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Synthesis and Complete Structure Determination of a Sperm-Activating and -Attracting Factor Isolated from the Ascidian *Ascidia sydneiensis*

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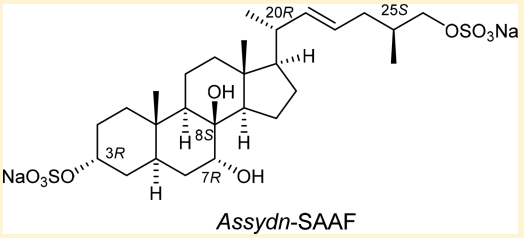
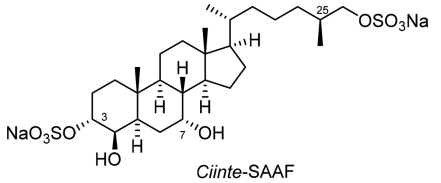
Supporting Information

ABSTRACT: For the complete structure elucidation of an endogenous sperm-activating and -attracting factor isolated from eggs of the ascidian *Ascidia sydneiensis* (*Assydn*-SAAF), its two possible diastereomers with respect to C-25 were synthesized. Starting from ergosterol, the characteristic steroid backbone was constructed by using an intramolecular pinacol coupling reaction and stereoselective reduction of a hydroxy ketone as key steps, and the side chain was introduced by Julia−Kocienski olefination. Comparison of the NMR data of the two diastereomers with those of the natural product led to the elucidation of the absolute configuration as 25*S*; thus the complete structure was determined and the first synthesis of *Assydn*-SAAF was achieved.

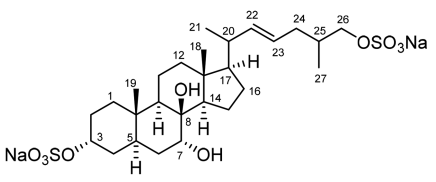


Assydn-SAAF

Sperm activation and chemotaxis, which ubiquitously occur in animals and plants, play an important role in fertilization.[1] Several nonpeptide small molecules are known to act as chemoattractants in fertilization. We have previously isolated the sperm-activating and -attracting factor (SAAF) from the eggs of the ascidian *Ciona intestinalis* (*Ciinte*-SAAF, Figure 1).[2] *Ciinte*-SAAF was the first example of a single agent concomitantly inducing both sperm activation and attraction. In addition, *Ciinte*-SAAF was proved to be active toward *C. savignyi*, another species in the same genus, indicating that there is tolerance on the species specificity.[2b] On the other hand, *Assydn*-SAAF (Figure 1) was isolated from the eggs of the ascidian *Ascidia sydneiensis*.[3] The molecular structure of *Assydn*-SAAF was determined by NMR and ESI/TOF-MS analysis using approximately 2.6 μg of sample to be 3,7,8,26-tetrahydroxycholest-22-ene-3,26-disulfate,[3] while the configurations at C-20 and C-25 remained unknown. Although the structure closely resembles that of *Ciinte*-SAAF, it was found that *Ciinte*-SAAF did not activate the sperm of *A. sydneiensis*, suggesting that there is high genus specificity.[3,4] Although the mechanisms underlying the sperm activation/attraction as well as genus specificity are not fully elucidated, unambiguous structure determination of *Assydn*-SAAF is necessary for further investigations. Herein we report the synthesis and complete structure determination of *Assydn*-SAAF based on the chemical synthesis of plausible diastereomers and comparison of the NMR data.



Ciinte-SAAF



Assydn-SAAF

Figure 1. Structure of *Ciinte*-SAAF and proposed structure of *Assydn*-SAAF.

RESULTS AND DISCUSSION

From the biosynthetic point of view, we assumed that the absolute configuration at C-20 of *Assydn*-SAAF (**1**) would be

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identical with that of *Ciinte*-SAAF, as shown in Figure 2, and supportive NOE correlations between H-12a–H_3-21 together with H_3-18–H-20 were observed.[3] To determine the complete structure of *Assydn*-SAAF, we planned to synthesize both diastereomers with respect to C-25, (25*S*)-**2a** and (25*R*)-**2b**, and compare the NMR data with those of the natural product. Although the structure of the steroid core was determined by NMR analysis, it was difficult to elucidate the configuration of the nonprotonated carbon at C-8. Therefore, prior to the synthesis of **2a** and **2b**, we planned to prepare model compound **3a** corresponding to the steroid core and its C-8-epimer **3b** (Figure 2) and compare the ^{1}H NMR data with those of the natural product.

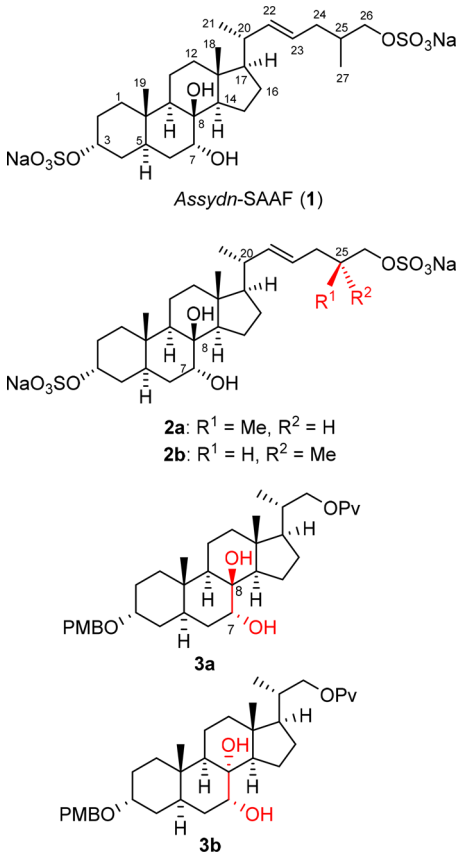
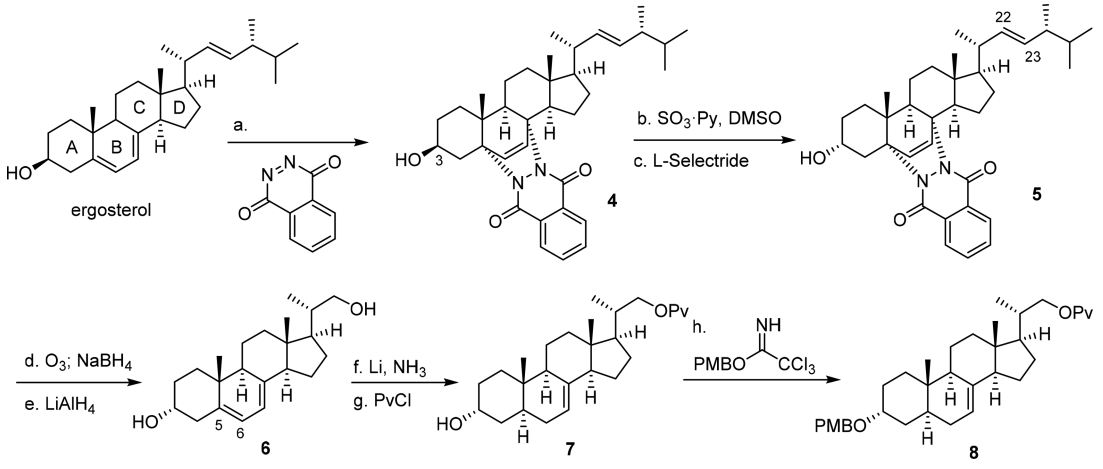


Figure 2. Proposed structure of *Assydn*-SAAF (**1**), two possible diastereomers with respect to C-25, (25*S*)-**2a** and (25*R*)-**2b**, and model compounds **3a** and **3b**.

Olefin **8**, the key intermediate of **3a** and **3b**, was synthesized from ergosterol as shown in Scheme 1. The diene moiety on the B-ring was protected via hetero-Diels–Alder reaction using phthalhydrazide in the presence of $Pb(OAc)_4$ to give cycloadduct **4** in 93% yield.[5] Inversion of the secondary alcohol at C-3 was carried out via Parikh–Doering oxidation (96%) followed by reduction with L-Selectride (lithium tri-*sec*-butylborohydride) to afford α-alcohol **5** as a single isomer (66%). The C-22/C-23 double bond was selectively cleaved by ozonolysis and following hydride reduction[6] gave the primary alcohol (71%). Removal of the phthalazide moiety with $LiAlH_4$[5] liberated the diene on the B-ring to afford **6**. Birch reduction of diene **6** proceeded regio- and stereoselectively[7] to furnish the 5α-cholest-7-ene skeleton. The primary hydroxy group of the diol was selectively protected as pivaloate **7** in 76% yield for three steps. The remaining secondary alcohol was protected as a *p*-methoxybenzyl (PMB) ether under acidic conditions[8] to provide olefin **8** in 83% yield.

Having the key intermediate in hand, we moved on to the synthesis of **3a** and **3b** as shown in Scheme 2. It is known that OsO_4-mediated dihydroxylation of 5α-cholest-7-ene and its derivatives provides α-diol selectively.[9] Thus, dihydroxylation of hindered olefin **8** with OsO_4 was carried out in the presence of 1,4-diazabicyclo[2.2.2]octane (DABCO),[10] and the reaction proceeded stereoslectively to afford the desired 7α,8α-diol **3b** in 80% yield as a single diastereomer. Because initial attempts to prepare **3a** directly from **8** via epoxidation/ring-opening sequence were unsuccessful, oxidative cleavage of the C-7/C-8 double bond followed by intramolecular pinacol coupling[11,12] of the resulting ketoaldehyde was examined. Although ozonolysis of olefin **8** was unsuccessful and gave a complex mixture, and attempts of oxidative cleavage of diol **3b** with

Scheme 1. Synthesis of Olefin 8 from Ergosterol[a]



[a]Reagents and conditions: (a) phthalhydrazide, $Pb(OAc)_4$, CH_2Cl_2, 0 °C to rt, 4 h, 93%; (b) SO_3·pyridine, dimethylsulfoxide (DMSO), Et_3N, CH_2Cl_2, rt, 2.5 h, 96%; (c) L-Selectride, tetrahydrofuran (THF), −78 °C, 30 min, 66%; (d) O_3, pyridine, CH_2Cl_2, −60 °C, 20 min; $NaBH_4$, MeOH, −60 °C to rt, 2 h, 71%; (e) $LiAlH_4$, THF, 80 °C, 1.5 h; (f) Li, NH_3, *tert*-amyl alcohol, THF, −78 °C, 1.5 h; (g) PvCl, pyridine, CH_2Cl_2, 0 °C, 2 h, 76% (3 steps); (h) *p*-methoxybenzyl trichloroacetimidate (PMBTCA), $La(OTf)_3$, toluene, 0 °C, 40 min, 83%.

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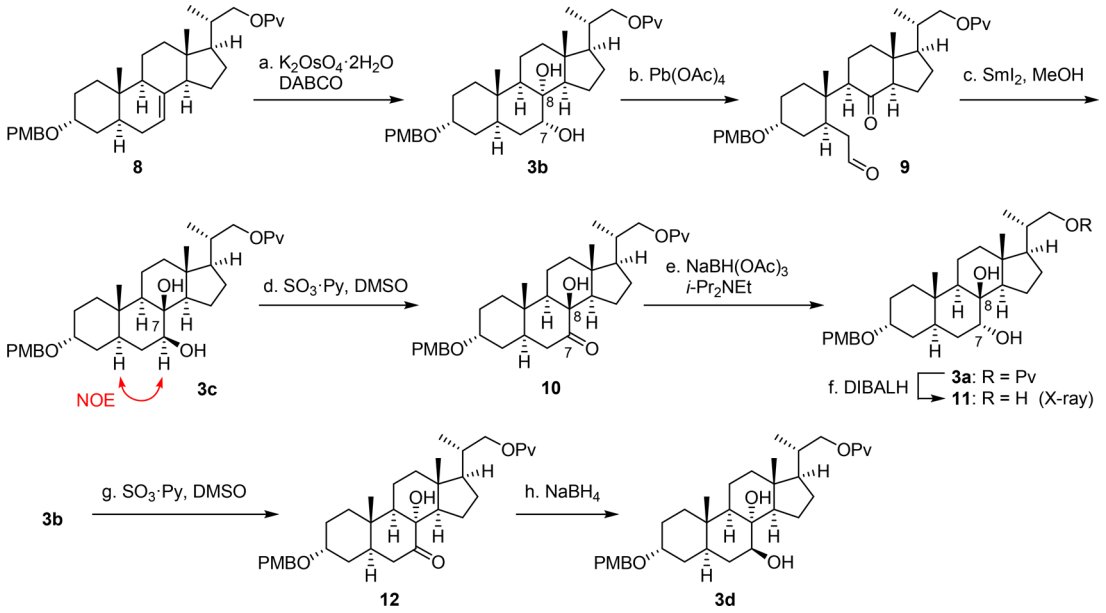
Scheme 2. Synthesis of 3a−3d from 8[a]



[a]Reagents and conditions: (a) $K_2OsO_4·2H_2O$, $K_3Fe(CN)_6$, K_2CO_3, $MeSO_2NH_2$, DABCO, *t*-BuOH/H_2O/CH_2Cl_2, rt, 36 h, 80%; (b) $Pb(OAc)_4$, CH_2Cl_2, 0 °C, 35 min, 92%; (c) SmI_2, MeOH, THF, 0 °C, 45 min, 85%; (d) SO_3·pyridine, DMSO, Et_3N, CH_2Cl_2, rt, 13 h, 85%; (e) $NaBH(OAc)_3$, *i*-Pr_2NEt, THF, 0 °C to rt, 15 h, 93%; (f) DIBALH, CH_2Cl_2, −78 °C to rt, 2 h, 91%; (g) SO_3·pyridine, DMSO, Et_3N, CH_2Cl_2, rt, 12 h, 76%; (h) $NaBH_4$, THF, MeOH, 0 °C, 1.5 h, 89%.

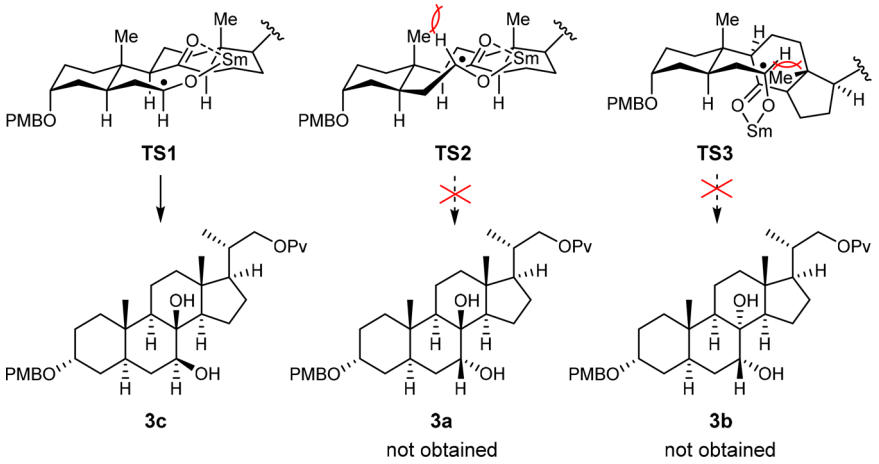


Figure 3. Plausible transition states of the intramolecular pinacol coupling of **9**.

