UNIVERSITY OF LIVERPOOL

A new chemoenzymatic approach to chiral bicyclic [2.2.2]octan-2,5-diones and diene ligands

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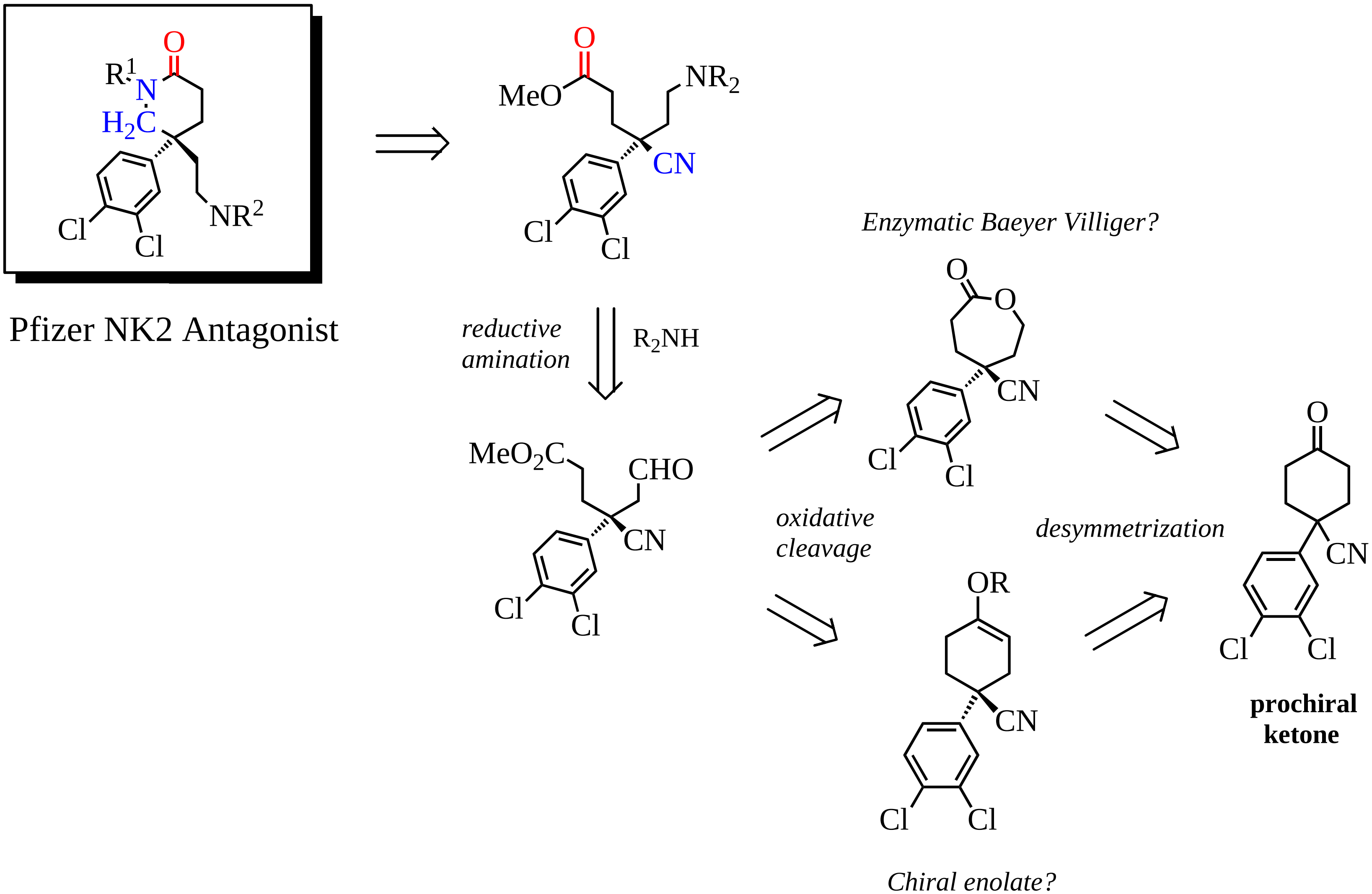
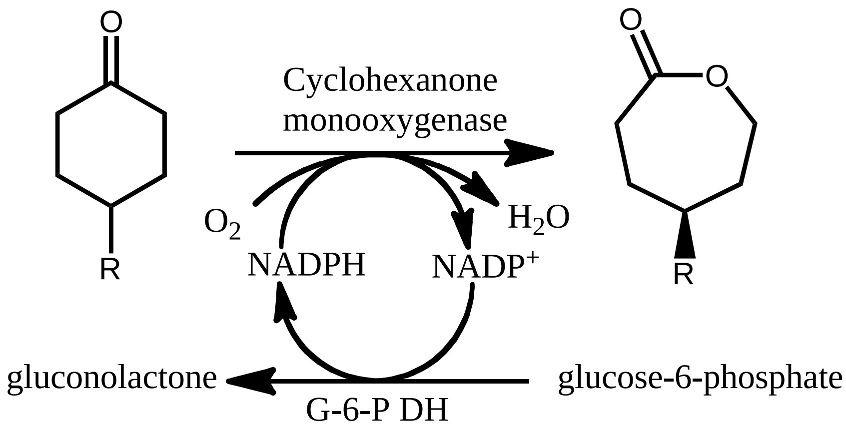
Biocatalysis: Challenges for Pharmaceuticals & Fine Chemicals
14th October 2010

Retrosynthesis of Pfizer NK-2 Antagonists

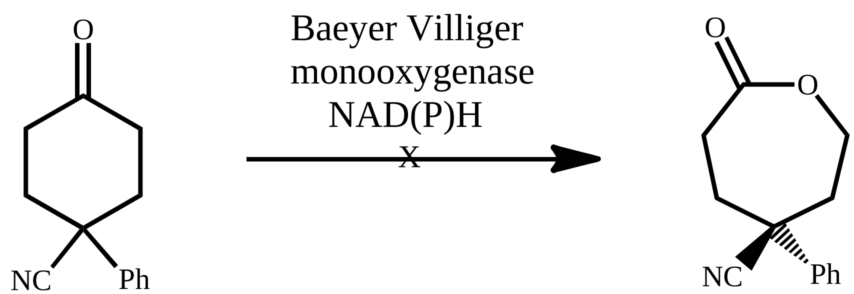


Baeyer Villiger Monooxygenases



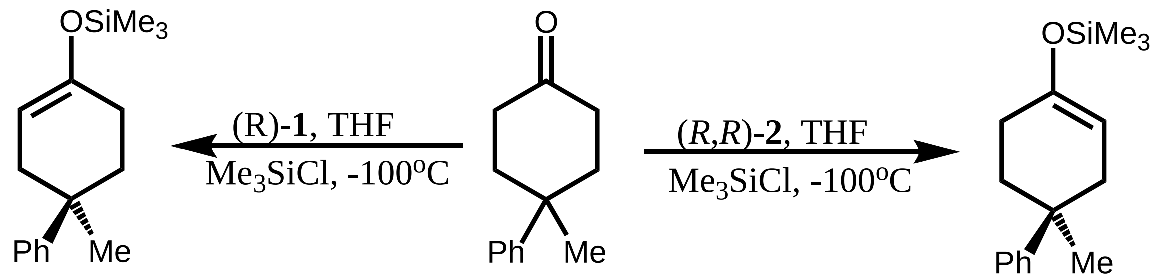
R = Me, Et, ^{i}Pr, n-Pr, ^{t}Bu
60-80% yield, >98% e.e.
R = n-Bu 70%, 52% e.e.
R = Ph no reaction

M. J. Tachner and D. J. Black, *J. Am. Chem. Soc.*, 1988, *110*, 6892



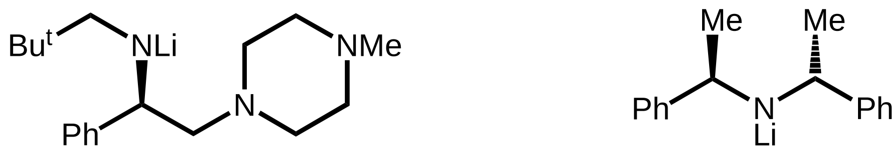
Cyclohexanone Monooxygenase
Diketocamphane Monooxygenase
2-oxo-Δ-^{3}-4,5,5-trimethylcyclopentenylacetylCoA MO

4,4-Disubstituted cyclohexanone with chiral lithium amide



(R) 66%, 66% e.e.

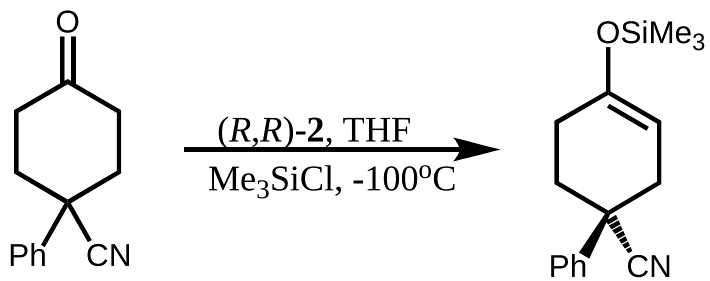
(S) 81%, 71% e.e.



