Automated Maskless Photolithography System for Peptide Microarray Synthesis on a Chip

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Received January 19, 2010

Maskless photolithographic peptide synthesis was performed on a glass chip using an automated peptide array synthesizer system. The peptide array synthesizer was built in a closed box, which contained optical and fluidic systems. The conditions for peptide synthesis were fully controlled by a computer program. For the peptide synthesis on a glass chip, 20 NVOC-protected amino acids were synthesized. The coupling efficiencies of two model peptide sequences were examined on ACA/APTS and PEG/CHI/GPTS chips. PEG/CHI/GPTS chip gave higher average stepwise yields of GIYWHHY (94%) and YIYGSFK (98%) than those of ACA/APTS chip. To quantify peptide−protein binding affinity, HPQ- or HPM-containing pentapeptides were synthesized on a PEG/CHI/GPTS chip and the binding event of Cy3 labeled-streptavidin was quantified. The peptide sequence of IQHPQ showed highest binding affinity with Cy3 labeled-streptavidin. The results demonstrated that the photolithographic peptide array synthesis method efficiently quantified the binding activities of protein-peptide interactions and it can be used for additional biological assay applications.

Introduction

Peptide microarrays have emerged for high-throughput screening of various biosystems including binding affinity and enzyme substrate screening.[1] Peptide microarrays can be used as the part of proteins for the elucidation of protein-biomolecule affinity.[2] Moreover, various enzyme substrates, such as protein kinase, phosphatase, and protease substrates have been screened using peptide microarrays.[3]

In general, there are two methods for the preparation of peptide microarrays: immobilization of pre-synthesized peptide derivatives and in situ synthesis of peptides on a chip.[4] An overview of peptide microarray preparation method is summarized in Table 1.[5] The method of spotting pre-synthesized peptide ensures a high quality chip because spotting materials can be obtained at high purity. However, chip design is highly restricted depending on the number of pre-synthesized peptides. Moreover, spots form with greater peptide density at the edges than in the middle because solution flows to the spot edge during solvent evaporation. The background is prone to contamination during washing step because of the activated background. The peptide should be modified to aminooxy acetyl group, cysteine residue, or cyclopentadiene for the proper chemoselective reaction.[6] In contrast, the in situ synthesis method is flexible for chip design so that the peptide microarray can be easily prepared with a well-established synthesis system. The spot shape of the in situ synthesis method is regular because solutions contact the chip surface homogeneously. The background signal from the in situ synthesis method is relatively lower than that produced by spotting pre-synthesized peptides because the background surface is selectively inert. However, the quality of peptide from the in situ synthesis method is lower than that of the spotting method because the peptide synthesized on a chip cannot be purified. Other than the SPOT synthesis method,[5c] light-directed, spatially addressable peptide array synthesis is a representative in situ synthesis method and was introduced by combining solid phase peptide synthesis with a semiconductor fabrication system.[5g] A photolabile protecting group on a chip is deprotected by selective UV irradiation with a photomask. Peptide microarray can be synthesized by repeated cycles of photodeprotection and coupling steps. Recently, the photomasks have been replaced with a digital micromirror array (MMA) for patterning of biomolecules.[5h,7] A method of using photogenerated acid (PGA) in the maskless photolithography system was reported by the Gao group to synthesize oligo-nucleotide and peptide microarrays.[5d−f] Instead of using a photolabile protecting group, they used commercially available, 4,4′-dimethoxytrityl (DMT) protected nucleotides and *tert*-butyloxycarbonyl (Boc) protected amino acids. However, for this method, a physical or chemical barrier should be constructed on a chip to prevent the diffusion of PGA to other regions.[8]

The present study focuses on peptide microarray synthesis on a glass chip using an automated maskless photolithography system. Twenty NVOC-amino acid monomers were synthesized, and peptide synthesis was optimized on a chip. To evaluate the photolithographic synthesis method, stepwise

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10.1021/cc100009g
Published on Web 05/18/2010

Table 1. Overview of Peptide Microarray Preparation Methods

		in situ synthesis		
			photolithographic synthesis	
	spotting pre-synthesized peptide	SPOT synthesis	PGA mediated method	photolabile protecting group mediated method
chemistry	chemoselective ligation	Fmoc chemistry	Boc chemistry	NVOC chemistry
synthesis efficiency	high	data not available	high	moderate
chip design	not flexible	flexible	flexible	flexible
spot size	~100 μm	~100 μm	>40 μm	~25 μm, ~200 μm (variable)
spot feature	coffee-ring shape	regular	regular	regular
background	activated surface	physical barrier	physical or chemical barrier	photolabile protecting group

Table 2. List of Chemical Abbreviations Used in the Article

abbreviation	chemical	abbreviation	chemical
ε-ACA	ε-aminocaproic acid	HOBt	1-hydroxybenzotriazole
APTS	3-aminopropyltriethoxysilane	HPM	His-Pro-Met
Boc	*tert*-butyloxycarbonyl	HPQ	His-Pro-Gln
BOP	benzotriazol-1-yloxy-tris (dimethylamino)phosphonium hexafluorophosphate	MeOH	methanol
CHI	chitosan	NMP	*N*-methylpyrrolidone
2-CTC resin	2-chlorotrityl chloride resin	NVOC	6-nitroveryloxycarbonyl
DCM	dichloromethane	PEG-SA	*O*,*O*′-bis-(2-aminopropyl) polypropylene glycol-block-polyethylene glycol 500-succinic acid
DIPEA	diisopropylethylamine	TEA	triethylamine
EtOAc	ethyl acetate	TFA	trifluoroacetic acid
RBITC	rhodamine B isothiocyanate	THF	tetrahydrofuran
GPTS	3-glycidoxypropyltrimethoxysilane	Trt	trityl
HATU	*O*-(7-azabenzo-triazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexa-fluorophosphate		

peptide coupling yields were checked on various surface-modified glass chips, and the protein binding assay was performed on each of the peptide microarray.

