Pergamon

Tetrahedron Letters 41 (2000) 6295–6298

TETRAHEDRON LETTERS

An improved synthesis of a selective $\alpha_v\beta_3$-integrin antagonist *cyclo*(-RGDfK-)

Xuedong Dai, Zhuang Su and Jun O. Liu*

Center for Cancer Research, Departments of Biology and Chemistry, Massachusetts Institute of Technology, 40 Ames Street, Cambridge, MA 02139, USA

Received 2 May 2000; revised 20 June 2000; accepted 21 June 2000

Abstract

The cyclic pentapeptide *cyclo*(-Arg-Gly-Asp-D-Phe-Lys-) is a highly potent and selective inhibitor for the $\alpha_v\beta_3$ integrin. A related compound, *cyclo*(-Arg-Gly-Asp-D-Phe-Val-), is a promising anticancer drug candidate; it inhibits angiogenesis and induces apoptosis in vascular cells. We have developed an improved solid-phase synthesis based on Kessler's procedure to afford *cyclo*(-RGDfK-) peptide in high purity and high yield in a multi-gram scale. This improved synthesis is environmentally friendly and may be generally applicable to other related cyclic peptide syntheses.

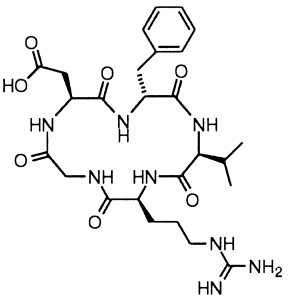
Keywords: $\alpha_v\beta_3$-integrin antagonist; cyclic RGD peptide; solid-phase synthesis.

The integrins are a class of cell surface adhesion proteins that play important roles in cell–cell and cell–matrix interactions.[1] They are involved in diverse physiological processes. In particular, it has been shown that the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ subgroups of integrins are required for tumor-induced angiogenesis. An inhibition of the binding of the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins to their native ligands by antibodies or cyclic peptides interferes with angiogenesis and induces tumor regression.[2] Antagonists of $\alpha_v\beta_3$ are undergoing clinical trials as potential cancer therapeutic agents.[2a]

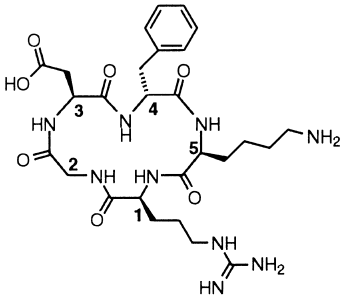
Integrins interact with their ligand proteins mainly through a tripeptide motif consisting of Arg-Gly-Asp (RGD). A number of RGD-containing peptides have been designed, synthesized and tested for their ability to serve as antagonists of integrins. Of the antagonists synthesized to date,[3,4] RGDfV (**1**)[3a] and its methylated analog by Kessler and co-workers are the most selective for $\alpha_v\beta_3$.[4]

* Corresponding author.

0040-4039/00/$ - see front matter
PII: S0040-4039(00)01060-1



cRGDfV (**1**)



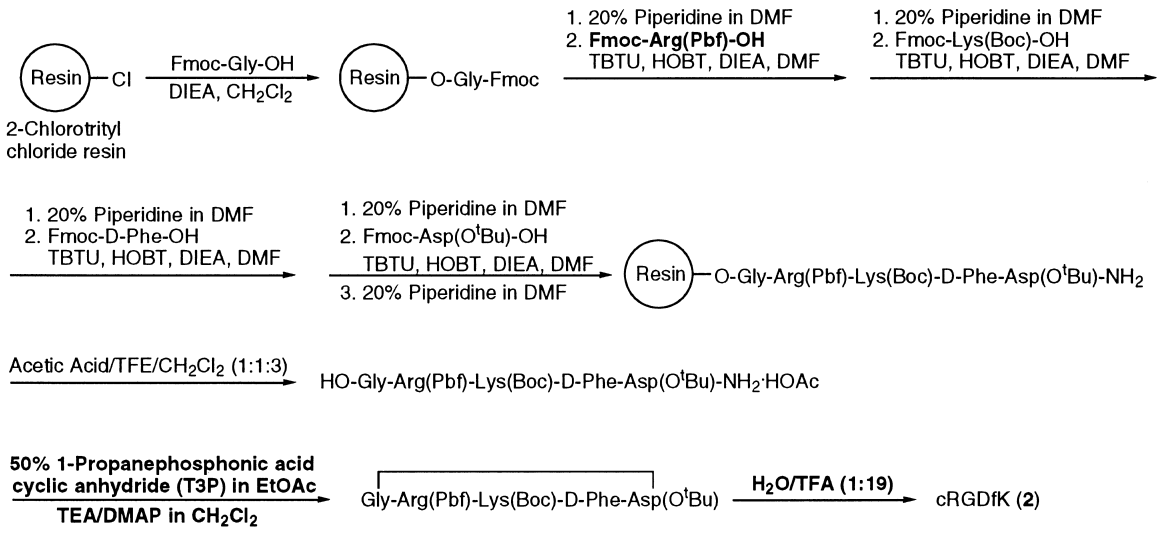
cRGDfK (**2**)

