Synthesis and Biological Activity of a Focused Library of Mitogen-Activated Protein Kinase Phosphatase Inhibitors

And

Investigation of the Utility of Benzothiazole and 5-Methylthiadiazole Sulfonamides as Nitrogen Atom Protecting Groups in Asymmetric Addition Reactions to Aldimines

David Arnold

4/28/07

Wipf Group: Research Topic Seminar

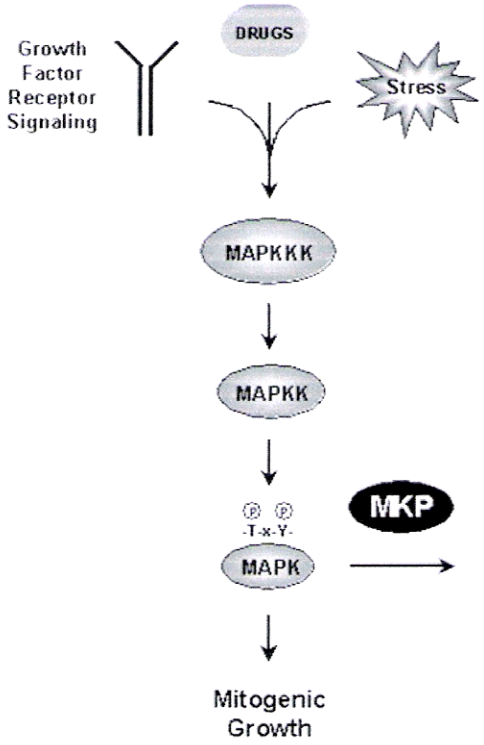
Outline for Part 1

- Library Synthesis:

  Brief Background
  Library Design
  Synthetic Routes
  Biological Results

Background: Mitogen-activated Protein Kinase Phosphatase-1 (MKP-1) as a Therapeutic Target

- MKP-1 is a dual-specificity phosphatase involved in tightly regulated signaling pathways responsible for cell growth, division and death.



T = Threonine

Y = Tyrosine

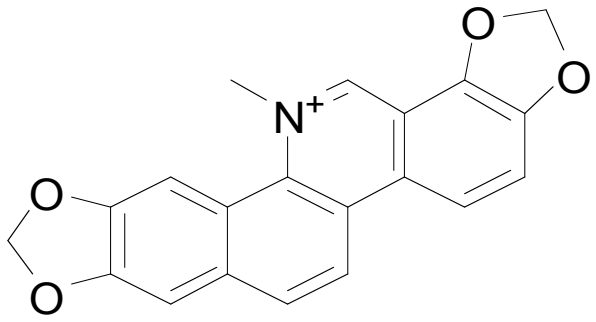
- *Annu. Rev. Pharmacol. Toxicol.* **2005**, *45*, 725.

Background: Mitogen-activated Protein Kinase Phosphatase-1 (MKP-1) as a Therapeutic Target

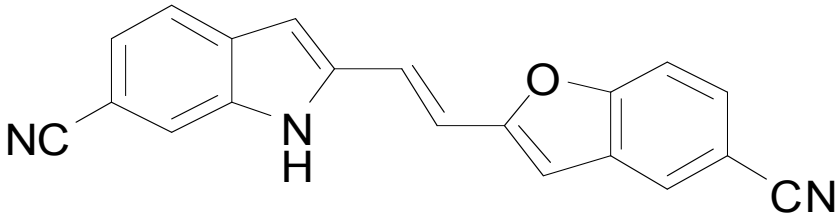
- MKP-1 was initially believed to be a tumor suppressor.
- MKP-1 has been found to be over expressed in prostate, gastric, breast and pancreatic cancer.
- To date, No x-ray crystal structures are available for Structure Activity Relationship (SAR) analysis to aid in inhibitor design.
- Selective and potent MKP-1 inhibitors may lead to therapeutic treatments for cancer.

• *Annu. Rev. Pharmacol. Toxicol.* **2005**, *45*, 725

Background: Two Known Inhibitors of MKP-1 Previously Discovered by the University of Pittsburgh



Sanguinarine



NU-126

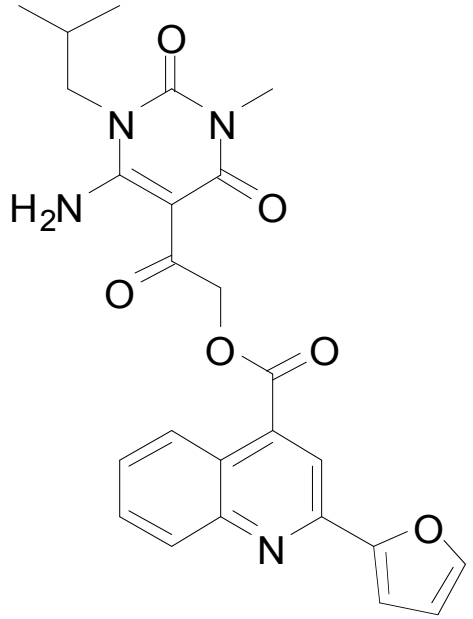
Comparison of Potency and Selectivity for Sanguinarine and NU-126

Average IC_{50} ± S.D. for inhibition of dual-specificity phosphatases

	MKP-1	MKP-3	Cdc25B	PTP1B	VHR
Sanguinarine	17.3 ± 1.2 μM	>>100	57.8 ± 11.6 μM	67.9 ± 11.7 μM	74.0 ± 5.3 μM
NU-126	28.8 ± 2.9 μM	>400	>400	>100	38.1 ± 2.8 μM

- *J. Biol. Chem.* **2005**, *280*, 19078.
- *Bioorg. Med. Chem.* **2006**, *14*, 5643.

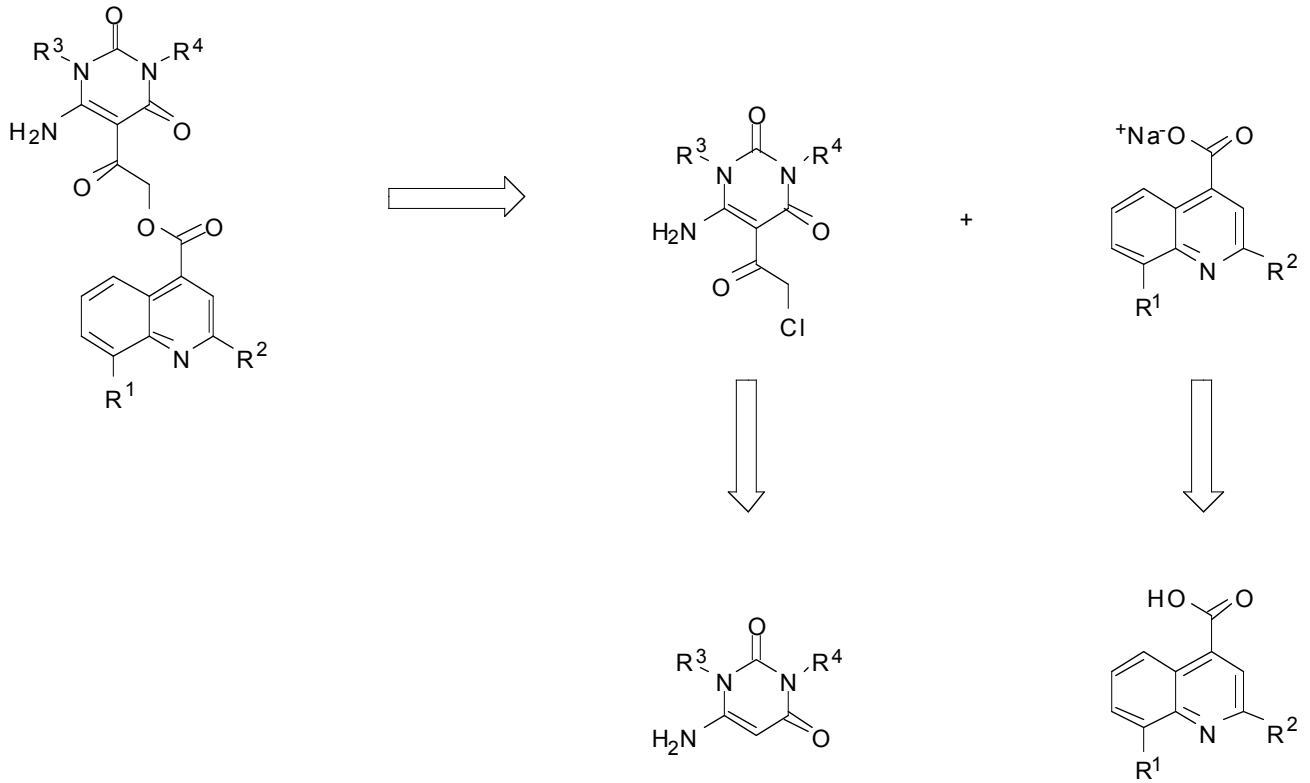
Pittsburgh Molecular Libraries Screening Center: High Throughput Screen of 13,309 Compounds for MKP-1 Inhibitors



IC_{50} = 19.2 $\pm$ 5.6 μM

- *Chem. Biol. Drug Des.* **2007**, *69*, 23.

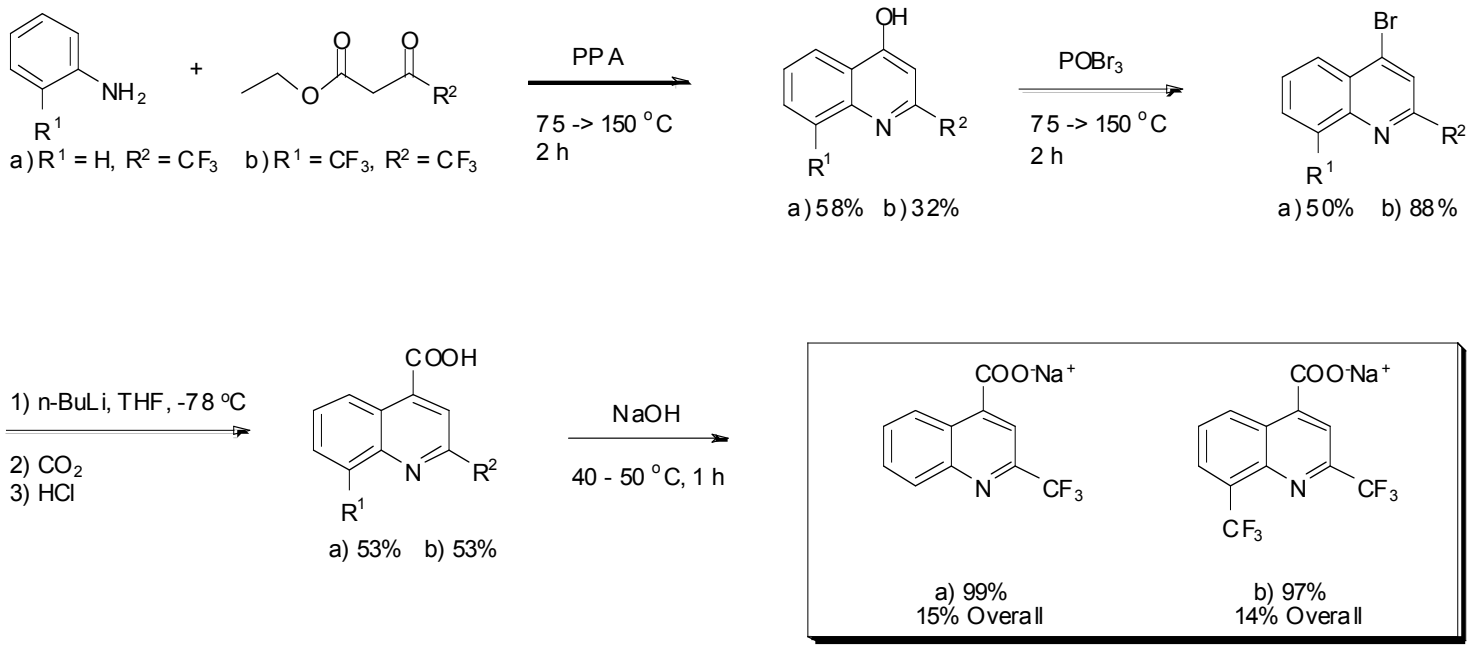
Library Design: Retro-Synthesis and Points of Diversification



R^1: H, CF_3
R^2: H, CF_3, Ph, Cyclopropyl, Furyl
R^3: Methyl, Methylcyclopropyl, Isobutyl, Benzyl
R^4: Methyl, Benzyl

Target Library:
26 Compounds

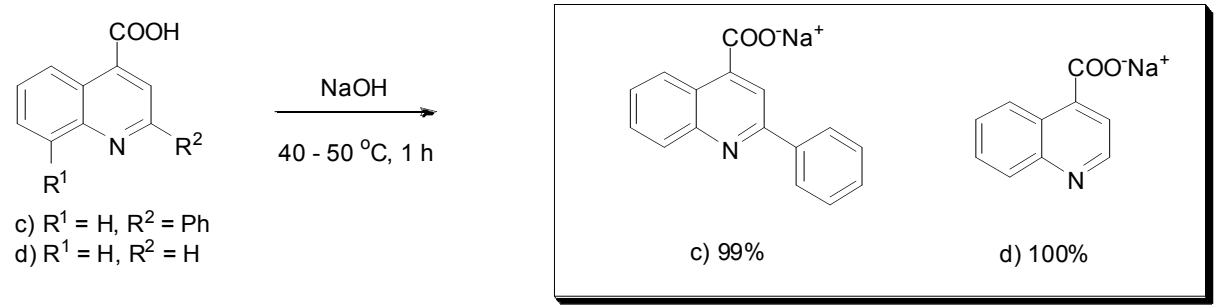
Synthesis of Mono and Bis-Trifluoromethyl Quinolinecarboxylic Acid Sodium Salts



- *Eur. J. Org. Chem.* **2003**, 1576–1588.
- *J. Med. Chem.* **2000**, *35*, 359–364.

