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The First Synthetic Receptor for the RGD Sequence

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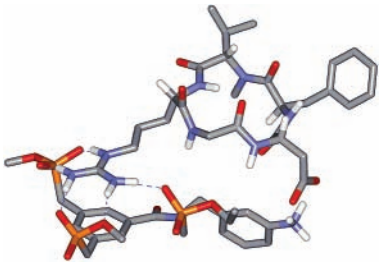
ABSTRACT



The combination of an optimized arginine receptor unit with a semirigid linker carrying a strategically placed primary ammonium group leads to the first synthetic RGD receptor. It binds to the free RGD peptide as well as to cyclo(RGDfV) in water with association constants around 1000 M^{-1}. RGD mimetics such as benzamidine 6 are not recognized, rendering the new host a prototype of a new class of receptors selective for the true RGD sequence in peptides.

Cell−cell and cell−matrix adhesion processes are vitally important for higher organisms. These interactions control essential body functions such as embryogenesis, cell differentiation, angiogenesis, heamostasis, wound healing, or immune response. One of the most important classes of proteins involved in these adhesion processes are the integrins, which are heterodimeric glycoproteins, anchored inside the cell membrane.[1] They interact only with proteins carrying the Arg-Gly-Asp sequence (RGD) on a solvent exposed loop. Proteins with such an RGD loop are fibrinogen,[2] fibronectin,[3] vitronectin,[4] thrombospondin,[5] osteospontin,[6] and a number of growth factors.

The specific complex formation of a relatively small peptide fragent with its binding site on a membrane-bound receptor thus constitutes a key element in many life processes. Inhibiton of this complex formation strongly influences all related events. Two alternatives may be chosen: conformationally well-defined synthetic RGD mimetics may recognize specific integrins[7] or small soluble receptors may bind to the RGD sequence itself, distinguishing between its extended or folded state. Whereas today almost all large pharmaceutical companies hold patents for the first pathway, virtually nothing is known about the second alternative.

We have recently published small optimized structures for the recognition of arginine in polar solvents (Figure 1).[8] They rely on the *m*-xylylene bisphosphonate recognition motif **1**, which combines electrostatic, hydrogen bond, and π-cation interactions in its 1:1 complex with N/C-protected arginine (800 M^{-1} in CD_3OD). Introduction of an additional phosphonate group on the 5-position with the appropriate rigid spacer led to improved binding because now the fifth NH proton of the guanidinium moiety was also bound (3500 M^{-1}

(1) Albelda, S. M.; Buck, C. A. *FASEB J.* **1990**, *4*, 2868.
(2) Gardner, J. M.; Hynes, R. O. *Cell* **1985**, *42*, 439.
(3) Pierschbacher, M. D.; Ruoslathi, E.; Sundelin, J.; Lind, P.; Peterson, P. A. *J. Biol. Chem.* **1982**, *257*, 9593.
(4) Schvartz, I.; Seger, D.; Shaltiel, S. *Int. J. Biochem. Cell Biol.* **1999**, *31*, 539.
(5) Lawer, J.; Weinstein, R.; Hynes, R. O. *J. Cell. Biol.* **1988**, *107*, 2351.
(6) Helfrich, M. H.; Nesbitt, S. A.; Dorey, E. L.; Horton, M. A. *J. Bone Miner. Res.* **1992**, *7*, 335.
(7) Haubner, R.; Finsinger, D.; Kessler, H. *Angew. Chem.* **1997**, *109*, 1440.
(8) (a) Schrader, T. *Chem. Eur. J.* **1997**, *3*, 1537. (b) Schrader, T. *Tetrahedron Lett.* **1998**, *39*, 517.

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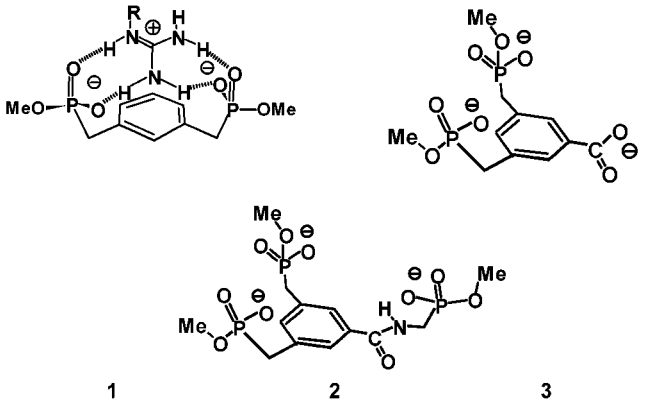

Figure 1. Basic *m*-xylylene recognition motif **1** for arginine in a peptidic environment, optimized structure **2** with an additional phosphonate arm, and carboxylate analogue **3**.

in CD_3OD).[9] No binding, however, could be observed in water.

