ORGANIC LETTERS

2004
Vol. 6, No. 14
2477−2480

Design and Synthesis of a Solid-Supported FR225659 Derivative for Its Receptor Screening

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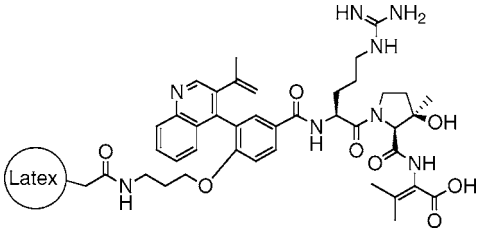
Received May 17, 2004

ABSTRACT



We describe the design and synthesis of latex particles attached to an FR225659 derivative to identify its receptor proteins. Two key building blocks were prepared by two-step degradation of FR225659 under basic conditions. The designed ligand showed an acceptable level of biological activity to make it of potential value for use in affinity-supported receptor identification. Affinity purification of FR225659-binding proteins using the latex nanoparticles provided three candidate receptor peptides for the biological activity.

Affinity chromatography technology[1] using solid-supported biologically active small molecules is a powerful methodology for identifying target receptors and proteins of small molecules from cell lysates. We have already reported effective latex nanoparticles composed of a styrene-glycidyl methacrylate copolymer for affinity chromatography.[2,3] The polyglycidyl surface shows relatively little nonspecific adsorption of proteins and can be reacted with amino groups to load ligands. Furthermore, the large total surface area is especially effective for purifying receptors from a small amount of cell lysate. As a result, latex nanoparticles attached to biologically active natural products are highly effective tools for chemical biology. Unfortunately, the immobilization reactions that attach the biologically active natural products

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(1) (a) Cuatrecasas, P.; Wilchek, M.; Anfinsen, C. B. *Proc. Natl. Acad. Sci. U.S.A.* **1968**, *61*, 636−643. (b) Cuatrecasas, P. *Annu. Rev. Biochem.* **1971**, *40*, 259−278. (c) Schreiber, S. L. *Bioorg. Med. Chem.* **1988**, *6*, 1127−1152. (d) Harding, M. W.; Galat, A.; Uehling, D. E.; Schreiber, S. L. *Nature* **1989**, *341*, 758−760. (e) Stockwell, B. R.; Hardwick, J. S.; Tong, J. K.; Schreiber, S. L. *J. Am. Chem. Soc.* **1999**, *121*, 10662−10663.

(2) (a) Wada, T.; Watanabe, H.; Kawaguchi, H.; Handa, H. *Method Enzymol.* **1995**, *254*, 595−604. (b) Shimizu, N.; Sugimoto, K.; Tang, J.; Nishi, T.; Sato, I.; Hiramoto, M.; Aizawa, S.; Hatakeyama, M.; Ohba, R.; Hatori, H.; Yoshikawa, T.; Suzuki, F.; Oomori, A.; Tanaka, H.; Kawaguchi, H.; Watanabe, H.; Handa, H. *Nat. Biotechnol.* **2000**, *18*, 877−881.

(3) For a review of latex nanoparticles, see: Kawaguchi, H. *Prog. Polym. Sci.* **2000**, *25*, 1171−1210.

10.1021/ol049100q CCC: $27.50
Published on Web 06/18/2004

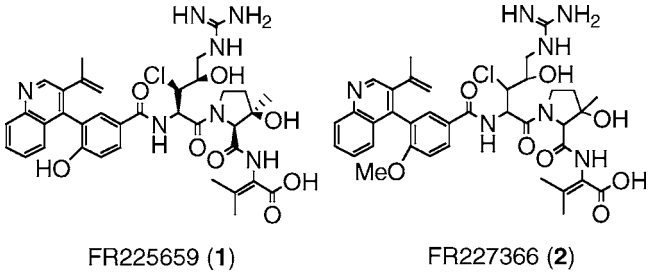


Figure 1. Structure of FR225659 (**1**) and FR227366 (**2**).

