

Synthesis of tetrazole analogues of amino acids: The Fmoc approach

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Introduction

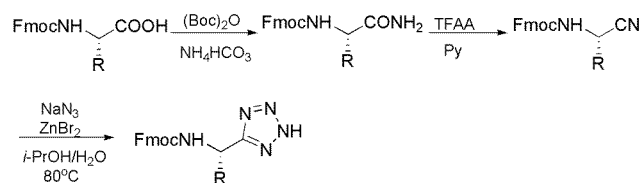
The chemistry of heterocycles has acquired immense importance in recent years. Tetrazoles represent an important class of heterocycles which exhibit a wide range of applications in medicinal as well as synthetic chemistry. They are used as *cis*-peptide bond mimics, drugs in pharmaceuticals and bioisosteres for carboxylic acids [1]. Since the acidity of tetrazole group corresponds closely with that of carboxylic acid, replacement of C-terminal amino acid residue with a tetrazole analogue often preserves or improves the biological activity of parent peptides. Alzheimer's β -secretase (BACE1) inhibitor [2] KMI-420 and its α -isomer are potent drug molecules that possess tetrazole sub-units. They are also employed as catalysts in asymmetric synthesis, peptide chelating agents and metalloprotein stabilizers. Proline-derived tetrazole is a powerful enantioselective catalyst used in conjugate addition reactions, asymmetric aldol, mannich reactions and multicomponent reactions. Tetrazoles exist as 1H and 2H tautomers of which the latter is found to be more stable.

The conventional synthesis of 5-substituted tetrazoles involves a [2+3] cycloaddition of an azide and a nitrile. But the major drawbacks of this approach are the *in situ* generation of hydrazoic acid which is highly toxic and explosive, use of expensive and toxic metals, etc. An alternate methodology to overcome these shortcomings was developed for the first time by Sharpless et al., [3] who reported a modified and efficient protocol by reacting alkyl and acyl nitriles with sodium azide in presence of zinc bromide in water/2-propanol mixture. The protocol was extended to peptide chemistry through the synthesis of tetrazole analogues using Z-chemistry.

Recently, free amino proline tetrazole has been used as an effective catalyst for asymmetric synthesis. Its preparation from Z-proline tetrazole is tedious requiring longer reaction time and use of expensive palladium for the Z-cleavage. The scale up process demands the use of special apparatus like H-Cube continuous flow hydrogenator [4] for hydrogenation reactions. In this context, the use of Fmoc-protection strategy attracted our attention since the properties of Fmoc group are ideally suited for simple and large scale synthesis of tetrazoles. The Fmoc group can be easily removed under mild basic conditions and also permits the use of routinely employed acid-labile protecting groups for side chain protection. We recently reported a simple four-step protocol for the synthesis of amino free tetrazole analogues of amino acids [5] employing Fmoc chemistry in solution phase.

Results and Discussion

As the first step, the Fmoc-amino acids were converted to their carboxamides using [(Boc₂O)-NH₄HCO₃] system [6]. The amides were then dehydrated to nitriles using trifluoroacetic anhydride and pyridine. A distinct IR peak at around 2240 cm⁻¹ confirmed the conversion into nitrile. The Fmoc-amino nitriles, isolated in high yields, were subjected to [2+3] cycloaddition (Scheme 1) with sodium azide in presence of catalytic amount of zinc bromide in water/2-propanol (2:1) mixture at reflux for 16 h. After acidification and extraction, the corresponding tetrazoles were obtained as pure solids in yields around 80-90% (Table 1).



Scheme 1. Synthesis of N^α-Fmoc-amino tetrazoles

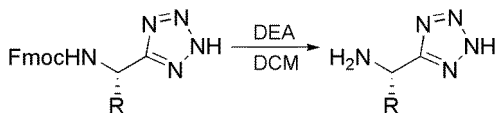
Table 1. N^α-Fmoc protected tetrazole analogues of amino acids

Fmoc-amino tetrazole ^a	Yield (%)	Mp (°C)	Mass ^b obtained
Fmoc-GlyT	89	181	321.3312
Fmoc-AlaT	91	189	335.3579
Fmoc-ValT	85	207	363.4147
Fmoc-LeuT	86	202	377.4379
Fmoc-IleT	88	219	377.4380
Fmoc-PheT	90	204	411.4526
Fmoc-Ser(OBz)T	82	223	441.4832
Fmoc-D-PhgT	84	211	397.4275
Fmoc-L-PhgT	82	186	397.4282
Fmoc-MetT	89	193	395.4764
Fmoc-ProT	81	180	361.3949
Fmoc-Asp(OBz)T	79	227	469.4902
Fmoc-Glu(OBz)T	81	232	483.5164

