JOC*Note*

Synthesis of (+)-Conagenin

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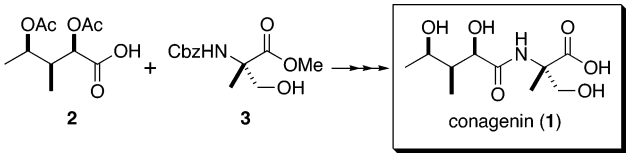
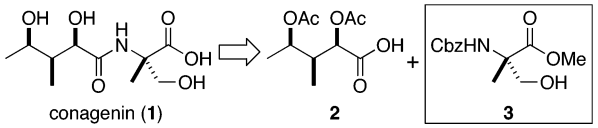


An efficient total synthesis of (+)-conagenin was achieved. The right fragment of conagenin, α-methylserine containing a quaternary stereocenter attached to nitrogen, was synthesized using allyl cyanate-to-isocyanate rearrangement. The left fragment, 2,4-dihydroxy-3-methylpentanoic acid, has three contiguous stereogenic centers, which was efficiently constructed by enantioselective monoreduction of 2-alkyl-1,3-diketones reported by Cossy, and chelation-controlled stereoselective reduction of β-hydroxy ketone. These two fragments were coupled through intramolecular amide bond formation with high efficiency.

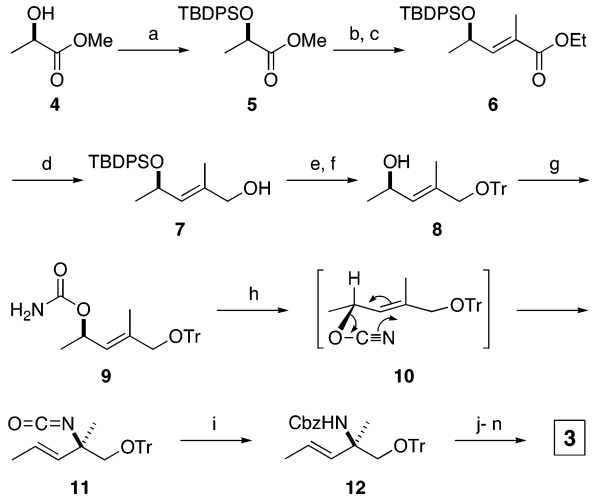
Conagenin (**1**) is a unique biologically important secondary metabolite isolated from the culture broths of *Streptomyces roseosporus* by Ishizuka and co-workers.[1] This compound stimulates activated T cells as a low molecular weight immunomodulator and was found to improve the antitumor efficacy of adriamycin and mitomycin C against murine leukemias, which suggest its potential utility for cancer chemotherapy.[2,3] Not surprisingly, therefore, conagenin has been an attractive target for the synthetic chemist, and two total syntheses of **1** have now been reported,[4a,b] in addition to formal synthesis[4c] and synthesis of its analogues.[5]

The synthesis of conagenin was to be assembled from two fragments **2** and **3** (Scheme 1), and a nitrogen-substituted quaternary stereocenter in the right α-methylserine moiety **3** was stereoselectively constructed with [3.3] sigmatropic rearrangement of allyl cyanate as represented in Scheme 2.[6]

SCHEME 1



SCHEME 2[a]



[a] Reagents and conditions: (a) TBDPSCl, imidazole, DMF, 95%; (b) DIBAL, CH_2Cl_2, −78 °C; (c) $Ph_3PC(CH_3)COOEt$, CH_2Cl_2, 85%, two steps; (d) DIBAL, CH_2Cl_2, −78 °C, 85%; (e) TrCl, pyridine, 70 °C, 98%; (f) Bu_4NF, CH_3CN, 60 °C, 95%; (g) CCl_3CONCO, CH_2Cl_2 then aq K_2CO_3/MeOH, quant; (h) PPh_3, CBr_4, Et_3N, CH_2Cl_2, −10 °C; (i) $PhCH_2ONa$, MS 4A, THF, 90%; two steps; (j) OsO_4, NMO, acetone/H_2O; (k) $NaIO_4$, THF/H_2O, 97%, two steps; (l) $NaClO_2$, 2-methyl-2-butene, KH_2PO_4, *t*-BuOH/H_2O; (m) MeI, K_2CO_3, DMF, 87%, two steps; (n) TFA, CH_2Cl_2, 92%.