Preparation of R^2 = H, Ph, Furyl and Cyclopropyl Quinolinecarboxylic Acid Sodium Salts

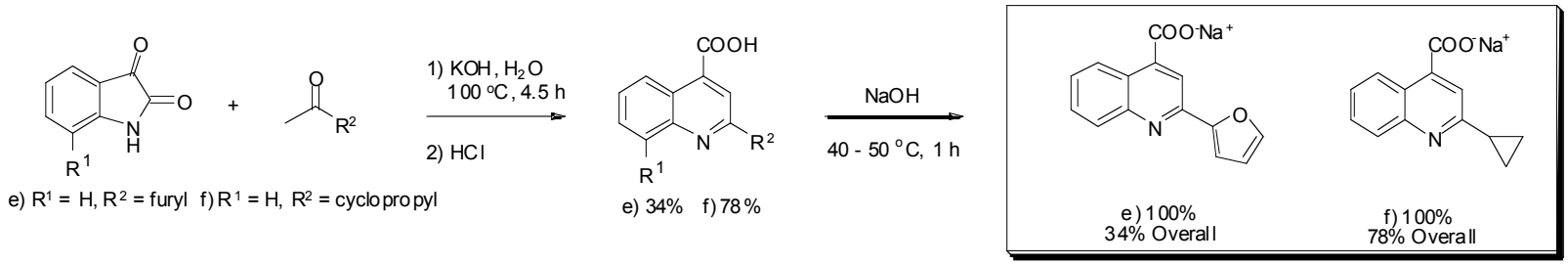
- Purchased quinolinecarboxylic acids



c) R^1 = H, R^2 = Ph
d) R^1 = H, R^2 = H

c) 99% d) 100%

- Synthesis of furyl and cyclopropyl quinolinecarboxylic sodium salts



e) R^1 = H, R^2 = furyl f) R^1 = H, R^2 = cyclopropyl

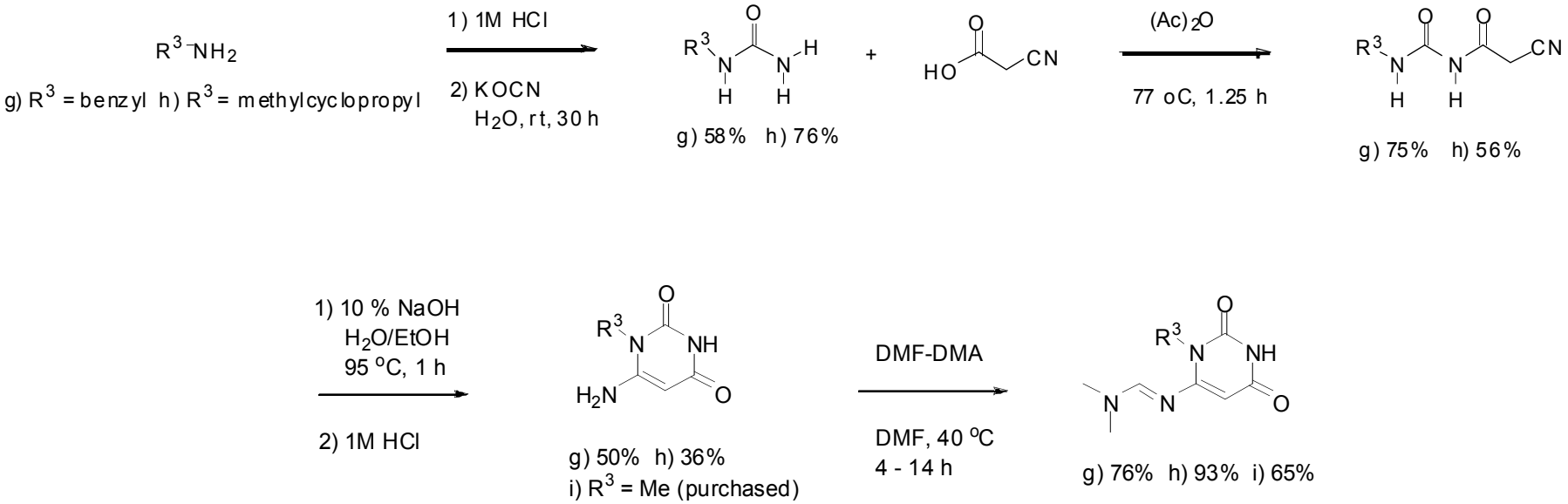
e) 34% f) 78%

e) 100%
34% Overall

f) 100%
78% Overall

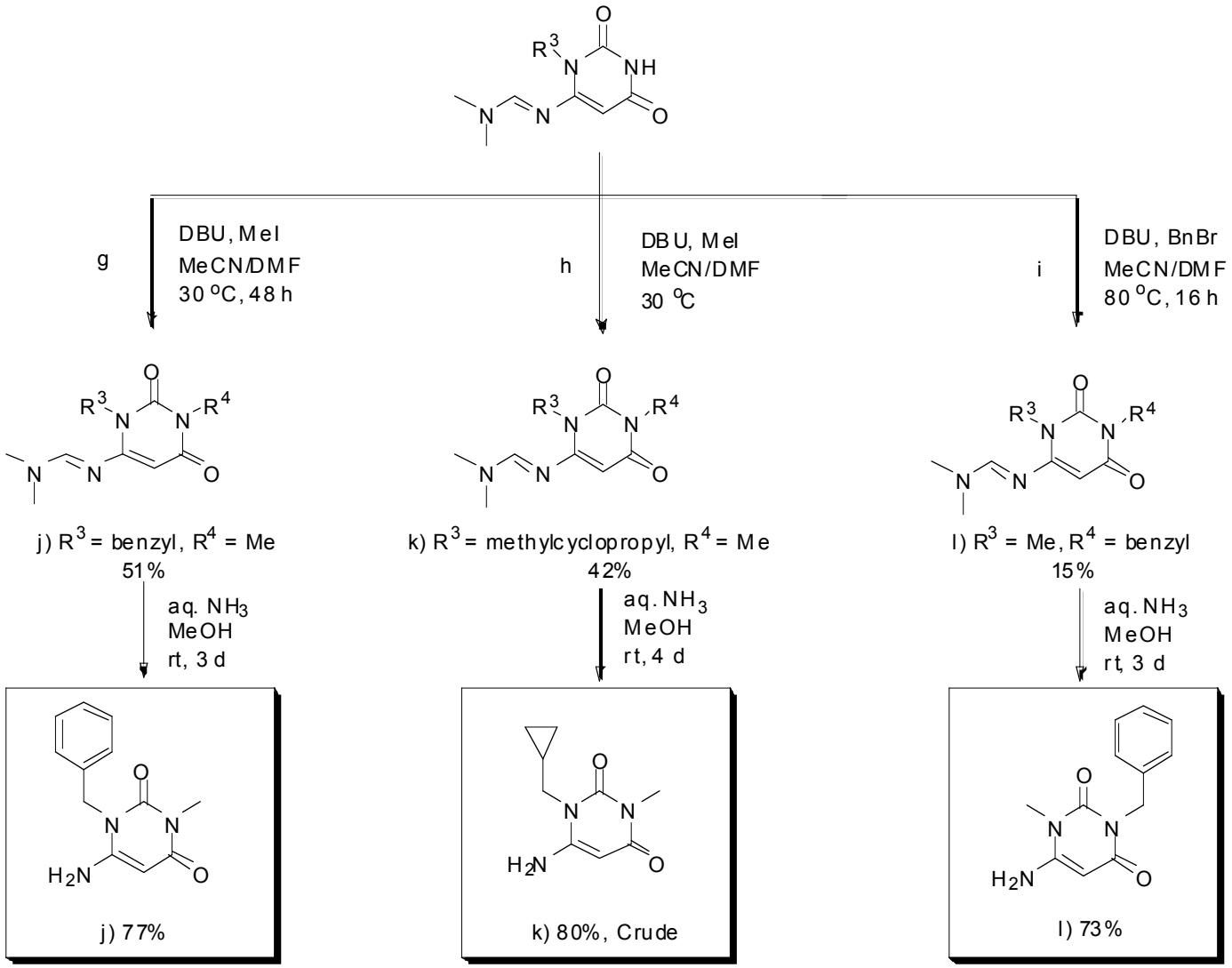
- *Russian Journal of Organic Chemistry*, **2003**, *39*, 1803.

Synthesis of N^6-[(Dimethylamino)methylene] Protected N^1-Alkylaminouracils



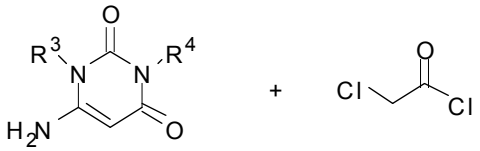
- *J. Org. Chem.* **1986**, *51*, 4180-4185.
- *J. Org. Chem.* **1951**, *16*, 1879-1890.
- *Synthesis*, **2001**, *3*, 478-482.