^a T corresponds to Tetrazole

^b HRMS

The Fmoc-group was deprotected using diethylamine in CH_2Cl_2 in 30 min in order to obtain free α -amino tetrazoles [6] (Scheme 2). The required compounds were easily isolable as stable solids with a simple precipitation using ether. The entire reaction sequence was found to be free from racemization as determined by chiral HPLC of the methylated derivatives of the corresponding tetrazoles.



Scheme 2. Synthesis of free amino tetrazoles

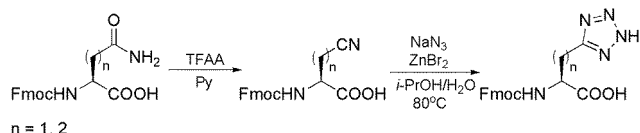
Table 2. List of free amino tetrazoles

Free amino tetrazole ^a	Yield (%)	Mp (°C)	Mass ^b obtained
GlyT	93	274	99.0932
AlaT	90	257	113.1237
ValT	88	273	141.1720
LeuT	89	262	155.2023
IleT	90	269	155.2001
PheT	94	278	189.2143
Ser(OBz)T	85	243	219.2405
D-PhgT	87	181	175.1933
L-PhgT	85	202	175.1919
MetT	87	233	173.2364
ProT	83	249	139.1559
Asp(OBz)T	81	227	247.2511
Glu(OBz)T	84	178	261.2768

^a T corresponds to Tetrazole

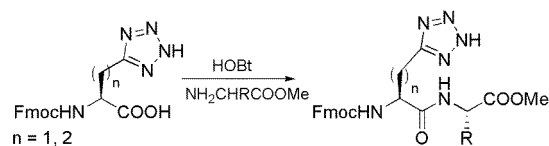
^b HRMS

The insertion of tetrazole unit at the β/γ -carboxylic position of aspartic and glutamic acids was undertaken. The carboxamide in the side chains of Asn and Gln presents a readily transformable group to incorporate tetrazole moiety following the above described simple reaction sequence.



Scheme 3. Synthesis of tetrazole containing unnatural amino acids

A similar protocol involving the conversion of carboxamide group to nitrile and its subsequent cycloaddition with sodium azide resulted in the Fmoc-protected side chain tetrazole containing unnatural amino acids (Fmoc-Asp(T)-OH and Fmoc-Glu(T)-OH) in an excellent overall yield (Scheme 3).



Scheme 4. Synthesis of peptides

These tetrazole containing acids were further incorporated into peptides (Scheme 4) by coupling them with amino acid methyl ester hydrochlorides using the standard HBTU and DIEA method. A simple work-up produced tetrazole containing peptides (Table 3) in yields of 66-76%.

Table 3. List of unnatural amino acids and peptides

Tetrazole analogues of Asp, Glu and peptides ^a	Yield (%)	Mp (°C)	Mass ^b obtained
Fmoc-Asp(T)-OH	76	202	379.3719
Fmoc-Glu(T)-OH	79	190	393.3991
Fmoc-Asp(T)-Leu-OMe	66	163	506.5563
Fmoc-Asp(T)-Ala-OMe	71	213	464.4765
Fmoc-Asp(T)-Gly-OMe	74	178	450.4301
Fmoc-Asp(T)-Phe-OMe	69	185	540.5726
Fmoc-Glu(T)-Leu-OMe	71	157	520.5812
Fmoc-Glu(T)-Ala-OMe	73	192	478.5023
Fmoc-Glu(T)-Gly-OMe	76	183	464.4741

^a T corresponds to Tetrazole, ^b HRMS

In summary, the tetrazole analogues of amino acids have been efficiently synthesized using Fmoc-protection strategy. The Fmoc protection enables easy isolation of intermediates and final amino free tetrazoles. Unnatural amino acids isosteric to Asp/Glu have also been synthesized by incorporating tetrazoles in the side chains and used as building blocks for peptide synthesis. The Fmoc-approach provides a simple, practical and scalable way to tetrazole synthesis.

Acknowledgements

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