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Conformationally Constrained Mimetics of Laminin Peptide YIGSR as Precursors for Antimetastatic Disintegrins

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Abstract: Conformationally constrained mimetics of the laminin cell-adhesion site, YIGSR, are described. The site is the natural antagonist of the integrin-associated laminin receptor 1 (LAMR1) known to mediate metastatic tumor adhesion. The attachment of selected metastatic cell lines toward the constrained antagonists has been assessed. Observed differential responses prompted by folding preferences of the mimetics revealed stronger attachment activities for turnlike structures. The results permit the conformational design of antimetastatic disintegrins.

The survival and metastasis of malignant cells strongly depend on their ability to invade and migrate through basement membranes.¹ The process is regulated by cell-surface receptors, integrins, and related non-integrin receptors, which mediate cellular adhesion to basement proteins.^{2,3} One of such proteins, laminin 1, is critical for the invasion and migration of metastasizing cells.⁴ A high-affinity receptor for laminin 1 has been identified as LAMR1, a 67 kDa protein.⁵ The protein appears to be associated with the $\alpha 6 \beta 1$ integrin subunit of $\alpha 6 \beta 1$ and $\alpha 6 \beta 4$ integrins.^{6,7} Malignant cells display increased expression of LAMR1 and the integrins compared to those observed in normal or dysplastic cells.^{2–4,8} Tumor cells also shed LAMR1 in a soluble form in amounts exceeding those proportional to the overexpression of the receptor.⁹ This abundance of free LAMR1 on cellular surfaces correlates well with the enhanced invasiveness of tumor cells reflected in their promoted aggressive malignant behavior.^{1–4} This is complementary to tumor blood vessels expressing receptors or receptor-associated proteins as specific markers for adhesion proteins during angiogenesis.¹⁰ Taken together, the findings imply that the site-specific targeting of LAMR1-laminin 1 interactions may sustain an efficient inhibition of metastasis. In this regard, a recognition site responsible for the receptor binding and identified in the $\beta 1$ chain of laminin as a nine amino acid sequence, CDPGYIGSR (**1**),¹¹ with the pharmacophore segment comprising five residues, YIGSR (**2**),¹² provides a straightforward platform for the specific inhibition of laminin-promoted cell adhesion. Indeed, synthetic **1** and **2**

have been shown to mediate metastatic tumor cell adhesion and to inhibit experimental metastasis without affecting other cellular activities implicated in cell invasion.¹³ The peptides neither influence proliferation rate or density of tumor cells nor alter their tumorigenicity, which supports a nontoxic mechanism of action.^{13,14} Thus, they act as disintegrins, receptor antagonists that specifically block cells from binding to the extracellular matrix.¹⁵ Consequently, designing synthetic disintegrins can confer a very attractive strategy for anticancer therapy.¹⁶ However, progress in this direction is precluded by the requirement for the folding of an intact protein to be “fixed” within a much smaller molecular space, that is, within its receptor-recognition site.¹⁶ In turn, this necessitates a conformation-oriented approach to ligand mimicry, which yet remains hampered by the conformational ambiguity of short peptide sequences. As a result, lower selectivity and poor activity of isolated recognition sites when compared with those of native proteins or, in fact, of the same peptides but structured within the native proteins are observed. In this context, conformational constraining of cell-adhesion motifs presents an obvious place to start.

Among different approaches, backbone or head-to-tail cyclization is intrinsically compatible with native folding and, therefore, is seen as the most efficient means toward conformational mimetics.¹⁶ One notable example of the approach is cilengitide, a superpotent $\alpha v \beta 3$ integrin antagonist developed by the Kessler group.^{17,18} This is a cyclic β -turn mimetic derived from the RGD sequence presenting a common and the most abundant motif for many adhesion proteins including snake venom disintegrins.¹⁵ RGD motif is located in loop regions of the proteins where it is arranged into a cyclic form by oxidized cysteine residues.^{15,16} Unlike RGD, the LAMR1-binding peptides are primarily found in the III domain of the $\beta 1$ chain of laminin 1, which renders their mimetics potentially more selective than RGD.^{13,14,19} The III domain contains homologous regions outfitted with consensus cysteine repeats that assemble into loop-rich structures through the formation of disulfide bonds.^{11,13,20} Additionally, **1** has three turn-inducing residues, one proline (P) and two glycines (G) which can provide two potential bending sites, DPG and IGS. Therefore, it can be assumed that **1** and **2** have inherent tendency for bend conformations. The assumption is further strengthened by the fact that in the native setting **1** and **2** can be spatially fixed by flanking cysteine residues²⁰ via oxidation.

