Peptidotriazoles on Solid Phase: [1,2,3]-Triazoles by Regiospecific Copper(I)-Catalyzed 1,3-Dipolar Cycloadditions of Terminal Alkynes to Azides

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The cycloaddition of azides to alkynes is one of the most important synthetic routes to 1H-[1,2,3]-triazoles. Here a novel regiospecific copper(I)-catalyzed 1,3-dipolar cycloaddition of terminal alkynes to azides on solid-phase is reported. Primary, secondary, and tertiary alkyl azides, aryl azides, and an azido sugar were used successfully in the copper(I)-catalyzed cycloaddition producing diversely 1,4-substituted [1,2,3]triazoles in peptide backbones or side chains. The reaction conditions were fully compatible with solid-phase peptide synthesis on polar supports. The copper(I) catalysis is mild and efficient (> 95% conversion and purity in most cases) and furthermore, the X-ray structure of 2-azido-2-methylpropanoic acid has been solved, to yield structural information on the 1,3-dipoles entering the reaction. Novel Fmoc-protected amino azides derived from Fmoc-amino alcohols were prepared by the Mitsunobu reaction.

Introduction

N-Heterocyclic compounds are broadly distributed in Nature, including amino acids, purines, pyrimidines, and many other natural products. N-Heterocyclic compounds such as [1,2,3]triazoles may display biological activities and there are numerous examples in the literature including anti-HIV activity, antimicrobial activity against Gram positive bacteria, selective β3-adrnergic receptor agonism, and more. [1,2,3]-Triazoles have also found wide use in industrial applications such as dyes, corrosion inhibition (of copper and copper alloys), photostabilizers, photographic materials, and agrochemicals. Therefore, it is important to develop new and more efficient solid-phase synthetic pathways to a diverse array of [1,2,3]-triazole pharmcophores and screen many analogues against relevant biological targets, possibly while attached to the solid support. The presented method is compatible with solid-phase combinatorial chemistry, so it is possible to make millions of compounds simultaneously using the split and combine method and screen the compounds on the solid phase.

Results and Discussion

Synthesis of [1,2,3]-Triazoles. Several different methods have been described for synthesis of [1,2,3]-triazoles, including the intramolecular cyclization of bishydrazones or mixed hydrazones, miscellaneous oxidations, as well as the 1,3-dipolar cycloaddition of azides to alkynes. The cycloaddition between azides and alkynes is typically carried out in refluxing toluene, but labile molecules may not survive these conditions. However, by using sodium, lithium, or magnesium salts of the alkyne, lower temperatures have been employed but often with limited or no success. L’abber reported the in situ generation of a propargyl azide by displacement of a sulfonate with lithium azide and copper(I) chloride. Instead of the expected product, an alkyl-substituted [1,2,3]triazole byproduct was isolated in low yield. This side reaction was not investigated further. One communication with limited scope and experimental details described the solid-phase synthesis of [1,2,3]triazoles by a diazo transfer reaction with tosyl azide. The present investigations...

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Scheme 1. Copper(I)-Catalyzed 1,3-Dipolar Cycloaddition of Alkynes to Azides Affording Peptidotriazoles or N-Substituted Histidine Analogs

Scheme 2. Copper(I)-Catalyzed [1,2,3]-Triazole Formation from 1 and Further Reactions to Yield Peptidotriazoles

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1) did not react even at elevated temperatures and prolonged reaction time. However, it has previously been reported that reactions with this azido group are much more difficult than with most other azido acids, supporting an argument of steric hindrance. 16,17 1,3-Dipolar cycloadditions with resin 7 (propargylic acid, Scheme 3) showed high conversions (>95%) but slightly lower purities (entry 8a-d, Table 1) than with resin 1 (propargylic acid, entry 3a-s, Table 1). Electron-deficient alkynes are more reactive in cycloaddition reactions, which explains why propargylic acid (resin 1) displayed slightly higher purities than proparglylglycine (resin 7) did. Furthermore, two peptidotriazoles were prepared in larger amount (26–42 mmol) for full characterization by 1H and 13C NMR as well as HR-MS. Compounds 9 and 10 were isolated in 79% and 87% yield (eight reaction steps; see Scheme 4), respectively. The reaction conditions for the catalyzed cycloaddition, N-ethylidioisopropylamine and copper(I) iodide at 25 °C, are mild and fully compatible with Fmoc- and Boc-peptide chemistry. Free amino groups, carboxylic acids, thioglycosides, and Fmoc, tert-butyl, trityl, Boc, and Pmc groups were found to be completely stable under the reaction conditions.

