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SYNTHESIS OF MOSESIN-4, A NATURALLY OCCURRING STEROID SAPONIN WITH SHARK REPELLENT ACTIVITY, AND ITS ANALOG 7-β-GALACTOSYL ETHYL CHOLATE

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ABSTRACT

The first synthesis of mosesin-4 (13), a naturally occurring steroid saponin with shark repellent activity, and its analog 19, is described. They both possess a free galactose residue attached axially at the 7α position of the steroidal aglycon. Methyl cholate 3-cathylate (2) was used as a model for exploring various several methods to glycosidate the severely hindered 7α-position. Best results were obtained with β-galactose pentacetate (3a), using trimethylsilyl triflate as a promoter, in 1,2-dichloroethane, at -20° C, for 14 hours.

INTRODUCTION

Certain species of fish have a self-defense mechanism consisting of the secretion of toxic substances that repel their predators.[1] Among these so-called ichthyocrinotoxic species are the Red Sea Moses sole *Pardachirus marmoratus* and its congener, the peacock Sole *Pardachirus pavoninus*, which repel sharks by emission of their toxic secretion at the moment when they are about to be bitten.[2] The chemical nature of these shark repellents has been clarified by Tachibana and coworkers, who have shown that the toxins consist of a mixture of peptides and steroidal saponins with detergent-like properties. They have eventually determined the structures of the peptidic pardaxins,[3a-c] and two classes of steroid glycosides, mosesins from *P. marmoratus*[4] and pavonins from *P. pavoninus*.[5a-b] These compounds were also found to be potent cell disrupters and hence should be important as physiological probes and exert interesting pharmacological activity.[3c, 6]

The biological activity of these toxins is believed to be related to their surfactant properties,[4,7] that in the case of saponins arises from a hydrophobic "top" and hydrophilic "bottom" regions (Fig.1). The structures of the lipophylic saponin toxins are collectively shown in Fig.1. The A/B ring can either be trans or cis, the oxygen functions at C-3 can be 3α-OH, 3β-OH or 3-one, and double bonds may or may not be present at C-4/C-5/C-6; in all cases a sugar moiety is attached axially at 7α or 15α position of the steroid skeleton. These molecule can interact through its large hydrophobic surfaces with the plasma membrane of a yet unidentified sense organ of the shark. The study of their mode of action, their general pharmacological activities, and the performance of proper tests as shark repellents, however, has been seriously hampered by the limited amount of compound available from natural sources. Furthermore, it is of interest to synthesize simpler steroid saponins that fit into the general pattern depicted in Fig.I, and to test their biological activity. This may yield potent and more readily accessible pharmacologically active compounds (and shark repellents). In

addition, the synthesis of steroid glycosides involving formation of sterically hindered β–glycosidic bonds between a sugar and a complex alcohol, is not a trivial matter.

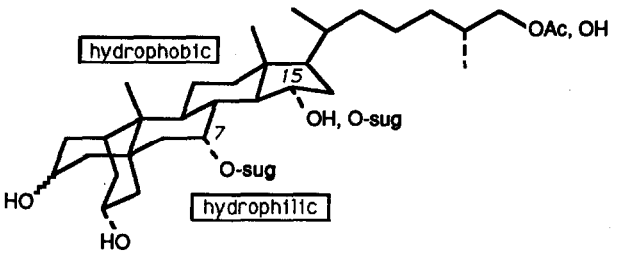


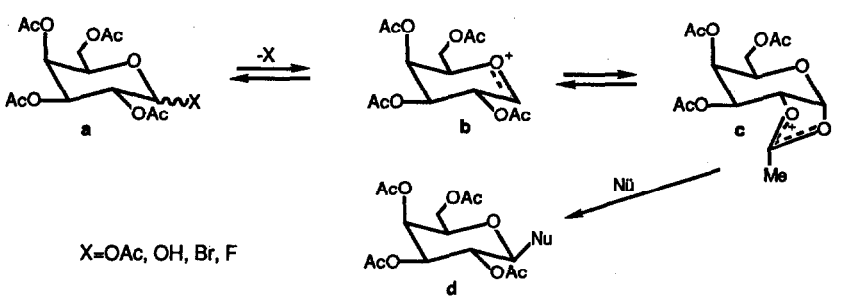
Fig. I

To the best of our knowledge, success in stereoselective glycosidation of cholic acid-type aglycones has been achieved only for the most reactive 3-hydroxyl position.[8a-f] We have therefore undertaken a systematic screening of the available glycosidation procedures[8f,9a-e] in order to arrive at a satisfactory route to achieve the 7α-glycosidation. In the following we report on the synthesis of mosesin-4 (13) and its analog (19).

RESULTS AND DISCUSSION

All glycosidation methods that we have investigated involve the activation of a 2-acetylgalactose through oxocarbenium ion formation. These methods can, in principle, overcome the low reactivity of the C_7-OH by transforming the sugar into a highly reactive species.

