Supporting Information

Enantioselective Synthesis of Dilignol Model Compounds and Their Stereodiscrimination Study with A Dye-Decolorizing Peroxidase

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1. General information

All chemicals were purchased at the highest purity grade. All solvents were anhydrous. All reactions were performed under argon atmosphere unless otherwise specified. Thin layer chromatography (TLC) was performed using 60 mesh silica gel plates and visualization was performed using short wavelength UV light (254 nm) and basic KMnO4 staining. HPLC was performed with a Waters Breeze 2 system consisting of a 1525 pump and a 2998 photodiode array detector. NMR spectra were recorded on a Varian 400 MHz spectrometer. Chemical shifts of proton (1H NMR) and carbon (13C NMR) were reported in ppm relative to the residual solvent peaks. High-resolution mass spectrometry (HRMS) was recorded on a Waters G2-XS QTof mass spectrometer. Surface plasmon resonance (SPR) was performed using a Biacore T200 instrument (GE Healthcare).

2. General procedures for preparing stereospecific dilignols

2-(2-methoxyphenoxy)acetic acid 2.1 To a solution of guaiacol (51.6 g, 1 equiv.) and ethyl bromoacetate (83.5 g, 1.2 equiv.) in acetone (206 mL) in a 500 mL three-neck flask was added K2CO3 (115 g, 2 equiv.) at r.t. The resulting suspension was refluxed overnight and then cooled to r.t. The solid was filtered off and washed with acetone. The solvent in the filtrate was removed under a reduced pressure. The residue was dissolved in MeOH (206 mL) and subjected to hydrolysis using 33 wt% NaOH (150 g). After the hydrolysis was complete as determined by TLC, the MeOH was removed under a reduced pressure. The residue was acidified by adding 6M HCl (aq.) until pH reached 2. The product would precipitate out in few hours to give 2 (62.4 g, 82%) as a white crystal. m.p.: 119−120°C; 1H NMR (400 MHz, CDCl3) δ: 11.35 (br, 1H), 7.03−6.99 (m, 1H), 6.92−6.88 (m, 3H),...
4.67 (s, 2H), 3.86 (s, 3H); 13C NMR (101 MHz, CDCl3) δ: 173.6, 149.5, 147.0, 123.0, 121.1, 115.0, 112.1, 66.8, 55.8.

General Procedures for preparation of compound 4. To a solution of compounds 2 (1.3 equiv.), 3 (1 equiv.), and Et3N (4 equiv.) in toluene was added pivaloyl chloride (1.5 equiv.) at 80 °C. The mixture was refluxed overnight and then quenched with water. The organic layer was separated, washed with water twice, and concentrated to dryness. The residue was purified by crystallization (for 4a, 4b, and 4g) using Et2O/toluene (10/1) or silica gel chromatography (for the rest) eluting with hexane/EtOAc (3/2) to yield 4.

(S)-3-(2-(2-methoxyphenoxy)acetyl)-4-phenyloxazolidin-2-one 4a. Product 4a was obtained as a white crystal (5.40 g, 82%) from compound 3c (3.26 g, 20 mmol). m.p.: 92 –93° C; [α]25D = +101° (c 1.33, CHCl3); 1H NMR (400 MHz, CDCl3) δ: 7.36–7.28 (m, 5H), 6.94–6.74 (m, 4H), 5.41 (dd, J = 8.7, 3.6 Hz, 1H), 5.33 (d, J = 17.8 Hz, 1H), 4.73 (t, J = 8.9 Hz, 1H), 4.33 (dd, J = 9.3, 3.6 Hz, 1H), 3.81 (s, 3H); 13C NMR (101 MHz, CDCl3) δ: 167.8, 153.8, 149.6, 147.2, 138.4, 129.2, 128.9, 126.2, 122.4, 120.6, 114.5, 112.1, 71.2, 68.6, 57.3, 55.9; HRMS: calc. for C18H17O5NNa+: [M+Na]+: 350.0999, found: 350.1010.

