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Total Synthesis of Celastrol, Development of a Platform to Access Celastroid Natural Products

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Supporting Information

ABSTRACT: Celastroid natural products, triterpenes, have been and continue to be investigated in clinical trials. Celastrol, and for that matter any member of the celastroid family, was prepared for the first time through chemical synthesis starting from 2,3-dimethylbutadiene. A triene cyclization precursor generated in 12 steps underwent a nonbiomimetic polyene cyclization mediated by ferric chloride to generate the generic celastroid pentacyclic core. In the cyclization, engagement of a tetrasubstituted olefin formed adjacent all carbon quaternary centers stereospecifically. With access to the carbocyclic core of the family of natural products, wilforic acid and wilforol A were prepared en route to racemic celastrol.

Celastrol was initially isolated from *Tripterygium wilfordii* (thunder of god vine) and later identified in a variety of plant species in the *Celastracae* family.[1] The natural product has a wide array of promising activities relevant to neuronal degeneration,[2,3] inflammation,[4] diseases caused by protein misfolding,[5,6] cancer,[7] and obesity.[8] The related celastroid natural product, tingenone (**2**), has been examined in clinical trials for skin, stomach, and uterine cancers and lymphoepithelioma and shown to possess moderate activity with minimal side effects (Figure 1).[9,10] Medicinal chemistry optimization of the triterpene oleanolic acid arrived at CDDO methyl ester (**5**). While not a member of the celastroid family, CDDO methyl ester is similarly related, as it possesses a strong Michael acceptor within a triterpene scaffold and continues to be used in multiple clinical trials. Recently, whole extracts from *T. wilfordii* containing celastrol and triptolide underwent clinical trials for HIV, Lupus, Crohn's, and kidney diseases, and rheumatoid arthritis which are at various stages of completion.[11] The therapeutic and biological effects of the celastroids are predominantly mediated by covalent modification of their targets by reaction of biological nucleophiles with the embedded quinone methide functionality.[1,12a] This reaction has been shown to be stereospecific, potentially engendering specificity for celastrol.[12b] Additionally, covalent modification has been examined in the context of celastrol interacting with DNA as well as modifying cysteines of different protein targets.[4,5,12,13]

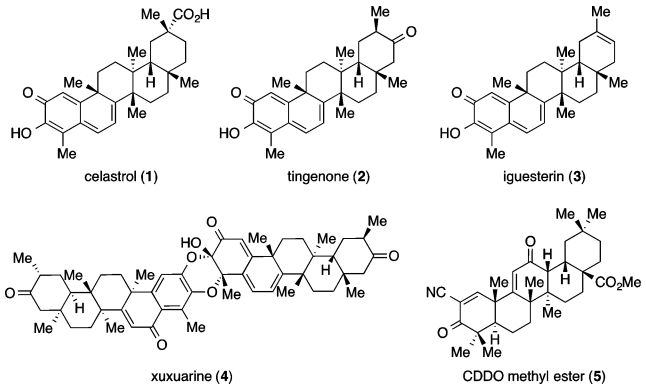

Figure 1. Structures of celastroid natural products and CDDO methyl ester.

The lack of an ability to synthetically access the celastroid natural products, including iguesterin (**3**) and xuxuarine A (**4**), has limited scientists' understanding of the compounds' chemistry and biology. The absence of a synthesis can, in part, be attributed to the incompatibility of biologically inspired polyene cyclization strategies for the assembly of the celastroids or, for that matter, any members of the friedelin-type of triterpenes. These reactions are highly desirable as they provide rapid access to relatively large molecules with multiple stereogenic centers in a single transformation from linear, polyunsaturated precursors.[14,15] Unfortunately, however, the biological polyene cyclization leading to celastrol is exceedingly difficult to reproduce in the laboratory due to a set of complex and energetically unfavorable methyl and hydride shifts.[16−18] The closest natural products to celastrol to be synthesized were the pentacyclic triterpenes, alnusenone, and friedelin, both of which were prepared by Ireland.[19−21] Attempts to implement polyene cyclizations for these natural products failed to improve access to the natural products, however, were inspirational for our studies.[22,23]

