Heteroatom Chemistry
Volume 23, Number 2, 2012

Synthesis and ^{1}H NMR Titration Study of 7-Deoxycholic Amide or Cholane Ionophores Containing Different Ion-Recognizing Groups at C3 and C12

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Received 6 September 2012; revised 28 November 2012

ABSTRACT: *Tweezer-type ionophores containing C3-carbamoylpropanamidoacetoxy and C12-dithiocarbamoyl groups on a 7-deoxycholic amide or cholane derivative were designed and synthesized. A representative ^{1}H NMR titration study indicated that newly synthesized ionophores form 1:1 complexes with the Ca^{2+} ion.* © 2012 Wiley Periodicals, Inc. Heteroatom Chem 23:187–194, 2012; View this article online at wileyonlinelibrary.com. DOI 10.1002/hc.21002

INTRODUCTION

Ion-selective electrodes are unique examples of chemical sensors that apply the concept of molecular recognition chemistry. Thus, we have developed and utilized the idea of molecular tweezer-type neutral carriers based on a deoxycholic acid backbone (DCAB) to yield highly selective ionophores for carbonate [1], chloride [2], calcium [3], silver [4], copper [5], and mercury [6], since the DCAB-based carriers are easy to design, easy to prepare from inexpensive deoxycholic acid (**1**; Fig. 1), and applicable to various ions by varying the types of ion recognizing groups attached to the hydroxyl linkers at C3 and C12. The hydroxyl linkers at the C3 and C12 carbons of the DCAB frame are approximately parallel and about 6 Å apart, a reasonable distance for various ions [7].

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More recently, we have found a synthetic method for introducing different functional groups in a step-by-step fashion at C3 and C12 on DCAB, which made possible the development of more DCAB-based neutral carriers with different ion-recognizing groups in the same molecule [4c,5,6]. Nonsymmetrically substituted tweezer-type DCAB-based ionophores, which contain a nitrogen or sulfur atom binding site at C3 and a sulfur atom binding site at C12, show excellent results for silver(I) ions [4c], whereas others that contain different ion-recognizing groups at the C3 position show outstanding results for copper and mercury [5,6]. Accordingly, our synthetic method for the step-by-step introduction of different functional groups at C3 and C12 on DCAB made it possible to be applicable to more ions.

Thus, we aimed to synthesize and study new types of DCAB-based ion-selective ionophores, which contain two different ion-recognizing functional groups with varied lengths at C3 and C12. Herein, we report the synthesis and ^{1}H NMR analysis of new nonsymmetrically substituted tweezer-type DCAB-based ionophores for use as highly selective metal ion hosts.

RESULTS AND DISCUSSION

Synthesis of Ionophores

As mentioned in the Introduction section, we recently found that mono-substituted 5β-cholan-24-amide (**4**) instead of di-substituted 5β-cholan-24-amide could be prepared from deoxycholic acid

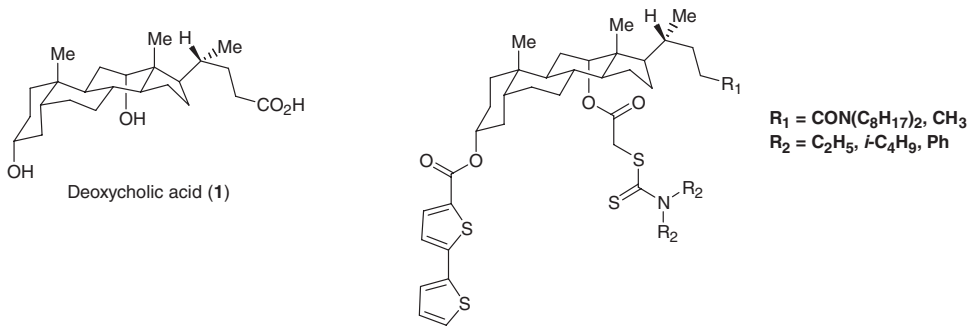
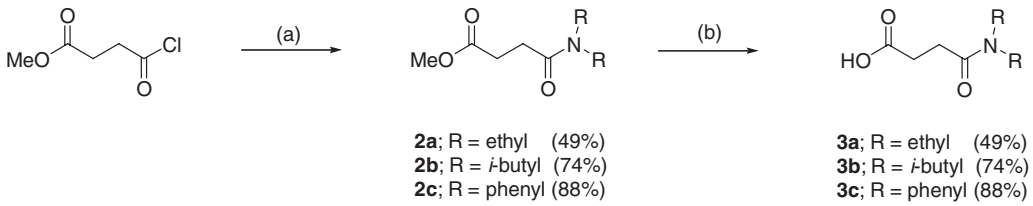


FIGURE 1 Structures of deoxycholic acid (**1**) and representative nonsymmetrically substituted DCAB-based ion-selective ionophores.

