Published in final edited form as:

Angew Chem Int Ed Engl. 2002 May 03; 41(9): 1603–1607.

In Situ Enzymatic Screening (ISES): A Tool for Catalyst Discovery and Reaction Development**

David B. Berkowitz, Mohua Bose, and Sungjo Choi

Department of Chemistry, University of Nebraska, Lincoln, NE 68588-0304 (USA), Fax: (+1)402-472-9402, dbb@unlserve.unl.edu

The move from the more deliberate, traditional approach to catalyst discovery to combinatorial approaches, has spurred great interest in the development of parallel screening methods. As Crabtree recently put it, ideally one seeks “an appropriate chemical sensor in a rapid parallel assay to detect *rate* and perhaps *selectivity*.”^[1] Herein, we describe the use of enzymes to report rapidly on relative reaction *rate* for a set of parallel reactions. In a complementary paper that was submitted parallel to this one, Seto recently described the use of enzymes to report on (enantio)*selectivity*.^[2] From a broader perspective, these developments may be regarded as adding to the versatility of enzymes as tools for the organic chemist, an area that has seen remarkable expansion in recent years from asymmetric processing of unnatural substrates,^[3–5] to protecting group cleavage under mild conditions,^[6] to the creation of artificial enzymatic pathways.^[7,8]

There is currently great interest in “combinatorial catalysis,”^[9] especially that involving transition metal (TM) catalyzed reactions, for which reaction discovery and optimization often involve varying (i) the metal; (ii) the ligand (type, structure and stoichiometry) and (iii) the substrate structure. By choosing such a model reaction, we sought to both establish proof of principle and to assess the ability of the screen to evaluate such variables one at a time.

In our approach, the organic reaction under study is coupled, in situ, to an enzymatic reaction that permits continuous UV-monitoring of the reaction. We term this approach in situ enzymatic screening (ISES). This method is complementary to previously communicated screening techniques,^[10–19] in that it provides for (i) evidence of product formation {not directly available using the elegant IR thermography method due to Morken and Reetz}^[11] and (ii) relative rate profiles {not easily available with time-point detection systems employing gas^[12] or liquid chromatography^[10,13] or mass spectrometry^[14]}, (iii) without the need to alter the substrate, by installing a chromophore,^[15] a fluorophore,^[16] or an azo-dye precursor.^[17]

[**]We thank D. Seebach and S.G. DiMaggio for valuable comments and thank the referees for suggesting a number of useful control experiments. Financial support from the National Institutes of Health (CA 62034) is gratefully acknowledged. D.B.B. is an Alfred P. Sloan Fellow. This research was facilitated by grants for NMR and GC/MS instrumentation from the NIH and the NSF, respectively.

Correspondence to: David B. Berkowitz.

Dedicated to Professor Samuel J. Danishefsky on the occasion of his 65th birthday

Supporting Information for this article is available on the WWW under <http://www.angewandte.com> or from the author.

Consonant with our interest in developing synthetic methodology toward densely functionalized β,γ -unsaturated amino acids,^[20] as inhibitors of PLP-dependent (PLP = pyridoxal phosphate) enzymes,^[21] we chose a TM-mediated allylic amination reaction as our model reaction here. We were influenced, in this regard, by an important precedent from Trost, scalemic vinylglycinol had been synthesized through Pd⁰-mediated allylic amination.^[22] We set out to use ISES to identify other TM's, including less expensive ones, capable of catalyzing the intramolecular allylic amination reaction illustrated in Scheme 1.^[23] Success here would, in principle, validate the use of ISES to screen other variants of the allylic displacement reaction.^[24]

A UV spectrophotometer fitted with an automated cell changer was chosen for parallel screening as this equipment is readily available commercially. Six non-Pd TM catalysts were simultaneously monitored for intramolecular allylic amination with model substrates **1a/b**. Because the TM complexes themselves are highly colored, these reactions are run in a biphasic fashion. The organic chemistry takes place in an upper organic layer, above the spectrophotometer beam, and the linked enzymatic chemistry takes place in a lower aqueous layer, squarely in the path of the light signal (Scheme 1). Turnover of the ethyl carbonate substrate in the organic layer leads to release of ethanol, which diffuses into the aqueous layer, where it undergoes enzymatic oxidation with concomitant formation of NADH ($\lambda_{\text{max}} = 340 \text{ nm}$). A coupled alcohol dehydrogenase (ADH)/aldehyde dehydrogenase (AIDH) enzyme assay was found to be optimal for screening (Figure 1).^[25]

