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Synthetic Approaches to N^{δ}-Methylated L-Arginine, N^{ω}-Hydroxy-L-arginine, L-Citrulline, and N^{δ}-Cyano-L-ornithine

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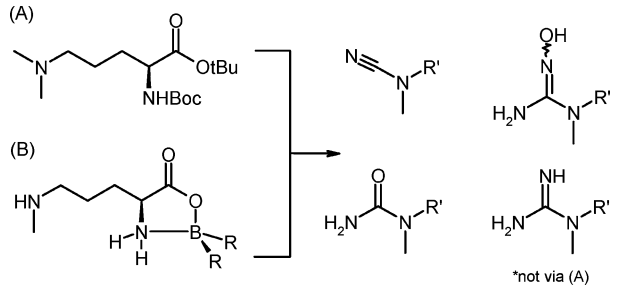

N^{ω}-Methylated arginines such as asymmetric dimethyl-L-arginine (ADMA) and monomethyl-L-arginine (NMMA) are known as potent physiological inhibitors of nitric oxide synthases (NOSs). To explore a possible physiological and pharmaceutical relevance of N^{δ}-methylated analogues, a synthetic scheme had to be developed that would not lead to N^{δ}-methyl-L-arginine only but also to its presumed metabolites of NOS catalysis. Two basic synthetic approaches have been pursued to obtain N^{δ}-methylated derivatives of L-ornithine, L-citrulline, L-arginine, and N^{ω}-hydroxy-L-arginine. A first attempt utilized conventionally protected L-ornithine, i.e., the *tert*-butyl ester and Boc-amine, and led to three end compounds in excellent yields. Simultaneous protection of the α-amino acid moiety by formation of boroxazolidinones, particularly by employing 9-borabicyclo[3.3.1]nonane (9-BBN-H), proved to be a convenient option to perform side chain modifications and led to all of the desired end compounds. Additionally, enantiomeric excess (ee, %) of crucial synthetic intermediates and end compounds was determined by chiral HPLC.

Introduction

Nitric oxide (NO) is a potent vasodilator that regulates vascular tone and tissue blood flow and inhibits platelet aggregation and leukocyte adhesion on the endothelial surface. In addition, it is generated in large quantities during host defense and immunological reactions and is synthesized in neurons of the central nervous system, where it acts as a neuromediator with many physiological functions.[1] Accordingly, it is vital that endogenous NO levels are strictly regulated. As a consequence it is not surprising that dysregulation leads or contributes to the development of numerous diseases.[2] To date, a number of strategies have emerged to provide rational control of NO levels by taking various metabolic pathways into consideration. Nevertheless, the predominant enzymes in this regulation process are nitric oxide synthases (NOSs) and arginases.[3]

Physiologically, the activity of NOSs can be regulated by the endogenous, competitive inhibitors asymmetric dimethyl-L-arginine (ADMA) and monomethyl-L-arginine (NMMA). These N^{ω}-methylated L-arginines are implicated in the development of diseases such as atherosclerosis and endothelial dysfunction.[4] The formation of N^{δ}-methyl-L-arginine **15** in yeast cells was described in 1998 and the responsible yeast enzyme was identified 1 year later.[5] However, there has not yet been reported further evidence of any physiological significance of **15**.

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(1) (a) Ignarro, L.; Murad, F., Eds. Nitric Oxide: Biochemistry, molecular biology, and therapeutic implications. In *Advances in Pharmacology*; Academic Press: San Diego, CA, 1995; Vol. 34. (b) Alderton, W. K.; Cooper, C. E.; Knowles, R. G. Nitric oxide synthases: structures, function and inhibition. *Biochem. J.* **2001**, *357*, 593.

(2) Vallance, P.; Leiper, J. *Nat. Rev. Drug Discovery* **2002**, *1*, 939.

(3) Boucher, J. L.; Moali, C.; Tenu, J. P. *Cell Mol. Life Sci.* **1999**, *55*, 1015.

(4) (a) Beltowski, J.; Kedra, A. *Pharmacol. Rep.* **2006**, *58*, 159. (b) Cardounel, A. J.; Cui, H.; Samouilov, A.; Johnson, W.; Kearns, P.; Tsai, A. L.; Berka, V.; Zweier, J. L. *J. Biol. Chem.* **2007**, *282*, 879.

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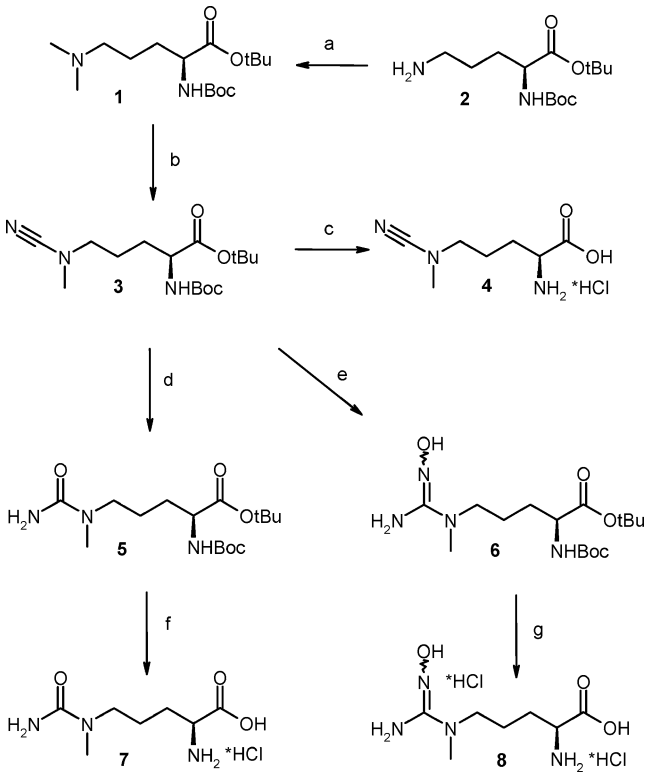
SCHEME 1. Synthetic Approach via Conventional Protecting Group Chemistry[a]



