

## Synthesis of tetrazole analogues of amino acids using Fmoc chemistry: isolation of amino free tetrazoles and their incorporation into peptides

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**Abstract**—An efficient synthesis of tetrazole analogues of amino acids starting from  $N^{\alpha}$ -Fmoc amino acid in a three-step protocol is reported. The free amino tetrazoles were obtained in good yields and with excellent purity after removal of the Fmoc group. The synthesis of analogues of aspartic and glutamic acids in which the 5-tetrazolyl moiety is inserted at the  $\beta/\gamma$  carboxyl group starting from Fmoc-Asn and Fmoc-Gln and the incorporation of these tetrazoles into peptides are also described.  
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Tetrazoles, being an interesting class of heterocycles, find a wide range of applications as drugs in pharmaceuticals,<sup>1</sup> cis-peptide bond mimics<sup>2</sup> and bioisosteres<sup>3</sup> for carboxylic acids. They are important precursors in medicinal chemistry owing to their increased resistance towards metabolic degradation pathways and have found increasing applications as catalysts<sup>4</sup> in asymmetric synthesis. Tetrazoles have also been utilized in organometallic chemistry as effective stabilizers of metalloprotein structures and as peptide chelating agents.<sup>5</sup>

The selective modification of amino acid chains in peptides and proteins is important in many areas of chemistry such as organic synthesis, the irreversible inhibition of enzymes and in the improvement of pharmacokinetic properties of peptide based drugs. Since, the acidity of the tetrazole group corresponds closely with that of a carboxylic acid, replacement of the C-terminal amino acid residue in biologically active peptides with a tetrazole analogue would create an interesting class of compounds for structure function relationship studies.

Indeed, isosteric displacement of the C-terminus carboxylic acid with a tetrazole often preserves or improves the biological activity of the parent peptides and their mimetics.

Conventionally, 5-substituted tetrazoles are synthesized<sup>6</sup> by [2+3] cycloaddition of an azide and a nitrile. However, disadvantages of these synthetic routes involve the use of expensive and toxic metals, strong Lewis acids and the in situ generation of hydrazoic acid which is highly toxic and explosive in nature. An alternative route to overcome some of these shortcomings was necessary. Sharpless et al.,<sup>7</sup> reported an efficient protocol for the synthesis of tetrazoles using sodium azide and zinc bromide in water/2-propanol mixture at reflux employing Z-protected amino acids as key synthons. Alternatively, tetrazole analogues of amino acids have been reported by Grzonka et al.,<sup>8</sup> from Z-protected amino nitriles using sodium azide and aluminium chloride in tetrahydrofuran at reflux. In continuation of our interest in incorporating a tetrazole heterocyclic moiety into amino acids and thereby peptides, we describe here our efforts to isolate amino free tetrazoles of  $\alpha$ -amino acids employing an Fmoc-protection strategy and their incorporation into peptides. Ley<sup>9</sup> recently reported the utility of free amine proline tetrazole as a catalyst in asymmetric synthesis. This catalyst was prepared after deprotection of the Z-group from Z-proline tetrazole

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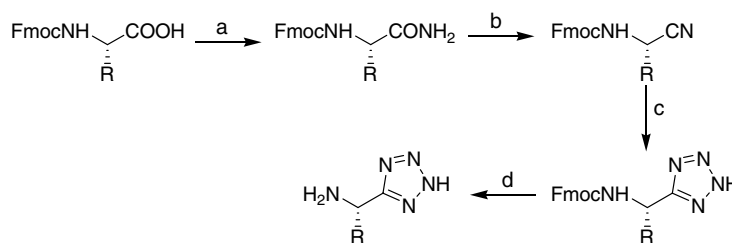
using a specifically designed H-cube continuous flow hydrogenator.<sup>10</sup> Although this process has led to complete removal of the Z-group in 4 h, the expensive nature of palladium, the special apparatus and the difficulties encountered in isolating the product are some of the limitations in scaling-up this protocol. In addition, our efforts to obtain amino free tetrazoles from Boc-Phe tetrazole using TFA were unsuccessful. In this context, the use of a Fmoc protection strategy was of particular interest since the Fmoc group can be easily removed under mild basic conditions in solution and also permits the use of Trt and *t*-butyl groups for side chain protection, which can also be cleaved under mild conditions.<sup>11</sup> We have envisaged a simple, four-step protocol for the synthesis of amino free tetrazole analogues of amino acids employing Fmoc chemistry in solution phase.

