Efficient Synthesis of Optically Pure N$^{\omega}$-Alkylated L-Arginines

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Abstract: A number of N$^{\omega}$-substituted L-arginines have been described to date, particularly with regard to nitric oxide synthase (NOS) modulators. Elaborate multistep syntheses and low yields limit the scope of preparing these modified L-arginines. We describe a synthetic methodology that delivers N$^{\omega}$-alkylated L-arginine derivatives from protected L-ornithine in a three-step sequence with excellent overall yields (81–90%) and in high purity. Analysis of the synthesized amino acids on a Crownpak Cr(+) column revealed no significant racemization, that is, >99.9% ee for all final compounds.

Key words: nitric oxide, *N*$^{\omega}$-alkyl, L-arginine, NOS, DDAH

Nitric oxide (NO) is involved in various physiological processes, such as maintenance of vascular tone, neuronal signaling, and host response to infection.[1] Over- or underproduction of NO contributes to numerous diseases.[2] Therefore, it is vital that endogenous NO levels are strictly regulated. Figure 1 depicts the predominant enzymes in this regulation process, which consequently present to be the major targets for potential NO modulators.

Due to its ubiquitous occurrence and versatile physiological roles, simple elevation or reduction of NO is likely to cause side-effects. Thus, it may be beneficial to rather smoothly affect NO levels by indirect modulation of dimethylarginine dimethylaminohydrolase (DDAH) activity, for example, inhibit DDAH and thereby attenuate NO production instead of completely inhibit NOSs.[5] However, the development of modulators of endogenous NO levels implies to derivatize L-arginine, the pivotal amino acid in NO-related metabolism. In particular, the investigation of novel inhibitors of NOSs has led to several L-arginine analogues, such as *N*$^{\omega}$-allyl-L-arginine or *N*$^{\omega}$-propargyl-L-arginine.[6,7] In contrast, not many potent inhibitors of the attractive molecular target DDAH, besides *N*$^{\omega}$-methoxyethyl-L-arginine, are known[8,9] (Table 1).

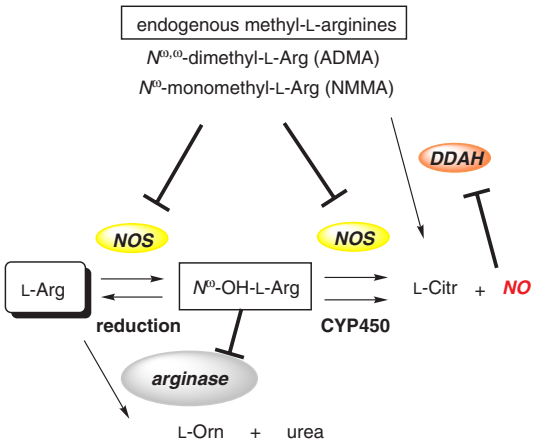
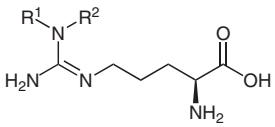


Figure 1 Physiological regulation of endogenous NO-levels. L-Citr = L-citrulline, L-Orn = L-ornithine, NOS = nitric oxide synthase (EC 1.14.13.39), DDAH = dimethylarginine dimethylaminohydrolase (EC 3.5.3.18), arginase (EC 3.5.3.1), microsomal/mitochondrial reduction: see ref. 3, CYP450-mediated oxidation: see ref. 4.

Table 1 Selected N$^{\omega}$-Substituted L-Arginines as Known NO Modulators



Entry	R^1	R^2	Compound
NOS inhibitors			
1	Me	H	NMMA[10]
2	Me	Me	ADMA[11]
3	H_2C=$CHCH_2$	H	**6c**[6]
4	HC≡CCH_2	H	**6e**[7]
NOS substrate			
5	MeO	H	**6a**[12]
DDAH inhibitor			
6	$MeOCH_2CH_2$	H	**6b**[8]

In our studies, we aim to investigate L-arginine based DDAH-inhibitors and also test selectivity of known NOS modulators for NOS over DDAH. A modification at the N$^{\delta}$-position of L-arginine on the NO-generating system has already been extensively investigated in our laboratory.[13] However, an efficient synthetic scheme is required that would deliver a set of differentially substituted L-arginines at the N$^{\omega}$-position.

There are several guanylating agents which deliver more or less substituted guanidines. Frequently used agents are pyrazole-1-carboxamidines, thioureas, and isothioureas.[14] The cyanamide-guanidine strategy is limited by relatively low reactivity of the cyanamide towards amines. For example, protected *N*$^{\omega}$-propargyl-L-arginine was prepared

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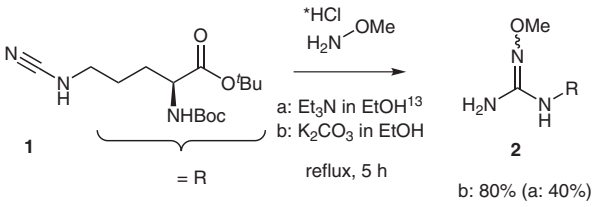
from its cyanamide in 32% yield.[7] We also attempted to prepare N^{ω}-methoxy-L-arginine from its cyanamide by reaction with methoxylamine hydrochloride according to Wagenaar et al., which was not reproducible with respect to the stated yield of 97%.[15] By using K_2CO_3 as the base and a different solvent system for column chromatography we were able to build up the *N*-methoxyguanidine moiety at most with an 80% yield (Scheme 1).



Scheme 1 'Cyanamide-guanidine' strategy: Preparation of methoxyguanidine moiety in protected N^{ω}-methoxy-L-arginine

Other groups reported yields for similar guanidines in the range of 60%.[16] Furthermore, within the syntheses of N^{δ}-substituted L-arginine analogues, we prepared hydroxyguanidines in 35–80% yields by treating cyanamides with hydroxylamine.[17] Consequently, this approach appears more or less limited to few N-nucleophiles.

