Supporting Information for *Angew. Chem. Int. Ed.* Z17085

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

David B. Berkowitz,* Mohua Bose and Sungjo Choi

General - *Organic Synthesis*

All reactions were conducted under an argon atmosphere using flame-dried glassware unless otherwise noted. $Cl_2Ni(PPh_3)_2$, $Pt(PPh_3)_4$, were purchased from Strem and $Ni(cod)_2$, $Mo(CO)_3(C_7H_7)$, $ClRh(PPh_3)_3$ and $CoCl_2$-hexahydrate were from Aldrich. The latter complex was dried (100 °C, overnight) on a Kugelrohr apparatus prior to use. Toluene and THF were distilled from sodium benzophenone ketyl. Acetonitrile, pyridine, methylene chloride and triethylamine were distilled from CaH_2. *n*-Butyllithium in hexanes (nominally 1.6 M) was purchased from Aldrich and titrated before use. NMR spectra were recorded on a Bruker-DRX-Avance-500 or a GE Omega-300 instrument. Chemical shifts are reported relative to (i) residual $CHCl_3$ (7.25 ppm, **^{1}H**); (77.0 ppm, **^{13}C**) or (ii) PPh_3 (-5.80 ppm, internal capillary standard, **^{31}P**). For HMBC (Heteronuclear Multiple Bond Correlation) experiments, ^{13}C chemical shifts were determined by summing projections over the indirect (^{13}C) dimension of a proton detected 2-dimensional, gradient selected spectrum. Infrared spectra were obtained using an Nicolet Avatar 360 FTIR spectrometer. Mass spectra were acquired at the Nebraska Center for Mass Spectrometry (University of Nebraska).

General - *Enzyme Assays*

All UV spectra were recorded on a Shimadzu UV-2101PC spectrophotometer equipped with a CPS-260 six-cell positioner with thermoelectric temperature control (set at 25°C for all experiments reported). Quartz cuvets were from Hellma. Pipetmen (P10, P200 and P1000) were from Rainin. (Yeast alcohol dehydrogenase (EC 1.1.1.1; lyophilized powder, nominally 280-440 U/mg solid depending upon the batch) was purchased from Sigma and yeast aldehyde dehydrogenase (EC 1.2.1.5; lyophilized powder, nominally 20-54 U/mg protein, depending on lot no.) from Boehringer-Mannheim. β-NAD^+(as the free acid) and β-NADH (disodium salt) were from Sigma.

Enzymatic Screening Procedures

1. Standard Assays

Solutions of both dehydrogenase enzymes were calibrated in terms of U/mL, using the standard assays delineated below. In each case, one S.I. unit is taken as the amount of enzyme catalyzing the formation of one μmol of NADH per minute. In a 1 mL final cuvet volume, this amounts to an absorbance change at 340 nm of 6.22 min^{-1}, or, more typically, 0.622 min^{-1} per 100 mU of enzyme.

Alcohol Dehydrogenase 100 mM EtOH, 7.4 mM NAD^+, 15 mM sodium pyrophosphate, pH 8.8. The final pH of the assay was 7.7.

Typically, the stock solution of ADH was prepared by dissolving 1.5 mg solid of the commercial enzyme lyophilisate in 660 μL of 25 mM $NaPO_4$, pH 7. Addition of 0.5 μL of this solution (5 μL of a 1:10 dilution) to a 1 mL standard assay solution gives rise to an

absorbance change of 0.44 ± 0.06 min^{-1} at 340 nm. This indicates the presence of 0.14 U/μL of stock solution.

Aldehyde Dehydrogenase 400 μM acetaldehyde, 7.4 mM NAD^+, 15 mM sodium pyrophosphate, pH 8.8. The final pH of the assay was 7.7.

Typically, the stock solution of AlDH was prepared by dissolving 5.2 mg solid of the commercial enzyme lyophilisate in 500 μL of 25 mM $NaPO_4$, pH 7. Addition of 10 μL of this to a 1 mL standard assay solution gives rise to an absorbance change of 0.13 ± 0.01 min^{-1} at 340 nm. This indicates the presence of 0.021 U/ 10 μL of stock solution.

2. Biphasic Screening Parameters

A. Optimal Interface Position

Quartz cuvets with a 1 cm light path and with a nominal one mL volume (actual filled volume = 1.6 mL) were used. To establish an appropriate position for the interface, the cuvet was initially filled to a 1 mL volume with the standard aqueous assay solution (vide supra) and the Abs_{340} vs. time was measured to establish a baseline value for the rate. This value was then compared to values for the same assay solution measured at cuvet volumes of 500 μL, 600 μL and 700 μL. No absorbance was seen with the 500 or the 600 μL solutions, whereas the expected baseline rate was observed for the 700 μL cuvet. We presume, therefore, that the spectrophotometer beam passes through the cuvet at approximately the 650 μL level. To insure that the organic/aqueous interface would be well-spaced from the beam, we chose to run biphasic assays with a rather "tall" 900 μL aqueous layer.

B. Organic Layer Composition

The "ideal" organic solvent for these screens was to satisfy several conditions: (1) It would be immiscible in the aqueous buffer solution chosen. (2) It would solubilize all organic substrates and TM complexes chosen. (3) It would promote the allylic displacement reaction under study (perhaps even by ligating to the metal). (4) It would permit the diffusion of the ethanolic byproduct into the aqueous buffer layer. With these considerations in mind, several water-immiscible solvents were examined, initially with a focus on the latter consideration.

In model experiments, 110 μmol (6.4 μL) of EtOH (as a model for the release of EtOH from 110 μmol of ethyl carbonate substrate) was added to the organic solvent (400 μL) in a 1.5 mL microcentrifuge tube. After vortexing the mixture, it was layered above the usual aqueous layer [900 μL; containing 7.4 mM NAD^+, ADH (1.3 U) and AlDH (0.12 U) in 15 mM sodium pyrophosphate, pH 8.8. The final assay pH was 7.7.]. Observed rates of NADH formation were as follows:

Solvent	ΔAbs(340 nm) {mAbs/min}
Hexane	142
THF	47
Toluene	14
THF/hexane (1:1)	109
THF/hexane/toluene (2:1:1)	*107*

We settled upon the latter solvent mixture. This solvent composition seems to provide a good balance between the need to promote allylic displacement and allow for significant EtOH diffusion into the aqueous layer, while also effectively solubilizing the substrate and TM complexes in the organic layer.

C. Aqueous Layer Composition

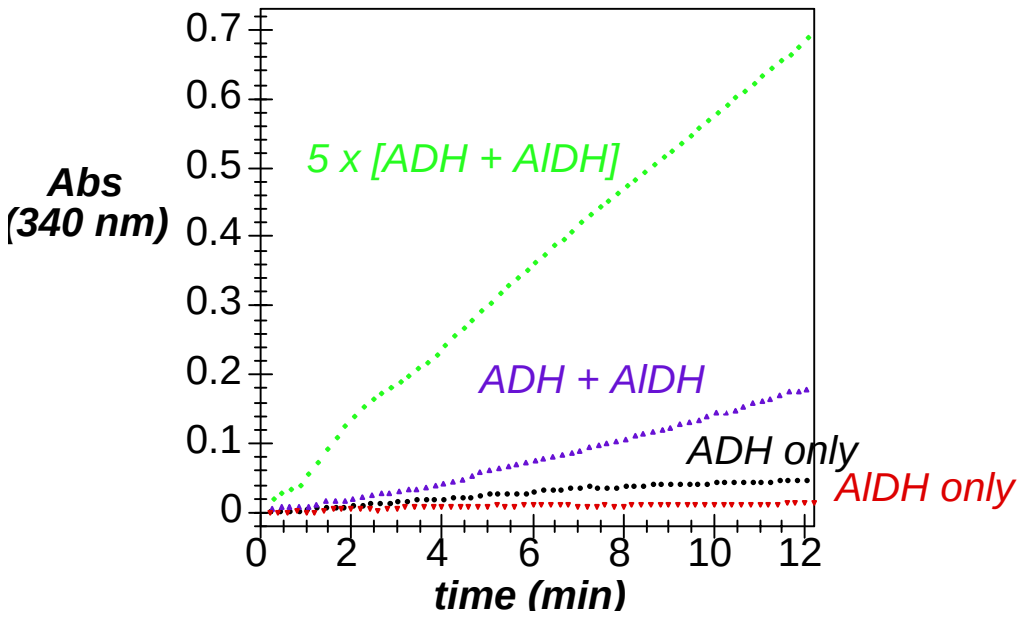
Note: Pyrophosphate buffer is compatible with both enzymes. A basic pH is employed to drive both oxidative equilbria more toward products. This is because three acidic protons are produced in the overall four-electron oxidation of EtOH to acetate by 2 NAD^+ (see Scheme 1 for an illustration of this). The 15 mM salt concentration is high enough to permit good buffering, yet low enough to allow for EtOH diffusion into the aqueous layer with a variety of organic layer compositions (vide supra).

D. Choice of Enzyme Couple

For the screening assay results presented in Figure 1 (left-hand plot), 1 x ADH corresponds to the addition of 1.8 μL (0.25 U) of the stock solution; and 5 x ADH corresponds to 9 μL (1.3 U). For the second enzyme, 1 x AlDH corresponds to the addition of 11 μL (0.024 U) of its stock solution; and 5 x AlDH corresponds to 55 μL (0.12 U).

- The AlDH enzyme alone gives no rate, as expected (no acetaldehyde present).
- Using both AlDH and ADH gives a significantly better observed rate than using the former enzyme alone, as is discussed in footnote 24 of the paper.
- Increasing both enzyme concentrations by a factor five gives approximately a fivefold increase in observed rate:

Enlargement of Figure 1 – left-hand plot



3. Typical Procedure for TM-Catalyzed Intramolecular Allylic Amination of <u>1a/b</u> with ISES

The aqueous layers are first prepared in the 6 cuvets to be screened, as follows:

<u>Stock solution</u>	<u>Vol. Pipetted</u>	<u>Final Aq. Cuvet Conc.</u>
37 mM, NAD^+ in 25 mM $NaPO_4$, pH 7	180 µL	7.4 mM
Yeast ADH (0.14 U/µL) in 25 mM $NaPO_4$, pH 7	9 µL	(1.3 U)
Yeast AlDH (0.021 U/10 µL) in 25 mM $NaPO_4$, pH 7	55 µL	(0.12 U)
15 mM sodium pyrophosphate, pH 8.8	656 µL	

The final pH of the buffer layer was 7.7.

Each cuvet is then sealed with a truncated septum (no. 4 as defined by Aldrich, p. T584 in the 2000-01 catalogue).

Note: A seventh control cuvet (double-beam instrument) is used, as well. This cuvet contains the same aqueous layer (900 μL, as described) over which has been layered the organic solvent being used [400 μl of THF/hexane/toluene (2:1:1) here].

The organic layers are prepared according to the following procedure, using either septum-covered vials or 1.5 mL microcentrifuge tubes (If the latter are chosen, to minimize air contact with the TM complexes, the plastic top is punctured with a 20 Ga needle for each transfer, and the hole resealed each time with electrical tape).

1. The substrate (110 μmol, 34 mg of **1a**) is dissolved in 100 μL of distilled THF in one vial.
2. The ligand [2-4 equivalents relative to TM, depending on the screen; i.e. 12 mg of PPh_3 (4 eq. case)] is dissolved in 100 μL of distilled THF in a second vial.
3. To the ligand vial is added TM complex [typically 11 μmol; i.e. 3.0 mg $Ni(cod)_2$- weighed out on an analytical balance in a glove bag] under Ar.*
4. To the ligand/TM mixture is added toluene (100 μL) via syringe.*

To the ligand/TM mixture is added LiHMDS (100 μL of a 1.0 M solution in hexane; 0.9 equiv. relative to substrate) via syringe.*

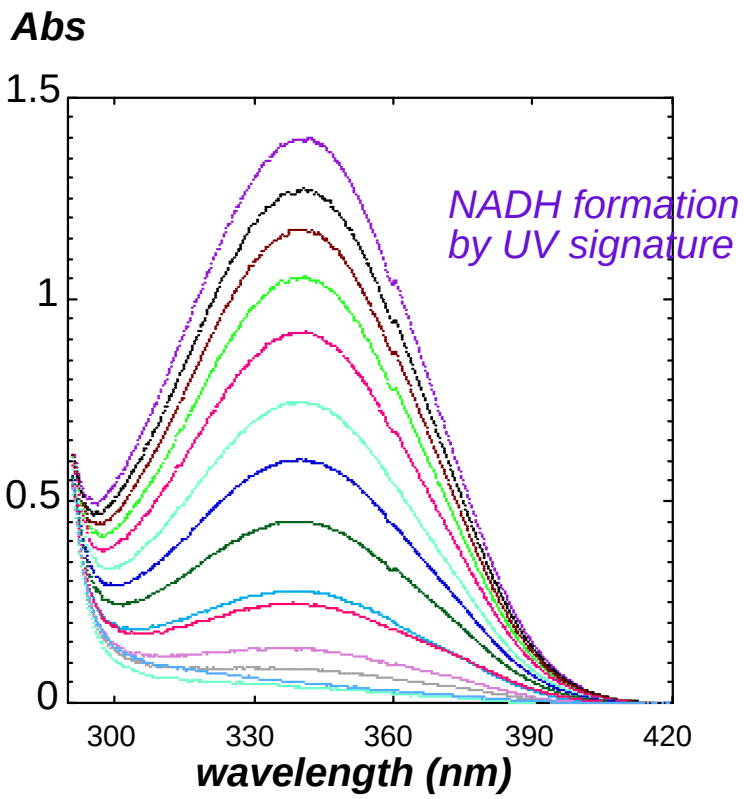
The substrate solution (vial one) is now added to the ligand/TM/base solution (vial two), via syringe and then the entire contents of vial two are immediately layered onto the aqueous layer of of the appropriate septum-covered quartz cuvet. The absorbance at 340 nm vs. time is recorded for six such cells in parallel, using the thermostatted (set to 25°C for all experiments), automatic, six-cell positioner.

(*Indicates that the solution is mixed by vortexing the vial at this stage)

4. Spectral Authentication of NADH as the Species at Being Observed at 340 nm

The assay, as described above for **1a** with $Ni(cod)_2$/TPP/LiHMDS is run against the usual control cuvet with the spectrophotometer in the UV spectral scanning mode. Thus a complete UV spectrum of the aqueous layer is taken every minute. The data are shown in Figure 1 (right-hand plot) and in an expanded version of that figure below. Note that, for clarity, only every second UV spectrum is actually displayed (i.e. Spectra shown are at 2 minute intervals). One sees the appearance with time of the characteristic UV spectrum of reduced nicontinamide (λ_{max} = 340 nm) as NADH is formed.

