Catalytic Antibodies in Organic Synthesis. Asymmetric Synthesis of (−)-α-Multistriatin

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Since its inception, not too long ago, the science of catalytic antibodies has undergone a remarkable maturation process.[1] From initial "proof of concept" and demonstration of fundamental, enzyme-like characteristics, antibodies have been shown to catalyze a broad scope of organic transformations, including difficult and unfavorable chemical reactions.[2] The relevance of the field to synthetic organic chemistry has been demonstrated recently by the ability to run these reactions with gram-scale quantities.[3] Here we go one step further and show for the first time that catalytic antibodies can be effectively used in natural product synthesis.

(−)-α-Multistriatin, **1**, is an essential component of the aggregation pheromone of the European elm bark beetle, *Scolytus multistriatus* (Marsham), which is the principal vector of Dutch elm disease.[4] The severe devastation of the elm population in the northeastern United States has resulted in extensive studies of the synthesis[5] and field utilization[6,7] of **1**. In order to achieve the required absolute configuration in all four asymmetric centers (1*S*,2*R*,4*S*,5*R*), most of the previous syntheses of **1** employ enantiomerically pure natural products as starting materials.[5] We focused on the opportunity to achieve the required chirality via asymmetric synthesis of ketone **2** using antibody catalysis. Monoclonal antibody 14D9 has already been proven an effective catalyst for hydrolysis of a variety of substrates that are structurally related to **2**, including a cyclic acetal,[8] ketals,[9] epoxides,[10] and enol ethers.[11,12] One could therefore expect that ketone **2** would be readily available by antibody-catalyzed enantioselective protonolysis of the corresponding enol ethers **3a** and **3b**.[13] This antibody-catalyzed step could be the only source of asymmetry in the molecule, provided that the following chemical steps proceed with control over the relative stereochemistry.

In acidic, aqueous media, both isomers are hydrolyzed to racemic ketone **2** at a comparable rate. Antibody 14D9 catalyzes this reaction under mildly acidic conditions (Scheme 1).[14] Interestingly, catalysis with the *Z* enol ether **3a** is much more effective (k_{cat}/k_{un} = 65 000) than with the *E* isomer, **3b** (k_{cat}/k_{un} = 5000).[15] This enzyme-like catalysis is evident from the observed Michaelis–Menten kinetics (**3a**, K_m = 230 μM, k_{cat} = 0.36 min^{-1} at pH 6.5; **3b**, K_m = 310 μM, k_{cat} = 0.044 min^{-1} at pH 6.0) and from the fact that catalysis is totally inhibited in the presence of stoichiometric quantities (with respect to 14D9) of the methylpiperidinium hapten against which this antibody has been elicited.[8] Both **3a** and **3b** are hydrolyzed by 14D9 to produce ketone **2** with the same absolute configuration (*S*), probably due to the structural similarity between both substrates.[16] The high rate acceleration in the case of **3a** allows the reaction to be driven to near completion to produce **2** in greater than 99% ee.

Because this specific reaction is not catalyzed by any known enzyme or other protein or any known biological component, there is no need to use a purified antibody.[17] Efficient catalysis is thus achieved with a partially purified 14D9 which was precipitated from the ascites fluid by saturated ammonium sulfate (SAS). We carried out the antibody-catalyzed reaction on a preparative scale using very simple organic-laboratory equipment according to a procedure that has been developed by Whitesides[18] for enzyme-catalyzed reactions and by Reymond[3a]

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(13) The two isomeric enol ethers **3a** (*Z*) and **3b** (*E*) were prepared in a simple, four-step sequence. Alkylation of 3-pentanone with methyl 4-(bromomethyl)benzoate afforded 1-[4-(methoxycarbonyl)phenyl]-2-methylpentan-3-one, which was then treated with trimethyl orthoformate and catalytic amounts of *p*-toluenesulfonic acid in methanol to give the corresponding ketal. The latter was heated in the presence of catalytic amounts of benzoic acid to produce, upon distillation (160 °C, 1 mm), four isomeric enol ethers. Heating with ethanolamine afforded a mixture of four isomers: *Z* and *E* tetrasubstituted enol ethers, **3a**, **3b**, along with the *Z* and *E* trisubstituted ones, **3c**, **3d** (in a ratio of 25:20:45:10, respectively), all of which were separated by chromatography.

