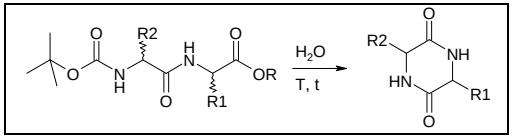
Microwave Assisted 2,5-Diketopiperazine Formation from Protected Dipeptides

Ronny Lundin and Panagiotis Ioannidis
Biotage Sweden AB, Kungsgatan 76, SE-753 18 Uppsala, Sweden

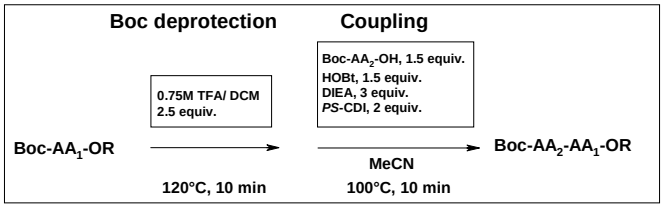
Introduction

The formation of 2,5-diketopiperazines from a water suspension of *N*-Boc protected dipeptide alkyl esters or dipeptide alkyl esters was induced by microwave assisted heating (***Scheme 1***). The products, which precipitated, were obtained in good yields. However, epimerisation/diastereomer formation was found to various extents depending on the amino acid sequence and the applied reaction temperature.



Scheme 1

Microwave assisted heating was also applied in the Boc deprotecting step as well as in the synthesis of the protected dipeptides, where polystyrene bound carbodiimide, with HOBt as additive, was used as the coupling agent (***Scheme 2***).



Scheme 2

Recently we reported the formation of the 2,5-diketopiperazine from a microwave heated water suspension of the *N*-Boc protected dipeptide ester Boc-D-Val-Gly-OMe.[1] The present study was undertaken in order to examine the generality of this procedure and to examine possible epimerisation.

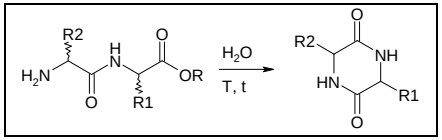
Procedures

Boc deprotection and coupling

The deprotections of either the *N*-Boc protected amino acid ester or the *N*-Boc protected dipeptide ester, and peptide coupling reactions were both performed using microwave heating (***Scheme 2***). 2.5 equiv. of 0.75 M TFA/DCM was used for the *N*-Boc deprotection of the amino acid/peptide esters. The coupling of the *N*-Boc protected amino acid to the *C*-terminal amino acid ester was performed in acetonitrile using polystyrene bound carbodiimide and HOBt as coupling agent. Efforts to perform the coupling reactions in dichloromethane gave chloro-methylation of HOBt as a by-product.[2] After an easy work-up, the protected dipeptides (***Table 1***) were obtained in 80-90% overall yields. The purities were generally >95% (by HPLC-MS). Detailed results will be presented elsewhere.

Cyclisation

The *N*-Boc protected dipeptide ester (***Scheme 1***) or N-unprotected dipeptide ester (***Scheme 3***) was suspended in water and heated to 150°C or 200°C by microwave irradiation for 20 minutes. Three alkyl esters were used: methyl, propyl and tert.-butyl. The 2,5-diketopiperazines formed were poorly soluble both in water and organic solvents. The isolation was performed by a simple filtration and washing of the solids. ^{1}H NMR spectroscopy and, in two cases, optical rotation measurements revealed the extent of epimerisation of the products, i.e. the diastereomeric excesses, de, and the enantiomeric excesses, ee, respectively.



