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Short communication

Conformationally flexible platelet aggregation inhibitors based on the tetrapeptide Arg–Gly–Asp–Arg

SI Klein[1]*, M Czekaj[1], BF Molino[2], V Chu[1]

[1]*Department of Cardiovascular Research, Rhone-Poulenc Rorer, 500 Arcola Rd, Collegeville, PA 19426;*
[2]*Department of Medicinal Chemistry, Helios Pharmaceuticals, 9800 Bluegrass Parkway, Louisville, KY 40299, USA*

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Summary — A series of nonpeptide fibrinogen receptor antagonists based upon the tetrapeptide Arg–Gly–Asp–Arg were prepared. These relatively simple derivatives incorporate a high degree of conformational flexibility that was anticipated to allow them to attain the requisite conformation for binding to the platelet fibrinogen receptor. Optimization of the distances between the required acidic and basic functional groups led eventually to compound **7**, which is a one hundred-fold more potent inhibitor of platelet aggregation than the peptide it is based upon.

RGD / platelet aggregation / GP IIb/IIIa / fibrinogen receptor antagonist

Introduction

Regardless of the stimulus involved, exposure of the platelet glycoprotein IIb/IIIa (GP IIb/IIIa) receptor is the final event that occurs during platelet activation and immediately prior to platelet aggregation [1]. The binding of the plasma protein fibrinogen to the exposed GP IIb/IIIa receptors on more than one platelet simultaneously, allows the process of platelet aggregation to go forward [2]. When this process occurs outside the bounds of normal hemostasis, thrombotic disorders such as unstable angina and myocardial infarction can result [3, 4].

Glycoprotein IIb/IIIa is a member of the integrin family of cell surface receptors [5]. It is well established that the binding interaction between fibrinogen and GP IIb/IIIa can be blocked by small molecules containing, or modeled after, the integrin receptor recognition sequence Arg–Gly–Asp (RGD) [6]. Many potent and selective RGD analogs have been reported that can block the processes of platelet aggregation and subsequent thrombus formation [7, 8]. Several of these are currently undergoing clinical evaluation [9-11].

One feature that most of these compounds share, along with a carboxylic acid and basic amine to mimic the side chains of the Asp and Arg residues in the peptide RGD, is the presence of a lipophilic group proximal to the carboxylic acid. Early work done with tetrapeptides of the structure RGD-X showed that lipophilic amino acids at the carboxyl terminus led to peptides with an enhanced ability to block platelet aggregation [12]. In many of the recently reported nonpeptides, this type of modification has generally provided an increase in in vitro potency [13, 14].

There have been a few reports of RGD-based analogs which contain an arginine or arginine-like residue at the carboxyl terminus [15, 16]. These structures are generally of a conformationally restricted nature, for example cyclic peptides. We report here a group of conformationally flexible compounds based on 1,1-diaminopropionic acid. Rather than incorporating a lipophilic group proximal to the carboxylic acid, these derivatives add a second basic functional group at this position and show a significant increase in in vitro potency over the peptides from which they are derived.

Chemistry

The synthesis of the compounds described in table I is illustrated in scheme 1. The guanidino acids needed to prepare the compounds in table I were produced by treating the corresponding amines with aminoiminomethanesulfonic acid [17]. Protection of the guanidine

