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A concise synthesis of (+)-conagenin and its isomer using chiral tricyclic iminolactones

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ABSTRACT

An efficient synthesis of (+)-conagenin, a novel immunomodulator produced by *Streptomyces roseosporus*, has been achieved via the shortest route at present. At the same time, 2-epiconagenin was synthesized according to the same methodology.

1. Introduction

(+)-Conagenin **1** (Fig. 1), a low molecular weight immunomodulator, was discovered in fermentation broths of *Streptomyces roseosporus* by Ishizuka et al.[1] The compound is an immunomodulator of antitumor activity stimulating activated T cells and enhancing the generation of antitumor cells. As conagenin itself does not show cytotoxicity to murine and human tumor cells, and was found to be effective *in vivo* in improving the antitumor activity of cyclophosphamide, mitomycin C, and adriamycin against murine leukemias, it may be useful in cancer chemotherapy.[2,3] Due to these biological activities and its unique and highly functionalized structure, (+)-conagenin **1** has attracted much attention from synthetic chemists. Five total syntheses[4] and a formal synthesis[5] of (+)-conagenin **1** have been reported, in addition to the synthesis of its analogues,[6] a partial synthesis,[7] and a review about synthesis of conagenin and α-methylserine.[8] Herein, we report the stereoselective synthesis of (+)-conagenin **1** and its isomer **2** which is for the first time reported (Fig. 1) in over 21.3% total yield using our chiral templates,[9] the glycine equivalents for the asymmetric synthesis of α-amino acid, which are derived from natural (1*R*)-(+)-camphor.

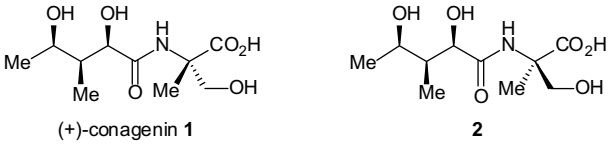

Figure 1.

2. Results and discussion

The synthesis of (+)-conagenin **1** was to be assembled from two fragments **3** and **4** (Scheme 1). Construction of the α-methylserine moiety **3**[10] was achieved starting from our chiral template, tricyclic iminolactone **5** in four steps, as represented in Scheme 2.

Monomethylation of tricyclic iminolactone **5** with CH_3I was carried out using a combination of lithium diisopropylamide (LDA) and *N*,*N*′-dimethylpropyleneurea (DMPU) at −78 °C (98% yield).[9] The monomethyl iminolactone **6** was then transformed into α,α-disubstituted iminolactone **7** and **7a** by treatment with an excess of formaldehyde and the combination of LDA and DMPU at −78 °C (94%, **7**:**7a** = 7:1), or −40 °C (95%, **7**:**7a** = 1.3:1). Hydrolysis of α,α-disubstituted iminolactone **7** in 6 *M* HCl solution at 90 °C for 6 h afforded (*S*)-methylserine **8** (92%). Methyl (*S*)-methylserinate **3** (91% crude product) was obtained by treating (*S*)-methylserine **8** with an excess of CH_2N_2 in Et_2O at room temperature.

Hatakeyama et al.[4a] reported the synthesis of carboxylic acid **4** effectively from diol **12**, which was obtained in a one-pot reaction. In order to improve the selectivity and the yield of diol **12**, it was prepared via a two-step sequence as shown in Scheme 3: (i) following the protocol reported by Paterson,[11] an enantio- and diastereoselective aldol reaction between propiophenone and acetaldehyde using (−)-$(Ipc)_2BOTf$/*i*-Pr_2NEt in dichloromethane proceeded smoothly to afford *syn*-2-methyl-3-hydroxyketone **11** (88%, de >99:1); (ii) the desired stereoselective reduction of **11** was accomplished by treatment with zinc borohydride[12,13] which was reported by Ichikawa[4c] in diethyl ether at 0 °C to afford the known diol **12** (de >25:1) in 85% yield {$[\alpha]_D^{27} = +35.0$ (*c* 1.05, $CHCl_3$), lit.[4a] $[\alpha]_D^{24} = +35.5$ (*c* 0.46, $CHCl_3$), lit.[4c] $[\alpha]_D^{18} = +41.0$ (*c* 1.05, $CHCl_3$), lit.[4f] $[\alpha]_D^{27} = +35.4$ (*c* 1.06, $CHCl_3$)}. According to the reported procedure,[4a,c,f] protection of both hydroxy groups as acetates and oxidation of the phenyl group with ruthenium tetraoxide afforded carboxylic acid **4** (81%).[14]

Finally, (+)-conagenin **1** was obtained using a Hatakeyama's procedure as shown in Scheme 4.[4a] Thereby, condensation of

