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Concise synthesis of Cannabisin G

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ABSTRACT

Cannabisin G (**1**), a naturally occurring lignanamide, was synthesized in 45% overall yield starting from 3-*tert*-butyl ethyl ferulate (**6**). An oxidative coupling by potassium ferricyanide in an alkaline media serves as the key step to construct the biphenylbutadiene skeleton of **1** with high regioselectivity.

Cannabisin G (**1**), a naturally occurring lignanamide, was isolated from the fruits of *Cannabis sativa* in 1995.[1] It has been used as a purgative in China and Japan. As shown in Figure 1, structurally, Cannabisin G and its other analogs such as Cannabisin D (**2**),[2] grossamide (**3**)[3] and Cannabisin E (**4**),[1] are the dimeric forms of *N*-*trans*-feruloyltyramine (**5**), formed via a single-electron oxidative coupling in vivo. The most concise biosynthetic approach is the direct oxidative dimerization of **5** (Fig. 2). However, literature reports[1,4] indicated that this approach was complicated due to the lack of regioselectively as several coupling modes[5] such as 8–5, 8–8 and 8-*O*-4 couplings could take place; furthermore, cyclization reactions could also occur making this approach impractical. As an example, the 8–8 coupling with further cyclization produces **2** while the similar 8–5 coupling produces **3**; both **2** and **3** were the dominant products formed when $FeCl_3$[1] was used as the reagent. The use of other oxidants such as horseradish peroxidase (HRP)-H_2O_2,[4] and $Cu(NO_3)_2{\cdot}3H_2O$[4] gave similar results. In all cases, only trace amounts of **1** were detected. Up till now, no other synthetic pathway toward **1** has been reported.

Recent work in our laboratory has been directed toward the synthesis of important natural products such as lignans[6,7] and stilbene dimers[8] using regioselective phenolic couplings. We introduced a *tert*-butyl as the protecting group into our coupling precursors which has greatly improved the 8–8 couplings in the lignan and stilbene dimer syntheses and significantly enhanced the yields of the desired products. In this study, we continued to apply the same strategy to synthesize Cannabisin G with outstanding regioselectivity and high yield.

As discussed above, there are two problems associated with the oxidative coupling of *N*-trans-feruloyltyramine: one is the regioselectivity of the oxidative coupling and another is the subsequent cyclization. We hope that the application of our *tert*-butyl protecting strategy would improve the yields of the desired 8–8 coupled products. When examining the conditions reported in the literature,[1,4] we discovered that all the reactions were carried out in acidic ($FeCl_3$ and $Cu(NO_3)_2{\cdot}3H_2O$) or neutral (horseradish peroxidase) conditions to produce the acyclic lignanamides including the desired **1**, but the subsequent cyclizations consumed the initially formed **1** making the reaction mixture complex.[1] We speculated that the 8–8 coupled product of quinone can give the **1** and **2** under alkaline and acidic conditions respectively, on the other hand, **1** is probably unstable under acidic conditions thus rearrange to **2** as shown in Figure 3. Therefore, we planned to carry out the coupling in alkaline conditions using potassium ferricyanide as the oxidant.

As depicted in Figure 4, our initial strategy involved the direct oxidative coupling of amide **7** that bears a *tert*-butyl group at C-3 and a subsequently debutylation of coupled dimer **8**. Compound **7** was successfully prepared from the 3-*tert*-butyl ferulic acid (**6**)[7] with tyramine hydrochloride using DCC as the reagent. Unfortunately, oxidative coupling reaction of **7** using alkaline potassium ferricyanide gave a complicated mixture, possibly due to the extraneous active phenolic substitute on the tyramine moiety. We therefore abandoned this route.

