Pergamon

Tetrahedron Letters 40 (1999) 2501–2504

TETRAHEDRON LETTERS

A Selective Method for the Preparation of Primary Amides: Synthesis of Fmoc-L-4-Carboxamidophenylalanine and other Compounds

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Received 18 January 1999; revised 27 January 1999; accepted 28 January 1999

Abstract: A new method for the synthesis of primary amides was developed in which carboxylic acids were treated with ammonium chloride in the presence of peptide synthesis coupling agents and base at room temperature. These mild conditions allow the conversion of carboxyl groups to primary amides on substrates possessing sensitive functional groups such as esters and the base-labile Fmoc protecting group.

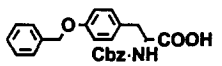
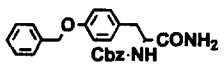
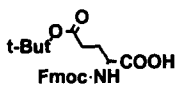
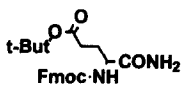
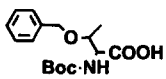
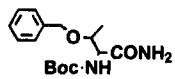
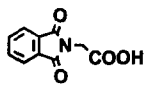
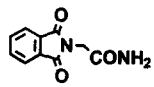
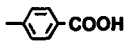
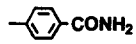
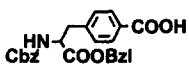
Traditional methods for the synthesis of unsubstituted carboxamides rely on the treatment of acyl halides, acid anhydrides, esters, or acids with ammonia.[1] However, there are difficulties associated with these procedures. Acylation of ammonia by acyl halides is highly exothermic and must be carefully controlled. Reaction of acid anhydrides with ammonia can be accompanied by the formation undesired imides.[2] Additionally, since ammonia can react with carboxylic esters, acids, and other functional groups,[3] these methods are sometimes not suitable for selective amide generation. Other modified methods have been reported: e.g. aminolysis of *N*-protected amino acid active esters with diaminomethane dihydrochloride,[4] and treatment of carboxylic acids with 25% aqueous ammonia in presence of HOBt and DCC.[5] For the latter, difficulties can arise from insolubility of starting materials and products or by competitive hydrolysis of the activated carboxyl group by hydroxide anion. We report a new, simple, and mild method for the conversion of carboxylic acids to primary amides using ammonium chloride, tertiary amine, and coupling agents typically used in peptide synthesis.

General procedure: Carboxylic acids were treated with PyBOP (1.5 eq.), HOBt (1.5 eq.), DIPEA (4 eq.), NH_4Cl (2 eq.) in DMF (4ml/mmol of acid) at room temperature to form the corresponding unsubstituted amides, followed by aqueous work-up. The results for the preparation of a series of amides of substrates possessing a variety of potentially labile groups are shown in Table 1. As monitored by reverse phase HPLC,[6] starting materials were converted quantitatively to products after 0.5 h when PyBOP was used as the coupling agent.[7] In contrast to ammonia, the base-labile Fmoc protecting group of Fmoc-Glu(OBzl)-OH (**2**) was stable to ammonium chloride. Under these conditions the carboxyl groups of **2** and Cbz-4-

0040-4039/99/$ - see front matter
PII: S0040-4039(99)00245-2

carboxylphenylalanine-OBzl[8] (6) underwent selective conversion to amides without undesired ammonolysis of either the *t*-butyl or benzyl esters of the starting materials.

Table 1

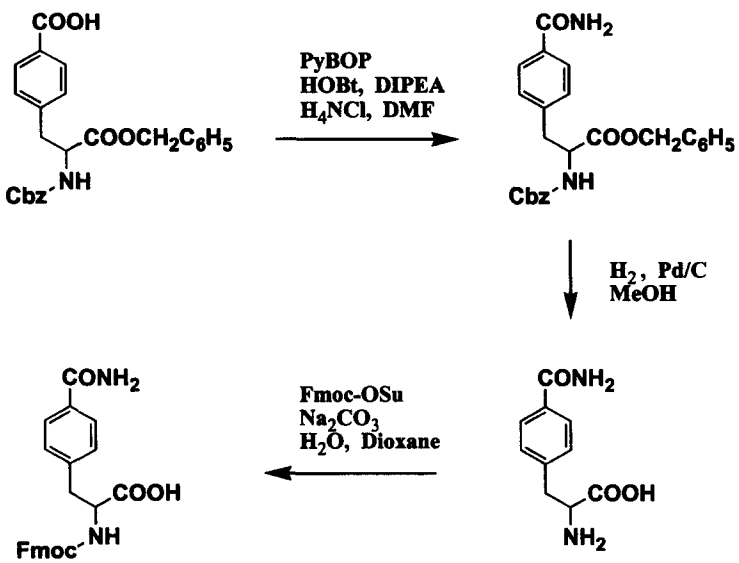
No.	Substrate	Product	Yield%
1			95
2			95
3			95
4			85
5			90
6			95

