Direct Synthesis of Chrysosplenol D

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An aldol condensation and an Algar−Flynn−Oyamada oxidative cyclization were key steps in the direct synthesis of chrysosplenol D, an efflux pump inhibitor that can potentiate the activity of commercially important antibiotics and antimalarials.

Chrysosplenol D (3′,4′,5-trihydroxy-3,6,7-trimethoxyflavone) (**1**) was initially isolated from the Chinese medicinal plant *Artemisia annua* L. (Asteraceae).[1] Later, it was characterized both from *Ehretia amoena*, a plant traditionally used in Uganda to treat sleeping sickness, and from *Vitex trifolia*, another Chinese folk medicine plant used to treat cancer.[2] It was also found in *Plectranthus cylindraceus* and in the African medicinal plant *Psiadia trinervi*a.[3] Chrysosplenetin (**2**) and vitexicarpin (**3**) are also members of this class of naturally occurring 3-methoxyflavones.[4,5]

The strong interest in chrysosplenol D stems from its ability to potentiate the already potent antimalarial activity of artemisinin and from its ability to potentiate the activity of norfloxacin. Presumably this activity is due to its inhibition of the multidrug resistance (MDR) efflux pumps in *Plasmodium falciparum* and in *Staphylococcus aureus.*[6] One limitation to the further evaluation of **1** is its low natural abundance. As part of our program to enhance plant-derived natural products,[7] we report herein a direct total synthesis of chrysosplenol D (**1**). Our strategy involved the synthesis of a chalcone intermediate, readily generated via an aldol condensation of ketone **4** with aldehyde **5**,[8] that would be subjected to oxidative cyclization, methylation, and deprotection.

Ketone **4** was synthesized by Friedel−Crafts acylation of commercially available 3,4,5-trimethoxyphenol.[9] An aldol reaction between aldehyde **5** and ketone **4** gave the known chalcone **6** in 81% yield as shown in Scheme 2.[10] Excess ketone was used for complete consumption of aldehyde in order to avoid purification problems due to the similar R_f values of **5** and **6** as well as the poor solubility of chalcone **6**. Oxidative cyclization of chalcone **6** was challenging. Apparently, the Algar−Flynn−Olyamada (AFO) reaction does not work well for the synthesis of *o*-methoxyflavones.[11] However, using excess hydrogen peroxide and sodium hydroxide and extending the reaction time, we were able to realize the cyclization of chalcone **6**.

Although we expected to obtain a flavonol, the usual product in the AFO oxidation, dihydroflavonol **7**, was formed in this reaction in 60% yield. Iodine oxidation[12] of **7** produced **8**, which was methylated to afford 3-methoxyflavone **9** in 77% yield. This compound had been previously prepared by Horie using the Allan−Robinson flavone synthesis.[13] Aluminum tribromide-mediated selective demethylation of **9** and debenzylation of **10** using a catalytic amount of 10% Pd−C afforded chrysosplenol D (**1**) in 84% yield from **9**. The ^{1}H NMR spectrum matched the literature spectrum.

In summary, we have achieved a direct synthesis of the biologically important 3-methoxyflavone chrysosplenol D. The synthesis of **1** was achieved in six steps from **5** in 31% overall yield. This synthetic route will make possible extensive evaluation of the efflux pump inhibitor **1**.

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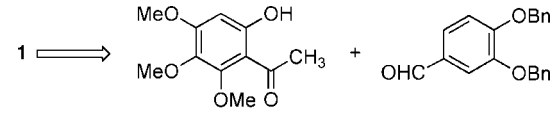
Scheme 1. Retrosynthetic Plan



Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded on either a Varian 300 MHz or a Varian 400 MHz Fourier transform NMR spectrometer. HRMS were recorded on a Kratos model MS-50 spectrometer, and LRMS were performed with a Finnigan TSQ700 mass spectrometer. All experiments were performed under an Ar atmosphere unless otherwise noted.

Compound 6. To a stirred solution of ketone **4** (1.09 g, 4.8 mmol) and aldehyde **5** (1.27 g, 4.00 mmol) in EtOH (40 mL) at 0 °C was added freshly powdered KOH (86.5%, 1.30 g, 20 mmol). The mixture was slowly allowed to warm to rt and stirred for 56 h. The solvent was evaporated to approximately one-fifth the original volume (∼8−10 mL). Ice-cold H_2O (∼2 mL) was added, and the mixture was neutralized with 2 N HCl. It was then extracted with EtOAc (3 × 50 mL) and dried over Na_2SO_4. The solvent was evaporated, and the crude residue was purified by Si gel cc (hexanes/EtOAc, 3:1) to yield compound **6** (1.70 g, 81%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.78 (s, 2H), 7.52−7.31 (m, 10H), 7.25−7.19 (m, 2H), 6.96 (d, 1H, J = 8.4 Hz), 6.31 (s, 1H), 5.24 (s, 2H), 5.22 (s, 2H), 3.89 (s, 3H), 3.87 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 192.7, 162.7, 160.1, 155.0, 151.2, 148.9, 143.5, 137.0, 136.8, 135.3, 128.8, 128.7, 128.0, 127.3, 127.2, 124.5, 123.5, 114.2, 114.1, 108.8, 96.7, 71.4, 70.9, 61.9, 61.3, 56.1; LRMS (EI) *m*/*z* 526 (M^+), 435, 318; HRMS (EI) calcd for $C_{32}H_{30}O_7$ 526.1992, found 526.2002.