Five Fmoc-protected amino alcohols [Fmoc-Arg(Pmc)-ol, Fmoc-Asp(tBu)-ol, Fmoc-Gly-ol, Fmoc-Met-ol, and Fmoc-Phe-ol] were subjected to Mitsunobu conditions with HN3 to convert them into Fmoc-protected amino azides that could be used directly in the cycloaddition reaction. Only the small nucleophile hydrazoic acid successfully converted the Fmoc-amino alcohols into their corresponding azides (48–98% yield), whereas TMS–N3,
Table 1. Puritya of the [1,2,3]-Triazoles Formed from Resin 1 and 7 in Schemes 2 and 3

<table>
<thead>
<tr>
<th>R</th>
<th>% puritya</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>C(CH3)2CO2H (no Cul)</td>
</tr>
<tr>
<td>3b</td>
<td>C(CH3)2COH</td>
</tr>
<tr>
<td>3c</td>
<td>C(CH3)(CH2)CO2H</td>
</tr>
<tr>
<td>3d</td>
<td>C(CH3)2CO2H</td>
</tr>
<tr>
<td>3e</td>
<td>(C-n-C3H7)2CO2H</td>
</tr>
<tr>
<td>3f</td>
<td>C(Ph)HCO2H</td>
</tr>
<tr>
<td>3g</td>
<td>CH2CO2H</td>
</tr>
<tr>
<td>3h</td>
<td>CH(n-C3H7)CO2H</td>
</tr>
<tr>
<td>3i</td>
<td>CH(n-C3H7)2CO2H</td>
</tr>
<tr>
<td>3j</td>
<td>CH(Ph)CO2H</td>
</tr>
<tr>
<td>3k</td>
<td>4-C6H4NH2</td>
</tr>
<tr>
<td>3l</td>
<td>2-amino-5-guanidinopyridinebc</td>
</tr>
<tr>
<td>3m</td>
<td>CH2CH(CH2CO2H)NH2c</td>
</tr>
<tr>
<td>3n</td>
<td>(C-n-C3H7)2CO2H</td>
</tr>
<tr>
<td>3o</td>
<td>CH2CH(CH2CH3)2NH2</td>
</tr>
<tr>
<td>3p</td>
<td>CH2CH(Ph)NH2c</td>
</tr>
<tr>
<td>3q</td>
<td>1-adamantyl</td>
</tr>
<tr>
<td>3r</td>
<td>2-(2-deoxy)-Gal-SPh</td>
</tr>
<tr>
<td>3s</td>
<td>H3</td>
</tr>
<tr>
<td>3a</td>
<td>C(CH3)2CO2H</td>
</tr>
<tr>
<td>3b</td>
<td>2-(2-deoxy)-Gal-SPh</td>
</tr>
<tr>
<td>3c</td>
<td>4-C6H4NH2</td>
</tr>
<tr>
<td>3d</td>
<td>CH2CH(Ph)NH-Fmocb</td>
</tr>
<tr>
<td>3e</td>
<td>H3</td>
</tr>
</tbody>
</table>

a Conversion was >95% except for 3a, 3f, and 8e, and the reported purities are from analytical HPLC traces (215 nm).

