Pergamon

Tetrahedron Letters 42 (2001) 7387–7391

TETRAHEDRON LETTERS

Solid-phase syntheses of constrained RGD scaffolds and their binding to the $\alpha_v\beta_3$ integrin receptor

Miriam Royo,[a,*] Wim Van Den Nest,[a] Montserrat del Fresno,[a] Ariadna Frieden,[a] Dror Yahalom,[b] Michael Rosenblatt,[b] Michael Chorev[b] and Fernando Albericio[a,*]

[a]*Departament de Química Orgànica, Universitat de Barcelona, E-08028 Barcelona, Spain*
[b]*Bone and Mineral Metabolism Unit, Charles A. Dana Thorndike Laboratories, Deparment of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02215, USA*

Received 20 July 2001; revised 17 August 2001; accepted 18 August 2001

Abstract—We report the solid-phase synthesis of two different families, containing either diketopiperazine or hydantoin structures, of rigidified RGD mimetics and their binding affinity to the $\alpha_v\beta_3$ receptor.

The important advances in molecular biology and gene technology are bringing important changes in biomedical sciences. Thus, the elucidation of the human genome followed by advancements in functional genomics and proteomics will revolutionize our understanding of the detailed molecular mechanisms underlying a broad spectrum of diseases. These developments will also identify new therapeutic targets and suggest novel mechanism-based therapeutic paradigms. To respond to these challenges we need to develop efficient, automatic, and rapid screening test systems ['high-throughput screening' (HTS)] in order to identify lead compounds with interesting biological activities. HTS systems require a large number of structurally diverse substances, which are being synthesized simultaneously or in parallel mode ['high-throughput organic synthesis' (HTOS)].[1] Therefore, there is a need for the development of efficient synthetic methods that can be applied in HTOS mode. Although HTOS may be conducted in solution, the solid-phase mode is very often the method of choice.[2] Herein, we report on the efficient solid-phase syntheses of constrained peptides containing the Arg-Gly-Asp (RGD) motif, as well as their binding affinity to the $\alpha_v\beta_3$ integrin receptor.

The family of integrin receptor participates in important cell–cell and cell–extracellular matrix interactions in many normal biological and pathophysiological processes.[3] These heterodimeric cell surface receptors are composed of α and β subunits. Currently, there are 15 α subunits and eight β subunits known. The $\alpha_v\beta_3$ integrin receptor is expressed in various cell types such as endothelial cells, melanoma, osteoclast and smooth muscle cells and plays an important role in angiogenesis and tumor cell migration.[4] The $\alpha_v\beta_3$ is the predominant integrin receptor expressed in osteoclasts, mediates its attachment to bone mineral matrix and plays a critical role in bone remodeling.[5] Inhibition of osteoclast binding to the bone mineral matrix by $\alpha_v\beta_3$ antagonists blocks osteoclast-mediated bone resorption.[5a–c,6] Thus establishing a proof-of-concept for and providing a novel paradigm for a mechanism-based therapy of osteoporosis.[7]

Many integrins, including $\alpha_v\beta_3$, are inhibited by small RGD-containing peptides, an ubiquitous receptor recognition motif.[3a,4a,8] Nevertheless, the adjacent amino acid residues flanking the RGD triad and the specific topology of the RGD side chains contribute to

Abbreviations: BAL, backbone amide linker; Bu, butyl; DBU, diazabicyclo[5.4.0]undec-7-ene; DIEA, *N*,*N*-diisopropylethylamine; DKP, diketopiperazine (2,5-piperazinedione); DMAP, 4-*N*,*N*-dimethylaminopiridine; DMF, *N*,*N*-dimethylformamide; Fmoc, 9-fluorenylmethoxycarbonyl; DSC, *N*,*N*′-disuccinimidyl carbonate; HATU, *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HBTU, *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HPLC, high-performance liquid chromatography; HTOS, high-throughput organic synthesis; HTS, high-throughput screening; Hyd, hydantoin; Mtt, 4-methyltrityl; MALDI-TOF, matrix-assisted laser desorption ionization time of flight; PAL, peptide amide linker; Pmc, 2,2,5,7,8-pentamethylchroman-6-sulfonyl; PyAOP, 7-azabenzotriazol-1-yl-oxytris(pyrrolidino)-phosphonium hexafluorophosphate; Reagent R, TFA–thioanisole–1,2-ethanedithiol–anisole (90:5:3:2); TFA, trifluoroacetic acid; amino acid symbols denote L-configuration unless otherwise noted.

