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Synthesis of arginine-containing hydroxamate dipeptidomimetics

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Abstract—The syntheses of arginine-containing hydroxamates using various peptide coupling methods are described. Fmoc-Arg(NO_2)-Cl prepared at low temperature did not undergo intramolecular δ-lactam formation and effectively provided desired hydroxamates (**8** and **10**) in good yields. Fmoc and *N*-nitro protecting groups can be easily removed. Therefore, this report provides a facile synthesis of arginine-containing peptidomimetic compounds.

1. Introduction

L-Arginine is a guanidino-containing basic amino acid,[1] which is positively charged at neutral pH, and is involved in many important physiological processes.[2] Many enzymes show a preference for arginine residues in natural substrates and synthetic inhibitors, for example, nitric oxide synthases[3] and trypsin-like serine proteases.[4] Therefore, peptidomimetics of arginine-containing biologically active molecules have been of interest for medicinal chemists and have provided molecules with better bioavailability, biostability, and potency.[1]

Despite the importance of arginine mimetics, the scope of their syntheses has been limited because of the difficulty of the chemistry associated with arginine. Firstly, the highly basic nature and nucleophilic character of the guanidino moiety in arginine normally requires appropriate protection of this group before subsequent chemical manipulation.[1] Ideally, protecting groups have to be removed easily under mild conditions and should be orthogonal to the other protecting groups. Secondly, acylation reactions of activated arginine usually compete with the side reaction of intramolecular δ-lactam formation (Fig. 1).[5] The latter case is a serious problem, especially when the reaction involves a weak nucleophile.

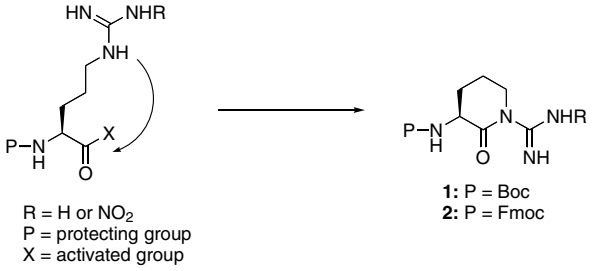

Figure 1. Intramolecular cyclization of arginine.

In the course of our research program of neuronal isoform selective nitric oxide synthase inhibitors discovery, we needed to synthesize nitroarginine-containing dipeptide peptidomimetic compounds (**8** and **10**, Scheme 2). The key feature in the inhibitor synthesis was the hydroxamate-forming coupling reaction. This reaction involves weakly nucleophilic *N*-alkyl hydroxylamines (**5** and **6**, Scheme 1), and the reaction requires strong activation of the other reactant, namely, protected arginine (**3** or **4**, Scheme 2). For this purpose, we investigated a way to activate nitroarginine, while avoiding common side reactions, and to provide a readily useful intermediate not only for the hydroxamate coupling, but also for the other arginine-containing pseudopeptides syntheses.