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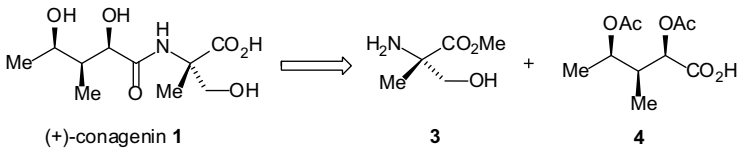
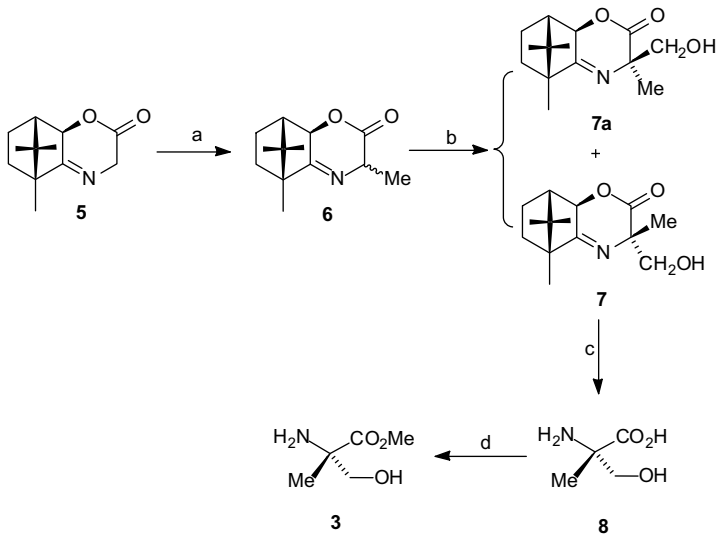
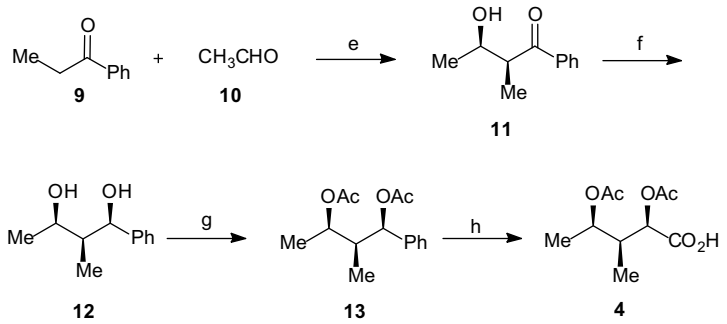


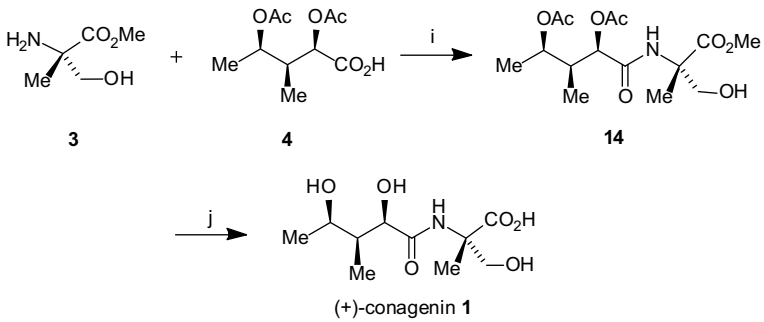
Scheme 1.



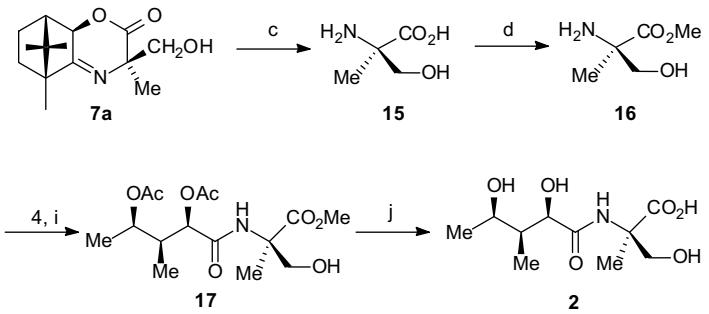
Scheme 2. Reagents and conditions: (a) LDA (1.1 equiv), DMPU (1.5 equiv), MeI (1.5 equiv), THF, −78 °C, 98%; (b) LDA (2 equiv), DMPU (1.5 equiv), HCHO/THF, −78 °C, 94%, **7**:**7a** = 7:1; or −40 °C, 95%, **7**:**7a** = 1.3:1; (c) (i) 6 M HCl, 90 °C, 6 h; (ii) EtOH, propylene oxide, 92%; (d) CH_2N_2/ether, rt, 91% (crude product).



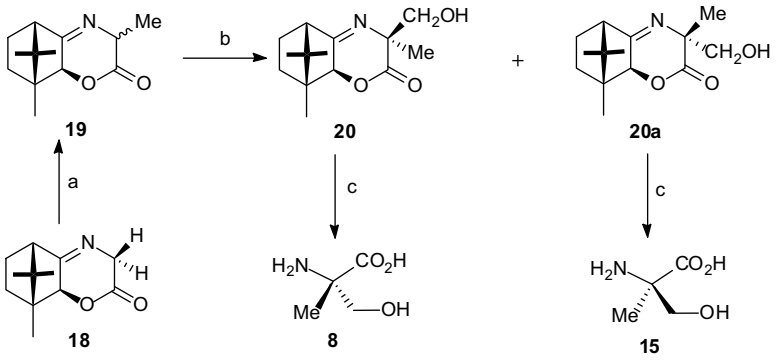
Scheme 3. Reagents and conditions: (e) (−)-$(Ipc)_2BOTf$, *i*-Pr_2NEt, CH_2Cl_2, −78 to −23 °C, 88%; (f) $Zn(BH_4)_2$–ether, 0 °C, 3 h, 85%; (g) Ac_2O, Et_3N, DMAP (cat.), CH_2Cl_2; (h) $RuCl_3$ (cat.), H_5IO_6, CH_3CN–CCl_4–H_2O (2:2:3), two steps 81%.



