Stereoelectronic Effects Dictate Mechanistic Dichotomy Between Cu(II)-Catalyzed and Enzyme-Catalyzed Reactions of Malonic Acid Half Thioesters

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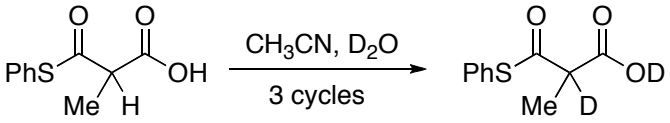
General. Reactions were performed in oven dry glassware, under an atmosphere of argon. Flash column chromatography was performed using a HPFC Biotage system (Biotage, Inc.) with pre-packed FLASH silica gel columns. Three independent runs of each experiment were performed except where noted and the results averaged.

Materials. Unless otherwise noted, solvents used were purified by passage through two packed columns of neutral alumina on a solvent dispensing system. Acetone was distilled over $CaSO_4$. Toluene-d8 and acetone-d6 were purchased from Aldrich and Cambridge Isotope Laboratories, Inc., respectively, and were used as received. Potassium cyanide-^{13}C was purchased from Cambridge Isotope Laboratories, Inc. and was reported by the manufacturer's certificate of analysis to contain 100.0% ^{13}C. Copper (II) trifluoromethanesulfonate, $Cu(OTf)_2$, was dried under high vacuum at ~110 °C for ≥ 12 hours prior to use. Dihydrocinnamaldehyde was purchased from Aldrich and was distilled freshly prior to each experiement. Other commercially available compounds were used without purification unless otherwise noted. TLC analyses were performed on a 250 μm Silica Gel $60F_{254}$ plates purchased from EM Science. MeMAHT was synthesized by the previously reported method[1] and trace water was azeotroped away from MeMAHT and Phbox with dry toluene before use in aldol reactions. All solutions for kinetics measurements were made while open to the atmosphere except where noted.

Performing the aldol reaction open to the atmosphere when using dihydrocinnamaldehyde had no deleterious effect on the yield or enantioselectivity of the reaction. Dihydrocinnamaldehyde, which provides yields of ≥ 90% of aldol product, was chosen as a representative aldehyde and was used for all of the experiments involving an aldehyde except where noted.

Instruments. IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian INOVA500, Mercury400, or Mercury300 spectrometer. Chemical shifts for proton and carbon resonances are reported in ppm (δ) relative to chloroform (δ 7.26, proton; 77.26, carbon), C_6D_6 (δ 7.16, proton; 128.00, carbon), CD_3CN (δ 1.93, proton), DMSO-d6 (δ 39.5, carbon), toluene-d8 (δ 2.09, Ph–CH_3, proton), or D_2O (δ 4.63, proton). Data are reported as follows: chemical shift, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet and m = multiplet).

2-Deuteriomonothiophenylmethylmalonic acid (MeMAHT-D).

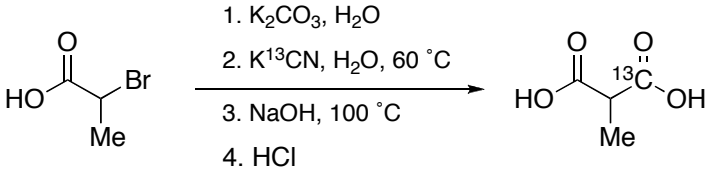


MeMAHT **MeMAHT-D**

2.00 g (9.51 mmol) of monothiophenylmethylmalonic acid (MeMAHT) was placed into a 100 mL flask and 20 mL of acetonitrile was added. When the MeMAHT had dissolved, 10 mL of D_2O was added and the solution was stirred for 6 hours under argon. The solvent was removed under vacuum and the process was repeated two more times to yield 1.98 g (9.33 mmol, 98% yield) of the product MeMAHT-D, which was chemically pure by NMR and contained < 1% unlabeled MeMAHT. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (s, 5H), 1.54 (s, 3H); ^{13}C NMR (100 MHz, C_6D_6) δ 192.9, 175.6, 134.8,

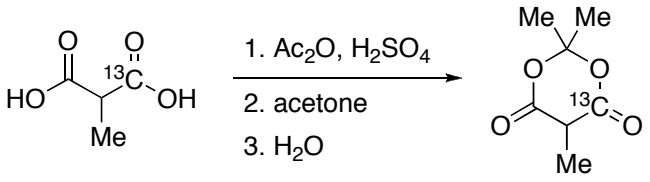
129.7, 129.4, 127.2, 53.2 (t, 1C, J = 20.2 Hz), 13.8; FTIR (neat, cm^{-1}) 2991 (br, small), 1720, 1478, 1442, 1280, 989.

1-^{13}C Methylmalonic acid.



2.62 g (17.13 mmol, 1.13 equiv) of 2-bromopropionic acid was placed into a 25 mL flask and a solution of 1.21 g (8.74 mmol, 0.58 equiv) of potassium carbonate in 3.5 mL of water was added dropwise with stirring. A solution of 1.00 g (15.13 mmol, 1.00 equiv) of potassium cyanide-^{13}C in 3.0 mL of water was added, and the solution was heated to 60 ˚C for 1 hour. 0.78 g (19.39 mmol, 1.28 equiv) of sodium hydroxide was added and the solution was heated to 100 ˚C overnight. Concentrated hydrochloric acid was added until the solution reached pH 1 and the solution was saturated with sodium chloride. The aqueous solution was extracted ten times with 25 mL of diethyl ether and the solvent was removed to give a mixture of the crude product and lactic acid. A saturated solution of the mixture in 1:1 benzene / acetone was layered with hexanes, causing small crystals to begin forming in a few minutes. The white crystals were filtered out by vacuum filtration and washed with a cold 9:1 hexanes / benzene solvent mixture to yield 1.53 g (12.84 mmol, 85% yield) of product. ^{1}H NMR (500 MHz, CD_3CN) δ 9.45 (br s, 2H), 3.40 (dq, 1H, J_1 = 8.5 Hz, J_2 = 7.5 Hz), 1.31 (dd, 3H, J_1 = 7.5 Hz, J_2 = 5.0 Hz); ^{13}C NMR (125 MHz, DMSO-d6) δ 171.7 (large), 45.7 (d, 1C, J = 55.5 Hz), 13.7; FTIR (neat, cm^{-1}) 2916 (br), 1693, 1666, 1467, 1404, 1296, 1239, 1205, 1092, 917; HRMS (ES-) calcd for $C_3{}^{13}CH_6O_4$ $(M-H)^-$: 118.0222, found 118.0223.

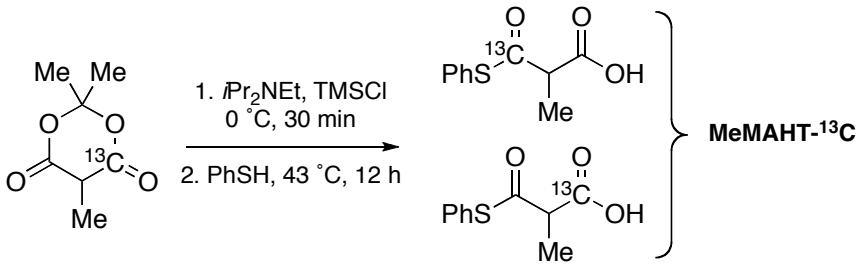
4-^{13}C-2,2,5-Trimethyl-1,3-dioxane-4,6-dione.



To a 25 mL flask containing 1.50 g (12.60 mmol, 1.00 equiv) of 1-^{13}C methyl malonic acid was added 2.0 mL of acetic anhydride and 5 drops of concentrated sulfuric acid to form a suspension. After stirring at room temperature for two hours, the solid had completely dissolved. 14.63 g (25.20 mmol, 2.00 equiv) of dry acetone was added dropwise and the solution was stirred for two hours. The reaction mixture was poured into a separatory funnel and 25 mL of water and 25 mL of diethyl ether was added. The organic layer was separated and the aqueous layer was extracted 3 times with 25 mL of diethyl ether. The organic layers were combined and extracted with 3 x 25 mL of saturated aqueous sodium bicarbonate solution. 4 M HCl was slowly added to the aqueous layer with vigorous stirring until the pH was 2-3. The aqueous layer was extracted three times with 50 mL of diethyl ether, and the organic layer was washed with brine, dried over anhydrous sodium sulfate, and evaporated to leave behind a white crystalline solid. The solid was taken up in 20 mL of chloroform and filtered to remove any unreacted 1-^{13}C methyl malonic acid. The filtrate was evaporated to give a white solid. A saturated solution of the solid in 2:1 benzene / acetone was layered with hexanes, causing colorless crystals to begin forming in a few minutes. The crystallization vessel was allowed to stand at room temperature for six hours and was then placed in a refrigerator overnight to complete the crystallization. The colorless crystals were filtered out by vacuum filtration and washed with a cold 9:1 hexanes / benzene solvent mixture to

yield 1.78 g (11.19 mmol, 89% yield) of product, which was pure by ^{1}H NMR. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.59 (dq, 1H, J_1 = 9.0 Hz, J_2 = 7.0 Hz), 1.81 (s, 3H), 1.76 (s, 3H), 1.57 (dd, 3H, J_1 = 7.0 Hz, J_2 = 4.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2 (large), 105.1, 41.6 (d, 1C, J = 50.3 Hz), 28.8, 26.6, 11.0; FTIR (neat, cm^{-1}) 3004, 2882, 1775, 1714, 1450, 1384, 1320, 1263, 1202, 1103, 1049, 974; HRMS (ES+) calcd for $C_6{}^{13}CH_{10}O_4$ $(M+H)^+$: 160.0691, found 160.0696.

1:1 Mixture of 1-^{13}C-Monothiophenylmethylmalonic Acid and 3-^{13}C-Monothiophenylmethylmalonic Acid (MeMAHT-^{13}C).



1.50 g (9.42 mmol, 1.00 equiv) of 4-^{13}C-2,2,5-trimethyl-1,3-dioxane-4,6-dione was weighed out into a 50 mL round bottom flask and 9.0 mL of acetonitrile was added. The mixture was stirred at room temperature until all of the starting material had dissolved. The flask was placed in an ice bath and 1.81 mL (10.37 mmol, 1.10 equiv) of dry *N*,*N*-diisopropylethylamine was added dropwise followed by 1.32 mL (10.37 mmol, 1.10 equiv) of trimethylsilylchloride. The clear, colorless reaction mixture was stirred at 0 ˚C for 15 minutes and 1.01 mL (9.89 mmol, 1.05 equiv) of thiophenol was added. The reaction mixture was heated to 43 ˚C for twelve hours. The reaction mixture was cooled to room temperature and 15 mL of diethyl ether and 15 mL of 0.3 M HCl was added to the flask. The organic layer was separated and the aqueous layer was extracted three

times with 25 mL of diethyl ether. The organic layers were combined and extracted with 3 x 25 mL of saturated aqueous sodium bicarbonate solution. 4 M HCl was slowly added to the aqueous layer with vigorous stirring until the pH was 2-3. The solution became cloudy and white as the pH fell below 5. The aqueous layer was extracted three times with 25 mL of diethyl ether, and the organic layer was washed with brine, dried over anhydrous sodium sulfate, and evaporated to leave behind a colorless oil, which changed to a white solid upon standing. A saturated solution of the crude product in 3:1 benzene / chloroform was layered with hexanes, causing crystals to begin forming in a few minutes. The crystallization vessel was allowed to stand at room temperature for six hours and was then placed in a refrigerator overnight to complete the crystallization. The colorless crystals were filtered out by vacuum filtration and washed with a cold 9:1 hexanes / benzene solvent mixture to yield 1.87 g (8.85 mmol, 94% yield) of product, which was pure by ^{1}H NMR. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.5-11.5 (br s, 1H), 7.44 (s, 5H), 3.81 (quintet, 1H, J = 7.0 Hz), 1.55 (dd, 3H, J_1 = 7.0 Hz, J_2 = 5.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 194.0 (large), 175.5 (large), 134.7, 130.0, 129.5, 126.8, 53.5 (d, 1C, J = 49.8 Hz), 14.4; FTIR (neat, cm^{-1}) 2992 (br), 1707, 1665, 1479, 1442, 1216, 935; HRMS (ES+) calcd for $C_9{}^{13}CH_{10}O_3S$ $(M+H)^+$: 212.0463, found 212.0458.

General Procedure for Kinetics Measurements.

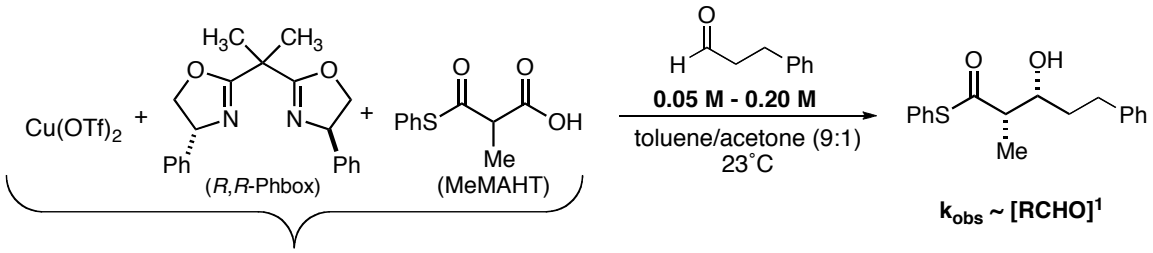
All kinetics measurements were performed at a controlled temperature of 23 ˚C by in situ NMR using a Varian INOVA500 NMR spectrometer. We calibrated a 90˚ pulse and used delays between pulses of > 5 x T_1 for the slowest decaying peak of interest. We acquired a spectrum approximately every 30-60 seconds for 30 minutes and

measured the increase of aldol product concentration over time by integrating the -CH_3 peak at 1.24 ppm corresponding to the aldol product versus the aromatic peak of the internal standard, 1,4-dimethoxybenzene, at 6.69 ppm both of which are well-resolved and sharp in the NMR spectrum despite the presence of paramagnetic Cu(II). The syn:anti ratio in the product was always 11:1. In each of the in situ NMR kinetics experiments, only the peak for the syn product was integrated. Henceforth, "aldol product" refers to the syn aldol product. From these integrals the concentration of aldol product, [aldol product], at each time point was calculated by the formula:

$$[\text{aldol product}] = \frac{4 * [\text{standard}] * \text{aldol product integral}}{3 * \text{standard integral}}$$

The slope of a plot of [aldol product] versus time gave the initial rate of the aldol reaction, d[aldol product]/dt. The slope of a plot of ln(initial rate) versus ln[starting material] gave the kinetic order in each starting material.

Procedure for Measuring Kinetic Order in Aldehyde.



$Cu(OTf)_2$ / (R)-Phbox / MeMAHT / Standard Solution.

45.2 mg (0.125 mmol, 0.10 equiv) $Cu(OTf)_2$ was weighed out into a 25 mL flask and 262.8 mg (1.25 mmol, 1.00 equiv) of MeMAHT was added to the flask. 2 mL of a 9:1 mixture of toluene-d8 and acetone-d6 was added and the resulting light green solution

was stirred for 15 minutes. A solution of 54.3 mg (0.162 mmol, 0.13 equiv) of (*R*,*R*)-Phbox in 2 mL of the 9:1 solvent mixture was added, and the solution was allowed to stir for 2 hours during which time it changed from green to blue. 86.4 mg (0.625 mmol, 0.50 equiv) of 1,4-dimethoxybenzene was added as an internal standard such that each sample solution would be 0.050 M in internal standard during the kinetics measurements. The solution was transferred to a 10 mL graduated cylinder and diluted with the solvent mixture to 5.00 mL.

RCHO Solution.

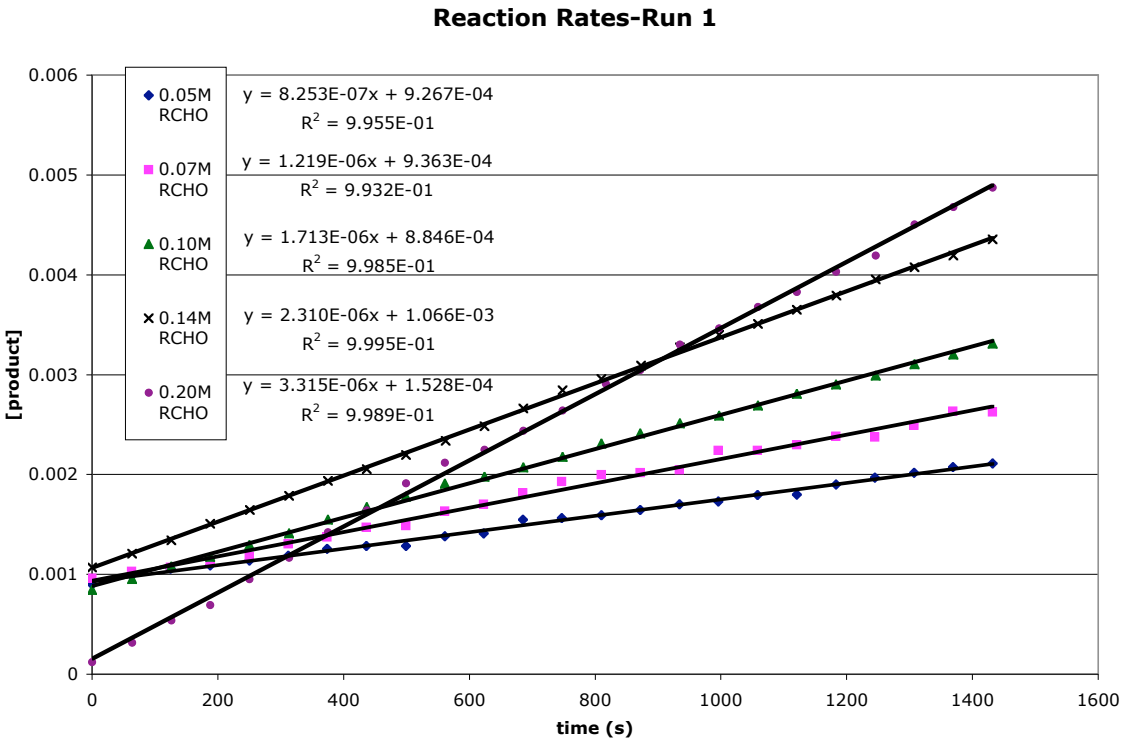
3.00 mL of a solution containing 198 μL (1.50 mmol) of dihydrocinnamaldehyde in the 9:1 solvent mixture was prepared by adding the aldehyde via microsyringe to 2 mL of the solvent and diluting the solution to 3.00 mL in a 10 mL graduated cylinder.

Sample Preparation.

Different amounts of each solution as shown in the chart below was added to separate NMR tubes in order from left to right in the chart by means of a 250 μL syringe. Immediately upon adding the RCHO solution, the NMR tube was capped, shaken, and placed into a dry ice / acetone bath. The samples were rapidly thawed by immersion in a room temperature water bath immediately prior to insertion into the NMR spectrometer. The kinetics measurements were performed as detailed in the general procedure.

sample #	μL of $Cu(OTf)_2$ solution	μL of solvent	μL of RCHO solution	equiv of RCHO (MeMAHT = 1.0 equiv)
1	200	250	50	0.5
2	200	230	70	0.7
3	200	200	100	1.0
4	200	160	140	1.4
5	200	100	200	2.0

Results.