SCHEME 1



Furthermore, the equatorial 2-acetyl group of galactose peracetylate is known to provide a way of controlling the stereochemistry of the process through the formation of the anchimerically stabilized cation c (Scheme 1) that directs the incoming nucleophile to the β position[9a,d]. Cholic acid methyl ester (1) was chosen as a model in order to develop a suitable method for the glycosidation reaction at the 7-hydroxyl group of the aglycone.

Since the reactivity order of the hydroxyl groups of cholic acid is 3α >7α >12α, only the 3α hydroxyl group needs to be protected.

This was achieved as described by Fieser et al.,[10] using ethyl chloroformate that yields the 3-cathyl ester (2) as the only reaction product (Scheme 2).

SCHEME 2

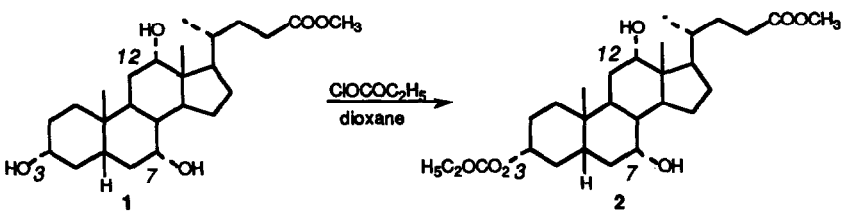


Table 1 summarizes the methods investigated for the glycosidation reaction (Scheme 3). The sugars used were all galactose 2,3,4,6-tetracetate derivatives.

Table 1. Results of the glycosidation reaction study[a]

ENTRY	AGLYCONE	SUGAR	PROMOTER	PRODUCT	YIELD (%)[b]
1	2	3a	Me_3SiOTf	4	30
2	2	3b	Me_3SiOTf	4	30
3	2	3c	AgOTf	4	20
4	2	3d	Me_3SiOTf	4	10
5	2a[c]	3d	Me_3SiOTf	4	10

a) Reaction conditions: $C_2H_4Cl_2$, -20° C, overnight, 3 eq sugar.

b) isolated yield.

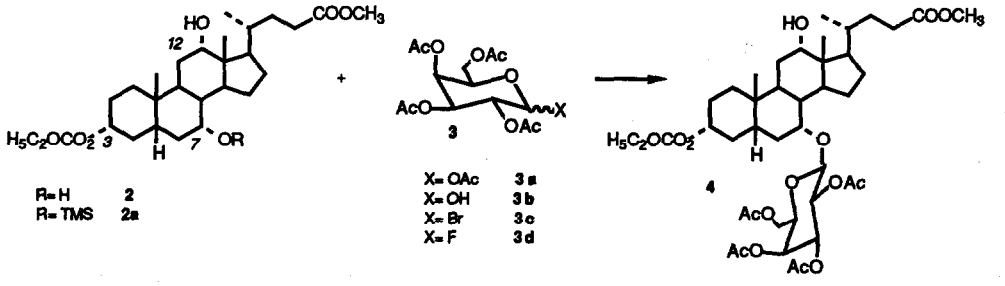
c) the silyl ether was readily hydrolized upon addition of Me_3SiOTf

As expected, in all cases the stereochemistry of the newly generated glycoside bond was β as determined by coupling constants in the proton NMR spectra.[11] Me_3SiOTf -promoted reaction of fluorosugar 3d with both aglycones 2 and 2a (entries 4 and 5) resulted in very low yields of the desired glycoside and generated a large number of by-products.A better scenario was offered by the AgOTf-promoted glycosidation with bromogalactose 3c.

Although the yield of 4 was still disappointingly low (entry 3), the reaction was not accompanied by large decomposition. In fact the only significant by-product observed was the 7-O-acetyl derivative (15%), and the starting aglycone could be recovered in 20%. Best

results were obtained using sugars **3a** and **3b** (entries 1 and 2). The glycoside **4** was isolated in 30% yield with 25% recovery of the starting aglycone.

SCHEME 3



As in the bromosugar case, the major by-product observed was the 7-O-acetyl derivative (10%). In all cases an increase in the reaction temperature caused a larger extent of by-product formation. Entry 1 was then chosen as the method of choice to be further investigated for the ready availability of the β-galactose pentacetate. The optimal conditions for the galactosidation reaction were found to require 1,2-dichloroethane (DCE) as solvent, at - 20°C, for 14 hours (Table 2).

The actual synthesis of mosesin-4 (**13**) started from the commercially available cholic acid (**5**) (Scheme 4).

