Synthesis and Biological Investigation of Novel Tricyclic Benzodiazepinedione-Based RGD Analogues

Elisabeth Addicks, Ralph Mazitschek, and Athanassios Giannis*[a]

Integrins, a widely expressed family of heterodimeric cell surface adhesion proteins, are expressed in a variety of cell types. They play a decisive role in cell–cell adhesion or cell to extracellular matrix adhesion events. Antagonists of $\alpha_v\beta_3$ or $\alpha_{IIb}\beta_3$ integrin may have a potential use in suppression of pathological processes. We present the synthesis of novel tricyclic benzodiazepinedione-based RGD analogues, which were subsequently tested in a solid-phase receptor assay in order to investigate their binding affinities towards $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ integrin.

KEYWORDS:

angiogenesis · benzodiazepines · drug research · integrins · peptidomimetics

Introduction

Integrins, a widely expressed family of heterodimeric cell surface adhesion proteins, are formed by various combinations of at least 17 α and 9 β subunits.[1] They integrate the cytoskeletal activities of a cell with its environment by participating in cell–cell adhesion or cell to extracellular matrix adhesion events.[2, 3] The combination of different α and β subunits determines the specificity and affinity for ligand binding.

The integrin $\alpha_v\beta_3$ (vitronectin receptor)[4] is expressed in a variety of cell types, such as osteoclasts, vascular smooth muscle cells, and endothelial cells. The vitronectin receptor, however, is expressed in most cell types at relatively low levels, whereas up-regulation occurs under pathophysiological conditions.[5] Among other integrins, this receptor recognizes the RGD tripeptide sequence (Arg-Gly-Asp) of extracellular ligands such as fibronectin, fibrinogen, vitronectin, laminin, von Willebrand factor, and osteopontin,[6, 7] and has been shown to mediate several biologically relevant processes such as adhesion of osteoclasts to the bone matrix and migration of vascular smooth muscle cells and endothelial cells. The specificity of the RGD-integrin interaction is generated by a combination of variation in the RGD conformation in different proteins and the contribution of sequences near the RGD moiety.[8]

Antagonists of $\alpha_v\beta_3$ integrin may have potential use[1] in suppression of angiogenesis (neovascularization), the process of formation of new blood capillaries from surrounding preexisting blood vessels. In the course of embryogenesis and of wound healing, this process plays an important role, whereas in healthy adults angiogenesis just occurs in the female reproductive tract. However, aberrant neovascularization emerges in chronic inflammatory diseases, diabetic retinopathy, and malignant processes.

Folkman observed that tumor growth requires an adequate blood supply and as a consequence postulated that inhibition of angiogenesis may represent a novel, powerful approach in antitumor therapy.[9, 10] Neovascularization necessitates a discrete morphological change of the parent vessel (that is, the vessel from which a new capillary sprout originates), characterized by a thinned endothelial cell lining, increased endothelial cell number, decreased pericyte number, and pericyte detachment followed by degradation of the basement membrane. Thereafter, endothelial cells migrate through the extracellular matrix towards the angiogenic stimulus.[11, 12] Tumor cells promote entry of vascular endothelial cells into the cell cycle and expression of integrin $\alpha_v\beta_3$.[13] After endothelial cells begin to move toward the angiogenic stimulus, $\alpha_v\beta_3$ ligation provides a survival signal, which finally results in differentiation and formation of mature blood vessels. Disruption of the $\alpha_v\beta_3$ ligation may lead to apoptosis with subsequent tumor regression as a result of lack of blood supply. In fact, the inhibition of binding of $\alpha_v\beta_3$ integrin to its native ligands by antibodies or cyclic peptides interferes with angiogenesis and results in tumor regression.[14] Disruption of tumor-associated blood vessel formation is a new strategy for cancer therapy, offering an important advantage: the targets of this novel therapy are endothelial cells. In contrast to the genetically unstable tumor cells, these possess a stable genome, so that development of drug resistance is unlikely.[15, 16] Furthermore, $\alpha_v\beta_3$ antagonists may have potential use in the suppression of proliferative diabetic retinopathy, which causes blindness in diabetic patients,[17, 18] and they may also be useful for the treatment of osteoclast-mediated osteoporosis.[19] It has been demonstrated that blocking of $\alpha_v\beta_3$ by a synthetic peptide mimetic diminished osteoclastic bone resorption in vitro and in vivo.[20–22]

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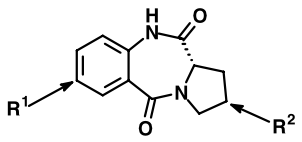
Integrin $\alpha_{IIb}\beta_3$, which is also a member of the integrin receptor family, possesses the same β chain as $\alpha_v\beta_3$. It is found on platelets and binds to the RGD plasma protein fibrinogen sequence after platelet activation. Thus, it is involved in the process of platelet aggregation, and $\alpha_{IIb}\beta_3$ antagonists consequently represent a promising approach in the treatment of thrombotic disorders.[23, 24]

Kessler et al. pointed out the structural properties required for selective binding to either $\alpha_{IIb}\beta_3$ or $\alpha_v\beta_3$ by using a library of cyclic stereoisomeric pentapeptides in which the RGD sequence was incorporated.[25] It was found that the cyclic pentapeptide c(RGDfV) is a potent and selective inhibitor of integrin $\alpha_v\beta_3$.[26] Investigation of isomers of c(RGDfV) revealed that not only the side chains but also the peptide backbone contribute to receptor binding by formation of at least one hydrogen bond.[27]

Several nonpeptide mimetics with high affinities for $\alpha_v\beta_3$ and with different central scaffolds (for example, indazole,[28] benzene,[19] isoxazoline,[29, 30] benzodiazepine,[31–33] and hydantoin:[34] for a review, see ref. [35]) were subsequently reported. We postulated that the tricyclic benzodiazepinedione scaffold (Scheme 1) should also be suitable for the development of new $\alpha_v\beta_3$ inhibitors. Here we describe the synthesis of several RGD mimetics based on this new scaffold. A solid-phase receptor assay was performed to examine the ability of the RGD analogues to inhibit binding of fibrinogen to $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ integrin.

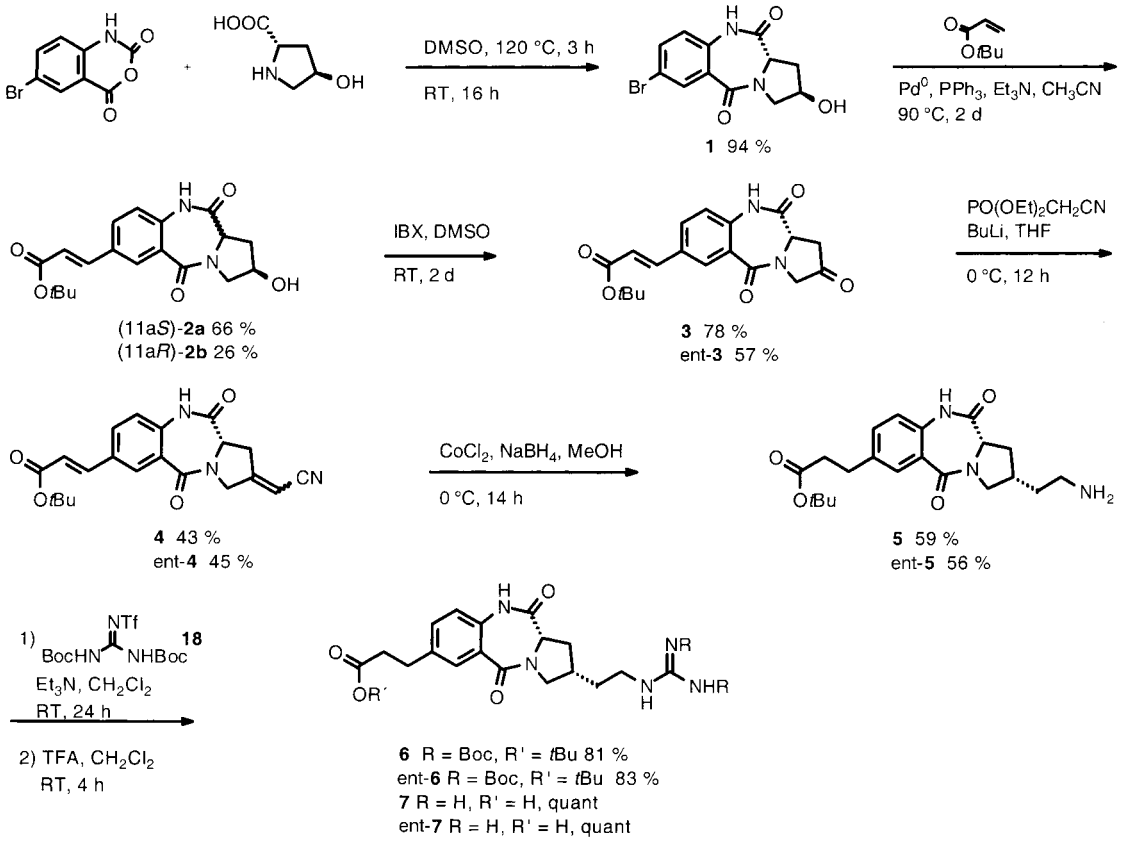


Scheme 1. *Tricyclic benzodiazepinedione scaffold: R^1, R^2 = side chains with functional groups.*

Results

Synthesis of the RGD analogues

RGD mimetics **7** and ent-**7** were prepared by the procedures outlined in Scheme 2. Treatment of bromoisatoic anhydride with (2*S*,4*R*)-4-hydroxypyrrolidine-2-carboxylic acid in dimethyl sulfoxide (DMSO) afforded benzodiazepinedione derivative **1**, which after a Heck reaction with *tert*-butyl acrylate in acetonitrile gave compound **2** as a mixture of diastereomers due to partial epimerization. The diastereoisomers were separated by flash chromatography. Subsequent steps were carried out with each isomer separately. Transformation of alcohol **2a** into the corresponding carbonyl compound **3** was performed by use of 1-hydroxy-(1*H*)-1,2-benziodoxol-3-one 1-oxide (IBX) as oxidant. Horner–Wadsworth–Emmons treatment of ketone **3** with diethyl cyanomethylphosphonate afforded α,β-unsaturated nitrile **4**. After reduction of derivative **4** with $CoCl_2/NaBH_4$,[36] the obtained amine **5** was transformed into the bis-*tert*-butoxycarbonyl-protected guanidine **6** by treatment with *N,N′*-bis-*tert*-butoxycarbonyl-*N″*-trifluoromethanesulfonyl-guanidine **18**.[37] Removal of the protective groups with trifluoroacetic acid



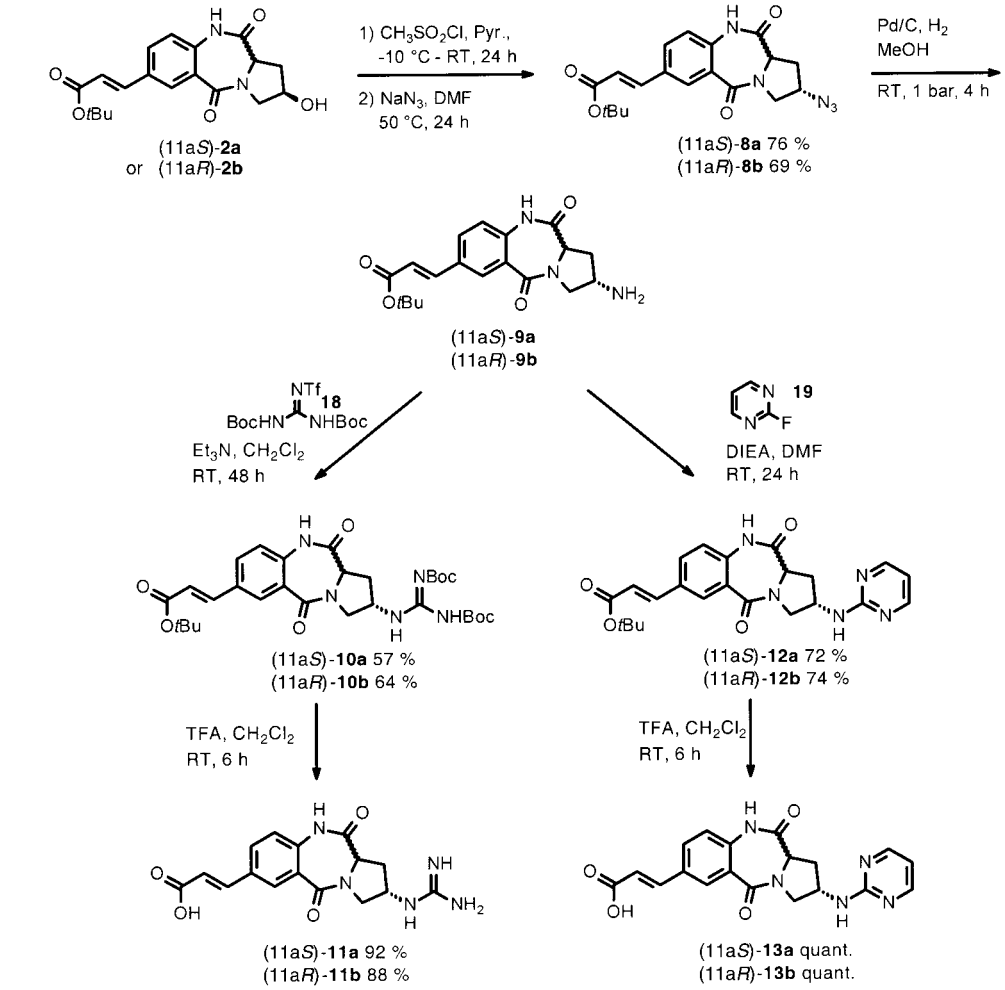
Scheme 2. *Synthesis of RGD analogues **7** and ent-**7**.*

(TFA) afforded the target molecule **7**. Similarly, the enantiomeric product ent-**7** was prepared from derivative **2b**.

