Pergamon

Tetrahedron Letters, Vol. 35, No. 25, pp. 4437-4440, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)00808-6

Solid-Phase Synthesis of Peptides Using Allylic Anchoring Groups 2. Palladium-Catalysed Cleavage of Fmoc-Protected Peptides[1, 2]

Paul Lloyd-Williams,[a] Ahmed Merzouk,[a, b] François Guibé,[b] Fernando Albericio,[a, c] and Ernest Giralt [a*]

[a] Departament de Química Orgànica, Universitat de Barcelona, E-08028 Barcelona, Spain

[b] Institut de Chimie Moléculaire d'Orsay, Laboratoire de Chimie Organique des Eléments de Transition, Orsay Cedex, France

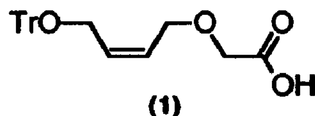
[c] Millipore Corporation, Peptide Research Group, 75A Wiggins Avenue, Bedford, MA 01730, USA

Abstract. High yields for the cleavage reaction of Fmoc-protected peptide segments from an allylic handle may be obtained using tributyltin hydride in the presence of $(Ph_3P)PdCl_2$ in a 1:1 mixture of DMF/DCM. Alternatively the cleavage reaction may be carried out using NMA as nucleophile in a 2:2:1 mixture of DMSO/THF/0.5M HCl in the presence of $(Ph_3P)_4Pd$. The Fmoc group is completely stable to both these cleavage methods.

The solid-phase synthesis of peptides using allyl handles was first reported by Kunz.[3] The use of such anchoring groups for the synthesis of protected peptide segments[4] is attractive because, in principle, they are compatible with both the Boc/Bzl- and Fmoc/*t*Bu- peptide synthesis strategies. Moreover for the Fmoc/*t*Bu strategy the allylic anchor provides a three-dimensional *orthogonal*[5, 6] protecting scheme.

Peptides may be cleaved from this type of solid support by an allyl transfer reaction brought about by treatment of a suspension of the the peptide-resin in a suitable solvent with a nucleophile (which acts an allyl acceptor) in the presence of the catalyst tetrakistriphenylphosphine palladium $[(Ph_3P)_4Pd]$. For Boc/Bzl-protected peptides we have reported on a method which gives almost quantitative cleavage yields and involves treating a suspension of the peptide-resin in 2:2:1 DMSO/THF/0.5M HCl, with morpholine as nucleophile, in the presence of $[(Ph_3P)_4Pd]$.[1] The use of a nucleophilic secondary amine as the allyl acceptor in the cleavage of Fmoc-protected peptides leads to deprotection of the Fmoc group,[7] but detachment of Fmoc-protected peptides from allyl resins has been reported using dimedone[3] or HOBt[8] as nucleophile. Our initial results using these compounds indicated that yields were not always high nor reproducible[1] and led us to investigate methods for bringing about more efficient cleavage of peptides incorporating the Fmoc group.

Cleavage of the peptide-resin bond by hydrostannolytic allyl transfer[9] using the handle **(1)** has been



(1)

reported by Loffet.[10] Since no base is required, this cleavage protocol is compatible with the use of the Fmoc group. As an alternative, use of the poorly nucleophilic base NMA[7, 11] in the method previously described by ourselves for the cleavage of Boc/Bzl protected peptides from allyl resins,[1] should provide another method for cleaving peptides protected with the Fmoc group from solid supports incorporating the handle **(1)**.

The Merrifield tetrapeptide (2), in addition to the protected peptides (3), (4) and (5) from the sequence of uteroglobin[12] were chosen as model compounds for exploring the usefulness of this chemistry in the synthesis of Fmoc-protected peptide segments.

