Synthesis of 4-ethyl-5-methyl-5,6-dihydrophenanthridine-8,9-diol as the key intermediate of potent agonists of the Wnt signalling pathway

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The synthesis of 4-ethyl-5-methyl-5,6-dihydro-phenanthridine-8,9-diol as the key intermediate of a series of potent Wnt signalling pathway agonists is reported. This synthesis features a consecutive aryl-aryl and N-aryl coupling, leading to 4-ethyl-5-methyl-8,9-dimethoxyphenanthridin-6-(5*H*)-one, whose structure was determined by spectroscopic analysis and X-ray crystallography, in a one-pot sequence with the presence of palladium catalyst and TFP (tri(2-furyl)phosphine) as the ligand.

Keywords: phenanthridin, palladium catalyst, aryl–aryl coupling, N-aryl coupling

The evolutionarily conserved Wnt signalling pathway plays an important role in the progress of embryonic development and human diseases. It regulates cell fate specification, proliferation, differentiation and survival during embryogenesis. Inappropriate regulation of the Wnt signalling pathway often links to many human diseases, such as cancer, Alzheimer's disease, familial exudative vitreoretinopathy and bone formation disorders. [1-4]

By screening a synthetic chemical library of phenanthridine derivatives, we previously identified one of the phenanthridine derivatives, hlyc 60 (Fig. 1), as an activator of the Wnt signalling pathway. It targets the DIX domain of Axin and potentiates the Axin–LRP6 association, thus promoting LRP6 phosphorylation and Wnt signalling transduction. Moreover, we identified that it may weaken the autoinhibition of Axin. Collectively, our study not only provides new insights into the regulation of the Wnt/b-catenin signalling pathway by a Wnt-specific small molecule but will also facilitate therapeutic applications, such as HSC expansion. [3]

Inspired by the above research results, further bioactivity and structural modification, of these type of compounds should be employed and it needs larger amounts of the phenanthridine-type derivatives than could be readily obtained through semi-synthesis are required to confirm and further explore their potent activities in bioassays, including *in vivo* assays. According to the structural analysis of hlyc-60, we found that a key intermediate, 4-ethyl-5-methyl-5,6-dihydrophenanthridine-8,9-diphenol (**1**), can simply transformed into hlyc-60 through a process of alkylation and click reaction. We designed a synthetic route for the production of this key intermediate **1** in the preparation of hlyc60.

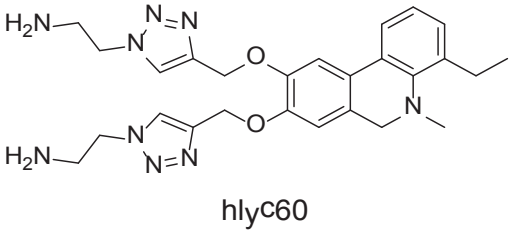


Fig. 1 The structure of hlyc-60.

Results and discussion

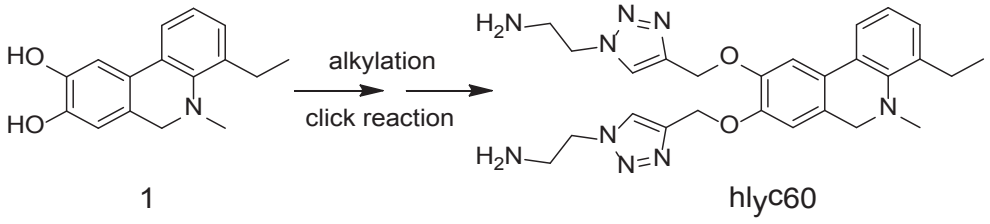
According to the retrosynthesis analysis of compound **1**, we concluded that **1** could be prepared from halogenated benzamide analogues and halogenated ethylbenzene through a consecutive aryl-aryl and N-aryl coupling process. Commercially purchased halogenated benzaldehyde can simply transform into halogenated benzamide by the processes of oxidation and amidation (Scheme 2).

As shown in Scheme 3, compound **1** was synthesised from 6-bromoveratraldehyde (**2**) in five steps. First, compound **2** was oxidised using $KMnO_4$ to afford **3**, which was amidated with methylamine to obtain **4**. Compound **4** was then condensed with iodoarene **5** using palladium catalysis to give **6**, followed by reduction of the C-6 carbonyl to afford **7**. Finally, compound **7** was obtained by deprotection of **1** in the presence of boron tribromide.