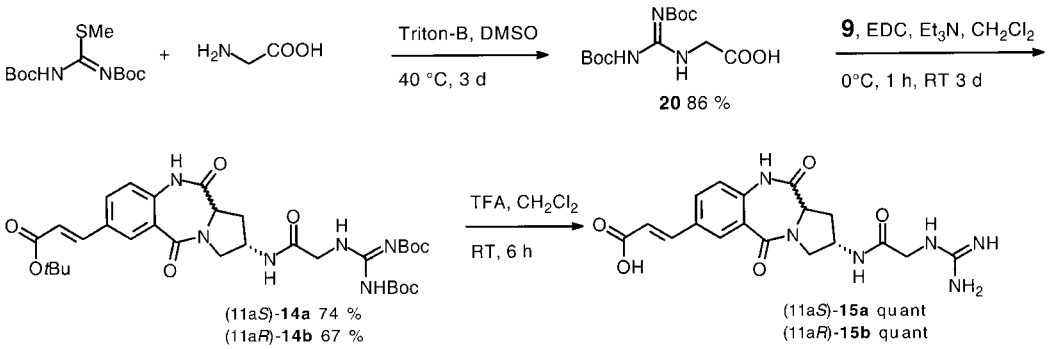
Conversion of the hydroxy group of alcohol **2a** into the mesylate, followed by treatment with sodium azide, afforded the azide **8a** (Scheme 3). Hydrogenolysis of this derivative yielded amine **9a**, which was subsequently converted into the corresponding bis-*tert*-butoxycarbonyl-protected guanidine **10a** by treatment with *N,N′*-bis-*tert*-butoxycarbonyl-*N″*-trifluoromethanesulfonyl guanidine (**18**). Removal of the protective groups with TFA afforded the target molecule **11a**. RGD analogue **11b** was prepared similarly, starting from alcohol **2b**.

Compound **12a**, which contains an aminopyrimidine moiety as a guanidine mimetic,[19, 38] was obtained by treatment of amine **9a** with 2-fluoropyrimidine **19**, which was available in one step from 2-aminopyrimidine.[39] Cleavage of the ester yielded RGD analogue **13a**. The diastereomer **13b** was prepared similarly, starting from amine **9b**.

The RGD analogues **15a** and **15b** were prepared according to the following procedure (Scheme 4). Treatment of glycine with



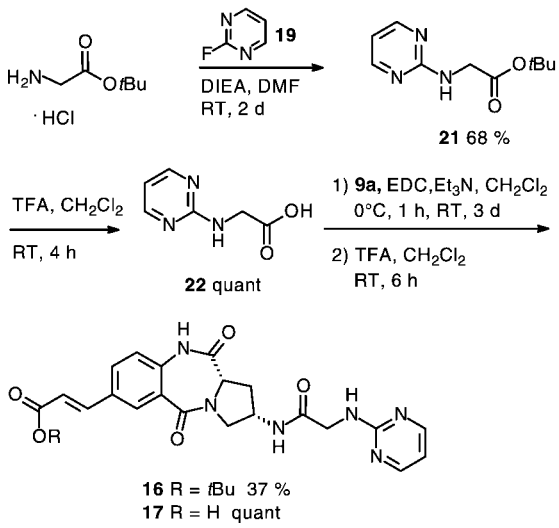
Scheme 3. *Synthesis of RGD analogues **11a**, **11b**, **13a**, and **13b**.*



Scheme 4. *Synthesis of RGD analogues **15a** and **15b**.*

1,3-bis-*tert*-butoxycarbonyl-2-methyl-2-thiopseudourea was carried out to obtain derivative **20.** Subsequent coupling with amines **9a** and **9b** and removal of the protective groups with TFA afforded RGD mimetics **15a** and **15b**, respectively.

Finally, compound **22** was synthesized by treatment of 2-fluoropyrimidine with glycine *tert*-butyl ester hydrochloride, followed by cleavage of the ester of compound **21** with TFA (Scheme 5). Subsequently, carboxylic acid **22** was coupled with amine **9a** to obtain amide **16**. Removal of the protective groups afforded RGD analogue **17**.



Scheme 5. *Synthesis of RGD analogue **17**.*

Solid-phase receptor assay

The abilities of the synthesized compounds to inhibit binding of fibrinogen to integrin $\alpha_{IIb}\beta_3$ or $\alpha_v\beta_3$ were characterized by a solid-phase competitive displacement assay. For this purpose, biotinylated fibrinogen was allowed to bind to immobilized integrin $\alpha_{IIb}\beta_3$ or $\alpha_v\beta_3$ in the presence of the synthesized RGD mimetics. The concentrations of compound **7** and ent-**7** required for half-maximal inhibition of ligand binding are shown in Figure 1 and Figure 2.

Discussion

We have presented the synthesis of novel tricyclic benzodiazepinediones, which were tested in a solid-phase receptor assay to identify compounds capable of recognizing integrins $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$. Benzodiazepines have been utilized as templates for peptide mimetics before, and there are many pharmacologically active substances that possess a benzodiazepine scaffold, such as neuropeptide mimetics (tifluadom, devazepide), *ras*-farnesyltransferase inhibitors, platelet-activating factor antagonists, HIV-protease inhibitors, antitumor antibiotics such as anthramycin and tomaymycin, or benzodiazepines such as diazepam (valium), which act as anxiolytics.[40, 41] The RGD analogues presented here feature a rigid scaffold, so they resist hydrophobic collapse and prevent the formation of pharmacologically inactive conformations in aqueous media.[42] They differ with respect to the distance between the carboxy and the guanidino functions. Furthermore, replacement of the guanidino moiety with 2-aminopyrimidine was performed to investigate the impact of an arginine mimetic.

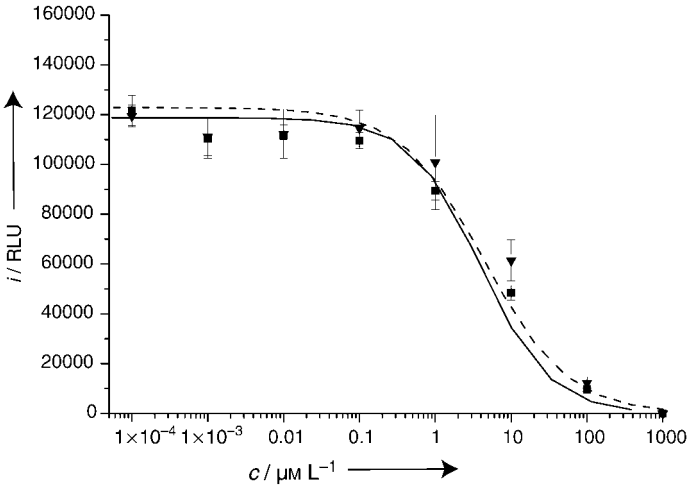


Figure 1. *Inhibition of the vitronectin receptor. Effect of compounds **7** and ent-**7** on ligand interaction with $\alpha_v\beta_3$ integrin: IC_{50} (**7**): 10 μM (▼,* solid line*); IC_{50} (*ent-**7**): 4 μM (■,* dotted line*);* i = *intensity,* RLU = *relative luminescence unit.*

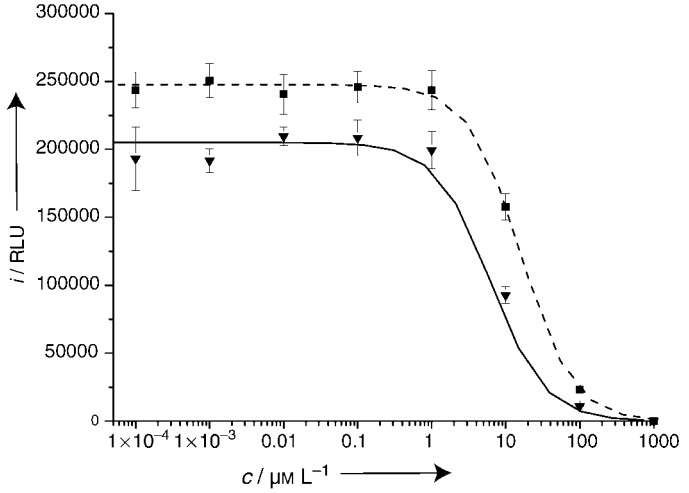


Figure 2. *Inhibition of the fibrinogen receptor. Effect of compounds **7** and ent-**7** on ligand interaction with $\alpha_{IIb}\beta_3$ integrin: IC_{50} (**7**): 8 μM (▼,* solid line*); IC_{50} (ent-**7**): 16 μM (■,* dotted line*);* i = *intensity,* RLU = *relative luminescence unit.*

The results of the receptor assay showed the importance of spacer length for potency. Compounds **11** and **15** turned out to be inactive; the IC_{50} values for neither $\alpha_v\beta_3$ nor $\alpha_{IIb}\beta_3$ inhibition were reached at the maximum concentration tested (100 μM). Substitution of the guanidino group by an aminopyrimidino function (compounds **13** and **17**) did not result in better binding properties. However, the RGD analogues **7** and ent-**7**, with a median distance (compared to the previously mentioned mimetics) between carboxy and guanidino functions, proved to be both good vitronectin receptor antagonists and also good fibrinogen receptor antagonists. As shown in Figure 1 and Figure 2, these compounds show only small selectivity: RGD

analogue **7** inhibits fibrinogen binding with IC_{50} values of 10 μM and 8 μM for $\alpha_v\beta_3$ integrin and $\alpha_{IIb}\beta_3$ integrin, respectively. Compound ent-**7** possesses IC_{50} values of 4 μM and 16 μM for $\alpha_v\beta_3$ integrin and $\alpha_{IIb}\beta_3$-integrin, respectively.

The derivative ent-**7** represents an interesting lead structure which may be used for further optimization. Additional functionalization may be performed (for example, alkylation at N10 and substitution of the guanidino moiety by various analogues) to improve both affinity and selectivity of receptor binding.

In summary, we present in this report the synthesis and biological investigation of several RGD analogues based on a tricyclic benzodiazepinedione scaffold. Through variation of the distance between the carboxy and the guanidino functions we found that compounds **7** and ent-**7** possess good affinities for $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ integrin. However, selectivity for $\alpha_v\beta_3$ integrin is still a challenge.

The presented syntheses demonstrate the variety of possibilities for functionalization and, as a consequence, benzodiazepinedione represents an interesting scaffold for the development of various peptidomimetics.

Experimental Section

General methods: Melting points were determined on a Büchi 530 apparatus and are uncorrected. ^{1}H NMR spectra were recorded on Bruker AC 250 or Bruker DRX 500 NMR spectrometers. The stereochemistries of the synthesized compounds were determined by NOE and 2D NMR spectroscopy. The solvents stated were used as internal standards. Elemental analyses were performed with a Heraeus CHN-Rapid instrument. High-resolution (HR) mass spectra were obtained with a Finnigan MAT MS 70 mass spectrometer. Optical rotation was measured on a Perkin–Elmer 241 polarimeter. Commercially available compounds were used without further purification unless otherwise noted. In general, reactions were carried out in dry solvents under argon atmosphere unless otherwise noted. All reactions were monitored by thin-layer chromatography (TLC) carried out on Merck F-254 silica aluminium sheets and viewed with UV light. Flash chromatography was performed on Merck silica gel 60.

