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Arginine thioacid in synthesis of arginine conjugates and peptides†

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Protected arginine thioacid enables convenient *N*-acylation with no detectable racemization. We report efficient syntheses of potentially biologically active arginine conjugates and novel arginine-containing di-, tri- and tetrapeptides in good yields without loss of chiral integrity.

Introduction

Arginine, an essential amino acid, is one of the most metabolically versatile amino acids and is involved in numerous biological processes.[1] Arginine is also the precursor for the synthesis of urea, ornithine, nitric oxide (NO), polyamines, proline, creatine, glutamate and agmatine.[2,3] Synthetic arginine-rich peptides are efficient transporters of diverse biomolecules including nucleic acids, peptides, and proteins.[4–6] Arginine conjugates are reported to enhance gene expression in tumors[7,8] and arginine peptides are known for targeted gene delivery.[9,10]

Arginine is the source of nitrogen in the biosynthesis of nitric oxide (NO),[2,11,12] which is important in the elimination of numerous pathogens. The synthesis of NO requires L-arginine (but not D-arginine) entering the binding site of NO synthase.[13,14] Overproduction of NO may lead to diseases like Alzheimer's or Parkinson,[15,16] and inflammatory diseases such as arthritis[17] or colitis.[18] The synthesis of NO from L-arginine can be inhibited by N^{ω}-methyl-L-arginine and N^{ω}-ethyl-L-arginine.[19] More selective inhibition was observed with dipeptides and dipeptide esters containing N^{ω}-nitroarginine and phenylalanine.[20]

The highly basic nature of the arginine guanidine moiety can cause serious problems during chemical transformations. Even protection of the guanidine moiety before chemical manipulation does not completely prevent side reactions. A major problem is lactam formation which frequently occurs during the activation of the arginine carboxyl group.[21,22] It has also been shown that urethanes as guanidino protecting groups do not hinder the acylation and as a result arginine is converted to ornithine.[23] One possible way to avoid this problem could be prior transformation of the carboxyl group to thioacid. Indeed during the last decade close attention has been paid to the potential of thioacids, and these compounds have been increasingly applied in the design of complex molecules particularly for peptide synthesis.[24] Danishefsky's group reported that thioacids can act as highly reactive acyl donors in peptide ligation reactions[25] and methods have been developed for peptide and amide bond formation using thioacids and azides[26,27] or isonitriles.[28,29] Recently, coupling procedures between thioacids and dithiocarbamate terminal amines has been investigated.[30] In general, the mild conditions and selectivity of thioacid reactions make them attractive intermediates in bioorganic and peptide chemistry. Synthetic methods using thioacids usually include pre-activation of the carboxyl group with carbodiimides,[31] CDI,[32] or conversion to such reactive intermediates as *p*-nitrophenyl esters,[33] *N*-hydroxysuccinimide esters,[34] mixed anhydrides,[35] or *N*-acylbenzotriazoles.[36] An alternative approach using Lawesson's reagent developed by Danishefsky avoids the activation step but requires harsh reaction conditions.[37] Melnyk's group recently reported the synthesis of peptide thioacids at neutral pH using bis-(2-sulfanylethyl)amido peptide precursors in moderate yield.[38]

Herein, we present a novel approach to arginine-containing compounds comprising the coupling of protected N^{ω}-nitroarginine thioacid to the N-termini of amino acids, peptides and other *N*-nucleophiles.

Results and discussion

The well-known intramolecular cyclization during carboxyl group activation of protected arginines has caused difficulty in the synthesis of the corresponding C-terminus derivatives. A recently reported benzotriazole-activated arginine, Cbz-protected L-$^{\omega}NO_2$-Arg-Bt (ref. 22) is potentially an attractive precursor for the synthesis of novel arginine conjugates. For this purpose, in our initial study we examined benzotriazole-activated arginine and encountered some limitations. First,