Enlargement of Figure 1 – right-hand plot



Other Control Experiments

1. Comparison of Relative Initial Rates of Reaction as Measured by a Time Point Assay (NMR Quantitation) vs. the Continuous ISES Assay

A *time point assay* (quench/work-up/NMR analysis) was undertaken in order to compare the relative rates obtained for the Ni^0- and Mo^0-catalyzed allylic aminations with those obtained in the *continuous* ISES assay presented herein. For each reaction, two trials were carried out. As can be seen from the data below, this time point assay estimates that the Ni^0-promoted reaction proceeds at a rate 8.7-13 times faster than the Mo^0-mediated reaction under the conditions used for the ISES screen (gives a relative rate ratio of 8.5-13; see Figure 2).

A. For the Ni^0 catalyzed reaction:

***Trial 1*:** To a solution of **1a** (68 mg, 220 μmol) dissolved in THF (200 μL) was added a solution of $Ni(cod)_2$ (6.0 mg, 22 μmol), triphenylphosphine (24 mg, 88 μmol) and LiHMDS (1 M in hexanes, 200 μL) in THF (200 μL)/toluene (200 μL). This was immediately layered over sodium pyrophosphate buffer (1.8 mL, 15 mM, pH 8.8) in a 2 mL volumetric flask (~2.8 mL total capacity), sealed with a septum and agitated using the same six-cell changer used for our ISES assays in the UV spectrophotometer.

For each time point, 100 μL of the reaction mixture was withdrawn using a microliter syringe and quenched with a mixture of saturated aqueous NH_4Cl (100 μL) and methanol (100 μL). Following extraction with EtOAc (200 μL), a 100 μL aliquot of the organic extract was withdrawn, mixed with 2'-acetonaphthone (0.45 μmol; 5 μL of a 0.09 M solution) as the internal standard for NMR.

After evaporation of the volatiles (rotary evaporator, HV pump) of $CDCl_3$ (500 μL) was added to each sample and a ^1H NMR spectrum acquired. The concentration of product

was calculated by comparing the integrals of the NMR signals at δ 2.87 (**H_3C**CO- standard, 3 H) and 5.4-5.5 (**H_2C**=CH- **2a** product, 2 H).

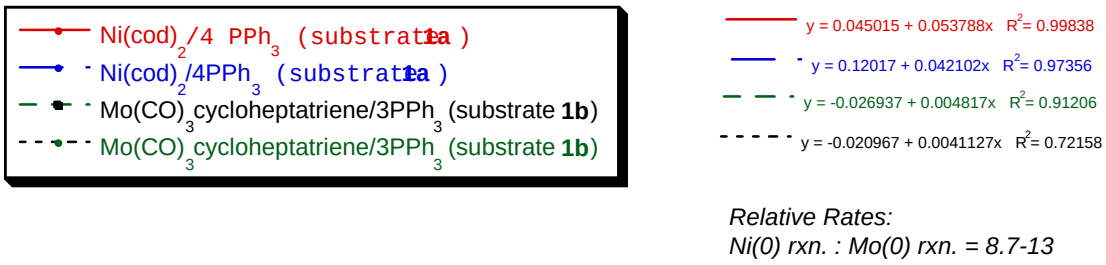
Trail 2: This experiment involves the same amounts of all reactants as in trial 1, but differs in order of addition. Namely, in this case, the organic phase was layered upon the aqueous buffer phase, and then the substrate solution was added to initiate the reaction. In the previous experiment, the substrate was added to the organic layer (containing all components) immediately before layering.

Thus, Ni $(cod)_2$/PPh_3 dissolved in THF:toluene (200 μL: 200 μL) and LiHMDS (200 μL, 1M in hexanes) was first layered over the buffer layer (1.8 mL) and then **1a** in THF (200 μL) was added to the organic layer. For each time point, a 100 μL aliquot was withdrawn, quenched, worked up and analyzed as in Trial 1.

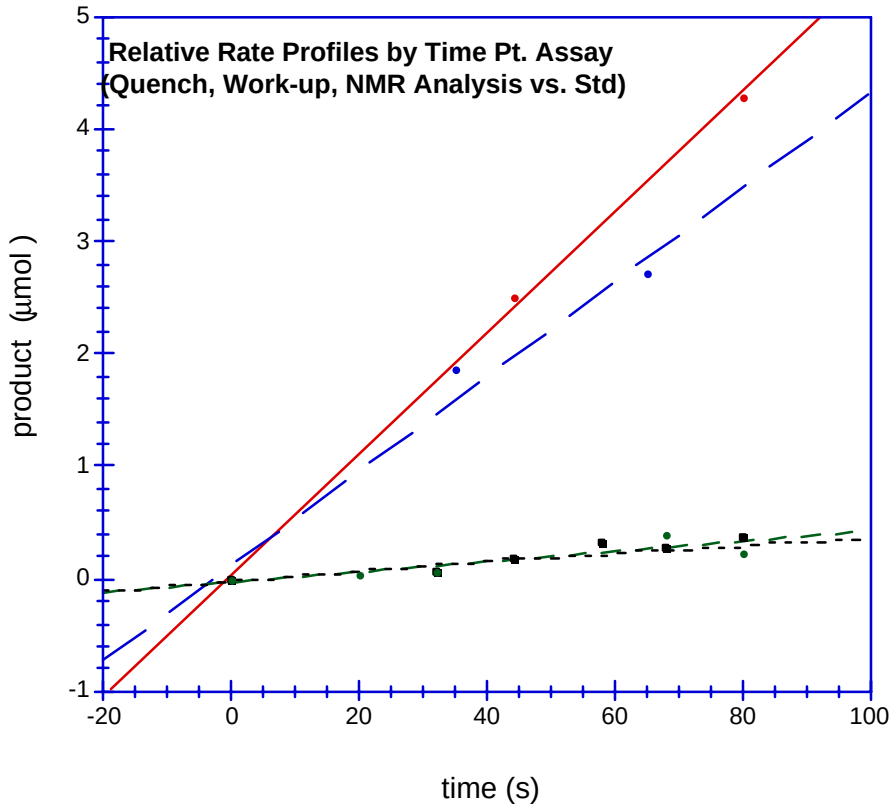
B. For the Mo^0 catalyzed reaction:

A solution of $Mo(CO)_3$cycloheptatriene (15 mg, 56 μmol) in THF:toluene (200 μL: 200 μL) and LiHMDS (110 μL, 1M in hexanes) was first layered over the sodium pyrophosphate buffer layer (1.8 mL, 15 mM, pH 8.8) in a 2 mL volumetric flask (~2.8 mL total capacity), sealed with a septum. Then a solution of **1b** (39 mg, 110 μmol) and PPh_3 (44 mg, 168 μmol) in THF:hexanes (200 μL: 90 μL) was added to the organic layer and agitated using the same six-cell changer used for our ISES assays in the UV spectrophotometer. For each time point, a 100 μL aliquot was withdrawn, quenched, worked up and analyzed as in Trial 1 of Ni^0 catalyzed allylic amination reaction. In this case, the concentration of product was calculated by comparing the integrals of the NMR signals at δ 2.87 (**H_3C**CO- standard, 3 H) and 5.5-5.6 (**H_2C**=CH- **2b** product, 2 H).

Figure S1



Relative Rates:
Ni(0) rxn. : Mo(0) rxn. = 8.7-13



2. Relative yields @ 1 h Reaction Times for Model RB Flask Reactions

To assess the correlation between relative rates observed by ISES and actual isolated yields, reactions were run under standard RB flask conditions. Concentrations of substrate **1a** (or **1b**), TM complex and ligand were as in the ISES screen. However, ca. 75% THF was employed as solvent, with the remainder being hexane from the LiHMDS

solution. A full equivalent of LiHMDS was employed. A typical model reaction is outlined below. The results of these experiments are tabulated in Figure 2.

To a 25 mL RB flask, fitted with a magnetic stir bar, under Ar, containing PPh_3 (25 mg, 96 μmol) and $Ni(cod)_2$ (6.6 mg, 24 μmol) in THF (320 μL) was added a solution of LiHMDS (240 μL, 1.0 M solution in hexane) dropwise, via syringe, at rt. To this was added, via cannula, a solution of **1a** (75 mg, 240 mmol) in THF (320 μL). The resulting reaction mixture was allowed to stir for 60 min, whereupon the reaction was quenched by addition of Et_2O (5 mL) and sat'd, aqueous NH_4Cl (5 mL). After partitioning, the aqueous layer was further extracted with Et_2O. The combined organic layers were dried ($MgSO_4$), filtered, evaporated and chromatographed (hexane/EtOAc 1:1) to provide **2a** (40 mg, 70 %).

3. Model Studies on the Ligand Dependence of Rh^I-Mediated Intermolecular Allylic Amination With a Simple Substrate

The aqueous layers were first prepared in the 6 cuvets to be screened identical to the typical composition as stated in the section "Typical Procedure for TM-catalyzed Intramolecular Allyic Amination of **1a/b** with ISES".

The organic layer was prepared according to the following procedure:

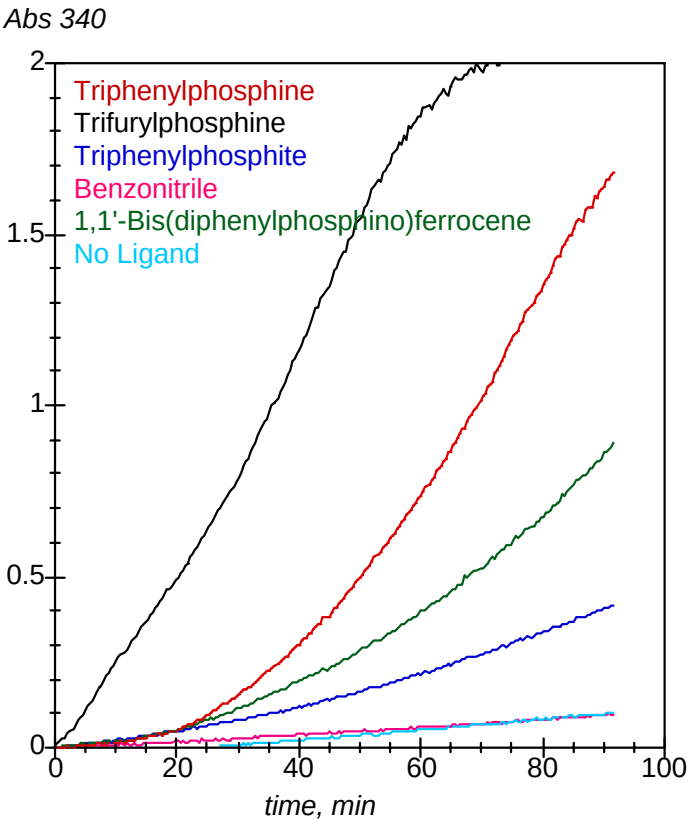
5. The substrate allyl ethyl carbonate (26 mg, 200 μmol) was dissolved in 100 μL of distilled THF in one vial.
6. The ligand [2 or 4 equivalents (for bidentate or monodentate ligands respectively) relative to the TM] was dissolved in 300 μL of distilled THF in one vial.
7. To the ligand vial was added the TM (Rh^I) as $[Rh(cod)Cl]_2$ (2 mg, 4 μmol).

8. To the TM/ligand vial was then added the nucleophile (dibenzylamine, 59 mg, 300 μmol), and the resultant mixture vortexed.

To 300 μL of the TM/ligand/nucleophilic amine mixture was then added 100 μL of the substrate solution via a syringe. Then the combined mixture was layered over the aqueous layer in the septum covered cuvette via a syringe. The absorbance at 340 nm vs time was thus recorded in parallel for six such cuvets which differ only in the type of ligand for Rh^{I}. For the selected ligand distribution, kinetic profiles were obtained over a longer time scale as shown below (Figure S2).

The rate profiles for Rh^{I} catalysis of this intermolecular version of the allylic amination parallel the experimental results of P. A. Evans that identifies Rh^{I} as a newly discovered TM catalyst for allylic amination purposes.

Figure S2



4. Extent to Which the Enzymatic Step(s) are Partially Rate-Limiting

The Ni^0- catalyzed allylic amination of **1a** was chosen for the desired study.

Top Organic layer: In all experiments, the upper organic layer had the same composition as in the *Typical Procedure for TM-Catalyzed Intramolecular Allylic Amination with ISES.*

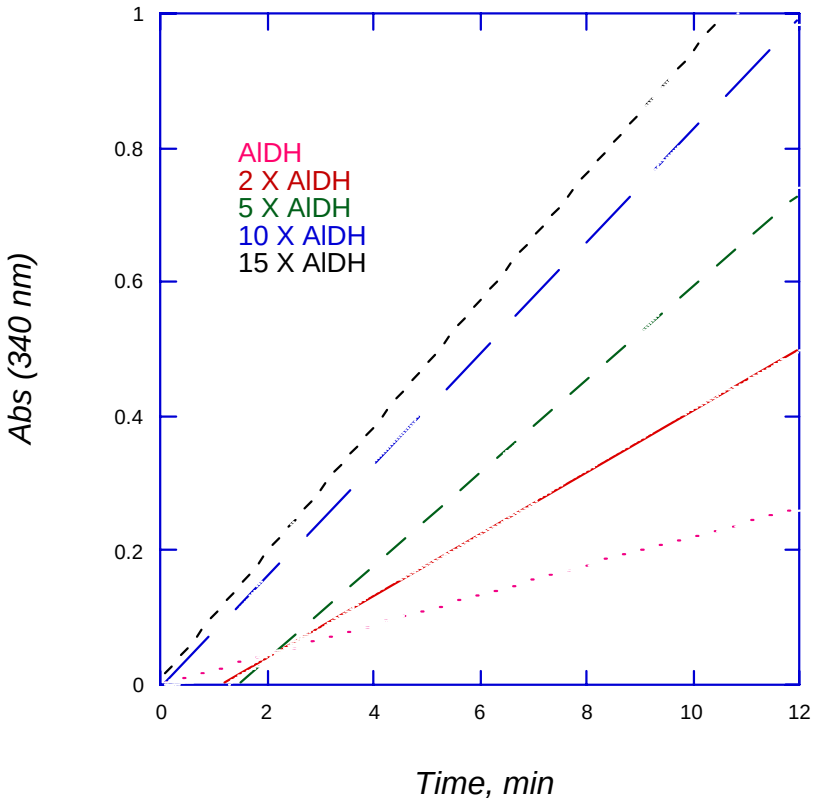
4A. Variation of AlDH:

Bottom Buffer layer (For Varying AlDH at Constant ADH): For each trial, β-NAD^+ (180 μL of a 37 mM stock sol'n; 7.4 mM final conc. in the aqueous layer) and ADH (29 μL of a 0.045 U/μL stock sol'n; 1.3 U) concentrations were held fixed. AlDH concentration was varied by taking 4, 8, 20, 40, 60 μL (0.006 U/μL stock sol'n) in five separate runs. In all cases, the final volume of the aqueous layer was adjusted to 900 μL with buffer (sodium pyrophosphate, 15 mM, pH 8.8). The final assay pH was adjusted to 7.7, wherever necessary with a 1M NaOH solution.

