A concise synthesis of (±)-methoxyfumimycin ethyl ester

Zhiwang Zhou[a]*, Yu Hu[b], Bin Wang[a], Xiaoru He[c], Gang Ren[c] and Lihua Feng[a]

[a]*School of Pharmaceutical Sciences, Nanchang University, Nanchang, 330006, Jiangxi, P.R. China*

[b]*Department of Chemistry, Nanchang University, Nanchang, 330031, Jiangxi, P.R. China*

[c]*Key Laboratory of Modern Preparation of TCM, Jiangxi University of Traditional Chinese Medicine, Ministry of Education, Nanchang 330004, Jiangxi, P.R. China*

The protecting-group-free synthesis of (±)-methoxyfumimycin ethyl ester, a potential bacterial peptide deformylase (PDF) inhibitor, is reported. The synthetic approach features a tandem Friedel–Crafts alkylation/lactonisation access as a key reaction to generate α, α-disubstituted amino acid unit.

Keywords: fumimycin, (±)-methoxyfumimycin ethyl ester, peptide deformylase inhibitors, Friedel–Crafts alkylation

(–)-Fumimycin (**1**), a fungal metabolite isolated from *Aspergillus fumisynnematus* F746, exhibits promising antibacterial activity.[1] It is a potent inhibitor of peptide deformylase (PDF),[2,3] a metalloenzyme essential for prokaryotic growth, but not for mammalian cells. PDF has been regarded as a nontoxic broad-spectrum antibacterial target.[4–6] Therefore, (–)-fumimycin and its analogue (–)-methoxyfumimycin (**2**) may represent lead structures for further optimisation and structure–activity relationship (SAR) studies to afford novel antibacterials (Fig. 1).

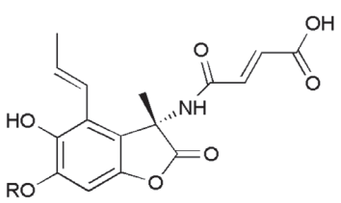
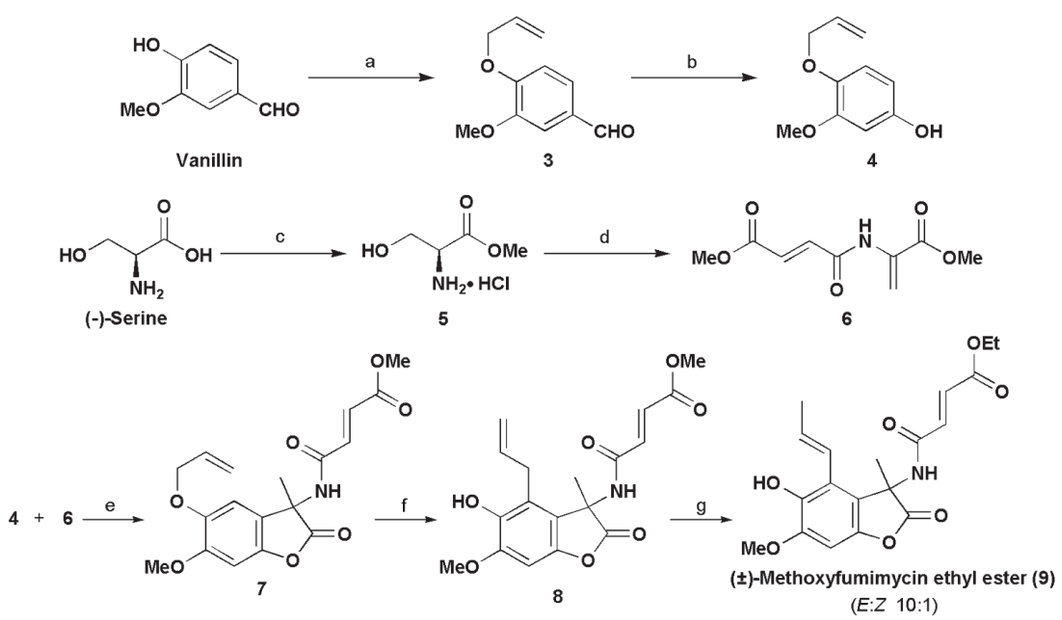


Fig. 1 Structure of (–)-fumimycin and (–)-methoxyfumimycin.

