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A NOVEL RGD CONTAINING DODECAPEPTIDOMIMETIC WHICH EXHIBITS SELECTIVE BINDING TO THE $\alpha_V\beta_3$ RECEPTOR

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Abstract: We report the synthesis, bioactivity and conformational analysis by ^{1}H NMR and computer simulations of a RGD containing dodecapeptidomimetic incorporating the β-turn mimetic BTD. This peptidomimetic exhibits potent binding to the $\alpha_V\beta_3$ receptor. The binding activity to the $\alpha_V\beta_5$ receptor is approximately tenfold weaker. Unlike most active RGD containing peptides reported to date this molecule does not show any binding to the fibronectin ($\alpha_V\beta_1$) or the IIb/IIIa receptors. © 1997 Elsevier Science Ltd.

The tripeptide Arg-Gly-Asp (RGD) sequence is one of the major cell-attachment domains of the extracellular matrix and platelet adhesion proteins.[1,2] Fibronectin, vitronectin, osteopontin, collagens, thrombospondin, fibrinogen, and von Willebrand factor contain the RGD sequence and their interaction with cells can be inhibited by RGD containing peptides.[3] Most RGD analogs bind to the integrins nonspecifically, which is one of the major drawbacks in their development as drugs. Since there are many types of RGD ligands, each carrying out specific biological functions, it is important for an RGD containing drug to maintain binding specificity to a given receptor so as not to interfere with other biological processes.

The RGD tripeptide sequence has been found to be at the tip of a hairpin in some small natural proteins with RGD activity or to comprise part of a β-turn in many conformationally constrained RGD peptides.[4–7] In particular, Muller et al. have synthesized a series of potent xanthene-coupled RGD containing peptides varying in the length of the peptide chains. Maximum inhibition of fibrinogen to the GP IIb/IIIa receptor was observed for the dodecapeptidomimetic derivative.[8] Our investigations are focused on the design and synthesis of novel conformationally constrained RGD containing analogs. For initial studies we employed the β-turn dipeptidomimetic BTD (Figure 1), which facilitates the hairpin bending of peptide molecules and therefore acts as a template for the folding of the RGD sequence.[9–11]

In this paper we report the design, synthesis, ^{1}H NMR, and computer simulations of a novel RGD containing peptidomimetic that exhibits potent binding to the $\alpha_V\beta_3$ integrin receptors. Following the design principles described above, we have synthesized the dodecapeptidomimetic **1**. The BTD was synthesized following the procedure by Bach et al.[12] The protected cyclic dodecapeptidomimetic was constructed via solid-phase synthesis on the Kaiser's oxime resin using standard Boc strategy.[13] After removal of the Boc group of the arginine residue, the amino group was allowed to react with the activated C-terminal attached to the resin, in the presence of acetic acid, thereby releasing the side chain protected cyclic dodecapeptidomimetic.[14] Palladium black in 80% formic acid/methanol removed the Cbz and Bzl protecting groups yielding the desired peptidomimetic **1**.[15]

