ACS chemical biology

Articles

pubs.acs.org/acschemicalbiology

The Fungal Sexual Pheromone Sirenin Activates the Human CatSper Channel Complex

Shameem Sultana Syeda,[†] Erick J. Carlson,[†] Melissa R. Miller,[‡,§] Rawle Francis,[†] David E. Clapham,[∥,⊥] Polina V. Lishko,[‡] Jon E. Hawkinson,[†] Derek Hook,[†] and Gunda I. Georg*[,†]

[†]Department of Medicinal Chemistry and Institute for Therapeutics Discovery and Development, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota 55414, United States

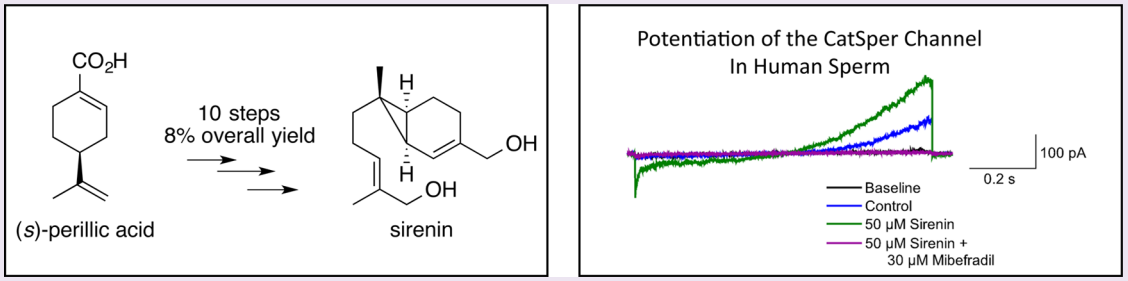
[‡]Department of Molecular and Cell Biology, University of California, Berkeley, California 94720, United States

[§]Department of Physiology, University of California, San Francisco, California 94158, United States

[∥]Department of Cardiology, Howard Hughes Medical Institute, Boston Children's Hospital, Boston, Massachusetts 02115, United States

[⊥]Department of Neurobiology, Harvard Medical School, Boston, Massachusetts 02115, United States

Supporting Information



ABSTRACT: The basal fungus *Allomyces macrogynus* (*A. macrogynus*) produces motile male gametes displaying well-studied chemotaxis toward their female counterparts. This chemotaxis is driven by sirenin, a sexual pheromone released by the female gametes. The pheromone evokes a large calcium influx in the motile gametes, which could proceed through the cation channel of sperm (CatSper) complex. Herein, we report the total synthesis of sirenin in 10 steps and 8% overall yield and show that the synthetic pheromone activates the CatSper channel complex, indicated by a concentration-dependent increase in intracellular calcium in human sperm. Sirenin activation of the CatSper channel was confirmed using whole-cell patch clamp electrophysiology with human sperm. Based on this proficient synthetic route and confirmed activation of CatSper, analogues of sirenin can be designed as blockers of the CatSper channel that could provide male contraceptive agents.

Upon deposition into the female reproductive tract, mammalian sperm must undergo a complex process known as capacitation before achieving successful fertilization.[1] While the intricate details surrounding capacitation remain largely unsolved, it has long been known that the intracellular concentration of calcium ions ($[Ca^{2+}]_i$) in sperm plays a pivotal role.[2−4] In mammalian sperm, $[Ca^{2+}]_i$ is predominantly controlled by the cation channel of sperm (CatSper).[5] CatSper is expressed exclusively in the principal piece of sperm[5] and is a heterotetrameric channel, comprising four pore-forming subunits (CatSper 1/2/3/4) and at least three auxiliary subunits, β, δ, and γ.[6,7] In mice, genetic disruption of any of these four sperm-specific CatSper channel subunits (CatSper1/2/3/4) ablates expression of the entire complex, leading to complete infertility.[8]

In 2011, it was shown that the sex hormone progesterone activates the human CatSper channel, giving new insight into mammalian sperm chemotaxis.[9,10] Although CatSper channels were originally thought to be animal-specific, it has been shown that the CatSper channel complex is present in the basal fungus *Allomyces macrogynus* (*A. macrogynus*).[11] *A. macrogynus* produces motile gametes whose chemotaxis is largely driven by the sexual pheromone sirenin (Figure 1).[12] It is not known if CatSper channels underlie the molecular mechanisms for sperm cell chemotaxis in *A. macrogynus*, as a receptor for sirenin has not yet been identified. However, since the mechanism of chemotaxis relies on calcium flux, sirenin may activate fungal

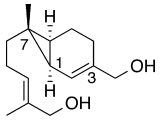
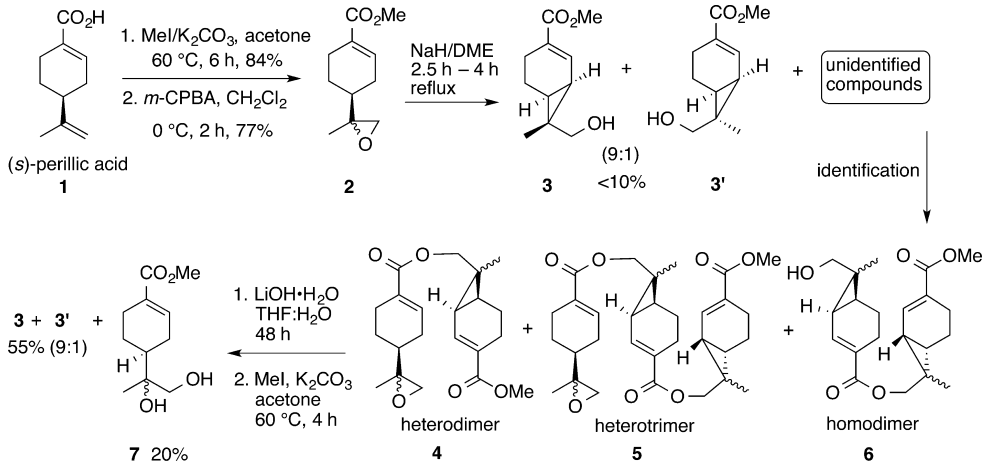


Figure 1. Structure of sirenin.

Received: June 24, 2015
Accepted: November 19, 2015
Published: December 16, 2015

DOI: 10.1021/acschembio.5b00748
ACS Chem. Biol. 2016, 11, 452−459

Scheme 1. NaH-Mediated Cyclopropanation, Identification of Side Products, and Their Conversion to 3



CatSper to enable gamete fertilization in *A. macrogynus*. Since it is believed that animals and fungi diverged from a common ancestor over 1 billion years ago, a compound that elicits a similar response in the gametes of both species would serve to demonstrate the high degree of conservation thought to govern most reproductive signaling.[11,13]

Sirenin has been an attractive target for the synthetic chemistry community given its unique properties and complex chemical structure. Sirenin is an oxygenated sesquiterpene [4.1.0] bicyclic ring system bearing two allylic hydroxyl groups. It was first isolated and characterized by Machlis and co-workers in the 1960s,[14−16] though Machlis had postulated its existence a decade earlier.[17] The pheromone displays attraction activity at 10 pM in chemotaxis bioassays involving male gametes of *A. macrogynus*.[16] To date, a number of syntheses have been developed for sirenin;[18−27] however, only three of them provided nonracemic sirenin. Rapoport and Plattner,[28] Corey *et al.*,[29] and Kitahara *et al.*[30] each used different chemistry to arrive at sirenin. We selected Kitahara's synthesis, which was derived from the method of Hortman and Ong,[31] as an attractive route to sirenin due to its inherent potential for analog synthesis. However, the key cyclopropanation reaction in this report is not efficient and generates significant amounts of previously uncharacterized side-products. Furthermore, the reactions used to extend the alkyl chain proved to be unreliable in our hands and also produced undesired reaction products. We therefore re-examined and then optimized the synthetic steps to develop a more efficient synthetic route for sirenin.