* Corresponding author.

0040-4039/01/$ - see front matter
PII: S0040-4039(01)01510-6

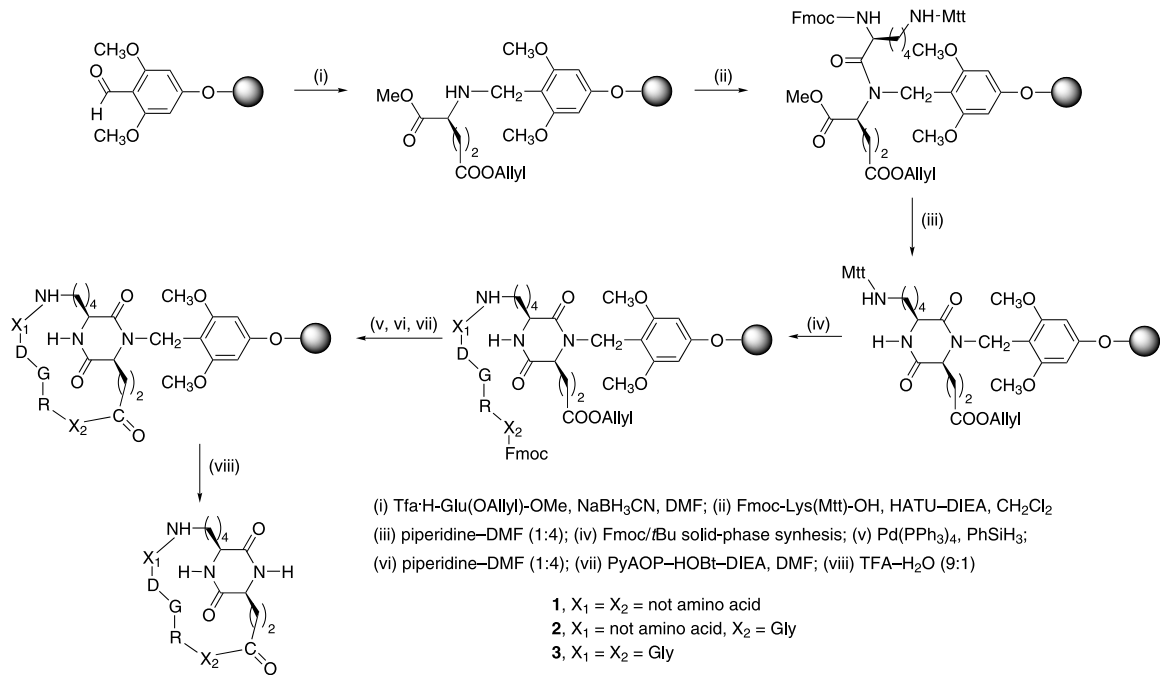
the specificity toward integrin receptor subtypes. Introduction of conformational constraints into the ligand may preferentially bias it to adopt a bioactive conformation specific to a particular integrin receptor subtype. Indeed, conformationally constrained RGD analogues, such as the cyclic(RGDfX) peptides and related *N*-methylated analogues, with high activity and selectivity toward $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin receptor subtypes, were reported.[9,10]

Herein, we report the development of efficient solid-phase-based synthetic methods to prepare RGD ligands with a greater degree of structural constraint than the one found in the previously reported 'head-to-tail' cyclic peptides. Initially, we synthesized a series of cyclic RGD peptides attached through the side chains of a [K-D]diketopiperazine (DKP), used as a relatively rigid scaffold.[11] The second series represents backbone rigidification introduced via an end-group-to-backbone cyclization in the form of an RG-derived hydantoin.[12]

