Synthesis F.-A. Litty et al. Paper

An Efficient Synthesis of Optically Pure N^{δ}-Monomethylated L-Arginine and L-Ornithine

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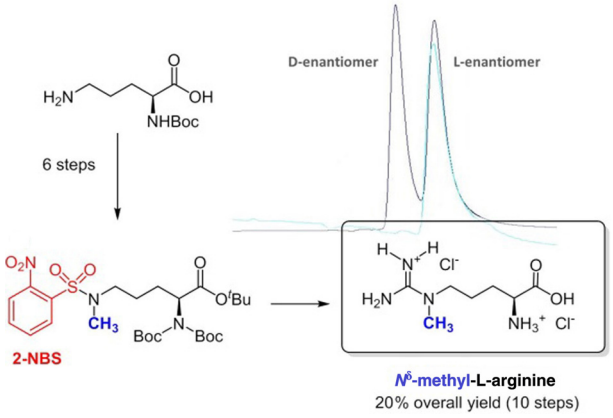

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Abstract N^{ω}-Methylated L-arginines such as asymmetric dimethyl-L-arginine (ADMA) and monomethyl-L-arginine (NMMA) are well-known endogenous modulators of the nitric oxide (NO) generating system. To understand the (patho)physiological role and impact of N^{δ}-methylation of L-arginine and L-ornithine an efficient synthesis of the pure enantiomers was needed. A synthetic approach that furnished both the desired amino acids in 8–10 steps from commercially available *N*-Boc-L-ornithine in good overall yields (20–21%) and with high optical purity (>99% ee) is reported.

Key words alkylation, amino acids, bioorganic chemistry, chiral pool, chiral resolution, protecting groups

Methylated arginines such as ADMA (asymmetric N^{ω},N^{ω}-dimethyl-L-arginine), NMMA (N^{ω}-monomethyl-L-arginine), and SDMA (symmetric N^{ω},$N^{\omega'}$-dimethyl-L-arginine) are formed by post-translational methylation of arginine residues in proteins (Scheme 1).[1,2] These modifications are catalyzed by protein arginine methyltransferases (PRMTs).[1] Within the proteolysis of these proteins, methylated arginines are liberated and metabolized by the dimethylarginine dimethylaminohydrolase (DDAH). They can act as physiological modulators of the nitric oxide (NO) generating system by inhibition of nitric oxide synthases (NOSs).[3] The degradation of L-arginine to L-ornithine and urea is catalyzed by arginases, which represents the final step of the urea cycle.[4] Nitric oxide is involved in a plethora of physiological processes, with the regulation of vascular tone and tissue blood flow as one of its many functions. The antiatherosclerotic potential of NO is largely due to inhibition of platelet aggregation and leukocyte adhesion on the endothelial surface. In addition, NO is a neuromediator with many physiological functions, including memory and pain modulation.[5] Therefore, not surprisingly, dysregulations of the NO modulating system are associated with the pathology of numerous diseases.[6]

Post-translational methylation of arginine residues in proteins can affect their physiological activity.[1] For example, in histones, arginine methylation can promote or prevent the docking of key effector molecules and thus regulates the transcription of specific genes (epigenetic regulations).[7] Furthermore, arginine methylation in non-histone proteins plays a crucial role in influencing distinct cellular functions as cellular development and tumorigenesis.[8]

While much is known about the above outlined N^{ω}-methyl-L-arginines, the (patho)physiological role of N^{δ}-methylation is not well understood. N^{δ}-Methyl-L-arginine (**11**, Schemes 1 and 2) was discovered in 1998 as a post-translational modification of arginine residues in yeast cells and is studied in our group regarding possible functions in the NO modulating system.[9] Moreover, N^{δ}-methyl-L-ornithine (**10**, Scheme 2) was identified as a degradation product of base-treated N^{δ}-methyl-L-arginine (**11**).[10] Strikingly, latest findings suggest a physiological relevance in humans since **11** has been detected and quantified in human plasma.[11]

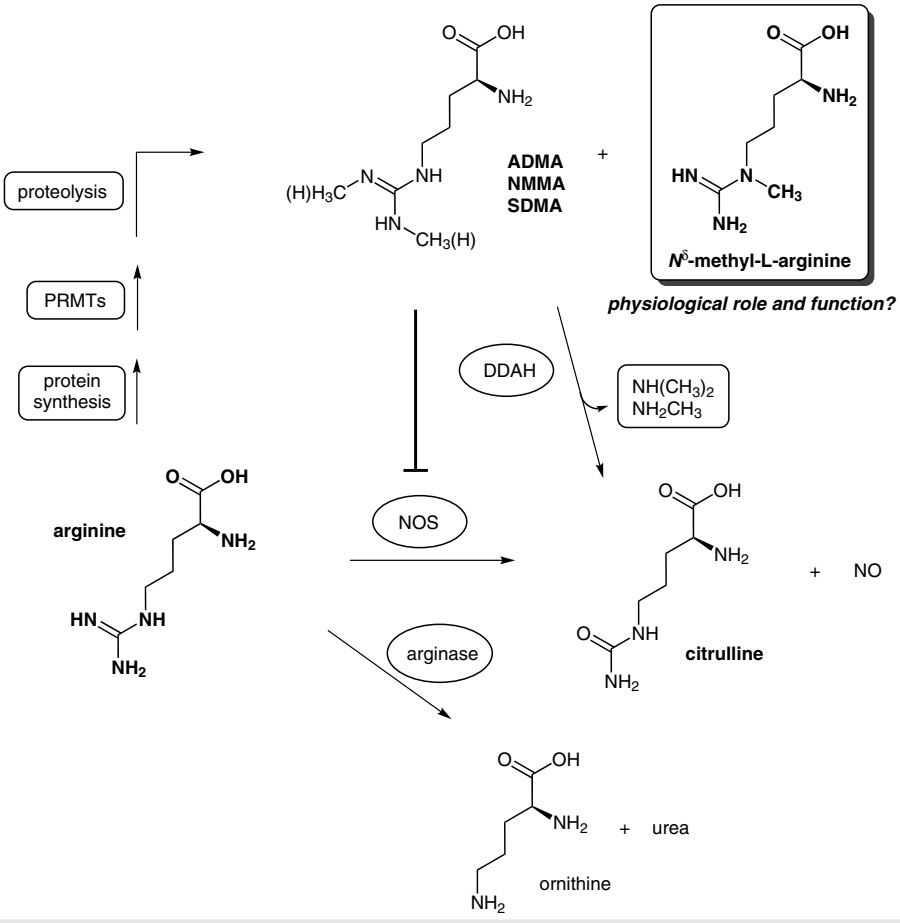
Therefore, to further study N^{δ}-methylated arginine **11** and its putative metabolite **10**, an efficient synthetic protocol is needed. However, selective methylation of the N^{δ}-nitrogen of arginine is a challenge and can only be tackled using a suitable protecting group strategy.

