Divergent synthesis of complex withanolides enabled by a scalable route and late-stage functionalization.

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Abstract: Withanolides are a group of naturally occurring C_{28} steroids based on an ergostane skeleton. They possess a high degree of polyoxygenation, and the abundance of *O*-functional groups has enabled various natural alterations to both the carbocyclic skeleton and the side chain. Consequently, these molecules possess intricate structural features that lead to their highly varied display of biological activities including anticancer, anti-inflammatory and immunomodulating properties. Despite being intriguing leads for further discovery research, synthetic access to the withanolides remains highly challenging—compounds for current biological research are mainly isolated from plants, often inefficiently. Here we report the divergent synthesis of eleven withanolides in 12 to 20 steps, enabled by a gram scale route and a series of late-stage functionalizations, most notably a bioinspired photooxygenation-allylic hydroperoxide rearrangement sequence. This approach sets stage for further biological research disconnected from a reliance on minute quantities of the parent natural products or their simple derivatives.

Introduction. Across the centuries, humanity has consistently turned to nature as a reliable source for fulfilling their fundamental requirements, including the provision of medicines essential for combating a diverse range of ailments.[1] Extracts from *Withania somnifera*, known by its Sanskrit name “Ashwagandha”, “Indian ginseng” or “Winter cherry”, have been used for over 3000 years in traditional medicine in India within the Ayuvedia system.[2] Many of the therapeutic properties of this extract may be associated with the presence of withanolides.[3,4] Since the isolation of the first withanolide, withaferin A, by Lavie in 1962,[5,6] this class of natural products has attracted enormous interest from biologists and chemists. To date, almost 1200 withanolides have been isolated and identified largely from the Solanaceae (nightshades) distributed widely among the world’s temperate and tropical zones.[3] The withanolides are a group of naturally occurring C_{28} steroids based on an ergostane skeleton in which C22/C26, or C23/C26, are oxidized to form a δ- or γ-lactone. One of the characteristic features of the plants producing withanolides is their extraordinary ability to introduce oxygen functionality at nearly every position of the carbocyclic skeleton and side chain (Fig. 1A). These intricate and stereochemically complex oxidation patterns have led to myriad biological activities that include anti-inflammatory, antitumor, immunomodulating, cancer chemopreventive, antibacterial and antifungal properties.[7,8] Further, some withanolides are reported to have the potential to be safely used as preventive and therapeutic interventions for COVID-19.[9,10] In recent years, there has been an explosion of interest in the West regarding the potential health benefits of the supplement Ashwagandha, particularly in the areas of stress management, cognitive function, and physical performance.[9]

Given the wide-ranging interest detailed above, it is unsurprising that a wealth of existing literature describes either the uses of withanolides in health supplements (>5250 publications) or in their biological activities, of both the natural products themselves and their close derivatives (>2000 publications) (Fig. 1B). In direct contrast, only five semi-syntheses have been reported, none of which were demonstrated to be either divergent or scalable: jaborosalactones A, B, and D,[11] withaferin A and 27-deoxywithaferin A,[12] withanolide D,[13] withanolide E,[14] and withanolide A.[15] There are also various reports of partial motif construction[16-23] and derivatization efforts.[8] As a consequence of the limited synthetic success, almost all primary material used in biological or clinical research are obtained from natural sources, which not only involves time-consuming extraction and separation protocols, but also limits effective medicinal chemistry and discovery studies beyond simple derivatizations of isolated natural products. An analysis of the structural characteristics of the withanolides reveals the majority of the

variations are derived from *O*-functionality, often on otherwise unactivated positions. For example, withanolide D and withaferin A are constitutional isomers, differentiated only by the placement of a single hydroxyl group (C20 vs. C27). However, in practice, this has resulted in the need for completely different, lengthy synthetic routes to these two molecules (20 and 32 steps to withanolide D and withaferin A, respectively). From the standpoint of chemical synthesis, the deliberate disruption of barriers between closely related molecules exhibiting only differences in oxygenation pattens is expected to significantly streamline the acquisition of withanolides, as opposed to pursuing a singular target-oriented approach for each molecule. The creation of a scalable, divergent and flexible pathway to withanolides would not only tackle the supply issue but also facilitate straightforward access to an array of unnatural analogs for conducting biological activity studies.