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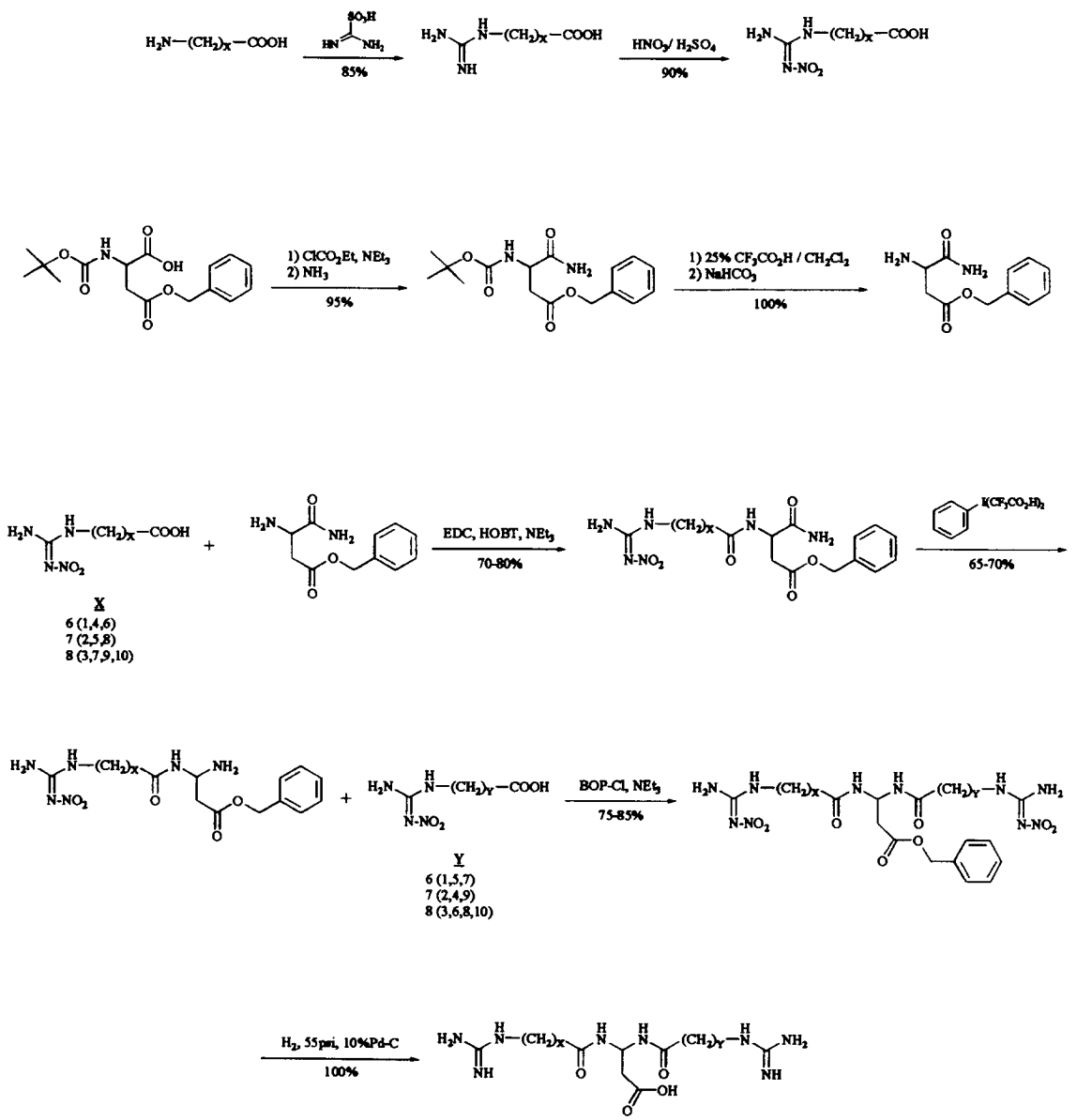
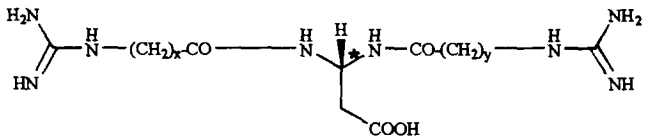


Scheme 1. Synthesis of 1,1-diaminopropionic acid-based RGD analogs.

was necessary in order to facilitate handling of these materials, and although nitroguanidines can be prepared directly from amines, we were able to obtain much higher yields by using a two-step process. Consequently, the requisite nitro-protected ω-guanidino carboxylic acids were prepared by treating the appropriate guanidino acids with a mixture of fuming nitric acid and sulfuric acid.

The nitro-protected guanidino acids were coupled to either aspartamide β-benzyl ester **1–9** or glutamide β-benzyl ester **10** using the well-known peptide coupling reagent 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC). The two carboxamides were prepared from suitably protected D- or L-amino acids using standard manipulations, as shown in scheme 1 for aspartic acid.

Table I. Arg–Gly–Asp–Arg-based fibrinogen receptor antagonists.



Compound	*X*	*Y*	*	*Aggregation, IC_{50} (μM)*	*Binding, IC_{50} (μM)*
1	6	6	–	2.80	0.63
2	7	7	–	0.12	0.07
3	8	8	–	0.14	0.11
4	6	7	S	0.74	0.68
5	7	6	R	0.32	0.13
6	6	8	S	2.80	0.70
7	8	6	R	0.12	nd
8	7	8	S	1.30	nd
9	8	7	R	0.30	0.13
10	8	8	– (Glu)	> 25.0	nd

Following acylation with the ω-nitroguanidino acids, the carboxamide was treated with I,I-bis(trifluoroacetoxy)iodobenzene, a mild reagent that induces the Hoffman rearrangement of primary carboxamides to amines [18]. This convenient method is reported to proceed with retention of configuration in the migrating group, and indeed there was no evidence of racemization of the chiral center, as demonstrated by the presence of a single material when analyzed by chiral HPLC. The resulting monoacylated geminal diamines are stable to isolation, purification by flash chromatography, and storage at room temperature.