$NaIO_4$ resulted in no reaction, treatment of diol **3b** with $Pb(OAc)_4$ proceeded smoothly to afford ketoaldehyde **9** in 92% yield. Then, intramolecular pinacol coupling of **9** was carried out by treating with SmI_2 in the presence of MeOH as a proton source to give 7*β*,8*β*-diol **3c**, the C-7 epimer of **3a**, in 85% yield as a single diastereomer. In the absence of MeOH, the yield of **3c** decreased to 52% with concomitant formation of byproducts. The structure of diol **3c** was confirmed by NOE experiments. The stereochemical outcome can be explained as shown in Figure 3. The SmI_2-mediated intramolecular pinacol coupling is thought to proceed via a chelation transition state bridging oxygen atoms at C-7 and C-8 with the samarium ion. Among the possible chelation transition states **TS1**−**TS3**, **TS1**, giving compound **3c**, appears to be most favored compared with **TS2** and **TS3**, in which severe steric repulsions exist. Although the stereochemistry at C-8 was successfully controlled, that at C-7 was opposite that of *Assydn*-SAAF. Therefore, inversion of configuration at C-7 was examined via an oxidation/reduction sequence. Parikh−Doering oxidation of diol **3c** gave hydroxy ketone **10** in 85% yield, and reduction of **10** with $NaBH(OAc)_3$ in the presence of *i*-Pr_2NEt resulted in the formation of diol **3a** in 93% yield as a single isomer, presumably due to neighboring participation of the C-8 hydroxy group. It is noteworthy that addition of *i*-Pr_2NEt is important to avoid the formation of the 8-deoxy byproduct. The absolute configuration of **3a** was confirmed by X-ray crystallographic analysis of the corresponding primary alcohol **11**, derived from **3a** by removing the pivaloyl group with diisobutylalminum hydride (DIBALH). The ORTEP drawing of **11** is depicted in Figure 4.

Then, we examined conversion of **3b** into **3d**, the remaining one of the four diastereomers at C-7 and C-8, via inversion of the secondary alcohol. Parikh−Doering oxidation of **3b** gave hydroxy ketone **12** in 76% yield, and successive reduction with

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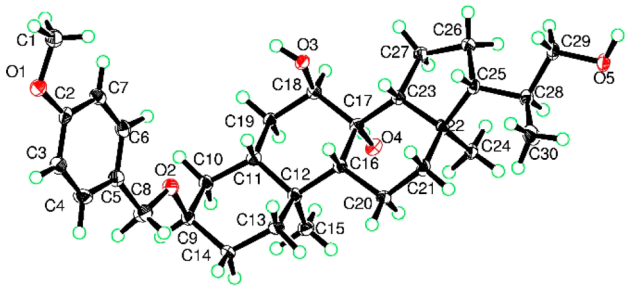


Figure 4. Structure of **11** in the crystalline state.

$NaBH_4$ provided 7β,8α-diol **3d** as the sole product, presumably due to attack of hydride on the less hindered side. Thus, we have established the synthetic route to construct the steroid core of *Assydn*-SAAF including all possible diastereomers at C-7 and C-8.

With all four diastereomers **3a**–**d** in hand, ^{1}H NMR coupling patterns of the H-7 proton were compared with that of *Assydn*-SAAF as shown in Figure 5. The H-7 proton signal of the natural product exhibited a characteristic splitting pattern reported as a broad triplet, J < 3 Hz.[3] Among the synthetic specimens **3a**–**d**, only **3a** showed a similar peak shape to the natural product, while larger J values than 3 Hz were observed for **3b**–**d**. Thus, the configuration of the *vic*-diol moiety of *Assydn*-SAAF was confirmed to be 7α-OH/8β-OH as reported.

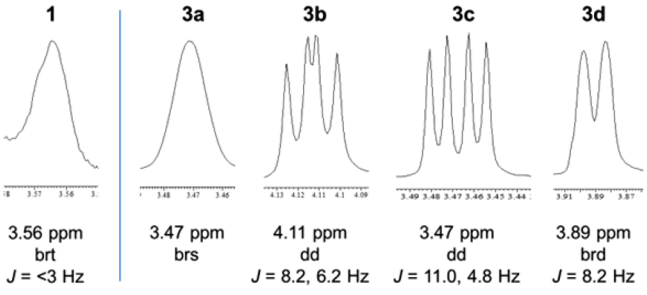
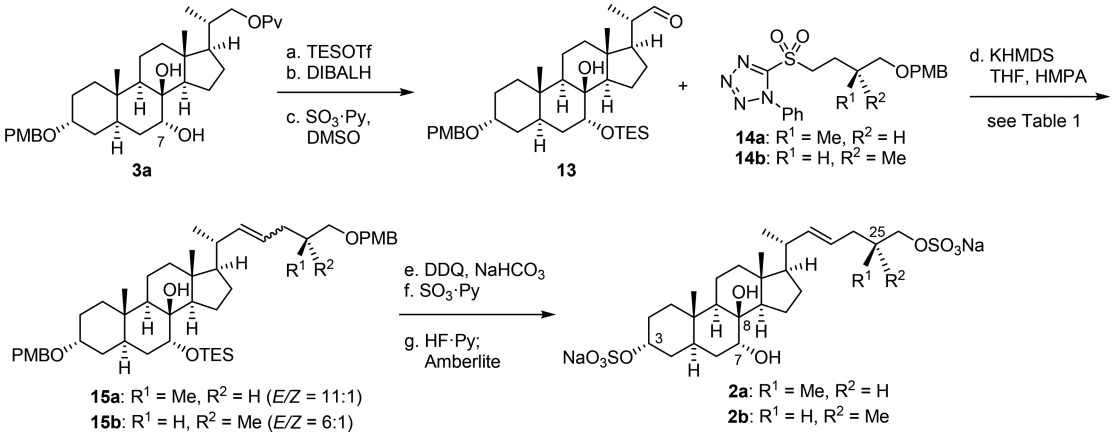


Figure 5. Comparison of ^{1}H NMR peak shapes of the H-7 proton between natural **1** and synthetic **3a**–**d**. **1**: 500 MHz in D_2O. **3a**–**d**: 600 MHz in $CDCl_3$.

Having confirmed the configuration at C-8, we moved on to the synthesis of (25*S*)-**2a** and (25*R*)-**2b** from **3a** (Scheme 3). The secondary alcohol at C-7 of **3a** was protected as a triethylsilyl (TES) ether (92%), the pivaloyl group was removed by reduction with DIBALH (99%), and oxidation of the resulting primary alcohol furnished aldehyde **13** (84%). Then, Julia–Kocienski olefination[13,14] of aldehyde **13** was examined by using sulfone (*S*)-**14a**, which was prepared from known alcohol (*S*)-**16a**[15] derived from (*S*)-2-methyl-1,4-butanediol (Scheme 4), via protection of the primary alcohol as a PMB ether (99%) and molybdate-H_2O_2-catalyzed oxidation of the corresponding tetrazol-5-yl thioether (89%). As shown in Table 1, a solution of aldehyde **13** in tetrahydrofuran (THF) was added to a solution of sulfone (*S*)-**14a** and potassium bis(trimethylsilyl)amide (KHMDS) in toluene, but resulted in the formation of a complex mixture (entry 1). Next, KHMDS was added to a premixed solution of aldehyde **13** and sulfone **14a** in THF to afford **15a** in 42% yield as an inseparable *E*/*Z* mixture in a 2:1 ratio (entry 2). When the base was changed to lithium bis(trimethylsilyl)amide (LHMDS), the yield was improved to 77%, but the *E*/*Z* ratio decreased to 1:1 (entry 3). Addition of Hexamethylphosphoric triamide (HMPA) as a cosolvent improved the *E*/*Z* ratio to 5:1, but the yield decreased to 28% (entry 4). When KHMDS was used in THF/HMPA, **15a** was obtained in 50% yield in a 7:1 ratio. Finally, the *E*/*Z* ratio was improved to 11:1 by using a THF solution of KHMDS to afford **15a** in 51% yield (entry 6). Then, the PMB groups of **15a** were removed with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in 86% yield. In this reaction, addition of aqueous $NaHCO_3$ was necessary to avoid the removal of the TES group. The resulting triol was converted to the 3,26-disulfate ester by treatment with a SO_3·pyridine complex. After removal of the TES group with HF·pyridine, the undesired *Z*-isomer was separated by preparative TLC. Finally, treatment with ion-exchange resin (Amberlite IR-120B) afforded (25*S*)-**2a** in 48% yield for two steps.[2c,d] On the other hand, (25*R*)-**2b** was synthesized in an analogous sequence from aldehyde **13** and sulfone (*R*)-**14b**, prepared

Scheme 3. Synthesis of *Assydn*-SAAF 2a and Its C-25-Epimer 2b[a]



[a]Reagents and conditions: (a) TESOTf, 2,6-lutidine, CH_2Cl_2, 0 °C to rt, 1.5 h, 92%; (b) DIBALH, CH_2Cl_2, −78 °C to rt, 1 h, 99%; (c) SO_3·pyridine, DMSO, Et_3N, CH_2Cl_2, 0 °C to rt, 7 h, 84%; (d) KHMDS, THF/HMPA, −78 °C to rt, 2 h, 51%, *E*/*Z* = 11:1 for (25*S*)-**15a**, 50%, *E*/*Z* = 6:1 for (25*R*)-**15b**; (e) DDQ, CH_2Cl_2, saturated $NaHCO_3$(aq), 0 °C, 20 min, 86% for (25*S*), 41% for (25*R*); (f) SO_3·pyridine, pyridine, rt, 1 h; (g) HF·pyridine, MeOH, 0 °C to rt, 7 h, then Amberlite IR-120B (Na^+), MeOH, 48% in two steps for (25*S*)-**2a**, 72% in two steps for (25*R*)-**2b**.

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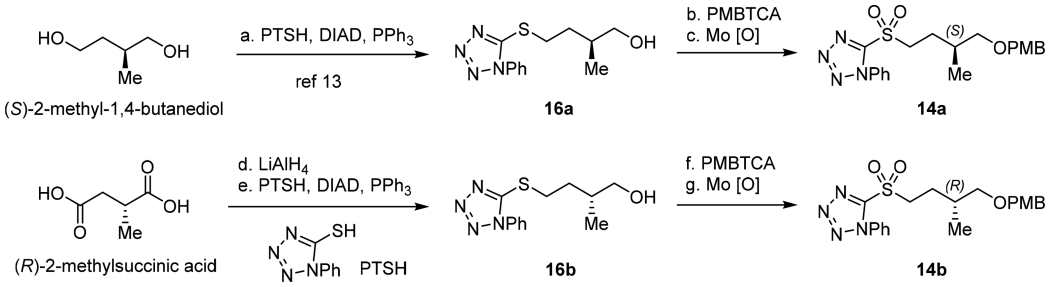
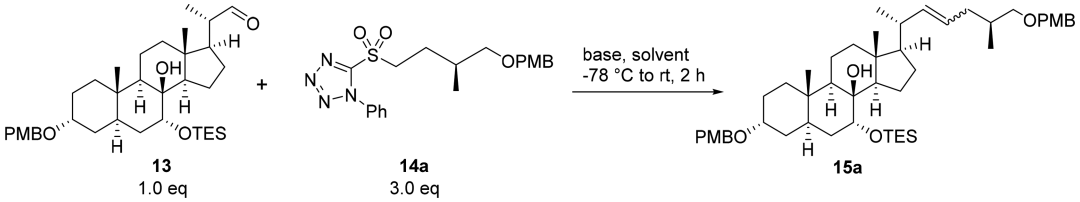
Scheme 4. Synthesis of Sulfone 14a and 14b[a]



[a]Reagents and conditions: (a) PTSH, diisopropyl azodicarboxylate (DIAD), PPh_3, THF, −78 °C to rt, 4 h, 25%; (b) PMBTCA, $La(OTf)_3$, toluene, rt, 23 h, 99%; (c) $(NH_4)_6Mo_7O_{24}·4H_2O$, H_2O_2, EtOH, rt, 5.5 h, 89%; (d) $LiAlH_4$, THF, −78 °C to rt, 17.5 h, 98%; (e) PTSH, DIAD, PPh_3, THF, −78 °C to rt, 7.5 h, 22%; (f) PMBTCA, $La(OTf)_3$, toluene, rt, 14 h, 90%; (g) $(NH_4)_6Mo_7O_{24}·4H_2O$, H_2O_2, EtOH, rt, 6 h, 91%.

Table 1. Optimization of Julia−Kocienski Reaction Using 13 and 14a



entry	base	solvent	yield/%	ratio/*E*:*Z*
1[a]	KHMDS in toluene	THF	0	
2[b]	KHMDS in toluene	THF	42	2:1
3[b]	LHMDS in THF	THF	77	1:1
4[b]	LHMDS in THF	THF/HMPA (4:1)	28	5:1
5[b]	KHMDS in toluene	THF/HMPA (4:1)	50	7:1
6[b]	KHMDS in THF	THF/HMPA (4:1)	51	11:1

[a]Aldehyde **13** was added to the mixture of sulfone **14a** and base. [b]Base was added to the mixture of aldehyde **13** and sulfone **14a**.

from (*R*)-2-methylsuccinic acid via alcohol (*R*)-**16b** (Scheme 4).

With both diastereomers (25*S*)-**2a** and (25*R*)-**2b** in hand, their ^1H and ^{13}C NMR chemical shifts were compared with those of the natural product (Table 2). The chemical shifts of (25*S*)-**2a** are identical with those of *Assydn*-SAAF (**1**), while there are noticeable differences in the ^1H NMR chemical shifts of (25*R*)-**2b** at the H_2-24 to H_2-26 positions. Therefore, the configuration at C-25 was determined to be *S*, and that at C-20 was also confirmed. Thus, complete structure of *Assydn*-SAAF was determined to be (3*R*,7*R*,8*S*,20*R*,25*S*)-3,7,8,26-tetrahydroxycholest-22-ene-3,26-disulfate, and the first synthesis of *Ascidia*-SAAF was achieved.

