[Chem. Pharm. Bull. 18(4) 784—788 (1970)]

UDC 547.964.4.04.07

Peptides. II. A New Synthetic Method for the Amino-protected Amino Acid Activated Esters[1,2]

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(Received December 8, 1969)

A facile procedure for the preparation of amino-protected amino acid activated ester was studied.

Dimethylaryloxyformimidium chloride (V), which was easily prepared from aryl chloroformate (II) and dimethylformamide under the evolution of carbon dioxide, was allowed to react with amino-protected amino acids to give the corresponding activated aryl esters.

Activated ester method is a one of very important procedures for the stepwise elongation of sequential peptides[4] so that a large number of investigations on the new activated esters has been reported.[4-11] Of these esters, some aryl esters such as *p*-nitrophenyl, trichlorophenyl and pentachlorophenyl esters are frequently utilized for practical peptide synthesis. For the preparation of these activated esters several procedures are known[4,5] and some improved procedures, for example, transesterification method using trifluoroacetate,[12,13] dichloroacetate or trichloroacetate,[14] or decomposition of mixed anhydride prepared from N-benzyloxycarbonylamino acids with succinimidoxycarbonyl chloride[15] have also been proposed.

On the other hand, Vilsmeier-Haack reagents, dimethyl chloroformimidium chloride anb related reagents, were utilized as coupling agent in peptide synthesis.[16-19] Furthermore, the reaction of methanol with dimethylaryloxyformimidium chlorides prepared from aryl chloroformates with dimethylformamide (DMF) were demonstrated recently their effectiveness for the protection and regeneration of phenolic hydroxyl group.[20] However, these use for ester synthesis has not been reported.

In the present paper a new facile procedure for the synthesis of amino-protected amino acid aryl esters using dimethylaryloxyformimidium chlorides are described. On mixing DMF

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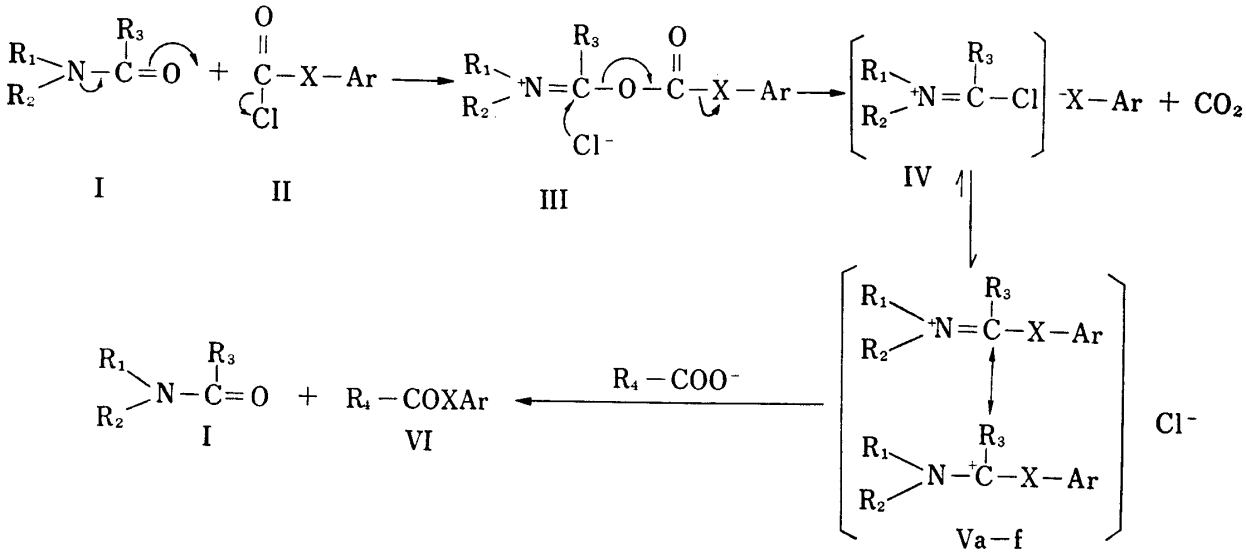
with a solution of pentachlorophenyl chloroformate in inert solvent a vigorous reaction takes place under evolution of carbon dioxide gas. When the same reaction was carried out on cooling, the precipitate was first formed and then carbon dioxide gas evolved gradually on warming to room temperature. The structure of precipitated hygroscopic salt was confirmed to be dimethylpentachlorophenoxyformimidium chloride (Vc) by the following data; infrared (IR) (Nujol, cm^{-1}): 1660 (C=N$^+$), and 1280 (aryl ether); nuclear magnetic resonance (NMR) (δ(ppm), in CD_3OD): two set of signals owing to tautomers appeared at 2.95 (singlet, =N–CH_3), 3.08 (singlet, =N–CH_3) and 8.10 (singlet, –$\overset{+}{C}$H–), and at 3.26 (singlet, =$\overset{+}{N}$–CH_3), 3.44 (singlet, =$\overset{+}{N}$–CH_3) and 8.75 (singlet, =CH–).[21]