Integrin $\alpha_v\beta_3$ was obtained from Chemicon; integrin $\alpha_{IIb}\beta_3$, purified human fibrinogen, bovine serum albumin (BSA) and peroxidase-labeled goat antibiotin antibody were obtained from Calbiochem Corp. (La Jolla, CA); tris(hydroxymethyl)aminomethane (Tris) buffer, Tween 20, and (+)-biotin *N*-succinimidyl ester were obtained from Fluka; BM chemiluminescence ELISA Substrate (POD) was obtained from Roche, Mannheim. C(RGDfV) was kindly provided by Prof. H. Kessler, TU München.

(2*R*,11 a*S*)-7-Bromo-2-hydroxy-1,2,3,11 a-tetrahydro-10*H*-benzo[e]pyrrolo[1,2-*a*][1,4]diazepine-5,11-dione (1): A stirred solution of bromoisatoic anhydride (10.0 g, 41.3 mmol) and (2*S*,4*R*)-4-hydroxypyrrolidine-2-carboxylic acid (6.5 g, 49.6 mmol) in DMSO (60 mL) was heated for 5 h at 140 °C. Stirring was continued at room temperature for 16 h. The solution was added to 300 mL ice-cooled water and the aqueous phase was extracted with ethyl acetate (4 × 100 mL). The organic layer was washed with water (2 × 50 mL), dried over Na_2SO_4, and evaporated to give **1** (12.1 g, 39.0 mmol, 94%) as a light brown solid. M.p.: 143 °C; $[\alpha]_D^{25} = +331.2$ ($c = 1.0$ in CH_3OH); ^{1}H NMR (500 MHz, [D$_6$]methanol, 25 °C): $\delta = 1.93$ (m, 1H; CH_2), 2.68 (dd, ^3J(H,H) = 5.65, ^2J(H,H) = 13.34 Hz, 1H; CH_2), 3.46 (dd, ^3J(H,H) = 4.89, ^2J(H,H) = 12.51 Hz, 1H; CH_2), 3.64 (ddd, ^3J(H,H) = 1.23 Hz, ^3J(H,H) = 3.67, ^2J(H,H) = 12.51 Hz, 1H; CH_2), 4.15 (dd, ^3J(H,H) = 6.10, ^3J(H,H) = 7.93 Hz, 1H; CH), 4.34 (m, 1H; CH), 6.90 (d, ^3J(H,H) = 8.55 Hz, 1H; Ar–H), 7.51 (dd, ^4J(H,H) = 2.45, ^3J(H,H) = 8.85 Hz, 1H; Ar–H), 7.83 (d, ^4J(H,H) = 2.45, 1H; Ar–H) ppm; HR-MS (EI, 70 eV): *m/z*: calcd for $C_{12}H_{11}BrN_2O_3$: 309.9953 $[M]^+$; found: 309.9935; elemental analysis calcd (%) for $C_{12}H_{11}BrN_2O_3 \cdot$ (DMSO): C 43.20, H 4.40, N 7.20; found: C 43.05, H 4.11, N 6.70.

***tert*-Butyl 3-((11 a*S*)-(2*R*)-hydroxy-5,11-dioxo-2,3,5,10,11,11 a-hexahydro-1*H*-benzo[e]pyrrolo[1,2-*a*][1,4]diazepin-7-yl)-acrylate (2)**: Compound **1** (500 mg, 1.6 mmol) was dissolved in acetonitrile (2 mL) and triethylamine (2 mL). Triphenylphosphine (21 mg, 0.08 mmol), palladium(II) acetate (4 mg, 0.018 mmol), and *tert*-butyl acrylate (0.5 mL, 3.4 mmol) were added. The mixture was stirred at 90 °C. After 24 h, a second portion of triphenylphosphine (21 mg, 0.08 mmol), palladium(II) acetate (4 mg, 0.018 mmol), and *tert*-butyl acrylate (0.5 mL, 3.4 mmol) was added and stirring was continued for a further 24 h at 90 °C. The solvent was removed in vacuo and the residue was purified by flash chromatography (CH_2Cl_2/CH_3OH 10:1, Rf = 0.26) to give **2** (527 mg, 1.47 mmol, 92%).

The diastereomers were separated by further flash chromatography (ethyl acetate/isopropanol 5:1, Rf (**2a**) = 0.67, Rf (**2b**) = 0.57) to yield 388 mg (1.08 mmol) of the 11 a*S* isomer **2a** and 110 mg (0.31 mmol) of the 11 a*R* isomer **2b**.

Isomer 2a: M.p.: 133 °C; $[\alpha]_d^{25} = +359.2$ ($c = 1.0$ in CH_2Cl_2); ^{1}H NMR (500 MHz, [D$_6$]chloroform, 25 °C): $\delta = 1.51$ (s, 9H; $C(CH_3)_3$), 2.19 (m, 1H; CH_2), 2.88 (m, 1H; CH_2), 3.62 (dd, ^3J(H,H) = 4.37, ^2J(H,H) = 12.71 Hz, 1H; CH_2), 4.00 (d, ^2J(H,H) = 13.11 Hz, 1H; CH_2), 4.31 (m, 1H; CH), 4.59 (m, 1H; CH), 6.32 (d, ^3J(H,H) = 15.90 Hz, 1H; CH=CH), 7.06 (d, ^3J(H,H) = 8.35 Hz, 1H; Ar–H), 7.46 (d, ^3J(H,H) = 15.89 Hz; CH=CH), 7.56 (dd, ^4J(H,H) = 1.93, ^3J(H,H) = 8.35, 1H; Ar–H), 7.98 (d, ^4J(H,H) = 1.93 Hz, 1H; Ar–H), 9.14 (s, 1H; NH) ppm; HR-MS (FAB, 70 eV): *m/z*: calcd for $C_{19}H_{23}N_2O_5$: 359.1607 $[M+H]^+$: found: 359.1627; elemental analysis calcd (%) for $C_{19}H_{22}N_2O_5 \cdot 0.25\,H_2O$: C 62.89, H 6.25, N 7.72; found: C 63.06, H 6.22, N 7.05.

Isomer 2b: M.p.: 142 °C; $[\alpha]_d^{25} = -274.8$ ($c = 1.0$ in CH_2Cl_2); ^{1}H NMR (500 MHz, [D$_6$]chloroform, 25 °C): $\delta = 1.51$ (s, 9H; $C(CH_3)_3$), 2.29 (m, 1H; CH_2), 2.83 (d, ^2J(H,H) = 12.93 Hz, 1H; CH_2), 3.66 (dd, ^3J(H,H) = 3.97, ^2J(H,H) = 12.82 Hz, 1H; CH_2), 3.86 (d, ^2J(H,H) = 12.93 Hz, 1H; CH_2), 4.20 (m, 1H; CH), 4.55 (m, 1H; CH), 6.34 (d, ^3J(H,H) = 15.89 Hz, 1H; CH=CH), 7.07 (d, ^3J(H,H) = 8.35 Hz, 1H; Ar–H), 7.53 (d, ^3J(H,H) = 15.89 Hz, 1H; CH=CH), 7.56 (dd, ^4J(H,H) = 2.13 Hz, ^3J(H,H) = 8.24 Hz, 1H; Ar–H), 8.12 (d, ^4J(H,H) = 1.93 Hz, 1H; Ar–H), 9.26 (br, 1H; NH) ppm; HR-MS (FAB, 70 eV): *m/z*: calcd for $C_{19}H_{23}N_2O_5$: 359.1607 $[M+H]^+$; found: 359.1627; elemental analysis calcd (%) for $C_{19}H_{22}N_2O_5 \cdot 0.5\,H_2O$: C 62.11, H 6.31, N 7.62; found: C 62.63, H 6.25, N 7.52.

***tert*-Butyl 3-((11 a*S*)-2,5,11-trioxo-2,3,5,10,11,11 a-hexahydro-1*H*-benzo[e]pyrrolo[1,2-a][1,4]diazepin-7-yl)-acrylate (3)**: Alcohol **2a** (780 mg, 2.18 mmol) was dissolved in DMSO (10 mL). IBX (793 mg, 2.83 mmol, 1.3 equiv) was added with stirring. After stirring for 3 d at room temperature, the solution was quenched with water (200 mL) and extracted with dichloromethane (4 × 40 mL). The combined organic layers were washed with water, dried over Na_2SO_4, and concentrated in vacuo. The product was purified by column chromatography (*n*-hexane/ethyl acetate 1:1) to yield ketone **3** (606 mg, 1.70 mmol, 78%). M.p.: 148 °C (dec.); $[\alpha]_d^{25} = +342.4$ ($c = 1.0$ in CH_2Cl_2); ^{1}H NMR (500 MHz, [D$_6$]chloroform, 25 °C): $\delta = 1.51$ (s, 9H; $C(CH_3)_3$), 2.87 (dd, ^3J(H,H) = 10.38 Hz, ^2J(H,H) = 18.92 Hz, 1H; CH_2), 3.60 (dd, ^3J(H,H) = 3.66, ^2J(H,H) = 19.53 Hz, 1H; CH_2), 3.96 (d, ^2J(H,H) = 20.14 Hz, 1H; CH_2), 4.32 (d, ^2J(H,H) = 20.15 Hz, 1H; CH_2), 4.64 (dd, ^3J(H,H) = 3.35, ^3J(H,H) = 10.07 Hz, 1H; CH), 6.40 (d, ^3J(H,H) =

16.03 Hz, 1H; CH=CH), 7.17 (d, ^3J(H,H) = 8.24 Hz, 1H; Ar–H), 7.55 (d, ^3J(H,H) = 16.18 Hz, 1H; CH=CH), 7.66 (dd, ^4J(H,H) = 2.14 Hz, ^3J(H,H) = 8.24 Hz, 1H; Ar–H), 8.11 (d, ^4J(H,H) = 1.83 Hz, 1H; Ar–H) 9.36 (br, 1H; NH) ppm; HR-MS (EI, 70 eV): *m/z*: calcd for $C_{19}H_{20}N_2O_5$: 356.1372 $[M]^+$; found: 356.1375.

***tert*-Butyl 3-((11 a*R*)-2,5,11-Trioxo-2,3,5,10,11,11 a-hexahydro-1*H*-benzo[e]pyrrolo[1,2-a][1,4]diazepin-7-yl)-acrylate (ent-3)**: Alcohol **2b** (820 mg, 2.29 mmol) was converted into the ketone ent-**3** (465 mg, 1.30 mmol, 57%) by the procedure reported for **3**. $[\alpha]_d^{25} = -355.0$ ($c = 1.0$ in CH_2Cl_2); HR-MS (FAB, 70 eV): *m/z*: calcd for $C_{19}H_{20}N_2O_5$: 357.1450 $[M+1]^+$; found: 357.1483.

