Multiple N-Methylation by a Designed Approach Enhances Receptor Selectivity

Jayanta Chatterjee,[†] Oded Ovadia,[⊥] Grit Zahn,[‡] Luciana Marinelli,[#] Amnon Hoffman,[⊥] Chaim Gilon,[£] and Horst Kessler*[,†]

Center for Integrated Protein Science at the Department Chemie, Lehrstuhl II für Organische Chemie, Technische Universität München, Lichtenbergstrasse 4, Garching D85747, Germany, Pharmaceutics and Organic Chemistry Departments, The Hebrew University of Jerusalem, Jerusalem 91904, Israel, Jerini A.G., Invalidenstrasse 130, 10115 Berlin, Germany, and Farmaceutica e Tossicologica, Università di Napoli 'Federico II', Via D. Montesano, 49-80131 Napoli, Italy

Received August 22, 2007

Abstract: An unselective cyclic peptide integrin ligand was sequentially N-methylated by a designed approach, where only the externally oriented (solvent exposed) amide bonds were N-methylated. The N-methylation resulted in tremendous enhancement in selectivity among the different integrin receptor subtypes (α5β1, αvβ3, and αIIbβ3). Conformational and docking studies were performed, which suggested that the receptor selectivity is principally caused by reduced backbone flexibility due to N-methylation.

Integrins are heterodimeric proteins that are important for cell−cell and cell−extracellular matrix interactions and are composed of α and β subunits.[1,2] Integrins serve as transmembrane linkers between their extracellular ligands and the cytoskeleton and modulate various signaling pathways; thus, they have the capacity to influence cell migration, differentiation, and survival during embryogenesis, angiogenesis, wound healing, immune and nonimmune defense mechanisms, hemostasis, and oncogenic transformation.[1] The combination of different α and β subunits determines the ligand specificity and therefore the biological function of the integrins. Many integrins are linked with pathological conditions; therefore, the specific inhibition of a certain integrin is a very promising tool for effective therapeutic intervention with limited side effects.

The αIIbβ3 is the most abundant integrin on the surface of platelets, which mediates aggregation of platelets to form thrombi. The activated αIIbβ3 in the final step of blood clot formation binds to blood glycoprotein fibrinogen to cross-link platelets in a growing thrombus. Thus, compounds that compete with fibrinogen in binding to αIIbβ3 can act as potent antithrombotic agents.[3] The peptidomimetic "Tirofiban" is already on the market for this purpose.[4,5] The vitronectin receptor αvβ3, which also recognizes other extracellular matrix proteins like fibronectin and osteopontin, is expressed on endothelial cells, osteoclasts, and tumor cells and is involved in processes of angiogenesis and bone remodeling.[6−8] A third important integrin which recognizes the RGD[a] tripeptide sequence is the fibronectin receptor α5β1, which is expressed on activated endothelial cells, immune cells, and fibroblasts.[9,10] It plays a key role during development of the vascular system and pathological angiogenesis, such as that in solid tumors.[11] We show here that a systematic multiple *N*-methyl scan can be employed to achieve surprisingly enhanced receptor selectivity between these receptor subtypes.

N-Methylation is a precious tool to modify lipophilicity,[12] proteolytic stability,[13] and bioavailability[3] and to induce conformational rigidity to the peptide backbone.[14] Mono-N-methylation of peptide ligands has been employed over the years to enhance potency,[15] new receptor subtype selectivity,[16] and tuning of an agonist to antagonist.[17] However, multiple N-methylation has been seldom employed,[18] probably owing to the availability of N-methylated amino acids, subsequent difficult coupling,[19] and unpredictable conformational change.[14,20]

