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A direct synthesis of atractylodinol, a potent inhibitor of PRRSV, and its biological evaluation

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ABSTRACT

A direct synthesis of atractylodinol from 2-furylbutenyne and bromoacetylene **6** is reported. Both compounds **1** and **8** showed greater than 99% virus inhibition.

Porcine reproductive and respiratory syndrome (PRRS) is caused by porcine reproductive and respiratory syndrome virus (PRRSV). PRRS is a widespread disease affecting domestic pigs, and is considered one of the most economically significant diseases in the global swine industry.[1] In 2005, the annual costs of PRRS for the American swine industry were approximately 560 million dollars.[2,3] Although vaccines are used to treat PRRSV, genetic diversity of PRRSV reduces the effectiveness of vaccines and contributes to PRRSV persistence in the field. There is an urgent need for alternate strategies to reduce the economic burden of PRRS. An alternative strategy is the development of potent antiviral drugs. Unlike vaccines, antiviral drugs can provide almost immediate treatment for animals. The antiviral drugs can also be used in periods of increased susceptibility. A few natural products have been reported to significantly inactivate the PRRS virus. Their structures are depicted in Figure 1.

Atractylodinol (**1**) and Ethoxysanguinarine (**2**) were reported as anti-PRRSV drugs by Li with IC_{50} values of 7.9 and 39.4 μmol/L, respectively.[4] Atractylodinol was isolated from rhizomes of *Atractylodes lancea*. *A. lancea* and is widely used in traditional Chinese and Japanese medicines against rheumatic diseases, digestive disorders, night blindness, and influenza.[5] The reported isolation procedure gives 9.6 mg of impure **1** from 570 g of dried and powered *A. lancea* rhizomes.[5] The low natural abundance inhibits future biological activity tests in animals. A patent using cobalt-complexed acetylenes to prepare dienediynes was reported in 2016.[6] The related hydrocarbon analog atractylodin was synthesized using a bis-silylated diacetylene.[7]

In our synthetic plan, compound **1** could be synthesized by coupling reaction of furyl enyne **4** and bromo alcohol **6**. Enyne **4** could be converted by a Corey–Fuchs reaction[8] from commercially available 3-(2-furyl)acrolein (**3**). Compound **6** is accessible through unsaturated aldehyde **5**.

Commercially available 3-(2-furyl)acrolein **3** was subjected to the Corey–Fuchs protocol as shown in Scheme 1 to obtain the terminal alkyne **4** in 67% overall yield. Treatment of unsaturated aldehyde **5** (generated from cis-butenediol in two steps)[9] with carbon tetrabromide and triphenylphosphine afforded a 1,1-dibromoalkene. Treatment of the 1,1-dibromoalkene with tetra-*n*-butylammonium fluoride in THF at 45 °C resulted in both deprotection of the TBS group and the elimination of bromide to make alcohol **6** in 66% yield.[10]

With the two coupling components in hand, we focused on finding the most suitable coupling condition. After several modifications, utilizing a copper (I) catalyst with ethylamine and hydroxylamine hydrochloride in methanol, the Cadiot–Chodkiewicz coupling reaction of compounds **4** and **6** furnished the desired natural product **1** in 25% isolated yield.[10] Although the yield was modest for the final step, it was the only effective conditions that we found for this two-component coupling. With the possible exception of the recent patent, no total synthesis route of atractylodinol

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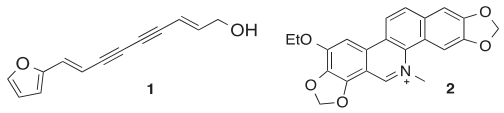
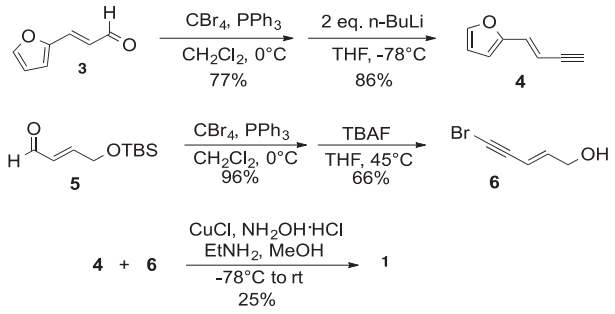
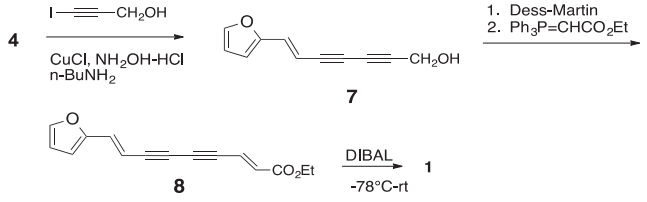


Figure 1. Natural product inhibitors of PRRSV.



