Supplementary Information

Suzuki-Miyaura cross-coupling catalyzed by protein-stabilized palladium nanoparticles under aerobic conditions in water: application to a one-pot chemoenzymatic enantioselective synthesis of chiral biaryl alcohols

Alessandro Prastaro,*a Pierpaolo Ceci,b,c Emilia Chiancone,b,c Alberto Boffi,b,c Roberto Cirilli,d Marisa Colone,e Giancarlo Fabrizi,a Annarita Stringaro,e and Sandro Cacchi*a

a Dipartimento di Chimica e Tecnologie del Farmaco, Sapienza, Università di Roma, P.le A. Moro 5, 00185 Rome, Italy. Fax: +39 (06) 4991-2780; Tel: +39 (06) 4991-2795; E-mail: sandro.cacchi@uniroma1.it
b Dipartimento di Scienze Biochimiche, Sapienza, Università di Roma, P.le A. Moro 5, 00185 Rome, Italy. Fax: +39 (06) 4440062; Tel: +39 (06) 49910990
c Istituto di Biologia e Patologia Molecolari CNR, P.le A. Moro 5, 00185 Rome, Italy. Fax: +39 (06) 4440062; Tel: +39 (06) 49910761; E-mail: emilia.chiancone@uniroma1.it
d Dipartimento del Farmaco, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy.
e Dipartimento di Tecnologia e Salute, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy

Experimental Section

General information

Melting points were determined with a Büchi B-545 apparatus and are uncorrected. All of the reagents and solvents are commercially available and were used as purchased, without further purification. Compounds were purified on axially compressed columns, packed with SiO2 25-40 µm (Macherey Nagel), connected to a Gilson solvent delivery system and to a Gilson refractive index detector, and eluting with n-hexane/AcOEt mixtures. 1H NMR (400.13 MHz) and 13C NMR (100.6 MHz) spectra were recorded with a Bruker Avance 400 spectrometer. Infrared (IR) spectra were recorded on a JASCO FT/IR-430 spectrophotometer. Mass spectra were determined with a QP2010 Gas Chromatograph Mass spectrometer (EI ion source). UV-Vis absorption spectra were recorded between 220-700 nm at rt with a spectrophotometer Varian Cary 50 and using a quartz cuvette with 0.1 cm path length. Transmission electron microscopy (TEM) analyses were made by Philips 208 transmission electron microscope (FEI Company) at 70 kV. The samples (10 µl) were applied to carbon coated copper grids (200-mesh) and after 30 s on the grid the samples were dried and visualized. In addition, some samples were negatively stained with (10 µl) phosphotungstic acid (PTA) 2% w/v solution buffered at pH = 7.3 to visualize the protein shell (Figure S1). The enantiomeric excess was determined by enantioselective HPLC on the Chiralpak AS-H chiral stationary phase using the mixture n-hexane-2-propanol 95/5 (v/v) as a mobile phase.
Cloning, protein expression and purification

Recombinant *Thermosynechococcus elongatus* Dps (TeDps) protein was obtained and purified as described by Franceschini et al. (2006), removing the two ammonium sulfate cuts. Typically yield was 200 mg of Dps protein per 1 L batch. Protein concentration were determined spectrophotometrically on the basis of an ε molar of 19940 M⁻¹ cm⁻¹ (Mw 214 kDa) at 280 nm.

Preparation of palladium nanoparticles stabilized by a thermophilic DNA binding protein from starved cells (Pd_{np}/Te-Dps)

A Dps solution (0.23 µmol, 0.15 M in NaCl) was brought to pH 8.5 using 30 mM NaOH (TITRINO, Metrohm AG). K₂PdCl₄ (969 µg, 29.7 µmol) was added to the protein solution under stirring at room temperature for 30 min and then NaBH₄ (156 µg, 41.4 µmol) for over 15 min. Any possible aggregates of Dps and/or palladium particles produced during nanoparticles formation were removed by filtration through 0.22 µm syringe filters. The solution was dialyzed against 0.15 M NaCl and concentrated for the following analyses. The samples containing Pd_{np}/Te-Dps was checked using Supherose 6 gel-filtration column (GE Healthcare), equilibrated with 0.15 M NaCl.