[a] Reagents and conditions: (a) $[CH_2O]_n$, Na_2CO_3, $NaBH_4$, MeOH, 85%. (b) BrCN, Na_2CO_3, dioxane, 94%. (c) HCl, dioxane, 91%. (d) H_2O_2 (3%), 1 M NaOH, EtOH, 86%. (e) NH_2OH, dioxane, 35%. (f) HCl, dioxane, 90%. (g) HCl, dioxane, 63%.

We aimed to examine a possible physiological role and pharmaceutical relevance of N^{δ}-methylated analogues by testing all enzymes that are involved in regulating endogenous NO levels, primarily NOSs and arginase.[6] The physiological process of NOS catalysis is a NADPH- and O_2-dependent five-electron oxidation of L-arginine to nitric oxide and L-citrulline via the intermediate N^{ω}-hydroxy-L-arginine,[7] requiring the synthesis of these N^{δ}-methyl analogues. The occurrence of an intermediately formed cyanamide is reported under certain conditions, also necessitating the synthesis of the respective N^{δ}-methyl analogue.[8]

Results and Discussion

From the preparative point of view it was desirable to develop (a) one common synthetic scheme that leads to all end compounds and (b) a synthetic sequence with minimum risk for racemization. Our first strategy started with the methylation of commercially available N^{α}-Boc-L-ornithine-*O*-*tert*-butyl ester **2** (Scheme 1).

Attempts to selectively monomethylate the protected L-ornithine with paraformaldehyde followed by reduction with sodium borohydride mainly resulted in the dimethylated compound **1**. This tertiary amine could then be reacted with cyanogen bromide (BrCN) in dry dioxane employing the von Braun degradation reaction.[9] The resulting cyanamide **3** was the key intermediate for the following conversion to three of the desired amino acids. Deprotection under anhydrous conditions with HCl in dioxane gave the hydrochloride of N^{δ}-cyano-N^{δ}-methyl-L-ornithine **4**, whereas partial hydrolysis of **3** under mild conditions by treatment with dilute alkaline hydrogen peroxide solution, followed by removal of the protecting groups with HCl in dioxane, led to the N^{δ}-methylated L-citrulline **7**. Building up the hydroxyguanidine moiety was achieved by reacting cyanamide **3** with hydroxylamine base[10] in dry dioxane. Eventually, deprotection yielded N^{ω}-hydroxy-N^{δ}-methyl-L-arginine as the dihydrochloride **8**. Owing to the fact that the oxygen in hydroxylamine exhibits nucleophilic properties, its reaction with the cyanamide cannot be ruled out, yielding an unstable aminoxyformamidine[11] derivative that would decompose to the respective urea. Thus, we performed ^{15}N NMR spectroscopy experiments with ^{15}N-labeled **8** to show that the reaction occurred via the nitrogen of hydroxylamine, which was the case under these reaction conditions, i.e., in aprotic solvent with hydroxylamine base (Supporting Information).[12] Essentially, all syntheses within this approach could be performed under conditions which did not significantly lead to racemization (≥98% ee), shown by enantiomeric separation of **7** and **8** on a Crownpak Cr(+) HPLC column (for details see the Supporting Information).[13] Nevertheless, N^{δ}-methyl-L-arginine as a pivotal target compound was not accessible by this route, since cyanamide **3** could not be converted to the respective guanidine compound with ammonia under various reaction conditions. We also tested a direct hydrogenation of hydroxyguanidine **8** using Pd/charcoal, H_2 in methanol (acetic acid 5% v/v) at atmospheric pressure, which proved difficult. Further attempts are underway considering successful reductions of other hydroxyguanidines described in the literature.[14]

Thus, a second methodology was established that utilized the simultaneous protection of the α-amino acid moiety of N^{δ}-methyl-L-ornithine **10** by intermediate formation of boroxazolidinones.[15] This approach was already described for the preparation of **8** and **15** by Luzzi and Marletta,[16b] who demonstrated the usability of *B*,*B*-diethyl boroxazolidinones. First, selective monomethylation of the side chain primary amine was performed in a four-step synthesis according to literature

(5) (a) Zobel-Thropp, P.; Gary, J. D.; Clarke, S. *J. Biol. Chem.* **1998**, *273*, 29283. (b) Niewmierzycka, A.; Clarke, S. *J. Biol. Chem.* **1999**, *274*, 814.

