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GLYCOLAMIDIC ESTER HANDLE USED WITH POLYSTYRENE SUPPORTS. APPLICATION TO THE SYNTHESIS OF TP5

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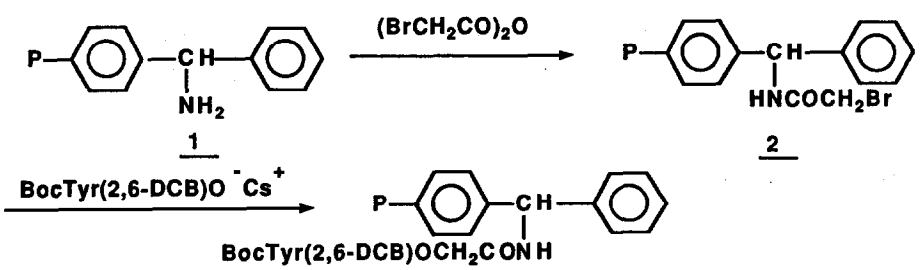
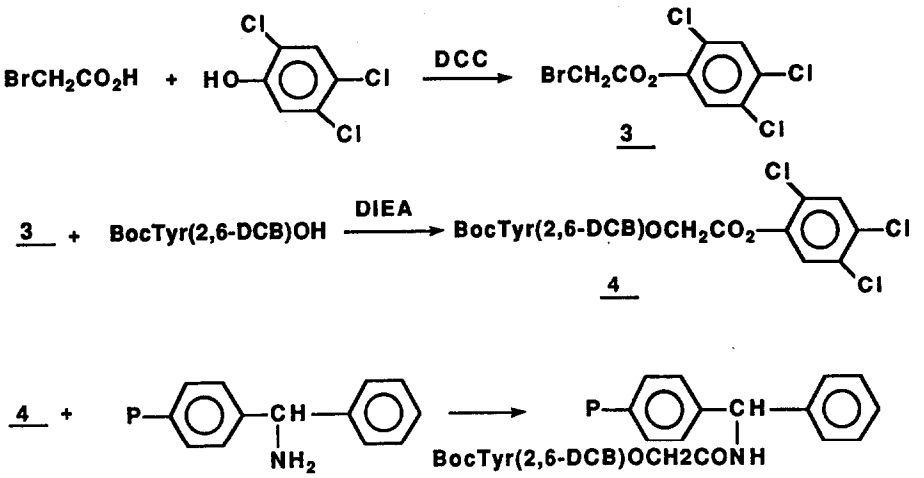
Abstract : Glycolamidic ester linkage of a peptide chain to a polystyrene resin can be easily cleaved under smooth alkaline conditions.

In a recent paper, Shekhani et al. (1) have attempted to synthesize the biologically active 32-36 thymopoietin II sequence : RKDVY (TP5) by using a polystyrene support and glycolamidic ester as base labile handle (2). These authors observed that cleavage of peptide required drastic alkaline conditions (warm alkali, extended period). However, we have previously reported (3) a convenient synthesis of TP5 starting from glycolamidic anchorage and polyamide resin. Detachment of peptide has been performed, with good yields, by using three equivalents of NaOH in isopropanol-water (70-30) at ambient temperature for 3 hours. The difference between the two syntheses lies in the nature of the support used : polystyrenes in contrast to polyamides are highly hydrophobic so that they shrink strongly in presence of water (4). If Shekhani et al. (1) have used the same cleavage conditions which are currently used with hydrophilic polyamides (2 or 3 equiv of NaOH in iPrOH-H_2O, 70-30), it appears normal that the peptide release would be very difficult. In our view, it is not the lack of lability of glycolamidic anchorage in basic media which is implicated, but the use of a support incompatible with water. Nevertheless, it seems possible to overcome this difficulty by using during the cleavage step, a solvent in which the polystyrene matrix would swell. In order to demonstrate this assumption, we have reinvestigated the solid-phase synthesis of TP5 carried out with a glycolamidic ester handle linked to a polystyrene matrix (i.e. BHA 1).