Typical procedure for the Suzuki-Miyaura cross-coupling reaction using 0.05 mol% of Pd_{np}/Te-Dps

4-Iodobenzoic acid (62 mg, 0.25 mmol), 0.05 mol% of Pd_{np}/Te-Dps, o-tolylboronic acid (34 mg, 0.25 mmol) in Tris (2 mL, pH = 8.9, 100 mM) were orbitally stirred for 24 h at 100 °C with a Heidolph Synthesis System. Then, after cooling, the liquid phase was extracted with CH₂Cl₂, the organic layer was washed with deionized water and dried over Na₂SO₄. The solvent was removed under vacuum and the residue was purified by chromatography (SiO₂, 35 g, n-Hexane/AcOEt/AcOH 96/3/1 v/v) to give 47.7 mg (90% yield) of 3a m.p: 187.5-188.8 °C; IR (KBr) 2949, 2905, 1673, 1608, 1427, 1321, 1291 cm⁻¹; ¹H NMR (DMSO-d₆) δ 8.02−7.99 (d, J = 8.6 Hz, 2H), 7.44-7.43(d, J = 8.0 Hz, 2H), 7.33-7.21 (m, 4H), 2.24 (s, 3H); ¹³CNMR (DMSO-d₆) δ 167.7, 146.24, 140.84, 135.17, 130.99, 129.86, 129.75, 129.73, 128.35, 126.56, 20.58. Anal Calcd for C₁₄H₁₂O₂₂ C, 79.22; H, 5.70; found C, 79.20; H, 5.68.

3b: m.p: 73.5-73.6 °C; IR (KBr) 2923, 2853, 1607, 1586, 1500, 1287, 1245, 806 cm⁻¹; ¹H NMR (CDCl₃) δ 7.68 (s, 4H), 7.58-7.56 (d, J = 8.0 Hz, 2H), 7.04-7.01 (d, J = 8.0 Hz, 2H); ¹³C NMR
(CDCl₃) δ 159.9, 144.35, 132.2, 128.4, 126.9, 125.77, 125.73, 125.7, 125.6, 123.1, 114.5, 55.4. Anal Calcd for C₁₄H₁₁F₃O C, 66.6; H, 4.4; found C, 66.7; H, 4.5.

3c: Oil; IR (neat) 2923, 2854, 2227, 1457 cm⁻¹; ¹H NMR (CDCl₃) δ 7.74−7.72 (d, J = 8.0 Hz, 2H), 7.47-7.45 (d, J = 8.0 Hz, 2H), 7.34-7.28 (m, 3H), 2.28 (s, 3H); ¹³C NMR (CDCl₃) δ 140.7, 137.2, 133.6, 132.7, 129.5, 128.6, 127.8, 127.6, 126.3, 115.7, 111.5, 21.7. Anal Calcd for C₁₄H₁₁N C, 87.01; H, 5.74; found C, 86.96; H, 5.01.

3d: Oil; IR (neat) 3030, 2925, 1901, 1611, 1484, 1438, 1330, 1258 cm⁻¹; ¹H NMR (CDCl₃) δ 7.83 (s, 1H), 7.79-7.74 (m, 1H), 7.62-7.48 (m, 4H), 7.29 (d, J = 7.9 Hz, 2H), 2.42 (s, 3H); ¹³C NMR (CDCl₃) δ 141.9, 137.9, 131.1 (d, J = 32.1 Hz), 130.2, 129.7, 129.2, 127.0, 124.2 (d, J = 272.0 Hz), 123.6 (q, J = 4.1 Hz), 21.1. Anal Calcd for C₁₄H₁₁F₃ C, 71.18; H, 4.69; found C, 71.12; H, 4.63.