The first synthesis of **10** and **11** was reported in 1983 by the Steglich group, who obtained the compounds in racemic form.[12] Since NOS, DDAH, and arginase are highly substrate-specific for naturally occurring L-amino acids, the establishment of a synthetic concept for optically pure **10** and **11** would thus be desirable.[13] We and others have described a synthetic approach for a series of N^{δ}-methylated L-arginine derivatives with already high enantiopurity (>98%

ee).[14,15] However, this strategy employed distinct boroxazolidinones as an orthogonal amino acid protecting group, which was highly dependent on the availability of optically pure **10**. Here, we present a conceptually different approach that avoids these shortcomings by furnishing **7**, a suitably protected form of **10**, in high yields and enantiopurity.

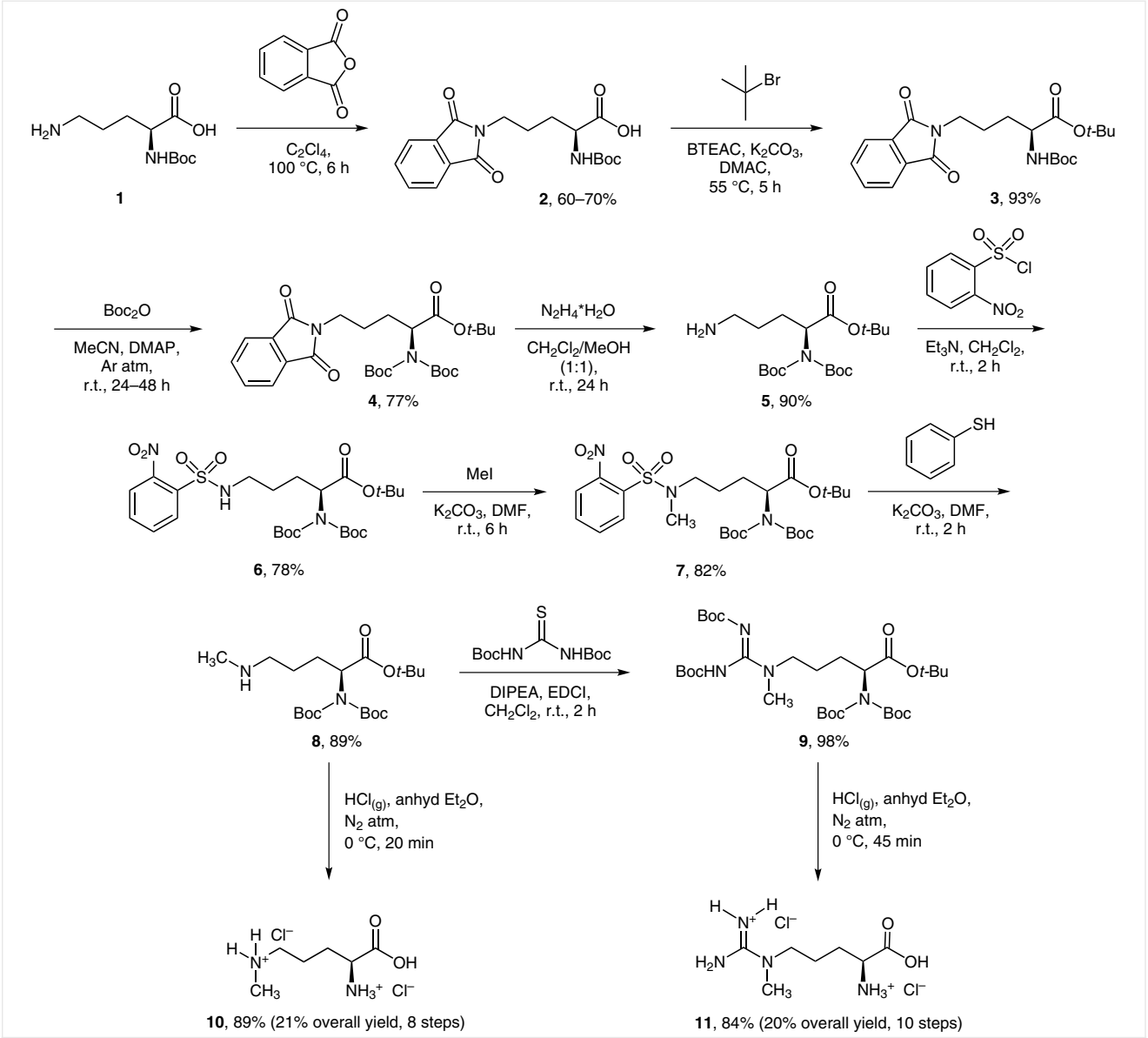
Previous work on the synthesis of **10** involved protecting group (PG) strategies that were associated with a high risk of racemization, such as the tosyl PG that allowed monomethylation of the N^{δ}-nitrogen in ornithine.[12,14,15] Cleavage of this PG can only be performed under rather harsh reaction conditions, namely treatment with HBr in glacial acetic acid and higher temperatures.[14] These acidic conditions cause rapid racemization of amino acids.[16] In fact, we determined that reaction times should not exceed 1.5 hours, and that HBr should be quickly removed under high vacuum (<1 mbar) at low temperature, not higher than 50–60 °C.[15]