Synthesis of N^1,N^3-Dialkyl-6-Aminouracils

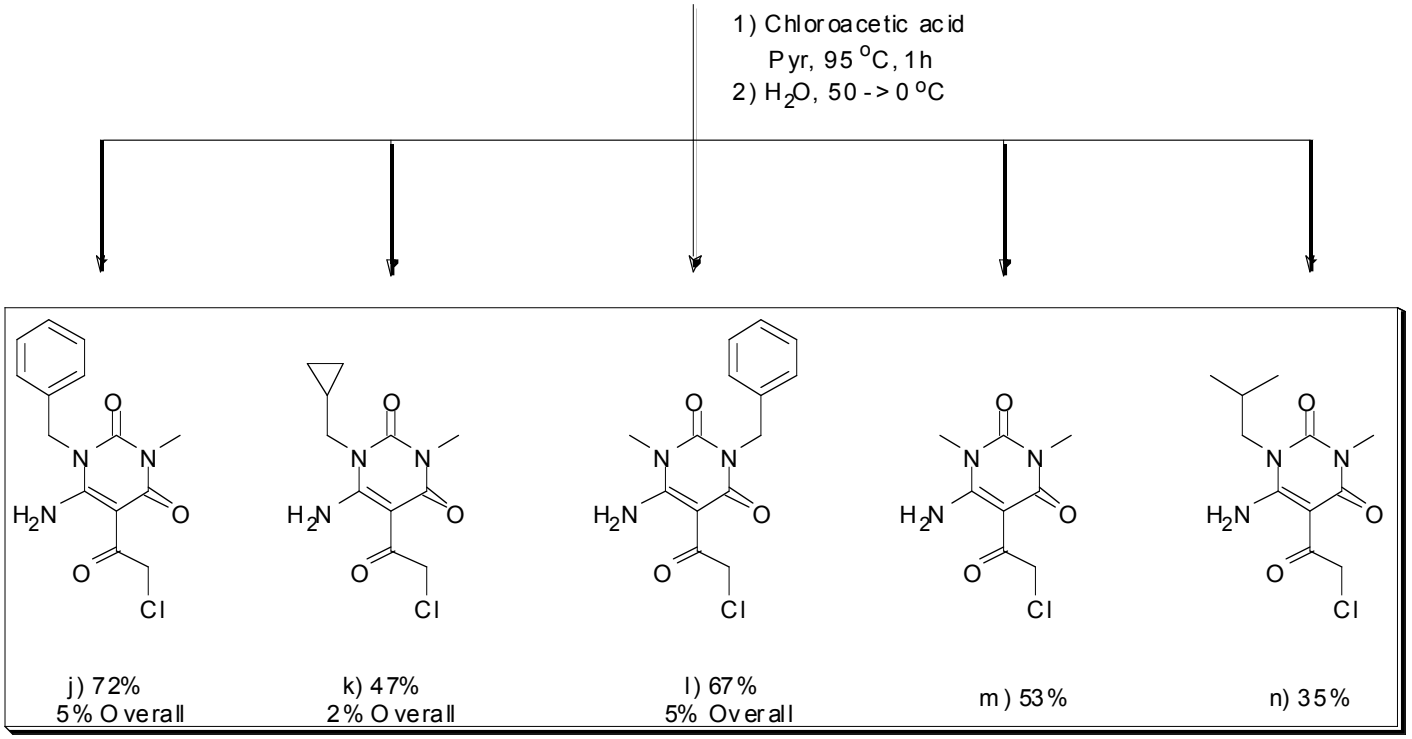


- *Synthesis*, **2001**, *3*, 478-482.

Synthesis of 5-Chloroacetyl-*N*[1],*N*[3]-Dialkyl-6-aminouracils

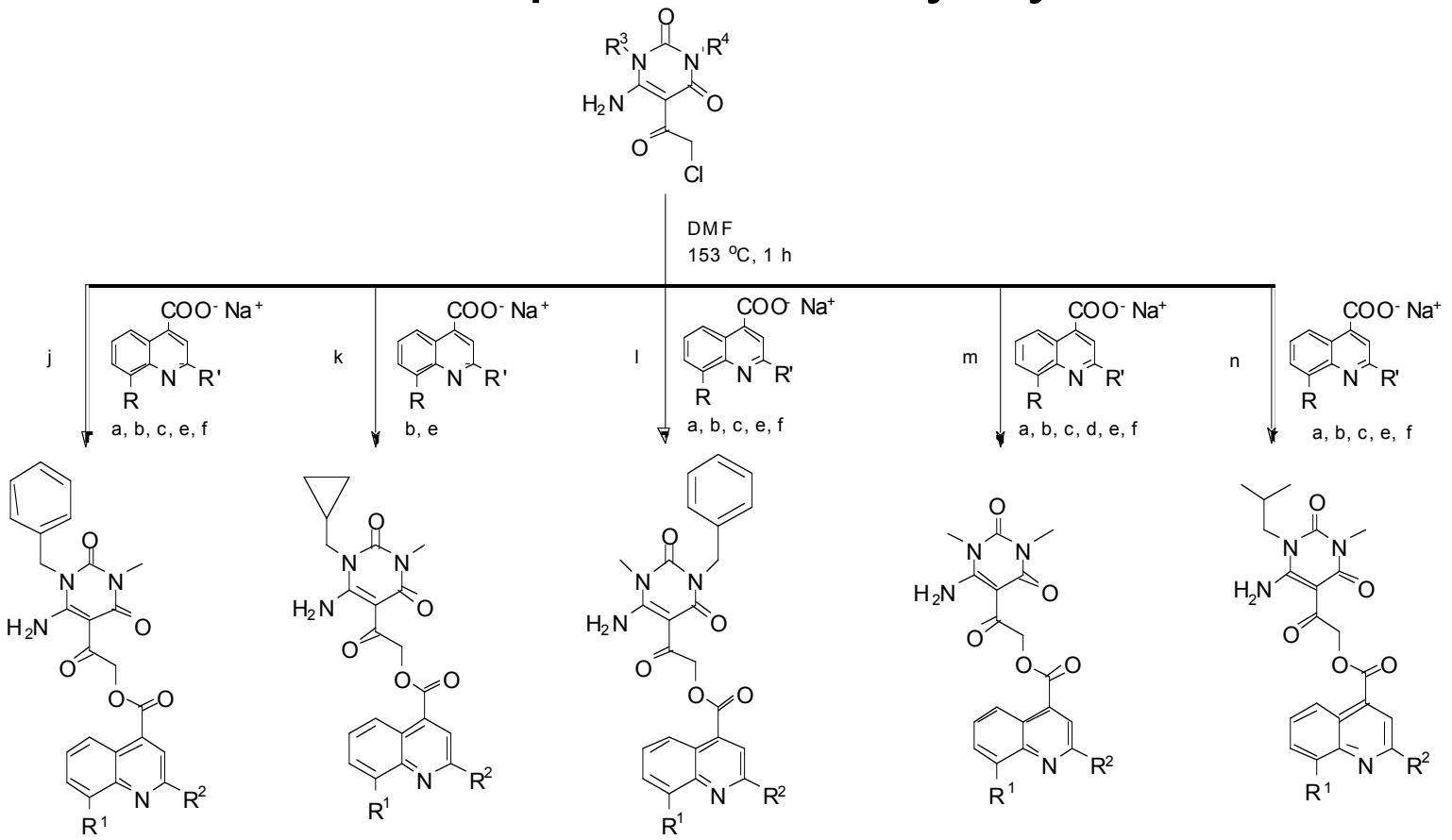


j) R^3 = benzyl, R^4 = Me k) R^3 = methylcyclopropyl, R^4 = Me
l) R^3 = Me, R^4 = benzyl m) R^3 = Me, R^4 = Me (Purchased)
n) R^3 = i-Bu, R^4 = Me (Purchased)



- *Chemistry of Heterocyclic Compounds*, **1978**, 443–446.

Final Compound Library Synthesis



o) R^1 = H, R^2 = CF_3: 51% (DMA-P154)
p) R^1 = CF_3, R^2 = CF_3: 58% (DMA-P155)
q) R^1 = H, R^2 = Ph: 39% (DMA-P148)
r) R^1 = H, R^2 = furan: 54% (DMA-P156)
s) R^1 = H, R^2 = cyclopropyl: 48% (DMA-P157)

t) R^1 = CF_3, R^2 = CF_3: 55% (DMA-P166)
u) R^1 = H, R^2 = furan: 56% (DMA-P165)

v) R^1 = H, R^2 = CF_3: 62% (DMA-P158)
w) R^1 = CF_3, R^2 = CF_3: 53% (DMA-P159)
x) R^1 = H, R^2 = Ph: 47% (DMA-P162)
y) R^1 = H, R^2 = furan: 59% (DMA-P160)
z) R^1 = H, R^2 = cyclopropyl: 50% (DMA-P161)

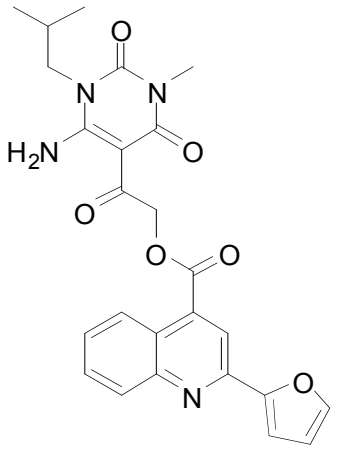
aa) R^1 = H, R^2 = CF_3: 45% (DMA-P145)
bb) R^1 = CF_3, R^2 = CF_3: 56% (DMA-P146)
cc) R^1 = H, R^2 = Ph: 66% (DMA-P100)
dd) R^1 = H, R^2 = H: 74% (DMA-P78)
ee) R^1 = H, R^2 = furan: 70% (DMA-P138)
ff) R^1 = H, R^2 = cyclopropyl: 59% (DMA-P140)

gg) R^1 = H, R^2 = CF_3: 58% (DMA-P151)
hh) R^1 = CF_3, R^2 = CF_3: 55% (DMA-P152)
ii) R^1 = H, R^2 = Ph: 60% (DMA-P101)
jj) R^1 = H, R^2 = furan: 61% (DMA-P153)
kk) R^1 = H, R^2 = cyclopropyl: 31% (DMA-P149)

- *J. Med. Chem.* **2000**, *35*, 359–364.

Library Results

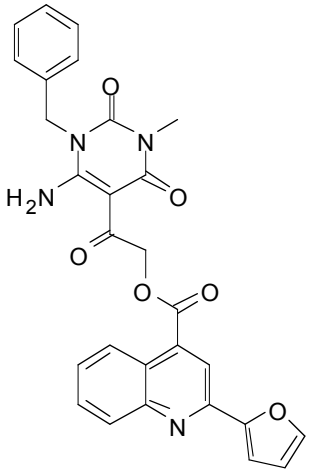
- 23 of the target 26 final library analogues were synthesized in yields ranging from 31-74%.
- A total library of 47 compounds was submitted for biological testing against MKP-1.
- None of the 24 uracil or quinoline intermediates tested were found to be biologically active against MKP-1.
- A remake of the original HTS hit showed an approximately three fold loss of potency against MKP-1.



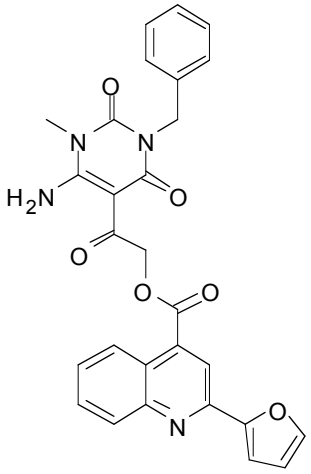
Original HTS Hit: IC_{50} = 19.2 $\pm$ 5.6 μM
Remake: IC_{50} = 50 μM

Library Results

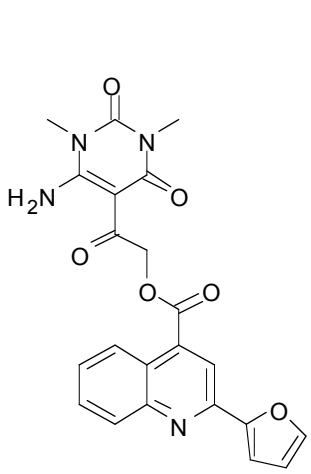
- All of the 23 final library analogues tested showed an IC_{50} >50 μM against MKP-3.
- Five analogues showed a potency against MKP-1 comparable to the original hit:



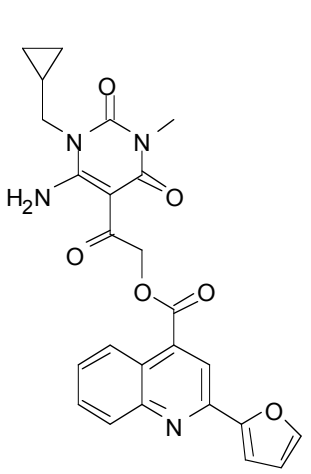
IC_{50} = 13.4 μM



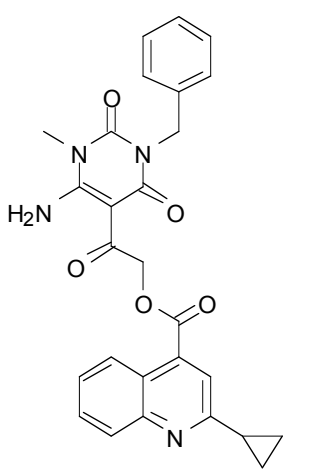
IC_{50} = 16.9 μM



IC_{50} = 20.6 μM



IC_{50} = 24.6 μM



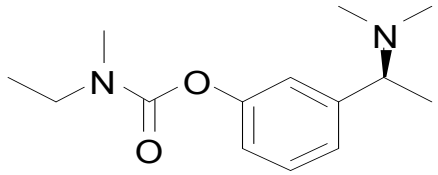
IC_{50} = 28.9 μM

Outline for Part 2

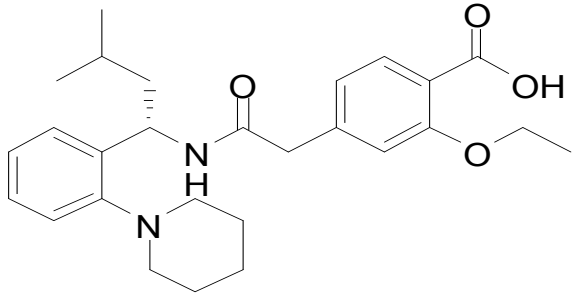
- Utility of Benzothiazole (Bts) and 5-Methylthiadiazole (Ths) Sulfonamides as Protecting Groups in the Synthesis of α-Chiral Amines:

Background
Sulfonamide Synthesis
Benzaldimine Synthesis
Racemic Additions
Asymmetric Additions
Deprotection
Future Directions

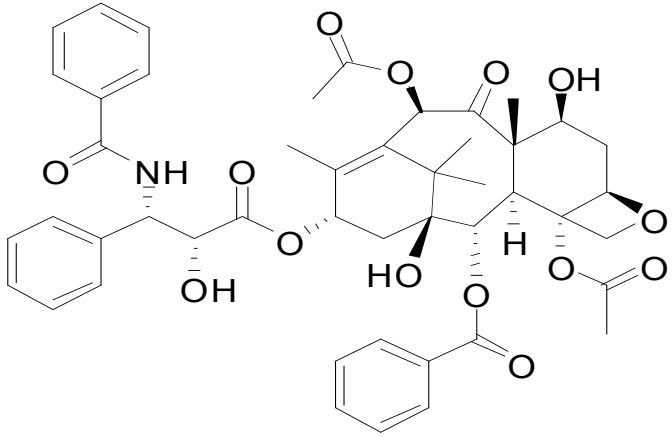
Background: Important Pharamacetucials Containing α-Chiral Amines



Rivastigmine
Treatment for mild to moderate dementia resulting from Alzheimer's or Parkinson's Disease



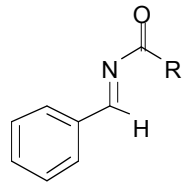
Repaglinide
Treatment for type II Diabetes



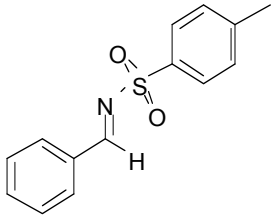
Taxol
Treatment for cancer

Background: Organometallic Additions to Aldimines

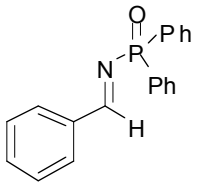
- Common Aldimines



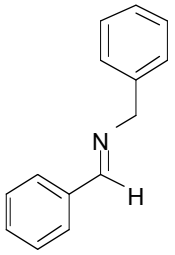
N-acylimines



N-sulfonylimines



N-phosphinoylimines

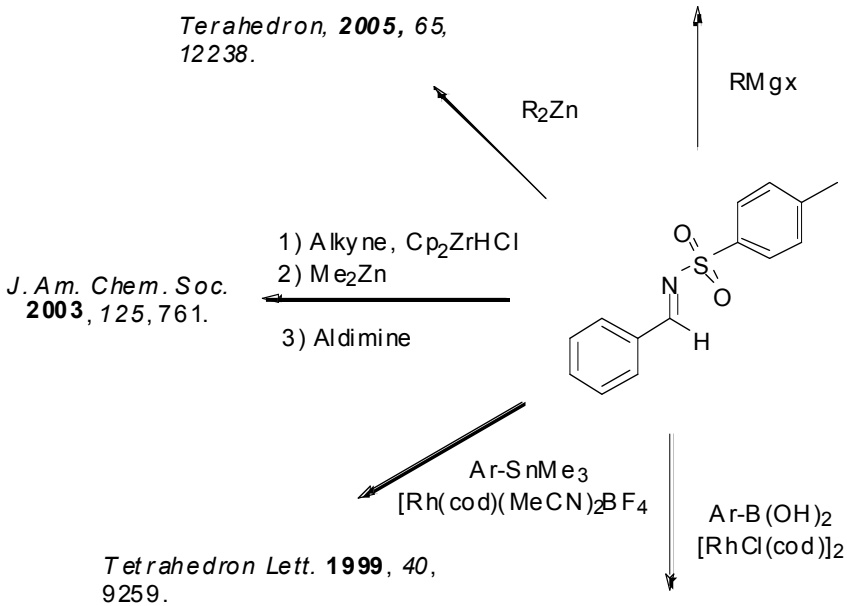


N-alkylimines

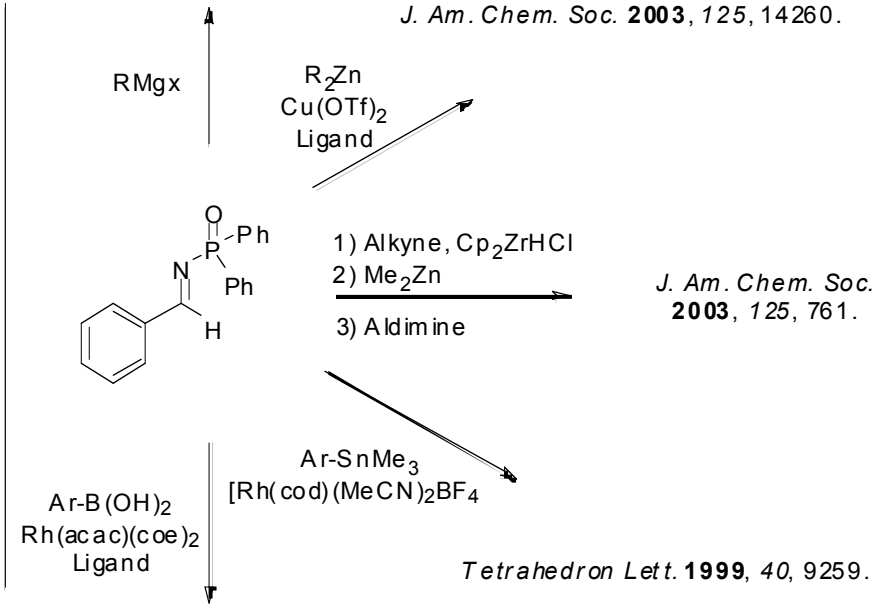
J. Org. Chem. **1990**, *55*, 393.

Synthesis **1999**, *6*, 930.

Terahedron, ***2005***, *65, 12238.*



J. Am. Chem. Soc. **2003**, *125*, 14260.

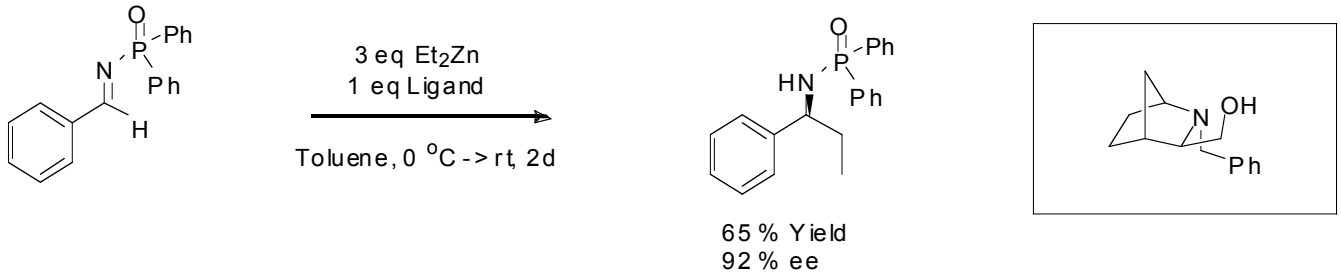


Synlett **2000**, *11*, 1637.

J. Am. Chem. Soc. **2005**, *127*, 1092.

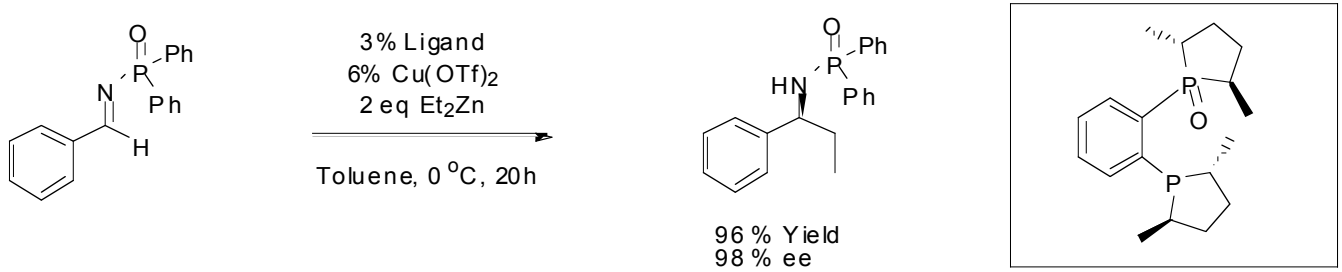
Background: Asymmetric Additions of Diethylzinc to N-phosphinoylaldimines

- Addition with a stoichiometric amount of ligand



- *J. Org. Chem.* **1998**, *63*, 2530.

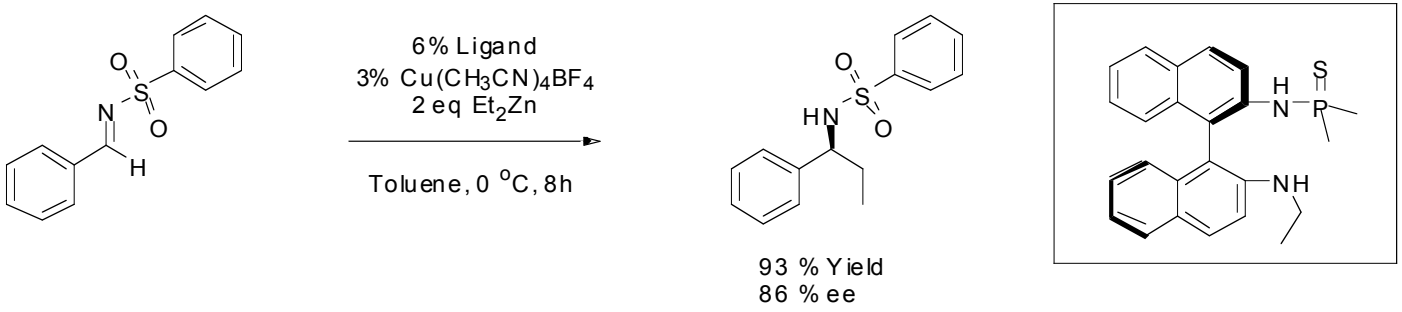
- Copper catalyzed additions



- *J. Am. Chem. Soc.* **2003**, *125*, 14260.

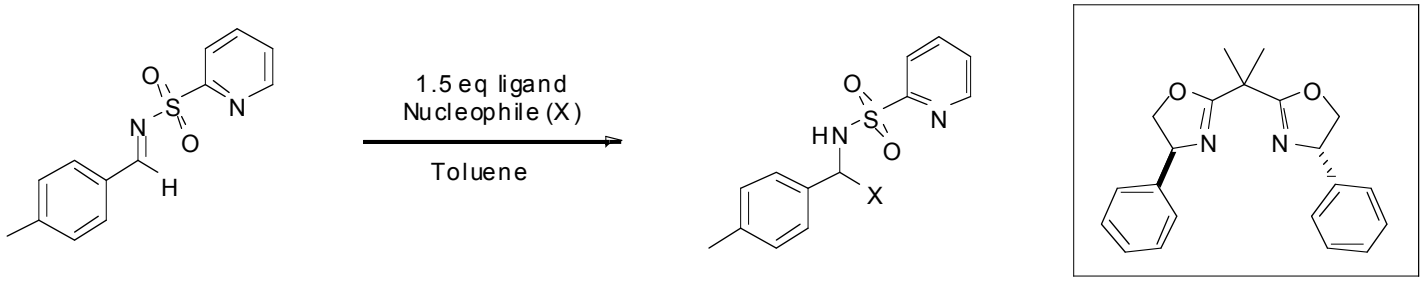
Background: Asymmetric Additions to N-Sulfonylaldimines

- Copper catalyzed asymmetric addition of Et_2Zn to sufonylaldimines



- *Synlett* **2007**, *1*, 19.

