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The Synthesis of Amino Acid Derivatives for Peptide Incorporation

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Abstract

This paper details the synthesis of amino acid derivatives for peptide incorporation. The molecules were synthesized following the Strecker synthesis, then conversion to a hydantoin, which is hydrolyzed to yield amine and carboxyl functions. The amino acid side chains are all positively charged cyclic amines and vary in ring size. The synthesized amino acid derivatives are achiral. The molecular structures have been determined through analytical techniques including nuclear magnetic resonance and infra-red spectroscopy.

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I. Introduction

Four families of lytic peptides have been discovered over the past decade. They were first observed by Boman and his coworkers while working on the humoral defense system of the giant silk moth, the *Hyalophora cecropia* [1]. The injection of bacteria into these moths induces the synthesis of certain specialized proteins that are capable of disturbing the bacterial cell membranes. The result is loss of membrane integrity, leading to lysis and cell death. This led to the discovery of several peptide classes with the ability to lyse bacterial cells. One aspect of lytic peptide investigation has focused on determining the method of cell lysis and duplicating that effect *in vivo*.

A structural motif found in lytic peptides is the amphipathic α helix. Its effect on lytic activity has been well studied [2-4]. An amphipathic α helix is defined as one with opposing polar and nonpolar faces oriented along the long axis of the helix. This structure is necessary for lytic activity, though it behaves differently in the different classes. Melittin is known to form transmembrane helical assemblages with the long axis vertical to the bilayer surface [5]. Magainin 2, on the other hand, is thought to associate with the membrane as a helix with its long axis parallel to the bilayer surface [6].

An interesting avenue of research involves seeking methods to both increase the lytic activity of the peptides and decrease their enzymatic degradation. All lytic peptides have the ability of forming amphipathic helices, which lead to transmembrane permeation and osmotically induced lysis of the infected cell [7]. Certain amino acids and their analogs are known to increase the α helical nature of the peptides [8-9]. One example is α-aminoisobutyric acid (AIB), a noncyclic α-α-dimethylated amino acid derivative known to favor α-helical peptide conformations (Figure 1). The amino acids synthesized here are designed to likewise enhance helix formation, which could lead to increased lytic behavior.

This paper focuses on the preparation of amino acids. Two synthetic schemes are used to prepare the molecules. An outline of the synthetic methodology is given in Figure 2. The amino acids are cyclic α-α-dialkylated derivatives and are non-natural molecules. The side chains of the amino acids vary as charged cyclic substituents (Figure 3). All are positively charged, possessing a charged tertiary nitrogen. The ring sizes are 6-membered and 4-membered rings. By incorporating these derivatives in key sequence positions, the effect of charged cyclic substituents on the activity can help determine the residues importance in maintaining and increasing lytic activity.

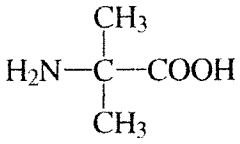
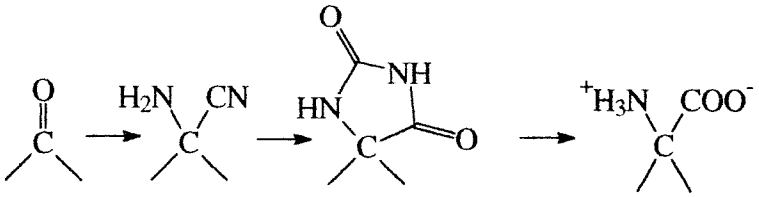
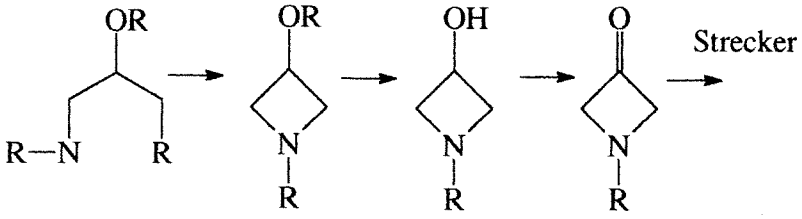