(14) Assay conditions: antibody (0.5 μM), substrate (50–500 μM), 1,3-bis[tris(hydroxymethyl)methylamino]propane buffered saline (50 mM, pH 6.5, 50 mM NaCl). All reactions were carried out at 24 °C and monitored at 254 nm by RP-HPLC (Hitachi L-6200A equipped with a Spherisorb column (25 cm × 4.6 mm, C18, 5μ) using 42:58 acetonitrile–water at 0.8 mL/min.

(15) In one experiment, a mixture of all four compounds, **3a–d**, was subjected to antibody-catalyzed hydrolysis, resulting in complete, selective consumption of **3a** with essentially no change in the concentration of **3b** and a small decrease of **3c,d**. However, since enol ethers **3c,d** are hydrolyzed approximately 10 times faster than **3a,b** under the same buffer conditions, in order to achieve high ee and avoid contamination of the product by racemic **2** it was necessary to remove **3b–d** from the mixture before the antibody-catalyzed step.

(16) Determination of absolute configuration and enantiomeric purity of ketone **2** was carried out by reduction with $NaBH_4$ to the corresponding diastereomeric mixture of alcohols, followed by HPLC analysis (Perkin-Elmer 410 equipped with a UV detector, 254 nm) using a Chiracell OD-H column, Daicel Chemical Industries) with 5:95 2-propanol–hexane at a flow rate of 1 mL/min. Absolute configuration was determined by comparison with an authentic sample which was synthesized from enantiomerically pure (1*S*,2*R*)-(+)-norephedrine using the Evans methodology, which is known to proceed with high diastereoselectivity and predictable absolute configuration: Evans, D. A.; Ennis, M. D.; Mathre, D. J. *J. Am. Chem. Soc.* **1982**, *104*, 1737.

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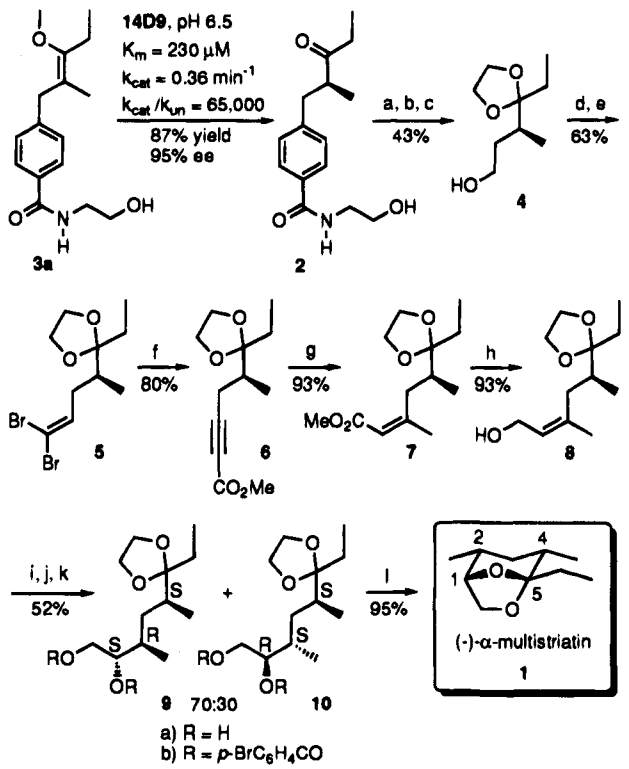
Scheme 1. Stereoselective Synthesis of α-Multistriatin[a]



[a] Key: (a) $RuCl_3$, $NaIO_4$, CCl_4, CH_3CN, H_2O. (b) Ethylene glycol, PPTS, benzene. (c) $LiAlH_4$, ether. (d) PCC, CH_2Cl_2. (e) CBr_4, PPh_3 CH_2Cl_2. (f) *n*-BuLi, $NCCO_2Me$, THF. (g) MeCu, TMEDA, ether. (h) DIBAL-H, THF, toluene. (i) BH_3-SMe_2, then H_2O_2/NaOH. (j) *p*-BrC_6H_4COCl, Et_3N, DMAP, CH_2Cl_2, column chromatography. (k) $LiAlH_4$, ether. (l) PPTS, CH_2Cl_2.

