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Use of a Robust Dehydrogenase from an Archaeal 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 organisms¹ may offer particular opportunities for asymmetric synthesis, complementary to approaches with mesophilic enzymes,² or those involving enzyme³ and pathway⁴ 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

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 (Fig. 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

The requisite, 2-arylpropionaldehydes were readily assembled via Pd(0)-catalyzed arylation of *t*-butyl propionate under Buchwald-Hartwig-type⁸ conditions, followed by reduction to the aldehyde (LDBBA⁹ or LAH/DMP oxid-*see* 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 gram @ 98% yield and 95% ee), ibuprofen (**3d**, IP), flurbiprofen (**3h**, FIP), 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 FIP.^{10b} Moreover, the recent observation that the profen-CoA thioester intermediates inhibit G6PDH,^{10c} argues for “chiral switching” to single (*S*)-antipodes.^{10d} Entries into (*S*)-profens^{11,12} include asymmetric

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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>.

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 H₂ 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 (FIP 97% ee;^{11c} Knochel arylation:^{11e} IP 97% ee). The hydrovinylation/oxidation approach is impressive (IP, FP, FIP, 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 (Fig. 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 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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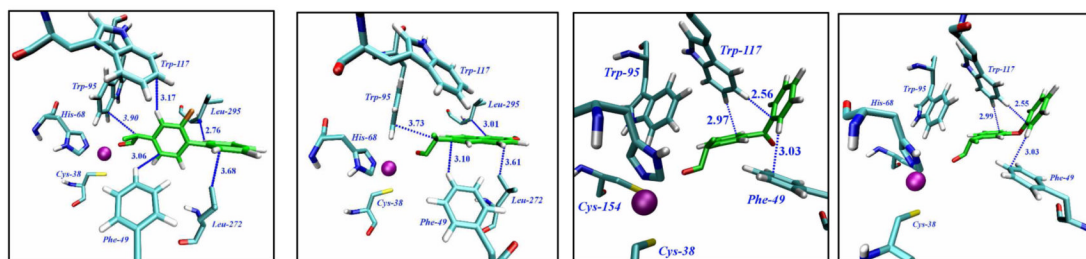
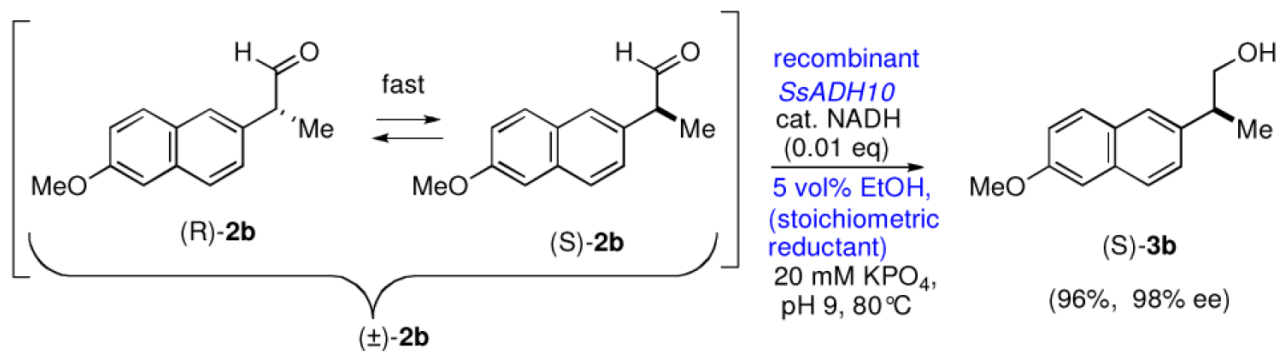


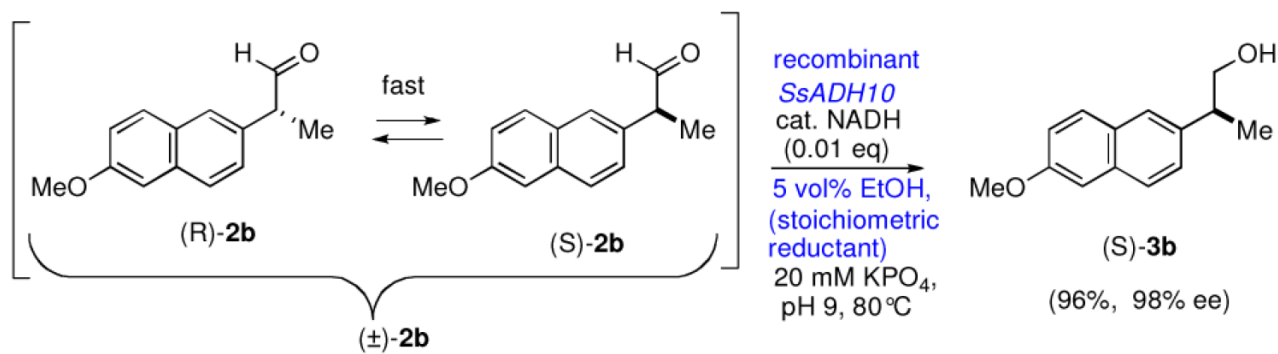
Figure 2. Structures of thermally relaxed (GROMACS 4.07) SsADH-10 (from 1R37) to which has been docked (Autodock Vina - left to right-): (i) (*S*)-flurbiprofen (ii) (*S*)-naproxen (iii) (*S*)-ketoprofen and (iv) (*S*)-fenoprofen (Zn ligation sphere: H68, C38, C154 and substrate carbonyl)