Hence, we aimed at utilizing a tosyl-like PG that similarly enables selective monomethylation and can be removed under mild conditions. 2-Nitrobenzenesulfonamides fulfill these requirements as they have been reported to be cleaved under mild conditions by treatment with nucleophilic agents like thiophenol.[17] However, before introducing the 2-nitrobenzenesulfonyl group, the first challenge was to efficiently protect the amino acid moiety from methylation. Commercially available N^{α}-Boc-protected L-ornithine **1** was treated with phthalic anhydride to protect the reactive N^{δ}-position and furnished **2** in good yields (60–70%) (Scheme 2).[18] Next, esterification of the α-carboxylic moiety was performed with *tert*-butyl bromide according to a literature-described protocol.[19] To avoid later methylation at the Boc-protected N^{α}-position, a second Boc group was introduced by treatment of **3** with di-*tert*-butyl dicarbonate (Boc_2O) in anhydrous acetonitrile and DMAP as a catalyst to furnish the fully protected ornithine **4**.[20] By treatment of **4** with hydrazine, the phthalimide PG could be efficiently removed, delivering **5** in very good yield (90%). This amino acid protected ornithine **5** served as an ideal building block for the subsequent synthetic sequence. Compound **5** was



Scheme 1 Biochemical pathways of L-arginine and its methylated analogues. PRMTs: protein arginine methyltransferases; ADMA: asymmetric N^{ω},N^{ω}-dimethyl-L-arginine; SDMA: symmetric N^{ω},$N^{\omega'}$-dimethyl-L-arginine; NMMA: N^{ω}-monomethyl-L-arginine, NO: nitric oxide; NOS: nitric oxide synthase (EC 1.14.13.39); DDAH: dimethylarginine dimethylaminohydrolase (EC 3.5.3.18); arginase (EC 3.5.3.1).

reacted with 2-nitrobenzenesulfonyl chloride and triethylamine in CH_2Cl_2 to afford the sulfonamide **6**, followed by methylation with MeI. Removal of the 2-nitrobenzenesulfonyl group was accomplished by reacting **7** with thiophenol in the presence of K_2CO_3 as a base. The reaction went to completion after 2 hours at room temperature to provide N^{δ}-methylated ornithine **8** in form of the free amine in very good yield (89%). *N*,*N*′-Bis-(*tert*-butyloxycarbonyl)thiourea was used as a guanylation reagent. This reaction was carried out with DIPEA for proton trapping and EDCI as the desulfuration agent, instead of the more toxic (and oftentimes less efficient) $HgCl_2$. Finally, for both **8** and **9**, protecting groups were carefully removed with gaseous HCl under moisture-free conditions in absolute diethyl ether at low temperatures to minimize risks for acid-catalyzed racemization. Under these conditions, the dihydrochloride salt (as confirmed by NMR spectroscopy, see Supporting Information) of N^{δ}-methyl-L-arginine precipitated and was further purified by flash chromatography on reversed-phase (RP-18) silica gel. In contrast to **11**, the dihydrochloride of N^{δ}-methyl-L-ornithine (**10**) did not readily precipitate but was isolated as a highly hygroscopic white solid after chromatographic workup.



Scheme 2 Linear synthesis of N^{δ}-methyl-L-arginine (**11**) and N^{δ}-methyl-L-ornithine (**10**). BTEAC: benzyltriethylammonium chloride; DMAC: *N*,*N*-dimethylacetamide.

There are different chromatographic (HPLC) methods for the discrimination of amino acid enantiomers. A conventional method is ligand exchange chromatography with chiral eluents on common RP-18 columns. One of these procedures was described by Gil-Av et al. and adapted to the herein reported compound of interest, N^{δ}-methyl-L-arginine (**11**).[21] L-Proline in combination with copper acetate forms stable diastereomeric complexes with N^{δ}-methylarginine enantiomers resulting in distinct retention times.

Using this system, injection of **11** showed a single peak, which corresponds to the second eluting peak (12.9 min, see Figure 1) from racemic N^{δ}-methylarginine that we obtained from the Steglich group.[12] Considering that the herein presented synthetic sequence started with enantiopure commercial material (**1**) with low risk for racemization, it can be assumed that the peak at 12.9 minutes corresponds to the L-enantiomer. To reinforce this assumption, we also analyzed D- and L-arginine and could confirm that the L-enantiomer eluted second (see Supporting Information). The limit of detection, determined for the D-enantiomer of racemic N^{δ}-methylarginine (10 mM), was 50 µM, based on a signal-to-noise-ratio of more than 3:1. Thus, the enantiomeric purity of **11** can be stated as ≥99% ee. Moreover, since **11** has been synthesized from **8**, it can also be concluded that N^{δ}-methyl-L-ornithine **10** exhibits >99% ee.

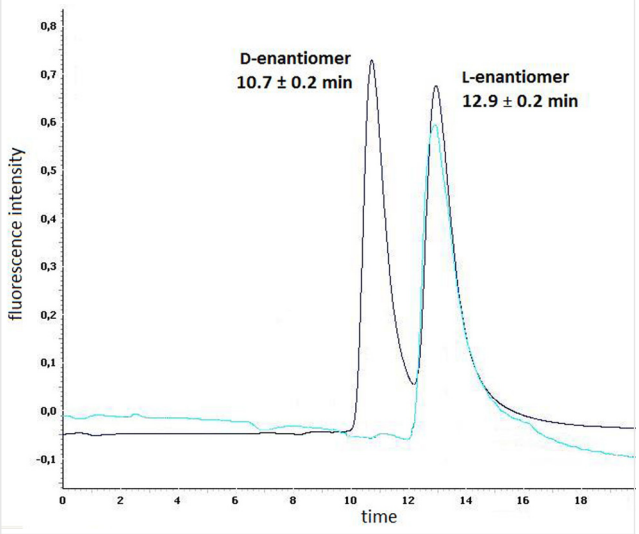


Figure 1 Representative chromatograms of racemic N^{δ}-methylarginine (black) and optically pure N^{δ}-methyl-L-arginine **11** (turquoise) on a VDS Optilab Nucleosil 100 C18 column with a chiral eluent composed of L-proline and aqueous $Cu(OAc)_2$ in ddH_2O (pH 4.5) and post-column *o*-PA derivatization (for details see the experimental section).

