Novabiochem®

Merck Biosciences AG · Switzerland
Weidenmattweg 4
4448 Läufelfingen
Phone +41 (62) 285 2525
Fax +41 (62) 285 2520

www.novabiochem.com

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Solid Phase Synthesis of Peptide Aldehydes

Sophie Barthélémy[1], Alexandre Petruka[1], Syed Tasadaque Ali Shah[2], Steffen Weik[2] and Jörg Rademann[2]

[1]Novabiochem, Merck Biosciences AG, Weidenmattweg 4, CH-4448 Läufelfingen, Switzerland

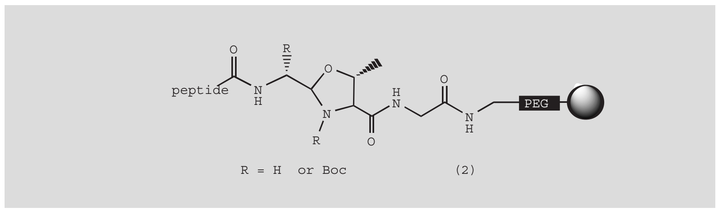
[2]Institute of Organic Chemistry, University of Tübingen, Auf der Morgenstelle 18, 72076 Tübingen, Germany

Introduction

Peptide aldehydes are potent inhibitors of serine, aspartyl and cysteinyl proteases and are valuable intermediates for the preparation of various important peptidomimetics and transition state isomers. There are three principle approaches to obtain peptide aldehydes: 1) oxidation of an appropriate peptide alcohol [1] or 1,2-diol [2]; 2) reduction of a peptide carboxylic acid derivative via a Weinreb amide formation [3, 4]; 3) step-wise or fragment synthesis using a masked amino acid aldehyde [5-10].

Results and Discussion

One of the simplest methods of protecting amino aldehydes on solid support involves the formation of an oxazolidine between an Fmoc-amino aldehyde and H-Thr-Gly-NovaSyn TG resin (1) *04-12-3710*.



Since oxazolidine formation is completely selective for aldehydes, even mixtures of amino alcohol and amino aldehyde obtained from incomplete oxidation reactions can be used to load the resin. Recently, the racemization-free oxidation of amino alcohols with polymer-supported IBX resin has been reported [11].
Two approaches were used to synthesize the amino acid aldehydes as outlined in Figure 1 and Figure 2.

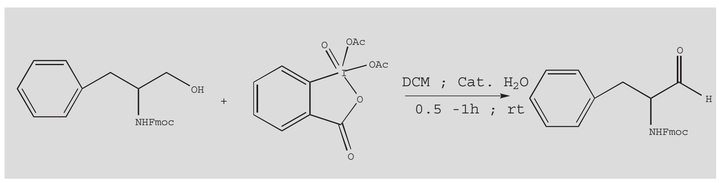


Figure 1. Oxidation of Fmoc amino alcohol by Dess-Martin periodinane.

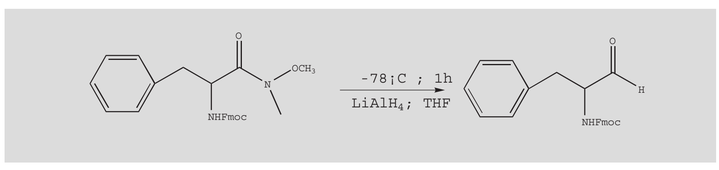


Figure 2. Reduction of Weinreb amide by $LiAlH_4$.

After loading of the resin with an aldehyde, it soon appeared necessary to protect the oxazolidine nitrogen (R = H) in order to prevent an untimely hydrolysis during the following synthesis steps. It can be blocked by treatment with Boc anhydride. The Boc-oxazolidine nitrogen is fully compatible with the Fmoc strategy. Although no acylation of this unprotected nitrogen during chain extension has been reported with mild activation methods, such as DIPCDI/HOBt. A quantitative difference between the protected and the unprotected version was observed. Fmoc-Lys(Fmoc)-Leu-Phe-H was obtained in 39% yield with unblocked resin and 80% yield using N-Boc oxazolidine resin.

