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Direct biooxidation of arenes to corresponding catechols with *E. coli* JM109 (pDTG602). Application to synthesis of combretastatins A-1 and B-1

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Abstract—Convergent syntheses of combretastatins A-1 and B-1 were accomplished via coupling of biocatalytically generated *p*-bromomethoxycatechol with trimethoxyphenylacetylene.

Oxygenated natural products containing a catechol subunit are ubiquitous in nature. Combretastatins A-1, B-1 and A-4 belong to this class of natural products and were isolated from the bark of an African willow, *Combretum caffrum*, in 1987.[1] Biological studies of these compounds have revealed that they are among the most cytotoxic agents tested so far against a series of cancer cell lines.[2] They share a common binding site on tubulin with the well-known antimitotic agents colchicine, podophyllotoxin, and steganancin; they are capable of inhibiting microtubule assembly at nanomolar concentrations. The high potency of combretastatins A-1 and A-4, Fig. 1, as angiogenesis inhibitors offers a new approach to cancer treatment. These natural products upon binding to tubulin, prevent tumors from metastasizing by inhibiting their ability to grow new blood vessels.[3] Structure–activity relationship analyses of combretastatins and their analogues have shown that the *cis*-olefin and the phenolic functional groups are essential for antitumor activity.[4] Such structural features can be fashioned quite easily through the use of *E. coli* JM109 (pDTG602)[5] in an environmentally friendly method of generating functionalized catechols for application to the synthesis of this class of natural products.

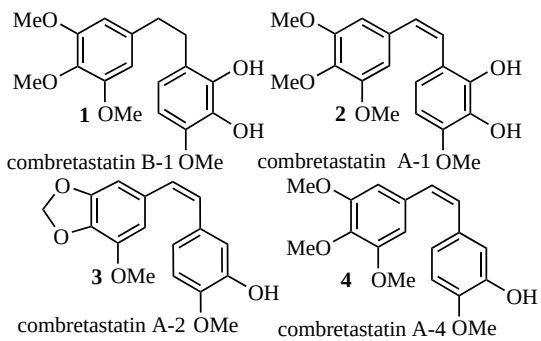

Figure 1. Structure variation in combretastatins.

After Gibson elucidated the degradation pathway, shown in Fig. 2, for aromatic compounds by *Pseudomonas* species,[6] he developed two sets of recombinant organisms for use in synthesis: one that expresses toluene dioxygenase (TDO) and one with plasmids for both TDO and the next enzyme in the sequence, dihydrodioldehydrogenase (DHDD). Application of the latter enzyme to either an arene or the diol derived from it yields the corresponding catechol as shown in Fig. 3.

We have already reported on the synthesis using recombinant organism JM109 (pDTG602) of several functionalized catechols[7] that are otherwise difficult to prepare by traditional means. In our approach to combretastatins we chose to prepare *p*-methoxybromocatechol (**9**)[8] and couple its protected form to 3,4,5-trimethoxyphenylethyne[2a] as shown in Scheme 1. The biooxidation of 4-bromoanisole provided ~1.5g/L of **9**, which was converted to the MOM-protected derivative **10** in ~90% yield. The coupling of aryl halide **10** with alkynylboronic ester of **11** under Suzuki–Miyaura conditions[9] provided acetylene **13**, which was hydrogenated completely to **14** and hydrolyzed to combretastatin B-1. Hydroboration of **13** to **15** was found

Keywords: biooxidation; catechol; combretastatins; Suzuki–Miyaura coupling.

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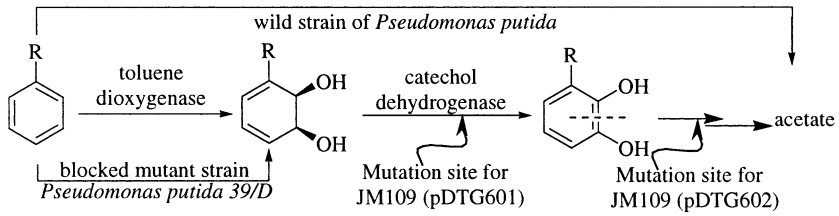

Figure 2. Degradation of aromatics by eukaryotic strains.

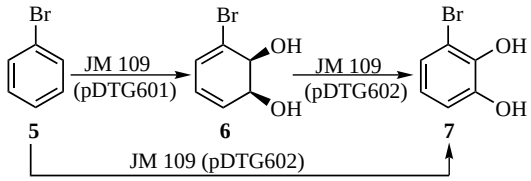

Figure 3. Biocatalytic synthesis of catechols.

superior to the use of Lindlar's catalyst and gave the required *cis*-olefin **15** in 84% yield. Final deprotection of MOM with diluted HCl provided for a concise preparation of combretastatin A-1 in an overall yield of 43–51% over five steps with no observed isomerization of the *cis*-olefin of the final product.

In summary, two related combretastatins were synthesized in a convergent manner and in yields competitive or superior to those of previous literature syntheses.[1,10] Current endeavors center around the efficient design of *para*-substituted analogs of A-1 as well as preparation of combretastatin A-4. Results of these endeavors will be reported in due course.

