ACS OMEGA

Open Access

http://pubs.acs.org/journal/acsodf

Article

Asymmetric Synthesis of Four Stereoisomers of 2,2-Dimethyl-3-hydroxy-4-(1′-angeloyloxy)-6-acetylchromane from *Ageratina grandifolia* and Plausible Absolute Stereochemistry of the Natural Product

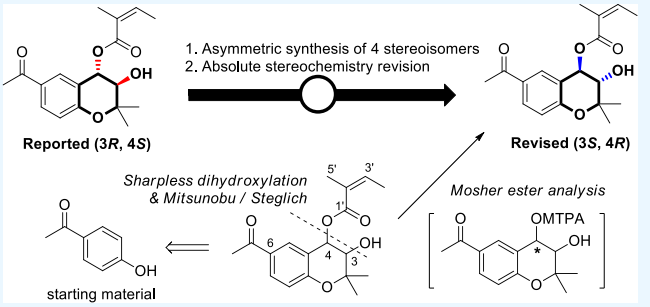
Changmin Oh, Ji Hyeon Im, Munhyung Bae, and Jong-Wha Jung*

Cite This: *ACS Omega* 2023, 8, 37384−37390

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: 2,2-Dimethyl-3-hydroxy-4-(1′-angeloyloxy)-6-acetylchromane is a natural product isolated from *Ageratina grandifolia* that exhibits inhibitory activity against yeast α-glucosidase. Initially, its structure was proposed to be 4-hydroxy-3-((*S*)-1′-angeloyloxy-(*R*)-2′,3′-epoxy-3′-methyl)butylacetophenone with an epoxide, but the structure was later revised to 2,2-dimethyl-3*R*-hydroxy-4*S*-(1-angeloyloxy)-6-acetylchromane. In this study, we present a total synthesis of 2,2-dimethyl-3-hydroxy-4-(1′-angeloyloxy)-6-acetylchromane from *A. gradifolia* and its stereoisomers. The key features of their synthesis include Sharpless asymmetric dihydroxylation of a readily available benzopyran substrate and subsequent Mitsunobu or Steglich reaction to provide both cis- and trans-isomers with chiral control. The absolute stereochemistry of the natural product was determined to be 2,2-dimethyl-3*S*-hydroxy-4*R*-(1′-angeloyloxy)-6-acetylchromane based on optical rotations of the synthesized compounds. The absolute configuration of the synthesized stereoisomers was confirmed by Mosher ester analysis. In addition, we provided ECD spectra for the four stereoisomers, which will allow verification of the absolute configuration of the natural product. Synthesis of all four stereoisomers of 2,2-dimethyl-3-hydroxy-4-(1′-angeloyloxy)-6-acetylchromane would facilitate the exploration of their potential biomedical applications.



INTRODUCTION

Computational nuclear magnetic resonance (NMR) methods have emerged as a convenient strategy for determining the structures of natural products.[1] Machine learning and artificial intelligence have further advanced computational prediction by simulating numerous complex molecules.[2] However, structural revisions are still not uncommon, and the revision and confirmation of the molecular architectures of natural products is fundamental for the discovery of novel bioactive compounds.[3] *Ageratina grandifolia* is a perennial herb used as a traditional remedy for treating stomach discomfort and skin afflictions.[4] 2,2-Dimethyl-3-hydroxy-4-(1′-angeloyloxy)-6-acetylchromane is a natural product isolated from the aerial parts of *A. grandifolia*, which exhibits inhibitory activity against yeast α-glucosidase.[5] Initially, its structure was proposed to be 4-hydroxy-3-((*S*)-1′-angeloyloxy-(*R*)-2′,3′-epoxy-3′-methyl)-butylacetophenone by Mata et al., and hereafter, Mata et al. revised the structure to 2,2-dimethyl-3*R*-hydroxy-4*S*-(1-angeloyloxy)-6-acetylchromane (Figure 1, **1a**).[6] The chemical structure of the natural product, including the absolute configuration, was determined based on the experimental NMR values, density functional theory (DFT) shielding tensors, and DP4+/J-DP4 statistical analyses.[6]

Navarro-Vázquez also revised the structure to *rel*-2,2-dimethyl-3*R*-hydroxy-4*S*-angeloyloxy-6-acetylchromane, independently.[7] Notably, Navarro-Vázquez calculated a conformationally averaged $[\alpha]_D$ value of −5.1 for 2,2-dimethyl-3*R*-hydroxy-4S-angeloyloxy-6-acetylchromane based on computed optical rotations,[7] although the originally reported $[\alpha]_D$ value for the natural product was +25.8.[5] Navarro-Vázquez noted that assigning an absolute configuration to the natural product would require detailed chiroptical studies.[7]

Owing to our interest in bioactive prenylated phenols and related compounds, such as chromanes, we employed a classical approach referred to as structural confirmation by the synthesis. Four stereoisomers of 2,2-dimethyl-3-hydroxy-4-(1′-angeloyloxy)-6-acetylchromane were synthesized asymmetrically to elucidate the chemical structure, including absolute configurations of C3 and C4 stereogenic carbons.

Received: July 24, 2023
Accepted: September 8, 2023
Published: September 26, 2023

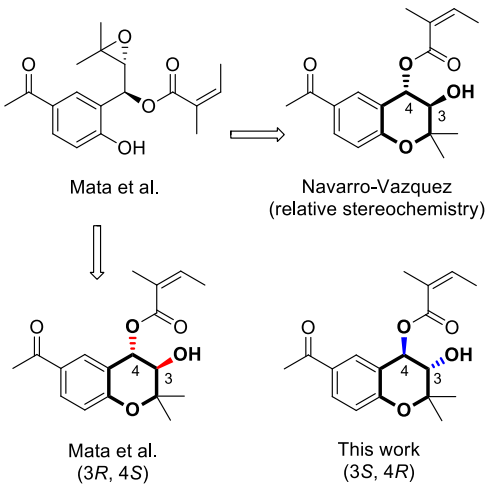


Figure 1. Proposed structures of the natural product from *A. grandifolia*.

