Enabling Technology

Asymmetric Synthesis of ENT-03 Enabled by Hydrogenation High-Throughput Experimentation

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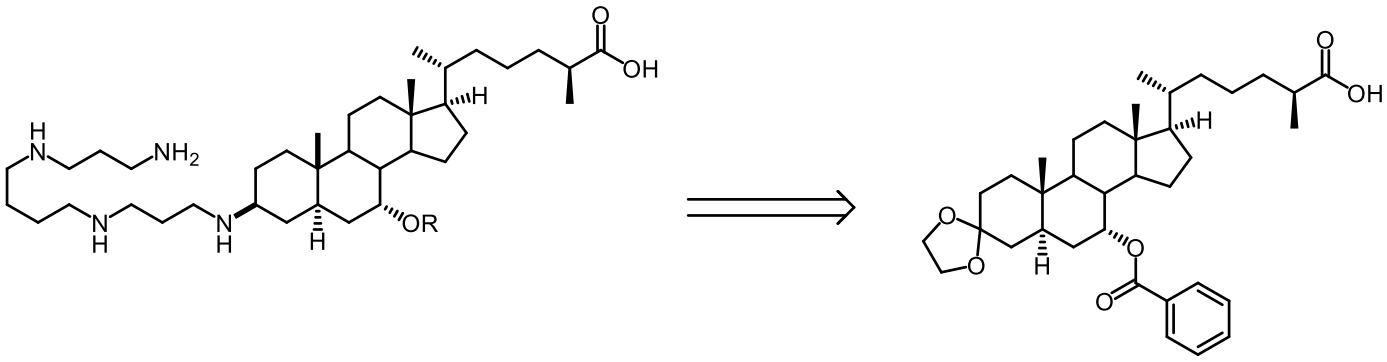
Introduction and Purpose

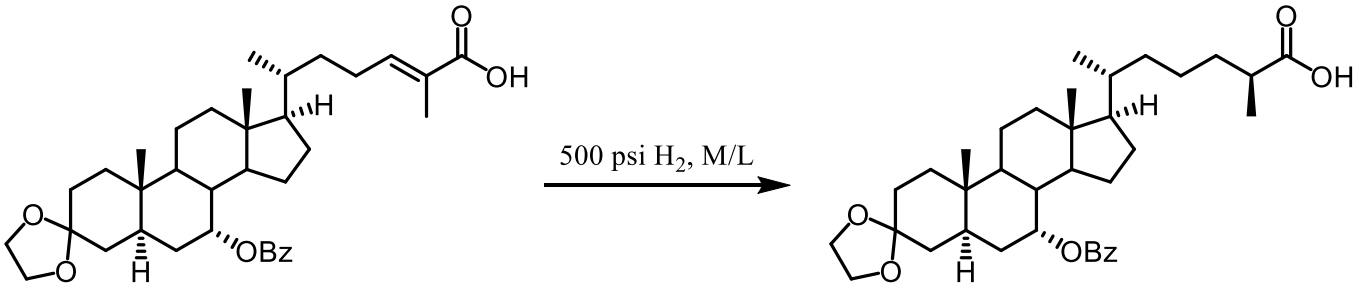


(25S)-ENT-03 (*S*)-10

ENT-03 was predicted to be the mammalian equivalent of trodusquemine, based on knowledge of the bile acids produced in mammals, such as 7-HOCA. The individual C-25 isomers of ENT-03 were prepared and both detected in mouse brain. Trodusquemine and ENT-03 have both demonstrated dramatic effects in obesity and insulin resistance. (25S)-ENT-03 was selected for development for the treatment of diabetes and obesity.

To establish a selective synthesis of (25S)-ENT-03, asymmetric hydrogenation of enoic acid 9 was explored as a key step to set the C25 stereocenter. After a screen of >200 reaction conditions was conducted, an in-situ formed ruthenium-Mandyphos complex was found effective in converting 9 to (S)-10 with 100% conversion and up to 95% de. Both enantiomers of the Mandyphos ligand are available, and similar conditions with the antipodal ligand delivered (R)-10 in 100% conversion and 94.4% de.

