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Synthesis and Evaluation of 4-Deacetoxyagosterol A as an MDR-Modulator

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Abstract—4-Deacetoxyagosterol A was synthesized from ergosterol by utilizing reductive regioselective epoxy cleavage as a key reaction. This synthesized congener of agosterol A, a spongean MDR-modulator, showed similar MDR-modulating activity against KB CV-60 cells overexpressing MRP.

Multidrug resistance (MDR), cross-resistance to several functionally and structurally diverse antitumor agents, is one of the most serious reasons for the failure of cancer chemotherapy.[1] Tumor cells with an MDR phenotype mainly overexpress membrane proteins serving as an energy-dependent drug efflux pump such as P-gp[2] and MRP.[3] Thus, suppression of the function of these membrane proteins is considered to be important to conquer the refractory behavior of tumors in clinical oncology. During the course of our search for biologically active substances from marine organisms, we isolated a polyhydroxysterol acetate, agosterol A (**1**), from a marine sponge of *Spongia* sp. and characterized it as an MDR modulator inhibiting the function of not only P-gp but also MRP.[4] Furthermore, extensive analysis of congeners from the same sponge and the chemical derivatization of agosterol A (**1**) disclosed some structure–activity relationships (SARs) in **1**.[5] In order to analyze more detailed SAR and access a more simplified lead compound, we undertook the synthesis of 4-deacetoxyagosterol A (**2**) from readily available ergosterol (**3**). Herein, we wish to describe the synthesis and the evaluation of MDR-modulating activity of **2**.

Our retrosynthetic analysis for 4-deacetoxyagosterol A (**2**) from **3** is illustrated in Chart 1. First, an oxidative disconnection of the $C_{22}-C_{23}$ double bond in **3** after protection of the diene portion affords a precursory aldehyde, which is further submitted to nucleophilic substitution by an alkyl metal reagent in accordance with Cram's rule, followed by inversion of the newly-formed hydroxyl group. Then, the 11α-hydroxyl group is built up by regioselective reductive epoxy cleavage of the 11α,12α-epoxide (ii) prepared from the 5,7,9(11)-triene (iii). Next, introduction of the 6α-OH group is conducted by regioselective hydroboration from the less hindered α-side. Finally, selective removal of the protecting group attached to the 3-OH function leads to the synthesis of **2**. This strategy was executed as follows.

The diene portion of the MOM ether of ergosterol (**3**) was masked with 1,4-dihydrophthalazine-1,4-dione prepared from the corresponding tetrahydro precursor and $Pb(OAc)_4$ in situ to give compound **4** in 82% yield from **3**. Selective ozonolysis of the $C_{22}-C_{23}$ double bond in the presence of pyridine provided an aldehyde in 86% yield, which was further subjected to Grignard reaction using 3-methylbutylmagnesium bromide to furnish a 22*S*-alcohol **5**[6] under control of Cram's rule. Inversion of the 22-hydroxyl group in **5** by the conventional Mitsunobu reaction[7] using triphenylphosphine, diethyl azodicarboxylate (DEAD), and 4-nitrobenzoic acid gave a 22*R* benzoate in poor yield (20%). As a result of examining several reaction conditions, the combined usage of *N*,*N*,*N*′,*N*′-tetramethylazodicarboxamide (TMAD), trimethylphosphine, and 4-methoxybenzoic acid[8] provided the desired ester[6] in 66% yield. In addition, the 18-Crown-6 catalyzed CsOAc treatment of the chloromethanesulfonate[9] of **5** also furnished the acetate of the desired alcohol in 56% yield. The 4-methoxybenzoate with 22*R*-configuration was subjected to

