

Synthesis and Biological Evaluation of Bile Acid Analogues Inhibitory to *Clostridium difficile* Spore Germination

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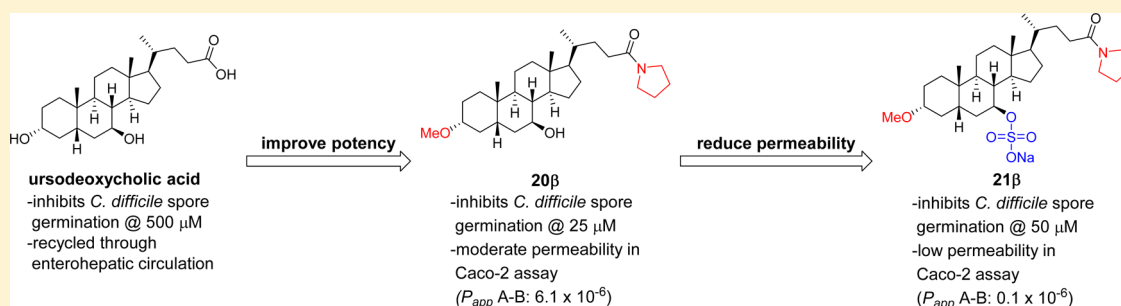
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S Supporting Information



ABSTRACT: Standard antibiotic-based strategies for the treatment of *Clostridium difficile* infections disrupt indigenous microbiota and commonly fail to eradicate bacterial spores, two key factors that allow recurrence of infection. As an alternative approach to controlling *C. difficile* infection, a series of bile acid derivatives have been prepared that inhibit taurocholate-induced spore germination. These analogues have been evaluated in a highly virulent NAP1 strain using optical density and phase-contrast microscopy assays. Heterocycle substitutions at C24 were well-tolerated and several tetrazole-containing derivatives were highly potent inhibitors in both assays, with complete inhibition of spore germination observed at 10–25 μ M. To limit intestinal absorption, C7-sulfated analogues designed to avoid active and passive transport pathways were prepared. One of these derivatives, compound **21b**, was found to be a potent inhibitor of *C. difficile* spore germination and poorly permeable in a Caco-2 model of intestinal epithelial absorption, suggesting that it is likely to be gut-restricted.

INTRODUCTION

Clostridium difficile is an anaerobic, spore-forming, Gram-positive bacterium that causes a potentially fatal infection of the colon. *C. difficile* infection (CDI) is the most common hospital-acquired infection, and in recent years CDI has also become increasingly acquired in the community.¹ The infection affects approximately 500000 patients annually and leads to death in 30000 patients per year in the U.S. alone.^{2–5} CDI typically occurs after the disruption of gut microbiota following a course of antibiotics and subsequent ingestion of *C. difficile* spores from the surrounding environment.⁶ The current standard of care also relies on antibiotics. However, this strategy can perpetuate recurrence of CDI because it fails to eliminate *C. difficile* spores and results in further suppression of indigenous microbiota. After the initial infection and antibiotic

treatment, relapse can occur in 20–40% of patients, with a higher risk of recurrence associated with each episode of infection.⁷ A large fraction of patients ultimately develops an indefinite syndrome of recurrent CDI (R-CDI), which becomes refractory to eradication with antibiotics alone.⁸

To prevent R-CDI, there has been a shift in the design of *C. difficile* therapies toward developing antibiotics that are more selective for this bacterium and minimize the suppression of gut microbiota.⁹ In addition to the recently approved drug fidaxomicin, there are several new compounds being evaluated as narrow-spectrum antibiotics in clinical trials for CDI and prevention of R-CDI, including CRS3123 (phase I),

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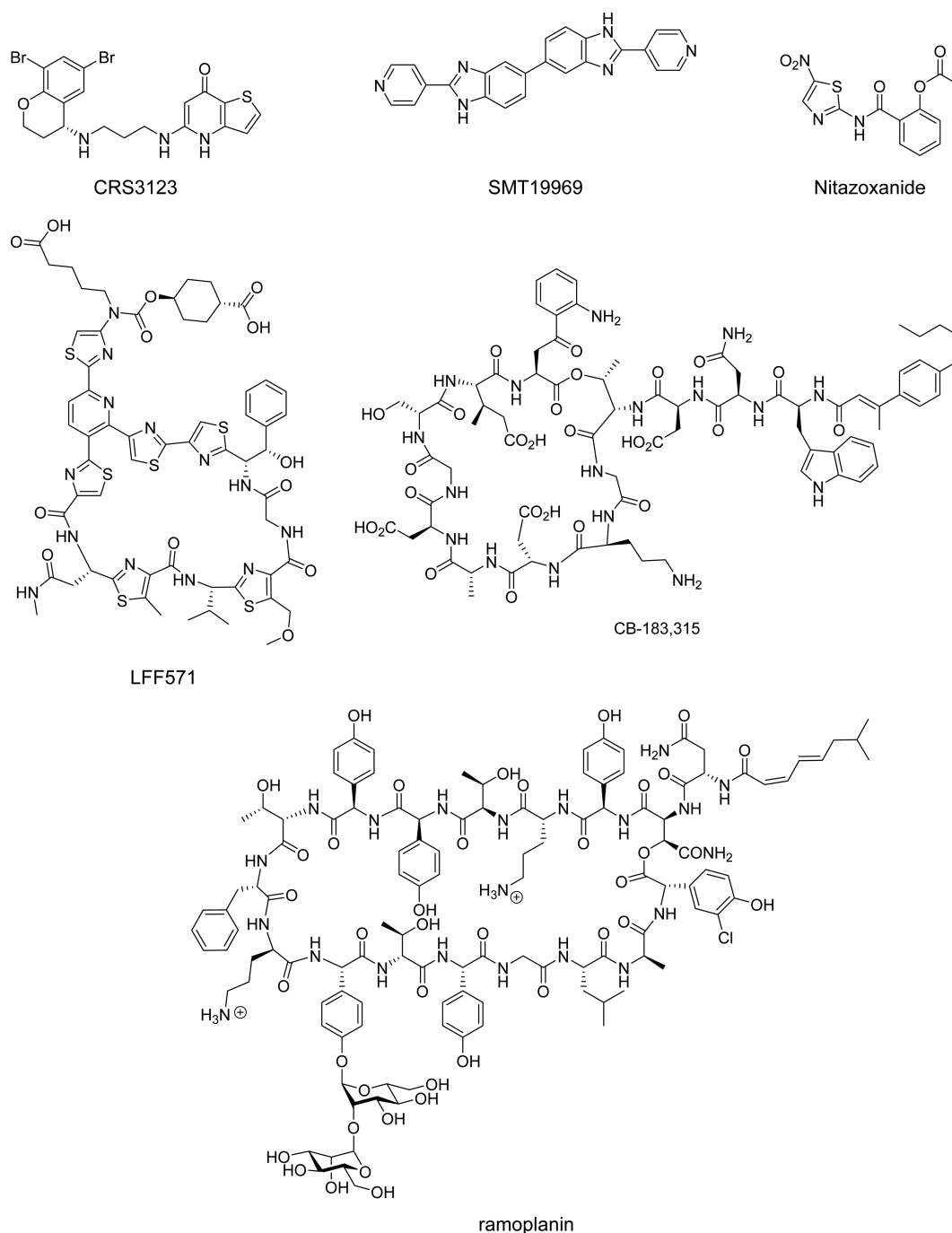


Figure 1. Structures of narrow spectrum antibiotics currently in clinical trials for the treatment of *C. difficile* infection.

SMT19969 (phase II), LFF571 (phase II), and ramoplanin (phase III, Figure 1).⁹ There are also existing drugs that are being repurposed for CDI in phase III trials (CB-183,315 and nitazoxanide, Figure 1).⁹ Outside of antibacterial therapeutics, recent evidence suggested that targeting the *C. difficile* toxins (TdcA and TdcB) through monoclonal antibody treatment reduces the recurrence of CDI.¹⁰

Recently, fecal microbiota transplantation (FMT) has emerged as a highly effective treatment in breaking the cycle of CDI recurrence. This procedure involves administration of fecal microbiota from healthy donors into the colon of patients.⁸ It results in prompt and sustained normalization of fecal microbial community structure in the recipients.¹¹ A number of mechanistic investigations of FMT in treatment of

R-CDI have converged on the critical role of secondary bile acid metabolism, which is normally mediated by gut microbiota, in inhibiting *C. difficile* spore germination and expansion in the colon.^{8,11–13} Spores of *C. difficile* germinate in the distal gut when they sense the appropriate host environment (signaled in large part by bile acids) and favorable nutrient conditions (signaled by glycine).^{14–17} Bile acids in the cholic acid (CA) family typically promote germination of *C. difficile* spores, while those in the chenodeoxycholic acid (CDCA) family generally inhibit *C. difficile* spore germination (Figure 2).^{13,17,18} Taurocholic acid (TCA), a potent pro-germinant, is routinely included in isolation media for *C. difficile*.^{19–21}

Under normal physiologic conditions, colonic microbiota mediate 7 α -dehydroxylation of primary bile acids, yielding

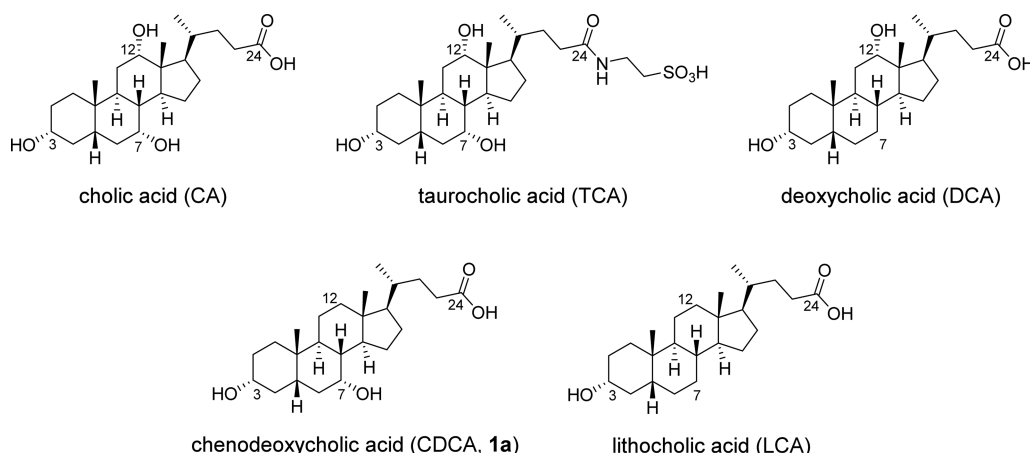


Figure 2. Structures of endogenous bile acids cholic acid (CA), taurocholic acid (TCA), deoxycholic acid (DCA), chenodeoxycholic acid (CDCA, **1a**), and lithocholic acid (LCA).

deoxycholic acid (DCA) and lithocholic acid (LCA) from CA and CDCA, respectively.^{22,23} This metabolism is disrupted by the administration of antibiotics. The fecal bile acid composition in patients with R-CDI syndrome promotes *C. difficile* spore germination and vegetative growth when evaluated using in vitro assays.¹³ FMT helps restore normal bile acid concentrations, leading to compositions that are inhibitory to spore germination and vegetative growth.¹³

However, some patients are not good candidates for FMT because they continue to require frequent antibiotics for non-*C. difficile* indications. Common examples in our clinic include patients with osteomyelitis, recurrent urinary tract infections, and recurrent sinusitis. A potentially complementary approach to preventing CDI without further disrupting the indigenous microbiota is to directly inhibit the germination of *C. difficile* spores.

In 2010, Sorg and Sonenshein reported that several commercially available synthetic analogues of CDCA were more potent than the natural bile acid at inhibiting TCA-promoted spore germination in the UK1 epidemic strain.²⁴ The most potent compound they reported was the C24 methyl ester derivative of CDCA (**2a**), with a K_i of 44 μM . Although this work provides one of the first examples of bile acid analogues successfully inhibiting *C. difficile* spore germination in vitro, the in vivo use of **2a** is potentially problematic because of the susceptibility of methyl esters to chemical or enzymatic hydrolysis.²⁵ In addition, bile acids are efficiently absorbed from the intestinal lumen by active and passive absorption mechanisms,^{26,27} and it is likely that enterohepatic recycling would prevent **2a** from reaching the concentration in the colon necessary to inhibit *C. difficile* spore germination. More recently, Sorg and co-workers have focused on investigating the mechanism by which bile acids inhibit or promote *C. difficile* spore germination. In their 2013 paper, these researchers hypothesized that the germination-specific protease, CspC, served as a bile acid receptor for *C. difficile* and found that mutations in this receptor could alter germinant specificity.¹⁴ In their originally proposed model, CspC then activates CspB protease, which in turn triggers the degradation of the spore cortex by the spore cortex lytic enzyme SleC.²⁸ In 2016, this model was revised based on data showing a potential inverse correlation between germination rate and CspC abundance. The researchers now hypothesize that CspC serves to both activate CspB and inhibit SleC but that the ability of CspC to

inhibit SleC may vary depending on the presence of an additional protein GerS.²⁸

Other examples of bile acid-based inhibitors of spore germination include compounds discovered by Abel-Santos and co-workers. Their lead compound (**3**) was a potent inhibitor of spore germination in ATCC strain 630, with an IC_{50} of $58 \pm 35 \mu\text{M}$.²⁹ These researchers also reported that compound **3** (Figure 3) eradicated CDI symptoms in a murine infection model of the same strain.³⁰

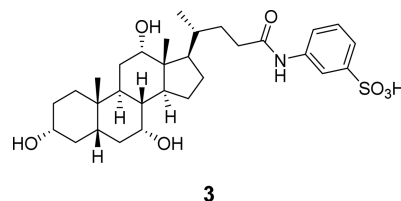


Figure 3. Likely structure of **3**, the most potent inhibitor of strain ATCC 630 described by Abel-Santos and co-workers.^{29,31}

The endogenous bile acid UDCA (**1b**, Figure 4) is another known inhibitor of spore germination and was active at ~ 500

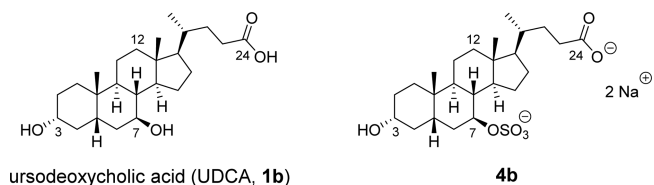
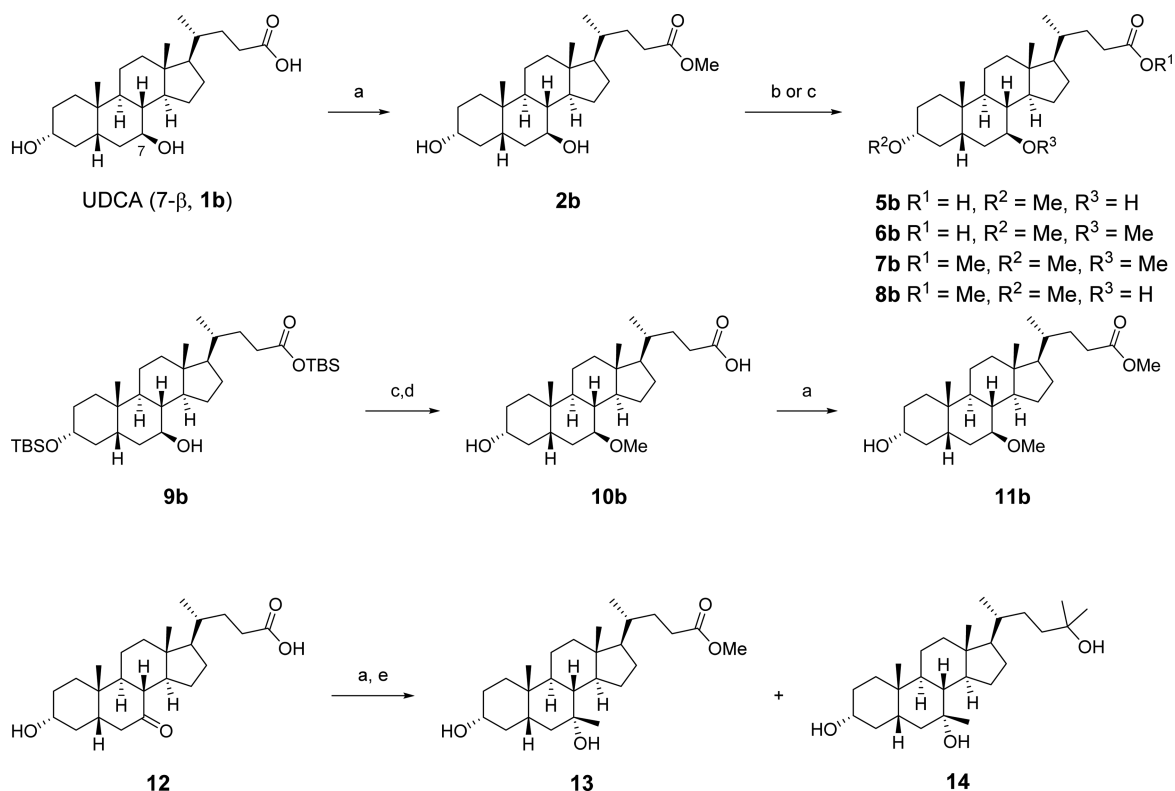


Figure 4. Structures of naturally occurring bile acids **1b** and **4b**.

μM across several strains of *C. difficile*, including a highly virulent NAP1 strain.^{24,32} Unfortunately, UDCA is unlikely to be a useful therapeutic agent in most patients because it is effectively reabsorbed into the enterohepatic circulation from the ileum, thus preventing the necessary intracolonic concentration needed to suppress *C. difficile* from being reached. However, we recently reported successful treatment of a patient with *C. difficile* ileal pouchitis with oral dosing of UDCA.³² We were able to achieve therapeutic levels of UDCA in the patient's ileal pouch because she had undergone a colectomy, which disrupts enterohepatic recycling. This result

Scheme 1. Synthesis of Methylated Esters and Carboxylic Acids^a

^aReagents and conditions: (a) *p*TSA·H₂O, MeOH; (b) NaH, MeI, THF; (c) MeOTf, 2,6-di-*tert*-butylpyridine, DCM; (d) TBAF, THF; (e) MeMgBr, Et₂O.

provides further evidence that bile acids can be used to treat R-CDI if adequate concentrations can be achieved clinically.

