

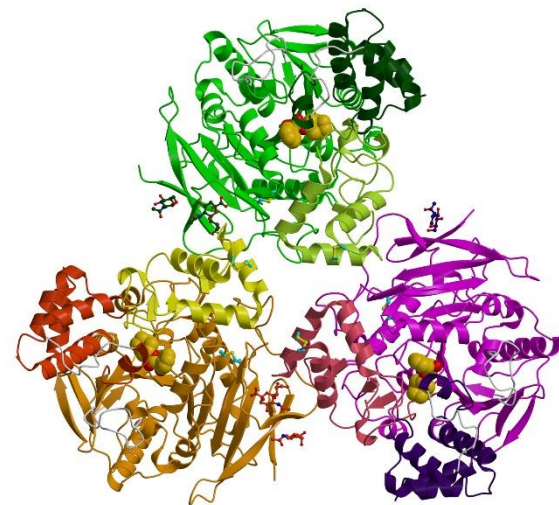
Biocatalysis in Organic Synthesis

Literature Review

Christian Kuper

Dphil Candidate, EAA group

12.11.2015





Outline



- **Introduction**
- **Enzymes for Carboxylic Acid Esters**
- **Transamination**
- **Glycosylation**
- **Enzymatic Halogenation**



Introduction



- **Biotransformation**
 - **Process that describes a reaction or a set of simultaneous reactions in which a pre-formed precursor molecule is converted using enzymes and/or whole cells, or combinations thereof, either free or immobilised.**

- **Considerations to take before using biocatalysis**
 - **Isolated enzymes or whole cells?**
 - **Cofactors? Cofactor recycling?**
 - **Organic solvents?**

Whittall, J.; Sutton, P. W. *Practical Methods for Biocatalysis and Biotransformations*, WILEY-VCH, **2012**.

Gröger, H.; Asano, Y. In *Enzyme Catalysis in Organic Synthesis*; Wiley-VCH Verlag GmbH & Co. KGaA: 2012, p 1.

Davis, B. G.; Boyer, V. *Natural Product Reports* **2001**, 18, 618



Introduction – Advantages and Limitations



- **Advantages**
 - **Highly selective operation in complex mixture**
 - **Stereo- and regiospecificity**
 - **Absence of side reactions → simpler separation, higher yields**
 - **Savings in energy and waste cost**

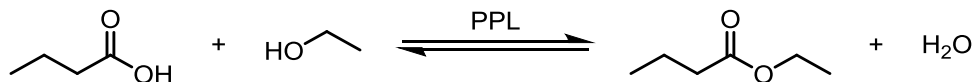
- **Limitations**
 - **Covering diversity of chemical reactions desired**
 - **Enzymes might not be readily available**
 - **Reaction conditions**
 - **pH, T, solvent, ...**



Carboxylic Acid Esters



- **Synthesis of esters is part of anabolic pathway**
- **Hydrolysis is part of catabolism (→digestion)**
 - **Enzymes responsible are esterases and lipases (subfamily of esterases)**
- **Enzymes don't influence the equilibrium of the reaction**
 - **Esterases should also catalyse formation of esters (shown in 1900)**



- **Enzymes to hydrolyse amides can often also hydrolyse esters, not the other way around**

Paravidino, M.; Böhm, P.; Gröger, H.; Hanefeld, U. In *Enzyme Catalysis in Organic Synthesis*; Wiley-VCH Verlag GmbH & Co. KGaA: 2012, p 249.

Kastle, J. H.; Loevenhart, A. S. *Amer. Chem. J.* **1900**, *24*, 491.

Chenevert, R.; Pelchat, N.; Jacques, F. *Current Organic Chemistry* **2006**, *10*, 1067.

Faber, K.; Riva, S. *Synthesis* **1992**, *1992*, 895.

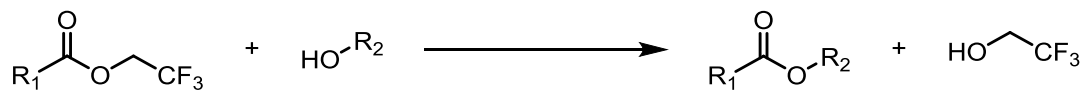
Hanefeld, U. *Organic & Biomolecular Chemistry* **2003**, *1*, 2405.



Carboxylic Acid Esters – water as a problem



- **Problem: equilibrium in organic solvents**
- **Control of water activity possible, but most enzymes show less activity at low water activities**
- **Many lipases have a hydrophobic “lid”, use of biphasic solvent systems improve reaction rate**
- **Esters can be used instead of carboxylic acids**
 - **transesterification rather than ester synthesis**



- **Trifluoroethanol seems not to be able to access active site**

Gedey, S.; Liljeblad, A.; Fülöp, F.; Kanerva, L. T. *Tetrahedron: Asymmetry* **1999**, *10*, 2573.

Liljeblad, A.; Kanerva, L. T. *Tetrahedron* **2006**, *62*, 5831.

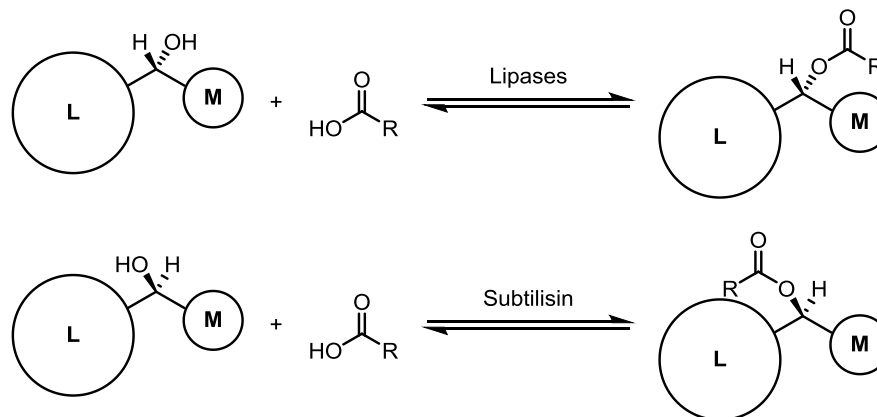
Paravidino, M.; Böhm, P.; Gröger, H.; Hanefeld, U. In *Enzyme Catalysis in Organic Synthesis*; Wiley-VCH Verlag GmbH & Co. KGaA: 2012, p 249.



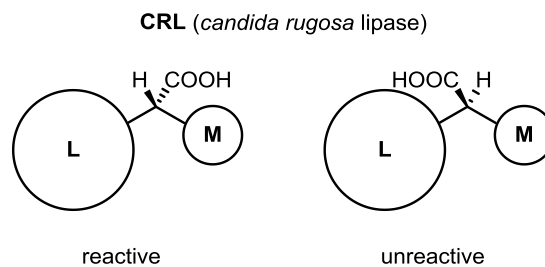
Carboxylic Acid Esters – Stereochemistry



- The rule of Kazlauskas describes the enantioselectivity of lipases for secondary alcohols



- A similar rule goes for enzyme CRL for acid substrates



Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *The Journal of Organic Chemistry* **1991**, *56*, 2656.

Paravidino, M.; Böhm, P.; Gröger, H.; Hanefeld, U. In *Enzyme Catalysis in Organic Synthesis*; Wiley-VCH Verlag GmbH & Co. KGaA: 2012, p 249.

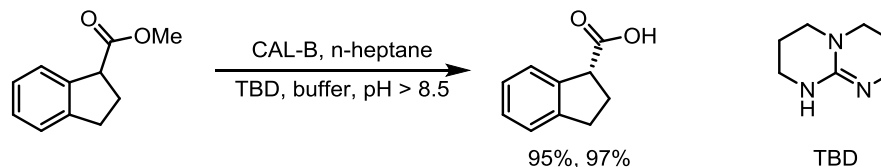
Savile, C. K.; Kazlauskas, R. J. *Journal of the American Chemical Society* **2005**, *127*, 12228.

Sharma, A.; Chattopadhyay, S. *Journal of Molecular Catalysis B: Enzymatic* **2000**, *10*, 531.

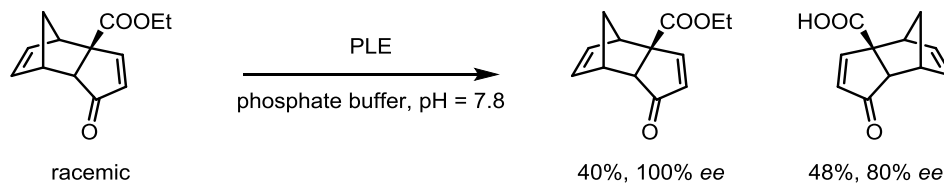
Tawaki, S.; Klibanov, A. M. *Journal of the American Chemical Society* **1992**, *114*, 1882.



Carboxylic Acid Esters



- Reaction done on up to 40 kg scale



- A number of different bicyclic structures can be used

Chakor, N. S.; Dallavalle, S.; Scaglioni, L.; Merlini, L. *European Journal of Organic Chemistry* **2010**, 2010, 6217.

Klunder, A. J. H.; Huizinga, W. B.; Sessink, P. J. M.; Zwanenburg, B. *Tetrahedron Letters* **1987**, 28, 357.

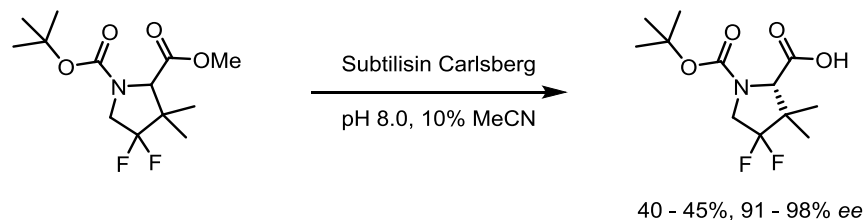
Klunder, A. J. H.; van Gastel, F. J. C.; Zwanenburg, B. *Tetrahedron Letters* **1988**, 29, 2697.