Reaction Rates-Run 1
0.05M RCHO
0.07M RCHO
0.10M RCHO
0.14M RCHO
0.20M RCHO
y = 8.253E-07x + 9.267E-04
R2 = 9.955E-01
y = 1.219E-06x + 9.363E-04
R2 = 9.932E-01
y = 1.713E-06x + 8.846E-04
R2 = 9.985E-01
y = 2.310E-06x + 1.066E-03
R2 = 9.995E-01
y = 3.315E-06x + 1.528E-04
R2 = 9.989E-01
[product]
time (s)
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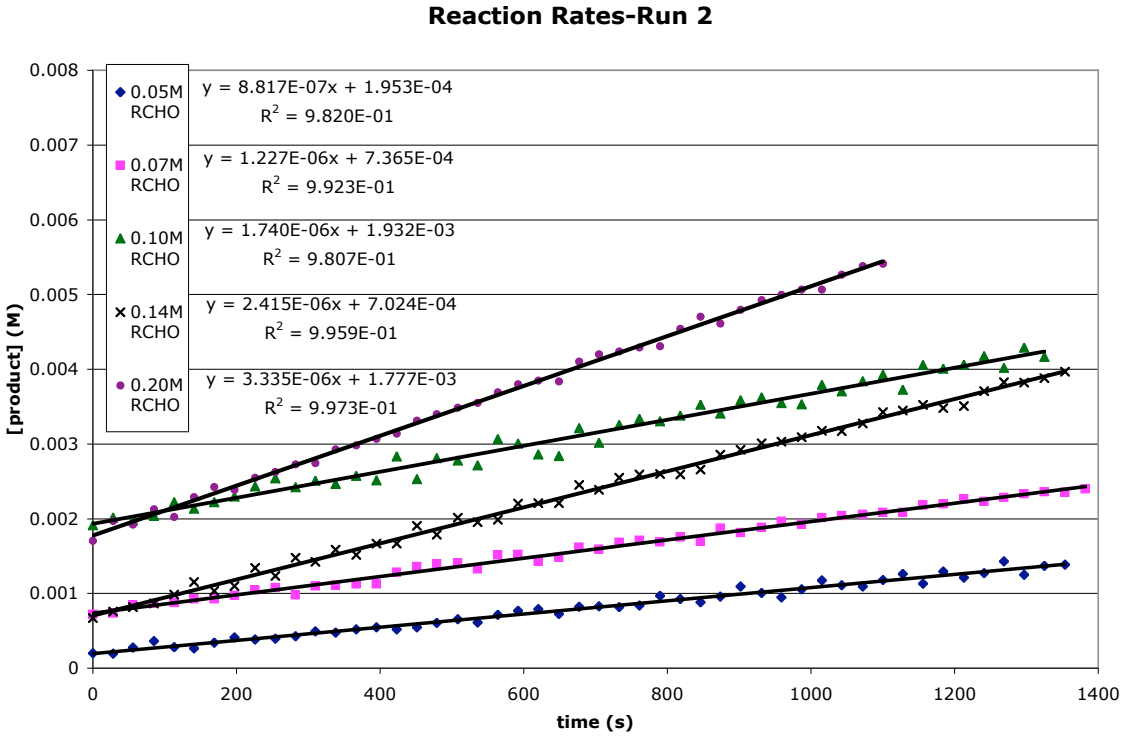
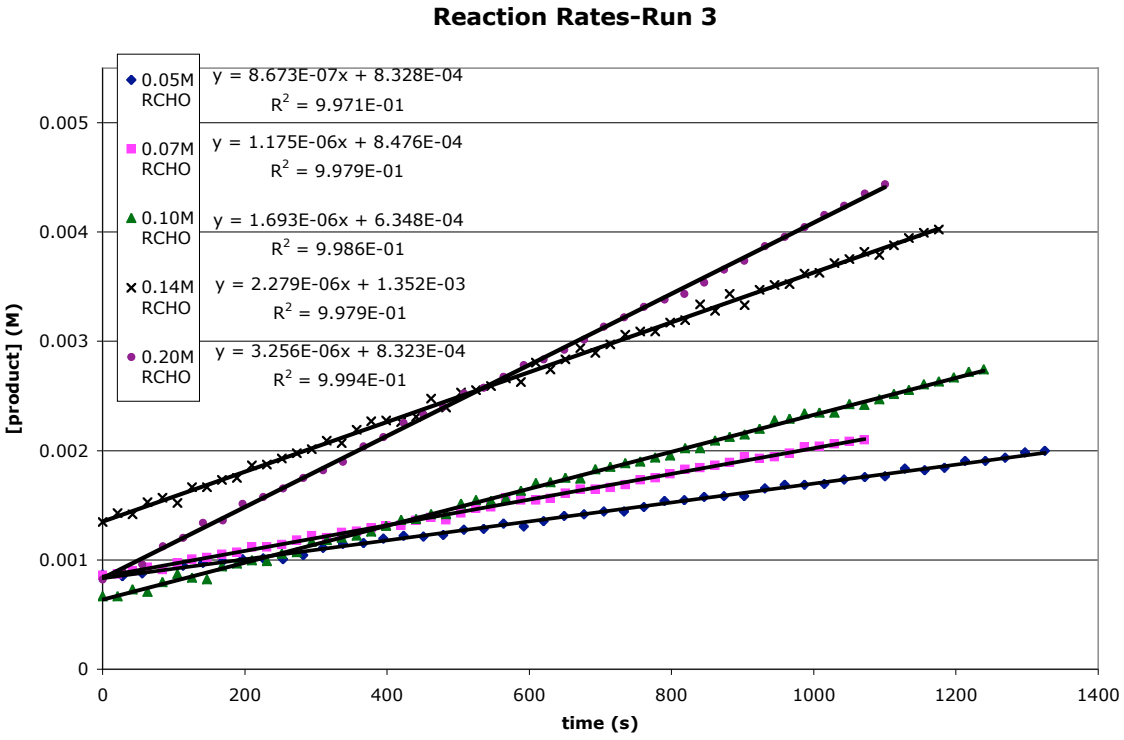
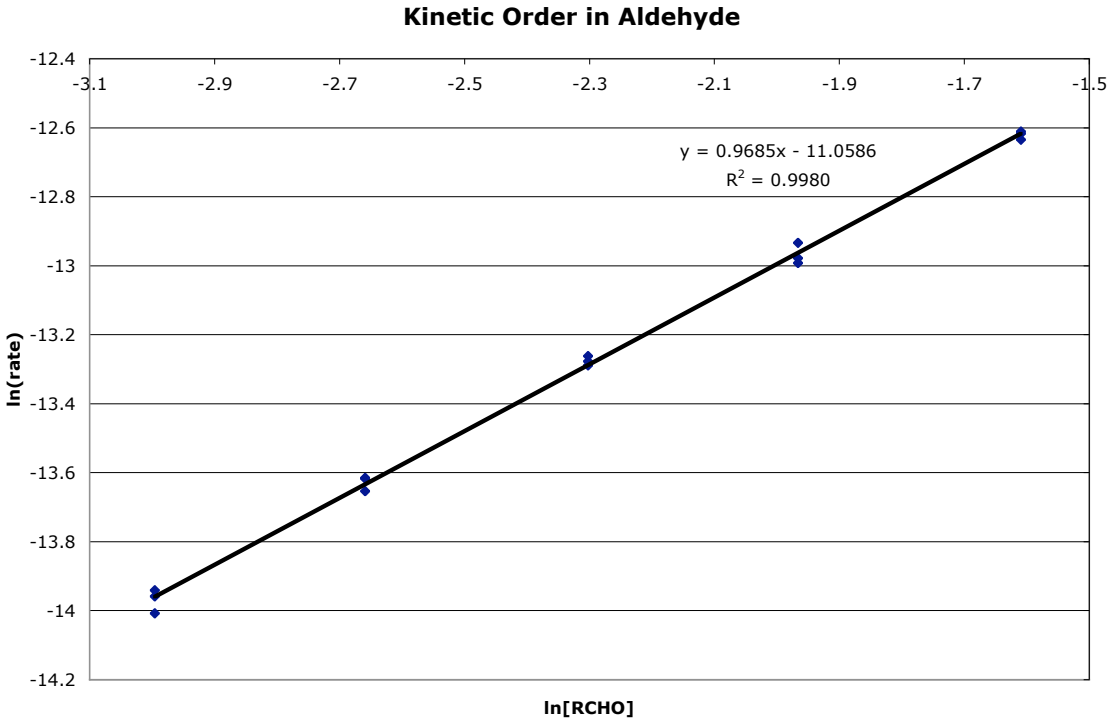
0.001
0.002
0.003
0.004
0.005
0.006
0
200
400
600
800
1000
1200
1400
1600

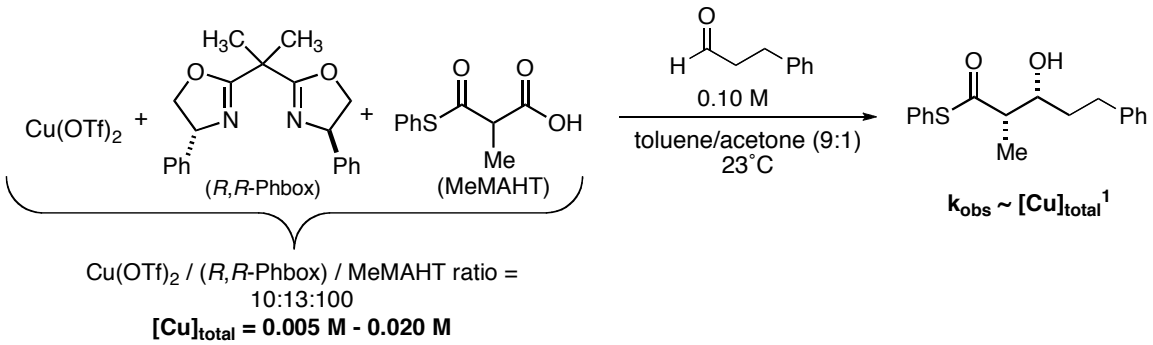

Reaction Rates-Run 2
0.05M RCHO
0.07M RCHO
0.10M RCHO
0.14M RCHO
0.20M RCHO
y = 8.817E-07x + 1.953E-04
R2 = 9.820E-01
y = 1.227E-06x + 7.365E-04
R2 = 9.923E-01
y = 1.740E-06x + 1.932E-03
R2 = 9.807E-01
y = 2.415E-06x + 7.024E-04
R2 = 9.959E-01
y = 3.335E-06x + 1.777E-03
R2 = 9.973E-01
[product] (M)
time (s)
0
0.001
0.002
0.003
0.004
0.005
0.006
0.007
0.008
0
200
400
600
800
1000
1200
1400





The slope of a plot of ln(initial rate) versus ln[RCHO] was 0.97±0.02 indicating that the aldol reaction is first-order in aldehyde.

Procedure for Measuring Kinetic Order in Catalyst ($Cu(OTf)_2$ + (*R*,*R*)-Phbox + MeMAHT; 10:13:100).



$Cu(OTf)_2$ / (R)-Phbox / MeMAHT solution.

45.2 mg (0.125 mmol) $Cu(OTf)_2$ was weighed out into a 25 mL flask and 262.8 mg (1.25 mmol) of MeMAHT was added to the flask. 2 mL of a 9:1 mixture of toluene-d8 and acetone-d6 was added and the resulting light green solution was stirred for 15 minutes. A solution of 54.3 mg (0.162 mmol) of (*R*,*R*)-Phbox in 2 mL of the 9:1 solvent mixture was added, and the solution was allowed to stir for 2 hours during which time it changed from green to blue. The solution was transferred to a 10 mL graduated cylinder and diluted with the solvent mixture to 5.00 mL.

RCHO / Standard Solution.

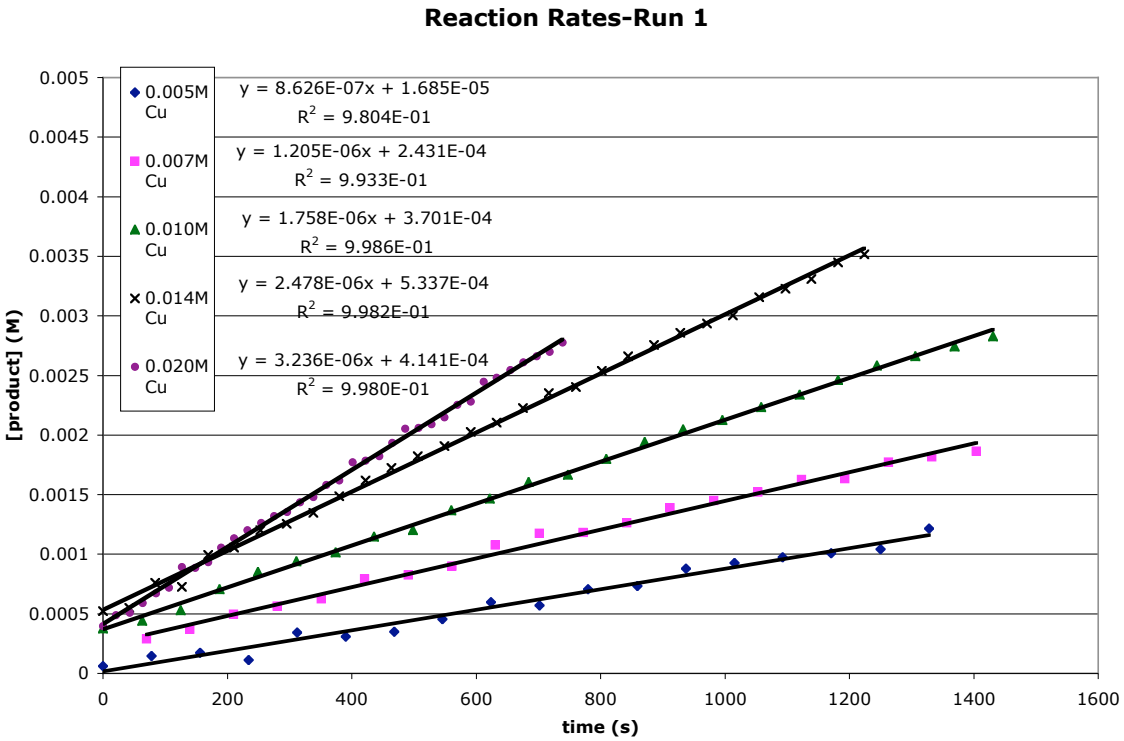
3.00 mL of a solution containing 198 μL (1.50 mmol, 1.00 equiv) of dihydrocinnamaldehyde and 103.6 mg (0.750 mmol, 0.50 equiv) of 1,4-dimethoxybenzene in a 9:1 mixture of toluene-d8 and acetone-d6 was prepared by dissolving the 1,4-dimethoxybenzene in 2 mL of the solvent, adding the aldehyde via microsyringe, and diluting the solution to 3.00 mL in a 10 mL graduated cylinder. Each sample solution would be 0.050 M in internal standard during the kinetics measurements.

Sample Preparation.

Different amounts of each solution as shown in the chart below was added to separate NMR tubes in order from left to right in the chart by means of 250 μL and 500 μL syringes. Immediately upon adding the RCHO solution, the NMR tube was capped, shaken, and placed into a dry ice / acetone bath. The samples were rapidly thawed by immersion in a room temperature water bath immediately prior to insertion into the NMR spectrometer. The kinetics measurements were performed as detailed in the general procedure.

sample #	μL of $Cu(OTf)_2$ solution	μL of solvent	μL of RCHO solution	equiv of catalyst (RCHO = 1.0 equiv.)
1	100	300	100	0.05
2	140	260	100	0.07
3	200	200	100	0.10
4	280	120	100	0.14
5	400	0	100	0.20

Results.


