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Nonpeptide RGD Antagonists: A Novel Class of Mimetics, the 5,8-Disubstituted 1-Azabicyclo[5.2.0]nonan-2-one Lactam

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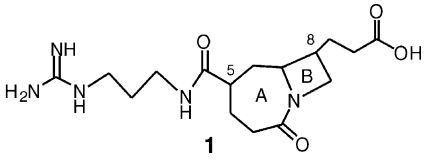
Abstract—The 1-azabicyclo[5.2.0]nonan-2-one lactam **1** adequately substituted on both cycles A and B as scaffolds mimics the conformationally constrained β-turn of the tripeptide RGD signaling motif of fibronectin. Using an in vitro assay, we establish that *trans* diastereoisomer **1b** dissociates a soluble fibronectin–integrin $\alpha_5\beta_1$ complex at concentrations comparable to those of a linear RGDS peptide as a competitor.

Attempts to mimic β-turn topologies of signaling peptide motifs, as is the case of the Arg-Gly-Asp (RGD) tripeptide in a variety of extracellular matrix proteins, have converged towards the synthesis of constrained non-peptidic molecules, several of them including a seven-membered benzodiazepine scaffold.[1–5] The conformational adaptability of a 7-membered ring is certainly part of the ability of such benzodiazepine-type compounds to interact efficiently with different cell adhesion receptors (integrins or IN).

The occurrence in these non-peptidic molecules of both guanidinium and carboxylate groups are essential elements for mimicking the Arg and Asp side chains of RGD, respectively.[6]

In this paper, we report on the synthesis of a new bicyclic lactam **1** (with cycle A being a seven-membered ring and B being a 4-membered ring) as a non-peptidic scaffold mimicking the RGD β-turn topology. Lactam **1** is substituted on both cycles A and B with the former bearing an analogue of the Arg side chain and the latter bearing an analogue of the Asp side chain. Finally, the CH_2 in the azetidine cycle might well mimic the CH_2 of Gly in the RGD motif. To maintain an Arg-to-Asp spatial relationship similar to that of the RGD motif in the crystal structure of an RGD-containing Fn fragment,[7] a suitably substituted amide group was added at position 5 of lactam **1**.



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The 1-azabicyclo[5.2.0]nonan-2-one bicyclic skeleton is not common and has been scarcely synthesized. The few reported synthetic routes[8] are not straightforward and usually result in poor yields. The synthetic approach we have developed (Scheme 1) takes advantage of several aspects of our recently reported synthesis of 1-azabicyclo[5.2.0]nonan-2-one itself and derivatives.[9] Among the considerations that led to such a synthesis were: (a) the lactam skeleton of compound **1** that is generated by photochemical rearrangement of the hexahydroindole oxaziridine **8** without interfering with the stereochemistry at the substituted positions; (b) the easy availability of such bicyclic structures from substituted indoles.

The non-peptide RGD mimetic **1** synthesized here was designed to compete with fibronectin for binding to the $\alpha_5\beta_1$ integrin. Evidence has been provided that $\alpha_5\beta_1$ and