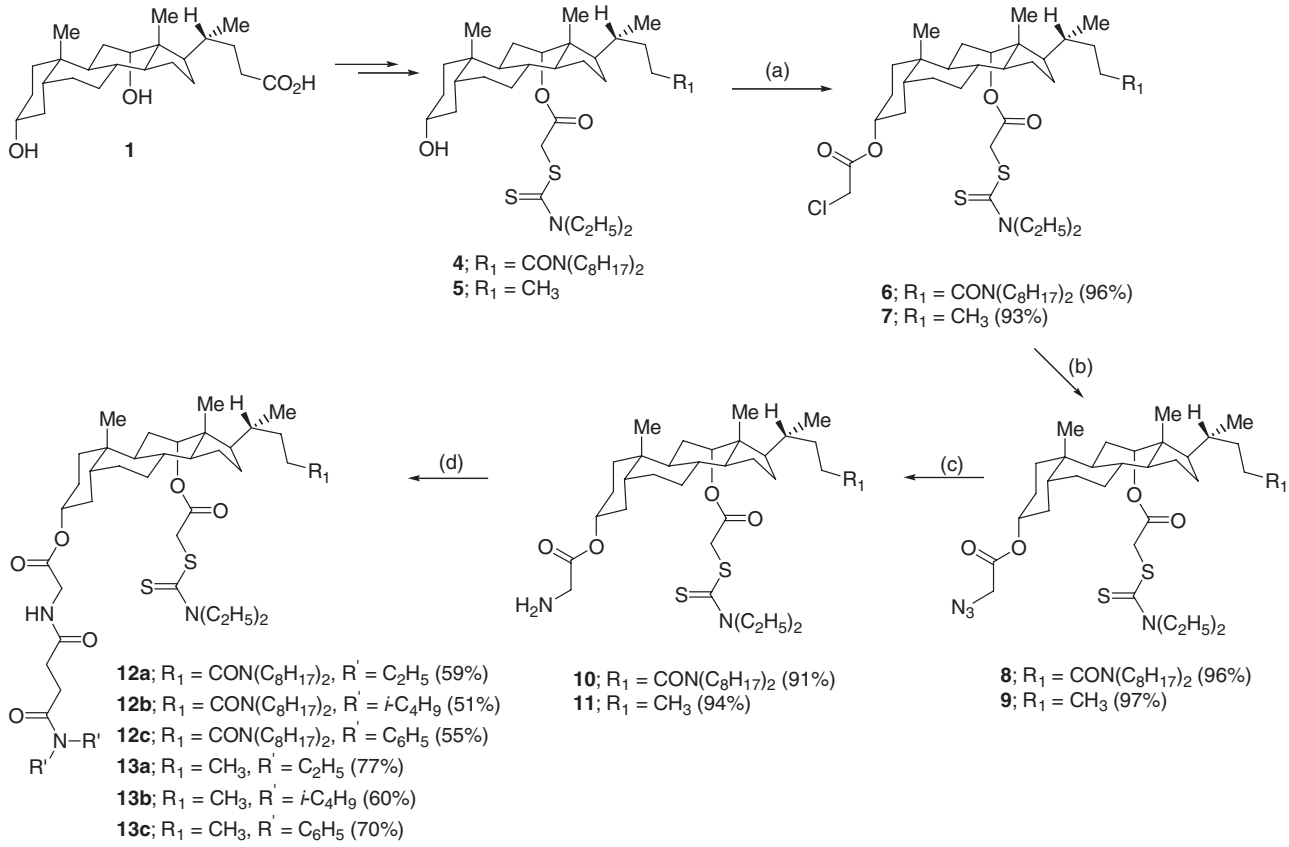
using a newly developed procedure [4c]. In addition to optimizing ion recognition groups at the 3α- and 12α-positions, the evaluation of lipophilic characteristics of the 5β-side chain is also important. Therefore, the preparation of 5β-cholan-24-amides with long alkyl chains on the amide group and 5β-cholane derivatives with an alkyl chain but no amide group should likewise be tested to obtain the best ionophore. Thus, **4** and **5**, containing 12α-dithiocarbamoyl groups on a 7-deoxycholic amide or cholane derivatives, were prepared from 7-deoxycholic acid (**1**) using the newly developed procedure and were used as starting substrates in this study.

Among the linking ion-recognizing moieties developed, glycolic diamide groups exhibited the best calcium ion recognition [3]. Thus, we introduced the suitably lengthened amide-related functional groups at C3 of DCAB with the expectation of new calcium ion-selective ionophores. For this purpose, *N*, *N*-di(alkyl, aryl)carbamoylpropanoic acid (**3**) was selected as an ion recognition linkage, was prepared through the two-step synthesis described in Scheme 1, and was introduced at the C3 positions in the starting substrates (**4** and **5**) as an ion-recognizing diamide group.

The starting substrates (**4** and **5**) were prepared from deoxycholic acid (**1**) using the procedure described in our previous paper [4c]. After preparation of the substrates, esterification of the 3α-hydroxyl group was performed with α-chloroacetyl chloride to convert the hydroxyl group into an α-chloroacetyl ester, which can be used as a precursor for the properly extended ion-recognizing diamide moiety. Thus, **4** or **5** was treated with α-chloroacetyl chloride in the presence of calcium hydride and tetrabutylammonium bromide in toluene at 90°C for 2 h to produce **6** or **7**, correspondingly (Scheme 2, (a)). Then, the chloro group of **6** or **7** was transformed into an amino group by a substitution reaction with sodium azide in DMF (50°C for 12 h), followed by the reduction of the azido group using nanosized zinc powder in acetic acid at room temperature (Scheme 2, (b) and (c)). Afterward, the target ionophores **12a–12c** or **13a–13c** were obtained in a reasonable yield by reacting **10a–10c** or **11a–11c** with *N*, *N*-di(alkyl, aryl)carbamoylpropanoic acid (**3**)/1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC)/1-hydroxybenzotriazole hydrate (HOBt) in dichloromethane at room temperature for 48 h (Scheme 2, (d)). Hence, six different prospective ionophores were successfully synthesized and their structures were fully identified with ^{1}H NMR, ^{13}C NMR, FTIR, and FAB MASS (**12a–12c**) or HRMS (**13a–13c**). Overall, the yields of the four-step synthesis from **4** to **12a–12c** or from **5** to **13a–13c** were 43%–49% and 50%–65%, respectively, and were shown to be excellent synthetic sequences.



SCHEME 1 (a) HNR_2, TEA, CH_2Cl_2, rt, 3 h; (b) LiOH, H_2O, THF, rt, 3 h.



