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Synthesis of Fmoc-protected amino ketones bearing *tert*-butyl based side-chain protecting groups

Jesús Vázquez and Fernando Albericio*

Barcelona Biomedical Research Institute-Barcelona Science Park and Department of Organic Chemistry, University of Barcelona, E-08028 Barcelona, Spain

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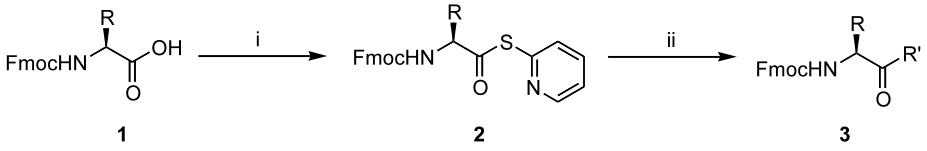
Abstract—A variety of Fmoc-protected amino ketones have been prepared in good yields from amino acids by their transformation into thioesters of 2-mercaptopyridine and reaction of the resulting products with dialkylcuprates.

Proteases, notably cysteine proteases, are implicated in the pathogenesis of many diseases; hence they have become targets of great pharmaceutical interest in recent years.[1] Several types of diseases are treated by inhibition of these enzymes, the most common strategy utilizing a peptide that can interact with the reactive centre of an enzyme's active site.[2–5] Both reversibly or irreversibly binding molecules can be used as cysteine protease inhibitors, although in almost all cases they feature an electrophilic functionality such as a carbonyl group or Michael acceptor that can react with the nucleophilic cysteine residue. Thus, ketones,[2] fluoroketones,[3] aldehydes[4] and sulfones[5] have been reported as cysteine protease inhibitors.

Caspases are a family of cysteine proteases that are involved in apoptotic cell death, and pharmacological caspase inhibition has been demonstrated to prevent neuronal cell death.[6] The syntheses of various inhibitors of caspases have been reported.[7] Among these molecules are compounds based on a family of tetrapeptides in which the C-terminal amino acid is a modified aspartic acid containing electrophilic groups such as ketones. Combinatorial chemistry has been used to discover caspase inhibitors by screening both amino acids and electrophillic groups. However, effective libraries require appropriate building blocks such as novel amino ketones.

While amino ketones have been synthesized in both solution and solid phase they have historically been simple molecules, such as amino acids with minimally functionalized side chains, and have required the use of protecting groups that are stable in basic media.[8]

An optimal strategy for the solid-phase synthesis of molecules containing a modified aspartic acid would involve the incorporation of an Fmoc-protected amino ketone to either Wang or Barlos resin through the β-carboxylic acid. Therefore, a convenient method for the preparation of Fmoc-derivatives of the modified aspartic acid would be useful.



1 2 3

Scheme 1. *Reagents and conditions*: (i) 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide (1 equiv.), 2-mercaptopyridine (1.1 equiv.), DCM, rt, 6 h; (ii) R'_2CuMgX (3 equiv.), THF, 0°C, 1–3 h.

Abbreviations: ^{t}Bu, *tert*-butyl; Fmoc, 9-fluorenylmethyloxycarbonyl; Boc, *tert*-butoxycarbonyl; DCM, dichloromethane; EtOAc, ethyl acetate; Ph, phenyl; THF, tetrahydrofuran; Amino acid symbols denote the L-configuration.

Keywords: amino ketones; ketone synthesis; combinatorial chemistry; high-throughput synthesis.

* Corresponding author.