The synthesis provided facile access to the natural product and its stereoisomers. In this study, we present the synthesis and the revision of the absolute stereochemistry of 2,2-dimethyl-3-hydroxy-4-(1′-angeloyloxy)-6-acetylchromane, a natural product obtained from *A. grandifolia*.[8]

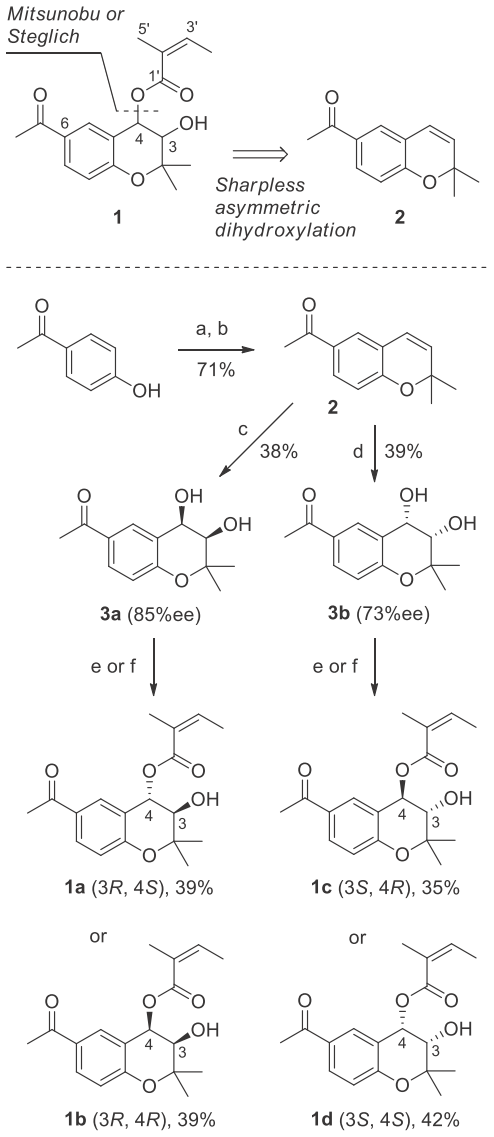
RESULTS AND DISCUSSION

The synthetic plan for the four stereoisomers of 2,2-dimethyl-3-hydroxy-4-(1′-angeloyloxy)-6-acetylchromane **1** is illustrated in Figure 1. The Sharpless asymmetric dihydroxylation was used to achieve enantioselective synthesis, thereby resulting in *cis*-hydroxyl groups at C3 and C4 starting from the benzopyran substrate **2**.[9] Subsequently, the stereochemistry at C4 was inverted through a Mitsunobu reaction[10] using an appropriate carboxylic acid, yielding trans-configuration for C3 and C4 in compounds **1a** or **1c**. Conversely, to maintain *cis*-configuration for C3 and C4 in compounds **1b** or **1d**, we employed the Steglich reaction[11] for selective esterification of *cis*-diol. The Sharpless asymmetric dihydroxylation,[12] Mitsunobu,[13] and Steglich[14] reactions are well-established methods known for their reliability in terms of reactivity and chiral control.

Our synthesis commenced with the preparation of benzopyran **2** as shown in Scheme 1. Commercially available 4-hydroxyacetophenone was reacted with 3-chloro-3-methyl-1-butyne to obtain the corresponding α,α-dimethyl propargyl ether. Subsequent cyclization upon heating resulted in the formation of benzopyran **2**. Afterward, enantiomerically enriched *cis*-diol **3a** and **3b** were successfully synthesized through osmium-catalyzed asymmetric dihydroxylation[9] using chiral ligands $(DHQ)_2PHAL$ and $(DHQD)_2PHAL$, respectively. The absolute configurations of *cis*-diol **3a** and **3b** were tentatively assigned using Sharpless's mnemonic device, and enantiomeric excesses were determined by chiral high-performance liquid chromatography (HPLC) analysis (Supporting Information). Notably, asymmetric dihydroxylation of 2,2-dimethyl-2*H*-chromene was included in Sharpless's original paper.[9]

Mitsunobu reaction of **3a** with angelic acid in the presence of PPh_3 and DIAD led to the inversion of stereochemistry at C4, in turn, yielding the desired 2,2-dimethyl-3*R*-hydroxy-4*S*-angeloyloxy-6-acetylchromane (**1a**). Steglich reaction of **3a** with angelic acid using DCC and DMAP yielded 2,2-dimethyl-3*R*-hydroxy-4*R*-angeloyloxy-6-acetylchromane (**1b**). Similar

Scheme 1. Synthetic Plan and Asymmetric Synthesis of Four Stereoisomers of 2,2-Dimethyl-3-hydroxy-4-(1-angeloyloxy)-chromane[a]



[a]Reagents and conditions: (a) 3-chloro-3-methyl-1-butyne, K_2CO_3, KI, CuI, acetone, 80 °C; (b) dimethylformamide (DMF), 155 °C; (c) $(DHQ)_2PHAL$, $K_2OsO_2(OH)_4$, $K_3Fe(CN)_6$, K_2CO_3, $MeSO_2NH_2$, *t*-BuOH, H_2O, 0 °C; (d) $(DHQD)_2PHAL$, $K_2OsO_2(OH)_4$, $K_3Fe(CN)_6$, K_2CO_3, $MeSO_2NH_2$, *t*-BuOH, H_2O, 0 °C; (e) angelic acid, PPh_3, DIAD, tetrahydrofuran (THF), 0 °C to rt; (f) angelic acid, DCC, DMAP, CH_2Cl_2, 0 °C.

synthetic approaches were employed to convert *cis*-diol **3b** to 2,2-dimethyl-3*S*-hydroxy-4*R*-angeloyloxy-6-acetylchromane (**1c**) and 2,2-dimethyl-3*S*-hydroxy-4*S*-angeloyloxy-6-acetylchromane (**1d**) under Mitsunobu and Steglich reactions, respectively.

After obtaining the four requisite stereoisomers, **1a**–**d**, we compared ^1H and ^{13}C NMR spectra of the natural product reported and synthetic **1a**–**d** to confirm its chemical structure (Tables S1 and S2, Supporting Information). The NMR spectra of **1a** and **1c** were in good accordance with those reported.[6] However, the optical rotation of **1a** ($[\alpha]_D^{24} = -56.3$, c 0.22, $CHCl_3$) contrasted with that of the natural product ($[\alpha]_D^{25} = +25.8$, c 1, $CHCl_3$) whose structure was proposed to

https://doi.org/10.1021/acsomega.3c05349
ACS Omega 2023, 8, 37384−37390

be 2,2-dimethyl-3*R*-hydroxy-4*S*-angeloyloxy-6-acetylchromane (**1a**) (Table 1). Consequently, we revised the structure of the natural product to 2,2-dimethyl-3*S*-hydroxy-4*R*-angeloyloxy-6-acetylchromane (**1c**) based on the optical rotations. Although Sharpless asymmetric dihydroxylation has been applied extensively in the asymmetric synthesis of various chiral compounds and its efficiency and reliability have demonstrated that it is one of the most predictable methods for chiral control, we aimed to verify the production of the desired chiral compounds.