An appropriate organic solvent for a biphasic ISES screen such as this should satisfy several criteria: (1) It should be essentially immiscible in the aqueous buffer. (2) It should solubilize all organic substrates and catalysts (TM complexes here) chosen. (3) It should be compatible with the reaction under study. (4) It should permit the diffusion of the (by)product (enzymatic substrate) into the aqueous buffer layer. Several organic solvents were examined here, and among them, a mixed organic solvent (THF:toluene:hexane - 2:1:1) was chosen as best meeting these criteria (Full details are provided in the Supporting Information).^{[26],[27]}

With this system, one can easily screen six reactions simultaneously, and obtain meaningful relative kinetic data within 10 min (Figure 2 and Table 1). Of course, all screening methods that rely upon the monitoring of a leaving group are susceptible to the false positive that would be observed via simple hydrolysis of substrate. This concern necessitates the independent testing "in the reaction flask" of apparent positive results in the cuvet. Only after a significant body of data of this kind is accumulated will it be possible to establish the extent of this potential limitation. In the case at hand, there is a good correlation between relative NADH formation rates observed and relative product formation rates in the upper organic layer, as examined independently by a NMR time point assay.^[28] There is also a correlation, though less direct, as expected, between relative rates observed in the cuvet and yields obtained for the same reactions in neat THF at 1 h reaction times (Figure 2).

Across a spectrum of non-Pd TM complexes screened, the most favorable results were obtained with Ni⁰ catalysts.^[29] To our knowledge, there have been only a couple of Ni⁰-catalyzed allylic aminations reported heretofore, and these employ simple secondary amines as nucleophiles. These methods require either stoichiometric nickel,^[23f] or elevated

temperatures.^[23d,e] *The cyclization uncovered here, in which a lithiated carbamate serves as intramolecular nucleophile^[32] apparently constitutes the first example of a room temperature allylic amination catalyzed by Ni⁰.* The screen also uncovered the first Mo⁰-mediated allylic amination, of which we are aware, albeit in modest yield.^[33]

With Ni⁰ showing promise, we next employed ISES to probe the ligand and nitrogen protecting group (PG) dependence of this reaction. The results are collected in Table 1 and correspond to data assimilated from three sets of 6-cuvet runs. Electron rich N-protecting groups are beneficial, with the PMP group giving the best rates. This PG was retained for the ligand screens, which turned up three hits (dppb, PPh₃, dppf), including the only two bidentate phosphine ligands examined.

Several other control experiments were run to more precisely define the optimal conditions and/or limitations of this biphasic ISES screen. In one, by varying the amount of one dehydrogenase enzyme with the other held constant,^[35] we were able to establish that, under the standard screening conditions chosen here, the ADH-mediated oxidation of ethanol to acetaldehyde is not at all rate-limiting. On the other hand, the AIDH-mediated oxidation of acetaldehyde to acetic acid is partially rate-limiting. So while, in general, relative rates are reproducible at ± 5 –15% under our standard conditions (Figure 2; points to consistency in AIDH-pipetting), this experimental uncertainty might be reduced even further by expending more AIDH (0.12 $\rightarrow$ 0.24 U) per cuvet, so as to make neither enzymatic step even partially rate-limiting.

Perhaps the central issue that arises when one considers the ISES concept is the co-compatibility of enzymatic chemistry and TM-mediated organic chemistry in a single vessel. While the biphasic nature of this assay is expected to offer a considerable degree of compartmentalization, for any reaction to be screened, it will be important to establish whether components of the aqueous phase (i.e. water and acetaldehyde) are diffusing into and inhibiting chemistry in the organic layer. Conversely, one must consider the possibility that components of the organic layer (e.g. metal complexes) are inhibitory toward the enzymatic reporter groups in the aqueous layer.

