Supporting Information

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SI Text

Synthesis of Tyrosinol-A488 (1, 2). The mixture of **1** and **2** for each enantiomer was synthesized as previously described (1). The **1** and **2** were separated by reverse-phase HPLC using a 9.4 × 250-mm 5 μM Zorbax Rx-C8 column (Agilent Technologies) and 100 mM triethylammonium acetate buffer (pH 7.0) (Calbiochem) as a loading buffer and acetonitrile as a mobile phase. The products were eluted with a 30-min, 4 ml/min gradient of 10–20% (vol/vol) acetonitrile with retention times of 9 and 13 min for **1** and **2,** respectively. Each product then underwent a second purification using the same conditions. The identity **1** and **2** was confirmed by electrospray ionization mass-spectrometry (ESI-MS). All chemicals from here onward were from Sigma–Aldrich, unless stated otherwise, and were of the highest purity available from that vendor.

Synthesis of Tyrosinol-A647. Each enantiomer of tyrosinol dissolved in 50 mM Na borate buffer (pH 8.6) was added in 10-fold molar excess to Alexa Fluor 647 carboxylic acid succinimidyl ester (Invitrogen). The mixture was stirred overnight at room temperature, and the product was purified as described in the preceding paragraph, except for elution with a 40-min, 4 ml/min gradient of 15–19% (vol/vol) acetonitrile.

The **3** and **4** were prepared by reacting L- or D-tyrosinol with *mono*-methyl *iso*phthalate or *tere*phthalate, followed by ester hydrolysis. In all cases, a solution of *mono*-methyl phthalate (90 mg, 0.5 mmol), 4-DMAP (43 mg, 0.35 mmol), and EDC HCl (115 mg, 0.6 mmol) in DMF (20 ml) was incubated at room temperature for 30 min and then added dropwise to a solution of L- or D-tyrosinol HCl (204 mg, 1.0 mmol) and triethylamine (2.5 mmol) in 10 ml of DMF. The resulting reaction mixture was stirred overnight at room temperature, evaporated, and redissolved in 20 ml of 0.1 M HCl. The mixture was extracted with ethyl acetate (3 × 60 ml), washed with saturated aqueous $NaHCO_3$ (3 × 60 ml) and with brine (60 ml), dried over anhydrous Na_2SO_4, and evaporated. The resulting residue was treated with 5 ml of 0.4 M NaOH in tetrahydrofuran/water (3:1, vol/vol) for 2 h. After the removal of tetrahydrofuran by evaporation, the solution was acidified to pH ≈2.0 and extracted with ethyl acetate (3 × 5 ml). The combined organic fractions were dried over anhydrous Na_2SO_4 and evaporated to give a crude product that was then purified by recrystallization from water. For **3**: ^{1}H NMR (300 MHz, CD_3OD) δ 2.72 (dd, J = 7.6, 13.6, 1H), 2.92 (dd, J = 6.5, 13.6, 1H), 3.62 (m, 2H), 4.24 (m, 1H), 6.70 (d, J = 8.4, 2H), 7.10 (d, J = 8.4, 2H), 7.53 (dd, J = 7.8, 7.8, 1H), 7.96 (ddd, J = 7.8, 1.8, 1.2, 1H), 8.14 (ddd, J = 7.8, 1.5, 1.4, 1H), 8.43 (dd, J = 1.7, 1.7, 1H). For **4**: ^{1}H NMR (300 MHz, CD_3OD) δ 2.77 (dd, J = 8.1, 13.8, 1H), 2.92 (dd, J = 6.3, 13.8, 1H), 3.64 (m, 2H), 4.29 (m, 1H), 6.70 (d, J = 8.0, 2H), 7.10 (d, J = 8.7, 2H), 7.81 (d, J = 8,7, 2H), 8.08 (d, J = 8.7, 2H).

Synthesis of *N*-Acetyl-Tyrosinol. *N*-acetyl-tyrosinol was synthesized as described in the literature (2). To a mixture of tyrosinol HCl (250 mg, 1.23 mmol) and triethylamine (3.0 mmol) in 10 ml of dry ethyl acetate on ice, acetyl chloride (1.5 mmol) in 10 ml of dry ethyl acetate was added dropwise with stirring. After the addition was completed (≈30 min), the reaction mixture was incubated for 2 h on ice. The white precipitate of triethylamine HCl was filtered and washed with ethyl acetate (2 × 20 ml). To remove unreacted tyrosinol, the combined filtrates were then washed with 0.1 M HCl (3 × 60 ml) and with brine (60 ml), dried over anhydrous Na_2SO_4, and evaporated. The purity of the product was confirmed by reverse-phase HPLC and determined to be >95%.

1. Lipovšek D, *et al.* (2007) Selection of horseradish peroxidase variants with enhanced enantioselectivity by yeast surface display. *Chem Biol* 14:1176–1185.

2. Dymicky M (1976) *N*-Acetyl-l-tyrosine ethyl ester. *Org Prep Proced Int* 8:219–222.