On the basis of these conventions, we set out to generate a series of **1/2** cyclopeptide mimetics and to explore correlations between their induced cell-adhesion responses and conformational preferences. In designing the mimetics the main emphasis was made on imposing conformational constraints within the pharmacophore site, YIGSR. Previous studies of the sequence by others revealed that tyrosine and arginine are essential for the cell-adhesion activity of the peptide.^{12,19,21} Indeed, substitutions at these positions invariably led to a marked loss of the biological activity. Isoleucine and serine residues are less defining, and their substitutions can give comparably active derivatives. Glycine may be important for a bend structure supporting the bioactive conformation.^{12,19,21} Collectively, the findings suggest that the spacing between

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a

b

X ₁	X ₂	Name	cyclo(Sequence)
N	G	3	NYIGSR
	P	4	NYIPSR
	a	5	NYIaSR
	s	6	NYIsSR
	e	7	NYIeSR
	k	8	NYIkSR
NN'	ss'	9	NYIsSRNYIsSR
	PP'	10	NYIPSRNYIPSR
DPG	G	11	DPGYIGSR
	s	12	DPGYIsSR
	e	13	DPGYIeSR
	k	14	DPGYIkSR
G	s	15	GYIsSR
p	s	16	pYIsSR

Figure 1. Designed LAMR1 antagonists: (a) cyclopeptide template $c(X_1YIX_2SR)$ with variables X_1 and X_2 ; (b) table summary of studied peptides. X' denotes the extra X positions ($X_1YIX_2SRX_1'YIX_2'SR$) for dimers **9** and **10**. D-Amino acids are in lower-case.

tyrosinyl and arginyl side chains and their spatial orientation are critical for the activity.

To establish whether these can be best matched in a restricted framework, we designed a cyclic hexapeptide template, $c(X_1YIX_2SR)$ (Figure 1). In this template tyrosine and arginine residues are separated by three (IX_2S) residues as in the native sequence and one (X_1) residue serving as a spacer. The only smaller cyclic arrangement for the peptide is cyclopentapeptide. However, in the cyclopentapeptide arrangement tyrosine and arginine residues are directly linked, which may compromise the match. By contrast, hexapeptide templates permit the degree of flexibility shown to be necessary sufficient for conformational design.^{16–18} For example, single glycine, proline, or D-amino acid residues in cyclohexapeptides induce turn arrangements and can be incorporated to allow for the conformational screening of bioactive forms.¹⁶ In our template, X_1 and X_2 are variables. X_2 is occupied by the native glycine, proline, or a D-amino acid to favor bending. X_1 is reserved for asparagine as a polar and neutral spacer and to afford the on-resin cyclization of the peptides (Scheme S1, Supporting Information). X_1 is also to be used to optimize the bend around X_2 , for which bend-promoting residues or motifs can be used.

