Organic & Biomolecular Chemistry

PAPER

View Article Online
View Journal | View Issue

Cite this: *Org. Biomol. Chem.*, 2014, **12**, 2273

Enzyme-promoted regioselective coupling oligomerization of isorhapontigenin towards the first synthesis of (±)-gnetulin†

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Received 10th December 2013,
Accepted 3rd February 2014

DOI: 10.1039/c3ob42456a

www.rsc.org/obc

We report the first synthesis of a natural (±)-gnetulin and an unnatural analogue of (±)-gnemonol M by using the regioselective oxidative coupling reactions of 5-*tert*-butyl-isorhapontigenin as the key step. Both the effects of different enzyme-catalyzed systems on the structures of coupling products and structural transformations of coupling products in the presence of several Lewis acids were systematically investigated.

1. Introduction

Natural oligostilbenes, a class of polyphenols derived from resveratrol (**1**) and isorhapontigenin (**7**), have attracted considerable interest due to their novel architectures and diverse bioactivities over the past three decades (Fig. 1).[1] Many synthetic chemists work on the synthesis of these oligomers, especially resveratrol dimers such as **2–6**, either through biotransformation strategies or chemically controlled approaches to intensively investigate their structure–activity relationship.[2] However, little attention has been given to the isorhapontigenin-based oligomers like **8–12**. These have the same construction patterns as **2–6**, but differ in OMe groups at the aromatic rings and few synthetic efforts towards them have been reported to date. This is because the traditional biomimetic synthesis strategy was not specific for the isorhapontigenin oligomers owing to the lack of regioselective control in the direct oxidative coupling of **7**, which led to diverse oligomeric structures.[3] Secondly, a variety of well-designed cascade reactions directed to the chemically controlled syntheses of resveratrol oligomers, reported by Snyder's group for example,[4] were not readily applicable to the syntheses of isorhapontigenin oligomers, because the phenolic hydroxyl groups of all the common building blocks were generally protected by methyl ethers before the crucial oligomeric skeletons were constructed. Thus, it was a significant challenge to develop effective synthetic routes for the preparation of various oligomeric isorhapontigenins. However, it was at present practical to produce these oligomers by improving the stereo-selectivity in the biomimetic synthesis methods.

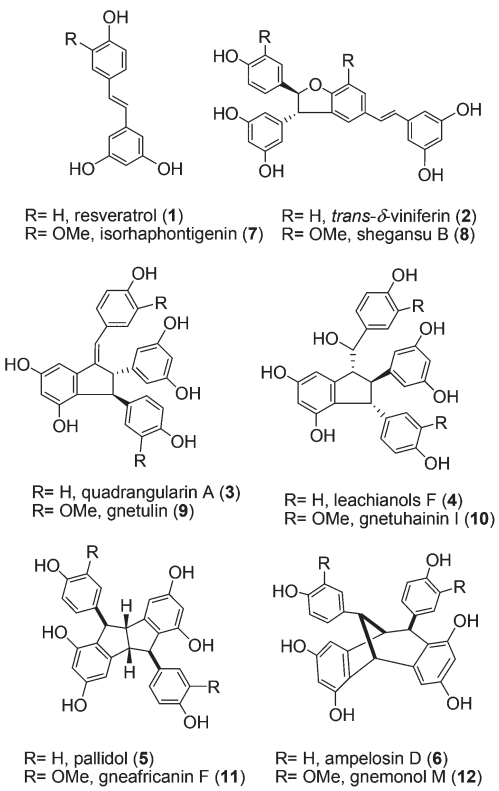

Fig. 1 Some representative natural oligostilbenes.

In 2006, Hou and coworkers succeeded in the first synthesis of (±)-quadrangularin A (**3**) by utilizing the enzyme-catalyzed regioselective oxidative coupling reaction of 2,5-di-*tert*-butyl-resveratrol as the key step.[5] To explore the validity of Hou's strategy in the syntheses of oligostilbenes, our group followed

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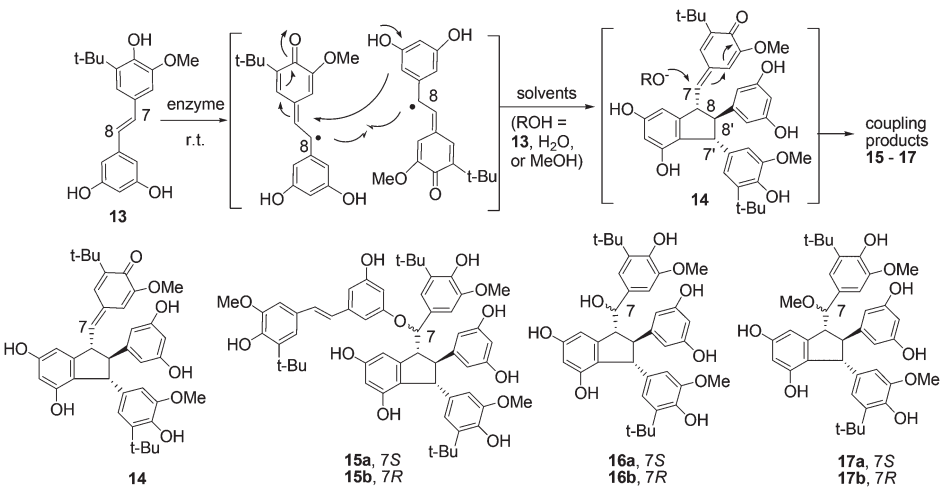
†Electronic supplementary information (ESI) available. See DOI: 10.1039/c3ob42456a

it and prepared several dimeric stilbenes including **3**, **5**, **6**, **11** and **12** in the presence of different metallic oxidants.[6] We then turned to a representative isorhapontigenin dimer—(±)-gnetulin (**9**). To the best of our knowledge, no attempt at the synthesis of **9** has been reported so far even though it was first isolated from the *Gnetum* species in early 1993, later in 2000 and 2001.[7] Herein the oxidative coupling reactions of 5-*tert*-butyl-isorhapontigenin (**13**) using different enzyme catalysts were studied to synthesize (±)-gnetulin (**9**).

