Synthesis of Ethylene-Bridged (N^{δ} to N^{ω}) Analogues of Arginine

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Introduction

Based on insights from earlier work,[1] we have designed and synthesized a series of nonnatural analogues of the cationic, ion-pairing amino acids L-arginine (**1**, Figure 1) and L-lysine, which feature incorporation of alkyl groups into the endogenous cationic side chains to increase overall lipophilicity and modulate electrostatic character.[2]

The analogues may improve several important parameters relevant to the in vivo activity of bioactive peptides such as binding potency and selectivity, lipophilicity (and thus bloodstream and blood−brain barrier access), and stability profiles.[3] Additional interest in structural variants of L-arginine arises from the ability of related compounds, such as N^{ω}-methyl-L-arginine (**2**, Figure 1),[4] to act as nitric oxide (NO) synthase (NOS) isoform inhibitors. These enzymes produce NO during conversion of L-arginine to L-citrulline, and L-arginine analogues typically serve as competitive inhibitors. While **2** and related compounds are potent in vitro inhibitors of different NOS isoforms, their use in vivo is confounded by deguanylation by the enzyme arginase to L-ornithine, which can be enzymatically converted back to L-arginine, the substrate for NOS.[5] We envisioned that N^{δ}-alkylated analogues of L-arginine (e.g., **3a**−**c**) would hinder deguanylation by arginase and result in enhanced in vivo inhibitory activity toward NOS. Further, identification of stable compounds that selectively inhibit different isoforms of NOS is of significant clinical interest, and substitution of larger alkyl groups for the N^{ω}-methyl of **2** improves the selectivity of this group of inhibitors.[6] The synthesis and characterization of arginine analogues **3a**−**c**, designed to improve peptide bioactivity and yield improved NOS inhibitors, are described in this paper.

Results and Discussion

We have produced various N^{ω} mono- and dialkylated analogues of arginine including **2** (Figure 1) through guanylation of ornithine with the requisite methylisothiourea salt.[2a] Analogues **3a**−**c** are not accessible by this route since the δ-nitrogen of ornithine must undergo both guanylation and alkylation to form the ethylene bridge. We first envisioned that alkylation of the δ-nitrogen of ornithine with an N-alkylated 1-bromo-2-aminoethane derivative or, less directly, with dibromoethane followed by substitution with ammonia or the requisite alkylamine would provide the ethylenediamino side chain. Diamine cyclization with cyanogen bromide (CNBr) as described by Ishikawa et al.[7] would then lead to facile production of the cylic guanidinium group. However, reaction of the δ-amino functionality of L-*N*-α-Boc-ornithine with Cbz-1-amino-2-bromoethane or dibromoethane under a variety of conditions failed to produce significant product yields.

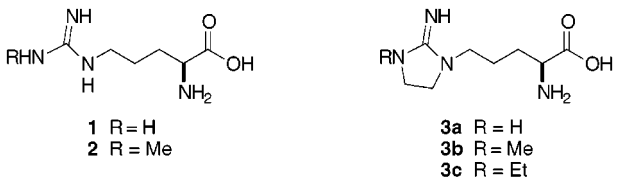
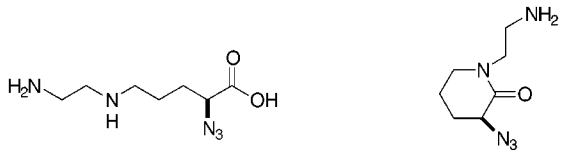


Figure 1. Arginine (**1**), N^{ω}-methylarginine (**2**), and ethylene-bridged analogues **3a**−**c**.

Reversing the alkylation procedure, with some elaboration, proved to be a more feasible route (Scheme 1). Previously, we synthesized (2*S*)-2-azido-5-bromovaleric acid (**4**) using the Evans chiral auxiliary[8] to stereoselectively introduce the α-azido group on the appropriate ω-bromo acid.[9] This strategy fixes the chiral center early, allowing the aqueous nucleophilic substitution reactions required for construction of the guanidino functionalities to be performed later in the syntheses. The α-amino group is masked as the azido functionality, a protective strategy necessary for the diamine cyclization reactions.