Initial screening for active conformations of **2** was performed using the $c(NYIX_2SR)$ template. In the assignment of X_2 position, two main criteria were applied. First, the site should bend; hence, glycine, proline, or D-amino acid is used. Second, the site should not interfere with the autonomous folding of the template; hence, sterically hindered or hydrophobic residues that are potentially conducive to insolubility or aggregation problems had to be avoided and only small and polar residues can be used. Following these criteria, the library comprising individually constructed peptides was generated. The ability of each peptide to mediate cell adhesion was tested at four concentrations using HT-1080 human fibrosarcoma cells. These cells are highly metastatic and exhibit a high affinity to laminin 1, which makes this cell line a standard model system for cell attachment.^{11–14}

The first peptide in the series, $c(NYIGSR)$ (**3**), was made to test the effect of the template on the activity of **2**. No increase in cell attachment was observed for the peptide when compared to **2**. The substitutions of glycine for proline (**4**) and D-alanine (**5**) led to similar activities. However, **5** proved to be poorly soluble in the cell culture medium at higher concentra-

tions, questioning the interpretation of its activity. To circumvent this, X_2 was made D-serine to give $c(NYIsSR)$ (**6**). Not only was this peptide readily soluble in the medium but it also showed dose-dependent increases in the activity (nearly 20% and 40% at the lowest and higher peptide concentrations respectively) (Figure 2a).

To reveal if the effect was conformation-driven, we probed the peptides using circular dichroism (CD^a) spectroscopy (Supporting Information). Intriguingly, spectra for **3**, **4**, and **5** peptides were similar and characteristic of random coil conformations (**5** not shown) (Figure S1 and Table S2). In marked contrast, spectra typical of the β -form with minimum and maximum at 216 and 196–200 nm, respectively, were recorded for **6** (Figure S1). Although certain ambiguity remains in the assessment of bend conformations, the spectra were consistent with those reported for β -turns.^{22–24} Therefore, it was reasonable to assume that the side chains of polar D-amino acids incorporated at the X_2 position can be involved in intramolecular interactions whereby more active conformations (presumably turns) are induced. To follow this, we made two other peptides, **7** and **8**, with X_2 positions occupied by anionic glutamate and cationic lysine residues, respectively. **8** was noticeably less active for cell attachment than **6**, but the activity of **7** was almost comparable with that of **6**. Interestingly, CD spectroscopy revealed characteristic β -structure bands at ~ 216 nm for **7** and **8**. However, unlike for **6** the maximum at 196 nm was not apparent in both peptides, suggesting a contribution of an open conformation.²⁴ The latter is featured by a distinctive minimum at 198–200 nm, the emergence of which from **6** to **8** was particularly evident (Figure S1). This suggests that the side chain of D-lysine may relieve the induced constraints, for example, as a result of eliminated intramolecular interactions that are otherwise stabilizing for **6** and **7**. One way to confirm this is to extend **6** into a more open cycle. With this in mind, a **6** dimer (**9**), in which two sequential copies of NYIsSR are cyclized, was made (Figure 1b, Tables S1 and S2). In this peptide both copies are in the ring, but constraints imposed in each are weaker allowing thus a greater, yet still limited, degree of flexibility. This resulted in a substantial increase in CD signals at 198 nm for **9** compared with **6** and **8**. The signals were similar to those of **3** and **4** and so were comparably weak cell-adhesion activities of the peptides (Figures 2 and S1, Table S2). Furthermore, the behavior of the **4** dimer (**10**) was almost identical to that of **2**: no appreciable secondary structure and comparable cell adhesion activity. Thus, taken together, the results emphasize the strong correlation between the cell attachment and conformational constraining of **2** within the cyclic template. In this respect, the ring size may play an equally important role. Indeed, it is generally proposed that small cycles provide more constrained and therefore more stable conformations.¹⁶ These are not necessarily bioactive, as the requirement for matching structure with activity remains dependent on mimicking active sites in native environments rather than on mimicking elementary motifs.^{16,21}

^a Abbreviations: OAll, allyl; CD, circular dichroism; DCM, dichloromethane; DIPEA, *N,N*-diisopropylethylamine; DMF, dimethylformamide; FAB, fast atom bombardment; HCTU, (2-(6-chloro-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylammonium hexafluorophosphate); RP-HPLC, reversed-phase high performance liquid chromatography; MALDI-ToF, matrix-assisted laser desorption ionization time-of-flight; MOPS, 3-(*N*-morpholino)propanesulfonic acid; Pbf, 2,2,4,6,7-pentamethylbenzofuran-5-sulfonyl; PBS, phosphate buffered saline; TFA, trifluoroacetic acid; TFE, trifluoroethanol; TIS, triisopropylsilane.