The reaction mechanism may be presented as in Chart 1.[20] Other immonium salts (Va—Vg) were also prepared from chloroformates and secondary amides, however, the formation of



Va: X=O, Ar=*p*-nitrophenyl, $R_1=R_2=CH_3$, $R_3=H$
Vb: X=O, Ar=2,4,5-trichlorophenyl, $R_1=R_2=CH_3$, $R_3=H$
Vc: X=O, Ar=pentachlorophenyl, $R_1=R_2=CH_3$, $R_3=H$
Vd: X=O, Ar=succinimidoxy, $R_1=R_2=CH_3$, $R_3=H$
Ve: X=S, Ar=phenyl, $R_1=R_2=CH_3$, $R_3=H$
Vf: X=O, Ar=*p*-nitrophenyl, $R_1=R_2=R_3=CH_3$
Vg: X=O, Ar=pentachlorophenyl, $R_1=R_2=R_3=CH_3$

Chart 1

those immonium salts were limited to aryl chloroformates or succinimidoxycarbonyl chloride because the reaction of alkyl chloroformates with DMF afforded only alkyl chlorides.[22] The reaction of those immonium salts with various acids was recognized to proceed smoothly in the presence of tertiary amine at room temperature and completed generally within 20 minutes to give activated esters. If the reaction is carried out in the absence of tertiary amine, it needs more longer reaction time and the yield is rather low.

This procedure has some advantages if the considerabler stable chloroformates is available: procedure is rather simple and suitable for large scale preparation, a short reaction time is required and formed components is very easily removable. Esters thus prepared are listed in the Table I. Direct coupling of formed activiated ester with amino acid and peptide esters are also examined (Table II).

21) NMR spectrum was measured with a Varian A-60 spectrometer using tetramethylsilane as an internal standard. DMF (in CD_3OD; δ (ppm)) shows signals at 2.86 (singlet, 3H), 3.00 (singlet, 3H) and 7.97 (singlet, 1H), so that immonium salt Vc may not be decomposed in CD_3OD.
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Table I. Activated Esters of Amino-protected Amino Acids[a] and Salicylic Acid

	Immonium salt	Base	Yield (%)	mp (°C) Found/Lit.	$[\alpha]_D^t$ (c, solvent) Found/Lit.
Z-Asp(OBzl)-ONP	Va	Et_3N	79	75 — 77	+12.0[23] (4, $CHCl_3$)
				75 — 76	+12.5[30] (4, $CHCl_3$)[23]
Z-CyS(Bzl)-ONP	Va	DMA[b]	74	92.5— 93.5	−42.4[26] (2, DMF)
				93 — 94	−43[20] (2, DMF)[24]
Z-CyS(Bzl)-OPCP	Vc	DMA[b]	77	168 —169	−34.0[26] (0.7, $CHCl_3$)
				171 —173	−34.3[24] (0.7, $CHCl_3$)[25]
Z-Gln-ONP[c]	Va	Et_3N	47	155 —157	−24.2[23] (2, DMF)
				155 —156	−24[20] (2, DMF)[24]
Z-Gly-OTCP	Vb	Et_3N	70	100 —101	
				102 —104[26]	
Z-Gly-OPCP	Vc	Et_3N	79	128 —130	
				133 —134[12]	
Z-Gly-SPh	Ve	Et_3N	55	72 — 73.5	
				70 — 72[26]	
Z-Phe-OHsu	Vd	Et_3N	81	136 —138.5	−53.2[23] (1, DMF)
				134 —136	−53.8[24] (1, DMF)[12]
Z-Phe-OTCP	Vb	Et_3N	75	140 —141	−40.1[23](1, DMF)
				140 —141	−39.4[24](1, DMF)[26]
Z-Phe-OPCP	Vc	Et_3N	75	154 —155	−49.5[25] (1, DMF)
	Vg	.....	55	154 —156	
				159 —160	−49.4[24] (1, DMF)[26]
Z-Pro-ONP	Va	Et_3N	82	94 — 96	−68.2[25] (2, DMF)
				94 — 96	−68[20] (2, DMF)[24]
Z-Tyr(Bzl)-ONP	Va	Et_3N	76	147 —148	−8.6[23](2, DMF)
				148 —150	−9[20] (2, DMF)[24]
Z-Val-ONP	Va	Et_3N	60	62 — 64	−25.0[23](2, DMF)
				63	−24.4[20] (2, DMF)[27]
o-HO-C_6H_4-ONP	Va	Py[d]	54	143 —145[e]	
				151.5—151.9[28]	