Bräse and coworkers carried out extensive work on the synthesis of fumimycin and its analogue including syntheses of racemic fumimycin,[7] methoxyfumimycin[8] and an enantioselective synthesis of (+)-fumimycin.[9] In their enantioselective work, the absolute configuration of (+)-fumimycin was assigned as *R* by CD-spectroscopy and TDDFT-calculations while the natural enantiomer was shown to possess the (*S*)-configuration.[9] However the key step in most of these studies was a 1, 2-addition of a Grignard reagent to a ketimine, which makes the reaction steps too long and reaction conditions too difficult. Recently, we succeeded in accomplishing the synthesis of a racemic intermediate for fumimycin with six steps from vanillin.[10] The key step for the formation of α, α-disubstituted amino benzofuran-2-one skeleton was a Friedel–Crafts alkylation/lactonisation cascade reaction.[11,12] We now report a convergent and protecting-group-free method resulting in a synthesis of racemic methoxyfumimycin ethyl ester, which might possess better antibacterial activity than the parent compound fumimycin and methoxyfumimycin as it is more lipophilic.[13]

Our synthetic work started from vanillin and L-serine respectively (Scheme 1). After refluxing with allylbromide



Scheme 1 Reagents and conditions: **a**, K_2CO_3, allylbromide, acetone, 98%; **b**, H_2O_2, H_2SO_4, $B(OH)_3$, THF, 92%; **c**, $SOCl_2$, MeOH, 99%; **d**, (i) monomethyl fumarate, $SOCl_2$, reflux. (ii) Et_3N, CH_2Cl_2, 60%; **e**, BF_3·Et_2O, THF, 50%; **f**, DMF, reflux, 80%; **g**, $RhCl_3$·$3H_2O$, EtOH, 68%.

* Correspondent. E-mail: zzw_308@163.com

and K_2CO_3 in acetone for 6 h, vanillin was transformed to the allyl ether **3** in 98% yield. It was then converted to the phenol derivative **4** *via* a Dakin oxidation reaction. L-serine methyl ester hydrochloride (**5)** was prepared from L-serine quantitatively by refluxing with $SOCl_2$ in methanol. It was then acylated and dehydrated by reacting with (*E*)-methyl 4-chloro-4-oxobut-2-enoate generated from monomethyl fumarate *in situ*.

A cascade Friedel–Crafts alkylation/lactonisation with intermediates **4** and **6** gave benzofuranone **7**, which was then smoothly converted to **8** by refluxing in DMF. Isomerisation of the terminal double bond was carried out by our previous method.[7] When **8** was heated under reflux for 8 h in the presence of $RhCl_3{\cdot}3H_2O$ in ethanol, the olefin isomerisation took place accompanied by transesterification to give (±)-methoxyfumimycin ethyl ester (**9**).

Conclusions

In conclusion, we have developed a concise synthetic route to (±)-methoxyfumimycin ethyl ester by a convergent and protecting-group-free strategy. In this approach, the Friedel–Crafts alkylation/lactonisation cascade reaction introduced the α,α-disubstituted amino benzofuran-2-one skeleton. The antibacterial activity and PDF inhibition assay of (±)-methoxyfumimycin ethyl ester are currently underway in our laboratory.

Experimental

Reagents and all solvents were analytically pure grade and were used without further purification. Column chromatography (CC) was performed on silica gel (200–300 mesh). Analytical TLC was performed on pre-coated silica gel GF_{254} plates (Qingdao Haiyang Chemical Co. Ltd, Qingdao, P.R. China). Visualisation on TLC was achieved by the use of UV light (254 nm) and treatment with I_2; ^1H (600 or 400 MHz) and ^{13}C (150 MHz) NMR spectra were recorded on a Bruker Avance 600 MHz spectrometer with $CDCl_3$ as solvent and TMS as internal standard. Chemical shifts are reported in ppm relative to the internal reference. ESIMS were obtained on a Waters ZQ4000/2695 HPLC-MS. HRMS were measured on a Finnigan MAT95 mass spectrometer.

4-Allyloxy-3-methoxybenzaldehyde **(3):**[8] A suspension of vanillin 3 (2.87 g, 18.86 mmol) and K_2CO_3 (3.64 g, 26.40 mmol) in acetone (28 mL), was treated with allylbromide (2.10 mL, 24.51 mmol). The mixture was heated to reflux for 6 h. After filtration, the filtrate was concentrated under reduced pressure. The crude product was purified by chromatography on silica gel (PE: AcOEt = 10: 1) to afford the allylether **3** as brown oil (3.55 g, 98%).