Initial Screening



9 **(*S*)-10**

Initial screen design: 12 catalysts, 4 solvents, with and without Et_3N

Conversion:

solvent	MeOH		EtOH		PhMe		DCE	
additive		NEt_3		NEt_3		NEt_3		NEt_3
Cat 1								
Cat 2								
Cat 3								
Cat 4								
Cat 5								
Cat 6								
Cat 7								
Cat 8								
Cat 9								
Cat 10								
Cat 11								
Cat 12								

Stereoselectivity:

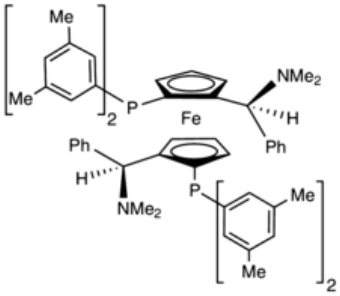
solvent	MeOH		EtOH		PhMe		DCE	
additive		NEt_3		NEt_3		NEt_3		NEt_3
Cat 1								
Cat 2								
Cat 3								
Cat 4								
Cat 5								
Cat 6								
Cat 7								
Cat 8								
Cat 9								
Cat 10								
Cat 11								
Cat 12								

Cat 2: *(p-cymene)$_2$RuCl$_2$/ SL-M009-1;*

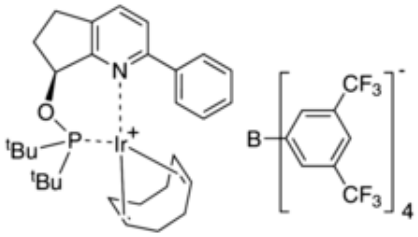
Cat 9: *Ir(cod)(Pfaltz)BArF;*

Cat 10: *Ir(cod)$_2$BArF / (S,S-DTBBnSIPHOX)*

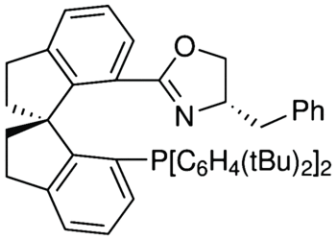
Ru/MandyPhos, Ir/Pfaltz and Ir/DTBBnSIPHOX were found to be effective in the transformation, and base additive tended to improve conversion. These two themes were further explored in subsequent screens.