Figure 1: α-aminoisobutyric acid (AIB)



Scheme 1: Strecker synthesis ⟶ hydantoin formation



Scheme 2: Preparation for Strecker synthesis

Figure 2: Methodology of Amino Acid Synthesis

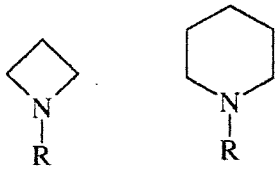
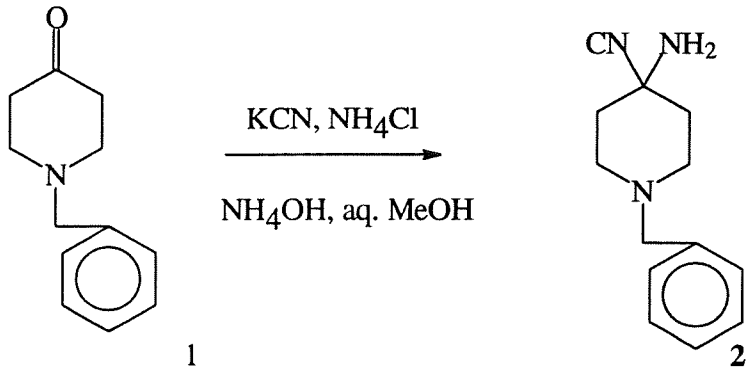


Figure 3: Side Chain Rings of Amino Acid Derivatives

II. Experimental

Nuclear magnetic resonance spectra were recorded with a Brucker AC 200 MHz in Fourier-transform mode. Peak positions are reported in parts per million with tetramethylsilane as an internal reference. Multiplet (m), quartet (q), triplet (t), doublet (d), or singlet (s) describe the multiplicity of resonances. Melting points were determined on a Hoover capillary melting point apparatus and are uncorrected. Infra-red spectra were recorded with a Perkin- Elmer 1760 apparatus in Fourier-transform mode and are reported in cm^{-1}.

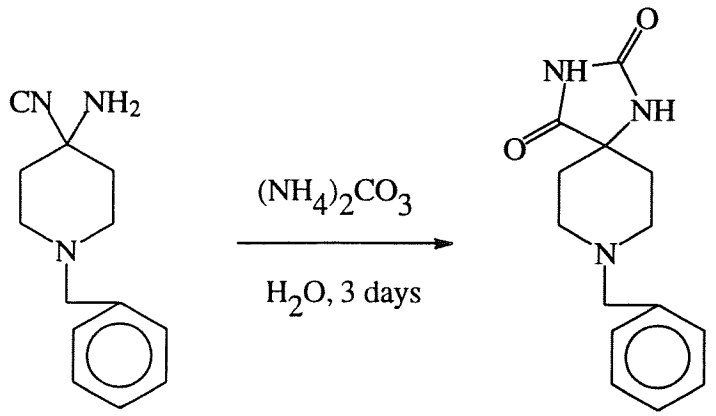


1 **2**

Preparation of 1-amino-4-benzyl piperdine-1-carbonitrile (2)

Procedure is modified from ref. 10. To a solution of benzyl piperdine (11.1 ml, 0.06 mole) in water (75 ml) and methanol (9 ml, 0.22 mol) were added ammonium chloride (9.0 g, 0.168 mol) and potassium cyanide (9.0 g, 0.138 mol). Ammonium hydroxide (13.8 ml, 0.35 mol) was then added to the reaction flask. The reactants were stirred for four days at room temperature and atmospheric conditions and turned light brown with time. The solution was diluted with water (75 ml) and compound **2** was