(R)-1

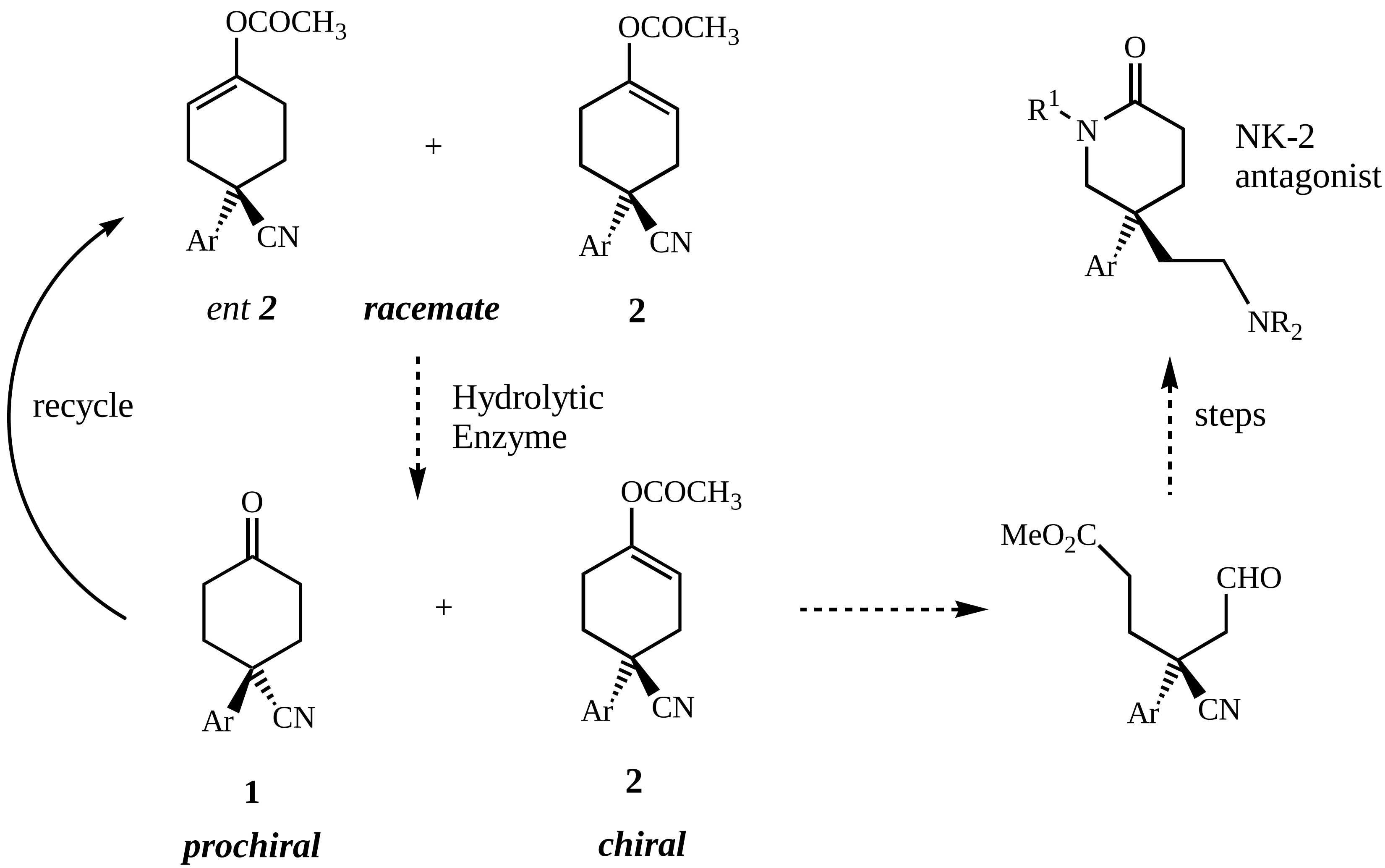
(*R*,*R*)-**2**

T. Honda, N. Kimura, M. Tsubuki, *Tetrahedron: Asymmetry*, 1993, *4*, 21

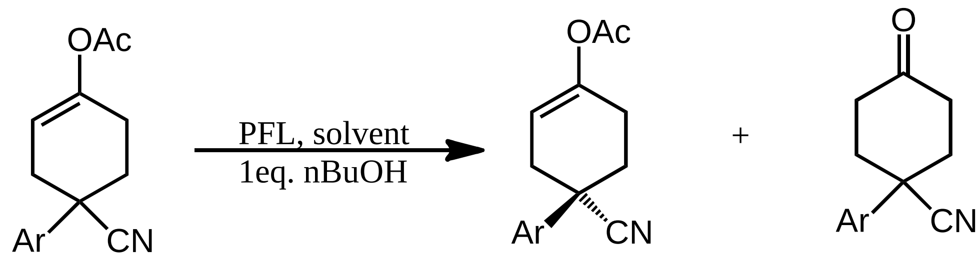


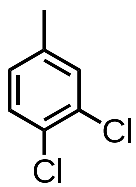
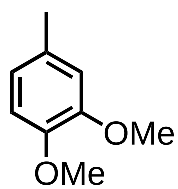
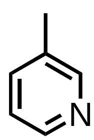
64% yield, 64% e.e.

Hydrolytic enzymes to desymmetrise the prochiral ketone



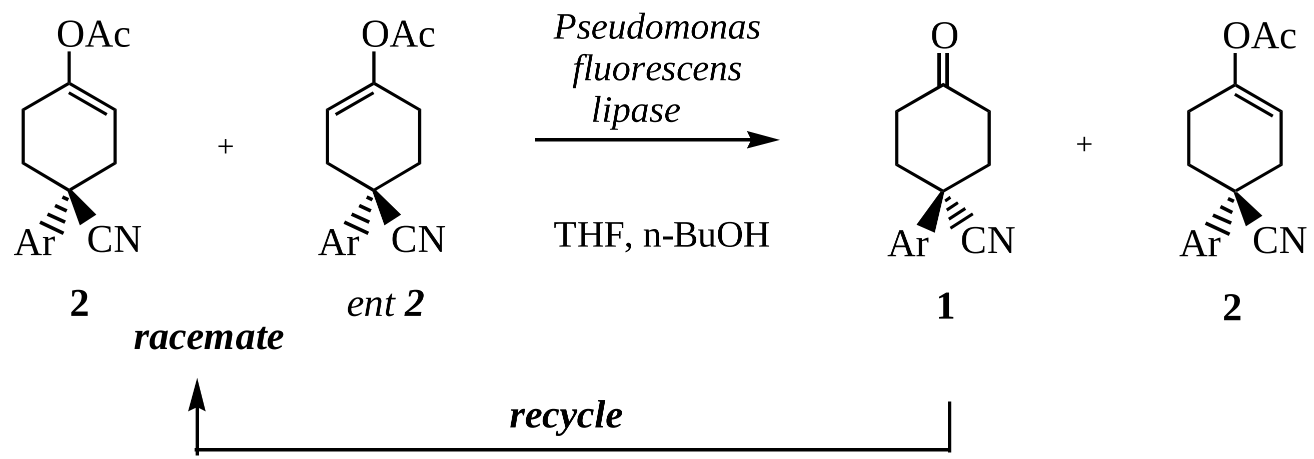
Biotransformation of 4-aryl-4-cyano enol esters



Ar	solvent time (h)	enol ester yield (%)	enol ester E.e. (%)	ketone (%)
	THF, 8.5h	30%	100%	70%
	THF, 3.5h	**38%**	**100%**	**62%**
	Toluene, 2h	48%	61%	52%
	THF, 22h	29%	96%	71%
	Toluene, 6h	30%	94%	70%
	THF, 6h	38%	78%	62%

G. Allan, A. J. Carnell, M. L. Escudero Hernandez and A. Pettman, *J. Chem. Soc. Perkin Trans 1*, 2000, 3382

Continuous Process for Deracemization without Work-up



Each cycle:

1. remove enzyme
2. ^{t}BuOK, isopropenyl acetate (3eq.)
3. Stir with Dowex H^{+} resin
4. remove resin
5. add Enzyme + nBuOH

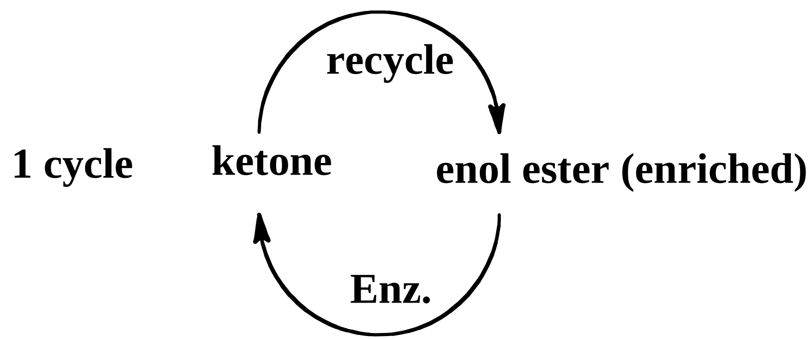
Enzyme tolerates ^{t}BuOH and acetone

Recycling the ketone

For a kinetic resolution $E = \dfrac{\ln[(1-c)(1-ee(S))]}{\ln[(1-c)(1+ee(S))]}$

E.e (max) for cyclic reaction = $\dfrac{(E - 1)}{(E + 1)}$

i.e. for E = 13, e.e.(max) = 85.7% for 100% yield

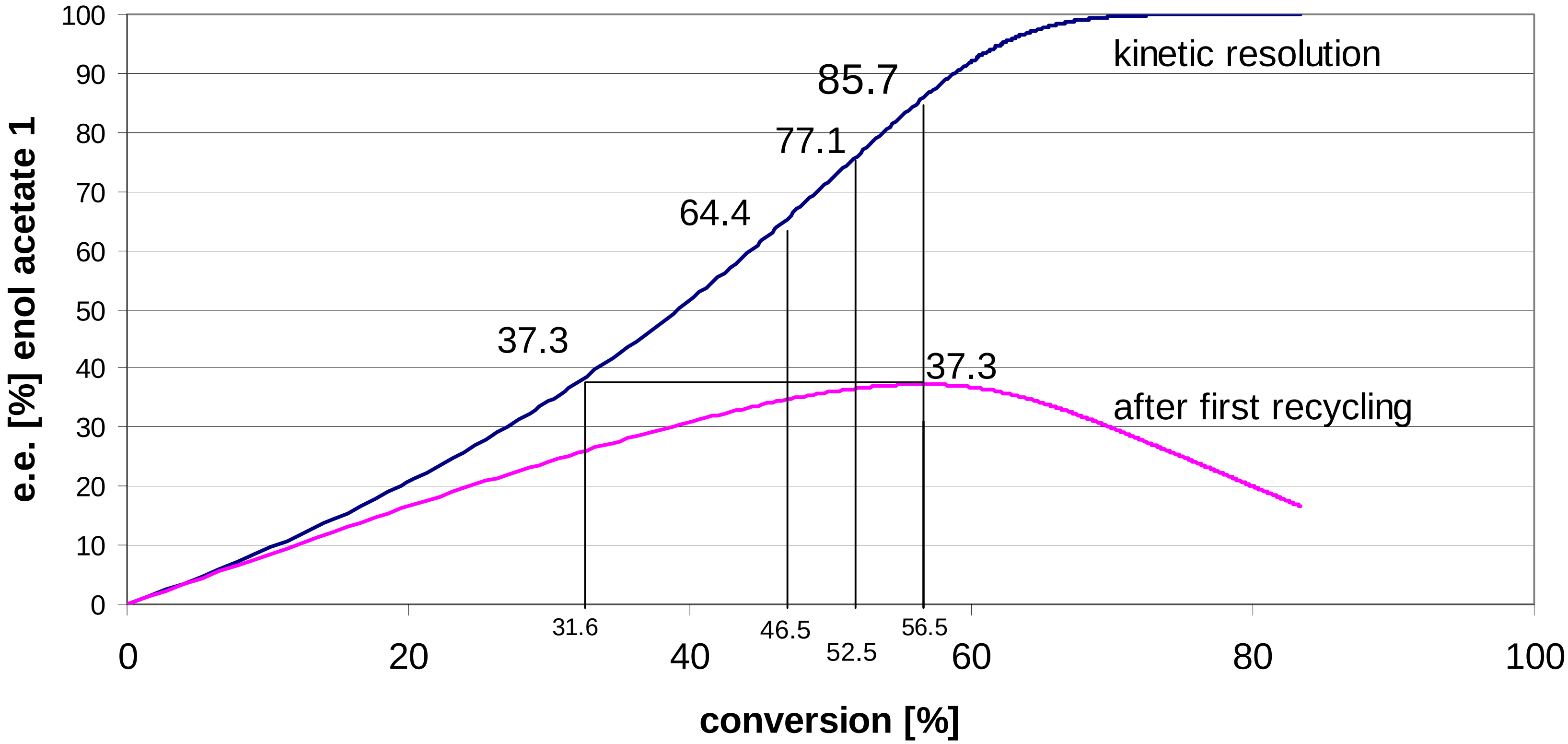


See: W. Kroutil and K. Faber, *Tetrahedron:Asymmetry*, 1998, *9*, 2901

Theoretical conversions and e.e.'s for deracemisation for E = 13

Cycle	% e.e. **1** (start)	% e.e. **1** (end)	% Conversion to ketone **2** needed
1	0	85.7	56.5
2	37.3	85.7	24.9
3	64.3	85.7	10
4	77.1	85.7	4
5	82.3		

Enantiomeric excess (e.e.) of enol acetate 1 after recycling of the ketone 2 *versus* conversion for E=13.

