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Properties and Applications of the (2-Nitrofluoren-9-yl)methoxycarbonyl Group

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Abstract: This paper presents a new protecting group, the (2-nitrofluoren-9-yl)methoxycarbonyl group. Investigations on the properties of this new modification of the Fmoc-system, such as the solvent-dependant photochemical cleavage, and enhanced lability towards bases, are described, as well as UV-kinetic measurements of the cleavage reaction. In addition, the incorporation of the (2-nitrofluoren-9-yl)methoxycarbonyl group into two peptides, and a sequence-dependant photochemical cleavage reaction are reported. Copyright © 2001 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: amino acid derivative; (2-nitrofluoren-9-yl)methoxycarbonyl amino acids; peptide synthesis; photochemical cleavage; protecting group

Twenty-five years ago, the 9-fluorenylmethoxycarbonyl-group (Fmoc) was introduced to organic chemistry and peptide synthesis as base-labile protecting group for amino-functions [1–4]. There are only few reports of modifications of the Fmoc-system [2,4–7]. A further modification of the Fmoc-system, the (2-nitrofluoren-9-yl)methoxycarbonyl, Fmoc(NO_2) protecting group has recently been described [8], and we now report further on its properties and applications.

The respective amino acid derivatives are prepared by facile nitration of Fmoc-amino acids in the solvent system 100% nitric acid/anhydrous dichloromethane. The conversions are nearly quantitative [8]. These amino acid derivatives have been used for photochemical investigations, and for investigations of the base-stability.

The photochemical experiments were performed with solutions of Fmoc(NO_2)-Ala-OH **1** (1 mg/mL) in various solvents. The solutions were irradiated at 366 nm. While no change was observed in ethyl acetate, acetone or acetonitrile, **1** decomposed relatively rapidly in DMF or DMSO to yield alanine and 2-nitrodibenzofulvene, as indicated by chromatographic and spectroscopic investigations (Figure 1). Irradiation at 366 nm for 6 h of more concentrated solutions (30 mg/mL) in ethyl acetate, acetonitrile or acetone led to about 10% decomposition. Comparative studies of the cleavage kinetics in DMSO relative to ethyl acetate gave a ratio of about 1:1000.

In order to explain this phenomenon, the possibility of a reaction analogous to the photolytic cleavage of *o*- or *p*-nitrobenzyl groups was considered [9]. Because, however, the nitro group in the fluorene system is in the 2-position ('meta' to the 9-position, from which the proton would be abstracted from in such a reaction), and no nitroso-compound was detectable after irradiation, this intramolecular mechanism could be excluded. Nonetheless, it can be assumed that the readily cleaved proton at the 9-position of the fluorene system migrates under solvent mediation to the photochemically excited nitro group, thus forming 2-nitrodibenzofulvene (Figure 2).

This suggestion is supported by the observation that addition of 10% acetic acid to DMF or DMSO solutions retards the cleavage reaction significantly,

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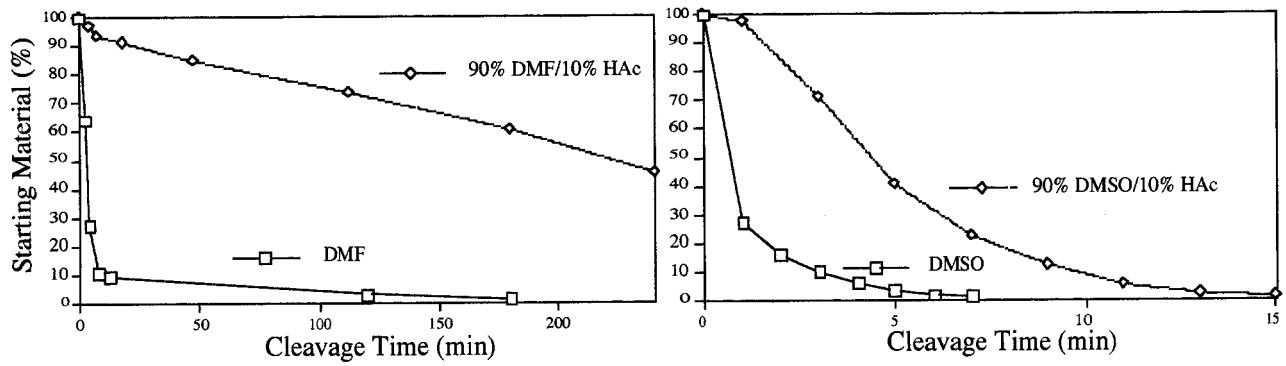

