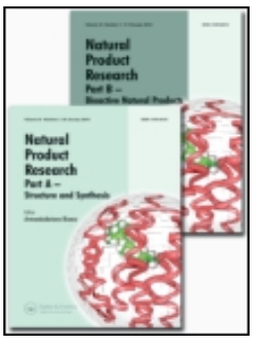

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SHORT SYNTHESIS OF NOVEL 9,11-SECOSTEROLS

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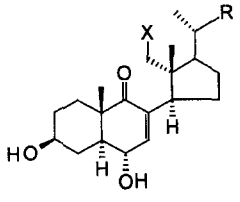
Starting from ergosterol two novel 9,11-secosterols with modified side chains (1a) and (1c) were synthesized via eight main transformations.

Keywords: Partial synthesis of sterols; 9,11-secosterols; Lipase-catalyzed regioselective acetylation

Marine invertebrates are a productive source of novel sterols. Many isolated novel compounds have unique biological properties [1]. These compounds also include cytotoxic 9,11-secosterols [2–8].

In our laboratory, 9,11-secosterols (**1a**)–(**1c**) were recently isolated from the soft coral *Gersemia fruticosa* (*Octocorallia, Alcyonacea, Nephtheidae*)



	X	R
1a	$-CH_2OAc$	
1b	$-CH_2OH$	
1c	$-CHO$	
1d	$-CH_2OH$	

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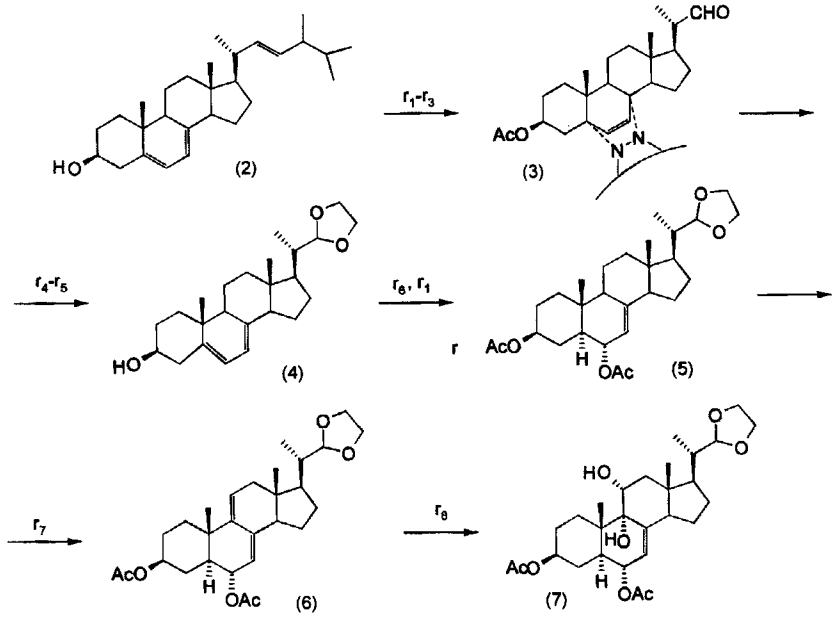
[9,10]. The inhibiting effect of (**1a**) on the cell cycle progression in the G_2/M phase was also demonstrated [11].

Starting from deoxycholic acid synthetic studies were carried out towards these secosterols [12,13]. However, this research topic has not yet been completed [14]. In our research community, studies on the total synthesis of these secosterols were started as well [15].



SCHEME 1 r_1: $(Ac)_2O$/Pyr. (1/2), r.t., 24 h; cryst. (MeOH–PhH); r_2 PTAD, acetone, – 70°C, 0.5 h; cryst. (EtOH–PhH); r_3: ozone, 1% Pyr. in CH_2Cl_2, – 70°C, 2 h; chrom. (PhH/acetone 20/1); r_4: $(CH_2OH)_2$, pTsOH, CH_2Cl_2, Δ, 3 h; chrom. (PE/acetone 8/1–6/1); r_5: $LiAlH_4$, THF, Δ, 18 h, chrom. ($CHCl_3$/acetone 50/1–40/1); r_6: a) BH_3 THF, 0°, 1 h; b) H_2O_2, NaOH, r.t., 1,5 h; chrom. (PE/acetone/CH_2Cl_2 15/1/1); r_7: $Hg(OCOCH_3)_2$, $CHCl_3$, CH_3COOH, r.t., 24 h, Δ, 0.5 h; chrom. (PE/Et_2O 5/1); r_8: OsO_4 (cat), $(CH_3)_3NO$, Pyr., *t*-BuOH, H_2O, Δ, 24 h; chrom. ($CHCl_3$/EtOH 9/1).