In recent experiments, we titrated the most basic amino acid esters as dicationic species with our new arginine binders.[10] Here we found that the new trisphosphonate receptor molecule **2** displayed a pronounced arginine selectivity, while a shorter carboxylate analogue turned out to be moderately selective for histidine. Thus, arginine methyl ester (36000 M^{-1} in CD_3OD) was bound 18 times better than histidine methyl ester and even 36 times better than lysine methyl ester (Table 1). In D_2O **2** can still bind the arginine

Table 1. Binding Constants Resulting from NMR Titration Experiments between Basic Amino Acid Esters and Optimized *m*-Xylylene Receptor Structures **2** and **3** in Methanol and Water at 25 °C

amino acid methyl ester x 2 HCl	**2** in CD_3OD	**2** in D_2O	**3** in D_2O
arginine	36000 ± 51%[a]	800 ± 43%[b]	300 ± 20%
histidine	2400 ± 55%	no saturation	500 ± 29%
lysine	1000 ± 22%	no shifts	no shifts

[a] Errors are standard deviations from the nonlinear regressions. [b] The large standard deviation in this case can be explained with small chemical shift differences.

ester (800 M^{-1}), but no saturation was found for the histidine derivative and no shifts at all for the lysine ester. Interestingly, the carboxylate **3** prefers histidine esters (500 M^{-1}) over arginine esters (300 M^{-1}) at the same conditions but cannot bind lysine esters.

(9) Rensing, S.; Springer, A.; Grawe, T.; Schrader, T. *J. Org. Chem.* **2001**, *66*, 5814. Other powerful arginine receptors: (a) Bell, T. W.; Khasanov, A. B.; Drew, M. G. B.; Filikov, A.; James, T. L. *Angew. Chem., Int. Ed.* **1999**, *38*, 2543. (b) Ngola, S. M.; Kearney, P. C.; Mecozzi, S.; Russell, K.; Dougherty, D. A. *J. Am. Chem. Soc.* **1999**, *121*, 1192.

(10) (a) Schneider, H. J.; Kramer, R.; Simova, S.; Schneider, U. *J. Am. Chem. Soc.* **1988**, *110*, 6442. (b) Wilcox, C. S. In *Frontiers in Supramolecular Chemistry*; Schneider, H. J., Ed.; Verlag Chemie: Weinheim, 1991; p 123.

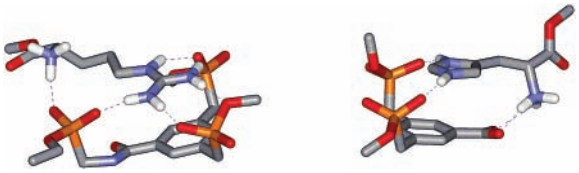

Figure 2. (a) Arginine ester recognition with **2**; note the extended side chain. (b) Histidine ester recognition with **3**; note the perfect structural complementarity. Monte Carlo simulations have been carried out in water (MacroModel 7.0, Amber*).

Monte Carlo simulations in water reach complex geometries with an intriguing complementarity between host and guest for the best binding partners described above.[11] The long trisphosphonate allows the arginine side chain to adopt a thermodynamically favorable extended conformation and still ensures a second electrostatic interaction with the α-ammonium group (Figure 2a). By contrast, the compact

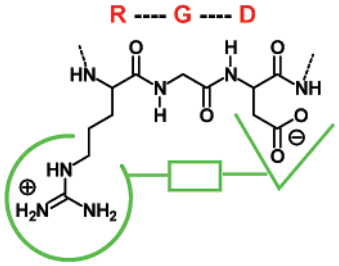

Figure 3. Schematic of the rational RGD receptor design. Recognition of the RGD sequence is achieved by a combination of an arginine host with an aspartate host interconnected by a rigid optimized spacer, which prevents self-association.

carboxylate can accommodate histidine's imidazolium ring just above its central benzene ring and still form an additional salt bridge between its carboxylate anion and histidine's α-ammonium group, which is in close proximity to its side chain (Figure 2b).

Its high arginine selectivity makes **2** an ideal candidate for a potential RGD receptor. Since lysine is not bound at all in water, we envisaged that an additional ammonium group attached to the skeleton of **2** at the correct distance via a rigid spacer could serve as the aspartate binding site. The spacer should prevent intramolecular dimerization, while the ammonium functionality is a bad guest for the trisphosphonate and should thus circumvent the unwanted intermolecular dimerization. Extensive modeling studies suggested the introduction of a *m*-aminobenzyl substituent at the amide nitrogen. This would also avoid the creation of a stereogenic center that would arise from attachment of this substituent at phosphonoglycine's methylene group.

