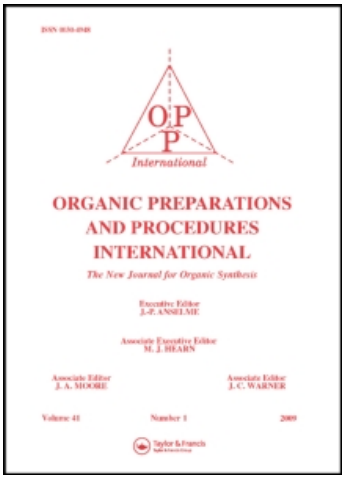

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SELECTIVE PROTECTION OF THE VARIOUS HYDROXY GROUPS OF CHOLIC ACID AND DERIVATIVES. A REVIEW

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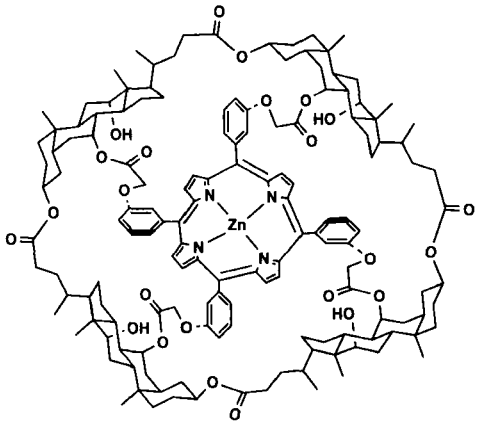
INTRODUCTION

Bile acids are naturally occurring and readily available natural compounds that play an important physiological role in all mammals.[1] The terminal carboxylic acid in C17-side chain can conjugated with taurine or glycine.[2-5] The direct coupling of cholic acid to both terminal amino groups of spermidine, a biogenic polyamine essential for tissue growth, yields a molecular system capable of functioning like a mechanical umbrella.[6] The structural rigidity of this steroid class of compounds and their amphiphilic properties, chirality, and the orientation of their hydroxyl groups toward the center of a concave face have made them interesting starting materials for the synthesis of a molecular bowl (*Figure 1*),[7-8] molecular tweezers,[9] crown ethers,[10-11] dimeric and oligomeric esters,[12] colaphanes,[13-16] cyclopeptides.[17]

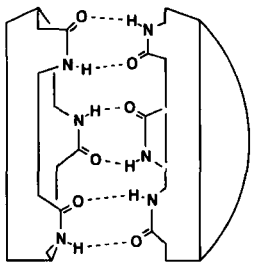
The utility of the bile acids derives not only because of their biological significance[18-19] but also because they are readily available, making them attractive starting materials for the production of other steroids.[20-24] Cholic acid is particularly inexpensive and useful. In the course of work on the use of cholic acid as a building-block for functionalized macrocyclic host molecules,[25] we have investigated a number of methods for selective protection of its various hydroxy groups. We now review some of these methods.

I. PROTECTION OF HYDROXYL GROUPS WITH ACETATE

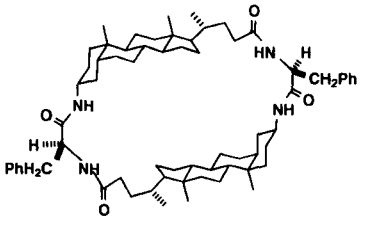
In the synthesis of bile acid derivatives, bile acids protected at hydroxyl groups are used very frequently. During 1960s, they have been used for the synthesis of C-24 labeled bile acids,[26-27] C-27 bile alcohols,[28-29] and norchol-22-enes.[30] In last decade, they have been used for the synthesis of cyclocholate derivatives.[12] The acetyl protecting group has generally been more widely used than other protecting groups because it is easily introduced and removed and possesses an acceptable stability under various conditions.



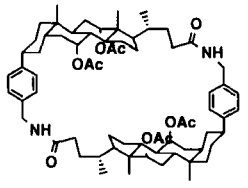
Molecular Bowl



Molecular Cylinder

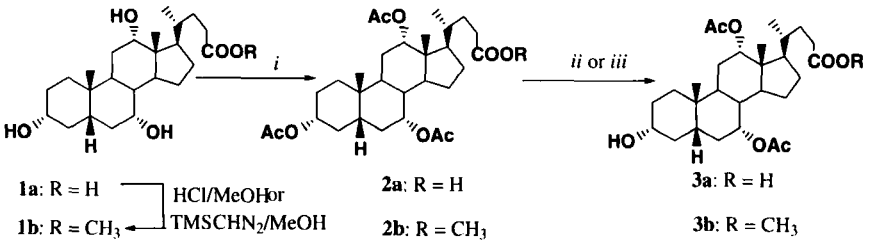


Cyclopeptide



Cyclophane

Fig. 1



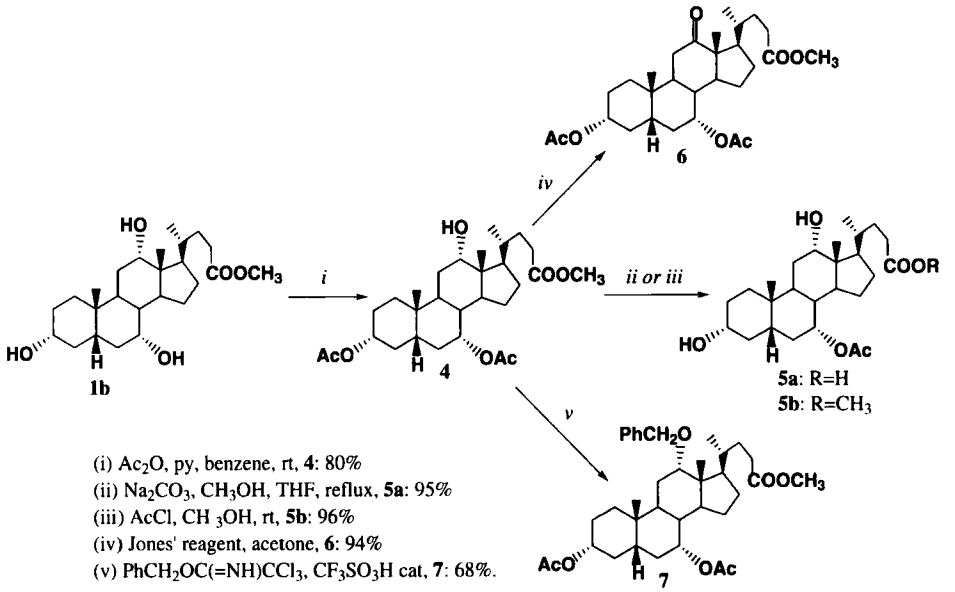
(i) Ac_2O, DMAP, py, rt, **2a**: 86% (ii) Na_2CO_3, CH_3OH, THF, reflux, **3a**: 95%
(iii) AcCl, CH_3OH, **3b**: 96% or K_2CO_3, CH_3OH, rt, **3b**: 99%