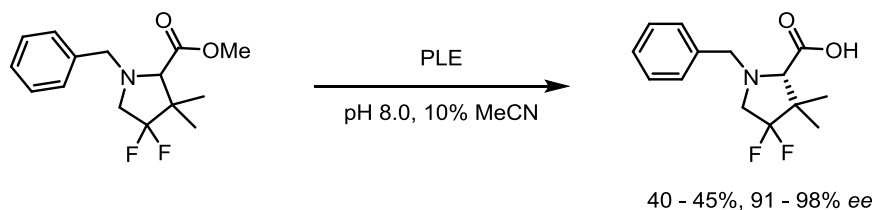
Carboxylic Acid Esters



- Enzyme use in the synthesis of HIV protease inhibitors
- Enzyme screening on substrate



- New enzyme screening on different substrate

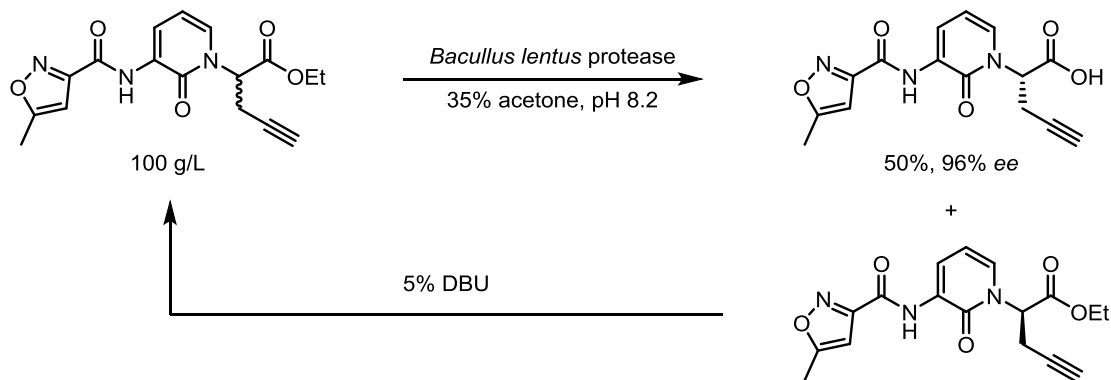




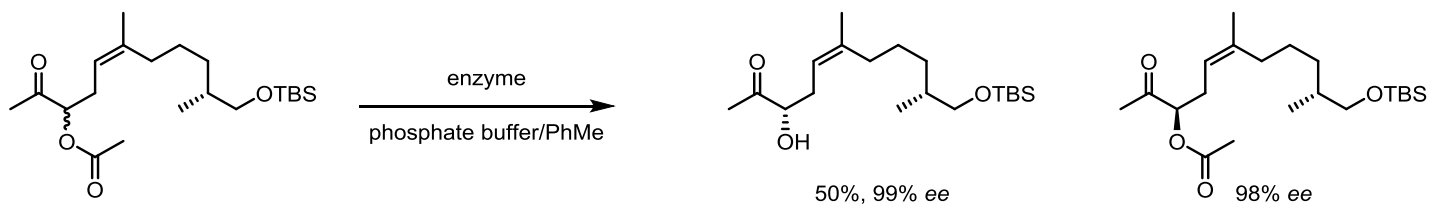
Carboxylic Acid Esters



- Enzymatic preparation of thiovirus protease inhibitor intermediates



- Enzymatic synthesis of a key building block for epothilones



Junhua; Zhao, L.; Ran, N. *Organic Process Research & Development* **2007**, *11*, 259.

Martinez, C. A.; Yazbeck, D. R.; Tao, J. *Tetrahedron* **2004**, *60*, 759.

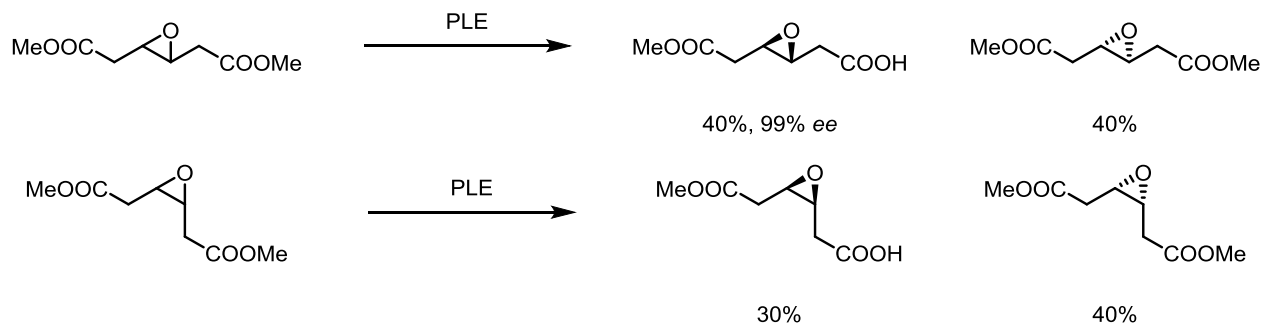
Scheid, G.; Ruijter, E.; Konarzycka-Bessler, M.; Bornscheuer, U. T.; Wessjohann, L. A. *Tetrahedron: Asymmetry* **2004**, *15*, 2861.



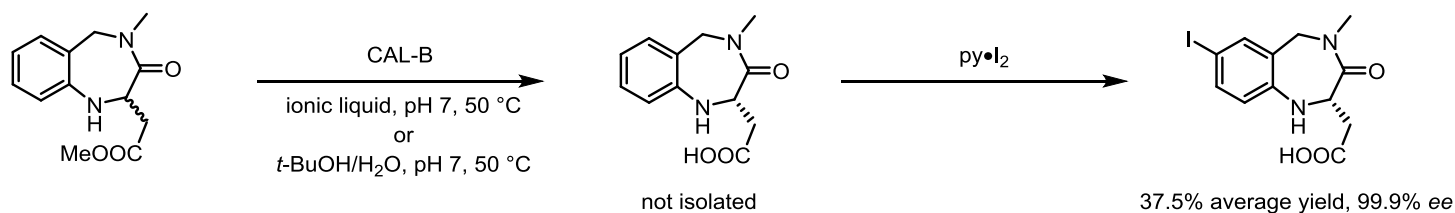
Carboxylic Acid Esters



- Epoxy esters



- Synthesis of key intermediate towards Lotrafiban



Mohr, P.; Rösslein, L.; Tamm, C. *Helvetica Chimica Acta* **1987**, *70*, 142.

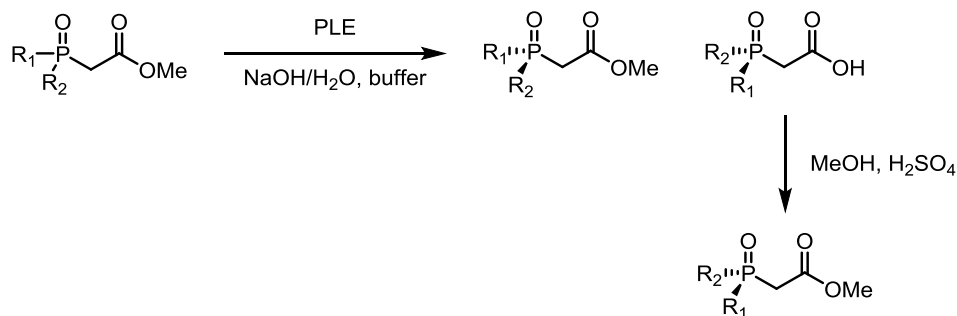
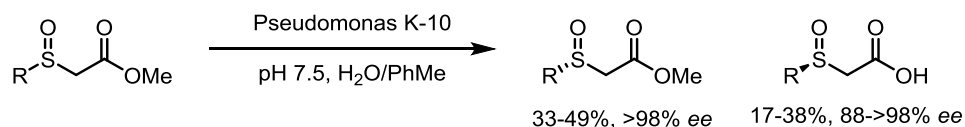
Roberts, N. J.; Seago, A.; Carey, J. S.; Freer, R.; Preston, C.; Lye, G. J. *Green Chemistry* **2004**, *6*, 475.



Carboxylic Acid Esters



- Heteroatoms can also be the chiral centre and still be substrates



Substrate	R ¹	R ²	pH	Time (h)	Recovered ester 1*				Acid 2*			
					Yield (%)	[α] _D (MeOH)	e.e. (%)	Abs. conf.	Yield (%)	[α] _D (MeOH)	e.e. (%)	Abs. conf.
1a	Ph	MeO	7.5	15	40	-16.1	-95	S	44	+9.1	64	R
										+10.8*		
1b	Ph	EtO	7.2	48	46	-11.3	67	S	40	+13.8	71	R
										+11.8*		
1c	Ph	<i>n</i> -PrO	7.2	120	69	-8.7	48	S	30*	+13.0	50	R
										+9.1*		
1d	Ph	<i>i</i> -PrO	7.5	120	50	-5.8	26	S	47*	+3.0*	14	R
1e	Ph	<i>n</i> -BuO	7.4	144	54	-3.0	30	S	33*	+4.0*	40	R
1f	Et	MeO	7.2	1.5	50	+8.5	38	-	34	-10*	42	-
1g	Et	EtO	7.2	48	40	+1.9	-	-	60*	-1.4*	-	-
1h	PhO	EtO	7.1	45	66	-3.0 ^b	20	-	22	+8.7 ^b	52	-
1i	Et ₂ N	MeO	7.5	7	20	-21.7	90	-	58	+5.8	-25	-

*after reesterification
^bin CHCl₃

Burgess, K.; Henderson, I. *Tetrahedron Letters* **1989**, *30*, 3633.

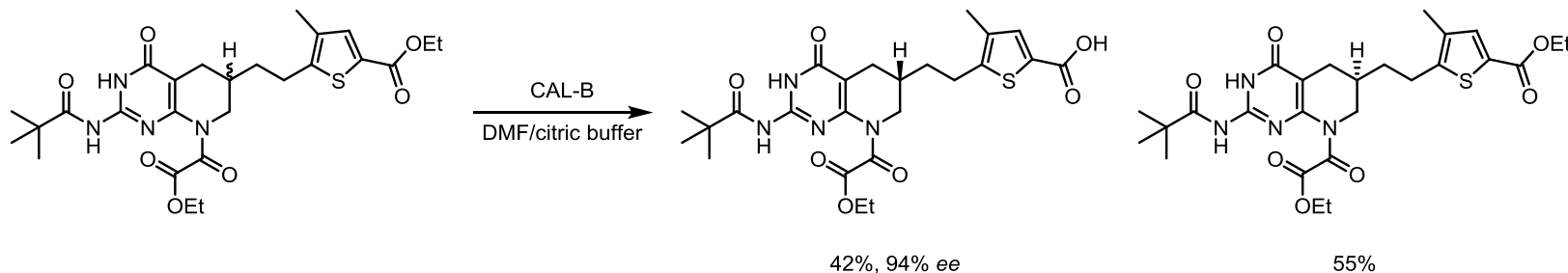
Kielbasiński, P.; Góralczyk, P.; Mikołajczyk, M.; Wieczorek, M. W.; Majzner, W. R. *Tetrahedron: Asymmetry* **1998**, *9*, 2641.