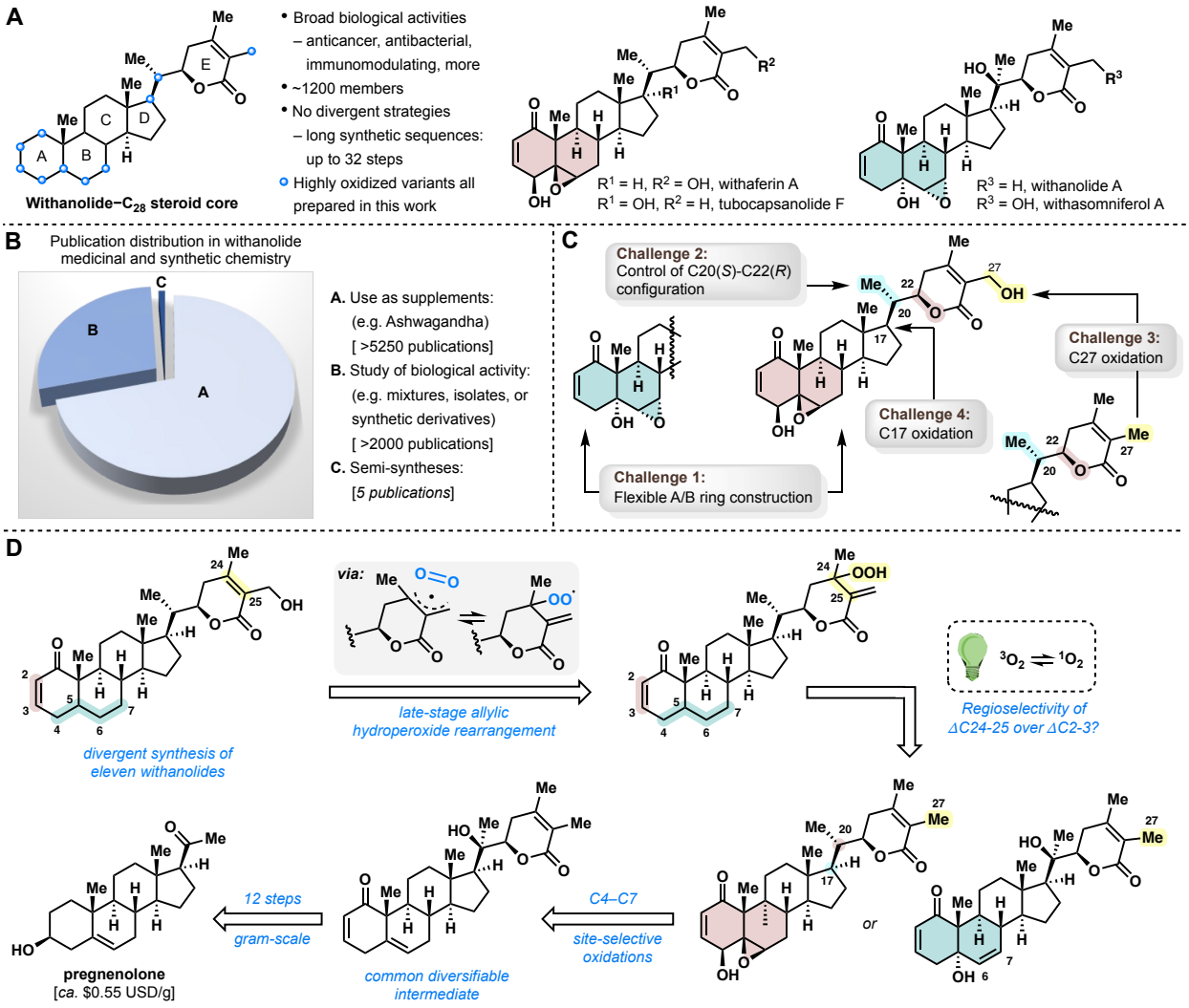

Fig. 1. Significance, challenges, and synthetic strategy of the withanolide natural products. (**A**) Withanolide core structure and representative examples. (**B**) Ratio of publication in withanolide medicinal and synthetic chemistry. (**C**) Key synthetic challenges. (**D**) Retrosynthetic analysis for a divergent synthesis of withanolides.

The synthetic challenges posed by the withanolides displayed in Fig. 1A are considerable and include: (1) construction of the highly oxidized variable A/B rings with concomitant assembly of the lactone side chain; (2) control of relative stereochemistry throughout the molecules, but particularly the δ-lactone construction with vicinal C20(*S*)-C22(*R*) configuration; (3) installation of C27 hydroxy group—which was responsible for much of the long synthetic route in the previous withaferin A synthesis;[12] and (4) site- and stereoselective introduction of a hydroxyl group at C17, allowing entrance into the tubocapsanolides (Fig. 1C). To address the above-mentioned

challenges, we propose a unified synthetic strategy that leverages a practical, scalable semi-synthesis to enable new, diversity-oriented routes through a series of late-stage functionalizations. In a retrosynthetic sense, a late-stage oxidation would greatly facilitate the introduction of the C17 and C27 hydroxy groups. We surmised that the oxidation at C27 in withanolides most likely occurs in nature by auto-oxidation with O_2 and rearrangement under sunlight. With this bioinspired hypothesis, a Schenck ene-allylic hydroperoxide rearrangement sequence was envisioned, which requires regioselective engagement of the lactone system over various permutations of the A/B ring. For a fully divergent synthesis, the two classical types of A/B ring systems (1-oxo-2-ene-4β-hydroxy-5β,6β-epoxy, shaded red, and 1-oxo-2-ene-5α-hydroxy-6α,7α-epoxy, shaded green) would need to be accessed by different oxidation reactions on C4-C7 with both regio- and stereochemical control from a common diversifiable intermediate, itself a natural product (Fig. 1D).[24] Historically, the steroid nucleus framework has served primarily for the development of new synthetic strategies in bioactive terpenoid and steroid synthesis,[25] however, in our context, we seek to harness the inherent practicality and cost-effectiveness of semi-synthesis. The common intermediate could be assembled in a scalable fashion by a sequence of choreographed oxidation reactions and a vinylogous aldol reaction-cyclization to construct the δ-lactone from pregnenolone, an economical and commercially available starting material (*ca.* US$0.55/g). The interplay of the combined strategies and practicality, which facilitated the synthesis of eleven withanolides, distinguishes this synthesis from previous reports and may serve as inspiration for future synthetic endeavors.