3e: m.p.: 104-105 °C; IR (neat) 3060, 2355, 1677, 1477, 748 cm⁻¹; ¹H NMR (CDCl₃) δ 8.29 (d, J = 8.0 Hz, 2H), 7.50 (d, J = 8.0 Hz, 2H), 7.35 (m, 3H), 7.22 (d, J = 7.2 Hz, 1H), 2.27 (s, 3H); ¹³C NMR (CDCl₃) δ 148.3, 143.7, 140.2, 135.6, 141.8, 138.8, 136.5, 134.3, 133.5, 133.4, 133.2, 133.1, 131.4, 129.2, 129.0, 127.4, 127.0, 123.1, 26.7, 20.2. Anal Calcd for C₁₃H₁₁NO₂ C, 73.23; H, 5.20; found C, 73.25; H, 5.25 (Known compound, see: W. Han, C. Liu, and Z. Jina, Adv. Synth. Catal. 2008, 350, 501).

3f: m.p.: 110.6-111.3 °C; IR (KBr) 1687, 1348, 821 cm⁻¹; ¹H NMR (CDCl₃) δ 8.22–8.21 (d, J = 4.0 Hz, 2H), 8.06-8.04 (d, J = 8.0 Hz, 2H), 7.77-7.67 (m, 3H), 7.45-7.43 (d, J = 8.0 Hz, 1H), 2.65 (s, 6H); ¹³C NMR (CDCl₃) δ 197.7, 149.6, 142.8, 135.6, 141.8, 138.8, 136.5, 134.3, 133.5, 133.4, 133.2, 133.1, 131.4, 129.2, 129.0, 127.4, 127.0, 123.1, 26.7, 20.2. Anal Calcd for C₁₅H₁₃NO₃ C, 70.58; H, 5.13; found C, 70.60; H, 5.17.

3g: m.p: 250.0-251.7 °C; IR (KBr) 2953, 2834, 2547, 1678, 1601, 1430, 1313, 1289 cm⁻¹; ¹H NMR (DMSO-d₆) δ 8.00–7.98 (d, J = 7.7 Hz, 2H), 7.76-7.68 (m, 4H), 7.07-7.05 (d, J = 6.4 Hz, 2H), 3.81 (s, 3H); ¹³C NMR (DMSO-d₆) δ 167.7, 160.07, 144.46, 131.76, 130.46, 129.37, 128.64, 126.62, 115.02, 55.73. Anal Calcd for C₁₄H₁₂O₃ C, 73.67; H, 5.30; found C, 73.65; H, 5.28

3h: m.p: 250.6 °C; IR (KBr) 2949, 2668, 2548, 1680 cm⁻¹; ¹H NMR (DMSO-d₆) δ 8.03–8.01 (d, J = 8.0 Hz, 2H), 7.81-7.75 (m, 4 H), 7.56-7.54 (d, J = 8.0 Hz, 2H); ¹³C NMR (DMSO-d₆) δ 169.3,
141.6, 133.7, 132.8, 130.8, 129.4, 128.8, 127.2. Anal. Calcd. for C\textsubscript{13}H\textsubscript{9}ClO\textsubscript{2} C, 67.11; H, 3.90; found C, 67.06; H, 3.84.

3i: mp: 218.0-219.4 °C; IR (KBr) 2949, 2668, 2548, 1680, 1607, 1422, 1318, 1288 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (DMSO-\textit{d}_6) \delta 12.9 (s, 1H), 8.04-8.02 (d, \textit{J} = 8.0 Hz, 2H), 7.81-7.79 (d, \textit{J} = 8.0 Hz, 2H), 7.74-7.72 (d, \textit{J} = 8.0 Hz, 2H), 7.75-7.48 (t, 2 H), 7.44-7.42 (d, \textit{J} = 8.0 Hz, 2H); \textsuperscript{13}C NMR (DMSO-\textit{d}_6) \delta 167.5, 144.7, 139.5, 130.4, 129.0, 128.7, 127.4, 127.2. Anal Calcd for C\textsubscript{13}H\textsubscript{10}O\textsubscript{2} C, 78.77; H, 5.09 found C, 78.78; H, 5.10. (Known compound, see: K. M. Dawood, A. Kirschning Tetrahedron 2005, \textbf{61}, 12121).