We studied the ability of sirenin to elicit calcium influx in human sperm cells, specifically via the CatSper channel. Sirenin-induced increases in whole cell $[Ca^{2+}]_i$ were monitored in human sperm with a calcium-sensitive dye using a FLIPR Tetra plate reader. Whole-cell patch clamp electrophysiology experiments were conducted to confirm that this rise in $[Ca^{2+}]_i$ was a result of direct CatSper channel activation rather than indirectly through activation of another ion channel located on human spermatozoa.[32−34]

RESULTS AND DISCUSSION

Synthesis of Sirenin. Esterification of (*S*)-perillic acid (**1**) with MeI/K_2CO_3 followed by epoxidation provided a diastereomeric mixture of methyl ester epoxides **2** in 77% yield (Scheme 1). The subsequent crucial cyclopropanation reaction to generate intermediate **3** provided poor yields and unidentified side products, which have not been isolated and characterized before. An attempt to carry out the NaH-mediated γ-anion generation followed by cyclopropanation with internal epoxide opening of **2** under reported conditions[30] provided less than 10% of the desired compound **3** and **3′** (9:1 ratio), along with an unidentified mixture of compounds, rather than the reported 46% yield. Changing reaction conditions such as base equivalents, concentration, and temperatures did not improve the yield of product **3** (Scheme 1).

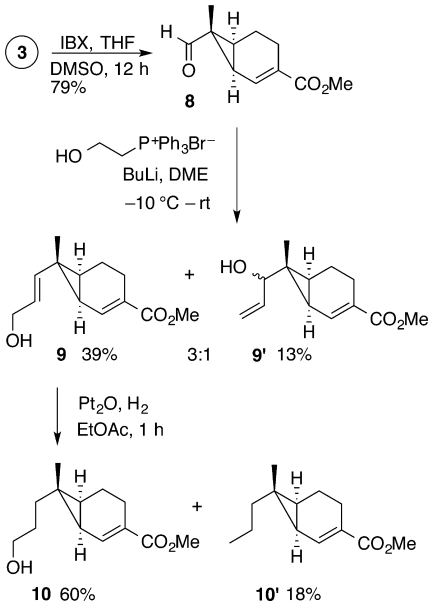
After extensive investigations toward an improved synthesis of the key intermediate **3**, all side products of the reaction were isolated and characterized and found to be heterodimer **4**, trimer **5**, and homodimer **6** (Scheme 1). These undesired products are the result of competing transesterification reactions at elevated temperature. Heterodimer **4** arises from the transesterification of **3** and **3′** with unreacted starting material **2**, and the dimer **4** undergoes transesterification with unreacted starting material **2** to generate trimer **5**. Homodimerization of the key intermediate **3** and **3′** by transesterification provided **6**. In order to obtain compounds **3** and **3′**, a reaction mixture consisting of **4**, **5**, and **6** was treated with LiOH to hydrolyze the esters followed by re-esterification with MeI/K_2CO_3 to provide an inseparable 9:1 mixture of the diastereomeric cyclopropanols **3** and **3′** (Scheme 1) with an improved overall yield of 55%. In addition, 20% of diol **7** was obtained. The ratio of **3** and **3′** was dependent on reaction time as reported earlier.[30] According to the literature,[30] the separation of these isomers was quite difficult and required conversion to their corresponding 3,5-dinitrobenzoates, separation by fractional crystallization, and subsequent hydrolysis and re-esterification to provide **3**.[30] In our synthesis, the diastereoisomers were separated during the subsequent oxidation and Wittig reaction steps (Scheme 2).

Next, we attempted to install the side chain under conditions developed previously as shown in Scheme 2.[30] Cyclopropanol **3** was oxidized to its corresponding aldehyde **8** in 50% yield with IBX. Wittig reaction of **8** with 2-hydroxyethyltriphenylphosphonium bromide in the presence of *n*-BuLi gave a rather disappointing 3:1 mixture of *E*-allyl alcohol **9** and the undesired regioisomer **9′**. Hydrogenation of **9** with PtO_2 provided the required compound **10** in 60% yield (Scheme 2) and the undesired deoxygenated compound **10′** in 18% yield.

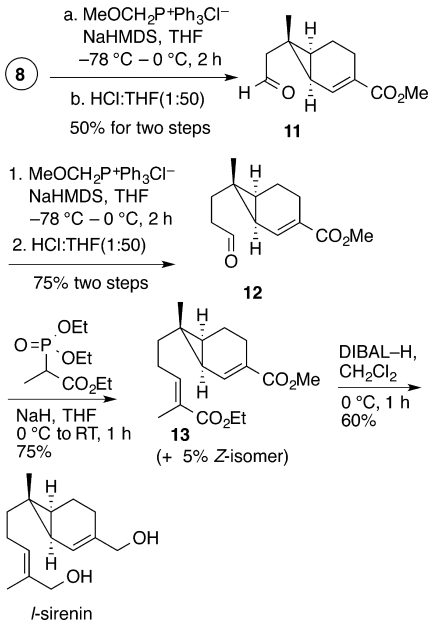
Because the reported Wittig/hydrogenation sequence yielded substantial amounts of undesired side products, we explored an

DOI: 10.1021/acschembio.5b00748
ACS Chem. Biol. 2016, 11, 452−459

Scheme 2. Side Chain Extension[30]



Scheme 3. Alternate Strategy for Side Chain Extension and Completion of the Sirenin Synthesis



alternative strategy for the side chain extension (Scheme 3). Elongation of aldehyde **8** by two iterative Wittig reactions with (methoxymethyl)triphenylphosphonium chloride followed by enol ether hydrolysis provided the two carbon chain-extended aldehyde **12** in 75% overall yield. To complete the synthesis of sirenin, a Horner–Wittig reaction of aldehyde **12** with methyl 2-diethylphosphonopropanoate in the presence of base provided the diene diester **13** in 75% yield along with 5% *Z*-isomer. Finally, the reduction of **13** with DIBAL-H yielded sirenin in 60%. The spectroscopic data of sirenin were in agreement with reported data.[14,30]

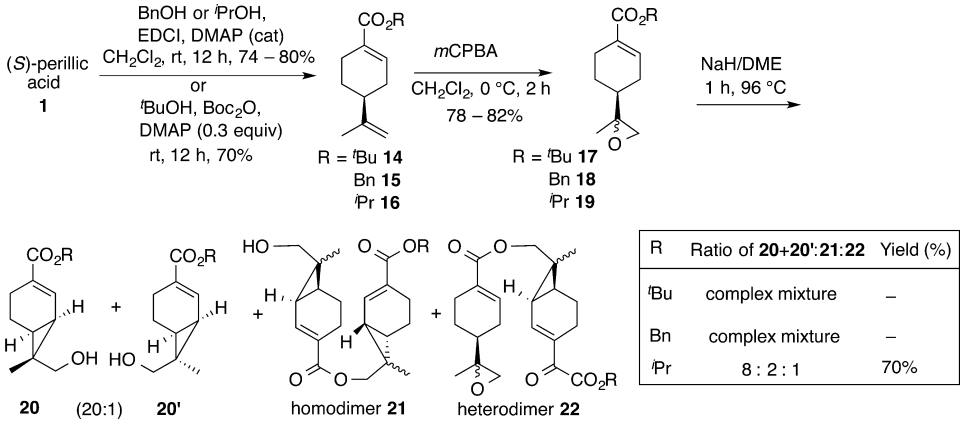
Although Kitahara's synthetic route provided an approach to sirenin, the key cyclopropanation transformation was beset by a low yield and substantial formation of side products. Therefore, we sought an improved procedure for this critical step. However, changes to the reaction conditions did not improve the product yields. We then decided to modify the cyclopropanation substrate by introducing a sterically hindered ester group to prevent transesterification. Hindered ester epoxides such as *tert*-butyl ester **17**, benzyl ester **18**, and isopropyl ester **19** were prepared as shown in Scheme 4. These ester epoxides were examined in the NaH-mediated cyclopropanation reaction. The *tert*-butyl ester **17** and the benzyl ester **18** epoxides provided a complex mixture of products, presumably due to thermal decomposition. However, isopropyl ester epoxide **19** produced the desired cyclopropylcarbinol **20** along with diastereomer **20′** (20:1) in 70% yield. It should be noted that the isopropyl ester diastereomers **20** and **20′** were easily separated by flash column chromatography, unlike the methyl ester isomers **3** and **3′**.[30] Thus, the use of the isopropyl ester not only improved the yield of cyclopropyl carbinol **20** but also allowed for the facile separation of isomers **20** and **20′**.