Figure 1 Determination of the photolytic cleavage kinetics of Fmoc(NO_2)-Ala-OH at 366 nm in different solvents (concentration 1 mg/mL).

and to a larger extent than would be expected from the dilution (Figure 1).

The cleavage reaction was also monitored by UV-spectroscopy. Prior to irradiation of a 0.5 mmol solution of **1** in DMF, the UV-spectrum showed only a single absorption band at 335 nm. The solution was irradiated at 366 nm for 1 min, and the irradiation time gradually increased up to 30 min to attain a total irradiation time of 45 min. After only 1 min's irradiation, a small band at 282 nm appeared. This increased in intensity with increasing irradiation time, while the band at 335 nm decreased in intensity. The UV-spectrum of pure 2-nitrodibenzofulvene was identical to that of the solution after 45 min of irradiation (Figure 3).

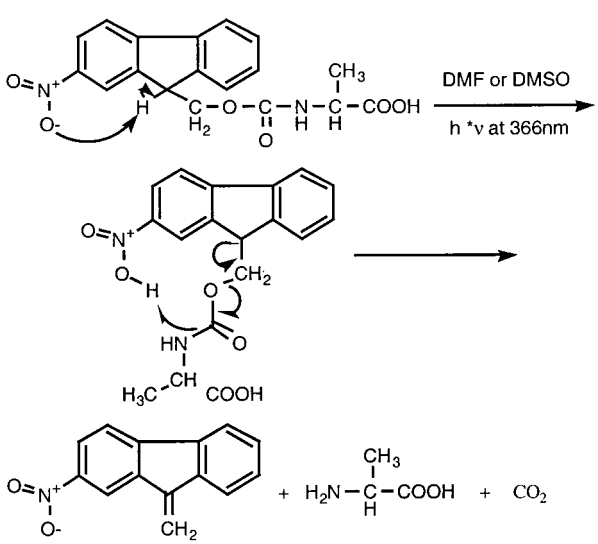

Figure 2 A schematic mechanism of the photochemical cleavage of the Fmoc(NO_2)-group. The proton-transfers are doubtless solvent-mediated.

In addition to the UV-lability of the new protecting group, the kinetics of cleavage of Fmoc(NO_2) under basic conditions was investigated. Both DIPEA and NMM were applied under conditions which would not lead to cleavage of the unsubstituted Fmoc-group. The cleavage of the Fmoc(NO_2) from **1** (1 mg/mL) with 5% DIPEA/DMF was complete within 10 min, whereas it was already completed in 3 min with 5% NMM/DMF. The measurements were performed in the dark to prevent photochemical cleavage.

With the experience gained from the above experiments, two peptides were synthesized on the PS-PEG resin TentaGel with *p*-(diphenylhydroxymethyl)benzoic acid as linker [10–12]. The synthesis was carried out on a Milligen 9050 continuous-flow peptide synthesizer with TBTU/NMM activation. Between the coupling and deblocking, there was a capping step with acetic anhydride. Finally, the Fmoc(NO_2)-amino acid was coupled outside the synthesizer using HOBt/DIC. Cleavage from the resin was accomplished with TFA.

