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Chemoenzymatic methods for the enantioselective preparation of sesquiterpenoid natural products from aromatic precursors*

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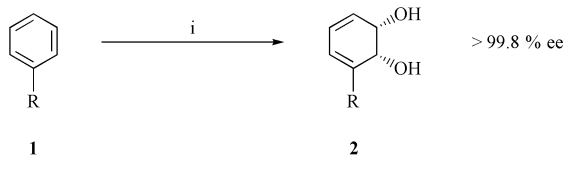
Abstract: The enantiomerically pure *cis*-1,2-dihydrocatechols **2**, which are generated by enzymatic dihydroxylation of the corresponding aromatic, engage in regio- and stereo-controlled Diels–Alder cycloaddition reactions to give a range of synthetically useful bicyclo[2.2.2]octenes. Certain examples of the latter type of compound have been used as starting materials in the synthesis of the sesquiterpenoids (–)-patchoulenone and (–)-hirsutene.

Dihydroxylation of monosubstituted benzenes (**1**) using a genetically engineered strain of *E. coli* that overexpresses the enzyme toluene dioxygenase (TDO) enables the large-scale production of enantiopure (>99.8 % ee) *cis*-1,2-dihydrocatechols of the general type **2** (Fig. 1) [1]. Like others [2], we [3] have been exploiting these densely functionalized and readily available metabolites as starting materials in the chemical synthesis of various classes of natural products. Such combined usage of chemical and enzymatic steps in the construction of target molecules is often referred to as chemoenzymatic synthesis. A concern often expressed about this approach is that since the enantiomeric enzyme is unlikely ever to be available then, correspondingly, the antipodal metabolite *ent*-**2** would seem inaccessible. However, through a combination of chemical and enzymatic methods, it has been possible to address this matter. For example, by presenting the *p*-iodinated derivative, **3**, of the original monosubstituted aromatic to TDO (Fig. 2) the sense of dihydroxylation is "reversed" and the *cis*-1,2-dihydrocatechol **4** becomes the predominant product [4]. Reductive removal of iodine, by either hydrogenolytic or elec-



1 **2**

R=Me, Br, Cl, CN, CH=CH_2, CF_3, CO_2H *etc*

Yields: up to 35g/litre fermentation broth

Fig. 1 *Reagents and conditions*: (i) *E. coli* JM109 (pDTG601).

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trochemical means, followed by an "enzymatic clean-up," to remove small quantities of compound **2**, then delivers **5** (≡ *ent*-**2**) in essentially enantiomerically pure form.

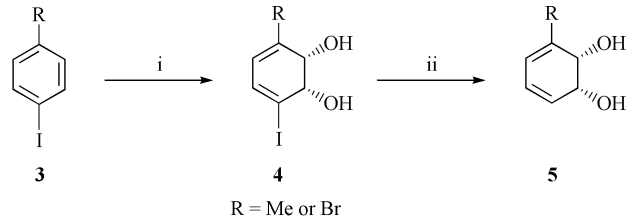


Fig. 2 *Reagents and conditions*: (i) *E. coli* JM109 (pDTG601); (ii) reductive deiodination.

Perhaps an even more attractive means for achieving enantiodivergence would be to start with a monochiral form of the *cis*-1,2-dihydrocatechol and convert this into either enantiomeric form of derivatives that would be useful building blocks for chemical synthesis. We have recently developed two Diels–Alder-based methods for achieving such outcomes, and these are shown in Figs. 3 and 4. In the first (Fig. 3), the high-pressure (19 kbar) promoted reaction of the so-called toluene diol **2** (R = CH_3) with lactone **6** affords, via an *endo*-transition state and operation of the *ortho*-rule, bicyclo[2.2.2]octene **7** (56 %) as the major product of reaction [5]. The selective formation of this "*syn*-adduct" via addition of the dienophile to the more hindered (α) face of the diene seems counterintuitive, but has precedents [6] and may be rationalized on the basis that there is a stabilizing interaction between the π-bond of **6** and the O–H bonds of **2** (R = CH_3) [7]. The use of high pressure is essential because the reaction cannot be effected at elevated temperatures and/or in the presence of Lewis acid catalysts owing to the ready dehydration and accompanying aromatization of **2**. If the acetonide derivative, **8**, of **2** (R = CH_3) is subjected to the same reaction with dienophile **6** then, owing to the steric demand of the acetal moiety, the exclusive product of reaction is the β-face addition product or "*anti*-adduct" **9** [5]. The pseudo-enantiomeric relationship between compounds **7** and **9** is readily discerned if one imagines hydrolysis of the latter to the corresponding diol **10**, then oxidation of this and stereoisomer **9** to the respective (and mirror image) diketones, **11** and *ent*-**11**. In another example (Fig. 4) of an enantiodivergent route to bicyclo[2.2.2]octanes, the iodo-diol (**2**, R = I) is converted into the corresponding bis-crotonate **12** which, upon heating, engages in the two possible and competing intramolecular Diels–Alder reactions to afford a ca. 3:5 mixture of adducts **13** and **14** [8]. Each of these products can be readily purified by a combination of fractional crystallization and chromatographic techniques. The pseudo-enantiomeric rela-

