

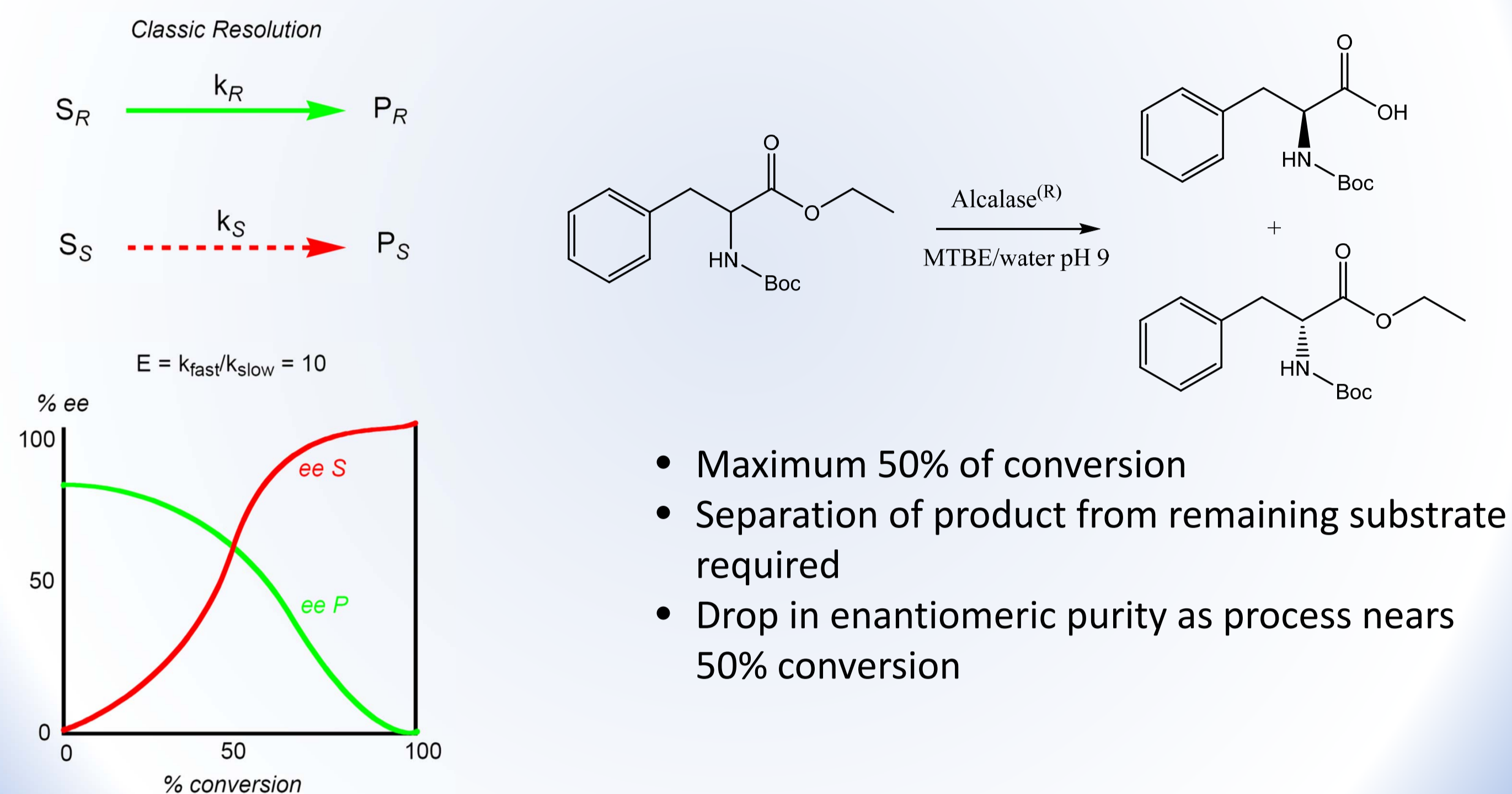


Aim of the work

To find an economically sound enzymatic process for the synthesis of enantiopure amino acid derivatives