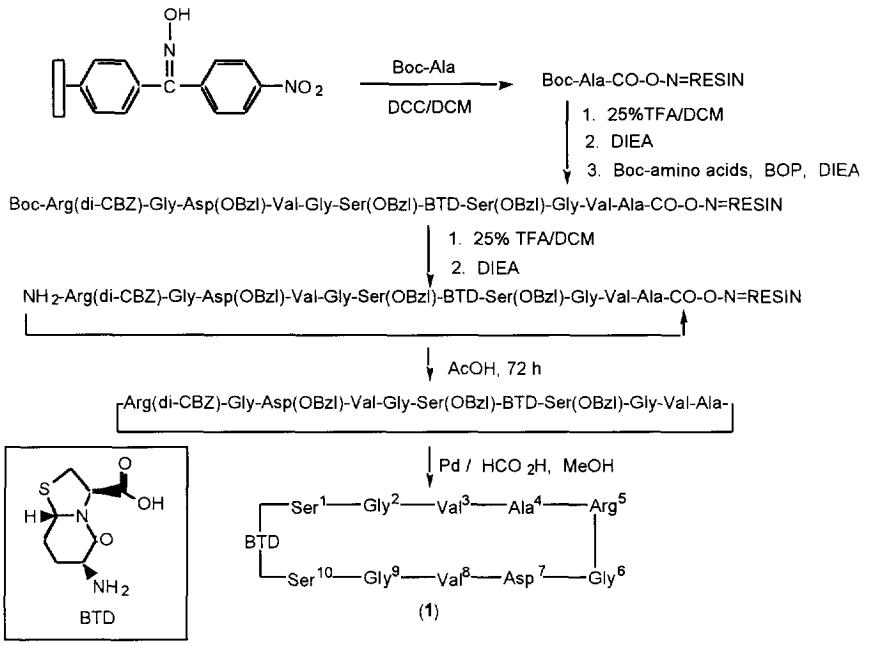

Figure 1: Synthesis of the dodecapeptidomimetic **1** and structure of the β-turn mimetic BTD

The conformation of the analog was studied by ^{1}H NMR spectroscopy in DMSO-d_6 and by computer simulations. The interproton distances, the ϕ torsion angles and the hydrogen bonding patterns were derived through the measurements of the nuclear Overhauser effects, the vicinal spin-spin coupling constants and the temperature coefficient of the amide protons. The distance geometry program DGEOM was used to generate 600 structures consistent with the NOE constraints.[16] These structures were minimized and structures not consistent with the NMR data were discarded. The remaining structures were subjected to molecular dynamics simulations using DISCOVER.[17]

Although the conformational search suggests that the molecule shows flexibility, the structures consistent with the NMR data share two common structural motifs; a type II' β-turn around the BTD template and a γ-turn at the other side of the molecule about the Asp^7 residue. There is a degree of distortion in the β II' turn with typical torsion angles ($\phi^{i+1} = 59$, $\Psi^{i+1} = -121$, $\phi^{i+2} = -86$, $\Psi^{i+2} = 42$). The presence of a β-turn is in agreement with the relatively low temperature coefficient of the NH of Ser^1 (2.9 -pbb/K). The lack of a NH in the position i+2 in the BTD template makes it impossible to observe the NH(i+2) NH(i+3) crosspeak in the ROESY experiment which is indicative for a β-turn.

The γ-turn about the Asp^7 residue (formed by the Val^8 NH and the Gly^6 CO) shows only minor variations throughout all structures consistent with the experimental data. The presence of a γ-turn about the Asp^7 residue is in agreement with NMR data, such as the temperature coefficient of the Val^8 NH (1.8 -ppb/K), which is the lowest temperature coefficient of all NH protons in this molecule. Furthermore the lack of a NH(Val^8)-NH(Asp^7) crosspeak in the ROESY experiment indicates that the Val^8 NH is not part of a β-turn,

a structural element frequently found in active RGD peptidomimetics in this portion of the molecule. The γ-turn about the Asp^7 is maintained during a 3 ns molecular dynamics simulation in vacuum. In addition to these two structural motifs, some of the structures consistent with the NMR data show a β-pleated substructure involving a hydrogen bond between the Gly^2 NH and Ser^{10} CO. This is supported by a relatively low temperature coefficient for Gly^2 NH (2.5 -ppb/K).

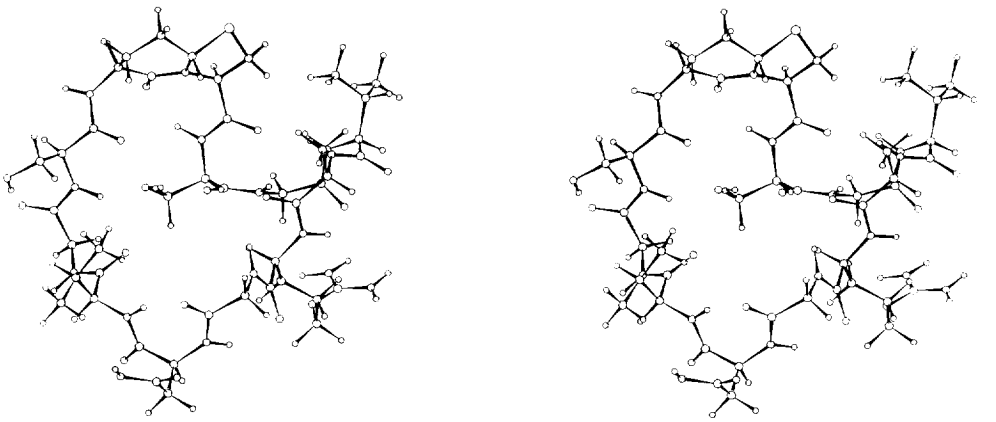

Figure 2: Stereoplot of conformation of the dodecapeptidomimetic **1** in solution as determined by ^1H NMR and computer simulations.