Biological activities of the synthetic specimens were evaluated based on the methods previously reported.[2a] Synthetic *Ascidia*-SAAF **2a** elicited sperm-activating and -attracting activity against the sperm of the ascidian *A. sydneiensis* at 5.0 μM. It is noteworthy that the C-25-epimer **2b** also elicited comparable activity to **2a**.[2c]

CONCLUSION

In conclusion, we have developed the stereodivergent synthetic route to construct the steroid core of *Assydn*-SAAF including all possible diastereomers at C-7 and C-8 via an intramolecular pinacol coupling reaction and stereoselective reduction of a hydroxy ketone as key steps, and syntheses of (25*S*)- and (25*R*)-*Assydn*-SAAF were achieved via a Julia−Kocienski reaction to introduce the side chain. Comparison of NMR data of the synthetic specimens with those of the natural product revealed that the structure of *Assydn*-SAAF is (3*R*,7*R*,8*S*,20*R*,25*S*)-3,7,8,26-tetrahydroxycholest-22-ene-3,26-disulfate. The pure synthetic specimen was also used to confirm the sperm-activating and -attracting activity. Further studies for identification of the receptor of SAAF as well as the relevant signal transduction pathway(s) are in progress.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were measured using a Yanaco MP-J3 micro melting point apparatus without correction. Optical rotations were recorded on a JASCO P-1010 polarimeter. IR spectra were recorded on a JASCO FT/IR-400. ^1H and ^{13}C NMR spectra were measured on JEOL JNM-ECA-600 or JNM-ECS400 instruments. Chemical shifts were reported in ppm from tetramethylsilane (TMS) with reference to internal residual solvent [^1H NMR: $CHCl_3$ (7.26), C_6HD_5 (7.16), acetone-d_5 (2.05), CD_2HOD (3.31); ^{13}C NMR: $CDCl_3$ (77.16), C_6D_6 (128.06), acetone-d_6 (29.84), CD_3OD (49.00)]. For compounds **1**, **2a**, and **2b**, NMR spectra were measured in D_2O with referring the chemical shifts to trace MeOH (3.34 ppm for ^1H NMR and 49.5 ppm for ^{13}C NMR). High-resolution ESI mass spectra (HRESIMS) were measured on a Bruker microTOFfocus spectrometer. All reactions sensitive to air or moisture were performed under an argon atmosphere with dry glassware unless otherwise noted. The anhydrous solvents, CH_2Cl_2, THF, and toluene were purchased from Kanto Chemical Co. Inc. or Wako Pure Chemical Industries Ltd. and used without further dehydration unless otherwise stated. 2,6-Lutidine, triethylsilyl trifluoromethanesulfonate (TESOTf), and HMPA were distilled before use. Amberlite IR-120B was ion-exchanged with 1 M aqueous NaOH, then washed with H_2O to neutrality, followed by conditioning with MeOH. Powdered Sm metal was purchased from Nippon

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Table 2. ^{13}C and ^{1}H NMR Chemical Shifts of 1, 2a, and 2b in D_2O[a]

	SAAF (1)[b]		(25S)-2a				(25R)-2b			
position	δ_C	δ_H	δ_C	$\Delta\delta_C$[c]	δ_H	$\Delta\delta_H$[c]	δ_C	$\Delta\delta_C$[c]	δ_H	$\Delta\delta_H$[c]
1	33.3	1.55	33.3	0.0	1.54	−0.01	33.4	0.1	1.56	0.01
		1.18			1.20	0.02			1.18	0.00
2	26.5	1.84	26.5	0.0	1.84	0.00	26.6	0.1	1.84	0.00
		1.80			1.81	0.01			1.80	0.00
3	78.5	4.65	78.5	0.0	4.66	0.01	78.4	−0.1	4.65	0.00
4	18.0	1.60	18.0	0.0	1.59	−0.01	18.0	0.0	1.60	0.00
		1.55			1.55	0.00			1.56	0.01
5	32.6	1.90	32.6	0.0	1.90	0.00	32.6	0.0	1.90	0.00
6	31.5	1.90	31.5	0.0	1.90	0.00	31.5	0.0	1.90	0.00
		1.18			1.18	0.00			1.18	0.00
7	72.0	3.56	72.1	0.1	3.56	0.00	72.0	0.0	3.56	0.00
8	n.d.		77.4				77.4			
9	50.2	1.25	50.2	0.0	1.24	−0.01	50.2	0.0	1.26	0.01
10	36.0		36.0	0.0			36.0	0.0		
11	32.9	1.59	32.9	0.0	1.57	−0.02	32.8	−0.1	1.60	0.01
12	40.7	1.99	40.8	0.1	2.00	0.01	40.8	0.1	2.00	0.01
		1.21			1.21	0.00			1.22	0.01
13	43.2		43.2	0.0			43.2	0.0		
14	53.9	1.54	53.9	0.0	1.55	0.01	53.9	0.0	1.56	0.02
15	18.6	1.47	18.7	0.1	1.47	0.00	18.7	0.1	1.48	0.01
		1.38			1.37	−0.01			1.37	−0.01
16	28.4	1.73	28.4	0.0	1.73	0.00	28.4	0.0	1.73	0.00
		1.28			1.28	0.00			1.28	0.00
17	56.6	1.14	56.6	0.0	1.15	0.01	56.6	0.0	1.15	0.01
18	13.5	0.90	13.5	0.0	0.90	0.00	13.6	0.1	0.90	0.00
19	11.5	0.90	11.5	0.0	0.90	0.00	11.5	0.0	0.90	0.00
20	39.7	2.07	39.9	0.2	2.07	0.00	39.9	0.2	2.06	−0.01
21	20.3	0.98	20.3	0.0	0.99	0.01	20.4	0.1	0.99	0.01
22	140.5	5.39	140.5	0.0	5.39	0.00	140.4	−0.1	5.39	0.00
23	125.5	5.39	125.3	−0.2	5.39	0.00	125.4	−0.1	5.39	0.00
24	36.0	2.02	36.0	0.0	2.02	0.00	36.0	0.0	2.08	0.06
		1.93			1.94	0.01			1.86	−0.07
25	33.5	1.87	33.5	0.0	1.87	0.00	33.5	0.0	1.89	0.02
26	74.1	3.95	74.1	0.0	3.95	0.00	74.2	0.1	3.92	−0.03
		3.83			3.83	0.00			3.85	0.02
27	16.4	0.92	16.4	0.0	0.93	0.01	16.2	−0.2	0.92	0.00

[a]Residual MeOH was adjusted to 49.5 ppm for ^{13}C NMR (150 MHz) and 3.34 ppm for ^{1}H NMR (600 MHz). [b]Data of **1** are adopted from the literature (ref 3). [c]$\Delta\delta = \delta$(synthetic **2a** or **2b**) − δ(natural **1**).

Yttrium Co., Ltd. All other chemicals were obtained from local vendors and used as supplied unless otherwise stated. Thin-layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} precoated plates (0.25 mm thickness) for the reaction analyses. Column chromatography was performed using Kanto silica gel 60N (spherical, neutral, 100−210 μm). Flash column chromatography was performed on Kanto silica gel 60N (spherical, neutral, 40−50 μm). For reversed-phase preparative TLC (RP-pTLC), Merck silica gel 60 RP-18 F_{254S} precoated plates (0.25 mm thickness) were used. X-ray crystallographic analysis was performed on a Bruker SMART APEX CCD-detector diffractometer.

Synthetic Procedures. *(22E)-5α,8α-(1,4-Dioxo-1,2,3,4-tetrahydrophthalazine-2,3-diyl)ergosta-6,22-dien-3β-ol (4).* Phthalhydrazide (16.4 g, 101 mmol), $Pb(OAc)_4$ (22.3 g, 40.2 mmol), and acetic acid (4.4 mL, 77 mmol) were added to a solution of ergosterol (10.0 g, 24.0 mmol) in dry CH_2Cl_2 (267 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 3 h and then at room temperature (rt) for 1 h. The reaction mixture was cooled to 0 °C and quenched with powdered Al_2O_3 (41.3 g, 405 mmol). After stirring at rt for 30 min, the resultant mixture was filtered through a pad of Celite. The filtrate was extracted with EtOAc, and the organic layer was washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 10/1 → 1/3) to give diene **4** (12.4 g, 22.3 mmol, 93%) as a yellow foam: $[\alpha]^{22}_D$ −137 (*c* 1.05, $CHCl_3$); R_f = 0.32 (hexane/EtOAc = 2/1); IR (neat) 3441, 2955, 2871, 1678, 1601, 1460, 1381, 1311, 1180, 1071, 1043, 1027, 974, 755, 725, 697 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.17−8.13 (m, 1H), 8.13−8.08 (m, 1H), 7.72−7.65 (m, 2H), 6.65 (d, *J* = 8.3 Hz, 1H), 6.27 (d, *J* = 8.3 Hz, 1H), 5.22 (dd, *J* = 15.6, 7.6 Hz, 1H), 5.15 (dd, *J* = 15.6, 8.4 Hz, 1H), 4.00−3.92 (m, 2H), 3.73−3.65 (m, 1H), 2.13−2.06 (m, 2H), 2.05−1.96 (m, 2H), 1.88−1.81 (m, 2H), 1.78−1.64 (m, 3H), 1.62−1.56 (m, 1H), 1.50−1.24 (m, 8H), 1.03 (s, 3H), 1.02 (d, *J* = 6.9 Hz, 3H), 0.90 (d, *J* = 6.9 Hz, 3H), 0.84 (s, 3H), 0.83 (d, *J* = 6.9 Hz, 3H), 0.81 (d, *J* = 6.9 Hz, 3H); the hydroxy proton was not observed probably due to the rapid exchange with contaminating H_2O; ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.9, 159.8, 138.2, 135.4, 132.9 (2C), 132.3, 130.7, 130.0, 129.1, 127.1, 127.0, 68.6, 67.7, 67.1, 56.7, 50.5, 49.0, 44.3, 42.8, 40.7, 40.0, 39.4, 35.6, 35.0, 33.2, 29.3, 28.3, 24.7, 21.9, 21.0, 20.0, 19.8, 18.7, 17.6, 13.4; HRESIMS *m*/*z* 579.3554 $[M + Na]^+$ (calcd for $C_{36}H_{48}N_2O_3Na$, 579.3557).

*(22E)-5α,8α-(1,4-Dioxo-1,2,3,4-tetrahydrophthalazine-2,3-diyl)-ergosta-6,22-dien-3-one (**S1**).* DMSO (7.0 mL, 99 mmol), Et_3N (7.0

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mL, 50 mmol), and SO_3·pyridine (4.74 g, 28.9 mmol) were added to a solution of alcohol **4** (5.53 g, 9.93 mmol) in dry CH_2Cl_2 (50 mL) at 0 °C. After stirring at rt for 3 h, the reaction was quenched with saturated aqueous NH_4Cl at 0 °C and extracted with Et_2O. The organic layer was washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 4/1 → 1/2) to give ketone **S1** (5.27 g, 9.50 mmol, 96%) as a yellow foam: $[\alpha]^{22}_D$ −25.4 (*c* 1.08, THF); R_f = 0.53 (hexane/EtOAc = 1/3); IR (neat) 2955, 2870, 1717, 1635, 1603, 1464, 1386, 1330, 1264, 1221, 1188, 1066, 1026, 975, 772, 694 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 8.17−8.14 (m, 2H), 7.72−7.67 (m, 2H), 6.59 (d, *J* = 8.4 Hz, 1H), 6.26 (d, *J* = 8.4 Hz, 1H), 5.26 (dd, *J* = 15.6, 7.2 Hz, 1H), 5.18 (dd, *J* = 15.6, 7.2 Hz, 1H), 4.02 (d, *J* = 18.0 Hz, 1H), 3.47 (dd, *J* = 12.0, 6.0 Hz, 1H), 2.80 (d, *J* = 18.0 Hz, 1H), 2.61−2.57 (m, 1H), 2.48−2.41 (m, 1H), 2.15−1.32 (m, 15H), 1.10 (s, 3H), 1.04 (d, *J* = 6.0 Hz, 3H), 0.92 (d, *J* = 6.6 Hz, 3H), 0.86 (s, 3H), 0.85 (d, *J* = 6.6 Hz, 3H), 0.83 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 204.6, 159.9, 156.9, 135.7, 135.3, 133.2, 132.9, 132.5, 131.6, 130.9, 129.2, 127.2, 127.1, 70.2, 67.5, 56.0, 51.1, 50.6, 44.8, 44.6, 42.9, 41.5, 39.9, 38.6, 36.2, 34.3, 33.2, 27.7, 24.3, 23.8, 21.3, 20.1, 19.8, 17.9, 17.6, 13.5; HRESIMS *m*/*z* 577.3408 $[M + Na]^+$ (calcd for $C_{36}H_{46}N_2O_3Na$, 577.3401).

*(22E)-5α,8α-(1,4-Dioxo-1,2,3,4-tetrahydrophthalazine-2,3-diyl)-ergosta-6,22-dien-3α-ol (**5**).* L-Selectride (1.0 M in THF, 0.235 mL, 0.235 mmol) was added to a solution of ketone **S1** (99.5 mg, 0.179 mmol) in dry THF (1.6 mL) at −78 °C. After stirring at −78 °C for 30 min, the reaction was quenched with 2 M aqueous NaOH (0.36 mL, 0.72 mmol) and H_2O_2 (30% in H_2O, 51 μL, 0.45 mmol). The resultant solution was stirred at rt for 20 min and extracted with Et_2O. The organic layer was washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc = 5/1 → 1/1) to give olefin **5** (65.7 mg, 0.118 mmol, 66%) as a yellow foam: $[\alpha]^{24}_D$ +271 (*c* 1.06, $CHCl_3$); R_f = 0.58 (hexane/EtOAc = 1/2); IR (neat) 3384, 2956, 2870, 1653, 1586, 1457, 1370, 1220, 1159, 1068, 971, 873, 772 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 8.14−8.08 (m, 2H), 7.69−7.63 (m, 2H), 6.63 (d, *J* = 8.3 Hz, 1H), 6.27 (d, *J* = 8.3 Hz, 1H), 5.22 (dd, *J* = 15.1, 7.6 Hz, 1H), 5.15 (dd, *J* = 15.1, 8.3 Hz, 1H), 4.31 (d, *J* = 16.5 Hz, 1H), 4.13 (brs, 1H), 4.00 (dd, *J* = 11.7, 6.9 Hz, 1H), 2.85 (s, 1H), 2.26 (dd, *J* = 16.5, 3.4 Hz, 1H), 2.16 (dd, *J* = 13.1, 4.8 Hz, 1H), 2.07−1.97 (m, 2H), 1.95−1.80 (m, 4H), 1.66−1.57 (m, 2H), 1.50−1.28 (m, 8H), 1.02 (d, *J* = 6.2 Hz, 3H), 0.99 (s, 3H), 0.90 (d, *J* = 6.9 Hz, 3H), 0.84 (s, 3H), 0.83 (d, *J* = 6.9 Hz, 3H), 0.81 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.0, 159.8, 138.9, 135.4, 132.9, 132.8, 132.3, 130.8, 130.3, 129.0, 127.0, 126.9, 68.3, 65.8, 64.6, 56.7, 50.9, 48.6, 44.2, 42.8, 41.5, 40.0, 39.3, 33.2, 32.3, 31.9, 28.3, 27.9, 24.4, 21.9, 21.0, 20.0, 19.8, 18.6, 17.6, 13.3; HRESIMS *m*/*z* 579.3557 $[M + Na]^+$ (calcd for $C_{36}H_{48}N_2O_3Na$ 579.3557).