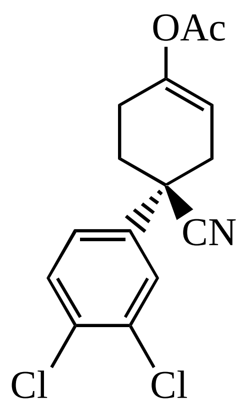


G. Allan, A.J. Carnell and W. Kroutil, *Tetrahedron Lett.*, 2001, *42*, 5959-5962.

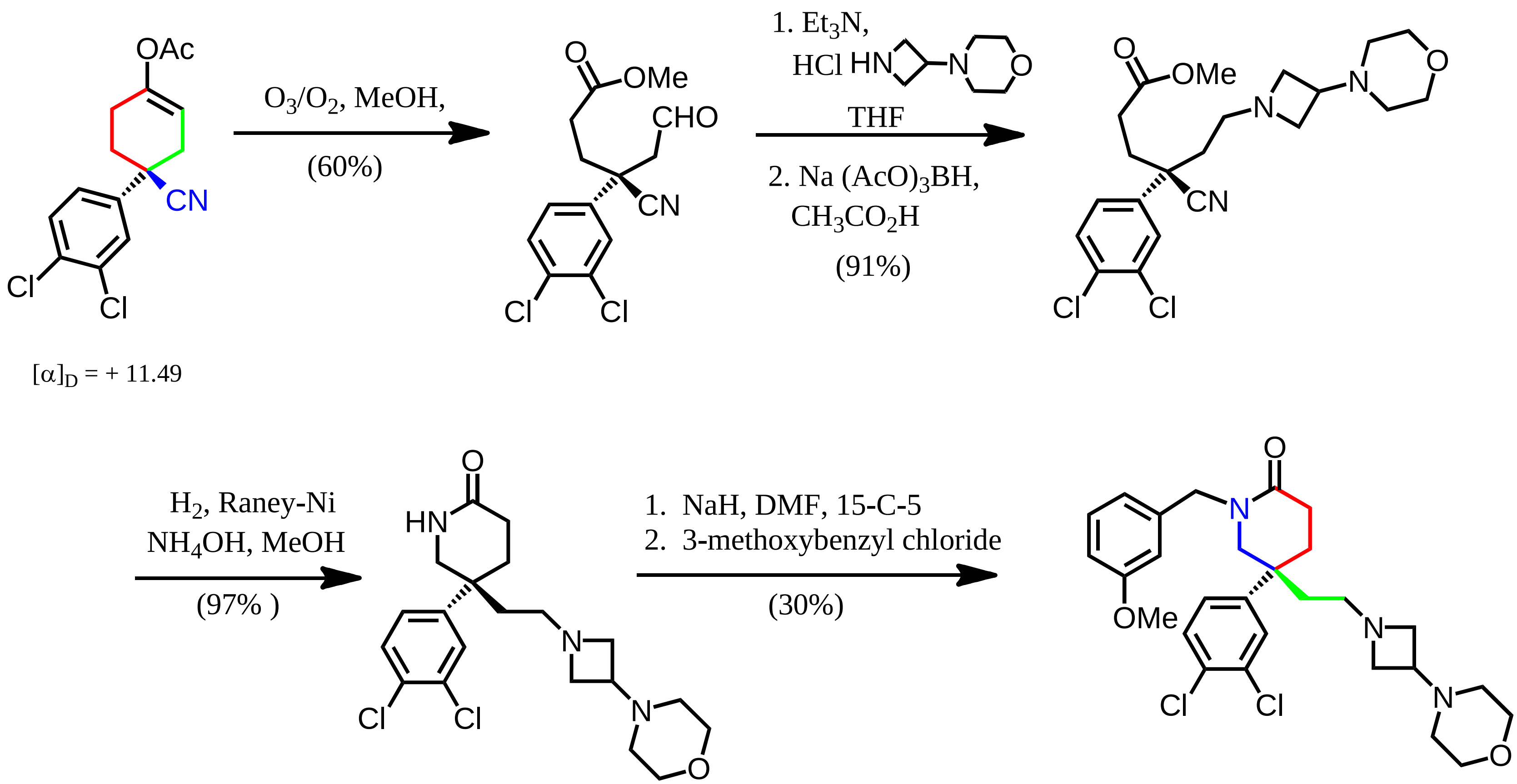
Deracemisation of enol ester **1** over 4 cycles

Cycle	% e.e. residual enol ester **1**	% conversion to ketone **2**
1	92	61
2	86	28
3	87	16
4	>99	12

Starting from 400 mg of the racemic material over 4 cycles 330 mg (82%) of the enol ester **1** in >99% e.e was isolated.



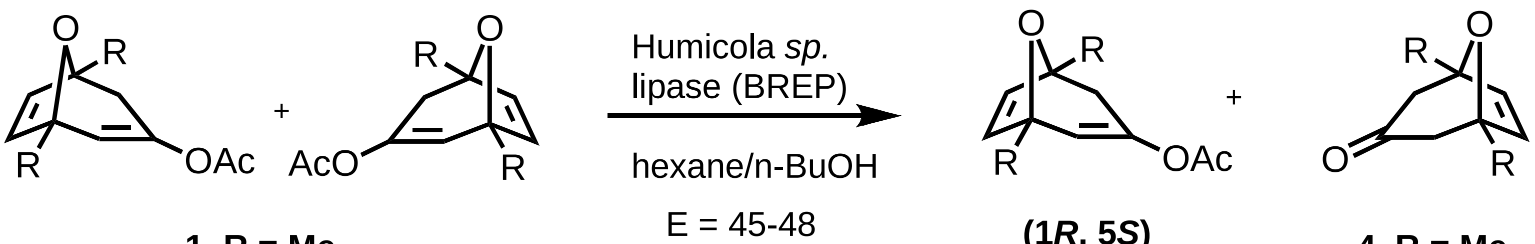
Synthesis of NK-2 antagonists Using Enol Ester



A.J. Carnell, M. L. Escudero Hernandez, A. Pettman and J.F. Bickley, *Tetrahedron Lett.*, 2000, 41, 6929

G. Allan, A. J. Carnell, M. L. Escudero Hernandez and A. Pettman, *Tetrahedron*, 2001, *57*, 81932.

Resolution of 8-oxabicyclic enol esters



1 R = Me,
2 R = Et
3 R = CH_2OMe

(1*R*, 5*S*)

91-99% e.e.

4 R = Me,
5 R = Et
6 R = CH_2OMe

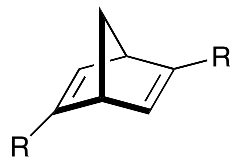
BREP = butanol rinsed enzyme preparation

- see Halling *et al.*, J.Chem. Soc., *Chem. Commun.*, 1998, *7*, 841

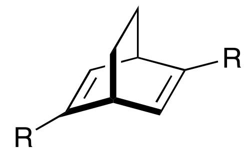
A.J. Carnell, S.A. Swain and J.F. Bickley, *Tetrahedron Lett.*, **1999**, *40*, 8633.

Chiral diene ligands for asymmetric catalysis

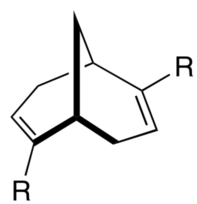
Hayashi



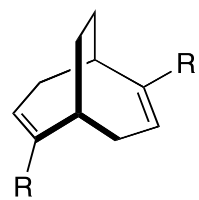
R-nbd* , R = Me, Ph, Bn



R-bod* , R = Ph, Bn

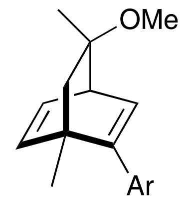


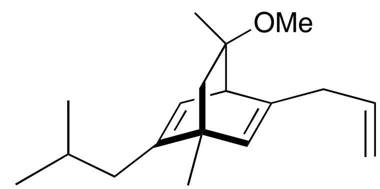
R- bnd* , R = Ph, Tol



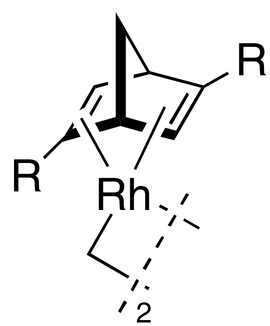
R-bdd* . R = Ph

Carreira



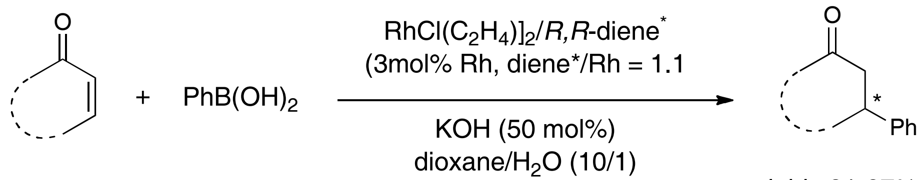


Catalyst:



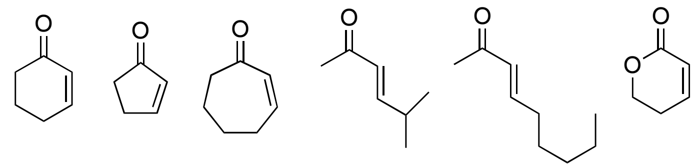
C. Defieber, H. Grutzmacher, E. M. Carreira, *Angew. Chem. Int. Ed.*, 2008, *24*, 4482.

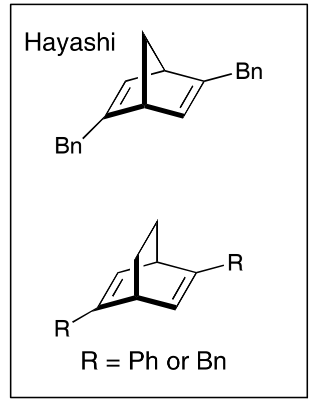
Rh-catalysed asymmetric reactions

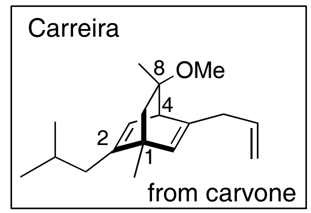


yields 81-97%

83- 99% e.e.



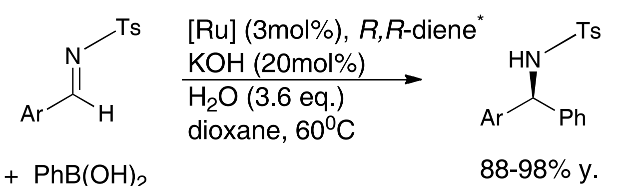




0.005% mol% rhodium can be used

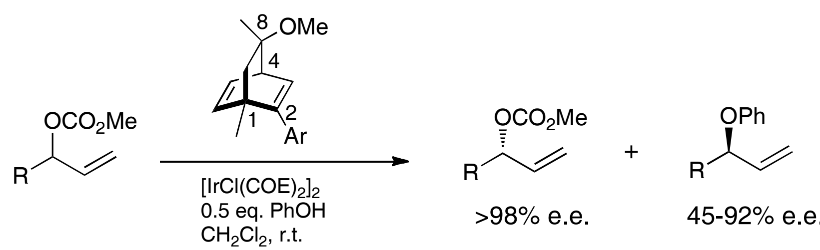
BINAP or chiral phorsphoramidite less effective

Hayashi *et al. J. Org. Chem.*, 2005, *70*, 2503-2508; *Tetrahedron: Asymmetry*, 2005, *16*, 1673-1679; Guillaume B.-G.; Hayashi, T. *J. Org. Chem.*, 2006, *71*, 8957-8960; Carreira E.M *et al.*,. *Org. Lett.* 2004, *6*, 3873



88-98% y.
81-99% e.e.

Hayashi *et al. Tetrahedron: Asymmetry*, 2005, *16*, 1673; *J. Org. Chem.*, 2006, *71*, 8957.

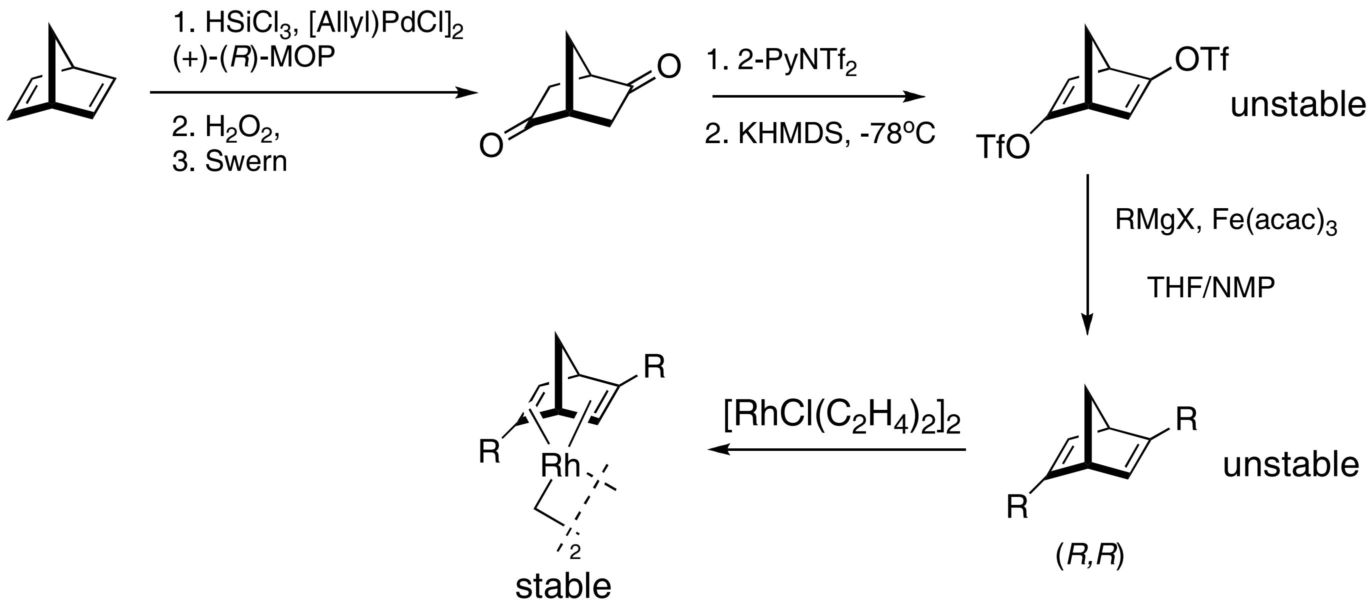


>98% e.e. 45-92% e.e.