Scheme 1

Methyl cholate (**1b**) in *Scheme 1* can be prepared from cholic acid (**1a**) by reacting with HCl/MeOH. In 1981, Hashimoto and co-workers[31] reported that trimethylsilyldiazomethane ($TMSCHN_2$) reacts with carboxylic acids in the presence of methanol rapidly to give methyl esters in quantitative yield at room temperature. This latter method has the advantage over HCl/MeOH in that acetyl protective groups of the 3α,7α,12α-OH are preserved. The $TMSCHN_2$ method can be applied efficiently in analytical work such as the determination of carboxylic acids by gas chromatography. Triacetylcholic acid (**2a**) was first prepared by acetic anhydride: acetic acid (2:3) containing a drop of perchloric acid.[32] In 1993, Amiet and co-workers[33] reported the preparation of **2a** by refluxing a

mixture of cholic acid with excess acetic anhydride in pyridine. These conditions were required for complete acetylation of the sterically crowded, axial C-12 hydroxy group. Recently, the reaction has been carried out in acetic anhydride with pyridine as the solvent using dimethylaminopyridine (DMAP) a catalyst (*Scheme 1*).[34] Methyl triacetoxycholanoate (**2b**) was synthesized by same method.[35] This reaction has also been reported when pyridine was replaced by methylene chloride.[13] Because the rate of hydrolysis of three acetate groups in decreasing order is 3α>>7α>12α (*vida infra*), selective removal of the 3a-acetoxy group can be achieved in two different ways. Methanolic HCl method produced compound **3b** in good yield (96%).[36] A more recent method using a mild base (K_2CO_3/CH_3OH) is also effective (99%).[37] Application of a Na_2CO_3/CH_3OH/ THF system under reflux conditions has been applied to remove both the 24-methyl and 3α-acetyl groups to give **3a**.[38]

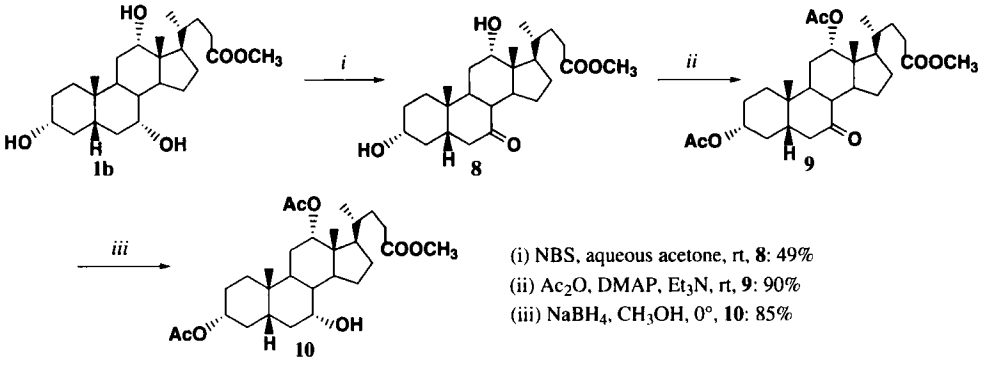
It has been shown that selective acetylation of the 3α- and 7α-hydroxy groups in cholic acid can be achieved by treatment with acetic anhydride and pyridine diluted in benzene at room temperature[39] and that the sterically hindered 12a-hydroxy group activates acetylation at the more sterically hindered 7α-hydroxy by transannular participation (*Scheme 2*).[40-41] Also, Fieser and Rajagopalan[39]



Scheme 2

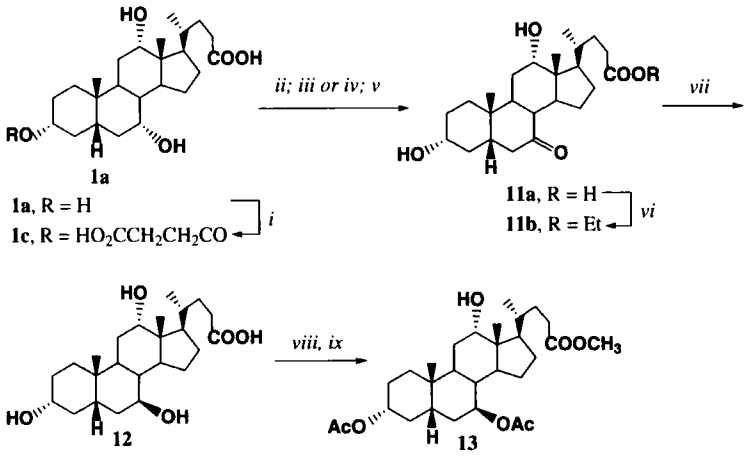
employed absolute CH_3OH through which dry gaseous HCl was passed for selective methanolysis of 3α-acetoxy group in **4** to give **5b**. The previously mentioned sodium carbonate method was also useful to convert **4** to **5a**.[38] Oxidation of **4** with potassium chromate[39,42] afforded methyl 3α,7α-diacetoxy-12-oxo-5β-cholan-24-oate **6**. Bonar-Law and co-workers[15] successfully benzylated the diacetate **4** employing benzyl trichloroacetimidate and trifluoromethanesulfonic acid.[43] The other attempts using (a) sodium hydride, benzyl bromide and tetrabutylammonium iodide in THF,[44] (b) benzyl bromide and silver oxide in DMF[45] and (c) hexabutyldistannoxane followed by benzyl bromide and tetrabutylammonium bromide[46]all failed to give the desired product **7**.

3,12-Diacetate **10** was synthesized from methyl cholate in three steps (overall yield 36%) as shown in *Scheme 3*. In the first step, the C-7 hydroxy group was protected as the corresponding ketone *via* selective oxidation.[39, 42] Ketoester **8** was acetylated at the C-3 and C-12 hydroxyls with Ac_2O/Et_3N/DMAP at room temperature to afford diacetoxyketo derivative **9** in 90% yield.[47-48]The reduction of ketoester **9** with $NaBH_4$ in MeOH at 0° (or with benzyltriethylammonium borohydride in refluxing CH_2Cl_2) regenerated the required 7α-OH group, affording alcohol **10** in 85% yield.[37]



Scheme 3

As part of a report in the synthesis of triamino methyl cholanoate, Davis and co-workers[49] reported the synthesis of 7-epi-cholic acid derivatives. Haslewood was the first to describe the partial oxidation of cholic acid (**1a**) at C_7 by addition of aqueous chromate to cholic acid in acetic acid buffered with sodium acetate (*Scheme 4*).[50] The oxidation of cholic acid with N-bromosuccinimide in

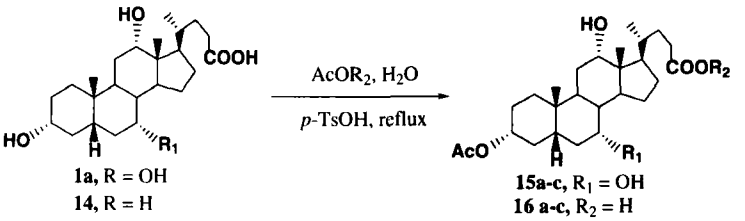


(i) succinic anhydride, py, CCl_4, reflux, 3h, **1c**: 91% (ii) K_2CrO_4, NaOAc, HOAc, 24h, rt, **11a**: 45%
(iii) NBA, acetone, H_2O, rt, 3h, **11a**: 57% (iv) NBS, 3% $NaHCO_3$, 70°, 1h, rt, overnight; 5% NaOH/MeOH, **11a**: 100%
(v) Br_2, NaOH, $NaHCO_3$, 2 days, rt (vi) EtOH, H_2SO_4, rt, overnight, **11b**: 41% (vii) K, *tert*-amyl alcohol
(viii) MeOH, H_2SO_4; (ix) Ac_2O, py, rt, 8h

Scheme 4

aqueous acetone described by Fieser and Rajagopalan[51] gave 7-oxo-3α,12α-dihydroxy-5β-cholanoic acid (**11a**) in 57% yield. Bromine oxidation of cholic acid followed by esterification with ethanol was reported by Hoehn and co-workers.[52] Subsequently, Batta and co-workers prepared **11a** from cholic acid in over 80% yield by the following modified route.[53] Cholic acid was first selectively protected by esterification with succinic anhydride at 3a-position to give compound **1c** which was selectively oxidized by N-bromosuccinimide in aqueous sodium bicarbonate. Finally, the hydrolysis was accomplished by 5% methanolic sodium hydroxide. This efficient procedure was confirmed by Davis and co-workers.[49] Reduction with potassium in *tert*-amyl alcohol yielded 7-epicholic acid **12** along with a trace of **1a**.[54] Esterification with methanol, followed by selective acetylation of the equatorial 3b,7b-hydroxyl groups, gave diacetates **13**.

