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Multigram Synthesis of Glyceollin I

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Supporting Information

ABSTRACT: Scaled-up procedures and preparation of glyceollin I in multigram quantities are described. The synthesis features construction of a cis-fused ring system in high enantiomeric excess after Sharpless asymmetric dihydroxylation of a key intermediate that is initially produced by an intramolecular Wittig reaction to afford the requisite alkene while simultaneously forming the first ring. The overall yield is 12% after 11 steps.

INTRODUCTION

Our collaborating laboratories have been examining the possibility for stress-induced enhancement of anticancer natural products within legumes that are staples of the U.S. agricultural industry.[1] Of particular note is the common soybean, *Glycine max*, which produces a family of phytoalexins[2] known as the glyceollins (GLYs; Figure 1). Because phytoalexins typically are present in very low amounts across select periods of time, supply issues represent an additional challenge toward their potential development as therapeutics. For example, the GLYs can be elicited in only trace amounts and isolated as a complex mixture when soybean plants are subjected to specific types of stress, such as when soy cotyledons are infected with *Aspergillus*.[3] Unlike their abundant isoflavonoid relatives genistein and daidzein which are normally present in soy, the GLYs exhibit marked antiestrogenic activity in some tissues[4] as well as unique anticancer properties[1,5] that hold promise for their use as selective estrogen receptor modulators (SERMs).[6] Among the GLYs, GLY I (**1**) is the most prevalent and appears to be the most interesting in terms of anticancer properties. To further characterize its promising activity, we have devised an advanced biological testing plan that requires several grams of **1**. Although attempts to optimize the isolation of **1** from natural sources remain ongoing, it was clear that a practical synthesis would be highly desirable in order to more quickly address the immediate supply needs.

The pterocarpans, as generally represented by **4** in Figure 1, are the second largest group of natural isoflavonoids.[7] Their 6a-hydroxy-substituted family, however, has only a few members. Thus, the 6a-hydroxy group represents a very distinctive molecular arrangement in these systems for which the accompanying asymmetry must be properly accounted for during synthesis. Owing in part to the lengthy, multistep routes needed to prepare these contiguous ring systems while maintaining rigorous stereocontrol, only four members of this family have been synthesized to date, namely pisatin,[8] variabilin,[9] and most recently in our laboratories GLY I[1,10] along with the GLY family's key phytochemical precursor glycinol[11] (respectively structures **5**, **6**, **1**, and **7** in Figure 1). Despite having already accomplished the total synthesis of **1**, it was additionally clear that scale-up from milligram to gram quantities of product would require a highly concerted team effort within our academic-based drug design and development center's laboratories in order to achieve this goal according to an intentionally ambitious time frame of ∼6 months.

BACKGROUND

From the onset of our synthetic studies in this area, we have recognized that introduction of the distinctive 6a-hydroxy group represents one of the most intricate chemical steps leading to these natural products. Previous investigators typically have utilized an isoflav-3-ene (**8** in Scheme 1) from which dihydroxylation and subsequent closure to the dihydrobenzofuran, **9**, produces the natural and more stable cis-fused ring system.[12] Alternatively, as also shown in retrosynthetic Scheme 1, we imagined that assembly of the GLYs' central skeleton might be accomplished by either of two routes. Both proceed from the same isoflavone, **10**, and subsequently take advantage of what we perceived to be very accessible alkene intermediates (**8** or **11**) for

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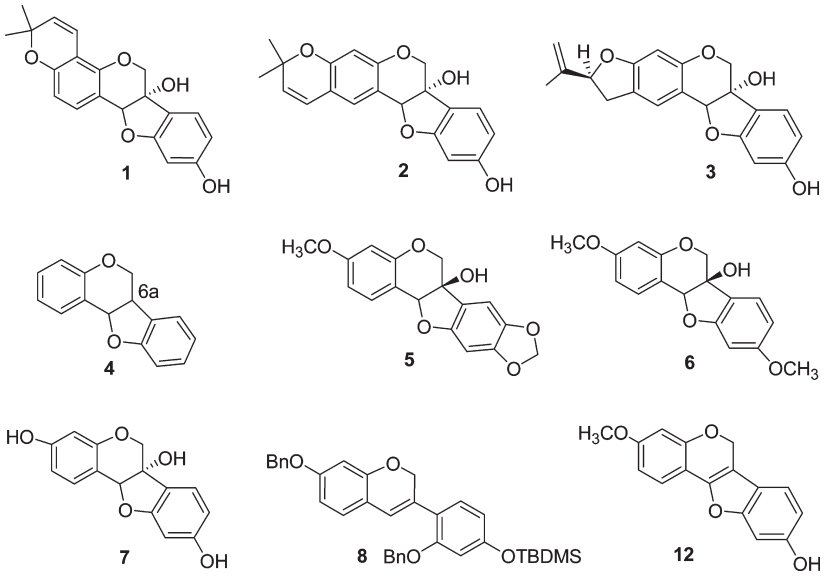
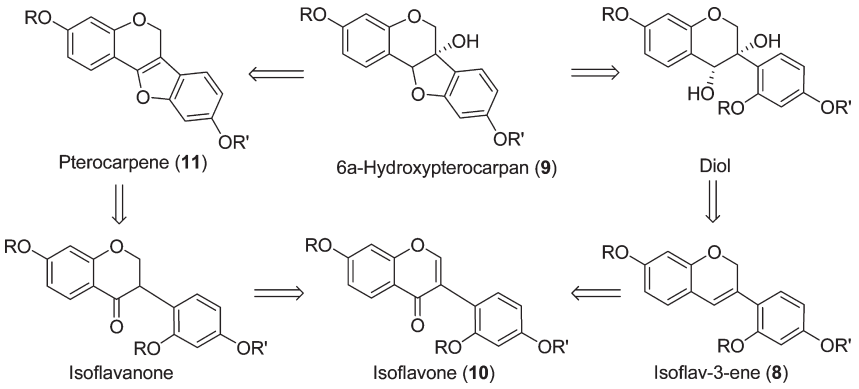


Figure 1. Selected structures. The glyceollins, such as GLY I **1**, GLY II **2**, and GLY III **3**, are phytoalexins produced by the soybean in response to specific stress conditions. Structure **4** depicts the typical pterocarpan ring system and specifically denotes the "6a" position. Structures **5** and **6** are respectively (+) pisatin and (+) variabilin; these compounds are 6a-hydroxypterocarpan natural products which have been previously synthesized by other investigators. All of the GLYs are derived from the phytochemical intermediate glycinol **7**. Structure **8** is an appropriately substituted isoflav-3-ene system that serves as a key intermediate for both of our former total syntheses and for the present scale-up synthesis of GLY I. Structure **12** is lespedezol A_1 which we previously prepared and used as a model system to explore alternative synthetic entries toward GLY I (see text for details).

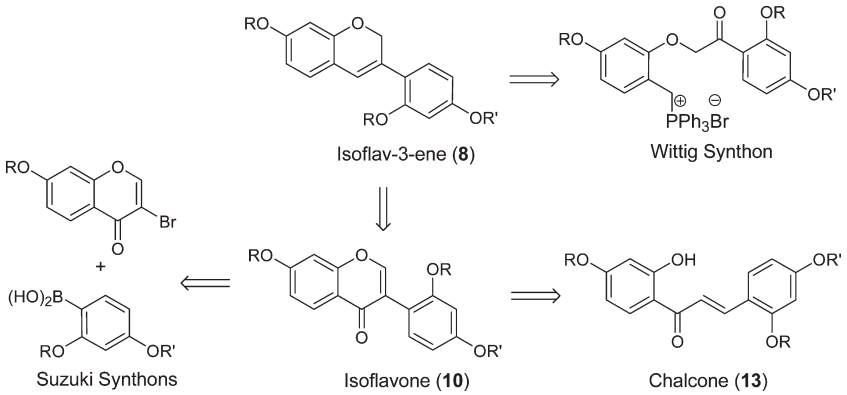
Scheme 1. Retrosynthetic approaches to the 6a-hydroxypterocarpan (9) required for GLY I



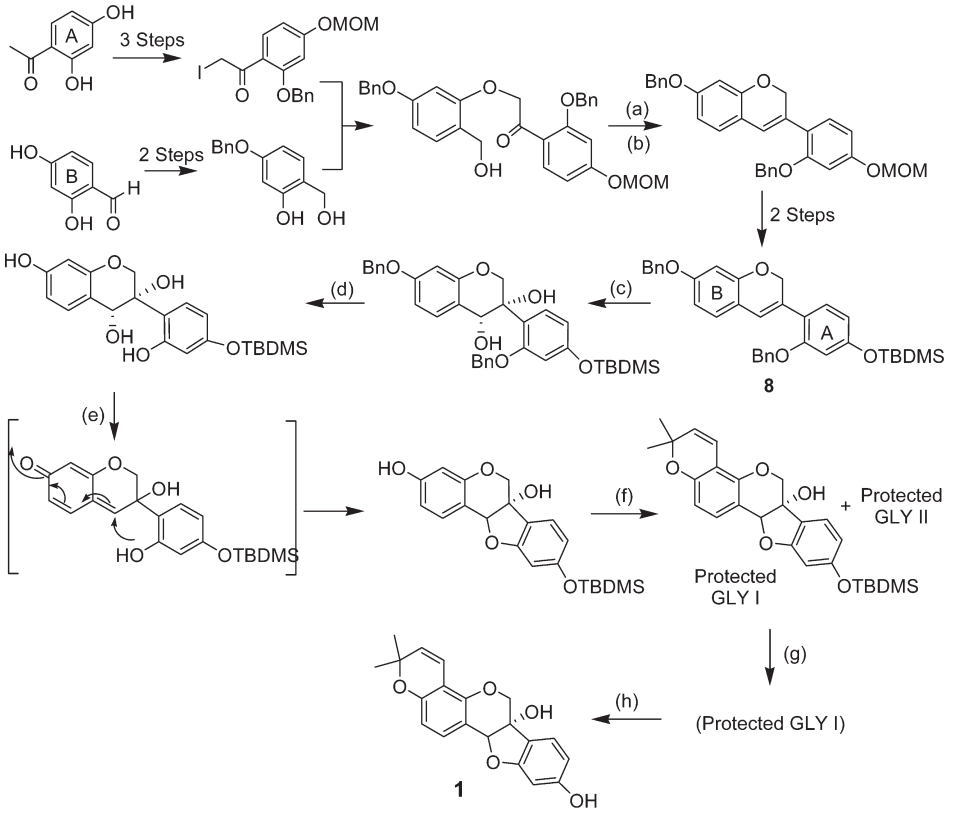
potential insertion of the 6a-hydroxy group. The diol route is similar to that in the literature, while the other necessitates adding a water molecule across the double bond of a pterocarpene (**11**) analogous to what has been reported for indene.[13] To examine the alternative strategy, we previously prepared lespedazol A_1 (**12** in Figure 1)[14] and used it as a model pterocarpene that we then subjected to several different types of conditions, none of which proved successful toward obtaining the 6a-hydroxy-pterocarpan system **9** (Scheme 1). We thus adopted the diol strategy and, in that regard, have previously explored several ways to obtain the key isoflav-3-ene intermediate **8**.

