POLYMERIC MACROPOROUS N-HYDROXYSUCCINIMIDE ESTERS OF *o*-NITROBENZENESULFENYLAMINO ACIDS IN THE SYNTHESIS OF PEPTIDES*

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Polymeric *o*-nitrobenzenesulfenylaminoacyl N-hydroxysuccinimide esters have been synthesized and used as active esters in the synthesis of model protected peptides.

The synthesis of a polymeric insoluble N-hydroxysuccinimide has been recently reported[1]. Thanks to the uniform structure and high permeability for substances of a high molecular weight, this imide has been successfully used as a polymeric reagent in the synthesis of peptides[2]. A number of protected peptides up to a tetrapeptide has been synthesized with the use of this activator on the basis of a maleic imide and styrene copolymer crosslinked with benzidine. In the earlier experiments, the amino group was protected by benzyloxycarbonyl and tert-butyloxycarbonyl residues. Removal of these protecting groups requires strongly acidic conditions that are not compatible with acid-labile amino acid side chains and the use of acid-labile blocking groups in side chains of amino acids. It was therefore desirable to look after such protecting groups that could be selectively removed under mild conditions. From protecting groups of this lability type, the *o*-nitrobenzenesulfenyl group[3] has been so far used along with activation by the N-hydroxysuccinimide ester in the synthesis in solution[4]. The same protecting group has been applied to the synthesis of bradykinin by means of polymeric active esters on the basis of poly(4-hydroxy-3-nitrostyrene)[5]. Furthermore, the chromophore of the *o*-nitrobenzenesulfenyl group makes possible spectrophotometric measurement of the kinetics operating in these reactions[6].

The present paper reports on the preparation of polymeric activated esters of *o*-nitrobenzenesulfenylamino acids on the basis of macroreticular poly(N-hydroxysuc-

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cinimide) beads mentioned above (Table I). In the synthesis of polymeric activated esters with the use of urethan-type protecting groups, best results were obtained[7] by the trifluoroacetate method[8] and by the mixed anhydride method[9]. In the synthesis of activated polymeric esters carrying the *o*-nitrobenzenesulfenyl protecting group, the trifluoroacetate method cannot be used because of the liberation of trifluoroacetic acid during the reaction. Only moderate yields of products were obtained by the

TABLE I

Synthesis of *o*-Nitrobenzenesulfenylamino Acid Poly(N-hydroxysuccinimide) Esters

In 8 : 2 (v/v) dichloromethane–dimethylformamide mixture as solvent and with 1 : 1·5 : 2·25 molar ratio of poly(N-hydroxysuccinimide) to *o*-nitrobenzenesulfenylamino acid to N,N′-dicyclohexylcarbodiimide.

Ester[a]	Polymer beads, diameter mm[b]	Yield, %[c]	Capacity W[d]	mmol/g S[e]
Ala[f]	0·1—0·2	97	1·96	1·92
Ala[f,g]	0·1—0·2	75	1·70	1·63
Ala	0·1—0·2	97	1·96	1·84
Ala	0·1—0·4	94	1·93	—
Gly[f]	0·1—0·2	97	1·98	1·93
Gly	0·2—0·4	82	1·85	—
Val	0·1—0·2	89	1·79	1·83
Val	0·4—0·5	89	1·78	—
Leu	0·1—0·2	90	1·75	1·85
Leu	0·1—0·4[h]	93	1·78	1·84
Leu[i]	0·1—0·4[h]	93	1·78	—
Ile	0·1—0·2	87	1·73	1·81
Pro	0·1—0·2	89	1·79	1·80
Pro	0·1—0·4[h]	94	1·84	1·86
Phe	0·1—0·2	94	1·69	1·69
Tyr(OBu^t)	0·1—0·2	90	1·48	1·48
Met[j]	0·1—0·2	63	1·43	1·21
Lys(Z)	0·1—0·2	90	1·39	1·39
Cys(Bzl)	0·1—0·2	95	1·58	1·55
Cys(Tr)	0·1—0·2	89	1·24	1·22
Asp(OBu^t)	0·1—0·2	82	1·52	1·62

[a] For abbreviations see ref.[11]; [b] crosslinked with 2 molar % of benzidine; [c] % of substitution of N-hydroxy groups available; [d] calculated from weight increase; [e] calculated from sulfur content; [f] 85 : 15 (v/v) dichloromethane–dimethylformamide; [g] 1 : 1·1 : 1·65 molar ratio; [h] crosslinked with 3 molar % of benzidine; [i] 1 : 1·4 : 2·0 molar ratio; [j] 1 : 1·3 : 2·25 molar ratio.

mixed anhydride method when compared with the N,N'-dicyclohexylcarbodiimide activation[10] which was finally used as the general method. However, no attempt was made to optimalize the conditions for the mixed anhydride method. The thus-obtained polymeric activated esters are shown in Table I. The amount of the bound *o*-nitrobenzenesulfenylamino acid was determined on the basis of the sulfur content and the weight increase. In some cases, the aminolysis was effected with excess cyclohexylamine and the N-cyclohexylamide of the appropriate *o*-nitrobenzenesulfenylamino acid was isolated. The results of all these determinations are in good agreement.