In this paper, we report on the partial synthesis of secosterols (**1a**) and (**1c**). Keeping in mind the C ring modification [3] of ergosterol (**2**) we started synthesis from (**2**) following Scheme 1. This molecule has a proper stereochemistry and carries functional groups necessary to perform modifications towards target compounds.

The first key intermediate (**5**) was prepared smoothly via routine 4-phenyl-1,2,4-triazolin-3,5-dione (PTAD) protection of a diene unit and side-chain ozonolysis [16] followed by the protection of the C-22 aldehyde

formed and deprotection of the diene unit in the B-ring. Unlike the hydroboration of steroidal homoallyl alcohol [12] the diene unit in hydroxydiene-aldehyde acetal (**4**) was hydroborated (followed by acetylation) with a good chemo- and regioselectivity. According to earlier results [3] the diacetoxy compound (**5**) obtained had the necessary α-oriented (pseudoequatorial) hydroxyl group at C-6 (9.8 Hz coupling between H-6 and H-5). The next principal step – dehydrogenation of diacetoxyacetal (**5**) to diene (**6**) – turned out to be a crucial one. In spite of an earlier work [17] demonstrating the dehydrogenation of 5,6-dihydroergosterol with mercuric acetate to the corresponding 7,9-diene with a satisfactory yield we encountered difficulties in performing this step efficiently. This was caused by the lability of the acetal protecting group under the conditions used [17]. The expected diene-acetal (**6**) was found with a poor 15% yield together with the starting acetal (**5**). The *cis*-dihydroxylation of diene-acetal (**6**) was performed using a catalytic amount of OsO_4[18]. This transformation resulted in a high yield of the second key intermediate – the *tetra*-substituted acetal (**7**). The preparation of the intermediate osmate ester, using a stoichiometric quantity of osmium tetroxide followed by its cleavage with $LiAlH_4$, appeared to be a less favourable procedure giving the corresponding tetrol with a low yield. Thus, the total yield of the second key intermediate (**7**) from ergosterol (**2**) was severely hampered by the dehydrogenation step.

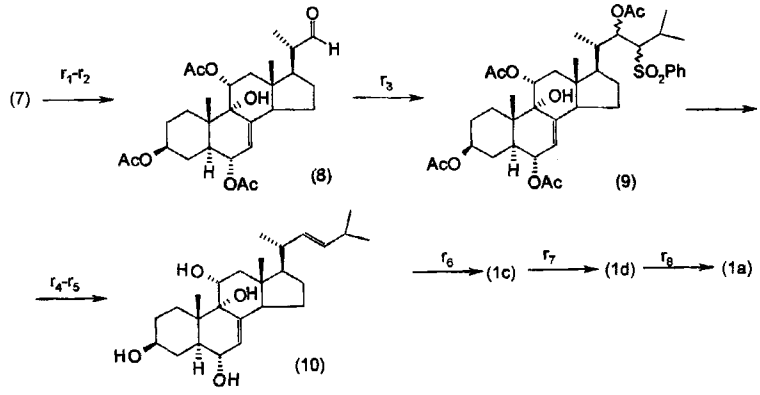
After the necessary protection/deprotection procedures the side chain was introduced by the Julia olefination [19] with a 30% yield (Scheme 2).

Notably, the sterically hindered hydroxyl group at C-9 was unaccessible to the acetylating agents [20].

The carbanion for alkylsulfonation was generated from the *i*-$BuSO_2Ph$ prepared smoothly from *i*-butyl iodide and thiophenol followed by the oxidation with *m*-chloroperbenzoic acid. The intermediate obtained – a diastereomeric mixture of acetoxysulfones (**9**), was reduced without separation. The product (**10**) obtained had a nor-cholestane side chain and a C-22 double bond with a E-geometry ($^3J_{HH}$ of olefinic protons 15.4 Hz; *trans* alkyl substituents from the ^{13}C chemical shifts). The selective cleavage of the carbon framework in diol (**10**) using $Pb(OAc)_4$ yielded smoothly the first target secosterol (**1c**) with a high yield.

The selective hydrogenation of the aldehyde moiety at C-11 with lithium *tris*[(3-ethyl-3-pentyl)oxy]aluminium hydride (LTEPA) [21] resulted in corresponding ketotriol (**1d**) with a 24% yield accompanied by the recovered (**1c**). The primary hydroxyl group formed was selectively acetylated with a customary acetic anhydride at lowered temperature [22] to obtain

the second target secosterol (**1c**) with an overall yield of 35%. The lipase-catalyzed regioselective acetylation [23] using Novozym® 435 (*Candida antarctica* lipase B) allowed us to perform this step with a 80% yield. The NMR data of the secosterols (**1a**) and (**1c**) prepared were identical to those of isolated natural secosterols [9,10].