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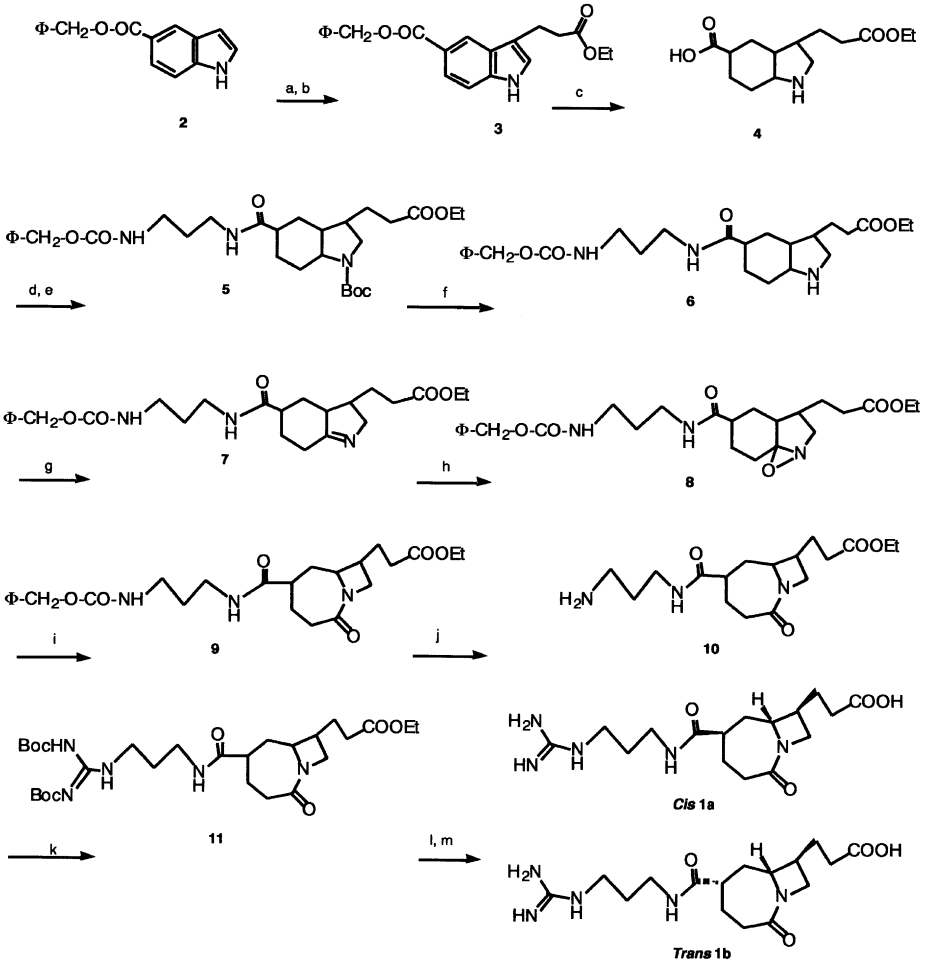

Scheme 1. Reagents and conditions (isolated yield): (a) CH_2O, L-proline, Meldrum's acid, CH_3CN, 22 h, 20 °C, 83%; (b) Cu, EtOH/pyridine, 16 h, reflux, 88%; (c) H_2, Pd/C (10%), AcOH/water, 70 kg/cm^2, 70 °C, 22 h, 91%; (d) Boc_2O, NaOH, dioxane/water, 16 h, 20 °C, 92%; (e) *N*-benzyloxycarbonyl-1,3-diaminopropane, DCC, DMAP, CH_2Cl_2, 21 h, 20 °C, 72%; (f) TFA, CH_2Cl_2, 1 h, 20 °C, 94%; (g) step 1: NCS, ether/CH_3CN, 1 h, 20 °C; step 2: KO_2, DMF, 4 h, 20 °C, 58% over the two steps; (h) *m*CPBA, CH_2Cl_2, 30 min, 0 °C, 86%; (i) hν, CH_3CN, 8 h, 18 °C, 45%; (j) H_2, $Pd(OH)_2$/C, EtOH, 6 h, 20 °C, 98%; (k) *N*,*N*′-bis-*t*-butoxycarbonylthiourea, $HgCl_2$, Et_3N, DMF, 30 min at 0 °C, 48 h at 20 °C, 79%; (l) NaOH, dioxane/water, 30 min at 0 °C, 20 h at 20 °C, 45%; (m) CF_3COOH, CH_2Cl_2, 4 h, 20 °C, 98%.

its ligand fibronectin play critical roles in angiogenesis, resulting in tumor growth in vivo.[10] Importantly, antibodies, peptide and non-peptide antagonists of integrin $\alpha_5\beta_1$ have been shown to block angiogenesis induced by several growth factors in both chick embryo and murine models.[10] Non-peptide $\alpha_5\beta_1$ antagonists could thus have a therapeutic application as either angiogenesis-blocking compounds or tumor-targeting molecules.