Experimental Section

Materials. 3-Aminopropyltriethoxysilane (APTS), 3-glycidoxypropyltrimethoxy-silane (GPTS), chitosan (CHI) (75−85% deacetylated chitin, M_N = 50,000−190,000), 4,5-dimethoxy-2-nitrobenzyl alcohol, biotin, 1-hydroxybenzotriazole (HOBt), diisopropylethylamine (DIPEA), triethylamine (TEA), trifluoroacetic acid (TFA), benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP), *O*-(7-azabenzo-triazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexa-fluorophosphate (HATU), succinic anhydride, magnesium sulfate (anhydrous), ninhydrin, phenol, pyridine, chloroform-*d*, methanol-*d*,[4] dimethylsulfoxide-*d*,[6] amino acid, and their derivatives were purchased from Aldrich. Streptavidin-Cy3 conjugates, Rhodamine B isothiocyanate (RBITC), ε-aminocaproic acid (ε-ACA), and polyoxyethylene sorbitan monolaurate (Tween 20) were purchased from Sigma. *O*,*O*′-Bis-(2-aminopropyl) polypropylene glycol-*block*-polyethylene glycol-*block*-polypropylene glycol 500 (Jeffamine ED-600) was purchased from Fluka. 2-Chlorotrityl chloride resin (2-CTC resin), Fmoc-amino acid, and their derivatives were purchased from BeadTech Inc. Sodium hydroxide, disodium hydrogen phosphate, and sodium dihydrogen phosphate were purchased from Oriental Chemical Industry. *N*-Methylpyrrolidone (NMP), chloroform, dichloromethane (DCM), dioxane, methanol (MeOH), tetrahydrofuran (THF), ethyl acetate (EtOAc), and hexane were purchased from Junsei Chemical Co. All solvents were purified by methods reported in the literature.[9] The glass slides (75 mm ×25 mm, Micro Slides # 2948) were purchased from Corning. Chemical abbreviations are summarized in Table 2.

Instruments. A microarray scanner (Axon Instrument, GenePix 4000B) was used for analyzing fluorescence intensity. NMR spectra were recorded on a JNM-LA300 spectrometer (Jeol Inc.) in deuterated solvents and were referenced to TMS (δ scale). For maskless photolithography, UV light passed through 365 nm band-pass filter from an illuminator (Hamamatsu Photonics, Mercury−Xenon lamp # L2570).

Peptide Array Synthesizer. The peptide array synthesizer consists of a UV light source, MMA, projection lenses, a UV bandpass filter, an optical stop, a chemical reaction chamber, and control valves for flow system as shown in Figure 1. MMA was fabricated with single crystalline silicon by micromachining a spatial UV light modulator.[10] The micromirrors were 210 μm × 210 μm of single crystalline silicon. The micromirrors were 16 × 16 arrayed and had a 5 μm gap from the bottom electrode (Figure 2). The reagents for amino acid coupling were injected through the inlet port by controlling valves and the pressure of nitrogen stream. The reaction chamber was sealed by a slide glass, on which surface peptides were synthesized. UV irradiation, solution delivery, reaction time, and washing condition were controlled by programming language, LABVIEW (National Instruments Co.) (Figure 3).

Syntheses of NVOC-Amino Acids.[11] Amino acids (2 mmol) were dissolved in 2 *N* aq. NaOH solution (1 mL). 6-Nitroveratryloxycarbonyl chloride (NVOC-Cl; 662 mg, 2.4 mmol) in 1,4-dioxane (1 mL) and DCM (4 mL) and 2 *N* aq. NaOH solution (1.1 mL) were added to the amino acid in NaOH solution in turns for 10 times in an ice bath. After

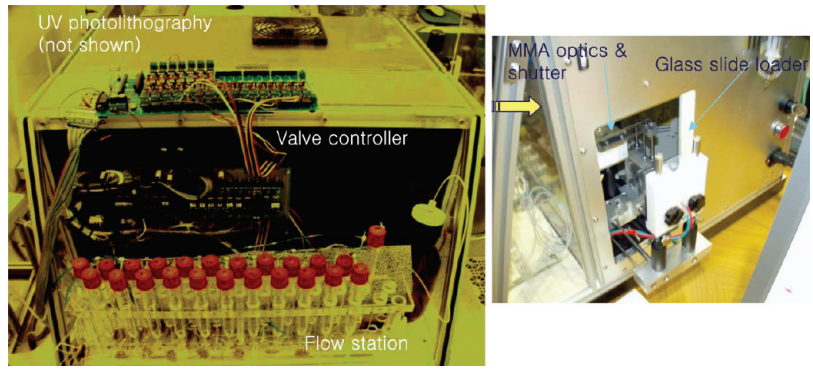


Figure 1. Photograph of peptide array synthesizer system.

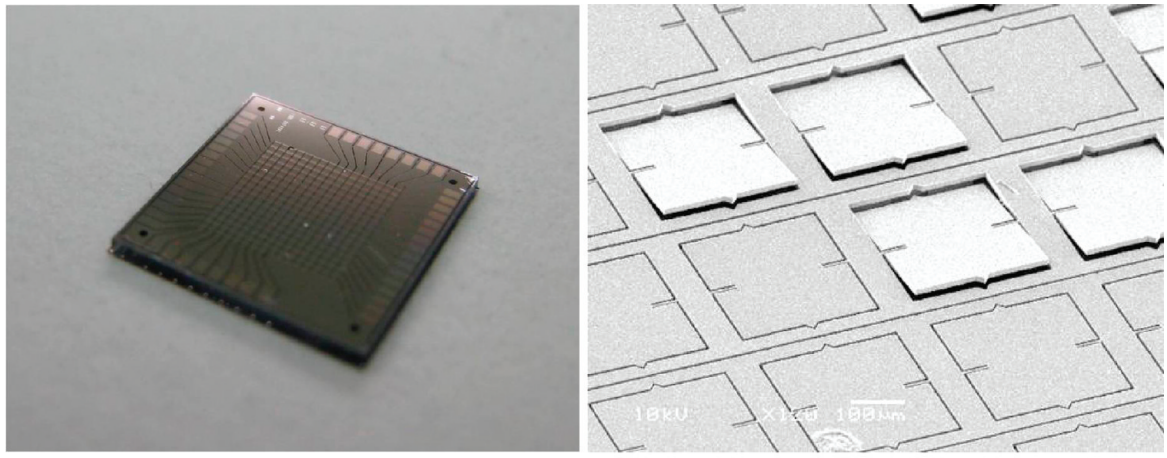


Figure 2. (a) Single crystalline silicon MMA (16 × 16 mirrors), (b) enlarged view of MMA (210 μm × 210 μm).