We first treated **4** with ethylenediamine and noted the formation of the desired N^{ω}-substituted product **8** with a major side product that proved to be the six-membered lactam[10] **9**, which was previously shown to be easily formed with analogous compounds in another laboratory.[11]



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(10) Evidence supporting lactamization was displayed in the test reaction of **4** with 30 equiv dimethylamine. The ^{1}H NMR spectra of the completed reaction mixture for the formation of (2*S*)-2-azido-N^{δ}-dimethylvaleric acid shows only one product since there is no exchangeable hydrogen on the δ-nitrogen available for cyclic condensation. This reaction follows the general nucleophilic substitution scheme used to form **7a** in the Experimental Section.

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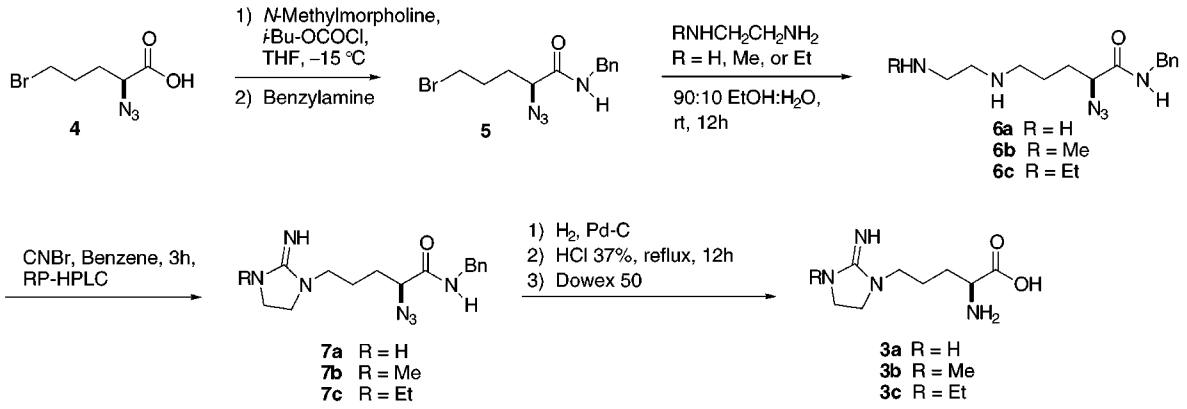
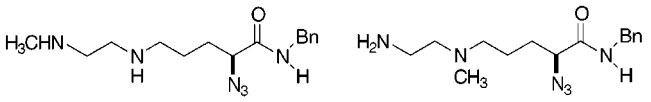
Scheme 1



We were unable to separate **8** and **9** by Dowex ion exchange and believe that further acid-catalyzed lactamization could occur during purification. This problem was circumvented by protecting the carboxylic acid of **4** as the benzyl amide **5** using the mixed anhydride method.[12] Next, **5** was treated with excess ethylenediamine or *N*-ethylethylenediamine in aqueous ethanol at room temperature overnight.[13] Products **6a** and **6c**, respectively, were extracted into butanol and treated immediately with CNBr to give crude cyclized products **7a** and **7c**. The benzyl amide enhances lipophilicity for the anhydrous cyclization and provides a means for facile RP-HPLC purification of the products. Reduction of the azido group and acid-catalyzed cleavage of the amide bond gives the cyclic arginine derivatives **3a**[14] and **3c**. Reaction of *N*-methylethylenediamine with **5**, however, resulted in significantly lower yields due to the production of a mixture of two isomers **6b** and **10**.