Fmoc-Leu-Ala-Gly-Val-OH (2)
Fmoc-Leu-Ser(*t*Bu)-Glu(O*t*Bu)-Lys(Boc)-Ile-Val-Lys(Boc)-Ser(*t*Bu)-Pro-OH (3)
Fmoc-Leu-Ser(Bzl)-Glu(OcHex)-Lys(ClZ)-Ile-Val-Lys(ClZ)-Ser(Bzl)-Pro-OH (4)
Fmoc-Gln(Trt)-Thr(*t*Bu)-Thr(*t*Bu)-Arg(Pmc)-Glu(O*t*Bu)-Asn(Trt)-Ile-Met-Lys(Boc)-OH (5)

Peptides (2) and (4) were synthesised on a polystyrene resin containing the handle (1) and Phe as an internal standard. Chain elongation was carried out using standard Boc chemistry and the third amino acid in each case was incorporated using a protocol designed to minimise the formation of DKPs.[13] For both peptides (2) and (4) leucine was incorporated at the end of the synthesis as its N^{α}-Fmoc derivative. Peptide (3) was also synthesised on the same resin incorporating (1) and Phe. Proline was incorporated by esterification using Fmoc-Pro-Cl and chain elongation proceeded using standard Fmoc/*t*Bu chemistry. Unfortunately all attempts to incorporate Fmoc-Lys(Boc)-OH led to severe DKP formation and this could only be solved in this case by incorporating the dipeptide Fmoc-Lys(Boc)-Ser(*t*Bu)-OH onto the resin. Thereafter chain elongation proceeded without incident. Peptide (5) was synthesised automatically by standard Fmoc/*t*Bu chemistry using a Millipore 9050 synthesiser on PEG-polystyrene[14] incorporating (1) and norleucine as an internal standard. The extended mode program was used incorporating a special cycle for the coupling of the third amino acid, in order to reduce DKP formation.

Peptides were cleaved from the solid support either hydrostannolytically (Method A) or using modifications of methods described by ourselves[1, 7] (Method B).

Method A-The peptide-resin (100 mg, ~75 μmol) and $PdCl_2(Ph_3P)_4$ (2 mg, 2.5 μmol) were suspended in a previously degassed mixture of 1:1 DMF/DCM (5 ml) and stirred vigorously under argon. The catalyst dissolved giving the suspension a yellow colour. Tributyltin hydride (75 μl, 0.25 mmol) in DCM (1 ml) was added over 30 min. and the mixture stirred for a further 10 min. After filtration, the resin was washed with 1:1 DMF/DCM, and the filtrate extracted repeatedly (*ca.* 4 times) with pentane.[15] 1M HCl was added to convert the tin carboxylate into the peptide free acid, followed by water to precipitate the crude peptide which was isolated by centrifugation and filtration.

Method B-The peptide-resin (100 mg, ~0.075 mmol) and $Pd(Ph_3P)_4$ (30 mg, 0.026 mmol) were suspended in a previously degassed mixture of 2:2:1 DMSO/THF/0.5 M HCl (5 ml) and stirred vigorously under argon. The catalyst dissolved giving the suspension a yellow colour. NMA (385 μl, 3.5 mmol) was added and the mixture stirred under argon for 12 h. Filtration, followed by washing the resin with DMF and chloroform, and solvent removal gave the crude peptide.

The results of the cleavage of peptides (2), (3), (4) and (5) from the solid support using both these methods are summarised in Table 1.

For peptide (2) cleavage yields were high irrespective of the cleavage method used. Method A gave almost quantitative cleavage of peptide from the resin in 30 min. Method B gave slightly lower yields. No removal of the Fmoc N^{α} protecting group was detected using either method as evidenced by the co-injection of the independently synthesised H-Leu-Ala-Gly-Val-OH with the cleavage mixture.

Table 1[a]

Peptide[b]	Method A	Method B
(2)	97	86
(3)	91	80
(4)	63/28[c]	70
(5)	87	86

[a] Cleavage yields are calculated with respect to the internal standard amino acid
[b] Peptides were characterised by amino acid analysis, FAB-MS and high field NMR after purification by semi-preparative HPLC.[16]
[c] Lower yield obtained when tributyltin hydride added all at once

Cleavage of peptide (3) provides a stiffer challenge to this type of methodology but yields of up to 91% were obtained using Method A. Method B gave somewhat lower, but still acceptable yields, although for both a certain degree of irreproducibility was observed[17] and those for Method B were found to depend upon the quantity of catalyst used - higher yields being obtained with larger amounts of catalyst. [$(Ph_3P)_4Pd$] is not stable to air and should be strored under argon but we believe that this dependency on the amount of catalyst used is not due to a deterioration in the quality of the commercial catalyst as several freshly-opened batches were used and in all cases a similar dependency was observed.