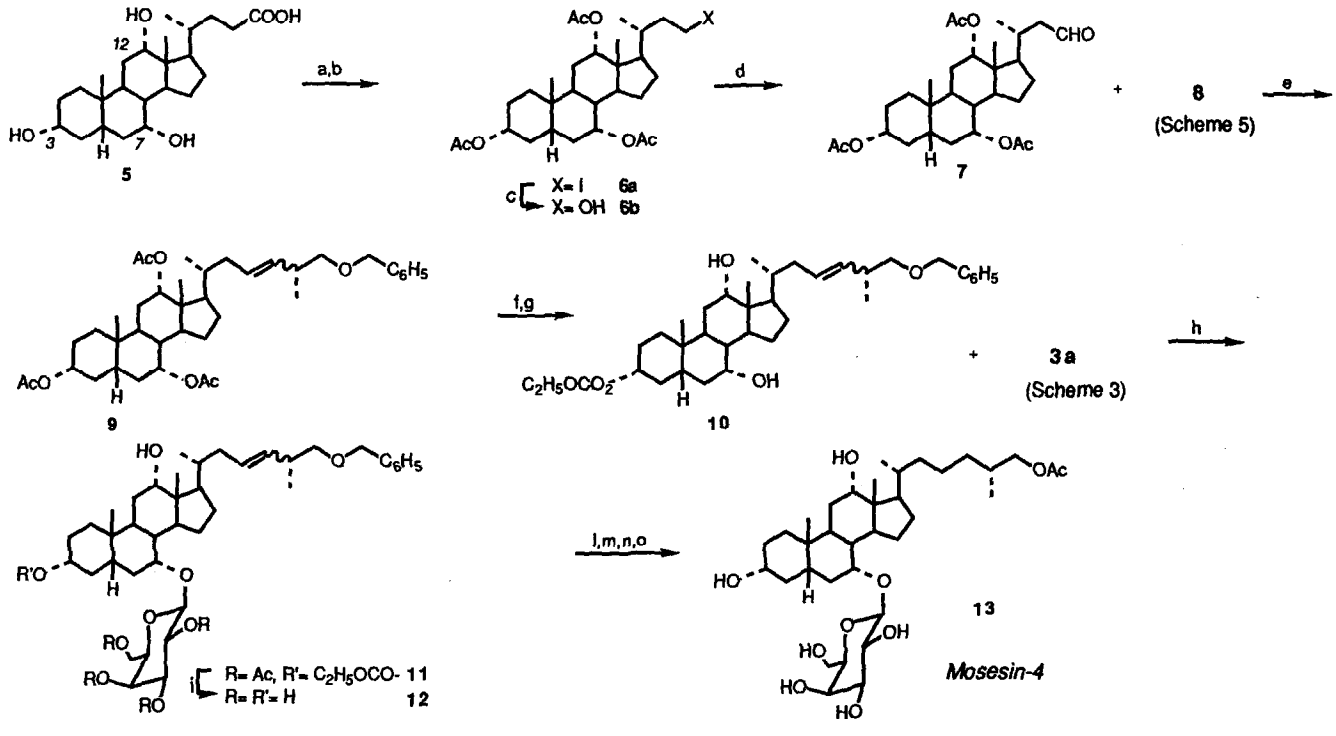
The first objective was elongation of the aglycone side chain, which we planned to achieve through a Wittig reaction between aldehyde **7** and the ylide of the phosphonium salt **8**. The synthesis of the aldehyde started with peracetylation of **5**. Successive photochemical decarboxylation /iodination reaction as reported by Concepcion et al.,[12] afforded iodide **6a** in which the side chain was shortened by one carbon atom. The iodide was selectively converted to alcohol **6b** retaining the acetates, using silver carbonate, and **6b** was then oxidized to the aldehyde **7** with PCC. Phoshonium salt **8** was synthesized from the commercially available S(+) methyl-3-hydroxy-2-methyl propionate (**15**) (Scheme 5).

Benzylation of the hydroxy group in **15** with freshly prepared silver oxide[13] gave the benzyl derivative **16**, which was smoothly reduced with $LiAlH_4$ affording the primary alcohol **17**. Treatment of **17** with NIS and triphenylphosphine gave alkyl iodide **18** which was subsequently converted to the phosphonium salt **8** with triphenylphospine in refluxing toluene. Deprotonation of **8** with n-BuLi in THF at -10°C led to the phosporous ylide that was immediately reacted with aldehyde **7** (Scheme 4) to afford olefin **9** as a pure isomer (possibly *Z*) in 60% yield.

After hydrolysis of peracetate **9**, the 3-hydroxyl group was selectively protected as the cathyl ester **10** with ethyl chloroformate. The galactosidation was carried out under the conditions optimized for cholic acid methyl ester (galactose peracetate, Me_3SiOTf, $C_2H_4Cl_2$, -20°C, 14h) to give glycoside **11** with the isolation yield of 40%. After alkaline hydrolysis of the cathyl and acetyl esters, the more reactive hydroxyl groups of the glycoside were protected as TBDMS-ethers using an excess of TBDMS-Cl.

Proton NMR integration of the resulting compound showed that this protocol resulted in the introduction of three TBDMS groups; no effort was made to establish their actual positions in the molecule. Hydrogenolysis of the 26-benzyl ether simultaneously accomplished hydrogenation of the 23-ene (**13**). Acetylation of the C-26 hydroxyl group, followed by cleavage of the TBDMS ethers with a 5% solution of 48% aq. HF in CH_3CN, afforded mosesin-4.