A Karl Fischer titration established that up to 1 wgt. % water diffuses into the organic layer under the screening conditions here.^[35] It is noteworthy that not only is this reaction apparently compatible with stoichiometric water, but, indeed, a considerable variety of organic transformations display aqueous compatibility (See the Supporting Information for a compilation of references to such reactions). Two experiments were carried out to assess the affect of enzymatically generated acetaldehyde on the TM-mediated chemistry in the organic phase. Neither introduction of acetaldehyde into the aqueous layer, nor into the organic layer directly (at 45 mol% levels) produced a decrease in the ISES-monitored reaction rate.^[35] As for the converse direction, control experiments revealed no inhibition of ethanol oxidation in the aqueous layer when standard levels of phosphine ligand and either Ni(cod)₂ or (C₇H₈)Mo(CO)₃ were introduced into the organic layer.^[35] These results are consistent with the appearance of a characteristic, unperturbed NADH spectrum in the aqueous layer with time, as the metal complex-mediated chemistry takes place in the upper organic layer

(Figure 1), suggesting that indeed little to no diffusion of these organometallic complexes into the aqueous layer is occurring here.

Having identified good conditions for this Ni⁰-mediated allylic amination at room temperature, these conditions were next applied to the syntheses of two important β,γ -unsaturated amino acids. Vinylglycine, a known inactivator of PLP-dependent transaminases for L-aspartate, L-alanine, L-serine and D-alanine^[36] is easily constructed as outlined in Scheme 2. It is both noteworthy that the key intramolecular allylic amination step proceeds in excellent yield and that the PPh₃-ligated Ni⁰ catalyst may be generated either via ligand exchange from Ni(cod)₂ or via Zn-mediated reduction of the corresponding dichloro-Ni^{II} complex.

The same strategy led to an expeditious synthesis of E-2-amino-5-phosphono-3-pentenoic acid (APPA).^[37] This β,γ -unsaturated phosphonate mimic of homoserine phosphate has been shown recently to inhibit two PLP-dependent enzymes, cystathionine γ -synthase (tight-binding reversible inhibitor; $K_i = 27 \mu\text{M}$)^[38a] and threonine synthase (irreversible inhibitor; $K_i = 400 \mu\text{M}$; $k_{\text{inact}} = 0.25 \text{ min}^{-1}$).^[38b] The requisite α -phosphonomethyl allylic carbonate **5** could be assembled in short order from aldehyde **4** (Scheme 2). Three operations are carried out in one pot initially. Aldehyde **4** is condensed with diethyl lithiomethylphosphonate at low *T*, followed sequentially by alkoxide trapping with ethyl chloroformate, and in situ desilylation. Pleasingly, with either dppb or TPP as ligand, the key Ni⁰-mediated allylic amination proceeds both efficiently and stereoselectively, yielding solely the E geometric isomer of **6**.

The ISES approach to catalyst screening demonstrated in this communication is expected to be applicable to a range of synthetically useful transformations, including others involving release of carbonates or alcohols and their detection with the enzyme couple detailed herein, and still others that involve monitoring entirely different products or leaving groups with other enzyme couples. Exploration of this as yet uncharted territory will be reported in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

- [1]. Loch JA , Crabtree RH Pure Appl. Chem 2001, 73, 119–128.
- [2]. Abato P , Seto CT J. Am. Chem. Soc 2001, 123, 9206–9207.11552847
- [3]. Jones JB , Wong C-H , Curr. Opin. Chem. Biol 1998, 2, 67–69.
- [4]. Klibanov AM Nature 2001, 409, 241–246.11196652
- [5]. Zhong G , Hoffmann T , Lerner R , Danishefsky SJ , Barbas CF J. Am. Chem. Soc 1997, 119, 8131–8132.
- [6]. Pathak T , Waldmann H , Curr. Opin. Chem. Biol 1998, 2, 112–120.9667905
- [7]. For the synthesis of industrial chemicals from glucose, see: Kambourakis S , Draths KM , Frost JW , J. Am. Chem. Soc 2000, 122, 9042–9043.
- [8]. For the synthesis of industrial sweeteners from starch, see: Moradian A , Benner SA , J. Am. Chem. Soc 1992, 114, 6980–6987.

