Novabiochem®
innovations 5/04

Strategies for the synthesis of peptide aldehydes

Peptide aldehydes

Peptide aldehydes are potent inhibitors of serine, aspartyl and cysteinyl proteases and are valuable intermediates for the preparation of reduced amide-bond peptidomimetics and transition-state isosteres.

There are three principle approaches for their preparation: firstly, oxidation of an appropriate peptide alcohol [1] or 1,2-diol [2]; secondly, reduction of a peptide carboxylic acid derivative, such as a Weinreb ester [3, 4]; finally, step-wise or fragment synthesis using a masked pre-formed aldehyde [5 - 10]. The last forms the basis of one of the simplest and most effective methods, which involves the solid-phase immobilization of an amino aldehyde by formation of an oxazolidine between a pre-formed Fmoc-amino aldehyde and H-Thr-Gly-NovaSyn® TG resin (Figure 1) [10].

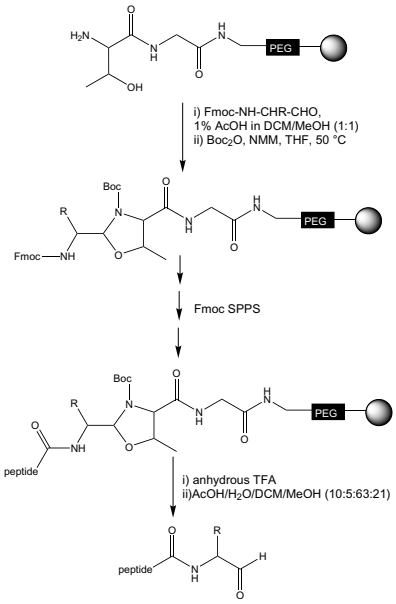

Figure 1: Preparation of peptide aldehydes using H-Thr-Gly-NovaSyn® TG resin.

After loading the resin, the oxazolidine nitrogen should be blocked by treatment with Boc-anhydride. The resultant acyloxazolidine is stable to base and is compatible with Fmoc protocols.

Cleavage from the resin and side-chain deprotection is carried out in two stages. Firstly, side-chain protecting groups are removed with anhydrous TFA. Secondly, the peptide aldehyde is cleaved from the resin with AcOH/water/DCM/MeOH (10:5:63:21) [11].

Novabiochem presently offers H-Thr-Gly-NovaSyn® TG resin pre-loaded with aldehydes of Arg, Asp, Leu, Phe, and Val.

If the appropriate pre-loaded resin is not available, the required Fmoc-amino aldehyde intermediate can be prepared by oxidation of the corresponding amino alcohol using Dess-Martin's periodinane [11]. A very promising variation of this method, particularly for small scale production of Fmoc-amino aldehydes, involves the use of IBX-polystyrene (Method 1, Figure 2) [12]. This reagent cleanly converts protected amino and peptide alcohols to aldehydes, and because it is attached to an insoluble polymer, it can be simply removed at the end of the reaction by filtration. Amino aldehydes produced in this way can be loaded onto H-Thr-Gly NovaSyn® TG resin and then used for standard peptide chemistry. Since oxazolidine formation is completely selective for aldehydes, even mixtures of amino alcohol and aldehyde obtained from incomplete oxidation reactions can be used to load the resin.

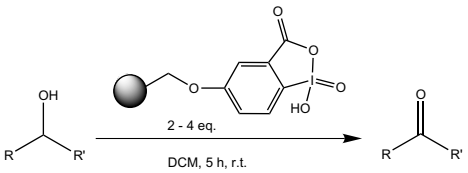

Figure 2: Oxidation of alcohols with IBX-PS.

Preparation of aldehydes using IBX-PS

Oxidation of Fmoc-Ile-ol and Boc-D-Phe-Cys(Trt)-Phe-D-Trp-Lys(Boc)-Thr(tBu)-Cys(Acm)-Thr(tBu)-ol

To exemplify the use of IBX-PS in the preparation of peptide and amino aldehydes, Fmoc-Ile-ol and a crude fully protected octreotide (prepared by $NaBH_4$ cleavage from HMBA-AM resin [13]) were oxidized as described in Method 1. In both cases, conversion to the aldehyde was complete in 24 h (Figure 3).