Gram-scale synthesis of withanolide D. To realize this goal, the development of a practical and scalable route to access both natural product **11** and withanolide D (**13**) was required (Fig. 2). The route commenced with a protecting group-free diastereoselective 1,2-addition of 1,3-dithiane to pregnenolone (**1**) on decagram scale. Oppenauer oxidation of diol **2** delivered enone **3** in near quantitative yield. The protection of hindered tertiary alcohol **3** with chloromethyl methyl ether (MOMCl) afforded both enone **4** (62% yield) and the globally protected product (32% yield), which, upon treatment with HCl, was recycled to **3** in 80% yield. After one series of recycling, enone **4** was obtained in 83% yield from **3** (see Supplementary Information). Various oxidation conditions (DDQ, IBX, and Saegusa oxidation) were then screened for their ability to deliver dienone **5** in high yield (see Table S1). Ultimately, the Mukaiyama dehydrogenation produced **5** in 87% yield while setting the stage for the kinetic migration of the C4-C5 double bond to C5-C6. The olefin isomerization exhibited sensitivity to reaction time, with longer reaction times (e.g. 1 hour) resulting in lower yields due to formation of the A ring aromatized product. Luche reduction produced alcohol **7** with >20:1 diastereoselectivity. After extensive experimentation, it was determined that the side chain needed to be installed at intermediate **8**, before the introduction of further electrophilic centers. Treatment with *N*-bromosuccinimide (NBS) at low temperature facilitated a seamless oxidative cleavage of the 1,3-dithiane, resulting in formation of aldehyde **8** without affecting the allylic alcohol in the A ring. Addition of the vinylogous enolate generated from 2,3-dimethylbut-2-enolate and lithium hexamethyldisilazide (LiHMDS) to aldehyde **8** and spontaneous cyclization delivered lactone **9** in 78% yield. Notably, this was done without protection of the A ring allylic alcohol.

With the lactone in place, modulation of the A/B ring oxidation state continued by the treatment of **9** where 2-nitrophenyl selenocyanate and tributylphosphine gave selenide **10** in 94% yield. A seleno-Mislow-Evans rearrangement under *meta*-chloroperoxybenzoic acid (*m*CPBA) conditions, followed by treatment with sodium methoxide (NaOMe) in one pot gave the corresponding allylic alcohol (not shown). Treatment with Dess-Martin and deprotection of the MOM group under acidic conditions delivered the common diversifiable intermediate (and natural product) **11** in 12 steps on multigram scale (2.0 grams obtained in a single pass). This three-step sequence required only one purification at the end, delivering enone **11** with 79% overall yield from selenide **10**. Installation of the C4 β-hydroxy group was required to occur, both chemo- and stereoselectively, without reaction at the four other available allylic positions on either the B ring or lactone. After careful optimization, selenium dioxide in dioxane was found to give complete stereoselectivity and the highest regioselectivity. Alcohol **12** was isolated in 71% yield on gram scale (92% yield, based on recovered starting material). Both the use of dioxane (instead of dichloromethane) and ceasing the reaction prior to full conversion avoided the undesirable C6 oxidized isomer. Next, we attempted to introduce the epoxide using Ikekawa's condition with *m*CPBA.[13] However, it was found that this condition gave the C5-C6 epoxide in a ratio of 2:1 (β:α) on larger scales. Instead, the titanium mediated epoxidation afforded withanolide D (**13**) in 75% yield as a single diastereomer with no

observed reaction at the C2-C3 olefin. The 14 step, scalable route allowed for 919 mg of withanolide D to be prepared in a single pass and set the stage for the forthcoming divergent synthesis.