One way to avoid bile acid reuptake into the enterohepatic circulation is with the addition of a sulfate group to the C7-position of UDCA (compound **4b** in Figure 4). In 1995, Rodrigues and co-workers reported that **4b** was gut restricted and when administered orally was found to be eliminated intact in the feces of rats.³³ This observation agrees with the later findings of Kolhatkar and Polli, who observed that C7-substituted bile acids typically do not undergo active transport by the apical sodium-dependent bile acid transporter (ASBT), the primary transporter of bile acids from the gut.³⁴ Additionally, the negatively charged sulfate group impedes passive absorption of the compound. This limited absorption is desirable for treating an infection in the lower intestine, as it provides a way to effectively increase the concentration of the therapeutic in the target area while minimizing unwanted systemic effects.³⁵ An additional advantage is that bile acids sulfated at C7 are resistant to desulfation and conjugation with taurine or glycine and thus are typically excreted unchanged in fecal matter.³⁶

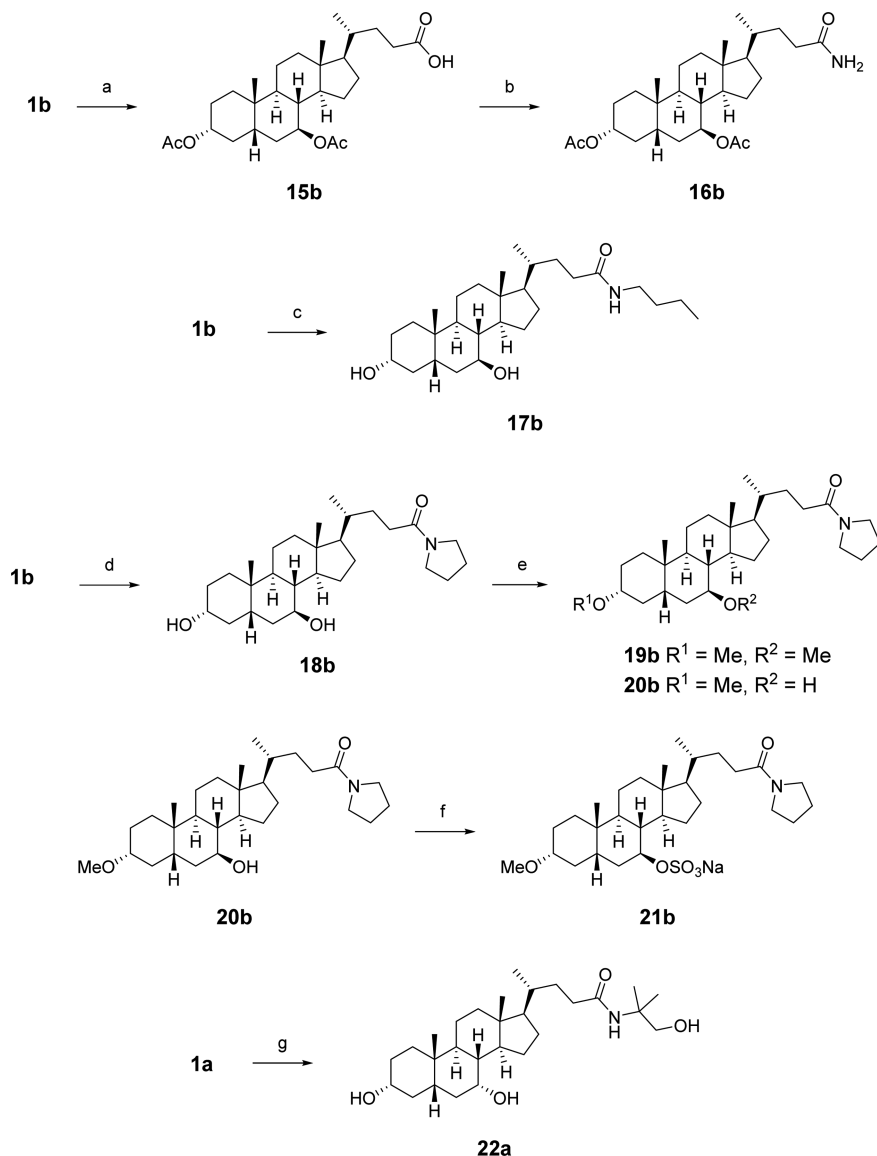
With these concepts in mind, we evaluated the ability of **4b** to prevent *C. difficile* spore germination. Unfortunately, while we determined that **4b** inhibits NAP1 spore germination, it is significantly less potent than UDCA (3000 μM, Supporting Information, Figure S1). Thus, any bile acid derivative containing a C7-sulfate group to restrict intestinal absorption is likely to need additional structural modifications to increase potency in order to counteract the detrimental effect on potency that comes from incorporating this moiety.

As part of our continued efforts to develop improved methods of treating CDI, we set out to develop novel, gut-restricted, bile acid analogues capable of inhibiting *C. difficile* spore germination. This approach builds on the natural mechanisms that control the lifecycle of *C. difficile* and has the advantage of not placing selective pressure on dividing cells, which may limit the potential of developing resistance.⁶ Herein, we report the synthesis of modified bile acid derivatives of CDCA and UDCA. These compounds have been evaluated in optical density and microscope-based spore germination assays, and we have identified several analogues that are highly potent inhibitors of *C. difficile* spore germination.

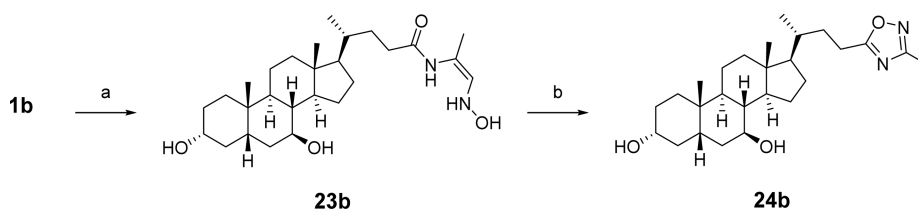
RESULTS AND DISCUSSION

Chemistry. CDCA (**1a**) and UDCA (**1b**) are epimeric endogenous bile acids, with the C7-hydroxyl group of **1a** in the α position and the C7-hydroxyl group of **1b** in the β position. The syntheses of analogues based on the **1b** scaffold are shown in the synthetic schemes below. Analogues of **1a** were prepared in the same manner, except for compound **11a** (see Scheme S1 in the Supporting Information). Initially, carboxylic acid and ester-containing derivatives were prepared to explore the SAR of modifying the C3- and C7-hydroxyl groups and for comparison with the inhibitory compounds described by Sorg and Sonenshein.²⁴ A series of analogues containing bioisosteric replacements³⁷ for carboxylic acids and esters, including amides and various heterocycles, were subsequently synthesized in order to obtain compounds that are less likely to be metabolized in the environment of the intestinal tract.

Synthesis of Esters and Carboxylic Acid Analogues. Treatment of **1b** with *p*-toluenesulfonic acid in methanol

Scheme 2. Synthesis of Amide Analogues^a

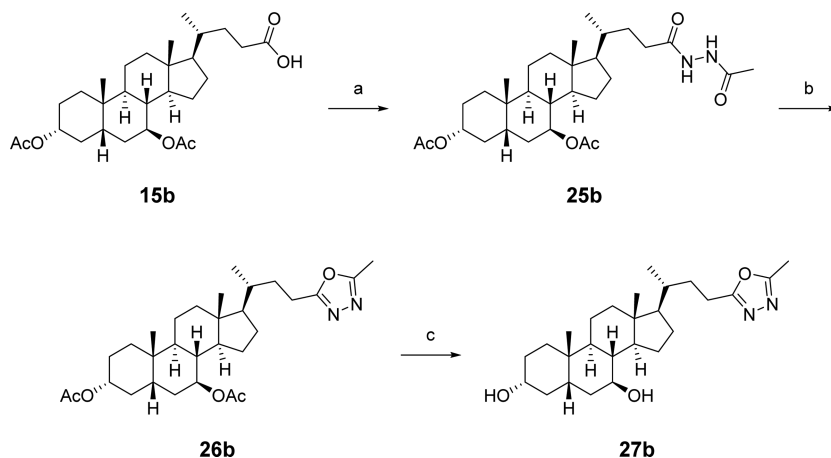
^aReagents and conditions: (a) Ac₂O, cat. DMAP, pyridine; (b) SOCl₂, benzene, 80 °C, then NH₃; (c) HATU, TEA, ⁿbutylamine, DCM; (d) HATU, TEA, pyrrolidine, DCM; (e) NaH, MeI, THF; (f) ClSO₃H, pyridine, 50 °C, ion exchange using Na⁺-Dowex resin; (g) HATU, TEA, 2-amino-2-methyl-propanol, DCM.

Scheme 3. Synthesis of 1,2,4-Oxadiazole^a

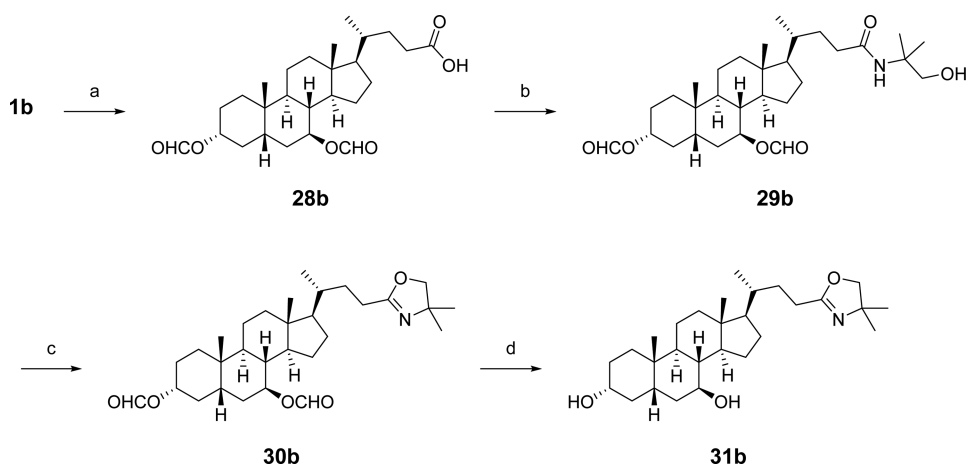
^aReagents and conditions: (a) HATU, DIPEA, (Z)-1-(hydroxyamino)prop-1-en-2-amine, DCM; (b) toluene, THF, 160 °C, 45 min.

afforded the methyl ester 2b (Scheme 1).³⁸ Surprisingly, methylation of the C3 and/or C7 alcohols of 2b with sodium hydride and methyl iodide in THF generated the carboxylic acid analogues 5b and 6b instead of the expected esters 7b and 8b. The methyl ester analogues 7b and 8b were instead obtained by methylation with methyl triflate and 2,6-di-*tert*-butylpyridine.³⁹ A three-step sequence of methylation, silyl

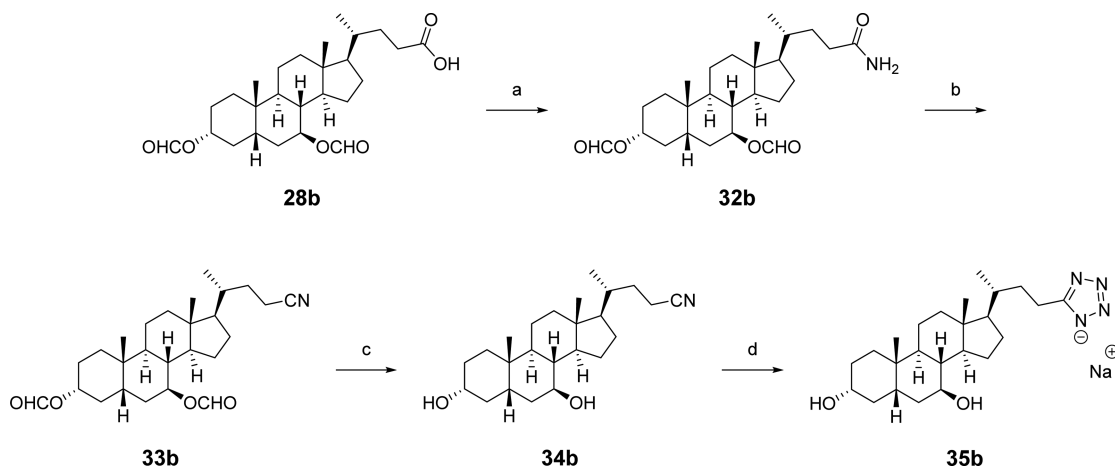
deprotection, and esterification provided 11b from compound 9b, which was prepared as previously described.⁴⁰ Addition of MeMgBr to the methyl ester of commercially available C7-ketone 12 produced the tertiary alcohols 13 and 14, with high stereoselectivity that matched the observed diastereomer reported by Une and co-workers.⁴¹

Scheme 4. Synthesis of 1,3,4-Oxadiazole^a

^aReagents and conditions: (a) HATU, TEA, acetohydrazone, DCM; (b) TsCl, TEA, DCM; (b) NaOH, MeOH, 75 °C.

Scheme 5. Synthesis of C24-Oxazoline 31b^a

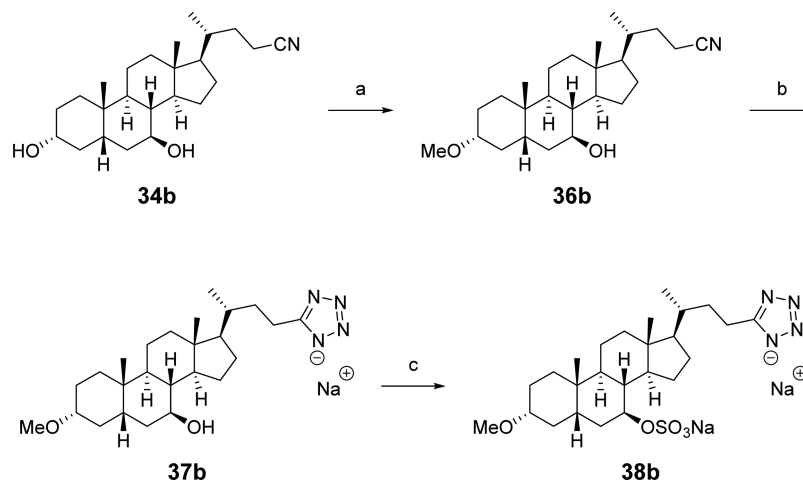
^aReagents and conditions: (a) cat. HClO₄, HCO₂H, 50 °C; (b) SOCl₂, benzene, 80 °C, then 2-amino-2-methylpropanol, DCM; (c) SOCl₂, DCM; (d) NaOH, MeOH, 75 °C.

Scheme 6. Synthesis of Tetrazole Analogue^a

^aReagents and conditions: (a) SOCl₂, benzene, 80 °C, then NH₃; (b) TFAA, pyridine, THF; (c) NaOMe, MeOH, 75 °C; (d) Et₃N·HCl, NaN₃, 1:1 toluene:DMF, 160 °C, ion-exchange using Na⁺-Dowex resin.

Synthesis of Amide Analogues. Primary amide **16b** was prepared by acylation of the C3 and C7 alcohols of **1b**,

followed by conversion to the acid chloride and amidation with ammonia gas (Scheme 2).⁴² Amides **17b** and **18b** were

Scheme 7. Synthesis of 3-Methoxy-7-sulfate Tetrazole Analogues^a

^aReagents and conditions: (a) NaH, MeI, THF; (b) ZnBr₂, NaN₃, H₂O/2-propanol, MW 170 °C, ion exchange using Na⁺-Dowex resin; (c) ClSO₃H, pyridine, 50 °C, ion exchange using Na⁺-Dowex resin.

synthesized through HATU-mediated coupling of **1b** with *n*-butylamine or pyrrolidine, respectively. Similarly, HATU coupling of **1a** with 2-amino-2-methyl-propanol afforded **22a** (the corresponding UDCA analogue **22b** was not prepared). Compound **18b** was methylated with sodium hydride and methyl iodide to afford bis-methylated amide **19b** and monomethylated amide **20b**. A sulfated derivative was synthesized by reaction of **20b** with chlorosulfonic acid in pyridine and was isolated as a triethylammonium salt through purification via reverse-phase column chromatography. The triethylammonium salt was then converted to the sodium salt (**21b**) by ion-exchange chromatography.⁴³

Synthesis of Oxadiazole Analogues. The 1,2,4-oxadiazole analogue was synthesized in a two-step procedure via a HATU-mediated amide coupling of **1b** to (Z)-1-(hydroxyamino)prop-1-en-2-amine, followed by microwave-assisted dehydrative cyclization to afford **24b** (Scheme 3).⁴⁴ The isomeric 1,3,4-oxadiazole was synthesized by conversion of carboxylic acid **15b** to hydrazine **25b**, followed by dehydrative cyclization using tosyl chloride and TEA and removal of the acetate protecting groups under basic conditions to obtain **27b** (Scheme 4).⁴⁵

Synthesis of the Oxazoline Analogue. To prepare the oxazoline analogue of **1b**, the C3 and C7 alcohols were first protected as formate esters (**28b**, Scheme 5). The carboxylic acid was converted to the corresponding acid chloride and reaction with 2-amino-2-methyl-propanol afforded the desired amide **29b**. Thionyl chloride-mediated dehydrative cyclization of the amide afforded the oxazoline **30b**, and the hydroxyl groups were deprotected under basic conditions to provide oxazoline analogue **31b**.

Synthesis of Tetrazole Analogues. Carboxylic acid **28b** was converted to the primary amide **32b** via treatment with thionyl chloride and then ammonia gas (Scheme 6). The amide was dehydrated with TFAA in pyridine to afford nitrile **33b**. The formate esters were hydrolyzed with sodium methoxide in refluxing methanol and the deprotected nitrile **34b** was heated with triethylammonium chloride and sodium azide to form the tetrazole,⁴⁶ which was converted into the sodium salt **35b** by ion-exchange chromatography.⁴³

To synthesize the 3-methoxy-7-sulfate tetrazole analogue, the C3-hydroxyl of **34b** was methylated with sodium hydride and

methyl iodide to afford **36b** (Scheme 7). The nitrile was heated with sodium azide and zinc(II) bromide to afford **37b** as a sodium salt after ion-exchange chromatography.^{43,47} Tetrazole **37b** was heated with chlorosulfonic acid in pyridine to incorporate a sulfate group, followed by ion-exchange chromatography using Dowex-50WX2 resin to obtain **38b** as the disodium salt.

Evaluation of Biological Activity of Compounds. Some of the key steps in spore germination are depolymerization of peptidoglycan in the spore cortex followed by hydration of the spore core, which can be observed as a phase change from bright to dark by phase-contrast microscopy. This change also can be observed by measuring the decrease in optical density of the spore suspension at 600 nm (OD₆₀₀) over time. An optical density assay was used as an initial screen to identify the most potent bile acid analogues. These compounds were then further evaluated using a phase-contrast microscopy assay to verify the initial findings.

Spores from a NAP1 strain of *C. difficile* were isolated from patient samples by previously reported methods.¹³ In the optical density assay, spores were incubated for 20 min on brain–heart infusion medium supplemented with 0.5% yeast extract and 0.1% L-cysteine (BHIS) along with 2000 μM TCA, a known germinant, under anaerobic conditions in the presence of the bile acid analogues. As a control for this experiment, spores were incubated with 2000 μM TCA for 20 min in the absence of test compounds, which resulted in a maximum change in OD₆₀₀ to approximately 60% of the initial value. Compounds showing significant potency at inhibiting germination in the optical density were retested, with similar results obtained between runs.

In the phase-contrast spore count assay, NAP1 spores were plated with either DMSO as a control or the test compounds at 10 μM. The number of spores and germinated cells were counted at *t*₀. The spores were then exposed to 2000 μM TCA for 20 min, and the number of spores and germinated cells were counted at *t*₂₀ for both the DMSO control and the test compound plates. Each experiment was performed in triplicate and the percentage of germinated cells at *t*₀ and *t*₂₀ determined. In the DMSO control samples, the percent of germinated spores increased from approximately 20% to 75% of the total number of cells after 20 min.

Results of Optical Density Assay. Spores were incubated in BHIS supplemented with 2000 μM of the germinant TCA and with varying concentrations of the bile acid analogues. Compounds were divided into three categories based on the lowest concentration at which complete inhibition of spore germination was observed (defined as a relative OD_{600} reading after 20 min greater than or equal to 0.95). Good inhibitors were effective at 50 μM or less, moderate inhibitors were effective at 100–500 μM , and poor inhibitors were effective at concentrations ≥ 1000 μM . Examples of compounds that were good or moderate inhibitors are shown in Figures 5 and 6, respectively.

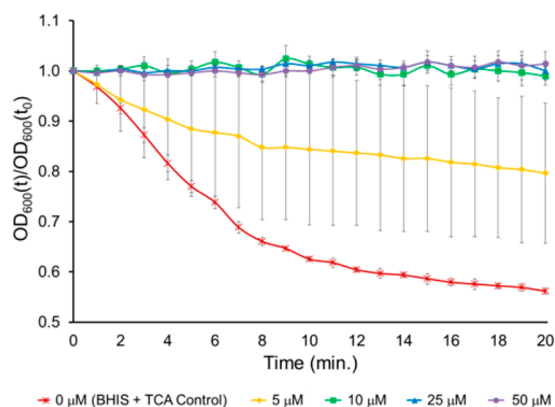


Figure 5. Example of a compound that was a potent inhibitor of *C. difficile* spore germination. The relative OD_{600} of spores in BHIS after 20 min exposure to 2000 μM TCA and 0, 5, 10, 25, or 50 μM 35a. $\text{OD}_{600}(t)/\text{OD}_{600}(t_0) = \text{OD}_{600}$ normalized to the initial OD_{600} (relative OD_{600}). Data represent mean \pm SEM.

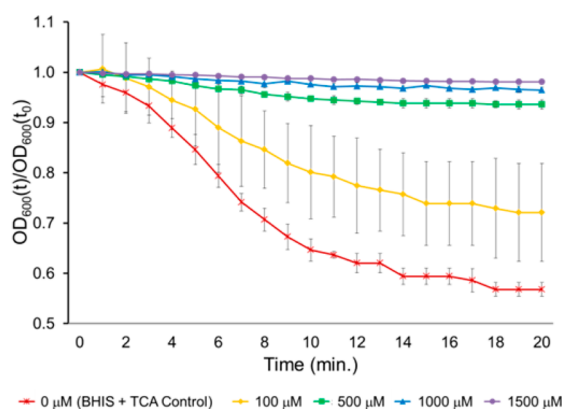


Figure 6. Example of a compound that was a moderate inhibitor of *C. difficile* spore germination. The relative OD_{600} of spores in BHIS after 20 min exposure to 2000 μM TCA and 0, 100, 500, 1000, or 1500 μM 19b. $\text{OD}_{600}(t)/\text{OD}_{600}(t_0) = \text{OD}_{600}$ normalized to the initial OD_{600} (relative OD_{600}). Data represent mean \pm SEM.