Cholic acid and deoxycholic acid can be converted to ethyl 3α-acetoxy derivatives in a one-pot by transesterification using EtOAc and *p*-TsOH (*Scheme 5*).[55] The success of these methods results from lower steric hindrance associated with 3α-OH compared to the 7α-OH and 12α-OH groups.



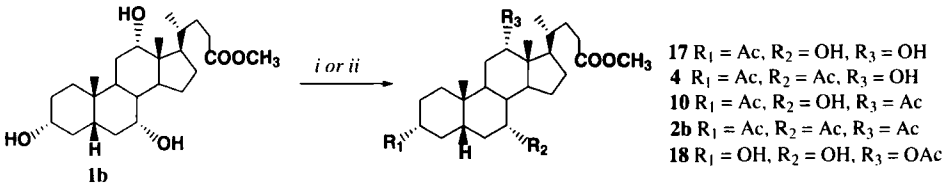
1a, R = OH
14, R = H

15a-c, R_1 = OH
16 a-c, R_2 = H

15a (R_2 = Et): 87%; **15b** (R_2 = *n*-Pr): 87%; **15c** (R_2 = *n*-Bu): 87%
16a (R_2 = Et): 87%; **16b** (R_2 = *n*-Pr): 87%; **16c** (R_2 = *n*-Bu): 87%

Scheme 5

Schwartz and coworkers[56] reported that treatment of compound **1b** with Ac_2O in toluene, with KOAc as base, resulted in preferential 3,12-diacetylation (**17**:**4**:**10**:**2b** = 10.3:7.6:51:26.2). Diacetate **10** was isolated in 33% yield (*Scheme 6*).



1b

17 R_1 = Ac, R_2 = OH, R_3 = OH
4 R_1 = Ac, R_2 = Ac, R_3 = OH
10 R_1 = Ac, R_2 = OH, R_3 = Ac
2b R_1 = Ac, R_2 = Ac, R_3 = Ac
18 R_1 = OH, R_2 = OH, R_3 = OAc

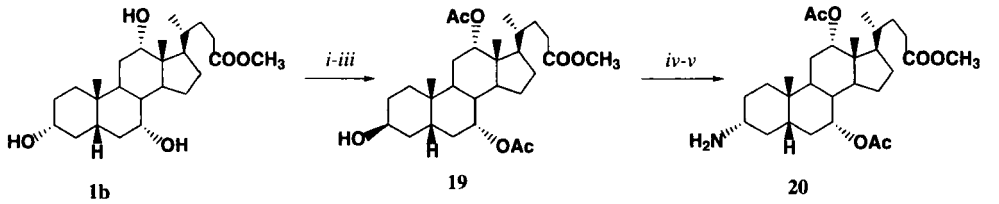
(i) Ac_2O, toluene, KOAc, rt, **17**: **4**: **10**: **2b** = 10.3: 7.6: 51: 26.2, **10**: 33%
(ii) Ac_2O, 1 month, rt, **17**: **4**: **10**: **2b** = 0: 26: 64: 10, then AcCl, MeOH, **18**: 29%

Scheme 6

Long-term equilibration conditions by Bonar-Law and co-workers[57] were aimed at an improved procedure for 3,12-diacetylation (**17**:**4**:**10**:**2b** = 0:26:64:10). The treatment of the crude product mixtures with MeOH/HCl (generated from MeOH/AcCl) not only led to deacetylation to give 3α-OH, but also showed some selectivity in favor of deacetylation at position 7. The end result was a proce-

dure for the synthesis of 12-monoacetate (**18**) in 29% yield from methyl cholate.

As part of the report of the synthesis of cyclopeptides, Albert and Feigel[17, 58] reported the conversion of the 3α-OH group of cholic acid to a 3α-amino group with retention of the configuration (*Scheme 7*). Methyl 7,12-diacetoxyl-3β-hydroxy-5β-cholan-24-oate (**19**) was prepared by three steps: 1) nucleophilic substitution by the formate group with inversion of the 3a-OH group activated with the



1b **19** **20**

(i) Ph_3P, DEAD, HCOOH; (ii) Ac_2O, py; (iii) CH_3ONa/CH_3OH, **19**: 70-80%

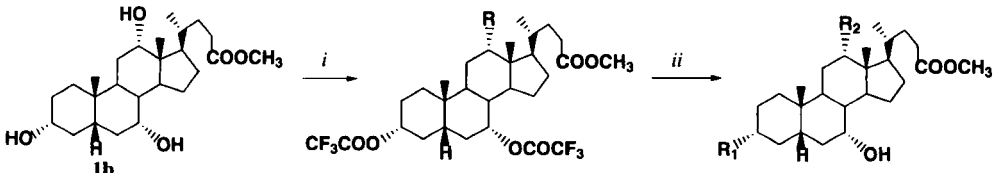
(iv) Ph_3P, DEAD, $Zn(N_3)_2.2py$; (v) $HSCH_2CH_2CH_2SH$, $NaCNBH_3$, isopropanol, **20**: 64%

Scheme 7

Ph_3P/DEAD (diethyl azodicarboxylate)-system; 2) acetylation of 7α,12α-dihydroxy groups was carried out in acetic anhydride-pyridine system; 3) The 3b-formyl group was cleaved to the 3β-hydroxy with 10% sodium methoxide in methanol. The configurational homogeneity was established by the different chemical shifts [($\delta(H3_{eq})$ = 4.10 ppm; $\delta(H3_{ax})$ = 3.64 ppm)] and different coupling constants ($J(H3_{eq})$: wide triplet of triplets; $\delta(H3_{ax})$: narrow multiplet). The return to the initial configuration was achieved with a second Mitsunobu substitution using azide as nucleophile. The azide was reduced with $NaCNBH_3$ and 1,3-propanedithiol in isopropanol to give the corresponding amine **20**.