Varying AlDH at ADH = 1.3 U

AlDH (U)		Slope (Abs min^{-1})
1	0.024	0.022 $\pm$ 0.002
2	0.048	0.052 $\pm$ 0.008
3	0.120	0.076 $\pm$ 0.014
4	0.240	0.090 $\pm$ 0.004
5	0.360	0.097 $\pm$ 0.004

Figure S3



Thus with the use of 1.3 U of ADH under the standard ISES conditions reported here, variation of AlDH level from 0.024 U to 0.360 U shows that the rate of NADH formation observed levels off at 0.24 U (showing that the AlDH-mediated step is no longer partially rate-limiting when 0.24 U or more of the enzyme is present).

4B. Variation of ADH:

Bottom Buffer layer (For Varying ADH at Constant AlDH): For each trial, β-NAD (180 µL of a 37 mM stock solution; 7.4 mM final aq. cuvet conc) and AlDH (180 µL of a 0.0033 U/µL; 0.60 U as aq. cuvet conc) were used as constant amounts. ADH (0.14 U/µL stock solution) of varying amounts (9 and 45 µL) was used in two separate recordings of

absorbance (340 nm) vs time. In both cases ,the final volume of the aqueous layer was adjusted to 900 μL with buffer (sodium pyrophosphate, 15 mM, pH 8.8). The final assay pH was 7.7.

By using 0.6 U of AlDH, we are well above the rate-limiting threshold for this second enzyme (see previous experiment). So, this experiment serves to directly measure the extent to which the first enzyme (ADH) is partially rate-limiting at its standard ISES assay concentration. Since, essentially no rate change (increases by 11%, which is within the experimental uncertainty) is observed upon increasing ADH conc. 5 X beyond its normal level, we conclude that the ADH-step is not at all rate limiting here.

Varying ADH at AlDH = 0.60 U

	ADH (U)	Slope (Abs min^{-1})
1	1.30	0.115 ± 0.017
2	6.50	0.128 ± 0.021

Taken together these experiments indicate that under the standard conditions that we chose to use for this ISES screen, the ADH-mediated step is not at all rate-limiting, but the AlDH-mediated step is partially rate-limiting. The data that we have acquired indicate that if care is taken to reproducibly add the same amount of AlDH to each cuvet (we used 0.12 U in our standard ISES assay), good relative rate data can be obtained. The control experiments performed here suggest that experimental uncertainties might be reduced even further if more AlDH is expended per assay.

5. Test for Level of Water Diffusing into the Organic Layer by Karl Fischer Titration

A Karl Fischer titration was performed using the coulometric method[1] with a calibrated Metrohm 684 KF coulometer. An aliquot of the organic solvent layer in question was taken up via syringe, and the syringe capped. It was then weighed on a high precision (five places beyond the decimal) Mettler balance. Following injection of the sample into the coulometer cell, the re-capped syringe was again weighed, to calculate the total sample weight. About one minute was usually sufficient to obtain a stable coulometer reading for the weight of water present in the sample. The Karl Fischer method is based on the oxidation of SO_2 by iodine using the residual water in the sample. Two types of organic layer samples were subjected to such analysis.

A. Initial Organic Solvent Layer

A sample of the THF:hexanes:toluene (2:1:1) mixture used in the ISES assay, when injected to the electrolytic cell, showed a residual water content of 0.09% or 41 mM (see data below). This gives an estimate of the initial water content in the organic layer prior to the allylic amination reaction.

B. Organic Solvent Layer in the Biphasic Assay System

An portion of the THF:hexanes:toluene (2:1:1) solvent mixture (4 mL total) was agitated over a sodium pyrophosphate buffer layer (15 mM, pH 8.8; 9 mL total) for 10 min. When an aliquot of this organic solvent layer was injected into the electrolytic cell, it showed a residual water content of 1.1% or 506 mM. This gives an estimate of the final water content in the organic layer after 10 min under the usual ISES assay conditions.

Note: To take into account experimental uncertainty in sample preparation, as well as analysis, each type of organic layer was prepared in triplicate, and each such sample

assayed in duplicate. This led to 6 readings of % water for each type of organic layer. A representative table of Karl Fischer results is presented below:

Figure S4

Sample	Syringe wgt. before injection (mg)	Syringe wgt. after injection (mg)	Weight of sample injected (mg)	Karl Fischer Reading (μg)	Percentage residual water (wt/wt)
A. a) i)	85.69	17.72	67.97	48.5	0.07
ii)	78.62	12.58	56.04	64.7	0.12
b) i)	64.22	21.62	42.60	50.7	0.12
ii)	123.65	14.92	108.73	78.3	0.07
c) i)	87.78	5.67	82.11	84.1	0.10
ii)	92.24	5.80	86.44	71.1	0.08
B. a) i)	50.64	4.97	45.67	463	1.01
ii)	95.62	5.52	90.12	886	0.98
b) i)	58.67	13.03	45.64	497	1.09
ii)	53.40	13.97	39.43	444	1.13
c) i)	72.46	20.58	51.88	623	1.20
ii)	34.15	10.33	23.82	284	1.19

6. Compilation of References to Organic Transformations Compatible with (Partially) Aqueous Media

Diels Alder : a) F. Fringuelli; O. Piermatti; F. Pizzo; L. Vaccaro Eur. *J. Org. Chem.* **2001**, *3*, 439-455; b) O. A. Attanasi; L. De Crescentini; P. Filippone; F. Fringuelli; F. Mantellini; M. Mattcucci; O. Piermatti; F. Pizzo *Helv. Chim. Acta* **2001**, *84*, 513-525; c) S. Otto; B. F.

N. Jan Engberts *J. Am. Chem. Soc.* **1999**, *121*, 6798-6806. d) A. Meijer; S. Otto; B. F. N. Jan Engberts *J. Org. Chem.* **1998**, *63*, 8989-8994; e) J. W. Wijnen; B. F. N. Jan Engberts, *J. Org. Chem.* **1997**, *62*, 2039-2044; f) Fringuelli, F.; Piermatti, O.; Pizzo, F. *Targets Heterocycl. Syst.* **1997**, 157-73.

Hetero-Diels Alder: a) F. Fringuelli; M. Matteucci; O. Piermatti; F. Pizzo; M. C. Burla *J. Org. Chem.* **2001**, *66*, 4661-4666; b) P. A. Grieco; D. T. Parker, W. F. Fobare, R. Ruckle, *J. Am. Chem. Soc.* **1987**, *109*, 5859-5961; c) P. A. Grieco, P. Galatsis, R. F. Spohn, *Tetrahedron* **1986**, *42*, 2847-2853.

C-C bond formation via conjugate addition: a*) Rhodium catalyzed conjugate addition of aryl-Pb*: R. Ding; Y. J. Chen; D. Wang; C. J. Li *Synlett* **2001**, *9*, 1470-1472; b) F. M. Da Silva; A. K. Gomes; J. Jones Jr. *Can. J. Chem.* **1999**, *77*, 624-627; c) F. M. Da Silva; A. K. Gomes; J. Jones Jr. *Can. J. Chem.* **1999**, *77*, 624-627; d)*C-C bond formation through conjugate addition of alkyl halides in presence of Zn-Cu couple*: C. Petrier; C. Dupuy; J. L. Luche *Tetrahedron Lett*. **1986**, *27*, 3149-52.

Aldol: *Lewis acid catalyzed (Mukaiyama aldol):* a) Y. Mori; K. Manabe; S. Kobayashi *Angew. Chem. Intl. Ed*. **2001**, *40*, 2815-2818; b) S. Kobayashi ; S. Nagayama ; T. Busujima *Tetrahedron* **1999**, *55*, 8739-8746; c) S. Kobayashi; T. Wakabayashi *Tetrahedron Lett*. **1998**, *39*, 5389-5392; d) T. P. Loh; J. Pei; K. S. Koh; G. Q. Cao; X. R. Li *Tetrahedron Lett.* **1997**, *38*, 3465-3468; *Base-catalyzed*: a) F. Fringuelli; O. Piermatti; F. Pizzo Editor(s): P. A. Grieco *Org. Synth. Water* **1998**, 250-261; b) F. Fringuelli; G. Pani; O. Piermatti; F. Pizzo *Tetrahedron* **1994**, *50*, 11499-508; *Pressure-promoted and under neutral conditions*: A. Lubineau; E. Meyer *Tetrahedron* **1988**, *44*, 6065-70.

Epoxidation: F. Fringuelli; O. Piermatti; F. Pizzo *Trends Org. Chem.* **1997**, *6*, 181-195.

C-X bond formation via epoxide opening: a) F. Fringuelli; F. Pizzo; L. Vaccaro *Journal of Organic Chemistry* **2001**, *66*, 3554-3558; b) D. Amantini; F. Fringuelli; F. Pizzo; L.

Vaccaro *J. Org. Chem.* **2001**, *66*, 4463-4467; c) F. Fringuelli,: F. Pizzo; L. Vaccaro. *J. Org. Chem*. **2001**, *66*, 4719-4722; d) F. Fringuelli,; F. Pizzo; L. Vaccaro *Tetrahedron Lett.* **2001**, *42*, 1131-1133; e) F. Fringuelli; F. Pizzo; L. Vaccaro *Synlett* **2000**, *3*, 311-314.

Addition of allyl/alkyl metals to carbonyls: a) A. Lubineau; J. Auge; Y. Queneau *Org. Synth. Water* **1998**, 102-140. b) *Allylindium*:J. A. Marshall *Chemtracts* **1997**, *10*, 481-496; c) P. Cintas, *Synlett* **1995**, 1087-1096; d) C.-J. Li, *Chem. Rev.* **1993**, *93*, 2023-2035; e) *Allyltin*: A. Boaretto; D. Marton; G. Tagliavini; A. Gambaro *J. Organomet. Chem.* **1985**, *286*, 9-16.

Oxidation/reduction: a) F. Fringuelli; F. Pizzo; L. Vaccaro Synthesis **2000**, *5*, 646-650; b) F. Fringuelli; O. Piermatti; F. Pizzo Editor(s): Grieco, Paul A. *Org. Synth. Water* **1998**, 223-249; b) *Oxidation of sulfides to sulfoxides*: F. Fringuelli; R. Pellegrino; F. Pizzo *Syn. Comm.* **1993**, *23*, 3157-63.

TM mediated cross-coupling reactions (Heck, Suzuki, Stille and Sonagashira couplings)*:* a) M. Lautens; A. Roy; K. Fukuoka; K. Fagnou; B. Martin-Matute *J. Am. Chem. Soc.* **2001**, *123*, 5358-5359; b) J. P. Genet, M. Savignac, *J. Organomet.* Chem. **1999**, *576*, 305-317; c) D. Badone; M. Baroni; R. Cardamone; A. Ielmini; U. Guzzi *J. Org. Chem*. **1997**, *62*, 7170-7173; d) J. M. Cuerva; E. Gomes-Begoa; M. Mendez; A. M. Echaveran *J. Org. Chem.* **1997**, *62*, 7540-41; d) T. Jeffery *Tetrahedron Lett*. **1994**, *35*, 3051-4.

Condensations of allylsilanes with iminium ions*:* S. D. Larsen, P. A. Grieco, W. F. Fobare, *J. Am. Chem. Soc.* **1986**, *108*, 3512-3513

Allylation of sulfonimines (In/Zn mediated): W. Lu; T. H. Chan, *J. Org. Chem.* **2000**, *65*, 8589-8594.

Water accelerated zirconocene-catalyzed methylalumination of terminal alkenes: P. Wipf, S. Ribe *Org. Lett*. **2000**, *2*, 1713-16.

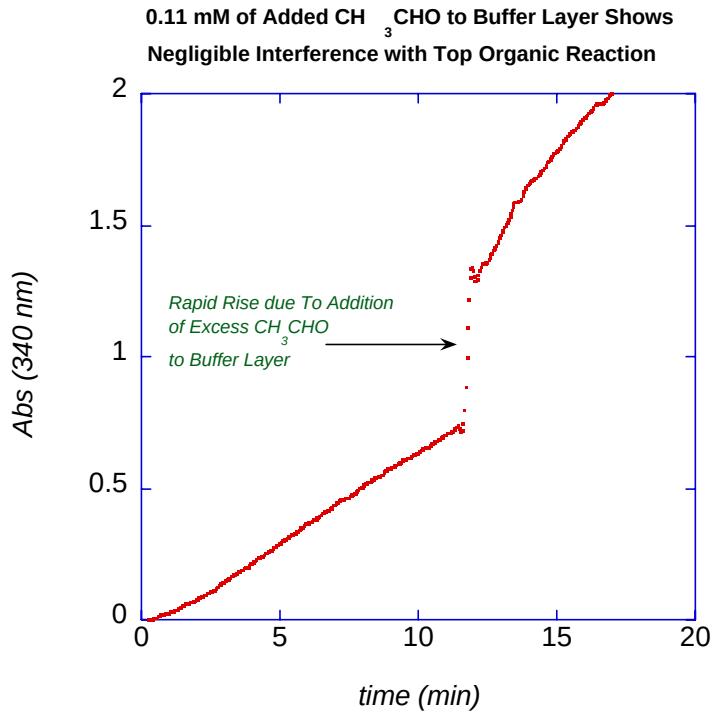
Reviews: a) S. Ribe; P. Wipf *Chem. Comm*. **2001**, *4*, 299-307; b) A. Lubineau, J. Auge in *Modern Solvents in Organic Synthesis* (Ed. P. Knochel), Springer Verlag, Berlin, 1999; c) P. A. Grieco, *Organic Synthesis in Water*, Blackie Academic & Professional, Hampshire, 1998; d) C. J. Chan; T. H. Chan, *Organic Reactions in Aqueous Media*, John Wiley, New York, 1997.

7. Effect of CH_3CHO on the TM-Catalyzed Allylic Amination Rate:

The $Ni(cod)_2/PPh_3$ catalyzed intramolecular allylic amination reaction of **1a** was chosen for this study.

7A. Direct Addition to Buffer Layer*:* CH_3CHO (10 μL of a 10 mM solution in deionized water; 0.11 mM final aq cuvet conc) was added via a microliter syringe to the lower buffer layer, at 11.6 min after the start of Ni^0 catalyzed intramolecular allylic amination reaction of **1a** in the top organic layer of the cuvet (this reaction of **1a** catalyzed by $Ni(cod)_2/PPh_3$ is identical to that described in the section *Typical Procedure for TM-Catalyzed Intramolecular Allylic Amination*). The normal rate profile (ADH-AlDH-NAD biphasic ISES assay) for the Ni^0 catalyzed reaction showed a sharp rise in absorbance (resulting from rapid oxidation of the added CH_3CHO to acetic acid) followed by gradual return to the rate prior to the addition.

Figure S5



7B. Addition to Organic Layer (Use of a ADH-APAD assay):

The buffer layer is prepared as follows:

Stock solution	Volume Pipetted	Final Aq. Cuvet Conc
37 mM, APAD in 25 mM $NaPO_4$, pH 7	50 µL	2.1 mM
Yeast ADH (0.11 U/µL) in 25mM $NaPO_4$, pH 7	12 µL	1.3 U
15 mM sodium pyrophosphate, pH 8.8	838 µL	

Final assay pH was 7.7.

