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NEW POLY(ETHYLENE GLYCOL) SUPPORTS FOR THE LIQUID-PHASE SYNTHESIS OF PROTECTED PEPTIDE HYDRAZIDES

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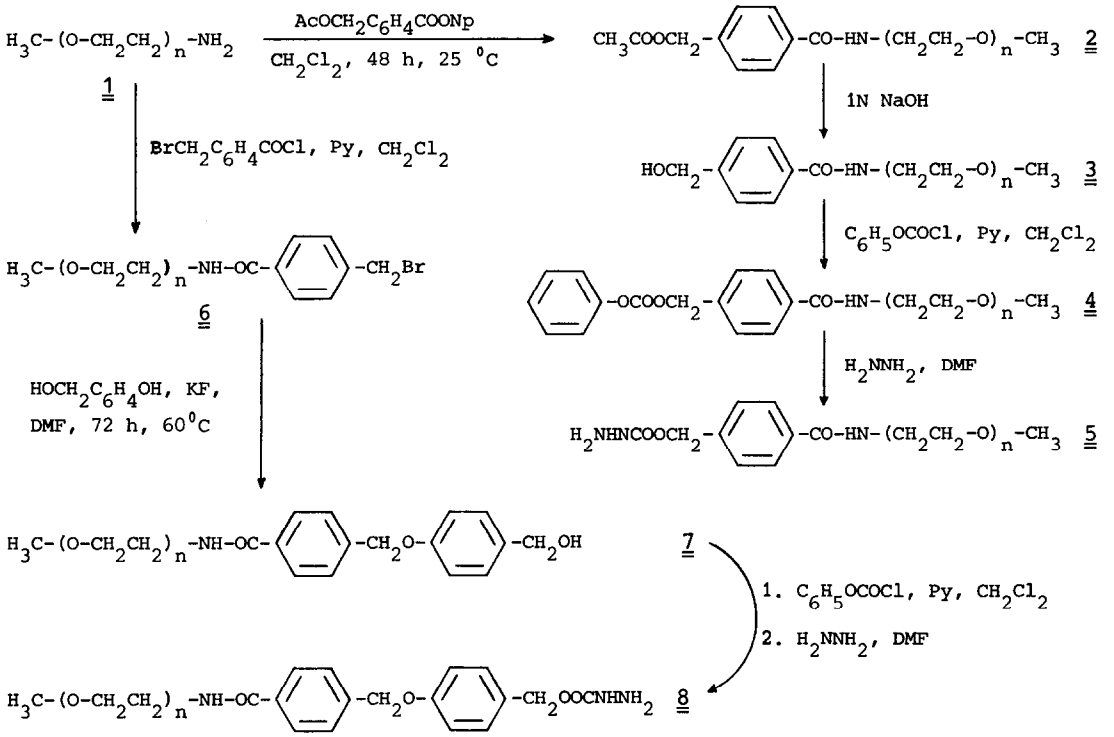
Summary. The liquid-phase synthesis of protected peptide hydrazides can be suitably performed on modified poly(ethylene glycol) supports containing benzyl-, p-benzyloxybenzyl-, and *tert*-alkyl-oxycarbonylhydrazide anchoring groups.

Protected peptide hydrazides[1] are useful precursors for the synthesis of the long peptide chains in that reaction with aqueous nitrous acid or alkyl nitrite in organic solvents gives the corresponding azides, which are suitable for segment condensation. The liquid-phase method[2] for peptide synthesis offers a rapid procedure, which combines strategic elements of both classical and solid-phase technique, for assembling small and medium-sized peptide chains on a polymeric soluble support. Consequently, protected peptide hydrazides may be obtained by either hydrazinolysis of peptide chains linked to poly(ethylene glycol) supports through an ester bond[1,3] or direct coupling of released protected peptide acids with hydrazine[4] . Though both of these procedures are attractive, they are not without some undesirable limitations and complications, such as low yields or modifications of Asp and Glu side chains protected as benzyl-type esters, and a more generally applicable method is desirable.