Table 1. Comparison of Optical Rotations of the Natural Products and Synthetic 1a−d

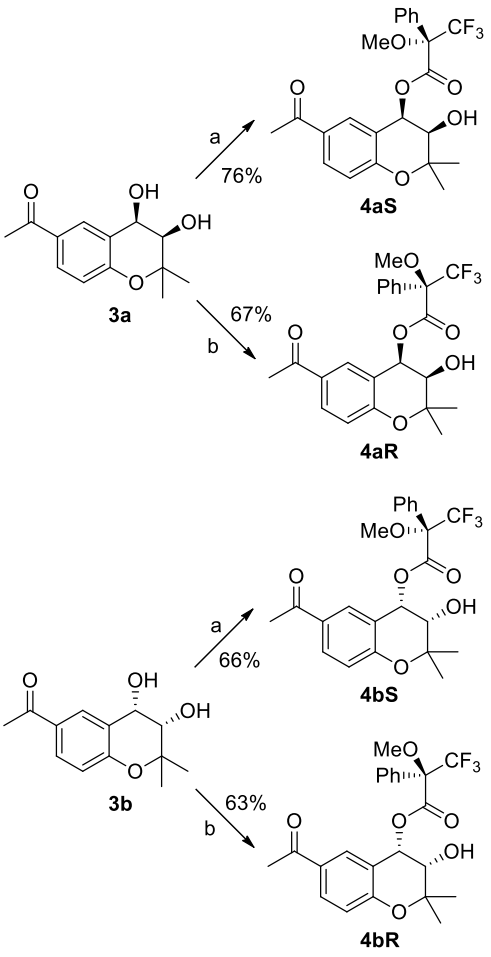
compound	absolute configuration at C3, C4	optical rotation
natural product (reported)	3*R*, 4*S*	$[\alpha]_D^{25}$ = +25.8 (*c* 1, $CHCl_3$)
1a	3*R*, 4*S*	$[\alpha]_D^{24}$ = −56.3 (*c* 0.22, $CHCl_3$)
1b	3*R*, 4*R*	$[\alpha]_D^{25}$ = +37.8 (*c* 0.16, $CHCl_3$)
1c	3*S*, 4*R*	$[\alpha]_D^{24}$ = +54.2 (*c* 0.22, $CHCl_3$)
1d	3*S*, 4*S*	$[\alpha]_D^{25}$ = −31.4 (*c* 0.25, $CHCl_3$)

Therefore, we conducted the Mosher ester analysis[15] to confirm the absolute configurations of Sharpless dihydroxylation products, *cis*-diol **3a** and **3b**. *cis*-Diol **3a** was treated with Mosher's acid chlorides, (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride, or (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride, which resulted in the formation of **4aS** and **4aR** esters, respectively (Scheme 2). Similarly, *cis*-diol **3b** was converted to **4bS** and **4bR** esters. Finally, the configurations of both *cis*-diol **3a** and **3b** were verified by comparative analysis of the ^{1}H NMR spectral data of **4aS**, **4aR**, **4bS**, and **4bR** (Tables S3).

Data from the ECD experiments and calculations also supported the assigned absolute configurations for **1a−d**. The energy minimized conformers of **1a−d** were calculated, and the ECD spectra for the conformers were calculated using time-dependent DFT calculations at the B3LYP/def-SV(P)//B3LYP/def-SV(P) level for all atoms (see Supporting Information, Tables S4−S7). The experimental ECD spectra of **1a−d** were consistent with the calculated ECD spectra of **1a−d** (Figure 2).

Finally, we tested the effects of four stereoisomers on several enzymes, including butyrylcholinesterase, acetylcholinesterase, and monoamineoxidase (MAO)-A and B, associated with central nervous system disorders. As a result, **1c** selectively inhibited MAO-B by 21.1% at 10 μM (Supporting Information, Figure S1). MAO-B inhibitors are used in the treatment of Parkinson's and Alzheimer's diseases while both MAO-A and -B inhibitors are used against anxiety and depression.[16] Clinically available MAO-B inhibitors suffer from significant side effects such as nausea, lightheadedness, headache, confusion, and hallucinations,[17] and there is an unmet medical need to develop novel MAO-B inhibitors without undesirable adverse effects. Although ours is a preliminary result, it shows potential use of these compounds for biomedical applications.

Scheme 2. Mosher Ester Synthesis From *cis*-Diol 3a and 3b[a]



[a]Reagents and conditions: (a) (*R*)-MTPA-Cl, pyridine, $CHCl_3$; (b) (*S*)-MTPA-Cl, pyridine, $CHCl_3$.

EXPERIMENTAL SECTION

General Experimental Procedure. Materials and reagents were purchased from Sigma-Aldrich, Alfa Aesar, and TCI, and used as received unless stated otherwise. Air- and moisture-sensitive reactions were conducted under Ar. Reactions were monitored by analytical thin layer chromatography using 0.25 mm silica gel plates (Merck & Co., Inc., Kenilworth, NJ, USA). Flash column chromatography was performed using Isolera (Biotage, Uppsala, Sweden) or manually using silica gel (260−400 mesh size; Merck & Co., Inc., Kenilworth, NJ, USA). NMR spectra were recorded by a spectrometer (Avance 500 MHz; Bruker Instruments Inc., Billerica, MA, USA) using deuterated solvents. Low-resolution mass spectra (MS) were recorded using Expression CMS (Advion, Ithaca, NY, USA), whereas high-resolution MS were recorded using a high-resolution mass spectrometer (JEOL JMS-700; Korean Basic Science Institute, Daegu, Korea). Enantiomeric ratios were determined by chiral HPLC using a chiral-phase column (Chiral ART Cellulose SC; YMC America, Devens, MA, USA). Optical rotations were recorded at the wavelength of sodium D-line (λ 589 nm) using a P-2000 polarimeter (JASCO, Tokyo, Japan).