II. PROTECTION OF HYDROXYL GROUPS WITH TRIFLUOROACETATES

Methyl cholate **1b** was treated with excess TFAA in THF. The two *bis*trifluoroacetates (**21:22** = 1:8) proved difficult to separate (*Scheme 8*).[57] However, after treatment with MeOH/HCl, the



1b

21 R = OH
22 R = CF_3COO

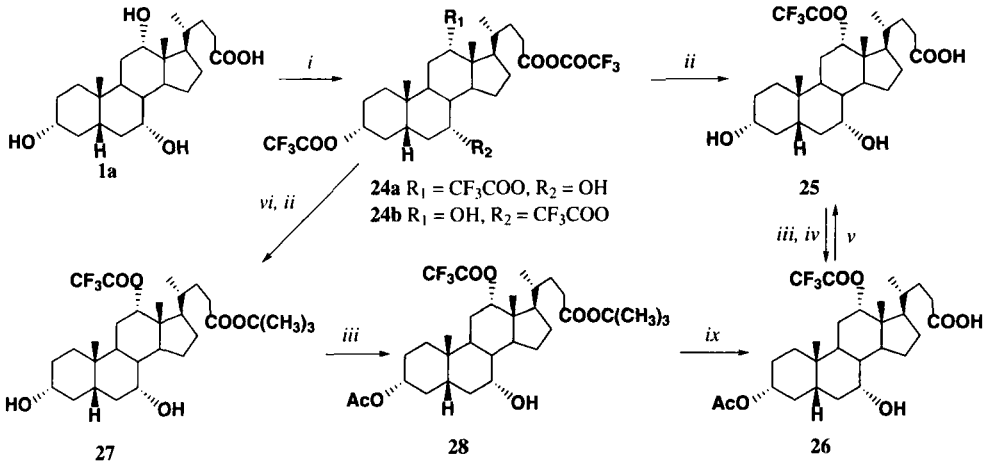
23a R_1 = CF_3COO, R_2 = OH
23b R_1 = OH, R_2 = CF_3COO

(i) TFAA, THF, -35 to -40°, **21:22** = 1:8 (ii) AcCl, CH_3OH, 0°, in **23b**: 55% overall yield from **1b**

Scheme 8

crude product was a mixture of the monotrifluoroacetates **23a** and **23b** in a ratio 1:10 from which the 12-protected derivative **23b** could be isolated by crystallization in 55% overall yield. In general, trifluoroacetate ester groups are more prone to hydrolysis than acetate ester. Under the same trifluoroacety-

lation conditions, cholic acid can be converted directly to the 12-trifluoroacetate **25** (*Scheme 9*) without the need for esterification of the carboxyl group. As shown in *Scheme 9*, treatment of cholic

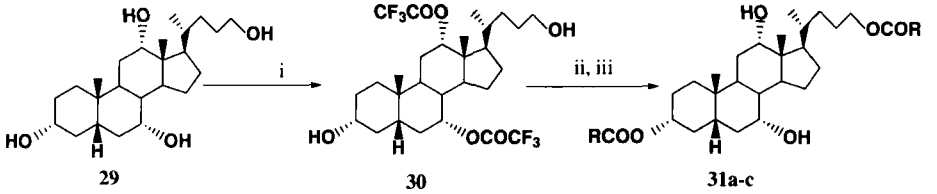


(i) TFAA, THF, **24a** and **24b** (ii) $NaHCO_3$ aq, MeOH, THF **25**: 65% overall yield from **1a**
(iii) Ac_2O, $CHCl_3$, 35-40°, 40h (iv) pH 7 buffer, rt, **26**: 60% overall yield from **25**
(v) HCl aq, MeCN **25**: 67% (vi) *t*-BuOH then (vii) **27**: 81% overall yield from **1a** (ix) TFA **26**: 81%

Scheme 9

acid **1a** with TFAA led to a mixture of partially trifluoroacetylated mixed anhydrides, principally **24a** and **24b**. Aqueous workup, mild base hydrolysis and crystallization gave the 12-trifluoroacetate **25** in 65% yield. Acetylation of **25** with Ac_2O gave **26**. Quenching the mixed anhydrides with *tert*-butanol proved to be an excellent method for *tert*-butyl esterification. As shown in *Scheme 9*, this method allowed the isolation of the 12-protected ester **27** in the highly satisfactory yield of 81%. The trifluoroacetate **27** was selectively acetylated at the 3-position to give the 3,12-diester derivative **28**, and the *t*-butyl ester cleaved with acid to provide the corresponding carboxylic acid in good yield.

During the synthesis of potential DNA binding compounds based on bile steroids, Brown and co-workers[59] first reduced the cholic acid (BH_3/THF or $LiAlH_4$) to the tetrahydroxy steroid **29** in 85% yield (*Scheme 10*). The conversion of **29** to the trifluoroacetate tetraester (trifluoroacetic



(i) $(CF_3CO)_2O$, ether; $MeOH/H_2O$, $NaHCO_3$
(ii) RCOCl, NEt_3, THF; THF/H_2O, NH_3

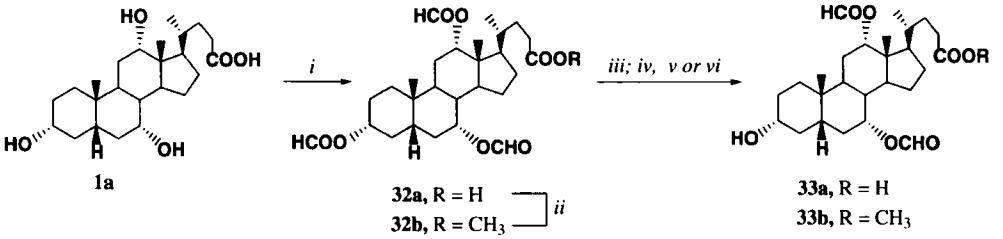
31a R = 
31b R = 
31c R = 

Scheme 10

anhydride/ether), followed by selective removal of the trifluoroacetyl groups at positions 3 and 24 ($MeOH/H_2O/NaHCO_3$) gave the 3,24-diol **30**. Treatment of **30** with 2 equivalents of the appropriate acid chloride (NEt_3/THF) afforded the corresponding diesters **31a-c**. A similar strategy was used to synthesize analogues of brassinosteroids from chenodeoxycholic acid.[60]

III. PROTECTION OF HYDROXYL GROUPS WITH FORMATE

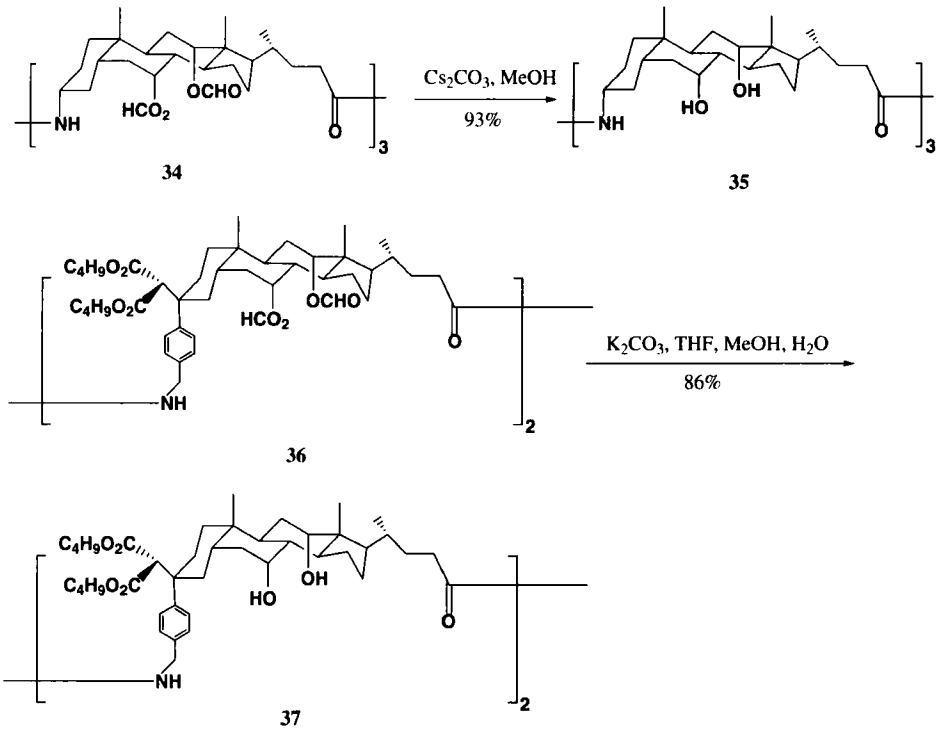
During the synthesis of bile acid acetates, Tserng and Klein found that pure products could not be isolated without column chromatography. However, the synthesis of bile acid formates resulted in products that were crystalline compounds with well-defined melting points.[61] During the synthesis of macrocyclic ìcholaphanesî, Davis and co-workers found that the removal of acetyl protection was troublesome and the formyl groups were more labile.[16] Bile acid formates were originally synthesized by Cortese and Baumann[62-69] and this synthesis was later modified by Hughes et al.[70] Tserng and Klein found these procedures gave impurities which were incompletely formylated bile acids other than the desired performylated bile acids.[61] Attempts to formylate bile acids with acetic-formic anhydride produced a mixture of formate and acetate esters.[71] The water originally present in the formic acid used and that produced during the reaction hindered the quantitative conversion to performylated bile acids. Tserng and Klein found that a catalytic amount of perchloric acid converted most of the bile acids into performylated bile acid **32a** and the addition of acetic anhydride at the final stage shifted the equilibrium toward complete formylation instead of reacting with the free hydroxyl group of the bile acid molecule (*Scheme 11*);[61] later work confirmed these results.[16, 72]



