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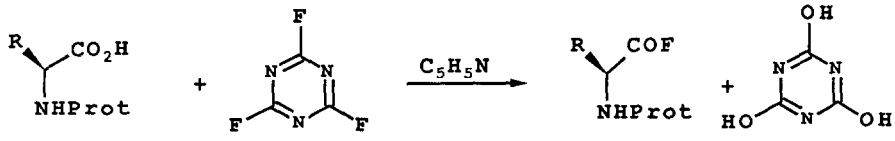
AMINO ACID FLUORIDES : THEIR PREPARATION AND USE IN PEPTIDE SYNTHESIS

Jean-Noël Bertho[a], Albert Loffet[b], Catherine Pinel[a], Florence Reuther[a] and Gérard Sennyey[a]
[a]SNPE, Centre de Recherches du Bouchet, F-91710 Vert-le Petit, France
[b]Propeptide, F-91710 Vert-le Petit, France

Abstract : Z or Fmoc amino acid fluorides have been prepared from the protected amino acids and cyanuric fluoride, and have been tested both in the condensation with simple amino acid esters and in Solid Phase Peptide Synthesis.

N-protected amino acid chlorides have been known since the beginning of the century[1]; they have been rarely used in peptide synthesis until recently[2], when they have been successfully applied to the rapid coupling of Fmoc-amino acids[2]. However, because of their high reactivity and sensitivity to hydrolysis, acid chlorides should be prepared from the parent amino acids immediately before use[2] and a large excess of reactant is needed for their synthesis. Acid fluorides, on the other hand, are known to be more stable to hydrolysis than the chlorides[3] but they have been rarely used in organic synthesis because of their presumed low reactivity toward common nucleophiles. However, fluorides like indole 2-carbonyl fluoride have been shown to react readily with silylated aliphatic and aromatic amines[4].

We have examined the potenty of amino acid fluorides in peptide synthesis. N-protected amino acid fluorides have been easily prepared from the reaction of the convenient amino acid derivative with cyanuryl fluoride[5].



Prot = Protecting group

In a typical experiment, equimolecular amount of the amino acid, pyridine and cyanuryl fluoride are mixed and stirred for 3-4 hours in dichloromethane at room temperature. At that time, ice-water is added to the reaction mixture and the precipitated cyanuric acid is filtered off. The organic phase is dried and evaporated to dryness, which

generally leaves the pure amino acid fluoride in cristalline form. This procedure is superior to that of Mukaiyama[6] which has been previously used for the synthesis of Z-glutamyl and Z-cystyl difluorides[7], in that by-products are readily eliminated by simple filtration thus allowing easy isolation of the fluorides. Side chain t-butyl protecting groups were not affected by the synthesis as could be seen from the NMR spectra of Fmoc-Lys(Boc) and Fmoc-Asp(O^tBu). Furthermore, most of these products are easily cristallized and have been found to be stable for up to six months under normal storage conditions (Table 1).

TABLE 1 : Preparation of amino acid fluorides

Amino Acid	Method	Yield %	mp °C	IR	$[\alpha]_D$
Z-Gly	a	89	50-51	1860	-
Z-Ala	b	80	liq	1850	-5 (3 AcOEt)
Z-Leu	b	80	liq	1850	-12 (1.6 AcOEt)
Z-Phe	a	70	80-83	1845	-30 (1.3 AcOEt)
Z-Phe	b	80			-30 (1.3 AcOEt)
Z-Val	b	75	liq	1850	+8 (3 AcOEt)
Fmoc-Ala	a	85	108	1850	-1 (2 AcOEt)
Fmoc-Ala	b	85			-1 (2 AcOEt)
Fmoc-Gly	b	91	134-135	1850	-
Fmoc-Ile	b	81	113-114	1845	+13 (0.6 AcOEt)
Fmoc-Leu	b	92	95-96	1850	-6 (1 AcOEt)
Fmoc-Lys(Boc)	b	80	120-121	1850	+3 (1 CH_2Cl_2)
Fmoc-Met	b	80	122-123	1850	-
Fmoc-Phe	b	75	110-111	1850	-23 (0.6 AcOEt)
Fmoc-Pro	b	91	75-77	1850	-26 (0.9 AcOEt)
Fmoc-Val	b	85	112-113	1845	+8 (1.85 AcOEt)
Fmoc-Tyr(OBzl)	b	80	128-130	1850	+25 (1 AcOEt)
Fmoc-Asp(O^tBu)	b	96	oil	1850	+25 (1 CH_2Cl_2)

solvent : a)CH_3CN ; b)CH_2Cl_2

Table 2 shows the results of the coupling of these fluorides with esters of amino acids in solution[8]. The absence of appreciable racemization was examined by comparison of physical data with the litterature and also by the absence of detectable amounts of diastereoisomers in the ^1H 200 MHz and ^{13}C NMR spectra of the dipeptides.