A revised synthetic strategy is formulated and illustrated in Figure 5. In the new route, we decided to use 3-*tert*-butyl ethyl

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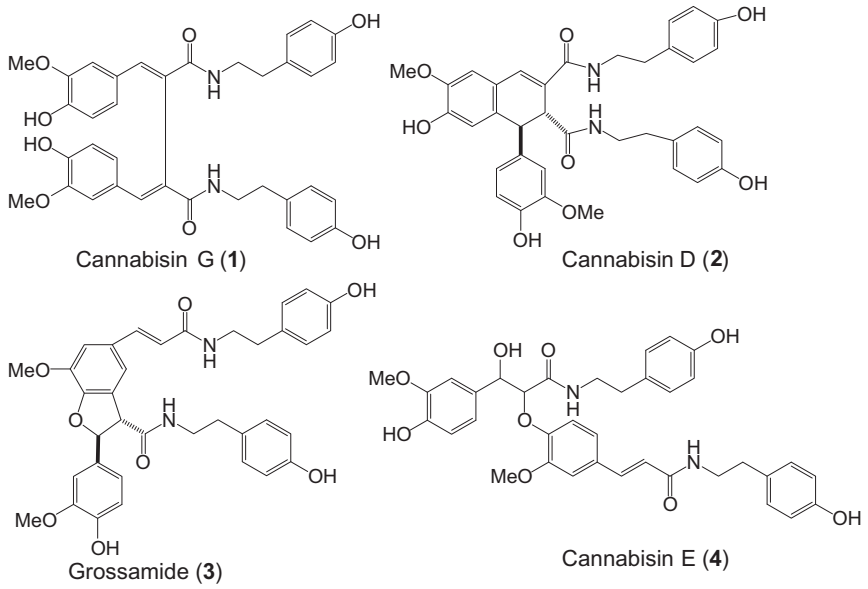

Figure 1. Chemical structures of lignanamides **1**, **2**, **3**, and **4**.

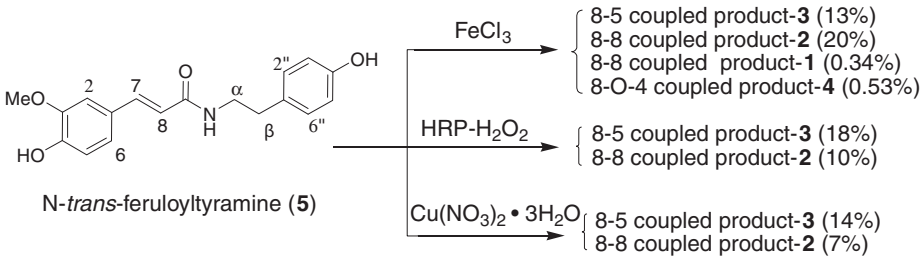

Figure 2. Product distribution profile from oxidation of **5**.[1,4]

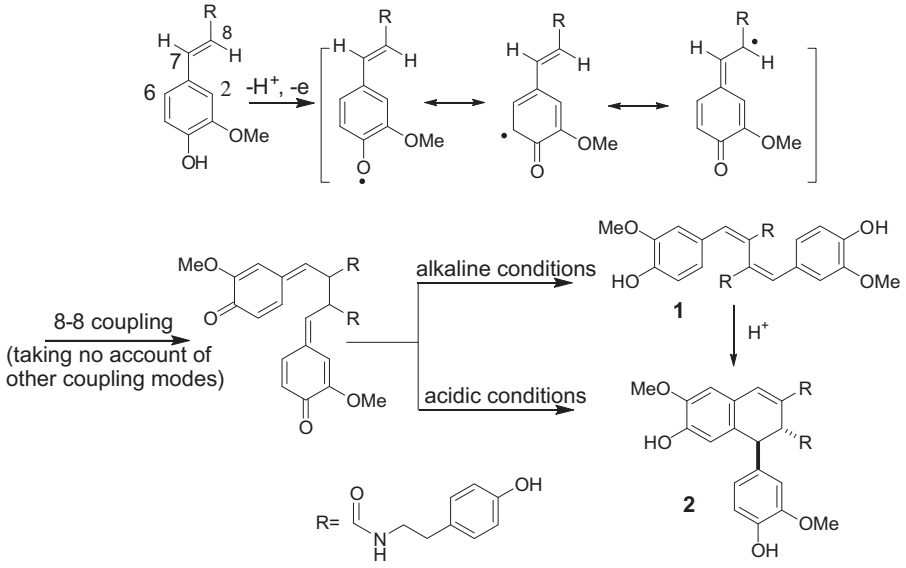

Figure 3. Purposed mechanism for 8–8 coupling in alkaline and acidic conditions.[5]