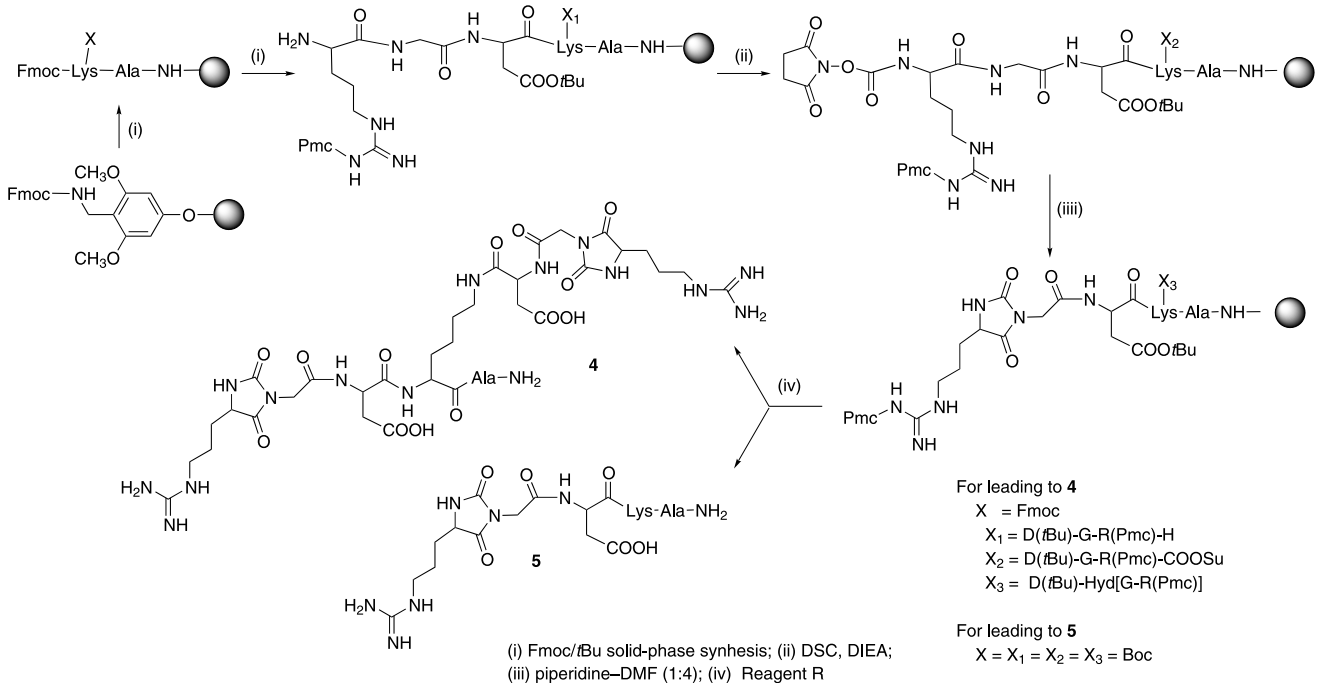
The DKP scaffold was generated by cyclization of on resin-bound orthogonally side chain protected dipeptide,[13] H-Lys(N^{ε}-Mtt)-Asp(βAllyl)-OMe, attached through the peptide bond nitrogen to a Backbone Amide Linker (BAL) resin[14] as outlined in Scheme 1. The synthetic steps included: (i) anchoring the H-Asp(βOAllyl)-OMe·HCl to the resin by reductive alkylation of the α-amino group using $NaBH_3CN$ in DMF; (ii) HATU/DIEA-mediated acylation of the secondary α-amino group, attached to the BAL-resin, with Fmoc-Lys(N^{ε}-Mtt)-OH in CH_2Cl_2–DMF (9:1); (iii) deprotection of the Fmoc-dipeptidyl BAL-resin with piperidine–DMF (1:4), which was followed by a spontaneous cyclization leading to essentially quantitative formation of [K(N^{ε}-Mtt)-D(βOAllyl)]DKP-BAL-resin; (iv) selective removal of the Mtt protecting group from the ε-amino function of the Lys with TFA–CH_2Cl_2 (1:99) and elongation of the peptidic chain employing the Fmoc/*t*Bu strategy; (v) removal of the allyl ester protecting group from the β-carboxyl function of Asp with $Pd(PPh_3)_4$ in the presence of $PhSiH_3$; (vi) removal of the Fmoc group from the N-terminal α-amino acid of the peptidic chain;[15] (vii) on-resin cyclization using PyAOP;[16] and (viii) final deprotection and cleavage of the bicyclic peptide from the resin, and purification.[17] Following this scheme we prepared three compounds *c*RGD-[K-D]DKP (**1**), *c*GRGD-[K-D]DKP (**2**), and *c*GRGDG-[K-D]DKP (**3**), which include increasing number of Gly residues within the sequence substituting the DKP scaffold. The additional glycines were introduced in order to study the effect of flexibility of the RGD-containing loop on the $\alpha_v\beta_3$ receptor binding affinity.