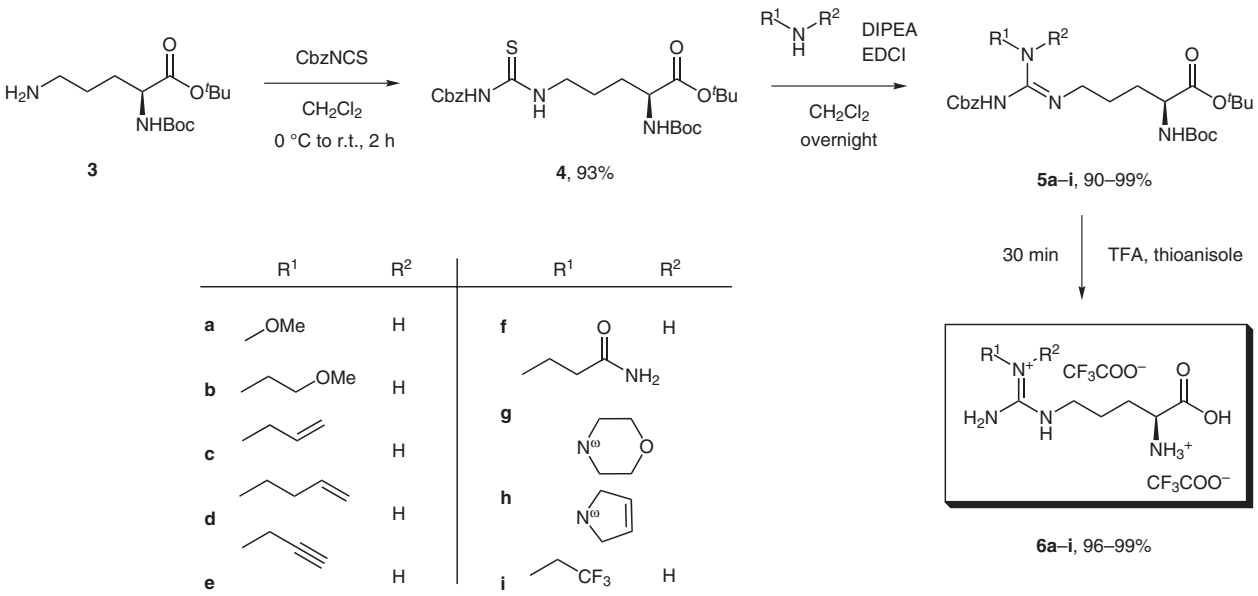
Rossiter et al. described the preparation of chain-shortened L-arginines by means of using *N*-alkyl-substituted *N*,*N*′-bis-Boc-1-carboxamidines.[8] The need to initially prepare different types of these reagents under Mitsunobu conditions and low overall yields (20–50%) may limit the scope of this approach. The most potent arginine-based DDAH-inhibitor N^{ω}-methoxyethyl-L-arginine was prepared this way in 41% overall yield. An optimized synthesis of N^{ω}-allyl-L-arginine was described by Bernatowicz et al. who also utilized a substituted pyrazole-1-carboxamidine.[18] Although overall yields were good (70%), disadvantages are the long reaction times (5 d) and again the required preparation of the guanylating reagent in two steps.

Another general procedure for the synthesis of N^{ω}-alkyl and N^{ω}-aryl-L-arginines was described by Chen et al. who employed *S*-methylisothioureas as the guanylating agent.[19] Although good overall yields were stated, the prerequisite to prepare the isothioureas in a two-step sequence from alkyl- or arylisothiocyanates seems disadvantageous. Additionally, ready commercial availability of the required isothiocyanates is limited and/or can be partially expensive.

An attractive approach to guanidines was described by Linton et al. who reacted carbamoylthioureas with alkyl- and arylamines in the presence of *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) as the desulfurizing agent.[20]

Martin et al. already successfully applied this strategy to the preparation of N^{ω}-hydroxy-L-arginines and very recently to N^{ω}-amino-L-arginines.[21,22] This prompted us to employ the concept for the preparation of several N^{ω}-alkyl-L-arginines. Initial isolation of N^{α}-Boc-N^{ω}-Cbz-L-thiocitrulline *tert*-butyl ester **4** provided a suitable building block for all guanidines (Scheme 2). The convenient reaction of thiourea **4** with 1.5 equivalents of amine and 1.5 equivalents of EDCI in anhydrous CH_2Cl_2 overnight allowed complete consumption of **4** as indicated by TLC.

Only the reaction of **4** with β-alanine amide to **5f** did not reach completion overnight – probably due to inferior solubility compared to the other amines employed – but an additional half-equivalent of the amine, EDCI and DIPEA resulted in quantitative conversion into the guanidine moiety. Protected L-arginines **5** could be subsequently deprotected under mild conditions with TFA/thioanisole within 30 minutes.[23] After removal of TFA in vacuo and separation of the amino acids between water and Et_2O to remove unpolar thioanisole and side-products, flash chro-



Scheme 2 Three-step synthesis of N^{ω}-substituted L-arginines

Table 2 Overall Yields for Nω-Substituted L-Arginines

Entry	Product **6**	Overall yield (%)[a]	Purity (%)[b]
1	**a**	87 (29[13])[c]	99.2
2	**b**	89 (44[6])	99.6
3	**c**	87 (70[16])	99.4
4	**d**	89	99.5
5	**e**	85 (32[5])	99.5
6	**f**	81	96.0
7	**g**	88	99.4
8	**h**	82	99.6
9	**i**	90	99.6

[a] Literature overall yield is given in parentheses.
[b] Determined by HPLC, using *o*-PA (*o*-phthaldialdehyde) precolumn derivatization (± 0.1%).
[c] Not reproducible in own attempts (see also Scheme 1).

matography on a RP-18 column provided Nω-substituted L-arginines **6** (Table 2).

For the accurate evaluation of enzymatic studies, such as the determination of K_i or K_m values, it is of pivotal importance that the tested compounds are enantiopure. Therefore, we tested all synthesized amino acids for enantiomeric purity on a Crownpak Cr(+) column. Furthermore, this should provide valuable information about the employed chemistry or, respectively, its risk for racemization (Figure 2). We did not synthesize D-enantiomers for this purpose but varied several chromatographic parameters that are known to affect the resolution of two enantiomers, that is, column temperature, pH of eluent, and flow-rate, and always observed highly symmetric peaks. In general, the separation factor of basic amino acids on this chiral stationary phase is very high (we determined α = 2.83 for D/L-arginine, α = 2.59 for N^{δ}-methyl-D/L-arginine[17]). Thus, it appears very unlikely that substituents at the Nω-position would dramatically impair the separation of the D/L-enantiomers. Essentially, based on the determined detection limit, we concluded optical purities of 99.9% ee for all the synthesized L-arginines **6**.The problematic nature of obtaining correct elemental analyses of highly hygroscopic amino acids also prompted us to carefully examine the general purity of the synthesized final compounds by HPLC. Therefore, L-arginines **6** were analyzed on a RP-18 column after precolumn-derivatization with *o*-phthaldialdehyde (*o*-PA). Only N^{ω}-(2-carboxamidoethyl)-L-arginine (**6f**) turned out to be less than 99% pure. This may be optimized by isolating its protected precursor **5f** in higher purity, but with concomitant losses in yields.