Reaction Rates-Run 1
0.005M Cu
0.007M Cu
0.010M Cu
0.014M Cu
0.020M Cu
y = 8.626E-07x + 1.685E-05
R2 = 9.804E-01
y = 1.205E-06x + 2.431E-04
R2 = 9.933E-01
y = 1.758E-06x + 3.701E-04
R2 = 9.986E-01
y = 2.478E-06x + 5.337E-04
R2 = 9.982E-01
y = 3.236E-06x + 4.141E-04
R2 = 9.980E-01
[product] (M)
time (s)
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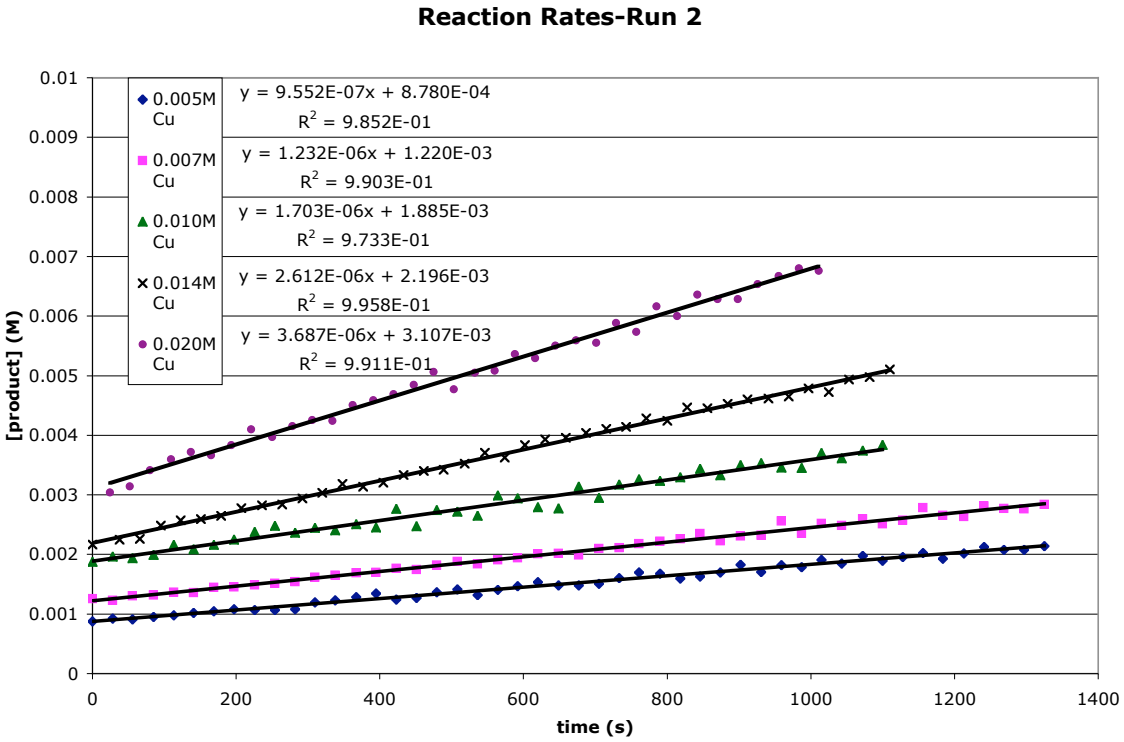
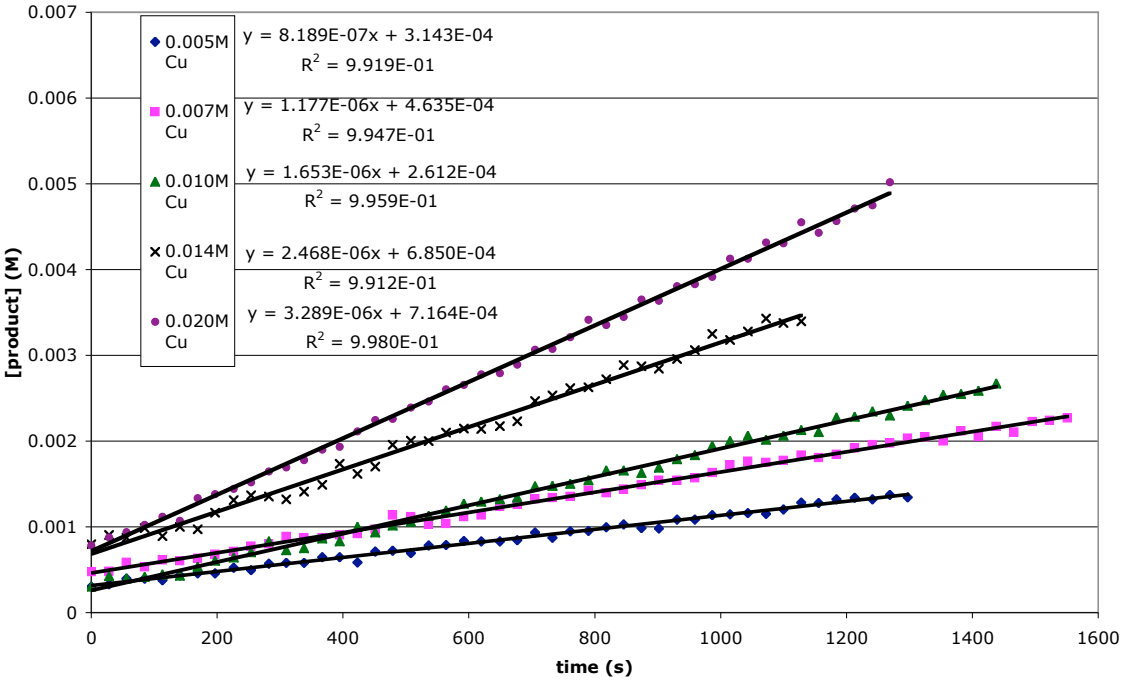
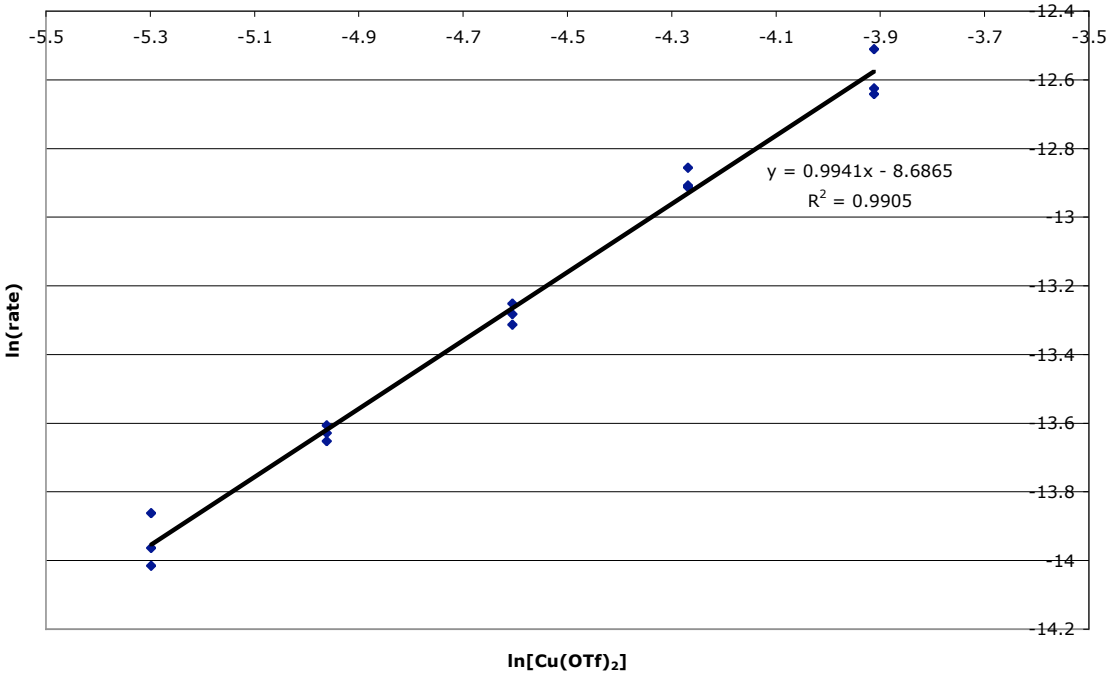
0.0005
0.001
0.0015
0.002
0.0025
0.003
0.0035
0.004
0.0045
0.005
0
200
400
600
800
1000
1200
1400
1600


Reaction Rates-Run 2
0.005M Cu
0.007M Cu
0.010M Cu
0.014M Cu
0.020M Cu
y = 9.552E-07x + 8.780E-04
R2 = 9.852E-01
y = 1.232E-06x + 1.220E-03
R2 = 9.903E-01
y = 1.703E-06x + 1.885E-03
R2 = 9.733E-01
y = 2.612E-06x + 2.196E-03
R2 = 9.958E-01
y = 3.687E-06x + 3.107E-03
R2 = 9.911E-01
[product] (M)
time (s)
0
0.001
0.002
0.003
0.004
0.005
0.006
0.007
0.008
0.009
0.01
0
200
400
600
800
1000
1200
1400

Reaction Rates-Run 3

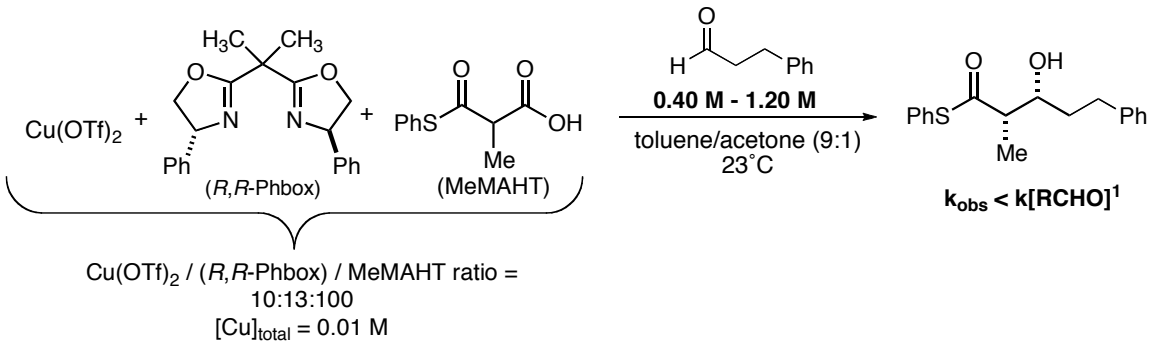


Kinetic Order in Catalyst



The slope of a plot of ln(initial rate) versus $\ln[Cu]_{total}$ was 0.99±0.03 indicating that the aldol reaction is first-order in Cu catalyst (a mixture of $Cu(OTf)_2$, (*R*,*R*)-Phbox, and MeMAHT in a fixed ratio of 10:13:100).

Procedure for Measuring Kinetic Order in Aldehyde at High Aldehyde Concentrations.



$Cu(OTf)_2$ / (R)-Phbox / MeMAHT / Standard Solution.

45.2 mg (0.125 mmol, 0.10 equiv) $Cu(OTf)_2$ was weighed out into a 25 mL flask and 262.8 mg (1.25 mmol, 1.00 equiv) of MeMAHT was added to the flask. 2 mL of a 9:1 mixture of toluene-d8 and acetone-d6 was added and the resulting light green solution was stirred for 15 minutes. A solution of 54.3 mg (0.162 mmol, 0.13 equiv) of (*R*,*R*)-Phbox in 2 mL of the 9:1 solvent mixture was added, and the solution was allowed to stir for 2 hours during which time it changed from green to blue. 86.4 mg (0.625 mmol, 0.50 equiv) of 1,4-dimethoxybenzene was added as an internal standard such that each sample solution would be 0.050 M in internal standard during the kinetics measurements. The solution was transferred to a 10 mL graduated cylinder and diluted with the solvent mixture to 5.00 mL.

RCHO Solution.

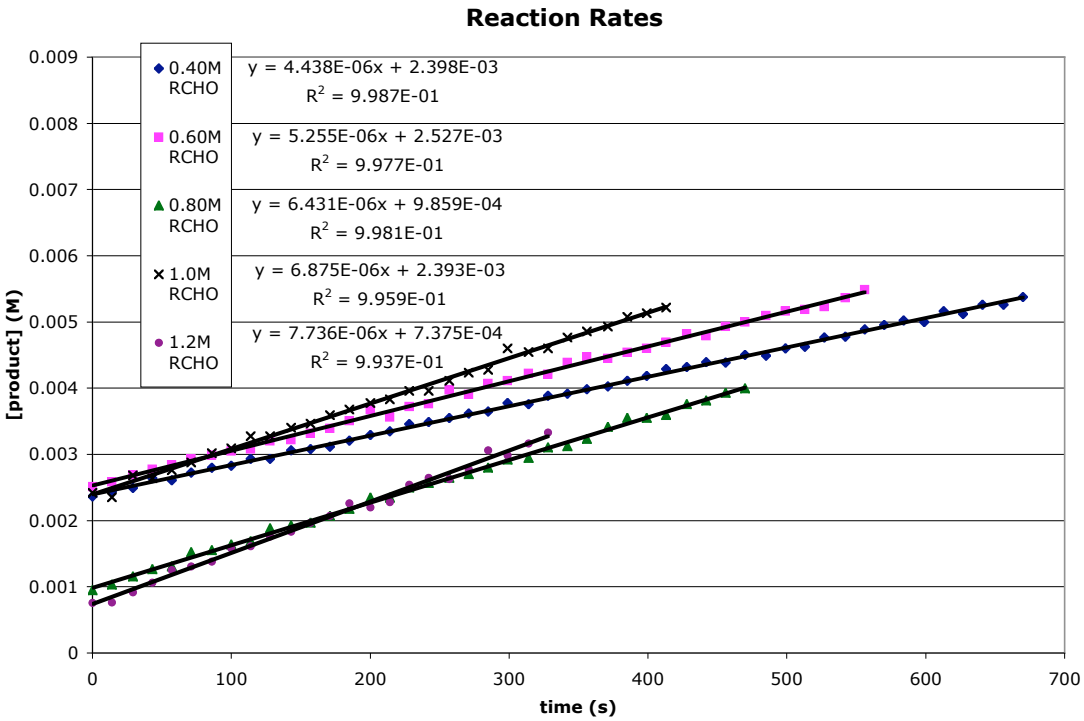
2.00 mL of a solution containing 658 μL (5.00 mmol) of dihydrocinnamaldehyde in the 9:1 solvent mixture was prepared by adding the aldehyde via microsyringe to 1 mL of the solvent and diluting the solution to 2.00 mL in a 10 mL graduated cylinder.

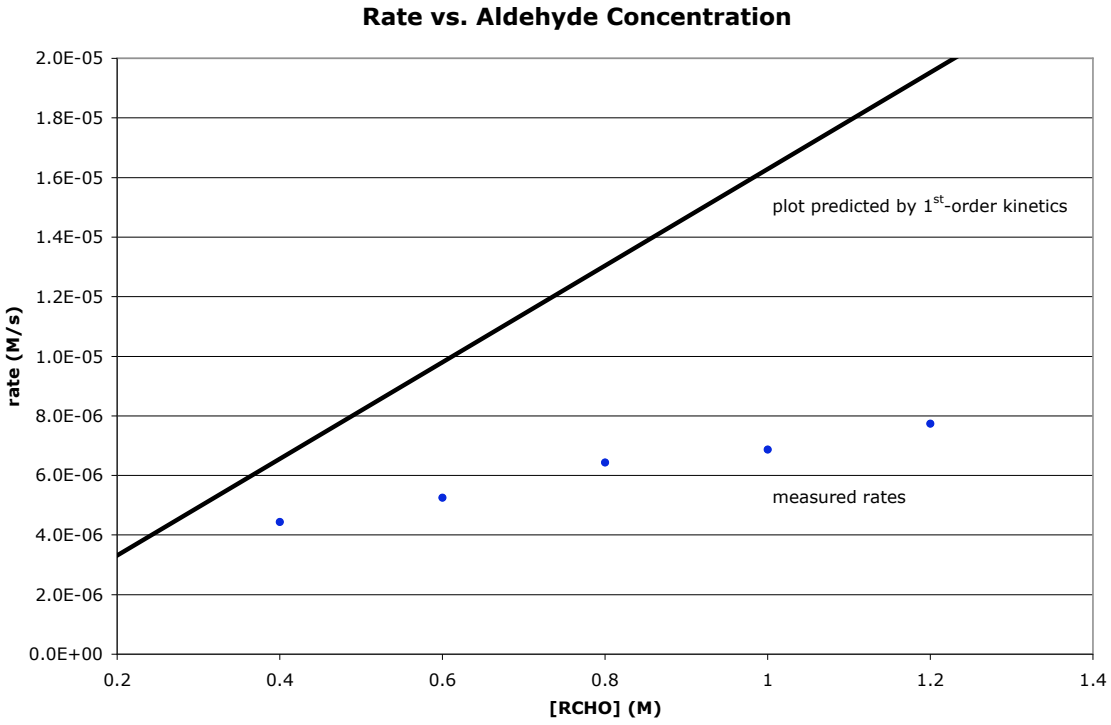
Sample Preparation.

Different amounts of each solution as shown in the chart below was added to separate NMR tubes in order from left to right in the chart by means of a 250 μL syringe. Immediately upon adding the RCHO solution, the NMR tube was capped, shaken, and placed into a dry ice / acetone bath. The samples were rapidly thawed by immersion in a room temperature water bath immediately prior to insertion into the NMR spectrometer. Only a single run of this experiment was performed.

sample #	μL of $Cu(OTf)_2$ solution	μL of solvent	μL of RCHO solution	equiv of RCHO (MeMAHT = 1.0 equiv)
1	200	220	80	4.0
2	200	180	120	6.0
3	200	140	160	8.0
4	200	100	200	10.0
5	200	60	240	12.0

Results.

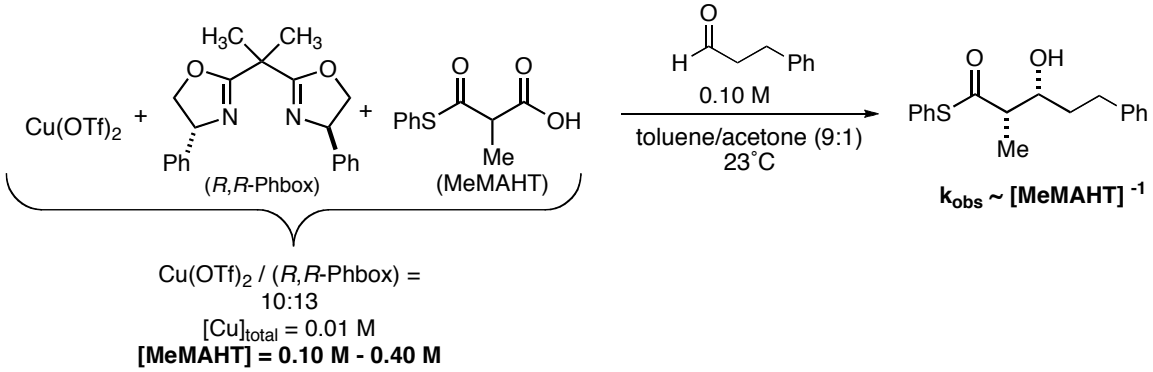




The above graph shows the rates of the aldol reaction versus [RCHO] with a

range of concentrations spanning 0.40 M to 1.20 M. It also displays a regression line extrapolated to higher concentrations based on the data measured for the rates of the aldol reaction versus [RCHO] with a range of concentrations spanning 0.05 M to 0.20 M, which was shown above to follow 1st-order kinetics. At higher concentrations of aldehyde, the reaction rate slows down from that predicted by a continuation of 1st-order kinetics. This suggests that aldol addition of a MeMAHT enolate to the aldehyde or possibly decarboxylation subsequent to aldol addition is rate-limiting at low aldehyde concentrations but that deprotonation to form a MeMAHT enolate may become at least partially rate-limiting at higher aldehyde concentrations.

Procedure for Measuring Kinetic Order in MeMAHT.



$Cu(OTf)_2$ / (R)-Phbox / MeMAHT / Standard Solution.

45.2 mg (0.125 mmol, 0.10 equiv) $Cu(OTf)_2$ was weighed out into a 25 mL flask and 262.8 mg (1.25 mmol, 1.00 equiv) of MeMAHT was added to the flask. 2 mL of a 9:1 mixture of toluene-d8 and acetone-d6 was added and the resulting light green solution was stirred for 15 minutes. A solution of 54.3 mg (0.162 mmol, 0.13 equiv) of (*R*,*R*)-Phbox in 2 mL of the 9:1 solvent mixture was added, and the solution was allowed to stir for 2 hours during which time it changed from green to blue. 48.7 mg (0.352 mmol,

0.282 equiv) of 1,4-dimethoxybenzene was added as an internal standard such that each sample solution would be 0.0282 M in internal standard during the kinetics measurements. The solution was transferred to a 10 mL graduated cylinder and diluted with the solvent mixture to 5.00 mL.

RCHO Solution.

3.00 mL of a solution containing 198 μL (1.50 mmol, 1.00 equiv) of dihydrocinnamaldehyde in a 9:1 mixture of toluene-d8 and acetone-d6 was prepared by adding the aldehyde via microsyringe to 2 mL of the solvent and diluting the solution to 3.00 mL in a 10 mL graduated cylinder.

MeMAHT Solution.

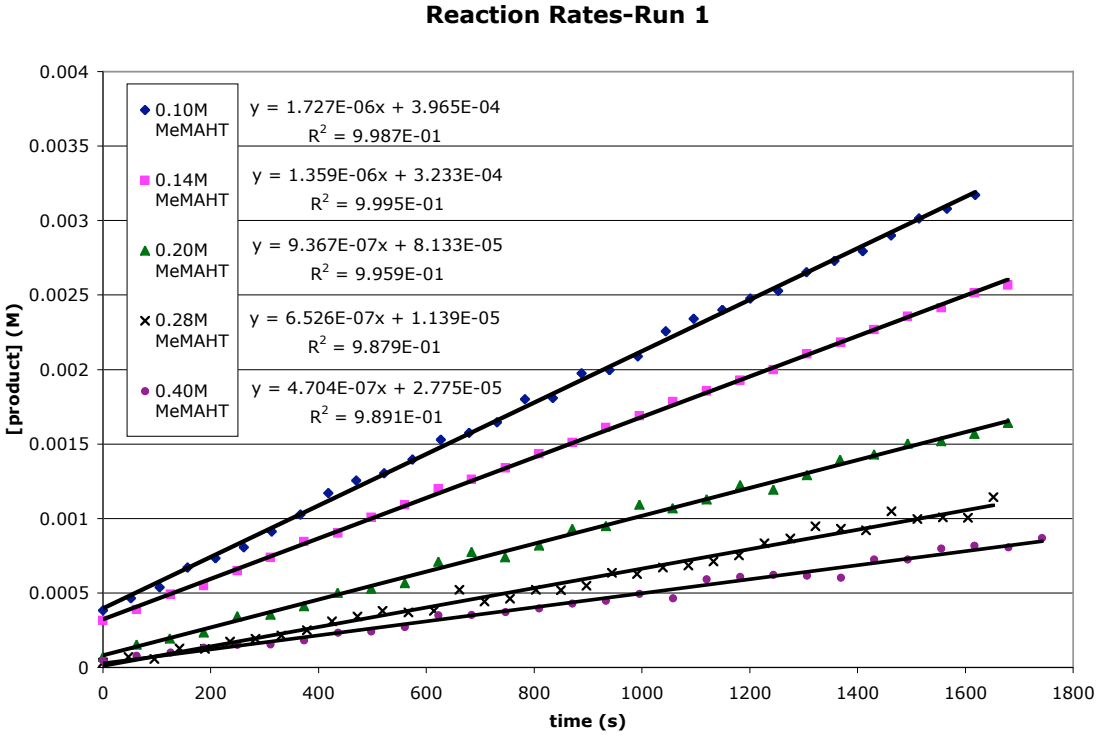
315.4 mg (1.50 mmol) of MeMAHT was dissolved in 1.5 mL of the 9:1 mixture of toluene-d8 and acetone-d6 and diluted to 2.00 mL in a 10 mL graduated cylinder to produce a solution with a MeMAHT concentration of 0.750 M.