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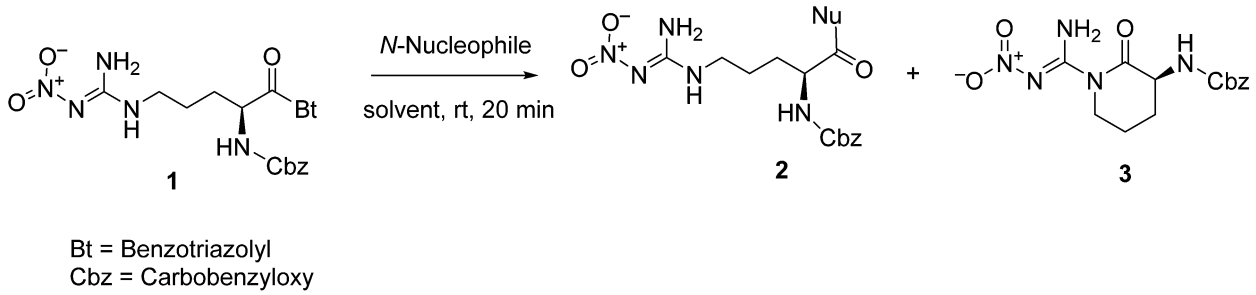
Table 1 Preparation of Cbz-protected arginine conjugates from Cbz-protected arginine benzotriazolide

N-Nucleophile	Solvent	Yield of product **2** (%)	Yield of by-product **3** (%)
n-Propylamine	THF	85%	11%
N-(3-Aminopropyl)imidazole	THF	83%	12%
Dipropylamine	THF	Traces	90%
Glycine	Acetonitrile–H_2O	80%	15%



Scheme 1 Synthesis of Cbz-protected arginine conjugates from Cbz-protected arginine benzotriazolide.

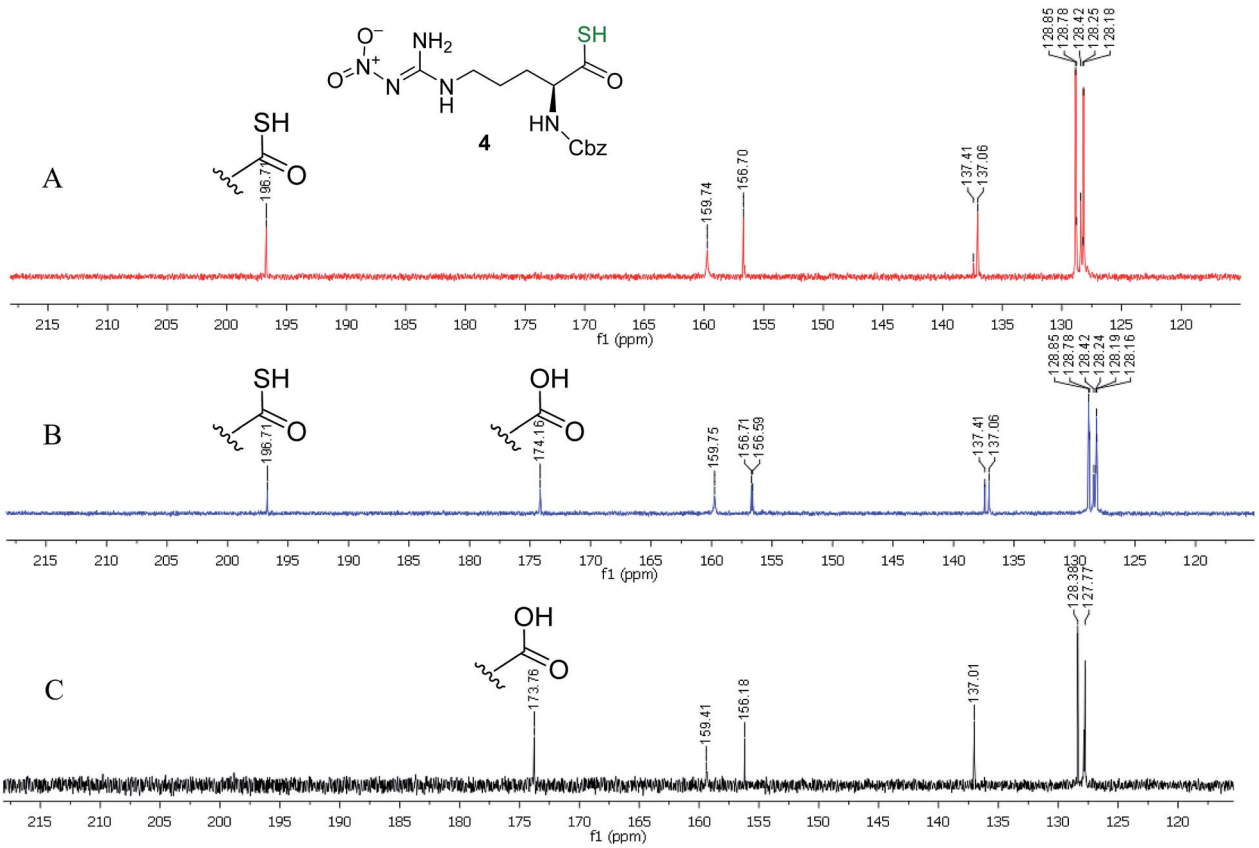


Fig. 1 Fragments of ^{13}C NMR (DMSO-d_6) of Cbz-L-Arg(NO_2)-SH **4** in DMSO-d_6 at rt: A – freshly prepared thioacid, B – after 24 h, C – pure protected arginine.

