Pergamon

Tetrahedron Letters, Vol. 37, No. 51, pp. 9195-9198, 1996

0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)02154-5

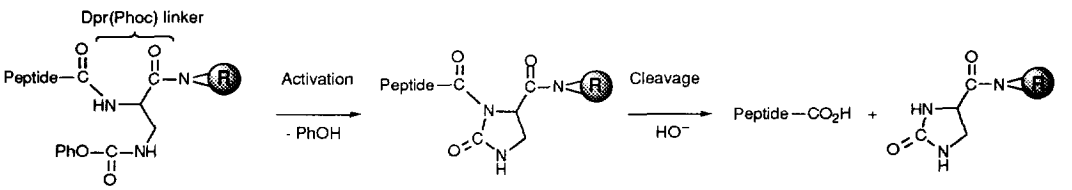
Fmoc-Based Solid-Phase Peptide Synthesis Using Dpr(Phoc) Linker. Synthesis of a *C*-terminal Proline Peptide

Régine Sola, Jean Méry, and Robert Pascal*

Centre de Recherches de Biochimie Macromoléculaire, CNRS and INSERM,
Route de Mende, BP 5051, 34033 Montpellier, France

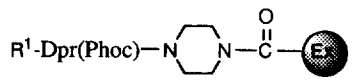
Abstract: Cyclic acylurea readily formed from Dpr(Phoc) linker was shown to provide linkage that is fully compatible with standard Fmoc-based solid-phase peptide synthesis protocols. The utility of this linkage was checked during Tyr-Asp-Pro-Ala-$(Pro)_6$-OH synthesis which is very prone to diketopiperazine formation at the dipeptide stage. This side-reaction was avoided by maintaining the Dpr(Phoc) linker until the tripeptide stage, while piperidine had to be temporary replaced with morpholine as reagent for Fmoc group removal. Copyright © 1996 Published by Elsevier Science Ltd

We previously described a safety-catch linker for solid-phase peptide synthesis [Dpr(Phoc) linker] specifically designed for Boc chemistry (Scheme 1).[1] We report here that particular features of this new linker are also of interest in Fmoc-chemistry.

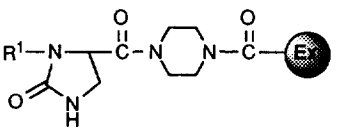


Scheme 1. Activation and cleavage of the Dpr(Phoc) linker (Dpr = L-2,3-diaminopropionic acid, Phoc = phenyloxycarbonyl).

Standard ester-based linkages can undergo a well known side-reaction at the dipeptide stage, leading to loss of peptides from the resin by diketopiperazine formation.[2] The extent of this side-reaction is generally limited but increases markedly when the cis conformation of the peptide bond is favoured, *e.g.* with peptides having a *C*-terminal proline.[2a-b] Moreover, this intramolecular aminolysis is promoted by the basic conditions of Fmoc protecting group removal.[2c] Various methods have thus been proposed for Fmoc-based solid-phase peptide synthesis, such as introduction of the second and third amino acids as a protected dipeptide segment,[3] use of anchorages based on hindered tertiary alcohols,[4-6] and α-amino protection of the second amino acid by a trityl group.[7] The presence of stable amide linkage in the Dpr(Phoc) linker instead of the usual ester linkage could be an alternative. To test this point, we selected Tyr-Asp-Pro-Ala-$(Pro)_6$-OH synthesis which is very prone to diketopiperazine formation.[6] A first attempt starting from the Fmoc-Pro resin **1a** (0.400 g, 5.2×10^{-4} equiv/g)[8] and using standard protocols[9] indicated no loss of dipeptide, as shown by the increased weight of the resin (0.558 g) and spectroscopic monitoring of Fmoc removal. However, after side-chain deprotection [TFA-H_2O (19:1), 1 h] and hydrolytic cleavage [0.04 M NaOH in ^{i}PrOH-H_2O (7:3), 24 h], the decapeptide



