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Silver(I) ion selective ionophores containing dithiocarbamoyl moieties on steroid backbone

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Abstract

Tweezer-type and non-tweezer-type ionophores containing dithiocarbamoyl groups on a 7-deoxycholic amide or cholane derivatives were designed and synthesized. Potentiometric evaluation of the poly(vinyl chloride) (PVC) membranes containing those deoxycholic amides/cholanes linked with tweezer-type dithiocarbamoyl moieties showed excellent affinity and selectivity to silver(I) ion over alkali, alkaline earth and other transition metal cations. On the other hand, deoxycholic amides/cholanes substituted with one dithiocarbamoyl group, i.e., non-tweezer-type ionophores, resulted in relatively poor potentiometric sensitivity and detection limits. The enhanced potentiometric properties of newly synthesized tweezer-type dithiocarbamoyl containing ionophores have been further improved by employing silver ion complexing reagent in the internal reference solution, which resulted in greatly reduced detection limit (∼100 ppt) for the electrodes based on them.

Keywords: Sensor; Silver(I); Ionophores; Deoxycholic amides/cholanes

1. Introduction

Molecular tweezer-type neutral carriers based on a cholic acid backbone (CAB) have been successfully utilized to yield highly selective ionophores for carbonate, chloride, silver and calcium [1–5]. The CAB-based carriers are easy to design and relatively straightforward to synthesize; previous examples show that the CAB frame may be as versatile as calix[*n*]arenes for developing selective ionophores. Many different CAB-based neutral carriers may be designed by substituting various types of ion-recognizing groups to the two hydroxyl linkers, which are approximately parallel at C3 and C12 carbons of the frame and about 6 Å apart [6]. It is expected that the selective ion-tweezing ability of a CAB-based neutral carrier be controlled by changing the cavity size between two binding sites and by changing the length of the linking chain between the substituent and the hydroxyl linker.

Our initial success in several CAB-based neutral carriers encouraged us to design improved 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-selective ionophores, possess sulfur and/or nitrogen atoms in their proposed binding sites [7]. In our previous report on silver selective ionophores, nitrogen containing 2,2′-bipyridine moieties as a binding site was adopted [4]. These ionophores, however, resulted in limited potentiometric performance when used in the construction of ion-selective electrodes. In addition to known binding moieties for the transition metal ions, dialkyl or diaryl dithiocarbamate and its analogues, one of the most utilized organic ligands, may be used to obtain ionophores with enhanced potentiometric properties. So new CAB-based ionophores that contain sulfur and nitrogen combined binding sites comprising vari-

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ous dialkyl or diaryl substituted dithiocarbamate were designed.

According to the hard-soft acid base (HSAB) concept [8], it is expected that improved selectivity and dynamic response behavior will be obtained with the ionophores that contain both nitrogen and softer sulfur atom in their binding sites. In the design of ionophores, long alkyl chains have been substituted to increase their lipophilicity in the membrane phase and to provide enhanced solvating ability around the binding sites. Herein, we report the synthesis and potentiometric evaluation of new silver ion-selective ionophores with substantially improved selectivity, which are the 7-deoxycholic amide or cholane based dialkyl or diaryl dithiocarbamate derivatives. The effect of silver ion-complexing reagent added to the internal reference solutions on the detection limits of the silver ion-selective electrodes is likewise reported [9].

2. Experimental

2.1. General

^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, on a Bruker or Jeol spectrometer. Chemical shifts are reported in ppm 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 various plasticizers [2-nitrophenyloctyl ether (NPOE), bis(2-ethylhexyl) sebacate (DOS), bis(1-butylpentyl) adipate (BBPA) 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). Compound **2** was prepared from 7-deoxycholic acid (**1**) using a known method [4].

2.2. Synthesis

2.2.1. General procedure for the preparation of N,N-dioctyl-3α,12α-bis(N,N-di(alkyl, aryl)thiocarbamoyl-sulfanylacetoxy)-5β-cholan-24-amides *3a–3c*

A solution of *N,N*-dioctyl-3α,12α-bis(chloroacetoxy)-5β-cholan-24-amide (**2**, 190 mg, 0.24 mmol) and sodium *N,N*-di(alkyl, aryl)dithiocarbamate (1.44 mmol) in 10 mL of THF was refluxed for 24 h and 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), washed with water (3 × 150 mL), dried over $MgSO_4$ and evaporated in vacuo. The residue was purified by chromatography on silica gel with ethyl acetate–hexane as eluent to yield **3a**–**3c** as waxy liquids.

2.2.2. N,N-Dioctyl-3α,12α-bis(N,N-diethylthiocarbamoylsulfanylacetoxy)-5β-cholan-24-amide *(3a)*

$C_{54}H_{95}N_3O_5S_4$; TLC (ethyl acetate:hexane = 2:3) R_f, 0.64; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.16 (br s, 1H, 12β-H), 4.85–4.70 (m, 1H, 3β-H), 4.35 (d, J = 16.1 Hz, 1H), 4.27 (d, J = 16.5 Hz, 1H), 4.15–3.95 (m, 6H), 3.90–3.72 (m, 4H), 3.40–3.20 (m, 4H), 2.40–0.70 (m, 77H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 193.6, 193.1, 172.8, 168.1, 167.9, 77.2, 75.7, 50.0, 49.7, 49.0, 47.8, 47.2, 46.8, 46.7, 45.7, 45.0, 41.7, 39.4, 39.1, 35.5, 34.9, 34.6, 34.1, 33.9, 31.9, 31.65, 31.63, 31.3, 29.8, 29.25, 29.23, 29.09, 29.05, 27.6, 27.3, 26.9, 26.8, 26.4, 25.8, 25.4, 23.4, 22.8, 22.5, 17.6, 14.0, 12.6, 12.4, 12.1, 11.4; IR (KBr) ν_{max} 3050, 2938, 1726, 1630, 1373, 1271, 1205, 1149 cm^{-1}; MS (FAB) *m*/*e* 995 (M^+), 788 ($M^+ - OCOCH_2SCSN(CH_2CH_3)_2$), 581 ($M^+ - 2OCOCH_2SCSN(CH_2CH_3)_2$).

2.2.3. N,N-Dioctyl-3α,12α-bis(N,N-diisobutylthiocarbamoylsulfanylacetoxy)-5β-cholan-24-amide *(3b)*

$C_{62}H_{111}N_3O_5S_4$; TLC (ethyl acetate:hexane = 1:3) R_f, 0.50; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.15 (br s, 1H, 12β-H), 4.85–4.65 (m, 1H, 3β-H), 4.45–4.05 (m, 4H), 3.95–3.70 (m, 4H), 3.70–3.45 (m, 4H), 3.40–3.10 (m, 4H), 2.50–0.70 (m, 93H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.4, 194.9, 172.8, 168.0, 167.9, 77.3, 75.7, 63.7, 63.1, 60.90, 60.89, 49.1, 47.8, 47.2, 45.7, 45.0, 41.7, 39.6, 39.4, 35.6, 34.9, 34.7, 34.2, 34.0, 31.9, 31.67, 31.64, 31.3, 29.9, 29.27, 29.23, 29.11, 27.7, 27.5, 27.2, 26.9, 26.8, 26.4, 26.2, 26.1, 25.8, 25.4, 23.4, 22.9, 22.5, 20.2, 20.1, 17.6, 14.0, 12.2; IR (KBr) ν_{max} 3051, 2960, 1731, 1637, 1383, 1270, 1154, 1090 cm^{-1}; MS (FAB) *m*/*e* 1107 (M^+), 844 ($M^+ - OCOCH_2SCSN(CH_2CH(CH_3)_2)$), 581 ($M^+ - 2OCOCH_2SCSN(CH_2CH(CH_3)_2)$).

