VIP Cyclopamine

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# Synthesis of Cyclopamine Using a Biomimetic and Diastereoselective Approach**

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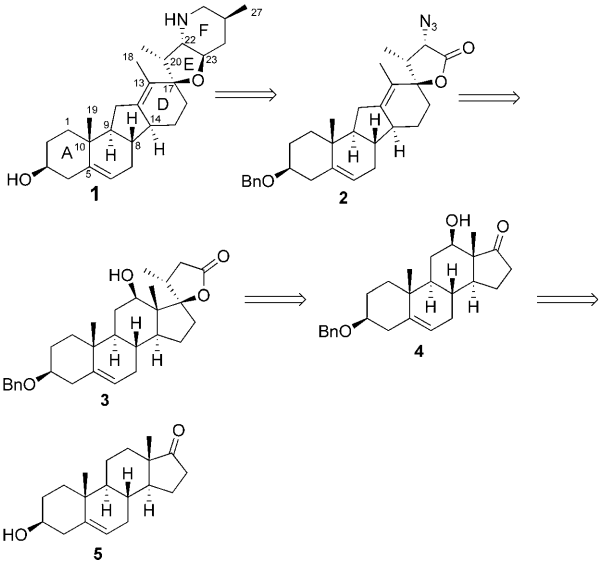
*Dedicated to Philip A. Beachy, Lynn F. James, and Richard F. Keeler*

The hedgehog signaling (Hh) pathway is a key regulator in embryogenesis and in the maintenance and generation of cells in adults.[1] Since aberrant activation of the Hh pathway leads to malignancies including basal cell carcinoma, medulloblastoma, rhabdomyosarcoma, and prostate, pancreatic and breast cancers, inhibition of Hh signaling provides a route to novel anticancer therapies.[2,3] The *Veratrum* alkaloid cyclopamine was the first known inhibitor of Hh signaling; it influences the balance between active and inactive forms of the protein Smoothened. Though high demand exists for cyclopamine as well as for metabolically more stable and potent analogues, an efficient chemical synthesis of this steroidal alkaloid is still missing.

We report herein an expeditious synthesis of cyclopamine starting from commercially available dehydroepiandrosterone and making use of biomimetic and diastereoselective transformations. While one total synthesis strategy can be envisioned based on previous publications,[4–6] this approach is tedious, non-stereoselective, and low yielding. Our synthesis, which entails a copper-mediated regioselective C–H activation/hydroxylation, cationic ring contraction/expansion, and finally an Alder-ene reaction, enables the rapid construction of this unusual molecule. This strategy also provides access to various cyclopamine analogues that do not exist in nature which might be useful in investigations of the biological activity of this unique natural product.

Cyclopamine (**1**; Scheme 1) was first reported in 1957, when sheepherders in Idaho, USA, were alarmed by the unsettling observation of newborn lambs with a single eye located, cyclops-like, in the middle of their foreheads.[7] Intense investigation led to the finding that the ingestion of the corn lily (*Veratrum californicum*) by pregnant sheep caused the birth of lambs with cyclopia. The *Veratrum* alkaloid cyclopamine was identified as the causative agent.




***Scheme 1.*** Structure and retrosynthetic analysis of cyclopamine (**1**). Bn = benzyl.

The malformation cyclopia has been known in humans since ancient times. It has been observed in severe cases of holoprosencephaly (HPE), a malformation of the brain in which the prosencephalon fails to cleave, resulting in undivided or incompletely divided cerebral hemispheres, telencephalon and diencephalon, and olfactory and optic nerves.[8] Associated with the brain abnormalities is a spectrum of midline craniofacial anomalies, including ocular hypotelorism and interorbital proboscis (ethmocephaly).[9] The etiology of HPE is heterogeneous and can include genetic and/or teratogenic factors.