In this work, we envisioned a "design approach" instead of the commonly used "library approach". For this purpose, the prerequisite is the knowledge of the bioactive conformation of the stem peptide (lead structure). Due to numerous studies of substituted cyclic penta- and hexapeptides containing the tripeptide sequence RGD, those peptides were chosen for this approach. We used the cyclic hexapeptide *cyclo* (-$G^1R^2G^3D^4f^5L^6$-), for which 63 different N-methylated analogues are possible (Figure 1A).[21] However, we synthesized a small library of seven derivatives in which only the externally oriented (solvent exposed) amide protons were N-methylated. This should lead to structures in which the overall conformation is only slightly modified, retaining at least some activity, but in addition, it may positively influence permeability when orally administered. So far, there is no orally available integrin antagonist in use; however, there are some compounds for αvβ3 reported by the Merck group which are orally available, but not yet as drugs on the market.[22]

The cyclic peptides merely act as a scaffold to hold the side chains in proper spatial orientation. Detailed study of the impact of N-methylation on cyclic pentapeptides[14] and hexapeptides (unpublished results) suggested to us that N-methylation of the externally oriented solvent-exposed amide protons may not drastically change the backbone peptide conformation (e.g., trans−cis peptide bond interconversion); however, it helps in rigidifying the backbone conformation by (i) restricting the peptide bond flip (180° rotation of the peptide bond about the adjacent C^{α}'s)[20,23] due to steric hindrance of the *N*-methyl group and (ii) ruling out the possibility of conformational equilibrium between interchangeable turn structures, for example, βII′ to γ and vice versa.[24] The reason for choosing the cyclic hexapeptide rather than the pentapeptide is the "rigidity", as cyclic hexapeptides unlike cyclic pentapeptides usually exhibit a conformation with two internally oriented peptide bridges (often two β turns). Cyclic hexapeptides correspond to cyclohexanes (see discussion in Heller et al.)[25] and prefer to adopt a chairlike conformation.[26]

The cyclic peptide *cyclo*(-$G^1R^2G^3D^4f^5L^6$-) (**1**), which was reported by Pfaff et al.[27] to be selective toward αIIbβ3 compared to αvβ3, reveals a βII′ turn about D-Phe−Leu and a βII turn about Arg−Gly,[21] which is the recognition motif, with two internal hydrogen bonds between $Asp^4CO-HNGly^1$ and $Gly^1-CO-HNAsp^4$. This stem peptide *cyclo*(-GRGDfL-) has some flexibility (due to the two Gly residues) but allows investigation of the effect of N-methylation on the backbone to obtain highly active and selective αIIbβ3[28] integrin antagonist.[29] We describe

* To whom correspondence should be addressed. E-mail: Kessler@ch.tum.de. Tel: 498928913300. Fax: 498928913210.
[†] Technische Universität München.
[⊥] Pharmaceutics Department, The Hebrew University of Jerusalem.
[£] Organic Chemistry Department, The Hebrew University of Jerusalem.
[‡] Jerini A.G..
[#] Università di Napoli 'Federico II'.

[a] Abbreviations: RGD, arginine glycine aspartic acid; HATU, *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridine-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; Caco-2, colon carcinoma cell monolayer; PAMPA, parallel artificial membrane permeation assay; Papp, apparent permeability.

10.1021/jm701044r CCC: $37.00
Published on Web 11/01/2007

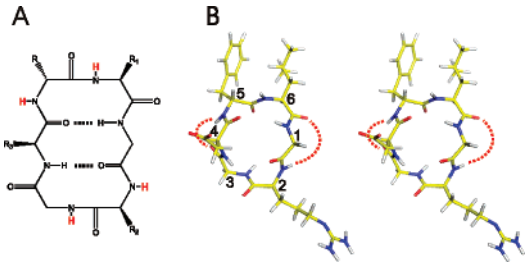

Figure 1. (A) Lead structure (**1**) with two β turns and the solvent-exposed amide protons (in red). (B) Stereoview of *cyclo*(-GRGDfL-) (**1**). Note the stabilizing γ turns about aspartic acid and glycine 1, presenting the peptide in a "folded conformation".