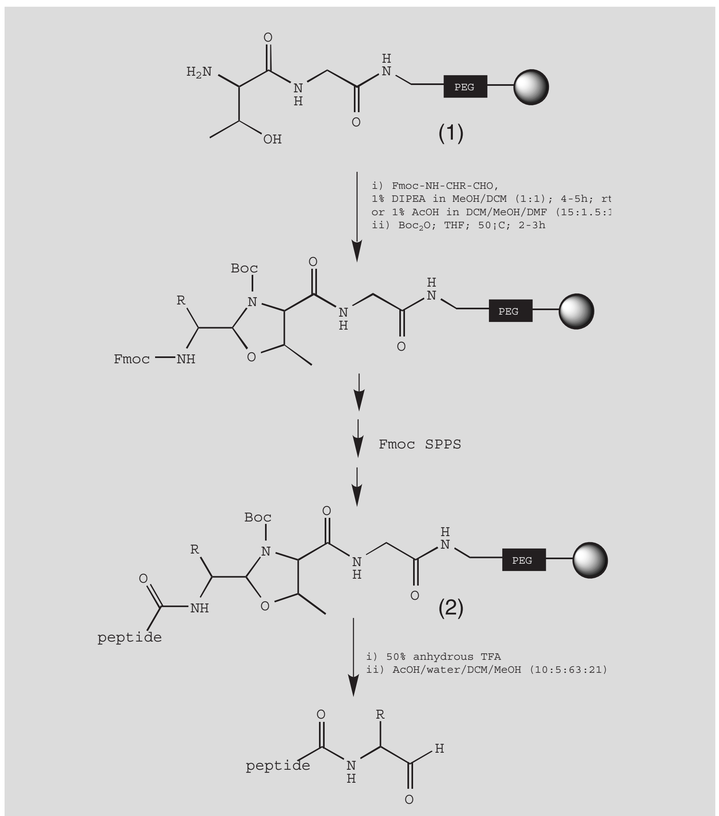
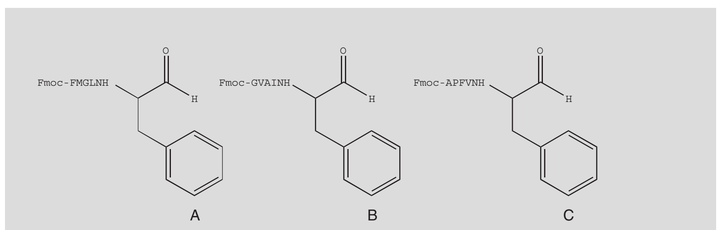


Figure 3. Preparation of peptide aldehydes using H-Thr-Gly-NovaSyn TG resin.

After standard peptide chain extension, the side-chain protecting groups including the Boc-oxazolidine are removed by using anhydrous TFA. Mild acidic conditions are applied to cleave the peptide aldehydes from the support.

Application 1

Three starting resins: Aminomethyl polystyrene, NovaGel and NovaSynTG were used according to the synthetic scheme described in Figure 3. Three heptapeptide aldehydes (A, B and C) were obtained. Quality and purity are compared in tables 1, 2 and 3.



No.	Loading mmol/g	% yield	% purity by HPLC
A	0.42	51	88
B	0.45	50	76
C	0.5	93	84

Table 1. Synthesis of peptide aldehydes via aminomethyl polystyrene, loading 1.28 mmol/g.

No.	Loading mmol/g	% yield	% purity by HPLC
A	0.32	52	92
B	0.35	60	87
C	0.29	78	79

Table 2. Synthesis of peptide aldehydes via aminomethyl NovaGel, loading 0.83 mmol/g.

No.	Loading mmol/g	% yield	% purity by HPLC
A	0.08	77	85
B	0.08	87	87
C	0.09	71	79

Table 3. Synthesis of peptide aldehydes via aminomethyl NovaSynTG, loading 0.28mmol/g.

Application 2

Fmoc-Lys(Fmoc)-Leu-Phe-H and Fmoc-Lys(Fmoc)-Leu-Leu-H were obtained using NovaSynTG resin as solid support. These two peptides were analyzed by HPLC and a second peak was observed in the case of Fmoc-Lys(Fmoc)-Leu-Leu-H. The ^1H NMR shown in Figure 4 gives only one aldehydic proton. An eventual racemization could not be proven. By mass spectroscopy, this second product was identified as an acetal derivative. The data obtained are: $C_{48}H_{56}N_4O_7$, Mw calcd. 800.4, $[M+1]^+$ m/z 801.5 for the first product and for the second $[M+hemiacetal]^+$ m/z 815.5 and $[M+acetal+Na]^+$ m/z 869.6 found.

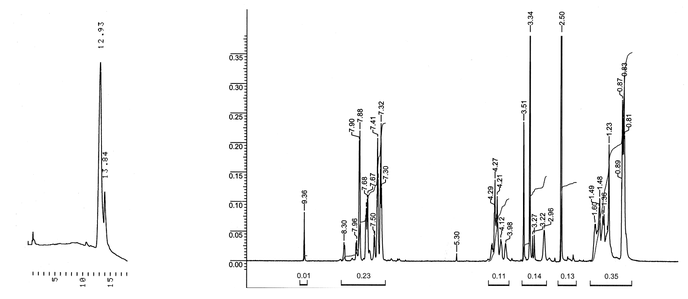


Figure 4. .HPLC elution of Fmoc-Lys(Fmoc)-Leu-Leu-H; column: Chromolith Speed ROD; gradient: 25%-97% B in 18 min; A: 0.1% TFA in water; B: 0.1% TFA in ACN and ^1H NMR (d_6DMSO, 400MHz).