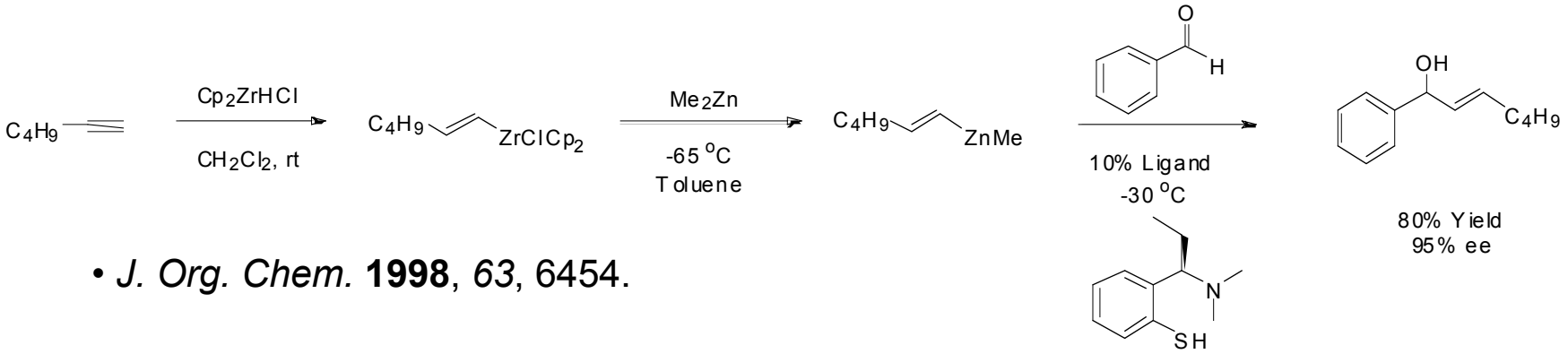
- Ligand catalyzed asymmetric additions to heteroaromatic sufonylaldimines



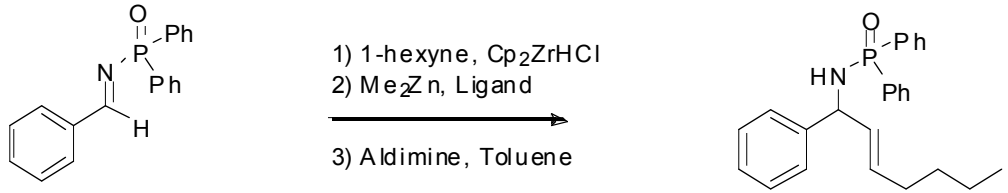
Nucleophile	Temperature	Yield	ee
CH_3MgBr	-95 °C	67%	83%
Et_2Zn	-40 °C	49%	46%

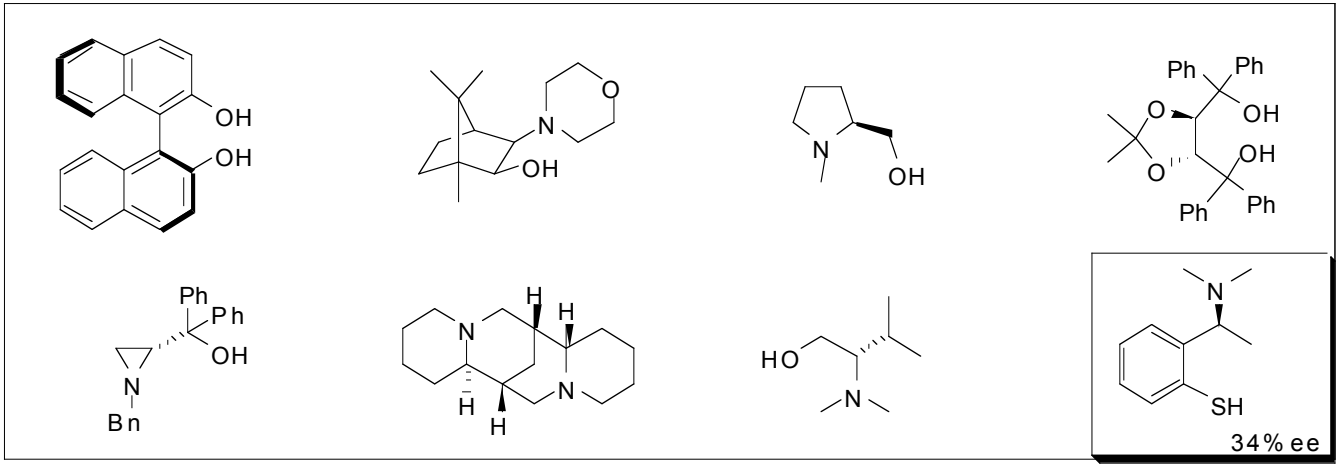
- *Tetrahedron Lett.* **2005**, *46*, 8941.

Background: Zr-Zn Transmetalation Followed by Catalytic Asymmetric Addition to Aldehydes and Aldimines



- *J. Org. Chem.* **1998**, *63*, 6454.

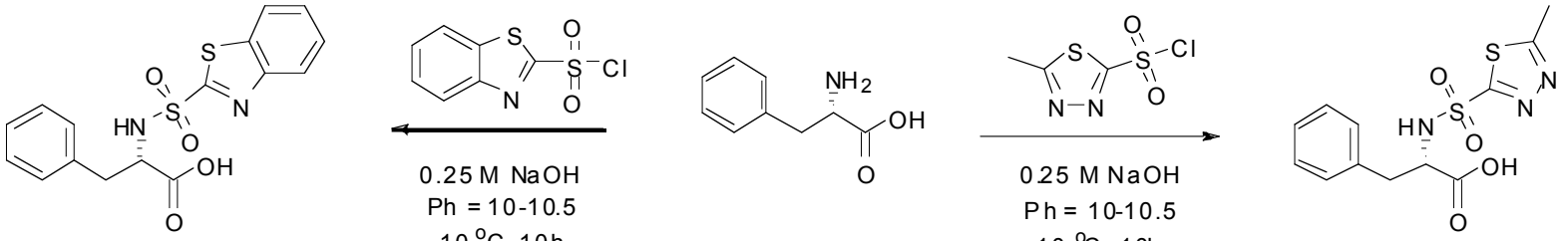




- Christopher Kendall, PhD. Thesis, University of Pittsburgh, **2004**.

Background: Introduction of the Benzothiazole (Bts) and 5-Methylthiadiazole (Ths) Sulfonamides as Nitrogen Atom Protecting Groups

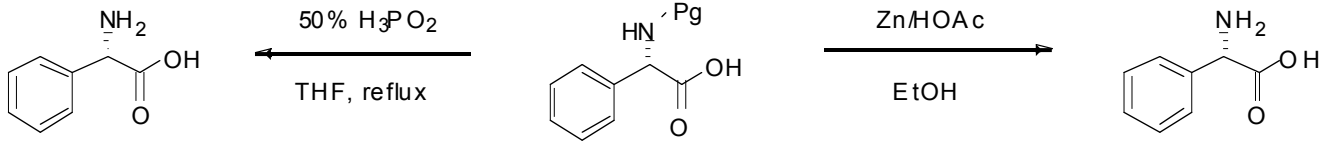
- Protection of Amino Acids

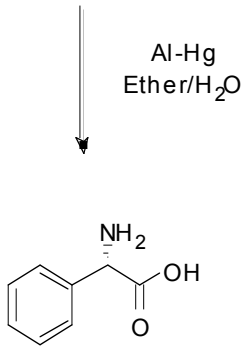


97% Yield

96% Yield

- Deprotection

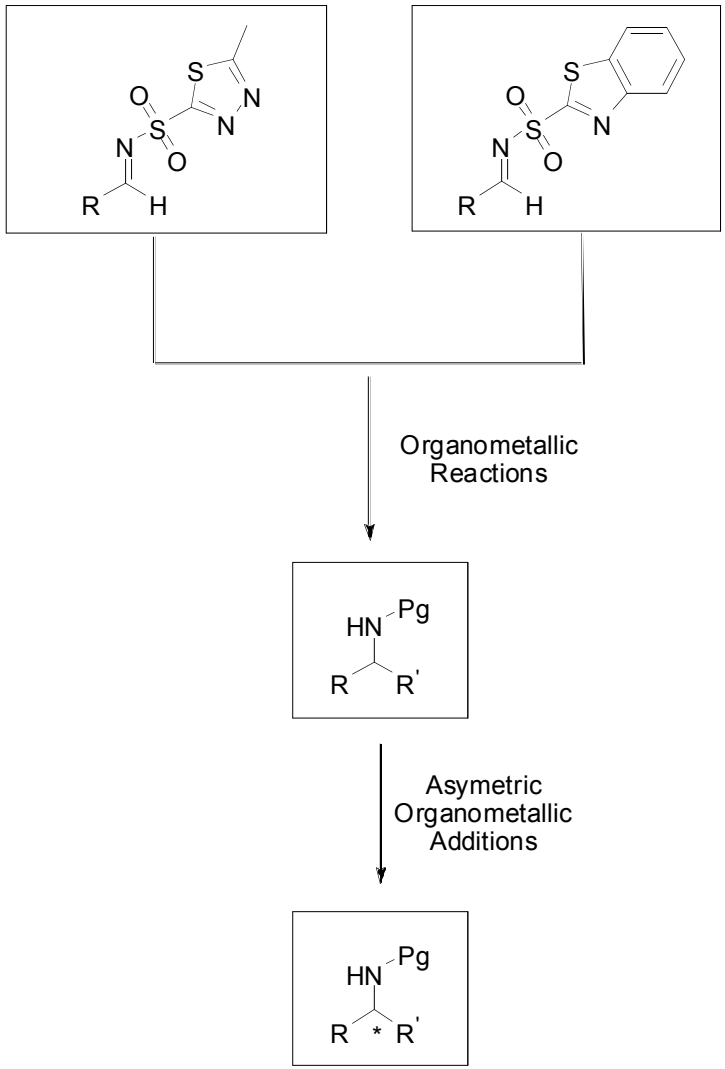




- Pg = Bts, Ths
- When Pg = Ts, No deprotection occurred
- >90% yields
- Retention of amino acid chirality

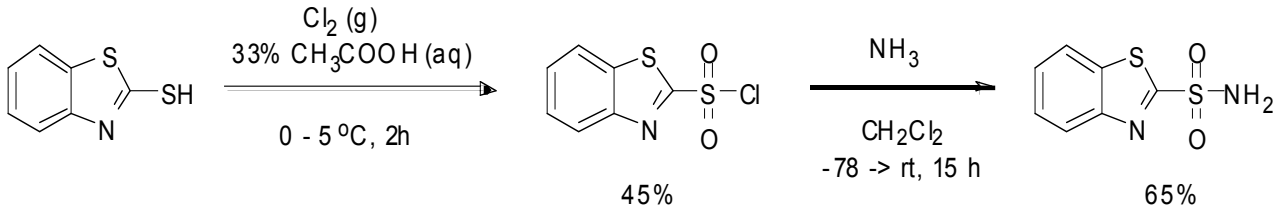
- J. Am. Chem. Soc. 1996, 118, 9796

Introduction To Research

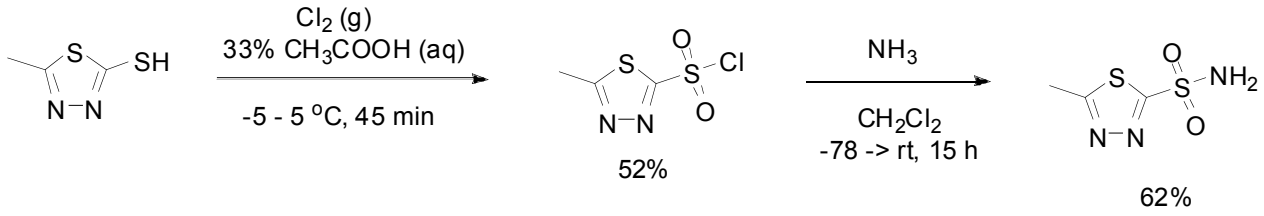


Synthesis of Benzothiazole-2-sulfonamides ($BtsNH_2$) and 5-Methyl-1,3,4-thidiazole-2-sulfonamides ($ThsNH_2$)

- $BtsNH_2$:



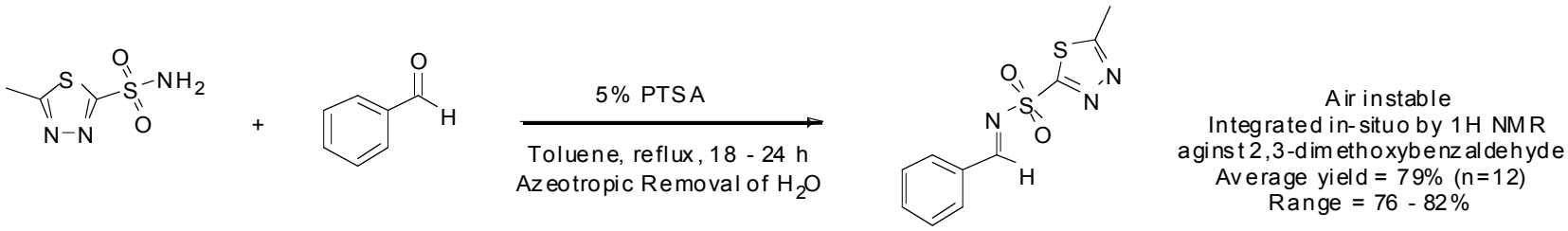
- $ThsNH_2$:



- *J. Am. Chem. Soc.* **1996**, *118*, 9796.