Modifications to the CDCA (1a) and UDCA (1b) scaffolds were made at the C24, C3, and C7 positions to determine how these changes affected the potency of the analogues at inhibiting TCA-promoted germination of *C. difficile* spores. Table 1 compares the lowest concentration of synthetic bile acids needed to obtain complete inhibition of *C. difficile* spore germination in the optical density assay (OD_{600} after 20 min. ≥ 0.95). Graphs of optical density vs time for all compounds tested can be found in the Supporting Information. Generally, most of the tested bile acid analogues demonstrated

concentration-dependent inhibition of spore germination in the NAP1 strain examined, with higher concentrations of test compound leading to greater inhibition. However, a small number of compounds showed abnormal responses, where lower concentrations appeared to be more effective at preventing germination than higher concentrations.⁴⁸ It is possible that these abnormal responses may be attributable to bile acid aggregation, micelle formation at the elevated concentrations,^{49,50} or were artifacts due to compound insolubility.^{51,52} Compounds showing significant potency in the optical density assay were subsequently tested in a phase contrast microscope assay to visually confirm that they actually inhibited *C. difficile* spore germination (see Microscope Spore Count Assay section below).

C24 Substitutions. Most synthetic ester derivatives (7a, 7b, 8a, 8b, 11a, 11b, and 40) had the same inhibitory activity as the most potent compound identified by Sorg and co-workers (2a, 500 μM in the optical density assay).²⁴ Two exceptions are 2b and 13, which more potently inhibited spore germination (50 μM). Replacing the methyl ester of 13 with the tertiary alcohol in 14 resulted in a large decrease in potency (50 μM compared to >1000 μM , respectively).

Acids 1a and 1b both inhibited TCA-promoted spore germination of the NAP1 strain used in our assay at 500 μM , consistent with previously reported data for these endogenous bile acids.^{18,21,24} Carboxylic acid analogues 5a, 5b, 10b, and 39 showed abnormal responses where greater germination appeared to be observed at higher concentrations. However, further evaluation of one of these compounds (39) in the optical density assay in the absence of spores also showed a change in optical density with time, suggesting what initially appeared to be germination was likely actually an artifact due to compound insolubility. As many better alternatives were identified, these carboxylic acid analogues were not further investigated.

In contrast, the pyrrolidine amide derivatives 18a, 18b, 20b, and 21b were among the most potent inhibitors synthesized, with complete inhibition of spore germination observed at concentrations ≤ 100 μM in the presence of 2000 μM TCA. Secondary amides 17a, 17b, and 22a were moderate inhibitors of the NAP1 strain (500 μM). Both primary amide 16b and pyrrolidine amide 20a had abnormal concentration responses and were not investigated further. To confirm that the activity of our analogues was not limited to just the NAP1 strain, compound 20b was also tested against Nap2 and Nap10 strains of *C. difficile* isolated from patients and found to completely inhibit spore germination at 100 μM . The sulfonated amide 3, reported by Abel-Santos and co-workers to be active against strain ATCC 630,²⁹ was also prepared but was inactive at concentrations up to 1000 μM in our assays (optical density and spore count data for 3 can be found in the Supporting Information, spectral data matched those previously reported for the compound⁵⁰).

Heterocyclic substitutions were well-tolerated at the C24 position. The oxazoline analogue 31b and the oxadiazole analogues 24a, 24b, 27a, and 27b inhibit NAP1 spore germination at 50 μM , although with oxadiazole 24b incomplete inhibition of germination seemed to occur at higher concentrations. However, the tetrazole analogue series (35a, 35b, 37a, 37b, 38a, and 38b) contained the most potent analogues, which inhibit spore germination at concentrations as low as 5 μM (37a) and did not promote germination under the assay conditions.

Table 1. Lowest Concentration of Bile Acid Analogues That Showed Complete Inhibition of NAP1 *C. difficile* Spore Germination in the Presence of 2000 μ M TCA in Optical Density Assay

Compound number	R ¹	R ²	R ³	Concentration Required for Complete Inhibition (μ M) ^a	Compound number	R ¹	R ²	R ³	Concentration Required for Complete Inhibition (μ M) ^a
1a		-OH	- α OH	500	27a		-OH	- α OH	50
1b		-OH	- β OH	500 ^b	27b		-OH	- β OH	50
4b ^d		-OH	- β OSO ₃ Na	3000	31b		-OH	- β OH	50
5a		-OMe	- α OH	>100 ^d	35a		-OH	- α OH	10
5b		-OMe	- β OH	>500 ^d	35b		-OH	- β OH	25
6b		-OMe	- β OMe	>1000	37a		-OMe	- α OH	5
10b		-OH	- β OMe	>500 ^d	37b		-OMe	- β OH	25
2a		-OH	- α OH	500	38a		-OMe	- α OSO ₃ Na	25
2b		-OH	- β OH	50	38b		-OMe	- β OSO ₃ Na	10
7a		-OMe	- α OMe	500					
7b		-OMe	- β OMe	500	3				>1000
8a		-OMe	- α OH	500	13				50
8b		-OMe	- β OH	500	14				>1000
11a		-OH	- α OMe	500	39				500 ^d
11b		-OH	- β OMe	500	40				500
16a		-OAc	- α OAc	50					
16b		-OAc	- β OAc	50 ^d					
17a		-OH	- α OH	500					
17b		-OH	- β OH	500					
18a		-OH	- α OH	50					
18b		-OH	- β OH	100					
19a		-OMe	- α OMe	500					
19b		-OMe	- β OMe	1000					
20a		-OMe	- α OH	100 ^d					
20b		-OMe	- β OH	25					
21b		-OMe	- β OSO ₃ Na	50					
22a		-OH	- α OH	500					
24a		-OH	- α OH	50					
24b		-OH	- β OH	50 ^d					

^aComplete inhibition = relative OD₆₀₀ greater than 0.95 in optical density assay after exposure of spores to BHIS and 2000 μ M TCA for 20 min with varying concentrations of bile acid analogues. ^bValue previously reported in Weingarden et al. (ref 32). ^cTested as the sodium carboxylate. ^dShows an abnormal concentration–response in the presence of TCA.

C3 and C7 Substitutions. In the UDCA methyl ester series, methoxy substitutions at C3, C7, or C3/C7 decreased the

potency of the compounds relative to **2b** (50 μ M). Conversely, trends in the CDCA methyl ester series showed that the

Table 2. Inhibitor Constants for Selected Compounds

Number	R ¹	R ²	R ³	K _i (μM) ^a
2a^b	-CO ₂ Me	-OH	-αOH	118.5 ± 22.3
20b^b		-OMe	-βOH	49.2 ± 4.8
21b^b		-OMe	-βOSO ₃ Na	20.0 ± 3.1
37a^b		-OMe	-αOH	17.9 ± 4.6
38b^c		-OMe	-βOSO ₃ Na	5.6 ± 3.3

^aData are averages of three experiments ± SEM. ^bEvaluated at 0.2 mM. ^cEvaluated at 0.05 mM.

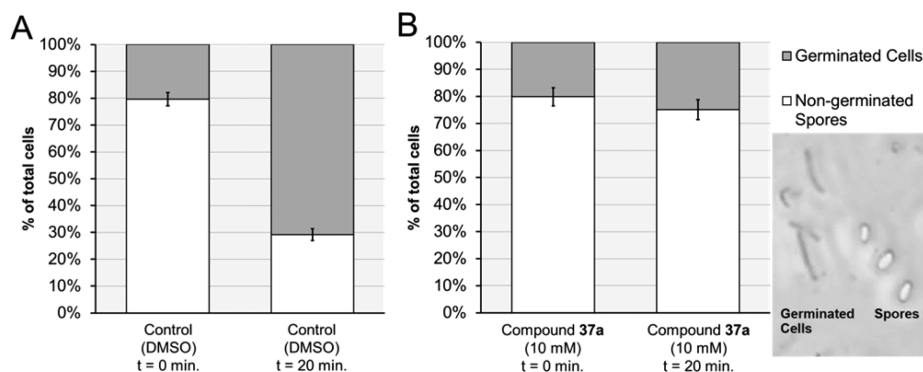


Figure 7. Cell count using phase-contrast microscopy to determine percentage of spore germination over 20 min. (A) Under the conditions of the control experiment (BHIS, 2000 μM TCA, and DMSO), the number of germinated spores rose from 20% to 71% over 20 min. (B) In contrast, far fewer spores germinated under the same conditions in the presence of 10 μM of 37a (from 20% to 25%). Inset: Phase-contrast microscope image of germinated cells (phase dark) and spores (phase bright).

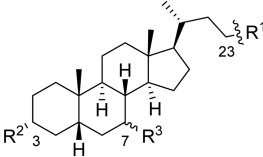
inclusion of a methoxy group at C3, C7, or C3/C7 had little to no effect on potency.



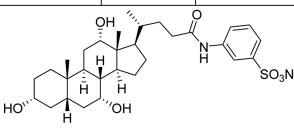
In the UDCA carboxylic acid series, the 3,7-dimethoxy compound **6b** did not inhibit spore germination at the highest concentration tested (1000 μM), whereas **1b** inhibited germination of the NAP1 strain at 500 μM. These results, and the weak inhibition demonstrated by **4b**, suggested that the C7-hydroxyl group may be important to inhibit spore germination in the UDCA carboxylic acid scaffold. The limited number of CDCA carboxylic acid analogues synthesized, and the abnormal concentration response exhibited by analogue **5a** makes it difficult to reach any similar conclusions about the effect of methylation in this series.⁵³

In the UDCA pyrrolidine amide series, 3,7-dimethoxy pyrrolidine amide **19b** was a less potent inhibitor (500 μM) than the monosubstituted C3 methoxy compound **20b** (25 μM) or diol **18b** (100 μM). Trends in the CDCA pyrrolidine amide series suggest that inclusion of a C7-methoxy substituent in **19a** (500 μM) decreases potency relative to the C7-hydroxyl group in **18a** or **20a** (50 and 100 μM, respectively).

Methylation of the C3-hydroxyl group in the tetrazole series did not seem to significantly affect the potency of the compounds for either **37a** or **37b** (5 and 25 μM, respectively) compared to **35a** or **35b**.

Effect of C7-Sulfation. As discussed above, the C7-sulfated analogue of UDCA (**4b**) is gut restricted but is a weaker germination inhibitor than UDCA (**1b**). With this in mind, we prepared C7-sulfated derivatives of some of our best inhibitors to determine whether potentially gut-restricted compounds that maintained good potency could be identified. Our results showed that this is indeed the case, as the pyrrolidine amide **20b** and its C7-sulfated analogue **21b** both showed similar potency in the optical density assay (25 and 50 μM, respectively). For the tetrazole series, a similar drop in potency was observed for the C7-sulfate **38a** (25 μM) compared to **37a** (5 μM). However, the UDCA tetrazole series showed a similar potency for compounds **37b** (25 μM) and **38b** (10 μM). These results confirm that by optimizing the rest of the scaffold, C7-sulfated analogues that are more potent inhibitors of spore germination than **4b** could be identified.

Table 3. Percent Germination of NAP1 Spores in the Presence of 2000 μ M TCA and 10 μ M Bile Acid Analogues after 20 min


Number	R ¹	R ²	R ³	Percent Spore Germination ^a		
				t ₀	t ₂₀	Relative to control ^b
2a	-CO ₂ Me	-OH	-αOH	25 ± 6	71 ± 1	73 ± 1
6b	-CO ₂ H	-OMe	-βOMe	25 ± 4	71 ± 2	75 ± 2
20b		-OMe	-βOH	18 ± 3	33 ± 6	15 ± 6
21b		-OMe	-βOSO ₃ Na	18 ± 2	30 ± 6	9 ± 5
35a		-OH	-αOH	15 ± 6	22 ± 6	12 ± 6
35b		-OH	-βOH	17 ± 4	29 ± 4	19 ± 4
37a		-OMe	-αOH	16 ± 4	21 ± 4	8 ± 4
37b		-OMe	-βOH	16 ± 4	31 ± 4	26 ± 4
38a		-OMe	-αOSO ₃ Na	20 ± 11	48 ± 11	51 ± 11
38b		-OMe	-βOSO ₃ Na	21 ± 3	44 ± 3	42 ± 3
3				25 ± 2	74 ± 2	78 ± 2

^aTotal germinated cells and nongerminated spores were counted at the initial time and after 20 min and reported as a percentage of the total cell count. BHIS + TCA control had 18–25% germination at t₀. Experiments were performed in triplicate (N = 3). Data represent mean ± standard deviation. ^bPercent germination (t₂₀ - t₀) with test compound/percent germination (t₂₀ - t₀) with control.

Determination of Inhibitor Constants. The optical density assay can be used to determine an inhibitor constant (K_i) when it is run with varying germinant concentrations while holding the inhibitor concentration constant (see Figures S56–S60 in the Supporting Information for a detailed description).²⁴ Selected compounds were evaluated at either 0.05 or 0.2 mM in this assay with varying concentrations of TCA and each experiment was repeated three times (Table 2). The results of this assay were similar to those obtained from the optical density assay using varying concentrations of inhibitor and a constant germinant concentration (Table 1), with tetrazole derivatives 37a and 37b showing greater potency than amides 20b and 21b, which in turn were more potent than ester derivative 2a. The results also confirmed that potent C7-sulfated derivatives could be identified.

Microscope Spore Count Assay. While the optical density assay allowed a large number of compounds to be evaluated at multiple concentrations in a reasonable amount of time, several compounds behaved unusually in this assay, most likely due to solubility issues. Therefore, a phase-contrast assay was used to directly confirm that the most potent analogues identified in Table 1 were actually inhibiting *C. difficile* spore germination. In this assay, spores appeared phase bright under the phase-contrast microscope, while germinated and vegetative cells

appeared phase dark (Figure 7). The percentage of germinated cells ranged from 15 to 25% in both the BHIS + TCA control plates and BHIS + TCA + test compound plates at time = 0 min. In a typical assay, an additional 54–63% of spores germinated after 20 min in the absence of any inhibitor (see Supporting Information for assay results for all compounds evaluated).

In Table 3, the percent of total spores germinated at t₀ was compared to the percent of total cells germinated at t₂₀ when spores were exposed to 2000 μ M TCA and bile acid analogues (10 μ M). Each experiment was repeated three times. Substantial germination of spores was observed for 2a,²⁴ 3,²⁹ and 6b at 10 μ M (73%, 78%, and 75% germinated cells relative to control, respectively), which was consistent with the data from the optical density assay and suggested that these compounds are poor inhibitors of *C. difficile* spore germination in this NAP1 strain. The pyrrolidine amides 20b and 21b had similar strong inhibitory effects on spore germination at this concentration (15% and 9% germinated cells, respectively). At a higher concentration (50 μ M), compound 21b showed complete inhibition of spore germination (see Table S1 in the Supporting Information). Tetrazoles 35a (12% germinated cells) and 35b (19% germinated cells) were similar in potency to the pyrrolidine amides. Methylation of the C3-hydroxyl

group had little effect on the inhibitory activity of the compounds: compound **37b** was slightly less potent (26% germinated cells) compared to the C3-hydroxyl compound **35b** (19% germinated cells), while **37a** showed a slight increase in potency (8% germinated cells) compared to **35a** (12% germinated cells). Unlike the similar activities observed for **20b** and **21b**, sulfation of the C7-hydroxyl group in the tetrazole series had a larger effect on potency, as compounds **38b** and **38a** were much less inhibitory at 10 μM than the other tetrazole analogues (42% and 51% germinated cells, respectively). The similar inhibition of spore germination for **20b** and its C7-sulfated derivative **21b** was unlike what was observed with **1b** and its C7-sulfated derivative **4b**, and **21b** may be a promising lead for further development due to its potency.

Cell Permeability. UDCA (**1b**) is a moderate inhibitor of *C. difficile* spore germination. However, it is both passively and actively absorbed from the intestine, resulting in much lower concentrations of **1b** in the colon than in the upper digestive tract.^{54,55} As discussed in the Introduction, we hypothesized that we could greatly reduce both the active and passive absorption of our bile acid analogues by incorporating a C7-sulfate group. To test this theory, compound **20b** and its sulfated derivative **21b** were selected for comparison in an in vitro Caco-2 model of intestinal epithelial permeability (Table 4). At 10 μM , **20b** was moderately permeable ($P_{\text{app}} = 6.1 \times 10^{-6}$ cm/s).

Table 4. In Vitro Absorption of Bile Acids at 10 μM in a Caco-2 Cell Monolayer Bidirectional Permeability Assay

compd	mean permeability A–B (10^{-6} cm/s)	mean permeability B–A (10^{-6} cm/s)
20b	6.1	0.5
21b	0.1	2.1
37a	10.7	1.9
38a	0.4	0.4

10^{-6} cm/s) in the apical to basolateral (A–B) direction and had low permeability ($P_{\text{app}} = 0.5 \times 10^{-6}$ cm/s) in the B–A direction, resulting in a transport ratio of approximately 12. This result suggests that **20b** may be actively transported, which is consistent with an earlier report from Hidalgo and Borchardt in which they concluded that the high transport ratio of TCA (greater than 10) was attributed to the expression of bile acid carriers on the Caco-2 monolayers.⁵⁶ In contrast, the sulfated compound **21b** has much lower permeability ($P_{\text{app}} = 0.1 \times 10^{-6}$ cm/s) in the A–B direction, suggesting that this sulfated derivative is unlikely to be extensively absorbed from the digestive tract. Additionally, the permeability data suggested that if **21b** is absorbed, it may be transported back into the gut as it is moderately permeable (P_{app} of 2.1×10^{-6} cm/s) in the B–A direction. Tetrazole CDCA derivatives **37a** and **38a** behaved similarly in the Caco-2 model (Table 4), with C7-hydroxy compound **37a** (P_{app} of 10.7×10^{-6} cm/s), demonstrating over 25 times greater permeability in the A–B direction than its C7-sulfated analogue **38a** (P_{app} of 0.4×10^{-6} cm/s).

CONCLUSIONS

We have identified several bile acid analogues that are potent inhibitors of *C. difficile* spore germination in a highly virulent NAP1 strain. Some of the most potent compounds include C24 amide or tetrazole derivatives of UDCA. Substitutions at C3 were well tolerated, while C7 substitutions generally decreased

the inhibitory activity of the compounds. To limit intestinal absorption, C7-sulfated analogues designed to avoid both active and passive transport pathways were prepared. One of these derivatives, compound **21b**, was found to be both a potent inhibitor of *C. difficile* spore germination and poorly permeable in a Caco-2 model of intestinal epithelial absorption, suggesting that it is likely to be gut-restricted. These compounds work by a different mechanism of action than existing medications such as vancomycin or fidaxomicin, which do not inhibit spore germination.⁵⁷ In future studies, we will evaluate the efficacy of our best compounds at preventing R-CDI in an animal model and investigate their effects on spore formation, outgrowth of vegetative cells, and toxin formation. We will also examine the effects of our bile acid derivatives on the indigenous gut microbiota. Our ultimate goal is to identify and clinically develop new bile acid inhibitors capable of preventing recurrent CDI in vulnerable patients.

EXPERIMENTAL SECTION

Chemistry. NMR spectra were recorded using a Bruker 400 spectrometer. ^1H NMR data are reported as follows: chemical shift in parts per million downfield of tetramethylsilane (TMS) or relative to the residual solvent peak, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, quint = quintet and m = multiplet), coupling constant (Hz), and integrated value. Unless otherwise specified, all materials, reagents, and solvents were obtained from commercial suppliers and were used without further purification. The progress of a synthetic procedure was monitored, where possible, by thin layer chromatography (TLC), and the compounds of interest were visualized using PMA/Ce(SO₄)₂ stain. TLC was conducted on silica gel 250 μm , F254 plates. Flash column chromatography was performed using Teledyne-Isco Combiflash Rf+PurIon with Redisep Rf silica gel columns. The purity of the compounds evaluated in the phase-contrast spore count assay was determined to be >95% by LC-MS/UV/ELSD analysis.