Scheme 4. Reagents and conditions: (i) DCC, HOBt, DMF, rt, 6 h, 69%; (j) 1 M K_2CO_3, MeOH, 85%.



Scheme 5. Reagents and conditions: (c) (i) 6 M HCl, 90 °C, 6 h; (ii) EtOH, propylene oxide, 92%; (d) CH_2N_2/ether, rt, 91% (crude product); (i) DCC, HOBt, DMF, rt, 6 h, 61%; (j) 1M K_2CO_3, MeOH, 83%.



Scheme 6. Reagents and conditions: (a) LDA (1.1 equiv), DMPU (1.5 equiv), MeI (1.5 equiv), THF, −78 °C, 95%; (b) LDA (2 equiv), DMPU (1.5 equiv), HCHO/THF, −78 °C, 91%, **20**:**20a** = 1:2; (c) (i) 6 M HCl, 90 °C, 6 h; (ii) EtOH, propylene oxide, 92%.

methyl (*S*)-methylserinate **3** with carboxylic acid **4** using dicyclohexylcarbodiimide (DCC) in the presence of 1-hydroxybenzotriazole (HOBt)[15] in DMF furnished the protected conagenin **14** in an enantiomerically pure form in 69% yield {colorless oil, $[\alpha]_D^{15} = +34.0$ (*c* 0.48, $CHCl_3$), lit.[4c] $[\alpha]_D^{21} = +32.9$ (*c* 0.35, $CHCl_3$)}. (Scheme 4). Deprotection was carried out under basic conditions to provide (+)-conagenin **1** in 85% yield {mp 156–158 °C, $[\alpha]_D^{23} = +50.0$ (*c* 0.45, MeOH), lit.[1] $[\alpha]_D^{27} = +55.4$, lit.[4a] $[\alpha]_D^{25} = +48.7$ (*c* 0.43, MeOH), lit.[4e] $[\alpha]_D^{31} = +50.2$ (*c* 0.38, MeOH)}. The accomplishment of this asymmetric total synthesis was confirmed by the identity of all physicochemical data of the synthesized and the natural (+)-conagenin **1**.

Based on the same methodology, compound **7a** provided the synthesis of methyl (*R*)-methylserinate **16** upon successive acidic hydrolysis and methylation. Compound **16** was then coupled with carboxylic acid **4**, and the resulting adduct was deprotected under similar conditions to give 2-epiconagenin **2** {colorless oil, $[\alpha]_D^{23} = +24.0$ (*c* 0.51, $CHCl_3$)} in 83% yield (Scheme 5).

(*S*)-Methylserine **8** and (*R*)-methylserine **15** could also be obtained from the other chiral template, tricyclic iminolactone **18**. In this case, the aldol reaction of monomethyl iminolactone **19** with an excess of formaldehyde occurred with low diastereoselectivity (**20**:**20a**, 1:2). Then acidic hydrolysis of **20** and **20a** afforded the desired compounds **8** and **15**, respectively (Scheme 6).

3. Conclusion

In conclusion, we have furnished the total synthesis of (+)-conagenin **1** and its isomer **2** using our chiral templates, tricyclic iminolactones **5** and **18** in the shortest route at present. The synthesis of 2-epiconagenin **2** was first reported. This methodology can also be applied to the synthesis of a variety of stereoisomers of (+)-conagenin **1** and its related natural products.

4. Experimental

4.1. General procedure

Solvents were dried by heating under reflux for at least 12 h over P_2O_5 (dichloromethane) or sodium/ benzophenone (toluene, THF, and *n*-hexane), and were freshly distilled prior to use. ^{1}H NMR spectra were recorded on a Bruker AM-400 Varian (400 MHz) or Mercury-300 (300 MHz) spectrometer, and are reported in ppm using a solvent as an internal standard ($CDCl_3$ at 7.26 ppm). Data are reported as (s = singlet, d = doublet, m = multiplet, br = broad); coupling constant (*J*) in Hertz; integration. Proton-decoupled ^{13}C NMR spectra were recorded on a Bruker AM-400 Varian (100 MHz) or Mercury-300 (75 MHz) spectrometer, and are reported in ppm using solvent as an internal standard ($CDCl_3$ at 77.0 ppm). Optical rotations were measured on the Perkin Elmer 341 polarimeter. Melting points were determined on an XT-4 melting point apparatus, and are uncorrected. HRMS were performed on Bruker Apex II mass instrument (ESI). MS were measured on a VG-7070E spectrometer (EI at 70 eV). For chiral diastereomeric products, the ratios were determined by integration of the ^{1}H NMR (Varian Mercury-400 MHz).