In summary, the establishment of an efficient synthesis for optically pure N^{δ}-methyl-L-arginine and -ornithine represents an important advance in arginine chemistry and will enable various studies of biological as well as pharmacological significance. Motivated by the necessity of high enantiomeric purity, we have developed a straightforward synthetic strategy, utilizing a protecting group concept (i.e., 2-nitrobenzenesulfonyl) that allowed installing the desired N^{δ}-methyl group under mild reaction conditions and minimal risk for racemization. We could ultimately verify optical purity by a reliable HPLC-based analytical method that employed a chiral eluent on a conventional reversed-phase column.

Melting points are uncorrected. ^1H (300 MHz), ^{13}C (75 MHz), and ^{35}Cl (29 MHz) NMR spectra were recorded on a Bruker Avance III 300 spectrometer at 298 K. Chemical shifts (δ values) were quoted in ppm relative to TMS as an internal standard, 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (TPS) as an external standard or alternatively, relative to the solvent residual signal. Spectra interpretation was performed by first order analysis. Signal assignment was done via ^1H, ^1H-COSY and ^{13}C-HSQC and -HMBC. Quantification of Cl^- by ^{35}Cl NMR was verified by a three-point calibration with NaCl in D_2O. Low-resolution mass spectra were recorded using a Bruker amazon SL system with LC coupling, electrospray ionization, in the positive mode. Recordings of exact mass spectra were performed at the Department of Physical Chemistry, Christian-Albrechts-University of Kiel, on a 7.05 Tesla Bruker APEX III FT-ICR mass spectrometer in ESI-positive mode; samples were dissolved in EtOH and diluted with a solvent mixture containing MeOH, H_2O, and formic acid (49.9:49.9:0.2) to a concentration of about 100 pmol/µL. Elemental analyses were performed on a CHNS analyzer (HEKAtech GmbH) at the Department of Inorganic Chemistry, Christian-Albrechts-University of Kiel. IR spectra were recorded on a Shimadzu IRAffinity-1S FTIR spectrometer equipped with MIRacle 10 Single Reflection ATR Accessory. Optical rotations were measured on a PerkinElmer 241 polarimeter at 20 °C. Reactions were monitored by TLC on precoated silica gel plates (SiO_2, 60, F_{254}). All compounds could be detected by either UV detection or by ninhydrin staining. Purification of synthesized compounds was carried out on a Combi Flash Rf, version 1.8.2, flash chromatography apparatus using Interchim PF-30SIHP-JP/12G or 40G or PF-15SIHP/12G silica gel columns. Reverse phase chromatography was performed by column chromatography using silica gel 60 silanized (0.063–0.200 mm, Merck). All starting materials were commercially available and used without further purification. N^{α}-(*tert*-Butyloxycarbonyl)-L-ornithine (**1**) was purchased from Bachem. *N*,*N*′-Bis-(*tert*-butyloxycarbonyl)thiourea was purchased from Sigma Aldrich. All solvents were distilled and dried according to standard procedures.

N^{α}-(*tert*-Butyloxycarbonyl)-N^{δ}-phthalimido-L-ornithine (2)[19]

N^{α}-(*tert*-Butyloxycarbonyl)-L-ornithine (**1**; 1.94 g, 8.4 mmol) and phthalic anhydride (2.22 g, 15.0 mmol) were suspended in $CHCl_3$ (20 mL) and tetrachloroethylene (150 mL). This suspension was heated for 2 h at 60 °C and an additional 6 h at 100 °C. The clear reaction mixture was concentrated in vacuo to afford the crude product. Purification was carried out by flash column chromatography (silica gel, CH_2Cl_2–MeOH, 0–5%); yield: 1.9 g (61%); white crystalline solid; mp 126 °C; R_f = 0.54 (CH_2Cl_2–MeOH, 9:1). The spectroscopic data were in agreement with those reported.[19]

IR (ATR): 2976, 1771, 1697, 1514, 1396, 1366, 1159, 1047, 718 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 9.36 (br s, 1 H, OH), 7.80–7.86 (m, 2 H, ArH), 7.68–7.75 (m, 2 H, ArH), 5.16 (d, ^3J = 8.2 Hz, 1 H, NH), 4.14–4.37 (m, 1 H, α-CH), 3.72 (t, ^3J = 6.8 Hz, 2 H, δ-CH_2), 1.65–2.00 (m, 4 H, β,γ-CH_2), 1.43 [s, 9 H, $C(CH_3)_3$].

^{13}C NMR (75 MHz, $CDCl_3$): δ = 176.5 (CO_2H), 168.4 (2 × *C*O-Pht), 155.6 (*C*O-Boc), 134.0 (2 × ArCH), 132.0 (2 × ArC), 123.3 (2 × ArCH), 80.3 [*C*(CH_3)$_3$], 53.0 (α-CH), 37.4 (δ-CH_2), 29.8 (β-CH_2), 28.3 [C(*C*H_3)$_3$], 24.8 (γ-CH_2).

MS (ESI): *m*/*z* = 725 [2 × M + H]$^+$, 625 [2 × M – C_4H_8 – CO_2 + H]$^+$, 363 [M + H]$^+$, 263 [M – C_4H_8 – CO_2 + H]$^+$.