Chemistry

The general synthetic route[11] to compound **1** is illustrated in Scheme 1. Compound **1** was prepared starting from the monosubstituted benzyl 5-indolecarboxylate **2**,[12] after introduction of a second substituent to afford the disubstituted compound **3**. The latter was obtained by addition of Meldrum's acid[13]-formaldehyde followed by cleavage of the Meldrum's group with copper powder. The newly introduced ester chain in **3** mimics the aspartyl side chain. The transformation of indole **3** into the octahydroindole compound **4**, was carried out by catalytic reduction under pressure with H_2 in the presence of 10% Pd/C in acetic acid/water, resulting in the concomitant deprotection of the benzyl group (90% yield; mixture of *cis* and *trans* diastereoisomers). The amino group of **4** was protected by Boc, before introducing the Arg-precursor chain at position 5 in cycle A upon reaction of the free carboxylic acid with *N*-benzyloxycarbonyl-1,3-diaminopropane (DMAP-catalyzed DCC coupling). Boc deprotection of **5**, by treatment with TFA, gave the octahydroindole **6** in 94% yield. To prepare oxaziridine **8**, we have demonstrated[9] that the most convenient strategy corresponds to the oxidation of the octahydroindole imine **7**. The chloramine, prepared by action of NCS on **6**, upon reaction with potassium superoxide (KO_2) in DMF, led to imine **7** in 58% yield. Oxidation of imine **7** to oxaziridine **8** was achieved by *m*-chloroperbenzoic acid (*m*CPBA). The photochemical rearrangement of oxaziridine **8** into lactam **9** is the critical step of the synthesis. As expected,[9] this photorearrangement was not influenced by disubstitutions. Upon UV irradiation (for conditions see ref 9), oxaziridine **8** afforded the expected 5,8-disubstituted 1-azabicyclo[5.2.0]nonan-2-one **9** (45% yield of isolated pure product).

Deprotection of the *N*-benzyloxycarbonyl chain by catalytic hydrogenolysis of lactam **9** with $Pd(OH)_2/C$ (Pearlmań's catalyst) gave **10** in 98% yield. Coupling of **10** in the presence of $HgCl_2$ with *N*,*N*′-bis-*t*-butoxycarbonylthiourea[14] resulted in the formation of compound **11** in 79% yield. Saponification of the ethyl ester by sodium hydroxide followed by deprotection of *N*-*t*-butoxycarbonyl by treatment with TFA gave 5-(guanidino carboxamido propyl) 1-azabicyclo[5.2.0]nonan-2-one 8-propionic acid **1**, as a mixture of two diastereoisomers, a major one **1a**, 85%, and a minor one **1b**, 15%. The two isomers, **1a** and **1b**, were then separated by preparative HPLC.[15] ^1H NMR spectra[15] provided clear evidence (*J*) that the major **1a** had the *cis* relative configuration and the minor **1b** the *trans* relative configuration.

Biological Results

The capacity of compounds **1** to act as RGD mimetics was evaluated using a miniaturized recombinant $\alpha_5\beta_1$ mini-integrin[16] that is soluble by only including part of the extracellular domain with different fibronectin recognition sites in both α and β subunits.

We previously described an in vitro assay to evaluate the fibronectin-binding properties of our mini-integrin.[16] This assay[17] was used here to determine the ability of compounds **1** to dissociate the soluble $\alpha_5\beta_1$/fibronectin complex. Briefly stated, the 1:1 complex (formed between the recombinant $\alpha_5\beta_1$ mini-integrin and a fibronectin 3Fn8-11 fragment that is produced as a GST fusion protein and encompasses the 8th–11th type III Fn modules with a single RGD motif in the 10th module), or IN/Fn complex, was immobilized on a Ni-nitriloacetic agarose support (His-tagged mini-integrin[16]) and the competitor compounds was added at increasing concentrations. This linear tetrapeptide was used as a reference compound since it has been previously shown to efficiently dissociate the $\alpha_5\beta_1$/fibronectin complex in vivo.[18] The dissociation of the IN:Fn complex was estimated by determining (by UV spectrophotometry; see ref 17) the amount of Fn eluted from the column at a given competitor concentration.