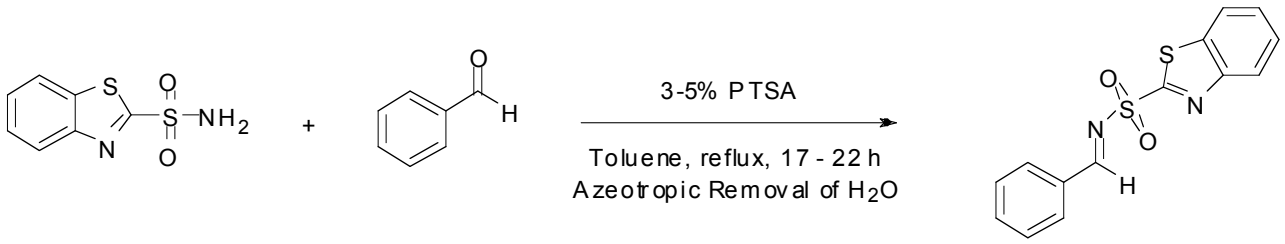
Preparation of Ths- and Bts-Benzaldimines

- Ths-Benzaldimine



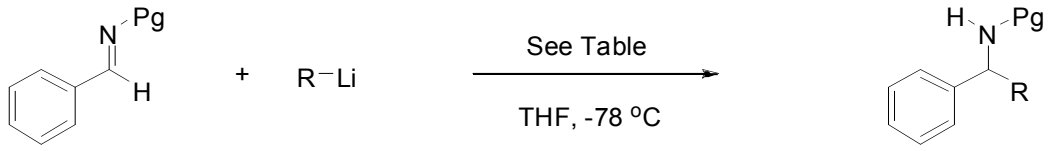
Air instable
Integrated in-situo by 1H NMR
aginst 2,3-dimethoxybenzaldehyde
Average yield = 79% (n=12)
Range = 76 - 82%

- Bts-Benzaldimine



Air stable solid
Average yield = 82% (n = 6)
Range = 80 - 87%

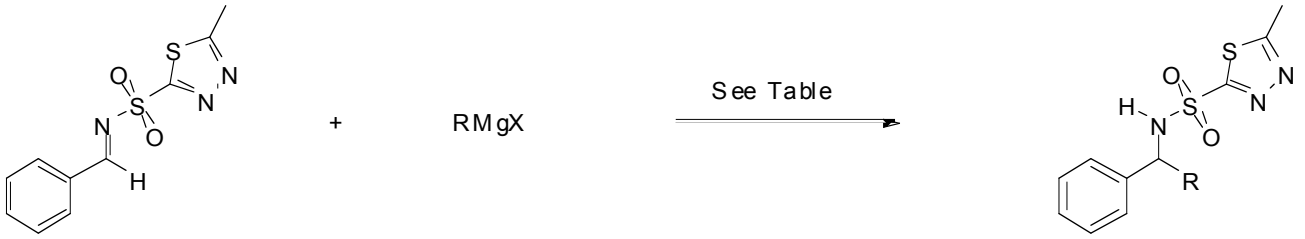
Racemic Additions: Lithium Reagents



Lithium Reactions

	Pg	R	Time	Yield
1	Ths	Me	2 h	23%
2	Ths	*t*-Butyl	2 h	dec.
3	Bts	Me	2 h	dec.

Racemic Additions: Grignard Additions to Ths-Benzaldimine



Grignard Reactions

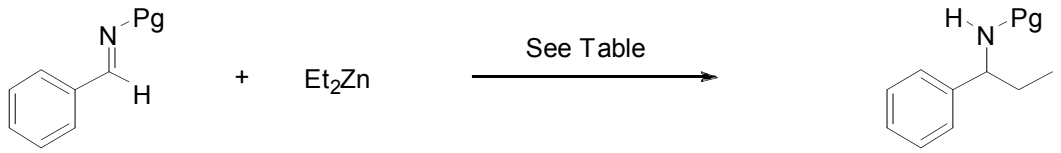
	Optimization Reactions					Rec. Yields	
	Solvent	**X**	**R**	**Time**	**Temp.**	**2 Step**	**1 Step**
1	CH_2Cl_2	Cl	i-Propyl	2.5 h	-78 °C	45%	X
2	**THF**	**Cl**	**i-Propyl**	**2 h**	**-78 °C**	**47%**	**X**
3	THF	Cl	i-Propyl	1.5 h	-78 -> rt	15%	X
Grignard Reactions Using Optimized Conditions							
1	THF	Cl	i-Propyl	2 h	-78 °C	46%	58%
2	THF	Br	Ph	2 h	-78 °C	51%	64%
3	THF	Br	Me	2 h	-78 °C	49%	61%
4	THF	Br	Vinyl	2 h	-78 °C	37%	46%
5	THF	Br	1-propynyl	2 h	-78 °C	39%	49%

Racemic Additions: Grignard Additions to Bts-Benzaldimine

Grignard Reactions

	Solvent	X	R	Time	Temp.	Yield
1	THF	Br	Me	2.5 h	-78 ^{o}C	87%
2	THF	Cl	i-Propyl	2.5 h	-78 ^{o}C	77%
3	THF	Br	Vinyl	3 h	-78 ^{o}C	85%
4	THF	Br	1-propynyl	3 h	-78 ^{o}C	71%
5	THF	Br	Ph	3 h	-78 ^{o}C	77%

Racemic Additions: Diethylzinc Additions to Bts- and Ths-Benzaldimines

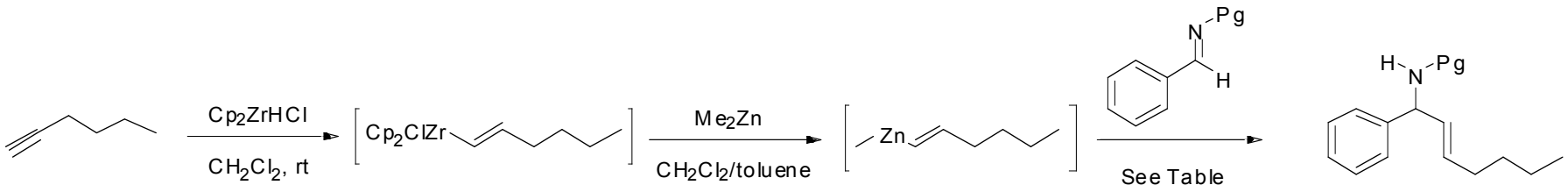


Diethylzinc Reactions

	Pg	Solvent	Eq. Et_2Zn	Temperature	Time	Yield
1	Ths	10% THF/Toluene	1	rt	2.5 h	46%
2	Ths	THF	1.2	rt	4 h	43%
3	Ths	Toluene	1.2	rt	2.5 h	53%
4	**Ths**	**Toluene**	**2**	**rt**	**2.5 h**	**70%**
5	Ths	Toluene	1	-78 °C	33 h	61%
6	**Bts**	**Toluene**	**2**	**rt**	**4 h**	**63%**
7	Bts	THF	2	rt	4 h	40%
8	Bts	Toluene	2	0 °C	6 h	40%

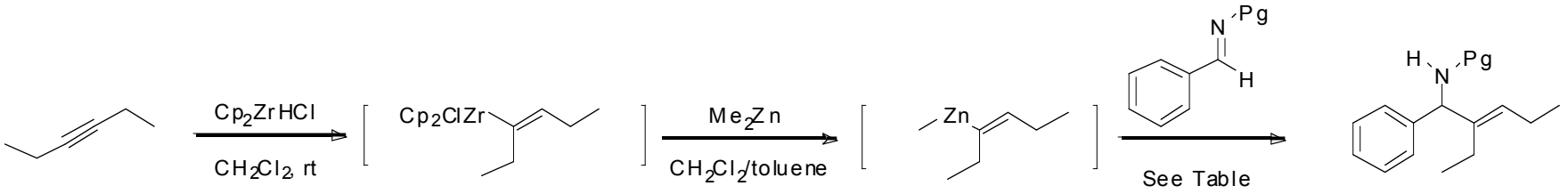
- *Tetrahedron* **2005**, *61*, 12238.

Racemic Reactions: Vinylzinc Additions to Bts- and Ths-Benzaldimines



1-Hexyne Derived Vinylzinc Additions

	Aldimine	Eq. Cp_2ZrHCl	Eq. Me_2Zn	Solvent	Temp	Time	Yield
1	Ths	1.6	1.5	Toluene 1:1 CH_2Cl_2	rt	2.5 h	35%
2	Ths	1.6	1.5	Toluene 1:1 CH_2Cl_2	-40 °C	10 h	57%
3	Bts	1.6	1.5	CH_2Cl_2	rt	4.5 h	71%

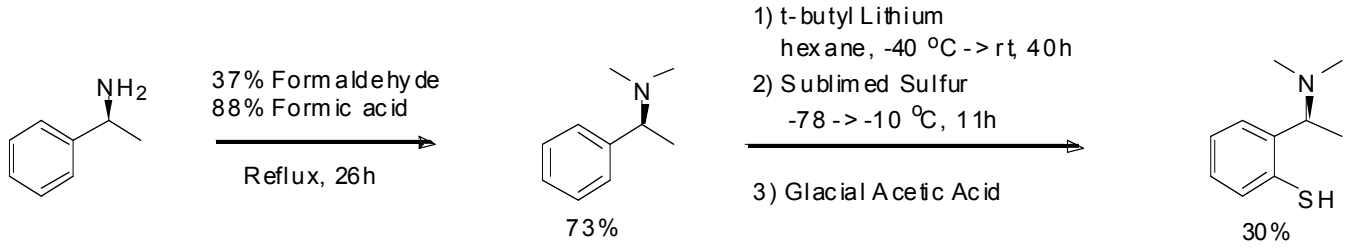


3-Hexyne Derived Vinylzinc Additions

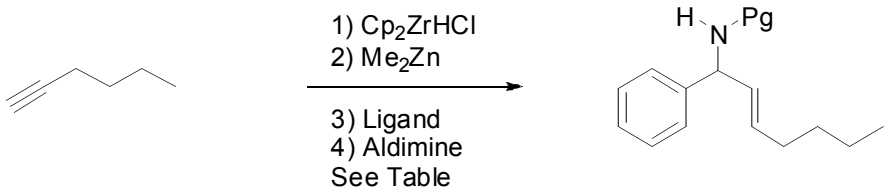
	Aldimine	Eq. Cp_2ZrHCl	Eq. Me_2Zn	Solvent	Temp	Time	Yield
1	Ths	1.6	1.5	Toluene 1:1 CH_2Cl_2	rt	2 h	47%
2	Bts	1.6	1.5	CH_2Cl_2	rt	4.5 h	63% (2 products)

- *J. Am. Chem. Soc.* **2003**, *125*, 761

Asymmetric Reactions: Vinylzinc Additions In the Presence of 2-[(R)-1-(Dimethylamino)ethyl]benzenethiol



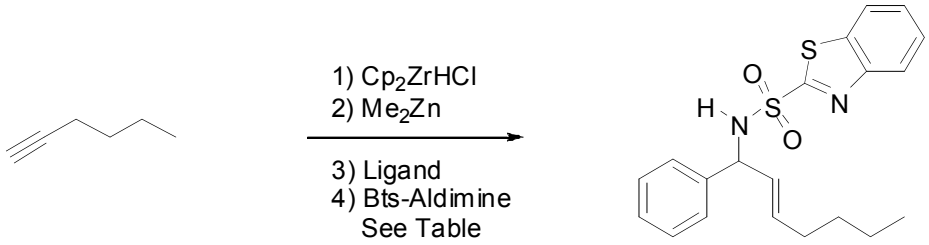
- *Org. Lett.* **2002**, *4*, 3619.