*(20S)-5α,8α-(1,4-Dioxo-1,2,3,4-tetrahydrophthalazine-2,3-diyl)-20-methylpregn-6-ene-3α,21-diol (**S2**).* O_3 was bubbled through a solution of olefin **5** (701 mg, 1.26 mmol) and pyridine (0.84 mL, 10 mmol) in dry CH_2Cl_2 (32 mL) at −60 °C for 20 min. O_2 was then bubbled through the solution for 10 min to remove excess O_3. $NaBH_4$ (383 mg, 10.1 mmol) and MeOH (30 mL) were added to the solution. After stirring at −60 °C for 1.5 h, the reaction mixture was warmed to rt and stirred for 40 min. The reaction was quenched with saturated aqueous NH_4Cl at 0 °C. The mixture was extracted with Et_2O, and the combined organic layer was washed with saturated aqueous $KHSO_4$, saturated aqueous $NaHCO_3$, and saturated aqueous NaCl. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc = 2/1 → 1/5) to give diol **S2** (438 mg, 0.893 mmol, 71%) as a yellow powder: $[\alpha]^{28}_D$ −14.7 (*c* 1.45, $CHCl_3$); R_f = 0.20 (hexane/EtOAc = 1/2); IR (neat) 3451, 2947, 2874, 1634, 1600, 1461, 1438, 1391, 1317, 1267, 1217, 1170, 1101, 1067, 1040, 1004, 975, 931, 906 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 8.15−8.07 (m, 2H), 7.71−7.64 (m, 2H), 6.65 (d, *J* = 8.3 Hz, 1H), 6.27 (d, *J* = 8.3 Hz, 1H), 4.31 (brd, *J* = 15.8 Hz, 1H), 4.14 (brs, 1H), 4.01 (dd, *J* = 11.7, 7.6 Hz, 1H), 3.68−3.63 (brd, *J* = 10.0 Hz, 1H), 3.39−3.34 (m, 1H), 2.83 (s, 1H), 2.26 (dd, *J* = 16.5, 3.4 Hz, 1H), 2.17 (dd, *J* = 13.1, 4.8 Hz, 1H), 2.02 (ddd, *J* = 13.1, 3.4, 3.4 Hz, 1H), 2.00−1.94 (m, 1H), 1.92−1.87 (m, 1H), 1.84 (ddd, *J* = 13.1, 13.1, 3.4 Hz, 1H), 1.65−1.28 (m, 11H), 1.06 (d, *J* = 6.2 Hz, 3H), 0.99 (s, 3H), 0.86 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.0, 159.8, 139.0, 132.97, 132.89, 130.8, 130.3, 128.8, 127.0, 126.9, 68.2, 67.8, 65.8, 64.6, 53.5, 50.8, 48.3, 44.5, 41.5, 39.3, 38.5, 32.3, 31.9, 27.9, 27.3, 24.4, 21.9, 18.5, 16.8, 13.2; HRESIMS *m*/*z* 513.2720 $[M + Na]^+$ (calcd for $C_{30}H_{38}N_2O_4Na$, 513.2724).

*(20S)-3α-Hydroxy-20-methyl-5α-pregn-7-en-21-yl Pivalate (**7**).* $LiAlH_4$ (233 mg, 6.13 mmol) was added to a solution of diol **S2** (792 mg, 1.61 mmol) in dry THF (23 mL) at 0 °C. After stirring at 80 °C for 2 h, the reaction mixture was cooled to 0 °C and treated sequentially with H_2O (0.25 mL), 2 M aqueous NaOH (0.50 mL), and H_2O (0.75 mL). The resultant solution was diluted with Et_2O and stirred for 10 min. The cake was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure. The crude diene **6** (594 mg) was used in the next reaction without further purification: R_f = 0.48 (hexane/EtOAc = 1/2); HRESIMS *m*/*z* 353.2461 $[M + Na]^+$ (calcd for $C_{22}H_{34}O_2Na$, 353.2451).

A solution of crude diene **6** (594 mg) and *tert*-amyl alcohol (1.05 mL, 9.66 mmol) in dry THF (20 mL + 10 + 4 mL rinse) was added to a stirred deep-blue solution of Li (113 mg, 16.3 mmol) in liquid NH_3 (20 mL) at −78 °C. After stirring at −78 °C for 1 h, the reaction mixture was quenched with solid NH_4Cl (8.65 g, 162 mmol), and the mixture was stirred at rt for 2 h. After addition of saturated aqueous NH_4Cl and H_2O, the reaction mixture was extracted with THF. The organic layer was washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude olefin **S3** (683 mg) was used in the next reaction without further purification.

Pivaloyl chloride (0.48 mL, 3.9 mmol) was added to a solution of crude olefin **S3** (683 mg) in pyridine (11 mL) and dry CH_2Cl_2 (8.0 mL) at 0 °C. After stirring at 0 °C for 2 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with saturated aqueous $KHSO_4$ and saturated aqueous NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc = 1/0 → 1/1) to give pivalate ester **7** (508 mg, 1.22 mmol, 76% for the three steps) as a colorless solid: $[\alpha]^{23}_D$ +8.80 (*c* 1.04, $CHCl_3$); mp 140−141 °C; R_f = 0.43 (hexane/EtOAc = 3/1); IR (neat) 3278, 2936, 2873, 1730, 1479, 1445, 1397, 1383, 1366, 1284, 1160, 1029, 1007, 979, 943, 905, 848, 794, 770 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 5.19−5.16 (m, 1H), 4.08−4.04 (m, 2H), 3.77 (dd, *J* = 11.0, 7.6 Hz, 1H), 2.03−1.99 (m, 1H) 1.90−1.79 (m, 2H), 1.79−1.49 (m, 12H), 1.49−1.39 (m, 4H), 1.39−1.22 (m, 3H), 1.20 (s, 9H), 1.02 (d, *J* = 6.9 Hz, 3H) 0.78 (s, 3H), 0.56 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 178.8, 139.2, 118.0, 69.4, 66.6, 54.9, 53.0, 49.6, 43.7, 39.6, 39.0, 36.4, 35.7, 34.87, 34.78, 32.1, 29.7, 28.9, 27.5, 27.4 (3C), 23.1, 21.4, 17.4, 12.2, 12.1; HRESIMS *m*/*z* 439.3178 $[M + Na]^+$ (calcd for $C_{27}H_{44}O_3Na$, 439.3183).

*(20S)-3α-(4-Methoxybenzyl)oxy-20-methyl-5α-pregn-7-en-21-yl Pivalate (**8**).* $La(OTf)_3$ (42.3 mg, 0.0722 mmol) was added to a solution of 4-methoxybenzyl-2,2,2-trichloroacetimidate (300 mg, 1.06 mmol) and alcohol **7** (298 mg, 0.714 mmol) in dry toluene (7.2 mL) at 0 °C. After stirring at 0 °C for 30 min, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ and then extracted with EtOAc. The organic layer was washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc = 1/0 → 1/1) to give olefin **8** (322 mg, 0.600 mmol, 83%) as a colorless foam: $[\alpha]^{22}_D$ +16.4 (*c* 1.23, $CHCl_3$); R_f = 0.45 (hexane/EtOAc = 7/1); IR (neat) 2933, 2872, 1723, 1613, 1586, 1513, 1463, 1397, 1362, 1283, 1246, 1161, 1072, 1036, 977, 942, 823, 771 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.26 (d, *J* = 7.8 Hz, 2H), 6.87 (d, *J* = 7.8 Hz, 2H), 5.19−5.16 (m, 1H), 4.41 (s, 2H), 4.07 (dd, *J* = 10.8, 3.0 Hz, 1H), 3.81−3.76 (m, 4H), 3.65 (brs, 1H), 2.03−1.98 (m, 1H), 1.90−1.75 (m, 5H), 1.75−1.64 (m,

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4H), 1.64−1.40 (m, 6H), 1.40−1.22 (m, 5H), 1.21 (s, 9H), 1.03 (d, J = 7.2 Hz, 3H), 0.79 (s, 3H), 0.57 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 178.7, 159.0, 139.3, 131.6, 128.9 (2C), 118.0, 113.8 (2C), 72.9, 69.4, 69.2, 55.3, 54.9, 52.9, 49.5, 43.6, 39.6, 39.0, 36.4, 35.2, 34.7, 32.7, 32.6, 29.7, 27.5, 27.4 (3C), 25.8, 23.1, 21.3, 17.4, 12.4, 12.0; HRESIMS m/z 559.3783 $[M + Na]^+$ (calcd for $C_{35}H_{52}O_4Na$, 559.3758).

*(20S)-7α,8α-Dihydroxy-3α-(4-methoxybenzyl)oxy-20-methyl-5α-pregnan-21-yl Pivalate (**3b**).* A solution of olefin **8** (51.0 mg, 95.1 μmol) in CH_2Cl_2 (0.05 mL + 0.05 mL rinse) was added to a stirred mixture of $K_2OsO_4{\cdot}2H_2O$ (1.9 mg, 5.2 μmol), DABCO (1.6 mg, 14 μmol), $K_3Fe(CN)_6$ (91.3 mg, 0.277 mmol), K_2CO_3 (39.6 mg, 0.287 mmol), and $MeSO_2NH_2$ (26.6 mg, 0.280 mmol) in t-BuOH/H_2O (1:1, v/v, 0.94 mL) at 0 °C. After stirring at rt for 36 h, the reaction mixture was quenched with solid $Na_2S_2O_3$ at 0 °C and stirred at rt for 10 h. The reaction mixture was extracted with Et_2O, and the organic layer was washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc = 3/1 → 1/3 with addition of 1 v/v % Et_3N) to give diol **3b** (42.5 mg, 74.5 μmol, 80%) as a colorless solid: $[\alpha]^{23}_D$ −14.7 (c 1.56, $CHCl_3$); mp 157−158 °C; R_f = 0.17 (hexane/EtOAc = 3/1); IR (neat) 3274, 2934, 2853, 1726, 1613, 1512, 1480, 1459, 1397, 1362, 1284, 1245, 1151, 1075, 1037, 973, 949, 827, 771 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.26 (d, J = 8.4 Hz, 2H), 6.86 (d, J = 8.4 Hz, 2H), 4.42 (d, J = 12.4 Hz, 1H), 4.41 (d, J = 12.4 Hz, 1H), 4.14−4.09 (m, 1H), 4.04 (dd, J = 10.8, 3.6 Hz, 1H), 3.82−3.77 (m, 1H), 3,79 (s, 3H), 3.61 (brs, 1H), 2.96−2.88 (m, 1H), 2.13−2.03 (m, 1H), 1.99−1.92 (m, 2H), 1.84−1.66 (m, 6H), 1.60−1.53 (m, 4H), 1.52−1.42 (m, 3H), 1.37−1.13 (m, 6H), 1.20 (s, 9H), 1.01 (d, J = 6.0 Hz, 3H), 0.85 (s, 3H), 0.79 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 178.8, 159.0, 131.7, 129.0 (2C), 113.8 (2C), 78.5, 72.6, 70.4, 69.3 (2C), 61.7, 55.7, 55.4, 53.7, 43.3, 40.3, 39.1, 38.5, 36.5, 36.2, 36.0, 32.3, 31.0, 27.4 (3C), 27.1, 25.8, 23.9, 22.9, 17.3, 15.0, 12.1; HRESIMS m/z 593.3801 $[M + Na]^+$ (calcd for $C_{35}H_{54}O_6Na$, 593.3813).

*(5R,20S)-3α-(4-Methoxybenzyl)oxy-20-methyl-7,8-dioxo-7,8-seco-5α-pregnan-21-yl Pivalate (**9**).* $Pb(OAc)_4$ (1.29 g, 2.33 mmol) was added to a solution of diol **3b** (838 mg, 1.47 mmol) in dry CH_2Cl_2 (15 mL) at 0 °C. After stirring at rt for 35 min, the reaction mixture was quenched with H_2O at 0 °C and filtered through a pad of Celite. The filtrate was extracted with Et_2O, and the organic layer was washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc = 2/1 → 0/1) to give ketoaldehyde **9** (773 mg, 1.36 mmol, 92%) as a colorless foam: $[\alpha]^{24}_D$ +2.7 (c 0.82, $CHCl_3$); R_f = 0.49 (hexane/EtOAc = 2/1); IR (neat) 2958, 2873, 1723, 1613, 1513, 1458, 1397, 1364, 1285, 1247, 1159, 1079, 1035, 976, 937, 823, 754 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.69 (dd, J = 3.4, 1.4 Hz, 1H), 7.25 (d, J = 8.9 Hz, 2H), 6.87 (d, J = 8.3 Hz, 2H), 4.41 (s, 2H), 4.04 (dd, J = 10.8, 3.6 Hz, 1H), 3.81−3.76 (m, 1H), 3.79 (s, 3H), 3.53 (brs, 1H), 2.66 (brs, 1H), 2.47−2.37 (m, 3H), 2.17−2.12 (m, 1H), 2.09−1.99 (m, 2H), 1.92−1.82 (m, 2H), 1.82−1.67 (m, 4H), 1.67−1.58 (m, 4H), 1.54−1.36 (m, 4H), 1.20 (s, 9H), 1.03 (s, 3H), 1.02 (d, J = 7.6 Hz, 3H), 0.61 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 210.7, 203.1, 178.7, 159.1, 131.2, 129.1 (2C), 113.9 (2C), 72.1, 69.5, 69.0, 63.1, 55.4 (2C), 53.5 (2C), 50.6, 44.3, 39.3, 39.0, 36.9, 35.7, 32.1, 27.5, 27.3 (3C), 26.2 (2C), 25.3, 19.3, 17.8, 17.3, 12.4; HRESIMS m/z 591.3659 $[M + Na]^+$ (calcd for $C_{35}H_{52}O_6Na$, 591.3656).

*(20S)-7β,8β-Dihydroxy-3α-(4-methoxybenzyl)oxy-20-methyl-5α-pregnan-21-yl Pivalate (**3c**).* Diiodomethane (1.30 mL, 16.2 mmol) was added dropwise to a vigorously stirred mixture of Sm metal (3.01 g, 20.0 mmol, treated in a glovebox) and dry THF (200 mL) at 0 °C. The mixture was vigorously stirred at 0 °C for 10 min and then at rt for 3 h to give a deep-blue suspension of SmI_2 (ca. 0.08 M in THF). This suspension (47.0 mL, 3.76 mmol) was added to a solution of ketoaldehyde **9** (537 mg, 0.945 mmol) in freshly distilled THF (47 mL) and MeOH (0.26 mL) at 0 °C. After stirring at 0 °C for 45 min, the reaction was quenched with saturated aqueous NH_4Cl and extracted with Et_2O. The organic layer was washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc = 7/1 → 1/1) to give diol **3c** (456 mg, 0.799 mmol, 85%) as a colorless solid: $[\alpha]^{24}_D$ +20.1 (c 1.03, $CHCl_3$); mp 163−164 °C; R_f = 0.31 (hexane/EtOAc = 3/1); IR (neat) 3533, 2933, 2870, 1725, 1613, 1513, 1458, 1397, 1362, 1286, 1247, 1169, 1057, 1036, 977, 946, 825, 774, 756 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.26 (d, J = 9.0 Hz, 2H), 6.87 (d, J = 9.0 Hz, 2H), 4.43 (d, J = 11.4 Hz, 1H), 4.39 (d, J = 11.4 Hz, 1H), 4.04 (dd, J = 10.2, 3.6 Hz, 2H), 3.80 (s, 3H), 3.76 (dd, J = 10.8, 7.2 Hz, 1H), 3.63−3.59 (m, 1H), 3.47 (dd, J = 11.4, 4.8 Hz, 1H), 2.06 (br, 1H), 2.02 (dt, J = 12.4, 3.4 Hz, 1H), 1.86−1.76 (m, 2H), 1.76−1.67 (m, 2H), 1.67−1.49 (m, 6H), 1.49−1.40 (m, 3H), 1.40−1.32 (m, 1H), 1.32−1.27 (m, 1H), 1.27−1.22 (m, 1H), 1.22−1.15 (m, 2H), 1.20 (s, 9H), 1.10 (ddd, J = 10.3, 9.6, 9.6 Hz, 1H), 0.99 (d, J = 6.6 Hz, 3H), 0.97 (s, 3H), 0.94 (s, 3H), 0.90 (dd, J = 13.1, 2.8 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 178.9, 159.1, 131.5, 129.1 (2C), 113.9 (2C), 76.2, 76.1, 72.9, 69.5, 69.4, 58.7, 55.6, 55.4, 52.7, 44.3, 41.1, 39.1, 37.4, 35.7, 35.5, 34.3, 33.7, 32.6, 27.8, 27.4 (3C), 25.3, 22.7, 18.0, 17.1, 13.8, 11.6; HRESIMS m/z 593.3808 $[M + Na]^+$ (calcd for $C_{35}H_{54}O_6Na$, 593.3813).