Having established an efficient synthetic route to cyclopropanation and side chain extensions, intermediate **20** was elaborated to the natural product sirenin (Scheme 5) in the same manner as described above in a five-step sequence beginning with IBX oxidation, iterative Wittig reactions, and the subsequent hydrolysis of enol ethers. Finally, Horner–Wadsworth–Emmons olefination and DIBAL-H reduction yielded *l*-sirenin in 8% overall yield in 10 steps linear from commercially available (*S*)-perillic acid. The spectroscopic data, including specific optical rotation, of the synthetic sirenin were in excellent agreement with the reported data for the isolated natural product[14] and for sirenin prepared by total synthesis.[14,28−30] In addition, we prepared the diastereomeric (*R*)- and (*S*)-Mosher bis-esters[35] of sirenin. ^{17}F NMR analysis indicated a diastereomeric ratio of 97:3 and 96:4, respectively. ^{1}H NMR analysis of the C7 methyl group of the two diasteroisomers indicated a ratio of 96:4.

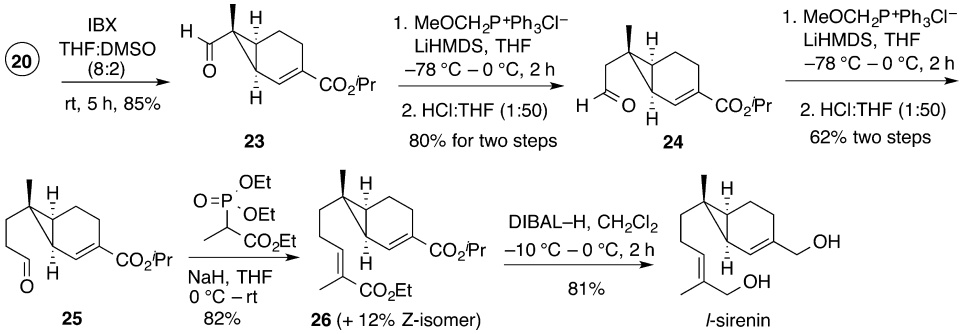
Biological Evaluation. The efficacy of sirenin acting at the CatSper channel in sperm obtained from healthy human volunteers was measured by two whole-cell methods: a calcium mobilization assay utilizing a calcium-specific dye (FLIPR assay) and patch clamp electrophysiology, which confirmed that this rise in $[Ca^{2+}]_i$ was a result of direct CatSper channel activation rather than indirectly through activation of another ion channel located on human spermatozoa.[32−34] In the FLIPR assay, sperm were collected and loaded with the calcium-specific fluorescent dye Fluo-4-AM and the fluorescence of the cells was continuously monitored. Increased fluorescence correlates with increased intracellular calcium levels produced by CatSper activation. It has been shown previously that this increase in calcium-specific fluorescence originates in the tail and propagates toward the sperm head.[36] Synthetic sirenin produced a concentration-dependent rise in $[Ca^{2+}]_i$ in human sperm with an EC_{50} of 2.9 ± 0.7 μM (Figure 2A, black traces). As sirenin was reported to increase chemotaxis of *A. macrogynus* gametes at concentrations as low as 10 pM,[37] this fungal pheromone has several orders of magnitude lower potency at the human channel, reflecting the billion years of separation between the two species. The time-course for increase in $[Ca^{2+}]_i$ elicited by sirenin was similar to that of progesterone (Figure 2A, red) and prostaglandin E_1 (PGE_1, Figure 2A, blue), two endogenous openers of the CatSper channel. Sirenin increased calcium fluorescence with the same maximum response as progesterone and PGE_1, although substantially higher concentrations of sirenin were required to reach

DOI: 10.1021/acschembio.5b00748
ACS Chem. Biol. 2016, 11, 452–459

Scheme 4. Synthesis of Ester Epoxides 17−19 and Cyclopropanation Optimization



Scheme 5. Completion of the Sirenin Synthesis



saturation (Figure 2B). Pretreatment with the known CatSper calcium channel blocker mibefradil[10] (30 μM) reduced the maximal sirenin-induced activation by 55% (Figure 2C, gray bar). Mibefradil also reduced the activity of progesterone and PGE_1 consistent with previous studies showing that mibefradil and the related T-type calcium channel blocker, NNC 55-0396, reduce progesterone-induced activation of the CatSper channel.[9,10] These observations indicate that sirenin increases sperm $[Ca^{2+}]_i$ by activation of the CatSper channel. Interestingly, in the presence of mibefradil, even high concentrations of sirenin failed to elicit a maximal activation, indicating that mibefradil produces an insurmountable inhibition, consistent with noncompetitive blockade of the CatSper channel (not shown).

To confirm that the sirenin-mediated rise in $[Ca^{2+}]_i$ observed in the calcium fluorescence assay was caused by activation of the CatSper channel, rather than by second messenger pathways[38] or other ion channels present in sperm,[39] whole cell patch clamp electrophysiology experiments were conducted according to established methods.[40,41] $I_{CatSper}$ is a pH-sensitive Ca^{2+}-selective ion current mediated by the CatSper channel and is the principal entry pathway for Ca^{2+} into the flagellum of mammalian sperm.[36] A step hyperpolarization of the sperm from 0 mV to −80 mV followed by a ramp depolarization from −80 to +80 mV elicited an inward current followed by an outward current mediated by CatSper. Sirenin (10 and 50 μM) potentiated both the inward and outward $I_{CatSper}$ currents (Figure 3A, left panel, green and Figure 3B,C) and in healthy human sperm in a similar manner to the potentiation produced by 1 μM progesterone (Figure 3A, right panel, red and Figure 3B). The magnitude of the current produced by sirenin was less than that elicited by progesterone, consistent with the lower potency of the compound in the calcium fluorescence assay. The sirenin-induced current was also completely inhibited by coapplication with 30 μM of the CatSper inhibitor mibefradil (Figure 3A, left panel, purple and Figure 3C), further supporting the conclusion that sirenin-stimulated increase in channel activity is the result of CatSper activation by sirenin and not the activation of another channel within sperm cells. Moreover, sirenin potentiates the inward current $I_{CatSper}$ to a greater degree than the outward current at both 10 and 50 μM in a similar manner to 1 μM progesterone (Figure 3B), consistent with the main effect of these activators to promote calcium entry into sperm. Taken together, these observations strongly suggest a similar mechanism of action for sirenin and progesterone to activate the CatSper channel complex.