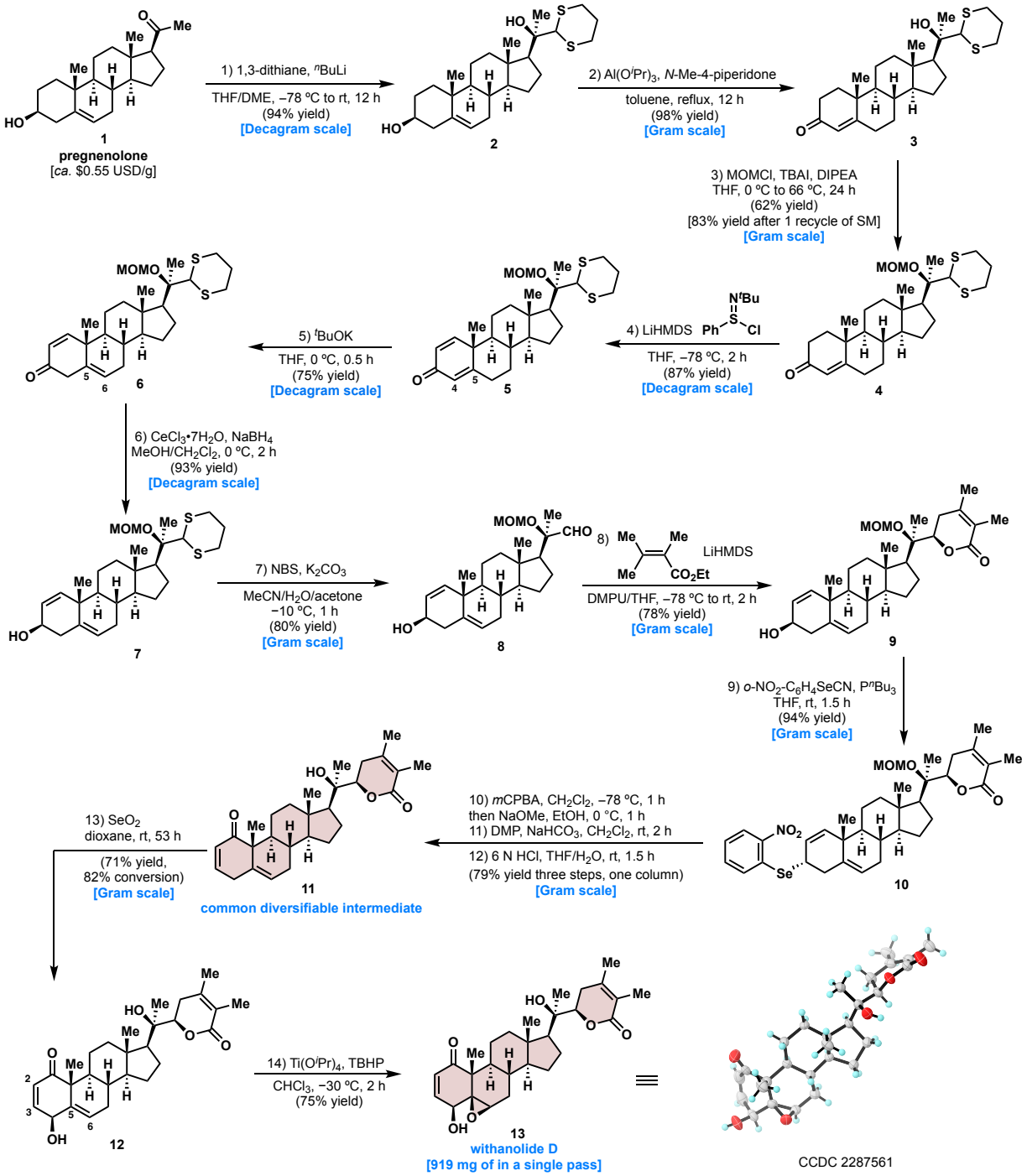

Fig. 2. Scalable synthesis of common diversifiable intermediate and withanolide D.

Further divergent synthesis and the development of the late-stage C27 oxidation. With an established practical route to both key intermediate **11** and withanolide D (**13**), we proceeded to synthesize nine other withanolides. Withanolide D (**13**) was converted to **14**[26] by regioselective epoxide-ring opening with $CeCl_3 \cdot 7H_2O$ in 86% yield. Selective hydrogenation of the C2-C3 double bond using palladium on carbon as a catalyst under ambient pressure delivered 2,3-dihydrowithanolide D (**15**)[27] in 93% yield (Fig. 3A).