Only recently was the cellular target of cyclopamine elucidated. This molecule binds to the plasma membrane protein smoothened, induces a conformational change, and inhibits the hedgehog signaling pathway.[3]

Cyclopamine (**1**) displays a plethora of unusual structural features, and therefore its synthesis poses significant challenges. Among them are the C-*nor*-D-*homo* steroid substructure and a highly substituted furan ring, which is attached through a spiro linkage to the D ring and also fused to a piperidine unit having a basic secondary nitrogen atom. Additionally, cyclopamine is quite acid sensitive: at pH < 3 or upon treatment of **1** with Lewis acids the furan ring is cleaved and the D ring aromatizes to form the toxic veratramine.

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Retrosynthetic considerations (Scheme 1) led us to define three objectives of paramount importance to our synthesis: 1) creation of the correct ABCD framework, 2) installation of the furan unit (ring E), and 3) synthesis of the piperidine ring (ring F).

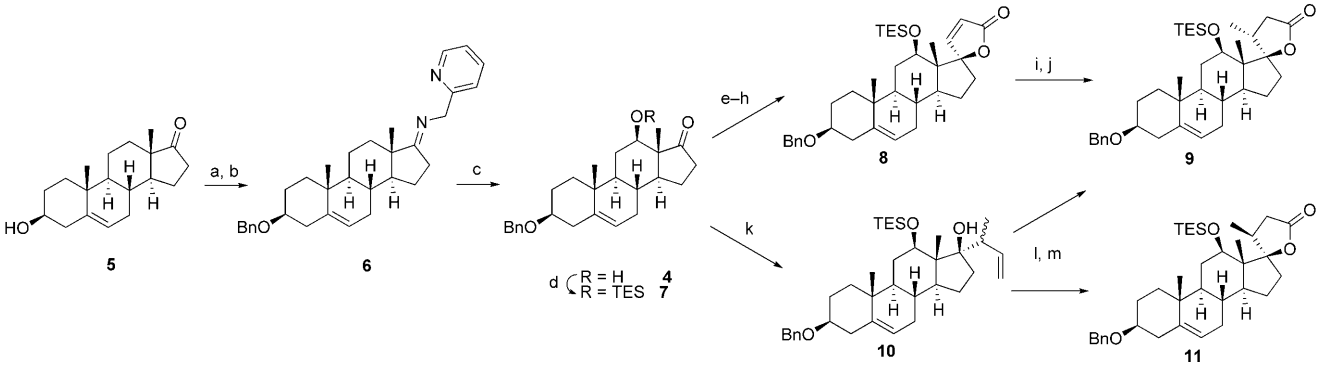
Although the total synthesis of the C-*nor*-D-*homo* steroid, for example from the (+)-Wieland–Miescher ketone, was a possibility we chose a semisynthetic route to this substructure for the sake of brevity and efficiency. We presumed that the cationic rearrangement of 12β-hydroxy steroids into their C-*nor*-D-*homo* counterparts[10] was a much easier approach. Since 12β-hydroxy steroids are rare and a properly functionalized one (like **4**) resembling the cyclopamine ABCD skeleton was not available, we envisioned the hydroxylation of commercially available steroids (e.g. dehydroepiandrosterone **5**) at position by 12 position by C–H activation. Fortunately Schönecker et al. recently published a method using copper salts and molecular oxygen for this purpose.[11] We planned that the furan with its spiro connection to the D ring would be installed by addition of a functionalized C nucleophile to the 17-keto group of **4**, thereby establishing the correct substitution at the C17 quaternary center in form of a tertiary alcohol. Oxidative cyclization could yield a lactone (as in **3**) as the furan precursor. This functionality was thought to be most suitable for further elaboration into the piperidine by proper C–N and C–C bond-forming reactions and for the final piperidine formation (ring F) by nucleophilic substitution.