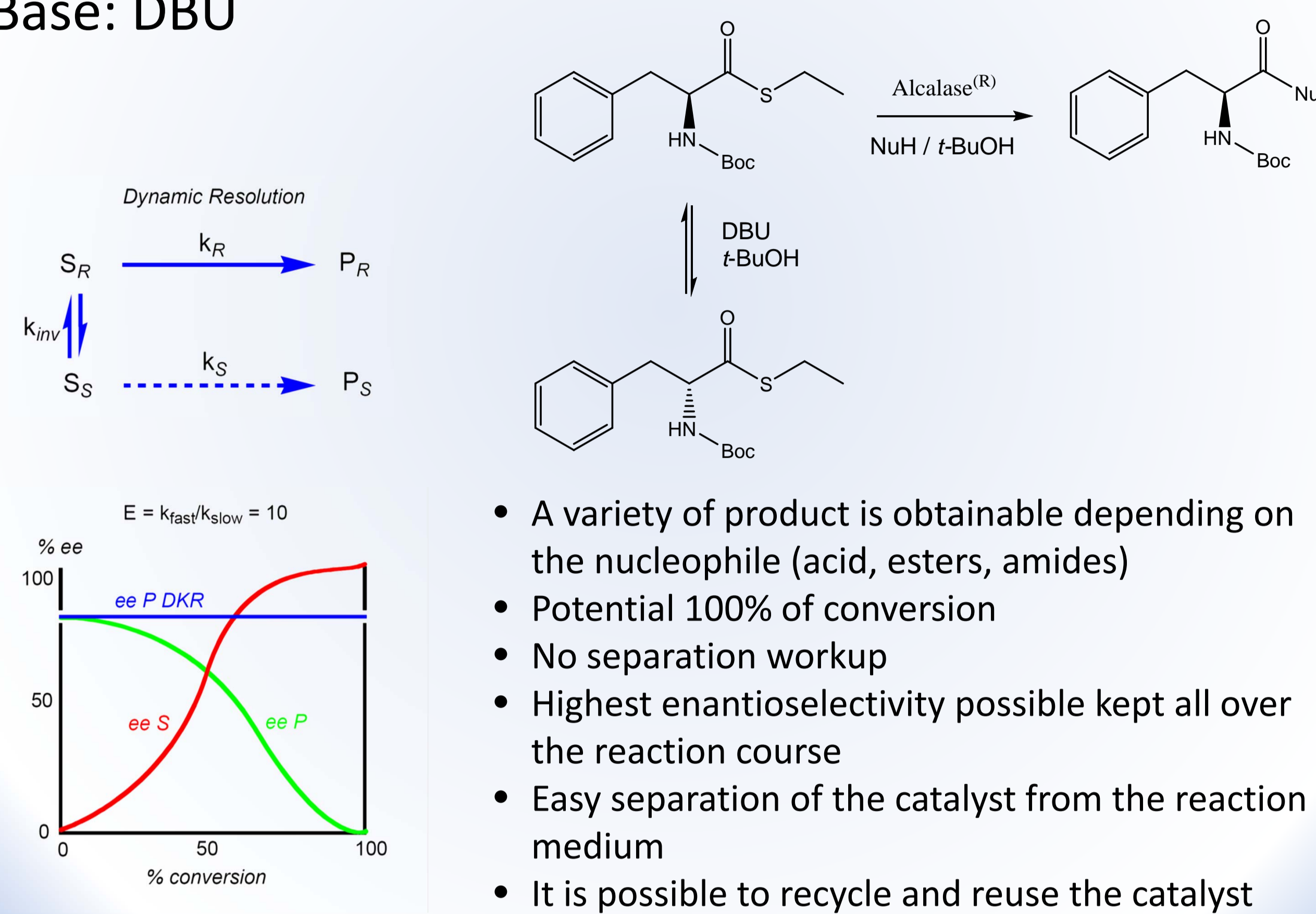
First generation system: Enzymatic Kinetic Resolution

- Solvent: MTBE/aqueous buffer (biphasic system)
- Catalyst: Alcalase[®] in liquid form