***tert*-Butyl 3-((11 a*S*)-2-cyanomethylene-5,11-dioxo-2,3,5,10,11,11 a-hexahydro-1*H*-benzo[e]pyrrolo[1,2-a][1,4]diazepin-7-yl)-acrylate (4)**: A solution of diethyl cyanomethylphosphonate (1.46 mL, 9.28 mmol) in tetrahydrofuran (25 mL) was cooled to 0 °C. *n*-Butyllithium (5.8 mL, 1.6 M in *n*-hexane, 9.28 mmol) was added dropwise over 15 min. After stirring for 0.5 h, the solution was cooled to −50 °C. A solution of ketone **3** (1.50 g, 4.21 mmol) in tetrahydrofuran (10 mL) was added with vigorous stirring. The reaction mixture was allowed to warm up to room temperature over 3 h and stirring was continued for 12 h at room temperature. Water (100 mL) was added, followed by extraction with dichloromethane. The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. The crude mixture was purified on silica gel (*n*-hexane/ethyl acetate 2:3) to give nitrile **4** (687 mg, 1.81 mmol, 43%). M.p.: 151 °C (dec.); $[\alpha]_d^{25} = +335.3$ ($c = 1.0$ in CH_2Cl_2); ^{1}H NMR (500 MHz, [D$_6$]chloroform, 25 °C): $\delta = 1.53$ (s, 9H; $C(CH_3)_3$), 2.96 (m, 1H; CH_2), 3.26 (dd, ^3J(H,H) = 1.20, ^2J(H,H) = 18.68 Hz, 1H; CH_2), 3.34 (dd, ^3J(H,H) = 1.19, ^2J(H,H) = 18.68 Hz, 1H; CH_2), 3.65 (m, 1H; CH_2), 4.61 (dd, ^3J(H,H) = 3.97, ^3J(H,H) = 11.13 Hz, 1H; CH), 6.41 (d, ^3J(H,H) = 15.90 Hz, 1H; CH=CH), 7.08 (m, 1H; CH–CN), 7.11 (d, ^3J(H,H) = 8.34 Hz, 1H; Ar–H), 7.56 (d, ^3J(H,H) = 15.90 Hz, 1H; CH=CH), 7.65 (dd, ^4J(H,H) = 1.99, ^3J(H,H) = 8.34 Hz, 1H; Ar–H), 8.14 (d, ^4J(H,H) = 1.99 Hz, 1H; Ar–H), 9.10 (s, 1H, NH) ppm; HR-MS (EI, 70 eV,): *m/z*: calcd for $C_{21}H_{21}N_3O_4$: 379.1532 $[M]^+$; found: 379.1527; elemental analysis calcd (%) for $C_{21}H_{21}N_3O_4 \cdot H_2O$: C 63.47, H 5.83, N 10.57; found: C 63.29, H 5.56, N 10.46.

***tert*-Butyl 3-((11 a*R*)-2-cyanomethylene-5,11-dioxo-2,3,5,10,11,11 a-hexahydro-1*H*-benzo[e]pyrrolo[1,2-a][1,4]diazepin-7-yl)-acrylate (ent-4)**: Ketone ent-**3** (608 mg, 1.71 mmol) was converted into the nitrile ent-**4** (291 mg, 0.77 mmol; 45%) with diethyl cyanomethylphosphonate (0.59 mL, 3.75 mmol) and *n*-butyllithium (2.35 mL, 1.6 M in *n*-hexane, 3.75 mmol) by the procedure reported for **4**. $[\alpha]_d^{25} = -343.6$ ($c = 0.5$ in CH_2Cl_2); HR-MS (FAB, 70 eV): *m/z*: calcd for $C_{21}H_{22}N_3O_4$: 380.1610 $[M+1]^+$; found: 380.1599; elemental analysis calcd (%) for $C_{21}H_{21}N_3O_4 \cdot 0.6\,H_2O$: C 64.64, H 5.73, N 10.77; found: C 64.29, H 5.45, N 10.33.

***tert*-Butyl 3-([(11 a*S*)-(2*S*)-2-(2-amino-ethyl)-5,11-dioxo-2,3,5,10,11,11 a-hexahydro-1*H*-benzo[*e*]pyrrolo[1,2-a][1,4]diazepin-7-yl]-propionate (5)**: Nitrile **4** (200 mg, 0.53 mmol) was dissolved in methanol (10 mL). After addition of $CoCl_2$ (137 mg, 1.05 mmol, 2 equiv), the mixture was cooled to 0 °C. $NaBH_4$ (195 mg, 5.27 mmol, 10 equiv) was added in small portions with stirring over 0.5 h, in the course of which Co_2B deposited as a black granular precipitate. The reaction mixture was warmed to room temperature over 3 h and stirring was continued for a further 12 h at room temperature. The reaction was worked up by addition of 10% citric acid to dissolve the Co_2B. A 25% NH_3 solution was added until pH = 9–10. The aqueous layer was extracted with ethyl acetate (4 × 30 mL), and the combined organic layers were washed with water and brine and dried over Na_2SO_4. Evaporation of the solvent yielded the amine **5** (120 mg, 0.31 mmol, 59%) as a slightly colored foam, which was used immediately for further reactions. ^{1}H NMR (500 MHz, [D$_6$]methanol, 25 °C): $\delta = 1.40$ (s, 9H; $C(CH_3)_3$), 1.66 (m, 2H), 2.28 (m, 2H), 2.44 (m, 1H), 2.55 (t, ^3J(H,H) = 7.15 Hz, 2H; CH_2), 2.77 (m, 2H), 2.91 (t, ^3J(H,H) = 7.15 Hz, 2H; CH_2), 3.10 (m, 1H), 4.14 (m, 2H), 7.04 (d, ^3J(H,H) = 8.34 Hz, 1H; Ar–H), 7.40 (dd, ^4J(H,H) = 1.99 Hz, ^3J(H,H) = 8.34 Hz, 1H; Ar–H), 7.72 (d, ^4J(H,H) = 1.99 Hz, 1H; Ar–H) ppm; HR-MS (FAB, 70 eV): *m/z*: calcd for $C_{21}H_{30}N_3O_4$: 388.2236 $[M+1]^+$; found: 388.2252.

***tert*-Butyl 3-[(11 a*R*)-(2*R*)-2-(2-amino-ethyl)-5,11-dioxo-2,3,5,10,11,11 a-hexahydro-1*H*-benzo[*e*]pyrrolo[1,2-a][1,4]diazepin-7-yl]-propionate (ent-5)**: Nitrile ent-**4** (284 mg, 0.75 mmol) was treated with $CoCl_2$ (194 mg, 1.50 mmol, 2 equiv) and $NaBH_4$ (277 mg, 7.49 mmol, 10 equiv) as reducing agent to afford amine ent-**5** (162 mg, 0.42 mmol, 56%) by the procedure reported for **5**. The crude product was used in the next step without any further purification. HR-MS (FAB, 70 eV): *m/z*: calcd for $C_{21}H_{30}N_3O_4$: 388.2236 $[M+1]^+$; found: 388.2246.

***tert*-Butyl 3-{(11 a*S*)-(2*S*)-2-[2-(*N*′,*N*″-Bis-*tert*-butoxycarbonyl-guanidino-ethyl)]-5,11-dioxo-2,3,5,10,11,11 a-hexahydro-1*H*-benzo[*e*]pyrrolo[1,2-a][1,4]diazepin-7-yl}-propionate (6)**: Amine **5** (314 mg, 0.81 mmol) was dissolved in dichloromethane (10 mL) and cooled to 0 °C. *N*,*N*′-Bis-*tert*-butoxycarbonyl-*N*″-trifluoromethanesulfonylguanidine (**18**; 382 mg, 0.98 mmol, 1.2 equiv) and triethylamine (0.14 mL, 0.98 mmol, 1.2 equiv) were added. The reaction mixture was then stirred at 0 °C for 1 h and at room temperature for 48 h. The solvent was removed under reduced pressure. The product was further purified by column chromatography (*n*-hexane/ethyl acetate 1:1) to give the bis-*tert*-butoxycarbonyl-protected guanidine **6** (369 mg, 0.59 mmol, 73%). M.p.: 190 °C (dec.); $[\alpha]_d^{25} = +160.8$ ($c = 1.0$ in CH_2Cl_2); ^{1}H NMR (500 MHz, [D$_6$]chloroform, 25 °C): $\delta = 1.40$ (s, 9H; $C(CH_3)_3$), 1.47 (s, 9H; $C(CH_3)_3$), 1.48 (s, 9H; $C(CH_3)_3$), 1.75 (m, 2H; CH_2), 2.26 (m, 1H; CH, 1H; CH_2), 2.50–2.55 (m, 1H; CH_2, m, 2H; CH_2), 2.90 (m, 2H; CH_2), 3.11 (dd, ^3J(H,H) = 7.55, ^2J(H,H) = 11.93 Hz, 1H; CH_2), 3.48 (m, 2H; CH_2), 4.06 (m, 1H; CH), 4.22 (dd, ^3J(H,H) = 7.55, ^2J(H,H) = 11.92 Hz, 1H; CH_2), 6.92 (d, ^3J(H,H) = 8.34 Hz, 1H; Ar–H), 7.31 (dd, ^4J(H,H) = 1.99, ^3J(H,H) = 7.95 Hz, 1H; Ar–H), 7.79 (d, ^4J(H,H) = 1.99 Hz, 1H; Ar–H), 8.33 (m, 1H, NH), 8.42 (s, 1H, NH), 11.47 (s, 1H, NH) ppm; HR-MS (FAB, 70 eV): *m/z*: calcd for $C_{32}H_{48}N_5O_8$: 630.3503 $[M+1]^+$; found: 630.3479.

***tert*-Butyl 3-{(11 a*R*)-(2*R*)-2-[2-(*N*′,N″-Bis-*tert*-butoxycarbonyl-guanidino)-ethyl]-5,11-dioxo-2,3,5,10,11,11 a-hexahydro-1*H*-benzo[*e*]-pyrrolo[1,2-a][1,4]diazepin-7-yl}-propionate (ent-6)**: Amine ent-**5** (170 mg, 0.44 mmol) was treated with *N*,*N*′-bis-*tert*-butoxycarbonyl-*N*″-trifluoromethanesulfonylguanidine (**18**, 207 mg, 0.53 mmol, 1.2 equiv) and triethylamine (0.08 mL, 0.53 mmol, 1.2 equiv) by the procedure reported for **6**, to yield the bis-*tert*-butoxycarbonyl-protected guanidine ent-**6** (224 mg, 0.36 mmol, 81%). $[\alpha]_d^{25} = -152.8$ ($c = 0.5$ in CH_2Cl_2); HR-MS (FAB, 70 eV): *m/z*: calcd for $C_{32}H_{48}N_5O_8$: 630.3503 $[M+1]^+$; found: 630.3478.

3-[(11 a*S*)-(2*S*)-2-(2-Guanidino-ethyl)-5,11-dioxo-2,3,5,10,11,11 a-hexahydro-1*H*-benzo[*e*]pyrrolo[1,2-a][1,4]diazepin-7-yl]-propionic acid trifluoroacetate (7): TFA (2 mL) was added to a solution of compound **6** (200 mg, 0.32 mmol) in dichloromethane (5 mL), followed by stirring for 4 h at room temperature. The solvent was removed in vacuo and the residue was triturated with diethyl ether to afford compound **7** in quantitative yield. M.p.: 124 °C (dec.); $[\alpha]_d^{25} = +185.0$ ($c = 0.5$ in CH_3OH); ^{1}H NMR (500 MHz, D_2O, 25 °C): $\delta =$ 1.74 (m, 2H; CH_2), 2.28 (m, 1H; CH_2), 2.37 (m, 1H; CH, 1H; CH_2), 2.71 (t, ^3J(H,H) = 7.29 Hz, 2H; CH_2), 2.95 (t, ^3J(H,H) = 7.35 Hz, 2H; CH_2), 3.21 (dd, ^3J(H,H) = 6.78, ^2J(H,H) = 12.05 Hz, 1H; CH_2), 3.30 (m, 2H; CH_2), 4.01 (dd, ^3J(H,H) = 6.60, ^2J(H,H) = 12.05 Hz, 1H; CH_2), 4.19 (m, 1H; CH), 7.10 (d, ^3J(H,H) = 8.29 Hz, 1H; Ar–H), 7.47 (dd, ^4J(H,H) = 2.26 Hz, ^3J(H,H) = 8.29 Hz, 1H; Ar–H), 7.66 (d, ^4J(H,H) = 2.26 Hz, 1H;

Ar–H) ppm; HR-MS (FAB, 70 eV): *m/z*: calcd for $C_{18}H_{24}N_5O_4$: 374.1828 $[M+1]^+$; found: 374.1819.

3-[(11 a*R*)-(2*R*)-2-(2-Guanidino-ethyl)-5,11-dioxo-2,3,5,10,11,11 a-hexahydro-1*H*-benzo[*e*]pyrrolo[1,2-a][1,4]diazepin-7-yl]-propionic acid trifluoroacetate (ent-7): The protective groups of ent-**6** (150 mg, 0.24 mmol) were removed by the procedure described above to give the enantiomer ent-**7** in quantitative yield. $[\alpha]_d^{25} = -172.4$ ($c = 0.5$ in CH_3OH); HR-MS (FAB, 70 eV): *m/z*: calcd for $C_{18}H_{24}N_5O_4$: 374.1828 $[M+1]^+$; found: 374.1816.