2.2.4. N,N-Dioctyl-3α,12α-bis(N,N-diphenylthiocarbamoylsulfanylacetoxy)-5β-cholan-24-amide *(3c)*

$C_{70}H_{95}N_3O_5S_4$; TLC (ethyl acetate:hexane = 2:3) R_f, 0.65; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–7.27 (m, 20H), 5.13 (br s, 1H, 12β-H), 4.75–4.60 (m, 1H, 3β-H), 4.30 (d, J = 16.7 Hz, 1H), 4.10 (d, J = 16.7 Hz, 1H), 3.99 (d, J = 16.5 Hz, 1H), 3.88 (d, J = 16.5 Hz, 1H), 3.35–3.02 (m, 4H), 2.30–0.70 (m, 65H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.0, 199.3, 172.8, 167.9, 167.6, 129.6, 128.4, 127.9, 75.7, 49.3, 47.9, 47.6, 45.9, 45.2, 41.8, 40.3, 40.1, 35.7, 35.0, 34.7, 34.2, 34.0, 32.0, 31.80, 31.76, 31.4, 29.9, 29.4, 29.3, 29.26, 29.21, 29.1, 27.8, 27.5, 27.1, 26.9, 26.5, 26.0, 25.4, 23.6, 23.0, 22.6, 17.7, 14.1, 12.3; IR (KBr) ν_{max} 3058, 2933, 1729, 1638, 1592, 1348, 1281, 1159, 1049, 732 cm^{-1}; MS (FAB) *m*/*e* 1186 (M^+), 883 ($M^+ - OCOCH_2SCSN(C_6H_5)_2$), 580 ($M^+ - 2OCOCH_2SCSN(C_6H_5)_2$).

2.2.5. *Methyl-3α,12α-dihydroxy-5β-cholan-24-oate (4)*

To a solution of deoxycholic acid (10.0 g, 25.5 mmol) in methanol (100 mL), acetyl chloride (5 mL) was added at 0 °C and was stirred for 20 h at room temperature. The solvent was removed under the reduced pressure. The residue was purified by chromatography on silica gel with ethyl acetate–hexane (3:7) as eluent to yield 8.91 g (86%) of **4** as a white solid: $C_{25}H_{42}O_4$; TLC (ethyl acetate) R_f, 0.70; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.97 (s, 1H, 12β-H), 3.65 (s, 3H, $COOCH_3$), 3.60 (br m, 1H, 3β-H), 2.37–2.22 (m, 2H), 1.87–0.67 (m, 35H); IR (NaCl) ν_{max} 3408 (br), 2940, 2875, 1749, 1453, 1381, 1262m 1170, 1052, 762 cm^{-1}.

2.2.6. *3α,12α-Bis(methoxymethoxy)-5β-cholan-24-ol (5)*

To a solution of methyl-3α,12α-dihydroxy-5β-cholan-24-oate (**4**, 1.00 g, 2.46 mmol) and dimethoxymethane (100 mL) in CH_2Cl_2 (100 mL), CF_3SO_3H (0.20 mL) was slowly added at room temperature and stirred for 5 h. After the completion, saturated aqueous NH_4OH solution (0.40 mL) was added slowly to the reaction mixture, dried over $MgSO_4$ and evaporated in vacuo. The resulting crude was diluted with diethyl ether, added to a solution of $LiAlH_4$ (196.50 mg, 4.92 mmol) in diethyl ether (5 mL) and was stirred for 2 h at room temperature. After 2 h, water (197 μL), 15% NaOH (aq) (197 μL) and water (394 μL) were added stepwise to the reaction mixture at 0 °C. The reaction mixture was filtered through a pad of celite (5 g) and the celite was washed with diethyl ether (150 mL). The combined filtrate and washings were dried over $MgSO_4$ and evaporated in vacuo. The residue was purified by chromatography on silica gel with ethyl acetate–hexane (3:7) as eluent to yield 1.01 g (88 %) of **4** as a white waxy liquid: $C_{28}H_{50}O_5$; TLC (ethyl acetate:hexane = 1:1) R_f, 0.59; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.71–4.64 (m, 4H, $OC\underline{H}_2OMe$), 3.79 (s, 1H, 12β-H), 3.60 (t, 2H, J = 6.3 Hz, $C\underline{H}_2OH$), 3.49 (br m, 1H, 3β-H), 3.41 (s, 3H, OCH_3), 3.34 (s, 3H, OCH_3), 1.87–0.92 (m, 30H), 0.89 (s, 3H, 19-CH_3), 0.68 (s, 3H, 18-CH_3); IR (NaCl) ν_{max} 3441 (br), 2940, 2868, 1479, 1453, 1387, 1223, 1150, 1111, 1052, 927, 762 cm^{-1}.

2.2.7. *3α,12α-Bis(methoxymethoxy)-5β-cholane (6)*

3α,12α-Di(methoxymethoxy)-5β-cholan-24-ol (**5**, 1.10 g, 0.39 mmol) and *p*-toluenesulfonyl chloride (1.30 g, 7.00 mmol) were dissolved in pyridine (2.50 mL) at 0 °C and stirred for 2 h. Water (20 drops) was added to the reaction mixture, which was stirred for 30 min. The reaction mixture was diluted with CH_2Cl_2 (60 mL) and was washed with 0.10 M HCl (aq) (3 × 60 mL), water (60 mL), saturated $NaHCO_3$ (aq) (3 × 60 mL) and water (60 mL). The organic layer was dried over $MgSO_4$ and evaporated in vacuo. The resulting crude was diluted with diethyl ether and was added to a solution of $LiAlH_4$ (130.80 mg, 3.45 mmol) in diethyl ether (25 mL) at 0 °C and was stirred for 2 h at room temperature. The reaction mixture was cooled to 0 °C. Water (130 μL), 15% NaOH (aq) (130 μL) and water (260 μL) were added stepwise to the mixture at 0 °C. The reaction mixture was filtered through a pad of celite (5 g) and the celite was washed with diethyl ether (150 mL). The combined filtrate and washings were dried over $MgSO_4$ and evaporated in vacuo. The residue was purified by chromatography on silica gel with ethyl acetate–hexane (3:7) as eluent to yield 750 mg (67%) of **6** as a white waxy liquid: $C_{28}H_{50}O_4$; TLC (ethyl acetate:hexane = 2:8) R_f, 0.53; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.71 (d, 1H, J = 6.8 Hz), 4.67 (s, 2H), 4.66 (d, 1H, J = 6.8 Hz), 3.80 (t, 1H, J = 2.6 Hz, 12-CH), 3.50 (m, 1H, 3-CH), 3.41 (s, 3H, OCH_3), 3.35 (s, 3H, OCH_3), 1.87–0.95 (m, 26H), 0.91 (d, 3H, J = 7.6 Hz, 21-CH_3), 0.90 (s, 3H, 19-CH_3), 0.85 (t, 3H, J = 7.0 Hz, 24-CH_3), 0.68 (s, 3H, 18-CH_3); IR (NaCl) ν_{max} 2934, 2868, 1467, 1449, 1373, 1213, 1147, 1104, 1046, 918 cm^{-1}.

2.2.8. *3α,12α-Dihydroxy-5β-cholane (7)*

To a solution of 3α,12α-bis(methoxymethoxy)-5β-cholane (**6**, 705 mg, 1.56 mmol) in methanol (25 mL) and CH_2Cl_2 (3 mL) co-solvent, *c*-HCl (0.20 mL) was added and warmed up to 50 °C, then stirred for 24 h. After 24 h, volatile materials were removed under the reduced pressure. The residue was diluted with diethyl ether (50 mL), washed with water (2 × 30 mL), dried over $MgSO_4$ and evaporated in vacuo. The residue was purified by chromatography on silica gel with ethyl acetate–hexane (3:7) as eluent to yield 460 mg (81%) of **7** as a white power: $C_{24}H_{42}O_2$; TLC (ethyl acetate:hexane = 5:5) R_f, 0.45; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.99 (s, 1H, 12-CH), 3.60 (m, 1H, 3-CH), 1.88–0.93 (m, 28H), 0.95 (d, 3H, J = 6.6 Hz, 21-CH_3), 0.90 (s, 3H, 19-CH_3), 0.86 (t, 3H, J = 7.0 Hz, 24-CH_3), 0.67 (s, 3H, 18-CH_3); IR (NaCl) ν_{max} 3376 (br), 2936, 2867, 1467, 1449, 1378, 1215, 1089, 1040, 760 cm^{-1}.