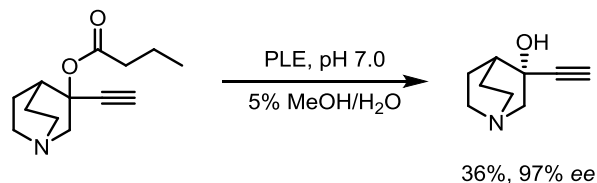
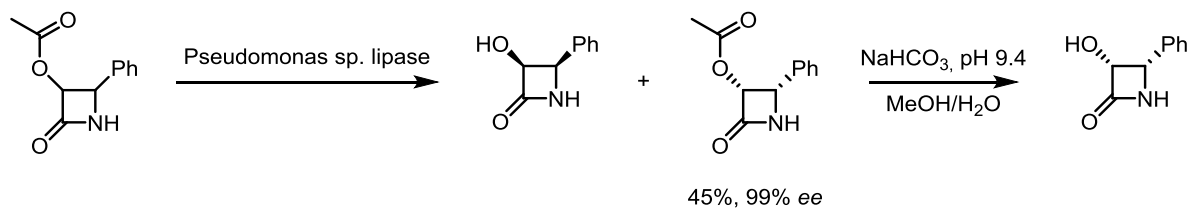
Carboxylic Acid Esters



- Remote chiral centres can be sufficient to distinguish between enantiomers



- Primary, secondary and tertiary alcohols are also viable substrates



Hu, S.; Kelly, S.; Lee, S.; Tao, J.; Flahive, E. *Organic Letters* **2006**, *8*, 1653.

Patel, R. N. *Coordination Chemistry Reviews* **2008**, *252*, 659.

Rn, P.; Banerjee, A.; Ry, K.; Jm, H.; Ws, L.; Ft, C.; Ra, P.; Ft, S. *Biotechnology and Applied Biochemistry* **1994**, *20*, 23.

Coope, J. F.; Main, B. G. *Tetrahedron: Asymmetry* **1995**, *6*, 1393.



Carboxylic Acid Esters



- Even axial chirality can be exploited

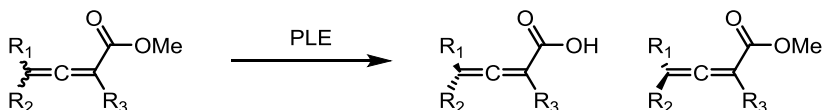
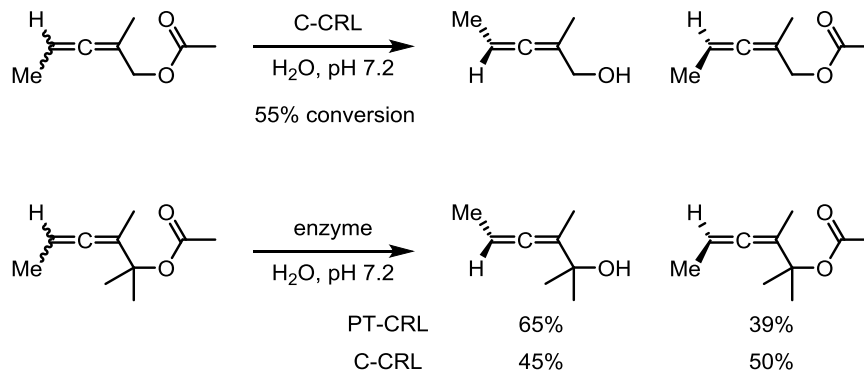


Table 1. PLE-catalysed hydrolyses^a of allenic esters (\pm)-(1a–m).

Substrate	Reaction time	% Hydrolysis	Acid product [% Yield, Abs. config., ^b % e.e. ^c]	Recovered ester [% Yield, Abs. config., ^b % e.e. ^c]
(1a)	1 day	31	(2a) [28, S(+), 38 ¹⁵ (45 ^d)]	(3a) [54, R(-), (9 ^d)]
(1b)	4 days	38	(2b) [20, S(+), 22 ¹⁵ (26 ^d)]	(3b) [44, R(-), (6 ^d)]
(1c)	13 h	50	(2c) [47, S(+), 7 ¹³ (6 ^d)]	(3c) [53, R(-), (5 ^d)]
(1d)	1.5 h	50	(2d) [47, S(+), 6 ¹⁰ (10 ^d)]	(3d) [31, R(-), 8 ¹⁰ (10 ^d)]
(1e)	22 h	50	(2e) [42, S(+), 21 ^c (22 ^d)]	(3e) [36, R(-), 23 ^c (23 ^d)]
(1f)	6 h	18	(2f) [17, (\pm)]	(3f) [71, (\pm)]
(1g)	6 h	10	(2g) [7, R(-), 16 ¹³ (16 ^d)]	(3g) [81, S(+), (1 ⁹)]
(1h)	22 h	50	(2h) [46, R(-), 31 ¹³ (34 ^d)]	(3h) [50, S(+), (38 ^d)]
(1i)	29 h	50	(2i) [33, R(-), 90 ¹³ (90 ^d)]	(3i) [50, S(+), (61 ^d)]
(1j)	4 days	44	(2j) [33, R(-), 88 ¹³ (88 ^d)]	(3j) [64, S(+), 36 ¹³ (39 ^d)]
(1k)	3 days ^f	54	(2k) [52, R(-), 63 ¹³ (78 ^d)]	(3k) [43, S(+), 73 ¹³ (83 ^d)]
(1l)	2 days ^f	35	(2l) [17, R(-), 93 ¹³ (100 ^d)]	(3l) [79, S(+), 22 ¹³ (22 ^d)]
(1m)	3 days	51	(2m) [42, R(-), 32 ^e]	(3m) [42, S(+), 31 ^e]

^a At 25 °C, pH 7. ^b Assigned by the method described in ref. 3, pp. 587–590, when correlations with literature rotations of known compounds were not possible. ^c Calculated on the basis of literature rotations unless designated otherwise. ^d By calculation.^{3,17} All calculated e.e.s are shown in parentheses. ^e By n.m.r.¹² Attempted n.m.r. determinations of e.e.s were unsuccessful for all other allenes in this Table. ^f At 35 °C, pH 7.



Ramaswamy, S.; Hui, R. A. H. F.; Jones, J. B. *Journal of the Chemical Society, Chemical Communications* **1986**, 1545.

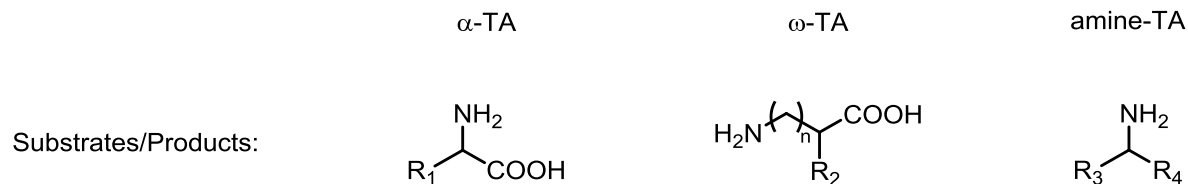
Cipiciani, A.; Bellezza, F. *Journal of Molecular Catalysis B: Enzymatic* **2002**, 17, 261.



Transamination

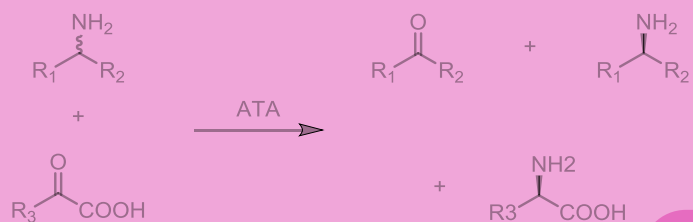


- **Transaminases, TAs (or Aminotransferases, ATs)**
- **Transfer of aminogroup from amino donor (usually amino acid) to substrates (ketones, aldehydes)**
- **Three groups of TAs**