Starting from commercially available **5** protection of the hydroxy moiety as a benzyl ether by reaction with Dudley's pyridinium triflate[12] and formation of the 2-picolylimine at position 7 by treatment of the ketone with 2-picolylamine under azeotropic removal of water afforded **6** in high yield and set the stage for the projected hydroxylation at position 12 (Scheme 2). Using tetrakis(acetonitrilo)copper(I) hexafluorophosphate and molecular oxygen, we were able to introduce the 12β-hydroxy group with complete regio- and diastereoselectivity in an encouraging yield. After protection of the 12β-hydroxy group as a triethylsily ether (→**7**), the construction of the lactone was the next objective.

The diastereoselective addition of lithium triethylsilyl propargylate to **7** gave the tertiary alcohol, which in turn was monodesilylated by treatment with acetic acid, reduced with the Lindlar catalyst to yield the *cis* isomer exclusively, and finally oxidatively cyclized by reaction with a catalytic amount of 2,2,6,6-tetramethylpiperidinoxy radical (TEMPO) and stoichiometric [bis(acetoxy)iodo]benzene (BAIB) to give the butenolide **8**. Treatment of **8** with the anion formed from trimethyl trithioorthoformate and *n*-butyllithium[13] followed by careful reduction with Raney nickel gave the properly functionalized lactone **9** as a single diastereoisomer (54% yield over six steps).

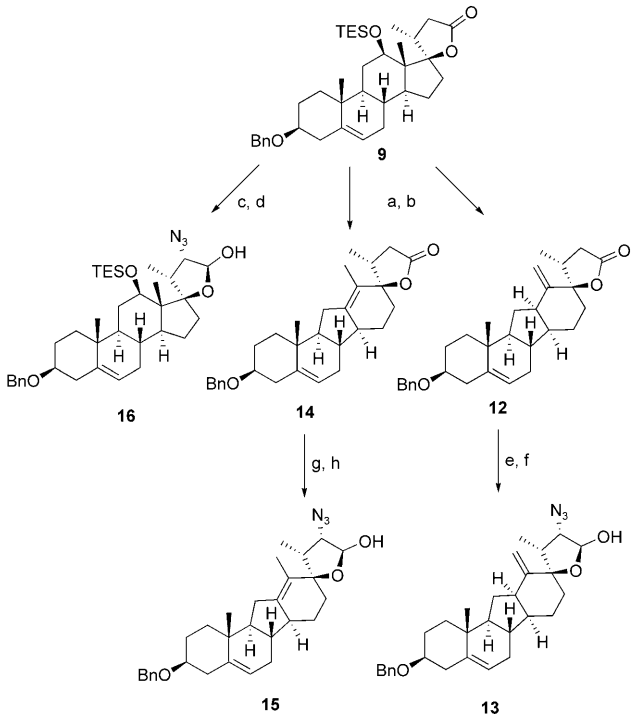
However, lactone **9** is available in similar yield by a more concise three-step synthesis. Treatment of the ketone with 2-methyl-1-propenylcerium chloride,[14] hydroboration/oxidation of the resulting alkenes with 9-borabicyclo[3.3.1]nonane (9-BBN) and sodium perborate, and finally oxidative cyclization of the intermediate diols using TEMPO/BAIB gave the lactones **9** and **11** as a mixture of epimers at C20. These in turn were easily separable by chromatography. Thus the desired 20*R* lactone **9** was obtained in 42% yield in only three steps. Furthermore the 20*S*-configurated lactone **11** was isolated in 30% yield. We used this much shorter sequence to access quantities of the lactone of up to 10 g per batch.

Before the construction of the piperidine commenced, it was time to rearrange the steroid skeleton. Cleavage of the triethylsilylether with hydrofluoric acid yielded the free alcohol, which was subjected to the action of trifluoromethanesulfonic anhydride ($Tf_2O$) in pyridine (Scheme 3). At elevated temperature this Wagner–Meerwein-type rearrangement gave the desired C-*nor*-D-*homo* skeleton in nearly quantitative yield as a 7:3 mixture of regioisomers **12** and **14** which were easily separable by chromatography. Though the isomer **12** with the exocyclic double bond dominated in the reaction mixture, we continued the next steps of the synthesis before attempting conversion into the endocyclic isomer.