Methyl (R)-4-((3R,5S,7S,8R,9S,10S,13R,14S,17R)-3,7-Dihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate (2b). To a solution of UDCA (1.00 g, 2.55 mmol) in MeOH (26 mL) was added pTSA·H₂O (0.048 g, 0.26 mmol) and the reaction stirred at room temperature for 24 h. The reaction was quenched by the addition of saturated aqueous NaHCO₃ (5 mL), and the solvent was removed by rotary evaporation. The residue was partitioned between saturated aqueous NaHCO₃ and EtOAc, and the aqueous layer was extracted with EtOAc (3 \times 15 mL). The combined organic layers were washed with saturated aqueous NaHCO₃ (3 \times 10 mL) and water (5 mL), dried over MgSO₄, filtered, and concentrated to obtain **2b** (0.933 g, 90% yield) as a white foam. ^1H NMR (400 MHz, CDCl₃) δ 3.67 (s, 3H), 3.60 (tt, J = 10.4, 4.6 Hz, 2H), 2.37 (ddd, J = 15.3, 10.1, 5.0 Hz, 1H), 2.23 (ddd, J = 15.6, 9.5, 6.5 Hz, 1H), 2.01 (dt, J = 12.5, 3.2 Hz, 1H), 1.95–1.88 (m, 1H), 1.86–1.75 (m, 4H), 1.72–1.65 (m, 2H), 1.65–1.57 (m, 2H), 1.57–1.50 (m, 2H), 1.51–1.40 (m, 6H), 1.40–1.21 (m, 5H), 1.20–0.98 (m, 3H), 0.96 (s, 3H), 0.94 (d, J = 6.4 Hz, 3H), 0.69 (s, 3H). ^{13}C NMR (100 MHz, CDCl₃) δ 174.7, 71.4, 71.3, 55.7, 54.9, 51.5, 43.8, 43.8, 42.4, 40.1, 39.2, 37.3, 36.8, 35.3, 34.9, 34.1, 31.1, 31.0, 30.3, 28.6, 26.9, 23.4, 21.2, 18.4, 12.1. HRMS (ESI): m/z calcd C₂₅H₄₂NO₄Na (M + Na⁺) 429.2981, found 429.2965. Compound **2a** was prepared by the same method. Data for methyl (R)-4-((3R,5S,7R,8R,9S,10S,13R,14S,17R)-3,7-dihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate (**2a**): ^1H NMR (400 MHz, CDCl₃) δ 3.85 (q, J = 3.0 Hz, 1H), 3.67 (s, 3H), 3.46 (tt, J = 11.1, 4.4 Hz, 1H), 2.36 (ddd, J = 15.3, 10.1, 5.1 Hz, 1H), 2.29–2.14 (m, 3H), 2.03–1.05 (m, 22H), 1.00 (dd, J = 14.3, 3.3 Hz, 1H), 0.95–0.91 (m, 1H), 0.93 (d, J = 6.4 Hz, 3H), 0.91 (s, 3H). ^{13}C NMR (100 MHz, CDCl₃) δ 174.7, 72.0, 68.5, 55.8, 51.5, 50.5, 42.7, 41.5, 39.9, 39.6, 39.4, 35.4, 35.3, 35.0, 34.6, 32.8, 31.0, 31.0, 30.7, 28.1, 23.7, 22.8, 20.6, 18.3, 11.8. HRMS (ESI): m/z calcd C₂₅H₄₂NO₄Na (M + Na⁺) 429.2981, found 429.2961.

(R)-4-((3R,5R,7S,8R,9S,10S,13R,14S,17R)-7-Hydroxy-3-methoxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoic Acid (**5b**) and (R)-4-((3R,5S,7S,8R,9S,10S,13R,14S,17R)-3,7-Dimethoxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoic Acid (**6b**). To a solution of **2b** (0.660 g, 1.62 mmol) in THF (8.1 mL) was added NaH (60% dispersion in mineral oil, 0.143 g, 3.57 mmol) and the reaction stirred at room temperature for 15 min. MeI (0.213 mL, 3.41 mmol) was added dropwise, and the mixture stirred for 3 h. The reaction was quenched by the addition of saturated aqueous NH_4Cl (5 mL), and the aqueous layer was extracted with EtOAc (3×10 mL). The combined organic layers were washed with water (10 mL), dried over MgSO_4 , filtered, and concentrated. The crude material was purified by flash column chromatography on silica gel (25–67% EtOAc in hexanes) to obtain **6b** (0.018 g, 3% yield), followed by the elution of **5b** (0.224 g, 34% yield). Data for **6b**: ^1H NMR (400 MHz, CDCl_3) δ 3.35 (s, 3H), 3.23 (s, 3H), 3.13 (qd, $J = 10.2, 9.5, 4.1$ Hz, 1H), 2.99 (dq, $J = 9.0, 5.0$ Hz, 1H), 2.39 (ddd, $J = 15.3, 10.2, 4.9$ Hz, 1H), 2.24 (ddd, $J = 15.5, 9.5, 6.3$ Hz, 1H), 1.96 (dt, $J = 12.7, 3.3$ Hz, 1H), 1.89–0.95 (m, 22H), 0.93 (s, 3H), 0.92 (d, $J = 5.7$ Hz, 3H), 0.84 (ddd, $J = 13.1, 9.5, 7.1$ Hz, 1H), 0.65 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 179.7, 80.6, 80.3, 56.3, 55.8, 55.6, 55.4, 43.8, 42.4, 41.7, 40.4, 39.5, 35.5, 35.1, 34.6, 33.9, 32.3, 31.2, 31.1, 28.8, 26.8, 26.6, 23.6, 21.5, 18.6, 12.4. HRMS (ESI): m/z calcd $\text{C}_{26}\text{H}_{44}\text{NaO}_4$ ($M + \text{Na}^+$) 443.3137, found 443.3111. Data for **5b**: ^1H NMR (400 MHz, CDCl_3) δ 3.60 (ddd, $J = 11.5, 8.6, 5.1$ Hz, 1H), 3.35 (s, 3H), 3.13 (tt, $J = 10.4, 4.8$ Hz, 1H), 2.40 (ddd, $J = 15.3, 10.1, 5.0$ Hz, 1H), 2.25 (ddd, $J = 15.8, 9.5, 6.5$ Hz, 1H), 2.05–1.03 (m, 23H), 1.00 (dd, $J = 14.5, 3.0$ Hz, 1H), 0.95 (s, 3H), 0.93 (d, $J = 7.12$ Hz, 3H), 0.68 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 179.4, 80.2, 71.5, 55.8, 55.7, 55.1, 43.9, 43.9, 42.5, 40.3, 39.2, 37.0, 35.4, 35.0, 34.5, 33.8, 31.1, 31.0, 28.8, 27.0, 26.7, 23.6, 21.3, 18.5, 12.3. HRMS (ESI): m/z calcd $\text{C}_{25}\text{H}_{42}\text{NO}_4\text{Na}$ ($M + \text{Na}^+$) 429.2981, found 429.2960. Compound **6a** was obtained in trace amounts and was not evaluated in the spore germination assays. Compound **5a** was prepared in the same manner as **5b**. Data for (R)-4-((3R,5R,7R,8R,9S,10S,13R,14S,17R)-7-hydroxy-3-methoxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoic acid (**5a**): ^1H NMR (400 MHz, CDCl_3) δ 3.84 (d, $J = 3.2$ Hz, 1H), 3.34 (s, 3H), 3.02 (td, $J = 10.9, 5.4$ Hz, 1H), 2.40 (ddd, $J = 15.5, 10.2, 5.1$ Hz, 1H), 2.25 (ddd, $J = 15.8, 9.7, 6.4$ Hz, 1H), 2.18–1.02 (m, 23H), 0.99 (d, $J = 3.3$ Hz, 1H), 0.94 (d, $J = 6.4$ Hz, 3H), 0.90 (s, 3H), 0.66 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 178.5, 80.8, 68.7, 56.0, 55.7, 50.6, 42.9, 41.6, 39.9, 39.7, 36.0, 35.6, 35.6, 35.5, 34.9, 33.0, 31.0, 30.9, 28.4, 27.2, 23.9, 23.1, 20.8, 18.5, 12.0. HRMS (ESI): m/z calcd $\text{C}_{25}\text{H}_{42}\text{NO}_4\text{Na}$ ($M + \text{Na}^+$) 429.2981, found 429.2998.

Methyl (R)-4-((3R,5S,7S,8R,9S,10S,13R,14S,17R)-3,7-Dimethoxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate (**7b**) and Methyl (R)-4-((3R,5R,7S,8R,9S,10S,13R,14S,17R)-7-Hydroxy-3-methoxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate (**8b**). To a solution of **2b** (0.260 g, 0.639 mmol) and 2,6-di-*tert*-butylpyridine (0.29 mL, 1.3 mmol) in DCM (6.4 mL) was added methyl triflate (approximately 0.1 mL). The reaction stirred at room temperature for 24 h and was quenched by the addition of 1 M aqueous HCl (5 mL). The aqueous layer was extracted with DCM (3×10 mL), and the combined organic layers were washed with saturated aqueous NaHCO_3 (2×10 mL), dried over MgSO_4 , filtered, and concentrated. The crude material was purified by flash column chromatography on silica gel (0–50% EtOAc in DCM) to obtain **7b** (0.147 g, 53% yield) as a clear colorless oil, followed by the elution of **8b** (0.054 g, 21% yield) as clear, colorless oil that solidified over time to a white amorphous solid. Data for **7b**: ^1H NMR (400 MHz, CDCl_3) δ 3.67 (s, 3H), 3.36 (s, 3H), 3.24 (s, 3H), 3.13 (tt, $J = 10.2, 4.4$ Hz, 1H), 2.99 (ddd, $J = 11.3, 8.8, 5.0$ Hz, 1H), 2.36 (ddd, $J = 15.2, 10.2, 4.9$ Hz, 1H), 2.22 (ddd, $J = 15.6, 9.6, 6.4$ Hz, 1H), 1.98 (dt, $J = 12.8, 3.3$ Hz, 1H), 1.90–1.71 (m, 6H), 1.71–1.61 (m, 2H), 1.53–1.37 (m, 7H), 1.37–1.30 (m, 1H), 1.30–1.09 (m, 5H), 1.09–0.97 (m, 2H), 0.94 (s, 3H), 0.92 (d, $J = 6.4$ Hz, 3H), 0.66 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 175.0, 80.6, 80.2, 56.4, 55.8, 55.7, 55.4, 51.7, 43.8, 42.4, 41.7, 40.5, 39.5, 35.6, 35.1, 34.6, 34.0, 32.3, 31.3, 31.3, 28.8, 26.9, 26.6, 23.6, 21.5, 18.6, 12.4. HRMS (ESI): m/z calcd $\text{C}_{27}\text{H}_{46}\text{NaO}_4$ (M

+ Na^+) 457.3294, found 457.3278. Data for **8b**: ^1H NMR (400 MHz, CDCl_3) δ 3.66 (s, 3H), 3.58 (s, 3H), 3.34 (s, 3H), 3.11 (tt, $J = 10.6, 4.5$ Hz, 1H), 2.36 (ddd, $J = 15.3, 10.2, 5.0$ Hz, 1H), 2.22 (ddd, $J = 15.6, 9.5, 6.5$ Hz, 1H), 2.02–0.96 (m, 24H), 0.94 (s, 3H), 0.92 (d, $J = 6.4$ Hz, 3H), 0.67 (s, 3H). ^{13}C NMR (400 MHz, $\text{DMSO}-d_6$) δ 3.87 (d, $J = 6.9$ Hz, 1H), 3.57 (s, 3H), 3.31–3.22 (m, 1H), 3.20 (s, 3H), 3.05 (tt, $J = 9.7, 5.3$ Hz, 1H), 2.32 (ddd, $J = 15.2, 9.7, 5.2$ Hz, 1H), 2.20 (ddd, $J = 15.7, 9.3, 6.8$ Hz, 1H), 1.91 (dd, $J = 11.4, 3.6$ Hz, 1H), 1.88–1.79 (m, 1H), 1.78–1.59 (m, 6H), 1.47–1.28 (m, 7H), 1.27–1.09 (m, 6H), 1.06–0.88 (m, 3H), 0.88 (s, 3H), 0.86 (d, $J = 6.8$ Hz, 3H), 0.61 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 174.9, 80.2, 71.6, 55.9, 55.8, 55.1, 51.7, 44.0, 44.0, 42.6, 40.3, 39.3, 37.2, 35.5, 35.1, 34.6, 33.9, 31.3, 31.2, 28.8, 27.1, 26.9, 23.6, 21.4, 18.6, 12.3. HRMS (ESI): m/z calcd $\text{C}_{26}\text{H}_{44}\text{NO}_4\text{Na}$ ($M + \text{Na}^+$) 443.3137, found 443.3146. Compounds **7a** and **8a** were prepared by the same method. Data for methyl (R)-4-((3R,5S,7R,8R,9S,10S,13R,14S,17R)-3,7-dimethoxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate (**7a**): ^1H NMR (400 MHz, CDCl_3) δ 3.66 (s, 3H), 3.33 (s, 3H), 3.23 (s, 3H), 3.17 (q, $J = 2.9$ Hz, 1H), 3.01 (tt, $J = 11.2, 4.3$ Hz, 1H), 2.35 (ddd, $J = 15.2, 10.2, 5.0$ Hz, 1H), 2.29–2.08 (m, 23H), 1.97–1.66 (m, 8H), 1.62 (ddd, $J = 15.0, 5.4, 3.2$ Hz, 1H), 1.57–1.39 (m, 5H), 1.37–1.10 (m, 8H), 1.08–0.99 (m, 1H), 0.92 (d, $J = 6.4$ Hz, 3H), 0.90 (s, 3H), 0.63 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 175.0, 80.8, 80.8, 68.7, 55.9, 55.7, 51.7, 50.6, 42.9, 41.6, 39.8, 39.7, 36.1, 35.6, 35.6, 35.5, 34.9, 33.0, 31.2, 31.2, 28.4, 27.2, 23.9, 23.1, 20.8, 18.5, 12.0. HRMS (ESI): m/z calcd $\text{C}_{27}\text{H}_{46}\text{NaO}_4$ ($M + \text{Na}^+$) 457.3294, found 457.3290. Data for methyl (R)-4-((3R,5R,7R,8R,9S,10S,13R,14S,17R)-7-hydroxy-3-methoxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate (**8a**): ^1H NMR (400 MHz, CDCl_3) δ 3.84 (q, $J = 3.0$ Hz, 1H), 3.67 (s, 3H), 3.35 (s, 3H), 3.02 (tt, $J = 11.1, 4.3$ Hz, 1H), 2.36 (ddd, $J = 15.3, 10.1, 5.2$ Hz, 1H), 2.23 (ddd, $J = 15.6, 9.6, 6.6$ Hz, 1H), 2.12 (td, $J = 13.2, 11.2$ Hz, 1H), 2.05–1.70 (m, 8H), 1.64 (dddd, $J = 12.5, 9.9, 7.1, 2.9$ Hz, 1H), 1.53–1.05 (m, 13H), 0.97 (dd, $J = 15.2, 4.3$ Hz, 1H), 0.93 (d, $J = 6.5$ Hz, 3H), 0.91 (s, 3H), 0.66 (s, 3H). ^{13}C NMR (400 MHz, $\text{DMSO}-d_6$) δ 4.10 (d, $J = 3.4$ Hz, 1H), 3.62 (q, $J = 3.1$ Hz, 1H), 3.57 (s, 3H), 3.20 (s, 3H), 2.92 (tt, $J = 11.1, 4.3$ Hz, 1H), 2.32 (ddd, $J = 15.2, 9.6, 5.3$ Hz, 1H), 2.26–2.09 (m, 2H), 1.94–1.86 (m, 1H), 1.86–1.57 (m, 9H), 1.46–1.31 (m, 5H), 1.29–1.15 (m, 4H), 1.15–1.03 (m, 3H), 0.99 (dq, $J = 11.8, 5.7$ Hz, 1H), 0.88 (d, $J = 6.4$ Hz, 3H), 0.85 (s, 3H), 0.60 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 175.0, 80.8, 68.7, 55.9, 55.7, 51.7, 50.6, 42.9, 41.6, 39.8, 39.7, 36.1, 35.6, 35.6, 35.5, 34.9, 33.0, 31.2, 31.2, 28.4, 27.2, 23.9, 23.1, 20.8, 18.5, 12.0. HRMS (ESI): m/z calcd $\text{C}_{26}\text{H}_{44}\text{NO}_4\text{Na}$ ($M + \text{Na}^+$) 443.3137, found 443.3121.

(R)-4-((3R,5S,7S,8R,9S,10S,13R,14S,17R)-3-Hydroxy-7-methoxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoic Acid (**10b**). To a solution of **9b** (0.300 g, 0.483 mmol) and 2,6-di-*tert*-butylpyridine (0.22 mL, 0.97 mmol) in DCM (5 mL) was methyl triflate (0.06 mL, 0.5 mmol). The reaction stirred at room temperature for 24 h and was quenched by the addition of 1 M aqueous HCl (5 mL). The aqueous layer was extracted with DCM (3×10 mL), and the combined organic layers were washed with saturated aqueous NaHCO_3 (2×10 mL), dried over MgSO_4 , filtered, and concentrated. The crude material was purified by flash column chromatography on silica gel (0–50% EtOAc in DCM) to obtain *tert*-butyldimethylsilyl (R)-4-((3R,5R,7S,8R,9S,10S,13R,14S,17R)-3-((*tert*-butyldimethylsilyl)oxy)-7-methoxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate (0.207 g, 68% yield) as a white amorphous solid. ^1H NMR (400 MHz, CDCl_3) δ 3.54 (tt, $J = 10.2, 4.7$ Hz, 1H), 3.24 (s, 3H), 3.01 (ddd, $J = 11.0, 9.1, 5.0$ Hz, 1H), 2.36 (ddd, $J = 15.2, 9.9, 5.1$ Hz, 1H), 2.22 (ddd, $J = 15.6, 9.4, 6.6$ Hz, 1H), 1.97 (dt, $J = 12.5, 3.1$ Hz, 1H), 1.90–0.97 (m, 23H), 0.94 (s, 9H), 0.93 (d, $J = 6.4$ Hz, 3H), 0.92 (s, 3H), 0.90 (s, 9H), 0.66 (s, 3H), 0.27 (s, 3H), 0.27 (s, 3H), 0.07 (s, 3H), 0.07 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 174.9, 80.5, 77.4, 72.6, 56.2, 55.6, 55.4, 43.8, 42.5, 41.7, 40.4, 39.4, 38.0, 35.4, 35.3, 34.2, 33.3, 32.3, 31.4, 31.0, 28.7, 26.5, 26.1, 26.1, 25.7, 25.7, 23.5, 21.5, 18.6, 18.5, 17.8, 12.3, –4.4, –4.5, –4.6, –4.6. To *tert*-butyldimethylsilyl (R)-4-((3R,5R,7S,8R,9S,10S,13R,14S,17R)-3-((*tert*-butyldimethylsilyl)oxy)-7-methoxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]

phenanthren-17-yl)pentanoate was added TBAF (0.58 mL, 0.58 mmol) in THF (2.7 mL) and the reaction mixture stirred for 3 h. Additional TBAF (0.58 mL, 0.58 mmol) was added, and the reaction mixture stirred at room temperature for 20 h. The solvent was evaporated, and the crude material was purified by flash column chromatography on silica gel (0–10% MeOH in DCM) to obtain **10b** (0.094 g, 84% yield). ¹H NMR (400 MHz, CDCl₃) δ 3.61 (tt, *J* = 10.5, 4.7 Hz, 1H), 3.25 (s, 3H), 3.01 (td, *J* = 12.9, 11.0, 4.9 Hz, 2H), 2.41 (ddd, *J* = 15.2, 10.2, 4.9 Hz, 1H), 2.27 (ddd, *J* = 15.6, 9.5, 6.3 Hz, 1H), 2.02–1.94 (m, 1H), 1.89–1.01 (m, 22H), 0.95 (d, *J* = 6.4 Hz, 3H), 0.94 (s, 3H), 0.67 (s, 3H). ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.94 (s, 1H), 4.45 (d, *J* = 4.5 Hz, 1H), 3.22 (d, *J* = 79.8 Hz, 6H), 2.94 (td, *J* = 10.3, 5.0 Hz, 2H), 2.23 (ddd, *J* = 15.2, 9.7, 5.3 Hz, 1H), 2.15–2.04 (m, 1H), 1.93 (dd, *J* = 11.6, 3.7 Hz, 1H), 1.82–1.64 (m, 3H), 1.64–1.52 (m, 2H), 1.52–1.45 (m, 2H), 1.36 (dddd, *J* = 26.7, 20.5, 12.5, 8.3 Hz, 8H), 1.26–1.09 (m, 5H), 0.88 (d, *J* = 6.7 Hz, 3H), 0.86 (s, 3H), 0.61 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 178.7, 80.6, 71.8, 56.4, 55.7, 55.4, 43.9, 42.4, 41.7, 40.5, 39.6, 37.5, 35.5, 35.1, 34.3, 32.2, 31.1, 30.6, 26.6, 25.5, 23.6, 20.3, 18.6, 13.8, 12.4. HRMS (ESI): *m/z* calcd C₂₅H₄₂NO₄Na (M + Na⁺) 429.2981, found 429.2965. Compound **10a** was not synthesized (see Scheme S2 for the synthetic route to **11a** in the Supporting Information).