Sample Preparation.

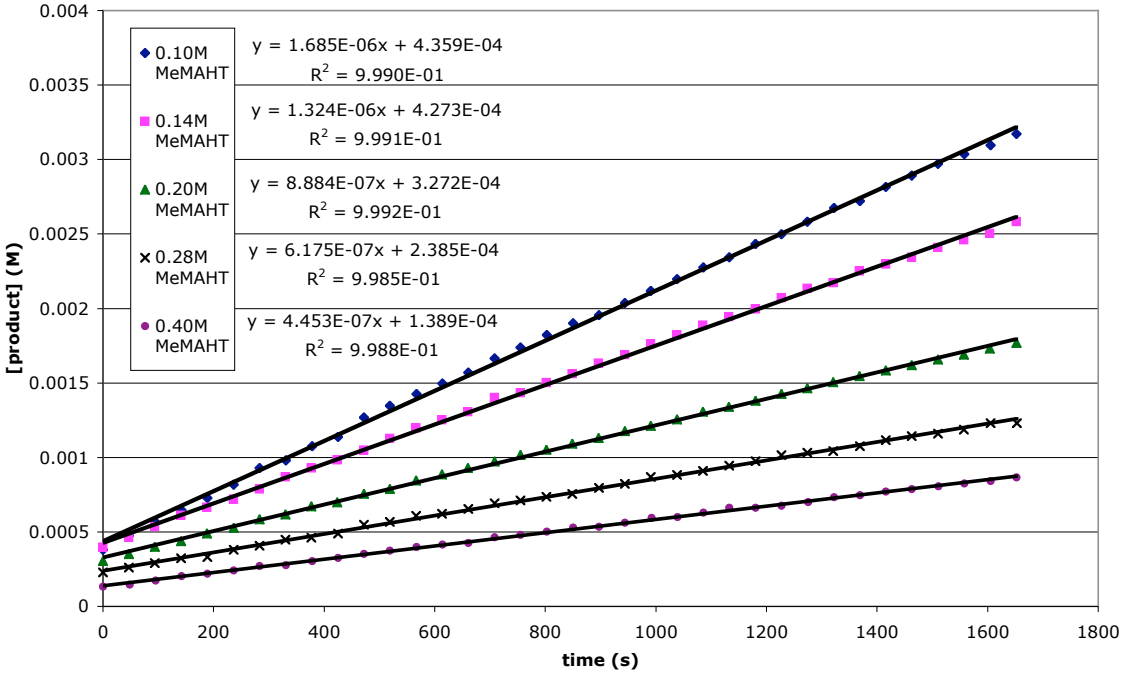
Different amounts of each solution as shown in the chart below was added to separate NMR tubes in order from left to right in the chart by means of a 250 μL syringe. Immediately upon adding the RCHO solution, the NMR tube was capped, shaken, and placed into a dry ice / acetone bath. The samples were rapidly thawed by immersion in a room temperature water bath immediately prior to insertion into the NMR spectrometer. The kinetics measurements were performed as detailed in the general procedure.

sample #	μL of $Cu(OTf)_2$ solution	μL of MeMAHT solution	μL of solvent	μL of RCHO solution	equiv of MeMAHT (RCHO = 1.0 equiv)
1	200	0	200	100	1.0
2	200	27	173	100	1.4
3	200	67	133	100	2.0
4	200	107	93	100	2.8
5	200	200	0	100	4.0

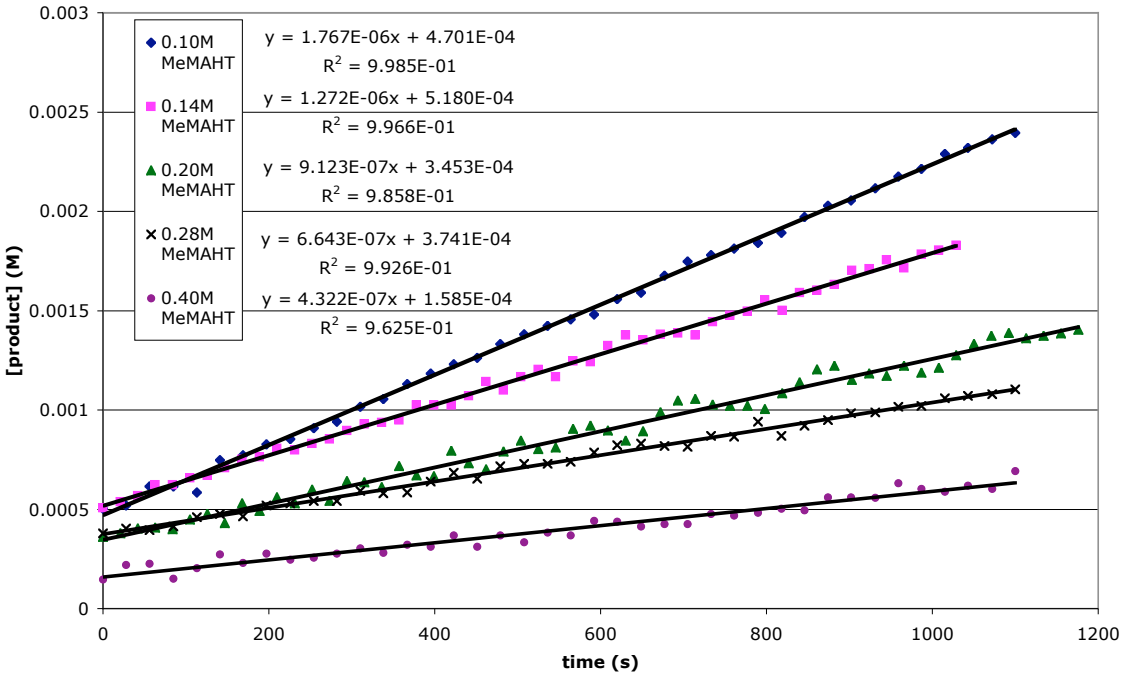
Results.

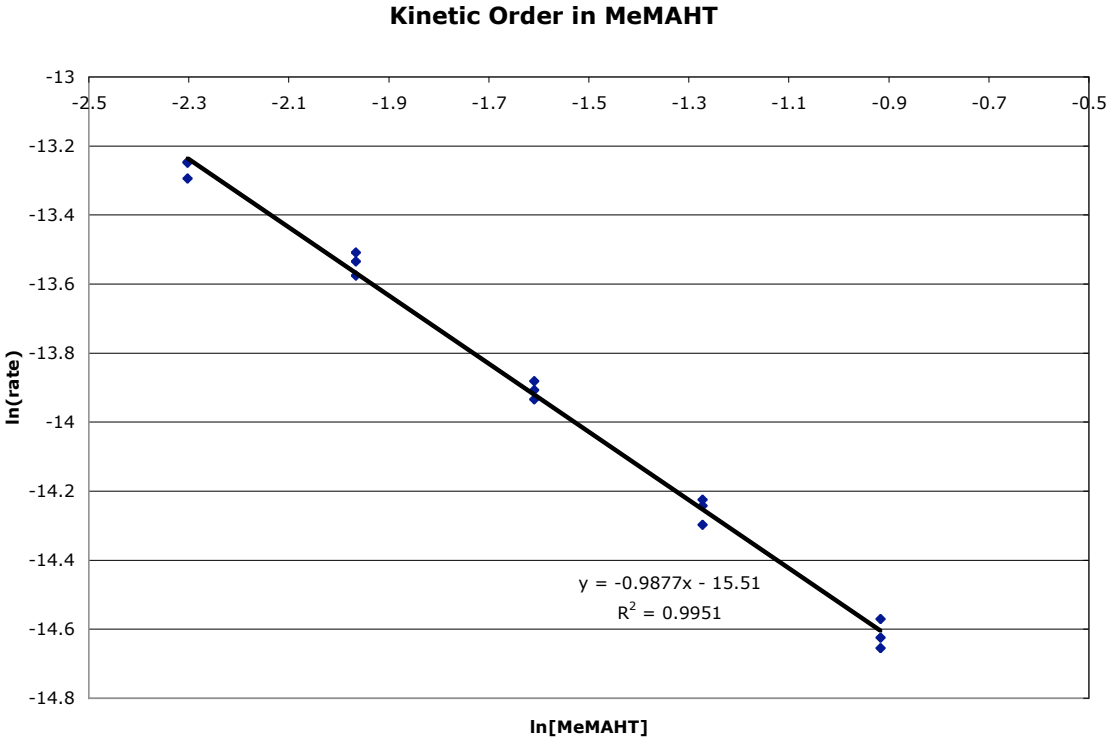


Reaction Rates-Run 2



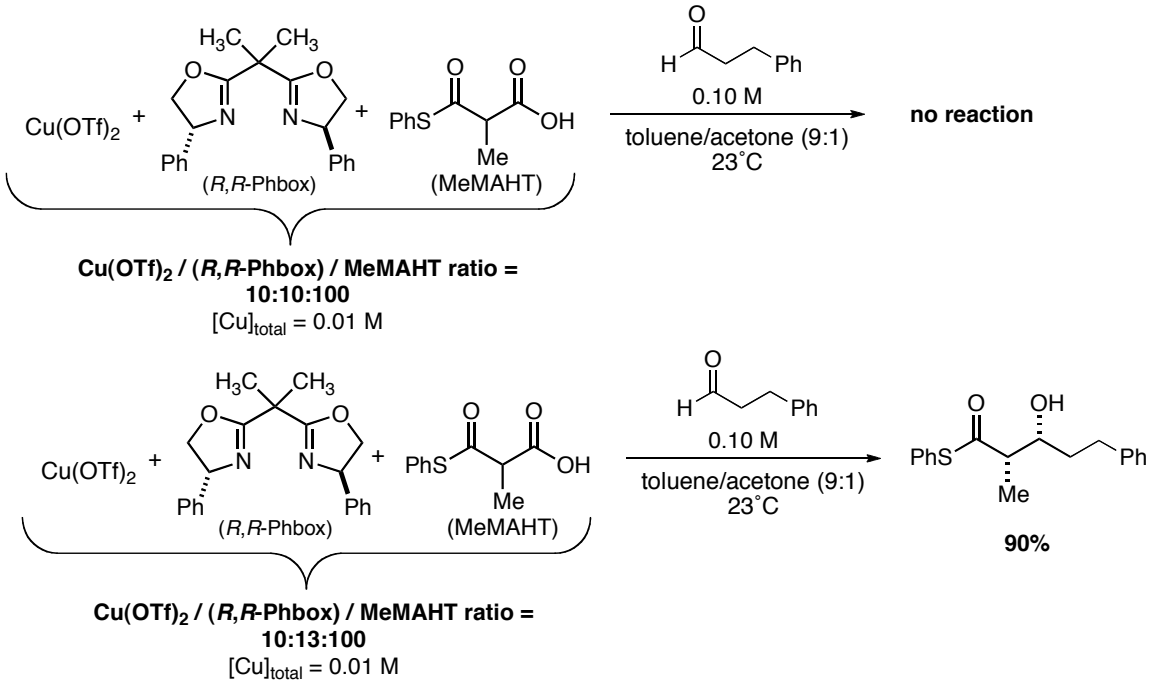
Reaction Rates-Run 3





The slope of a plot of ln(initial rate) versus ln[MeMAHT] was -0.99 ± 0.02 indicating that the aldol reaction is inverse first-order in MeMAHT.

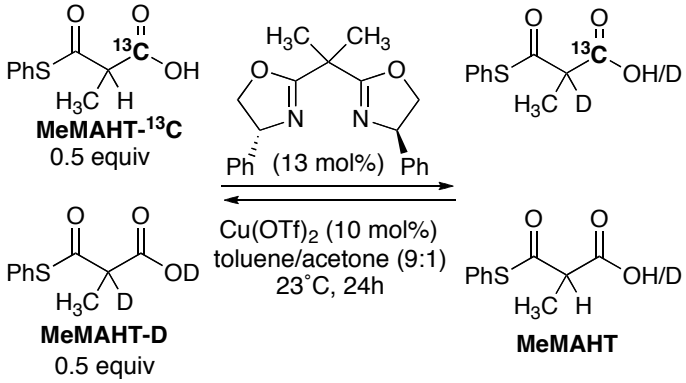
Demonstration of the Necessity for a Slight Excess of (R,R)-Phbox Over $Cu(OTf)_2$.



18.1 mg (0.050 mmol, 0.10 equiv) $Cu(OTf)_2$ was weighed out into a 25 mL flask and 105.1 mg (0.500 mmol, 1.00 equiv) of MeMAHT was added to the flask. 2.0 mL of a 9:1 mixture of toluene / acetone was added and the resulting light green solution was stirred for 15 minutes. A solution of 21.7 mg (0.065 mmol) of (*R,R*)-Phbox in 4.0 mL of the 9:1 solvent mixture was made, and 3.0 mL of this solution (0.10 equiv) was added to the reaction mixture. The resulting solution was allowed to stir for 2 hours during which time it changed from green to blue. 66 μL (0.500 mmol, 1.00 equiv) of dihydrocinnamaldehyde was added by syringe. After the reaction mixture had stirred for 4 hours, TLC analysis showed that no reaction had occurred. At this time the remaining 1.0 mL of the (*R,R*)-Phbox solution was added to bring the total equivalents of (*R,R*)-Phbox up to 0.13. After 10 minutes TLC analysis indicated the presence of aldol product. Subsequent TLC analysis revealed that the reaction proceeded as normal and went to completion after 24 hours. The reaction was quenched by shaking it with 5 mL of 0.5 M

HCl. The product mixture was extracted out with diethyl ether. The ether solution was washed with brine, dried over anhydrous sodium sulfate, and evaporated to leave behind a slightly yellowish oil. The crude product mixture was loaded onto a silica gel column which was eluted with 90% hexanes / 10% ethyl acetate. The fractions containing the aldol product were combined and the solvent was evaporated to yield 135 mg (90%) of aldol product which was pure by ^{1}H NMR.

Measurement of the Rate of MeMAHT Enolization.



Note: 50% of the MeMAHT-^{13}C contains a ^{13}C atom at the carboxylate position and 50% contains a ^{13}C atom at the thioester position. Only one isomer is drawn.

$k_{enolization}$ = 5.2 ± 0.2 x 10^{-5} M/s

$t_{1/2}$ = 5.5 ± 0.2 min.

Cu(OTf)$_2$ / (*R*,*R*)-Phbox / MeMAHT-^{13}C Solution.

36.2 mg (0.100 mmol, 0.10 equiv) Cu(OTf)$_2$ was weighed out into a 50 mL flask and 52.8 mg (0.250 mmol, 0.25 equiv) of MeMAHT-^{13}C was added to the flask. 4.0 mL of a 9:1 mixture of toluene / acetone was added and the resulting light green solution was stirred for 15 minutes. A solution of 43.5 mg (0.130 mmol, 0.13 equiv) of (*R*,*R*)-Phbox in 3.0 mL of the 9:1 solvent mixture was added, and the solution was allowed to stir for 2 hours during which time it changed from green to blue.

Cu(OTf)$_2$ / (*R*,*R*)-Phbox / MeMAHT-D Solution.

A Cu(OTf)$_2$ / (*R*,*R*)-Phbox / MeMAHT-D solution was prepared identically to the Cu(OTf)$_2$ / (*R*,*R*)-Phbox / MeMAHT-^{13}C solution except that the MeMAHT-^{13}C was

replaced with 53.1 mg (0.250 mmol, 0.25 equiv) of MeMAHT-D.

MeMAHT-^{13}C Solution.

158.4 mg (0.750 mmol) of MeMAHT-^{13}C was dissolved in 3.0 mL of the 9:1 mixture of toluene / acetone.

MeMAHT-D Solution.

159.2 mg (0.750 mmol) of MeMAHT-D was dissolved in 3.0 mL of the 9:1 mixture of toluene / acetone.

Experimental Procedure.

