Isolation of Nor-secofriedelanes from the Sedative Extracts of *Galphimia glauca*§

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Preparative-scale recycling HPLC was used for the complete resolution of a complex mixture of nor-secofriedelanes into five major peaks (I−V) from the sedative methanolic extracts prepared from the aerial parts of *Galphimia glauca*. Argentation chromatography was used to show peaks I, II, IV, and V to be mixtures of isomers around the E-ring double bond, represented by the endocyclic C-20, C-21 double-bond isomers, galphimines A (**3**), B (**1**), D (**4**), and E (**2**), and the C-20, C-29 exocyclic forms, galphimines F−I (**5**−**8**). Galphimine C (**9**), isolated from peak III, corresponded to the C-19, C-20 double-bond isomer of the previously known major sedative constituent galphimine B. The characterization of all the new triterpenes (**3**−**9**) was performed primarily by high-field NMR spectroscopy. Comparison between experimental and calculated $^1H-^1H$ vicinal coupling constants and the analysis of molecular mechanics structures revealed that the ring B of these compounds exists in a boatlike conformation. The absolute configuration for the stereogenic carbinol center at C-4 was established by the application of the Mosher ester derivatization technique carried out in NMR tubes.

Galphimine B (**1**), a nor-secotriterpene and the main sedative component of *Galphimia glauca* (Cav.) Kuntze (Malpighiaceae), was isolated from the aerial parts of this plant, which is traditionally utilized in Mexico for the treatment of central nervous system disorders.[1,2] The first report on the isolation of this active principle was published about a decade ago when a product was obtained by fractional crystallization from a complex mixture of nor-secofriedelanes. The structure of compound **1** has been resolved by X-ray diffraction studies,[3] but no reference to any physical constants (mp and $[\alpha]_D$) nor to spectral data (NMR and MS) has been made. Recently, a second investigation conducted on *G. glauca* tissue cultures led to not only the isolation of the above-mentioned major constituent but also a second related compound, 6-acetoxygalphimine B (**2**).[4] The critical factor in achieving total purification of both samples was the development of a suitable HPLC procedure as a simple routine analytical process able to detect and quantify the in vitro production of the sedative galphimines **1** and **2** in cell suspension cultures.[5]

For further biotechnological in vitro studies, a more complete knowledge of the friedelane profile present in the wild plant is necessary, since pure samples of the triterpenes involved are needed for use as chromatographic standards to monitor their biosynthetic production. Therefore, an important prerequisite to accomplish their isolation is the standardization of analytical HPLC procedures, which can be scaled up easily for preparative purposes. This was achieved by using reversed-phase chromatography operating in a recycling mode. The present study describes the isolation of five major HPLC peaks from the original sedative MeOH extract that was prepared from the aerial parts. Four of the eluted peaks represent diastereomeric mixtures of Δ^{20} and $\Delta^{20(29)}$ isomers. Through the use of argentation chromatography,[6] for both silica gel TLC and column chromatography, their complete resolution is described. In addition, a fifth peak corresponding to the Δ^{19} isomer of galphimine B is also mentioned in this paper.

Results and Discussion

The mixture of galphimines present in the sedative triterpene fraction of *G. glauca* aerial parts has remained unresolved after a decade of extensive pharmacological investigation in Mexico.[1,2] Due to our present biotechnological approach focus on the in vitro production of the main active constituent galphimine B (**1**),[4,5] the separation and characterization of the additional major components of the mixture has become a high-priority phytochemical issue. These pure samples are needed for use as HPLC standard compounds for the quantification of the in vitro triterpene production by cell and hairy root suspension cultures. In addition, the identification of novel compounds will be the starting point for the elucidation of the complete metabolic pathway, which would allow the future implementation of molecular biology-based methods for manipulation of the friedelane biosynthesis. It is hoped that this approach will lead to an increase in the in vitro production of the sedative principles of *G. glauca*.

The fractionation by reversed-phase HPLC of the nor-secofriedelane mixture present in the MeOH extract of *G. glauca* resulted in the collection of five subfractions (peaks I−V), which were further purified to chromatographic homogeneity by application of preparative-scale recycling HPLC.[7,8] In each of these analyzed eluates, one individual spot was observed by silica gel TLC. This chromatographic behavior was maintained even after 10 consecutive HPLC cycles (Figure S1, Supporting Information). However, despite the observed TLC homogeneity in all the NMR spectra of the eluted peaks, with the exception of peak III, a group of olefinic signals was recognized, suggesting the presence of a mixture. These signals were representative

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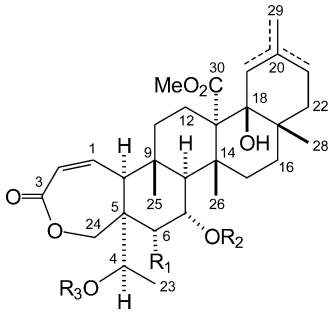
of an exomethylene vinylic moiety (H_2-29: δ 4.5 and 4.7; δ_C 109) and a trisubstituted endocyclic double bond (H-21: δ 5.1; δ_C 118). The results of the present investigation demonstrated, with the use of silver nitrate-impregnated silica gel plates,[6] that each of these peaks was a mixture of isomers around the E-ring double bond. The eluates represented different pairs of double-bond isomers, of the C-20, C-21 endocyclic double bond (**1**−**4**) and of the C-20, C-29 exocyclic forms (**5**−**8**), which could be completely separated from each other by $AgNO_3$-impregnated silica gel column chromatography (Figure S1, Supporting Information).



	R_1	R_2	R_3
Δ^{20}			
1	H	H	H
2	OAc	H	H
3	OH	H	H
4	OH	Ac	H
10	OAc	Ac	Ac
12r	H	(*R*)-MTPA	(*R*)-MTPA
12s	H	(*S*)-MTPA	(*S*)-MTPA
13r	OAc	(*R*)-MTPA	(*R*)-MTPA
13s	OAc	(*S*)-MTPA	(*S*)-MTPA
$\Delta^{20(29)}$			
5	H	H	H
6	OAc	H	H
7	OH	H	H
8	OH	Ac	H
11	OAc	Ac	Ac
Δ^{19}			
9	H	H	H

Deduction of the basic skeleton as a nor-secofriedelane type for all of the isolated galphimines was achieved through comparative analysis of their NMR data ($^1H-^1H$ COSY, $^1H-^{13}C$ HMBC, and NOESY) with those previously reported for related triterpenes[9−11] and, in particular, 6-acetoxygalphimine B or galphimine E (**2**).[4] The following common structural features for all the compounds were observed: the presence of three tertiary methyl groups (Me-25, Me-26, and Me-28); one secondary methyl group at C-4 (Me-23); a carbomethoxy group (C-30); one tertiary alcohol at C-18; and the seven-membered α,β-unsaturated lactone ring A. The last-mentioned functionality was confirmed through an HMBC-connectivity analysis where the carbonyl lactone resonance (δ 169) showed correlations with the vinylic protons H-1 (δ 6.4) and H-2 (δ 6.0). The latter two were also part of an ABX spin-system with H-10 (δ 2.5−2.7). The oxidation pattern of ring B was likewise deduced by comparison with **2**.[4] Whereas the methylene signal for C-6 (δ 37) in compounds **1**, **5**, and **9** was missing in the ^{13}C NMR spectra of compounds **2**−**4** and **6**−**8**, a hydroxylated (δ 65) or acetoxylated (δ 69) methine carbon appeared instead.