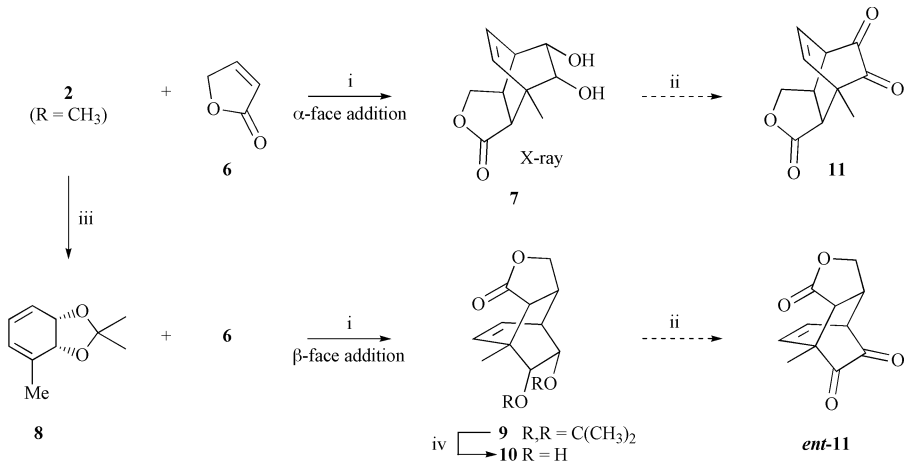


Fig. 3 *Reagents and conditions*: (i) CH_2Cl_2, 19 kbar, 18 °C, 16 h; (ii) oxidation; (iii) $(CH_3)_2C(OCH_3)_2$, *p*-TsOH (cat.), –10 to 18 °C, 24 h; (iv) acetonide hydrolysis.

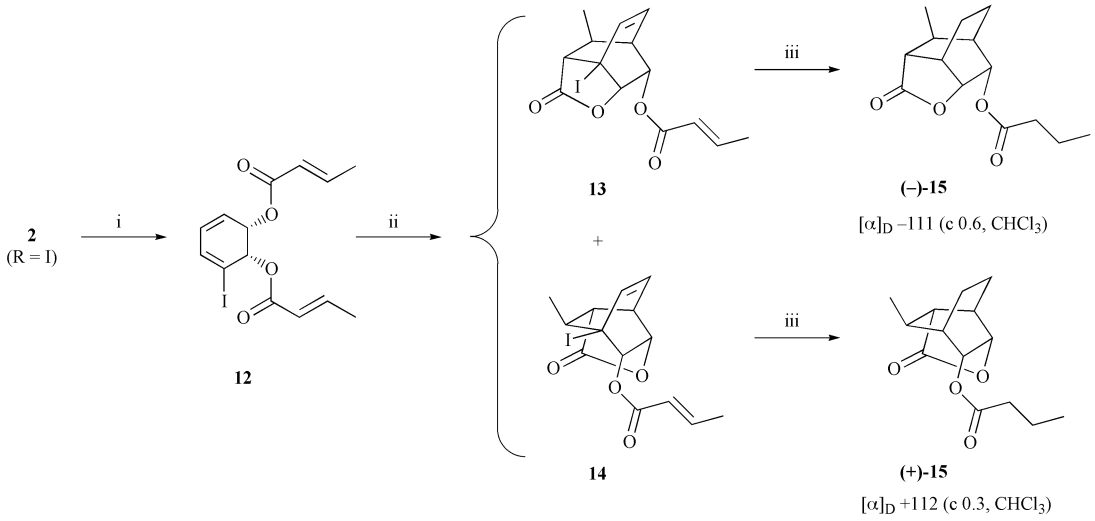
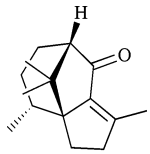


Fig. 4 *Reagents and conditions*: (i) BuLi (2.1 mol equiv), (*E*)-$CH_3CHCHCOCl$ (2.0 mol equiv), THF, –78 to 0 °C, 3 h; (ii) C_6H_6, 80 °C, 4 h; (iii) H_2 (1 atm), 10 % Pd on C, $^iPr_2NEt$, EtOH, 18 °C, 16 h.

tionship between them is highlighted by the exhaustive hydrogenation/hydrogenolysis of each to give (–)-**15** and (+)-**15**, respectively.