partial decomposition of L-Cbz-$^{\omega}NO_2$-Arg-Bt was observed in solution although this compound was stable in the solid state for at least 6 months at room temperature.[22] Moreover, attempts to couple benzotriazole derivatives with a bulky amine such as dipropylamine, led to formation of cyclic lactam **3**. However, benzotriazole-activated arginine reacted smoothly with primary amine or amino acids to form the products shown in Table 1.

The tendency of benzotriazole-activated arginine **1** to undergo cyclization prompted us to find a new synthetic derivative of arginine which could be easily obtained and handled during synthetic manipulations (Scheme 1).

The ability to efficiently convert Cbz-L-$^{\omega}NO_2$-Arg-Bt to the corresponding thioacid with retention of chirality[36] without any side reactions, inspired use of the arginine thioacid as a new precursor for the synthesis of arginine C-terminus derivatives.

The thioacid of protected L-arginine, Cbz-L-$^{\omega}NO_2$-Arg-SH **4**, is a stable white crystalline compound readily soluble in EtOAc, MeOH, THF and CH_3CN. However, arginine thioacid **4** hydrolysed to corresponding carboxylic acid in wet-DMSO-d_6 at room temperature (Fig. 1).

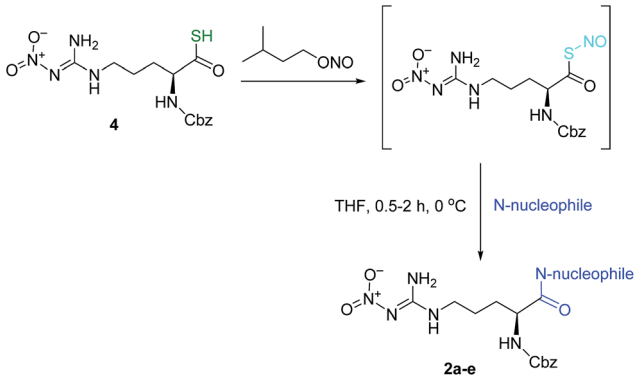
Preparation of arginine conjugates

Recently several strategies of amide bond formation have been developed using simple thioacid and amine as starting materials. Thus *S*-nitrosation of thioacids results in very reactive *S*-nitrosothioacid intermediates which react with a wide range of amines.[39] Another very mild and fast method of converting thioacids to the corresponding amides includes simple treatment of a thioacid with amine in presence of Cu(II) salts.[40,41] These carboxylic acid derivatives did not show any reactivity towards hydroxyl groups.

Both methods were tried, but in our hands *S*-nitrosation is more efficient (Table 2). The optimized standard conditions[39] were found to be slow addition of isoamyl nitrite (2.0 equiv.) to a solution of arginine thioacid, Cbz-L-$^{\omega}NO_2$-Arg-SH (1.0 equiv.), and amine (1.2 equiv.) in THF at 0 °C and in the dark. Although, formation of the arginine amide products was observed immediately, consumption of starting arginine thioacid required 0.5–2 h depending on the amine substrate. Thus, bulky dipropylamine reacts slowly under these conditions. In this approach formation of the cyclic side product **3** was not observed, and the desired arginine conjugates were easily obtained in pure form by precipitation from ether (Scheme 2) (Table 3).

Table 2 Preparation of Cbz-protected arginine conjugates from Cbz-protected arginine thioacid

Conjugates, **2**	*N*-Nucleophile	Yield (%)
2a	*n*-Propylamine	80
2b	*N*-(3-Aminopropyl)imidazole	78
2c	Dipropylamine	45
2d	Benzylamine	66
2e	Piperidine	56



Scheme 2 Synthesis of Cbz-protected arginine conjugates from Cbz-protected arginine thioacid **4**.

