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Sanger's Reagent: An Excellent End-Capping and/or Labeling Agent for SPPS

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Introduction

Acetylation of N^{α}-non-acylated amino groups in course of SPPS is widely used method which is fairly fast and almost quantitative. However, acetylated side products do not markedly differ by separation characteristics from the main product. Several authors suggested other end-capping reagents which gave to N-terminus such physico-chemical properties that made separation of both products easier [1]. For the same purpose we examined 2,4-dinitrofluorobenzene (DNFB), introduced by Sanger [2] for structural characterization of protein fragments. This reagent is the potent electrophile which arylates quantitatively primary and secondary amines.

Results and Discussion

Model peptides were synthesized on MeBHA resin with Rink-amide linker by Fmoc/Bu^t strategy with DIC/HOBt activation and cleaved from the resin with TFA/EDT/triisopropylsilane/water (87.5:2.5:5:5) mixture. Protected peptide fragments were prepared on 2-chlorotrityl chloride resin and cleaved with a mixture of TFA/AcOH/DCM (1:1:8). The outcome of end-capping reaction was followed by HPLC with monitoring at 222 and 360 nm (Dnp-peptides). All products were characterized by FAB MS and amino acid analysis. Dinitrophenylation and acetylation were accomplished either in a mixture of DNFB/DIEA (10:5 eq.) in DCM, or in acetanhydride/DIEA (10:10 eq.) in DCM, respectively.

Dinitrophenylation was compared with acetylation on several model peptides. The results of syntheses using the DNFB end-capping were significantly better (peptides were purer and did not contain any by-products derived from the action of acetanhydride on the peptide). This was demonstrated by the parallel syntheses of a non-complicated sequence (H-Ala-Thr-Leu-Asn-Phe-Pro-Ile-Ser-Pro-Trp-NH_2), both with acetylation and dinitrophenylation, where almost no side products were observed. The target product prepared according to a protocol using the DNFB method was obtained in higher HPLC purity (97% vs. 89%).

Efficiency of DNFB method was studied on the synthesis of a part of a "difficult sequence" (H-Ile-Ile-Ile-His-Abu-Thr-Leu-Asn-Phe-NH_2). After the condensation of His derivative and

subsequent dinitrophenylation, besides the main product also the deletion peptide (Dnp-Abu-Thr-Leu-Asn-Phe-NH_2) was found. In each of further synthetic steps one more Dnp-labeled deletion by-product was detected (both at 222 nm and 360 nm). Any other side product was not observed. The main product was obtained in 87% yield. The content of Dnp-peptides varied, according to HPLC, from 2 to 6%.

The dinitrophenylation was also tested for quantitative end-capping in fragment condensation. Fragment Fmoc-Arg(Pmc)-Asn(Trt)-Leu-Leu-Thr(Bu^t)-Gln(Trt)-Ile-Gly-OH was condensed to H-Abu-Thr(Bu^t)-Leu-Asn(Trt)-Phe-resin. Both products of the condensation, H-Arg-Asn-Leu-Leu-Thr-Gln-Ile-Gly-Abu-Thr-Leu-Asn-Phe-OH and Dnp-Abu-Thr-Leu-Asn-PheOH, were isolated. The Dnp-labeled deletion product was easily identified by HPLC at 360 nm. No other significant side product was detected.

Besides simple HPLC separation also novel affinity "filtration" technique based on specific interaction of Dnp groups with anti-Dnp antibody was examined. The method was applied to purification of acyclic V3-loop (envelope glycoprotein fragment of HIV-1). The sequence was assembled from the following protected fragments on 2-chlorotrityl chloride resin (DIC/HOBt) by use of the DNFB end-capping method:

(1) Fmoc-Cys(Acm)-Thr(Bu^t)-Arg(Pmc)-Pro-Asn-Asn-Asn-Thr(Bu^t)-OH

(2) Fmoc-Arg(Pmc)-Lys(Boc)-Ser(Bu^t)-Ile-His(Trt)-Ile-Gly-OH

(3) Fmoc-Pro-Gly-Arg(Pmc)-Ala-Phe-Tyr(Bu^t)-Thr(Bu^t)-Thr(Bu^t)-Gly-OH

(4) H-Glu(OBu^t)-Ile-Ile-Gly-Asp(OBu^t)-Ile-Arg(Pmc)-Gln(Trt)-Ala-His(Trt)-Cys(Acm)-resin.

After cleavage crude product was dissolved in a buffer (0.5 M TRIS.HCl, pH 8) and poured on a column of Sepharose binding anti-Dnp antibody [3]. The column was then eluted with the same buffer and eluent was analyzed by HPLC. Dnp-labeled side products of the synthesis were specifically bound to Dnp-antibody on the column and the desired peptide was eluted.

We found DNFB as a superior reagent for termination of N^{α}-non-acylated peptide chains during solid-phase peptide synthesis. The reaction is fast, pure and quantitative. No side-reactions with soft nucleofiles (Met, Cys(Acm,Trt), Trp(Boc)) were observed. In comparison to commonly used acetylation the dinitrophenylated side products offer better properties in terms of separation from a target peptide. DNP-peptides can be easily separated from the desired product either by conventional HPLC or affinity techniques.

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