Racemization-Free Chemoenzymatic Peptide Synthesis Enabled *via* a Combination of Transition Metal and Enzyme Catalysis

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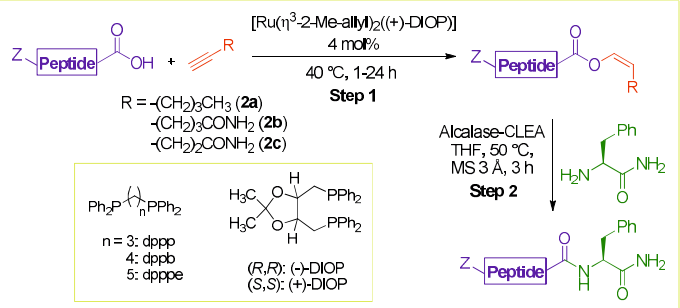
Introduction

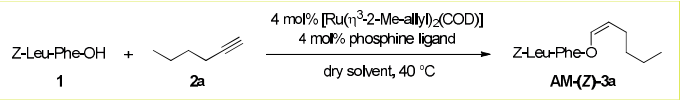
In recent years, the scalable synthesis of peptides has gained significant importance, as such products have been increasingly investigated and marketed in industry. Although some excellent methods for the assembly of peptides are available, the issue of racemization-free fragment coupling remains a problem without a general solution. On this poster we present a convenient and atom-economical method to prepare peptide enolesters without racemization using transition metal catalysis and their subsequent use for enzymatic peptide synthesis.[1-3]



Scheme 1: (*Z*)-*anti*-MARKOVNIKOV Ru-catalyzed addition of alkynes to peptide fragments followed by Alcalase-CLEA-catalyzed condensation of peptide enolesters with H-Phe-NH_2.

Results and Discussion

The key challenge in our approach was to develop a mild, racemization-free method for the synthesis of enolesters of quite complex peptide fragments which are significantly more prone to racemization than single amino acids.[1-2] Therefore we chose Z-Leu-Phe-OH (**1**) as test substrate, as Phe is known as the amino acid to be most prone towards racemization.



Scheme 2: Ru-catalyzed addition of 1-hexyne (**2a**) to Z-Leu-Phe-OH (**1**).

In an extensive screening of different types of ligands, using *in situ* prepared Ru-complexes, DIOP gave superior results. Interestingly, (+)-DIOP showed higher activity than (-)-DIOP indicating a possible cooperative interaction between the chiral catalyst and the chiral substrate (**Table 1**).

Table 1: Screening of bidentate phosphines in the Ru-catalyzed addition of 1-hexyne (**2a**) to Z-Leu-Phe-OH (**1**, 0.50 M) in THF (**Scheme 2**).

Entry	Ligand[a]	Conversion [%][b]	AM-(*Z*)-3a/AM-(*E*)-3a/M-3a
1	**dppp**	57	29/-/71
2	**dppb**	97	98/-/2
3	**dpppe**	57	84/-/16
4	(-)-**DIOP**	98	96/-/4
5	(+)-**DIOP**	99	>99/-/<1

[a]4 mol% of ligand used based on **1**. [b]Determined after 24 h.
AM: *anti*-MARKOVNIKOV-product, M: MARKOVNIKOV-product.

Surprisingly, the use of polar protic solvents such as MeOH, EtOH and 2-PrOH led to the fastest conversion and the lowest amounts of racemization (**Table 2**).

Table 2: Screening of various solvents in the Ru-catalyzed addition of 1-hexyne (**2a**) to Z-Leu-Phe-OH (**1**, 0.50 M) (**Scheme 2**).

Entry	Solvent	Conversion [%][a]			Yield [%]	D-Phe [%]
		1 h	3 h	24 h		
1	CH_2Cl_2	39	73	99	88	1.5
2	$CHCl_3$	66	98	-	87	0.2
3	MeOH[b]	26	51	95	85	<0.1
4	EtOH	73	91	-	86	<0.1
5	2-PrOH	99	-	-	88	<0.1

[a]Sum of isomers. [b]Reaction carried out at 0.25 M.

Taking into account that Alcalase-CLEA is an endoprotease, we introduced amide motifs within the peptide enolester moiety which allow a better recognition of our substrates by the enzyme and lead to faster reaction (**Table 3**, **Figure 1**).

Table 3: Racemization-free Ru-catalyzed synthesis of peptide enolesters using [Ru(η3-2-Me-allyl)2((+)-DIOP)] at 40 °C in 2-PrOH (**Scheme 1**).

Entry	Peptide/Alkyne	Time [h]	Conversion/Yield [%]	D-AA [%][a]
1	Z-Leu-Phe-OH **1**/**2a**	1	99/88	<0.1
2	Z-Ile-Ser-OH/**2a**	24	97[b]/83	0.1
3	Z-Leu-Phe-Ala-OH/**2a**	1	99/87	0.1
4	Boc-Phe-Tyr-OH/**2a**	3	99/86	0.2
5	Z-Leu-Phe-OH **1**/**2b**	2	98/80	<0.1
6	Z-Leu-Phe-OH **1**/**2c**	2	97/82	<0.1

[a]Amount of *C*-terminal racemization. [b]Ethanol used as solvent.

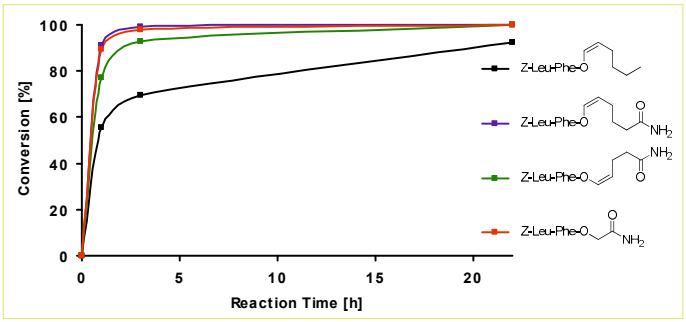


Figure 1: Kinetic comparison of Alcalase-CLEA-catalyzed peptide coupling of activated esters with H-Phe-NH_2 in THF at 50 °C (**Scheme 1**, **Step 2**).

Conclusion

We demonstrated that under proper conditions the Ru-catalyzed addition of alkynes using (+)-DIOP as a ligand is a convenient method for the racemization-free preparation of peptide enolesters. These compounds are excellent substrates in chemoenzymatic peptide coupling reactions using Alcalase-CLEA.

Acknowledgements

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