Pozdnev et al.<sup>12</sup> reported the synthesis of Boc/Z-amino acid amides including Fmoc-Phe and Leu using the di-*tert*-butyl pyrocarbonate [(Boc)<sub>2</sub>O]-pyridine system. A similar protocol with required modifications was employed for our synthesis of Fmoc-protected amino acid carboxamides (Scheme 1). Subsequent dehydration of the amides to the corresponding nitriles was achieved in excellent yields employing trifluoroacetic anhydride.<sup>13</sup> This reagent was best suited for our protocol since DCC mediated dehydration requires the use of pyridine as a solvent, a strategy that does not fully support Fmoc-chemistry. Another dehydrating reagent, cyanuric chloride was not used because the synthetic protocol was

aimed at keeping the  $\alpha$ -carboxyl group free in the case of side chain-derived tetrazole analogues. A characteristic peak around 2240 cm<sup>-1</sup> in the IR spectrum confirmed the presence of a nitrile group. All the Fmoc- $\alpha$ -amino nitriles prepared were obtained as crystalline solids. The methodology introduced by Sharpless et al.,<sup>7</sup> for the conversion of  $\alpha$ -amino nitriles to 5-substituted tetrazoles<sup>14</sup> was found to be applicable in the case of the Fmoc-amino nitriles described herein. The reaction proceeds by addition of sodium azide and a catalytic amount of zinc bromide to  $\alpha$ -amino nitriles in water/2-propanol (2:1) mixture at reflux for 16 h. A simple work-up involving acidification and extraction yielded the corresponding tetrazoles as pure solids in yields around 80–90%.

The Fmoc-group was deprotected using diethylamine in CH<sub>2</sub>Cl<sub>2</sub> in 30 min to yield  $\alpha$ -amino tetrazoles.<sup>15</sup> After evaporation of the solvent and trituration with ether, the compounds were isolated as pure solids (Table 1). The reaction sequence was found to be free from racemization<sup>16</sup> as determined by chiral HPLC of the methylated derivatives of the corresponding tetrazoles.

Further, the utility of Fmoc chemistry for inserting the tetrazole unit into the  $\beta/\gamma$  carboxylic position of aspartic and glutamic acids was demonstrated (Scheme 2). It was envisaged that the carboxamido group present in the side chains of asparagine and glutamine could be readily exploited for the incorporation of a tetrazole moiety in



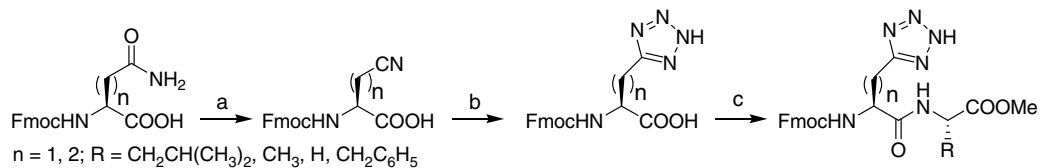
**Scheme 1.** Reagents and conditions: (a) (Boc)<sub>2</sub>O, pyridine, NH<sub>4</sub>HCO<sub>3</sub>, rt, 5 h, 70–80%; (b) trifluoroacetic anhydride–pyridine (1:1), THF, 0 °C, 3 h, 85–95%; (c) NaN<sub>3</sub>, ZnBr<sub>2</sub>, water/2-propanol (2:1), 80 °C, 16 h, 80–90%; (d) diethylamine, CH<sub>2</sub>Cl<sub>2</sub>, 30 min, 80–94%.