Poor results with C_2-symmetric ligands

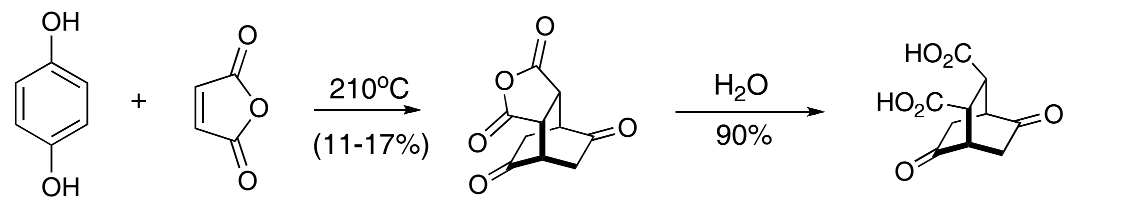
Carreira *et al. J. Am. Chem. Soc.* 2004, *126*, 1628-1629

Chiral Synthesis of [2.2.1] Bicyclic Diene Ligand

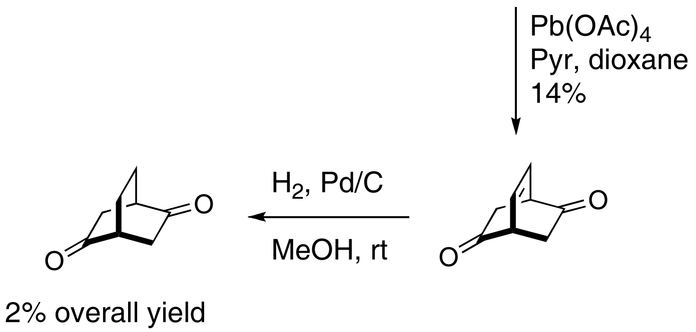


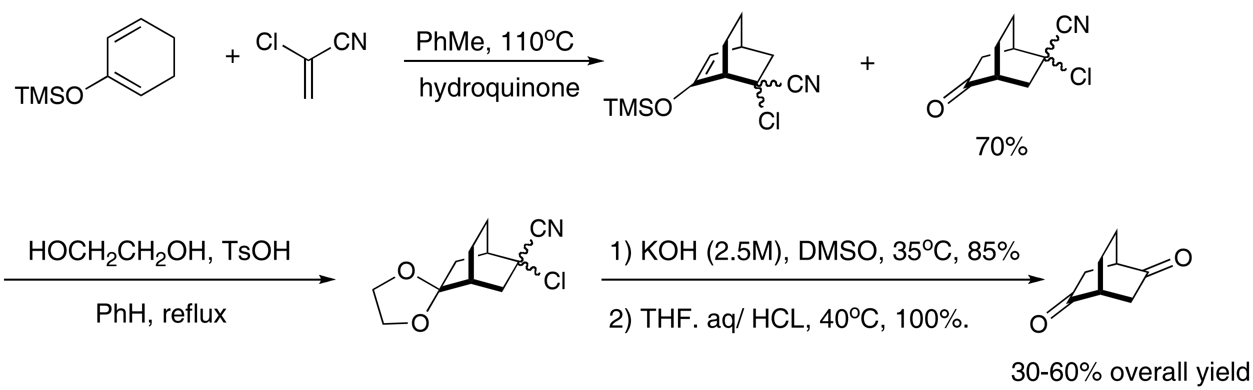
Guillaume B.-G.; Hayashi, T. *J. Org. Chem.*, 2006, *71*, 8957.

Synthesis of bicyclic [2.2.2] diketone



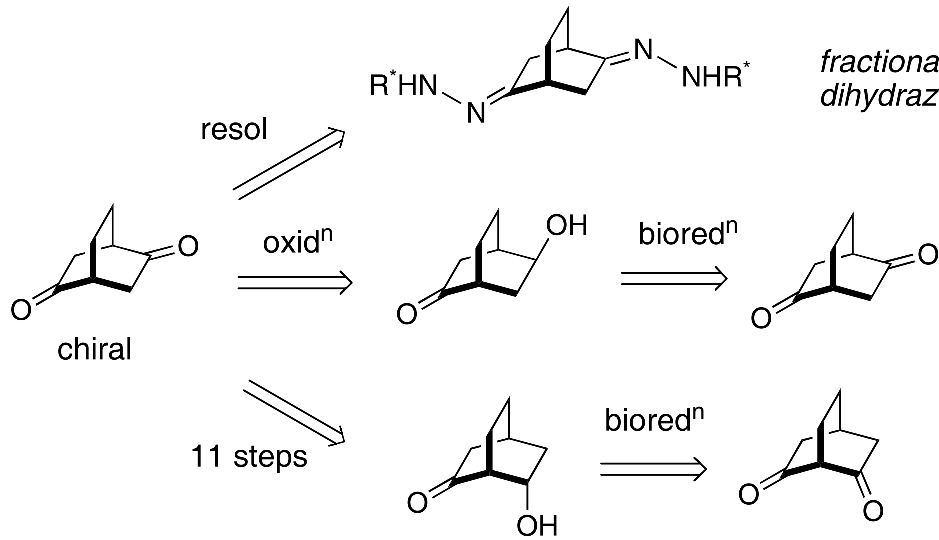
Paquette *et al. J. Org.Chem.* 1985, *50*, 5528;
J. Org. Chem. 1988, *53*, 1969





N. H. Werstiuk *et al. Can. J. Chem.* 1992, *70*, 974. .

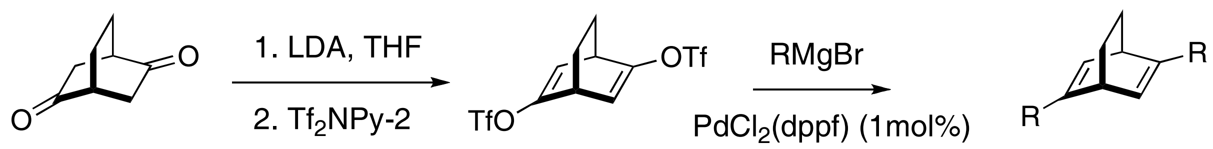
Accessing the Chiral Hayashi [2.2.2] Diene



fractional recryst of chiral dihydrazone (4.5% yield)

whole cell asymmetric bioreduction then re-oxidation using engineered yeast

11 step transposition from chiral 2,6-hydroxyketone

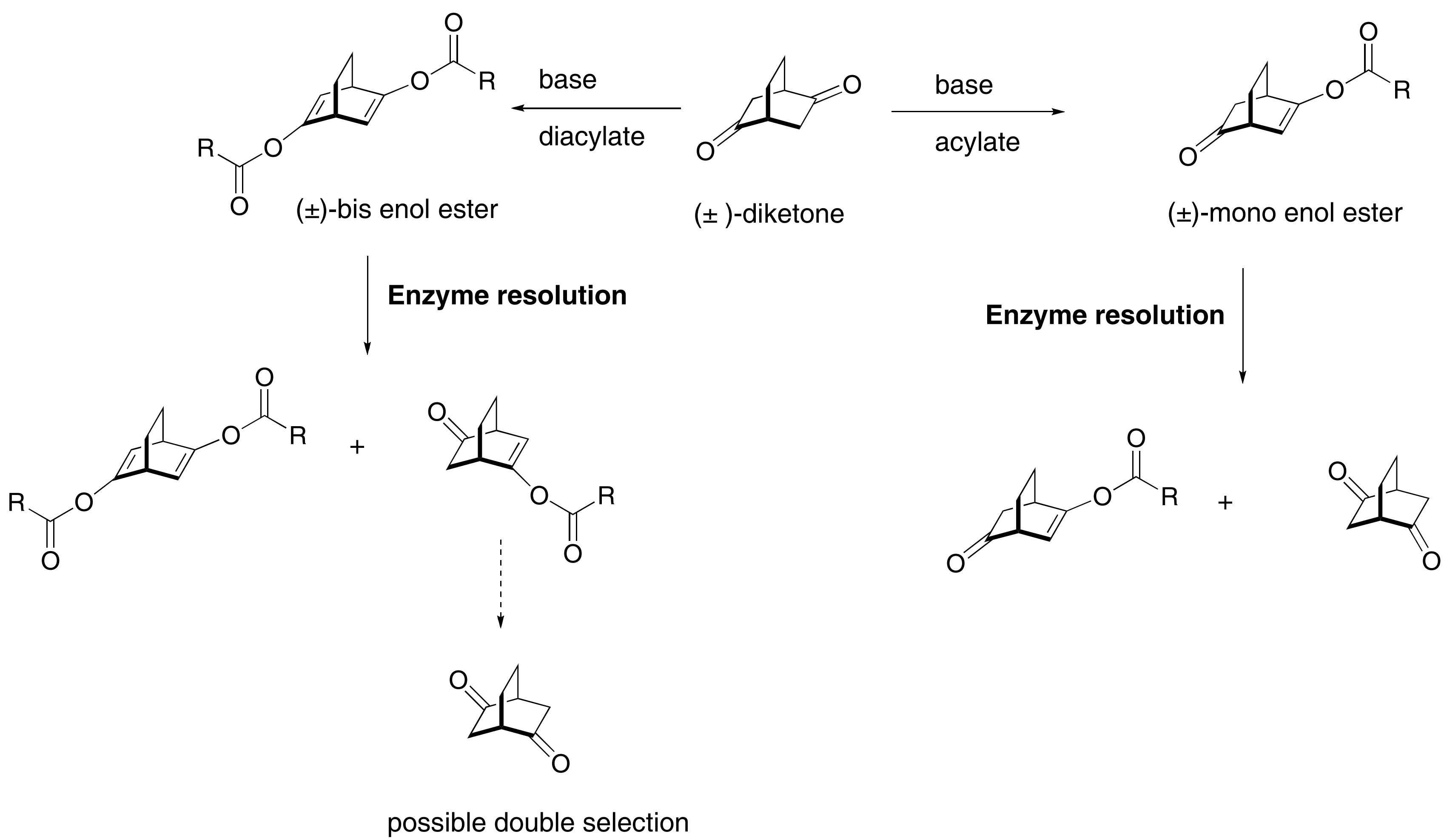


racemic

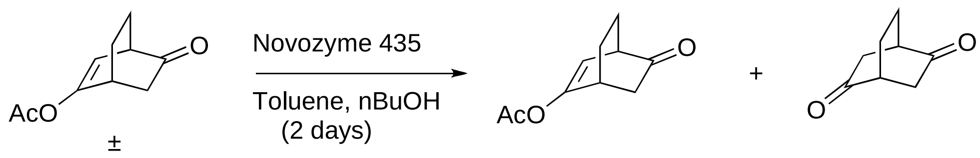
Chiral diene obtained by chiral HPLC of diene or triflate

Otomaru *et al.*, *J. Org. Chem.*, 2005, *70*, 2503
Friberg *et al.*, *Org. Biomol. Chem.*, 2006, *4*, 2304.
Almqvist *et al.*, *J. Org. Chem.*, 1996, *61*, 3794.