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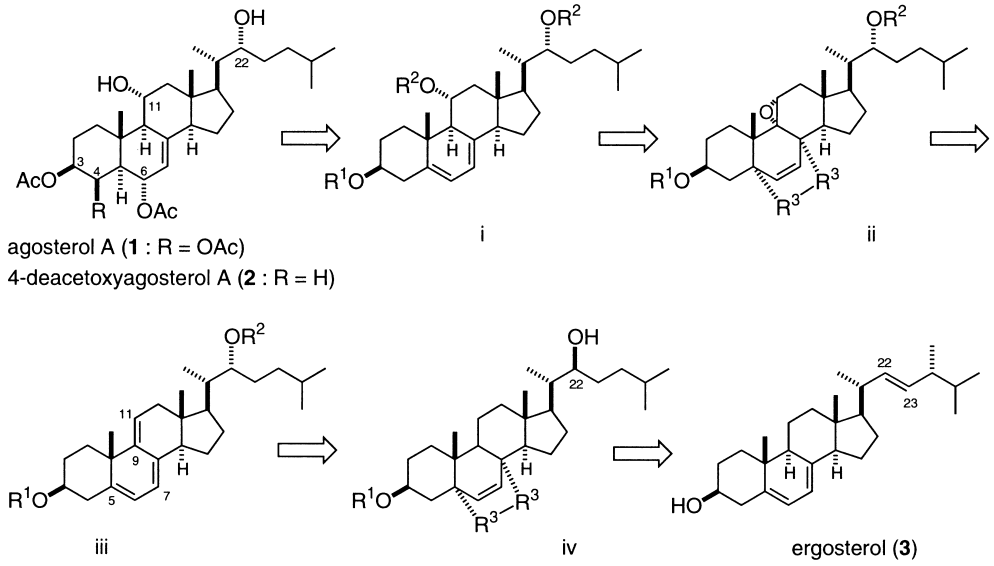
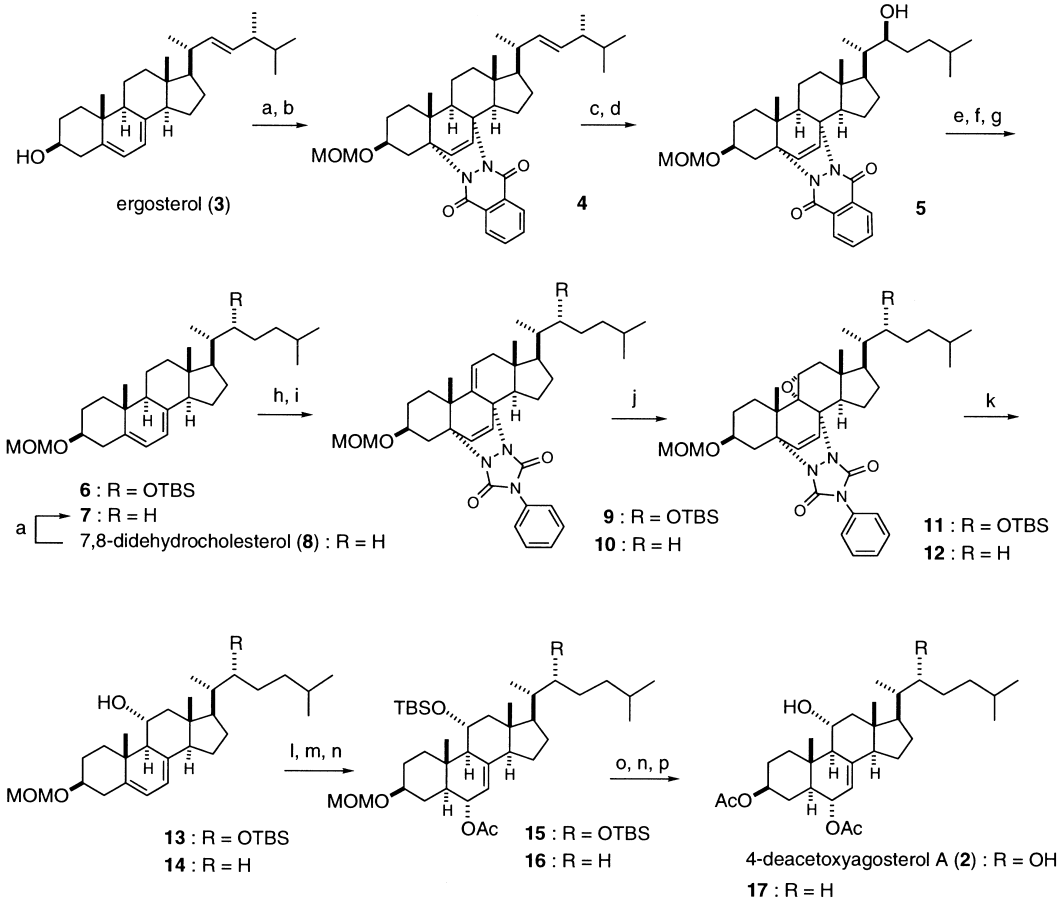


Chart 1.