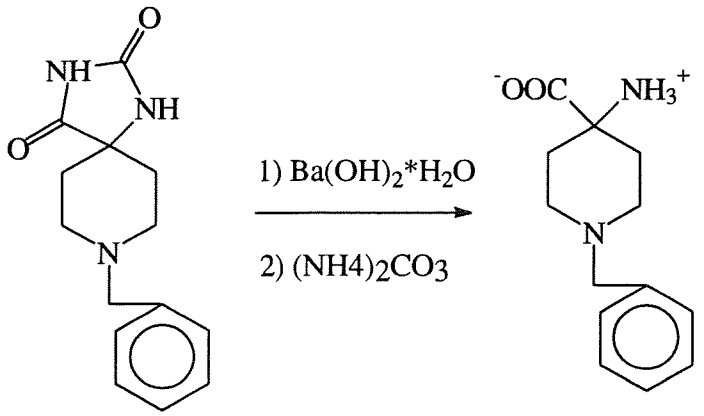
extracted into chloroform (4× 100 ml). The organic layer was then evaporated, resulting in a thick brown oil that crystallized under reduced pressure. Pure **2** was obtained as light brown crystals through sublimation at 75°C (7.10g 0.328 mol, 55%); mp 65- 66 °C; lit. mp 69 °C ^{1}H NMR (200 MHz, d-DMSO) δ 1.882-1.89 (m, 2H); 2.12-2.22 (m, 2H); 2.75-2.83 (m, 2H); 3.5 (d, NH_2); 7.28 (s, 6H).



2 **3**

Preparation of 8-phenylmethyl-1,3,8-triazospiro[4,5] decane 2,4 dione (3)

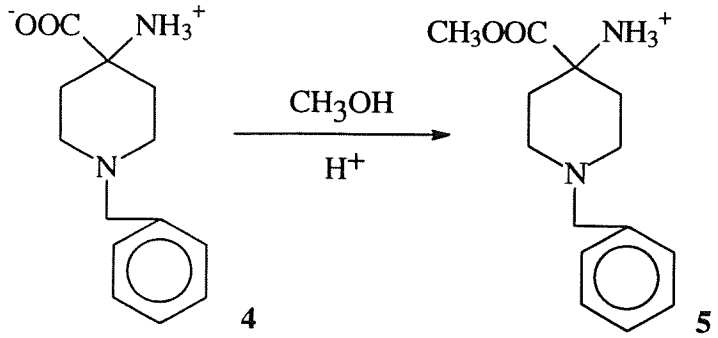
Procedure modified from ref. 10. Compound **2** (3.61g, 0.0165 mol) and excess ammonium carbonate (10g, 0.104 mol) were added to water (50 ml) and stirred at room temperature for three days. The resulting white solution was then filtered and washed with water (6 × 100 ml). White solid **3** was then placed under reduced pressure to dry. Compound **3** was isolated as white crystals. (4.2g, 0.0162 mol, 98%) mp 237-238 °C (dec), lit. mp 243 °C; ^{1}H NMR (200 MHz, d-DMSO) δ 0.68-0.71 (m, 4H); 1.38-1.52 (m, 4H); 2.23-2.34 (d, 2H); 6.36 (s, 6H); 7.55 (s, NH_2); 9.32 (s, NH_2); ^{13}C NMR (200 MHz, d-DMSO) 10C: δ33.145, 48.1, 60.15, 61.8, 126.79, 128.14, 128.55, 138.53, 156.23, 178.065 FT-IR: 1411.8, 1736.5, 2359.6, 3195.2 cm^{-1}.