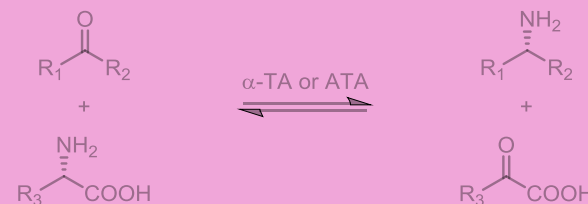




Transamination – general strategies

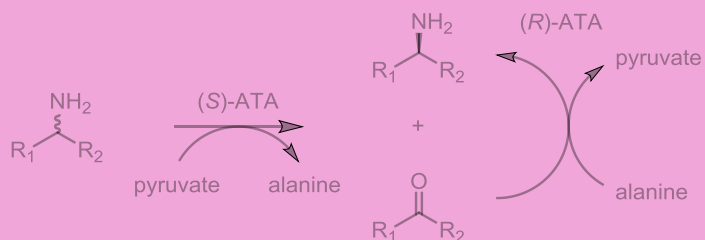


Asymmetric synthesis

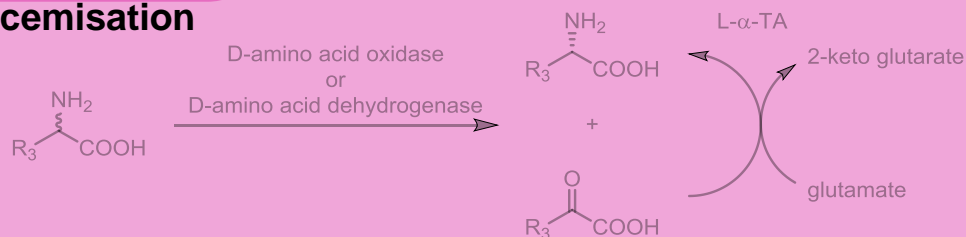


1st step

2nd step



Deracemisation

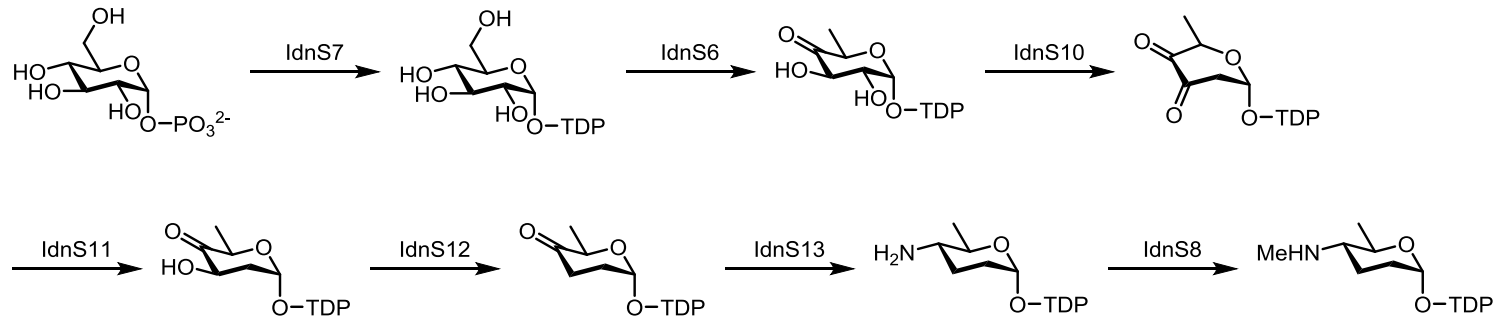




Transamination – Incednine sugar



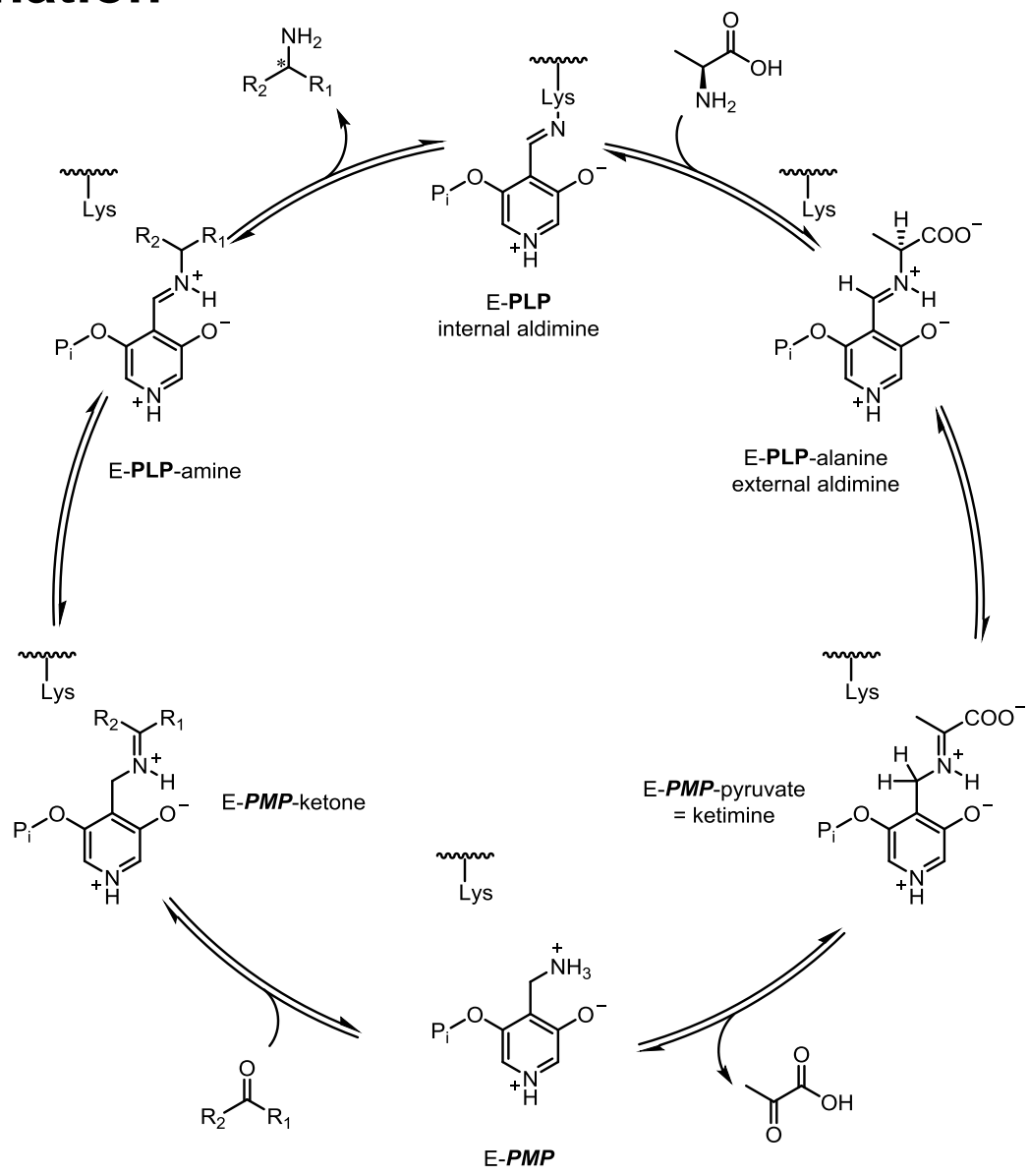
- **Transamination is also involved in the biosynthesis of the incednine-sugar**



- **Problems for this strategy: availability of appropriate enzymes,**

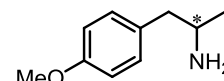
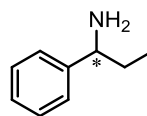
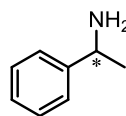
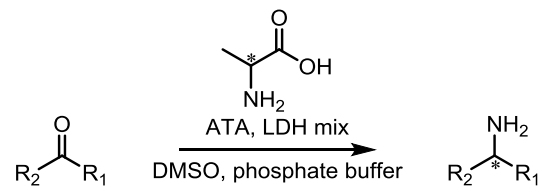


Transamination

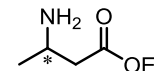
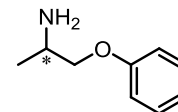
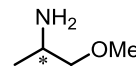
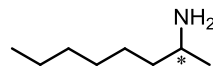
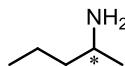




Transamination

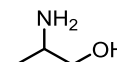
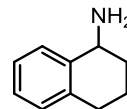


ATA 117	43, 96% ee (R)	3, 42% ee (R)	98, >99% ee (R)	98, >99% ee (R)
ATA 103	21, >99% ee (S)	3, >99% ee (S)	52, 40% ee (S)	
ATA 113	5, >99% ee (S)	28, >99% ee (S)	21, 40% ee (S)	97, 98% ee (S)



ATA 117	89, >99% ee (R)	70, 90% ee (R)	>99, >99% ee (R)	>99, >99% ee (R)	>99, 98% ee (R)
ATA 103	19, 13% ee (S)	24, 90% ee (S)	86, 5% ee (R)	29, 81% ee (S)	83, 50% ee (S)
ATA 113	82, >99% ee (S)	52, 98% ee (S)	>99, 77% ee (S)	>99, 96% ee (S)	98, 61% ee (S)

unsuccessful substrates



Koszelewski, D.; Lavandera, I.; Clay, D.; Rozzell, D.; Kroutil, W. *Advanced Synthesis & Catalysis* **2008**, 350, 2761.

Mutti, F. G.; Fuchs, C. S.; Pressnitz, D.; Sattler, J. H.; Kroutil, W. *Advanced Synthesis & Catalysis* **2011**, 353, 3227.

Schätzle, S.; Steffen-Munsberg, F.; Thontowi, A.; Höhne, M.; Robins, K.; Bornscheuer, U. T. *Advanced Synthesis & Catalysis* **2011**, 353, 2439.

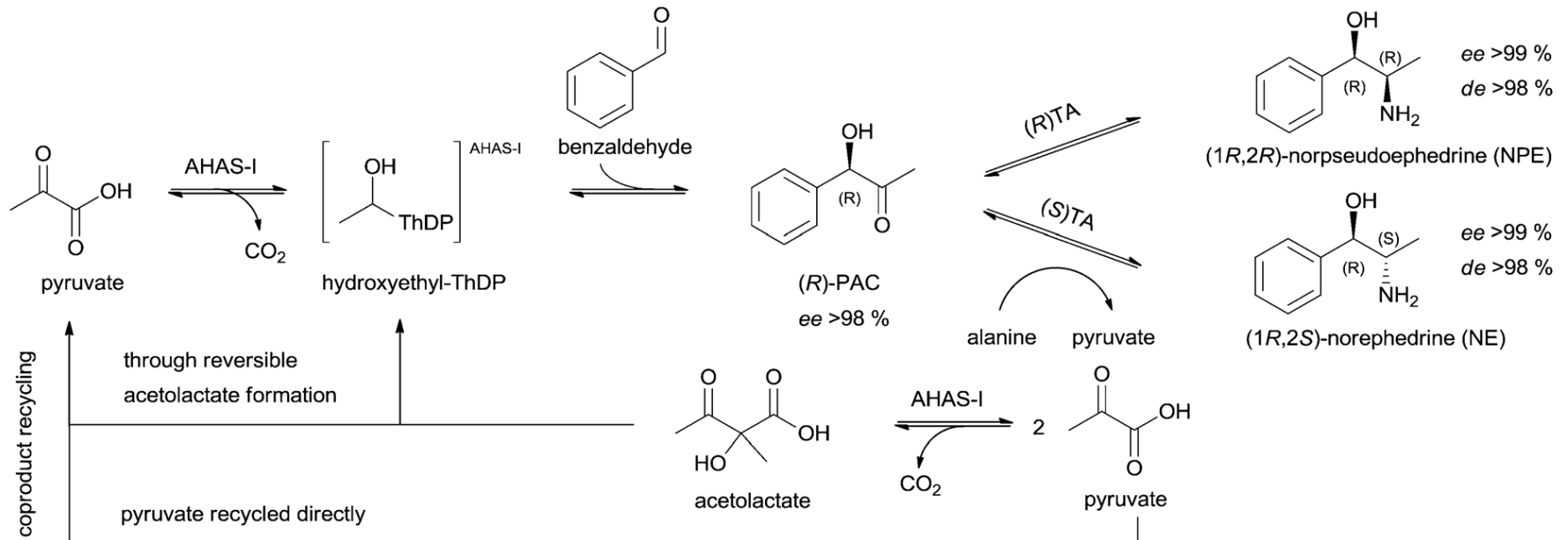


Transamination



step 1: carboligation with intrinsic pyruvate decarboxylation
(hydroxyethyl-ThDP remains bound in AHAS-I active site)