It was more difficult to achieve satisfactory cleavage of peptide (4) from the resin and indeed only moderate yields were obtained in the best of cases. Method B gave the best results for this peptide but was again dependent upon the quantity of catalyst used. When morpholine was substituted for NMA,[1] no improvement in cleavage yield was observed. This latter variation of course leads to deprotection of the Fmoc group. Method A in this case gave lower yields and these dropped substantially if the tributyltinhydride was added all at once rather than over 30 min.

Cleavage yields for peptide (5) were good and little difference with respect to yield was observed between Methods A and B. Method B did however lead to appreciable oxidation of the Met residue to the sulfoxide which was presumably caused by the DMSO used in the medium. This oxidation may be avoided by carrying out the cleavage reaction by Method B in chloroform containing NMA and acetic acid (50µl NMA, 100µl acetic acid per 1 ml $CHCl_3$).[7] Again Method B (both variants) showed a degree of dependence on the quantity of catalyst used.

The yields shown in Table 1 indicate that the use of the allyl handle (1) in conjunction with Method A for cleavage from the solid support is a viable method for the synthesis of protected peptide segments. Cleavage yields range from moderate to excellent. Method B provides a useful alternative method which gives better yields in some cases but does suffer from the disadvantages that it appears to depend, to some extent, on the quantity of catalyst (better yields being obtained when more catalyst is used) and that oxidation of unprotected Met residues can occur. This latter problem may be avoided by changing to the chloroform acetic acid NMA system.[7]

These results demonstrate that the palladium-mediated cleavage of allyl handles can be brought about in high yield either hydrostannolytically or by using NMA as a nucleophile under conditions which do not remove the Fmoc group. This peptide synthesis strategy comprised of N^{α} Fmoc protecting group, *t*Bu-based side-chain protection and allyl anchoring group provides a three dimensional orthogonal peptide synthesis scheme which merits further investigation for the synthesis of protected peptide segments. The mild conditions under which the allyl group can be removed in the presence of the Fmoc group and *t*Bu-based protection might also be useful in the construction of other complex molecules.

Acknowledgments

We thank Albert Loffet of Propeptide Ltd, France, for discussions which initiated the project, for financial support to A. M. and for a gift of handle (**1**). This work was supported by grant no. PB 92-0864 from CICYT and by grant no. ERB4050PL930394 from the Commission of the European Communities.

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2 Abbreviations used in this paper for amino acids and for the designations of peptides follow the rules of the IUPAC-IUB Comission of Biochemical Nomenclature in *European J. Biochem.*, **1984**, *138*, 9-37 and *J. Biol. Chem.*, **1989**, *264*, 633-673. The following additional abbreviations are used: Boc, *tert*-butoxycarbonyl; *t*Bu, *tert*-butyl; Bzl, benzyl; cHex, cyclohexyl; ClZ, 2-chlorobenzyloxycarbonyl; DCM, dichloromethane; DKP, diketopiperazine; DMF, *N,N*-dimethylformamide; DMSO, dimethyl sulfoxide; FAB-MS, fast atom bombardment-mass spectrometry; Fmoc, 9-fluorenylmethoxycarbonyl; HOBt, 1-hydroxybenzotriazole; HPLC, high performance liquid chromatography; NMA, *N*-methylaniline; NMR, nuclear magnetic resonance; PEG, polyethyleneglycol; Ph, phenyl; Pmc, 2,2,5,7,8-pentamethylchroman-6-sulfonyl; THF, tetrahydrofuran; Trt, triphenylmethyl (trityl). The amino acid symbols used denote the L configuration except in the case of Gly.

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(*Received in UK* 29 *March* 1994; *accepted* 22 *April* 1994)