Peptide 1: Fmoc(NO_2)-Leu-Thr-Gly-Lys-Ser-Leu-Glu-Ala-Asp-Gly-OH
Peptide 2: Fmoc(NO_2)-Ile-Asn-Arg-Arg-Met-Lys-Trp-Lys-Lys-Glu-Asn-OH

Fmoc(NO_2)-Leu-OH **2** in the assembly of peptide 1 was coupled at a fourfold excess in DMF for a period of 2.5 h. This led to double coupling of Leu, as is clearly visible from the mass spectrum. Obviously, the Fmoc(NO_2)-group was cleaved from Fmoc(NO_2)-Leu-peptide 1 to a slight extent under these coupling conditions, resulting in a second coupling of **2**. Chromatograms were taken at various detector wavelengths (Figure 4).

Coupling of Fmoc(NO_2)-Ile-OH **3** in the assembly of peptide 2 was performed in both DMF and in

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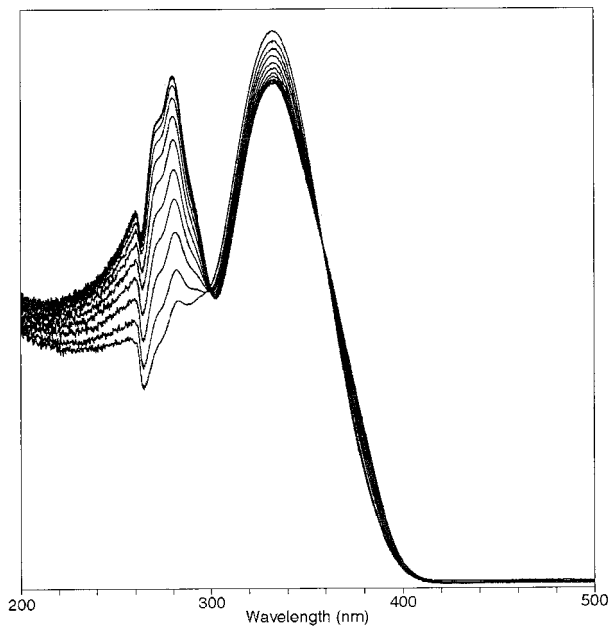

Figure 3 UV-spectroscopic monitoring of the photolytic cleavage of the Fmoc(NO_2)-group.

THF. Under otherwise identical conditions, coupling in DMF was complete within 2 h, whereas this was not the case for THF (Figure 5). On the other hand, the chromatogram showed that the proportion of peptide with doubly coupled Ile was considerably lower (Figures 5 and 6). The peak detected at 220 nm with a retention time of 8.5 min (Figure 6) was not visible at higher wavelengths. This peak represents the failure sequence peptide 2 minus Ile.

Finally, the Fmoc(NO_2)-group was cleaved from both peptides photochemically by dissolving them in DMF (1 μmol/mL) and irradiating at 366 nm (Figure 7).

The Fmoc(NO_2)-group was cleaved considerably slower from the 'acidic' peptide 1 than from the 'basic' peptide 2, although the amino groups of the latter are protonated after cleavage from the resin. This observation is in agreement with the postulated mechanism (Figure 2).

In summary, the Fmoc(NO_2)-group is a new protecting-group for amino functions which can be cleaved under both basic and solvent-dependant under photolytic conditions. It should be possible to apply this protecting-group to the *N*-terminus of difficult peptide sequences, to purify the resulting peptides by HPLC using a detection wavelength of 380 nm to locate the desired peptide, with final removal of the Fmoc(NO_2)-group photolytically to give the pure peptide.

EXPERIMENTAL PART

Peptide synthesis was carried out with the continuous-flow peptide synthesizer Milligen 9050. The PS-PEG-resin TentaGel [10] was used as polymeric support and *p*-(diphenylhydroxymethyl)benzoic acid [11,12] as linker (loading Fmoc-Gly-OH = 0.2 mmol/g; loading Fmoc-Asn(Trt)-OH = 0.18 mmol/g). Activation was performed with TBTU/NMM. The couplings were carried out with a sixfold excess of Fmoc-amino acids. Coupling times were 12 min, capping with acetic anhydride/DIPEA (3:1) at 4 min,