SCHEME 2 (a) $ClCH_2COCl$, CaH_2, Bu_4NBr, toluene, 90°C, 2 h; (b) NaN_3, DMF, 50°C, 12 h; (c) nanosized Zn powder, acetic acid, rt, 24 h; (d) **3**, EDC, HOBt, CH_2Cl_2, DMF, rt, 48 h.

NMR Titration Experiments of Ionophores with the Ca^{2+} Ion

The calcium ion, one of the most important electrolytes in physiological systems, is known to form 1:2 and 1:3 complexes with the non-cyclic diamides, i.e., (–)-(*R*, *R*) – *N*,*N*′-[bis(11-ethoxycarbonyl)undecyl]-*N*,*N*′-4,5-tetramethyl-3,6-dioxaoctanediamide (ETH 1001) and *N*,*N*,*N*′,*N*′-tetracyclo-3-oxapantanediamide (ETH 129), respectively [8]. Our previously synthesized tweezer -type ionophores, such as *N*,*N*-dioctyl-3α,12α-bis (*N*-heptyl-*N*-methylcarbamoyl-methoxyacetamido-acetoxy)-5β-cholan-24-amide ionophore [3] and ionophores that have a bithiophene moiety on the 3α-position and diphenylaminothioxomethylthioacetyloxy group on the 12α-position of cholan-24-amide/cholane [4c], which exhibited excellent selectivity and sensitivity to calcium and silver ions, respectively, formed 1:1 complexes using two ion-recognizing groups that were properly oriented in a neutral carrier. Consequently, whether an ionophore will form a proper complex with the specific ion is the initial step in the development of a new ionophore.

Therefore, ^{1}H NMR titration experiments were examined after the syntheses of target neutral carriers to verify the recognition properties of the newly synthesized calcium ion-selective ionophores toward the calcium ion. ^{1}H NMR titrations were performed with the synthesized ionophore and $Ca(SCN)_2$ in methanol-d_4/acetone-d_6 (v/v = 1/1) co-solvent, which was determined to be the optimum condition according to the titration experiments. The representative best results of the NMR titrations with **13a** are shown in Fig. 2.

The ^{1}H NMR spectrum of the free ionophore **13a** shown in Fig. 2a was altered with the addition of the Ca^{2+} ion; following the addition of 1 equiv of $Ca(SCN)_2$ to the ionophore in methanol-d_4/acetone-d_6 solution, changes in the chemical shifts of several peaks were clearly observed (Fig. 2b). While considerable downfield shifts of the H_a, H_c, H_d, and H_e peaks that are adjacent to the carbonyl π-bond or next to the nitrogen of the amide group were observed, negligible shifts of the H_b peaks on the