3 4

Preparation of 4-amino-1--phenylmethyl piperdine 4-carboxylic acid (4)

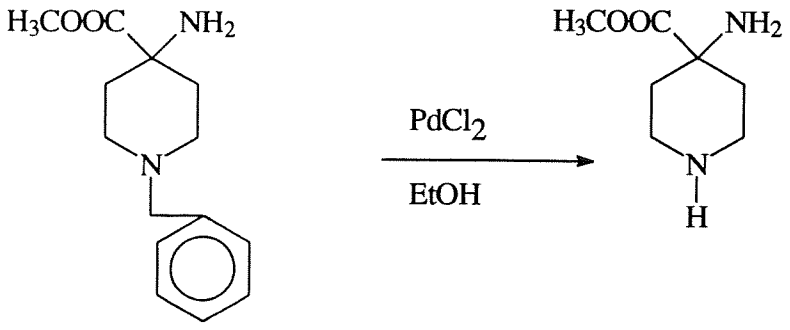
Procedure modified from ref. 10. Compound **4** was prepared by the hydrolysis of **3**. Compound **3** (3.0g, 11.48 mmol) and barium hydroxide (6.0 g, 31.57 mmol) were placed in a Parr high pressure bomb calorimeter with 150 ml water. The mixture was reacted for six hours under heat (153°C) and constant pressure. The resulting solution was treated with an excess amount of ammonium carbonate in an agitated water bath for 0.5h. The excess barium carbonate was filtered off and was washed with H_2O (2 × 10 ml). The supernatant was then treated with activated carbon and filtered through Celite. The water was gently boiled off and the white residue was redissolved in water. Methanol (50 ml) was then added until the solution became cloudy. The solution was filtered and the resulting amino acid was obtained as white crystals (1.43 g, 0.0061 mol, 53 %). mp 314-316 °C, ^{1}H NMR (200 MHz, D_2O) δ 1.32-1.34 (m, 4H), 3.42 (d, 2H), 5.65 (d, N*H*$_2$) , 7.02 (s, 6H), 9.82 (s, COO*H*). FTIR: 1381.5, 1554.6, 1754.5, 3380.1 cm^{-1}.



4 5

Protecting amino acid (5)

Procedure followed from ref. 11. The carboxyl group of the amino acid **4** was protected by esterification. Compound **4** (1g, 4.3 mmol) was added to methyl alcohol (50 ml) and a small amount of concentrated HCl. The reaction was refluxed for 5 h and water was removed by azeotropic distillation. The product was isolated as white crystals (1.02g, 4.1 mmol, 95%) ^{1}H NMR (200 MHz, D_20) δ 1.32-1.34 (m, 4H); 2.58-2.59 (s, 3H); 3.42 (d, 2H); 5.65 (d, NH_2);7.02(s, 6H); FT-IR: 1381.5, 1554.6, 1754.5, 3380.1 cm^{-1}.

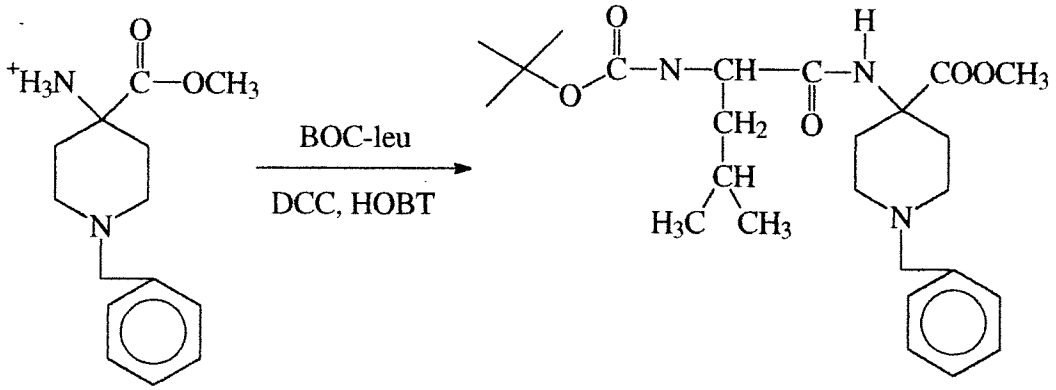


5 6

Deprotecting 3° N of protected amino acid (6)

Procedure modified from ref. 12. A solution of the blocked amino acid (1.5g, 4.5 mmol) in 95% ethanol (75 ml) was diluted with water up to 350 ml total volume until the solution became milky. Palladium chloride (0.8g, 4.5 mmol) was added and the solution was shaken on a Parr apparatus for 30 min. under an inert atmosphere. The mixture was vacuum filtered and placed under reduced pressure to remove organic volatiles. The

mixture was lyophilized to yield white solid. ^1H and FTIR analysis of the product showed that the 3° N protecting group, -methylphenyl, was not removed.

