Tetrahedron Letters, Vol.31, No.50, pp 7363-7366, 1990
Printed in Great Britain

0040-4039/90 $3.00 + .00
Pergamon Press plc

Use of BOP[1] Reagent for the Suppression of Diketopiperazine Formation in Boc/Bzl Solid-Phase Peptide Synthesis

Margarida Gairí, Paul Lloyd-Williams, Fernando Albericio and Ernest Giralt*
Departament de Química Orgànica, Universitat de Barcelona, Martí i Franquès 1-11,
E-08028 Barcelona, Spain.

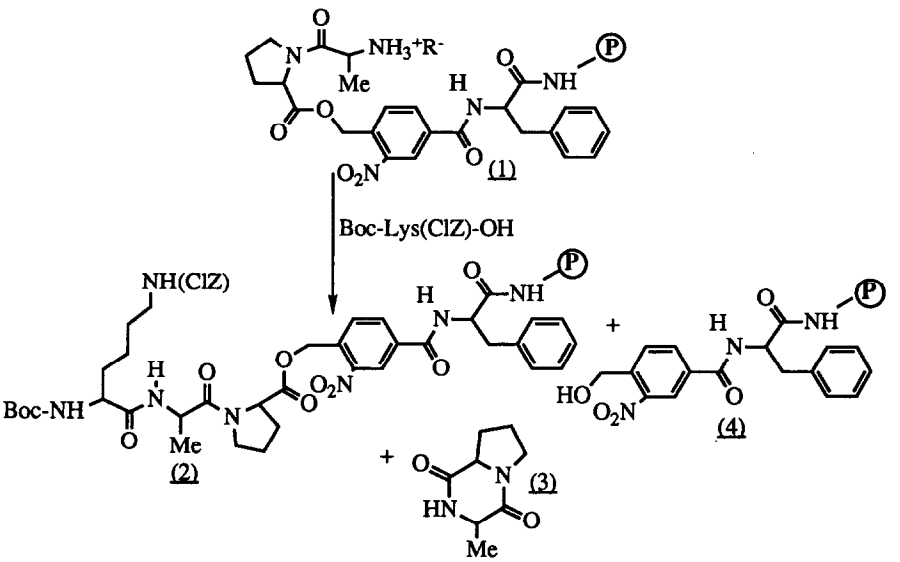
Abstract : BOP coupling reagent suppresses the formation of diketopiperazines in the solid-phase coupling of the third amino acid to dipeptides when a nitro-benzylic "handle" is used to link the peptide to the resin. The amino function of the second amino acid may be deprotected with TFA and the coupling carried out without a prior neutralisation step.

The formation of diketopiperazines (DKPs) is a well-known, undesired side-reaction in solid-phase peptide synthesis (2,3) and can be troublesome when benzyl-type peptide-resin linkages are used(4,5). Formation of diketopiperazines is sequence-dependent (2,3,6), and either acid-(3) or base-catalysed (2,5,6). DKP formation leads not only to a reduction in the overall yield but also to the formation of hydroxyl groups (7) which can then give rise to other side-reactions. Suzuki et al (8) have described a procedure for suppressing DKP formation in the Boc/Bzl strategy for solid-phase peptide synthesis. The method entails Boc-deprotection with 4 N HCl in dioxane followed by addition, without prior neutralisation of the resin, of the N-methyl morpholine salt of the subsequent amino acid of the sequence along with DCC. The base is necessary to liberate the amino group of the dipeptide.The method suppresses DKP formation but has the drawbacks of requiring freshly prepared HCl solution in dioxane, a lengthy reaction time (often 4-5 hours) and of being unamenable to use in automated peptide synthesis.

The use of Nbb-resin (α-[3-nitrobenzamido]benzylpolystyrene) has attracted us for some time (9,10) because it is orthogonal (11) with the Boc/Bzl strategy for solid-phase peptide synthesis and allows photolytic detachment of fully-protected peptides from the resin. Such protected peptide fragments may then be purified and coupled in a convergent peptide synthesis methodology (12).The electron-withdrawing nature of the nitrobenzyl linkage makes the strategy especially prone to DKP formation (5). The Suzuki procedure suppresses DKP formation with this resin but a simpler protocol would be advantageous. Recently Castro et al (13) have described the use of the benzotriazol-1-yl-oxy-tris-(dimethylamino)phosphonium hexafluorophosphate (BOP) (14) coupling reagent in solid-phase peptide synthesis (15), without prior neutralisation of the amino component of the peptide resin,and this prompted the present investigation of an alternative to the Suzuki method when Nbb-resin is used.

