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9-Hydroxy-9-(4-carboxyphenyl)xanthene - A New Linker for the Synthesis of Peptide Amides

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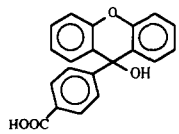
Abstract: The easy synthesis of 9-hydroxy-9-(4-carboxyphenyl)xanthene **1** a new linker for the solid phase peptide synthesis of peptide amides is reported. The cleavage conditions were checked and several peptide amides were synthesized using the TentaGel-resin™ on the Milligen 9050 continuous flow peptide synthesizer. © 1997 Elsevier Science Ltd.

The synthesis of peptide amides via solid phase peptide synthesis is difficult although some linkers[1-6] for this purpose have been generated. It was reported[5] that some of these handles require either a high concentration of TFA (trifluoracetic acid) or a long reaction time for the complete cleavage of the peptide amides with the possibility of side-reactions[3]. In addition, some of these linkers are hard to synthesize[3,4,5] or have to be reduced on the resin[1,2] prior to the loading of an amino acid derivative. To overcome these problems the linker 9-hydroxy-9-(4-carboxyphenyl)xanthene **1** was created (Scheme 1).



Scheme 1. 9-Hydroxy-9-(4-carboxyphenyl)xanthene

Xanthone was treated with 4-bromotoluene in a Grignard reaction to give 9-hydroxy-9-(4-methylphenyl)xanthene in a yield of 75%. This intermediate was oxidized using $KMnO_4$ resulting in **1** in a yield of 65% after recrystallization from ethyl acetate. The purity of **1** was checked: mp 178-181°C; FD-MS (field desorption): 318.1, $C_{20}H_{14}O_4$ = 318.3; ^{13}C NMR (in $CDCl_3$, d/ppm): 207.2 (COOH), 167.7, 155.1, 150.4, 129.9, 129.7, 129.6, 128.4, 126.8, 124.1, 116.8 (aromatic carbons), 70.0 (C_9 of xanthydrol).

1 was coupled to the amino groups of the TentaGel-resin™ [7] according to the common procedure which employs hydroxybenzotriazole/diisopropylcarbodiimide followed by chlorination of the 9-position using acetyl chloride/dichloromethane = 1:1 overnight. After the removal of the solvents, the resin was washed twice with absolute dichloromethane and suspended in THF saturated with NH_3. After 2 h reaction time the resin then was washed with DMF, methanol and diethyl ether and dried. The obtained resin-bound amino-linker was reacted with a Fmoc-amino acid fluoride[8] in an eight fold excess and with a 1.5 fold excess of diisopropylethylamine overnight. A coupling yield of 70-80% was obtained depending on the amino acid

derivative. The remaining amino-groups were capped with acetanhydride. In further investigations the cleavage kinetics of Fmoc-Gly amide from the resin bound linker was checked. Firstly, a 1%-solution of TFA in dichloromethane containing 1.5% triisopropylsilane and, secondly, a 10%-solution of TFA in dichloromethane with the same amount of scavenger were tried. 1% TFA cleaves about 55 % Fmoc-Gly amide in 60 min whereas 10% TFA cleaves about 88% in 5 min.

Peptide synthesis was performed on a Milligen 9050 continous-flow synthesizer with TBTU/NMM activation. The following four peptide amides were synthesized without difficulty: [Leu^5]-enkephalin amide (H-Tyr-Gly-Gly-Phe-Leu-NH_2), Pneumadin (H-Ala-Gly-Glu-Pro-Lys-Leu-Asp-Ala-Gly-Val-NH_2), Buccalin (H-Gly-Met-Asp-Ser-Leu-Ala-Phe-Ser-Gly-Gly-Leu-NH_2) and Leukokinin I (H-Asp-Pro-Ala-Phe-Asn-Ser-Trp-Gly-NH_2). All peptides were cleaved from the resin (500 mg) with a mixture of 0.4 g phenol, 0.125 ml ethanedithiol, 0.25 ml thioanisol, 0.25 ml water and 5 ml TFA. The cleavage time was 1h because all the side-chain protecting groups also had to be removed. The results of the four syntheses are depicted in Table 1.

Table 1. Results of the Syntheses of the four Peptide Amides

Peptide amide	Loading (%)	Molecular weight	Yield of crude product (%)	Purity/HPLC[1] (%)
[Leu^5]-enkephalin amide	76	554.6	83.3	96.7
Pneumadin	71	955.1	75.4	86.7
Buccalin	76	1053.2	90.4	77.1
Leukokinin I	80	891.9	84.4	82.1

[1]The purity of the crude pruduct was checked by RP-HPLC on a C18-column (gradient: 0 min 5% B, 30 min 50% B, 35 min 100% B, 40 min 5% B; A=water+0.1%TFA, B=acetonitrile+0.08% TFA; detection at 220 nm)

The advantages of this new linker-compound are obvious: **1** can be easily synthesized, the resin-bound amino-linker can be loaded using Fmoc-amino acid fluorides in good yields, the peptide amides can be cleaved from the linker in good yields, no side reactions were detected.

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