Scheme 1. Reagents and conditions: (a) MOMCl, $^{i}Pr_2NEt$, CH_2Cl_2, 96% from **8**; (b) phthalhydrazide, $Pb(OAc)_4$, CH_2Cl_2–AcOH, 2 steps, 82% from **3**; (c) O_3, CH_2Cl_2–Py; (d) 3-methylbutylmagnesium bromide, THF, 2 steps, 77%; (e) TMAD, PMe_3, *p*-OCH_3-BzOH, THF, 66%; (f) $LiAlH_4$, THF, 89%; (g) TBSOTf, 2,6-lutidine, DMF, 91%; (h) $Hg(OAc)_2$, EtOH–$CHCl_3$–AcOH; (i) 4-phenyl-1,2,4-triazoline-3,5-dione, CH_2Cl_2, **9**: 2 steps, 71%, **10**: 2 steps, 74%; (j) *m*CPBA, $CHCl_3$, **11**: 65%, **12**: 60%; (k) $LiAlH_4$, Et_2AlCl, THF, 84% from **11**, 90% from **12**; (l) TBSOTf, 2,6-lutidine, toluene, **13**: 95%, **14**: 96%; (m) BH_3–Me_2S, THF; (n) Ac_2O–Py, **15**: 2 steps, 65%, **16**: 2 steps, 73%; (o) TMSBr, CH_2Cl_2, 96% from **15**, 85% from **16**; (p) HF–Py, THF, **2**: 2 steps, 94%, **17**: 2 steps, 81%.

$LiAlH_4$ reduction to furnish a diene alcohol, which was further treated with *t*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) and 2,6-lutidine to give a TBS ether **6**.

Dehydrogenation of **6** with $Hg(OAc)_2$[10] followed by selective protection of the homoannular 5,7-diene by 4-phenyl-1,2,4-triazoline-3,5-dione gave **9** in 71% yield for two steps. Epoxidation of **9** with *m*-chloroperbenzoic acid in $CHCl_3$ predominantly afforded the 9α,11α-epoxide **11**,[11] which was further subjected to reductive cleavage of the epoxy ring and diene-deprotection by use of $LiAlH_4$ to give the desired 11α-alcohol **13** in unsatisfactory yield (48%). For the purpose of overcoming this undesirable outcome, we examined carefully this reaction condition utilizing **12** as a model substrate. The epoxide **12** was prepared from 7,8-didehydrocholesterol (**8**) through the same three-step transformation after protection of the 3-OH group as a MOM ether. Intensive investigation of the reaction conditions led us to find a facile Et_2AlCl-catalyzed $LiAlH_4$-reductive epoxy cleavage method, which proceeded with concomitant removal of the triazolidine group to furnish a 11α-hydroxy-5,7-diene (**14**) from **12** in 90% yield. Namely, after pre-treatment of **12** with Et_2AlCl in THF under reflux, the reaction mixture was refluxed with $LiAlH_4$ to furnish **14**. Application of this method to **11** resulted in generation of **13** in nearly the same yield (84%). The stereochemistry at C-9 in **13** was confirmed to be *R* by the coupling constant (11 Hz) between 9-H and 11-H.

The 11α-hydroxyl group in **13** was protected as a TBS ether; then, the resulting diene with fully protected hydroxyl functions was submitted to hydroboration by a BH_3–Me_2S complex, followed by oxidation with H_2O_2 to afford an allyl alcohol with 6α-hydroxy-7-ene structure. Usual acetylation of the allyl alcohol provided **15** in 65% yield for two steps. The configuration at C-5 and C-6 was elucidated by the following spectral features: (1) in the ^1H NMR spectrum of **15**, the signal due to 6-H appeared at 5.02 ppm as a broad doublet with a coupling constant of 10 Hz; (2) the NOESY spectrum of **15** showed a distinct cross-peak between 6-H and 10-CH_3. Selective cleavage of the MOM group in **15** was achieved using TMSBr to give a monoalcohol in 96% yield. The monoalcohol was further subjected to successive acetylation using Ac_2O/pyridine and removal of the TBS group with HF–pyridine to furnish 4-deacetoxyagosterol A (**2**)[12] in 7.3% of total yield for 17 steps. With a view to our exploration for more straightforward lead compounds, the chemical transformation from **12** into a 22-deoxyanalogue (**17**)[13] of **2** was also carried out by the same procedure (Scheme 1).

Table 1. Reversal of MDR in KB-C2 and KB CV-60 cells by agosterol A and its derivatives

Compound	Dose (μg/mL)	Growth inhibition (%)		
		KB-3-1[a]	KB-C2[b]	KB CV-60[c]
1	10	13 ± 7	84 ± 2	76 ± 2
	3		82 ± 0	73 ± 0
	1		75 ± 4	66 ± 0
2	10	19 ± 5	87 ± 1	69 ± 2
	3	13 ± 4	76 ± 3	64 ± 1
	1		44 ± 1	59 ± 2
17	10	39 ± 7	88 ± 1	42 ± 7
	3	5 ± 10	69 ± 1	25 ± 4
	1		37 ± 0	15 ± 5

[a]Cytotoxicity of each compound.
[b]Growth inhibition in the presence of colchicine (0.1 μg/mL).
[c]Growth inhibition in the presence of vincristine (0.1 μg/mL).
Each value presents mean ± S.D. Colchicine and vincristine show no cytotoxicity against KB-C2 and KB CV-60 at 0.1 μg/mL dose, respectively.