SL-M009-1



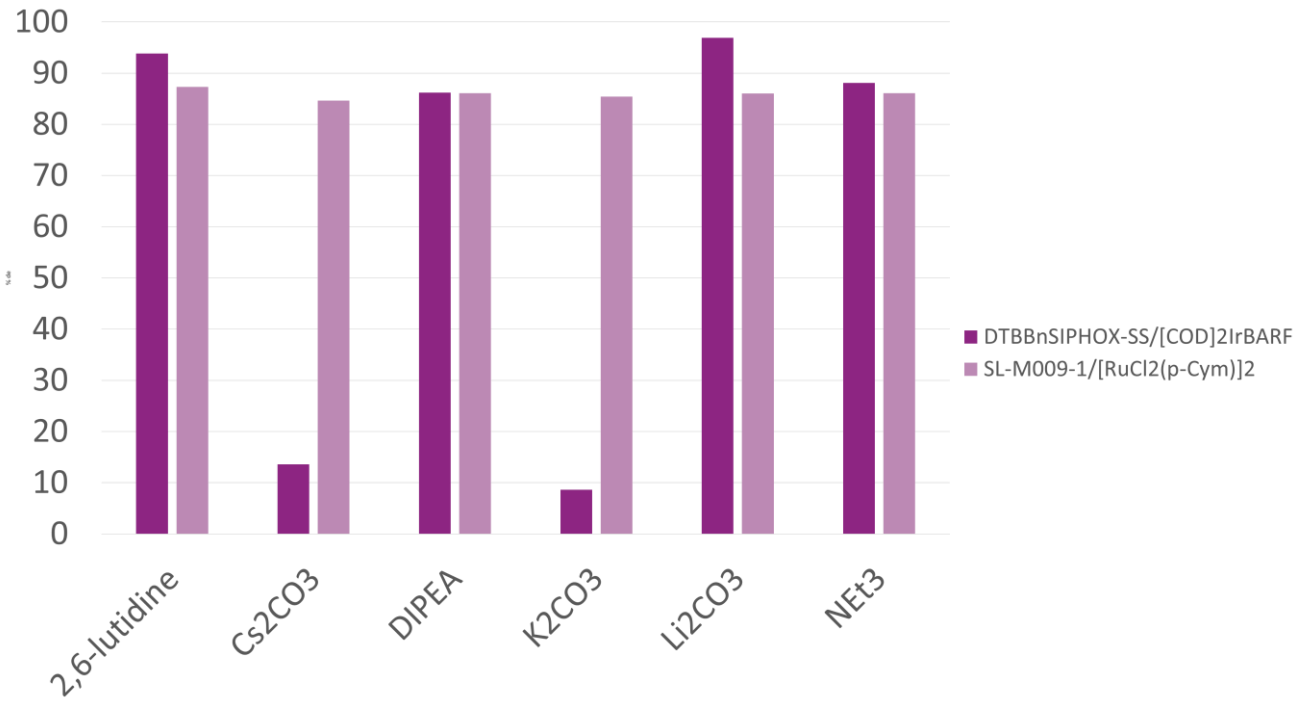
Ir(cod)(Pfaltz)BArF



(*S,S*-DTBBnSIPHOX)

Base comparison

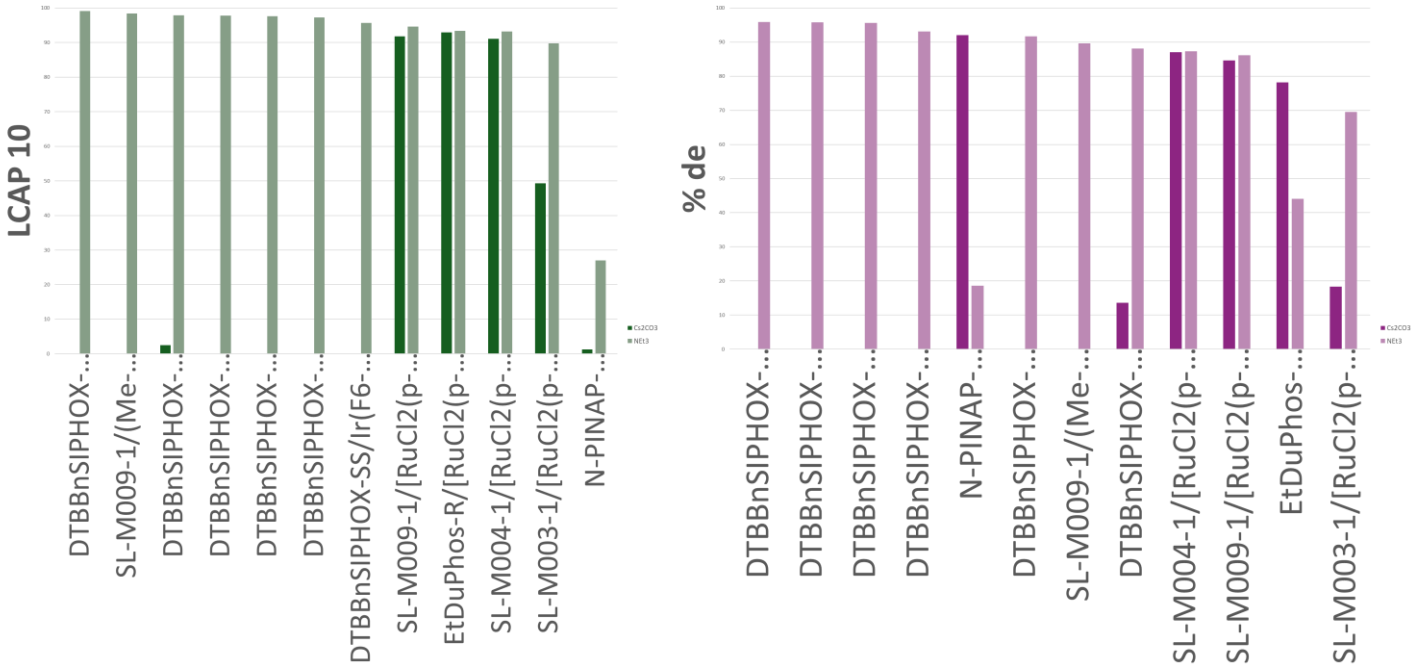
2,6-lutidine, iPr_2NEt, K_2CO_3 and Li_2CO_3 were tested in addition to Et_3N and Cs_2CO_3.



