) HCOOH, Ac_2O, 55 °C, 1.5 h, 99%; (b) ClCOOMe, NEt_3, $HN(C_8H_{17})_2$, 0 °C, 3 h, 89%; (c) K_2CO_3, MeOH, THF, 0 °C, 24 h, 98%; (d) AcCl, MeOH, 0 °C, 20 h, 86%; (e) $CH_2(OCH_3)_2$, CF_3SO_3H, CH_2Cl_2, rt, 5 h; (f) LAH, Et_2O, rt, 2 h, 88% (for two steps); (g) TsCl, Py, 75 °C, 1.5 h; (h) LAH, Et_2O, 0 °C, 1 h, 73% (for two steps); (i) HCl, MeOH, CH_2Cl_2, 50 °C, 2 days, 96%; (j) $ClCH_2COCl$, CaH_2, Bu_4NBr, Tol, 90 °C, 5 h; (k) (i) $NaSC(S)NR_2$, EtOH, 70 °C, 12 h, (ii) saturated LiOH (aq) 2 mL, 5 min ([a]**3c**, [a]**5c**; saturated LiOH (aq) 10 mL, 10 min).

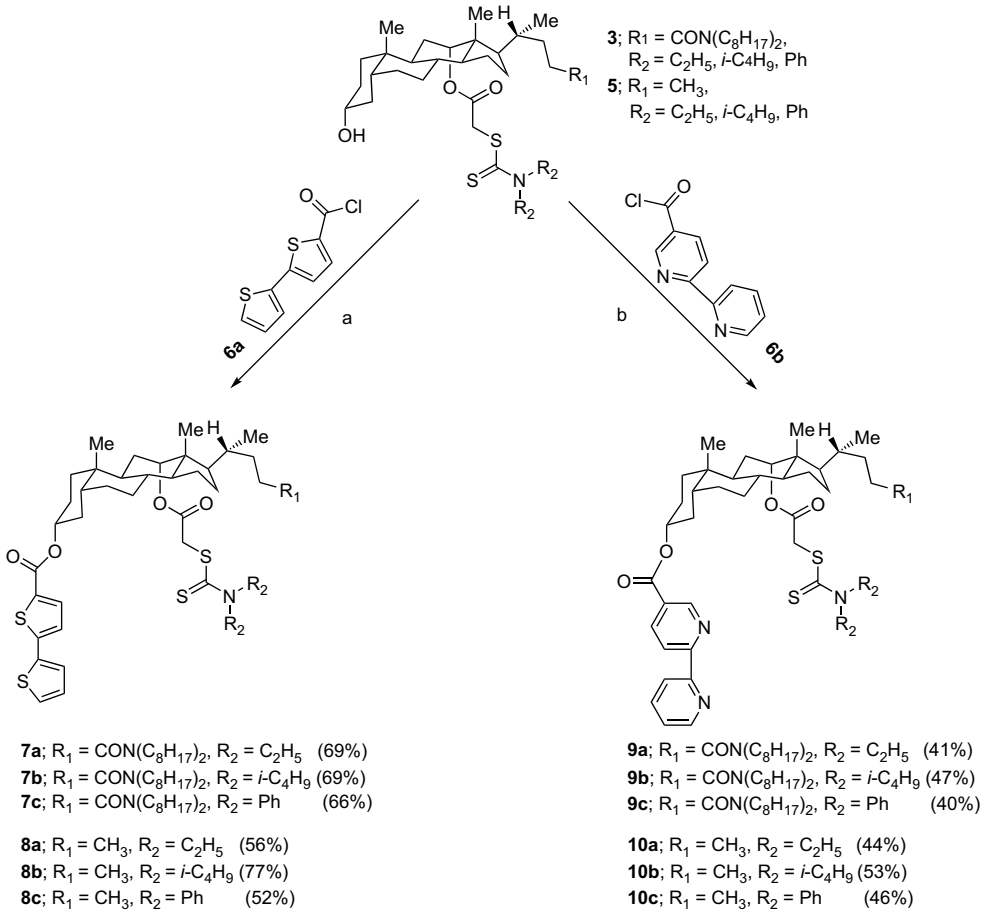
2. Results and discussion

2.1. Synthesis of ionophores

As mentioned earlier in the introduction, our previously reported CAB-based neutral carriers have the same ion-recognizing groups at C3 and C12 because introducing the different functional groups at C3 and C12 independently was not easy to control. However, recently we found that 3α,12α-bis(chloroacetoxy)-*N*,*N*-dioctyl-5β-cholan-24-amide (**2**) could be transformed into mono-substituted 5β-cholan-24-amides, **3a**–**3c**, instead of di-substituted 5β-cholan-24-amide using the modified procedure.[6] Although **2** and sodium *N*,*N*-di-R-dithiocarbamate (R=ethyl, isobutyl, phenyl) in THF at reflux (bath temp, 70 °C) produced di-R-substituted 5β-cholan-24-amide, such as *N*,*N*-dioctyl-3α,12α-bis[[[[(di-R-amino)-thioxomethyl]thio]acetyl]oxy]-5β-cholan-24-amide (R=ethyl, isobutyl, phenyl), **2** and sodium *N*,*N*-di-R-dithiocarbamate (R=ethyl, isobutyl, phenyl) in EtOH at 70 °C produced 3α-hydroxy-*N*,*N*-dioctyl-12α-[[[[(di-R-amino)thioxomethyl]thio]acetyl]oxy]-5β-cholan-24-amides (**3a**–**3c**) with good yields. In EtOH, the formation of *N*,*N*-dioctyl-3α,12α-bis[[[[(di-R-amino)thioxomethyl]thio]acetyl]oxy]-5β-cholan-24-amide was observed first, which is similar to the reaction in THF. However, 3α-hydroxy-*N*,*N*-dioctyl-12α-[[[[(di-R-thioxomethyl]thio]acetyl]oxy]-5β-cholan-24-amide (**3**) gradually appeared and increased as the reaction proceeded. After 24 h, *N*,*N*-dioctyl-3α,12α-bis[[[[(di-R-amino)thioxomethyl]thio]acetyl]oxy]-5β-cholan-24-amide disappeared and **3** was obtained as a final product. It seemed that highly selective trans-esterification had occurred. Although a more sterically hindered secondary alkoxy group of 12α-position is safe for trans-esterification in EtOH, the relatively less hindered secondary alkoxy group of 3α-position is cleaved to the hydroxyl group via a trans-esterification reaction even in the neutral condition.

To improve the time-consuming trans-esterification reaction condition, several control experiments were carried out in this study. As a result, we found that hydrolysis of 3α-position was accelerated the reaction to completion appropriately by adding aqueous LiOH to the reaction mixture (Scheme 1, step k), which improved the reaction time and handiness compared with the results from the previous trans-esterification method. As it has a free 3α-hydroxy group, it can be used as a precursor for the synthesis of tweezer-type CAB-based ionophores that has a different ion recognition group on 3α- and 12α-position, respectively, which would be a valuable precursor for developing a new type of ionophores.

In addition to optimizing ion recognition groups on 3α- and 12α-position, the evaluation of lipophilic characteristics of the 5β-side chain is also important.[6] Therefore, the preparation of 5β-cholan-24-amides with long alkyl chains on the amide group and 5β-cholane derivatives with an alkyl chain should likewise be optimized in order to obtain the best ionophore. Thus, to prepare 5β-cholane precursors, the similar reaction condition was applied to 3α,12α-bis(chloroacetoxy)-5β-cholane (**4**) and it produced 3α-hydroxy-12α-[[[[(di-R-amino)thioxomethyl]thio]acetyl]oxy]-5β-cholanes (**5a**–**5c**, R=ethyl, isobutyl, phenyl) as well (Scheme 1, step l). Accordingly, **3a**–**3c** and **5a**–**5c**, containing 12α-dithiocarbamoyl groups on a 7-deoxycholic amide or cholane derivatives were



7a; R_1 = $CON(C_8H_{17})_2$, R_2 = C_2H_5 (69%)
7b; R_1 = $CON(C_8H_{17})_2$, R_2 = i-C_4H_9 (69%)
7c; R_1 = $CON(C_8H_{17})_2$, R_2 = Ph (66%)

8a; R_1 = CH_3, R_2 = C_2H_5 (56%)
8b; R_1 = CH_3, R_2 = i-C_4H_9 (77%)
8c; R_1 = CH_3, R_2 = Ph (52%)

9a; R_1 = $CON(C_8H_{17})_2$, R_2 = C_2H_5 (41%)
9b; R_1 = $CON(C_8H_{17})_2$, R_2 = i-C_4H_9 (47%)
9c; R_1 = $CON(C_8H_{17})_2$, R_2 = Ph (40%)

10a; R_1 = CH_3, R_2 = C_2H_5 (44%)
10b; R_1 = CH_3, R_2 = i-C_4H_9 (53%)
10c; R_1 = CH_3, R_2 = Ph (46%)

Scheme 2. (a) **6a**, DMAP, TEA, CH_2Cl_2, rt, 24 h, (b) **6b**, pyridine, reflux, 12 h.

prepared from 7-deoxycholic acid (**1**) as described in Scheme 1, which used as precursors in this study.