The hydroxyl group of D-lactic acid methyl ester (**4**) was protected as *t*-butyldiphenylsilyl (TBDPS) ether (95% yield). Reduction of the ester **5** with diisobutylaluminum hydride (DIBAL) followed by chain extension using ethyl 2-(triphenylphosphoranylidene)propionate furnished unsaturated ester **6** exclusively in 85% yield over two steps.[7] Treatment of the ester **6** with DIBAL in dichloromethane at −78 °C afforded allyl alcohol **7** (85% yield), and protection of the hydroxyl group in **7** as a triphenylmethyl ether and removal of the TBDPS group with tetrabutylammonium fluoride gave allyl alcohol **8** (93% yield, over two steps). This allyl alcohol **8** was then transformed into

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(2) (a) Kawatsu, M.; Yamashita, T.; Osono, M.; Ishizuka, M.; Takeuchi, T. *J. Antibiot*. **1993**, *46*, 1687. (b) Kawatsu, M.; Yamashita, T.; Ishizuka, M.; Takeuchi, T. *J. Antibiot*. **1994**, *47*, 1123. (c) Ishizuka, M.; Kawatsu, M.; Yamashita, T.; Ueno, M.; Takeuchi, T. *Int. J. Immunopharmacol*. **1995**, *17*, 133.
(3) (a) Kawatsu, M.; Yamashita, T.; Ishizuka, M.; Takeuchi, T. *J. Antibiot*. **1995**, *48*, 222. (b) Hamada, M.; Yamamoto, S.; Moriguchi, S.; Kishino, Y. *J. Antibiot*. **2001**, *54*, 349. (c) Hamada, M.; Sonotake, E.; Yamamoto, S. Moriguchi, S. *J. Antibiot*. **1999**, *53*, 548.
(4) (a) Hatakeyama, S.; Fukuyama, H.; Mukugi, Y.; Irie, H. *Tetrahedron Lett*. **1996**, *37*, 4047. (b) Sano, S. Miwa, T.; Hayashi, K.; Nozaki, K.; Ozaki, Y.; Nagao, Y. *Tetrahedron Lett*. **2001**, *42*, 4029. Formal asymmetric synthesis: (c) Enders, D.; Bartsch, M.; Runsink, J. *Synthesis* **1999**, 243.
(5) Diastereomers synthesis: (a) Kovács-Kulyássa, Á.; Herczegh, P.; Sztaricskai, F. J. *Tetrahedron Lett*. **1996**, *37*, 4047. (b) Kovács-Kulyássa, Á.; Herczegh, P.; Sztaricskai, F. J. *Tetrahedron* **1997**, *53*, 13883. Synthetic study: (c) Rodrigues, J. A. R.; Moran, P. J. S.; Milagre, C. D. F.; Ursini, C. V. *Tetrahedron Lett*. **2004**, *45*, 3579.
(6) (a) Ichikawa, Y. *Synlett* **1991**, 238. (b) Ichikawa, Y.; Tsuboi, K.; Isobe, M. *J. Chem. Soc., Perkin Trans. 1* **1994**, 2791. (c) Ichikawa, Y.; Osada, M.; Ohtani, I.; Isobe, M. *J. Chem. Soc., Perkin Trans. 1* **1997**, 1449.
(7) (*Z*)-Isomer was not detectable by ^1H NMR analysis.