**Table 1.** *N*<sup>2</sup>-protected tetrazole analogues of amino acids and amine free tetrazoles

Entry	Fmoc-amino tetrazole <sup>a</sup>	Yield (%)	Mp (°C)	Mass <sup>b</sup> (obs/calc)	Free amino tetrazole <sup>a</sup>	Yield (%)	Mp (°C)	Mass <sup>b</sup> (obs/calc)
1	Fmoc-GlyT	89	181	321.3312 (321.3333)	GlyT	93	274	99.0932 (99.0946)
2	Fmoc-AlaT	91	189	335.3579 (335.3598)	AlaT	90	257	113.1237 (113.1211)
3	Fmoc-ValT	85	207	363.4147 (363.4130)	ValT	88	273	141.1720 (141.1743)
4	Fmoc-LeuT	86	202	377.4379 (377.4396)	LeuT	89	262	155.2023 (155.2009)
5	Fmoc-IleT	88	219	377.4380 (377.4396)	IleT	90	269	155.2001 (155.2009)
6	Fmoc-PheT	90	204	411.4526 (411.4558)	PheT	94	278	189.2143 (189.2171)
7	Fmoc-Ser (OBz)T	82	223	441.4832 (441.4818)	Ser(OBz)T	85	243	219.2405 (219.2431)
8	Fmoc- <i>D</i> -PhgT	84	211	397.4275 (397.4292)	<i>D</i> -PhgT	87	181	175.1933 (175.1905)
9	Fmoc- <i>L</i> -PhgT	82	186	397.4282 (397.4292)	<i>L</i> -PhgT	85	202	175.1919 (175.1905)
10	Fmoc-MetT	89	193	395.4764 (395.4780)	MetT	87	233	173.2364 (173.2393)
11	Fmoc-ProT	81	180	361.3949 (361.3971)	ProT	83	249	139.1559 (139.1584)
12	Fmoc-Asp (OBz)T	79	227	469.4902 (469.4919)	Asp(OBz)T	81	227	247.2511 (247.2532)
13	Fmoc-Glu (OBz)T	81	232	483.5164 (483.5185)	Glu(OBz)T	84	178	261.2768 (261.2798)

<sup>a</sup> T corresponds to Tetrazole.

<sup>b</sup> High resolution mass spectrometry (HRMS).



**Scheme 2.** Reagents and conditions: (a) Trifluoroacetic anhydride–pyridine (1:1), THF, 0 °C, 6 h, 70–80%; (b)  $\text{NaN}_3$ ,  $\text{ZnBr}_2$ , water/2-propanol (2:1), 80 °C, 16 h, 65–75%; (c) HBTU, DIEA, MeOH, DCM, 0 °C  $\rightarrow$  rt, 5 h, 80–90%.

**Table 2.** Synthesis of side chain derived tetrazole analogues of aspartic and glutamic acids and peptides

Entry	Compound	Yield (%)	Mp (°C)	Mass <sup>a</sup> (obs/calc)
1	Fmoc-Asp(T)-OH	76	202	379.3719 (379.3734)
2	Fmoc-Glu(T)-OH	79	190	393.3991 (393.4008)
3	Fmoc-Asp(T)-Leu-OCH <sub>3</sub>	66	163	506.5563 (506.5581)
4	Fmoc-Asp(T)-Ala-OCH <sub>3</sub>	71	213	464.4765 (464.4789)
5	Fmoc-Asp(T)-Gly-OCH <sub>3</sub>	74	178	450.4301 (450.4513)
6	Fmoc-Asp(T)-Phe-OCH <sub>3</sub>	69	185	540.5726 (540.5758)
7	Fmoc-Glu(T)-Leu-OCH <sub>3</sub>	71	157	520.5812 (520.5855)
8	Fmoc-Glu(T)-Ala-OCH <sub>3</sub>	73	192	478.5023 (478.5058)
9	Fmoc-Glu(T)-Gly-OCH <sub>3</sub>	76	183	464.4741 (464.4785)

<sup>a</sup> High resolution mass spectrometry (HRMS).

the side chains. Although, initially we had started our synthesis using Fmoc-Asn-OMe and Fmoc-Gln-OMe, it was found that the entire protocol could be carried out starting from Fmoc-Asn/Gln.

A two-step protocol including conversion of a carbox-amido group to a nitrile followed by cycloaddition resulted in Fmoc-Asp(T)-OH and Fmoc-Glu(T)-OH in 76% and 79% yields, respectively, which were fully characterized by <sup>1</sup>H NMR, <sup>13</sup>C NMR and mass spectral studies (Table 2). The tetrazolyl acids were employed as building blocks to prepare peptides possessing a tetrazole unit in the side chain. Fmoc-Asp(T)-OH or Fmoc-Glu(T)-OH was coupled with amino acid methyl ester hydrochlorides using the standard HBTU and DIEA methods. The coupling was carried out at 0 °C in DCM and the reaction mixture was allowed to warm to room temperature over 5 h. After work-up, the products were isolated as solids in yields around 80–90%.