step 2: reductive amination



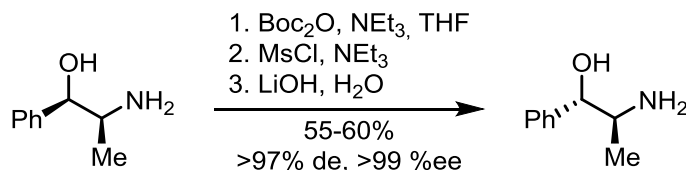
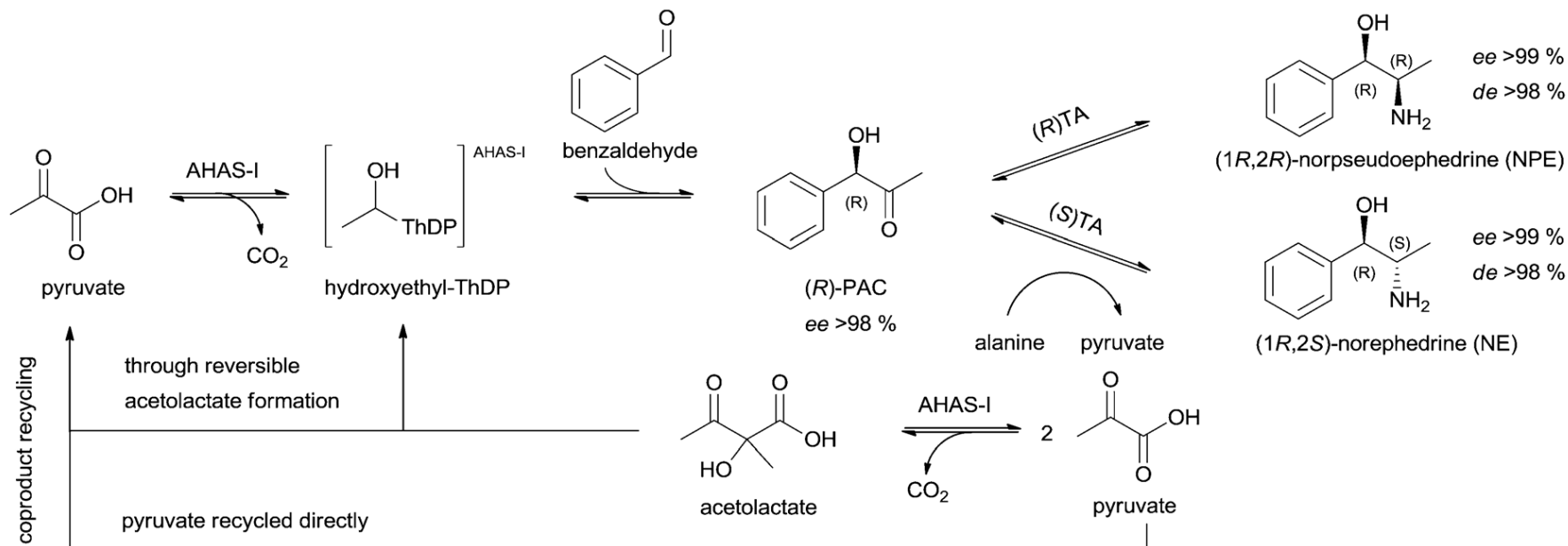


Transamination



step 1: carboligation with intrinsic pyruvate decarboxylation
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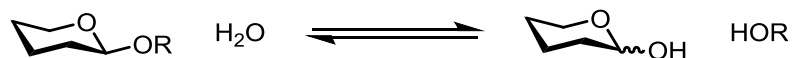




Glycosidic Bonds



- Carbohydrates fill important role in many biological events
- Glycosidic bonds link sugars to other groups
- Glycosidases hydrolyse the glycosidic bond
- Glycosyltransferases catalyse glycosylation reactions

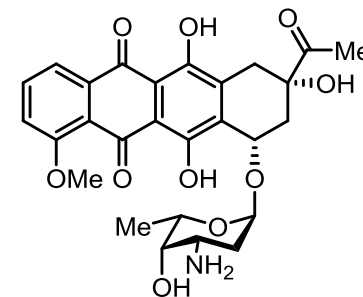




Glycosidic Bonds

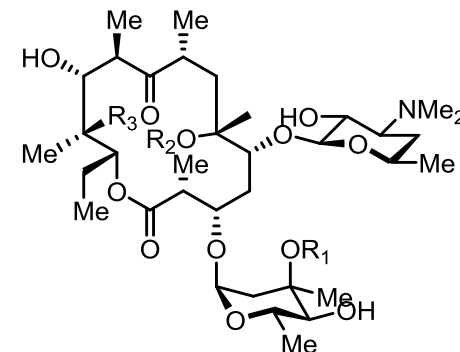


- **Daunomycin shows antitumor activity**
 - Inhibits DNA replication and RNA transcription
 - Only α -glycoside linked compound is active



daunomycin

- **Erythromycin is used clinically as an antibiotic**
 - Sugar moieties are essential for microbial activity
 - Monodemethylated erythromycin only retains 5% of antibacterial activity



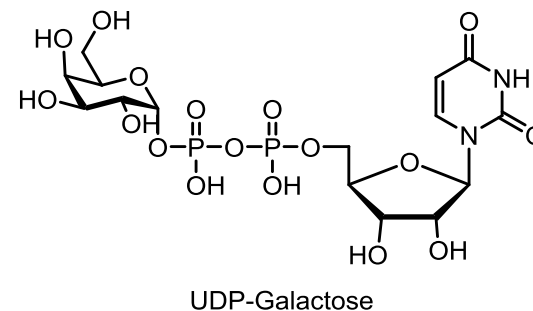
erythromycin A: R₁ = Me, R₂ = H, R₃ = OH
erythromycin B: R₁ = Me, R₂ = R₃ = H
erythromycin C: R₁ = R₂ = H, R₃ = OH
erythromycin D: R₁ = R₂ = R₃ = H



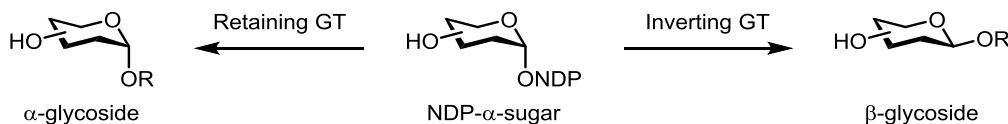
Glycosyltransferases



- Over 195 000 GT sequences comprising 97 families in CAZy database
- Glycosylation reaction needs activated sugar substrates
 - nucleoside diphosphate sugars (NDP-sugars)

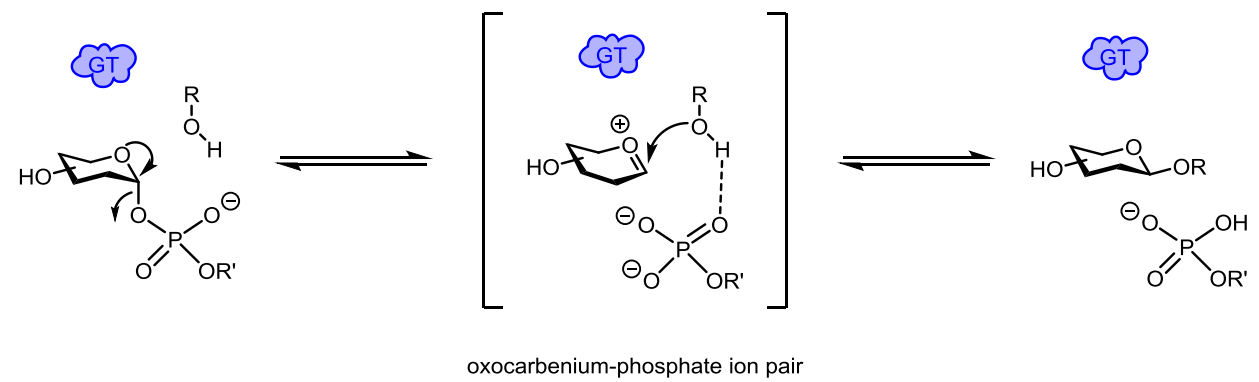
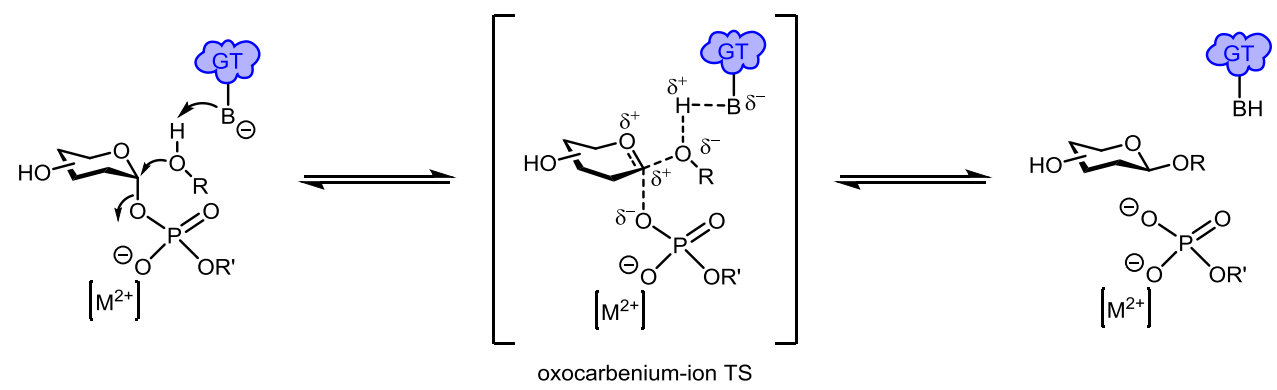
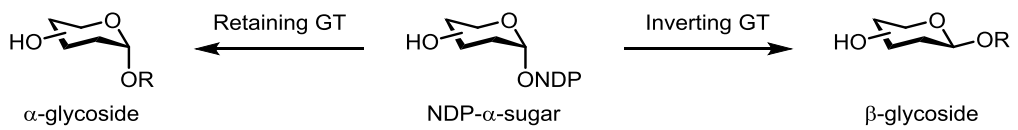