In addition to PyBOP, other peptide synthesis coupling agents such as HBTU, EDAC, and DCC were usable in this reaction (Table 2). Therefore, purification can be simplified by means of the differient solubilities of the coupling agent and products, e.g. the organic soluble coupling agent DCC could be used to synthesize water-soluble amides. On the other hand, if the product is soluble in organic solvent, water-soluble coupling agents such as EDAC or PyBOP should be chosen, since the by-products can be removed by aqueous extraction. Reaction time varied with the coupling agent. As is generally the case in peptide synthesis, the carbodiimides DCC and EDAC took longer than the phosphonium and uronium agents. For derivatization of the α-carboxyl group of Cbz-Tyr(Bzl)-OH, reactions using PyBOP and HBTU were complete in 30 min whereas EDAC and DCC required 2 and 5 hr, respectively. Conversion of the side chain carboxyl group of Cbz-4-Cpa-OBzl using PyBOP was completed after 0.5h, while the reaction using EDAC required 3h. Thus PyBOP and HBTU were the most efficient reagents for this reaction.

This procedure was used to synthesize Fmoc-4-carboxamidophenylalanine from Cbz-4-Cpa-OBzl[8] as shown in the Scheme.[9] This new amino acid combines features of both phenylalanine and glutamine and may serve as a neutral replacement for 4-carboxyphenylalanine[10,11] in peptide research and drug discovery efforts.

Table 2

Substrate	Coupling reagent	Reaction time (h)	Yield (%)
Cbz-Tyr(Bzl)-OH	PyBOP	0.5	100
	HBTU	0.5	100
	EDAC	2	100
	DCC	5	100
Cbz-4-Cpa(OBzl)-OH	PyBOP	0.5	100
	EDAC	3	100



Scheme

In summary, treatment of carboxylic acids with ammonium chloride and peptide synthesis coupling reagents in the presence of tertiary amines constitutes a straightforward method for the preparation of primary amides. Amides were prepared in excellent yields under very mild conditions which allowed selective aminolysis of activated carboxyl groups while susceptable functional groups such as esters and the base-labile Fmoc protecting group remained intact. Although our focus was on amino acids in this work, it is expected that this procedure will be useful in other areas of synthetic chemistry as well as solid phase organic synthesis.

Acknowledgements: FAB-MS analysis was carried out by Annie Bellatore and William Siefert of the Analytical Chemistry Center of The University of Texas Health Science Center at Houston. We are grateful to the National Cancer Institute (CA53617) for support of this work.

Abbreviations: Boc, *t*-butyloxycarbonyl; Bzl, benzyl; Cbz, benzyloxycarbonyl; Cpa, carboxyphenylalanine; DCC, *N,N'*-dicyclohexylcarbodiimide; DIPEA, diisopropylethylamine; DMF, dimethylformamide; EDAC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; FAB-MS, fast atom bombardment mass spectrometry; Fmoc, 9-fluorenylmethoxycarbonyl; Fmoc-OSu, *N*-(9-fluorenylmethoxycarbonyloxy)-succinimide; HBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; PyBOP, benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate.

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4. Galaverva G., Corradini R., Cossena A. and Marchelli R. *Int. J. Peptide Protein Res.* **1993**, *42*, 53-57.
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6. Analytical HPLC was performed on a Hewlett Packard 1090 liquid chromatograph using a Vydac C4 column (4.6 x 250 mm). The gradient was 15-75% B/25min with a flow rate of 1.5ml/min (A: 0.1% TFA in water; B: 0.1%TFA in acetonitrile). Elution was monitored with a diode array detector at both 230 nm and 274 nm.
7.

Substrate				Product				
No.		HPLC* (min)	IR (OH band)		HPLC* (min)	IR (NH band)	FAB-MS calculated	found
1	acid	17.396	3380-2500	amide	16.151	3375, 3321	405.18	405.2
2	acid	17.639	3380-2500	amide	16.399	3377, 3321	425.21	425.3
3	acid	13.768	3380-2500	amide	11.799	3391, 3334	309.18	309.2
4	acid	4.68	3350-2500	amide	3.26	3416	205.06	205.0
5	acid		3170-2000	amide		3391		
6	acid	17.249	3380-2505	amide	15.993	3385, 3332	433.18	433.2

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9. 0.43g (1mmol) of Cbz-4-Cpa-Bzl, 0.78g (1.5 mmol) of PyBOP, and 0.20g (1.5 mmol) of HOBt were dissolved in 4 ml of DMF. 0.58ml (4 mmol) of DIPEA and 0.01g (2 mmol) of NH_4Cl were added successively. The mixture was stirred for 0.5h at room temperature. Hydogenolysis in the presence of 10% Pd/C removed the both benzyl based protecting groups, and was followed by treatment with Fmoc-OSu (1eq.) and Na_2CO_3 (3eq.) in water:dioxane (1:1) at room temperature for 4 h to afford 0.21 gm (0.5 mmol) of Fmoc-4-carboxyamidophenylalanine-OH.
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