MATERIALS AND METHODS

Human Sperm Calcium Fluorescence Assay. Semen from healthy human donors was collected and incubated in a shaker at 37 °C until complete liquefaction was observed. Semen samples were analyzed for motility/cell count/morphology according to updated WHO parameters for semen analysis.[42] The sample was diluted to 50 mL in low pH/low K^+ (low/low) buffer containing (in mM): 101 NaCl, 4.69 KCl, 0.2 $MgSO_4$, 0.36 KH_2PO_4, 25 $NaHCO_3$, 0.32 sodium pyruvate, 2.78 glucose, 94 sodium lactate, and 0.2 $CaCl_2$ at a pH of 6.7 adjusted with HCl. The sample was washed by dilution to 50 mL of low/low buffer and centrifugation at 800 × g for 10 min at 10 °C followed by aspiration of the supernatant. The pellet was then resuspended with 10 mL of low/low buffer containing 10 μM Fluo-4-AM (Life Technologies, Grand Island, NY) with 1 mM probenicid (to reduce dye efflux) and incubated for 30 min at ambient temperature. The sample was again diluted to 50 mL with low/low buffer, centrifuged, and the supernatant aspirated to remove extracellular dye; the pellet was resuspended in ca. 10 mL of low/low buffer. The dye-loaded sperm were then plated into black clear-bottom 384-well assay

DOI: 10.1021/acschembio.5b00748
ACS Chem. Biol. 2016, 11, 452−459

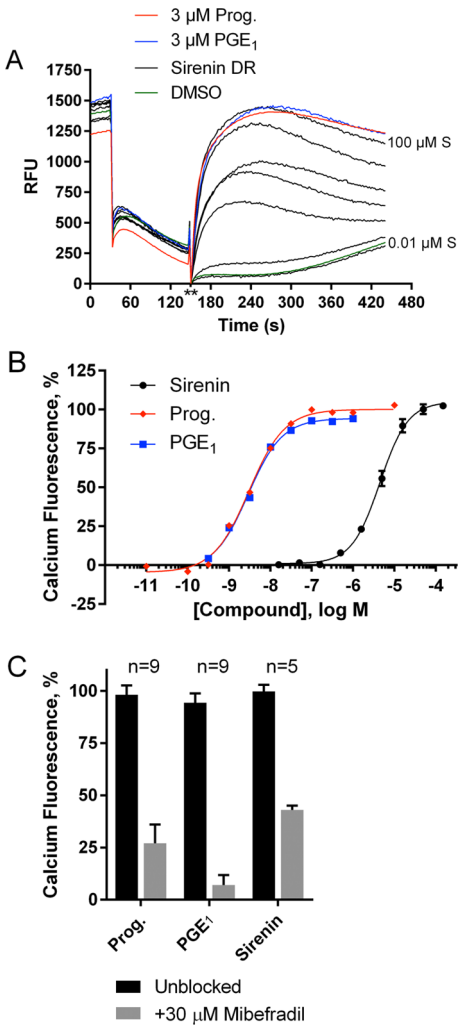


Figure 2. Sirenin activates CatSper in human sperm measured by calcium fluorescence. (A) Raw FLIPR traces showing increases in $[Ca^{2+}]_i$ elicited by 3 μM progesterone (Prog; red), 3 μM PGE_1 (blue), and increasing concentrations of sirenin (black) compared to the low pH/low K^+ buffer (green) control. The sirenin (S) dose response increases from 10 nM to 100 μM by half-log concentrations. Cells were treated with compounds at 150 s (**). (B) Concentration-dependent increases in $[Ca]^{2+}_i$ elicited by sirenin (black, EC_{50} = 2.9 ± 0.7 μM), progesterone (red, EC_{50} = 7.7 ± 0.9 nM), and PGE_1 (blue, EC_{50} = 4.2 ± 0.7 nM). (C) Sirenin elicits the same level of calcium influx as two endogenous activators of the CatSper channel, progesterone and PGE_1. Human sperm were treated with 30 μM sirenin or 1 μM progesterone or 1 μM PGE_1 (black), and the rise in $[Ca^{2+}]_i$ was measured. Mibefradil (gray bar; 30 μM) reduced the calcium influx for all three compounds. Pretreatment with 30 μM mibefradil decreased the sirenin-induced rise in $[Ca^{2+}]_i$ by 55%. Calcium fluorescence is expressed as the percent RFU produced by a saturating concentration of progesterone (3 μM). EC_{50} values determined using Prism v6.05.

plates (Corning Inc., Tewksbury, MA) and transferred to the FLIPR Tetra platform (Molecular Devices, Sunnyvale, CA). The calcium-induced fluorescence signal was continuously monitored for 7 min at 2 s intervals. When present, mibefradil was added 2 min prior to sirenin addition, and the signal was monitored for 5 min after sirenin addition. Calcium fluorescence is expressed as a percent of the RFU produced by 3 μM progesterone, and the activation EC_{50} and inhibition IC_{50} values were calculated using Prism (v 6.0, GraphPad).

Human Sperm Electrophysiology. Whole cell electrophysiology was employed as reported in Lishko *et al.*[41] Gigaohm seals were formed at the cytoplasmic droplet[43] of highly motile human sperm cells in standard high saline (HS) buffer containing (in mM) 130 NaCl, 20 HEPES, 10 sodium lactate, 5 glucose, 5 KCl, 2 $CaCl_2$, 1 $MgSO_4$, and 1 sodium pyruvate at a pH of 7.4 adjusted with NaOH, 320 mOsm/L. The intrapipette solution contained 130 Cs-methanesulfonate ($CsMeSO_3$), 70 HEPES, 3 EGTA, 2 EDTA, and 0.5 TrisHCl at a pH of 7.4 adjusted with CsOH, 330 mOsm/L. Divalent-free bath solution (CsDVF) was used for recording monovalent current through CatSper containing (in mM) 140 $CsMeSO_3$, 40 HEPES, and 1 EDTA at a pH of 7.4 adjusted with CsOH, 320 mOsm/L. The sirenin effect on CatSper was evaluated by diluting stock in DMSO (10 mM) in CsDVF to test concentration. HS solution was used for baseline measurements. All electrophysiology experiments were performed at ambient temperature. Data were analyzed with Origin 9.0 and Clampfit 10.3. Statistical data are presented as the mean ± SEM, where *n* indicates the number of individual experiments determined using Prism. Electrophysiology experiments used sperm cells from four individual healthy donors.

Synthetic Intermediates and Sirenin. (*S*)-Perillic acid (**1**) is commercially available, but due to its high costs we started the synthesis with the less expensive (*S*)-perillic aldehyde. (*S*)-Perillic aldehyde was oxidized to (*S*)-perillic acid (**1**) following a previously reported procedure.[30] The synthetic procedure and the data for compound **1** can be found in the SI.

General Procedure for the Synthesis of the Esters 14−16. A solution of perillic acid (1 equiv), 4-(dimethylamino)pyridine (0.1 equiv), and the appropriate alcohol (^{t}BuOH, BnOH, and ^{i}PrOH (1.1 equiv)) dissolved in CH_2Cl_2 (10 mL) was cooled with stirring at 0 °C for 10 min. L-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDCI) (1 equiv) was added, and the reaction mixture was stirred at RT for 24 h. After the completion of the reaction (monitored by TLC), the reaction mixture was diluted with CH_2Cl_2 and washed successively with saturated sodium bicarbonate (10 mL) and water (2 × 15 mL) and brine and then dried over Na_2SO_4. The solvent was removed *in vacuo* and the products **14**, **15**, and **16** were purified by flash column chromatography (silica gel, 0−5% ethyl acetate in hexanes). The data for compounds **14** and **15** can be found in the SI.

*Isopropyl (S)-4-(Prop-1-en-2-yl)cyclohex-1-enecarboxylate (**16**).* The compound was purified by flash column chromatography (silica gel, 0−5% ethyl acetate in hexanes) to obtain **16** (2.78 g, 74% yield) as a colorless oil. $[\alpha]_D^{22}$ − 96.5 (*c* 1.61, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.96 (qd, *J* = 3.0, 2.0, 1.5 Hz, 1H), 5.06 (p, *J* = 6.3 Hz, 1H), 4.78−4.67 (m, 2H), 2.54−2.40 (m, 1H), 2.38−2.31 (m, 1H) 2.29−2.01 (m, 3H), 1.95−1.82 (m, 1H), 1.74 (s, 3H), 1.53−1.37 (m, 1H), 1.26 (d, *J* = 6.3 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.9, 148.9, 138.4, 130.5, 109.1, 67.3, 40.1, 31.0, 27.1, 24.6, 21.9, 20.7. HRMS (ESI-TOF) *m*/*z* calcd for $C_{13}H_{20}Na_1O_2$ $[M + Na]^+$: 231.1356. Found: 231.1355.

