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Synthesis of Antimicrobial Natural Products Targeting FtsZ: (+)-Totarol and Related Totarane Diterpenes

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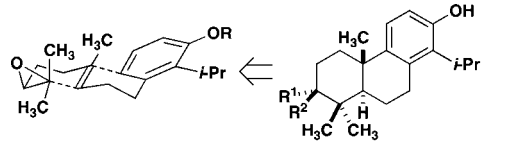
ABSTRACT



totarol, (R^1 = R^2 = H)
totaradiol, (R^1 = H, R^2 = OH)
totarolone, (R^1, R^2 = O)

An efficient, convergent synthesis of totarol by a diastereoselective epoxide/alkene/arene bicyclization is described. The reported synthesis enables the preparation of related diterpenes totaradiol and totarolone as well as previously unavailable derivatives that exhibit comparable inhibition of the bacterial cell division protein FtsZ.

Bacterial cell division is a novel target for the development of new antibiotics to fight infections that are resistant to current therapies.[1] FtsZ is the central protein of bacterial cell division that forms the Z-ring at midcell and enables septation.[2] This GTPase is structurally related to eukaryotic tubulin, which has been successfully targeted as a treatment for cancer. Although tubulin is targeted by many small molecules, and several are in clinical use as drugs, inhibitors of FtsZ are significantly lower in number and in vitro potency. More significantly, three separate allosteric sites of inhibition on tubulin have been identified for paclitaxel, colchicine, and the *Vinca* alkaloids. Similar knowledge regarding FtsZ is lacking, and no direct evidence for inactivation outside the GTP binding site has been reported.[3] Our group is interested in developing efficient syntheses of natural products that target FtsZ with the long-term goal of using synthesis to elucidate the mechanism by which these compounds act on this protein.[4,5]

Totarol is a diterpene produced in the sap of *Podocarpus totara*, a conifer native to New Zealand. The wood from the tree is prized for its resistance to rot, and the antimicrobial properties of the secondary metabolites in the sap are well-

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(3) Indirect techniques, including site-directed mutagenesis and mapping of mutations to resistant strains, have provided evidence for the mode of inactivation of one FtsZ inhibitor: Haydon, D. J.; Stokes, N. R.; Ure, R.; Galbraith, G.; Bennett, J. M.; Brown, D. R.; Baker, P. J.; Barynin, V. V.; Rice, D. W.; Sedelnikova, S. E.; Heal, J. R.; Sheridan, J. M.; Aiwale, S. T.; Chauhan, P. K.; Srivastava, A.; Taneja, A.; Collins, I.; Errington, J.; Czaplewski, L. G. *Science* **2008**, *321*, 1673–1675.

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(5) Curcumin has been reported to target many proteins, including both FtsZ and tubulin: (a) Rai, D.; Singh, J. K.; Roy, N.; Panda, D. *Biochem. J.* **2008**, *410*, 147–155. (b) Gupta, K. K.; Bharne, S. S.; Rathinasamy, K.; Naik, N. R.; Panda, D. *FEBS J.* **2006**, *273*, 5320–5332.

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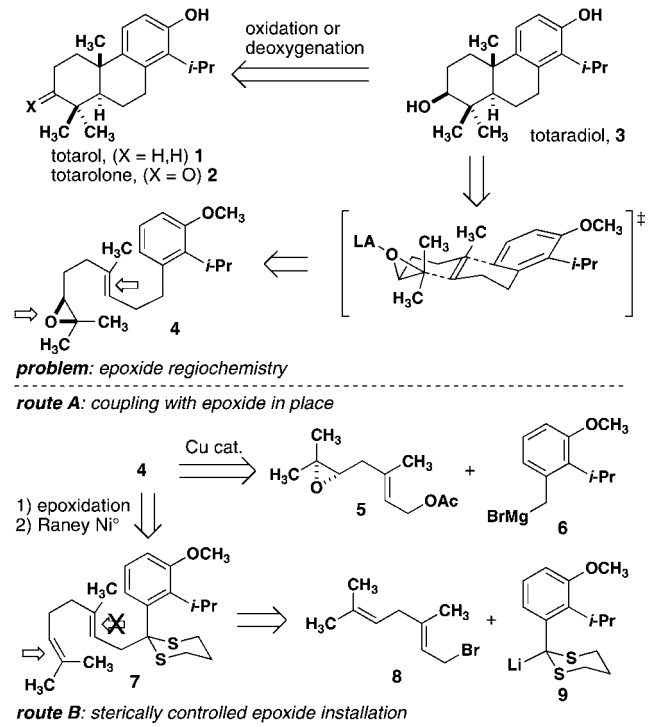
established.[6] Totarol is approved for use as an antimicrobial additive in several consumer products, including toothpaste and acne treatments.[7] Although several previous studies have probed the origin of totarol's antimicrobial activity,[8] FtsZ was only recently identified as a discrete molecular target.[9]

We have undertaken the synthesis of totarol and related diterpenes as part of a broader research program aimed at discerning the mechanism by which FtsZ can be inactivated by small molecules. Previous syntheses of totarol include routes to racemic material[10] and semisyntheses[11] from chiral terpenoid precursors. Recent routes to related tricyclic systems have focused on electrophile-induced cyclization of polyene-derived precursors. Efficient syntheses of analogous tricyclic compounds have been reported using enantioselective protonation[12] and halogenation[13] of alkenes to effect polycyclization reactions. None of these efforts to date has resulted in the synthesis of the related diterpenoids totaradiol and totarolone. As a result, medicinal studies of totarol rely on preparing derivatives of the natural material, largely limiting these studies to modifications of the B and C rings.[14] We recognized that an epoxide-initiated polycyclization would provide access to all three natural products and enable the synthesis of previously inaccessible A-ring derivatives for biochemical studies.

Retrosynthetic analysis reveals that a suitable precursor (**4**, Scheme 1) is produced by benzylic attachment of epoxygeraniol to a substituted arene. Cyclizations of related substrates have been described,[15] and these substrates are produced by either copper- or palladium-catalyzed cross-coupling reactions to allylic acetates or halides, respectively.[16,17] Alternatively, direct coupling of Grignard reagents to allylic phosphonates has also been employed.[18] A consistent synthetic challenge is the installation of the epoxide at one of the two trisubstituted

Scheme 1. Retrosynthesis of Totarol, Totaradiol and Totarolone



alkenes. The established lack of regiocontrol in the Sharpless asymmetric dihydroxylation (SAD) reaction[19] would necessitate installation of the epoxide before the coupling reaction (Scheme 1, route A). Given the liability posed by the use of benzylic Grignard reagents, we also considered an alternate coupling using dithiane **9** (Scheme 1, route B). The latter route would rely on steric control of the dihydroxylation.[20] This synthetic scheme provides protected totaradiol directly from the polycyclization and totarol or totarolone by subsequent deoxygenation or oxidation, respectively.

We initially explored route A by preparing the precursor to **4** in seven steps (Scheme 2). Acid **10** was converted to the amide precursor of **12** via the reaction of the mixed anhydride with amide **11**.[21] The resultant amide was cyclized to oxazoline **12** using triethylamine and methanesulfonyl

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(16) (a) Baeckvall, J. E.; Sellen, M. *Chem. Commun.* **1987**, 827–829. (b) Baeckvall, J. E.; Sellen, M.; Grant, B. *J. Am. Chem. Soc.* **1990**, *112*, 6615–6621. (c) Gansaeuer, A.; Justicia, J.; Rosales, A.; Rinker, B. *Synlett* **2005**, 1954–1956.