We have had previous experience of the problem of DKP formation in the synthesis of the tripeptide Lys-Ala-Pro which corresponds to the 4-6 fragment of the neurotoxin apamin (16) and therefore chose this tripeptide as our first model. Boc-Phe-OH was attached directly to a *p*-methylbenzhydrylamine resin by a standard coupling protocol (17) and served as an internal standard for estimating DKP formation (18). 4-Bromomethyl-3-nitrobenzoic acid was converted to its symmetrical anhydride and then coupled to the N-group of Phe (9). Boc-Pro-OH was incorporated as the cesium salt (19) in a yield of 87%

and Boc-Ala-OH using a standard coupling protocol (17). The incorporation of the third amino acid was carried out by deprotecting the dipeptide amino group with TFA, [(1) in Scheme 1] and then without prior neutralisation, coupling Boc-Lys(ClZ)-OH using BOP reagent both in the presence and in the absence of additional HOBt (20), using either DIEA or NMM as base. The formation of the DKP [(3) in Scheme 1] competes with the formation of the new peptide bond leading to the tripeptide resin [(2) in Scheme 1]. This BOP-mediated coupling was then compared with both the Suzuki procedure (21) and a standard protocol (17) for the same amino acid coupling step. (See Table 1)



SCHEME 1

An exactly analogous comparison of the same coupling procedures was carried out for the sequence D-Val -L-Pro (21), which is known to be particularly prone to DKP formation (3) (See Table 1). A standard coupling protocol gave rise to 59% formation of DKPs in the case of the dipeptide L-Ala-L-Pro and 91% for the sequence D-Val-L-Pro. The Suzuki procedure suppressed their formation completely in the former case and led to less than 10% DKP formation in the latter. When BOP was used as the coupling reagent, for both sequences the amount of DKP formed, under these conditions, was only slightly dependent upon the presence or absence of additional HOBt, slightly less DKP formation being observed when additional HOBt was used. However the amount of DKP formation was influenced by the base employed. The base is necessary in order to liberate the amino group of the dipeptide, to form the anion of the carboxylic acid for formation of the active ester and also to neutralise acidic species formed during the coupling(13), hence the use of a larger excess of base with BOP couplings than was used in the Suzuki procedure. With the sequence L-Ala-L-Pro use of NMM gave rise to 20% DKP formation , but use of DIEA led to significantly reduced amounts of DKPs (comparable to those amounts formed using the Suzuki

procedure) and a considerably shorter reaction time. No trifluoroacetylation of the amino component was detected in this study.

Coupling Procedure for the third amino acid[a]	Amino acid ratio[b]			Formation of DKPs (%)[c]
	Phe	Ala	Lys	
Standard protocol	1.00	0.36	0.26	59
Suzuki	1.00	0.88	0.92	0
BocaaOH/BOP/NMM[d] 1eq 1eq 2eq	1.00	0.70	0.68	20
BocaaOH/HOBt/BOP/NMM[d] 1eq 1eq 1eq 2eq	1.00	0.70	0.69	20
BocaaOH/BOP/NMM[d] 1eq 0.5eq 2eq	1.00	0.76	0.77	12
BocaaOH/BOP/DIEA[e] 1eq 1eq 2eq	1.00	0.82	0.94	5
BocaaOH/HOBt/BOP/DIEA[e] 1eq 1eq 1eq 2eq	1.00	0.85	0.83	2
	Phe	D-Val	Lys	
Standard protocol	1.00	0.07	0.05	91[f]
Suzuki	1.00	0.72	0.68	8[f]
BocaaOH/BOP/DIEA[e] 1eq 1eq 2eq	1.00	0.71	0.72	9[f]
BocaaOH/HOBt/BOP/DIEA[e] 1eq 1eq 1eq 2eq	1.00	0.71	0.73	9[f]

[a]Qualitative ninhydrin test was used to determine completion of coupling.
[b]Determined by amino acid analysis.
[c]Calculated from the ratio of Ala to Phe after incorporation of Lys, divided by the same ratio before incorporation (0.87) and expressed as a percentage.
[d]General procedure for these couplings - deprotection with TFA, followed by rapid washing with DCM. Each reagent (3 fold excess relative to amino component) was dissolved separately in DMF and added to the resin in the order shown in Table 1. All couplings were complete within 90 minutes.
[e]General procedure for these couplings - as for note d but complete within 60 minutes.
[f]Calculated by determining the ratio of D-Val to Phe after incorporation of Lys and then dividing this ratio by the ratio of D-Val to Phe before incorporation of Lys (0.78) and expressing this number as a percentage.

TABLE 1

These results indicate that BOP reagent used with DIEA as base is a useful alternative to the Suzuki procedure for minimising DKP formation in cases where this secondary reaction is troublesome in solid-phase peptide synthesis. Use of the BOP reagent has the advantages of ease of use (no freshly prepared HCl solution in dioxane required), short (~60 minutes) reaction times and of being amenable to use in automated protocols for solid-phase peptide synthesis.