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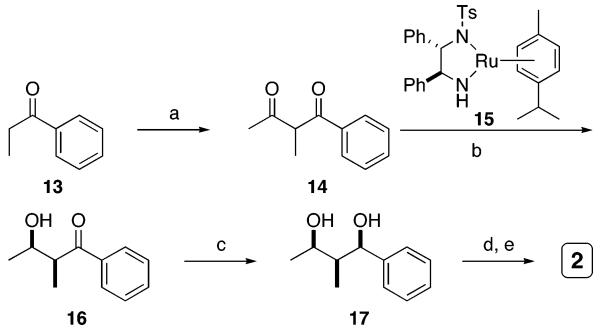
SCHEME 3[a]

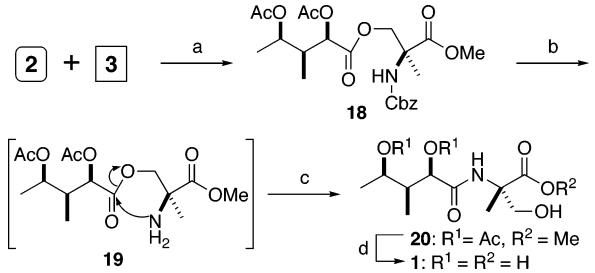


[a] Reagents and conditions: (a) Ac_2O, TsOH, $2BF_3{\cdot}3AcOH$, 89%; (b) **15**, HCOOH, Et_3N, CH_2Cl_2, 40 °C, 89%; (c) $Zn(BH_4)_2$, Et_2O, 0 °C, 70%; (d) Ac_2O, DMAP, pyridine, 98%; (e) $RuCl_3{\cdot}nH_2O$, H_5IO_6, $CCl_4/CH_3CN/H_2O$, 78%.

allyl carbamate **9** by treatment with trichloroacetyl isocyanate followed by hydrolysis of the resultant *N*-trichloroacetyl carbamate with potassium carbonate in aqueous methanol. Dehydration of allyl carbamate **9** was carried out by using the modified Appel conditions (PPh_3, CBr_4, Et_3N, CH_2Cl_2, −10 °C)[8] to generate allyl cyanate **10**, which immediately underwent [3.3] sigmatropic rearrangement at −10 °C to afford allyl isocyanate **11**. After careful workup, the resultant isocyanate **11** was immediately treated with sodium benzyl alkoxide in THF. Benzyl carbamate **12**[9] was obtained in 90% overall yield from **8**. Transformation of the double bond in **12** into the methoxycarbonyl group was accomplished in 84% overall yield by a four-step sequence: (i) catalytic osmylation with *N*-methylmorpholine *N*-oxide, (ii) diol cleavage using sodium periodate, (iii) sodium chlorite oxidation of the resultant aldehyde, and (iv) esterification with methyl iodide and potassium carbonate in DMF. Finally, removal of triphenylmethyl group with trifluoroacetic acid in dichloromethane gave the requisite α-methylserine **3**).

For the synthesis of left fragment of conagenin (Scheme 3), it was envisioned that Hatakeyama's intermediate **17**[4a] could be expeditiously accessed from 2-methyl-1,3-diketone **14**, which was prepared by Lewis acid catalyzed acylation of propiophenone (**13**) in 89% yield. Following the protocol reported by Cossy, enantioselective monoreduction of **14** using chiral ruthenium catalyst, (*S*,*S*)-Ru-[*N*-(tosyl)-1,2-diphenylethylenediamine] (*p*-cymene) (**15**),[10] proceeded smoothly to afford *syn*-2-methyl-3-hydroxyketone **16** in good 89% yield.[11] The chelation-controlled reduction of **16** using zinc borohydride in ether[12] gave the desired *syn*-1,3-diol **17** (*syn*/*anti* → 30:1),[13] which was purified by column chromatography and recrystallization

SCHEME 4[a]



[a] Reagents and conditions: (a) DCC, DMAP, HOBt, CH_2Cl_2; (b) H_2, 10% Pd−C, THF; (c) aq $NaHCO_3$, 90%, three steps; (d) 1 M K_2CO_3, MeOH, 87%.

from hexane (70% yield). After protection of the two hydroxyl groups in **17** as acetates (Ac_2O, DMAP, pyridine), the phenyl group was oxidatively cleaved with ruthenium tetraoxide using periodic acid as the stoicheometric oxidant in a biphasic system ($CCl_4/CH_3CN/H_2O$) to afford the left fragment of conagenin **2** (76% yield in two steps).[14]