SCHEME 4



SCHEME 5. *Reagents and conditions:* a) Ac_2O, pyridine, r.t., (>90%); b) hν, iodosobenzene diacetate (IBDA), I_2,70°C, (45-50%); c) Ag_2CO_3, aq. acetone, 50°C, (85-90%); d) PCC,CH_2Cl_2, r.t., (85%); e) nBuLi, THF, 0°C, 1h, (60%); f) NaOH, aq. MeOH, *reflux*, (>90%); g) $ClCO_2C_2H_5$, dioxane, (90%); h) TMSOTf, $C_2H_4Cl_2$, -20°, 14h, (40%); i) NaOH, aq. MeOH, *reflux*, (>90%); l) TBDMS-Cl, DMAP, CH_2Cl_2, r.t., 5h, (90%); m) H_2 / Pd, MeOH, (>95%); n) Ac_2O, pyr/THF, (83%); o) 95:5 CH_3CN / 48% HF, 4h, (>90%).

The synthetic compound proved to be identical to natural mosesin-4 by spectroscopic and chromatographic analysis (^{1}H and ^{13}C NMR, HPLC, $[\alpha]_D$).

Compound 19 (Scheme 6), corresponding to mosesin-4 but with a simpler side chain, was synthesized as an analog to be tested as shark repellent and other activities. It was easily prepared from 4 by transesterification with sodium ethoxide in ethanol (Scheme 6). Mosesin-4 and its analog 19, are to be tested for their general pharmacological activities, shark repellency, etc..

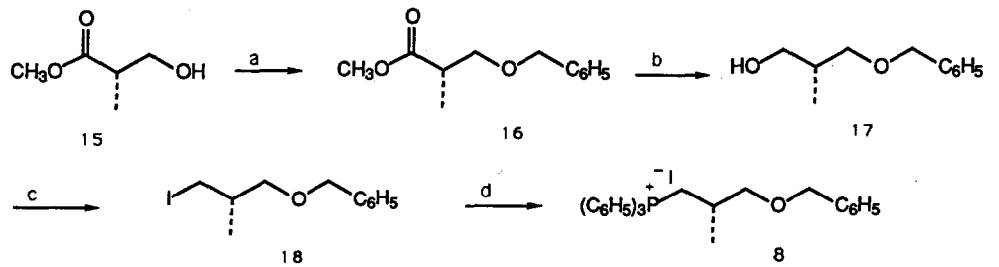
TABLE 2. Results of different reaction conditions of 2 with 3a.

ENTRY	SOLVENT	TEMP (°C)	TIME	Eq. of Me_3SiOTf	YIELD (%)[a]
1	$C_2H_4Cl_2$	-20°	14h	2.5	30
2	$C_2H_4Cl_2$	0°	3h	2.5	20
2	$C_2H_4Cl_2$	r.t.	3-4h	2.5	20[b]
3	THF	-20°→ r.t.[c]	24h	2.5	0
4	benzene	5°→ r.t.[d]	8h	2.5	trace
5	CH_3CN	-20°→ r.t.[c]	24h	2.5	0
6	CH_2Cl_2	r.t.	20h	2.5	15
7	$C_2H_4Cl_2$	-20°	14h	1.0	25
8	$C_2H_4Cl_2$	-20°	14h	0.1	10

a) isolated yield; b) Compound 4 easily decomposed. Large extent of by-product formation;

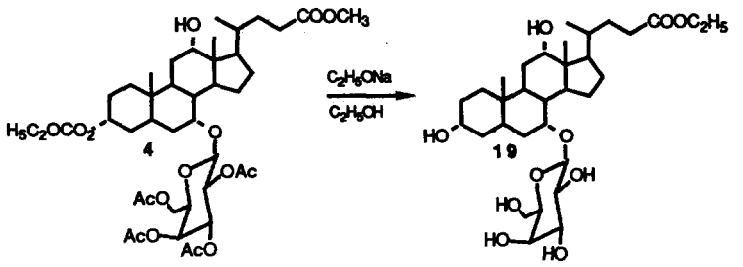
c) 20h at -20°C, 4h at r.t.; d) reagents mixed at 5°C, temperature slowly raised to r.t..

SCHEME 5



Scheme 5. Reagents and Conditions: a) $C_6H_5CH_2Br$, Ag_2O, ether, (50-55%); b) $LiAlH_4$, THF, 0°C, (>90%); c) NIS, $(C_6H_5)_3P$, CH_2Cl_2,0°C, (75-80%); d) $(C_6H_5)_3P$, toluene, reflux, (90%).

SCHEME 6



EXPERIMENTAL SECTION

^1H and ^{13}C NMR spectra were recorded on a Bruker WM-250 spectrometer, unless otherwise specified. FAB spectra were measured on a VG-7070 EQ spectrometer (Xe ionizing gas, 3-nitrobenzyl alcohol matrix); The rotations of the polarized light were recorded at 589 nm on a JASCO DIP-181 Polarimeter.