*(20S)-8β-Hydroxy-3α-(4-methoxybenzyl)oxy-20-methyl-7-oxo-5α-pregnan-21-yl Pivalate (**10**).* DMSO (0.15 mL, 2.1 mmol), Et_3N (0.15 mL, 1.1 mmol), and SO_3·pyridine (103 mg, 0.627 mmol) were added to a solution of diol **3c** (69.9 mg, 0.122 mmol) in dry CH_2Cl_2 (1.2 mL) at 0 °C. After stirring at rt for 13 h, the reaction mixture was quenched with saturated aqueous NH_4Cl at 0 °C and extracted with Et_2O. The organic layer was washed with saturated aqueous $KHSO_4$ and saturated aqueous NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 10/1 → 1/1) to give hydroxy ketone **10** (59.1 mg, 0.104 mmol, 85%) as a colorless foam: $[\alpha]^{24}_D$ −17.0 (c 1.17, $CHCl_3$); R_f = 0.35 (hexane/EtOAc = 3/1); IR (neat) 3502, 2934, 1712, 1612, 1513, 1462, 1397, 1362, 1340, 1285, 1247, 1168, 1063, 1036, 975, 946, 824, 754, 666 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.22 (d, J = 8.4 Hz, 2H), 6.85 (d, J = 8.4 Hz, 2H), 4.37 (s, 2H), 4.05 (dd, J = 10.2, 3.0 Hz, 1H), 3.78 (s, 3H), 3.78−3.74 (m, 1H), 3.62 (brs, 1H), 3.04 (dd, J = 14.4, 12.0 Hz, 1H), 2.11−2.04 (m, 1H), 2.02−1.93 (m, 2H), 1.93−1.85 (m, 1H), 1.85−1.75 (m, 4H), 1.74−1.66 (m, 1H), 1.66−1.51 (m, 5H), 1.49 (brd, J = 13.1 Hz, 1H), 1.39−1.31 (m, 1H), 1.30−1.08 (m, 5H), 1.19 (s, 9H), 1.15 (s, 3H), 0.99 (d, J = 6.0 Hz, 3H), 0.94 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 211.7, 178.7, 159.0, 131.2, 128.9 (2C), 113.8 (2C), 81.4, 72.7, 69.5, 69.2, 58.9, 55.3, 52.4, 52.1, 43.3, 43.1, 41.7, 39.9, 39.0, 36.9, 35.3, 33.9, 32.8, 27.3 (4C), 25.3, 19.9, 17.8, 17.1, 14.0, 11.5; HRESIMS m/z 591.3656 $[M + Na]^+$ (calcd for $C_{35}H_{52}O_6Na$, 591.3656).

*(20S)-7α,8β-Dihydroxy-3α-(4-methoxybenzyl)oxy-20-methyl-5α-pregnan-21-yl Pivalate (**3a**).* $NaBH(OAc)_3$ (827 mg, 3.12 mmol) was added to a solution of hydroxy ketone **10** (59.1 mg, 0.104 mmol) and i-Pr_2NEt (2.5 mL, 14 mmol) in dry THF (5.2 mL) at 0 °C. After stirring at rt for 15 h, the reaction mixture was quenched with saturated aqueous NH_4Cl at 0 °C and extracted with EtOAc. The organic layer was washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc = 10/1 → 1/3) to give diol **3a** (55.3 mg, 0.0969 mmol, 93%) as a colorless foam: $[\alpha]^{22}_D$ +6.82 (c 1.11, $CHCl_3$); R_f = 0.12 (hexane/EtOAc = 3/1); IR (neat) 3525, 2936, 1714, 1613, 1513, 1458, 1397, 1362, 1287, 1246, 1169, 1124, 1065, 1034, 971, 943, 897, 821, 756 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.27 (d, J = 8.4 Hz, 2H), 6.87 (d, J = 8.4 Hz, 2H), 4.45 (d, J = 11.4 Hz, 1H), 4.40 (d, J = 11.4 Hz, 1H), 4.06 (dd, J = 10.8, 3.0 Hz, 1H), 3.80 (s, 3H), 3.75 (dd, J = 10.2, 7.2 Hz, 1H), 3.63 (brs, 1H), 3.48 (brs, 1H), 2.07−2.02 (m, 1H), 1.98 (dt, J = 13.2, 3.6 Hz, 1H), 1.92 (td, J = 13.2, 1.8 Hz, 1H), 1.91−1.84 (m, 1H), 1.80 (brd, J = 13.8 Hz, 1H), 1.75−1.68 (m, 2H), 1.68−1.54 (m, 4H), 1.48−1.42 (m, 3H), 1.41−1.21 (m, 8H), 1.20 (s, 9H), 1.13 (dt, J = 14.4, 2.8 Hz, 1H), 0.99 (d, J = 6.0 Hz, 3H), 0.95 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 178.9, 159.1, 131.7, 129.1 (2C), 113.9 (2C), 76.5, 73.1, 72.6, 69.5, 69.3, 55.4, 53.4, 53.2, 49.8, 43.2, 40.4, 39.1, 36.4, 35.5, 33.5, 32.5 (2C), 32.4, 27.4 (3C), 27.2, 25.4, 18.7,

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17.8, 17.0, 13.7, 11.5; HRESIMS m/z 593.3806 $[M + Na]^+$ (calcd for $C_{35}H_{54}O_6Na$, 593.3813).

*(20S)-3α-(4-Methoxybenzyl)oxy-20-methyl-5α-pregnane-7α,8β,21-triol (**11**).* DIBALH (1.0 M in toluene, 0.80 mL, 0.80 mmol) was added to a solution of pivalate **3a** (55.3 mg, 0.0969 mmol) in dry CH_2Cl_2 (1.0 mL) at −78 °C. After stirring at −78 °C for 1 h, the reaction mixture was warmed to rt. After stirring at rt for 1 h, the reaction mixture was quenched with MeOH at 0 °C. After addition of Et_2O and saturated aqueous Na^+/K^+ tartrate, the mixture was stirred at rt for 1 h and then extracted with EtOAc. The organic layer was washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc = 2/1 → 1/3) to give triol **11** (42.7 mg, 0.0877 mmol, 91%) as a colorless solid: $[\alpha]^{23}_D$ +7.51 (*c* 1.42, $CHCl_3$); mp 188−189 °C; R_f = 0.22 (hexane/EtOAc = 1/1); IR (neat) 3416, 2934, 1612, 1586, 1512, 1443, 1361, 1302, 1246, 1171, 1145, 1125, 1032, 990, 968, 896, 822, 755, 666 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.26 (d, *J* = 8.4 Hz, 2H), 6.87 (d, *J* = 8.4 Hz, 2H), 4.45 (d, *J* = 11.4 Hz, 1H), 4.39 (d, *J* = 11.4 Hz, 1H), 3.80 (s, 3H), 3.63−3.59 (m, 2H), 3.46 (brs, 1H), 3.33 (dd, *J* = 10.2, 7.2 Hz, 1H), 2.07−2.01 (m, 1H), 1.98 (dt, *J* = 12.4, 3.4 Hz, 1H), 1.90 (td, *J* = 13.8, 2.8 Hz, 1H), 1.89−1.83 (m, 1H), 1.80 (brd, *J* = 15.1 Hz, 1H), 1.77−1.49 (m, 8H), 1.49−1.40 (m, 3H), 1.38−1.15 (m, 7H), 1.12 (dt, *J* = 13.8, 2.8 Hz, 1H), 1.01 (d, *J* = 7.2 Hz, 3H), 0.94 (s, 3H), 0.93 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 159.1, 131.6, 129.1 (2C), 113.9 (2C), 76.5, 73.1, 72.5, 69.5, 67.9, 55.4, 53.1 (2C), 49.8, 43.2, 40.4, 38.3, 36.4, 33.5, 32.5, 32.42, 32.38, 27.3, 25.3, 18.6, 17.7, 16.6, 13.7, 11.5; HRESIMS m/z 509.3239 $[M + Na]^+$ (calcd for $C_{30}H_{46}O_5Na$, 509.3237).

*(20S)-8α-Hydroxy-3α-(4-methoxybenzyl)oxy-20-methyl-7-oxo-5α-pregnan-21-yl Pivalate (**12**).* *i*-Pr_2NEt (0.063 mL, 0.36 mmol) and SO_3·pyridine (28.6 mg, 0.179 mmol) were added to a solution of diol **3b** (20.5 mg, 0.0359 mmol) in dry CH_2Cl_2/DMSO (4:1, v/v, 0.90 mL) at 0 °C. After stirring at rt for 12 h, the reaction mixture was quenched with water at 0 °C and extracted with EtOAc. The organic layer was washed with water and saturated aqueous NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 9/1 → 4/1 with 1% v/v Et_3N) to give hydroxy ketone **12** (15.5 mg, 0.0273 mmol, 76%) as a colorless foam: $[\alpha]^{29}_D$ −14.3 (*c* 0.77, benzene); R_f = 0.31 (EtOAc/hexane = 1/3); IR (neat) 3342, 2969, 2934, 2912, 2868, 1726, 1712, 1612, 1513, 1285, 1244, 1167, 1088, 1036 cm^{-1}; ^1H NMR (600 MHz, C_6D_6) δ 7.31 (d, *J* = 8.3 Hz, 2H), 6.86 (d, *J* = 8.9 Hz, 2H), 4.36 (d, *J* = 11.7 Hz, 1H), 4.33 (d, *J* = 11.7 Hz, 1H), 4.08 (dd, *J* = 11.0, 3.4 Hz, 1H), 3.87 (dd, *J* = 11.0, 6.9 Hz, 1H), 3.59−3.52 (m, 1H), 3.48−3.45 (m, 1H), 3.33 (s, 3H), 2.59 (dd, *J* = 17.2, 6.9 Hz, 1H), 2.27−2.17 (m, 1H), 1.80 (dd, *J* = 17.2, 11.7 Hz, 1H), 1.79−1.68 (m, 4H), 1.67−1.61 (m, 1H), 1.62−1.56 (m, 1H), 1.55−1.44 (m, 2H), 1.43−1.36 (m, 2H), 1.34−1.23 (m, 4H), 1.22 (s, 9H), 1.16 (dd, *J* = 13.1, 7.6 Hz, 1H), 1.10−1.01 (m, 2H), 1.00 (d, *J* = 6.9 Hz, 3H), 0.95−0.88 (m, 1H), 0.88 (s, 3H), 0.64 (s, 3H); ^{13}C NMR (150 MHz, C_6D_6) δ 210.0, 177.8, 159.7, 132.0, 129.2 (2C), 114.1 (2C), 78.8, 72.5, 69.7, 69.2, 59.3, 58.8, 54.8, 53.7, 44.2, 43.1, 39.9, 39.0, 36.5, 36.2, 35.5, 32.5, 32.4, 27.5 (3C), 27.4, 26.0, 22.9, 19.9, 17.4, 14.4, 13.7; HRESIMS m/z 591.3650 $[M + Na]^+$ (calcd for $C_{35}H_{52}O_6Na$, 591.3656).

*(20S)-7β,8α-Dihydroxy-3α-(4-methoxybenzyl)oxy-20-methyl-5α-pregnan-21-yl Pivalate (**3d**).* $NaBH_4$ (15.5 mg, 0.410 mmol) was added to a solution of hydroxy ketone **12** (15.5 mg, 0.0273 mmol) in dry THF/MeOH (4:1, v/v, 1.0 mL) at 0 °C. After stirring at 0 °C for 1.5 h, the reaction mixture was quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with water and saturated aqueous NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc = 9/1 → 3/2 with 1% v/v Et_3N) to give diol **3d** (13.9 mg, 0.0244 mmol, 89%) as a colorless solid: $[\alpha]^{29}_D$ +5.8 (*c* 0.69, $CHCl_3$); R_f = 0.18 (hexane/EtOAc = 3/1); IR (neat) 3333, 2918, 1710, 1513, 1236, 1165, 1052 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.25 (d, *J* = 8.9 Hz, 2H), 6.85 (d, *J* = 8.9 Hz, 2H), 4.41 (s, 2H), 4.04 (dd, *J* = 11.0, 3.4 Hz, 1H), 3.89 (brd, *J* = 8.3 Hz, 1H), 3.79 (s, 3H), 3.78 (dd, *J* = 10.3, 6.9 Hz, 1H), 3.63 (brs, 1H), 2.69−2.61 (m, 1H), 2.04 (ddd, *J* = 14.4, 8.3, 6.2 Hz, 1H), 1.94 (ddd, *J* = 12.4, 3.4, 3.4 Hz, 1H), 1.89−1.81 (m, 1H), 1.80−1.73 (m, 3H), 1.72−1.65 (m, 2H), 1.61 (dd, *J* = 13.8, 6.9 Hz, 1H), 1.58−1.50 (m, 4H), 1.47−1.34 (m, 4H), 1.34−1.20 (m, 4H), 1.20 (s, 9H), 1.17−1.09 (m, 1H), 1.03 (s, 3H), 1.01 (d, *J* = 6.2 Hz, 3H), 0.90 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 178.9, 159.0, 131.7, 129.0 (2C), 113.8 (2C), 76.7, 73.0, 69.30, 69.25, 69.17, 62.8, 55.4, 55.2, 54.4, 43.4, 40.0, 39.1, 37.2, 36.9, 36.1, 35.0, 32.6, 30.3, 27.4 (3C), 26.9, 25.9, 23.9, 20.6, 17.1, 14.0, 13.5; HRESIMS m/z 593.3820 $[M + Na]^+$ (calcd for $C_{35}H_{54}O_6Na$, 593.3813).