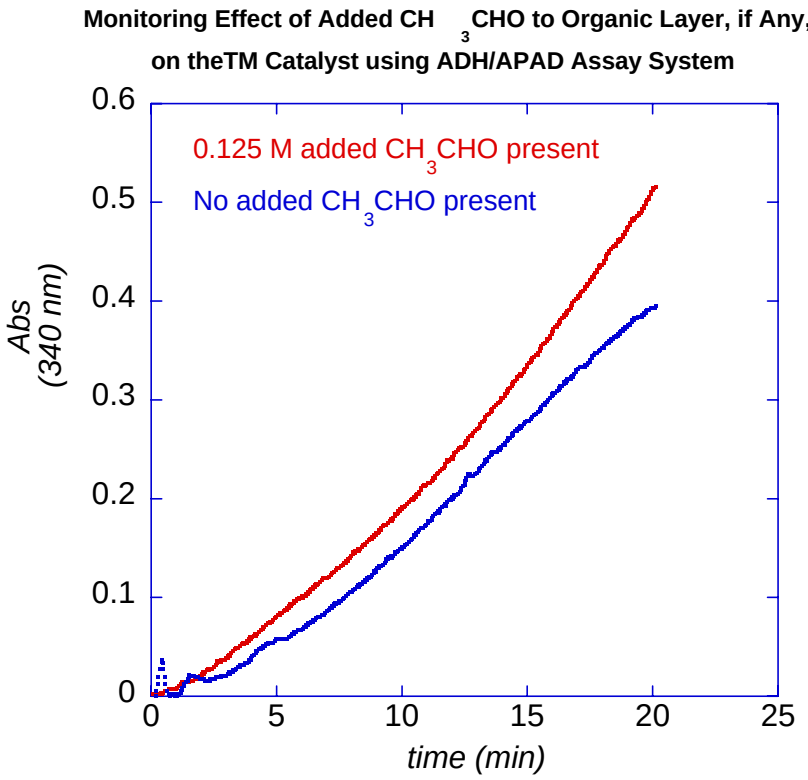
The organic layer is identical in scale and composition to that in the $Ni(cod)_2/PPh_3$ catalyzed intramolecular allylic amination reaction of **1a** described in the section *Typical Procedure for TM-Catalyzed Intramolecular Allylic Amination.* This formed the top layer in the cuvet. Two consecutive runs were made in duplicate:

a) The absorbance at 365 nm vs time is recorded first without any added CH_3CHO. Slope obtained was: 0.020 ± 0.004 Abs min^{-1}; b) In the second run CH_3CHO (50 μL of a 1 M solution in toluene; 0.125 M final concentration in the organic layer) was added to the organic layer immediately following the addition of **1a** and increase in absorbance at 365 nm vs. time was recorded. Slope obtained was: $(0.020 \pm 0.005$ Abs min^{-1}.

Hence, no inhibitory effect of added CH_3CHO on the rate of Ni^0 catalyzed intramolecular allylic amination reaction of **1a** was observed.

Experiment **7B** serves as a more stringent test of potential acetaldehyde interference with the Ni^0-mediated allylic substitution reaction where the acetaldehyde was introduced directly into the organic layer. Since a much higher concentration of acetaldehyde was considered for this addition , we chose to remove the second reporting enzyme, AlDH. This would prevent the formation of a huge NADH spike from oxidation of the acetaldehyde. For this purpose, we were able to employ a single reporting enzyme by simply substituting APAD+ (3-Acetylpyridine Adenine Dinucleotide) in place of NAD^+. $APAD^+$ (redox potential = -258 mV, λ_{max} = 365 nm) is a better oxidizing agent than NAD^+ ((redox potential = -320 mV) and so, though a bit more costly, provides for a single enzyme assay of released EtOH that gives acceptable rates. Even when 50 μmol of acetaldehyde was added directly to the organic layer, no reduction in the rate of NADH oxidation (of released EtOH) was observed. This experiment indicates that even 125 mM concentrations of acetaldehyde in the organic layer apparently do not interfere with the Ni^0-mediated allylic substitition reaction under study.

Figure S6

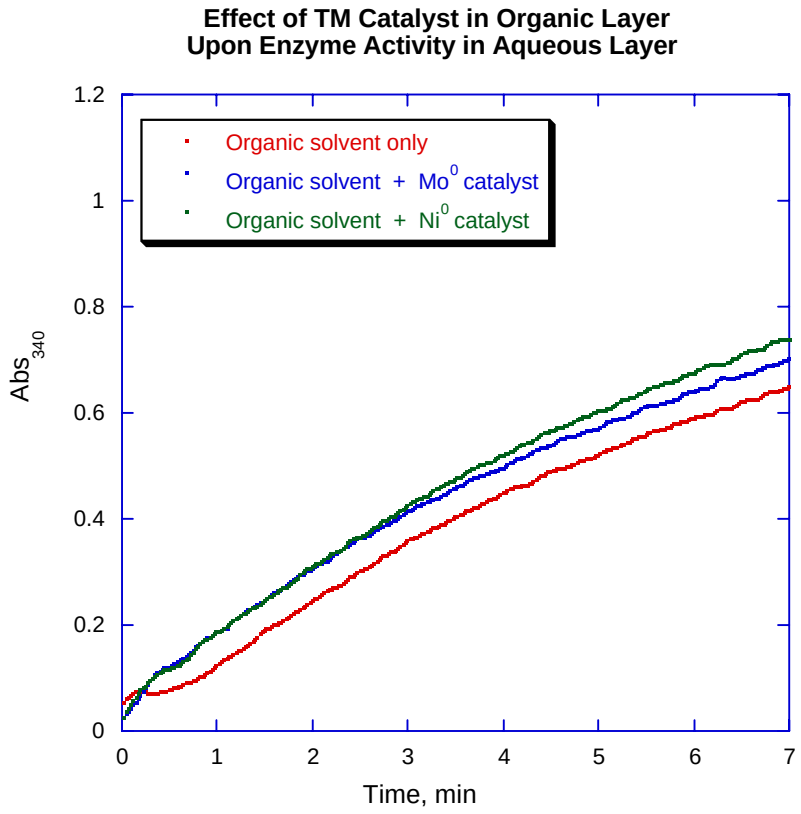


8. Test for Enzyme Inhibition by TM Catalysts in the Organic Layer

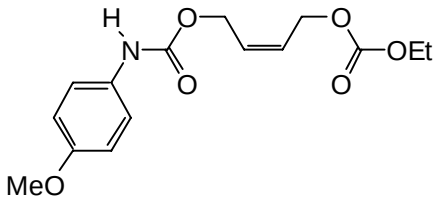
In order to assess the possible effect of a TM complex in organic layer upon enzyme activity in the neighboring organic layer, the following control experiment was performed. Two cuvets –one containing TM complex-ligand and the other containing only solvent in the organic layer were compared side-by-side for their relative rates of EtOH oxidation in the aqueous layer. The control cuvet contained a 400 µL organic layer composed exclusively of solvent (THF:toluene:hexane 2:1:1) and a 900 µL aqueous layer identical to that of a typical screen (vide supra). The TM-cuvets also contained either 11 µmol $[Ni(cod)_2]$/4 PPh_3 or 55 µmol $[Mo(C_7H_8)(CO)_3]$/3 PPh_3, but were otherwise identical to

the control cuvet. Reactions were initiated by injection of 0.18 µmol (200 µM final conc in the aqueous layer) of EtOH into the buffer layer. No inhibition of the rate of formation of NADH was seen in the cuvets containing either the Ni^0 complex or the Mo^0 complex.

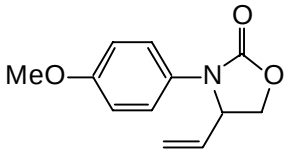
Figure S7



Synthetic Chemistry



Ethyl (2Z)-4-[(p-methoxyanilino)carbonyloxy]-2-butenyl Carbonate (1a). To a solution of ethyl (2Z)-4-hydroxy-2-butenyl carbonate[2] (5.13 g, 32 mmol) in THF (40 mL) at 0°C were added sequentially pyridine (3.89 mL, 48 mmol) and p-methoxyphenyl isocyanate (6.23 mL, 48 mmol) via syringe. The solution was allowed to warm slowly to rt over 12 h. Ethyl ether was added into the reaction mixture and the organic layer was washed with sat'd $CuSO_4$ solution. After drying ($MgSO_4$), filtration and evaporation, the crude product was purified by SiO_2 chromatography (33→50% EtOAc-hexanes) to provide **1a** (9.67 g, 98%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.28 (t, J = 7 Hz, 3 H), 3.75 (s, 3 H), 4.18 (q, J = 7 Hz, 2 H), 4.71-4.76 (m, 4 H), 5.72-5.84 (m, 2 H), 6.75 (br s, 1 H), 6.79-6.84 (m, 2 H), 7.26 (br d, J = 7 Hz, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2, 55.5, 60.4, 63.0, 64.1, 114.1, 120.7, 127.3, 128.8, 130.7, 153.5, 154.9, 156.0; HRMS (FAB, 3-NBA) calcd for $C_{15}H_{19}NO_6$ [$(M+H)^+$] 310.1290, obsd 310.1282; [M^+] 309.1212, obsd 309.1211.

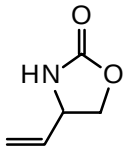


N-(p-methoxyphenyl)-4-vinyl-2-oxazolidinone (2a).

Method A. To a solution of $Ni(cod)_2$ (13.2 mg, 48 μmol) and TPP (25 mg, 96 μmol) in THF (2 mL) was cannulated **1a** (75 mg, 0.24 mmol) in THF (1 mL), and then LiHMDS

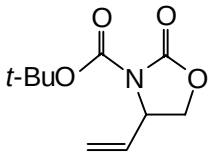
(1.0M in hexane, 0.24 mL, 0.24 mmol) was added slowly via syringe. The reaction mixture was stirred for 1 h and quenched with NH_4Cl (aq.) followed by extraction with ethyl ether. The organic layer was dried ($MgSO_4$), filtered, concentrated and chromatographed (25→33% EtOAc-hexanes) to yield **2a** (47 mg, 89%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.77 (s, 3 H), 4.08 (dd, J = 7, 9 Hz, 1 H), 4.56 (t, J = 9 Hz, 1 H), 4.71-4.78 (m, 1 H), 5.27 (dd, J = 0.7, 10 Hz, 1 H), 5.30 (dd, J = 0.7, 17 Hz, 1 H), 5.76 (ddd, J = 8, 10, 17 Hz, 1 H); 6.87 (d, J = 9 Hz, 2 H), 7.27 (d, J = 9 Hz, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.3, 60.2, 66.9, 114.1, 120.6, 124.0, 129.6, 134.7, 156.0, 157.1; IR (ATR) 1752, 2934 cm^{-1}; HRMS (FAB, 3-NBA, NaI) calcd for $C_{12}H_{13}NO_3Na$ ($M+Na^+$) 242.0793, obsd 242.0790.

Method B. A mixture of $Ni(PPh_3)_2Cl_2$ (423 mg, 0.65 mmol), TPP (339 mg, 1.29 mmol) and zinc dust (83 mg, 1.29 mmol) in THF (20 mL) was stirred under Ar for 0.5 h at rt to generate Ni(0), resulting in a change of color from green-blue to reddish brown. To this was added a solution of **1a** (1 g, 3.23 mmol) in THF (10 mL) via cannula, followed by LiHMDS (1.0 M in hexane, 3.23 mL, 3.23 mmol), slowly via syringe. The reaction mixture was stirred for 30 min, quenched with NH_4Cl (aq.) and then extracted with ethyl ether. The organic layer was dried ($MgSO_4$), filtered, concentrated, and chromatographed (25→33% EtOAc-hexanes) to yield **2a** (598 mg, 85%).

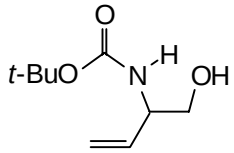


4-Vinyl-2-oxazolidinone. To a solution of **2a** (1.73 g, 7.90 mmol) in CH_3CN (175 mL) was added CAN (13.0 g, 23.7 mmol) in H_2O (87 mL) dropwise at 0 °C. The reaction

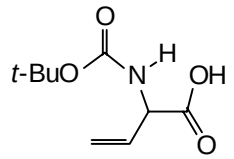
mixture was stirred at 0 °C for 20 min and then quenched with sat'd aqueous sodium sulfite followed by extraction with ethyl acetate. After drying ($MgSO_4$) and evaporation, the residue was purified by silica gel column chromatography (33→50% EtOAc-hexanes) to afford the vinyl oxazolidinone (694 mg, 78%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.97 (dd, J = 6, 8 Hz, 1 H), 4.28-4.36 (m, 1H), 4.45 (appt, J = 8 Hz, 1 H), 5.15 (dt, J = 1, 10 Hz, 1 H), 5.24 (dt, J = 1, 17 Hz, 1 H), 5.74 (ddd, J = 7, 10, 17 Hz, 1 H), 6.81 (br s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 54.9, 69.8, 118.1, 135.6, 160.0; HRMS (FAB, 3-NBA) calcd for $C_5H_8NO_2$ [$(M+H)^+$] 114.0555, obsd 114.0551.



N-(*tert*-Butoxycarbonyl)-4-vinyl-2-oxazolidinone. To a solution of vinyl oxazolidinone (0.35 g, 3.12 mmol) in CH_2Cl_2 (30 mL) and Et_3N (0.65 mL, 4.68 mmol) was added a solution of Boc_2O (2 g, 9.37 mmol) in CH_2Cl_2 (10 mL) at rt. The reaction mixture was stirred for 60 h. The reaction was quenched with H_2O and extracted with CH_2Cl_2, dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by silica gel column chromatography (20% EtOAc-hexanes) to yield the Boc-protected vinyl oxazolidinone (583 mg, 88%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (s, 9 H), 3.98 (dd, J = 4, 9 Hz, 1 H), 4.39 (appt, J = 9 Hz, 1 H), 4.67 (ddd, J = 4, 7, 9 Hz, 1 H), 5.26 (d, J = 10 Hz, 1 H), 5.27 (d, J = 17 Hz, 1 H), 5.82 (ddd, J = 7, 10, 17 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.7, 57.1, 66.6, 83.7, 118.5, 134.4, 148.9, 152.1; IR (ATR) 1724, 1811, 2981 cm^{-1}; HRMS (FAB, 3-NBA) calcd for $C_{10}H_{16}NO_4$ [$(M+H)^+$] 214.1079, obsd 214.1073.

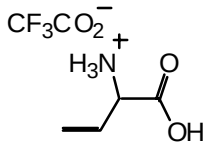


N-(*tert*-Butoxycarbonyl)-2-vinylglycinol. To a solution of Boc-protected vinyl oxazolidinone (1.13 g, 5.30 mmol) in MeOH (50 mL) was added Cs_2CO_3 (345 mg, 1.06 mmol). After the reaction mixture was stirred for 1.5 h, saturated aqueous NH_4Cl solution was added and MeOH was evaporated. The product was extracted with CH_2Cl_2, dried ($MgSO_4$) and concentrated. Flash chromatography (25→33% EtOAc-hexanes) gave the title compound (872 mg, 88%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 (s, 9 H), 2.49 (br s, 1 H), 3.60 (dd, J = 5, 11 Hz, 1 H), 3.69 (dd, J = 4, 11 Hz, 1 H), 4.22 (br s, 1 H), 4.94 (br s, 1 H) 5.21 (d, J = 10 Hz, 1 H), 5.25 (d, J = 16 Hz, 1 H), 5.79 (ddd, J = 5, 10, 16 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.1, 54.4, 64.4, 115.9, 135.6, 155.9; IR (ATR) 1683, 2977, 3337 cm^{-1}; HRMS (FAB, 3-NBA, LiI) calcd for $C_9H_{17}NO_3Li$ ($M+Li^+$) 194.1368, obsd 194.1359.

