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3-HYDROXYPROPIONITRILE: A NEW REAGENT FOR CARBOXYL PROTECTION IN PEPTIDE SYNTHESIS[+]

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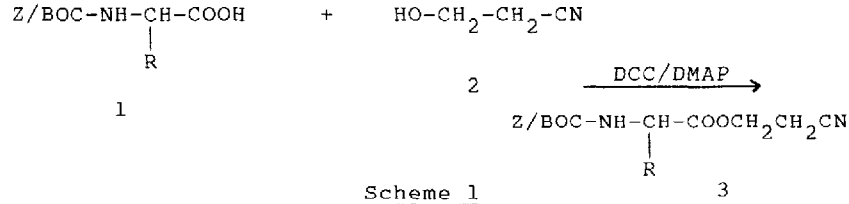
Summary: 3-Hydroxypropionitrile has been employed as a convenient reagent for carboxyl protection in peptide synthesis. Deblocking of this group is extremely facile and proceeds without side reactions.

A large number of amino protecting groups have been described in peptide syntheses. But there is a limited choice in case of carboxyl protecting groups. The commonly used alkyl and aralkyl esters have some limitations[1]. The use of benzyl ester precludes Z-protection at the amino terminus, deblocking of methyl ester is usually incomplete in case of oligopeptides. This has necessitated search for new carboxyl protecting groups. In this paper we report the use of a new carboxyl protecting group in peptide synthesis.

3-Hydroxypropionitrile (2-cyanoethanol, HO-CE) has been widely used to protect phosphate residues in oligonucleotide synthesis[2]. However, its application in peptides has not been investigated. This prompted us to explore cyanoethyl as carboxyl protecting group in peptide chemistry. We have synthesized many cyanoethyl esters of N-protected amino acids. Esterification was done by DCC/DMAP[3] using suitably protected amino acids as shown in Scheme 1.



Scheme 1

[+]CDRI Communication No.4470

Compound	Yield (%)	m.p. (°C)	$[\alpha]_D$ (C-1,MeOH)	I.R.(cm^{-1}) -CN
1. Boc-Gly-OCE	88	61-64	-	2300
2. Z-Gly-OCE	95	Oil	-	2290
3. Boc-Ala-OCE	93	68-69	-41	2310
4. Z-Ala-OCE	91	48-50	-33	2295
5. Boc-Pro-OCE	85	Oil	-59	2290
6. Z-Pro-OCE	90	Oil	-51	2300
7. Boc-Phe-OCE	95	84-86	- 8	2300
8. Z-Leu-OCE	82	Oil	-30	2290
9. Boc-Met-OCE	92	57-59	-37	2290
10. Z-Trp-OCE	89	94-97	-18.5	2310
11.++Boc-Asn-OCE	63	117-119	-23	2290
12. Boc-Glu(OBzl)-OCE	79	69-70	-25	2285
13. Z-Gln-OCE	58	128-130	-26	2280
14. Z-Lys(Z)-OCE	92	Oil	-13	2300
15. Boc-Thr(Bzl)-OCE	75	Oil	-17.8	2290
16. Boc-Arg(NO_2)-OCE	65	115-117	-17	2290
17.**Z-Gly-Gly-OCE	78	92-95	-	2300
18.**Boc-Gly-Gly-OCE	67	Oil	-	2290
19.**Boc-Phe-Met-OCE	81	137-138	-25	2300
20.**Z-Phe-Leu-OCE	68	130-132	-24	2280
21.**Z-Ala-Gly-Gly-OCE	70	82-84	- 4	2300
22.**Boc-Gly-Gly-Phe-Met-OCE	58	109-111	- 3	-
23.**Z-Gly-Gly-Phe-Leu-OCE	60	116-118	-15	-
24.**Z-Tyr-Gly-Gly-Phe-Leu-OCE	56	78-80	-17	-

Table 1

++Deblocking was complete in 3 hrs.

**Yield refers to peptide coupling reaction

The cyanoethyl esters were obtained in almost quantitative yields[4] (Table 1). We have found that cyanoethyl moiety is stable to acidolytic (cleavage of BOC) and hydrogenolytic (cleavage of Z) conditions, normally used in peptide synthesis[5,6].The Z-group was deblocked from compounds 2 and 20 under neutral conditions. The products obtained (H_2N-Gly-OCE and H_2N-Phe-Leu-OCE) were stable at r.t. and did not form diketopiperazine as seen by TLC. Moreover subsequent coupling of these fragments with carboxyl component gave pure peptides in satisfactory yields. The

application of -OCE group in peptide synthesis has been demonstrated by synthesising a biologically active peptide Leu-Enkephaline.

The deblocking of cyanoethyl group is extremely facile and proceeds without side reactions[7]. Deblocking has been achieved by 10% aq. potassium carbonate. In all the cases deblocking was complete in less than ten minutes as monitered by TLC. Whereas complete deblocking of -OCE group in case of tetra and pentapeptides was achieved in 1 hr. In this regard cyanoethyl is superior to other protecting groups removable by β-elimination reported in the literature[8-11]

Our observations can be summarised as follows: (1) cyanoethyl esters of amino acids can be easily prepared in good yields, (2) deblocking is selective and quantitative, (3) mild and selective deblocking conditions employed here would be ideal in orthogonal protection strategy[12], often preferred in peptide synthesis.

General procedure for esterification: A solution of suitably protected amino acid (1, 1.0 mmol) in methylene chloride/dimethylformamide (10 ml) was stirred with cyanoethanol (2, 2.0 mmol) in presence of catalytic amount of DMAP. To this was added a solution of DCC (1.1 mmol) in methylene chloride dropwise. Reaction was stirred at room temperature overnight. At the end of the reaction, after filtering DCU the solvent was removed under reduced pressure. The residual mass was dissolved in ethyl acetate and successively washed with 5% aq. $NaHCO_3$, water, 5% citric acid and brine. The organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified by flash chromatography using a linear gradient of chloroform → methanol (10%) as the eluant. Appropriate fractions were pooled, and the product was crystallized wherever possible.

General procedure for deblocking: To a stirred solution of amino acid/peptide ester (3, 1.0 mmol) in methanol (5 ml) was added 10% aq. potassium carbonate solution (2-3 ml) at room temperature. Deblocking was complete in less than ten minutes. Reaction was quenched by adding 5% citric acid (5 ml). After the usual work up free acid was obtained from organic layer in more than 80% yield. This was directly used in subsequent coupling reactions.

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4. All the compounds gave satisfactory, IR, NMR and elemental analysis. Homogeniety of the compounds was checked by silica gel TLC in 10% MeOH:$CHCl_3$ as the developing solvent. In the PMR spectra all the compounds exhibited a pair of triplets in region 2.2-2.7 δ and 3.8-4.3 δ (J=7.0 Hz) corresponding to α and β methylene protons of cyanoethyl moiety respectively.
5. 5N HCl in dioxane (1 ml) was added to a solution of Z-Gly-OCE (1 mmole) in CH_2Cl_2 (2 ml). The reaction was stirred at r.t. for 2 hrs. After work up starting material was recovered in quantitative yield. Similar results were obtained with TFA/CH_2Cl_2, suggesting that -OCE is stable to acidolytic conditions.
6. Boc-Gly-OCE (0.5 mmole) was taken in 25 ml MeOH subjected to hydrogenolysis over 10% Pd/C (100 mg) for 2 hrs. After work up starting material was recovered quantitatively indicating stability of --OCE group to hydrogenolysis.
7. The products obtained after deblocking of -OCE group have been characterized by comparing with the authentic samples with the help of Co-TLC, superimposable IR and NMR.
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