Scheme 1. Total synthesis of atractylodinol.



Scheme 2. Alternate synthetic route.

had been reported in the literature. Despite the modest yield of the coupling reaction, the overall yield from commercially available materials was 11%.

To improve the synthetic route for natural product **1**, as well as to make analogs **7** and **8**, we designed an alternate route as shown below in Scheme 2. Coupling of 3-iodopropargyl alcohol[11] with **4** provided alcohol **7** in 37% isolated yield. Alcohol oxidation using the Dess–Martin reagent followed immediately by a Wittig reaction using commercially available phosphorane generated ester **8** in 80% yield over two steps. Reduction of ester **8** using DIBAL[12] from −78 °C to room temperature afforded **1** in 88% yield. The advantages of this route included a significantly improved coupling reaction with easily prepared 3-iodopropargyl alcohol and the high yield ester reduction with DIBAL. This route would be the preferred route for the scaling up of **1**.[14–17]

The antiviral activity of compounds **1**, **4**, and **8** was evaluated in vitro using 10 μg of each compound and 10^2 focus forming units (FFU) of PRRSV strain NVSL97-7895. DMSO was used as a control.[18] The compound plus virus mixtures were incubated at 37° for one hour, and then inoculated in triplicate onto MARC-145 cells. At 24 h post infection, cells were fixed and immunocytochemistry performed to detect foci of PRRSV-infected cells. Virus inhibition was calculated as the percent reduction in FFU in compound-treated wells compared to virus-only control wells. The percent virus inhibition (PVI) for compound **1** was 100.0 ± 0.7%, which demonstrates that our synthetic compound **1** is effective toward PRRSV as was reported. The PVI of compounds **4** and **8** were 93.4 ± 4.0% and 99.3 ± 0.7%, respectively. The DMSO had little to no inhibitory effect on PRRSV, with PVI of 0.78 ± 14.3%, indicating the inhibitory activity is due to the compounds, and not DMSO (Fig. 2).

Atractylodinol (**1**) was successfully synthesized in seven steps and all the characterization spectra (NMR and MS) of final product were identical to the literature spectra. One more efficient six-step

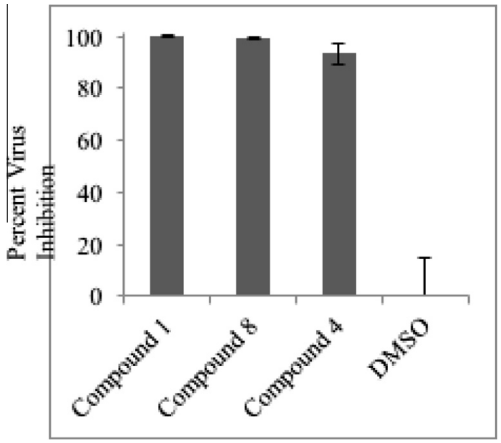


Figure 2. Antiviral activity was tested using 10 ug of each compound against 10^2 FFU PRRSV. Percent virus inhibition is reported as the mean percent reduction in compound-treated wells compared to virus-only control wells. Error bars represent ± one standard deviation of the mean of replicates.

synthetic route through ethyl ester **8** was also reported. To the best of our knowledge, neither of these two efficient synthetic routes has been reported.