The molecular mechanics global minimum of compound **3** (E_{MMX} = 97.70 kcal/mol) displayed a conformation similar

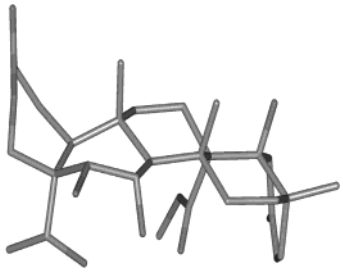


Figure 1. Minimum energy structure of galphimine A (**3**).

Table 1. ^1H NMR Chemical Shift Data for Diagnostic Signals of the (*S*)- and (*R*)-MTPA-Ester Derivatives **12** and **13**

MTPA ester	proton chemical shifts ($\Delta\delta_H = \delta_S - \delta_R$)[a] H-23	$\Delta\delta_H$	H-24a	$\Delta\delta_H$	H-24b	$\Delta\delta_H$	C-4 config
12	1.361	+0.062	3.809	−0.056	4.293	−0.110	*R*
	1.299		3.865		4.403		
13	1.483	+0.072	4.182	−0.048	4.274	−0.089	*R*
	1.411		4.230		4.363		

[a] Data measured in $CDCl_3$ at 300 MHz.

to that found in the X-ray structure of galphimine B (**1**)[3] in that ring B in both substances adopts a classical boat conformation (Figure 1). The observed vicinal coupling constants ($J_{6,7} \cong 5.5$ Hz and $J_{7,8} \cong 8$ Hz) found in compounds **2**−**4** and **6**−**8** were similar to the calculated values generated from the molecular model of **3** ($J_{6,7} = 4.6$ Hz and $J_{7,8} = 8$ Hz). These values were obtained from the corresponding molecular mechanics dihedral angles H-6−C-6−C-7−H-7 = +40° and H-7−C-7−C-8−H-8 = +151° by using the Altona equation,[12,13] thus defining an α-configuration for the functionalities (hydroxyl or acetyloxy groups) on these chiral centers. In addition, a NOESY cross-peak was clearly recorded between H-6 and Me-25, supporting the discussed structural assignment for all the above-mentioned cases. The molecular model for the opposite stereochemistry at C-6 (i.e., β-OH) registered a dihedral angle of −82°, which allowed for the calculation of $J_{6,7}$ = 1.4 Hz.

Through acetylation, all members of each isomeric series, i.e., compounds **2**−**4** and **6**−**8**, afforded the same peracetylated derivative **10** or **11**, respectively. This chemical correlation, as well as that obtained by application of the Mosher esters methodology,[14] provided conclusive evidence for the same C-4 absolute configuration in all galphimines. This reaction was performed in NMR tubes in deuterated pyridine, which allowed heating the solution to promote the esterification. In the NMR spectra of the (*S*)-MTPA ester of the galphimines B and E (MTPA derivatives **12s** and **13s**, respectively), the H-23 methyl group protons were relatively deshielded compared to those signals in the (*R*)-MTPA derivatives (**12r** and **13r**). The chemical shift difference ($\Delta\delta = \delta_S - \delta_R$) between corresponding Me-23 protons was positive (Table 1), allowing the confirmation of a C-4 (*R*) absolute configuration through the application of the configurational model proposed by Kakisawa and associates.[15]

The major HPLC peak II (t_R = 26 min; Figure S1, Supporting Information) afforded an isomeric pair of compounds both with the molecular formula $C_{30}H_{44}O_7$, which was subsequently resolved by argentation chromatography on a silica gel column into pure compounds **1** and **5**. The second most prominent peak, eluate V (t_R = 33.5 min), afforded a pair of compounds with the molecular formula $C_{32}H_{46}O_9$, representing galphimine E (**2**) and its isomer **6**. The molecular formula calculated for peak IV (t_R = 30 min) was the same as that of peak V but was resolved

into pure compounds **4** and **8**. The isomerism of peaks IV and V was a consequence of the position interchange of the acetyl group residue between the hydroxylated C-6 and C-7 of the basic skeleton. Peak I (t_R = 19 min) yielded **3** and **7**, whose FABMS led to the molecular formula $C_{30}H_{44}O_8$ and indicated that these pure compounds represented the deacetylated form of galphimine D as well as galphimine E. Galphimine C (peak III; t_R = 27.5 min) was present in only one isomeric form (**9**) and posessed the same molecular formula as galphimine B ($C_{30}H_{44}O_7$). For compounds in both isomeric series, their structure elucidation was in total agreement with the NMR data included in the Experimental Section.

Galphimine C (**9**) represents the C-19, C-20 endocyclic double-bond isomer of compound **1**. The NMR experiments allowed unambiguously for the identification of all resonances in ring E and the placement of the double bond between C-19 and C-20 (Figure S2, Supporting Information). The line width at half-height for the olefinic proton signal H-19 ($W_{1/2}$ = 5 Hz) supported this assignment, since a higher value was measured for compounds in the C-20, C-21 endocyclic series ($W_{1/2}$ = 9 Hz). In the $^1H-^1H$ COSY spectrum, the vinylic proton H-19 (δ 5.3) showed allylic couplings with the Me-29 group (δ 1.7) and the H-21 α-proton (δ 1.9). Additionally, mutual $^3J_{H-H}$ correlations were observed between both ring E methylene groups, i.e., CH_2-21 and CH_2-22, both of which were absent in the isomeric Δ^{20} endocyclic series. Also observed were HMBC correlations for the Me-29 group (δ 0.9) and C-19 (δ 127; $^3J_{CH}$), C-20 (δ 133; $^2J_{CH}$), and C-21 (δ 27; $^3J_{CH}$). A long-range coupling between C-18 (δ 77) and H-22β (δ 1.0) as well as cross-peaks between the Me-28 signal (δ 0.9), C-18 (δ 77), and C-22 (δ 32) were also detected. The hydroxyl group, as an allylic substituent in galphimine C (**9**), through a molecular conformational change, produced a better interaction with the reversed-phase C_{18} adsorbent in HPLC. This situation favored its separation from the two additional isomers of galphimine B, i.e., the eluted peak II (**1** and **5**) (Figure S1, Supporting Information).