Scheme 1



Scheme 2

Deprotection	TFA (40 %) in CH_2Cl_2	1 x 2 min
	idem	6 x 30 "
Washings	CH_2Cl_2	6 x 2 "
Neutralization	5 % DIEA in CH_2Cl_2	2 x 3 "
Washings	CH_2Cl_2	
Couplings	symmetrical anhydride (2.3) (3 equiv) in CH_2Cl_2. For Boc Arg (Mts) OH a minimum of DMF was added to ensure a complete dissolution. After 15 min DIEA (1 equiv) was added to the reaction mixture. Kaiser's test (5) was negative after 1 hour of shaking	
Washings	CH_2Cl_2	4 x 1 min

Table 1

Bromoacetylation of BHA resin 1 (0.6 mmole NH_2/g, from Peptide Institute, Osaka, Japan) with bromoacetic anhydride (2) afforded the bromoacetamido support 2 (Scheme 1). Reaction with the cesium salt of BocTyr(2,6-DCB)OH (2) anchored the first amino acid (0.57 mmole Tyr/g, determined by amino acid analysis).

It is also possible to incorporate tyrosine by using the preformed BocTyr(2,6-DCB)-glycolic acid block 4 (Scheme 2). This product was prepared by reacting the 2,4,5-trichlorophenyl ester of bromoacetic acid 3 with DIEA salt of BocTyr(2,6-DCB)OH. 4 was condensed with BHA resin 1 in presence of hydroxybenzotriazole. The amount of Tyr incorporated was 0.58 mmol/g (determined by amino acid analysis).

The TP5 sequence was built by using the protocol reported in Table 1.

Side-chain protections used were : Asp : Chx, Lys : 2 ClZ, Arg : Mts. After the completion of the synthesis, the protected peptide-resin adduct was dried in vacuo and divided in two equal parts (0.5 g each). The first was suspended in isopropanol-water (70-30), 1M NaOH (2 equiv, 0.6 ml) was added and the mixture shaken. Despite an extended reaction time (36 h), no protected peptide was detected in solution. The second part was suspended in ice-cooled DMF, 1M NaOH (2 equiv, 0,6 ml) was added and the mixture shaken at 0°C. The release of peptide was monitored by HPLC (Aquapore RP-300, 250 x 4 mn ,A : H_2O 0.1 % H_3PO_4, B : 90 % CH_3CN 0.1 % H_3PO_4, 100 % A to 100 % B in 35 min) ; after 2 h the reaction mixture did not evolve any longer. The resin was filtered off and washed twice with DMF (6). The filtrates were pooled and the pH of the solution was brought to 7 with 1M HCl. DMF was removed in a rotatory evaporator, the white residue was triturated with water and dried for 12 h in vacuo (P_2O_5). The side-chains were deprotected by using 1M TFMSA in TFA containing thioanisole, metacresol and ethanedithiol (7). After 1 h at 0°C, TP5 was precipitated with cold diethylether. It was purified by Sephadex G.10 (0.1M HCl) followed by semi-preparative RP/HPLC (Nucleosil C8 5m , 250 x 20 mm, A : H_2O 0.1 % TFA, B : 60 % CH_3CN 0.1 % TFA, 100 A to 100 B in 3 h, 5 ml/min). The yield in purified peptide was 62 % based on the amount of tyrosine linked to BHA (Amino Acid Analysis : R 0.98, K 1.02, D 0.99, V 1.0, Y 0.89, Molecular weight in FAB-MS : 680 Da). TP5 coinjected in RP-HPLC with an authentic sample (3) gave a single peak.

As expected, with BHA resins the yield of cleavage is completely dependent of the nature of solvent. The use of DMF in place of iPrOH-H_2O ensures the completion of the reaction.

This work shows that it is possible (i) to introduce easily the glycolamidic anchorage in a commercialy available BHA resin and (ii) to cleave it by using smooth alkaline conditions (2 equiv NaOH 1M, 0°C DMF, 2h). It is not necessary to employ $Li^+S^-CH_2CH_2OH$ as recommended by Shekhani et al. (1).

Abbreviations : DMF : dimethylformamide ; BHA-resin : benzhydrylamine-resin ; TFMSA : trifluoromethanesulfonic acid ; TFA : trifluoroacetic acid ; 2,6-DCB : 2,6-dichlorobenzyl ; Chx ; cyclohexyl; Mts : Mesitylene-2-sulfonyl ; DIEA : diisopropylethylamine ; 2 ClZ : (2-chlorobenzyl)oxycarbonyl ; DCC: dicyclohexylcarbodiimide.

References and Notes

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