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Chemoenzymatic total synthesis of (+)-conagenin, a low-molecular-weight immunomodulator

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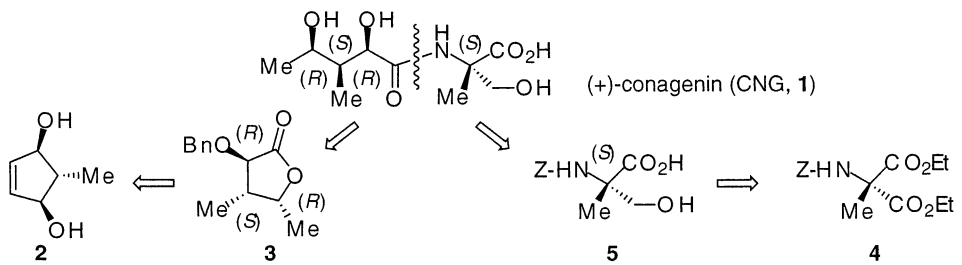
Abstract—Total synthesis of (+)-conagenin, a low-molecular-weight immunomodulator, was achieved based on enantioselective enzymatic reactions followed by chemoselective reductions.

(+)-Conagenin (CNG, **1**), a natural product originally isolated from fermentation broths of *Streptomyces roseosporus*, has been found to stimulate activated T cells by producing lymphokines as a low-molecular-weight immunomodulator.[1–3] In addition, the improvement of efficacy of antitumor agents, such as mitomycin C and adriamycin, by CNG (**1**) has also been reported.[4–9] These biological activities of CNG (**1**), possessing an α-substituted serine moiety, has made this compound an intriguing target for total synthesis. An asymmetric total synthesis[10] and an asymmetric formal synthesis[11] of CNG (**1**) have been reported, as have investigations of further syntheses[12,13] of diastereoisomers of CNG (**1**). We report herein a successful convergent method for the total synthesis of CNG (**1**) based on enantioselective enzymatic reactions followed by chemoselective reductions.

The retrosynthetic analysis of CNG (**1**) is outlined in Scheme 1. (*S*)-Z-α-Methylserine (**5**) was synthesized in 94% ee {$[\alpha]_D^{26}$ +10.1° (*c* 0.88, EtOH)} as a colorless amorphous powder from σ-symmetric prochiral diethyl α-aminomalonate (**4**) by utilizing our chemoenzymatic methodology based on enantioselective enzymatic hydrolysis followed by chemoselective enantiodivergent reduction.[14] Methylation of **5** with (trimethylsilyl)-diazomethane gave the (*S*)-Z-α-methylserine methylester (**6**) in 66% yield. Hydrogenolytic deprotection of the *N*-Z group of **6**, followed transesterification of the resultant (*S*)-α-methylserine methylester in BnOH and Et_3N (10:1) at 60°C, afforded (*S*)-α-methylserine benzyl ester (**7**) {colorless oil, $[\alpha]_D^{26}$ −9.0° (*c* 0.66, $CHCl_3$)} in 44% yield from **6**, as shown in Scheme 2.

Pentanoic acid **13** was also prepared by the chemoenzymatic sequence illustrated in Scheme 3. Photosensitized oxygenation of 5-methylcyclopentadiene (**8**),[15,16] followed by in situ reduction with thiourea, furnished σ-symmetric prochiral 5-methyl-2-cyclopentene-1,4-diol (**2**) in 35% yield.[17] Enzymatic acetylation of **2** with lipase AK (Amano Pharmaceutical Co. Ltd) in the



Scheme 1. Retrosynthetic analysis of (+)-conagenin (CNG, **1**).

Keywords: amino acids and derivatives; asymmetric synthesis; enzymes and enzyme reactions; natural products; reduction.
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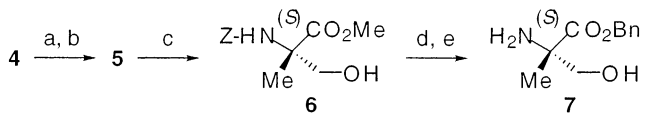


Scheme 2. (a) Porcine liver esterase (PLE)/1/15 M phosphate buffer (pH 7.0)–MeCN (10:1)/rt/12 h; (b) $LiBH_4/Et_2O$–THF (3:1)/reflux/1.5 h; (c) $TMSCHN_2$/MeOH–benzene (2:7)/rt/4 h; (d) H_2/10% Pd–C/MeOH/rt/12 h; (e) BnOH–Et_3N (10:1)/60°C/2 days.