Keywords: Arginine; Hydroxamate; Peptidomimetics; Acid chloride.
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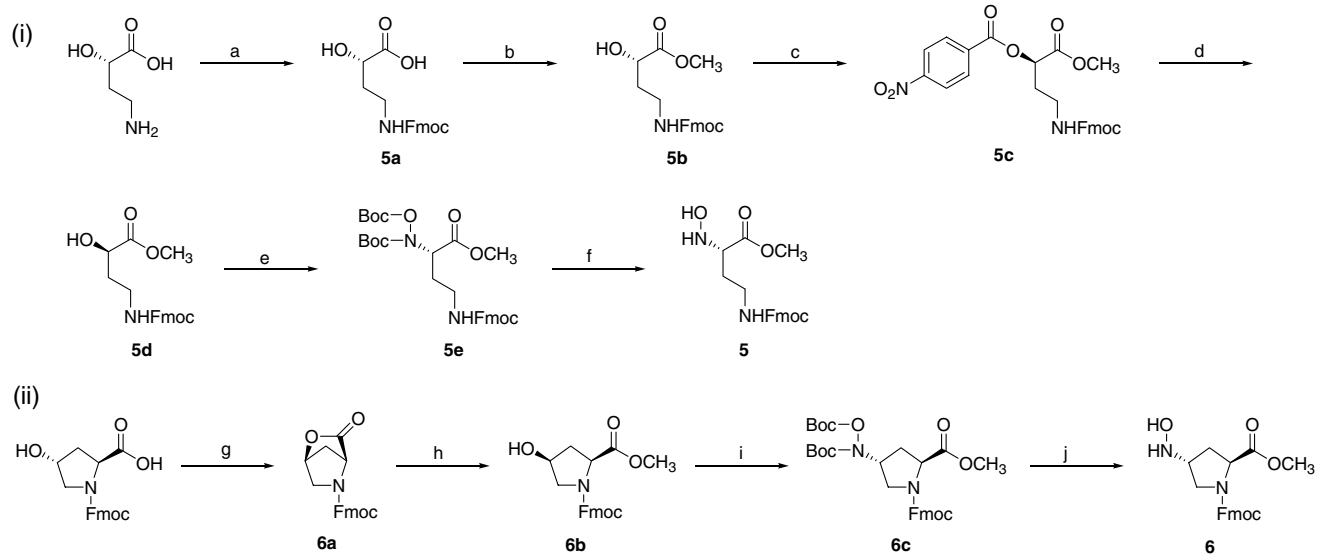
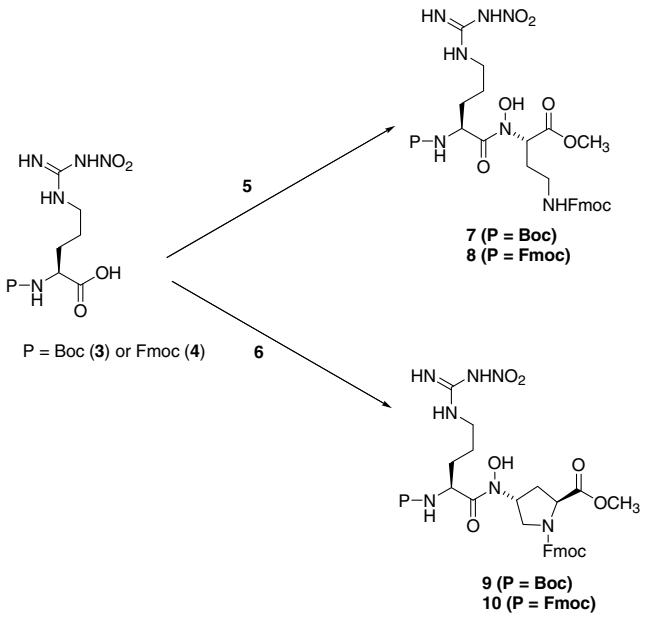


Scheme 1. Synthesis of *N*-alkylhydroxylamines **5** and **6**. Reagents and conditions: (a) Fmoc-Cl, 10% Na_2CO_3, dioxane, 86%; (b) MeI, $NaHCO_3$, DMF, 95%; (c) *p*-nitrobenzoic acid, DEAD, PPh_3, THF, 94%; (d) MeOH, NaN_3, 40 °C, 98%; (e) Boc-NH-O-Boc, DEAD, PPh_3, THF, 60%; (f) 50% TFA, CH_2Cl_2, 96%; (g) DIAD, PPh_3, THF, 85%; (h) MeOH, NaN_3, 40 °C, 96%; (i) Boc-NH-O-Boc, DEAD, PPh_3, THF, 45%; (j) 50% TFA, CH_2Cl_2, 98%.



Scheme 2. Nitroarginine-containing dipeptidomimetic intermediate syntheses.