The newly formed free amine was coupled to a second nitroguanidino acid of the same or different chain length. Use of the more reactive coupling agent, bis(2-oxo-3-oxazolidinyl) phosphonic chloride (BOP-Cl), was required in this case to obtain reasonable yields of the diacylated materials. Subsequent hydrogenolysis of the benzyl ester and concomitant reduction of the nitro groups provided the final compounds, which were purified by reverse-phase HPLC using an acetonitrile/water gradient, buffered with 0.1% trifluoroacetic acid. Lyophilization of the fractions containing the final product then gave each material as a white powder.

The synthesis of des-NH_2,des-COOH-Arg-(ΨCH_2CH_2)Gly–Asp–Arg (fig 1) was accomplished in a similar fashion by coupling 8-nitroguanidinooctanoic acid to aspartic acid α-*t*-butyl-β-benzyl ester. Removal of the *t*-butyl ester with trifluoroacetic acid and coupling of the free carboxyl group to nitroagmatine gave the penultimate product.

Results and discussion

All IC_{50} values reported are for the inhibition of the ADP-induced aggregation of fixed, activated human platelets [19, 20]. During the course of our work with RGD-containing tetrapeptides, we observed that the

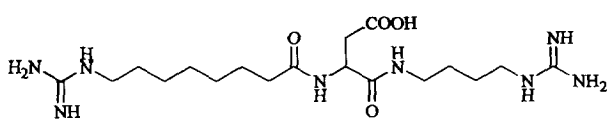


Fig 1. des-NH_2, des-COOH, R(ΨCH_2CH_2)GDR (IC_{50} = 1.0 mM).

peptide Arg–Gly–Asp–Arg was slightly more potent than the peptide Arg–Gly–Asp–Val insofar as inhibiting the aggregation of fixed, activated human platelets was concerned. By contrast, acidic side chains at this position (for example Arg–Gly–Asp–Glu) led to completely inactive peptides (fig 2). This seemed to imply that whatever the exact mechanism of binding of RGD-containing peptides to GP IIb/IIIa was, the receptor could accommodate either a lipophilic or basic side chain at the C-terminal position.

It is known that peptides of the structure RGD-X are amenable to specific modifications that allow for either retention of, or improvement in, in vitro antiaggregatory activity. These modifications include deletion of both the amino and acidic termini and elimination of the Arg–Gly amide bond [21]. When applied to the peptide RGDR, these modifications led to a sevenfold improvement in potency.

Further modification of this structure by reversing the direction of the remaining amide bond led to the compounds described in table I. These structures may be viewed as one acidic and two basic functional groups tethered together in a highly flexible manner, which should allow them to attain the conformation necessary for binding to the GP IIb/IIIa receptor. That these compounds do indeed function as inhibitors of platelet aggregation by binding to GP IIb/IIIa, rather than in some nonspecific manner, is demonstrated by the good IC_{50} values generated upon measuring their ability to inhibit the binding of radiolabelled fibrinogen to activated platelets [22, 23], as well as the clear preference for a single stereochemistry at the one chiral center present in compounds **4**–**9**.

In addition, it is well known that in contrast to tetrapeptides of the structure RGD-X, tetrapeptides of the structure RGE-X do not bind to GP IIb/IIIa and do not inhibit platelet aggregation [24]. Compound **10**, which is based on glutamic acid rather than aspartic acid, is also inactive. The relationship between the activity of **3** and **10** mirrors the relationship between the activity of RGD-X and RGE-X peptides.

The symmetric compounds **1**–**3** seem to suggest that a total distance of 19 atoms between the two basic termini of this series is optimal. Both 17 atoms (**1**) and 21 atoms (**3**) are less potent inhibitors in vitro. A group of asymmetric compounds containing between 18 and 20 atoms was then synthesized, of either possible stereochemistry.

Here again a distance of 19 atoms is seen to be optimal and **7** shows an almost tenfold increase in potency over des-NH_2,des-COOH-R(ΨCH_2CH_2)GDR. Both **5** and **9** are two- to three-fold less potent than **7**. The R stereochemistry at the chiral center derived from D-aspartic acid is consistently preferred.

Thus, starting with the tetrapeptide RGDR and first eliminating most of the functionality except for the amino acid sidechains and then optimizing chain lengths, in vitro potency for inhibiting platelet aggregation was increased by almost two orders of magnitude.