SCHEME 2 r_1: Ac_2O, Pyr., r.t., 24 h; chrom. ($CHCl_3$/acetone 40/1); r_2: H_2O, AcOH, 75°C, 20 min, chrom. (PE/Et_2O 4/1); r_3: n-BuLi, *i*-$BuSO_2Ph$, THF, −70°C, 2 h; r_4: Na/Hg, MeOH, EtOAc, −40°C/−20°C, 8 h/40 h; r_5: 0.6 N KOH (in EtOH/H_2O 19/1), Δ, 15 min; chrom. ($CHCl_3$/Et_2O 10/1–8/1); r_6: $Pb(OAc)_4$, AcOH, r.t., 10 min; chrom. ($CHCl_3$/EtOH 20/1); r_7: LTEPA, THF, −78°C, 3 h; chrom. ($CHCl_3$/EtOH 5/1); r_8: Ac_2O (1.2 eq.), Pyr., $CHCl_3$, −7°C, 48 h; or r_8: vinyl acetate (1.5 eq.), Novozym® 435, $CHCl_3$, r.t., 48 h.

The described synthetic scheme consists of eight principal steps allowing the preparation of these and related secosterols. This will enable further insights into antiproliferative and cytotoxic properties of these natural compounds. The limiting step of the scheme – the transformation of monoene (**5**) to diene (**6**) using a novel chemoenzymatic approach – is currently under study in our laboratory.

EXPERIMENTAL

All the reaction products were purified over silica (if not stated otherwise) using flash chromatography under low pressure. The yields were not optimized. ^{13}C- and ^{1}H-NMR spectra were recorded at 125.7 and 500.17 MHz on a Bruker AMX-500 spectrometer. For the assignments 2D FT ^{1}H–^{1}H and ^{1}H–^{13}C COSY correlation diagrams were used. MS measurements were performed (at EI −70 eV; CI – isobutane; gas chromatographic separation: 200–300°C, RLS-150, 10 m) on a Hitachi M-80B spectrometer. IR and

UV spectra were recorded on a Specord IR-75 and a DU-7 (Beckman) device, respectively. The optical rotation was measured using a polarimeter "Polamat A"; a cuvette with a path length of 10 cm was used. For TLC plates from Merck were used (DC-Alufolien Kieselgel 60 F_{254}). The reagents used were from Merck and Aldrich.

3β,6α-Diacetoxy-5α-pregn-7-ene-20(S)-carbaldehyde ethylene acetal (**5**) Ergosterol (8.0 g) (recrystallized from MeOH/PhH) was acetylated followed by recrystallization from EtOH/PhH and the adduct with PTAD was prepared (yield: 8.6 g; 95%). The adduct was ozonolized in a 1% pyridine in CH_2Cl_2 and the 20(S)-aldehyde formed (yield: 2.2 g 30%, diastereomeric purity controlled by TLC, *n*-hexane/*i*-propanol 5/1) was protected as ethylene acetal. PTAD protecting group was cleaved off (as described earlier [15]) and after borohydration [3] and acetylation acetal (**5**) (yield: 0.71 g; 64%) was obtained: m.p. 205–207°C, $[\alpha]_D^{23}$ +76 (c 2.6, $CHCl_3$); ^{13}C- and ^{1}H-NMR (in $CDCl_3$): C-1 36.63 (1.24 and 1.81); C-2 27.11 (1.49 and 1.83); C-3 72.62 (4.68); C-4 29.63 (1.34 and 1.93); C-5 44.80 (1.58); C-6 73.06 (5.06); C-7 118.03 (5.05); C-8 142.68; C-9 48.76 (1.78). C-10 35.30; C-11 22.83 (1.43 and 1.61); C-12 38.93 (1.31 and 2.04); C-13 43.89; C-14 54.13 (1.87); C-15 21.22 (1.47 and 1.63); C-16 26.97 (1.45 and 1.96); C-17 52.20 (1.51); C-18 11.75 (0.55); C-19 13.83 (0.92); C-20 39.47 (1.78); C-21 11.66 (0.94); C-22 105.88 (4.85); -O-CH_2CH_2-O- 65.02 (3.85 and 3.97) and 65.18 (3.85 and 3.93); -OAc 21.31 (2.05) and 21.36 (2.03); 170.54 and 171.26.