The capacity to extend the sequences just mentioned to a wide variety of related examples means that a significant range of enantiomerically pure and synthetically useful bicyclo[2.2.2]octanes is now available. Our recent completion of total syntheses of the structurally complex sesquiterpenes (–)-patchoulenone (**16**) [3d,m] and (–)-hirsutene (**17**) [3n] from such precursors by the routes detailed below should serve to highlight the potential of this approach.



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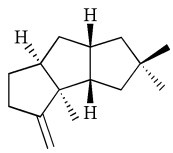

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The reaction sequence leading from the C7 synthon **2** (R = CH_3) to the medicinally interesting cyperene-type sesquiterpene **16** is shown in Fig. 5 and begins with formation of the crystalline acetal **18** (53 %). A thermally promoted and diastereofacially selective Diels–Alder reaction of the latter compound with Corey's ketene-equivalent followed by base-promoted hydrolysis of the resulting epimeric mixture of α-chloronitriles gave bicyclo[2.2.2]octenone **19** in 86 % yield. *gem*-Dimethylation of the last compound afforded ketone **20** (89 %), which was hydrogenated to give the saturated equivalent **21** (100 %). Wittig olefination of compound **21** could not be achieved under conventional conditions because of the hindered nature of the carbonyl group, but use of high pressure allowed the target olefin **22** to be obtained in 86 % yield. Straightforward manipulation of the PMB acetal within substrate **22** then led, over four steps, to ketone **23** (81 % from **22**), which was reacted with isopropenyl lithium to give, in a completely diastereoselective manner, the 1,5-dienol **24**. This last compound engaged in an anionic oxy-Cope rearrangement on exposure to sodium hydride to give, after work-up, the bicyclo[5.3.1]octenone **25** in 76 % yield (from **23**). The X-ray crystal structure of the latter compound reveals that the molecule adopts a distinctly U-shaped conformation, which brings the carbonyl and olefinic moieties into such close contact that samarium iodide-mediated one-electron reduction of this compound creates a ketyl radical anion that cyclizes onto the adjacent olefin. When thiophenol is used