Cyclization was conducted on this mixture since the two products could not be resolved by RP-HPLC. Apparently, while the *N*-ethyl group provided enough steric hindrance to afford predominantly the desired ω-alkyl diamine isomer **6c**, this was not the case with **6b**. The overall yields of analogues **3a**−**c** were thus 61%, 25%, and 49%, respectively, from **4**.

Synthesis of analogues **3a**−**c** provides compounds with interesting differences in properties when compared to arginine. We have measured pK_a's of approximately 11 for **3a**−**c** by potentiometric titration,[15] which is about two units lower than arginine. The lowered basicity can be attributed to decreased resonance for the alkylated guanidinium groups[16] and the lipophilicity imparted by alkyl substituents that favors protonation on the more easily solvated exocyclic 2-imino group.[17] All of these compounds show more nonpolar character in octanol−water partitioning and HPLC experiments. Each of the analogues has been N^{α}-Fmoc-protected[18] and incorporated into short (<10 residue) peptides of potential biological interest.[3] It was not necessary to utilize the side-chain protecting groups usually required for solid-phase peptide synthesis of guanidino-containing amino acids. Currently, we are attempting to protect the side-chain functionalities so that these analogues will be better suited for lengthy solid-phase syntheses. Potency and selectivity of these compounds as NOS isoform inhibitors will be reported shortly.

Experimental Section

^{1}H and ^{13}C NMR spectra were acquired on a 300 or 400 MHz spectrometer. All chemical reagents and reagent-grade solvents were obtained from commercial suppliers. Flash chromatography was performed using Whatman silica gel 60 (230−400 mesh) and monitored using Whatman UV_{254} thin-layer chromatography plates. Semipreparative HPLC was performed on a Waters dual pump HPLC system in combination with a Kontrosorb 10C18 column using the following mobile phase system: 0.1% trifluoroacetic acid in water (solvent A) and 0.084% trifluoroacetic acid in acetonitrile (solvent B). Optical rotations were measured in 1 dm cells of 1 or 10 mL capacity. Optical purity was assessed at >95% by the use of a modified Mosher's method.[19]

(2*S*)-2-Azido-*N*-benzyl-5-bromovaleramide (5). Following the method of Anderson et al.,[12] (2*S*)-2-azido-5-bromovaleric acid (**4**) (0.96 g, 4.32 mmol) was dissolved in dry THF (27.9 mL) and equilibrated to −15 °C, at which time *N*-methylmorpholine (0.48 mL, 4.32 mmol) was added followed by isobutyl chloroformate (0.59 mL, 4.54 mmol). The temperature was maintained at −15 °C for 4 min when benzylamine (0.50 mL, 4.54 mmol) was added. The reaction was stirred mechanically at this temperature for 1 min before being allowed to warm to room temperature over 60 min. The reaction mixture was concentrated in vacuo and then partitioned between 0.1 M pH 7.5 phosphate buffer and dichloromethane. The organic fractions were pooled, dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (ethyl acetate/hexanes 1:1, $R_f = 0.32$), giving 1.21 g of the clear oil (**5**) in 90% yield: $[\alpha]^{25}_D = +10.4$ ($c = 1.0$ in MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42−7.21 (m, 5H), 4.47 (d, $J = 5.8$ Hz, 2H), 4.10 (t, $J = 5.7$ Hz, 1H), 3.44 (t, $J = 6.1$ Hz,

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2H), 2.25−1.90 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.6, 137.4, 128.8, 127.8, 127.8, 63.6, 43.6, 32.5, 30.9, 28.3.