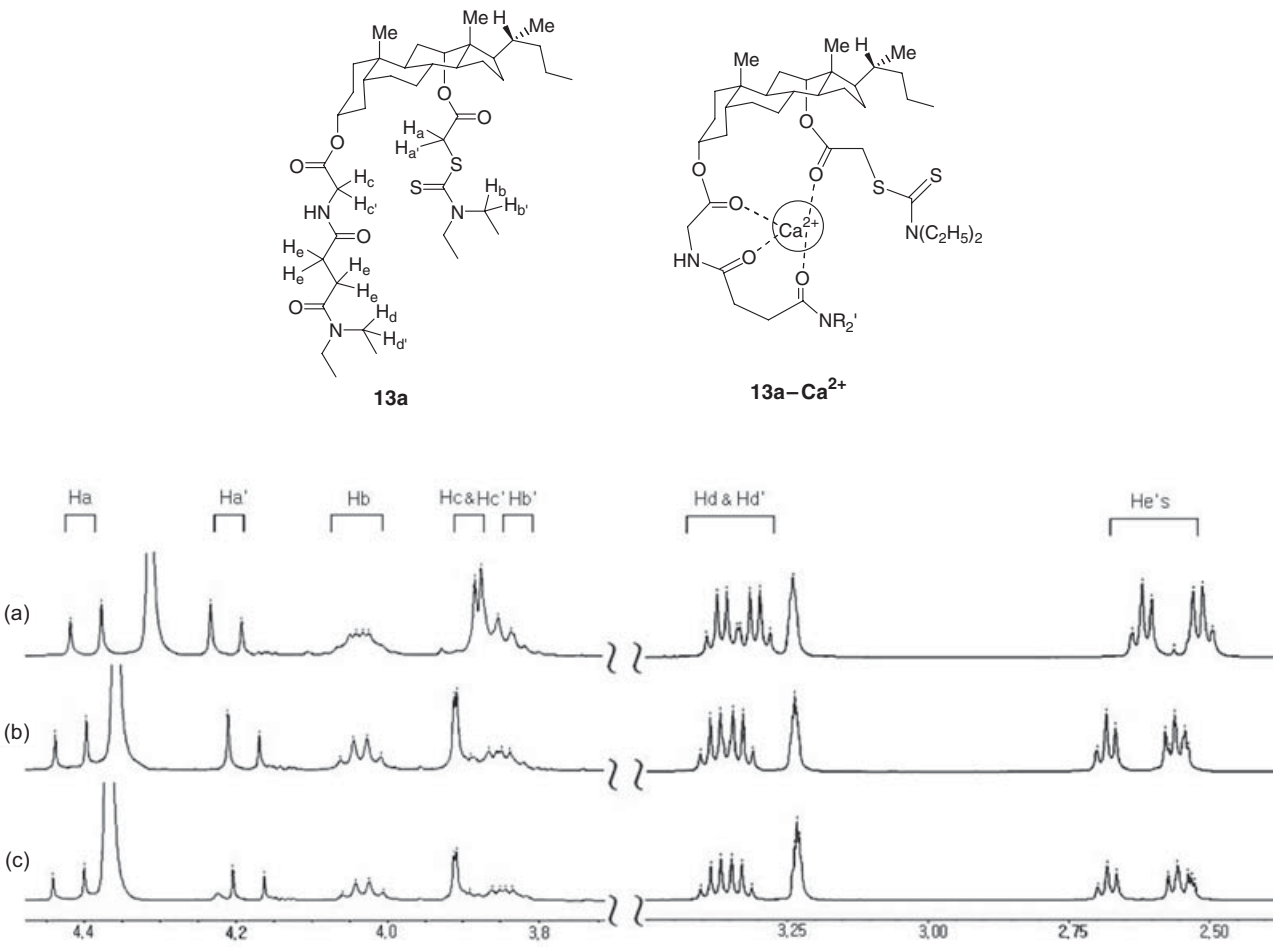


FIGURE 2 ^{1}H NMR spectra (400 MHz) of the ionophores, (a) free **13a** measured in methanol-d_4/acetone-d_6 (v/v = 1/1), (b) **13a** after addition of 1 equiv of $Ca(SCN)_2$, and (c) **13a** after addition of 2 equiv of $Ca(SCN)_2$.

dithiocarbamoyl moiety were observed. Moreover, interesting upfield and downfield shifts are observed at the peaks of H_a or H_a' when a Ca^{2+} ion is added to the free ionophore solution. While one of the diastereotopic protons, H_a, is shifted downfield due to the addition of 1 equiv of $Ca(SCN)_2$ to the free ionophore, the other proton, H_a', experiences an upfield shift. Obviously, H_a is on the same plane as the adjacent carbonyl π-bond, and its σ-bond donates electrons to the carbonyl group via the hyperconjugation effect during Ca^{2+} ion complexation with the ionophore. These ^{1}H NMR changes can be attributed to the complexation between the Ca^{2+} ion and heteroatoms in the dithiocarbamoyl and carbamoylpropanamidoacetoxy moieties in the **13a** ionophore, especially the oxygen heteroatom of the carbonyl group.

Unlike the Ag^+ ion ionophores [4c], sulfur heteroatoms on the dithiocarbamoyl group seemed to scarcely participate in the tweezering of Ca^{2+} ions as there was no observable chemical shift change at H_b. This occurrence is in some way expected on the basis of the hard–soft acid base concept. In our previous work, the ionophores that contained softer sulfur atoms in their binding sites were well tweezered for the soft ions, such as Ag^+, Hg^{2+}, and Co^{2+}. However, a relatively minor contribution to complexation is expected between a soft sulfur atom and a relatively harder Ca^{2+} ion. It is surprising that complexation occurred almost exclusively between a Ca^{2+} ion and oxygen atoms of the carbonyl groups of the ionophores, with no noticeable participation of the softer sulfur atom. These results strongly imply that the oxygen atoms in the dithiocarbamoyl and carbamoylpropanamidoacetoxy moieties are largely responsible for the calcium ion recognition. In addition, an increase in the amount of $Ca(SCN)_2$, up to 2 equiv, did not notably influence the chemical shifts of the H_a, H_c, H_d, and H_e peaks for **13a** (Fig. 2c). This indicates that ionophore **13a** forms a 1:1 complex with the Ca^{2+} ion, as shown in Fig. 2 (**13a**–**Ca**$^{2+}$).

CONCLUSION

In this article, we synthesized uniquely designed calcium ion-selective ionophores based on nonsymmetrically substituted tweezer-type ionophores containing a carbamoylpropanamidoacetoxy moiety at the C3-position and dithiocarbamoyl groups at the C12-position of a 7-deoxycholic amide or cholane derivative. ^{1}H NMR titration experiments implied that the synthesized ionophore forms a 1:1 complex with the Ca^{2+} ion using oxygen atoms from the carbonyl groups of the ion-recognizing linkage at the C3- and C12-positions of the neutral ionophore.

EXPERIMENTAL

General Considerations

^{1}H and ^{13}C NMR spectra were recorded at 400 (JEOL, Tokyo, Japan) and 100 MHz, respectively. Chemical shifts are reported in parts per million relative to the residual solvent as an internal standard. High-resolution mass was recorded mostly on a JEOL JMS-DX303 mass spectrometer, and GC/MS spectra were recorded on an Agilent 6890N GC connected to an Agilent 5975 mass selective detector. IR spectra were recorded using an MB104 FTIR (ABB Bomem, Inc., Zurich, Switzerland). Most of the chemical reagents were purchased from Sigma Aldrich Co. (St. Louis, Missouri) unless noted otherwise and were used without purification in most cases. Solvents were purchased and dried using normal laboratory techniques. All major products were isolated by flash column chromatography on silica gel (230–400 mesh ATSM, purchased from Merck & Co., Inc. (Whitehouse Station, New Jersey)) using ethyl acetate/hexane cosolvent as the eluent.

Syntheses

General Procedure for the Preparation of 3α-Chloroacetoxy-12α-[[[[(diethylamino)thioxomethyl]thio]acetyl]-oxy]-N,N-dioctyl-5β-cholan-24-amide **6** *and 3α-Chloroacetoxy-12α-[[[[(diethylamino)thioxomethyl]thio]-acetyl]oxy]-5β-cholane* **7**. 12α-[[[[(diethylamino)thioxomethyl]thio]acetyl]oxy]-3α-hydroxy-*N*,*N*-dioctyl-5β-cholan-24-amide (**4**) or 12α-[[[[(diethylamino)thioxomethyl]thio]acetyl]oxy]-3α-hydroxy-5β-cholane (**5**) (0.61 mmol), calcium hydride (130 mg, 3.05 mmol), tetrabutylammonium bromide (25 mg, 0.08 mmol), and toluene (10 mL) were mixed and stirred. Chloroacetyl chloride (533 μL, 6.72 mmol) was slowly added and stirred for 2 h at 90°C. The mixture was filtered through a pad of Celite (5 g) after cooling and was then 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 (200 mL) and washed with saturated $NaHCO_3$(aq) (2 × 200 mL), washed with water (1 × 200 mL), dried over $MgSO_4$, and evaporated in vacuo. The crude product was purified by silica gel chromatography with ethyl acetate/hexane (2:8) as an eluent to yield **6** and ethyl acetate–hexane (1.5:8.5) as an eluent to yield **7** as waxy liquids.