(i) HCOOH, $HClO_4$, **32a**: 95% (ii) CH_2N_2, CH_2Cl_2, MeOH (iii) $NaHCO_3$, MeOH, 0°, **33b**: 92%
(iv) NH_3, CH_3OH, **33a**: *ca*, 100% (v) 0.2 *N* NaOH, water/acetone (vi) $NaOCH_3$, MeOH

Scheme 11

The selective hydrolysis of 3α-formate **32a** using saturated methanolic ammonia, two equivalents of sodium hydroxide in water, or slightly more than one equivalent of sodium methoxide in methanol at room temperature can be achieved in quantitative yield.[61] Esterification of **32a** with diazomethane followed by selective deformylation of 3a-formate gave **33b** in 92% yield.[16] The removal of 7α-formate and 12α-formate groups of cyclocholates can be achieved by $Cs_2CO_3/MeOH$[73-74] or K_2CO_3/THF/MeOH/ H_2O (*Scheme 12*).[16]



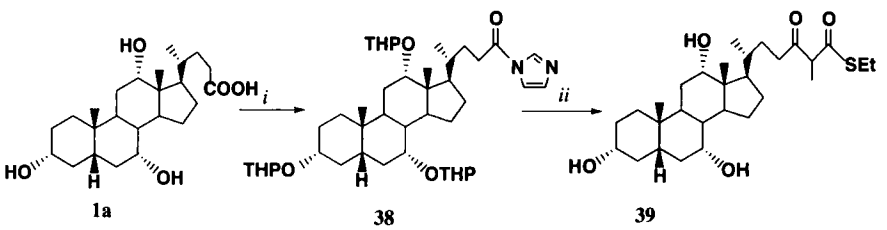
34 35 36 37

Scheme 12

IV. PROTECTION OF HYDROXYL GROUPS WITH OTHER MOIETIES

1. Tetrahydropyranyl (THP) Method

In the course of the synthesis of 24-oxo-3α,7α,12α-trihydroxy-5β-cholestan-26-oyl CoA, a postulated intermediate of bile acid biosynthesis, Korenaga and coworkers[75] employed *tris*-tetrahydropyranyl (THP) ether derivative of cholic acid **1a**. (*Scheme 13*). Treatment of **1a** with 1,1'-carbonyldiimidazole gave the imidazolide **38**, which without isolation, was treated with the magnesium salt of 2-methylmalonic acid ethanethiol half ester. The condensation proceeded well, and the ethanethiol ester of 24-oxo-THCA **39** was obtained in 71% yield after acidic deprotection of THP groups.



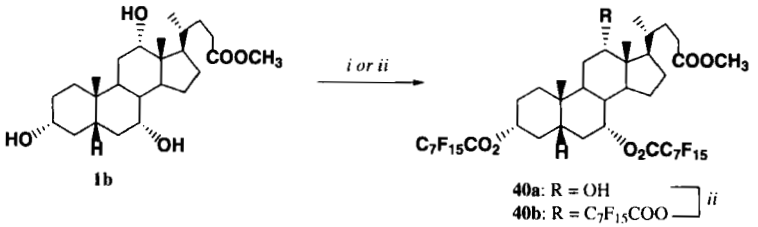
1a 38 39

(i) DHP/H$^+$; CDI (ii) $Mg^{2+}(^-OOCCH(CH_3)COSEt)_2$; H^+

Scheme 13

2. Perfluorooctanoyl Ester Method

In 1987, Malik and Sharts[76] reported the synthesis of a series of mono-, *bis*, and *tris*-perfluorooctanoyloxy derivatives of sterols and bile acids. The reaction of methyl cholate **1b** with a slight excess of perfluorooctanoyl chloride in a 5% solution of triethylamine (TEA) in methylene chloride in the presence of DMAP at room temperature gave the corresponding perfluorooctanoyl esters **40b** in 81% yield (*Scheme 14*). Without DMAP, no reaction at C_{12}-OH was observed. The preparation of



(i) $C_7F_{15}COCl$, TEA, or DMAP, CH_2Cl_2, 25°, **40a**: 78%

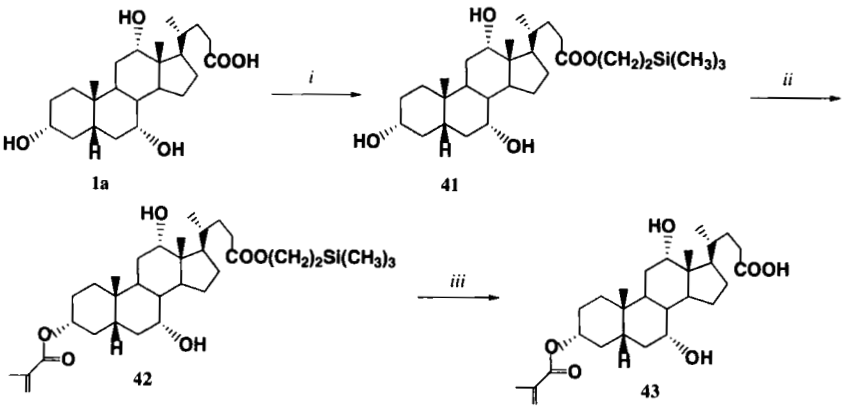
(ii) $C_7F_{15}COCl$, TEA, DMAP, CH_2Cl_2, 25°, **40b**: 81%

Scheme 14

perfluorooctanoyl chloride was done by heating perfluorooctanoic acid in thionyl chloride. The use of perfluorooctanoic acid anhydride would be unacceptably expensive since two moles of perfluorocarboxylic acid are required to give one mole of ester.

3. Methacryloyl Ester Method

In 1992, Ahlheim and Hallensleben described the synthesis of polymerizable derivatives of cholic acid containing a methacrylic group directly attached to C_3 or via a C_{11} cinnamic ester spacer.[77] The carboxylic acid group in cholic acid **1a** was first protected by 2-trimethylsilylethanol to give **41** in 73% yield (*Scheme 15*). After reacting with methacryloyl chloride, tetrabutylammonium fluoride finished the deprotection.