Isoflav-3-enes such as **8** are generally obtained from their corresponding isoflavones **10**. While several routes can be taken to the latter, they are historically obtained, and seemingly still so in the most generally consistent manner, from their correspondingly substituted chalcones (**13** in Scheme 2) through an acetal adduct by using a thallium-mediated oxidative rearrangement.[15] In addition to successfully utilizing this route, we attempted the rearrangement via a newer method that employs Koser's reagent[16] but found that its net yields of 10−15% were considerably lower than that for the thallium-mediated approach at ∼70%. Similarly, attempts to assemble **10** via a Suzuki coupling[17] also proved less efficient, likely due to steric hindrance when R is a benzyl group on the boron-containing partner (Scheme 2).Other seemingly attractive condensations such as that involving a deoxybenzoin,[18] require more elaborate preassembly of the appropriately substituted coupling partners needed for eventual elaboration into our specific target compound, GLY I (**1**). Alternatively, as also shown in retrosynthetic Scheme 2, we imagined that assembly of **8** might instead be accomplished without having to traverse the isoflavone, namely via an intramolecular Wittig reaction somewhat analogous to the latter's deployment in several other types of ring-forming systems.[19] Examination of this alternative strategy was investigated directly with very accessible starting materials that would be needed later for synthesis of **1**. All of these early-stage reactions, including the novel Wittig-driven ring-closure, proved quite

Scheme 2. Retrosynthetic approaches to the key isoflav-3-ene intermediate 8



Scheme 3. Previous[10] total synthesis of GLY I utilizing a nonbiomimetic Wittig reaction (steps (a) and (b)) to obtain the key intermediate isoflav-3-ene 8 (after adjustment of protecting groups)[a]



[a] Reagents and conditions: (a) $PPh_3 \cdot HBr$, CH_3CN, rt; (b) *t*-BuONa, MeOH, reflux, 78% (over two steps); (c) OsO_4, $(DHQD)_2PHAL$, CH_2Cl_2, −78 °C; (d) Pd−C (10%), H_2, Me_2CO, 80%; (e) polymeric base, EtOH, molecular sieves 4 Å, 64%; f) 1,1-diethoxy-3-methyl-2-butene, picoline, *p*-xylene, 130 °C, 61%; (g) silica gel column chromatography, (CH_2Cl_2/MeOH); (h) $Et_3N \cdot 3HF$, CH_3CN, −20 to 4 °C, 77%.

successful. Thus, from this backdrop two strategies became available for potential scale-up of **1**: a 'biomimetic route' in which the initial intermediates involved chalcone and isoflavone-related materials; and a 'nonbiomimetic route' in which the early assembly utilized a Wittig reaction. Our early Wittig and biomimetic routes to GLY I are depicted in Schemes 3 and 4, respectively.[1,10]

Interestingly, while both of these routes begin with the same two starting materials such that this aspect of overall cost considerations is not a factor, their initial building blocks become reversed within the contiguous ring system during assembly of the key isoflav-3-ene (note locations of rings "A" and "B" within **8** between Schemes 3 and 4). Likewise, both routes require a similar number of overall synthetic steps and column chromatography purifications, again equally balancing these types of practical comparisons. Alternatively and much to its favor, the Wittig approach was accomplished in ∼3% overall yield while

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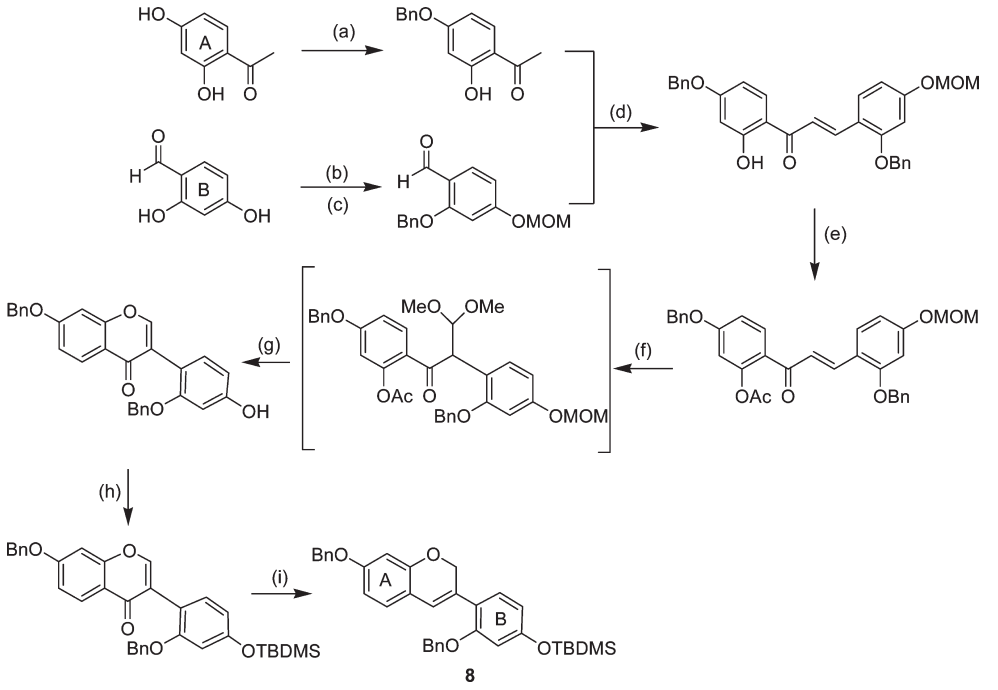
Scheme 4. Previous[1] biomimetic route to the key isoflav-3-ene intermediate 8[a]



[a] Reagents and conditions: (a) BnBr, K_2CO_3, CH_3CN, reflux, 10 h, 88%; (b) MOMCl, K_2CO_3, Me_2CO, rt, 24 h, 70%; (c) BnBr, K_2CO_3, CH_3CN, reflux, 4 h, 80%; (d) piperidine, MeOH, 60 °C, 6 h, 80%; (e) acetic anhydride, Et_3N, rt, 92%; (f) $Tl(NO_3)_3$, $3H_2O$, MeOH:TMOF (1:1), rt, 8 h; (g) 10% HCl, THF, reflux, 6 h, 68% (over two steps); (h) TBDMSCl, Et_3N, CH_2Cl_2, 90%; (i) 1) $LiBH_4$, THF, 0 °C, 6 h; 2) 10% HCl, reflux, 2 h, 45% (over two steps).

that for the biomimetic approach was only about 1%. In addition, the Wittig route eliminates the use of thallium which we felt would be a very advantageous step closer to a 'greener' overall procedure immediately from the outset of our scale-up activities.

STRATEGIC SCALE-UP STUDIES

Before embarking on a step-by-step enhancement of the Wittig route, five strategic questions were entertained experimentally on small scale: (1) Could the initial MOM protecting group be retained through the entire sequence even though we previously had trouble removing it at the end due to concomitant decomposition under acidic conditions (note that while the TBDMS protecting group behaves very nicely during its removal at the end of the overall synthesis, it is not compatible with our earlier Wittig step such that its singular use across the entire route is precluded)? (2) What is the chemical nature and physical scope of the instability observed for certain of the intermediates and, especially, for the final product GLY I? (3) Although preliminary results had not been encouraging, could we perform the asymmetric dihydroxylation step with less than an equal stoichiometry of chiral ligand? (4) Could we coax spontaneous ring-closure to the dihydrobenzofuran system as part of the debenzylation step, particularly since preliminary data suggested that this might be feasible? (5) By taking another look at the late-stage formation of the final chromene ring needed for GLY I, could we improve the regiochemistry so as to provide an even larger ratio of the desired GLY I product over the GLY II side-product? Each of these questions is briefly addressed below.

(1) An attempt was made to find a selectivity window that would allow for removal of MOM while minimizing decomposition of GLY I, still suspecting that the latter may be particularly sensitive to acidic conditions. These studies used the less precious racemic version of the penultimate intermediate that we were able to take through the entire synthetic route to that point uneventfully with a MOM protecting group. Several experiments[20−23] were then conducted on small scale so as to examine a range of acidic pH levels, cold temperatures and abbreviated reaction times, as well as in some cases deploying alternative reagents such as magnesium bromide which is known to be highly selective toward deprotection of MOM groups.[23] Table 1 provides a further listing of the various deprotection studies. None of these experiments were able to circumvent decomposition while simultaneously delivering enhanced product ratios to the extent that it would become advantageous to eliminate the earlier two-step switch of MOM to a TBDMS group immediately after the Wittig-driven ring closure that forms the key isoflav-3-ene **8a**.

Table 1. MOM deprotection experiments using the racemic form of GLY I's penultimate intermediate

reagent	pH	temp. (°C)	yield (%)[a]	time (h)	ref
$PPh_3 \cdot HBr$	4−6	rt	0−5	2−4	20
$PPh_3 \cdot HBr$	4−6	0	5−10	4−6	
$NaHSO_4 \cdot SiO_2$	4−6	rt	5−10	3−5	21
$NaHSO_4 \cdot SiO_2$	4−6	0	10−15	4−6	
0.01 M HCl (MeOH)	2−3	rt	0	1−2	22
0.01 M HCl (MeOH)	2−3	0	0−5	3−4	
$MgBr_2$/EtSH	–	rt	0−5	1−3	23
$MgBr_2$/EtSH	–	0	0−5	4−5	

[a] Based on TLC and ^1H NMR peak ratios. Major side product is dehydro GLY I initially, followed by further degradation with time.