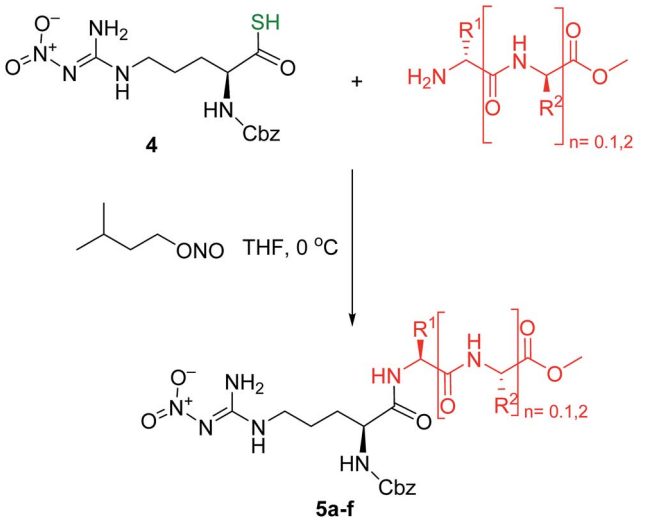
Table 3 Preparation of Cbz-protected arginine peptides

Product	Amino acid/peptide	Yield (%)	Mp (°C)	$[\alpha]_D^{20a}$
5a	D-Ala-OMe	55	149–151	+15.8
5b	L-Ala-OMe	58	158–160	−18.3
5c	DL-Ala-OMe	56	140–142	Racemic
5d	L-Val-OMe	68	138–139	−25.0
5e	L-Ala-L-Phe-OMe	71	152–154	−44.3
5f	Gly-L-Ala-L-Phe-OMe	56	103–105	−35.2

[a] $c = 1.0$, MeOH.

Preparation of arginine di- and tri-peptides

As a further application of arginine thioacid **4** in peptide synthesis, different esters of amino acids, dipeptides and tripeptides were used in the above *S*-nitrosation reaction resulting in the synthesis of arginine di-, tri- and tetrapeptides in good yields without loss of chiral integrity (Scheme 3). Arginine containing peptides are known for their better penetration ability and bioactivity.



Scheme 3 Synthesis of Cbz-protected arginine peptides.