There seems to be a significant geographically based difference between the semiarid highland specimens of *G. glauca* and those of the tropical coastal plains[11] of Mexico, in that the galphimines (**1**−**9**) have only been isolated in plant material collected from the former, although the production of nor-secofriedelanes is found in plants from both areas. Finally, the evaluation of galphimines in a panel of four human tumor cell lines (KB, HCT-15, OVCAR, and SQC-1) indicated a lack of significant cytotoxicity (ED_{50} > 4 μg/mL), despite their potentiality as alkylating agents due to their α,β-unsaturated lactone ring. This lack of toxic cellular effects could also imply the safety in future sedatives developed for human consumption in over-the-counter remedies or phytomedicines containing this type of bioactive principle.

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer model 241 polarimeter. ^1H (500 and 300 MHz) and ^{13}C (125.7 and 75.4 MHz) NMR experiments were recorded on a Bruker AMX-500 or a Varian XL-300GS spectrometer in $CDCl_3$ or pyridine-d_5 solution containing TMS as internal standard. Positive-ion LRFABMS and HRFABMS were registered using a glycerol or poly(ethylene glycol) matrix on a JEOL SX102A spectrometer. Cytotoxicity was conducted at UNAM (Facultad de Química) using cultured KB (nasopharyngeal carcinoma), HCT-15 (colon cancer), OVCAR (ovarian adenocarcinoma), and SQC-1 (squamous cell cervical carcinoma) cells, according to previously described protocols.[16,17]

Plant Material. Leaves of *Galphimia glauca* were collected at the "Municipio Doctor Mora", Guanajuato, Mexico, in July 2001. The voucher specimens were identified by Abigail Aguilar and deposited at Instituto Mexicano del Seguro Social Herbarium (IMSSM: 11061), Centro Médico Nacional Siglo XXI, Mexico City.

Extraction and Isolation. The dried leaves (800 g) were powdered and defatted by maceration at room temperature with hexane. The residual material was extracted exhaustively with $CHCl_3$ and MeOH to afford, after removal of the solvent, a dark green syrup (104 g) and a brownish oily residue (69 g), respectively. The crude mixture of galphimines was obtained after fractionation of the MeOH extract by open column cromatography over silica gel eluted with a gradient of MeOH in $CHCl_3$. A total of 52 fractions (150 mL each) were collected and combined (fractions 11−16) to give a complex pool constituted by compounds **1**−**9** (2.5 g).

Recycling HPLC Separation. The instrumentation used for HPLC analysis consisted of a Waters (Millipore Corp., Waters Chromatography Division, Milford, MA) 600E multisolvent delivery system equipped with a Waters W996 diode array detector (232 nm). Control of the equipment, data acquisition, processing, and management of chromatographic information were performed by the Millennium 32 software program (Waters). The analytical HPLC separations were done on a Symmetry C_{18} column (Waters; 5 μm, 4.6 × 250 mm) with an isocratic elution of CH_3CN-H_2O (45:55), a flow rate of 0.65 mL/min, and a sample injection of 10 μL (1 mg/mL). The crude galphimine fraction was subjected to preparative HPLC on a reversed-phase C_{18} column (7 μm, 19 × 300 nm). The elution was isocratic with CH_3CN-H_2O (45:55) using a flow rate of 10 mL/min. Eluates across the peaks with t_R of 18.9 min (peak I: 28 mg; **3** and **7**), 26.2 min (peak II: 80 mg; **1** and **5**), 27.7 min (peak III: 3 mg; **9**), 29.9 min (peak IV: 20 mg; **4** and **8**), and 33.4 min (peak V: 50 mg; **2** and **6**) were collected by the technique of heart cutting[7,8] and independently reinjected in the apparatus operated in the recycle mode.[18] The complete separation of HPLC peaks I−V was achieved to homogeneity (HPLC; Figure S1, Supporting Information) after five to ten consecutive cycles employing an isocratic elution with CH_3CN-H_2O (7:3).

Argentation Chromatography. TLC: precoated Si gel 60 F_{254} aluminum sheets (20 × 20 cm) and HPTLC plates (10 × 10 cm) were impregnated with silver nitrate by immersion (3×) in a solution of 2 g of $AgNO_3$ in acetone−H_2O (3:2; 50 mL). After each immersion, the plates were dried at 100 °C. Final activation was performed by heating overnight at 90 °C. Plates were appropriately stored to avoid deactivation by moisture and oxidation by exposure to air and light. Argentation TLC, using $CHCl_3$−EtOAc (1:2), satisfactorily separated the galphimine mixture (TLC; Figure S1, Supporting Information). Spraying the plates with vanillin−sulfuric acid solution (0.1 g in 10 mL of H_2SO_4 98%) allowed the visualization of compounds.

Column Chromatography. Silica gel 60 (40−60 μm) was prepared by mixing the adsorbent (50 g) in a 2% $AgNO_3$ solution of acetone−H_2O (3:2, 120 mL). The impregnated adsorbent was dried following the above-described procedures for TLC plates. Complete resolution of all diastereomeric pairs (10 mg) was achieved by normal column chromatography using 5 g of the impregnated adsorbent and $CHCl_3$−EtOAc (1:1) as elution solvent. These argentation techniques afforded pure compounds **3** (2 mg) and **7** (6 mg) from peak I; **1** (3 mg) and **5** (5 mg) from peak II; **4** (3 mg) and **8** (5 mg) from peak IV; and finally, **2** (3 mg) and **6** (5 mg) from peak V.