In our laboratory, the synthesis of celastrol was initiated by bromination of 2,3-dimethylbutadiene producing a single isomer of the crystalline *trans* 1,4-dibromide **6** in high yield after recrystallization (Scheme 1).[24] Treatment of the lithium anion of *N*,*N*-dimethylacetamide with the dibromide proceeded well on 15 g scale to generated the bis-amide **7** in 91% yield after recrystallization (mp 69−71 °C). Slow addition of a methyl lithium solution into a mixture of the amide in THF at

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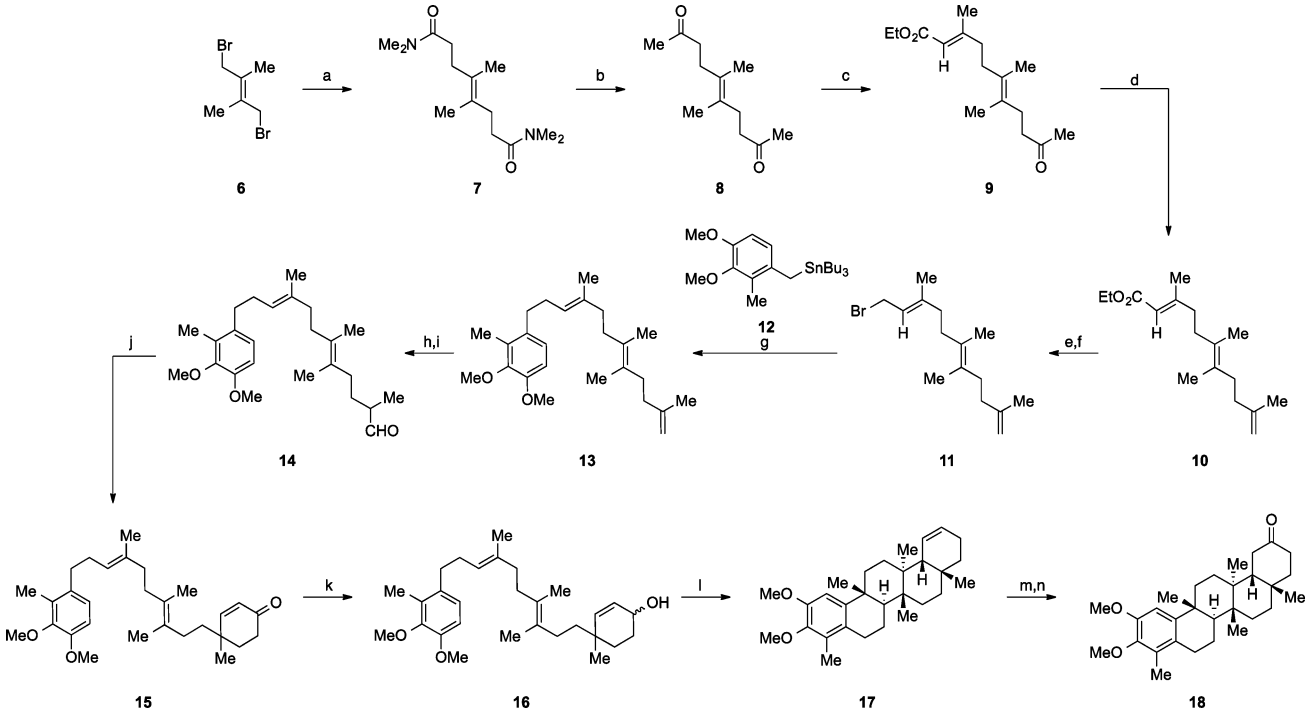
Scheme 1. Synthesis of Ketone 18[a]



[a]Reagents and conditions: (a) LDA, Me_2NCOMe, THF, −78 °C, 3.5 h, 91%. (b) MeLi, THF, −78 °C, 30 min, 89%. (c) Triethyl phosphonoacetate, NaHMDS, PhMe, 0 to 60 °C, 24 h, 59%, 6:1 *E*/*Z*. (d) *n*-BuLi, Ph_3PMeBr, THF, 0 °C, 1.5 h, 85%. (e) $LiAlH_4$, Et_2O, −78 °C to −20 °C, 5 h, 93%. (f) PBr_3, Et_2O, −20 to 0 °C, 2 h. (g) **12**, *n*-BuLi, THF, − 78 °C, 78% 2-steps. (h) 9-BBN, THF, 0 °C; then H_2O_2, NaOH, THF/H_2O, 0 to 23 °C, 60 h, 88%. (i) DMP, $NaHCO_3$, CH_2Cl_2, 0 °C, 80 min, 88%. (j) C_4H_9N, PhMe, 23−110 °C, 24 h, then methyl vinyl ketone, PhMe, 23−82 °C 72 h, 83%. (k) $LiAlH_4$, Et_2O, 0 °C, 5 min, 99%, dr 1.4:1. (l) $FeCl_3$, CH_2Cl_2, −78 °C to −20 °C, 6 h, 38%. (m) BH_3, THF, −78 to 23 °C, then H_2O_2, NaOH, THF/H_2O, 0 to 23 °C. (n) Jones reagent, Me_2CO, −78 °C to −10 °C, 2 h, 60% 2-steps.