4-Allyloxy-3-methoxyphenol **(4):**[8] Boric acid (5.72 g, 92.30 mmol) was suspended in a mixture of THF (50 mL), H_2O_2 (30% in H_2O, 6 mL) and H_2SO_4 (2.66 mL, 98%). After stirring for 30 min, **3** (3.55 g**,** 18.47 mmol) was added as solution in THF (20 mL) over 15 min. After additional stirring for 5 h, the mixture was filtered. The filtrate was neutralised by the addition of sat. $NaHCO_3$ solution; the aqueous layer was extracted with EtOAc (3 × 90 mL). The combined organic extracts were washed with brine (50 mL), dried over Na_2SO_4 and concentrated under reduced pressure. Flash chromatography (PE: AcOEt = 4 : 1) afforded the phenol **4** as brown oil (3.06 g, 92%). ^1H NMR δ ($CDCl_3$, ppm, 600 MHz): 3.68 (s, 3H), 4.49 (d, *J* = 6 Hz, 2H), 5.21 (dd, *J* = 10.2, 1.2 Hz, 1H), 5.32 (dd, *J* = 17.4, 1.2 Hz, 1H), 5.99–6.05 (m, 1H), 6.34 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.46 (d, *J* = 2.4 Hz, 1H), 6.72 (d, *J* = 8.4 Hz, 1H), 7.20 (brs, 1H).

L-serine methyl ester hydrochloride **(5)**:[14] At 0 °C, thinly chloride (20 mL, 253.30 mmol) was added dropwise to anhydrous methanol (100 mL). The solution was stirred at 0 °C for 30 min and then L-serine (24.50 g, 233.13 mmol) was added. The reaction mixture was stirred at room temperature for 24 h and TLC ($CHCl_3/CH_3OH$, 9 : 1) indicated complete disappearance of L-Serine. The reaction mixture was evaporated under reduced pressure and the residue was triturated with petroleum ether repeatedly to provide 35.91 g (99%) of HCl·L-Ser-OCH_3 (**5)** as a colourless powder which was used directly for the next reaction.

(E)-Methyl 4-(1-methoxy-1-oxoprop-2-en-2-ylamino)-4-oxobut-2-en-oate **(6):** Under argon, thionyl chloride (0.20 mL, 2.90 mmol) was added to monomethyl fumarate (37.3 mg, 0.29 mmol) in a 10 mL round-bottomed flask and then heated to reflux for 2 h. The reaction mixture was then evaporated to remove excessive thionyl chloride to give the acyl chloride, which was used directly for the next step. Under argon, a three-necked flask equipped with a dropping funnel was charged with **5** (44.6 mg, 0.29 mmol) and anhydrous CH_2Cl_2 (5 mL). Et_3N (0.28 mL) in CH_2Cl_2 (2 mL) were added at 0 °C, followed by the dropwise addition of the acyl chloride in CH_2Cl_2 (5 mL). The reaction mixture was stirred 30 min at 0 °C and overnight at room temperature. Water (10 mL) are added to the flask, and, after separation, the organic phase was washed with water (2 × 10 mL) and brine (2 × 10 mL) then dried over $MgSO_4$. The suspension was filtered and the solvent removed under vacuum. Flash chromatography (PE: AcOEt = 5: 1) afforded **6** (37.1 mg, 60%). ^1H NMR δ ($CDCl_3$, ppm, 400 MHz): 3.82 (s, 3H), 3.88 (s, 3H), 6.01 (s, 1H), 6.76 (s, 1H), 6.89 (d, *J* = 15.6 Hz, 1H), 6.99 (d, *J* = 15.6 Hz, 1H), 8.05 (brs, 1H, NH).