Method 1: Oxidation with IBX-PS

1. Dissolve protected peptide or amino alcohol (1 eq.) in DCM. Add IBX-PS (3 eq.).
2. Gently agitate mixture o/n.
3. Remove resin by filtration, wash with DCM and evaporate filtrates to dryness.

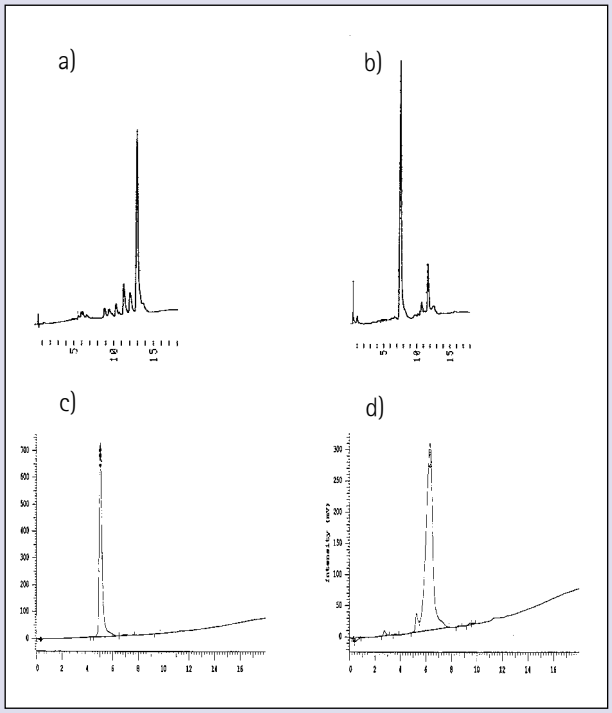

Figure 3: HPLC elution profile of a) crude protected octreotide; b) crude protected octreotide aldehyde; column: Merck Chromolith Speed ROD; gradient: 20%-90% B in 15 min; A: 0.1% TFA aq. ; B: 0.1 % TFA in MeCN; c) Fmoc-Ile-ol; d) Fmoc-Ile-H; column: Merck Chromolith SpeedROD; gradient: 25%-97% B in 18 min; A: 0.1% TFA aq. ; B: 0.1 % TFA in MeCN.

Solid phase synthesis of peptide aldehydes

The synthesis of peptide aldehydes using H-Thr-Gly-NovaSyn® TG resin is illustrated in Applications 1-3. The resin can be loaded with an Fmoc-protected amino aldehyde as described in Method 2, or alternatively, for peptides containing a C-terminal aspartal, argininal, leucinal, phenylalaninal, or valinal residue, pre-loaded resins are available.

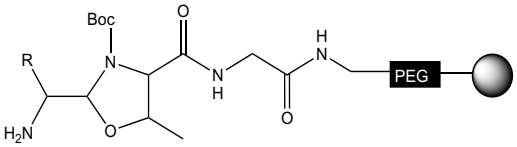

Figure 4: Amino aldehydes attached to H-Thr-Gly-NovaSyn® TG resin.

Method 2: Loading of H-Thr-Gly-NovaSyn® TG resin

1. Suspend H-Thr-Gly-NovaSyn® TG resin in 1% AcOH in MeOH/DCM (1:1) containing Fmoc-amino aldehyde (5 eq. relative to resin substitution) in DCM.
2. Gently agitate mixture at rt for 4 h and monitor by TNBS test.
3. Remove resin by filtration, wash with DCM, DMF, and THF.
4. Treat resin with Boc_2O (5 eq.) and NMM (5eq.) in THF at 50°C for 3 h to cap oxazolidine nitrogen.
5. Remove resin by filtration and wash with THF, DCM, and DMF.

Synthesis of Fmoc-Lys(Fmoc)-Leu-Phe-H

Fmoc-Lys(Fmoc)-Leu-Phe-H was prepared using H-Thr-Gly-NovaSyn® TG resin as described in Application 1.