In summary, we have demonstrated the applicability of the 'carbamoylthiourea-guanidine' strategy to the preparation of various Nω-substituted L-arginines in excellent overall yields and high purity. Chromatography on a chiral stationary phase demonstrated no risk for racemization within this synthetic sequence and thereby underlines its utility for the preparation of various L-arginine analogues as probes for enzymes of the NO-generating system. It is further noteworthy that the described methodology proved suitable for the preparation of a series of unsymmetrically substituted, nonamino acid guanidines (data not shown).

Melting points are uncorrected. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker ARX 300 spectrometer at 300 K. Chemical shifts (δ values) are reported in ppm relative to TMS or 3-(trimethylsilyl)-1-propanesulfonic acid-d_6 sodium salt (TPS) as an internal standard, or in ^{1}H spectra of compounds **6** relative to the residual solvent signal. All coupling constants (*J* values) were obtained by first order analysis of the multiplets and are quoted in Hz. Low-resolution mass spectra were recorded on a Bruker-Esquire-LC with an electrospray ionization (ESI). Recordings of high-resolution mass spectra were conducted at the Institute of Analytical Chemistry, University of Leipzig, Germany on a Bruker 7.4 Tesla FTICR mass spectrometer BioApex II equipped with an ESI-ion source (Agilent). Chromatography on chiral stationary phase: Waters Breeze™ HPLC system: Waters 1525 binary pump, Waters 2487 dual wave-

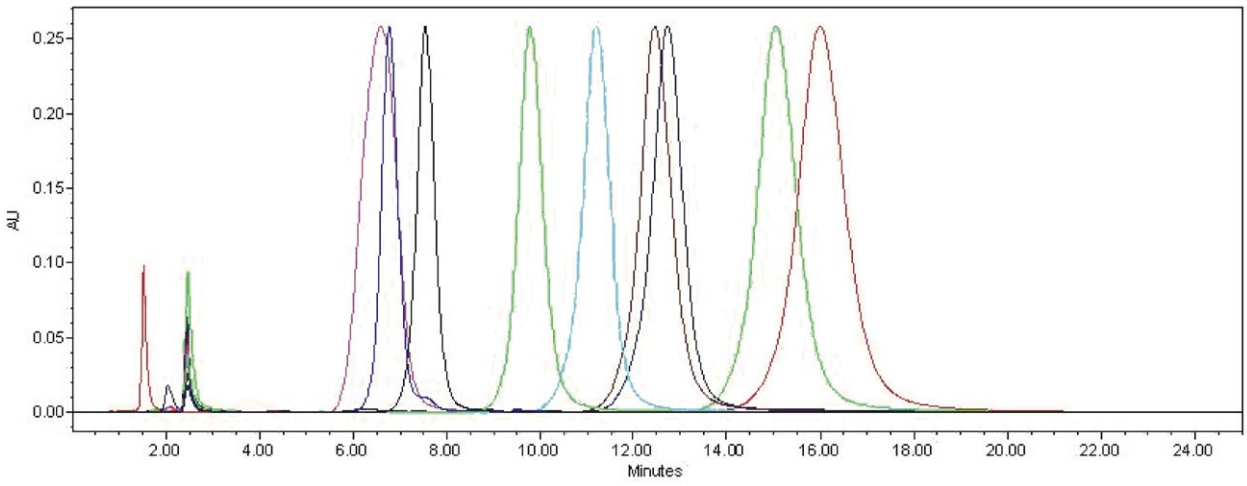


Figure 2 Chromatogram of optically pure Nω-substituted L-arginines **6** on a Crownpak Cr(+) column: retention times: **6a**, 6.6 min; **6f**, 6.8 min; **6g**, 7.5 min; **6e**, 9.8 min; **6b**, 11.2 min; **6c**, 12.5 min; **6h**, 12.7 min; **6i**, 15.1 min; **6d**, 16.0 min (for details see the experimental section).

length absorbance detector, Waters 717 Plus autosampler; stationary phase: Crownpak Cr(+) column (250 × 4.6 mm) thermostated at 15 °C; the eluent consisted of *aqua bidest.* and $HClO_4$, pH 1.5; flow rate was kept at 0.6 mL/min, except for **6d** (1 mL/min); detection at 200 nm. For determination of % ee, L-arginines **6** were dissolved in *aqua bidest.* (1 mM) and 10 μL was injected. Purity assessment of L-arginines **6** by RP-HPLC: autosampler Waters 717 plus, Waters 600 Controller, Waters 470 scanning fluorescence detector, set at λ_{ex}: 338 nm, λ_{em}: 425 nm; stationary phase: NovaPak RP_{18} (4 × 150 mm, VDS Optilab, 4 μm) with a Phenomenex C18 (4 × 3.0 mm) guard column, thermostated at 30 °C; eluent A consisted of 86% 10 mM K_3PO_4 buffer (pH 4.65), 8% MeCN, 6% MeOH; eluent B consisted of 40% MeCN, 30% MeOH, 30% *aqua bidest.*; elution conditions: flow rate was kept at 1 mL/min; 0–2 min, isocratic with 100% eluent A; 2–3.5 min, linear gradient to 90% eluent A and 10% eluent B; 3.5–12 min, isocratic with 90% eluent A and 10% eluent B; 12–16 min, linear gradient to 25% eluent A and 75% eluent B; 16–25 min, isocratic with 25% eluent A and 75% eluent B; 25–28 min, linear gradient to 100% eluent A; 28–35 min, reequilibration with 100% eluent A; precolumn derivatization: the autosampler was set to mix 7 μL of *o*-PA reagent with 8 μL of sample (0.1 mM) and allowed to react for 4 min at r.t. before injection. *o*-PA reagent was prepared by dissolving *o*-PA (50 mg) in MeOH (1 mL), followed by adding 0.2 M of potassium borate buffer (pH 9.4, 9 mL) and 2-mercaptoethanol (53 μL). Elemental analyses were performed on a CHNS analyzer (HEKAtech GmbH) and were within 0.4% for all synthesized compounds, except for **5f** and the highly hygroscopic L-arginines **6**.