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13. Experimental procedure for the synthesis of **(*E*)-2-(But-1-en-3-yn-1-yl)furan (4):** To a solution of CBr_4 (2.653 g, 8.0 mmol) in CH_2Cl_2 (50 mL) at 0 °C, PPh_3 (4.197 g, 16 mmol) was added in portions over 3 minutes. The color of solution changed to dark brown. After adding, the mixture was stirred for 10 minutes under argon gas protection. Acrolein **3** (0.489 g, 4 mmol) was added to the resulting solution over 5 minutes portionwise. After another 30 minutes stirring at 0 °C, TLC was checked to make sure the reaction was done. Evaporation of CH_2Cl_2, followed by a quick flash column chromatography (silica gel, EtOAc:hexanes 1:1) to remove most of salt gave a pale gray crude solid product. The purification of crude product by flash column chromatography (silica gel, EtOAc:hexanes 1:4) afforded dibromo compound as a light yellow solid in 77% yield; ^1H NMR (300 MHz, $CDCl_3$) δ = 7.47–7.40 (m, 1H), 7.02 (dd, *J* = 10.4, 0.5 Hz, 1H), 6.68 (dd, *J* = 15.5, 10.4 Hz, 1H), 6.54–6.36 (m, 3H).
To a solution of prepared dibromo compound (0.560 g, 2.02 mmol) in 15 mL THF, *n*-BuLi (2.5 M in hexane, 1.62 mL, 4.03 mmol) was added dropwise over 10 min at −78 °C under argon protection. The resulting mixture stirred at −78 °C for 1 h, the temperature was increased to rt. After 1 h stirring at rt, saturated NH_4Cl aqueous solution was added slowly to quench the reaction. The reaction mixture was extracted with diethyl ether (3 × 30 mL). The organic layers were collected, dried with anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (pentane)

to afforded **4** as colorless oil in 86% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.38 (d, *J* = 1.8 Hz, 1H), 6.78 (d, *J* = 16.1 Hz, 1H), 6.45–6.31 (m, 2H), 6.03 (d, *J* = 18.4 Hz, 1H), 3.09 (d, *J* = 2.4 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 152.0, 143.4, 130.3, 112.1, 110.7, 105.3, 83.0, 80.3;
HRMS (ESI-QTOF) calcd for C_8H_6O $[M+H]^+$ 119.0491, found 119.0493.