***tert*-Butyl 3-((11 a*S*)-(2*S*)-2-azido-5,11-dioxo-2,3,5,10,11,11 a-hexahydro-1*H*-benzo[e]pyrrolo[1,2-*a*][1,4]diazepin-7-yl)-acrylate (8 a):** Compound **2 a** (460 mg, 1.28 mmol) was dissolved in dichloromethane (10 mL). After addition of pyridine (3 mL) the solution was cooled to $-10\,^\circ C$. Methanesulfonyl chloride (0.13 mL, 1.67 mmol) was slowly added with stirring. The mixture was then stirred for 1 h at $-10\,^\circ C$ and for 10 h at room temperature. The solution was washed with sat. $NaHCO_3$ solution and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with 10% citric acid and water and dried over $MgSO_4$. The solvent was removed under reduced pressure. The crude mesylate was dissolved in *N*,*N*-dimethylformamide (8 mL). Sodium azide (1.25 g, 19.23 mmol) was added, followed by stirring at 50 °C for 48 h. Afterwards, the mixture was poured into ice-cooled water and the aqueous phase was extracted with dichloromethane. After the organic layer had been dried over Na_2SO_4, the solvent was evaporated. Purification by flash chromatography (CH_2Cl_2/CH_3OH 10:1, Rf = 0.53) afforded product **8 a** (374 mg, 0.98 mmol, 76%). Mp.: 109 °C (dec.); $[\alpha]_d^{25} = +391.2$ ($c = 1.0$ in CH_2Cl_2); ^1H NMR (500 MHz, [D$_6$]chloroform, 25 °C): $\delta = 1.52$ (s, 9H; $C(CH_3)_3$), 2.38 (m, 1H; CH_2), 3.10 (dd, $^3J(H,H) = 1.99$, $^2J(H,H) = 13.91$ Hz, 1H; CH_2), 3.73 (m, 1H; CH_2), 3.82 (dd, $^3J(H,H) = 5.14$, $^2J(H,H) = 12{,}98$ Hz, 1H; CH_2), 4.20 (dd, $^3J(H,H) = 1.99$, $^3J(H,H) = 9.14$ Hz, 1H; CH), 4.37 (m, 1H; CH), 6.39 (d, $^3J(H,H) = 15.90$ Hz, 1H, CH=CH), 7.10 (m, 1H; Ar–H), 7.55 (d, $^3J(H,H) = 15.89$ Hz, 1H, CH=CH), 7.62 (dd, $^4J(H,H) = 1.99$, $^3J(H,H) = 8.34$ Hz, 1H; Ar–H), 8.13 (d, $^4J(H,H) = 1.99$ Hz, 1H; Ar–H), 9.10 (br, 1H; NH) ppm; HR-MS (EI, 70 eV): *m/z*: calcd for $C_{19}H_{21}N_5O_4$: 383.1593 $[M]^+$; found: 383.1595; elemental analysis calcd (%) for $C_{19}H_{21}N_5O_4 \cdot 0.5\,H_2O$: C 58.16, H 5.65; found: C 58.30, H 5.88.

***tert*-Butyl 3-((11 a*R*)-(2*S*)-2-azido-5,11-dioxo-2,3,5,10,11,11 a-hexahydro-1*H*-benzo[e]pyrrolo[1,2-*a*][1,4]diazepin-7-yl)-acrylate (8 b)**: Alcohol **2 b** (300 mg, 0.84 mmol) was converted into the azide by the procedure reported for **8 a**, to yield compound **8 b** (221 mg, 0.58 mmol, 69%). M.p.: 114 °C (dec.); $[\alpha]_d^{25} = -371.4$ ($c = 1.0$ in CH_2Cl_2); ^1H NMR (500 MHz, [D$_6$]chloroform, 25 °C): $\delta = 1.50$ (s, 9H; $C(CH_3)_3$), 2.18 (m, 1H; CH_2), 3.01 (m, 1H; CH_2), 3.77 (dd, $^3J(H,H) = 5.56$, $^2J(H,H) = 12.32$ Hz, 1H; CH_2), 3.86 (dd, $^3J(H,H) = 4.77$, $^2J(H,H) = 12.32$ Hz, 1H; CH_2), 4.23 (dd, $^3J(H,H) = 5.17$, $^3J(H,H) = 8.34$ Hz, 1H; CH), 4.30 (m, 1H; CH), 6.37 (d, $^3J(H,H) = 15.90$ Hz, 1H; CH=CH), 7.10 (dd, $^3J(H,H) = 8.35$ Hz, 1H; Ar–H), 7.53 (d, $^3J(H,H) = 15.93$ Hz, 1H; CH=CH), 7.59 (dd, $^4J(H,H) = 1.99$, $^3J(H,H) = 8.35$ Hz, 1H; Ar–H), 8.11 (d, $^4J(H,H) = 1.99$ Hz, 1H; Ar–H), 9.66 (br, 1H; NH) ppm; HR-MS (FAB, 70 eV): *m/z*: calcd for $C_{19}H_{22}N_5O_4$: 384.1671 $[M+1]^+$; found: 384.1680; elemental analysis calcd (%) for $C_{19}H_{21}N_5O_4 \cdot 0.5\,H_2O$: C 58.16, H 5.65; found: C 57.74, H 5.65.

***tert*-Butyl 3-((11 a*S*)-(2*S*)-2-amino-5,11-dioxo-2,3,5,10,11,11 a-hexahydro-1*H*-benzo[e]pyrrolo[1,2-*a*][1,4]diazepin-7-yl)-acrylate (9 a)**: Azide **8 a** (1 g, 2.61 mmol) was dissolved in methanol (degassed and flushed with argon, 10 mL). After addition of palladium on charcoal the mixture was degassed again and afterwards flushed with hydrogen. The mixture was stirred for 4 h under hydrogen (1 bar), followed by separation of the palladium on charcoal by filtration over Celite. The solvent was removed under reduced pressure to afford amine **9 a** (931 mg, 2.61 mmol) in quantitative yield ($CH_2Cl_2/CH_3OH/NH_3$ 5:1:0.01, Rf = 0.22). M.p.: 151 °C; $[\alpha]_d^{25} = +360.8$ ($c = 1.0$ in CH_3OH); ^1H NMR (500 MHz, [D$_6$]methanol, 25 °C): $\delta = 1.52$ (s, 9H; $C(CH_3)_3$), 2.40 (m, 1H; CH_2), 2.64 (m, 1H; CH_2), 3.45 (dd, $^3J(H,H) = 3.78$, $^2J(H,H) = 12.12$ Hz, 1H; CH_2), 3.68 (m, 1H; CH), 3.98 (dd, $^3J(H,H) = 5.96$, $^2J(H,H) = 12{,}31$ Hz, 1H; CH_2), 4.29 (dd, $^3J(H,H) = 3.98$, $^3J(H,H) = 8.74$ Hz, 1H; CH), 6.43 (d, $^3J(H,H) = 15.89$ Hz, 1H; CH=CH), 7.17 (d, $^3J(H,H) = 8.35$ Hz, 1H; Ar–H), 7.56 (d, $^3J(H,H) = 15.89$ Hz, 1H; CH=CH), 7.77 (dd, $^4J(H,H) = 1.99$, $^3J(H,H) = 8.34$ Hz, 1H; Ar–H), 8.04 (d, $^4J(H,H) = 1.99$ Hz, 1H; Ar–H) ppm; HR-MS (FAB, 70 eV): *m/z*: calcd for $C_{19}H_{24}N_3O_4$: 358.1769 $[M+1]^+$; found: 358.1746; elemental analysis calcd (%) for $C_{19}H_{23}N_3O_4 \cdot 0.67\,H_2O$: C 61.78, H 6.64, N 11.37; found: C 61.92, H 6.47, N 11.26.

***tert*-Butyl 3-((11 a*R*)-(2*S*)-2-amino-5,11-dioxo-2,3,5,10,11,11 a-hexahydro-1*H*-benzo[e]pyrrolo[1,2-*a*][1,4]diazepin-7-yl)-acrylate (9 b)**: Azide **8 b** (500 mg, 1.30 mmol) was reduced to the amine by the procedure reported for **9 a** to yield amine **9 b** (463 mg, 1.30 mmol) in quantitative yield ($CH_2Cl_2/CH_3OH/NH_3$ 5:1:0.01, Rf = 0.24). M.p.: 158 °C; $[\alpha]_d^{25} = -352.5$ ($c = 1.0$ in CH_3OH); ^1H NMR (500 MHz, [D$_6$]methanol, 25 °C): $\delta = 1.52$ (s, 9H; $C(CH_3)_3$), 2.04 (m, 1H; CH_2), 2.93 (m, 1H; CH_2), 3.53 (dd, $^3J(H,H) = 7.15$, $^2J(H,H) = 11.92$ Hz, 1H; CH_2), 3.76 (m, 1H; CH), 3.88 (dd, $^3J(H,H) = 6.75$, $^2J(H,H) = 11.92$ Hz, 1H; CH_2), 4.34 (dd, $^3J(H,H) = 3.18$, $^3J(H,H) = 8.35$ Hz, 1H; CH), 6.41 (d, $^3J(H,H) = 16.29$ Hz, 1H; CH=CH), 7.15 (d, $^3J(H,H) = 8.74$ Hz, 1H; Ar–H), 7.55 (d, $^3J(H,H) = 15.89$ Hz, 1H; CH=CH), 7.74 (dd, $^4J(H,H) = 1.99$ Hz, $^3J(H,H) = 8.34$, 1H; Ar–H), 8.03 (d, $^4J(H,H) = 1.99$ Hz, 1H; Ar–H) ppm; HR-MS (FAB, 70 eV): *m/z*: calcd for $C_{19}H_{24}N_3O_4$: 358.1769 $[M+1]^+$; found: 358.1746; elemental analysis calcd (%) for $C_{19}H_{23}N_3O_4 \cdot H_2O$: C 60.79, H 6.71, N 11.19; found: C 61.92, H 6.47, N 11.26.

***tert*-Butyl 3-[(11 a*S*)-(2*S*)-2-(*N*′,*N*″-bis-*tert*-butoxycarbonyl-guanidino)-5,11-dioxo-2,3,5,10,11,11 a-hexahydro-1*H*-benzo[e]-pyrrolo[1,2-*a*][1,4]diazepin-7-yl]-acrylate (10 a)**: *N*,*N*′-Bis-*tert*-butoxycarbonyl-*N*″-trifluoromethanesulfonyl guanidine (**18**; 285 mg, 0.73 mmol, 1.3 equiv) was added to a solution of amine **9 a** (200 mg, 0.56 mmol) and triethylamine (0.08 mL) in dichloromethane (5 mL) at 0 °C. The solution was allowed to warm up, with stirring, to room temperature over 1 h. After the mixture had been stirred for 3 d at room temperature the solvent was removed under vacuum. Purification by flash chromatography (CH_2Cl_2/CH_3OH 10:1, Rf = 0.61) gave compound **10 a** (190 mg, 0.32 mmol, 57%). M.p.: 149 °C (dec.); $[\alpha]_d^{25} = +194.7$ ($c = 1.0$ in CH_2Cl_2); ^1H NMR (500 MHz, [D$_6$]chloroform, 25 °C): $\delta = 1.12$ (s, 9H; $C(CH_3)_3$), 1.47 (s, 9H; $C(CH_3)_3$), 1.51 (s, 9H; $C(CH_3)_3$), 2.40 (m, 1H; CH_2), 2.96 (d, $^2J(H,H) = 14.04$ Hz, 1H; CH_2), 3.91 (m, 2H; CH_2), 4.24 (dd, $^3J(H,H) = 1.22$, $^3J(H,H) = 8.48$ Hz, 1H; CH), 4.75 (m, 1H; CH), 6.38 (d, $^3J(H,H) = 16.17$ Hz, 1H; CH=CH), 7.23 (d, $^3J(H,H) = 8.55$ Hz, 1H; Ar–H), 7.55 (d, $^3J(H,H) = 15.87$ Hz, 1H; CH=CH), 7.64 (dd, $^3J(H,H) = 8.55$, $^4J(H,H) = 1.83$ Hz, 1H; Ar–H), 8.12 (d, $^4J(H,H) = 1.83$ Hz, 1H; Ar–H), 8.73 (d, $^3J(H,H) = 6.10$ Hz, 1H; NH), 10.62 (br, 1H; NH), 11.32 (s, 1H; NH) ppm; HR-MS (FAB, 70 eV): *m/z*: calcd for $C_{30}H_{42}N_5O_8$: 600.3033 $[M+1]^+$; found: 600.3020; elemental analysis calcd (%) for $C_{30}H_{41}N_5O_8 \cdot H_2O$: C 58.33, H 7.02, N 11.34; found: C 58.71, H 6.69, N 11.21.