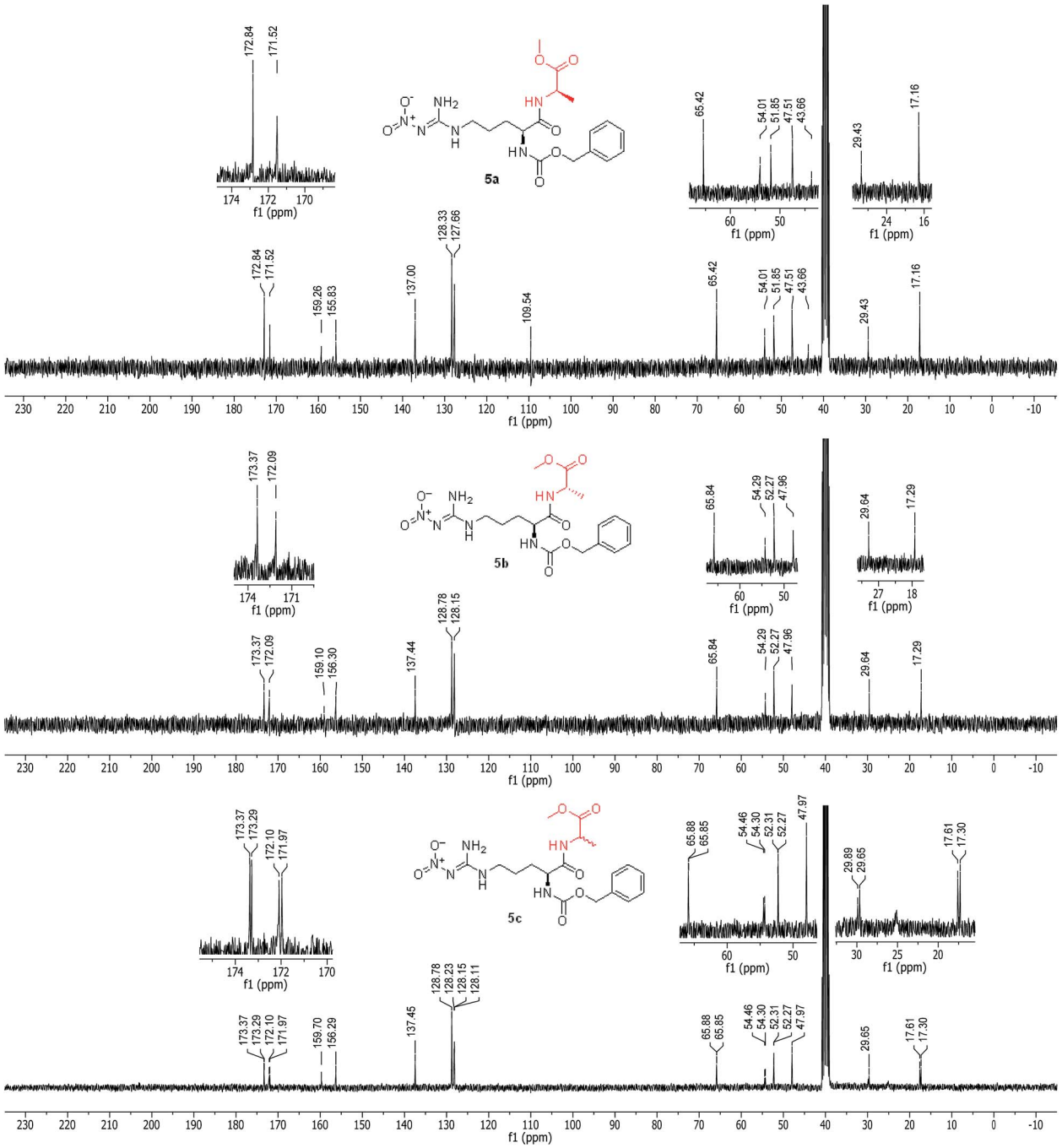


Fig. 2 ^{13}C NMR (DMSO-d_6) of compounds **5a–c**.

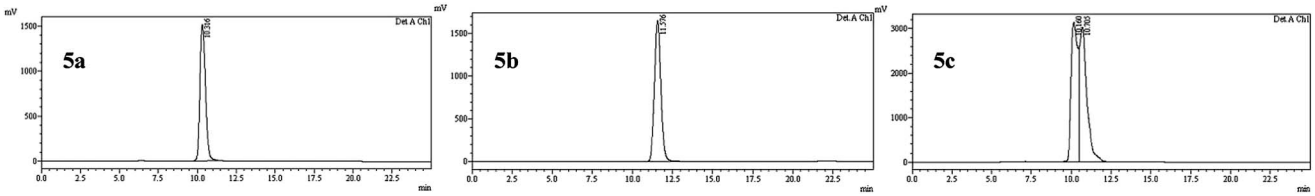


Fig. 3 HPLC spectra of compounds **5a–c** (chiral column, 50% ACN/H_2O, 1.0 mL min^{-1}).

To prove the retention of chirality in our described method we coupled the protected arginine thioacid **4** with D-Ala-OMe, L-Ala-OMe and dl-Ala-OMe. Compound **5c** displayed duplicate peaks in the NMR spectra. However, duplication of peaks in cases of **5a** and **5b** was not observed (Fig. 2). The chiral integrity of other synthesized products was supported by NMR data. For further proof, HPLC analysis of **5a–c** using a chiral column showed single peak for **5a** and **5b** but two peaks in the case of **5c**, this indicates **5c** is mixture of diastereoisomers of **5a** and **5b** (Fig. 3).

Conclusion

In conclusion, Cbz-protected arginine thioacid is a convenient coupling reagent, sufficiently reactive to form amide bonds at ambient temperature. This reagent offers an efficient method to prepare N-protected arginine conjugates and arginine peptides in synthetically useful yields without detectable racemization or intramolecular cyclization.

Experimental section

General

All solvents were reagent grade. Tetrahydrofuran was distilled from sodium/benzophenone under dry nitrogen. Melting points were determined on a capillary point apparatus equipped with a digital thermometer. NMR spectra were recorded in DMSO-d_6 on Mercury, Gemini or Varian NMR spectrometers operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C. Elemental analyses were performed on a Carlo Erba-EA1108 instrument and mass spectrometry was done with electro spray ionization (ESI). HPLC spectra were recorded on a Shimadzu instrument using Supelco chiral HPLC column.