When the four above solutions had been prepared, the MeMAHT-^{13}C solution was added by syringe to the rapidly stirring $Cu(OTf)_2$ / (*R,R*)-Phbox / MeMAHT-^{13}C solution. Likewise, the MeMAHT-D solution was added by syringe to the rapidly stirring $Cu(OTf)_2$ / (*R,R*)-Phbox / MeMAHT-D solution. The two resulting solutions were mixed by rapidly syringing the $Cu(OTf)_2$ / (*R,R*)-Phbox / MeMAHT-^{13}C solution into the $Cu(OTf)_2$ / (*R,R*)-Phbox / MeMAHT-D solution at which time a stopwatch was started. 1.0 mL aliquots were withdrawn by syringe according to the table below and quenched by shaking the solution rapidly with a saturated solution of $NaHCO_3$ in D_2O. The aqueous layer was separated and added to an NMR tube. The isotope distribution in the MeMAHT mixture was analyzed by ^{1}H NMR (300 MHz, D_2O) by integrating the peak at δ 3.60 (quintet, 1H, J = 6.5 Hz) corresponding to MeMAHT-^{13}C versus the peak at δ 3.60 (q, 1H, J = 6.5 Hz) corresponding to MeMAHT, the lines of which are sufficiently well-resolved for separate integration. The percent of MeMAHT and MeMAHT-^{13}C relative to the total of the two was calculated from the integrals and are listed in the table below along with the % exchange calculated by the following formula:

$$\%\text{exchange} = \frac{2[\text{MeMAHT}]}{[\text{MeMAHT}-^{13}\text{C}] + [\text{MeMAHT}]} = 2 * \%\text{MeMAHT}$$

Run 1

time (s)	% MeMAHT	% MeMAHT-^{13}C	% exchange
60	13.2	86.8	26.4
120	18.5	81.5	37
180	24.2	75.8	48.4
300	28.4	71.6	56.8
360	31.5	68.5	63
420	34.3	65.7	68.6
540	37	63	74

Run 2

time (s)	% MeMAHT	% MeMAHT-^{13}C	% exchange
60	12.7	87.3	25.4
120	19.1	80.9	38.2
180	23.6	76.4	47.2
240	26.7	73.3	53.4
300	29.5	70.5	59
360	32.9	67.1	65.8
480	36	64	72
600	38.9	61.1	77.8
720	40.9	59.1	81.8

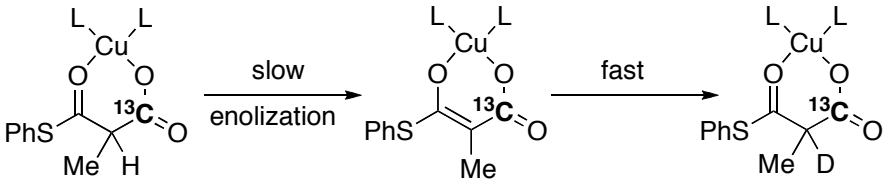
Run 3

time (s)	% MeMAHT	% MeMAHT-^{13}C	% exchange
60	11.1	88.9	22.2
180	20.6	79.4	41.2
300	26	74	52
420	30.6	69.4	61.2
540	35.7	64.3	71.4

The McKay equation[2] for exchange kinetics was used to calculate the exchange rate:

$$\ln(1 - \%\text{exchange}) = -\text{exchange rate}\left(\frac{[\text{MeMAHT - D}]_{\text{init}} + [\text{MeMAHT-}^{13}\text{C}]_{\text{init}}}{[\text{MeMAHT - D}]_{\text{init}}[\text{MeMAHT-}^{13}\text{C}]_{\text{init}}}\right)t$$

Isotopic Exchange



∴ **isotopic exchange rate = enolization rate**

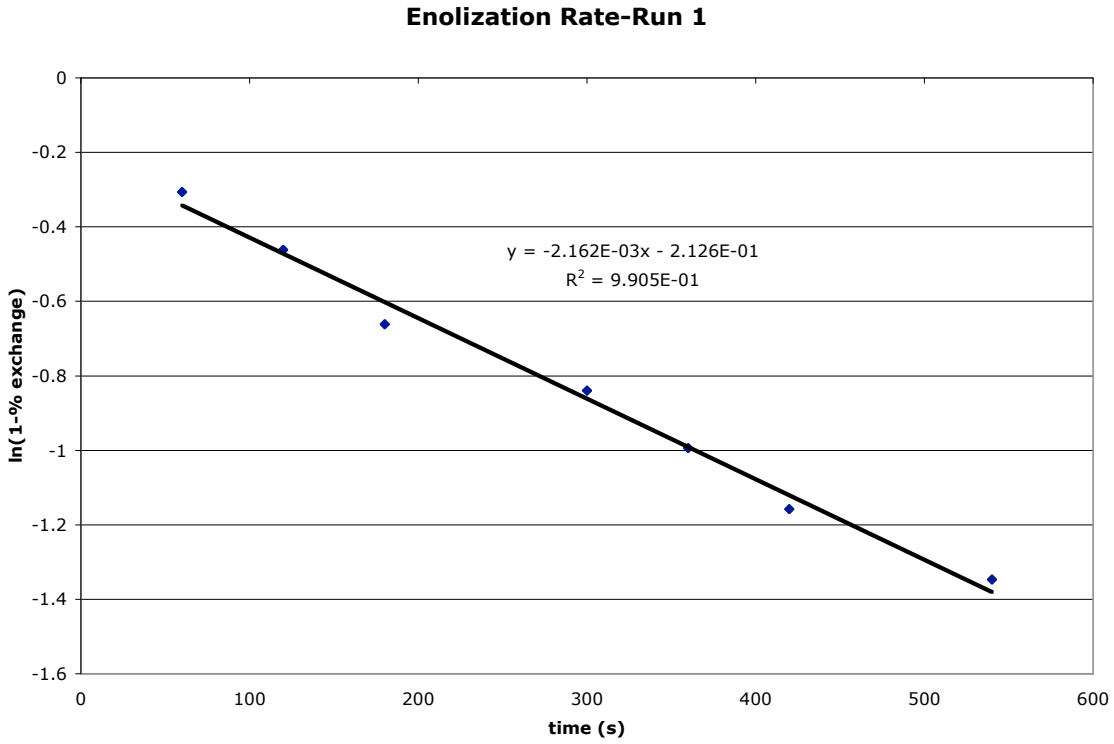
Since MeMAHT exists predominantly in its nonenolized form in 9:1 toluene / acetone solution containing $Cu(OTf)_2$ / (*R*,*R*)-Phbox (see below) and isotopic exchange between MeMAHT-^{13}C and MeMAHT-D occurs by reversible enolization, the rate of isotopic exchange should be equal to the enolization rate of MeMAHT (the rate-limiting step for isotopic exchange). ln(1-%exchange) was plotted versus time to give a line with a slope equal to:

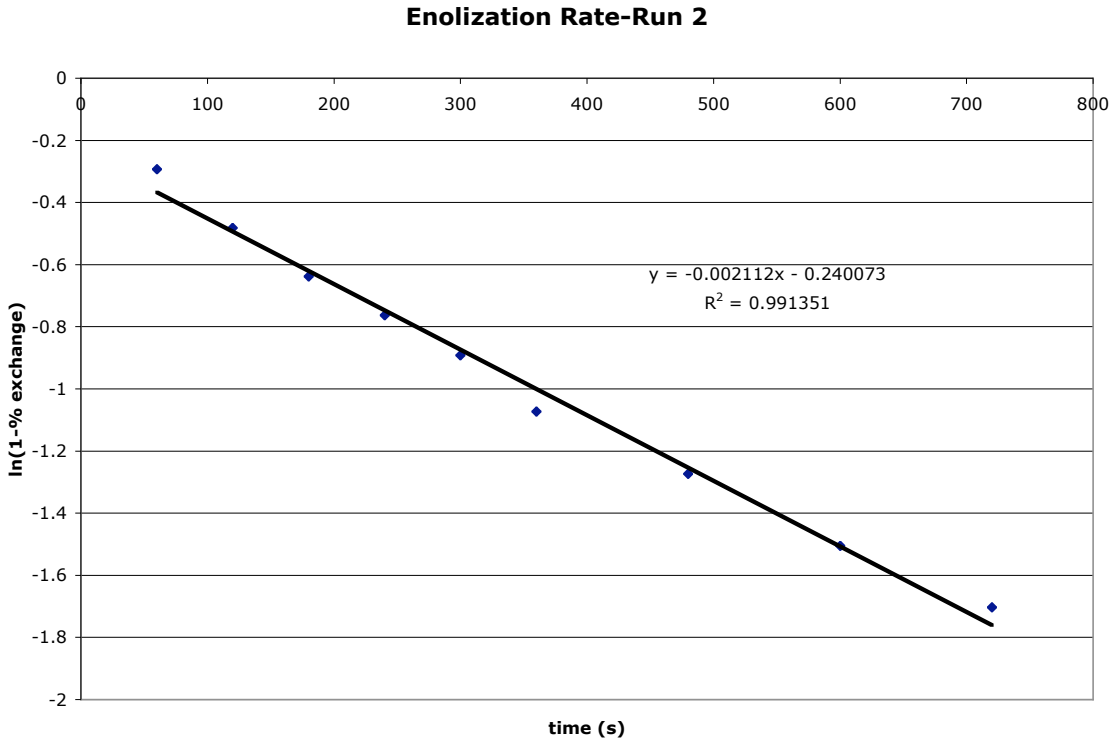
$$-\text{enolization rate}\left(\frac{[\text{MeMAHT - D}]_{\text{init}} + [\text{MeMAHT-}^{13}\text{C}]_{\text{init}}}{[\text{MeMAHT - D}]_{\text{init}}[\text{MeMAHT-}^{13}\text{C}]_{\text{init}}}\right) = -40\ \text{M}^{-1} * \text{enolization rate}$$

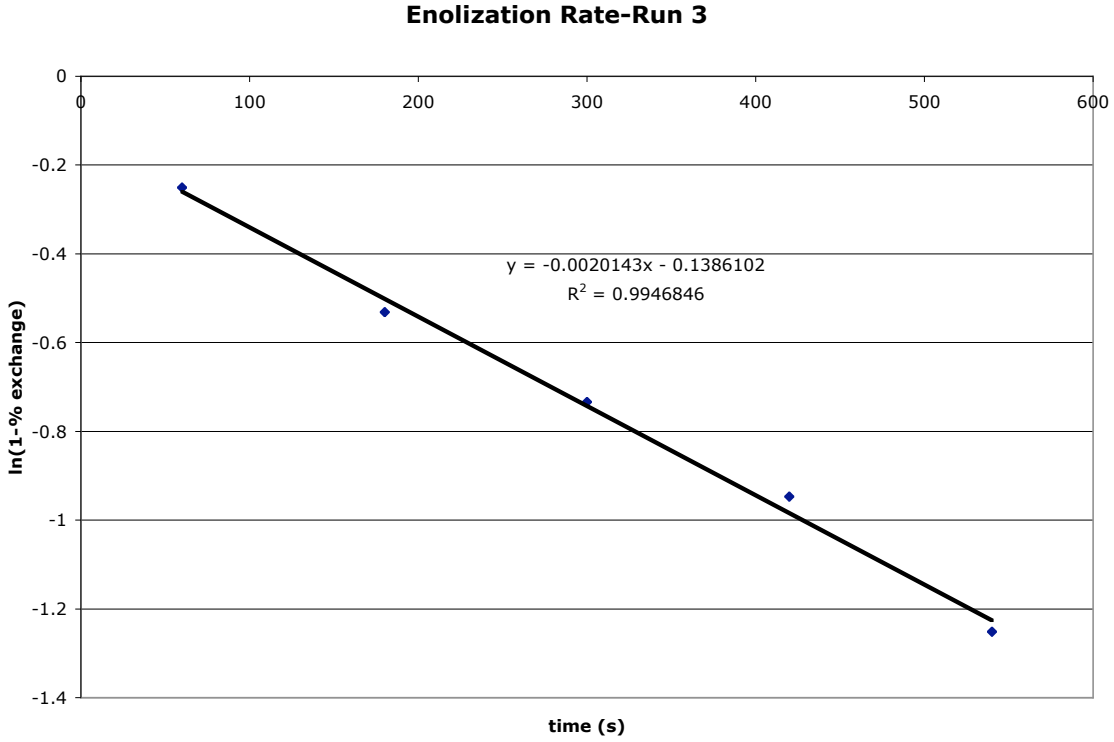
The half-time of exchange, $t_{1/2}$, was calculated using the following formula:

$$t_{1/2} = \left(\frac{[\text{MeMAHT - D}]_{\text{init}}[\text{MeMAHT-}^{13}\text{C}]_{\text{init}}}{[\text{MeMAHT - D}]_{\text{init}} + [\text{MeMAHT-}^{13}\text{C}]_{\text{init}}}\right)\left(\frac{\ln 2}{\text{enolization rate}}\right)$$

Results.


Enolization Rate-Run 1
y = -2.162E-03x - 2.126E-01
R2 = 9.905E-01
ln(1-% exchange)
time (s)
0
-0.2
-0.4
-0.6
-0.8
-1
-1.2
-1.4
-1.6
0
100
200
300
400
500
600


Enolization Rate-Run 2
y = -0.002112x - 0.240073
R2 = 0.991351
ln(1-% exchange)
time (s)
0
-0.2
-0.4
-0.6
-0.8
-1
-1.2
-1.4
-1.6
-1.8
-2
0
100
200
300
400
500
600
700
800

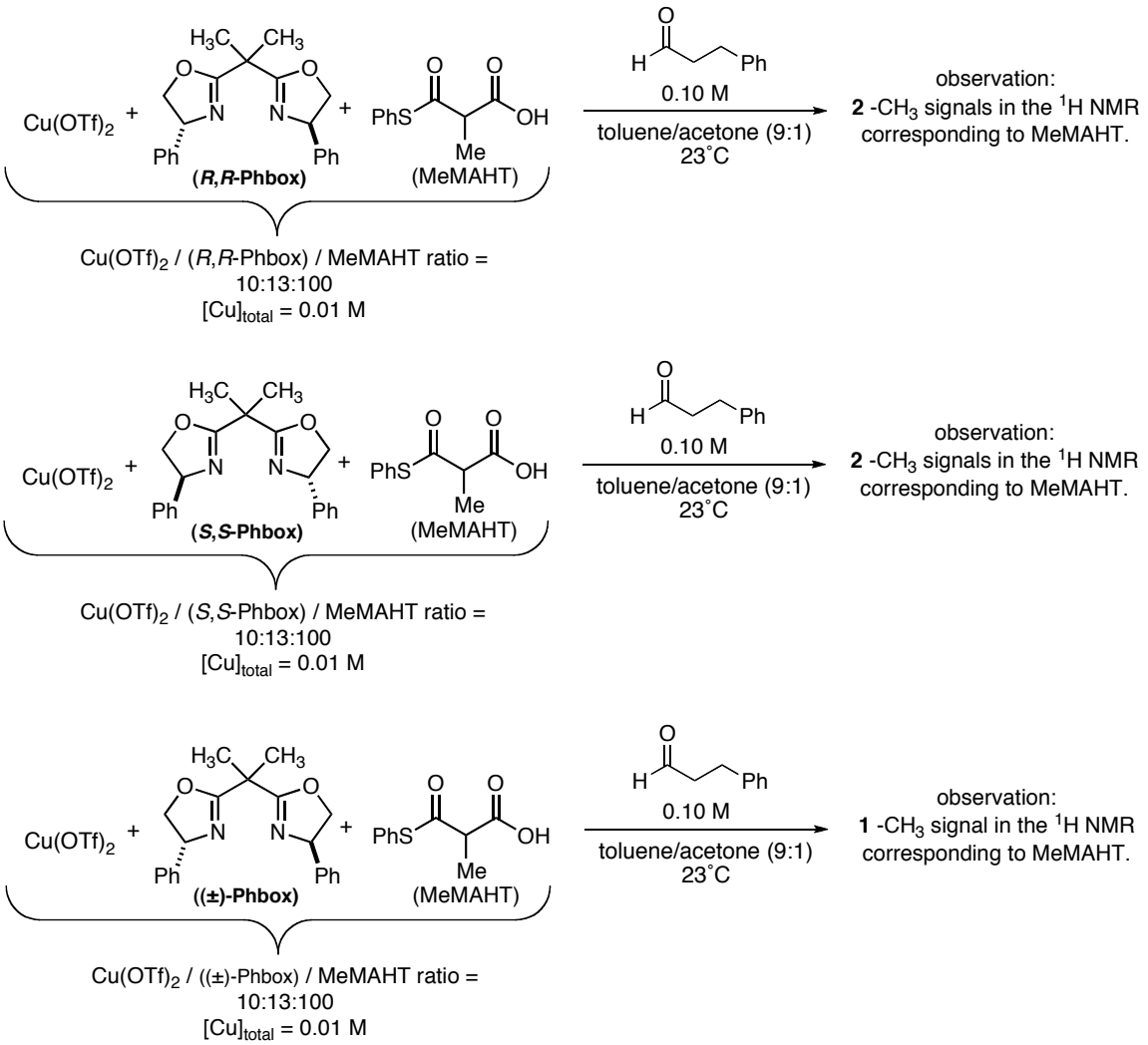


	run 1	run 2	run 3	average	stdev
slope	-2.16E-03	-2.11E-03	-2.01E-03	-2.10E-03	7.51E-05
enolization rate (M/s)	5.41E-05	5.28E-05	5.04E-05	**5.24E-05**	**1.88E-06**
$t_{1/2}$ (min)	5.34	5.47	5.74	**5.52**	**0.20**

The above results indicate that the MeMAHT enolization rate = 5.2 ± 0.2 x 10^{-5} M/s with a half-time equal to 5.5 ± 0.2 minutes. Based on all of our kinetics runs measured at the same starting material concentrations used in the above isotope exchange experiment and using 1.0 equivalents of aldehyde (0.10 M), the initial rate of the aldol reaction (rate = -d[MeMAHT]/dt) is 1.72 ± 0.04 x 10^{-6} M/s (1.88 ± 0.04 x 10^{-6} M/s if the anti stereoisomer of the product is included). The enolization rate is about 30 times faster than the aldol reaction at standard reaction conditions indicating that enolization is too fast to be the rate-limiting step; however, it is not so much faster than the aldol reaction

rate that it would be expected to be completely nonrate-limiting at high aldehyde concentrations.

Observation of Nonenolized MeMAHT Under Aldol Reaction Conditions.



$Cu(OTf)_2$ / (*R*,*R*)-Phbox / MeMAHT Solution.

22.6 mg (0.0625 mmol, 0.10 equiv) $Cu(OTf)_2$ was weighed out into a 25 mL flask and 131.4 mg (0.625 mmol, 1.00 equiv) of MeMAHT was added to the flask. 2 mL of a 9:1 mixture of toluene-d8 and acetone-d6 was added and the resulting light green solution was stirred for 15 minutes. A solution of 27.2 mg (0.0812 mmol, 0.13 equiv) of (*R*,*R*)-

Phbox in 2 mL of the 9:1 solvent mixture was added, and the solution was allowed to stir for 2 hours during which time it changed from green to blue. The solution was transferred to a 10 mL graduated cylinder and diluted with the solvent mixture to 5.00 mL.

$Cu(OTf)_2$ / (*S*,*S*)-Phbox / MeMAHT Solution.

A solution was prepared identically to the $Cu(OTf)_2$ / (*R*,*R*)-Phbox / MeMAHT solution except that (*S*,*S*)-Phbox was substituted for (*R*,*R*)-Phbox.

RCHO Solution.

3.00 mL of a solution containing 198 μL (1.50 mmol, 1.00 equiv) of dihydrocinnamaldehyde in a 9:1 mixture of toluene-d8 and acetone-d6 was prepared by adding the aldehyde via microsyringe to 2 mL of the solvent and diluting the solution to 3.00 mL in a 10 mL graduated cylinder.

Sample Preparation.

Different amounts of each solution as shown in the chart below was added to separate NMR tubes in order from left to right in the chart by means of a 500 μL syringe. Immediately upon adding the RCHO solution, the NMR tube was capped, shaken, and placed into a dry ice / acetone bath. The samples were rapidly thawed by immersion in a room temperature water bath immediately prior to insertion into the NMR spectrometer.

sample #	μL of $Cu(OTf)_2$/ (R,R)-Phbox solution	μL of $Cu(OTf)_2$/ (S,S)-Phbox solution	μL of RCHO solution	ee of Phbox	number of CH_3 peaks for MeMAHT
1	400	0	100	99%	**2**
2	0	400	100	-99%	**2**
3	200	200	100	0%	**1**

NMR Observations.

Samples 1 and 2 made from Phbox of > 99% optical purity displayed two broad peaks corresponding to the CH_3 protons of MeMAHT at 1.53 and 1.71 ppm. Sample 3 containing a 1:1 mixture of (*R,R*)-Phbox and (*S,S*)-Phbox displayed only one broad peak corresponding to the CH_3 protons of MeMAHT at 1.56 ppm. During our in situ NMR kinetics experiments, we observed that the two broad peaks are shifted downfield and become broader with increasing $Cu(OTf)_2$ / Phbox concentrations and are shifted upfield and become sharper (and larger) with increasing MeMAHT concentrations. This phenomenon indicates that exchange between MeMAHT bound to the copper catalyst and free MeMAHT is rapid on the NMR timescale. If the exchange process were slow, then one would expect to observe two extremely broad downfield MeMAHT resonances corresponding to the two diastereomeric catalyst complexes, (*R*)-MeMAHT:Cu:(*R,R*)-Phbox and (*S*)-MeMAHT:Cu:(*R,R*)-Phbox, provided that the resonances for the Cu complexes are not too broad to be visible, and one large, sharp resonance for free (±)-MeMAHT. In any case, if MeMAHT exchange were slow, then increasing the $Cu(OTf)_2$ / Phbox concentrations or the MeMAHT concentration would increase the integral of the corresponding NMR resonances but would have no effect on the chemical shift.