After the preparation of precursors, **3a**–**3c** and **5a**–**5c**, the esterification of the 3α-hydroxy group was performed with proper carbonyl chloride to transform the 3α-hydroxy to the proper ester group that has ion-recognizing functional groups such as bithiophenyl or bipyridyl moiety. Thus, **3a**–**3c** or **5a**–**5c** was treated with [2,2′-bithiophene]-5-carbonyl chloride (**6a**) in the presence of DMAP and TEA in CH_2Cl_2 at room temperature for 24 h to produce 12α-[[[[(di(alkyl, aryl)amino)thioxomethyl]thio]acetyl]oxy]-3α-[[[2,2′-bithiophen]-5-yl]carboxy]-*N*,*N*-dioctyl-5β-cholan-24-amides (**7a**–**7c**) or 12α-[[[[(di(alkyl, aryl)amino)thioxomethyl]thio]acetyl]oxy-3α-[[[2,2′-bithiophen]-5-yl]carboxy]-5β-cholanes (**8a**–**8c**) correspondingly (Scheme 2, (a)). Likewise, 12α-[[[[(di(alkyl, aryl)amino)thioxomethyl]thio]acetyl]oxy]-3α-[[[2,2′-bipyridin]-5-yl]carboxy]-*N*,*N*-dioctyl-5β-cholan-24-amides (**9a**–**9c**) or 12α-[[[[(di(alkyl, aryl)amino)-thioxomethyl]thio]acetyl]oxy-3α-[[[2,2′-bipyridin]-5-yl]carboxy]-5β-cholanes (**10a**–**10c**) were obtained by treating **3a**–**3c** or **5a**–**5c** with [2,2′-bipyridine]-5-carbonyl chloride (**6b**) in pyridine at reflux for 12 h, respectively (Scheme 2, (b)). Hence, 12 different prospective ionophores, (i.e., **7a**–**7c**, **8a**–**8c**, **9a**–**9c**, and **10a**–**10c**) were synthesized successfully and their structures were fully identified with ^{1}H NMR, ^{13}C NMR, FTIR, and FAB MASS (**7b**, **7c**, **9b**, and **9c**) or HRMS (**3a**–**3c**, **5a**–**5c**, **7a**, **8a**–**8c**, **9a**, and **10a**–**10c**).

2.2. Potentiometric properties of ionophores

DOA was chosen as the plasticizer for the preparation of ion-selective membranes; it provided the best potentiometric performance among four different plasticizers (NPOE, DOS, DOA, and BBPA) when used for the preparation of transition metal ion-selective electrodes.[5] Membranes consisting of 1:2 ratio of PVC and DOA were prepared as described in the Experimental section and all 12 ionophores were then evaluated with the DOA-plasticized PVC membranes. No further efforts were made to improve the potentiometric performance of each membrane to compare the effect of structural variations in the binding properties of CAB-based ionophores under the same conditions. The transition metal ion selectivities of all ionophores were evaluated for 10 different ions (Ag^+, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+}, and Hg^{2+}), and the results are compared with respect to the Ag^+.

Figures 1 and 2 show the typical calibration plots of the electrodes based on ionophores **7a**–**7c** and **8a**–**8c** (Fig. 1) and **9a**–**9c** and **10a**–**10c** (Fig. 2), respectively. The plots include only silver ion responses of the electrodes for clarity, and the responses were measured in the 10^{-9} to 10^{-3} M range. Responses to other transition metal ions were negligible except for Hg^{2+} in case of the electrodes based on **9a**–**10c**. The selectivity coefficients with respect to silver ions were determined by the matched potential method; the concentration of the primary ion that brings about the same response potential corresponding to 10^{-2} M of interfering ions was determined and their activities were compared. Tables 1 and 2 summarize the potentiometric characteristics (response slopes, detection limits, and selectivity coefficients) of all membrane electrodes prepared with the ionophores **7a**–**10c**.

The ISEs prepared with the ionophores **7a**–**8c** with bithiophenyl moiety on 3α-position and dithiocarbamoyl moiety on 12α-position exhibited near Nernstian response to Ag^+, resulting in the

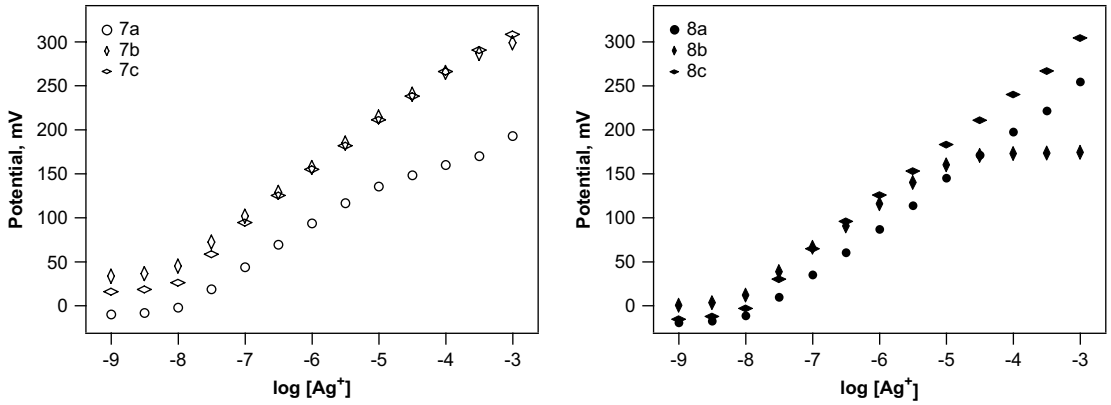


Figure 1. Calibration plots of the electrodes based on ionophores **7a**–**8c**. Only the representative responses are presented for clarity. Responses to other transition metal ions (M^{z+}=Zn^{2+}, Pb^{2+}, Ni^{2+}, Co^{2+}, Cd^{2+}, Fe^{2+}, Cu^{2+}, and Mn^{2+}) are negligible ($\log K^{POT}_{Ag^+,M^+} < -5.5$).

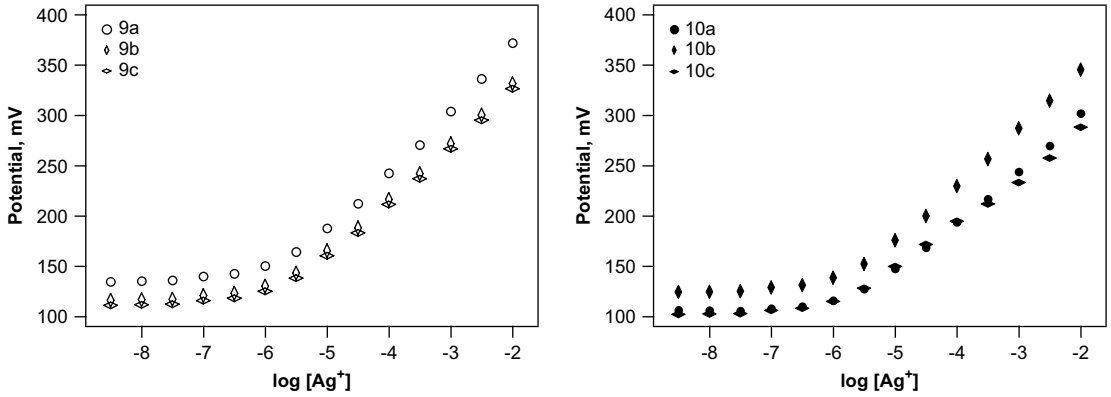


Figure 2. Calibration plots of the electrodes based on ionophores **9a**–**10c**. Only the representative responses are presented for clarity. Responses to other transition metal ions (M^{z+}=Zn^{2+}, Pb^{2+}, Ni^{2+}, Co^{2+}, Cd^{2+}, Fe^{2+}, Cu^{2+}, and Mn^{2+}) are negligible ($\log K^{POT}_{Ag^+,M^+} < -5.0$).

slopes of 42–59 mV/decade and the detection limits of 10^{-7} to 10^{-8} M. It is interesting to note that the ISEs based on compounds **7c** and **8c** exhibit ideal response, 59 mV/decade. Both ionophores contain diphenylaminothioxomethylthioacetyloxy group on 12α-position of cholan-24-amide (**7c**) and cholane (**8c**). Presumably, resonance participation of phenyl groups (R_2 in Scheme 2) on dithiocarbamoyl moiety of **7c** and **8c** may reduce the electron density of the sulfur atom, resulting in sulfur atoms softer than that of compounds **7a**, **7b**, **8a**, and **8b** that have hyperconjugative e-donating groups on R_2. It could result in ISE membranes with improved silver ion responses, which is indicative of increased interaction with silver ions. A similar tendency was observed in ionophores, **9a**–**10c**, which has bipyridyl moiety on the 3α-position and dithiocarbamoyl moiety on the 12α-position (Table 2). That is, ISEs based on **9c** and **10c** that contain the same type of phenyl groups on R_2 as those in **7c** and **8c** also show theoretical response slopes to Ag^+.

