Total Synthesis of Agosterol A: an MDR-Modulator from a Marine Sponge

Nobutoshi Murakami, Masanori Sugimoto, Mari Morita, and Motomasa Kobayashi*[a]

Abstract: The first total synthesis of agosterol A, a modulator of multidrug resistance (MDR) mediated by P-gp and MRP1, and isolated from a marine sponge, was achieved from ergosterol by utilizing a regioselective epoxy-cleavage reaction and regioselective dehydroxylation as the key reactions.

Keywords: drug research · multidrug resistance · MRP1 · steroids · total synthesis

Introduction

The development of multidrug resistance (MDR), which is observed as a response to chemotherapy, is a very serious problem in the treatment of cancer.[1] The major documented mechanism by which tumor cells acquire this MDR phenotype is the increased expression of an ATP-dependent membrane glycoprotein, which serves as an efflux pump for antitumor agents.[2] Besides a representative and well-characterized glycoprotein called P-glycoprotein (P-gp),[2] an increased expression of a multidrug-resistance-associated protein (MRP1)[3] has been found in many non-P-gp mediated MDR cells. Since there have been few MDR-modulating substances mediated by MRP1, we have been searching for new modulators from extracts of marine invertebrates on the basis of bioassay. Recently, we characterized a potent modulator of MDR mediated by P-gp and MRP1 named agosterol A, which was isolated from a marine sponge: *Spongia sp.*, and investigated the structure–activity relationship by the use of naturally occurring congeners and synthetic analogues.[4, 5] Limited availability from natural sources compelled us to explore a synthetic protocol of agosterol A. As a result of preliminary study, we recently achieved the synthesis of a 4-deacetoxyl analogue of agosterol A.[6] In this report, we wish to present the first total synthesis of agosterol A from ergosterol.

Results and Discussion

Scheme 1 delineates our retrosynthetic analysis for agosterol A (**1**) from commercially available ergosterol (**2**); this includes the sequential, stereoselective introduction of four hydroxyl functions. In the first instance, an oxidative cleavage of the double bond between C22 and C23 in **2**, after protection of the diene portion, would afford a precursory aldehyde, which is further submitted to nucleophilic substitution by a Grignard reagent to give an undesired 22*(S)*-alcohol (**vi**) under the direction of Cram's rule. Thereafter, the 22*(S)*-hydroxyl group would be inverted to a 22*(R)*-hydroxyl group by the Mitsunobu reaction. The 11α-hydroxyl group would be built up by a regioselective, reductive epoxy-cleavage of the 9α,11α-epoxide (**iv**) prepared from the 5,7,9(11)-triene (**v**), which is obtained by dehydrogenation from the homoannular diene. After protection of the homoannular diene of **iii**, regioselective introduction of a C3=C4 double bond, followed by selective dihydroxylation would afford 3β,4β-dihydroxyl functions. Continuing on from this, a 6β-hydroxyl group would be introduced by regio- and stereo-selective hydroboration from the less-hindered α-side. Finally, differentiated removal of the protecting group and acetylation would lead to agosterol A (**1**). This strategy was executed as follows:

After protection of the hydroxyl group in ergosterol (**2**) as a methoxymethyl (MOM) ether, masking of the 5,7-diene portion with 4-phenyl-1,2,4-triazoline-3,5-dione,[7] a typical protecting group of *cisoid* dienes, provided a 6,22-diene derivative. However, further selective ozonolysis of the C22=C23 double bond proved to be unsuccessful. Therefore, the 5,7-diene portion of the MOM ether of **2** was protected with 1,4-dihydrophthalazine-1,4-dione,[8] prepared from the corresponding tetrahydro precursor and $Pb(OAc)_4$ in situ, to give compound **3** in 82% yield from **2** (Scheme 2). In contrast, ozonolysis of **3** in the presence of pyridine cleaved the C22=C23 double bond selectively to give an aldehyde in 90% yield. The aldehyde was further subjected to a Grignard reaction by using 3-methylbutylmagnesium bromide to furnish an undesired 22*(S)*-alcohol **4** under the direction of Cram's rule. Inversion of the 22-hydroxyl group in **4** by the conventional Mitsunobu reaction[9] with triphenylphosphine, diethyl azodicarboxylate (DEAD), and 4-nitrobenzoic acid gave a 22*(R)*-benzoate in poor yield (20%). As a result of

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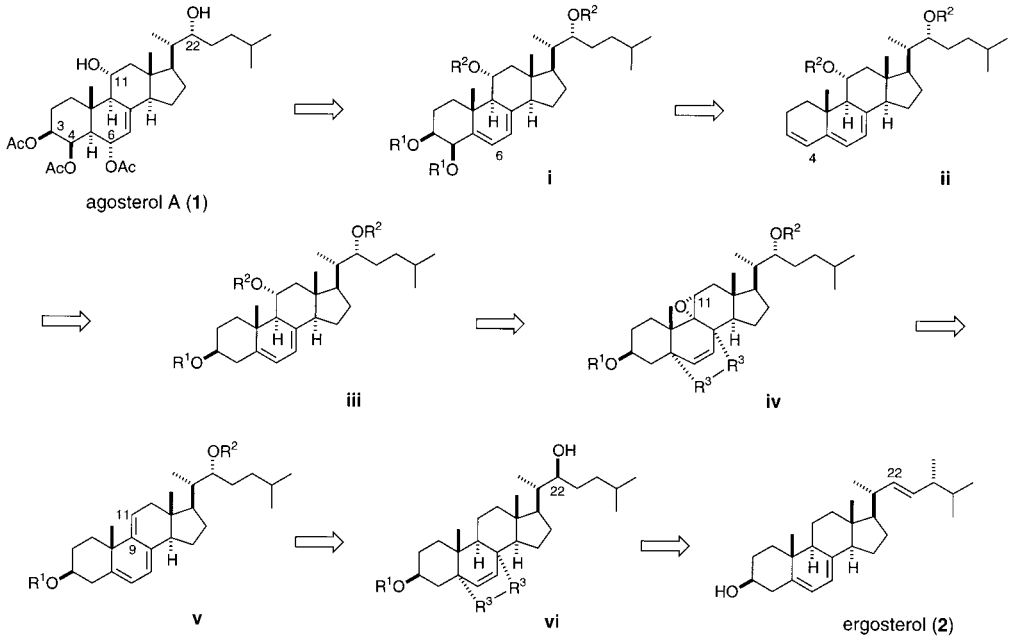
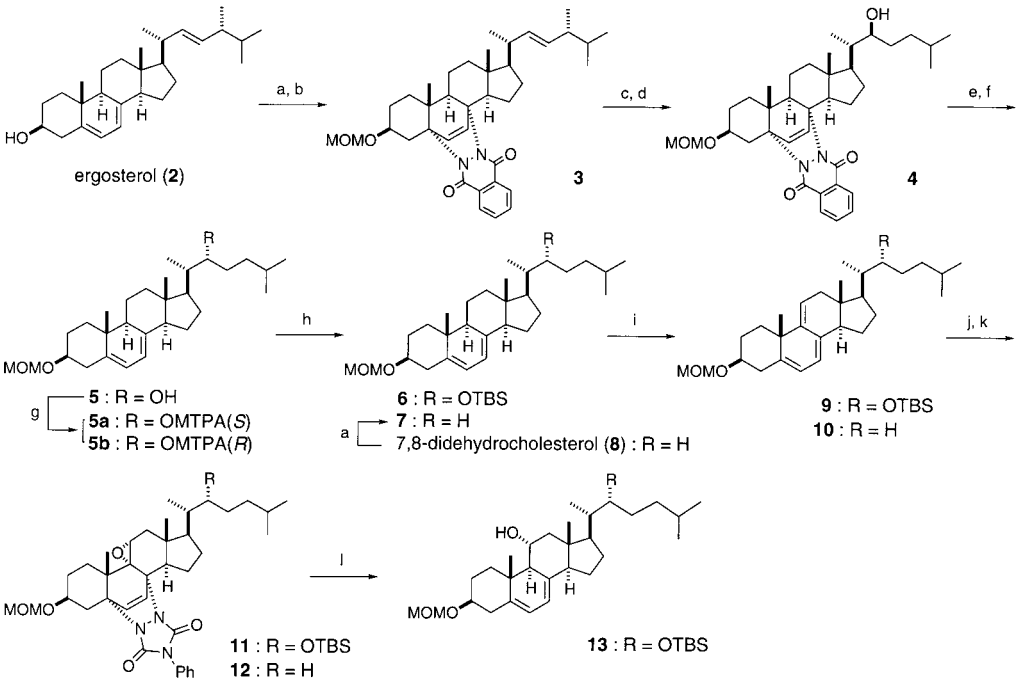


Scheme 1. Retrosynthetic steps for the synthesis of agosterol A from ergosterol.



