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Second-generation synthesis of endogenous sperm-activating and attracting factor (SAAF) isolated from the ascidian *Ciona intestinalis*

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ABSTRACT

Stereoselective synthesis of the chemoattractant sperm-activating and attracting factor (SAAF), isolated from the eggs of the ascidian *Ciona intestinalis*, was achieved via reductive 1,3-transposition of an allylic alcohol and the axial opening of an epoxide as key steps. This second-generation synthesis improved the total yield of SAAF over that of the first-generation synthesis and provided a key intermediate for synthesizing molecular probes of SAAF.

Chemotaxis of sperm toward an egg is a critical step in reproduction, particularly in aquatic environments, where sperm frequently travel long distances to contact an egg.[1] Yoshida et al. isolated a non-peptidic chemoattractant called sperm-activating and attracting factor (SAAF) from eggs of the ascidian *Ciona intestinalis*. The structure of SAAF was determined to be a novel polyhydroxysterol sulfate (Fig. 1, **1**).[2,3] SAAF is the first steroid possessing chemotactic activity, and the first example of a single agent that possesses both sperm activation and attraction activities, which are reportedly elicited through different mechanisms.[4]

Thus, SAAF may serve as a key compound for biological studies on signal transduction pathways leading to sperm flagellum movement. Because of the limited availability of SAAF from natural sources, the ability to produce the compound by chemical synthesis is needed for further biological investigations. The first synthesis of **1** by our group indicated that construction of the A ring was problematic due to the low stereoselectivity and/or poor reproducibility of reduction of the 3-keto-4-enol intermediate.[3] Herein, a highly stereoselective synthesis of **1** is described in which construction of the contiguous stereogenic centers at C3, C4, and C5 is completely controlled. The present synthesis also provides a key intermediate for synthesizing molecular probes of SAAF (Fig. 1, **2**) for identification of the target proteins.[5]

Stereoselective synthesis of the steroid core **11** is shown in Scheme 1. The known alcohol **4**, prepared from chenodeoxycholic acid **3** in two steps (93%),[6] was protected as BOM ether **5**. Dehydro-

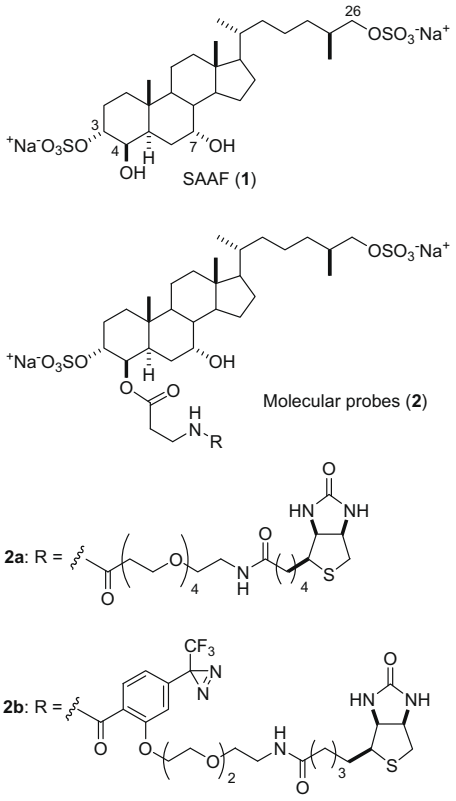

Figure 1. Structures of SAAF (**1**) and its molecular probes (**2a**–**b**).