Table 1. The Seven N-Methylated Cyclic Analogues with the Stem Peptide *cyclo*(-G^{1}R^{2}G^{3}D^{4}f^{5}L^6-) and Their Binding Affinity (IC_{50} in nM) toward Three Different Integrins; the N-Methylated Residues Are Highlighted in Bold

no.	analogue	$\alpha_5\beta_1$	$\alpha_v\beta_3$	$\alpha_{IIb}\beta_3$	$\alpha_v\beta_3/\alpha_{IIb}\beta_3$
1	c(-GRGDfL-)	740	100	195	0.5
2	c(-GRGDf**L**-)	3900	103	560	0.2
3	c(-GRGD**f**L-)	4300	490	2000	0.2
4	c(-G**R**GDfL-)	1200	770	12	64
5	c(-GRGD**f**L-)	>20000	1200	620	2
6	c(-G**R**GDf**L**-)	~20000	1300	15	86
7	c(-G**R**GD**f**L-)	>20000	2730	165	16
8	c(-G**R**GD**f**L-)	>20000	12200	30	406

here a biased small library in which all externally oriented amide bonds except Gly^3, which is involved in the receptor binding,[30] were N-methylated (Table 1).

The linear peptides were obtained by standard solid-phase techniques, with N-methylation either in solution[31] or on a solid support,[32] and finally, the cyclization was carried out in solution using HATU/HOBt.[33]

It is worth noting that, despite our previous report, we found no receptor selectivity for **1**, and there was an inclination toward $\alpha v\beta 3$ selectivity with N-methylated leucine (**2**) or D-phenylalanine (**3**). The different IC_{50} values and, consequently, the different receptor selectivity can be explained by other experimental conditions used in this work compared to the work by Pfaff and colleagues.[27] The main difference is the composition of used integrins and thus the applied protein concentration, which causes other IC_{50} values (details in Supporting Information).

Significant selectivity was first obtained in **4** with the N-methylated arginine residue, which corroborates with previous results.[34] Extending further the N-methylation of **4** to leucine, there was almost no loss in the activity in **6** but a further gain in selectivity. Exchanging the site of N-methylation from leucine to phenylalanine in **7**, there was a sudden loss in the activity. However, interestingly, the activity was gained back with an additional N-methylation of **7**, giving rise to **8**, with a tremendous enhancement in the selectivity and still high activity for $\alpha IIb\beta 3$. It is really surprising that a single *N*-methyl group when present at phenylalanine is responsible for the loss of activity in **7** and the gaining back the activity and enormous selectivity when present at leucine in **8**.

These results prompted us to study the solution conformation of these analogues. It is well-known that the selectivity between $\alpha v\beta 3$ and $\alpha IIb\beta 3$ can be achieved by fine-tuning the distance between the carboxyl group of aspartic acid and the guanidine group of arginine in the ligands.[35] In our case, the selectivity first arose by N-methylation of the arginine residue (**4**), which was primarily owing to the reduction in the flexibility about arginine and glycine 3 (which resembles a βI′ turn), presenting

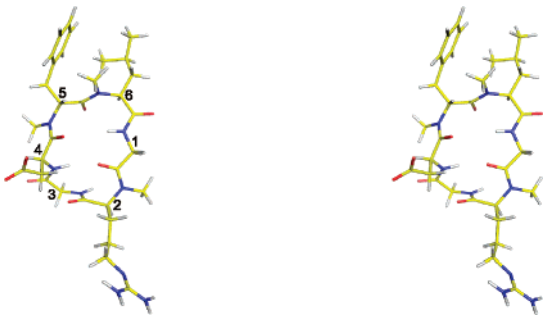

Figure 2. Stereoview of *cyclo*(-G**R**GD**f**L-) (**8**). The stabilizing γ turns about aspartic acid and glycine 1 are absent, resulting in a flat structure. Note the βI′ turn about Arg and Gly.[3]

the peptidic backbone in an extended orientation. It is to be noted that the side chain of arginine is very flexible and could fit into $\alpha v\beta 3$ and $\alpha IIb\beta 3$; thus, the main selectivity was brought about by the rigidity in the scaffold by N-methylation.