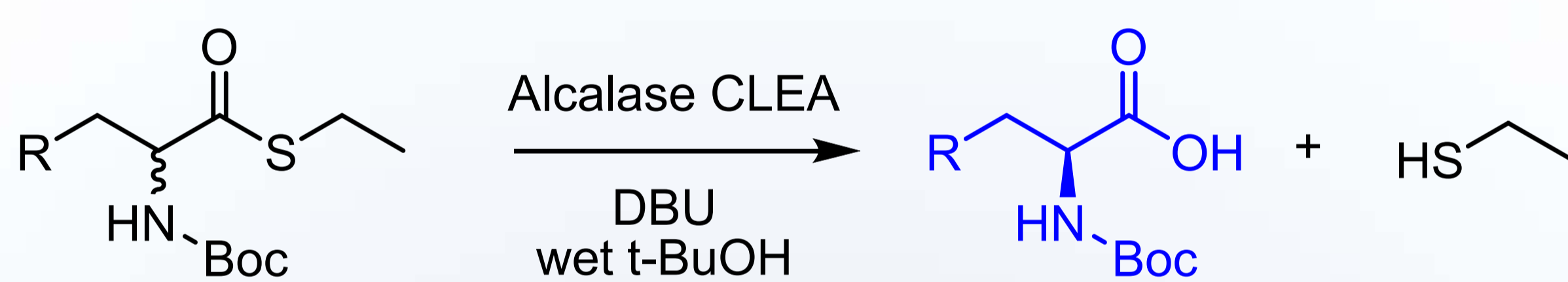


Second generation system: Enzymatic Dynamic kinetic Resolution

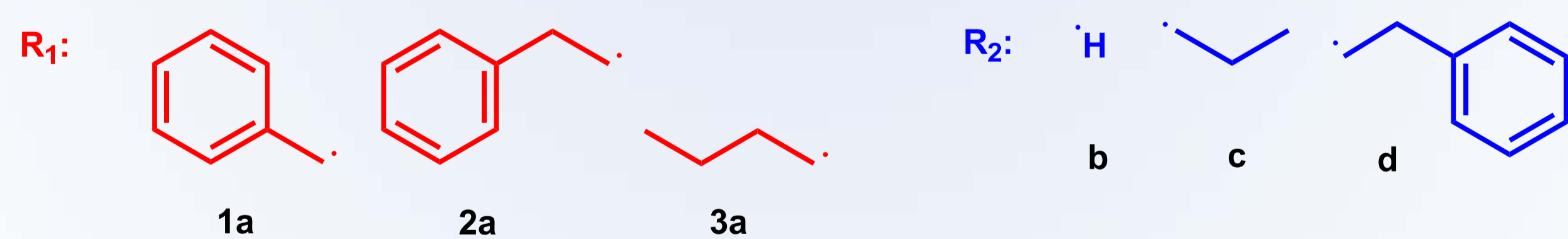
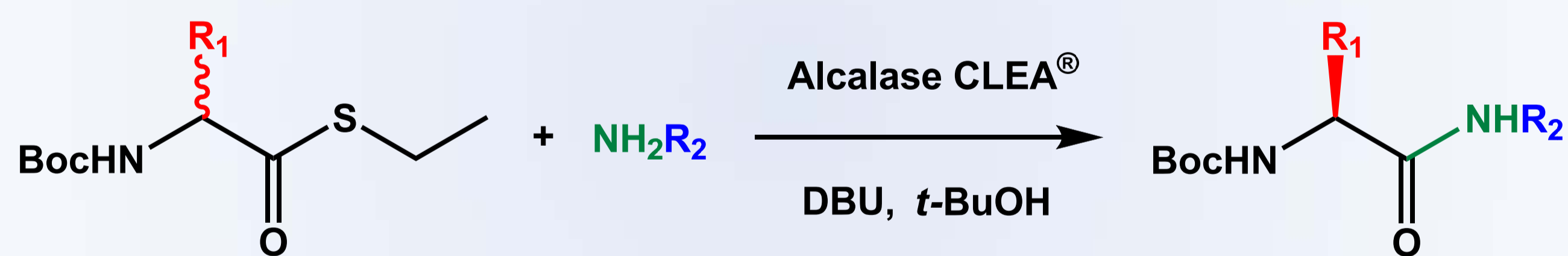
- Solvent: Wet t-BuOH (monophasic system)
- Catalyst: Alcalase[®] in solid form
- Base: DBU



The results: hydrolysis and amidation



R	Time (h)	Yield (%)	e.e. (S) (%)
Ph-	12	99	>99
PhCH ₂ -	40	94	99
CH ₃ CH ₂ CH ₂ -	48	91	99
CH ₃ CH ₂ -	24	99	>99
CH ₃ (CH ₂) ₃ CH ₂ -	110	96	>99



Substrate	Product (isolated yields, %)	Product ee%	DBU [equiv.]	Enzyme Loading [w/w]	Amine [equiv.]	Reaction time [h]
1a	1b (82)	98	2	1:1	2	7
1a	1c (76)	99	4	1:1	1.2	7
1a	1d (89)	98	3	2:1	1.2	5
2a	2b (60)	99	2	1:1	3	30
2a	2c (63)	99	4	4:1	1.2	24
2a	2d (87)	99	2	4:1	1.2	24
3a	3b (66)	99	2	2.5:1	3	168
3a	3c (65)	98	2	2:1	1.2	30
3a	3d (69)	96	2	1:1	1.2	30

The catalyst



Alcalase CLEA[®] is employed as catalyst:

- high activity
- high stability
- easy separation and reuse

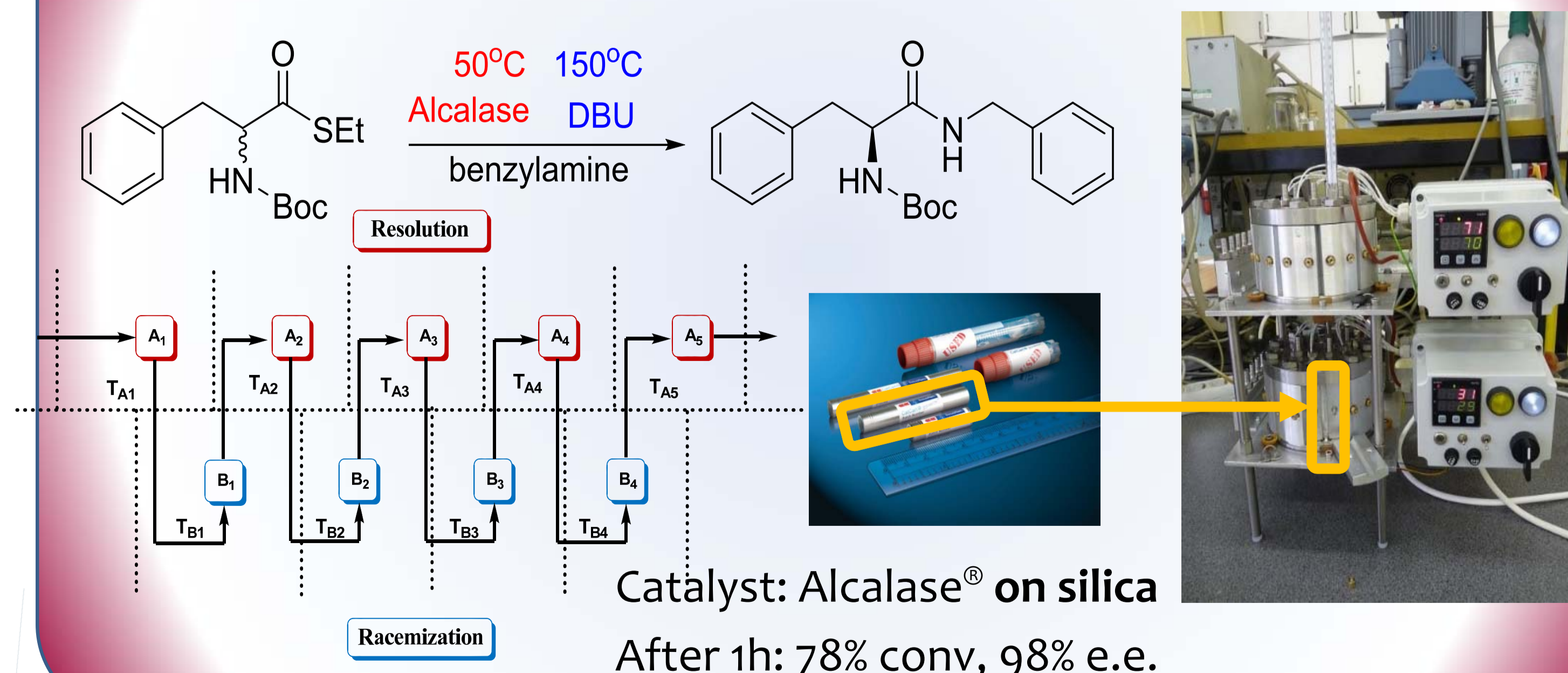
Cycle	t (h)	conv %	ee(S) %
1	20	92	99
5	20	82	99
15	20	76	99

Substrate: racemic NBoc-nVal-SEt

The catalyst is separated by centrifugation and directly added to the successive batch

It is possible to restore the full activity through a simple protocol

...in continuous-flow



1. Agosta, E; Caligiuri, A; D'Arrigo, P; Servi, S; Tessaro, D; Canevotti, R; «Enzymatic approach to both enantiomers of N-Boc hydrophobic amino acids», *Tetrahedron: Asymmetry*, 17, 13, 1995-1999, **2006**
2. Arosio, D; Caligiuri, A; D'Arrigo, P; Pedrocchi-Fantoni, G; Rossi, C; Saraceno, C; Servi, S; Tessaro, D, «Chemo-Enzymatic Dynamic Kinetic Resolution of Amino Acid Thioesters», *Adv Synth Catal*, 349, 8-9, 1345-1348, **2007**
3. D'Arrigo, P; Cerioli, L; Servi, S; Viani, F; Tessaro, D; «Synergy between catalysts: enzymes and bases. DKR of non-natural amino acids derivatives», *Catal Sci Technol*, 2, 8, 1606-1616, **2012**