Note

Zinc promoted rapid and efficient synthesis of Fmoc- and Z- α , α -dialkylamino acids under neutral conditions

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The introduction of N^{α} -9-fluorenylmethoxycarbonyl (Fmoc) and benzyloxycarbonyl (Z) groups into α , α -dialkylamino acids is described at neutral pH using Fmoc-Cl or Z-Cl as an acylating agent respectively in the presence of activated zinc powder. The reaction is simple, fast and clean. It also permits the scale up with high yields. It is completely free from protected oligomer formation, which is a known side-reaction when Schotten-Baumann procedure is followed. All the Fmoc- and Z-amino acids prepared have been fully characterized.

The invention of the carbobenzoxy group (benzyloxycarbonyl or Z or Cbz) group by Max Bergmann and Lenoidas Zervas in 1932 promoted a strong expansion of peptide chemistry¹. The chemical synthesis of peptides progressed by leaps and bounds with the discovery of the base labile, mild, orthogonal alternative 9-fluorenylmethoxycarbonyl (Fmoc) group by Han and Carpino in 1970²⁻⁴. Benzyl chloroformate (or benzyloxycarbonyl chloride or $C_6H_5CH_2OCOCl$) and 9-fluorenylmethoxycarbonyl chloride (Fmoc-Cl) are the reagents routinely employed for the introduction of Z and Fmoc groups respectively^{5,6}. They are readily obtained through the reaction of phosgene with the respective alcohol (benzyl alcohol or 9-fluorenyl methanol) with or without a diluent.

Recently metal mediated reactions have gained a lot of scope in organic synthesis because of their simple work-up, catalysis property and selectivity. Several new methods have been developed based on the use of a variety of metals such as magnesium, tin and zinc. In this context, the use of zinc seems to be quite promising. The utility of zinc for the synthesis of β , γ -unsaturated ketones by a reaction of an acid chloride with allyl bromide and homoallylic alcohols has been described^{7,8}. The zinc mediated synthesis of stabilized triphenylphosphonium ylides⁹, amide bond formation¹⁰ and carbamates preparation¹¹ has been reported. Recently, we have demonstrated the use of zinc as a catalyst for the racemization free synthesis of several

dipeptides employing acid chlorides¹² and acid fluorides¹³ of Fmoc-amino acids as coupling agents.

The introduction of non-coded or nonribosomal α , α -dialkylamino acids into peptide sequences have pronounced effects on the conformation of peptide backbone¹⁴⁻¹⁸. They rigidify the peptide backbone through the formation of helices and β -turns. These organized structures are responsible for the interesting biological activity of the peptaibols, a group of peptide antibiotics isolated from soil fungi and characterized by a large percentage of Aib residues. In bilayer membranes the peptaibols form voltage dependent channels that are reminiscent of those found at neuronal synapses and, at high cellular concentrations, can cause cell lysis. α , α -Disubstituted residues are also found in nonpeptaibol peptides such as chlamydocin¹⁹ where they may have similar conformational effects.

The Z and Fmoc groups have been introduced into α , α -dialkylamino acids by using Z-Cl and Fmoc-Cl respectively as acylating agents under Schotten-Baumann conditions (**Scheme I**). The use of an inorganic base like Na_2CO_3 or $NaHCO_3$ in the synthesis of carbamates employing alkyloxycarbonyl chlorides has a dual purpose. It not only helps in dissolution of an amino acid but also for the abstraction of the liberated HCl eliminating the formation of unreacted amino acid as its hydrochloride salt. However, it is important to note that acylation reactions carried out under these conditions are found to be always accompanied by some side-reactions. Thus, the formation of protected oligomers like Z-dipeptide and Z-tripeptide and Fmoc-di and tripeptides during the synthesis of Z- and Fmoc-amino acids respectively is well documented²⁰⁻²⁹ (**Scheme II**). Similar reactions have been noticed in the case of dialkylamino acids like Aib as well³⁰. In this context, less reactive acylating reagents like Z-OBt, Z-ONp and Z-OPhNO₂ (*p*) and Fmoc-ODSp, Fmoc-ONSu²⁵, Fmoc-N₃, Fmoc-ONp and Fmoc-OPh have been tried with limited success. The synthesis of Z- α -amino acids catalyzed by zinc dust has been reported by us recently³¹. This paper describes the zinc promoted simple synthesis of oligomer free N^{α} -Z- and Fmoc- α , α -dialkylamino acids using Z-Cl and Fmoc-Cl as an acylating agent respectively under neutral conditions.