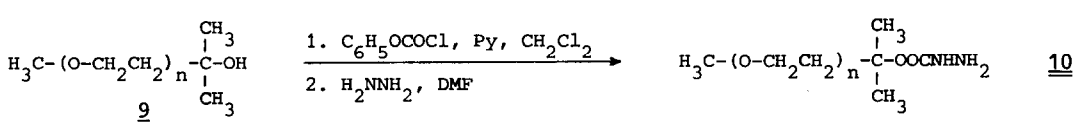
Recently a number of modified poly(ethylene glycol) supports were described[3,5] , which can be used for the liquid-phase synthesis of free and protected peptides and of peptide amides[5] , and are cleavable from the finished product by acidolysis, hydrogenolysis, and photolysis; I wish now to report the preparation and use of a new type of modified soluble polymers, bearing an oxycarbonylhydrazyde functional group and designed for the direct liquid-phase synthesis of peptide hydrazides.

Poly(ethylene glycol) monomethyl ether (M_r= 5000) was converted to amino-poly(ethylene glycol) monomethyl ether 1 according to the procedure of Mutter[6] , and 1 was allowed to react with the p-nitrophenyl ester of p-acetyloxymethyl-

benzoic acid (prepared in the usual manner[20]); the product, 2, was deacetylated with 1 N NaOH to yield 3, which was acylated with phenyl chloroformate, and the phenyl carbonate (4) obtained was then hydrazinolyzed to give the benzyloxycarbonylhydrazide polymer 5. The amino-polymer 1 was also converted to its p-bromomethylbenzoyl derivative 6 by treatment with p-bromethylbenzoyl chloride[5], and the substituted benzyl bromide 6 was allowed to react with a large excess (5-8 equiv.) of 4-hydroxymethylphenol in the presence of KF[7] to give 7; the reaction of the alkoxybenzyl alcohol 7 with phenyl chloroformate first and then with hydrazine yielded the p-alkoxybenzyloxycarbonylhydrazide polymer 8.



The monofunctional poly(ethylene glycol) bearing a tertiary alcohol group 9, prepared as described by Anzinger *et al.*[8], was converted to the *tert*-alkyloxycarbonylhydrazide polymer 10 by treatment with phenyl chloroformate followed by hydrazinolysis. Polymers 5, 8, and 10 were repeatedly crystallized from CH_2Cl_2 and DMF solutions by slow addition of anhydrous diethyl ether untill chromatographically homogeneous. Hydrazide contents were determined by nitrogen microanalysis.



The modified poly(ethylene glycol) supports 5, 8, and 10 were employed for assembling a number of protected tri-, tetra-, and pentapeptide hydrazides *(Table*

Table 1. Protected Peptide Hydrazides prepared by Liquid-Phase Method.

PRODUCT [a]	Support	CLEAVAGE [b]: Agent	Yield %	overall yield [c] %	mp (°C)	$[\alpha]_D^{25}$, deg		Ref.
Boc-Ala-Ala-Ala-$NHNH_2$	5	H_2	96	53	247-249	-18.1±0.4	(c=1, DMF)	(d)
Boc-Leu-Leu-Gly-$NHNH_2$	5	H_2	91	48	oil [e]			(f)
Boc-Gln-Ala-Ala-Thr-Gly-$NHNH_2$	5	H_2	95	35	207-209	-15.2±0.5	(c=0.5, DMF)	(g)
Boc-Ala-Glu(OBu^t)-Lys(N^{ε}-Boc)-Lys(N^{ε}Boc)-$NHNH_2$	5	H_2	90	27	167-168	-23.8±0.3	(c=1, CH_3OH)	(h)
Fmoc-Gly-Ala-Val-Leu-$NHNH_2$	8	TFA	92	43	218-222	-26.5±0.5	(c=1, DMF)	(i)
Fmoc-Gly-Phe-Phe-Tyr(Bzl)-Thr(Bzl)-$NHNH_2$	8	TFA	85	41	190-193	- 1.2±0.2	(c=1, DMF)	(j)
Z-Gly-Leu-Phe-$NHNH_2$	8	TFA	93	49	193-194	-29.7±0.3	(c=0.5, DMF)	(k)
Z-Val-Lys(N^{ε}-Z)-Pro-Gly-$NHNH_2$	8	TFA	89	37	202-203	-44.1±0.5	(c=1, CH_3OH)	(l)
	10	TFA	95	35	203-204	-43.0±0.4	(c=1, CH_3OH)	(l)
Z-Gly-Phe-Phe-Tyr(Bzl)-Thr(Bzl)-$NHNH_2$	8	TFA	87	31	226-227	- 2.1±0.5	(c=1, DMF)	(m)
	10	TFA	94	30	225-227	- 2.9±0.4	(c=1, DMF)	(m)