The identity of the base critically impacted the conversion and selectivity of the Ir system, but the Ru system tolerated a wide variety of bases without significant change in % de or conversion.

Follow-up screen: catalyst systems

Top 12 out of 48 catalyst systems for stereoselectivity:

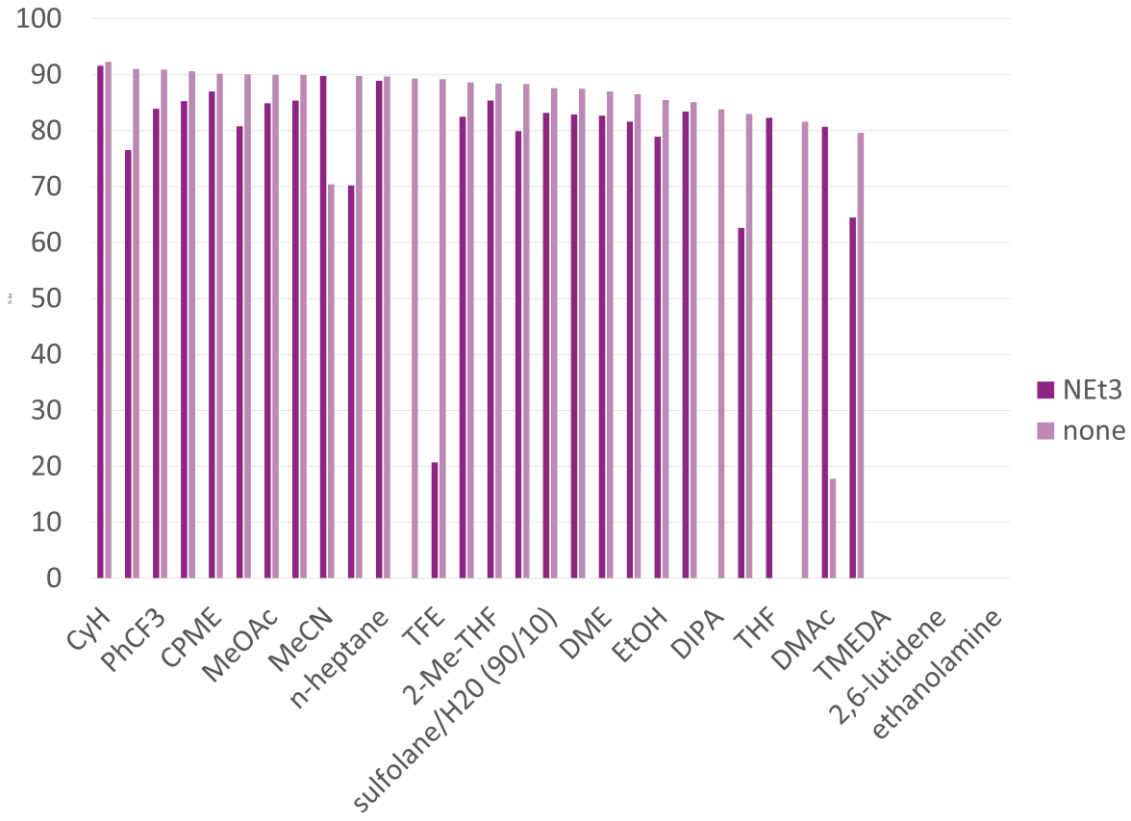


Different metal precursors and ligands were screened, though the best ligands were the same as in the initial screen. 98% LCAP and 90% de were observed with Ru(methallyl)$_2$(cod) / M009-1 whereas 98% LCAP and 96% ee were observed with Ir(cod)(acac) and (*S,S*)-DTBBnSIPHOX.