Anal. Calcd for $C_{18}H_{22}N_2O_6$ (362.38): C, 59.66; H, 6.12; N, 7.73. Found: C, 59.21; H, 6.15; N, 7.58.

N^α-(*tert*-Butyloxycarbonyl)-*N*^{δ}-phthalimido-L-ornithine *tert*-Butyl Ester (3)[19]

Compound **2** (1.87 g, 5.2 mmol), benzyltriethylammonium chloride (1.17 g, 5.2 mmol, 1 equiv), *tert*-butyl bromide (27 mL, 239 mmol, 46 equiv), and K_2CO_3 (19 g, 135 mmol, 26 equiv) were added to DMAC (40 mL). This reaction mixture was heated for 5 h at 55 °C and cooled to r.t. The salts were filtered and washed with EtOAc (3 × 25 mL). The filtrate was washed with H_2O (3 × 20 mL) and the combined aqueous phases were extracted with EtOAc (1 × 25 mL). The combined organic layers were dried (Na_2SO_4) and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (silica gel, cyclohexane–EtOAc, 0–20%); yield: 2.0 g (93%); colorless oil; R_f = 0.48 (cyclohexane–EtOAc, 2:1). The spectroscopic data were in agreement with those reported.[19]

IR (ATR): 3399, 2978, 1771, 1740, 1694, 1508, 1393, 1150, 718 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.81–7.88 (m, 2 H, ArH), 7.68–7.75 (m, 2 H, ArH), 5.06 (d, ^3J = 8.3 Hz, 1 H, NH), 4.14–4.27 (m, 1 H, α-CH), 3.71 (t, ^3J = 6.9 Hz, 2 H, δ-CH_2), 1.62–1.91 (m, 4 H, β,γ-CH_2), 1.45 [s, 9 H, C(CH_3)$_3$], 1.43 [s, 9 H, C(CH_3)$_3$].

^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.6 (CO_2*t*-Bu), 168.3 (2 × *C*O-Pht), 155.3 (*C*O-Boc), 133.9 (2 × ArCH), 132.1 (2 × ArC), 123.3 (2 × ArCH), 79.7, 80.3 [2 × *C*(CH_3)$_3$], 53.6 (α-CH), 37.6 (δ-CH_2), 30.3 (β-CH_2), 28.3 [C(*C*H_3)$_3$], 28.0 [C(*C*H_3)$_3$], 24.5 (γ-CH_2).

MS (ESI): *m*/*z* = 419 [M + H]$^+$, 319 [M – C_4H_8 – CO_2 + H]$^+$, 263 [M – 2 × C_4H_8 – CO_2 + H]$^+$.

Anal. Calcd for $C_{22}H_{30}N_2O_6$ (418.49): C, 63.14; H, 7.23; N, 6.69. Found: C, 62.70; H, 7.56; N, 7.12.

N^α,*N*^{α}-Bis(*tert*-Butyloxycarbonyl)-*N*^{δ}-phthalimido-L-ornithine *tert*-Butyl Ester (4)

Compound **3** (2.0 g, 4.8 mmol), DMAP (61 mg, 0.5 mmol), and di-*tert*-butyl dicarbonate (10.9 g, 48 mmol, 10 equiv) were dissolved in anhydrous MeCN (25 mL) under an argon atmosphere. This reaction mixture was stirred magnetically until the reaction was completed (TLC). The mixture was diluted with CH_2Cl_2 (25 mL) and washed with sat. aq $NaHCO_3$ (20 mL), H_2O (20 mL), and brine (20 mL). The organic phase was dried (Na_2SO_4) and concentrated in vacuo. Purification was carried out by flash column chromatography (silica gel, cyclohexane–EtOAc, 0–20%); yield: 1.9 g (77%); colorless oil; R_f = 0.56 (cyclohexane–EtOAc, 2:1).

IR (ATR): 2978, 1773, 1736, 1711, 1366, 1126, 1026, 719 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.82–7.84 (m, 2 H, ArH), 7.69–7.73 (m, 2 H, ArH), 4.78 (dd, ^3J = 9.3, 5.1 Hz, 1 H, α-CH), 3.72 (dt, ^3J =6.9 Hz, ^4J = 1.9 Hz, 2 H, δ-CH_2), 2.04–2.16 (m, 1 H, β-CH_2), 1.83–1.97 (m, 1 H, β-CH_2), 1.68–1.79 (m, 2 H, γ-CH_2), 1.49 [2 s, 18 H, C(CH_3)$_3$], 1.43 [s, 9 H, C(CH_3)$_3$].

^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.6 (CO_2*t*-Bu), 168.3 (2 × *C*O-Pht), 152.4 (2 × *C*O-Boc), 133.8 (2 × ArCH), 132.2 (2 × ArC), 123.2 (2 × ArCH), 82.9 [2 × *C*(CH_3)$_3$], 81.3 [*C*(CH_3)$_3$], 58.4 (α-CH), 37.5 (δ-CH_2), 28.0 [2 × C(*C*H_3)$_3$], 27.9 [C(*C*H_3)$_3$], 26.5 (β-CH_2), 25.6 (γ-CH_2).

MS (ESI): *m*/*z* = 541 [M + Na]$^+$, 419 [M – C_4H_8 – CO_2 + H]$^+$, 319 [M – 2 × C_4H_8 – 2 × CO_2 + H]$^+$ 263 [M – 3 × C_4H_8 – 2 × CO_2 + H]$^+$.

Anal. Calcd for $C_{27}H_{38}N_2O_8$ (518.61): C, 62.53; H, 7.39; N, 5.40. Found: C, 62.00; H, 7.34; N, 5.49.