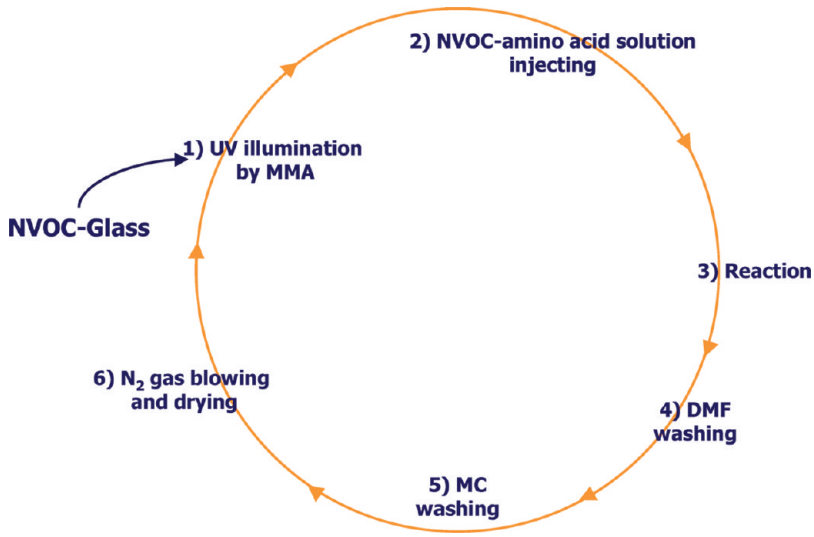


Figure 3. Reaction cycle of peptide synthesis.

stirring for 1 h, the ice bath was removed, and the solution was vigorously stirred for 4−12 h. The progress of the reaction was monitored by TLC. The organic layer was removed, and the aqueous layer was acidified to pH 3−4 using 5 *N* aq. HCl solution. Side chain-protected amino acids were acidified by acetic acid. The product was extracted by EtOAc and dried by $MgSO_4$, and the solvent was evaporated. When acetic acid was added, the remaining acetic acid residue in the product was evaporated azeotropically with hexane three times.

Synthesis of NVOC-PEG-SA. 6-Nitroveratryloxycarbonyl-Jeffamine ED-600-succinic acid (NVOC-PEG-SA) was synthesized using solid phase chemistry. 2-CTC resin (10 g, 1.0 mmol/g) was swollen in TEA (50 mmol, 7.0 mL)/DCM (100 mL), and Jeffamine ED-600 (100 mmol, 60 g) was added. The reaction mixture was stirred at 25 °C for 12 h. The solution, which contained excess reagents, was filtered out, and the resin was washed with DCM (×3) and MeOH (×3). The resin was swollen in DIPEA (25 mmol, 4.4 mL) and DCM (100 mL). The solution of DIPEA (25

mmol, 4.4 mL) and NVOC-Cl (20 mmol, 5.5 g) in DCM was added into the resin mixture and stirred at 25 °C for 12 h. After washing with the above method, 5% (v/v) TFA in DCM (120 mL) was added and stirred at 25 °C for 30 min. The resin was filtered, and the filtrated solution was collected. After the solvent was completely evaporated, the remaining residue was dissolved in DIPEA (15 mmol, 2.6 mL)/THF (25 mL). Succinic anhydride (15 mmol, 1.5 g) in THF (25 mL) was added to the solution, and the solution was magnetically stirred. The product was separated by flash column (chloroform/MeOH = 5:1).

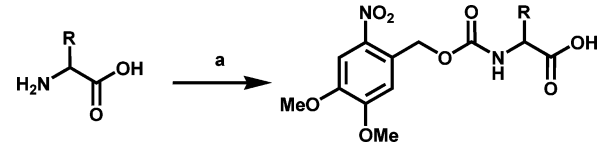
Surface Modification of Glass Slides.[12] The glass slides were precleaned in a mixture of H_2SO_4 and H_2O_2 (4:1) for 10 min and rinsed with H_2O, ethanol, and dried in vacuo. The amination or epoxidation process was performed on the glass surface by two different routes. Silanizations with 3-aminopropyltriethoxysilane (APTS) or 3-glycidoxypropyltrimethoxysilane (GPTS) was carried out at 45 °C in 5% (v/v) chloroform solution for 2 or 12 h, respectively. To remove the non-covalently adsorbed silane molecules, sonication in chloroform was performed for 10 min. The glass slides were rinsed with ethanol and blown dry with nitrogen gas. The attachment of the hydrophilic polymer, chitosan (CHI), was carried out on a GPTS treated glass slide with 1% (w/v) CHI in 1% AcOH-H_2O at 25 °C for 12 h. The slides were then sonicated in H_2O for 10 min, rinsed extensively with H_2O, and blown dry with nitrogen gas. To generate a photolabile NVOC-protected surface, the aminated surface or CHI-treated surface was exposed to a 5 mM solution of NVOC-ε-aminocaproic acid (NVOC-ε-ACA−OH) or NVOC-PEG-SA, BOP, HOBt, and DIPEA in DMF at 25 °C for 2 h. The samples were then rinsed with DMF and DCM, and dried by a nitrogen stream.

Optimization of Peptide Synthesis on Glass Slides. The reaction chamber was prefilled with 5 mM sulfuric acid/dioxane to reduce side reactions during the photocleavage reaction.[13] By using MMA, UV light was irradiated on the specific region of the NVOC-protected glass surface for 20 min, as previously reported.[10] Stepwise coupling efficiencies of the synthesized peptides on the glass slides were calculated from the ratios of fluorescence intensities of RBITC using the following procedures.[14] After UV light was irradiated on the NVOC-protected glass surface using MMA, a 5 mM solution of NVOC-amino acid, HATU, and DIPEA in NMP were injected and coupled to the exposed free amino groups on the surfaces for 20 min. The unreacted amino groups were capped by 10% acetic anhydride/10% pyridine in NMP for 10 min. As the number of on-state micromirrors was reduced through proper direction of the UV irradiation, the coupling and capping steps were repeated. Finally, droplets of 5 mM DIPEA and RBITC/NMP were added to the free amino groups to quantify stepwise peptide coupling efficiency by the equation below:[15]

$$\%\text{Yield (step } n) = (I_n/I_{n-1}) \times 100$$

where I_n = fluorescence intensity for peptide oligomer of length n and I_{n-1} = fluorescence intensity for peptide oligomer of length $n - 1$.

Scheme 1. Synthesis of NVOC-Amino Acid[a]



[a] Reagents and conditions: (a) NVOC-Cl (1.2 equiv), 2 N aq. NaOH (2.1 equiv) in (1:4) 1,4-dioxane and DCM, 0 °C→RT, 4−12 h.

Table 3. Yield of NVOC-Amino Acids

NVOC-amino acid	yield (%)	NVOC-amino acid	yield (%)
NVOC-Gly-OH	76	NVOC-Ser(*t*Bu)−OH	94
NVOC-Ala-OH	82	NVOC-Thr(*t*Bu)−OH	54 (92[a])
NVOC-Leu-OH	88	NVOC-Cys(Trt)−OH	21
NVOC-Ile-OH	78	NVOC-His(Trt)−OH	88 (94[a])
NVOC-Phe-OH	74	NVOC-Gln(Trt)−OH	52 (94[a])
NVOC-Val-OH	83	NVOC-Asn(Trt)−OH	25
NVOC-Pro-OH	90	NVOC-Glu(*t*Bu)−OH	23 (94[a])
NVOC-Trp-OH	96	NVOC-Asp(*t*Bu)−OH	94
NVOC-Met-OH	64	NVOC-Lys(Boc)−OH	24
NVOC-Arg(Pbf)−OH	45	NVOC-ε-ACA−OH	91
NVOC-Tyr(*t*Bu)−OH	93		

[a] Products were acidified with acetic acid before extracting with EtOAc.