Though conversion and selectivity were better for the Ir system, the relative cost of Ir versus Ru and relative availability of each respective optimal ligand drove a decision to focus on the Ru/MandyPhos system in further screens.

Solvent deep dive

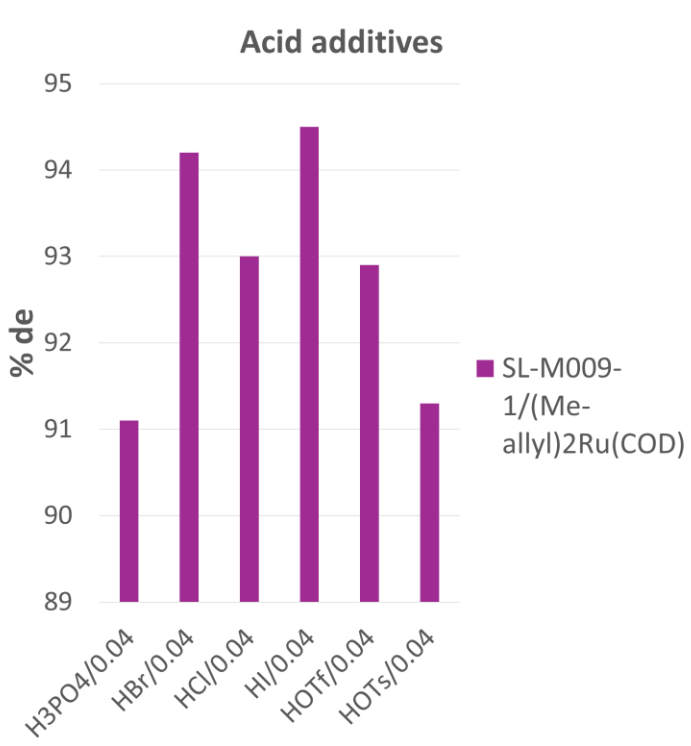
>30 solvents were investigated at 2% loading of Ru/M-009-1

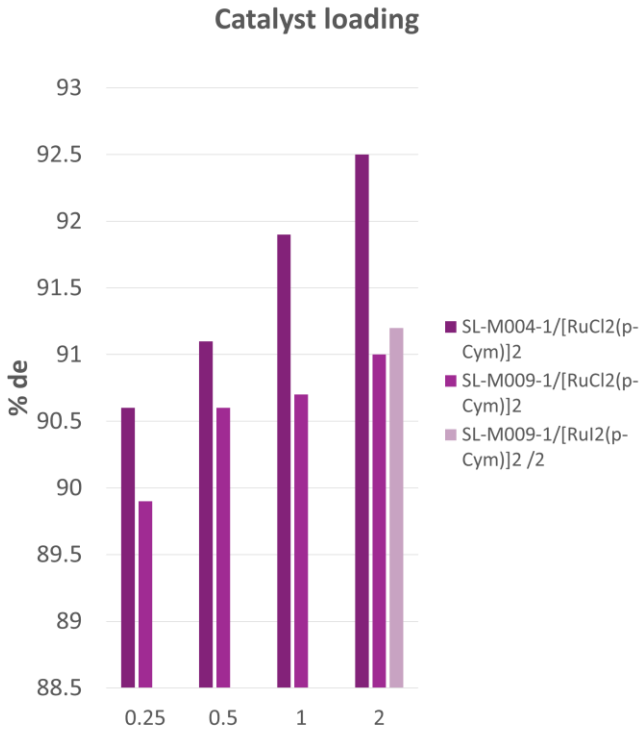


The best selectivity was obtained in cyclohexane, followed by DCE. Suitable crude product formation (>90%) was achieved with most solvents, with DCE at >95% and cyclohexane at 92.5%. Generally, addition of base had a negative effect on selectivity.

Acid additives and catalyst loading

A screen of acid additives and reduced catalyst loading in DCE was performed. Selected results are shown below.



