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SELECTIVE CLEAVAGE OF PROTECTED AMINO ACIDS AND PEPTIDES FROM OXYACYL RESINS BY AN 18-CROWN-6 COMPLEX OF POTASSIUM CYANIDE

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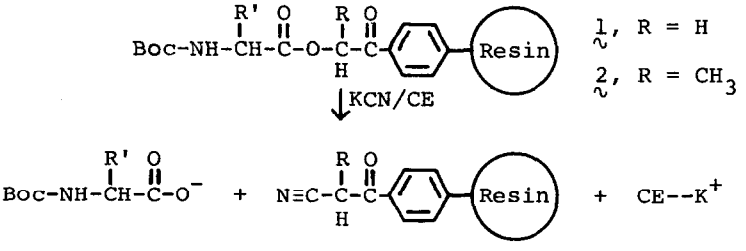
Condensation of peptide fragments on solid supports has achieved notable success through the recent synthesis of several complex peptides[1-3]. But the generality of this approach depends on the ease of obtaining suitably masked derivatives of the desired peptide fragments, especially a convenient method for preparing Boc-peptide acids by solid-phase peptide synthesis[4]. We have found that an 18-crown-6 complex of potassium cyanide is a suitable reagent for removal of protected peptide fragments from the Boc-aminoacyl-oxyacyl-polystyrene resins $\underset{\sim}{1}$[5] and $\underset{\sim}{2}$[6].



$\underset{\sim}{1}$, R = H

$\underset{\sim}{2}$, R = CH_3

The synthetic strategy of masking the α-amino groups with Boc and the side-chain functionalities with benzylic groups requires a mild method for cleavage from the resin in order to obtain the fully protected peptide acids needed for fragment condensation. Hydrazinolysis[5] of the phenacyl ester bond of resin $\underset{\sim}{1}$ or $\underset{\sim}{2}$ proceeds efficiently, but deprotection of side-chain benzyl ester groups also occurs. In addition, attempts to remove peptides from the

Table I. Use of Various Reagents to Cleave Peptide from Boc-Gly-Gly-Leu-Val-Gln-Pro-Gly-OCH(CH_3)CO-Polystyrene

Reagent	Solvent	Time (hr)	Yield[a] (%)
1.0 M $NaSC_6H_5$	DMF	24	22
0.5 M NaOH	Dioxane--H_2O	24	57
Photolysis (> 320 nm)	DMF	72	48-82
1.0 M KCN	DMF	72	5-6

[a] Based on amino acid analysis of the peptide remaining bound to the resin.

oxyacyl resins by thiolysis[5], saponification[5], or photolysis[6] have not been entirely satisfactory (Table I). More desirable would be a cleavage reagent as efficient as hydrazine but more selective. The reactivity of KCN with phenacyl bromide[7] suggested that its reaction with phenacyl esters might be useful if could be made a better nucleophile. Since crown ethers[8] (CE) can enhance the nucleophilicity of anions such as fluoride and cyanide[9], the dicyclohexyl-18-crown-6 complex of KCN (KCN/CE) in aprotic polar solvents was examined for its ability to displace protected amino acid or peptide carboxylates from resin-bound phenacyl esters.

High yields of peptide were obtained with 0.1 to 1.0 M KCN/CE (1:1 complex) at room temperature in dimethylformamide (DMF), N-methylpyrrolidone (NMP) or CH_3CN (Table II). Similar treatment with KCN in the absence of CE gave only 5-6% yields (Table I). Little difference was seen between the reactivity of resins $\underset{\sim}{1}$ and $\underset{\sim}{2}$. The KCN/CE reagent was quite selective, being unreactive toward most of the usual side-chain protecting groups. For example, Boc-Ser(Bzl), Boc-Thr(Bzl), Boc-Tyr(Bzl), Boc-Cys(MeBzl), Boc-His(Bzl), Boc-His(Tos), Boc-Arg(Tos), and For-Gly were stable after treatment with 0.5 M KCN/CE in DMF for 3 days. This reagent effectively removed the formyl group from Boc-Trp(For), however, and gave Boc-His as the major product from Boc-His(Dnp) along with

Table II. Cleavage of Boc-Amino Acids and Peptides from Oxyacyl Resins by KCN/CE

Oxyacyl Resin	KCN/CE (M)	Time (hr)	Yield[a] (%)
Boc-Gly-OCH(CH_3)-CO-Resin	1.0	16	94 (96)
"	0.4	8	92
"	0.2	8	95
"	0.1	8	90
Boc-Gly-OCH_2-CO-Resin	1.0	16	97 (98)
"	0.4	8	93
"	0.2	8	89
"	0.1	8	89
Boc-Gly-Gly-Leu-Val-Gln-Pro-Gly-OCH(CH_3)-CO-Resin	0.4	18	(93)
"	0.2	2x12	86
"	0.1	2x12	87

[a] Solvent was NMP except DMF for values in parentheses.

other minor ninhydrin-positive products. Boc-Asp(OBzl)-Gly-NH_2 was treated with KCN/CE under similar conditions and 95% of the starting material was recovered by column chromatography, indicating that very little if any of the β-benzyl ester was cleaved. Protected amino acids attached as benzyl esters to resin supports were also relatively stable to KCN/CE; prolonged treatment (3 days) produced less than 10% cleavage of Boc-Gly-OCH_2-resin, Boc-Val-OCH_2-resin, Boc-Gly-OCH_2-Pam-resin[10] and Boc-Val-OCH_2-Pam-resin. This new procedure is quite mild and selective and will complement existing methods for the preparation of fully protected peptide fragments by solid-phase synthesis[11].

A typical example is illustrated by the synthesis of Boc-Gly-Gly-Leu-Val-Gln-Pro-Gly-OH. Boc-Gly-2-oxypropionyl-copoly(styrene--1% divinylbenzene)[7] (2, R' = H; 2 g, 0.35 mmol/g) was extended to the Boc-heptapeptide-resin[12] by standard solid-phase methods[4,13]. A solution of dicyclohexyl-18-crown-6 ether (0.744 g, 2 mmol; Fluka AG) and dry, finely pulverized KCN (0.13 g, 2 mmol) in dry DMF (20 ml) was stirred with the peptide-resin for 12 hr at 25°. Additional 2 mmol portions of KCN and the crown ether were added and stirring was continued for 12 hr. The resin was filtered and washed with warm DMF. The filtrate was

concentrated and diluted with water. The precipitated peptide was suspended in 50% aqueous DMF and adjusted to pH 2.5 with HCl. After evaporation of the solvent, the residue was triturated with petroleum ether and dried. The protected peptide was dissolved in 19:1 (v/v) $CHCl_3$--CH_3CO_2H, applied to a silica gel-60 column[14], and eluted with a linear gradient from 19:1 $CHCl_3$--CH_3CO_2H to 19:20:1 $CHCl_3$--CH_3OH--CH_3CO_2H to provide homogeneous Boc-Gly-Gly-Leu-Val-Gln-Pro-Gly-OH (0.21 g) in 49% yield.[12]

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12 We have observed that about 30% of H-Gly-oxyacyl-resins 1 and 2 became terminated during peptide synthesis. A similar observation with one of these resins was recently reported by C. Birr et al. at the Fifth American Peptide Symposium, San Diego, California, June 1977. An intramolecular cyclization to a dihydro-1,4-oxazin-2-one was thought to be involved.

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