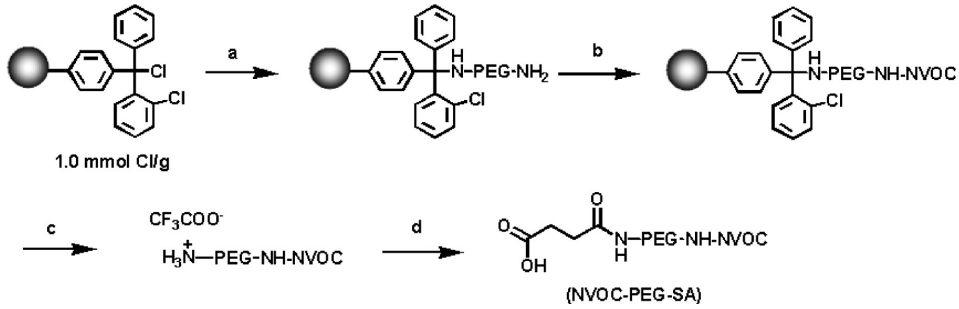
Preparation of Peptide Microarray. UV light was irradiated for 20 min on the NVOC-protected surface placed in the peptide array synthesizer. The exposed surface amino groups were coupled with 5 mM NVOC-amino acid, HATU, and DIPEA in DMF at 25 °C for 20 min. The slide was then rinsed with DMF and DCM, and it was dried by a nitrogen stream. After repeating UV irradiation, coupling of monomer, and surface washing, the side chain-protecting groups of each amino acid were removed by Reagent K (85% TFA, 5% phenol, 5% thioanisol, 2.5% ethandithiol, 2.5% H_2O) at 25 °C for 30 min, and the slide was rinsed and dried.

Binding of Cy3-Conjugated Streptavidin on Peptide Microarray. The peptide microarray was exposed to the phosphate buffer solution of Cy3-conjugated streptavidin (1 μg/mL) at 25 °C for 30 min. The glass slide was rinsed with 0.5% (v/v) Tween 20 dissolved in the phosphate buffer and dried by a nitrogen stream. The protein-patterned images were observed using a microarray scanner.

Results and Discussion

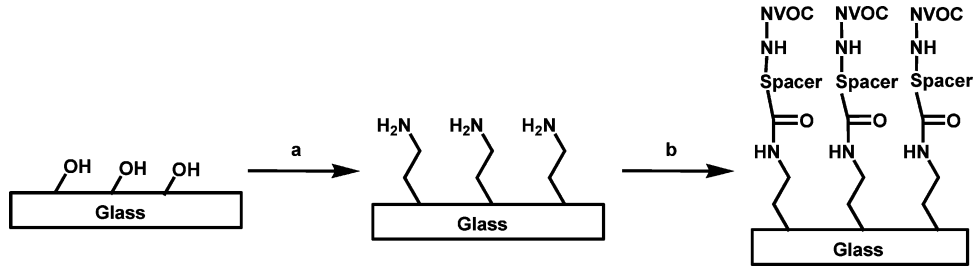
Synthesis of NVOC-Amino Acid. The 6-Nitroveratryloxycarbonyl (NVOC) group is a representative photolabile protecting group in solid phase peptide synthesis. Twenty NVOC-protected amino acids and NVOC-ε-aminocaproic acid were synthesized from NVOC-Cl and the corresponding amino acids under the conventional Schötten-Baumann conditions (Scheme 1). After introducing the NVOC-group, extraction with EtOAc gave good results of final products, especially for hydrophobic side chain-containing amino acids. Their yields were 64−96% (Table 3). Some amino acids with acid-sensitive side chain-protecting groups (e.g., trityl or *t*-butyl containing amino acids) were hardly extracted by EtOAc after acidification by aq. HCl solution. They formed insoluble materials, which were suspended in solution after treatment with aq. HCl solution. As a result, the normal extraction procedure produced poor yields. In particular, Cys(Trt), Asn(Trt), Glu(*t*Bu), and Lys(Boc) gave yields of

Scheme 2. Synthesis of NVOC-PEG-SA [a]



[a] Reagents and conditions: (a) Jeffamine ED-600 (10 equiv), TEA (5 equiv) in DCM, 25 °C, 12 h; (b) NVOC-Cl (2 equiv), DIPEA (2.5 equiv) in DCM, 25 °C, 12 h; (c) 5% (v/v) TFA in DCM, 25 °C, 30 min; (d) succinic anhydride (1.5 equiv), DIPEA (1.5 equiv) in THF, 25 °C, 12 h.

Scheme 3. Coupling of NVOC-Spacers to the Aminated Glass Surface[a]



[a] Reagents and conditions: (a)(i) 5% APTS/chloroform, 45 °C, 2 h, (ii) 5% GPTS/chloroform, 45 °C, 12 h and then 1% (w/v) CHI in 1% AcOH-H_2O, 25 °C, 12 h; (b) 5 mM NVOC-ε-ACA−OH or NVOC-PEG-SA, BOP, HOBt, DIPEA in DMF, 25 °C, 12 h.

approximately 20%. As side chain-protecting groups were acid labile, the strong acid probably caused some side reaction during the workup. However, by acidifying them with acetic acid, the products were extracted more easily than with hydrochloric acid. All yields increased to greater than 90%.

Synthesis of NVOC-PEG-SA. The 6-nitroveratryloxycarbonyl-*O*,*O*′-bis-(2-aminopropyl)polypropylene glycol-*block*-polyethylene glycol 500-succinic acid (NVOC-PEG-SA) was synthesized via two steps; monoprotection of diamino PEG (Jeffamine ED-600) and subsequent succinylation of the remaining amino group (Scheme 2). First, monoprotection of the diamino group was performed on the 2-chlorotritylchloride (2-CTC) resin. It was reported that solution phase synthesis produced the monoprotected diamino group at less than 50% yield because the isolation and purification steps were troublesome. However, the efficiency of monoprotection via the solid phase method turned out to be higher than the solution phase method when an excess of diamino PEG was used for attachment to the 2-CTC resin. By virtue of the mild acid cleavage conditions (1 to 5% TFA in DCM) of 2-CTC resin, monoprotection of the bifunctional group can be achieved even with an acid labile Boc group. Excess reagent (Jeffamine ED-600) could be reused after evaporation of the solvent. After succinylation, the product was separated by flash column chromatography, and the overall yield was 91%.