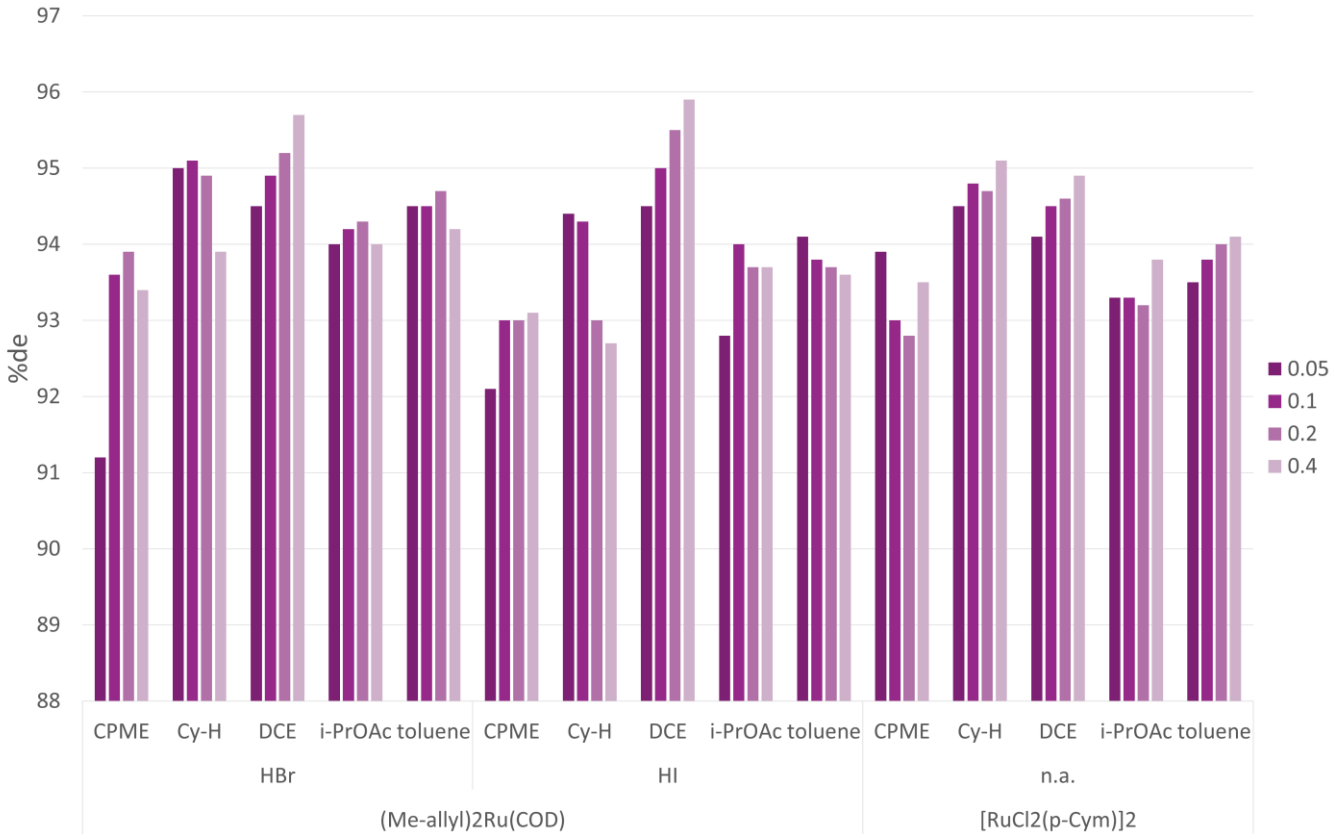


Exogenous HBr or HI was found to improve selectivity of the Ru/M-009-1 system.

Catalyst loading as low as 0.25% was investigated with M-009 and M-004. Decreased selectivity was observed at lower loading, with M004-1 showing slightly better selectivity and conversion.