(R)-3-(2-(2-methoxyphenoxy)acetyl)-4-phenyloxazolidin-2-one 4b. NMR data are the same as compound 4a.

(S)-4-benzyl-3-(2-(2-methoxyphenoxy)acetyl)oxazolidin-2-one 4c. Product 4c was obtained as a viscous oil (12.72 g, 93%) from compound 3e (7.09 g, 40 mmol). [α]25D= +112 ° (c 3.54, MeOH); 1H NMR (400 MHz, CDCl3) δ: 7.33–7.24 (m, 3H), 7.20–7.17 (m, 2H), 7.00–6.86 (m, 4H), 5.29 (s, 2H), 4.72–4.66 (m, 1H), 4.30–4.21 (m, 2H), 3.88 (s, 3H), 3.31 (dd, J = 13.5, 3.3 Hz, 1H), 2.83 (dd, J = 13.5, 9.3, 1H); 13C NMR (101 MHz, CDCl3) δ: 168.1, 153.3, 149.4, 147.0, 134.7, 129.1, 128.7, 127.1, 122.2, 120.4, 114.4, 112.0, 68.3, 67.2, 55.6, 54.5, 37.2; HRMS: calc. for C19H19O5NNa+: [M+Na]+: 364.1155, found: 364.1179.

(R)-4-benzyl-3-(2-(2-methoxyphenoxy)acetyl)oxazolidin-2-one 4d. NMR data are the same as compound 4c.

(S)-4-isopropyl-3-(2-(2-methoxyphenoxy)acetyl)oxazolidin-2-one 4e. Product 4e was obtained as a viscous oil (2.68 g, 46%) from compound 3a (2.58 g, 20 mmol). [α]25D= +77.3° (c 1.71, CHCl3); 1H NMR (400 MHz, CDCl3) δ: 6.99–6.84 (m, 4H), 5.29 (s, 2H), 4.48–4.44 (m, 1H), 4.37 (t, J = 8.8 Hz, 1H), 4.28 (dd, J = 9.1, 3.2 Hz, 1H), 3.88 (s, 3H), 2.48–2.40 (m, 1H), 0.90 (dd, J = 8.4, 7.0 Hz, 6H); 13C NMR (101 MHz, CDCl3) δ: 167.7, 153.7, 149.1, 146.8, 135.4, 128.3, 128.1, 125.9, 121.8, 120.1, 113.9, 111.7, 67.8, 64.1, 57.6, 55.3, 27.7, 17.1, 14.0.

(S)-3-(2-(2-methoxyphenoxy)acetyl)-5,5-dimethyl-4-phenyloxazolidin-2-one 4f. Product 4f was obtained as a white crystal (0.96 g, 90%) from compound 3b (0.57 g, 3 mmol). m.p.: 94 –95° C; [α]25D= +80.4° (c 1.10, CHCl3); 1H NMR (400 MHz, CDCl3) δ: 7.33–7.24 (m, 3H), 7.20–7.17 (m, 2H), 7.00–6.86 (m, 4H), 5.29 (s, 2H), 4.72–4.66 (m, 1H), 4.30–4.21 (m, 2H), 3.88 (s, 3H), 1.55 (s, 3H), 0.96 (s, 3H); 13C NMR (101 MHz, CDCl3) δ: 167.6, 152.7, 149.1, 146.8, 135.4, 128.3, 128.1, 125.9, 121.8, 120.1, 114.0, 111.7, 83.7, 68.1, 65.9, 55.3, 28.1, 23.1; HRMS: calc. for C20H21O5NNa+: [M+Na]+: 378.1312, found: 378.1321.