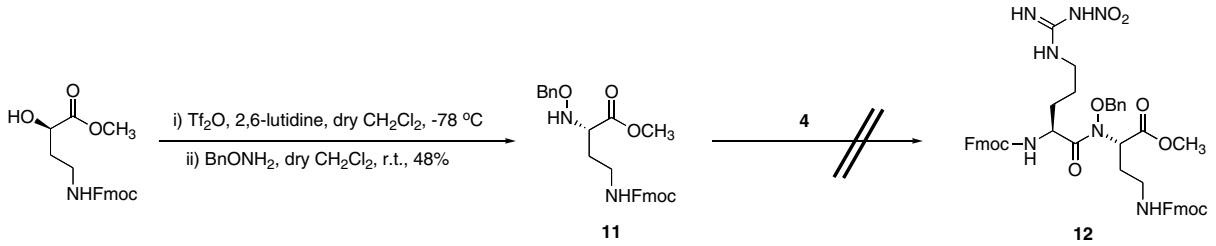
2. Results and discussion

TFA salts of *N*-alkylhydroxylamines **5** and **6** were prepared according to Scheme 1. For the synthesis of **5**, commercially available (*S*)-(−)-4-amino-2-hydroxybutyric acid was protected followed by a series of two Mitsunobu reactions to obtain the hydroxylamine functionality with the desired chirality. Fmoc-*trans*-4-Hydroxy-L-proline was converted to **6** by the following successive reactions: an intramolecular Mitsunobu reaction, a sodium azide catalyzed methanolysis, a Mitsunobu reaction with *N*,*O*-diBoc protected hydroxylamine, and a Boc deprotection.[6]

Attempted reactions to obtain **7** or **9** using uronium- or phosphonium-based peptide coupling reagents[7] resulted in no isolable product formation.[8] In these reactions, most of the starting material (**5** or **6**) remained unchanged, indicating that **5** or **6** are weak nucleophiles; consequently, side product **1** (Fig. 1)[9] was observed. Because it has been reported that one of the halophosphonium salts, PyBroP,[10] is highly efficient for difficult coupling reactions with essentially no epimerization,[11] we employed this reagent for the coupling of **4** with **5**. PyBroP/DIEA reagent provided an 8% isolated yield of **8**. The yield was slightly improved by using the in situ acid fluoride forming reagent TFFH/Na_2CO_3. Acid fluoride is reported to be an effective reagent for sterically demanding peptide coupling reactions.[12] However, the yield of **8** did not exceed 20% with TFFH, and **2** was recovered as a major product.

To compare the reactivity of hydroxylamine **5** with an *O*-protected hydroxylamine analogue, a coupling reaction using *O*-benzyl protected hydroxylamine **11** (Scheme 3) was attempted. Under peptide coupling conditions such as EDC/HOAt, HATU/DIEA, and PyBroP/DIEA, compound **11** did not couple with **4**. Therefore, we concluded that free hydroxylamine **5** was more reactive than protected hydroxylamine **11** in the coupling reaction with **4**, and investigated further for a better coupling system with **5**.

To overcome the low reactivity problem of hydroxylamines **5** or **6**, a highly activated protected nitroarginine, such as an acid chloride, seemed desirable, if not necessary, for the coupling reaction. Previously, the use of sulfonamide (i.e., tosyl) protected arginines for the



Scheme 3. Hydroxamate coupling reaction with benzyl protected hydroxylamine **11**.

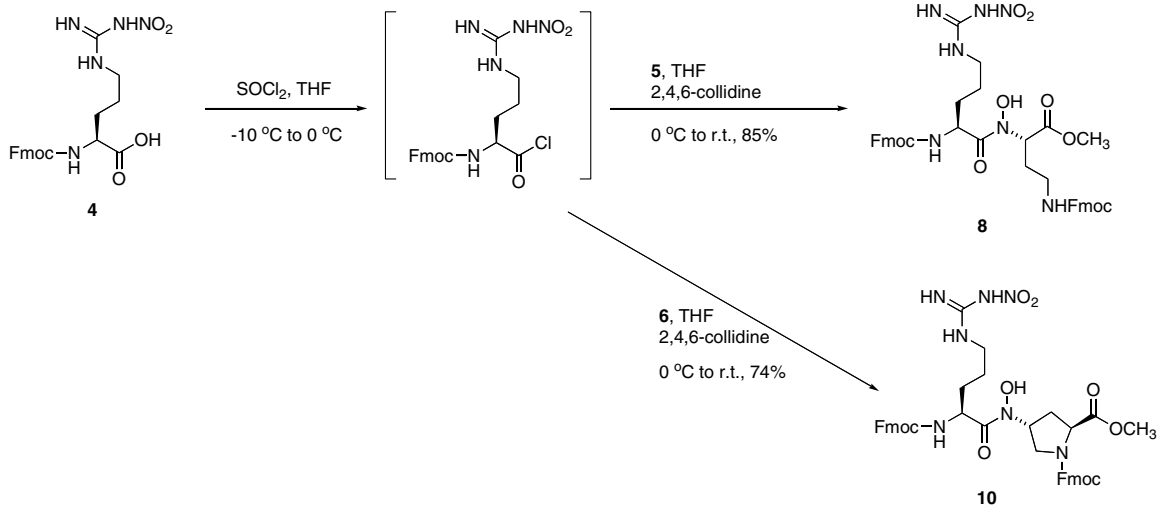
preparation of the corresponding acid chlorides was reported.[13] However, sulfonamide is one of the most stable nitrogen protecting groups, so methods for deprotection usually suffer from poor yields and/or harsh reaction conditions.[14] In peptide syntheses, harsh conditions can lead to unwanted side reactions such as a loss of stereogenic integrity or the deprotection of other protecting groups.[15] Additionally, a nitro group attached to arginine at the ω-nitrogen was necessary for our nitric oxide synthase inhibitor synthesis, so we used the conditions[13c] to prepare Fmoc-Arg(NO_2)-Cl. Using this activated amino acid, we investigated proper conditions for the hydroxamate coupling reaction. Fmoc and *N*-nitro protecting groups can be deprotected by secondary amine treatment and hydrogenation,[16] respectively. Furthermore, as Carpino et al.[17] reported, Fmoc-amino acid chloride coupling occurs without loss of chirality at the α-carbon under appropriate conditions. Wang et al. proved minimal loss of stereointegrity during an acid chloride mediated hydroxamate-forming reaction.[18]

