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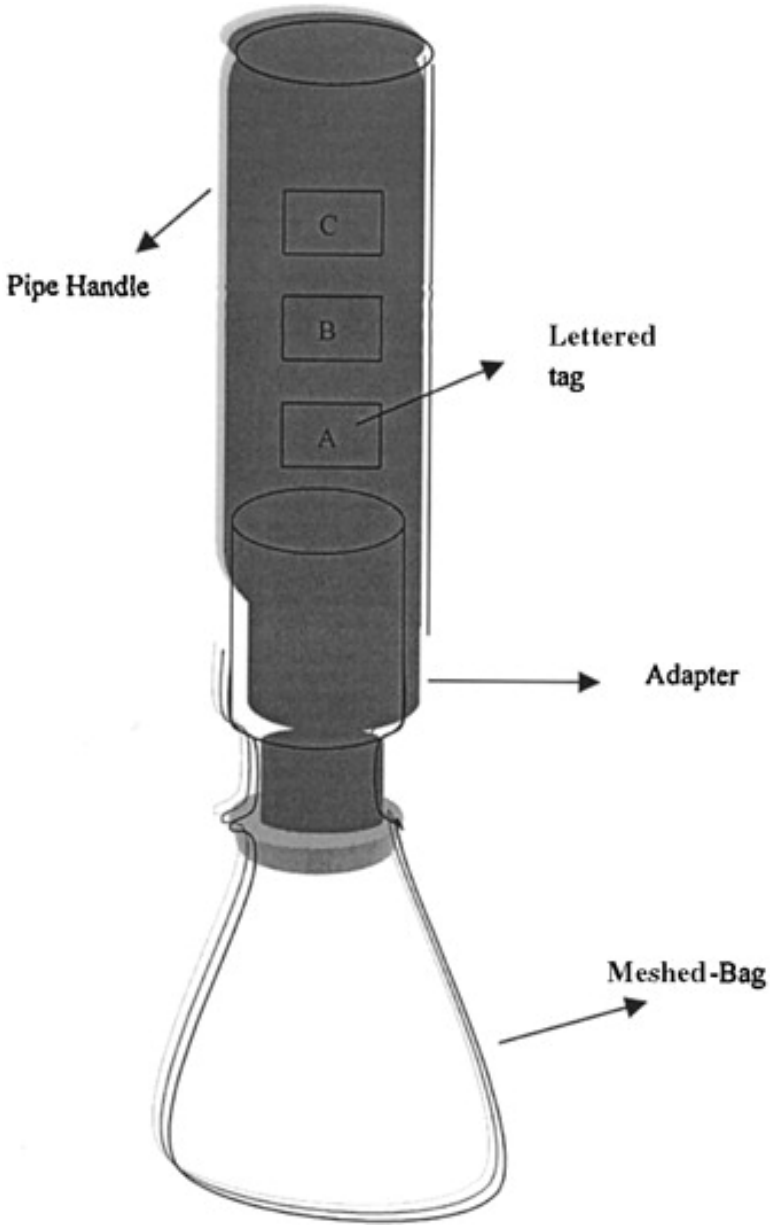

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"Meshed-Bag Gathered-Bunch" Method for Solid-Phase Synthesis of Small Molecular Diverse Compounds

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A new "Meshed-Bag Gathered-Bunch" technology for the solid-phase synthesis of chemical libraries was developed. Using such technology, we synthesized muramyl dipeptide mimetics including derivatives at the N- and C-terminus, cyclic muramyl dipeptide mimetics, muramyl dipeptide and Tuftsin's analogue conjugates. The advantages of such a method include ease of manufacture, low unit cost of production, the physical encoding method, and the compatibility with both parallel and "split−mix" approaches.

Introduction

The identification and optimization of new drug candidates are greatly facilitated by combinatorial small-molecule synthesis.[1−9] The term "combinatorial chemistry" embodies three critical processes: generation of a chemical library, a deconvolution strategy to identify the precise active compound, and a high-throughput screening system. Four general approaches have been developed for the purpose of synthesis and evaluation of chemical libraries: (i) libraries derived from biological sources;[6] (ii) a parallel synthetic approach, such as a parallel solid-phase method in a final products-off-polymer or solution-phase synthesis of a small-molecule compound libraries; (iii) "split−mix" synthesis; (iv) affinity binding selection approach.[10−12] Each of these approaches has advantages and disadvantages. For example, the "split−mix" technology requires an encoding strategy such as the "one-bead, one-compound" method,[7a] the "one-bead, two-compound" option,[13] the radio frequency (rf) tag,[14−15] and chemical encoding.[16−19] If mixture libraries are synthesized using approach ii, a deconvolution strategy is required. Some examples would be iteration,[20] positional scanning,[21,22] orthogonal partitioning,[23] and recursive deconvolution.[24] A very large number of compounds can be generated using approach i; however, there is typically low diversity within each library.