- [9]. a) Reviews: Reetz MT *Angew. Chem. Int. Ed* 2001, 40, 284–310; b) Brandeleit B, Schaefer JD, Powers TS, Turner HW, Weinberg WH, *Angew. Chem* 1999, 111, 2648–2689; *Angew. Chem. Int. Ed.* **1999**, 38, 2494–2532; b) Shimizu KD, Snapper ML, Hoveyda AH, *Chem.-Eur. J* 1998, 4, 1885–1189; c) Francis MB, Jamison TF, Jacobsen EN, *Curr. Op. Chem. Biol* 1998, 2, 422–428.
- [10]. Porte M, Reibenspies J, Burgess K, *J. Am. Chem. Soc* 1998, 120, 9180–9187.
- [11]. a) Taylor SJ, Morken JP, *Science* 1998, 280, 267–270; 9535652b) Reetz MT, Becker MH, Kühlung KM, Holzwarth A *Angew. Chem* 1998, 110, 2792–2795; *Angew. Chem. Int. Ed.* **1998**, 37, 2647–2650; c) Reetz MT, Becker MH, Leibl M, Fürstner A, *Angew. Chem. Int. Ed* 2000, 39, 1236–1239.
- [12]. GC-Monitoring: a) Taylor SJ, Morken JP, *J. Am. Chem. Soc* 1999, 121, 12202–12203; b) Gennari C, Ceccarelli S, Piarulli U, Montalbetti CAGN, Jackson RFW, *J. Org. Chem* 1998, 63, 5312–5313; c) Shimizu KD, Cole BM, Krueger CA, Kuntz KW, Snapper ML, Hoveyda AH, *Angew. Chem* 1997, 109, 1782–1785; *Angew. Chem. Int. Ed. Engl.* **1997**, 36, 1704–1707.
- [13]. HPLC-Monitoring: Porter JR, Wirschun WG, Kuntz KW, Snapper ML, Hoveyda AH, *J. Am. Chem. Soc* 2000, 122, 2657–2658.
- [14]. a) Reetz MT, Becker MH, Klein H-W, Stöckigt D, *Angew. Chem* 1999, 111, 1872–1875; *Angew. Chem. Int. Ed.* **1999**, 38, 1758–1761; b) Hinderling C, Chen P, *Angew. Chem* 1999, 111, 2393–2396; *Angew. Chem. Int. Ed.* **1999**, 38, 2253–2256.
- [15]. Berg T, Vandersteen AM, Janda KD, *Bioorg. Med. Chem. Lett* 1998, 8, 1221–1224. 9871739
- [16]. a) Su H, Yeung ES, *J. Am. Chem. Soc* 2000, 122, 7422–7423; b) Stambuli JP, Stauffer SR, Shaughnessy KH, Hartwig J, *J. Am. Chem. Soc* 2001, 123, 2677–2678; 11456943c) Cooper AC, McAlexander LH, Lee DH, Torres MT, Crabtree RH, *J. Am. Chem. Soc* 1998, 120, 9971–9972; d) Moreira R, Hovranek M, Sames D, *J. Am. Chem. Soc* 2001, 123, 3927–3931; For fluorogenic assays to screen catalytic antibodies, see: 11457142e) Klein G, Reymond JL, *Helv. Chim. Acta* 1999, 82, 400–407; f) Reymond JL, Koch T, Schroer J, Tierney E, *Proc. Natl. Acad. Sci. USA*, 1996, 93, 4251–4256. 8633050
