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Use of a Robust Dehydrogenase from an Archael Hyperthermophile in Asymmetric Catalysis−Dynamic Reductive Kinetic Resolution Entry into (*S*)-Profens

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Hyperthermophilic archaea are of great interest in evolutionary microbiology, owing to their ability to withstand high temperatures and often extremes of pressure, pH, and salinity. Enzymes from these organisms[1] may offer particular opportunities for asymmetric synthesis, complementary to approaches with mesophilic enzymes,[2] or those involving enzyme[3] and pathway[4] reengineering. However, perhaps due to a bias that hyperthermophilic enzymes have "narrow substrate specificities,"[5] archaeal extremophiles remain a largely untapped resource in asymmetric synthesis.[6]

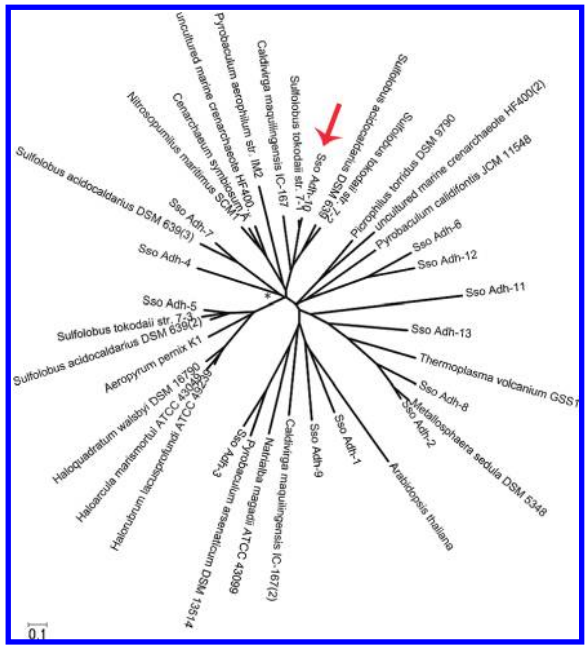
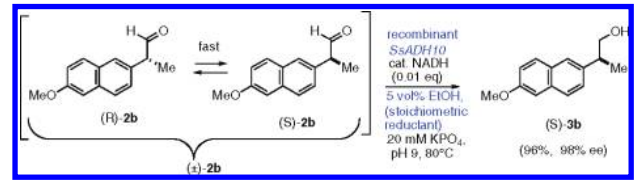
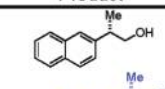
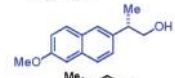
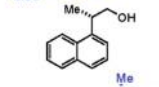
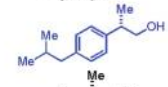
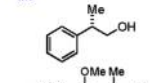
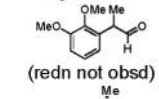
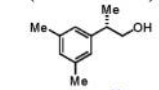
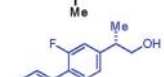
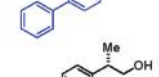
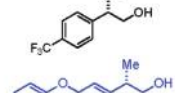
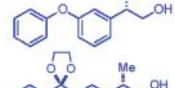
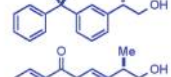


Figure 1. Protein phylogeny of the SsADH proteins. A consensus neighbor joining distance tree is shown of all SsADHs and homologues of highest sequence identity in related taxa. Distances are indicated by the bar (lower left corner) and represent 10 substitutions per 100 residues. Percent occurrence among 100 trees was greater than 50% for all nodes except those indicated with an asterisk.

Herein, we disclose a remarkably general Dynamic Reductive Kinetic Resolution (DYRKR) entry into (*S*)-profens, including several important NSAIDs. The enzyme employed is alcohol dehydrogenase (ADH)-10, one of 13 annotated ADHs in the hyperthermophile *Sulfolobus solfataricus*. Protein phylogenetic analysis of this paralogous family indicates SsADH-10 is most closely related to homologues in distant taxa (Figure 1). The highest identity between SsADH-10 and any other SsADHs is only 34%, suggesting that the SsADH family was established prior to the emergence of other archaeal lineages. Though not described as such, the SsADH-10 appears to be the only SsADH isozyme for which structural information is available in the pdb.[7]

Table 1. SsADH10-Mediated DYRKR Entry into Profenols



Cpd No.	DYRKR Product[a]	t(h)	Yield (%)[b]	ee (%)[c]
3a		18	57%	94%
3b		18	96%	98%
3c		18	90%	80%
3d		18	92%	99%
3e		18	74%	98%
2f		18	recvd SM	NA
3g		18	99%	90%
3h		18	77%	97%
3i		24	55%	98%
3j		12	85%	95%
3k		18	95%	61%
3l		18	85%	95%

[a] DYRKR performed on a 1 mmol scale (1 mol % NADH; 5 vol% EtOH). [b] Isolated yields. [c] ee's by chiral LC or GC. Blue - Profen drug precursor.