(6) Kotthaus, J.; Schade, D.; Töpker-Lehmann, K.; Beitz, E.; Clement, B. *Bioorg. Med. Chem.* 2007, doi: 10.1016/j.bmc.2007.11.066.

(7) Stuehr, D. J.; Kwon, N. S.; Nathan, C. F.; Griffith, O. W.; Feldman, P. L.; Wiseman, J. *J. Biol. Chem.* **1991**, *266*, 6259.

(8) (a) Clague, M. J.; Wishnok, J. S.; Marletta, M. A. *Biochemistry* **1997**, *36*, 14465. (b) Rusche, K. M.; Spiering, M. M.; Marletta, M. A. *Biochemistry* **1998**, *37*, 15503.

(9) Braun, V. J *Ber. Dtsch. Chem. Ges.* **1900**, *33*, 1438.

(10) Hydroxylamine base was set free from its hydrochloride with sodium ethanolate. Steudel, R.; Schenk, P. W. In *Handbuch der präparativen anorganischen Chemie*, 3rd ed.; Brauer, G., Ed.; Ferdinand Enke Verlag: Stuttgart, Germany, 1975; Vol. 1, p 464.

(11) (a) Zinner, G.; Kleinau, V. *Chem.-Z.* **1977**, *10*, 451. (b) Belzecki, C.; Hintze, B.; Kwiatkowska, S. *Bull. Acad. Pol. Sci. Chem.* **1970**, *18*, 379.

(12) Similar results were obtained for $^{15}N^{\omega}$-hydroxy-L-arginine: Clement, B.; Schnörwangen, E.; Kämpchen, T.; Mordvintcev, P.; Mülsch, A. *Arch. Pharm.* **1994**, *327*, 793.

(13) Enantiomeric excess of cyanamide **4** was not determined due to its hydrolytic instability in the required eluent, i.e. aq $HClO_4$, pH 1.5.

(14) (a) Looper, R. E.; Runnegar, M. T.; Williams, R. M. *Tetrahedron* **2006**, *62*, 4549. (b) Nordmann, R.; Loosli, H.-R. *Helv. Chim. Acta* **1985**, *68*, 1025.

(15) Nefkens, G. H. L.; Zwanenburg, B. *Tetrahedron* **1983**, *39*, 2995.

(16) (a) Diethylboryl complexation procedure under these conditions first described by Garcia et al. *Tetrahedron*: *Asymmetry* **2000**, *11*, 991. (b) Luzzi, S. D.; Marletta, M. A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3934. (c) Described by Luzzi and Marletta for the diethylboryl complexes.

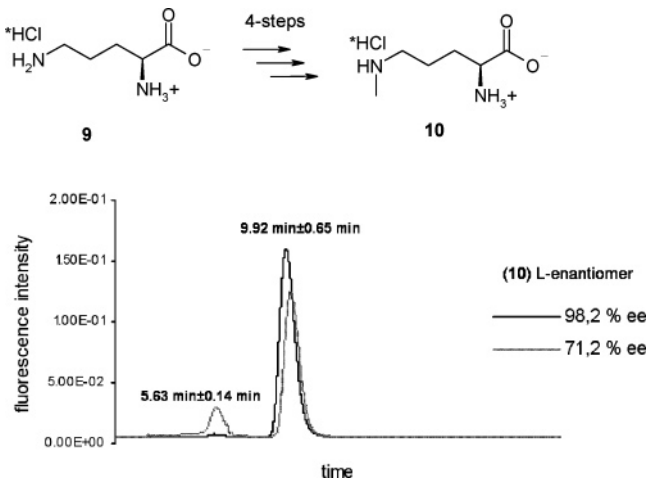


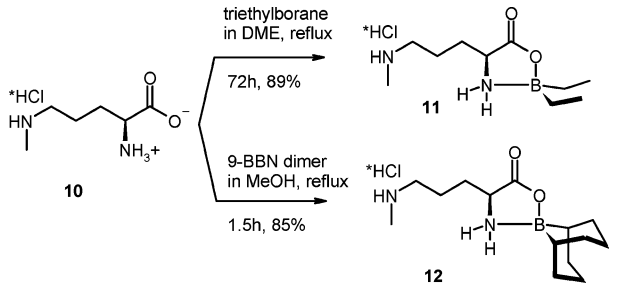
FIGURE 1. Synthesis and chiral chromatography of N^{δ}-methylornithine **10** on a Crownpak Cr(+) column using *o*-PA for postcolumn derivatization. See the Supporting Information for details.

procedures[16b,17−23] with minor modifications, after evaluating the problematic nature of racemization (Figure 1).[24]

Several authors reported on the synthesis of **10** but only a few specified α_D-values, leaving open the question of enantiomeric purity. In particular, the final deprotecting step with refluxing HBr (47%) turned out to have a major impact on racemization, which was confirmed by determining two direct synthetic precursors to be >98% enantiomerically pure (Supporting Information). Reaction times should not exceed 1.5 h and in addition, HBr should be quickly removed in high vacuum (<1 mbar) at low temperature, i.e., not exceeding 50−60 °C. This way 98% ee of the L-enantiomer could be prepared in good yields (84% for the last deprotecting step). Figure 1 also demonstrates the consequence of longer reaction times (2 h) and distillation at higher temperatures (80−90 °C), which was a 71% ee N^{δ}-methylornithine batch.