(R)-1-(4-benzyl-2-thioxothiazolidin-3-yl)-2-(2-methoxyphenoxy)ethan-1-one 4g. Product 4g was obtained as a yellow crystal (0.67 g, 60%) from compound 3e (0.63 g, 3 mmol). m.p.: 133 –134° C; [α]25D= +138° (c 1.10, CHCl3); 1H NMR (400 MHz, CDCl3) δ: 7.36–7.26 (m, 5H), 6.94–6.74 (m, 4H), 5.30 (q, J = 17.8 Hz, 2H), 5.05 (s, 1H), 3.76 (s, 3H), 1.55 (s, 3H), 0.96 (s, 3H); 13C NMR (101 MHz, CDCl3) δ: 200.9, 169.3, 149.6, 146.8, 135.4, 128.3, 128.1, 125.9, 121.8, 120.1, 114.0, 111.7, 83.7, 68.1, 65.9, 55.3, 28.1, 23.1; HRMS: calc. for C19H19O3S2NNa+: [M+Na]+: 396.0699, found: 396.0706.

General procedures for preparation of 5. To a mixture of compound 4 (1 mmol), vanillin (1.2 equiv.), MgCl2 (0.2 equiv.) and organic base (4 equiv.) in a solvent (5 mL) was added TMSCl (3 equiv.) at 0–
5 °C. The slurry was stirred at 0–5 °C for 24 h and then the water was added. The mixture was stirred for additional 30 min. The organic layer was separated and washed with water 3 times. Fifty µL organic layer was withdrawn and the solvent was evaporated. The residue was dissolved in 200 µL MeOH and analyzed by HPLC for diastereoselectivity. HPLC was performed with an analytical column (Luna C18-2, 5 µm, 4.6 mm × 250 mm) that was eluted at 1.00 mL/min using a linear gradient from 60 to 73% methanol in water over 65 min. A typical HPLC is shown in Figure 2 and results are summarized in Table 1. The remaining organic extractions were combined, dried with anhydrous MgSO₄, and concentrated to dryness. The residue was purified by silica gel chromatography eluting with hexane/EtOAc (2/1) to yield 5.

(S)-3-((2R,3R)-3-(3-methoxy-4-((trimethylsilyl)oxy)phenyl)-2-(2-methoxyphenoxy)-3-((trimethylsilyl)oxy)propanoyl)-4-phenyloxazolidin-2-one (referred as di-TMS-protected aldol product in main text; see its NMR spectra on pages S27-29). ¹H NMR (400 MHz, CDCl₃) δ: 7.43–7.30 (m, 5H), 7.09 (d, J = 2.0 Hz, 1H), 6.99 (dd, J = 8.1, 2.0 Hz, 1H), 6.86–6.76 (m, 2H), 6.69 (dd, J = 8.1, 1.5 Hz, 1H), 6.55 (td, J = 7.7, 1.5 Hz, 1H), 6.33 (dd, J = 8.0, 1.6 Hz, 1H), 6.10 (d, J = 8.5 Hz, 1H), 5.53 (dd, J = 8.4, 2.8 Hz, 1H), 4.89 (d, J = 8.5 Hz, 1H), 4.66 (t, J = 8.7 Hz, 1H), 4.40 (dd, J = 8.9, 2.8 Hz, 1H), 3.80 (s, 3H), 3.38 (s, 3H), 0.22 (s, 9H), -0.02 (s, 9H); ¹³C NMR (101 MHz, CDCl₃) δ: 171.4, 153.7, 150.8, 147.6, 144.5, 139.2, 135.0, 129.2, 128.7, 126.4, 123.4, 120.7, 120.4, 118.9, 112.7, 111.7, 81.3, 76.9, 70.2, 57.6, 55.7, 55.6, 0.4, 0.2; HRMS: calc. for C₃₂H₄₁O₈Si₂NNa⁺ [M+Na]⁺: 646.2263, found: 646.2320.