2.2.9. *3α,12α-Bis(chloroacetoxy)-5β-cholane (8)*

Chloroacetyl chloride (1.36 mL, 17.22 mmol) was slowly added to a solution of **7** (1.49 g, 2.42 mmol), CaH_2 (345.22 mg, 8.20 mmol) and Bu_4NBr (67.70 mg, 0.21 mmol) in toluene (10 mL). The mixture was stirred for 5 h at 90 °C, cooled to room temperature and filtered through a pad of celite (5 g). The celite was washed with ethyl acetate (100 mL) and the combined filtrate and washings were washed with saturated aqueous $NaHCO_3$ solution (2 × 100 mL) and water (100 mL). The organic layer was dried over $MgSO_4$ and evaporated in vacuo. Purification of the residue by chromatography on silica gel with ethyl acetate–hexane (1:9) yielded 385 mg (91%) of **8** as a waxy liquid: $C_{28}H_{44}Cl_2O_4$; TLC (ethyl acetate:hexane = 2:8) R_f, 0.50; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.20 (br s, 1H, 12β-H), 4.85–4.73 (m, 1H, 3β-H), 4.07 (s, 2H), 4.03 (s, 2H), 2.00–0.50 (m, 38H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.7, 166.5, 78.1, 76.3, 49.2, 47.7, 45.0, 41.7, 41.17, 41.13, 38.0, 35.5, 34.8, 34.5, 34.3, 33.9, 31.9, 27.3, 26.7, 26.3, 25.8, 25.4, 23.3, 22.9, 19.0, 17.8, 14.4, 12.2; IR (NaCl) ν_{max} 2954, 2870, 1743, 1468, 1410, 1311, 1203, 1180 cm^{-1}; MS m/e 420 ($M^+ - ClCH_2CO_2, H_2$), 349 ($M^+ - ClCH_2CO_2$, H_2, $CH_3CH_2CHCH_3$), 326 ($M^+ -$ 2($ClCH_2CO_2$, H_2)).

2.2.10. *General procedure for the preparation of N,N-dioctyl-3α,12α-bis(N,N-di(alkyl,aryl) thiocarbamoylsulfanylacetoxy)-5β-cholanes **9a–9c***

A solution of 3α,12α-bis(chloroacetoxy)-5β-cholane (**8**, 199 mg, 0.39 mmol) and sodium *N,N*-di(alkyl, aryl)dithiocarbamate (1.95 mmol) in 10 mL of THF was refluxed for 24 h and 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), washed with water (3 × 150 mL), dried over $MgSO_4$ and evaporated in vacuo. The residue was purified by chromatography on silica gel with ethyl acetate–hexane as eluent to give **9a**–**9c** as colorless liquids.

2.2.11. *3α,12α-Bis(N,N-diethylthiocarbamoylsulfanylacetoxy)-5β-cholane **(9a)***

$C_{38}H_{64}N_2O_4S_4$; TLC (ethyl acetate:hexane = 2:3) R_f, 0.62; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.15 (br s, 1H, 12β-H), 4.88–4.70 (m, 1H, 3β-H), 4.36 (d, J = 16.2 Hz, 1H), 4.27 (d, J = 16.2 Hz, 1H), 4.19 (d, J = 16.5 Hz, 1H), 4.09 (d, J = 16.5 Hz, 1H), 4.05–3.95 (m, 4H), 3.90–3.70 (m, 4H), 2.00–0.68 (m, 50H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 193.7, 193.3, 168.1, 167.9, 77.4, 75.8, 50.0, 49.7, 49.0, 47.5, 46.9, 46.7, 45.0, 41.8, 39.5, 39.2, 38.0, 35.6, 35.0, 34.7, 34.2, 34.0, 32.0, 27.4, 26.8, 26.5, 25.8, 25.4, 23.4, 22.9, 19.2, 17.7, 14.5, 12.6, 12.5, 12.1, 11.4; IR (KBr) ν_{max} 3056, 2937, 1728, 1355, 1270, 1207, 1159 cm^{-1}; HRMS calc. for $C_{38}H_{64}N_2O_4S_4$ *m/z* 740.3749, found 740.3749.

2.2.12. *3α,12α-Bis(N,N-diisobutylthiocarbamoylsulfanylacetoxy)-5β-cholane **(9b)***

$C_{46}H_{80}N_2O_4S_4$; TLC (ethyl acetate:hexane = 1:4) R_f, 0.58; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.15 (br s, 1H, 12β-H), 4.85–4.65 (m, 1H, 3β-H), 4.44 (d, J = 16.3 Hz, 1H), 4.25–4.07 (m, 3H), 3.95–3.75 (m, 4H), 3.70–3.50 (m, 4H), 2.60–2.20 (m, 4H), 2.10–0.60 (m, 62H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.5, 195.0, 168.1, 167.9, 75.7, 63.8, 63.1, 60.9, 49.2, 47.5, 45.0, 41.8, 39.6, 39.5, 38.0, 35.6, 35.0, 34.7, 34.2, 34.0, 32.0, 27.7, 27.6, 27.5, 26.9, 26.5, 26.2, 26.1, 25.8, 25.5, 23.4, 22.9, 20.3, 20.2, 19.1, 17.7, 14.5, 12.2; IR (KBr) ν_{max} 3051, 2959, 1727, 1385, 1265, 1154, 1090 cm^{-1}; HRMS calc. for $C_{46}H_{80}N_2O_4S_4$ *m/z* 852.5001, found 852.5023.

2.2.13. *3α,12α-Bis(N,N-diphenylthiocarbamoylsulfanylacetoxy)-5β-cholane **(9c)***

$C_{54}H_{64}N_2O_4S_4$; TLC (ethyl acetate:hexane = 1:3) R_f, 0.42; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50–7.10 (m, 20H), 5.13 (br s, 1H, 12β-H), 4.80–4.60 (m, 1H, 3β-H), 4.33 (d, J = 16.6 Hz, 1H), 4.10 (d, J = 16.6 Hz, 1H), 3.99 (d, J = 16.4 Hz, 1H), 3.89 (d, J = 16.4 Hz, 1H), 2.00–0.60 (m, 38H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.1, 199.5, 167.9, 167.6, 145.1, 129.6, 128.4, 128.0, 77.6, 75.8, 49.4, 47.7, 45.2, 41.8, 40.3, 40.2, 38.1, 35.7, 35.2, 34.8, 34.3, 34.1, 32.0, 27.7, 26.9, 26.5, 26.0, 25.4, 23.6, 23.0, 19.3, 17.8, 14.6, 12.3; IR (KBr) ν_{max} 3060, 2953, 1732, 1589, 1355, 1281, 1197, 1154, 743 cm^{-1}; HRMS calc. for $C_{54}H_{64}N_2O_4S_4$ *m/z* 932.3749, found 932.3744.

2.2.14. *General procedure for the preparation of N,N-dioctyl-12α-(N,N-di(alkyl, aryl)thiocarbamoylsulfanylacetoxy)-3α-hydroxy-5β-cholan-24-amides **10a–10c***

A solution of *N,N*-dioctyl-3α,12α-bis(chloroacetoxy)-5β-cholan-24-amide (**2**, 395 mg, 0.51 mmol) and sodium *N,N*-di(alkyl, aryl)dithiocarbamate (1.53 mmol) in 12 mL of EtOH was refluxed for 24 h and filtered through a pad of celite (5 g) after cooling. The celite was washed with dichloromethane (200 mL) and combined filtrate and washings were evaporated in vacuo. The residue was diluted with CH_2Cl_2 (150 mL), washed with saturated $NaHCO_3$ (2 × 150 mL) and water, dried over $MgSO_4$ and evaporated in vacuo. The residue was purified by chromatography on silica gel with ethyl acetate–hexane as eluent to yield **10a**–**10c** as waxy liquids.