4.2. (1*S*,2*R*,5*S*,8*R*)-5,8,11,11-Tetramethyl-3-oxa-6 azatri-cyclo-[6.2.1.0^{2,7}]undec-6-en-4-one 6

Diisopropylamine (156 μL, 1.1 mmol, 1.1 equiv) was added to a solution of dry THF (3 mL) and *n*-BuLi (2.1 M, 520 μL, 1.1 mmol, 1.1 equiv) at −78 °C in flame-dried modified flasks under an argon

atmosphere. The reaction mixture was stirred at −78 °C for 30 min under an argon atmosphere. Compound **5** (207 mg, 1.0 mmol) in dry THF (10 mL) was added dropwise over a period of 10 min into the above freshly prepared LDA solution via a syringe, and the resulting solution was stirred at −78 °C for 30 min 0.35 mL DMPU (3 equiv) was then added to the reaction mixture. A solution of CH_3I (1.2 mmol, 1.2 equiv) in dry THF (10 mL) was then added to the reaction mixture via a syringe with the needle contacting the wall of the neck over 10 min. The solution was stirred at −78 °C for 1 h. Subsequently, aqueous ammonium chloride (10%, 2 mL) was added to the mixture to quench the reaction. The reaction mixture was warmed up to room temperature, washed with saturated aqueous $NaHSO_3$ (3 × 5 mL), $NaHCO_3$, and water; dried ($MgSO_4$), and concentrated to give the crude product. The crude product was purified by column chromatography to yield desired compound **6** (432 mg, 98%, dr >99:1). $[\alpha]_D^{22} = +100.7$ (*c* 1.08, $CHCl_3$), mp 78–80 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.63 (d, *J* = 7.6 Hz, 1H), 4.59 (s, 1H), 2.24 (d, *J* = 4.8 Hz, 1H), 2.16–2.04 (m, 1H), 1.82–1.73 (m, 1H), 1.62–1.54 (m, 1H), 1.44 (d, *J* = 7.6 Hz, 3H), 1.42–1.38 (m, 1H), 1.06 (s, 3H), 0.98 (s, 3H), 0.81 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.1, 171.9, 78.5, 57.6, 52.6, 48.4, 47.9, 29.1, 25.8, 20.1, 19.5, 16.3, 10.0; MS: *m*/*z* 221 (M^+, 26.4), 193 (47.9), 177 (100.0), 162 (91.1), 149 (46.4), 124 (38.8), 110 (14.1), 96 (55.7), 69 (14.5), 68 (15.3), 55 (14.1). IR (NaCl, $CHCl_3$): 2968 (m), 1744 (s), 1691 (m) cm^{-1}. HRMS (ESI): *m*/*z* calcd for $C_{13}H_{19}NO_2$ M^+ 221.1410, found M^+ 221.1416. Anal. Calcd for $C_{13}H_{19}NO_2$: C, 70.56; H, 8.65; N, 6.33. Found: C, 70.54; H, 8.56; N, 6.24.

4.3. (1*R*,4*S*,7*R*,8*S*)-1,4,11,11-Tetramethyl-4-hydroxymethyl-6-oxa-3-aza-tricyclo[6.2.1.0^{2,7}]undec-2-en-5-one 7

4.3.1. (1*R*,4*R*,7*R*,8*S*)-1,4,11,11-Tetramethyl-4-hydroxymethyl-6-oxa-3-aza-tricyclo[6.2.1.0^{2,7}]undec-2-en-5-one 7a