***tert*-Butyl 3-[(11 a*R*)-(2*S*)-2-(*N*′,*N*″-bis-*tert*-butoxycarbonyl-guanidino)-5,11-dioxo-2,3,5,10,11,11 a-hexahydro-1*H*-benzo[e]-pyrrolo[1,2-*a*][1,4]diazepin-7-yl]-acrylate (10 b)**: Amine **9 b** (150 mg, 0.42 mmol) was converted into the bis-*tert*-butoxycarbonyl-protected guanidino-benzodiazepinedione **10 b** (161 mg, 0.27 mmol, 64%) by the procedure reported for **10 a**. M.p.: 126 °C (dec.); $[\alpha]_d^{25} = -199.6$ ($c = 1.0$ in CH_2Cl_2); ^1H NMR (500 MHz, [D$_6$]chloroform, 25 °C): $\delta = 1.47$ (s, 9H; $C(CH_3)_3$), 1.48 (s, 9H; $C(CH_3)_3$), 1.51 (s, 9H; $C(CH_3)$), 2.08 (m, 1H; CH_2), 3.05 (m, 1H; CH_2), 3.52 (dd, $^3J(H,H) = 7.33$, $^2J(H,H) = 12.21$ Hz, 1H; CH_2), 4.11 (dd, $^3J(H,H) = 6.56$, $^2J(H,H) = 12{,}21$ Hz, 1H; CH_2), 4.20 (dd, $^3J(H,H) = 2.75$, $^3J(H,H) = 8.24$ Hz, 1H; CH), 4.69 (m, 1H;

CH), 6.33 (d, ^3J(H,H) = 16.18 Hz, 1H; CH=CH), 7.10 (d, ^3J(H,H) = 8.24 Hz, 1H; Ar–H), 7.49 (d, ^3J(H,H) = 16.18 Hz, 1H; CH=CH), 7.60 (dd, ^4J(H,H) = 2.04, ^3J(H,H) = 8.24 Hz, 1H; Ar–H), 8.06 (d, ^4J(H,H) = 2.04 Hz, 1H; Ar–H), 8.52 (d, ^3J(H,H) = 6.72 Hz, 1H; NH), 9.13 (br, 1H; NH), 11.46 (s, 1H; NH) ppm; HR-MS (FAB, 70 eV): *m/z*: calcd for $C_{30}H_{42}N_5O_8$: 600.3033 $[M+1]^+$; found: 600.3055; elemental analysis calcd (%) for $C_{30}H_{42}N_5O_8 \cdot H_2O$: C 58.33, H 7.02, N 11.34; found: C 58.68, H 6.71, N 11.20.

3-((11 a*S*)-(2*S*)-2-Guanidino-5,11-dioxo-2,3,5,10,11,11 a-hexahydro-1*H*-benzo[e]pyrrolo[1,2-*a*][1,4]diazepin-7-yl)-acrylic acid trifluoroacetate (11 a): Compound **10 a** (65 mg, 0.11 mmol) was dissolved in dichloromethane (6 mL). TFA (1.5 mL) was slowly added with stirring. After the mixture had been kept for 6 h at room temperature, the solvent was removed. Purification of the product by semipreparative HPLC (1% B to 20% B in 10 min; A: H_2O + 0.1% TFA; B: acetonitrile + 0.1% TFA) gave the target molecule **11 a** as the trifluoroacetate in 92% yield (46 mg, 0.10 mmol). M.p.: 195 °C (dec.); $[\alpha]_d^{25} = +305.2$ ($c = 0.5$ in CH_3OH); ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): $\delta = 2.35$ (m, 1H; CH_2), 2.62 (m, 1H; CH_2), 3.43 (d, ^2J(H,H) = 12.36 Hz, 1H; CH_2), 3.96 (dd, ^2J(H,H) = 12.36, ^3J(H,H) = 5.65 Hz, 1H; CH_2), 4.16 (m, 1H; CH), 4.29 (dd, ^3J(H,H) = 3.53 Hz, ^3J(H,H) = 8.83 Hz, 1H; CH), 6.48 (d, 1H; ^3J(H,H) = 15.89 Hz, CH=CH), 7.19 (d, ^3J(H,H) = 8.47 Hz, 1H; Ar–H), 7.55 (d, ^3J(H,H) = 15.89 Hz, 1H; CH=CH), 7.79 (br, 1H; NH), 7.89 (dd, ^4J = 1.77, ^3J(H,H) = 8.48 Hz, 1H; Ar–H), 7.97 (d, ^4J = 1.77 Hz, 1H; Ar–H) 10.79 (br, 1H; NH) ppm; HR-MS (FAB, 70 eV): *m/z*: calcd for $C_{16}H_{18}N_5O_4$: 344.1359 $[M+1]^+$; found: 344.1343.

3-((11 a*R*)-(2*S*)-2-Guanidino-5,11-dioxo-2,3,5,10,11,11 a-hexahydro-1*H*-benzo[e]pyrrolo[1,2-*a*][1,4]diazepin-7-yl)-acrylic acid trifluoroacetate (11 b): The protective groups of compound **10 b** (150 mg, 0.25 mmol) were removed by the procedure reported for **11 a**, to obtain product **11 b** (99 mg, 0.22 mmol, 88%). M.p.: 176 °C (dec.); $[\alpha]_d^{25}$ −224.9 ($c = 1.0$ in CH_3OH); ^{1}H NMR (500 MHz, [D$_6$]methanol, 25 °C): $\delta = 2.40$ (m, 1H; CH_2), 3.19 (m, 1H; CH_2), 3,80 (dd, ^3J(H,H) = 6.71, ^2J(H,H) = 11.91 Hz, 1H; CH_2), 4.20 (dd, ^3J(H,H) = 6.41, ^2J(H,H) = 11.91 Hz, 1H; CH_2), 4.49 (m, 1H; CH), 4.61 (m, 1H; CH), 6.71 (d, ^3J(H,H) = 16.17 Hz, 1H; CH=CH), 7.39 (d, ^3J(H,H) = 8.54 Hz, 1H; Ar–H), 7.88 (d, ^3J(H,H) = 15.87 Hz, 1H; CH=CH), 8.02 (dd, ^4J(H,H) = 2.13, ^3J(H,H) = 8.54 Hz, 1H; Ar–H), 8.27 (d, ^4J(H,H) = 2.14 Hz, 1H; Ar–H) ppm; HR-MS (FAB, 70 eV): *m/z*: calcd for $C_{16}H_{18}N_5O_4$: 344.1359 $[M+1]^+$; found: 344.1344.

***tert*-Butyl 3-((11 a*S*)-(2*S*)-5,11-dioxo-2-pyrimidin-2-ylamino-2,3,5,10,11,11 a-hexahydro-1*H*-benzo[e]pyrrolo[1,2-*a*][1,4]diazepin-7-yl)-acrylate (12 a)**: Amine **9 a** (180 mg, 0.50 mmol) and 2-fluoropyrimidine **19** (200 mg, 2.0 mmol) were dissolved in *N*,*N*-dimethylformamide (6 mL). After addition of diisopropylethylamine (0.36 mL, 2.0 mmol) the solution was stirred for 24 h at room temperature. The solution was added to cold water and the aqueous phase was extracted with dichloromethane. The combined organic layers were dried over Na_2SO_4. Afterwards, the solvent was removed in vacuo and the residue was purified by flash chromatography (CH_2Cl_2/CH_3OH 10:1, Rf = 0.41) to afford compound **12 a** (158 mg, 0.36 mmol, 72%). M.p.: 175 °C; $[\alpha]_d^{25} = +304.1$ ($c = 1.0$ in CH_2Cl_2); ^{1}H NMR (500 MHz, [D$_6$]chloroform, 25 °C): $\delta = 1.48$ (s, 9H, $C(CH_3)_3$), 2.39 (m, 1H; CH_2), 2.86 (m, 1H; CH_2), 3.82 (dd, ^3J(H,H) = 4.75, ^2J(H,H) = 12.72 Hz, 1H; CH_2), 3.90 (d, ^2J(H,H) = 12.81 Hz, 1H; CH_2), 4.19 (d, ^3J(H,H) = 8.46 Hz, 1H; CH), 4.58 (m, 1H; CH), 6.28 (d, ^3J(H,H) = 15.89 Hz, 1H; CH=CH), 6.37 (d, ^3J(H,H) = 5.97 Hz, 1H; NH), 6.51 (dd, ^3J(H,H) = 4.76, ^3J(H,H) = 4.76 Hz, 1H; Ar–H), 7.00 (d, ^3J(H,H) = 8.74 Hz, 1H; Ar–H), 7.44 (d, ^3J(H,H) = 15.89 Hz, 1H, CH=CH), 7.53 (dd, ^4J(H,H) = 1.99, ^3J(H,H) = 8.34 Hz, 1H; Ar–H), 7.99 (s, 1H; NH), 8.00 (d, ^4J(H,H) = 1.98 Hz, 1H; Ar–H), 8.25 (d, ^3J(H,H) = 4.77 Hz, 2H; Ar–H), 10.08 (s, 1H; NH) ppm; HR-MS (FAB, 70 eV): *m/z*: calcd for $C_{23}H_{26}N_5O_4$: 436.1984 $[M+1]^+$; found: 436.1995; elemental analysis calcd (%) for $C_{23}H_{25}N_5O_4$: C 63.44, H 5.79, N 16.08; found: C 63.16, H 5.82, N 16.10.

***tert*-Butyl 3-((11 a*R*)-(2*S*)-5,11-dioxo-2-pyrimidin-2-ylamino-2,3,5,10,11,11 a-hexahydro-1*H*-benzo[e]-pyrrolo[1,2-*a*][1,4]diazepin-7-yl]-acrylate (12 b)**: Amine **9 b** (130 mg, 0.36 mmol) was converted into the aminopyrimidino-benzodiazepinedione **12 b** (117 mg, 0.27 mmol, 74%) by the procedure reported for **12 a**. M.p.: 181 °C; $[\alpha]_d^{25} = -281.8$ ($c = 0.5$ in CH_2Cl_2); ^{1}H NMR (500 MHz, [D$_6$]chloroform, 25 °C): $\delta = 1.51$ (s, 9H, $C(CH_3)_3$), 2.12 (m, 1H; CH_2), 2.95 (m, 1H; CH_2), 3.65 (dd, ^3J(H,H) = 6.56, ^2J(H,H) = 12.12 Hz, 1H; CH_2), 4.06 (dd, ^3J(H,H) = 6.36, ^2J(H,H) = 11.92 Hz, 1H; CH_2), 4.30 (dd, ^3J(H,H) = 3.97, ^3J(H,H) = 8.34 Hz, 1H; CH), 4.66 (m, 1H; CH), 6.02 (d, ^3J(H,H) = 6.76 Hz, 1H; NH), 6.36 (d, ^3J(H,H) = 15.89 Hz, 1H; CH=CH), 6.58 (dd, ^3J(H,H) = 4.88, ^3J(H,H) = 4.88 Hz, 1H; Ar–H), 7.00 (d, ^3J(H,H) = 8.34 Hz, 1H; Ar–H), 7.53 (d, ^3J(H,H) = 15.87 Hz, 1H; CH=CH), 0 (dd, ^4J(H,H) = 1.99, ^3J(H,H) = 8.34 Hz, 1H; Ar–H), 8.11 (d, ^4J(H,H) = 1.99 Hz, 1H; Ar–H), 8.20 (d, ^3J(H,H) = 4.77 Hz, 2H; Ar–H), 9.56 (br, 1H; NH) ppm; HR-MS (FAB, 70 eV): *m/z*: calcd for $C_{23}H_{26}N_5O_4$: 436.1984 $[M+1]^+$; found: 436.1964.