The hydantoin family[18] was prepared as outlined in Scheme 2 by: (i) elongation of the peptidic chain on a peptide amide linker (PAL) resin[19] using a Fmoc/*t*Bu strategy; (ii) reaction of the α-amino function of arginine with DSC in the presence of DMAP; (iii) treatment with piperidine–DMF (1:4);[20] and (iv) final deprotection and cleavage of the peptide from the resin, and purification (Fig. 1).[17]

One of the goals of the study was the preparation of a model product with two copies of the hydantoin-RGD in order to study the possibility of using this synthetic strategy for obtaining multiple copies of hydantoin-RGD ligands. Thus, for the preparation of compound **4**, containing two copies of hydantoin-RGD we coupled Fmoc-Lys(N^{ε}-Fmoc)-OH to the Ala-PAL resin, and for the preparation of compound **5**, the monomeric hydantoin-RGD, we used Fmoc-Lys(N^{ε}-Boc)-OH.



Scheme 1. Solid-phase synthesis of RGD containing DKP's.



Scheme 2. Solid-phase synthesis of RGD containing hydantoins.

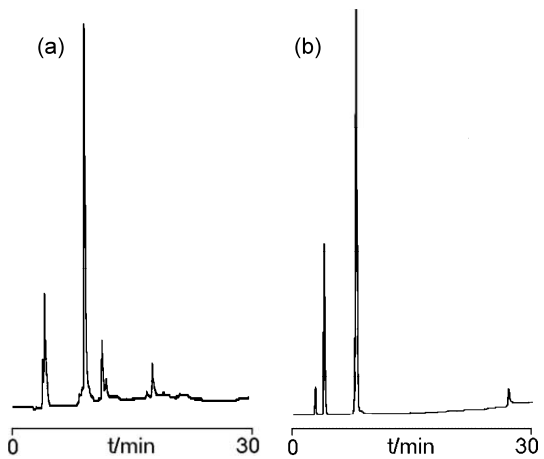


Figure 1. HPLC chromatograms of **5** as representative example of the series; (a) crude product; (b) after reverse-phase purification. Analytical conditions: reverse-phase C_{18} column; elution by a linear gradient over 30 min of 0.036% TFA in ACN and 0.045% TFA in H_2O from 0:1 to 1:0, flow rate 1.0 mL min^{-1}, UV detection at 220 nm.

The affinity of the five RGD containing ligands towards the $\alpha_v\beta_3$ receptor was measured in a radioreceptor binding assay by competition with [^{125}I]echistatin, according to a previously published protocol.[21] Results shown in Table 1 indicate that in the case of DKP analogues **1**–**3**, the binding affinity to the $\alpha_v\beta_3$ receptor is affected by the size of the RGD-containing ring. Thus, compound **1**, where the loop contains just the sequence RGD, shows the lowest IC_{50}, which corresponds to the highest binding affinity. These results correspond very well with previous reports associating higher conformational constraint of the RGD motif with higher affinity to the $\alpha_v\beta_3$ receptor.[9] The results obtained for the hydantoin-based analogues show higher binding affinity for compound **4**, the one presenting two copies of the hydantoin-RGD structure, than for compound **5**, containing only one copy. The bivalent nature of analogue **4** may partially explain its enhanced potency as compared to the monovalent analogue **5**.[22]