In the stem peptide **1** or when leucine (**2**) and/or phenylalanine (**3**) were N-methylated, there was considerable flexibility in the βII turn, resulting in unspecific binding. In all of the peptides lacking the N-methylated arginine residue, the NMR and the MD showed the possibility of forming a γ turn about the glycine in position 3, which ultimately brought the side chains of the aspartic acid and arginine close to each other, resulting in a comparatively better fit into the $\alpha v\beta 3$ binding pocket. In addition to the γ turn, we observed a "kink" in the backbone conformations of **1** and **2**, giving rise to a "folded" structure,[36] resulting in two further γ turns about Asp and Gly^1 (Figure 1B).

This kinked conformation is probably favored for $\alpha v\beta 3$ as both **1** and **2** bind better to $\alpha v\beta 3$ than to $\alpha IIb\beta 3$. This kink was lost by N-methylation of D-Phe and/or Arg, which blocked the γ turns and presented the peptide in a flattened conformation. In the case of **8**, there was no indication of any γ turn about Gly^3, and the peptide was in a flat conformation (Figure 2), which eventually resulted in holding the aspartic acid and arginine side chains apart, fitting well into the $\alpha IIb\beta 3$ pocket.

The conformation of peptide **8** is very similar to that of **4**; the only difference is the slight clockwise rotation of the phenyl ring and counterclockwise rotation of the isopropyl group of the leucine side chain (as suggested by the ROE's). In addition, we observed a close resemblance to a βI′ turn about Arg and Gly^3 in **8** owing to the reduced flexibility in this region (due to the *N*-Me-D-Phe), and thus, there is no indication of the formation of γ turn about Gly^3, whereas the βI′ turn in **4** was flexible, and there was a close resemblance to a γ turn about Gly^3 (Figure 3). This is probably one of the reasons for low binding of **8** to $\alpha v\beta 3$, in contrast to that for **4**.

To have an insight into the binding modes of **4** and **8** into $\alpha IIb\beta 3$,[37] docking studies were performed using the software Autodock. In both peptides, the carboxylate group of Asp was found to coordinate the metal ion at the MIDAS region, whereas the Arg side chain extended into the deep β-propeller pocket, forming a hydrogen bond to the αIIb-Asp224 carboxylate group. The main difference between the **4** and **8** binding modes comes from the upper part of the peptides in the region from D-Phe^5–Leu^6 (Figures 2 and 3). Due to multiple N-methylation introduced in **8**, and especially due to the N-methylation of D-Phe residue, **8**, when compared to **4**, seems to lower its $\pi-\pi$ interaction with β3-Tyr122 (shown by the yellow arrow in Figure 4) and did not properly orient the Leu carbonyl group to hydrogen bond with the R214 side chain (shown by the white arrow in Figure 4).

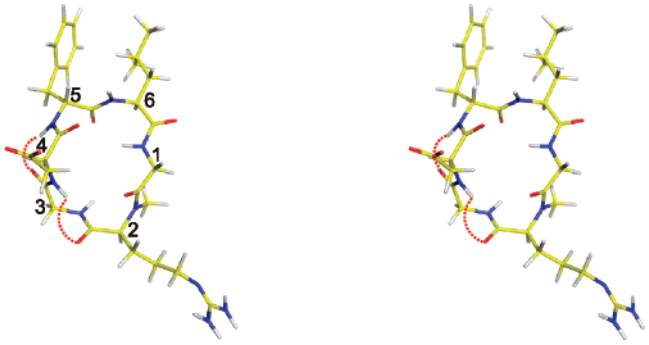

Figure 3. Stereopicture of *cyclo*(-G**R**GDfL-) (**4**). About Gly^3, the molecule has a tendency to adopt a γ turn (shown by the curved line), forming a hydrogen bond between AspNH and ArgCO. Also interesting to note is the kink in the peptide backbone about Asp. This kink is stabilized by formation of a closed γ turn involving the PheNH and Gly^3CO.