Options for enzyme resolution of [2.2.2] bicyclic diketone via enol esters



Enzyme Screening



Entry	Enzyme	% Conversion	ee% enolester (enolester left%)		E
1	*Canadida Rugosa* (Chiralzyme L-3, Europa Lipase AY)	0	--	(100)	
2	*Pseudomonas fluorescens* (PFL, Amano AK30)	0	--	(100)	
3	***Canadida antartica* (CAL-B, Novozyme 435)**	11.8	12.4	(88.2)	**17**
4	*Pseudomonas cepacia* (PCL, Lipase PS Amano)	0	--	(100)	
5	Lipase Amano 10	0	--	(100)	
6	*Penicillium camembertii* (Lipase G Amano 50)	0	--	(100)	
7	*Rhizopus oryzae* (Lipase F AP-15)	0	--	(100)	
8	*Mucor Meihei* (Lipase M Amano,Europa lipase 15(RS))	0	--	(100)	
9	**Chirazyme L-2 (Cal-B)**	11.3	14.8	(88.7)	**16**
10	Europa Lipase 20	3.5	5.0	(98.5)	

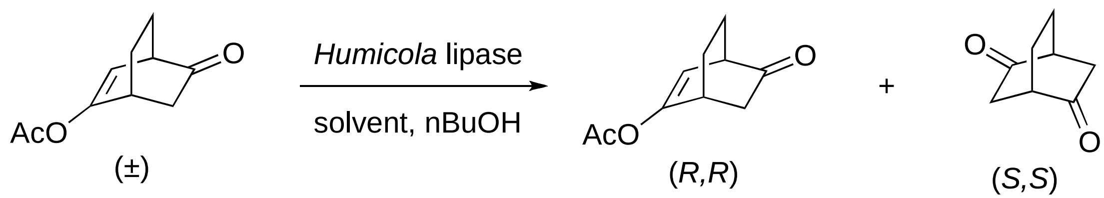
Reactions in Buffer or hexane less selective

Silica-absorbed *Humicola* Lipase Solvent Screen

entry	Solvents	Conversion	E.E.	E value
1	Hexane	28	26	6
2	Toluene	No reaction	-	-
3	TBME	41	43	6
4	Et_2O	27	13	2
5	DME	No reaction	-	-
6	Acetone	No reaction	-	-

entry	Solvents	Conversion	E.E.	E value
1	Hexane	28	26	6
2	Cyclohexane	31	28	5
3	Heptane	29	27	6
4	Pentane	31	37	15
5	Petroether	27	26	5

Humicola Lipase - support screening



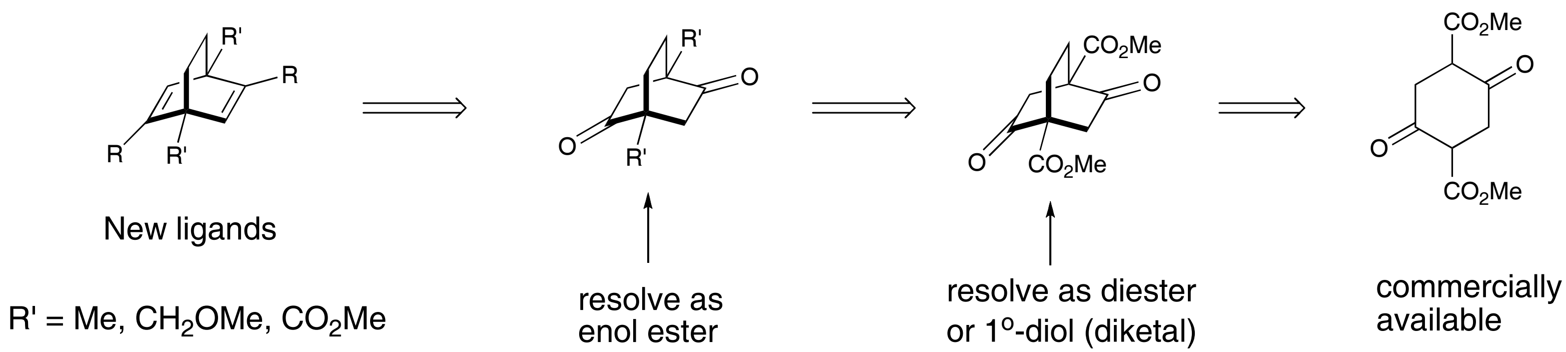
Entry	Solvent	Enzyme Form	E Value
1	toluene	Silica adsorbed	No reaction
2	TBME	Silica absorbed	6
3	Et_2O	Silica absorbed	2
4	Hexane/cyclohexane/ heptane/petroleum ether	Silica absorbed	5/6
5	Pentane	Silica absorbed	15
6	Pentane	lyophilised	6
7	Pentane	Protein coated microcrystals	10
8	Pentane	Sol gel entrapped	20
9	Pentane	GS01-immobilised	23
10	Pentane	GS02- immobilised	40*
11	**Pentane**	**GS03-immobilised**	**270**
12	**Pentane**	**Accurel**	**177**
13	Pentane	Accurel (dried)	33
14	Pentane	Eupergit	4

Increasing Hydrophobicity Of GSOX surface

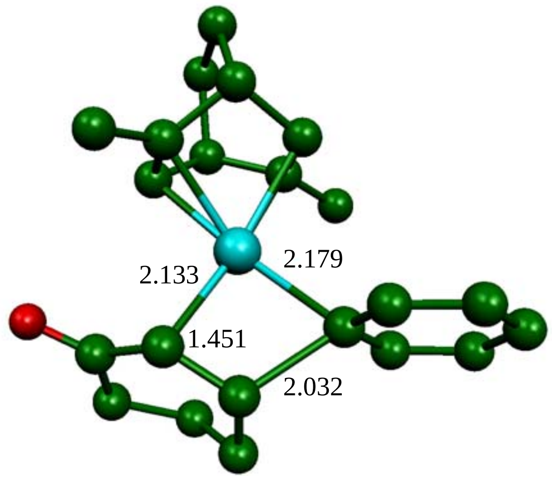
GS0X modified silica supports from PhosphonicS Ltd

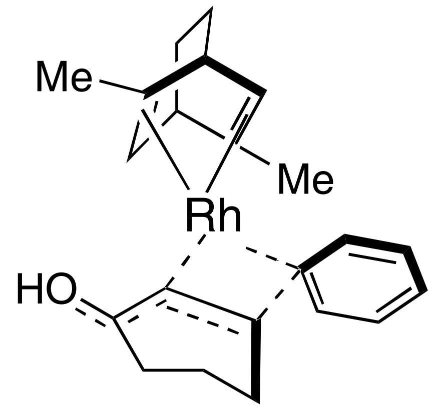
Luo and Carnell, J. Org. Chem., 2010, *75*, 2057-2060

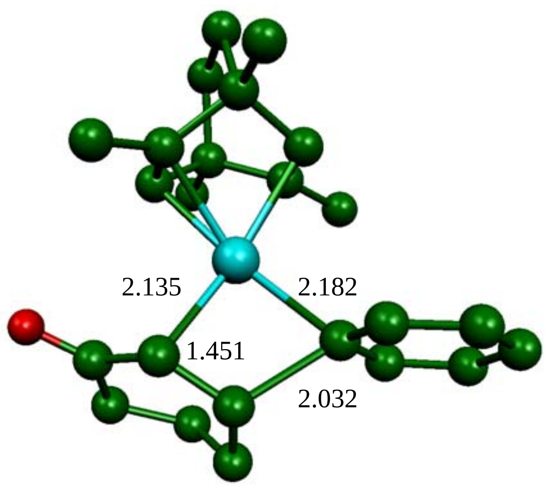
New Range of C_2-Symmetric Chiral Diene Ligands

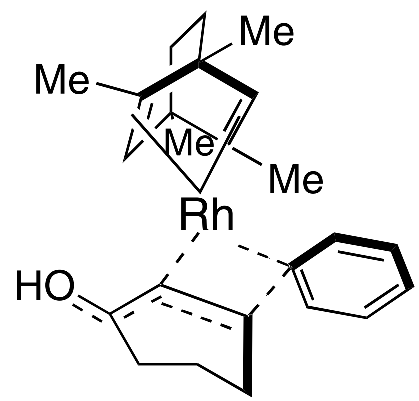


Transition states for conjugate additions



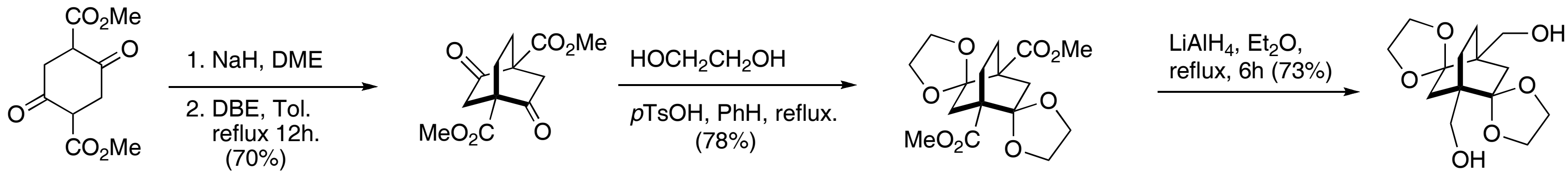




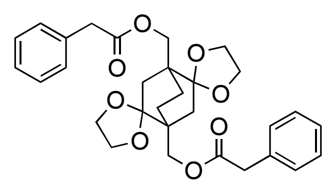


DFT calculations using PCGAMESS predict similar reaction rates and e.e.'s
N. Berry, A. J. Carnell, Y. Luo unpublished results

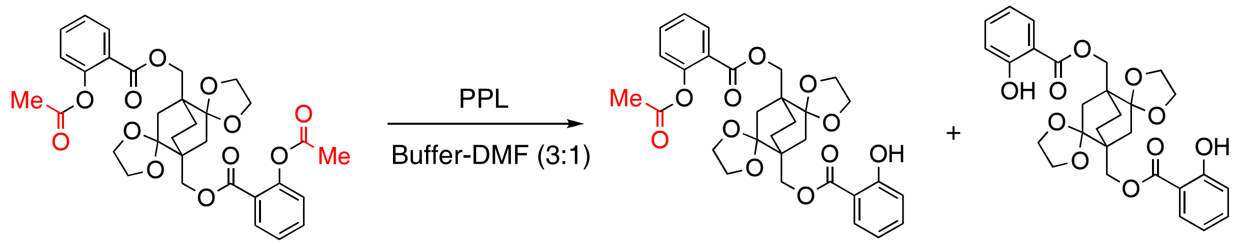
Resolution of Diol Diketal



Substrates - Introduction of chromophore into hydrolysable group



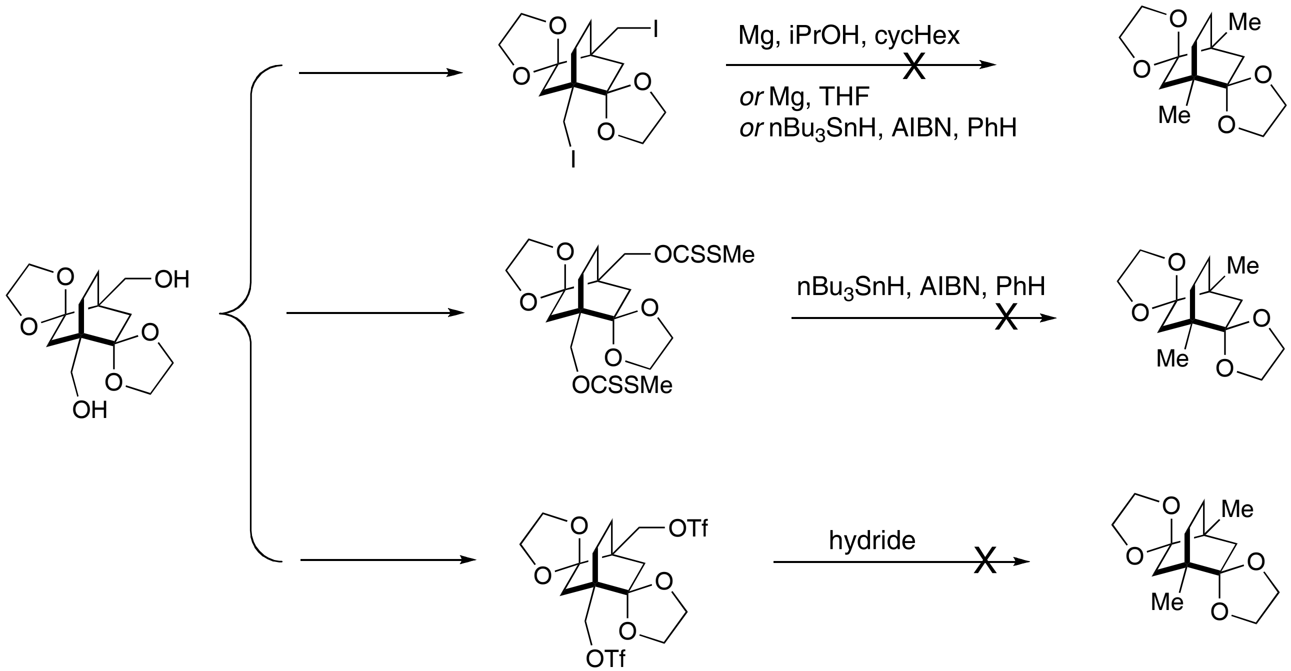
Penicillin acylase/buffer - no reaction



54%, 73% e.e.