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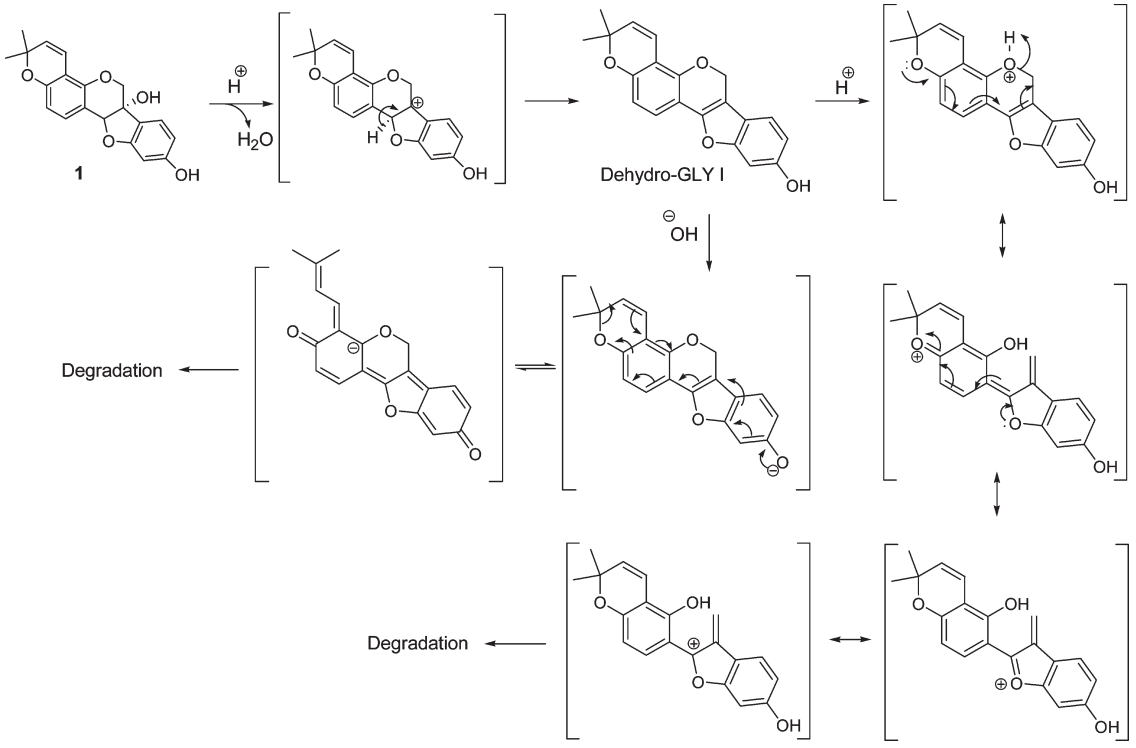
Scheme 5. Degradation of GLY I (1) and its dehydrated product[a]



[a] Note that the initial transient formation of the tertiary carbocation (additionally stabilized by its benzylic nature) via an E1 process is required because the cis-ring fusion in GLY I precludes a simple E2 type of collapse in a concerted manner.

Accepting that it remains most desirable overall to start with a MOM protecting group and then replace it with a TBDMS group, we next investigated the possibility for accomplishing this exchange in a 'one-pot' fashion. This would eliminate the need to isolate the first isoflav-3-ene intermediate for which susceptibility to spontaneous decomposition during workup was at least suspected to contribute to lower yields (see further discussion below). After taking advantage of our previously devised procedure that uses $PPh_3 \cdot HBr$ in dichloromethane to gently remove MOM within the course of a few hours,[10] we added triethylamine followed by TBDMSCl to the same flask and allowed the mixture to stir overnight. This facile manipulation proved successful while retaining a similar net yield across the former two-step procedure. Its utility was then further demonstrated by successfully deploying it during our reported synthesis of glycinol, **7**.[11]

(2) Prompted by the experimentally confirmed sensitivity toward acidic conditions noted above, coupled with theoretical concerns from the start that GLY I (**1**) might readily loose a water molecule to form an inherently more stable material because the resulting double bond then conjugates two aryl groups (Scheme 5), we undertook HPLC stability studies of **1** to better define the scope of its liability. GLY I appears to be adequately stable (for synthetic-related purification manipulations) in protic media above pH 4 and below pH 10 providing that temperatures are not raised significantly past 40 °C for prolonged periods, e.g. during solvent removal on a rotavap.[24] Quite unexpectedly, however, even though the conjugated dehydro-GLY I may be more thermodynamically stable, its chemical sensitivity appears to be enhanced and its resulting solution stability becomes diminished. The latter material is apparently capable of collapsing across essentially the entire pH range with just a modest amount of warming such that its manipulations in basic or acidic solvents should be kept to a minimum. Proposed mechanisms for all of these decomposition pathways are provided within Scheme 5. Verification of the postulated reactive intermediates has not been undertaken experimentally.

Importantly, an appreciation of the propensity for these dehydro- (or 'ene-containing') materials to undergo spontaneous decomposition must also be extended to include analogous structures such as the key intermediate **8** being envisioned for scale-up. Even though **8** lacks the additional ring strain present in GLY I and, itself, had not previously demonstrated any particularly problematic stability issues during our prior total syntheses studies, subsequent isoflavene-related molecules synthesized more recently in our laboratories have demonstrated significant sensitivity toward acidic conditions when subjected to, albeit, somewhat harsher conditions.[25]

(3) Previous attempts to simultaneously reduce the levels of both osmium tetroxide (OT) and chiral ligand utilized during the Sharpless dihydroxylation step were unsuccessful, with ee falling-off proportionately when anything less than full stoichiometric amounts were deployed. However, these investigations were of a preliminary nature and utilized a convenient chiral shift reagent NMR protocol as an expeditious but crude (~± 5% precision) assay method.[26] The diastereomeric proton shifts that become diagnostic for the diol enantiomers in this practical, front-line assay are tabulated within the Supporting Information. To follow-up, we felt it would be worthwhile to develop an HPLC assay method at this step for future quality control (QC) use, as well as for repeating some of our prior efforts toward potentially reducing the load of chiral ligand, if not also reducing the OT load. Table 2 provides the distinct chromatographic retention

Table 2. Chiral HPLC data for the diol intermediate and for GLY I (1)[a]

cmpd	RT_1[b]	RT_2[b]
racemic (±)-diol	10.38	15.31
chiral (+)-diol[c]	10.35	–
chiral (−)-diol[d]	–	15.27
racemic (±)-**1**[e]	11.74	13.51
natural (−)-**1**[f]	11.75	–
unnatural (+)-**1**[e]	–	13.35

[a] Methods for all compounds utilized a Chiracel OD 25 cm × 0.46 cm column with UV detection at 230 nm on a Waters HPLC system. Mobile phase for the diols was hexane/isopropanol (25:75) at 1.0 mL/min. Mobile phase for **1** was hexane/isopropanol (10:90) at 1.5 mL/min. [b] RT = retention time in minutes. [c] Having stereochemistry eventually needed for natural (−)-GLY I. [d] Having stereochemistry eventually needed for unnatural (+)-GLY I. [e] Prepared as previously described.[1,10] [f] Prepared as previously described[1,10] and identical to authentic sample isolated from elicited soybeans.

times for each of the diol enantiomers when examined by an HPLC method that utilizes a chiral column and, ultimately, provided ∼± 1% precision.

Even though stoichiometric amounts of OT are typically utilized during these types of dihydroxylations, and especially so for the case of isoflavenes which bear neighboring oxygen substituents that are thought to additionally chelate with the OT intermediate complex,[12b] we previously were able to reduce the amount of OT required during this reaction to just catalytic amounts by adding *N*-methylmorpholine oxide as a secondary oxidant plus methanesulfonamide[27] as an agent to facilitate cleavage of the osmate−ester complexes so as to expedite recycling of osmium.[10] These conditions proved to be extremely useful when we prepared racemic materials but became disappointing when we tried to prepare the individual enantiomers by adding chiral ligands at various equivalents in that no asymmetric bias was observed. Thus, we adopted the more traditional approach of using stoichiometric amounts of OT and the chiral ligand wherein we were then able to achieve greater than 95% ee. Our initial choice for the ligand upon using the Sharpless mnemonic model[27] was $(DHQD)_2PHAL$, and it did produce the appropriate stereochemistry ultimately needed for natural GLY 1. Repetition of these earlier experiments with less OT and various ligand loads performed on small scale and utilizing the more refined HPLC assay method, produced the same negative results relative to loss in ee. For example, the data recorded in Table 3 indicates that while other catalysts can be utilized, higher equivalents and lower temperatures are required to obtain greater than 95% ee.

We next evaluated the possibility of reducing the quantity of chiral ligand while still maintaining the full stoichiometry of OT, but again this did not provide for adequately biased asymmetry during the reaction. Presumably a subtle extension of our earlier explanation[10] for these negative results also pertains to these new studies, namely that the chiral ligand needs to remain fully engaged with the osmate ester-substrate complex in order to display its enantioselectivity during a given molecular-level reaction event. If this molecular arrangement is disrupted by either reducing OT (in an attempt to allow for recycling) or a lack of chiral ligand, then the asymmetric bias quickly becomes lost. Finally, an experimental survey of other potential asymmetric ligands was conducted in stoichiometric and even excess amounts, paying particular attention to materials that are considerably less expensive

Table 3. Different reaction conditions using chiral ligand DHQD-CLB

OsO_4 (equiv)	DHQD-CLB[a] (equiv)	addition duration	reac. temp. (°C)	% ee
1.5	1.1	all at once	−20	80
1.5	2.5	10−15 min	−20	83
1.5	4	10−15 min	−20	82
1.5	4	10−15 min	−80	89
1.5	5	10−15 min	−80	92
1.5	5	5 h	−80	94.5
1.5	6	10 h	−80	98.2
1.5	6	5 h	−80	97.8

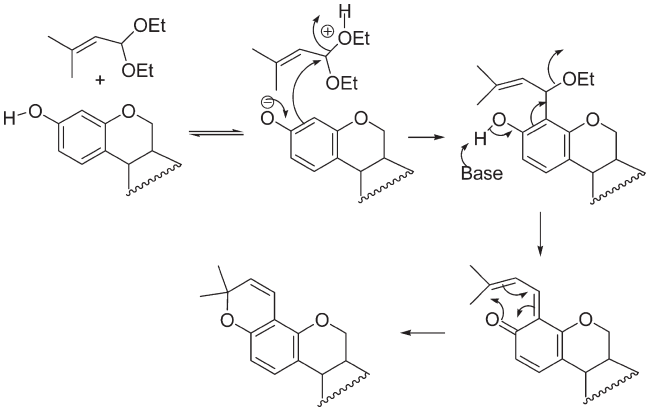
[a] Dihydroquinidine *p*-chlorobenzoate.

such as hydroquinidine 4-chlorobenzoate (exemplified in Table 3). None of these studies identified a better ligand than $(DHQD)_2$-PHAL which was shown to consistently deliver enantiomeric excess at 97% or higher and, wherein, we ultimately set our QC ee specification (by HPLC assay) for this critical step in the planned overall synthesis.

