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Sets of Aldolase Antibodies with Antipodal Reactivities. Formal Synthesis of Epothilone E by Large-Scale Antibody-Catalyzed Resolution of Thiazole Aldol

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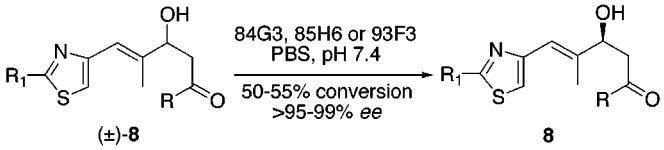
ABSTRACT



Three monoclonal aldolase antibodies, generated against a β-diketone hapten by reactive immunization, catalyzed rapid and highly enantioselective retro-aldol reactions of *ent*-8a–k, providing optically pure 8a–k by kinetic resolution. Compounds (±)-8a, (±)-8g, and (±)-8k have been resolved in multigram quantities using 0.003, 0.005, and 0.0004 mol % antibody catalysts, respectively. Resolved compounds 8a–k are useful synthons for the construction of epothilones A–E (2–6) and their analogues. Here, a formal synthesis of epothilone E, 6 has been achieved starting from compound 8g.

Reactive immunization provides a unique opportunity to generate catalytic antibodies[1] that are efficient yet broad in scope. Recently, two aldolase antibodies 33F12 and 38C2 were generated against a β-diketone hapten, 6-(4-glutaramidophenyl)hexane-2,4-dione, using reactive immunization.[2] These antibody catalysts were found to be useful synthetic catalysts in that they catalyze a wide range of aldol- and retro-aldol reactions, typically with a very high degree of enantioselectivity.[3] Recently, nine new catalytic antibodies were generated against hapten **1**. Like 33F12 and 38C2, these new antibodies also catalyze aldol reactions with a wide variety of aldehydes and ketones via an enamine mechanism.[4] However, the aldol products prepared with these new antibodies are antipodal as compared to those obtained by catalysis with the antibodies 33F12 and 38C2. Two of the new catalysts, 84G3 and 93F3, operate with the highest

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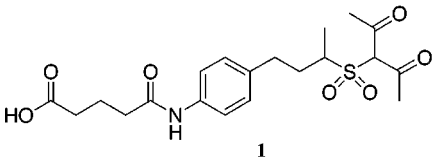

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Figure 1. Structure of hapten **1** for monoclonal aldolase antibodies 84G3, 85H6, and 93F3.

catalytic proficiencies yet observed with antibodies, $(k_{cat}/K_m)/k_{un} > 10^{13}$ M^{-1}. These new catalysts present significant advantages to our continued efforts on the application of antibody catalysis to the synthesis of epothilones and their analogues.

Epothilones A−E (**2**−**6**, Figure 2) are sixteen-membered macrolides isolated from myxobacteria (*Sorangium cellulosum* strain 90).[5,6] Several total syntheses of epothilones A−E, as well as their analogues, have been achieved[7−10] and their biological properties have been recorded.[6,11] Recently, we reported a synthesis of the naturally occurring epothilones A−D, **2**−**5**, starting from aldol products **7** and **8a**.[12] Compound **7** was obtained in 96% ee by resolution of (±)-**7** using antibody 38C2. Compound **8a** was prepared by the antibody 38C2-catalyzed aldol reaction of acetone with the aldehyde **9a**; however, it had a modest enantiomeric purity (75% ee). With the newly discovered monoclonal catalytic aldolase antibodies with antipodal reactivities, we anticipated that compound (±)-**8a** could be resolved to afford the compound **8a**. Here, we report the resolution of compound (±)-**8a** and its analogues, (±)-**8b**−**k**, using antibodies 84G3, 85H6, or 93F3. Compounds (±)-**8a**, (±)-**8g**, and (±)-**8k** have been resolved in multigram quantities using 0.003, 0.005, and 0.0004 mol %, respectively, of antibody 84G3. We also present a formal synthesis of epothilone E, **6**, starting from **8g**.

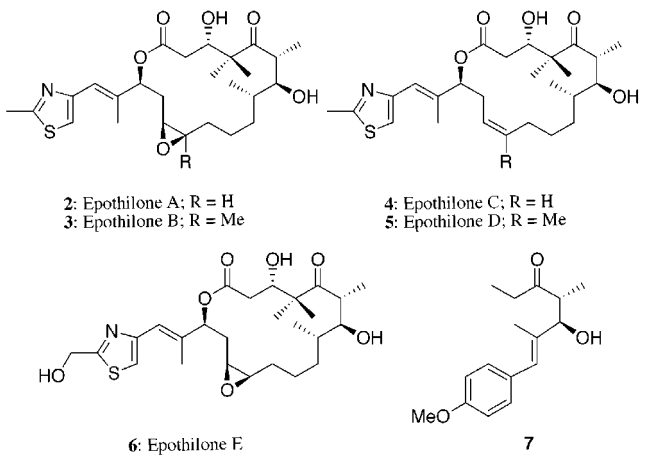

Figure 2. Structures of epothilones A−E (**2**−**6**) and the antibody 38C2 resolved strating material **7**.