Methyl (R)-4-((3R,5S,7S,8R,9S,10S,13R,14S,17R)-3-Hydroxy-7-methoxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate (11b). To a solution of **10b** (50 mg, 0.12 mmol) in MeOH (2.6 mL) was added pTSA·H₂O (0.050 g, 0.26 mmol) and the reaction stirred at room temperature for 24 h. The solvent was removed by rotary evaporation. The crude material was purified by flash column chromatography on silica gel (33% EtOAc in DCM as eluent) to obtain **11b** (0.031 g, 60% yield) as a white amorphous solid. ¹H NMR (400 MHz, CDCl₃) δ 3.67 (s, 3H), 3.59 (tt, *J* = 10.5, 4.7 Hz, 1H), 3.24 (s, 3H), 2.99 (ddd, *J* = 11.3, 9.0, 5.2 Hz, 1H), 2.36 (ddd, *J* = 15.2, 10.3, 4.9 Hz, 1H), 2.22 (ddd, *J* = 15.5, 9.6, 6.4 Hz, 1H), 1.98 (dt, *J* = 12.5, 3.3 Hz, 1H), 1.90–0.97 (m, 23H), 0.93 (s, 3H), 0.92 (d, *J* = 6.4 Hz, 3H), 0.66 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 175.0, 80.5, 71.7, 56.4, 55.4, 51.7, 43.8, 42.4, 41.7, 40.5, 39.6, 37.5, 35.5, 35.1, 34.3, 32.2, 31.3, 30.6, 28.7, 26.6, 23.5, 21.5, 18.6, 12.4. HRMS (ESI): *m/z* calcd C₂₆H₄₄NaO₄ (M + Na⁺) 443.3137, found 443.3119. Compound **11a** was prepared by a different method than **11b** (see Scheme S2 in the Supporting Information).

Methyl (R)-4-((3R,5S,7R,8R,9S,10S,13R,14S,17R)-3,7-Dihydroxy-7,10,13-trimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate (13) and **(3R,5S,7R,8R,9S,10S,13R,14S,17R)-17-((R)-5-Hydroxy-5-methylhexan-2-yl)-7,10,13-trimethylhexadecahydro-1H-cyclopenta[a]phenanthrene-3,7-diol (14)**. 3- α -7-oxo CDCA (0.800 g, 2.05 mmol) was dissolved in MeOH (20 mL), and pTSA·H₂O (0.051 g, 0.27 mmol) was added. The reaction mixture stirred at room temperature for 12 h, and the solvent was removed under reduced pressure. The crude material was purified by flash column chromatography on silica gel (0–40% EtOAc in DCM) to obtain the methyl ester (0.671 g, 81% yield) as a white foam. ¹H NMR (400 MHz, CDCl₃) δ 3.67 (s, 3H), 3.61 (dq, *J* = 10.7, 5.1 Hz, 1H), 2.86 (dd, *J* = 12.5, 6.0 Hz, 1H), 2.37 (qd, *J* = 10.7, 9.9, 6.2 Hz, 2H), 2.29–2.12 (m, 2H), 2.04–1.22 (m, 16H), 1.20 (s, 3H), 1.19–1.06 (m, 3H), 0.98 (dd, *J* = 12.3, 6.3 Hz, 1H), 0.93 (d, *J* = 6.3 Hz, 3H), 0.94–0.93 (m, 1H), 0.66 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 212.1, 174.9, 71.2, 55.0, 51.7, 49.7, 49.1, 46.3, 45.6, 42.9, 42.9, 39.2, 37.6, 35.4, 35.4, 34.4, 31.3, 31.2, 30.1, 28.5, 25.0, 23.3, 21.9, 18.6, 12.3. To a solution of the methyl ester (0.671 g, 1.66 mmol) in Et₂O (17 mL) at room temperature was added MeMgBr (1.6 mL, 4.9 mmol) dropwise. The reaction was stirred for 2 h and was quenched by the addition of 1 M aqueous HCl (10 mL). The aqueous layer was extracted with Et₂O (3 × 10 mL), and the combined organic layers were dried over MgSO₄, filtered, and concentrated. The crude material was purified by flash column chromatography on silica gel (0–100% EtOAc in DCM) to obtain recovered starting material (160 mg, 24% recovery), followed by the elution of **13** (0.107 g, 15% yield) and **14** (0.130 g, 19% yield) as white foams. Data for **13**: ¹H NMR (400 MHz, DMSO-*d*₆) δ 4.30 (s, 1H), 3.57 (s, 3H), 3.36 (d, *J* = 18.1 Hz, 1H), 3.21 (tt, *J* = 10.3, 4.2 Hz, 1H), 2.32 (ddd, *J* = 15.2, 9.7, 5.2 Hz, 1H), 2.26–2.12 (m, 2H),

1.97–0.93 (m, 25H), 0.89 (d, *J* = 6.3 Hz, 3H), 0.85 (d, *J* = 2.8 Hz, 1H), 0.80 (s, 3H), 0.62 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 173.7, 70.9, 70.9, 70.2, 54.4, 51.2, 50.8, 44.2, 43.3, 43.1, 41.9, 38.2, 35.6, 35.3, 34.8, 34.3, 33.1, 30.6, 30.4, 30.4, 28.0, 27.3, 22.8, 20.8, 18.3, 12.0. HRMS (ESI): *m/z* calcd C₂₆H₄₄NO₄Na (M + Na⁺) 443.3137, found 443.3116. Data for **14**: ¹H NMR (400 MHz, DMSO-*d*₆) δ 4.31 (d, *J* = 4.7 Hz, 1H), 4.01 (s, 1H), 3.65 (s, 1H), 3.32 (s, 3H), 3.20 (tt, *J* = 9.5, 4.7 Hz, 1H), 2.18 (q, *J* = 12.7 Hz, 1H), 1.96–1.06 (m, 23H), 1.05 (s, 3H), 1.04 (s, 3H), 1.04–1.03 (m, 1H), 0.89 (d, *J* = 6.5 Hz, 3H), 0.86 (d, *J* = 2.3 Hz, 1H), 0.80 (s, 3H), 0.62 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 70.9, 70.2, 68.8, 64.9, 54.5, 50.8, 44.2, 43.2, 43.1, 41.9, 38.2, 35.6, 35.5, 35.3, 34.3, 33.2, 30.4, 29.8, 29.5, 29.1, 28.1, 27.3, 22.8, 20.8, 18.9, 15.2, 12.1. HRMS (ESI): *m/z* calcd C₂₇H₄₈NaO₃ (M + Na⁺) 443.3501, found 443.3483.

(R)-4-((3R,5S,7S,8R,9S,10S,13R,14S,17R)-3,7-Diacetoxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoic Acid (15b). UDCA (1.00 g, 2.55 mmol) was dissolved in pyridine (3 mL, 37 mmol) and Ac₂O (2 mL, 21 mmol). The reaction stirred at room temperature 12 h and was diluted with Et₂O (100 mL). The organic layer was washed with 1 M aqueous HCl (3 × 20 mL), dried over Na₂SO₄, filtered, and concentrated. The crude material was purified by flash column chromatography on silica gel (0–100% EtOAc in DCM as eluent) to obtain **15b** (1.19 g, 98% yield) as a white foam. ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.94 (s, 1H), 4.65 (td, *J* = 11.0, 4.9 Hz, 1H), 4.55 (tt, *J* = 11.0, 4.9 Hz, 1H), 2.22 (ddd, *J* = 15.1, 9.6, 5.3 Hz, 1H), 2.09 (ddd, *J* = 15.7, 9.3, 7.1 Hz, 1H), 1.98 (s, 3H), 1.93 (s, 3H), 1.82–0.99 (m, 24H), 0.92 (s, 3H), 0.88 (d, *J* = 6.4 Hz, 3H), 0.63 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 174.8, 169.8, 169.8, 73.0, 72.8, 64.9, 54.4, 54.3, 43.1, 41.2, 38.6, 34.7, 33.9, 33.5, 32.6, 32.5, 30.7, 30.7, 28.0, 26.0, 25.3, 22.8, 21.5, 21.0, 20.8, 18.2, 15.2, 11.8. TLC-MS (ESI): *m/z* calcd C₂₈H₄₄NaO₆ (M + Na⁺) 499.3, found 499.9. Compound **15a** was prepared by the same method. Data for **(R)-4-((3R,5S,7R,8R,9S,10S,13R,14S,17R)-3,7-diacetoxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoic acid (15a)**: ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.94 (s, 1H), 4.76 (t, *J* = 3.0 Hz, 1H), 4.47 (tt, *J* = 11.0, 4.3 Hz, 1H), 2.23 (ddd, *J* = 15.0, 9.6, 5.3 Hz, 1H), 2.10 (ddd, *J* = 15.8, 9.1, 6.9 Hz, 1H), 1.98 (s, 3H), 1.97 (s, 3H), 1.95–1.90 (m, 3H), 1.83–0.95 (m, 21H), 0.90 (s, 3H), 0.88 (d, *J* = 6.7 Hz, 3H), 0.62 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 174.8, 169.8, 169.7, 73.4, 70.5, 64.9, 55.3, 50.0, 42.2, 37.1, 34.8, 34.7, 34.4, 34.3, 34.3, 33.6, 30.8, 30.7, 30.6, 27.5, 26.4, 23.0, 22.3, 21.3, 21.1, 20.2, 18.1, 11.5. TLC-MS (ESI): *m/z* calcd C₂₈H₄₃O₆ (M – H[–]) 475.6, found 475.9.

(3R,5S,7S,8R,9S,10S,13R,14S,17R)-17-((R)-5-Amino-5-oxopentan-2-yl)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthrene-3,7-diyl Diacetate (16b). Compound **15b** (0.840 g, 1.76 mmol) was dissolved in benzene (8.8 mL), and SOCl₂ (0.52 mL, 7.1 mmol) was added dropwise. The reaction was heated to reflux for 4 h and cooled to room temperature. The solvent was removed under reduced pressure, and excess SOCl₂ was removed by coevaporation with benzene (3 × 5 mL). The residue was dissolved in ammonia solution (0.5 M in THF, 15 mL) and stirred for 12 h. The solvent was removed under reduced pressure, and the crude material was purified by flash column chromatography on silica gel (15–100% EtOAc in DCM as eluent) to obtain **16b** (0.581 g, 69% yield) as a white foam. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.20 (s, 1H), 6.63 (s, 1H), 4.66 (td, *J* = 10.7, 4.8 Hz, 1H), 4.55 (tt, *J* = 10.7, 4.8 Hz, 1H), 2.05 (ddd, *J* = 14.6, 9.9, 5.3 Hz, 1H), 1.98 (s, 3H), 1.93 (s, 3H), 1.95–1.89 (m, 1H), 1.86–0.96 (m, 26H), 0.92 (s, 3H), 0.88 (d, *J* = 6.5 Hz, 3H), 0.63 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 174.6, 169.8, 169.8, 73.0, 72.8, 54.9, 54.5, 54.4, 43.1, 41.2, 38.6, 34.8, 33.9, 33.5, 32.7, 32.5, 32.0, 31.4, 28.0, 26.0, 25.3, 22.8, 21.5, 21.0, 20.8, 18.4, 11.8, 0.1. TLC-MS (ESI): *m/z* calcd C₂₈H₄₇NO₆ (M + H₂O⁺) 493.7, found 493.1. Compound **16a** was prepared by the same method. Data for **(3R,5S,7R,8R,9S,10S,13R,14S,17R)-17-((R)-5-amino-5-oxopentan-2-yl)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthrene-3,7-diyl diacetate (16a)**: ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.21 (s, 1H), 6.63 (s, 1H), 4.76 (d, *J* = 3.2 Hz, 1H), 4.47 (dt, *J* = 11.5, 6.7 Hz, 1H), 2.12–1.87 (m, 4H), 1.98 (s, 3H), 1.97 (s, 3H), 1.87–0.96 (m, 21H), 0.91 (s, 3H), 0.89–0.87 (m, 1H), 0.88 (d, *J* = 6.6 Hz, 3H), 0.62 (s,

3H). ^{13}C NMR (100 MHz, CDCl_3) δ 176.0, 170.8, 170.6, 74.3, 71.4, 55.9, 42.8, 41.1, 39.6, 38.0, 35.5, 35.0, 34.9, 34.8, 34.2, 32.9, 31.7, 31.4, 28.2, 26.9, 23.7, 22.8, 21.7, 21.6, 20.8, 18.5, 11.8. TLC-MS (ESI): m/z calcd $\text{C}_{28}\text{H}_{43}\text{O}_6$ ($\text{M} - \text{H}^-$) 475.6, found 475.9.

(*R*)-*N*-Butyl-4-((3*R*,5*S*,7*S*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-3,7-Dihydroxy-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)pentanamide (**17b**). To a solution of UDCA (0.393 g, 1.00 mmol) in THF (10 mL) was added carbonyldiimidazole (0.243 g, 1.50 mmol) and the reaction stirred for 1 h at room temperature. To the solution was added butan-1-amine (0.198 mL, 2.00 mmol) and the reaction stirred for 18 h at room temperature. The reaction was quenched with saturated aqueous NH_4Cl (10 mL). The aqueous and organic layers were separated, and the aqueous layer was extracted with EtOAc (3 \times 10 mL). The combined organic layers were washed with saturated aqueous NH_4Cl (3 \times 5 mL) and water (5 mL), and then dried over MgSO_4 , filtered, and concentrated. The crude material was purified by flash column chromatography on silica gel (2–10% MeOH in EtOAc as eluent) to obtain **17b** (0.162 g, 36% yield) as an off-white amorphous solid. ^1H NMR (400 MHz, CDCl_3) δ 5.45 (s, 1H), 3.59 (tq, J = 10.0, 4.5, 4.1 Hz, 2H), 3.25 (td, J = 7.1, 5.7 Hz, 2H), 2.23 (ddd, J = 15.0, 10.4, 4.8 Hz, 1H), 2.12–0.97 (m, 28H), 0.96–0.90 (m, 9H), 0.68 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 173.8, 72.0, 68.4, 56.1, 50.5, 42.8, 41.7, 39.8, 39.5, 39.3, 35.6, 35.5, 35.2, 34.8, 33.7, 32.9, 32.1, 31.8, 30.7, 29.8, 28.4, 23.8, 22.9, 20.7, 20.2, 18.5, 13.9, 11.9. HRMS (ESI): m/z calcd $\text{C}_{28}\text{H}_{49}\text{NO}_3\text{Na}$ ($\text{M} + \text{Na}^+$) 470.3610, found 470.3615. Compound **17a** was prepared by the same method. Data for (*R*)-*N*-butyl-4-((3*R*,5*S*,7*R*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-3,7-dihydroxy-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)pentanamide (**17a**): ^1H NMR (400 MHz, CDCl_3) δ 5.57 (t, J = 5.8 Hz, 1H), 3.83 (q, J = 2.9 Hz, 1H), 3.44 (tt, J = 11.0, 4.3 Hz, 1H), 3.23 (q, J = 6.7 Hz, 2H), 2.30–1.03 (m, 27H), 1.03–0.83 (m, 11H), 0.65 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 173.8, 72.0, 68.4, 56.1, 50.5, 42.8, 41.7, 39.9, 39.8, 39.5, 39.3, 35.6, 35.5, 35.2, 34.8, 33.7, 32.9, 32.1, 31.8, 30.7, 28.4, 23.8, 22.9, 20.7, 20.2, 18.5, 13.9, 11.9. HRMS (ESI): m/z calcd $\text{C}_{28}\text{H}_{49}\text{NO}_3\text{Na}$ ($\text{M} + \text{Na}^+$) 470.3610, found 470.3614.

(*R*)-4-((3*R*,5*S*,7*S*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-3,7-Dihydroxy-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)-1-(pyrrolidin-1-yl)pentan-1-one (**18b**). To a solution of UDCA (0.500 g, 1.27 mmol), TEA (0.53 mL, 3.8 mmol), and pyrrolidine (0.21 mL, 2.6 mmol) in DCM (13 mL) was added HATU (0.533 g, 1.40 mmol). The reaction mixture was stirred at room temperature for 16 h. The reaction was quenched by the addition of saturated aqueous NaHCO_3 (6 mL), and the aqueous layer was extracted with DCM (3 \times 5 mL). The combined organic layers were dried over MgSO_4 , filtered, and concentrated. The crude material was purified by flash column chromatography on silica gel (2–5% MeOH in EtOAc as eluent) to obtain **18b** (0.495 g, 87% yield) as an off-white foam. ^1H NMR (400 MHz, CDCl_3) δ 3.48 (dp, J = 10.2, 4.2 Hz, 2H), 3.14 (q, J = 6.7 Hz, 2H), 2.12 (ddd, J = 14.9, 10.5, 4.9 Hz, 1H), 2.00–0.86 (m, 26H), 0.86–0.77 (m, 11H), 0.57 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 172.2, 76.7, 71.5, 71.4, 55.7, 55.0, 46.6, 45.6, 43.8, 43.8, 42.4, 40.1, 39.2, 37.3, 36.8, 35.5, 34.9, 34.1, 31.7, 31.0, 30.3, 28.6, 26.9, 26.2, 24.4, 23.4, 21.2, 18.6. HRMS (ESI): m/z calcd $\text{C}_{28}\text{H}_{47}\text{NNaO}_3$ ($\text{M} + \text{Na}^+$) 468.3454, found 468.3479. Compound **18a** was prepared by the same method. Data for (*R*)-4-((3*R*,5*S*,7*R*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-3,7-dihydroxy-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)-1-(pyrrolidin-1-yl)pentan-1-one (**18a**): ^1H NMR (400 MHz, CDCl_3) δ 3.85 (q, J = 3.0 Hz, 1H), 3.44 (dt, J = 13.8, 6.7 Hz, 6H), 2.30 (ddt, J = 16.7, 10.9, 5.5 Hz, 1H), 2.21–1.06 (m, 27H), 1.00 (dd, J = 14.2, 3.3 Hz, 1H), 0.95 (d, J = 6.4 Hz, 3H), 0.91 (s, 3H), 0.67 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 172.2, 72.0, 68.5, 55.8, 50.5, 46.6, 45.6, 42.7, 41.5, 39.9, 39.7, 39.4, 35.5, 35.3, 35.0, 34.6, 32.8, 31.6, 30.9, 30.7, 28.2, 26.2, 24.4, 23.7, 22.8, 20.6, 18.5, 11.8. HRMS (ESI): m/z calcd $\text{C}_{28}\text{H}_{47}\text{NO}_3\text{Na}$ ($\text{M} + \text{Na}^+$) 468.3454, found 468.3454.