Surface Modification and Peptide Coupling Efficiency. We previously reported that a hydrophilic polymer (e.g., chitosan (CHI)) grafted surface reduced nonspecific binding.[12] In this study, CHI was grafted on an epoxy group modified surface, which was produced by GPTS. The APTS modified surface was also prepared to compare peptide synthesis efficiency to that of the CHI surface. In addition, the resulting aminated surfaces were introduced by two types of spacers, NVOC-ε-ACA−OH and NVOC-PEG-SA. NVOC-ε-ACA−OH was coupled as a hydrophobic spacer and NVOC-PEG-SA as a hydrophilic spacer (Scheme 3). The surfaces were named ACA/APTS and PEG/CHI/GPTS, respectively.

Two peptide sequences were synthesized to assess the stepwise coupling efficiency during peptide synthesis on a glass chip. Two model peptides, GIYWHHY and YIYGSFK, which are active substrates for tyrosine kinase $p60^{c\text{-}src}$, were synthesized on the ACA/APTS and PEG/CHI/GPTS glass surfaces. Figure 4 shows the procedure of coupling efficiency measurement. UV light was irradiated on a specific surface region from MMA. The NVOC-amino acid was coupled, and the remaining free amino groups were acetylated. The irradiation, coupling, and capping steps were repeated as the number of on-state micromirrors was reduced through the proper direction during the UV irradiation step. Finally, after all the protecting groups were removed, RBITC was coupled to the remaining amino groups. The resulting surfaces provided information for the stepwise coupling efficiency, and the ratios of fluorescence intensities were calculated. In GIYWHHY synthesis, the coupling efficiency was prominently lowered, particularly in the step where the surface amino groups were coupled with NVOC-His(Trt) which possessed a bulky trityl group. On the ACA/APTS glass surface, the overall coupling yield was 25%, and the average stepwise coupling yield was 82% (Figure 5 (a)). The PEG/CHI/GPTS surface gave a higher yield than the ACA/APTS glass surface (overall coupling yield: 62%, average coupling yield: 94%) (Figure 5 (b)). Since the amino groups of the ACA/APTS glass surface were distributed more densely than

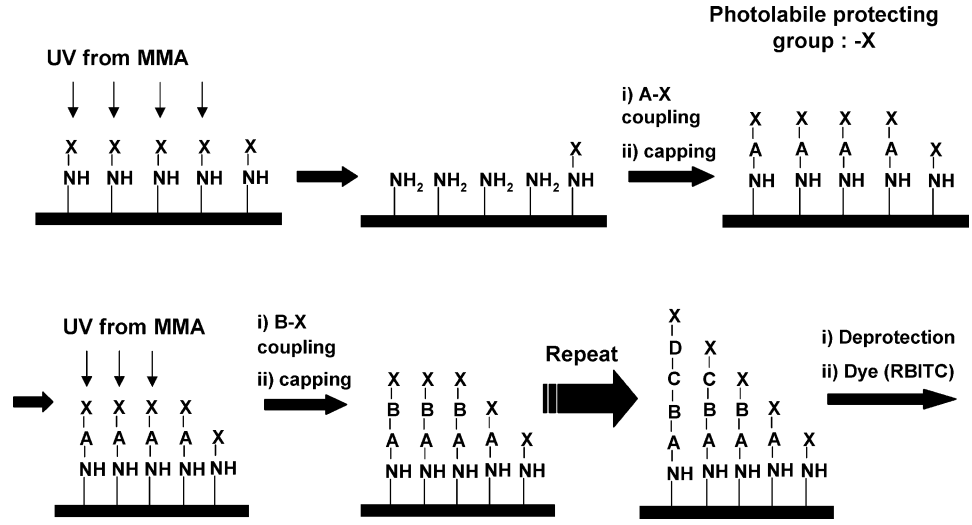


Figure 4. Method of coupling efficiency measurement.