In a search for novel anti-angiogenic agents, we needed to obtain the cyclic pentapeptide cRGDfK (**2**). First designed and synthesized by Kessler's group,[3a] cRGDfK binds to $\alpha_v\beta_3$ with high potency and selectivity. As the fifth amino acid has little influence on the activity, the primary amino group of the lysine in cRGDfK offers an ideal site for further functionalization of this cyclic peptide. Indeed, the amino group on the lysine side chain has been coupled with fluorescein, and the resulting conjugate was shown to be enriched in retina undergoing neovascularization.[5]

The original synthesis of cRGDfK employed the Fmoc solid-phase chemistry to build a linear, protected RGDfK peptide, followed by cleavage from the solid resin, cyclization of the linear peptide, and removal of the protecting groups.[3a] Despite its high efficiency, several disadvantages remain: (1) After the cleavage of the linear peptide from the resin, the cyclization of the acid terminal of glycine and the α-amino terminal of lysine was performed via in situ activation using diphenylphosphoryl azide (DPPA), requiring aqueous work-up; (2) the removal of the guanidine-protecting group from arginine, the 4-methoxy-2,3,6-trimethylbenzenesulfonyl (Mtr) group, necessitated the use of a mixture of several toxic and malodorous reagents, including trifluoroacetic acid (TFA), phenol, thioanisole, and ethanedithiol; and (3) purification of the final product requires HPLC separation.

We set out to improve the original synthesis in all three aspects: (1) We decided to use 1-propanephosphonic acid cyclic anhydride (T3P) for the cyclization of the linear peptide. This reagent is highly effective as a coupling reagent in peptide synthesis.[6] The product should be easily purified by silica gel chromatography, eliminating the need for the toxic DPPA; (2) we decided to change the protecting group of arginine from Mtr to 2,2,5,7,8-pentamethylchroman-6-sulfonyl (Pbf), which requires much milder deprotection conditions (1:19 water:trifluoroacetic acid (TFA)).[7] An added advantage is that all the other protecting groups can be removed under the same condition; and (3) as the final deprotection step does not involve the aforementioned toxic and high-boiling reagents, purification of the final product by HPLC is unnecessary. Here we report the improved synthesis of cRGDfK (Scheme 1).

Similar to the literature procedure, the synthesis of the linear peptide started with the incorporation of glycine onto *o*-chlorotrityl chloride resin by mixing the resin (16.72 g, 1.35 mmol/g, 22.57 mmol) with a solution of Fmoc-Gly-OH (16.78 g, 56.44 mmol) and *N*,*N*-diisopropylethylamine (DIEA, 9.04 mL, 51.90 mmol) in dry dichloromethane (DCM) in a 500 mL peptide synthesis vessel (ChemGlass). After shaking the reaction mixture for 2.5 h using a Burrell Wrist-Action shaker, 9.0 mL of DIEA and 50 mL of methanol were added to cap the unreacted sites of the resin. After 30 min, the resin was washed with dimethylformamide (DMF, 2×), DCM (2×), methanol (2×), and diethylether (2×) and dried in vacuo. The loading of the resin was



Scheme 1. Improved synthesis of cRGDfK

calculated as 17.60 mmol Fmoc-glycine. The Fmoc-protecting group was then removed by shaking with a 20% solution of piperidine in DMF twice. The deprotection reaction was monitored by ninhydrin test. The deprotected resin was washed with DMF six times.

To the resin was added a solution of Fmoc-Arg(Pbf)-OH acids (25.00 g, 38.53 mmol), 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU, 10.16 g, 31.64 mmol), 1-hydroxybenzotriazole (HOBt, 4.28 g, 31.67 mmol), and DIEA (19.68 mL, 112.98 mmol) in DMF. The mixture was shaken for 1.5 h. The resulting resin was washed with DMF six times, deprotected by shaking with a 20% solution of piperidine in DMF twice, and washed with DMF for an additional six times. Subsequently, Fmoc-Lys(Boc)-OH, Fmoc-D-Phe-OH, and Fmoc-Asp(O^{t}Bu)-OH were coupled to the resin in the same manner.

The linear RGDfK peptide was cleaved from the resin without affecting other protecting groups by treating with 250 mL of a mixture of acetic acid, 2,2,2-trifluoroethane (TFE), and DCM (1:1:3) for 1 h at room temperature. The resin was washed twice with 250 mL of the same mixture and then with DCM three times. The eluents were combined and concentrated. The excess acetic acid was azeotroped off with toluene.

