Tetrahedron Letters, Vol. 33, No. 13, pp. 1775-1778, 1992
Printed in Great Britain

0040-4039/92 $3.00 + .00
Pergamon Press Ltd

A New Fluorene-Derived Anchor for Solid-Phase Synthesis of Protected Peptides

Francesc Rabanal, Ernest Giralt, and Fernando Albericio*

Department de Química Orgànica, Facultat de Química, Universitat de Barcelona, E-08028 Barcelona, Spain

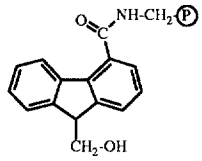
Keywords: base-labile handle, convergent solid-phase peptide synthesis, protected peptides.

Abstract: *N*-[9-(hydroxymethyl)-2-fluorenyl] succinamic acid may be used as an anchoring linkage for the preparation of protected peptide segments in combination with Boc/Bzl[1] protection scheme. The new handle proved to be stable to the usual conditions of solid-phase peptide synthesis and gave the protected peptide in high yields and purities by treatment with piperidine or morpholine.

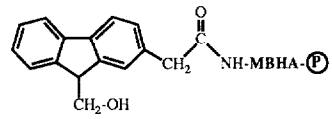
Convergent solid-phase peptide synthesis appears to be the most plausible strategy for the synthesis of large peptides and proteins.[2] This strategy involves the preparation by solid-phase methodology of fully protected peptide segments, their purification in solution followed by their assembly on a new solid support and finally, the simultaneous cleavage of the side chain blocking groups and detachment of the target peptide from the resin. Ideally, preparation of protected peptide fragments requires a three-dimensional *orthogonal* scheme of protection involving both temporal and semipermanent blocking groups as well as a bifunctional linkage agent, *handle* which serves to attach the growing peptide to the resin.[3] Detachment of the peptide from the resin under sufficiently mild conditions leaves protecting groups unaffected and affords a fully protected intermediate suitable for later segment condensation.

Up to now, several handles have been designed for this convergent strategy using the Boc/Bzl and Fmoc/*t*-Bu peptide synthesis chemistries. As far as the Boc/Bzl scheme of protection is concerned, protected peptides can be prepared by use of the photolabile *ortho*-nitrobenzyl[4] and α-methylphenacyl[5] handles, allyl-derived anchoring linkages [cleaved with Pd(0) in the presence of nucleophiles][6] and nucleophile- and base-labile handles.[7] These last ones offer the advantage that no special equipment is required (e. g. photolysis reactor for photolabile handles) and the use of sensitive reaction conditions is avoided (e.g. as those described for the cleavage of allyl-type handles).[6d] Furthermore, the scale up should not represent any inconvenience.

Our aim concentrates on the development of a new base-labile fluorene-derived handle which is easy to synthesize, stable to solid-phase peptide synthesis conditions, and capable of yielding the protected peptide quantitatively at the cleavage step.



1



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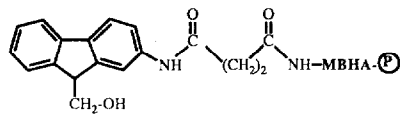


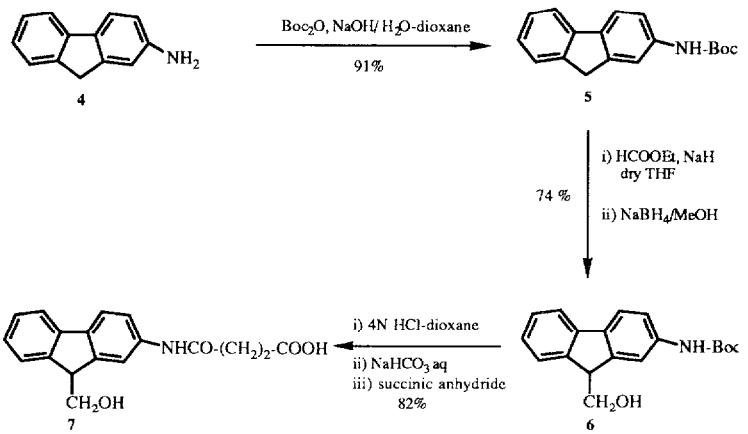
3

Previous anchor structures based in the fluorene ring (**1**[7c,d,f] and **2**[7e] see above) have been reported not to be trouble-free, as it will be discussed later. As an alternative, we propose the new anchor-resin **3**, which contains the handle, *N*-[9-hydroxymethyl)-2-fluorenyl] succinamic acid (HMFS handle). This new anchoring linkage proved to fullfill the above mentioned requirements.

