

REACTIONS RATES OF POLYMERIC N-HYDROXYSUCCINIMIDE ESTERS OF *o*-NITROBENZENESULPHENYLAMINO ACIDS WITH ESTERS OF AMINO ACIDS*

V.GUT^a and Yu.A.DAVIDOVICH^b

^a *Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6, and*

^b *Institute Khimii Elementoorganicheskikh Soedinenij, Soviet Academy of Sciences, Moscow, USSR*

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Reaction rates of the synthesis of dipeptides with polymeric activated N-hydroxysuccinimide-type esters of selected N-*o*-nitrobenzenesulphenylamino acids in dichloromethane or dimethylformamide and their dependence on reaction conditions were examined.

In a succeeding paper¹ of this series there will be reported a synthesis of esters of *o*-nitrobenzenesulphenylamino acids and a macroporous polymer containing the structure of a N-hydroxysuccinimide unit along with the applicability and advantageous properties of these esters in the synthesis of peptides. The potential use of excess polymeric agent in the synthesis could negatively affect the purity of the product owing to those side reactions the by-product of which would not remain bound to the polymer. The reactivity of these agents has been therefore quantitatively examined with the aim to obtain data for optimum reaction conditions. Reaction rates were measured by means of a continuous method recommended earlier² for measurement of reaction rates in the solid phase peptide synthesis and based on the technique of Merrifield. Reactions were followed by the change of absorption of the *o*-nitrobenzenesulphenyl protecting group at 384 nm.

As it may be seen from Table I (for abbreviations see ref.³), the apparent half-time in reactions of esters of the particular amino acids varied within almost three orders of magnitude. The greatest effect on the slow-down of the reaction may be ascribed to the bulky side chains of valine and isoleucine in the neighbourhood of the amino acid α -carbon atom and then to proline with a secondary amino group. The effect of aliphatic residues unbranched at the β -carbon atom on retardation of the aminolysis is lower, as exemplified by leucine and lysine. The lowest retardation is observed in the case of aralkyl substituents. On the other hand, the reaction is accelerated

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by polarisable substituents of the β -carbon atom. The fact that alanine deviates from this series may be due to a different course of the reaction curve. None of the reactions is of the 1st order with respect to the activated ester; the apparent 1st order reaction constant decreases in the course of the reaction. Such an observation was expected in view of the similar character of reactions in the solid phase synthesis². With the polymeric activated ester of *o*-nitrobenzenesulphenylalanine, the retardation is strikingly high as shown on comparison with the valine ester in Table II (ap-

TABLE I

Apparent Half-Times $t_{0.5}$ (min) in Aminolysis of the Activated Polymeric Esters of N-*o*-Nitrobenzenesulphenylamino Acids with Alanine and Valine Tert-butyl Esters in Dichloromethane

Amino acid	H-Ala-OBu ^t 0.00225M	H-Val-OBu ^t 0.00195M	Amino acid	H-Ala-OBu ^t 0.00225M	H-Val-OBu ^t 0.00195M
Asp(OBu ^t)	1.18	—	Leu	20.50	52.67
Cys(Bzl)	4.63	—	Lys(Z)	21.67	—
Cys(Tr)	5.12	—	Pro	62.83	136
Gly	6.80	6.40	Val	452	1215
Phe	14	35	Val		160 ^a
Tyr(Bu ^t)	15.40	—	Ile	584	240 ^a
Ala	18.80	29.90	Ile		371 ^b

^a $t_{0.1}$, ^b $t_{0.15}$.

TABLE II

Velocity Constant k_1 for Various Periods in Aminolysis of Polymeric Activated Esters in Dichloromethane

Nps-activated ester Amino ester	Period of reaction, %	k_1 , min ⁻¹	Nps-activated ester Amino ester	Period of reaction, %	k_1 , min ⁻¹
Ala	4—28	0.47	Val	9—25	0.0161
+ H-Ala-OBu ^t (0.0225M)	28—39	0.23	+ H-Ala-OBu ^t (0.103M)	46—52	0.0139
	41—45	0.15		54—60	0.0125
	46—51	0.047		61—66	0.0099
	57—70	0.0094		68—82	0.0058
	71—77	0.0049			
	78—83	0.0015			
	83—94	0.0011			