ferulate (**9**) as the coupling precursor and subsequently introduce the tyramine moieties to the coupled intermediate. Compound **9** was reported in our previous work.[6] As expected, the oxidation of **9** in a benzene–water two-phase system using alkaline potassium ferricyanide as the oxidation reagent afforded regioselectively the desired 8–8 coupling product **10** in excellent yield. The *tert*-butyl group was then successfully removed from **10** via a Friedel–Crafts reaction using $AlCl_3$ as the regent in moderate yield.[9]

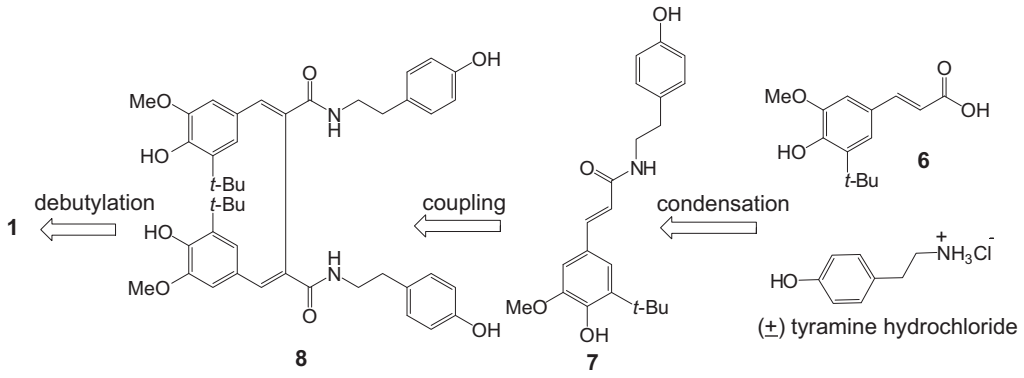

Figure 4. A failed trial path to prepare **1**.

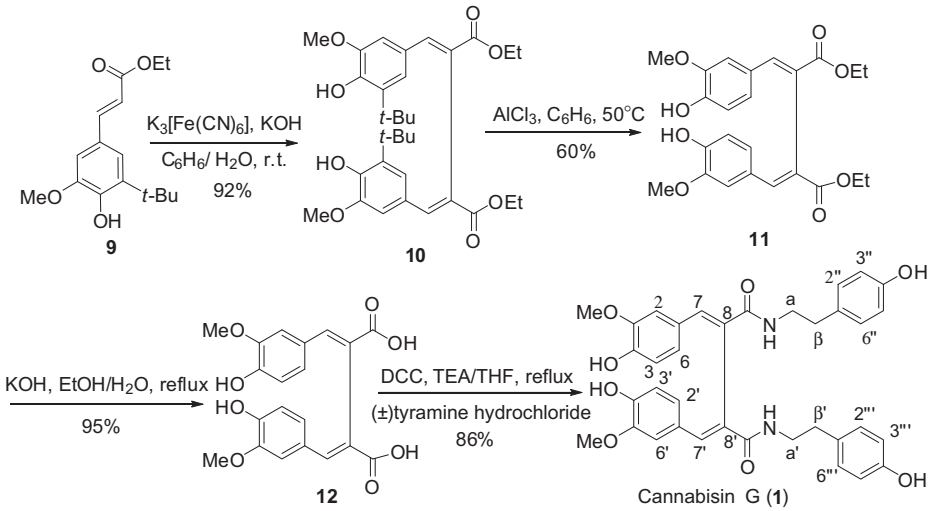

Figure 5. The optimized path to synthesize **1**.