Compound **10** was converted to the diethylboryl complex **11** by refluxing with triethylborane (1 M solution in THF) in DME[16a,b] for 72 h (Scheme 2). However, rather long reaction times, the fact that **11** could not be isolated in high purity, and difficulties in subsequent reactions prompted us to choose 9-borabicyclo[3.3.1]nonane (9-BBN-H) as an alternate complexing reagent. 9-BBN-H was easily and efficiently introduced by refluxing it with **10** in methanol for 1.5 h according to the protocol of Dent et al.[25]

SCHEME 2. Formation of Boroxazolidinone Complexes

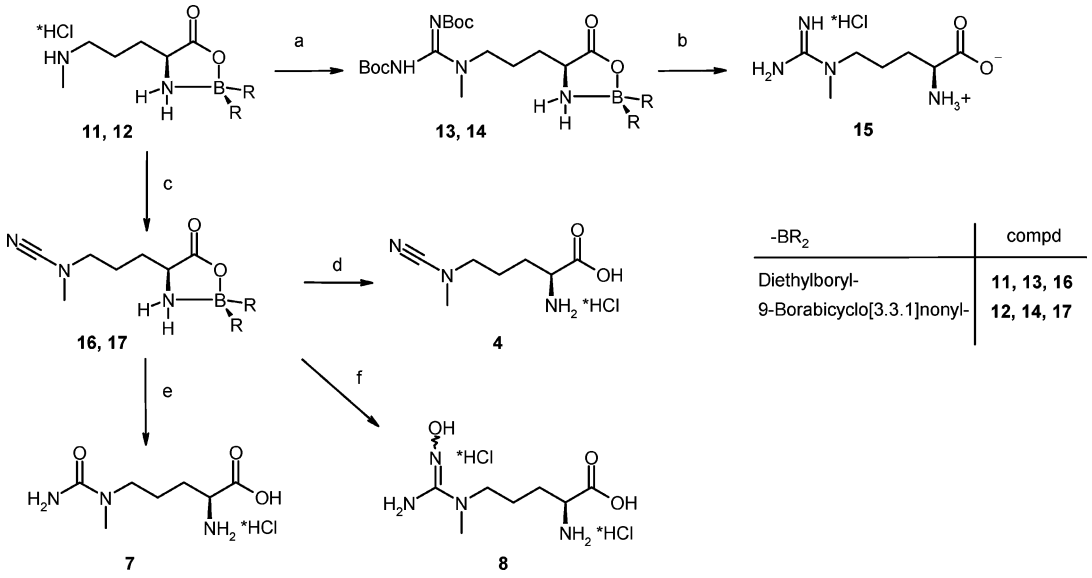


Both boron complexes were submitted to the following side chain manipulations and identical chemical procedures were employed (Scheme 3). Building up the guanidine group from **11** or **12** was accomplished with *N*,*N*′-bis(*tert*-butyloxycarbonyl)thiourea in DMF with triethylamine as the base.[16c] The protected intermediates **13** and **14** could be isolated and completely characterized.[26] Removal of both the Boc-groups and the boron complexes was carried out under mild conditions in one pot by treating **13** or **14** with TFA and subsequently with aqueous 1.5 M HCl, since boroxazolidinones appeared to be quite stable to TFA.[27] N^{δ}-Methyl-L-arginine was isolated as the monohydrochloride **15** after cation exchange chromatography. Converting boroxazolidinone-protected N^{δ}-methyl-L-ornithine to the corresponding cyanamides **16** and **17** was performed in dry DME[16c] with BrCN after unsuccessfully testing a variety of other etherial solvents to improve yields of this step. Particularly, the 9-BBN-strategy was expected to significantly increase solubility in Et_2O, THF, or dioxane and thereby improve this yield-limiting step. However, compound **4** precipitated as the hydrochloride by flushing gaseous HCl over a solution of **16** or **17** in dry dioxane. Partial hydrolysis to the citrulline derivative was realized by treating **16** and **17** with TFA (60%, aqueous) in acetone, taken the hydrolytic instability of boron complexes in neutral to alkaline aqueous media into consideration.[27]

N^{ω}-Hydroxy-N^{δ}-methyl-L-arginine **8** was obtained directly by reacting hydroxylamine hydrochloride with **16** or **17** in dry methanol.[16c] Thereby, the boroxazolidinones are cleaved, but building up the hydroxyguanidine moiety apparently proceeds faster. Under these conditions it cannot be circumvented that the respective citrulline derivative **7** originates as a side product (1−1.5%) via the mechanism discussed above. This contamination increases with prolonged reaction times, and 2.5 h was found to be the ideal compromise between yield and side product. Attempts to avoid this side reaction were undertaken by changing to aprotic solvents but with concomitant, major losses in yield again. Chromatographic workup was then performed on a cellulose column and afforded N^{ω}-hydroxy-N^{δ}-methyl-L-arginine as the free amino acid that was subsequently isolated as the dihydrochloride **8** by flushing gaseous HCl over a suspension in dioxane.[28]