1



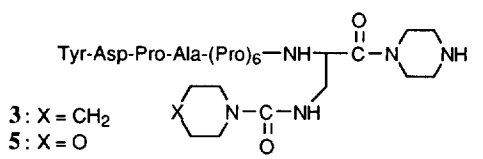
2

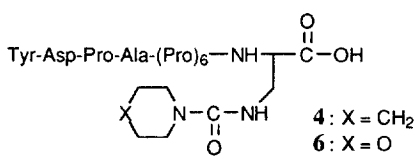
Ex : Expansin resin

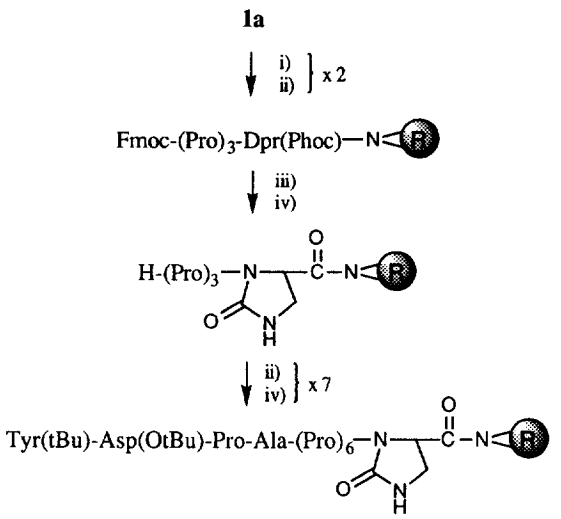
a : R^1 = Fmoc-Pro
b : R^1 = Bz-Pro
c : R^1 = Pro-Pro

was released in very low yield (< 5%). Extended cleavage (120 h) allowed the release of two major side-products that were characterized, by electrospray ionization-mass spectrometry after HPLC purification, as derivatives with molecular weights (M = 1243 and 1311) higher than that of the parent peptide (M = 1046). Piperidine derivatives **3** and **4** are consistent with the observed mass spectra. Formation of their polymer-bound precursors can be explained by a side-reaction of the phenyl carbamate moiety of Dpr(Phoc) residue with piperidine, which makes cleavage of the linkage impossible. Their release in solution is accounted for by alkaline hydrolysis of both piperazine amide bonds with rates higher than that of peptide bonds.

Further investigations were aimed at retaining the advantage of amide linkage while limiting the extent of phenyl carbamate side-reaction. Firstly, this side-reaction was shown to be reduced when using morpholine instead of piperidine as Fmoc removal reagent: after 1 h treatment of resin **1b**[10] in morpholine-DMF (1:1), the amount of Bz-Pro and phenol released was 80% of the initial value, instead of *ca.* 25 % after 30 min in piperidine-DMF (1:4). Secondly, we demonstrated that the Dpr(Phoc) linker was quantitatively converted into the cyclic acylurea form, **2**, without cleavage of the anchorage by dilute solutions of PhONa-PhOH in DMF[11]: 2 h treatment of resin **1b** with a solution containing PhONa (10^{-2} M) and PhOH (10^{-3} M) in DMF allowed the conversion into resin **2b** with no loss of Bz-Pro (HPLC analysis of the solutions obtained after alkaline hydrolysis of resin **2b** indicated the absence of PhOH and the amount of Bz-Pro was identical to that obtained from resin **1b**). Lastly, the cyclic form of the linker, **2**, was shown to be stable in piperidine-DMF (1:4) since the resin obtained after 44 h treatment of resin **2b** was still able to release benzoylproline in 85 % yield upon hydrolytic cleavage. As diketopiperazine formation is not suppressed by hindrance of the cyclic form **2**,[12] we retained the synthesis protocols of Scheme 2 which involve (i) use of the Dpr(Phoc) linker associated with Fmoc group removal by morpholine-DMF 1:1 for the first and second aminoacids, (ii) cyclization of the linker at the tripeptide stage, (iii) standard Fmoc chemistry for the following steps. Two syntheses of the decapeptide were carried out using a manual batch apparatus and a Milligen 9050 continuous flow synthesizer. The resin **1a** (0.402 g, 5.2 x 10^{-4} equiv/g) was submitted to the manual synthesis protocol, giving 0.529 g peptide resin. After side-chain deprotection [TFA-H_2O (19:1), 1 h], the peptide was released from the resin using a new procedure[13] involving $CaCl_2$ catalysis [0.6 M $CaCl_2$ in ^{i}PrOH-H2O (7:3), 5 ml to which 0.51 ml 1 N NaOH, 2.4 equiv,[14] was added, 2 x 3 h]. The solution was neutralized with acetic acid, desalted on a Sephadex G-10 column and freeze-dried, giving a white solid (0.120 g, 55%) containing the decapeptide at 90% purity, as shown by HPLC analysis (Fig. 1) together with a major impurity (6%) identified as the des-proline nonapeptide (the only side-product resulting from the non-completion of 10 coupling or deprotection steps). Further cleavage under strongly alkaline conditions (0.2 N NaOH in ^{i}PrOH-H_2O 7:3, 147 h) released several side-products resulting from the degradation of the Dpr(Phoc) linker by morpholine (40% overall yield, Fig. 1).[15] Continuous flow synthesis gave similar results, except that the des-proline nonapeptide was obtained with increased yield (20%), probably because of the shorter deprotection time used for the morpholine-DMF (1:1) reagent (20 min[16] instead of 35 min).