The polymeric activated esters form yellow spherical particles that readily swell in dimethylformamide, tetrahydrofuran, dioxane, and dichloromethane. Thus for example, the swelling power of *o*-nitrobenzenesulfenyl-L-alanine ester with the capacity of 1·9 mmol per 1 gram of the polymer is 1·67 g of dichloromethane per 1 gram of the polymer.

The higher reactivity of the *o*-nitrobenzenesulfenyl protecting group in comparison with the benzyloxycarbonyl and tert-butyloxycarbonyl groups prompts to caution in handling the present polymeric esters. Acidic contaminants that are often present in dimethylformamide and dichloromethane, slowly impart a yellow colour to the supernatant in suspensions of polymeric esters in these solvents. An additional purification is therefore inevitable. The beads are satisfactorily stable when stored at −15°C in the presence of silica gel.

The peptides synthesized with the use of polymeric agents reported in the present paper are shown in Scheme 1. In order to achieve complete acylation of the amino component, an excess of the polymeric agent was used. Completeness of the acylation was checked by disappearence of the amino component by means of thin-layer chromatography. The resin was filtered off and the filtrate evaporated to afford the corresponding protected peptides of chromatographical homogeneity and in high yield.

Nps-L-Ala-O(P) + H-Gly-OMe → Nps-L-Ala-Gly-OMe
Nps-L-Ile-O(P) + H-Gly-OMe → Nps-L-Ile-Gly-OMe
Nps-L-Ala-O(P) + H-L-Phe-NH_2 → Nps-L-Ala-L-Phe-NH_2
Nps-Gly-O(P) + H-L-Pro-L-Leu-Gly-OMe → Nps-Gly-L-Pro-L-Leu-Gly-OMe

SCHEME 1

(P) Poly(N-hydroxysuccinimide).

EXPERIMENTAL

The *o*-nitrobenzenesulfenylamino acids were activated by means of macroreticular poly(N-hydroxysuccinimide) beads which were prepared by the earlier reported[1] crosslinking of the linear alternating maleic anhydride–styrene copolymer by means of two to three mol % of benzidine in dimethylformamide as solvent. The product was then allowed to react with acetic anhydride

and finally with hydroxylamine hydrochloride. The thus-obtained water-insoluble polymeric N-hydroxysuccinimide contained approximately 4·0 mmol of N-hydroxy groups per 1 gram of the polymer. The polymer formed faintly yellow beads of 0·1 to 0·4 millimeter in diameter, swelling in dimethylformamide, tetrahydrofuran, dichloromethane, and some other polar solvents.

The starting *o*-nitrobenzenesulfenylamino acids were synthesized according to the reported procedure[3]. The homogeneity of peptides was checked by thin-layer chromatography on ready-for-use Silufol silica gel sheets (Kavalier Glassworks, Votice, Czechoslovakia) in the solvent system 1-butanol–acetic acid–water (4 : 1 : 1). Optical rotation was determined on a Jouan spectropolarimeter. Dichloromethane was passed through alumina (Brockmann activity I) and the effluent distilled. Dimethylformamide was dried over phosphorus pentoxide, distilled at 15 Torr, the distillate stored over barium oxide, and redistilled at 15 Torr.

Preparation of Polymeric N-Hydroxysuccinimide Esters of *o*-Nitrobenzenesulfenylamino Acids

To a suspension of poly(N-hydroxysuccinimide) (1 equivalent) in a solution of 1·1 to 1·5 equivalent of the appropriate *o*-nitrobenzenesulfenylamino acid in a 4 : 1 (v/v) mixture of dichloromethane and dimethylformamide there was added at −10°C N,N′-dicyclohexylcarbodiimide (2·25 equivalent) dissolved in the same solvent mixture. The whole mixture was stirred without cooling for 15 min (the temperature raised to 0°C), then for one hour with ice-cooling, thereafter for 30 min without cooling (the temperature raised to 22°C), and finally for 6 h at 22°C. The suspension was stored at 0°C overnight, the solid collected with suction, and washed with ethanol, acetone, and finally with ether. The weight increase was determined after drying to constant weight at 15 Torr. The polymer was stored at −15°C.