(*R*)-4-((3*R*,5*S*,7*S*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-3,7-Dimethoxy-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)-1-(pyrrolidin-1-yl)pentan-1-one (**19b**) and (*R*)-4-((3*R*,5*S*,7*S*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-7-Hydroxy-3-methoxy-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)-1-(pyrrolidin-1-yl)pentan-1-one (**20b**). To a solution of **18b** (0.480 g, 1.08 mmol) in THF (10 mL) was added NaH (60% dispersion in

mineral oil, 0.065 g, 1.6 mmol). The reaction stirred at room temperature for 15 min, and MeI (0.08 mL, 1.3 mmol) was added in one portion. The reaction stirred overnight 13 h and quenched with saturated aqueous NH_4Cl (8 mL). The organic and aqueous layers were separated, and the aqueous layer was extracted with EtOAc (3 \times 10 mL). The combined organic layers were washed with brine (10 mL), dried over MgSO_4 , filtered, and concentrated. The crude material was purified by flash column chromatography on silica gel (1/3/0.1 DCM/EtOAc/MeOH) to obtain **19b** (0.041 g, 8% yield) as white amorphous solid, followed by the elution of **20b** (0.327 g, 66% yield) as a white amorphous solid. Data for **19b**: ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 3.38 (t, J = 6.8 Hz, 2H), 3.24 (t, J = 6.9 Hz, 2H), 3.22 (s, 3H), 3.17 (d, J = 5.3 Hz, 2H), 3.13 (s, 3H), 3.07 (td, J = 10.6, 5.0 Hz, 1H), 3.00–2.89 (m, 1H), 2.23 (ddd, J = 15.5, 10.4, 5.1 Hz, 1H), 2.09 (ddd, J = 15.5, 10.1, 5.8 Hz, 1H), 1.97–1.01 (m, 24H), 1.00–0.91 (m, 2H), 0.89 (d, J = 6.6 Hz, 3H), 0.87 (s, 3H), 0.61 (s, 3H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 170.7, 79.4, 79.3, 55.5, 54.9, 54.8, 54.7, 54.7, 48.6, 45.9, 45.2, 43.1, 41.5, 40.9, 35.0, 34.4, 33.9, 33.2, 31.7, 30.8, 30.6, 28.1, 26.4, 26.1, 25.7, 24.0, 23.2, 20.9, 18.5, 12.0. TLC-MS (ESI): m/z calcd $\text{C}_{30}\text{H}_{52}\text{NO}_4$ ($\text{M} + \text{OH}^-$) 490.4, found 490.2. Data for **20b**: ^1H NMR (400 MHz, CDCl_3) δ 3.59 (q, J = 7.0, 4.6 Hz, 1H), 3.44 (dt, J = 16.0, 6.8 Hz, 4H), 3.35 (s, 3H), 3.12 (dp, J = 10.6, 4.5 Hz, 1H), 2.31 (ddd, J = 15.4, 10.7, 5.0 Hz, 1H), 2.16 (ddd, J = 15.2, 10.3, 5.7 Hz, 1H), 2.04–1.04 (m, 27H), 1.01 (dd, J = 14.2, 3.0 Hz, 1H), 0.95 (s, 3H), 0.94 (d, J = 6.4 Hz, 3H), 0.68 (s, 3H). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 3.86 (d, J = 6.8 Hz, 1H), 3.38 (t, J = 6.7 Hz, 2H), 3.24 (t, J = 6.9 Hz, 3H), 3.20 (s, 3H), 3.04 (td, J = 10.4, 4.6 Hz, 1H), 2.23 (ddd, J = 15.5, 10.5, 5.2 Hz, 1H), 2.09 (ddd, J = 15.5, 9.9, 5.9 Hz, 1H), 1.98–0.98 (m, 27H), 0.94 (dd, J = 14.3, 2.7 Hz, 1H), 0.89 (d, J = 6.3 Hz, 3H), 0.88 (s, 3H), 0.62 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 172.4, 80.2, 71.6, 55.9, 55.8, 55.2, 46.8, 45.8, 44.0, 42.6, 40.3, 39.3, 37.1, 35.7, 35.1, 34.6, 34.6, 33.9, 31.9, 31.2, 28.8, 27.1, 26.9, 26.4, 24.6, 23.6, 21.4, 18.8, 12.4. HRMS (ESI): m/z calcd $\text{C}_{29}\text{H}_{49}\text{NO}_3\text{Na}$ ($\text{M} + \text{Na}^+$) 482.3610, found 482.3612. Compounds **19a** and **20a** were prepared by the same method. Data for (*R*)-4-((3*R*,5*S*,7*R*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-3,7-dimethoxy-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)-1-(pyrrolidin-1-yl)pentan-1-one (**19a**): ^1H NMR (400 MHz, CDCl_3) δ 3.45 (dt, J = 16.5, 6.8 Hz, 4H), 3.34 (s, 3H), 3.24 (s, 3H), 3.18 (q, J = 2.8 Hz, 1H), 3.01 (tt, J = 11.1, 4.2 Hz, 1H), 2.31 (ddd, J = 15.5, 10.9, 5.0 Hz, 1H), 2.24–2.08 (m, 2H), 2.01–1.67 (m, 12H), 1.63 (ddd, J = 15.0, 5.5, 3.2 Hz, 1H), 1.59–1.41 (m, 5H), 1.42–1.15 (m, 8H), 1.05 (tt, J = 10.3, 5.7 Hz, 1H), 0.94 (d, J = 6.6 Hz, 3H), 0.91 (s, 3H), 0.65 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 172.5, 80.9, 80.9, 56.1, 55.9, 55.6, 50.4, 46.8, 45.8, 42.7, 42.2, 39.8, 39.7, 35.7, 35.6, 35.5, 34.9, 33.9, 31.7, 31.1, 28.4, 28.1, 26.9, 26.4, 24.6, 23.9, 23.2, 21.1, 18.7, 11.9. HRMS (ESI): m/z calcd $\text{C}_{30}\text{H}_{51}\text{NNaO}_3$ ($\text{M} + \text{Na}^+$) 496.3767, found 496.3769. Data for (*R*)-4-((3*R*,5*S*,7*R*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-7-hydroxy-3-methoxy-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)-1-(pyrrolidin-1-yl)pentan-1-one (**20a**): ^1H NMR (400 MHz, CDCl_3) δ 3.85 (s, 1H), 3.44 (dt, J = 16.8, 7.0 Hz, 4H), 3.35 (d, J = 1.0 Hz, 3H), 3.02 (td, J = 11.0, 5.4 Hz, 1H), 2.31 (ddd, J = 15.5, 10.8, 5.1 Hz, 1H), 2.24–2.09 (m, 2H), 2.03–1.03 (m, 26H), 0.99–0.95 (m, 1H), 0.95 (d, J = 6.5 Hz, 3H), 0.91 (s, 3H), 0.67 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 172.4, 80.8, 68.7, 56.0, 55.7, 50.7, 46.8, 45.8, 42.9, 41.6, 39.9, 39.7, 36.1, 35.7, 35.6, 35.5, 34.9, 33.0, 31.7, 31.1, 28.4, 27.2, 26.4, 24.6, 24.0, 23.1, 20.8, 18.7, 12.0. HRMS (ESI): m/z calcd $\text{C}_{29}\text{H}_{49}\text{NO}_3\text{Na}$ ($\text{M} + \text{Na}^+$) 482.3610, found 482.3624.

Sodium (3*R*,5*S*,7*S*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-3-Methoxy-10,13-dimethyl-17-((*R*)-5-oxo-5-(pyrrolidin-1-yl)pentan-2-yl)-hexadecahydro-1*H*-cyclopenta[*a*]phenanthren-7-yl Sulfate (**21b**). To a solution of **20b** (0.050 g, 0.11 mmol) in pyridine (0.54 mL) was added dropwise chlorosulfonic acid (0.05 mL, 0.7 mmol). The reaction mixture was heated to 50 $^\circ\text{C}$ for 30 min, then cooled to room temperature. The reaction was terminated by the addition of water (1 mL), and the solvents were removed by rotary evaporation. The crude material was dissolved in DMSO (0.5 mL) and 1 M triethylammonium acetate buffer (0.1 mL) and purified by flash column chromatography (5–100% 20 mM triethylammonium acetate buffer in acetonitrile in water as eluent, C_{18} column) to yield a white solid after lyophilization.

To prepare the sodium salt of **21b**, a 1 cm wide column was filled with 12 cm of Dowex-50WX2 (50–100 mesh, strongly acidic) ion-exchange resin. The column was prepared by sequentially washing with 1:1 acetonitrile/water, ~1 M aqueous NaHCO₃ (caution: gas evolution), water, and finally 1:1 acetonitrile/water. The reaction product was dissolved in 1:1 acetonitrile/water and loaded onto the column, which was eluted with 1:1 acetonitrile/water. The fractions containing the product were lyophilized to furnish **21b** as an off-white solid (0.054 g, 88% yield). ¹H NMR (400 MHz, D₂O) δ 4.32 (app q, J = 9.9, 8.5 Hz, 1H), 3.51 (t, J = 6.9 Hz, 2H), 3.40 (d, J = 7.0 Hz, 2H), 3.36 (s, 3H), 3.31 (s, 1H), 2.37 (d, J = 10.4 Hz, 1H), 2.32–2.16 (m, 1H), 2.14–1.05 (m, 28H), 1.01 (s, 3H), 1.00 (d, J = 6.6 Hz, 6H), 0.72 (s, 3H). ¹H NMR (400 MHz, DMSO-*d*₆) δ 3.93 (s, 1H), 3.38 (t, J = 6.7 Hz, 2H), 3.25 (t, J = 6.8 Hz, 2H), 3.21 (s, 3H), 3.05 (s, 1H), 2.29–2.00 (m, 4H), 1.99–1.80 (m, 3H), 1.80–1.53 (m, 8H), 1.37 (d, J = 7.8 Hz, 6H), 1.29–0.97 (m, 8H), 0.94 (d, J = 14.8 Hz, 1H), 0.89 (d, J = 6.4 Hz, 3H), 0.89 (s, 3H), 0.60 (s, 3H). ¹³C NMR (100 MHz, D₂O) δ 174.5, 81.0, 80.0, 54.8, 54.5, 54.3, 47.5, 46.1, 43.4, 41.7, 41.1, 39.8, 39.2, 35.2, 34.4, 33.8, 33.5, 32.8, 31.1, 30.9, 28.1, 26.0, 25.8, 25.5, 24.1, 23.1, 21.4, 18.7, 11.9. TLC-MS (ESI): *m/z* calcd C₂₉H₄₇NO₆S (M – H)[–] 537.3, found 537.2.

(*R*)-4-((3*R*,5*S*,7*R*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-3,7-Dihydroxy-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)-*N*-(1-hydroxy-2-methylpropan-2-yl)pentanamide (**22a**). To a suspension of CDCA (0.589 g, 1.50 mmol) in DCM (15 mL) was added TEA (0.63 mL, 4.5 mmol), 2-amino-2-methylpropan-1-ol (0.147 g, 1.65 mmol), and HATU (0.627 g, 1.65 mmol). The reaction was stirred at room temperature for 2 h and was quenched by the addition of water (10 mL). The aqueous and organic layers were separated, and the organic layer was washed with water (2 × 10 mL). The organic layer was dried over MgSO₄, filtered, and concentrated. The crude material was purified by flash column chromatography on silica gel (10–100% EtOAc in DCM as eluent) to obtain **22a** (0.375 g, 54% yield) as an off-white foam. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.22 (s, 1H), 4.88 (t, J = 5.9 Hz, 1H), 4.30 (d, J = 4.7 Hz, 1H), 4.10 (d, J = 3.4 Hz, 1H), 3.62 (s, 1H), 3.42–3.33 (m, 2H), 3.17 (dd, J = 10.5, 5.0 Hz, 1H), 2.32–1.15 (m, 22H), 1.15 (s, 6H), 1.13–0.93 (m, 3H), 0.88–0.86 (m, 1H), 0.87 (d, J = 6.6 Hz, 3H), 0.83 (s, 3H), 0.60 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 175.0, 72.0, 70.9, 68.5, 56.2, 55.9, 50.5, 42.8, 41.6, 39.9, 39.8, 39.5, 35.5, 35.4, 35.2, 34.7, 34.1, 32.9, 31.9, 30.7, 28.4, 24.8, 24.8, 23.8, 22.9, 20.7, 18.6, 11.9. TLC-MS (ESI): *m/z* calcd C₂₈H₄₃O₆ (M + OH + 2H₂O)⁺ 516.4, found 516.0.

(*R*)-4-((3*R*,5*S*,7*S*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-3,7-Dihydroxy-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)-*N*-((*Z*)-1-(hydroxyamino)prop-1-en-2-yl)pentanamide (**23b**). To a suspension of UDCA (1.00 g, 2.55 mmol) in DCM (8.5 mL) was added (*Z*)-*N*'-hydroxyacetimidamide (0.189 g, 2.55 mmol), HATU (1.07 g, 2.80 mmol), and diisopropylethylamine (1.3 mL, 7.6 mmol). The reaction stirred at room temperature for 24 h and was quenched with water (5 mL). The organic phase was washed with water (2 × 10 mL), dried over MgSO₄, filtered, and concentrated. The crude material was purified by flash column chromatography on silica gel (1–10% MeOH in EtOAc as eluent) to obtain **23b** (0.639 g, 56% yield) as a white amorphous solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 6.29 (s, 2H), 4.43 (d, J = 4.6 Hz, 1H), 3.87 (d, J = 6.8 Hz, 1H), 3.31–3.22 (m, 2H), 2.37 (ddd, J = 15.2, 9.8, 5.2 Hz, 1H), 2.24 (ddd, J = 15.7, 9.3, 6.7 Hz, 2H), 1.94 (d, J = 11.2 Hz, 1H), 1.90–1.73 (m, 2H), 1.72 (s, 3H), 1.70–1.58 (m, 4H), 1.56–0.93 (m, 16H), 0.90 (d, J = 6.6 Hz, 3H), 0.87 (s, 3H), 0.62 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 171.1, 155.7, 69.7, 69.5, 64.9, 55.9, 54.6, 43.1, 43.0, 42.2, 38.7, 37.7, 37.3, 34.9, 34.8, 33.8, 30.7, 30.2, 29.3, 28.1, 26.7, 23.3, 20.8, 18.4, 16.3, 12.0. TLC-MS (ESI): *m/z* calcd C₂₆H₄₄N₂O₄ (M[–]) 448.3, found 448.2. Compound **23a** was prepared by the same method. Data for (*R*)-4-((3*R*,5*S*,7*R*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-3,7-dihydroxy-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)-*N*-((*Z*)-1-(hydroxyamino)prop-1-en-2-yl)pentanamide (**23a**): ¹H NMR (400 MHz, DMSO-*d*₆) δ 4.43 (d, J = 4.6 Hz, 1H), 3.87 (d, J = 6.8 Hz, 1H), 3.30–3.21 (m, 2H), 2.90 (ddd, J = 15.1, 10.0, 4.4 Hz, 1H), 2.84–2.70 (m, 1H), 2.29 (s, 3H), 1.97–1.00 (m, 25H), 0.94 (d, J = 5.9 Hz, 3H), 0.89–0.87 (m, 1H), 0.84 (s, 3H), 0.61 (s, 3H). ¹³C NMR (100 MHz,

DMSO-*d*₆) δ 179.8, 166.6, 69.6, 69.3, 64.8, 55.7, 54.3, 43.0, 42.9, 42.1, 38.8, 38.6, 37.6, 37.2, 34.7, 33.6, 32.2, 30.1, 28.0, 26.6, 23.2, 22.5, 20.7, 18.1, 11.9, 11.0. TLC-MS (ESI): *m/z* calcd C₂₆H₄₃N₂O₄ (M – H)[–] 447.3, found 447.4.

(3*R*,5*S*,7*S*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-10,13-Dimethyl-17-((*R*)-4-(3-methyl-1,2,4-oxadiazol-5-yl)butan-2-yl)hexadecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,7-diol (**24b**). Compound **23b** (0.300 g, 0.669 mmol) was dissolved in toluene (2.2 mL) and THF (2.5 mL). The solution was heated in the MW at 160 °C for 45 min. The solvent was removed under reduced pressure, and the crude residue was purified by flash column chromatography on silica gel (0–10% MeOH in EtOAc as eluent) to obtain **24b** (0.155 g, 54% yield) as a white foam. ¹H NMR (400 MHz, DMSO-*d*₆) δ 4.43 (d, J = 4.6 Hz, 1H), 3.87 (d, J = 6.8 Hz, 1H), 3.31–3.23 (m, 2H), 2.90 (ddd, J = 15.1, 10.0, 4.4 Hz, 1H), 2.86–2.72 (m, 2H), 2.29 (s, 3H), 2.01–1.59 (m, 7H), 1.57–1.00 (m, 16H), 0.94 (d, J = 5.9 Hz, 3H), 0.87 (s, 3H), 0.61 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 180.0, 166.8, 69.8, 69.5, 55.9, 54.5, 43.2, 43.1, 42.2, 38.8, 38.8, 37.8, 37.3, 34.9, 33.8, 33.8, 32.4, 30.3, 28.2, 26.8, 23.4, 22.7, 20.9, 18.3, 12.1, 11.2. HRMS (ESI): *m/z* calcd C₂₆H₄₂N₂NaO₃ (M + Na⁺) 453.3093, found 453.3074. Compound **24a** was prepared by the same method. Data for (3*R*,5*S*,7*R*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-10,13-dimethyl-17-((*R*)-4-(3-methyl-1,2,4-oxadiazol-5-yl)butan-2-yl)hexadecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,7-diol (**24a**): ¹H NMR (400 MHz, DMSO-*d*₆) δ 4.30 (d, J = 4.7 Hz, 1H), 4.11 (d, J = 3.4 Hz, 1H), 3.62 (t, J = 3.2 Hz, 1H), 3.25–3.10 (m, 1H), 2.90 (ddd, J = 14.8, 10.1, 4.5 Hz, 1H), 2.84–2.73 (m, 1H), 2.29 (s, 3H), 2.19 (q, J = 13.0 Hz, 1H), 1.97–1.61 (m, 7H), 1.40 (tdd, J = 21.4, 13.9, 10.1 Hz, 8H), 1.29–1.06 (m, 7H), 1.00 (td, J = 11.8, 6.1 Hz, 1H), 0.94 (d, J = 5.6 Hz, 3H), 0.84 (s, 3H), 0.60 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 180.3, 167.1, 72.0, 68.5, 55.7, 50.5, 42.8, 41.6, 39.9, 39.7, 39.5, 35.5, 35.4, 35.1, 34.8, 32.9, 32.8, 30.8, 28.3, 23.8, 23.5, 22.9, 20.7, 18.4, 11.9, 11.7. HRMS (ESI): *m/z* calcd C₂₆H₄₂N₂NaO₃ (M + Na⁺) 453.3093, found 453.3075.

(3*R*,5*S*,7*S*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-17-((*R*)-5-(2-Acetylhydrazinyl)-5-oxopentan-2-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,7-diyl Diacetate (**25b**). To a solution of **15b** (1.19 g, 2.50 mmol) in DCM (25 mL) was added HATU (1.23 g, 3.25 mmol), DIPEA (1.2 mL, 6.7 mmol), and aceto-hydrazide (0.222 g, 3.00 mmol). The reaction stirred at room temperature for 18 h and was quenched by the addition of saturated aqueous NaHCO₃ (20 mL). The aqueous layer was extracted with DCM (3 × 20 mL), and the combined organic layers were washed with saturated aqueous NaHCO₃ (3 × 20 mL), dried over Na₂SO₄, filtered, and concentrated. The crude material was purified by flash column chromatography on silica gel (1–10% MeOH in DCM as eluent) to obtain **25b** (0.633 g, 48% yield) as a yellow foam. ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.66 (d, J = 2.0 Hz, 1H), 9.62 (d, J = 2.0 Hz, 1H), 4.66 (td, J = 10.7, 4.9 Hz, 1H), 4.55 (tt, J = 10.6, 4.9 Hz, 1H), 2.12 (ddd, J = 14.3, 9.5, 5.1 Hz, 1H), 2.07–1.99 (m, 1H), 1.98 (s, 3H), 1.93 (s, 3H), 1.83 (s, 3H), 1.80–0.96 (m, 24H), 0.93 (s, 3H), 0.89 (d, J = 6.4 Hz, 3H), 0.63 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 171.3, 169.8, 169.8, 167.9, 73.0, 72.8, 54.9, 54.5, 54.4, 43.1, 41.3, 39.2, 38.6, 34.7, 33.9, 33.5, 32.7, 32.5, 31.3, 30.1, 28.0, 26.0, 25.3, 22.8, 21.5, 21.0, 20.8, 20.5, 18.4, 11.8. TLC-MS (ESI): *m/z* calcd C₃₀H₄₈N₂O₆ (M[–]) 532.4, found 532.5. Compound **25a** was prepared by the same method. Data for (3*R*,5*S*,7*R*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-17-((*R*)-5-(2-acetylhydrazinyl)-5-oxopentan-2-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,7-diyl diacetate (**25a**): ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.67 (d, J = 2.0 Hz, 1H), 9.63 (d, J = 2.0 Hz, 1H), 4.76 (q, J = 2.9 Hz, 1H), 4.47 (tt, J = 11.2, 4.4 Hz, 1H), 2.13 (ddd, J = 14.5, 9.6, 5.2 Hz, 1H), 2.08–1.88 (m, 10H), 1.82 (s, 3H), 1.81–0.97 (m, 20H), 0.90 (s, 3H), 0.89 (d, J = 7.6 Hz, 3H), 0.88–0.84 (m, 1H), 0.62 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 171.3, 169.8, 169.7, 167.9, 73.4, 70.5, 55.3, 50.0, 42.2, 40.2, 39.0, 37.1, 34.8, 34.4, 34.3, 34.3, 33.6, 31.2, 30.8, 30.0, 27.6, 26.4, 23.0, 22.3, 21.3, 21.1, 20.5, 20.2, 18.2, 11.6. TLC-MS (ESI): *m/z* calcd C₃₀H₄₈N₂O₆ (M + H⁺) 533.4, found 533.3.