a) Used amino acids are all L-configuration except glycine. b) DMA=dimethylaniline
c) DMF was used as solvent. d) Py=pyridine
e) *Anal.* Calcd. for $C_{13}H_9O_5N$: C, 60.23; H, 3.50; N, 5.40. Found: C, 60.58; H, 3.25; N, 4.99

Table II. Preparation of Acylated Peptide Esters *via* Activated Esters[a]

	Immonium salt	Base	Yield (%)	mp (°C) Found/Lit.	$[\alpha]_D^t$ (c, solvent) Found/Lit.
Z-Leu-Gly-OEt	Va	Et_3N	71	102 —104	−27.4[22](5, EtOH)
				102.5—103.5	−27.5[20] (5, EtOH)[29]
Z-Trp-Gly-OBzl	Va	DMA[b]	67	122 —124	−21.1[23] (0.95, MeOH)
				117 —118	−19±1[25] (0.95, MeOH)[30]
Z-Pro-Leu-Gly-OEt	Va	Et_3N	67	150 —152	−81.2[24] (2.5, EtOH)
				151 —152	−82.6[23] (2.5, EtOH)[24]

a) Used amino acids are all L-configuration except glycine. b) DMA=dimethylaniline

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Unfortunately, this procedure is not suitable for the preparation of peptidic activated esters, because Anderson's racemization test[31] shows complete racemization even if mild bases[29,32] is utilized at lower temperature. The mechanism for ester formation may be presented as in Chart 2.

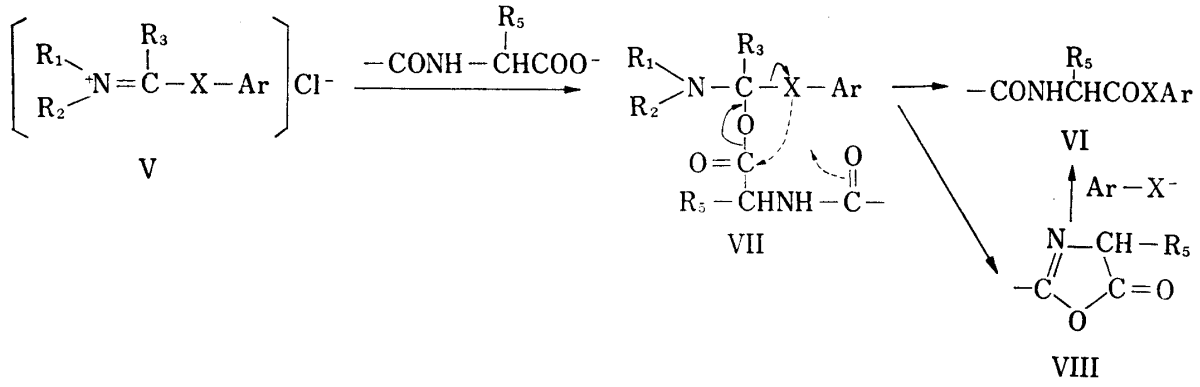

Chart 2

When the amino-protecting group is urethane type which has not been observed oxazolone formation, nucleophilic attack of carboxyl carbon atom by the Ar–X$^-$ anion preferentially affords the optically activated esters. On the contrary, when amino-protected peptides is used, the reaction proceeds through the oxazolone intermediate (VIII) to give racemized product.