3i: mp: 97.9-98.4 °C; IR (KBr) 2923, 2360, 2341, 1673, 815 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (CDCl\textsubscript{3}) \delta 8.06−8.0 (d, \textit{J} = 8.0 Hz, 2H), 7.68-7.66 (d, \textit{J} = 8 Hz, 2H), 7.59-7.57 (d, \textit{J} = 8.0 Hz, 2H), 7.47-7.45 (d, \textit{J} = 8.0 Hz, 2H) 2.66 (s, 3H); \textsuperscript{13}C NMR (CDCl\textsubscript{3}) \delta 197.8, 141.2, 136.6, 135.1, 134.2, 129.4, 127.6, 29.3. Anal Calcd for C\textsubscript{14}H\textsubscript{11}ClO C, 72.89; H, 4.81; found C, 72.85; H, 4.78

3m: mp: 120.6-121.4 °C; IR (KBr) 1685, 1605, 1396, 1356, 1322, 823 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (CDCl\textsubscript{3}) 8.09-8.07 (d, \textit{J} = 8.0 Hz 2H), 7.75-7.70 (m, 6H), 2.67 (s, 3H); \textsuperscript{13}C NMR (CDCl\textsubscript{3}) \delta 197.6, 144.2, 143.4, 136.6, 129.1, 127.6, 127.5, 125.9, 26.73. Anal Calcd for C\textsubscript{15}H\textsubscript{11}F\textsubscript{3}O C, 68.19; H, 4.20 found. C, 68.20; H, 4.21. (Known compound, see: B. H. Lipshultz et al. \textit{Org. Lett.}, 2008, \textbf{10}, 4279).

3n: mp: 153.3-154.5 °C; IR (KBr) 1673, 1600, 1294, 1818 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (CDCl\textsubscript{3}) \delta 8.03−8.01 (d, \textit{J} = 8.0 Hz, 2 H), 7.67-7.65 (d, \textit{J} = 8.0 Hz, 2 H), 7.61-7.58 (d, \textit{J} = 8.0 Hz, 2 H), 7.03-7.01 (d, \textit{J} = 8.0 Hz, 2 H), 3.88 (s, 3H), 2.64 (s, 3H); \textsuperscript{13}C NMR (CDCl\textsubscript{3}) \delta 197.7, 159.9, 145.4, 135.4, 132.3, 129.0, 128.4, 126.6, 114.4, 55.4, 26.6; Anal Calcd for C\textsubscript{15}H\textsubscript{11}F\textsubscript{3}O C, 69.44; H, 5.30; found C, 69.50; H, 5.35. (Known compound, see: C. C. Tzschucke et al, \textit{Angew.Chem. Int. Ed.}. 2002, \textbf{41}, 4500).

3o: mp:152.7-153.9 °C; IR (KBr) 1683, 1594, 1508, 1338, 1263 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (CDCl\textsubscript{3}) \delta 8.34−8.32 (d, \textit{J} =8.0 Hz, 2H), 8.11-8.09 (d, \textit{J} = 8.0 Hz, 2H), 7.80-7.83 (d, \textit{J} =8.0 Hz, 2H), 7.75-7.73 (d, \textit{J} =8.0 Hz, 2H), 2.67 (s, 3H); \textsuperscript{13}C NMR (CDCl\textsubscript{3}) \delta 197.4, 147.7, 146.2,143.1, 137.2, 129.2, 128.1, 127.7, 124.3, 26.5. Anal Calcd for C\textsubscript{14}H\textsubscript{11}NO\textsubscript{3} C, 69.70; H, 4.60; found C, 69.73; H, 4.64.