−78 °C proved optimal to afford the crystalline diketone **8** in 89% yield (mp 55−57 °C). Horner−Wadsworth−Emmons olefination desymmetrized the diketone providing the enoate **9** as an inseparable isomeric mixture of olefins in 59% yield with an *E*/*Z* ratio of 6:1. Notably, the reaction exceeded a statistical derived maximum of 50%. Wittig olefination, using methyl triphenylphosphorane, cleanly provided triene **10**. The *cis*/*trans* isomers were separated at this stage by silica gel chromatography accessing isomerically pure **10**. The undesired *cis*-enoate ester can be isomerized to the desired *trans*-enoate ester with freshly prepared sodium ethoxide in ethanol to form the desired isomer in 51% yield. Reduction with $LiAlH_4$ provided the allylic alcohol which was treated with PBr_3 to afford the corresponding activated bromide **11** which was used in the subsequent displacement reaction without purification. Tin−lithium exchange of stannane **12** (see Supporting Information for synthesis, five steps from vanillyl alcohol,[25] 54% overall) using *n*-butyllithium generated a red-orange solution of the toluyl anion in THF which was caused to react with **11** at −78 °C to form the triene **13** on gram scale. The tin−lithium exchange proved superior compared to Grignard formation from the benzylic bromide due to competitive Wurtz coupling.

Selective hydroboration of the least substituted alkene of **13** using crystalline 9-BBN followed by oxidation generated the corresponding primary alcohol which was oxidized with buffered Dess−Martin periodinane[26] to generate aldehyde **14** in 76% overall yield. Stork-enamine Robinson annulation using methyl vinyl ketone provided the desired enone **15** in 83% yield.[23,27] $LiAlH_4$ reduction provided an inconsequencial diasteromeric mixture of allylic alcohols **16**.

The allylic alcohols **16** were subjected to polyene cyclizations using a variety of Lewis and Brønsted acids. Of the reagents used, $FeCl_3$ was singular in the yields obtained.[28] After optimization, we observed that allowing the temperature to warm from −78 to −30 °C with a 500 μM solution of **16** in CH_2Cl_2 using $FeCl_3$ (150 mol %) formed the pentacyclic alkene **17**. While the cyclization step is intramolecular, dilute solutions were imperative to obtaining acceptable yields and reproducibility. Gratifyingly, the cyclization was successfully performed on gram scale with an acceptable yield of 38%. The same cyclization reaction employing $SnCl_4$ provided **17** in 15% yield.[23] Hydroboration of the olefin at −78 °C followed by oxidation provided the alcohol which was directly oxidized to yield the ketone **18**, structurally confirmed through single crystal X-ray diffraction (see Supporting Information).

The ketone was converted to the corresponding unsaturated ester **19** after carbonylation of the enol triflate using $Pd(dppf)Cl_2$ (Scheme 2). While **19** was resistant to reduction using several reagents, dissolving metal reduction successfully reduced the enoate and afforded the amide. Therefore, saponification of **19** followed by reduction using dissolved sodium in ammonia at −78 °C avoided amide formation to provide the unsaturated acid **20** as a 3:1 mixture of diasteromers. Methylation of the enolate, forming the last quaternary carbon, was challenging due to significant steric impediment, and the acid **20** required subjection to two rounds of alkylation to produce the C-methylated acid in 47% yield. This acid was converted to the methyl ester using $TMSCHN_2$ in methanol/benzene to afford the ester **21**. Esterification was necessary to simplify purification and to provide the structural

