# An Efficient Substrate-Controlled Approach Towards Hypoestoxide, a Member of a Family of Diterpenoid Natural Products with an Inside-Out [9.3.1]Bicyclic Core**

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Hypoestoxide (**1**, Scheme 1) was isolated from the tropical shrub *Hypoestes rosea*, found in the Nigerian rainforests.[1] Extracts from these shrubs have been used in folk medicine
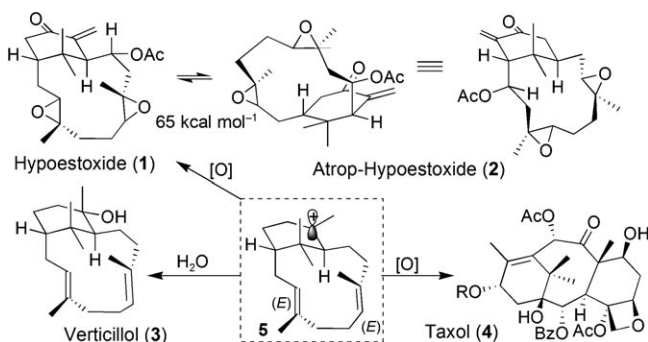

Scheme 1. Hypoestoxide and verticillol.

for generations, to treat various skin rashes and infections. Hypoestoxide has been shown in recent studies to exhibit promising anticancer,[2] antimalarial,[3] and anti-inflammatory activity.[4] Our interest stems primarily from encouraging antiangiogenic activities, in which hypoestoxide was shown to inhibit the growth of a number of human and murine tumor cell lines in vivo. In terms of angiogenesis, hypoestoxide inhibited vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). Hypoestoxide is a bicyclo[9.3.1]pentadecane diterpenoid containing a rigid "inside–outside" ring system decorated with an exocyclic enone, two epoxide moieties, and an acetate group. This rare ring system has also been described for the verticillanes,[5] of which verticillol (**3**, Scheme 1)[6] is the most well known. As a more oxygenated variant of verticillol, it is tempting to propose that hypoestoxide is formed from the same common

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cationic precursor (**5**) as both verticillol[7] and taxol (**4**),[8] which in turn originates from consecutive cyclizations of geranylgeranyl pyrophosphate. In the case of verticillol, the cation **5** is trapped with water, whereas, for taxol and hypoestoxide, it undergoes endocyclic and exocyclic eliminations, followed by oxygenations and cyclizations. As part of our efforts to evaluate the molecular mechanisms[9] of promising natural product anticancer agents we have focused our investigations on hypoestoxide and the verticillanes.

Several factors needed to be considered before beginning our synthetic efforts. First, for a *trans*-[9.3.1]bicyclic framework, two different atropisomers are possible. Calculations (B3LYP/6-311 + G(d,p)) indicated that hypoestoxide is 4.1 kcal mol^{-1} more stable than the atropisomer (**2**). Therefore, we imagined that any such macrocyclization would preferentially form the naturally occurring atropisomer. In addition, the energy change attributed to the process of interconverting hypoestoxide and the atropisomer was estimated to be 65 kcal mol^{-1}. Diene **6** seemed an ideal target because it allows access to all known verticillanes, and we attempted its synthesis using a conformationally controlled ring-closing metathesis (Scheme 2). This diene provides four
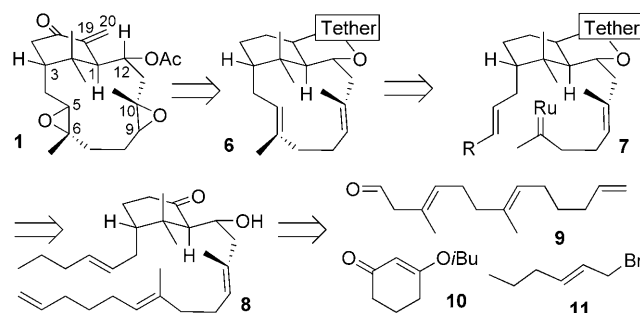
Scheme 2. Retrosynthesis of hypoestoxide.

different options for ring-closing metathesis. Both the C5=C6 and C9=C10 bonds can originate from either a standard monosubstituted carbene or, alternatively, from a disubstituted carbene, such as **7**, which can be accessed by relay metathesis.[10] Our analysis suggested that, in closing the macrocycle, it would be advantageous to bring together a more-substituted carbene with a less-hindered terminus to minimize competing dimerization pathways. We further postulated that ruthenium carbene **7** (C5=C6 disconnection) would be the better candidate for macrocyclization, since the

equivalent C10-disubstituted carbene would be more sterically hindered and suffer from unfavorable interactions with the C12 hydroxy group. Concurrently, we proposed to rigidify the macrocyclization substrate to bring the two olefin termini closer together[11] and ensure formation of the correct atropisomer. Ketone **8** (Scheme 2) serves as the branch-point that would allow us, at a later stage, to evaluate several fused ring sizes. Cyclization precursor **8** would be assembled from three simple building blocks (**9–11**). Our endgame towards hypoestoxide would rely on substrate-controlled bisepoxidation.