proximations of reaction periods are expressed by 1st order rate constants). The reaction course was also similar in dimethylformamide as solvent. Reactions of polymeric activated esters may be considered to be of the 1st order with respect to the amino component (Table III). This finding is important for application of the observed rate characteristics in the preparative utilisation of the present agents since it makes possible extrapolation in determinations of reaction conditions. Dependence on the structure of the amino component is considerably lower than that on the structure of the activated ester (Table I), quite in accordance with observations on activated esters in homogeneous solutions⁴. The aminolysis is more retarded by the secondary amino group of proline than by the bulky side chain of valine (*cf.* Tables I and IV). Concerning dependence of the reaction rate on solvents, a close similarity has been observed between reaction rates of polymeric leucine and valine derivatives in dichloromethane and dimethylformamide (Table IV) except for the alanine derivative. Reactions in dimethylformamide are about 2.7 times faster. The reactivity is thus inverse with respect to that observed with N-hydroxysuccinimide derivatives in homogeneous solutions⁵. The aminolysis of proline tert-butyl ester was however slower in dimethylformamide; a maximum rate has been observed in a mixture of the two solvents (dichloromethane and dimethylformamide) as shown by initial rate values in Table IV. A comparison with some other amino esters could not be performed because of troublesome measurements in this solvent mixture. Measurement of the reaction rate dependence on the bead size of the polymer esterified with *o*-nitrobenzenesulphenylvaline and alanine tert-butyl ester (0.206M in dichloromethane) affords a rough information on the carrier properties with respect to diffusion:

Diameter, mm	0.1–0.2	0.4–0.5
Apparent half-time, min	44.75	56

Owing to a great difference in particle dimensions of the tested polymeric activated esters, the dependence on the bead size is relatively small, indicating thus high diffusion rates on the level of the polymer particle dimensions.

The influence of the polymer on the reactions examined asserts itself especially by a relative retardation of the reaction during its course. Owing to a low dependence of reaction rates on the bulkiness of the amino component and on dimensions of the polymer particles, this slow-down cannot be due to diffusion on the level of particle dimensions. A gradient of the diffusion coefficient might however exist in the polymer; when sheer enough, it could considerably affect the local reactivity. In a limit case, when the immediate neighbourhood of the reacting group on the polymer is affected, such a system could be designated as a continuum of parallel reactions. In both the cases, a dynamic effect probably asserts itself in the course of the reaction on the basis of changed physical properties of the polymer owing to removal of the amino acid residues previously attached to this polymer.

Such an explanation would be supported by quantitative differences in the relative retardation of the reaction course in the case of alanine. The reaction rate gradually decreases by more than two orders of magnitude while the amount of the bound protected amino acid differs to a negligible extent with the particular derivatives. A substitution of similar N-hydroxysuccinimide residues on the polymer may be thus assumed.

Concerning the preparative reaction conditions, it is advantageous to use about 50% of the capacity of the polymeric activated ester, *i.e.*, to perform the reaction in the presence of about 100% excess of the polymer with respect to the amino component. Some time ago⁶, an analogous synthesis (with benzyloxycarbonyl and

TABLE III

Dependence of Apparent Reaction Half-Times in the Aminolysis of Activated Polymeric Esters on Amine Concentration in Dichloromethane

Nps-activated ester Amino ester	Concentration M	Half-time min
Val	0.0225	452.5
+ H-Ala-OBu ^t	0.0492	202
	0.103	91.75
	0.206	44.75
Ile	0.0195	240 ^a , 371 ^b
+ H-Val-OBu ^t	0.195	22.5 ^a , 36 ^b
		165

^a $t_{0.1}$, ^b $t_{0.15}$.

TABLE IV

Effect of Solvent on the Rate of Aminolysis of Polymeric Activated Esters

Nps-activated ester and amino ester	Dichloromethane $t_{0.5}$, min	Dimethylformamide $t_{0.5}$, min
Ala, H-Ala-OBu ^{t a}	18.75	8.3
Leu, H-Ala-OBu ^{t a}	20.5	7.6
Val, H-Ala-OBu ^{t a}	452	170
Val, H-Pro-OBu ^{t b}	30 ^c , 26.5 ^{c,d}	41 ^c

^a c 0.0225M; ^b c 0.1M; ^c $t_{0.025}$; ^d 1 : 1 mixture of dichloromethane and dimethylformamide.

tert-butyloxycarbonyl residues as protecting groups) has been successfully performed under such conditions.

EXPERIMENTAL

Materials. Preparation of polymeric activated esters was performed according to methods to be reported¹. Dichloromethane was purified by passage through a column of alumina and distillation of the effluent. Dimethylformamide was dried over phosphorus pentoxide, distilled, shaken with barium oxide, and distilled under diminished pressure.

Methods. Measurements were performed with the use of a continuous spectrophotometric technique² and a shaken reaction vessel. Prior to measurements, the polymeric esters were always washed with three portions of the particular solvent. The half-times were directly read from graphical reaction records. The 1st order constants were evaluated by calculation².

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REFERENCES

1. Davidovich Yu. A., Gut V.: This Journal, in press.
2. Gut V.: This Journal 40, 129 (1975).
3. For symbols of amino acids derivatives and peptides see *Biochem. J.* 126, 773 (1972).
4. Błotny G.: *Rocz. Chem.* 41, 299 (1967).
5. Kemp D. S.: Proc. 11th Europ. Peptide Symposium, Vienna, Austria, April 1971 (H. Nesvadba, Ed.), p. 1. North-Holland, Amsterdam 1973.
6. Rogozhin S. V., Davidovich Yu. A., Andreev S. M., Jurtanov A. N.: *Dokl. Akad. Nauk SSSR* 212, 108 (1973).

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