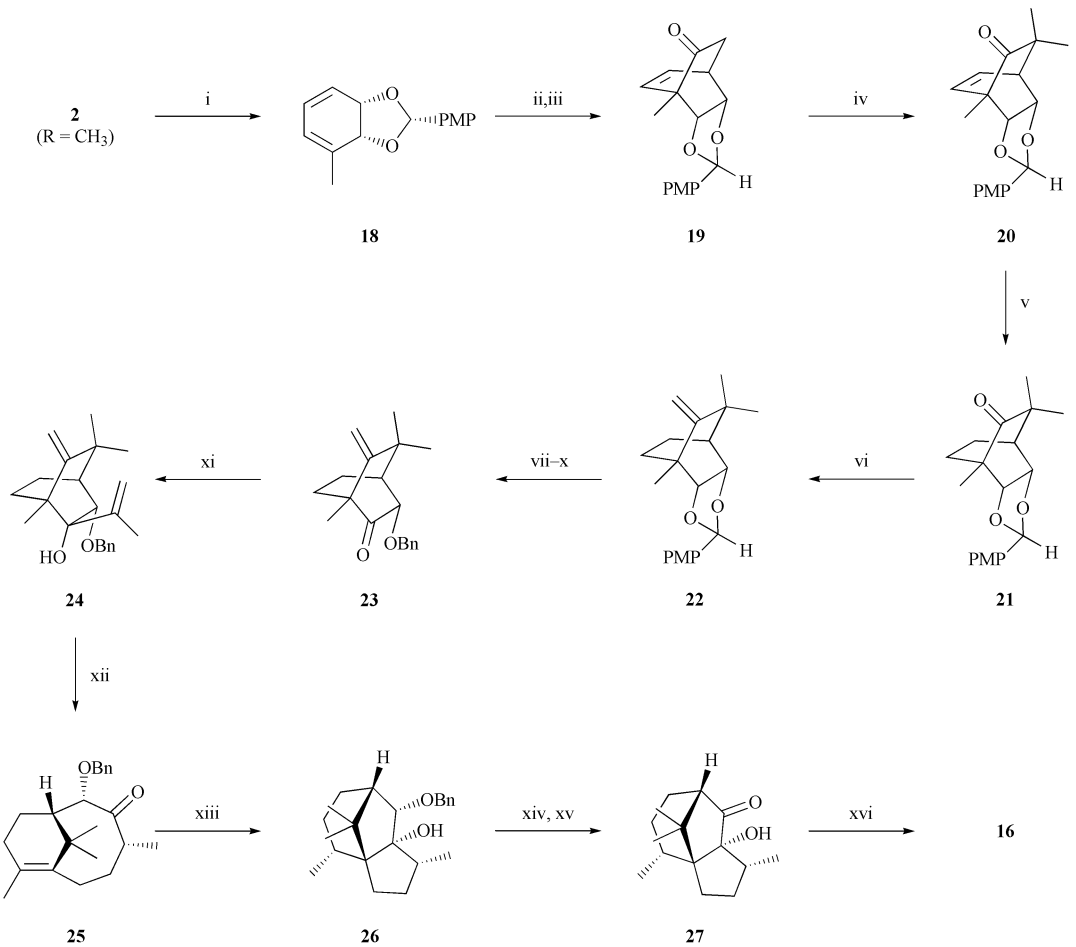


Fig. 5 *Reagents and conditions*: (i) *p*-MBDMA (1.1 mol equiv), (+)-CSA.H_2O (0.01 mol equiv), CH_2Cl_2, –20 °C, 0.25 h, then recrystallization (60–80 petroleum spirit); (ii) α-chloroacrylonitrile (3 mol equiv), C_6H_6, reflux, 48 h; (iii) KOH (5 mol equiv), ^{t}BuOH, 70 °C, 1.5 h; (iv) MeI (2 mol equiv), NaHMDS (2 mol equiv), THF, 0 °C, 2 h then repeat × 2; (v) PtO_2 (0.2 mol equiv), H_2, THF, 18 °C, 6.5 h; (vi) $Ph_3P{=}CH_2$ (1.5 mol equiv), THF, 19 kbar, 18 °C, 2 h; (vii) DIBAL-H (5 mol equiv, 1 M in hexane), 1:1 CH_2Cl_2/pentane, –60 °C, 4 h then –40 °C, 1.5 h; (viii) BnBr (2 mol equiv), NaH (3.5 mol equiv), Bu_4NI (0.1 mol equiv), DMF, 18 °C, 15 h; (ix) DDQ (1.5 mol equiv), 9:1 CH_2Cl_2/H_2O, 18 °C, 0.25 h; (x) TPAP (0.05 mol equiv), NMO (3 mol equiv), 4 Å molecular sieves, CH_2Cl_2, 18 °C, 2.5 h; (xi) isopropenyl lithium (5 mol equiv), diethyl ether, –78 to 18 °C, 0.2 h; (xii) NaH (1.5 mol equiv), THF, 66 °C, 3 h; (xiii) SmI_2, (2.5 mol equiv), PhSH (20 mol equiv), 1:3 HMPA/THF, 0 °C, 2 h; (xiv) H_2 (1 atm), 10 % Pd on C, THF, 18 °C, 3 h; (xv) SO_3/pyridine (1 mol equiv), Et_3N (14 mol equiv), CH_2Cl_2/DMSO, 18 to 35 °C, 48 h; (xvi) $SOCl_2$ (50 mol equiv), pyridine, 40 °C, 18 h.

as a hydrogen atom source to "quench" the ensuing 3°-radical, then serviceable yields (74 %) of the compound **26**, which incorporates the cyperene framework, can be obtained. Hydrogenolytic cleavage of the benzyl group within this product revealed a diol, the 2°-hydroxyl of which was oxidized to give acyloin **27** (65 % over two steps). Thionyl chloride/pyridine-mediated dehydration of compound **27** then gave (–)-patchoulenone **16** (72 %), the derived spectral data for which matched, in all respects, those reported [9] for the natural product.