If MeMAHT existed as the enolized form in $Cu(OTf)_2$ / Phbox solution, then only one peak for the CH_3 protons of MeMAHT would be observed because the achiral enolized MeMAHT unit would not create diastereomers when bound to the C_2-symmetric Cu:Phbox catalyst. If enolization were rapid on the NMR timescale, then the enolization process would render the MeMAHT effectively achiral on the NMR timescale, i.e. the rapid enolization process would cause the peaks corresponding to the diastereomeric

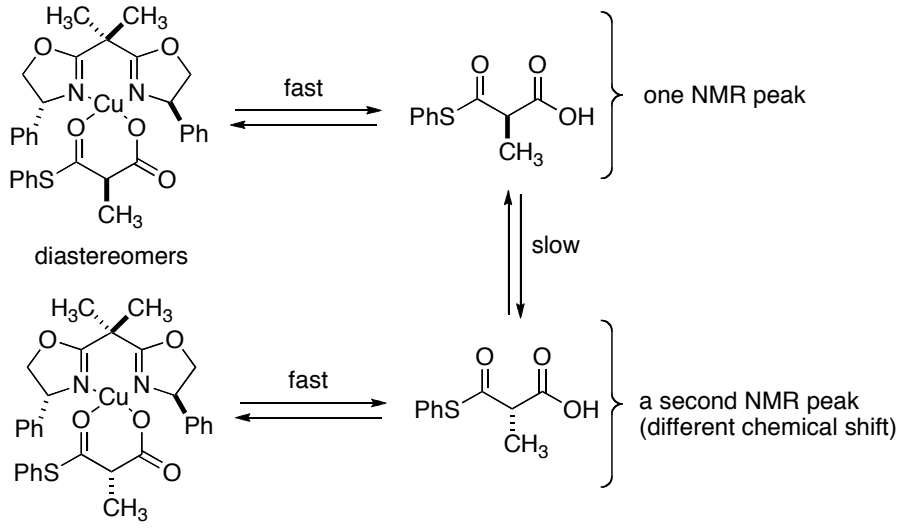
complexes between the Cu:Phbox catalyst and each enantiomer of the MeMAHT to coalesce into a single peak.

However, if MeMAHT exchange is rapid and enolization is slow on the NMR timescale, then the optically pure Cu:(*R,R*)-Phbox catalyst would form diastereomeric complexes with each enantiomer of the MeMAHT giving rise to two distinct sets of NMR peaks whose chemical shifts would depend upon the equilibrium constants between free MeMAHT and each enantiomer of MeMAHT bound to the Cu:(*R,R*)-Phbox catalyst, the relative amounts of MeMAHT and Cu:(*R,R*)-Phbox, as well as the chemical shifts of the (*R*)-MeMAHT:Cu:(*R,R*)-Phbox and (*S*)-MeMAHT:Cu:(*R,R*)-Phbox catalyst complexes. One NMR peak would correspond to (*R*)-MeMAHT in rapid equilibrium with (*R*)-MeMAHT:Cu:(*R,R*)-Phbox. The chemical shift of this peak would be the weighted-average chemical shift of the two species, because the rapid MeMAHT exchange process causes the chemically-inequivalent peaks for (*R*)-MeMAHT and (*R*)-MeMAHT:Cu:(*R,R*)-Phbox to coalesce into a single time-averaged peak. A second NMR peak would correspond to (*S*)-MeMAHT in rapid equilibrium with (*S*)-MeMAHT:Cu:(*R,R*)-Phbox again occurring at the weighted-average chemical shift of the two species. These two peaks would have different chemical shifts because the diastereomeric components that give rise to the time-averaged peaks have different chemical shifts or are present in different concentrations or both. This situation is analogous to the use of lanthanide complexes with chiral ligands to determine the optical purity of mixtures of enantiomers.

Although rapid MeMAHT exchange equilibrates (*R*)-MeMAHT with (*R*)-MeMAHT:Cu:(*R,R*)-Phbox and (*S*)-MeMAHT with (*S*)-MeMAHT:Cu:(*R,R*)-Phbox to

give two NMR peaks instead of the possible three peaks, only MeMAHT enolization can equilibrate (*R*)-MeMAHT:Cu:(*R,R*)-Phbox with (*S*)-MeMAHT:Cu:(*R,R*)-Phbox and cause these two peaks to coalesce into one. Rapid enolization would cause a molecule of (*R*)-MeMAHT to be in the same time-averaged magnetic environment as a molecule of (*S*)-MeMAHT. Because MeMAHT exchange is fast and enolization is slow on the NMR time scale, two peaks are observed when (*R,R*)-Phbox is used.

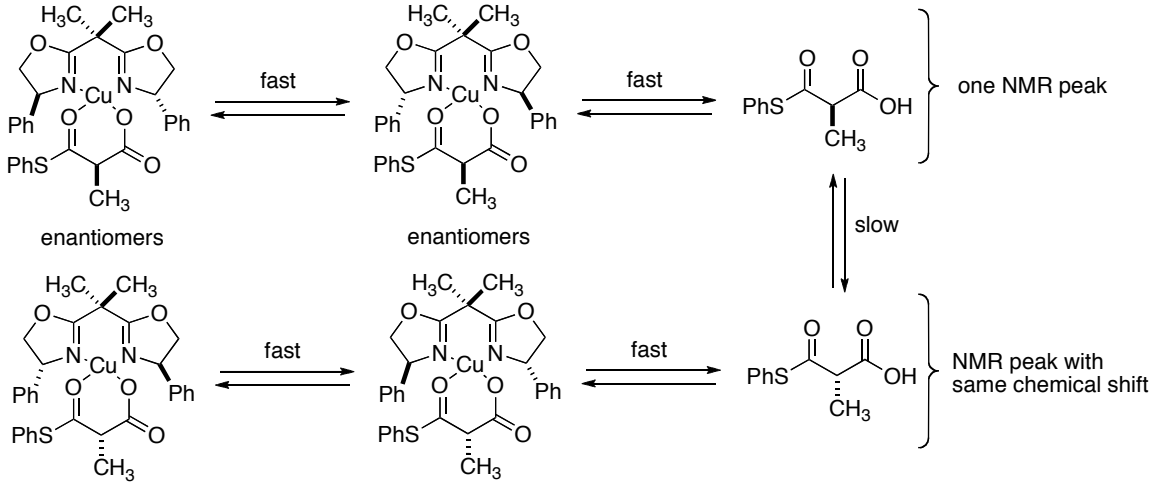
Equilibria with (*R,R*)-Phbox only



If racemic Phbox is used, then there is a mechanism that causes a molecule of (*R*)-MeMAHT to be in the same time-averaged magnetic environment as a molecule of (*S*)-MeMAHT. Rather than requiring rapid enolization to achieve this effect, a molecule of (*R*)-MeMAHT can dissociate from Cu:(*R,R*)-Phbox and associate with Cu:(*S,S*)-Phbox to form (*R*)-MeMAHT:Cu:(*S,S*)-Phbox. Likewise, a molecule of (*S*)-MeMAHT can dissociate from Cu:(*S,S*)-Phbox and associate with Cu:(*R,R*)-Phbox to form (*S*)-MeMAHT:Cu:(*R,R*)-Phbox. Although this exchange process does not interconvert (*R*)-MeMAHT and (*S*)-MeMAHT, it does allow (*R*)-MeMAHT to form an enantiomeric complex corresponding to each complex formed by (*S*)-MeMAHT, e.g. (*R*)-

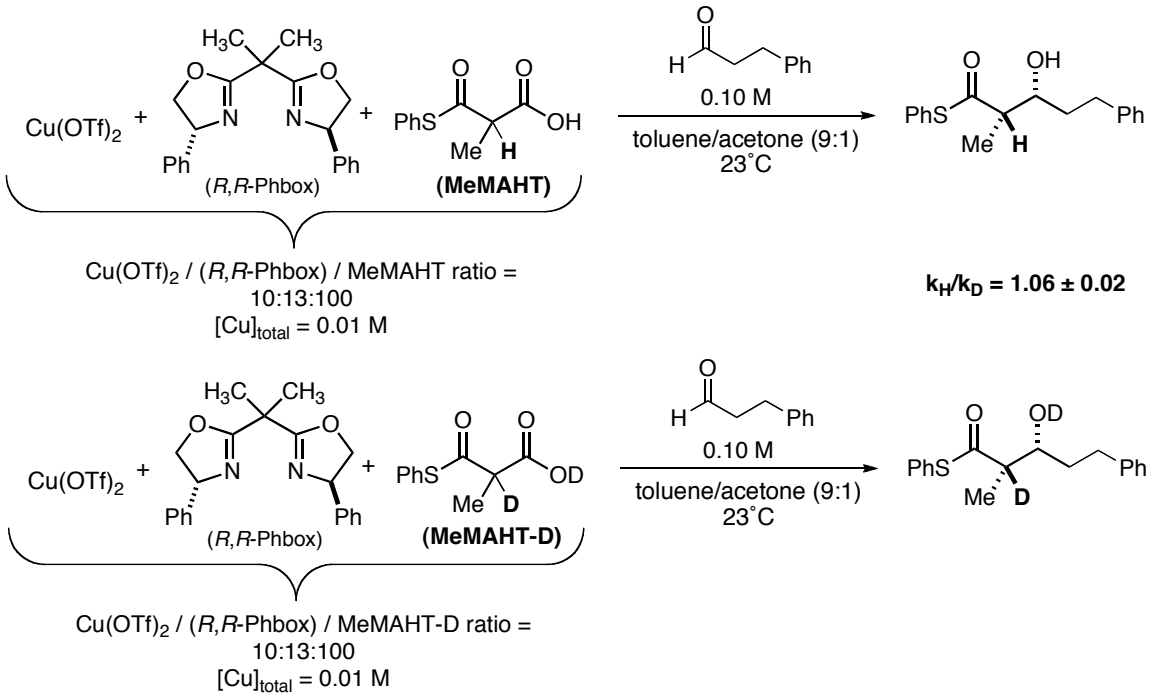
MeMAHT:Cu:(*S*,*S*)-Phbox and (*S*)-MeMAHT:Cu:(*R*,*R*)-Phbox. Using racemic Phbox allows for both MeMAHT enantiomers to exist in equivalent time-averaged environments and causes the two peaks seen when only (*R*,*R*)-Phbox is used to coalesce into a single peak.

Equilibria with (±)-Phbox



Because two NMR peaks corresponding to the CH_3 protons of MeMAHT are observed when (*R*,*R*)-Phbox is used and only one peak when racemic Phbox is used, we conclude that the resting state of the MeMAHT:Cu:(*R*,*R*)-Phbox catalyst contains MeMAHT in its nonenolized form. Furthermore, enolization must be slow on the NMR timescale. This was confirmed by measurement of the enolization rate by observing the isotopic exchange rate between MeMAHT-^{13}C and MeMAHT-D (see above). It was demonstrated above that the aldol reaction is kinetically inverse first-order in [MeMAHT] at MeMAHT concentrations spanning 0.10 M to 0.40 M. For this reason we believe that a second molecule of MeMAHT binds reversibly to the Cu catalyst in its resting state and renders it inactive.

Isotope Effect at the α-hydrogen (MeMAHT vs. MeMAHT-D) by Rate Comparison.



$Cu(OTf)_2$ / (*R,R*)-Phbox / MeMAHT / Standard Solution.

45.2 mg (0.125 mmol, 0.10 equiv) $Cu(OTf)_2$ was weighed out into a 25 mL flask and 65.7 mg (0.312 mmol, 0.25 equiv) of MeMAHT was added to the flask. 2 mL of a 9:1 mixture of toluene-d8 and acetone-d6 was added and the resulting light green solution was stirred for 15 minutes. A solution of 54.3 mg (0.162 mmol, 0.13 equiv) of (*R,R*)-Phbox in 2 mL of the 9:1 solvent mixture was added, and the solution was allowed to stir for 2 hours during which time it changed from green to blue. 86.4 mg (0.625 mmol, 0.50 equiv) of 1,4-dimethoxybenzene was added as an internal standard such that each sample solution would be 0.050 M in internal standard during the kinetics measurements. The solution was transferred to a 10 mL graduated cylinder and diluted with the solvent mixture to 5.00 mL.

$Cu(OTf)_2$ / (*R*,*R*)-Phbox / MeMAHT-D / Standard Solution.

A $Cu(OTf)_2$ / MeMAHT-D solution was prepared identically to the $Cu(OTf)_2$ / (*R*,*R*)-Phbox / MeMAHT solution except that the MeMAHT was replaced with 66.3 mg (0.312 mmol, 0.25 equiv) of MeMAHT-D.

MeMAHT Solution.

78.8 mg (0.375 mmol) of MeMAHT was dissolved in 1.5 mL of the 9:1 mixture of toluene-d8 and acetone-d6 and diluted to 2.00 mL in a 10 mL graduated cylinder to produce a solution with a MeMAHT concentration of 0.188 M.

MeMAHT-D Solution.

79.6 mg (0.375 mmol) of MeMAHT-D was dissolved in 1.5 mL of the 9:1 mixture of toluene-d8 and acetone-d6 and diluted to 2.00 mL in a 10 mL graduated cylinder to produce a solution with a MeMAHT-D concentration of 0.188 M.

RCHO Solution.

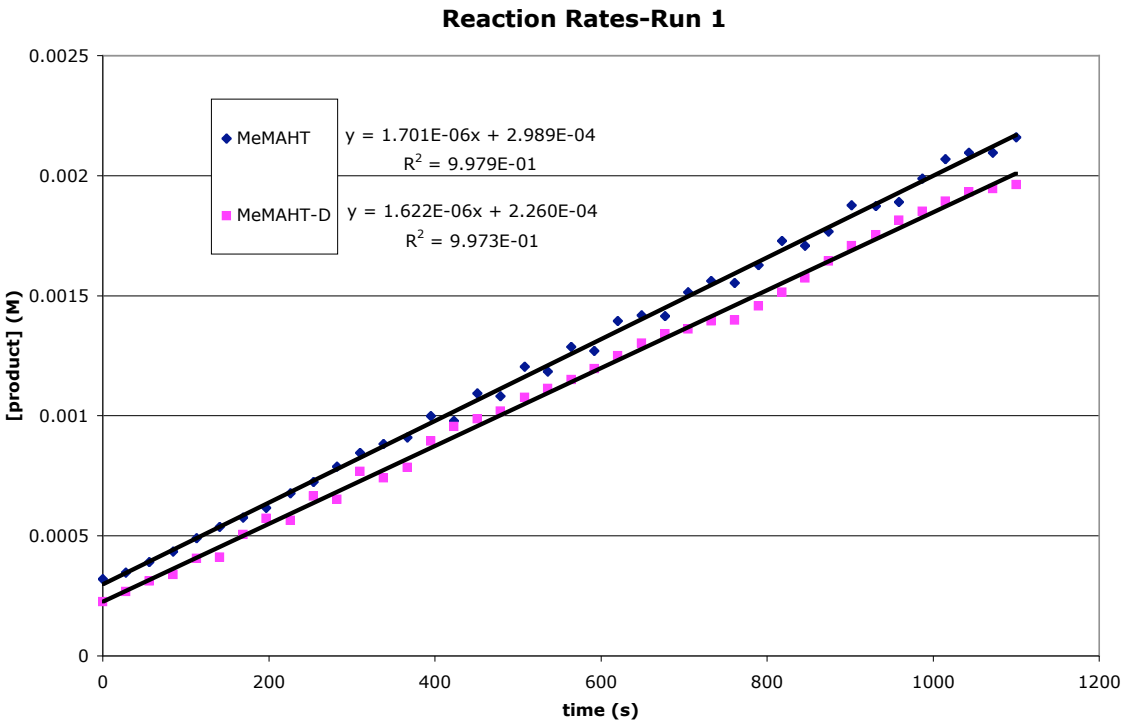
3.00 mL of a solution containing 198 μL (1.50 mmol, 1.00 equiv) of dihydrocinnamaldehyde in a 9:1 mixture of toluene-d8 and acetone-d6 was prepared by adding the aldehyde via microsyringe to 2 mL of the solvent and diluting the solution to 3.00 mL in a 10 mL graduated cylinder.

Sample Preparation.

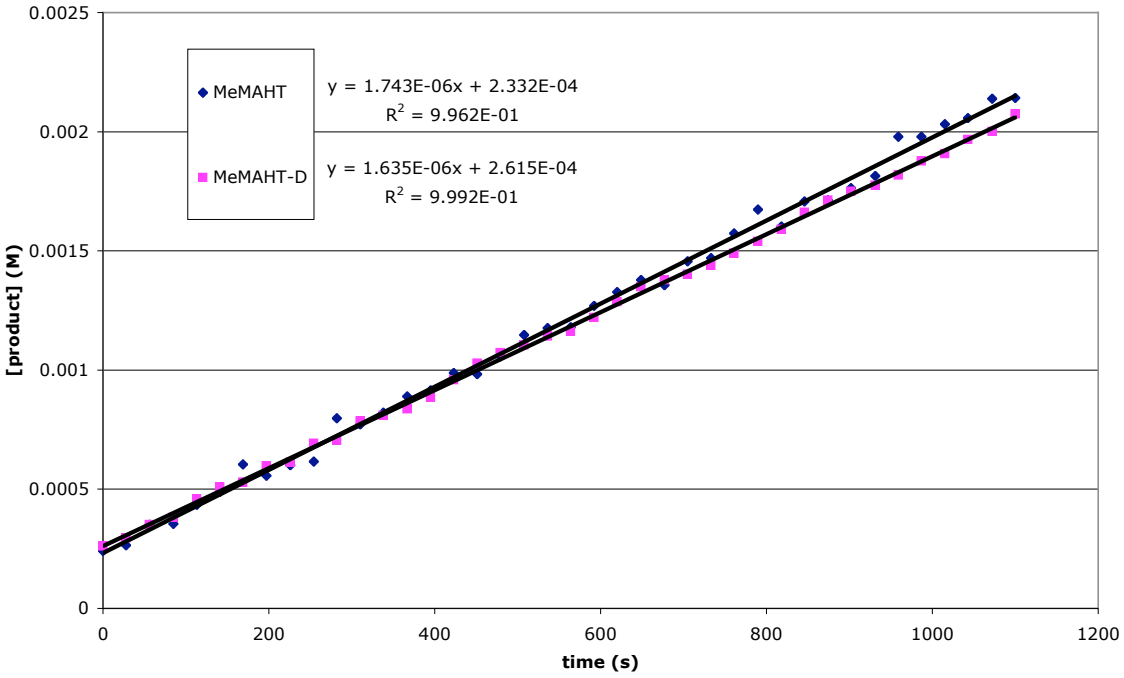
Samples for measuring the aldol reaction rate when using MeMAHT were prepared by mixing 200 μL of $Cu(OTf)_2$ / (*R*,*R*)-Phbox / MeMAHT solution, 200 μL of MeMAHT solution, and 100 μL of RCHO solution. Samples for measuring the aldol reaction rate when using MeMAHT-D were prepared by mixing 200 μL of $Cu(OTf)_2$ / (*R*,*R*)-Phbox / MeMAHT-D solution, 200 μL of MeMAHT-D solution, and 100 μL of

RCHO solution. The MeMAHT-D solution is added immediately before the RCHO solution to minimize the loss of deuterium from the MeMAHT-D in the presence of $Cu(OTf)_2$ and (*R*,*R*)-Phbox during the sample preparation. The MeMAHT sample is prepared in exactly the same manner for consistency. Immediately upon adding the RCHO solution, the NMR tube was capped, shaken, and placed into a dry ice / acetone bath. The samples were rapidly thawed by immersion in a room temperature water bath immediately prior to insertion into the NMR spectrometer. The kinetics measurements were performed as detailed in the general procedure.