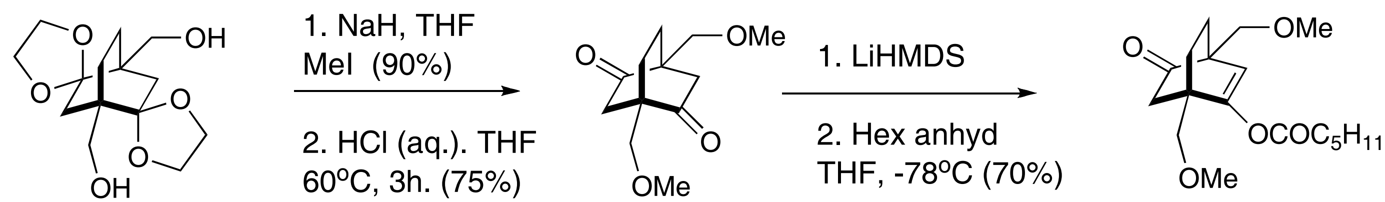
46%, 77% e.e.

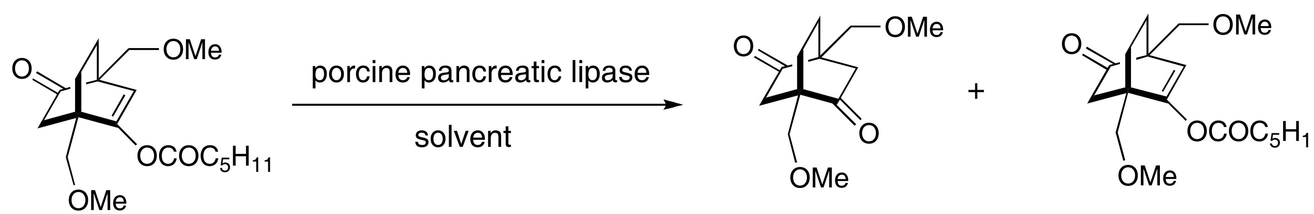
Attempted Deoxygenation



$LiAlH(OEt)_3$,	THF	No reaction
$NaBH_4$	EtOH, THF	No reaction
$LiAlH_4$	THF	Mixture of starting material and product
$LiEt_3HB$	THF	trace product

Resolution of Methoxy Ether Enol Hexanoate

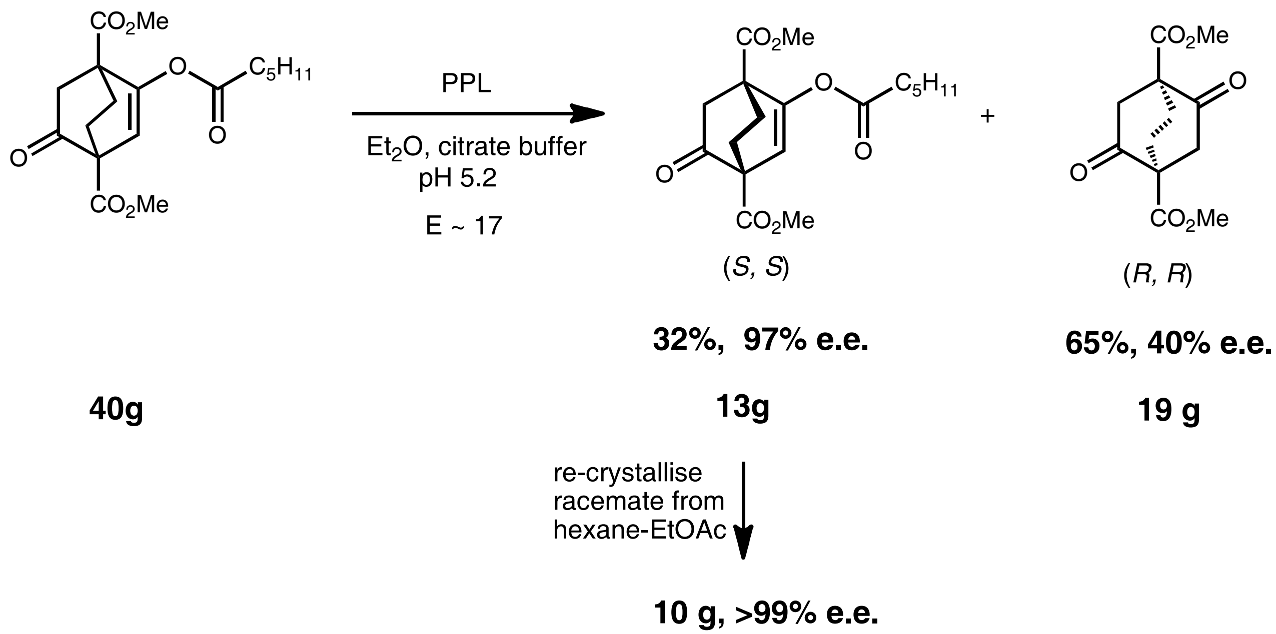




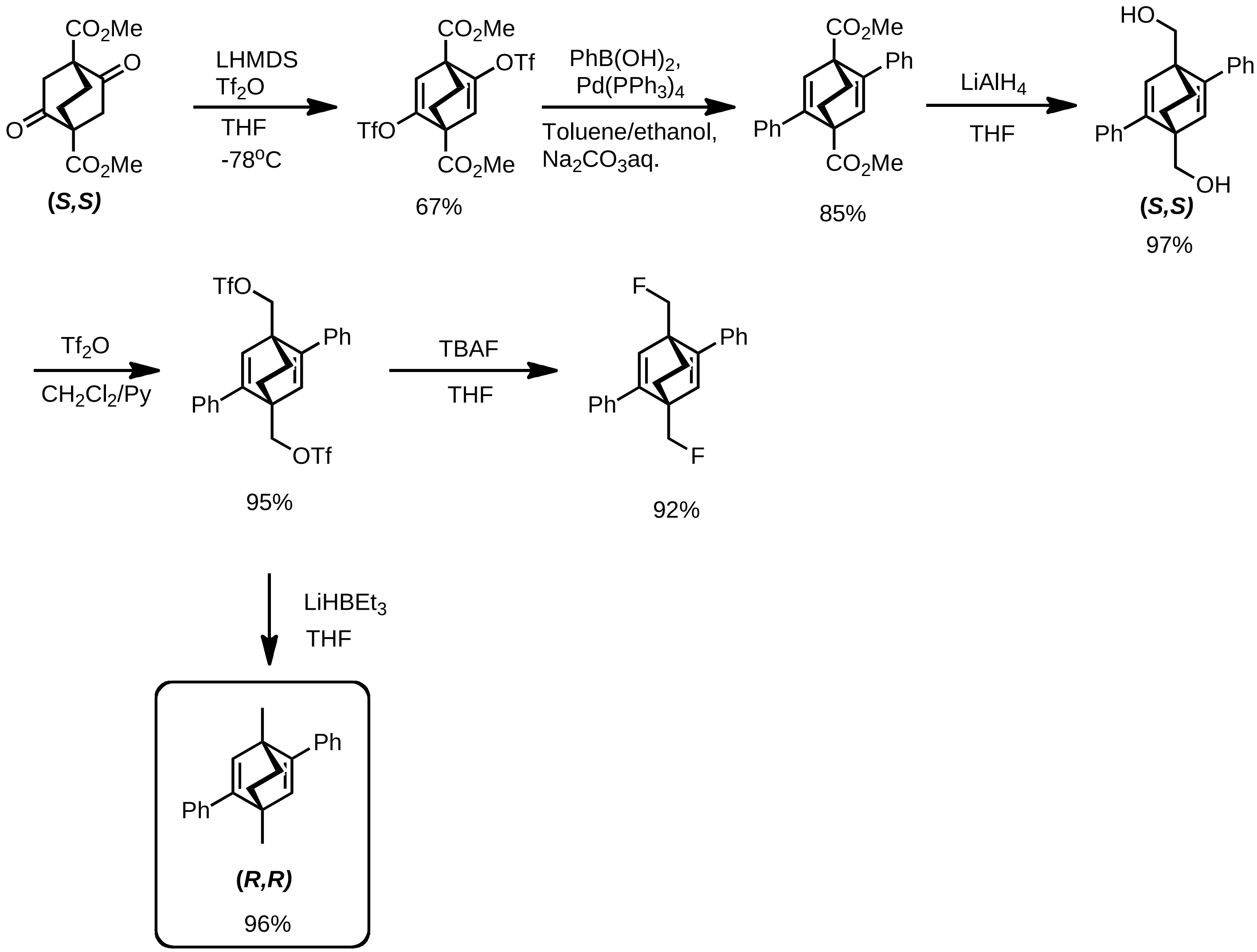
	Solvent		Buffer-co-solvent (4:1)		
	Toluene	Buffer (pH 7.5)	DME	DMF	DMSO
E value	nr	4-7	1	25-30	2

…too many steps!

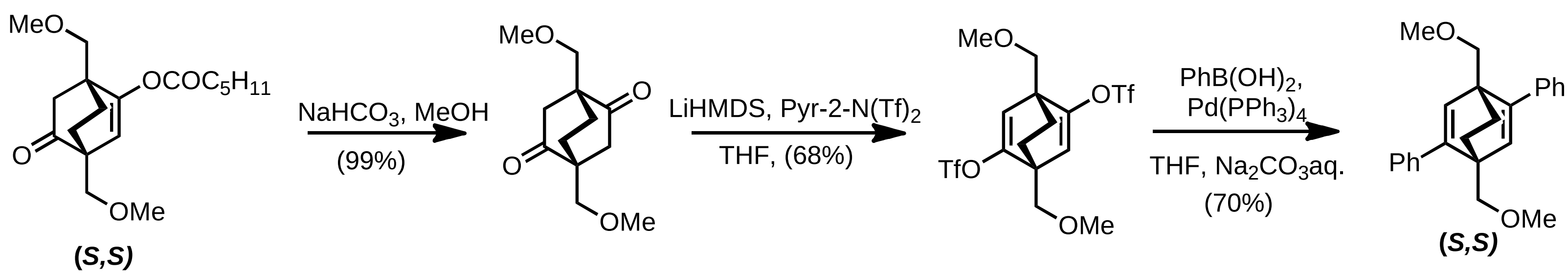
Resolution of Dimethyl Ester Enol Hexanoate