The ethyl esters of **11** were saponified using KOH in refluxing aqueous EtOH to produce the intermediate diacid **12** in 95% yield. Condensation of the diacid with tyramine hydrochloride in refluxing THF using DCC as the coupling reagent afforded the targeted product **1** in high yields. All spectral data of **1** obtained were in good agreement with those reported in literature.[1]

In conclusion, a facile method for the synthesis of Cannabisin G (**1**) was reported by using a highly regioselective coupling reaction, the benefit of using *tert*-butyl as a transient protecting group is cleared demonstrated by suppressing the undesired coupling modes. Furthermore, we have effectively eliminated the subsequent cyclizations by conducting the coupling in an alkaline media using potassium ferricyanide as the reagent. The overall yield was 45%. We expect that the new synthetic strategy could be applied to the synthesis of other lignanamides with similar structures.

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General experimental procedures

10. Structural determinations of the isolated compounds were based on ^{1}H, ^{13}C NMR, MS, IR, and UV analysis. All NMR spectra were recorded on a Varian Mercury 300 or 400 MHz instrument. EIMS (70 eV) spectra were obtained on a VG ZAB-HS spectrometer. FAB HRMS spectra were obtained on an Autostec-3090 mass spectrometer. Melting points were measured on a Kofler hot stage without calibration. IR spectra were obtained on a Nicolet NEXUS 670 FT-IR spectrometer. UV spectra were measured using TU-1901 UV–vis spectrophotometer. All solvents were ACS grade or higher and freshly purified and dried by standard techniques prior to use as needed. Silica gel (200–300 mesh) was purchased from QingDao Marine Chemical Co. (QingDao, China).

Synthesis of E-3-(3-tert-butyl-4-hydroxy-5-methoxyphenyl)-N-2-[4-hydroxylphenylethyl]-2-propenamide (**7**)*:* TEA (5 mL) was added dropwise to a suspension of (±) tyramine hydrochloride (345 mg, 0.20 mmol) in THF (20 mL), then compound **6** (500 mg, 0.20 mmol) and DCC (412 mg, 0.20 mmol) were added to the solution. The reaction mixture was stirred at room temperature for 2 h, and then the solvent was evaporated in vacuo. The residue was purified by column chromatography using petroleum ether and ethyl acetate (3:1, v/v) as eluent to afford **7** (605 mg, 82%) as an amorphous solid; ^{1}H NMR (CD_3COCD_3, 300 MHz) δ 1.39 (9H, s, $C(CH_3)_3$), 2.76 (2H, t, J = 7.2 Hz, H-β), 3.51 (2H, dd, J = 7.2, 13.2 Hz, H-α), 3.86 (3H, s, OCH_3), 6.54 (1H, d, J = 15.6 Hz, H-7), 6.77 (2H, d, J = 8.1 Hz, H-3″, H-5″), 7.06 (2H, d, J = 8.1 Hz, H-2″, H-6″), 7.07 (2H, s, H-2, H-6), 7.37 (1H, br t, J = 13.2 Hz, NH), 7.49 (2H, d, J = 15.6 Hz, H-7); ^{13}C NMR (CD_3COCD_3, 75 MHz) δ 30.1 (CH_3, $C(CH_3)_3$), 35.2 (C, $C(CH_3)_3$), 35.7 (CH_2, C-β), 41.9 (CH_2, C-α), 56.3 (CH_3, OCH_3), 108.4 (CH, C-2), 116.0 (CH, C-3″, C-5″), 119.4 (CH, C-6), 120.4 (CH, C-8), 126.6 (C, C-1), 130.4 (CH, C-2″, C-6″), 130.9 (C, C-1″), 136.1 (C, C-5), 141.1 (CH, C-7), 147.3 (C, C-2), 148.4 (C, C-3), 156.7 (C, C-4″), 166.6 (C, C═O); EIMS *m*/*z*: 369 (M^+), 354, 260, 151,137.