Diisopropylamine (156 μL, 1.1 mmol, 1.1 equiv) was added to a solution of dry THF (3 mL) and *n*-BuLi (2.1 M, 520 μL, 1.1 mmol, 1.1 equiv) at −78 °C in flame-dried modified flasks under an argon atmosphere. The reaction mixture was stirred at −78 °C for 30 min under an argon atmosphere. Compound **6** (221 mg, 1.0 mmol) in dry THF (10 mL) was added dropwise over a period of 10 min into the above freshly prepared LDA solution via a syringe, and the resulting solution was stirred at −78 °C for 30 min after which 0.35 mL DMPU (3 equiv) was added to the reaction mixture. A solution of saturated HCHO (gas) in dry THF (10 mL) was then added to the reaction mixture via a syringe with the needle contacting the wall of the neck over 10 min. The solution was stirred at −78 °C for 2 h. after which aqueous ammonium chloride (10%, 2 mL) was added to the mixture to quench the reaction. The reaction mixture was warmed up to room temperature, washed with saturated aqueous $NaHSO_3$ (3 × 5 mL), $NaHCO_3$, and water; dried over $MgSO_4$ and concentrated to give the crude product. The crude product was purified by column chromatography to yield the desired compounds **7** and **7a** (236 mg, 94%, **7**:**7a** = 7:1). Compound **7**: $[\alpha]_D^{15} = +86$ (*c* 0.40, $CHCl_3$); mp 156–157 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.79 (3H, s), 0.95 (3H, s), 1.06 (3H, s), 1.35–1.42 (1H, m), 1.54 (3H, s), 1.64–1.80 (2H, m), 1.99–2.05 (1H, m), 2.17 (1H, d, *J* = 4.8 Hz), 3.74 (1H, d, *J* = 10.8 Hz), 3.99 (1H, d, *J* = 10.8 Hz), 4.79 (1H, s); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 10.3, 19.4, 20.2, 24.6, 26.0, 29.2, 47.8, 48.3, 52.9, 65.3, 69.0, 80.7, 174.2, 181.2; MS: *m*/*z* 251 (M^+, 1.0), 223 (1.3), 220 (3.2), 207 (54.8), 192 (100.0), 179 (59.9), 164 (16.8), 148 (25.0), 124 (74.0), 91 (40.2), 84 (39.4), 77 (33.7), 69 (47.3), 55 (38.2), 41 (87.6); HRMS (ESI): *m*/*z* calcd for $C_{14}H_{21}NO_3$: $[M+H]^+$ 252.1594, found 252.1595. Compound **7a**: $[\alpha]_D^{15} = +124$ (*c* 0.30, $CHCl_3$); mp 123–124 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.76 (3H, s), 0.94 (3H, s), 1.01 (3H, s), 1.33 (3H, s), 1.35–1.41 (1H, m), 1.51–1.61 (1H, m), 1.71–1.80 (1H, m), 2.00–2.10 (1H, m), 2.22 (1H, d, *J* = 4.8 Hz), 2.84 (1H, br s), 3.81 (1H, d, *J* = 10.5 Hz), 3.91 (1H, d, *J* = 10.5 Hz), 4.58 (1H, s); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 10.0, 18.4, 19.4, 20.0, 25.7, 28.9, 47.7, 48.3, 52.8, 63.1, 68.8, 78.8, 173.3, 181.6; MS: *m*/*z* 251 (M^+, 1.94), 223 (1.18), 220 (13.3), 207 (54.7), 192 (100.0), 179 (59.6), 164 (13.8), 148 (24.7), 124 (60.0), 91 (39.3), 84 (36.1), 77 (27.7), 69 (29.9), 55 (28.1), 41 (66.6); HRMS (ESI): *m*/*z* calcd for $C_{14}H_{21}NO_3$: $[M+H]^+$ 252.1594, found 252.1595.

4.4. (1*S*,4*S*,7*S*,8*R*)-4,8,11,11-Tetramethyl-4-hydroxymethyl-6-oxa-3-aza-tricyclo[6.2.1.0^{2,7}]undec-2-en-5-one 20

4.4.1. (1*S*,4*R*,7*S*,8*R*)-1,4,11,11-Tetramethyl-4-hydroxymethyl-6-oxa-3-aza-tricyclo[6.2.1.0^{2,7}]undec-2-en-5-one 20a

Diisopropylamine (156 μL, 1.1 mmol, 1.1 equiv) was added to a solution of dry THF (3 mL) and *n*-BuLi (2.1 M, 520 μL, 1.1 mmol, 1.1 equiv) at −78 °C in flame-dried modified flasks under an argon atmosphere. The reaction mixture was then stirred at −78 °C for 30 min under an argon atmosphere. Compound **19** (221 mg, 1.0 mmol) in dry THF (10 mL) was added dropwise over a period of 10 min into the above freshly prepared LDA solution via a syringe, and the resulting solution was stirred at −78 °C for 30 min after which 0.35 mL DMPU (3 equiv) was added to the reaction mixture. A solution of saturated HCHO (gas) in dry THF (10 mL) was then added to the reaction mixture via a syringe with the needle contacting the wall of the neck over 10 min. The solution was then stirred at −78 °C for 2 h. Subsequently, aqueous ammonium chloride (10%, 2 mL) was added to the mixture to quench the reaction. The reaction mixture was warmed up to room temperature, washed with saturated aqueous $NaHSO_3$ (3 × 5 mL), $NaHCO_3$, and water; dried over $MgSO_4$; and concentrated to give the crude product. The crude product was purified by column chromatography to yield desired compounds **20** and **20a** (228 mg, 91%, **20**:**20a** = 1:2). Compound **20**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.82 (3H, s), 0.96 (3H, s), 1.07 (3H, s), 1.36 (3H, s), 1.36–1.43 (1H, m), 1.52–1.60 (1H, m), 1.73–2.08 (2H, m), 2.42 (1H, d, *J* = 4.8 Hz), 2.83 (1H, br s), 3.83 (1H, d, *J* = 10.8 Hz), 3.94 (1H, d, *J* = 10.8 Hz), 4.41 (1H, s); MS: *m*/*z* 251 (M^+, 1.2), 223 (1.4), 220 (3.2), 207 (60.0), 192 (100.0), 179 (61.2), 164 (16.8), 148 (25.0), 124 (74.0), 91 (40.2), 84 (39.4), 77 (33.7), 69 (47.3), 55 (38.2), 41 (88.6); Compound **20a**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.83 (3H, s), 0.97 (3H, s), 1.05 (3H, s), 1.36–1.43 (1H, m), 1.54 (3H, s), 1.59–1.68 (1H, m), 1.80–1.96 (1H, m), 1.97–2.09 (1H, m), 2.41 (1H, d, *J* = 3.9 Hz), 3.73 (1H, d, *J* = 10.8 Hz), 4.00 (1H, d, *J* = 10.8 Hz), 4.62 (1H, s); MS: *m*/*z* 251 (M^+, 1.93), 223 (1.20), 220 (13.3), 207 (54.7), 192 (100.0), 179 (59.6), 164 (13.8), 148 (24.7), 124 (60.0), 91 (39.3), 84 (36.1), 77 (27.7), 69 (30.0), 55 (28.0), 41 (66.5).