(17) Rudinger, J.; Gut, V. *Tetrahedron* **1968**, *24*, 6351.
(18) Erlanger, B. F.; Sachs, H.; Brand, E. *J. Am. Chem. Soc.* **1954**, *76*, 1806.
(19) Yamamoto, H.; Yang, J. T. *Biopolymers* **1974**, *13*, 1093.
(20) Benoiton, L. *Can. J. Chem.* **1964**, *42*, 2043.
(21) Thomas, K.; Kapfhammer, J.; Flaschenträger, B. *Hoppe-Seyler's Z. Physiol. Chem.* **1923**, *125*, 75.
(22) Klein, C.; Schulz, G.; Steglich, W. *Liebigs Ann. Chem.* **1983**, 1623.
(23) Paik, W. K.; Paik, K. P.; Sangduk, K. *Anal. Biochem.* **1980**, 104, 343.
(24) Protocols of different authors for synthetic intermediates leading to **10** and the final deprotecting step were considered and evaluated. Detailed synthetic protocols are provided in the Supporting Information along with the references. Note: So far reported characterization data were in most cases incomplete and are summarized in the Supporting Information.
(25) (a) Dent, W. H., III; Erickson, W. R.; Fields, S. C.; Parker, M. H.; Tromiczak, E. G. *Org. Lett.* **2002**, *4*, 1249. (b) Further articles reporting about similar application of 9-BBN-H: Cudic, M.; Mari, F.; Fields, G. B. *J. Org. Chem.* **2007**, *72*, 5581. Syed, B. M.; Gustafsson T.; Kihlberg, J. *Tetrahedron* **2004**, *60*, 5571. Walker, W. H., IV; Rokita, S. E. *J. Org. Chem.* **2003**, *68*, 1563.
(26) A complete spectroscopic characterization of the herein described complexed intermediates is given in the Supporting Information, including 2D NMR experiments with 9-BBN complexes.
(27) 9-BBN complex **12** was examined in terms of pH stability (HPTLC experiments, data not shown). It withstands even concentrated TFA over several hours, making it suitable in orthogonal sets, but hydrolyzes rapidly in dilute HCl as well as at pH >6. Maximum stability was observed at pH 2−4.
(28) Hydroxyguanidines are known to be most stable as salts of strong acids. Literature: (a) hydroxyguanidine: Walker, J. B.; Walker, M. S. *J. Biol. Chem.* **1959**, *234*, 1481. (b) N^{ω}-hydroxy-L-arginine: Fukuto, J. M. *Meth. Enzymol.* **1996**, *268*, 365.

SCHEME 3. Synthetic Approach via Boron Complexing Strategy[a]



[a] Reagents and conditions: (a) *N*,*N*′-Bis(*tert*-butyloxycarbonyl)thiourea, $HgCl_2$, TEA, DMF, 41%. (b) Concentrated TFA, 1.5 N HCl, 85%. (c) BrCN, TEA, DME, 29%. (d) $HCl_{(g)}$, dioxane, 75−85%. (e) 60% $TFA_{(aq)}$, acetone; $HCl_{(g)}$, dioxane, 60−80%. (f) $NH_2OH{\cdot}HCl$, TEA, MeOH; $HCl_{(g)}$, dioxane, 80%.

TABLE 1. Overall Yields (%) for End Compounds

entry	route A[a]	route B[b]
4	72.7 (30.8)	8.9
7	61.8 (26.2)	8.4
8	17.6 (7.5)	8.4
15	N.A.	12.6

[a] Conventional protecting strategy starting from commercially available N^{α}-Boc-L-ornithine-*O*-*tert*-butyl ester **2**. It is to be noted that **2** is prepared in a four-step synthesis from L-ornithine in a 42.4% overall yield.[28b,29,30] Thus, for comparibility reasons, % values in parentheses represent yields from L-ornithine. [b] Boron complexing strategy (9-BBN-H approach).

In conclusion, the presented boron complexing methodology proved to be suitable for the herein described side chain modifications and therefore underlines its specific applicability in amino acid chemistry. 9-BBN-H as the complexing reagent was superior to the respective diethylboryl complexes in terms of ease of introduction, purity of intermediates, stability, and yield in places. Importantly, no significant racemization took place, either after obtaining optically pure N^{δ}-methyl-L-ornithine **10** or starting from **2**. This fact is vital for the accurate determination of K_i values for NOS inhibition and testings with all other enzymes involved in the nitric oxide pathway. Besides overall yields, the conventional protecting scheme might be preferred with respect to building up a hydroxyguanidine moiety, considering its stability toward hydroxylamine and the discussed formation of the unwanted urea derivative.