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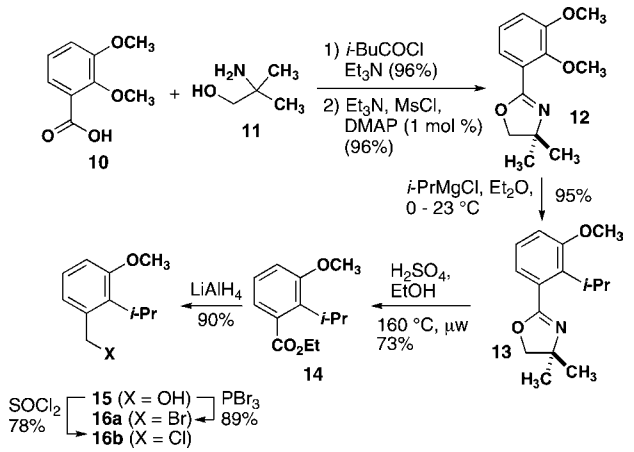
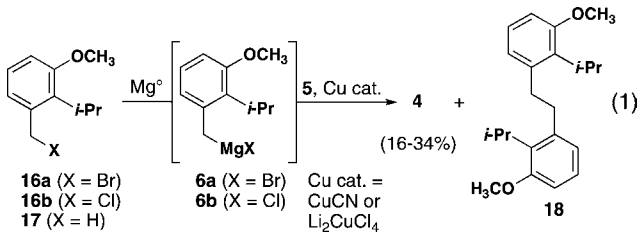
Scheme 2. Synthesis of Grignard Reagents **6a** and **6b**



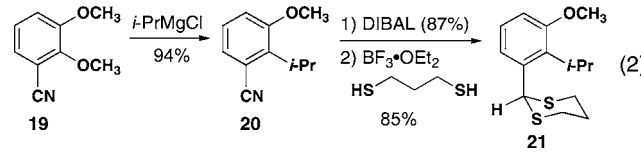
chloride[22] in 96% overall yield from **10**. Regioselective displacement of the *o*-methoxy group was achieved in high yield using conditions reported by Myers.[23] Although many conditions have been reported for the solvolysis of dimethyloxazolines related to **13**, this substrate proved problematic.[21,24] We found that acidic hydrolysis in ethanol was more consistently high yielding for conversion to ester **14**.[25] Reduction to alcohol **15** and conversion to either bromide **16a** or chloride **16b** were straightforward.

Synthesis of cyclization precursor **4** was attempted using a copper-catalyzed coupling (eq 1).[15] Although conversion of chloride **16b** in to the requisite Grignard reagent **6b** has been reported,[26] we observed sluggish consumption of magnesium turnings under standard conditions. Next, bromide **16a** was used to form Grignard reagent **6a**. Benzylic bromides often provide significant quantities of Würtz coupling products when allowed to react with magnesium metal. In this case, we had hoped that the bulky ortho substituent would suppress this side reaction. Transfer of **6a** into a solution of epoxygeranyl acetate (**5**) and Li_2CuCl_4 or CuCN provided **4** in highly variable yields along with Würtz product **18**.[27] The long sequence to prepare **16** paired with the variable yields in the coupling reaction prompted us to consider an alternate route to **4**.



(1)

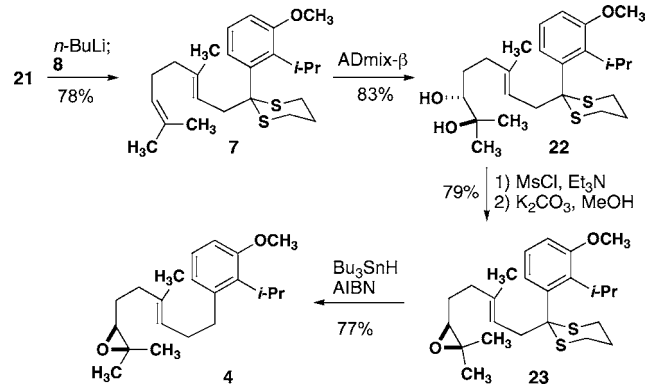
We began route B by preparing the dithiane precursor of **9**. Although a sequence analogous to Scheme 2 would be suitable, an alternative route from the benzonitrile would avoid excessive changes in oxidation state. Direct displacement of methoxy groups ortho to aryl cyano groups is possible.[28] Myers noted that this reaction is often lower yielding than the analogous reactions of oxazolines.[29] That said, the introduction of an additional methoxy group seems to increase the yield of displacement. Nitrile **19** was treated with *i*-PrMgCl to produce the displacement product **20** in high yield (eq 2). Reduction and dithioacetal formation proceeded smoothly to produce **21** in three steps from commercially available benzonitrile **19**.