The pharmaceutical industry and the scientific community have recently become more interested in small-molecule libraries because there is wide structural diversity within these libraries and greater potential for bioavailability. Small-molecule libraries are currently generated using solution-phase or solid-phase methodologies or a combination of the two. The advantages of solid-phase synthesis are obvious, including greatly simplified sample purification and the ease of removing unwanted side products. These significant advantages allow well-reaction-optimized libraries to be synthesized automatically. However, there are many types of chemical reaction that do not work as well on solid supports as in solution and give products with a lower purity. Furthermore, solid-phase synthesis using the current set of commercial linkers requires an attachment point on the target compound that may be undesirable. The solid support may even have to be modified such that the product yield and purity are acceptable. The big advantage of working in solution is that a large number of optimal reaction conditions have already been published, thereby greatly facilitating large-scale syntheses. The disadvantages include the difficulties associated with the removal of excess reagents, catalysts, and side products. This may be partly circumvented through the use of solid-support scavengers that assist with the removal of other species.

The concepts associated with solid-phase synthesis allow much of the process to be automated. The introduction of robotic systems into the laboratory has, to some extent, liberated the scientific community from routine operations. In the ideal world, the complete synthesis of chemical libraries would be fully automated and would include a problem-solving capacity. However, until such a time occurs, the development and application of cheaper solutions to achieve successful combinatorial chemistry are still greatly in demand. This paper describes a new technique, "meshed-bag gathered-bunch" (MBGB), that combines all the features of the "tea-bag" method[25] with physical encoding strategy. We have successfully applied this new technology to the solid-phase synthesis of a new, nonspecific macrophage modulator of the muramyl dipeptide[26] scaffold library, based on our previous work[27] in this area.

Results and Discussion

Design and Application of "MBGB" Technique. The "MBGB" unit is illustrated in Figure 1. There are three parts: a meshed-bag, a pipe handle, and an adapter. All

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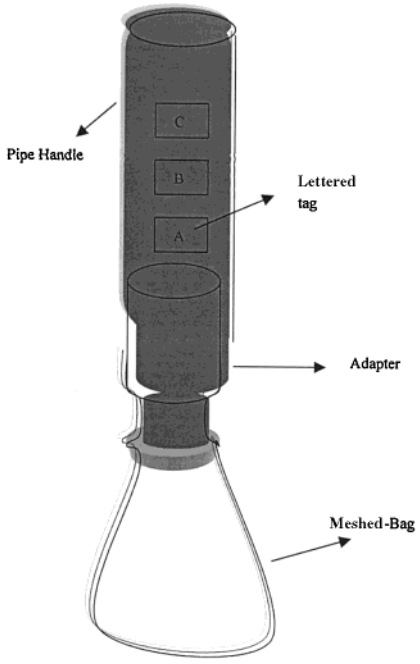


Figure 1. Illustration of basic structure of "MBGB" unit. It is composed of three parts, which are the meshed-bag, pipe handle, and adapter.

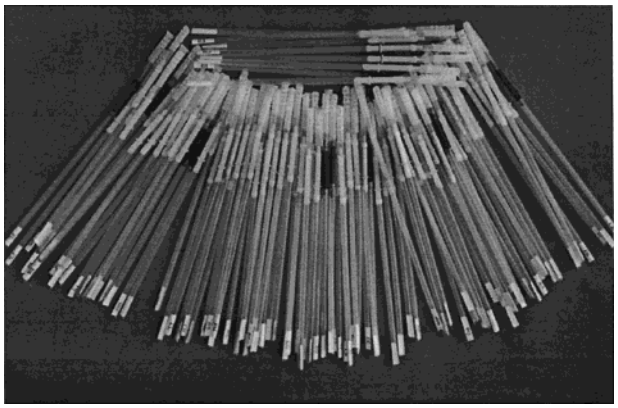


Figure 2. "MBGB" units that have been applied to our library synthesis.

components are made from polypropylene, which is inert to many organic solvents. The meshed-bags are produced easily and cheaply using an electric iron. The "MBGB" unit is capable of containing 10−100 mg of resin for synthesizing 5−50 mg of the final product. The 75 μm mesh size permits reactants and solvents to freely move across the membrane while retaining resin particles of between 100 and 200 mesh. The pipe handle serves two functions: handling of the bags and tag encoding. The adapter connects the meshed-bag to the handle. We used both numbered tag and lettered tag options for our synthesis. The tags are printed, then inserted in sequence into the handle as the building blocks are assembled. These tags can be seen from the outside of the handle. When the synthesis is complete, the synthetic information pertaining to each MBGB is obtained by simply reading the encoded tags and matching them up with the specific building blocks selected. The MBGB units are demonstrated in the photograph in Figure 2. This technique is compatible with both parallel synthesis and "split−mix" combinatorial synthesis. For instance, the whole set or subset of "MBGB"s can react with one chosen building block. On the other hand, an individual "MBGB" can also react with a series of building blocks in a parallel approach. All washing steps may be performed using a gathered-bunch approach so that the synthesis ends with a combinatorial step. In our experiments, several hundred "MBGB"s were handled simultaneously.