The two diastereoisomers, **1a** and **1b**, were assayed for their capacity to dissociate the IN:Fn complex. As shown in Table 1, it appears that the *trans* diastereoisomer **1b** dissociates the IN/Fn complex at an IC_{50} value close to that observed for RGDS. A remarkable observation is that both *cis* **1a** and *trans* **1b** diastereoisomers, differ by two orders of magnitude in efficiency to compete with Fn for binding to the $\alpha_5\beta_1$ soluble receptor. We anticipate that one of the enantiomers of **1b** might still display an enhanced potency in releasing Fn from its receptor. Exact knowledge of the positioning of **1b** within the RGD-binding site of the integrin receptor is of crucial importance for adapting the syntheses of our non-peptide RGD mimetics. Since our synthetic strategy is intimately linked to the indole chemistry (Scheme 1) this offers a powerful strategy for introducing a variety of structural modulations in a regio- and stereoselective manner that could be exploited for enhancing the affinity, as well as the selectivity of recognition of the RGD-type antagonist by a given integrin receptor. In vivo degradation by proteolysis of a non-peptide antagonist as **1b** might be substantially reduced in comparison to peptidyl RGD-analogues, thus offering a real advantage in a therapeutical perspective. In conclusion, we have developed a versatile and novel route to 5,8-disustituted 1-azabicyclo[5.2.0]nonan-2-one lactams as compound **1b**. *trans* diastereoisomer **1b** diplays an efficacy at dissociating the IN/Fn interactions in our in vitro assay comparable to that observed for a linear RGD peptide. This suggests that this bicyclic lactam is a convenient framework as a non-peptidic compound mimicking the β-turn topology of the RGD signal in fibronectin, and likely other RGD-containing extra-cellular matrix proteins involved in cell adhesion.

Table 1. Competition[a] between fibronectin (3Fn8-11 fragment) and different RGD analogues for binding[16] to soluble integrin $\alpha_5\beta_1$ (IC_{50} values in M)

RGDS	Mimetic **1**	Mimetic **1b**	Mimetic **1a**
$2.2\pm0.3\times10^{-6}$	$1.3\pm0.4\times10^{-3}$	$6.5\pm0.5\times10^{-5}$	$3.1\pm0.4\times10^{-3}$

[a]Competition between the RGD mimetics, the RGDS linear peptide and fibronectin GST-3Fn8-11 fragment; (see text) for binding to the recombinant $\alpha_5\beta_1$ mini-integrin. The stoichiometric ratio of the $\alpha_5\beta_1$:GST-3Fn8-11 complex is 1:1 while the stoichiometric ratio is 1:2 with RGDS (Banères et al., unpublished results). For K_d values see ref 17.

Acknowledgements

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17. A standard competition assay was carried out as follows. Step 1: a 400 μL volume of an equimolecular mixture (5 μM) of the His-tagged mini-integrin $\alpha_5\beta_1$ and of the human fibronectin fragment 3Fn8-11 (see ref 15 for the definition of these macromolecules) in buffer A (12.5 mM Na-borate, 150 mM NaCl, 5 mM $MgCl_2$ at pH 8.3) was added to a 400 μL volume of Ni-nitriloacetic agarose resin in buffer A previously loaded on a Pasteur pipet and stopped with glass wool; the total mixture was incubated for 30 min at room temperature. Step 2: elution is started and the resin is washed once with a 400 μL volume of buffer A (no UV absorbance detected in the eluate). Step 3: a 400 μL volume of a given RGD analogue at varying concentration was loaded onto the column; after a 30 min incubation, elution was started and the resin washed with an additional 400 μL volume of buffer A. The UV absorbance of the total volume eluted (2×400 μL) was measured at 276 nm (thus giving the quantity of 3Fn8-11 eluted; a molar absorptivity of 45,800 L mol^{-1} cm^{-1} was used[15]), as well as at 320 nm (no correction for scattering effects was necessary). Competition profiles included 10–12 points each and were analyzed by a computer-assisted program (PRISM, San Diego, CA, USA) with $K_d(Fn)=2.5\times10^{-7}$ M, $n=1$, $K_d(RGDS)=1.7\times10^{-5}$ M, $n=2$ (thermodynamic data with n being the stoichiometric ratios: Banères et al., not yet published).
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