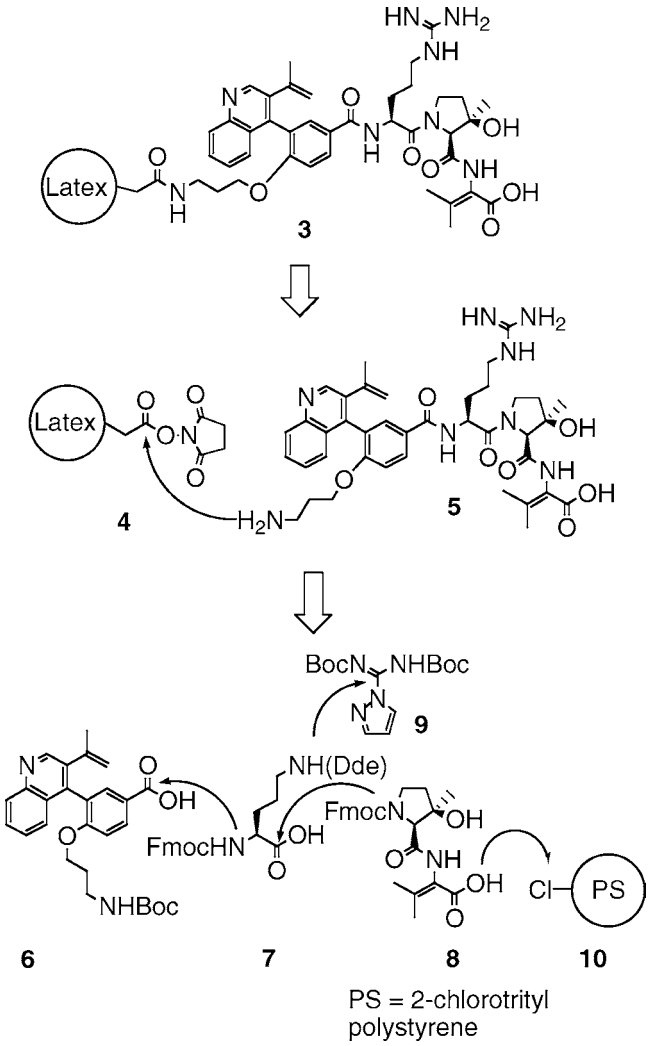
to nanoparticles are often problematic, particularly when the supporting molecule contains several unstable functional groups.

FR225659 (**1**) and FR227366 (**2**) were isolated from the cultured broth of fungal strain no. 19353, which originated from a decayed leaf.[4] Both molecules share a unique structure that consists of a carboxylic acid and three unusual amino acids (Figure 1). They also potently inhibit glucagon-induced gluconeogenesis in vitro. Although their tripeptide structures might cause them to be easily metabolized in vivo, they could nevertheless serve as useful ligands for identifying new antidiabetic drug targets. Therefore, we planned to synthesize latex nanoparticles coupled to an FR225659 derivative for receptor screening. Herein we describe the synthesis of an FR225659 derivative bound via an aminoalkyl group onto the solid-phase of latex nanoparticles and its use for identifying candidate receptors.

The design of the solid-supported FR225659 derivative **3** is shown in Scheme 1. The supported tripeptide **3** was selected for affinity chromatography because the biological activity of the methyl ether **2** was comparable to that of the phenol **1**. It was envisaged that the tripeptide amine **5** would undergo chemoselective acylation with the solid-supported activated ester **4** to provide the solid-supported FR225659 derivative **3**. An arginine moiety was selected instead of the 3-chloro-4-hydroxyl arginine to improve the chemical stability of the resulting ligand.

The solid-phase strategy employed for the synthesis of amine **5** was based upon the sequential coupling of four building blocks **6**−**9**; 2-chlorotrityl polystyrene **10** would be used as the solid-support for the synthesis. The unique carboxylic acid **6** and dipeptide **8** would both be obtained by degradation of naturally occurring FR225659 (**1**). α-*N*-Fmoc and *δ*-*N*-[1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl] (Dde)[5] ornithine (**7**) were selected instead of the arginine moiety, as the direct coupling of arginine derivatives

Scheme 1. Strategy for the Synthesis of Solid-Supported FR225659 for Receptor Screening



with solid-supported amines often results in low yields due to generation of the cyclic product. Selective deprotection of the *δ*-amino group, followed by coupling of amidine **9** would provide the desired arginine derivatives.