2. Results and discussion

Our synthetic route commenced with the preparation of the coupling precursor **13** according to our previous procedure.[6b] Two commercially available enzymes with different activity grades were selected as the catalysts under variable solvent conditions to conduct the single-electron-transfer mediated oxidative coupling reactions of **13**. As illustrated in Scheme 1 and Table 1, stilbene **13** was first treated with horseradish peroxidase (HRP) with lower activity (250 units mg^{-1}) in the acetone–water system and the coupling reaction oxidized by H_2O_2 proceeded slowly. Surprisingly, the expected 8–8 coupling intermediate **14** was very unstable and only detected initially by thin layer chromatography (TLC). Two diastereoisomeric trimers **15a** and **15b** were formed gradually as major products when dimer **14** was attacked by monomer **13** in the reaction system. However, isomers **16a** and **16b** with a 2 : 1 ratio were isolated from the coupling products in 63% yield when **13** was subjected to HRP with higher activity (≥300 units mg^{-1}) in the acetone–water (2 : 1) system. The dimers' structures were unaffected, but their yields obviously decreased by changing the ratio of acetone–water from 2 : 1, 1 : 1 to 1 : 2. Additionally, acetone was replaced by methanol to survey the solvent effects on the coupling reaction as shown in entry 3, in which similar dimers **17a** and **17b** were obtained correspondingly in 32% yield. To our delight, the desired coupling intermediate **14** was relatively stable and isolated in 48% yield when the coupling reaction of **13** was conducted under slightly acidic conditions (pH = 6). Apart from HRP, laccase was also employed to catalyze the oxidative coupling of **13** in the neutral environment, and diastereoisomers **16a** and **16b** with a 1 : 1.5 ratio were afforded in lower yield than in the HRP–H_2O_2 system.

With these coupling products in hands, we hoped to synthesize several natural oligomers including **9** and **10** through the respective deprotection reactions of **14–17**. Nevertheless, none of them succeeded under our standard debutylation conditions (6 equiv. $AlCl_3$, 50 °C) due to the complicated products that resulted. Inspired by Hou's work,[5] the protonation rearrangement of **14** by treating with activated neutral Al_2O_3 to afford the key precursor **18** was attempted, but failed unexpectedly after numerous trials. Species **14** was almost unchanged with a small quantity of Al_2O_3, while only trace amounts of **18**

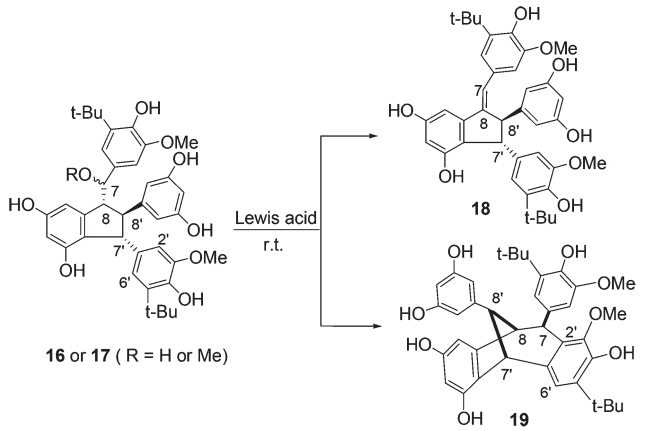


Scheme 1

Table 1 Treatment of **13** with different enzyme-catalyzed conditions

Entry	Enzyme oxidants	Solvent systems (volume ratio)	Time (hour)	Coupling products (molar ratio)	Conversion (%)	Recovered **13** (%)
1	HRP–H_2O_2 [a]	Acetone–H_2O (3 : 1)	60	**15a : 15b** (1.5 : 1)	44	40
2	HRP–H_2O_2 [b]	Acetone–H_2O (2 : 1)	48	**16a : 16b** (2 : 1)	63	11
3	HRP–H_2O_2 [b]	Methanol–H_2O (4 : 1)	48	**17a : 17b** (1 : 1)	32	12
4	HRP–H_2O_2 [b]	Acetone–pH 6.0 buffer (2 : 1)	60	**14**	48	27
5	Laccase[c]	Acetone–H_2O (2 : 1)	60	**16a : 16b** (1 : 1.5)	37	21

[a] RZ ≈ 3, activity = 250 units mg^{-1}. [b] RZ > 3, activity ≥ 300 units mg^{-1}. [c] From *Trametes versicolor*, activity ≥ 0.5 unit mg^{-1}.



Scheme 2

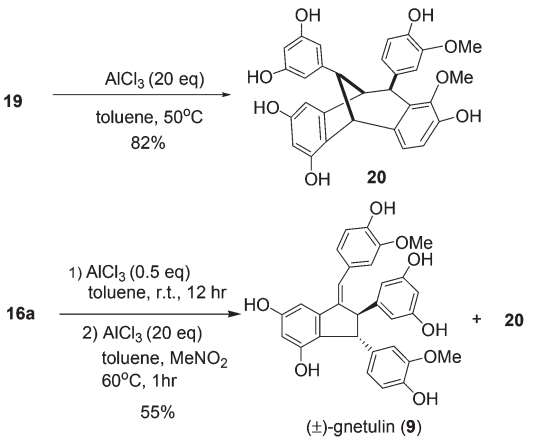
and unconverted **14** were detected in reaction mixtures if **14** was absorbed onto excess Al_2O_3. This was unfavorable for the accumulation of **18**. Thus, we had to revise our preliminary strategy for the synthesis of **9**. As depicted in Scheme 2 and Table 2, several Lewis acids were selected to eliminate H_2O or MeOH from **16** or **17** to produce **18**.