Synthesis of Z- and Fmoc- α,α -dialkylamino acids under Schotten - Baumann conditions

Scheme I

Formation of Fmoc-dipeptide.
(similar dipeptide formation is also rationalized in the preparation of Z-amino acids).

Scheme II

Synthesis of Z- and Fmoc- α,α -dialkylamino acids catalyzed by zinc dust.

Scheme III

It is now found that Z- and Fmoc groups can be introduced into amino acids easily using Z-Cl and Fmoc-Cl respectively in the presence of commercial, activated zinc dust (**Scheme III**). The amino acid to be acylated was dissolved by using suitable procedure. It was done by the dropwise addition of 1 M HCl to a suspension of an amino acid in acetonitrile till a

clear solution obtained. The pH of the resulting solution was then brought to neutral using zinc dust. In other cases, the amino acid was dissolved by the slow addition of an organic base (triethylamine) and then the pH adjusted to neutral. In the presence of an equimolar quantity of zinc dust, the solution of Z-Cl in acetone or Fmoc-Cl in acetonitrile was added in

Table I—Fmoc- α , α -dialkylamino acids*

Sl. No.	Amino acid	Yield (%)	M.p. (°C)	TLC R _f Value	R _t Value	¹ H NMR
1	Fmoc-Ac ₅ c	70	192-94	0.73	15.0	1.5-2.0 (8H, m, Ac ₅ c protons), 4.1 (1H, d, CH ₂ O), 4.3 (2H, t, CHFMoc), 5.9 (1H, s, NH), 7.2-7.8 (8H, m, aryl).
2	Fmoc-Ac ₆ c	71	191-92	0.75	16.0	1.2-1.8 (10H, m, Ac ₆ c protons), 4.2 (1H, d, CH ₂ O), 4.4 (2H, t, CHFMoc), 6.1 (1H, s, NH), 7.2-7.8 (8H, m, aryl).
3	Fmoc-Ac ₇ c	68	178-80	0.77	15.8	1.4-2.0 (12H, m, Ac ₇ c protons), 4.0 (1H, d, CH ₂ O), 4.2 (2H, t, CHFMoc), 5.8 (1H, s, NH), 7.2-7.8 (8H, m, aryl).
4	Fmoc-Ac ₈ c	69	210-12	0.71	15.4	1.1-1.8 (14H, m, Ac ₈ c protons), 4.1 (1H, d, CH ₂ O), 4.3 (2H, t, CHFMoc), 5.9 (1H, s, NH), 7.2-7.8 (8H, m, aryl).
5	Fmoc-Aib	70	179-80 (179-80) ³²	0.79	14.7	1.47 (6H, m, alkyl protons), 4.1 (1H, d, CH ₂ O), 4.3 (2H, t, CHFMoc), 6.33 (1H, s, NH), 7.2-7.8 (8H, m, aryl).
6	Fmoc-Deg	66	130-32	0.80	14.9	0.9-2.0 (10H, m, alkyl protons), 4.2 (1H, d, CH ₂ O), 4.4 (2H, t, CHFMoc), 6.8 (1H, s, NH), 7.2-7.8 (8H, m, aryl).
7	Fmoc-Dpg	69	143-45	0.79	14.8	0.82-2.2 (14H, m, alkyl protons), 4.1 (1H, d, CH ₂ O), 4.3 (2H, t, CHFMoc), 6.9 (1H, s, NH), 7.2-7.8 (8H, m, aryl).
8	Fmoc-Dbg	68	110-12	0.83	14.9	0.8-2.1 (18H, m, alkyl protons), 4.2 (1H, d, CH ₂ O), 4.3 (2H, t, CHFMoc), 7.2 (1H, s, NH), 7.3-7.9 (8H, m, aryl).
9	Fmoc-Dbzg	67	216-18	0.86	15.2	3.9 (1H, d, CH ₂ O), 4.19 (2H, t, CHFMoc), 5.0 (4H, s, benzyl), 6.9 (1H, s, NH), 7.2-7.8 (18H, m, aryl).
10	Fmoc-Dphg	69	182-84	0.88	15.0	3.95 (1H, d, CH ₂ O), 4.3 (2H, t, CHFMoc), 6.8 (1H, s, NH), 7.2-7.8 (18H, m, aryl).