*Regiospecificity.* Thermal 1,3-dipolar cycloaddition of alkenes to azides is not a regiospecific reaction.9 This could be advantageous if both regioisomers were desired but would be considered a disadvantage in preparative work. Fmoc-Phe-[[CH2N3] (19) and resin 1 gave two isomers under thermal conditions (reflux in toluene), 13a and 13b in a 2:1 ratio (analytical HPLC and 1H MAS NMR of the two regioisomers from the thermal cycloaddition are presented in Figure 1). The analogous copper(I)-catalyzed reaction gave only one regioisomer, the 1,4-substituted [1,2,3]-triazole (entry 3p, Table 1). All the other azides, primary, secondary, and tertiary alkyl azides, aryl azides, Fmoc-protected amino azides, and azido sugars proved that the catalysis was generally regiospecific in forming only the 1,4-substituted [1,2,3]-triazole. The triazole proton in 13a was found at 8.50 ppm, whereas in 13b it was shifted upfield to 8.23 ppm (Figure 1). In a similar 1,4- and 1,5-substituted [1,2,3]-triazole system, it was concluded that the triazole proton in 1,4-substituted triazoles was always shifted considerably downfield compared to 1,5-substituted triazoles.22 This supports the evidence that the copper(I)-catalyzed reaction only gives the 1,4-substituted triazole 13a in Figure 1 and is in full agreement with HPLC data from coinjection of reaction mixtures from the thermal and the copper(I)-catalyzed 1,3-dipolar cycloaddition. Furthermore, strong NOE effects have been observed between the triazole proton and the N-substituted alkyl group in 9 and 10 (Figure 2), suggesting that the triazole proton and N-substituent are in close proximity as in the 1,4-substituted triazole.

In contrast, the uncatalyzed thermal reaction of 2-azido-2-methylpropanoic acid (a tertiary alkyl azide) with resin 1 afforded only one regioisomer, the 1,4-substituted triazole, probably due to steric effects. This was substantiated by the X-ray crystal structure of 2-azido-2-methylpropanoic acid (Figure 3), where the two methyl groups and the carboxyl group effectively shield one side of the azido group, thereby blocking the cycloaddition to yield the sterically more crowded 1,5-substituted triazole.

*Compatibility.* To test the generality of the copper(I)-catalyzed reaction, 13 representative protected tripeptides acylated with propargylic acid at the N-terminus were synthesized and subjected to the reaction conditions for the copper(I)-catalyzed 1,3-dipolar cycloaddition with 2-azido-2-methylpropanoic acid (Scheme 5). Alanine, proline, tert-butyl-protected threonine/tyrosine/aspartic acid, trityl-protected asparagine/histidine/cysteine, me-
thionine, Boc-protected lysine/tryptophan, and Pmc-protected arginine were used and all showed conversions above 95% and 80–95% purity of the resulting peptidotriazoles (12a–l, Table 2). The following functional groups were included: thioethers; esters; amides; ethers; Fmoc, Boc, tert-butyl, trityl, and Pmc groups; and oxidation sensitive residues such as methionine, tryptophan, and cysteine.

Since all peptides gave the expected products without side reactions, the copper(I)-catalyzed 1,3-dipolar cycloaddition was fully compatible with solid-phase peptide synthesis. All reactions have been carried out on PEGA800 resin,23 a hydrophilic tertiary amide–poly(ethylene glycol) based resin, but the reaction conditions were also tested on SPOCC1500,24 a completely inert resin with only primary ether bonds, and it performed equally well (data not shown).

**Solid/Solution Phase.** Both solution- and solid-phase chemistry have their respective advantages and disadvantages. In the case of the copper(I)-catalyzed 1,3-dipolar cycloaddition, the solution-phase reaction is complicated by cross-coupling products between two terminal alkynes such as the Glaser coupling and Straus coupling.25 Furthermore, PEGA resin acylated with 2-azido-2-methylpropionic acid was subjected to the reaction conditions with the modification that the reactants were inversely immobilized, i.e., the terminal alkyne in solution and the azide on the resin. Prolonged reaction time, elevated temperature, and a large excess of alkyne gave only starting material because of alkyne cross-coupling. The advantage of solid-phase reactions is the highly solvated state of the PEG-resin-bound intermediates such as the copper acetylide and that cross-couplings do not occur, thereby allowing the copper(I)-catalyzed reaction to proceed smoothly when the alkyne is attached to the resin.

**Perspective.** The [1,2,3]-triazole can be viewed as a peptide isoster that, when incorporated into a peptide, is displaying hydrogen-bonding capability, aromaticity, and backbone restriction. Compounds 4a and 4b (Scheme 4).