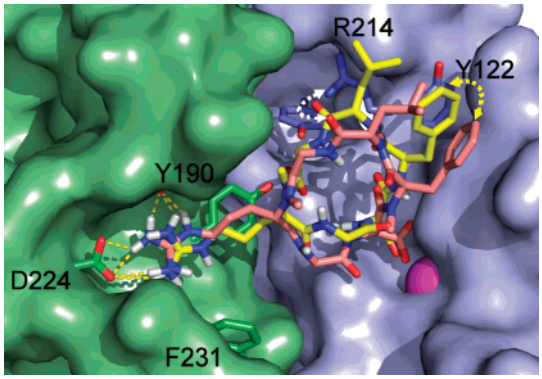

Figure 4. Docked **4** (yellow) and **8** (pink) in the αIIbβ3 integrin. The αIIb subunit of the receptor is represented by the green surface, while the β3 subunit is represented by the violet surface. In both subunits, important side chains are highlighted as sticks. The metal ion in the MIDAS region is represented by a magenta sphere. The loss of $\pi-\pi$ interaction of D-Phe residue of **8** with Tyr122 is shown by the yellow arrow, and the improper orientation of Leu CO of **8** to form a hydrogen bond with the Arg214 side chain is shown by the white arrow.

N-Methylation was suggested also to affect permeability characteristics of peptides.[38] Therefore, the permeability of the library was assessed by the Caco-2 model.[39] All of the analogues had lower permeability (Papp between 3.0×10^{-8} and 1.5×10^{-7} cm/sec) compared to that of mannitol (9.0×10^{-7} cm/sec) as the standard.

Since N-methylation affects the hydrophobicity of the molecules, we examined whether N-methylation could improve the permeation of the compounds through biological membranes via the transcellular pathway. For that, we used the PAMPA,[40] which is a non-cell-based in vitro assay system that evaluates passive transcellular permeation. To our surprise, none of the analogues penetrated across the lipid artificial membrane, suggesting that these peptides have poor intestinal permeability, which is limited exclusively to the paracellular pathway (via tight junctions), and N-methylation did not change or improve the transport in this series.

In conclusion, we demonstrate that a systematic multiple N-methylation by knowing the bioactive conformation of the stem peptide can be employed for enhancing receptor selectivity and activity of a moderately active ligand, though in this case, we failed to improve the bioavailability of the analogues. Using this conformational design approach, one can minimize the size of the library considerably. In this case, the selectivity of the analogue arises predominantly due to the reduced flexibility of the peptide. Multiple N-methylation results also in a better understanding of the bioactive conformation. Thus, multiple N-methylation of peptides could be a straightforward and simplistic approach to obtain highly potent and selective ligands.

Acknowledgment. We gratefully acknowledge the Humboldt Foundation for the support via the Max-Planck-Forschungspreis. This work was also, in part supported, by the German−Israel Foundation. We thank Thorsten Lanz for performing ELISA assays.