Scheme 2. Reagents and conditions: a) MOMCl, iPr$_2$NEt, CH_2Cl_2, 96% from **8**; b) phthalhydrazide, $Pb(OAc)_4$, CH_2Cl_2-AcOH, two steps 82%; c) O_3, CH_2Cl_2-Py, then, Me_2S; d) 3-methylbutylmagnesium bromide, THF, two steps 90%; e) TMAD, PMe_3, pOCH$_3$BzOH, THF, 66%; f) $LiAlH_4$, THF, 89%; g) (*S*)-(−)-MTPA[(*R*)-(+)-MTPA], EDCI·HCl, DMAP, CH_2Cl_2, **5a**: 82%, **5b**: 76%; h) TBSOTf, 2,6-lutidine, DMF-CH_2Cl_2, 91%; i) $Hg(OAc)_2$, EtOH/$CHCl_3$/AcOH, **9**: 80%, **10**: 85%; j) 4-phenyl-1,2,4-triazoline-3,5-dione, CH_2Cl_2, 89% from **9**, 94% from **10**; k) mCPBA, $CHCl_3$, **11**: 71%, **12**: 64%; l) $LiAlH_4$, THF, 48% from **11**.

examining several reaction conditions, a combination of *N*,*N*,*N*′,*N*′-tetramethylazodicarboxamide (TMAD), trimethylphosphine, and 4-methoxybenzoic acid[10] provided the desired 22*(R)*-benzoate in 66% yield. On the other hand, CsOAc treatment of the chloromethanesulfonate[11] of **4** in the presence of [18]crown-6 furnished the acetate with 22*(R)* configuration in 56% yield. Reductive cleavage of both the 4-methoxybenzoyl group and the protecting group of the diene portion occurred spontaneously, and the configuration at the C22 hydroxyl group in **5** was confirmed by the modified Mosher method.[12] Namely, the proton signals due to the 23-H_2, 24-H_2, 26-H_3, and 27-H_3 of the (*R*)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) ester (**5b**) appeared at higher field than those of the (*S*)-MTPA ester

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(**5a**), whereas the signals ascribable to the protons 20-H, 21-H_3, and 18-H_3, settled in the left-side region of the 22-hydroxyl group in **5b**, were observed at lower field, as can be seen in Figure 1.

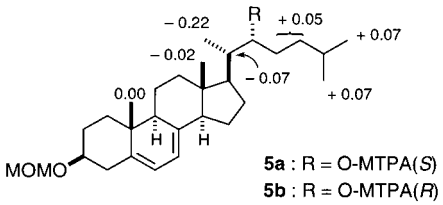


Figure 1. Distribution of $\Delta\delta(=\delta S-\delta R)$ values (ppm, 500 MHz).

After protection of the 22-hydroxyl group in **5** with *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) and 2,6-lutidine, dehydrogenation with $Hg(OAc)_2$[13] afforded a triene **9** in 73% yield over two steps. Protection of the homoannular 5,7-diene of **9** by using 4-phenyl-1,2,4-triazoline-3,5-dione, and subsequent epoxidation with *meta*-chloroperbenzoic acid in $CHCl_3$ predominantly afforded a 9α,11α-epoxide **11** in 71% yield. The stereochemistry on the epoxy moiety in **11** was established by NOE observation between the 11β-H, 19-H, and 18-H. Reductive cleavage of the epoxy ring and deprotection of the triazoline group in **11** by $LiAlH_4$ gave the desired 11α-alcohol **13** in unsatisfactory yield (48%).

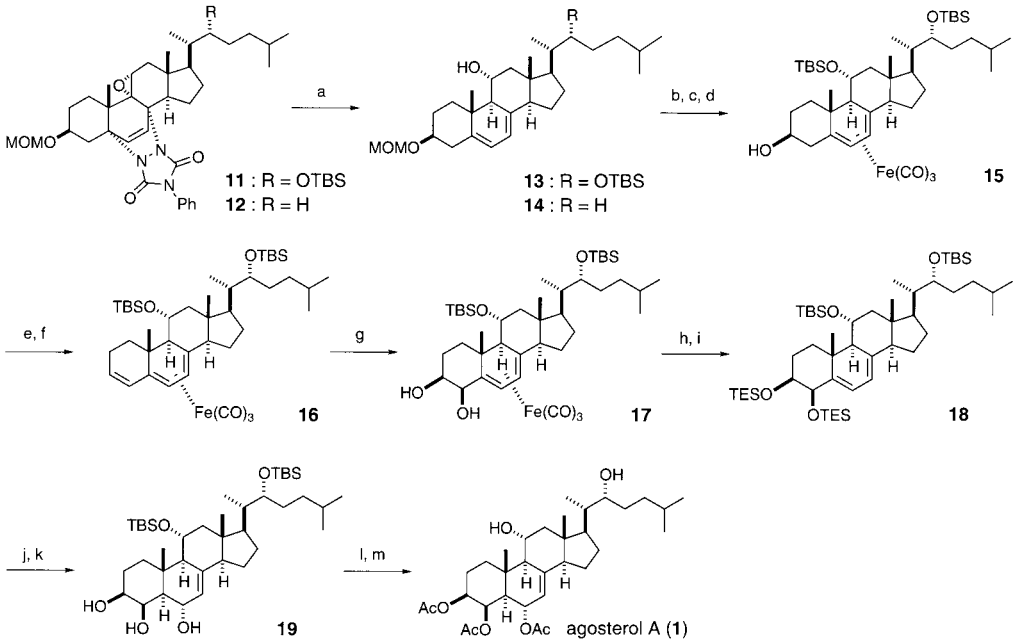
To overcome this undesirable outcome, we carefully examined the reaction conditions utilizing **12** as a model substrate. The epoxide **12** was prepared from 7,8-didehydrocholesterol (**8**) through the same three-step transformation, after protection of the 3-hydroxyl group in **8** as an MOM ether. The reported procedures to lead from the epoxide to an alcohol, in which BH_3-$LiBH_4$,[14] AlH_3,[15] potassium triphenylborohydride-Ph_3B,[16] and Cp_2TiCl_2-Mn-1,4-cyclohexadiene[17] were used, did not afford any satisfactory outcome. We also attempted the use of representative metal-hydride reagents such as Super-Hydride®, L-Selectride®, and Red-Al® for this conversion. Among them, only Red-Al gave the expected 11α-alcohol **14** in poor yield (16%).

This finding directed us to examine the cooperation of a Lewis acid with $LiAlH_4$. Three of the four Lewis acids tested (the exception being $TiCl_4$) afforded the 11α-alcohol **14** (Table 1, Scheme 3). In particular, Et_2AlCl enhanced the epoxy ring cleavage along with removal of the triazolidine group to furnish the desired 11α-alcohol **14** as compared with the reaction conditions in the absence of a Lewis acid (run 5). Intensive investigation of the reaction conditions revealed that the treatment of **12** with Et_2AlCl under reflux prior to addition of $LiAlH_4$ improved the yield of **14**, bringing it up to 90% (run 7). Replacement of $LiAlH_4$ by diisobutylaluminum hydride (DIBAL-H) or Red-Al resulted in a slight decrease in the yields of **14** (DIBAL-H: 80%; Red-Al: 52%). Hence,

Table 1. Investigation on regioselective reductive epoxy cleavage of **12**.

Run	Lewis Acid	Hydride	Pretreatment	Yield
1	–	$LiAlH_4$	–	46%
2	$BF_3\cdot Et_2O$	$LiAlH_4$	6 h	35%
3	$Al(OiPr)_3$	$LiAlH_4$	6 h	18%
4	$TiCl_4$	$LiAlH_4$	6 h	0%
5	Et_2AlCl	$LiAlH_4$	1 min	58%
6	Et_2AlCl	$LiAlH_4$	2 h	70%
7	Et_2AlCl	$LiAlH_4$	6 h	90%
8		$LiAlH_4$ + Et_2AlCl	–	10%

Each reaction was carried out in THF under reflux. After pre-treatment by 2 mol equiv. of Lewis acid, 10 mol equiv. of hydride reagent was added.