*Isopropyl (S)-4-((R)-2-Methyloxiran-2-yl)cyclohex-1-ene-1-carboxylate and Its 2S Isomer (**19**).* In a 200 mL round-bottomed flask equipped with a magnetic stir bar was dissolved perillic acid isopropyl ester (2.69 g, 12.9 mmol) in CH_2Cl_2 (110 mL) and cooled to 0 °C. To this solution was added *m*-chloroperbenzoic acid (*m*-CPBA; 2.81 g, 16.2 mmol, activity 77%) in four portions over 2 h at 0 °C. After stirring for another 30 min at 0 °C, the precipitated *m*-chlorobenzoic acid was removed by filtration. The filtrate was washed with saturated $Na_2S_2O_3$ (20 mL) and extracted with CH_2Cl_2 (4 × 20 mL). The combined organic layers were washed once with saturated $NaHCO_3$ (30 mL) and dried over Na_2SO_4. Solvent removal *in vacuo* gave a crude oil that was purified by column chromatography (silica gel, 0−10% ethyl acetate in hexanes) to obtain title compound **19** (2.38 g, 82%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.93 (ddd, *J* = 8.8, 5.6, 2.5 Hz, 1H), 5.05 (p, *J* = 6.3 Hz, 1H), 2.65 (dd, J = 6.7, 4.8 Hz, 1H), 2.57 (t, *J* = 4.8 Hz, 1H), 2.49 (dtd, *J* = 15.6, 5.0, 2.7 Hz, 1H), 2.39−1.85 (m, 4H), 1.54 (ddt, *J* = 13.2, 8.2, 2.5 Hz, 1H), 1.46−1.32 (m, 0.5H), 1.29 (s, 3H), 1.26 (d, *J* = 6.3 Hz, 6.5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.7, 137.8, 137.6, 130.8, 130.7, 67.4, 58.9, 58.7, 52.9, 52.7, 39.1, 39.0, 27.7, 24.5, 24.3, 24.1, 21.8, 18.4, 18.2. HRMS (ESI-TOF) *m*/*z* calcd for $C_{13}H_{20}Na_1O_3$ $[M + Na]^+$: 247.1305.

DOI: 10.1021/acschembio.5b00748
ACS Chem. Biol. 2016, 11, 452−459

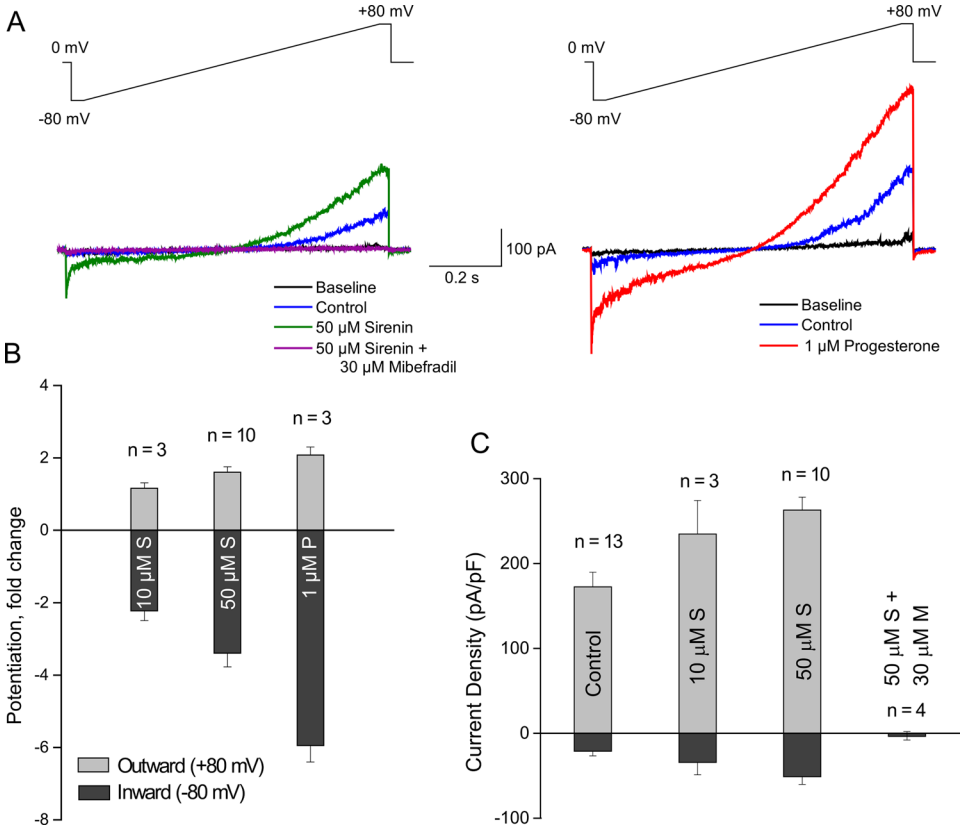


Figure 3. Sirenin increases intracellular calcium in human sperm through activation of the CatSper channel. (A) Representative monovalent $I_{CatSper}$ whole-cell recordings from human spermatozoa using divalent free bath solution (DVF) in the absence (control; blue) or presence of test compound. Currents were elicited in response to indicated voltage ramp. Left panel, 50 μM sirenin (S; green) and 50 μM sirenin (S) with 30 μM mibefradil (M; purple). Right panel, 1 μM progesterone (P; red). Baseline indicates recordings performed in HS bath solution. (B) Averaged fold amplitude change of $I_{CatSper}$ recorded from human spermatozoa in the presence of indicated test compound. Potentiation was determined by dividing current amplitudes of $I_{CatSper}$ at −80 mV (negative, inward current) and +80 mV (positive, outward current) by the amplitude of $I_{CatSper}$ in the absence of the corresponding compound from the same cell. (C) Averaged current density of $I_{CatSper}$ recorded from human spermatozoa in the presence of indicated test compound. Where appropriate, data are represented as mean ± SEM with *n* indicating the number of individual cells recorded.

Found: 247.1301. The data for compounds **17** and **18** can be found in the SI.

*Isopropyl (1R,6S,7R)-7-(Hydroxymethyl)-7-methylbicyclo[4.1.0]-hept-2-ene-3-carboxylate (**20**).* The solution of epoxy esters mixture **19** in DME was added to the stirred suspension of NaH in DME under N_2. The mixture was stirred and heated under reflux for 1 h. The reaction mixture was cooled, and isopropanol was added to destroy the excess NaH. The reaction mixture was poured into ice water and adjusted to pH 6 with AcOH and extracted with EtOAc. The aqueous solution was extracted with EtOAc, and the extracts were washed with saturated $NaHCO_3$ and saturated NaCl solution and dried over Na_2SO_4. Evaporation of the solvent afforded the crude product, which was purified by flash column chromatography (silica gel, 0−40% ethyl acetate in hexanes) to obtain **20** (0.49 g, 49%) and **20′** (0.02 g, 2%), **21** (0.13 g, 13%), and **22** (0.06 g, 6%) in 70% over all yield. Compound **20**, colorless oil. $[\alpha]_D^{22}$ + 74.6 (*c* 1.05, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.18 (dt, *J* = 5.5, 1.6 Hz, 1H), 5.05 (p, *J* = 6.3 Hz, 1H), 3.46 (d, *J* = 10.9 Hz, 1H), 3.37 (d, *J* = 11.0 Hz, 1H), 2.59−2.31 (m, 1H), 2.07−1.69 (m, 3H), 1.51 (brs, 1H), 1.40 (dd, *J* = 8.2, 5.5 Hz, 1H), 1.26 (d, *J* = 6.2 Hz, 7H), 1.01 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.7, 137.6, 128.8, 72.3, 67.4, 34.3, 21.9, 21.8, 21.6, 20.3, 16.7, 11.4. HRMS (ESI-TOF) *m*/*z* calcd for $C_{13}H_{20}Na_1O_3$ $[M + Na]^+$: 247.1305. Found: 247.1304. The data for compounds **20′**, **21**, and **22** can be found in the SI.