The naturally occurring linear triquinane (+)-hirsutene [*ent*-(**17**)] has been a popular synthetic target for showcasing new methodologies [10] but, remarkably, only one total synthesis of enantiomeri-

cally pure material has been disclosed in over 20 years of activity in the area. Figure 6 delineates a synthesis of (–)-hirsutene (**17**) from toluene diol **2** (R = CH_3), and because the enantiomeric form of this starting material is also known (see Fig. 2) the work described constitutes a formal total synthesis of the natural material as well. In keeping with earlier observations, the high-pressure-promoted Diels–Alder reaction of diol **2** (R = CH_3) with cyclopentenone (**28**) afforded the α-face or "*syn*-addition" product **29** (70 %) as the major product of reaction. *gem*-Dimethylation of the acetonide derivative then gave compound **30** (98 %), and the now superfluous carbonyl unit was deleted by a stepwise reduction process involving a Barton–McCombie deoxygenation in the final step. The acetonide group associated with product **31** (75 %) was cleaved with aqueous acid, and the hydroxyl moiety remote from the bridgehead methyl group in the ensuing diol was selectively oxidized using the sterically demanding oxoammonium salt derived from 4-acetamidoTEMPO. The remaining hydroxyl group was then protected as the MEM-ether to give compound **32** (55 % from **31**). In a pivotal step of the reaction sequence, and following on from concepts originally enunciated by Demuth and Schaffner [11] and recently exploited by Singh et al. [10] in his synthesis of (±)-**17**, bicyclo[2.2.2]octenone **32** was subject to a photochemically promoted oxa-di-π-methane rearrangement reaction thereby affording the cyclopropa-fused triquinane **33** (66 %). The structure of this photoproduct follows from NMR spectroscopic data and was confirmed by single-crystal X-ray analysis. Elaboration of compound **33** to the final target involved reductive cleavage of the cyclopropane ring, deletion of the carbonyl group using the same methods as employed earlier, then hydrolytic cleavage of the MEM-ether to give alcohol **34** (36 % over four steps). Completion of the synthesis required oxidation of the last compound to the corresponding ketone, then Wittig methylenation to give (–)-hirsutene (**17**) (98 % at 33 % conversion), the specific rotation for which was similar in magnitude (but opposite sign) to that reported by Hua et al. [12] for the natural product. While conceptually straightforward, these last few steps proved especially demanding from a

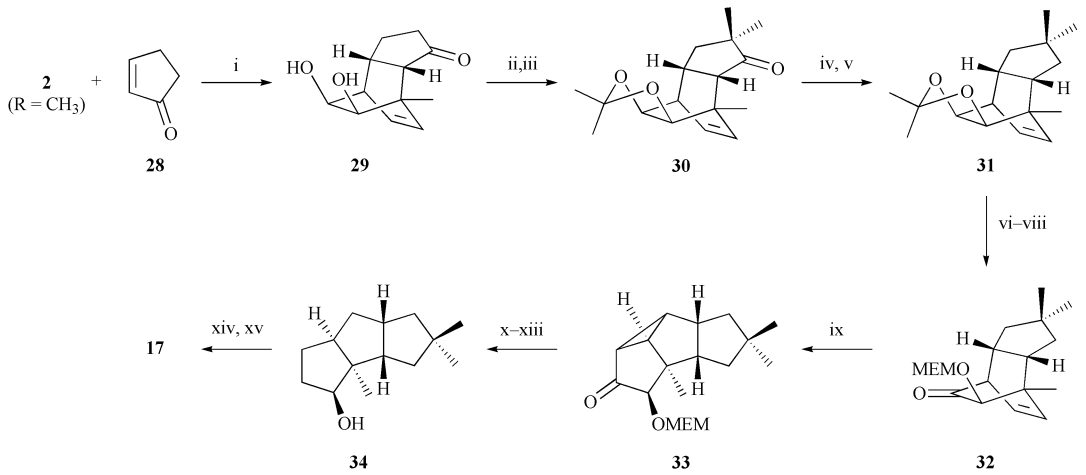
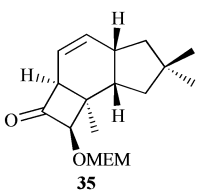