Experimental Section

N^{α}-(*tert*-Butyloxycarbonyl)-N^{δ}-dimethyl-L-ornithine *tert*-Butyl Ester (1) N^{α}-(*tert*-Butyloxycarbonyl)-L-ornithine *tert*-butyl ester hydrochloride (1.14 g, 3.5 mmol) was dissolved in CH_2Cl_2 and the corresponding base was set free by treatment with gaseous NH_3. The suspension was filtered and the organic solvent removed in vacuo. The resulting yellow oil was dissolved in 30 mL of dry MeOH and 1.8 g of Na_2CO_3 and 440 mg of paraformaldehyde (13.9 mmol) was added. This suspension was stirred for 2 h before adding a 3-fold excess of $NaBH_4$ (364 mg, 10.4 mmol). After 24 h the reaction was quenched by adding water and adjusting the pH with 1 M HCl to 7−8. MeOH was removed under reduced pressure, the water phase was adjusted to pH 4 with acetic acid, and unreacted starting material was extracted with CH_2Cl_2. The product was obtained by another extraction with CH_2Cl_2 at pH 8. The organic phases were dried with Na_2SO_4 and evaporated in a vacuum. Further purification was realized by chromatography on silica gel (CH_2Cl_2/MeOH, 8.5:1.5). The title compound crystallized from the collected fractions in the refrigerator. Yield 941 mg (85%). Mp 46 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.38 (s, 9H), 1.39 (s, 9H), 1.46−1.53 (m, 4H), 2.28 (s, 6H), 2.44 (t, 2H, ^3J = 6.5 Hz), 3.76 (t, 1H, ^3J = 6.0 Hz), 7.18 (d, 1H, ^3J = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 23.3, 28.0, 28.4, 30.7, 45.2, 53.9, 59.2, 79.2, 81.5, 155.5, 172.0.

N^{α}-(*tert*-Butyloxycarbonyl)-N^{δ}-cyano-N^{δ}-methyl-L-ornithine *tert*-Butyl Ester (3). 1 (500 mg, 1.58 mmol) was dissolved in 3 mL of dry 1,4-dioxane and the solution was added dropwise to a solution of 270 mg of Na_2CO_3 and 178 mg (1.75 mmol) of cyanogen bromide in 3 mL of 1,4-dioxane under nitrogen atmosphere. After 20 h of stirring Na_2CO_3 was filtered off and the reaction mixture was concentrated in a vacuum to give an oily residue. The crude product was purified by flash chromatography (EtOAc/*n*-pentane, 10:7). Yield 486 mg (94%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.39 (s, 9H), 1.40 (s, 9H), 1.57−1.75 (m, 4H), 2.78 (s, 3H), 2.95 (t, 2H, ^3J = 6.5 Hz), 3.79 (m, 1H), 7.15 (d, 1H, ^3J = 7.9 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 23.0, 27.9, 28.2, 29.8, 38.8, 52.4, 53.2, 79.7, 82.2, 118.2, 155.3, 171.3.

N^{δ}-Cyano-N^{δ}-methyl-L-ornithine Hydrochloride (4). From 3: A 291 mg (0.889 mmol) sample of the protected cyanamide **3** was dissolved in 7.5 mL of 4 M HCl in dry 1,4-dioxane and the solution was allowed to stand for 12 h under nitrogen atmosphere. The precipitated hydrochloride was filtered under a nitrogen stream, washed with dry EtOAc, and stored under nitrogen at −20 °C due to its high hygroscopicity and hydrolytic instability. Yield 168 mg (91%). **From 16, 17:** A 0.288 mmol sample of thoroughly dried **16** or **17** was dissolved in 14 mL of dry 1,4-dioxane and a stream of HCl was flushed over the liquid. After 24 h in the refrigerator the desired compounds precipitated. For complete precipitation, dry Et_2O was added and **4** filtered under a nitrogen stream. Yield 44.8−50.8 mg (75−85%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.64−1.86 (m, 4H), 2.81 (s, 3H), 3.01 (t, 2H, ^3J = 6.9 Hz), 3.90 (m, 1H), 8.58 (s, 3H), 8.97 (br s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ

22.4, 26.8, 38.1, 51.4, 51.6, 118.4, 170.6; HRMS *m*/*z* calcd for $C_7H_{14}N_3O_2$ (MH^+) 172.10805, found 172.10805.

***N*$^{\alpha}$-(*tert*-Butyloxycarbonyl)-*N*$^{\delta}$-methyl-L-citrulline *tert*-Butyl Ester (5).** A 196 mg (0.6 mmol) sample of the protected cyanamide **3** were dissolved in 17 mL of EtOH. After adding 58 mL of a dilute (3%) aqueous H_2O_2 solution and 1.15 mL of 1 M NaOH the reaction mixture was stirred for 4 h. The title compound was extracted twice with CH_2Cl_2, then the combined organic phases were dried with Na_2SO_4 and evaporated under reduced pressure. The resulting oil was subjected to flash chromatography (CH_2Cl_2/MeOH, 7:1) and the purified product crystallized upon drying in vacuo. Yield 178 mg (86%). Mp 40 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.38 (s, 9H), 1.39 (s, 9H), 1.46−1.59 (m, 4H), 2.73 (s, 3H), 3.10 (t, 2H, ^3J = 6.4 Hz), 3.76 (m, 1H), 5.71 (s, 2H), 7.11 (d, 1H, ^3J = 7.6 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 23.4, 28.0, 28.3, 29.7, 34.5, 48.5, 53.2, 79.8, 82.1, 155.7, 158.9, 171.8.