3p: Oil °C; IR (neat) 3345, 3060, 1681, 1604, 1267 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (DMSO-\textit{d}_6) \delta 13.23 (s, 1H), 8.04−8.02 (d, \textit{J} = 8.0 Hz, 1H), 7.99-7.92 (m, 3H), 7.73-7.71 (m, 1H), 7.50-7.25 (s,
1H), 7.23-7.21 (s, 1H) 2.60 (s, 3H); 13C NMR (DMSO-d6) δ 198.6, 168.6, 140.9, 137.4, 134.7, 134.0, 132.8, 130.5, 128.6, 127.6, 127.4, 94.5, 27.3. Anal Calcd for C15H12O3, C, 74.99; H, 5.03; found C, 74.97; H, 5.06.

3q: m.p.: 97.8-98.6 °C; IR (KBr) 2923, 2853, 1607, 1586, 1500, 1287, 1245, 806 cm⁻¹; 1H NMR(CDCl3) δ 7.25-7.21 (m, 6H), 6.95 (d, J = 8.8 Hz, 2H), 3.85 (s, 3H), 2.27 (s, 3H); 13C NMR (DMSO) δ 158.5, 141.5, 135.5, 134.4, 130.3, 129.2, 129.9, 127.6, 127.5, 27.0; MS (m/e) 196 (M⁺), 181, 152, 127, 102, 91, 76. Anal Calcd for; found. C14H14O C, 84.81; H, 7.12; found C, 84.75; H, 7.07. (Known compound, see: Tao, Bin; J. Org. Chem, 2004, 69, 4330).

3r: m.p.: 47.8-48.2: °C; IR (KBr) 3340, 1409, 1084 cm⁻¹; 1H NMR (CDCl3) δ, 8.03 (d, J = 8.5 Hz, 2H), 7.69 (d, J = 8.5 Hz, 2H), 7.62–7.65 (m, 2H), 7.46–7.49 (m, 2H), 7.36–7.39 (m, 5H), 2.64 (s, 3H); 13C NMR δ 198.1, 146.1, 140.2, 136.2, 129.3, 129.2, 128.6, 127.6, 127.5, 27.0; MS (m/e) 196 (M⁺), 181, 152, 127, 102, 91, 76. Anal Calcd for; found. C14H14O C, 84.81; H, 7.12; found C, 84.75; H, 7.07. (Known compound, see: G. E. Robinson, J. M. Vernon, J. Chem. Soc. C. 1971, 3363.).

Typical procedure for the one-pot chemoenzymatic synthesis of chiral biaryl alcohols

1-Chloro-4-iodobenzene (59.61 mg, 0.25 mmol), 0.05 mol% of Pdnp/Te-Dps, and 4-acetylphenylboronic acid (40.9 mg, 0.25 mmol.) in TRIS (2 mL, pH = 8.9, 100 mM) were orbitally stirred for 24 h at 100 °C with a Heidolph Synthesi System. After cooling the reaction to room temperature, 500 µL of 2-propanol, (R)-LB-ADH (200 U/mg, 414 µL), and NADP⁺ (8.6 mg, 0.01mmol) were added and the mixture was stirred for 24h and at room temperature. After this time, the liquid phase was extracted with CH₂Cl₂, the organic layer was washed with deionized water and dried over Na₂SO₄. The solvent was removed under vacuum and the residue was purified by chromatography (SiO₂, 35 g, n-Hexane/AcOEt 70/30 v/v) to give 51.6 mg (89% yield) of (R)-4a: m.p.:42.5-43.5 °C; IR (KBr) 3338, 1405, 1085, 898, 835 cm⁻¹; 1H NMR (CDCl3) δ, 7.62-7.60 (d, J = 8.5 Hz, 2H), 7.46–7.41 (m, 5H), 4.97–4.93 (m, 1H), 1.72 (s, 1H), 1.56 (s, 3H); 13C NMR δ 144.8, 140.8, 140.5, 128.7, 127.3, 127.1, 125.8, 70.2, 25.1. Anal Calcd for; found. C14H14O C, 84.81; H, 7.12; found C, 84.77; H, 7.09. [α]D²⁰ +50 (0.1, CHCl₃), ee>99% ( [α]D²⁰ –43.7 for the (S)-enantiomer, 99% ee; J.-i. Ito, S. Ujiie, and H. Nishiyama Organometallics 2009, 28, 630.)

