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Synthesis of peptides employing Fmoc-amino acid chlorides and commercial zinc dust

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Abstract : Coupling of Fmoc-amino acid chlorides mediated by activated commercial zinc dust for the synthesis of peptides is described. The reaction is carried out in an organic medium. The necessity of using an inorganic base like $NaHCO_3/Na_2CO_3$ or an organic base like DIEA/NMM/TEA/pyridine is circumvented. The coupling is reasonably fast, clean, racemization free and high yielding.

The activation of the carboxylic group of Fmoc-amino acid as its chloride significantly enhances its reactivity with amino component in peptide synthesis leading to the rapid and efficient incorporation of acid moiety. However, such acylation reactions have to be carried out in the presence of a base for abstraction of the liberated HCl. A biphasic system like 5% Na_2CO_3/10%$NaHCO_3$-CH_2Cl_2/$CHCl_3$ or an organic base like diisopropylethylamine (DIEA), triethylamine (TEA), *N*-methylmorpholine (NMM) or pyridine-CH_2Cl_2/$CHCl_3$ have to be used.[2] The use of an equimolar quantity of an organic base is known to result in (i) 2-(9-fluorenylmethoxy)-5(4H)-oxazolone formation and epimerization, (ii) premature deblocking of Fmoc group leading to the formation of dibenzofulvene.[3] In this context, the use of potassium salts of 1-hydroxybenzotriazole (KOBt)[4] or 1-hydroxy-7-azabenzotriazole (KOAt)[4] were found to be a suitable additives, eliminating the above side-reactions and hence allowed the coupling process to continue for longer durations. [5,6]

Recently metal mediated reactions has been found to have wide scope in organic synthesis because of their simple work-up and selectivity. Several methods have been developed based on the use of a variety of metals such as magnesium,[7] tin[8] and zinc.[9] The utility of zinc for the synthesis of β,γ-unsaturated ketones by a reaction of an acid chloride with allyl bromide[10] and homoallylic alcohols[11] has been demonstrated. Further, the zinc-mediated preparation of stabilised

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triphenylphosphonium ylides,[12] amide bond formation,[13] Friedel-Crafts acylation,[14] carbamate formation[14] and esterification of acid chlorides[14] has been demonstrated recently. This communication describes the synthesis of peptides employing Fmoc-amino acid chlorides in presence of activated, commercial zinc dust (Scheme 1).

$$\text{Fmoc-NH-CHR}_1\text{-COCl} + \text{HCl.NH}_2\text{-CHR}_2\text{-COOX}$$

$$\Big\downarrow \text{ Zn (2 equiv.)}$$

$$\text{Fmoc-NH-CHR}_1\text{-CO-NH-CHR}_2\text{-COOX}$$

(X = methyl or ethyl group)

Scheme 1

The coupling of Fmoc-amino acid chlorides in presence of equimolar quantity of activated zinc (pretreated with HCl and thoroughly washed with water prior to use) was complete within 10-15 min. The course of the reaction was monitored by TLC and IR. The IR band corresponding to the –CO of the acid chloride at around 1790 cm^{-1} disappeared completely within 15 min. The work-up and isolation of the products were easy. Thus all the peptides made (Table 1) by this procedure were obtained in good yield (79 to 90%). The purity of the crude protected peptides, as checked by HPLC was found to be satisfactory.[15] It was also found that the acylation reactions can be carried out not only in tetrahydrofuran but also in toluene. It indicates that tetrahydrofuran is not mopping up HCl. During these studies, the amino acid ester hydrochloride salt was initially neutralized *in situ* using activated zinc (checked by pH and solubility of ester) prior to coupling. The coupling was found to be free from racemization by NMR analysis.[16] It was determined by the C-methylene doublets and the methyl ester singlets of the NMR spectra of the diastereomeric protected dipeptides 9-fluorenylmethoxycarbonyl-L-(or D-) phenylglycyl-L-phenylalanyl methyl esters {Fmoc-Phg-Phe-OMe [δ, 3.18 (d, $CHCH_2C_6H_5$), 3.64 (s, OCH_3)] and Fmoc-D-Phg-Phe-OMe [δ, 2.96 (d, $CHCH_2C_6H_5$), 3.74 (s, OCH_3)]}. Neither the oxazolone formation (IR analysis) nor the premature deblocking of the Fmoc group (ninhydrin test) was observed during coupling. A control experiment carried out using Fmoc-amino acid chloride with amino component without zinc does not yield the desired peptide.