Experimental protocols

In vitro platelet aggregation studies

Blood was obtained from human volunteers, all of whom had been free of any medications for at least 14 days prior to blood donation. In all cases the first 1–2 mL of blood obtained were discarded in order to avoid the traces of thrombin that have been shown to be generated during venipuncture. The remainder of each blood sample was mixed with 10% of its volume of a 3.8% sodium citrate solution. Gel-filtered platelets were isolated following the procedures of Marguerie [19] and Ruggeri [20]. For the preparation of fixed, activated platelets, washed platelets were activated with human α-thrombin

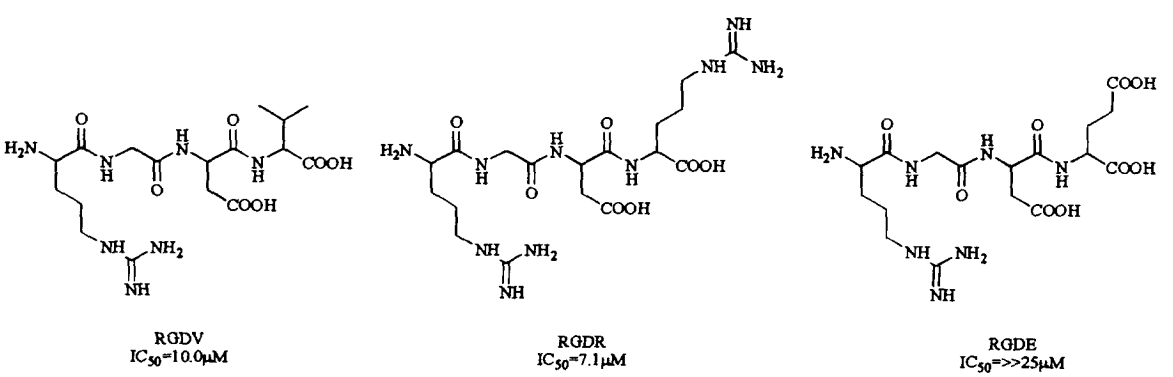


Fig 2. Arg–Gly–Asp–Xaa tetrapeptides.

(Enzyme Research Lab, South Bend, IN) at a final concentration of 1U/mL for 2 min at room temperature, followed by the addition of the thrombin inhibitor I-2581 (Kabi, Pharmacia Harper, Franklin, OH) at a final concentration of 20 μM. To the activated platelets, paraformaldehyde (Sigma) was added to a final concentration of 0.5% and incubated for 30 min at room temperature. The fixed, activated platelets were then collected by centrifugation at 650 *g* for 15 min. Platelet pellets were washed four times with Tyrode's-HSA buffer and resuspended to 2 x 10^8 cells/mL in the same buffer.

Platelet aggregation was performed using fixed, activated platelets according to the turbidometric method of Born [25]. Various doses of a given compound were incubated with 0.4 mL of platelet suspension for 1 min and aggregation was initiated by the addition of fibrinogen (Calbiochem) to a final concentration of 250 μg/mL (0.72 μM). A platelet aggregation profiler model PAP-4 (Bio Data, Hatsboro, PA) was used to record platelet aggregation. Inhibition of aggregation was expressed as the percentage of the rate of aggregation in the presence of antagonists compared to that observed in the absence of antagonists. IC_{50} was then calculated for each compound [26].

Competitive ^{125}I-fibrinogen binding studies

Fibrinogen (Kabi, Stockholm, Sweden) was purified according to Hawiger and Timmons[15a] and radioiodinated using a modification of the procedure of Fraker and Speck[15b]. Competitive binding assays were performed according to Hawiger and Timmons (1992) with minor modifications. Reactions were carried out in duplicate in Tyrode's buffer and 1 x 10^8 platelets/mL, 100 nM [^{125}I]-fibrinogen and either 100 μM TRAP (SFLLRN-NH_2) (Peninsula Laboratories, Belmont, CA) or 10 μM ADP. When inhibitors of [^{125}I]-fibrinogen binding were tested, both inhibitor and [^{125}I]-fibrinogen were added prior to agonist addition. Following a 30 min incubation at room temperature, the reactions were layered onto a 20% sucrose cushion and centrifuged at 10 000 *g* for 3 min. The reaction tubes were frozen with liquid N_2 and the tips of each tube clipped off and counted in a γ-counter.