General Procedure for Diamine Substitution and Subsequent Cyclization. (2*S*)-2-Azido-*N*-benzyl-5-(2-iminoimidazolidine)valeramide (7a). Compound **5** (342 mg, 1.10 mmol) was dissolved in EtOH/H_2O (90:10, 6.5 mL). Neat ethylenediamine (991 mg, 16.50 mmol) was added to the stirring solution and allowed to react overnight in a closed container at ambient temperature. Next, the EtOH was evaporated and the crude product extracted from 1.5 N NH_4OH into saturated butanol. The butanol fractions were combined, washed with NH_4-OH, and concentrated under reduced pressure with mild heating at 50 °C. This procedure removes most of the aqueous soluble ethylenediamine while ensuring the newly formed diamine side chain of **6a** is in the un-ionized form for subsequent cyclization. The product **6a** (ca. 95% purity) was used without further purification in the guanidinium-forming cyclization reaction following the procedure of Ishikawa et al.[7] Briefly, CNBr (112 mg, 1.10 mmol) was dissolved in benzene (3.0 mL) and stirred at room temperature. Next, **6a**, dissolved in benzene (1.0 mL), was added dropwise over 5 min to the CNBr solution. The formation of **7a** was noted by precipitation of the insoluble HBr salt. The reaction was stirred for a minimum of 3 h, upon which time the benzene was removed under reduced pressure and the product purified by RP-HPLC. Purification of about 400 mg of crude product was effected by elution at a flow rate of 6 mL/min using a linear gradient from 3 to 35% B over 60 min and monitoring the effluent by UV absorbance at 254 nm. The yield of **7a**, a yellowish oil, from **5** was 77%: $[\alpha]^{25}_D = -1.8$ ($c = 1.0$ in MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 7.28−7.12 (m, 5H), 4.33 (s, 2H), 3.90 (t, $J = 6.3$ Hz, 1H), 3.53 (s, 4H), 3.23 (t, $J = 7.0$ Hz, 2H), 1.85−1.65 (m, 2H), 1.65−1.49 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 171.8, 160.4, 139.7, 129.6, 128.7, 128.4, 64.1, 48.8, 45.0, 44.2, 41.9, 29.7, 24.0.

(2*S*)-2-Azido-*N*-benzyl-5-(2-imino-3-methylimidazolidine)valeramide (7b). The synthesis of **7b** follows the general procedure described above except that *N*-methylethylenediamine was used in place of ethylenediamine. The mixture of isomers **6b** and **10**, which could not be separated by chromatography, was treated with CNBr to give crude **7b**. Approximately 350 mg of crude product was purified by RP-HPLC as above using a linear gradient from 8 to 35% B over 60 min to give pure **7b** as a yellowish oil in 30% yield from **5**: $[\alpha]^{25}_D = +8.0$ ($c = 1.0$ in MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 7.28−7.10 (m, 5H), 4.31 (s, 2H), 3.88 (t, $J = 6.3$ Hz, 1H), 3.55−3.38 (m, 4H), 3.21 (t, $J = 7.0$ Hz, 2H), 2.86 (s, 3H), 1.83−1.63 (m, 2H), 1.63−1.46 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 171.8, 159.4, 139.7, 129.6, 128.7, 128.4, 64.0, 49.2, 46.7, 45.8, 44.2, 32.4, 29.6, 24.0.

(2*S*)-2-Azido-*N*-benzyl-5-(3-ethyl-2-iminoimidazolidine)valeramide (7c). The synthesis of **7c** follows the general procedure described above except that *N*-ethylethylenediamine was used in place of ethylenediamine. Approximately 375 mg of crude product was purified by RP-HPLC as above using a linear gradient from 8 to 30% B over 60 min. The yield was 60% for the yellowish oil **7c** from **5**: $[\alpha]^{25}_D = +15.8$ ($c = 1.0$ in MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 7.30−7.13 (m, 5H), 4.32 (s, 2H), 3.90 (t, $J = 6.3$ Hz, 1H), 3.61−3.42 (m, 4H), 3.33−3.16 (m, 4H), 1.87−1.65 (m, 2H), 1.65−1.48 (m, 2H), 1.12 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 171.8, 158.5, 139.7, 129.6, 128.7, 128.4, 64.0, 46.7, 46.2, 45.7, 44.2, 41.0, 29.6, 23.9, 12.0.