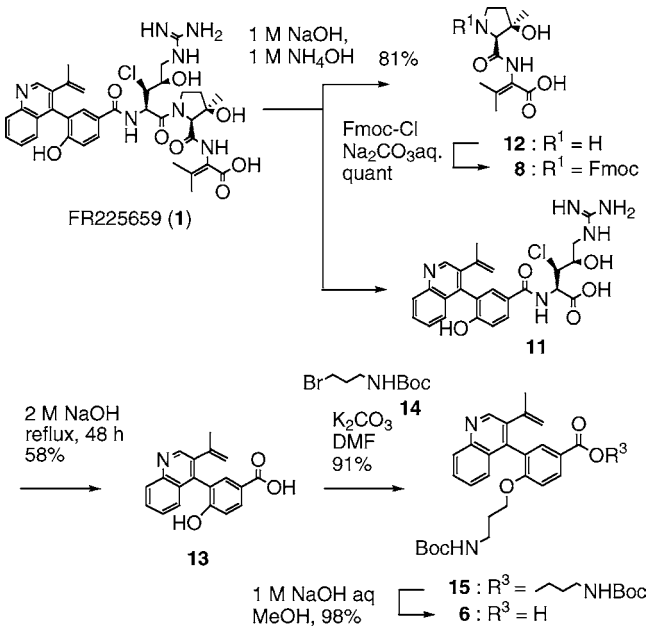
The preparation of building blocks **6** and **8** is shown in Scheme 2. Hydrolysis of FR225659 (**1**) under mildly basic conditions effected amide bond cleavage between the arginine and proline moieties. Purification of the crude mixture was accomplished by reverse-phase column chromatography to afford dehydroamino acid derivative **12** in 81% yield and a mixture containing the aromatic acyl derivative **11**. The dipeptide **12** was converted to the Fmoc derivative **8** by acylation with Fmoc-Cl in quantitative yield. Further degradation of the mixture containing **11** was achieved by heating it under basic conditions; this provided the acid **13** in 58% yield from **1**. Treatment of **13** with bromide **14** in the presence of K_2CO_3 afforded the dialkylated product **15** in 91% yield, whose ester could be hydrolyzed to provide acid **6** in 98% yield.

The solid-phase synthesis of amine **5** is disclosed in Scheme 3. Loading of the dipeptide **8** onto 2-chlorotrityl

(4) (a) Ohtsu, Y.; Sasamura, H.; Tsurumi, Y.; Yoshimura, S.; Takase, S.; Hashimoto, M.; Shibata, T.; Hino, M.; Fujii, T. *J. Antibiot.* **2003**, *56*, 682−688. (b) Ohtsu, Y.; Sasamura, H.; Shibata, T.; Nakajima, H.; Hino, M.; Fujii, T. *J. Antibiot.* **2003**, *56*, 689−693. (c) Zenkoh, T.; Ohtsu, Y.; Yoshimura, S.; Shigematsu, N.; Takase, S.; Hino, M. *J. Antibiot.* **2003**, *56*, 694−699.

(5) (a) Bycroft, B. W.; Chan, W. C.; Chhabra, S. R.; Hone, N. D. *J. Chem. Soc., Chem. Commun.* **1993**, 778−779. (b) Chhabra, S. R.; Hothi, B.; Evans, D. J.; White, P. D.; Bycroft, B. W.; Chan, W. C. *Tetrahedron Lett.* **1998**, *39*, 1603−1606.

Scheme 2



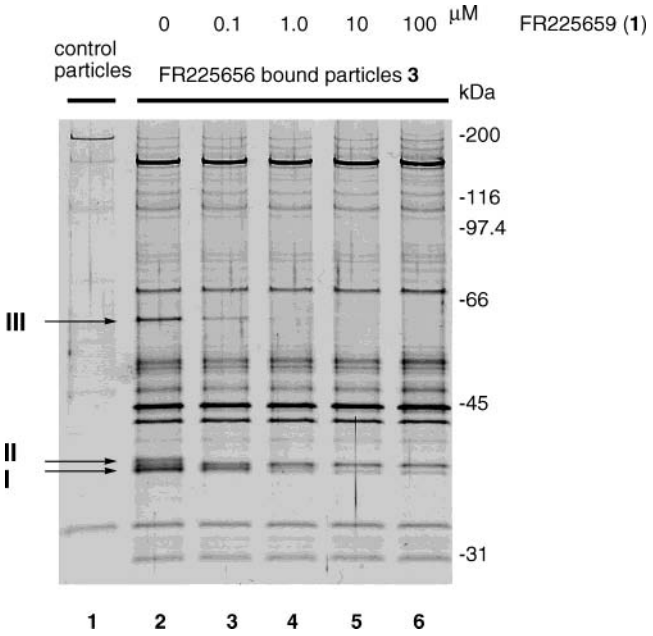


Figure 2. Elucidation of FR225659-specific binding proteins in rat hepatocyte cell lysates. Lane 1 showed binding of proteins to control particles in buffer (10mM Tris-HCl pH 7.4, 100 mM NaCl, 1.0 mM $MgCl_2$, 0.2 mM EDTA, 10% glycerol, 0.1% Nonidet P-40, 1.0 mM $CaCl_2$ and 0.5 mM DTT) at 4 °C for 1 h. Lane 2 showed binding of proteins to FR225659-bound particles **3**. Lanes 3−6 showed binding of proteins to FR225659-bound particles **3** in the presence of FR225659 (**1**). The concentrations of **1** were 0.1 μg/mL (lane 3), 1 μg/mL (lane 4), 10 μg/mL (lane 5), and 100 μg/mL (lane 6).