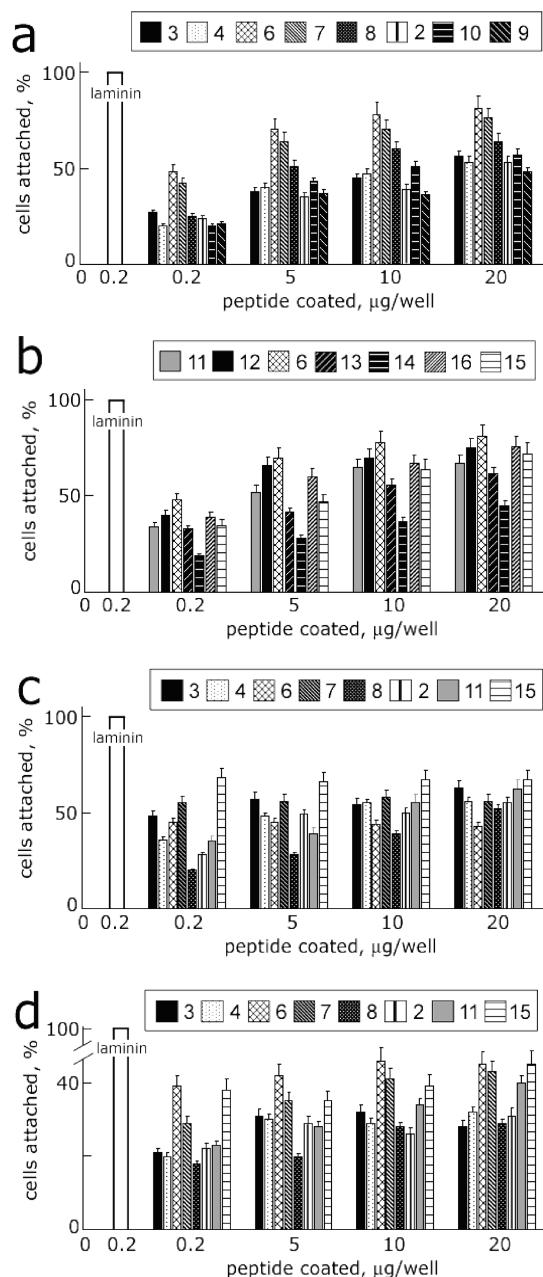


Figure 2. Attachment of HT-1080 (a, b), B16-F10 (c), and HeLa (d) cells to peptide-coated plates. Cell attachments to laminin 1 and BSA are taken as 100% and 0%. Each bar represents the mean of two independent experiments done in triplicate.

Admittedly, this varies from one case to another. However, one main principle in finding matched mimetics is always to consider the secondary structure within and near the active site. This may require more extended cyclic systems providing the stabilizing contribution of added residues. Related to the LAMR1 antagonists, the aforementioned DPG in **1** may impose additional constraints on **2** to tighten the bioactive conformation. To probe this, X_1 was made DPG to give a series of cyclooctapeptide derivatives c(DPGYIX₂SR), where X_2 = glycine (**11**), D-serine (**12**), D-glutamate (**13**), and D-lysine (**14**) (Figure 1). All peptides, except **11**, were found to be less active for cell attachment than their cyclohexapeptide counterparts (Figure 2b, Tables S1 and S2). **13** and **14** showed weaker activities than **7** and **8**. The activities of **11** and **12** were higher and similar to those of **3** and **6**, correspondingly. The

