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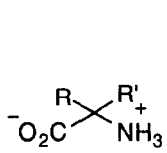
Synthesis of a Series of Polar, Orthogonally Protected, α,α–Disubstituted Amino Acids

T. Scott Yokum, Matthew G. Bursavich, Sarina A. Piha-Paul, David A. Hall, and Mark L. McLaughlin*

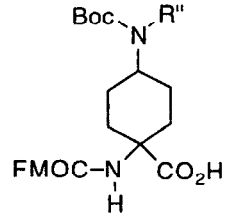
Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana 70803 USA

Abstract: The synthesis of four novel, "cationic", α,α-disubstituted amino acids is described. The new amino acids use an orthogonal protection scheme making them suitable for incorporation via solid-phase peptide synthesis. © 1997 Elsevier Science Ltd.

α,α-Disubstituted amino acids (ααAAs), **1** (where R, R' ≠H), are widely used in peptide design because of their structure promoting effects.[1] α–Aminoisobutyric acid (Aib) and Aib-like residues (where R, R' are not bulky) have been incorporated into peptides to form 3_{10} or α–helices depending upon the design,[2] percentage of ααAAs,[3] and location of the ααAAs.[4] Unfortunately, most ααAAs that have been incorporated are hydrophobic which precludes spectroscopic or crystallization experiments of peptides containing high percentages of ααAAs in aqueous media.[1,2b] Very few examples of polar ααAAs suitable for incorporation into peptides have been reported in the literature.[5,6] Polar ααAAs are a must for the synthesis of short, highly helical, water soluble peptides containing high percentages of ααAAs. These peptides are integral to the study of the 3_{10}/α-helix equilibrium, the investigation of the stability of the 3_{10}-helix in aqueous media, and in the design of short antimicrobial peptides.[2,7] Herein we report the synthesis of a series of polar ααAAs suitable for incorporation into peptides.



1



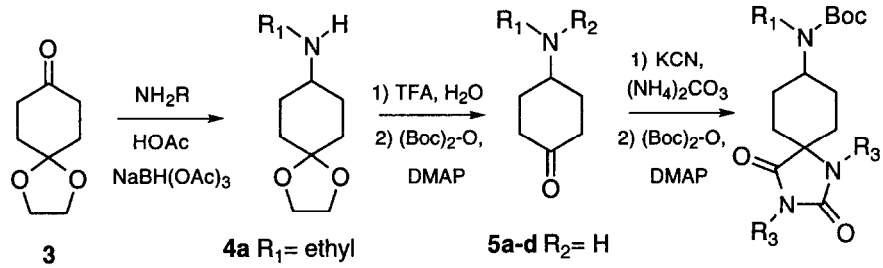
2

R"= ethyl, butyl, benzyl, or 2-naphthylmethyl

The general structure of the amino acids is shown above, **2**. The amino acids are constructed from a 6-membered ring backbone due to the high helix promoting effects previously exhibited by cyclic ααAAs in short peptides.[2a,7] The polar functionality is introduced via reductive amination which provides

flexibility for the incorporation of a wide variety of R" groups, such as hydrophobic chains, additional polar groups, or fluorescent probes. This side chain nitrogen will be protonated under physiological conditions and under conditions normally used for aqueous spectroscopic characterization.

The synthesis begins with a reductive amination on the commercially available 1,4-cyclohexanedione monoethylene ketal **3** with the amine of choice (1.1 equiv.), acetic acid (1.0 equiv.), and sodium triacetoxyborohydride (1.6 equiv.) in 1,2-dichloroethane to give **4**.[8] Sodium cyanoborohydride was also tried as the reducing agent but gave unsatisfactory yields as compared to the acetoxy compound. The ketone functionality is unmasked by treating **4** with a 20% trifluoroacetic acid/water solution and heating to reflux in THF for 24 hours to yield **5**. To obtain an amino acid suitable for Fmoc solid-phase peptide synthesis, the side chain must be orthogonally protected with respect to the α–nitrogen. Thus, the nitrogen on ketone **5** is protected with *t*-butyloxycarbonyl (Boc) using Et_3N (0.95 equiv.), $(Boc)_2$-O (1.14 equiv.) and a catalytic amount of DMAP in THF to give **6**.