Our synthetic efforts commenced with a Grignard addition to methacrolein **12**, followed by a Johnson–Claisen rearrangement[12] to generate ethyl ester **13** (Scheme 3). This
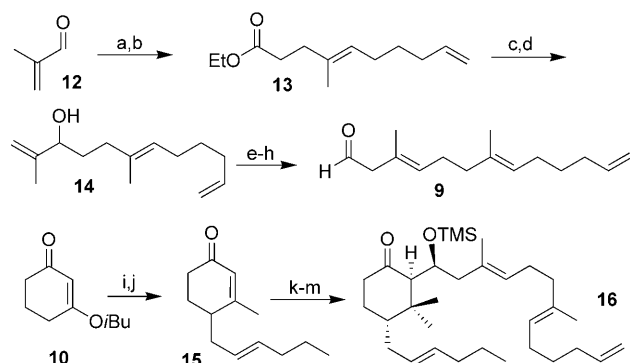

Scheme 3. Synthesis of a common macrocyclization precursor. Reagents and conditions: a) CH_2=$CH(CH_2)_3MgBr$ (0.7 equiv), Et_2O, −10 to 23 °C, 3 h, 90%; b) $CH_3C(OEt)_3$ (5 equiv), propionic acid (0.03 equiv), 140 °C, 2 h, 92%; c) DIBAL-H (1.05 equiv), hexanes, −78 °C, 2 h, 95%; d) CH_2=$C(CH_3)MgBr$ (1.5 equiv), THF, −10 to 23 °C, 2 h, 62%; e) NaH (4.5 equiv), $BrCH_2CO_2H$ (1.05 equiv), THF, 85 °C, 6 h; f) LDA (3.6 equiv), THF, −45 °C, 3.5 h; g) $LiAlH_4$ (2.5 equiv), Et_2O, 50 °C, 1.5 h, 80% (3 steps); h) $Pb(OAc)_4$ (1.05 equiv), Na_2CO_3 (1.05 equiv), CH_2Cl_2, 0 °C, 1 h, 95%; i) LDA (1.05 equiv), (*E*)-1-bromo-2-hexene (1.2 equiv), THF, −78 to 0 °C, 2 h; j) MeLi (2.7 equiv), THF then HCl, −78 to 23 °C, 12 h, 72% (2 steps); k) MeLi (2.5 equiv), CuI (1.25 equiv), Et_2O, then TMSCl (5 equiv), Et_3N (5 equiv), −5 °C, 2.5 h, 88%; l) MeLi (1.06 equiv), Et_2O, then $ZnCl_2$ (1.1 equiv) then **9** (0.5 equiv), −45 °C, 2 h; m) TMSCl (1.9 equiv), Et_3N, CH_2Cl_2, 0 to 23 °C, 18 h, 65% (2 steps). DIBAL-H = diisobutylaluminum hydride; LDA = lithium diisopropylamine; TMS = trimethylsilyl.

ester was reduced to the aldehyde with DIBAL-H, and another Grignard addition afforded allylic alcohol **14**. We next utilized a [2,3] rearrangement[13] to stereoselectively install the second trisubstituted olefin. The hydroxy acid was reduced and the resulting diol was cleaved with lead tetraacetate to give aldehyde **9**. The other key component, enone **15**, was readily assembled using the Stork–Danheiser method.[14] This enone was then subjected to a conjugate addition and in situ trapping to form the trimethylsilyl enol ether[15] to couple with aldehyde **9**. The addition of $ZnCl_2$[16] was required to promote the desired aldol reaction to form tetraene **16** with the desired *trans* arrangement[17] on the six-membered ring. This route efficiently assembles the versatile synthetic intermediate **16** in only ten steps from methacrolein.

