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A NEW APPROACH TO THE POLYMER REAGENT FOR PEPTIDE SYNTHESIS: PREPARATION OF N-HYDROXYSUCCINIMIDE ESTER POLYMERS VIA POLYMERIZABLE ACTIVE ESTERS

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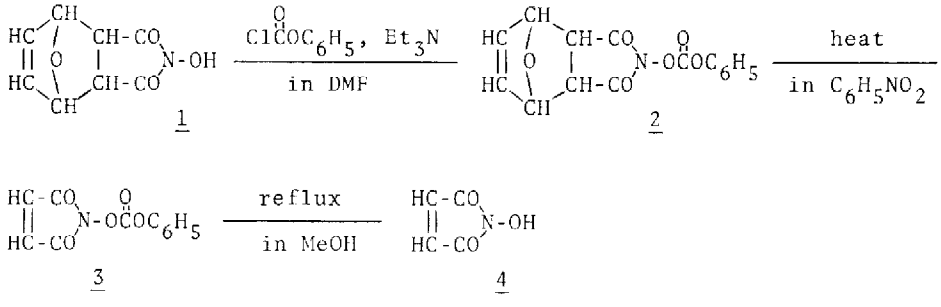
Active ester polymer reagents have been utilized for simplification of the procedures in peptide synthesis.[1,2] Among these reagents, N-hydroxysuccinimide ester polymers[3-7] are particularly important, since the corresponding monomeric derivatives have proved potentially useful.[8] All the synthetic methods used for the active ester polymers have required a polymer reaction between an amino acid component and a hydroxyl polymer as a key condensation step. However, this kind of polymer reaction sometimes leaves contaminations which deteriorate the quality of the resulting polymer reagent.[9]

We wish to report a new route to the N-hydroxysuccinimide ester polymers. The route consists of synthesis of N-hydroxymaleimide esters of N-protected amino acids and copolymerization of these esters with suitable vinyl monomers. The pure active ester polymers will be obtained, and, in contrast to the usual method, the composition of the polymers can be pre-adjusted, that is, the regulations of the polymer environment are possible by proper use of the comonomers. These are additional merits of this new route.

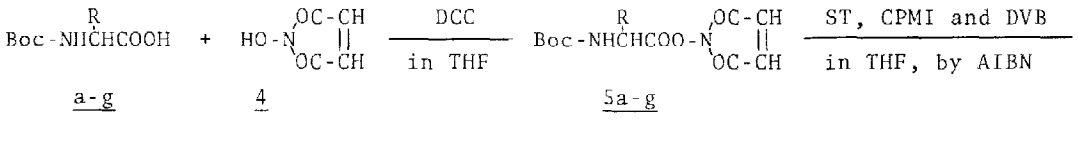
The preparation of N-hydroxymaleimide 4 once claimed by Russian workers[10] was found to give rather N-hydroxyisomaleimide.[11] The present synthesis is carried out successfully by a new protection of the N-hydroxyl group. The reaction sequence to 4 is shown in Scheme I.



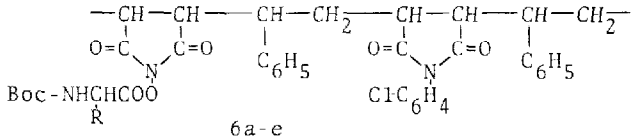
1 2 3 4

Scheme I

Compound 1[5] reacted with phenyl chloroformate in DMF at 5°C in the presence of triethylamine, to give 2 in 95% yield, mp 134-136°C.[12] When 2 was heated in nitrobenzene at 160°C for 0.5 hr with t-butylcatechol as polymerization inhibitor, 3 was obtained in 78% yield, mp 98-99°C. The phenoxycarbonyl group of 3 was cleaved cleanly on treatment with boiling methanol for 2 hr, giving 4 in 67% yield. It was recrystallized from toluene, mp 125-126°C; NMR(acetone-D_6) δ 6.80 (2H s); IR 3150, 1785 and 1725 cm^{-1}.



a-g 4 5a-g



6a-e

Esters 5a-g of 4 with Boc-amino acids were prepared by condensing an equimolar mixture of the both components with dicyclohexylcarbodiimide(DCC) in THF at ice bath temperature for 10-20 hr. The common carbonyl absorptions of the esters were 1810, 1790 and 1740 cm^{-1}. The results are shown in Table I.

Copolymerization of these purified esters was conducted in THF(30 ml) at 50°C for 4 hr in the presence of divinylbenzene(DVB, 3 mol%) by azobisisobutyronitrile (AIBN, 6 mol%), using a mixture of 5.0:4.0:1.0 molar ratio of styrene(ST, 1.50 g), N-(p-chlorophenyl)maleimide(CPMI, 2.50 g) and the ester(5, ca 1 g). The polymer precipitated was collected and washed in Soxhlet extractor with dichloromethane. The copolymerization results are summarized in Table II. It is seen that the copolymer compositions reflect closely the initially feeded monomer ratio.

Table I. Synthesis of Boc-amino Acid N-Hydroxymaleimide Esters

Hydroxymaleimide ester		Yield[a] %	M.p. °C	$[\alpha]_D^{27}$ (c=1, THF)
5a	Boc-L-Pro	37	112-113	-57.0°
5b	Boc-L-Ala	35	136-137	-24.0
5c	Boc-L-Phe	38	139-140	-16.8
5d	Boc-L-Lue	36	84-85	-22.2
5e	Boc-Sar	63	96-97	
5f	Boc-Gly	51	134-135	
5g	Boc-L-Ile	22	69-70	-27.3

[a]The yields were not optimized.

