Tetrahedron Letters No. 20, pp 2021 - 2024, 1972. Pergamon Press. Printed in Great Britain.

APPLICATION OF ELECTROLYTIC REDUCTION FOR REMOVAL OF 2-HALOGENOETHOXYCARBONYL GROUPS IN PEPTIDE SYNTHESIS

E. Kasafírek

Research Institute for Pharmacy and Biochemistry, Prague 3

(Received in UK 27 March 1972; accepted for publication 7 April 1972)

The application of 2-halogenoethoxycarbonyl residues as protecting groups of amino acids and peptides in synthesis was described by Woodward et al.[1] (2,2,2-trichloroethoxycarbonyl TCE), Grimshaw[2] (2-chloroethoxycarbonyl CE, 2-bromoethoxycarbonyl BE and 2-iodoethoxycarbonyl IE) and now by Yajima et al.[3] (TCE). So far protecting groups of this type can be easily removed by electrolytic reduction in mild acid medium on zinc and even more rapidly on mercury electrodes [4].

For the purpose of this study, we synthesized further new 2-halogenoethoxycarbonyl derivatives, i.e. 2,2,2-tribromoethoxycarbonyl (TBE) and 2,2,2-trifluoroethoxycarbonyl (TFE). All protected amino acids* were prepared using appropriate chloroformate in aqueous-organic medium as acylating agent. The protecting groups under study are as follows:

$X\text{-}CH_2\text{-}O\text{-}CO\text{-}$, where X = $ClCH_2$, $BrCH_2$, ICH_2, F_3C, Cl_3C and Br_3C. (see table 1.)

We studied the conditions for electrolytic reduction on model substances (CE-Gly, BE-Gly, IE-Gly, TFE-Gly, TCE-Phe and TBE-Gly) at a temperature of 20°C and current density 21 mA/cm^2. We used graphite as anode. The electrolyte was lithium chloride, possibly sodium iodide, in a mixture of methanol and acetic acid. We studied the course of the electrolytic reduction on a polarograph in

* All amino acids used in this work (with the exception of glycine) are of the L configuration.

McIlvain buffer of pH 6 chromatographically and electrophoretically on a thin layer. The electrolytic removal of the individual protecting groups in relation to the nature of electrodes is shown in table 2. The IE, TCE and TBE groups are of practical importance for peptide synthesis, as their quantitative removal is easy. Furthermore the difference of their half-wave potentials affords, by using a potentiostat, the selective removal of the individual protecting groups: $(E_{1/2})_{IE}$ = -1.28 V, $(E_{1/2})_{TCE}$ = -0.89 V or $(E_{1/2})_{TBE}$ = -0.09 V.

Table 1.

Acylated amino acids and their derivatives

Amino acid	2-Halogenoethoxycarbonyl derivative (m.p.°C)
Gly	TFE(94-96), TBE(113-114)
Ala	IE(103-104)
Phe	IE(84-85), TCE(127-128), TBE(84-88)
Tyr(Bzl)	CE(110-112), IE(95-100), TCE(110-113)
Trp	CE(108-109), IE(103-105), TBE(96-100)
δ-Orn	IE(210-213)
ε-Lys	IE(200-202)
Lys(Tos)	IE(91-93)
Arg(NO_2)	IE(109-112)
His(Bzl)	CE(186-187), IE(190-192)
Pro	TCE(60-62), TBE(94-97)
Met	IE(76-77), TCE(83-85), TBE(159-161)*
Asp	BE(122-123), IE(135-137), TCE(148-150)
Glu	IE(98-100), TCE(55-60)
Asn	IE(154-157)
Gln	IE(126-127), TCE(116-118), TBE(140-142)

* Dicyclohexyl ammonium salt.

It is possible to remove the CE group in the presence of sodium iodide. Be derivatives may be easily converted to reducible IE derivatives. The tosyl group and benzyloxycarbonyl group are stable under these conditions of reduction. Quantitative removal of the IE group is also possible under conditions of electrolytic reduction according to Horner [5], i.e. in the presence of tetramethylammonium chloride.

We verified the applicability of this method by synthesis of peptides listed in table 3. No undesirable products are formed in reduction of methionine derivatives. Isolation of free peptide ester was carried out either chromatographically on Zerolit FF or by extraction with ammonia in chloroform; the isolated peptide ester was chromatographically pure.

Table 2.

Electrolytic reduction of protecting groups (1 mmol, period 120 min.)

Protecting group	Electrodes		Protecting group	Electrodes	
	Zn	Hg		Zn	Hg
CE	- *	- *	TFE	-	-
BE	+	+	TCE	++ **	+++(quant.)
IE	+++(95-98%)	+++(quant.)	TBE	+++	+++(quant.)

\- not reduced, + reduced very little, ++ partly reduced, +++ reduced.

* Reduced in the presence of sodium iodide. ** Reduction is accompanied by formation of an side product, i.e. by 2,2-dichloroethoxycarbonyl derivative ($E_{1/2}$) = -1.27 V. Its amount increases during reduction and reaches limit values after all trichloro derivatives had been reduced; its reduction is rather difficult with zinc cathode. Under these conditions the monochloro derivative (CE) is neither reduced, nor does it afford a polarographic wave.

Table 3.

Peptide synthesis *

Starting compound	M.p. °C	Acylating agent	Product	M.p. °C
IE-Leu-GlyOEt	89-91	Z-Pro	Z-Pro-Leu-GlyOEt	143-145
IE-Lys(Tos)-GlyOEt	116-119	Z-ProONp	Z-Pro-Lys(Tos)-GlyOEt	115-118
IE-Met-GlyOEt	119-121	TCE-Tyr(Bzl)	TCE-Tyr(Bzl)-Met-GlyOEt	109-111
IE-Phe-TyrOMe	137-138	CE-Gly	CE-Gly-Phe-TyrOMe	98-101

* Application of mixed anhydrides, N,N´-dicyclohexylcarbodiimide or active esters were used in coupling procedures.

The products were isolated in yields of 60-75% (calculated on the starting protected dipeptide).

The full paper will be published in Collection Czechoslovak Chem. Communications.

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