The bioactivity data (Table 1) show that the molecule binds specifically to the $\alpha_v\beta_3$ receptor and that the binding activity to the $\alpha_v\beta_5$ receptor is approximately tenfold weaker. The binding activity to the $\alpha_v\beta_3$ receptor was determined by the PMA stimulated human-derived B lymphocyte (JY) cell assay. No binding to the fibronectin receptor or to the IIb/IIIa receptor has been detected. Since RGD containing peptides that bind to the fibronectin and to the IIb/IIIa receptors often show a β-turn around the RGD portion of the molecule, it has been postulated that the presence of such a turn might be important for binding. The lack of binding activity of compound **1** to these receptors and the absence of structures containing a β-turn spanning the RGD portion of the molecule supports the importance of a β-turn in the RGD unit for binding to these receptors.

The presence of a γ-turn within the RGD portion of the molecule in all structures consistent with the NMR data makes it reasonable to relate this turn to the specific binding to the $\alpha_v\beta_3$ receptor . It is noteworthy that the conformation of the cyclic pentapeptide c[-Arg-Gly-Asp-Phe-D-Val-] which shows a similiar binding pattern adopts a γ-turn about the Asp residue (formed between the Phe NH and Gly CO).[18] This would support the hypothesis that the presence of a γ-turn in the RGD unit is important for the specific binding to the $\alpha_v\beta_3$ receptors.

Table 1: Binding of GRGDSP and compound **1** to the isolated receptors and PMA stimulated human-derived B lymphocyte (JY) cell adhesion to vitronectin-coated microtiter plates

Peptides	Fibronectin ELISA 1X Tris	IIb/IIIa ELISA	$\alpha_v\beta_5$ ELISA	JY[a]	$\alpha_v\beta_3$ [b] (estimated)
GRGDSP	0.02 μM	0.8 μM	0.582 μM	50μM	5 μM
1	> 10.0 μM	> 10.0μM	0.4 μM	0.5 μM	0.05 μM

[a]see reference 19. [b]the binding activity to the $\alpha_v\beta_3$ ELISA was estimated from the results of the JY cell assay.

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Experimental Section

Boc amino acids were purchased from Bachem California. Synthesis of Boc-BTD-OH was carried out according to literature procedure.[12]

