Use of *o*-Hydroxyphenyl Esters for the Preparation of an Optically Pure Polytripeptide

By Y. TRUDELLE

(*Centre de Biophysique Moléculaire, 45-Orléans-La Source, France*)

Summary An improvement of the *o*-hydroxyphenyl ester method in polypeptide synthesis is reported.

THE *o*-hydroxyphenyl esters of peptides were first suggested by Young,[1] as a new, racemization-free method of coupling.

Despite the fact that catechol is weakly acidic (pK_a 9·85), coupling is still very fast, for the reactivity of *o*-hydroxyphenyl esters towards amines is enhanced by intramolecular base catalysis (anchimeric assistance). The same explanation has been advanced for example in the case of 8-hydroxyquinoline esters, which, however, are not very convenient derivatives because of the hygroscopic nature of their hydrochlorides.

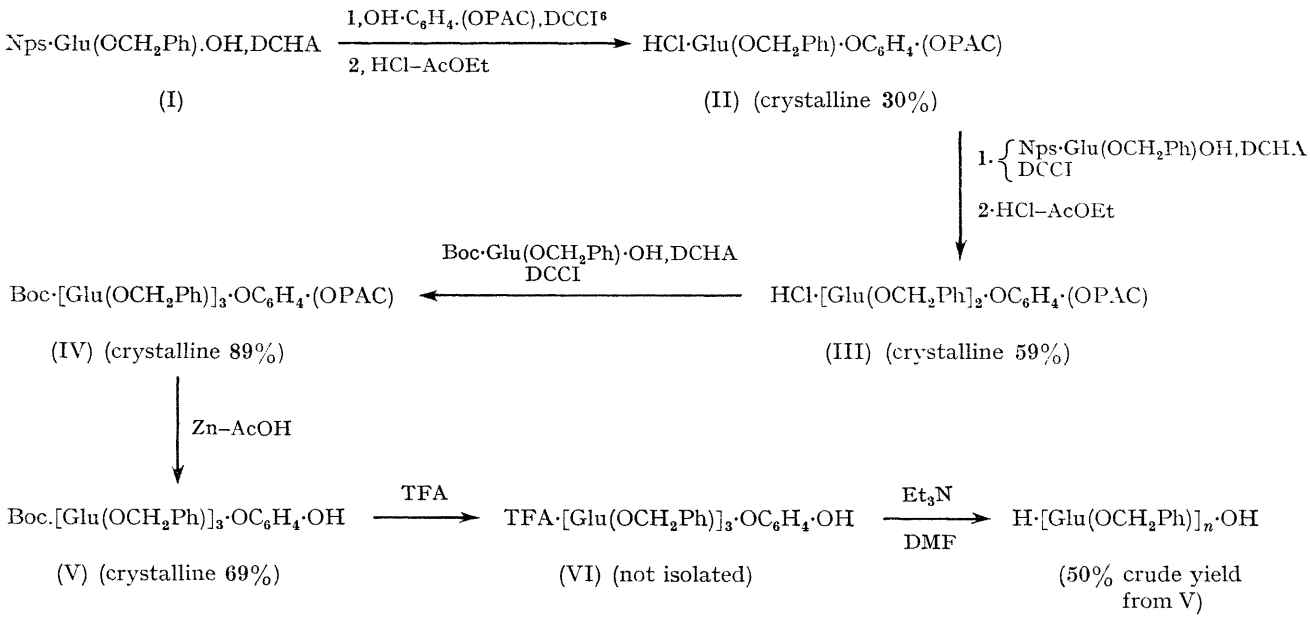
The main difficulty encountered in using *o*-hydroxyphenyl esters is the necessity of protecting the free hydroxy-group. Jones and Young[2,3] used the benzyl ether. By this route, an optically pure polytripeptide, poly(glycyl-L-prolyl-L-alanyl-), has been prepared.[3] However, removal of the benzyl ether protecting group requires rather drastic conditions (HBr 6·5 N in AcOH), not always compatible with the protection of side-chains.

We have investigated other methods for protecting the hydroxy-group to find one more convenient. The t-butyl group was thought promising, but all attempts to synthesise *o*-(t-butoxy)phenol failed.† The *p*-methoxybenzyl group showed the same disadvantages as the benzyl group.

† The reaction of t-butyl bromide with the sodium salt of catechol, and of t-butyl alcohol with catechol in the presence of zinc chloride have failed. Moreover, the reaction of isobutene and catechol in the presence of sulphuric acid leads to substitution on the aromatic ring. As shown by atomic models, *o*-(t-butoxy)phenol must be highly strained.