3β,6α-Diacetoxy-5α-pregn-7,9(11)-diene-20(S)-carbaldehyde ethylene acetal (**6**) Acetal (**5**) (710 mg) was treated with mercuric acetate [3]. The reaction product was chromatographed over silica and diene-acetal (**6**) was isolated as a 1/1 mixture with the starting compound (**5**) (yield: 210 mg; 15%) along with the corresponding mono- and triacetyldienes (**6a**) and (**6b**). (**6**) – NMR ($CDCl_3$): C-1 34.54 (1.46a, 1.94e); C-2 27.38 (1.61a, 1.97e); C-3 72.63 (4.68a); C-4 29.34 (1.36a, 1.96e); C-5 43.61 (1.61a); C-6 72.23 (5.15a); C-7 120.21 (5.21); C-8 137.91; C-9 142.05; C-10 35.66; C-11 121.25 (5.56); C-12 41.92 (2.15 and 2.32); C-13 42.80; C-14 50.71 (2.24); C-15 23.14 (1.40 and 1.76); C-16 27.44 (1.48 and 2.02); C-17 52.29 (1.59); C-18 11.11 (0.53); C-19 20.64 (1.02); C-20 39.30 (1.83); C-21 11.32 (0.94); C-22 105.87 (4.87); -OAc 21.29 (2.11), 21.34 (2.03), 170.52, 171.15; -O-CH_2 -CH_2-O- 65.04 and 65.17 (3.85–3.95); MS: 472 (M^+), 412 ($M^+ - 60$), 352 ($M^+ - 120$); UV, λ_{max}: 251.5, 242.0, 235.0 nm; (**6a**) – MS: 414 (M^+), 354

($M^+ - 60$); UV, λ_{max}: 250.5 nm and (6b); MS, 531 ($M^+ + 1$), 471 ($M^+ - 59$), 410 ($M^+ - 120$), 350 (M^+ 180); UV, λ_{max}: 249.5, 242.5, 236.0.

3β,6α-Diacetoxy-9α,11α-dihydroxy-5α-pregn-7-ene-20(S)-carbaldehyde ethylene acetal (**7**) 103 µl of a 2.5% solution of OsO_4 in *tert*-BuOH was added to 210 mg of the mixture of acetal (**5**) and diene (**6**), 65 mg of trimethylamine N-oxide dihydrate, 37 µl of pyridine, 0.25 ml of water and 80 ml of *tert*-BuOH. The solution was refluxed under argon for 24 h and then worked up [17]. By column chromatography the recovered acetal (**5**) was first isolated (82 mg) and then, 76 mg of diacetoxy-dihydroxyacetal (**7**) was obtained (yield: 0.76 g; 82%): m.p. 223–224°C; $[\alpha]_D^{22}$ +46 (c 5.2, $CHCl_3$); NMR ($CDCl_3$): C-1 31.51 (1.80a, 2.08e); C-2 27.01 (1.48a, 1.85e; C-3 72.19 (4.65a); C-4 29.97 (1.32a, 2.00e); C-5 38.59 (2.07a); C-6 72.52 (4.98a); C-7 123.69 (5.29); C-8 141.32; C-9 74.07; C-10 40.47; C-11 69.11 (4.12); C-12 46.33 (1.57a, 2.16e); C-13 43.17; C-14 50.05 (2.38); C-15 22.90 (1.42 and 1.58); C-16 26.87 (1.45 and 2.00); C-17 51.81 (1.59); C-18 12.07 (0.59); C-19 15.52 (1.08); C-20 39.35 (1.77); C-21 11.65 (0.95); C-22 105.65 (4.83); -OAc 21.19 (2.06), 21.35 (2.02), 170.54, 171.16; -O-CH_2-CH_2-O- 64.99 and 65.11 (3.79, 3.82, 3.95, 3.97).