N-[9-hydroxymethyl-2-fluorenyl] succinamic acid (**7**) was synthesized in four steps from commercially available 2-aminofluorene in an overall yield of 55% after purification (scheme).[8] The HMFS handle (1.5 equiv.) was attached to an MBHA polystyrene resin (0.56 mmol/g) containing Phe as internal reference with *N, N'*-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) using *N,N*-dimethylformamide (DMF) as solvent (25° C, overnight reaction) in quantitative yield. Next, the *C*-terminal Boc-amino acid (5 equiv) was anchored to the hydroxymethyl group of the resin-bound handle using DCC in the presence of *N,N*-dimethylaminopyridine (DMAP; 0.5 equiv.) in DMF (double coupling, 1 h, 25° C) again in quantitative yield. The following amino acid residues of the sequence were incorporated stepwise according to Boc/Bzl protocols used in our laboratory.[4b]

Boc-Val-OH was attached to handle-resin **3** and used as a model to evaluate the stability of the linkage towards DMF and *N, N*-diisopropylethylamine (DIPEA). Amino analysis of both the acid hydrolysates of the resin before and after the cleavage and of the filtrates showed that handle-resin **3** is stable to DMF (24 h, 25°C) and 5% DIPEA in dichloromethane (no loss of Val after 2 h, <8% loss of Val after 24 h, 25 °C). The usefulness of the new handle was demonstrated by the synthesis of the Merrifield peptide Boc-Leu-Ala-Gly-Val-OH and the peptide corresponding to the sequence 31-38 of uteroglobin, Boc-Asp(OcHx)-Asp(OcHx)-Thr(Bzl)-Met-Lys(ClZ)-Asp(OcHx)-Ala-Gly-OH. The peptides were successfully cleaved either with piperidine or morpholine (20% in DMF, 2h, 25°C). However, we observed that morpholine cleavage rendered cleaner HPLC profiles than those afforded by piperidine. Thus, purities of >95% and 93% were achieved with morpholine for Merrifield peptide and Uteroglobin (31-38), respectively, whereas 92% and 85% purity were obtained with piperidine.

Scheme



According to these experimental data, HMFS handle proves to be superior to other fluorene-derived handles previously reported. Thus, incomplete removal of the protected peptide from the resin has been described occasionally for handle **1**[7d] and slight lability to basic amino groups of the growing peptide chain has been detected for anchor **2**.[7e] The electron-withdrawing effect provided by substituents such as -CONH- and -CH_2-CONH- may increase the acidity of the hydrogen at position 9 of the fluorene ring (handles **1** and **2**) thus facilitating the elimination process by bases such as amino groups of the peptide chain or DIPEA, commonly used in peptide synthesis at the neutralization step after Boc deprotection. In contrast, the fluorene nucleus in the HMFS handle (**3**) has been conveniently substituted with an electron-donating *N*-amide group to *fine-tune* its base lability. Thus, stability to those bases is achieved and consequently, the growing peptide chain is not prematurely lost. However, lability towards secondary bases like piperidine or morpholine is preserved, allowing the release of the target peptide from the resin.

In conclusion, the new handle *N*-[9-(hydroxymethyl)-2-fluorenyl] succinamic acid is easily synthesized and can be used with any amino-functionalized resin. The anchor is entirely stable throughout the assembly of peptide chains by Boc/Bzl chemistry. Final cleavage with 20% piperidine or morpholine provides protected peptide in high yields and homogeneities, though cleavage with morpholine is the best choice.

Acknowledgements. This work was supported by funds from ICI-Farma and Comision Interdepartamental para la Ciencia y la Tecnologia (CICYT grant PB 89-257). We thank Ronald Cotton (ICI-Pharmaceuticals) for fruitful discussions which helped us to start this work. The comments of Paul Lloyd-Williams during the preparation of this manuscript are also appreciated.

References and Notes

1. Abbreviations used are: Boc, *tert* -butyloxycarbonyl; Bzl, benzyl; ClZ, 2-chlorobenzyloxycarbonyl; DCC, *N*,*N'*-dicyclohexylcarbodiimide; DIPEA, *N*,*N*-diisopropylethylamine; DMAP,

N,N-dimethyl-4-aminopyridine; DMF, *N,N*-dimethylformamide; Fmoc, 9-fluorenylmethyloxycarbonyl; HMFS, *N*-[9-hydroxymethyl)-2-fluorenyl] succinamic acid; HOBt, 1-hydroxybenzotriazole; MBHA, 4-methylbenzhidrylamine resin;. OcHx, O-cyclohexyl.

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8. . The hydroxymethylation followed a modified version of Carpino's procedure for fluorene [L. A. Carpino, *J. Org. Chem.*, **45**, 4250-4252 (1980)]. The reaction is best carried out in dry THF under argon atmosphere at 35°C. The reaction time should not exceed 25-30 minutes since a byproduct of unknown structure is formed in appreciable yield.

9. The stability of the most common Bzl-derived protecting groups has been tested and these have been shown to be stable to both cleavage conditions with the exception of Tosyl and 2, 4-dinitrophenyl for His. M. Gairí, unpublished results.

(Received in UK 13 December 1991)