resin[6] (1.25 mmol/mg) was achieved using diisopropylethylamine (DIEA) in dichloromethane to provide the solid-supported dipeptide **16**. Loading was determined to be quantitative by cleavage from the resin under acidic conditions, and by measurement of the mass recovery of **8**. Deprotection of the Fmoc group, followed by coupling with ornithine **7** in the presence of tetramethylfluoroformidinium hexafluorophosphate (TFFH)[7] and DIEA, provided the solid-supported tripeptide **17**. Removal of the Fmoc group, followed by acylation with benzoic acid derivative **6** using DIC−HOBt in *N*-methylpyrrolidinone, afforded the solid-supported acyl tripeptide **18**. Finally, conversion of the ornithine residue into an arginine was examined. Selective deprotection of the Dde protecting group was achieved with hydrazine. This was followed by coupling of the resulting amine with amidine **9**[8] to provide the Boc-protected guanidine **19**. Cleavage of the product from the resin with acetic acid−trifluoroethanol−dichloromethane (1:1:8), followed by purification by preparative TLC, provided the tri-Boc-protected carboxylic acid **20** in 82% yield from **16**. Removal of the tri-*N*-Boc groups in 40% TFA−dichloromethane solution at ambient temperature provided the amine derivative **5** as its TFA salt in 91% yield with 95% purity. Its purity was estimated by HPLC on the basis of total UV absorption from 190 to 600 nm (interval, 2.0 nm) using a diode array detector.

Glucagon-induced gluconeogenesis inhibitory activity in primary cultured rat hepatocytes was examined.[4b] In this assay, the IC_{50} value of the amine derivative **5** (IC_{50} = 0.830 μM) was comparable to that of FR225659 (**1**) (IC_{50} = 0.530 μM) as a positive control. These results indicated that the amino derivative **5** could be used to prepare the affinity particles for its receptor identification.

Treatment of the activated ester **4**[9] on the latex nanoparticles with a 0.2 M solution of **5** in 0.2 M acetic acid sodium salt buffer (500 μL) for 1 h at room temperature, followed by amidation of the remaining activated esters with 2-hydroxyethylamine, provided the affinity particles **3**. Conversion of any remaining activated esters to the 2-hydroxyethylamides was effective for reducing nonspecific interactions of the particles with proteins. HPLC analysis of the acid degradation product of **3** showed a loading amount of 38.9 nmol/mg. The results of the FR225659 affinity binding experiments using a cytoplasmic fraction of rat hepatocytes are shown in Figure 2. Control latex particles[10] showed no binding proteins (lane 1). In contrast, FR225659-bound particles **3** showed binding to a number of proteins (lane 2). FR225659 (**1**) specifically inhibited the binding of particle **3** to three peptides I−III in a concentration-dependent manner

(6) 2-Chlorotrityl resin (UniSphere 200) was purchased from IRORI, San Diego, CA.