The initial attempt to convert **16** to **18** through the dehydration with *p*-TsOH resulted in complex products. Then $BF_3{\cdot}Et_2O$ was selected to catalyze the dehydration of **16a** as shown in entries 2 and 3.[8] Surprisingly, no new spots appeared on the TLC plates in these two reactions. However, the ^1H NMR spectrum of the recovered starting materials showed the mixture of the desired **18** and the unreacted **16a** (1 : 2), or the bridged structure **19**. The formation of **19** may be explained as a result of further intramolecular Friedel–Crafts alkylation of **16a** and **18**, in which the electron-rich C-2′ underwent an electrophilic attack by the carbocation at C-7 formed in the presence of an excess amount of $BF_3{\cdot}Et_2O$. It is worth noting that the dimers **16**, **18** and **19** possessed the same polarity and were difficult to distinguish on TLC, except on the ^1H NMR spectrum. The treatment of **17** with $BF_3{\cdot}Et_2O$ (5 equiv.) also provided **19** as the single product in high yield.

Subsequently, **16a** was subjected to 8 equivalents of $AlCl_3$ at room temperature to give the mixture of **18** and **16a** as well as a few big polar unidentified products. By decreasing the amount of $AlCl_3$ and prolonging the reaction time, the molar ratio of **18** and **16a** and their yields increased significantly and reached the acceptable values (3 : 1 and 71%) when 0.5 equivalent of $AlCl_3$ was used for 20 hours as shown in entry 8.

Finally, the debutylation reaction of **19** was conducted with 20 equivalents of $AlCl_3$ at 50 °C for an hour and produced the unnatural dimeric isorhapontigenin **20**, an analogue of gnemonol M (**12**), in 82% yield (Scheme 3). Crucially, a mixture of the desired products (±)-gnetulin (**9**) and **20** (1 : 1) was obtained in 55% yield in a one-pot reaction of **16a**, in which 0.5 equivalent of $AlCl_3$ was used first in toluene at room temperature for 12 hours and 20 equivalents of $AlCl_3$ were added at 60 °C for another hour. The spectrum of **9** was in good agreement with the values reported in the literature.[7] The formation of **20** in this process can be explained as that the unreacted **16a** at the first stage underwent intramolecular Friedel–Crafts alkylation and subsequent debutylation at the second stage. It should be mentioned that (±)-**9** was difficult to purify from the mixture of **9** and **20** using the conventional chromatographic methods because of their totally identical polarity.

In addition, we also synthesized **17a** or **17b** through the reaction of **16a** or **16b** with HBr in MeOH solution at room temperature in 82%–86% yield, respectively (Scheme 4). The unexpected dimer **21** was obtained in 84% yield when the reaction mixture of **16a** was heated to 40 °C accidently. Interestingly, when **16a** reacted with 2 equivalents of $BF_3{\cdot}Et_2O$ for 8 hours, a small quantity of **21** was isolated from the products including **18** and **19**. The existence of **21** should be attributable

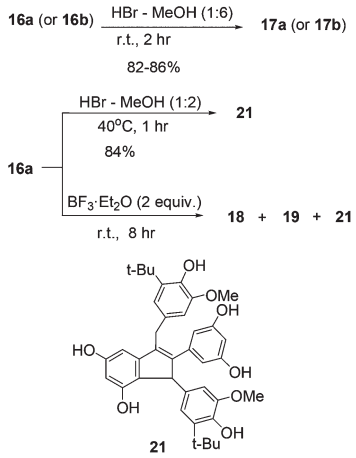


Scheme 3

Table 2 Reactions of coupling products with several Lewis acids

Entry	Coupling products	Lewis acid (equiv.)	Solvent	Time (hour)	Products (molar ratio)	Isolated yield (%)
1	**16a** (or **16b**)	*p*-TsOH (1)	CH_2Cl_2	0.5	Complex	—
2	**16a**	$BF_3{\cdot}Et_2O$ (2)	CH_2Cl_2	1	**18 : 16a** (1 : 2)[a]	27
3	**16a** (or **16b**)	$BF_3{\cdot}Et_2O$ (4)	CH_2Cl_2	1	**19**	90
4	**17a** (or **17b**)	$BF_3{\cdot}Et_2O$ (5)	CH_2Cl_2	1	**19**	92
5	**16a**	$AlCl_3$ (8)	Toluene	1.5	**18 : 16a** (1 : 3)[a]	15
6	**16a**	$AlCl_3$ (4)	Toluene	2	**18 : 16a** (1 : 2)[a]	22
7	**16a**	$AlCl_3$ (1.5)	Toluene	4	**18 : 16a** (2 : 1)[a]	43
8	**16a**	$AlCl_3$ (0.5)	Toluene	20	**18 : 16a** (3 : 1)[a]	71

[a] Determined by a crude ^1H NMR spectrum.



Scheme 4

to the further migration rearrangement of double bonds of **18** in an acidic environment, which clearly affirmed the instability of **16** (or **18**) under the acidic conditions.