Synthesis of Cyclic Dodecapeptidomimetic c[-Arg-Gly-Asp-Val-Gly-Ser-BTD-Ser-Gly-Val-Ala-]. The peptide synthesis was carried out on a manually operated solid-phase synthesis apparatus. Boc-Ala-OH (76 mg, 0.4 mmol) was attached to oxime resin (2.00 g) with DCC (86 mg, 0.4 mmol) in 20 mL of DCM at room temperature overnight, followed by acylation with a mixture of trimethyl acetic anhydride (6.0 mL) and DIEA (1.0 mL) for two days. The substitution level tested was 0.178 mmol/g of resin based on picric acid titration. The obtained Boc-Ala-resin was deprotected with 25 % TFA/DCM (30 min) and neutralized with 5% DIEA/DCM. A DMF solution of Boc-Val-OH (260 mg, 1.2 mmol), BOP (530 mg, 1.2 mmol), and DIEA (0.36 mL, 2.0 mmol) was poured into the reaction vessel. The coupling was complete in 6 h. The peptide chain elongation was continued by consecutive deprotections of Boc groups and coupling of the following amino acids: Boc-Gly-OH, Boc-Ser(OBzl)-OH, Boc-BTD-OH, Boc-Ser(OBzl)-OH, Boc-Gly-OH, Boc-Val-OH, Boc-Asp(OBzl)-OH, Boc-Gly-OH, Boc-Arg(di-CBz)-OH. The product, Boc-Arg(di-CBz)-Gly-Asp(OBzl)-Val-Gly-Ser(OBzl)-BTD-Ser(OBzl)-Gly-Val-Ala-resin, was obtained. After the last Boc group was removed from the N-terminal residue, the cyclization was carried out on the resin in DMF (15 mL) and DCM (15 mL) in the presence of acetic acid (0.24 mL, 4.0 mmol) and DIEA (0.72 mL, 4.0 mmol) at room temperature for three days. The resin was removed by filtration and washed twice with DMF and twice with DCM. The crude fully protected cyclic dodecapeptidomimetic was obtained after filtration and evaporation (70.9 mg, 12% yield based on Boc-Ala-resin), FABMS *m/z* 1646 (M + H)$^+$. To a stirring solution of 50 mg of the protected cyclic dodecapeptidomimetic in 40 mL of 80% formic acid/methanol was added 50 mg of Pd black in 10 mL of 80% formic acid/methanol. The reaction was stirred under nitrogen at room temperature for 2.5 h. After filtration, the solvents were evaporated under reduced pressure. Purification of the final product was carried out on RP-HPLC to give 20.5 mg (56 % yield) of c[-Arg-Gly-Asp-Val-Gly-Ser-BTD-Ser-Gly-Val-Ala-] R_t = 14.38 min (Vydac C_{18} proteins analytical column; eluent 14% CH_3CN / H_2O with 0.1% TFA; flow rate 1 mL/min.; detection at 215 nm): R_f = 0.34 (*n*-BuOH/H_2O/AcOH/EtOAc, 1/1/1/1), FABMS *m/z* 1084 (M+H)$^+$, 1106 (M+Na)$^+$, 1122 (M+K)$^+$, 1236 (M+NBA)$^+$; ^{1}H NMR (DMSO-d_6) δ 8.57 (d, *J* = 7.1, 1H, BTD-NH), 8.44 (t, *J* = 5.6, 1H, Gly^{6}NH), 8.26 (d, *J* = 7.4, 1H, Asp^{7}NH), 8.14 (t, *J* = 5.2, 1H, Gly^{9}NH), 8.07 (d, *J* = 6.9, 1H, Ala^{4}NH), 8.04 (d, *J* = 7.1, 1H, Ser^{1}NH), 8.03 (d, *J* = 6.1, 1H, Arg^{5}NH), 7.98 (t, *J* = 5.6, 1H, Gly^{7}NH), 7.91 (d, *J* = 7.7, 1H, Ser^{10}NH), 7.76 (d, *J* = 8.5, 1H, Val^{3}NH), 7.44 (d, *J* = 8.9, 1H, Val^{8}NH), 7.38 (dd, *J* = 5.3, 10.7, 1H, Arg^{5}H$^\varepsilon$), 4.96 (bt, *J* = 7.0, 1H, BTD-H^9), 4.92 (dd, *J* = 4.0, 11.9, 1H, BTD-H^6), 4.47 (q, *J* = 7.2, 1H, Asp^{7}H$^\alpha$), 4.38 (q, *J* = 7.1, 1H, Ser^{10}H$^\alpha$), 4.30 (q, *J* = 7.0, 1H, Ala^{4}H$^\alpha$), 4.25