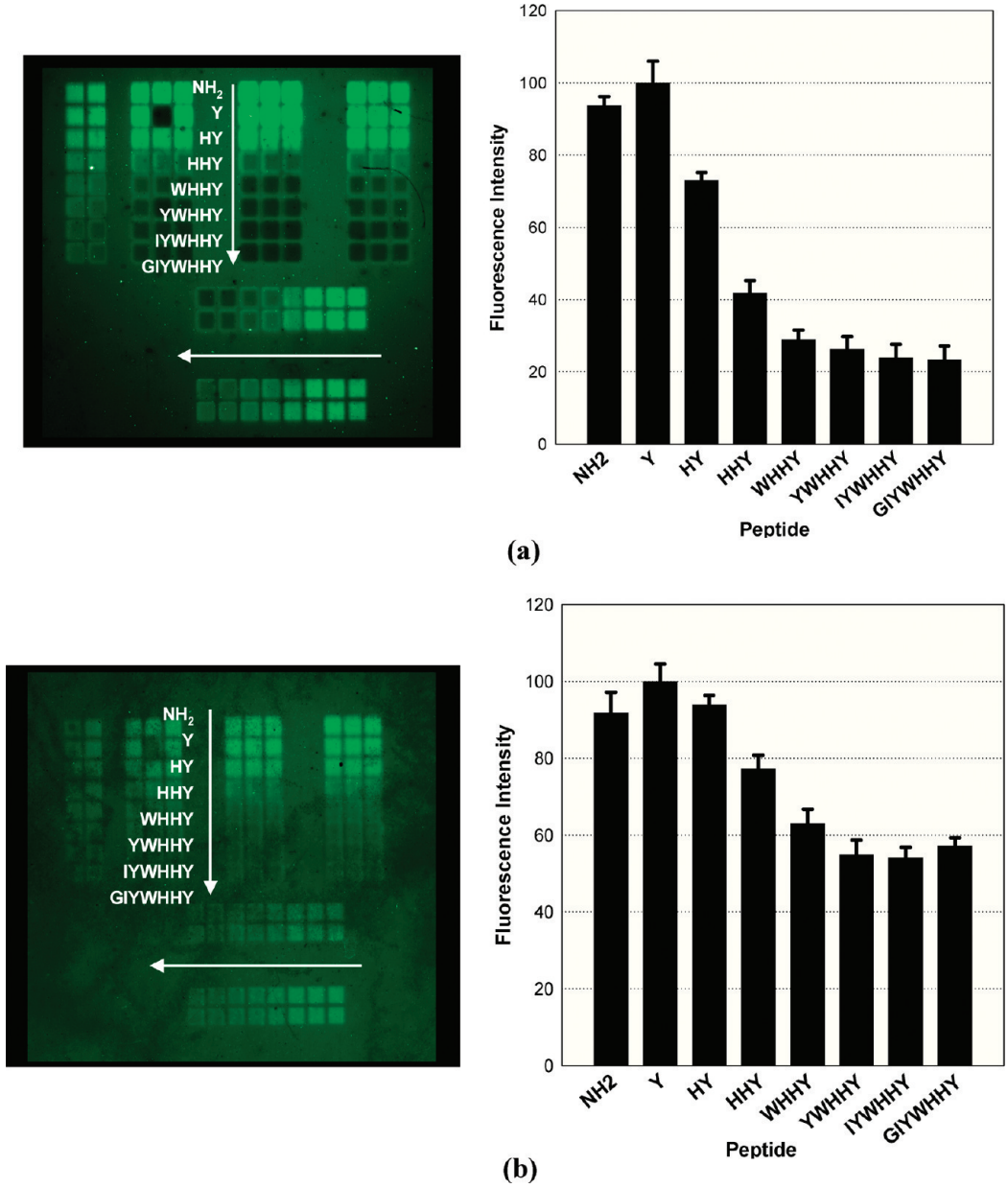


Figure 5. RBITC-labeled surface for checking coupling efficiency of GIYWHHY: (a) fluorescence image and graph on the ACA/APTS glass surface, (b) fluorescence image and graph on the PEG/CHI/GPTS glass surface.

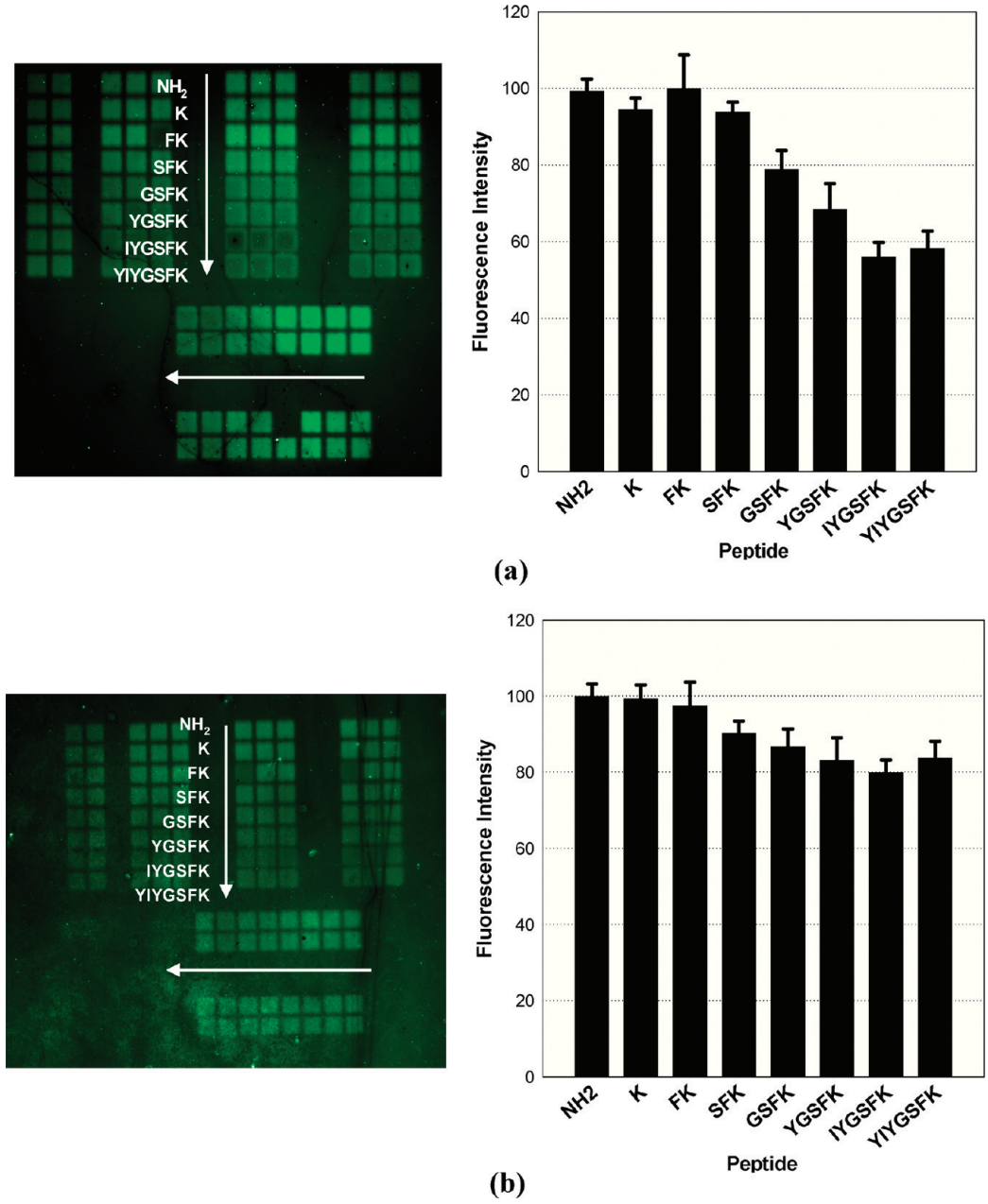


Figure 6. RBITC-labeled surface for checking coupling efficiency of YIYGSFK: (a) fluorescence image and graph on the ACA/APTS glass surface, (b) fluorescence image and graph on the PEG/CHI/GPTS glass surface.

those of PEG/CHI/GPTS, there would have been more steric hindrance when synthesizing the peptide on the ACA/APTS glass surface. In YIYGSFK synthesis, the coupling efficiency was relatively higher than that of GIYWHHY. On the ACA/APTS glass surface, the overall coupling yield was 59%, and the average stepwise coupling yield was 93% (Figure 6 (a)). The PEG/CHI/GPTS glass surface also gave a higher yield than the ACA/APTS glass surface (overall coupling yield: 84%, average coupling yield: 98%) because of the same reason as above (Figure 6 (b)). It can be concluded that peptide synthesis on a glass chip is more favorable on the PEG/CHI/GPTS glass surface. However, the efficiency of peptide coupling on a chip was still lower than that of conventional solid phase peptide synthesis on polymer beads. The solution of NVOC-amino acid activated by HATU was unstable for long storage time in the automated synthesis system. The problem of low coupling efficiency could be overcome by using more stable NVOC-amino acid pentafluorophenyl esters, which will be reported in the near future.[11]

Synthesis of Protein Binding Peptide Microarray. A peptide microarray was prepared on the glass chip using NVOC-amino acids. To measure binding affinity between peptide and protein, streptavidin was chosen as a model protein to study the binding assay, since HPQ- or HPM-containing pentapeptides are known to bind streptavidin.[16] Thirteen NVOC-amino acids (Ala, Gly, Ile, Met, Gln, Pro, His, Glu, Val, Asn, Trp, Phe, Arg) were used for synthesizing peptides on the PEG/CHI/GPTS glass surface. UV light was irradiated on the NVOC-protected surface with a proper pattern generated from MMA. Then, NVOC-amino acid was coupled to the irradiated site by using a coupling agent. UV irradiation and NVOC-amino acid coupling steps were repeated until the predesigned peptide sequences were built