differences in the activity were reflected in the conformational preferences of the peptides (Figure S1 and Table S2). CD signals for **14** were associated with an increased minimum of ~200 nm and represented a typical random coil conformation. In the case of **13** the minimum was also apparent but shifted to 208 nm which with another minimum at 222 nm can be characteristic of a helical conformation. In this light, the transition from a β -structure in **6** to a helix in **13** indicates that **7** may be prone to adopting a helical structure, which is proven to lead to the impairing of adhesion. This is also supported by the folding of **12** into a β -form with retention of the cell attachment activity of **6**. Further, CD spectra of **11** may represent an intermediate state including the features of a β -form and a helix. The activity of the peptides also seems to be intermediate compared to those of **3** and **6**. Although not fully conclusive with regard to which structural type is responsible for the increase in the activity of **3**, this is clearly attributable to DPG. Nevertheless, being fairly moderate, the effect poses a question of whether more efficient constraints can be afforded by a single bending residue in X_1 position. To clarify this, we made two peptides derived from **6**, with asparagine replaced with bend-promoting glycine and D-proline residues, **15** and **16**, respectively (Figure 1b, Scheme S2, and Table S1). Gratifyingly, CD signatures for **15** and **16** were remarkably similar to those for **11** and **12** and so were the corresponding cell attachment activities (Figures 2b and S1).

To this end, the ability of the designed mimetics to mediate adhesion of HT-1080 cells that are known to adhere well to laminin and laminin-like peptides was studied. To test if the same applies to other cell types, we probed mimetics using mouse melanoma B16-F10 cells. Cells of this type are highly metastatic and have been used as tumor cell adhesion and metastasis models.^{11–13} The cells have reportedly been shown to readily adhere to laminin 1. However, their attachment to **2** or laminin-like peptides is less pronounced and often lacks consistency between different design series.^{11–13,19,25} This can yet be beneficial for estimating the strength of the effect observed for HT-1080 cells and their selective response to the designed mimetics. With this in mind, we tested several mimetics for their ability to support the adhesion of B16-F10 cells. Generally, the extent of the attachment was found to be comparable to that for HT-1080. Yet a few distinctive features in the behavior of B16-F10 cells were observed. Consistent with the data for HT-1080, B16-F10 gave dose response attachments (Figure 2c). However, these were only for **3**, **4**, **8**, and **2**, the activities of which increased with increasing concentrations. In contrast, responses to **6** and **7** were not concentration-dependent, with **6** being notably less active for B16-F10 than for HT-1080. Structurally similar **11** and **15** exhibited different attachment patterns that appear to be only in part consistent with those observed for HT-1080 cells: **11** can be grouped with **3**, **4**, and **8**, whereas **15** gave responses comparable with those of **6** and **7** (Figures 2c and S1). Additionally, the activities within the **6** < **7** < **15** row were reverse to those found for HT-1080 cells (Figure 2c and Table S2).

Overall, the observed differences were not substantial prompting the conclusion that B16-F10 cells are less responsive to conformational changes in **2**. Thus, consistent with earlier observations,^{12,19,25} HT-1080 and B16-F10 readily adhere to laminin 1 but respond to **2**-derived peptides differently. Related to our case, it can be speculated that HT-1080 cells provide differential attachment responses to the designed mimetics, while B16-F10 cells react nonspecifically, that is, strong adherence to laminin 1 and nondifferential response to

the mimetics. In this vein, a cell type with less specific adhesion properties toward laminin 1 may give a lower cut-off in the activity, within which differential or no response to the conformational mimetics may be observed. To establish this, we performed the same set of tests using HeLa cells. These are human cervical carcinoma cells that were shown to be conditionally adherent to laminin and its components.^{26,27} Little has been reported on the binding of HeLa cells to 2-like peptides, which may partly be attributed to the moderate affinity of the cells to the peptides.²⁸ In our case, HeLa adhered to laminin 1 less efficiently than did HT-1080 and B16-F10 cells (Figure 2d). Likewise, lower but medially dose-dependent attachments were observed for the mimetics. On the basis of the expressed activities, two groupings could be identified. In one, peptides that gave more appreciable β -form CD spectra (**6**, **7**, **15**) also gave stronger attachment responses (Figures 2d and S1, Table S2). In the other, structurally amorphous **3**, **4**, and **8** yielded lower responses that were also comparable with those for **2**, **11**, which showed intermediate activities in HT-1080 and B16-F10, could also be placed between the two groups in HeLa.