(q, J = 6.7, 1H, Ser^{1}H$^\alpha$), 4.16 dd, J = 6.3, 8.5, 1H, Val^{3}H$^\alpha$), 4.14 (q, J = 6.8, 1H, Arg^{5}H$^\alpha$), 4.11 (q, J = 6.9, 1H, Val^{8}H$^\alpha$), 4.04 (q, J = 7.2, 1H, BTD-H^3), 3.86 (dd, J = 5.5, 11.8, 1H, Gly^{2}H$^\alpha$), 3.84 (dd, J = 5.1, 13.2, 1H, Gly^{6}H$^\alpha$), 3.80 (m, 2H, Gly^{9}H$^\alpha$), 3.72 (dd, J = 6.4, 10.8, 1H, Ser^{1}H$^\beta$), 3.69 (dd, J = 5.85, 11.8, 1H, Gly^2 H$^\alpha$), 3.68 (dd, J = 6.9,10.8, 1H, Ser^{1}Hβ), 3.55 (dd, J = 5.5, 1H, Gly^{6}H$^\alpha$), 3.51 (m, 2H, Ser^{10}H$^\beta$), 3.32 (dd, J = 6.6, 11.4, 1H, BTD-H^8), 3.14 (dd, J = 8.3, 11.4, 1H, BTD-H^8), 3.06 (m, 2H, Arg^5-H$^\delta$), 2.83 (dd, J = 5.2, 16.8, 1H, Asp^7 H$^\beta$), 2.58 (dd, J = 8.3, 16.8, 1H, Asp^7 H$^\beta$), 2.27 (m, 1H, BTD-H^5), 1.96 (m, 1H, BTD-H^4), 1.96 (m, 1H, Val^{8}H$^\beta$), 1.94 (m, 1H, Val^{3}H$^\beta$), 1.93 (m, 1H, BTD-H^4), 1.76 (m, 1H, BTD-H^5), 1.63 (m, 1H, Arg^5 H$^\beta$), 1.56 (m, 1H, Arg^5 H$^\beta$), 1.44 (m, 2H, Arg^5 H$^\gamma$), 1.21 (d, J = 6.8, 3H, Ala^4 H$^\beta$), 0.86 (d, J = 6.9, 6H, Val^{8}H$^\gamma$), 0.83 (d, J = 6.7, 6H, Val^{3}H$^\gamma$). HR-FABMS: calcd. for $C_{43}H_{70}N_{15}O_{16}S$ $(M + H)^+$ m/z 1084.4846 found 1084.4851.

^{1}H NMR Measurements. The ^{1}H NMR spectra were recorded on a Bruker AMX 500 spectrometer operating at 500 MHz. All experiments were carried out in DMSO-d_6 with the solvent peak as internal standard. The peak assignments were made using DQF-COSY and the rotating frame nuclear Overhauser enhancemen (ROESY) experiments.[20] The ROESY experiments were carried out using mixing times of 100 and 200 ms with a spin locking field of 2.5 KHz. All of the two-dimensional spectra were obtained using 2K data points in the f2 domain and 400 points in the f1 domain for ROESY experiment and 512 data points in f1 domain for the DQF-COSY. The time proportional phase increment was used. Applying zero filling procedures resulted in a final matrix of 2K x 2K data points. Multiplication with a 30^O shifted sine bell function was used for the DQF-COSY multiplication with a 90^O shifted sine bell function was applied for the ROESY to enhance the spectra.[21–23] Coupling constants were obtained from the one-dimensional spectra containing 16 K data points in 6300 Hz or DQF-COSY experiment. NOEs were assigned as strong, medium and weak relative to each other according to their intensities. The ϕ angles were calculated by use of a Karplus-type relationship reported by Bystrov and Cung.[24–26]

Computer Simulations. The computer simulation includes distance geometry, energy minimization, cluster analysis and molecular dynamics simulation. The distance geometry program DGEOM was used to generate structures consistent with the distance constraints derived from the NOEs. The structures were minimized applying the VA09A algorithm until the maximum derivative was less than 0.001 Kcal/mol and the Φ torsional angles and the hydrogen bonding patterns of these structures were compared with the values derived form the NMR measurements. Structures not consistent with the experimental values were discarded. Those structures consistent with the experimental data and with energies not higher than 10 Kcal/mol compared to the lowest energy were subjected to cluster analysis and resulted in preferred conformational families. The minimum energy conformation of each cluster was subjected to molecular dynamics.

Molecular dynamics and mechanics calculations were carried out in vacuo employing the DISCOVER program with the CVFF force field. A distance dependent dielectric constant was used. The molecular dynamics simulation were carried out at 300 K.

References and Notes

Abbreviations used are those recommended by the IUPAC-IUB Commission: Bzl, benzyl; Boc, (tertbutyloxy)carbonyl; Cbz, benzyloxycarbonyl; DCC, dicyclohexylcarbodiimide; BOP, (benzotriazol-1-yloxy)tris(dimethyl-amino)phosphonium hexafluorophoshate; TFA, Trifluoroacetic acid; DIEA, diisopropylethylamine; DCM, dichloromethane; DMF, dimethylformamide.

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