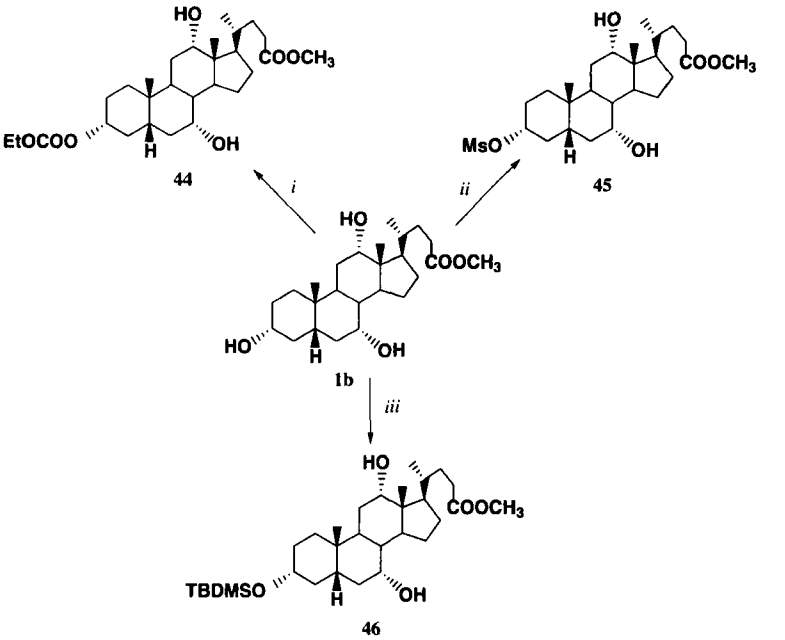


(i) $(CH_3)_3Si(CH_2)_2OH$, *p*-TsOH, **41**: 73% (ii) Et_3N, $CHCl_3$, **42**: 68% (iii) TBAF, $CHCl_3$, **43**: 44%

Scheme 15

4. Carbethoxy Ester Method

Methyl 3-carbethoxycholate (**44**) was synthesized from methyl cholate with EtOCOCl/pyridine at room temperature (*Scheme 16*).[39, 42] Small amounts of by-products, 3,7-dicarbethoxycholate and 3α,7α,12α-tricarbethoxycholate, can be detected using TLC. Hoshita and coworkers first applied the *tert*-butyldimethylsilyl group (*Scheme 16*) to selectively protect 3-OH of cholic acid (58%).[78] Sander



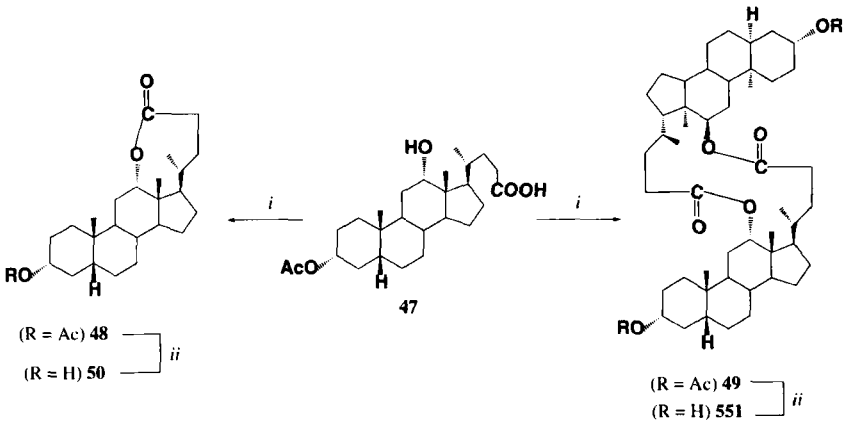
(i) EtOCOCl, py, rt, **44**: 80% (ii) MsCl, py, rt, **45**: 100% (iii) TBDMSCl, Et_3N, DMAP, rt, **46**: 89%

Scheme 16

and coworkers successfully repeated this method on similar compounds with better yield (89%).[7] Schmitt and coworkers synthesized **45** in quantitative yield by treating methyl cholate with 1.2 equivalent of methanesulfonyl chloride in pyridine at 0° for 30 min and at room temperature for 2 h.[79]

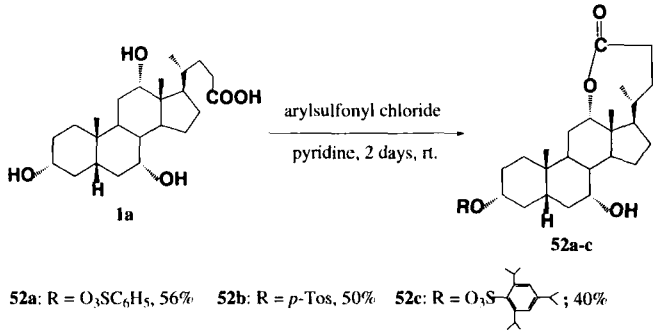
5. Intramolecular Cyclization

In our study of the synthesis of cyclocholates, it was observed that when the cyclization was carried out on deoxycholic acid derivatives having an unprotected 12α-OH group, lactone **48** (*Scheme 17*) was the major product.[38] The facile formation of this lactone system offers a way to selectively protect the 12α-OH group in cholic acid. According to these studies, the previously reported results on the formation of *bis*-lactone from 3α-acetoxy-12α-hydroxy-5β-cholanoic acid (**47**) is questionable (*Scheme 17*).[80] The reported MS data (M^+: 416) supports our suspicions.[80] After the hydrolysis of the intermediate **49**, compound **51** showed the same data (^1H NMR, ^{13}C NMR and MS) as compound **50** which was synthesized independently. Upon standing at room temperature for 2 days, a mixture of arylsulfonyl chloride and cholic acid in pyridine solution gave 7α-hydroxy-3α-arylsulfonyloxy-5β-cholanoic-24,12-lactone (*Scheme 18*).[81] This is the reason why 12α-OH needs to be protected in the macrolactonization of cholic acid derivatives to cyclocholates.



(i) $Cl_2P(O)OEt$, $(Et)_3N$, CH_2Cl_2, 88h, rt, 60% (ii) $NaOCH_3$, MeOH, benzene, 1h, rt, 83%