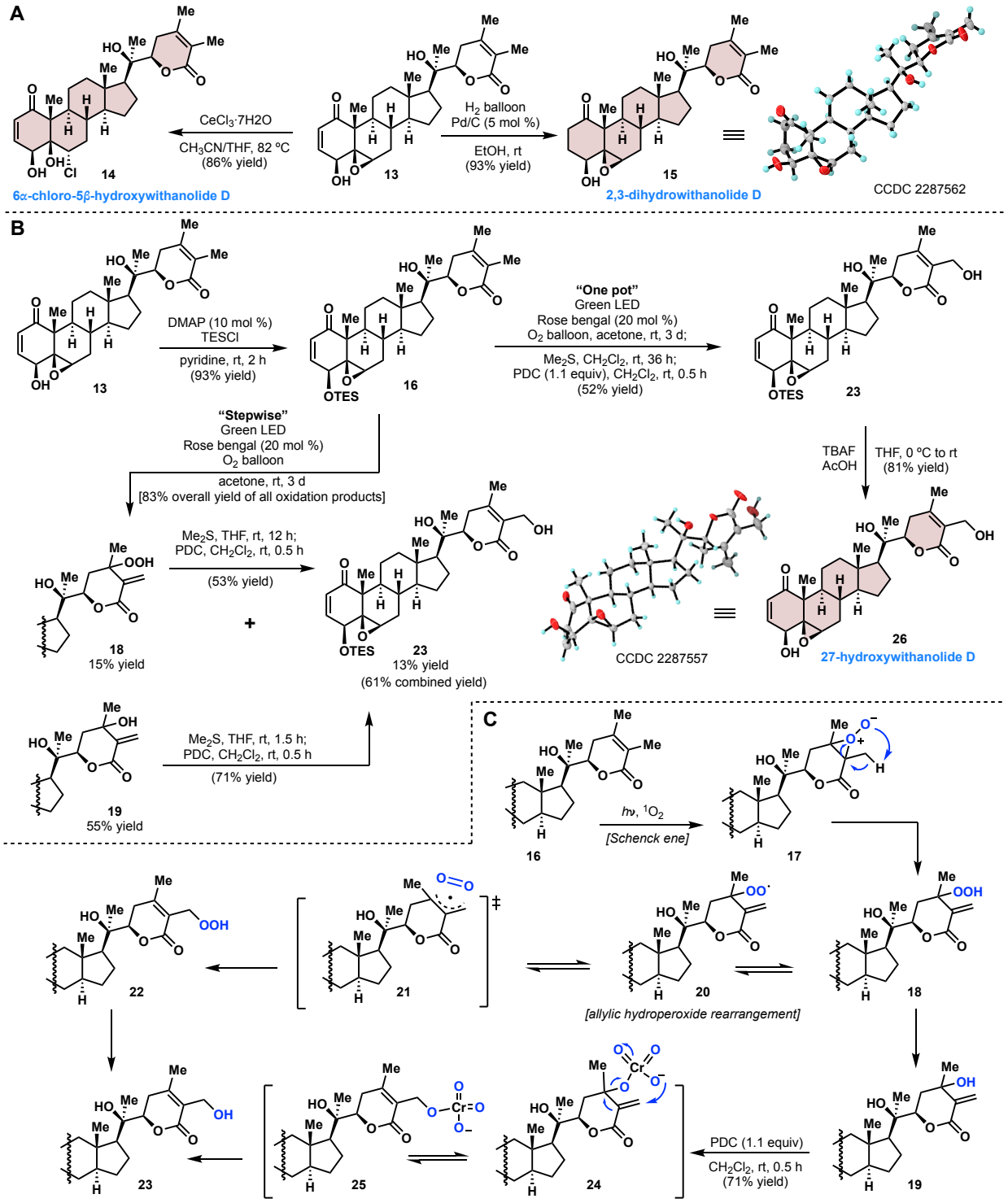

Fig. 3. Divergent synthesis of the withanolides and late-stage C27 oxidation. (**A**) Diversification of withanolide D. (**B**) Development of C27 oxidation and synthesis of 27-hydroxywithanolide D. (**C**) Mechanism of the Schenck ene-allylic hydroperoxide rearrangement.

The oxidation state of C27 is a common modification among naturally occurring withanolides. Therefore, a key challenge is the late-stage oxidation of C27 in the presence of not only a substantial amount of sensitive functionality, but also multiple competing sites for potential oxidation. Since C27 is both a β-position of the lactone and an allylic position, initial attempts were made under radical conditions such as NBS with AIBN or light. However, despite much optimization, only complex mixtures resulted. Inspired by the work of Foote and

coworkers,[28] we instead turned to the photooxygenation of α,β-unsaturated lactones. Initial triethylsilyl (TES) protection of the C4 β-hydroxy group afforded **16** in 93% yield. Subjection of **16** to Schenck ene conditions using Rose bengal as catalyst under green LED light showed significant consumption of the starting material. Analysis of the reaction mixture revealed three main products: hydroperoxide **18**, oxygen addition product **19** and the desired C27 oxidized product **23**. The reaction required three days to achieve full conversion during which time hydroperoxide **18** gradually converted to **19** and **23**. Despite the extensive investigation of photosensitized oxygenation reactions in alkenes and enol ethers,[29] reports involving unsaturated ketones or esters are scarce,[30] particularly in the context of natural product synthesis. We speculated that with an appropriate allylic alcohol transposition method, both **18** and **19** could be isolated and transferred to desired product **23**. Indeed, a pyridinium dichromate (PDC)-mediated transposition afforded **23** in 53% and 71% yield, from **18** and **19**, respectively. C27 hydroxy **23** was obtained in a 61% overall combined yield from **16** (Fig. 3B). The mechanism for this reaction presumably involves two stages (Fig. 3C). Singlet oxygen reacts with the electron-deficient olefin in the lactone of **16** forming perepoxide intermediate **17**, which is followed by abstraction of the β-proton that gives rise to the hydroperoxide intermediate **18**.[29] The allylic hydroperoxide rearrangement involves a caged allylic radical-dioxygen pair transition state followed by a [3C,2O] sigmatropic shift, which delivers rearranged hydroperoxide product **22**.[31,32] The same overall transformation of **19** to **23** was achieved through a chromate ester upon treatment with PDC (Fig. 3C). A streamlined one-pot procedure was subsequently developed where **16** was sequentially treated with the photooxygenation condition, dimethyl sulfide (DMS), and PDC to afford **23** directly in 52% yield. After successful oxidation of the C27 position, attempted removal of the TES group under conventional *tetra-n*-butylammonium fluoride (TBAF) condition led to decomposition. It was eventually determined that the basicity of the TBAF solution led to detrimental effects (Table S14). Thus, treatment of **23** with acetic acid-buffered TBAF furnished 27-hydroxywithanolide D (**26**)[33] in 81% yield.