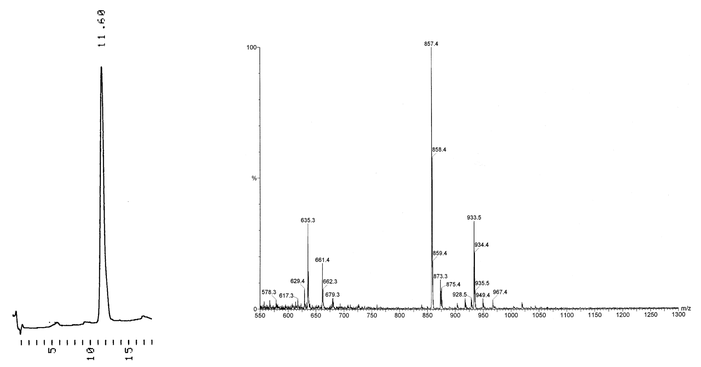


Figure 5. HPLC elution of Fmoc-Lys(Fmoc)-Leu-Phe-H; column: Chromolith Speed ROD; gradient: 35%-97% B in 15 min; A: 0.1% TFA in water; B: 0.1% TFA in ACN and ES-MS, $C_{51}H_{54}N_4O_7$, Mw calcd. 834.4, $[M+Na]^+$ m/z 857.4 found.

Methods

Method 1: Oxidation of Fmoc amino alcohol using Dess-Martin periodinane

Fmoc-amino alcohol (5.36 mmol, obtained from Fmoc-amino acid according to Liskamp et al. [12]) was dissolved in dry DCM (200 ml). Dess-Martin periodinane (12 mmol, 2.2 eq.) was added and dissolved almost completely. Water (200 µl, 11.1 mmol) was added and after 1 h all starting material was consumed (TLC). The reaction was diluted with Et_2O (150 ml) and stirred 30 min with an aqueous 80 % saturated bicarbonate solution (150 ml) containing sodium thiosulfate-pentahydrate (60.12 mmol). The aqueous phase was extracted with Et_2O (300 ml). The combined organic phases were washed with saturated bicarbonate, water (2x), and brine (2x) and dried over Na_2SO_4. Solvents were removed in vacuo. Fmoc amino acid aldehyde is obtained in 99 % yield and the optical rotation $[\alpha]_{D,20}$ = + 51,2 ° ($CHCl_3$, c=1g pro 100 ml) [13]. The amino acid aldehydes were obtained without racemization [11].

Method 2: Reduction of Weinreb amide using $LiAlH_4$

Under nitrogen atmosphere, 950 mg (25 mmol, 5eq.) of $LiAlH_4$ were suspended in 20 ml dry THF and cooled to -78°C. 2.15 g (5 mmol, 1eq.) of Fmoc Weinreb amino acid dissolved in 20 ml dry THF was slowly added to the suspension. The reaction was stirred for 1h and controlled by TLC. 60 ml of Et_2O was added before the quenching process with 20 ml of 10 % citric acid. After extraction, the organic phase was dried over Na_2SO_4, filtered and concentrated. The amino acid aldehyde should be stored at -20°C.

Method 3: Synthesis of peptide aldehydes

H-Thr-Gly-NovaSyn TG resin (1eq.) was suspended in 1% DIPEA in DCM/MeOH (1/1) containing 5 eq. of Fmoc-Phe-H. The mixture was shaken at rt for 4-5h under Argon. The loading of the resin was determined by the Fmoc UV assay to be 0.20 mmol/g. Boc protection may be effected using 5 eq. of $(Boc)_2O$ in THF at 50°C for 3-4h. The Fmoc group was removed with 20% piperidine in DMF and the peptide chain was extended using PyBOP® activation. After assembly of the target sequence, the resin was washed with i-PrOH, THF, i-PrOH, MeOH and dried o/n. The side-chain protecting groups were removed by treatment with 50% TFA in DCM (2*10 min), after which the resin was washed with DCM. Cleavage from the resin was effected by three treatments with AcOH/water/DCM/MeOH (10:5: 63: 21) for 30 min, to afford the product in 80 % yield with the HPLC profile shown in Figure 5. ES-MS (dansylhydrazone) expected 1081.5, found M+H = 1082.7.

Conclusions

High quality and purity of peptide aldehydes successfully synthesized
N-Boc protecting group of oxazolidine nitrogen needed and fully comptatible with Fmoc strategy
NovaGel and NovaSynTG starting resins suitable for peptide aldehyde synthesis
Polystyrene resin not recommended
Acetal formation can occur and be observed by LC-MS, this reversible reaction can be avoided by reducing the quantity of methanol

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