![Figure 1. Analytical HPLC profile of 1,4- and 1,5-substituted [1,2,3]-triazole (13a and 13b) and 1H MAS NMR of the two regiosomers' amide protons and triazole protons (singlet signals at 8.50 and 8.23 ppm).](image1)

![Figure 2. 2D NOESY spectra of 9 and 10. The NOEs marked with arrows prove the regiospecific 1,4-substitution of the [1,2,3]-triazoles.](image2)

*Scheme 4. Synthesis of Peptidotriazoles 9 and 10*

(i) 2-Azido-2-methylpropanoic acid, DIPEA, CuI; (ii) 1-azidoadamantane, DIPEA, CuI; (iii) 0.1 M NaOH (aq).

Conclusion. The described copper(I)-catalyzed 1,3-dipolar cycloaddition of terminal alkenes to azides gave access to one specific regiosomer, the 1,4-substituted [1,2,3]-triazole, worked excellently on solid support (~95% conversion and purity at 25 °C in most cases), and was fully compatible with solid-phase peptide synthesis, all of the amino acids, and their protecting groups. Cross-coupling reactions in solution were not a problem on solid support, because of the highly solvated PEG-resin-bound copper acetylide. A diverse set of 1,4-substituted [1,2,3]-triazoles have been prepared, and synthesis of large libraries is in progress. The X-ray structure of 2-azido-2-methylpropanoic acid has been solved and illustrates the steric environment in α,α-disubstituted azido acids.

Experimental Section

4H and 13C NMR spectra were recorded on a Bruker DRX250 (250 MHz) and MAS NMR spectra on a Varian Unity Inova 500 MHz spectrometer equipped with a 4 mm 4H-observe Nano NMR-probe. Electrospray mass spectrometry was performed in the positive mode on a Fisons VG Quattro instrument. Analytical and preparative reverse-phase HPLC separations were performed on a Waters HPLC system using analytical Zorbax 300SB-C18 (4.5 × 50 mm) and Delta PAK (47 × 300 mm) C18 columns with a flow rate of 1 and 20 cm3 min⁻¹, respectively. Detection was at 215 nm on a multilength detector (Waters 490E) for analytical purposes, and a photodiode array detector (Waters M991) was used for preparative separations. A solvent system consisting of (A) 0.1% TFA in water (B) 0.1% TFA in 90% acetonitrile–10% water was used. 1R spectra were recorded on a Perkin-Elmer 1600 FTIR instrument as neat liquids or as KBr pellets. Optical rotations were measured on a Perkin-Elmer 241 polarimeter at 25 °C. Melting points were determined with a Büchi B-540 apparatus and were uncorrected.

General Procedures. Coupling of Fmoc-amino acid-OPfp-esters to amino groups was performed with 3 equiv Fmoc-AA-OPfp and 1 equiv D Hind-OH in DMF. Fmoc deprotection was effected with 20% piperidine in DMF for 2 + 18 min followed by washing of the resin six times with DMF. The resin was washed with six times with the appropriate solvent between each reaction step. Amino acid couplings were followed by the Kaiser test. Two equivalents of copper(I) iodide was used for practical reasons because of the small-scale reactions (ca. 5 mg of resin), but lower stoichiometry can be used (0.01 equiv dissolved in pyridine).

Analysis. Cleavage of the peptide for analytical purposes was effected with 0.1 M NaOH (aq) for 2 h in a small Eppendorf tube followed by neutralization with 0.1 M HCl (aq) and centrifugation. The supernatant was analyzed by analytical HPLC and collected fractions by ESI-MS. Preparation of the α-azido acids used in the experimental work has been described previously. Fmoc-Gly-ol and phenyl-3,4,6-tri-O-acetyl-2-azido-2-deoxy-1-thio-α-D-galactopyranoside were kindly provided by Dr. Jürgen Beyer and Dr. Shiro Komba, respectively. All amino acids were L-amino acids.