Chemistry

All amino acids, solvents and other reagents were used as received from commercial sources without additional purification. Proton NMR spectra were recorded on a Bruker ARX 300 MHz spectrometer. Mass spectra were obtained from a Varian VG-70SE spectrometer. Preparative reverse-phase HPLC was performed with a Rainin SD-1 Dynamax system and a 2 inch C-18 reverse-phase Dynamax 60A column using a gradient of 20% acetonitrile/0.1% TFA in water to 100% acetonitrile and a flow rate of 50 mL/min. Analytical reverse-phase HPLC was performed with a Rainin HPX system and an analytical C-18 reverse-phase Dynamax 60 A column using the same gradient system used for preparative work and a flow rate of 1 mL/min.

Work-up means drying over magnesium sulfate, filtering and concentrating in vacuo. Compounds **1–10** were obtained as hygroscopic solids following lyophilization after final reverse-phase HPLC purification. Consequently, elemental analysis was not obtained for these compounds and purity was gauged by analytical reverse-phase HPLC of the lyophilized samples using the gradient system described below. Retention time (RT) in this system and area percentage (A%) is given for each compound.

Preparation of ω-guanidinocarboxylic acids (general procedure)

To a solution of potassium carbonate (20 mmol) in 25 mL of water is added the amino acid (10 mmol) in a single portion at room temperature. To the resulting solution is added aminoiminomethanesulfonic acid (10 mmol) portionwise at room temperature. The reaction mixture is allowed to stir overnight at room temperature, filtered and the precipitate was washed with water and dried under vacuum.

A mixture of 20 mL of fuming nitric acid and 12 mL of sulfuric acid was cooled to 0 °C and the guanidine (1 g) was carefully added portionwise. Stirring was continued for 1 h at 0 °C and the reaction mixture was poured onto ice and brought to pH 8 with concentrated ammonium hydroxide. The pH was readjusted to 5 and the mixture allowed to stand at room temperature for several hours. The precipitate was collected, washed with ethanol, and dried under vacuum.

7-Nitroguanidinoheptanoic acid
^{1}H-NMR (DMSO–d_6) δ 3.15 (m, 2H), 2.18 (t, *J* = 4.2 Hz, 2H), 1.40 (m, 8H).

8-Nitroguanidinooctanoic acid
^{1}H-NMR (DMSO–d_6) δ 3.15 (m, 2H), 2.16 (t, *J* = 4.2 Hz, 2H), 1.40 (m, 10H).

9-Nitroguanidinononanoic acid
^{1}H-NMR (DMSO–d_6) δ 3.15 (m, 2H), 2.16 (t, *J* = 4.2 Hz, 2H), 1.40 (m, 12H).

Aspartic acid amide β-benzyl ester
To a solution of *N*-α-Boc-aspartic acid β-benzyl ester (2.0 g, 6.2 mmol) in 50 mL of methylene chloride was added triethylamine (1.3 mL, 9.3 mmol) in a single portion at room temperature. The resulting solution was cooled to 0 °C and ethyl chloroformate (0.7 mL, 6.8 mmol) was added dropwise via a syringe. After addition was complete, stirring was continued for 5 min at 0 °C. Ammonia gas was bubbled through the reaction mixture for 5 min at 0 °C and stirring continued for an additional hour. The reaction mixture was diluted with methylene chloride (100 mL) and washed with water (3 x 75 mL). Work-up provided the desired carboxamide.

The *N*-α-Boc carboxamide was redissolved in 30 mL of methylene chloride and trifluoroacetic acid was added in a single portion at 0 °C. Stirring was continued for 1 h at 0 °C and all solvents were removed in vacuo. The residue was partitioned between ethyl acetate and saturated sodium bicarbonate. The organic layer was separated and subjected to work-up to provide the desired product, which was used without further purification.

^{1}H-NMR ($CDCl_3$) δ 7.35 (s, 5H), 5.12 (s, 2H), 4.50 (m, 1H), 3.08 (dd, *J* = 7.5, 2.4 Hz), 2.70 (dd, *J* = 7.5, 3.6 Hz).

Glutamic acid amide β-benzyl ester
Prepared starting from *N*-α-Boc-glutamic acid-γ-benzyl ester using an otherwise identical procedure.

^{1}H-NMR ($CDCl_3$) δ 7.35 (s, 5H), 5.07 (s, 2H), 4.28 (m, 1H), 2.46 (t, *J* = 7.0 Hz, 2H), 1.22 (m, 2H).