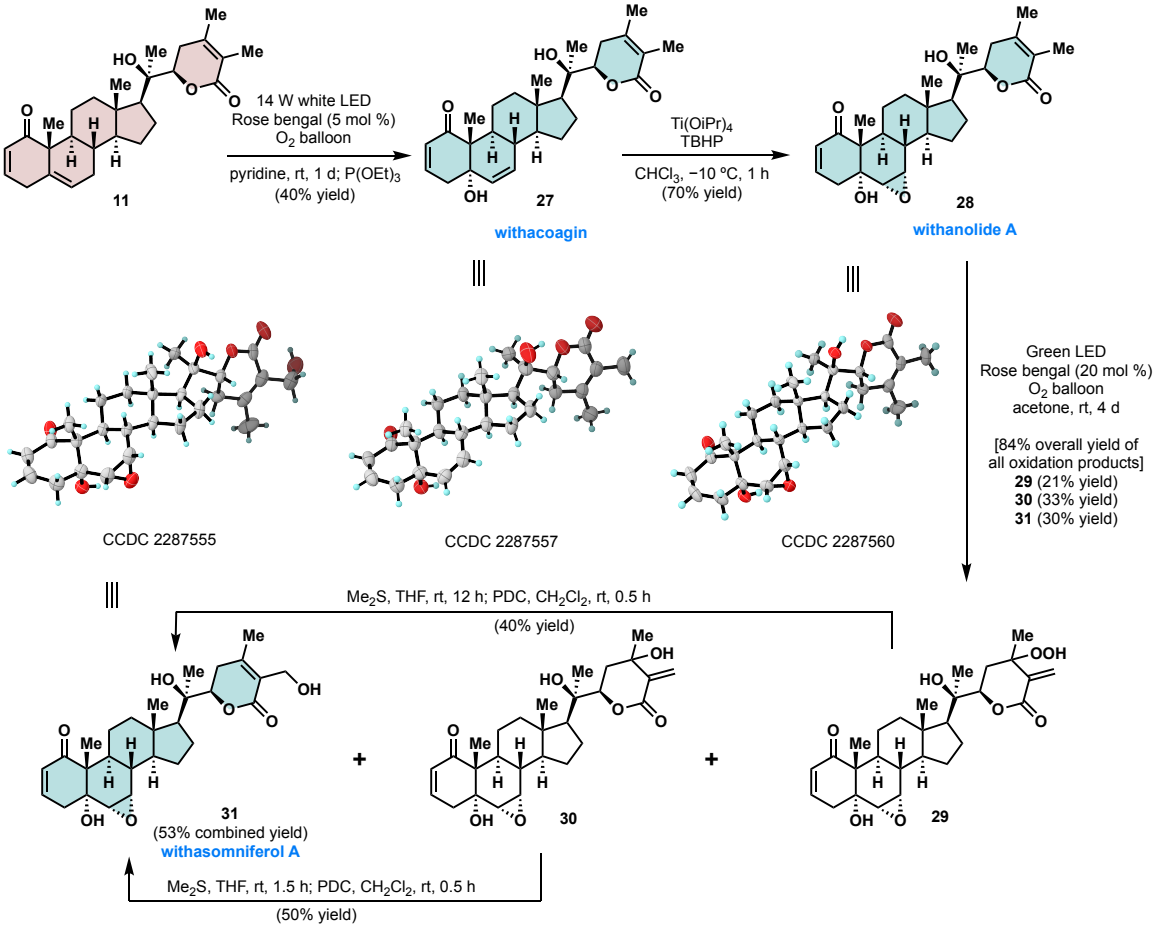

Fig. 4. Synthesis of withacoagin, withanolide A, and withasomniferol A.

Diversification to alternate A/B ring withanolides. After establishing the A/B ring oxidation pattern of withanolide D and the C27 late-stage hydroxylation, we turned our attention towards other representative withanolides that contained an alternative A/B ring structure (exemplified by withanolide A), further highlighting the versatility of readily accessible intermediate **11** (Fig. 4). Installation of the C5 α-hydroxy proved to be the key transformation. Inspired by the pioneering work of Gademann,[15] common intermediate **11** was subjected to photooxygenation conditions; using pyridine as solvent with white LEDs afforded withacoagin (**27**)[34] in 40% yield. Attempts to further optimize the oxidation conditions including changing solvents, photocatalysts, light sources, as well as varying reductants led to no significant improvement of the yield (see Table S15). A byproduct was identified in which the peroxide bridge formed between C2 and C5, however, photooxygenations of a starting material without the C2-C3 olefin led to complex mixtures with no trace of the desired product. Withacoagin was then treated with titanium isopropoxide and *tert*-butyl hydroperoxide (TBHP) to deliver withanolide A (**28**)[35] in 70% yield as a single diastereomer. Withanolide A (**28**) was then subjected, without protection, to the Schenck ene-rearrangement conditions developed in the synthesis of 27-hydroxywithanolide D (**26**). This resulted in an 84% yield of hydroperoxide **29**, alcohol **30** and desired natural product withasomniferol A (**31**).[36] In a similar fashion to the conversion of **16** to **23**, hydroperoxide **29** formed first and gradually transformed to **30** and **31**. Peroxide **29** and alcohol **30** were treated separately with DMS followed by PDC to afford withasomniferol A (**31**) in 53% combined overall yield. It is notable that withasomniferol A was prepared from **11** without the aid of protecting groups, highlighting the mild and effective conditions of the bioinspired C27 oxidation method.