(R)-4b: m.p: 97.5-96.5 °C; IR (KBr) 3313, 2973, 1486, 1390, 815 cm⁻¹; 1H NMR (CDCl3) δ 7.58–7.52 (m, 4H), 7.48–7.46 (d, J = 8.0 Hz, 2H), 7.43–7.41 (d, J = 8.0 Hz, 2H), 4.98 (m, 1H), 1.84 (s, 1H), 1.52 (s, 3H); 13C NMR (CDCl3) δ 145.4, 139.4, 139.2, 133.4, 128.9, 128.3, 127.1, 126.0,
70.1, 25.2 Anal Calcd for C\textsubscript{14}H\textsubscript{13}ClO C, 72.89; H, 4.81; found C, 72.84; H, 4.77. $\left[\alpha\right]_{D}^{20} +32$ (0.1, CHCl\textsubscript{3}), ee>99%

(R)-4c: m.p: 115.7-116.5 °C; IR (KBr) 3369, 2979, 2360, 1396, 1128, 823 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (CDCl\textsubscript{3}) δ 7.71 (s, 4H), 7.62-7.60 (d, $J = 8.0$ Hz, 2H), 7.51-7.49 (d, $J = 8.0$ Hz, 2H), 5.00 (m, 1H), 2.08 (s, 1H), 1.58-1.56 (d, $J = 8.0$ Hz, 3H); \textsuperscript{13}C NMR (CDCl\textsubscript{3}) δ 145.9, 144.4, 138.9, 129.5, 129.2, 127.4, 127.3, 126.1, 125.7, 125.6, 122.9, 70.0, 25.2 Anal Calcd for C\textsubscript{15}H\textsubscript{13}F\textsubscript{3}O C, 67.66; H, 4.92; found C, 67.60; H, 4.94. $\left[\alpha\right]_{D}^{20} +27$ (0.1, CHCl\textsubscript{3}), ee>99%.

Typical procedure for the determination of the enantiomeric excess of 4a and 4c
The enantiomeric excess was determined by enantioselective HPLC on the Chiralpak AS-H chiral stationary phase using the mixture n-hexane-2-propanol 95/5 (v/v) as a mobile phase. In these conditions, the (R)-enantiomers of 4a-4c were eluted before the (S)-enantiomers. The enantiomeric peak identification was carried out by spiking racemic forms of biaryl alcohols with the enantiomers obtained from asymmetric reaction. The chromatographic data were: $k_1$ (R)-4a = 1.25, $k_2$ (S)-4a = 1.51, $\alpha$ = 1.21, $R_s$ = 2.63; $k_1$ (R)-4b = 1.57, $k_2$ (S)-4b = 1.97, $\alpha$ = 1.25, $R_s$ = 3.34; (R)-4c = 1.76, $k_2$ (S)-4c = 2.08, $\alpha$ = 1.18, $R_s$ = 2.58. $k_1$: retention factor of the first eluted enantiomer, defined as $(t_1 - t_0)/t_0$ where $t_0$ is the void time of the column; $\alpha$: enantioselectivity factor defined as $k_2/k_1$; $R_s$: resolution factor defined as $2(t_2-t_1)/(w_1+w_2)$ where $t_1$ and $t_2$ are retention times and $w_1$ and $w_2$ are band widths at the baseline in time units. Other analytical chromatographic conditions: flow-rate, 1.0 mL/min; temperature, 25 °C; detector: UV at 254 nm.

**Figure S1.** TEM image of Pd\textsubscript{np}/Te-Dps negatively stained with PTA and 2x magnification in the inset.
Figure S2. CD spectra of 4a-4c in ethanol