Table 1. $\alpha_v\beta_3$ Competitive binding assay results

Compound	Displacement (%)[a]		IC_{50} (μM)[b]
	10^{-6} M	10^{-5} M	
1	40	78	4 ± 1
2	23	63	8 ± 2
3	10	34	nd
4	43	80	3 ± 1
5	15	40	nd

[a] The two concentrations were tested in two independent experiments. Each value is mean of a duplicate, with less than 10% difference within the duplicate.

[b] Values are means of two experiments, each performed at six concentration points (in duplicates) for each compound; nd, not determined.

In conclusion, we report the development of two efficient solid-phase methods for the synthesis of constrained RGD containing ligands that suggest novel and interesting leads for constructing potent $\alpha_v\beta_3$ receptor antagonists. The binding affinities observed for both DKP- and hydantoin-based RGD-containing compounds are very encouraging. We therefore plan the development of a solid-phase based combinatorial approach to generate DKP- and hydantoin-based

RGD-containing libraries that will extend and elaborate the principles demonstrated in this study.

Acknowledgements

This work was partially supported by CICYT (BQU2000-0235), Generalitat de Catalunya [Grup Consolidat, and Centre de Referència en Biotecnologia], and Grant AR42833 from the National Institute for Arthritis and Musculoskeletal Diseases, National Institutes of Health.