- Mechanism of glycosylation can either be retaining or inverting



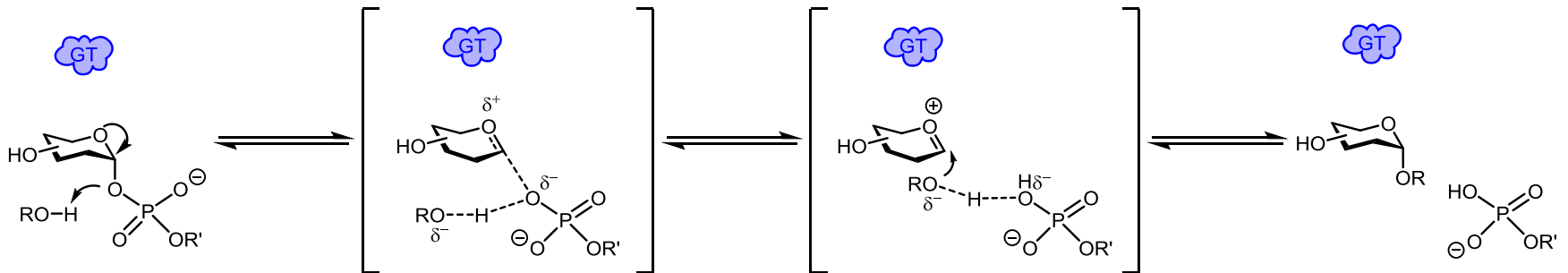
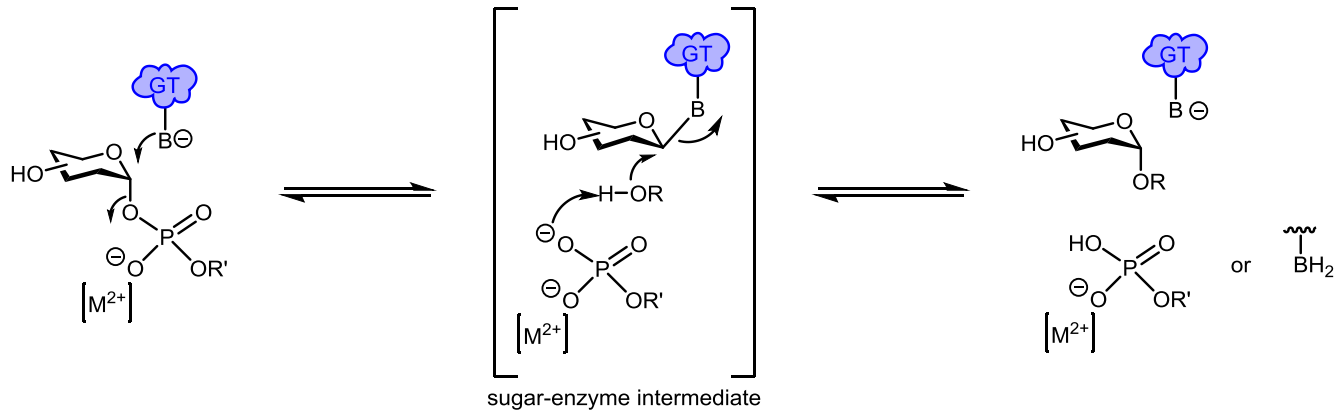
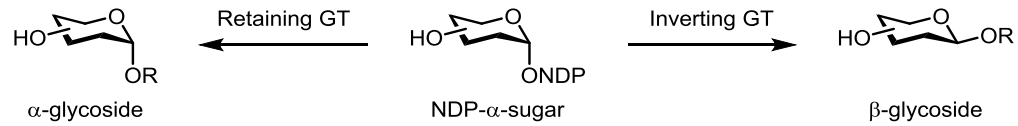


Glycosyltransferases – Mechanism I





Glycosyltransferases – Mechanism II

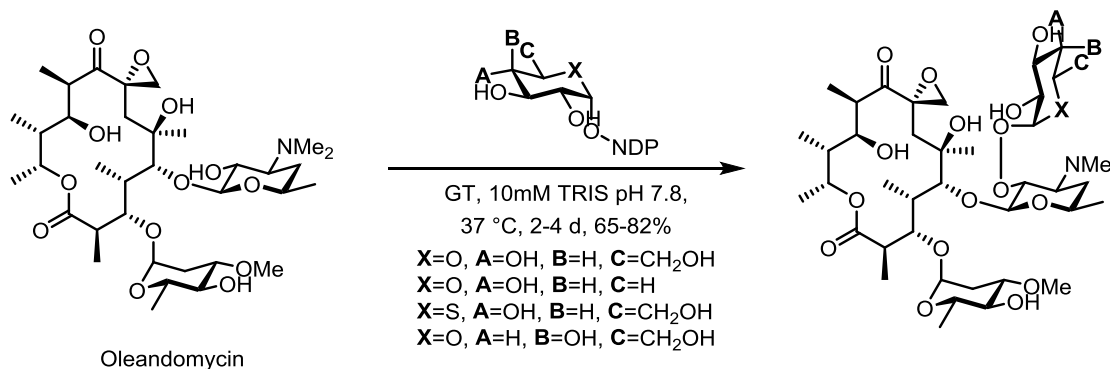




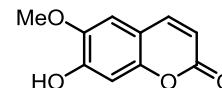
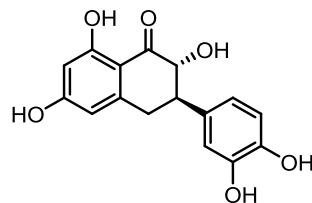
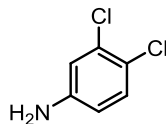
Glycosyltransferases – Commercial Enzymes



- **Macrolide glycosyltransferase, EC 2.4.1.-**
 - **Source: *Streptomyces lividans* DSMZ 46482**



- **Library screening: 18 sugar donors to 64 acceptors**
- **Other acceptor substrates (selection)**

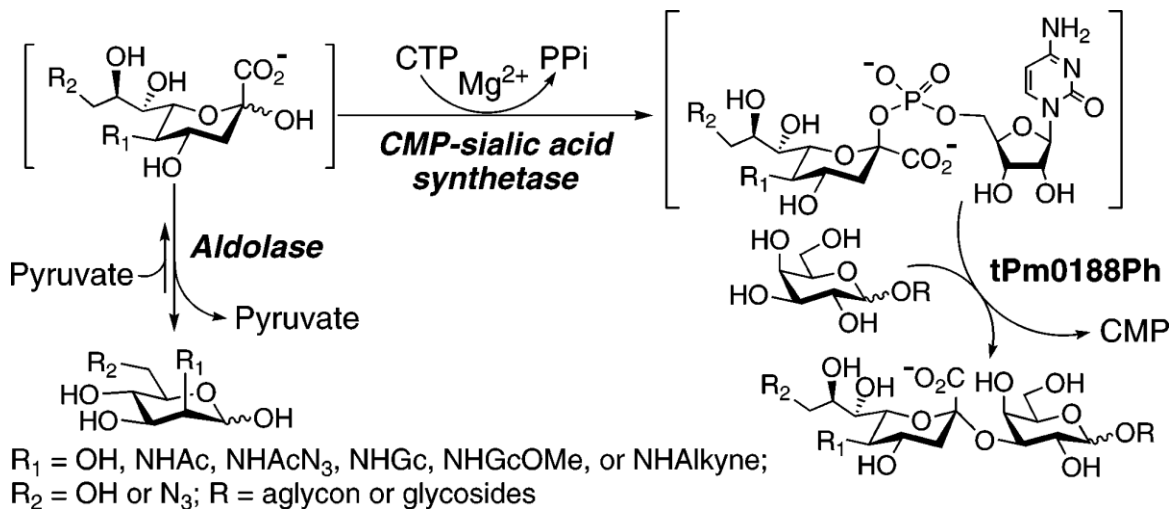




Glycosyltransferase – Commercial Enzymes



- **A-2,3/2,6-Sialyltransferase/sialidase, EC 2.4.99.1/4**
– **Source: *Pasteurella multocida***



Donor Precursors	Entry	Product	Yield (%)
	a		95
	b		79
	c		92
	d		84
	e		75
	f		91
	g		76
	h		79
	i		82
	j		84
	k		62
	l		80
	m		75
	n		75
	o		80
	p		81
	q		90
	r		82
	s		65
	t		91
	u		88
	v		90



Glycosidases



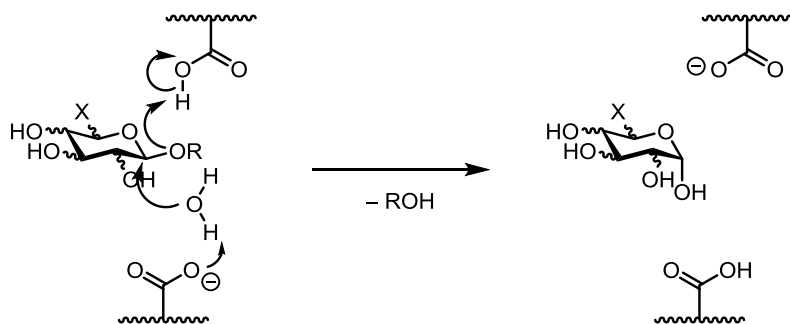
- **Hydrolyse glycosidic bond**
- **Breakdown of glycan structures is of great importance in many processes in agricultural, pulp and paper, textile, and food industries**
- **115 sequence based families**
- **α -glycosidases cleave α -glycosidic bonds, β -glycosidases...**
- **Glycosidases can also be used to synthesise oligosaccharides by transglycosylation or reverse hydrolysis reactions**