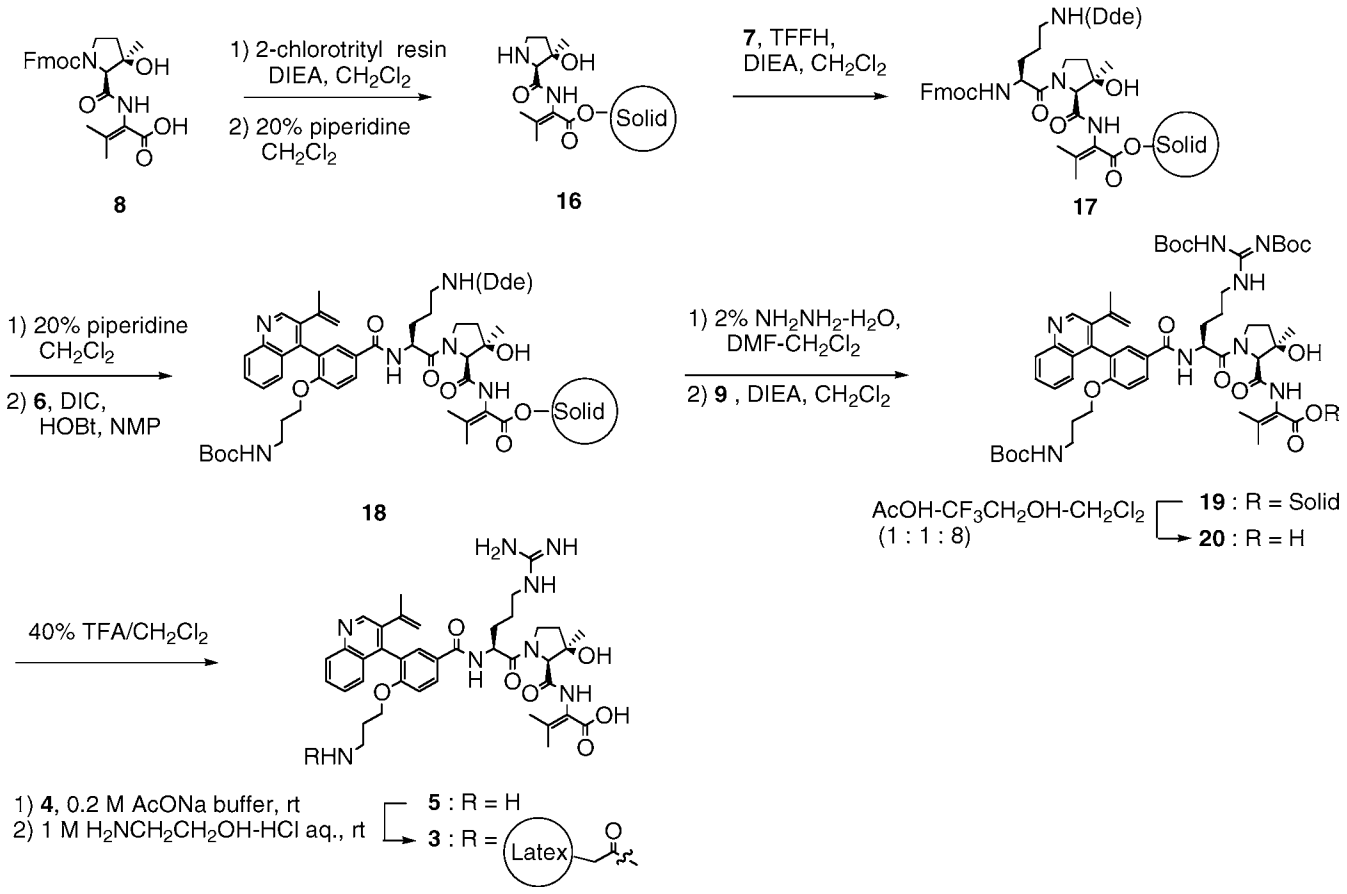
(7) Carpino, L. A.; El-Faham, A. *J. Am. Chem. Soc.* **1995**, *117*, 5401−5402.

(8) (a) Robinson, S.; Roskamp, E. J. *Tetrahedron* **1997**, *53*, 6697−6705. (b) Yong, Y. F.; Kowalski, J. A.; Thoen, J. C.; Lipton, M. A. *Tetrahedron Lett.* **1999**, *40*, 53−56.

(9) Activated ester **4** was prepared by acylation of the amino-bearing latex particles (SGNEGDEN)[2b] with succinic anhydride, followed by esterification of resulting acids with *N*-hydroxysuccinimide.

(10) Control resin was prepared by amidation of the activated ester **4** with 2-hydroxyethylamine.

Scheme 3



(lanes 3−6). They possess apparent molecular masses of 36, 37, and 60 kDa, respectively. The three peptides, which appear to specifically bind to FR225659 in a dose-dependent manner (Figure 2), may serve as candidate receptors for the inhibition of glucagon-induced gluconeogenesis. Current studies include sequence determination for the peptides, which may play an important role in diabetes, and elucidation of the biological outcomes induced by the binding of **1**.

In conclusion, we have described the synthesis of latex nanoparticles **3** coupled to the bioactive FR225659 derivative (loading 38.9 nmol/mg). Affinity purification of FR225659-binding proteins using the latex nanoparticles **3** provided three candidate peptides for the biological activity. Elucidation of the biological roles of these peptides is in progress.

Acknowledgment. This work was performed under the management of the Research Association for Biotechnology as a part of the Industrial Science and Technology Frontier Program supported by NEDO (New Energy and Industrial Technology Development Organization).

Supporting Information Available: Experimental procedures for the degradation and the solid-phase synthesis and full characterization for compounds **5**, **6**, **8**, **12**, **13**, **15** and **20**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL049100Q