Supplementary Material (ESI) for Chemical Communications
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Supplementary data

Biosynthesis of hydroxydiphenylacetylene by regiospecific monooxygenation

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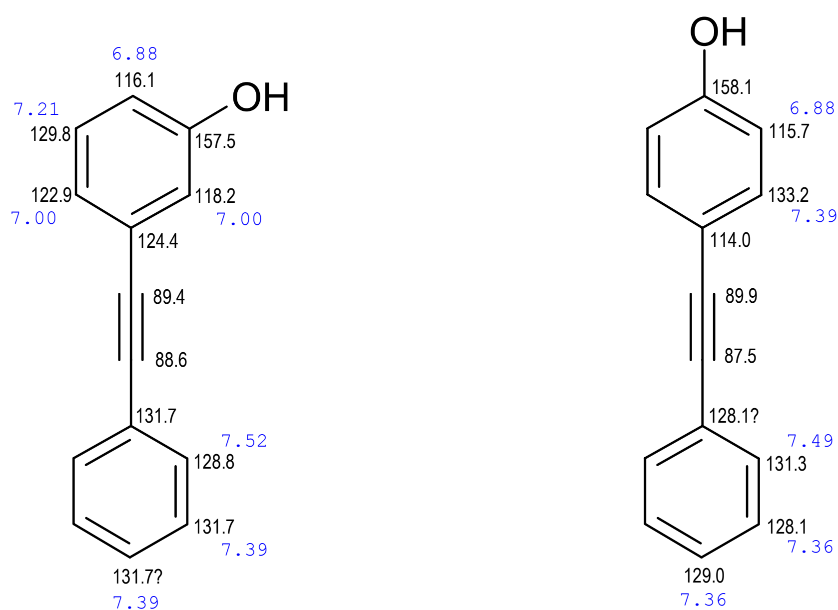
Fig. S1; ^{13}C and ^{1}H NMR data for *meta* and *para*-hydroxydiphenylacetylene

The samples were dissolved in acetone–*d6*. NMR spectra were recorded on a Varian Inova spectrometer equipped with a 5 mm indirect detection probe, operating at 500 MHz for ^{1}H and at 125 MHz for ^{13}C. Chemical shifts are reported in ppm relative to TMS.
The ^{1}H and ^{13}C chemical shifts assignments presented below were based on the ^{1}H-^{13}C one-bond and long-range correlations seen in the ghmbc (gradient heteronuclear multiple bond correlation) spectra. (Personal communication, Dr Ion Ghiviriga, University of Florida)



S2. Construction of expression plasmids pJS407 and pJS409

Plasmids pJS407 and pJS409 containing the genes for the toluene-2- and toluene-4-monooxygenases were derived from recombinant plasmid pRO2016 (Johnson and Olsen, 1997) (Fig. S2)). The two oxygenase operons were subcloned into expression vector pSE380 (*bla* pMB1ori(ColE1 pBR322) p*trc lac* I^{q})(Invitrogen, Carlsbad, Calif. USA) using stepwise approaches. The native promoter regions of the operons were deleted by using polymerase chain reaction (PCR). Standard methods and conditions were followed for DNA manipulations and analysis and cell transformations (Ausbel *et al*., 1998).