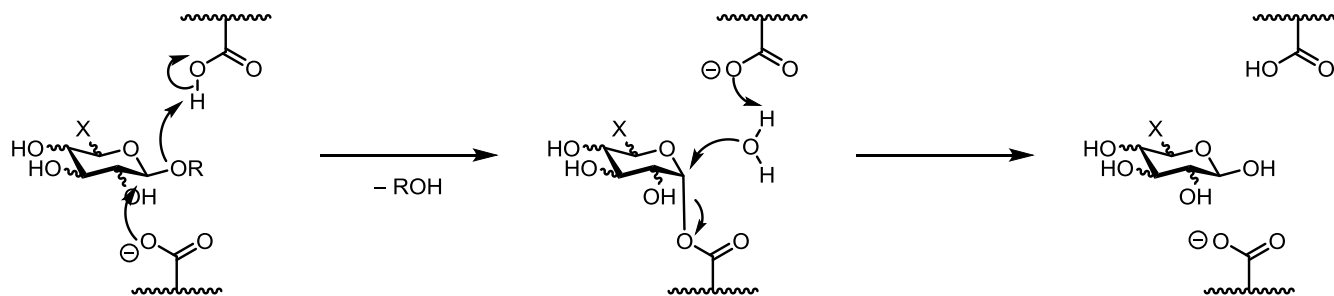
Glycosidases - Mechanism



- Inverting enzymes**

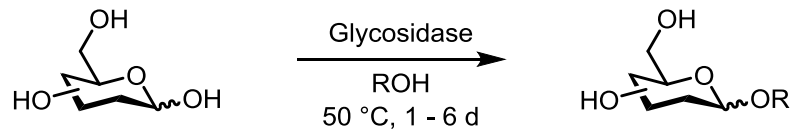


- Retaining enzymes**

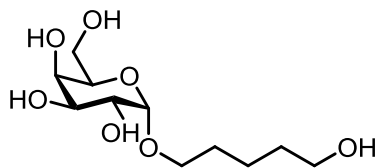




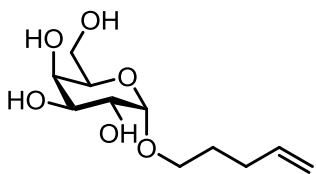
Glycosidases



α -Galactosidase (*Aspergillus niger*)

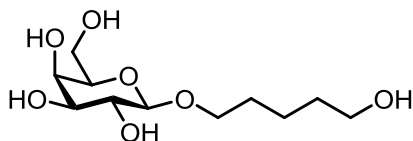


47%

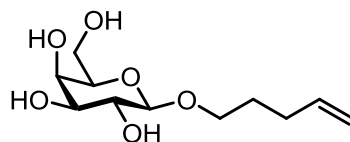


37%

β -Galactosidase (*Aspergillus oryzae*)

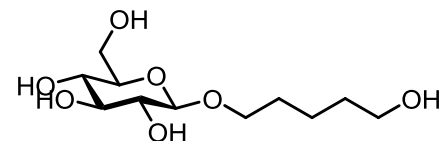


48%

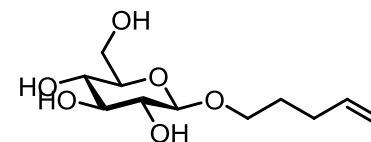


22%

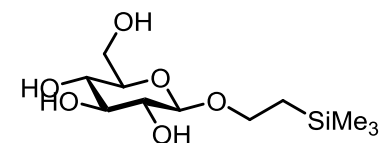
β -Glucosidase (almond)



61%



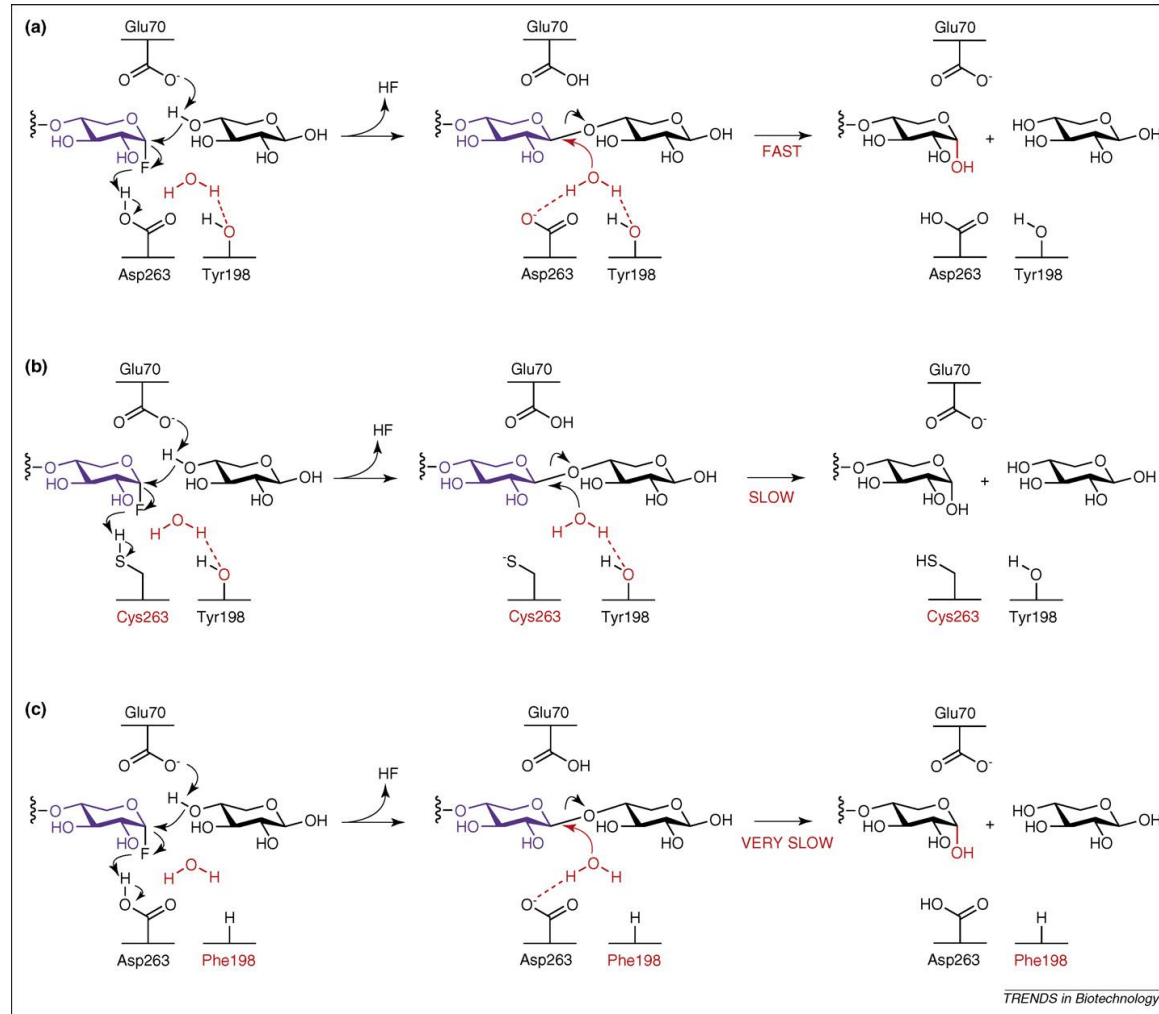
50%



11%



Glycosynthase



Bojarová, P.; Křen, V. *Trends in Biotechnology* **2009**, 27, 199.

Perugino, G.; Cobucci-Ponzano, B.; Rossi, M.; Moracci, M. *Advanced Synthesis & Catalysis* **2005**, 347, 941.

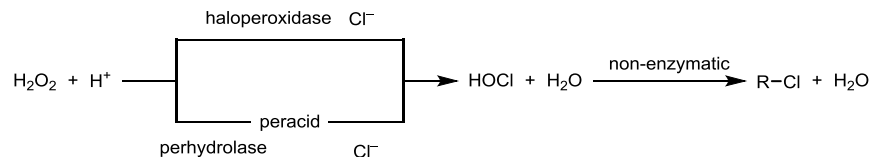
Hancock, S. M.; Vaughan, M. D.; Withers, S. G. *Current Opinion in Chemical Biology* **2006**, 10, 509.



Enzymatic Halogenations



- **Haloperoxidases and Perhydrolases**



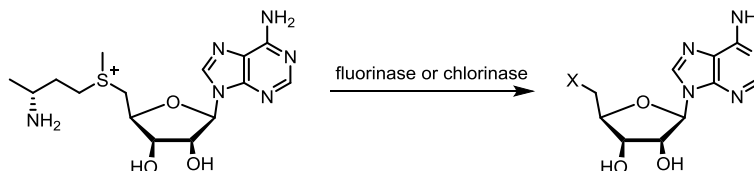
- **FADH₂ dependent halogenases**

- Hypohalous acid is formed from Flavin hydroperoxide

- **Non-heme Iron, α -Ketoglutarate, O₂-dependent Halogenases**

- Formation of a substrate radical by hydrogen atom abstraction

- **S-Adenosylmethionine-dependent Halogenases**



Itoh, N.; Izumi, Y.; Yamada, H. *Biochemistry* **1987**, *26*, 282.

Hohaus, K.; Altmann, A.; Burd, W.; Fischer, I.; Hammer, P. E.; Hill, D. S.; Ligon, J. M.; van Pée, K.-H. *Angew. Chem. Int. Ed.* **1997**, *36*, 2012.

Sitachitta, N.; Márquez, B. L.; Thomas Williamson, R.; Rossi, J.; Ann Roberts, M.; Gerwick, W. H.; Nguyen, V.-A.; Willis, C. L. *Tetrahedron* **2000**, *56*, 9103.

Dong, C.; Huang, F.; Deng, H.; Schaffrath, C.; Spencer, J. B.; O'Hagan, D.; Naismith, J. H. *Nature* **2004**, *427*, 561.

Eustaquio, A. S.; Pojer, F.; Noel, J. P.; Moore, B. S. *Nat Chem Biol* **2008**, *4*, 69.

Flecks, S.; Patallo, E. P.; Zhu, X.; Ernyei, A. J.; Seifert, G.; Schneider, A.; Dong, C.; Naismith, J. H.; van Pée, K. H. *Angew Chem Int Ed Engl* **2008**, *47*, 9533.