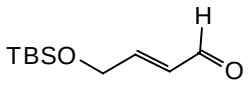


N-(*tert*-Butoxycarbonyl)-2-vinylglycine. To a solution of N-(*tert*-butoxycarbonyl)-2-vinylglycinol (50 mg, 0.267 mmol) in acetone (5 mL) was added Jones reagent (4 M, 0.2 mL, 0.8 mmol) at 0 °C over 10 min and the reaction mixture was then stirred for 3 h at rt. Excess Jones reagent was quenched by addition of *i*-PrOH. The acetone and *i*-PrOH were removed under reduced pressure, and the residue was partitioned between water and ethyl

acetate. The organic phase was separated and the product was extracted into saturated aqueous Na_2CO_3 solution. Acidification of the aqueous phase to pH 4 with acetic acid followed by extraction with ethyl acetate, drying ($MgSO_4$), filtration and evaporation gave Boc-protected vinylglycine (44 mg, 82%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.37 (s, 9 H), 4.51 (dd, *J* = 6, 7 Hz, 1 H), 5.17 (d, *J* = 10 Hz, 1 H), 5.28 (d, *J* = 17 Hz, 1 H), 5.87 (ddd, *J* = 6, 10, 17 Hz, 1 H), 7.30 (d, *J* = 7 Hz, 1 H), 12.65 (br s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.2, 56.2, 78.2, 117.1, 133.1, 155.2, 172.0; HRMS (FAB, 3-NBA, NaI) calcd for C9H15NO4 Na (M+Na+) 224.0899, obsd 224.0893.

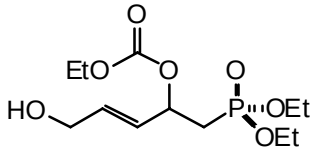


α-Vinylglycine, Trifluoroacetate Salt (3). To a solution of Boc-protected vinylglycine (82 mg, 0.41 mmol) in CH_2Cl_2 (10 mL) at 0 °C was added CF_3COOH (3 mL). After 3 h at room temperature, H_2O was added, followed by extraction with CH_2Cl_2 and Et_2O. Evaporation of the aqueous layer, followed by thorough drying in vacuo (Δ, P_2O_5 sidearm) provided **3**[3] (74 mg, 84%): ^{1}H NMR (300 MHz, D_2O) δ 4.58 (d, *J* = 7 Hz, 1 H), 5.54 (d, *J* = 17 Hz, 1 H), 5.55 (d, *J* = 110 Hz, 1 H), 5.97 (ddd, *J* = 7, 10, 17 Hz, 1 H); HRMS (FAB, 3-NBA) calcd for $C_4H_{18}NO_2$ [$(M+H)^+$] 102.0555, obsd 102.0554.



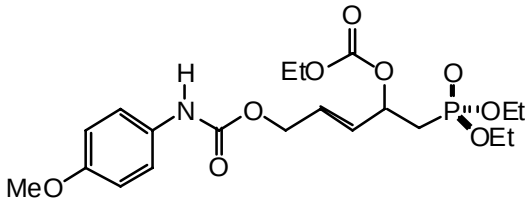
(E)-4-*tert*-Butyldimethylsilyloxy-2-butenal (4). To a solution of oxalyl chloride (21 mL, 24.7 mmol, 2.0 M in CH_2Cl_2) in CH_2Cl_2 (30 mL) at -78 °C was added a solution of DMSO (3.3 mL, 49.4 mmol) in CH_2Cl_2 (30 mL) via cannula. After stirring for 10 min

at -78 °C, a solution of starting alcohol[4] (5.0 g, 24.7 mmol) in CH_2Cl_2 (50 mL) was added, dropwise via cannula. After an additional 30 min at -78 °C, a solution of NEt_3 (12.05 mL, 86.48 mmol) in CH_2Cl_2 (20 mL) was added in the same manner. After 1 h at -78 °C, Et_2O was then added at -78 °C and the reaction mixture was allowed to warm to rt. The crude reaction mixture was then poured into Et_2O and extracted sequentially with H_2O and sat'd NH_4Cl. After drying ($MgSO_4$), filtration and evaporation, column chromatography (10% EtOAc/hexane) provided aldehyde **4** (4.01 g, 81 %): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.06 (s, 6 H), 0.89 (s, 9 H), 4.43 (dd, J = 2, 3 Hz, 2 H), 6.37 (ddt, J = 2, 8, 16 Hz, 1 H), 6.86 (dt, J = 5, 16 Hz, 1 H), 9.85 (d, J = 8 Hz, 1 H); IR (ATR) 1690, 2955 cm^{-1}; ^{13}C NMR (75 MHz, $CDCl_3$) δ 5.5, 18.3, 25.7, 62.2, 130.5, 156.4, 193.3.



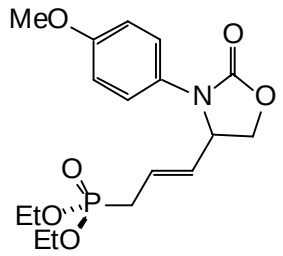
Ethyl (2E)-1-(Diethylphosphonomethyl)-4-hydroxy-2-butenyl Carbonate. To a solution of diethyl methylphosphonate (292 mg, 1.92 mmol) in THF (5 mL) at -78°C was added n-BuLi (1.34 mL, 1.92 mmol, 1.43 M in hexanes) dropwise via syringe. After stirring for 30 min at -40°C, a solution of **4** (320 mg, 1.60 mmol) in THF (5 mL) was added, dropwise, via cannula. After 30 min at the same temperature, ethyl chloroformate (0.38 mL, 2.40 mmol) was added slowly, and the reaction was allowed to warm to rt. Then 0.3 M HCl solution (5 mL) was added and stirring continued overnight. The reaction mixture was extracted with CH_2Cl_2 and dried over $MgSO_4$ and concentrated in vacuo. Purification by a silica gel column chromatography (EtOAc) to yielded the title carbonate

(336 mg, 68%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26-1.33 (m 9 H), 2.07 (s, 1 H), 2.13 (ddd, *J* = 7, 15, 19 Hz, 1 H), 2.30 (ddd, *J* = 7, 15, 118 Hz, 1 H), 4.04-4.21 (m, 8 H), 5.35-5.45 (m, 1 H), 5.78 (ddt, *J* = 2, 7, 16 Hz, 1 H), 5.98 (ddt, *J* = 1, 5, 16 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.8, 15.8, 15.9, 31.*1* (d, J = 140.8 Hz), 61.2, *6*1.58 (d, J = 5.5 *H*z), 61.65 (d, J = 5.5 Hz), 63.6, 72.5, 126.27 (d, J = 8.8 Hz), 134.0, 153.6; 31P NMR (121 MHz, $CDCl_3$) δ 25.17; HRMS (FAB, 3-NBA) calcd for $C_{12}H_{23}O_7P$ [$(M+H)^+$] 311.1260, obsd 311.1267.



Diethyl 2-Ethoxycarbonyloxy-5-[(p-methoxyanilino]carbonyloxy]-(3E)-pentenylphosphonate (5). To a solution of the preceding alcohol (1.14 g, 3.67 mmol) in THF (30 mL) at 0 °C were added sequentially pyridine (0.45 mL, 5.51 mmol) and p-methoxyphenyl isocyanate (0.57 mL, 4.41 mmol), via syringe. After allowing the reaction mixture to warm to rt and stir for 36 h, ether was added. The organic phase was washed with sat'd $CuSO_4$ solution. After drying ($MgSO_4$), filtration and evaporation, the crude product was purified by SiO_2 chromatography (50→100% EtOAc-hexanes) to provide **5** (1.63 g, 96%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20 (t, *J* = 7 Hz, 3 H), 1.23 (t, *J* = 7 Hz, 6 H), 2.06 (ddd, *J* = 6, 15, 19 Hz, 1 H), 2.22 (ddd, *J* = 7, 15, 17 Hz, 1 H), 3.68 (s, 3 H), 4.02 (overlapping app q, *J* = 7 Hz, 4 H), 4.10 (q, *J* = 7 Hz, 2 H), 4.56 (d, *J* = 5 Hz, 2 H), 5.31-5.41 (m, 1 H), 5.77 (dd, *J* = 6, 16 Hz, 1 H), 5.87 (dt, *J* = 5, 16 Hz, 1 H), 6.72-6.77 (m, 2H), 7.26 (br d, *J* = 9 Hz, 2 H), 7.52 (br s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 16.0, 16.1, 31.24 (d, *J* = 140.8 Hz), 55.2, 61.74 (d, *J* = 5.5 Hz), 63.79 (d, *J* = 17.6 Hz), 72.2, 113.9,

120.4, 128.3, 129.94 (d, J = 9.9 Hz), 131.1, 153.5, 153.7, 155.6; ^{31}P NMR (121 MHz, $CDCl_3$) δ 24.95; IR (ATR) 1250, 1730, 2981, 3250 cm^{-1}; HRMS (FAB, 3-NBA, LiI) calcd for $C_{20}H_{30}NO_9PLi$ ($M+Li^+$) 466.1818, obsd 466.1835.

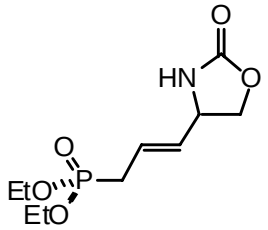


N-(p-Methoxyphenyl)-4-[(3'-diethylphosphono)-1'E-propenyl]-2-oxazolidinone (6). *Method A.* To a solution of $Ni(cod)_2$ (12 mg, 44 μmol) and dppb (19 mg, 44 μmol) in THF (3.5 mL), was cannulated **5** (100 mg, 0.22 mmol) and then LiHMDS (1 M in hexane, 0.22 mL, 0.22 mmol) was added slowly via syringe. The reaction mixture was stirred for 90 min and quenched with NH_4Cl (aq.) followed by extraction with ethyl ether. The organic layer was dried ($MgSO_4$), filtered, concentrated, and chromatographed (EtOAc) to yield **6** (64 mg, 79%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.13 (t, J = 7 Hz, 3 H), 1.16 (t, J = 7 Hz, 3 H), 2.47 (dd, J = 7, 21 Hz, 2 H), 3.69 (s, 3 H), 3.78-4.02 (m, 5 H), 4.49 (appt, J = 8 Hz, 1 H), 4.67-4.75 (m, 1 H), 5.50 (ddd, J = 5, 8, 15 Hz, 1 H), 5.60-5.72 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.1, 16.2, 29.9 (d, J = 139.7 Hz), 55.2, 59.3, 61.7 (d, J = 4.4 Hz), 61.8 (d, J = 4.4 Hz), 66.89 (d, J = 4.4 Hz), 114.0, 124.0, 126.1 (d, J = 11.0 Hz), 129.4, 131.5 (d, J= 14.3 Hz), 155.8, 157.0; ^{31}P NMR (121MHz, $CDCl_3$) δ 25.30; IR (ATR) 1245, 1747, 2981 cm^{-1}; HRMS (FAB, 3-NBA, LiI) calcd for $C_{17}H_{24}NO_6PLi$ ($M+Li^+$) 376.1501, obsd 376.1513.

Method B. To a solution of $Ni(cod)_2$ (12 mg, 44 μmol) and TPP (23 mg, 88 μmol) in THF (3.5 mL) was cannulated **5** (100 mg, 0.22 mmol) and then LiHMDS (1 M in hexane, 0.22

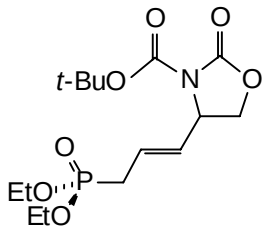
mL, 0.22 mmol) was added slowly via syringe. The reaction mixture was stirred for 90 min and quenched with NH_4Cl (aq.) followed by extraction with ethyl ether. The organic layer was dried ($MgSO_4$), filtered, concentrated, and chromatographed (EtOAc) to yield **6** (63 mg, 77%).

Method C. A mixture of $Ni(PPh_3)_2Cl_2$ (142 mg, 0.22 mmol), TPP (114 mg, 0.44 mmol), zinc dust (28 mg, 0.44 mmol), in THF (8 mL) were stirred for 0.5 h at rt under Ar to generate Ni(0) (color change from green-blue to reddish brown). Then **5** (0.5 g, 1.09 mmol) in THF (8 mL) was added, via cannula, followed by LiHMDS (1 M in hexane, 1.09 mL, 1.09 mmol). The reaction mixture was stirred for 2 h and then quenched with NH_4Cl (aq.) followed by extraction with ethyl ether. The organic layer was dried ($MgSO_4$), filtered, concentrated, and chromatographed (EtOAc) to yield **6** (280 mg, 70%).

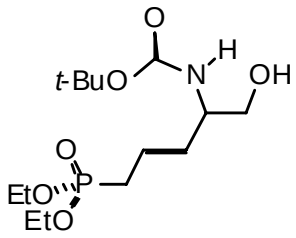


4-[(3'-Diethylphosphono)-1'E-propenyl]-2-oxazolidinone. To a solution of **6** (360 mg, 0.98 mmol) in CH_3CN (23 mL) was added CAN (1.60 g, 2.92 mmol) in H_2O (11 mL) dropwise at 0°C. The reaction mixture was stirred at 0°C for 20 min and quenched with sat'd aqueous sodium sulfite, followed by extraction with ethyl acetate. After drying ($MgSO_4$) and evaporation, the residue was purified by silica gel column chromatography (5% MeOH-EtOAc) to afford the title oxazolidinone (229 mg, 89%): ^1H NMR (300 MHz, $CDCl_3$) δ 1.27 (t, J = 7 Hz, 6 H), 2.55 (dd, J = 7, 22 Hz, 2 H), 3.95-4.10 (m, 5 H), 4.31-4.39 (m, 1 H), 4.67 (appt, J = 8 Hz, 1 H), 5.58 (ddd, J = 4, 7, 16 Hz, 1 H), 5.63-5.75 (m, 2 H);

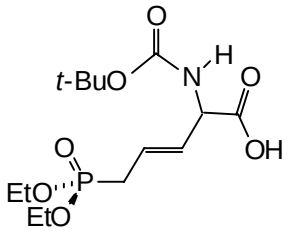
^{13}C NMR (75 MHz, $CDCl_3$) δ 16.0, 16.1, 29.55 (d, J = 139.7 Hz), 54.1(d, J = 2.2 Hz), 61.75 (d, J = 4.4 Hz), 61.82 (d, J = 4.4 Hz), 69.46 (d, J = 4.4 Hz), 123.22 (d, J = 11.0 Hz), 132.8 (d, J = 14.3 Hz), 155.8, 157.0; ^{31}P NMR (121 MHz, $CDCl_3$) δ 25.84; IR (ATR) 1224, 1746, 3237 cm^{-1}; HRMS (FAB, 3-NBA) calcd for $C_{10}H_{19}NO_5P$ [$(M+H)^+$] 264.1000, obsd 264.0992.