Typical procedure : To a solution of amino acid ester hydrochloride salt (1 mmol) and zinc dust (1 mmol) in tetrahydrofuran (3 mL) was added a solution of Fmoc-amino acid chloride (1 mmol) and zinc dust (1 mmol) in tetrahydrofuran (3 mL) and the mixture stirred for 10-15 min at room temperature. After the completion of the reaction (monitored by TLC), the mixture was filtered. The organic layer was evaporated and the residue dissolved in chloroform was washed twice with 5 mL portions of 5% HCl, 5% Na_2CO_3, water and dried over Na_2SO_4. **Evaporation of solvent *in vacuo* and** recrystallization of residue from suitable solvents gave the **product as a white solid.**

Table 1 Protected dipeptide esters*

Sl. No.	Peptide	Yield (%)	M.p. (°C)	$[\alpha]^{25}_D$ (c1, $CHCl_3$)	R_f Value**	Elemental analysis : Found (Calcd.) %		
						C	H	N
1.	Fmoc-Phg#-Phe-OMe	89	194-96	+24.0	0.89	74.16	5.90	5.34
						(74.15	5.61	5.24)
2.	Fmoc-D-Phg-Phe-OMe	90	193-95	-24.0	0.91	73.96	5.47	5.18
						(74.15	5.61	5.24)
3.	Fmoc-Phe-Leu-OMe	90	163-65	-21.6	0.88	72.64	6.38	5.20
						(72.35	6.65	5.44)
4.	Fmoc-Phe-Phg-OMe	88	158-60	+22.6	0.84	74.08	5.72	5.36
						(74.15	5.61	5.24)
5.	Fmoc-Phe-Ala-OMe	89	183-85	-18.0	0.91	71.24	5.71	5.99
						(71.17	5.97	5.92)
6.	Fmoc-Phe-Phe-OEt	87	169-71	-22.3	0.86	74.74	6.14	4.74
						(74.71	6.08	4.97)
7.	Fmoc-Ile-Gly-OEt	89	110-12	-30.4	0.88	68.21	6.56	6.51
						(68.47	6.89	6.38)
8.	Fmoc-Ala-Leu-OMe	90	125-27	-28.6	0.81	68.53	6.92	6.42
						(68.47	6.89	6.38)
9.	Fmoc-Gly-Val-OMe	89	97-99	+18.6	0.92	67.61	6.46	6.58
						(67.30	6.38	6.82)
10.	Fmoc-Pro-Pro-OMe	88	118-20	+40.1	0.79	69.59	6.34	6.31
						(69.62	6.29	6.24)
11.	Fmoc-Ile-Pro-OMe	86	65-67	+19.6	0.89	69.56	6.98	6.24
						(69.80	6.94	6.03)
12.	Fmoc-Tyr(Bzl)-Phe-OMe	87	171-73	+16.2	0.86	75.44	6.05	4.38
						(75.21	5.54	4.27)

* all the peptides fully characterised by ^{1}H NMR;

** TLC analysis carried out using chloroform : methanol : acetic acid (40 : 2 : 1, v/v/v);

Phg, 2-amino-2-phenyl acetic acid (phenyl glycine).

Thus the coupling of Fmoc-amino acid chlorides can be accomplished in presence of commercial zinc dust instead of KOBt or KOAt. The neutralization of amino acid ester hydrochloride salt was also carried out using zinc dust. As there is no addition of any additional base, the coupling is free from racemization and other side reactions. The peptides are isolated as white solids in good purity. The application of this method for the synthesis of peptides employing urethane protected amino acid fluorides is in progress.

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