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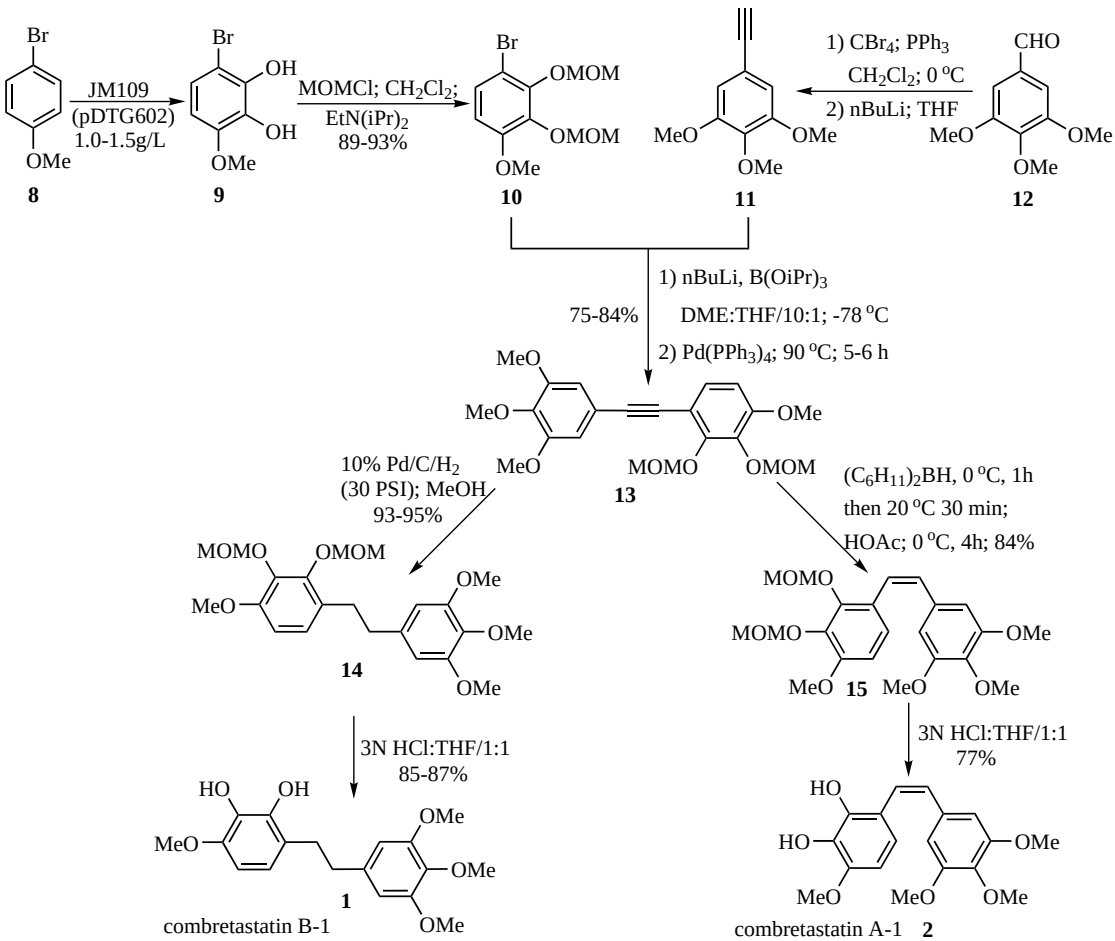

Scheme 1. Chemoenzymatic synthesis of combretastatins A-1 and B-1.

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8. General procedure for the large-scale (15 L) fermentation with *E. coli* JM109 (pDTG602): A mineral salts broth (MSB, 600 mL) containing K_2HPO_4 (9.6 g), KH_2PO_4 (8.4 g), $(NH_4)_2SO_4$ (3 g), yeast extract (9 g), glucose (18 g), and $MgSO_4{\cdot}7H_2O$ (1.2 g) was divided into two 2.8 L Fernbach flasks and sterilized. After the broth had cooled to ambient temperature, ampicillin (100 mg L^{-1}) was added, and each flask inoculated with a single colony of *E. coli* JM109 (pDTG 602) from a fully grown plate. The inoculum was grown overnight on an orbital shaker (35°C, 150 rpm) and added to sterilized production medium (8 L) in a 15 L fermentor. The production medium consists of an aqueous mixture of KH_2PO_4 (60 g), citric acid (16 g), $MgSO_4{\cdot}7H_2O$ (40 g), trace metal solution (16 mL) including: [Na_2SO_4 (1 g L^{-1}), $MnSO_4$ (2 g L^{-1}), $ZnCl_2$ (2 g L^{-1}), $CoCl_2{\cdot}6H_2O$ (2 g L^{-1}), $CuSO_4{\cdot}5H_2O$ (0.3 g L^{-1}), $FeSO_4{\cdot}7H_2O$ (10 g L^{-1}), pH 1.0], conc. H_2SO_4 (9.6 mL) and ferric ammonium citrate (9.6 mL, 270 g L^{-1}). The mixture was neutralized with conc. ammonia and supplemented with ampicillin (800 mg) and thiamine hydrochloride (2.69 g). The culture was grown for 24 h with concentrated glucose solution (720 g L^{-1}) as the carbon source, then induced with 80 mg isopropyl β-D-thiogalactopyranoside (IPTG) after the optical density (OD) had reached at least 15 of the blank. Once the OD was 45 or higher, bromoanisole was added at a rate of 10 g h^{-1}. After 2 h of substrate addition, cells were removed via centrifugation and the supernatant was extracted with ethyl acetate. The metabolite, bromoanisole catechol, was purified by flash chromatography to afford 5 g of bromoanisole catechol (**9**) as white solid whose physical properties matched those reported in the literature (see Ref. 2c).
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