Galphimine B (1): white amorphous powder; mp 149−152 °C; $[\alpha]^{25}_D$ −51° (*c* 0.16, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 6.41 (1H, dd, *J* = 12.0, 9.0 Hz, H-1), 6.05 (1H, d, *J* = 12.0 Hz, H-2), 5.15 (1H, m, H-21), 4.22 (1H, m, H-7β), 4.22 (1H, d, *J* = 12.0, H-24b), 3.52 (1H, m, H-4), 3.52 (1H, m, H-24a), 3.51 (3H, s, OCH_3), 2.92 (1H, dd, *J* = 13.1, 3.3 Hz, H-15α), 2.67 (1H, dd, *J* = 15.9, 6.6 Hz, H-6α), 2.65 (1H, m, H-19b), 2.54

(1H, d, J = 9.0 Hz, H-10α), 2.59 (1H, m, H-22α), 2.27 (1H, d, J = 7.9 Hz, H-8α), 2.05 (1H, m, H-16*β*), 2.03 (2H, m, H-12), 1.80 (1H, m, H-19a), 1.53 (3H, s, CH_3-29), 1.52 (1H, m, H-22*β*), 1.47 (1H, m, H-6*β*), 1.47 (1H, m, H-11b), 1.36 (3H, s, H-26), 1.22 (1H, m, H-11a), 1.22 (1H, m, H-15*β*), 1.22 (1H, m, H-16α), 1.08 (3H, d, J = 6.0 Hz, CH_3-23), 0.97 (3H, s, CH_3-28), 0.93 (3H, s, CH_3-25); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 175.1 (C, C-30), 169.9 (C, C-3), 145.7 (CH, C-1), 131.6 (C, C-20), 118.8 (CH, C-21), 123.5 (CH, C-2), 76.6 (C, C-18), 72.4 (CH, C-4), 73.0 (CH_2, C-24), 66.5 (CH, C-7), 57.5 (C, C-13), 53.8 (CH, C-8), 53.3 (CH, C-10), 51.3 (CH_3, OCH_3), 48.3 (C, C-5), 42.1 (C, C-14), 40.5 (C, C-9), 40.3 (CH_2, C-19), 40.0 (CH_2, C-11), 38.6 (CH_2, C-22), 37.3 (CH_2, C-6), 37.1 (C, C-17), 31.8 (CH_2, C-16), 29.7 (CH_2, C-15), 26.6 (CH_3, C-28), 23.4 (CH_2, C-12), 22.9 (CH_3, C-29), 21.6 (CH_3, C-26), 20.4 (CH_3, C-25), 18.1 (CH_3, C-23); positive FABMS m/z 517 $[M + H]^+$, 499 $[M + H - H_2O]^+$; HRFABMS m/z 517.3148 ($[M + H]^+$, calcd for $C_{30}H_{45}O_7$ −3.4 ppm error).

Galphimine E (6-acetoxygalphimine B, 2): white amorphous powder; mp 177−179 °C; $[\alpha]^{25}_D$ −22.4° (c 2.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.40 (1H, dd, J = 12.0, 8.7 Hz, H-1), 6.07 (1H, d, J = 12.0 Hz, H-2), 5.53 (1H, d, J = 5.7 Hz, H-6*β*), 5.15 (1H, brs, H-21), 4.42 (1H, dd, J = 7.8, 6.0 Hz, H-7*β*), 4.10 (1H, d, J = 12.2 Hz, H-24b), 3.95 (1H, q, J = 6.6 Hz, H-4), 3.72 (1H, d, J = 12.2 Hz, H-24a), 3.52 (3H, s, OCH_3), 2.89 (1H, td, J = 13.5, 4.5 Hz, H-15α), 2.75 (1H, d, J = 8.7 Hz, H-10α), 2.63 (1H, d, J = 15.0 Hz, H-19b), 2.59 (1H, m, H-22α), 2.53 (1H, d, J = 7.8 Hz, H-8α), 2.15 (3H, s, OAc), 2.04 (2H, m, H-12), 1.97 (1H, m, H-16*β*), 1.82 (1H, d, J = 15.0 Hz, H-19a), 1.53 (3H, s, CH_3-29), 1.51 (1H, m, H-22*β*), 1.46 (1H, m, H-11b), 1.36 (1H, s, CH_3-26), 1.23 (1H, m, H-11a), 1.18 (1H, m, H-15*β*), 1.18 (1H, m, H-16α), 1.00 (3H, d, J = 6.3 Hz, CH_3-23), 1.00 (3H, s, CH_3-25), 0.96 (3H, s, CH_3-28); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 175.1 (C, C-30), 169.7 (C, OAc), 169.3 (C, C-3), 144.7 (CH, C-1), 131.6 (C, C-20), 123.9 (CH, C-2), 118.8 (CH, C-21), 76.6 (C, C-18), 69.9 (CH, C-6), 69.4 (CH_2, C-24), 65.3 (CH, C-7), 63.8 (CH, C-4), 57.6 (C, C-13), 52.7 (CH, C-8), 52.0, (CH, C-10), 51.5 (C, C-5), 51.3 (OCH_3), 42.1 (C, C-14), 40.4 (CH_2, C-11), 40.2 (CH_2, C-19), 39.3 (C, C-9), 38.7 (CH_2, C-22), 37.1 (C, C-17), 31.9 (CH_2, C-16), 29.2 (CH_2, C-15), 26.6 (CH_3, C-28), 23.4 (CH_2, C-12), 22.9 (CH_3, C-29), 21.7 (CH_3, C-26), 21.4 (CH_3, C-25), 20.9 (CH_3, OAc), 18.0 (CH_3, C-23); positive FABMS m/z 597 $[M + Na]^+$, 557 $[M + H - H_2O]^+$; HRFABMS m/z 557.3117 ($[M + H - H_2O]^+$, calcd for $C_{32}H_{45}O_8$ +0.5 ppm error).

Galphimine A (3): white amorphous powder; mp 117−119 °C; $[\alpha]^{25}_D$ −37° (c 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.37 (1H, dd, J = 12.2, 9.0 Hz, H-1), 6.05 (1H, d, J = 12.2 Hz, H-2), 5.27 (1H, brs, H-21), 4.35 (1H, m, H-6*β*), 4.17 (1H, m, H-7*β*), 4.03 (2H, m, H-24), 3.92 (1H, q, J = 6.3 Hz, H-4), 3.54 (3H, s, OCH_3), 2.96 (1H, td, J = 14.4, 4.9 Hz, H-15α), 2.90 (1H, d, J = 9.0 Hz, H-19b), 2.59 (1H, d, J = 9.0 Hz, H-10α), 2.59 (1H, m, H-22α), 2.40 (1H, d, J = 7.9 Hz, H-8α), 2.03 (2H, m, H-12), 2.00 (1H, m, H-16*β*), 1.81 (1H, d, J = 15.3 Hz, H-19a), 1.54 (3H, s, CH_3-29), 1.51 (1H, m, H-22*β*), 1.46 (1H, m, H-11b), 1.37 (1H, m, H-11a), 1.33 (3H, s, CH_3-26), 1.32 (1H, m, H-15*β*), 1.20 (1H, m, H-16α), 1.09 (3H, s, CH_3-28), 0.98 (3H, d, J = 6.3 Hz, CH_3-23), 0.92 (3H, s, CH_3-25); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 175.6 (C, C-30), 169.7 (C, C-3), 144.8 (CH, C-1), 132.0 (C, C-20), 123.8 (CH, C-2), 118.5 (CH, C-21), 77.5 (C, C-18), 70.0 (CH_2, C-24), 67.4 (CH, C-7), 66.2 (CH, C-6), 62.9 (CH, C-4), 57.5 (C, C-13), 52.8 (CH, C-8), 52.5 (C, C-5), 52.4 (CH, C-10), 51.6 (OCH_3), 42.1 (C, C-14), 40.5 (CH_2, C-11), 40.1 (CH_2, C-19), 38.8 (C, C-9), 38.5 (CH_2, C-22), 38.1 (C, C-17), 32.3 (CH_2, C-16), 29.1 (CH_2, C-15), 26.6 (CH_3, C-28), 23.3 (CH_2, C-12), 22.8 (CH_3, C-29), 21.4 (CH_3, C-26), 21.2 (CH_3, C-25), 17.6 (CH_3, C-23); positive FABMS m/z 533 $[M + H]^+$; HRFABMS m/z 533.3137 ($[M + H]^+$, calcd for $C_{30}H_{45}O_8$ +4.3 ppm error).