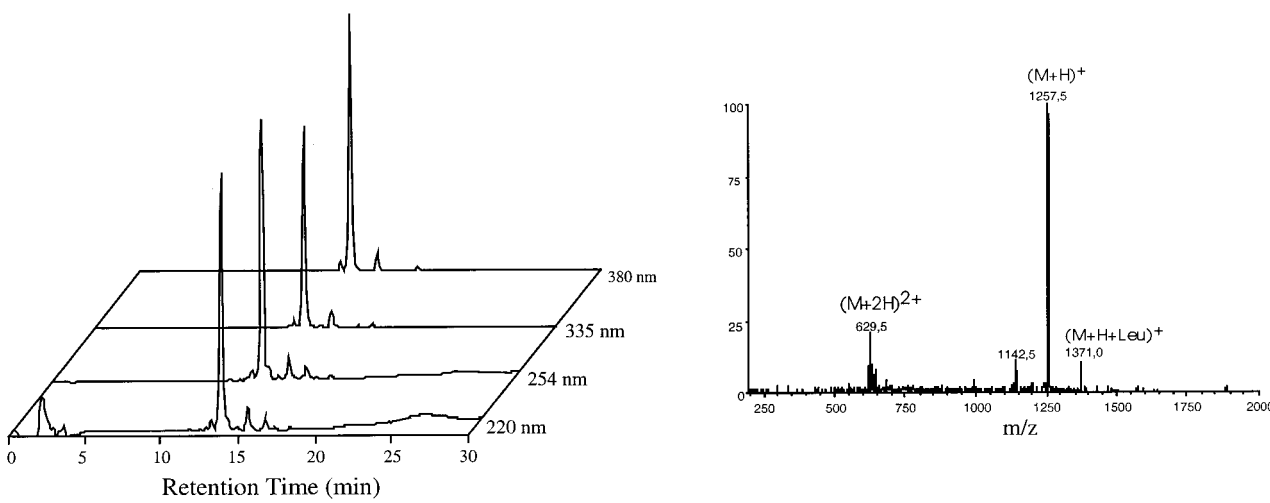

Figure 4 HPLC-chromatograms of peptide 1 with detection at different wavelengths; ionspray mass spectrum of peptide 1.

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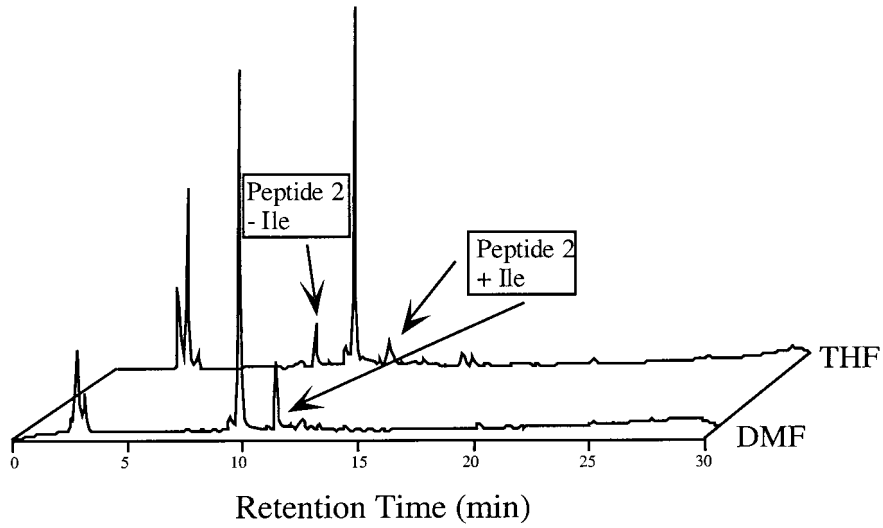

Figure 5 HPLC-comparison of the products from two syntheses of peptide 2 differing only in the solvent (THF vs. DMF) used for coupling the terminal Fmoc(NO_2)-amino acid (detection wavelength = 220 nm).