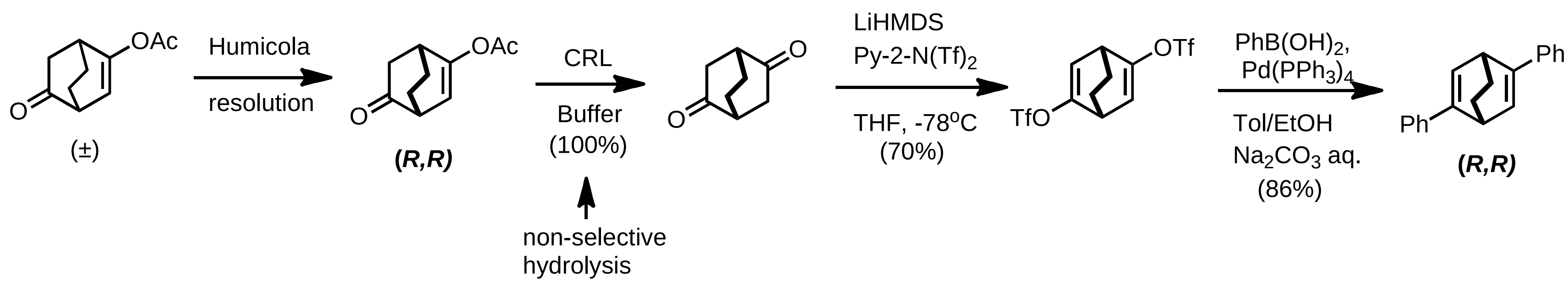


Diene Ligand Synthesis

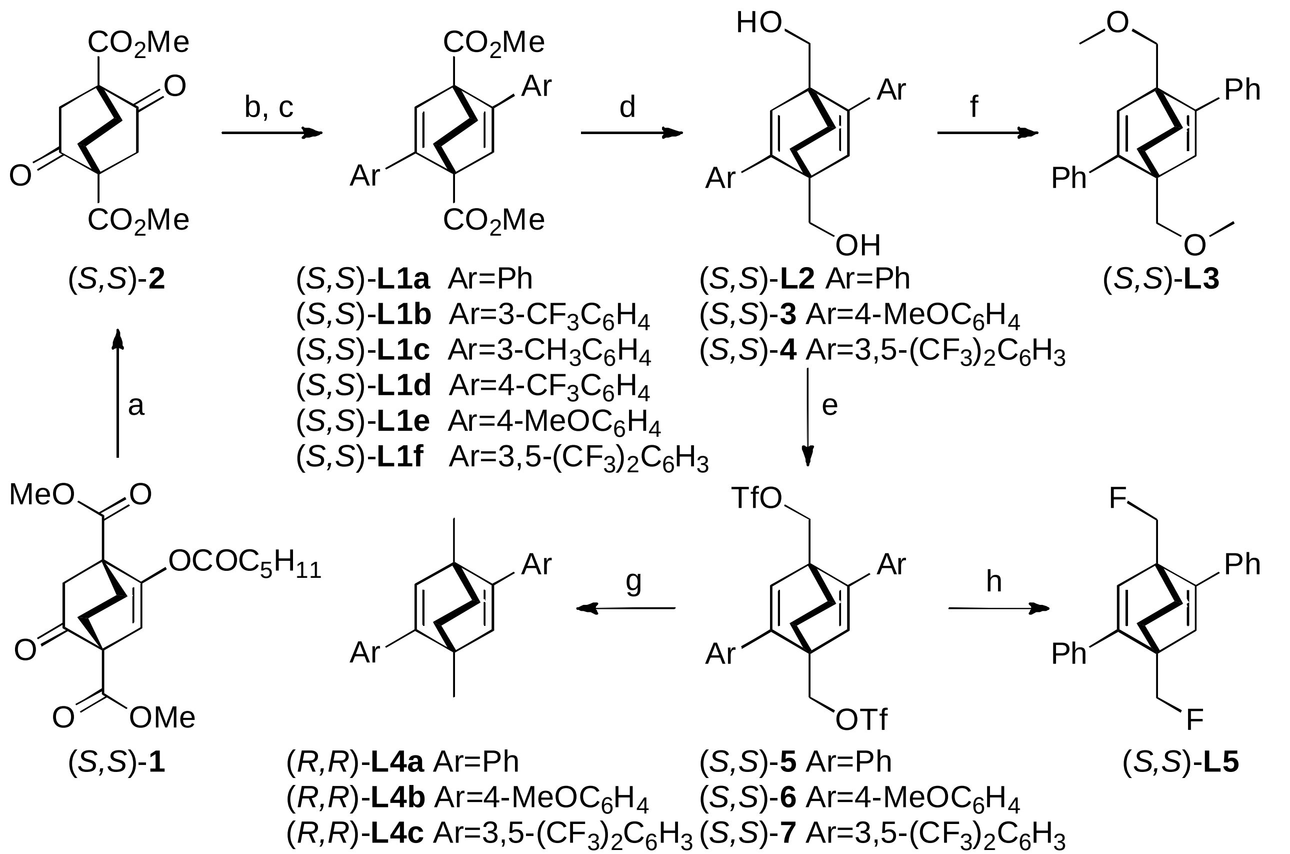


Further Ligand Synthesis





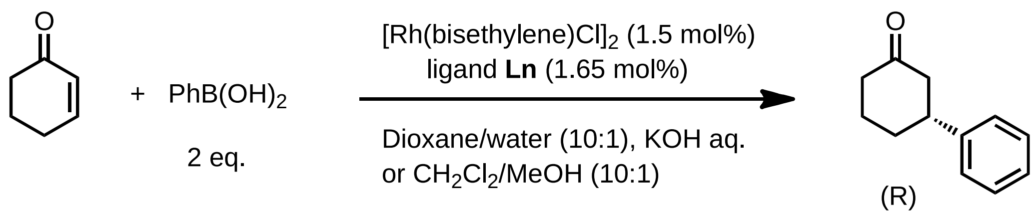
Ligand Synthesis



a) Na_2CO_3, MeOH (99%); b) LHMDS, Tf_2O, THF, -78ºC (70%); c) $ArB(OH)_2$, $Pd(PPh_3)_4$, Tol/EtOH/aq, Na_2CO_3, rt. (95%) d) $LiAlH_4$, THF, (99%); e) Tf_2O, CH_2Cl_2/Pyr, -78ºC – rt (99%); f) NaH, MeI, THF (95%); g) $LiHBEt_3$, THF, rt (99%); h) TBAF, THF, 40ºC (95%).

Luo and Carnell, *Angew. Chem. Int. Ed.,* 2010, *49*, 2750-2754

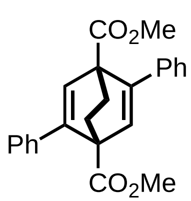
Catalytic Asymmetric Conjugate Addition



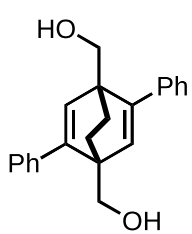
Entry[a]		Ligand		Yield (%)	e.e. (%)
	Ln	1,4-R	2,5- Aryl		
1	**L1a**	CO_2Me	Ph	95	89
2	**L2**	CH_2OH	Ph	94	97
3	**L3**	CH_2OMe	Ph	95	88
4	**L4a**	Me	Ph	96	98
5[b]	**L4a**	Me	Ph	98	99
6	**L5**	CH_2F	Ph	96	98
7[c]	**L6**	H	Ph	97	96

← 1.2 eq. $PhB(OH)_2$ with **L4a**

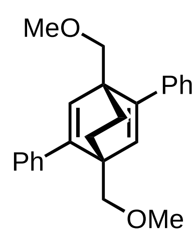
Ligands Ln



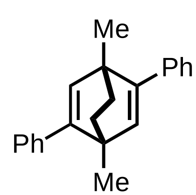
L1a



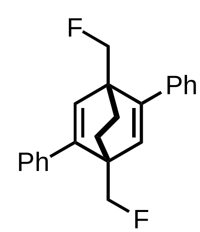
L2



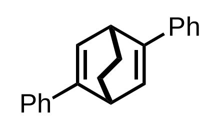
L3



L4a

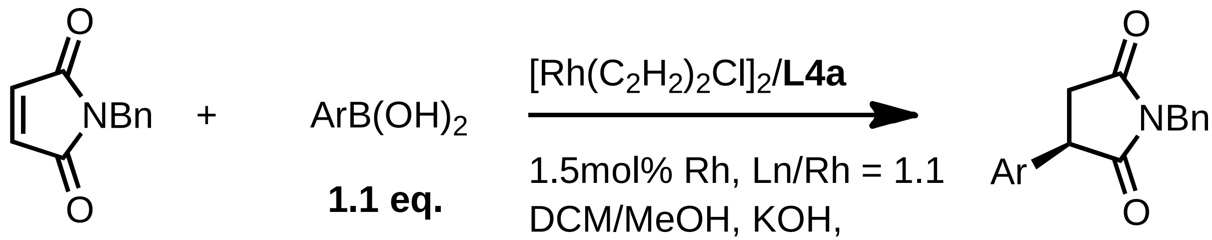


L5

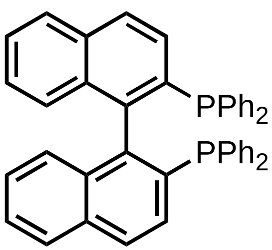


L6

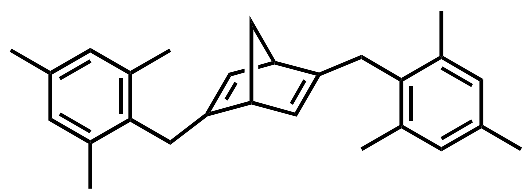
Asymmetric Conjugate Addition to N-benzyl maleimide



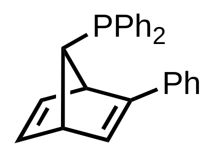
9 examples
92-99% yield
91-99% e.e.



70%, 58% e.e.



88%, 69% e.e.

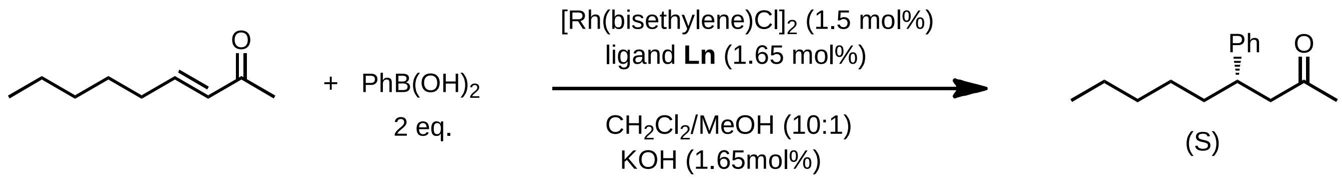


98%, 93% e.e.

all used 3 eq. of $PhB(OH)_2$

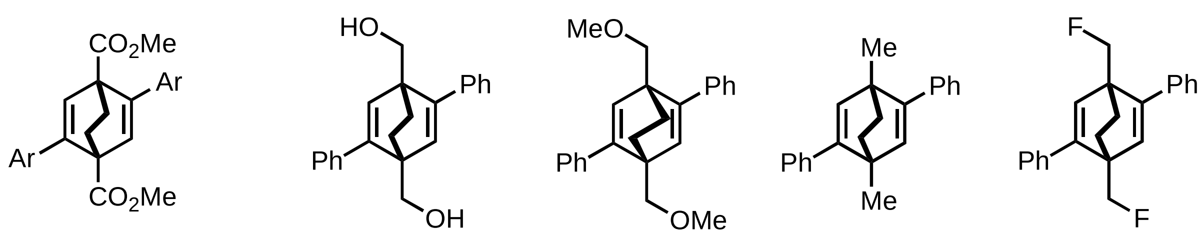
Luo and Carnell, *Angew. Chem. Int. Ed.,* 2010, *49*, 2750-2754

Acyclic substrate ligand optimisation



		Ligand			
Entry	**Ln**	1,4-R	2,5- Aryl	Yield (%)	e.e. (%)
1	**L4a**	Me	Ph	99	52
2	**L2**	CH_2OH	Ph	98	57
3	**L3**	CH_2OMe	Ph	98	71
4	**L5**	CH_2F	Ph	95	67
5	**L1a**	CO_2Me	Ph	95	75
6	**L1b**	CO_2Me	3-$CF_3C_6H_4$	93	83
7	**L1c**	CO_2Me	3-$CH_3C_6H_4$	98	73
8	**L1d**	CO_2Me	4-$CF_3C_6H_4$	95	82
9	**L1e**	CO_2Me	4-$MeOC_6H_4$	99	74
10	**L1f**	CO_2Me	3,5- $(CF_3)_2C_6H_3$	43	89

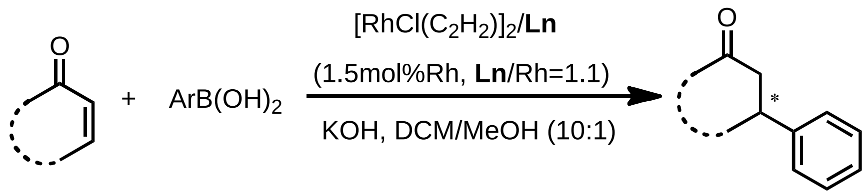
← 1.2 eq. PhB(OH)$_2$ with **L4a** (entry 1)

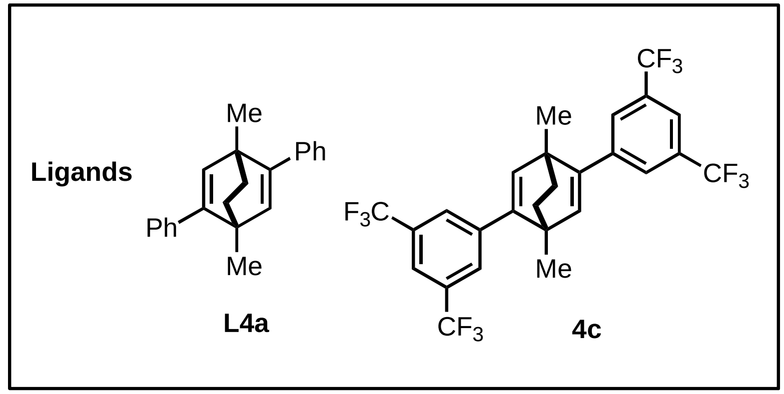


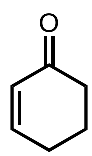
L1a Ar=Ph
L1b Ar=3-$CF_3C_6H_4$
L1c Ar=3-$CH_3C_6H_4$
L1d Ar=4-$CF_3C_6H_4$
L1e Ar=4-$MeOC_6H_4$
L1f Ar=3,5-$(CF_3)_2C_6H_3$

L2 **L3** **L4a** **L5**

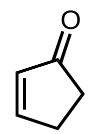
Tuning electron density of diene ligand for acyclic enones



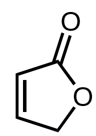




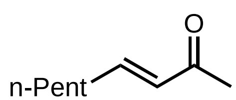
4a 98%, 99% e.e.
4c 92%, 98% e.e.