All starting materials were commercially available and used without further purification. N^{α}-Boc-L-ornithine *tert*-butyl ester hydrochloride **3** and β-alanine amide hydrochloride were purchased from Bachem. All solvents were distilled and dried according to standard procedures. *Aqua bidest.* refers to doubly distilled water from a Finnaqua 75-E-4 system. CbzNCS[19] was prepared according to a procedure of Martin et al. but optimized using freshly powdered KSCN,[18] which resulted in higher yields and shorter reaction times. Reactions were monitored by TLC on precoated silica gel plates (SiO_2 60, F_{254}). All compounds could be visualized by ninhydrin spray and heating at 120 °C. Purification of synthesized compounds was performed by column chromatography using silica gel (particle size 40–63 μm). L-Arginines **6** were purified by flash chromatography (CombiFlash®RETRIEVE) on a RP-18 Redi*Sep*® column (43 g) using *aqua bidest.* (containing 0.1% TFA) as the eluent (30 mL/min).

N^{ω}-Benzyloxycarbonyl-N^{α}-*tert*-butyloxycarbonyl-L-thiocitrulline *tert*-Butyl Ester (4)[21]

N^{α}-*tert*-Butyloxycarbonyl-L-ornithine *tert*-butyl ester hydrochloride (**3**; 2.28 g, 7 mmol) was dissolved in anhyd CH_2Cl_2 (20 mL) 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 oil was dissolved in anhyd CH_2Cl_2 (250 mL) and cooled to 0 °C. A 0.5 M CH_2Cl_2 solution of CbzNCS (14 mL, 7 mmol) were added dropwise over 30 min and the mixture was stirred for 2 h while warming to r.t. until TLC indicated complete consumption of starting material. CH_2Cl_2 was evaporated to one-third of the volume and was washed with 1% aq HCl (25 mL), H_2O (25 mL), and brine (25 mL). The organic phase was dried (Na_2SO_4) and concentrated to a yellow oil. The crude product (>96% pure by TLC) was purified by column chromatography (cyclohexane–EtOAc, 4:1) to obtain 3.13 g (93%) of a light-yellow oil that solidified upon standing in the refrigerator.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.44 [s, 9 H, $C(CH_3)_3$], 1.46 [s, 9 H, $C(CH_3)_3$], 1.64–1.75 (m, 4 H, β,γ-CH_2), 3.66 (m, 2 H, NCH_2), 4.20 (m, 1 H, α-CH), 5.08 (m, 1 H, NH), 5.10 (s, 2 H, CH_2Ph), 7.36 (m, 5 H, ArH), 8.15 (br s, 1 H, NHCbz), 9.65 (br s, 1 H, NHBoc).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 24.8 (γ-CH_2), 28.7 [C(CH_3)$_3$], 29.0 [C(CH_3)$_3$], 30.9 (β-CH_2), 45.8 (NCH_2), 54.2 (α-CH), 68.8 (CH_2Ph), 80.4 [$C(CH_3)_3$], 82.8 [$C(CH_3)_3$], 129.0, 129.4, 129.5 (ArCH), 135.2 (ArC), 153.2 (thiourea-C), 156.0 (CO-Boc), 172.2 (CO_2-*t*-Bu), 179.8 (CO-Cbz).

MS (ESI): *m*/*z* = 482 [M + H]$^+$, 426 [M – C_4H_8 + H]$^+$, 370 [M – 2 × C_4H_8 + H]$^+$, 326 [M – 2 × C_4H_8, – CO_2 + H]$^+$.

Protected L-Arginines 5; General Procedure

A literature protocol for the preparation of carbamoylguanidines was used for the herein presented protected L-arginines.[20] DIPEA (1.5 equiv), amine (1.5 equiv), and EDCI (1.5 equiv) were reacted with thiourea **5** (0.5 mmol) in anhyd CH_2Cl_2 (5 mL). For amines that were applied as hydrochlorides (i.e., methoxylamine, but-3-enylamine, and β-alanine amide) 3 equiv of DIPEA were used. Unless otherwise noted, reactions were complete after stirring overnight. The organic phase was diluted with CH_2Cl_2 (10 mL) and washed with 1% aq HCl (5 mL), H_2O (5 mL), and brine (5 mL). The resulting oils were purified by column chromatography using CH_2Cl_2–MeOH as the eluent.

N^{ω}-Benzyloxycarbonyl-N^{α}-*tert*-butyloxycarbonyl-$N^{\omega'}$-methoxy-L-arginine *tert*-Butyl Ester (5a)

Eluent: CH_2Cl_2–MeOH (99:1); R_f = 0.3; yield: 235 mg (95%); colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.44 [s, 9 H, $C(CH_3)_3$], 1.46 [s, 9 H, $C(CH_3)_3$], 1.58–2.02 (m, 4 H, β,γ-CH_2), 3.11 (m, 2 H, NCH_2), 3.66 (s, 3 H, OCH_3), 4.18 (m, 1 H, α-CH), 5.11 (m, 1 H, NH), 5.13 (s, 2 H, CH_2Ph), 6.25 (m, 1 H, NH), 7.36 (m, 5 H, ArH), 7.91 (br s, 1 H, NHBoc).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 25.6 (γ-CH_2), 28.7 [C(CH_3)$_3$], 29.0 [C(CH_3)$_3$], 30.9 (β-CH_2), 41.2 (NCH_2), 54.5 (α-CH), 62.0 (OCH_3), 68.3 (OCH_2), 80.3 [$C(CH_3)_3$], 82.5 [$C(CH_3)_3$], 129.0, 129.3, 129.4 (ArCH), 135.8 (ArC), 148.8, 153.6, 156.0, 172.5 (CO_2-*t*-Bu).

MS (ESI): *m*/*z* = 495 [M + H]$^+$.