Scheme 3. Reagents and conditions: a) $LiAlH_4$, Et_2AlCl, THF, 84% from **11**, 90% from **12**; b) TBSOTf, 2,6-lutidine, toluene, 95% from **13**; c) $Fe(CO)_5$, 1-(4-methoxyphenyl)-4-phenyl-1-azabuta-(*E*, *E*)-1,3-diene, $PhCH_3$, 94%; d) $MgBr_2$-Et_2O, Me_2S, CH_2Cl_2, 73%; e) TsCl, Py, quant.; f) DBN, PhH, quant.; g) OsO_4, Py, then aq. $NaHSO_3$, 80%; h) TESOTf, DMAP, Py, quant.; i) Me_3NO, PhH, 95%; j) BH_3Me_2S, THF, then, H_2O_2, aq. NaOH, 62%; k) TBAF, THF, 90%; l) Ac_2O-Py, 91%; m) HF-Py, THF, 84%.

chelation between the epoxy oxygen and the aluminum atom might participate in this reductive epoxy-cleavage reaction. The stereochemistry at C9 in **14** was confirmed to be *R* by the coupling constant ($J = 9.3$ Hz) between 9-H and 11-H. This stereoselectivity indicates that the 11α-alcohol **14** will be given through protonation from the less hindered α-side to the C9 carbocation intermediate.

Application of this conversion condition to **11** furnished **13** in nearly the same yield (84%) as the preparation for **14**, without losing the TBS group.[18] After protection of the 11-hydroxyl group in **13** as a TBS ether, the 5,7-diene portion was masked by using pentacarbonyl iron and 1-(4-methoxyphenyl)-4-phenyl-1-azabuta-(*E*,*E*)-1,3-diene[19] to give a tricarbonyl iron complex in 90% yield for two steps. In contrast, replacement of the protecting group by phthalazine and triazoline afforded no desired 3-ene compounds because of the lability of both under basic conditions. The MOM group in the complex was removed by TMSBr[20] treatment to give an alcohol **15** in unsatisfactory yield (50%). In contrast, treatment with $MgBr_2$-etherate[21] and Me_2S afforded **15** in 73% yield without deprotection of the tricarbonyl iron moiety. Next, quantitative treatment of **15** with *para*-toluenesulfonyl chloride in pyridine provided the corresponding tosylate, which was further subjected to an elimination reaction in 2,6-lutidine solution to selectively give an undesired 2-ene derivative. On the other hand, treatment of the tosylate with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and 2,6-lutidine in toluene gave a 3-ene **16** and a 2-ene derivative in a ratio of 2:1 quantitatively. Detailed examination of the reaction conditions disclosed that the treatment of the tosylate with 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) in benzene under reflux furnished only the desired **16** in excellent yield.

Dihydroxylation of **16** with osmium tetraoxide proceeded stereoselectively from the β-side free from interruption by the tricarbonyl iron function to give a *cis*-3β,4β-diol **17** in 80% yield. The ^{1}H NMR spectrum of **17** exhibited two oxymethine signals due to the 3-H (br d, $J = 11.1$ Hz) and the 4-H (br s), indicating construction of the desired 3*(S)*, 4*(R)*-configurations. The newly generated hydroxyl groups in **17** were protected by triethylsilyl trifluoromethanesulfonate (TESOTf) and 4-dimethylaminopyridine (DMAP) in pyridine to give a di-TES ether. Subsequent cleavage of the tricarbonyl iron residue by using trimethylamine *N*-oxide[22] afforded a diene **18** (95% for two steps). Hydroboration of **18** by use of a borane dimethyl sulfide complex, followed by H_2O_2 treatment proceeded with moderate stereo- and regio-selectivity (62%) to give a 6α-alcohol. Deprotection of the TES groups by using tetrabutylammonium fluoride (TBAF)[23] provided a triol **19** in 90% yield. The stereochemistry on the C5 and C6 carbons in **19** was established to be α by the physico-chemical feature; three characteristic signals assignable to the oxymethine protons ($\delta = 3.56$, m, H3; $\delta = 4.29$, br s, H4; $\delta = 4.32$, br d, $J = 9.5$ Hz, H6) were observed in the ^{1}H NMR spectrum, and the chemical shifts and coupling constants resembled those of trideacetylagosterol A.[5] On acetylation with Ac_2O in pyridine, the triol **19** was converted to a triacetate, which was further subjected to HF-pyridine treatment[24] to furnish agosterol A (**1**) in 76% yield for two steps. The synthesized agosterol A was shown to be superimposable with the natural product[4] isolated from the marine sponge in comparison of the spectroscopic features such as ^{1}H NMR, ^{13}C NMR, IR, MS, and optical rotation data.

Conclusion

We have achieved the first total synthesis of agosterol A (**1**) from ergosterol (**2**), a readily available, naturally occurring sterol, in 3.5% total yield through 23 steps. The present synthetic protocol includes a regioselective, reductive epoxy-cleavage reaction and regioselective dehydroxylation as the key reactions.

Experimental Section

General: The following instruments were used to obtain physical data: a Jasco DIP-370 digital polarimeter for specific rotations, a JASCO FT/IR-5300 infrared spectrometer for IR spectra, a JEOLJMS SX-102 mass spectrometer for FAB-MS, a JEOLJNM LA-500 (500 MHz), a JEOLJNM-AL300 (300 MHz) spectrometer for ^{1}H NMR spectra (^{1}H NMR: $CDCl_3$ solution with tetramethylsilane (TMS) as internal standard unless otherwise specified), and a Yanaco CHN CORDER(MT-5) for elemental analysis. All new compounds bearing the 5,7-conjugated diene portion were characterized by high-resolution FAB-MS because of the lability in light; HPLC was performed by using an Hitachi L-6000 pump equipped with an Hitachi L-4000H UV detector. Silica gel (Merck 60–230 mesh) and precoated thin-layer chromatography plates (Merck, Kiesel gel, $60F_{254}$) were used for column chromatography and TLC, respectively. Spots on TLC plates were detected by spraying $Ce(SO_4)_2/H_2SO_4$ [$Ce(SO_4)_2 \cdot nH_2O$ (10 g) in 6.3% aqueous H_2SO_4 (1.0 L)] or acidic *para*-anisaldehyde solution [*para*-anisaldehyde (25 mL), *c*-H_2SO_4 (25 mL), AcOH (5 mL), EtOH (425 mL)] with subsequent heating.

Conversion from ergosterol (2) to compound 3: iPr$_2$NEt (20 mL, 115 mmol) was added to a solution of ergosterol (15.2 g, 38.3 mmol) in CH_2Cl_2 (190 mL) at 0 °C, then the whole mixture was stirred for 5 min. Methoxymethyl (MOM) chloride (7.2 mL, 96 mmol) was added to the reaction mixture at 0 °C, then the whole mixture was stirred at room temperature in the dark for a further 36 h. The reaction mixture was poured into saturated aqueous NH_4Cl, then the whole mixture was extracted with EtOAc. The EtOAc extract was successively washed with 5% aqueous HCl and saturated aqueous NaCl, then dried over $MgSO_4$. Removal of the solvent from the EtOAc extract under reduced pressure gave a MOM ether (19.4 g). A solution of $Pb(OAc)_4$ (28.4 g, 64 mmol) in CH_2Cl_2 (420 mL) and glacial AcOH (5.0 mL) was slowly added to a solution of the MOM ether (18.8 g) and phthalhydrazide (27.9 g, 172 mmol) in CH_2Cl_2 (630 mL), keeping the temperature between -5 °C and 0 °C. Then the whole mixture was stirred at 0 °C for 1 h. The reaction was quenched with Al_2O_3 (76 g, 745 mmol) and stirred for 30 min. After filtration, the filtrate was successively washed with H_2O, saturated aqueous $NaHCO_3$, and saturated aqueous NaCl, then dried over $MgSO_4$. Removal of the solvent from the CH_2Cl_2 layer under reduced pressure gave a crude product, which was purified by column chromatography (SiO_2 240 g, *n*-hexane/EtOAc 4:1) to furnish compound **3** as a yellow amorphous solid. Yield: 18.2 g, 82%; $[\alpha]_D^{23} = -126.8°$ ($c = 1.89$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 8.13$ (m, 2H; Ph*H*), 7.32 (m, 2H; Ph*H*), 6.65, 6.27 (both d, $J = 7.9$ Hz, 1H; H6,7), 5.22 (dd, $J = 15.2$, 7.9 Hz, 1H; H22 or 23), 5.14 (dd, $J = 15.2$, 7.3 Hz, 1H; H22 or 23), 4.72, 4.63 (ABq, $J = 6.7$ Hz, 2H; 3-OCH_2OCH$_3$), 3.54 (m, 1H; H3), 3.34 (s, 3H; 3-OCH$_2$OCH_3), 1.02 (s, 3H; H19), 1.02, 0.90 (both d, $J = 6.7$ Hz, 3H; H21,28), 0.84 (s, 3H; H18), 0.83, 0.81 (both d, $J = 6.7$ Hz, 3H; H26,27); IR $\tilde{\nu}_{max}$ (KBr): 2955, 1653, 1310, 1044 cm^{-1}; FAB-MS *m*/*z*: 601 $[M+H]^+$; FAB-HRMS *m*/*z*: calcd for $C_{38}H_{53}N_2O_4$: 601.4005, found: 601.3994; elemental analysis calcd (%) for $C_{38}H_{52}N_2O_4$ (600.8): C 75.96, H 8.72, N 4.66; found C 76.31, H 8.63, N 4.88.