References

1. (a) *Combinatorial Peptide and Non-peptide Libraries*; Jung, G., Ed.; VCH: Weinheim, 1996; (b) *Combinatorial Libraries. Synthesis, Screening and Application Potential*; Cortese, R., Ed.; Walter de Gruyter & Co.: Berlin, 1996; (c) *Molecular Diversity and Combinatorial Chemistry. Libraries and Drug Discovery*; Chaiken, I. M.; Janda, K. D., Eds.; ACS Books: Washington, 1996; (d) *Combinatorial Chemistry: Synthesis and Applications*; Wilson, S. H.; Czarnik, A. W., Eds.; Wiley & Sons: New York, 1997; (e) *A Practical Guide to Combinatorial Chemistry*; Czarnik, A. W.; DeWitt, S. H., Eds.; ACS Books: Washington, 1997; (f) *Solid-Supported Combinatorial and Parallel Synthesis of Small-Molecular-Weight Compound Libraries*; Obrecht, D.; Villalgordo, J. M., Eds.; Pergamon: Oxford, 1998; (g) *Combinatorial Chemistry: A Practical Approach*; Bannwarth, W.; Felder, E., Eds.; Wiley-VCH: Weinheim, 2000; (h) Seneci, P. *Solid-Phase Synthesis and Combinatorial Technologies*; John Wiley & Sons: New York, 2001.
2. (a) Merrifield, R. B. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 799–810; (b) Fields, G. B.; Tian, Z.; Barany, G. In *Synthetic Peptides. A Users Guide*; Grant, G. A., Ed.; W. H. Freeman: New York, 1992; pp. 77–183; (c) Blackburn, G. M.; Gait, M. J. In *Nucleic Acids in Chemistry and Biology*, 2nd ed.; Oxford University Press: Oxford, 1996; (d) Lloyd-Williams, P.; Albericio, F.; Giralt, E. In *Chemical Approaches to the Synthesis of Peptides and Proteins*; CRC: Boca Raton, FL, 1997; (e) Kates, S. A.; Albericio, F. In *Practical Solid-Phase Synthesis*: *A Book Companion*; Marcel Dekker: New York, 2000.
3. (a) Rouslahti, E.; Pierschbacher, M. D. *Science* **1987**, *238*, 491–497; (b) Hynes, R. O. *Cell* **1992**, *69*, 11–25.
4. (a) Brooks, P. C.; Clark, C. F.; Cheresh, D. A. *Science* **1994**, *264*, 569–571; (b) Brooks, P. C.; Montgomety, A. M. P.; Rosenfeld, M.; Reisfeld, R. A.; Hu, T.; Klier, G.; Cheresh, D. A. *Cell* **1994**, *79*, 1157–1164; (c) Mousa, S. A.; Cheresh, D. A. *Drug Discov. Today* **1997**, *2*, 187–199.
5. (a) Sato, M.; Sardana, M. K.; Grasser, W. A.; Garsky, V. M.; Murray, J. M.; Gould, R. J. *J. Cell. Biol.* **1990**, *111*, 1713–1723; (b) Fisher, J. E.; Caulfield, M. P.; Sato, M.; Quartuccio, H. A.; Gould, R. J.; Garsky, V. M.; Rodan, G. A.; Rosenblatt, M. *Endocrinology* **1993**, *132*, 1411–1413; (c) Rodan, S. B.; Rodan, G. A. *J. Endocrinol.* **1997**, *10*, 456.
6. (a) Fisher, J. E.; Caulfield, M.; Sato, M. *Endocrinoogy* **1993**, *132*, 1411–1413; (b) Masarachia, P.; Yamamoto, M.; Leu, C. T.; Rodan, G.; Duong, L. *Endocrinology* **1998**, *139*, 1401–1410; (c) Yamamoto, M.; Fischer, J. E.; Gentile, M.; Leu, C. T.; Rodan, S. B.; Rodan, G. A. *Endocrinology* **1998**, *139*, 1411–1419.
7. For a review on the role of $\alpha_v\beta_3$ in osteoporosis, see: Robey, P. G. *Ann. Rep. Med. Chem.* **1993**, *28*, 227–236.
8. Cox, D.; Aoki, T.; Seri, J.; Motoyama, Y.; Yoshida, K. *Med. Res. Rev.* **1994**, *14*, 194–195.
9. (a) Peishoff, C. E.; Ali, F. E.; Bean, J. W.; Calvo, R.; D'Ambrosio, C. A.; Eggleston, D. S.; Hwang, S. M.; Kline, T. P.; Koster, P. F.; Nichols, A.; Powers, D.; Romoff, T.; Samanen, J. M.; Stadel, J.; Vasko, J.; Kopple, K. D. *J. Med. Chem.* **1992**, *35*, 3962–3969; (b) Bach, A. C.; Espina, J. R.; Jackson, S. A.; Stouten, P. F. W.; Dure, J. L.; Mousa, S. A.; DeGrado, W. F. J. *J. Am. Chem. Soc.* **1996**, *118*, 293–294; (c) Burguess, K.; Kim, D. *J. Med. Chem.* **1996**, *39*, 4520–4526.
10. (a) Haubner, R.; Finsinger, D.; Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1374–1389; (b) Dechantsreiter, M. A.; Planker, E.; Matha, B.; Lohof, E.; Holzeman, G.; Jonczyk, A.; Goodman, S. L.; Kessler, H. *J. Med. Chem.* **1999**, *42*, 3033–3040.
11. Bisag, Ch.; Weber, Ch.; Robinson, J. A. *Helv. Chim. Acta* **1996**, *79*, 1825–1841.
12. (a) Stilz, H. V.; Jablonka, B.; Just, M.; Knolle, J.; Paulus, E. F.; Zoller, G. *J. Med. Chem.* **1996**, *39*, 2118–2122; (b) Peyman, A.; Wehner, V.; Knolle, J.; Stilz, H. V.; Carniato, D.; Ruxer, J. M.; Gourvest, J. F.; Gadek, T. R.; Bodary, S. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 179–182.
13. The synthesis of the DKP scaffold has been previously reported by our group: (a) del Fresno, M.; Alsina, J.; Royo, M.; Barany, G.; Albericio, F. *Tetrahedron Lett.* **1998**, *39*, 2639–2642. Other solid-phase strategies for DKP synthesis have been also described previously, but are not as versatile as the strategy used in the present work and will not allow the preparation of the target compounds; (b) Krchnák, V.; Weichsel, A. S.; Cabel, D.; Lebl, M. in Ref. 1c pp. 99–117; (c) Gordon, D. W.; Steele, J. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 47–50; (d) Szardenings, A. K.; Burkoth, T. S.; Lu, H. H.; Tien, D. W.; Campbell, D. A. *Tetrahedron* **1997**, *53*, 6573–6593.
14. (a) Jensen, K. J.; Alsina, J.; Songster, M. F.; Vágner, J.; Albericio, F.; Barany, G. *J. Am. Chem. Soc.* **1998**, *120*, 5441–5452; (b) Alsina, J.; Jensen, K. J.; Albericio, F.; Barany, G. *Chem. Eur. J.* **1999**, *5*, 2787–2795.
15. If these two steps are reversed (first removal of the Fmoc group and then of the allyl group) allylation of the free amine can easily occur. See: Gómez-Martínez, P.; Dessolin, M.; Guibé, F.; Albericio, F. *J. Chem. Soc., Perkin Trans. 1* **1999**, 22871–22874.
16. Use of aminium salts such as HBTU or HATU for the cyclization step can provoke guanylation of the free amine. See: (a) Albericio, F.; Bofill, J. M.; El-Faham, A.; Kates, S. A. *J. Org. Chem.* **1998**, *63*, 9678–9683; (b) del Fresno, M.; El-Faham, A.; Carpino, L. A.; Royo, M.; Albericio, F. *Org. Lett.* **2000**, *2*, 3539–3542.
17. Cleavage yields from the resin were in all cases >75%. Purity of crude products was 50–70% for the DKP family and 70–90% for the Hyd one. All compounds after reverse-phase purification showed a purity of >98% and a correct mass upon MALDI-TOF mass spectrometry.
18. Solid-phase synthesis of hydantoin can be carried out using a similar approach as the one described in this