2.2.15. *N,N-Dioctyl-12α-(N,N-diethylthiocarbamoylsulfanylacetoxy)-3α-hydroxy-5β-cholan-24-amide **(10a)***

$C_{47}H_{84}N_2O_4S_2$; TLC (ethyl acetate:hexane = 1:1) R_f, 0.45; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.13 (br s, 1H, 12β-H), 4.39 (d, J = 16.5 Hz, 1H), 4.19 (d, J = 16.5 Hz, 1H), 4.12–3.90 (m, 2H), 3.90–3.70 (m, 2H), 3.62–3.48 (m, 1H, 3β-H), 3.29–3.19 (m, 4H), 2.34–0.72 (m, 72H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 193.3, 172.9, 168.0, 77.6, 71.7, 50.1, 49.1, 48.0, 47.4, 46.9, 45.8, 45.2, 42.0, 39.1, 36.5, 35.6, 35.2, 35.0, 34.2, 34.1, 31.8, 31.7, 31.4, 30.6, 30.0, 29.4, 29.3, 29.21, 29.18, 27.8, 27.4, 27.0, 26.9, 26.1, 25.3, 23.5, 23.0, 22.6, 17.7, 14.0, 12.6, 12.2, 11.5; IR (KBr) ν_{max} 3425, 3056, 2985, 1727, 1627, 1488, 1263 cm^{-1}; HRMS calc. for $C_{47}H_{84}N_2O_4S_2$ *m/z* 804.5873, found 804.5895.

2.2.16. *N,N-Dioctyl-12α-(N,N-diisobutylthiocarbamoylsulfanylacetoxy)-3α-hydroxy-5β-cholan-24-amide **(10b)***

$C_{51}H_{92}N_2O_4S_2$; TLC (ethyl acetate:hexane = 2:3) R_f, 0.49; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.12 (br s, 1H, 12β-H), 4.45 (d, J = 16.7 Hz, 1H), 4.15 (d, J = 16.7 Hz, 1H), 3.95–3.74 (m, 2H), 3.71–3.48 (m, 3H), 3.37–3.13 (m, 4H), 2.53–2.11 (m, 4H), 1.95–0.72 (m, 76H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 194.9, 172.8, 167.8, 77.6, 71.7, 63.9, 61.1, 49.1, 47.9, 47.4, 45.8, 45.1, 42.0, 39.2, 36.5, 35.5, 35.3, 34.9, 34.1, 34.0, 31.69, 31.65, 31.3, 30.6, 30.0, 29.3, 29.2, 29.1, 27.7, 27.3, 27.0, 26.8, 26.2, 26.0, 25.2, 23.5, 22.8, 22.5, 20.3, 20.2, 17.7, 14.0, 12.2; IR (KBr) ν_{max} 3420, 3052, 2956, 1727, 1631, 1469, 1273 cm^{-1}; HRMS calc. for $C_{51}H_{92}N_2O_4S_2$ *m/z* 860.6499, found 860.6508.

2.2.17. N,N-Dioctyl-12α-(N,N-diphenylthiocarbamoylsulfanylacetoxy)-3α-hydroxy-5β-cholan-24-amide ***(10c)***

$C_{55}H_{84}N_2O_4S_2$; TLC (ethyl acetate:hexane = 2:3) R_f, 0.40; ^1H NMR (300 MHz, $CDCl_3$) δ 7.58–7.30(m, 10H), 5.10 (br s, 1H, 12β-H), 4.36 (d, *J* = 16.8 Hz, 1H), 4.04 (d, *J* = 16.8 Hz, 1H), 3.52–3.42 (m, 1H, 3β-H), 3.33–3.07 (m, 4H), 2.25–0.73 (m, 66H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.4, 172.7, 167.3, 129.5, 128.4, 127.8, 77.6, 71.5, 49.3, 47.8, 47.5, 45.8, 45.2, 42.0, 39.8, 36.6, 35.6, 35.2, 34.8, 34.1, 34.0, 31.7, 31.6, 31.2, 30.6, 29.7, 29.3, 29.2, 29.1, 29.07, 29.00, 27.7, 27.4, 27.0, 26.8, 26.1, 25.2, 23.5, 22.8, 22.5, 17.6, 14.0, 12.1; IR (KBr) ν_{max} 3450, 3052, 2964, 1711, 1628, 1252 cm^{-1}; HRMS calc. for $C_{55}H_{84}N_2O_4S_2$ *m/z* 900.5873, found 900.5867.

2.2.18. General procedure for the preparation of N,N-dioctyl-12α-(N,N-di(alkyl, aryl)thiocarbamoylsulfanylacetoxy)-3α-hydroxy-5β-cholanes ***11a–11c***

A solution of 3α,12α-bis(chloroacetoxy)-5β-cholane (**8**, 121 mg, 0.24 mmol) and sodium *N,N*-di(alkyl, aryl)dithiocarbamate (1.18 mmol) in 8 mL of EtOH was refluxed for 24 h and filtered through a pad of celite (5 g) after cooling. The celite was washed with dichloromethane (200 mL) and combined filtrate and washings were evaporated in vacuo. And the residue was diluted with CH_2Cl_2 (150 mL), washed with saturated $NaHCO_3$ (2 × 150 mL) and water, dried over $MgSO_4$ and evaporated in vacuo. The residue was purified by chromatography on silica gel with ethyl acetate–hexane as eluent to yield **11a** and **11b** and as a waxy liquid.

2.2.19. 12α-(N,N-Diethylthiocarbamoylsulfanylacetoxy)-3α-hydroxy-5β-cholane ***(11a)***

$C_{31}H_{53}NO_3S_2$; TLC (ethyl acetate:hexane = 1:1) R_f, 0.43; ^1H NMR (300 MHz, $CDCl_3$) δ 5.13 (br s, 1H, 12β-H), 4.38 (d, *J* = 16.4 Hz, 1H), 4.20 (d, *J* = 16.4 Hz, 1H), 4.10–3.98 (m, 2H), 3.87–3.72 (m, 2H), 3.62–3.48 (m, 1H, 3β-H), 1.86–0.71 (m, 45H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 193.4, 167.9, 77.7, 71.7, 50.1, 49.0, 47.5, 46.8, 45.1, 42.0, 39.2, 38.0, 36.5, 35.6, 35.2, 35.0, 34.1, 34.0, 30.6, 27.4, 27.0, 26.0, 25.2, 23.4, 22.9, 19.1, 17.8, 14.4, 12.5, 12.1, 11.5; IR (KBr) ν_{max} 3460, 3057, 2989, 1720, 1262 cm^{-1}; HRMS calc. for $C_{31}H_{53}NO_3S_2$ *m/z* 551.3466, found 551.3455.

2.2.20. 12α-(N,N-Diisobutylthiocarbamoylsulfanylacetoxy)-3α-hydroxy-5β-cholane ***(11b)***

$C_{35}H_{61}NO_3S_2$; TLC (ethyl acetate:hexane = 3:7) R_f, 0.43; ^1H NMR (300 MHz, $CDCl_3$) δ 5.11 (br s, 1H, 12β-H), 4.48 (d, *J* = 16.5 Hz, 1H), 4.13 (d, *J* = 16.5 Hz, 1H), 3.96–3.76 (m, 2H), 3.72–3.48 (m, 3H), 2.57–2.30 (m, 2H), 1.91–0.76 (m, 51H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.1, 167.8, 77.7, 71.8, 64.0, 61.1, 49.2, 47.5, 45.1, 42.0, 39.3, 38.0, 36.6, 35.6, 35.3, 34.9, 34.1, 34.0, 30.7, 27.7, 27.4, 27.0, 26.2, 26.0, 25.2, 23.5, 22.9, 20.2, 19.0, 17.8, 14.5, 12.1; IR (KBr) ν_{max} 3425, 3052, 2961, 1727, 1469, 1268 cm^{-1}; HRMS calc. for $C_{35}H_{61}NO_3S_2$ *m/z* 607.4093, found 607.4084.