(*S*)-2-(((Benzyloxy)carbonyl)amino)-5-(2-nitro guanidino) pentanethioic-*S*-acid (4). Thio arginine **4** was prepared by following our previous protocol[36] with a modified workup procedure. After completion of the reaction, THF was evaporated and residue was vigorously stirred with a mixture of diethyl ether and 1 N HCl (2 : 1) at 0 °C for 2 h. The resulting white solid was filtered, washed with cold diethylether and dried under high vacuum at room temperature. White microcrystals (344 mg, 93%); mp 153–155 °C (lit[36] mp 153–155 °C); $[\alpha]_D^{20}$ −9.0 (c = 0.5, MeOH); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.47 (s, 1H), 8.14–7.79 (m, 3H), 7.30–7.42 (m, 5H), 5.04–5.21 (m, 2H), 4.28–4.41 (m, 1H), 3.07–3.22 (m, 2H), 1.51–1.82 (m, 4H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 196.3, 159.4, 156.3, 136.7, 128.5, 128.0, 127.8, 66.1, 63.6, 60.9, 32.5, 28.0; anal. calcd for $C_{14}H_{19}N_5O_5S$: C, 45.52; H, 5.18; N, 18.96. Found: C, 45.85; H, 5.15; N, 18.62%.

General procedure for the synthesis of N-protected arginine conjugates. To a stirred solution of arginine thioacid, Cbz-L-$^{\omega}$NO$_2$-Arg-SH (1.0 mmol), and amine (1.2 equiv.) (or 1.2 mmol amino acid/peptide hydrochloride and 1.2 mmol TEA) in 10 mL THF, isoamyl nitrite (2.0 mmol) was added at 0 °C in the dark. The reaction was stirred for 2 h while slowly warming to rt. The reaction mixture was then concentrated under reduced pressure, diluted with ethyl acetate, and washed with sat. $NaHCO_3$, 1 N HCl and brine. The organic layer was dried with anhydrous $MgSO_4$ and solvent was removed under reduced pressure. Treatment of the resulting crude product with ether followed by filtration and washing 2–3 times with ether afforded the desired product in analytical purity.

Benzyl-(*S*)-(5-(2-nitroguanidino)-1-oxo-1-(propyl amino) pentan-2-yl)carbamate (2a). White solid (316 mg, 80%); mp 133–135 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.60–7.60 (m, 4H), 7.28–7.50 (br s, 5H), 5.01 (br s, 2H), 4.01–3.85 (m, 1H), 3.19–3.07 (m, 2H), 2.93–3.05 (m, 2H), 1.69–1.43 (m, 4H), 1.44–1.31 (m, 2H), 0.82 (t, J = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.5, 155.9, 137.1, 128.5, 128.4, 127.8, 127.7, 65.5, 54.5, 39.5, 29.4, 22.3, 11.4. HRMS (ESI) calcd for $C_{17}H_{27}N_6O_5$ $[M + H]^+$ 395.2037, found: 395.2033, calcd for $C_{17}H_{26}N_6O_5Na$ $[M + Na]^+$ 417.1857, found: 417.1865%.

Benzyl-(*S*)-(1-((3-(1*H*-imidazol-1-yl)propyl)amino)-5-(2-nitroguanidino)-1-oxopentan-2-yl)carbamate (2b). White solid (359 mg, 78%); mp 157–159 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.61–8.20 (br s, 1H), 8.19–7.65 (m, 3H), 7.58 (br s, 1H), 7.42 (d, J = 8.4 Hz, 1H), 7.39–7.24 (m, 5H), 7.13 (br s, 1H), 6.86 (br s, 1H), 5.01 (br s, 2H), 4.00–3.84 (m, 3H), 3.18–3.05 (m, 2H), 3.04–2.93 (m, 2H), 1.86–1.72 (m, 2H), 1.68–1.38 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.8, 156.0, 137.3, 137.0, 128.4, 128.3, 127.8, 127.7, 119.3, 65.4, 54.5, 43.4, 35.6, 30.7, 29.1. Anal. calcd for $C_{20}H_{28}N_8O_5$: C, 52.17; H, 6.13; N, 24.33. Found: C, 52.25; H, 6.07; N, 24.12%.

Benzyl-(*S*)-(1-(dipropylamino)-5-(2-nitroguanidino)-1-oxopentan-2-yl)carbamate (2c). White solid (196 mg, 45%); mp 95–97 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.66–7.55 (m, 3H), 7.48 (d, J = 8.3 Hz, 1H), 7.40–7.26 (m, 5H), 5.01 (br s, 2H), 4.41–4.27 (m, 1H), 3.28–3.18 (m, 2H), 3.16–3.00 (m, 4H), 1.62–1.50 (m, 4H), 1.52–1.32 (m, 4H), 0.84 (t, J = 7.8 Hz, 3H), 0.78 (t, J = 7.8 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.6, 159.8, 156.4, 137.5, 128.8, 128.2, 128.1, 65.8, 50.9, 48.9, 47.3, 29.4, 22.5, 20.9, 11.6, 11.4. Anal. calcd for $C_{20}H_{32}N_6O_5$: C, 55.03; H, 7.39; N, 19.25. Found: C, 54.65; H, 7.80; N, 19.12%.