4.5. (*S*)-Methylserine 8 and (*R*)-methylserine 15

Compound **7** (**7a**) (100 mg, 0.4 mmol) was treated with 6 *M* HCl (2 mL) in THF (2 mL) at 90 °C for 4 h and then concentrated under reduced pressure. The mixture was extracted with diethyl ether. The aqueous layer was evaporated under reduced pressure. The residue was dissolved in EtOH (4 mL), after which propylidene oxide (3 mL) was added, and the mixture was stirred at room temperature for 30 min during which time, fine white solids precipitated. The precipitate was collected by filtration, washed successively with cold EtOH and Et_2O, and dried to afford the desired (*S*)-methylserine **8** or (*R*)-methylserine **15** (44 mg, 92%). Compound **8**: $[\alpha]_D^{15} = +6.0$ (*c* 1.0, H,O); mp 237–239 °C; ^{1}H NMR (D_2O, 300 MHz): δ 1.23 (3H, s), 3.47 (1H, d, *J* = 11.7 Hz), 3.72 (1H, d, *J* = 11.7 Hz); ^{13}C NMR (D_2O, 75 MHz): δ 18.6, 62.6, 64.9, 175.6; HRMS (ESI): *m*/*z* calcd for $C_4H_9NO_3$: $[M+H]^+$ 120.0655, found 120.0651. Compound **15**: $[\alpha]_D^{15} = -6.0$ (*c* = 1.0, H,O); mp 237–239 °C; ^{1}H NMR (D_2O, 300 MHz): δ 1.23 (3H, s), 3.47 (1H, d, *J* = 11.7 Hz), 3.72 (1H, d, *J* = 11.7 Hz); ^{13}C NMR (D_2O, 75 MHz): δ

18.6, 62.6, 64.9, 175.6; HRMS (ESI): *m*/*z* calcd for $C_4H_9NO_3$: $[M+H]^+$ 120.0655, found 120.0651.

4.6. Methyl (*S*)-methylserinate 3 and methyl (*R*)-methylserinate 16

To a solution of (*S*)-methylserine **8** or (*R*)-methylserine **15** in MeOH was added an excess solution of CH_2N_2 in Et_2O at 0 °C. The solution was stirred at 0 °C for 20 min. The precipitate was filtrated by EtOAc, and the residue was concentrated under reduced pressure. The crude product was not purified for the following next step.

4.7. (2*S*,3*R*)-3-Hydroxy-2-methyl-1-phenylbutan-1-one 11

The prepared diisopinocampheylborane triflate solution (2.05 mL, 3.9 mmol, 1.9 M in hexane) was diluted with DCM (16 mL) and cooled to −78 °C under argon. To the stirred solution was added dropwise diisopropylethylamine (1.04 mL, 6 mmol), followed by the propiophenone (402 mg, 3 mmol). If the boron triflate solution was faintly yellow, it usually became colorless upon the addition of the amine. After 2–3 h of enolization at −78 °C, freshly distilled acetaldehyde (8 mmol) was added dropwise after which the reaction mixture was stirred at −78 °C for a further hour before being stirred at an increased temperature (−23 °C) for 16 h. The reaction mixture was then partitioned between ether (3 × 20 mL) and pH 7 buffer (20 mL). The combined ether extracts were concentrated in vacuo, and the residue was dissolved in methanol (15 mL) and pH 7 buffer (3 mL). The solution was cooled to 0 °C, after which 30% hydrogen peroxide (4 mL) was added, and stirring was continued at room temp. for 2 h. The mixture was then poured into water (30 mL) and extracted with dichloromethane (3 × 30 mL). The combined extracts were washed in turn with $NaHCO_3$ solution and brine, then dried over $MgSO_4$, and concentrated in vacuo to yield a yellow-orange oil. The crude product was purified by column chromatography on silica gel eluting with AcOEt/hexane (1:8) to afford the compound **11** (470 mg, 88%, *syn*/*anti* >99:1) as a colorless oil. Compound **11**: $[\alpha]_D^{15} = +17$ (*c* 0.50, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.24 (3H, d, *J* = 6.6 Hz), 1.28 (3H, d, *J* = 7.2 Hz), 3.13 (1H, br s), 3.43 (1H, qd, *J* = 6.9 Hz, 3 Hz), 4.26 (1H, m), 7.48 (2H), 7.60 (1H), 7.95 (2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 11.1, 20.2, 45.6, 67.5, 128.4, 128.7, 133.5, 135.7, 205.9; MS: *m*/*z* 178 (M^+, 0.3), 160 (5.0), 133 (25.3), 123 (15.6), 105 (100.0), 77 (56.1), 56 (17.4), 51 (18.2), 43 (10.5).