1-(2,2-Dimethyl-2*H*-chromen-6-yl)ethan-1-one (2). 3-Chloro-3-methyl-1-butyne (3.9 mL, 35 mmol) was added dropwise to a stirred solution of 4′-hydroxyacetophenone

https://doi.org/10.1021/acsomega.3c05349
ACS Omega 2023, 8, 37384−37390

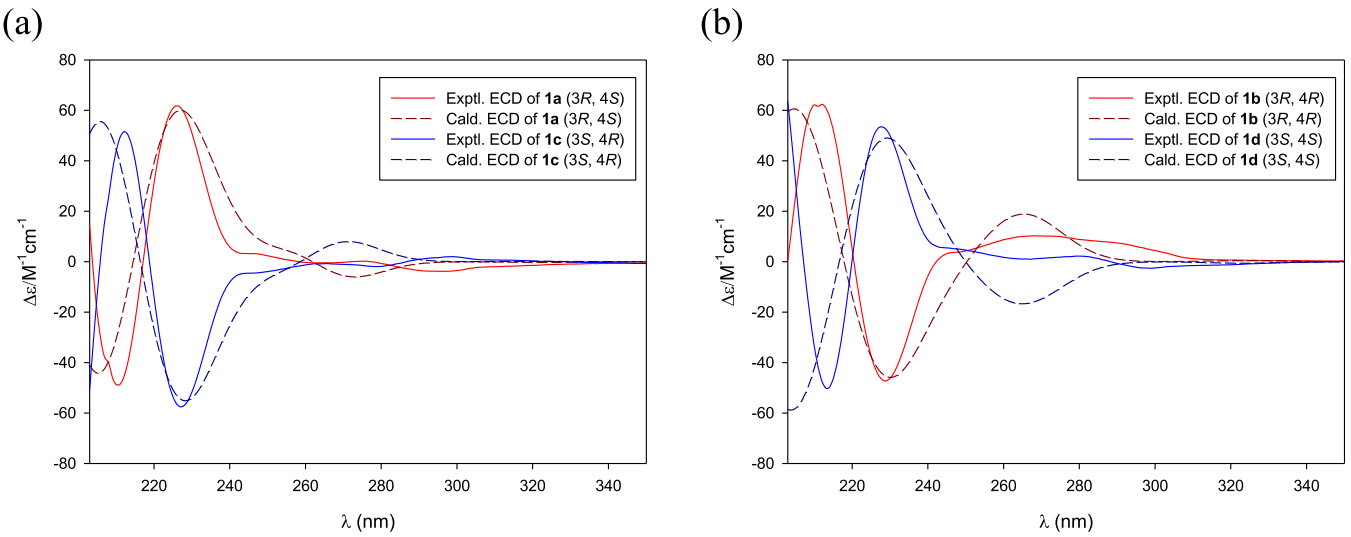


Figure 2. Experimental and calculated ECD spectra of (a) **1a** vs **1c** and (b) **1b** vs **1d**.

(1.361 g, 10 mmol), potassium carbonate (4.146 g, 30 mmol), potassium iodide (2.772 g, 16.7 mmol), and copper(I) iodide (38 mg, 0.2 mmol) in acetone (30 mL). The reaction mixture was refluxed at 80 °C for 3 h and then cooled. Afterward, an aqueous solution of 1*N* sodium hydroxide was added to the mixture and extracted with ethyl acetate. The combined organic phase was washed with brine, dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was passed through a silica gel plug (eluted in hexanes/ethyl acetate = 4:1) to obtain 1-(4-((2-methylbut-3-yn-2-yl)oxy)phenyl)ethan-1-one as a yellow oil (1.602 g) after drying in vacuo. The yellow oil was used for the subsequent step without further purification. Rf = 0.52 (hexanes/ethyl acetate = 4:1); ^{1}H NMR (500 MHz, CD_3OD): δ 7.92 (d, J = 9.0 Hz, 2H), 7.27 (d, J = 8.9 Hz, 2H), 3.13 (s, 1H), 2.53 (s, 3H), 1.67 (s, 6H); ^{13}C NMR (126 MHz, CD_3OD): δ 199.4, 161.6, 132.2, 131.1, 120.2, 86.0, 76.6, 73.6, 30.0, 26.5; ESIMS m/z 203.4 $[M + H]^+$.

The yellow oil was dissolved in DMF (50 mL). The reaction mixture was stirred at 155 °C for 15 h, cooled, and extracted with ethyl acetate. The combined organic phase was washed with brine, dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to obtain 1-(2,2-dimethyl-2*H*-chromen-6-yl)ethan-1-one (**2**, 1.432 g, 70.8% for two steps) as a light yellow oil. Rf = 0.52 (hexanes/ethyl acetate = 4:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.74 (dd, J = 8.5, 2.2 Hz, 1H), 7.61 (d, J = 2.2 Hz, 1H), 6.78 (d, J = 8.0 Hz, 1H), 6.35 (d, J = 9.6 Hz, 1H), 5.66 (d, J = 9.9 Hz, 1H), 2.53 (s, 3H), 1.45 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 196.9, 157.6, 131.3, 130.5, 130.4, 127.0, 121.8, 120.8, 116.3, 77.7, 28.5, 26.4; HREIMS m/z 202.0993 $[M]^+$ (calcd for $C_{13}H_{14}O_2$, 202.0994).

1-((3*R*,4*R*)-3,4-Dihydroxy-2,2-dimethylchroman-6-yl)-ethan-1-one (3a). A mixture of $K_3Fe(CN)_6$ (2.23 g, 6.77 mmol), K_2CO_3 (936 mg, 6.77 mmol), $(DHQ)_2PHAL$ (36 mg, 0.045 mmol), and $K_2OsO_2(OH)_4$ (17 mg, 0.045 mmol) was dissolved in *t*-BuOH (15 mL) and H_2O (15 mL). The solution was cooled to 0 °C, and methanesulfonamide (215 mg, 2.26 mmol) was added to the solution, and stirred. A solution of **2** (456 mg, 2.26 mmol) in *t*-BuOH (8 mL) and H_2O (8 mL) was added to the mixture after 15 min. The reaction mixture was vigorously stirred at 0 °C for 4.5 days. The reaction was quenched with an aqueous $Na_2S_2O_5$ solution and then extracted with $CHCl_3$. The combined organic phase was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to obtain 1-((3*R*,4*R*)-3,4-dihydroxy-2,2-dimethylchroman-6-yl)ethan-1-one (**3a**, 201 mg, 37.6, 85% ee) as a white solid; Rf = 0.20 (hexanes/ethyl acetate = 1:1); [chiral-phase HPLC analysis] T_R = 5.319 min (YMC Chiral Art Cellulose-SZ, 4.6 mm i.d. × 75 mm, 3 μm), 15% *i*-propanol in hexane, flow rate = 0.7 mL/min); Enantiomeric excess = 85% ee. $[\alpha]_D^{25}$ = +77.8 (c 0.04, $CHCl_3$); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.06 (d, J = 1.4 Hz, 1H), 7.73 (dd, J = 8.5, 2.3 Hz, 1H), 6.76 (d, J = 8.5 Hz, 1H), 5.35 (d, J = 6.8 Hz, 1H), 4.98 (d, J = 3.9 Hz, 1H), 4.74 (t, J = 4.8 Hz, 1H), 3.62 (t, J = 3.4 Hz, 1H), 2.49 (s, 3H), 1.38 (s, 3H), 1.22 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6): δ 196.2, 157.0, 129.5, 129.2, 128.8, 124.1, 115.8, 79.2, 69.9, 63.7, 26.3, 24.7, 24.4. HREIMS m/z 236.1048 $[M]^+$ (calcd for $C_{13}H_{16}O_4$, 236.1049).