(4) Debenzylation of the diol to the tetrol affords the possibility for spontaneous cyclization to form the requisite dihydrobenzofuran via the dehydrated quinone-methide intermediate shown within brackets in Scheme 3. Indeed, a 'one-pot' procedure has been described[28] and previously used during the synthesis of pisatin.[12b] However, prior experience with this approach during our total syntheses of GLY I produced only small amounts of the cyclized product, and we eventually deemed it more advantageous to first isolate and purify the tetrol. During subsequent cyclization, we used a dilute solution in anhydrous ethanol to promote the desired intramolecular reaction, a polymer-bound base,[29] and molecular sieves to trap the water side product so as to additionally drive the cyclization forward. In the end, a respectable 64% yield across the two steps was obtained.[10] Nevertheless, upon additionally exploring this situation, we found that by simply changing the solvent used during the hydrogenolysis reaction from acetone to anhydrous ethanol, and then increasing the hydrogen pressure from 15 to 35 psi so as to maintain a similar net reaction time, at least 85% yields of the cyclized material were obtained with the remaining mass balance also being isolable and still available for separate cyclization. Again, the improved utility of this modified procedure was further demonstrated by deploying it successfully during our reported synthesis of glycinol, 7, wherein we also noted that the pronounced solvent effect may be due to enhanced solvation of the polar transition state during nucleophilic attack by the resorcinolic ortho hydroxy-oxygen atom on the quinone-methide, as well as from the anhydrous conditions being better able to accommodate the side-product water.[11] In some subsequent runs during further scale-up (later discussion), the desired cyclization was found to be essentially quantitative.

(5) Finally, we took another look at both the stage and method where we assemble the chromene ring. For our previous total syntheses and related medicinal chemistry structure−activity relationship (SAR) studies, we felt that it was advantageous to reserve construction of the isoprenyl-related chromene ring system until the end because late-stage divergent chemistry could then allow for GLY I, GLY II, and potentially GLY III analogues to be generated from the same overall route. This incentive is not present for our strategic scale-up considerations directed toward

Scheme 6. Mechanism of the isoprenylation reaction utilized to form the final chromene ring system within GLY I



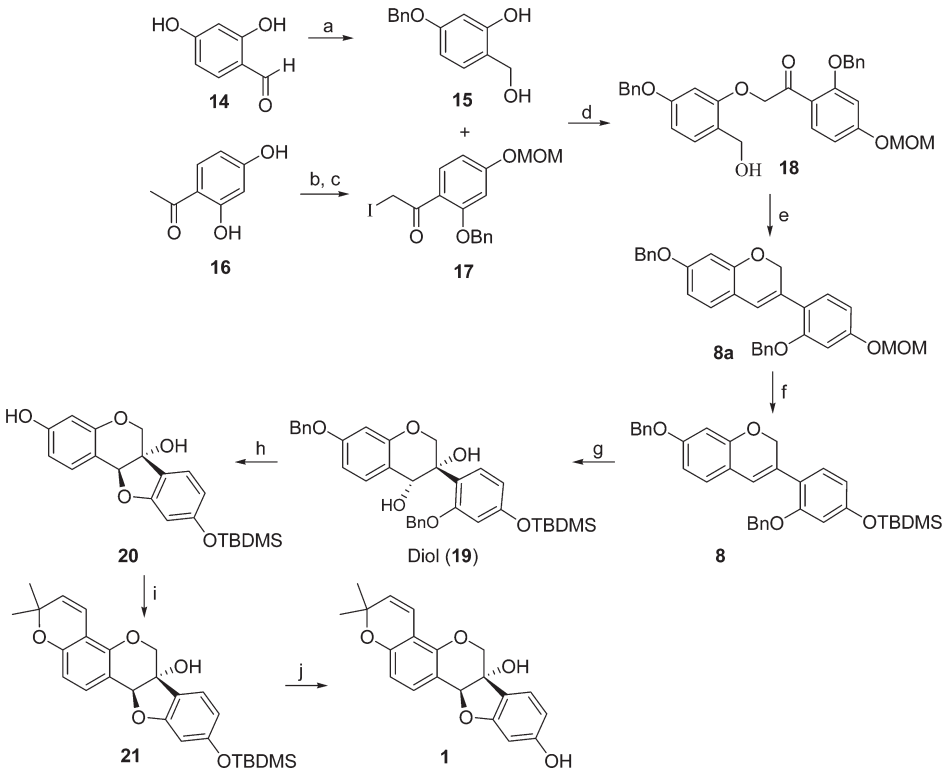
just the production of GLY I. Instead, for scale-up purposes it would initially appear to be advantageous to move this promiscuous ring-forming reaction to the earliest stage possible in the overall process with the thought that it might even then play the role of a protecting group for the appropriate phenolic hydroxyl functionality immediately from the start of the synthesis. Indeed, we have deployed this type of strategy successfully in our prior total synthesis of xanthohumol by an overall method that is quite amenable to process chemistry, although in that case xanthohumol contains a noncyclized isoprene unit linked directly to an aryl-ring within the molecular framework of a chalcone.[30] Adding to the challenge within the context of GLY I's structure, however, is the chromene's requirement for itself to ultimately display a double bond. That type of double bond would be susceptible to both the OT reaction at the dihydroxylation stage and to potential reduction during hydrogenolysis of a remaining orthogonally generated benzyl protecting group at the stage of the dihydrobenzofuran ring formation, the latter now having been nicely worked-out according to the experiments discussed above. While temporarily masking the double bond in a preassembled chromene system additionally can be imagined, we felt that these extra steps and their less precedented behavior during scale-up chemistry, detracted from moving the chromene ring's construction to an earlier stage of the planned synthesis. Alternatively, we did reinvestigate the two common methods used to form this ring, namely a modified Aldol condensation[31] and a Harfenist−Thom rearrangement of the propargyl ether.[32] As before,[10] introduction of a dimethylpropargyl group remained tedious such that the Aldol route was still preferred. As shown in Scheme 6, this reaction proceeds by condensing the tautomeric keto-form of the phenol with the unsaturated aldehyde masked as its acetal. Because this can occur with the keto-form by involving either of the α-carbons, the reaction produces both the GLY I and GLY II penultimate intermediates. However, GLY I is produced in ∼5-fold higher amounts. Various attempts to modify this to even higher ratios by adjustment of the reaction conditions were not fruitful.

Benefiting from these initial investigations, the intended scale-up chemistry route was upgraded to that shown in Scheme 7. It is two steps shorter than the original Wittig route, certain of its reactions have already been improved in yield, and the need for column chromatography purifications in at least some instances have been completely removed. In addition, QC specifications have been firmly established for the critical asymmetric step, and both a convenient front-line NMR assay and a practical follow-up HPLC assay have been developed to assess ee. Performing a retro-yield analysis suggested that in order to obtain ∼5 g of GLY I, we would need to prepare ∼250 g of the central isoflav-3-ene intermediate starting from ∼2 kg of the initial commercially available building blocks. Toward such amounts, we felt that scale-up for the early steps could proceed in increments of 100−250 mg initially and quickly move across gram into 100 g quantities with limitations in glassware ultimately preventing scale-up much past the 250−500 g level (2−5 L flasks) within our academic-based center's laboratories. We felt that the middle steps could then be comfortably explored at the 10-mg range with scale-up proceeding to the 100-mg and finally 1−50 g level. However, because of the size limitations of our specialized glassware and equipment, as well as for safety reasons within our multipurpose academic laboratories, we envisioned caps on the OT and hydrogenation reactions being set at the ∼10 g scale. The last several steps would likely not be faced with such logistic problems as the accumulated falloff in material will become more limiting than either glassware size or safety considerations. Our team approach placed two chemists at the front-end of the composite of operations who were charged with optimizing reactions and initiating enough scale-up to deduce satisfactory material specifications for achieving high performance in each subsequent step. One chemist was dedicated to developing analytical methods and performing much of the sample assays with assistance for the latter from any of the others as needed. The remaining four lab-based chemists performed scale-up reactions and repetitive large-scale purification operations. 'All-team-member' meetings were held weekly. In addition to continually monitoring and potentially upgrading the technological considerations while appropriately balancing the distributions of effort, these meetings also ensured that coverage of critical overnight reactions and column purifications were tended to in an equitably shared manner across all of the team members. A summary of the actual scale-up follows in the next section. A singular focus is provided for the critical establishment of asymmetry associated with the 6a-hydroxy functionality which is so distinctive for all of the glyceollin family members inclusive of our specific target compound GLY I.

MULTIGRAM PREPARATION OF GLY I

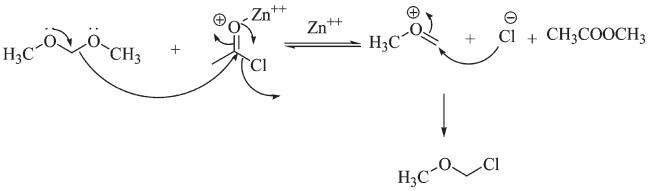
Scale-Up to the Key Isoflav-3-ene Intermediate (8). Initial attempts to regioselectively protect **14** had resulted in significant amounts of dibenzylated material (30%) which we previously were able to largely circumvent (<5%)[10] by specifically deploying sodium bicarbonate in acetonitrile.[33] Likewise, subsequent reduction to alcohol **15** using sodium borohydride followed by conventional workups also had proven to be problematic, likely owing to ready formation of the quinone-methide under basic and even acidic conditions. A review of the literature indicated that despite being more elaborate, alternative methods are usually employed for making salicylalcohol from salicylaldehyde.[34] Nevertheless, during our prior small scale syntheses[10] we eventually were able to conduct a standard sodium borohydride reduction in methanol by adopting a workup wherein after evaporation of solvent, 0.1 N sulfuric acid was carefully added so as to achieve an acidic pH of not less than 6.0. Addition of water then conveniently precipitated high purity product in nearly 80% yield. This convenient two-step process was found to be quite

Scheme 7. Final scale-up chemistry route used to prepare GLY 1 (1)[a]



[a] Specific reaction conditions: (a) i) BnBr, $KHCO_3$, CH_3CN, reflux, 15 h; ii) $NaBH_4$, MeOH, 0 °C then rt, 6 h, 61%, over two steps; (b) i) DIPEA, CH_3COCl, $CH_3OCH_2OCH_3$, cat. $Zn(OAc)_2$, EtOAc, 0 °C then rt, 18 h; ii) BnBr, K_2CO_3, Me_2CO, reflux, 18 h, 74% over two steps; (c) I_2, Selectfluor, CH_2Cl_2/MeOH, rt, 20 h, 79%; (d) K_2CO_3, Me_2CO, reflux, 20 h, 72%; (e) i) $PPh_3 \cdot HBr$, CH_3CN, rt, 1 h; ii) *t*-BuOK, MeOH, reflux, 24 h, 76% over two steps; (f) $PPh_3 \cdot HBr$, CH_2Cl_2, rt, 2 h, then Et_3N, TBDMS-Cl, rt, 12 h, 70%; (g) OsO_4, $(DHQD)_2PHAL$, CH_2Cl_2, −20 °C, 20 h, 95%; (h) Cat. Pd/C, H_2, EtOH, rt, 2 h, 100%; (i) 1,1-diethoxy-3-methylbut-2-ene, 3-picoline, *p*-xylene, 110 °C, 18 h, 60%; (j) $Et_3N \cdot 3HF$, pyridine, CH_2Cl_2, rt, 5 h, 90%.