- [17]. Lavastre O, Morken JP, *Angew. Chem* 1999, 111, 3357–3359; *Angew. Chem. Int. Ed.* **1999**, 38, 3163–3165.
- [18]. For an interesting approach involving “chemosensors”, see: a) Copeland GT, Miller SJ, *J. Am. Chem. Soc* 2001, 123, 6496–6502; 11439035b) Harris RF, Nation AJ, Copeland GT, Miller SJ, *J. Am. Chem. Soc* 2000, 122, 11270–11271; c) Copeland GT, Miller SJ, *J. Am. Chem. Soc* 1999, 121, 4306–4307.
- [19]. For the use of laser-induced, resonance-enhanced multi-photon ionization, see: Senkan SM, *Nature* 1998, 394, 350–353.
- [20]. (a) Berkowitz DB, Chisowa E, McFadden JM, *Tetrahedron Symposia-in-Print* 2001, 57, 6329–6343; (b) Berkowitz DB, McFadden JM, Chisowa E, Semerad CL, *J. Am. Chem. Soc* 2000, 123, 11031–11032; (b) Berkowitz DB, McFadden JM, Sloss MK, *J. Org. Chem* 2000, 65, 2907–2918 and refs therein. 10814177
- [21]. Berkowitz DB, Pedersen ML, Jahng WJ, *Bioorg. Med. Chem. Lett* 1996, 6, 2151–2156 and refs therein. 29123334
- [22]. a) Trost BM, Bunt RC, Lemoine RC, Calkins TL, *J. Am. Chem. Soc* 2000, 122, 5968–5976; b) Larksarp C, Alper H, *J. Am. Chem. Soc* 1997, 119, 3709–3715; c) Hayashi T, Yamamoto A, Ito Y, *Tetrahedron Lett* 1988, 29, 99–102.
- [23]. In general, there are relatively few examples of aminations upon allyl-X electrophiles mediated by metals other than Pd, though this is an area of considerable current interest: Rh^I a) Evans PA, Robinson JE, Nelson JD, *J. Am. Chem. Soc* 1999, 121, 6761–6762; b) Evans PA, Robinson JE, *Org. Lett* 1999, 1, 1929–1931; Ir^I 10836050c) Takeuchi R, Shiga N, *Org. Lett* 1999, 1, 265–267; Ni⁰ d) Bricout H, Carpentier J-F, Mortreux A, *Tetrahedron* 1998, 54, 1073–1084; e) Bricout H, Carpentier J-F, Mortreux A, *J. C. S. Chem. Commun* 1995, 1863–1864; f) Moberg C, *Tetrahedron Lett* 1980, 21, 4539–4542; Ru^I (stoichiometric) g) Kondo T, Ono H, Satake N, Mitsudo T, Watanabe Y, *Organometallics* 1995, 14, 1945–1953; For entirely different chemistry, that nonetheless is postulated to proceed via an intramolecular amination upon a cationic π -allylic-Ru^{IV} intermediate, see: h) Trost BM, Pinkerton AB, Kremzow D, *J. Am.*