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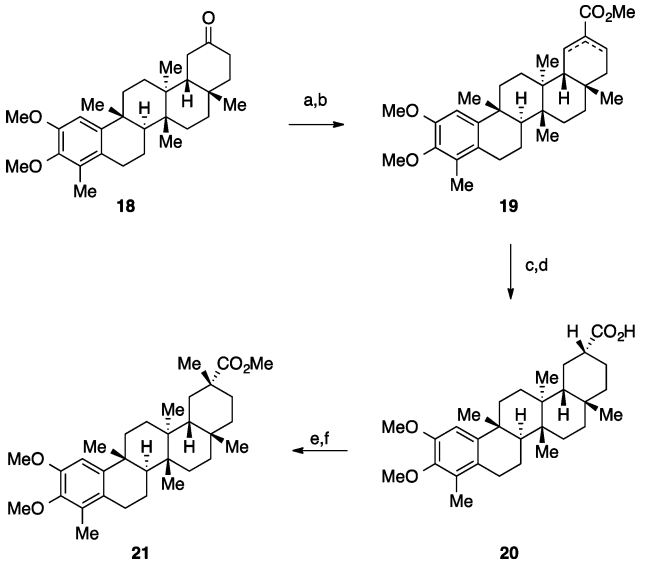
Scheme 2. Synthesis of Methyl Ester 21[a]



[a]Reagents and conditions: (a) LiHMDS, $PhNTf_2$, THF, −78 to 23 °C, 1.5 h, (2:1). (b) $Pd(dppf)Cl_2$, Et_3N, CO, MeOH, 65 °C, 12 h, 83%, 2-steps. (c) KOH, $MeOH/H_2O$, 65 °C, 2 h. (d) Na, NH_3, *t*-BuOH, THF, −78 °C, 60 min, 98%, dr 3:1, 2-steps. (e) Et_2NLi, MeI, THF, 23−70 °C, 90 min; then Et_2NLi, MeI, THF, 23−70 °C, 90 min (f) $TMSCHN_2$, MeOH/PhH, 5 min, 47% (3-steps).

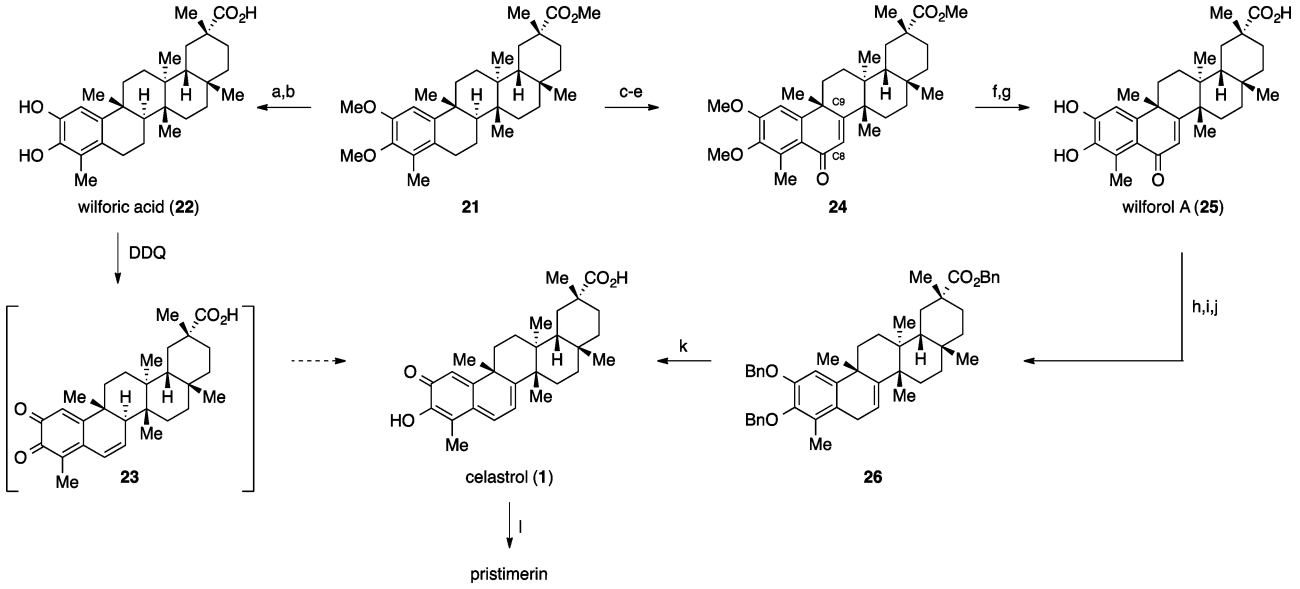
assignment of both diastereomers. Unambiguous confirmation of the desired product was attained through single crystal X-ray diffraction (see Supporting Information).