4.8. (1*R*,2*S*,3*R*)-2-Methyl-1-phenylbutane-1,3-diol 12

To a solution of β-hydroxyketone **11** (178 mg, 1.00 mmol) in Et_2O cooled to 0 °C was added a solution of $Zn(BH_4)_2$ in THF (0.16 M, 8 mL) at 0 °C. The solution was stirred at 0 °C for 1 h. The reaction mixture was diluted with 10% HCl, extracted with Et_2O, dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel eluting with AcOEt/hexane (1:4) to afford 1,3-diol **12** (155 mg, 85%, *syn*/*anti* >25:1) as a colorless oil. Recrystallization: the 1,3-diol (155 mg) was dissolved in hexane (2 ml) at 60 °C. The solution was cooled down to −30 °C and gradually warmed up to room temperature to give white crystals (122 mg, 79%). Compound **12**: mp 50–52 °C; $[\alpha]_D^{15} = +38.0$ (*c* 0.20, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 082 (3H, d, *J* = 7.1 Hz), 1.21 (3H, d, *J* = 6.5 Hz), 1.73 (1H, m), 2.56 (1H, s), 3.31 (1H, s), 4.23 (1H, m), 5.03 (1H, d, *J* = 3.0 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 4.0, 21.5, 45.0, 72.1, 78.5, 125.6, 127.0, 128.1, 143.3; MS: *m*/*z* 162 (4.5), 117 (6.1), 107 (100.0), 91 (6.6), 77 (29.1), 51 (8.1), 43 (12.3).

4.9. (2*R*,3*S*,4*R*)-2,4-Diacetyloxy-3-methylpentanoic acid 4

To a mixture of 1,3-diol **12** (90 mg, 0.5 mmol), pyridine (1.0 mL), and Ac_2O (1.0 mL) was added 4-dimethylaminopyridine (2 mg, 0.016 mmol). The solution was stirred at room temperature for 30 min and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel eluting with AcOEt/hexane (1:9 and 1:4) to afford the acetate (125 mg, 95%) as a colorless oil. To a solution of acetate (53 mg, 0.2 mmol) and $RuCl_3{\cdot}nH_2O$ (9 mg, 0.4 mmol) in $CCl_4/CH_3CN/H_2O$ (2:2:3, 2.0 mL) charged in a snapped vial was added periodic acid (832 mg, 4 mmol) in a single portion at room temperature. The mixture was stirred vigorously at room temperature for 36 h, and 2-propanol (0.5 mL) was added. After the mixture was stirred for 30 min, CH_2Cl_2 (10 mL) and H_2O (5 mL) were added. The separated aqueous layer was extracted with CH_2Cl_2 (3 times). The combined organic extracts were washed with brine and dried over Na_2SO_4. Concentration under reduced pressure afforded pentanoic acid **4** (37 mg, 81%) as a yellow oil. Compound **4**: $[\alpha]_D^{15} = +1.5$ (*c* 0.50, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.07 (3H, d, *J* = 7.2 Hz), 1.25 (3H, d, *J* = 7.2 Hz), 2.04 (3H, s), 2.15 (3H, s), 2.28 (1H, qd, *J* = 7.2 Hz, 3.6 Hz), 4.96 (1H, q, *J* = 7.2 Hz), 5.14 (1H, d, *J* = 3.6 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 10.4, 17.3, 20.5, 21.1, 39.2, 71.1, 72.4, 170.5, 170.8, 174.4.