Cholic acid peracetate: To a cooled(0°) suspension of **5** (10g; 0.0245 mol) in acetic anhydride (20ml), pyridine (30ml) and 4-dimethylaminopyridine (DMAP) (1.80g; 0.0147 mol; 0.2 eq.) were added. The reaction mixture was then stirred for 3.5 hours at r.t.. The solvent was concentrated under reduced pressure, the mixture diluted with ether and washed with 0.15 M HCl. The organic layer was dried over Na_2SO_4 and the solvent evaporated down to afford **6a** as a white solid (12.4 g; 94.7 %).

^1H NMR ($CDCl_3$) : δ 0.71 (s, 18-H_3); 0.80 (d, J=5.0 Hz, 21-H_3); 0.89 (s, 19-H_3); 2.00, 2.05 and 2.10 (3s, OAc's); 4.55 (m, 3-H); 4.89 (m, 7-H); 5.08 (m, 12-H).

23-Iodo-24-nor-5β-cholan-3α,7α,12α-triol-3α,7α,12α-triacetate (6a): A stirring solution of cholic acid peracetylate (12.4 g; 0.0232 mol; 1eq.) in CCl_4 (1l), containing iodosobenzene diacetate (IBDA) (4.11 g; 0.0128 mol) and iodine (2.15 g; 0.0116 mol), was irradiated with two 100 W tungsten filament lamps for 1 hour at refluxing temperature. Another portion of IBDA (0.0128 mol) and iodine (0.0116 mol) was then added, and the irradiation at the same temperature was continued for 1 more hour. The reaction mixture was washed with 20% sodium thiosulfate. Flash silica-gel column chromatography of the residue (eluant 75:25 n-Hexane-EtOAc) gave the alkyl iodide **6a** in 48% yield. ^1H NMR ($CDCl_3$): δ 0.73 (s, 18-H_3); 0.79 (d, J= 5 Hz, 21-H_3); 0.86 (s, 19-H_3); 2.03, 2.06, 2.11 (3s, OAcs); 3.05 and 3.28 (m, 2H, 23-H_2).

23-hydroxy-24-nor-5β-cholan-3α,7α,12α-triol-3α,7α,12α-triacetate (6b): The alkyl iodide **6a** (11g; 0.0178 mol; 1 eq.) was dissolved in aqueous acetone (10 ml) and silver carbonate (10 g; 0.0363 mol; 2 eq.) was added. The mixture was sonicated for ~2.5 hours and then stirred at refluxing temperature overnight. After filtration and solvent evaporation, the filtrate residue was flash chromatographed on a silica-gel column (eluant n-Hexane-EtOAc 1:1) to afford compound **6b** as a white solid (8.0g; 89%).

23-al-24-nor-5β-cholan-3α,7α,12α-triol-3α,7α,12α-triacetate (7): To a solution of **6b** (7.7g; 0.0152 mol) in dry CH_2Cl_2 (30 ml), PCC in large excess was added. After 1.5 hour the mixture was filtered through florisil and the solvent evaporated to give **7** as a white solid in 85% yield. ^1H NMR ($CDCl_3$): δ 0.72 (s, 18-H_3); 0.90 (d, J= 6.6 Hz, 21-H_3); 0.90 (s, 19-H_3); 2.41 (dd, 2H, J= 2.5, 16, 22 H_2); 9.70 (d, J= 2.1 Hz, aldehyde).

26-benzyloxy-25R-Δ_{22}-5β-cholestan-3α,7α,12α-triacetate (9): To a cooled (-10°) suspension of the phosphonium salt (7.8g; 0.0142 mol; 1.3 eq.) in dry THF (60 ml), n-BuLi (0.0142 mol; 9.5 ml) was slowly added under argon. After 20 min. the aldehyde in solution was added and the temperature let to raise to 0°C. The reaction was quenched after 1 hour by addition of a saturated NH_4Cl solution and washed with Et_2O-H_2O. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was

flash chromatographed through a silica-gel column (n-Hexane-EtOAc 75:25 as eluant) to give 9 as a sticky solid (3.54 g; 50%).

^{1}H NMR ($CDCl_3$): δ 0.70 (s, 18-H_3); 0.78 (d, J= 5.6 Hz, 25-H_3); 0.90 (s, 19-H_3); 0.97 (d, J= 5.6 Hz 21-H_3); 2.03, 2.06 and 2.11 (3s, OAc's); 2.72 (m, 1H, 25-H); 3.27 (ddd, J_{gem}= 8.4 Hz, J_{vic}= 5.6 Hz, 2H, 26-H_2); 4.48 (dd, 2H, J= 11 Hz, -O$\underline{CH_2}C_6H_5$); 5.10-5.40 (m, 2H, $\Delta_{23,24}$); 7.28-7.35 (m, phenyl).

26-benzyloxy-25R-Δ_{22}-5β-cholestan-3α,7α,12α-triol-3α-cathylate (10): 9 was reacted with NaOH in a THF/ MeOH /H_2O solution at refluxing temperature for 6.5 hours. To a cooled solution of the resulting triol (3.0g; 5.72 mmol) in dioxane (15ml) and pyridine (4ml), ethyl chloroformate (3 ml) was slowly added. The reaction mixture was stirred for 30 min at r.t. and then quenched by addition of H_2O. After extraction with Et_2O, the organic residue was flash chromatographed on a silica-gel column (n-Hexane-EtOAc 75:25 as eluant) to give 10 as a white solid (3.2g; 91%).