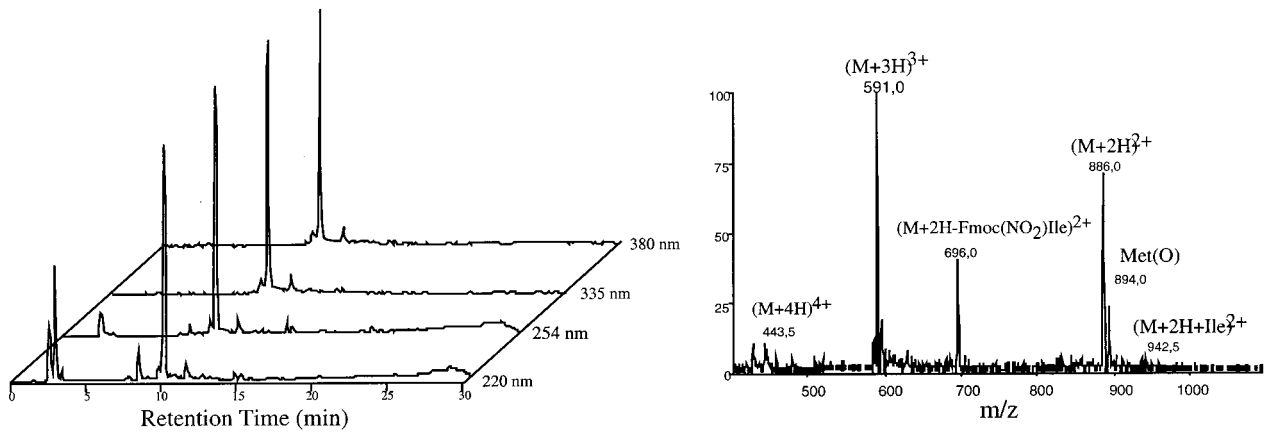

Figure 6 HPLC-chromatograms of peptide 2 with detection at different wavelengths; ionspray mass spectrum of peptide 2.

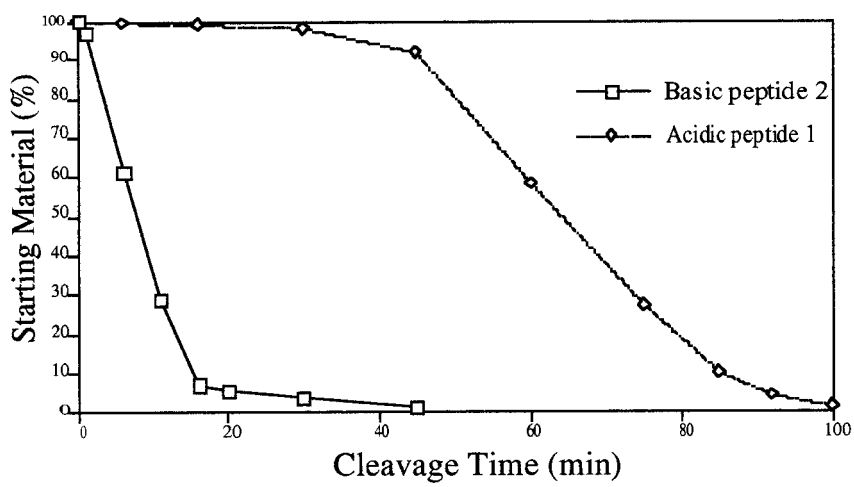

Figure 7 Determination of the photolytic cleavage kinetics of the Fmoc(NO_2)-group of both peptides (concentration 1 μmol/mL).

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and the deprotection with 20% piperidine in DMF also at 4 min. The *N*-terminal Fmoc(NO_2)-amino acid was coupled in a fourfold excess using HOBt/DIC and times as above. Cleavage of peptide 1 from the resin (500 mg) was accomplished with 5% water in TFA; cleavage of peptide 2 from the resin (500 mg) was performed with 0.38 g phenol, 0.12 mL ethane dithiol, 0.25 mL thioanisole, 0.25 mL water and 5 mL TFA. After 3 h, the peptides were precipitated with *t*-butyl methyl ether. The resulting suspensions were centrifuged, and the peptides were dried *in vacuo*. Yield of peptide 1: 51 mg; yield of peptide 2: 59 mg.

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