The requisite 2-arylpropionaldehydes were readily assembled via Pd(0)-catalyzed arylation of *tert*-butyl propionate under

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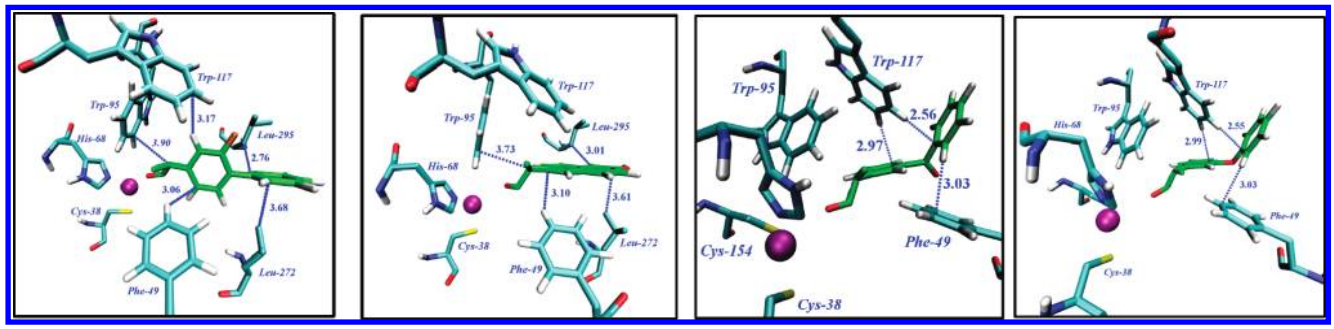

Figure 2. Structures of thermally relaxed (GROMACS 4.07) SsADH-10 (from 1R37) to which has been docked (Autodock Vina, left to right): (i) (*S*)-flurbiprofenal, (ii) (*S*)-naproxenal, (iii) (*S*)-ketoprofenal, and (iv) (*S*)-fenoprofenal (Zn ligation sphere: H68, C38, C154, and substrate carbonyl).

Buchwald−Hartwig-type[8] conditions, followed by reduction to the aldehyde (LDBBA[9] or LAH/DMP oxid; see Supporting Information (SI)). Optimal DYRKR conditions (Table 1, 80 °C, pH 9) led to efficient throughput of *rac*-aldehyde to the (*S*)-2-arylpropionaldehyde, particularly with *m*- and *p*-substitution. Notably, (*S*)-profenols corresponding to the NSAIDs naproxen (**3b**, scaled to 1 g @ 98% yield and 95% ee), ibuprofen (**3d**, IP), flurbiprofen (**3h**, FlP), fenoprofen (**3j**, FP), and ketoprofen (**3l**, KP) were obtained in excellent yields (up to 96%) and high enantioselectivity (up to 99%).

Naproxen is FDA-approved as the active (*S*)-antipode. While most individuals can invert (*R*)-ibuprofen to the (*S*)-antipode, the pathway is inefficient for KP[10a] and FlP.[10b] Moreover, the recent observation that the Profen-CoA thioester intermediates in this pathway inhibit G6PDH[10c] argues for "chiral switching" to single (*S*)-antipodes.[10d] Entries into (*S*)-profens[11,12] include asymmetric hydrogenation (NP 98% ee, IP 97% ee)[11g] and hydroformylation (IP, 92% ee).[11f] DKR processes include enantioselective crystallization (NP >99% ee),[11b] DYRKR with H_2 as reductant under Ru(II) catalysis (IP 92% ee),[11d] and lipase/Ru(II)-mediated DKR of allylic acetates, followed by Cu-mediated Grignard arylation (FlP 97% ee;[11c] Knochel arylation:[11e] IP 97% ee). The hydrovinylation/oxidation approach is impressive (IP, FP, FlP, NP >96% ee),[11a] but access to KP requires late stage arylation. Thus, the broad side chain tolerance of SsADH-10 makes the method presented here among the most generally (*S*)-selective.

To explore how these extended hydrophobic substrates bind to SsADH-10, docking was carried out (Figure 2) for the (*S*)-antipodes of flurbiprofenal, naproxenal, ketoprofenal, and fenoprofenal. A detailed discussion of the approach and results is provided in the SI. Briefly, W95 is seen as enforcing (*S*)-selectivity, with ligands clustering into two distinct distal ring binding modes. "Channel-gating" L272 and L295 appear to form a hydrophobic pocket for naproxenal and flurbiprofenal. For the more flexible ketoprofenal and fenoprofenal, edge-to-face π-π-interactions with W117 and F49 are proposed.

From a practical viewpoint, we have also found that SsADH-10 may be engaged in a "thermal recycling" approach that may be generalizable to other hyperthermophilic enzymes. Namely, while 30 vol% cosolvent is often needed to dissolve hydrophobic DH substrates,[13] we use a higher *T* (80 °C) @ just 5% EtOH (solvent and biorenewable reductant). Importantly, upon completion of the reaction, cooling to rt allows the product to precipitate and be collected by filtration (see TOC graphic and SI). Reclaimed SsADH may be recycled (5 cycles @ 94−96% ee). Given the growing interest in thermophilic enzymes in synthesis,[1,14] and in engineering thermostability into mesophilic enzymes,[15] this "thermal switching" approach is likely to find broad application, well beyond the domain of geothermal dehydrogenases.

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Supporting Information Available: Details of SsADH-10 expression, synthesis, spectra, DYRKR, and modeling. This material is available free of charge via the Internet at http://pubs.acs.org.

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