3α-Chloroacetoxy-12α-[[[[(diethylamino)thioxomethyl]thio]acetyl]oxy]-N,N-dioctyl-5β-cholan-24-amide (**6**). Waxy liquid; TLC (silica gel, 35% ethyl acetate/hexane) R_f 0.57; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.13 (br s, 1H, 12β-H), 4.85–4.74 (m, 1H, 3β-H), 4.39 (d, J = 16.5 Hz, 1H), 4.22 (d, J = 16.5 Hz, 1H), 4.10–4.01 (m, 4H), 3.81–3.78 (m, 2H), 3.32–3.17 (m, 4H), 2.35–2.18 (m, 2H), 1.99–0.72 (m, 88H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.3, 172.8, 167.9, 166.8, 77.4, 76.5, 50.1, 49.1, 47.9, 47.5, 46.8, 45.8, 45.2, 41.7, 41.3, 39.2, 35.6, 35.0, 34.7, 34.2, 34.0, 31.9, 31.8, 31.7, 31.4, 30.1, 29.4, 29.3, 29.2, 29.17, 29.14, 27.8, 27.4, 27.0, 26.9, 26.8, 26.4, 25.9, 25.4, 23.4, 22.9, 22.58, 22.57, 17.7, 14.0, 12.6, 12.2, 11.5; IR (CH_2Cl_2) ν_{max} 2937, 2862, 1731, 1641, 1459, 1418, 1270, 1183, 1006 cm^{-1}; HRMS calcd. for $C_{49}H_{85}ClN_2O_5S_2$ *m/z* 880.5588, found 880.5599.

3α-Chloroacetoxy-12α-[[[[(diethylamino)thioxomethyl]thio]acetyl]oxy]-5β-cholane (**7**). Waxy liquid; TLC (silica gel, 15% ethyl acetate/hexane) R_f 0.24; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.13 (br s, 1H, 12β-H), 4.83–4.75 (m, 1H, 3β-H), 4.39 (d, J = 16.4 Hz, 1H), 4.22 (d, J = 16.4 Hz, 1H), 4.10–3.97 (m, 4H), 3.83–3.78 (m, 2H), 2.00–0.72 (m, 52H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.4, 167.9, 166.9, 77.5, 76.5, 50.1, 49.1, 47.6, 46.8, 45.1, 41.8, 41.3, 39.3, 38.1, 35.6, 35.0, 34.7, 34.2, 34.1, 31.9, 27.5, 26.9, 26.4, 25.9, 25.4, 23.5, 22.9, 19.2, 17.8, 14.5, 12.6, 12.2, 11.5; IR (CH_2Cl_2) ν_{max} 2958, 2869, 1753, 1732, 1490, 1418, 1271, 1186, 1009 cm^{-1}; HRMS calcd. for $C_{33}H_{54}ClNO_4S_2$ *m/z* 627.3183, found 627.3179.

General Procedure for the Preparation of 3α-Azidoacetoxy-12α-[[[[(diethylamino)thioxomethyl]thio]acetyl]-oxy]-N,N-dioctyl-5β-cholan-24-amide **8** *and 3α-Azidoacetoxy-12α-[[[[(diethylamino)thioxomethyl]thio]acetyl]-oxy]-5β-cholane* **9**. 3α-Chloroacetoxy-12α-[[[[(diethylamino)thioxomethyl]thio]acetyl]oxy]-*N*,*N*-dioctyl-5β-cholan-24-amide(**6**) or 3α-chloroacetoxy-12α-[[[[(diethylamino)thioxomethyl]thio]acetyl]oxy]-5β-cholane(**7**) (0.59 mmol) and sodium azide (60 mg, 0.88 mmol) were dissolved in DMF (15 mL) and stirred for 12 h at 50°C. The reaction mixture was evaporated in vacuo, diluted with dichloromethane (150 mL), washed with 1 N HCl (1 × 150 mL) and then with water (1 × 150 mL), dried over $MgSO_4$, and evaporated in vacuo.

The crude product was purified by chromatography on silica gel with ethyl acetate–hexane (3:7) as an eluent to yield **8** and ethyl acetate–hexane (1:9) as an eluent to yield **9** as waxy liquids.

*3α-Azidoacetoxy-12α-[[[[(diethylamino)thioxomethyl]thio]acetyl]oxy]-N,N-dioctyl-5β-cholan-24-amide (***8**). Waxy liquid; TLC (silica gel, 35% ethyl acetate/hexane) R_f 0.57; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.13 (br s, 1H, 12β-H), 4.85–4.78 (m, 1H, 3β-H), 4.40 (d, J = 16.6 Hz, 1H), 4.19 (d, J = 16.6 Hz, 1H), 4.11–3.95 (m, 2H), 3.91–3.78 (m, 4H), 3.35–3.14 (m, 4H), 2.36–2.14 (m, 2H), 2.00–0.72 (m, 88H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.4, 172.8, 167.93, 167.86, 77.4, 76.2, 50.5, 50.1, 49.1, 48.0, 47.5, 46.8, 45.8, 45.2, 41.8, 39.2, 35.6, 35.1, 34.7, 34.2, 34.0, 32.0, 31.8, 31.7, 31.4, 30.1, 29.4, 29.3, 29.22, 29.19, 29.16, 27.8, 27.4, 27.0, 26.9, 26.8, 26.5, 25.9, 25.4, 23.5, 22.9, 22.6, 17.8, 14.1, 12.6, 12.2, 11.5; IR (CH_2Cl_2) ν_{max} 2933, 2860, 2106, 1743, 1641, 1465, 1417, 1267, 1201 cm^{-1}; HRMS calcd. for $C_{49}H_{85}N_5O_5S_2$ m/z 887.5992, found 887.6002.