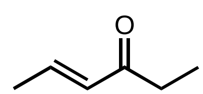
4a 100%, 99% e.e.
4c 80%, 96% e.e.



4a 95%, 98% e.e.
4c 76%, 92% e.e.

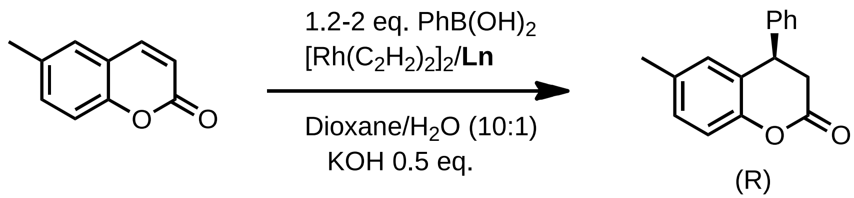


4a 99%, **52% e.e.**
4c 99%, **97% e.e.**



4a 99%, **67% e.e.**
4c 99%, **95% e.e.**

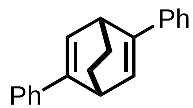
Asymmetric conjugate addition to 6-methylcoumarin



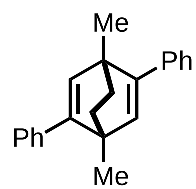
Entry	Ligand	temp. (^{o}C)	Time (hr)	conv. (%)	Yield (%)	e.e. (%)
1	**L6**	30	24	<5	**--**	n.d.
2	**L6**	50	6	40	**32**	98
3	**L4a**	30	6	55	**50**	98
4	**L4a**	50	6	85	**72**	98
5	**L4b**	30	3	100	**95**	98

← (entry 5) 1.2 eq. $PhB(OH)_2$
25mol% KOH

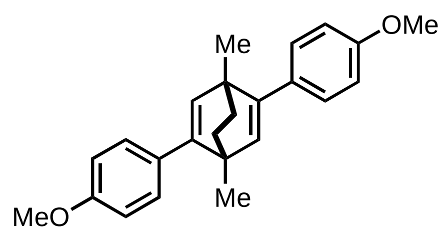
Ligands



L6 Hayashi Ligand

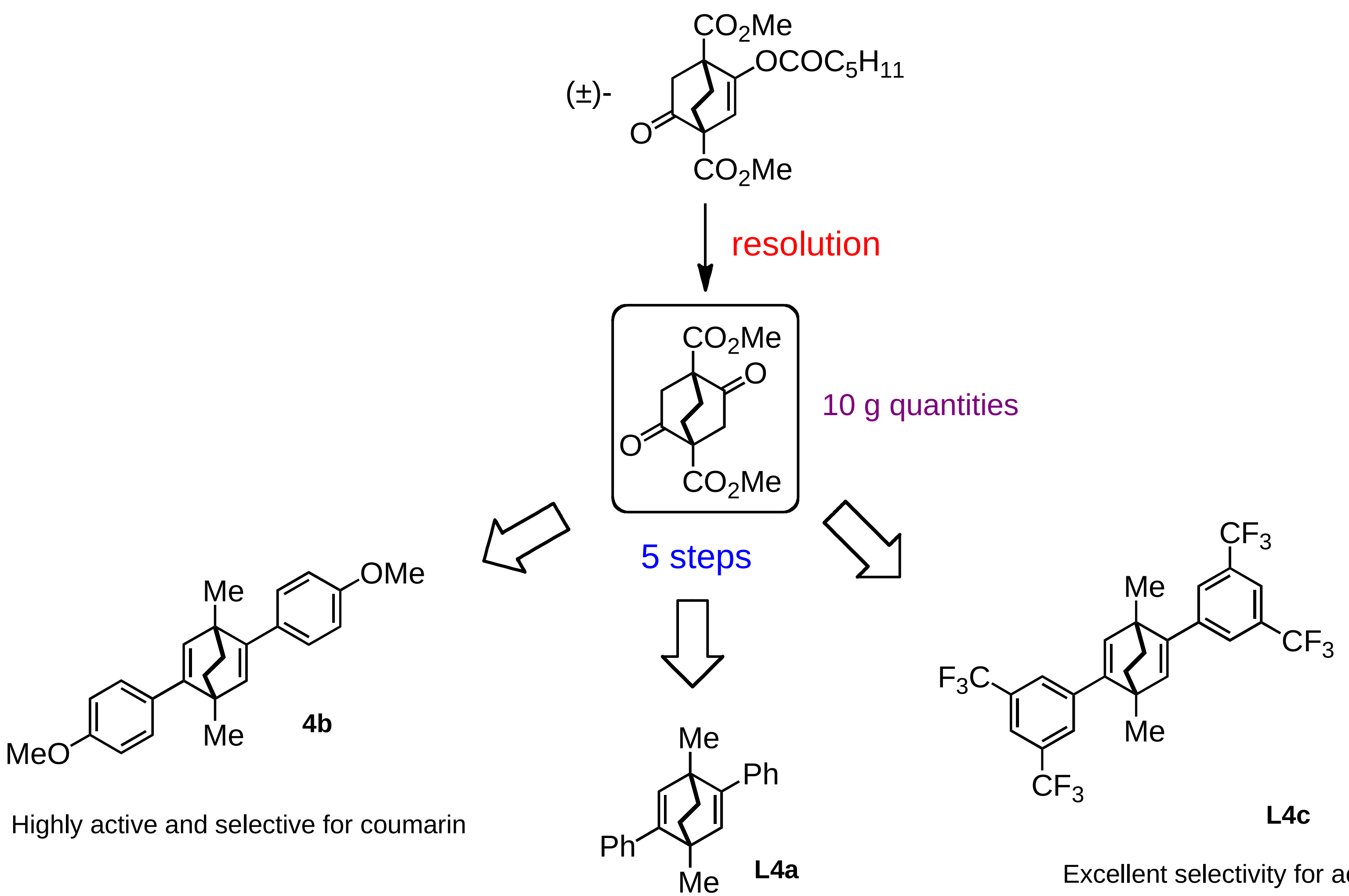


L4a



4b

Chemoenzymatic Synthesis of New Bicyclic Ligands



Highly active and selective for coumarin

Excellent selectivity for acyclic enones

Faster than Hayashi's
and others (1hr at rt vs 3hrs at 30^{o}C)
minimal deboronation - only 1.2 eq. $PhB(OH)_2$ required *cf*
2 eq. or more with Hayashi diene

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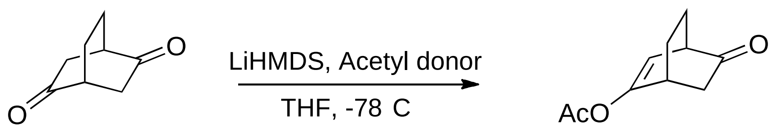
John Whittall

PhosphonicS ltd

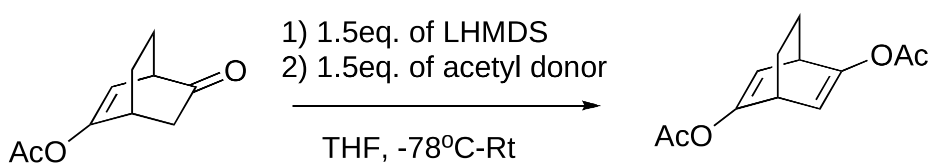
Funding: Pfizer, Dorothy Hodgkin Postgraduate Award (Y.L.), EPSRC (DTAs), BBSRC (PDRA to G.A.).

Synthesis of Enol Ester



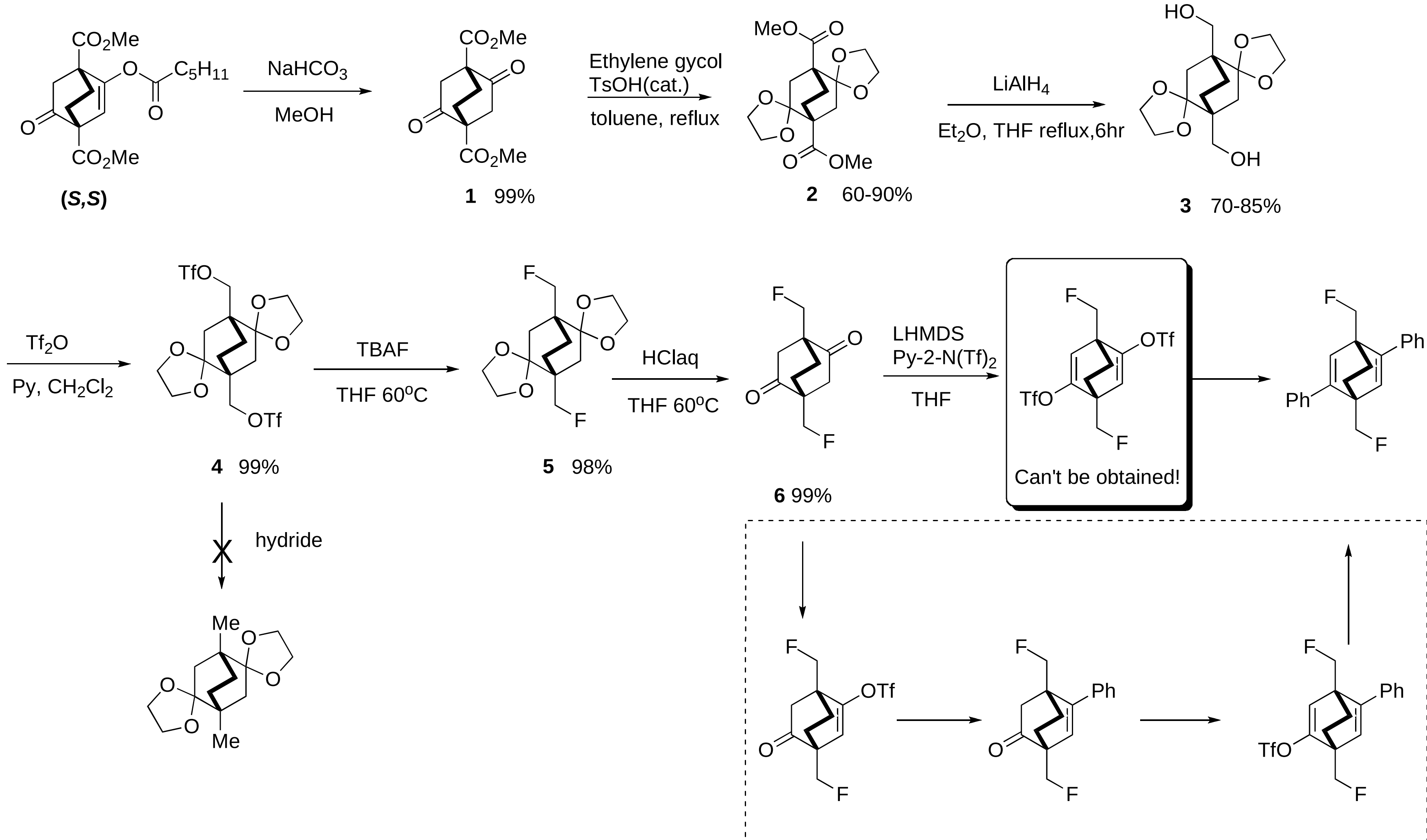
Conditions screening for synthesis of enolacetate

Base	Quantity	Time	Acetyl donor	yield	Temperature
LHMDS	3eq.	12hr	Acetyl chloride	39.7	-78
LHMDS	3eq.	48hr	Acetyl chloride	42.9	-78
LDA	3eq.	12hr	Acetyl chloride	7.9	-78
LHMDS	3eq.	48hr	Acetyl chloride	47.6	-78 - rt
LDA	3eq.	48hr	Acetic anhydride	12.7	-78 - rt
LHMDS	3eq.	48hr	Acetic anhydride	47.6	-78 - rt
LHMDS	**1.5eq.**	**48hr**	**Acetyl chloride**	**90**	**-78 - rt**
LHMDS	1.5eq.	48hr	Acetic anhydride	88	-78 - rt



low yield

Diene Ligand Synthesis



…..too long!!

X-ray Structure of Rh Complex with ligand L4a