Divergent synthesis of withaferin A and tubocapsanolide F. Due to its promising therapeutic potential, withaferin A has garnered significant and enduring interest from discovery chemists. One of the most promising hallmarks of withaferin A is its anticancer and chemo-preventive potential, which has been delineated by multiple studies from various research groups.[37] Despite this, the 32-step synthesis by Ikekawa remains the only known report of its preparation.[12] With both the correct A/B ring pattern and C27 oxidation in hand, it remained to excise the C20 hydroxyl group with retention of the correct stereochemistry. It is well-precedented that conducting deoxygenations at positions with significant steric hindrance is highly challenging; in this specific case, the requirement to do so in a complex molecular environment further increases the difficulty. To this end, numerous deoxygenation methods were investigated. Protocols based on acid-mediated activation of the tertiary alcohol followed by reductive quenching gave either no conversion (at room temperature) or decomposition (at elevated temperatures). Radical-associated deoxygenation strategies proved troublesome due to the aforementioned challenge of functionalizing the C20 hydroxyl group. Specifically, attempts to convert it into conventional precursors such as xanthates, thiocarbamates, *m*-CF_3 benzoates or *N*-phthalimidoyl oxalate derivatives were unsuccessful—all resulted in either no conversion or the decomposition of starting material (see Fig. S14, S15). Confronted with these failures, we elected to pursue an elimination-reduction approach. Initial attempts with thionyl chloride ($SOCl_2$) in pyridine led to decomposition of starting material, while Burgess' reagent gave no conversion. Instead, Martin's sulfurane delivered exo-double bond product **32** from TES-protected withanolide D **16** in 86% yield within 1 h (Fig. 5A). With **32** in hand, different hydrogenation catalysts were investigated. Palladium on carbon, homogenous Crabtree's catalyst and Wilkinson's catalyst resulted in reduction of the C2-C3 double bond while leaving the C20-C21 alkene intact. Various metal hydride atom transfer reactions led to either recovery of the starting material or decomposition (see Table S24). To our delight, platinum dioxide produced desired product **33** in 75% yield with a 2:1 d.r. The C2-C3 double bond reduced first, within ~6 h, while the C20-C21 alkene reacted more slowly. Attempts to improve the d.r. with solvent (MeOH, EtOAc) or additives were unsuccessful. Regeneration of the C2-C3 unsaturation proved more recalcitrant than expected: the previously used Mukaiyama dehydrogenation failed and treatment with IBX resulted in either no conversion or cleavage of the B ring epoxide at various temperatures. Surprisingly, treatment of **33** with benzeneseleninic acid anhydride delivered enone **34** in 88% yield. A telescoped deprotection using TBAF provided 27-deoxywithaferin A (**35**) in 73% yield from **34** in one-pot. Exposure of the TES-protected 27-deoxywithaferin A **34** to the Schenck ene-rearrangement conditions gave TES-protected withaferin A **38** through an identical pathway as described before resulting in a 63% combined yield. Deprotection with a buffered TBAF solution completed the synthesis of withaferin A (**39**).