In summary, the obtained results reveal the general tendency of the designed mimetics with more appreciable β -conformations to generate stronger cell attachment responses. Although the observed effect is not sufficiently discriminative to single out one particular construct, the findings stress the conformational constraining of isolated cell-adhesion sites as an efficient means toward the development of selective anti-metastatic disintegrins.

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Supporting Information Available: Experimental procedures including synthesis, characterization, spectroscopic data, and biological assays. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) Engbring, J. A.; Kleinman, H. K. The basement membrane matrix in malignancy. *J. Pathol.* **2003**, *200*, 465–470.
- (2) Leber, M. F.; Efferth, T. Molecular principles of cancer invasion and metastasis. *Int. J. Oncol.* **2009**, *34*, 881–895.
- (3) Luo, B. H.; Springer, T. A. Integrin structures and conformational signaling. *Curr. Opin. Cell Biol.* **2006**, *18*, 579–586.
- (4) Kleinman, H. K.; Cannon, F. B.; Laurie, G. W.; Hassell, J. R.; Aumailley, M.; Terranova, V. P.; Martin, G. R.; DuBois-Dalcq, M. Biological activities of laminin. *J. Cell. Biochem.* **1985**, *27*, 317–325.
- (5) Nelson, J.; McFerran, N. V.; Pivato, G.; Chambers, E.; Doherty, C.; Steele, D.; Timson, D. J. The 67 kDa laminin receptor: structure, function and role in disease. *Biosci. Rep.* **2008**, *28*, 33–48.
- (6) Ardini, E.; Tagliabue, E.; Magnifico, A.; Butò, S.; Castronovo, V.; Colnaghi, M. I.; Ménard, S. Co-regulation and physical association of the 67-kDa monomeric laminin receptor and the $\alpha 6 \beta 4$ integrin. *J. Biol. Chem.* **1997**, *272*, 2342–2345.
- (7) Belkin, A. M.; Stepp, M. A. Integrins as receptors for laminins. *Microsc. Res. Tech.* **2000**, *51*, 280–301.
- (8) Hand, P. H.; Thor, A.; Schlom, J.; Rao, C. N.; Liotta, L. Expression of laminin receptor in normal and carcinomatous human tissues as defined by a monoclonal antibody. *Cancer Res.* **1985**, *45*, 2713–2719.
- (9) Karpátová, M.; Tagliabue, E.; Castronovo, V.; Magnifico, A.; Ardini, E.; Morelli, D.; Belotti, D.; Colnaghi, M. I.; Ménard, S. Shedding of the 67-kD laminin receptor by human cancer cells. *J. Cell. Biochem.* **1996**, *60*, 226–234.
- (10) Ruoslahti, E. Specialization of tumour vasculature. *Nat. Rev. Cancer* **2002**, *2*, 83–90.
- (11) Graf, J.; Iwamoto, Y.; Sasaki, M.; Martin, G. R.; Kleinman, H. K.; Robey, F. A.; Yamada, Y. Identification of an amino acid sequence in laminin mediating cell attachment, chemotaxis, and receptor binding. *Cell* **1987**, *48*, 989–996.
- (12) Graf, J.; Ogle, R. C.; Robey, F. A.; Sasaki, M.; Martin, G. R.; Yamada, Y.; Kleinman, H. K. A pentapeptide from the laminin B1 chain mediates cell adhesion and binds the 67,000 laminin receptor. *Biochemistry* **1987**, *26*, 6896–6900.
- (13) Iwamoto, Y.; Robey, F. A.; Graf, J.; Sasaki, M.; Kleinman, H. K.; Yamada, Y.; Martin, G. R. YIGSR, a synthetic laminin pentapeptide, inhibits experimental metastasis formation. *Science* **1987**, *238*, 1132–1134.