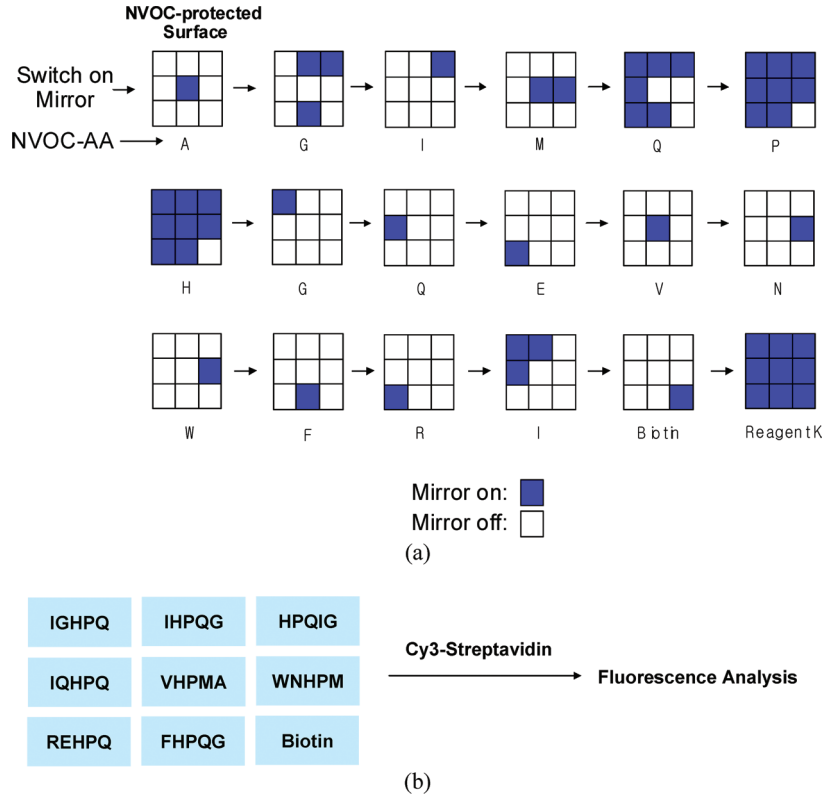


Figure 7. Synthesis of streptavidin-binding peptide sequence: (a) mask design and NVOC-amino acid coupling, (b) resulting peptide array and Cy3-streptavidin binding.

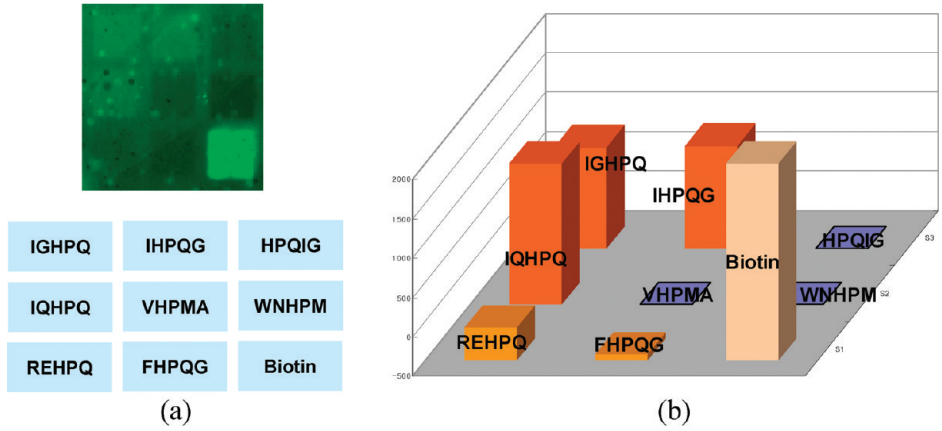


Figure 8. Fluorescence analysis of surface-bound Cy3-streptavidin on the peptide microarray; (a) fluorescence image, (b) analysis of fluorescence intensity.

up (Figure 7 (a)). Eight sequences of HPQ- or HPM-containing pentapeptides were synthesized on the glass chip, and biotin was coupled as a control. Thereafter, the glass surface was treated with Reagent K to remove the side chain-protecting groups from the peptides. After treating the prepared peptide microarray with Cy3-streptavidin, fluorescence intensity was measured in the region of each peptide (Figure 7 (b)).

The fluorescence image of streptavidin binding is depicted in Figure 8. The binding affinity with Cy3-streptavidin was in the following order: Biotin > IQHPQ > IGHPQ > IHPQG > > REHPQ > FHPQG ≫ VHPMA ≈ HPQIG ≈ WNHPM. In our previous study, the binding affinity of streptavidin-alkaline phosphatase conjugate was spectrophotometrically quantified on TentaGel resin with four kinds of ligands, and the order of binding affinity was Biotin > IGHPQ > IHPQG > HPQIG.[17] The assay result from the peptide chip was the same as the one from resin beads. As these results show, the peptide microarray synthesis method in this research is quite efficient for quantifying the binding activities of various peptides to a protein, and it is easy to apply other bioassay systems.

Conclusion

The maskless photolithographic synthesis method using micromirror array (MMA) was used on a glass chip. For the preparation of peptide microarray, an automated peptide array synthesizer was built in a closed box. A computer program controlled photolithography, monomer injection, and washing conditions. For the peptide synthesis on a glass chip, the ACA/APTS glass surface and the CHI/GPTS glass surface were prepared, and NVOC-protected 20 amino acids

and their analogues were synthesized as building blocks using the photolithographic synthesis method. The coupling efficiencies of GIYWHHY and YIYGSFK were verified to determine efficient peptide synthesis conditions on a glass chip. Peptide synthesis on a glass chip was more favorable on the PEG/CHI/GPTS surface than on the ACA/APTS surface. To examine peptide−protein binding profile, HPQ- or HPM-containing pentapeptides were synthesized on a glass chip and a Cy3-streptavidin binding assay was performed. The peptide sequence of IQHPQ showed the highest binding affinity with Cy3 labeled-streptavidin. From these results, we have proven that photolithographic peptide synthesis is an efficient method to quantify the binding activities of various peptide ligands.

Acknowledgment. This work was supported by the Nano-Systems Institute-National Core Research Center (NSI-NCRC) program and Korea Biotech R&D Group of Next-generation growth engine project of the Ministry of Education, Science and Technology (F104AB01005-07A0201-00510).