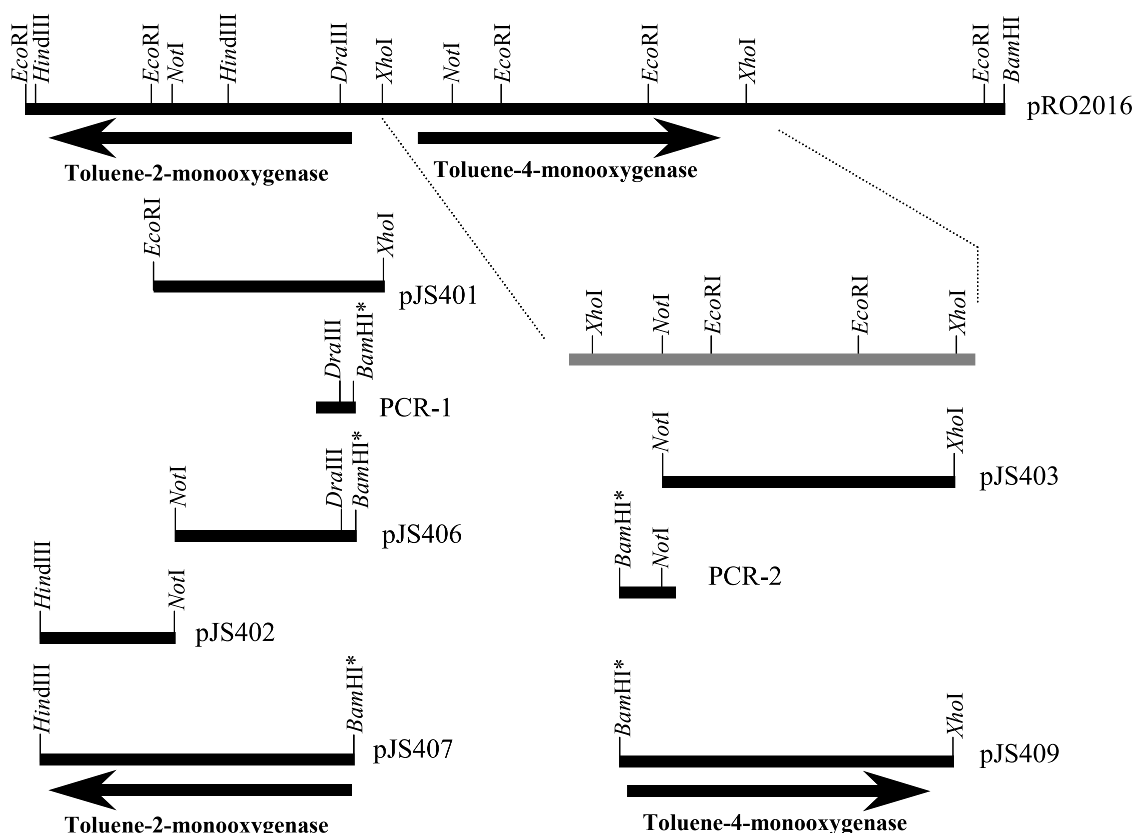


Fig. S2. Restriction maps and summary of the derivation of pJS406 and pJS409. Arrows below pRO2016 map depict regions encoding the monooxygenases and their transcription direction. Asterisk indicates restriction sites added using PCR.

***i*) pJS407 –** The 3' end of the toluene-2-monooxygenase was subcloned into pSE380 as a 1.9-kb *Hin*dIII-*Not*I restriction fragment to yield pJS402 and then transferred to competent *E. coli* Top10F' (F' {*lac*I^{q}, Tn10 (Tet^{R})} *mcr*A Δ (*mrr-hsd*RMS-*mcr*BC) O80 *lac*ZΔM15 Δ*lac*X74 *deo*R *rec*A1 *ara*D139 Δ(*ara-leu*) *7697 gal*U *gal*K *rps*L (str^{R}) *end*A1 *nup*G). by using electroporation. The 5' end of the operon was subcloned into pBlueScript KS+ (*bla* ColE1ori *lac*Z f1(+) origin) (Strategene, La Jolla, Calif, USA) by excising the 3.2-kb *Eco*RI-*Xho*I from pRO2016 and ligating to compatibly cleaved pBluescript to form pJS401 which was transferred to *E. coli* Top10F'. The PCR was used to amplify a 550-bp portion of the toluene-2-monooxygenase operon extending from 33-bp upstream of the *tbmA* translational start site to point past the unique *Dra*III restriction recognition site within the operon. A *Bam*HI restriction recognition site was engineered in the upstream primer to ease subsequent subcloning. PCR was done using pRO2016 as template DNA and oligonucleotides designated, *tbmA*+ (5' ATC GGA TCC GTG GCA AAC CCG ACC TCA ACA GC 3') and *tbmA*- (5' GGT CAT GGT CCA GGT GGC GTA ATA 3') as primers. The PCR product (PCR-1, Fig. S2) was treated with *Dra*III then ligated to the 2.0-kb *Not*I-*Dra*III fragment of the pJS401 DNA insert. The ligation product was subsequently treated with *Bam*HI and *Not*I; ligated to compatibly-cleaved pBluescript to form pJS406, and then transferred to competent *E. coli* Top10F'. Finally, to reform a functional operon encoding the toluene-2-monooxygenase, the 2.8-kb *Not*I-*Bam*HI insert from pJS406 was excised and ligated to compatibly cleaved pJS402 to yield recombinant plasmid pJS407, which was transferred to competent *E. coli* Top10F'. Nucleotide sequence determinations were done to confirm that the intended construct was obtained.

***ii*) pJS409 –** The 3' end of the toluene-4-monooxygenase operon was obtained as a 4.0-kb *Not*I-*Xho*I restriction fragment, subcloned into pBluescript to form pJS403 and transferred to *E. coli* Top10F'. As with the toluene-2-monooxygenase the 5'end of the toluene-4-monooxygenase, minus the native promoter region, was obtained using PCR. Oligonucleotide primers were synthesized that annealed downstream from the *Not*I restriction site within the toluene-4-monooxygenase (*tbmL*- 5' AAC TGC CCG ACG ATC CAC TCA G) and an upstream region near the translation start site of the operon (*tbmL*+ 5' GTCGGA TCC AAA AAC ACT ACA GAC CCT ACC A 3'). Following the PCR using pRO2016 as template and *tbmL+ and tbmL-* as primers, the 0.8-kb product (PCR-2, Fig. S2) was gel purified, then treated with *Not*I. The *Not*I-cleaved PCR-product was ligated to the 4.0-kb *Not*I:*Xho*I DNA insert from pJS403; treated with restriction enzymes *Bam*HI and *Xho*I, and then ligated to compatibly cleaved pSE380 to yield recombinant plasmid pJS409, which was transferred to competent *E. coli* Top10F'. Again, nucleotide sequence determinations were done to confirm that the intended construct was obtained.

Ausbel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (ed.) 1998 *Current protocols in molecular biology*. New York: John Wiley and Sons, Inc.

Johnson, G. R. & Olsen, R. H. 1997 Multiple pathways for toluene degradation in *Burkholderia* sp. strain JS150. *Applied and Environmental Microbiology* **63**, 4047-4052.