Asymmetric Additions Using 2-[(R)-1-(Dimethylamino)ethyl]benzenethiol Ligand

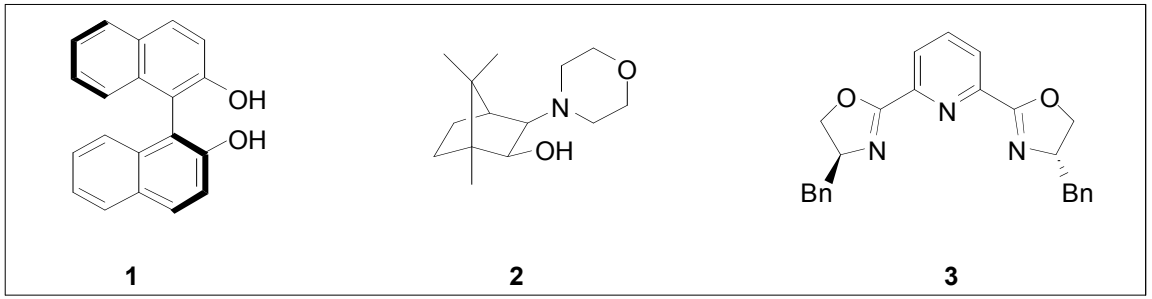
	Aldimine	Eq. Cp_2ZrHCl	Eq. Me_2Zn	Ligand	Solvent	Temp	Time	Yield	ee
1	Ths	3.2	3	100%	Toluene 1:2 CH_2Cl_2	-40 °C	12 h	63%	10 %
2	Ths	1.5	1	15%	Toluene 1:1 CH_2Cl_2	-40 °C	15 h	33%	0%
3	Bts	2.5	2.5	100%	CH_2Cl_2	-30 °C	20 h	62%	5%
4	Bts	2.5	2.5	100%	Toluene	-30 °C	16 h	80%	3%

Asymmetric Reactions: Vinylzinc Additions Using a Diverse Set of Ligands



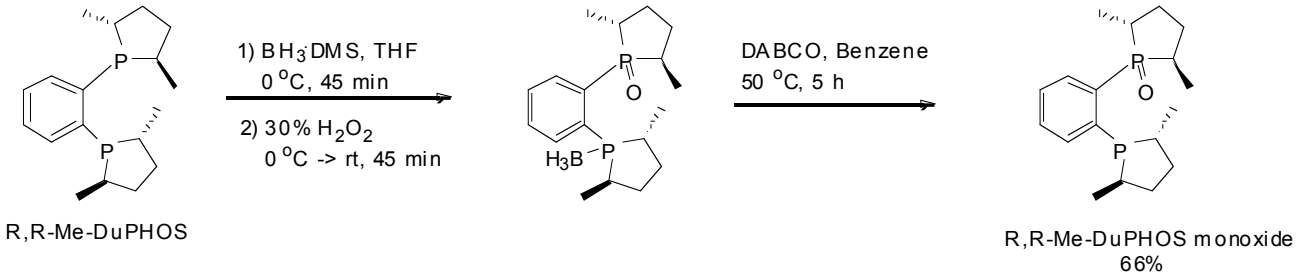
Asymmetric Additions Using a Diverse Set of Ligands

	Eq. Cp_2ZrHCl	Eq. Me_2Zn	Eq. Ligand	Solvent	Temp	Time	Yield	ee
1	2.5	2.5	100% of **1**	Toluene	-30 °C	20 h	X	X
2	2.5	2.5	100% of **2**	Toluene	-30 °C	13 h	84%	1%
3	1.5	1.5	100% of **3**	Toluene	-30 °C	9 h	13%	3%

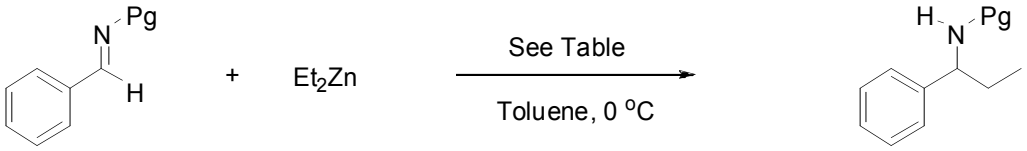


1 **2** **3**

Asymmetric Reactions: Diethylzinc Additions Using $Cu(OTf)_2$ Catalysis and Me-DuPHOS Derived Ligands



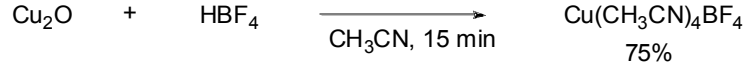
- *J. Am. Chem. Soc.* **2003**, *125*, 14260.



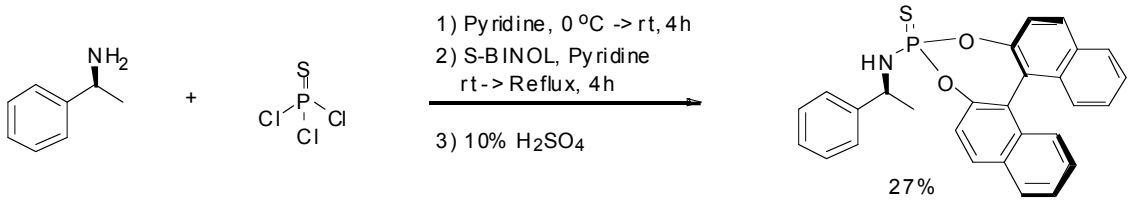
Copper Catalyzed Reactions

	Pg	% $Cu(OTf)_2$	% Ligand	Time	Yield	% ee
1	$P(O)Ph_2$	6%	3% of Me-DuPHOS monoxide	19 h	86%	97%
2	Bts	10%	5% of Me-DuPHOS	15 h	48%	0%
3	Bts	10%	5% of Me-DuPHOS monoxide	15 h	60%	0%

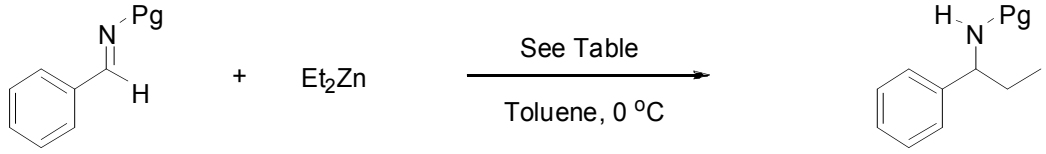
Asymmetric Reactions: Diethylzinc Additions Using $Cu(CH_3CN)_4BF_4$ Catalysis and BINOL Derived Thiophosphoramide Ligands



- *J. Org. Chem.* **2002**, *67*, 3450.



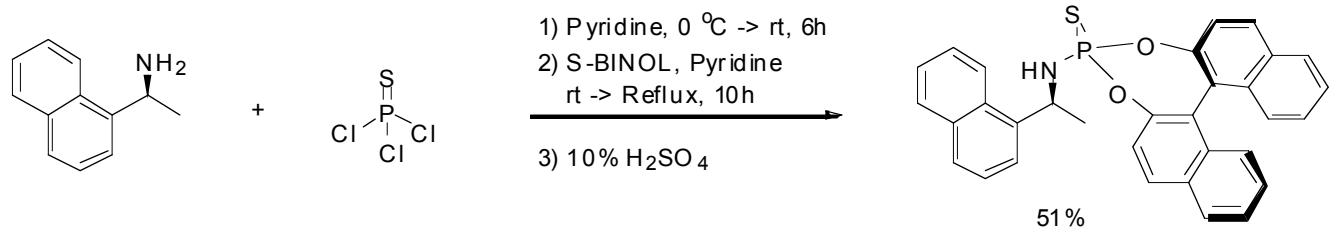
- *J. Org. Chem.* **1993**, *58*, 1748.

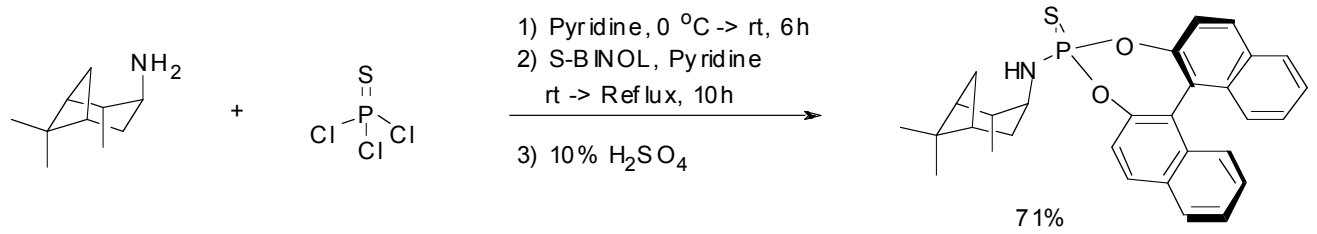


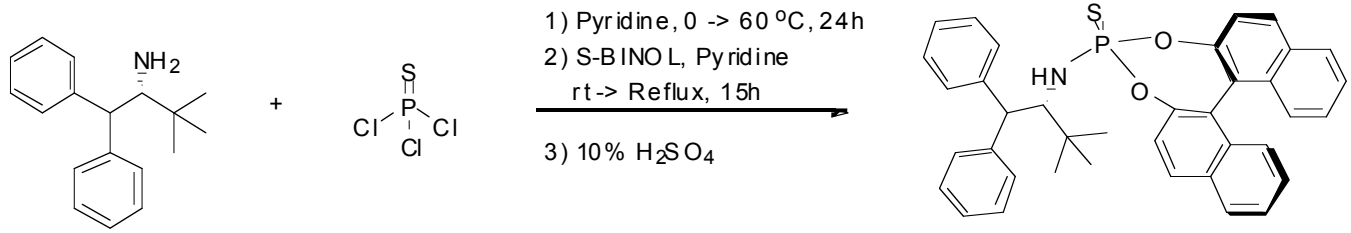
Copper Catalyzed Reactions

	Pg	% $Cu(CH_3CN)_4BF_4$	% Ligand	Time	Yield	% ee
1	Bts	3%	6%	15 h	83%	9%
2	Bts	6%	12%	17 h	85%	11%
3	Bts	25%	50%	16 h	74%	8%
4	Ths	5%	10%	17 h	70%	10%

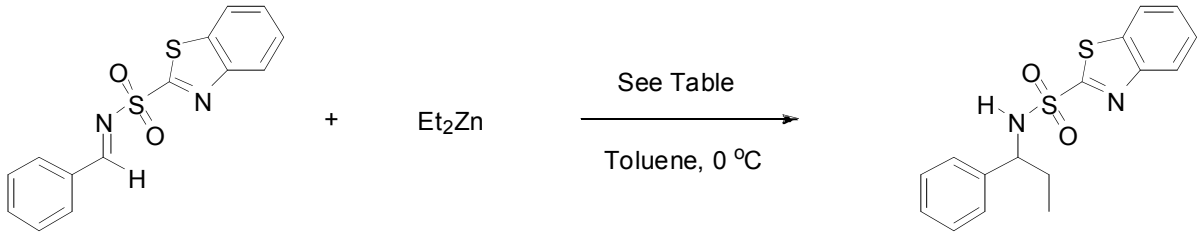
Asymmetric Reactions: Synthesis of BINOL Derived Thiophosphoramide Ligands Using a Variety of Chiral Amines