- (14) Sakamoto, N.; Iwahana, M.; Tanaka, N. G.; Osada, Y. Inhibition of angiogenesis and tumor growth by a synthetic laminin peptide, CDPGYIGSR-NH₂. *Cancer Res.* **1991**, *51*, 903–906.
- (15) McLane, M. A.; Joerger, T.; Mahmoud, A. Disintegrins in health and disease. *Front. Biosci.* **2008**, *13*, 6617–6637.
- (16) Heckmann, D.; Kessler, H. Design and chemical synthesis of integrin ligands. *Methods Enzymol.* **2007**, *426*, 463–503.
- (17) Wermuth, J.; Goodman, S. L.; Jonczyk, A.; Kessler, H. Stereoisomerism and biological activity of the selective and superactive $\alpha v \beta 3$ integrin inhibitor cyclo-(RGDfV-) and its retro-inverso peptide. *J. Am. Chem. Soc.* **1997**, *119*, 1328–1335.
- (18) Aumailley, M.; Gurrath, M.; Müller, G.; Calvete, J.; Timpl, R.; Kessler, H. Arg-Gly-Asp constrained within cyclic pentapeptides. Strong and selective inhibitors of cell adhesion to vitronectin and laminin fragment P1. *FEBS Lett.* **1991**, *291*, 50–54.
- (19) Zhao, M.; Kleinman, H. K.; Mokotoff, M. Synthetic laminin-like peptides and pseudopeptides as potential antimetastatic agents. *J. Med. Chem.* **1994**, *37*, 3383–3388.
- (20) Pikkariainen, T.; Eddy, R.; Fukushima, Y.; Byers, M.; Shows, T.; Pihlajaniemi, T.; Saraste, M.; Tryggvason, K. Human laminin B1 chain. *J. Biol. Chem.* **1987**, *262*, 10454–10462.
- (21) Ostheimer, G. J.; Starkey, J. R.; Lambert, C. G.; Helgeson, S. L.; Dratz, E. A. NMR constrained solution structures for laminin peptide 11. Analogs define structural requirements for inhibition of tumor cell invasion of basement membrane matrix. *J. Biol. Chem.* **1992**, *267*, 25120–25128.
- (22) Brahms, S.; Brahms, J.; Spach, G.; Brack, A. Identification of beta, beta-turns and unordered conformations in polypeptide chains by vacuum ultraviolet circular dichroism. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 3208–3212.
- (23) Perczel, A.; Fasman, G. Quantitative analysis of cyclic β -turn models. *Protein Sci.* **1992**, *1*, 378–395.
- (24) *Circular Dichroism and the Conformational Analysis of Biomolecules*; Fasman, G. D., Ed.; Plenum Press: New York, 1996; 738 pp.
- (25) Yamamura, K.; Kibbey, M. C.; Kleinman, H. K. Melanoma cells selected for adhesion to laminin peptides have different malignant properties. *Cancer Res.* **1993**, *53*, 423–428.
- (26) Kawahara, E.; Imai, K.; Kumagai, S.; Yamamoto, E.; Nakanishi, I. Inhibitory effects of adhesion oligopeptides on the invasion of squamous carcinoma cells with special reference to implication of αv integrins. *J. Cancer Res. Clin. Oncol.* **1995**, *121*, 133–140.
- (27) Sugihara, K.; Saito, T.; Okadome, M.; Sonoda, K.; Kobayashi, H.; Kamura, T.; Tsukamoto, N.; Nakano, H. The promotion of invasion through the basement membrane of cervical carcinoma cells by fibronectin as a chemoattractant. *Cancer Lett.* **1994**, *79*, 167–173.
- (28) Hirano, Y.; Okuno, M.; Hayashi, T.; Goto, K.; Nakajima, A. Cell-attachment activities of surface immobilized oligopeptides RGD, RGDS, RGDV, RGDT, and YIGSR toward five cell lines. *J. Biomater. Sci., Polym. Ed.* **1993**, *4*, 235–243.