In conclusion, tetrazole analogues of amino acids/peptides have been synthesized through an Fmoc-amino protection strategy. The use of Fmoc for amine protection facilitated the easy isolation of intermediate nitriles and final tetrazoles. Amino free tetrazoles were obtained by deprotection of the Fmoc group under mild reaction conditions. The tetrazole-containing unnatural amino acids isosteric to Asp/Glu have been similarly synthesized from Fmoc-Asn/Gln and utilized in the synthesis of peptides. In all cases, the final tetrazole products were isolated in high yields and purities.

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14. *General procedure for the synthesis of tetrazole analogues of N<sup>α</sup>-Fmoc-amino acids*: N<sup>α</sup>-Fmoc-amino nitrile (1 mmol), sodium azide (2 mmol) and zinc bromide (0.5 mmol) were dissolved in a mixture of 2-propanol (15 mL) and water (30 mL), and stirred at reflux for 16 h. After completion of the reaction [as monitored by TLC, using chloroform:methanol (9:1) as eluant], 5 mL of 3 N HCl and 30 mL of ethyl acetate were added and stirring was continued until no solid remained. The aqueous layer was extracted twice with ethyl acetate. The combined organic layer was washed with water and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The solvent was removed in vacuo and the residue was recrystallized from EtOAc/hexane (1:4).  
*Compound characterization data for Fmoc-ValT* (Table 1, entry 3): IR (KBr) 1692, 1533, 1263 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 0.91 (t, *J* = 6.8 Hz, 6H), 1.06 (m, 1H), 4.20 (t, *J* = 6.6 Hz, 1H), 4.54 (d, *J* = 6.6 Hz, 2H), 5.07 (br d, *J* = 5.8 Hz, 2H), 7.24–7.78 (m, 8H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz): δ 19.1, 26.3, 37.2, 39.1, 45.7, 48.5, 66.2, 120.4, 124.6, 126.4, 127.1, 140.9, 148.6, 154.5.  
*Fmoc-LeuT* (Table 1, entry 4): IR (KBr) 1693, 1538, 1264 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 0.94–0.96 (t, *J* = 6.8 Hz, 6H), 1.67 (m, 1H), 1.97 (t, *J* = 8.4 Hz, 2H), 4.18 (t, *J* = 5.9 Hz, 1H), 4.52 (d, *J* = 5.9 Hz, 1H), 5.13 (d, *J* = 6 Hz, 2H), 5.40 (br d, *J* = 5.9 Hz, 1H), 7.22–7.79 (m, 8H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz): δ 21.9, 24.1, 36.5, 39.5, 40.6, 46.6, 65.6, 120.0, 125.1, 126.9, 127.5, 140.7, 143.6, 143.8, 155.8.
15. *General procedure for deprotection of the Fmoc group*: To a solution of N<sup>α</sup>-Fmoc-protected amino tetrazole (1 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> was added diethylamine (2 mL) and the mixture was stirred at room temperature for 30 min. After completion of the reaction (as monitored by TLC), the solvent was removed in vacuo. The crude product was recrystallized from EtOAc/hexane (1:4) to obtain the free α-amino tetrazole as a solid.  
*Compound characterization data for: Alanine tetrazole* (Table 1, entry 2): IR (KBr) 1535, 1442, 1266 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 1.49 (d, *J* = 6.1 Hz, 3H), 4.15 (m, 1H), 4.93 (br d, *J* = 6.6 Hz, 2H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz): δ 21.5, 46.6, 151.3.  
*Leucine tetrazole* (Table 1, entry 4): IR (KBr) 1532, 1444, 1263 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 0.86–0.88 (d, *J* = 4.8 Hz, 6H), 1.65 (m, 1H), 1.97 (t, *J* = 5.8 Hz, 2H), 2.81 (br d, *J* = 6.6 Hz, 2H), 3.72 (q, *J* = 6.2 Hz, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz): δ 23.8, 24.2, 42.5, 46.1, 150.3.
16. *Racemization studies*: The enantiomeric purities of the synthesized tetrazoles were preserved appreciably following the present method. The reaction sequence yields tetrazoles with ee's exceeding 80% as determined by chiral HPLC studies of the corresponding methylated derivatives.