Ozonolysis of compound 3 followed by Grignard reaction giving compound 4: Ozone gas was bubbled through a solution of compound **3** (15.5 g, 25.8 mmol) in dry CH_2Cl_2 (1.5 L) and pyridine (44 mL, 541 mmol) at

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$-60\,^{\circ}C$ for 3 h. The excess O_3 was removed by bubbling with Ar, then the whole mixture was treated with Me_2S (9.4 mL, 129 mmol) at $-60\,^{\circ}C$ for 30 min. Removal of the solvent from the reaction mixture under reduced pressure gave a crude aldehyde (18.3 g). Mg (22.7 g, 0.95 mol) was activated by heating with a burner then cooled, and THF (630 mL) was added. 3-Methylbutylbromide (78.6 mL, 0.63 mol) was slowly added to this suspension, and the mixture was stirred for 1 h to give a solution of 3-methylbutylmagnesium bromide (1.0 M in THF). Some of this solution (153 mL, 153 mmol) was added to a solution of the crude aldehyde (18.3 g) in THF (220 mL) at $-78\,^{\circ}C$, then the whole mixture was stirred for 1 h. The reaction mixture was quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The EtOAc extract was successively washed with saturated aqueous $Na_2S_2O_3$ and saturated aqueous NaCl, then dried over $MgSO_4$. Removal of the solvent from the EtOAc extract under reduced pressure gave a crude product, which was purified by column chromatography (SiO_2 200 g, PhH/acetone 10:1) to furnish compound **4** as a yellow amorphous solid. Yield: 12.1 g, 90 %; $[\alpha]_D^{23} = -114.6^{\circ}$ ($c = 0.61$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 8.14$ (m, 2H; Ph*H*), 7.64 (m, 2H; Ph*H*), 6.66, 6.27 (both d, $J = 7.9$ Hz, 1H; H6,7), 4.72, 4.63 (ABq, $J = 6.7$ Hz, 2H; 3-OCH_2OCH$_3$), 3.63 (m, 1H; H22), 3.54 (m, 1H; H3), 3.35 (s, 3H; 3-OCH$_2$OCH_3), 1.03 (s, 3H; H19), 0.91 (d, $J = 6.7$ Hz, 3H; H21), 0.89 (d, $J = 6.7$ Hz, 6H; H26, 27), 0.81 (s, 3H; H18); IR $\tilde{\nu}_{max}$ (KBr) = 3482, 2951, 1651, 1312 cm^{-1}; FAB-MS *m*/*z*: 605 $[M+H]^+$; FAB-HRMS *m*/*z*: calcd for $C_{37}H_{53}N_2O_5$: 605.3954, found: 605.3959; elemental analysis calcd (%) for $C_{37}H_{52}N_2O_5$ (604.8): C 73.48, H 8.67, N 4.63; found C 73.73, H 8.73, N 4.81.

Inversion of hydroxyl group in compound 4 followed by $LiAlH_4$ reduction: PMe_3 (4.5 mL, 43 mmol) was added to a solution of compound **4** (11.9 g, 19.6 mmol) and *para*-anisic acid (6.6 g, 43 mmol) in THF (56 mL), then TMAD (7.4 g, 43 mmol) was added to the reaction mixture at 0 °C and this was stirred for 10 min. The whole mixture was stirred at room temperature for 20 min, then warmed to 50 °C and stirred at this temperature for 1.5 h. Removal of the solvent from the reaction mixture under reduced pressure gave a crude product, which was purified by column chromatography (SiO_2 150 g, *n*-hexane/EtOAc 3:1) to furnish a *para*-anisyl ester (9.6 g, 66 %). A solution of the *para*-anisyl ester (9.5g, 12.9 mmol) in THF (193 mL) was treated with $LiAlH_4$ (4.9 g, 129 mmol) under reflux for 2 h in the dark. The reaction mixture was slowly quenched with 0.5 N aqueous potassium sodium tartrate at 0 °C, then the whole mixture was extracted with EtOAc. The EtOAc extract was washed with saturated aqueous NaCl, then dried over $MgSO_4$. Removal of the solvent from the EtOAc extract under reduced pressure gave a crude product, which was purified by column chromatography (SiO_2 120 g, *n*-hexane/EtOAc 6:1) to furnish compound **5** as a colorless amorphous solid. Yield: 5.1 g, 89 %; $[\alpha]_D^{23} = -46.2^{\circ}$ ($c = 1.13$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 5.56$ (dd, $J = 5.5$, 1.9 Hz, 1H; H6), 5.38 (dt, $J = 5.5$, 2.6 Hz, 1H; H7), 4.72, 4.70 (ABq, $J = 6.7$ Hz, 2H; 3-OCH_2OCH$_3$), 3.63 (m, 1H; H22), 3.49 (m, 1H; H3), 3.38 (s, 3H; 3-OCH$_2$OCH_3), 0.95 (d, $J = 7.1$ Hz, 3H; H21), 0.94 (s, 3H; H19), 0.91, 0.90 (both d, $J = 6.4$ Hz, 3H; H26,27), 0.64 (s, 3H; H18); IR $\tilde{\nu}_{max}$ (KBr) = 3482, 2946, 2876, 1462, 1044 cm^{-1} ; FAB-MS *m*/*z*: 467 $[M+Na]^+$; FAB-HRMS *m*/*z*: calcd for $C_{29}H_{48}O_3Na$: 467.3502, found: 467.3503.

Preparation of (*S*)- and (*R*)-MTPA esters of compound 5: DMAP (2.7 mg, 0.022 mmol), EDCI·HCl (6.3 mg, 0.033 mmol) and (*S*)-MTPA (6.6 mg, 0.028 mmol) were successively added to a solution of compound **5** (5.0 mg, 0.011 mmol) in CH_2Cl_2 (1.0 mL), then the whole mixture was stirred at room temperature for 9 h in the dark. The reaction mixture was poured into saturated aqueous NH_4Cl, then the whole mixture was extracted with EtOAc. The EtOAc extract was washed with saturated aqueous NaCl, then dried over $MgSO_4$. Removal of the solvent from the EtOAc extract under reduced pressure gave a crude product, which was purified by column chromatography (SiO_2 2 g, *n*-hexane/EtOAc 10:1) to furnish an (*S*)-MTPA ester as a colorless amorphous solid. Yield: 5.8 mg, 82 %; $[\alpha]_D^{23} = -67.3^{\circ}$ ($c = 0.44$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 7.54$ (m, 2H; (OCH$_3$)*Ph*CF$_3$), 7.39 (m, 3H; (OCH$_3$)*Ph*CF$_3$), 5.58 (dd, $J = 5.5$, 1.9 Hz, 1H; H6), 5.40 (dt, $J = 5.5$, 2.5 Hz, 1H; H7), 5.08 (br d, $J = 10.5$ Hz, 1H; H22), 4.72, 4.70 (ABq, $J = 6.6$ Hz, 2H; 3-OCH_2OCH$_3$), 3.56 (s, 3H; (OCH_3)PhCF$_3$), 3.52 (m, 1H; H3), 3.38 (s, 3H; 3-OCH$_2$OCH_3), 1.83 (m, 1H; H20), 1.48 (m, 4H; H23, 24), 0.95 (s, 3H; H19), 0.87, 0.85 (both d, $J =$ 6.6 Hz, 3H; H26,27), 0.75 (d, $J = 6.6$ Hz, 3H; H21), 0.62 (s, 3H; H18); IR $\tilde{\nu}_{max}$ (KBr) = 2949, 1742, 1454, 1269, 1169 cm^{-1}; FAB-MS *m*/*z*: 683 $[M+Na]^+$; FAB-HRMS *m*/*z*: calcd for $C_{39}H_{55}F_3O_5Na$: 683.3900, found: 683.3889.