However, ISEs based on bipyridine containing ionophores, compounds **9a**–**10c**, exhibited substantially increased responses to Hg^{2+} compared with those based on bithiophene, **7a**–**8c**, resulting in reduced Ag^+ selectivity over that of Hg^{2+}. It appears that the bidentate ligand, bipyridine, provides a favorable binding site to Hg^{2+} compared with bithiophene, which may be explained by Pearson's hard and soft acid base classification. The ISE results provide some insights on the role of the functional groups in compounds **7a**–**10c**. The sensitive variations in the Ag^+ response characteristics with the change in the R_2 functional group on

Table 1
Potentiometric properties of silver ion-selective electrodes based on ionophores containing bithiophene moiety

Compound	Slope[a]	Detection limit[b]	Selectivity coefficient ($\log K^{POT}_{Ag^+,Hg^{2+}}$)[c]
7a	42.9	−7.2	−4.7
7b	53.5	−7.6	−4.1
7c	58.1	−7.6	−4.5
8a	49.5	−7.5	−5.0
8b	45.5	−7.6	−3.5
8c	58.6	−7.7	−3.6

[a] Range : 10^{-7} to 10^{-3} M, mV/decade.
[b] log[Ag^+], M.
[c] The sliver ion selectivity overall other transition metal ions ($\log K^{POT}_{Ag^+,M^+}$; M^+ = Zn^{2+}, Pb^{2+}, Ni^{2+}, Co^{2+}, Cd^{2+}, Fe^{2+}, Cu^{2+}, Mn^{2+}) tested is less than −5.5.

Table 2
Potentiometric properties of silver ion-selective electrodes based on ionophores containing bipyridine moiety

Compound	Slope[a]	Detection limit[b]	Selectivity coefficient ($\log K^{POT}_{Ag^+,Hg^{2+}}$)[c]
9a	48.8	−5.8	−0.2
9b	52.2	−5.8	−0.9
9c	58.6	−5.3	−1.7
10a	53.0	−5.6	−0.7
10b	48.9	−5.8	−0.7
10c	54.4	−5.9	−1.3

[a] Range : 10^{-7} to 10^{-2} M, mV/decade.
[b] log[Ag^+], M.
[c] The sliver ion selectivity overall other transition metal ions ($\log K^{POT}_{Ag^+,M^+}$; M^+ = Zn^{2+}, Pb^{2+}, Ni^{2+}, Co^{2+}, Cd^{2+}, Fe^{2+}, Cu^{2+}, Mn^{2+}) tested is less than −5.0.

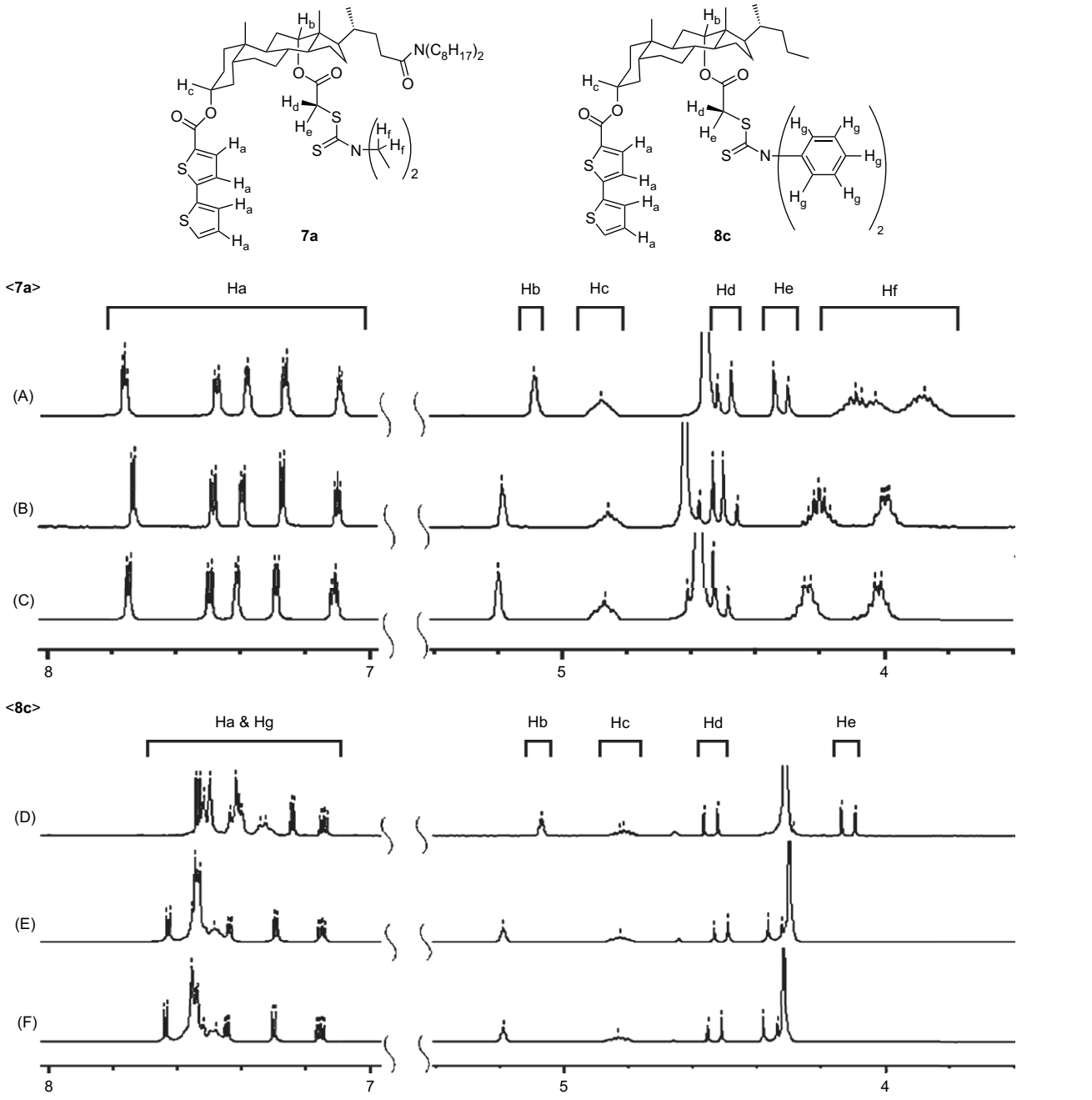


Figure 3. ^{1}H NMR spectra (400 MHz) of the ionophores, (A) free **7a** measured in methanol-d_4/acetone-d_6 (v/v=5:1), (B) **7a** after addition of 1 equiv of $AgNO_3$, (C) **7a** after addition of 2 equiv of $AgNO_3$, (D) free **8c** measured in methanol-d_4/acetone-d_6 (v/v=5:6), (E) **8c** after addition of 1 equiv of $AgNO_3$, and (F) **8c** after addition of 2 equiv of $AgNO_3$.

dithiocarbamoyl moiety but not with the type of ligands on 3α-position indicate that the sulfur atom in the thiocarbamoyl moiety is mostly responsible for the silver ion recognition. On the other hand, the functional groups of bithiophene and bipyridine on the 3α-position, which are introduced as the tweezers of **7a**–**8c** and **9a**–**10c** on the 12α-position together with the dithiocarbamoyl group, seem to affect the silver binding ability secondarily.

2.3. NMR titration experiments of ionophores with Ag^+ ion

To confirm the recognition properties of the newly synthesized silver ion-selective ionophores toward Ag^+ ion, ^{1}H NMR titration experiments were examined. Titrations were done with the ionophore and $AgNO_3$ in methanol-d_4/acetone-d_6 (v/v=5:1 to 5:6) co-solvent. Among the NMR titration experiments we were carried out, two representative results of NMR titrations (**7a** and **8c**) are shown in Fig. 3; one (**7a**) is that showed a minimally good result from ISE test and the other (**8c**) is that exhibited the most excellent result with a theoretical slope value of 59 mV/decade.

^{1}H NMR spectra of free ionophores, **7a** and **8c** shown in Figure 3 A and D, were altered with the Ag^+ ion addition; following the addition of 1 equiv of $AgNO_3$ to the ionophore in methanol-d_4/acetone-d_6 solution, changes in chemical shifts of several peaks were clearly observed by the influence of Ag^+ ion Fig. 3 B and E. While significant downfield shifts of the H_e peak (diastereotopic proton that is on the same plane with adjacent carbonyl π-bond), the H_f on dithiocarbamoyl moiety and the 12β-H_c peaks were observed, relatively small shifts of the H_a peaks on bithiophene moiety were

noted. Definitely, these changes in NMR peaks are attributed to the complexation between Ag^+ ion and hetero atoms in dithiocarbamoyl and bithiophene moiety. However, the extent of downfield shifts of the H_a peaks on bithiophene moiety of **7a** and **8c** differed. While there were some observable downfield shifts of the H_a peaks on bithiophene moiety of **8c**, there were insignificant downfield shifts observed for that of **7a**. However, in the case of **7c** that was as good as **8c** as an ionophore, comparable downfield shifts for the H_a, H_c, H_e, and H_f peaks were observed from NMR titration experiments similar to **8c**. This proves that the effective tweezer-type ion capture by dithiocarbamoyl and bithiophene moieties is important for ISEs even though bithiophene moiety appears to have a relatively small effect compared to dithiocarbamoyl moiety.