Design and Synthesis of Muramyl Dipeptide and Tuftsin's Analogue Conjugates by Application of "Meshed-Bag Gathered-Bunch" Technology. Tuftsin's analogue (Thr-Arg-Pro-Lys-OH) is another stimulant of macrophage.[28] It displays biological activities similar to that of muramyl dipeptide, such as activating the macrophage to kill tumor cell, inducing immuno response, being antibacterial, etc. We expect that Tuftsin's analogue may stimulate the macrophage with muramyl dipeptide synergistically. A total of 10 conjugates of Tuftsin's analogue and muramyl dipeptide were designed as the following sequences:

M1, MurNAc-Ala-D-isoGln-Thr-Arg-Pro-Lys-OH

M2, MurNAc-Ala-D-isoGln-Lys-Thr-Arg-Pro-Lys-OH

M3, Ala-D-isoGln-Thr-Arg-Pro-Lys-OH

M4, Ala-D-isoGln-Lys-Thr-Arg-Pro-Lys-OH

M5, MurNAc-Thr-Arg-Pro-Lys-OH

M6, MurNAc-Lys-Thr-Arg-Pro-Lys-OH

M7, MurNAc-Thr-D-isoGln-Thr-Arg-Pro-Lys-OH

M8, MurNAc-Thr-d-isoGln-Lys-Thr-Arg-Pro-Lys-OH

M9, MurNAc-Ala-D-Glu(OBzl)-Thr-Arg-Pro-Lys-OH

M10, MurNAc-D-Glu(OBzl)-Lys-Thr-Arg-Pro-Lys-OH

where MurNAc is the muramic acid's residue.

Applying our "meshed-bag gathered-bunch" technique and standard Fmoc strategy, we have successfully generated each of the muramyl dipeptide and Tuftsin's analogue conjugates listed as above. All cleaved peptides are greater than 85% pure. A typical HPLC and mass spectrum of the peptide M6 is shown in Figure 3.

Design and Synthesis of Derivatives of L-Ala-D-isoGln Dipeptide. The dipeptide (L-Ala-D-isoGln) of muramyl dipeptide is responsible for its immunobiological activities.[29] Its multiple biological activities may be attributed to different binding receptors in the host immune system.[30] Muramic acid is considered as a resource of pyrogenicity.[31] Derivatives of muramyl dipeptide that do not have a sugar moiety present have been found to display a lower pyrogenic side effect than muramyl dipeptide.[32] Therefore, the chemical synthesis and biological evaluation of more muramyl dipeptide targeted scaffolds may help us to discover new potential clinical agents with less pyrogenic side effects. Figure 4 illustrates six series of new dipeptide scaffolds that have been designed. All of the dipeptide derivatives are non-peptide-acylated through various linkers at both the N-terminus and C-terminus of the peptide.

Forty-two carboxylic acid building blocks (Figure 5) were selected for a chemical library comprising 2100 individual

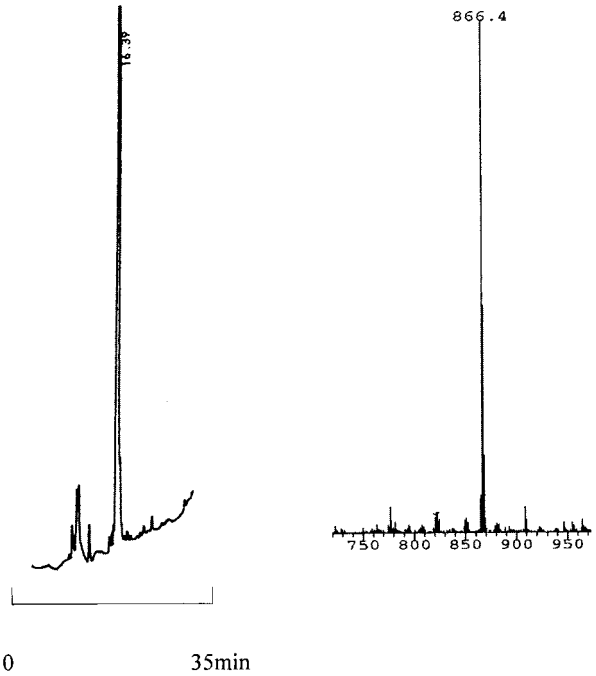


Figure 3. HPLC and MS profiles of a typical peptide of muramyl dipeptide and Tuftsin's analogue conjugates (M6) that has been synthesized by application of "meshed-bag gathered-bunch" technology.