[a] Satisfactory elemental and amino acids analyses have been obtained for all products. [b] The reported cleavage yields are based on quantitative amino acid analysis of the peptide remaining on the polymer after removal of the cleaved peptide hydrazide. [c] Based on starting protected aminoacyl-polymer. [d] Ref. *9* : mp 249-250, OR -17.3±0.3. [e] T.l.c. on silica gel plates (Merck, F-254) gave one chlorine positive, picryl-positive spot, R_f 0.58 (n-BuOH/AcOH/H_2O 5:1:4); microanalysis was performed on the tripeptide hydrazide obtained after removal of the Boc N^{α}-protecting group by 50% TFA in CH_2Cl_2. [f] Ref. *10*. [g] Ref. *11* : mp 210-212, OR -16.2 (c=0.4, DMF). [h] Ref. *12* : mp 165-167, OR -25.0 (c=1.1, CH_3OH). [i] Ref. *13* : mp 220-225, OR -24.25. [j] Ref. *13* : mp 196-198, OR -0.40. [k] Ref. *14* : mp 191-192, OR -28.3. [l] Ref. *15* : mp 200-201, OR -47.7. [m] Ref. *16* : mp 215-218, OR -3.94.

1), which after cleavage from the polymers and crystallization from a suitable solvent (CH_3OH, C_2H_5OH, or DMF by addition of water or diethyl ether) were obtained in satisfactory yields and homogeneous form. At the start N^{α}-protected amino acids were condensed with the hydrazide supports by means of DCC in the presence of 1 equiv. of HOBt[17], and the stepwise syntheses were then performed by the DCC/HOBt procedure according to the general techniques of liquid-phase method[2].

The anchoring bond between the peptide chain and polymer 5 was cleaved, at the end of the synthesis, by catalytic hydrogenolysis (5% Pd on $BaSO_4$, 40 psi,

room temperature, 18-22 h), thus leaving *tert*-butyl based protecting groups unaffected. Bonds formed by supports 8 and 10, which are structurally similar to those devised by Wang and Merrifield[18] and by Wang[19] for the solid-phase synthesis of peptide hydrazides, are acid-labile and were selectively cleaved by 50% TFA in CH_2Cl_2 (30-60 min, 25 °C); under these conditions the benzyl based protecting groups are normally stable as well as the Fmoc group, thus allowing the preparation of fully protected peptide hydrazides. Syntheses on support 5 were performed employing, as N^{α} temporary protection, the Boc group (if no side chain protecting groups were present) or the Bpoc group (if *tert*-butyl based protecting groups were introduced during the chain assembly); the last amino acid was coupled as its N^{α}-Boc derivative. The synthesis on support 8 is compatible with Bpoc and Fmoc N^{α} protection, while polymer 10 can be used with a strategy of synthesis based on the use of N^{α}- Bpoc, Fmoc, or Z groups.

REFERENCES AND NOTES

Abbreviations: Boc, *tert*-butyloxycarbonyl; Bpoc, 2-(4-biphenylyl)propyl(2)oxycarbonyl; Bu^t, *tert*-butyl; Bzl, benzyl; DCC, dicyclohexylcarbodiimide; Fmoc, 9-fluorenylmethyloxycarbonyl; HOBt, 1-hydroxybenzotriazole; ONp, 4-nitrophenyl ester; OR, optical rotation; Py, pyridine; TFA, trifluoroacetic acid; Z, benzyloxycarbonyl.

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