Scheme 8. Proposed mechanism for in situ generation of MOM-Cl

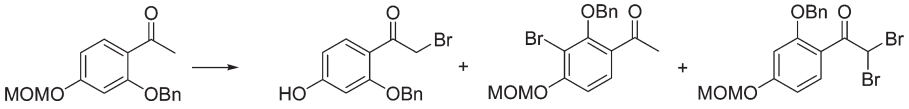


scalable up to the 100 g level. A recrystallization of the protected aldehyde intermediate from methanol to remove traces of unreacted benzylbromide was found to be conducive to this material's better performance in the next step. During larger-scale runs, sodium borohydride was added portion-wise and 0.5 N sulfuric acid was used for the workup to carefully adjust the pH to 6−7. Over 900 g of intermediate **15** was conveniently prepared in this manner with average yields across the two steps consistently running at about 61%.

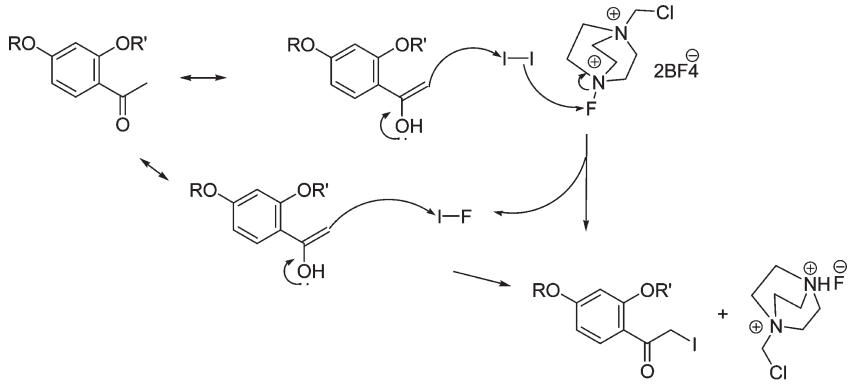
Orthogonal protection of the acetophenone **16** began with regioselective introduction of a MOM group. We previously had demonstrated on small scale while using MOM-Cl that this can be accomplished with high selectivity for the para position because the ortho hydroxy group forms a tight hydrogen bond with the adjacent carbonyl so as to render it less reactive.[10] At larger scale, however, we regarded the direct manipulation of MOM-Cl as being undesirable. Thus, this reagent was generated in situ from 'methylal' and Zn^{2+}.[35] A proposed mechanism for the latter is shown in Scheme 8. This safer procedure did not alter regioselectivity, was scalable to the 200-g level, and in the end, increased the yield for this step by nearly 20%. The ortho hydroxyl was protected next by treatment with benzylbromide using sodium carbonate in acetone to deprotonate the phenol. This veritably useful reagent/solvent pair[36] behaved appropriately for ready incorporation of the second protecting group up to similar 200 g levels of reaction.

In order to reduce costs related to using the expensive catalyst Selectfluor, alternative reaction conditions for selective halogenation of the diprotected ketone were quickly reinvestigated[10] at the gram level. Bromination by conventional methods such as bromine in acetic acid, cupric bromide in dioxane, phenyltrimethylammonium bromide, and polymer-supported tribromide,[37] all resulted in a complex mixture of products associated with additions to the electron rich aromatic ring and dibromination at the α-position, as well as loss of the MOM protecting group (Scheme 9). Among such approaches, bromine in acetic acid was the only one that delivered a single, α-brominated product but the latter had also lost MOM. Extensive investigations were then directed toward altering the reaction conditions for selective iodination, including an experimental survey to completely replace the expensive catalyst.[38] None of these studies identified a superior system. Alternatively, upon scale-up from the 1- and 2-g levels to the 5- and 10-g levels, it was found that use of

Scheme 9. Problematic bromination of diprotected ketone



Scheme 10. Proposed mechanism for Selectfluor-assisted selective α-iodination[a]



[a] It is also suggested that the Selectfluor molecule may tend to form a 'stacking' arrangement with the benzene ring depending upon the nature of the solvent and solutes' concentrations.

methylene chloride and methanol (1:5) in 0.2 M concentration was important for obtaining high yields of regioselectively iodinated product **17**. Addition of freshly prepared saturated aqueous solutions of sodium thiosulfate pentahydrate to neutralize traces of unused iodine was also found to be important during workup. Scale-up proceeded in 2- to 5-fold increments up to 124-g runs where apparatus size (larger than 2 L round-bottom flask) with adequate stirring of the nonhomogeneous reaction mixture became a limiting factor. Another problem encountered during scale-up of this reaction was the observation that there were periodic losses in yield which appeared to be attributable to variable quality of the Selectfluor even from new bottles having an identical batch number from the same commercial source, perhaps reflecting sensitivity of the reagent to historically undefined variations in storage and shipping conditions. Because there were no readily discernible analytical differences in these reagent batches, to circumvent this issue we devised a standardized small-scale reaction (5 g, 17.5 mmol) protocol as a functional QC measure to be undertaken in our laboratories for each new bottle prior to its use in larger-scale reactions. The progress of this reaction was followed by both TLC and ^{1}H NMR, with the appearance of a peak at ca. δ 4.4 for the protons on the carbon bearing the iodine atom accompanied by the loss of the methylketone peak at ca. δ 2.5 being particularly diagnostic. A proposed mechanism for this interesting reaction is shown in Scheme 10. Beyond knowing that the transformation proceeds via a reactive iodinium species,[39] further mechanistic details have not been reported. Regioselective iodination at the α-carbon in the presence of a benzene ring is very delicate, especially when the latter's electron density is increased by substitution with electron-donating benzyloxy- and methoxymethyleneoxy groups. Perhaps in addition to just activating elemental iodine toward electrophilic attack (Scheme 10), the positive charges on this catalyst also serve to counter the overall electron density on the benzene ring by residing in a stacking arrangement with the latter. While we have not pursued mechanistic studies per se, the specific solvent ratio and concentration range found to be optimal for this reaction are thus less surprising because such an intermolecular arrangement would be expected to be highly sensitive to even subtle modifications of these solvation shell variables.[40]

Table 4. Stability data determined by ^{1}H NMR for 17 in different solvents at different temperatures (*t* is time after reaction set up)

solvent		approx. % degradation			
		t = 0 h	*t* = 6 h	*t* = 17 h	*t* = 24 h
CH_2Cl_2	reflux	0	0	0	—[a]
CH_3CN	70 °C	0	60	70	—[a]
Me_2CO	rt	0	0	2.0	6
	reflux	0	0	60	95
DMF	70 °C	0	—[b]	95	—[a]

[a] Reaction was not performed up to this duration. [b] No observation was made until *t* = 17 h time point.

The ether linkage between **15** and **17** was formed under basic conditions after refluxing the reaction mixture for 20−24 h in acetone. Solutions of **17** were observed to undergo decomposition at elevated temperatures. Stability studies were undertaken to ascertain the least harsh conditions needed to form the ether. Although the identities of the degradation products were not discernible from these studies, the integrity of **17** could be readily assessed by proton NMR. The results are summarized in Table 4. After examination of several different solvents, acetone remained preferable across variations in temperature and time relative to **17**'s stability. Scale-up readily proceeded from the ∼1−115 g

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(~140 mmol) level. Reaction progress was again followed by both TLC and ^{1}H NMR, with complete disappearance of the diagnostic peak at about δ 4.4 for the protons on the carbon bearing the iodine atom in **17**, serving to signal stoppage of the reaction. Purification of **15** and **17** as well as the ether intermediate **18** can be accomplished by crystallization in high yields.

As discussed in the Background section, cyclization of **18** to **8a** was accomplished by an intramolecular Wittig olefination reaction. At the 1 g scale, substantial amounts of **18** were found to degrade during initial preparation of the Wittig salt upon treatment with triphenylphosphine hydrobromide. This occurred even when formation of this precursor was attempted in freshly distilled acetonitrile at room temperature.[10] Analysis of data collected for the major side-product confirmed that the MOM protecting group was gradually being cleaved, an event under these types of conditions that also has at least some precedent within the literature.[21] Careful monitoring of this initial part of the reaction by TLC revealed that formation of the Wittig salt could be completed in just 1 h of stirring at room temperature. Even more importantly, as scale-up proceeded we found that portionwise addition of the triphenylphosphine hydrobomide was preferable to adding the entire reagent in one lot. Thus, this reagent was added in five separate fractions of 0.2 equiv at ~15−20 min apart. The overall timing of this reaction can be easily monitored visually because **18** has limited solubility in acetonitrile, whereas its Wittig salt is very soluble such that, by addition of the last 0.2 equiv, the reaction mixture becomes a homogeneously clear solution. Ultimately, we were able to achieve complete conversion of **18** to its Wittig salt with negligible formation of any side products including that involving loss of MOM. This material was then used without further purification in the intramolecular condensation by treatment with sodium *tert*-butoxide in refluxing methanol, after which **8a** precipitates from the reaction medium. Scale-up proceeded to the 30 g (58 mmol) level averaging ~76% yields, somewhat higher than the ~70% observed during our early scale-up chemistry efforts and comparable to those at 78% observed previously during our initial total syntheses of GLY I that had been accomplished at the mg scale.[1,10]

As already described in the prior section pertaining to our initial strategic chemistry studies, protecting group modification of **8a** to **8** was optimized so as to become a high-yielding, one-pot procedure. Scale-up proceeded to the 20 g (41 mmol) level with average yields being maintained around 70%. Triphenylphosphine was removed by filtering a slurry of the reaction mixture in CH_2Cl_2/TFA/activated silica. Product purification was then accomplished by silica pad filtration followed by crystallization such that, to this point in the overall synthesis, the need for column chromatography purification procedures had been completely removed.