An (*R*)-MTPA ester (**5b**), a colorless amorphous solid, was prepared from **5** (4.7 mg, 0.0106 mmol) in the same manner. Yield: 5.1 mg, 76 %; $[\alpha]_D^{20} = -14.5^{\circ}$ ($c = 0.19$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 7.54$ (m, 2H; (OCH$_3$)*Ph*CF$_3$), 7.39 (m, 3H; (OCH$_3$)*Ph*CF$_3$), 5.58 (dd, $J = 5.5$, 1.9 Hz, 1H; H6), 5.41 (dt, $J = 5.5$, 2.5 Hz, 1H; H7), 5.10 (br d, $J = 10.2$ Hz, 1H; H22), 4.72, 4.70 (ABq, $J = 6.6$ Hz, 2H; 3-OCH_2OCH$_3$), 3.58 (s, 3H; (OCH_3)PhCF$_3$), 3.53 (m, 1H; H3), 3.38 (s, 3H; 3-OCH$_2$OCH_3), 1.90 (m, 1H; H20), 1.43 (m, 4H; H23,24), 0.97 (d, $J = 6.6$ Hz, 3H; H21), 0.95 (s, 3H; H19), 0.80 (d, $J = 6.4$ Hz, 3H; H27), 0.75 (d, $J = 6.4$ Hz, 3H; H26), 0.64 (s, 3H; H18),; IR $\tilde{\nu}_{max}$ (KBr) = 2949, 1736, 1454, 1269, 1167 cm^{-1}; FAB-MS *m*/*z*: 683 $[M+Na]^+$; FAB-HRMS *m*/*z*: calcd for $C_{39}H_{55}F_3O_5Na$: 683.3899, found: 683.3901.

Conversion from compound 5 to compound 9: 2,6-Lutidine (2.4 mL, 20.4 mmol), then TBSOTf (4.2 mL, 18.4 mmol) were added to a solution of compound **5** (4.55 g, 10.2 mmol) in DMF/CH_2Cl_2 (2:1, 93 mL) at room temperature and the whole mixture was stirred for 1 h in the dark. The reaction mixture was poured into saturated aqueous NH_4Cl, then the whole mixture was extracted three times with EtOAc/$PhCH_3$ (1:1). The organic layer was washed with saturated aqueous NaCl, then dried over $MgSO_4$. Removal of the solvent from the organic layer under reduced pressure gave a crude product, which was purified by column chromatography (SiO_2 100 g, *n*-hexane/EtOAc 30:1) to furnish compound **6** (5.2 g, 91 %). A solution of compound **6** (5.2 g, 9.25 mmol) in EtOH/AcOH/$CHCl_3$ (100:0.056:70, 263 mL) was treated with $Hg(OAc)_2$ (14.7 g, 46.3 mmol) at room temperature for 17 h in the dark. After filtration through a SiO_2 column (25 g) with EtOAc/$CHCl_3$ (1:1) as eluant, removal of the solvent from the filtrate under reduced pressure gave a crude product, a solution of which in $CHCl_3$ was passed through a Florisil® column to give a triene **9** (5.1 g). Since **9** was partly degraded in the SiO_2 column chromatography, the next transformation was carried out without further purification, except for separating the sample for characterization. An aliquot of the crude product (21 mg) was purified by SiO_2 column chromatography (SiO_2 2 g, *n*-hexane/EtOAc 20:1) to furnish a triene **9** as a colorless amorphous solid. Yield: 17 mg, 80 %; $[\alpha]_D^{22} = +152.9^{\circ}$ ($c = 1.49$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 5.68$, 5.50, 5.40 (all br d, $J =$ ca. 6 Hz, 1H; H6,7,11), 4.69 (br s, 2H; 3-OCH_2OCH$_3$), 3.90 (br d, $J = 8.7$ Hz, 1H; H22), 3.49 (m, 1H; H3), 3.37 (s, 3H; 3-OCH$_2$OCH_3), 1.25 (s, 3H; H19), 0.87 (m, 18H; 22-OSi(CH$_3$)$_2$C(CH_3)$_3$, H21,26,27), 0.56 (s, 3H; H18), 0.03 (s, 6H; 22-OSi(CH_3)$_2$C(CH$_3$)$_3$); IR $\tilde{\nu}_{max}$ (KBr) = 2951, 1464, 1254, 1044 cm^{-1}; FAB-MS *m*/*z*: 579 $[M+Na]^+$; FAB-HRMS *m*/*z*: calcd for $C_{35}H_{60}O_3SiNa$: 579.4210, found: 579.4194.

Conversion from 7,8-didehydrocholesterol (8) to compound 10: The same treatment of compound **8** (2.9 g, 7.8 mmol) as in the preparation for the MOM ether of compound **2** gave a crude product, which was purified by column chromatography (SiO_2 70 g, *n*-hexane/EtOAc 30:1) to furnish **7** (3.07 g, 96 %). The MOM ether **7** (1.65 g, 3.86 mmol) was transformed to a triene **10** (1.4 g) in the same manner as for the preparation for compound **9**. Because of the same property as compound **9**, the triene **10** was also converted without further purification. An aliquot of the crude product (20 mg) was purified by column chromatography (SiO_2 2 g, *n*-hexane/EtOAc 20:1) to furnish compound **10** as a colorless amorphous solid. Yield: 16 mg, 85 %; $[\alpha]_D^{23} = +162.5^{\circ}$ ($c = 4.59$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 5.67$, 5.50, 5.40 (all br d, $J =$ ca. 6 Hz, 1H; H6,7,11), 4.68 (br s, 2H; 3-OCH_2OCH$_3$), 3.49 (m, 1H; H3), 3.37 (s, 3H; 3-OCH$_2$OCH_3), 1.24 (s, 3H; H19), 0.92 (d, $J = 6.1$ Hz, 3H; H21), 0.87, 0.86 (both d, $J = 6.7$ Hz, 3H; H26,27), 0.56 (s, 3H; H18); IR $\tilde{\nu}_{max}$ (KBr) = 2949, 1466, 1148, 1105, 1044 cm^{-1} ; FAB-MS *m*/*z*: 449 $[M+Na]^+$; FAB-HRMS *m*/*z*: calcd for $C_{29}H_{46}O_2Na$: 449.3395, found: 449.3385.

Conversion from compound 9 to compound 11: A solution of compound **9** (4.8 g) in CH_2Cl_2 (121 mL) was treated with 4-phenyl-1,2,4-triazoline-3,5-dione (1.53 g, 8.72 mmol) at room temperature for 1 h. Removal of the solvent from the reaction mixture under reduced pressure gave a crude product, which was purified by column chromatography (SiO_2 100 g, *n*-hexane/EtOAc 5:1) to furnish a Diels–Alder adduct (4.8 g, 71 % from compound **6**). A solution of the Diels–Alder adduct of compound **9** (4.6 g, 6.34 mmol) in $CHCl_3$ (127 mL) was treated with 3-chloro peroxybenzoic acid (*m*CPBA) (10.9 g, 63.4 mmol) at room temperature for 17 h. The reaction mixture was poured into 20 % aqueous K_2CO_3, then the whole mixture was extracted with EtOAc. The EtOAc extract was successively washed with saturated aqueous $NaHCO_3$ and saturated aqueous $Na_2S_2O_3$/saturated aqueous NaCl (1:1), then dried over $MgSO_4$. Removal of the

solvent from the EtOAc extract under reduced pressure gave a crude product, which was purified by column chromatography (SiO_2 120 g, *n*-hexane/EtOAc 2:1) to furnish a mixture of compound **11** and an 9(11)-en-6,7-epoxide isomer in a ratio of 3:1 (4.5 g, 95%). An aliquot of this mixture (20 mg) was purified by HPLC [column: COSMOSIL 5C18-AR-II (20 mm ø × 250 mm), mobile phase: MeOH/H_2O 99:1, detection: UV ($\lambda = 230$ nm), flow rate: 5.0 mL min^{-1}] to furnish compound **11** (14 mg) and the 9(11)-en-6,7-epoxide isomer (4.7 mg) both as colorless amorphous solids.

Compound **11**: $[\alpha]_D^{22} = -58.3^\circ$ ($c = 1.22$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 7.42$ (m, 4H; Ph*H*), 7.31 (m, 1H; Ph*H*), 6.53, 6.30 (both d, $J =$ 8.5 Hz, 1H; H6,7), 4.80, 4.72 (ABq, $J = 6.6$ Hz, 2H; 3-OCH_2OCH$_3$), 4.27 (m, 1H; H3), 3.57 (dt, $J = 8.2$, 3.6 Hz, 1H; H22), 3.38 (s, 3H; 3-OCH$_2$OCH_3), 3.25 (br d, $J = 5.6$ Hz, 1H; H11), 1.09 (s, 3H; H19), 0.90 (d, $J = 6.6$ Hz, 3H; H21), 0.88 (s, 9H; 22-OSi(CH$_3$)$_2$C(CH_3)$_3$), 0.86, 0.85 (both d, $J = 6.6$ Hz, 3H; H26,27), 0.75 (s, 3H; H18), 0.03, 0.02 (both s, 3H; 22-OSi(CH_3)$_2$C(CH$_3$)$_3$); IR $\tilde{\nu}_{max}$ (KBr) = 2953, 1759, 1711, 1399, 1046 cm^{-1}; FAB-MS *m*/*z*: 770 [*M*+Na]$^+$; FAB-HRMS *m*/*z*: calcd for $C_{43}H_{65}N_3O_6SiNa$: 770.4540, found: 770.4513; elemental analysis calcd (%) for $C_{43}H_{65}N_3O_6Si$ (748.1): C 69.04, H 8.76, N 5.62; found C 68.74, H 8.73, N 5.29.