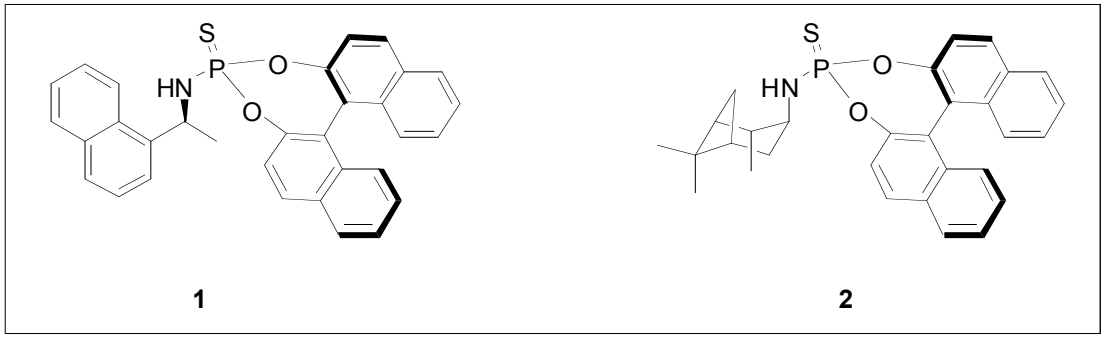


Asymmetric Reactions: Diethylzinc Additions Using $Cu(CH_3CN)_4BF_4$ Catalysis and BINOL Derived Thiophosphoramide Ligands



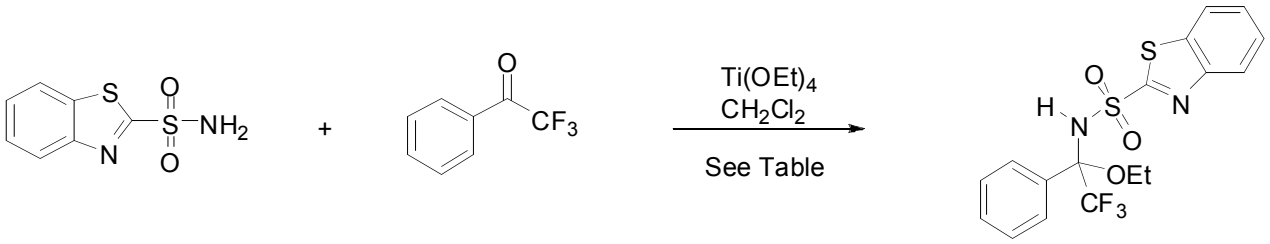
Copper Catalyzed Reactions

	Addition	% $Cu(CH_3CN)_4BF_4$	% Ligand	Time	Yield	% ee
1	Aldimine First	5%	10% of **1**	14 h	76%	5%
2	Aldimine First	5%	10% of **2**	14 h	83%	7%
3	Aldimine First	25%	50% of **2**	15 h	83%	12%
4	Et_2Zn First	5%	10% of **2**	15 h	69%	0%



1 **2**

Synthesis of Trifluoromethyl Hemiaminals

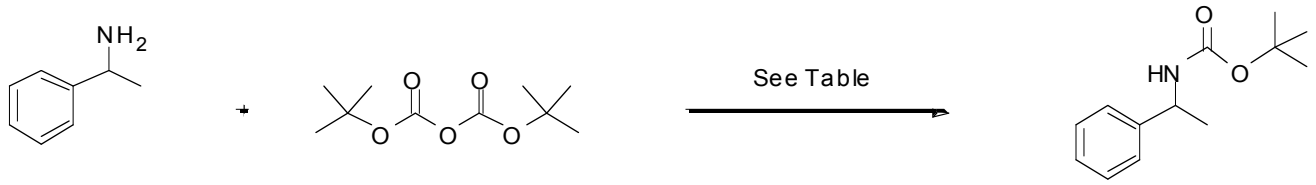


Trifluormethyl Hemiaminals

	Eq. Ketone	Eq. $Ti(OEt)_4$	Additive	Temp	Time	^{19}F NMR % Conversion	Yield
1	3	2	X	rt	4 d	47%	12%
2	2	1.5	X	MW 110 °C	1.5 h	dec.	X
3	2	1.5	15% DMAP	rt	2 h	60%	Trace
4	2	1.5	20% DMAP	rt	5 h / 4 d	57%	13%

• *Org. Lett.* **2006**, *8*, 2743.

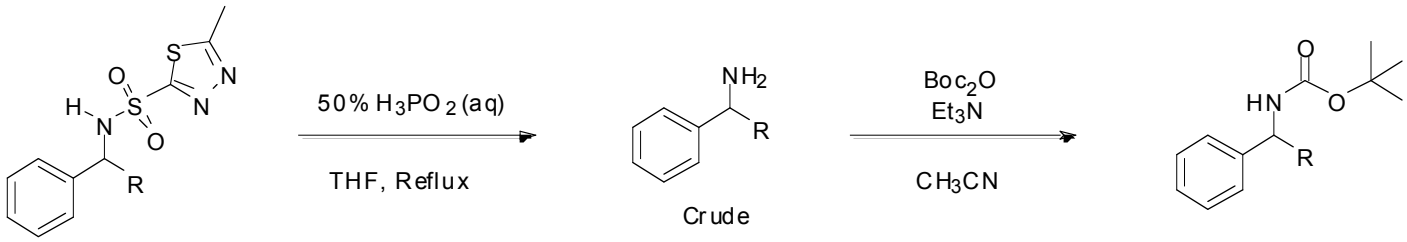
Deprotection Reactions: Optimization of Boc Anhydride Conditions for Amine Protection



Optimization of Boc Protection at Room Temp

	Eq. $(Boc)_2O$	Base/Catalyst	Solvent	Time	% Yield
1	1.2	10 % DMAP	CH_2Cl_2	24 h	11%
2	1.2	2.5 M NaOH	Dioxane	2.75 h	82%
3	1.25	2.5 M NaOH	THF	1 h	89%
4	**1.25**	**1 eq Et_3N**	**CH_3CN**	**1 h**	**99%**

Deprotection Reactions: Hypophosphorous Acid

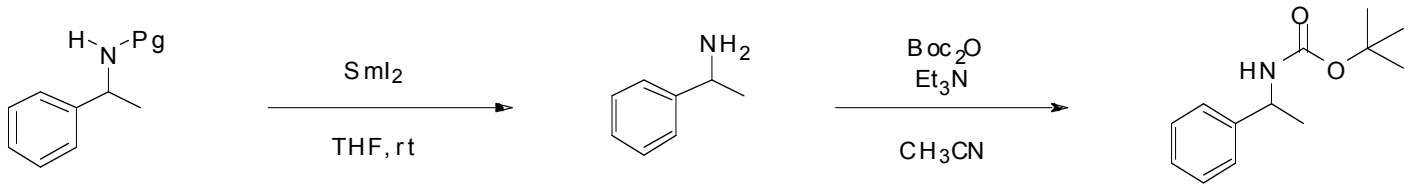


H_3PO_2 Deprotection and Boc Protection

		Deprotection				Protection		
	R	**Eq. H_3PO_2**	**Additon Time**	**Time**	**Workup pH**	**Eq. $(Boc)_2O$**	**Time**	**% Yield**
1	Me	30	3 h	4.5 h	>13	1.25	1 h	84%
2	Me	30	3 h	4.5 h	>13	1.25	1 h	81%
3	Ph	30	3 h	4.5 h	>13	1.25	1 h	83%
4	1-propynyl	30	3 h	4.5 h	>13	1.25	1 h	88%

- *J. Am. Chem. Soc.* **1996**, *118*, 9796

Deprotection Reactions: Samarium(II) Iodide

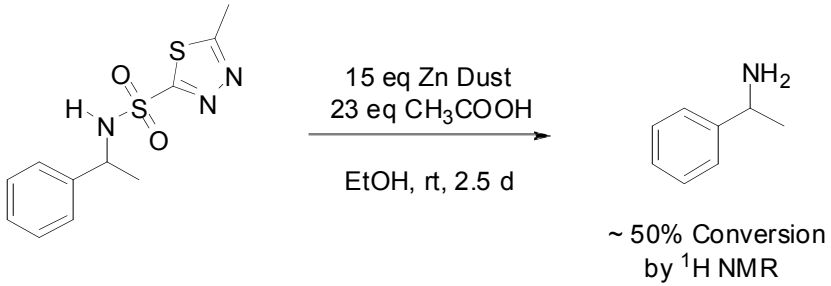


SmI_2 Deprotection and Boc Protection

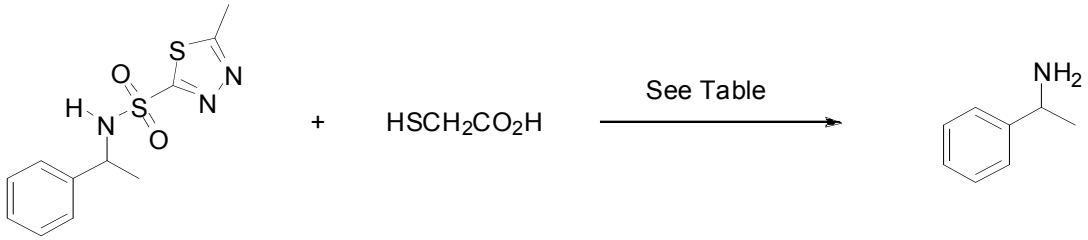
	Deprotection				Protection	
	Pg	**Eq. SmI_2**	**Time**	**Amine**	**Conditions**	**Yield**
1	Ths	5	15 h	Crude	Optimized	51%
2	Bts	5	15 h	Crude	Optimized	44%
3	Ths	5	30 h	Crude	Optimized	48%
4	Ths	7	8 h	Crude	Optimized	84%
5	Bts	9	8 h	Crude	Optimized	83%

- *J. Org. Chem.* **1995**, *60*, 5969

Deprotection Reactions: Zn/HOAc and Mercaptoacetic Acid



- *J. Am. Chem. Soc.* **1996**, *118*, 9796.



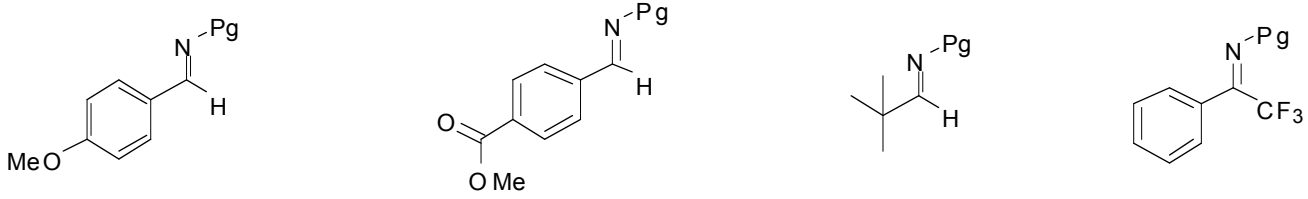
Mercaptoacetic Acid Deprotection

	Base	Eq. $HSCH_2CO_2H$	Solvent	Temp.	Time	^{1}H NMR % Conversion
1	Et_3N	1.3	CH_2Cl_2	40 °C	30 h	40%
2	LiOH	1.3	CH_2Cl_2	rt	48 h	10%
3	Et_3N	1.3	THF	66 °C	23 h	45%
4	Et_3N	1.3	CH_2Cl_2	μW 70-80 °C	4 h	50%
5	Et_3N	3	CH_2Cl_2	40 °C	40 h	67%

- *Chem. Commun.* **2004**, 353.

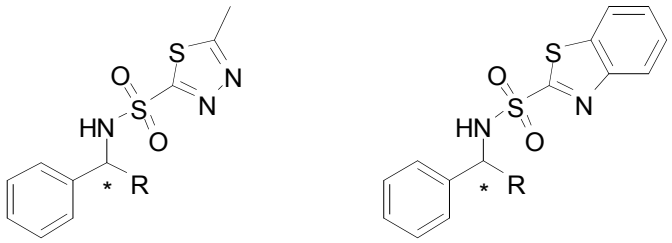
Future Directions

- Increase substrate scope



Pg = Bts, Ths

- Continue to screen ligands and catalytic organometallic conditions to Improve the enantiomeric excess of the aforementioned reactions.



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