Encouraged by the rapid assembly of metathesis precursor **16**, we decided to evaluate five- and seven-membered ring tethers. Lactone **17** was formed by converting ketone **16** into an enol triflate, followed by deprotection and carbonylation (Scheme 4).[18] However, this lactone and its reduced variants
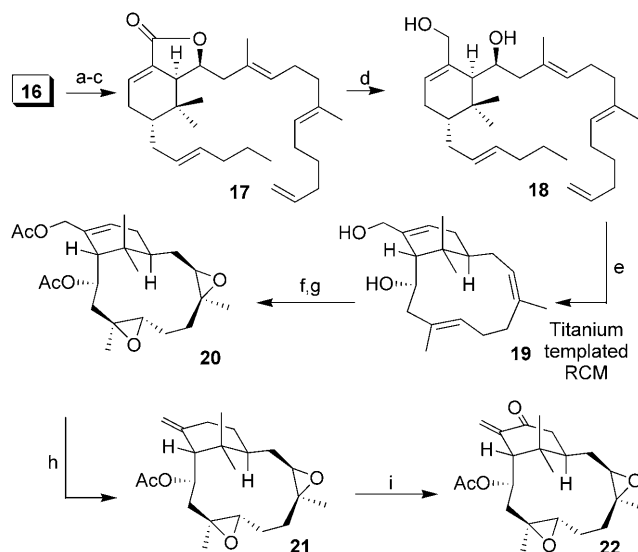

Scheme 4. Efficient assembly of a hypoestoxide isomer. Reagents and conditions: a) LHMDS (2.9 equiv), Comins reagent (1.9 equiv), THF, −78 °C, 5 h, 95%; b) Amberlyst-15 (cat.), MeOH, THF, 23 °C, 3 h, 95%; c) [Pd(PPh$_3$)$_4$] (0.25 equiv), CO (60 psi), Et_3N (12 equiv), DMF, 50 °C, 15 h, 92%; d) DIBAL-H (8 equiv), toluene, −78 to 23 °C, 1 h, 93%; e) Ti(O*i*Pr)$_4$ (10 equiv), then Grubbs II catalyst (20 mol%), toluene, reflux, 8 min, 95%; f) Ac_2O (10 equiv), DMAP (2 equiv), Et_3N (20 equiv), CH_2Cl_2, 23 °C, 1 h, 92%; g) DMDO (2 equiv), acetone, 23 °C, 10 min, 93%; h) [Pd(PPh$_3$)$_4$], (0.04 equiv) Et_3N, HCOOH, THF, 75 °C, 15 h, 87%; i) SeO_2, (2 equiv), CH_2Cl_2/CH_3OH (1:1), 65 °C, 15 h, then DMP (2 equiv), CH_2Cl_2, 23 °C, 1 h, 77%. DMAP = 4-dimethylaminopyridine; DMDO = dimethyldioxirane; LHMDS = lithium hexamethyldisilazide; DMP = Dess–Martin periodinane.

(1,4- and 1,2-reductions) did not undergo ring-closing metathesis. The seven-membered-ring series was easily accessed by reducing **17** to the diol **18** and tethering the two hydroxy groups. A carbonate tether, obtained by treating **18** with triphosgene, turned out to be an ideal cyclization substrate. Optimized conditions using the Grubbs second generation catalyst[19] in refluxing toluene afforded bicyclic substrate **19** in excellent yield.[20] Having developed a successful cyclization substrate, we sought to eliminate two steps from the synthetic sequence by tethering the diol in situ, using titanium additives.[21] Gratifyingly, adding excess titanium isopropoxide prior to the addition of the catalyst led to the formation of triene **19** in equally high yield, directly from diol **18**.

Surprisingly, extensive NMR spectroscopic analysis[22] of **19** revealed additional problems with the structure. The ring-closing metathesis not only gave the undesired *Z* olefin, but also took place with the C12-bearing tether in an axial position instead of the more stable equatorial position, thus forming the wrong atropisomer of the natural product. Although this unexpected result demonstrated that the metathesis catalyst had overridden the planned effect of the

facial bias, it did confirm the applicability of our tethered-cyclization strategy. The metathesis product **19** was then bisacetylated, and the two trisubstituted macrocyclic olefins were subjected to substrate-controlled bisepoxidation, which afforded **20** as the only product (Scheme 4). Tsuji and co-workers' reductive allylic transposition[23] was used to form the desired *exo*-methylene moiety in **21**. Allylic oxidation was achieved with selenium dioxide and the resulting alcohol was oxidized with Dess–Martin periodinane to enone **22**. This 19-step synthesis of an isomer of hypoestoxide highlights the efficiency of our synthetic assembly.[24]