N^{ω}-Benzyloxycarbonyl-N^{α}-*tert*-butyloxycarbonyl-$N^{\omega'}$-(2-methoxyethyl)-L-arginine *tert*-Butyl Ester (5b)

Eluent: CH_2Cl_2–MeOH (95:5); R_f = 0.3; yield: 259 mg (99%); colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.44 [s, 9 H, $C(CH_3)_3$], 1.45 [s, 9 H, $C(CH_3)_3$], 1.51–1.93 (m, 4 H, β,γ-CH_2), 3.29 (m, 2 H, NCH_2), 3.37 (s, 3 H, OCH_3), 3.42, 3.50 (2 m, 4 H, NCH_2CH_2O), 4.16 (m, 1 H, α-CH), 5.10 (br s, 3 H, CH_2Ph, NH), 6.21 (br s, 1 H, NH), 7.28 (m, 3 H, ArH), 7.39 (m, 2 H, ArH), 9.11 (br s, 1 H, NHBoc).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 25.8 (γ-CH_2), 28.7 [C(CH_3)$_3$], 29.0 [C(CH_3)$_3$], 31.2 (β-CH_2), 41.3 (NCH_2), 54.2 (α-CH), 59.6 (OCH_3), 67.1 (CH_2Ph), 80.5 [$C(CH_3)_3$], 82.8 [$C(CH_3)_3$], 128.2, 128.6, 128.9 (ArCH), 138.5 (ArC), 156.2 (guanidine-C), 162.0 (CO-Boc), 164.8 (CO-Cbz), 172.2 (CO_2-*t*-Bu).

MS (ESI): *m*/*z* = 523 [M + H]$^+$.

N^{ω}-Allyl-$N^{\omega'}$-benzyloxycarbonyl-N^{α}-*tert*-butyloxycarbonyl-L-arginine *tert*-Butyl Ester (5c)

Eluent: CH_2Cl_2–MeOH (95:5); R_f = 0.3; yield: 240 mg (95%); colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.45 [s, 9 H, $C(CH_3)_3$], 1.47 [s, 9 H, $C(CH_3)_3$], 1.59–1.92 (m, 4 H, β,γ-CH_2), 3.37 (m, 2 H, NCH_2), 3.92 (m, 2 H, NCH_2CH=CH_2), 4.18 (m, 1 H, α-CH), 5.13 (s, 2 H, CH_2Ph), 5.16–5.38 (m, 3 H, CH=CH_2, NH), 5.89 (ddt, *J* = 17.2, 10.3, 5.2 Hz, 1 H, C*H*=CH_2), 7.32 (m, 3 H, ArH), 7.42 (m, 2 H, ArH), 8.79 (br s, 1 H, NHBoc).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 25.8 (γ-CH_2), 28.7 [C(CH_3)$_3$], 29.0 [C(CH_3)$_3$], 31.5 (β-CH_2), 41.6 (NCH_2), 44.5 (NCH_2), 53.8 (α-CH),

67.3 (CH_2Ph), 80.6 [$C(CH_3)_3$], 83.0 [$C(CH_3)_3$], 117.7 (CH=CH_2), 128.3, 128.7, 129.0 (ArCH), 134.5 (CH=CH_2), 138.3 (ArC), 156.2 (guanidine-C), 160.8 (CO-Boc), 164.8 (CO-Cbz), 172.2 (CO_2-*t*-Bu).

MS (ESI): *m*/*z* = 505 $[M + H]^+$.

N^{ω}-Benzyloxycarbonyl-$N^{\omega'}$-(but-3-enyl)-N^{α}-*tert*-butyloxycarbonyl-L-arginine *tert*-Butyl Ester (5d)

Eluent: CH_2Cl_2–MeOH (93:7); R_f = 0.17; yield: 251 mg (97%); colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.45 [s, 9 H, $C(CH_3)_3$], 1.47 [s, 9 H, $C(CH_3)_3$], 1.57–1.92 (m, 4 H, β,γ-CH_2), 2.35 (pseudo q, *J* = 6.8 Hz, 2 H, CH_2CH=CH_2), 3.31 (m, 4 H, 2 × NCH_2), 4.17 (m, 1 H, α-CH), 5.10–5.19 (m, 3 H, CH=CH_2, NH), 5.12 (s, 2 H, CH_2Ph), 5.80 (ddt, *J* = 17.1, 10.2, 6.8 Hz, 1 H, CH=CH_2), 7.29 (m, 3 H, ArH), 7.39 (m, 2 H, ArH), 8.91 (br s, 1 H, NHBoc).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 25.7 (γ-CH_2), 28.7 [C(CH_3)$_3$], 29.0 [C(CH_3)$_3$], 31.5 (β-CH_2), 34.2 (CH_2CH=CH_2), 41.0 (NCH_2), 41.2 (NCH_2), 53.8 (α-CH), 67.1 (CH_2Ph), 80.6 [$C(CH_3)_3$], 83.0 [$C(CH_3)_3$], 118.3 (CH=CH_2), 128.2, 128.6, 128.9 (ArCH), 135.5 (CH=CH_2), 138.4 (ArC), 156.3 (guanidine-C), 160.8 (CO-Boc), 164.8 (CO-Cbz), 172.1 (CO_2-*t*-Bu).

MS (ESI): *m*/*z* = 519 $[M + H]^+$.

N^{ω}-Benzyloxycarbonyl-N^{α}-*tert*-butyloxycarbonyl-$N^{\omega'}$-propargyl-L-arginine *tert*-Butyl Ester (5e)

Eluent: CH_2Cl_2–MeOH (97:3); R_f = 0.21; yield: 233 mg (93%); white foamy wax.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.46 [s, 9 H, $C(CH_3)_3$], 1.48 [s, 9 H, $C(CH_3)_3$], 1.54–1.90 (m, 4 H, β,γ-CH_2), 2.30 (br s, 1 H, C≡CH), 3.37 (m, 2 H, NCH_2), 4.12 (m, 3 H, NCH_2C≡CH, α-CH), 5.13 (s, 2 H, CH_2Ph), 5.18 (m, 1 H, NH), 7.31 (m, 3 H, ArH), 7.41 (m, 2 H, ArH), 9.03 (br s, 1 H, NHBoc).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 25.8 (γ-CH_2), 28.7 [C(CH_3)$_3$], 29.0 [C(CH_3)$_3$], 31.6, 41.3 (NCH_2), 53.7 (α-CH), 67.3, 73.0 (C≡CH), 80.7 [$C(CH_3)_3$], 83.1 [$C(CH_3)_3$], 128.2, 128.7, 128.9 (ArCH), 138.3 (ArC), 156.4 (guanidine-C), 160.5 (CO-Boc), 164.7 (CO-Cbz), 172.1 (CO_2-*t*-Bu).

MS (ESI): *m*/*z* = 503 $[M + H]^+$.