^{1}H NMR ($CDCl_3$): δ 0.68 (s, 18-H_3); 0.88 (s, 19-H_3); 0.97 (2d, J= 5.6 Hz, 21-H_3 and 27-H_3); 1.28 (t, J= 6.2 Hz, 3H, CH_3 cathylate); 2.75 (m, 1H, H-25); 3.25 (ddd, J_{gem}= 8.4 Hz, J_{vic}= 5.6 Hz, 2H, 26-H_2); 3.84 (m, 7-H); 3.97 (m, 12-H); 4.15 (q, J= 6.2 Hz, 2H CH_2, cathylate); 4.42 (m, 3-H); 4.50 (dd, 2H, J=11 Hz, 0$\underline{CH_2}C_6H_5$) 5.12-5.45 (m, 2H, $\Delta_{23,24}$); 7.25-7.30 (m, phenyl). ^{13}C (pyridine-d_5, 62 MHz): δ 13.15, 18.12, 23.35, 24.00, 27.55, 28.48, 29.76, 31.76, 32.93, 34.67, 35.45, 35.98, 36.38, 37.14, 40.85, 41.07, 42.66, 42.86, 47.12, 47.69, 67.87, 71.99, 72.59, 73.10, 75.79, 76.13, 80.62, 127.79, 127.89, 128.75, 129.51, 133.73, 139.70, 174.

26-benzyloxy-25R-Δ_{22}-3α-cathyl-7α-β-galactoperacetyl-5β-cholestane-12α-ol (11): To a chilled (-20°) solution of b-galactose peracetylated (4.78g; 0.0122mol; 3 eq.) in dry $C_2H_4Cl_2$ (40 ml), in presence of dry Molecular sieves 4 Å, TMSOTf (2.27g; 0.0102 mol; 1.97 ml; 2.5 eq.) was added under argon. After stirring 1 hour at the same temperature, the aglycon 10 (2.5g; 4.08 mmol; 1 eq.), in $C_2H_4Cl_2$ solution, was added, and the mixture stirred at the same temperature for 14 hours. The reaction was quenched by direct extraction with EtOAc / $NaHCO_3$ 1N. The organic layer was dried over $NaSO_4$ and the solvent evaporated under reduced pressure. The residue was chromatographed on a silica-gel column (eluant n-Hexane-EtOAc 80:20 to recover the starting material and 65:35 to recover the glycoside). The recovered starting material was reacted again in the same conditions. Totally 1.3g of glycoside were obtained (40%).

^{1}H NMR ($CDCl_3$): d 0.65 (s, 18-H_3); 0.90 (s, 19-H_3); 0.94 (d, J= 5.6 Hz, 21-H_3); 0.96 (d, J= 5.6 Hz, 27-H_3); 1.24 (t, J= 6.2 Hz, CH_3 cathylate); 1.98, 2.0, 2.08, 2.13 (4s, OAc's); 2.78 (m, 1H, 25-H); 3.25 (ddd, J_{gem}= 8.4 Hz, J_{vic}= 5.6 Hz, 2H, 26-H_2); 3.95 (m, 2H, 7-H and 12-H); 3.98 (m, 1H, 5'-H); 4.15 (q, J= 6.2 Hz, 2H, CH_2O cathylate); 4.02-4.12 (m, 2H, 6'-H_2); 4.40 (m, 3-H); 4.50 (dd, 2H, J= 11 Hz, O$\underline{CH_2}C_6H_5$); 4.58 (d, J= 7.0 Hz, 1'-H); 5.01 (dd, J= 10.5 Hz, 3.3 Hz, 3'-H); 5.10-5.25 (multiplicity unclear, 4H, 2'-H, 4'-H, D23,24); 7.22-7.38 (m, phenyl). ^{13}C (pyridine-d_5, 62 MHz): δ 13.01, 14.41, 18.02, 20.49, 21.23, 22.73, 23.35, 26.85, 27.42, 28.27, 29.30, 32.88, 34.54, 35.25, 36.91, 39.97, 41.35, 41.58, 43.86, 47.15, 47.45, 55.87, 56.26, 56.65, 62.06, 63.47, 68.38, 70.16, 71.32, 72.09, 72.45, 73.05, 75.17, 75.74, 78.27, 98.91, 127.73, 127.88, 129.55, 133.66, 139.70, 155.11, 170.13, 170.59. FAB$^+$-MS (3-nitrobenzyl alcohol matrix): m /e 950 (10%) $(M+H)^+$; 928 (10%) $(M+H)^+$; 820 (10%) (M-benzylic alcohol)$^+$; 561 (40%) [$(M+H)^+$-sugar-$2H_2O$]; 472 (68%) (M^+-sugar-$2H_2O$-cathylate).