(R)-3-((2S,3S)-3-hydroxy-3-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propanoyl)-4-phenyloxazolidin-2-one 5a. Product 5a was obtained as a viscous oil (0.38 g, 70%) from compound 4a (0.33 g, 1.00 mmol). ¹H NMR (400 MHz, CDCl₃) δ: 7.35–7.28 (m, 5H), 7.11 (d, J = 1.9 Hz, 1H), 7.04 (dd, J = 8.1, 1.9 Hz, 1H), 6.84 (dd, J = 17.7, 7.9 Hz, 2H), 6.70 (dd, J = 8.3, 1.5 Hz, 1H), 6.60 (dd, J = 7.9, 1.5 Hz, 1H), 6.41 (dd, J = 7.9, 1.5 Hz, 1H), 6.11 (d, J = 8.4 Hz, 1H), 5.59 (s, 1H), 5.52 (dd, J = 8.4, 2.8 Hz, 1H), 4.91 (d, J = 8.3 Hz, 1H), 4.67 (t, J = 8.7 Hz, 1H), 4.39 (dd, J = 8.9, 2.9 Hz, 1H), 3.87 (s, 3H), 3.43 (s, 3H), 0.00 (s, 9H); ¹³C NMR (101 MHz, CDCl₃) δ: 171.3, 153.7, 150.5, 147.3, 146.4, 145.6, 139.1, 133.3, 129.2, 128.7, 126.2, 123.1, 121.2, 120.7, 118.1, 113.8, 112.7, 110.3, 80.9, 77.5, 77.1, 76.8, 76.8, 70.2, 57.6, 56.0, 55.7, 0.2; HRMS: calc. for C₂₉H₃₃O₈SiNNa⁺ [M+Na]⁺: 574.1868, found: 574.1882.

General procedures for preparation of 6. The organic layer containing 5 was added 2 drops of TFA and stirred for 30 min. The resulting mixture was quenched with saturated NaHCO₃ (aq.) and extracted with CH₂Cl₂. The organic extractions were combined and concentrated to dryness. The residue was purified by silica gel chromatography eluting with hexane/EtOAc (2/1) to yield 6.

(R)-3-((2S,3S)-3-(4-hydroxy-3-methoxyphenoxy)-2-(2-methoxyphenoxy)propanoyl)-4-phenyloxazolidin-2-one 5b. NMR data are the same as compound 5a.

General procedures for preparation of compounds 1a and 1b. To a solution of compound 6 in THF was added 25 wt% aqueous solution of NaBH₄ (4 equiv.) at 0–5 °C. The mixture was stirred at 0–5 °C for 1 h and then quenched with 1M HCl (aq). Solvents were removed under a reduced pressure. The crude product was extracted with CH₂Cl₂ (50 mL×2). The organic layers were combined and concentrated to dryness. The residue was purified by silica gel chromatography eluting with.
hexane/EtOAc (1/1) to yield 1. Enantiomeric excess (ee) was determined by HPLC using a Chiralpak® AD(-H) column (4.6 mm×250 mm, Daicel Chemical Industries, Exton, PA) eluted at 0.5 mL/min with 15% isopropanol in hexane. HPLC profiles are shown in Figure S1. Optical rotations are summarized in Table S1.

(1R, 2S)-1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propane-1,3-diol 1a. Product 1a was obtained as a viscous oil (0.95 g, 83%) from compound 6a (1.71 g, 3.54 mmol). ee>99%; 1H NMR (400 MHz, CDCl$_3$) δ: 7.08–7.04 (m, 1H), 6.98–6.81 (m, 6H), 5.64 (s, 1H), 4.97 (t, $J = 3.7$ Hz, 1H), 4.16 (td, $J = 5.5, 3.7$ Hz, 1H), 3.93–3.87 (m, 7H), 3.69–3.65 (m, 1H), 3.51 (d, $J = 3.3$ Hz, 1H), 2.78 (t, $J = 6.6$ Hz, 1H); 13C NMR (101 MHz, CDCl$_3$) δ: 150.7, 146.8, 146.6, 144.9, 132.0, 123.0, 123.3, 121.2, 119.5, 119.0, 114.3, 112.0, 109.1, 86.0, 72.6, 60.6, 55.5 (2); HRMS: calc. for C$_{17}$H$_{20}$O$_6$Na$^+$ [M+Na]$^+$: 343.1152, found: 343.1143.