Chem. Soc, 2000, 122, 12007–12008; Mo^{IV}i) Malkov AV , Spoor P , Vinader V , Kocovsky P , J. Org. Chem 1999, 64, 5308–5311.

- [24]. Trost BM , Van Vranken DL , Chem. Rev 1996, 96, 395–422.11848758
- [25]. The second enzymatic reaction not only provides for the formation of a second molecule of NADH for each EtOH equivalent oxidized, but it also converts an unfavorable equilibrium into a highly favorable one. Thus, even at pH 7.7, $G' = +4.6$ kcal/mol for the (ADH-mediated) oxidation of ethanol with NAD⁺, whereas $G' = -15.2$ kcal/mol for the subsequent (AIDH-mediated) oxidation of acetaldehyde to acetate [For a discussion of the equilibrium constants for these oxidations, see: Alberty RA , Arch. Biochem. Biophys 1993, 307, 8–14.8239668]. The G' values given here assume an ionic strength = 0.1 and have been corrected for the fixed assay pH}. Note: The thermodynamics for the ADH-step can be improved by substitution of APAD+ (a better oxidant, but more expensive cofactor; redox potential = -258 mV, $\lambda_{\text{max}} = 365$ nm) for NAD+ (redox potential = -320 mV; $\lambda_{\text{max}} = 340$ nm). For experimental details of this alternative single dehydrogenase ISES assay, see the Supporting Information under the heading: Effect of Acetaldehyde on TM-Catalyzed Allylic Amination Rate.
- [26]. One referee has pointed out that with ISES one might encounter a “leveling effect” wherein turnover rates that exceed the “instantaneous” release rate of EtOH into the aqueous layer from the solvent of choice cannot be differentiated. Indeed, we do see this “topping out effect” with dppb as ligand for the reaction under study here (Table 1, entry 14). However, we find that we are able to observe faster catalysts [such as Pd2(dba)3] by the simple expedient of reducing the mole percentage of catalyst (down to 2 % in this case). So while we can circumvent “leveling effect” issues in the biphasic assay, the need for a viable biphasic and for an acceptable leaving group release rate into the aqueous layer does put limits on the types of organic solvents that can be employed in biphasic ISES.
- [27]. Restrictions on solvent composition also arise in the IR thermography (ref 11a) and gel-embedded fluorescent chemosensor (ref 18b) screens, where other practical issues (“bead floatability” and gel-swelling, respectively) arise. While a THF/PhCH3/hexane mixed solvent proved best for ISES with this chemistry, for other reactions, in an inverted configuration, it should be possible to run ISES assays with solvents more dense than water.
- [28]. As a control experiment, for the cuvet screens of both the Ni0- and Mo0-catalyzed reactions (first and third entries, Figure 2), aliquots were drawn from the upper organic layers of the cuvetts at early times. After quenching, and extractive workup, NMR analysis versus an internal standard (2'-acetonaphthone), showed a ratio of initial rates ([P]/time) of ~ 8.7–13:1, consistent with the ratio of slopes observed for NADH formation (~ 8.5:1) in the aqueous layer of the same cuvetts. Full details of these experiments are provided in the Supporting Information. We simply note here that these experiments are both tedious (one time point is obtained for each aliquot taken, worked up and analyzed by NMR; in contrast to the continuous relative [product] vs. time data provide by the ISES screen) and difficult (to get a valid time point one needs an instantaneous quenching of the reaction, which may be difficult to obtain, and lead to considerable experimental uncertainty, especially for rapid reactions at short times).
- [29]. In light of Evans' work (refs. 23a,b), one might have expected better results for the Rh(I) catalyst here. In fact, whereas the unmodified Wilkinson catalyst proved to be much less efficient than the Ni0 catalysts found for this reaction involving a lithiated carbamate as internal nitrogen nucleophile, it proved to be a good catalyst for the reaction of a simple substrate (allyl ethyl carbonate) with a neutral, external nitrogen nucleophile (dibenzylamine; see the Supporting Information for details).
- [30]. With one exception, all cuvetts contain an organic layer: 110 μmol 1a, 100 μmol LiHMDS, 10 mol % catalyst, with added PPh3, where noted, in 400 μL total organic solvent {THF:PhMe:hexane (2:1:1)} all over an aqueous layer containing: 7.4 mM NAD⁺; ADH (1.3 U) and AIDH (0.12 U) in 15 mM pH 8.8 sodium pyrophosphate buffer; final pH 7.7 in 900 μL total aqueous volume. The lone exception is entry 3 which was run at higher catalyst loading (50 mol% catalyst) on substrate 1b at half the usual concentration (i.e. 55 μmol in the same volume) for solubility reasons.
- [31]. RB flask reactions contained the same concentrations of 1a/b and catalyst as the cuvet reactions, with 1 eq. LiHMDS in THF/hexane (73:27). All reactions were stopped after 60 min reaction time and, where appropriate, product (2a/b) was isolated by SiO2 chromatography.