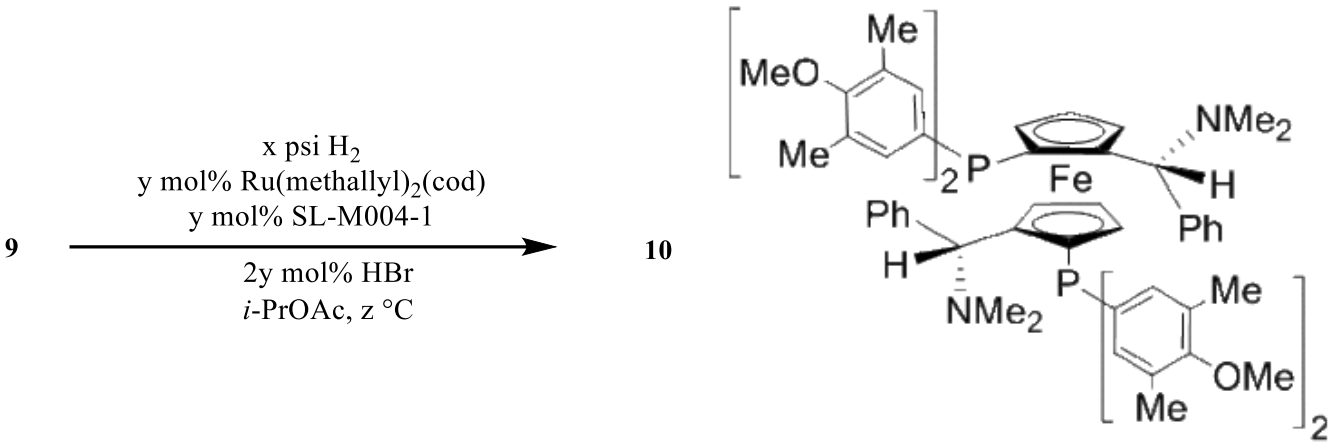
Further screening

Further reduction in catalyst loading of Ru/M-004-1 was investigated along with solvent and acid additive.



Conversion was still acceptable even at 0.05% catalyst loading. However, selectivity did suffer at lower catalyst loading. Overall, the best results were obtained in DCE; however, more process-friendly solvents also gave >90% conversion and >93% de. Finally, *i*-PrOAc and HBr were chosen for final optimization as more scalable options.