(1S, 2R)-1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propane-1,3-diol 1b. NMR and HRMS data are the same as compound 1a.

Table S1. Optical rotations of stereoisomers 1a-1d in MeOH

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<th>compound</th>
<th>configuration</th>
<th>concentration (g/100 mL)</th>
<th>our study</th>
<th>$[\alpha]_D^{25}$ reported$^a$</th>
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<tr>
<td>1a</td>
<td>1R, 2S</td>
<td>1.76</td>
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<td>-39.2°</td>
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<td>1b</td>
<td>1S, 2R</td>
<td>1.66</td>
<td>7.3°</td>
<td>8.7°</td>
</tr>
<tr>
<td>1c</td>
<td>1R, 2R</td>
<td>1.56</td>
<td>-9.8°</td>
<td>-8.5°</td>
</tr>
<tr>
<td>1d</td>
<td>1S, 2S</td>
<td>1.44</td>
<td>40.4°</td>
<td>43.8°</td>
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</table>

$^a$See reference 3.

General procedures for preparation of compound 7. To a solution of compound 4 (1 mmol) in CH$_2$Cl$_2$ (3 mL) was added a solution of nBu$_2$BOTf (1.2 equiv.) in CH$_2$Cl$_2$ (1.0 M) at 0–5 °C. After 15 min, DPEA (1.5 equiv.) was added to the red solution and the red color faded immediately. After stirring at 0–5 °C for additional 15 min, the reaction mixture was cooled to −78 °C and a solution of protected vanillin (1.2 equiv.) in CH$_2$Cl$_2$ (2 mL) was added slowly. After stirring for 1 h, the mixture was warmed to r.t. and stirred for additional 3 h. The reaction was quenched with water. The organic layer was separated and 50 µL was withdrawn. After the solvent was evaporated, the residue was dissolved in 200 µL MeOH and analyzed by HPLC for diastereoselectivity as described above. A typical HPLC is shown in Figure S2 and the results are summarized in Table S2. The remaining organic layer was concentrated to dryness. The residue was purified by silica gel chromatography eluting with hexane/EtOAc (2/1) to yield 7.
(S)-4-benzyl-3-((2S,3R)-3-(4-(benzyloxy)-3-methoxyphenyl)-3-hydroxy-2-(2-methoxyphenoxy)propanoyl)oxazolidin-2-one 7c. Product 7c was obtained as a viscous oil (3.85 g, 56%) from compound 4c (3.60 g, 11.46 mmol). [α]D = +128° (c 0.88, MeOH); 1H NMR (400 MHz, CDCl3) δ: 7.43–7.34 (m, 3H), 7.28–7.22 (m, 4H), 7.09–6.81 (m, 10H), 6.15 (d, J = 6.5 Hz, 1H), 5.13–5.06 (m, 4H), 4.13–4.09 (m, 1H), 3.88 (s, 3H), 3.86 (s, 3H), 3.83 (d, J = 1.8 Hz, 1H), 3.33 (t, J = 8.3 Hz, 1H), 3.11 (dd, J = 13.5, 3.3 Hz, 1H), 2.70 (dd, J = 13.5, 9.3 Hz, 1H); 13C NMR (101 MHz, CDCl3) δ: 169.8, 152.8, 150.0, 149.5, 147.9, 146.9, 136.9, 134.7, 130.9, 129.4, 128.8, 128.5, 127.8, 127.3, 127.2, 123.4, 121.0, 119.2, 117.2, 113.2, 112.5, 110.6, 81.6, 75.9, 70.7, 66.4, 55.9, 55.8, 55.7, 37.6; HRMS: calc. for C34H33O8NNa+ [M+Na]+: 606.2098, found: 606.2144.