Table 1

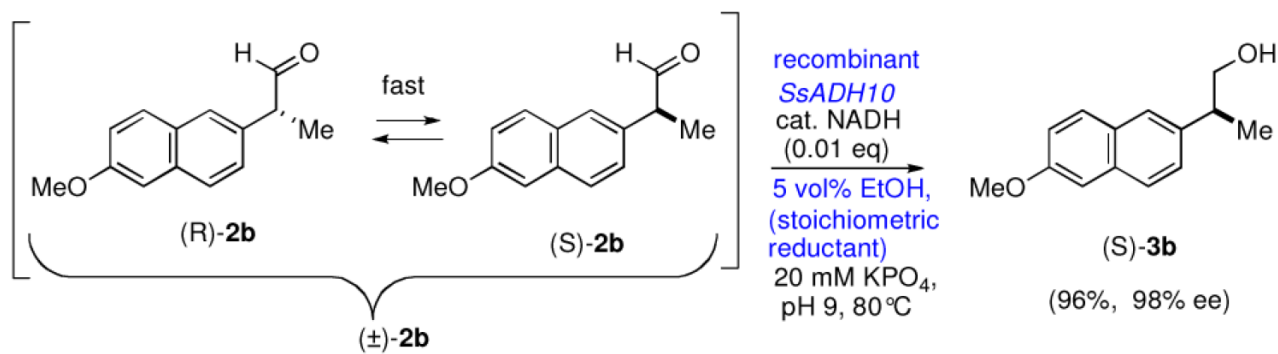
SsADH10-Mediated DYRKR Entry into Profenols



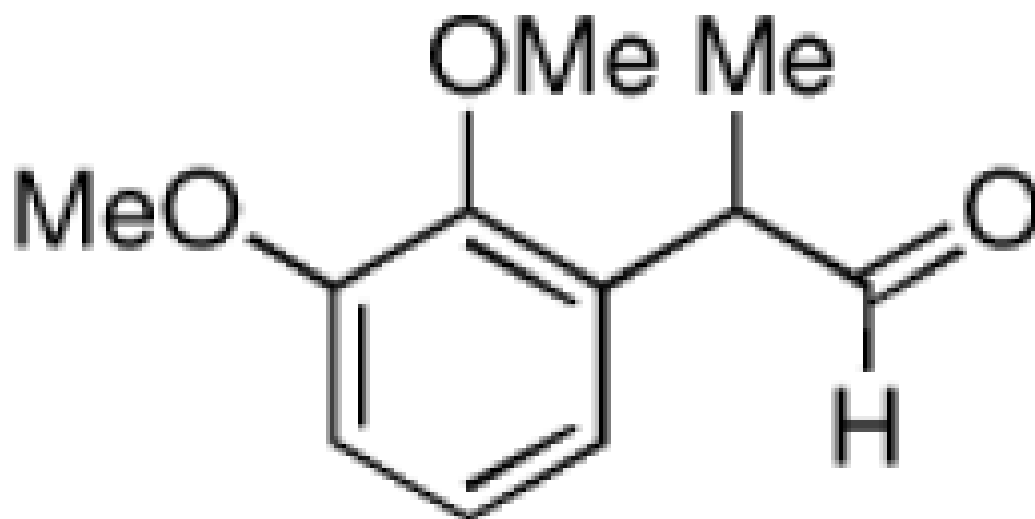
Cpd No.	DYRKR Product ^a	t(h)	Yield (%) ^b
3a		18	57%
3b		18	96%