Compound 7. To a stirred solution of chalcone **6** (527 mg, 1 mmol) in MeOH (45 mL) at 0 °C was added 10% aqueous NaOH (15 mL) followed by 30% aqueous H_2O_2 (10 mL). The mixture was stirred at 0 °C for 1 h, then slowly allowed to warm to rt and stirred for 16 h. Cold H_2O (∼1 mL) was added, and then it was acidified with 2 N aqueous HCl. Solid NaCl was added to the solution, and the mixture was then extracted with CH_2Cl_2 (5 × 50 mL). The combined organic phase was dried over Na_2SO_4. The solvent was evaporated and the crude residue was purified by Si gel cc (hexanes/EtOAc, 1:1) to yield the dihydroflavonol **7** (325 mg, 60%) as a yellowish solid: mp 168−170 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.50−7.32 (m, 10H), 7.19−6.95 (m, 3H), 6.32 (s, 1H), 5.20 (s, 4H), 4.96 (d, 1H, J = 12.3 Hz), 4.45 (d, 1H, J = 12.0 Hz), 4.00 (s, 3H), 3.88 (s, 3H), 3.85 (s, 3H); LRMS (EI) *m*/*z* 542 (M^+), 360, 334 (100%), 303, 269, 181; HRMS (EI) calcd for $C_{32}H_{30}O_8$: 542.1941, found: 542.1948.

Compound 8. To a stirred mixture of dihydroflavonol **7** (108 mg, 0.2 mmol) and anhydrous KOAc (884 mg, 9 mmol) in glacial acetic acid (7 mL) at 0 °C was added dropwise I_2 (115 mg, 0.45 mmol) dissolved in HOAc (7 mL) over 20 min. The mixture was then boiled for 5 h. It was cooled to rt, and ice-cold H_2O (∼20 mL) was added. The excess I_2 was removed by addition of solid $Na_2S_2O_7$. It was extracted with EtOAc (3 × 40 mL), and the combined organic phases were dried over Na_2SO_4. The solvent was evaporated and the crude residue was purified by Si gel cc (hexanes/EtOAc, 1:1) to yield flavonol **8** (84 mg, 78%) as a yellowish oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ

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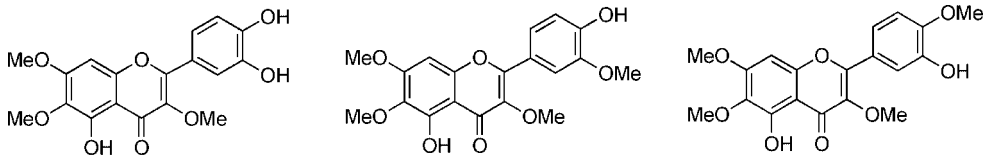
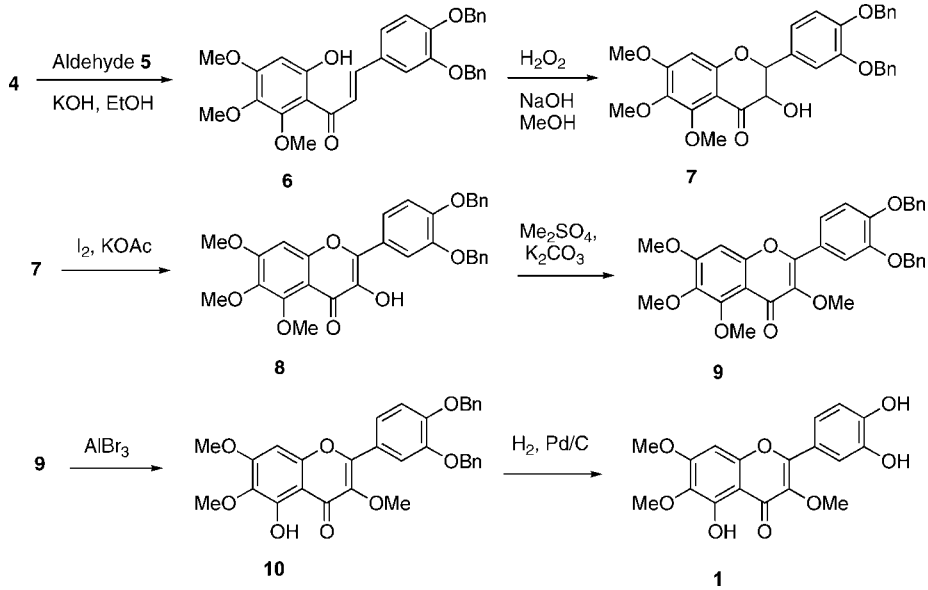


Figure 1. Structures of **1**−**3**.