Abbreviations: 

DHbt-OH, 595–596

DHbt-OH, 595–596


Table 2. Purity of Peptidotriazoles with Protected Amino Acids from Scheme 5

<table>
<thead>
<tr>
<th>Xxx</th>
<th>% purity</th>
<th>Xxx</th>
<th>% purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala</td>
<td>95</td>
<td>His(Trt)</td>
<td>80</td>
</tr>
<tr>
<td>Pro</td>
<td>95</td>
<td>Cys(Trt)</td>
<td>81</td>
</tr>
<tr>
<td>Thr(Bu)</td>
<td>95</td>
<td>Met</td>
<td>85</td>
</tr>
<tr>
<td>Tyr(Bu)</td>
<td>95</td>
<td>Lys(Boc)</td>
<td>95</td>
</tr>
<tr>
<td>Asp(Bu)</td>
<td>95</td>
<td>Trp(Boc)</td>
<td>95</td>
</tr>
<tr>
<td>Asn(Trt)</td>
<td>90</td>
<td>Arg(Pmc)</td>
<td>98</td>
</tr>
</tbody>
</table>

Conversion was >95% in all cases, and the reported purities are from analytical HPLC traces (215 nm).

34, 36, 38

34, 36, 38

References:


fluorophosphate. One- and three-letter codes are used for the amino acids according to IUPAC rules.

**Propynoyl-Phe-Gly-Phe-HMBAPEGA**

Methanaminium tetrafluoroborate, 1,2,3-triazole-4-carboxylic acid. HPLC: \( t_{R} = 14.1 \) and 14.3 min (the starting material was racemic). ESI-MS: \( \text{calc} (M^+ = C_{12}H_{11}N_{2}O_{4}) \), 656.2 Da; found (\( MH^+ \)), 656.3 for both peaks.

**Propynoyl-Phe-Gly-Phe-HMBAPEGA**

Methanaminium tetrafluoroborate, 2-azido-2-phenylethanoic acid. HPLC: \( t_{R} = 14.1 \) and 14.3 min (the starting material was racemic). ESI-MS: \( \text{calc} (M^+ = C_{12}H_{11}N_{2}O_{4}) \), 656.2 Da; found (\( MH^+ \)), 656.3 for both peaks.

**Propynoyl-Phe-Gly-Phe-HMBAPEGA**

Methanaminium tetrafluoroborate, 1,2,3-triazole-4-carboxylic acid. HPLC: \( t_{R} = 14.1 \) and 14.3 min (the starting material was racemic). ESI-MS: \( \text{calc} (M^+ = C_{12}H_{11}N_{2}O_{4}) \), 656.2 Da; found (\( MH^+ \)), 656.3 for both peaks.
and NEM (4 equiv) were added to the resin after 5 min of preactivation in DMF. The resin was washed with DMF and lyophilized. A sample of the resin was Fmoc-deprotected, cleaved, and analyzed by HPLC and MS. HPLC: \( t_{R} = 11.0 \) min, ESI-MS: calcd (M+Na\(^+\) = C\(_{30}\)H\(_{34}\)N\(_{4}\)O\(_{4}\)Na\(^+\)), 522.2 Da; found (M+Na\(^+\)), m/z 522.4.

**General Procedure to N-Substituted Histidine Analogues** (8a-e) (50 equiv), C\(_{2}\) (2 equiv), and 50 (2 equiv) were added to resin \( 7 \) (0.41 mmol/g, 5 mg of resin, ca. 2 \( \mu \)mol swollen in 200 \( \mu \)L of THF). Each reaction was left for 16 h and then washed with THF, water, and DMF. Fmoc deprotection was effected, a sample of each resin was cleaved, and the product was analyzed by HPLC and MS.

(8a) Phe-Gly-Phe-Gly-OH (8a).

(8b) Peptidotriazoles on Solid Phase and the product was analyzed by HPLC and MS.

**Deprotection** was effected, a sample of each resin was cleaved, and the product was analyzed by HPLC and MS.

**Deprotection** was effected, a sample of each resin was cleaved, and the product was analyzed by HPLC and MS.

(8c) Phe-Gly-Phe-Gly-OH (8c).

(8d) Peptidotriazoles on Solid Phase and the product was analyzed by HPLC and MS.

(8e) Phe-Gly-Phe-Gly-OH (8e).