N-tert-Butoxycarbonyl-4-[(3'-diethylphosphono)-1'E-propenyl]-2-oxazolidinone. To a solution of the preceding oxazolidinone (220 mg, 0.84 mmol) in THF (15 mL) was added LiHMDS (1.0 M in THF, 1.25 mL) and then a solution of Boc_2O (547 mg, 2.50 mmol) in THF (3 mL) at rt. The reaction mixture was stirred for 30 min and quenched with H_2O. Extraction (EtOAc), drying ($MgSO_4$), concentration and chromatography (5% MeOH-EtOAc) yielded the Boc-protected oxazolidinone (258 mg, 85%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26 (t, J = 7 Hz, 6 H), 1.46 (s, 9 H), 2.56 (dd, J = 6, 22 Hz, 2 H), 3.95-4.09 (m, 4 H), 3.97 (dd, J = 4, 9 Hz, 1 H), 4.37 (appt, J = 9 Hz, 1 H), 4.63-4.70 (m, 1 H), 5.62 (ddd, J = 4, 7, 15 Hz, 1 H), 5.68-5.78 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.05, 16.10, 27.5, 29.7 (d, J = 139.7 Hz), 56.2, 61.6 (d, J = 6.6 Hz), 61.7 (d, J = 5.5 Hz), 66.5 (d, J = 3.3 Hz), 124.3 (d, J = 11.0 Hz), 131.0 (d, J = 14.3 Hz); ^{31}P NMR (121 MHz, $CDCl_3$) δ 25.58; IR (ATR) 1243, 1723, 1803, 2981 cm^{-1}; HRMS (FAB, 3-NBA) calcd for $C_{15}H_{27}NO_7P$ [$(M+H)^+$] 364.1525, obsd 364.1532.

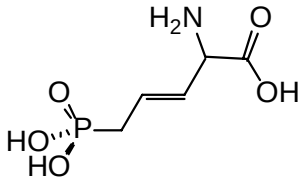


2-(tert-Butoxycarbonyl)amino-5-diethylphosphono-3E-pentenol. To a solution of Boc-protected oxazolidinone (250 mg, 0.69 mmol) in EtOH (20 mL) was added Cs_2CO_3 (45 mg, 0.14 mmol). After the reaction mixture was stirred for 1.5 h, saturated, aqueous NH_4Cl was added and the EtOH was evaporated. Following extraction CH_2Cl_2, drying ($MgSO_4$) and concentration, flash chromatography (5% MeOH-EtOAc) provided the title compound (220 mg, 95%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27 (t, J = 7 Hz, 6 H), 1.39 (s, 9 H), 2.56 (dd, J = 5, 22 Hz, 2 H), 3.50 (br s, 1 H), 3.56 (dd, J = 5, 11 Hz, 1 H), 3.61 (dd, J = 4, 11 Hz, 1 H), 3.99-4.12 (m, 1 H), 5.28 (br s, 1 H), 5.51-5.68 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.11, 16.19, 28.1, 29.7 (d, J = 139.7 Hz), 53.8, 61.95 (2 C), (d, J = 6.6 Hz), 64.37 (d, J = 2.2 Hz), 79.0, 119.9 (d, J = 9.9 Hz), 133.9 (d, J = 14.3 Hz), 155.5; ^{31}P NMR (121 MHz, $CDCl_3$) δ 27.20; IR (ATR) 1165, 1708, 2979, 3361 cm^{-1}; HRMS (FAB, 3-NBA) calcd for $C_{14}H_{29}NO_6P$ [$(M+H)^+$] 338.1733, obsd 338.1744.



2-(tert-Butoxycarbonyl)amino-5-diethylphosphono-3E-pentenoic Acid. To a solution of the Boc-protected alcohol (100 mg, 0.30 mmol) in acetone (5 mL) was added Jones reagent (4 M, 0.22 mL, 0.89 mmol) at 0 °C over 10 min, and the reaction mixture was stirred for 3 h at rt. Excess Jones reagent was quenched with *i*-PrOH. The acetone and

i-PrOH were removed under reduced pressure, and the residue was partitioned between water and ethyl acetate. The organic phase was separated and the product was extracted into saturated aqueous Na_2CO_3 solution. Acidification (pH 4) with acetic acid followed by extraction with EtOAc, drying ($MgSO_4$), filtration and evaporation gave the product (79 mg, 76%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.19 (t, J = 7 Hz, 6 H), 1.36 (s, 9 H), 2.60 (dd, J = 7, 22 Hz, 2 H), 3.95 (overlapping app q, J = 7 Hz, 1 H), 4.30-4.39 (m, 1 H), 5.43-5.55 (m, 1 H), 5.62-5.74 (m, 1 H), 6.80-6.89 (m, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 16.20, 16.27, 28.2, 29.0 (d, J = 152.9 Hz), 56.1, 61.23 (d, J = 2.2 Hz), 61.31 (d, J = 3.3 Hz), 120.3 (d, J = 12.1 Hz), 131.6 (br), 154.9, 171.7; ^{31}P NMR (121 MHz, DMSO-d_6) δ 27.23; HRMS (FAB, 3-NBA, NaI) calcd for $C_{14}H_{26}NO_7P$ [$(M+Na)^+$] 374.1345, obsd 374.1332.

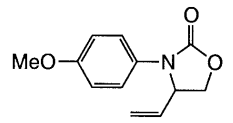


(E)-2-Amino-5-phosphono-3-pentenoic Acid (7). To a solution of Boc-acid (30 mg, 0.085 mmol) in CH_3CN (2 mL) at 0 °C was added TMSI (0.11 mL, 0.77 mmol). After allowing to warm to rt and stirring for 6 h, H_2O (≈ 200 μL) was added. Evaporation of the volatiles yields **7** (17 mg, ca. 70%). If desired, further purification may be achieved via flash chromatography {CH_3COCH_3/CH_2Cl_2/MeOH(45:45:10)→*i*-PrOH/CH_3CN/50mM NH_4HCO_3 (aq) (2:1:1)} followed by several lyophilization cycles, and then thorough drying in vacuo (P_2O_5 sidearm, 50°C), to provide a sample of **7** as the ammonium salt: ^{1}H NMR (500 MHz, D_2O) δ 2.53 (dd, J = 8, 21 Hz, 2 H), 4.29 (d, J = 9 Hz, 1 H), 5.67 (ddd, J

= 5, 9, 14 Hz, 1 H), 5.99-6.07 (m, 1 H); ^{13}C NMR (125 MHz, D_2O; HMBC) δ 33.8, 57.1; 124.3, 133.8, 173.9; ^{31}P NMR (202 MHz, D_2O) δ 18.44.

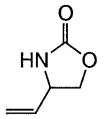
References for Supporting Information:

1. The United States Pharmacopeia/ The National Formulary (USP 24/NF 19), (National Publishing, Philadelphia, 1999; ISBN 1-889788-03-1), pp. 2004-5.
2. This monocarbonate ester may be synthesized in 82% yield from 2Z-butene-1,4-diol: C. Fernandez-Rivas, M. Mendez, A. M. Echavarren, *J. Am. Chem. Soc.* **2000**, *122*, 1221-1222.
3. D. B. Berkowitz, M. K. Smith, *Synthesis* **1996**, 39-41.
4. This monosilylated alcohol is available in 87% yield from 2Z-butene-1,4-diol: W. R. Roush, M. L. Reilly, K. Koyama, B. B. Brown, *J. Org. Chem.* **1997**, *62*, 8708-8721.

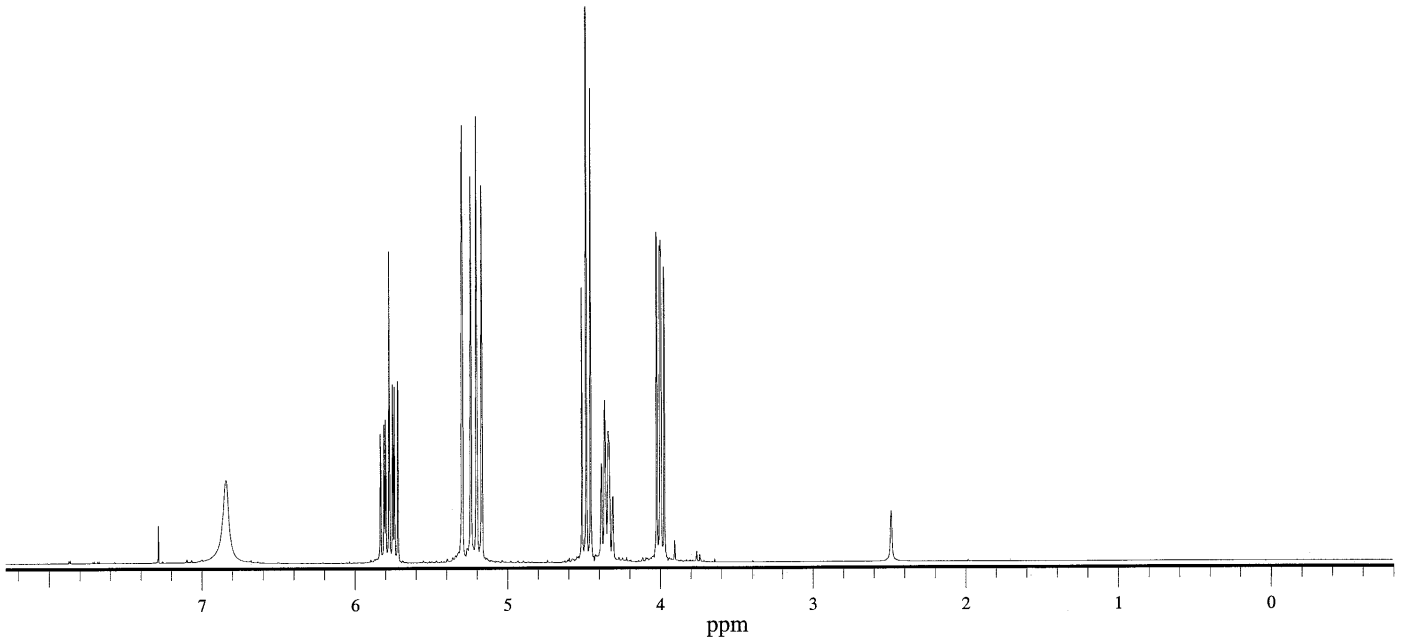


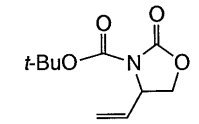
2a

^{1}H NMR (300 MHz, $CDCl_3$)

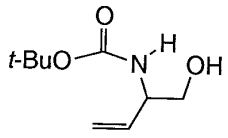


^{1}H NMR (300 MHz, $CDCl_3$)

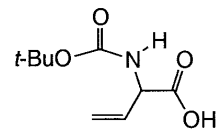




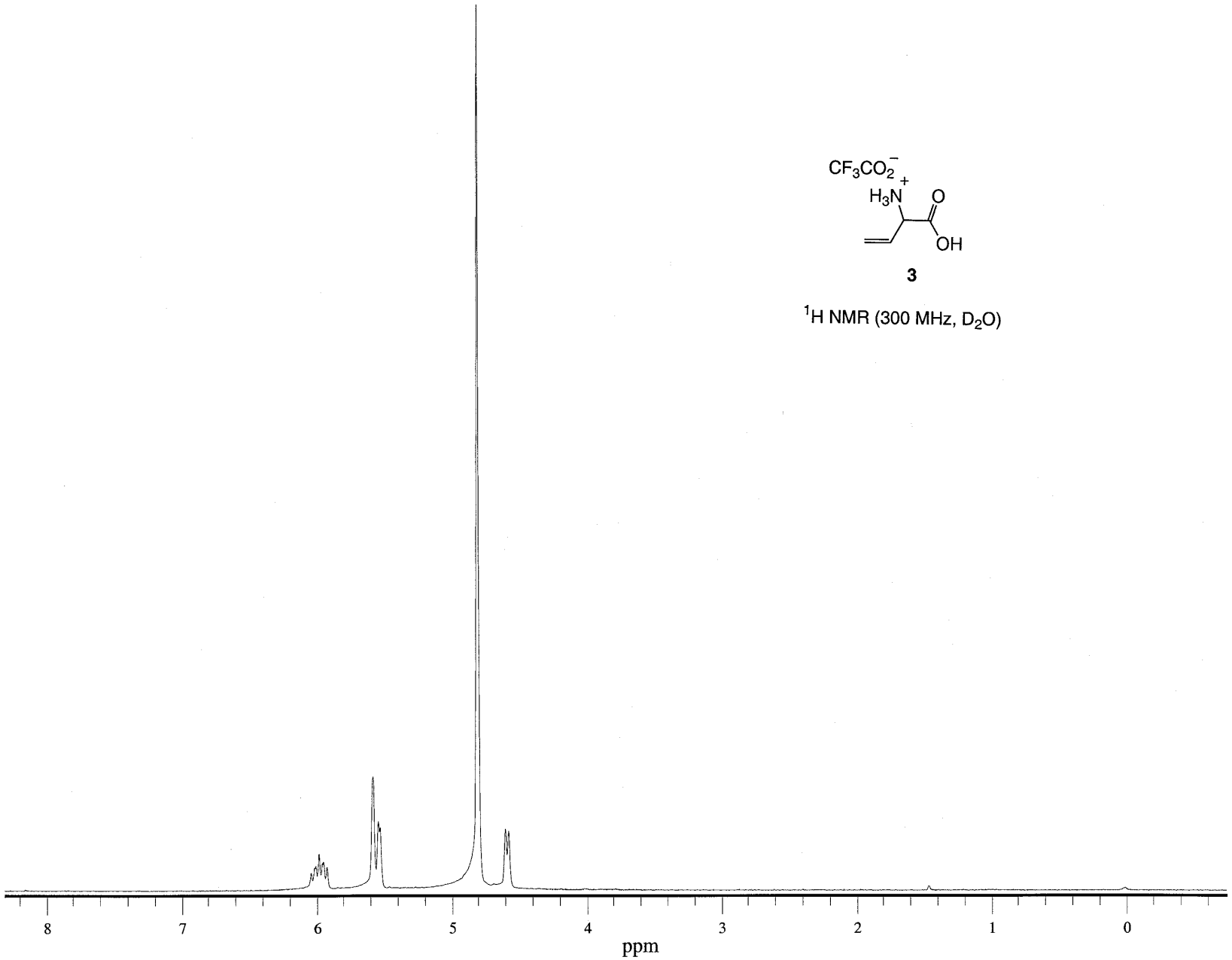
^{1}H NMR (300 MHz, $CDCl_3$)



^{1}H NMR (300 MHz, $CDCl_3$)