N-α-(7-(Nitroguanidino)heptanoyl)aspartic acid amide β-benzyl ester (general procedure for acylation using EDC)
To a solution of 7-guanidinoheptanoic acid (250 mg, 1 mmol) in 5 mL of dimethylsulfoxide (DMF) was added triethylamine (0.28 mL, 2 mmol), EDC (193 mg, 1 mmol), and hydroxybenzotriazole (HOBT) (136 mg, 1 mmol) sequentially at room temperature. Stirring was continued for 20 min at room tempe-

rature and aspartic acid amide β-benzyl ester (262 mg, 1 mmol) was added in a single portion. Stirring was allowed to continue overnight at room temperature. The reaction mixture was diluted with ethyl acetate (100 mL) and washed with water (3 x 50 mL), 1 N HCl (1 x 50 mL), saturated sodium bicarbonate (1 x 50 mL) and brine (1 x 50 mL). The organic layer was worked up and subjected to flash chromatography (1:4 ethyl acetate/hexanes) to give *N*-α-(7-guanidinoheptanoyl)aspartic acid amide β-benzyl ester as a white solid (yield 81%).

^{1}H-NMR (DMSO–d_6) δ 7.35 (s, 5H), 5.08 (s, 2H), 4.59 (m, 1H), 3.60 (t, J = 2.6 Hz, 2H), 2.75 (dd, J = 7.5, 2.8 Hz, 1H), 2.58 (dd, J = 7.5, 3.8 Hz, 1H), 2.08 (t, J = 5.9 Hz, 2H), 1.47 (m, 3H), 1.20 (m, 5H).

N-α-(8-(Nitroguanidino)octanoyl)aspartic acid amide β-benzyl ester

^{1}H-NMR (DMSO–d_6) δ 7.35 (s, 5H), 5.08 (s, 2H), 4.63 (m, 1H), 3.62 (t, J = 2.8 Hz, 2H), 2.75 (dd, J = 7.5, 2.5 Hz, 1H), 2.55 (dd, J = 7.5, 4.6 Hz, 1H), 2.28 (t, J = 5.3 Hz, 2H), 1.47 (m, 4H), 1.20 (m, 6H).

N-α-(9-(Nitroguanidino)nonanoyl)aspartic acid amide β-benzyl ester

^{1}H-NMR (DMSO–d_6) δ 7.35 (s, 5H), 5.08 (s, 2H), 4.68 (m, 1H), 3.62 (t, J = 2.8 Hz, 2H), 2.70 (dd, J = 7.8, 3.0 Hz, 1H), 2.49 (dd, J = 7.8, 4.9 Hz, 1H), 2.20 (t, J = 4.5 Hz, 2H), 1.47 (m, 4H), 1.20 (m, 8H).

N-α-(9-(Nitroguanidino)nonanoyl)glutamic acid amide γ-benzyl ester

^{1}H-NMR (DMSO–d_6) δ 7.35 (s, 5H), 5.18 (s, 2H), 4.74 (m, 1H), 3.62 (t, J = 3.5 Hz, 2H), 2.67 (t, J = 5.1 Hz, 2H), 2.20 (t, J = 4.5 Hz, 2H), 1.47 (m, 4H), 1.20 (m, 10H).

3-(7-(Nitroguanidino)heptanoyl)amino-3-aminopropionic acid benzyl ester (general procedure for Hoffman rearrangement)

I,I-bis(trifluoroacetyl)iodobenzene (78 mg, 0.18 mmol) was dissolved in 2 mL of acetonitrile at room temperature and 2 mL of distilled water was added. To the resulting solution was added *N*-α-(7-guanidinoheptanoyl)aspartic acid amide β-benzyl ester in a single portion at room temperature. Following the addition of the carboxamide, stirring was continued for 5 h at room temperature. The reaction mixture was diluted with 10 mL of water and 1 mL of concentrated hydrochloric acid was added dropwise. The aqueous mixture was extracted with ether (5 x 10 mL). The combined organic extracts were dried over magnesium sulfate and filtered. The resulting solution was concentrated until a precipitate began forming, and then allowed to stand overnight in the refrigerator. The white solid, 3-(7-guanidinoheptanoyl)amino-3-aminopropionic acid benzyl ester hydrochloride, was filtered off and dried under vacuum (yield 70%).

^{1}H-NMR (DMSO–d_6) δ 7.39 (s, 5H), 5.22 (m, 1H), 5.12 (s, 2H), 3.12 (m, 2H), 2.98 (m, 2H), 2.07 (t, J = 8.0 Hz, 2H), 1.48 (m, 3H), 1.25 (m, 5H).