Galphimine D (4): white amorphous powder; mp 247−248 °C; $[\alpha]^{25}_D$ −8° (c 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.40 (1H, dd, J = 12.2, 8.7 Hz, H-1), 6.07 (1H, d, J = 12.2 Hz, H-2), 5.48 (1H, dd, J = 8.4, 6.3 Hz, H-7*β*), 5.16 (1H, m, H-21), 4.57 (1H, m, H-6*β*), 4.14 (2H, m, H-24), 4.03 (1H, q, J = 6.6 Hz, H-4), 3.52 (3H, s, OCH_3), 2.82 (1H, td, J = 13.6, 4.9 Hz, H-15α), 2.75 (1H, d, J = 8.8 Hz, H-10α), 2.64 (1H, d, J = 17.5 Hz, H-19b), 2.59 (1H, d, J = 8.4 Hz, H-8α), 2.59 (1H, m, H-22α), 2.17 (3H, s, OAc), 2.03 (2H, m, H-12), 1.98 (1H, m, H-16*β*), 1.83 (1H, m, H-19a), 1.55 (1H, m, H-22*β*), 1.54 (3H, s, CH_3-29), 1.47 (2H, m, H-11), 1.37 (3H, s, CH_3-26), 1.20 (1H, m, H-16α), 1.05 (3H, CH_3-23), 0.98 (3H, s, CH_3-25), 0.95 (3H, s, CH_3-28), 0.83 (1H, ddd, J = 13.6, 4.9, 2.3 Hz, H-15*β*); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 175.0 (C, C-30), 171.8 (C, OAc), 169.2 (C, C-3), 144.2 (CH, C-1), 131.6 (C, C-20), 123.7 (CH, C-2), 118.8 (CH, C-21), 77.1 (C, C-18), 70.8 (CH, C-7), 69.3 (CH_2, C-24), 65.3 (CH, C-6), 64.3 (CH, C-4), 57.4 (C, C-13), 53.3 (CH, C-10), 53.2 (C, C-5), 51.3 (OCH_3), 49.4 (CH, C-8), 42.2 (C, C-14), 40.5 (CH_2, C-11), 40.2 (CH_2, C-19), 39.0 (C, C-17), 38.6 (CH_2, C-22), 38.5 (C, C-9), 31.8 (CH_2, C-16), 27.4 (CH_2, C-15), 26.5 (CH_3, C-28), 23.2 (CH_2, C-12), 22.9 (CH_3, C-29), 22.0 (CH_3, C-25), 21.5 (CH_3, OAc), 20.9 (CH_3, C-26), 18.2 (CH_3, C-23); positive FABMS m/z 575 $[M + H]^+$, 557 $[M + H - H_2O]^+$; HRFABMS m/z 575.3203 ($[M + H]^+$, calcd for $C_{32}H_{47}O_9$ −2.9 ppm error).

Galphimine F (5): white amorphous powder; mp 148−150 °C; $[\alpha]^{25}_D$ −38° (c 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.43 (1H, dd, J = 12.4, 8.6 Hz, H-1), 6.04 (1H, d, J = 12.4 Hz, H-2), 4.68 (1H, q, J = 2.4 Hz, H-29b), 4.46 (1H, q, J = 2.4 Hz, H-29a), 4.23 (1H, d, J = 12.0 Hz, Hb-24), 4.20 (1H, m, H*β*-7), 3.52 (1H, q, J = 6.3 Hz, H-4), 3.52 (1H, m, H-24a), 3.50 (3H, s, OCH_3), 3.01 (1H, td, J = 12.0, 4.5 Hz, H-15α), 2.92 (1H, d, J = 15.0 Hz, H-19b), 2.67 (1H, dd, J = 15.9, 6.6 Hz, H-6α), 2.54 (1H, d, J = 8.6 Hz, H-10α), 2.27 (1H, m, H-21*β*), 2.14 (1H, m, H-22α), 2.10 (1H, m, H-8α), 2.05 (1H, d, J = 15.0 Hz, H-19a), 2.02 (2H, m, H-12), 2.01 (1H, m, H-16*β*), 1.98 (1H, m, H-21α), 1.46 (1H, m, H-6*β*), 1.44 (1H, m, H-11b), 1.35 (3H, s, CH_3-26), 1.28 (1H, m, H-16α), 1.24 (1H, m, H-11a), 1.20 (1H, ddd, J = 12.0, 4.5, 2.4 Hz, H-15*β*), 1.10 (3H, s, CH_3-28), 1.05 (1H, m, H-22*β*), 1.04 (3H, d, J = 6.3 Hz, CH_3-23), 0.95 (3H, s, CH_3-25); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 175.0 (C, C-30), 169.9 (C, C-3), 145.8 (CH, C-1), 144.3 (C, C-20), 123.6 (CH, C-2), 109.5 (CH_2, C-29), 77.3 (C, C-18), 73.0 (CH_2, C-24), 72.4 (CH, C-4), 66.5 (CH, C-7), 57.5 (C, C-13), 54.4 (CH, C-8), 53.6 (CH, C-10), 51.4 (OCH_3), 48.3 (C, C-5), 42.5 (CH_2, C-19), 42.1 (C, C-14), 40.3 (C, C-9), 39.9 (CH_2, C-11), 38.2 (C, C-17), 37.3 (CH_2, C-6), 36.0 (CH_2, C-22), 34.0 (CH_2, C-16), 29.3 (CH_2, C-21), 29.0 (CH_2, C-15), 25.6 (CH_3, C-28), 23.4 (CH_2, C-12), 21.0 (CH_3, C-25), 21.0 (CH_3, C-26), 18.0 (CH_3, C-23); positive FABMS m/z 517 $[M + H]^+$, 499 $[M + H - H_2O]^+$; HRFABMS m/z 517.3148 ($[M + H]^+$, calcd for $C_{30}H_{45}O_7$ −3.4 ppm error).