1-((3*S*,4*S*)-3,4-Dihydroxy-2,2-dimethylchroman-6-yl)-ethan-1-one (3b). A mixture of $K_3Fe(CN)_6$ (3.292 g, 10 mmol), K_2CO_3 (1.382 g, 10 mmol), $(DHQD)_2PHAL$ (53 mg, 0.067 mmol), and $K_2OsO_2(OH)_4$ (25 mg, 0.067 mmol) was dissolved in *t*-BuOH (20 mL) and H_2O (20 mL). The solution was cooled to 0 °C, and methanesulfonamide (317 mg, 3.333 mmol) was added to the solution, and stirred. A solution of **2** (674 mg, 3.333 mmol) in *t*-BuOH (13 mL) and H_2O (13 mL) was added to the mixture after 15 min. The reaction mixture was vigorously stirred at 0 °C for 4.5 days. The reaction was quenched with aqueous $Na_2S_2O_5$ solution and then extracted with $CHCl_3$. The combined organic phase was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to obtain 1-((3*S*,4*S*)-3,4-dihydroxy-2,2-dimethylchroman-6-yl)ethan-1-one (**3b**, 306 mg, 38.9%, 73% ee) as a white solid; Rf = 0.20 (hexanes/ethyl acetate = 1:1); [chiral-phase HPLC analysis] T_R = 8.069 min (YMC Chiral Art Cellulose-SZ, 4.6 mm i.d. × 75 mm, 3 μm), 15% *i*-propanol in hexane, flow rate = 0.7 mL/min); Enantiomeric excess = 73% ee. $[\alpha]_D^{25}$ = −61.5 (c 0.11, $CHCl_3$); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.06 (d, J = 1.3 Hz, 1H), 7.73 (dd, J

https://doi.org/10.1021/acsomega.3c05349
ACS Omega 2023, 8, 37384−37390

= 8.5, 2.1 Hz, 1H), 6.76 (d, J = 8.5 Hz, 1H), 5.34 (d, J = 7.2 Hz, 1H), 4.97 (d, J = 4.2 Hz, 1H), 4.74 (dd, J = 7.1, 3.9 Hz, 1H), 3.62 (t, J = 3.9 Hz, 1H), 2.49 (s, 3H), 1.38 (s, 3H), 1.22 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6): δ 196.1, 157.0, 129.5, 129.2, 128.8, 124.1, 115.8, 79.2, 69.9, 63.7, 26.3, 24.7, 24.4; HREIMS m/z 236.1051 $[M]^+$ (calcd for $C_{13}H_{16}O_4$, 236.1049).

(3*R*,4*S*)-2,2-Dimethyl-3-hydroxy-4-(1′-angeloyloxy)-6-acetylchromane (1a). Diisopropyl azodicarboxylate (0.075 mL, 0.381 mmol) was added to a triphenylphosphine solution (100 mg, 0.381 mmol) in THF (1.3 mL). After stirring for 30 min at 0 °C, **3a** (30 mg, 0.127 mmol) was added to the solution and then stirred for 15 min at 0 °C. Thereafter, angelic acid (115 mg, 1.143 mmol) was added to the solution and stirred for another 15 min at 0 °C. The reaction mixture was stirred for 2.5 h at room temperature. Volatiles were removed under reduced pressure and the residue was purified by silica gel column chromatography to obtain (3*R*,4*S*)-2,2-dimethyl-3-hydroxy-4-(1′-angeloyloxy)-6-acetylchromane (**1a**, 15.7 mg, 38.8%) as a colorless oil; Rf = 0.5 (hexanes/ethyl acetate = 2:1); $[\alpha]_D^{24}$ = −56.3 (c 0.22, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.87 (dd, J = 2.2 Hz, 0.9 Hz, 1H), 7.84 (dd, J = 8.6, 2.1 Hz, 1H), 6.89 (d, J = 8.6 Hz, 1H), 6.24 (qq, J = 7.3, 1.5 Hz, 1H), 5.96 (d, J = 7.6 Hz, 1H), 3.88 (dd, J = 7.5 Hz, 3.6 Hz, 1H), 3.38 (d, J = 4.2 Hz, 1H), 2.52 (s, 3H), 2.05 (dq, J = 7.3, 1.5 Hz, 3H), 1.94 (quin, J = 1.5 Hz, 3H), 1.51 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 196.6, 169.6, 157.3, 141.3, 130.6, 129.9, 127.0, 119.7, 117.6, 79.8, 74.3, 71.9, 26.4, 26.1, 20.7, 19.9, 16.2; HREIMS m/z 318.1470 $[M]^+$ (calcd for $C_{18}H_{22}O_5$, 318.1467).

(3*R*,4*R*)-2,2-Dimethyl-3-hydroxy-4-(1′-angeloyloxy)-6-acetylchromane (1b). Angelic acid (76.3 mg, 0.762 mmol), DMAP (12.4 mg, 0.102 mmol), and DCC (157 mg, 0.762 mmol) were subsequently added to a stirred solution of **3a** (60 mg, 0.254 mmol). The reaction mixture was stirred for 1.5 days at 0 °C. The white precipitate that formed was then filtered off and washed with CH_2Cl_2. The combined filtrate was dried under reduced pressure. The residue was purified by silica gel column chromatography to obtain (3*R*,4*R*)-2,2-dimethyl-3-hydroxy-4-(1′-angeloyloxy)-6-acetylchromane (31.1 mg, 38.5%) as a colorless oil. Rf = 0.46 (hexanes/ethyl acetate = 2:1); $[\alpha]_D^{25}$ = +37.8 (c 0.16, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.91 (dd, J = 2.2 Hz, 1.0 Hz, 1H), 7.85 (dd, J = 8.6, 2.1 Hz, 1H), 6.90 (d, J = 8.6 Hz, 1H), 6.23 (qq, J = 7.3, 1.3 Hz, 1H), 6.19 (d, J = 4.1 Hz, 1H), 4.05 (d, J = 4.1 Hz, 1H), 2.52 (s, 3H), 2.16 (br s, 1H), 2.07 (dq, J = 7.3, 1.6 Hz, 3H), 1.97 (quin, J = 1.5 Hz, 3H), 1.51 (s, 3H), 1.41 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 196.6, 167.8, 157.5, 140.9, 130.6, 130.5, 130.1, 127.1, 118.2, 117.6, 78.9, 69.7, 67.6, 26.4, 24.7, 23.8, 20.8, 16.2; HREIMS m/z 318.1464 $[M]^+$ (calcd for $C_{18}H_{22}O_5$, 318.1467).