Along with histidine, arginine is one of the amino acids that is not stable in an acid halide form at room temperature.[19] At room temperature the δ-amino group of N^{ω}-nitroarginine possesses sufficient reactivity for intramolecular lactam formation to occur. In fact, the attempted hydroxamate coupling reaction between **5** and Fmoc-Arg(NO_2)-Cl was unsuccessful at ambient temperature. However, by running the reaction at a lower temperature (−10 °C), as reported by Inouye et al.,[13c] it was found that the side reaction could be suppressed significantly when the acid chloride was used in situ. In our hydroxamate coupling reaction, we found that 2,4,6-collidine performed superior to pyridine or triethylamine as the base, and THF provided excellent solubility. Using these optimized conditions, Fmoc-Arg(NO_2)-Cl and hydroxylamines **5** and **6** coupled to form hydroxamates **8** and **10** in 85% and 74% isolated yields, respectively (Scheme 4).[20] The extent of epimerization during the coupling reaction was determined by HPLC (Supplementary data). Results obtained from this experiment indicate that the loss of stereointegrity was about 5%. We have found that anhydrous conditions are a prerequisite for the success of this reaction, including the suppression of racemization.

In summary, the conditions for arginine-containing hydroxamate synthesis were investigated. We found conditions that allowed the coupling of Fmoc-Arg(NO_2)-Cl and free hydroxylamine analogs **5** and **6** to be carried out effectively without significant side reactions. This method should broaden the scope of arginine containing peptidomimetic compound preparation.

3. Typical procedure for Fmoc-Arg(NO_2)-Cl preparation and hydroxamate coupling

A completely dissolved solution of Fmoc-Arg(NO_2)-OH (486 mg, 1.10 mmol) in freshly distilled anhydrous THF (3 mL) was chilled in an ice-acetone bath



Scheme 4. Fmoc-Arg(NO_2)-Cl and hydroxamates **5** and **6** coupling reactions.