Fig. 6 *Reagents and conditions*: (i) 19 kbar, CH_2Cl_2, 18 °C, 24 h; (ii) 2,2-DMP, *p*-TsOH.H_2O (cat.), 18 °C, 16 h; (iii) MeI (4.2 mol equiv), LiHMDS (3.15 mol equiv), THF, 0–18 °C, ca. 4 h; (iv) $LiAlH_4$ (1.1 mol equiv), THF, 0–50 °C, 29 h; (v) (a) NaH (5 mol equiv), CS_2 (10 mol equiv), THF, 0–66 °C, 18 h, then MeI (20 mol equiv), 18–66 °C, 56 h; (b) *n*-Bu_3SnH (5 mol equiv), AIBN (cat.), toluene, 111 °C, 18 h; (vi) 3:2 v/v AcOH–H_2O, 60 °C, 96 h; (vii) 4-AcNHTEMPO (2.1 mol equiv), *p*-TsOH.H_2O (2.1 mol equiv), CH_2Cl_2, 0–18 °C, 16 h; (viii) MEM-Cl (2 mol equiv), Hünig's base (2.5 mol equiv), CH_2Cl_2, 18 °C, 16 h; (ix) irradiation with Phillips 125-W HPL-N lamp, acetophenone (2.5 mol equiv), acetone, ca. 10 °C, 32 h; (x) *n*-Bu_3SnH (6 mol equiv), AIBN (cat.), C_6H_6, 80 °C, 8 h; (xi) $NaBH_4$ (2.25 mol equiv), MeOH, 18 °C, 4 h; (xii) (a) NaH (5 mol equiv), CS_2 (10 mol equiv), THF, 0–66 °C, 18 h then MeI (16 mol equiv), 18–66 °C, 9 h; (b) *n*-Bu_3SnH (2 mol equiv), AIBN (cat.), toluene, 111 °C, 2 h; (xiii) PPTS (2.6 mol equiv), ^{t}BuOH, 82 °C, 8 h; (xiv) PCC (2 mol equiv), CH_2Cl_2, 18 °C, 16 h; (xv) $Ph_3P{=}CH_2$ (2 mol equiv), toluene, 0–66 °C, 1.5 h.

practical viewpoint because of the exceptionally high vapor pressure of the final product, the precursor ketone, and the alcohol **34**.

While investigating the photochemical conversion **32** → **33**, we noted formation of small amounts (5 %) of the isomeric cyclobutanone **35**, which presumably arises via "leakage" through a singlet reaction pathway. Indeed, direct irradiation of ketone **32** with light from a medium-pressure mercury lamp afforded compound **35** in 66 % yield, and this was accompanied by ca. 5 % of the photodecarbonylation product **36**. Significantly, these "new" photoproducts bear a strong structural resemblance to the protoilludane and marasmane sesquiterpenoids such as tsugicoline A (**37**) [13] and isovelleral (**38**) [14], respectively. Of course, as synthesis chemists it is very tempting to try and effect the conversion **32** → **37** by chemical means because of the intriguing biological properties of the latter. Clearly, implementation of this idea requires, inter alia, the devising of a method for incorporation of a hydroxymethyl group. Even better, therefore, might be to try and develop procedures for the whole-cell biotransformation of *m*-methylbenzylalcohol (or an equivalent) into the corresponding *cis*-1,2-dihydrocatechol such that the 15th carbon of target sesquiterpene **37** is in place from the beginning of the chemical parts of the synthesis. These sorts of considerations highlight another possible area for development in the field of chemoenzymatic synthesis. Rather than simply "making do" with already available *cis*-1,2-dihydrocatechols as starting materials, can we generate new sets of metabolites that



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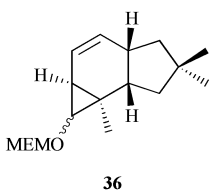

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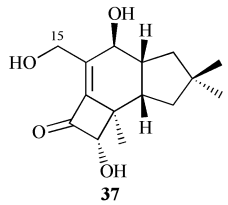

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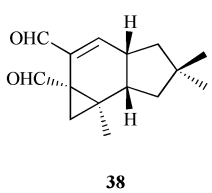

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are "customized" for a particular synthetic task? While that is easier said than done, especially in cases where TDO cannot effectively dihydroxylate the relevant substrate, it may be possible, through application of gene shuffling and directed evolution techniques [15], to generate mutant enzymes/organisms capable of performing the required task. So, it would be nice to think that one day synthesis chemists might have access to a vast range of mutant cell lines that enable the selective biotransformation of any one of the large number of abundant aromatic feed stocks into any synthetically desirable dihydrocatechol or related metabolite. Such possibilities, when considered in conjunction with the above-mentioned "tricks" (Figs. 3 and 4) available for enantiomeric switching, suggest a promising future for the chemoenzymatic synthesis of terpenoids and other natural products from aromatic metabolites.

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