N^{ω}-Benzyloxycarbonyl-N^{α}-*tert*-butyloxycarbonyl-$N^{\omega'}$-(2-carbamoylethyl)-L-arginine *tert*-Butyl Ester (5f)

After stirring overnight, additional 0.5 equiv of β-alanine amide hydrochloride, EDCI, and 1 equiv DIPEA were added and stirred for 4 h. Eluent: CH_2Cl_2–MeOH (92:8); R_f = 0.34; yield: 241 mg (90%); white foamy wax.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.45 [s, 9 H, $C(CH_3)_3$], 1.48 [s, 9 H, $C(CH_3)_3$], 1.56–1.92 (m, 4 H, β,γ-CH_2), 2.50 (t, ^3J = 5.6 Hz, 2 H, CH_2CONH$_2$), 3.24 (m, 2 H, NCH_2), 3.28 (m, 2 H, NCH_2), 4.15 (m, 1 H, α-CH), 5.12 (s, 2 H, CH_2Ph), 5.36 (br s, 1 H, NH), 6.11 (br s, 2 H, CONH$_2$), 7.32 (m, 3 H, ArH), 7.45 (m, 2 H, ArH), 9.05 (br s, 1 H, NHBoc).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 25.7 (γ-CH_2), 28.6 [C(CH_3)$_3$], 28.9 [C(CH_3)$_3$], 30.7 (β-CH_2), 36.2 (CH_2), 37.6 (CH_2), 41.3 (NCH_2), 54.3 (α-CH), 67.1 (CH_2Ph), 80.5 [$C(CH_3)_3$], 82.7 [$C(CH_3)_3$], 128.2, 128.5, 128.9 (ArCH), 138.3 (ArC), 156.3 (guanidine-C), 160.9 (CO-Boc), 164.5 (CO-Cbz), 172.4 (CO_2-*t*-Bu), 175.5 (CONH$_2$).

MS (ESI): *m*/*z* = 536 $[M + H]^+$.

N^{δ}-{[(*N*-Benzyloxycarbonylamino)morpholino]methylidene}-N^{α}-*tert*-butyloxycarbonyl-L-ornithine *tert*-Butyl Ester (5g)

Eluent: CH_2Cl_2–MeOH (95:5); R_f = 0.25; yield: 265 mg (98%); yellow wax.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.46 [s, 9 H, $C(CH_3)_3$], 1.48 [s, 9 H, $C(CH_3)_3$], 1.56–1.92 (m, 4 H, β,γ-CH_2), 3.23 (m, 2 H, NCH_2), 3.42 (pseudo t, 4 H, 2 × NCH_2), 3.71 (pseudo t, 4 H, 2 × OCH_2), 4.16 (m, 1 H, α-CH), 5.09 (br s, 1 H, NH), 5.13 (s, 2 H, CH_2Ph), 7.34 (m, 3 H, ArH), 7.42 (m, 2 H, ArH), 8.27 (br s, 1 H, NHBoc).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 26.8 (γ-CH_2), 28.7 [C(CH_3)$_3$], 29.0 [C(CH_3)$_3$], 30.9 (β-CH_2), 45.8 (NCH_2), 48.4 (NCH_2), 53.9 (α-CH), 67.2 (OCH_2), 67.5 (OCH_2), 80.6 [$C(CH_3)_3$], 82.9 [$C(CH_3)_3$], 128.4, 128.9, 129.0 (ArCH), 138.0 (ArC), 155.4 (guanidine-C), 163.2 (CO-Boc), 165.0 (CO-Cbz), 171.4 (CO_2-*t*-Bu).

MS (ESI): *m*/*z* = 535 $[M + H]^+$.

N^{δ}-{[(*N*-Benzyloxycarbonylamino)-2,5-dihydro-1*H*-pyrrol-1-yl]methylidene}-N^{α}-*tert*-butyloxycarbonyl-L-ornithine *tert*-Butyl Ester (5h)

Eluent: CH_2Cl_2–MeOH (95:5); R_f = 0.31; yield: 240 mg (92%); light yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.45 [s, 9 H, $C(CH_3)_3$], 1.47 [s, 9 H, $C(CH_3)_3$], 1.55–1.90 (m, 4 H, β,γ-CH_2), 3.23 (m, 2 H, NCH_2), 4.15 (m, 1 H, α-CH), 4.26 (s, 4 H, 2 × NCH_2CH=), 5.13 (br s, 3 H, CH_2Ph, NH), 5.80 (s, 2 H, 2 × CH_2CH=), 7.30 (m, 3 H, ArH), 7.42 (m, 2 H, ArH).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 26.4 (γ-CH_2), 28.7 [C(CH_3)$_3$], 29.0 [C(CH_3)$_3$], 31.0 (β-CH_2), 44.1 (NCH_2), 54.0 (α-CH), 55.6 (NCH_2CH=), 67.3 (CH_2Ph), 80.5 [$C(CH_3)_3$], 82.9 [$C(CH_3)_3$], 125.8 (CH_2CH=), 128.1, 128.6, 128.9 (ArCH), 138.7 (ArC), 156.1 (guanidine-C), 161.1 (CO-Boc), 161.7 (CO-Cbz), 172.2 (CO_2-*t*-Bu).

MS (ESI): *m*/*z* = 517 $[M + H]^+$.

N^{ω}-Benzyloxycarbonyl-N^{α}-*tert*-butyloxycarbonyl-$N^{\omega',\omega'}$-(2,2,2-trifluoroethyl)-L-arginine *tert*-Butyl Ester (5i)

Eluent: CH_2Cl_2–MeOH (98:2); R_f = 0.43; yield: 271 mg (98%); colorless wax.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.45 [s, 9 H, $C(CH_3)_3$], 1.48 [s, 9 H, $C(CH_3)_3$], 1.64–1.94 (m, 4 H, β,γ-CH_2), 3.27 (m, 1 H), 3.43 (m, 1 H), 3.94 (m, 1 H), 4.18 (m, 1 H, α-CH), 4.36 (m, 1 H), 5.14 (s, 2 H, CH_2Ph), 5.26 (m, 1 H), 5.99 (br s, 1 H, NH), 7.31 (m, 3 H, ArH), 7.41 (m, 2 H, ArH), 9.19 (br s, 1 H, NHBoc).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 25.1 (γ-CH_2), 27.9 [C(CH_3)$_3$], 28.2 [C(CH_3)$_3$], 31.2 (β-CH_2), 40.8 (NCH_2), 42.4 (q, $J_{C,F}$ = 34.5 Hz, CH_2CF_3), 52.7 (α-CH), 67.0 (CH_2Ph), 80.3 [$C(CH_3)_3$], 82.6 [$C(CH_3)_3$], 124.1 (q, $J_{C,F}$ = 278.9 Hz, CF_3), 127.8, 128.0, 128.3 (ArCH), 137.1 (ArC), 156.0 (guanidine-C), 159.4 (CO-Boc), 163.0 (CO-Cbz), 171.3 (CO_2-*t*-Bu).