3-((11 a*S*)-(2*S*)-5,11-Dioxo-2-pyrimidin-2-ylamino-2,3,5,10,11,11 a-hexahydro-1*H*-benzo[e]pyrrolo[1,2-*a*][1,4]diazepin-7-yl)-acrylic acid trifluoroacetate (13 a): Compound **12 a** (100 mg, 0.23 mmol) was dissolved in dichloromethane (5 mL). TFA (0.5 mL) was added with stirring. Stirring was continued until complete cleavage of the ester. The solvent was removed in vacuo, and the residue was triturated with diethyl ether to afford RGD mimetic **13 a** in quantitative yield. M.p.: 133 °C (dec.); $[\alpha]_d^{25} = +364.0$ ($c = 0.5$ in DMSO); ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): $\delta = 2.3$ Hz, ^2J(H,H) = 11.93 Hz, 1H; CH_2), 3.99 (dd, ^3J(H,H) = 5.56, ^2J(H,H) = 11.92 Hz, 1H; CH_2), 4.32 (dd, ^3J(H,H) = 3.97, ^3J(H,H) = 8.34 Hz, 1H; CH), 4.39 (m, 1H; CH), 6.50 (d, ^3J(H,H) = 15.89 Hz, 1H; CH=CH), 6.66 (dd, ^3J(H,H) = 4.76, ^3J(H,H) = 4.76 Hz, 1H; Ar–H), 7.00 (br, 1H; NH), 7.18 (d, ^3J(H,H) = 8.75 Hz, 1H; Ar–H), 7.60 (d, ^3J(H,H) = 15.89 Hz, 1H, CH=CH), 7.89 (dd, ^4J(H,H) = 1.99, ^3J(H,H) = 8.35 Hz, 1H; Ar–H), 8.00 (d, ^4J(H,H) = 1.98 Hz, 1H; Ar–H), 8.31 (d, ^3J(H,H) = 4.45 Hz, 2H; Ar–H), 10.84 (s, 1H; NH) ppm; HR-MS (FAB, 70 eV): *m/z*: calcd for $C_{19}H_{18}N_5O_4$: 380.1358 $[M+1]^+$; found: 380.1341; elemental analysis calcd (%) for $C_{19}H_{17}N_5O_4 \cdot 2$TFA: C 46.24, H 3.72; found: C 46.91, H 3.22.

3-((11 a*R*)-(2*S*)-5,11-Dioxo-2-pyrimidin-2-ylamino-2,3,5,10,11,11 a-hexahydro-1*H*-benzo[e]pyrrolo[1,2-*a*][1,4]diazepin-7-yl)-acrylic acid trifluoroacetate (13 b): The protective groups of compound **12 b** (110 mg, 0.25 mmol) were removed with TFA by the procedure reported for **13 a**, to obtain the RGD mimetic **13 b** in quantitative yield. $[\alpha]_d^{25} = -325.2$ ($c = 0.5$ in DMSO); M.p.: 124 °C (dec.); ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): $\delta = 2.07$ (m, 1H; CH_2), 2.81 (m, 1H; CH_2), 3.55 (dd, ^3J(H,H) = 6.41, ^2J(H,H) = 11.90 Hz, 1H; CH_2), 3.79 (dd, ^3J(H,H) = 6.26, ^2J(H,H) = 11.60 Hz, 1H; CH_2), 4.41 (dd, ^3J(H,H) = 3.81, ^3J(H,H) = 7.93 Hz, 1H; CH), 4.50 (m, 1H; CH), 6.51 (d, ^3J(H,H) = 16.18 Hz, 1H; CH=CH), 6.68 (dd, ^3J(H,H) = 4.74, ^3J(H,H) = 4.74 Hz, 1H; Ar–H), 7.17 (d, ^3J(H,H) = 8.54 Hz, 1H; Ar–H) 7.61 (d, ^3J(H,H) = 16.17 Hz, 1H; CH=CH), 7.90 (dd, ^4J(H,H) = 1.94 Hz , ^3J(H,H) = 8.55 Hz, 1H; Ar–H), 7.94 (br, 1H; NH), 8.00 (d, ^4J(H,H) = 2.13 Hz, 1H; Ar–H), 8.36 (d, ^3J(H,H) = 4.58 Hz, 2H; Ar–H), 10.77 (s, 1H; NH) ppm; HR-MS (FAB, 70 eV): *m/z*: calcd for $C_{19}H_{18}N_5O_4$: 380.1358 $[M+1]^+$; found: 380.1362.

(Bis-*tert*-butoxycarbonyl-guanidino)acetic acid (20): 1,3-Bis-*tert*-butoxycarbonyl-2-methyl-2-thiopseudourea (3.0 g, 10.3 mmol, 0.95 equiv) and Triton B (4.8 mL, 10 mmol, 40% methanolic solution) were added with stirring to a solution of glycine (816 mg, 10.9 mmol) in DMSO (10 mL). After the mixture had been stirred for 3 d at room temperature, aqueous citric acid (5%, 150 mL) was added to the solution, which was afterwards extracted with ethyl acetate. The

combined organic layers were washed with brine and water and dried over Na_2SO_4. The solvent was evaporated to obtain compound **20** (2.38 g, 7.5 mmol, 69%). M.p: 140 (dec.); ^{1}H NMR (250 MHz, [D$_6$]chloroform, 25 °C): $\delta = 1.47$ (s, 9H; $C(CH_3)_3$), 1.49 (s, 9H; $C(CH_3)_3$), 4.18 (s, 2H; CH_2), 8.92 (br, 1H; NH), 9.80 (br, 1H; NH), 11.35 (br, 1H; NH) ppm; HR-MS (FAB, 70 eV): *m/z*: calcd for $C_{13}H_{24}N_3O_6$: 318.1665 $[M+1]^+$; found: 318.1651; elemental analysis calcd (%) for $C_{13}H_{23}N_3O_6$: C 49.20, H 7.31, N 13.24; found: C 49.21, H 7.24, N 13.24.

***tert*-Butyl 3-{(11a*S*)-(2*S*)-2-[(2-(*N*′,*N*″-bis-*tert*-butoxycarbonyl-guanidino)-acetylamino]-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1*H*-benzo[e]pyrrolo[1,2-*a*][1,4]diazepin-7-yl}-acrylate (14a)**: Amine **9a** (150 mg, 0.42 mmol) and (bis-*tert*-butoxycarbonyl-guanidino)-acetic acid (**20**, 160 mg, 0.50 mmol) were dissolved in dichloromethane (10 mL) and cooled to 0 °C. *N*-(3-dimethylaminopropyl)-*N*′-ethyl-carbodiimide hydrochloride (EDC; 116 mg, 60 mmol) and triethylamine (0.11 mL, 0.79 mmol) were added, with stirring. Stirring was continued for 1 h at 0 °C and for 14 h at room temperature. The solvent was removed and the residue was dissolved in ethyl acetate. After washing with water, 5% citric acid, $NaHCO_3$ (sat.), and water, the organic phase was dried over Na_2SO_4. The solvent was evaporated and the oily residue was purified by flash chromatography (CH_2Cl_2/CH_3OH 10:1, Rf = 0.51) to afford compound **14a** (204 mg, 0.31 mmol, 74%). M.p.: 145 °C (dec.); $[\alpha]_d^{25} = +240.2$ ($c = 1.0$ in CH_2Cl_2); ^{1}H NMR (500 MHz, [D$_6$]chloroform, 25 °C): $\delta = 1.36$ (s, 9H; $C(CH_3)_3$), 1.48 (s, 9H; $C(CH_3)_3$), 1.50 (s, 9H; $C(CH_3)_3$), 2.34 (m, 1H; CH_2), 2.72 (d, ^2J(H,H) = 14.30 Hz, 1H; CH_2), 3.76 (dd, ^3J(H,H) = 5.17, ^2J(H,H) = 13.12 Hz, 1H; CH_2), 3.94 – 4.00 (m, 1H; CH_2, 1H; CH_2), 4.09 (dd, ^3J(H,H) = 4.17, ^2J(H,H) = 17.29 Hz, 1H; CH_2), 4.19 (d, ^3J(H,H) = 8.74 Hz, 1H; CH), 4.53 (m, 1H; CH), 6.37 (d, ^3J(H,H) = 15.89 Hz, 1H; CH=CH), 7.12 (d, ^3J(H,H) = 8.35 Hz, 1H; Ar–H), 7.27 (d, ^3J(H,H) = 5.96 Hz, 1H, NH), 7.53 (d, ^3J(H,H) = 15.89 Hz, 1H, CH=CH), 7.60 (dd, ^4J(H,H) = 1.99, ^3J(H,H) = 8.35 Hz, 1H; Ar–H), 8.09 (d, ^4J(H,H) = 1.99 Hz, 1H; Ar–H), 9.10 (s, 1H, NH), 9.52 (s, 1H, NH), 11.37 (s, 1H, NH) ppm; HR-MS (FAB, 70 eV): *m/z*: calcd for $C_{32}H_{45}N_6O_9$: 657.3248 $[M+1]^+$; found: 657.3232; elemental analysis calcd (%) for $C_{32}H_{44}N_6O_9 \cdot 2H_2O$: C 55.48, H 6.98, N 12.13; found: C 54.63, H 6.23, N 12.23.

***tert*-Butyl 3-{(11a*R*)-(2*S*)-2-[(2-(*N*′,*N*″-bis-*tert*-butoxycarbonyl-guanidino)-acetylamino]-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1*H*-benzo[e]pyrrolo[1,2-*a*][1,4]diazepin-7-yl}-acrylate (14b)**: Amine **9b** (200 mg, 0.56 mmol) was converted into the bis-*tert*-butoxycarbonyl-protected guanidine **14b** (246 mg, 0.37 mmol, 67%) by the procedure reported for **14a**. M.p.: 144 °C (dec.); $[\alpha]_d^{25} = -216.0$ ($c = 1.0$ in CH_2Cl_2); ^{1}H NMR (500 MHz, [D$_6$]chloroform, 25 °C): $\delta = 1.42$ (s, 9H; $C(CH_3)_3$), 1.46 (s, 9H; $C(CH_3)_3$), 1.49 (s, 9H; $C(CH_3)_3$), 2.12 (m, 1H; CH_2), 3.00 (m, 1H; CH_2), 3.62 (dd, ^3J(H,H) = 6.41, ^2J(H,H) = 12.21 Hz, 1H; CH_2), 3.79 – 3.89 (m, 2H; CH_2, 1H; CH_2), 4.25 (dd, ^3J(H,H) = 3.80, ^3J(H,H) = 8.08 Hz, 1H; CH), 4.49 (m, 1H; CH), 6.33 (d, ^3J(H,H) = 16.18 Hz, 1H; CH=CH), 7.10 (d, ^3J(H,H) = 8.56 Hz, 1H; Ar–H), 7.50 (d, ^3J(H,H) = 16.18 Hz, 1H, CH=CH), 7.59 (dd, ^4J(H,H) = 2.14, ^3J(H,H) = 8.92 Hz, 1H; Ar–H), 7.63 (d, ^3J(H,H) = 6.71 Hz, 1H; NH), 8.01 (d, ^4J(H,H) = 2.14 Hz, 1H; Ar–H), 8.92 (br, 1H; NH), 9.40 (s, 1H; NH), 11.27 (s, 1H, NH) ppm; HR-MS (FAB, 70 eV): *m/z*: calcd for $C_{32}H_{45}N_6O_9$: 657.3248 $[M+1]^+$; found: 657.3259; elemental analysis calcd (%) for $C_{32}H_{44}N_6O_9 \cdot 2H_2O$: C 55.48, H 6.98, N 12.13; found C 54.98, H 6.87, N 12.54.

3-[(11a*S*)-(2*S*)-2-(2-Guanidino-acetylamino)-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1*H*-benzo[e]pyrrolo[1,2-*a*][1,4]diazepin-7-yl]-acrylic acid trifluoroacetate (15a): Compound **14a** (150 mg, 0.23 mmol) was dissolved in dichloromethane (5 mL). TFA (0.7 mL) was added with stirring, and stirring was continued until cleavage of the protective groups was complete. The solvent was removed under reduced pressure. The residue was triturated with diethyl ether to afford RGD mimetic **15a** in quantitative yield. M.p.: 163 °C (dec.); $[\alpha]_d^{25} = +265.0$ ($c = 0.5$ in CH_3OH); ^{1}H NMR (500 MHz, [D$_6$]methanol, 25 °C): $\delta = 2.41$ (m, 1H; CH_2), 2.82 (dd, ^3J(H,H) = 1.59, ^2J(H,H) = 13.91 Hz, 1H; CH_2), 3.74 (d, ^2J(H,H) = 12.72 Hz, 1H; CH_2), 3.89 (dd, ^3J(H,H) = 5.56, ^2J(H,H) = 13.11 Hz, 1H; CH_2), 3.91 (d, ^3J(H,H) = 3.58 Hz, 2H; CH_2), 4.36 (dd, ^3J(H,H) = 1.59, ^3J(H,H) = 8.74 Hz, 1H; CH), 4.46 (m, 1H; CH), 6.52 (d, ^3J(H,H) = 15.89 Hz, 1H; CH=CH), 7.21 (d, ^3J(H,H) = 8.34 Hz, 1H; Ar–H), 7.68 (d, ^3J(H,H) = 15.90 Hz, 1H, CH=CH), 7.83 (dd, ^4J(H,H) = 1.99, ^3J(H,H) = 8.35 Hz, 1H; Ar–H), 8.10 (d, ^4J(H,H) = 1.98 Hz, 1H; Ar–H) ppm; HR-MS (FAB, 70 eV): *m/z*: calcd for $C_{18}H_{21}N_6O_5$: 401.1573 $[M+1]^+$; found: 401.1590.