Mild deprotonation of the amide in dipolar aprotic solution, followed by addition of the phthaloylprotected spacer as a

(11) MacroModel 7.0, Schrödinger Inc., force-field: Amber*, 1000 steps, water.

benzyl bromide proceeded smoothly to give the secondary amide **4** as an E/Z mixture of ~4:1.[12] Subsequent 3-fold monodealkylation with LiBr[13] followed by removal of the phthalimide with hydrazine afforded the free base **5**, which was finally back-titrated with 1 equiv of HCl to give the desired receptor molecule **5a**. It is highly soluble in water and shows no appreciable self-association in this solvent according to a dilution experiment.

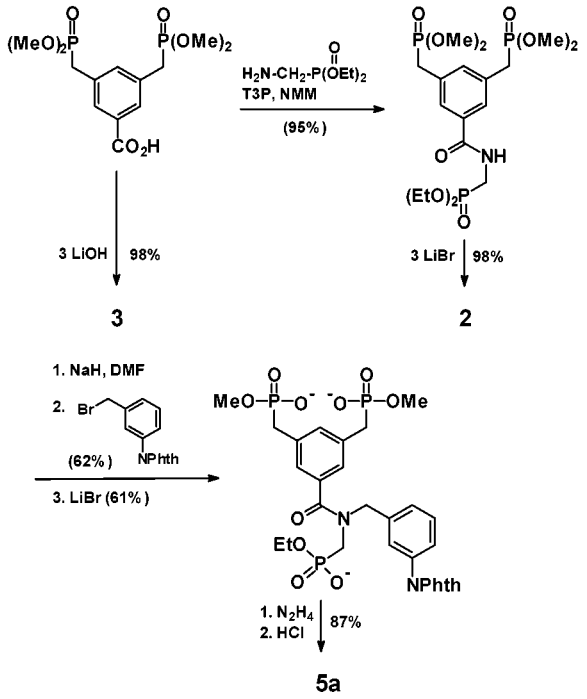

Figure 4. Synthesis of **2**, **3**, and **5a** by selective N-alkylation.

Before we examined its potential to bind RGD, we used the trisphosphonate **2** as a model compound. Since the free RGD tripeptide also contains a dicationic C-protected arginine, the trisphosphonate might also show some affinity for it in water. Indeed, a certain interaction was found (300 M^{-1}), probably weakened by the strong electrostatic repulsion between the trisphosphonate anion and the two aspartate carboxylate groups. However, for our new RGD binder **5a** the interaction with the free tripeptide produced a K_a value half an order of magnitude higher than that of **2**. Distinct complexation-induced shifts were found in the aspartate region, indicating that the additional salt bridge had indeed been formed. Next we titrated **5a** with Kessler's cyclopeptide cyclo(RGDfV), which is a model compound for the RGD loop in fibronectin.[14] Here, only one cationic and one anionic

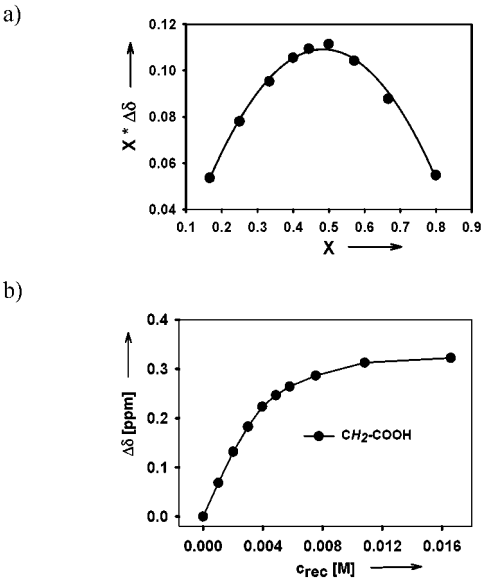

Figure 5. (a) Job plot and (b) NMR titration curve for the complexation of free RGD peptide with **5a** in water.

group are present just as in the real RGD loop of the target proteins. To our great pleasure, a clear 1:1 stoichiometry with a binding constant of 700 M^{-1} was detected for **5a**.[15] Again, upfield shifts were found in the aspartate region of the cyclopeptide. This shows that the concept of combining an arginine with an aspartate binder is valid even in the simplest case of an ammonium group.

Table 2. Binding Constants Resulting from NMR Titration Experiments between RGD-Containing Peptides or Mimetics and Trisphosphonate Receptor Molecules **2** and **5a** in Water at 25 °C

RGD peptide or mimetic	**2** (M^{-1})	**5a** (M^{-1})	self-association (dilution)
free RGD	300 ± 15%[a]	1300 ± 5%	no shifts
cyclo(RGDfV)		700 ± 14%	no shifts
benzamidine **6**		no shifts	**5a** alone: no shifts

[a] Errors are standard deviations from the nonlinear regressions.