2.2.21. 12α-(N,N-Diphenylthiocarbamoylsulfanylacetoxy)-3α-hydroxy-5β-cholane ***(11c)***

A solution of 3α,12α-bis(chloroacetoxy)-5β-cholane (**8**, 111 mg, 0.22 mmol) and sodium *N,N*-diphenyldithiocarbamate (0.64 mmol) in THF (2 mL) and EtOH (9 mL) was refluxed for 24 h and filtered through a pad of celite (5 g) after cooling. The celite was washed with dichloromethane (200 mL) and combined filtrate and washings were evaporated in vacuo. The residue was diluted with CH_2Cl_2 (150 mL), washed with saturated $NaHCO_3$ (2 × 150 mL) and water, dried over $MgSO_4$ and evaporated in vacuo. The residue was purified by chromatography on silica gel with ethyl acetate–hexane as eluent to yield **11c** as a waxy liquid. $C_{39}H_{53}NO_3S_2$; TLC (ethyl acetate:hexane = 3:7) R_f, 0.31; ^1H NMR (300 MHz, $CDCl_3$) δ 7.54–7.25 (m, 10H), 5.10 (br s, 1H, 12β-H), 4.38 (d, *J* = 16.7 Hz, 1H), 4.03 (d, *J* = 16.7 Hz, 1H), 3.52–3.40 (m, 1H, 3β-H), 1.98–0.72 (m, 39H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.6, 167.4, 145.1, 129.6, 128.4, 127.8, 77.7, 71.6, 49.3, 47.6, 45.1, 42.0, 39.9, 38.0, 36.6, 35.6, 35.2, 35.0, 34.2, 34.0, 30.6, 27.6, 27.0, 26.2, 25.2, 23.6, 22.9, 19.1, 17.7, 14.5, 12.1; IR (KBr) ν_{max} 3423, 3056, 2933, 1722, 1490, 1264 cm^{-1}; HRMS calc. for $C_{39}H_{53}NO_3S_2$ *m/z* 647.3467, found 647.3466.

2.3. 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 [NPOE, DOA, DOS or BBPA]). 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 initial evaluation of the ionophores, but replaced with various mixtures of $AgNO_3$ (10^{-5} to 10^{-1} M) and Na_2EDTA (5×10^{-3} to 10^{-1} M) to enhance the detection limits of the electrodes. 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 response of the electrodes to pH changes was tested by adding aliquots of sodium solution to a solution of 11.4 mM boric acid, 6.7 mM citric acid and 10.0 mM NaH_2PO_4 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 [10–12]; the concentration of the primary ion that brings about the same response potential corresponding to 10^{-3} 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 [11,12].

3. Results and discussion

3.1. Synthesis of ionophores

Newly designed molecules for silver ion-selective ionophores were prepared, starting with 7-deoxycholic acid (**1**), as described in Scheme 1. As enough hydrophobicity of the compound is required for ion-selective membranes, the acid group of deoxycholic acid was first converted into a long chain-containing dialkyl amide through the formylation of hydroxyl groups, along with the introduction of amide moiety followed by deformylation to regenerate hydroxyl groups. With this key intermediate in hand, chloroacetyl groups were introduced on two hydroxyl groups (**2**) for an easy and convenient nucleophilic substitution reaction. The dithiocarbamate derivatives were prepared using a known method [9].

To keep the moderate hydrophobicity and to diminish the interference of the amide group in the frame, the short hydrocarbon-chain introduced at 24-position of the frame ionophores was also synthesized as described in Scheme 1. The acid moiety of **1** was converted into a short hydrocarbon-chain through the esterification of the acid group (**4**), the MOM protection of the hydroxyl groups, the reduction of the ester group (**5**), the tosylation of the alcohol moiety, the detosylation to generate short hydrocarbon-chain (**6**) and deprotection of the MOM protected group to regenerate the hydroxyl group (**7**). With this key intermediate in hand, chloroacetyl groups were introduced on two hydroxyl groups (**8**) to link the binding sites. Six different prospective ionophores, (i.e., **3a**–**3c** and **9a**–**9c**) were synthesized and their structures were fully identified with ^{1}H NMR, ^{13}C NMR, FT-IR and FAB-MASS (**3a**–**3c**) or HRMS (**9a**–**9c**).

For the comparison of the tweezering effect of tweezer-type ionophores versus non-tweezer-type ionophores, non-tweezer-type ionophores were synthesized as shown in Scheme 2. Non-tweezer-type ionophores that contain only one dithiocarbamoyl group at C12 position of the frame backbone were easily prepared by changing the solvent system from THF (Scheme 2, a) to EtOH. Ionophores **10a**, **10b**, **10c**, **11a**, **11b** and **11c** were obtained through nucleophilic substitution reaction in EtOH or THF/EtOH solution as shown in Scheme 2. The structures were also fully identified with ^{1}H NMR, ^{13}C NMR, FT-IR and HRMS.

3.2. Potentiometric properties of ionophores

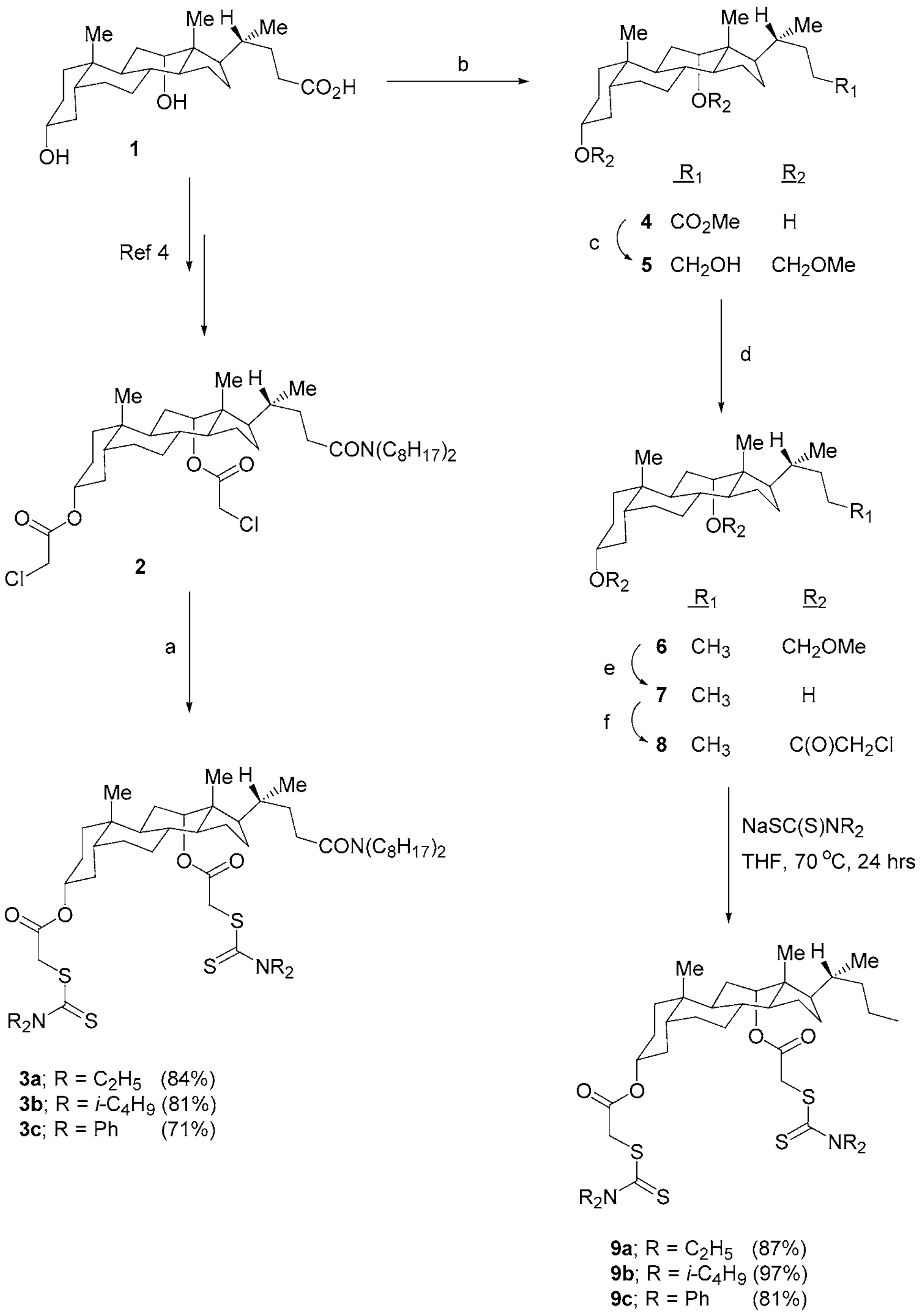
To examine the potentiometric properties of newly synthesized silver ion-selective ionophores in an optimal matrix condition, the effect of several common plasticizers on the overall performance of PVC-based membranes doped with compound **3a** were first examined. Membranes composed of a 1:3 ratio of PVC and plasticizer were prepared as described in Section 2 with four different plasticizers: NPOE, DOS, DOA and BBPA. The results are shown in Fig. 1; the dynamic response curves obtained in the 10^{-7} to 10^{-3} M range clearly shows the difference in potentiometric responses depending on the type of plasticizers employed.