*(20S)-8β-Hydroxy-3α-(4-methoxybenzyl)oxy-20-methyl-7α-(triethylsilyl)oxy-5α-pregnan-21-yl Pivalate (**S4**).* TESOTf (0.16 mL, 0.71 mmol) was added to a solution of diol **3a** (199 mg, 0.348 mmol) and 2,6-lutidine (0.16 mL, 1.4 mmol) in dry CH_2Cl_2 (3.5 mL) at 0 °C. After stirring at rt for 1.5 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ at 0 °C and extracted with EtOAc. The organic layer was washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc = 30/1 → 10/1) to give silyl ether **S4** (220 mg, 0.321 mmol, 92%) as a colorless foam: $[\alpha]^{23}_D$ +2.21 (*c* 1.00, $CHCl_3$); R_f = 0.57 (hexane/EtOAc = 4/1); IR (neat) 3527, 2937, 2843, 1727, 1614, 1513, 1458, 1397, 1363, 1285, 1246, 1168, 1129, 1086, 1036, 1015, 974, 946, 824, 792, 772, 741 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.26 (d, *J* = 8.4 Hz, 2H), 6.86 (d, *J* = 8.4 Hz, 2H), 4.44 (d, *J* = 11.4 Hz, 1H), 4.37 (d, *J* = 11.4 Hz, 1H), 4.00 (dd, *J* = 10.2, 2.4 Hz, 1H), 3.82 (dd, *J* = 10.8, 7.2 Hz, 1H), 3.80 (s, 3H), 3.60 (brs, 1H), 3.48 (brs, 1H), 2.21 (brt, *J* = 13.1 Hz, 1H), 1.96 (dt, *J* = 12.4, 2.8 Hz, 1H), 1.86−1.66 (m, 5H), 1.65−1.56 (m, 2H), 1.56−1.49 (m, 2H), 1.46−1.40 (m, 2H), 1.40−1.23 (m, 5H), 1.23−1.16 (m, 2H), 1.20 (s, 9H), 1.08 (dt, *J* = 14.4, 2.8 Hz, 1H), 1.01−0.99 (m, 4H), 0.934 (t, *J* = 8.3 Hz, 9H), 0.930 (s, 3H), 0.92 (s, 3H), 0.65−0.54 (m, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 178.8, 158.9, 131.9, 128.7 (2C), 113.7 (2C), 77.2, 73.2, 73.1, 69.4, 69.2, 55.4, 53.4, 52.8, 49.3, 42.9, 40.5, 39.1, 36.4, 35.4, 33.6, 32.7, 32.0, 31.9, 27.3 (3C), 27.0, 26.1, 18.6, 17.7, 17.2, 13.7, 11.6, 7.2 (3C), 5.5 (3C); HRESIMS m/z 707.4700 $[M + Na]^+$ (calcd for $C_{41}H_{68}O_6SiNa$, 707.4677).

*(20S)-3α-(4-Methoxybenzyl)oxy-20-methyl-7α-(triethylsilyl)oxy-5α-pregnane-8β,21-diol (**S5**).* DIBALH (1.0 M in toluene, 1.7 mL, 1.7 mmol) was added to a solution of silyl ether **S4** (136 mg, 0.199 mmol) in dry CH_2Cl_2 (2.0 mL) at −78 °C. After stirring at −78 °C for 40 min, the reaction mixture was warmed to rt. After stirring at rt for 20 min, the reaction mixture was quenched with MeOH at 0 °C. After addition of Et_2O and saturated aqueous Na^+/K^+ tartrate, the reaction mixture was stirred for 1 h and then extracted with EtOAc. The organic layer was washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc = 7/1 → 1/1) to give diol **S5** (119 mg, 0.198 mmol, 99%) as a colorless foam: $[\alpha]^{26}_D$ +5.44 (*c* 1.02, $CHCl_3$); R_f = 0.16 (hexane/EtOAc = 4/1); IR (neat) 3430, 2935, 2873, 1613, 1513, 1463, 1362, 1301, 1246, 1220, 1170, 1149, 1086, 1037, 973, 943, 825, 772, 742, 675 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.26 (d, *J* = 8.3 Hz, 2H), 6.86 (d, *J* = 8.3 Hz, 2H), 4.44 (d, *J* = 11.7 Hz, 1H), 4.37 (d, *J* = 11.7 Hz, 1H), 3.80 (s, 3H), 3.63 (dd, *J* = 10.3, 3.4 Hz, 1H), 3.59 (brt, *J* = 2.8 Hz, 1H), 3.48 (brs, 1H), 3.34 (dd, *J* = 10.3, 7.2 Hz, 1H), 2.20 (tt, *J* = 13.1, 2.8 Hz, 1H), 1.96 (dt, *J* = 12.4, 3.4 Hz, 1H), 1.86−1.69 (m, 4H), 1.66−1.49 (m, 6H), 1.46−1.40 (m, 2H), 1.40−1.22 (m, 6H), 1.18 (td, *J* = 12.4, 3.4 Hz, 1H), 1.12 (ddd, *J* = 10.3, 9.6, 9.6 Hz, 1H), 1.08 (dt, *J* = 13.8, 2.8 Hz, 1H), 1.02 (d, *J* = 6.2 Hz, 3H), 0.94 (t, *J* = 8.3 Hz, 9H), 0.93 (s, 3H), 0.92 (s, 3H), 0.65−0.54 (m, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 158.9, 131.9, 128.7 (2C), 113.7 (2C), 77.3, 73.2, 73.1, 69.2, 68.0, 55.4, 53.2, 52.7, 49.3, 43.0, 40.5, 38.5, 36.4, 33.7, 32.7, 32.0, 31.9, 27.2, 26.1, 18.7, 17.7, 16.6, 13.7, 11.6, 7.2 (3C), 5.5 (3C); HRESIMS m/z 623.4099 $[M + Na]^+$ (calcd for $C_{36}H_{60}O_5SiNa$, 623.4102).

*(20S)-8β-Hydroxy-3α-(4-methoxybenzyl)oxy-7α-(triethylsilyl)oxy-5α-pregnane-20-carbaldehyde (**13**).* DMSO (55 μL, 0.78 mmol), Et_3N (55 μL, 0.39 mmol), and SO_3·pyridine (37.5 mg, 0.229 mmol)

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were added to a solution of diol **S5** (29.8 mg, 0.0496 mmol) in dry CH_2Cl_2 (0.50 mL) at 0 °C. After stirring at rt for 7 h, the reaction mixture was quenched with saturated aqueous NH_4Cl at 0 °C and extracted with Et_2O. The organic layer was washed with saturated aqueous $KHSO_4$ and saturated aqueous NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc = 20/1 → 7/1) to give aldehyde **13** (24.8 mg, 0.0414 mmol, 84%) as a colorless foam: $[\alpha]^{22}_D$ +4.67 (*c* 1.08, $CHCl_3$); R_f = 0.57 (hexane/EtOAc = 3/1); IR (neat) 3545, 2936, 2873, 1722, 1613, 1513, 1457, 1362, 1301, 1246, 1170, 1148, 1128, 1086, 1037, 1011, 977, 943, 824, 791, 772, 742 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 9.56 (d, *J* = 2.8 Hz, 1H), 7.26 (d, *J* = 8.4 Hz, 2H), 6.86 (d, *J* = 8.4 Hz, 2H), 4.45 (d, *J* = 11.4 Hz, 1H), 4.37 (d, *J* = 11.4 Hz, 1H), 3.80 (s, 3H), 3.60 (brs, 1H), 3.48 (brs, 1H), 2.36−2.30 (m, 1H), 2.25−2.17 (m, 1H), 1.93 (dt, *J* = 12.4, 2.8 Hz, 1H), 1.87−1.79 (m, 2H), 1.79−1.73 (m, 2H), 1.66−1.53 (m, 4H), 1.47−1.20 (m, 9H), 1.11−1.06 (m, 1H), 1.10 (d, *J* = 6.6 Hz, 3H), 0.95 (s, 3H), 0.94 (t, *J* = 7.6 Hz, 9H), 0.93 (s, 3H), 0.65−0.55 (m, 6H); the hydroxy proton was not observed probably due to the rapid exchange with contaminating H_2O; ^{13}C NMR (150 MHz, $CDCl_3$) δ 205.3, 158.9, 131.9, 128.7 (2C), 113.7 (2C), 77.2, 73.3, 73.0, 69.2, 55.4, 52.4, 51.6, 49.4, 49.3, 43.5, 40.4, 36.4, 33.7, 32.6, 32.0, 31.9, 26.6, 26.1, 19.0, 17.7, 14.0, 13.3, 11.7, 7.2 (3C), 5.5 (3C); HRESIMS *m*/*z* 621.3944 $[M + Na]^+$ (calcd for $C_{36}H_{58}O_5SiNa$, 621.3946).

*(S)-2-Methyl-4-((1-phenyl-1H-tetrazol-5-yl)thio)butan-1-ol (**16a**).*[1] PPh_3 (269 mg, 1.02 mmol) and 1-phenyl-1*H*-tetrazole-5-thiol (PTSH) (179 mg, 1.00 mmol) were added to a solution of (*S*)-(−)-2-methyl-1,4-butanediol (110 mg, 1.03 mmol) in dry THF (5.4 mL) at rt. The solution was cooled to −78 °C, and Diisopropyl azodicarboxylate (DIAD) (1.9 M in toluene, 0.56 mL, 1.06 mmol) was added. After stirring at −78 °C for 1 h, the reaction mixture was warmed to −20 °C over 1 h. After stirring at −20 °C for 2 h, the reaction mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc = 5/1 → 1/3) to give sulfide **16a** (65.7 mg, 0.249 mmol, 25%) as a colorless oil: $[\alpha]^{24}_D$ −8.02 (*c* 1.08, $CHCl_3$); R_f = 0.55 (hexane/EtOAc = 1/2); IR (neat) 3415, 2930, 2873, 1720, 1597, 1499, 1461, 1387, 1279, 1243, 1174, 1075, 1041, 1014, 983, 912, 762, 693 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.57−7.52 (m, 5H), 3.60 (dd, *J* = 11.0, 5.5 Hz, 1H), 3.53 (dd, *J* = 11.0, 6.9 Hz, 1H), 3.48−3.38 (m, 2H), 2.14 (br, 1H, OH), 2.01−1.94 (m, 1H), 1.87−1.79 (m, 1H), 1.78−1.71 (m, 1H), 0.97 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 154.7, 133.8, 130.3, 129.9 (2C), 124.0 (2C), 67.4, 35.0, 33.0, 30.9, 16.5; HRESIMS *m*/*z* 287.0937 $[M + Na]^+$ (calcd for $C_{12}H_{16}N_4OSNa$, 287.0937).

*(S)-5-((4-((4-Methoxybenzyl)oxy)-3-methylbutyl)thio)-1-phenyl-1H-tetrazole (**S6a**).* $La(OTf)_3$ (14.3 mg, 0.0244 mmol) was added to a mixture of 4-methoxybenzyl-2,2,2-trichloroacetimidate (108 mg, 0.380 mmol) and sulfide **16a** (65.7 mg, 0.249 mmol) in dry toluene (2.5 mL) at rt. After stirring at rt for 19 h, $La(OTf)_3$ (30.1 mg, 0.0514 mmol) was added to the reaction mixture. After stirring at rt for 4 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ at 0 °C and extracted with EtOAc. The organic layer was washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc = 10/1 → 3/1) to give sulfide **S6a** (94.9 mg, 0.247 mmol, 99%) as a colorless foam: $[\alpha]^{24}_D$ −1.27 (*c* 1.01, $CHCl_3$); R_f = 0.49 (hexane/EtOAc = 2/1); IR (neat) 2956, 1726, 1611, 1513, 1499, 1462, 1387, 1302, 1246, 1174, 1089, 1035, 1015, 915, 824, 760, 694 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.59−7.51 (m, 5H), 7.24 (d, *J* = 8.4 Hz, 2H), 6.86 (d, *J* = 8.4 Hz, 2H), 4.42 (s, 2H), 3.79 (s, 3H), 3.50−3.44 (m, 1H), 3.41−3.35 (m, 1H), 3.34−3.27 (m, 2H), 2.00−1.90 (m, 2H), 1.72−1.65 (m, 1H), 0.98 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 159.3, 154.6, 133.9, 130.6, 130.2, 129.9 (2C), 129.3 (2C), 124.0 (2C), 113.9 (2C), 74.9, 72.9, 55.4, 33.3, 33.0, 31.4, 16.9; HRESIMS *m*/*z* 407.1506 $[M + Na]^+$ (calcd for $C_{20}H_{24}N_4O_2SNa$, 407.1512).

*(S)-5-((4-((4-Methoxybenzyl)oxy)-3-methylbutyl)sulfonyl)-1-phenyl-1H-tetrazole (**14a**).* A solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (66.2 mg, 0.0534 mmol) in H_2O_2 (30% in H_2O, 0.60 mL) was added to a solution of sulfide **S6a** (205 mg, 0.534 mmol) in EtOH (0.76 mL) at rt. After stirring at rt for 5.5 h, the reaction mixture was extracted with EtOAc. The organic layer was washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc = 7/1 → 3/1) to give sulfone **14a** (198 mg, 0.475 mmol, 89%) as a colorless foam: $[\alpha]^{24}_D$ −6.90 (*c* 1.01, $CHCl_3$); R_f = 0.54 (hexane/EtOAc = 2/1); IR (neat) 3354, 2959, 1730, 1611, 1513, 1497, 1462, 1340, 1302, 1247, 1174, 1151, 1091, 1034, 918, 824, 770, 763, 689 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.68 (d, *J* = 7.2 Hz, 2H), 7.65−7.57 (m, 3H), 7.24 (d, *J* = 9.0 Hz, 2H), 6.87 (d, *J* = 9.0 Hz, 2H), 4.42 (s, 2H), 3.84−3.73 (m, 5H), 3.37 (dd, *J* = 9.6, 4.8 Hz, 1H), 3.26 (dd, *J* = 9.6, 7.2 Hz, 1H), 2.10−2.02 (m, 1H), 2.00−1.92 (m, 1H), 1.90−1.82 (m, 1H), 0.98 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 159.4, 153.6, 133.2, 131.6, 130.3, 129.8 (2C), 129.4 (2C), 125.2 (2C), 114.0 (2C), 74.5, 73.0, 55.4, 54.5, 32.7, 26.4, 17.0; HRESIMS *m*/*z* 439.1411 $[M + Na]^+$ (calcd for $C_{20}H_{24}N_4O_4SNa$, 439.1410).