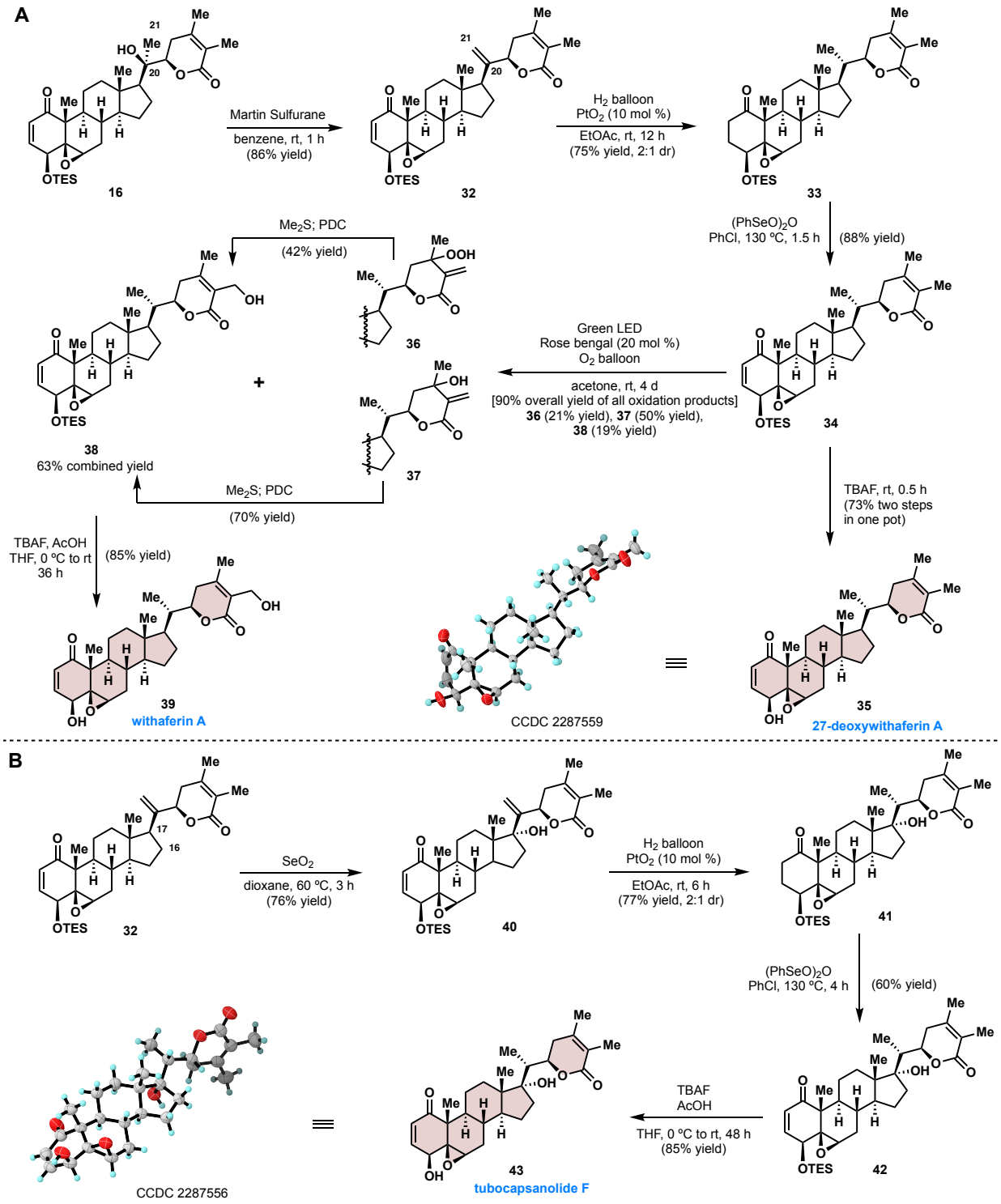

Fig. 5. Synthesis of withaferin A and C17 oxidation en route to tubocapsanolide F. (**A**) Synthesis of withaferin A and 27-deoxywithaferin A. (**B**) Synthesis of tubocapsanolide F.

Tubocapsanolide F was first isolated in 2007 and represents a sub-family of withanolides in which both C16 and C17 are oxidized.[38] It has shown significant cytotoxic activity against numerous liver, breast, and lung cancer cell lines. The C16 and C17 C–H bonds of withanolides and related scaffolds are often difficult to manipulate due to the lack of proximate chemical handles, although Breslow's elegant remote functionalization approach represents one alternative solution to this problem.[39] Here we leveraged our divergent route through intermediate

32 to prepare tubocapsanolide F (Fig. 5B). Exposure of **32** to selenium dioxide in dioxane at 60 ºC resulted in the site-selective and stereospecific oxidation of C17 to afford tertiary allylic alcohol **40** in 76% yield. Hydrogenation by platinum dioxide at ambient pressure delivered product **41** in 77% yield (2:1 d.r.). Regeneration of the C2-C3 double bond occurred in 60% yield followed by deprotection to give tubocapsanolide F.

In summary, a gram-scale route to both a common intermediate and withanolide D enabled eleven withanolides to be synthesized in 12 to 20 steps with eight structures confirmed by X-ray crystallography. Two types of classical A/B ring structures, typified by withaferin A and withanolide A, were prepared via selective allylic oxidations and photochemical Schenck ene reactions. A bioinspired photochemical oxygenation-allylic hydroperoxide rearrangement sequence enabled the late-stage C27 C–H oxidation, which significantly improved the step economy of the syntheses compared to previous reports. A site- and stereoselective allylic oxidation on C17 resulted in the late-stage functionalization of the D ring without the use of a pre-installed templated directing group. The brevity, scalability, modularity, and diversity-oriented nature of this route is expected to provide a wide range of withanolides and allow for the deliberate preparation of novel analogs that can be more appropriately tuned with an eye toward the creation of novel therapeutics.

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Competing interests: A patent application naming JML and WC as inventors has been filed by H. Lee Moffitt Cancer Center and Research Institute, which covers the synthetic routes to the withanolides.

Data and materials availability: All data including the experimental procedures, and compound characterization data are available in the main text or supplementary materials. Crystallographic data for compounds **13**, **15**, **26**, **27**, **28**, **31, 35** and **43** are available free of charge from Cambridge Crystallographic Data Center.

Supplementary Information
Materials and Methods
Figures. S1 to S24
Tables S1 to S40
NMR spectra
X-ray data
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