3-(8-(Nitroguanidino)octanoyl)amino-3-aminopropionic acid benzyl ester

^{1}H-NMR (DMSO–d_6) δ 7.39 (s, 5H), 5.28 (m, 1H), 5.12 (s, 2H), 3.17 (m, 2H), 2.98 (m, 2H), 2.12 (t, J = 7.8 Hz, 2H), 1.48 (m, 4H), 1.25 (m, 6H).

3-(9-(Nitroguanidino)nonanoyl)amino-3-aminopropionic acid benzyl ester

^{1}H-NMR (DMSO–d_6) δ 7.39 (s, 5H), 5.28 (m, 1H), 5.16 (s, 2H), 3.16 (m, 2H), 3.04 (m, 2H), 2.10 (t, J = 8.1 Hz, 2H), 1.48 (m, 5H), 1.25 (m, 7H).

4-(9-Nitroguanidinononanoyl)amino-4-aminobutanoic acid benzyl ester

^{1}H-NMR (DMSO–d_6) δ 7.36 (s, 5H), 5.35 (m, 1H), 5.18 (s, 2H), 3.26 (m, 2H), 3.00 (m, 2H), 2.10 (t, J = 8.1 Hz, 2H), 1.48 (m, 5H), 1.25 (m, 9H).

*3,3-bis(7-Guanidinoheptanoyl)aminopropionic acid **1** (general procedure for acylation using BOP–Cl and deprotection of the penultimate product)*

To a solution of 7-nitroguanidinoheptanoic acid (54 mg, 0.22 mmol) in 5 mL of dimethylformamide was added triethylamine (0.06 mL, 0.44 mmol) in a single portion at room temperature. The solution was cooled to 0 °C and BOP–Cl (56 mg, 0.22 mmol) was added in a single portion. Stirring was continued for 5 min at 0 °C and 3-(7-guanidinoheptanoyl)amino-3-aminopropionic acid benzyl ester hydrochloride (100 mg, 0.22 mmol) was added in a single portion. The reaction mixture was allowed to come to room temperature and stirred overnight. The reaction mixture was diluted with ethyl acetate (100 mL) and sequentially washed with water (3 x 50 mL), 1 N hydrochloric acid (1 x 25 mL), saturated sodium bicarbonate (1 x 25 mL) and brine (1 x 50 mL). The organic layer was worked up to provide 3,3-bis((7-nitroguanidino)heptanoyl)-aminopropionic acid benzyl ester, which was used without further purification.

This material was dissolved in 18 mL of methanol and 2 mL of glacial acetic acid. Approximately 10 mg of 10% palladium on charcoal was added and the mixture shaken under a positive pressure of hydrogen (55 psi) for 14 h. Catalyst was filtered off and the filtrate concentrated in vacuo. The crude product was purified by reverse-phase HPLC on a C18 column, using a gradient of 20% to 80% acetonitrile in water buffered with 0.1% trifluoroacetic acid. Fractions containing the desired product were combined. Acetonitrile was removed in vacuo and the remaining solution diluted with water and lyophilized to provide 3,3-bis(7-guanidinoheptanoyl)aminopropionic acid as a white powder.

^{1}H-NMR (DMSO–d_6) δ 4.60 (m, 1H), 3.56 (m, 1H), 3.46 (m, 1H), 3.12 (t, J = 6.7 Hz, 4H), 2.23 (t, J = 10.6 Hz, 4H), 1.40 (m, 7H), 1.15 (m, 9H). MS *m/z* (FAB) 443 (M + H)$^+$. HPLC RT 17.90 min, 96 A%.

*3,3-Bis(8-guanidinooctanoyl)aminopropionic acid **2***

^{1}H-NMR (DMSO–d_6) δ 4.60 (m, 1H), 3.54 (m, 1H), 3.42 (m, 1H), 3.30 (t, J = 8.5 Hz, 4H), 2.42 (t, J = 9.2 Hz, 4H), 1.40 (m, 9H), 1.20 (m, 11H). MS *m/z* (FAB) 471 (M + H)$^+$. HPLC RT 17.07 min, 96 A%.

*3,3-Bis(9-guanidinononanoyl)aminopropionic acid **3***

^{1}H-NMR (DMSO–d_6) δ 4.62 (m, 1H), 3.54 (m, 1H), 3.44 (m, 1H), 3.35 (t, J = 8.2 Hz, 4H), 2.40 (t, J = 9.7 Hz, 4H), 1.40 (m, 11H), 1.20 (m, 13H). MS *m/z* (FAB) 499 (M + H)$^+$. HPLC RT 15.19 min, 95 A%.

