Enzyme-promoted oxidative cross-coupling for the synthesis of oxyresveratrol-related heterodimers

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Abstract
Horseradish peroxidase–H₂O₂-mediated oxidative cross-coupling reactions of oxyresveratrol 2-methyl ether and brominated isorhapontigenin or brominated resveratrol efficiently produce two 8-5′-coupled dihydrobenzofuran-type heterodimers. The LiAlH₄-catalysed reductive debrominations of these cross-coupled dimeric intermediates provided the first synthesis of two unnatural oxyresveratrol-related heterodimers.

Keywords
biomimetic synthesis, heterodimers, oxidative cross-coupling, oxyresveratrol, regioselectivity

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Introduction
Natural polyphenolic oligostilbenes possess complex structures and have diverse biological properties as potential therapeutic agents in human health. Most of them are the homooligomers of stilbene units including resveratrol (1), isorhapontigenin (2), piceatannol (3) and oxyresveratrol (4) (Figure 1).¹⁻⁶ Numerous efforts have been devoted to synthesizing these homooligomeric products, especially oligomers of 1 for investigating their structure–activity relationships, and significant achievements had been made over the past 40 years.¹⁰⁻¹⁴ However, little attention has been paid to the small number of heterooligomers polymerized from different stilbene monomers, such as the heterodimers 5⁻⁸,¹⁵ Only a few efforts to synthesize these dimers have been made so far, which included FeCl₃·6H₂O-catalysed oxidative cross-coupling reactions of stilbenes 1 and 2 conducted by Lin’s group,² and enzyme-mediated biotransformation of the mixture of 1 and pterostilbene studied by Queiroz’s team.¹⁶ Both methods invariably afforded various complex homodimers and heterooligomers of two monomers in very low isolated yields, which resulted from the lack of regioselective control in the oxidative cross-coupling reactions of stilbenes. Recently, our group efficiently synthesized two resveratrol-related...
heterodimers including 5 using FeCl₃·6H₂O-catalysed or horseradish peroxidase (HRP)–H₂O₂-promoted oxidative cross-coupling reactions of 1 and 5-bromo-isorhapontigenin,¹¹ based on our research on the regioselective biometric synthesis of natural homodimers of 1 or 2.¹²−¹⁴ This preliminary success inspired us to prepare other natural stilbene heterooligomers such as oxyresveratrol-related heterodimers 6–8 using a similar synthetic strategy.

The key to synthesis of oxyresveratrol-related heterodimers 6–8 is the selection of stilbene precursors. We recently found that the direct oxidation of 4 promoted by different catalysts invariably led to a complex product mixture and the selective methyl protection of the reactive 2-OH in 4 remarkably improved the regioselectivity of the oxidative coupling reactions.¹² Hence, oxyresveratrol 2-methyl ether (9), as one of two hetero-monomers in the oxidative cross-coupling dimerization, should be more suitable than stilbene 4 for the synthesis of dimers 6–8.

**Results and discussion**

The oxidative coupling reactions of monomer 9 and stilbenes 1, 2 or 3 are believed to be the most concise approach to the heterooligomeric structures 6–8. We initially explored the direct cross-coupling reaction of 9 and 2 or 1 in the HRP–H₂O₂–aqueous acetone system, which resulted in complex and indistinguishable coupling product mixtures consisting of several homodimers and heterodimers of the two precursors. This result encouraged us to apply brominated isorhapontigenin 10 and brominated resveratrol 11 as the other precursor used with 9 to improve the regioselective control in the cross-coupling reactions.

As shown in Scheme 1, when monomer 9 was added slowly into an acetone–water–solution of stilbene 10 and HRP–H₂O₂, a small amount of heterodimer 12 and a large quantity of homodimer 13 were formed as the major coupling products. Consistent with our earlier work,⁵ the yield of heterodimer 12 was largely influenced by the molar ratios and the addition sequences of the two coupling precursors (9 and 10), as well as the amount and the addition rate of oxidant (HRP–H₂O₂). Hence, we optimized the cross-coupling reaction conditions to increase the yield of 12. When HRP and 3% H₂O₂ were added in batch within 20 min to a solution of equivalent amounts of 9 and 10 in acetone–water and the reaction continued for 1 h at room temperature until precursor 9 had disappeared, the heterodimer 12 with a 43% maximum yield and only a trace amount of homodimer 13 were isolated from the product mixture.