*3α - Azidoacetoxy - 12α - [[[[(diethylamino)thioxomethyl]thio]acetyl]oxy]-5β-cholane (***9**). Waxy liquid; TLC (silica gel, 15% ethyl acetate/hexane) R_f 0.23; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.13 (br s, 1H, 12β-H), 4.86–4.78 (m, 1H, 3β-H), 4.40 (d, J = 16.4 Hz, 1H), 4.20 (d, J = 16.4 Hz, 1H), 4.11–3.96 (m, 2H), 3.91–3.77 (m, 4H), 2.01–0.72 (m, 46H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.5, 167.91, 167.87, 77.5, 76.2, 50.5, 50.1, 49.1, 47.6, 46.8, 45.1, 41.8, 39.2, 38.1, 35.6, 35.0, 34.7, 34.2, 34.1, 32.0, 27.5, 26.8, 26.5, 25.9, 25.4, 23.5, 22.9, 19.2, 17.8, 14.5, 12.6, 12.2, 11.5; IR (CH_2Cl_2) ν_{max} 2953, 2869, 2107, 1745, 1492, 1418, 1355, 1267, 1204 cm^{-1}; HRMS calcd. for $C_{33}H_{54}N_4O_4S_2$ *m/z* 634.3587, found 634.3570.

General Procedure for the Preparation of 3α-Aminoacetoxy - 12α - [[[[(diethylamino)thioxomethyl]thio]acetyl]-oxy]-N,N-dioctyl-5β-cholan-24-amide **10** *and 3α-Aminoacetoxy-12α-[[[[(diethylamino)thioxomethyl]thio] - acetyl]oxy]-5β-cholane* **11**. 3α-Azidoacetoxy - 12α-[[[[(diethylamino)thioxomethyl]thio]acetyl]oxy]-*N*,*N*-dioctyl - 5β - cholan - 24-amide (**8**) or 3α-azidoacetoxy-12α-[[[[(diethylamino)-thioxomethyl]thio]acetyl]oxy]-5β-cholane (**9**) (0.56 mmol) and nanosized Zn powder (500 mg, 7.61 mmol) were dissolved in glacial acetic acid (20 mL) and stirred for 24 h at room temperature. The reaction mixture was filtered through a pad of Celite (5 g) and washed with dichloromethane (200 mL). The crude product was purified by chromatography on silica gel with NH_4OH/MeOH/CH_2Cl_2 (0.1:1:15) as an eluent to yield **10** and NH_4OH/MeOH/CH_2Cl_2 (0.2:2:15) as an eluent to yield **11** as waxy liquids.

*N,N - Dioctyl - 3α-aminoacetoxy-12α-(N,N-diethylthiocarbamoylsulfanylacetoxy) - 5β - cholan - 24 - amide (***10**). Waxy liquid; TLC (silica gel, NH_4OH:MeOH:CH_2Cl_2 = 0.1:1:15) R_f 0.43. Its NMR spectrum could not be verified due to its instability. The identity was confirmed after the next step reaction.

*3α - Aminoacetoxy - 12α-(N,N-diethylthiocarbamoylsulfanylacetoxy)-5β-cholane (***11**). Waxy liquid; TLC (silica gel, NH_4OH:MeOH:CH_2Cl_2 = 0.1:1:15) R_f 0.33. Its NMR spectrum could not be verified due to its instability. The identity was confirmed after the next step reaction.

General Procedure for the Preparation of α-[[[[4-(Di(alkyl, aryl)amino)-1,4-dioxobutyl]amino]acetyl]oxy]-12α-[[[[(diethylamino)thioxomethyl]thio]acetyl]-oxy]-N,N-dioctyl-5β-cholan-24-amides **12a, 12b,** *and* **12c**. *N*, *N*-Di(alkyl, aryl)carbamoylpr opanoic acid (0.79 mmol) and 1-hydroxybenzotriazole hydrate (HOBt) (110 mg, 0.79 mmol) were dissolved in dichloromethane (10 mL), DMF (1 mL) at room temperature, which was stirred for 10 min. EDC (150 mg, 0.79 mmol) was added to the reaction mixture and was stirred for 30 min. 3α-Aminoacetoxy-12α-[[[[(diethylamino)thioxo-methyl]thio]acetyl]-oxy]-*N*,*N*-dioctyl-5β-cholan-24-amide (**10,** 140 mg, 0.16 mmol) was dissolved in dichloromethane (10 mL), slowly added to the reaction mixture at 0°C and stirred for 48 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and was washed with saturated $NaHCO_3$(aq) (1 × 100 mL), washed with water (1 × 100 mL), dried over $MgSO_4$, and evaporated in vacuo. The crude product was purified by silica gel chromatography with ethyl acetate as an eluent to yield **12a** and ethyl acetate–hexane (6:4) as an eluent to yield **12b** or **12c** as waxy solid.