Finally, the phenacyl group was found to be suitable; *o*-(phenacyloxy)phenol,[4] when esterified by amino-acids or peptides leads in most cases to easily crystallisable derivatives and removal of this ether requires only mild conditions. For example, Hendrickson and Kandall,[5] described removal with zinc dust in aqueous or anhydrous acetic acid. Such conditions allow selective removal of the phenacyl group, leaving intact most other side-chain protecting groups. However, it has been found that removal of the phenacyl group must be performed on a peptide whose amino-group is protected. If it is in the form of hydrochloride, a complex mixture contaminated by zinc is obtained, so that isolation of the pure peptide may be unsuccessful.

As amino-protecting group, *o*-nitrophenylsulphenyl exhibits a high sensitivity towards hydrogenolysis by zinc–acetic acid, but t-butoxycarbonyl is suitable.



SCHEME

TABLE

Comparison of several PBLG *samples*

Method of preparation	Method of purification	M_w [e]	$[\alpha]^{25}$ (546 nm)	Extent of racemization
NCA	C	9,200	−19·45	0
NCA	C	12,500	−19·65	0
NCA	A	15,000	−18·95	0
	C	16,500	−19·60	0
NCA	A	50,000	−19·90	0
o-Hydroxyphenyl ester[a] ..	A		−18·90	
	C	11,000	−19·60	Not detectable
N-Hydroxysuccinimide ester[b] ..	B	12,000	−18·60	?
DCCI + HOSu[c]	B	3,000	−16	?
Pentachlorophenyl ester[d] ..	B	25,000	−13·75	about 90%

A Precipitation with ethanol; B precipitation with ethanol followed by dialysis against dimethyl formamide; C precipitation with ethanol followed by extraction with boiling ethanol in a hot Soxhlet extractor'.

[a] Polycondensation of the tripeptide *o*-hydroxyphenyl ester in DMF. [b] Polycondensation of the dipeptide *N*-hydroxysuccinimide ester in DMF. [c] Polycondensation performed by mixing in DMF the free dipeptide with DCCI (dicyclohexylcarbodi-imide) and *N*-hydroxysuccinimide. The yield of PBLG is very low (5%). [d] Polycondensation of the tripeptide pentachlorophenyl ester in DMF. [e] M_w is estimated from intrinsic viscosity according to Doty.[8]

In order to detect the extent of racemization by the different methods which may occur during polycondensation, we have polycondensed the dipeptide or tripeptide active esters:

$$H\text{–}(\gamma\text{-benzyl-L-glutamyl})_{2\text{ or }3}\text{–}OX$$

and compared the optical rotations in dichloroacetic acid of PBLG obtained by such a way, with that of optically pure PBLG prepared by the *N*-carboxyanhydride (NCA) method.

However, the resulting polymer may contain oligopeptides and low molecular weight polypeptides, the

specific rotations of which differ significantly from that of a high molecular weight and optically pure PBLG. Therefore, we have compared PBLG samples of similar molecular weight which had been purified similarly.

The main features and results are summarized in the Scheme and the Table.

From the Table it appears that PBLG prepared using the *o*-hydroxyphenyl ester exhibits the same optical rotation as the corresponding PBLG obtained by the NCA method. It can be therefore concluded that polycondensation *via* the *o*-hydroxyphenyl ester does not induce any detectable racemization. PBLG samples obtained *via* *N*-hydroxysuccinimide have lower optical rotations than standard PBLG. However, such a difference might be due to the presence of smaller polypeptides since purification was restricted to precipitation by ethanol followed by a dialysis.‡ Polycondensation *via* pentachlorophenyl ester however leads to considerable racemization; this contrasts with the results of Kovacs,[7] obtained in dimethyl sulphoxide.

I thank Dr. G. Spach for his advice and Dr. A. Brack for a gift of low molecular weight PBLG prepared by the NCA method.

(*Received, March 10th*, 1971; *Com.* 198.)

‡ Purification of PBLG by extraction in a hot Soxhlet extractor with boiling ethanol has been found more efficient than dialysis for removing small polypeptides.

[1] G. T. Young, in 'Peptides', eds. H. C. Beyermann, A. Van De Linde, and W. Massen Van Der Brink, North Holland Publishing Co., Amsterdam, 1967, p. 55.
[2] J. H. Jones and G. T. Young, *J. Chem. Soc.* (*C*), 1968, 436.
[3] J. H. Jones, *Chem. Comm.*, 1969, 1436.
[4] I. Lazennec, *Bull. Soc. chim., France*, 1903, 502.
[5] J. B. Hendrickson and C. Kandall, *Tetrahedron Letters*, 1970, **5**, 343.
[6] J. S. Morley in 'Peptides', ed. L. Zervas, Pergamon Press, Oxford, 1966, p. 351.
[7] J. Kovacs, R. Giannotti, and A. Kapoor, *J. Amer. Chem. Soc.*, 1966, **88**, 2282.
[8] J. C. Mitchell, A. E. Woodward, and P. Doty, *J. Amer. Chem. Soc.*, 1957, **79**, 3955.