(2)

The cyclization precursor was prepared by regio- and enantioselective dihydroxylation (Scheme 3). Lithiation of **21** under standard conditions followed by alkylation with geranyl bromide produced alkene **7** in high yield. Although previous attempts at regioselective dihydroxylation of related substrates have only been modestly successful, treatment of **22** with ADmix-β produced diol **22** as a single regioisomer in 90−95% ee.[30] Desulfurization[31] and dehydration provided epoxide **4** in seven steps from commercially available materials.

Scheme 3. Synthesis of Cyclization Precursor **4**



Benzyl geraniol derivative **4** was converted into diterpenes **1**−**3** by a short sequence. Cyclization of **4** proceeded

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(27) In one run, **18** was isolated as an mixture with bromide **16a** and **17** (from protonation of **6a**) in 43% yield.

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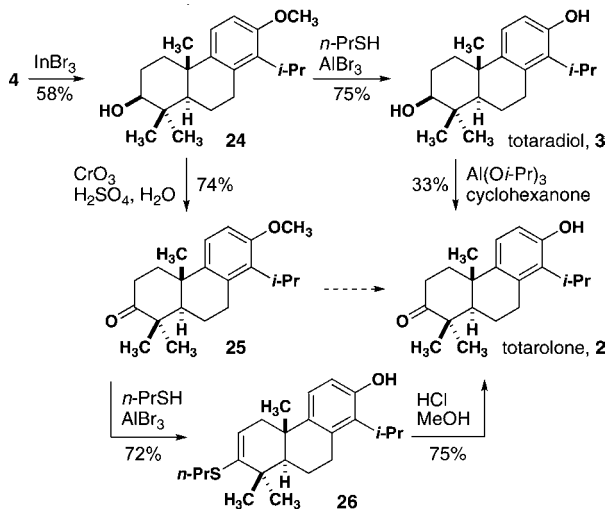
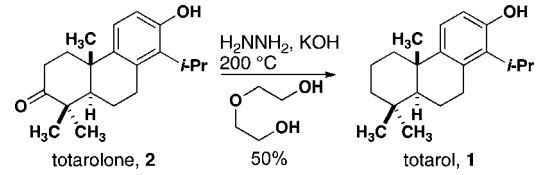
Scheme 4. Synthesis of Totaradiol and Totarolone



smoothly in the presence of indium tribromide (2.0 equiv) to give tricyclic alcohol **24** in 58% yield as a single detectable diastereomer (Scheme 4).[14] This result is in marked contrast to the acid-mediated cyclization of an alkene substrate in a recent syntheses of totarol in which 25% of the cis diastereomer is formed and the overall yield is 50%.[9]

Two combinations of oxidation and demethylation of **24** were investigated. Deprotection of **24** produced totaradiol in good yield.[32] Subsequent Oppenauer oxidation to totarolone (**2**) was achieved in a maximum yield of 33%.[33] Given the limitations placed on oxidation conditions with the free phenol present, the order of operation was reversed. Jones oxidation of **24** to **25** proceeded in 74% yield.[34] Demethylation under conditions used for **24** were complicated by the formation of vinyl sulfide **26**, which was isolated in 72% yield. This material could be hydrolyzed to **2** in high yield.[35] Other attempts at direct conversion of **25** to **2** were unsuccessful, seemingly due to side reaction of the ketone. Synthetic totaradiol (**3**) and totarolone (**2**) each exhibited ^{13}C and ^{1}H NMR spectra and optical rotations in good agreement with reported values.

Scheme 5. Synthesis of Totarol from Totarolone



Final conversion of totarolone to totarol was achieved using the Wolff−Kischner reduction in modest yield (Scheme 5).[36] The synthetic sample of (+)-totarol (**1**) produced by this exhibited identical chromatographic properties (TLC, GC−MS) when compared to an authentic sample, and the ^{1}H NMR spectrum was also identical. The longest linear sequence of reactions was 11 steps from commercially available nitrile **19**.

In summary, we have reported a concise, enantioselective route to (+)-totarol and the first syntheses of the related diterpenes (+)-totaradiol and (+)-totarolone. This route sets the stage for a detailed study of the mechanism by which FtsZ is inactivated by this natural product.

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Supporting Information Available: Experimental procedures for the preparation of all new compounds. This information is available free of charge via the Internet at http://pubs.acs.org.

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