work, but using a less friendly reagent than DSC. See: (a) Xiao, X.-Y.; Ngu, K.; Chao, C.; Patel, D. V. *J. Org. Chem.* **1997**, *62*, 6968–6973; (b) Nefzi, A.; Ostresh, J. M.; Giulianotti, M.; Houghten, R. A. *Tetrahedron Lett.* **1998**, *39*, 8199–8202; (c) Chong, P. Y.; Petillo, P. A. *Tetrahedron Lett.* **1999**, *40*, 2493–2496. Furthermore, other methods described involve a simultaneous hydantoin formation and cleavage from the resin, and therefore these will not allow the preparation of the target compounds. See: (d) Boeijen, A.; Kruijtzer, J. A. W.; Liskamp, R. M. J. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2375–2380; (e) Park, K.-H.; Olmstead, M. M.; Kurth, M. J. *J. Org. Chem.* **1998**, *63*, 6579–6585; (f) Stadlwieser, J.; Ellmerer-Müller, E. P.; Takó, A.; Maslouh, N.; Bannwarth, W. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 1402–1404.

19. Bardaji, E.; Torres, J. L.; Clapes, P.; Albericio, F.; Barany, G.; Rodriguez, R. E.; Sacristan, M. P.; Valencia, G. *J. Chem. Soc., Perkin Trans. 1* **1991**, 1755–1759.
20. Although, in the present work the use of piperidine solutions to provoke the hydantoin formation did not lead to appreciable amount of the corresponding piperidide urea, further work involving the use of hydantoin as a scaffold showed that piperidine can lead to the above mentioned side-reaction. This side-reaction, which is more important when reaction is scaled-up, can be avoided by the use of a non nucleophilic base such as DBU.
21. The assay was performed using purified soluble $\alpha_v\beta_3$ receptor. Results were analyzed by Scatchard analysis, performed using a program written on Microsoft Excel spreadsheet version 5.0. See: Greenberg, Z.; Stoch, S. A.; Traianedes, K.; Teng, H.; Rosenblatt, M.; Chorev, M. *Anal. Biochem.* **1999**, *266*, 153–164.
22. (a) Minton, A. P. *Mol. Pharmacol.* **1981**, *19*, 1–14; (b) Erez, M.; Takemori, A. E.; Portoghese, P. S. *J. Med. Chem.* **1982**, *25*, 847–849.