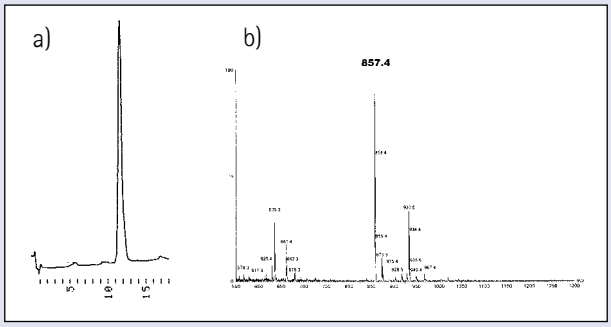

Figure 5: a) HPLC elution profile of Fmoc-Lys(Fmoc)-Leu-Phe-H. Merck Chromolith Speed ROD; gradient: 35%-97% B in 15 min; A: 0.1% TFA aq.; B: 0.1% TFA in MeCN; b) ES mass spectrum of crude Fmoc-Lys(Fmoc)-Leu-Phe-H.

Application 1: Synthesis of Fmoc-Lys(Fmoc)-Leu-Phe-H

H-Thr-Gly-NovaSyn® TG resin (0.23 mmole/g) was loaded with Fmoc-Phe-H according to Method 2. The loading of the resin was determined by the Fmoc UV assay to be 0.20 mmole/g. The Fmoc group was removed with 20% piperidine in DMF and the chain was extended using 3-fold excesses of Fmoc-amino acids activated with PyBOP® (3 eq.) in the presence of HOBt (0.5 eq.) and DIPEA (6 eq.). After assembly of the target sequence, the resin was washed with i-PrOH, THF, i-PrOH, MeOH and dried o/n. Cleavage from the resin was effected by two treatments with anhydrous TFA for 10 min and then three treatments with AcOH/water/DCM/MeOH (10:5:63:21) for 30 min, to afford the product in 40% yield with the HPLC profile shown in Figure 5. ESMS expected ($M+Na^+$) 857.4, found 857.4.

Synthesis of Fmoc-Lys(Fmoc)-Leu-Leu-H

Fmoc-Lys(Fmoc)-Leu-Leu-H was prepared using H-Leu-H NovaSyn® TG resin as described in Application 2.

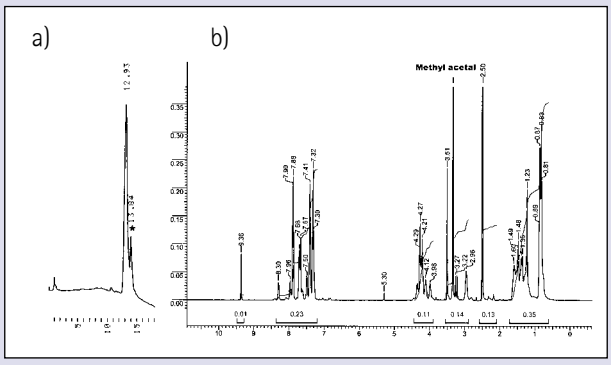

Figure 6: a) HPLC elution profile of Fmoc-Lys(Fmoc)-Leu-Leu-H. Merck Chromolith Speed ROD; gradient: 25%-97% B in 18 min; A: 0.1% TFA aq.; B: 0.1% TFA in MeCN; b) 1H nmr spectrum of crude Fmoc-Lys(Fmoc)-Leu-Leu-H.

Application 2: Synthesis of Fmoc-Lys(Fmoc)-Leu-Leu-H

H-Leu-H NovaSyn® TG resin (200 mg, 0.1 mmole) was used to prepare Fmoc-Lys(Fmoc)-Leu-Leu-H NovaSyn® TG resin according to Application 1. After assembly of the target sequence, the resin was washed with i-PrOH, THF, i-PrOH, MeOH and dried o/n. Cleavage from the resin was effected by two treatments with anhydrous TFA for 10 min and then 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 6. Peak* was identified by ESMS to be the dimethyl acetal. ESMS expected ($M+H^+$) 800.4, found 801.5.