for this reaction with 14D9. In each catalytic cycle a solution of the enol ether **3a** (180 mg, 0.65 mmol) in DMF (1 mL) was added to a solution of a crude SAS fraction of antibody 14D9 (22.5 mL containing 225 mg of protein, 0.0015 mmol) in bis-tris buffer (50 mM, pH 6.5) and the mixture was stirred at 24 °C. Progress of the reaction was monitored by HPLC. It could also be seen visually, as the starting mixture was turbid-white (due to relatively lower solubility of the starting material relative to that of the product) and became clear as the reaction reached completion. Recovery of the catalyst after each cycle was achieved using cellulose dialysis bags (allowing diffusion of molecules smaller than 12−14 kDa).[3a] The reaction was interrupted after 60 h at 80% conversion by transfer of the mixture into a dialysis bag, and the mixture was dialyzed into 500 mL of the same buffer over 16 h. The antibody solution was taken to the next catalytic cycle with a fresh solution of **3a**. The buffer solution was saturated with sodium chloride and extracted with dichloromethane. As reported in the previous large-scale use of 14D9, only minor deterioration of catalytic activity could be observed over the first five cycles of the reaction.[3a,19]

Ketone **2** was converted to **1** in a sequence of 12 chemical reactions (Scheme 1). The aromatic portion of **2** was exhaustively degraded with $RuCl_3$ and sodium periodate.[20] The resultant keto acid was then converted to ketal alcohol **4** by reaction with ethylene glycol and catalytic amounts of pyridinium *p*-toluenesulfonate (PPTS) in benzene followed by reduction with $LiAlH_4$ in ether. An NMR spectrum of the Mosher ester of **4**[21] indicated 92% ee, reflecting only minor loss of enantiomeric purity throughout these three steps. Alcohol **4** was oxidized with pyridinium chlorochromate in dichloromethane to the corresponding aldehyde. The latter was treated with a solution of triphenylphosphine and carbon tetrabromide in dichloromethane to give the dibromoalkene, **5**.[22] Treatment of **5** with *n*-butyllithium and methyl cyanoformate produced the substituted methyl propargylate **6**. Reaction of the latter with "MeCu" (prepared from methyllithium and CuI in THF and tetramethylethylenediamine) afforded geometrically pure (*Z*) α,β-unsaturated ester **7**.[23] Reduction of this ester with diisobutylaluminum hydride in toluene−THF afforded the corresponding allylic alcohol, **8**, with retention of the (*Z*) geometry. Treatment of **8** with a solution of borane−dimethyl sulfide complex in THF followed by oxidation with basic (NaOH, 3 N) hydrogen peroxide produced a 70:30 mixture of two diastereomeric products: **9** (having the required 4*S*,6*R*,7*S* configuration) and its 4*S*,6*S*,7*R* diastereomer, **10**, respectively. Esterification with 4-bromobenzoyl chloride produced the corresponding bis-bromobenzoate derivatives, which were easily separated by column chromatography. The purified diol **9** was then recovered via reductive cleavage with $LiAlH_4$. Finally, treatment of **9** with catalytic amounts of PPTS in dichloromethane followed by Kugelrohr distillation at 110 °C afforded (−)-α-multistriatin, **1**, in the form of a colorless oil.[24]

In conclusion, the relevance of antibody catalysis to synthetic organic chemistry has been demonstrated here by an efficient total synthesis of an important, biologically active natural product. All four asymmetric centers originate from a key, antibody-catalyzed protonolysis of an enol ether. That specific step is a unique example of a chemical transformation which is difficult to achieve either by an available synthetic methodology or via catalysis with a known enzyme.[25]

The synthesis of natural products remains the ultimate testing ground for new concepts in organic chemistry. This has been the case, for example, with the advent of organometallic chemistry throughout the past four decades. Thus, the key point in the present study is not simply that one can make α-multistriatin, or even that this is now the best way to synthesize the compound, but rather that catalytic antibodies perform competitively in the important testing ground of natural product synthesis.

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Supplementary Material Available: NMR and analytical data for **2**, **3a**, and **4**−**9** (2 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(24) The synthetic pheromone **1** has been checked in field experiments and found as active as the naturally occurring compound in attracting the European-elm bark-beetles into traps loaded with a mixture of **1** with (−)-α-cubebene (a host-produced component) and (−)-4-methylheptan-3-ol.

(25) The closest literature precedents involve enantioselective protonation of various metal enolates. See, for example: (a) Fehr, C.; Galindo, J. *J. Am. Chem. Soc.* **1988**, *110*, 6909. (b) Matsumoto, K.; Ohta, H. *Tetrahedron Lett.* **1991**, *32*, 4729. (c) Yasukata, T.; Koga, K. *Tetrahedron: Asymmetry* **1993**, *4*, 35. (d) Vedejs, E.; Lee, N.; Sakata, S. T. *J. Am. Chem. Soc.* **1994**, *116*, 2175 and references cited therein.