The dihydrobenzofuran skeleton of heterodimer 12 was established on the basis of ¹H and ¹³C NMR spectroscopy as well as by HRMS. The connectivity of the two heteromonomer units in dimer 12 was further confirmed by X-ray crystallographic analysis. This is the first report on the X-ray crystal structure of benzofuran-type oligostilbenes (Figure 2). The formation of the 8-5’-coupled heterodimer 12 verified the higher reactivity of the M₈ radical mesomer of stilbene 10 in comparison to that of 9, which preferentially participated in the cross-coupling dimerization under the optimized reaction conditions.

Based on the above result, we further studied the oxidative cross-coupling reaction of 9 and 11 under the HRP–H₂O₂-catalysed conditions (Scheme 1). When equivalent amounts of precursors 9 and 11 were added simultaneously into the reaction system, the oxidation of 9 was obviously faster than that of 11 and mostly produced the homodimer 13, and a very small amount of the 8-5’-coupled heterodimer 14. Under the optimized reaction conditions, 1.5 equiv. of 9 and 3% H₂O₂ were added dropwise into the reaction mixture within 30 min and stirring was continued for 1 h at room temperature until precursor 9 had disappeared. The homodimer 13 in 17% yield and heterodimer 14 in a 30% maximum yield were isolated from the product mixture. These results further confirmed that the yield of heterodimer 14 can be effectively increased by the optimization of the molar ratio and the addition sequence of precursors 9 and 11.

We next attempted to remove the bromine protecting groups and methyl groups from the heterodimeric intermediates 12 and 14 in order to synthesize several oligostilbenes. As shown in Scheme 2, the LiAlH₄-catalysed reductive debromination reaction of dimer 12 in tetrahydrofuran at 50°C smoothly led to unnatural product 15 in 65%
yield. Under the same deprotection condition, the bromine atoms of dimer 14 were removed and the heterodimer 16 was obtained in 70% yield. The subsequent direct demethylation of 12 for the synthesis of natural 7 or 8 was not successful because of the insolubility of 12 in the BBr3-dichloromethane system. The global acetylation of 12 followed by deacetylation and demethylation in one-pot finally led to an inseparable product mixture.

Conclusion

In conclusion, we have investigated the biomimetic cross-coupling reactions of two different stilbene monomers under HRP–H2O2–acetone–H2O oxidative conditions. The key to the regioselective synthesis of heterodimers 12 and 14 lays first in the distinct difference in the reactivity of 2-methylated oxyresveratrol (9) and brominated isorhapontigenin 10 or brominated resveratrol 11 under the same HRP–H2O2-catalysed oxidative conditions, and second in the application of appropriate molar ratios and addition sequences of the two coupling precursors. The X-ray crystal structure of 12 was reported, which confirms the dihydrobenzofuran skeleton of the oligostilbenes. Two unnatural oligostilbenes 15 and 16 were synthesized by the reductive debromination reactions of heterodimer 12 or 14. The formation of dihydrobenzofuran-type heterodimers 15 and 16 confirmed that this regioselective synthetic strategy is applicable to the preparation of other stilbene heterooligomers.

Experimental

General methods

Structural determinations of the isolated compounds were based on 1H, 13C NMR, NOESY, 1H-1H COSY and 1H-1H NOSEY spectra, and HRMS analysis. All NMR spectra were recorded on Varian Mercury 500 or 600 MHz instruments in the indicated solvent. 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 on silica gel (200–300 mesh), purchased from Qingdao Marine Chemical Co. (Qingdao, China). The monomer 9, 10 and 11 were chemically synthesized according to the procedure we previously reported.17,21,25

Figure 2. X-ray crystal structure of the heterodimer 12.

Scheme 2. Reductive debromination products of heterodimers 12 or 14.