Having obtained two fragments, pentanoic acid **2** and α-methylserine **3**, we next turned our attention to the amide bond construction by an intramolecular ester-to-amide exchange reaction. Condensation of **2** with **3** was carried out cleanly using dicyclohexylcarbodiimide (DCC) in the presence of DMAP and 1-hydroxybenzotriazole (HOBt) in dichloromethane to afford ester **18** (Scheme 4). Removal of the Cbz group by hydrogenolysis (H_2, 10% Pd−C, THF) and subsequent treatment of the resultant ester **19** with aqueous sodium bicarbonate gave rise to the amide **20** in 90% yield over three steps.[15] Finally, hydrolytic removal of the two acetyl and methyl ester protecting groups (1M K_2CO_3, MeOH) furnished (+)-conagenin (**1**), which exhibited spectroscopic and physical properties identical to those of the authentic samples.[16]

Experimental Section

(1*R*,2*E*)-1,3-Dimethyl-4-(trityloxy)but-2-en-1-yl carbamate (9). To a solution of allyl alcohol **8** (270 mg, 0.75 mmol) in CH_2Cl_2 (5.0 mL) at 0 °C was added trichloroacetyl isocyanate

(8) (a) Ichikawa, Y. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2135. (b) O'Neil, I. A. In *Comprehensive Organic Functional Group Transformations*; Katritzky, A., Meth-Cohn, O., Ress, C. W., Eds.; Pergamon: Oxford, 1995; Vol. 3, p 696.

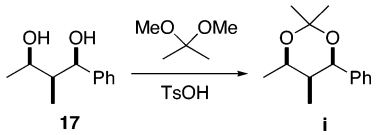
(9) Enantiomeric purity of **12** was determined to be 97% ee by ^1H NMR analysis of the MTPA esters, which were prepared by removal of triphenylmethyl group in **12** followed by esterification with (+)- and (−)-MTPA chlorides. This result indicates that [1.3]-chirality transfer of allyl cyanate **10** was achieved with excellent selectivity.

(10) Hashiguchi, S.; Fujii, A.; Takehara, J.; Ikariya, T.; Noyori, R. *J. Am. Chem. Soc.* **1995**, *117*, 7562.

(11) This highly enantio- and diastereoselective reduction of **14** has been reported by Cossy. See: Eustache, F.; Dalko, P. I.; Cossy, J. *Org. Lett.* **2002**, *4*, 1263.

(12) Nakata, T.; Tani, Y.; Hatozaki, M.; Oishi, T. *Chem Pharm. Bull.* **1984**, *32*, 1411.

(13) The stereochemistry of this reduction, initially assigned on the basis of a chelation-control model, was confirmed by ^{13}C NMR analysis of the acetonide **i** derived from 1,3-diol **17**. In general, the *syn*-1,3-diol acetonides have carbon resonances for the acetonide methyl groups at 30 and 19 ppm, while the *anti*-isomers show methyl resonances around 25 ppm. The ^{13}C NMR spectra of **i** showed two methyl signals at 19.6 and 30.1 ppm, which unambiguously determined the configuration of **17**. See: (a) Rychnovsky, S. D.; Skalitzky, D. J. *Tetrahedron Lett.* **1990**, *31*, 945. (b) Evans, D. A.; Rieger, D. L.; Gage, J. R. *Tetrahedron Lett.* **1990**, *31*, 7099..



(14) (a) Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936. (b) Nu-ez, M. T.; Martin, V. S.; *J. Org. Chem.* **1990**, *55*, 1928.

(15) Similar intramolecular acyl migration using byproduct was noted in the previous synthesis of conagenin, and our experiments followed its procedure. See ref 4a.

(16) The NMR analysis of synthetic conagenin (**1**) in D_2O was complicated by sample dependent variability in the chemical shifts of the methylene protons in the α-methylserine unit. This problem was avoided by measurement of the spectrum in CD_3OD.