Synthesis of E-3-(3-tert-butyl-4-hydroxy-5-methoxyphenyl)-propenoate (**9**)*:* Compound **9** was prepared in three steps in 65% overall yield using commercially available creosol as the starting material.[6]

Synthesis of diethyl (E,E)-bis(3-tert-butyl-4-hydroxy-5-methoxybenzylidene) succinate (**10**)*:* Compound **9** (200 mg, 0.72 mmol) in benzene (7.2 mL) was vigorously stirred with an aqueous solution (1.45 mL) containing potassium ferricyanide (600 mg) and potassium hydroxide (220 mg) for 0.5 h under nitrogen. The organic layer was washed with water, brine, and dried over $MgSO_4$. The solvent was evaporated under vacuum and the residue was purified by column chromatography using petroleum ether and ethyl acetate (5:1, v/v) as eluent to afford **10** (183 mg, 92%) as a yellow oil; IR (KBr) ν_{max}: 3412, 2957, 1700, 1366, 978 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.10 (6H, t, J = 6.9 Hz, CH_3CH_2), 1.34 (18H, s, $C(CH_3)_3$), 3.77 (6H, s, OCH_3), 4.14 (4H, q, J = 6.9 Hz, CH_3CH_2), 6.17 (2H, s), 7.03 (2H, s), 7.11 (2H, s), 7.85 (2H, s, H-7, H-7′). EIMS m/z 554 (M^+), 278, 57. HRMS: m/z 577.2790 (calcd for $C_{32}H_{42}O_8$ + Na, 577.2772).

Synthesis of diethyl (E,E)-bis(4-hydroxy-3-methoxybenzylidene) succinate (**11**)*:* To a solution of compound **10** (1.8 g, 0.32 mmol) in dry benzene (40 mL) was added $AlCl_3$ (8 equiv, 3.5 g) at 50 °C. The reaction mixture was stirred for 1 h, and then quenched with ice-water. After extraction with benzene, the combined organic layers were washed with brine and dried over Na_2SO_4. After evaporation under vacuum, the residue was purified by column chromatography using petroleum ether and ethyl acetate (3:1, v/v) as eluent to afford **11** (858 mg, 60%) as an amorphous solid; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.11 (6H, t, J = 6.9 Hz, CH_3CH_2), 3.74 (6H, s, OCH_3), 4.15 (4H, q, J = 6.9 Hz, CH_3CH_2), 6.84 (2H, d, J = 8.1 Hz, H-5, H-5′), 7.06 (2H, d, J = 8.1 Hz, H-6, H-6′), 7.86 (2H, s, H-2, H-2′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 13.9 (CH_3, CH_3CH_2O), 55.5 (CH_2, CH_3CH_2O), 60.9(CH_3, OCH_3), 111.3 (CH, C-2, C-2'), 114.5 (CH, C-5, C-5′), 124.6 (CH, C-6, C-6′), 124.5 (C, C-1, C-1′), 127.1 (C, C-8, C-8′), 142.2 (CH, C-7, C-7′), 146.4 (C, C-4, C-4′), 147.3 (C, C-3, C-3′), 167.3 (C, C═O); EIMS m/z 422 (M^+), 296, 260, 151, 137.