Overall, the results strongly imply that the sulfur and oxygen atoms in the dithiocarbamoyl moiety are mostly responsible for the silver ion recognition together with an assist of the sulfur atoms in bithiophene. Increase in the amount of $AgNO_3$ up to 2 equiv did not notably influence the chemical shifts of the H_a, H_c, H_d, and H_e peaks anymore for both **7a** and **8c** (Fig. 3 C and F). Certainly, this indicates that ionophores, both **7a** and **8c**, form a 1:1 complex with the Ag^+ ion.

3. Conclusion

In this article, we synthesized uniquely designed silver ion-selective ionophores based on unsymmetric tweezer-type ionophores containing bithiophenyl or bipyridyl moiety on the C3-position and dithiocarbamoyl groups on a 7-deoxycholic amide or cholane derivative. Their potentiometric properties were evaluated with the PVC membrane-based electrodes. Newly synthesized unsymmetrically substituted tweezer-type CAB-based 12 ionophores linked with unsymmetric tweezer-type bipyridyl, bithiophenyl, and dithiocarbamoyl moieties showed excellent affinity and selectivity to silver(I) ion over alkali, alkaline earth, and other transition metal ions. Overall, most ionophores showed slopes that were near Nernstian responses with 10^{-6} to 10^{-7} M detection limit. Especially, ionophores **7c** and **8c** that have bithiophene moiety on the 3α-position and diphenylaminothioxomethylthioacetyloxy group on the 12α-position of cholan-24-amide (**7c**) or cholane (**8c**) reveal the most excellent result with the theoretical slope value of 59 mV/decade. Compounds with bipyridyl moiety and diphenylaminothioxomethylthioacetyloxy group also showed similar properties, but, exhibited reduced Ag^+ selectivity over that of Hg^{2+}. The results show that the sensitive variations in the responses to silver ion are the most sensitive to the change in substituted functional groups on the dithiocarbamoyl moiety.

4. Experimental

4.1. General

^1H and ^{13}C NMR spectra were recorded at 300 (Bruker) or 400 (Jeol) and 75 or 100 MHz, respectively. Chemical shifts are reported in parts per million relative to residual solvent as an internal standard. High-resolution mass was recorded mostly on a JEOL JMS-DX303 mass spectrometer. Infrared (IR) spectra were recorded using MB104 FTIR (ABB Bomem Inc.).

Chemical reagents were purchased from Aldrich unless noted otherwise and were used without purification in most cases. Solvents were purchased and dried using the usual laboratory techniques. All anhydrous reactions were carried out under nitrogen atmosphere. Poly(vinyl chloride) (PVC) and bis(2-ethylhexyl) adipate (DOA) were purchased from Fluka Chemie AG (Buch, Switzerland). All other chemicals for analytical experiments were analytical-reagent grade. Standard solutions and buffers were prepared with freshly deionized water (18 MΩ cm). Compounds **2** and **4** were prepared from 7-deoxycholic acid (**1**) using our previous method as shown in Scheme 1.[4–6]

4.2. Synthesis

*4.2.1. General procedure for the preparation of 12α-[[[[(di(alkyl, aryl)amino)thioxomethyl]thio]acetyl]oxy]-3α-hydroxy-N,N-dioctyl-5β-cholan-24-amides **3a–3c***

A solution of *N,N*-dioctyl-3α,12α-bis(chloroacetoxy)-5β-cholan-24-amide (**2**, 218 mg, 0.27 mmol) and sodium *N,N*-di(alkyl, aryl)dithiocarbamate (1.36 mmol) in 10 mL of EtOH was refluxed for 12 h and then saturated LiOH(aq) (2–10 mL) was added to reaction mixture and stirred for 5–10 min. It was filtered through a pad of Celite (5 g) after cooling. The Celite was washed with dichloromethane (200 mL) and the combined filtrate and washings were collected and evaporated in vacuo. The residue was diluted with CH_2Cl_2 (150 mL), extracted with saturated $NaHCO_3$(aq) (2×150 mL), washed with water (1×150 mL), dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by chromatography on silica gel with ethyl acetate/hexane as an eluent to yield **3a–3c** as waxy liquids.

*4.2.1.1. 12α-[[[[(Diethylamino)thioxomethyl]thio]acetyl]oxy]-3α-hydroxy-N,N-dioctyl-5β-cholan-24-amide (**3a**).* Waxy liquid; TLC (silica gel, 50% ethyl acetate/hexane) R_f 0.45; ^1H NMR (300 MHz, $CDCl_3$) δ 5.13 (1H, br s, 12β-*H*), 4.39 (1H, d, *J*=16.5 Hz, RSCH_2CO$_2$R′), 4.19 (1H, d, *J*=16.5 Hz, RSCH_2CO$_2$R′), 4.12–3.90 (2H, m, RCSN(CH_2R′)$_2$), 3.90–3.70 (2H, m, RCSN(CH_2R′)$_2$), 3.62–3.48 (1H, m, 3β-*H*), 3.29–3.19 (4H, m, RCON(CH_2R′)$_2$), 2.34–0.72 (72H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 193.34, 172.91, 167.96, 77.58, 71.70, 50.11, 49.07, 47.96, 47.44, 46.88, 45.82, 45.17, 42.02, 39.13, 36.51, 35.65, 35.25, 35.04, 34.18, 34.05, 31.76, 31.72, 31.43, 30.61, 30.03, 29.36, 29.31, 29.21, 29.18, 27.75, 27.41, 27.03, 26.89, 26.06, 25.28, 23.50, 22.94, 22.58, 17.74, 14.05, 12.57, 12.20, 11.51; IR (KBr) ν_{max} 3425, 3056, 2985, 1727, 1627, 1488, 1263 cm^{-1}; HRMS calcd for $C_{47}H_{84}N_2O_4S_2$ *m*/*z* 804.5873, found 804.5895.

*4.2.1.2. 3α-Hydroxy-12α-[[[[(diisobutylamino)thioxomethyl]thio]-acetyl]oxy]-N,N-dioctyl-5β-cholan-24-amide (**3b**).* Waxy liquid; TLC (silica gel, 40% ethyl acetate/hexane) R_f 0.49; ^1H NMR (300 MHz, $CDCl_3$) δ 5.12 (1H, br s, 12β-*H*), 4.45 (1H, d, *J*=16.7 Hz, RSCH_2CO$_2$R′), 4.15 (1H, d, *J*=16.7 Hz, RSCH_2CO$_2$R′), 3.95–3.74 (2H, m, RCSN(CH_2R′)$_2$), 3.71–3.48 (3H, m, 3β-*H* and RCSN(CH_2R′)$_2$), 3.37–3.13 (4H, m, RCON(CH_2R′)$_2$), 2.53–2.11 (4H, m, RCH_2CONR′$_2$ and RCSN(CH$_2$C*H*R′$_2$)$_2$), 1.95–0.72 (76H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 194.94, 172.80, 167.82, 77.58, 71.67, 63.89, 61.05, 49.10, 47.90, 47.44, 45.76, 45.08, 42.97, 39.23, 36.52, 35.55, 35.26, 34.92, 34.09, 33.98, 31.69, 31.65, 31.35, 30.59, 29.95, 29.29, 29.24, 29.11, 27.69, 27.26, 26.96, 26.84, 26.18, 25.95, 25.18, 23.45, 22.83, 22.45, 20.32, 20.20, 17.67, 13.98, 12.16; IR (KBr) ν_{max} 3420, 3052, 2956, 1727, 1631, 1469, 1273 cm^{-1}; HRMS calcd for $C_{51}H_{92}N_2O_4S_2$ *m*/*z* 860.6499, found 860.6508.

*4.2.1.3. 3α-Hydroxy-N,N-dioctyl-12α-[[[[(diphenylamino)thioxomethyl]thio]acetyl]oxy]-5β-cholan-24-amide (**3c**).* Waxy liquid; TLC (silica gel, 40% ethyl acetate/hexane) R_f 0.40; ^1H NMR (300 MHz, $CDCl_3$) δ 7.58–7.30 (10H, m, *Ph*), 5.10 (1H, br s, 12β-*H*), 4.36 (1H, d, *J*=16.8 Hz, RSCH_2CO$_2$R′), 4.04 (1H, d, *J*=16.8 Hz, RSCH_2CO$_2$R′), 3.52–3.39 (1H, m, 3β-*H*), 3.37–3.07 (4H, m, RCON(CH_2R′)$_2$), 2.25–0.73 (66H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.41, 172.66, 167.34, 129.54, 128.41, 127.76, 77.59, 71.51, 49.26, 47.84, 47.47, 45.78, 45.16, 41.96, 39.84, 36.55, 35.60, 35.18, 34.81, 34.14, 33.97, 31.68, 31.63, 31.24, 30.56, 29.74, 29.28, 29.18, 29.12, 29.07, 29.00, 27.70, 27.37, 26.95, 26.78, 26.12, 25.16, 23.52, 22.81, 22.50, 17.64, 13.98, 12.13; IR (KBr) ν_{max} 3450, 3052, 2964, 1711, 1628, 1252 cm^{-1}; HRMS calcd for $C_{55}H_{84}N_2O_4S_2$ *m*/*z* 900.5873, found 900.5867.