3. Conclusions

In summary, the enzyme-catalyzed oxidative coupling reactions of 5-*tert*-butyl-isorhapontigenin (**13**) in various solvents were studied extensively and several 8–8 coupling oligomers including **14–17** were prepared. The treatments of dimer **16** with different Lewis acids led to **18** as the dehydrated product or **19** as the alkylated product, which depended on the type and the amount of Lewis acid used. The first synthesis of (±)-gnetulin (**9**) and unnatural dimeric isorhapontigenin (**20**) was successfully accomplished by the global removal of *tert*-butyl groups in **18** and **19**, respectively. Our synthetic strategy for this family of interesting natural products is simple, rapid, and applicable to the preparation of other analogues of **9**, which is ongoing in our laboratory.

4. Experimental

General methods

Structural determinations of the isolated compounds were based on ^{1}H, ^{13}C NMR, NOESY, ^{1}H–^{1}H COSY, HMBC spectra, and HRMS analysis. All NMR spectra were recorded on a Varian Mercury 400 MHz instrument in a solvent as indicated. HRMS spectra were measured on an Autostec-3090 mass spectrometer. All solvents were freshly purified and dried by standard techniques prior to use. Purification of products was performed by column chromatography (CC) on silica gel (200–300 mush), purchased from Qingdao Marine Chemical Co. (Qingdao, China).

The enzyme-catalyzed coupling reaction of stilbene 13

Method A: synthesis of coupling trimers 15a and 15b. A solution of compound **13** (0.60 g, 1.9 mmol) and horseradish peroxidase (13 mg, RZ ≈ 3, activity = 250 units mg^{-1}) in acetone (24 mL) and water (8 mL) was treated with hydrogen peroxide (3%, 10 mL) at room temperature under an argon atmosphere and continuously stirred for 60 hours. Acetone was removed and the aqueous reaction mixture was extracted with EtOAc (60 mL), washed with saturated brine and then dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified on a silica gel (CH_2Cl_2–MeOH = 20 : 1) to give unchanged **13** (260 mg), and two trimers **15a** (90 mg, 26.5%) and **15b** (60 mg, 17.6%).

15a: R_f = 0.25 (CH_2Cl_2–MeOH = 15 : 1); yellow amorphous powder; ^{1}H NMR (CD_3COCD_3, 400 MHz) δ: 1.36 (s, 9H), 1.38 (s, 9H), 1.44 (s, 9H), 3.52 (t, J = 3.0 Hz, 1H), 3.60 (dd, J = 8.1, 3.0 Hz, 1H), 3.71 (s, 3H), 3.76 (s, 3H), 3.87 (s, 3H), 3.84 (d, J = 3.0 Hz, 1H), 5.04 (d, J = 8.1 Hz, 1H), 5.69 (brs, 1H), 6.26 (brs, 5H), 6.32 (d, J = 1.8 Hz, 1H), 6.35 (brs, 1H), 6.48 (s, 1H), 6.60 (d, J = 1.2 Hz, 1H), 6.66 (d, J = 1.2 Hz, 1H), 6.68 (s, 1H), 6.77 (d, J = 14.4 Hz, 1H), 6.82 (d, J = 14.4 Hz, 1H), 6.91 (s, 1H), 6.98 (d, J = 1.8 Hz, 1H), 7.11 (s, 1H), 7.13 (s, 1H); ^{13}C NMR (CD_3COCD_3, 100 MHz) δ: 30.5 (9C), 35.2 (3C), 56.4 (3C), 59.3, 61.4, 84.3, 101.3, 102.5, 103.9, 105.2, 106.1 (3C), 107.1, 107.2, 108.3, 109.4, 118.0, 119.4, 120.4, 123.0, 126.4, 128.7, 129.9, 130.4, 134.9, 135.2, 135.8, 136.7, 140.3, 143.6, 145.0, 145.6, 145.9, 147.9, 148.1, 148.4, 150.7, 155.0, 158.4, 159.1, 159.3 (3C), 160.6; HRMS (ESI): m/z calcd for $[M - H]^+$: 939.4325, found: 939.4341.

15b: R_f = 0.24 (CH_2Cl_2–MeOH = 15 : 1); yellow amorphous powder; ^{1}H NMR (CD_3COCD_3, 400 MHz) δ: 1.31 (s, 9H), 1.33 (s, 9H), 1.43 (s, 9H), 2.89 (s, 1H), 3.50 (d, J = 8.4 Hz, 1H), 3.68 (s, 3H), 3.79 (s, 3H), 3.90 (s, 3H), 4.34 (s, 1H), 5.01 (d, J = 8.4 Hz, 1H), 5.84 (d, J = 1.8 Hz, 2H), 6.14 (t, J = 1.8 Hz, 1H), 6.26 (d, J = 1.5 Hz, 1H), 6.37 (d, J = 1.5 Hz, 1H), 6.49 (d, J = 1.8 Hz, 2H), 6.51(s, 2H), 6.56 (s, 1H), 6.63 (s, 1H), 6.80 (d, J = 16.8 Hz, 1H), 6.81 (d, J = 1.5 Hz, 1H), 6.86 (d, J = 16.8 Hz, 1H), 6.97 (s, 1H), 7.05 (s, 1H); ^{13}C NMR (CD_3COCD_3, 100 MHz) δ: 29.8 (3C), 30.0 (6C), 35.2 (3C), 55.5, 56.4 (3C), 58.4, 61.3, 85.1, 101.0, 102.5, 104.0, 105.3, 105.5 (2C), 106.0, 107.0 (2C), 108.6, 109.3, 117.9, 119.2, 119.5, 122.1, 126.5, 128.7, 129.9, 131.3, 135.1, 135.4, 135.9, 136.8 (2C), 140.3, 143.9, 145.0, 145.6, 148.2, 148.1, 148.5, 149.0, 151.2, 155.1, 159.1 (3C), 160.9; HRMS (ESI): m/z calcd for $[M - H]^+$: 939.4325, found: 939.4315.