Table II—Z- α , α -dialkylamino acids

Sl. No.	Amino acid	Yield (%)	M.p. (°C)	TLC R _f Value	R _t Value	¹ H NMR
1	Z-Ac ₅ c	69	91-2 (91-2) ³³	0.70	14.9	1.4-2.0 (8H, m, Ac ₅ c protons), 5.1 (2H, s, benzyl), 5.9 (1H, s, NH), 7.4 (10H, m, aryl).
2	Z-Ac ₆ c	73	148-50 ³⁴	0.63	14.8	1.2-1.8 (10H, m, Ac ₆ c protons), 5.07 (2H, s, benzyl), 6.2 (1H, s, NH), 7.5 (10H, m, aryl).
3	Z-Ac ₇ c	68	95-8 (109-110) ³⁵	0.68	15.0	1.5 (12H, m, Ac ₇ c protons), 4.6 (1H, s, NH), 5.06 (4H, s, benzyl), 7.4 (10H, m, aryl).
4	Z-Ac ₈ c	70	147-148	0.71	15.2	1.1-1.8 (14H, m, Ac ₈ c protons), 5.08 (2H, s, benzyl), 5.9 (1H, s, NH), 7.5 (1H, m, aryl).
5	Z-Ac ₉ c	69	146-47 (146-47) ³⁵	0.69	15.0	1.4-1.9 (16H, m, Ac ₉ c protons), 4.4 (1H, s, NH), 5.05 (4H, s, benzyl), 7.37 (10H, m, aryl).
6	Z-Aib	71	68-70 (68-70) ³⁶	0.70	14.9	1.58 (6H, m, alkyl), 5.09 (2H, s, benzyl), 6.33 (1H, s, NH), 7.2 (10H, m, aryl).
7	Z-Deg	70	91-2	0.71	15.3	0.9-2.0 (10H, m, alkyl), 4.99 (2H, s, benzyl), 6.9 (1H, s, NH), 7.3 (10H, m, aryl).
8	Z-Dpg	68	87-9 (89-90) ³⁷	0.75	15.7	0.8-2.1 (14H, m, alkyl), 4.8 (2H, s, benzyl), 7.2 (1H, s, NH), 7.4 (10H, m, aryl).
9	Z-Dbg	67	82-3	0.72	15.5	0.82 (18H, m, alkyl), 5.01 (2H, s, benzyl), 7.1 (1H, s, NH), 7.5 (10H, m, aryl).
10	Z-phg	69	127-28 (128-29) ³⁸	0.69	15.1	5-09 (2H, s, benzyl), 6.22 (1H, s, NH), 7.33 (15H, m, aryl).

* Abbreviations used are : Ac₅c, 1-aminocyclopentane-1-carboxylic acid; Ac₆c, 1-aminocyclohexane-1-carboxylic acid; Ac₇c, 1-aminocycloheptane-1-carboxylic acid; Ac₈c, 1-aminocyclooctane-1-carboxylic acid; Ac₉c, 1-aminocyclononane-1-carboxylic acid; Aib, α -aminoisobutyric acid; Deg, Diethylglycine; Dpg, dipropylglycine; Dbg, dibutylglycine; Dbzg, dibenzylglycine; Dphg, diphenylglycine;

Figure 1—Analytical RP-HPLC of (a) Fmoc-Aib prepared from Fmoc-Cl/ Na_2CO_3 -(b) Fmoc-Aib prepared from Fmoc-Cl/Zn; Delta pack C-18 column (3.9×300 mm) was used; flow rate: 0.75 ml/min; U>V> monitoring at 252 nm; eluant: Isocratic 70% acetonitrile.

one portion. The course of the reaction was monitored by TLC. The formation of the urethane group was also identified by the presence of a peak at round $1688\text{--}1710\text{ cm}^{-1}$ in IR. The acylation was complete in about 45 min to 1 hr. After the completion of the reaction, the mixture was subjected to an aqueous work-up to yield the corresponding Z- and Fmoc- α , α -dialkyl-amino acids in good yield and purity (**Tables I and II**).