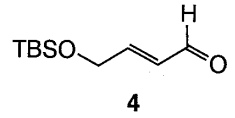


^{1}H NMR (300 MHz, DMSO-d_6)



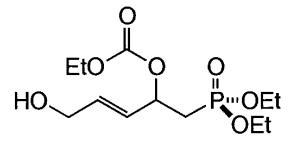
3

^{1}H NMR (300 MHz, D_2O)

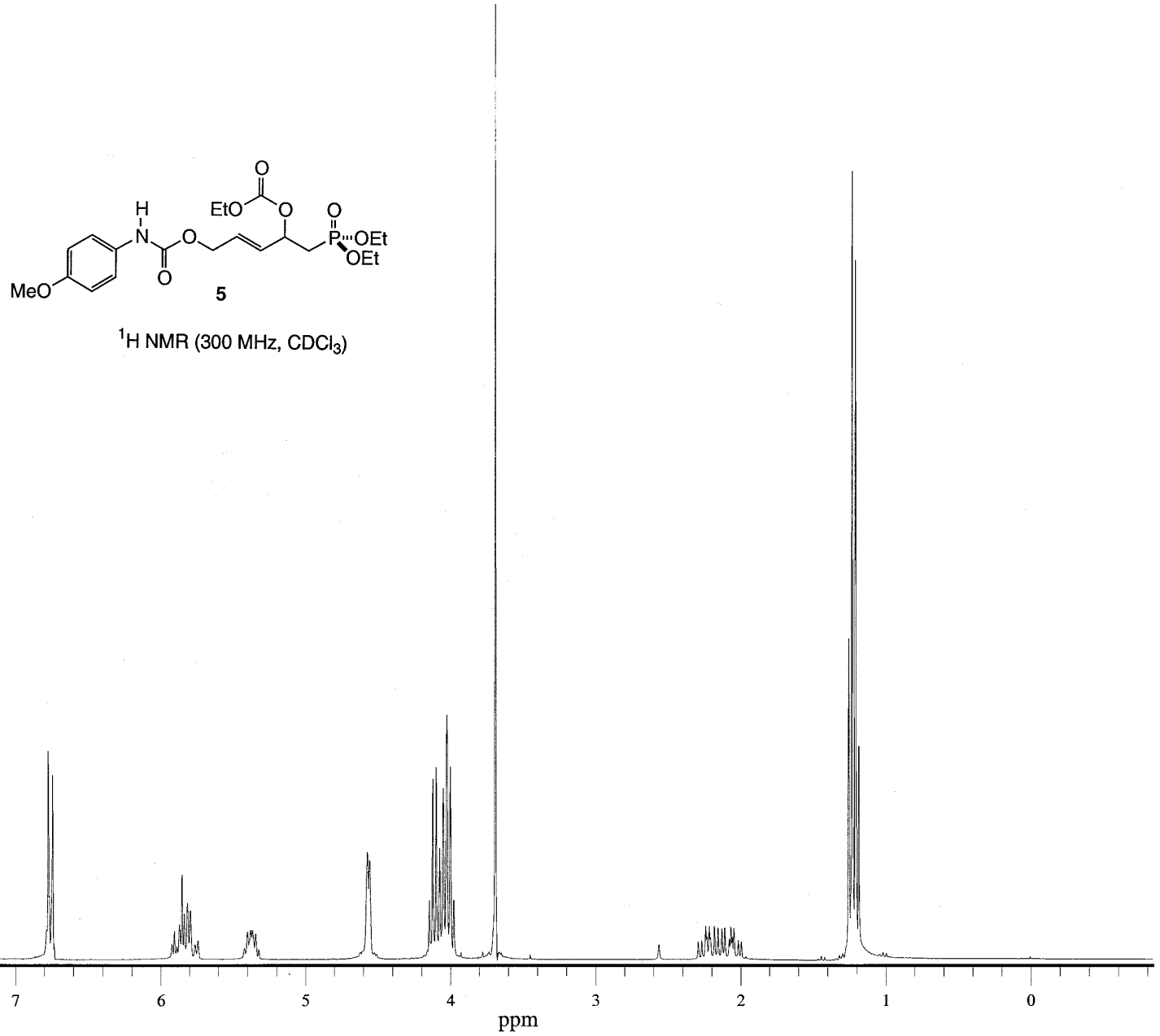


4

^{1}H NMR (300 MHz, $CDCl_3$)

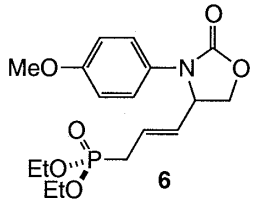


^{1}H NMR (300 MHz, $CDCl_3$)



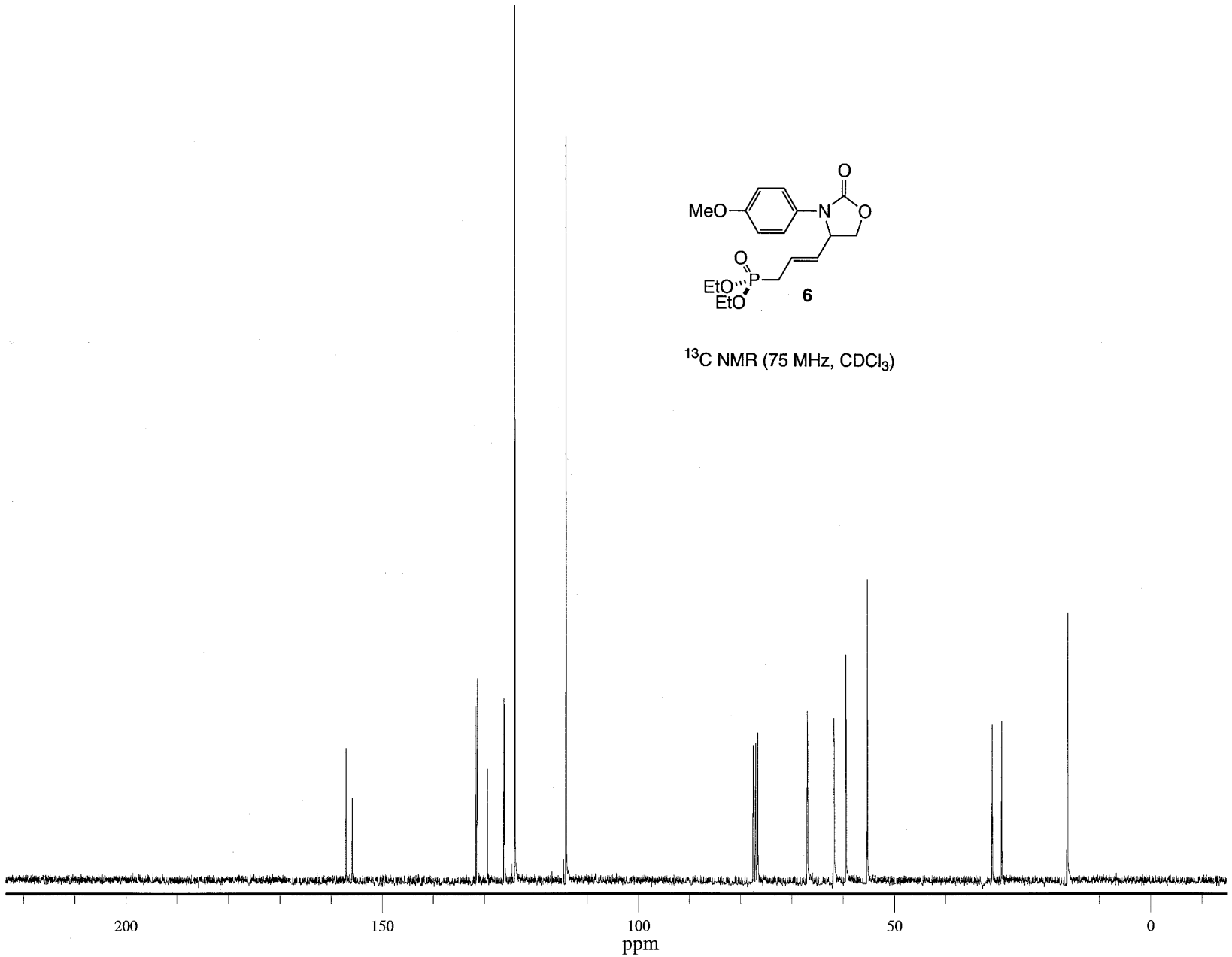
5

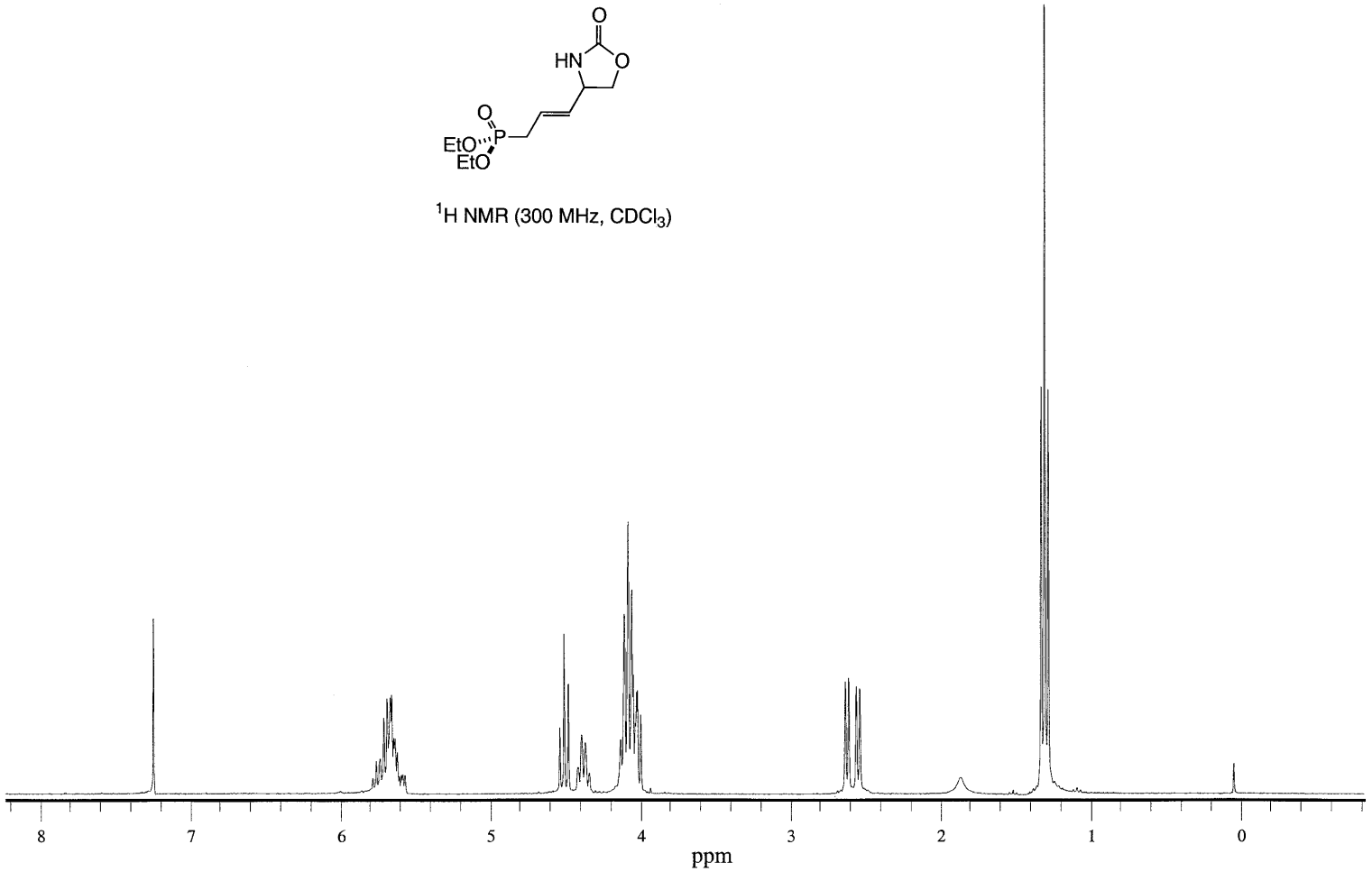
^{1}H NMR (300 MHz, $CDCl_3$)



6

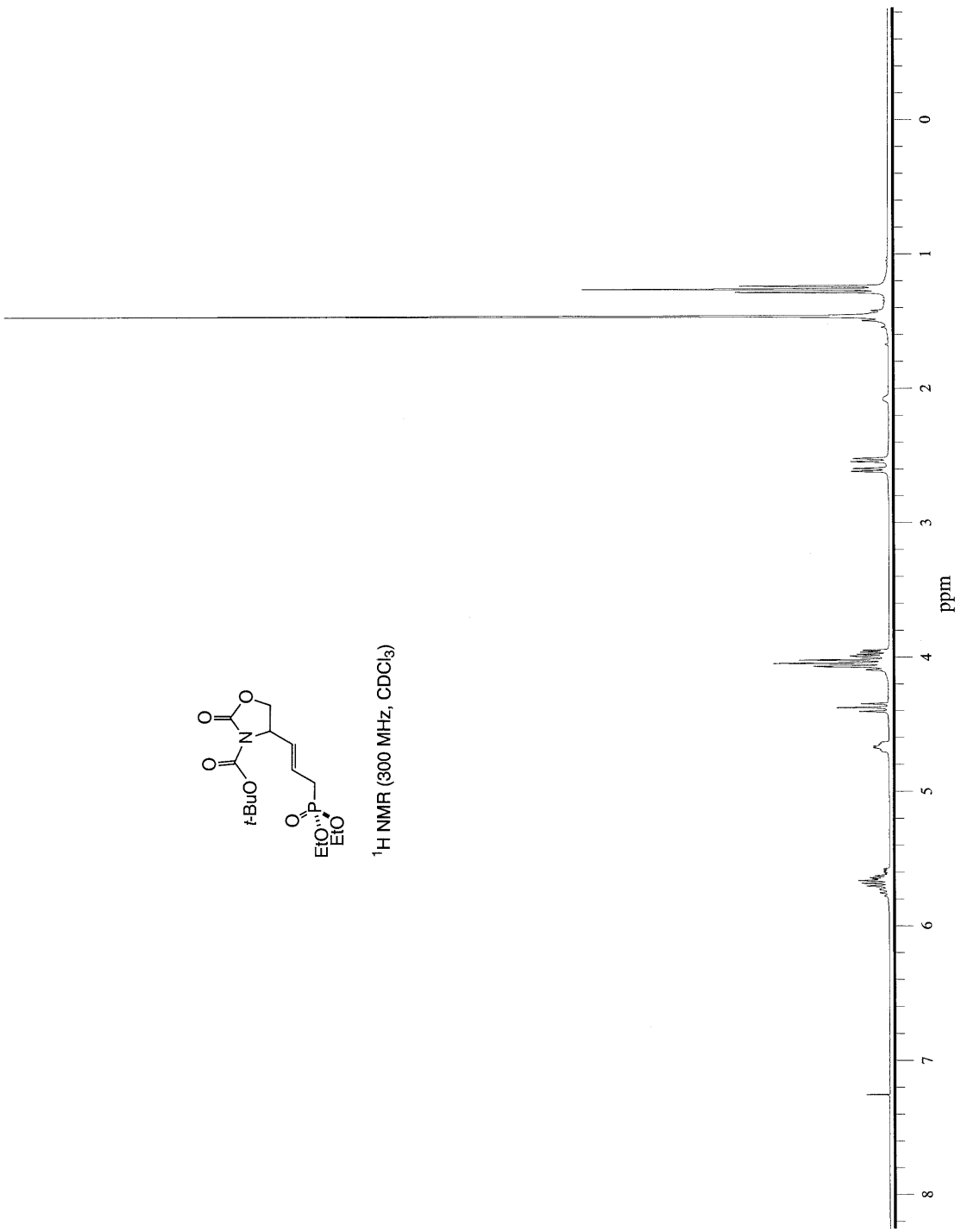
^{1}H NMR (300 MHz, $CDCl_3$)