Matthews, M. L.; Krest, C. M.; Barr, E. W.; Vaillancourt, F. H.; Walsh, C. T.; Green, M. T.; Krebs, C.; Bollinger, J. M. *Biochemistry* **2009**, *48*, 4331.

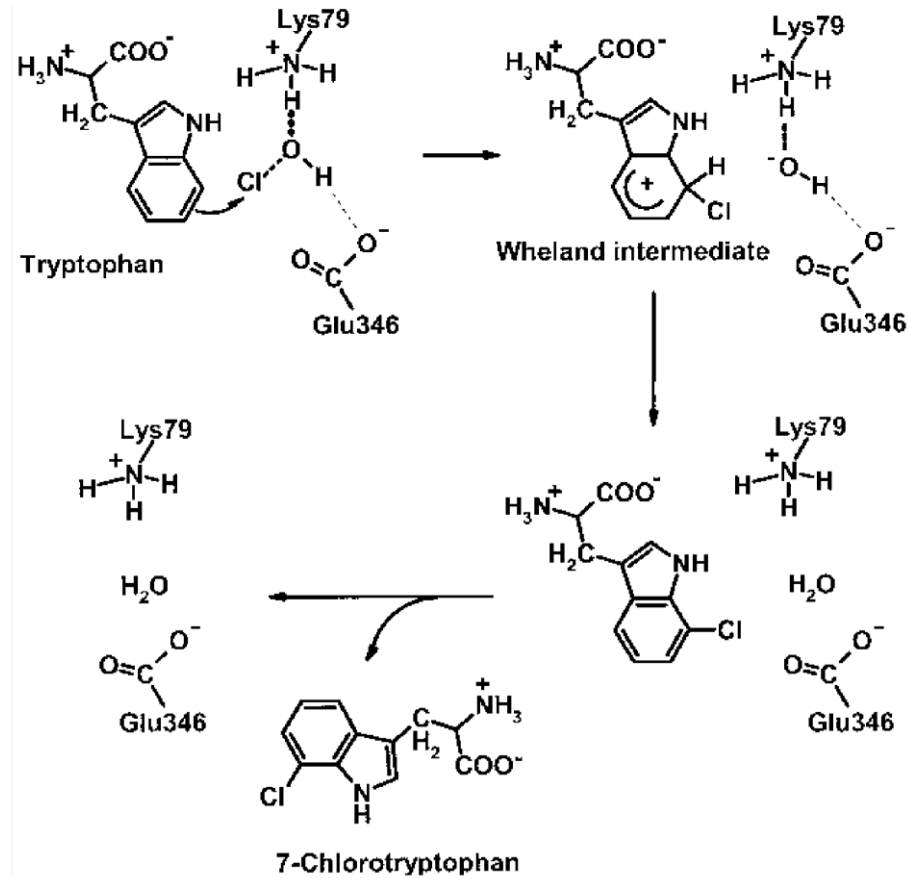
van Pée, K.-H. In *Enzyme Catalysis in Organic Synthesis*; Wiley-VCH Verlag GmbH & Co. KGaA: 2012, p 1569.



Enzymatic Halogenation



- FADH₂ dependent halogenases

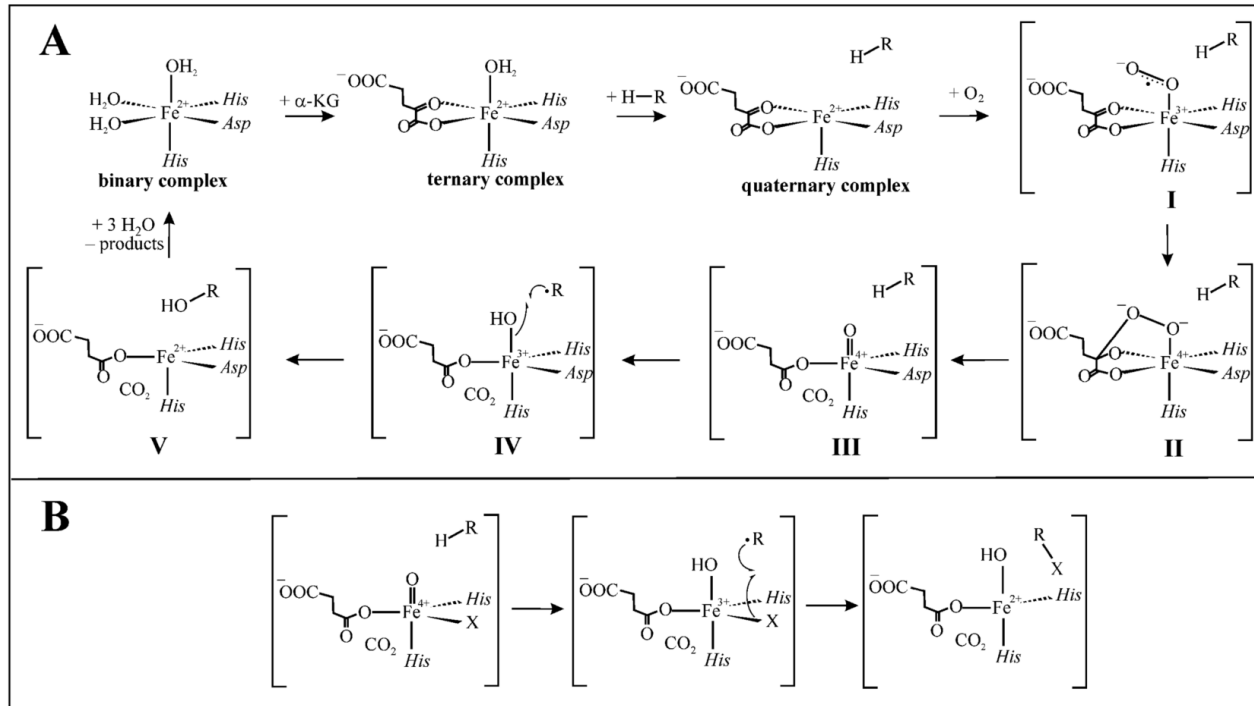




Enzymatic Halogenation



- **Non-heme Iron, α -Ketoglutarate, O_2 -dependent Halogenases**



– **HAG mechanism and hypothesis for halogenase mechanism**



Enzymatic Halogenation



- **Haloperoxidases and Peroxidases**
 - Same selectivity as chemical halogenation using hypohalous acid
- **FADH₂ dependent halogenases**
 - Good regioselectivity because hypohalous acid cannot leave active site
- **Non-heme Iron, α -Ketoglutarate, O₂-dependent Halogenases**
 - So far only halogenations of terminal methyl groups reported
- **S-Adenosylmethionine-dependent Halogenases**
 - Only S-adenosylmethionine is accepted as substrate

Itoh, N.; Izumi, Y.; Yamada, H. *Biochemistry* **1987**, *26*, 282.

Hohaus, K.; Altmann, A.; Burd, W.; Fischer, I.; Hammer, P. E.; Hill, D. S.; Ligon, J. M.; van Pée, K.-H. *Angew. Chem. Int. Ed.* **1997**, *36*, 2012.

Sitachitta, N.; Márquez, B. L.; Thomas Williamson, R.; Rossi, J.; Ann Roberts, M.; Gerwick, W. H.; Nguyen, V.-A.; Willis, C. L. *Tetrahedron* **2000**, *56*, 9103.

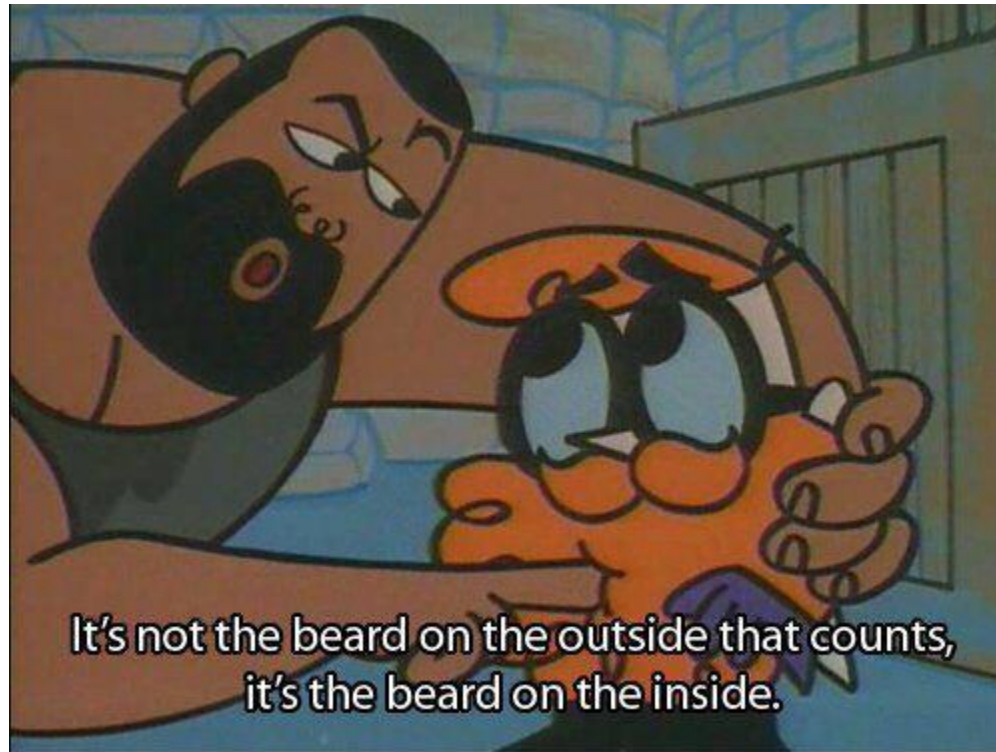
Dong, C.; Huang, F.; Deng, H.; Schaffrath, C.; Spencer, J. B.; O'Hagan, D.; Naismith, J. H. *Nature* **2004**, *427*, 561.

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van Pée, K.-H. In *Enzyme Catalysis in Organic Synthesis*; Wiley-VCH Verlag GmbH & Co. KGaA: 2012, p 1569.



It's not the beard on the outside that counts,
it's the beard on the inside.



Thank you for your kind Attention

Questions?