The preparation of **4** was the key step of the whole route, so the reaction conditions were optimised. We studied the correlations between the yield and the quantity of TFP and from the results, the highest yield was with 0.1 molar equiv. of TFP (Fig. 2).

Experimental

ESI and high-resolution mass spectra were recorded using a Finnigan Mat 90 instrument and VG Auto Spec-3000 spectrometer, respectively. Melting points were measured using X-4 apparatus (Yingyu Yuhua Instrument Factory, Gongyi, Henan Province, China). NMR



Scheme 1 The transformation from key intermediate **1** to hlyc60.

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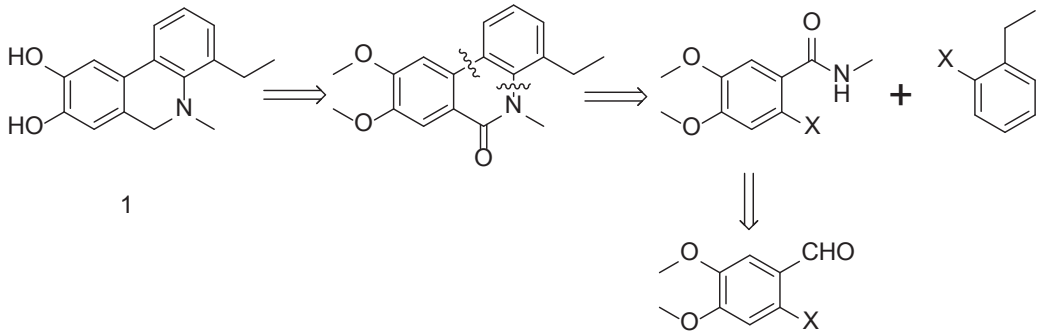
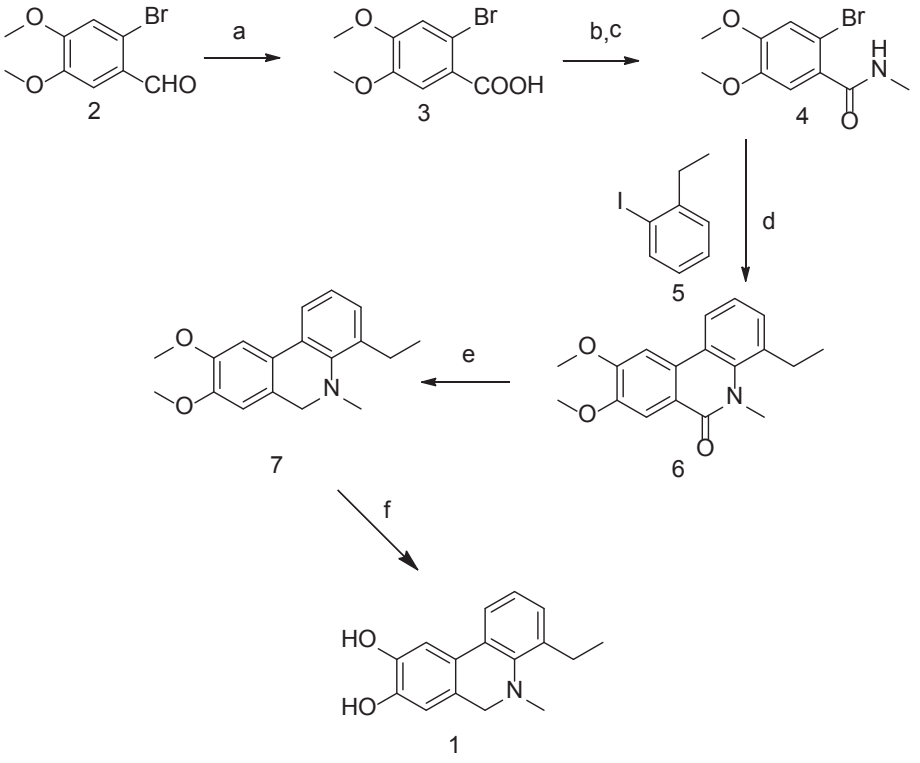


Scheme 2 The retrosynthesis analysis of hlyc60.



