Pergamon

Tetrahedron Letters 40 (1999) 377–380

TETRAHEDRON LETTERS

4-(3-Hydroxy-4-methylpentyl)phenylacetic Acid as a New Linker for the Solid Phase Synthesis of Peptides with Boc Chemistry

Katri Rosenthal, Mikael Erlandsson and Anders Undén*

Department of Neurochemistry & Neurotoxicology, Stockholm University, S-10691 Stockholm, Sweden

Received 15 September 1998; accepted 26 October 1998

Abstract: The anchoring the first amino acid in Boc chemistry to a 4-(3-hydroxy-4-methylpentyl)phenylacetic acid linker is described and compared to the conventional Pam resin. The peptidyl-4-(4-methyl-3-pentoxy)phenylacetamide linkage is slightly more stable to TFA than the Pam linker but in contrast to the Pam linker stable to cleavage of benzylic protective groups with TFMSA/DMS/TFA mixtures. This allows a mild and convenient two step deprotection procedure using the "low TFMSA-high HF". In HF this new linker reacts preferentially in an intramolecular reaction forming a tetrahydronaphthalene derivative.

The recommended method for anchoring the first amino acid in Boc/benzyl solid phase peptide synthesis has, for two decades, been coupling the first amino acid in the form of 4-(acyloxymethyl)phenylacetic acid (OMPA) derivative to an aminomethyl resin forming a phenylacetamidomethyl (Pam) bridge between the peptide and the resin as suggested by Merrifield and co-workers.[1] Although the Pam linkage has been successfully employed in the synthesis of a large number of both long peptides and proteins, this type of linkage is not without problems. During the acidolytic cleavage of the protected peptide attached to the resin, reactive carbocations are formed that can alkylate sensitive residues on the peptide. This type of side-reaction can be significantly reduced if the reaction mechanism for cleavage is changed by using a superstrong acid together with a nucleophile where the benzylic protective groups are cleaved by an S_N2 mechanism using HF or TFMSA together with dimethylsulphide ("low HF" and "low TFMSA"), methods developed by Tam and Merrifield.[2,3] As these methods do not cleave the commonly used protective groups for aspartic acid, arginine, and cysteine the peptide usually has to be subject to a second cleavage step, preferentially anhydrous HF.

Unfortunately, both the "low HF" and the "low TFMSA" methods result in extensive cleavage (more than 90 %) of the peptide-Pam linkage.[4] This leaves the partially deprotected peptide in a complex acidic solution from which it has to be precipitated as in the case of the "low TFMSA" procedure, or together with a large amount of reactive dimethylbenzyl sulfonium salts that rapidly have to be removed after the final cleavage as in the case of "low HF". Moreover, both distillation and evaporation of HF are complicated when DMS is present in the HF reaction vessel.

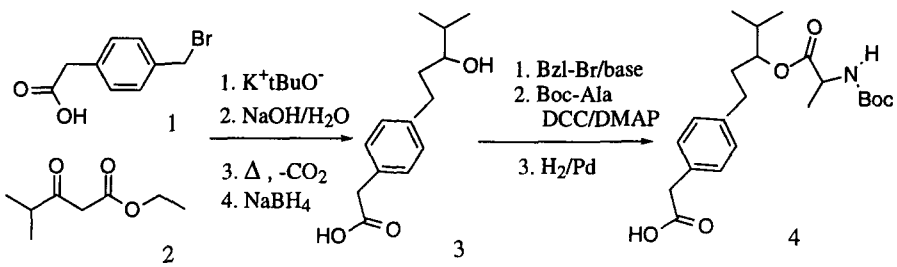
It can therefore be concluded that the optimal and most convenient two-step deprotection procedure for the synthesis of peptides with Boc/benzyl strategy would be treatment with the "low TFMSA" procedure where most of the benzylic protective groups are cleaved by an S_N2 mechanism and Met(SO) is reduced in

0040-4039/99/$ - see front matter
PII: S0040-4039(98)02316-8

the TFMSA step allowing the removal of dimethylsulfonium salts by filtration, followed by anhydrous HF where residual protective groups and the peptide-resin linkage are cleaved.