Galphimine G (6): white amorphous powder; mp 176−178 °C; $[\alpha]^{25}_D$ −21° (c 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.41 (1H, dd, J = 12.2, 8.8 Hz, H-1), 6.07 (1H, d, J = 12.2 Hz, H-2), 5.51 (1H, d, J = 5.9 Hz, H-6*β*), 4.67 (1H, q, J = 2.4 Hz, H-29b), 4.46 (1H, q, J = 2.4 Hz, H-29a), 4.42 (1H, dd, J = 7.8, 5.9 Hz, H-7*β*), 4.11 (1H, d, J = 12.2 Hz, H-24b), 3.95 (1H, q, J = 6.4 Hz, H-4), 3.71 (1H, d, J = 12.2 Hz, H-24a), 3.49 (3H, s, OCH_3), 2.94 (1H, td, J = 13.2, 3.9 Hz, H-15α), 2.89 (1H, d, J = 14.7 Hz, H-19b), 2.75 (1H, d, J = 8.8 Hz, H-10α), 2.33 (1H, d, J = 7.8 Hz, H-8α), 2.25 (1H, m, H-21*β*), 2.16 (1H, m, H-22α), 2.13 (3H, s, OAc), 2.09 (1H, m, H-19a), 2.02 (2H, m, H-12), 2.00 (1H, m, H-16*β*), 1.97 (1H, m, H-21α), 1.43 (2H, m, H-11), 1.35 (3H, s, CH_3-26), 1.27 (1H, m, H-16α), 1.14 (1H, m, H-15*β*), 1.07 (3H, s, CH_3-28), 1.03 (1H, m, H-22*β*), 1.01 (3H, s, CH_3-25), 0.98 (3H, d, J = 6.3 Hz, CH_3-23); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 174.8 (C, C-30), 169.6 (C, OAc), 169.3 (C, C-3), 144.8 (C, C-20), 144.4 (CH, C-1), 124.0 (CH, C-2), 109.4 (CH_2, C-29), 76.6 (C, C-18), 69.8 (CH, C-6), 69.4 (CH_2, C-24), 65.4 (CH, C-7), 63.8 (CH, C-4), 57.6 (C, C-13), 53.2 (CH, C-8), 52.3 (CH, C-10), 51.5 (C, C-5), 51.4 (OCH_3), 42.5 (CH_2, C-19), 42.0 (C, C-14), 40.4 (CH_2, C-11), 39.0 (C, C-9), 38.2 (C, C-17), 36.1 (CH_2, C-22), 34.1 (CH_2, C-16), 29.0 (CH_2, C-21), 28.8 (CH_2, C-15), 25.6 (CH_3, C-28), 23.4 (CH_2, C-12), 22.0 (CH_3, C-25), 21.1 (CH_3, C-26), 20.9 (CH_3, OAc), 18.0 (CH_3, C-23); positive FABMS m/z 575 $[M + H]^+$, 557 $[M + H - H_2O]^+$; HRFABMS m/z 575.3243 ($[M + H]^+$, calcd for $C_{32}H_{47}O_9$ +3.9 ppm error).

Galphimine H (7): white amorphous powder; mp 116−118 °C; $[\alpha]^{25}_D$ −35° (c 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.40 (1H, dd, J = 12.2, 9.0 Hz, H-1), 6.07 (1H, d, J = 12.2 Hz, H-2), 4.71 (1H, q, J = 2.2 Hz, H-29b), 4.47 (1H, q, J = 2.2 Hz, H-29a), 4.38 (1H, m, H-6*β*), 4.16 (1H, m, H-7*β*), 4.04 (2H, m, H-24), 3.94 (1H, q, J = 6.4 Hz, H-4), 3.54 (3H, s, OCH_3), 2.96

(1H, td, J = 14.2, 4.7 Hz, H-15α), 2.92 (1H, d, J = 15.0 Hz, H-19b), 2.60 (1H, d, J = 9.0 Hz, H-10α), 2.24 (1H, d, J = 7.9 Hz, H-8α), 2.30 (1H, m, H-21β), 2.16 (1H, m, H-22α), 2.12 (1H, m, H-19a), 2.03 (2H, m, H-12), 2.00 (1H, m, H-16β), 1.98 (1H, m, H-21α), 1.45 (1H, m, H-11b), 1.39 (1H, m, H-11a), 1.35 (3H, s, CH_3-26), 1.30 (1H, m, H-15β), 1.27 (1H, m, H-16α), 1.10 (3H, s, CH_3-28), 1.05 (1H, m, H-22β), 1.00 (3H, d, J = 6.4 Hz, CH_3-23), 0.95 (3H, s, CH_3-25); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 175.5 (C, C-30), 169.7 (C, C-3), 144.8 (CH, C-1), 144.2 (C, C-20), 123.8 (CH, C-2), 109.3 (CH_2, C-29), 77.5 (C, C-18), 70.0 (CH_2, C-24), 67.3 (CH, C-7), 65.9 (CH, C-6), 62.9 (CH, C-4), 57.5 (C, C-13), 52.8 (CH, C-8), 52.4 (C, C-5), 52.4 (CH, C-10), 51.5 (OCH_3), 42.3 (CH_2, C-19), 42.0 (C, C-14), 40.5 (CH_2, C-11), 38.8 (C, C-9), 38.1 (C, C-17), 35.9 (CH_2, C-22), 34.4 (CH_2, C-16), 28.9 (CH_2, C-21), 28.7 (CH_2, C-15), 25.5 (CH_3, C-28), 23.2 (CH_2, C-12), 21.7 (CH_3, C-25), 20.7 (CH_3, C-26), 17.8 (CH_3, C-23); positive FABMS m/z 533 $[M + H]^+$, 515 $[M + H - H_2O]^+$; HRFABMS m/z 533.3114 ($[M + H]^+$, calcd for $C_{30}H_{45}O_8$).