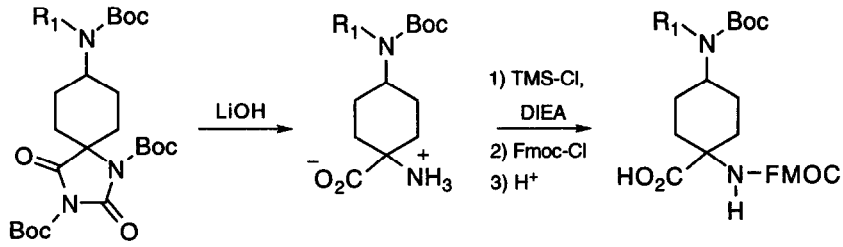
3

4a R_1= ethyl
4b R_1= butyl
4c R_1= benzyl
4d R_1= 2-naphthylmethyl

5a-d R_2= H
6a-d R_2=Boc

7a-d R_3= H
8a-d R_3= Boc

Ketone **6** is then converted to hydantoin **7** using a Bucherer-Bergs procedure.[5,9] At this point, conventionally the hydantoin would be hydrolyzed using harsh conditions (i.e. barium hydroxide in a Parr™ Bomb) which could effect the Boc protecting group. Therefore, we use a mild hydrolysis method developed by Rebek in which the hydantoin nitrogens are activated by reaction with $(Boc)_2$-O to give **8**.[5,10] The selective hydrolysis is accomplished with 1N lithium hydroxide (8 equiv..) and THF as a co-solvent at room temperature to give amino acid **9**. The α–nitrogen is protected with fluorenylmethyloxycarbonyl (Fmoc) using TMS-Cl (2.5 equiv.), DIEA (3.0 equiv.), and Fmoc-Cl (1.1 equiv.) in methylene chloride. Protecting the α–nitrogen of a very hydrophobic amino acid of this type is difficult using traditional methods with aqueous/organic mixtures because of the extreme non-polar nature of the side chain (protected). The method we use, developed by Bolin and co-workers,[11] solubilizes the amino acid by forming the silyl ester in neat organic media thereby allowing protection of hydrophobic amino acids. The crude product obtained from the Fmoc reaction is purified over silica gel using $CHCl_3$/MeOH mixtures to give pure **2**.[12]



8a-d **9a-d** **2a-d**

The method provides a general route to polar ααAAs with R groups of varying hydrophobicity. This method could also be easily adapted to the synthesis of fluorescent or other tagged ααAAs. We plan to study the effects of the hydrophobic chains on antimicrobial activity and helicity.

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12. **2a**; 1-amino(N^{α}-Fmoc)-4-(N^{δ}-Boc, N^{δ}-ethyl)cyclohexane-1-carboxylic acid. ^1H NMR (250 MHz, DMSO *d6*) δ 7.94 (d, 2H), 7.79 (d, 2H), 7.62 (s, 1H), 7.45 (t, 2H), 7.38 (t, 2H), 4.32 (m, 3H), 3.82 (bm, 1H), 3.16 (bm, 2H), 2.25 (bm, 2H), 1.74 (bm, 4H), 1.50 (bm, 2H), 1.46 (s, 9H), 1.12 (t, 3H). ^{13}C NMR (100 MHz, DMSO *d6*) δ 175.88, 155.24, 154.24, 143.82, 140.73, 127.65, 127.00, 125.33, 120.01, 78.26, 65.20, 57.28, 54.20, 46.75, 43.80, 31.02, 28.15, 25.05, 16.02. FAB-MS (glycerol) *m/z* 509.3 $(M+H)^+$. 15.1% overall yield. **2b**; 1-amino(N^{α}-Fmoc)-4-(N^{δ}-Boc, N^{δ}-butyl)cyclohexane-1-carboxylic acid.^1H NMR (300 MHz, DMSO *d6*) δ 7.88 (d, 2H), 7.73 (d, 2H), 7.56 (s, 1H),7.42 (t, 2H), 7.31 (t, 2H), 4.26 (m, 3H), 3.70 (bm, 1H), 3.02 (bm, 2H), 2.16 (bm, 2H), 1.69 (bm, 4H), 1.42 (bm, 4H), 1.40 (s, 9H), 1.24 (m, 2H), 0.89 (t, 3H). ^{13}C NMR (50MHz, $CDCl_3$) δ 178.54, 155.68, 155.38, 143.73, 141.28, 127.64, 127.01, 124.96, 119.89, 79.47, 66.63, 58.16, 54.00, 47.19, 43.56, 32.76, 31.64, 28.47, 25.21, 20.25, 13.88. FAB-MS (glycerol) *m/z* 537.5 $(M+H)^+$. 28.4% overall yield. **2c**; 1-amino(N^{α}-Fmoc)-4-(N^{δ}-Boc, N^{δ}-benzyl)cyclohexane-1-carboxylic acid. ^1H NMR (250 MHz, $CDCl_3$) δ 7.73 (d, 2H), 7.57 (d, 2H), 7.36 (t, 2H), 7.29 (m, 7H), 4.89 (s, 1H) 4.41 (d, 2H), 4.33 (s, 2H), 4.16 (t, 1H), 4.00 (bm, 1H), 2.11 (bm, 2H), 1.79 (bm, 2H), 1.54 (bm, 4H), 1.40 (s, 9H). ^{13}C NMR (100 MHz, DMSO *d6*) δ 175.87, 155.40, 155.25, 143.81, 140.72, 140.02, 128.15, 127.63, 127.02, 126.70, 126.49, 125.35, 120.08, 79.19, 65.21, 57.25, 54.16, 46.76, 46.10, 31.02, 28.01, 25.07. FAB-MS (glycerol) *m/z* 571.2 $(M+H)^+$. 13.7% overall yield. **2d**; 1-amino(N^{α}-Fmoc)-4-(N^{δ}-Boc, N^{δ}-2-naphthylmethyl)cyclohexane-1-carboxylic acid. ^1H NMR (300 MHz, DMSO *d6*) δ 8.48 (s, 1H), 7.96 (m, 2H), 7.80 (m, 2H), 7.69 (m, 3H), 7.30 (bm, 8H), 4.92 (s, 2H), 4.85 (d, 1H), 4.75 (m, 2H), 3.60 (bm, 1H), 2.13 (bm, 2H), 1.74 (bm, 4H), 1.51 (bm, 2H)1.46 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.93, 155.99, 155.85, 143.86, 141.41, 134.68, 133.79, 130.92, 129.01, 127.75, 127.14, 126.20, 125.99, 125.78, 125.65, 125.40, 125.10, 123.44, 119.99, 80.29, 66.61, 58.20, 54.50, 47.40, 45.00, 32.72, 28.52, 25.55. FAB-MS (glycerol) *m/z* 621.0 $(M+H)^+$. 18.7% overall yield.

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