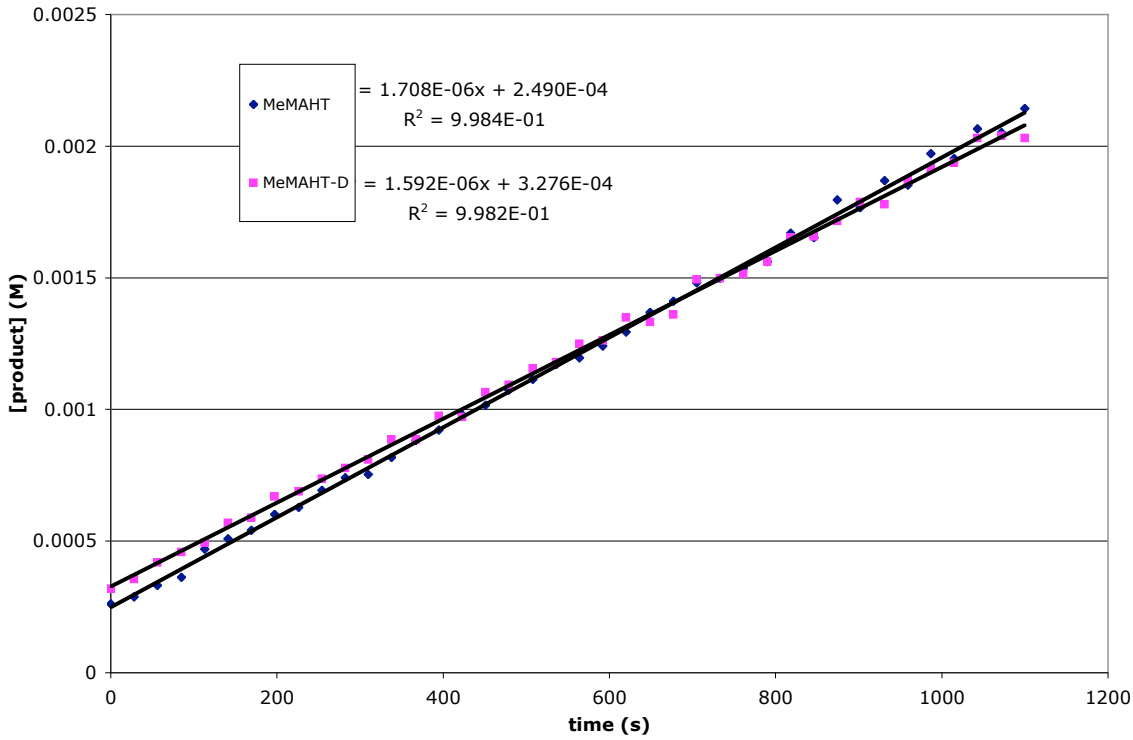
Results.



Reaction Rates-Run 2



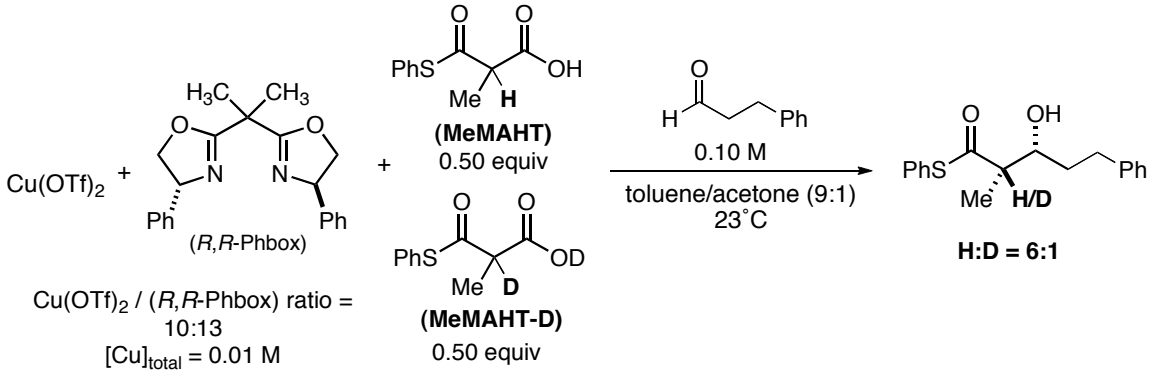
Reaction Rates-Run 3



	run 1 rate	run 2 rate	run 3 rate	average	stdev
MeMAHT	1.701E-06	1.743E-06	1.708E-06	1.717E-06	2.253E-08
MeMAHT-D	1.622E-06	1.635E-06	1.592E-06	1.616E-06	2.171E-08
k_H/k_D	1.049	1.066	1.073	**1.063**	**0.012**

Based on the above data, the isotope effect on the initial rate of an aldol reaction using unlabeled MeMAHT compared to MeMAHT-D, $k_H/k_D = 1.06 \pm 0.02$.

Measurement of the H:D Ratio in the Aldol Product Arising from 1:1 MeMAHT / MeMAHT-D.



$Cu(OTf)_2$ / (*R,R*)-Phbox / MeMAHT Solution.

18.1 mg (0.050 mmol, 0.10 equiv) $Cu(OTf)_2$ was weighed out into a 25 mL flask and 105.1 mg (0.500 mmol, 1.00 equiv) of MeMAHT was added to the flask. 3.0 mL of a 9:1 mixture of toluene / acetone was added and the resulting light green solution was stirred for 15 minutes. A solution of 21.7 mg (0.065 mmol, 0.13 equiv) of (*R,R*)-Phbox in 2.0 mL of the 9:1 solvent mixture was made, and 3.0 mL of this solution (0.10 equiv) was added to the reaction mixture. The resulting solution was allowed to stir for 2 hours during which time it changed from green to blue.

$Cu(OTf)_2$ / (*R,R*)-Phbox / MeMAHT-D solution.

A $Cu(OTf)_2$ / (*R,R*)-Phbox / MeMAHT-D solution was prepared identically to the $Cu(OTf)_2$ / (*R,R*)-Phbox / MeMAHT solution except that the MeMAHT was replaced with 106.1 mg (0.500 mmol, 1.00 equiv) of MeMAHT-D.

Experimental Procedure.

The $Cu(OTf)_2$ / (*R,R*)-Phbox / MeMAHT solution was added by syringe to the $Cu(OTf)_2$ / (*R,R*)-Phbox / MeMAHT-D solution. 66 μL (0.500 mmol, 1.00 equiv) of dihydrocinnamaldehyde was added by microsyringe and the reaction mixture was allowed to stir for 10 minutes. The reaction was quenched by shaking it with 10 mL of 0.5 M HCl. (Using 0.5 M DCl in D_2O instead of 0.5 M HCl had no effect on the isotope distribution in the product.) The product was extracted out with diethyl ether and washed with saturated aqueous $NaHCO_3$ to remove unreacted MeMAHT. The ether solution was washed with brine, dried over anhydrous sodium sulfate, and evaporated to leave behind a colorless liquid. The liquid was loaded onto a silica gel column which was eluted with 90% hexanes / 10% ethyl acetate. The fractions containing the aldol product were combined and the solvent was evaporated. The isotope distribution in the aldol product was analyzed by ^1H NMR (500 MHz, C_6D_6). The CH_3 peak for the aldol product containing a deuterium at the α-position appears as a singlet at 1.12 ppm while the CH_3 peak for the unlabeled product appears as a doublet at 1.13 ppm. This causes the singlet of the deuterated product to be perfectly overlapped with the upfield (right) line of the doublet of the unlabeled product. Each of these lines can be integrated separately and the ratio of H:D at the α-position in the product was calculated according to the following equations:

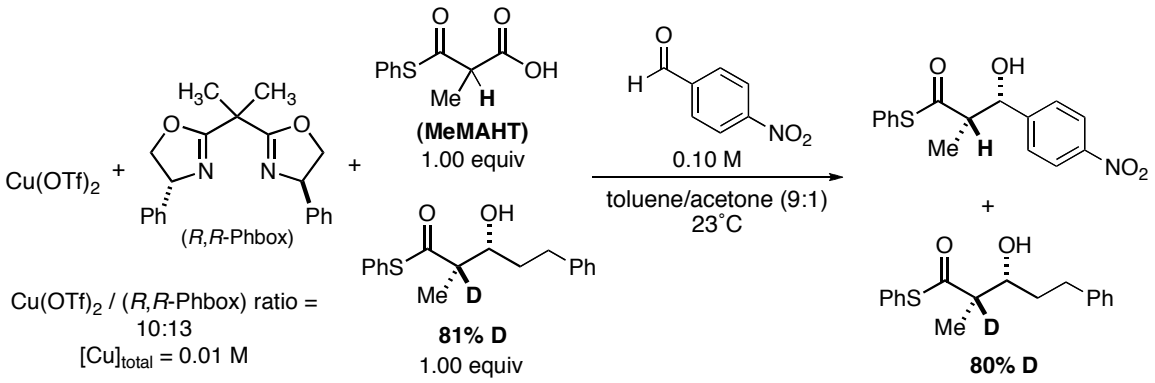
$$\%D = \frac{\text{integral}_{\text{right}} - \text{integral}_{\text{left}}}{\text{integral}_{\text{total}}}$$

$$\%H = 100\% - \%D$$

$$H : D \text{ ratio} = \frac{\%H}{\%D}$$

Three independent runs of this experiment gave H:D ratios of 6.0:1, 6.3:1 and 5.9:1, respectively. We conclude that the observed 6:1 H:D ratio must be caused by a protonation step following the rate-limiting step. If the elementary step responsible for the 6:1 ratio preceded the rate-limiting step or was the rate-limiting step, then one would expect that an aldol reaction using MeMAHT would be considerably faster than one using MeMAHT-D which was shown not to be the case (see above).

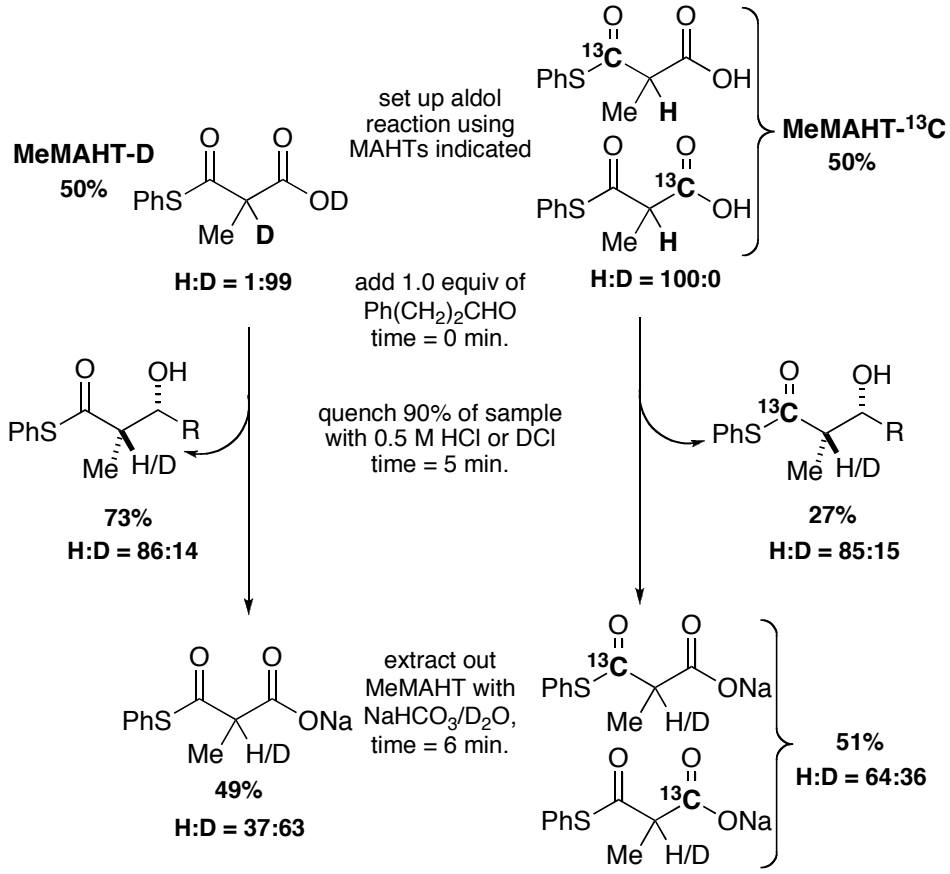
Demonstration of Configurational Stability of the Aldol Product to the Aldol Reaction Conditions.



18.1 mg (0.050 mmol, 0.10 equiv) $Cu(OTf)_2$ was weighed out into a 25 mL flask and 105.1 mg (0.500 mmol, 1.00 equiv) of MeMAHT was added to the flask. 2.0 mL of a 9:1 mixture of toluene / acetone was added and the resulting light green solution was stirred for 15 minutes. A solution of 21.7 mg (0.065 mmol, 0.13 equiv) of (*R,R*)-Phbox in 2.0 mL of the 9:1 solvent mixture was added to the reaction mixture. The resulting

solution was allowed to stir for 2 hours during which time it changed from green to blue. 150.7 mg (0.500 mmol, 1.00 equiv) of aldol product (made from MeMAHT-D and dihydrocinnamaldehyde with a few drops of D_2O added to the reaction mixture) containing 81% D at the α-position and 75.6 mg (0.500 mmol, 1.00 equiv) of 4-nitrobenzaldehyde dissolved in 1.0 mL of the 9:1 solvent mixture was added by syringe and the solution was allowed to stir for 24 hours. The reaction was quenched by shaking it with 5 mL of 0.5 M HCl. The product mixture was extracted out with diethyl ether. The ether solution was washed with brine, dried over anhydrous sodium sulfate, and evaporated to leave behind a yellow oil containing some solids. The crude product mixture was loaded onto a silica gel column which was eluted with 90% hexanes / 10% ethyl acetate. The fractions containing the aldol product made from dihydrocinnamaldehyde were combined and the solvent was evaporated. By the method described in the previous experiment, the α-position was determined to contain 80% D indicating that the deuterium content remained essentially unchanged during the aldol reaction and purification process. We therefore conclude that the aldol product is configurationally stable to the aldol reaction conditions. Note: This experiment was performed only once.

MeMAHT-^{13}C / MeMAHT-D Crossover Experiment.



$Cu(OTf)_2$ / (*R,R*)-Phbox / MeMAHT-^{13}C Solution.

36.2 mg (0.100 mmol, 0.10 equiv) $Cu(OTf)_2$ was weighed out into a 50 mL flask and 52.8 mg (0.250 mmol, 0.25 equiv) of MeMAHT-^{13}C was added to the flask. 4.0 mL of a 9:1 mixture of toluene / acetone was added and the resulting light green solution was stirred for 15 minutes. A solution of 43.5 mg (0.130 mmol, 0.13 equiv) of (*R,R*)-Phbox in 3.0 mL of the 9:1 solvent mixture was added, and the solution was allowed to stir for 2 hours during which time it changed from green to blue.

$Cu(OTf)_2$ / (*R,R*)-Phbox / MeMAHT-D Solution.

A $Cu(OTf)_2$ / MeMAHT-D solution was prepared identically to the $Cu(OTf)_2$ / MeMAHT-^{13}C solution except that the MeMAHT-^{13}C was replaced with 53.1 mg (0.250

mmol, 0.25 equiv) of MeMAHT-D.

MeMAHT-^{13}C Solution.

158.4 mg (0.750 mmol) of MeMAHT-^{13}C was dissolved in 3.0 mL of the 9:1 mixture of toluene / acetone.

MeMAHT-D Solution.

159.2 mg (0.750 mmol) of MeMAHT-D was dissolved in 3.0 mL of the 9:1 mixture of toluene / acetone.

Experimental Procedure.

When the four above solutions had been prepared, the MeMAHT-^{13}C solution was added by syringe to the rapidly stirring $Cu(OTf)_2$ / MeMAHT-^{13}C solution. Likewise, the MeMAHT-D solution was added by syringe to the rapidly stirring $Cu(OTf)_2$ / MeMAHT-D solution. The two resulting solutions were mixed by rapidly syringing the $Cu(OTf)_2$ / MeMAHT-^{13}C solution into the $Cu(OTf)_2$ / MeMAHT-D solution. After 30 seconds of stirring, 125 μL (0.947 mmol, 1.00 equiv) of dihydrocinnamaldehyde was added by syringe and a timer was started immediately thereafter.

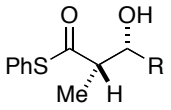
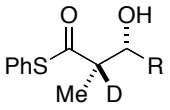
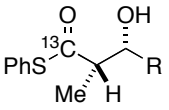
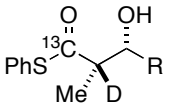
After 5 minutes had elapsed, about 90% of the solution was removed by syringe and quenched by shaking it with 50 mL of 0.5 M HCl. (Using 0.5 M DCl in D_2O instead of 0.5 M HCl had no effect on the isotope distribution in the product.) The product was extracted out with diethyl ether and washed with saturated aqueous $NaHCO_3$ to remove unreacted MeMAHT. The ether solution was washed with brine, dried over anhydrous sodium sulfate, and evaporated to leave behind a colorless liquid. The liquid was loaded onto a silica gel column which was eluted with 90% hexanes / 10% ethyl acetate. The

fractions containing the aldol product were combined and the solvent was evaporated. The isotope distribution in the aldol product was analyzed by ^{1}H NMR (400 MHz, C_6D_6). The peaks corresponding to the $-CH_3$ protons in each isotopic isomer are sufficiently resolved that the proportion of each isomer could be determined from the integrals. Because of the additional coupling to ^{13}C, there is no overlap between the ^{13}C-containing isomers and isomers containing ^{12}C only. The peaks corresponding to isomers with a deuterium in the α-position overlap perfectly with the upfield (right) line of the doublet corresponding to isomers with a proton in the α-position and so the following formulas are used to obtain the percent of each isomer:

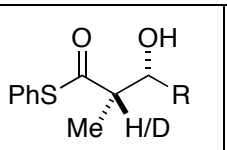
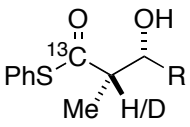
$$\%D = \frac{\text{integral}_{\text{right}} - \text{integral}_{\text{left}}}{\text{integral}_{\text{total}}}$$

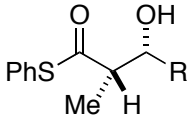
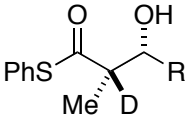
$$\%H = 100\% - \%D$$

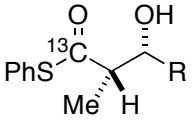
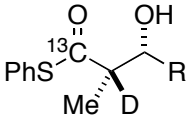
The results are summarized in the table below:

				
-CH_3 NMR peak	δ 1.13 (d, J = 7.0)	δ 1.12 (s)	δ 1.13 (dd, J_1 = 7.0, J_2 = 6.0)	δ 1.12 (d, J = 6.0)
product run 1	63%	10%	24%	4%
product run 2	64%	11%	22%	4%
product run 3	62%	10%	24%	4%

Averaging the results above gives the following ratios:

	
73%	27%

	
86%	14%

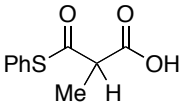
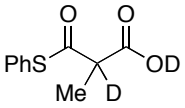
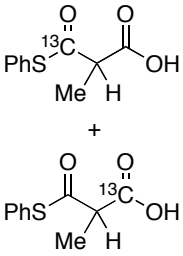
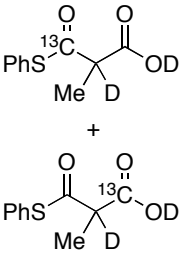
	