- [32]. Among several bases examined for carbamate deprotonation (includes n-BuLi, NaHMDS, KHMDS, LDA, NaOTMS), LiHMDS gave the best amination rates/yields. Interestingly, Evans also apparently settled upon this base for his RhI-mediated aminations (refs 23a,b).
- [33]. While Mo⁰-mediated allylic alkylation is well-developed chemistry (For leading references, see; a) Belda O , Kaiser NF , Bremberg U , Larhed M , Hallberg A , Moberg C , J. Org. Chem 2000, 65, 5868–5870;10970341b) Trost BM , Hildbrand S , Dogra K , J. Am. Chem. Soc 1999, 121, 10416–10417;c) Glorius F , Pfaltz A , Org. Lett 1999, 1, 141–144), we have been unable to find a report of Mo⁰-mediated allylic amination. Kocovsky has developed a mechanistically distinct MoIV-mediated allylic amination reaction upon allylic alcohols (ref 23i).
- [34]. The PG screens were run at half the usual substrate concentration (138 mM), but at the usual concentrations of Ni(cod)₂ (27.5 mM) and PPh₃ (110 mM). On the other hand, the ligand screens (below the dashed line) were run at the usual concentrations of substrate (275 mM) and metal (27.5 mM), but at half of the typical ligand concentration (55 mM for monodentate L's or 27.5 mM for bidentate L's). These changes are apparently approximately compensatory. For example, for the PPh₃ ligand/PMP PG case, the PG screen conditions gave a rate of 35 mAbs/min, whereas the ligand screen conditions gave slopes of 36 and 41 mAbs/min, in two independent runs.
- [35]. A complete description of these experiments is included in the Supporting Information.
- [36]. a) Cho C , Ishii R , Hyeon S , Suzuki A , Agric. Biol. Chem 1987, 51, 2597–2598;b) Cornell NW , Zuurendonk PF , Kerich MJ , Straight CB , Biochem. J 1984, 220, 707–716;6466297c) Soper TS , Manning JM , Marcotte PA , Walsh CT , J. Biol. Chem 1977, 252, 1571–1575;402367d) Rando RR , Relyea N , Cheng L , J. Biol. Chem 1976, 251, 3306–3312;6451e) Rando RR , Biochemistry 1974, 13, 3859–3863.4472160
- [37]. For other syntheses of APPA, see: a) Fukuari M , Ichimoto I , Mitsunori K , Biosci. Biotech. Biochem 1996, 60, 680–682;b) Allgeier H , Angst C , Bold G , Duthaler R , Heckendorn R , Togni A , Eur. Pat. Appl 1989, EP 302826 A2 19890208.
- [38]. a) Clausen T , Wahl MC , Messerschmidt A , Huber R , Fuhrmann JC , Laber B , Streber W , Steegborn C , Biol. Chem 1999, 380, 1237–1242;10595588b) Laber B , Gerbling KP , Harde C , Neff KH , Nordhoff E , Pohlenz HD , Biochemistry 1994, 33, 3413–3423.7907888

Figure 1.

UV traces showing (left) the dependence of NADH formation rate observed upon enzymatic couple used and (right) a clear spectroscopic signature indicating that NADH is the species giving rise to the 340 nm absorbance increase observed. All traces are for the reaction depicted in Scheme 1. Spectral scans shown (right) are at 2 min intervals. The AIDH only experiment (left) is a negative control and shows no rate, as expected.

Catalyst	Slope [mAbs/min] [a]	Isolated Yield [%]
$\text{Ni}(\text{cod})_2/4\text{PPh}_3$	58 [b]	70
$[\text{Ni}(\text{cod})_2/4\text{PPh}_3]$ [c]		89
$\text{Mo}(\text{CO})_3(\text{C}_7\text{H}_8)/3\text{PPh}_3$ [d]	6.8	18
$[\text{Mo}(\text{CO})_3(\text{C}_7\text{H}_8)]$ [e]		35
$\text{Mo}(\text{CO})_3(\text{C}_7\text{H}_8)/4\text{PPh}_3$	0.6	[f]
$\text{Pt}(\text{PPh}_3)_4$	2.5	15 [g]
$\text{RhCl}(\text{PPh}_3)_3$	1.5	≈ 5 [f]
$\text{Co}(\text{Cl})_2/4\text{PPh}_3$	0.7	[e]

Figure 2.

^a) UV traces (above) showing the relative rates of turnover of substrate **1**, as inferred from enzymatically coupled EtOH oxidation. ^b) Expansion of the left-hand graph. For each metal, the table provides a comparison between the NADH-formation rate observed in the cuvet^[30] screen and isolated yield of **2** for the same reaction run in an RB flask.^[31] [a] Slopes of linear least squares fit lines to the UV traces shown above. Note: The entries in parentheses represent RB flask results only, run at higher catalyst loadings. [b] For this entry, three runs gave values of 55, 58 and 60 mAbs/min, consistent with an experimental uncertainty of ± 5 –15% for these ISES slopes. [c] Reaction run at 20 mol% catalyst. [d] In both the cuvet and the RB flask, this reaction was run on **1b** at 138 mM substrate (half the usual concentration) and at 50 mol% catalyst. [e] Reaction run on **1b** at 100 mol% catalyst at the usual concentration (275 mM). [f] These cases showed little to no product by crude ^1H NMR. [g] For the Pt^0 catalyzed reaction, the rate observed by ISES increases somewhat beyond the initial 12 min window.