N^α,*N*^{α}-Bis(*tert*-butyloxycarbonyl)-L-ornithine *tert*-Butyl Ester (5)

Compound **4** (1.88 g, 3.6 mmol) was dissolved in a mixture of CH_2Cl_2 and MeOH (1:1, 40 mL). Hydrazine hydrate (2.7 mL, 54 mmol, 15 equiv) was added and the reaction mixture was stirred magnetically at r.t. After 24 h, the mixture was filtered to remove the precipitated phthalhydrazide and quenched by adding sat. aq $NaHCO_3$ (30 mL). The organic phase was separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were washed with brine (30 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Compound **5** was at this point already pure (TLC) and used in the following reaction without any purification; yield: 1.27 g (90%); colorless oil; R_f = 0.37 (CH_2Cl_2–MeOH–aq NH_3, 8.5:1.5:0.5).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.72 (dd, ^3J = 9.6, 5.2 Hz, 1 H, α-CH), 2.64–2.79 (m, 2 H, δ-CH_2), 2.03–2.15 (m, 1 H, β-CH_2), 1.80–1.94 (m, 1 H, β-CH_2), 1.40–1.56 (m, 2 H, γ-CH_2), 1.51 [2 s, 18 H, C(CH_3)$_3$], 1.45 [s, 9 H, C(CH_3)$_3$], 1.21 (br s, 2 H, NH_2).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.9 (CO_2*t*-Bu), 152.5 (2 × *C*O-Boc), 82.7 [2 × *C*(CH_3)$_3$], 81.2 [*C*(CH_3)$_3$], 58.8 (α-CH), 42.0 (δ-CH_2), 30.7 (β-CH_2), 28.1 [2 × C(*C*H_3)$_3$], 28.0 [C(*C*H_3)$_3$], 26.6 (γ-CH_2).

MS (ESI): *m*/*z* = 777 [2 × M + H]$^+$, 389 [M + H]$^+$, 289 [M – C_4H_8 – CO_2 + H]$^+$.

Anal. Calcd for $C_{19}H_{36}N_2O_6$ (388.51): C, 58.74; H, 9.34; N, 7.21. Found: C, 58.49; H, 9.61; N, 6.91.

N^α,*N*^{α}-Bis(*tert*-butyloxycarbonyl)-*N*^{δ}-(2-nitrophenylsulfonyl)-L-ornithine *tert*-Butyl Ester (6)

Compound **5** (1.27 g, 3.3 mmol) and 2-nitrosulfonyl chloride (0.95 g, 4.3 mmol, 1.3 equiv) were dissolved in anhydrous CH_2Cl_2 (60 mL). Et_3N (589 µL, 4.3 mmol, 1.3 equiv) was added and the reaction mixture was stirred magnetically at 0 °C for 30 min and at r.t. for an additional 2 h. The solvent was removed in vacuo to afford the crude product. Purification was performed by flash column chromatography (silica gel, cyclohexane–EtOAc, 0–25%); yield: 1.46 g (78%); colorless oil; R_f = 0.69 (cyclohexane–EtOAc, 1:1).

IR (ATR): 2978, 1734, 1697, 1554, 1366, 1155, 1130, 851 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.10–8.16 (m, 1 H, ArH), 7.82–7.89 (m, 1 H, ArH), 7.69–7.77 (m, 2 H, ArH), 5.35 (t, ^3J = 6.2 Hz, 1 H, NH), 4.63 (dd, ^3J = 9.0, 5.5 Hz, 1 H, α-CH), 3.12 (q, ^3J = 6.7 Hz, 2 H, δ-CH_2), 2.02–2.14 (m, 1 H, β-CH_2), 1.78–1.91 (m, 1 H, β-CH_2), 1.54–1.67 (m, 2 H, γ-CH_2), 1.49 [2 s, 18 H, C(CH_3)$_3$], 1.43 [s, 9 H, C(CH_3)$_3$].

^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.4 (CO_2*t*-Bu), 152.5 (2 × *C*O-Boc), 148.1 (ArC–N), 133.8 (ArC–S), 125.4, 131.1, 132.8, 133.5 (ArCH), 83.0 [2 × *C*(CH_3)$_3$], 81.5 [*C*(CH_3)$_3$], 58.2 (α-CH), 43.5 (δ-CH_2), 28.0 [2 × C(*C*H_3)$_3$], 27.9 [C(*C*H_3)$_3$], 26.8 (β-CH_2), 26.5 (γ-CH_2).

MS (ESI): *m*/*z* = 596 [M + Na]$^+$, 362 [M – 3 × C_4H_8 – CO_2 + H]$^+$, 318 [M – 3 × C_4H_8 – 2 × CO_2 + H]$^+$.

N^α,*N*^{α}-Bis(*tert*-butyloxycarbonyl)-*N*^{δ}-methyl-*N*^{δ}-(2-nitrophenylsulfonyl)-L-ornithine *tert*-Butyl Ester (7)

To a solution of **6** (1.45 g, 2.5 mmol) in DMF (15 mL) were added K_2CO_3 (1.05 g, 7.6 mmol, 3 equiv) and MeI (475 µL, 7.6 mmol, 3 equiv). The reaction mixture was stirred magnetically at r.t. for 6 h. The mixture was quenched by adding 10% aq ammonia (10 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were dried

(Na_2SO_4) and concentrated under reduced pressure. The resulting oil was purified by flash column chromatography (silica gel, cyclohexane–EtOAc, 0–30%); yield: 1.22 g (82%); colorless oil; R_f = 0.67 (toluene–EtOAc, 7:3).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.95–8.01 (m, 1 H, ArH), 7.63–7.71 (m, 2 H, ArH), 7.57–7.63 (m, 1 H, ArH), 4.70 (dd, ^3J = 9.1, 5.5 Hz, 1 H, α-CH), 3.11–3.34 (m, 2 H, δ-CH_2), 2.89 (s, 3 H, NCH_3), 2.02–2.14 (m, 1 H, β-CH_2), 1.79–1.92 (m, 1 H, β-CH_2), 1.58–1.74 (m, 2 H, γ-CH_2), 1.50 [2 s, 18 H, $C(CH_3)_3$], 1.44 [s, 9 H, $C(CH_3)_3$].