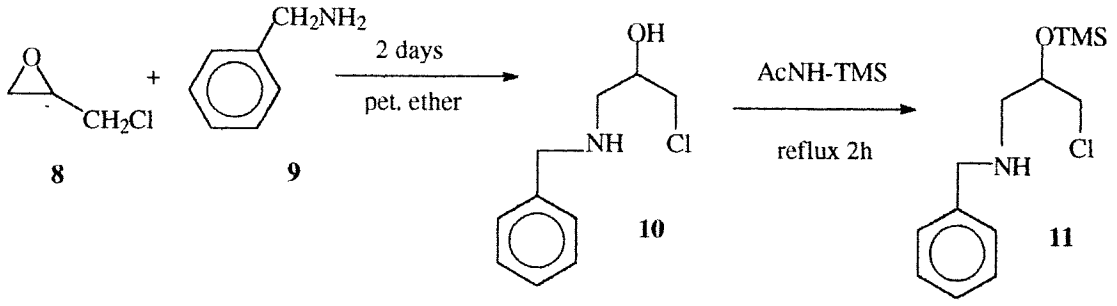


5 9

Coupling of protected -Amino Acid to BOC-L-Leucine (7)

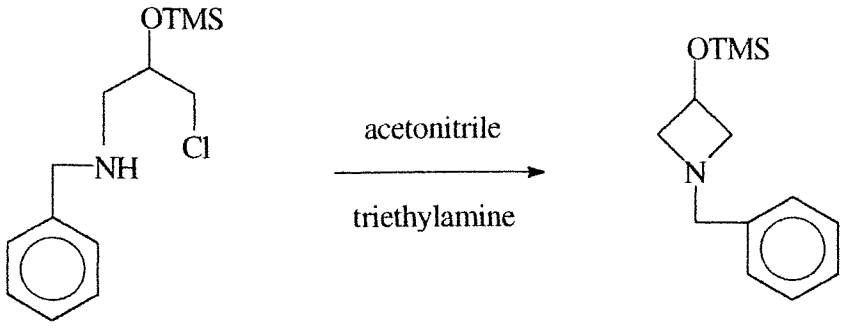
Procedure was followed from ref. 11. Compound **5** (16.2g, 64.9 mmole) in 250 ml of dichloromethane was neutralized with triethylamine (6.5g, 65 mmol) at 0°C. The protected L-leucine (15g, 75 mmol) was dissolved in 200 ml of DCM and added to the reaction, followed by the addition of solid 1-hydroxy-benzotriazole (8.8g, 65 mmole). The reation was stirred for five minutes. 1,3-dicyclohexylcarbodiimide (13.4g, 65 mmole) in 75 ml of DCM was then added to the solution and the reaction stirred for four hours at 0°C. The white mixture was then stirred at room temperature overnight. The resulting white solution was filtered and the DCM evaporated off. The resulting yellow oil was then resuspended in DCM. The organic layer was washed successively with (1) 1N HCl (2) $NaHCO_3$ and (3) salt water. The organic layer was then dried over $MgSO_4$

and evaporated. The resulting product was a thick yellow oil. Through analytical techniques, it was determined that the coupling reaction was unsuccessful.