(180 μL, 1.51 mmol), and the reaction mixture was stirred at 0 °C for 30 min. To this solution was added an aqueous potassium carbonate (410 mg in 1.0 mL of water) and MeOH (4.0 mL), and CH_2Cl_2 was removed by evaporation. The reaction mixture was stirred at room temperature for 2 h and then diluted with AcOEt. The mixture was washed with H_2O, and the combined aqueous layer was extracted with AcOEt. The combined organic layers were washed with brine, 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 and 1:3) to afford allyl carbamate **9** (300 mg, quant) as a colorless gum: $[\alpha]^{17}_D = +12.1$ (*c* 1.92, $CHCl_3$); IR (KBr) ν_{max} = 3486, 3347, 1718 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.32 (3H, d, J = 6.0), 1.67 (3H, s), 3.50 (2H), 4.62 (2H), 5.56 (1H, dq, J = 9.0, 6.5), 5.62 (1H, d, J = 9.0), 7.20−7.47 (15H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.5, 21.1, 68.1, 68.5, 86.8, 124.7, 127.0, 127.8, 128.6, 136.2, 144.1, 156.5; HRMS (FAB) calcd for $C_{26}H_{28}NO_3$ $[M + H]^+$ 402.2069, found 402.2057.

Benzyl {(1*R*,2*E*)-1-Methyl-1-[(trityloxy)methyl]but-2-en-1-yl}carbamate (12). To a solution of allyl carbamate **9** (96 mg, 0.24 mmol), triphenylphosphine (157 mg, 0.60 mmol), and triethylamine (120 μL, 0.87 mmol) in CH_2Cl_2 (3.0 mL) cooled to −10 °C was added a solution of carbon tetrabromide (222 mg, 0.67 mmol) in CH_2Cl_2 (0.50 mL). After being stirred at −10 °C for 30 min, the reaction mixture was diluted with hexane (20 mL). The resulting reaction mixture was washed with H_2O, 1 M $KHSO_4$, aqueous saturated $NaHCO_3$, and brine and dried over Na_2SO_4. Concentration under reduced pressure gave crude allyl isocyanate **11**, which was immediately dissolved in THF (2.0 mL). Powdered molecular sieves 4A were added to the solution, and the resulting suspension was treated at 0 °C with a solution of sodium benzyl alkoxide [prepared from benzyl alcohol (52 mg, 0.48 mmol) and sodium hydride (20 mg, 60% dispersion in mineral oil, 0.50 mmol) in 1.0 mL of THF]. After being stirred at room temperature for 1 h, the reaction mixture was diluted with AcOEt, washed with saturated NH_4Cl and brine, and dried over Na_2SO_4. Concentration under reduced pressure and purification by column chromatography on silica gel eluting with AcOEt/hexane (0:1 and 1:20) furnished benzyl carbamate **12** (100 mg, 85%) as a colorless syrup: $[\alpha]^{20}_D = +5.7$ (*c* 1.00, $CHCl_3$); IR (KBr) ν_{max} = 3428, 3354, 1731 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.44 (3H, s), 1.68 (3H, d, J = 4.4), 3.05 (1H, d, J = 9.0), 3.13 (1H, d, J = 9.0), 5.04 (2H, s), 5.14 (1H, s), 5.46−5.60 (2H), 7.20−7.44 (20H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 17.8, 22.3, 56.4, 66.1, 69.0, 86.5, 124.6, 127.0, 127.8, 127.9, 128.0, 128.4, 128.7, 133.8, 136.7, 143.7, 154.7; HRMS (FAB) calcd for $C_{33}H_{34}NO_3$ $[M + H]^+$ 492.2534, found 492.2527.