Galphimine I (8): white amorphous powder; mp 246−247 °C; $[\alpha]^{25}_D$ −9° (c 2.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.41 (1H, dd, J = 12.2, 8.8 Hz, H-1), 6.07 (1H, d, J = 12.2 Hz, H-2), 5.46 (1H, m, H-7β), 4.69 (1H, q, J = 2.4 Hz, H-29b), 4.56 (1H, m, H-6β), 4.46 (1H, q, J = 2.4 Hz, H-29a), 4.12 (2H, m, H-24), 4.02 (1H, m, H-4), 3.52 (3H, s, OCH_3), 2.91 (1H, d, J = 15.6 Hz, H-19b), 2.82 (1H, td, J = 13.2, 4.9 Hz, H-15α), 2.76 (1H, d, J = 8.8 Hz, H-10α), 2.59 (1H, m, H-8α), 2.28 (1H, m, H-21β), 2.16 (3H, s, OAc), 2.12 (1H, m, H-22α), 2.12 (1H, m, H-19a), 2.04 (2H, m, H-12), 1.99 (1H, m, H-21α), 1.99 (1H, m, H-16β), 1.46 (2H, m, H-11), 1.36 (3H, s, CH_3-26), 1.20 (1H, m, H-16α), 1.08 (3H, s, CH_3-28), 1.03 (3H, d, J = 6.4 Hz, CH_3-23), 1.03 (1H, m, H-22β), 0.98 (3H, s, CH_3-25), 0.81 (1H, ddd, J = 13.2, 4.9, 2.9 Hz, H-15β); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 175.0 (C, C-30), 171.7 (OAc), 169.6 (C, C-3), 144.8 (C, C-20), 144.4 (CH, C-1), 123.8 (CH, C-2), 109.4 (CH_2, C-29), 76.6 (C, C-18), 70.9 (CH, C-7), 69.4 (CH_2, C-24), 65.4 (CH, C-6), 64.4 (CH, C-4), 57.4 (C, C-13), 53.5 (CH, C-10), 52.1 (C, C-5), 51.4 (OCH_3), 50.1 (CH, C-8), 42.5 (CH_2, C-19), 42.2 (C, C-14), 40.6 (CH_2, C-11), 38.8 (C, C-9), 38.1 (C, C-17), 36.0 (CH_2, C-22), 34.1 (CH_2, C-16), 28.9 (CH_2, C-21), 27.4 (CH_2, C-15), 25.5 (CH_3, C-28), 23.3 (CH_2, C-12), 22.0 (CH_3, C-25), 21.6 (CH_3, OAc), 20.9 (CH_3, C-26), 18.2 (CH_3, C-23); positive FABMS m/z 575 $[M + H]^+$, 557 $[M + H - H_2O]^+$; HRFABMS m/z 575.3220 ($[M + H]^+$, calcd for $C_{32}H_{47}O_9$ −0.1 ppm error).

Galphimine C (9): white amorphous powder; mp 119−121 °C; $[\alpha]^{25}_D$ −55° (c 2.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.42 (1H, dd, J = 12.2, 8.8 Hz, H-1), 6.02 (1H, d, J = 12.2 Hz, H-2), 5.29 (1H, br. s, H-19), 4.22 (1H, m, H-7β), 4.22 (1H, d, J = 11.7 Hz, H-24b), 3.53 (1H, m, H-4), 3.53 (3H, s, OCH_3), 3.51 (1H, m, H-24a), 2.98 (1H, td, J = 13.2, 4.4 Hz, H-15α), 2.68 (1H, dd, J = 16.1, 6.4 Hz, H-6α), 2.58 (1H, d, J = 8.8 Hz, H-10α), 2.25 (1H, d, J = 7.8 Hz, H-8α), 2.16 (1H, m, H-22α), 2.10 (1H, dd, J = 13.7, 4.4 Hz, H-12α), 2.05 (1H, m, H-16β), 1.98 (1H, m, H-21β), 1.97 (1H, m, H-12β), 1.72 (1H, m, H-21α), 1.68 (3H, s, CH_3-29), 1.47 (1H, m, H-6β), 1.42 (1H, m, H-11b), 1.36 (3H, s, CH_3-26), 1.36 (1H, m, H-16α), 1.21 (1H, m, H-15β), 1.18 (1H, m, H-11a), 1.09 (3H, d, J = 6.4 Hz, CH_3-23), 1.02 (1H, m, H-22β), 0.94 (3H, s, CH_3-25), 0.93 (1H, s, CH_3-28); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 176.2 (C, C-30), 169.9 (C, C-3), 145.9 (CH, C-1), 133.4 (C, C-20), 126.8 (CH, C-19), 123.5 (CH, C-2), 76.6 (C, C-18), 73.0 (CH_2, C-24), 72.4 (CH, C-4), 66.6 (CH, C-7), 60.2 (C, C-13), 54.2 (CH, C-8), 53.2 (CH, C-10), 51.2 (OCH_3), 48.3 (C, C-5), 41.5 (C, C-14), 40.6 (C, C-9), 40.1 (CH_2, C-11), 37.3 (CH_2, C-6), 36.7 (C, C-17), 33.6 (CH_2, C-16), 32.5 (CH_2, C-22), 29.6 (CH_2, C-15), 27.0 (CH_2, C-21), 25.5 (CH_3, C-28), 23.6 (CH_2, C-12), 23.1 (CH_3, C-29), 20.3 (CH_3, C-25), 20.2 (CH_3, C-26), 18.1 (CH_3, C-23); positive FABMS m/z 517 $[M + H]^+$, 499 $[M + H - H_2O]^+$; HRFABMS m/z 517.3165 ($[M + H]^+$, calcd for $C_{30}H_{45}O_7$ −0.1 ppm error).

Acetylation of Galphimines 2−4 and 6−7. Each individual compound (1−2 mg) was dissolved in Ac_2O−pyridine (1:4) (2.5 mL) and stood at room temperature for 24 h. The derivatives were precipitated by addition of cold water, and their purification was performed by semipreparative HPLC on a reversed-phase C_{18} column (300 × 7.8 mm, 6 μm), using CH_3CN−H_2O (7:3, flow rate = 3 mL/min), to give derivatives **10** (1.5 mg) and **11** (2 mg).

Peracetylated derivative 10: white amorphous powder; $[\alpha]^{25}_D$ −14° (c 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.30 (1H, dd, J = 12.6, 8.4 Hz, H-1), 6.06 (1H, d, J = 12.6 Hz, H-2), 5.66 (1H, d, J = 4.8 Hz, H-6), 5.53 (1H, m, H-7), 5.46 (1H, m, H-4), 5.15 (1H, brs, H-21), 4.40 (1H, d, J = 12.4 Hz, H-24b), 4.08 (1H, d, J = 12.4 Hz, H-24a), 3.52 (3H, s, OCH_3), 2.50 (1H, d, J = 7.8 Hz, H-8), 2.42 (1H, d, J = 8.4 Hz, H-10), 2.10 (3H, s, OAc), 2.08 (3H, s, OAc), 2.00 (3H, s, OAc), 1.56 (3H, s, CH_3-29), 1.36 (3H, s, CH_3-26), 1.23 (3H, d, J = 6.3 Hz, CH_3-23), 1.07 (6H, s, CH_3-25, 28).