Experimental

All melting points are uncorrected and taken on a Hoover "Uni-Melt" apparatus. Optical rotations were measured with a Yanagimoto photo-magnetic polarimerter, Model OR-10. Reaction mixture was examined by thin-layer chromatography on silica gel G in chloroform–acetic acid–methanol (95:3:5). Spots were demonstrated by the combination of hydrogen bromide and ninhydrin for N-benzyloxycarbonyl derivatives.

Materials——*p*-Nitrophenyl chloroformate,[33] 2,4,5-trichlorophenyl chloroformate,[34] pentachlorophenyl chloroformate[35] and succinimidoxycarbonyl chloride[15] were prepared according to the literature.

Dimethylpentachlorophenoxyformimidium Chloride (Vc)——Dry DMF (3 ml) was added all at once into a well stirred solution of pentachlorophenyl chloroformate (4.0 g, 0.012 mole) in dry tetrahydrofuran (20 ml; dry benzene, toluene, DMF *etc* is also suitable as solvent) on ice–cooling. White precipitate appeared soon and then carbon dioxide gas evolved gradually on warming to room temperature. The suspension was used directly for ester formation without further treatment. For analysis filtered precipitate was washed with dry tetrahydrofuran, dried over phosphorous pentoxide and measured its IR and NMR spectra.

Other immonium salts, dimethyl-*p*-nitrophenoxyformimidium chloride (Va), dimethyl-2,4,5-trichlrophenoxyformimidium chloride (Vb), dimethylsuccinimidoxyformimidium chloride (Vd), dimethylphenylthioformimidium chloride (Ve), dimethyl-*p*-nitrophenoxyacetoimidium chloride (Vf) and dimethylpentachlorophenoxyacetoimidium chloride (Vg) were also prepared in a similar manner as described above for Vc. These immonium salts are unstable toward moisture.

General Procedure for the Preparation of Activated Esters——To the suspension of immonium salt (0.01—0.012 mole) prepared as described above a solution of N-benzyloxycarbonylamino acid (0.01 mole) and tertiary amine (0.01—0.012 mole) in suitable solvent (10—20 ml; chloroform, tetrahydrofuran, ethyl acetate or DMF *etc*) was added dropwise at room temperature. The reaction was completed generally within 10—20

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34) W. Broadbent, J.S. Morley and B.E. Stone, *J. Chem. Soc.* (*C*), **1967**, 2632.
35) *a*) M. Fujino and C. Hatanaka, *Chem. Pharm. Bull.* (Tokyo), **15**, 2015 (1967); *b*) M. Itoh and D. Morino, *Experientia*, **24**, 101 (1968).

minutes. The formed ester was taken up to ethyl acetate and the extract was washed with water and dried over $MgSO_4$. The crude product was treated as described in the literature (see Table I).

General Procedure for the Direct Coupling of Activated Ester with Amine Component——N-Benzyloxycarbonylamino acid *p*-nitrophenyl ester taken up to ethyl acetate as described above was coupled directly with amine component. A typical example is as follows: To a suspension of Va prepared from *p*-nitrophenyl chloroformate (1.2 g) with DMF (1.0 ml) in dry benzene (10 ml) a solution of N-benzyloxycarbonyltryptophan (1.6 g) and dimethylaniline (0.73 g) in dry tetrahydrofuran (10 ml) was added dropwise with stirring below 10°, and stirred further 2 hr at room temperature. The product was extracted with ethyl acetate and the extract was washed with water, dried over $MgSO_4$ and concentrated to about 20 ml. A solution of glycine benzyl ester *p*-toluenesulfonate (1.6 g) and triethylamine (0.7 ml) in chloroform (15 ml) was added to the above mentioned solution and the mixture was treated as described in the literature (see Table II).

Acknowledgement The author express his deep gratitude to Dr. S. Sakakibara of Institute for Protein Research Osaka Univercity, and Dr. H. Nakano, director of this laboratory for their kind advices and encouragements. Thanks are also to Mr. D. Morino for his skillful technical assistance.