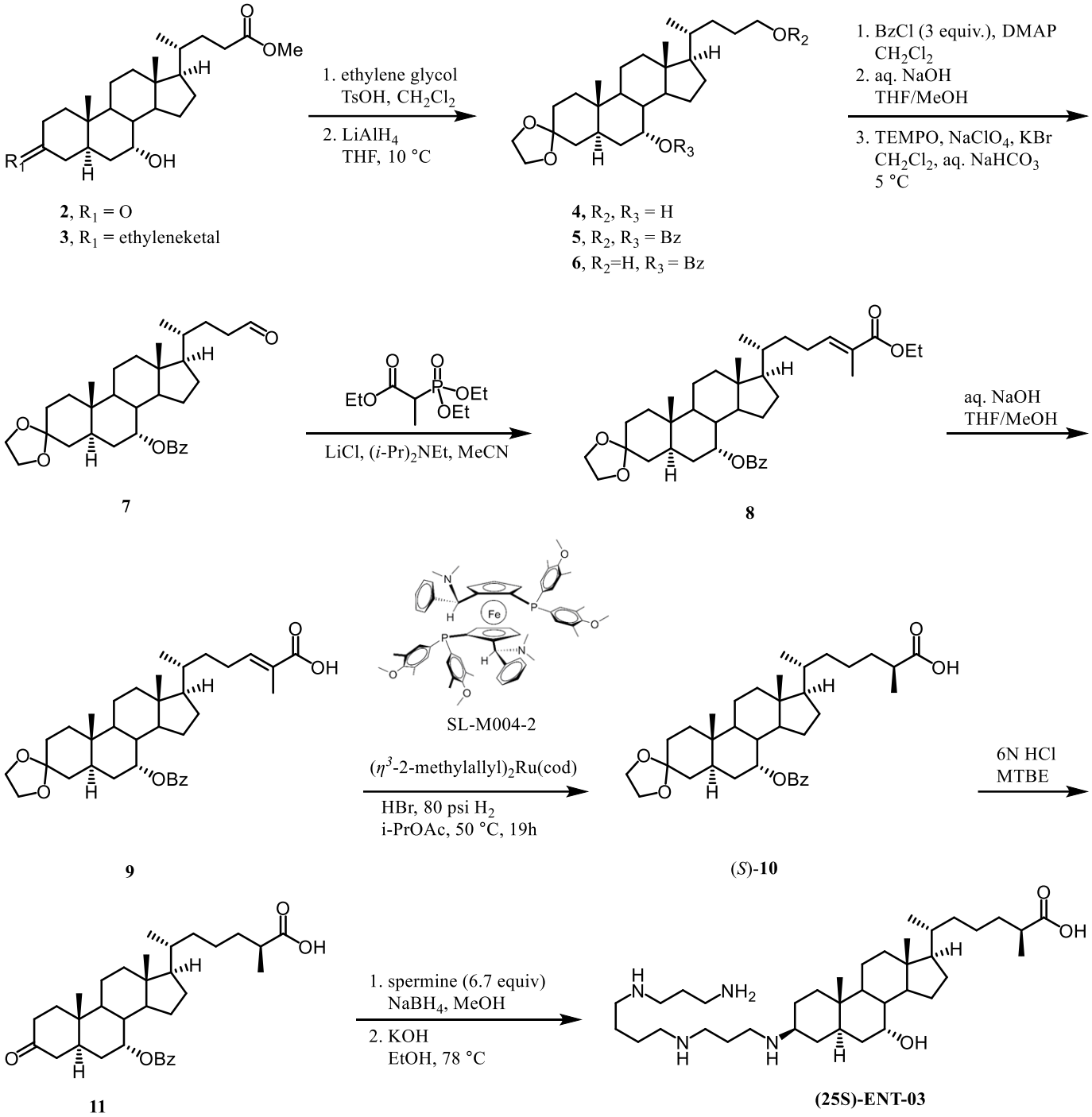
Temperature and Pressure Effect



SL-M004-1

T (°C)	P (psi)	Conc. (mM)	Mol % Ru	AP 9	AP 10	% de 10
50	520	1.2	2.0	0	91.2	94.4
35	520	1.2	2.0	0	90.9	94.7
70	450	1.2	2.0	0	92.0	92.6
50	450	1.2	2.0	0	90.1	93.9
50	115	1.2	2.0	0	90.7	94.4
50	500	0.01	0.4	0	90.5	94.0

ENT-03 Synthesis Including Key Asymmetric Hydrogenation



2, R_1 = O
3, R_1 = ethyleneketal

4, R_2, R_3 = H
5, R_2, R_3 = Bz
6, R_2=H, R_3 = Bz

7

8

9

(*S*)-**10**

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(25S)-ENT-03

We would like to thank the team at Enterin Inc. for the opportunity to work on this project and for permission to share this work.

The Porton J-STAR team is excited to share our significant contributions to the development of ENT-03, a promising mammalian analog of trodusquemine for treating diabetes and obesity. The asymmetric hydrogenation development was led by Dr. Thorsten Rosner, and published along with several other industry experts including Dr. Hui Chen, Dr. Qi Gao, Dr. Andrew Thompson, and Ian Crouch. A critical breakthrough in this synthesis involved the asymmetric hydrogenation of an enoic acid, achieving the desired enantiomer with up to 95% enantiomeric excess using only 0.05 mol% of a commercially available ruthenium-Mandyphos catalyst.

This success resulted from a meticulous series of high-throughput screening experiments, where we evaluated a variety of catalysts, solvents, and additives across more than 200 reaction conditions. Our rigorous evaluation and optimization process pinpointed (Me-allyl)2Ru(COD)/SL-M004-1 as the superior catalyst. We also selected the eco-friendly solvent i-PrOAc, which provided optimal scalability and efficiency in the reaction.

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25 Years Exp. in the Pharmaceutical Industry;
Drug Substance Enabling Technology Leader

The Porton J-STAR team is dedicated to bringing this level of scientific rigor to all our clients. Contact us at business@portonusa.com to see how we can help your molecule advance in development.

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