The membrane with the NPOE plasticizer exhibited a reduced response slope (29.0 mV/decade), implying that the relatively high dielectric solvent does not provide a favorable environment for silver ion–ligand complexing. The membrane with BBPA resulted in a near Nernstian response, 55.0 mV/decade, in the 10^{-7} to 10^{-3} M range. However, it exhibited very slow responses (over 20 s); BBPA tends to smear out of the PVC membrane and impede the transfer of ions at the membrane/sample interface. Both membranes with DOA and DOS exhibit Nerenstian slopes (57.0 and 56.8 mV/decade, respectively) with fast responses. The DOA-plasticized membrane, however, required less time for stabilization and faster responses. The test has been extended to the **3b** and **3c** as well as the **9a**–**9c** compounds; the trends were almost the same for all cases. For this reason, DOA was selected as the plasticizer for preparing silver ion-selective membranes.

It was also noticed that the silver ion-selective membranes with DOA, DOS and BBPA plasticizers exhibit over-Nernstian responses in the region below the 10^{-7} M range: the magnitude of the response was about 1.5 decades (80 mV) in the range. It apparently implies that the newly synthesized ionophores have high complex formation constants with silver ions and may result in the subnanomolar detection limit with an appropriately optimized membrane composition and/or electrode configuration.

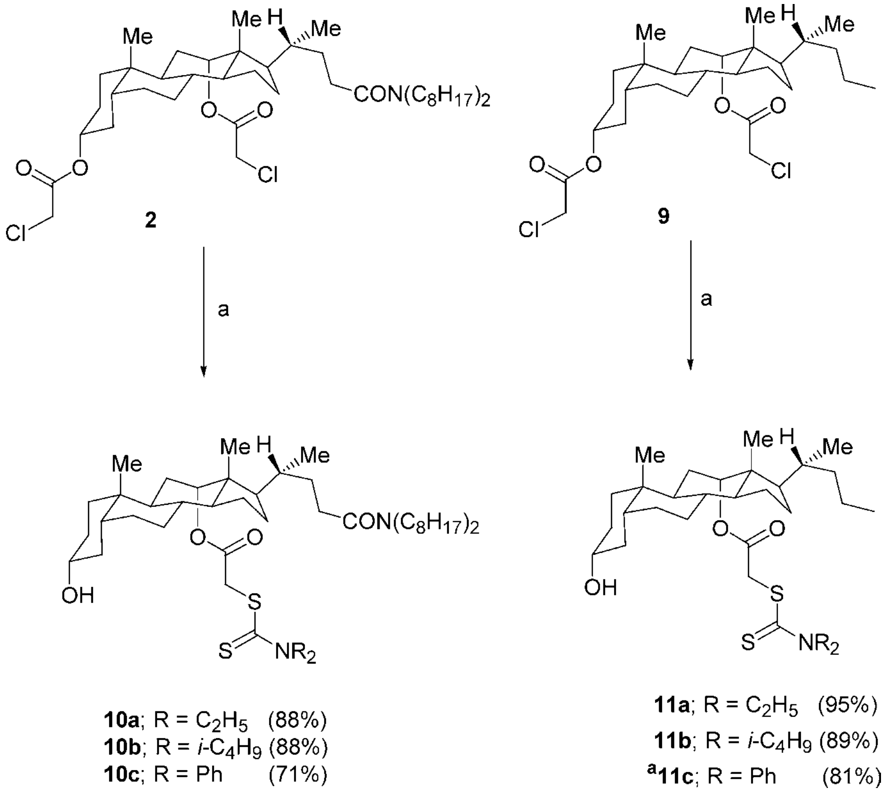
All 12 ionophores were then evaluated with the DOA-plasticized PVC membranes to find the effect of structural variations in the binding sites of CAB-based ionophores on their potentiometric characteristics. The silver selectivity of all ionophores was evaluated over nine transition metal ions (Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+} and Hg^{2+}). Fig. 2 shows the four typical calibration plots; they include the responses of the electrodes to two most responsive ions for clarity. It was noticed that only Cu^{2+} and Hg^{2+} are two most interfering ions, while the other ions hardly give rise to discernable changes.

Table 1 lists the potentiometric characteristics of all ionophores doped in the DOA-plasticized membranes with the 0.1 M KCl internal reference solution. Again, Table 1 includes the silver ion selectivity coefficients only of Hg^{2+}, as the coefficient ($K^{POT}_{Ag^+,j}$) values for all other ions are negligible (less than −4.5). While the detection limits are cer-



Scheme 1. (a) $NaSC(S)NR_2$, THF, 70 °C, 24 h; (b) AcCl, MeOH, 0 °C, 86%; (c) (i) $CH_2(OCH_3)_2$, CF_3SO_3H, CH_2Cl_2, 5 h, rt (ii) $LiAlH_4$, Et_2O, 2 h, rt (88% for two steps); (d) (i) TsCl (3 equivalent), pyridine, 1.5 h, 75 °C (ii) $LiAlH_4$, Et_2O, 1 h, 0 °C (73% for two steps); (e) HCl, MeOH, CH_2Cl_2, 96%; (f) $ClCH_2COCl$, CaH_2, Bu_4NBr, toluene, 90 °C, 5 h, 91%.

tainly lower than 10^{-8} M, the exact values were not evaluated because of a large over-Nernstian response observed below 10^{-7} M. Table 1 also compares the performance of four different groups of CAB-based ionophores; CAB with two alkyl and phenyl dithiocarbarmates with long dioctyl amide (**3a**–**3c**) and short pentyl side chains (**9a**–**9c**) and the CAB with single alkyl and phenyl dithiocarbarmates with long dioctyl amide (**10a**–**10c**) and short pentyl side chains (**11a**–**11c**). The silver ion selectivity of **3a** and **3b** over mercury ion is significantly improved (5–50 times) when the side chain did not contain any amide group. Comparing the response slopes of the electrodes based on **3** and **10**, and **9** and **11**, respectively, it appears that the complexing ability of the tweezer-type ionophore is significantly better than those with a single binding site; those with two binding sites give rise to Nernstian slopes (55–60 mV/decade),



Scheme 2. (a) $NaSC(S)NR_2$, EtOH, 70 °C, 24 h (^{a}**11c**; THF/EtOH).

while those with a single binding site produce sub-Nernstian slopes (48–54 mV/decade). The enhanced sensitivity of the two dithiocarbamate group containing compounds (**3** and **9**) to silver ion also increased their selectivity over mercury ion. These results clearly demonstrate the advantage of CAB-based tweezer-type ionophores over those with a single binding site.