(−10 °C), and to this was added $SOCl_2$ (262 mg, 2.20 mmol) dropwise. The mixture was stirred under nitrogen for 1 h. Chilled anhydrous ether (20 mL) was then introduced to yield a syrupy precipitate. Maintaining low temperature, the solvent was removed under reduced pressure, and the residue was triturated with chilled anhydrous ether (20 mL). A white syrupy solid was obtained after evaporation under reduced pressure. Immediately after evaporation, an ice-cold solution of **6** (275 mg, 0.55 mmol) and 2,4,6-collidine (67 mg, 0.55 mmol) in THF (5 mL) was added to the acid chloride via a cannula. Stirring continued for 20 min at 0 °C and 20 min more at room temperature. The solvent was removed under reduced pressure, and the residue was treated with EtOAc (10 mL). The organic mixture was washed twice with 5% $NaHCO_3$, water, 0.5 N HCl, and brine, and dried over Na_2SO_4. The solution was concentrated in vacuo, and the residue was purified by flash column chromatography (EtOAc/MeOH = 19:1, $R_f = 0.45$) to afford **10** (330 mg, 74%) as a white foamy solid. ^{1}H NMR (400 MHz, acetone-d_6) δ 7.84 (d, $J = 5.6$ Hz, 4H), 7.64 (m, 4H), 7.40 (d, $J = 7.2$ Hz, 4H), 7.32 (d, $J = 5.2$ Hz, 4H), 6.73 (m, 1H), 5.26 (m, 1H), 4.88 (s, 1H), 4.51 (d, $J = 5.6$ Hz, 1H), 4.29 (m, 6H), 3.70 (m, 4H), 3.39 (s, 2H), 2.62 (m, 1H), 2.19 (d, $J = 6.4$ Hz, 1H), 2.05 (q, $J = 2.5$ Hz, 2H), 1.80 (m, 4H); ^{13}C NMR (125 MHz, acetone-d_6) 172.73, 170.40, 160.19, 156.60, 154.65, 144.41, 144.24, 141.45, 127.95, 127.43, 127.36, 125.54, 125.32, 120.22, 67.58, 66.71, 59.98, 58.51, 57.94, 53.43, 52.10, 51.87, 49.93, 47.32, 47.22, 40.89, 40.72, 33.88, 32.94, 20.28; HRMS (ES) (m/z): $M+H^+$ calcd for $C_{42}H_{44}N_7O_{10}$ 806.3150, found 806.3156. Anal. Calcd for $C_{42}H_{43}N_7O_{10}$: C, 62.60, H, 5.38, N, 12.17. Found: C, 62.40, H, 5.38, N, 11.75.

Acknowledgment

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Supplementary data

Synthetic procedures for all intermediates and HPLC experiment data. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.03.190.

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9. TLC (hexane/EtOAc, 1:1) $R_f = 0.50$; ^{1}H NMR (500 MHz, CD_3OD) δ 4.36 (t, $J = 7.5$ Hz, 1H), 4.27 (t, $J = 6.5$ Hz, 1H), 3.69 (s, 1H), 2.21 (q, $J = 6.5$ Hz, 1H), 1.94 (t, $J = 7.0$ Hz, 2H), 1.77 (t, $J = 7.0$ Hz, 1H), 1.46 (s, 9H); MS (APCI, CH_2Cl_2) $[M+H^+] = 301.7$.
10. Reagent abbreviations used: PyBroP = bromo-tris-pyrrolidinophosphonium hexafluorophosphate; DIEA = *N*,*N*-diisopropylethylamine; TFFH = fluoro-*N*,*N*,*N*′,*N*′-tetramethylformamidinium hexafluorophosphate; EDC = *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide; HATU = *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HBTU = *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HOBt = 1-hydroxybenzotriazole; BOP = benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate; PyBOP = benzotriazole-1-yl-oxy-tris-(pyrrolidino)-phosphonium.
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20. **Compound 8:** TLC (EtOAc/MeOH, 19:1) $R_f = 0.45$; ^{1}H NMR (500 MHz, acetone-d_6) δ 7.83 (d, $J = 7.5$ Hz, 4H), 7.66 (m, 4H), 7.37 (d, $J = 6.0$ Hz, 4H), 7.29 (s, 4H), 6.76 (d, $J = 8.0$ Hz, 1H), 6.54 (s, 1H), 5.22 (dd, $J = 4.5$ Hz, 1H), 4.94 (d, $J = 4.5$ Hz, 1H), 4.29 (m, 6H), 4.17 (d, $J = 6.5$ Hz, 1H), 3.70 (s, 3H), 3.41 (s, 2H),

3.31 (d, $J = 5.0$ Hz, 1H), 3.19 (d, $J = 6.0$ Hz, 1H), 2.26 (d, $J = 6.5$ Hz, 1H), 2.09 (s, 1H), 1.83 (m, 4H); ^{13}C NMR (125 MHz, acetone-d_6) 173.84, 170.29, 160.19, 156.84, 144.53, 144.49, 144.35, 144.20, 141.44, 141.41, 127.90, 127.85, 127.30, 125.61, 125.53, 125.46, 120.15, 66.78, 66.41, 56.71, 54.39, 52.08, 51.18, 47.36, 47.31, 40.99, 37.56, 27.88, 25.11; ESI (MeOH) $[M+H^+] = 794.4$.