(3*S*,4*R*)-2,2-Dimethyl-3-hydroxy-4-(1′-angeloyloxy)-6-acetylchromane (1c). 1c was synthesized in analogy to **1a**, except that **3b** was used instead of **3a**, which yielded a colorless oil (34.6%); Rf = 0.5 (hexanes/ethyl acetate = 2:1); $[\alpha]_D^{24}$ = +54.2 (c 0.22, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.88 (dd, J = 2.2 Hz, 0.9 Hz, 1H), 7.84 (dd, J = 8.6, 2.1 Hz, 1H), 6.89 (d, J = 8.6 Hz, 1H), 6.24 (qq, J = 7.3, 1.3 Hz, 1H), 5.96 (d, J = 7.6 Hz, 1H), 3.88 (d, J = 7.6 Hz, 1H), 3.32 (br s, 1H), 2.52 (s, 3H), 2.05 (dq, J = 7.3, 1.6 Hz, 3H), 1.94 (quin, J = 1.5 Hz, 3H), 1.51 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 196.6, 169.7, 157.4, 141.3, 130.6, 130.6, 129.9, 127.0, 119.7, 117.7, 79.8, 74.3, 72.0, 26.4, 26.1, 20.7, 19.9, 16.2; HREIMS m/z 318.1466 $[M]^+$ (calcd for $C_{18}H_{22}O_5$, 318.1467).

(3*S*,4*S*)-2,2-Dimethyl-3-hydroxy-4-(1′-angeloyloxy)-6-acetylchromane (1d). 1d was synthesized in analogy to **1b**, except that **3b** was used instead of **3a**, which yielded a colorless oil (42.0%); Rf = 0.46 (hexanes/ethyl acetate = 2:1); $[\alpha]_D^{25}$ = −31.4 (c 0.25, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.91 (dd, J = 2.2 Hz, 1.0 Hz, 1H), 7.85 (dd, J = 8.6, 2.0 Hz, 1H), 6.90 (d, J = 8.6 Hz, 1H), 6.23 (qq, J = 7.2, 1.4 Hz, 1H), 6.18 (d, J = 4.2 Hz, 1H), 4.05 (d, J = 4.1 Hz, 1H), 2.52 (s, 3H), 2.20 (br s, 1H), 2.06 (dq, J = 7.3, 1.6 Hz, 3H), 1.97 (quin, J = 1.6 Hz, 3H), 1.50 (s, 3H), 1.41 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 196.6, 167.8, 157.5, 140.9, 130.6, 130.5, 130.1, 127.1, 118.2, 117.6, 78.9, 69.6, 67.6, 26.4, 24.7, 23.8, 20.8, 16.2; HREIMS (EI) m/z 318.1470 $[M]^+$ (calcd for $C_{18}H_{22}O_5$, 318.1467).

(3*R*,4*R*)-6-Acetyl-3-hydroxy-2,2-dimethylchroman-4-yl (*S*)-3,3,3-Trifluoro-2-methoxy-2-phenylpropanoate (4aS). *R*-(−)-MTPA-Cl (10 μL, 0.053 mmol) was added to a stirred solution of diol **3a** (10 mg, 0.042 mmol) and pyridine (14 μL, 0.176 mmol) in CH_2Cl_2 (300 μL), and the mixture was stirred for 6 h at room temperature. Afterward, the reaction mixture was quenched with water and extracted with diethyl ether. The combined organic layers were dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography to obtain **4aS** (14.4 mg, 75.7%); Rf = 0.48 (hexanes/ethyl acetate = 2:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.89−7.83 (m, 2H), 7.62−7.56 (m, 2H), 7.46−7.40 (m, 3H), 6.89 (d, J = 9.4 Hz, 1H), 6.29 (d, J = 4.1 Hz, 1H), 4.05 (dd, J = 5.9, 4.2 Hz, 1H), 3.62 (s, 3H), 2.47 (s, 3H), 1.87 (d, J = 6.0 Hz, 1H), 1.41 (s, 3H), 1.36 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 196.2, 167.1, 157.5, 132.0, 131.0, 130.7, 130.5, 130.1, 128.9, 127.3, 126.9, 124.6, 122.3, 120.0, 118.0, 116.9, 85.3, 85.0, 84.8, 84.6, 78.8, 70.3, 69.9, 55.8, 26.3, 25.1, 22.8; HREIMS (EI) m/z 452.1450 $[M]^+$ (calcd for $C_{23}H_{23}F_3O_6$, 452.1447).

(3*R*,4*R*)-6-Acetyl-3-hydroxy-2,2-dimethylchroman-4-yl (*R*)-3,3,3-Trifluoro-2-methoxy-2-phenylpropanoate (4aR). *S*-(−)-MTPA-Cl (10 μL, 0.053 mmol) was added to a stirred solution of diol **3a** (10 mg, 0.042 mmol) and pyridine (14 μL, 0.176 mmol) in CH_2Cl_2 (300 μL) and stirred for 6 h at room temperature. Thereafter, the reaction mixture was quenched with water and extracted with diethyl ether. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography to obtain **4aR** (12.8 mg, 67.4%) as a colorless oil; Rf = 0.50 (hexanes/ethyl acetate = 2:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.85 (dd, J = 8.7, 2.2 Hz, 1H), 7.68−7.63 (m, 2H), 7.59−7.58 (m, 1H), 7.48−7.42 (m, 3H), 6.88 (d, J = 8.7 Hz, 1H), 6.24 (d, J = 4.2 Hz, 1H), 4.15 (d, J = 4.3 Hz, 1H), 3.63 (s, 3H), 2.34 (s, 3H), 1.99 (br s, 1H), 1.45 (s, 3H), 1.42 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 196.3, 167.1, 157.1, 132.1, 130.8, 130.6, 130.4, 130.1, 128.8, 127.4, 126.9, 124.6, 122.3, 120.0, 117.9, 116.4, 85.4, 85.2, 84.9, 84.7, 79.0, 70.3, 69.4, 55.8, 26.3, 24.6, 23.5; HREIMS (EI) m/z: 452.1442 $[M]^+$ (calcd for $C_{23}H_{23}F_3O_6$, 452.1447).