The HPLC analysis of Fmoc-Aib, made by this method, has been shown to be completely free from Fmoc-Aib-Aib (**Figure 1**). Compared to the standard procedure using Fmoc-Cl in dioxane/aqueous Na_2CO_3 at 0°C , the reaction of Fmoc-Cl in presence of zinc proceeds rapidly. It is because of the trapping of the liberated HCl by zinc dust. The chromatographic analysis of Fmoc- α , α -dialkylamino acids was carried out using the literature TLC system toluene : acetic acid, (10:1) which is known to separate Fmoc-amino acid and its oligomers²¹. It revealed that all the amino acids made by this method are free from the corresponding oligomers. As described in **Scheme II**, the sodium salt of amino acid reacts with unreacted Fmoc-Cl leading to the formation of dipeptides. In the present method, the formation of amino acid salts is circumvented.

Our studies have shown that the oligomer free Z- and Fmoc- α , α -dialkylamino acids can be made by using Z-Cl and Fmoc-Cl as an acylating agent in the presence of zinc dust under neutral conditions. The formation of oligomeric peptides is completely circumvented. It is a simple and convenient procedure. There is no need to convert Z-Cl or Fmoc-Cl to their corresponding carbonates of 1-hydroxybenzotriazole, *N*-hydroxysuccinimide etc. All the Z- and Fmoc- α , α -dialkylamino acids after recrystallization have been obtained in good yield and purity. By using the same procedure other groups like isobutyloxycarbonyl group can also be introduced. However, our attempts to introduce p-toluenesulphonyl group using Tos-Cl into Aib by this method were unsuccessful.

Experimental Section

The melting points were determined by using capillary tubes and are uncorrected. TLC analysis was carried on precoated silica gel plates using solvent system CHCl_3 : methanol : acetic acid (40:2:1 v/v/v) and R_f value is designated as R_f A. I.R. spectra were recorded on a Nicolet model Impact 400D FT-IR spectrometer (KBr pellets, 3 cm^{-1} resolution). Analytical RP-HPLC was performed with a water LC-3000 system using a bondopak C-18-Å Column

(3.9×300 μ m) (10 μ , spherical) using as eluent acetonitrile (0.1% TFA)/H₂O (65/35, isocratic) flow rate (0.75 mL/min, 252 nm). ¹H NMR spectra were recorded on a Bruker ACF 200 MHz spectrometer using Me₄Si as an internal standard. The α,α -dialkylamino acids were synthesized starting from the appropriate ketones. The hydantoins were first prepared from ketones and then hydrolysed using 60% H₂SO₄ according to the reported procedures³⁹. DPG (diphenylglycine) was synthesized by refluxing the mixture of benzil and urea in alcoholic NaOH using the reported procedure⁴⁰. Commercial zinc powder was preactivated by treating with 5% HCl and then thoroughly washed with water prior to use.

The general procedure for the synthesis of Fmoc- or Z- α,α -dialkylamino acids. The α,α -dialkylamino acid (10 mmole) was suspended in acetonitrile (20 mL) and 1M HCl was added slowly till it dissolves completely. The activated zinc powder was added slowly in portions till the reaction mixture attains neutral pH. Fmoc-Cl or Z-Cl (10 mmole) in acetonitrile was added in one portion and the reaction mixture was stirred. The progress of the reaction was monitored by TLC and IR. After the completion of the reaction, the mixture was filtered and evaporated the organic solvent *in vacuo*. The residue was dissolved in 10 mL portions of 10% Na₂CO₃ and washed with ether (3 × 10 mL) and then the aqueous phase was acidified to pH 2. It was extracted with ethyl acetate (3 × 10 mL), washed with water (3 × 10 mL) and brine and dried then over anhyd. Na₂SO₄. Evaporation of the resulting organic layer to an oil which on recrystallization using suitable solvent gave Fmoc- or Z- α,α -dialkylamino acids in good yield and purity (as checked by HPLC).

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