Saponification and methyl ether cleavage mediated by BBr_3 in CH_2Cl_2 at 0 °C afforded wilforic acid (**22**) in 68% yield from **21** (Scheme 3). Initially, we hypothesized that wilforic acid simply needed to undergo double oxidation followed by tautomerization to afford celastrol (**1**) in a single operation. However, while sequential oxidations succeeded forming **23**, attempts to induce tautomerization to form celastrol failed. From these results, unfortunately, we surmised that the C8−C9 olefin must be installed prior to oxidation.

Jones reagent oxidized ester **21** at the benzylic position to produce the corresponding ketone which was subjected to further oxidation through a selenoxide elimination to provide the enone **24**. Saponification followed by deprotection using $AlBr_3$ in toluene generated wilforol A (**26**) which was then benzylated to afford the perbenzyl ether. Subsequent $LiAlH_4$ reduction with careful monitoring of the reaction's progress by TLC with gradual warming from −78 to −30 °C allowed access to the desired alcohol while limiting reduction of the hindered E-ring ester. The resulting active benzyl-allylic alcohol was deoxygenated via ionic reduction with TFA and Et_3SiH to provide the perbenzyl ester **26** in moderate yield over two steps.[29] In a single operation, perbenzyl derivative **26** was subjected to hydrogenation using Pd/C, and upon indication (by TLC) of completion, the flask was opened to atmosphere and stirred vigorously to afford celastrol (**1**) as a red-orange solid in 75% yield. Celastrol in turn was esterified using $TMSCHN_2$ in methanol/benzene to produce the closely related natural product pristimerin (**27**).[30]

In conclusion a platform utilizing a polyene cascade was developed to provide access to the pentacyclic framework of the celastroid class of triterpenoids, lending to the total synthesis of celastrol and related natural products wilforic acid, wilforol A, and pristimerin. The allylic alcohol cyclization precursor **16** is accessed in >5 g quantities in 12 steps (longest linear) commencing from 2,3-dimethylbutadiene with an overall yield of 21%. The developed cascade employs ferric chloride as an activator in a dilute solution of CH_2Cl_2 to generate the pentacycle in 38% yield on gram scale and

Scheme 3. Synthesis of Celastrol (1)[a]



[a]Reagents and conditions: (a) KOH, 1,4-dioxane/H_2O, 100 °C, 4 h, 99%. (b) BBr_3, CH_2Cl_2, 0 °C, 5 min, 69%. (c) Jones reagent, Me_2CO, 0−23 °C, 5 min, 96%. (d) *i*-Pr_2NEt, TMSOTf, CH_2Cl_2, 23 °C, 60 min. (e) PhSeCl, THF, −78 to 23 °C, then H_2O_2, THF/H_2O, 20 min, 92% 2-steps. (f) KOH, 1,4-dioxane/H_2O, 100 °C, 24 h, 95%. (g) $AlBr_3$, PhMe, 110 °C, 2 h, 87%. (h) K_2CO_3, NaI, BnBr, Me_2CO, 60 °C, 48 h, 90% (i) $LiAlH_4$, Et_2O, −78 °C to −30 °C, 2 h. (j) Et_3SiH, TFA, CH_2Cl_2, 0 °C, 10 min, 53% 2-steps. (k) Pd/C, H_2, EtOAc, 23 °C, 2 h; then air, EtOAc, 23 °C, 5.5 h, 75%. (l) $TMSCHN_2$, MeOH/PhH, 23 °C, 2 min, 99%.

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showcases the utility of this reagent for polyene cyclizations. Through this intermediate, the first syntheses of celastrol and pristimerin were completed in 31 and 32 (longest linear steps, respectively) as well as wilforic acid and wilforol A.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b06261.

Experimental methods and spectral data (PDF)
(CIF)
(CIF)

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Notes

The authors declare no competing financial interest.

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