*3α - [[[[4 - (Diethylamino) - 1,4 - dioxobutyl]amino]acetyl] oxy] - 12α - [[[[(diethylamino) thioxomethyl]thio]-acetyl]oxy] - N,N - dioctyl-5β-cholan-24-amide (***12a**). Waxy solid; TLC (silica gel, 20% acetone/CH_2Cl_2) R_f 0.45; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.67 (t, J = 5.0 Hz, 1H), 5.13 (br s, 1H, 12β-H), 4.81–4.74 (m, 1H, 3β-H), 4.38 (d, J = 16.5 Hz, 1H), 4.22 (d, J = 16.5 Hz, 1H), 4.06–3.95 (m, 4H), 3.86–3.75 (m, 2H), 3.40–3.16 (m, 8H), 2.69–2.59 (m, 4H), 2.36–0.72 (m, 84H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.4, 172.8, 172.7, 170.8, 169.4, 168.0, 77.3, 75.6, 50.2, 49.1, 48.0, 47.5, 46.9, 45.9, 45.2, 41.89, 41.84, 41.75, 40.4, 39.2, 35.7, 35.1, 34.8, 34.3, 34.1, 32.1, 31.83, 31.79, 31.5, 31.3, 30.1, 29.43, 29.38, 29.28, 29.25, 29.22, 28.6, 27.8, 27.4, 27.1, 27.0, 26.9, 26.6, 25.9, 25.5, 23.5, 23.0, 17.8, 14.1, 13.1, 12.7,

12.3, 11.5; IR (CH_2Cl_2) ν_{max} 3450, 2926, 2860, 1742, 1724, 1636, 1463, 1418, 1271, 1203, 1143 cm^{-1}; MS (FAB) *m/e* 1018 (M^+), 581 (M^+-$C_{40}H_{71}NO$).

3α-[[[[4-(Diisobutylamino)-1,4-dioxobutyl]amino]acetyl]oxy]-12α - [[[[(diethylamino)thioxomethyl]thio] - acetyl]oxy] - N,N - dioctyl-5β-cholan-24-amide (**12b**). Waxy solid; TLC (silica gel, 15% acetone/CH_2Cl_2) R_f 0.52; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.67 (t, J = 5.0 Hz, 1H), 5.13 (br s, 1H, 12β-H), 4.80–4.72 (m, 1H, 3β-H), 4.38 (d, J = 16.5 Hz, 1H), 4.22 (d, J = 16.5 Hz, 1H), 4.04–3.98 (m, 4H), 3.84–3.78 (m, 2H), 3.33–3.10 (m, 8H), 2.70 (t,J = 6.3 Hz, 2H), 2.61 (t,J = 6.3 Hz, 2H), 2.33–0.72 (m, 92H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.4, 172.9, 172.7, 172.1, 169.4, 168.0, 77.4, 75.5, 55.3, 53.3, 50.2, 49.1, 48.0, 47.5, 46.8, 45.9, 45.2, 41.8, 41.7, 39.2, 35.7, 35.1, 34.8, 34.1, 32.1, 31.81, 31.77, 31.52, 31.46, 30.1, 29.41, 29.36, 29.25, 29.23, 29.20, 28.9, 27.8, 27.7, 27.4, 27.1, 27.0, 26.9, 26.53, 26.50, 25.9, 25.5, 23.5, 23.0, 22.6, 20.2, 20.1, 17.8, 14.1, 12.7, 12.3, 11.5; IR (CH_2Cl_2) ν_{max} 3323, 2958, 2927, 2865, 1745, 1729, 1636, 1466, 1419, 1272, 1202, 1144 cm^{-1}; MS (FAB) *m/e* 1074 (M^+), 581 (M^+-$C_{40}H_{71}NO$).

3α-[[[[4-(Diphenylamino)-1,4-dioxobutyl]amino]acetyl]oxy]-12α-[[[[(diethylamino)thioxomethyl]thio] - acetyl]oxy] - N,N - dioctyl-5β-cholan-24-amide (**12c**). Waxy solid; TLC (silica gel, 10% acetone/CH_2Cl_2) R_f 0.40; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (br s, 10H), 6.51 (t, J = 5.2 Hz, 1H), 5.13 (br s, 1H, 12β-H), 4.81–4.73 (m, 1H, 3β-H), 4.39 (d, J = 16.5 Hz, 1H), 4.22 (d, J = 16.5 Hz, 1H), 4.03–3.93 (m, 4H), 3.81–3.79 (m, 2H), 3.35–3.18 (m, 4H), 2.59 (s, 4H), 2.36–0.72 (m, 85H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.3, 172.8, 172.3, 172.0, 169.3, 167.9, 142.5, 129.7, 128.8, 127.9, 126.4, 77.2, 75.5, 60.3, 50.1, 49.0, 47.9, 47.4, 46.8, 45.8, 45.1, 41.8, 39.1, 35.6, 35.0, 34.7, 34.2, 34.0, 32.0, 31.73, 31.70, 31.4, 31.2, 31.0, 30.0, 29.33, 29.28, 29.18, 29.15, 29.13, 27.7, 27.34, 27.0, 26.9, 26.8, 26.5, 25.8, 25.4, 23.4, 22.9, 22.6, 17.7, 14.1, 14.0, 12.6, 12.2, 11.5; IR (CH_2Cl_2) ν_{max} 3445, 3065, 2931, 2857, 2083, 1722, 1670, 1644, 1488, 1419, 1377, 1358, 1265, 1203, 756, 702 cm^{-1}; MS (FAB) *m/e* 1114 (M^+), 581 (M^+-$C_{40}H_{71}NO$).