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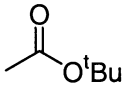
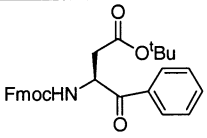
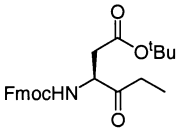
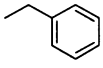
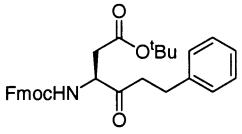
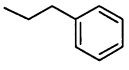
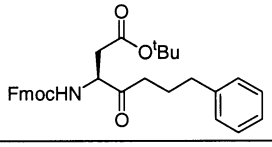
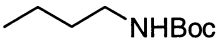
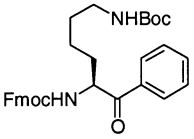
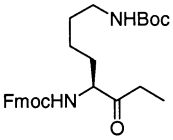
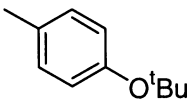
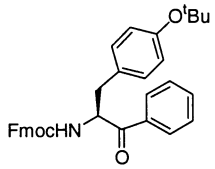
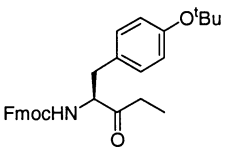
In our search for ketone-forming conditions in which both the Fmoc and ^{t}Bu ester protecting groups of Fmoc-Asp(O^{t}Bu)-OH would be stable, we initially explored Grignard chemistry on Weinreb amides. As one would expect, the Fmoc group proved to be unstable under these conditions.[9,10] Although we were able to obtain the ketone product using the Weinreb derivative of Boc-Asp(^{t}Bu)-OH,[9] this process required the elimination of the Boc group and subsequent Fmoc protection of the amine.

Organocuprates, which are used in the synthesis of ketones from acid chlorides or thioesters, are widely regarded as weak bases and so we hypothesized that they would be compatible with syntheses in the presence of Fmoc-protecting groups. We report here a general method for the preparation of Fmoc-protected amino ketones from amino acids in good yields (Scheme 1).

We based our studies on thioesters because the traditional preparation of acid chlorides is incompatible with the presence of Boc or ^{t}Bu protecting groups.[11] Furthermore, in solution the PPh_3/CCl_4 procedure generates triphenylphosphine oxide, which is difficult to separate from the desired product, and the alternative use of PPh_3 resin is rather expensive.

We chose 2-mercaptopyridine as the thiol since it is odorless, relatively inexpensive and generates highly reactive thioesters. The synthesis of these thioesters was

Table 1. Preparation of Fmoc-protected amino ketones from Fmoc-protected amino thioesters