Scheme 3 Total synthesis of compound **1.** Reagents and conditions: (a) $NaHCO_3$, $KMnO_4$, H_2O, 90 °C, 3 h, 85%; (b) $SOCl_2$, DMF, THF, 50 °C, 2 h; (c) CH_3NH_2 (30%), 5 °C, 1 h, 75%; (d) K_2CO_3, norbornene, $Pd(OAc)_2$, TFP, MeCN, 85°C, 6 h, 75%; (e) LAH, THF, –78 °C, 2 h, 60%; g. BBr_3, CH_2Cl_2, –78 °C, 4 h, 80%.

Equivalent of TFP	Yield
0.01	24%
0.02	32%
0.05	46%
0.08	54%
0.1	75%
0.12	73%
0.15	74%
0.2	70%

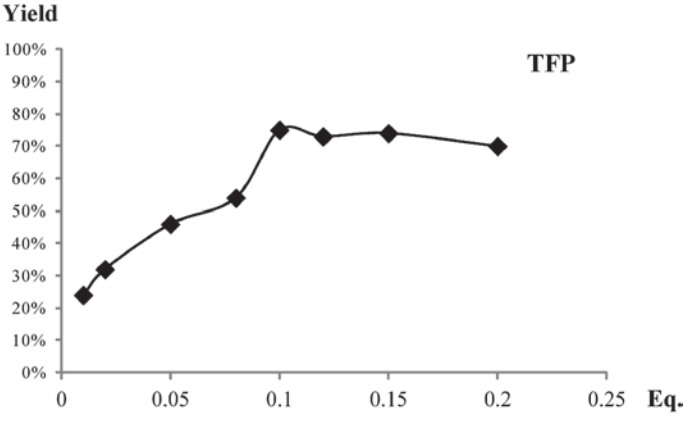


Fig. 2 Correlations between yields and the dosage of TFP.

experiments were carried out on a Bruker AM-400 spectrometer or a DRX-500 spectrometer or an Avance III 600 spectrometer with the solvents $CDCl_3$ and DMSO-d_6, and Me_4Si as internal standard. Column chromatography was performed on Silica gels (60–80 mesh, 200–300 mesh, 300–400 mesh, Qingdao Haiyang Chemical Co. Ltd, Qingdao, China). Pre-coated silica gel 60 F254 (Merck, Darmstadt, Germany) was used for TLC. Semipreparative HPLC was performed on a Hypersil Gold RP-C18 column (i.d. 10 × 250 mm; Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) developed with CH_3CN–H_2O at r.t. All regular solvents and reagents were reagent grade and purchased from Aldrich-Sigma Chemical Co., Acros Organics and J&K Scientific. The purities of all compounds were more than 95% as determined by HPLC. All yields reported are for dry compounds that require no further purification for use in other reactions.

2-Bromo-4,5-dimethoxybenzoic acid (**3**): A solution of 6-bromoveratraldehyde (**2**, 250 mg, 1 mmol), $NaHCO_3$ (200 mg), and $KMnO_4$ (500 mg) in H_2O (20 mL) was heated to 90 °C with stirring for 3 h, then extracted with CH_2Cl_2 (20 mL) twice. The organic phase was washed with saturated NH_4Cl and brine, dried over $MgSO_4$, filtered and concentrated. The residue was then purified by column chromatography to give **3** as a pale yellow solid (220 mg, 85% yield). m.p. 183–185 °C, (lit.[6] 184–185 °C); IR (KBr) ν_{max} 2960, 1702, 1598, 1568, 1514, 1436, 1263, 663, 511 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.54 (s, 1H), 7.52 (s,1H), 3.87 (s, 3H), 3.81 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.87, 153.3, 152.14, 121.85, 116.73, 115.31, 111.64, 56.81, 56.64; ESI^+MS *m/z* 261 $[M + H]^+$; HREIMS *m/z* 259.9690 $[M]^+$ (calcd for $C_9H_9BrO_4$, 259.9684).