^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.5 (CO_2t-Bu), 152.5 (2 × *C*O-Boc), 148.0 (ArC–N), 132.5 (ArC–S), 124.0, 130.9, 131.5, 133.4 (ArCH), 83.0 [2 × *C*$(CH_3)_3$], 81.4 [*C*$(CH_3)_3$], 58.3 (α-CH), 49.7 (δ-CH_2), 34.4 (NCH_3), 28.0 [2 × C(*C*$H_3)_3$], 27.9 [C(*C*$H_3)_3$], 26.4 (β-CH_2), 24.8 (γ-CH_2).

MS (ESI): *m*/*z* = 610 $[M + Na]^+$, 388 $[M - 2 \times C_4H_8 - 2 \times CO_2 + H]^+$, 376 $[M - 3 \times C_4H_8 - CO_2 + H]^+$, 332 $[M - 3 \times C_4H_8 - 2 \times CO_2 + H]^+$.

Anal. Calcd for $C_{26}H_{41}N_3O_{10}S{\cdot}0.4\ H_2O$ (594.90): C, 52.49; H, 7.08; N, 7.06. Found: C, 52.74; H, 7.67; N, 6.85.

N^α,*N*^{α}-Bis(*tert*-butyloxycarbonyl)-*N*^{δ}-methyl-L-ornithine *tert*-Butyl Ester (8)

To a solution of **7** (1.21 g, 2.1 mmol) in DMF (15 mL) were added K_2CO_3 (0.85 g, 6.2 mmol, 3 equiv) and thiophenol (509 µL, 4.1 mmol, 2 equiv). The reaction mixture was stirred magnetically at r.t. for 2 h and washed with 10% aq ammonia (10 mL). The mixture was extracted with CH_2Cl_2 (3 × 10 mL) and the combined organic phases were dried (Na_2SO_4). The solvent was removed under reduced pressure and purification was carried out by flash column chromatography (silica gel, CH_2Cl_2–MeOH, 0–30%); yield: 0.74 g (89%); colorless oil; R_f = 0.52 (CH_2Cl_2–MeOH–aq NH_3, 8.5:1.5:0.5).

IR (ATR): 2978, 1734, 1697, 1366, 1130, 849 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.72 (dd, ^3J = 9.5, 5.3 Hz, 1 H, α-CH), 2.97 (br s, 1 H, NH), 2.61–2.70 (m, 2 H, δ-CH_2), 2.46 (s, 3 H, NCH_3), 2.04–2.16 (m, 1 H, β-CH_2), 1.82–1.94 (m, 1 H, β-CH_2), 1.53–1.66 (m, 2 H, γ-CH_2), 1.50 [2 s, 18 H, $C(CH_3)_3$], 1.44 [s, 9 H, $C(CH_3)_3$].

^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.8 (CO_2t-Bu), 152.5 (2 × *C*O-Boc), 82.8 [2 × *C*$(CH_3)_3$], 81.2 [*C*$(CH_3)_3$], 58.7 (α-CH), 51.1 (δ-CH_2), 35.8 (NCH_3), 28.0 [2 × C(*C*$H_3)_3$], 27.9 [C(*C*$H_3)_3$], 26.9 (β-CH_2), 26.0 (γ-CH_2).

MS (ESI): *m*/*z* = 425 $[M + Na]^+$, 303 $[M - C_4H_8 - CO_2 + H]^+$.

HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{20}H_{38}N_2O_6$: 403.2803; found: 403.2798.

Anal. Calcd for $C_{20}H_{38}N_2O_6$ (402.53): C, 59.68; H, 9.52; N, 6.96. Found: C, 59.31; H, 9.89; N, 6.63.

N^ω,*N*$^{ω'}$-Bis(*tert*-butyloxycarbonyl)-*N*^{α},*N*^{α}-bis(*tert*-butyloxycarbonyl)-*N*^{δ}-methyl-L-arginine *tert*-Butyl Ester (9)

To a solution of compound **8** (0.71 g, 1.88 mmol) in anhydrous CH_2Cl_2 (20 mL) was added *N*,*N*′-bis-(*tert*-butyloxycarbonyl)thiourea (0.59 g, 2.13 mmol, 1.2 equiv) and DIPEA (611 µL, 3.6 mmol, 2 equiv). The reaction mixture was stirred magnetically at 0 °C for 30 min; EDCI was then added (0.69 g, 3.6 mmol, 2 equiv) and the mixture was stirred for an additional 2 h at r.t. The solvent was removed in vacuo and purification was performed by flash column chromatography (silica gel, cyclohexane–EtOAc, 0–30%); yield: 1.12 g (98%); colorless oil; R_f = 0.51 (cyclohexane–EtOAc, 1:1).

IR (ATR): 2978, 1740, 1699, 1608, 1366, 1231, 1047, 847 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.6 (br s, 1 H, NH), 4.72 (dd, ^3J = 9.0, 5.4 Hz, 1 H, α-CH), 3.35–3.63 (m, 2 H, δ-CH_2), 3.00 (s, 3 H, NCH_3), 1.99–2.12 (m, 1 H, β-CH_2), 1.77–1.92 (m, 1 H, β-CH_2), 1.60–1.74 (m, 2 H, γ-CH_2), 1.51 [2 s, 18 H, $C(CH_3)_3$], 1.48 [2 s, 18 H, $C(CH_3)_3$], 1.45 [s, 9 H, $C(CH_3)_3$].

^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.6 (CO_2t-Bu), 155.8 (C=N), 152.5 (4 × *C*O-Boc), 82.9 [4 × *C*$(CH_3)_3$], 81.3 [*C*$(CH_3)_3$], 58.5 (α-CH), 50.6 (δ-CH_2), 36.8 (NCH_3), 28.2 [2 × C(*C*$H_3)_3$], 28.1 [2 × C(*C*$H_3)_3$], 28.0 [C(*C*$H_3)_3$], 26.6 (β-CH_2), 24.3 (γ-CH_2).