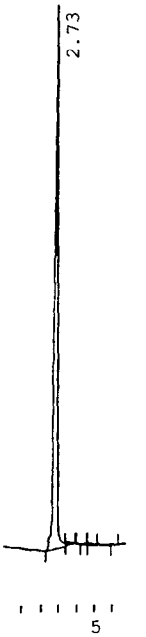
We have also explored the potentiality of these fluorides in solid phase synthesis using p-(benzyloxy)benzylalcohol Wang's resin. The first amino acid is easily linked to the resin by reaction of the fluoride (3 eq., 1h30, r.t.) in the presence of a catalytic amount of DMAP.

TABLE 2 : Coupling of amino acid fluorides

Dipeptide	Method	Yield %	mp °C	$[\alpha]_D$
Z-Gly-Phe OMe	A	82	oil	+ 34 (1 AcOEt)
Z-Gly-Ala-OMe	A	60	oil	- 6 (1 AcOEt)
Z-Ala-Gly-OEt	A	50	95-96	-21 (1.0 EtOH)
Z-Val-Phe-OEt	A	84	128-130	-
Z-Val-Ala-OEt	A	74	159-160	- 47(1.6 MeOH)
Z-Leu-Phe-OMe	A	95	79-80	- 26 (1.3 MeOH)
Z-Phe-Ala-OMe	A	91	128-130	- 22 (1.25 EtOH)
Fmoc-Ala-Ala-OMe	A	66	195-196	- 28 (1.35 AcOH)
Fmoc-Ala-Ala-OMe	B	87	id	- 31 (0.63 AcOH)
Fmoc-Phe-Phe-OEt	B	86	171-173	+18 (0.97 CH_2Cl_2)
Fmoc-Ile-Pro-OMe	B	77	63-65	-60 (0.28 CH_2Cl_2)

Method[8] : A) NMM/CH_3CN B) CH_2Cl_2/aq. Na_2CO_3

After conventionnal capping with acetic anhydride (5 eq., 30 min., r.t.) and careful washings of the resin, the Fmoc-amino acid has been cleaved from the resin with trifluoroacetic acid and the amount of recovered Fmoc-amino acid determined by UV titration. Substitution of the resin usually range from 81 to 94% and the recovered Fmoc-amino acid does not show any significant impurity on examination by TLC and HPLC. The coupling step is carried out with an excess of the amino acid fluoride (3 eq.) at room temperature until the Kaiser test is negative (usually 20 min.). Washings and cleavage of the peptide from the resin are run as usual.



HPLC of crude Leu-Ala-Val-Gly[9]

With this technique, we have prepared the model peptide Leu-Ala-Val-Gly with a 95% yield based on the resin capacity and a chromatographic purity of 99%.

Thus, amino acid fluorides are easily prepared and suitable for use in peptide synthesis. They can be isolated in pure form and are mostly cristalline compounds (especially the Fmoc derivatives) which are stable for a long period of time under normal conditions.

References and Notes

1-Fischer, E., Otto, E., *Ber.*, **1903**, *36*, 2106.
2-Carpino, L.A., Cohen, B.J., Stephens, K.E., Sadat-Aalee, D., Tien, J.H., Landridge, D.C., *J. Org. Chem.*, **1986**, *51*, 3732.
3-Olah, G.A., *J. Org. Chem.*, **1961**, *26*, 225.
4-Rajeswari, S., Jones, R.J., Cava, M.P., *Tetrahedron Letters*, **1987**, *28*, 5099.
5-Olah, G.H., *Synthesis*, **1973**, 487.
6-Mukayama, T., Tanaka, T., *Chem. Lett.*, **1976**, 303.
7-Picard, C., Cazaux, L., Tisner, P., *Tetrahedron Letters*, **1984**, *25*, 3809.
8-A) The N-protected amino acid fluoride (1 mmole) is reacted with the chlorhydrate of an α-amino ester (1.1 mmole) and N-methylmorpholine (2.2 mmoles) in acetonitrile (10 ml) until the acyl fluoride is consumed (approximately 4 h at 25°C). The reaction is then worked up as usual.

-B) A solution of the acyl fluoride (1.2 mmole) in CH_2Cl_2 (5 ml) and a 10% $NaHCO_3$ aqueous solution (10 ml) are added simultaneously to a stirred solution of the ester (1 mmole) in CH_2Cl_2 (5 ml) at room temperature. After completion of the reaction (generally 1 to 2 hours), the mixture is worked up as usual.

9-Reversed phase C 18 ; H_2O Pic B7(Waters)/CH_3CN (60/40) ; 1 ml/min ; λ=210 nm

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