4.2.2. General procedure for the preparation of 12α-[[[[(di-(alkyl, aryl)amino)thioxomethyl]thio]acetyl]oxy]-3α-hydroxy-5β-cholanes ***5a–5c***

A solution of 3α,12α-bis(chloroacetoxy)-5β-cholane (**4**, 121 mg, 0.24 mmol) and sodium *N,N*-di(alkyl, aryl)dithiocarbamate (1.18 mmol) in 10 mL of EtOH was refluxed for 12 h and then saturated LiOH(aq) (2–10 mL) was added to reaction mixture and stirred for 5–10 min. It was filtered through a pad of Celite (5 g) after cooling. The Celite was washed with dichloromethane (200 mL) and the combined filtrate and washings were evaporated in vacuo. The residue was diluted with CH_2Cl_2 (150 mL), extracted with saturated $NaHCO_3$(aq) (2×150 mL), washed with water (1×150 mL), dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by chromatography on silica gel with ethyl acetate/hexane as an eluent to give **5a–5c** as colorless waxy liquids.

*4.2.2.1. 12α-[[[[(Diethylamino)thioxomethyl]thio]acetyl]oxy]-3α-hydroxy-5β-cholane (**5a**).* Waxy liquid; TLC (silica gel, 50% ethyl acetate/hexane) R_f 0.43; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.13 (1H, br s, 12β-*H*), 4.38 (1H, d, *J*=16.4 Hz, RSCH_2CO$_2$R′), 4.20 (1H, d, *J*=16.4 Hz, RSCH_2CO$_2$R′), 4.10–3.98 (2H, m, RCSN(CH_2R′)$_2$), 3.87–3.72 (2H, m, RCSN(CH_2R′)$_2$), 3.62–3.48 (1H, m, 3β-*H*), 1.86–0.71 (45H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 193.41, 167.88, 77.65, 71.71, 50.06, 49.04, 47.53, 46.79, 45.06, 42.00, 39.17, 38.02, 36.49, 35.62, 35.22, 34.97, 34.15, 34.01, 30.60, 27.44, 27.01, 26.04, 25.20, 23.44, 22.88, 19.11, 17.76, 14.44, 12.51, 12.10, 11.46; IR(KBr) ν_{max} 3460, 3057, 2989, 1720, 1262 cm^{-1}; HRMS calcd for $C_{31}H_{53}NO_3S_2$ *m*/*z* 551.3467, found 551.3454.

*4.2.2.2. 3α-Hydroxy-12α-[[[[(diisobutylamino)thioxomethyl]thio]-acetyl]oxy]-5β-cholane (**5b**).* Waxy liquid; TLC (silica gel, 30% ethyl acetate/hexane) R_f 0.43; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.11 (1H, br s, 12β-*H*), 4.48 (1H, d, *J*=16.5 Hz, RSCH_2CO$_2$R′), 4.13 (1H, d, *J*=16.5 Hz, RSCH_2CO$_2$R′), 3.96–3.76 (2H, m, RCSN(CH_2R′)$_2$), 3.72–3.48 (3H, m, 3β-*H* and RCSN(CH_2R′)$_2$), 2.57–2.30 (2H, m, RCSN(CH$_2$C*H*R′$_2$)$_2$), 1.91–0.76 (51H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.06, 167.82, 77.71, 71.77, 64.00, 61.12, 49.21, 47.49, 45.06, 42.04, 39.34, 38.04, 36.58, 35.60, 35.32, 34.92, 34.14, 34.02, 30.66, 27.74, 27.40, 27.00, 26.23, 26.00, 25.20, 23.47, 22.86, 20.22, 19.00, 17.76, 14.48, 12.14; IR (KBr) ν_{max} 3425, 3052, 2961, 1727, 1469, 1268 cm^{-1}; HRMS calcd for $C_{35}H_{61}NO_3S_2$ *m*/*z* 607.4093, found 607.4084.

*4.2.2.3. 3α-Hydroxy-12α-[[[[(diphenylamino)thioxomethyl]thio]acetyl]oxy]-5β-cholane (**5c**).* Waxy liquid; TLC (silica gel, 30% ethyl acetate/hexane) R_f 0.31; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54–7.25 (10H, m, *Ph*), 5.10 (1H, br s, 12β-*H*), 4.38 (1H, d, *J*=16.7 Hz, RSCH_2CO$_2$R′), 4.03 (1H, d, *J*=16.7 Hz, RSCH_2CO$_2$R′), 3.52–3.40 (1H, m, 3β-*H*), 1.98–0.72 (39H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.56, 167.40, 145.1, 129.55, 128.44, 127.83, 77.73, 71.62, 49.32, 47.60, 45.12, 42.00, 39.91, 38.00, 36.58, 35.62, 35.22, 34.97, 34.19, 34.01, 30.62, 27.55, 27.04, 26.16, 25.17, 23.56, 22.86, 19.06, 17.71, 14.48, 12.11; IR (KBr) ν_{max} 3423, 3056, 2933, 1722, 1490, 1264 cm^{-1}; HRMS calcd for $C_{39}H_{53}NO_3S_2$ *m*/*z* 647.3467, found 647.3466.

4.2.3. General procedure for the preparation of 12α-[[[[(di(alkyl, aryl)amino)thioxomethyl]thio]acetyl]oxy]-N,N-dioctyl-3α-[[[2,2′-bithiophen]-5-yl]carboxy]-5β-cholan-24-amides ***7a–7c***

*4.2.3.1. 12α-[[[[(Diethylamino)thioxomethyl]thio]acetyl]oxy]-3α-hydroxy-N,N-dioctyl-5β-cholan-24-amide (**3a**).* Compound **3a** (175 mg, 0.22 mmol), 4-(dimethylamino)pyridine (32 mg, 0.26 mmol), and [2,2′-bithiophene]-5-carbonyl chloride (152 mg, 0.66 mmol) were dissolved in dichloromethane (10 mL) at room temperature. TEA (93 μL, 0.66 mmol) was slowly added to the reaction mixture, which was stirred for 24 h. The reaction mixture was filtered through a pad of Celite (5 g). The Celite was washed with dichloromethane (200 mL) and the combined filtrate and washings were collected and evaporated in vacuo. The residue was diluted with CH_2Cl_2 (150 mL), extracted with saturated $NaHCO_3$(aq) (2×150 mL), washed with water (1×150 mL), dried over $MgSO_4$, and evaporated in vacuo. The crude was purified by chromatography on silica gel with ethyl acetate/hexane (1:3) as an eluent to yield **7a–7c** as waxy liquids.

*4.2.3.2. 12α-[[[[(Diethylamino)thioxomethyl]thio]acetyl]oxy]-N,N-dioctyl-3α-[[[2,2′-bithiophen]-5-yl]carboxy]-5β-cholan-24-amide (**7a**).* Waxy liquid; TLC (silica gel, 40% ethyl acetate/hexane) R_f 0.58; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (1H, d, *J*=3.8 Hz, *Th*), 7.35–7.20 (2H, m, *Th*), 7.12 (1H, d, *J*=3.8 Hz, *Th*), 7.10–7.01 (1H, m, *Th*), 5.15 (1H, br s, 12β-*H*), 5.00–4.83 (1H, m, 3β-*H*), 4.44 (1H, d, *J*=16.5 Hz, RSCH_2CO$_2$R′), 4.25 (1H, d, *J*=16.5 Hz, RSCH_2CO$_2$R′), 4.10–3.93 (2H, m, RCSN(CH_2R′)$_2$), 3.90–3.72 (2H, m, RCSN(CH_2R′)$_2$), 3.40–3.10 (4H, m, RCON(CH_2R′)$_2$), 2.40–0.68 (71H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 193.16, 172.76, 167.86, 161.56, 143.66, 136.38, 133.85, 132.44, 127.93, 125.74, 124.92, 123.69, 77.33, 75.17, 50.00, 48.97, 47.86, 47.37, 46.64, 45.72, 45.08, 41.76, 39.08, 35.57, 34.93, 34.74, 34.11, 34.02, 32.16, 31.67, 31.63, 31.32, 29.94, 29.28, 29.22, 29.12, 29.09, 27.68, 27.29, 26.94, 26.80, 26.59, 25.86, 25.32, 23.38, 22.83, 22.49, 17.65, 13.97, 12.59, 12.14, 11.37; IR (KBr) ν_{max} 3078, 2952, 1703, 1638, 1452, 1420, 1270 cm^{-1}; HRMS calcd for $C_{56}H_{88}N_2O_5S_4$ *m*/*z* 996.5576, found 996.5570.