(R)-4-benzyl-3-((2R,3S)-3-(4-(benzyloxy)-3-methoxyphenyl)-3-hydroxy-2-(2-methoxyphenoxy)propanoyl)oxazolidin-2-one 7d. NMR data are the same as compound 7c.

Figure S2. HPLC profile of diastereoselectivity using methods B. The straight line indicates MeOH gradient. The peak at 10.8 min represents unreacted aldehyde.

Table S2. Optimization of aldol reactions using method B

<table>
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<td>4a</td>
<td>Bn</td>
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<td>Bn</td>
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<tr>
<td>7</td>
<td>4c</td>
<td>TBDMS</td>
<td>trace product</td>
</tr>
</tbody>
</table>

aProtecting group; bDiastereoselectivity was determined by HPLC. See Experimental for details.
General procedures for preparation of compound 8. The synthetic procedures were the same as the one for preparing compounds 1a and 1b described above, using compound 7 as the starting material. The residue from organic extractions was used directly for next step without purification.

(1R,2R)-1-(4-(benzyloxy)-3-methoxyphenyl)-2-(2-methoxyphenoxy)propane-1,3-diol 8c. Product 8c was obtained as a viscous oil (1.50 g, 83%) from compound 7c (2.58 g, 4.42 mmol). 1H NMR (400 MHz, CDCl3) δ: 7.44–7.26 (m, 5H), 7.13–7.01 (m, 3H), 6.94–6.84 (m, 4H), 5.14 (s, 2H), 4.96 (d, J = 7.8 Hz, 1H), 4.05–4.02 (m, 1H), 3.88 (d, 6H), 3.76 (s, 1H), 3.63 (d, J = 12.6 Hz, 1H), 3.49–3.46 (m, 1H), 2.85–2.80 (m, 1H); 13C NMR (101 MHz, CDCl3) δ: 150.8, 149.5, 147.7, 147.5, 147.0, 128.3, 127.7, 127.2, 123.6, 121.5, 121.4, 120.1, 119.3, 113.7, 112.0, 110.5, 88.3, 73.5, 70.8, 60.8, 55.8, 55.6; HRMS: calc. for C24H26O6Na⁺ [M+Na]⁺: 433.1622, found: 433.1624.

(1S,2S)-1-(4-(benzyloxy)-3-methoxyphenyl)-2-(2-methoxyphenoxy)propane-1,3-diol 8d. NMR data are the same as compound 8c.

General procedures for preparation of compounds 1c and 1d. To a solution of crude 8 in MeOH was added 10% Pd/C powder. The reaction was hydrogenated at atmospheric pressure and vigorously stirred for 12 hours at r.t. The powder was removed by Celite filtration. After the solvent was evaporated, the residue was purified by silica gel chromatography eluting with hexane/EtOAc (1/1) to yield 1. Chiral HPLC was performed as described for 1a/1b to determine ee and is shown in Figure S1. Their optical rotations are summarized in Table S1.

(1R, 2R)-1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propane-1,3-diol 1c. Product 1c was obtained as a viscous oil (0.65 g, 93%) from compound 8c (0.90 g, 2.18 mmol). ee>99%; 1H NMR (400 MHz, CDCl3) δ: 7.14–7.04 (m, 2H), 6.97–6.87 (m, 5H), 5.74 (s, 1H), 4.90 (d, J = 8.0 Hz, 1H), 4.04–4.00 (m, 1H), 3.90 (s, 3H), 3.87 (s, 3H), 3.71 (br, 1H), 3.63 (d, J = 12.5, 12.3 Hz, 1H), 3.48 (dd, J = 12.4, 4.0 Hz, 1H), 2.79 (br, 1H); 13C NMR (101 MHz, CDCl3) δ: 151.2, 147.7, 146.8, 145.6, 131.6, 124.1, 121.7, 112.8, 120.2, 114.5, 112.2, 109.6, 89.2, 74.0, 61.1, 56.0, 55.9; HRMS: calc. for C17H20O6Na⁺ [M+Na]⁺: 343.1152, found: 343.1143.