3β,6α,11α-Triacetoxy-9α-hydroxy-pregn-7-ene-20(S)-carbaldehyde (**8**) After the routine protection of hydroxyl groups and deprotection [24] of the aldehyde moiety starting from 87 mg of (**7**) 54 mg of (**8**) was obtained (yield: 68%): m.p. 176–177°C; $[\alpha]_D^{21}$ +38 (c 6.5, MeOH); TLC–R_f 0.32 (n-hexane/acetone 2/1); NMR ($CDCl_3$): C-1 31.20 (1.81a, 1.41e); C-2 27.09 (1.49a, 1.86e); C-3 71.68 (4.63a); C-4 29.89 (1.39a, 2.01e); C-5 38.40 (2.13a); C-6 72.00 (4.98a); C-7 124.53 (5.33); C-8 139.72; C-9 74.53; C-10 40.60; C-11 72.06 (5.36); C-12 41.71 (1.64a, 2.09e); C-13 43.10; C-14 49.97 (2.50); C-15 23.10 (1.47 and 1.64); C-16 26.80 (1.43 and 2.03); C-17 50.45 (1.73); C-18 12.48 (0.70); C-19 15.42 (1.05); C-20 49.50 (2.36); C-21 13.49 (1.12); C-22 204.13 (9.57); -OAc 21.17 (2.06), 21.32 (2.03), 21.76 (2.07), 169.06, 170.54, 171.16; IR (KBr), ν_{max} 2720 (-CHO), 1730, 1715 (-CHO, $-COCH_3$), 1670 (-C=C-), 1390 (-$OCOCH_3$), 1235, 1015 (-C–O-), 970, 920, 900 (-CH=CH-) cm^{-1}.

24-nor-3β,6α,9α,11α-Tetrahydroxy-5α-cholesta-7,22(E)-diene (**10**) From 54 mg of aldehydes (**8**) 11 mg (yield: 30%) of tetrol (**10**) was obtained using the Julia olefination procedure [19]. (10): m.p. 192–194°C; TLC – R_f 0.39 ($CHCl_3$/EtOH 5/1); $[\alpha]_D^{19}$ +10 (c 2.0, MeOH); NMR (pyridine-d5): C-1 32.96 (2.31a, 2.69e); C-2 32.58 (1.89a, 2.18e); C-3 70.13 (3.97a); C-4 35.89 (1.83a, 3.16e); C-5 43.40 (2.51a); C-6 69.46 (4.19a); C-7 129.34

(5.90); C-8 139.63; C-9 75.07; C-10 41.17; C-11 69.44 (4.47a); C-12 47.36 (1.96a, 2.37e); C-13 43.08; C-14 51.10 (2.77a); C-15 23.15 (1.47); C-16 28.46 (1.27, 1.65); C-17 55.86 (1.29); C-18 12.74 (0.70); C-19 16.14 (1.30); C-20 40.41 (1.98); C-21 20.94 (1.03); C-22 133.63 (5.15); C-23 135.20 (5.30); C-25 31.11 (2.20); C-26,27 22.80 (1.96); IR (KBr) – 3400 (-OH), 1660 (-C=C-), 1415 (-OH), 1010, 970, 830, 805 (-CH=CH-) cm^{-1}.

24-nor-9,11-seco-11-Oxo-3β,6α-dihydroxycholesta-7,22(E)-dien-9-one (**1c**) 11 mg of tetrol (**10**) was treated with lead tetraacetate [3] and 10 mg (yield: 90%) of (**1c**) as an oil was obtained: TLC – R_f 0.52 ($CHCl_3$/EtOH 5/1); $[\alpha]_D^{19}$ +26 (c 2.7, MeOH); NMR data of (**1c**) were identical to those of the natural secosterol isolated from the coral [10].

24-nor-9,11-seco-11-Hydroxy-3β,6α-dihydroxycholesta-7,22(E)-dien-9-one (**1d**) 10 mg of aldehyde (**1c**) was reduced with LTEPA [21] and 2.5 mg of triol (**1d**) (conversion 25%) was obtained: m.p. 83–84°C; TLC – R_f 0.58 ($CHCl_3$/EtOH); ^{1}H-NMR: in comparison with the spectrum of starting (**1c**) the product showed no resonance characteristic to aldehyde hydrogen; characteristic 4 spin system from H-11 (3.68 m, 3.88 m) and H-12 (1.0 m, 1.59 m) appeared. At the same time, other functional groups were not modified: H-3 at 3.60 (m), H-6 at 4.29 (dm), H-7 at 6.58 (d), H-18 at 0.64 (s), H-19 at 1.14 (s), H-21 at 1.03 (d), H-22 at 5.25 (m), H-23 at 5.28 (m), H-25 and H-26 at 0.95 (d).

24-nor-9,11-seco-11-Acetoxy-3β,6α-dihydroxycholesta-7,22(E)-dien-9-one (**1a**) To 2.5 mg of (**1d**) 1 ml of 0.05% vinyl acetate in $CHCl_3$ (1.5 eq.) and 100 mg of Novozym® 435 [25] were added and the solution was stored for 2 days. After filtration and purification 2 mg of the target secosterol was obtained (yield: 80%). TLC R_f value and NMR data of the product were identical to those of the original sample isolated from the coral [9].

Acknowledgements

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