Scale-Up of the Asymmetric Dihydroxylation Reaction to Produce the Chiral Diol Intermediate (19). Optimization of this critical step has already been described above in the discussions about our strategic chemistry studies. The following comments convey additional hurdles encountered during further scale-up. Somewhat similar to our previous observations for the iodination reagent, we also noted variation in the quality of performance by the chiral catalyst needed for this step, although in this case pertaining more toward when it was purchased from different suppliers rather than from lot-to-lot and within lot variations. Even more problematic, however, was the fact that we seemed to have quickly depleted the commercial world supply of $(DHQD)_2PHAL$ with all of our orders suddenly being placed on indefinite back-order status. Given our overall time frame requirement, this situation made an in-house synthesis of the chiral ligand imperative. The latter was accomplished by following a two-step literature procedure[41] further adapted to allow for preparation of large quantities. QC measures were adopted to establish the integrity of the synthesized ligand, namely by comparison of melting point and NMR spectra with data from the literature.[41] Because of the observed potential for variation in catalytic performance, however, a functional QC measure was additionally established. This QC assay involved conducting a 50 mg standard reaction using separately QC-approved starting material **8** and separately QC-approved OT, noting that OT is itself capable of showing differences in performance between lots (further discussed below). Satisfactory assessment of the product from the standard reaction had to meet a specification of >97% ee using our chiral HPLC assay as described above. Interestingly, in the end, not only were the supply issues overcome by preparing this catalyst ourselves, but a cost comparison also indicated that after our synthesis of ~200 g of chiral catalyst, we were able to effect a savings of nearly $4000.00.

Another concern arising during scale-up at this step pertained to safety issues for handling the OT because we would need to use levels as high as 5 g during a given larger-scale reaction run. OT was purchased as crystalline material in 1-g sealed vials. To manipulate multiples of this highly toxic material into repeated reaction runs, we devised a procedure wherein several vials could be individually broken by a plunger extending into a small-mouthed, thick-walled bottle already containing a specified volume of toluene so as to make 100 mg/mL solutions of reagent. An upper range of 50 to 100 mL volumes was typically prepared in this manner. A sodium bisulfite 'bath' was additionally deployed around the bottle to protect the environment should any breakage of the device itself occur, although this never became an issue across numerous deployments of this convenient process. Scale-up proceeded to the 10 g (18 mmol) level with average yields being ~95%, somewhat higher than our lower scale efforts at ~86% and substantially higher than our prior milligram-range total synthesis efforts at ~70%.[1,10] In addition, for the highest scale runs **19** exhibited >98% ee even though the temperature during the asymmetric dihydroxylation was able to be raised to the more convenient −20 °C compared to the previous conditions that utilized −78 °C.[1,10] Purification again exploited a high-yielding crystallization rather than column chromatography. Final product specifications included: melting point; TLC; proton NMR; elemental analysis data for carbon and hydrogen; and importantly, >97% ee by HPLC analysis.

Formation of the Last Two Rings and Final Deprotection to Provide GLY I (1). Optimizations for debenzylation of **19** with concomitant cyclization to the *cis*-fused dihydrobenzofuran system in **20**, and for formation of the final chromeme ring system in **21**, already have been discussed above in the strategic chemistry development studies section. The following comments convey additional observations encountered during further scale-up. The latter was performed up to the range of ~5 g (8.5 mmol) and 3 g (6.6 mmol) in ~93% and 60% yields for **20** and **21**, respectively. Because the chromene regioisomers for both GLY I and GLY II are produced in an ~5:1 ratio during the course of this last ring-forming reaction, their separation by gravity column chromatography at this late stage represents the first use of this purification technique needed during the course of the entire synthesis. Specifications for both **20** and **21** include melting point, TLC,

Table 5. Optical rotation data for key chiral intermediates and for GLY I

cmpd	$[\alpha]^{25}_{D}$	solvent	conc. [*c*, (g/100 mL)]
diol **19**	+6.7	MeOH	1.6
20	−209.5	MeOH	0.3
synthetic **1**	−202.6	EtOAc	0.15
natural[a] **1**	−201.0	EtOAc	0.10

[a] Authentic sample obtained by isolation from stressed soybean plants.

Table 6. Yield and scale comparison for synthesis of GLY I

reaction	before optimization		after optimization	
step	av % yield[a]	largest scale[a]	av % yield	largest scale
a	61[b]	1 mmol (0.14 g)	61[b]	0.72 mol (100 g)
b	56	1 mmol (0.15 g)	74	1.3 mol (200 g)
c	70	1 mmol (0.29 g)	79	0.43 mol (124 g)
d	72	1 mmol (0.41 g)	72	0.28 mol (116 g)
e	78	1 mmol (0.51 g)	76	58 mmol (30 g)
f	69	1 mmol (0.48 g)	70	41 mmol (20 g)
g	70	1 mmol (0.54 g)	95	18 mmol (10 g)
h	54	0.1 mmol (39 mg)	100	8.5 mmol (5 g)
i	61	0.1 mmol (40 mg)	60	6.6 mmol (3 g)
j	77	0.1 mmol (43 mg)	90	2.2 mmol (1 g)
overall	2.7[c]	∼25 mg after 13 steps	11.5[c]	∼5 g after 11 steps

[a] Data from previous synthesis.[10] [b] Across two steps. [c] Across longest linear flow of steps.

proton NMR and chiral HPLC data with preservation of at least 97% ee. In the final step, deprotection with basic reagents like silica-supported TBAF or TAS-F[42] had largely resulted in degradation of the initially formed product.[10] Subsequent stability studies (see prior discussion above) have shown that formation of a dehydro-species can occur which, in turn, is even more susceptible toward additional decomposition (Scheme 5). Mildly acidic reagents were further explored as part of the scale-up. These studies indicated that while $NEt_3 \cdot 3\ HF$[43] should be retained as the reagent of choice, it is important to buffer the reaction mixture with excess pyridine. The latter significantly reduces formation of the dehydro-GLY I side-product and raises the yield for this step to ∼90% of the target compound **1** when it is run at the 1-g (2.2 mmol) level. Purification of the final material was accomplished by either flash column chromatography or by deployment of a Combi*flash* companion unit. After combination and evaporation of eluted samples, residues can be conveniently manipulated via methanol which, in turn, can be either lyophilized or evaporated and dried so as to provide a free-flowing solid product. Complete specifications for **1** are provided in the Experimental Section. A summary of the optical rotation data for the asymmetric intermediates and final compound **1** are provided in Table 5. Chiral HPLC chromatograms for **1** are provided within the Supporting Information.

SUMMARY

Table 6 provides a side-by-side comparison of the yields and reaction amounts for each of the steps before and after optimization and scale-up. The overall yields are ∼3% versus nearly 12% with the former route requiring 13 steps plus several column chromatography purifications, and the optimized synthesis requiring 11 steps with only two column chromatography purifications that occur at the end of the overall route. For the key asymmetric dihydroxylation step, it is necessary to have a stoichiometric amount of both OT and chiral ligand present throughout the course of the reaction. Favorable to deploying this type of asymmetric reaction within these types of molecular constructs, scale-up from milligram to low-gram and then to mid-gram range was accompanied by increasing yields while retaining excellent ee. Preparation of the chiral ligand can be highly advantageous from both a supply and cost perspective. Safe ways to handle both of the reagents needed for this reaction in a very practical manner on multigram scale have been devised and conveyed. In the end, nearly 5 g of material was prepared after several runs conducted by a team of six synthetic medicinal chemists and one bioanalytical chemist working over a period of ∼6 months. It is felt that by *following this* [now] *well-trodden path*,[44] the overall procedure should be quite amenable to even further scale-up within quicker timeframes while demanding less person-power. Of course, alternative paths always remain inviting as well.

EXPERIMENTAL SECTION

General. Chemical reactions were conducted under nitrogen in anhydrous solvents unless stated otherwise. Anhydrous solvents were purchased from commercial sources and were used without additional purification except for: (i) acetone (Me_2CO) which was further dried over 3 Å molecular sieves; and (ii) tetrahydrofuran (THF) which was further distilled under nitrogen over sodium benzophenone. All other reagents obtained from commercial suppliers were used without further purification. Thin layer chromatography (TLC) was done on 250 μm fluorescent TLC plates (Baker-flex, Silica Gel IB-F from VWR International, LLC) and visualized by using UV light or iodine vapor. Normal-phase flash and gravity column chromatography purifications were performed using silica gel (200−425 mesh 60 Å pore size) and ACS grade solvents. A Redi*Sep* flash column (12 g) was used for flash separation on a Combi*flash* companion unit from Teledyne Isco, Inc. Optical rotations were determined on a Rudolph Research model AUTOPOL III automatic polarimeter. Melting points (mps) were determined on an Electrothermal digital melting point apparatus and are uncorrected. NMR spectra were recorded on either a Varian Inova-600 spectrometer at 600 MHz, or a Unity-400 spectrometer at 400 MHz. Peak locations were referenced using either tetramethylsilane (TMS) or residual nondeuterated solvent as an internal standard. Proton coupling constants are expressed in hertz. ^{13}C NMR chemical shifts are reported to the first decimal place unless peaks are very close, wherein for such instances values are reported to a second decimal place. Spin multiplicity for ^{1}H NMR are reported using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, quin = quintet, br = broadened, dd = doublet doublet, dt = doublet triplet, dq = doublet quartet and other combinations derived from those listed. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA, U.S.A., and were regarded as acceptable when experimental values are within ±0.4% of the calculated values.

4-Benzyloxy-2-hydroxybenzaldehyde (15a). To a solution of the dihydroxybenzaldehyde **14** (100 g, 0.67 mol) in CH_3CN (1 L), BnBr (112.4 g, 0.67 mol) and $NaHCO_3$ (90.8 g, 0.67 mol) were added. The reaction mixture was refluxed for 48 h and was monitored by TLC. Upon completion, the white precipitate was

filtered and the filtrate was evaporated under vacuum. The residue was dissolved in MeOH (500 mL) and was precipitated at 0 °C. The precipitates were further recrystallized from MeOH to obtain **15a** (127.5 g, 0.56 mol, 84%) as an off-white solid: mp 80−82 °C (lit.[10] 78−80 °C); ^{1}H NMR (DMSO, 400 MHz) δ 10.99 (1H, s), 9.99 (1H, s), 7.37 (5H, m), 6.61 (1H, m), 6.54 (1H, d), 5.15 (2H, s).