(3*R*,5*S*,7*S*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-10,13-Dimethyl-17-((*R*)-4-(5-methyl-1,3,4-oxadiazol-2-yl)butan-2-yl)hexadecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,7-diyl Diacetate (**26b**). To a solution of **25b** (0.623 g, 1.17 mmol) and triethylamine (0.41 mL, 2.9 mmol)

in DCM (12 mL) was added tosyl chloride (0.223 g, 1.17 mmol) and the reaction stirred at room temperature for 24 h. The solvent was removed under reduced pressure, and the crude material was purified by flash column chromatography on silica gel (1–5% MeOH in DCM as eluent) to obtain **26b** (0.491 g, 0.954 mmol, 82% yield) as a white foam. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 4.66 (td, $J = 10.7, 4.9$ Hz, 1H), 4.55 (dq, $J = 10.4, 5.2, 4.8$ Hz, 1H), 2.81 (ddd, $J = 14.2, 9.7, 4.2$ Hz, 1H), 2.70 (dt, $J = 15.5, 7.7$ Hz, 1H), 2.44 (s, 3H), 1.97 (s, 3H), 1.93 (s, 3H), 1.84–0.99 (m, 23H), 0.94 (d, $J = 5.9$ Hz, 3H), 0.92 (s, 3H), 0.88–0.76 (m, 1H), 0.63 (s, 3H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 169.8, 169.7, 166.7, 163.3, 72.9, 72.8, 54.3, 54.2, 43.1, 41.8, 41.2, 39.2, 38.6, 34.6, 33.9, 33.5, 32.6, 32.5, 32.1, 27.9, 26.0, 25.2, 22.8, 21.4, 21.0, 20.9, 18.1, 14.1, 11.8, 10.4. TLC-MS (ESI): m/z calcd $\text{C}_{30}\text{H}_{47}\text{N}_2\text{O}_5$ ($M + \text{H}^+$) 515.3, found 515.7. Compound **26a** was prepared by the same method. Data for (3*R*,5*S*,7*R*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-10,13-dimethyl-17-((*R*)-4-(5-methyl-1,3,4-oxadiazol-2-yl)butan-2-yl)hexadecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,7-diyl diacetate (**26a**): ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 4.76 (t, $J = 3.1$ Hz, 1H), 4.47 (tt, $J = 10.9, 4.6$ Hz, 1H), 2.82 (ddd, $J = 14.6, 9.7, 4.4$ Hz, 1H), 2.70 (dt, $J = 15.5, 7.6$ Hz, 1H), 2.44 (s, 3H), 2.07–1.87 (m, 2H), 1.98 (s, 3H), 1.97 (s, 3H), 1.86–0.98 (m, 22H), 0.94 (d, $J = 6.0$ Hz, 3H), 0.91 (s, 3H), 0.62 (s, 3H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 169.8, 169.7, 166.8, 163.3, 73.4, 70.5, 55.0, 50.0, 42.2, 41.8, 39.0, 37.1, 34.7, 34.4, 34.3, 34.3, 33.6, 32.0, 30.8, 27.5, 26.4, 23.0, 22.3, 21.4, 21.3, 21.1, 20.2, 18.0, 11.5, 10.4. TLC-MS (ESI): m/z calcd $\text{C}_{30}\text{H}_{47}\text{N}_2\text{O}_5$ ($M + \text{OH}^-$) 531.3, found 531.3.

(3*R*,5*S*,7*S*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-10,13-Dimethyl-17-((*R*)-4-(5-methyl-1,3,4-oxadiazol-2-yl)butan-2-yl)hexadecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,7-diol (**27b**). To a solution of **26b** (0.491 g, 0.954 mmol) in MeOH (5 mL) was added NaOH (0.095 g, 2.4 mmol), and the reaction was heated to reflux for 12 h. The solution was cooled to room temperature, and the solvent was removed under reduced pressure. The residue was partitioned between water (10 mL) and EtOAc (10 mL). The aqueous layer was extracted with EtOAc (2 \times 10 mL), and the combined organic layers were dried over Na_2SO_4 , filtered, and concentrated. The crude material was purified by flash column chromatography on silica gel to obtain **27b** (0.250 g, 61% yield) as a white foam. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 4.43 (d, $J = 4.5$ Hz, 1H), 3.87 (d, $J = 6.8$ Hz, 1H), 3.32–3.22 (m, 2H), 2.82 (ddd, $J = 14.4, 10.0, 4.3$ Hz, 1H), 2.70 (ddd, $J = 15.5, 9.1, 6.6$ Hz, 1H), 2.44 (s, 3H), 2.01–1.00 (m, 23H), 0.94 (d, $J = 5.7$ Hz, 3H), 0.93–0.91 (m, 1H), 0.87 (s, 3H), 0.62 (s, 3H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 166.8, 163.3, 69.7, 69.4, 55.8, 54.9, 54.4, 43.1, 43.0, 42.2, 38.7, 37.7, 37.3, 34.8, 34.8, 33.7, 32.2, 30.2, 28.1, 26.7, 23.3, 21.5, 20.8, 18.2, 12.0, 10.4. TLC-MS (ESI): m/z calcd $\text{C}_{26}\text{H}_{43}\text{N}_2\text{O}_3$ ($M + \text{H}^+$) 431.3, found 431.6. Compound **27a** was prepared by the same method. Data for (3*R*,5*S*,7*S*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-10,13-dimethyl-17-((*R*)-4-(5-methyl-1,3,4-oxadiazol-2-yl)butan-2-yl)hexadecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,7-diol (**27a**): ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 4.30 (d, $J = 4.6$ Hz, 1H), 4.11 (d, $J = 3.4$ Hz, 1H), 3.74–3.52 (m, 1H), 3.18 (qt, $J = 9.5, 4.4$ Hz, 1H), 2.82 (ddd, $J = 14.4, 9.8, 4.4$ Hz, 1H), 2.70 (ddd, $J = 15.6, 9.2, 6.7$ Hz, 1H), 2.44 (s, 3H), 2.19 (app q, $J = 12.8$ Hz, 1H), 1.96–1.05 (m, 21H), 1.00 (td, $J = 12.2, 11.7, 6.2$ Hz, 1H), 0.94 (d, $J = 6.0$ Hz, 3H), 0.88 (dd, $J = 14.4, 3.2$ Hz, 1H), 0.83 (s, 3H), 0.60 (s, 3H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 166.8, 163.3, 70.3, 66.1, 59.7, 55.3, 50.0, 42.0, 41.4, 39.6, 35.3, 34.9, 34.8, 34.7, 32.3, 32.1, 30.6, 27.7, 23.1, 22.7, 21.5, 20.8, 20.2, 18.1, 11.6, 10.4. TLC-MS (ESI): m/z calcd $\text{C}_{26}\text{H}_{43}\text{N}_2\text{O}_3$ ($M + \text{H}^+$) 431.3, found 431.2.

(3*R*,5*S*,7*S*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-17-((*R*)-5-((1-Hydroxy-2-methylpropan-2-yl)amino)-5-oxopentan-2-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,7-diyl Diformate (**29b**). UDCA (1.00 g, 2.55 mmol) was dissolved in formic acid (20 mL), and perchloric acid (0.05 mL, 0.7 mmol) was added. The solution was heated to 50 $^\circ\text{C}$ for 12 h, then cooled to 40 $^\circ\text{C}$. Acetic anhydride (10 mL) was added over 10 min, and the solution was cooled to room temperature. The reaction was quenched with water (100 mL), and the aqueous layer was extracted with Et_2O (3 \times 100 mL). The combined organic layers were washed with water (10 \times 50 mL), dried over Na_2SO_4 , filtered, and concentrated. Crude (*R*)-4-

((3*R*,5*S*,7*S*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-3,7-bis(formyloxy)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)pentanoic acid (**28b**) was used for the next step without further purification. Thionyl chloride (1.71 mL, 23.4 mmol) was added dropwise to a solution of the crude material in benzene (12 mL), and the reaction was heated to reflux for 4 h. The reaction was cooled to room temperature, and the solvent was evaporated. The residual thionyl chloride was removed by coevaporation with benzene (2 \times 6 mL), and the crude material was dissolved in DCM (5 mL). A solution of 2-amino-2-methylpropan-1-ol (0.498 g, 5.59 mmol) in DCM (1 mL) was added dropwise at 0 $^\circ\text{C}$. After 1.5 h, the reaction was filtered and the solids were rinsed with DCM. The filtrate was evaporated to dryness, and the crude material was purified by flash column chromatography on silica gel (0–40% EtOAc in DCM as eluent) to obtain **29b** (0.824 g, 62% yield) as a white foam. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.19 (s, 1H), 8.16 (s, 1H), 4.87 (t, $J = 5.9$ Hz, 1H), 4.77 (td, $J = 10.8, 4.9$ Hz, 1H), 4.66 (dq, $J = 10.6, 5.3, 4.7$ Hz, 1H), 3.35 (d, $J = 5.3$ Hz, 2H), 2.07 (ddd, $J = 14.5, 9.8, 5.2$ Hz, 1H), 1.98–1.87 (m, 2H), 1.84–1.49 (m, 12H), 1.46–1.16 (m, 8H), 1.15 (s, 6H), 1.13–0.96 (m, 4H), 0.94 (s, 3H), 0.88 (d, $J = 6.5$ Hz, 1H), 0.63 (s, 3H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 162.7, 152.1, 151.7, 63.1, 63.0, 57.6, 54.9, 44.5, 44.3, 44.1, 33.1, 31.2, 24.7, 23.8, 23.5, 23.0, 22.8, 22.5, 21.6, 17.9, 16.1, 15.6, 13.7, 13.7, 12.8, 10.8, 8.5, 5.2, 1.8, –9.9. TLC-MS (ESI): m/z calcd $\text{C}_{30}\text{H}_{48}\text{NO}_3$ ($M - \text{H}^+$) 518.4, found 518.9.

(3*R*,5*S*,7*S*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-17-((*R*)-4-(4,4-Dimethyl-4,5-dihydrooxazol-2-yl)butan-2-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,7-diyl Diformate (**30b**). To a solution of **29b** (0.824 g, 1.59 mmol) in THF (4 mL) was added dropwise SOCl_2 (0.64 mL, 8.7 mmol) at 0 $^\circ\text{C}$. After stirring for 18 h overnight, the reaction was quenched with saturated aqueous NaHCO_3 (10 mL). The aqueous layer was extracted with Et_2O (5 \times 10 mL), and the combined organic layers were dried over MgSO_4 , filtered, and concentrated. The crude material was purified by flash column chromatography on silica gel (0–30% EtOAc in DCM as eluent) to obtain **30b** (0.505 g, 64% yield) as an off-white foam. ^1H NMR (400 MHz, CDCl_3) δ 8.02 (s, 1H), 7.98 (s, 1H), 4.90 (td, $J = 10.8, 5.2$ Hz, 1H), 4.81 (dq, $J = 10.2, 5.4$ Hz, 1H), 2.37–1.02 (m, 34H), 0.99 (s, 3H), 0.93 (d, $J = 6.4$ Hz, 3H), 0.69 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 174.8, 161.2, 160.8, 79.1, 73.8, 73.6, 71.1, 56.4, 55.5, 55.2, 43.9, 43.8, 42.2, 40.1, 40.0, 39.6, 35.5, 34.6, 34.4, 34.2, 33.1, 33.0, 32.0, 28.6, 26.6, 26.0, 23.4, 21.4, 18.7, 12.3. TLC-MS (ESI): m/z calcd $\text{C}_{32}\text{H}_{51}\text{N}_2\text{O}_3$ ($M + \text{H}^+$) 543.4, found 543.1.

(3*R*,5*S*,7*S*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-17-((*R*)-4-(4,4-Dimethyl-4,5-dihydrooxazol-2-yl)butan-2-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,7-diol (**31b**). NaOH (0.078 g, 1.9 mmol) was added to a solution of **30b** (0.390 g, 0.777 mmol) in MeOH (4 mL), and the reaction was heated to reflux for 2 h. The reaction was cooled to room temperature and diluted with EtOAc (25 mL). The organic layer was washed with water (3 \times 10 mL), dried over MgSO_4 , filtered, and concentrated. The crude material was purified by flash column chromatography on silica gel (0–100% EtOAc in DCM) to obtain **31b** (0.254 g, 73% yield) as a white foam. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 4.43 (dd, $J = 4.7, 2.7$ Hz, 1H), 3.86 (d, $J = 6.8$ Hz, 1H), 3.83 (d, $J = 1.0$ Hz, 2H), 3.32–3.21 (m, 2H), 2.18 (ddd, $J = 14.8, 9.6, 5.1$ Hz, 1H), 2.11–1.99 (m, 1H), 1.98–1.60 (m, 6H), 1.57–0.98 (m, 24H), 0.89 (d, $J = 6.5$ Hz, 3H), 0.87 (s, 3H), 0.61 (s, 3H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 164.7, 77.9, 69.7, 69.4, 66.7, 64.9, 55.8, 54.5, 43.1, 43.0, 42.2, 38.7, 37.7, 37.3, 34.8, 34.7, 33.7, 31.8, 30.2, 28.3, 28.2, 28.2, 26.7, 24.3, 23.3, 20.8, 18.4, 12.0. HRMS (ESI): m/z calcd $\text{C}_{28}\text{H}_{48}\text{NO}_3$ ($M + \text{H}^+$) 446.3634, found 446.3608.

(3*R*,5*S*,7*S*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-17-((*R*)-5-Amino-5-oxopentan-2-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,7-diyl Diformate (**32b**). To a solution of **28b** (3.30 g, 7.36 mmol) in benzene (38 mL) was added SOCl_2 (2.8 mL, 38 mmol), followed by 3 drops of DMF. The reaction was heated to 80 $^\circ\text{C}$ for 4 h, then cooled to room temperature. The excess SOCl_2 was removed by coevaporation with benzene (3 \times 5 mL). The residue was dissolved in benzene (20 mL), and ammonia gas was bubbled through the solution for 20 min. The solvent was removed under reduced pressure, and the residue was partitioned between water (50 mL) and

EtOAc (50 mL). The aqueous phase was extracted with EtOAc (2 × 20 mL), and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated. The crude material was purified by flash column chromatography on silica gel (5–100% EtOAc in hexanes) to obtain **32b** (1.72 g, 52% yield) as a white foam. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.19 (s, 1H), 8.16 (s, 1H), 7.37–7.10 (m, 1H), 6.63 (s, 1H), 4.77 (td, *J* = 10.7, 4.9 Hz, 1H), 4.68 (dq, *J* = 11.0, 5.9, 5.3 Hz, 1H), 2.04 (dt, *J* = 10.0, 5.0 Hz, 1H), 1.99–0.96 (m, 25H), 0.94 (s, 3H), 0.88 (d, *J* = 6.4 Hz, 3H), 0.63 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 174.6, 162.1, 161.7, 73.1, 73.0, 54.5, 54.3, 43.1, 41.2, 39.2, 38.5, 34.8, 33.8, 33.5, 32.8, 32.5, 32.0, 31.4, 30.7, 27.9, 26.1, 25.6, 22.8, 20.8, 18.4, 11.8. TLC-MS (ESI): *m/z* calcd C₂₆H₄₁NNaO₅ (M + Na⁺) 470.3, found 470.9. Compound **32a** was prepared by the same method. Data for (3*R*,5*S*,7*R*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-17-((*R*)-5-amino-5-oxopentan-2-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,7-diyl diformate (**32a**): ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.24 (s, 1H), 8.17 (s, 1H), 7.20 (s, 1H), 6.63 (s, 1H), 4.88 (d, *J* = 3.2 Hz, 1H), 4.59 (tt, *J* = 10.9, 4.5 Hz, 1H), 2.11–0.99 (m, 26H), 0.92 (s, 3H), 0.88 (d, *J* = 6.4 Hz, 3H), 0.62 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 174.7, 161.9, 161.7, 73.3, 70.8, 55.3, 49.9, 42.2, 42.2, 39.0, 37.0, 34.8, 34.3, 34.2, 33.5, 31.9, 31.3, 31.1, 27.5, 26.5, 22.9, 22.3, 20.8, 20.2, 18.3, 11.6. TLC-MS (ESI): *m/z* calcd C₂₆H₄₁NNaO₅ (M + Na⁺) 470.3, found 470.8.

(3*R*,5*S*,7*S*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-17-((*R*)-4-cyanobutan-2-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,7-diyl Diformate (**33b**). To a solution of **32b** (0.780 g, 1.74 mmol) in THF (17 mL) was added pyridine (0.28 mL, 3.4 mmol) and TFAA (0.50 mL, 3.5 mmol). The reaction was stirred overnight at room temperature, and the solvent was removed under reduced pressure. The residue was partitioned between 1 M aqueous HCl (50 mL) and EtOAc (50 mL). The aqueous layer was extracted with EtOAc (2 × 50 mL), and the combined organic layers were washed with brine (30 mL), dried over Na₂SO₄, filtered, and concentrated. The crude material (**33b**) was used for the next reaction without further purification. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.19 (s, 1H), 8.16 (s, 1H), 4.77 (td, *J* = 10.8, 4.9 Hz, 1H), 4.67 (tt, *J* = 10.7, 4.8 Hz, 1H), 2.60–2.33 (m, 2H), 1.98–0.98 (m, 24H), 0.94 (s, 3H), 0.91 (d, *J* = 6.5 Hz, 3H), 0.65 (s, 3H). ¹H NMR (400 MHz, CD₃OD) δ 3.57–3.40 (m, 2H), 2.56–2.31 (m, 2H), 2.09–1.01 (m, 24H), 0.99 (d, *J* = 6.5 Hz, 3H), 0.97 (s, 3H), 0.74 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 161.1, 160.7, 120.2, 73.6, 73.5, 55.4, 54.9, 43.8, 42.1, 39.9, 39.9, 39.5, 35.1, 34.5, 34.1, 33.0, 32.9, 31.6, 28.5, 26.5, 25.9, 23.3, 21.3, 18.1, 14.4, 12.2. HRMS (ESI): *m/z* calcd C₂₆H₃₉NNaO₄ (M + Na⁺) 452.2777, found 452.2747. Compound **33a** was prepared by the same method. Data for (3*R*,5*S*,7*R*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-17-((*R*)-4-cyanobutan-2-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,7-diyl diformate (**33a**): ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.24 (s, 1H), 8.17 (s, 1H), 4.89 (q, *J* = 3.0 Hz, 1H), 4.59 (tt, *J* = 11.0, 4.5 Hz, 1H), 2.52 (m, 2H), 2.41 (dt, *J* = 16.8, 8.1 Hz, 1H), 2.14–0.98 (m, 22H), 0.92 (s, 3H), 0.91 (d, *J* = 6.7 Hz, 3H), 0.92–0.87 (m, 1H), 0.64 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 160.8, 160.8, 120.2, 74.1, 71.4, 55.6, 50.2, 42.9, 41.0, 39.5, 37.9, 35.2, 34.9, 34.9, 34.7, 34.0, 31.5, 31.5, 28.1, 26.8, 23.5, 22.7, 20.7, 18.0, 14.3, 11.8. TLC-MS (ESI): *m/z* calcd C₂₆H₃₉NNaO₄ (M + Na⁺) 452.3, found 452.0.

(*R*)-4-((3*R*,5*S*,7*S*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-3,7-Dihydroxy-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)-pentanenitrile (**34b**). To a suspension of **33b** (0.749 g, 1.74 mmol) in MeOH (8.72 mL) was added NaOH (0.174 g, 4.36 mmol), and the reaction was heated to reflux for 12 h. The reaction was cooled to room temperature, and the solvent was removed under reduced pressure. The residue was partitioned between 1 M aqueous HCl (15 mL) and EtOAc (30 mL). The aqueous layer was extracted with EtOAc (2 × 30 mL), and the combined organic layers were washed with water (10 mL) and brine (10 mL), dried over Na₂SO₄, filtered, and concentrated. The crude material was purified by flash column chromatography on silica gel (50–100% EtOAc in hexanes as eluent) to obtain **34b** as a white amorphous solid (0.445 g, 68% yield over 2 steps). ¹H NMR (400 MHz, DMSO-*d*₆) δ 4.43 (d, *J* = 4.5 Hz, 1H), 3.88 (d, *J* = 7.0 Hz, 1H), 3.33–3.19 (m, 2H), 2.62–2.30 (m, 2H), 2.00–0.96 (m, 24H), 0.91 (d, *J* = 6.5 Hz, 3H), 0.87 (s, 3H), 0.63 (s,

3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 121.1, 69.7, 69.4, 55.8, 54.3, 43.2, 43.0, 42.2, 38.7, 37.7, 37.3, 34.8, 34.8, 33.8, 33.8, 31.0, 30.2, 28.1, 26.7, 23.3, 20.8, 17.8, 13.4, 12.0. TLC-MS (ESI): *m/z* calcd C₂₄H₃₉NO₂ (M⁺) 373.3, found 373.5. Compound **34a** was prepared by the same method. Data for (*R*)-4-((3*R*,5*S*,7*R*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-3,7-dihydroxy-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)pentanenitrile (**34a**): ¹H NMR (400 MHz, DMSO-*d*₆) δ 4.31 (d, *J* = 4.7 Hz, 1H), 4.12 (d, *J* = 3.4 Hz, 1H), 3.63 (q, *J* = 3.0 Hz, 1H), 3.19 (ddp, *J* = 15.5, 9.5, 4.7, 4.3 Hz, 1H), 2.60–2.31 (m, 2H), 2.19 (q, *J* = 12.8 Hz, 1H), 1.94–0.95 (m, 22H), 0.91 (d, *J* = 6.5 Hz, 3H), 0.87 (d, *J* = 3.1 Hz, 1H), 0.84 (s, 3H), 0.62 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 120.3, 71.8, 68.3, 55.6, 42.7, 41.5, 40.9, 39.7, 39.7, 39.4, 35.4, 35.2, 35.1, 34.8, 32.8, 31.5, 30.6, 28.2, 23.6, 22.8, 20.6, 17.9, 14.3, 11.8. TLC-MS (ESI): *m/z* calcd C₂₄H₄₁NO₃ (M + H₂O⁺) 391.3, found 391.6.