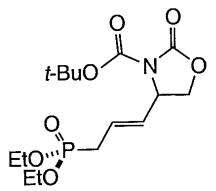

MeO
N
O
O
O
P
EtO
EtO
6
^{13}C NMR (75 MHz, $CDCl_3$)
200
150
100
50
0
ppm



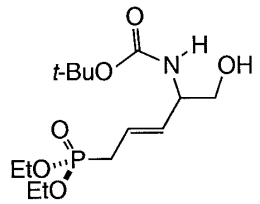
^{1}H NMR (300 MHz, $CDCl_3$)

^{13}C NMR (75 MHz, $CDCl_3$)
ppm
0
50
100
150
200

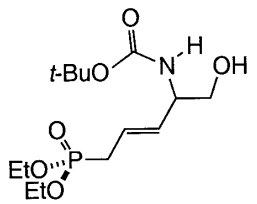

t-BuO
EtO
EtO
^{1}H NMR (300 MHz, CDCl$_3$)
ppm
0
1
2
3
4
5
6
7
8



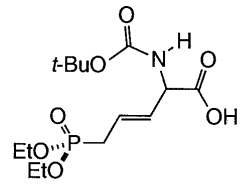
^{13}C NMR (75 MHz, $CDCl_3$)



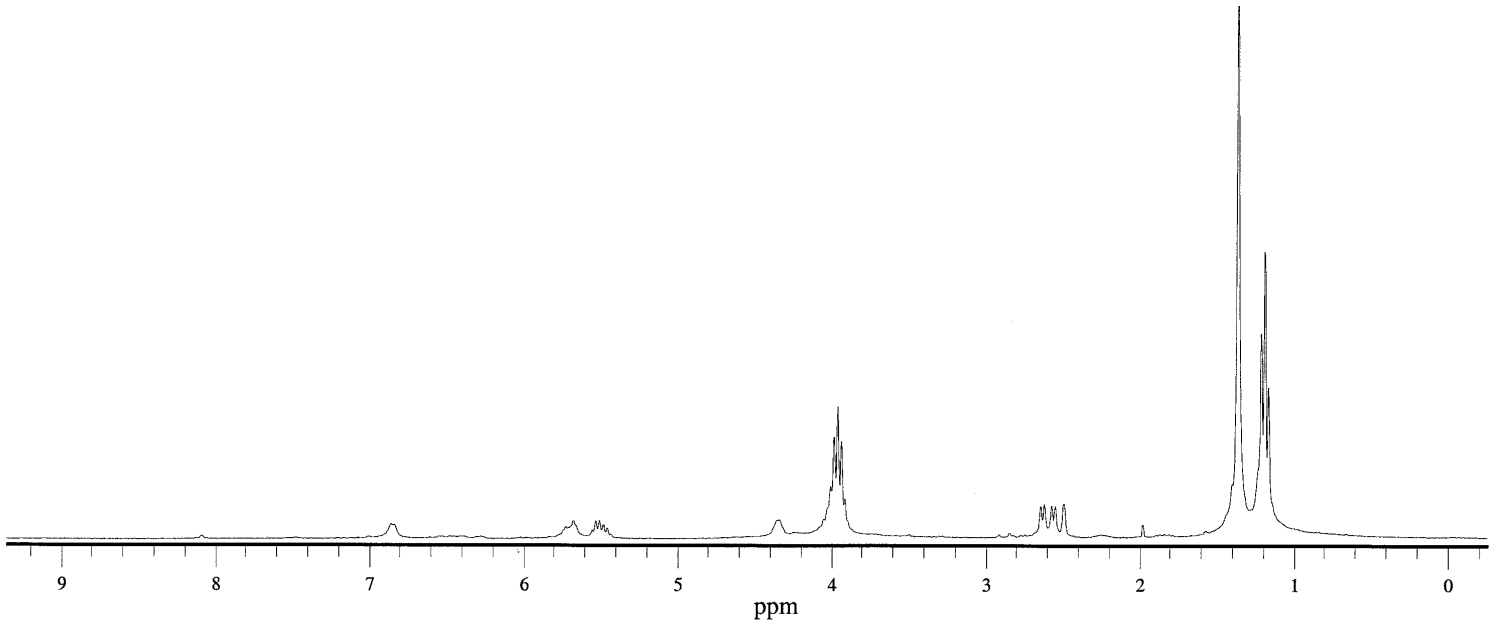
^{1}H NMR (300 MHz, $CDCl_3$)

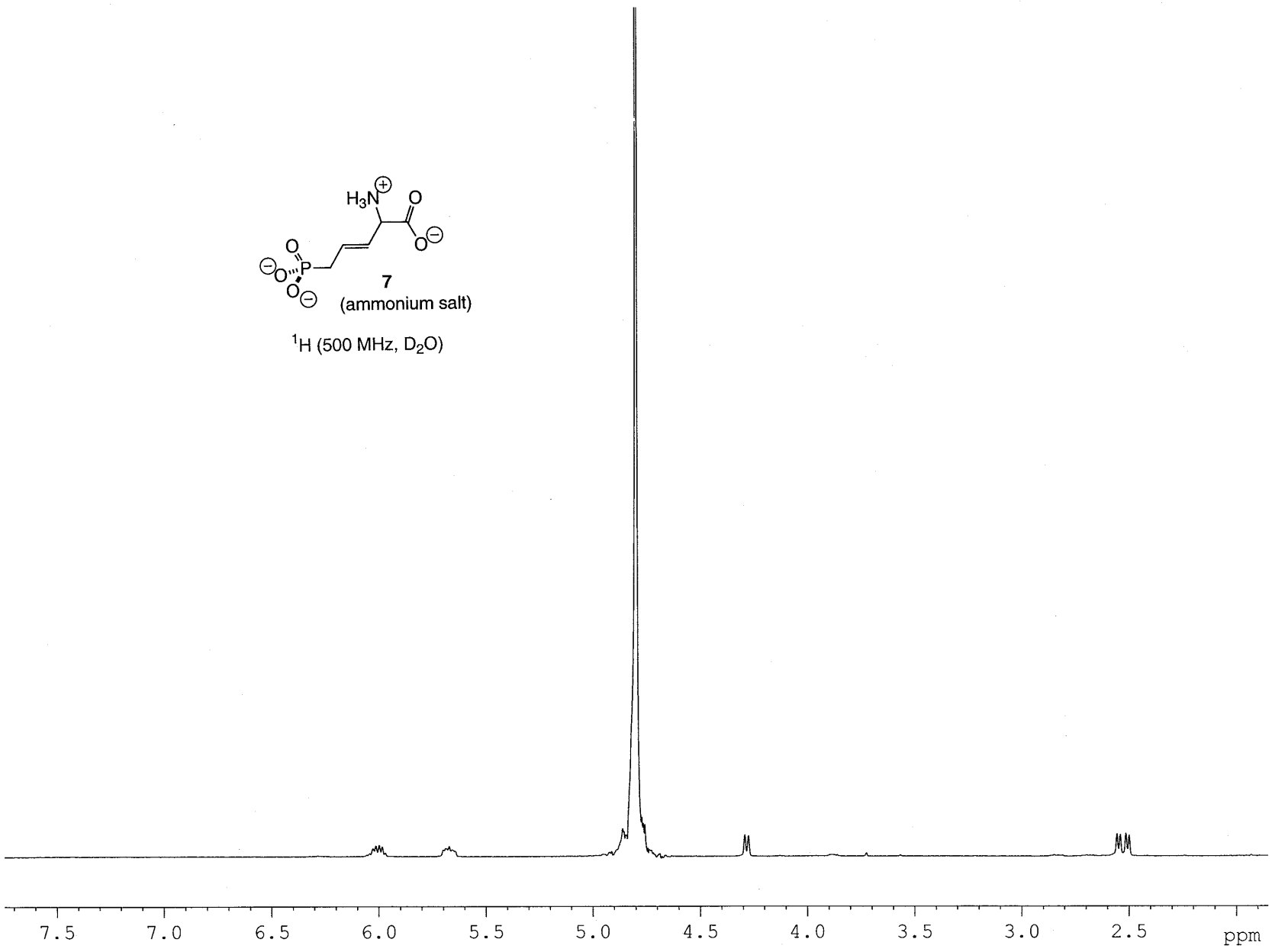


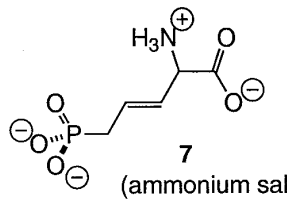
^{13}C NMR (75 MHz, $CDCl_3$)



^{1}H NMR (300 MHz, DMSO-d_6)

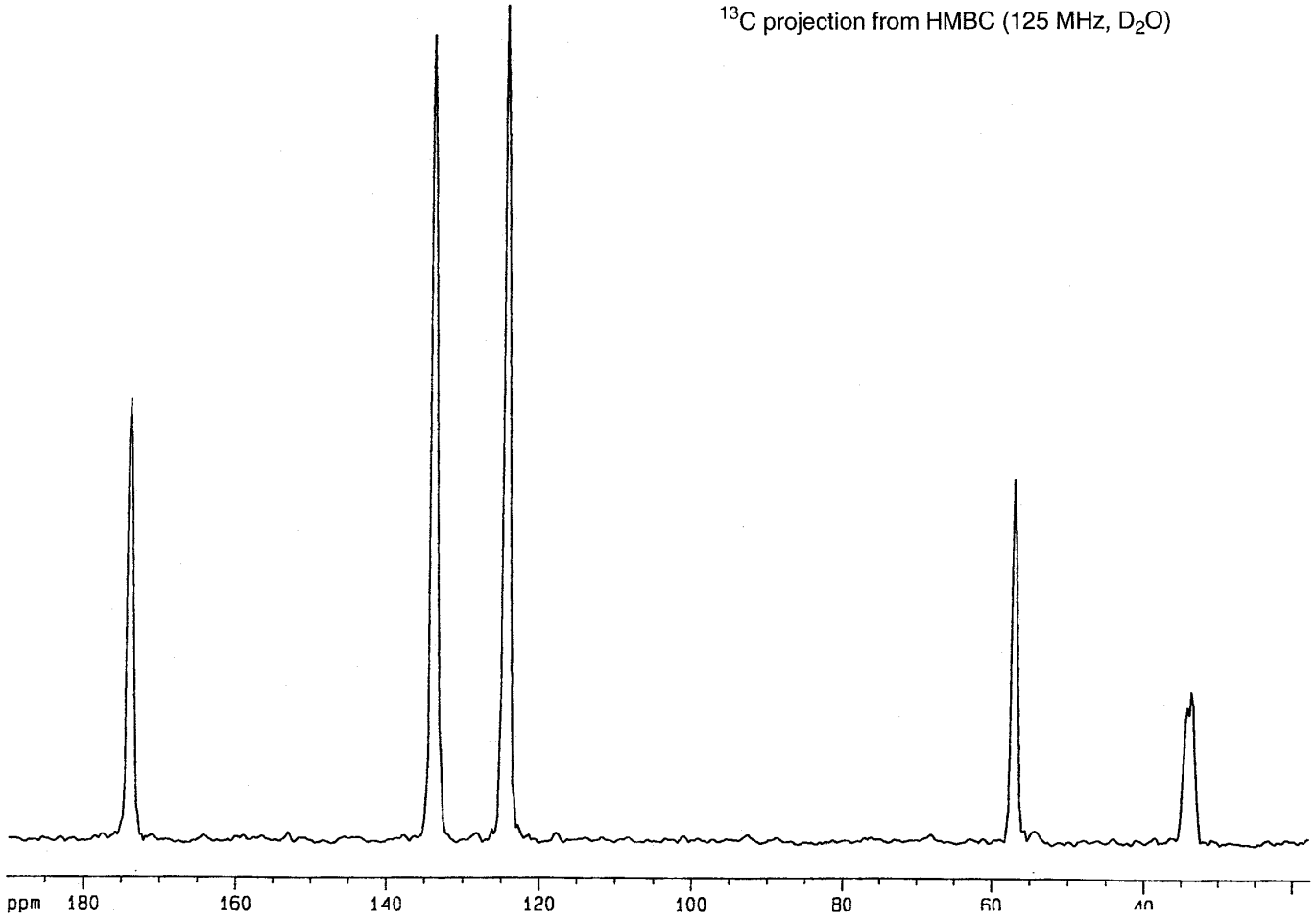



7
(ammonium salt)
^{1}H (500 MHz, D_2O)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
ppm



7
(ammonium salt)

^{13}C projection from HMBC (125 MHz, D_2O)




```
Current Data Parameters
NAME        MB-14-5mix_HC
EXPNO                 20
PROCNO                 5

F2 - Acquisition Parameters
Date_          20001117
Time               17.06
INSTRUM            spect
PROBHD     5 mm TXI 1H-
PULPROG       inv4gslrnd
TD                  1024
SOLVENT             CDCl3
NS                    80
DS                    32
SWH            2948.113 Hz
FIDRES         2.879017 Hz
AQ             0.1737204 sec
RG               14596.5
DW               169.600 usec
DE                 6.00 usec
TE                300.0 K
d0             0.00000300 sec
D1             1.93630695 sec
D6             0.07000000 sec
d13            0.00000300 sec
D16            0.00025000 sec
IN0            0.00001590 sec

============ CHANNEL f1 =============
NUC1                  1H
P1                12.40 usec
p2                24.80 usec
PL1                6.00 dB
SFO1         500.1323526 MHz

============ CHANNEL f2 =============
NUC2                 13C
P3                12.90 usec
PL2                0.00 dB
SFO2         125.7702341 MHz

============ GRADIENT CHANNEL ========
P16              1000.00 usec

F2 - Processing parameters
SI                  1024
SF           125.7577390 MHz
WDW                 SINE
SSB                    0
LB                  0.00 Hz
GB                     0
PC                  1.00

1D NMR plot parameters
CX                 20.00 cm
F1P               189.917 ppm
F1              23883.50 Hz
F2P                17.565 ppm
F2              2208.89 Hz
PPMCM           8.61760 ppm/cm
```