Initially, we studied catalysis of the retro-aldol reaction of compound (±)-**8a** with all nine catalytic aldolase antibodies raised against the hapten **1**. We found that three of these antibodies, 84G3, 85H6, and 93F3, efficiently catalyzed the retro-aldol reaction of (±)-**8a** to aldehyde **9a** and acetone. The kinetic resolution of compound (±)-**8a** by antibody catalyzed retro-aldol reaction was studied using chiral reverse-phase HPLC. We found that when the reactions reached ∼50% conversion, the remaining aldols could be isolated in essentially enantiomerically pure form.[13] The unreacted aldol compound was identified as **8a** by comparison with the synthetic sample of **8a**[14] as well as one derived from the resolution of (±)-**8a** with the antibody 38C2.[12a]

On the basis of these results, several analogues of (±)-**8a** were prepared and the relative rates of their reaction[15] with

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(9) For the synthesis of epothilone E, see: Nicolaou, K. C.; He, Y.; Roschangar, F.; King, N. P.; Vourloumis, D. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 84.

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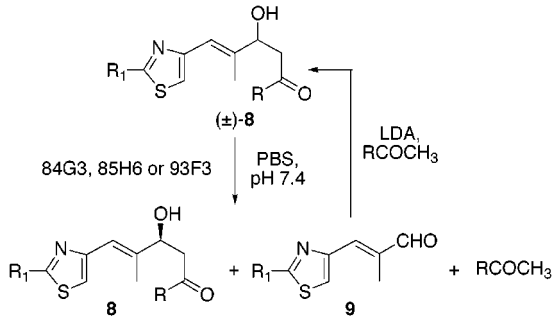
(11) For biological studies of epothilones and their analogues, see: Chou, T.-C.; Zhang, X. G.; Balog, A.; Su, D. S.; Meng, D. F.; Savin, K.; Bertino, J. R.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 9642 and references therein.

(12) (a) For the total syntheses of **2** and **4**, see: Sinha S. C.; Barbas, C. F., III.; Lerner, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 14603. (b) Total or formal syntheses of **3** and **5**, unpublished results.

(13) A set of reaction mixtures (100 μL each) containing compound (±)-**8a** (0.01 M solution in CH_3CN, 10 μL) and aldolase antibodies (6 μM solution in PBS, 90 μL) was kept at room temperature for 12 h and then analyzed by HPLC (ODR column, see Supporting Information). Reaction mixtures containing antibodies 84G3, 85H6, and 93F3 were found to possess only one enantiomer of (±)-**8a**.

(14) By using the methodology of Patterson (Patterson, I.; Goodman, J. M.; Lister, M. A.; Schumann, R. C.; McClure, C. K.; Norcross, R. D. *Tetrahedron* **1990**, *46*, 4663), a sample of **8a** for comparison was prepared by the reaction of prop-1-en-2-ol diisocamphenylborinate (prepared from (+)-$(Ipc)_2$BOTf and acetone) with the aldehyde **9a**. (For HPLC conditions, see the Supporting Information.)

Table 1. Resolution of Compounds (±)-**8a**−**k** by Antibody 84G3, 85H6, and 93F3 Catalyzed Retro-Aldol Reactions[a]



compounds	84G3 ee (%)	85H6 ee (%)	93F3 ee (%)
8a: R = R_1 = Me	98	94	98
8b: R = Et, R_1 = Me	99[b]	99	99
8c: R = Pr, R_1 = Me	99	99	99
8d: R = Bu, R_1 = Me	99	99	99
8e: R = Pen, R_1 = Me	97	97[f]	97[d]
8f: R = But-1-ene, R_1 = Me	98[c]	98	99
8g: R = Me, R_1 = CH_2OH	99	99	99
8h: R = CH_2F, R_1 = Me	96[e]	99[f]	98[c]
8i: R = Me, R_1 = OMe	95[b,g]	95[c,g]	95[c,g]
8j: R = Me, R_1 = SMe	99[d]	99	99[e]
8k: R = Et, R_1 = SMe	99	99	99[c]

[a] Enantiomeric excesses were recorded at 50% conversions unless otherwise mentioned. (For HPLC conditions, see the Supporting Information.) [b] 51% conversion. [c] 52% conversion. [d] 53% conversion. [e] 54% conversion. [f] 55% conversion. [g] Peaks of the two enantiomers on HPLC trace were not baseline separable.