Assessment of the MDR-modulating activity was made from the ability to potentiate the respective cytotoxicity of colchicine against KB-C2 cells[14] and that of vincristine against KB CV-60 cells[3] as compared with parental human epidermoid carcinoma KB-3-1 cells. The former MDR cell line was shown to overexpress P-gp, while the latter to overexpress MRP. Table 1 summarizes the MDR-modulating potency for agosterol A (**1**), 4-deacetoxy- (**2**), and 4-deacetoxy-22-deoxy-congener (**17**). In a previous study of SAR,[5] we found that both the three acetoxyl groups in ring AB and the two hydroxyl groups are crucial for the MDR-modulating activity. Compound **17** lacking both 4-acetoxy and 22-hydroxyl groups showed not only serious reduction of MDR reversal properties but also enhancement of cytotoxicity against parental KB-3-1 cells. On the other hand, 4-deacetoxyagosterol A (**2**) mostly preserves the MDR-modulating potency of agosterol A (**1**). On the basis of these findings, the following SAR of **1** is assumed: (1) a 22-hydroxyl group is an important structural function for the MDR-modulating activity of **1**; (2) the MDR-modulating activity of **1** is minimally affected by an acetoxyl group on C-4. So far, there are few MDR-modulators inhibiting the function of MRP.[15,16] In this context, it should be noted that the activity of 4-deacetoxyagosterol A (**2**) to restore the sensitivity of KB CV-60 cells against vincristine is nearly as potent as that of agosterol A (**1**).

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6. The configurations at C-22 in **5** and the inversed 4-methoxybenzoate were confirmed by the modified Mosher method.[17] In the case of 4-methoxybenzoate, this methodology was applied to the diene alcohol obtained by $LiAlH_4$ treatment.
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11. The stereochemistry on the epoxy moiety was elucidated by NOE enhancements between 11β-H and 10, 13-CH_3 protons.
12. Colorless amorphous solid, $[\alpha]_D$ +47.6° ($c=0.39$, $CHCl_3$, 27 °C). IR ν_{max} (KBr) cm^{-1}: 3476, 1732, 1252, 1030. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.57 (3H, s, H-18), 0.90, 0.91 (both 3H, d, $J=6.7$ Hz, H-26, 27), 0.95 (3H, d, $J=6.7$ Hz, H-21), 1.04 (3H, s, H-19), 2.03 (3H, s, 3-*O*-Ac), 2.07 (3H, s, 6-*O*-Ac), 2.33 (1H, dd, $J=12.1$, 5.1 Hz, H-12β), 2.51 (1H, dt, $J=14.0$, 3.5 Hz, H-1β), 3.60 (1H, brd, $J=11.1$ Hz, H-22), 3.97 (1H, brs, $W_{h/2}=23$ Hz, H-11), 4.65 (1H, m, H-3), 5.04 (1H, brd, $J=9.9$ Hz, H-6), 5.15 (1H, d, $J=1.6$ Hz, H-7). FAB-MS *m*/*z*: 541 $(M+Na)^+$. FAB-HRMS *m*/*z*: calcd for $C_{31}H_{50}O_6Na$: 541.3506, found: 541.3499.
13. Colorless amorphous solid, $[\alpha]_D$ +53.9° ($c=0.41$, $CHCl_3$, 27 °C). IR ν_{max} (KBr) cm^{-1}: 3470, 1734, 1244, 1030. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.55 (3H, s, H-18), 0.86, 0.87 (both 3H, d, $J=6.6$ Hz, H-26, 27), 0.93 (3H, d, $J=6.3$ Hz, H-21), 1.04 (3H, s, H-19), 2.03 (3H, s, 3-*O*-Ac), 2.07 (3H, s, 6-*O*-Ac), 2.33 (1H, dd, $J=12.1$, 5.3 Hz, H-12β), 2.51 (1H, dt, $J=14.1$, 3.5 Hz, H-1β), 3.96 (1H, brs, $W_{h/2}=24$ Hz, H-11), 4.68 (1H, m, H-3), 5.04 (1H, brd, $J=10.0$ Hz, H-6), 5.14 (1H, brs, H-7). FAB-MS *m*/*z*: 525 $(M+Na)^+$. FAB-HRMS *m*/*z*: calcd for $C_{31}H_{50}O_5Na$: 525.3556, found: 525.3550.
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