Having confirmed that the ISEs based on CAB-based ionophores (**3** and **9**) exhibit a high silver ion selectivity with a low detection limit, attempts to develop ISE systems similar to the one based on *o*,*o*″-bis[2-(methylthio)ethyl]-*tert*-butylcalix[4]arene were made. Bakker and co-workers proposed that the contamination of the membrane surface by transmembrane diffusion of the primary ions from the inner filling solution increases the detection limits [13–15]. To find an optimal composition of the internal reference solution according to the theory, several silver ion-selective electrodes were prepared, as listed in Table 2, with the membranes based on compound **9a**.

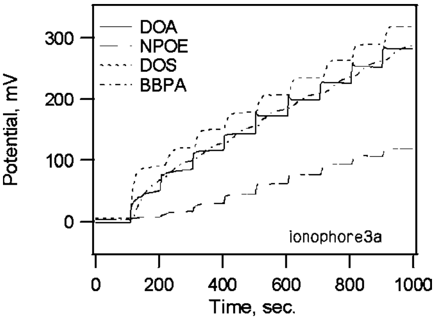


Fig. 1. Potentiometric response of ionophore **3a**-based membranes prepared with different plasticizers.

The mixture of $AgNO_3$ and Na_2EDTA was used to reduce the free primary ion concentration in the inner filling solution while maintaining the thermodynamic stability of the symmetric ISE system. The results are summarized in the same table. It was noticed that the slopes and detection limits of the electrodes varied greatly with the concentration of free silver ion in the internal filling solution. As expected, the response slope and the detection limit of the electrodes were worse when the silver ion concentration of the internal filling solution was high (37.0 mV/decade when $[Ag^+] = 10^{-1}$ M). Since the detection limit and slope of the electrode remain almost constant as long as the $[Ag^+]$ is less than 10^{-3} M, the available free silver ion in the internal solution was decreased by adding EDTA. The lowest detection limit with a response slope approaching a theoretical value (59.5 mV/decade at 25 °C) was obtained when the internal filling solution had 10^{-9} M of free Ag^+ (composition 8 in Table 2). Using this internal solution composition, the electrodes were constructed based on **3a**–**3c** and **9a**–**9c**. Their potentiometric properties are listed in Table 3. All electrodes produced the detection limits near the nano molarity range with a near Nernstian response slope. It is interesting to note herein that the electrodes based on compounds **9a**–**9c**, which

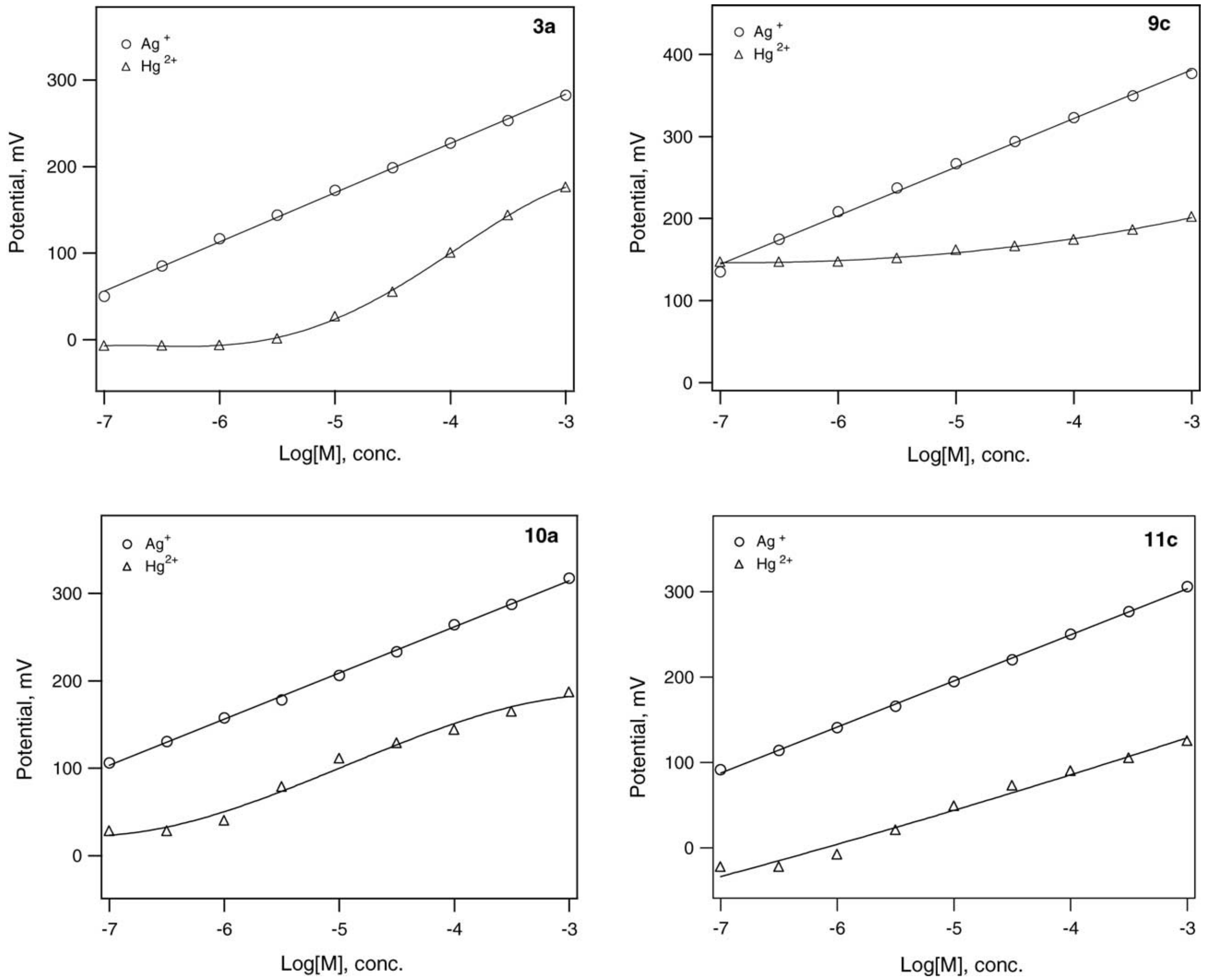


Fig. 2. Calibration plots of the electrodes based on ionophores **3a**–**11c**. Only representative responses are presented for clarity. Responses to other transition metal ions ($M^{z+} = Zn^{2+}$, Pb^{2+}, Ni^{2+}, Co^{2+}, Cd^{2+}, Fe^{2+}, Cu^{2+}, Mn^{2+}) are negligible ($\log K^{POT}_{Ag^+,M^+} < -4.5$).

have a shorter side chain in the cholic acid frame, exhibit improved potentiometric properties. On the other hand, the compounds **3b** and **9b**, which have a longer alkyl chain on the amine (R_2 = isobutyl versus ethyl or phenyl) in the same series of compounds, produced the high response slopes and low detection limits. This seems to indicate that improved potentiometric properties can be obtained by providing a hydrophobic environment around the binding site while eliminating electronegative atoms containing functional groups from the non-binding sites [16,17].