Mosesin-4 (13) :11 (1.3g; 1.4 mol was reacted with NaOH (3 eq.) overnight at r.t. in a THF /MeOH /H_2O solution (10 ml) to get the free glycoside 12 (828 mg; 86%). This (828 mg; 1.21 mmol, 1 eq.) was reacted with TBDMS-Cl (3.27g; 21.7 mmol; 3 eq.) in dry CH_2Cl_2 (40 ml), in presence of DMAP (3.54 g; 29 mmol; 4 eq.), in order to get the sylilation of the most reactive hydroxyl groups. After 5 hours, TLC showed only one major spot. The reaction was quenched by direct extraction with water. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography (eluant CH_2Cl_2-Me_2CO 96:4) afforded the sylil ether as a very dense liquid. ^{1}H NMR is not reported for the high congestion of the signals, but the valuation of the integrated areas shows that 3 hydroxyl groups were protected, probably 3, 6' and 3'. The subsequent hydrogenation / hydrogenolysis with 0.2 eq. of 5% H_2 / Pd in EtOAc, afforded the 26 free hydroxyl group glycoside. This was reacted with 10 eq of acetic anhydride and

pyridine, in THF for 17 hours, to give the 26 monoacetylated glycoside. The cleavage of the silyl protecting groups was then achieved by reaction of the monoacetylated compound with 10ml of a 95:5 CH_3CN / 48% HF solution, for 30 min. at r.t.. The reaction was quenched by direct extraction with EtOAc / $NaHCO_3$ 1N. The organic layer was dried over Na_2SO_4 and the solvent evaporated down. The residue was h.p.l.c. chromatographed on a reverse phase column (YMC-PACK S-343 I15ODS) (MeOH 90% as eluant) to give 180 mg of pure mosesin-4 (overall yield from step i is 60%).

^1H NMR (CD_3OD): δ 0.69 (s, 18-H_3); 0.92 (s, 19-H_3); 0.92 (d, J= 6.7 Hz, 27-H_3); 1.0 (d, J= 6.5 Hz, 21-H_3); 2.02 (s, 26 OAc); 3.37 (m, 3-H); 3.44 (td, J= 6 Hz, 1 Hz, 5'-H); 3.48 (dd, J= 10 Hz, 3 Hz, 3'-H); 3.53 (dd, J= 10 Hz, 7 Hz, 2'-H); 3.68 (dd, J= 11 Hz, 6 Hz, 6'-H_a) and 3.74 (dd, J= 11 Hz, 6 Hz, 6'-H_b); 3.85 (dd, J= 3 Hz, 1 Hz, 4'-H); 3.92-3.96 (m, 2H, 7-H and 12-H); 4.29 (d, J= 7 Hz, 1'-H). ^{13}C (CD_3OD, 62 MHz): δ 13.12, 17.03, 18.19, 20.92, 23.01, 23.98, 24.48, 28.21, 28.81, 29.59, 30.24, 31.33, 34.87, 35.89, 36.52, 36.94, 37.04, 39.95, 40.84, 42.32, 43.11, 47.41, 62.24, 68.66, 70.26, 72.92, 74.12, 74.42, 75.20, 76.45, 101.42, 121.81, 122.16, 122.51, 170.82. FAB$^+$-MS (3-nitrobenzyl alcohol matrix): m/z 663 (100%) $(M+Na)^+$; 443 (25%) [$(M+H)^+$- galactose - H_2O]; 425 (52%) [$(M+H)^+$- galactose - $2H_2O$]; $[\alpha]_D$ = +8°, C 0.7 (C_2H_5OH).

(S)-methyl-3-benzyloxy-2-methylpropionate (16): To a sonicating solution of S(+)methyl-3-hydroxy-2-methylpropionate (15) (10g; 0.0846 mol; 9.34 ml; 1 eq.) and benzyl bromide (43.4 g; 0.254 mol; 30.2 ml; 3 eq.) in dry ether (15 ml), Ag_2O (39.2g 0.169 mol; 2 eq.) was slowly added under argon. The reaction mixture was sonicated for 10 min. and then stirred at r.t. for 20 hours. After filtration, the solvent was concentrated and the mixture chromatographed on a silica-gel column (eluant n-Hexane-Et_2O 95:5) to afford 16 as a liquid (9.3g; 52%).

^1H NMR ($CDCl_3$): δ 1.18 (d, J= 7 Hz, CH_3); 2.79 (m, 1H, $\underline{CH}CH_3$); 3.47 (dd, J_{gem}= 9 Hz, J_{vic}= 5.5 Hz, 1H, $O\underline{CH_2}CH$); 3.65 (dd, J_{gem}= 9 Hz, J_{vic}= 7.5 Hz, 1H, $O\underline{CH_2}CH$); 3.68 (s, OCH_3); 4.50 (s, 2H, $O\underline{CH_2}C_6H_5$); 7.10-7.30 (m, 5H, phenyl).