Preparation of 1-phenylmethylamino-3-chloro-2-trimethylsilyl ether (11)

Procedure was followed from ref. 13. To 200 ml of petroleum ether were added epichlorohydrin (**8**, 15.6 ml, 0.2 mol) and benzylamine (**9**, 21.8 ml, 0.2 mol). The mixture was stirred at room temperature for two days. Compound **10** was not isolated. N-(trimethylsilyl)-acetamide (26.2g, 0.2 mol) was added to the reaction mixture of **10**. The reaction temperature was raised to 190°C and the mixture was refluxed for 2h. The cooled mixture was then filtered and resulting white crystals of compound **13** were placed under vacuum overnight. (37.2g, 0.14 mol, 68%); mp 119-120 °C lit mp 127 °C; ^{1}H NMR (200 MHz, $DCCl_3$) δ 1.32-1.34 (m, 4H); 3.64 (t, 2'C*H*$_2$); 3.32 (t, 4' C*H*$_2$); 4.41-4.45 (s, N*H*); 7.02 (s, 6H). FT-IR: 698.1, 737.2, 2899.3, 3.084.4, 3492.5 cm^{-1}.



11 **12**

Preparation of 1-phenylmethyl-3-azetidinyl trimethylsilyl ether (12)

Procedure was followed from ref. 13. 1-phenylmethylamino-3-chloro-2-trimethylsilyl ether (36.5g, 0.13mol) was placed in a solution of acetonitrile (200 ml, 3.65 mol) and triethylamine (85 ml, 0.6 mol). The reaction was stirred with a mechanical stirrer and refluxed at 140°C for three days. As the mixture cooled, white crystals of the product precipitated out. These crystals were filtered and dried under reduced pressure. (5.07g, 0.0215 mol, 16%) mp 91-93°C lit mp 88-89°C; ^{1}H NMR (200 MHz, $DCCl_3$) δ 3.62-3.65 (t, 2'CH_2, 4' CH_2); 2.71 (t, 2H); 7.15 (s, 6H).

III. Results

The synthesis of 4-amino-4-carboxylic acid-1-phenylmethyl piperdine (**4**) is reported with reasonably good yields (42 - 53%). The structure of this molecule has been determined by NMR and IR analysis.

The structure of compound **4** can be verified through NMR and IR spectroscopy. The NMR spectra show the phenyl (s, δ 7.02) and methyl (d, δ 3.42) portions of the 3° N protecting group. The carboxyl (s, δ 9.82) and amine (d, δ 5.65-5.74) protons also appear. The carboxylic acid proton shows up as a singlet, whereas the amine protons again appear as a wide band. The protons on the piperdine show up at δ 1.32-1.34 as multiplets. The IR spectrum shows a carboxyl stretch at 1754.4 cm^{-1} and an amine stretch at 3380.1 cm^{-1}.

The removal of the 3° N protecting group was unsuccessful. The molecule is highly hindered sterically, with the protecting group itself being bulky. The removal is this group was expected to be tricky. After the reaction was performed, analysis showed the presence of the protecting group still intact and almost none of the unprotected 3° N. The NMR showed the phenyl (δ 7.09) and the methyl (δ 3.54) still intact. In fact the NMR and IR analysis of the before and after reaction product were identical.

NMR	*Before deprotection*	*After deprotection*
11' CH_2	δ 3.42	δ 3.54
6*H*	δ 7.02	δ 7.09
COO*H*	δ 9.82	δ 10.03
NH_3	δ 5.65	δ 5.79

Through these analysis, it was determined that the protecting group was not removed. The amino acid **4** was therefore coupled to leucine with the 3° N protecting group still intact.

IV. Discussion

The preparation of 4-amino-4-carboxylic acid-1-phenylmethyl piperdine has been outlined in this paper. The yields of the molecule are moderately good, but improvements in overall yield can be made. The failure to remove the 3° N of the side chain hampered the coupling ability of the amino acid with the leucine.

V. Acknowledgments

I would like to thank Dr. Mark McLaughlin for supporting all this work, Cal Becker for teaching me the laboratory techniques and Tamara Schaller for being an encouragement throughout. Thanks to Scott Yokum for giving me the procedure for the coupling reactions. Dr. Seay and Ms. Hood of the Honors College also deserve much credit for motivating me to finish what I started.

VI. References

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