14. Experimental procedure for the synthesis of **(*E*)-5-Bromopent-2-en-4-yn-1-ol (6):** Dibromo Compound was prepared using previous described procedure for compound **4** in 96% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 6.96 (d, *J* = 10.3 Hz, 1H), 6.42–6.26 (m, 1H), 6.14–6.00 (m, 1H), 4.00 (d, *J* = 7.8 Hz, 2H), 0.91 (s, 9H), 0.08 (d, *J* = 7.5 Hz, 3H).
To a solution of the dibromoalkene obtained above (0.383 g, 1.08 mmol) in THF was added TBAF (1.0 M solution in THF, 0.4 mL, 0.4 mmol) at rt, then the mixture was stirred at 45 °C for 18 h and diluted with Et_2O. The mixture was washed with saturated NH_4Cl, H_2O, and brine and then dried over $MgSO_4$. Concentration gave the mixture of the corresponding TBS- deprotected bromoacetylene **6**; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 6.38–6.23 (m, 1H), 5.72 (dt, *J* = 15.9, 1.9 Hz, 1H), 4.20 (dd, *J* = 5.0, 1.9 Hz, 2H).
15. **Atractylodinol (1):** To a solution of $EtNH_2$ (70% aqueous solution, 2.2 mL) in MeOH (3 mL) was added CuCl (13.0 mg, 131.4 μmol) at rt that resulted in the formation of a blue solution. To the resulting mixture was added $NH_2OH{\cdot}HCl$ (54.8 mg, 0.788 mmol) at room temperature to discharge the blue color. The resulting colorless solution indicated the presence of Cu(I) salt. To the resulting mixture was added **4** (108.6 mg, 0.920 mmol) in MeOH (2 mL) at rt, and the mixture was stirred at rt for 10 min that resulted in the formation of a yellow suspension. To the resulting mixture was added bromoacetylene **6** (42.3 mg, 0.263 mmol) in MeOH (2 mL) at −78 °C, and mixture was stirred at the same temperature for 30 min. The mixture was allowed to warm to rt for 3 h. The mixture was diluted with Et_2O, washed with H_2O and brine, and then dried over $MgSO_4$. Concentration and flash column chromatography (silica gel, EtOAc:hexanes 1:4) gave the corresponding product **1**; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.39 (s, 1H), 6.81 (d, *J* = 16.0 Hz, 1H), 6.41 (dt, *J* = 11.1, 4.5 Hz, 3H), 6.11 (d, *J* = 15.9 Hz, 1H), 5.88 (d, *J* = 15.9 Hz, 1H), 4.26 (d, *J* = 3.3 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 152.0, 145.4, 143.9, 131.3, 112.4, 111.6, 109.3, 104.8, 81.3, 81.1, 77.1, 75.0, 63.0. HRMS (ESI-QTOF) calcd for $C_{13}H_8O$ $[M+H-H_2O]^+$ 181.0848, found 181.0646.
16. Experimental procedure for the synthesis of **(E)-7-(furan-2-yl)hepta-6-en-2,4-diyn-1-ol (7):** Alcohol compound was prepared using similar procedure for compound **1** in 37% yield; ^{1}H NMR (600 MHz, $CDCl_3$) δ = 7.39 (s, 1H), 6.82 (d, J = 16.0 Hz, 1H), 6.40 (d, J = 18.0 Hz, 2H), 6.07 (d, J = 16.0 Hz, 1H), 4.40 (s, 2H), 2.00 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ = 151.9, 143.9, 131.8, 112.3, 111.7, 104.4, 81.6, 78.5, 76.3, 70.9, 51.9; HRMS (ESI-QTOF) calcd for $C_{11}H_8O_2$ $[M+H]^+$ 173.0597, found 173.0599.
17. Experimental procedure for the synthesis of **Ethyl (2E,8E)-9-(furan-2-yl) nona-2,8-dien-4,6-diynoate (8):** To a solution of **7** (50.0 mg, 0.29 mmol) in CH_2Cl_2 (4 mL) was added Dess-Martin Periodinane (135 mg, 0.32 mmol) at 0 °C, the mixture was warm up to room temperature and stirred for an additional hour. (Carbethoxymethylene)triphenylphosphorane (121.8 mg, 0.35 mmol) in CH_2Cl_2 (1 mL) was then added to the mixture and stirred for another hour. Concentration and flash column chromatography (silica gel, EtOAc:hexanes 1:10) gave compound **8** in 80% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.41 (s, 1H), 6.87 (m, 2H), 6.44 (m, 2H), 6.32 (d, J = 15.8 Hz, 1H), 6.14 (d, J = 15.9 Hz, 1H), 4.23 (q, J = 7.1 Hz, 2H), 1.30 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 165.7, 151.8, 144.2, 132.8, 132.3, 124.1, 112.5, 112.3, 104.2, 85.3, 82.9, 79.4, 76.5, 61.2, 14.4; HRMS (ESI-QTOF) calcd for $C_{15}H_{12}O_3$ $[M+H]^+$ 241,0859, found 241.0856.
18. **Antiviral Activity** Compounds were diluted in DMSO and screened for anti-PRRSV activity using a focus-reduction assay adapted from Wu et al.[13] Briefly, MARC-145 cells were seeded at 3×10^5 cells/well in a 12-well plate 24 h prior to the anti-viral assay, and media changed to 1 ml/well directly before infection. For each compound, 35 μg (35 μl) was added to 700 focus-forming units (FFU) of PRRSV in a volume of 1.2 ml (incubation volume), for an incubation concentration of 29.17 μg/ml of the compound. In addition, a virus-only sample with 700 FFU PRRSV in 1.2 ml media was used as a control. The virus-compound mixtures and virus-only control were incubated at 37° for one hour. Samples were brought to a total volume of 3.5 ml, and 1 ml was inoculated per well in triplicate, resulting in each well containing 10 μg compound and 200 FFU in a well volume of 2 ml for a final well concentration of 5 μg/ml of compound. The plates were incubated at 37° supplemented with 5% CO_2. At 24 h post infection, cells were fixed in ice-cold methanol:acetone and immunocytochemistry performed using the PRRSV N protein specific monoclonal antibody SDOW17 (Rural Technology) as the primary antibody and sheep anti-mouse IgG conjugated to HRP (Jackson ImmunoResearch) as the secondary antibody. Following addition of the HRP substrate, cells were rinsed with distilled water, air-dried, and foci of infected cells enumerated by light microscopy. Percent virus inhibition was calculated compared to virus-only control wells. Standard deviations were calculated using the means of repeated experiments.