*2-Bromo-4,5-dimethoxy-*N*-methyl-benzamide* (**4**): Compound **3** (260 mg, 1mmol) was dissolved in THF (10 mL), to which DMF (0.1 mL) and $SOCl_2$ (0.5 mL, 4mmol) were added. The reaction solution was stirred for 2 h at 50 °C and then concentrated to remove THF. The residue was then added to a 30% solution of methylamine in water (20 mL) at 5°C and filtered. The cake was purified by column chromatography to give **4** as a pale yellow solid (205 mg, 75% yield). m.p. 119–121 °C; IR (KBr) ν_{max} 3422, 2961, 1597, 1551, 1505, 1456, 1259, 1159, 1014, 865 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.46 (s, 1H), 7.33 (s, 1H), 6.63 (s, 1H), 3.87 (s, 3H), 3.85 (s, 3H), 2.79 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.31, 152.32, 151.16, 131.95, 116.69, 116.31, 112.86, 56.81, 56.78, 26.33; ESI^+MS *m/z* 274 $[M+H]^+$; HREIMS *m/z* 273.0004 $[M]^+$ (calcd for $C_{10}H_{12}BrNO_3$, 273.0001).

*4-Ethyl-5-methyl-8,9-dimethoxyphenanthridin-6(5*H*)-one* (**6**): A flask was charged under nitrogen with $Pd(OAc)_2$ (3.0 mg, 0.013 mmol), tri-2-furylphosphine (6.2 mg, 0.027 mmol), K_2CO_3 (72.3 mg, 0.52 mmol), the amide **4** (0.26 mmol), a solution of norbornene (26.9 mg, 0.286 mmol) in anhydrous solvent (5.8 mL), and 1-ethyl-2-iodobenzene (**5**, 0.26 mmol). The reaction mixture was heated with stirring at 85 °C for 6 h and then cooled to room temperature. After the addition of saturated NH_4Cl (30 mL) and extraction with EtOAc (3×15 mL), the combined organic extracts were washed with brine (30 mL) and dried over Na_2SO_4. Removal of the solvent under reduced pressure gave the crude product, which was purified by flash chromatography on silica gel to furnish **6** as white wax (58 mg, 75% yield). IR (KBr) ν_{max} 3446, 2923, 1642, 1550, 1512, 1464, 1360, 1029, 691, 563, cm^{-1}; ^1H NMR δ 8.21 (d, *J* = 7.6 Hz, 1H), 7.71 (s, 1H), 7.60 (s, 1H), 7.32 (d, *J* = 7.4 Hz, 1H), 7.29–7.25 (m, 1H), 3.92 (s, 3H), 3.89 (s, 3H), 3.76 (s, 3H), 3.01–3.03 (m, 2H), 1.25 (t, *J* = 8.1 Hz, 3H); ^{13}C NMR: δ 166.1, 139.4, 134.5, 132.5, 132.1, 131.3, 128.7, 127.5, 125.1, 129.6, 122.2, 121.1, 121.0, 58.3, 58.1, 38.6, 28.1, 15.5; HREIMS *m/z* 297.1361 $[M]^+$ (calcd for $C_{18}H_{19}NO_3$, 297.1365).

4-Ethyl-5-methyl-8,9-dimethoxy-5,6-dihydrophenanthridine (**7**): A solution of **6** (30 mg, 0.1 mmol) in THF (5 mL) was added to LAH (20 mg) at –78 °C. The reaction was stirred for 2 h and then quenched using H_2O (5 mL). The mixture was then extracted with Et_2O (20 mL) twice. The organic phase was washed with brine and concentrated, and the residue was purified by column chromatography to give **7** as a colourless solid (21 mg, 75% yield), its X-ray crystal structure was determined (Fig. 3), m.p. 72–74 °C; IR (KBr) ν_{max} 2912, 1653, 1638, 1542, 1509, 1451, 1260, 643, 576 cm^{-1};^1H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, *J* = 7.1 Hz, 1H), 7.53 (s, 1H), 7.49 (s, 1H), 7.18 (d, *J* = 6.9 Hz, 1H), 6.75 (m,1H), 4.06 (s, 2H), 4.02 (s, 3H), 3.98 (s, 3H), 2.96 (q, *J* = 7.4 Hz, 2H), 2.41 (s, 3H), 1.23 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.4 (C), 146.5 (C), 140.5 (C), 138.8 (C), 132.9 (C), 131.3 (CH), 130.0 (C), 129.0 (C), 123.2 (CH), 120.5 (CH), 108.9 (CH), 103.1 (CH), 52.3 (CH_2), 56.8 (CH_3), 56.4 (CH_3), 39.9 (CH_3), 28.5 (CH_2), 15.8 (CH_3). HREIMS *m/z* 283.1567 $[M]^+$ (calcd for $C_{18}H_{21}NO_2$, 283.1572).