Peracetylated derivative 11: white amorphous powder; $[\alpha]^{25}_D$ −9° (c 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.30 (1H, dd, J = 12.6, 8.4 Hz, H-1), 6.06 (1H, d, J = 12.6 Hz, H-2), 5.66 (1H, d, J = 4.8 Hz, H-6), 5.53 (1H, m, H-7), 5.46 (1H, m, H-4), 4.69 (1H, q, J = 2.3 Hz, H-29b), 4.44 (1H, q, J = 2.3 Hz, H-29b), 4.40 (1H, d, J = 12.4 Hz, H-24b), 4.08 (1H, d, J = 12.4 Hz, H-24a), 3.52 (3H, s, OCH_3), 2.50 (1H, d, J = 7.8 Hz, H-8), 2.42 (1H, d, J = 8.4 Hz, H-10), 2.10 (3H, s, OAc), 2.08 (3H, s, OAc), 2.00 (3H, s, OAc), 1.34 (3H, s, CH_3-26), 1.23 (3H, d, J = 6.3 Hz, CH_3-23), 1.08 (6H, s, CH_3-25, 28).

Determination of the Absolute Configuration. Each individual solution (2.0 mg) of galphimines B (**1**) and E (**2**) was treated with 4-(dimethylamino)pyridine (3 mg, previously heated at 70 °C for 3 h) and dry pyridine-d_5 (0.75 mL) in NMR tubes. (*S*)-(−)-α-Methoxy-α-trifluoromethylphenylacetyl (MTPA) chloride was added (20 μL). The reactions were allowed to stand at 70−75 °C for 5 h under an atmosphere of N_2. NMR spectra were then recorded at 300 MHz by acquiring the reaction mixtures. Further purification was performed as follows.[18] The mixtures were transferred from the NMR tubes into vials. Saturated aqueous $NaHCO_3$ and Et_2O were added to the mixtures and stirred vigorously for 5 min. Water (5 mL × 2) was added and extracted with $CHCl_3$. The organic phases were washed with 0.5 N HCl, dried with anhydrous Na_2SO_4, and concentrated. Each crude residue was purified by column chromatography over silica gel using $CHCl_3$−EtOAc (4:1) as eluent to give the (*R*)-MTPA ester (1.5−2.0 mg). NMR spectra in $CDCl_3$ were recorded after purification. Treatment of the same galphimines with (*R*)-(+)-MTPA chloride as described above yielded the (*S*)-MTPA esters.

(*R*)-MTPA ester of galphimine B (12r): white amorphous powder; $[\alpha]^{25}_D$ +18° (c 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.52 (2H, m, phenyl-MTPA), 7.44 (3H, m, phenyl-MTPA), 5.60 (1H, q, J = 6.6 Hz, H-4), 5.17 (1H, brs, H-21), 4.40 (1H, d, J = 12.0 Hz, H-24b), 3.86 (1H, d, J = 12.0 Hz, H-24a), 1.30 (3H, d, J = 6.6 Hz, CH_3-23); ^{1}H NMR (C_5D_5N, 300 MHz) δ 6.15 (1H, q, J = 6.6 Hz, H-4), 4.55 (1H, d, J = 11.7 Hz, H-24b), 4.02 (1H, d, J = 11.7 Hz, H-24a), 1.44 (3H, d, J = 6.6 Hz, CH_3-23).

(*S*)-MTPA ester of galphimine B (12s): white amorphous powder; $[\alpha]^{25}_D$ −24° (c 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.52 (2H, m, phenyl-MTPA), 7.44 (3H, m, phenyl-MTPA), 5.53 (1H, q, J = 6.6 Hz, H-4), 5.19 (1H, brs, H-21), 4.29 (1H, d, J = 12.0 Hz, H-24b), 3.81 (1H, d, J = 12.0 Hz, H-24a), 1.36 (3H, d, J = 6.6 Hz, CH_3-23); ^{1}H NMR (C_5D_5N, 300 MHz) δ 6.07 (1H, m, H-4), 4.44 (1H, d, J = 12.3 Hz, H-24b), 3.98 (1H, d, J = 12.3 Hz, H-24a), 1.51 (3H, d, J = 6.3 Hz, CH_3-23).

(*R*)-MTPA ester of galphimine E (13r): white amorphous powder; $[\alpha]^{25}_D$ +12° (c 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.52 (2H, m, phenyl-MTPA), 7.44 (3H, m, phenyl-MTPA), 5.81 (1H, q, J = 6.6 Hz, H-4), 5.16 (1H, brs, H-21), 4.36 (1H, d, J = 11.5 Hz, H-24b), 4.23 (1H, d, J = 11.5 Hz, H-24a), 1.41 (3H, d, J = 6.6 Hz, CH_3-23); ^{1}H NMR (C_5D_5N, 300 MHz) δ 6.38 (1H, q, J = 6.6 Hz, H-4), 4.64 (1H, d, J = 12.3 Hz, H-24b), 4.48 (1H, d, J = 12.3 Hz, H-24a), 1.70 (3H, d, J = 6.6 Hz, CH_3-23).

(*S*)-MTPA ester of galphimine E (13s): white amorphous powder; $[\alpha]^{25}_D$ −14° (c 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.52 (2H, m, phenyl-MTPA), 7.44 (3H, m, phenyl-MTPA), 5.71 (1H, q, J = 6.3 Hz, H-4), 4.27 (1H, d, J = 12.3 Hz, H-24b), 4.18 (1H, d, J = 12.3 Hz, H-24a), 1.48 (3H, d, J = 6.3 Hz, CH_3-23); ^{1}H NMR (C_5D_5N, 300 MHz) δ 6.27 (1H, q, J = 6.0 Hz,

H-4), 4.55 (1H, d, J = 12.3 Hz, H-24b), 4.44 (1H, d, J = 12.3 Hz, H-24a), 1.77 (3H, d, J = 6.0 Hz, CH_3-23).

Molecular Modeling Calculations. The molecular mechanics minimum energy structure of galphimine A (**3**) was generated using the MMX force field as implemented in the PCMODEL molecular modeling program V 6.00 (Serena Software, Box 3076, Bloomington, IN 47402-3076). The X-ray Cartesian coordinates of galphimine B (**1**)[3] were used as the starting point for the molecular modeling calculations. A systematic conformational search for all the rings of **3**, according to Dreiding models, was achieved considering dihedral angle rotations of ca. 20° for those bonds that allowed such a movement. The E_{MMX} values were monitored throughout the calculation process.

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Supporting Information Available: Chromatographic profiles (HPLC and TLC) of the galphimine mixture investigated and NMR spectra for galphimine ring E double-bond isomers. This information is available free of charge via the Internet at http://www.pubs.acs.org.

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