***N*$^{\alpha}$-(*tert*-Butyloxycarbonyl)-*N*$^{\omega}$-hydroxy-*N*$^{\delta}$-methyl-L-arginine *tert*-Butyl Ester (6). 3** (373 mg, 1.14 mmol) was dissolved in 4 mL of dry 1,4-dioxane and the solution was added dropwise to a solution of 57 mg (1.7 mmol) of hydroxylamine in 2 mL of 1,4-dioxane. The solution was then stirred for 12 h. The solvent was evaporated under reduced pressure and the crude product was further purified by flash chromatography (CH_2Cl_2/MeOH/AcOH, 8.5:1.5:0.05). Pooled fractions were concentrated on a rotary evaporator. To remove remaining acetic acid, toluene was added and evaporated again. The purified compound was obtained as a yellow oil. Yield 144 mg (35%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.37 (s, 18H), 1.51−1.59 (m, 4H), 2.88 (s, 3H), 3.24 (t, 2H), 3.78 (m, 1H), 7.11 (d, 1H, ^3J = 7.5 Hz), 7.71 (s, 2H), 9.83 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 23.0, 27.9, 28.0, 28.4, 36.3, 50.4, 53.7, 80.0, 82.3, 156.2, 158.1, 172.0.

***N*$^{\delta}$-Methyl-L-citrulline Hydrochloride (7). From 5:** The protected compound **5** (311 mg, 0.9 mmol) was dissolved in 9 mL of 4 M HCl in dry 1,4-dioxane and the solution was allowed to stand for 20 h under nitrogen atmosphere. For complete precipitation, dry Et_2O was added and the highly hygroscopic title compound was obtained by filtration under a nitrogen stream. The product was washed with dry EtOAc and stored at −20 °C under nitrogen due to its high hygroscopicity. Yield 183 mg (90%). **From 16, 17: 16** or **17** (0.144 mmol) was dissolved in 3 mL of acetone and 1 mL of TFA (60%) was added. The solution was stirred for 2−3 h at room temperature and concentrated in vacuo. Completion of the reaction was monitored via TLC (isopropanol/water/AcOH, 6:3:1, R_f 0.68). $HCl_{(aq)}$ (10 mL, 1.5 M) was added and the mixture was stirred for another 2 h at room temperature. The product was purified by column chromatography on microgranular cellulose (ACN/water, 5:3). After removal of the solvent the oily product was dispersed in 1,4-dioxane and gaseous HCl was flushed over the liquid to obtain the hydrochloride **5**. Yield 122−163 mg (60−80%). Ee 98.1% (Crownpak Cr(+)). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.51−1.76 (m, 4H), 2.76 (s, 3H), 3.18 (t, 2H, ^3J = 6.7 Hz), 3.90 (m, 1H), 5.18 (s, 2H), 8.38 (s, 3H), 13.68 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 22.8, 27.0, 34.3, 47.3, 51.7, 159.0, 170.8; HRMS *m*/*z* calcd for $C_7H_{16}N_3O_3$ (MH^+) 190.11862, found 190.11849.

***N*$^{\omega}$-Hydroxy-*N*$^{\delta}$-methyl-L-arginine Dihydrochloride (8). From 6:** The protected L-arginine derivative **6** (319 mg, 0.885 mmol) was dissolved in 6.4 mL of 4 M HCl in dry 1,4-dioxane and left for 18 h under nitrogen. Complete precipitation was achieved by adding dry Et_2O. The highly hygroscopic compound was filtered under a nitrogen stream and washed several times with dry EtOAc. It was best stored under nitrogen at −20 °C. Yield 147 mg (63%). **From 16, 17:** Synthesis from **16** and **17** was performed according to ref 15b with the following alterations: reaction times were 2.5 h at room temperature. After purification by column chromatography on microgranular cellulose (ACN/0.1% $TFA_{(aq)}$, 5:3) the oil was dispersed in 1,4-dioxane and gaseous HCl was flushed over the liquid. The product crystallized after standing in the refrigerator and was isolated after 24 h by filtration. Yield 76.4 mg (80%). Ee 98.1% (Crownpak Cr(+)). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.62−1.79 (m, 4H), 2.92 (s, 3H), 3.32 (m, 2H), 3.91 (m, 1H), 7.90 (s, 1H), 7.71 (s, 2H), 8.47 (s, 3H), 9.98 (s, 1H), 10.53 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 22.2, 26.6, 35.7, 48.5, 51.5, 157.9, 170.6; ^{15}N NMR (DMSO-d_6, ^{15}N-labeled compound, measuring time 5 h, INEPT 3 h) δ 136.6 (d, $^1J_{(^{15}N-^1H)}$ = 97.5 Hz, NHOH); HRMS *m*/*z* calcd for $C_7H_{17}N_4O_3$ (MH^+) 205.12952, found 205.12956.