***Scheme 2.*** Synthesis of 12β-hydroxy lactones **9** and **11**: Reaction conditions: a) 2-benzyloxymethylpyridinium triflate, MgO, $PhCF_3$, 85 °C; b) 2-picolyl amine, *p*TsOH (2.5 mol%), toluene, reflux, 90% (95% b.r.s.m.) over two steps; c) $[Cu(MeCN)_4]PF_6$, acetone, then $O_2$ (1 atm), then $NH_4OH$, then HOAc, MeOH, 48% (57% brsm); d) TESOTf, 2,6-lutidine, $CH_2Cl_2$, 0 °C, 93%; e) triethylsilylpropargyllithium, THF, −15 °C; f) HOAc, THF, $H_2O$; g) Lindlar catalyst (3 mol%), pyridine, THF; h) BAIB, TEMPO (20 mol%), $CH_2Cl_2$, 64% (77% b.r.s.m.) over four steps; i) tris(methylthio)methyllithium, THF, −78 °C; j) Raney nickel (W2), THF, $H_2O$, 69% over two steps; k) 1-methyl-2-propenylcerium chloride, THF, 0 °C, 95%; l) 9-BBN, THF, reflux, then $NaBO_3$, $H_2O$, 50 °C; m) BAIB, TEMPO (20 mol%), $CH_2Cl_2$, 77% over two steps, d.r. 6:4. brsm = based on recovered starting material, OTf = triflate, *p*TsOH = *p*-toluenesulfonic acid, TES = triethylsilyl

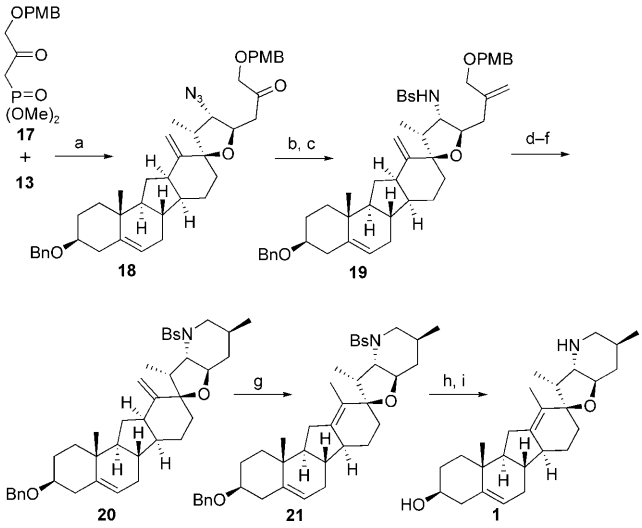



***Scheme 3.*** Synthesis of azido lactoles **13**, **15**, and **16**. Reaction conditions: a) HF, MeCN, $H_2O$, 85%; b) $Tf_2O$, pyridine, 0°C→50°C, 94% (7:3 ratio of **12** and **14**); c) LDA, THF, −78°C, then trisyl azide, −78°C, then HOAc, KOAc, −78°C→22°C, 78% (d.r. 8:1); d) DIBAH, THF, −78°C→−65°C, 82%; e) LDA, THF, −78°C, then trisyl azide, −78°C, then HOAc,−78°C→22°C, 93% (d.r. 5:4); f) DIBAH, THF, −78°C→−65°C, 95%; g) LDA, THF, −78°C, then trisyl azide, −78°C, then HOAc, −78°C→22°C, 75% (d.r. 3:1); h) DIBAH, THF, −78°C→−65°C, 90%.

To incorporate the nitrogen in the molecule, we formed the lactone enolate by treatment with excess lithium diisopropylamide (LDA) at −30°C and reacted it with trisyl azide[15] to give the azidolactones in 75% yield as 3:1 mixture of diastereomers, which were separable by chromatography. Since the undesired azide could be isomerized into the desired one by treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in $CH_2Cl_2$, no material was lost at this stage.