*(25S)-3α,26-Bis(4-methoxybenzyl)oxy-7α-(triethylsilyl)oxy-5α-cholest-22-en-8β-ol (**15a**).* Aldehyde **13** (71.9 mg, 0.120 mmol) and sulfone **14a** (150 mg, 0.360 mmol) were dissolved in freshly distilled THF (4.1 mL) and HMPA (1.2 mL) and cooled to −78 °C. A freshly prepared solution of KHMDS (0.5 M in freshly distilled THF, 0.72 mL, 0.36 mmol) was added to the above solution, and the mixture was stirred at −78 °C for 1 h. After stirring at rt for 1 h, the reaction mixture was cooled to 0 °C and quenched with saturated aqueous NH_4Cl. The resultant mixture was extracted with Et_2O, and the combined organic layer was washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc = 24/1 → 4/1) to give olefin **15a** (*E*/*Z* = 11:1, 48.2 mg, 0.0611 mmol, 51%) as a colorless foam: $[\alpha]^{25}_D$ −15.0 (*c* 1.39, $CHCl_3$); R_f = 0.48 (hexane/EtOAc = 5/1); IR (neat) 3566, 2935, 2872, 1717, 1613, 1586, 1513, 1457, 1362, 1301, 1246, 1207, 1171, 1149, 1086, 1038, 1012, 974, 945, 908, 822, 792, 741, 724, 677 cm^{-1}; ^1H NMR of *E*-isomer (600 MHz, $CDCl_3$) δ 7.28−7.24 (m, 4H), 6.89−6.84 (m, 4H), 5.29−5.17 (m, 2H), 4.45 (d, *J* = 11.7 Hz, 1H), 4.42 (s, 2H), 4.37 (d, *J* = 11.7 Hz, 1H), 3.80 (s, 6H), 3.59 (brs, 1H), 3.48 (brs, 1H), 3.29 (dd, *J* = 8.9, 6.2 Hz, 1H), 3.21 (dd, *J* = 8.9, 6.2 Hz, 1H), 2.23−2.18 (tt, *J* = 13.1, 2.8 Hz, 1H), 2.10−2.04 (m, 1H), 2.01−1.95 (m, 1H), 1.94 (dt, *J* = 12.4, 3.4 Hz, 1H), 1.86−1.70 (m, 5H), 1.68−1.50 (m, 5H), 1.50−1.41 (m, 3H), 1.41−1.27 (m, 3H), 1.25−1.14 (m, 3H), 1.11−1.04 (m, 2H), 0.97 (d, *J* = 6.9 Hz, 3H), 0.94 (t, *J* = 7.6 Hz, 9H), 0.93 (s, 3H), 0.91 (s, 3H), 0.89 (d, *J* = 6.9 Hz, 3H), 0.65−0.54 (m, 6H); ^{13}C NMR of *E*-isomer (150 MHz, $CDCl_3$) δ 159.2, 158.9, 138.7, 131.9, 131.1, 129.3 (2C), 128.7 (2C), 125.4, 113.9 (2C), 113.7 (2C), 77.4, 75.3, 73.2, 73.1, 72.8, 69.2, 56.5, 55.4 (2C), 53.0, 49.3, 42.8, 40.5, 39.9, 36.7, 36.4, 34.0, 33.7, 32.7, 32.0, 31.9, 28.2, 26.1, 20.6, 18.6, 17.7, 16.9, 13.8, 11.6, 7.2 (3C), 5.6 (3C); HRESIMS *m*/*z* 811.5303 $[M + Na]^+$ (calcd for $C_{49}H_{76}O_6SiNa$, 811.5303).

*(25S)-7α-(Triethylsilyl)oxy-5α-cholest-22-ene-3α,8β,26-triol (**S7a**).* DDQ (57.5 mg, 0.246 mmol) was added to a mixture of olefin **15a** (48.2 mg, 0.0611 mmol, *E*/*Z* = 11:1) in dry CH_2Cl_2 (1.2 mL) and saturated aqueous $NaHCO_3$ (0.12 mL) at 0 °C. After stirring at rt for 20 min, the reaction mixture was cooled to 0 °C and quenched with saturated aqueous $NaHCO_3$ and saturated aqueous $Na_2S_2O_3$. After extraction with EtOAc, the combined organic layer was washed with H_2O and saturated aqueous NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc = 10/1 → 1/1) to give triol **S7a** (28.9 mg, 0.0526 mmol, 86%, *E*/*Z* = 11:1) as a colorless foam: $[\alpha]^{26}_D$ −28.2 (*c* 1.44, $CHCl_3$); R_f = 0.10 (hexane/EtOAc = 5/1); IR (neat) 3394, 2935, 2873, 1716, 1508, 1457, 1414, 1372, 1231, 1216, 1146, 1087, 1002, 974, 944, 902, 868, 841 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 5.34−5.22 (m, 2H), 4.02 (brt, *J* = 2.5 Hz, 1H), 3.51 (dd, *J* = 10.3, 6.2 Hz, 1H), 3.48 (brs, 1H), 3.43 (dd, *J* = 10.3, 6.2 Hz, 1H), 2.17−2.10 (m, 1H), 2.07−1.97 (m, 2H), 1.95 (dt, *J* = 13.1, 3.4 Hz, 1H), 1.91−1.85 (m, 1H), 1.84 (td, *J* =

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13.6, 2.1 Hz, 1H), 1.77−1.67 (m, 3H), 1.66−1.50 (m, 6H), 1.50−1.42 (m, 2H), 1.35−1.12 (m, 8H), 1.11−1.06 (m, 1H), 1.05 (dt, J = 13.8, 2.8 Hz, 1H), 0.98 (d, J = 6.4 Hz, 3H), 0.97 (t, J = 7.9 Hz, 9H), 0.92 (s, 6H), 0.90 (d, J = 6.9 Hz, 3H), 0.64−0.55 (m, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 138.8, 125.5, 77.3, 73.2, 68.2, 66.7, 56.5, 53.0, 49.4, 42.8, 40.5, 39.9, 36.6, 36.5, 36.2, 35.7, 33.1, 32.5, 31.5, 28.4, 28.2, 20.6, 18.6, 17.7, 16.6, 13.8, 11.3, 7.2 (3C), 5.5 (3C); HRESIMS m/z 571.4152 $[M + Na]^+$ (calcd for $C_{33}H_{60}O_4SiNa$, 571.4153).

*Dipyridinium (25S)-8β-Hydroxy-7α-(triethylsilyl)oxy-5α-cholest-22-ene-3α,26-diyl Disulfate (**S8a**).* SO_3·pyridine (11.1 mg, 0.0676 mmol) was added to a solution of triol **S7a** (3.8 mg, 6.9 μmol, E/Z = 5:1) in freshly distilled pyridine (0.28 mL) at rt. After stirring at rt for 1 h, the solvent was removed under reduced pressure. Et_2O was then added to the residue and decanted to remove excess SO_3·pyridine. The precipitates were dissolved in $CHCl_3$, and insoluble impurities were removed by decantation. The supernatant was concentrated under reduced pressure. Crude disulfate **S8a** (6.0 mg) thus obtained was used in the next reaction without further purification: R_f = 0.27 ($CHCl_3$/MeOH = 5/2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.92 (d, J = 4.8 Hz, 4H), 8.33 (t, J = 7.2 Hz, 2H), 7.87 (t, J = 7.2 Hz, 4H), 5.31−5.21 (m, 2H), 4.75 (brs, 1H), 3.98 (dd, J = 9.6, 6.6 Hz, 1H), 3.90 (dd, J = 9.6, 6.6 Hz, 1H), 3.45 (brs, 1H), 2.22−2.11 (m, 2H), 2.05−1.96 (m, 2H), 1.96−1.85 (m, 2H), 1.85−1.77 (m, 2H), 1.74−1.54 (m, 5H), 1.54−1.41 (m, 4H), 1.35−1.23 (m, 3H), 1.23−1.16 (m, 2H), 1.16−1.01 (m, 3H), 0.96 (d, J = 6.6 Hz, 3H), 0.94−0.88 (m, 18H), 0.62−0.50 (m, 6H). The two pyridinium protons were not observed probably due to the rapid exchange with contaminating H_2O.

*Disodium (22E,25S)-7α,8β-Dihydroxy-5α-cholest-22-ene-3α,26-diyl Disulfate (**2a**).* HF·pyridine (70% HF in pyridine, 7 μL, 0.3 mmol) was added to a solution of crude disulfate **S8a** (6.0 mg) in MeOH (0.14 mL) at 0 °C. After stirring at rt for 7 h, the reaction mixture was quenched with Et_3N at 0 °C and concentrated under reduced pressure. Purification by RP-pTLC (MeOH/H_2O = 2/1) afforded ammonium salt (4.6 mg) as a colorless foams. Undesired Z-isomer was separated and removed at this point. A solution of the above ammonium salt (4.6 mg) in MeOH was passed through a column packed with Amberlite IR-120B cation exchange resin (Na^+ form) pre-equilibrated with 1 M aqueous NaOH, H_2O, and MeOH. The column was eluted with MeOH, and the eluate was concentrated under reduced pressure to obtain (25S)-SAAF **2a** (2.1 mg, 3.3 μmol, 48% for the three steps) as a colorless foam: $[\alpha]^{26}_D$ −7.6 (c 0.23, MeOH); IR (neat) 3676, 2937, 2923, 2865, 2844, 1716, 1698, 1653, 1558, 1507, 1473, 1373, 1213, 1055, 1033, 1012, 908 cm^{-1}; ^{1}H NMR (600 MHz, D_2O) [chemical shifts were referred to trace MeOH (3.34 ppm for ^{1}H NMR, and 49.5 ppm for ^{13}C NMR) as originally reported[3]] δ 5.42−5.36 (m, 2H), 4.66 (brs, 1H), 3.95 (dd, J = 9.6, 6.0 Hz, 1H), 3.83 (dd, J = 9.6, 6.0 Hz, 1H), 3.56 (brs, 1H), 2.10−2.06 (m, 1H), 2.04−1.99 (m, 2H), 1.96−1.94 (m, 1H), 1.92−1.79 (m, 5H), 1.76−1.70 (m, 1H), 1.63−1.53 (m, 6H), 1.50−1.45 (m, 1H), 1.40−1.33 (m, 1H), 1.32−1.12 (m, 6H), 0.99 (d, J = 6.2 Hz, 3H), 0.93 (d, J = 6.9 Hz, 3H), 0.901 (s, 3H), 0.895 (s, 3H). The two hydroxy protons were not observed due to H/D exchange with the solvent; ^{13}C NMR (150 MHz, D_2O)[a] δ 140.5, 125.3, 78.5, 77.4, 74.1, 72.1, 56.6, 53.9, 50.2, 43.2, 40.8, 39.9, 36.02, 35.99, 33.5, 33.3, 32.9, 32.6, 31.5, 28.4, 26.5, 20.3, 18.7, 18.0, 16.4, 13.5, 11.5; [a]chemical shifts were referred to trace MeOH (3.34 ppm for 1H NMR, and 49.5 ppm for ^{13}C NMR) as originally reported;[3] HRESIMS m/z 296.1184 $[M - 2Na]^{2-}$ (calcd for $C_{27}H_{44}O_{10}S_2$, 296.1182).

(R)-(+)-2-Methyl-1,4-butanediol. $LiAlH_4$ (1.13 g, 29.8 mmol) was added to a solution of (*R*)-(+)-methylsuccinic acid (1.20 g, 9.11 mmol) in dry THF (30 mL) at −78 °C. After stirring at rt for 5 h, the reaction mixture was cooled to 0 °C and treated with $LiAlH_4$ (700 mg, 18.4 mmol). After stirring at rt for 1 h, the reaction mixture was warmed to 60 °C. After stirring at 60 °C for 11.5 h, the reaction mixture was cooled to 0 °C and treated sequentially with H_2O (2.0 mL), 3 M aqueous NaOH (2.5 mL), and H_2O (6.0 mL). The resultant mixture was diluted with Et_2O and stirred for 15 min. The cake was filtered through a pad of Celite, and the filtrate was extracted with THF. The organic layer was washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc = 1/1 → 0/1) to give diol (932 mg, 8.95 mmol, 98%) as a colorless oil: $[\alpha]^{26}_D$ +22.1 (c 1.05, $CHCl_3$); R_f = 0.30 (hexane/EtOAc = 0/1); IR (neat) 3318, 2928, 2359, 1458, 1220, 1036, 836, 773, 671 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.77−3.73 (m, 1H), 3.65−3.61 (m, 1H), 3.54 (dd, J = 10.2, 4.2 Hz, 1H), 3.40 (dd, J = 10.2, 7.2 Hz, 1H), 3.35 (brs, 2H), 1.83−1.75 (m, 1H), 1.63−1.52 (m, 2H), 0.91 (d, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 68.3, 61.1, 37.7, 34.3, 17.4; HRESIMS m/z 127.0714 $[M + Na]^+$ (calcd for $C_5H_{12}O_2Na$, 127.0730).

*(R)-2-Methyl-4-((1-phenyl-1H-tetrazol-5-yl)thio)butan-1-ol (**16b**).* PPh_3 (1.77 g, 6.75 mmol) and PTSH (1.20 g, 6.75 mmol) were added to a solution of (*R*)-(+)-2-methyl-1,4-butanediol (740 mg, 7.11 mmol) in dry THF (18 mL) at rt. The resultant solution was cooled to −78 °C, and DIAD (1.9 M in toluene, 3.8 mL, 7.2 mmol) was added to the mixture. After stirring at −78 °C for 1 h, the reaction mixture was warmed to −20 °C over 2 h. After stirring at −20 °C for 2 h, the mixture was warmed to rt over 2 h. After stirring at rt for 30 min, the reaction mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc = 5/1 → 1/3) to give sulfide **16b** (392 mg, 1.48 mmol, 22%) as a colorless oil: $[\alpha]^{26}_D$ +8.95 (c 1.02, $CHCl_3$); R_f = 0.57 (hexane/EtOAc = 1/2); IR (neat) 3396, 2929, 2874, 1597, 1499, 1461, 1387, 1279, 1243, 1075, 1041, 1015, 984, 771, 763, 693 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.59−7.51 (m, 5H), 3.61 (dd, J = 11.4, 6.6 Hz, 1H), 3.54 (dd, J = 11.4, 6.6 Hz, 1H), 3.48−3.39 (m, 2H), 2.01 (br, OH), 2.01−1.95 (m, 1H), 1.87−1.80 (m, 1H), 1.78−1.72 (m, 1H), 0.98 (d, J = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 154.7, 133.8, 130.3, 129.9 (2C), 124.0 (2C), 67.4, 35.0, 33.1, 31.0, 16.5; HRESIMS m/z 287.0938 $[M + Na]^+$ (calcd for $C_{12}H_{16}N_4OSNa$, 287.0937).