We also tried to bind the powerful mimetic **6** for the RGD conformation in fibrinogen with **5a** but failed completely.[16] Not even the slightest shift was observable in any of the host or guest protons. This could have two explanations: (a) **5a** recognizes only the correct folded conformation of the arginine and asparate side chain in fibronectin, and (b) the benzamidine group present in the synthetic drug does not

(12) (a) Hoffmann, R. V.; Nayyar, N. K. *J. Org. Chem.* **1994**, *59*, 3530. (b) Suezawa, H.; Tsuchiya, K.; Tahara, E.; Hirota, M. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 11, 3973.

(13) (a) Karaman, R.; Goldblum, A.; Breuer, E. *J. Chem. Soc., Perkin Trans.* **1989**, 765. (b) Krawczyk, H. *Synth. Commun.* **1997**, *27*, 3151.

(14) Dechantsreiter, M. A.; Plancker, E.; Mathä, B.; Lohof, E.; Hölzemann, G.; Jonczyk, A.; Goodmann, S. L.; Kessler, H. *J. Med. Chem.* **1999**, *42*, 3033.

(15) By means of a Job plot: (a) Job, P. *Compt. Rend.* **1925**, *180*, 928. (b) Blanda, M. T.; Horner, J. H.; Newcomb, M. *J. Org. Chem.* **1989**, *54*, 4626.

(16) Stilz, H. U.; Guba, W.; Jablonka, B.; Just, M.; Klingler, O.; König, W.; Wehner, V.; Zoller, G. *J. Med. Chem.* **2001**, *44*, 1158.

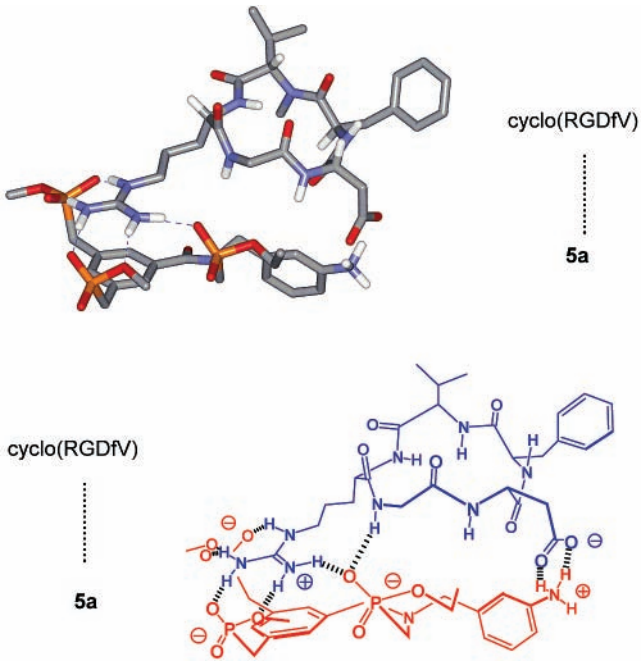

Figure 6. Proposed structure for the complex between the new RGD ligand **5a** and cyclo(RGDfV) in water (MacroModel 7.0, OPLS-AA). Bottom right: Lewis structure.

fit into the receptor's binding site designed for the slim alkylguanidinium moiety. Preliminary molecular mechanics calculations support especially the second argument. This is encouraging, because for a potential therapeutic use no side effects should occur with other drugs in the patient's body. In the future, we will combine our optimized arginine binder with the powerful aspartate ligand introduced by the Schmuck group[17] to achieve highly efficient and selective RGD binding in water.

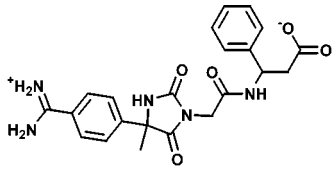

Figure 7. RGD mimetic **6**, a fibrinogen receptor antagonist.

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Supporting Information Available: Experimental procedures, NMR titration tables and curves, and Job plots. This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) (a) Schmuck, C. *Chem. Eur. J.* **2000**, *4*, 709. Other powerful aspartate receptors: (b) Aït-Haddou, H.; Wiskur, S. L.; Lynch, V. M.; Anslyn, E. V. *J. Am. Chem. Soc.* **2001**, *123*, 11296. (c) Albert, J. S.; Goodman, M.; Hamilton, A. D. *J. Am. Chem. Soc.* **1995**, *117*, 1143.