Synthesis of 4,4′-dihydroxy-3,3′-dimethoxy-β,β′-bicinnamic acid (**12**)*:* Compound **11** (1.0 g, 0.23 mmol) was dissolved in EtOH–H_2O (4:1, v/v, 50 mL) and KOH (0.39 g, 3.5 equiv) was added. The reaction mixture was refluxed for 8 h. EtOH was removed under reduced pressure and the syrup was diluted with H_2O (5 mL). The mixtures were acidified to pH 1 using HCl (2.0 N) in an ice-bath. White solid precipitated from the solution. The solid was extracted with ethyl ether (5 × 15 mL). Evaporation of the ether solution afforded the crude product which was recrystallized from EtOH–H_2O to yield the diacid **12** (0.88 g, 96%) as white needles. Mp: 171–172 °C; ^1H NMR (DMSO, 300 MHz) δ 3.64 (6H, s, OCH_3), 6.71 (2H, dd, J = 4.8, 5.1 Hz, H-5, H-5′), 7.01 (2H, dd, J = 1.8, 4.8 Hz, H-6, H-6′), 7.19 (2H, d, J = 1.8 Hz, H-2, H-2′), 7.65 (2H, s, H-7, H-7′), 9.56 (2H, s, COOH); ^{13}C NMR (DMSO, 75 MHz) δ 55.9 (CH_3, OCH_3), 113.8 (CH, C-2, C-2′), 116.2 (CH, C-5, C-5′), 124.8 (CH, C-6, C-6′), 125.6 (C, C-1, C-1′), 126.8 (C, C-8, C-8′), 138.3 (CH, C-7, C-7′), 147.9 (C, C-4, C-4′), 149.0 (C, C-3, C-3′), 169.0 (C, C═O); HRMS: m/z 387.1072 (calcd for $C_{20}H_{18}O_8$+H, 387.1074).

Synthesis of Cannabisin G (**1**)*:* Triethylamine (5 mL) was added dropwise to a suspension of (±) tyramine hydrochloride (224 mg, 0.13 mmol) in THF (20 mL), then compound **12** (500 mg, 0.13 mmol) and DCC (268 mg, 0.13 mmol) were added. The reaction mixture was heated to reflux for 2 h. The solvent was removed under reduced pressure, and the residue was purified by column chromatography using petroleum ether and ethyl acetate (2:1, v/v) as eluent to afford **1** (694 mg, 86%) as a pale yellow solid; IR (KBr) ν_{max}: 3360, 2948, 1658, 1560 cm^{-1}; ^1H NMR (CD_3COCD_3, 400 MHz) δ 2.42 (2H, ddd, J = 13.6, 8.4, 2.0 Hz, H-β), 2.52 (2H, ddd, J = 13.6, 8.4, 2.0 Hz, H-β′), 3.25 (2H, dddd, J = 13.6, 6.4, 8.4, 2.0 Hz, H-α), 3.48 (2H, dddd, J = 13.6, 8.4, 2.0 Hz, H-α′), 3.74 (6H, s, OCH_3), 6.68 (4H, d, J = 8.4 Hz, H-3″, H-5″, H-3‴, H-5‴), 6.83 (2H, d, J = 8.0 Hz, H-5, H-5′), 6.84 (4H, d, J = 8.4 Hz, H-2″, H-2‴, H-6″, H-6‴), 7.08 (2H, dd, J = 8.4, 2.0 Hz, H-6, H-6′), 7.12 (2H, br t, J = 5.6 Hz, NH), 7.28 (2H, d, J = 2.0 Hz, H-2, H-2′), 7.88 (2H, s, H-7, H-7′); ^{13}C NMR (CD_3COCD_3,100 MHz) δ 35.3 (CH_2, C-β, C-β′), 42.6 (CH_2, C-α, C-α′), 56.0 (CH_3, OCH_3), 113.2 (CH, C-2,C-2′), 116.0 (CH, C-5, C-5′), 125.8 (CH, C-6, C-6′), 127.9 (C, C-8, C-8′), 130.3 (CH, C-2″, C-2‴, C-6″, C-6‴), 130.8 (C, C-1″, C-1‴), 140.3 (CH, C-7, C-7′), 148.2 (C, C-4, C-4′), 149.1 (C, C-3, C-3″), 156.5 (C, C-4″, C-4‴), 166.0 (C, C═O); HRMS: m/z 623.2390 (calcd for $C_{36}H_{36}N_2O_8$−H, 623.2399).