*Isopropyl (1R,6S,7R)-7-Formyl-7-methylbicyclo[4.1.0]hept-2-ene-3-carboxylate (**23**).* To a suspension of 2-iodoxybenzoic acid (IBX; 0.93 g, 3.34 mmol) in DMSO (2 mL) was added alcohol **20** (0.50 g, 2.23 mmol) in anhydrous THF (10 mL) at ambient temperature. After stirring for 5 h, the reaction mixture was diluted with ether (40 mL) and filtered over Celite. The filtrate was washed with water, saturated $NaHCO_3$, brine, dried (Na_2SO_4), and evaporated *in vacuo*. The crude aldehyde **23**, a colorless oil, (0.42 g, 85%) was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.89 (s, 1H), 7.05 (dt, *J* = 5.5, 1.8 Hz, 1H), 5.06 (p, *J* = 6.3 Hz, 1H), 2.62−2.35 (m, 1H), 2.18−1.75 (m, 5H), 1.26 (d, *J* = 6.2 Hz, 6H), 1.15 (s, 3H). ^{13}C NMR (100 MHz, CDCl3): δ 200.5, 166.1, 133.2, 131.3, 67.9, 41.6, 23.8, 23.6, 21.8, 21.2, 15.8, 7.5.

*Isopropyl (1R,6S,7R)-7-Methyl-7-(2-oxoethyl)bicyclo[4.1.0]hept-2-ene-3-carboxylate (**24**).* To a stirred suspension of (methoxymethy1)-triphenylphosphonium chloride (2.07 g, 6.05 mmol) in THF (20 mL) was added LiHMDS (1.0 M in THF, 5.00 mL, 5.04 mmol) at −78 °C. The resulting red solution was stirred at 4 °C for 15 min before being treated with a solution of aldehyde **23** (0.44 g, 2.01 mmol) in THF (5 mL). The reaction mixture was stirred at RT for 1 h, and saturated aqueous NH_4Cl was added. The aqueous phase was extracted with EtOAc (3 × 20 mL), and the combined organics were dried over Na_2SO_4 and evaporated to form a residue, which was purified by column chromatography (silica gel, 0−10% ethyl acetate in hexanes) to obtain the corresponding enol ether. To a stirred solution of the enol ether in THF (10 mL) was added 4 N HCl (0.20 mL) at 0 °C. The resulting solution was allowed to stir at RT for 30 min and was then poured into water (40 mL) and extracted with ether (4 × 30 mL). The combined organic layers were washed with saturated $NaHCO_3$ and brine, dried, and concentrated. Purification of the resulting residue by flash chromatography (silica gel, 0−10% ethyl acetate in hexanes) furnished aldehyde **24** (0.38 g, 80%) as a colorless

DOI: 10.1021/acschembio.5b00748
ACS Chem. Biol. 2016, 11, 452−459

oil. $[\alpha]_D^{22}$ + 42.6 (*c* 2.95, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 9.75 (s, 1H), 7.15 (dt, *J* = 5.4, 1.6 Hz, 1H), 5.01 (p, *J* = 6.2 Hz, 1H), 2.40−2.36 (m, 1H), 2.34 (t, *J* = 2.1 Hz, 2H), 1.97−1.75 (m, 3H), 1.38 (dd, *J* = 8.2, 5.4 Hz, 1H), 1.36−1.22 (m, 1H), 1.22 (d, *J* = 6.3 Hz, 6H), 0.94 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 201.7, 166.4, 136.8, 129.0, 67.3, 55.9, 27.1, 23.4, 21.9, 21.8, 21.7, 21.3, 16.6, 13.8.

*Isopropyl (1R,6S,7R)-7-Methyl-7-(3-oxopropyl)bicyclo[4.1.0]hept-2-ene-3-carboxylate (**25**).* The title compound, a colorless oil, was prepared similar to compound **24**. $[\alpha]_D^{22}$ + 92.7 (*c* 2.55, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 9.78 (s, 1H), 7.15 (dt, *J* = 5.6, 1.6 Hz, 1H), 5.03 (p, *J* = 6.3 Hz, 1H), 2.53 (td, *J* = 7.7, 1.8 Hz, 2H), 2.47−2.26 (m, 1H), 2.01−1.43 (m, 5H), 1.24 (d, *J* = 6.3 Hz, 7H), 1.13 (td, *J* = 6.2, 3.1 Hz, 1H), 0.87 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 202.1, 166.7, 137.8, 128.4, 67.3, 41.6, 35.2, 31.6, 24.6, 23.1, 21.9, 21.8, 21.5, 16.9, 12.9. HRMS (ESI-TOF) *m*/*z* calcd for $C_{15}H_{22}Na_1O_3$ $[M + Na]^+$: 273.1461. Found: 273.1462.

*Isopropyl (1R,6S,7R)-7-((E)-5-Ethoxy-4-methyl-5-oxopent-3-en-1-yl)-7-methylbicyclo[4.1.0]hept-2-ene-3-carboxylate (**26**).* To a stirred suspension of NaH (60% dispersion in mineral oil, 0.06 g, 1.58 mmol) in THF (5 mL) at 0 °C was added 2-phosphonopropionate (0.42 g, 1.76 mmol) in THF (3 mL). The resulted mixture was stirred for 1 h at RT. Aldehyde **25** (0.22 g, 0.88 mmol) in THF (2 mL) was added to the above mixture at 0 °C and stirred for 1 h at RT. The reaction mixture was quenched with saturated NH_4Cl, extracted with EtOAc, and washed with water and brine. The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo* to furnish a mixture of *E* and *Z* diene esters. The resulting residue was purified by flash column chromatography (silica gel, 0−10% ethyl acetate in hexanes) to afford *E*-isomer **26** as a colorless oil (0.24 g, 82% yield) and *Z*-isomer (0.03 g, 12% yield). $[\alpha]_D^{22}$ + 51.1 (*c* 2.77, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 7.17 (dt, *J* = 5.6, 1.6 Hz, 1H), 6.73 (tt, *J* = 7.4, 1.5 Hz, 1H), 5.03 (p, *J* = 6.3 Hz, 1H), 4.17 (q, *J* = 7.1 Hz, 2H), 2.45−2.30 (m, 1H), 2.26 (q, *J* = 7.8 Hz, 2H), 2.02−1.68 (m, 3H), 1.82 (s, 3H), 1.58−1.41 (m, 1H), 1.27 (t, *J* = 7.1 Hz, 4H), 1.24 (d, *J* = 6.3 Hz, 7H), 1.13 (td, *J* = 6.2, 3.0 Hz, 1H), 0.89 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.1, 166.8, 141.6, 138.3, 128.1, 127.7, 67.3, 60.5, 41.9, 32.3, 26.1, 24.8, 23.2, 21.9, 21.8, 21.6, 16.9, 14.2, 13.0, 12.2. HRMS (ESI-TOF) *m*/*z* calcd for $C_{20}H_{30}Na_1O_4$ $[M + Na]^+$: 357.2036. Found: 357.2031.