*4.2.3.3. 12α-[[[[(Diisobutylamino)thioxomethyl]thio]acetyl]oxy]-N,N-dioctyl-3α-[[[2,2′-bithiophen]-5-yl]carboxy]-5β-cholan-24-amide (**7b**).* Waxy liquid; TLC (silica gel, 30% ethyl acetate/hexane) R_f 0.68; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (1H, d, *J*=3.9 Hz, *Th*), 7.35–7.25 (2H, m, *Th*), 7.12 (1H, d, *J*=3.9 Hz, *Th*), 7.03 (1H, dd, *J*=5.0, 3.8 Hz, *Th*), 5.15 (1H, br s, 12β-*H*), 5.00–4.85 (1H, m, 3β-*H*), 4.50 (1H, d, *J*=16.8 Hz, RSCH_2CO$_2$R′), 4.22 (1H, d, *J*=16.8 Hz, RSCH_2CO$_2$R′), 3.93–3.75 (2H, m, RCSN(CH_2R′)$_2$), 3.70–3.52 (2H, m, RCSN(CH_2R′)$_2$), 3.40–3.12 (4H, m, RCON(CH_2R′)$_2$), 2.52–0.70 (79H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 194.83, 172.72, 167.79, 161.55, 143.60, 142.44, 136.41, 133.84, 132.47, 127.89, 127.01, 125.66, 124.86, 123.99, 123.64, 77.32, 75.10, 63.91, 60.94, 49.08, 47.87, 47.41, 45.72, 45.06, 41.76, 39.30, 35.56, 34.88, 34.77, 34.08, 34.00, 32.09, 31.66, 31.62, 31.33, 29.94, 29.26, 29.21, 29.08, 27.68, 27.22, 26.93, 26.80, 26.59, 26.16, 25.82, 25.32, 23.39, 22.81, 22.48, 20.17, 17.64, 14.05, 13.96, 12.18; IR (KBr) ν_{max} 3054, 2960, 1700, 1630, 1453, 1422, 1261 cm^{-1}; MS (FAB) *m*/*z* 1051 (M^+), 790 (M^+−OCOCH$_2$SC(S)N(C$_6$H$_5$)$_2$), 580 (M^+−OCOCH$_2$SC(S)N(C$_6$H$_5$)$_2$, OCOC$_8$H$_5$S$_2$).

*4.2.3.4. N,N-Dioctyl-12α-[[[[(diphenylamino)thioxomethyl]thio]acetyl]oxy]-3α-[[[2,2′-bithiophen]-5-yl]carboxy]-5β-cholan-24-amide (**7c**).* Waxy liquid; TLC (silica gel, 30% ethyl acetate/hexane) R_f 0.66; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (1H, d, *J*=3.9 Hz, *Th*), 7.43–7.22 (12H, m, *Th* and *Ph*), 7.10–7.05 (2H, m, *Th*), 5.13 (1H, br s, 12β-*H*), 4.92–4.82 (1H, m, 3β-*H*), 4.41 (1H, d, *J*=16.6 Hz, RSCH_2CO$_2$R′), 4.12 (1H, d, *J*=16.6 Hz, RSCH_2CO$_2$R′), 3.38–3.02 (4H, m, RCON(CH_2R′)$_2$), 2.30–0.72 (66H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.38, 172.75, 167.45, 161.65, 143.69, 136.60, 134.03, 132.57, 129.56, 128.39, 128.08, 127.86, 125.81, 125.05, 123.80, 77.57, 75.07, 49.43, 47.95, 47.59, 45.91, 45.28, 41.81, 40.14, 35.72, 34.90, 34.20, 34.09, 32.13, 31.80, 31.75, 31.34, 29.83, 29.41, 29.31, 29.25, 29.19, 29.13, 27.84, 27.50, 27.09, 26.90, 26.63, 26.06, 25.38, 23.60, 22.89, 22.62, 17.76, 14.09, 12.30; IR (KBr) ν_{max} 3069, 2955, 1697, 1635, 1493, 1452, 1284 cm^{-1}; MS (FAB) *m*/*z* 1093 (M^+), 790 (M^+−OCOCH$_2$SC(S)N(C$_6$H$_5$)$_2$), 580 (M^+−OCOCH$_2$SC(S)N(C$_6$H$_5$)$_2$, OCOC$_8$H$_5$S$_2$).

4.2.4. General procedure for the preparation of 12α-[[[[(di(alkyl, aryl)amino)thioxomethyl]thio]acetyl]oxy]-N,N-dioctyl-3α-[[[2,2′-bithiophen]-5-yl]carboxy]-5β-cholanes ***8a–8c***

*4.2.4.1. 12α-[[[[(Diethylamino)thioxomethyl]thio]acetyl]oxy]-3α-hydroxy-5β-cholane (**5a**).* Compound **5a** (68 mg, 0.12 mmol),

4-(dimethylamino)pyridine (18 mg, 0.15 mmol), and [2,2′-bithiophene]-5-carbonyl chloride (84 mg, 0.37 mmol) were dissolved in dichloromethane (6 mL) at room temperature. TEA (50 μL, 0.37 mmol) was slowly added to the reaction mixture, which was stirred for 24 h. The reaction mixture was filtered through a pad of Celite (5 g). The Celite was washed with dichloromethane (150 mL) and the combined filtrate and washings were collected and evaporated in vacuo. The residue was diluted with CH_2Cl_2 (100 mL), extracted with saturated $NaHCO_3$(aq) (2×100 mL), washed with water (1×100 mL), dried over $MgSO_4$, and evaporated in vacuo. The crude was purified by chromatography on silica gel with ethyl acetate/hexane (1:4) as an eluent to yield **8a**–**8c** as waxy liquids.

*4.2.4.2. 12α-[[[[(Diethylamino)thioxomethyl]thio]acetyl]oxy]-3α-[[[2,2′-bithiophen]-5-yl]carboxy]-5β-cholane (**8a**).* Waxy liquid; TLC (silica gel, 20% ethyl acetate/hexane) R_f 0.50; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (1H, d, *J*=3.9 Hz, *Th*), 7.31–7.26 (2H, m, *Th*), 7.12 (1H, d, *J*=3.9 Hz, *Th*), 7.04 (1H, dd, *J*=5.0, 3.7 Hz, *Th*), 5.15 (1H, br s, 12β-*H*), 4.97–4.86 (1H, m, 3β-*H*), 4.45 (1H, d, *J*=16.3 Hz, RSCH_2CO$_2$R′), 4.24 (1H, d, *J*=16.3 Hz, RSCH_2CO$_2$R′), 4.01 (2H, q, *J*=7.0 Hz, RCSN(CH_2R′)$_2$), 3.79 (2H, q, *J*=7.1 Hz, RCSN(CH_2R′)$_2$), 2.12–0.70 (44H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 193.38, 167.98, 161.72, 143.77, 136.51, 133.97, 132.60, 128.06, 125.84, 125.05, 123.82, 77.56, 75.33, 60.34, 50.11, 49.10, 47.61, 46.74, 45.14, 41.91, 39.30, 38.10, 35.70, 35.05, 34.87, 34.26, 34.15, 32.30, 27.52, 26.98, 26.71, 25.98, 25.42, 23.50, 22.97, 21.01, 19.23, 17.83, 14.54, 14.18, 12.69, 12.21, 11.50 ppm; IR (KBr) ν_{max} 3070, 2952, 1703, 1489, 1452, 1270 cm^{-1}; HRMS calcd for $C_{40}H_{57}NO_4S_4$ *m*/*z* 743.3170, found 743.3176.

*4.2.4.3. 12α-[[[[(Diisobutylamino)thioxomethyl]thio]acetyl]oxy]-3α-[[[2,2′-bithiophen]-5-yl]carboxy]-5β-cholane (**8b**).* Waxy liquid; TLC (silica gel, 20% ethyl acetate/hexane) R_f 0.49; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (1H, d, *J*=3.8 Hz, *Th*), 7.28 (1H, d, *J*=5.0 Hz, *Th*), 7.26 (1H, d, *J*=3.7 Hz, *Th*), 7.11 (1H, d, *J*=3.8 Hz, *Th*), 7.03 (1H, dd, *J*=5.0, 3.7 Hz, *Th*), 5.14 (1H, br s, 12β-*H*), 4.98–4.85 (1H, m, 3β-*H*), 4.57 (1H, d, *J*=16.5 Hz, RSCH_2CO$_2$R′), 4.17 (1H, d, *J*=16.5 Hz, RSCH_2CO$_2$R′), 3.92–3.76 (2H, m, RCSN(CH_2R′)$_2$), 3.60 (2H, d, *J*=7.5 Hz, RCSN(CH_2R′)$_2$), 2.52–2.32 (2H, m, RCSN(CH$_2$C*H*R′$_2$)$_2$), 2.10–0.70 (50H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 194.97, 167.88, 161.69, 143.68, 136.52, 133.94, 132.60, 127.96, 125.72, 124.96, 123.73, 77.49, 75.23, 64.06, 61.05, 49.18, 47.49, 45.06, 41.87, 39.44, 38.06, 35.64, 34.95, 34.85, 34.18, 34.10, 32.17, 27.77, 27.42, 26.93, 26.65, 26.26, 25.90, 25.37, 23.46, 22.91, 20.24, 19.04, 17.75, 14.50, 14.13, 12.19; IR (KBr) ν_{max} 3070, 2957, 1703, 1456, 1282 cm^{-1}; HRMS calcd for $C_{44}H_{65}NO_4S_4$ *m*/*z* 799.3796, found 799.3819.