To complete a total synthesis of atrop-hypoestoxide (Scheme 1) we needed to invert both the C12[25] stereocenter and the C5=C6 bond geometry. Towards that end, the primary alcohol of metathesis product **19** (Scheme 5) was selectively protected and the C12 alcohol was converted into a ketone
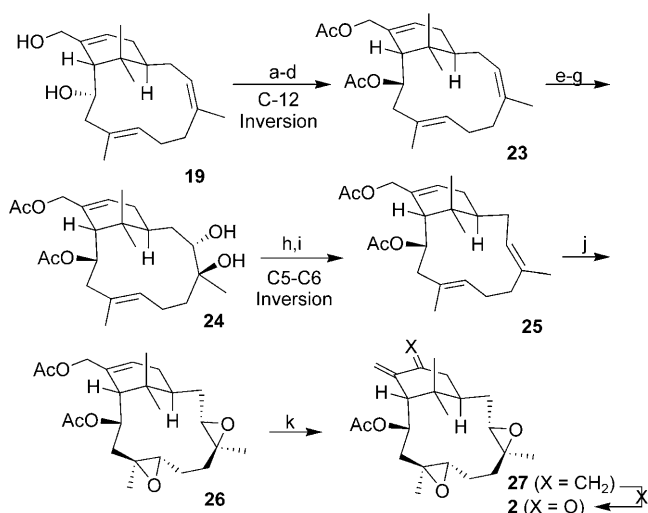
Scheme 5. Synthesis of 18-desoxy-atrop-hypoestoxide. Reagents and conditions: a) TBSCl (3 equiv), imidazole (9 equiv), CH$_2$Cl$_2$, 23 °C, 30 min, 62 %; b) TPAP (0.05 equiv), NMO (1.5 equiv), CH$_2$Cl$_2$, 23 °C, 45 min; c) LiAlH$_4$ (25 equiv), THF, then HCl, 0 to 23 °C, 1 h, 96 % (2 steps); d) Ac$_2$O (10 equiv), Et$_3$N (20 equiv), DMAP (2 equiv), CH$_2$Cl$_2$, 23 °C, 1.5 h, 98 %; e) OsO$_4$ (1.1 equiv), THF, 0 to 23 °C, 3 h, 74 %; f) (COCl)$_2$ (9 equiv), Et$_3$N (36 equiv), DMSO (18 equiv), CH$_2$Cl$_2$, −78 to 0 °C, 1 h, 80 %; g) NaBH$_4$ (10 equiv), MeOH, 23 °C, 30 min, 80 %; h) Cl$_2$C=S (10.5 equiv), DMAP (40 equiv), CH$_2$Cl$_2$, 45 °C, 20 h, 91 %; i) P(OEt)$_3$ (500 equiv), 160 °C, 15 h, 86 %; j) DMDO (2 equiv), acetone, 23 °C, 4 h, 99 %; k) [Pd(PPh$_3$)$_4$] (1 equiv), Et$_3$N (10 equiv), HCOOH (10 equiv), THF, 75 °C, 15 h, 55 %. TBS = *tert*-butyldimethylsilyl; TPAP = tetrapropylammonium perruthenate; NMO = *N*-methylmorpholine-*N*-oxide.

using the Ley oxidation.[26] Substrate-controlled reduction of the ketone and acetylation of the resulting diol gave **23** as a single product.[27] We then turned our attention to the more challenging task of inverting the C5=C6 trisubstituted bond.

Given the rigid semispherical shape of the bicycle, this double bond inversion is a particularly challenging task because nucleophiles can not approach from inside the macrocyclic unit. We proposed that a diol could alleviate this problem by internal cyclization/inversion of the tertiary alkoxide of the resulting secondary mesylate.[28] To investigate

this proposition, it was essential to find oxidation conditions that discriminated between the C5=C6 bond and the C9=C10 bond. Gratifyingly, the C5=C6 bond was selectively dihydroxylated using standard conditions. Unfortunately, the resulting monomesylate did not form the desired inverted epoxide, but instead underwent a facile ring contraction to an 11-membered ring, incorporating a methyl ketone.[29] This inversion conundrum was solved by instead forming diol **24** (Scheme 5) by using a substrate-controlled oxidation/reduction sequence. The hydroxy groups of diol **24** were then converted into a cyclic thiocarbonate, which, when subjected to the Corey–Winter deoxygenation conditions,[30] afforded the desired *E,E,E*-triene **25**.[22] Following bisepoxidation of the macrocyclic diene moiety, palladium-mediated allylic transposition was again successfully employed to form the desired *exo*-methylene moiety. Unfortunately, all efforts to oxidize **27** to atrop-hypoestoxide **2** proved unsuccessful.[31]