The head-to-tail cyclization was performed by slowly adding a solution of the linear peptide acetate salt in 200 mL of DCM to a solution of 50% 1-propanephosphonic acid cyclic anhydride (T3P) in EtOAc (54.24 mL), triethyl amine (TEA, 63.2 mL), and DMAP (200 mg) in 8 L of DCM. After stirring overnight, the starting material could not be detected by RP-HPLC. The reaction mixture was concentrated and purified by flash chromatography (methanol:ethyl acetate, 1:10) to afford 17.23 g (MW = 1012.23, 96.7%, 17.02 mmol, relative to the amount of Gly coupled to the resin) of the protected cyclic peptide as light yellow solid.[8]

The protecting groups of the above cyclic peptide (16.05 g) were removed with a mixture of water and trifluoroacetic acid (TFA) (1:19). TFA was removed by azeotroping with toluene. Trituration of a DCM solution of the product with diethyl ether followed by filtration yielded cRGDfK·2TFA salt (MW = 831.72, 10.81 g, 82.0%) as a light yellow powder. The overall yield (79.3% relative to the amount of Gly coupled to the resin) is significantly higher than the reported yield (44%).[3a] The cyclic peptide salt obtained this way is pure and shows only one peak in

reverse phase high-performance liquid chromatography (RP-HPLC). ESI mass spectrum and NMR data of the compound were identical to those previously reported.[3a]

In conclusion, we have developed an improved multi-gram-scale solid-phase synthesis of the cRGDfK. In comparison with the original synthesis, the current procedure not only eliminates the use of several toxic reagents, but also significantly improves the yield of the synthesis. These improvements may find applications in practical, large-scale synthesis of cRGDfV and related cyclic peptides.

References

1. (a) *Integrins, Molecular and Biological Responses to the Extracellular Matrix*; Cheresh, D. A.; Mecham, R. P., Eds.; Academic Press: London, 1994. (b) *Cell Adhesion Molecules in Cancer and Inflammation*; Epenetos, A. A.; Pignatelli, M., Eds.; Harwood Academic Publishers: Chur, 1995. (c) Hynes, R. O. *Cell* **1992**, *69*, 11–25.
2. (a) Brooks, P. C.; Montgomery, A. M. P.; Rosenfeld, M.; Reisfeld, R. A.; Hu, G.; Klier, G.; Cheresh, D. A. *Cell* **1994**, *79*, 1157–1164. (b) Brooks, P. C.; Clark, C. F.; Cheresh, D. A. *Science* **1994**, *264*, 569–571. (c) Friedlander, M.; Brooks, P. C.; Schaffer, R. W.; Kincaid, C. M.; Varner, J. A.; Cheresh, D. A. *Science* **1995**, *270*, 1500–1502. (d) Lode, H. N.; Moehler, T.; Xiang, R.; Jonczyk, A.; Gillies, S. D.; Cheresh, D. A.; Reisfeld, R. A. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 1591–1596.
3. (a) Haubner, R.; Gratias, R.; Diefenbach, B.; Goodman, S. L.; Jonczyk, A.; Kessler, H. *J. Am Chem. Soc.* **1996**, *118*, 7461–7472. (b) Wermuth, J.; Goodman, S. L.; Jonczyk, A.; Kessler, H. *J. Am. Chem. Soc.* **1997**, *119*, 1328 – 1335. (c) Bach, A. C.; Espina, J. R.; Jackson, S. A.; Stouten, P. F. W.; Duke, J. L.; Mousa, S. A.; DeGrado, W. F. *J. Am Chem. Soc.* **1996**, *118*, 293–294. (d) Peishoff, C. E.; Ali, F. E.; Bean, J. W.; Calvo, R.; D'Ambrosio, C. A.; Eggleston, D. S.; Kline, T. P.; Koster, P.; Nicols, A.; Powrs, D.; Romoff, T.; Samanen, J. M.; Stadel, J.; Vasco, J.; Wong, A.; Kopple, K. D. *J. Med. Chem.* **1992**, *35*, 3962–3969. (e) Burgess, K.; Lim, D. *J. Med. Chem.* **1996**, *39*, 4520–4526. (f) Tran, T.-A.; Mattern, R.-H.; Zhu, Q.; Goodman, M. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 997–1002.
4. 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.
5. Hammes, H.-P.; Brownlee, M.; Jonczyk, A.; Sutter, A.; Preissner, K. T. *Nature Med.* **1996**, *2*, 529–533.
6. (a) Wissmann, H.; Hoechst, A.-G. *Phosphorus Sulfur* **1987**, *30*, 645–648. (b) Wissmann, H.; Kleiner, H. J.; Hoechst, A.-G. *Angew. Chem.* **1980**, *92*, 129–130
7. (a) Fields, C. G.; Fields, G. B. *Tetrahedron Lett.* **1993**, *34*, 6661–6664. (b) Carpino, L. A.; Shroff, H.; Triolo, S. A.; Mansour, E.-S. M. E.; Wenschuh, H.; Albericio, F. *Tetrahedron Lett.* **1993**, *34*, 7829–7832.
8. Retention time $t_R = 38$ min on a reverse phase C-18 column. Elution with a linear gradient over 60 min of buffer A and buffer B from 49:1 to 1:49 (buffer A = 4 mM ammonium acetate and 0.1% formic acid in water; buffer B = 4 mM ammonium acetate, 80% acetonitrile and 0.1% formic acid in water). Flow rate: 50 μL/min. Detection wavelength: 220 nm. ESI MS for $C_{49}H_{73}N_9O_{12}S$, calculated: 1011.5; found: 1011.7.