4-Benzyloxysalicyl alcohol (15). To a solution of the benzaldehyde **15a** (127.5 g, 0.52 mol) in MeOH (1 L) at 0 °C, $NaBH_4$ (16 g, 0.42 mol) was added. The reaction mixture was stirred for 18 h at rt. Reaction progress was monitored by TLC. Upon completion, the reaction mixture was reduced to one-fourth volume and was treated with 0.5 N aq H_2SO_4 to adjust pH to about 6−7 followed by addition of H_2O (1 L). Upon vigorous stirring white precipitate formed. After filtration, the precipitate was lyophilized, washed using a chilled toluene (∼−50 °C) and dried under vacuum to obtain **15** (89 g, 0.38 mol, 73%) as an off-white solid: mp 86−88 °C (lit.[10] 88−90 °C); ^{1}H NMR (DMSO, 400 MHz) δ 9.33 (1H, s), 7.35 (5H, m,) 7.08 (1H, d, J = 8.4 Hz), 6.42 (2H, m), 5.01 (2H, s), 4.80 (1H, m), 4.39 (2H, s).

2-Benzyloxy-4-methoxymethylenoxyacetophenone (16a). To a solution of dimethoxymethanal (150.5 g, 1.97 mol, 175 mL) and $Zn(OAc)_2$ (43.88 mg, 0.24 mmol) in EtOAc (350 mL), CH_3COCl (154.7 g, 1.97 mol) was added over 2−3 h. The reaction mixture was stirred for an additional 2−3 h and then cooled to 0 °C. A solution of **16** (200.0 g, 1.31 mol) in EtOAc (615 mL) was then added slowly, followed by dropwise addition of Hunig's base (211.6 g, 1.63 mol). The reaction mixture was stirred for 18 h while being monitored by TLC. After completion, H_2O (300 mL) was added and the mixture further stirred for 1 h. The organic phase was washed with 1 M NaOH, brine and dried over anhyd Na_2SO_4. Solvents were evaporated under vacuum to obtain a yellowish oily residue which was utilized directly in the next step.

To a solution of the above product (128.3 g) in Me_2CO (1.3 L), BnBr (168.6 g, 0.98 mol) and K_2CO_3 (96.74 g) were added under a flow of N_2. The reaction mixture was stirred under reflux while progress was monitored by TLC. After completion, the mixture was filtered and solvents were evaporated under vacuum. The product was recrystallized from MeOH to obtain **16a** (249.0 g, 0.97 mol, 74% in two steps) as off-white crystals: mp 71−73 °C (lit.[10] 70−71 °C); TLC R_f = 0.63 [EtOAc/hexanes (1:2)]; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.82 (1H, d, J = 8.4 Hz), 7.40 (5H, m), 6.68 (2H, m), 5.19 (2H, s), 5.14 (2H, s), 3.47 (3H, s), 2.55 (3H, s).

1-(2′-Benzyloxy-4′-methoxymethylenoxy)phenyl-2-iodoethanone (17). To a solution of acetophenone **16a** (124.26 g, 0.43 mol) in anhyd CH_2Cl_2 (280 mL) and anhyd MeOH (1.7 L), Selectfluor (100 g, 0.26 mol) was added followed by elemental I_2 (49.88 g, 0.22 mol) under a flow of N_2. The reaction mixture was stirred for 24 h. Reaction progress was monitored by TLC and ^{1}H NMR. After completion, the mixture was filtered and the precipitate was washed extensively with CH_2Cl_2. The combined organic phase was evaporated under vacuum at 25−30 °C. The residue was again dissolved in CH_2Cl_2. The organic layer was washed with freshly prepared $Na_2S_2O_3$ solution (10% w/v), dried over anhyd Na_2SO_4 and evaporated under vacuum. The residue was recrystallized from MeOH to obtain **17** (140.1 g, 0.34 mol, 79%) as yellowish crystals: mp 76−78 °C (lit.[10] 66−68 °C); TLC R_f = 0.65 [EtOAc/hexanes (1:2)]; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.88 (1H, d, J = 9.6 Hz), 7.49 (2H, m), 7.41 (3H, m), 6.71 (2H, m), 5.20 (2H, s), 5.17 (2H, s), 4.40 (2H, s), 3.48 (3H, s).

2-(5′-Benzyloxy-2′-hydroxymethyl)phenoxy-1-(2′-benzyloxy-4′-methoxy-methy-lenoxy)phenylethanone (18). To a solution of salicyl alcohol **15** (107.5 g, 0.46 mol) and α-iodo ketone **17** (115.7 g, 0.28 mol) in Me_2CO (1.4 L) was added K_2CO_3 (46.32 g, 0.34 mol). The reaction mixture was stirred at reflux for 18−20 h. Reaction progress was followed by TLC and ^{1}H NMR. After completion, solvents were evaporated under vacuum, and the residue was dissolved in EtOAc/H_2O (1:1) mixture. The organic layer was washed with 0.1 M HCl, saturated $NaHCO_3$, H_2O and brine, dried over anhyd Na_2SO_4, and evaporated to dryness under vacuum. The solid residue was recrystallized from EtOAc/hexane (700 mL, 1:1) to obtain **18** (107.8 g, 0.21 mol, 72%) as a white solid: mp 122−124 °C [lit.[10] 115−118 °C]; TLC R_f = 0.3 [EtOAc/hexane (1:2)]; ^{1}H NMR ($(CD_3)_2CO$, 400 MHz) δ 7.87 (1H, d, J = 8.8 Hz), 7.64 (2H, d, J = 7.6 Hz), 7.41 (6H, m), 7.33 (1H, m), 7.24 (2H, m), 6.93 (1H, d, J = 2 Hz), 6.77 (1H, dd, J = 2, 8.8 Hz), 6.56 (1H, dd, J = 2.4, 8.4 Hz), 6.26 (1H, m), 5.32 (2H, s), 5.31 (2H, s), 5.22 (2H, s), 4.57 (2H, d, J = 6.4 Hz), 4.11 (1H, t, J = 6.4 Hz), 3.45 (3H, s).

2′,7-Dibenzyloxy-4′-(methoxymethylenoxy)isoflav-3-ene (8a). To a suspension of **18** (30.1 g, 58.5 mmol) in anhyd CH_3CN (1 L), $PPh_3 \cdot HBr$ (20.1 g, 58.4 mmol) was added in five portions of ∼4.0 g each at intervals of ∼15−20 min. By the last addition, the reaction mixture became a clear solution. The progress of the reaction was checked by TLC (CH_2Cl_2:MeOH [15/1] as developing system). The reaction was complete in 1−2 h. Solvents were then evaporated to dryness under vacuum at rt to obtain an off-white residue which was used in the next step without further purification.

To a solution of product from the previous step in anhyd MeOH (1.5 L), potassium *tert*-butoxide (13.1 g, 0.12 mol) was added with stirring. The reaction mixture was refluxed for 18−24 h. Reaction progress was monitored by TLC. After completion, the mixture was cooled to rt and filtered. The precipitate was dissolved in CH_2Cl_2. The organic layer was washed with H_2O and dried over anhyd Na_2SO_4. After filtration, the solvents were evaporated under vacuum to obtain **8a** (21.4 g, 44.5 mmol, 76% over two steps) as an off-white solid: mp 126 −131 °C (lit.[10] 115−118 °C) ; TLC R_f 0.39 (hexanes/EtOAc [5:1]); ^{1}H NMR ($(CD_3)_2CO$, 600 MHz) δ 7.42 (10H, m), 7.28 (1H, d, J = 8.4 Hz), 7.02 (1H, d, J = 8.4 Hz), 6.81 (1H, d, J = 2.4 Hz), 6.69 (1H, dd, J = 2.4, 8.4 Hz), 6.61 (1H, s) 6.57 (1H, dd, J = 2.4, 8.4 Hz), 6.47 (1H, d, J = 2.4 Hz).15 (2H, s), 5.10 (2H, s), 4.94 (2H, d, J = 1.2 Hz), 0.97 (9H, s), 0.19 (6H, s);^{13}C NMR ($(CD_3)_2CO$, 150 MHz) δ 160.4, 159.2, 158.1, 155.6, 138.2, 137.7, 129.98, 129.91, 129.3, 129.2, 128.8, 128.7, 128.5, 128.34, 128.30, 122.5, 121.8, 118.0, 108.9, 108.8, 102.9, 102.4, 95.0, 90.1, 71.0, 70.4, 68.9, 56.0, 3.37, 3.35, −11.4.

2′,7-Dibenzyloxy-4′-(*tert*-butyldimethylsilyloxy)isoflav-3-ene (8). To a solution of 2′,7-dibenzyloxy-4′-(methoxymethalenoxy)-isoflav-3-ene **8a** (19.6 g, 40.8 mmol) in anhyd CH_2Cl_2 (200 mL) $PPh_3 \cdot HBr$ (17.6 g, 51.2 mmol) was added. The reaction mixture was stirred at rt for 1−2 h while followed by TLC (EtOAc/hexanes 1:2). After completion, Et_3N (7.8 g, 75.6 mmol, 10 mL) and TBDMS-Cl (7.6 g, 50.4 mmol) were added. The reaction was stirred at rt for 12−15 h. After completion, solvents were evaporated under vacuum at 30 °C. The solid residue was dissolved in CH_2Cl_2 (2 L), and oven-dried silica (340 g, dried overnight at 120 °C in oven and cooled in a desiccator) and a small amount of TFA were added; the mixture was gently stirred until complete disappearance of PPh_3 (TLC). After filtration, the filtrates were passed through a pad of silica. The solvents

were evaporated under vacuum, and the residue was recrystallized from CH_2Cl_2/MeOH (1:5) to obtain **8** (15.9 g, 28.6 mmol, 70%) as white crystals: mp 104−106 °C [lit.[10] 106−107 °C]; TLC R_f = 0.42 [EtOAc/hexanes (1:2)]; ^{1}H NMR ($(CD_3)_2CO$, 600 MHz) δ 7.40 (10H, m), 7.25 (1H, d, J = 8.4 Hz), 7.02 (1H, d, J = 8.4 Hz), 6.62 (2H, m), 6.57 (1H, dd, J = 2.4, 8.4 Hz), 6.51 (1H, dd, J = 2.4, 8.4 Hz), 6.47 (1H, d, J = 2.4 Hz), 5.15 (2H, s), 5.10 (2H, s), 4.94 (2H, d, J = 1.2 Hz), 0.97 (9H, s), 0.19 (6H, s);^{13}C NMR ($(CD_3)_2CO$, 150 MHz) δ 160.4, 158.1, 157.5, 155.6, 138.2, 137.9, 130.0, 129.8, 129.3, 129.2, 128.7, 128.57, 128.52, 128.3, 128.29, 128.28, 121.8, 118.1, 113.1, 108.9, 105.9, 102.9, 90.1, 70.9, 70.4, 68.9, 25.9, −4.3, −11.4; Anal. (%) calcd for $C_{17}H_{18}O_7$, C 76.30, H 6.95, found, C 76.02, H 7.09.