Sodium 5-((*R*)-3-((3*R*,5*S*,7*S*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-3,7-dihydroxy-10,13-dimethylhexadeca-hydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)butyl)tetrazol-1-ide (**35b**). To a suspension of **34b** (0.200 g, 0.535 mmol) in toluene (1.1 mL) in a MW vial was added NaN₃ (0.104 g, 1.61 mmol) and triethylamine hydrochloride (0.258 g, 1.87 mmol). The reaction was heated via MW at 130 °C for 3 h, then cooled to room temperature. DMF (1 mL) was added, and the reaction was heated via MW at 160 °C for 4 h, then cooled to room temperature. The reaction was quenched with water (1 mL), and 1 M aqueous HCl (2 mL) was added. The precipitate was collected via vacuum filtration and rinsed with water (3 mL), then dried on the frit. To prepare the sodium salt of **35b**, a 1 cm wide column was filled with 12 cm of Dowex-50WX2 (50–100 mesh, strongly acidic) ion-exchange resin. The column was prepared by sequentially washing with 1:1 acetonitrile/water, ~1 M aqueous NaHCO₃ (caution: gas evolution), water, and finally 1:1 acetonitrile/water. The reaction product was dissolved in 1:1 acetonitrile/water and loaded onto the column, which was eluted with 1:1 acetonitrile/water. The fractions containing the product were lyophilized to furnish **35b** as a white solid (98 mg, 42% yield). ¹H NMR (400 MHz, CD₃OD) δ 3.48 (t, *J* = 6.9 Hz, 2H), 2.88 (ddd, *J* = 14.5, 10.7, 4.2 Hz, 1H), 2.72 (dt, *J* = 14.8, 8.4 Hz, 1H), 2.05 (d, *J* = 12.4 Hz, 1H), 1.99–1.74 (m, 4H), 1.70–1.09 (m, 18H), 1.06–1.02 (m, 1H), 1.04 (d, *J* = 5.9 Hz, 3H), 0.96 (s, 3H), 0.69 (s, 3H). ¹³C NMR (100 MHz, CD₃OD) δ 163.8, 72.2, 72.0, 57.5, 56.6, 44.8, 44.5, 44.1, 41.6, 40.7, 38.6, 38.0, 36.8, 36.7, 36.1, 35.2, 31.1, 29.7, 28.0, 23.9, 22.9, 22.4, 19.2, 12.6. LC/MS (ESI): *m/z* calcd C₂₄H₃₉N₄O₂ (M⁺) 415.3, found 415.6. Compound **35a** was prepared by the same method. Data for sodium 5-((*R*)-3-((3*R*,5*S*,7*R*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-3,7-dihydroxy-10,13-dimethylhexadeca-hydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)butyl)tetrazol-1-ide (**35a**): ¹H NMR (400 MHz, CD₃OD) δ 3.79 (d, *J* = 2.8 Hz, 1H), 3.38 (td, *J* = 11.0, 5.5 Hz, 1H), 2.99 (ddd, *J* = 15.0, 10.4, 4.3 Hz, 1H), 2.93–2.78 (m, 1H), 2.27 (q, *J* = 12.9 Hz, 1H), 2.06–1.16 (m, 21H), 1.16–1.07 (m, 1H), 1.05 (d, *J* = 5.9 Hz, 3H), 1.00 (dd, *J* = 14.2, 3.3 Hz, 1H), 0.93 (s, 3H), 0.68 (s, 3H). ¹³C NMR (100 MHz, CD₃OD) δ 159.2, 72.8, 69.0, 57.1, 51.5, 43.7, 43.2, 41.0, 40.7, 40.5, 36.8, 36.5, 36.2, 35.9, 35.4, 34.0, 31.3, 29.2, 24.6, 23.4, 21.8, 21.3, 18.8, 12.1. LC/MS (ESI): *m/z* calcd C₂₄H₃₉N₄O₂ (M⁺) 415.3, found 415.4.

(*R*)-4-((3*R*,5*R*,7*S*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-7-Hydroxy-3-methoxy-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)pentanenitrile (**36b**). To a suspension of NaH (60% dispersion in mineral oil, 0.072 g, 1.8 mmol) in THF (3 mL) was added a solution of **34b** (0.270 g, 0.723 mmol) in THF (3 mL). After 1 h at room temperature, MeI (0.050 mL, 0.79 mmol) was added as a solution in THF (3 mL). After 12 h at room temperature, additional MeI (0.050 mL, 0.79 mmol) was added and the reaction stirred an additional 24 h. The reaction was quenched by the addition of 1 M aqueous HCl (5 mL), and the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with saturated aqueous sodium thiosulfate (10 mL), saturated aqueous NaHCO₃ (10 mL), water (10 mL), and brine (10 mL). The organic layer was dried over Na₂SO₄, filtered, and concentrated. The crude material was purified by flash column chromatography on silica gel (5–100% EtOAc in hexanes as eluent) to obtain **36b** (0.200 g, 71% yield) as a white amorphous solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 3.88 (d, *J* = 6.7 Hz, 1H),

3.32–3.23 (m, 1H), 3.20 (s, 3H), 3.05 (dd, $J = 10.6, 5.1$ Hz, 1H), 2.56–2.52 (m, 2H), 2.41 (dt, $J = 16.8, 8.1$ Hz, 1H), 1.99–0.94 (m, 22H), 0.91–0.89 (m, 1H), 0.90 (d, $J = 6.9$ Hz, 3H), 0.89 (s, 3H), 0.63 (s, 3H). ^1H NMR (400 MHz, CD_2Cl_2) δ 3.52 (ddd, $J = 11.5, 9.0, 5.1$ Hz, 1H), 3.28 (s, 3H), 3.07 (dp, $J = 10.2, 4.7$ Hz, 1H), 2.36 (ddd, $J = 17.0, 8.8, 5.2$ Hz, 1H), 2.25 (dt, $J = 16.7, 8.2$ Hz, 1H), 2.07–0.97 (m, 24H), 0.92 (d, $J = 7.4$ Hz, 3H), 0.91 (s, 3H), 0.68 (s, 3H). ^{13}C NMR (100 MHz, CD_2Cl_2) δ 120.7, 80.5, 71.5, 56.1, 55.7, 55.1, 44.2, 44.1, 42.9, 40.5, 39.4, 37.7, 35.6, 35.2, 34.7, 34.2, 32.0, 28.9, 27.3, 27.1, 23.6, 21.5, 18.2, 14.6, 12.3. Compound **36a** was prepared by the same method. Data for (R)-4-((3R,5R,7R,8R,9S,10S,13R,14S,17R)-7-hydroxy-3-methoxy-10,13-dimethylhexadecahydro-1H-cyclopenta[*a*]-phenanthren-17-yl)pentanenitrile (**36a**): ^1H NMR (400 MHz, CDCl_3) δ 3.84 (d, $J = 3.4$ Hz, 1H), 3.34 (s, 3H), 3.02 (tt, $J = 11.1, 4.3$ Hz, 1H), 2.37 (ddd, $J = 16.9, 8.5, 5.1$ Hz, 1H), 2.27 (dt, $J = 16.8, 8.2$ Hz, 1H), 2.17–1.05 (m, 23H), 1.00–0.93 (m, 1H), 0.97 (d, $J = 6.6$ Hz, 3H), 0.91 (s, 3H), 0.68 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 120.2, 80.5, 68.4, 55.5, 55.5, 50.4, 42.8, 41.4, 39.6, 39.4, 35.9, 35.4, 35.3, 35.2, 34.8, 32.8, 31.5, 28.2, 27.0, 23.7, 22.8, 20.5, 17.9, 14.3, 11.8. TLC-MS (ESI): m/z calcd $\text{C}_{25}\text{H}_{42}\text{NO}_2$ ($\text{M} + \text{H}^+$) 388.3, found 388.7.

Sodium 5-((R)-3-((3R,5R,7S,8R,9S,10S,13R,14S,17R)-7-Hydroxy-3-methoxy-10,13-dimethyl-hexadecahydro-1H-cyclopenta[*a*]-phenanthren-17-yl)butyl)tetrazol-1-ide (37b). To a suspension of **36b** (0.150 g, 0.387 mmol) in *i*PrOH (2 mL) was added zinc bromide (0.096 g, 0.43 mmol) and sodium azide (0.028 g, 0.43 mmol) in water (2 mL). The reaction was heated via MW at 160 °C for 9 h. Starting material remained, and additional zinc bromide (0.096 g, 0.43 mmol) and sodium azide (0.028 g, 0.43 mmol) were added and the reaction was heated for 5 h. The reaction was poured into 1 M aqueous HCl (10 mL), and the aqueous layer was extracted with EtOAc (4 × 10 mL). The combined organic layers were dried over Na_2SO_4 , filtered, and concentrated. The crude material was purified by flash column chromatography on silica gel (2–10% MeOH in DCM as eluent) to obtain the tetrazole (0.086 g, 52% yield) as a white solid. To prepare the sodium salt of **37b**, a 1 cm wide column was filled with 12 cm of Dowex-50WX2 (50–100 mesh, strongly acidic) ion-exchange resin. The column was prepared by sequentially washing with 1:1 acetonitrile/water, ~1 M aqueous NaHCO_3 (caution: gas evolution), water, and finally 1:1 acetonitrile/water. The reaction product was dissolved in 1:1 acetonitrile/water and loaded onto the column, which was eluted with 1:1 acetonitrile/water. The fractions containing the product were lyophilized to furnish **37b** as a white solid. ^1H NMR (400 MHz, CD_3OD) δ 3.50–3.39 (m, 1H), 3.33 (s, 3H), 3.16 (td, $J = 10.7, 4.8$ Hz, 1H), 3.02–2.92 (m, 1H), 2.81 (ddd, $J = 15.0, 9.4, 6.6$ Hz, 1H), 2.74–2.62 (m, 1H), 2.04 (dt, $J = 12.4, 3.1$ Hz, 2H), 1.98–1.09 (m, 20H), 1.04 (d, $J = 6.0$ Hz, 3H), 1.00 (dd, $J = 10.8, 3.4$ Hz, 1H), 0.97 (s, 3H), 0.70 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 160.6, 81.7, 71.9, 57.4, 56.4, 55.9, 44.8, 44.5, 43.9, 41.5, 40.7, 38.6, 36.7, 35.9, 35.8, 35.4, 34.7, 29.6, 27.9, 27.7, 23.9, 22.4, 21.8, 19.0, 12.6. TLC-MS (ESI): m/z calcd $\text{C}_{25}\text{H}_{41}\text{N}_4\text{O}_2$ (M^+) 429.3, found 429.7. Compound **37a** was prepared by the same method. Data for sodium 5-((R)-3-((3R,5R,7R,8R,9S,10S,13R,14S,17R)-7-hydroxy-3-methoxy-10,13-dimethyl-hexadecahydro-1H-cyclopenta[*a*]phenanthren-17-yl)butyl)-tetrazol-1-ide (**37a**): ^1H NMR (400 MHz, CD_3OD) δ 3.80 (s, 1H), 3.33 (s, 3H), 3.06 (dq, $J = 10.7, 5.4, 4.1$ Hz, 1H), 3.00–2.88 (m, 1H), 2.88–2.75 (m, 1H), 2.28–1.16 (m, 22H), 1.14–1.08 (m, 1H), 1.05 (d, $J = 5.8$ Hz, 3H), 1.03–0.96 (m, 1H), 0.94 (s, 3H), 0.69 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 156.0, 82.4, 69.0, 57.3, 55.6, 51.5, 43.7, 43.1, 41.0, 40.8, 37.0, 36.8, 36.5, 36.3, 36.1, 35.8, 34.0, 29.3, 28.0, 24.6, 23.4, 22.3, 21.8, 18.9, 12.1. HRMS (ESI): m/z calcd $\text{C}_{25}\text{H}_{42}\text{N}_4\text{NaO}_2$ ($\text{M} + \text{Na}^+$) 453.3206, found 453.3164.

Sodium 5-((R)-3-((3R,5S,7S,8R,9S,10S,13R,14S,17R)-3-Methoxy-10,13-dimethyl-7-(sulfonato-oxy)hexadecahydro-1H-cyclopenta[*a*]phenanthren-17-yl)butyl)tetrazol-1-ide (38b). To a solution of **37b** (0.060 g, 0.14 mmol) in pyridine (0.7 mL) was added dropwise chlorosulfonic acid (0.06 mL, 0.9 mmol). The reaction was heated to 50 °C for 30 min and was cooled to room temperature. The reaction was quenched by the addition of 1 mL of water, and the solvents were removed by rotary evaporation. The crude material was dissolved in DMSO (0.5 mL) and 1 M triethylammonium acetate buffer (0.1 mL)

and purified by flash column chromatography (5–100% 20 mM triethylammonium acetate buffer in acetonitrile in water as eluent, C_{18} column) to yield a white solid after lyophilization. To prepare the sodium salt of **38b**, a 1 cm wide column was filled with 12 cm of Dowex-50WX2 (50–100 mesh, strongly acidic) ion-exchange resin. The column was prepared by sequentially washing with 1:1 acetonitrile/water, ~1 M aqueous NaHCO_3 (caution: gas evolution), water, and finally 1:1 acetonitrile/water. The reaction product was dissolved in 1:1 acetonitrile/water and loaded onto the column, which was eluted with 1:1 acetonitrile/water. The fractions containing the product were lyophilized to furnish **38b** as an off-white solid (0.025 g, 32% yield) as the disodium salt. ^1H NMR (400 MHz, CD_3OD) δ 4.28 (td, $J = 10.8, 5.0$ Hz, 1H), 3.33 (s, 3H), 3.16 (dt, $J = 10.8, 5.8$ Hz, 1H), 2.93 (ddd, $J = 14.7, 10.7, 4.4$ Hz, 1H), 2.86–2.63 (m, 1H), 2.28–1.10 (m, 21H), 1.06 (s, 1H), 1.04 (d, $J = 5.8$ Hz, 3H), 0.98 (s, 3H), 0.69 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 161.4, 81.6, 80.8, 56.5, 56.4, 55.8, 44.9, 43.7, 42.7, 41.2, 40.7, 36.8, 36.0, 35.8, 35.3, 35.0, 34.4, 29.7, 27.8, 27.1, 23.9, 22.4, 22.2, 19.1, 12.5. TLC-MS (ESI): m/z calcd $\text{C}_{25}\text{H}_{41}\text{N}_4\text{O}_5\text{S}$ (M^+) 509.3, found 509.5. Compound **38a** was prepared by the same method. Data for sodium 5-((R)-3-((3R,5S,7R,8R,9S,10S,13R,14S,17R)-3-methoxy-10,13-dimethyl-7-(sulfonatooxy)hexadecahydro-1H-cyclopenta[*a*]phenanthren-17-yl)-butyl)tetrazol-1-ide (**38a**): ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 4.11 (d, $J = 3.1$ Hz, 1H), 3.19 (s, 3H), 2.98–2.82 (m, 2H), 2.76 (dt, $J = 15.1, 8.0$ Hz, 1H), 2.21–1.02 (m, 23H), 0.95 (d, $J = 5.7$ Hz, 3H), 0.90–0.79 (m, 1H), 0.85 (s, 3H), 0.58 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 158.8, 82.4, 78.2, 56.9, 55.6, 51.2, 43.8, 43.0, 40.8, 40.7, 36.7, 36.4, 36.2, 36.0, 35.2, 34.7, 31.8, 29.2, 28.3, 24.4, 23.3, 21.8, 21.1, 18.8, 12.2. HRMS (ESI): m/z calcd $\text{C}_{25}\text{H}_{41}\text{N}_4\text{Na}_2\text{O}_5\text{S}$ ($\text{M} + \text{H}^+$) 555.2593, found 555.2586.

Biology. Isolation and characterization of *C. difficile* isolates was performed as previously reported.¹³

***C. difficile* Spore Germination Assays. Optical Density Assay.** BHIS media was spiked with TCA to a final concentration of 2000 μM to test compounds for inhibition of germination. Either 130 μL of BHIS media (negative control) or the BHIS with 2000 μM TCA was added to separate wells of a 96-well plate in triplicate. The test compound (10 μL) in solvent (typically DMSO or DMSO/ethanol) was added to both the BHIS and BHIS with TCA in triplicate. In addition, BHIS with 2000 μM TCA (positive control) was amended, in triplicate, with solvent (DMSO or DMSO/ethanol). BHIS with DMSO or DMSO/ethanol and filter sterilized water were also run in duplicate as a negative control. The 96-well plates containing the solutions were reduced in an anaerobic chamber for 2–3 h. Spores, at a ~0.5 McFarland standard concentration, were heated to 65 °C for 30 min before being inoculated into the test media, with or without spiked bile acid analogues solutions, within an anaerobic bag under nitrogen gas. The OD_{600} was measured once every minute for 20 min with an EL808 microplate reader (Biotek Instruments, Inc., Winooski, VT) and normalized using the OD_{600} obtained at time zero [relative $\text{OD}_{600} = \text{OD}_{600}(t)/\text{OD}_{600}(t_0)$].

Microscope Spore Count Assay. Media and spore preparation for phase contrast assay was performed in the same method as outlined in the optical density assay above. t_0 samples were immediately placed on ice, while the t_{20} samples were incubated at room temperature for 20 min. Following the 20 min incubation, the t_{20} samples were also placed on ice to prevent further germination. Then 10 μL of each of the triplicate samples were removed and counted on a hemocytometer under a Plan 40/0.65 Ph2 lens for counts of spores and germinated cells. Experiments were performed in triplicate and average percentage of germination was determined.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.7b00295.

Inhibitory activity of 7-UDCA in a NAP1 strain of *C. difficile*, synthetic scheme for **11a**, and graphs of relative

OD₆₀₀ vs time and phase-contrast microscopy data for all compounds tested (PDF)
Molecular formula strings (CSV)

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

A-B, apical to basolateral; app, apparent; ASBT, apical sodium-dependent bile acid transporter; BHIS, brain–heart infusion supplemented; *C. difficile*, *Clostridium difficile*; CDAD, *C. difficile*-associated disease; CDI, *C. difficile* infection; CA, cholic acid; CDCA, chenodeoxycholic acid; DCM, dichloromethane; DIPEA, diisopropylethylamine; DMA, dimethylacetamide; DMAP, dimethylaminopyridine; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; ELSD, evaporative light scattering detection; EtOAc, ethyl acetate; Et₂O, diethyl ether; FMT, fecal microbiota transplantation; HATU, 1-[bis(dimethylamino)-methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate; HRMS, high resolution mass spectrometry; LC-MS, liquid chromatography–mass spectrometry; MeOTf, methyl trifluoromethanesulfonate; MW, microwave; NAP1, North American pulsotype 1; NMR, nuclear magnetic resonance; P_{app} , apparent permeability; pTSA, *para*-toluenesulfonic acid; R-CDI, recurrent *C. difficile* infection; SAR, structure–activity relationship; SEM, standard error of the mean; TBAF, tetrabutylammonium fluoride; TBS, tertbutyldimethylsilyl; TCA, taurocholic acid, taurocholate; TEA, triethylamine; TFAA, trifluoromethylacetic anhydride; THF, tetrahydrofuran; TLC, thin layer chromatography; TMS, trimethylsilane; UDCA, ursodeoxycholic acid; UV, ultraviolet

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