**General Procedure to 1-(1-Carboxy-1-methylthyl)-1H-[1,2,3]-triazol-4-yl]propionyl-Phe-Gly-Phe-Gly-OH (8a).** R \( \equiv \) N\(_\equiv\) 2-azido-2-methylpropionic acid. HPLC: \( t_{R} = 11.3 \) min. ESI-MS: calcd (M+Na\(^+\) = C\(_{30}\)H\(_{34}\)N\(_{4}\)O\(_{4}\)Na\(^+\)), 651.3 Da; found (M+Na\(^+\)), m/z 651.5.

(8f) 1-(1-Carboxy-1-methylthyl)-1H-[1,2,3]-triazol-4-yl]propionyl-Phe-Gly-Phe-Gly-OH (8h).

**Deprotection** was effected, a sample of each resin was cleaved, and the product was analyzed by HPLC and MS.

(8g) 1-(1-Carboxy-1-methylthyl)-1H-[1,2,3]-triazol-4-yl]propionyl-Phe-Gly-Phe-Gly-OH (8d).

**Deprotection** was effected, a sample of each resin was cleaved, and the product was analyzed by HPLC and MS.

(8h) 1-(1-Carboxy-1-methylthyl)-1H-[1,2,3]-triazol-4-yl]propionyl-Phe-Gly-Phe-Gly-OH (8d).

**Deprotection** was effected, a sample of each resin was cleaved, and the product was analyzed by HPLC and MS.

(8i) 1-(1-Carboxy-1-methylthyl)-1H-[1,2,3]-triazol-4-yl]propionyl-Phe-Gly-Phe-Gly-OH (8d).

**Deprotection** was effected, a sample of each resin was cleaved, and the product was analyzed by HPLC and MS.

(8j) 1-(1-Carboxy-1-methylthyl)-1H-[1,2,3]-triazol-4-yl]propionyl-Phe-Gly-Phe-Gly-OH (8d).

**Deprotection** was effected, a sample of each resin was cleaved, and the product was analyzed by HPLC and MS.

(8k) 1-(1-Carboxy-1-methylthyl)-1H-[1,2,3]-triazol-4-yl]propionyl-Phe-Gly-Phe-Gly-OH (8d).

**Deprotection** was effected, a sample of each resin was cleaved, and the product was analyzed by HPLC and MS.

(8l) 1-(1-Carboxy-1-methylthyl)-1H-[1,2,3]-triazol-4-yl]propionyl-Phe-Gly-Phe-Gly-OH (8d).

**Deprotection** was effected, a sample of each resin was cleaved, and the product was analyzed by HPLC and MS.
of amide region (500 MHz, DMSO-<sup>d6</sup>): δ = 8.13 (d, 1 H, J = 8.4 Hz, Phe-NH), 8.23 (s, 1 H, triazole-H<sup>4</sup>), 8.31 (m, 1 H, Gly-NH), 8.35 (t, 1 H, J = 5.4 Hz, Gly-NH).

**Propynyl-Phe-Gly-HMBA-PEGA<sub>800</sub> (14).** Resin 14 was prepared in a manner similar to that of resin 1 until H-Phe-Gly-HMBA-PEGA<sub>800</sub> was obtained, and it was then washed with CH<sub>2</sub>Cl<sub>2</sub>. Propargylic acid (3 equiv) and EEDQ (3.1 equiv) were mixed in CH<sub>2</sub>Cl<sub>2</sub>, transferred to the resin, and reacted for 16 h. The resin was washed with CH<sub>2</sub>Cl<sub>2</sub>, cleaved, and analyzed by HPLC and MS. HPLC: t<sub>b</sub> = 8.9 min. ESI-MS: calc'd (M + Na<sup>+</sup>) = C<sub>40</sub>H<sub>30</sub>N<sub>1</sub>O<sub>40</sub>Na<sup>+</sup>, 297.1 Da; found (M + Na<sup>+</sup>), m/z 297.0.

**General Procedure for Conversion of Fmoc-Amino Alcohols to Fmoc-Amino Azides (15–19).** The Fmoc-amino alcohol (1 equiv, 1 mmol), Ph<sub>3</sub>P (1.5 equiv), and H<sub>2</sub>N<sub>2</sub> in toluene (5 equiv, 1.5 M) were dissolved in dry THF (12 mL) under argon and cooled to 0 °C. Disopropyl azodicarboxylate (1.6 equiv) was added dropwise, and the reaction was stirred at 25 °C for 1.5 h. Everything was concentrated in vacuo and purified by flash chromatography.

