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CARBOXAMIDOMETHYL ESTER AS USEFUL HANDLE IN POLYSTYRENE-BASED PEPTIDE SYNTHESIS: CLEAVAGE OF PEPTIDE WITH MERCAPTIDE

Mohammed Saleh Shekhani,[a] Gerald Grübler,[b] Hartmut Echner,[b] and Wolfgang Voelter.*[b]

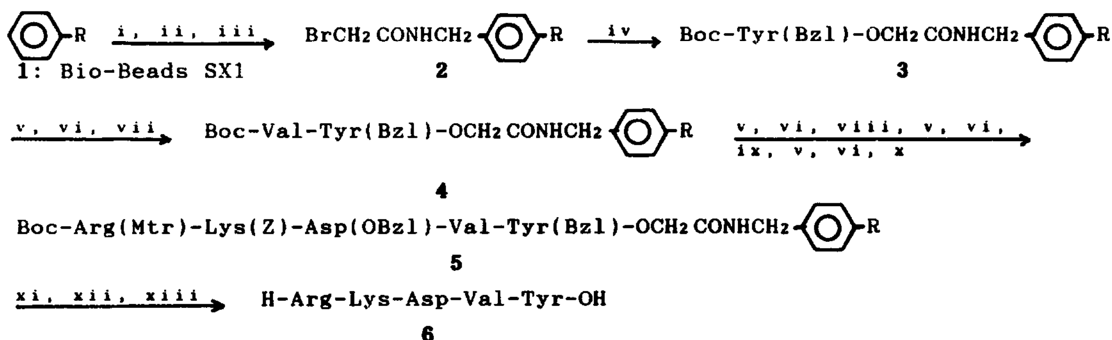
(a) HEJ Research Institute of Chemistry, University of Karachi, Karachi-32 Pakistan.

(b) Abteilung für Physikalische Biochemie des Physiologisch-chemischen Instituts der Universität Tübingen, Hoppe-Seyler-Str.4, D-7400 Tübingen, FRG.

Abstract: Glycolamidic ester linkage of a peptide chain to a polystyrene resin can be cleaved with the lithium salt of mercaptoethanol, allowing utilization of this handle for Boc strategy of solid phase peptide synthesis.

Benzyl ester-type linkages have dominated polystyrene-based peptide synthesis since its inception.[1,2] There has, however, been a growing realization that this linkage is not ideal[2,3] and especially cleavable linkages are desirable. This consideration in relation to the protecting group problem as a whole has led to the development of a number of handles.[2,4] We describe here the carboxamidomethyl ester as a useful handle on a polystyrene solid support and the release of the peptide chain under essentially neutral conditions at the end of the synthesis.

The introduction of the carboxamidomethyl(CAM) ester as a base labile carboxyl protective group,[5] led us[6] to the utilization of the glycolamidic ester as a base labile handle on a polystyrene support, while others[4h-j] have used the same handle on a polyamide support. In our hands, however, the cleavage of peptides from the support required rather drastic alkaline conditions (warm alkali, extended period), limiting the utility of this handle. We, therefore, undertook an investigation of alternative cleavage conditions. As expected,[5] the linkage was stable to a 12h treatment with 50% TFA in CH_2Cl_2. When the tyrosine-bearing resin **3** was treated with Li^+ $^-SCH_2CH_2OH$ in a mixture of mercaptoethanol and THF at room temperature, the protected tyrosine was quantitatively cleaved within 2h, as shown by the isolation of the protected amino acid from the solution and by the amino acid analysis of the resin. These findings suggested that the carboxyamidomethyl ester linkage was suitable for Boc strategy of solid phase peptide synthesis. This fact was demonstrated by synthesizing the biologically active 32-36 thymopoietin II sequence H-Arg-Lys-Asp-Val-Tyr-OH (TP 5), (Scheme I).



Scheme I: (i) N-chlorometylphthalimide/$SnCl_4$(cat.)/TFA-CH_2Cl_2(1:1)/5h. (ii)hydrazine/EtOH/reflux/10h. (iii)$(BrCH_2CO)_2O$/*i*-Pr_2NEt/DMF. (iv)(Boc-Tyr(Bzl)-O^-)$_2$Cs/DMF. (v)TFA-CH_2Cl_2-anisole (8:8:1). (vi)N-methylmorpholine-CH_2Cl_2(2:15). (vii)Boc-Val-OBt/CH_2Cl_2. (viii)Boc-Asp(O-Bzl)-OBt/CH_2Cl_2. (ix)Boc-Lys(Z)-OBt. (x)Boc-Arg(Mtr)-OBT. (xi)$LiSCH_2CH_2OH$/$HSCH_2CH_2OH$/THF. (xii)90%TFA in MeSPh. (xiii)H_2/10%Pd-C/MeOH-H_2O.

Phthalimidomethylation[7] of Bio-Beads SX1[8](200-400 mesh), hydrazinolysis,[7] and bromoacetylation with bromoacetic anhydride[9] (monitored by the Kaiser test[10]) afforded the bromoacetamido resin **2**. Reaction with the caesium salt (3 equiv.) of Boc-Tyr(Bzl)-OH in DMF anchored the first amino acid.[11] Deblocking (TFA-CH_2Cl_2-anisole 8:8:1, then N-

methylmorpholine-CH_2Cl_2, 2:15, followed by reaction with the N-hydroxybenzotriazole ester[12] (3 equiv.) of Boc-Val-OH in CH_2Cl_2 introduced the second amino acid (reaction was monitored by the Kaiser test[10]). The cycle was repeated for the subsequent amino acids with appropriate side chain protection, (cf. scheme I). After the incorporation of all the five residues, the amino acid analysis of the resin gave satisfactory results.

The peptide was cleaved from the resin as follows: To a mixture of mercaptoethanol (1 ml, 1.11 g, 10.43 mmol) and anhydrous THF (3 ml) under N_2 was added *n*-BuLi (1.21 *M*, 1 ml) at 0°C. The mixture was stirred for 10 min and then warmed up to the room temperature. TP5-bearing resin (100 mg, 0.94 mmol of TP5/g of resin) was added and the mixture was stirred for 3h. The resin was filtered off, washed with MeOH, and water. The washings were combined and concentrated *in vacuo* to 10 ml (the pH of the solution was 7.5 at this stage), and acidified with 2*N* citric acid to pH 3.0. The protected peptide was extracted with EtOAc. The organic layer was washed several times with water, sat. aq. NaCl, dried (Na_2SO_4) and evaporated *in vacuo*. The side chains of the crude product were deblocked under standard conditions (scheme I) and the TP5 was characterized by direct comparison with an authentic sample.[13,14]

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11. The resin was analyzed for Br and tyrosine in order to establish the completion of reaction.
12. Prepared by the reaction of protected amino acid (1 equiv.) with N-hydroxybenzotriazole (1 equiv.) in the presence of DCC (1.05 equiv.) in CH_2Cl_2.
13. Purchased from Calbiotech, Copenhagen, Denmark.
14. HPLC conditions: LiChrospher 100 RP-18-5, 250x4mm, A: 60% CH_3CN(0.05% TFA), B: H_2O(0.05% TFA).A-B 5/95(2min), A-B 95/5(30min), flow rate: 1ml/min. Retention time of TP5: 16.5 min.

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