*Isopropyl (1R,6S,7R)-7-((Z)-5-Ethoxy-4-methyl-5-oxopent-3-en-1-yl)-7-methylbicyclo[4.1.0]hept-2-ene-3-carboxylate (**26′**).* ^1H NMR (400 MHz, $CDCl_3$): δ 7.19 (dt, *J* = 5.6, 1.6 Hz, 1H), 5.90 (td, *J* = 7.7, 1.7 Hz, 1H), 5.04 (p, *J* = 6.2 Hz, 1H), 4.20 (q, *J* = 7.1 Hz, 2H), 2.55 (qd, *J* = 8.0, 1.4 Hz, 2H), 2.49−2.29 (m, 1H), 1.97−1.66 (m, 3H), 1.88 (s, 3H), 1.57−1.37 (m, 1H), 1.30 (t, *J* = 7.2 Hz, 4H (one H merged with triplet)), 1.25 (d, *J* = 6.3 Hz, 7H (one H merged with doublet)), 1.14 (ddd, *J* = 8.4, 4.3, 1.7 Hz, 1H), 0.89 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.0, 166.9, 142.3, 138.6, 127.9, 127.2, 67.2, 60.0, 42.8, 32.6, 27.0, 24.9, 23.2, 21.96, 21.92, 21.6, 20.6, 17.0, 14.3, 13.0.

Sirenin. To a solution of diene ester **26** (0.15 g, 0.45 mmol) in CH_2Cl_2 (6 mL) was added DIBAL-H (1.0 M in hexanes, 2.69 mL, 2.69 mmol) at −10 °C. The reaction mixture was stirred for 2 h at 0 °C. The reaction mixture was quenched by dropwise addition of MeOH (3 mL) and saturated sodium potassium tartrate (15 mL) and stirred at RT for 1 h. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated to form a residue, which was purified by flash column chromatography (silica gel, 0−10% ethyl acetate in hexanes) to provide sirenin (0.08 g, 81%) as a colorless oil. The spectroscopic data and specific rotation of the synthetic sirenin were in agreement with reported data. Found: $[\alpha]_D^{22}$ − 45.6 (*c* 1.48, $CHCl_3$), literature value[14] for the isolated natural product: $[\alpha]_D^{22}$ − 45.0 (*c* 1.0, $CHCl_3$), literature value[28] $[\alpha]_D^{23}$ − 43.3 (*c* 1.42, $CHCl_3$), literature value[29] $[\alpha]_D^{23}$ − 48.0 (*c* 0.80, $CHCl_3$), literature value[30] $[\alpha]_D^{24}$ − 44.6 (*c* 1.07, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 5.83 (d, *J* = 4.5 Hz, 1H), 5.39 (td, *J* = 7.4, 1.8 Hz, 1H), 3.99 (d, *J* = 8.4 Hz, 4H), 2.11 (q, *J* = 7.9 Hz, 2H), 2.06−1.97 (m, 1H), 1.90−1.79 (m, 1H), 1.78−1.69 (m, 2H), 1.67 (s, 3H), 1.54 (s, 2H), 1.41−1.30 (m, 1H), 1.26−1.14 (m, 1H), 1.02 (dd, *J* = 8.6, 4.6 Hz, 1H), 0.95−0.88 (m, 1H), 0.86 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 137.1, 134.4, 126.3, 121.4, 69.0, 67.5, 42.5, 28.8, 25.1, 23.4, 21.7, 21.6, 17.4, 13.6, 12.6. HRMS (ESI-TOF) *m*/*z* calcd for $C_{15}H_{24}Na_1O_2$ $[M + Na]^+$: 259.1669. Found: 259.1673.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschembio.5b00748.

General chemistry methods, synthesis procedures, and data for compounds **1**−**3**, **3′**, **4**−**15**, **17**, **18**, **21**, and **22** and the MTPA esters of sirenin. ^1H and ^{13}C NMR spectra of all new compounds and ^{19}F NMR spectra for the Mosher esters of sirenin (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Telephone: (612) 626-6320. E-mail: georg@umn.edu.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We gratefully acknowledge the support from NIH/NICHD: 1U01HD076542 and HHSN275201300017C (to G.I.G), 1U01 HDO45857 (to D.E.C.), and R21HD081403 (to P.V.L.), as well as from NIH/NIGMS R01GM111802 (to P.V.L.). We thank K. Miki (Boston Children's Hospital) for pilot experiments. The protocol for the human sperm studies was approved by the Institutional Review Board of the University of Minnesota: IRB protocol #1102M96152. The protocol for the human sperm studies was approved by the Committees on Human Research at the University of California, Berkeley: protocol number 2013-06-5395.

■ ABBREVIATIONS

CsDVF, divalent-free bath solution; DIBAL-H, diisobutylaluminum hydride; DME, dimethoxyethane; DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol tetraacetic acid; EtOAc, ethyl acetate; Fluo-4-AM, glycine, *N*-[4-[6-[(acetyloxy)methoxy]-2,7-difluoro-3-oxo-3*H*-xanthen-9-yl]-2-[2-[2-[bis[2-[(acetyloxy)methoxy]-2-oxoethyl]amino]-5-methylphenoxy]ethoxy]phenyl]-*N*-[2-[(acetyloxy)methoxy]-2-oxoethyl]-,(acetyloxy)methyl ester; FLIPR, Fluorometric Imaging Plate Reader; HEPES, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid; HS, high salt solution; LiHMDS, lithium hexamethyldisilazide; M, mibefradil; MTPA-Cl, α-methoxy-α-(trifluoromethyl)phenylacetyl chloride; NaHMDS, sodium hexamethyldisilazide; P and Prog., progesterone; RFU, relative fluorescence units; S, sirenin; Tris, tris(hydroxymethyl)aminomethane hydrochloride

■ REFERENCES

(1) Yanagimachi, R. (1994) Fertility of mammalian spermatozoa: its development and relativity. *Zygote 2*, 371−372.

(2) Singh, J. P., Babcock, D. F., and Lardy, H. A. (1978) Increased calcium-ion influx is a component of capacitation of spermatozoa. *Biochem. J. 172*, 549−556.

(3) Reyes, A., Goicoechea, B., and Rosado, A. (1978) Calcium ion requirement for rabbit spermatozoal capacitation and enhancement of fertilizing ability by ionophore A23187 and cyclic adenosine 3′:5′-monophosphate. *Fertil. Steril. 29*, 451−455.

(4) Mahanes, M. S., Ochs, D. L., and Eng, L. A. (1986) Cell calcium of ejaculated rabbit spermatozoa before and following in vitro capacitation. *Biochem. Biophys. Res. Commun. 134*, 664−670.