Scheme 2. Solid-phase synthesis of the decapeptide.
(a) Batch synthesis: (i) Morpholine-DMF (1:1) (5 ml, 1 x 1 min + 1 x 4 min + 1 x 10 min + 1 x 20 min); DMF washes (5 ml, 6 x 0.5 min); (ii) 55 min Fmoc amino acid coupling in DMF after 7 min preactivation (Fmoc-AA 3.1 equiv, TBTU 3.0 equiv, HOBt 3.0 equiv, *N,N*-diisopropylethylamine 3.6 equiv); DMF washes (5 ml, 4 x 0.5 min); (iii) 10^{-2} M PhONa - 5 x 10^{-3} M PhOH in DMF (25 ml, 1 x 5 min + 1 x 15 min + 4 x 25 min); DMF washes (5 ml, 6 x 0.5 min); (iv) piperidine-DMF (1:4) (2 x 1 min + 1 x 5 min).
(b) Continuous flow synthesis: (i) Morpholine-DMF (1:1) (1.5 ml/min, 20 min) introduced as "auxiliary wash"; DMF wash (2.5 ml/min, 7 min); (ii) 45 min Fmoc amino acid (4 equiv) coupling in DMF [activation with TBTU (4 equiv), HOBt (4 equiv), *N*-ethylmorpholine (6 equiv)]; DMF wash (6 min); (iii) 10^{-2} M PhONa - 2 x 10^{-3} M PhOH in DMF (1.5 ml/min, 120 min) introduced as "auxiliary wash"; DMF wash (6 min); (iv) piperidine-DMF (1:4) (2.5 ml/min, 9 min).

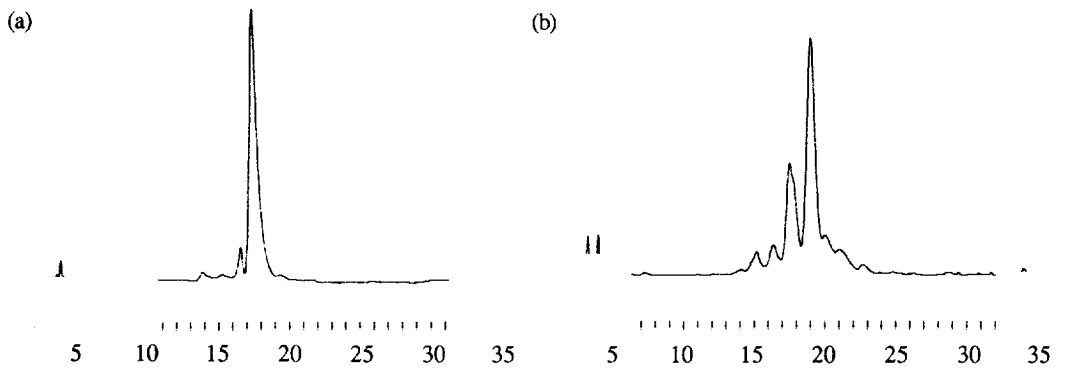


Fig. 1. HPLC profiles of (a) the Sephadex G-10 purified peptide and (b) the crude solution obtained from extended 0.2 N NaOH cleavage. HPLC conditions: Brownlee Aquapore RP-300 7 µm column 220 x 4.6 mm; buffer A, 0.1 % aq. TFA; B, MeCN (0.06 % TFA); linear gradient 5-25 % B over 20 min and then 25-90 % B over 15 min; detection 220 nm. FAB-MS identification of HPLC peaks: (a) r.t. 17.3 : *m/z* 1047 ($M+H^+$), Tyr-Asp-Pro-Ala-(Pro)6-OH; r.t. 16.5 : *m/z* 950 ($M+H^+$), Tyr-Asp-Pro-Ala-(Pro)5-OH; (b) r.t. 17.6 : *m/z* 1314 ($M+H^+$), **5** and *m/z* 1149 ($M+H^+$), [des-Pro]**6**; r.t. 19.0 : *m/z* 1246 ($M+H^+$), **6**.