9(11)-En-6,7-epoxide: $[\alpha]_D^{23} = -19.8^\circ$ ($c = 0.64$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 7.44$ (m, 4H; Ph*H*), 7.34 (m, 1H; Ph*H*), 5.78 (br d, $J = 5.5$ Hz, 1H; H11), 4.79, 4.68 (ABq, $J = 6.6$ Hz, 2H; 3-OCH_2OCH$_3$), 4.03 (m, 1H; H3), 3.59 (m, 1H; H22), 3.37 (s, 3H; 3-OCH$_2$OCH_3), 3.62, 3.35 (both d, $J = 4.6$ Hz, 1H; H6,7), 1.36 (s, 3H; H19), 0.91 (d, $J = 6.8$ Hz, 3H; H21), 0.89 (s, 9H; 22-OSi(CH$_3$)$_2$C(CH_3)$_3$), 0.86, 0.85 (both d, $J = 6.4$ Hz, 3H; H26,27), 0.81 (s, 3H; H18), 0.03 (s, 6H; 22-OSi(CH_3)$_2$C(CH$_3$)$_3$); IR $\tilde{\nu}_{max}$ (KBr) = 2951, 1765, 1713, 1399, 1044 cm^{-1}; FAB-MS *m*/*z*: 748 [*M*+H]$^+$; FAB-HRMS *m*/*z*: calcd for $C_{43}H_{66}N_3O_6Si$: 748.4721, found: 748.4724; elemental analysis calcd (%) for $C_{43}H_{65}N_3O_6Si$ (748.1): C 69.04, H 8.76, N 5.62; found C 69.19, H 8.81, N 5.72.

Conversion from compound 10 to compound 12: The same treatment of compound **10** (1.36 g, 3.18 mmol) as in the preparation for compound **11** gave a crude product, which was purified by column chromatography (SiO_2 36 g, *n*-hexane/EtOAc 7:2) to furnish a Diels–Alder adduct of compound **10** (1.8 g, 94%). Epoxidation of the 22-deoxy congener was conducted in the same manner as for the preparation of compound **11** to furnish compound **12** and an 6,7-epoxide isomer in a ratio of 4:1 (1.3 g, 80%) through chromatographic purification (SiO_2 40 g, *n*-hexane/EtOAc 5:2). An aliquot of this mixture (21 mg) was purified by HPLC [column: COSMOSIL 5C18-AR-II (20 mm ø × 250 mm), mobile phase: MeOH/H_2O 99:1, detection: UV ($\lambda = 230$ nm), flow rate: 5.0 mL min^{-1}] to furnish compound **12** (15 mg) and the 9(11)-en-6,7-epoxide isomer (3.8 mg) as colorless amorphous solids.

Compound **12**: $[\alpha]_D^{21} = -91.9^\circ$ ($c = 1.07$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 7.42$ (m, 4H; Ph*H*), 7.31 (m, 1H; Ph*H*), 6.54, 6.29 (both d, $J =$ 8.4 Hz, 1H; H6,7), 4.81, 4.72 (ABq, $J = 6.8$ Hz, 2H; 3-OCH_2OCH$_3$), 4.27 (m, 1H; H3), 3.38 (s, 3H; 3-OCH$_2$OCH_3), 3.25 (br d, $J = 5.5$ Hz, 1H; H11), 1.09 (s, 3H; H19), 0.92 (d, $J = 6.5$ Hz, 3H; H21), 0.86, 0.85 (both d, $J =$ 6.7 Hz, 3H; H26,27), 0.75 (s, 3H; H18); IR $\tilde{\nu}_{max}$ (KBr): 2953, 1757, 1707, 1399, 1046 cm^{-1}; FAB-MS *m*/*z*: 618 [*M*+H]$^+$; FAB-HRMS *m*/*z*: calcd for $C_{37}H_{52}N_3O_5$: 618.3907, found: 618.3922; elemental analysis calcd (%) for $C_{37}H_{51}N_3O_5$ (617.8): C 71.93, H 8.32, N 6.80; found C 72.13, H 8.62, N 6.85.

9(11)-Ene-6,7-epoxide: $[\alpha]_D^{20} = -26.2^\circ$ ($c = 0.36$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 7.43$ (m, 4H; Ph*H*), 7.33 (m, 1H; Ph*H*), 5.77 (dd, $J = 6.7$, 1.6 Hz, 1H; H11), 4.80, 4.68 (ABq, $J = 6.8$ Hz, 2H; 3-OCH_2OCH$_3$), 4.08 (m, 1H; H3), 3.63, 3.35 (both d, $J = 4.8$ Hz, 1H; H6,7), 3.37 (s, 3H; 3-OCH$_2$OCH_3), 1.35 (s, 3H; H19), 0.93 (d, $J = 6.5$ Hz, 3H; H21), 0.86 (d, $J = 6.8$ Hz, 6H; H26,27), 0.82 (s, 3H; H18); IR $\tilde{\nu}_{max}$ (KBr) = 2930, 1763, 1711, 1412, 1044 cm^{-1}; FAB-MS *m*/*z*: 618 [*M*+H]$^+$; FAB-HRMS *m*/*z*: calcd for $C_{37}H_{52}N_3O_5$: 618.3907, found: 618.3906; elemental analysis calcd (%) for $C_{37}H_{51}N_3O_5$ (617.8): C 71.93, H 8.32, N 6.80; found C 72.09, H 8.43, N 6.93.

Reductive cleavage of the epoxide 11: A solution of the epoxide **11** (21 mg, 0.028 mmol) in THF (1.8 mL) was treated with $LiAlH_4$ (10.6 mg, 0.28 mmol) under reflux for 15 h in the dark. Work-up in the same manner as for the preparation of compound **5** gave a crude product, which was purified by column chromatography (SiO_2 2 g, *n*-hexane/EtOAc 4:1) to furnish compound **13** (6.0 mg, 48%).

Reductive cleavage of the epoxide 11 by using Et_2AlCl: A solution of Et_2AlCl (1.0 M in *n*-hexane; 8.0 mL, 80.0 mmol) was added to a solution of the epoxide **11** (3.0 g, 4.0 mmol) in THF (251 mL), then the whole mixture was refluxed for 6 h in the dark. The reaction mixture was treated with $LiAlH_4$ (1.5 g, 40 mmol) under reflux for 1 h in the dark. Work-up in the same manner as for the preparation of compound **5** above gave a crude product, which was purified by column chromatography (SiO_2 50 g, *n*-hexane/EtOAc 4:1) to furnish compound **13** as a colorless amorphous solid. Yield: 1.5 g, 84%; $[\alpha]_D^{21} = -20.1^\circ$ ($c = 0.16$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 5.54$ (dd, $J = 5.5$, 1.9 Hz, 1H; H6), 5.38 (dt, $J = 5.5$, 2.6 Hz, 1H; H7), 4.72, 4.70 (ABq, $J = 6.7$ Hz, 2H; 3-OCH_2OCH$_3$), 4.21 (m, 1H; H11), 3.55 (m, 2H; H3,22), 3.38 (s, 3H; 3-OCH$_2$OCH_3), 2.03 (br d, $J = 8.5$ Hz, 1H; H9), 1.11 (s, 3H; H19), 0.95 (d, $J = 6.7$ Hz, 3H; H21), 0.88 (s, 9H; 22-OSi(CH$_3$)$_2$C(CH_3)$_3$), 0.87 (d, $J = 6.4$ Hz, 6H; H26,27), 0.61 (s, 3H; H18), 0.03, 0.02 (both s, 3H; 22-OSi(CH_3)$_2$C(CH$_3$)$_3$); IR $\tilde{\nu}_{max}$ (KBr) = 3401, 2948, 1464, 1042 cm^{-1}; FAB-MS *m*/*z*: 597 [*M*+Na]$^+$; FAB-HRMS *m*/*z*: calcd for $C_{35}H_{62}O_4SiNa$: 597.4315, found: 597.4321.

