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Novel Synthesis of N^{α}-Methyl-arginine and N^{α}-Methyl-ornithine Derivatives

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Abstract: N^{α}-Boc-N^{α}-methyl-$N^{\omega,\omega'}$-bis(benzyloxycarbonyl)-*L*-arginine and N^{α}-Boc-N^{α}-methyl-N^{δ}-benzyloxycarbonyl-*L*-ornithine have been synthesized starting with Boc-*L*-Gln. The γ-carboxamide of Boc-*L*-Gln was dehydrated to a nitrile group and the resulting compound is selectively methylated providing *N*-Boc-2-methylamino-4-cyanobutyric acid. The nitrile is then reduced to an amine furnishing the key intermediate N^{α}-Boc-N^{α}-methyl-*L*-ornithine.

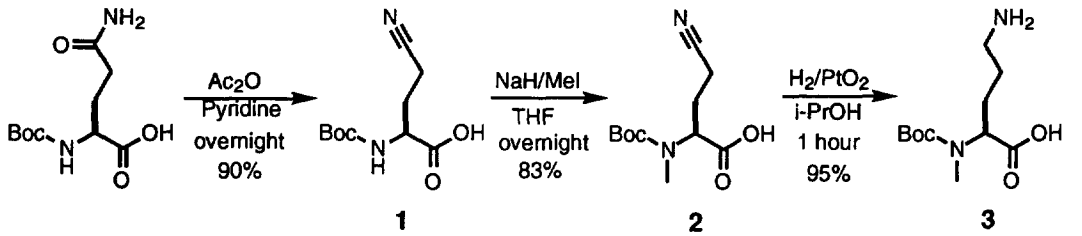
N-methyl amino acids are a group of important modified amino acids which have been widely used in medicinal chemistry and biochemistry to change the conformation, restrict the flexibility and enhance the potency of the molecule.[1-2] For example, recently, a series of cyclic Arg-Gly-Asp containing peptides have been developed as potential antithrombotics.[3-6] Among these, DMP-728, a cyclic *N*MeArg-Gly-Asp containing peptide is one of the most potent compounds.[5] A key feature required for the high potency of this compound is the N^{α}-methyl group of the arginine residue. This modification has been shown to increase the rigidity of the peptide macrocycle and also to restrict the number of low-energy rotomers of the arginine side chain.[7]

The only N^{α}, N^{ω}-diprotected N^{α}-methyl-arginine derivative synthesized in the literature is N^{α}-Boc-N^{α}-methyl-N^{ω}-tosyl-arginine. This compound is prepared in very low overall yield from N^{ω}-tosyl-arginine by: i) benzylation of the α-amine using benzaldehyde and $NaCNBH_3$; ii) monomethylation of the resulting secondary amine using formaldehyde and formic acid; iii) hydrogenolysis of the benzyl group; iv) addition of *t*-butyloxycarbonyl (Boc) group using di-*t*-butyl-dicarbonate. This compound is highly expensive due to the low overall yield and the expense of the starting material N^{ω}-tosyl-arginine. In addition, the very harsh acidic conditions required to remove the tosyl group and the concomitant side reactions[8] makes this compound less useful in large scale synthesis of *N*MeArg containing peptides, suggesting that a synthetic route to another N^{α}-Boc-N^{α}-methyl-arginine derivative with a different protecting group at guanidine should be investigated.