these antibodies and the enantiomeric purity of the remaining unreacted aldols were determined. We found that compounds **8b**−**k** could also be obtained with very high enantiomeric purity at 50% conversion (unless otherwise mentioned) by kinetic resolution of (±)-**8b**−**k** with the antibodies 84G3, 85H6, or 93F3 (Table 1). All three catalysts gave similar results with antibodies 84G3 and 93F3, demonstrating a rate enhancement slightly greater than that observed with 85H6. Increasing the chain length from methyl in (±)-**8a** to ethyl and propyl group in (±)-**8b** and (±)-**8c**, respectively, also increased the relative rate of reaction. Further increases in the chain length to butyl and pentyl groups as in compounds (±)-**8d** and (±)-**8f** decreased the rate. Substrates with a C_5 chain length were very slow and the catalysts did not tolerate further extension at this position. Nevertheless, the resolved products **8a**−**k** were obtained with very high enantiomeric purity at 50−54% conversion. Interestingly, compound (±)-**8k** was the fastest reacting substrate with all of the antibodies and could be resolved with >99% enantioselectivity *in less than 10 min employing 0.5 mol % antibody 93F3*. When we reduced the catalyst concentration to 0.01 mol %, the reaction was complete after overnight incubation.

(15) Compounds (±)-**8a**−**k** (10^{-2} M solution in CH_3CN, 10 μL) were incubated with the antibody 84G3, 85H6, or 93G3 (90 μL of 6 μM antibody solution in PBS buffer, pH 7.4) and progress of the reaction was followed by HPLC equipped with a chiral reverse phase ODR column (Daicel Chemical Industries). (For HPLC conditions, see the Supporting Information.)

Next we studied the feasibility of resolving compound (±)-**8a** and its analogues with these antibodies on a synthetically useful scale. Here we used antibody 84G3 to perform the resolution of compounds (±)-**8a**, (±)-**8g**, and (±)-**8k**. Compound (±)-**8a** (16.8 g, 74.7 mmol) was incubated with 0.003 mol % of antibody 84G3 (340 mg, 0.00227 mmol) in PBS buffer (pH 7.4) at 37 °C. Progress of the reaction was followed by disappearance of the peak corresponding to *ent*-**8a** in the HPLC trace. In this way, the racemic mixture was resolved in 7 days, affording **8a** (>97% enantiomeric purity) in 45% isolated yield (see Supporting Information). Aldehyde **9a** was recovered in 42% isolated yield and converted back to the racemic aldol for subsequent recycling.[16] Thus, even though the process is a kinetic resolution the overall yield could be enhanced because the products could be recycled.

Similarly, compound (±)-**8g** (1.45 g, 6.02 mmol) was resolved using 0.005 mol % of antibody 84G3 (45 mg, 0.0003 mmol) with an overall turnover number of more than 10 000 to afford enantiomerically pure **8g** (0.7 g, 48%) and aldehyde **9g** (516 mg, 47%) respectively (see Supporting Information). The fastest substrate in this series, (±)-**8k** (8.4 g, 31 mmol), was resolved with as little as 0.0004 mol % of the antibody 84G3 (20 mg, 0.000 133 mmol) in a week to afford **8k** in 49% and the corresponding aldehyde **9k** in 45% isolated yields (see Supporting Information).