Scheme 3

Results and discussion

The outcome of the cyclisation reactions is summarised in ***Table 1***. In entry **1** & **2**, *N*-Boc protected dipeptide esters containing only one chiral amino acid, phenylalanine, were subjected to microwave heating at 200°C for 20 minutes. In entry **1**, phenylalanine was positioned at the *N*-terminal and in entry **2** at the *C*-terminal position. Epimerisation of either of them would give rise to scalemic diketopiperazine different only in the degree of epimerisation. Epimerisation occurred at both the *C*- (entry **1**) and at the *N*-terminal position (entry **2**). The measured optical rotation indicates that the epimerisation occurs to a higher degree at the *N*- (entry **2**) than at the *C*-terminal position (entry **1**). In entries **3-5**, fully protected dipeptides consisting of two phenylalanines but with different ester groups were heated to 200°C for 20 minutes. The de values of the resulting products, measured by ^{1}H NMR, showed the lowest degree of epimerisation with the methyl ester and the highest with the propyl ester. Heating the same fully protected peptides to only 150°C for 20 minutes, entries **6-8**, gave considerably lower epimerisation, but also very low conversions. Dipeptide esters with free *N*-terminals, showed after heating at 200°C (entries **9-10**) and 150°C (entries **11-12**) a similar degree of epimerisation as the corresponding *N*-Boc protected dipeptides at identical conditions. Heating at 150°C, however, resulted in higher yields compared to the corresponding *N*-Boc peptides. Changing the sequence in the *N*-Boc protected dipeptide metyl ester from diphenylalanine to dileucine (entry **13**) gave no significant difference in the degree of epimerisation at 200°C of the corresponding cyclised product, but the yield was slightly lower. At the same conditions, substitution of diphenylalanine with divaline (entry **14**) resulted in less epimerised diketopiperazine. The yield was, however, heavily reduced.

Conclusions

From the results it can be concluded that epimerisation occurs during the cyclisation and is dependant on the applied temperature. The results of the experiments indicate that both the *N*- and the *C*-terminal amino acid are epimerised. The extent of epimerisation observed is dependant on the dipeptide sequence and the ester attached. It was also found that microwave heating to 150 or 200°C of an optically pure 2,5-diketopiperazine in water, entries **15-16**, resulted in epimerisation as well. The degree of epimerisation again depends on the temperature applied.

Cyclisation

Entry	Protected dipeptide	T, °C	t, min	Diketopiperazine Yield, %	ee, % *	de, % **
1	Boc-Gly-Phe-OPr	200	20	77	61	-
2	Boc-Phe-Gly-OPr	200	20	90	40	-
3	Boc-Phe-Phe-OMe	200	20	88	-	60
4	Boc-Phe-Phe-OPr	200	20	83	-	24
5	Boc-Phe-Phe-OtBu	200	20	74	-	36
6	Boc-Phe-Phe-OMe	150	20	16	-	92
7	Boc-Phe-Phe-OPr	150	20	12	-	68
8	Boc-Phe-Phe-OtBu	150	20	<1	-	ND
9	H-Phe-Phe-OMe	200	20	74	-	36
10	H-Phe-Phe-OPr	200	20	78	-	24
11	H-Phe-Phe-OMe	150	20	54	-	88
12	H-Phe-Phe-OPr	150	20	41	-	84
13	Boc-Leu-Leu-OMe	200	20	77	-	62
14	Boc-Val-Val-OMe	200	20	42	-	76

* Calculated from specific optical rotation measurements. Comparison with the optically pure reference cyclo(-Gly-L-Phe)[3].

** Calculated from ^{1}H NMR spectroscopy.

Table 1

Epimerisation of diketopiperazine by microwave heating

Entry	Diketopiperazine Reference[3], de=100%	T (°C)	t (min)	Yield, %	de, % **
15	Cyclo(-L-Phe-L-Phe)	200	20	84	68
16	Cyclo(-L-Phe-L-Phe)	150	20	88	94

** Calculated from ^{1}H NMR spectroscopy.

Table 2

General cyclisation / epimerisation procedure

0.25 mmol protected dipeptide or diketopiperazine was suspended in 2.5 mL water in a 2-5 mL microwave reaction vial. The vial was capped and heated with stirring in Initiator™ Sixty for 20 minutes at the temperatures stated in ***Tables 1 and 2***. After cooling, the vial was decapped and the precipitated 2,5-diketopiperazine was filtered off, washed with diluted hydrochloric acid and acetonitrile and dried *in vacuo*. All products were weighed and analysed by HPLC-MS. Products which consisted of mixtures of diastereomers were further analysed by ^{1}H NMR, and the de values were calculated. Products consisting of enantiomeric mixtures were subjected to specific optical rotation measurement from which the ee values were calculated by comparison with an optically pure reference.[3]

References & notes:

1. Carlsson, A-C.; Jam, F.; Tullberg, M.; Pilotti, Å.; Ioannidis, P.; Luthman, K. and Grøtli, M., Tetrahedron Lett. 2006, **47**, 5199
2. Jian-guo Ji; De-yi Zhang; Yun-hua Ye and Qi-yi Xing, Tetrahedron Lett. 1998, **36**, 6515
3. Bachem AG, Switzerland

Biotage