(+)-4′-*tert*-Butyldimethylsilyloxy-2′,7-(dibenzyloxy)isoflavan-3,4-diol (19). To a solution of chiral ligand $(DHQD)_2PHAL$ (15.6 g, 20.0 mmol) in CH_2Cl_2 (80 mL), OsO4 (5 g, 20.0 mmol) was added. After stirring at −20 °C for 1 h, a solution of **8** (10 g, 18.1 mmol) in CH_2Cl_2 (80 mL) was slowly added over 10−15 min, and the mixture was stirred at −20 °C for 18−20 h. Reaction progress was monitored by TLC. After completion, the reaction was allowed to warm to rt, and 10% sodium sulfite (100 mL, pH ∼9.0) and 10% sodium bisulfite (100 mL, pH ∼4) solution was added. After stirring at rt for 2 h, a mixture of THF/EtOAc (1:4, 1 L) was added to the reaction mixture and further stirred at 55 °C (external oil bath temp) for an additional 3−4 h. The reaction mixture was cooled to rt and filtered. The aq phase was extracted with EtOAc. The combined organic phase was washed wit 0.1 M HCl and brine and dried over anhyd Na_2SO_4 and evaporated under vacuum. The product was recrystallized from EtOAc/hexanes to obtain **19** (10.2 g, 17.3 mmol, 95%, >98% ee) as a white solid: mp 75−77 °C; $[\alpha]^{25}_D$ +6.7 (c 1.6, MeOH); TLC R_f = 0.28 [EtOAc/hexanes (1:3)]; Chiral HPLC RT = 10.35 min [Standard racemate RT = 10.38 and 15.31 min], mobile phase was 2-propanol/hexanes (25:75) at 1.0 mL/min; ^{1}H NMR ($(CD_3)_2CO$, 600 MHz) δ 7.59 (1H, dd, J = 2.4. 8.4 Hz), 7.39 (11H, m), 6.58 (2H, m), 6.49 (1H, d, J = 2.4 8.4 Hz), 6.38 (1H, d, J = 2.4 Hz), 5.52 (1H, d, J = 6.6 Hz), 5.20 (2H, s), 5.07 (2H, s), 4.73 (1H, d, J = 11.4 Hz), 4.26 (1H, m), 4.21 (1H, m), 4.02 (1H, d, J = 11.4 Hz), 0.96 (9H, s), 0.17 (6H, s); ^{13}C NMR ($(CD_3)_2CO$, 100 MHz) δ 159.9, 157.4, 157.2, 155.7, 138.4, 137.8, 130.7, 130.1, 129.3, 129.2, 128.6, 128.6, 128.4, 128.2, 128.1, 123.5, 118.3, 112.4, 108.7, 106.1, 102.1, 72.0, 70.8, 7.2, 67.6, 67.4, 25.9, 18.6, −4.3; Anal. (%) calcd for $C_{35}H_{40}O_6Si$, C 71.89, H 6.89, found, C 71.83, H 6.92.

(−)-9-(*tert*-Butyldimethylsilyloxy)glycinol (20). To a solution of **19** (5.01 g, 8.5 mmol) in anhydrous EtOH (110 mL) at 0 °C, 10% Pd/C (1.01 g) was added. The mixture was stirred at rt for 4 h under hydrogen atmosphere (35 psi). Progress was followed by TLC. Prolonged reaction times can cause losses in overall yield. After completion, the reaction mixture was passed through a pad of Celite which was then washed with EtOH (3 × 50 mL). The combined solvents were evaporated under vacuum to obtain **20** (3.27 g, 8.5 mmol, 100%) as an off-white powder: mp 196−198 °C; $[\alpha]^{25}_D$ −209.5 (c 0.3, MeOH); TLC R_f = 0.41 [MeOH/CH_2Cl_2/hexanes (1:10:10)]; ^{1}H NMR ($(CD_3)_2CO$, 600 MHz) δ 8.57 (1H, s), 7.31 (1H, d, J = 8.4 Hz), 7.26 (1H, d, J = 7.8 Hz), 6.56 (1H, dd, J = 8.4 Hz, J = 2.4 Hz), 6.46 (1H, dd, J = 2.4, 8.4 Hz), 6.33 (1H, d, J = 2.4 Hz), 6.27 (1H, d, J = 1.8 Hz), 5.28 (1H, s), 5.03 (1H, s), 4.13 (1H, d, J = 11.4 Hz), 4.01 (1H, d, J = 11.4 Hz), 0.97 (9H, s), 0.20 (6H, s); ^{13}C NMR ($(CD_3)_2CO$, 150 MHz) δ 161.6, 159.6, 158.6, 157.0, 133.1, 125.0, 123.6, 113.3, 113.0, 110.7, 103.7, 103.2, 90.1, 85.8, 76.6, 70.5, 25.9, −4.39, −4.40, −11.45, −11.46; Anal. (%) calcd for $C_{21}H_{26}O_5Si$, C 65.26, H 6.78, found, C 65.75, H 6.76.

9-(*tert*-Butyldimethylsilyloxy)glyceollin I (21). To a mixture of 6a-hydroxypterocarpan **20** (3.07 g, 7.94 mmol) in anhyd *p*-xylene (40 mL) were added 1,1-diethoxy-3-methyl-2-butene (2.6 g, 16.4 mmol, 3.1 mL) and 3-picoline (0.2 g, 2.1 mmol, 0.3 mL) successively under a flow of N_2. The reaction flask was fitted with a distillation assembly and was stirred at 125 °C (internal temp. 120 °C). Progress of the reaction was followed by TLC. After completion, the reaction mixture was directly applied to a column and was purified by gravity column chromatography using a step gradient [first hexanes/CH_2Cl_2 (2:1), 450 mL then was changed to hexanes/CH_2Cl_2/EtOAc (20: 10:1) to obtain **21** (2.2 g, 4.8 mmol, 60%) as off-white solid: mp 69−75 °C; TLC R_f 0.57 [EtOAc/hexanes (1:2)]; ^{1}H NMR ($(CD_3)_2CO$, 600 MHz) δ 7.28 (1H, d, J = 8.4 Hz), 7.24 (1H, d, J = 8.4 Hz), 6.57 (1H, d, J = 10.2 Hz), 6.46 (1H, m), 6.28 (1H, d, J = 2.4 Hz), 5.66 (1H, d, J = 10.2 Hz), 5.28 (1H, s), 5.09 (1H, s,), 4.20 (1H, d, J = 11.4 Hz), 4.08 (1H, d, J = 11.4 Hz), 1.39 (3H, s), 1.35 (3H, s), 0.97 (9H, s), 0.20 (6H, s); Anal. (%) calcd for $C_{21}H_{26}O_5Si$, C 68.99, H 7.13, found, C 68.91, H 7.27.

(−)-Glyceollin I (1). To a solution of **21** (1 g, 2.2 mmol) in CH_2Cl_2 (30 mL) were added $Et_3N\cdot 3HF$ (30 mmol) and excess pyridine (45 mmol). The reaction mixture was stirred at rt for 6 h. Progress was followed by TLC. After completion, solvents were evaporated under vacuum at 20 °C and directly applied to a flash column (silica ∼20 g) using 'dry sample' loading techniques [EtOAc/hexanes (1:1)]. The eluting fractions were collected, solvents were removed under vacuum, and the resulting residue was lyophilized after dissolving in a minimal amount of MeOH to obtain **1** (0.67 g, 1.97 mmol, 90%) as pinkish-white solid: mp 95−101 °C; $[\alpha]^{25}_D$ −202.6 (c 0.15, EtOAc); TLC R_f = 0.33 (MeOH/CH_2Cl_2/hexanes (1:10:10)); Chiral HPLC RT = 11.75 min [standard racemate RT = 11.74 and 13.51 min], mobile phase was 2-propanol/hexanes (10:90) at 1.5 mL/min; ^{1}H NMR (CD_3OD, 600 MHz) δ 7.21 (1H, d, J = 8.4 Hz), 7.16 (1H, d, J = 8.0 Hz), 6.60 (1H, d, J = 10 Hz), 6.46 (1H, d, J = 8.4 Hz), 6.40 (1H, dd, J = 2.0, 8.4 Hz), 6.22 (1H, d, J = 2 Hz), 5.62 (1H, d, J = 10 Hz), 5.16 (1H, s), 4.16 (1H, d, J = 11.6 Hz), 3.93 (1H, d, J = 11.2 Hz), 1.38 (3H, s), 1.35 (3H, s); Anal. (%) calcd for $C_{20}H_{18}O_5\cdot 0.1H_2O$, C 70.62, H 5.39, found, C 70.35, H 5.65.

Chiral NMR Shift Reagent Studies.[26]. Europium(III) tris-[3-(heptafluoro-propylhydroxy-methylene)-*l*-camphorate was deployed in 20% mole ratio by dissolving it in the same deuterated solvent as for the routine NMR sample, adding the solution directly to the latter, and then rerunning the NMR spectrum.

Chiral Column Chromatography.[11,45] In addition to the method delineated in Table 2, a chiral Cyclobond column from ASTEC Inc. can be used in a Waters HPLC equipped with a model 2659 separation module, a quaternary pump, a degasser, an auto sampler/injector (syringe volume =100 μL), a column oven and a model 2996 photodiode array detector. The mobile phase in this case used a gradient solvent system having water, acetonitrile, and methanol in the following percentages with time: 60, 0, 40 at the start; 45, 0, 55 at 30 min; and 60, 10, 30 at 48 min, after which the system was stepped back to start conditions and flushed for 12 min. Temperature was 35 °C, flow rate was 0.5 mL/min, and the detector was set at 254 nm. Retention times (min) were: 52.7 for authentic GLY I standard from stressed soybean; 53.2 for synthesized material; 49.4 for synthesized unnatural (+) material; and 49.5 + 53.3 for synthesized racemic material.

■ ASSOCIATED CONTENT

Supporting Information. Experimental details for synthesis of chiral ligand ($(DHQD)_2PHAL$); chiral HPLC chromatograms of **1**, its racemate, and opposite enantiomer; NMR chiral shift reagent data pertaining to the enantiomeric diols. This material is available free of charge via the Internet at http://pubs.acs.org.

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