(1S, 2S)-1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propane-1,3-diol 1d. NMR and HRMS data are the same as compound 1c.

3. Enzyme purification

His-tagged TcDyP was purified by following the published protocol.4 Its SDS-PAGE after Ni-NTA purification is shown in Figure S3. The molecular weight of TcDyP was calculated at 43.6 KDa. Its specific activity (SA) with Reactive Blue 19 at r.t. was determined to be 122 µmol min⁻¹mg⁻¹.

Figure S3. SDS-PAGE of purified TcDyP.
4. Surface plasmon resonance (SPR) experiments

Direct binding of stereospecific model lignin compounds with TcDyP was measured by SPR using a Biacore T200 instrument (GE Healthcare) at 25°C. To begin, a CMD-500M sensorchip (XanTec Bioanalytics GmbH) was used to create TcDyP biosensor by conventional, random amine coupling chemistry. Briefly, an equal volume mixture of 0.1 M N-hydroxysuccinimide (NHS) and 0.4 M ethyl(dimethylaminopropyl) carbodiimide (EDC) was injected for 7 min at 5 µl•min\(^{-1}\) over a single flow cell. Then, 50 µg•ml\(^{-1}\) DyP protein diluted in 10 mM sodium acetate (pH 6.0) was injected for 15 min and immediately followed by a 7-min injection of 1M ethanolamine (pH 9.0) to quench remaining reactive groups. A final immobilization level of 13,500 response units (RU) was achieved. A reference surface was created by EDC/NHS activation followed by immediate quenching using ethanolamine.

All binding experiments were performed in a running buffer consisting of 20 mM HEPES (pH 7.4), 140 mM NaCl, 0.005% (v/v) Tween 20 using a flow rate of 30 µl•min\(^{-1}\). To evaluate lignin model compounds as prospective ligands for TcDyP, a working stock of each compound was diluted with running buffer to prepare solutions at 500, 250, 125, 62.5, 31.25, and 7.8 µM. Running buffer alone was used as a blank injection. Samples were injected over the biosensor for 30 s, followed by 30 s of dissociation phase. A wash step consisting of 2 M NaCl in ddH\(_2\)O (30 s contact time) was used to regenerate the surface. Quadruplicate injections were performed for all samples, including the buffer controls.

The sensorgrams obtained from each experimental series were analyzed using Biacore T200 Evaluation software v3.0. Reference corrected curves were generated by subtracting the signal from the reference surface from each experimental surface. The signal just prior to injection stop of these subtracted sensorgrams was treated as the DyP-binding response. Because the on-rates of analytes (dilignols) were fast and the surface density was high, kinetic fitting was not performed. Instead, steady-state affinity analysis was used to characterize the apparent binding affinity (\(K_D\)). Thus, the five seconds of each curve immediately prior to injection stop were averaged and plotted against analyte concentrations. Representative sensorgrams and global fittings based on a 1:1 binding model are shown in Figures S4 and S5, respectively. They were used to determine the parameters reported in Table 2. The \(K_D\) value is defined as the concentration of ligand, at which a half-maximal \(RU_{\text{max}}\) is achieved.
Figure S4. Representative sensorgrams of TcDyP with stereoisomers 1a (A), 1b (B), 1c (C), and 1d (D) at a series of concentrations.