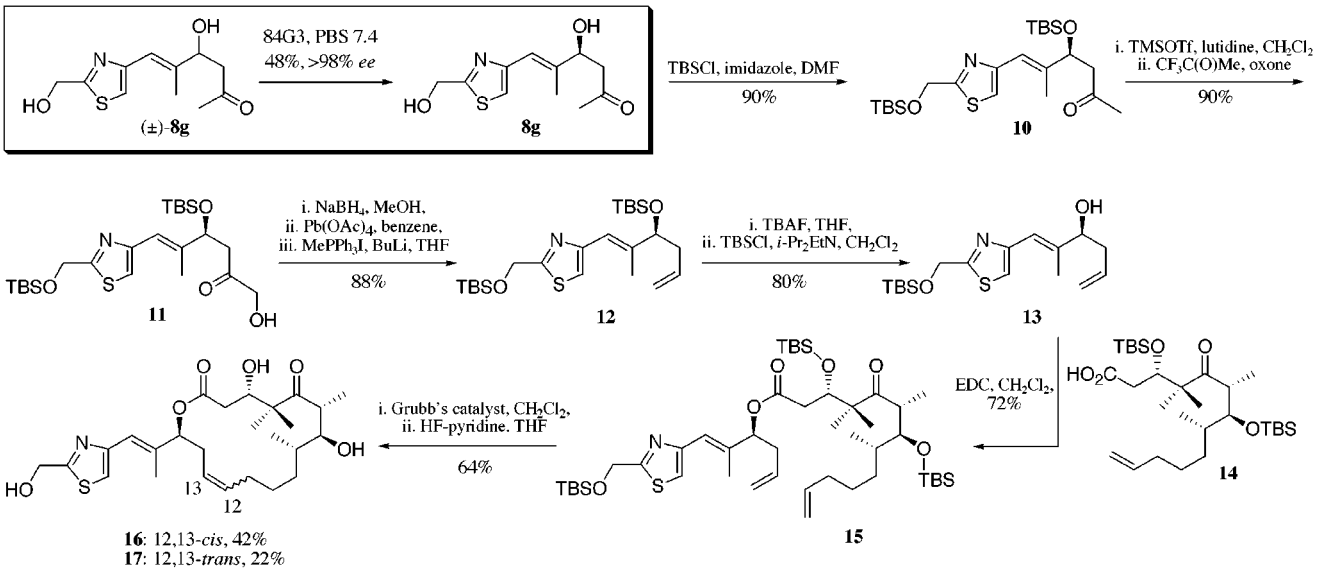
Synthesis of Epothilone E, 6. Compounds **8a**−**k** are useful synthons for the construction of the naturally occurring epothilones **2**−**6** as well as their analogues modified in the thiazole ring. Compounds with a methyl ketone function, such as in **8a**, were converted in a sequence of five simple and high-yielding steps to the key alkene or iodoalkene precursors used in the syntheses of epothilones A−D, **2**−**5**, by metathesis and/or macrolactonization approaches.[12] Here, we used a similar strategy and synthesized epothilone E, **6**, starting from the compound **8g** by the metathesis approach (Scheme 1).

Compound **8g**, isolated from the antibody reaction was first protected as the bis-TBS ether to give **10**. The methyl ketone functionality of compound **10** was converted to its TMS-enol ether with TMSOTf and lutidine at −78 °C, and then reacted with CF_3COCH_3/oxone to yield the primary hydroxy ketone **11**. Compound **11** was reduced to the corresponding vicinal diol, which was then cleaved with $Pb(OAc)_4$ to provide the corresponding aldehyde. Wittig reaction of the produced aldehyde with methyltriphenylphosphorane afforded the olefin **12**. Both the TBS groups of compound **12** were removed by reaction with TBAF and subsequently the primary alcohol was selectively protected as the TBS ether to yield the allylic alcohol **13**.[17] Compound **13** was esterified with acid **14**,[12] providing ester **15**. Metathesis of compound **15** with Grubb's catalyst yielded the cyclized product as a mixture of *cis* and *trans* olefins in a ratio of 3:2. Subsequent deprotection yielded compound **16**

(16) Antibodies recovered after one reaction cycle retained their catalytic activity.

(17) **Physical data of compound 13:** $[\alpha]_D$= −13.42° (c = 0.37, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.01 (s, 1H), 6.52 (s, 1H), 5.80 (m, 1H), 5.12 (d, J = 17.4 Hz, 1H), 5.09 (d, J = 11.3 Hz, 1H), 4.93 (s, 2H), 4.18 (t, J = 6.7 Hz, 1H), 2.38 (m, 3H), 2.02 (s, 3H), 0.93 (s, 9H), 0.11 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.1, 153.0, 141.4, 134.6, 119.0, 117.8, 115.7, 76.4, 63.2, 40.0, 25.7, 18.2, 14.4, −5.5; MS 340 (MH^+), 362 (MNa^+).

Scheme 1



and its *trans* isomer, **17**. The spectral data (^{1}H NMR, ^{13}C NMR, MS, optical rotation) of **16** were identical to the published data.[9,18] Conversion of **16** to epothilone E, **6**, has been reported.[9]

In conclusion, we have shown that the antibodies 84G3, 85H6, or 93F3 are very powerful catalysts for the resolution of the thiazole aldols (±)-**8a**−**k** on a preparative scale. This antibody-based synthon approach provides an attractive synthetic route to natural epothilones and their 13-alkyl derivatives as exemplified here with synthesis of desoxyepothilone E, **16**, and its *trans* isomer. Large-scale resolutions of thiazole aldols and synthesis of 13-alkyl analogues of **2**−**6** are in progress.

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Supporting Information Available: HPLC data for compounds (±)-**8a**−**k**, physical and/or spectral data for compounds **8a**−**k** and **15**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) **Physical data of compound 16:** $[\alpha]_D$ −43.7° (c = 0.3, $CHCl_3$), lit.[9] −44.2° (c = 0.6, $CHCl_3$).