Cpd No.	DYRKR Product ^a	t(h)	Yield (%) ^b
3c		18	90%
3d		18	92%
3e		18	74%



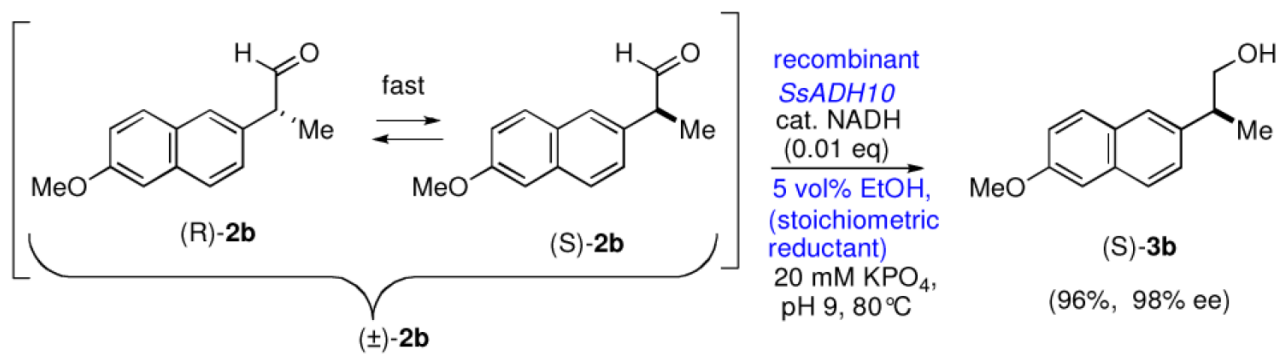
Cpd No.	DYRKR Product ^a	t(h)	Yield (%) ^b
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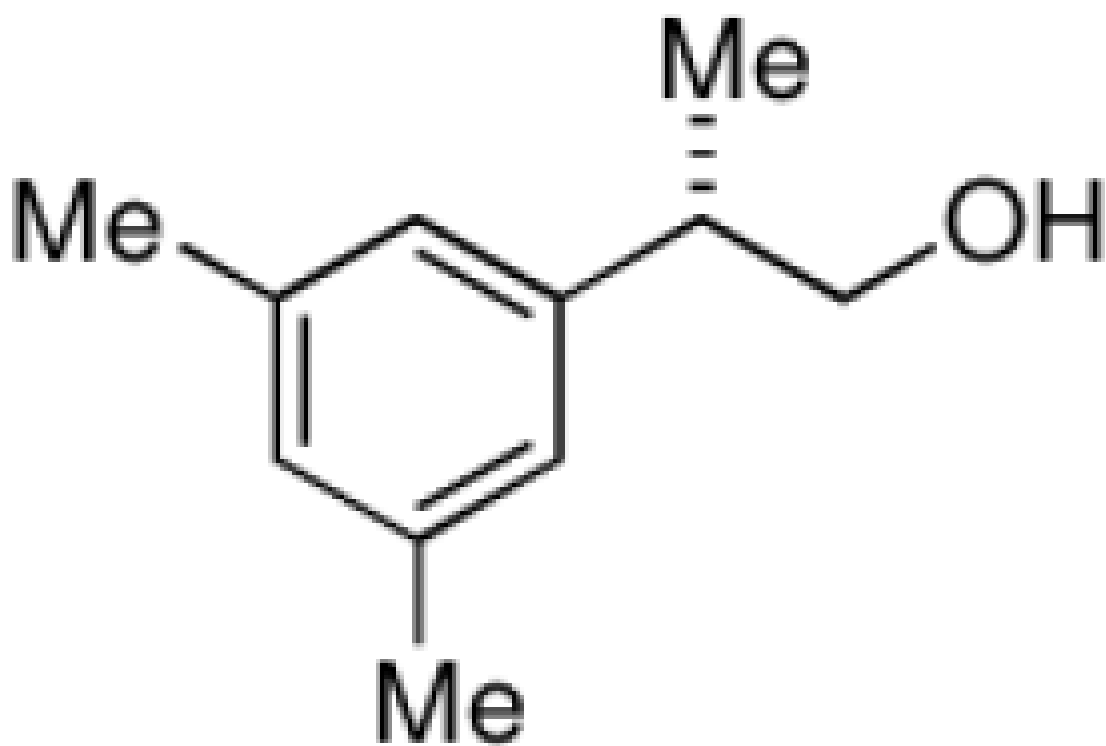
2f

(redn not obsd)

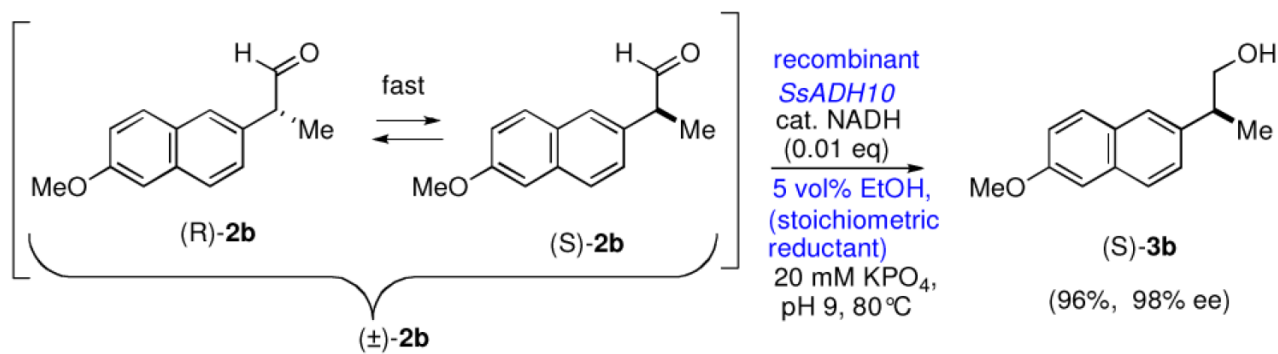
18 recvd SI



Cpd No.	DYRKR Product ^a	t(h)	Yield (%) ^b
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18 99%



Cpd No.	DYRKR Product ^a	t(h)	Yield (%) ^b
3h		18	77%
3i		24	55%
3j		12	85%
3k		18	95%
3l		18	85%

^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.