Method B: synthesis of coupling dimers 16a and 16b. (1) A solution of compound **13** (0.43 g, 1.37 mmol) and horseradish peroxidase (21 mg, RZ > 3, activity ≥ 300 units mg^{-1}) in acetone (12 mL) and water (6 mL) was treated with hydrogen peroxide (3%, 4 mL) at room temperature under an argon atmosphere and continuously stirred for 48 hours. Acetone was removed and the aqueous reaction mixture was extracted with EtOAc (50 mL), washed with saturated brine and then dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified on a silica gel (25 : 1 CH_2Cl_2–MeOH) to give unchanged **13** (47 mg), and two dimers **16a** (160 mg, 42%) and **16b** (80 mg, 21%).

(2) 24 mg laccase (from *Trametes versicolor*, activity ≥ 0.5 unit mg^{-1}) was dissolved in water (2 mL) and added to the solution of compound **13** (0.10 g, 0.32 mmol) in acetone

(4 mL) at room temperature under an argon atmosphere. The reaction mixture was continuously stirred for 60 hours. Acetone was removed and the aqueous reaction mixture was extracted with EtOAc (20 mL), washed with saturated brine and then dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified on a silica gel (CH_2Cl_2–MeOH = 25 : 1) to give unchanged **13** (21 mg), and two minor trimers **16a** (12 mg, 15%) and **16b** (17 mg, 22%).

16a: R_f = 0.33 (CH_2Cl_2–MeOH = 15 : 1); brown amorphous powder; ^{1}H NMR (CD_3COCD_3, 400 MHz) δ: 1.33 (s, 9H), 1.34 (s, 9H), 3.34 (dd, J = 8.0, 3.6 Hz, 1H), 3.60 (t, J = 3.6 Hz, 1H), 3.76 (s, 3H), 3.77 (s, 3H), 4.18 (d, J = 4.4 Hz, 1H), 4.27 (d, J = 3.6 Hz, 1H), 4.47 (dd, J = 8.0, 4.4 Hz, 1H), 5.66 (d, J = 2.0 Hz, 1H), 6.16 (d, J = 2.0 Hz, 2H), 6.17 (d, J = 2.0 Hz, 1H), 6.23 (t, J = 2.0 Hz, 1H), 6.58 (d, J = 2.0 Hz, 2), 6.66 (d, J = 2.0 Hz, 1H), 6.88 (d, J = 2.0 Hz, 1H), 7.15(s, 1H), 7.21 (s, 1H), 7.35 (s, 1H), 7.93 (s, 1H), 8.10 (s, 2H); ^{13}C NMR (CD_3COCD_3, 100 MHz) δ: 30.1 (6C), 34.8, 34.9, 56.0, 56.1, 56.3, 58.7, 62.4, 77.7, 100.7, 101.8, 105.7, 105.9 (2C), 107.8, 108.9, 118.2, 119.1, 122.6, 134.2, 134.3, 134.8, 136.6, 143.2, 144.2, 147.0, 147.4, 147.6, 151.2, 154.6, 158.1, 158.9 (2C); HRMS (ESI): m/z calcd for $[M - H]^+$: 643.2913, found: 643.2919.

16b: R_f = 0.32 (CH_2Cl_2–MeOH = 15 : 1); brown amorphous powder; ^{1}H NMR (CD_3COCD_3, 400 MHz) δ: 1.28 (s, 9H), 1.33 (s, 9H), 2.81 (dd, J = 3.2, 2.8 Hz, 1H), 3.33 (dd, J = 8.8, 3.2 Hz, 1H), 3.66 (s, 3H), 3.75 (s, 3H), 4.08 (d, J = 4.8 Hz, 1H), 4.24 (d, J = 2.8 Hz, 1H), 4.50 (dd, J = 8.8, 4.8 Hz, 1H), 5.85 (d, J = 2.4 Hz, 2H), 6.16 (d, J = 2.0 Hz, 2H), 6.12 (t, J = 2.4 Hz, 1H), 6.34 (d, J = 2.4 Hz, 1H), 6.46 (d, J = 1.6 Hz, 1H), 6.49 (d, J = 2.0 Hz, 1H), 6.54 (d, J = 2.0 Hz, 1H), 6.56 (d, J = 2.0 Hz, 1H), 7.14 (s, 1H), 7.16 (s, 1H), 7.49 (s, 1H), 8.02 (s, 2H), 8.10 (s, 1H); ^{13}C NMR (CD_3COCD_3, 100 MHz) δ: 29.6 (6C), 34.7, 34.9, 55.8, 56.0, 56.1, 59.1, 61.8, 88.1, 100.7, 102.0, 105.5 (2C), 106.0, 108.2, 108.9, 118.0, 118.3, 121.9, 134.6, 134.7, 135.0, 136.3, 143.4, 144.0, 147.3, 147.7, 149.2, 151.0, 154.7, 158.5, 158.8 (2C); HRMS (ESI): m/z calcd for $[M - H]^+$: 643.2913, found: 643.2907.

Method C: synthesis of coupling dimers 17a and 17b. A solution of compound **13** (95 mg, 0.30 mmol) and horseradish peroxidase (8.9 mg, RZ > 3, activity ≥ 300 units mg^{-1}) in methanol (4 mL) and water (1 mL) was treated with hydrogen peroxide (3%, 3 mL) at room temperature under an argon atmosphere and stirred continuously for 48 hours. Acetone was removed and the aqueous reaction mixture was extracted with EtOAc (20 mL), washed with saturated brine and then dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified on a silica gel (CH_2Cl_2–MeOH = 25 : 1) to give unchanged **13** (11 mg), and two dimers **17a** (13.4 mg, 16%) and **17b** (13.6 mg, 16%).