General Procedure for Final Deprotection. (2*S*)-2-Amino-5-(2-iminoimidazolidine)valeric Acid (3a). Compound **7a** (362 mg, 0.84 mmol) was dissolved in MeOH (30 mL) to which palladium, 10 wt % on activated carbon (Pd−C 10%) (25 mg), was added under nitrogen. The hydrogenation flask was placed on a Parr reactor at 30 psi and shaken overnight. Next, the Pd−C 10% was removed by filtration and the MeOH removed in vacuo. The resulting amine was then refluxed in HCl 37% overnight, at which time the reaction was cooled and evaporated to dryness under reduced pressure. The crude material was dissolved in 1.5 N NH_4OH and washed with dichloromethane to remove residual benzylamine. The final product was purified on a Dowex-50 ion-exchange column eluting with 1.5 N NH_4OH while monitoring by TLC (phenol/H_2O 3:1) with ninhydrin detection. The yield of the white solid **3a** from **7a** was 88%: $[\alpha]^{25}_D = +16.9$ ($c = 1.0$ in 6 N HCl); mp 275−300 °C dec; ^{1}H NMR (300 MHz, D_2O) δ 4.15 (t, $J = 6.2$ Hz, 1H), 3.78−3.58 (m, 4H), 3.37 (t, $J = 6.8$ Hz, 2H) 2.12−1.90 (m, 2H), 1.90−1.66 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 178.3, 165.5, 59.1, 54.2, 50.3, 47.3, 33.4, 28.7; ESI MS calcd for $C_8H_{16}N_4O_2$ (MH^+) 201.3, found 201.2.

(2*S*)-2-Amino-5-(2-imino-3-methylimidazolidine)valeric Acid (3b). Compound **7b** was used in the general procedure above to produce arginine analogue **3b**, a white solid, in 93% yield: $[\alpha]^{25}_D = -4.4$ ($c = 1.0$ in 6 N HCl); mp 275−300 °C dec; ^{1}H NMR (300 MHz, D_2O) δ 3.78 (t, $J = 5.8$ Hz, 1H), 3.64 (s, 4H), 3.34 (t, $J = 6.9$ Hz, 2H), 2.93 (s, 3H), 1.96−1.82 (m, 2H), 1.82−1.59 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 178.4, 160.6, 57.4, 50.8, 48.6, 47.4, 34.5, 30.8, 25.1; ESI MS calcd for $C_9H_{18}N_4O_2$ (MH^+) 215.3, found 215.2.

(2*S*)-2-Amino-5-(3-ethyl-2-iminoimidazolidine)valeric Acid (3c). Compound **7c** was used in the general procedure above to produce arginine analogue **3c**, a white solid, in 90% yield: $[\alpha]^{25}_D = -6.6$ ($c = 1.0$ in 6 N HCl); mp 275−300 °C dec; ^{1}H NMR (300 MHz, D_2O) δ 4.16 (t, $J = 5.5$ Hz, 1H), 3.68 (s, 4H), 3.45−3.27 (m, 4H), 2.14−1.91 (m, 2H), 1.91−1.68 (m, 2H), 1.19 (t, $J = 7.1$ Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ 178.4, 163.4, 59.2, 52.2, 51.6, 50.8, 46.4, 33.3, 28.7, 17.6; ESI MS calcd for $C_{10}H_{20}N_4O_2$ (MH^+) 229.3, found 229.2.

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Supporting Information Available: 300 MHz ^{1}H NMR spectra for **5**, **6a**, the mixture of **6b** and **10**, **6c**, **7a**−**c**, 2-imino-1-imidazolidineacetic acid, and **3a**−**c**, 400 mHz ^{1}H NMR spectrum of Mosher's derivatized **3a**, 100 MHz ^{13}C NMR spectra for **5**, **7a**−**c**, and **3a**−**c**, and ESI MS for **3a**−**c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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