(3*S*,4*S*)-6-Acetyl-3-hydroxy-2,2-dimethylchroman-4-yl (*S*)-3,3,3-Trifluoro-2-methoxy-2-phenylpropanoate (4bS). **4bS** was synthesized from diol **3b** (10 mg, 0.042 mmol) using the same procedure for synthesizing **4aS**; 12.5 mg, 65.8%; colorless oil; Rf = 0.50 (hexanes/ethyl acetate = 2:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.85 (dd, J = 8.7, 2.2 Hz,

https://doi.org/10.1021/acsomega.3c05349
ACS Omega 2023, 8, 37384−37390

1H), 7.69−7.63 (m, 2H), 7.59−7.58 (m, 1H), 7.48−7.42 (m, 3H), 6.88 (d, J = 8.7 Hz, 1H), 6.24 (d, J = 4.2 Hz, 1H), 4.15 (dd, J = 7.1, 4.3 Hz, 1H), 3.63 (s, 3H), 2.34 (s, 3H), 1.99 (d, J = 7.3 Hz, 1H), 1.45 (s, 3H), 1.42 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 196.3, 167.1, 157.1, 132.1, 130.8, 130.6, 130.4, 130.1, 128.8, 127.4, 126.9, 124.6, 122.3, 120.0, 117.9, 116.4, 85.4, 85.2, 85.0, 84.7, 79.0, 70.3, 69.4, 55.8, 26.3, 24.6, 23.5; HREIMS m/z 452.1451 $[M]^+$ (calcd for $C_{23}H_{23}F_3O_6$, 452.1447).

(3*S*,4*S*)-6-Acetyl-3-hydroxy-2,2-dimethylchroman-4-yl (*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (4bR). **4bR** was synthesized from diol **3b** (10 mg, 0.042 mmol) using the same procedure for synthesizing **4aR**; 12 mg, 63.1%; colorless oil; Rf = 0.48 (hexanes/ethyl acetate = 2:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.89−7.84 (m, 2H), 7.62−7.56 (m, 2H), 7.46−7.40 (m, 3H), 6.89 (d, J = 9.3 Hz, 1H), 6.29 (d, J = 4.2 Hz, 1H), 4.05 (d, J = 4.2 Hz, 1H), 3.62 (s, 3H), 2.47 (s, 3H), 1.85 (br s, 1H), 1.41 (s, 3H), 1.36 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 196.2, 167.1, 157.5, 132.0, 131.0, 130.7, 130.5, 130.1, 128.9, 127.3, 126.9, 124.6, 122.3, 120.0, 118.0, 116.9, 85.4, 85.2, 85.0, 84.8, 78.8, 70.3, 69.9, 55.9, 26.3, 25.1, 22.8; HREIMS m/z 452.1448 $[M]^+$ (calcd for $C_{23}H_{23}F_3O_6$, 452.1447).

ECD Calculation of 1a−d. Based on the NMR data, the possible isomers of the compound were predicted, and their structural energy minimizations were conducted using Avogadro 1.2.0 with the MMFF force field. Subsequently, DFT calculations were performed by Turbomole X 4.3.2 with the DFT settings (functional B3-LYP/gridsize m^3), and utilizing the 6-31G basis set for all atoms, and geometry optimization options (energy 10^{-6} Hartree, gradient norm $|dE/dxyz| = 10^{-3}$ Hartree/Bohr). The ECD spectra of the four isomers were calculated by using DFT at the functional B3LYP/DFT level and def-SV(P) basis set. The calculated ECD spectra were simulated by overlapping each transition, where σ represents the width of the band at $1/e$ height. ΔEi and Ri correspond to the excitation energies and rotatory strengths for transition i, respectively. In this calculation, the σ value was set at 0.10 eV.

CONCLUSIONS

We successfully accomplished the total synthesis of the four stereoisomers of 2,2-dimethyl-3-hydroxy-4-(1′-angeloyloxy)-6-acetylchromane. The key features of our synthesis involved the utilization of Sharpless asymmetric dihydroxylation of a readily accessible benzopyran substrate, followed by Mitsunobu or Steglich reaction to achieve chiral control. The absolute stereochemistry of the natural product isolated from *A. grandifolia* has been revised to 2,2-dimethyl-3*S*-hydroxy-4*R*-(1′-angeloyloxy)-6-acetylchromane (**1c**) based on the NMR data and optical rotations of the synthesized compounds. The absolute configuration of the synthesized stereoisomers was confirmed through Mosher ester analysis. In addition, we provided ECD spectra for the four stereoisomers which will allow verification of the absolute configuration of the natural product. Synthesis of all four stereoisomers of 2,2-dimethyl-3-hydroxy-4-(1′-angeloyloxy)-6-acetylchromane would facilitate the exploration of their potential biomedical applications.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c05349.

Comparison of the ^{1}H NMR for the reported natural product and synthetic **1a−d**, comparison of the ^{13}C NMR for the reported natural product and synthetic **1a−d**, Mosher ester analysis of 3a (a) and 3b (b), in vitro enzyme inhibition assay, copies of ^{1}H and ^{13}C NMR spectra for **2**, **3a−b**, **1a−d**, **4aS**, **4aR**, **4bS**, and **4bR**, and chiral HPLC analysis for **3a−b** (PDF)

AUTHOR INFORMATION

Corresponding Author

Jong-Wha Jung − *College of Pharmacy, Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 41566, Republic of Korea; Vessel-Organ Interaction Research Center, Kyungpook National University, Daegu 41566, Republic of Korea;* orcid.org/0000-0001-9885-3447; Phone: +82-53-950-8578; Email: jungj@knu.ac.kr; Fax: +82-53-950-8557

Authors

Changmin Oh − *College of Pharmacy, Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 41566, Republic of Korea; Vessel-Organ Interaction Research Center, Kyungpook National University, Daegu 41566, Republic of Korea;* orcid.org/0009-0002-8434-3870

Ji Hyeon Im − *Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea;* orcid.org/0009-0001-1874-4441

Munhyung Bae − *College of Pharmacy, Gachon University, Incheon 21936, Republic of Korea*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c05349

Author Contributions

J.-W. J. designed this study. C. O., J. H. I., and M. B. performed the experiments. C. O., M. B., and J.-W. J. analyzed the data and wrote the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This study was supported by the National Research Foundation of Korea (NRF-2020R1C1C1013670, NRF-2020R1A5A2017323, and NRF-2022M3E5E8081209, to J.-W.J., RS-2023-00211757129820768700001 and 2022R1F1A1076487111298207687010l, to M.B.) funded by the Ministry of Science and ICT. Financial support from the fourth BK21 project funded by the Ministry of Education (5199990614732 to C.O.) is gratefully acknowledged. We thank YMC Korea for chiral HPLC analysis.