*4.2.4.4. 12α-[[[[(Diphenylamino)thioxomethyl]thio]acetyl]oxy]-3α-[[(2,2′-bithiophen)-5-yl]carboxy]-5β-cholane (**8c**).* Waxy liquid; TLC (silica gel, 20% ethyl acetate/hexane) R_f 0.49; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (1H, d, *J*=3.9 Hz, *Th*), 7.49–7.23 (12H, m, *Th* and *Ph*), 7.08 (1H, d, *J*=3.9 Hz, *Th*), 7.05 (1H, dd, *J*=5.1, 3.7 Hz, *Th*), 5.12 (1H, br s, 12β-*H*), 4.92–4.79 (1H, m, 3β-*H*), 4.44 (1H, d, *J*=16.6 Hz, RSCH_2CO$_2$R′), 4.10 (1H, d, *J*=16.6 Hz, RSCH_2CO$_2$R′), 2.00–0.72 (38H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.44, 167.42, 161.64, 143.64, 136.56, 134.01, 132.55, 129.47, 128.32, 128.04, 127.86, 125.76, 125.01, 123.78, 77.60, 75.07, 49.39, 47.65, 45.17, 41.79, 40.14, 38.05, 35.67, 35.04, 34.80, 34.19, 34.06, 32.11, 27.62, 26.92, 26.59, 26.01, 25.32, 23.56, 22.87, 19.13, 17.73, 14.50, 12.18; IR (KBr) ν_{max} 3062, 2952, 1739, 1695, 1452, 1266 cm^{-1}; HRMS calcd for $C_{48}H_{57}NO_4S_4$ *m*/*z* 839.3170, found 839.3166.

*4.2.5. General procedure for the preparation of 12α-[[[[(di(alkyl, aryl)amino)thioxomethyl]thio]acetyl]oxy]-N,N-dioctyl-3α-[[[2,2′-bipyridin]-5-yl]]carboxy]-5β-cholan-24-amides **9a**–**9c***

[2,2′-Bipyridine]-5-carboxylic acid (100 mg, 0.50 mmol) was dissolved in thionyl chloride (5 mL, 68.85 mmol), which was stirred for 3 h. The reaction mixture was evaporated in vacuo. 12α-[[[[(Diethylamino)thioxomethyl]thio]acetyl]oxy]-3α-hydroxy-*N*,*N*-dioctyl-5β-cholan-24-amide (**3a**, 210 mg, 0.26 mmol) was dissolved in pyridine (10 mL) and was added to the reaction mixture at 0 °C, which was stirred for 12 h at reflux. The reaction mixture was filtered through a pad of Celite (5 g). The Celite was washed with dichloromethane (200 mL) and the combined filtrate and washings were collected and evaporated in vacuo. The residue was diluted with CH_2Cl_2 (150 mL), extracted with saturated $NaHCO_3$(aq) (2×150 mL), washed with 1 N HCl (2×150 mL), washed with water (1×150 mL), dried over $MgSO_4$, and evaporated in vacuo. The crude was purified by chromatography on silica gel with ethyl acetate/hexane (4:6) as eluent to yield **9a**–**9c** as waxy liquids.

*4.2.5.1. 3α-[[[2,2′-Bipyridin]-5-yl]carboxy]-12α-[[[[(diethylamino)-thioxomethyl]thio]acetyl]oxy]-N,N-dioctyl-5β-cholan-24-amide (**9a**).* Waxy liquid; TLC (silica gel, 40% ethyl acetate/hexane) R_f 0.22; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.31–9.33 (1H, m, *Py*), 8.71–8.69 (1H, m, *Py*), 8.51–8.47 (3H, m, *Py*), 7.84 (1H, td, *J*=7.8, 1.8 Hz, *Py*), 7.35 (1H, ddd, *J*=7.8, 4.8, 1.2 Hz, *Py*), 5.14 (1H, br s, 12β-*H*), 5.05–4.99 (1H, m, 3β-*H*), 4.50 (1H, d, *J*=16.5 Hz, RSCH_2CO$_2$R′), 4.19 (1H, d, *J*=16.5 Hz, RSCH_2CO$_2$R′), 4.06–3.95 (2H, m, RCSN(CH_2R′)$_2$), 3.78 (2H, m, RCSN(CH_2R′)$_2$), 3.35–3.15 (4H, m, RCON(CH_2R′)$_2$), 2.38–2.04 (2H, m, RCH_2CONR′$_2$), 2.38–0.71 (68H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.17, 172.77, 167.97, 164.86, 159.01, 155.14, 150.59, 149.21, 138.06, 136.91, 126.30, 124.26, 121.76, 120.25, 77.41, 75.43, 50.12, 48.97, 47.89, 47.49, 46.67, 45.78, 45.14, 41.83, 39.02, 35.62, 35.01, 34.82, 34.12, 32.15, 31.72, 31.69, 31.36, 30.04, 29.32, 29.28, 29.18, 29.15, 29.10, 27.72, 27.36, 26.99, 26.86, 26.57, 25.89, 25.32, 23.43, 22.88, 22.55, 17.70, 14.02, 12.60, 12.17, 11.40; IR (KBr) ν_{max} 3053, 2925, 1716, 1639, 1462, 1271 cm^{-1}; HRMS calcd for $C_{58}H_{90}N_4O_5S_2$ *m*/*z* 986.6353, found 986.6370.

*4.2.5.2. 3α-[[[2,2′-Bipyridin]-5-yl]carboxy]-12α-[[[[(diisobutylamino)-thioxomethyl]thio]acetyl]oxy]-N,N-dioctyl-5β-cholan-24-amide (**9b**).* Waxy liquid; TLC (silica gel, 30% ethyl acetate/hexane) R_f 0.22; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.35–9.33 (1H, m, *Py*), 8.71–8.70 (1H, m, *Py*), 8.51–8.47 (3H, m, *Py*), 7.84 (1H, td, *J*=7.8, 1.8 Hz, *Py*), 7.35 (1H, ddd, *J*=7.7, 4.9, 1.2 Hz, *Py*), 5.15 (1H, br s, 12β-*H*), 5.06–4.96 (1H, m, 3β-*H*), 4.57 (1H, d, *J*=16.8 Hz, RSCH_2CO$_2$R′), 4.15 (1H, d, *J*=16.8 Hz, RSCH_2CO$_2$R′), 3.86–3.79 (2H, m, RCSN(CH_2R′)$_2$), 3.60–3.59 (2H, m, RCSN(CH_2R′)$_2$), 3.34–3.18 (4H, m, RCON(CH_2R′)$_2$), 2.54–0.70 (79H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.76, 172.78, 167.95, 164.90, 159.98, 155.15, 150.65, 149.22, 138.09, 136.91, 126.28, 124.28, 121.74, 120.23, 77.41, 75.44, 64.12, 61.11, 49.05, 47.91, 47.53, 45.79, 45.11, 41.84, 39.24, 35.61, 34.95, 34.84, 34.15, 32.08, 31.74, 31.70, 31.36, 30.02, 29.34, 29.29, 29.19, 29.13, 27.78, 27.73, 27.28, 26.99, 26.88, 26.59, 26.19, 25.88, 25.35, 23.46, 22.89, 22.56, 20.32, 20.21, 17.71, 14.05, 12.24; IR (KBr) ν_{max} 3058, 2955, 1716, 1637, 1466, 1280 cm^{-1}; MS (FAB) *m*/*z* 1044 (M^+), 781 (M−OCOCH$_2$SC(S)N(*i*-C$_4$H$_9$)$_2$), 580 (M−OCOCH2SC(S)N(*i*-C$_4$H$_9$)$_2$, OCOC$_{10}$H$_7$N$_2$).

*4.2.5.3. 3α-[[[2,2′-Bipyridin]-5-yl]carboxy]-12α-[[[[(diphenylamino)-thioxomethyl]thio]acetyl]oxy]-N,N-dioctyl-5β-cholan-24-amide (**9c**).* Waxy liquid; TLC (silica gel, 30% ethyl acetate/hexane) R_f 0.22; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.18–9.16 (1H, m, *Py*), 8.75–8.72 (1H, m, *Py*), 8.51 (1H, d, *J*=8.1 Hz, *Py*), 8.43 (1H, dd, *J*=8.1, 0.7 Hz, *Py*), 8.29 (1H, dd, *J*=7.9, 2.1 Hz, *Py*), 7.88 (1H, ddd, *J*=7.9, 7.7, 1.8 Hz, *Py*), 7.45–7.19 (11H, m, *Py* and *Ph*), 5.13 (1H, br s, 12β-*H*), 4.98–4.88 (1H, m, 3β-*H*), 4.47 (1H, d, *J*=16.8 Hz, RSCH_2CO$_2$R), 4.07 (1H, d, *J*=16.8 Hz, RSCH_2CO$_2$R), 3.35–3.01 (4H, m, RCON(CH_2R′)$_2$), 2.30–0.73 (65H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.29, 172.66, 167.46, 164.80, 158.89, 155.27, 150.70, 149.28, 138.20, 137.00, 129.55, 127.85, 126.26, 124.30, 121.79, 120.26, 77.54, 75.20, 60.34, 49.40, 47.90, 47.56, 45.88, 45.27, 41.78, 39.84, 35.69, 34.80, 34.18, 34.14, 31.98, 31.78, 31.72, 31.25, 29.69, 29.39, 29.28, 29.22, 29.18, 29.08, 27.82, 27.48, 27.05, 26.93, 26.88, 26.50, 26.05, 25.31, 23.60, 22.84, 22.59, 17.71, 14.07, 12.24; IR

(KBr) ν_{max} 3057, 2923, 1714, 1638, 1590, 1491, 1279 cm^{-1}; MS (FAB) *m*/*z* 1084 (M^+), 781 (M−OCOCH$_2$SC(S)N(C$_6$H$_5$)$_2$), 580 (M−OCOCH$_2$SC(S)N(C$_6$H$_5$)$_2$, OCOC$_{10}$H$_7$N$_2$).