Benzyl-(*S*)-(1-(benzylamino)-5-(2-nitroguanidino)-1-oxopentan-2-yl)carbamate (2d). White solid (292 mg, 66%); mp 133–135 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.56–7.51 (m, 3H), 7.43 (d, J = 8.0 Hz, 2H), 7.36–7.17 (m, 10H), 5.02 (s, 2H), 4.31–4.20 (m, 2H), 4.06–3.93 (m, 1H), 3.17–3.05 (m, 2H), 1.73–1.39 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.8, 159.3, 156.0, 139.3, 137.0, 128.4, 128.3, 127.8, 127.7, 127.1, 127.1, 126.8, 126.7, 65.5, 54.5, 42.1, 39.5, 29.2. Anal. calcd for $C_{21}H_{26}N_6O_5$: C, 57.00; H, 5.92; N, 18.99. Found: C, 56.70; H, 6.30; N, 19.11%.

Benzyl-(*S*,*E*)-(5-(2-nitroguanidino)-1-oxo-1-(piperidin-1-yl) pentan-2-yl)carbamate (2e). White solid (235 mg, 56%); mp 86–88 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.70–7.47 (m, 3H), 7.45 (d, J = 8.3 Hz, 1H), 7.40–7.24 (m, 5H), 5.02 (s, 2H), 4.50–4.35 (m, 1H), 3.51–3.33 (m, 4H), 3.19–3.07 (m, 2H), 1.65–1.32 (m, 10H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.8, 159.69, 156.3, 137.5, 128.8, 128.2, 128.1, 65.8, 65.4, 50.6, 46.2, 43.0, 29.1, 26.5, 25.8, 24.5, 15.6. Anal. calcd for $C_{19}H_{28}N_6O_5$: C, 54.27; H, 6.71; N, 19.99. Found: C, 53.90; H, 7.03; N, 20.32%.

Methyl-N^2-((benzyloxy)carbonyl)-$N^{\omega\prime}$-nitro-L-arginyl-D-alaninate (5a). White solid (241 mg, 55%); mp 149–151 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.56–7.53 (br s, 1H), 8.32 (d, J = 7.2 Hz,

1H), 7.43–7.25 (m, 5H), 5.03 (s, 2H), 4.27 (q, J = 7.2 Hz, 1H), 4.11–3.98 (m, 1H), 3.62 (s, 3H), 3.17–3.07 (m, 2H), 1.68–1.41 (m, 4H), 1.26 (d, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.8, 171.5, 159.3, 155.8, 137.0, 128.3, 127.7, 109.5, 65.4, 54.0, 51.9, 47.5, 43.7, 29.4, 17.2. Anal. calcd for $C_{18}H_{26}N_6O_7$: C, 49.31; H, 5.98; N, 19.17. Found: C, 49.23; H, 6.37; N, 19.54%.

Methyl-N^2-((benzyloxy)carbonyl)-$N^{\omega\prime}$-nitro-L-arginyl-L-alaninate (5b). White solid (232 mg, 58%); mp 158–160 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.56–7.53 (m, 1H), 8.35 (d, J = 7.2 Hz, 1H), 7.43–7.25 (m, 5H), 5.02 (br s, 2H), 4.26 (q, J = 7.2 Hz, 1H), 4.09–3.94 (m, 1H), 3.60 (s, 3H), 3.15–3.04 (m, 2H), 1.71–1.44 (s, 4H), 1.28 (d, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.4, 172.1, 159.1, 156.3, 137.4, 128.8, 128.2, 128.1, 65.8, 54.3, 52.3, 48.0, 29.6, 17.3. Anal. calcd for $C_{18}H_{26}N_6O_7$: C, 49.31; H, 5.98; N, 19.17. Found: C, 49.00; H, 6.23; N, 18.81%.