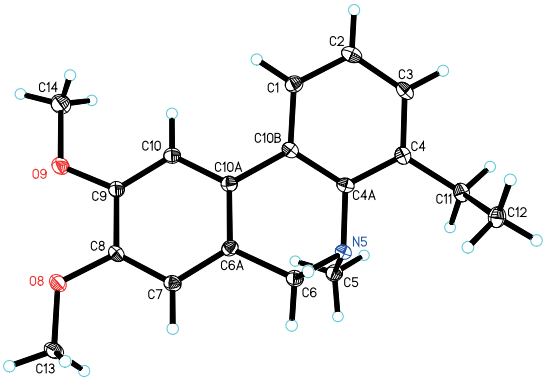


Figure 3 X-ray crystal structure of compound **7** (CCDC 948062), crystal data: $C_{18}H_{21}NO_2$, *M* = 283.36, monoclinic, *a* = 20.412(7) Å, *b* = 9.126(3) Å, *c* = 8.164(3) Å, α = 90.00°, β = 95.825(5)°, γ = 90.00°, *V* = 1513.0(9) Å^3, *T* = 100(2) K, space group *P*21/*c*, *Z* = 4, *μ*(MoKα) = 0.081 mm^{-1}, 12550 reflections measured, 3649 independent reflections (R_{int} = 0.0612). The final R_1 values were 0.0789 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1941 ($I > 2\sigma(I)$). The final R_1 values were 0.1238 (all data). The final $wR(F^2)$ values were 0.2230 (all data). The goodness of fit on F^2 was 1.089.

4-Ethyl-5-methyl-5,6-dihydrophenanthridine-8,9-diol (**1**): Compound **7** (29 mg, 0.1 mmol) was dissolved in 5 mL CH_2Cl_2. The reaction solution was then cooled to –78 °C and BBr_3 (100 μL, 0.2 mmol) was added. The mixture was then stirred for 4 h after which it was diluted in 10 mL saturated $NaHCO_3$. The solution was extracted twice with CH_2Cl_2 (15 mL), and the organic layer was washed with brine, concentrated, and then purified by column chromatography using chloroform-methanol (20:1) as the eluent to give **1** as a pale yellow powder (20 mg, 80% yield) m.p. 139–141 °C; IR (KBr) ν_{max} 3384, 2965, 1613, 1513, 1451, 1411, 1377, 1304, 1248, 1122, 929, 867, 804, 755 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, *J* = 7.1 Hz, 1H), 7.27 (d, *J* = 2.7 Hz, 1H), 7.17 (dt, *J* = 15.0, 7.5 Hz, 1H), 7.00 (s, 1H), 6.75 (s, 1H), 3.96 (s, 2H), 2.82–2.76 (m, 2H), 2.31 (s, 3H), 1.28 (dd, *J* = 14.7, 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.8 (C), 143.0 (C), 141.2 (C), 139.4 (C), 128.8 (C), 127.6 (CH), 126.3(C), 125.1 (C), 124.6 (CH), 120.7 (CH), 113.8 (CH), 110.4 (CH), 53.6 (CH_2), 40.2 (CH_3), 23.2 (CH_2), 14.8 (CH_3)。HREIMS *m/z* 255.1250 $[M]^+$ (calcd for $C_{16}H_{17}NO_2$, 255.1259).

Conclusions

To enable the production of phenanthridine-type derivatives in sufficient quantities to enable their use in further bioassays, a totally synthetic route to the key intermediate **8** was developed. This new route hinges on an efficient palladium-catalysed coupling reaction involving sequential C–N and C–C bond formation with C–H activation. In conclusion, an efficient and convenient synthesis of the key intermediate of potent phenanthridins Wnt agonists has been reported. This route featured a one-step palladium catalysed preparation of 4-ethyl-5-methyl-8,9-dimethoxyphenanthridin-6(5*H*)-one (**6**). The relatively simple reaction procedure, utilisation of cheap and readily available reagents, and ideal yields (23%) of products are the main advantages of the present approach.

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