Acknowledgements.

M.G. and P.L.W thank Ministerio de Educación y Ciencia, Madrid for financial support. We thank Isidre Casals of the Servei Científic-Tècnic de la Universitat de Barcelona and Francisco Cárdenas, for amino acid analyses.This work was supported by funds from CICYT (grant BT 86-18) and from SKF.

References.

1) Abbreviations used in this article:- Boc-tert-butoxycarbonyl-, BOP-benzotriazol-1-yl-oxy-tris-(dimethylamino)phosphonium hexafluorophosphate, Bzl-benzyl-, ClZ-2-chlorobenzyloxycarbonyl-, DCC-dicyclohexylcarbodiimide,DCM-dichloromethane, DIEA-diisopropylethylamine, DMF-dimethylformamide, HOBt -1-hydroxybenzotriazole, NMM - N-methyl morpholine, TFA - trifluoroacetic acid.

2) M.C.Khosla, R.R.Smeby and F.M.Bumpus, *J. Am. Chem. Soc.*, **94**, 4721- 4724 , (1972).

3) B.F.Gisin and R.B.Merrifield, *J. Am. Chem. Soc.*, **94**,3102-3106, (1972).

4) E.Giralt, R.Eritja and E.Pedroso, *Tetrahedron Letts.*, **22**,3779-3782, (1981).

5) E.Pedroso, A.Grandas, X.de las Heras, R.Eritja and E.Giralt, *Tetrahedron Lett.*, **27**, 743-746, (1986).

6) M.Rothe and J.Mazánek, *Angew. Chem. Int. Ed. Engl.*, **11**, 293 , (1972).

7) S.B.H.Kent, A.R.Mitchell, M.Engelhard and R.B.Merrifield, *Proc. Natl. Acad. Sci. USA.*, **76**, 2180-2184, (1979).

8) K.Suzuki, K.Nitta and N.Endo, *Chem.Pharm. Bull.*, **23**, 222-224, (1975).

9) E.Giralt, F.Albericio, E.Pedroso, C.Granier, J.van Rietschoten, *Tetrahedron*, **38**, 1193-1201, (1982).

10) A.Grandas, F.Albericio, J.Josa, E.Giralt, E.Pedroso, J.M.Sabatier, and J.van Rietschoten, *Tetrahedron*, **45**, 4637-4648, (1989).

11) G.Barany and R.B.Merrifield, *J.Am. Chem. Soc.*, **99**,7363-7365, (1977).

12) E. Pedroso, A Grandas, M.A. Saralegui, E. Giralt, C.Granier, and J.van Rietschoten, *Tetrahedron* **38**, 1183-1192, (1982).

13) D.Le-Nguyen, A.Heitz and B.Castro, *J. Chem. Soc., Perkin Trans. I*, 1915-1919, (1987).

14) B.Castro, J.R.Dormoy, G.Evin, et C.Selve, *Tetrahedron Letts.*,**14**,1219-1222, (1975).

15) Although BOP is widely used, it is important to stress that the carcenogenic HMPA is a side product and the reagent must be handled with caution.

16) P.Haux, H.Sawerthal and E.Habermann, *Hoppe-Seyler's Z. Physiol. Chem.*, **348**, 737, (1968).

17) A standard coupling protocol was as follows :- The resin was swollen by washing with DCM (4 x 1') and deprotected with 30% TFA/DCM (1 x1', 1 x 30'), washing with DCM (4 x 1'), followed by neutralisation with 5% DIEA/DCM (4 x 2') and washing again with DCM (4 x 1'). The amino acid (3 fold excess relative to the amino function) was added as a solution in DCM followed by DCC also as a solution in the same solvent. The mixture was left 60 minutes and then filtered, washing successively with DCM (4 x 1') and methanol (4 x 1').

18) E. Atherton, D.L.Clive, R.C.Sheppard, *J.Am.Chem.Soc.*, **97**, 6584-6585, (1975).

19) B.F.Gisin, *Helv. Chim. Acta* , **56**, 1476-1482, (1973).

20) D.Hudson, *J.Org.Chem.*, **53**, 617- 624, (1988).

21) Suzuki *et al.* have proposed more than one experimental procedure, the one we have used is as follows:-Deprotection of the amino function with *ca.* 4 N HCl in dioxane (1 x 1', 1 x 30'), washing with dioxane (4 x 1'), DCM (4 x 1'). The NMM - salt of the amino acid in DCM (3 fold excess relative to the amino function) was then added to the resin followed immediately by the DCC solution also in DCM. The coupling was allowed to stand at room temperature with occasional agitation for 4-5 hours. The resin was then washed well with DCM.

22) In this case the yield for incorporation of the Boc-Pro-OH was 65%.

(Received in UK 2 July 1990)