17a: R_f = 0.36 (CH_2Cl_2–MeOH = 15 : 1); yellow amorphous powder; ^{1}H NMR (CD_3COCD_3, 400 MHz) δ: 1.28 (s, 9H), 1.34 (s, 9H), 2.69 (brs, 1H), 3.05 (s, 3H), 3.34 (d, J = 8.0 Hz, 1H), 3.66 (s, 3H), 3.78 (s, 3H), 3.93 (d, J = 8.0 Hz, 1H), 4.25 (brs, 1H), 5.77 (d, J = 2.0 Hz, 2H), 6.09 (d, J = 2.0 Hz, 1H), 6.32 (d, J = 2.0 Hz, 1H), 6.33 (d, J = 2.0 Hz, 1H), 6.36 (d, J = 2.0 Hz, 1H), 6.51 (d, J = 2.0 Hz, 1H), 6.56 (d, J = 2.0 Hz, 1H), 6.71 (d, J = 2.0 Hz, 1H), 7.24 (s, 1H), 7.31 (s, 1H), 7.71 (s, 1H), 8.05 (s, 2H), 8.17 (s, 1H); ^{13}C NMR (CD_3COCD_3, 100 MHz) δ: 30.2 (6C), 35.0, 35.2, 55.8, 56.3, 56.4, 56.7, 59.0, 60.8, 89.0, 101.0, 102.4, 105.6 (2C), 106.2, 108.9, 109.4, 118.2, 119.4, 122.1, 131.4, 134.8, 135.3, 136.7, 143.7, 144.9, 147.9, 148.1, 149.7, 151.3, 155.0, 159.0, 159.1 (2C); HRMS (ESI): m/z calcd for $[M - H]^+$: 657.3069, found: 657.3075.

17b: R_f = 0.35 (CH_2Cl_2–MeOH = 15 : 1); yellow amorphous powder; ^{1}H NMR (CD_3COCD_3, 400 MHz) δ: 1.29 (s, 9H), 1.31 (s, 9H), 2.99 (s, 3H), 3.34 (dd, J = 8.0, 3.6 Hz, 1H), 3.41 (t, J = 3.6 Hz, 1H), 3.75 (s, 3H), 3.78 (s, 3H), 3.95 (d, J = 8.0 Hz, 1H), 4.25 (d, J = 3.6 Hz, 1H), 5.69 (brs, 1H), 6.16 (d, J = 2.0 Hz, 2H), 6.18 (d, J = 2.0 Hz, 1H), 6.23 (d, J = 2.0 Hz, 1H), 6.53 (d, J = 2.0 Hz, 1H), 6.55 (d, J = 2.0 Hz, 1H), 6.64 (d, J = 2.0 Hz, 1H), 6.77 (d, J = 2.0 Hz, 1H), 7.12 (s, 1H), 7.27 (s, 2H), 7.90 (s, 1H), 8.07 (s, 2H); ^{13}C NMR (CD_3COCD_3, 100 MHz) δ: 30.2 (6C), 35.1, 35.2, 56.3, 56.4, 56.6, 59.2, 60.5, 61.1, 88.0, 101.2, 102.2, 106.2, 106.3 (2C), 108.5, 109.2, 118.5, 120.6, 122.8, 130.6, 134.8, 135.2, 136.6, 143.6, 145.0, 146.8, 147.9, 148.1, 150.7, 155.0, 158.5, 159.3 (2C); HRMS (ESI): m/z calcd for $[M - H]^+$: 657.3069, found: 657.3065.

Method D: synthesis of coupling dimer 14. Horseradish peroxidase (5.2 mg, RZ > 3, activity ≥ 300 units mg^{-1}) was dissolved in buffer solution (pH = 6), to which a solution of compound **13** (50 mg, 0.16 mmol) in acetone (6 mL) was added at room temperature under an argon atmosphere. After stirring for 10 min the mixture was treated with hydrogen peroxide (3%, 0.5 mL) and continuously stirred for 60 hours. The reaction mixture was quenched with water and was extracted with EtOAc (20 mL), washed with saturated brine and then dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified on a silica gel (CH_2Cl_2–MeOH = 35 : 1) to give unchanged **13** (13 mg), and dimer **14** (18 mg, 48%).

14: R_f = 0.46 (CH_2Cl_2–MeOH = 15 : 1); yellow amorphous powder; ^{1}H NMR (CD_3COCD_3, 400 MHz): δ: 1.30 (s, 9H), 1.34 (s, 9H), 3.11 (dd, J = 9.0, 8.4 Hz, 1H), 4.36 (d, J = 8.4 Hz, 1H), 4.63 (dd, J = 9.2, 9.0 Hz, 1H), 6.17 (brs, 1H), 6.22 (brs, 1H), 6.27 (brs, 2H), 6.31 (brs, 1H), 6.49 (d, J = 9.2 Hz, 1 H), 6.61 (brs, 1 H), 6.63 (brs, 1 H), 6.64 (brs, 1 H), 7.08 (s, 1H), 7.19 (s, 1H), 7.21 (s, 1H), 8.17 (s, 2H), 8.21 (s, 1 H); ^{13}C NMR (CD_3COCD_3, 100 MHz): δ: 30.4 (6 C), 34.8, 34.9, 56.0, 56.1, 58.7, 60.2, 62.4, 100.7, 101.9, 105.7, 105.9 (2 C), 106.0, 108.2, 108.9, 118.2, 119.1, 122.6, 134.2, 134.8, 136.6, 143.2, 144.3, 147.1, 149.8, 151.0, 153.6, 154.6, 156.9, 158.2, 159.0 (2C); HRMS (ESI): m/z calcd for $[M - H]^+$: 625.2796, found: 625.2787.