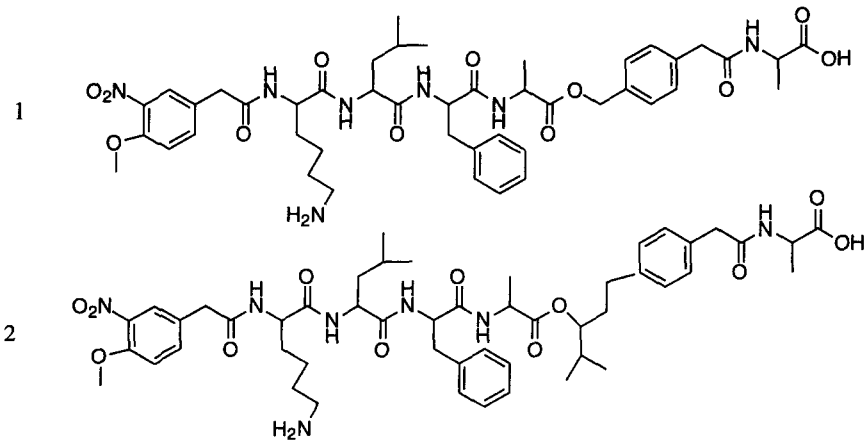
In this communication we describe the synthesis and properties of a new linker where these problems associated with conventional Pam-linker are avoided by anchoring the first amino acid residue as an ester of 4-(3-hydroxy-4-methylpentyl)phenylacetic acid **3** .

The synthesis of Boc-alanyl-4-(3-oxy-4-methyl-pentyl)phenylacetic acid (Boc-alanyl-OMPPA) is outlined in Scheme 1. α-Bromotolylacetic acid **1**, prepared as described,[5] was used to alkylate ethyl isobutyrylacetate **2** followed by saponification of the ester and thermal decarboxylation under reduced pressure of the resulting oil. The ketone was reduced and carboxylic acid group protected as a benzylester. Boc-Ala was coupled by DCC/DMAP in DCM and the benzyl protective group removed by catalytic hydrogenation to give the Boc-OMPPA handle **4**.[6]



Scheme 1. Synthesis of Boc-alanyl-OMPPA handle.

The relative stability of the linker as compared to the corresponding (acyloxymethyl)phenylacetic (OMPA) derivative forming the Pam linker was studied by treatment of the model peptide Mnp-Lys-Leu-Phe-X-Ala-OH (Scheme 2) by neat TFA (X= alanyl-OMPA or alanyl-OMPPA).



Scheme 2. Structures of Mnp-Lys-Leu-Phe-Ala-OMPA-Ala-OH (1) and Mnp-Lys-Leu-Phe-Ala-OMPPA-Ala-OH (2).

The α-amino group of lysine was acylated by (4-methoxy-3-nitrophenyl)acetic acid; Mnp. The HPLC profiles were monitored at 340 nm which is the absorbency maximum for (4-methoxy-3-nitrophenyl)acetic acid. After 5 days at 40°C 33.6 % of the Pam type of linker was cleaved as compared to 4.9 % in the case of the alanine anchored to the OMPPA linker **4**.

The stability of the Boc-OMPA and Boc-OMPPA to "low TFMSA" reaction conditions (TFMSA:DMS:TFA:*p*-cresol 1:3:5:1 v/v; 0 °C, 6 h) was studied using the same model peptide (Scheme 2). We have found that 88.9 % of the OMPA linkage and only 1.3 % of the new OMPPA linkage was cleaved, probably by a S_N1 mechanism as a result of the relatively high acid strength ($H_O \approx -5.2$).[4]

During acidolytic cleavage of protective groups and peptide-resin linkage with standard liquid HF in the presence of 10% scavenger, reactive carbocations are generated that can irreversibly alkylate the sensitive side chain functional groups on the peptide. Carbocations formed from the peptide linker do not normally result in decreased purity of the product but can decrease the yield by irreversibly alkylating cysteine, methionine, tyrosine and tryptophan residues.

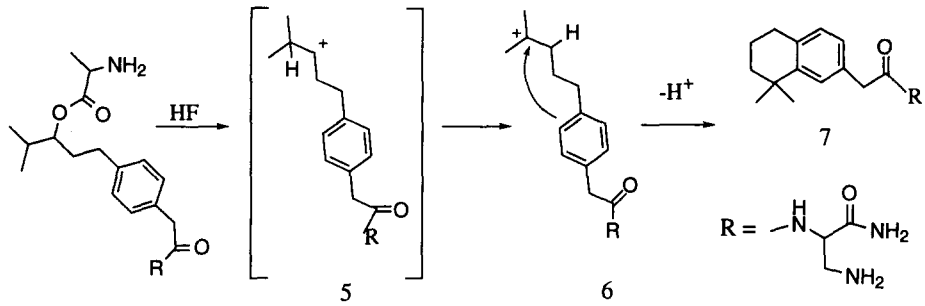
In order to investigate the relative reactivity of the carbocations formed upon cleavage of the Pam type of linker as compared to the new linker, Boc-Ala-OMPA and Boc-Ala-OMPPA were coupled to the N-terminus of the model peptide Lys(ClZ)-Leu-Phe(NO_2)-Ala synthesised on a Merrifield resin, and the peptide was finally cleaved by HF in the presence of 10 % *p*-cresol or *p*-thiocresol as scavengers. In the presence of *p*-cresol more than 99% of the peptide could be recovered as a *p*-cresol adduct in the case of OMPA linker while no analogous product could be detected when Boc-Ala-OMPPA was incorporated.