To construct the furan motif we considered a tandem Horner–Wadsworth–Emmons reaction/intramolecular Michael addition between β-ketophosphonate **17** (its synthesis is given in the Supporting Information) and azidolactol **15** (accessible from the azidolactone by treatment with diisobutylaluminum hydride (DIBAH) at −65°C). After extensive experimentation we realized that neither azidolactol **15** (with a C12–C13 double bond) nor azidolactol **16** (directly synthesized from lactone **9** without rearrangement) gave the desired product even at elevated temperatures and prolonged reaction times. Much to our relief the regioisomer **12** with a C13–C18 double bond after conversion into the azidolactol **13** gave the desired furan **18** as a single product in good yield under mild reaction conditions (base: barium hydroxide, solvent system: THF/water[16]).

With the properly functionalized furan in hand, we focussed on the construction of the piperidine ring. The 25-ketone **18** was transformed into an alkene by a two-step Peterson olefination sequence (including reaction with (trimethylsily)methylcerium chloride[17] then elimination by brief treatment with diluted hydrofluoric acid). Subsequent Staudinger reduction[18] of the azide and protection of the amine formed with benzenesulfonyl chloride furnished the sulfonamide **19**, which in turn was treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to give the corresponding allyl alcohol (Scheme 4). Ring closure by a modified Mitsunobu protocol[19] proceeded with high efficiency and furnished the piperidine in a convincing yield (57% over six steps).




***Scheme 4.*** Synthesis of cyclopamine (**1**). Reaction conditions: a) **17**, $Ba(OH)_2$, THF, $H_2O$, then **13**, 48% (59% b.r.s.m.); b) (trimethylsilyl)-methylcerium chloride, THF, −78°C, then HF, MeCN, $H_2O$; c) $PPh_3$, THF, $H_2O$, 50°C, then BsCl, $Et_3N$, $CH_2Cl_2$, 40°C, 80% over two steps; d) DDQ, pH 7.0 buffer, $CH_2Cl_2$; e) $Bu_3P$, ADDP, toluene; f) [RhCl-$(PPh_3)_3$], $H_2$ (1 atm), benzene, 71% (d.r. 3:1) over three steps; g) *N*-sulfinylbenzenesulfonamide, benzene, 60°C, then Raney nickel (W2), $H_2$ (5 atm), benzene, 57%; h) Raney nickel (W2), EtOH, 78°C; i) sodium naphthalenide, DME, −78°C, 79% over two steps. ADDP = azodicarboxylic dipiperidide, Bs = benzenesulfonyl, DME = dimethoxyethane, PMB = *para*-methoxybenzyl.

Completely regioselective hydrogenation of the C25–C27 double bond using Wilkinson's catalyst gave the protected cyclopamine **20** with an exocyclic double bond between C13 and C18. Fortunately this isomer could be converted into protected cyclopamine **21** by treatment with *N*-sulfinylbenzenesulfonamide[20] in an Alder-ene reaction followed by desulfurization of the corresponding intermediate using excess Raney nickel.[21] Under the adopted reaction conditions neither the C5–C6 double bond nor the benzyl ether protecting group was affected. This two-step preparation proceeded in a good yield of 57%. Final removal, first of the benzyl ether (Raney nickel in refluxing ethanol) and then of the benzenesulfonamide using excess sodium naphthalenide at −78°C[22] gave the natural product, which was spectroscopically identical to a previously reported sample[23] in every respect.

We have developed a concise and efficient strategy (20 steps, 1% overall yield) for the synthesis of cyclopamine.

Its features are a regio- and stereoselective hydroxylation by C–H activation, a biomimetic ring contraction/expansion, a tandem Horner–Wadsworth–Emmons reaction/Michael addition, and an Alder-ene reaction. Since we also devised methods to access several diastereoisomers and isomers with an exocyclic double bond, we are confident that implementation of this synthetic approach will broaden our knowledge on cyclopamine's structure–activity relationships and will furnish analogues with fine-tuned bioactivity. Studies and biological evaluations are currently being conducted and will be reported in due course.

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