Glycine ester. N,N′-Dicyclohexylcarbodiimide (3·70 g) in a mixture of dichloromethane (8·5 ml) and dimethylformamide (1·5 ml) was added to poly(N-hydroxysuccinimide) (2·03 g; particle size, 0·1—0·2 mm) and *o*-nitrobenzenesulfenylglycine (2·73 g) in CH_2Cl_2 (10·2 ml) and dimethylformamide (1·8 ml). Yield, 3·70 g; weight increase, 1·67 g, *i.e.*, 7·36 mmol of bound glycine (1·98 mmol/g). Found: 7·03% N, 6·18% S; on the basis of the sulfur content, the capacity is 1·93 mmol/g.

L-*Alanine ester.* N,N′-Dicyclohexylcarbodiimide (3·10 g) in dichloromethane (8 ml) and dimethylformamide (2 ml) was added at −15°C to a mixture of poly(N-hydroxysuccinimide) (1·70 g; particle size, 0·1—0·2 mm), *o*-nitrobenzenesulfenyl-L-alanine (2·42 g), dichloromethane (7·2 ml), and dimethylformamide (1·8 ml). Yield, 3·11 g; weight increase, 1·47 g (1·96 mmol/g of bound alanine). Found: 6·52% N, 5·87% S (1·84 mmol/g).

L-*Proline ester.* N,N′-Dicyclohexylcarbodiimide (2·57 g) in dichloromethane (6·4 ml) and dimethylformamide (1·6 ml) was added at −15°C to a mixture of poly(N-hydroxysuccinimide) (1·41 g; particle size, 0·1—0·2 mm), *o*-nitrobenzenesulfenyl-L-proline (2·22 g), dichloromethane (6·4 ml), and dimethylformamide (1·6 ml). Yield, 2·61 g; weight increase, 1·47 g (1·79 mmol/g of bound L-proline). Found: 6·79% N, 5·78% S (1·80 mmol/g).

S-*Benzyl*-L-*cysteine ester.* N,N′-Dicyclohexylcarbodiimide (3·10 g) in dichloromethane (5·6 ml) and dimethylformamide (1·4 ml) was added at −20°C to a mixture of poly(N-hydroxysuccinimide) (1·70 g; particle size, 0·1—0·2 mm), *o*-nitrobenzenesulfenyl-S-benzyl-L-cysteine (3·64 g), dichloromethane (10·4 ml), and dimethylformamide (2·6 ml). Yield, 3·81 g; weight increase 2·18 g (1·58 mmol/g of bound L-cysteine. Found: 9·88% S (1·55 mmol/g).

O-*Tert-butyl*-L-*tyrosine ester.* N,N′-Dicyclohexylcarbodiimide (1·55 g) in dichloromethane (2·8 ml) and dimethylformamide (0·7 ml) was added at −20°C to a mixture of poly(N-hydroxy-

succinimide) (0·85 g; particle size, 0·1—0·2 mm), *o*-nitrobenzenesulfenyl-O-tert-butyl-L-tyrosine (1·95 g), dichloromethane (5·2 ml), and dimethylformamide (1·3 ml). Yield, 1·93 g; weight increase, 1·11 g (1·48 mmol/g of bound L-tyrosine). Found: 6·07% N, 4·72% S (1·48 mmol/g).

Preparation of Polymeric N-Hydroxysuccinimide Esters of *o*-Nitrobenzenesulfenylamino Acids by the Mixed Anhydride Method

L-*Alanine ester.* Sec-butyl chloroformate (0·85 g) was added at −20°C to a stirred solution of *o*-nitrobenzenesulfenyl-L-alanine (1·5 g) and N-ethylmorpholine (0·63 g) in tetrahydrofuran (5 ml). The mixture was stirred at −15°C for 10 min and then poly(N-hydroxysuccinimide) (1·7 g; particle size, 0·1—0·2 mm) was added. The whole mixture was stirred at 40°C for 40 min, the polymer collected with suction, washed successively with tetrahydrofuran, acetone, methanol, and ether, and dried to constant weight at 15 Torr. Yield, 2·40 g (1·32 mmol/g of bound L-alanine).

L-*Isoleucine ester.* Ethyl chloroformate (1·0 ml) was added at −15°C to a stirred solution of *o*-nitrobenzenesulfenyl-L-isoleucine (3·00 g) and N-ethylmorpholine (1·1 ml) in dimethylformamide (25 ml). After 10 min, poly(N-hydroxysuccinimide) (3·00 g) was added and the mixture processed as above to afford 5·25 g of the polymer; weight increase, 2·25 g (1·61 mmol/g of bound L-isoleucine).