Table II. Copolymerization of the N-Hydroxymaleimide Esters

Copolymer	Yield %	Polymer composition, mol% ST	CPMI	5a-e	Analyses (Calc.)[a] C, %	H, %	N, %
6a	79	0.49	0.42	0.09[b]	67.41 (67.47	5.06 4.86	4.68 5.06)
6b	72	0.50	0.41	0.09	67.93 (67.83	5.05 4.86	4.83 5.08)
6c	70	0.50	0.41	0.09	68.56 (68.70	5.12 4.87	4.59 4.87)
6d	75	0.49	0.41	0.10	67.73 (67.83	5.19 5.11	4.86 5.07)
6e	79	0.50	0.40	0.10	67.82 (67.65	5.12 4.90	4.92 5.15)

[a]The calculated values are derived on the basis of the composition.
[b]The ester content of 0.54 mmol/g from the composition was agreed with a value 0.52 mmol/g of amino acid analysis.

In order to test these polymer reagents obtained, known peptides were prepared under conditions of only slight excess reagents to amino components. Thus, polymer 6c (1.00 g, 0.531 mmol) was suspended in THF (20 ml). To this was added ethyl glycinate free base in DMF (3.0 ml) which was prepared from the hydrochloride (0.067 g, 0.48 mmol), and the mixture was stirred for 16 hr at 25°C. Separation of the wasted polymer by filtration followed by evaporation of the solvent gave almost pure Boc-L-Phe-Gly-OEt in 86% yield. One recrystallization gave a pure sample. These results are presented in Table III.

Table III. Preparation of Peptide Derivatives

Copolymer	Peptide formed	Yield %	M.p. [lit.] °C	$[\alpha]_D^{18}$ [lit.]
6b	Boc-L-Ala-L-Ala-OBzl	89	70-71 [73-74][a]	-54.9° (c=1, MeOH)
6c	Boc-L-Phe-Gly-OEt	86	89.5-90 [89.5-90][b]	-4.8 (c=1, EtOH) [-4.3 (c=2, EtOH)][b]
6d	Boc-L-Leu-L-Val-OMe	99	143-144 [144-147][c]	-40.0 (c=1, MtOH) [-41.1 (c=0.5, MeOH)][c]
6d	Boc-L-Leu-Gly-OEt	94	80-81 [78-79][d]	-25.7 (c=1, EtOH)

[a]Ref. 3. [b]G. W. Anderson and A. C. McGregor, J. Am. Chem. Soc., 79, 6180 (1957). [c]D. A. Laufer and E. R. Blout, J. Am. Chem. Soc., 89, 1246 (1967). [d]Ref. 7.

Partial replacement of the ester unit by CPMI made the polymers sufficiently to swell in dichloromethane or THF as well as DMF, and served to prevent their drastic solubility changes during peptide forming reactions.

In conclusion, once N-hydroxymaleimide is secured, its amino acid or peptide esters may be readily prepared and purified. The subsequent rather mild polymerization does not harm the amino acid moiety, and the polymer reagents derived will have well defined structures and provide potential advantages in handling during peptide synthesis.

Further studies on these polymerizable active esters are now in progress.

References and Notes

1. M. Fridkin, A. Patchornik, and E. Katchalski, J. Am. Chem. Soc., 87, 4646 (1965).
2. C. C. Leznoff, Chem. Soc. Rev., 3, 65 (1974); J. H. Jones and B. Ridge, in "Amino-acid, Peptides, and Proteins," Vol. 5, The Chemical Society, London, 1974, p. 292.
3. D. A. Laufer, T. M. Chapman, D. I. Marlborough, V. M. Vaidya, and E. R. Blout, J. Am. Chem. Soc., 90, 2696 (1968).
4. M. Akiyama, M. Narita, and M. Okawara, J. Polymer Sci., 7A-1, 1299 (1969); M Akiyama, Y. Yanagisawa, and M. Okawara, J. Polymer Sci., 7A-1, 1905 (1969).
5. M. Narita, T. Teramoto, and M. Okawara, Bull. Chem. Soc. Jpn., 44, 1084 (1971); 45, 3149 (1972).
6. M. Fridkin, A. Patchornik, and E. Katchalski, Biochemistry, 11, 466 (1972).
7. S. V. Rogozin, Yu. A. Davidovich, S. M. Andreev, and A. I. Yurtanov, Dokl. Akad. Nauk SSSR, 212, 108 (1973).
8. G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, J. Am. Chem. Soc., 86, 1839 (1964).
9. Side reactions were noted during the formation of the N-hydroxysuccinimide esters of amino acids; see H. Gross and L. Bilk, Tetrahedron, 24, 6935 (1968).
10. V. S. Ivanov, V. K. Smirnova, A. E. Semenova, and T. Yune, Zh. Organ. Khim., 1, 1705 (1965).
11. M. Narita, M. Akiyama, and M. Okawara, Bull. Chem. Soc. Jpn., 44, 437 (1971)
12. Satisfactory elemental analyses were obtained for these and other new compounds described here. We thank Mrs. M. Asuke for these analyses.