Synthesis of 18. $AlCl_3$ (4.6 mg, 0.035 mmol) was added to a solution of compound **16a** (45 mg, 0.07 mmol) in dry toluene (3 mL) at room temperature. The reaction mixture was stirred for 20 hours and then quenched with ice-water, and extracted with EtOAc (20 mL). The combined organic layer was washed with saturated brine and then dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue purified on a silica gel (CH_2Cl_2–MeOH = 25 : 1) to afford the mixture of unreacted **16a** and **18** (3 : 1, 71%).

18: R_f = 0.33 (CH_2Cl_2–MeOH = 15 : 1); yellow amorphous powder; ^{1}H NMR (CD_3COCD_3, 400 MHz): δ: 1.31 (s, 18H), 3.62 (s, 3H), 3.70 (s, 3H), 4.22 (brs, 1H), 4.29 (brs, 1H), 6.20 (d, J =

2.0 Hz, 1H), 6.30 (d, J = 2.0 Hz, 1H), 6.35 (d, J = 2.0 Hz, 2H), 6.59 (d, J = 2.0 Hz, 1H), 6.80 (d, J = 2.0 Hz, 1H), 6.81 (d, J = 2.0 Hz, 1H), 6.88 (d, J = 2.0 Hz, 1H), 7.09 (s, 2H), 7.38 (brs, 1 H), 7.89 (s, 1H), 8.21 (s, 2H), 8.26 (s, 1H); ^{13}C NMR (CD_3COCD_3, 100 MHz): δ: 30.4 (6C), 35.2 (2C), 56.3, 56.4, 58.1, 60.8, 98.5, 101.4, 103.6, 106.5, 107.8, 108.7, 109.8, 118.1, 121.6, 122.4, 123.9, 124.5, 128.9, 135.5, 135.6, 136.9, 142.8, 143.8, 144.8, 147.4, 147.9, 149.2, 155.9, 159.6, 159.8 (2C); HRMS (ESI): m/z calcd for $[M - H]^+$: 625.2807, found: 625.2809.

Synthesis of 19. A solution of $BF_3{\cdot}Et_2O$ (4 equiv.) was added to a solution of compound **16a** (45 mg, 0.06 mmol) in dry dicholoromethane (5 mL). The reaction mixture was stirred for 1 hour at room temperature and then quenched with ice-water (5 mL), and extracted with EtOAc. The combined organic layer was washed with saturated brine and then dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue purified on a silica gel (CH_2Cl_2–MeOH = 25 : 1) to afford compound **19** (40 mg, 90%).

19: R_f = 0.33 (CH_2Cl_2–MeOH = 15 : 1); yellow amorphous powder; ^{1}H NMR (CD_3COCD_3, 400 MHz): δ: 1.35 (s, 9H), 1.38 (s, 9H), 3.24 (s, 3H), 3.35 (brs, 1H), 3.72 (brs, 1H), 3.81 (s, 3H), 4.23 (brs, 1H), 4.26 (brs, 1H), 6.00 (d, J = 2.0 Hz, 2H), 6.03 (d, J = 2.0 Hz, 2H), 6.47 (d, J = 2.0 Hz, 1H), 6.71 (d, J = 2.0 Hz, 1H), 6.83 (d, J = 2.0 Hz, 1H), 7.05 (s, 1H), 7.15 (s, 1 H), 7.22 (s, 1 H), 7.88 (s, 1H), 7.89 (s, 1H), 7.99 (s, 2H); ^{13}C NMR (CD_3COCD_3, 100 MHz): δ: 30.4 (6C), 35.1 (2C), 49.0, 52.0, 56.5, 57.7, 59.9, 60.5, 101.0, 101.9, 104.2, 105.8, 107.1 (2C), 109.8, 110.4, 119.5, 119.6, 128.5, 135.0, 135.2, 136.5, 137.8, 143.5, 143.6, 147.2, 147.9, 148.3, 152.8, 158.4, 158.8 (2C); HRMS (ESI): m/z calcd for $[M - H]^+$: 625.2796, found: 625.2789.

Synthesis of 20. $AlCl_3$ (92 mg, 0.69 mmol) was added to a solution of compound **19** (24 mg, 0.04 mmol) in dry toluene (8 mL) at room temperature. The mixture was heated to 50 °C and stirred for 2 hours. The reaction was quenched with ice-water and extracted with EtOAc. The combined organic layer was washed with saturated brine and then dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue purified on a silica gel (CH_2Cl_2–MeOH = 20 : 1) to afford compound **20** (16 mg, 82%).

20: R_f = 0.28 (CH_2Cl_2–MeOH = 10 : 1); brown amorphous powder; ^{1}H NMR (CD_3COCD_3, 400 MHz): δ: 3.23 (s, 3H), 3.33 (brs, 1H), 3.71 (brs, 1H), 3.82 (s, 3H), 4.22 (brs, 1H), 4.29 (brs, 1H), 5.98 (d, J = 2.0 Hz, 2H), 6.03 (d, J = 2.0 Hz, 2H), 6.60 (dd, J = 8.0, 2.0 Hz, 1H), 6.68 (d, J = 8.0 Hz, 1H), 6.76 (d, J = 8.0 Hz, 1H), 6.93 (d, J = 8.0 Hz, 1H), 6.98 (d, J = 2.0 Hz, 1H), 7.45 (brs, 1H), 7.77 (brs, 1 H), 7.88 (brs, 1 H), 7.94 (brs, 1H), 8.03 (brs, 2H); ^{13}C NMR (CD_3COCD_3, 100 MHz): δ: 48.2, 48.7, 51.5, 56.3, 57.7, 59.2, 100.9, 101.8, 104.1, 106.9, 107.0, 113.0, 115.0, 115.1, 115.2, 121.5, 128.5, 130.5, 139.6, 139.9, 145.6, 146.8, 147.0, 148.0, 149.4, 152.6, 152.7, 158.4, 158.8, 158.9; HRMS (ESI): m/z calcd for $[M + H]^+$: 515.1700, found: 515.1704.