Figure S5. Steady-state affinity analysis of stereoisomers 1a (A), 1b (B), 1c (C), and 1d (D). The solid lines represent global fitting of the four trials based on a 1:1 binding model.
5. Enzyme assays

All enzymatic reactions were carried out at r.t. In a final volume of 1000 µL consisting of 50 mM sodium citrate (pH 3.0), 1.4 mM H₂O₂, 1 mg/mL BSA and 0.7mM model compound 1, enzyme was added to a final concentration of 0.5 µM to initiate the degradation. At defined time points, 90 µL aliquots were removed from the mixture and the reaction was quenched by heating the sample at 95 °C for 5 min. Each sample was added an internal standard, phenol, to a final concentration of 1.5 mM and then centrifuged to remove protein precipitates. The supernatant was analyzed by HPLC using a column (Luna C18–2, 5 µm, 4.6 mm x 250 mm) eluted at 1.00 ml/min with a linear gradient from 30 to 90% methanol in ddH₂O over 30 min. Degradation of the model compounds were monitored and reaction rates were determined by the slope of the initial phase. Enzyme specific activities (SAs) were calculated based on the reaction rate and protein concentration, which are summarized in Table 2.

6. References

$^1$H NMR (400 MHz, CDCl$_3$)
$^{13}$C NMR (100.6 MHz, CDCl$_3$)

![Chemical Structure](image)

2
$^1$H NMR (400 MHz, CDCl$_3$)

![NMR Spectra Image]

4a
gCOSY $^1$H NMR (400 MHz, CDCl$_3$)
$^{13}$C NMR (100.6 MHz, CDCl$_3$)

![Chemical Structure](image)

4a
gCOSY $^1$H NMR (400 MHz, CDCl$_3$)
$^{13}$C NMR (100.6 MHz, CDCl$_3$)

![Chemical Structure](image)

**4c**

**NMR Spectra**

- Chemical shifts in ppm:
  - 168.102
  - 153.273
  - 149.427
  - 146.999
  - 134.708
  - 129.148
  - 127.081
  - 123.227
  - 120.412
  - 114.388
  - 111.967
  - 68.73
  - 67.80
  - 54.457
  - 55.593
  - 37.237

**f1 (ppm)**

- Ranges: 0 to 180
gCOSY $^1$H NMR (400 MHz, CDCl$_3$)
$^{13}$C NMR (100.6 MHz, CDCl$_3$)

![Chemical structure](image)

4e
gCOSY $^1$H NMR (400 MHz, CDCl$_3$)
$^{13}$C NMR (100.6 MHz, CDCl$_3$)

![Chemical Structure](image)

4f
$^1$H NMR (400 MHz, CDCl$_3$)

![Chemical Structure](4g)

f1 (ppm)
$^{13}$C NMR (100.6 MHz, CDCl$_3$)
$^1\text{H NMR (400 MHz, CDCl}_3$)
$^{13}$C NMR (100.6 MHz, CDCl$_3$)
$^1$H NMR (400 MHz, CDCl$_3$)

5a
gCOSY $^1$H NMR (400 MHz, CDCl$_3$)
$^{13}$C NMR (100.6 MHz, CDCl$_3$)
$^1$H NMR (400 MHz, CDCl₃)
gCOSY $^1$H NMR (400 MHz, CDCl$_3$)
$^{13}$C NMR (100.6 MHz, CDCl$_3$)
$^1$H NMR (400 MHz, CDCl$_3$)

1a

H$_2$O
$^{1}H$ NMR (400 MHz, CDCl$_3$)
$^{13}$C NMR (100.6 MHz, CDCl$_3$)

1a
$^1$H NMR (400 MHz, CDCl$_3$)

![Chemical Structure](image)

**7c**
gCOSY $^1$H NMR (400 MHz, CDCl$_3$)
$^1$H NMR (400 MHz, CDCl$_3$)
$^{13}$C NMR (100.6 MHz, CDCl$_3$)

8c
$^1$H NMR (400 MHz, CDCl$_3$)

1c
gCOSY $^1$H NMR (400 MHz, CDCl$_3$)
$^{13}$C NMR (100.6 MHz, CDCl$_3$)

1c