Conversion from compound 12 to compound 14: The same treatment of compound **12** (542 mg, 0.877 mmol) as in the preparation for compound **13** was carried out to furnish compound **14** (351 mg, 90%) through chromatographic purification (SiO_2 20 g, *n*-hexane/EtOAc 6:1) as a colorless amorphous solid: $[\alpha]_D^{21} = -44.6^\circ$ ($c = 0.34$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 5.54$ (dd, $J = 5.5$, 1.9 Hz, 1H; H6), 5.37 (dt, $J = 5.5$, 2.6 Hz, 1H; H7), 4.71, 4.69 (ABq, $J = 6.9$ Hz, 2H; 3-OCH_2OCH$_3$), 4.21 (ddd, $J = 10.8$, 9.3, 5.5 Hz, 1H; H11), 3.54 (m, 1H; H3), 3.38 (s, 3H; 3-OCH$_2$OCH_3), 2.02 (d, $J = 9.3$ Hz, 1H; H9), 1.11 (s, 3H; H19), 0.96 (d, $J = 6.5$ Hz, 3H; H21), 0.87, 0.86 (both d, $J = 6.7$ Hz, 3H; H26,27), 0.62 (s, 3H; H18); IR $\tilde{\nu}_{max}$ (KBr) = 3405, 2955, 1466, 1107, 1042 cm^{-1}; FAB-MS *m*/*z*: 467 [*M*+Na]$^+$; FAB-HRMS *m*/*z*: calcd for $C_{29}H_{48}O_3Na$: 467.3501, found: 467.3530.

Conversion from compound 13 to compound 15: 2,6-Lutidine (1.07 mL, 9.2 mmol), then TBSOTf (1.75 mL, 7.64 mmol) were added to a solution of compound **13** (1.36 g, 3.06 mmol) in $PhCH_3$ (99 mL) at room temperature and the whole mixture was stirred for 1 h in the dark. Work-up in the same manner as for the preparation of the TBS ether of compound **5a** gave a crude product, which was purified by column chromatography (SiO_2 50 g, *n*-hexane/EtOAc 30:1) to furnish an TBS ether as a colorless amorphous solid. Yield: 2.0 g, 95%; $[\alpha]_D^{22} = -15.7^\circ$ ($c = 0.10$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 5.50$ (dd, $J = 5.5$, 1.9 Hz, 1H; H6), 5.33 (dt, $J = 5.5$, 2.6 Hz, 1H; H7), 4.71, 4.69 (ABq, $J = 6.7$ Hz, 2H; 3-OCH_2OCH$_3$), 4.27 (td, $J = 10.7$, 5.4 Hz, 1H; H11), 3.55 (m, 2H; H3, 22), 3.38 (s, 3H; 3-OCH$_2$OCH_3), 2.14 (d, $J = 10.7$ Hz, 1H; H9) 1.08 (s, 3H; H19), 0.94 (d, $J = 6.7$ Hz, 3H; H21), 0.89 (s, 18H; 11, 22-OSi(CH$_3$)$_2$C(CH_3)$_3$), 0.87 (d, $J =$ 6.4 Hz, 6H; H26,27), 0.59 (s, 3H; H18), 0.10, 0.09 (both s, 3H; 11,22-OSi(CH_3)$_2$C(CH$_3$)$_3$), 0.03 (s, 6H); IR $\tilde{\nu}_{max}$ (KBr) = 2953, 1466, 1256, 1047 cm^{-1}; FAB-MS *m*/*z*: 711 [*M*+Na]$^+$; FAB-HRMS *m*/*z*: calcd for $C_{41}H_{76}O_4Si_2Na$: 711.5180, found: 711.5170.

1-(4-Methoxyphenyl)-4-phenyl-1-azabuta-(*E*,*E*)-1,3-diene (190 mg, 0.8 mmol) and $Fe(CO)_5$ (18 mL, 138 mmol) were added to a solution of the TBS ether (1.9 g, 2.76 mmol) in $PhCH_3$ (18 mL), and the whole mixture was refluxed for 27 h in the dark. Removal of the solvent from the reaction mixture under reduced pressure gave a crude product, which was purified by column chromatography (SiO_2 50 g, *n*-hexane/acetone 30:1) to furnish a tricarbonyl iron complex (2.1 g, 94%). $MgBr_2 \cdot Et_2O$ (2.3 g, 9.0 mmol) was gradually added to a solution of this complex (1.24 g, 1.5 mmol) in CH_2Cl_2 (15 mL) and Me_2S (3.3 mL, 45 mmol) at room temperature, then the whole mixture was stirred for 4 h in the dark. Work-up in the same manner as for the preparation of the TBS ether of compound **5a** gave a crude product, which was purified by column chromatography (SiO_2 50 g, *n*-hexane/EtOAc 5:1) to furnish compound **15** as a yellow amorphous solid. Yield: 860 mg, 73%; $[\alpha]_D^{23} = -21.6^\circ$ ($c = 0.13$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 5.22$, 4.92 (both d, $J = 4.4$ Hz, 1H; H6,7), 3.68 (m, 1H; H3), 3.53 (m, 2H; H11,22), 1.09 (s, 3H; H19), 0.90 (d, $J = 7.0$ Hz, 3H; H21), 0.88 (m, 15H; 11 or 22-OSi(CH$_3$)$_2$C(CH_3)$_3$, H26,27), 0.85 (s, 9H; 11 or 22-OSi(CH$_3$)$_2$C(CH_3)$_3$), 0.69 (s, 3H; H18), 0.08 (s, 3H), 0.02 (s, 6H), 0.01 (s, 3H) [11,22-OSi(CH_3)$_2$C(CH$_3$)$_3$]; IR $\tilde{\nu}_{max}$ (KBr) = 3414, 2957, 2033, 1962, 1470, 1254, 1063 cm^{-1}; FAB-MS *m*/*z*: 807 [*M*+Na]$^+$; FAB-HRMS *m*/*z*: calcd for $C_{42}H_{72}FeO_6Si_2Na$: 807.4115, found: 807.4088.

Conversion from compound 15 to compound 16: TsCl (2.07 g, 10.9 mmol) was added to a solution of compound **15** (850 mg, 1.08 mmol) in pyridine (21.7 mL), then the whole mixture was stirred at room temperature for 12 h in the dark. The reaction mixture was poured into 5% aqueous HCl, then the whole mixture was extracted with EtOAc. The EtOAc extract was washed with saturated aqueous NaCl, then dried over $MgSO_4$. Removal of the solvent from the EtOAc extract under reduced pressure gave a crude

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product, which was purified by column chromatography (SiO_2 50 g, *n*-hexane/EtOAc 15:1) to furnish a tosylate (1.1 g, quant.). A solution of the tosylate (884 mg, 0.94 mmol) in PhH (56 mL) was heated under reflux with DBN (11.7 mL, 94 mmol) for 26 h in the dark. Work-up in the same manner as for the preparation of the TBS ether of compound **5a** gave a crude product, which was purified by column chromatography (SiO_2 30 g, 100% *n*-hexane) to furnish compound **16** as a yellow amorphous solid. Yield: 722 mg, quant.; $[\alpha]_D^{23} = -152.3^\circ$ ($c = 0.34$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 5.59$ (br s, 2H; H3,4), 5.19, 4.99 (both d, $J = 4.3$ Hz, 1H; H6,7), 3.62 (td, $J = 10.5$, 4.9 Hz, 1H; H11), 3.54 (m, 1H; H22), 0.97 (s, 3H; H19), 0.92 (d, $J = 6.9$ Hz, 3H; H21), 0.88 (m, 24H; 11,22-OSi$(CH_3)_2$C$(CH_3)_3$, H26,27), 0.72 (s, 3H; H18), 0.07, 0.02 (both s, 6H; 11,22-OSi$(CH_3)_2$C$(CH_3)_3$); IR $\tilde{\nu}_{max}$ (KBr) = 2959, 2033, 1960, 1468, 1256, 1059 cm^{-1}; FAB-MS *m*/*z*: 789 $[M+Na]^+$; FAB-HRMS *m*/*z*: calcd for $C_{42}H_{70}FeO_5Si_2Na$: 789.4009, found: 789.3998.