Supporting Information Available. Screen capture of the LABVIEW program and characterization of NVOC-amino acids by ^{1}H NMR. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Henderson, G.; Bradley, M. *Curr. Opin. Biotechnol.* **2007**, *18*, 326–330. (b) Uttamchandani, M.; Yao, S. Q. *Curr. Pharm. Design* **2008**, *14*, 2428–2438.

(2) (a) Andresen, H.; Zarse, K.; Grötzinger, C.; Hollidt, J. M.; Ehrentreich-Förster, E.; Bier, F. F.; Kreuzer, O. J. *J. Immunol. Methods* **2006**, *315*, 11–18. (b) Tapia, V.; Bongartz, J.; Schutkowski, M.; Bruni, N.; Weiser, A.; Ay, B.; Volkmer, R.; Or-Guil, M. *Anal. Biochem.* **2007**, *363*, 108–118.

(3) (a) Schutkowski, M.; Reimer, U.; Panse, S.; Dong, L.; Lizcano, J. M.; Alessi, D. R.; Schneider-Mergener, J. *Angew. Chem., Int. Ed.* **2004**, *43*, 2671–2674. (b) Wang, H.; Brautigan, D. L. *Mol. Cell. Proteomics* **2006**, *5*, 2124–2130. (c) Gosalia, D. N.; Salisbury, C. M.; Ellman, J. A.; Diamond, S. L. *Mol. Cell. Proteomics* **2005**, *4*, 626–636. (d) Sun, H.; Lu, C. H. S.; Uttamchandani, M.; Xia, Y.; Liou, Y. C.; Yao, S. Q. *Angew. Chem., Int. Ed.* **2008**, *47*, 1698–1702. (e) Sun, H.; Lu, C. H. S.; Shi, H.; Gao, L.; Yao, S. Q. *Nat. Protoc.* **2008**, *3*, 1485–1493.

(4) Shin, D. S.; Kim, D. H.; Chung, W. J.; Lee, Y. S. *J. Biochem. Mol. Biol.* **2005**, *38*, 517–525.

(5) (a) Pirrung, M. C. *Angew. Chem., Int. Ed.* **2002**, *41*, 1276–1289. (b) Falsey, J. R.; Renil, M.; Park, S.; Li, S.; Lam, K. S. *Bioconjugate Chem.* **2001**, *12*, 346–353. (c) Frank, R. *J. Immunol. Methods* **2002**, *267*, 13–26. (d) Gao, X.; Gulari, E.; Zhou, X. *Biopolymers* **2004**, *73*, 579–596. (e) Pellois, J. P.; Wang, W.; Gao, X. *J. Comb. Chem.* **2000**, *2*, 355–360. (f) Pellois, J. P.; Zhou, X.; Srivannavit, O.; Zhou, T.; Gulari, E.; Gao, X. *Nat. Biotechnol.* **2002**, *20*, 922–926. (g) Fodor, S. P. A.; Read, J. L.; Pirrung, M. C.; Stryer, L.; Lu, A. T.; Solas, D. *Science* **1991**, *251*, 767–773. (h) Singh-Gasson, S.; Green, R. D.; Yue, Y.; Nelson, C.; Blattner, C. F.; Sussman, M. R.; Cerrina, F. *Nat. Biotechnol.* **1999**, *17*, 974–978.

(6) (a) Reimer, U.; Reineke, U.; Schneider-Mergener, J. *Curr. Opin. Biotechnol.* **2002**, *13*, 315–320. (b) Buss, H.; Dörrie, A.; Schmitz, M. L.; Frank, R.; Livingstone, M.; Resch, K.; Kracht, M. *J. Biol. Chem.* **2004**, *279*, 49571–49574. (c) Takahashi, M.; Nokihara, K.; Mihara, H. *Chem. Biol.* **2003**, *10*, 53–60. (d) Houseman, B. T.; Huh, J. H.; Kron, S. J.; Mrksich, M. *Nat. Biotechnol.* **2002**, *20*, 270–274.

(7) (a) Lee, K. N.; Shin, D. S.; Lee, Y. S.; Kim, Y. K. *J. Micromech. Microeng.* **2003**, *13*, 18–25. (b) Lee, K. N.; Shin, D. S.; Lee, Y. S.; Kim, Y. K. *J. Micromech. Microeng.* **2003**, *13*, 474–481. (c) Kim, J. K.; Shin, D. S.; Chung, W. J.; Jang, K. H.; Lee, K. N.; Kim, Y. K.; Lee, Y. S. *Colloid Surf. B.* **2004**, *33*, 67–75.

(8) Srivannavit, O.; Gulari, M.; Gulari, E.; LeProust, E.; Pellois, J. P.; Gao, X.; Zhou, X. *Sens. Actuators A.* **2004**, *116*, 150–160.

(9) Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*, 3rd ed.; Pergamon Press: Oxford, 1988; pp 57−64.

(10) Liu, Z. C.; Shin, D. S.; Shokouhimehr, M.; Lee, K. N.; Yoo, B. W.; Kim, Y. K.; Lee, Y. S. *Biosens. Bioelectron.* **2007**, *22*, 2891–2897.

(11) Shin, D. S.; Lee, Y. S. *Synlett* **2009**, 3307–3310.

(12) Shin, D. S.; Lee, K. N.; Jang, K. H.; Kim, J. K.; Chung, W. J.; Kim, Y. K.; Lee, Y. S. *Biosens. Bioelectron.* **2003**, *19*, 485–494.

(13) (a) Patchornik, A.; Amit, B.; Woodward, R. B. *J. Am. Chem. Soc.* **1970**, *92*, 6333–6335. (b) Holmes, C. P.; Adams, C. L.; Kochersperger, L. M.; Mortensen, R. B.; Aldwin, L. A. *Biopolymers* **1995**, *37*, 199–211.

(14) (a) McGall, G. H.; Barone, A. D.; Diggelmann, M.; Fodor, S. P. A.; Gentalen, E.; Ngo, N. *J. Am. Chem. Soc.* **1997**, *119*, 5081–5090. (b) Komolpis, K.; Srivannavit, O.; Gulari, E. *Biotechnol. Prog.* **2002**, *18*, 641–646.

(15) Kim, D. H.; Shin, D. S.; Lee, Y. S. *J. Pept. Sci.* **2007**, *13*, 625–633.

(16) Lam, K. S.; Salmon, S. E.; Hersh, E. M.; Hruby, V. J.; Kazmierski, W. M.; Knapp, R. J. *Nature* **1991**, *354*, 82–84.

(17) Lee, Y. S.; Kim, D. H. *Peptide 1996*; Ramage, R., Epton, R., Eds.; Mayflower Scientific Ltd.: Birmingham, 1997; pp 571−572.

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