presence of vinyl acetate as an acyl donor afforded a chiral mono ester **9** {colorless oil, $[\alpha]_D^{25}$ −76.3° (*c* 0.98, $CHCl_3$)} in 94% yield with high enantiomeric excess (99% ee).[18] The absolute configuration of **9** was determined by ^1H NMR analysis (400 MHz, $CDCl_3$) of the Mosher ester [(*R*)- and (*S*)-methoxy(trifluoromethyl)phenylacetate (MTPA)] derivatives of **9**.[19] After benzylation of **9** with BnBr in the presence of Ag_2O in 87% yield, oxidative cleavage of the double bond of the resulting benzyl ether **10** {colorless oil, $[\alpha]_D^{26}$ −4.1° (*c* 1.01, $CHCl_3$)}, followed by alkaline hydrolysis of the acetoxy group, lactonization with cyanuric chloride, and chemoselective reduction of the activated carboxyl group,[20] provided the desired primary alcohol **11** {colorless column from Et_2O, mp 61–63°C, $[\alpha]_D^{26}$ +182.4° (*c* 1.02, $CHCl_3$)} without isolation of each intermediate (23% yield, four steps). Radical-induced deoxygenation of **11** was performed using Barton's method[21] to give the corresponding chiral lactone **3** {colorless powder from Et_2O, mp 91–94°C, $[\alpha]_D^{25}$ +146.7° (*c* 1.00, $CHCl_3$)} (46% yield, two steps). The desired stereochemistry of **3** was revealed by NOE analysis (300 MHz, $CDCl_3$) as follows. When C3-H of **3** was irradiated, an NOE enhancement of the C4- and C5-Me protons was recognized, as shown in Fig. 1. A similar NOE enhancement between the C4- and C5-Me protons of **3** was also observed. Treatment of the lactone **3** with aqueous NaOH in MeOH followed by methylation with MeI gave methyl ester **12** {colorless oil, $[\alpha]_D^{26}$ +89.5° (*c* 1.00, $CHCl_3$)} in 85% yield. After benzylation of **12** with benzyl 2,2,2-trichloroacetimidate in the presence of $BF_3{\cdot}Et_2O$,[22] the resulting ester was subjected to alkaline hydrolysis to give pentanoic acid **13** {colorless oil, $[\alpha]_D^{26}$ +3.1° (*c* 1.04, $CHCl_3$)} in 63% yield from **12**.

Condensation of α-methylserine derivative **7** with pentanoic acid **13** accompanied by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) in the presence of *N*-methylmorpholine (NMM) and 1-hydroxybenzotriazole (HOBt) in DMF furnished the protected CNG **14** {colorless oil, $[\alpha]_D^{26}$ +22.7° (*c* 0.52, $CHCl_3$)} as an enantiomerically pure form in 57% yield (Scheme 4). Finally, compound **14** was submitted to catalytic hydrogenolysis on Pd–C to give CNG (**1**) {colorless powder, $[\alpha]_D^{25}$ +48.4° (*c* 0.40, MeOH), lit.[1] $[\alpha]_D^{27}$ +55.4°, lit.[10] $[\alpha]_D^{26}$ +48.7° (*c* 0.43, MeOH)} in 95% yield. The accomplishment of this total asymmetric synthesis was confirmed by the identity of all physicochemical data of the synthesized and the natural CNG (**1**).[23]

In summary, we have described a convergent and chemoenzymatic total synthesis of (+)-conagenin (CNG, **1**). This methodology will also be applicable for the synthesis of a variety of stereoisomers of CNG (**1**).