Scheme 17



52a: R = $O_3SC_6H_5$, 56% **52b**: R = *p*-Tos, 50% **52c**: R = O_3S-; 40%

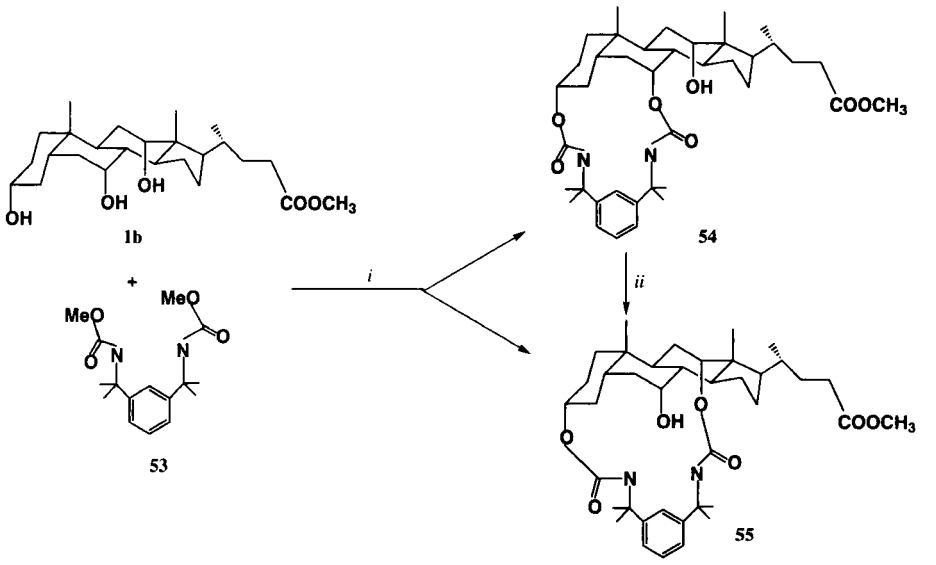
Scheme 18

6. a,a,a',a'-Tetramethyl-*m*-xylylenedicarbamate Transesterification

A facile regioselective macrocyclization of methyl cholate (**1b**) by transesterification using α,α,α',α'-teramethyl-*m*-xylylenedicarbamate **53** by was reported by Yamada and coworkers.[82] This double transesterification was performed in refluxing toluene using 3% $SnCl_2$ and 1% glycoluril (acetyleneurea) as additives (*Scheme 19*). After 2h, the kinetically favored product **54** was obtained selectively in excellent yield (94%), and **55** was not detected. After establishment of equilibrium (7 days), the thermodynamically favored cyclophane **55** was obtained as a major product in yield (68%). The conversion of the cyclophane **54** to **55** was performed in refluxing toluene, using an excess amount of methanol as a promoter.

7. Nitrate Ester Method

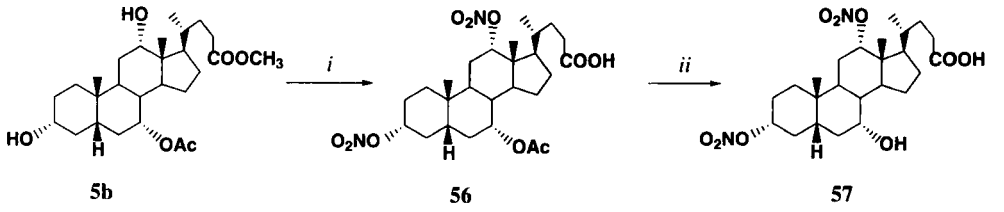
In the course of synthesis of the B-ring 5b-enone steroid skeleton characteristic of the insect moulting hormone ecdysone from cholic acid, the methyl ester 7a-acetyl derivative **5b** (*Scheme 2*) was nitrated at the 3α- and 12α-OH groups using conditions previously described.[83, 84] Saponification restored the 7α-OH and 24-oic acid groups while leaving the 3α- and 12α-nitrate groups intact leading



(i) $SnCl_2$, glycoluril, toluene, reflux, 2h, **54**: 94%; $SnCl_2$, glycolril, toluene, reflux, 7 days, **55**: 68%
(ii) $SnCl_2$, glycoluril, MeOH, reflux, 2 days, **55**: 14%

Scheme 19

to **57** (*Scheme 20*). The nitrate groups could selectively be converted back to OH groups with Zn dust/glacial HOAc reduction in the presence of a variety of other functional groups.[84]

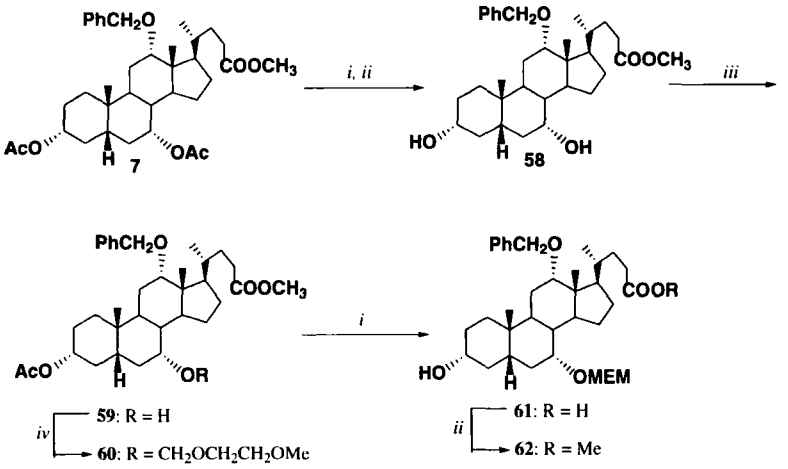


(i) HNO_3, Ac_2O, $CHCl_3$, -5°, 1h, **56**: 89% (ii) KOH/CH_3OH, reflux, **57**: 95%

Scheme 20

8. Benzyl Ether Method

In the study of thermodynamically-controlled cyclization and interconversion of oligocholates, Brady and Sanders applied a series of benzyl derivatives to protect 7α- and 12α-hydroxy groups.[85] Two acetyl protecting groups and the methyl ester in **7** were removed under basic conditions (*Scheme 21*). The methyl ester was regenerated by stirring in methanolic HCl. The benzyl ether **58** was next acetylated at 3a-position with acetic anhydride and pyridine to give the monoacetate **59** in 88% yield; this monoacetylation was made possible because the 12α-benzyl ether does not assist the acetylation of the 7α-OH group.[40-41] Compound **59** was then alkylated with MEM chloride and base to form MEM ether **60** in 85% yield. After basic hydrolysis of the 3a-acetyl and the methyl ester groups,

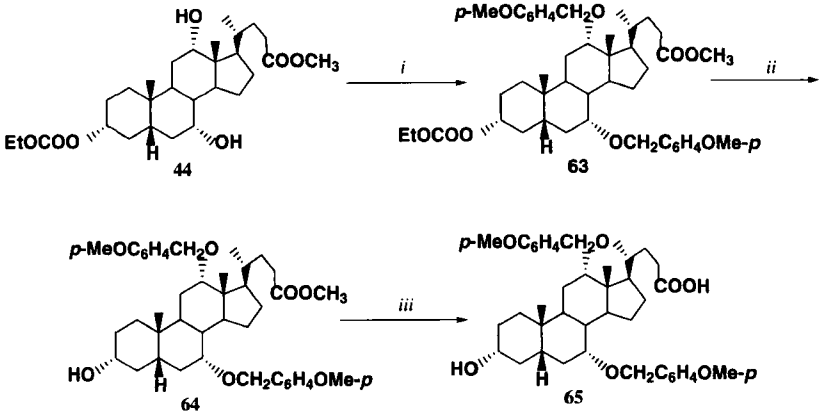


(i) NaOH, THF, reflux, 14h (ii) HCl/MeOH, rt., 12h, **58**: 60% for two steps.
(iii) Ac_2O, py, rt, 48h, **59**: 88% (iv) MEMCl, *i*-Pr_2EtN, CH_2Cl_2, 50°, 14h, **60**: 85%

Scheme 21

the methyl ester was regenerated to yield the methyl 3α-hydroxy-7α-(2-methoxyethoxymethyloxy)-12α-benzyloxycholanoate **62** in 52% overall yield from **58**.