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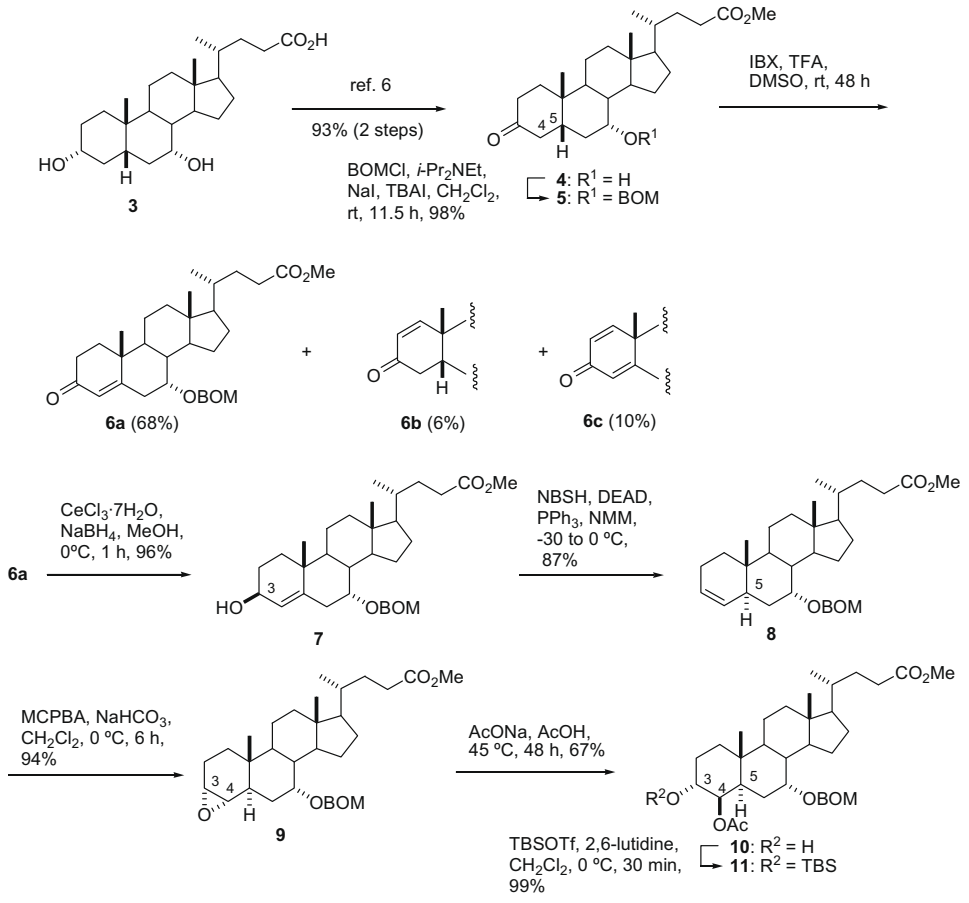


Scheme 1. Synthesis of the steroid core of SAAF (**11**).

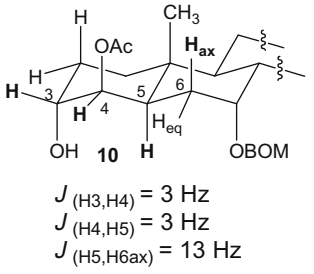
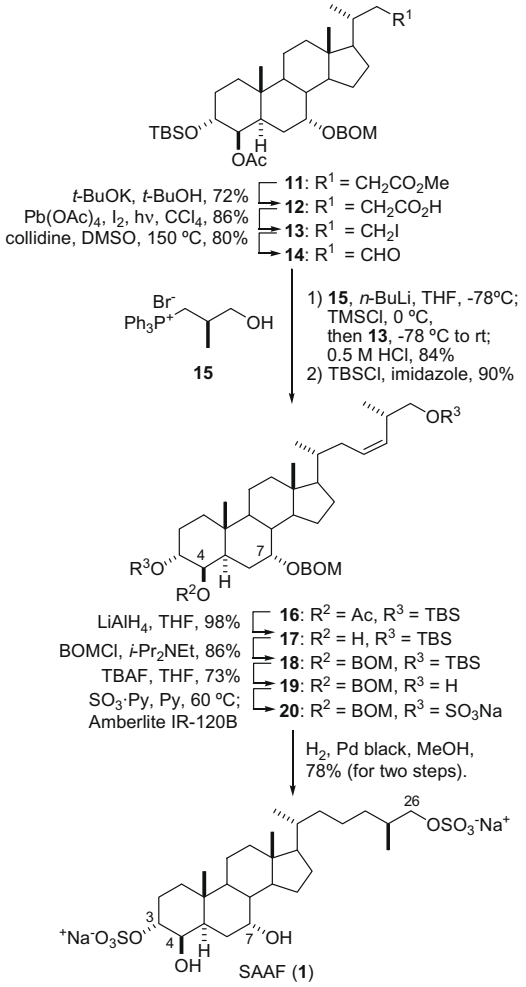


Figure 2. Selected ^{1}H NMR coupling constants of **10**.

genation of ketone **5** proceeded in a regioselective manner by treatment with *o*-iodoxybenzoic acid (IBX)[7] in the presence of trifluoroacetic acid (TFA) to afford the C4–C5 unsaturated ketone **6a** in 68% yield with concomitant formation of regioisomer **6b** (C1–C2 unsaturated, 6%) and dienone **6c** (C1–C2 and C4–C5 unsaturated, 10%). Luche reduction[8] of enone **6a** resulted in the formation of β-alcohol **7** as a single isomer. Reductive 1,3-transposition of allylic alcohol **7** was achieved successfully according to the Myers protocol[9] [i.e., **7** was subjected to Mitsunobu reaction conditions using diethyl azodicarboxylate (DEAD), triphenylphosphine, and *o*-nitrobenzenesulfonylhydrazine (NBSH) in the presence of *N*-methylmorphorine (NMM) in toluene at −30 to 0 °C] to afford olefin **8** in 87% yield as a single isomer. Since stereocontrol at the C5 position was complete, the stereoselective introduction of a 3,4-diol unit in *trans*-diaxial orientation was conducted. Epoxidation of **8** with *m*-chloroperbenzoic acid (MCPBA) proceeded stereoselectively to furnish α-epoxide **9** as a single isomer.[10] Regioselective opening of epoxide **9** was achieved by treatment with sodium acetate in acetic acid at 45 °C for two days to yield **10** (67%) via diaxial opening (Fürst-Plattner rule)[11] with recovery of **9** (20%). The structure of **10** was confirmed by ^{1}H NMR analysis as shown in Figure 2. The resulting secondary alcohol was protected as the TBS ether **11**. Thus, stereoselective construction of the contiguous stereogenic centers of the steroid core at C3, C4, and C5 was achieved. The overall yield from chenodeoxycholic acid for nine steps was 32%, a dramatic improvement over that of the corresponding intermediate in the first-generation synthesis (9.2%, nine steps).[3]