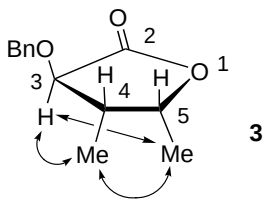
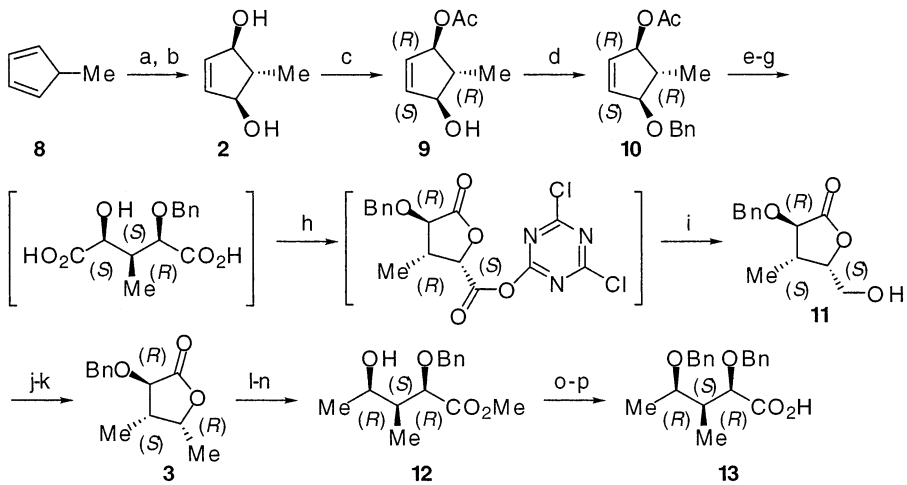
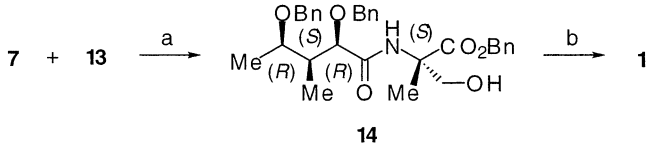


Figure 1. Selected ^1H–^1H NOE enhancements (300 MHz ^1H NMR, $CDCl_3$) for **3**.



Scheme 3. (a) $O_2/h\nu$/rose bengal/AcONa/MeOH/−78°C/80 min; (b) $(NH_2)_2CS$/MeOH/rt/13 h; (c) lipase AK/CH_2=CHOAc/THF/rt/7 h; (d) Ag_2O/BnBr/KI/toluene/rt/15.5 h; (e) $NaIO_4/KMnO_4/Na_2CO_3$/acetone–H_2O (5:6)/rt/1 h; (f) 1N NaOH/MeOH/rt/1 h; (g) H^+; (h) cyanuric chloride/NMM/DME/rt/3 h; (i) $NaBH_4$/0°C/10 min; (j) PhOCSCl/DMAP/CH_2Cl_2/rt/4 h; (k) AIBN/*n*-Bu_3SnH/toluene/75°C/1 h; (l) 1N NaOH/MeOH/rt/1 h; (m) H^+ (pH 6–7); (n) K_2CO_3/MeI/acetone/rt/3.5 h; (o) $BnOCNHCCl_3/BF_3{\cdot}Et_2O$/cyclopentane/rt/2 h; (p) 1N K_2CO_3/MeOH/reflux/2 h.



Scheme 4. (a) EDC·HCl/NMM/HOBt/DMF/rt/6 h; (b) H_2/10% Pd–C/MeOH/rt/5 days.

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23. Synthesized (+)-conagenin (CNG, **1**): ^{1}H NMR (400 MHz, CD_3OD) δ 0.93 (3 H, d, $J=7.1$ Hz), 1.22 (3 H, d, $J=6.3$ Hz), 1.50 (3 H, s), 1.89 (1 H, dq, $J=2.4$, 6.3 Hz), 3.84 (1 H, d, $J=11.0$ Hz), 3.85 (1 H, q, $J=6.1$ Hz), 4.04 (1 H, d, $J=11.0$ Hz), 4.15 (1 H, d, $J=2.4$ Hz); ^{13}C NMR (75 MHz, CD_3OD) δ 8.4, 20.0, 21.2, 43.7, 62.8, 66.1, 71.2, 75.1, 175.8, 176.9. The synthesized methyl ester of (+)-conagenin (CNG, **1**) also had spectroscopic properties identical to the authentic sample:[10] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92 (3 H, d, $J=7.1$ Hz), 1.26 (3 H, d, $J=6.3$ Hz), 1.55 (3 H, s), 1.8 (2 H, brs), 2.09 (1 H, tq, $J=2.1$, 7.1 Hz), 2.40 (1 H, brs), 3.81 (3 H, s), 3.84 (1 H, d, $J=11.2$ Hz), 3.9 (1 H, brs), 4.06 (1 H, d, $J=11.2$ Hz), 4.35 (1 H, d, $J=2.2$ Hz), 7.51 (1 H, brs); ^{13}C NMR (75 MHz, $CDCl_3$) δ 4.9, 20.5, 21.9, 40.3, 53.0, 61.9, 66.8, 72.2, 77.2, 173.0, 173.3