Dihydroxylation of compound 16 with OsO_4: A solution of compound **16** (617 mg, 0.805 mmol) in pyridine (8.6 mL) was treated with OsO_4 (1.0 M in Py, 1.17 mL, 1.17 mmol) at room temperature for 5.5 h in the dark. The reaction mixture was successively treated with H_2O (34 mL) and $NaHSO_3$ (11.7 g, 97.5 mmol), then the whole mixture was stirred at room temperature for 24 h in the dark. The mixture was extracted with EtOAc, then the EtOAc extract was washed with saturated aqueous NaCl and dried over $MgSO_4$. Removal of the solvent from the EtOAc extract under reduced pressure gave a crude product, which was purified by column chromatography (SiO_2 15 g, *n*-hexane/EtOAc 3:1) to furnish compound **17** as a yellow amorphous solid. Yield: 493 mg, 80%; $[\alpha]_D^{23} = -35.2^\circ$ ($c = 0.79$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 5.41$, 5.30 (both d, $J = 4.5$ Hz, 1H; H6,7), 3.84 (br d, $J = 11.1$ Hz, 1H; H3), 3.80 (br s, 1H; H4), 3.52 (m, 2H; H11,22), 1.23 (s, 3H; H19), 0.90 (d, $J = 6.8$ Hz, 3H; H21), 0.89 (d, $J = 7.2$ Hz, 3H; H26, 27), 0.87 (d, $J = 7.2$ Hz, 3H), 0.88 (s, 9H), 0.85 (s, 9H; [11, 22-OSi$(CH_3)_2$C$(CH_3)_3$]), 0.68 (s, 3H; H18), 0.04 (s, 3H), 0.02 (s, 6H), 0.01 (s, 3H) [11,22-OSi$(CH_3)_2$C$(CH_3)_3$]; IR $\tilde{\nu}_{max}$ (KBr) = 3405, 2953, 2035, 1964, 1466, 1256, 1061 cm^{-1}; FAB-MS *m*/*z*: 823 $[M+Na]^+$; FAB-HRMS *m*/*z*: calcd for $C_{42}H_{72}FeO_7Si_2Na$: 823.4064, found: 823.4060.

Conversion from compound 17 to compound 18: DMAP (6.0 mg, 0.049 mmol) was added to a solution of compound **17** (42.7 mg, 0.053 mmol) in pyridine (1.0 mL), then the reaction mixture was treated with TESOTf (89 μL, 0.39 mmol) at room temperature for 1 h in the dark. Work-up in the same manner as for the preparation of compound **5a** gave a crude product, which was purified by column chromatography (SiO_2 2 g, *n*-hexane/EtOAc 100:1) to furnish a di-TES ether (56.0 mg, quant.). A solution of the di-TES ether (55.0 mg, 0.052 mmol) in PhH (5.2 mL) was treated with Me_3NO (78 mg, 1.04 mmol) at room temperature for 28 h in the dark. Work-up in the same manner as for the preparation of compound **5a** gave compound **18** as a colorless amorphous solid. Yield: 44 mg, 95%; $[\alpha]_D^{22} = -76.1^\circ$ ($c = 1.46$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 5.66$ (d, $J = 5.6$ Hz, 1H; H6), 5.40 (dt, $J = 5.6$, 2.8 Hz, 1H; H7), 4.28 (m, 1H; H11), 4.10 (br s, 1H; H4), 3.57 (br d, $J = 8.4$ Hz, 1H; H22), 3.51 (br d, $J = 11.4$ Hz, 1H; H3), 1.20 (s, 3H; H19), 0.95 (3,4-OSi$(CH_2CH_3)_3$, 21H; H21), 0.90, 0.89 (both s, 9H; 11 or 22-OSi$(CH_3)_2$C$(CH_3)_3$), 0.88 (d, $J = 6.6$ Hz, 6H; H26,27), 0.60 (s, 15H; 3,4-OSi$(CH_2CH_3)_3$, H18), 0.10, 0.09 (both s, 3H; 11 or 22-OSi$(CH_3)_2$C$(CH_3)_3$), 0.03 (s, 6H); IR $\tilde{\nu}_{max}$ (KBr) = 2953, 1462, 1254, 1090, 835 cm^{-1}; FAB-MS *m*/*z*: 911 $[M+Na]^+$; FAB-HRMS *m*/*z*: calcd for $C_{51}H_{100}O_4Si_4Na$: 911.6597, found: 911.6587.

Hydroboration followed by deprotection of TES groups giving compound 19: A solution of $BH_3 \cdot SMe_2$ (2.0 M, 0.048 mmol) in THF, (27 μL) was added to a solution of compound **18** (21.0 mg, 0.024 mmol) in THF (0.8 mL) at 0 °C, then the whole mixture was stirred at room temperature for 2 h in the dark. The reaction mixture was treated with MeOH (50 μL) at 0 °C, then 1.0 N aqueous NaOH (66 μL) and 30% aqueous H_2O_2 (17 μL) were successively added to the reaction mixture at 0 °C. After being stirred at room temperature for 1 h, the whole mixture was poured into saturated aqueous NaCl, and was then extracted with EtOAc. The EtOAc extract was washed with saturated aqueous $Na_2S_2O_3$/saturated aqueous NaCl (1:1), then dried over $MgSO_4$. Removal of the solvent from the EtOAc extract under reduced pressure gave a crude product, which was purified by column chromatography (SiO_2 2 g, *n*-hexane/EtOAc 40:1) to furnish an 6α-alcohol (13.4 mg, 62%). A solution of the 6α-alcohol (11.0 mg, 0.012 mmol) in THF (1.84 mL) was treated with TBAF (1.0 M in THF, 120 μL, 0.12 mmol) at 20 °C for 24 h. The reaction mixture was poured into saturated aqueous NaCl, then the whole mixture was extracted with EtOAc. The EtOAc extract was washed with saturated aqueous NaCl, then dried over $MgSO_4$. Removal of the solvent from the EtOAc extract under reduced pressure gave a crude product, which was purified by column chromatography (SiO_2 2 g, *n*-hexane/EtOAc 1:3) to furnish compound **19** as a colorless amorphous solid. Yield: 7.4 mg, 90%; $[\alpha]_D^{22} = +15.0^\circ$ ($c = 0.18$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 5.33$ (br s, 1H; H7), 4.32 (br d, $J = 9.5$ Hz, 1H; H6), 4.29 (br s, 1H; H4), 3.99 (td, $J = 10.9$, 5.2 Hz, 1H; H11), 3.56 (m, 2H; H3, 22), 1.14 (s, 3H; H19), 0.91 (d, $J = 6.6$ Hz, 3H; H21), 0.89, 0.88 (both s, 9H; 11 or 22-OSi$(CH_3)_2$C$(CH_3)_3$), 0.87 (d, $J = 6.6$ Hz, 6H; H26,27), 0.53 (s, 3H; H18), 0.09, 0.08 (both s, 3H; 11, 22-OSi$(CH_3)_2$C$(CH_3)_3$), 0.02 (s, 6H); IR $\tilde{\nu}_{max}$ (KBr) = 3409, 2957, 1472, 1254, 1063, 835 cm^{-1}; FAB-MS *m*/*z*: 701 $[M+Na]^+$; FAB-HRMS *m*/*z*: calcd for $C_{39}H_{74}O_5Si_2Na$: 701.4973, found: 701.4948.

Conversion from compound 19 to agosterol A (1): A solution of compound **19** (5.6 mg, 0.0082 mmol) in pyridine (1.6 mL) was treated with Ac_2O (0.4 mL) at 50 °C for 24 h. Work-up in the same manner as for the preparation of compound **5a** gave a crude product, which was purified by column chromatography (SiO_2 2 g, *n*-hexane/EtOAc 3:1) to furnish a triacetate (6.0 mg, 91%). A solution of the triacetate (4.0 mg, 0.005 mmol) in THF (1.5 mL) was treated with 70% HF-pyridine (0.3 mL) at room temperature for 24 h. The reaction mixture was poured into saturated aqueous $NaHCO_3$, then the whole mixture was extracted with EtOAc. The EtOAc extract was successively washed with saturated aqueous NaCl, saturated aqueous $NaHCO_3$, and saturated aqueous NaCl, then dried over $MgSO_4$. Removal of the solvent from the EtOAc extract under reduced pressure gave a crude product, which was purified by column chromatography (SiO_2 2 g, *n*-hexane/EtOAc 1:1) to furnish agosterol A (**1**) (2.4 mg, 84%). The synthesized agosterol A was identical with the authentic sample as shown by ^{1}H NMR, ^{13}C NMR, IR, MS, and optical rotation.

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[18] Epoxidation with *m*CPBA for the triazolidine gave a mixture of the 6-en-9,11-epoxide (**11**) and a 9(11)-en-6,7-epoxide in a ratio of 3:1, as shown by ^{1}H NMR analysis, that was inseparable by ordinary SiO_2 column chromatography. Since isolation of the resulting 11α-alcohol **13** was very easy, this conversion was executed as a mixture in the synthesis of agosterol A (**1**).
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