Table 1. HF, 0°C, 1h Reactivity of OMPA and OMPPA Handles in the Presence of Scavengers.

Scavenger	OMPA linker	OMPPA linker
p-cresol	>99% of cresol adduct	no cresol adduct
p-thiocresol	>99% of thiocresol adduct	73 % of thiocresol adduct

When the more reactive *p*-thiocresol was used as a scavenger again more than 99 % of the peptide was recovered as a scavenger adduct in the case of the OMPA linker but only 73 % of the OMPPA linker. As the reactivities of these scavengers are similar to tyrosine and cysteine residues in a peptide these results suggest that the danger of reattachment of the peptide to the resin upon HF cleavage is significantly reduced with this new linkage. Massspectrometrical analysis of the products formed upon cleavage of Ala-OMPPA in HF/*p*-cresol and partially in HF/*p*-thiocresol indicated that instead of reacting with the scavenger, the linker had undergone an cyclisation or elimination reaction. To characterise the product formed upon cleavage, Boc-Ala-OMPPA was coupled to the MBHA resin bound diaminopropinoic acid (Dap) where the side-chain amino group of Dap was protected by a benzyloxycarbonyl group and treated with HF in the presence of *p*-cresol. NMR analysis of the resulting product showed that 3-amino-2-[2-(8,8-dimethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)-acetylamino]-propionamide **7**[7] had been formed. Based on these results we therefore suggest that upon cleavage of OMPPA handle the initially formed secondary cation (**5**, Scheme 3) rearranges

to the more stable tertiary carbocation **6** which undergoes an intramolecular cyclisation forming the dimethyltetrahydronaphthalene derivative **7**.



Scheme 3. Proposed mechanism for the reaction of the OMPPA linker in HF.

ACKNOWLEDGEMENTS

This work was supported by Swedish Research Council for Engineering Sciences, Swedish Natural Science Research Council and Magnus Bergwall stiftelse.
The authors wish to thank Peter Damberg at the Department of Biophysics for excellent NMR assistance.

REFERENCES AND NOTES

1. Mitchell, A.R.; Kent, S. B. H.; Engelhard, M.; Merrifield, R. B. J. Org. Chem. 1978, 43, 2845-2852.

2. Tam, J. P.; Heath, W. F.; Merrifield, R. B. J. Am. Chem. Soc. 1983, 105, 6442-6455.

3. Tam, J. P. J. Org. Chem. 1985, 50, 5291-5298.

4. Tam, J. P.; Merrifield, R. B. in *The Peptides*, Vol.9, eds. Udenfreund, S.; Meienhofer, J., Academic Press, New York, 1983, pp. 187, 232-233.

5. Chauffe, L.; Andrews, L. J.; Keefer, R. M. J. Org. Chem. 1966, 31, 3758.

6. NMR spectra were recorded in DMSO-d6 on a Varian 400 MHz spectrometer.
^{13}C NMR (100 MHz, DMSO-d6) δ 174.6, 172.7, 155.2, 139.9, 132.3, 129.2, 128.1, 77.9, 77.2, 50.1, 49.4, 48.7, 31.0, 28.1, 27.3, 18.3, 17.0 (two CH_3)

7. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.26 (1H), 7.95 (2H), 7.35 (1H), 7.23 (1H), 6.93 (2H), 4.47 (1H), 3.44 (2H), 3.40 (1H), 2.95 (1H), 2.68 (2H), 1.73 (2H), 1.62 (2H), 1.23 (6H)
^{13}C NMR (100 MHz, DMSO-d6) δ 171.0 (two CO), 144.8, 133.5, 132.9, 128.5, 127.2, 126.2, 49.9, 41.8, 41.3, 33.3, 31.6 (two CH_2), 29.7, 19.2. Analysis performed in acetone-d6 showed an additional peak at δ 38.8