The '*bis*(*p*-methoxybenzyl)' monomer **65** was synthesized as a control for the'MEM' monomer because it has a different UV spectrum and no metal-binding ability (*Scheme 22*). The



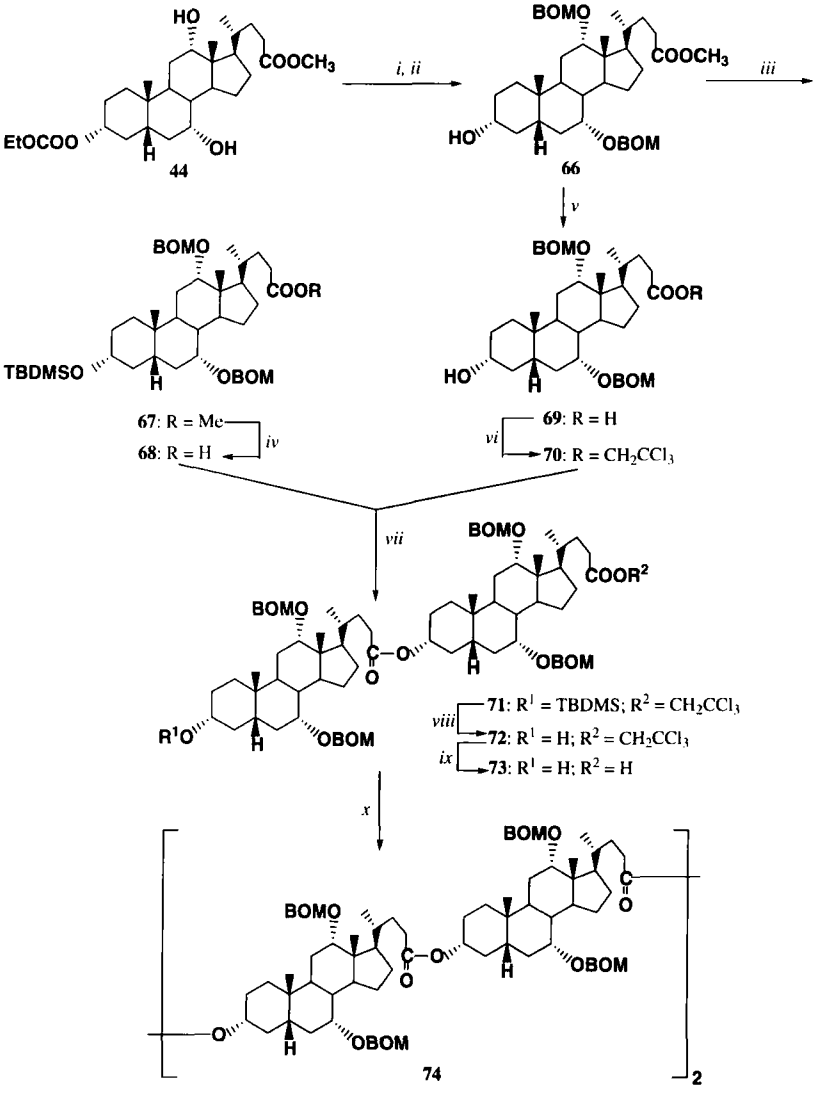
(i) *p*-methoxybenzyl 2,2,2-trichloroacetimidate, camphorsulfonic acid, 40°, 2.5h, **63**: 74%
(ii) KOMe, THF, rt., 2h, **64**: 81% (iii) NaOH, THF, MeOH, reflux, 4h, **65**: 93%

Scheme 22

p-methoxybenzyl group was introduced by similar trichloroacetimidate methodology as used for the benzyl group. The *p*-methoxybenzyl 2,2,2-trichloroacetimidate was synthesized from *p*-methoxybenzyl alcohol and trichloroacetonitrile by method described by Cramer *et al.*[86] As found by Yonemitsu *et. al.*,[87] a weaker acid is required for the addition of this reagent to hydroxy groups. Catalysis with 5 mol% camphorsulfonic acid gave the dibenzylated product **63** in 74% yield. Two successive deprotec-

tion steps furnished 3α-hydroxy-7α,12α-*bis*(*p*-methoxybenzyloxy)cholanoic acid **65** in 54% overall yield from **44**.

In order to synthesize the '*bis*(BOM)' monomer **66**, **44** was alkylated with (benzyloxymethoxy)methyl chloride and Hünig's base according to the procedure of Stork and Isobe (*Scheme 23*).[88] The dialkylated product was deprotected at the 3α-position by addition of methanolic



(i) BOMCl, *i*-Pr_2NEt, toluene, 90°, 19h (ii) KOMe, THF, rt, 1h, **66**: 58% for two steps
(iii) TBDMSCl, DMAP, Et_3N, CH_2Cl_2, 20h, **67**: 63% (iv) NaOH, THF, MeOH, 50°, 2h, **68**: 87%
(v) NaOH, THF, MeOH, reflux, 90 min, **69** (vi) $HOCH_2CCl_3$, DCBC, DMAP, toluene, 16h, **70**: 94%
(vii) DCBC, Et_3N, DMAP, CH_2Cl_2, 1.5h, **71**: 65% (viii) HF, THF, 3h, **72**: 93%
(ix) Zn, HOAc, 4h, **73**: 75% (x) DCBC, DMAP, CH_2Cl_2, 48h, **74**: 37%

Scheme 23

potassium methoxide, to yield the methyl 7α,12α-*bis*(benzyloxymethoxy)-3α-hydroxy cholanoate **66** in 58% yield from **44**. Synthesis of the *bis*(benzyloxymethoxy) cyclic tetramer **74** was carried out by the coupling and cyclization of the linear dimer **73**. To synthesize the linear dimer **73**, suitable protocols were required for the protection of the 24-oic acid and 3α-hydroxy of the '*bis*(BOM)' monomer **66**. The *tert*-butyldimethylsilyl group was found to be suitable for the protection of the 3α-hydroxy group and 2,2,2-trichloroethyl ester was developed to protect the 24-carboxylic acid. The 2,2,2-trichloroethyl ester **70** with a free 3α-hydroxy group was synthesized from the acid **69** by a Yamaguchi reaction in 94% yield. The 3α-TBDMS protected 24-free acid **68** was obtained from the methyl ester **66** and TBDMS chloride followed by hydrolysis of the methyl ester by refluxing in THF and sodium hydroxide in 55% overall yield. The two fragments were then coupled by a Yamaguchi esterification to give the protected linear dimer **71** in 65% yield. This dimer was deprotected at the tail 3a-position with aqueous HF in 93% yield to give **72**. No significant decomposition of the BOMO acetal units were observed in this step. The 2,2,2-trichloroethyl ester was then removed by reaction with zinc powder in aqueous potassium phosphate buffer to give the free acid **73** in 75% yield. The linear dimer was then cyclized using the modified Yamaguchi procedure. This gave several macrocycles, the main product being cyclic tetramer **74** which was isolated in 37% yield.

V. CONCLUSION

The various selective functionalizations of the hydroxy groups at the 3α, 7α, and 12α positions in cholic acid offer a number of ways of protecting these groups under different reaction conditions until they need to be regenerated. The 7α- and 12α-hydroxy groups can be simultaneously protected as acetate (*Scheme 1 and 7*), triflate (*Scheme 10*), or formate (*Scheme 11*) esters leaving the 3α-OH free for chemical modification. Both the 3α- and 12α-hydroxyl groups can be protected as acetate (*Scheme 3 and 9*) or nitrate (*Scheme 20*) esters while leaving the 7α-OH available for chemical modification. Simultaneous protection of the 3α- and 7α-hydroxyl groups as acetate esters (*Scheme 2*) leaves the 12α-OH open for chemical transformation.

Selective protection of the 3α-OH group as carbethoxy or mesyl esters (*Scheme 16*) leaves both the 7α- and 12α-hydroxyl group open for chemical modification. Hydrolysis was the general method for deprotection of the esters, except for nitrate esters which were deprotected by Zn dust/glacial acetic acid reduction. Tetrahydropyranyl and *t*-butyldimethylsilyl ethers were deprotected by mild acid hydrolysis. In addition, *t*-butyldimethylsilyl ethers are easily deprotected by fluoride anion. Benzyl and related protecting ethers are easily removed by catalytic hydrogenation.

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