(E)-Methyl-4-(5-(allyloxy)-6-methoxy-3-methyl-2-oxo-2,3-dihydrobenzofuran-3-ylamino)-4-oxobut-2-en-oate **(7):** $BF_3{\cdot}Et_2O$ (0.4 mL, 1.38 mmol) was added to a solution of **4** (99 mg, 0.55 mmol) and **6** (117 mg, 0.55 mmol) in anhydrous THF (2 mL). The mixture was then heated to reflux for 48 h. Work-up began by quenching with saturated $NaHCO_3$ aqueous solution followed by filtration. The solvent was removed under reduced pressure and the residue was extracted with CH_2Cl_2. The combined organic extracts were washed with brine (20 mL), dried over Na_2SO_4 and concentrated under reduced pressure. Flash chromatography (PE: acetone = 4: 1 → 3: 1) afforded **7** as a white solid (99.4 mg, 50%). ^1H NMR δ ($CDCl_3$, ppm, 600 MHz): 1.68 (s, 3H), 3.78 (s, 3H), 3.87 (s, 3H), 4.53 (m, 2H), 5.26 (d, *J* = 10.8 Hz, 1H), 5.38 (d, *J* = 16.8 Hz, 1H), 6.03–6.07 (m, 1H), 6.75 (d, *J* = 15.6 Hz, 1H), 6.78 (s, 1H), 6.81 (s, 1H), 6.91 (d, *J* = 15.6 Hz, 1H), 7.44 (brs, 1H, NH), ^{13}C NMR δ ($CDCl_3$, ppm): 176.2, 166.0, 162.8, 151.5, 147.7, 145.5, 134.4, 133.2, 131.5, 119.0, 118.1, 108.8, 96.6, 71.1, 57.8, 56.3, 52.4, 24.1.

(E)-Methyl-4-(4-allyl-5-hydroxy-6-methoxy-3-methyl-2-oxo-2,3-dihydrobenzofuran-3-ylamino)-4-oxobut-2-en-oate **(8):** A solution of **7** (61 mg, 0.17 mmol) in DMF (3 mL) was heated at 170 °C to reflux for 16 h. The solvent was removed under reduced pressure; and 10 mL water was added to the residue. The mixture was extracted with EtOAc (3 × 10 mL). The organic phase was washed with saturated brine, dried over Na_2SO_4 and concentrated under reduced pressure. Flash chromatography (PE : acetone = 4 : 1 → 3 : 1) afforded **8** as a white solid (48.8 mg, 80%). ^1H NMR δ ($CDCl_3$, ppm, 600 MHz): 1.74 (s, 3H), 3.38 (dd, *J* = 15.6, 6.0 Hz, 1H), 3.47 (dd, *J* = 15.6, 5.4 Hz, 1H), 3.78 (s, 3H), 3.90 (s, 3H), 4.87 (dd, *J* = 16.8, 1.2 Hz, 1H), 4.96 (dd, *J* = 10.2, 1.2 Hz, 1H), 5.51 (brs, 1H, OH), 5.88–5.94 (m, 1H), 6.69 (s, 1H), 6.73 (d, *J* = 15.6 Hz, 1H), 6.88 (d, *J* = 15.6 Hz, 1H), 7.16 (brs, 1H, NH).

(E)-Ethyl-4-(5-hydroxy-6-methoxy-3-methyl-2-oxo-4-((E)-prop-1-enyl)-2,3-dihydrobenzofuran-3-ylamino)-4-oxobut-2-enoate ((±)methoxyfumimycin ethyl ester **(9):** A mixture of **8** (38 mg, 0.11 mmol) and $RhCl_3{\cdot}3H_2O$ (5 mg, 19 μmol) in EtOH (2 mL) was heated to reflux. After 8 h, the reaction was allowed to cool to room temperature and filtered through a 3 cm pad of Celite. The filtrate was concentrated under reduced pressure. Flash chromatography (PE : acetone = 5: 1 → 3 : 1) afforded **9** as a white solid (*E* : *Z* 10 : 1, 26 mg, 68%). ^1H NMR δ ($CDCl_3$, ppm, 600 MHz): 1.30 (t, *J* = 7.2 Hz, 3H), 1.75 (s, 3H), 1.92 (dd, *J* = 6.6, 1.8 Hz, 3H), 3.91 (s, 3H), 4.24 (q, *J* = 7.2 Hz, 2H), 5.82 (s, 1H, OH), 6.31 (dd, *J* = 16.2, 1.8 Hz, 1H), 6.65 (s, 1H), 6.68 (dd, *J* = 16.2, 6.6 Hz, 1H), 6.74 (d, *J* = 15.6 Hz, H), 6.86 (brs, 1H, NH), 6.92 (d, *J* = 15.6 Hz, 1H). ^{13}C NMR ($CDCl_3$, ppm): 175.8, 165.3, 162.7, 147.4, 146.3, 140.8, 134.5, 133.8, 132.4, 121.2, 120.5, 116.4, 93.9, 61.4, 58.4, 56.5, 23.5, 19.8, 14.1. (−) ESI-MS (MeOH): *m/z* 374.1 $[M-H]^-$, 749.3 $[2M-H]^-$. HRMS (ESI): *m/z* $[M-H]^-$ calcd for $C_{19}H_{20}NO_7$: 374.1245; found: 374.1248.

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