Towards an even more expedient synthetic assembly and a general route towards both hypoestoxide and verticillol, we investigated an additional tethering strategy. We were interested in learning how this six-membered ring tether would affect the selectivity of the ring-closing metathesis (Scheme 6). Grignard addition to ketone **16** afforded a
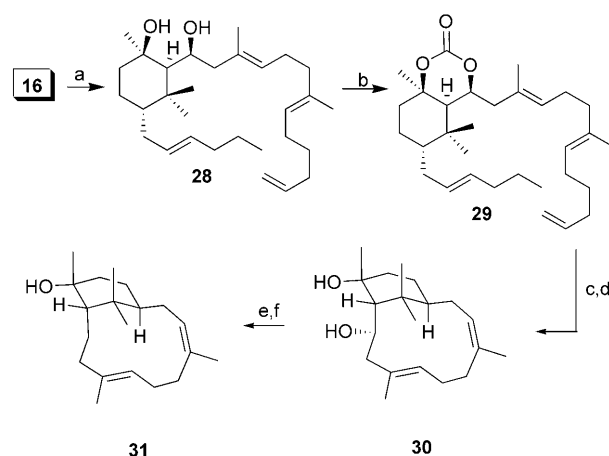
Scheme 6. Synthesis of a verticillol isomer. Reagents and conditions: a) MeMgBr (10 equiv), THF, 0 °C, 1 h, 60 %; b) triphosgene (1 equiv), pyridine (15 equiv), CH$_2$Cl$_2$, 23 °C, 18 h, 75 %; c) Grubbs II catalyst (0.3 equiv), toluene, reflux, 8 min, 40 %; d) NaOH, dioxane, 23 °C, 6 h, 71 %; e) MsCl (10 equiv), DMAP (1 equiv), pyridine (10 equiv), CH$_2$Cl$_2$, 23 °C, 20 h, 51 %; f) lithium naphthalenide, −78 °C, 10 min, 93 %. Ms = methanesulfonyl.

single diol diastereoisomer **28**, which was readily tethered as a cyclic carbonate.[32] This carbonate, when treated with the Grubbs catalyst, formed a single macrocyclic isomer which, upon deprotection, afforded diol **30**. NMR spectroscopic analysis indicated that the undesired *Z* olefin and incorrect atropisomer were again formed as the only bicyclic product in the ring-closing metathesis reaction. This diol could, however, be converted into an isomer of verticillol by selective mesylation of the secondary alcohol and reductive removal of the resulting sulfonate ester. This synthetic route to an isomer of verticillol constitutes only 16 synthetic steps from methacrolein **12**.

In summary, we have reported the first synthetic efforts towards the natural product hypoestoxide. An efficient flexible synthetic route that also provides access to the verticillane family of natural products has been devised. This synthetic roadmap has been utilized to accomplish the synthesis of 18-desoxy-atrop-hypoestoxide, as well as isomers of both hypoestoxide and verticillol. Efforts are underway to utilize a similar titanium-templated macrocyclization approach to synthesize hypoestoxide, verticillol, and additional analogues thereof.

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[24] Independently of this study, we have converted diol **28** into **22** in five steps (bisepoxidation, acetylation, dehydration, allylic oxidation, and oxidation).

[25] To further highlight the fine balance needed for a successful cyclization, it is worth noting that the correct C12-hydroxy isomer does not undergo ring-closing metathesis.

[26] The substrate-controlled reduction of the ketone can be explained by the geminal dimethyl group on the ring, which blocks the hydride delivery from what might otherwise be considered the less-hindered face.

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[29] Interestingly, the C9=C10 trisubstituted bond could be cleanly inverted to the *cis* epoxide using this same sequence after initially epoxidizing C5=C6. This bisepoxide was subjected to Tsuji's allylic transposition and readily underwent allylic oxidation.

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[31] Over 20 different allylic oxidations were attempted without success, using reagents including: SeO_2, $C_6F_5Se(O)OH$, $NaClO_2$/T-Hydro, $Mn(OAc)_3$, $Co(OAc)_2$/TBHP, $[Rh_2(cap)_4]$/TBHP, and Pd/C/TBHP.

[32] All nonrigidified substrates failed to cyclize using any of the known ruthenium metathesis catalysts. Other diol diastereoisomers were also accessed and evaluated, but these also failed to cyclize.