(4-(Amino-(2,2,5,6,8-pentamethylchroman-7-sulfonylimino)methyl)-aminoo)ethyl ester (16). Fmoc-Gly-OH (0.717 mmol) afforded 15 (350 mg, 72%) after flash chromatography (PE/EA 2:1). 1H NMR (250 MHz, CDCl<sub>3</sub>): δ = 1.30 (s, 6H, C<sub>6</sub>(CH<sub>3</sub>)<sub>3</sub>), 1.50 (m, 4H, CH<sub>2</sub>) and CH<sub>2</sub>), 1.78 (m, 2H, Ar-CH<sub>2</sub>), 2.12 and 2.62 (s, 9H, Ar-CH<sub>3</sub>), 2.59 (m, 2H, Ar-CH<sub>2</sub>), 3.21 (m, 2H, CH<sub>2</sub>), 3.29 (m, 2H, CH<sub>2</sub>), 3.72(m, 1H, CH<sup>3</sup>), 4.16 (m, 1H, CHCHO), 4.38 (m, 2H, CHCHO), 5.42 (d, J = 8 Hz, 1H, CH<sub>3</sub>), 6.36 (br s, 3H, NH and NH<sub>2</sub> of guanidine group), 7.28–7.78 (8H, Fmoc-aminoprotons). 13C NMR (62.5 MHz, CDCl<sub>3</sub>): δ = 13.4, 15.5 and 18.8 (3Ar-CH<sub>3</sub>), 19.9 (Ar-CH<sub>2</sub>), 27.0 (CH<sub>2</sub>), 28.0 (C<sub>6</sub>(CH<sub>3</sub>)<sub>3</sub>), 30.6 (CH<sub>2</sub>), 34.0 (Ar-CH<sub>2</sub>), 42.0 (CH<sub>2</sub>), 48.5 (CHCHO), 52.1 (CH<sup>3</sup>), 56.1 (CH<sub>3</sub>), 68.1 (CH<sub>2</sub>), 74.9 (Ar-O<sub>2</sub>Ar), 113.3–145.1 (aromatic carbons), 154.9 (C=NH), 157.6 (Fmoc-CHO). Mp: 80–82 °C. IR: 1719, 2101 cm<sup>-1</sup>. [α]<sup>D</sup> = −5° (c = 2.0, CHCl<sub>3</sub>).

4-Azido-(S)-3-(9H-fluoren-9-ylmethoxy carbonylamino)-butyric acid tert-Butyl Ester, Fmoc-Asp(Bu)-<sup>3</sup>[CH<sub>2</sub>N<sub>3</sub>]<sup>16</sup>. Fmoc-Asp(Bu)-ol (2.68 mmol) afforded 16 as a syrup (1.11 g, 98%) after flash chromatography (PE/EA 5:1). 1H NMR (250 MHz, CDCl<sub>3</sub>): δ = 1.46 (s, 9H, tert-butyl), 2.53 (d, 2H, J = 5 Hz, CH<sub>2</sub>), 3.51 (m, 2H, CH<sub>2</sub>), 4.15 (m, 1H, CH<sup>3</sup>), 4.22 (t, J = 7 Hz, 1H, CHCHO), 4.42 (d, J = 7 Hz, 2H, CHCHO), 5.43 (d, J = 8 Hz, 1H, NH), 7.28–7.78 (8H, Fmoc aminoprotons). 13C NMR (62.5 MHz, CDCl<sub>3</sub>): δ = 28.4 (CH<sub>3</sub>), 37.7 (CHCHO), 47.6 (CH<sub>2</sub>), 48.3 (CH<sub>3</sub>), 54.1 (CH<sub>3</sub>), 67.3 (CH<sub>2</sub>), 82.1 (C<sub>2</sub>), 120.4–144.2 (aromatic carbons), 156.0 (Fmoc-CHO), 170.5 (Asp-CHO). IR: 1732, 2103 cm<sup>-1</sup>. [α]<sup>D</sup> = −3° (c = 1.0, CHCl<sub>3</sub>).