Synthesis of H-Ala-Arg-Gly-Leu-Pro-Tyr-Ala-Glu-Leu-H

H-Ala-Arg-Gly-Leu-Pro-Tyr-Ala-Glu-Leu-H was prepared using H-Leu-H NovaSyn® TG resin as described in Application 3.

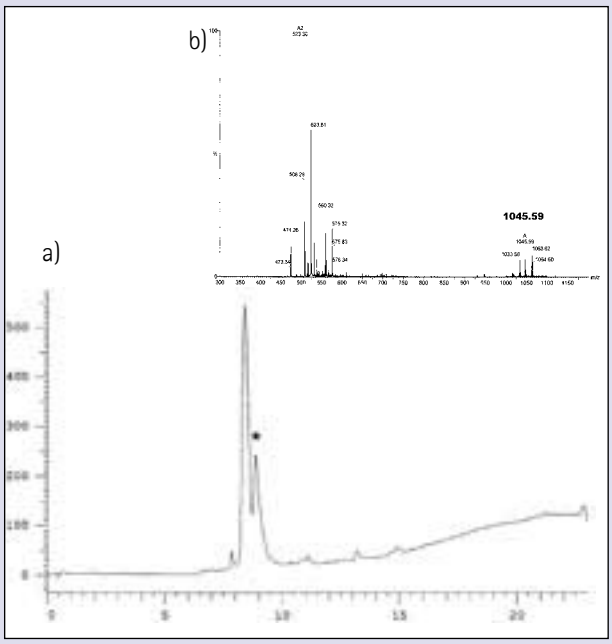

Figure 7: a) HPLC elution profile of crude H-Ala-Arg-Gly-Leu-Pro-Tyr-Ala-Glu-Leu-H. Column: Merck Chromolith Speed ROD; gradient: 0%-100% B in 20 min; A: 0.1% TFA aq.; B: 0.1% TFA in MeCN; b) ES mass spectrum of crude H-Ala-Arg-Gly-Leu-Pro-Tyr-Ala-Glu-Leu-H.

Application 3: Synthesis of H-Ala-Arg-Gly-Leu-Pro-Tyr-Ala-Glu-Leu-H

H-Leu-H NovaSyn® TG resin (1 g, 0.23 mmole) was suspended in DMF and left to swell for 30 min. Chain extension was then carried out for 1 h using a 3-fold excess of Fmoc-amino acid activated with PyBOP® (3 eq.) in the presence of HOBt (0.5 eq.) and DIPEA (6 eq.), with the exception of the N-terminal residue (Ala), which was introduced using a Boc-protected derivative. Fmoc group removal was effected with DBU/piperidine/DMF (2:2:96). After assembly of the target sequence, the resin was washed with DMF, i-PrOH, THF, and MeOH and dried o/n. The side-chain protecting groups were removed by treatment with 100% TFA (2 x10 min). The resin was then washed with DCM and the product was cleaved from the resin by three treatments with AcOH/water/DCM/MeOH (10:5:63:21) for 30 min, to afford the product in 38% yield with the HPLC profile shown in Figure 7. Peak* was identified by ESMS to be the dimethyl acetal. ESMS expected ($M+H^+$)1044.56, found 1045.59.

References

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Ordering information

01-64-0445	IBX-polystyrene	1 g
		5 g
		25 g
04-12-3710	H-Thr-Gly-NovaSyn® TG resin	1 g
		5 g
04-12-3722	H-Asp(OtBu)-H NovaSyn® TG resin	1 g
		5 g
04-12-3720	H-Leu-H NovaSyn® TG resin	1 g
		5 g
04-12-3721	H-Phe-H NovaSyn® TG resin	1 g
		5 g
04-12-3725	H-Val-H NovaSyn® TG resin	1 g
NEW		5 g
04-12-3723	H-Arg(Boc)$_2$-H NovaSyn® TG resin	1 g
NEW		5 g

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