MS (ESI): *m*/*z* = 547 $[M + H]^+$.

Deprotected L-Arginines 6; General Procedure[21]

Protected L-arginines **5** (0.4 mmol) were stirred in a mixture of TFA (8 mL) and thioanisole (2.4 mL, 50 equiv) for 30 min. TFA was distilled out under reduced pressure and H_2O (5 mL) and Et_2O (15 mL) were added. The organic phase was extracted with H_2O (2 × 5 mL). The aqueous phase was concentrated on a rotary evaporator and the resulting oil was dissolved in *aqua bidest.* containing 0.1% of TFA (1 mL) and purified by flash chromatography on a RP-18 column. Ninhydrin-positive fractions were combined, concentrated in vacuo to ca. 10 mL and lyophilized.

N^{ω}-Methoxy-L-arginine Bis(trifluoroacetate) (6a)[15]

Yield: 171 mg (99%); colorless oil; R_f = 0.56 (*i*-PrOH–H_2O–AcOH, 6:3:1). The spectroscopic data were in good agreement with those reported.

^{1}H NMR (300 MHz, D_2O): δ = 1.78 (m, 2 H, γ-CH_2), 1.99 (m, 2 H, β-CH_2), 3.31 (t, 2 H, ^3J = 6.8 Hz, NCH_2), 3.75 (s, 3 H, OCH_3), 4.06 (t, ^3J = 6.2 Hz, 1 H, α-CH).

^{13}C NMR (75 MHz, D_2O/TPS): δ = 26.4 (γ-CH_2), 29.7 (β-CH_2), 43.0 (NCH_2), 55.5 (α-CH), 67.3 (OCH_3), 160.1 (guanidine-C), 174.8 (CO_2H).

HRMS (ESI): *m*/*z* calcd for $C_7H_{17}N_4O_3$ $[M + H]^+$: 205.12952; found: 205.12951.

N^{ω}-(2-Methoxyethyl)-L-arginine Bis(trifluoroacetate) (6b)[8]
Yield: 179 mg (97%); colorless oil; R_f = 0.5 (*i*-PrOH–H_2O–AcOH, 6:3:1). The spectroscopic data were in good agreement with those reported.

^{1}H NMR (300 MHz, D_2O): δ = 1.76 (m, 2 H, γ-CH_2), 2.00 (m, 2 H, β-CH_2), 3.29 (m, 2 H, NCH_2), 3.39 (s, 3 H, OCH_3), 3.40 (m, 2 H, $N^{\omega}CH_2$), 3.62 (m, 2 H, OCH_2), 4.09 (m, 1 H, α-CH).

^{13}C NMR (75 MHz, D_2O/TPS): δ = 26.6 (γ-CH_2), 29.7 (β-CH_2), 43.1 (NCH_2), 43.8 ($N^{\omega}CH_2$), 55.4 (α-CH), 60.9 (OCH_3), 73.1 (OCH_2), 159.0 (guanidine-C), 174.7 (CO_2H).

HRMS (ESI): *m*/*z* calcd for $C_9H_{21}N_4O_3$ $[M + H]^+$: 233.16082; found: 233.16073.

N^{ω}-Allyl-L-arginine Bis(trifluoroacetate) (6c)[24]
Yield: 173 mg (98%); colorless oil; R_f = 0.54 (*i*-PrOH–H_2O–AcOH, 6:3:1). The spectroscopic data were in good agreement with those reported.

^{1}H NMR (300 MHz, D_2O): δ = 1.74 (m, 2 H, γ-CH_2), 2.00 (m, 2 H, β-CH_2), 3.30 (t, 2 H, 3*J* = 6.7 Hz, NCH_2), 3.87 (d, ^3J = 4.6 Hz, 2 H, $N^{\omega}CH_2$), 4.11 (t, ^3J = 6.3 Hz, 1 H, α-CH), 5.25 (m, 1 H, CH=CH_2), 5.29 (m, 1 H, CH=CH_2), 5.90 (ddt, *J* = 17.3, 10.4, 4.8 Hz, 1 H, C*H*=CH_2).

^{13}C NMR (75 MHz, D_2O/TPS): δ = 26.5 (γ-CH_2), 29.6 (β-CH_2), 43.0 (NCH_2), 45.7 ($N^{\omega}CH_2$), 55.3 (α-CH), 119.0 (CH=*C*H_2), 135.1 (*C*H=CH_2), 158.6 (guanidine-C), 174.5 (CO_2H).

HRMS (ESI): *m*/*z* calcd for $C_9H_{19}N_4O_2$ $[M + H]^+$: 215.15025; found: 215.15029.

N^{ω}-(But-3-enyl)-L-arginine Bis(trifluoroacetate) (6d)
Yield: 181 mg (99%); colorless oil; R_f = 0.61 (*i*-PrOH–H_2O–AcOH, 6:3:1).

^{1}H NMR (300 MHz, D_2O): δ = 1.76 (m, 2 H, γ-CH_2), 1.98 (m, 2 H, β-CH_2), 2.34 (br pseudo q, 2 H, CH_2CH=CH), 3.27 (t, ^3J = 6.7 Hz, 2 H, NCH_2), 3.29 (t, ^3J = 6.7 Hz, 2 H, NCH_2), 4.11 (t, ^3J = 6.3 Hz, 1 H, α-CH), 5.16 (m, 2 H, C*H*=CH_2), 5.84 (ddt, *J* = 17.2, 10.3, 6.8 Hz, C*H*=CH_2).

^{13}C NMR (75 MHz, D_2O/TPS): δ = 26.6 (γ-CH_2), 29.6 (β-CH_2), 35.1 (*C*H_2CH=), 42.98 (NCH_2), 43.0 (N-CH_2), 55.3 (α-CH), 120.2 (CH=*C*H_2), 137.6 (*C*H=CH_2), 158.5 (guanidine-C), 174.4 (CO_2H).

HRMS (ESI): *m*/*z* calcd for $C_{10}H_{21}N_4O_2$ $[M + H]^+$: 229.16590; found: 229.16596.