4.10. Methyl *N*-[(2*R*,3*S*,4*R*)-2,4-diacetyloxy-3-methylva-leryl]-2-methyl-L-serinate 14

4.10.1. Methyl *N*-[(2*R*,3*S*,4*R*)-2,4-diacetyloxy-3-methylvaleryl]-2-methyl-D-serinate 17

At first DCC (35 mg, 0.17 mmol) was added to a solution of (*S*)-methylserinate **3** or (*R*)-methylserinate **16** (12 mg, 0.10 mmol), crude pentanoic acid **4** (17 mg, 0.073 mmol), and HOBt (20 mg, 0.14 mmol) in DMF (2 mL) at 0 °C. After the mixture was stirred for 30 min, DMAP (9 mg, 0.072 mmol) was added in one portion. After being stirred at room temperature for 11 h, the reaction mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was chromatographed on silica gel (30% EtOAc/hexane = 1:1) to give **14** (24 mg, 69%) or **17** (21 mg, 61%) as a colorless oil. Compound **14**: $[\alpha]_D^{15} = +34.0$ (*c* 0.48, $CHCl_3$) {lit.[4c] $[\alpha]_D^{21} = +32.9$ (*c* 0.35, $CHCl_3$)}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.01 (3H, d, *J* = 7.2 Hz), 1.25 (3H, d, *J* = 6.4 Hz), 1.54 (3H, s), 2.06 (3H, s), 2.17 (3H, s), 2.27 (1H, qt, *J* = 7.2 Hz, 5.2 Hz), 3.21 (1H), 3.79 (3H, s), 3.81 (1H, dd, *J* = 11.4 Hz, 6.8 Hz), 4.13 (1H, dd, *J* = 11.4 Hz, 5.4 Hz), 4.97–5.00 (1H, m), 5.01 (1H, d, *J* = 5.2 Hz), 7.14 (1H, s); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 9.6, 17.9, 19.5, 20.7, 21.1, 39.7, 52.9, 62.2, 65.3, 71.1, 75.0, 168.8, 170.3, 170.8, 173.3; MS: *m*/*z* 329 (0.1), 317 (0.8), 288 (0.6), 257 (0.9), 215 (1.5), 197 (2.1), 182 (3.3), 173 (17.1), 102 (22.5), 85 (14.9), 69 (21.7), 55 (22.3), 43 (100.0); HRMS (ESI): *m*/*z* calcd for $C_{15}H_{26}NO_8^+$ $[M+H]^+$ 348.1653, found $[M+H]^+$ 348.1656. Compound **17**: $[\alpha]_D^{15} = +13.0$ (*c* 0.62, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.05 (3H, d, *J* = 7.2 Hz), 1.24 (3H, d, *J* = 6.8 Hz), 1.53 (3H, s), 2.06 (3H, s), 2.17 (3H, s), 2.24 (1H, qt, *J* = 7.2 Hz, 6.0 Hz), 3.17 (1H), 3.80 (3H, s), 3.81 (1H, dd, *J* = 11.6 Hz, 8.0 Hz), 4.15 (1H, dd, *J* = 11.6 Hz, 5.2 Hz), 4.91–4.93 (1H, m), 4.93 (1H, d, *J* = 6.0 Hz), 6.97 (1H, s); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 9.8, 17.8, 19.3, 20.8, 21.2, 39.7, 53.1, 62.5, 65.4, 70.8, 75.7, 168.8, 170.5, 170.7, 173.5; MS: *m*/*z* 329 (0.1), 317 (1.7), 288 (0.7), 257 (0.9), 215 (1.6), 197 (2.1), 182 (2.7), 173 (21.6), 102 (19.4), 85 (11.0), 69 (15.7), 55 (7.3), 43 (100.0); HRMS (ESI): *m*/*z* calcd for $C_{15}H_{26}NO_8^+$ $[M+H]^+$ 348.1653, found $[M+H]^+$ 348.1650.

4.11. (+)-Conagenin 1 and 2-*epi*-conagenin 2

Aqueous K_2CO_3 (1.0 M, 0.4 mL) was added to a solution of **14** or **17** (35 mg, 0.1 mmol) in MeOH (1.2 mL) at 0 °C, and the mixture

was stirred at room temperature for 2 h. The reaction mixture was then neutralized with aqueous $KHSO_4$ (1.0 M, 1 mL). The reaction mixture was concentrated and the residue was purified by column chromatography (followed by 8:1 CH_2Cl_2–MeOH as an eluent) to give **1** (21.1 mg, 85%) or **2** (20.6 mg, 83%) as colorless crystals. Compound **1**: mp 156–158 °C; $[\alpha]_D^{23} = +50.0$ (*c* 0.45, MeOH) {lit.[1] $[\alpha]_D^{27} = +55.4$, lit.[4a] $[\alpha]_D^{25} = +48.7$ (*c* 0.43, MeOH), lit.[4e] $[\alpha]_D^{31} = +50.2$ (*c* 0.38, MeOH)}; ^{1}H NMR (CD_3OD, 400 MHz): δ 0.92 (3H, d, J = 7.2 Hz), 1.23 (3H, d, J = 6.0 Hz), 1.49 (3H, s), 1.90 (1H, qdd, J = 7.2, 6.0, 2.8 Hz), 3.83 (1H, d, J = 10.8 Hz), 3.86–3.83 (1H, m), 4.10 (1H, d, J = 10.8 Hz), 4.15 (1H, d, J = 2.8 Hz): ^{13}C NMR (CD_3OD, 100 MHz): δ 8.2, 20.1, 21.2, 43.6, 63.0, 66.3, 71.3, 75.4, 175.7, 176.6; HRMS (ESI): *m*/*z* calcd for $C_{10}H_{20}NO_6^-$ $[M-H]^-$248.1140, found $[M-H]^-$ 248.1146. Compound **2**: mp 172–174 °C; $[\alpha]_D^{23} = +24.0$ (*c* 0.51, MeOH); ^{1}H NMR (CD_3OD, 400 MHz): δ 0.95 (3H, d, J = 6.8 Hz), 1.22 (3H, d, J = 4.8 Hz), 1.50 (3H, s), 1.89 (1H, qdd, J = 6.8, 4.8, 2.4 Hz), 3.85 (1H, d, J = 10.4 Hz), 3.90–3.84 (1H, m), 4.02 (1H, d, J = 10.4 Hz), 4.12 (1H, d, J = 2.4 Hz): ^{13}C NMR (CD_3OD, 100 MHz): δ 8.3, 20.2, 21.2, 43.8, 63.3, 66.9, 70.9, 75.5, 175.7, 179.3; HRMS (ESI): *m*/*z* calcd for $C_{10}H_{20}NO_6^-$ $[M-H]^-$ 248.1140, found $[M-H]^-$ 248.1146.

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