Supporting Information Available: NMR spectrum, HRMS, and detailed experimental and computational procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Hynes, R. O. Integrins: Bidirectional, allosteric signaling machines. *Cell* **2002**, *110*, 673−687.
(2) Humphries, J. D.; Byron, A.; Humphries, M. J. Integrin ligands at a glance. *J. Cell Sci.* **2006**, *119*, 3901−3903.
(3) Barker, P. L.; Bullens, S.; Bunting, S.; Burdick, D. J.; Chan, K. S.; Deisher, T.; Eigenbrot, C.; Gadek, T. R.; Gantzos, R.; Lipari, M. T.; Muir, C. D.; Napier, M. A.; Pitti, R. M.; Padua, A.; Quan, C.; Stanley, M.; Struble, M.; Tom, J. Y. K.; Burnier, J. P. Cyclic RGD peptide analogs as antiplatelet antithrombotics. *J. Med. Chem.* **1992**, *35*, 2040−2048.
(4) Gould, R. J.; Chang, C. T. C.; Lynch, R. J.; Manno, P. D.; Zhang, G. X.; Bednar, B.; Friedman, P. A.; Egbertson, M.; Turchi, L. M.; Anderson, P. S.; Hartman, G. MK-0383 is a potent nonpeptide mimic of RGD that inhibits glycoprotein-IIB/IIIA. *Thromb. Haemostasis* **1993**, *69*, 976−976.
(5) Egbertson, M. S.; Chang, C. T. C.; Duggan, M. E.; Gould, R. J.; Halczenko, W.; Hartman, G. D.; Laswell, W. L.; Lynch, J. J.; Lynch, R. J.; Manno, P. D.; Naylor, A. M.; Prugh, J. D.; Ramjit, D. R.; Sitko, G. R.; Smith, R. S.; Turchi, L. M.; Zhang, G. X. Nonpeptide fibrinogen receptor antagonists. 2. Optimization of a tyrosine template as a mimic for Arg−Gly−Asp. *J. Med. Chem.* **1994**, *37*, 2537−2551.
(6) Horton, M. A.; Taylor, M. L.; Arnett, T. R.; Helfrich, M. H. Arg−Gly−Asp (RGD) peptides and the anti-vitronectin receptor antibody 23C6 inhibit dentin resorption and cell spreading by osteoclasts. *Exp. Cell Res.* **1991**, *195*, 368−375.
(7) Varner, J. A.; Brooks, P. C.; Cheresh, D. A. The integrin alpha(V)-beta(3): Angiogenesis and apoptosis. *Cell Adhes. Commun.* **1995**, *3*, 367−374.
(8) Meyer, A.; Auemheimer, J.; Modlinger, A.; Kessler, H. Targeting RGD recognizing integrins: Drug development, biomaterial research, tumor imaging and targeting. *Curr. Pharm. Des.* **2006**, *12*, 2723−2747.
(9) Collo, G.; Pepper, M. S. Endothelial cell integrin alpha 5 beta 1 expression is modulated by cytokines and during migration in vitro. *J. Cell Sci.* **1999**, *112*, 569−578.
(10) Burns, J. A.; Issekutz, T. B.; Yagita, H.; Issekutz, A. C. The alpha-(4)beta(1) (very late antigen (VLA)-4, CD49d/CD29) and alpha(5)-beta(1) (VLA-5, CD49e/CD29) integrins mediate beta(2) (CD11/CD18) integrin-independent neutrophil recruitment to endotoxin-induced lung inflammation. *J. Immunol.* **2001**, *166*, 4644−4649.
(11) Kim, S.; Bell, K.; Mousa, S. A.; Varner, J. A. Regulation of angiogenesis in vivo by ligation of integrin alpha 5 beta 1 with the central cell-binding domain of fibronectin. *Am. J. Pathol.* **2000**, *156*, 1345−1362.
(12) Fairlie, D. P.; Abbenante, G.; March, D. R. Macrocyclic peptidomimetics—forcing peptides into bioactive conformations. *Curr. Med. Chem.* **1995**, *2*, 654−686.
(13) Cody, W. L.; He, J. X.; Reily, M. D.; Haleen, S. J.; Walker, D. M.; Reyner, E. L.; Stewart, B. H.; Doherty, A. M. Design of a potent combined pseudopeptide endothelin-A/endothelin-B receptor antagonist, Ac-DBhg(16)−Leu−Asp−Ile-[*N*Me]Ile−Trp(21) (PD 156252): Examination of its pharmacokinetic and spectral properties. *J. Med. Chem.* **1997**, *40*, 2228−2240.
(14) Chatterjee, J.; Mierke, D.; Kessler, H. N-Methylated cyclic pentaalanine peptides as template structures. *J. Am. Chem. Soc.* **2006**, *128*, 15164−15172.
(15) Dechantsreiter, M. A.; Planker, E.; Mathä, B.; Lohof, E.; Hölzemann, G.; Jonczyk, A.; Goodman, S. L.; Kessler, H. N-Methylated cyclic RGD peptides as highly active and selective alpha(v)beta(3) integrin antagonists. *J. Med. Chem.* **1999**, *42*, 3033−3040.
(16) Rajeswaran, W. G.; Hocart, S. J.; Murphy, W. A.; Taylor, J. E.; Coy, D. H. Highly potent and subtype selective ligands derived by N-methyl scan of a somatostatin antagonist. *J. Med. Chem.* **2001**, *44*, 1305−1311.