Scheme 1.
ISES of catalysts for allylic displacement

Scheme 2.

a) $\text{Ni}(\text{cod})_2$, PPh_3 , LiHMDS (89%) *or* Zn^0 , $\text{Cl}_2\text{Ni}(\text{PPh}_3)_2$, LiHMDS (84%); b) CAN , $\text{MeCN}/\text{H}_2\text{O}$ (2:1) (78%); c) Boc_2O , NEt_3 , CH_2Cl_2 (88%); d) Cs_2CO_3 , MeOH (88%); e) CrO_3 , $\text{H}_2\text{SO}_4(\text{aq})$, acetone (82%); f) TFA , CH_2Cl_2 (84%, isolated as the TFA salt); g) $\text{MeP}(\text{O})(\text{OEt})_2$, $n\text{-BuLi}$, THF , -40°C , 30 min; EtO_2CCl , 30 min; then 0.3N HCl , warm to rt (68%); h) $p\text{-MeO-C}_6\text{H}_4\text{NCO}$, pyr, (96%); i) 20 mol% $\text{Ni}(\text{cod})_2$, dppb , LiHMDS (79%); *or* 20 mol% $\text{Ni}(\text{cod})_2$, PPh_3 , LiHMDS (77%); Zn^0 , *or* 20 mol% $\text{Cl}_2\text{Ni}(\text{PPh}_3)_2$, LiHMDS (70%); j) CAN , $\text{MeCN}/\text{H}_2\text{O}$ (2:1) (89%); k) Boc_2O , LiHMDS , THF (85%); l) Cs_2CO_3 , EtOH (95%); m) CrO_3 , $\text{H}_2\text{SO}_4(\text{aq})$, acetone (76%); n) TMSI , CH_2Cl_2 (80%; isolated as the ammonium salt)

Table 1.

Nitrogen Protecting Gp/Ligand Screens

Ligand	N-Protecting Group (R)	Slope ^a (mAbs/min)
PPh_3	H (no protection)	7
PPh_3	CHPh_2 (benzhydryl)	9
PPh_3	$\text{C}_6\text{H}_2\text{-3,4,5-(OMe)}_3$	18
PPh_3	$\text{C}_6\text{H}_4\text{-p-NO}_2$ (PNP)	0.2
PPh_3	$\text{CO}_2\text{t-Bu}$ (Boc)	1
PPh_3	$\text{C}_6\text{H}_4\text{-p-OMe}$ (PMP)	35
<hr/>		
P(OMe)_3	$\text{C}_6\text{H}_4\text{-p-OMe}$ (PMP)	1
$\text{P(2,6-di-OMe-C}_6\text{H}_3)_3$	$\text{C}_6\text{H}_4\text{-p-OMe}$ (PMP)	7
AsPh_3	$\text{C}_6\text{H}_4\text{-p-OMe}$ (PMP)	0.3
$2,2'\text{-CH}_2\text{-(4S)-Ph-box}^b$	$\text{C}_6\text{H}_4\text{-p-OMe}$ (PMP)	0.6
dppf^c	$\text{C}_6\text{H}_4\text{-p-OMe}$ (PMP)	26
$\text{P(C}_6\text{H}_4\text{-p-NMe}_2)_3$	$\text{C}_6\text{H}_4\text{-p-OMe}$ (PMP)	1
$\text{P(C}_6\text{F}_5)_3$	$\text{C}_6\text{H}_4\text{-p-OMe}$ (PMP)	0.3
$\text{Ph}_2\text{P(CH}_2)_4\text{PPh}_2$	$\text{C}_6\text{H}_4\text{-p-OMe}$ (PMP)	118
P(2-fur)_3	$\text{C}_6\text{H}_4\text{-p-OMe}$ (PMP)	0.4
P(t-Bu)_3	$\text{C}_6\text{H}_4\text{-p-OMe}$ (PMP)	0.4

^[a] Slopes of least-squares fitted lines to the Abs vs. time data over ca. 10 min.^[34]

^[b] 2,2'-methylenebis[(4S)-4-phenyl-2-oxazoline].

$[c]_{1,1}$ -bis(diphenylphosphino)ferrocene.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript