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Total Synthesis of (±)-Glyceollin II and a Dihydro Derivative

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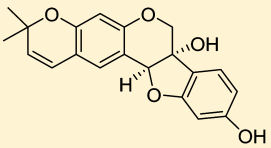
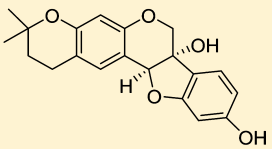
Supporting Information

ABSTRACT: Stressed soybeans produce a group of phytoalexins that belong to the 6a-hydroxypterocarpan family of flavonoids. Certain of the more prominent members, such as the glyceollins I, II, and III, have demonstrated potential antidiabetic properties and promising cytotoxicity in both human breast and prostate cancer cell cultures with preliminary studies in animals further demonstrating antitumor effects in estrogen-dependent, human breast cancer cell implants. Although syntheses of glyceollin I have been reported previously, this work constitutes the first total directed synthesis of (±)-glyceollin II. It involves 12 steps with an overall yield of 7% using practical methods that should be readily scalable to produce quantities needed for advanced biological characterization. Highlights include a novel intramolecular benzoin condensation, a chelation-controlled lithium aluminum hydride-mediated reduction, and an intramolecular cyclization via the formation of a transient epoxide intermediate to cap the construction of the 6a-hydroxypterocarpan system. Additionally, a dihydro analogue has been obtained, and several isolated intermediates have been made available for evaluation of their biological properties and possible contributions toward elaborating key structure−activity relationship data among this family of promising phytoalexins elicited from stressed soybeans.



(±)-Glyceollin II



(±)-Dihydroglyceollin II

Stressed soybeans produce a group of phytoalexins that belong to the 6a-hydroxypterocarpan family of flavonoids.[1] To date, 10 members have been identified of which three are the most abundant.[2] Called glyceollins (GLYs; Figure 1), the most prominent member of this group, GLY I, has been shown to have potential antidiabetic properties[3] and promising cytotoxicity in both human breast and prostate cancer cell cultures.[4] Preliminary studies in animal models further demonstrate antitumor effects on estrogen-dependent human breast cancer cell implants.[5]

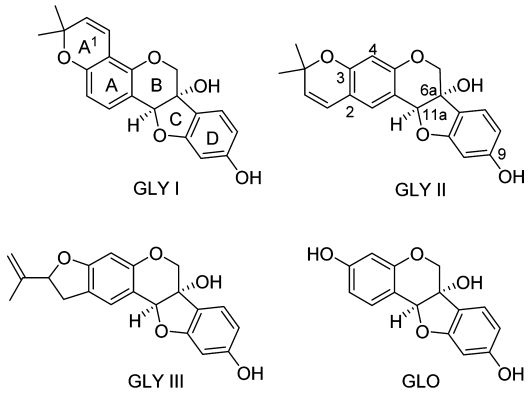


Figure 1. Structures of the three most abundant 6a-hydroxypterocarpans elicited from stressed soybeans, glyceollins I, II, and III, shown as GLY I, GLY II, and GLY III, respectively. Ring designations are indicated on GLY I and pertinent scaffold numbering is depicted on GLY II. Also shown is the key phytochemical intermediate of the GLYs, glycinol (GLO). The phytochemical pathways leading to all of soy's normal and elicited 6a-hydroxypterocarpans are depicted in the Supporting Information.

We are developing synthetic routes to several of the GLY family members to provide larger quantities of material to enable more advanced pharmacological studies and confirm the preliminary biological findings. At this point, both a biomimetic[4] and novel[6,7] route to GLY I, as well as two different methods to prepare the key phytochemical intermediate GLO, have been devised.[8,9] Although GLY II is obtained in low yield as a side-product during the synthesis of GLY I, no direct synthesis of GLY II has been developed. Herein, the first direct syntheses of racemic GLY II and its dihydro analogue by a new route that should be generally applicable to all of the GLYs using practical methodology are reported.

RESULTS AND DISCUSSION

The prior two syntheses of GLY I are depicted in Scheme 1. After eight steps, both routes take advantage of the same isoflav-3-ene intermediate for introduction of the 6a-hydroxy group by treatment with osmium tetroxide (OsO_4) to form the diol before closure of the benzofuran C-ring. The latter occurs via *cis*-fusion of the quinone-methide with the neighboring phenolic hydroxy group that is simultaneously exposed during hydrogenolysis. Construction of ring A[1], by aldol reaction with

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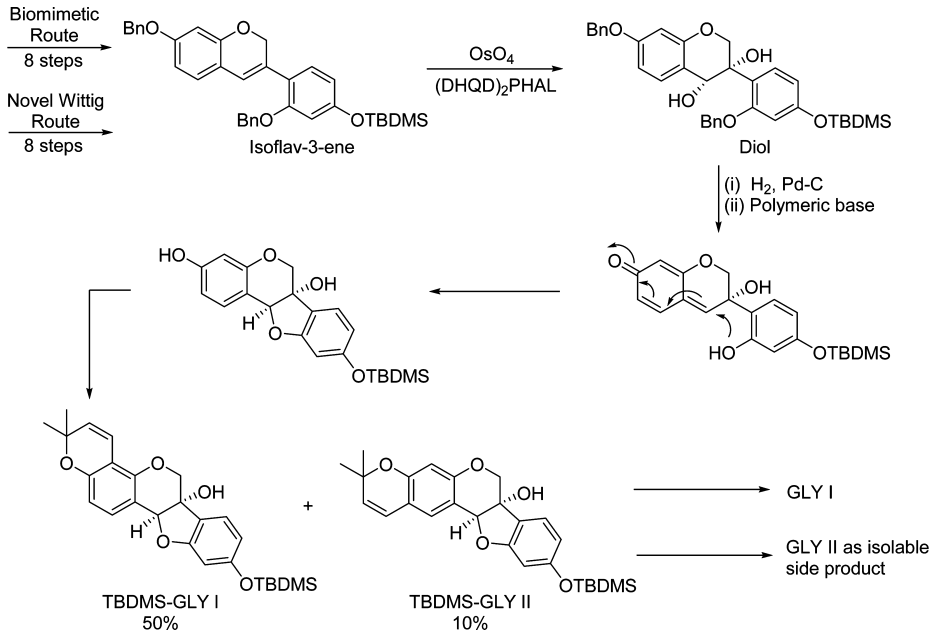
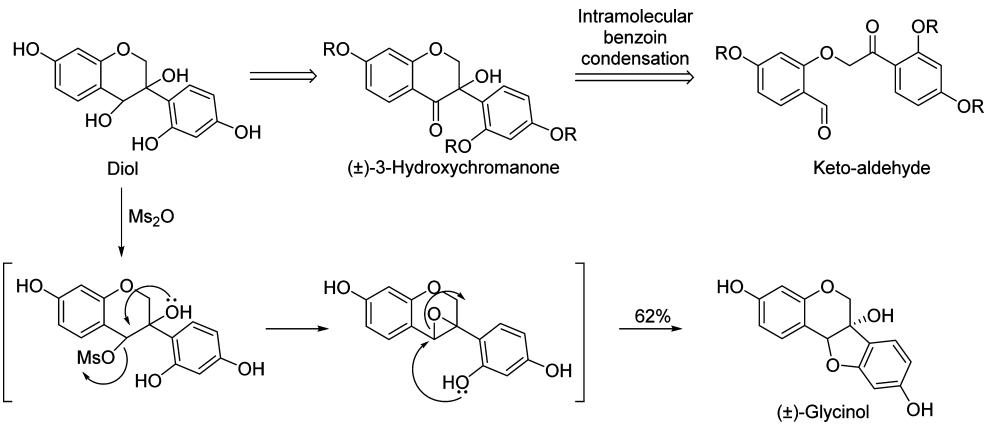
Scheme 1. Previous Syntheses of GLY I Accompanied by Isolation of GLY II as a Side Product[3,6,7]



Scheme 2. Successful Strategy Devised to Eliminate the Use of OsO_4 that Fortuitously Allowed for Closure of Ring C without Relying upon a Quinone-Methide Intermediate when Deploying Glycinol (GLO) as a Model Compound



an unsaturated aldehyde deployed as its acetal, occurs in approximately 50% isolated yield for TBDMS-protected GLY I. Approximately 10% of TBDMS-GLY II can also be obtained as a side-product after chromatographic separation. In principle, two steps common to both routes could be altered advantageously while devising a new synthetic strategy. The first was to eliminate the need for deploying osmium tetroxide, and the second involved direct construction of the specific types of A^1 rings present in the various GLYs at the outset rather than as the penultimate step of the overall synthesis. To accomplish the latter, an alternate way to generate ring C was needed because a quinone-methide cannot be formed when the 7-oxygen is alkylated as part of a preformed A^1-ring system.

The strategy to eliminate osmium tetroxide is depicted in Scheme 2, where the retro-synthesis arrows represent retrosynthetic manipulations, and the regular arrows are the typical experimental manipulations, in this case afforded by the unanticipated result during formation of the diol during model studies. The retrosynthetic strategy involved reduction of an appropriate 3-hydroxychromanone with the latter potentially being accessible by an intramolecular benzoin condensation. This chemistry was explored within the framework of racemic GLO as a convenient model.[9] Not only were the conditions for these novel steps refined, but the reduction with lithium aluminum hydride (LAH) occurs via chelation control to produce a racemic mixture of only the *anti*-1,2-diol. Having some literature precedent,[10] this fortuitous outcome permitted the formation of an epoxide from the diols that could then be opened by the neighboring phenolic hydroxy group to form ring C. Thus, the need to rely upon a quinone-methide-driven ring closure was eliminated. In the case of the model GLO, this closure actually became significantly higher yielding than that obtained by the quinone-methide approach.[9]

The approach to address a specific construction of the A^1-A ring system of GLY II at the start of the synthesis is depicted in retrosynthetic Scheme 3. On the basis of a literature precedent,[11,12] this involves manipulating 7-hydroxycoumarin to form the target ring system and then takes advantage of the previous model chemistry to produce a keto-aldehyde that is poised for an intramolecular benzoin condensation.

With the understanding that final deprotection in the presence of the 6a-hydroxy group would need to be

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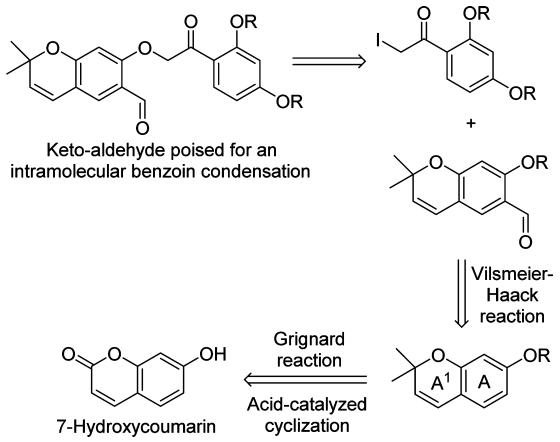
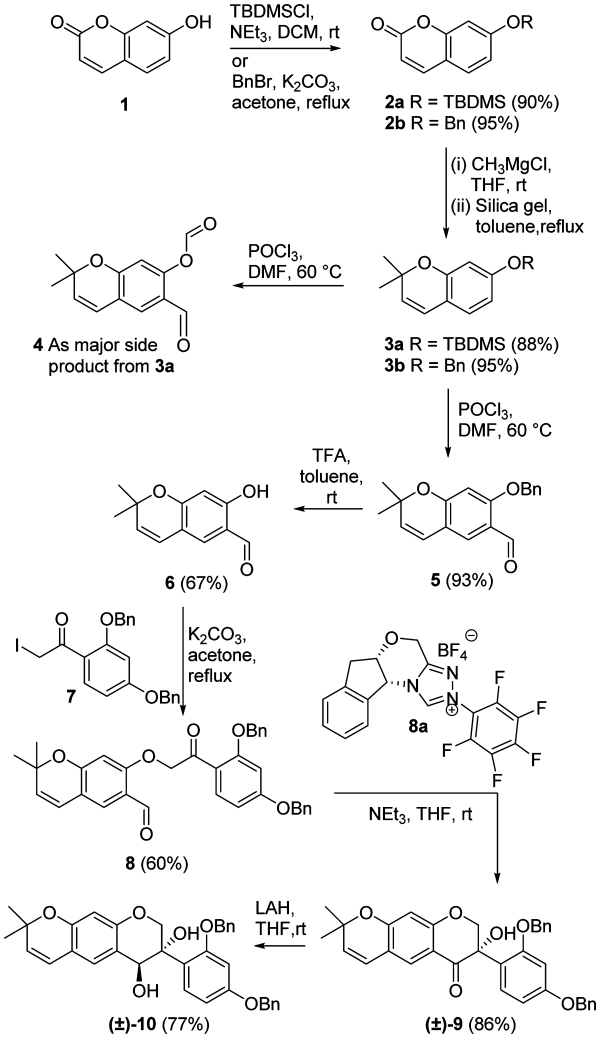
Scheme 3. Retrosynthetic Strategy Proposed for Constructing the A^1-A Ring System at the Outset of the Overall Synthetic Path



accomplished under mild conditions,[6] a TBDMS group was deployed first. As shown in Scheme 4, commercially available 7-hydroxycoumarin **1** was protected using *t*-butyldimethylsilyl chloride (TBDMSCl) and base to give **2a** in high yield. As

Scheme 4. Synthesis of the Reduced Benzoin Condensation Product Needed for Cyclization to Form Ring C in GLY II



envisioned in retrosynthetic Scheme 3, the A^1-A ring system was successfully constructed using an excess of Grignard reagent followed by acid-catalyzed cyclization. Initially, catalytic amounts of 15% H_2SO_4 were used to prompt cyclization but yields were low and accompanied by degradation. Thus, mildly acidic silica gel was employed in refluxing toluene, which afforded **3a** in 88% yield across the two steps. A Vilsmeier–Haack reaction[13] of **3a**, however, proved problematic in that it caused removal of the silyl protecting group followed by formylation of the resulting hydroxy group to generate side-product **4** as observed by NMR data of the crude reaction mixture. This type of event has literature precedent, where the Vilsmeier reagent has been used as an electrophile for one-pot deprotection and formylation of *O*-silylated ethers to form *O*-formates.[14] As an alternative, **1** was protected as its benzyl ether, which successfully underwent both the A^1-A ring construction and subsequent Vilsmeier–Haack formylation to afford **5** as the exclusive product in greater than 80% yield across all four steps.

Deprotection of **5** was necessary for the next coupling reaction with **7** to form keto-aldehyde **8**. Variously protected forms of partner **7**, including the dibenzyl derivative, have been used in several of the prior 6a-hydroxypterocarpan-related syntheses.[6−9] Because conventional hydrogenolysis over palladium will also reduce the double bond in ring A^1, excess trifluoroacetic acid (TFA) in toluene was used to cleave the benzyl group in **5** where it is thought to be effective in this specific context because it chelates with the *ortho*-keto-phenol functionality.[15] Although the model chemistry[9] suggested that the formyl group in **6** might need to be masked as an acetal prior to coupling, this was found to be unnecessary, and keto-aldehyde **8** was obtained directly in 60% yield after recrystallization. This is attributed to the lower reactivity of the formyl group toward an intramolecular aldol side-reaction due to the presence of the electron-donating group present on the A ring. Nevertheless, caution was taken while performing this step by monitoring the reaction periodically with TLC and quenching it upon the first appearance of any suspected aldol product. In line with the model chemistry,[9] the key intramolecular benzoin condensation to form **9** as a racemic mixture proceeded efficiently by again deploying the Rovis triazolium catalyst **8a**,[16] and then this was successfully followed by reduction of the C-4 carbonyl group with LAH to afford *anti*-diol **10** with yields of 86 and 77%, respectively. The final steps leading to both racemic GLY II and its dihydro analogue are conveyed in Scheme 5.

Deprotection of **10** was required next to construct the C-ring. As anticipated, removal of the benzyl groups by catalytic hydrogenolysis was accompanied by reduction of the double bond in ring A^1 to produce **11**, which could be utilized as an intermediate to obtain an interesting dihydro derivative of racemic GLY II. The latter underwent cyclization smoothly upon generation of the epoxide and provided the target analogue in 40% overall yield from **10**. The C-11a proton shift at 5.24 ppm in the ^{1}H NMR spectrum was diagnostic for the anticipated *cis*-fusion of ring C into the pterocarpan skeleton.[6,17] Alternatively, boron trichloride (BCl_3) in pentamethylbenzene (PMB) represents a gentle method that might be used to selectively remove the benzyl groups in **10** without losing the ring A^1 double bond.[18] However, even at low temperature (−78 °C), complete decomposition of the starting material and generation of a complex mixture within 15 min after the addition of BCl_3 were observed. This may be

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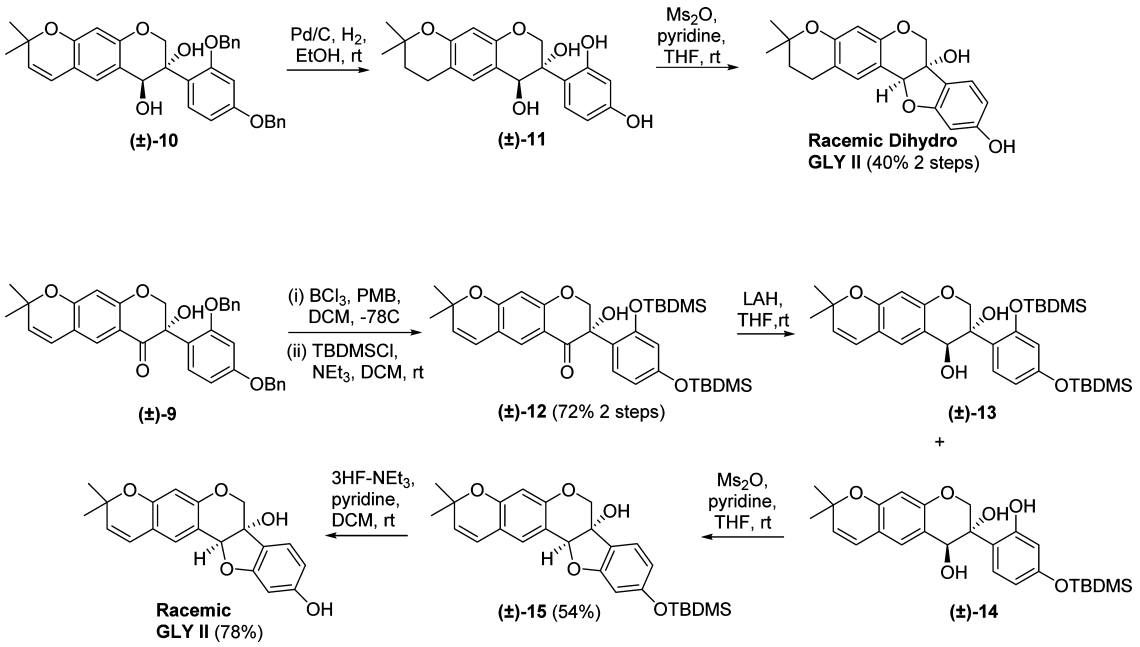
Scheme 5. Completion of Syntheses Leading to Racemic GLY II and its Dihydro Analogue



attributed to the propensity of the *trans*-diol functionality to readily undergo dehydration by an E1 mechanism that is initially driven by relieving the steric strain associated with the tertiary hydroxy group while simultaneously extending the conjugation across the two aromatic systems. The resulting transient keto−enol species subsequently participates in other side reactions. Given the latter and this outcome from one of the more gentle methods, other Lewis acid-mediated conditions for selective debenzylation were not pursued. Instead, the benzyl functionality in precursor **9** was removed, and the phenolic hydroxy groups were subsequently protected with TBDMS groups because the latter can be cleaved selectively in the presence of double bonds using mild conditions. Deprotection of **9** proceeded uneventfully, as did subsequent reprotection with TBDMS to provide **12** in 72% yield after both steps.

Reduction of **12** with 1.1 equiv of LAH produced both the anticipated diol **13** and its partially deprotected version **14**. It is not unusual for LAH to cleave TBDMS protecting groups under these conditions.[19] Again fortuitously, the *para*-substitution in diol **13** always remained intact, suggesting that the *ortho*-TBDMS moiety is much more susceptible to such a reaction. This is similar to an earlier observation where LAH tends to chelate with the tertiary hydroxy group[9] and, in the present context, allows for preferential removal of the nearby *ortho*-situated moiety compared to the distant *para*-moiety due to neighboring group participation.[19] Taking advantage of this inherent preference for reactivity, conditions were optimized to provide **14** as the only product and from which closure of ring C to the 6a-hydroxypterocarpan **15** occurred smoothly in 54% when scaled to the 50 mg level. Final deprotection of the remaining TBDMS group was carried out with extreme care due to the possibility for an analogous E1 loss of water from the 6a−11a positions. During a previous multigram total synthesis of GLY I,[7] a mild protocol was devised for the deprotection of TBDMS ethers using 3HF-NEt_3[20] in pyridine. Pyridine served as a buffer to maintain the reaction pH between ∼6−7, which was demonstrated to be optimal for preserving the 6a-hydroxy group within this family of pterocarpans. These conditions were employed in the present case and provided racemic GLY II in 78% yield.

Structural confirmations of (±)-GLY II and its dihydro derivative were initially assessed by ^{1}H and ^{13}C NMR comparison to samples of GLO and GLY I and to the GLY II material previously obtained as a side product during synthesis of GLY I. The C-11a proton shift at 5.23 ppm and its splitting pattern are particularly diagnostic of the *cis*-fused 6a-hydroxypterocarpan system.[3,6,7,17] Reverse phase LC-MS chromatograms for standard samples of GLY I and GLY II produced a single peak in each case. The retention time for synthetic (±)-GLY II at 9.6 min matched that for the natural enantiomer isolated from stressed soybeans (chromatograms available in the Supporting Information). As has been found previously for other members of this family, elemental analyses indicate the presence of water entrained within the matrices for both solid products with 0.6 mol equivalents for the dihydro derivative and 1.1 mol equivalents for (±)-GLY II.

This work constitutes the first total directed synthesis of racemic GLY II. The synthesis involves 12 steps with an overall yield of 7%. It is highly practical and should be readily scalable to amounts required for preliminary biological characterizations, including in vivo studies in small animals. Highlights include a novel intramolecular benzoin condensation that allows the overall synthesis to be free of the need for an osmium-mediated dihydroxylation step, chelation-controlled LAH-mediated reduction that affords only the *trans*-diols and bypasses the need for a quinone-methide cyclization step to form the C-ring, which can also be used to conveniently effect selective removal of a neighboring TBDMS protecting group, and an intramolecular cyclization via the formation of a transient epoxide intermediate to cap the construction of the 6a-hydroxypterocarpan system. In addition to providing an analytical standard for quantitative determination of GLY II in plant samples by LC-MS, a dihydro analogue has been obtained along with several isolated intermediates that can be evaluated for their biological properties and potential contributions to

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structure−activity relationships (SAR) within this unique family of promising phytoalexins derived from stressed soybeans.

EXPERIMENTAL SECTION

General Experimental Procedures. All chemical reactions were conducted in oven-dried glassware under a gentle flow of argon in anhydrous solvents unless stated otherwise. Reagents obtained from commercial suppliers were used without purification except pentamethylbenzene (PMB), which was recrystallized from MeOH. Acetone was dried over 4 Å molecular sieves, and THF was distilled under N_2 over sodium-benzophenone. Hydrogenation reactions were performed using a Parr hydrogenator and by shaking the hydrogenation flask at pressures indicated. TLC was performed on 250 μm fluorescent SiO_2 TLC plates and visualized by UV light or iodine vapor. Normal-phase column chromatography was performed using silica gel (200−425 mesh 60 Å pore size) and ACS grade solvents. Melting points are uncorrected. Optical rotation was measured on a Rudolf Research Automatic Polarimeter (Autopol IV). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance600 spectrophotometer, chemical shifts are reported to second and first decimal places, respectively. Peak locations were referenced using either TMS or residual nondeuterated solvent as an internal standard. Proton coupling constants are expressed in Hz. In some cases, overlapping signals occurred in the ^{13}C NMR spectra. The following abbreviations were used to denote spin multiplicity for ^{1}H NMR: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, m = multiplet. Elemental analyses [anal. (%)] were determined by Atlantic Microlabs Inc., Norcross, GA. All results were within ±0.4% of the theoretical values.

*7-(tert-Butyl-dimethylsilyloxy)-chromen-2-one (**2a**).* TBDMSCl (181 mg, 1.2 mmol) was added to a stirred mixture of 7-hydroxycoumarin **1** (162 mg, 1 mmol) and NEt_3 (0.28 mL, 2 mmol) in DCM (2 mL). The reaction mixture was stirred at RT for 4 h. Saturated NH_4Cl (5 mL) was added to the mixture, and the layers were separated. The aqueous layer was extracted with DCM (10 mL). The combined organic layer was washed with H_2O (5 mL) and brine (5 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give an oil that was purified by a short silica gel column [hexanes, then EtOAc/hex (1:5)] to yield the target product as a white solid (249 mg, 90%): mp 53−55 °C; TLC R_f 0.84 [EtOAc/hex (1:2)]; ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.25 (s, 6 H), 0.99 (s, 9 H), 6.26 (d, J = 9.5 Hz, 1 H), 6.76−6.78 (m, 1 H), 6.78−6.79 (m, 1 H), 7.34 (d, J = 8.3 Hz, 1 H), 7.64 (d, J = 9.5 Hz, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ −4.4, 18.3, 25.6, 107.8, 113.2, 113.4, 117.5, 128.7, 143.4, 155.6, 159.4, 161.2; anal. (%) C 65.18, H 7.29, calcd for $C_{15}H_{20}O_3Si$, C 65.25, H 7.17.

*7-Benzyloxychromen-2-one (**2b**).* Benzyl bromide (1.3 mL, 11 mmol) was added to a stirred mixture of 7-hydroxycoumarin **1** (1.62 g, 10 mmol) and anhydrous K_2CO_3 (2.07 g, 15 mmol) in acetone (20 mL). The reaction mixture was heated under reflux for 4 h. Upon cooling, 1 N HCl (~15 mL) was added to neutralize the mixture (pH 7.0−7.5). The solid obtained was collected by vacuum filtration, washed with H_2O (20 mL), and dried to yield the target product as an off-white solid (2.4 g, 95%): mp 154−156 °C (lit. mp:[21] 153−155 °C); TLC R_f 0.63 [EtOAc/hex (2:1)]; ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.13 (s, 2 H), 6.26 (d, J = 9.3 Hz, 1 H), 6.89 (d, J = 2.4 Hz, 1 H), 6.92 (dd, J = 8.6, 2.4 Hz, 1 H), 7.34−7.45 (m, 6 H), 7.64 (d, J = 9.5 Hz, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 70.5, 101.9, 112.7, 113.2, 113.3, 127.5, 128.4, 128.8, 128.7, 135.7, 143.4, 155.8, 161.2, 161.8; anal. (%) C 76.18, H 4.79, calcd for $C_{16}H_{12}O_3$, C 76.06, H 5.02.

*2,2-Dimethyl-7-(tert-Butyl-dimethylsilyloxy)-2H-1-benzopyran (**3a**).* Methyl magnesium chloride (9 mL, 27 mmol, 3 M solution in THF) was added dropwise to a solution of **2a** (2.5 g, 9 mmol) in THF (65 mL) at 0 °C. The resulting yellow solution was stirred at RT for 12 h. Saturated NH_4Cl (30 mL) was then added and the layers separated. The aqueous layer was extracted twice with EtOAc (20 mL). The organic layers were combined and washed with H_2O (50 mL), brine (50 mL) and dried over anhydrous Na_2SO_4, and evaporated to give the dialkylated acyclic intermediate as a yellow oil that was used in the next step immediately by dissolving it in toluene (30 mL) and adding silica gel to the solution (2.5 g). The reaction mixture was heated under reflux for 12 h. The reaction was then cooled to RT and filtered. The silica gel was extensively washed with EtOAc (50 mL). The filtrate was evaporated to give the crude product which was purified using a short silica gel column (hexanes) to yield the desired product **3a** as a clear oil (2.3 g, 88%): TLC R_f 0.67 [EtOAc/hex (1:4)]; ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.19 (s, 6 H), 0.97 (s, 9 H), 1.41 (s, 6 H), 5.47 (d, J = 9.8 Hz, 1 H), 6.27 (d, J = 9.8 Hz, 1 H), 6.31−6.32 (m, 1 H), 6.33−6.35 (m, 1 H), 6.82 (d, J = 8.1 Hz, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ −4.5, 18.2, 25.6, 27.9, 76.2, 108.4, 112.6, 115.3, 122.0, 126.7, 128.1, 153.9, 156.6; anal. (%) calcd for $C_{17}H_{26}O_2Si$, C 70.29, H 9.02, found C 70.38, H 8.89.

*7-(Benzyloxy)-2,2-dimethyl-2H-1-benzopyran (**3b**).* Methyl magnesium chloride (4.4 mL, 13.2 mmol, 3 M solution in THF) was added dropwise to a solution of 7-benzyloxycoumarin **2b** (1.12 g, 4.4 mmol) in THF (30 mL) at 0 °C. The resulting yellow solution was stirred at RT overnight. Saturated NH_4Cl (20 mL) was added, and the layers were separated. The aqueous layer was extracted twice with EtOAc (20 mL). The organic layers were combined and washed with H_2O (20 mL), brine (20 mL), dried over anhydrous Na_2SO_4, and evaporated to give a yellow oil that was used immediately in the next step. The oil was dissolved in toluene (30 mL), and silica gel was added to the solution (2 g). The reaction mixture was heated under gentle reflux for 12 h. Upon cooling to RT, the reaction was filtered, and the silica gel was washed extensively with EtOAc (50 mL). The filtrate was evaporated to give a crude product that was purified using a short silica gel column (hexanes) to yield the target product as a clear oil that solidified to a white amorphous solid upon cooling at 4 °C (1.23 g, 95%): TLC R_f 0.71 [EtOAc/hex (2:1)]; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.42 (s, 6 H), 5.01 (s, 2 H), 5.47 (d, J = 9.8 Hz, 1 H), 6.27 (d, J = 10.0 Hz, 1 H), 6.45−6.46 (m, 1 H), 6.46−6.48 (m, 1 H), 6.88 (d, J = 8.1 Hz, 1 H), 7.30−7.33 (m, 1 H), 7.36−7.39 (m, 2 H), 7.40−7.43 (m, 2 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 28.0, 69.9, 76.4, 102.9, 107.4, 114.8, 121.9, 121.8, 126.9, 127.5, 127.9, 127.9, 128.5, 136.9, 154.1, 159.8; anal. (%) C 81.17, H 6.81, calcd for $C_{18}H_{18}O_2$, C 81.07, H 7.13.

*7-(Benzyloxy)-2,2-dimethyl-2H-1-benzopyran-6-carboxaldehyde (**5**).* $POCl_3$ (0.45 mL, 4.79 mmol) was added dropwise to a flask containing DMF (0.32 mL, 4.13 mmol) at 0 °C. The resulting solution was stirred at 0 °C for 15 min followed by the addition of benzopyran **3b** (423 mg, 1.6 mmol) in DMF (1.5 mL). The ice bath was removed, and the reaction mixture was stirred at 60 °C for 3 h. Upon cooling, EtOAc (10 mL) and H_2O (15 mL) were added to the reaction mixture. The layers were separated, and the aqueous layer was extracted with EtOAc (10 mL). The combined organic layer was washed with brine (15 mL), dried over anhydrous Na_2SO_4, and evaporated to a give a residue that was purified by a short silica gel column [hexanes then EtOAc/hex (1:4)] to give the target product as a yellow oil that solidified to a yellow solid upon cooling overnight at 4 °C (433 mg, 93%): mp 56−58 °C; TLC R_f 0.46 [EtOAc/hex (1:5)]; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.45 (s, 6 H), 5.13 (s, 2 H), 5.56 (d, J = 9.8 Hz, 1 H), 6.31 (d, J = 10.0 Hz, 1 H), 6.45 (s, 1 H), 7.34−7.45 (m, 5 H), 7.53 (s, 1 H), 10.35 (s, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 28.5, 28.6, 70.5, 78.1, 100.7, 114.6, 118.9, 121.1, 126.5, 127.3, 128.3, 128.7, 128.9, 135.9, 160.3, 162.9, 188.1; anal. (%) C 77.18, H 6.22, calcd for $C_{19}H_{18}O_3$·0.06 EtOAc, C 76.80, H 6.31.

*2,2-Dimethyl-7-Hydroxy-2H-1-benzopyran-6-carboxaldehyde (**6**).* To a solution of aldehyde **5** (3.5 g, 11.85 mmol) in toluene (60 mL) was added TFA (30 mL) in small portions at RT. The reaction mixture was allowed to stir at RT for 12 h. Upon completion (^{1}H NMR), saturated $NaHCO_3$ (75 mL) was added carefully. EtOAc (50 mL) was then added, and the layers were separated. The organic layer was washed with saturated $NaHCO_3$ (30 mL × 2) and H_2O (30 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give an oily residue. The residue was taken up in EtOAc (20 mL) and washed with 10% NaOH (20 mL × 4). The combined NaOH extract was neutralized to pH 6.5−7.0 using 1 N HCl (~100 mL). EtOAc (50 mL) was then added, and the layers were separated. The aqueous layer was washed with EtOAc (25 mL), and the combined organic layers were washed with H_2O (25 mL × 4) and brine (25 mL),

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dried over anhydrous Na_2SO_4, and evaporated under high vacuum to yield the target product as a light brown solid (1.61 g, 67%): mp 94–96 °C; TLC R_f 0.63 [EtOAc/hex (1:3)]; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.45 (s, 6 H), 5.59 (d, J = 9.9 Hz, 1 H), 6.29 (d, J = 9.9 Hz, 1 H), 6.33 (s, 1 H), 7.11 (s, 1 H), 9.66 (s, 1 H), 11.43 (s, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 28.6, 104.2, 114.4, 115.2, 120.6, 128.9, 131.4, 161.2, 164.3, 194.1; anal. (%) C 70.57, H 5.92, calcd for $C_{12}H_{12}O_3$, C 70.58, H 5.90.

*7-[2-(2,4-Bisbenzyloxy)phenyl-2-oxoethoxy]-2,2-dimethyl-2H-chromene-6-carboxaldehyde (**8**).* To a solution of aldehyde **6** (467 mg, 2.26 mmol) in acetone (5 mL) was added anhydrous K_2CO_3 (317 mg, 2.29 mmol). The solution was heated. Upon reflux, α-iodoketone **7** (prepared separately according to refs 6–9; 0.89 g, 1.93 mmol) was added, and the mixture was refluxed for 4–5 h (monitoring the reaction progress by TLC every hour). The reaction mixture was cooled to 0 °C, and H_2O (5 mL) was added. The crude solid was collected by vacuum filtration and recrystallized from EtOAc/hex [1:4, 5 mL] to yield compound **8** as an off-white solid (619 mg, 60%): mp 170–172 °C; TLC R_f 0.36 [EtOAc/hex (1:3)]; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.44 (s, 6 H), 5.13 (s, 4 H), 5.14 (s, 2 H), 5.53 (d, J = 9.9 Hz, 1 H), 5.91 (s, 1 H), 6.28 (d, J = 9.9 Hz, 1 H), 6.67 (d, J = 2.2 Hz, 1 H), 6.70 (dd, J = 8.8, 2.2 Hz, 1 H), 7.36–7.39 (m, 1 H), 7.40–7.46 (m, 9 H), 7.50 (s, 1 H), 8.03 (d, J = 8.8 Hz, 1 H), 10.37 (s, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 28.7, 70.4, 71.2, 74.3, 77.9, 100.0, 100.3, 107.1, 114.5, 118.3, 118.9, 121.2, 126.4, 127.6, 128.1, 128.4, 128.6, 128.8, 128.9, 129.1, 133.4, 135.2, 135.9, 160, 160.5, 162.6, 164.5, 188.4, 191.8; anal. (%) C 74.63, H 5.78, calcd for $C_{34}H_{30}O_6 \cdot 0.7\ H_2O$, C 74.23, H 5.54.

*(±)-7-[2,4-Bis(benzyloxy)phenyl]-7-hydroxy-2,2-dimethyl-7,8-dihydro-2H,6H-pyrano[3,2-g]chromen-6-one (**9**).* To a solution of keto-aldehyde **8** (534 mg, 1 mmol) in THF (16 mL) was added Rovis triazolium salt **8a** (47 mg, 0.1 mmol) followed by the addition of NEt_3 (30 μL, 0.18 mmol). The mixture was stirred at RT for 8 h. After the reaction was complete (monitored by TLC and ^{1}H NMR), H_2O (5 mL) and EtOAc (10 mL) were added, and the layers were separated. The organic layer was washed with brine (10 mL), dried over anhydrous Na_2SO_4, concentrated under reduced pressure, and subjected to silica gel column chromatography [EtOAc/hex (1:3)] to afford compound **9** as a pale yellow solid (460 mg, 86%): mp 69–72 °C; $[\alpha]^{22}_D$ 0 (c 0.5, $CHCl_3$); TLC R_f 0.58 [EtOAc/hex (1:1)]; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.43 (s, 3 H), 1.45 (s, 3 H), 3.49 (s, 1 H), 4.24 (d, J = 11.7 Hz, 1 H), 4.87 (d, J = 11.7 Hz, 1 H), 4.94–5.00 (m, 2H), 5.02 (s, 2 H), 5.59 (d, J = 9.9 Hz, 1 H), 6.26 (d, J = 9.9 Hz, 1 H), 6.27 (s, 1 H), 6.58 (dd, J = 8.4, 2.2 Hz, 1 H), 6.59–6.61 (m, 1 H), 7.25 (d, J = 1.5 Hz, 2 H), 7.27–7.30 (m, 2 H), 7.31–7.35 (m, 1 H), 7.36–7.42 (m, 6 H), 7.45 (d, J = 8.4 Hz, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 28.5, 28.6, 70.2, 70.7, 73.9, 74.3, 77.9, 101.3, 104.1, 105.8, 113.7, 116.3, 119.8, 121.2, 125.7, 127.5, 127.4, 127.9, 128.1, 128.4, 128.5, 128.6, 129.4, 135.9, 136.6, 156.9, 160.1, 160.3, 162.6, 190.9; anal. (%) C 76.26, H 5.67, calcd for $C_{34}H_{36}O_6 \cdot 0.05\ H_2O$, C 75.88, H 5.80.

*(±)-7-[2,4-Bis(benzyloxy)phenyl]-2,2-dimethyl-7,8-dihydro-2H-pyrano[3,2-g]chromene-6,7-diol (**10**).* To a solution of hydroxyketone **9** (267 mg, 0.5 mmol) in THF (5 mL) was added dropwise LAH (0.55 mL, 0.55 mmol, 1 M solution in THF) at 0 °C. The reaction was brought to RT and stirred for 4 h. The reaction was quenched with 0.1 N HCl (5 mL); EtOAc (10 mL) was added, and the layers were separated. The organic layer was washed with 0.1 N HCl (5 mL), 10% $NaHCO_3$ (5 mL), and brine (5 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The resulting residue was subjected to a short silica gel column [EtOAc/hex (1:3)] to afford compound **10** as a white solid (206 mg, 77%): mp 79–81 °C; TLC R_f 0.36 [EtOAc/Hex (1:3)]; ^{1}H NMR (600 MHz, acetone-d_6) δ 1.36 (s, 6 H), 4.22 (d, J = 5.1 Hz, 1 H), 4.28 (dd, J = 10.6, 1.5 Hz, 1 H), 4.33 (s, 1 H), 4.69–4.74 (m, 1 H), 4.83–4.86 (m, 1 H), 5.11 (s, 2 H), 5.27 (s, 2 H), 5.56 (d, J = 9.5 Hz, 1 H), 6.14 (s, 1 H), 6.33 (d, J = 9.5 Hz, 1 H), 6.60 (dd, J = 8.4, 2.6 Hz, 1 H), 6.81 (d, J = 2.6 Hz, 1 H), 6.90 (s, 1 H), 7.24 (d, J = 8.4 Hz, 1 H), 7.32–7.37 (m, 2 H), 7.38–7.44 (m, 4 H), 7.47 (d, J = 7.3 Hz, 2 H), 7.57 (d, J = 7.3 Hz, 2 H); ^{13}C NMR (150 MHz, acetone-d_6) δ 27.9, 68.3, 69.9, 70.2, 71.1, 72.4, 76.4, 101.7, 103.6, 106.3, 115.3, 118.1, 122.4, 123.1, 128.3, 128.4, 128.5, 128.7, 129.0, 129.1, 129.2, 137.5, 138.0, 154.1, 155.4, 158.8, 159.9; anal. (%) C 76.10, H 6.01, calcd for $C_{34}C_{32}O_6$, C 75.91, H 6.10.

Racemic Dihydroglyceollin II [(±)-Dihydro-GLY II]. To a solution of diol **10** (120 mg, 0.22 mmol) in EtOH (5 mL) was added 10% Pd/C (30 mg). The resulting mixture was shaken at RT for 12 h under H_2 atmosphere (35 psi). The mixture was filtered through Celite, and the latter was washed with EtOAc (10 mL). The filtrate was evaporated, and the residue obtained was passed through a short silica gel column [EtOAc/hex (1:1)] to afford debenzylated intermediate **11**, which was used in the next step without further purification. To a solution of the debenzylated diol **11** (80 mg, 0.22 mmol) in THF (3 mL) were added Ms_2O (77 mg, 0.44 mmol) and anhydrous pyridine (50 μL, 0.66 mmol). The resulting suspension was stirred at RT for 12 h after which the solvents were evaporated under reduced pressure. The residue obtained was purified by silica gel column chromatography [EtOAc/hex (1:3)]. The eluting solvents were evaporated under reduced pressure and then lyophilized to obtain the product, (±)-dihydro-GLY II as an off-white solid (30 mg, 40%): mp 105–108 °C; TLC R_f 0.39 [EtOAc/hex (1:1)]; ^{1}H NMR (600 MHz, acetone-d_6) δ 1.27 (s, 3 H), 1.29 (s, 3 H), 1.80 (t, J = 6.8 Hz, 2 H), 2.76 (t, J = 6.6 Hz, 2 H), 4.02 (d, J = 11.4 Hz, 1 H), 4.08 (d, J = 11.4 Hz, 1 H), 4.92 (s, 1 H), 5.26 (s, 1 H), 6.17 (s, 1 H), 6.24 (d, J = 1.8 Hz, 1 H), 6.42 (dd, J = 8.1, 2.2 Hz, 1 H), 7.16 (s, 1 H), 7.20 (d, J = 8.4 Hz, 1 H), 8.45 (s, 1 H); ^{13}C NMR (150 MHz, acetone-d_6) δ 22.0, 26.6, 26.8, 33.0, 70.3, 74.8, 76.5, 85.8, 98.3, 104.5, 108.5, 113.4, 115.7, 121.2, 124.9, 132.5, 154.9, 155.7, 160.4, 161.7; anal. (%) C 68.40, H 6.08, calcd for $C_{20}H_{20}O_5 \cdot 0.6\ H_2O$, C 68.11, H 5.96.

*(±)-7-[2,4-Bis(t-butyldimethylsilyloxy)phenyl]-7-hydroxy-2,2-dimethyl-7,8-dihydro-2H,6H-pyrano[3,2-g]chromen-6-one (**12**).* A solution of **9** (535 mg, 1 mmol) and pentamethylbenzene (371 mg, 2.5 mmol) in DCM (20 mL) was stirred for 15 min at −78 °C. To this mixture was added BCl_3 (2 mL, 2 mmol, 1 M solution in DCM) dropwise over 10 min via syringe at −78 °C. The reaction mixture was left to stir at this temperature for 30 min upon which the TLC indicated complete consumption of the starting material. The reaction was quenched with saturated $NaHCO_3$:MeOH (1:1, 20 mL) at −78 °C, and the resulting solution was stirred at 0 °C for 15 min. The MeOH and DCM were evaporated, and the resulting suspension was dissolved in EtOAc (10 mL). The organic layer was washed with H_2O (5 mL) and brine (5 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give a residue that was subjected to silica gel column chromatography [EtOAc/hex (1:2)] to yield the debenzylated 3-hydroxychromanone intermediate as an amorphous solid (265 mg), which was then used in the next step. NEt_3 (0.14 mL, 1 mmol) and TBDMSCl (148 mg, 1 mmol) were added to a solution of deprotected 3-hydroxychromanone intermediate (173 mg, 0.5 mmol) in DCM (4 mL) at 0 °C. The reaction mixture was warmed to RT and stirred for 8 h. Additional TBDMSCl (37 mg, 0.25 mmol) and NEt_3 (40 μL, 0.29 mmol) were added followed by stirring of the mixture at RT for another 8 h. Upon disappearance of starting material (TLC), H_2O (5 mL) and DCM (5 mL) were added, and the layers were separated. The organic layer was washed with 0.1 N HCl (5 mL), H_2O (5 mL), and brine (5 mL), dried over anhydrous Na_2SO_4, and evaporated to give a residue that was purified by silica gel column chromatography [EtOAc/hex (1:4)] to afford compound **12** as a clear oil (419 mg, 72% combined for both steps): TLC R_f 0.67 [EtOAc/Hex (1:3)]; ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.17 (s, 6 H), 0.22 (s, 3 H), 0.27 (s, 3 H), 0.95 (s, 18 H), 1.43 (s, 3 H), 1.45 (s, 3 H), 3.75 (s, 1 H), 4.25 (d, J = 11.4 Hz, 1 H), 4.96 (d, J = 11.7 Hz, 1 H), 5.60 (d, J = 9.9 Hz, 1 H), 6.30 (d, J = 0.7 Hz, 1 H), 6.32 (d, J = 9.9 Hz, 1 H), 6.35 (d, J = 2.2 Hz, 1 H), 6.36–6.38 (m, 1 H), 7.15 (d, J = 8.4 Hz, 1 H), 7.58 (s, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ −4.4, −4.1, −3.8, 18.2, 18.5, 25.6, 25.7, 25.8, 25.9, 28.5, 28.6, 73.5, 74.2, 77.9, 104.2, 110.9, 112.5, 113.6, 116.4, 121.1, 125.7, 128.5, 129.6, 154.6, 156.6, 160.3, 162.8, 190.9; anal. (%) C 64.45, H 8.03, calcd for $C_{32}H_{46}O_6Si_2 \cdot 0.75\ H_2O$, C 64.10, H 8.22.

*(±)-7-(2′-Hydroxy-4′-t-butyldimethylsilylphenyl)-2,2-dimethyl-7,8-dihydro-2H-pyrano[3,2-g]chromene-6,7-diol (**14**).* To a solution of TBDMS-protected hydroxyketone **12** (100 mg, 0.17 mmol) in THF

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(1.5 mL) was added dropwise LAH (0.3 mL, 0.3 mmol, 1 M solution in THF) at 0 °C. The reaction was brought to RT and stirred for 6 h. The reaction was quenched with 0.1 N HCl (5 mL); EtOAc (10 mL) was added, and the layers were separated. The organic layer was washed with 0.1 N HCl (5 mL), 10% $NaHCO_3$ (5 mL), and brine (5 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The resulting residue was subjected to a short silica gel column [EtOAc/hex (1:2)] to afford compound **14** as a white solid (60 mg, 75%): mp 175−177 °C; TLC R_f 0.53 [EtOAc/hex (1:2)]; ^{1}H NMR (600 MHz, acetone-d_6) δ 0.22 (s, 6 H), 0.99 (s, 9 H), 1.38 (s, 3 H), 1.39 (s, 3 H), 4.19 (dd, J = 11.2, 1.7 Hz, 1 H), 4.66−4.70 (m, 1 H), 4.74 (d, J = 11.4 Hz, 1 H), 5.59 (d, J = 9.5 Hz, 1 H), 6.20 (s, 1 H), 6.32−6.35 (m, 2 H), 6.35 (d, J = 7.7 Hz, 1 H), 6.96 (s, 1 H), 7.03 (d, J = 8.4 Hz, 1 H); ^{13}C NMR (150 MHz, acetone-d_6) δ −4.2, 18.8, 26.1, 28.3, 28.4, 67.9, 69.6, 74.4, 76.9, 104.2, 109.5, 111.8, 116.1, 117.81, 120.2, 122.7, 129.1, 129.2, 129.8, 154.8, 155.3, 157.2, 158.8; anal. (%) calcd for $C_{26}H_{34}O_6Si$·0.8 H_2O, C 64.38, H 7.40, found C 64.12, H 7.01.

*Racemic 9-(t-Butyldimethylsilyloxy)glyceollin II [(±)-TBDMS-GLY II] (**15**).* To a solution of diol **14** (60 mg, 0.13 mmol) in THF (2.5 mL) was added Ms_2O (45 mg, 0.26 mmol) and anhydrous pyridine (32 μL, 0.4 mmol). The resulting suspension was stirred at RT for 12 h after which the solvents were evaporated. The residue obtained was purified by silica gel column chromatography [EtOAc/hex (1:3)] to yield the target product as an amorphous white solid (32 mg, 54%): TLC R_f 0.68 [EtOAc/hex (1:3)]; ^{1}H NMR (600 MHz, acetone-d_6) δ 0.20 (s, 6 H), 0.97 (s, 9 H), 1.37 (s, 3 H), 1.39 (s, 3 H), 4.03 (d, J = 11.4 Hz, 1 H), 4.15 (dd, J = 11.4, 0.7 Hz, 1H), 5.07 (s, 1 H), 5.27 (s, 1 H), 5.66 (d, J = 9.9 Hz, 1 H), 6.22 (s, 1 H), 6.28 (d, J = 2.2 Hz, 1 H), 6.41 (d, J = 9.9 Hz, 1 H), 6.47 (dd, J = 8.1, 2.2 Hz, 1H), 7.15 (s, 1 H), 7.27 (d, J = 8.1 Hz, 1 H); ^{13}C NMR (150 MHz, acetone-d_6) δ −4.3, 18.8, 26.1, 28.5, 28.40, 70.7, 76.8, 77.3, 85.8, 103.4, 104.9, 113.6, 114.3, 117.3, 122.4, 123.6, 125.2, 129.9, 130.1, 155.4, 156.9, 158.9, 161.8; anal. (%) C 68.99, H 7.13, calcd for $C_{26}H_{32}O_5Si$, C 68.72, H 6.94.

Racemic Glyceollin II [(±)-GLY II]. To a solution of **15** (30 mg, 0.06 mmol) in DCM (1 mL) were added Et_3N·3 HF (28 μL, 0.16 mmol) and excess anhydrous pyridine (40 μL, 0.6 mmol). The reaction mixture was stirred at RT for 6 h. Upon disappearance of starting material (TLC), the solvents were evaporated, and the resulting residue was directly applied to a silica gel column [EtOAc/hex (1:1)]. The eluting fractions were collected; the solvents were removed under reduced pressure, and the resulting residue was lyophilized to obtain the target product (±)-GLY II as a white solid (16 mg, 78%): mp 61−65 °C; TLC R_f 0.52 [EtOAc/hex (1:1)]; ^{1}H NMR (600 MHz, acetone-d_6) δ 1.36 (s, 3 H), 1.39 (s, 3 H), 4.04 (d, J = 11.3 Hz, 1 H), 4.13 (d, J = 11.7 Hz, 1 H), 4.96 (s, 1 H), 5.25 (s, 1 H), 5.65 (d, J = 9.5 Hz, 1 H), 6.21 (s, 1 H), 6.25 (d, J = 2.2 Hz, 1 H), 6.40−6.44 (m, 2 H), 7.14 (s, 1 H), 7.21 (d, J = 8.1 Hz, 1 H), 8.47 (s, 1 H); ^{13}C NMR (150 MHz, acetone-d_6) δ 28.3, 70.5, 76.6, 77.2, 85.7, 98.7, 104.8, 108.9, 114.5, 117.1, 121.3, 122.2, 125.1, 129.8, 155.2, 156.8, 160.7, 161.9; anal. (%) C 67.07, H 5.68, calcd for $C_{20}H_{18}O_5$·1.1 H_2O, C 66.86, H 5.57; HPLC was essentially a single peak (>97% AUC) with a retention time identical to the authentic sample of the natural form from the soybeans (retention time = 9.6 min).

Glyceollins Extraction and Sample Handling. Dried soybean plant powder (100 mg) was placed in a 25 mL round-bottom flask fitted with a water-cooled condenser. HPLC grade MeOH (10 mL) was added, and the slurry was gently refluxed at 64 °C for 2 h. The slurry was filtered, and the residue was extracted again with fresh MeOH (10 mL). This process was repeated a third time. The extracts were combined, filtered with a Titan2 PTFE syringe filter consisting of a glass wool prefilter and a 0.45 μm final filter, and then a 500 μL aliquot was vacuum centrifuged (the remaining sample can be stored at 4 °C for several months without deterioration). The resulting residue was dissolved in 150 μL mobile phase and placed in a 0.3 mL HPLC microvial for LC-MS analysis. Quantification of GLY I can be accomplished using authentic material to prepare a stock solution in MeCN at 8×10^{-4} M from which dilutions can afford a standard curve and various QC samples. The calibration curve prepared for GLY I was linear from 5−3,000 ng/mL, and the three QC samples were 30, 300, and 1000 ng/mL.

LC-MS Conditions. An Alliance LC (model Waters 2795) equipped with a quaternary pump, a degasser, an autosampler, and a column oven from Waters Corporation (Milford, MA, USA) were used. The detector was a mass spectrometry MICROMASS Quatro Micro API from Waters Corporation. MassLynx V4.1 software from Waters Corporation (Milford, MA, USA) was used for data acquisition and processing. Quattro Micro V4.1 software from Waters Corporation was used to set up MS, and Inlet Method V4.1 software from Waters Corporation was used to set up LC. The LC method used a flow rate of 350 μL/min with an injection volume of 10 μL. The total run time was around 15 min. The samples were run through an Ascentis Express C_{18} analytical column (reversed-phase, 2.1× 75 mm, 2.7 μm) purchased from Sigma-Aldrich (St. Louis, MO, USA) with a guard column XBridge C_{18} (2.1× 10 mm, 3.5 μm). The mobile phase was 55% 0.1% formic acid buffer and 45% MeOH. Column and sample temperatures were set at 35 ± 5 and 5 ± 5 °C, respectively. MS detection used an electrospray ionization (ESI) source in positive mode; nitrogen was used as the desolvation gas and was set at a flow rate of 750 and 50 L/h, respectively. The source and desolvation gas temperature were 100 and 400 °C, respectively. The ESI source tip (capillary) voltage was 3.5 kV, extractor was 3.0 V, ion energy was 0.5 V, cone voltage was 45 V, and collision energy was 2 V.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00607.

Soybean's phytochemical pathways leading to normal natural products and stressed phytoalexins, ^{1}H and ^{13}C NMR spectra for all synthesized compounds, and LC-MS chromatogram for final material compared to natural materials (PDF)

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Notes

The authors declare no competing financial interest.

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