R	R'	Product	Time (h)	Yield (%)	^{1}H-NMR data (δ)
	Ph		3	54	7.98 (d, J=7.3 Hz, 2H) 7.72 (d, J=7.3 Hz, 2H) 7.65-7.12 (m, 9H) 5.67 (d, J=8.8 Hz, 1H) 4.50-4.00 (m, 4H) 2.71 (dd, J=5.8, 16.1 Hz, 1H) 2.50 (dd, J=5.7, 16.1 1H) 1.40 (s, 9H)
	Et		1	77	7.76 (d, J=7.8 Hz, 2H) 7.59 (d, J=7.4 Hz, 2H) 7.41 (t, J=7.8 Hz, 2H) 7.32 (t, J=7.4 Hz, 2H) 5.86 (d, J=8.6 Hz, 1H) 4.55-4.35 (m, 3H) 4.23 (t, J=6.6 Hz, 1H) 2.91 (dd, J=4.3, 16.8 Hz, 1H) 2.71 (dd, J=4.4, 16.8 Hz, 1H) 2.60-2.48 (m, 2H) 1.43 (s, 9H) 1.07 (t, J=7.2 Hz, 3H)
			3	71	7.76 (d, J=7.3 Hz, 2H) 7.59 (d, J=7.3 Hz, 2H) 7.50-7.10 (m, 9H) 5.86 (d, J=8.8 Hz, 1H) 4.55-4.33 (m, 3H) 4.23 (t, J=6.6 Hz, 1H) 2.90 (dd, J=4.3, 16.8 Hz, 1H) 2.83-2.47 (m, 5H) 1.41 (s, 9H)
			3	73	7.76 (d, J=7.3 Hz, 2H) 7.59 (d, J=7.3 Hz, 2H) 7.48-7.09 (m, 9H) 5.88 (d, J=8.8 Hz, 1H) 4.55-4.3 (m, 3H) 4.22 (t, J=6.6 Hz, 1H) 2.88 (dd, J=4.4, 16.8 Hz, 1H) 2.79-2.45 (m, 5H) 1.92 (q, J=7.3Hz, 2H) 1.42 (s, 9H)
	Ph		3	49	7.97 (d, J=7.3 Hz, 2H) 7.77 (d, J=6.6 Hz, 2H) 7.70-7.20 (m, 9H) 5.84 (d, J=8.1 Hz, 1H) 5.45-5.25 (m, 1H) 4.80-4.30 (m, 3H) 4.24 (dd, J=7.3, 6.6 Hz, 1H) 3.20-2.90 (m, 2H) 2.10-1.80 (m, 2H) 1.72-1.25 (m, 4H) 1.42 (s, 9H)
	Et		1	75	7.76 (d, J=6.6 Hz, 2H) 7.60 (d, J=7.3 Hz, 2H) 7.41 (t, J=6.6 Hz, 2H) 7.32 (t, J=7.3 Hz, 2H) 5.58 (d, J=7.3 Hz, 1H) 4.67-4.47 (m, 1H) 4.45-4.30 (m, 3H) 4.22 (t, J=6.6 Hz, 1H) 3.20-3.00 (m, 2H) 2.66-2.38 (m, 2H) 1.75-2.00 (m, 2H) 1.70-1.18 (m, 4H) 1.43 (s, 9H) 1.09 (t, J=7.3 Hz, 3H)
	Ph		3	46	7.91 (d, J=7.3 Hz, 2H) 7.77 (d, J=7.3 Hz, 2H) 7.58 (t, J=6.6 Hz, 2H) 7.53-7.23 (m, 7H) 6.86 (d, J=8.8 Hz, 2H) 6.79 (d, J=8.8 Hz, 2H) 5.73 (d, J=8.8 Hz, 1H) 5.67-5.50 (m, 1H) 4.52-4.28 (m, 2H) 4.22 (t, J=6.6 Hz, 1H) 3.22 (dd, J=5.8, 13.9 Hz, 1H) 3.04 (dd, J=5.1, 13.9 1H) 1.27 (s, 9H)
	Et		1	74	7.76 (d, J=7.3 Hz, 2H) 7.57 (d, J=7.3 Hz, 2H) 7.40 (t, J=6.6 Hz, 2H) 7.31 (t, J=7.3 Hz, 2H) 7.01 (d, J=8.8 Hz, 2H) 6.90 (d, J=8.5 Hz, 2H) 5.44 (d, J=7.3 Hz, 1H) 4.59 (dt, J=6.6, 7.3 Hz, 1H) 4.50-4.28 (m, 2H) 4.19 (t, J=6.6 Hz, 1H) 2.99 (d, J=6.6 Hz, 2H) 2.48-2.20 (m, 2H) 1.31 (s, 9H) 0.98 (t, J=7.3 Hz, 3H)

carried out with 1-ethyl-3-(3′-dimethylamino propyl)-carbodiimide because its urea byproduct can be easily removed by aqueous extraction.[12] Treatment of thioesters **2** with dialkylcuprates in anhydrous THF under an Ar atmosphere afforded the Fmoc-protected amino ketones **3** in good yields (Table 1). Besides the Asp derivatives, this methodology was also used for the preparation, with similar results (Table 1), of Tyr and Lys derivatives from the corresponding Fmoc/^{t}Bu and Fmoc/Boc protected amino acids.[13]

In conclusion, we have developed a general and straightforward methodology to prepare Fmoc-protected amino ketones. These derivatives, after removal of the ^{t}Bu-based protecting groups with TFA, are currently being incorporated into solid supports through the side-chain for the preparation of libraries.[14] The results of these studies will be published elsewhere.

Experimental

General. THF was distilled from sodium/benzophenone immediately before use. Column chromatography was performed with silica gel (Silica 60 A, 35–70 μm). Thin-layer chromatography (TLC) was performed on aluminum-backed silica plates (Merck 60/F254) to follow the reactions. ^{1}H NMR spectra were obtained on a Varian Gemini 200 MHz spectrometer in $CDCl_3$ unless stated otherwise. Chemical shifts are reported in ppm (δ units) downfield from internal tetramethylsilane or the appropriate solvent signal [$(CD_3)_2CO$].