4.2.6. General procedure for the preparation of 12α-[[[[(di(alkyl, aryl)amino)thioxomethyl]thio]acetyl]oxy]-3α-[[[2,2′-bipyridin]-5-yl]carboxy]-5β-cholanes ***10a–10c***

[2,2′-Bipyridine]-5-carboxylic acid (100 mg, 0.50 mmol) was dissolved in thionyl chloride (5 mL, 68.85 mmol), which was stirred for 3 h. The reaction mixture was evaporated in vacuo. 12α-[[[[(Diethylamino)thioxomethyl]thio]acetyl]oxy]-3α-hydroxy-5β-cholane (**5a**, 170 mg, 0.31 mmol) was dissolved in pyridine (10 mL) and was added to the reaction mixture at 0 °C, which was stirred for 12 h at reflux. The reaction mixture was filtered through a pad of Celite (5 g). The Celite was washed with dichloromethane (200 mL) and the combined filtrate and washings were collected and evaporated in vacuo. The residue was diluted with CH_2Cl_2 (150 mL), extracted with saturated $NaHCO_3$(aq) (2×150 mL), washed with 1 N HCl (2×150 mL), washed with water (1×150 mL), dried over $MgSO_4$, and evaporated in vacuo. The crude was purified by chromatography on silica gel with ethyl acetate/hexane (2:8) as an eluent to yield **10a–10c** as waxy liquids.

*4.2.6.1. 3α-[[[2,2′-Bipyridin]-5-yl]carboxy]-12α-[[[[(diethylamino)-thioxomethyl]thio]acetyl]oxy]-5β-cholane (**10a**).* Waxy liquid; TLC (silica gel, 20% ethyl acetate/hexane) R_f 0.20; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.33 (1H, dd, *J*=1.9, 1.1 Hz, *Py*), 8.72–8.68 (1H, m, *Py*), 8.51–8.42 (3H, m, *Py*), 7.84 (1H, td, *J*=7.7, 1.7 Hz, *Py*), 7.35 (1H, ddd, *J*=7.7, 4.9, 1.2 Hz, *Py*), 5.14 (1H, br s, 12β-*H*), 5.08–4.96 (1H, m, 3β-*H*), 4.50 (1H, d, *J*=16.5 Hz, RSC*H*$_2$CO$_2$R′), 4.19 (1H, d, *J*=16.5 Hz, RSC*H*$_2$CO$_2$R′), 4.15–3.93 (2H, m, RCSN(C*H*$_2$R′)$_2$), 3.78 (2H, q, *J*=7.1 Hz, RCSN(C*H*$_2$R′)$_2$), 2.17–0.73 (44H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.31, 168.00, 164.91, 159.01, 155.18, 150.64, 149.23, 138.11, 136.94, 126.34, 124.29, 121.79, 120.27, 77.54, 75.48, 60.32, 50.13, 49.01, 47.57, 46.66, 45.09, 41.87, 39.11, 38.06, 35.66, 34.96, 34.85, 34.18, 34.16, 32.18, 27.46, 26.93, 26.59, 25.93, 25.34, 23.45, 22.92, 19.15, 17.77, 14.50, 14.14, 12.61, 12.14, 11.43; IR (KBr) ν_{max} 3057, 2926, 1715, 1636, 1269 cm^{-1}; HRMS calcd for $C_{42}H_{59}N_3O_4S_2$ *m*/*z* 733.3947, found 733.3919.

*4.2.6.2. 3α-[[[2,2′-Bipyridin]-5-yl]carboxy]-12α-[[[[(diisobutylamino)-thioxomethyl]thio]acetyl]oxy]-5β-cholane (**10b**).* Waxy liquid; TLC (silica gel, 20% ethyl acetate/hexane) R_f 0.20; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.35–9.33 (1H, m, *Py*), 8.71–8.69 (1H, m, *Py*), 8.52–8.45 (3H, m, *Py*), 7.84 (1H, td, *J*=7.7, 1.7 Hz, *Py*), 7.35 (1H, ddd, *J*=7.6, 4.8, 1.1 Hz, *Py*), 5.13 (1H, br s, 12β-*H*), 5.08–4.97 (1H, m, 3β-*H*), 4.63 (1H, d, *J*=16.7 Hz, RSC*H*$_2$CO$_2$R′), 4.12 (1H, d, *J*=16.7 Hz, RSC*H*$_2$CO$_2$R′), 3.90–3.76 (2H, m, RCSN(C*H*$_2$R′)$_2$), 3.60 (2H, d, *J*=7.3 Hz, RCSN(C*H*$_2$R′)$_2$), 2.55–2.30 (2H, m, RCSN(CH$_2$C*H*R′$_2$)$_2$), 2.18–0.69 (50H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.94, 167.95, 164.39, 155.81, 153.14, 150.60, 147.16, 139.59, 138.88, 127.27, 125.02, 123.13, 121.47, 77.49, 75.85, 64.22, 61.14, 60.32, 49.12, 47.44, 45.04, 41.88, 39.27, 38.04, 35.63, 34.86, 34.16, 32.05, 27.77, 27.39, 26.93, 26.54, 26.24, 25.87, 25.35, 23.44, 22.89, 20.32, 20.21, 18.92, 17.75, 14.52, 14.12, 12.17; IR (KBr) ν_{max} 3052, 2956, 1715, 1594, 1280 cm^{-1}; HRMS calcd for $C_{46}H_{67}N_3O_4S_2$ *m*/*z* 789.4573, found 789.4566.

*4.2.6.3. 3α-[[[2,2′-Bipyridin]-5-yl]carboxy]-12α-[[[[(diphenylamino)-thioxomethyl]thio]acetyl]oxy]-5β-cholane (**10c**).* Waxy liquid; TLC (silica gel, 20% ethyl acetate/hexane) R_f 0.21; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.19–9.17 (1H, m, *Py*), 8.77–8.70 (1H, m, *Py*), 8.52 (1H, d, *J*=8.1 Hz, *Py*), 8.43 (1H, d, *J*=8.1 Hz, *Py*), 8.29 (1H, dd, *J*=8.0, 2.2 Hz, *Py*), 7.87 (1H, ddd, *J*=8.0, 7.7, 1.8 Hz, *Py*), 7.47–7.17 (11H, m, *Py* and *Ph*), 5.12 (1H, br s, 12β-*H*), 5.00–4.79 (1H, m, 3β-*H*), 4.50 (1H, d, *J*=16.8 Hz, RSC*H*$_2$CO$_2$R), 4.05 (1H, d, *J*=16.8 Hz, RSC*H*$_2$CO$_2$R), 2.05–0.70 (48H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.49, 167.50, 164.87, 158.82, 155.28, 150.72, 149.26, 138.27, 137.08, 129.52, 128.37, 127.94, 126.34, 124.34, 121.87, 120.34, 77.65, 75.29, 49.45, 47.69, 45.24, 41.85, 39.91, 38.09, 35.74, 35.03, 34.88, 34.26, 34.19, 32.04, 27.67, 26.99, 26.54, 26.07, 25.32, 23.63, 22.89, 19.07, 17.75, 14.56, 12.20; IR (KBr) ν_{max} 3066, 2956, 1713, 1587, 1281 cm^{-1}; HRMS calcd for $C_{50}H_{59}N_3O_4S_2$ *m*/*z* 829.3947, found 829.3954.

4.3. Preparation and evaluation of polymer membranes

The ion-selective membranes were prepared with 12 different ionophores (composition of membrane cocktails: 2 mg of ionophore, 66 mg of PVC and 132 mg of plasticizer [DOA]). The cocktail solutions dissolved in 1.0 mL THF were then poured into a glass ring (i.d. 22 mm) placed on a slide glass and dried for a day at room temperature. After curing, 5.5 mm-diameter disks were punched out of the master membrane and placed in Philips electrode bodies (IS-561) (Glasbläserei MÖller, Zürich, Switzerland). The inner filling solutions were 0.1 M KCl for evaluation of the ionophores. An Orion (Cambridge, MA, USA) sleeve-type double-junction Ag/AgCl electrode (Model 90–02) was used as the external reference. The potential differences between the silver ISEs and the reference electrode were measured using an IBM AT-type computer equipped with a custom-built high-impedance input 16-channel analog-to-digital converter. Before being used, all electrodes were presoaked in 0.01 M (or 0.001 M at times and deionized water) magnesium acetate buffer (pH 4.5). Dynamic response curves and calibration plots for transition metal ions in the 10^{-9} to 10^{-3} M range were obtained by adding calculated volume of standard solutions stepwise to 200 mL of background electrolyte (0.01 M Magnesium acetate, pH 4.5) at room temperature. The solutions were magnetically stirred during the recording of all potential measurement. The selectivity coefficients were determined by using the matched potential method;[9–11] the concentration of the primary ion that brings about the same response potential corresponding to 10^{-2} M interfering ion was determined and their activities have been compared. The detection limits of the electrodes were obtained from the calibration curves, as described in the references.[10,11]

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