(R)-(-)-3-Benzyloxy-2-methyl-propanol (17): To a cooled (0°C) solution of 16 (7.2g; 0.0347 mol; 1 eq.) in dry THF (200 ml), $LiAlH_4$ (2.64 g; 0.0695 mol; 2 eq.) was slowly added under argon. After 20 min. the reaction was quenched by adding H_2O and EtOAc. Filtration through a celite bed afforded 17 as liquid in quantitative yield (6.2 g). Spectral data of 17 are identical to those reported by Branca et al.[14] for the same compound. The optical purity of 17 was proved by adding tris[3-(heptafluoropropyl-hydroxymethylene)-(+)-camphorato], europium (III) to the NMR sample.

(S)-(+)-3-Benzyloxy-2-methyl-propyliodide (18): To a cooled (0°C) solution of 17 (6.8g; 0.0378 mol; 1 eq.) in dry CH_2Cl_2 (55 ml), triphenylphosphine (10.9g; 0.0416 mol; 1.1 eq.) and N-iodosuccinimide (NIS) (9.35g; 0.0416 mol; 1.1 eq.) were added. The reaction time was 20 min.. The solvent was concentrated under reduced pressure and the mixture chromatographed on a silica-gel column (nHexane- EtOAc 95:5) to give 8.3g (76%) of the desired alkyl iodide as liquid.

^1H NMR ($CDCl_3$): δ 1.0 (d, J= 6.7 Hz, CH_3); 1.77 (m, 1H, $\underline{CH}CH_3$); 3.25-3.45 (m, 4H, CH_2I and $O\underline{CH_2}CH$); 4.50 (s, 2H, $O\underline{CH_2}C_6H_5$); 7.24-7.40 (m, 5H, phenyl).

(S)-(-)-3-Benzyloxy-2-methyl-triphenylphosponium iodide (8): To a solution of 18 (8.3g; 0.0286 mol; 1 eq.) in dry toluene (28.6 ml), triphenylphosphine (9.0g; 0.0343 mol; 1.2 eq.) was added, and the solution was stirred at refluxing temperature for 18 hours. The white precipitate was filtered and washed with ether. Yield 85% (13.3g).

^1H NMR (DMSO-d_6): δ 0.78 (d, J=6.7 Hz, CH_3); 2.12 (m, 1H, $\underline{CH}CH_3$); 3.25-3.35 (m, 2H, OCH_2CH); 3.35-3.80 (m, 2H, $P\underline{CH_2}CH$); 4.22 (d, J_{AB}=12.2 Hz, 1H, $O\underline{CH_2}C_6H_5$); 4.30 (d, J_{AB}=12.2 Hz, 1H, $O\underline{CH_2}C_6H_5$); 7.15-7.40 (m, 5H, phenyl); 7.65-7.90 (m,15H, $(C_6H_5)_3P$). ^{13}C (DMSO-d_6, 62 MHz): δ 18.0, 23.71, 28.78, 28.84, 71.98, 73.98, 74.14, 118.4, 119.8, 127.4, 127.5, 128.1, 129.9, 130.1, 133.4, 133.5, 134.6, 134.7, 137.8. MS (EI / CH_4): m /z 425 $(M-I^-)^+$; 263 $[(C_6H_5)_3P]^+$. $[\alpha]_D$ =-28°, C= 3.4 (DMSO).

7α-β-galactosyl-ethyl cholate (19): Compound 4 (662 mg; 0.802 mmol) was reacted with a solution of C_2H_5ONa / C_2H_5OH (10 ml) for two hours at r.t.. The reaction was quenched by direct extraction with EtOAc-HCl 0.2N. The organic layer was dried over

Na_2SO_4 and concentrated under reduced pressure. Purification on h.p.l.c. (YMC-PACK S-343 I 15ODS) (MeOH 90%), afforded **19** as a white solid (300 mg; 63%).

^1H NMR, 300 MHz (CD_3OD): δ 0.69 (s, 18-H_3); 0.92 (s, 19-H_3); 1.0 (d, J= 6.6 Hz, 21-H_3); 1.23 (t, J=6.8 Hz, 3H, $OCH_2\underline{CH_3}$); 3.44 (t,d, J= 6 Hz, 1 Hz, 5'-H); 3.48 (dd, J= 9.6 Hz, 3.3 Hz, 3'-H); 3.53 (dd, J= 10 Hz, 7 Hz, 2'-H); 3.68 (dd, J= 11 Hz, 1 Hz, 6'-H_a); and 3.75 (dd, J= 11 Hz, 1 Hz, 6'-H_b); 3.86 (dd, J= 3 Hz, 1Hz, 4'-H); 3.92-3.96 (m, 2H, 7-H and 12-H); 4.50 (q, J= 7 Hz, 2H, $O\underline{CH_2}CH_3$); 4.30 (d, J= 7.2 Hz, 1'-H). ^{13}C (pyridine-d_5, 75 MHz): δ 13.00, 14.39, 17.56, 23.11, 27.93, 28.02, 29.67, 30.02, 31.38, 31.53, 35.22, 35.77, 36.15, 40.07, 40.43, 41.85, 42.36, 46.83, 47.05, 60.09, 61.92, 70.00, 71.89, 72.32, 72.54, 73.93, 75.85, 76.89, 102.04, 174.00.

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