N^{ω}-Propargyl-L-arginine Bis(trifluoroacetate) (6e)[7]
Yield: 173 mg (98%); colorless oil; R_f = 0.57 (*i*-PrOH–H_2O–AcOH, 6:3:1). The spectroscopic data were not in full agreement with those reported.

^{1}H NMR (300 MHz, D_2O): δ = 1.75 (m, 2 H, γ-CH_2), 1.99 (m, 2 H, β-CH_2), 2.76 (m, 1 H, C≡CH), 3.28 (t, ^3J = 6.8 Hz, 2 H, NCH_2), 4.03 (d, ^4J = 2.3 Hz, 2 H, NCH_2C≡CH), 4.08 (t, ^3J = 6.2 Hz, 1 H, α-CH).

^{13}C NMR (75 MHz, D_2O/TPS): δ = 26.5 (γ-CH_2), 29.6 (β-CH_2), 33.3 (N*C*H_2C≡CH), 43.2 (NCH_2), 55.3 (α-CH), 76.4 (C≡*C*H), 80.6 (*C*≡CH), 158.4 (guanidine-C), 174.6 (CO_2H).

HRMS (ESI): *m*/*z* calcd for $C_9H_{17}N_4O_2$ $[M + H]^+$: 213.1346; found: 213.13448.

N^{ω}-(2-Carbamoylethyl)-L-arginine Bis(trifluoroacetate) (6f)
Yield: 185 mg (97%); colorless oil; R_f = 0.43 (*i*-PrOH–H_2O–AcOH, 6:3:1).

^{1}H NMR (300 MHz, D_2O): δ = 1.73 (m, 2 H, γ-CH_2), 1.98 (m, 2 H, β-CH_2), 2.57 (t, ^3J = 6.4 Hz, 2 H, CH_2CONH$_2$), 3.26 (t, ^3J = 6.5 Hz, 2 H, NCH_2), 3.48 (t, ^3J = 6.3 Hz, 2 H, $N^{\omega}CH_2$), 4.07 (t, ^3J = 6.1 Hz, 1 H, α-CH).

^{13}C NMR (75 MHz, D_2O/TPS): δ = 26.5 (γ-CH_2), 29.6 (β-CH_2), 36.8 (*C*H_2CONH$_2$), 40.1 ($N^{\omega}CH_2$), 43.1 (NCH_2), 55.4 (α-CH), 158.6 (guanidine-C), 174.6 ($CONH_2$), 179.0 (CO_2H).

HRMS (ESI): *m*/*z* calcd for $C_9H_{20}N_5O_3$ $[M + H]^+$: 246.15607; found: 246.15587.

N^{δ}-[(Aminomorpholino)methylidene]-L-ornithine Bis(trifluoroacetate) (6g)
Yield: 184 mg (97%); colorless oil; R_f = 0.38 (*i*-PrOH–H_2O–AcOH, 6:3:1).

^{1}H NMR (300 MHz, D_2O): δ = 1.70 (m, 2 H, γ-CH_2), 1.96 (m, 2 H, β-CH_2), 3.30 (t, 2 H, ^3J = 6.8 Hz, NCH_2), 3.44 (t, ^3J = 4.9 Hz, 4 H, 2 × $N^{\omega}CH_2$), 3.76 (t, ^3J = 4.9 Hz, 4 H, 2 × OCH_2), 4.09 (t, ^3J = 6.2 Hz, 1 H, α-CH).

^{13}C NMR (75 MHz, D_2O/TPS): δ = 26.5 (γ-CH_2), 29.6 (β-CH_2), 43.9 (NCH_2), 48.5 ($N^{\omega}CH_2$), 55.1 (α-CH), 68.3 (OCH_2), 159.1 (guanidine-C), 174.4 (CO_2H).

HRMS (ESI): *m*/*z* calcd for $C_{10}H_{21}N_4O_3$ $[M + H]^+$: 245.16082; found: 245.16070.

N^{δ}-[(Amino-2,5-dihydro-1*H*-pyrrol-1-yl)methylidene]-L-ornithine Bis(trifluoroacetate) (6h)
Yield: 175 mg (96%); light yellow oil; R_f = 0.44 (*i*-PrOH–H_2O–AcOH, 6:3:1).

^{1}H NMR (300 MHz, D_2O): δ = 1.75 (m, 2 H, γ-CH_2), 1.98 (m, 2 H, β-CH_2), 3.33 (t, ^3J = 6.5 Hz, 2 H, NCH_2), 4.10 (t, ^3J = 6.2 Hz, 1 H, α-CH), 4.20 (s, 4 H, $N^{\omega}CH_2$), 5.90 (s, 2 H, CH_2C*H*=).

^{13}C NMR (75 MHz, D_2O/TPS): δ = 26.7 (γ-CH_2), 29.6 (β-CH_2), 43.6 (NCH_2), 55.3 (α-CH), 56.4 (*C*H_2CH=), 127.4 (CH_2*C*H), 155.9 (guanidine-C), 174.5 (CO_2H).

HRMS (ESI): *m*/*z* calcd for $C_{10}H_{19}N_4O_2$ $[M + H]^+$: 227.15025; found: 227.15026.

$N^{\omega,\omega}$-(2,2,2-Trifluoroethyl)-L-arginine Bis(trifluoroacetate) (6i)
Yield: 192 mg (99%); colorless oil; R_f = 0.70 (*i*-PrOH–H_2O–AcOH, 6:3:1).

^{1}H NMR (300 MHz, D_2O): δ = 1.77 (m, 2 H, γ-CH_2), 1.91 (m, 2 H, β-CH_2), 3.31 (m, 2 H, NCH_2), 4.0 (q, ^3J = 8.8 Hz, 2 H, $N^{\omega}CH_2$), 4.09 (m, 1 H, α-CH).

^{13}C NMR (75 MHz, D_2O/TPS): δ = 26.4 (γ-CH_2), 29.6 (β-CH_2), 45.1 (q, $^3J_{C,F}$ = 34.9 Hz, *C*H_2CF_3), 43.3 (NCH_2), 55.2 (α-CH), 126.5 (q, $J_{C,F}$ = 278.8 Hz, CF_3), 158.9 (guanidine-C), 174.4 (CO_2H).

HRMS (ESI): *m*/*z* calcd for $C_8H_{16}F_3N_4O_2$ $[M + H]^+$: 257.12199; found: 257.12193.

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