Next, side chain elongation was accomplished in a sequence analogous to that in the first-generation synthesis (Scheme 2).[3] Selective hydrolysis of the methyl ester in the presence of acetate was achieved by treatment with *t*-BuOK in *t*-BuOH to yield carboxylic acid **12** (72%) with recovery of **11** (20%). Conversion of carboxylic acid **12** to aldehyde **14** was achieved through decarboxylative iodination (86%),[12] followed by treatment of the resulting iodide **13** with DMSO in the presence of collidine (80%).[13] Wittig olefination using phosphonium salt **15**[14,15] gave a *Z*-olefin (*Z*:*E* = 20:1) in 84% yield, with protection of the resulting primary alcohol as a TBS ether (**16**) in 90% yield. Removal of the acetyl group with $LiAlH_4$ provided alcohol **17** (98%),[16] a common intermediate of SAAF (**1**) and its molecular probes (**2**).[5] Protection of the secondary alcohol as a BOM ether provided **18** (86%) followed by removal of the TBS groups with TBAF to give diol **19** (73%) which was converted to sulfate **20** by successive treatment with sulfur trioxide–pyridine complex and ion exchange resin (Amberlite IR-120B). Finally, removal of the BOM groups using palladium black as a catalyst under



Scheme 2. Synthesis of SAAF (**1**).

hydrogen and concomitant hydrogenation of the double bond afforded SAAF (**1**) in 78% yield over two steps.

In conclusion, stereocontrolled synthesis of SAAF (**1**) was achieved via an improved route that completely controlled the contiguous stereogenic centers at C3, C4, and C5. The present synthesis also provided a key intermediate (**17**) for synthesizing molecular probes of SAAF. Syntheses of molecular probes to elucidate signal transduction pathways induced by SAAF are currently in progress.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.03.011.

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16. *Physical data of* **17:** Colorless amorphous; $[\alpha]_D^{28}$ +11.3 (*c* 0.91, $CHCl_3$); R_f = 0.47 (hexane/EtOAc = 5/1); IR (film) 3501, 2886, 2857, 1471, 1460, 1361, 1255, 1149, 1083, 1046, 1027, 1004, 836, 774, 734, 697, 673 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.32–7.24 (5H, m, Ph), 5.37–5.32 (1H, m, H23), 5.17 (1H, t, *J* = 9.5 Hz, H24), 4.85 (1H, d, *J* = 7.5 Hz, –OCH_2O–), 4.75 (1H, d, *J* = 7.5 Hz, –OCH_2O–), 4.63–4.53 (2H, m, $PhCH_2$–), 3.78 (1H, d, *J* = 2.5 Hz, H4), 3.76 (1H, d, *J* = 2.5 Hz, H3), 3.47–3.45 (1H, m, H26), 3.41 (1H, d, *J* = 1.5 Hz, H7), 3.34–3.30 (1H, m, H26), 2.57–1.02 (23H, m, H1, 2, 5, 6, 8, 9, 11, 12, 14, 15, 16, 17, 20, 22, 25), 1.00 (3H, s, H19), 0.93 (3H, d, *J* = 7.5 Hz, H27), 0.91 (3H, d, *J* = 7.5 Hz, H21), 0.84 (18H, s, –Si*t*-Bu), 0.63 (3H, s, H18), 0.02 (6H, s, –$SiCH_3$), 0.01 (6H, s, –$SiCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.95, 133.18, 128.55, 128.26, 127.63, 127.47, 94.74, 77.26, 76.42, 69.73, 67.97, 55.99, 50.14, 47.11, 42.61, 42.58, 39.94, 39.14, 36.67, 36.53, 35.91, 34.85, 34.03, 31.74, 30.85, 28.32, 26.06, 26.03, 25.89, 25.73, 25.03, 24.00, 20.05, 18.89, 18.48, 18.11, 17.58, 17.02, 13.58, 11.89, −4.67, −4.87, −5.14, −5.17; HRMS (ESI-TOF) calcd for $C_{47}H_{82}O_5Si_2$ [M+Na^+] 805.5593, found: 805.5601.