The results described here show that the Dpr(Phoc) linker, orthogonal with a Boc/benzyl strategy for solid-phase peptide synthesis,[1] is compatible with Fmoc/^{t}Bu chemistry, provided that piperidine is avoided as deprotection reagent and replaced with the morpholine-DMF (1:1) reagent, which is acceptable for a few steps. Furthermore, complete compatibility with the piperidine reagent was observed after conversion into the acylurea form **2**, which can be carried out as soon as the first residue has been coupled to the linker or two residues later to suppress diketopiperazine formation. Compatibility of the Dpr(Phoc) linker with both Boc and Fmoc strategies for solid-phase peptide synthesis would be of interest for the preparation of peptide conjugates or cyclic peptides that require multidimensional protection schemes.

REFERENCES AND NOTES

1. Sola, R.; Saguer, P.; David, M.-L.; Pascal, R. *J. Chem. Soc., Chem. Commun.* **1993**, 1786-1788.
2. a) Gisin, B. F.; Merrifield, R. B. *J. Am. Chem. Soc.* **1972**, *94*, 3102-3106. b) Khosla, M. C.; Smeby, R. R.; Bumpus, F. M. *J. Am. Chem. Soc.* **1972**, *94*, 4721-4724. c) Pedroso, E.; Grandas, A.; de las Heras, X.; Eritja, R.; Giralt, E. *Tetrahedron Lett.* **1986**, *27*, 743-746.
3. Dalcol, I.; Rabanal, F.; Ludevid, M.-D.; Albericio, F.; Giralt, E. *J. Org. Chem.* **1995**, *60*, 7575-7581.
4. Barlos, K.; Gatos, D.; Kallitsis, J.; Papaphotiou, G.; Wenquing, Y.; Schäfer, W. *Tetrahedron Lett.* **1989**, *30*, 3943-3946; Barlos, K.; Gatos, D.; Kapolos, S.; Papaphotiou, G.; Schäfer, W.; Wenquing, Y. *Tetrahedron Lett.* **1989**, *30*, 3947-3950
5. Akaji, K.; Kiso, Y.; Carpino, L. A. *J. Chem. Soc., Chem. Commun.* **1990**, 584-586.
6. Kochansky, J.; Wagner, R. M. *Tetrahedron Lett.* **1992**, *33*, 8007-8010.
7. Alsina, J.; Giralt, E.; Albericio, F. *Tetrahedron Lett.* **1996**, *37*, 4195-4198.
8. The starting resin was prepared from Expansin resin using Boc chemistry as previously described [ref 1]. Expansin resin was a generous gift from Expansia, F-30390 Aramon, France.
9. Milligen 9050 peptide synthesizer. Fmoc protecting group removal by piperidine-DMF (1:4), 7 min.
10. Bz-Pro resin **1b** was prepared from Fmoc-Pro resin **1a** by deprotection with piperidine-DMF (1:4) (7 min) followed by the reaction of benzoyl chloride in the presence of pyridine in DMF.
11. Preparation of PhONa-PhOH solutions: a 1.5 molar excess of PhOH was dissolved in 1N NaOH, the addition of dry DMF followed by removal of the solvent under reduced pressure were repeated twice and the residue was diluted with a 1×10^{-3} - 5×10^{-3} M solution of PhOH in dry DMF.
12. A 70 % loss of dipeptide was observed during 9 min treatment of Pro-Pro-resin **2c** with piperidine-DMF (1:4), followed by DMF washes (6 x 0.5 min).
13. Pascal, R; Sola, R. in preparation.
14. A 20% excess only because of the presence of β-aspartyl and *C*-terminal carboxyl groups.
15. The extent of the side-reaction with morpholine might be reduced by *ca*. 50% if the first residue is introduced as a Boc-protected amino acid provided that it has no side-chain protection.
16. A 20 min deprotection time has been recommended for glycopeptide synthesis [Meldal, M.; Bielfeldt, T.; Peters, S.; Jensen, K., J.; Paulsen, H.; Bock, K. *Int. J. Pept. Protein Res.* **1994**, *43*, 529-536].

(*Received in France* 8 *October* 1996; *accepted* 4 *November* 1996)