DOI: 10.1021/acschembio.5b00748
ACS Chem. Biol. 2016, 11, 452−459

(5) Quill, T. A., Ren, D., Clapham, D. E., and Garbers, D. L. (2001) A voltage-gated ion channel expressed specifically in spermatozoa. *Proc. Natl. Acad. Sci. U. S. A. 98*, 12527−12531.
(6) Chung, J. J., Navarro, B., Krapivinsky, G., Krapivinsky, L., and Clapham, D. E. (2011) A novel gene required for male fertility and functional CATSPER channel formation in spermatozoa. *Nat. Commun. 2*, 153.
(7) Liu, J., Xia, J., Cho, K. H., Clapham, D. E., and Ren, D. (2007) CatSper beta, a novel transmembrane protein in the CatSper channel complex. *J. Biol. Chem. 282*, 18945−18952.
(8) Qi, H., Moran, M. M., Navarro, B., Chong, J. A., Krapivinsky, G., Krapivinsky, L., Kirichok, Y., Ramsey, I. S., Quill, T. A., and Clapham, D. E. (2007) All four CatSper ion channel proteins are required for male fertility and sperm cell hyperactivated motility. *Proc. Natl. Acad. Sci. U. S. A. 104*, 1219−1223.
(9) Lishko, P. V., Botchkina, I. L., and Kirichok, Y. (2011) Progesterone activates the principal Ca2+ channel of human sperm. *Nature 471*, 387−391.
(10) Strunker, T., Goodwin, N., Brenker, C., Kashikar, N. D., Weyand, I., Seifert, R., and Kaupp, U. B. (2011) The CatSper channel mediates progesterone-induced Ca2+ influx in human sperm. *Nature 471*, 382−387.
(11) Cai, X., Wang, X., Patel, S., and Clapham, D. E. (2015) Insights into the early evolution of animal calcium signaling machinery: A unicellular point of view. *Cell Calcium 57*, 166−173.
(12) Pommerville, J. C., Strickland, J. B., and Harding, K. E. (1990) Pheromone interactions and ionic communication in gametes of aquatic fungusAllomyces macrogynus. *J. Chem. Ecol. 16*, 121−131.
(13) Cai, X., Wang, X., and Clapham, D. E. (2014) Early evolution of the eukaryotic Ca2+ signaling machinery: conservation of the CatSper channel complex. *Mol. Biol. Evol. 31*, 2735−2740.
(14) Machlis, L., Nutting, W. H., Williams, M. W., and Rapoport, H. (1966) Production, isolation, and characterization of sirenin. *Biochemistry 5*, 2147−2152.
(15) Machlis, L. (1968) The response of wild type male gametes of allomyces to sirenin. *Plant Physiol. 43*, 1319−1320.
(16) Nutting, W. H., Rapoport, H., and Machlis, L. (1968) The Structure of Sirenin. *J. Am. Chem. Soc. 90*, 6434−6438.
(17) Machlis, L. (1958) Evidence for a sexual hormone in Allomyces. *Physiol. Plant. 11*, 181−192.
(18) Corey, E. J., Achiwa, K., and Katzenellenbogen, J. A. (1969) Total synthesis of dl-sirenin. *J. Am. Chem. Soc. 91*, 4318−4320.
(19) Grieco, P. A. (1969) Total Synthesis of dl-sirenin. *J. Am. Chem. Soc. 91*, 5660−5661.
(20) Mori, K., and Matsui, M. (1969) Synthesis of racemic sirenin, a plant sex hormone. *Tetrahedron Lett. 10*, 4435−4438.
(21) Plattner, J. J., Bhalerao, U. T., and Rapoport, H. (1969) Synthesis of dl-sirenin and dl-isosirenin. *J. Am. Chem. Soc. 92*, 3429−3433.
(22) Corey, E. J., and Achiwa, K. (1970) Simple synthetic route to dl-sirenin. *Tetrahedron Lett. 11*, 2245−2246.
(23) Bhalerao, U. T., Plattner, J. J., and Rapoport, H. (1970) Synthesis of dl-sirenin and dl-isosirenin. *J. Am. Chem. Soc. 92*, 3429−3433.
(24) Garbers, C. F., Steenkamp, J. A., and Visagie, H. E. (1975) Terpenoid synthesis. III. Chloromethyl ethers in the hydroxymethylation of olefins and the synthesis of sirenin. *Tetrahedron Lett. 16*, 3753−3756.
(25) Kitatani, K., Hiyama, T., and Nozaki, H. (1976) Stereoselective one-pot dialkylation of gem-dihalocyclopropanes - simple route to D,L-sesquicarene and D,L-sirenin. *J. Am. Chem. Soc. 98*, 2362−2364.
(26) Dupreez, H. E., Garbers, C. F., and Steenkamp, J. A. (1980) Terpenoid synthesis.VII. Electrophilic addition-reactions of chloromethyl ethers in the hydroxymethylation of olefins and the synthesis of sirenin. *S. Afr. J. Chem. 33*, 21−26.
(27) Mandai, T., Hara, K., Kawada, M., and Nokami, J. (1983) A new total synthesis of dl-sirenin. *Tetrahedron Lett. 24*, 1517−1518.
(28) Rapoport, H., and Plattner, J. J. (1971) Synthesis of d- and l-sirenin and their absolute configurations. *J. Am. Chem. Soc. 93*, 1758−1761.
(29) Gant, T. G., Noe, M. C., and Corey, E. J. (1995) The first enantioselective synthesis of the chemotactic factor sirenin by an intramolecular [2 + 1] cyclization using a new chiral catalyst. *Tetrahedron Lett. 36*, 8745−8748.
(30) Kitahara, T., Horiguchi, A., and Mori, K. (1988) Synthetic microbial chemistry.19. The synthesis of (−)-sirenin, sperm attractant of the water mold Allomyces-Macrogynus. *Tetrahedron 44*, 4713−4720.
(31) Hortmann, A. G., and Ong, A. (1970) New route to 8- 9-substituted carenes. *J. Org. Chem. 35*, 4290−4292.
(32) Navarro, B., Kirichok, Y., and Clapham, D. E. (2007) KSper, a pH-sensitive K+ current that controls sperm membrane potential. *Proc. Natl. Acad. Sci. U. S. A. 104*, 7688−7692.
(33) Lishko, P. V., Kirichok, Y., Ren, D., Navarro, B., Chung, J. J., and Clapham, D. E. (2012) The control of male fertility by spermatozoan ion channels. *Annu. Rev. Physiol. 74*, 453−475.
(34) Zeng, X. H., Yang, C., Xia, X. M., Liu, M., and Lingle, C. J. (2015) SLO3 auxiliary subunit LRRC52 controls gating of sperm KSPER currents and is critical for normal fertility. *Proc. Natl. Acad. Sci. U. S. A. 112*, 2599−2604.
(35) Dale, J. A., Dull, D. L., and Mosher, H. S. (1969) α-Methoxy-α-trifluoromethylphenylacetic acid, a versatile reagent for the determination of enantiomeric composition of alcohols and amines. *J. Org. Chem. 34*, 2543−2549.
(36) Xia, J. S., Reigada, D., Mitchell, C. H., and Ren, D. (2007) CATSPER channel-mediated Ca2+ entry into mouse sperm triggers a tail-to-head propagation. *Biol. Reprod. 77*, 551−559.
(37) Pommerville, J. C., Strickland, J. B., Romo, D., and Harding, K. E. (1988) Effects of Analogs of the Fungal Sexual Pheromone Sirenin on Male Gamete Motility in Allomyces-Macrogynus. *Plant Physiol. 88*, 139−142.
(38) Strunker, T., Weyand, I., Bonigk, W., Van, Q., Loogen, A., Brown, J. E., Kashikar, N., Hagen, V., Krause, E., and Kaupp, U. B. (2006) A K+-selective cGMP-gated ion channel controls chemosensation of sperm. *Nat. Cell Biol. 8*, 1149−1154.
(39) Darszon, A., Nishigaki, T., Beltran, C., and Trevino, C. L. (2011) Calcium channels in the development, maturation, and function of spermatozoa. *Physiol. Rev. 91*, 1305−1355.
(40) Lishko, P. V., and Kirichok, Y. (2010) The role of Hv1 and CatSper channels in sperm activation. *J. Physiol. 588*, 4667−4672.
(41) Lishko, P., Clapham, D. E., Navarro, B., and Kirichok, Y. (2013) Sperm patch-clamp. *Methods Enzymol. 525*, 59−83.
(42) Cooper, T. G., Noonan, E., von Eckardstein, S., Auger, J., Baker, H. W. G., Behre, H. M., Haugen, T. B., Kruger, T., Wang, C., Mbizvo, M. T., and Vogelsong, K. M. (2010) World Health Organization reference values for human semen characteristics. *Hum. Reprod. Update 16*, 231−245.
(43) Cooper, T. G. (2011) The epididymis, cytoplasmic droplets and male fertility. *Asian J. Androl. 13*, 130−138.

DOI: 10.1021/acschembio.5b00748
ACS Chem. Biol. 2016, 11, 452−459