Methyl *N*-[(2*R*,3*S*,4*R*)-2,4-Diacetyloxy-3-methylvaleryl]-2-methyl-L-serinate (20). Dicyclohexylcarbodiimide (35 mg, 0.17 mmol) was added to a solution of α-methylserine (**3**) (30 mg, 0.11 mmol), pentanoic acid (**2**) (30 mg, 0.13 mmol), and HOBt (23 mg, 0.17 mmol) in CH_2Cl_2 (1.0 mL) at room temperature. After the mixture was stirred for 30 min, 4-(dimethylamino)pyridine (16 mg, 0.13 mmol) was added in one portion. After being stirred at room temperature for 1 h, the reaction mixture was filtered and concentrated under reduced pressure. Purification by column chromatography on silica gel eluting with AcOEt/hexane (1:3) gave crude ester **18**, which was dissolved in THF (2.0 mL). Palladium on carbon (10%, 10 mg) was added, and the solution was stirred vigorously under hydrogen atmosphere for 30 min. After the deprotection of the Cbz-group in **18** was checked by TLC analysis, aqueous $NaHCO_3$ (0.20 mL) was added. After being stirred at room temperature for 10 h, the reaction mixture was filtered and concentrated under reduced pressure. Purification by column chromatography on silica gel eluting with AcOEt/hexane (1:1 and 2:1) gave the protected conagenin (**20**) (35 mg, 90%, three steps) as a clear gum: $[\alpha]^{21}_D = +32.9$ (*c* 0.35, $CHCl_3$); IR (KBr) ν_{max} = 3404, 1739, 1683 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.02 (3H, d, J = 7.1), 1.26 (3H, d, J = 6.3), 1.55 (3H, s), 2.06 (3H, s), 2.18 (3H, s), 2.28 (1H, qt, J = 7.1, 5.4), 3.27 (1H), 3.80 (3H, s), 3.82 (1H, dd, J = 11.0, 6.5), 4.13 (1H, dd, J = 11.0, 4.5), 4.99 (1H, d, J = 11.5), 5.02 (1H, qd, J = 6.3, 5.4), 7.14 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 9.6, 18.0, 19.6, 20.8, 21.2, 39.7, 53.0, 62.4, 65.4, 71.0, 75.1, 168.9, 170.4, 170.9, 173.5; HRMS (FAB) calcd for $C_{15}H_{26}NO_8$ $[M + H]^+$ 348.1658, found 348.1667.

(+)-Conagenin (1). To a solution of protected conagenin (**20**) (35 mg, 0.10 mmol) in MeOH (1.20 mL) cooled to 0 °C was added aqueous K_2CO_3 (1.0 M, 0.40 mL), and the cooling bath was removed. After being stirred at room temperature for 2 h, the reaction mixture was neutralized with aqueous $KHSO_4$ (1.0 M, 1.0 mL). The reaction mixture was concentrated under reduced pressure to afford the residue, which was purified by ODS column chromatography (Cosmosil 75 C_{18}-OPN, H_2O followed by 19:1 H_2O/MeCN as eluent) to furnish (+)-conagenin (22 mg, 87%) as a colorless crystal: mp 153−155 °C; $[\alpha]^{20}_D = +56.8$ (*c* 0.44, MeOH) (lit.[1] $[\alpha]^{27}_D = +55.4$); IR (KBr) ν_{max} = 3488, 3369, 3325, 3059, 1703, 1635, 1530, 1457, 1250 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 0.94 (3H, d, J = 7.0), 1.22 (3H, d, J = 6.0), 1.51 (3H, s), 1.89 (1H, qdd, J = 7.0, 6.0, 2.5), 3.82 (1H, d, J = 11.0), 3.85 (1H, quint, J = 6.0), 4.02 (1H, d, J = 11.0), 4.16 (1H, d, J = 2.5), 8.10 (1H, s, exchanged with CD_3OD); ^{13}C NMR (CD_3OD, 100 MHz) δ 8.2, 19.9, 21.2, 43.7, 62.5, 66.0, 71.2, 75.2, 175.8, 176.4; HRMS (FAB) calcd for $C_{10}H_{20}NO_6$ $[M + H]^+$ 250.1291, found 250.1286.

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Supporting Information Available: Experimental procedures and spectral data for all relevant compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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