o-Nitrobenzenesulfenyl-L-isoleucylglycine Methyl Ester

A mixture of poly(N-hydroxysuccinimide) ester of *o*-nitrobenzenesulfenyl-L-isoleucine (3·00 g), glycine ethyl ester hydrochloride (0·30 g), triethylamine (0·4 ml), and tetrahydrofuran (15 ml) was stirred at 22°C for 24 h. The polymer was filtered off and washed with acetone and ether. The filtrate and washings were evaporated under diminished pressure and the residue taken up into ethyl acetate and water. The organic layer was washed with water, 0·6M aqueous sodium hydrogen carbonate, and water again, dried over anhydrous sodium sulfate, and evaporated to afford 0·65 g (76%) of the title methyl ester, m.p. 79°C; $[\alpha]_D^{22}$ +22·0° (*c* 1, methanol). For $C_{15}N_{21}N_3O_5S$ (355·9) calculated: 50·69% C, 5·96% H, 11·85% N; found: 50·74% C, 6·13% H, 11·88% N.

o-Nitrobenzenesulfenyl-L-alanyl-L-phenylalanine Amide

A suspension of poly(N-hydroxysuccinimide) ester of *o*-nitronitrobenzenesulfenyl-L-alanine (5·40 g), L-phenylalanine amide (0·50 g), and dimethylformamide (30 ml) was stirred at 22°C for 18 h. The polymer was filtered off and washed with acetone and methanol. The filtrate and washings were processed as above to afford a yellow solid which was washed with three 20 ml portions of ether and dried at 15 Torr. Yield, 1·20 g (100%) of the title amide, m.p. 185—186°C; $[\alpha]_D^{24}$ −25·6° (*c* 0·8, methanol); R_F value, 0·7. For $C_{18}H_{20}N_4O_4S$ (388·4) calculated: 55·65% C, 5·15% H, 14·40% N; found: 55·70% C, 4·96% H, 14·15% N.

o-Nitrobenzenesulfenyl-L-alanylglycine Methyl Ester

A suspension of poly(N-hydroxysuccinimide) ester of *o*-nitrobenzenesulfenyl-L-alanine (4·75 g), glycine methyl ester hydrochloride (0·40 g), triethylamine (0·5 ml), and tetrahydrofuran (20 ml) was stirred at 22°C for 18 h, the polymer filtered off and washed with methanol, acetone, and ether. The filtrate and washings were processed as above. The yellow product was washed on the

filter with n-hexane (50 ml) and dried at 15 Torr. Yield, 0·63 g (66%) of the title methyl ester, m.p. 122–123°C; $[\alpha]_D^{22}$ +32° (*c* 1, methanol). For $C_{12}H_{15}N_3O_5S$ (313·3) calculated: 46·00% C, 4·82% H, 13·40% N; found: 47·84% C, 4·99% H, 13·89% N.

o-Nitrobenzenesulfenylglycyl-L-prolyl-L-leucylglycine Methyl Ester

A suspension of poly(N-hydroxysuccinimide) ester of *o*-nitrobenzenesulfenylglycine (1·9 g), L-prolyl-L-leucylglycine methyl ester hydrochloride (0·39 g), N-methylmorpholine (0·3 ml), and dimethylformamide (10 ml) was stirred at 22°C for 24 h, the polymer filtered off, and washed with acetone and methanol. The filtrate and washings were concentrated under diminished pressure to the volume of 5 ml and the concentrate was diluted with water. The yellow precipitate was filtered off, washed with water (100 ml), methanol (20 ml), and ether, and dried to constant weight at 15 Torr. Yield, 0·61 g (100%) of the title methyl ester, m.p. 158–160°C; $[\alpha]_D^{25}$ −3·6° (*c* 0·5, acetone); R_F value, 0·59.

REFERENCES

1. Rogozhin S. V., Davidovich Yu. A., Andreev S. M., Yurtanov A. I.: Dokl. Akad. Nauk SSSR *211*, 1356 (1973).
2. Rogozhin S. V., Davidovich Yu. A., Andreev S. M., Yurtanov A. I.: Dokl. Akad. Nauk SSSR *212*, 108 (1973).
3. Zervas L., Borovas D., Gazis E.: J. Amer. Chem. Soc. *85*, 3660 (1863).
4. Meienhofer J.: Nature (London) *205*, 73 (1965).
5. Fridkin M., Patchornik A., Katchalski E.: J. Amer. Chem. Soc. *90*, 2953 (1968).
6. Gut V., Davidovich Yu. A.: This Journal *41*, 780 (1976).
7. Rogozhin S. V., Davidovich Yu. A., Samoylova M. A., Mironova N. V., Yurtanov A. I., Andreev S. M.: Izv. Akad. Nauk SSSR *1975*, 428.
8. Sakakibara S., Inukai N.: Bull. Chem. Soc. Jap. *38*, 1979 (1965).
9. Anderson G. W., Callahan F. M., Zimmerman J. E.: J. Amer. Chem. Soc. *89*, 178 (1967).
10. Anderson G. W., Zimmerman J. E., Callahan F. M.: J. Amer. Chem. Soc. *86*, 1839 (1964).
11. Symbols for Amino Acid Derivatives and Peptides in: Biochem. J. *126*, 773 (1972).

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