Since bis(benzyloxycarbonyl)guanidine is stable to the conditions used for the deprotection of Boc group[9] and can be deprotected by hydrogenolysis,[10] we were interested in N^{α}-Boc-N^{α}-methyl-arginine with bis(benzyloxycarbonyl)-protection at the guanidine. Such a compound would be useful in both solution phase and solid phase peptide synthesis. Since guanylation of the δ-amine of ornithine has been developed,[10a] a convenient and efficient synthesis of this compound is via the corresponding N^{α}-methyl-ornithine. Selective methylation of N^{α}-Boc-ornithine using sodium hydride/iodomethane[9] requires bisprotection of the δ-amine. A phthalyl group would be suitable for this purpose. However, the synthetic sequence of N^{α}-Boc-N^{δ}-phthalyl-ornithine is lengthy starting with ornithine by i) reaction of ornithine with copper sulfate followed by

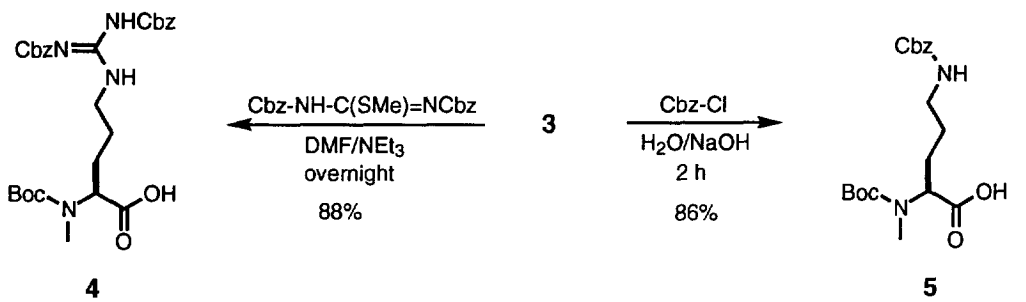
phthalylation of the resulting complex; ii) decomplexation using 6 N HCl; iii) addition of Boc group using di-*t*-butyl-dicarbonate. We therefore explored the use of a nitrile as a masked form of the δ-nitrogen of ornithine.

Scheme 1



The synthesis of N^{α}-Boc-N^{α}-methyl-$N^{\omega,\omega'}$-bis(benzyloxycarbonyl)-*L*-arginine started with an inexpensive N^{α}-Boc-*L*-glutamine (BocGln) as illustrated in Scheme 1. The δ-carboxamide of BocGln was dehydrated to a nitrile group. Among those dehydrating agents reported in the literatures,[11-13] we found that acetic acid/pyridine is the most efficient and convenient. Compound **1** was obtained as a syrup in 90% yield after treatment of BocGln with acetic acid in pyridine and silica gel chromatography. The dehydration was indicated by the disappearance of the characteristic proton NMR signals of the δ-carboxamide in compound **1**.[14] Selective methylation of compound **1** using sodium hydride and iodomethane in THF[9] produced crystalline *N*-Boc-2-methylamino-4-cyano-*L*-butyric acid (**2**) in 83% yield.[15] Hydrogenation of compound **2** using PtO_2 in isopropanol in the presence of HCl afforded the key intermediate N^{α}-Boc-N^{α}-methyl-*L*-ornithine HCl salt (**3**) in 95% yield.[16]

Scheme 2



Guanylation of compound **3** using bis(benzyloxycarbonyl)-*S*-methyl-isothiourea[17,10a] in DMF in the presence of triethylamine afforded N^{α}-Boc-N^{α}-methyl-$N^{\omega,\omega'}$-bis(benzyloxycarbonyl)-*L*-arginine (**4**) in 88% yield (Scheme 2) as a white foam.[18] The intermediate **3** is also useful for the synthesis of N^{α}-methyl-ornithine derivatives. For example, acylation of compound **3** using benzyl chloroformate in water in the presence of

sodium hydroxide at 0°C produced N^{α}-Boc-N^{α}-methyl-N^{δ}-benzyloxycarbonyl-*L*-ornithine in 86% yield (Scheme 2) as a white foam.[19,20]

The rate of hydrogenolysis of bis(benzyloxycarbonyl) group in compound **4** is solvent and acid dependent. In general, hydrogenolysis using palladium on charcoal as the catalyst is much faster in the presence of strong acids such as HCl and $MeSO_3H$ than weak acids such as acetic acid and formic acid. Hydrogenation is sluggish and requires an overnight reaction in methanol in the presence of HCl as reported in the literatures,[10] but the reaction was complete in 2 hours at atmospheric pressure in DMF in the presence of HCl.