*(R)-5-((4-((4-Methoxybenzyl)oxy)-3-methylbutyl)thio)-1-phenyl-1H-tetrazole (**S6b**).* $La(OTf)_3$ (87.4 mg, 0.149 mmol) was added to a solution of 4-methoxybenzyl-2,2,2-trichloroacetimidate (616 mg, 2.18 mmol) and sulfide **16b** (385 mg, 1.45 mmol) in dry toluene (14 mL) at rt. After stirring at rt for 14 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ at 0 °C and then extracted with EtOAc. The organic layer was washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc = 10/1 → 5/1) to give sulfide **S6b** (499 mg, 1.30 mmol, 90%) as a colorless foam: $[\alpha]^{26}_D$ +1.41 (c 1.05, $CHCl_3$); R_f = 0.52 (hexane/EtOAc = 2/1); IR (neat) 2932, 1730, 1612, 1513, 1499, 1462, 1387, 1302, 1246, 1174, 1089, 1034, 1015, 914, 822, 774, 762, 693 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.58−7.51 (m, 5H), 7.24 (d, J = 9.0 Hz, 2H), 6.87 (d, J = 9.0 Hz, 2H), 4.42 (s, 2H), 3.80 (s, 3H), 3.50−3.45 (m, 1H), 3.41−3.36 (m, 1H), 3.33−3.28 (m, 2H), 1.99−1.90 (m, 2H), 1.71−1.65 (m, 1H), 0.98 (d, J = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 159.3, 154.6, 133.9, 130.7, 130.2, 129.9 (2C), 129.3 (2C), 124.0 (2C), 113.9 (2C), 75.0, 72.9, 55.4, 33.4, 33.0, 31.5, 17.0; HRESIMS m/z 407.1534 $[M + Na]^+$ (calcd for $C_{20}H_{24}N_4O_2SNa$, 407.1512).

*(R)-5-((4-((4-Methoxybenzyl)oxy)-3-methylbutyl)sulfonyl)-1-phenyl-1H-tetrazole (**14b**).* A solution of $(NH_4)_6Mo_7O_{24}$·$4H_2O$ (65.4 mg, 0.0529 mmol) in H_2O_2 (30% in H_2O, 0.59 mL) was added to a solution of sulfide **S6b** (204 mg, 0.529 mmol) in EtOH (0.76 mL) at rt. After stirring at rt for 6 h, the reaction mixture was extracted with EtOAc. The organic layer was washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc = 7/1 → 3/1) to give sulfone **14b** (201 mg, 0.482 mmol, 91%) as a colorless foam: $[\alpha]^{27}_D$ +6.83 (c 1.01, $CHCl_3$); R_f = 0.54 (hexane/EtOAc = 2/1); IR (neat) 2958, 1731, 1612, 1513, 1498, 1463, 1340, 1302, 1247, 1175, 1152, 1092, 1034, 822, 783, 768, 760, 689 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.66 (d, J = 7.8 Hz, 2H), 7.61−7.56 (m, 3H), 7.24 (d, J = 7.8 Hz, 2H), 6.87 (d, J = 7.8 Hz, 2H), 4.42 (s, 2H), 3.82−3.73 (m, 5H), 3.37 (dd, J = 9.6, 4.8 Hz, 1H), 3.26 (dd, J = 9.6, 4.8 Hz, 1H), 2.09−2.03 (m, 1H), 1.98−1.93 (m, 1H), 1.88−1.81 (m, 1H), 0.97 (d, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 159.2, 153.5, 133.1, 131.5,

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130.3, 129.7 (2C), 129.3 (2C), 125.2 (2C), 113.9 (2C), 74.4, 72.8, 55.3, 54.4, 32.5, 26.3, 16.8; HRESIMS m/z 439.1423 $[M + Na]^+$ (calcd for $C_{20}H_{24}N_4O_4SNa$, 439.1410).

*(25R)-3α,26-Bis(4-methoxybenzyl)oxy-7α-(triethylsilyl)oxy-5α-cholest-22-en-8β-ol (**15b**).* A mixture of aldehyde **13** (15.9 mg, 0.0265 mmol) and sulfone **14b** (34.2 mg, 0.0821 mmol) was dissolved in freshly distilled THF (0.90 mL) and HMPA (0.27 mL) and cooled to −78 °C. A solution of KHMDS (0.5 M in freshly distilled THF, 0.16 mL, 0.080 mmol) was added to the above mixture and stirred at −78 °C for 1 h. The reaction mixture was warmed to rt and stirred for 1 h. The reaction mixture was cooled to 0 °C and quenched with saturated aqueous NH_4Cl. After extraction with Et_2O, the combined organic layers were washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc = 24/1 → 7/1) to give olefin **15b** ($E/Z \approx 6:1$, 10.5 mg, 0.0133 mmol, 50%) as a colorless foam: $[\alpha]^{25}_D$ −14.7 (c 0.53, $CHCl_3$); R_f = 0.46 (hexane/EtOAc = 5/1); IR (neat) 3566, 2930, 2872, 1731, 1613, 1586, 1513, 1457, 1443, 1372, 1362, 1301, 1246, 1220, 1171, 1149, 1087, 1038, 1012, 973, 945, 908, 822, 772, 744, 724, 673 cm^{-1}; ^1H NMR of E-isomer (600 MHz, $CDCl_3$) δ 7.28−7.24 (m, 4H), 6.89−6.84 (m, 4H), 5.28−5.15 (m, 2H), 4.45 (d, J = 11.7 Hz, 1H), 4.42 (s, 2H), 4.37 (d, J = 11.7 Hz, 1H), 3.80 (s, 6H), 3.59 (brs, 1H), 3.48 (brs, 1H), 3.28 (dd, J = 8.9, 6.2 Hz, 1H), 3.21 (dd, J = 8.9, 6.2 Hz, 1H), 2.20 (tt, J = 13.1, 2.8 Hz, 1H), 2.13−2.05 (m, 1H), 2.03−1.91 (m, 2H), 1.86−1.70 (m, 5H), 1.68−1.50 (m, 5H), 1.50−1.41 (m, 3H), 1.41−1.24 (m, 6H), 1.12−1.04 (m, 2H), 0.96 (d, J = 6.2 Hz, 3H), 0.94 (t, J = 8.3 Hz, 9H), 0.93 (s, 3H), 0.91 (s, 3H), 0.89 (d, J = 6.6 Hz, 3H), 0.65−0.54 (m, 6H); ^{13}C NMR of E-isomer (150 MHz, C_6D_6) δ 159.7, 159.5, 138.9, 132.3, 131.6, 129.3 (2C), 128.9 (2C), 126.1, 114.1 (2C), 114.0 (2C), 77.2, 75.2, 73.9, 73.6, 73.0, 69.7, 57.0, 54.8 (2C), 53.5, 49.9, 43.2, 41.0, 40.4, 37.1, 36.8, 34.5, 34.2, 33.1, 32.9, 32.4, 28.8, 26.1, 21.0, 18.8, 18.2, 17.2, 14.0, 12.0, 7.4 (3C), 5.9 (3C); HRESIMS m/z 811.5326 $[M + Na]^+$ (calcd for $C_{49}H_{76}O_6SiNa$, 811.5303).

*(25R)-7α-(Triethylsilyl)oxy-5α-cholest-22-ene-3α,8β,26-triol (**S7b**).* DDQ (17.5 mg, 0.0748 mmol) was added to a solution of olefin **15b** (19.6 mg, 0.0248 mmol, $E/Z \approx 6:1$) in dry CH_2Cl_2 (0.50 mL) and pH 7 buffer (0.10 mL) at 0 °C. After stirring at rt for 20 min, the reaction was quenched with saturated aqueous $NaHCO_3$ and saturated aqueous $Na_2S_2O_3$ at 0 °C and then extracted with EtOAc. The organic layer was washed with H_2O and saturated aqueous NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc = 14/1 → 1/1) to give triol **S7b** (5.6 mg, 0.010 mmol, 41%, $E/Z \approx 6:1$) as a colorless foam: $[\alpha]^{25}_D$ −52.2 (c 0.28, $CHCl_3$); R_f = 0.12 (hexane/EtOAc = 5/1); IR (neat) 3405, 2935, 2873, 1717, 1457, 1414, 1372, 1219, 1146, 1087, 1002, 974, 944, 902, 868, 841, 791 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 5.33−5.23 (m, 2H), 4.02 (brs, 1H), 3.50 (dd, J = 10.8, 6.0 Hz, 1H), 3.48 (brs, 1H), 3.44 (dd, J = 10.8, 6.0 Hz, 1H), 2.14 (tt, J = 13.1, 2.8 Hz, 1H), 2.06 (dt, J = 13.4, 6.2 Hz, 1H), 2.04−1.98 (m, 1H), 1.95 (dt, J = 12.4, 2.8 Hz, 1H), 1.89−1.80 (m, 2H), 1.78−1.43 (m, 12H), 1.33 (dd, J = 13.1, 3.4 Hz, 1H), 1.30−1.15 (m, 5H), 1.14−1.30 (m, 2H), 1.02 (brs, 1H), 0.98 (d, J = 6.2 Hz, 3H), 0.96 (t, J = 8.3 Hz, 9H), 0.91 (s, 6H), 0.90 (d, J = 6.9 Hz, 3H), 0.65−0.55 (m, 6H); ^{13}C NMR (150 MHz, C_6D_6) δ 138.8, 126.2, 77.2, 73.9, 67.8, 66.3, 56.9, 53.5, 49.9, 43.2, 41.0, 40.4, 36.9, 36.8, 36.5, 35.9, 33.5, 33.0, 31.7, 29.1, 28.7, 20.9, 18.8, 18.2, 16.7, 13.9, 11.7, 7.4 (3C), 5.8 (3C); HRESIMS m/z 571.4167 $[M + Na]^+$ (calcd for $C_{33}H_{60}O_4SiNa$, 571.4153).

*Dipyridinium (25R)-8β-Hydroxy-7α-(triethylsilyl)oxy-5α-cholest-22-ene-3α,26-diyl Disulfate (**S8b**).* SO_3·pyridine (17.0 mg, 0.104 mmol) was added to a solution of triol **S7b** (5.6 mg, 0.010 mmol, $E/Z \approx 6:1$) in freshly distilled pyridine (0.40 mL) at rt. After stirring at rt for 2 h, the solvent was removed under reduced pressure. Et_2O was added to the residue and decanted to remove excess SO_3·pyridine. The precipitates were dissolved in $CHCl_3$, and insoluble impurities were removed by decantation. The supernatant was concentrated under reduced pressure. The crude disulfate **S8b** (17.1 mg, $E/Z \approx 6:1$) was used in the next reaction without further purification: R_f = 0.20 ($CHCl_3$/MeOH = 5/2); ^1H NMR (600 MHz, CD_3OD) δ 8.90 (brs, 4H), 8.72−8.66 (m, 2H), 8.17−8.11 (m, 4H), 5.31−5.20 (m, 2H), 4.61 (brs, 1H), 3.89−3.84 (m, 1H), 3.82−3.76 (m, 1H), 3.44 (brs, 1H), 2.25−2.07 (m, 2H), 2.07−1.78 (m, 6H), 1.76−1.58 (m, 5H), 1.58−1.35 (m, 5H), 1.35−1.02 (m, 6H), 1.02−0.91 (m, 21H), 0.65−0.60 (m, 6H); three protons (the pyridinium and the hydroxy protons) were not observed probably due to the H/D exchange with the solvent.

*Disodium (22E,25R)-7α,8β-Dihydroxy-5α-cholest-22-ene-3α,26-diyl Disulfate (**2b**).* HF·pyridine (70% HF in pyridine, 20 μL, 0.76 mmol) was added to a solution of crude disulfate **S8b** (17.1 mg) in MeOH (0.20 mL) at 0 °C. After stirring at rt for 17.5 h, the reaction was quenched with Et_3N at 0 °C and concentrated under reduced pressure. Purification by RP-pTLC (MeOH/H_2O = 2/1) afforded an ammonium salt of desilylated product (6.4 mg) as a colorless foam. Undesired Z-isomer was separated and removed at this point. A solution of the above ammonium salt (6.4 mg) in MeOH was passed through a column packed with Amberlite IR-120B cation exchange resin (Na^+ form) pre-equilibrated with 1 M aqueous NaOH, H_2O, and MeOH. The column was eluted with MeOH, and the eluate was concentrated under reduced pressure to obtain (25R)-SAAF **2b** (4.7 mg, 7.4 μmol, 72% for the three steps) as a colorless foam: $[\alpha]^{23}_D$ −4.1 (c 0.24, MeOH); IR (neat) 3445, 2939, 2871, 1716, 1647, 1558, 1541, 1507, 1456, 1373, 1227, 1055, 1033, 1011 cm^{-1}; ^1H NMR (600 MHz, D_2O) [chemical shifts were referred to trace MeOH (3.34 ppm for ^1H NMR and 49.5 ppm for ^{13}C NMR) as originally reported[3]] δ 5.42−5.36 (m, 2H), 4.65 (brs, 1H), 3.92 (dd, J = 9.6, 5.4 Hz, 1H), 3.85 (dd, J = 9.6, 5.4 Hz, 1H), 3.56 (brs, 1H), 2.09−2.06 (m, 2H), 2.01−1.99 (m, 1H), 1.94−1.79 (m, 6H), 1.76−1.70 (m, 1H), 1.62−1.55 (m, 6H), 1.51−1.46 (m, 1H), 1.40−1.33 (m, 1H), 1.28−1.13 (m, 6H), 0.99 (d, J = 6.6 Hz, 3H), 0.92 (d, J = 6.0 Hz, 3H), 0.904 (s, 3H), 0.900 (s, 3H); two hydroxy protons were not observed probably due to the H/D exchange with the solvent; ^{13}C NMR (150 MHz, D_2O)[a] δ 140.4, 125.4, 78.4, 77.4, 74.2, 72.0, 56.6, 53.9, 50.2, 43.2, 40.8, 39.9, 36.0 (2C), 33.5, 33.4, 32.8, 32.6, 31.5, 28.4, 26.6, 20.4, 18.7, 18.0, 16.2, 13.6, 11.5; HRESIMS m/z 296.1193 $[M - 2Na]^{2-}$ (calcd for $C_{27}H_{44}O_{10}S_2$, 296.1182). [a]Chemical shifts were referred to trace MeOH (3.34 ppm for ^1H NMR, and 49.5 ppm for ^{13}C NMR) as originally reported.[3]

*X-ray Crystallographic Analysis of **11**.* A colorless crystal of **11** was obtained from a mixture of CH_2Cl_2/n-hexane. Mo Kα radiation (λ = 0.710 73 Å) was used for X-ray diffraction data collection (Supporting Information Table S1 for details): $C_{30}H_{46}O_5$, M = 486.67, orthorhombic, space group $P2_12_12_1$, a = 6.7944(14) Å, b = 13.003(3) Å, c = 29.832(6) Å, α = 90°, β = 90°, γ = 90°, V = 2635.6(9) Å^3, Z = 4, ρ_{calc} = 1.226 g/m^3, μ = 0.081 mm^{-1}, crystal size 0.10 × 0.08 × 0.05 mm^3, $F(000)$ = 1064, 15 400 reflections collected, 6024 independent reflections (R_{int} = 0.0512), R_1 = 0.0459 [$I > 2\sigma(I)$], wR_2 = 0.0757 [$I > 2\sigma(I)$], R_1 = 0.0751 (all data), wR_2 = 0.0848 (all data), goodness of fit = 1.030, Flack parameter = 0.8(9). The crystal structure was solved with SHELXS-97 and refined using SHELXL-97 with the assistance of KENX. Crystallographic data for **11** have been deposited with the Cambridge Crystallographic Data Centre (deposition number 1813183). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: + 44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.7b01052.

^1H and ^{13}C NMR spectra of new synthetic compounds; details about X-ray crystallographic analysis of **11** (PDF)

X-ray crystallographic data for **11** (CIF)

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Notes

The authors declare no competing financial interest.

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