(17) Laufer, R.; Wormser, U.; Friedman, Z. Y.; Gilon, C.; Chorev, M.; Selinger, Z. Neurokinin-B is a preferred agonist for a neuronal substance-P receptor and its action is antagonized by enkephalin. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 7444−7448.
(18) Holladay, M. W.; Kopecka, H.; Miller, T. R.; Bednarz, L.; Nikkel, A. L.; Bianchi, B. R.; Witte, D. G.; Shiosaki, K.; Lin, C. W.; Asin, K. E.; Nadzan, A. M. Tetrapeptide CCK-A agonists−effect of backbone N-methylations on in-vitro and in-vivo CCK activity. *J. Med. Chem.* **1994**, *37*, 630−635.
(19) Teixido, M.; Albericio, F.; Giralt, E. Solid-phase synthesis and characterization of N-methyl-rich peptides. *J. Pept. Res.* **2005**, *65*, 153−166.
(20) Chatterjee, J.; Mierke, D. F.; Kessler, H. Conformational preference and potential templates of N-methylated cyclic pentaalanine peptides. *Chem.−Eur. J.* **2007**, in press.
(21) Gurrath, M.; Müller, G.; Kessler, H.; Aumailley, M.; Timpl, R. Conformation activity studies of rationally designed potent antiadhesive RGD peptides. *Eur. J. Biochem.* **1992**, *210*, 911−921.
(22) Coleman, P. J.; Brashear, K. M.; Askew, B. C.; Hutchinson, J. H.; McVean, C. A.; Duong, L. T.; Feuston, B. P.; Fernandez-Metzler, C.; Gentile, M. A.; Hartman, G. D.; Kimmel, D. B.; Leu, C. T.; Lipfert, L.; Merkle, K.; Pennypacker, B.; Prueksaritanont, T.; Rodan, G. A.; Wesolowski, G. A.; Rodan, S. B.; Duggan, M. E. Nonpeptide alpha(v)beta(3) antagonists. Part 11: Discovery and preclinical evaluation of potent alpha v beta(3) antagonists for the prevention and treatment of osteoporosis. *J. Med. Chem.* **2004**, *47*, 4829−4837.
(23) Mierke, D. F.; Kurz, M.; Kessler, H. Peptide flexibility and calculations of an ensemble of molecules. *J. Am. Chem. Soc.* **1994**, *116*, 1042−1049.
(24) Kessler, H. Peptide Conformations. 19. Conformation and biological-activity of cyclic-peptides. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 512−523.
(25) Heller, M.; Sukopp, M.; Tsomaia, N.; John, M.; Mierke, D. F.; Reif, B.; Kessler, H. The conformation of *cyclo*(-D-Pro-Ala(4)-) as a model for cyclic pentapeptides of the DL4 type. *J. Am. Chem. Soc.* **2006**, *128*, 13806−13814.
(26) Kessler, H.; Gratias, R.; Hessler, G.; Gurrath, M.; Müller, G. Conformation of cyclic peptides. Principle concepts and the design of selectivity and superactivity in bioactive sequences by "spatial screening". *Pure Appl. Chem.* **1996**, *68*, 1201−1205.
(27) Pfaff, M.; Tangemann, K.; Müller, B.; Gurrath, M.; Müller, G.; Kessler, H.; Timpl, R.; Engel, J. Selective recognition of cyclic RGD peptides of NMR defined conformation by Alpha-II-Beta-3, Alpha-V-Beta-3, and Alpha-5-Beta-1 integrins. *J. Biol. Chem.* **1994**, *269*, 20233−20238.
(28) Phillips, D. R.; Charo, I. F.; Parise, L. V.; Fitzgerald, L. A. The platelet membrane glycoprotein-IIB-IIIA complex. *Blood* **1988**, *71*, 831−843.