Scheme 2. Synthesis of Chrysosplenol D



7.89 (d, 1H, J = 1.5 Hz), 7.79 (d, 1H, J = 8.7 Hz), 7.54−7.31 (m, 10H), 7.05 (d, 1H, J = 8.4 Hz), 6.73 (s, 1H), 5.27 (s, 2H), 5.26 (s, 2H), 4.03 (s, 3H), 4.00 (s, 3H), 3.93 (s, 3H); LRMS (EI) *m*/*z* 540 (M^+), 449, 421, 333 (100%); HRMS (EI) calcd for $C_{32}H_{28}O_8$ 540.1784, found 540.1792.

Compound 9. To a stirred mixture of flavonol **8** (60 mg, 0.11 mmol) and anhydrous K_2CO_3 (138 mg, 1 mmol) in dry acetone (3 mL) at rt was added dropwise Me_2SO_4 (101 mg, 1 mmol). The mixture was refluxed for 9 h. The acetone was evaporated and the residue was purified by Si gel cc (initially, hexanes; then hexanes/EtOAc, 1:1) to yield compound **9** (59 mg, 97%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.77 (d, 1H, J = 2.0 Hz), 7.66 (dd, 1H, J = 9.8, 2.2 Hz), 7.51−7.48 (m, 4H), 7.42−7.38 (m, 4H), 7.36−7.32 (m, 2H), 7.03 (d, 1H, J = 8.8 Hz), 6.70 (s, 1H), 5.27 (s, 4H), 4.00 (s, 3H), 3.97 (s, 3H), 3.92 (s, 3H), 3.72 (s, 3H); LRMS (EI) *m*/*z* 554 (M^+), 539, 463 (100%); HRMS (EI) calcd for $C_{33}H_{30}O_8$ 554.1941, found 554.1950.

Compound 10. To a mixture of 3-methoxyflavone **9** (55 mg, 0.1 mmol) in MeCN (1 mL) at 0 °C was added dropwise a solution of $AlBr_3$ (186 mg, 0.7 mmol) in MeCN (3.5 mL). The mixture was stirred at 0 °C for 20 min, and then 2% aqueous HCl solution (7.5 mL) was added. The solution was refluxed at 75 °C for 25 min and then cooled to rt. The solvent was evaporated, and the residue was then extracted with CH_2Cl_2 (2 × 25 mL) and dried over Na_2SO_4. The solvent was evaporated and the residue was purified by chromatography on Si gel (hexanes/EtOAc, 3:2) to yield flavone **10** (49 mg, 91%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.77 (d, 1H, J = 3.0 Hz), 7.67 (dd, 1H, J = 7.5, 3.0 Hz), 7.51−7.47 (m, 4H), 7.44−7.33 (m, 6H), 7.04 (d, 1H, J = 9.0 Hz), 6.44 (s, 1H), 5.29 (s, 2H), 5.27 (s, 2H), 3.96 (s, 3H), 3.92 (s, 3H), 3.71 (s, 3H); LRMS (EI) *m*/*z* 540 (M^+), 449; HRMS (EI) calcd for $C_{32}H_{28}O_8$ 540.1784, found 540.1792.

Chrysosplenol D (1). A mixture of 3-methoxyflavone **10** (16 mg, 0.03 mmol) and a catalytic amount of 10% Pd/C in MeOH/EtOAc (6 mL, 1:1) was stirred in H_2 atmosphere at rt for 45 min. The mixture was purified by Si gel cc (hexanes/EtOAc, 1:1) to yield the natural product **1** (10 mg, 92%) as a sticky yellowish solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 12.43 (brs, 1H), 7.90 (d, 1H, J = 2.0 Hz), 7.59 (dd, 1H, J = 8.4, 2.0 Hz), 7.03 (d, 1H, J = 8.8 Hz), 6.53 (s, 1H), 3.97 (s, 3H), 3.93 (s, 3H), 3.83 (s, 3H); LRMS (EI) *m*/*z* 360 (M^+, 100%), 345, 317; HRMS (EI) calcd for $C_{18}H_{16}O_8$ 360.0845, found 360.0853.

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