REFERENCES

(1) (a) Zhu, H.; Wang, Y.; Nafie, L. A. Computational methods and points for attention in absolute configuration determination. *Front. Nat. Prod.* **2023**, *1*, 1086897. Review (b) Semenov, V. A.; Krivdin, L. B. Computational NMR of natural products. *Russ. Chem. Rev.* **2022**, *91* (5), RCR5027.

(2) Cortés, I.; Cuadrado, C.; Daranas, A. H.; Sarotti, A. M. Machine learning in computational NMR-aided structural elucidation. *Front. Nat. Prod.* **2023**, *2*, 1122426. Review
(3) Marcarino, M. O.; Cicetti, S.; Zanardi, M. M.; Sarotti, A. M. A critical review on the use of DP4+ in the structural elucidation of natural products: the good, the bad and the ugly. A practical guide. *Nat. Prod. Rep.* **2022**, *39* (1), 58−76.
(4) Hinojosa-Espinosa, O.; Villaseñor, J. L.; Ortiz, E. On the identity of two Mexican species of Ageratina (Eupatorieae, Asteraceae): A. grandifolia and A. rivalis. *Bot. Sci.* **2019**, *97* (2), 250−259.
(5) Gutierrez-Gonzalez, J. A.; Pérez-Vásquez, A.; Torres-Colín, R.; Rangel-Grimaldo, M.; Rebollar-Ramos, D.; Mata, R. α-Glucosidase Inhibitors from Ageratina grandifolia. *J. Nat. Prod.* **2021**, *84* (5), 1573−1578.
(6) Gutierrez-Gonzalez, J. A.; Pérez-Vásquez, A.; Torres-Colín, R.; Rangel-Grimaldo, M.; Rebollar-Ramos, D.; Mata, R. Correction to "α-Glucosidase Inhibitors from Ageratina grandifolia". *J. Nat. Prod.* **2021**, *84* (7), 2065−2066.
(7) Navarro-Vazquez, A. Computational Structural Revision of a 4-Hydroxy-3-(1'-angeloyloxy-2',3'-epoxy-3'-methyl)butylacetophenone Compound from Ageratina grandifolia. *J. Nat. Prod.* **2021**, *84* (7), 2043−2047.
(8) We realized that Monica et al. reported the synthesis and absolute configuration determination of this natural product when preparing our manuscript. Their synthetic strategy, employing Jacobsen's asymmetric epoxidation as a key step, is different from ours, but their revision of absolute configuration is consistent with our findings: Dandawate, M.; Choudhury, R.; Krishna, G. R.; Reddy, D. S. Total Synthesis and Absolute Configuration Determination of the α-Glycosidase Inhibitor (3S,4R)-6-Acetyl-3-hydroxy-2,2-dimethylchroman-4-yl (Z)-2-Methylbut-2-enoate from Ageratina grandifolia. *J. Nat. Prod.* **2023**, *86* (7), 1878−1883.
(9) Wang, Z.-M.; Kakiuchi, K.; Sharpless, K. B. Osmium-catalyzed asymmetric dihydroxylation of cyclic cis-disubstituted olefins. *J. Org. Chem.* **1994**, *59* (23), 6895−6897.
(10) Mitsunobu, O.; Yamada, M. Preparation of esters of carboxylic and phosphoric acid via quaternary phosphonium salts. *Bull. Chem. Soc. Jpn.* **1967**, *40* (10), 2380−2382.
(11) Neises, B.; Steglich, W. Simple method for the esterification of carboxylic acids. *Angew. Chem., Int. Ed. Engl.* **1978**, *17* (7), 522−524.
(12) (a) Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. Catalytic asymmetric dihydroxylation. *Chem. Rev.* **1994**, *94* (8), 2483−2547. (b) Heravi, M. M.; Zadsirjan, V.; Esfandyari, M.; Lashaki, T. B. Applications of sharpless asymmetric dihydroxylation in the total synthesis of natural products. *Tetrahedron: Asymmetry* **2017**, *28* (8), 987−1043. (c) Mushtaq, A.; Zahoor, A. F.; Bilal, M.; Hussain, S. M.; Irfan, M.; Akhtar, R.; Irfan, A.; Kotwica-Mojzych, K.; Mojzych, M. Sharpless Asymmetric Dihydroxylation: An Impressive Gadget for the Synthesis of Natural Products: A Review. *Molecules* **2023**, *28* (6), 2722.
(13) (a) But, T. Y. S.; Toy, P. H. The Mitsunobu reaction: origin, mechanism, improvements, and applications. *Chem.—Asian J.* **2007**, *2* (11), 1340−1355. (b) Swamy, K. K.; Kumar, N. B.; Balaraman, E.; Kumar, K. P. Mitsunobu and related reactions: advances and applications. *Chem. Rev.* **2009**, *109* (6), 2551−2651. (c) Munawar, S.; Zahoor, A. F.; Ali, S.; Javed, S.; Irfan, M.; Irfan, A.; Kotwica-Mojzych, K.; Mojzych, M. Mitsunobu Reaction: A Powerful Tool for the Synthesis of Natural Products: A Review. *Molecules* **2022**, *27* (20), 6953.
(14) Jordan, A.; Whymark, K. D.; Sydenham, J.; Sneddon, H. F. A solvent-reagent selection guide for Steglich-type esterification of carboxylic acids. *Green Chem.* **2021**, *23* (17), 6405−6413.
(15) Hoye, T. R.; Jeffrey, C. S.; Shao, F. Mosher ester analysis for the determination of absolute configuration of stereogenic (chiral) carbinol carbons. *Nat. Protoc.* **2007**, *2* (10), 2451−2458.
(16) Tripathi, R. K. P.; Ayyannan, S. R. Monoamine oxidase-B inhibitors as potential neurotherapeutic agents: An overview and update. *Med. Res. Rev.* **2019**, *39* (5), 1603−1706.
(17) Baweja, G. S.; Gupta, S.; Kumar, B.; Patel, P.; Asati, V. Recent updates on structural insights of MAO-B inhibitors: A review on target-based approach. *Mol. Divers.* **2023**, 1.

https://doi.org/10.1021/acsomega.3c05349
ACS Omega 2023, 8, 37384−37390