In conclusion, the use of a nitrile group as a masked form for the racemization-free[21] synthesis of N^{α}-methyl-arginine and N^{α}-methyl-ornithine is novel, and the synthetic route to compounds **4** and **5** is efficient and convenient. These two compounds were synthesized using an inexpensive starting material, BocGln, and were obtained in high overall yields (62% for compound **4** and 61% for compound **5**).

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14. A solution of Boc-*L*-Gln (36 mmol, 8.9 g) in 100 mL pyridine and 4.4 mL acetic anhydride (43 mmol) was stirred at room temperature overnight and concentrated. The residue was taken up in ethyl acetate and the solution was washed with 5% citric acid 3 times and brine 3 times, dried ($MgSO_4$), and concentrated. Purification on a silica gel column using 10% methanol/CH_2Cl_2 gave 7.4 g (90%) syrupy product **1**. ^{1}H-NMR (DMSO-d_6): δ 6.77 (d, 1H, NH); 3.86 (m, 1H, CαH); 2.45 (m, 2H, CH_2); 1.99 (dd, 1H, CH_2);

1.84 (dd, 1H, CH_2); 1.39 (s, 9H, Boc). $[\alpha]_D^{25}$ -6.60° (c 0.424, methanol). FAB-MS $(M+H)^+$: calcd 229.2, found 229.2.