ISEs that exhibited a super-Nernstian response to transition metal ions often lacked potentiometric reproducibility and rarely returned to their background potential once they were tested with selective ions. This is attributed to the formation of a strong ligand–ion complex, which results in an irreproducible ISE. The potentiometric reproducibility of the CAB-based ionophore electrode was then examined by repeating the potentiometric titration from 10^{-7} to 10^{-3} M several times. Membranes of the electrodes were washed briefly with deionized water in each measurement. Their background

Table 1
Potentiometric properties of silver ion-selective electrodes based on ionophores **3a**–**11c**

Compound	Slope[a]	Detection limit[b]	Selectivity coefficient ($\log K^{POT}_{Ag^+,Hg^{2+}}$)[c]
3a	56.8	7.5	−1.9
3b	54.9	7.1	−2.6
3c	56.0	7.2	−3.6
9a	60.0	7.6	−3.6
9b	63.6	7.5	−3.3
9c	59.2	7.7	−3.7
10a	52.7	−7.8	−2.3
10b	52.6	−7.8	−2.1
10c	53.1	−8.0	−2.9
11a	47.5	−8.1	−2.3
11b	50.6	−8.1	−2.1
11c	53.9	−7.7	−3.3

[a] Range: 10^{-9} to 10^{-3} M, mV/decade.

[b] $\log[Ag^+]$, M.

[c] The sliver ion selectivity over all 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 −4.5.

Table 2
Composition of inner reference electrolyte solutions of silver ion-selective electrodes based on **3a** and their potentiometric properties

Entry	Internal filling solution[a]			
	$AgNO_3$	Na_2EDTA	Slope[b]	Detection limit[c]
1	10^{-1}		37.0	−7.2
2	10^{-2}		52.8	−7.8
3	10^{-3}		55.7	−8.1
4	10^{-4}		55.1	−8.1
5	10^{-5}		55.5	−8.1
6	10^{-3}	5×10^{-3}	53.5	−8.1
7	10^{-3}	10^{-2}	53.8	−8.7
8	10^{-3}	5×10^{-2}	60.0	−8.9
9	10^{-3}	10^{-1}	58.6	−8.7

[a] mol/L.
[b] mV/decade.
[c] $\log[Ag^+]$.

Table 3
Electrochemical properties of the silver-selective electrodes based on optimized inner reference electrolyte solution (composition 8 in Table 2)

Ionophore	Slope[a]	Detection limit[b]
3a	56.3	−8.5
3b	54.9	−8.7
3c	52.6	−8.5
9a	60.0	−8.9
9b	63.6	−9.2
9c	54.8	−9.1

[a] mV/decade.
[b] $\log[Ag^+]$.

potentials have increased substantially after the first test and gradually until the fourth test to a level of 10^{-6} M of silver ion [5]. The reproducibility over 10^{-6} M Ag^+ was excellent and the background potential was recovered to about 5×10^{-8} M when the electrode was washed with a high concentration of sodium chloride after use. This result suggests that silver ions partitioned into the membrane phase are tightly complexed with the CAB-based ionophore and remain as cationic sites in the membrane. The design of improved transition metal ion-selective ionophores, therefore, requires a very subtle manipulation of complex formation constants within the structure.

4. Conclusion

In this article, a rational design of transition metal ion-selective neutral carriers was shown based on tweezer-type and non-tweezer-type ionophores containing dithiocarbamoyl groups on a 7-deoxycholic amide or cholane derivatives. Their potentiometric properties were evaluated with the PVC membrane-based electrodes. The results reveal that the ionophores containing deoxycholic amides/cholanes linked with tweezer-type dithiocarbamoyl moieties showed excellent affinity and selectivity to silver(I) ion over alkali, alkaline earth and other transition metal cations. To investigate the effect of structural variation at the side chain, which is introduced to control lipophilicity and in binding sites on the potentiometric properties of resulting ISE membranes, 12 different analogues were synthesized. Consequently, we observed that the ionophores with shorter side chains at non-binding sites and with a hydrophobic environment in the binding site exhibit improved potentiometric properties, i.e., low detection limit and high response slope. The enhanced potentiometric properties of newly synthesized tweezer-type dithiocarbamoyl containing ionophores have been further improved by employing silver ion complexing reagent (EDTA) in the internal reference solution, which resulted in a greatly reduced detection limit (∼100 ppt) for the electrodes based on them.

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References

[1] H.J. Lee, I.J. Yoon, C.L. Yoo, H. Pyun, G.S. Cha, H. Nam, Anal. Chem. 72 (2000) 4694.

[2] Y.S. Choi, L. Lvova, J.H. Shin, S.H. Oh, C.S. Lee, B.H. Kim, G.S. Cha, H. Nam, Anal. Chem. 74 (2002) 2435.

[3] M.H. Lee, C.L. Yoo, J.S. Lee, In. Cho, B.H. Kim, G.S. Cha, H. Nam, Anal. Chem. 74 (2002) 2603.

[4] B.H. Kim, C.S. Lee, J.H. Shim, H.P. Hong, G.S. Cha, Y.M. Jun, H. Nam, Talanta 61 (2003) 393.

[5] J.H. Shim, I.S. Jeong, H.P. Hong, J.H. On, K.S. Kim, H.S. Kim, B.H. Kim, G.S. Cha, H. Nam, Talanta 63 (2004) 61.

[6] (a) For representative examples, see: A.P. Davis, Chem. Soc. Rev. (1993) 243;
(b) R.P. Bonar-Law, J.K.M. Sanders, J. Am. Chem. Soc. 117 (1995) 259;
(c) R.P. Bonar-Law, L.G. Mackay, C.J. Walter, V. Marvaud, J.K.M. Sanders, Pure Appl. Chem. 66 (1994) 803;
(d) U. Maitra, S. Balasubramanian, J. Chem. Soc., Perkin Trans. 1 (1995) 83;
(e) R.P. Bonar-Law, J.K.M. Sanders, J. Chem. Soc., Perkin Trans. 1 (1995) 3085;
(f) L.J. D’Souza, U. Maitra, J. Org. Chem. 61 (1996) 9494.

[7] (a) S. Chung, W. Kim, S.B. Park, D.Y. Kim, S.S. Lee, Talanta 44 (1997) 1291;
(b) S.S. Lee, M.K. Ahn, S.B. Park, Analyst 123 (1998) 383.

[8] (a) R.G. Pearson, J. Chem. Educ. 45 (1968) 581;
(b) S. Kumar, M.S. Hundal, N. Kaur, R. Singh, H. Singh, J. Org. Chem. 61 (1996) 7819.

[9] (a) S. Kamata, H. Murata, Y. Kubo, A. Bhale, Analyst 114 (1989) 1029;
(b) G. Kaugars, V.L. Rizzo, J. Heterocycl. Chem. 18 (1981) 411;
(c) E.J. Kupchik, P.J. Calabretta, Inorg. Chem. 4 (1965) 973.

[10] V.P.Y. Gadzekpo, G.D. Christian, Anal. Chim. Acta 164 (1984) 279.

[11] IUPAC recommendations for nomenclature of ion-selective electrodes, Pure Appl. Chem. 66 (1994) 2528.

[12] IUPAC selectivity coefficients for ion-selective electrodes: recommended methods for reporting K_{AB}^{pot} values, Pure Appl. Chem. 67 (1995) 507.
[13] T. Sokalski, T. Zwickl, E. Bakker, E. Pretsch, Anal. Chem. 71 (1999) 1204.
[14] T. Sokalski, A. Ceresa, M. Fibbioli, T. Zwickl, E. Bakker, E. Pretsch, Anal. Chem. 71 (1999) 1210.
[15] A. Ceresa, A. Radu, S. Peper, E. Bakker, E. Pretsch, Anal. Chem. 74 (2002) 4027.
[16] E. Bakker, P. Bühlmann, E. Pretsch, Chem. Rev. 97 (1997) 3083.
[17] P. Bühlmann, E. Pretsch, E. Bakker, Chem. Rev. 98 (1998) 1593.