Preparation of *N*$^{\delta}$-Methyl-L-ornithine (10): Summary. First, the preparation of *N*$^{\delta}$-(*p*-toluenesulfonyl)-L-ornithine **10a** was accomplished according to the literature[17] but using Na_2EDTA for the removal of Cu(II) instead of H_2S. Yield 71%. α^{20}_D +21.0 (2%, 6 N HCl) [lit.[18,19] α^{23}_D +20.8 (2%, 6 N HCl)]. Subsequent conversion to *N*$^{\alpha}$-benzoyl-*N*$^{\delta}$-(*p*-toluenesulfonyl)- L-ornithine **10b** was performed with benzoylchloride in 1 M NaOH.[16b,20] Yield 79%. Ee 99.2% ((*R*,*R*)-Whelk O1); α^{20}_D −5.0 (2%, DMF) [lit.[20] α^{23}_D −3.0 (2%, DMF)]. Methylation to *N*$^{\alpha}$-benzoyl-*N*$^{\delta}$-methyl-*N*$^{\delta}$-(*p*-toluenesulfonyl)- L-ornithine **10c** was realized using dimethyl sulfate in 2 equiv of NaOH.[21,22] Yield 90%. Ee 98.8% ((*R*,*R*)-Whelk O1); α^{20}_D −4.2 (2%, DMF) [lit.[20] α^{23}_D −3.0 (2%, DMF)]. *N*$^{\delta}$-Methyl-L-ornithine was obtained by deprotection in refluxing hydrobromic acid (47%).[16b,23] In terms of racemization it is of importance to terminate the reaction after 1.5 h and to remove HBr in a high vacuum (<1 mbar), not above 50−60 °C. The product was recrystallized from ethanol. Yield 84%. Mp 243 °C; Ee 98.2% (Crownpak Cr(+)); α^{20}_D +23.2 (2%, 6N HCl) [lit.[20] α^{23}_D +19.7 (2%, 6 N HCl); lit.[31] α^{20}_D +25.5 (2%, 6 N HCl)].

9-Borabicyclo[3.3.1]non-9-yl[*N*$^{\delta}$-methyl-L-ornithinato-*O*,*N*]-boron Hydrochloride (12). 10 (2.0 g, 11 mmol) was added to a stirred, hot solution of 1.61 g of crystalline, dimeric 9-borabicyclo-[3.3.1]nonane (13.2 mmol monomer) in 24 mL of MeOH under nitrogen atmosphere and refluxed for 1.5 h. The suspension was filtered and the residue was washed two times with *n*-hexane and Et_2O. Yield 2.83 g (85%). Mp 275 °C dec. ^{1}H NMR (DMSO-d_6/$CDCl_3$ 1:1, 300 MHz) δ 0.51 (s, 1H), 0.56 (s, 1H), 1.43 (m, 2H), 1.58 (m, 2H), 1.62−2.07 (m, 12H), 2.59 (s, 3H), 2.94 (m, 2H), 3.57 (m, 1H), 5.87 (br t, 1H), 6.45 (dd, 1H), 9.02 (s, 2H); ^{13}C NMR (DMSO-d_6/$CDCl_3$ 1:1, 75 MHz) δ 22.3, 22.5, 23.6, 23.9, 24.3, 27.4, 30.97, 31.04, 31.2, 31.3, 32.6, 48.0, 53.9, 173.7; LRMS (ESI) 267 (MH^+).

9-Borabicyclo[3.3.1]non-9-yl[*N*$^{\omega}$,*N*$^{\omega'}$-bis(*tert*-butyloxycarbonyl)-*N*$^{\delta}$-methyl-L-argininato-*O*,*N*]boron (14). Guanylation of **12** was performed according to ref 15b. Yield 302 mg (41%). Mp 110 °C (foaming; 270 °C dec). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 0.48 (s, 1H), 0.52 (s, 1H), 1.27−1.50 (m, 20H), 1.50−1.90 (m, 14H), 2.90 (s, 3H), 3.33 (m, 2H), 3.53 (m, 1H), 5.81 (br dd, 1H), 6.38 (br dd, 1H), 9.52 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 22.2, 23.4, 23.5, 23.8, 24.2, 27.4, 27.9, 30.7, 31.1, 31.2, 35.6, 48.9, 54.2, 151.1, 152.0, 159.9, 173.3; LRMS (ESI) 457 (MH^+).

9-Borabicyclo[3.3.1]non-9-yl[*N*$^{\delta}$-cyano-*N*$^{\delta}$-methyl-L-ornithinato-*O*,*N*]boron (17). Reaction of **12** with cyanogen bromide was performed according to ref 15b. Yield 135 mg (29%). Mp 235 °C. ^{1}H NMR (DMSO-d_6) δ 0.49 (s, 1H), 0.53 (s, 1H), 1.42 (m, 2H), 1.50−1.97 (m, 14H), 2.59 (s, 3H), 3.00 (qt, 2H), 3.54 (m, 1H), 5.84 (br t, 1H), 6.43 (br dd, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 22.2, 23.4, 23.6, 23.8, 24.1, 27.3, 30.7, 31.1, 31.2, 38.2, 51.7, 54.0, 118.5, 173.3; LRMS (ESI) 292 (MH^+).

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Supporting Information Available: General experimental methods, reagents, and material; complete synthetic protocols and spectral data for compounds **1**−**17**; elemental analyses and HPLC

(29) Harris, J. I.; Work, T. S. *Tetrahedron Lett.* **2006**, *47*, 5159.
(30) Feldman, P. L. *Tetrahedron Lett.* **1991**, *32*, 875.
(31) Belyaev, A. A.; Krasko, E. V. *Synthesis* **1991**, *5*, 417.

chromatograms for purity assessment; chiral chromatography on Crownpak Cr(+) and (*R*,*R*)-Whelk O1; 1D ^{1}H, ^{13}C NMR spectra; 2D NMR spectra of **12** and **14**; IR spectra of boron-complexed cyanamides **16** and **17**; and ^{15}N NMR spectrum of ^{15}N-labeled **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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