Preparation of thioesters. A sample procedure is as follows: To a stirred suspension of Fmoc-Asp(O′Bu)-OH (1.43 mmol) in DCM (20 mL) was added 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide (1.43 mmol). Upon complete dissolution, 2-mercaptopyridine (1.57 mmol) was added to the reaction and stirring was continued for 6 h. The resulting mixture was diluted with DCM (20 mL) and the organic phase was washed with 0.1 N aq. HCl (2×20 mL), 10% aq. Na_2CO_3 (2×20 mL) and sat. brine (1×20 mL), dried over $MgSO_4$ and filtered. The solvent was removed (rotary evaporation) to give a pale yellow foamy solid (598 mg, 83%). ^{1}H NMR δ 8.63 (d, $J=5.9$ Hz, 1H) 7.8–7.2 (m, 11H) 6.10 (d, $J=9.5$ Hz, 1H) 4.85 (ddd, $J=9.5$, 5.1, 4.4 Hz, 1H) 4.65–4.25 (m, 3H) 3.04 (dd, $J=5.1$, 17.6 Hz, 1H) 2.74 (dd, $J=4.4$, 16.8 Hz, 1H) 1.46 (s, 9H); MS (MALDI-TOF): m/z calcd: $[M+Na]^+=527.16$ $[M+K]^+=543.27$. Found: $[M+Na]^+=527.39$ $[M+K]^+=543.39$.

Preparation of ketones. A sample procedure is as follows: In a dry, Ar-purged flask was added anhydrous THF (15 mL) to CuI (567 mg, 2.98 mmol) and the resulting suspension cooled to −78°C. The Grignard reagent of 1-bromo-3-phenylpropane (1 M in THF, 6 mL, 5.95 mmol) was added. Once the addition was finished the reaction was heated to 0°C and stirring was continued for 2 h. A solution of the previously prepared thioester (500 mg, 0.99 mmol) in anhydrous THF (15 mL) was then added and stirring was continued for 3 h. The reaction mixture was quenched with saturated aqueous ammonium chloride (2×20 mL) and diluted with diethyl ether (30 mL). After several washes with saturated aqueous ammonium chloride (2×20 mL), 10% aq. Na_2CO_3 (2×20 mL) and sat. brine (1×20 mL) the THF–ether solution was dried over $MgSO_4$, filtered and concentrated by rotary evaporation. Purification by column chromatography (silica gel, hexane–EtOAc 8:2) gave 342 mg of the desired product (73%) as a yellow oil. See Table 1 for NMR data. MS (ESI, positive ion): m/z calcd: 513.3 $[M+1]^+$ Found: 513.6 $[M+1]^+$.

Acknowledgements

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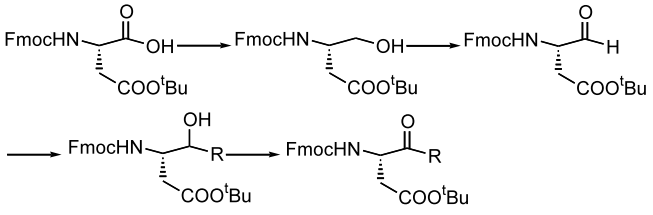
9. Reactions with commercial phenyl and tolyl Grignard derivatives should be carried out for 16 h at 0°C for complete conversion of the Weinreb amide into the ketone.
10. Research at Merck Frosst Canada & Co has been published in a patent [WO 00/32620 (*Chem. Abstr.* 133:17829)], which describes a longer, more complicated method for the preparation of Fmoc-Asp(O^{t}Bu)-R:



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12. These thioester derivatives are not stable in the presence of TFA, as demonstrated when an attempt was made to remove the ^{t}Bu group with the aim of anchoring the aspartic derivative through the side chain to a solid support to carry out the Grignard reaction on solid phase.
13. Reaction of derivatives **2** with Grignard reagents, first, at −78°C for 2 h and then at −30°C for 1.5–3 h, led to intractable mixtures of crude products.
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