General Procedure for the Preparation of 3α-[[[[4-(Di(alkyl, aryl)amino)-1,4-dioxobutyl]amino]acetyl]oxy]- 12α-[[[[(diethylamino)thioxomethyl]thio]acetyl]oxy]-5β-cholanes **13a, 13b,** *and* **13c**. *N*, *N*-Di(alkyl, aryl)carbamoylpropanoic acid (1.78 mmol) and HOBt (240 mg, 1.78 mmol) were dissolved in dichloromethane (10 mL), DMF (1 mL) at room temperature and stirred for 10 min. EDC (340 mg, 1.78 mmol) was added to the reaction mixture and stirred for 30 min. 3α-Aminoacetoxy-12α-[[[[(diethylamino)thioxo - methyl] thio]-acetyl]oxy]-5β-cholane (**11,** 220 mg, 0.36 mmol) was dissolved in dichloromethane (10 mL), slowly added to the reaction mixture at 0°C and stirred for 48 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and was washed with saturated $NaHCO_3$(aq) (1 × 100 mL), washed with water (1 × 100 mL), dried over $MgSO_4$, and evaporated in vacuo. The crude product was purified by silica gel chromatography with ethyl acetate as an eluent to yield **13a** and ethyl acetate–hexane (6:4) as an eluent to yield **13b** and **13c** as a waxy solid.

3α-[[[[4-(Diethylamino)-1,4-dioxobutyl]amino]acetyl] oxy] - 12α - [[[[(diethylamino) thioxomethyl]thio]-acetyl]oxy]-5β-cholane (**13a**). Waxy solid; TLC (silica gel, 20% acetone/CH_2Cl_2) R_f 0.44; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.78 (t, J = 5.2 Hz, 1H), 5.13 (br s, 1H, 12β-H), 4.81–4.73 (m, 1H, 3β-H), 4.39 (d, J = 16.4 Hz, 1H), 4.21 (d, J = 16.4 Hz, 1H), 4.06–3.94 (m, 4H), 3.81 (m, 2H), 3.40–3.30 (m, 4H), 2.69–2.59 (m, 4H), 1.97–0.71 (m, 56H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.5, 172.7, 170.8, 169.4, 168.0, 77.4, 75.5, 50.2, 49.1, 47.6, 46.8, 45.1, 41.89, 41.86, 41.7, 40.4, 38.1, 35.7, 35.1, 34.8, 34.3, 34.1, 32.1, 31.3, 28.5, 27.5, 26.9, 26.5, 25.9, 25.5, 23.5, 23.0, 19.2, 17.8, 14.6, 14.1, 13.1, 12.7, 12.2, 11.5; IR (CH_2Cl_2) ν_{max} 3379, 2955, 2936, 2872, 1741, 1673, 1626, 1543, 1487, 1420, 1270, 1203 cm^{-1}; HRMS calcd. for $C_{41}H_{69}N_3O_6S_2$ *m/e* 763.4628, found 763.4627.

3α-[[[[4-(Diisobytylamino)-1,4-dioxobutyl]amino]acetyl]oxy]-12α-[[[[(diethylamino)thioxomethyl]thio]-acetyl]oxy]-5β-cholane (**13b**). Waxy solid; TLC (silica gel, 15% acetone/CH_2Cl_2) R_f 0.53; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.62 (t, J = 5.0 Hz, 1H), 5.13 (br s, 1H, 12β-H), 4.80–4.72 (m, 1H, 3β-H), 4.39 (d, J = 16.4 Hz, 1H), 4.21 (d, J = 16.4 Hz, 1H), 4.07–3.94 (m, 4H), 3.81 (m, 2H), 3.19 (d, 2H), 3.11 (d, 2H), 2.70 (t, 2H), 2.60 (t, 2H), 2.05–0.71 (m, 64H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.5, 172.7, 172.0, 169.4, 168.0, 77.5, 75.6, 55.3, 53.3, 50.2, 49.1, 47.6, 46.8, 45.1, 41.9, 41.7, 39.3, 38.1, 35.7, 35.1, 34.8, 34.3, 34.1, 32.1, 31.6, 29.0, 27.7, 27.5, 26.9, 26.53, 26.51, 25.9, 25.5, 23.5, 23.0, 20.2, 20.1, 19.2, 17.8, 14.6, 12.7, 12.2, 11.5; IR (CH_2Cl_2) ν_{max} 3322, 2961, 2931, 2871, 1744, 1676, 1638, 1544, 1420, 1269, 1203, 1146 cm^{-1}; HRMS calcd. for $C_{45}H_{77}N_3O_6S_2$ *m/e* 819.5254, found 819.5253.

3α-[[[[4-(Diphenylamino)-1,4-dioxobutyl]amino]acetyl]oxy]-12α-[[[[(diethylamino)thioxomethyl]thio]-acetyl]oxy]-5β-cholane (**13c**). Waxy solid; TLC (silica gel, 10% acetone/CH_2Cl_2) R_f 0.45; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (br s, 10H), 6.55 (t, J = 5.1 Hz, 1H), 5.11 (br s, 1H, 12β-H), 4.78–4.71 (m, 1H, 3β-H), 4.38 (d, J = 16.3 Hz, 1H), 4.19 (d, J = 16.3 Hz, 1H), 4.04–3.94 (m, 4H), 3.77 (m, 2H), 2.57 (s, 4H),

1.92–0.69 (m, 49H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.4, 172.3, 172.1, 169.4, 168.0, 142.5, 129.8, 128.8, 128.1, 126.4, 77.5, 75.6, 60.4, 50.2, 49.1, 47.6, 46.8, 45.1, 44.9, 41.8, 41.7, 39.2, 38.1, 35.7, 35.1, 34.8, 34.2, 34.1, 34.0, 32.1, 31.2, 31.0, 27.5, 26.9, 26.5, 25.9, 25.4, 23.5, 22.9, 21.0, 19.2, 17.8, 14.6, 14.2, 12.7, 12.2, 11.5; IR (CH_2Cl_2) ν_{max} 3341, 3061, 2955, 2936, 2871, 1951, 1890, 1871, 1739, 1667, 1493, 1269, 1205, 739, 699 cm^{-1}; HRMS calcd. for $C_{49}H_{69}N_3O_6S_2$ *m/e* 859.4628, found 859.4614.

ACKNOWLEDGMENT

This work was supported by Kwangwoon University in 2010.

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