85%	15%

The remaining 10% of the reaction mixture was quenched after 6 minutes by shaking it rapidly with a saturated solution of $NaHCO_3$ in D_2O. The aqueous layer was separated and added to an NMR tube. The isotope distribution in the reisolated product was analyzed by ^1H NMR (400 MHz, D_2O). The peaks corresponding to the $-CH_3$ protons in each isotopic isomer are sufficiently resolved that the proportion of each isomer could be determined from the integrals. Because of the additional coupling to ^{13}C, there is no overlap between the ^{13}C-containing isomers and isomers containing ^{12}C only. The peaks corresponding to isomers with a deuterium in the α-position overlap perfectly with the upfield (right) line of the doublet corresponding to isomers with a proton in the α-position and so the following formulas are used to obtain the percent of each isomer:

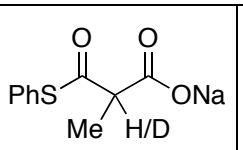
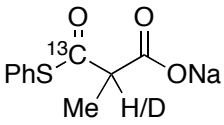
$$\%D = \frac{\text{integral}_{\text{right}} - \text{integral}_{\text{left}}}{\text{integral}_{\text{total}}}$$

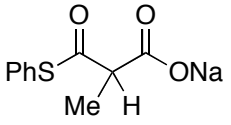
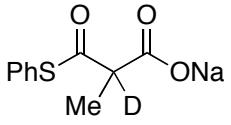
$$\%H = 100\% - \%D$$

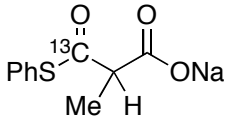
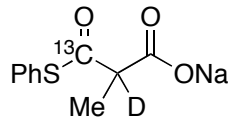
The results are summarized in the table below:

				
-CH_3 NMR peak	δ 1.18 (d, J = 7.0)	δ 1.17 (s)	δ 1.18 (dd, J_1 = 7.0, J_2 = 6.0)	δ 1.17 (d, J = 6.0)
original starting material	0.5%	49.5%	50%	0%
reisolated starting material run 1	19%	29%	32%	20%
reisolated starting material run 2	19%	31%	31%	19%
reisolated starting material run 3	16%	33%	34%	16%

Averaging the results above gives the following ratios:

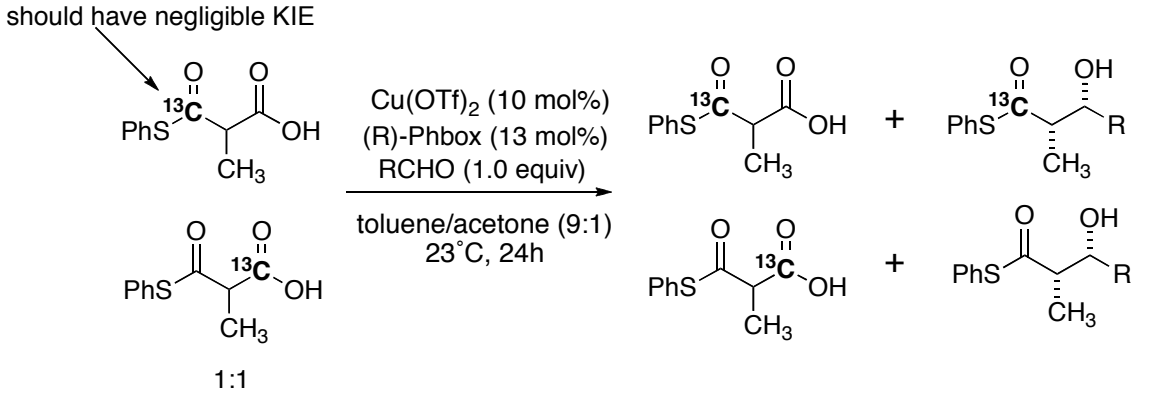
	
49%	51%

	
37%	63%

	
36%	64%

The results above indicate that after 5 minutes the original isotopic labeling in the starting materials has become completely scrambled in the product. Because the products are configurationally stable to the reaction and purification conditions (see above), the scrambling cannot have occurred after the aldol product was first formed. Because even after an additional minute of exposure to the reaction conditions about 27% of the isotopic label in the starting material is still unscrambled, the complete scrambling of the product could not have occurred prior to the aldol reaction. Therefore, we conclude that the scrambling must have occurred as part of one or more of the elementary steps of the aldol reaction itself as a deprotonation / reprotonation process. Furthermore, since both the MeMAHT-^{13}C and the MeMAHT-D led to products containing an identical H:D ratio, they presumably proceeded through essentially identical intermediates, i.e. enolates, each having lost all memory and the corresponding influence of its original isotopic label.

Determination of the ^{13}C Kinetic Isotope Effect (KIE) at the Carboxylate Carbon of MeMAHT.



90.4 mg (0.250 mmol, 0.10 equiv) $Cu(OTf)_2$ was weighed out into a 100 mL flask and 528.1 mg (2.500 mmol, 1.00 equiv) of MeMAHT-^{13}C was added to the flask. 15 mL of a 9:1 mixture of toluene / acetone was added and the resulting light green solution was stirred for 15 minutes. A solution of 108.7 mg (0.325 mmol, 0.13 equiv) of (*R*,*R*)-Phbox and 138.2 mg (1.000 mmol, 0.40 equiv) of 1,4-dimethoxybenzene as an internal standard in 10 mL of the 9:1 solvent mixture was added, and the solution was allowed to stir for 2 hours during which time it changed from green to blue. 329 μL (2.500 mmol, 1.00 equiv) of dihydrocinnamaldehyde was added by syringe, and the reaction mixture allowed to stir overnight. After 12 hours the progress of the reaction was monitored by TLC until the spot for the MeMAHT-^{13}C was barely visible.

The reaction mixture was quenched with 50 mL of 0.5 M HCl. The mixture was extracted with 5 x 50 mL diethyl ether, and the ether solution was washed with 5 x 50 mL of saturated aqueous $NaHCO_3$. 4 M HCl was slowly added to the aqueous layer with vigorous stirring until the pH was 2-3. The aqueous layer was extracted with 5 x 50 mL diethyl ether, and the organic layer was washed with 50 mL of brine. The brine was then extracted with 2 x 50 mL diethyl ether and the ether solutions were combined, dried over anhydrous sodium sulfate, and evaporated to leave behind a colorless oil which was ≥ 95% pure MeMAHT-^{13}C. The sample was weighed and the percent conversion was calculated by the following equation:

$$\%\ \text{conversion} = 100\% - \left(\frac{\text{MeMAHT-}^{13}\text{C weight}_{\text{final}}}{\text{MeMAHT-}^{13}\text{C weight}_{\text{initial}}} \right)$$

The MeMAHT-^{13}C was converted to its methyl ester and purified because using the MeMAHT-^{13}C directly for ^{13}C NMR analysis occasionally led to broadened peaks presumably due to paramagnetic impurites. The reisolated MeMAHT-^{13}C was dissolved

in 5 mL of 9:1 benzene / methanol and (trimethylsilyl)diazomethane (2.0 M in hexanes) was added dropwise until the yellow color persisted. The solvent was evaporated and the crude product was loaded onto a silica gel column which was eluted with 90% hexanes / 10% ethyl acetate. The fractions containing the methyl ester of MeMAHT-^{13}C were combined and evaporated to give a product that was pure by ^{1}H NMR. A sample of the original, unreacted MeMAHT-^{13}C was converted to its methyl ester in an identical manner to serve as a standard for ^{13}C NMR analysis.

NMR samples of the standard and reisolated starting material each having been converted to their methyl esters were prepared identically. 30 mg of the methyl ester was dissolved in $CDCl_3$ and transferred to an NMR tube, and the solution was diluted with $CDCl_3$ to the level of 5 cm in the tube. We carried out a T_1 determination by the inversion-recovery method for the sample and then acquired the ^{13}C NMR spectrum of the sample at 100.603 MHz with 120 s (120 s > 5 x the longest T_1) delays between calibrated 90° pulses. An acquisition time of 5.00 s was used and 78,000 points were collected. The spectrum was zero-filled to 131,072 points before Fourier transformation. A zeroth order baseline correction was applied and the integrations of the peaks for the thioester carbon at 193.9 ppm and the ester carbon at 169.5 ppm were determined numerically using a constant region equal to five times the linewidth at half the maximum peak height on either side of each peak. The sum of the integrals was set to 100.00 and the integrals of the peaks for the thioester carbons are included in the following table:

thioester integrals	standard	run 1	run 2	run 3
measurement 1	49.31	46.48	46.46	47.45
measurement 2	49.34	46.50	46.45	47.50
measurement 3	49.45	46.41	46.48	47.44
measurement 4	49.38	46.35	46.47	47.41
measurement 5	49.40	46.43	46.42	47.43
average	**49.38**	**46.43**	**46.46**	**47.45**

The ^{13}C kinetic isotope effect for the carboxylate position in the MeMAHT-^{13}C was calculated using the following formula[3]:

$$KIE = \frac{\ln(1 - F)}{\ln[(1 - F)R/R_o]}$$

F is the percent conversion of the MeMAHT-^{13}C during the aldol reaction, and R/R_o is the proportion of ^{13}C in the recovered starting material compared to that in the original starting material. The ^{13}C isotope effect for the thioester carbon is assumed to be equal to 1 since no bonds to this carbon are formed or broken during the course of the aldol reaction. Since all of the MeMAHT-^{13}C molecules contain exactly one ^{13}C (half at the thioester and half at the carboxylic acid), the integral corresponding to the thioester peak cannot be used as a standard against which to measure the integral corresponding to the ester (carboxylate in MeMAHT-^{13}C) peak. This is because of the fact that as the ^{12}C in the carboxylate position is depleted preferentially to ^{13}C by the aldol reaction, the ^{12}C content at the thioester position is enriched by an equal amount. Therefore, R_o and R are determined by measuring the ratio of the integral corresponding to the ester peak to the total of the integrals for the ester and thioester peaks before and after the reaction, respectively. The total of the integrals for the ester and thioester peaks can be used as a

standard because the total ^{13}C content at the two positions remains the same regardless of the percent conversion and KIE (one ^{13}C atom per molecule of MeMAHT-^{13}C). This leads to the following formula for R/R_o that is applicable when the integrals for the ester and thioester peaks are both determined and the sum of the two is set to be equal to a constant:

$$R/R_o = \frac{\text{ester carbon integral}_{\text{final}}}{\text{ester carbon integral}_{\text{initial}}}$$

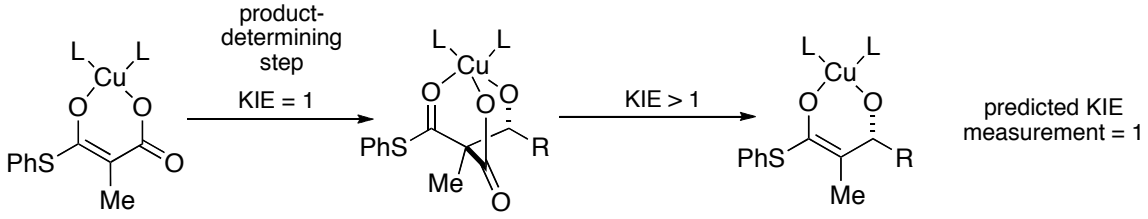
Using the above formulas and the formulas for calculating the error, which were obtained from reference 3, a ^{13}C KIE of of 1.020 ± 0.002 was calculated as shown in the following table:

	run 1	**run 2**	**run 3**
mg of MeMAHT-^{13}C reisolated	35.9	29.6	73.4
% conversion	93.2	94.4	86.1
R/R_o	1.0581	1.0577	1.0381
R/R_o error	0.0029	0.0048	0.0039
KIE	1.0213	1.0200	1.0193
KIE error	0.0015	0.0017	0.0014
average KIE	**1.0202**		
error	**0.0019**		

Because the above experiment determines the ^{13}C KIE by measuring the preferential depletion of ^{12}C at the carboxylate position in the starting material, the elementary step in the aldol reaction that determines the rate of irreversible starting material depletion, i.e. the product-determining step, must have a significant intrinsic ^{13}C KIE in order to give rise to a measured value of 1.020 ± 0.002. Addition of a MeMAHT enolate to an aldehyde should have a negligible intrinsic ^{13}C KIE at the carboxylate position because no bonds to the carboxylate carbon are formed or broken during an aldol

addition step. If addition to the aldehyde were irreversible, then this step would have to be responsible for any preferential depletion of ^{12}C at the carboxylate position in the starting material. A decarboxylation step subsequent to irreversible addition to the aldehyde would have no opportunity to select ^{12}C over ^{13}C and would consequently have no effect on the measured ^{13}C KIE value. Because the ^{13}C KIE is substantially different from one, addition to the aldehyde must be reversible. In that case, the intermediate formed after addition to the aldehyde but before decarboxylation would be able to either decarboxylate or undergo a retroaldol reaction to regenerate starting material. Such an intermediate with ^{12}C at the carboxylate position would have a greater preference to decarboxylate versus reverting to starting material compared to an intermediate with ^{13}C at the carboxylate position. This scenario (reversible aldol addition) provides a way for the decarboxylation step to influence the rate of ^{12}C depletion in the starting material and give rise to the substantial ^{13}C KIE value of 1.020 ± 0.002.

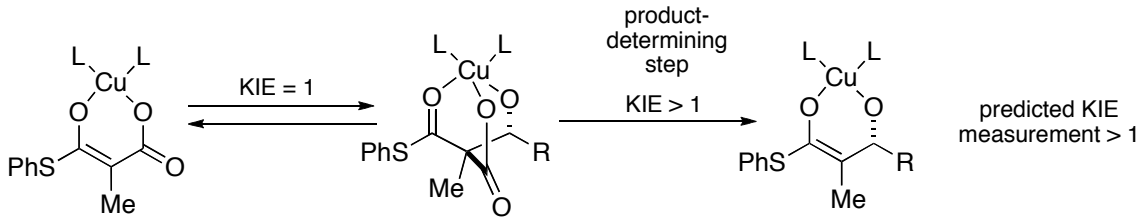
Irreversible Aldol Addition



This intermediate has no opportunity to select ^{12}C over ^{13}C. Once formed it must decarboxylate regardless of the carbon isotope at the carboxylate position.

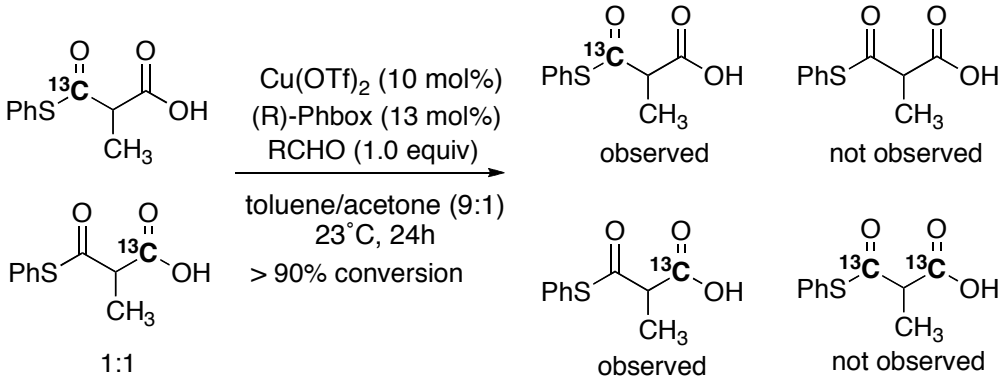
actual KIE measurement = **1.020 ± 0.002**

Reversible Aldol Addition



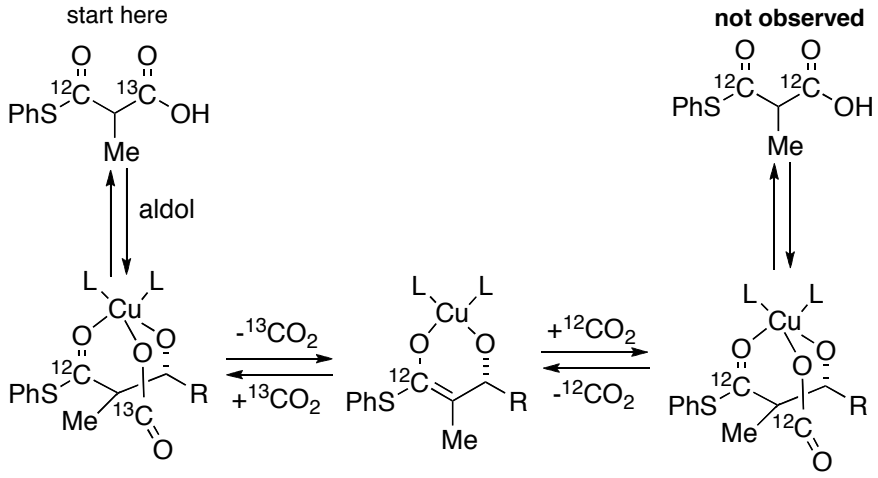
This intermediate may either decarboxylate or undergo retroaldol and regenerate starting material. The partitioning between decarboxylation and retroaldol will be influenced by the carbon isotope's effect on the decarboxylation rate.

Determination of the Reversibility of Decarboxylation.



reisolated starting material

The samples of MeMAHT-^{13}C reisolated at high conversion for use in the previous experiment for determining the ^{13}C KIE at the carboxylate position were analyzed by ^{1}H NMR (500 MHz, $CDCl_3$). The peak at 3.81 ppm corresponding to the hydrogen on the α-carbon in the original standard sample of MeMAHT-^{13}C appears as a quintet due to 7.0 Hz coupling to the three –CH_3 hydrogens and 7.0 Hz coupling to a exactly one ^{13}C atom at either the thioester or the carboxylate positions. If the MeMAHT sample included noticeable amounts of MeMAHT molecules with either zero or two ^{13}C atoms, then the ^{1}H NMR spectrum would show a superposition of a quartet, quintet, and sextet at 3.81 ppm corresponding to MeMAHT with zero, one, and two ^{13}C atoms, respectively. Both the original sample and the reisolated MeMAHT-^{13}C gave rise to identical ^{1}H NMR spectra with exclusively a quintet at 3.81 ppm. No traces of a quartet or a sextet were detectable.



If decarboxylation were reversible in the aldol reaction, then the $^{13}CO_2$ lost by decarboxylation of MeMAHT-^{13}C with a ^{13}C at the carboxylate position could recarboxylate an enolate derived from MeMAHT-^{13}C with a ^{13}C at the thioester position giving rise to a MeMAHT molecule containing two ^{13}C atoms. A MeMAHT molecule

containing zero ^{13}C atoms could be formed in similar fashion. Because we do not observe any reisolated MeMAHT-^{13}C containing zero or two ^{13}C atoms, we conclude that decarboxylation must be irreversible.

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[1] Magdziak, D.; Lalic, G.; Lee, H. M.; Fortner, K. C.; Aloise, A. D.; Shair, M. D. *J. Am. Chem. Soc.* **2005**, *127*, 7284-7285.

[2] (a) McKay, H. A. C. *Nature* **1938**, *142*, 997-998. (b) McKay, H. A. C. *J. Am. Chem. Soc.* **1943**, *65*, 702-706.

[3] Singleton, D. A.; Thomas, A. A. *J. Am. Chem. Soc.* **1995**, *117*, 9357-9358.