3-[(11a*R*)-(2*S*)-2-(2-Guanidino-acetylamino)-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1*H*-benzo[e]pyrrolo[1,2-*a*][1,4]diazepin-7-yl)-acrylic acid trifluoroacetate (15b): The protective groups of compound **14b** (100 mg, 0.15 mmol) were removed by the procedure reported for **15a** to obtain product **15b** in quantitative yield. M.p.: 168 °C (dec.); $[\alpha]_d^{25} = -255.6$ ($c = 0.5$ in CH_3OH); ^{1}H NMR (500 MHz, [D$_6$]methanol, 25 °C): $\delta = 2.14$ (m, 1H; CH_2), 2.95 (m, 1H; CH_2), 3.56 (dd, ^3J(H,H) = 7.15, ^2J(H,H) = 11.92 Hz, 1H; CH_2), 3.94 (m, 2H; CH_2, 1H; CH_2), 4.37 (dd, ^3J(H,H) = 3.17, ^3J(H,H) = 8.30 Hz, 1H; CH), 4.55 (m, 1H; CH), 6.51 (d, ^3J(H,H) = 16.12 Hz, 1H; CH=CH), 7.18 (d, ^3J(H,H) = 8.54 Hz, 1H; Ar–H), 7.68 (d, ^3J(H,H) = 16.11 Hz, 1H; CH=CH), 7.82 (dd, ^4J(H,H) = 2.07, ^3J(H,H) = 8.42 Hz, 1H; Ar–H), 8.07 (d, ^4J(H,H) = 2.20 Hz, 1H; Ar–H) ppm; HR-MS (FAB, 70 eV): *m/z*: calcd for $C_{18}H_{21}N_6O_5$: 401.1573 $[M+1]^+$; found: 401.1602.

***tert*-Butyl (pyrimidin-2-ylamino)-acetate (21)**: 2-Fluoropyrimidine (**19**; 200 mg, 2.04 mmol) and diisopropylethylamine (0.71 mL, 4.08 mmol) were added to glycine *tert*-butyl ester hydrochloride (171 mg, 1.02 mmol) in *N*,*N*-dimethylformamide (6 mL). The solution was stirred for 24 h at room temperature. After addition of water (50 mL) the mixture was extracted with dichloromethane, followed by removal of the solvent in vacuo. The crude product was purified by flash chromatography (CH_2Cl_2/CH_3OH 10:1, Rf = 0.48) to yield compound **21** (145 mg, 0.69 mmol, 68%). M.p.: 96 °C; ^{1}H NMR (500 MHz, [D$_6$]chloroform, 25 °C): $\delta = 1.42$ (s, 9H; $C(CH_3)_3$), 4.04 (d, ^3J(H,H) = 5.49 Hz, 2H; CH_2), 5.84 (br, 1H; NH), 6.52 (dd, ^3J(H,H) = 4.73, ^3J(H,H) = 4.73 Hz, 1H; Ar–H), 8.25 (d, ^3J(H,H) = 4.57 Hz, 2H; Ar–H) ppm; HR-MS (EI, 70 eV): *m/z*: calcd for $C_{10}H_{15}N_3O_2$: 209.1164 $[M]^+$; found: 209.1169.

(Pyrimidin-2-ylamino)-acetic acid trifluoroacetate (22): *tert*-Butyl (pyrimidin-2-ylamino)-acetate (**21**; 100 mg, 0.48 mmol) was treated with TFA (5 mL, 10% in dichloromethane) for 4 h. The solvent was removed to obtain product **22** in quantitative yield. M.p.: 130 °C (dec.); ^{1}H NMR (500 MHz, [D$_6$]methanol, 25 °C): $\delta = 4.14$ (s, 2H; CH_2), 6.85 (dd, ^3J(H,H) = 5.19, ^3J(H,H) = 5.19 Hz, 1H; Ar–H), 8.43 (br, 2H; Ar–H) ppm; HR-MS (EI, 70 eV): *m/z*: calcd for $C_6H_7N_3O_2$: 153.0538 $[M]^+$; found: 153.0551.

***tert*-Butyl 3-{(11a*S*)-(2*S*)-5,11-dioxo-2-[2-(pyrimidin-2-ylamino)-acetylamino]-2,3,5,10,11,11a-hexahydro-1*H*-benzo[e]pyrrolo[1,2-*a*][1,4]diazepin-7-yl}-acrylate (16)**: Amine **9a** (195 mg, 0.55 mmol) and (pyrimidin-2-ylamino)-acetic acid trifluoroacetate (**23**; 145 mg, 0.55 mmol) were dissolved in dichloromethane (10 mL) and cooled to 0 °C. EDC (126 mg, 0.66 mmol) and triethylamine (0.12 mL, 0.82 mmol) were added, with stirring. Stirring was continued for 1 h at 0 °C and for 14 h at room temperature. The solvent was then removed and the residue was dissolved in ethyl acetate. After washing with water, 5% citric acid, $NaHCO_3$ (sat.), and water, the organic phase was dried over Na_2SO_4. The solvent was evaporated and the oily residue was purified by flash chromatography (CH_2Cl_2/CH_3OH 10:1, Rf = 0.36) to afford compound **16** (99 mg, 0.20 mmol, 37%). M.p.: 202 °C (dec.); $[\alpha]_d^{25} = +366.4$ ($c = 0.5$ in DMSO); ^{1}H NMR (500 MHz, [D$_6$]chloroform, 25 °C): $\delta = 1.40$ (s, 9H; $C(CH_3)_3$), 2.22 (m, 1H; CH_2), 2.59 (d, ^2J(H,H) = 14.30 Hz, 1H; CH_2), 3.63 (dd, ^3J(H,H) =

4.96, ^2J(H,H) = 12.92 Hz, 1H; CH_2), 3.76 (d, ^2J(H,H) = 12.71 Hz, 1H; CH_2), 3.85 (d, ^2J(H,H) = 17.09 Hz, 1H; CH_2), 3.95 (d, ^2J(H,H) = 17.09 Hz, 1H; CH_2), 4.03 (d, ^3J(H,H) = 8.35 Hz, 1H; CH), 4.41 (m, 1H; CH), 6.27 (d, ^3J(H,H) = 15.89 Hz, 1H; CH=CH), 6.43 (dd, ^3J(H,H) = 4.97, ^3J(H,H) = 4.97 Hz, 1H; Ar–H), 6.93 (d, ^3J(H,H) = 8.34 Hz, 1H; Ar–H), 7.43 (d, ^3J(H,H) = 15.89 Hz, 1H; CH=CH), 7.51 (dd, ^4J(H,H) = 1.99, ^3J(H,H) = 8.35 Hz, 1H; Ar–H), 7.63 (d, ^3J(H,H) = 6.76 Hz, 1H; NH), 7.95 (d, ^4J(H,H) = 2.38 Hz, 1H; Ar–H), 8.12 (d, ^3J(H,H) = 4.76 Hz, 2H; Ar–H) ppm; HR-MS (EI, 70 eV): *m/z*: calcd for $C_{25}H_{28}N_6O_5$: 492.2121 $[M]^+$; found: 492.2117; elemental analysis calcd (%) for $C_{25}H_{28}N_6O_5 \cdot H_2O$: C 58.81, H 5.92; found: C 59.04, H 5.51.

3-{(11 a*S*)-(2*S*)-5,11-Dioxo-2-[2-(pyrimidin-2-ylamino)-acetylamino]-2,3,5,10,11,11 a-hexahydro-1*H*-benzo[e]pyrrolo[1,2-*a*][1,4]diazepin-7-yl}-acrylic acid trifluoroacetate (17): Compound **16** (35 mg, 0.07 mmol) was treated with TFA (5 mL, 10% in dichloromethane) for 6 h. The solvent was removed and the residue was triturated with diethyl ether to afford **17** in quantitative yield. M.p.: 158 °C (dec.); $[\alpha]_d^{25} = +365.0$ ($c = 0.5$ in DMSO); ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): $\delta = 2.24$ (m, 1H; CH_2), 2.50 (m, 1H; CH_2), 3.33 (dd, ^3J(H,H) = 4.17, ^2J(H,H) = 12.12 Hz, 1H; CH_2), 3.81 – 3.88 (m, 2H; CH_2, 1H; CH_2), 4.24 (dd, ^3J(H,H) = 3.98, ^3J(H,H) = 8.75 Hz, 1H; CH), 4.28 (m, 1H; CH), 6.49 (d, ^3J(H,H) = 15.89 Hz, 1H; CH=CH), 6.67 (dd, ^3J(H,H) = 4.77, ^3J(H,H) = 4.77 Hz, 1H; Ar–H), 7.15 (d, ^3J(H,H) = 8.75 Hz, 1H; Ar–H), 7.59 (d, ^3J(H,H) = 16.30 Hz, 1H; CH=CH), 7.88 (dd, ^4J(H,H) = 1.99, ^3J(H,H) = 8.74 Hz, 1H; Ar–H), 7.90 (d, ^3J(H,H) = 6.76 Hz, 1H; NH), 7.98 (d, ^4J(H,H) = 2.39 Hz, 1H; Ar–H), 8.35 (d, ^3J(H,H) = 4.77 Hz, 2H; Ar–H), 10.73 (s, 1H; NH) ppm; HR-MS (FAB, 70 eV): *m/z*: calcd for $C_{21}H_{21}N_6O_5$: 437.1573 $[M+1]^+$; found: 437.1592; elemental analysis calcd (%) for $C_{21}H_{20}N_6O_5 \cdot 0.9$ TFA: C 52.53, H 4.04; found: C 52.26, H 4.24.

Solid-phase receptor assay:

Biotinylation of fibrinogen: Human fibrinogen (100 mg) was dissolved in aqueous NaCl (0.3 M, 5 mL) at 37 °C according to the manufacturer's instructions and then further diluted with $NaHCO_3$ (0.1 M, pH 9.6) to a final concentration of 1 mg mL^{-1}. (+)-Biotin *N*-succinimidyl ester was dissolved in *N*,*N*-dimethylformamide at 1 mg mL^{-1}. The biotinylation reagent and the fibrinogen were mixed in a ratio of 1:3 and incubated at room temperature for 90 min. The reaction mixture was subsequently dialyzed for 4 h at room temperature against Tris buffer (20 mM Tris, 150 mM NaCl, pH 7.4). After dialysis, the solution was centrifuged for 10 min at 5000 g to separate precipitates. Tween 20 (0,005%) was added and the solution produced could be stored at 4 °C for up to one week. The biotinylated fibrinogen prepared by this procedure contained about 200 – 250 μg protein mL^{-1} as determined by UV/Vis spectroscopy.

Integrins $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ were diluted in Tris+ (20 mM Tris, 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM $MnCl_2$, pH 7.4) at final concentrations of 0.2 μg mL^{-1} and 1.0 μg mL^{-1}, respectively. Subsequently, integrins (100 μL/well) were immobilized overnight at 4 °C on Lumitrac 600 96-well microtiter plates. The coating solution was flicked off and free binding sites were blocked with 1% BSA (300 μL/well) in Tris+ for one hour at room temperature. The blocking solution was flicked off and the plates were washed twice with TBST (Tris+ containing 0.01% Tween 20).

The inhibitors were serially diluted in biotinyl fibrinogen solution (Tris+, 10 μg mL^{-1} fibrinogen). The individual inhibitors (100 μL each) were added to each well and the system was incubated at RT for 2 h. The plates were then washed twice with TBST. Peroxidase-conjugated antibiotin antibody (goat, Calbiochem; 1:1000 in TBST + 0,1% BSA; 100 μL) was added and the system was incubated for another hour. Finally, the microtiter plates were washed three times for at least 5 min with TBST. For quantification, chemiluminescence substrate (100 μL) was added and the resulting light emission was detected with an Orion luminescence reader (Berthold). Positive controls received no inhibitors. The cyclopentapeptide c(RGDfV) was used as internal standard.

All experiments were performed at least in triplicate. IC_{50} values were calculated by sigmoid fitting of the dose – response relationship (Origin).

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