MS (ESI): *m*/*z* = 645 $[M + H]^+$.

HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{31}H_{56}N_4O_{10}$: 645.4069; found: 645.4072.

Anal. Calcd for $C_{31}H_{56}N_4O_{10}$ (644.81): C, 57.74; H, 8.75; N, 8.69. Found: C, 57.72; H, 9.01; N, 8.60.

N^δ-Methyl-L-ornithine Dihydrochloride (10)

Compound **8** (0.22 g, 0.54 mmol) was dissolved in absolute Et_2O (20 mL) under a N_2 atmosphere and stirred magnetically at 0 °C for 30 min. Gaseous HCl was carefully bubbled through the solution for 20 min and the mixture was stirred at 0 °C for an additional 2 h. The reaction mixture was left in the refrigerator for 24 h and then concentrated under vacuum. The white solid was taken up in double distilled H_2O (2 × 1 mL) and purified by RP-18 column chromatography with double distilled H_2O as eluent. The product containing fractions were combined and concentrated under reduced pressure; yield: 105 mg (89%); white, crystalline and very hygroscopic solid (mp not measurable); $[\alpha]_D^{20}$ +11.5 (*c* 2.00, H_2O); R_f = 0.37 (*i*-PrOH–H_2O–AcOH, 6:3:1).

^{1}H NMR (300 MHz, D_2O/TPS): δ = 4.02 (t, ^3J = 6.1 Hz, 1 H, α-CH), 3.06 (t, ^3J = 7.5 Hz, 2 H, δ-CH_2), 2.69 (s, 3 H, NCH_3), 1.70–2.08 (m, 4 H, β,γ-CH_2).

^{13}C NMR (75 MHz, D_2O/TPS): δ = 173.8 (CO_2H), 54.4 (α-CH), 49.7 (δ-CH_2), 34.6 (NCH_3), 28.7 (β-CH_2), 23.3 (γ-CH_2).

MS (ESI): *m*/*z* = 461 $[3 \times M + Na]^+$, 293 $[2 \times M + H]^+$, 147 $[M + H]^+$.

HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_6H_{14}N_2O_2$: 147.1128; found: 147.1127.

N^δ-Methyl-L-arginine Dihydrochloride (11)

Compound **9** (1.02 g, 1.58 mmol) was dissolved in absolute Et_2O (80 mL) under a N_2 atmosphere and stirred magnetically at 0 °C for 30 min. Gaseous HCl was carefully bubbled through the solution for 45 min and the reaction mixture was stirred at 0 °C for an additional 6 h. The product **11** precipitated during storage in the refrigerator and was isolated after 24 h by careful solvent evaporation. The white solid was taken up in double distilled H_2O (2 × 1 mL) and purified by RP-18 column chromatography with double distilled H_2O as eluent. The product containing fractions were combined and concentrated under vacuum; yield: 369 mg (84%); white solid; mp 210 °C (Lit.[12] mp 210–212 °C for DL-compound); $[\alpha]_D^{20}$ +11.0 (*c* 2.00, H_2O); R_f = 0.51 (*i*-PrOH–H_2O–AcOH, 6:3:1).

IR (ATR): 3361, 3167, 2947, 1720, 1622, 1504, 1427, 1231, 752 cm^{-1}.

^{1}H NMR (300 MHz, D_2O/TPS): δ = 4.04 (t, ^3J = 6.1 Hz, 1 H, α-CH), 3.37 (t, ^3J = 7.3 Hz, 2 H, δ-CH_2), 2.99 (s, 3 H, NCH_3), 1.62–2.02 (m, 4 H, β,γ-CH_2).

^{13}C NMR (75 MHz, D_2O/TPS): δ = 173.8 (CO_2H), 158.3 (C=N), 54.6 (α-CH), 51.3 (δ-CH_2), 37.7 (NCH_3), 28.5 (β-CH_2), 24.0 (γ-CH_2).

^{35}Cl NMR (29 MHz, D_2O, NaCl): Calcd for $C_7H_{18}Cl_2N_4O_2$: Cl, 27.15; found: Cl, 24.66 (91%).

MS (ESI): m/z = 753 [4 × M + H]$^+$, 565 [3 × M + H]$^+$, 377 [2 × M + H]$^+$, 189 [M + H]$^+$.

HRMS (ESI): m/z [M + H]$^+$ calcd for $C_7H_{16}N_4O_2$: 189.1346; found: 189.1348.

HPLC-Based Chiral Separation of N^{δ}-Methyl-D/L-arginine

Discrimination of the enantiomers was carried out on a Waters HPLC system equipped with Waters W600 pump, Waters W474 fluorescence detector, and Waters 717 Plus autosampler; stationary phase: VDS Optilab Nucleosil 100 C18, 300 × 4.0 mm, 10 µm column. Eluent consisted of 17 mM L-proline and 4 mM $Cu(OAc)_2$ in double distilled H_2O (pH 4.5) with a flow rate of 0.5 mL/min. For detection of N^{δ}-methylarginine an online post-column derivatization equipped with a 2.5 m Teflon reaction coil was installed. Derivatization reagent contained 120 mL of *o*-phthalaldehyde (dissolved in 2 mL of MeOH), 0.3 mL of 2-mercaptoethanol, 0.1 M H_3BO_3, and 3 mM EDTA adjusted to pH 9.25 with NaOH. The flow rate for post-column derivatization was set to 1.0 mL/min. Detection of derivatized amino acids was carried out by a Waters W474 fluorescence detector, set to λ_{ex}: 340 nm, λ_{em}: 455 nm. Chromatography was performed isocratically at r.t. Reproducibility of peak areas of three sequential injections was assessed with a relative standard deviation of less than 1.2% for both enantiomers.

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Supporting Information

Supporting information for this article is available online at http://dx.doi.org/10.1055/s-0035-1561303.

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