*S-3-(7-guanidinoheptanoyl)amino-3-(8-guanidinooctanoyl)-aminopropionic acid **4***

^{1}H-NMR (DMSO–d_6) δ 4.60 (m, 1H), 3.54 (m, 1H), 3.46 (m, 1H), 3.19 (t, J = 6.6 Hz, 2H), 2.97 (t, J = 6.9 Hz, 2H), 2.23 (t, J = 11.7 Hz, 2H), 2.05 (t, J = 7.2 Hz, 2H), 1.40 (m, 8H), 1.15 (m, 10H). MS *m/z* (FAB) 457 (M + H)$^+$. HPLC RT 9.78 min, 97 A%.

*R-3-(7-guanidinoheptanoyl)amino-3-(8-guanidinooctanoyl)-aminopropionic acid **5***

^{1}H-NMR (DMSO–d_6) δ 4.60 (m, 1H), 3.56 (m, 1H), 3.46 (m, 1H), 3.19 (t, J = 6.7 Hz, 2H), 2.97 (t, J = 6.9 Hz, 2H), 2.23 (t,

J = 11.6 Hz, 2H), 2.05 (t, J = 7.2 Hz, 2H), 1.40 (m, 8H), 1.15 (m, 10H). MS *m/z* (FAB) 457 (M + H)$^+$. HPLC RT 10.17 min, 98 A%.

S-3-(7-guanidinoheptanoyl)amino-3-(9-guanidinononanoyl)-aminopropionic acid ***6***
^{1}H-NMR (DMSO-d_6) δ 4.60 (m, 1H), 3.60 (m, 1H), 3.45 (m, 1H), 3.19 (t, J = 7.0 Hz, 2H), 2.87 (t, J = 7.2 Hz, 2H), 2.20 (t, J = 11.4 Hz, 2H), 1.90 (t, J = 7.3 Hz, 2H), 1.40 (m, 9H), 1.15 (m, 11H). MS *m/z* (FAB) 471 (M + H)$^+$. HPLC RT 12.03 min, 95 A%.

R-3-(7-guanidinoheptanoyl)amino-3-(9-guanidinononanoyl)-aminopropionic acid ***7***
^{1}H-NMR (DMSO-d_6) δ 4.60 (m, 1H), 3.62 (m, 1H), 3.44 (m, 1H), 3.18 (t, J = 6.7 Hz, 2H), 2.84 (t, J = 7.2 Hz, 2H), 2.23 (t, J = 10.6 Hz, 2H), 1.98 (t, J = 7.3 Hz, 2H), 1.40 (m, 9H), 1.15 (m, 11H). MS *m/z* (FAB) 471 (M + H)$^+$. HPLC RT 11.10 min, 97 A%.

S-3-((8-nitroguanidino)octanoyl)amino-3-((9-nitroguanidino)-nonanoyl)aminopropionic acid ***8***
^{1}H-NMR (DMSO-d_6) δ 4.60 (m, 1H), 3.56 (m, 1H), 3.46 (m, 1H), 3.26 (t, J = 6.9 Hz, 2H), 2.86 (t, J = 7.0 Hz, 2H), 2.20 (t, J = 9.8 Hz, 2H), 1.98 (t, J = 7.4 Hz, 2H), 1.40 (m, 10H), 1.15 (m, 12H). MS *m/z* (FAB) 485 (M + H)$^+$. HPLC RT 12.01 min, 97 A%.

R-3-((8-nitroguanidino)octanoyl)amino-3-((9-nitroguanidino)-nonanoyl)aminopropionic acid ***9***
^{1}H-NMR (DMSO-d_6) δ 4.60 (m, 1H), 3.62 (m, 1H), 3.42 (m, 1H), 3.24 (t, J = 7.0 Hz, 2H), 2.84 (t, J = 7.0 Hz, 2H), 2.23 (t, J = 10.1 Hz, 2H), 1.98 (t, J = 7.3 Hz, 2H), 1.40 (m, 10H), 1.15 (m, 12H). MS *m/z* (FAB) 485 (M + H)$^+$. HPLC RT 14.42 min, 99 A%.

4,4-Bis(9-guanidinononanoyl)aminobutanoic acid ***10***
^{1}H-NMR (DMSO-d_6) δ 4.62 (m, 1H), 3.30 (t, J = 6.2 Hz, 2H), 3.35 (t, J = 8.2 Hz, 4H), 2.40 (t, J = 9.7 Hz, 4H), 1.40 (m, 11H), 1.20 (m, 15H). MS *m/z* (FAB) 513 (M + H)$^+$. HPLC RT 13.10 min, 98 A%.

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