Methyl-N^2-((benzyloxy)carbonyl)-$N^{\omega\prime}$-nitro-L-arginyl-DL-alaninate (5c). White solid (246 mg, 56%); mp 140–142 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.12–7.40 (m, 3H), 8.36–8.28 (m, 1H), 7.37–7.25 (m, 5H), 5.07–4.95 (m, 2H), 4.26 (q, J = 7.2 Hz, 1H), 4.10–3.95 (m, 1H), 3.59 (s, 3H), 3.15–3.04 (m, 2H), 1.70–1.44 (s, 4H), 1.31–1.20 (m, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.4, 173.3, 172.1, 172.0, 159.7, 156.3, 137.4, 128.8, 128.2, 128.1, 128.1, 65.9, 65.8, 54.5, 52.3, 52.3, 48.0, 29.6, 25.3, 17.6, 17.3. Anal. calcd for $C_{18}H_{26}N_6O_7$: C, 49.31; H, 5.98; N, 19.17. Found: C, 49.27; H, 6.16; N, 19.45%.

Methyl-N^2-((benzyloxy)carbonyl)-$N^{\omega\prime}$-nitro-L-arginyl-L-valinate (5d). White solid (317 mg, 68%); mp 138–139 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.62–7.51 (m, 3H), 8.10 (d, J = 8.1 Hz, 1H), 7.43 (d, J = 7.8 Hz, 1H), 7.40–7.23 (m, 5H), 5.02 (s, 2H), 4.25–4.02 (m, 2H), 3.62 (s, 3H), 3.20–3.04 (m, 2H), 2.12–1.94 (m, 1H), 1.71–1.41 (m, 4H), 0.97–0.75 (m, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.6, 172.3, 159.7, 156.3, 137.5, 128.8, 128.8, 128.3, 128.2, 128.1, 65.8, 57.8, 54.4, 52.1, 30.4, 29.6, 25.3, 25.2, 19.4, 18.6. Anal. calcd for $C_{20}H_{30}N_6O_7$: C, 51.49; H, 6.48; N, 18.02. Found: C, 51.16; H, 6.79; N, 18.09%.

Methyl-N^2-((benzyloxy)carbonyl)-$N^{\omega\prime}$-nitro-L-arginyl-L-alanyl-L-phenylalaninate (5e). White solid (416 mg, 71%); mp 152–154 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.55–8.37 (br s, 1H), 8.31 (d, J = 7.8 Hz, 1H), 7.93 (d, J = 7.9 Hz, 1H), 7.43 (d, J = 8.0 Hz, 1H), 7.38–7.16 (m, 10H), 5.02 (s, 2H), 4.50–4.38 (m, 1H), 4.32–4.24 (m, 1H), 4.04–3.95 (m, 1H), 3.57 (s, 3H), 3.16–3.04 (m, 2H), 3.05–2.87 (m, 2H), 1.68–1.40 (m, 4H), 1.18 (d, J = 6.8 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.7, 172.2, 171.6, 159.7, 156.4, 137.4, 129.5, 128.8, 128.7, 128.2, 128.1, 127.0, 65.9, 54.5, 54.1, 52.2, 48.2, 37.0, 29.6, 25.1, 18.8. HRMS (ESI$^+$) calcd for $C_{27}H_{36}N_7O_8$ [M + H]$^+$ 586.2620, found: 586.2619, calcd for $C_{27}H_{35}N_7O_8Na$ [M + Na]$^+$ 608.2439, found: 608.2439%.

Methyl-N^2-((benzyloxy)carbonyl)-$N^{\omega\prime}$-nitro-L-arginylglycyl-L-alanyl-L-phenylalaninate (5f). White solid (360 mg, 56%); mp 103–105 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.49 (br s, 1H), 8.31 (d, J = 7.7 Hz, 1H), 8.21–8.16 (m, 1H), 7.88 (d, J = 7.6 Hz, 1H), 7.51 (d, J = 7.4 Hz, 1H), 7.39–7.11 (m, 10H), 5.07–4.96 (m, 2H), 4.49–4.40 (m, 1H), 4.35–4.27 (m, 1H), 4.04–3.96 (m, 1H), 3.73–3.64 (m, 2H), 3.57 (s, 3H), 3.17–3.07 (m, 2H), 3.05–2.90 (m, 2H), 1.72–1.43 (m, 4H), 1.16 (d, J = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 172.7, 172.5, 172.2, 168.6, 159.7, 156.5, 137.5, 137.4, 129.5, 128.8, 128.7, 128.3, 128.2, 127.0, 66.0, 54.8, 54.1, 52.3, 48.2, 42.4, 37.0, 29.4, 25.1, 18.7, 15.7. HRMS (ESI$^+$) calcd for $C_{29}H_{39}N_8O_9$ [M + H]$^+$ 643.2835, found: 643.2830, calcd for $C_{29}H_{38}N_8O_9Na$ [M + Na]$^+$ 665.2654, found: 665.2658%.

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