Synthesis of (±)-gnetulin (9). $AlCl_3$ (5 mg, 0.037 mmol) was added to a solution of compound **16a** (51 mg, 0.079 mmol) in dry toluene (5 mL) and was stirred for 24 hours at room temperature. Then $AlCl_3$ (210 mg, 1.58 mmol) and CH_3NO_2 (0.5 mL) were added to the reaction mixture, which was heated to 60 °C and stirred for another 1 hour. The reaction was quenched with ice-water and extracted with EtOAc. The combined organic layer was washed with saturated brine and then dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue purified on a silica gel (CH_2Cl_2–MeOH = 20 : 1) to afford the mixture of (±)-**9** and **20** (1 : 1, 55%) as a brown amorphous powder.

(±)-**9**: R_f = 0.28 (CH_2Cl_2–MeOH = 10 : 1); ^{1}H NMR (CD_3COCD_3, 400 MHz): δ: 3.56 (s, 3H), 3.71 (s, 3H), 4.19 (brs, 1H), 4.27 (brs, 1H), 6.20 (t, J = 2.0 Hz, 1H), 6.30 (d, J = 2.0 Hz, 1H), 6.33 (d, J = 2.0 Hz, 2H), 6.50 (dd, J = 8.0, 2.0 Hz, 1H), 6.64 (d, J = 8.0 Hz, 1H), 6.69 (d, J = 8.0 Hz, 1H), 6.74 (d, J = 2.0 Hz, 1H), 6.78 (d, J = 2.0 Hz, 1H), 6.83 (dd, J = 8.0, 2.0 Hz, 1H), 6.89 (d, J = 2.0 Hz, 1H), 7.05 (s, 1H); ^{13}C NMR (CD_3COCD_3, 100 MHz): δ: 56.2 (2C), 58.0, 60.8, 98.5, 101.6, 103.9, 106.4 (2C), 112.2, 115.6 (2C), 120.1, 120.4, 123.3, 123.9, 124.6, 130.3, 138.5, 142.9, 145.9, 146.7, 147.3, 148.1 (2C), 149.2, 156.0, 159.8, 159.9 (2C); HRMS (ESI): m/z calcd for $[M + H]^+$: 515.1700, found: 515.1705.

Synthesis of 17 from 16. Aqueous 40% HBr (2 mL) was added dropwise to the solution of **16a** (or **16b**) (90 mg, 0.14 mmol) in methanol (12 mL). The reaction mixture was stirred continuously at room temperature for 2 hours and quenched with aqueous $NaHCO_3$. Methanol was removed and the aqueous reaction mixture extracted with EtOAc (20 mL), washed with saturated brine and then dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue purified on a silica gel (25 : 1 CH_2Cl_2–MeOH) to give **17a** (or **17b**) (82% or 86%) as a yellow amorphous powder.

Synthesis of 21. Aqueous 40% HBr (6 mL) was added dropwise to the solution of **16a** (88 mg, 0.14 mmol) in methanol (12 mL). The reaction mixture was stirred at 40 °C for 1 hour and quenched with aqueous $NaHCO_3$. Methanol was removed and the aqueous reaction mixture extracted with EtOAc (20 mL), washed with saturated brine and then dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue purified on a silica gel (25 : 1 CH_2Cl_2–MeOH) to give **21** (77 mg, 84%).

21: R_f = 0.40 (CH_2Cl_2–MeOH = 15 : 1); yellow amorphous powder; ^{1}H NMR (CD_3COCD_3, 400 MHz): δ: 1.20 (s, 9H), 1.34 (s, 9H), 3.62 (s, 3H), 3.73 (s, 3H), 3.88 (s, 2H), 4.91 (s, 1H), 6.17 (d, J = 2.0 Hz, 1H), 6.20 (d, J = 2.0 Hz, 1H), 6.35 (d, J = 2.0 Hz, 1H), 6.37 (d, J = 2.0 Hz, 2H), 6.53 (d, J = 2.0 Hz, 1H), 6.75 (brs, 2H), 6.86 (brs, 2H), 7.01 (s, 1H), 7.19 (s, 1 H), 7.69 (brs, 1 H), 8.00 (brs, 1H), 8.20 (brs, 2H); ^{13}C NMR (CD_3COCD_3, 100 MHz): δ: 30.2 (6C), 32.6, 35.0, 35.2, 56.4 (2C), 56.5, 100.9, 101.0, 102.0, 108.5, 108.6, 109.4, 109.9, 110.6, 119.5 (2C), 120.3, 129.9, 130.6, 134.8, 135.6, 137.2, 139.6, 143.6, 143.8, 148.0, 148.8, 150.1, 153.9, 158.8, 159.0 (2C); HRMS (ESI): m/z calcd for $[M - H]^+$: 625.2796, found: 625.2805.

Acknowledgements

This research work was financially supported by the National Natural Science Foundation of China (no. 21062010) and

'QingLan' Talent Engineering Funds of Lanzhou Jiaotong University (no. QL-08-06A).

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