5-[5-[(1E)-2-(3,5-dihydroxyphenyl)ethenyl]-2-(3-methoxy-4-hydroxyphenyl)-6-methoxy-2,3-dihydrobenzofuran-1-yl]-1,3-benzenediol (12). The precursor 9 (25.0 mg, 0.097 mmol) and stilbene 10 (32.6 mg, 0.097 mmol) were dissolved in acetone (3.0 mL). A solution of horseradish peroxidase (5.7 mg, RZ >3, activity ≥300 u/mg) in water (1.7 mL) and hydrogen peroxide (3% H2O2, 0.3 mL) were slowly added in batch to the reaction system within 40 min. The reaction was continued at room temperature under an argon atmosphere for 1 h. The acetone was removed and the aqueous reaction mixture was extracted with EtOAc (3 × 10 mL), washed with saturated brine and then dried over anhydrous MgSO4. The solvent was removed under reduced pressure and the residue was purified on a silica gel column chromatography (CH2Cl2/MeOH = 20:1) to give heterodimer 12 (24.7 mg, 43%) as a yellowish amorphous powder. This compound was recrystallized from a mixture of THF and petroleum ether (1:5:2) at 10°C to afford pale yellowish-like needle crystals. 12: m.p. 188–191°C; IR (KBr): νmax 3411, 2960, 1598, 1491, 1280, 1156, 1030 cm−1; 1H NMR (500 MHz, d-acetone): δ 3.73 (s, 3H), 3.80 (s, 3H), 4.33 (d, J = 8.5 Hz, 1H), 5.29 (d, J = 8.5 Hz, 1H), 6.07 (d, J = 2.0 Hz, 2H), 6.11 (t, J = 2.0 Hz, 1H), 6.15 (t, J = 2.0 Hz, 1H), 6.38 (d, J = 2.0 Hz, 2H), 6.52 (s, 1H), 6.74 (d, J = 16.5 Hz, 1H), 6.89 (d, J = 2.0 Hz, 1H), 6.99 (d, J = 2.0 Hz, 1H), 7.18 (s, 1H), 7.23 (d, J = 16.5 Hz, 1H), 8.10 (br s, 5H); 13C NMR (125 MHz, d-acetone): δ 56.2, 56.8, 57.5, 93.8, 94.5, 102.5, 102.6, 105.6 (2C), 107.3 (2C), 109.1, 109.7, 120.3, 122.9, 123.1 (2C), 123.8, 127.1, 134.0, 141.3, 145.0, 145.4, 149.1, 159.2, 159.6 (2C), 159.8 (2C), 161.7. HRMS (ESI): m/z [M+H]+ calc for C30H25BrO8Cl: 591.06600; found: 591.06616. HRMS (ESI): m/z [M+Cl]+ calc for C30H23BrO7Cl: 629.04063; found: 629.04201. CCDC 2105346 for compound 12 contains the supplementary data.
crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

5-[[1E]-2-(3,5-dihydroxyphenyl)ethenyl]-2-(3,5-dibromo-4-hydroxyphenyl)-6-methoxy-2,3-dihydrobenzofuranyl]-1,3-benzenedi 

The combined organic layer was washed with brine, dried over anhydrous MgSO4 and evaporated in vacuum. The resulting mixture was extracted with EtOAc (3 × 10 mL). The combined organic layer was washed with brine, dried over anhydrous MgSO4 and evaporated in vacuum. The crude residue was subjected to silica gel column chromatography (CH2Cl2/MeOH = 30:1) to recover stilbene as a pale yellowish oil.

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5-[[1E]-2-(3,5-dihydroxyphenyl)ethenyl]-2-(3-methoxy-4-hydroxyphenyl)-6-methoxy-2,3-dihydrobenzofuranyl]-1,3-benzenedi 

The precursor (d, 1H) = 7.0 Hz, 1H), 6.50 (d, J = 2.5 Hz, 1H), 6.68 (s, 1H), 6.87 (d, J = 16.5 Hz, 1H), 7.32 (s, 1H), 7.36 (d, J = 16.5 Hz, 1H), 7.57 (s, 2H); 13C NMR (125 MHz, d-acetone): δ 56.3, 57.4, 92.4, 94.5, 102.5, 102.6, 105.6 (2C), 107.2 (2C), 111.6, 120.5, 122.6, 123.2, 123.7, 127.2, 130.9 (3C), 136.5, 141.3, 145.4, 151.5, 159.3, 159.5 (2C), 159.8 (2C), 161.4. HRMS (ESI): m/z [M + H]+ cycled for C30H25O8Br2: 513.1569; found: 513.1567.

Declaration of conflicting interests

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