15. Compound **1** (20 mmol, 4.57 g) was dissolved in 100 mL THF and the solution was cooled in an ice bath. To it was added iodomethane (160 mmol, 10 mL) and NaH (60 mmol, 2.4 g) and the mixture was stirred at room temperature overnight. Ethyl acetate (20 mL) was added followed by 50 mL water. The pH of the solution was adjusted to 9 by adding 0.1 N HCl and the solution was concentrated to a small amount (~50 mL). Sodium thiosulfate (0.1 N, 5 mL) was added followed by 50 mL water. The solution was extracted with ether twice and the water layer was acidified to pH 3 with 5% citric acid with stirring. The solution was extracted with ethyl acetate 3 times, and the combined ethyl acetate solution was washed with brine 4 times, dried ($MgSO_4$), and concentrated. Crystallization from ethyl acetate/petroleum ether gave 4.02 g (83%) crystalline product **2**. ^{1}H-NMR (DMSO-d_6): δ 4.50 & 4.32 (m, 1H, *cis &trans* CαH);[20] 2.74 (s, 3H, NCH_3); 2.45 (m, 2H, CH_2); 2.14 (dd, 1H, CH_2); 2.02 (dd, 1H, CH_2); 1.41 & 1.38 (s, 9H, *cis & trans* Boc). m.p. 116-118°C. $[\alpha]_D^{25}$ -51.60° (c 0.500, methanol). FAB-MS $(M+H)^+$: calcd 243.2, found 243.2.
16. Compound **2** (11.5 mmol, 2.8 g) was dissolved in 30 mL cooled isopropanol containing 0.84 mL concentrated HCl and 280 mg platinum(IV) oxide was added. The mixture was hydrogenated at 50 psi for 1 hour and filtered through celite. The filtrate was concentrated and triturated with ether to give 3.08 g (95%) solid product **3**. ^{1}H-NMR (DMSO-d_6): δ 8.40 (b, 3H, NH_3^+); 4.51 & 4.24 (m, 1H, *cis & trans* CαH); 2.78 (m, 2H, CH_2); 2.72 (s, 3H, NCH_3); 1.84 (m, 1H, CH_2); 1.75 (m, 1H, CH_2); 1.54 (m, 2H, CH_2); 1.41 & 1.38 (s, 9H, Boc). m.p. 147-149°C. $[\alpha]_D^{25}$ -30.23° (c 0.526, methanol). FAB-MS $(M+H)^+$: calcd 247.2, found 247.3.
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18. Compound **3** (6 mmol, 1.7 g) and bis(benzyloxycarbonyl)-*S*-methylisothiourea (5 mmol, 1.7 g) were dissolved in 10 mL DMF containing 1.8 mL triethylamine and the mixture was stirred overnight. Ethyl acetate (100 mL) was added followed by 40 mL 5% citric acid. The organic layer was separated, washed with 5% citric acid twice, brine 3 times, dried ($MgSO_4$), and concentrated to give an oily product **4**. The product was triturated with petroleum ether and dried under high vacuum to give a white foam. Yield 2.4 g (88%). ^{1}H-NMR (DMSO-d_6): δ 11.60 (s, 1H, NH); 8.46 (t, 1H, NH); 7.38 (m, 10H, $2C_6H_5$); 5.20 (s, 2H, OCH_2); 5.02 (s, 2H, OCH_2); 4.48 & 4.28 (m, 1H, *cis &trans* CαH); 3.34 (m, 2H, CH_2); 2.70 & 2.68 (s, 3H, *cis & trans* NCH_3); 1.80 (m, 1H, CH_2); 1.68 (m, 1H, CH_2); 1.46 (m, 2H, CH_2); 1.38 & 1.32 (s, 9H, *cis & trans* Boc). m.p. 51-53°C. $[\alpha]_D^{25}$ -17.18° (c 0.576, methanol). FAB-MS $(M+H)^+$: calcd 557.3, found 557.3.
19. Compound **3** (5 mmol, 1.4 g) was dissolved in 10 mL 1 N NaOH and the solution was cooled in an ice bath. To it was added benzyl chloroformate (6 mmol, 0.86 mL) and 10 mL 1 N NaOH over 1 hour. After stirring for one more hour, the solution was extracted with ether twice. The water layer was acidified to pH 3 with 5% citric acid and extracted with ethyl acetate twice. The combined organic phase was washed with brine 3 times, dried ($MgSO_4$), and concentrated to give a white foam. Yield 1.6 g (86%). ^{1}H-NMR (DMSO-d_6): δ 7.34 (m, 6H, C_6H_5 & NH); 5.01 (s, 2H, OCH_2); 4.42 & 4.24 (m, 1H, *cis &trans* CαH); 3.00 (m, 2H, CH_2); 2.68 (s, 3H, NCH_3); 1.82 (m, 1H, CH_2); 1.60 (m, 1H, CH_2); 1.40 & 1.37 (s, 9H, *cis & trans* Boc); 1.36 (m, 2H, CH_2). m.p. 80-84°C. $[\alpha]_D^{25}$ -19.18° (c 0.584, methanol). FAB-MS $(M+H)^+$: calcd 381.3, found 381.2.
20. It is necessary to note that the Boc group, the CαH and/or the N-CH_3 each exhibits two signals in compounds **2**, **3**, **4** and **5** in DMSO-d_6 and $CDCl_3$ due to the *trans* and *cis* conformations of the N^{α}-Boc amide bond. We observed similar proton NMR patterns with other N^{α}-methyl-arginine and N^{α}-methyl-arginine-containing peptides. These phenomena were not reported previously in the literatures.[9b, 3]
21. The possibility of significant racemization was excluded by coupling N^{α}-Boc-N^{α}-methyl-N^{δ}-benzyloxycarbonyl-*L*-ornithine (Boc-NMeOrn(Cbz)) to Phe-t-butyl ester, giving dipeptide NMeOrn(Cbz)-Phe (after deprotection with trifluoroacetic acid). The crude product contained undetectable quantities (< 0.5 %) of the D,L dipeptide, whose elution time on HPLC was obtained through independent synthesis. The synthesis of Boc-NMeArg$(Cbz)_2$ was similarly shown to occur with < 0.5% racemization.

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