(29) Hoekstra, W. J.; Maryanoff, B. E.; Damiano, B. P.; Andrade-Gordon, P.; Cohen, J. H.; Costanzo, M. J.; Haertlein, B. J.; Hecker, L. R.; Hulshizer, B. L.; Kauffman, J. A.; Keane, P.; McComsey, D. F.; Mitchell, J. A.; Scott, L.; Shah, R. D.; Yabut, S. C. Potent, orally active GPIIb/IIIa antagonists containing a nipecotic acid subunit. Structure−activity studies leading to the discovery of RWJ-53308. *J. Med. Chem.* **1999**, *42*, 5254−5265.
(30) Xiong, J. P.; Stehle, T.; Zhang, R. G.; Joachimiak, A.; Frech, M.; Goodman, S. L.; Arnaout, M. A. Crystal structure of the extracellular segment of integrin alpha V beta 3 in complex with an Arg−Gly−Asp ligand. *Science* **2002**, *296*, 151−155.
(31) Freidinger, R. M.; Hinkle, J. S.; Perlow, D. S.; Arison, B. H. Synthesis of 9-fluorenylmethyloxycarbonyl-protected *N*-alkyl amino-acids by reduction of oxazolidinones. *J. Org. Chem.* **1983**, *48*, 77−81.
(32) Biron, E.; Chatterjee, J.; Kessler, H. Optimized selective N-methylation of peptides on solid support. *J. Pept. Sci.* **2006**, *12*, 213−219.
(33) Carpino, L. A.; Imazumi, H.; El-Faham, A.; Ferrer, F. J.; Zhang, C. W.; Lee, Y. S.; Foxman, B. M.; Henklein, P.; Hanay, C.; Mugge, C.; Wenschuh, H.; Klose, K.; Beyermann, M.; Bienert, M. The uronium/guanidinium peptide coupling reagents: Finally the true uronium salts. *Angew. Chem., Int. Ed.* **2002**, *41*, 442−445.
(34) Bach, A. C.; Espina, J. R.; Jackson, S. A.; Stouten, P. F. W.; Duke, J. L.; Mousa, S. A.; DeGrado, W. F. Type II′ to type I beta-turn swap changes specificity for integrins. *J. Am. Chem. Soc.* **1996**, *118*, 293−294.
(35) Müller, G.; Gurrath, M.; Kessler, H. Pharmacophore refinement of GPIIB/IIIA antagonists based on comparative-studies of antiadhesive cyclic and acyclic RGD peptides. *J. Comput.-Aided Mol. Des.* **1994**, *8*, 709−730.
(36) He, Y. B.; Huang, Z. W.; Raynor, K.; Reisine, T.; Goodman, M. Syntheses and conformations of somatostatin-related cyclic hexapeptides incorporating specific alpha-methylated and beta-methylated residues. *J. Am. Chem. Soc.* **1993**, *115*, 8066−8072.
(37) Xiao, T.; Takagi, J.; Coller, B. S.; Wang, J. H.; Springer, T. A. Structural basis for allostery in integrins and binding to fibrinogen-mimetic therapeutics. *Nature* **2004**, *432*, 59−67.
(38) Conradi, R. A.; Hilgers, A. R.; Ho, N. F. H.; Burton, P. S. The influence of peptide structure on transport across Caco-2 Cells. 2. Peptide-bond modification which results in improved permeability. *Pharm. Res.* **1992**, *9*, 435−439.
(39) Artursson, P.; Karlsson, J. Correlation between oral-drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells. *Biochem. Biophys. Res. Commun.* **1991**, *175*, 880−885.
(40) Kansy, M.; Senner, F.; Gubernator, K. Physicochemical high throughput screening: Parallel artificial membrane permeation assay in the description of passive absorption processes. *J. Med. Chem.* **1998**, *41*, 1007−1010.

JM701044R