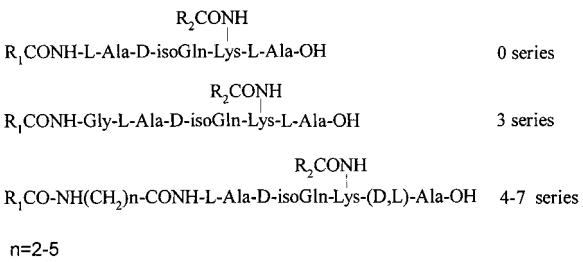


Figure 4. Scaffolds of designed muramyl dipeptide derivatives.

compounds. All peptide coupling and non-peptide acylation were carried out using "MBGB" technology. The solid support used was Wang resin. Fifty compounds were randomly selected for analysis. The correct molecular weight (FAB-MS) was detected for each cleaved compound. The average purity of the 50 compounds was >70% by HPLC.

Design and Synthesis of MDP Cyclic Derivatives. Since the structure of cyclic compounds is more restricted, cyclic peptides have a greater conformational stability and exhibit higher binding specificities than the corresponding linear peptide. Cyclic peptides might also display greater proteolytic-stable properties. Thus, the design and synthesis of cyclic peptides are of much interest. To the best of our knowledge, cyclic muramyl dipeptides have not been reported yet. This paper describes several new cyclic MDP mimetics obtained from the coupling of the side chain of both isoGln and Lys of the sequence, as shown in Figure 6.

The success of cyclic peptide synthesis is determined by several factors such as the extent of intermolecular cross coupling, peptide sequence, peptide length, and coupling reagents. It was found that Rink amide resin with a loading of 0.2−0.3 mmol/g gave good results using BOP/HOBt/NMM as the coupling reagent. Ten cyclic muramyl dipeptide mimetics were then synthesized according to Scheme 1 using "MBGB" technology. The structures of the target compounds are listed in Table 1. The correct molecular weight was detected for each compound. The purity of the compounds ranged from 20% to 80% (by HPLC).

Conclusions

A new "meshed-bag gathered-bunch" technology for the solid-phase synthesis of chemical libraries was developed. Using such technology, we synthesized muramyl dipeptide mimetics including derivatives at the N- and C-terminus, cyclic muramyl dipeptide mimetics, muramyl dipeptide and Tuftsin's analogue conjugates. The advantages of such a method include ease of manufacture, low unit cost of production, the physical encoding method, and the compatibility with both parallel and "split−mix" approaches.

Experimental Section

NMR spectra were measured on 300 MHz spectrometers with [D_6]DMSO as solvent. Tetramethylsilane (TMS) served as internal standard ($\delta = 0$). Mass spectra were performed on a Zabspec high-resolution magnetic mass spectrometer (Micromass Ltd., U.K.) equipped with fast atom bombardment (FAB) ionization. Reverse-phase high-performance liquid chromatography (HPLC) was carried out on 25 cm × 0.46 cm reverse-phase C-18 columns employing a Waters 600E liquid chromatography system. HPLC experiments were performed using gradient and isocratic conditions. Eluents used include solvent A (H_2O with 0.1% TFA), solvent B (70% CH_3CN in water with 0.1% TFA), solvent C (90% CH_3CN in water with 0.1% TFA), solvent D (90% methanol in water with 0.1% TFA), and solvent E (95% methanol in water with 0.1% TFA).

The "Meshed-Bag Gathered-Bunch" apparatus was made from polypropylene fabric from Synthetic Industries, Inc. A piece of the fabric was cut, folded into a pouch, then sealed along three edges with an electric iron. After the resin is placed inside the bag, one end of polypropylene adaptor is inserted into the bag and then this edge is also sealed with the iron. The other end of the adaptor is attached to a polypropylene pipe using a similar procedure. Numbered or lettered tags can be inserted into the pipe handle. In our laboratory, the numbers or letters that coded for each building block were printed onto paper. When the reactions are complete, the structural information can be obtained by just reading the combination of tags.

"One-Pot" Synthesis of the Fmoc-D-isoGln Building Block. Carbobenzyloxy chloride [Bz-OCOCl] (94 mL) and aqueous 4 N NaOH solution (120 mL) was added dropwise, with stirring, into a 1 L three-necked flask containing D-glutamic acid (60 g) in aqueous 4 N NaOH solution (208 mL) over 30 min. The reaction solution was then heated to 60 °C and stirred for 15 min. Then an additional 25 mL of 4 N NaOH solution was added, and the mixture was stirred for another 15 min. The resulting solution was extracted with Et_2O (4 × 350 mL). The aqueous layer was cooled to 0 °C and acidified with 2 N HCl to pH 2.0, then extracted again

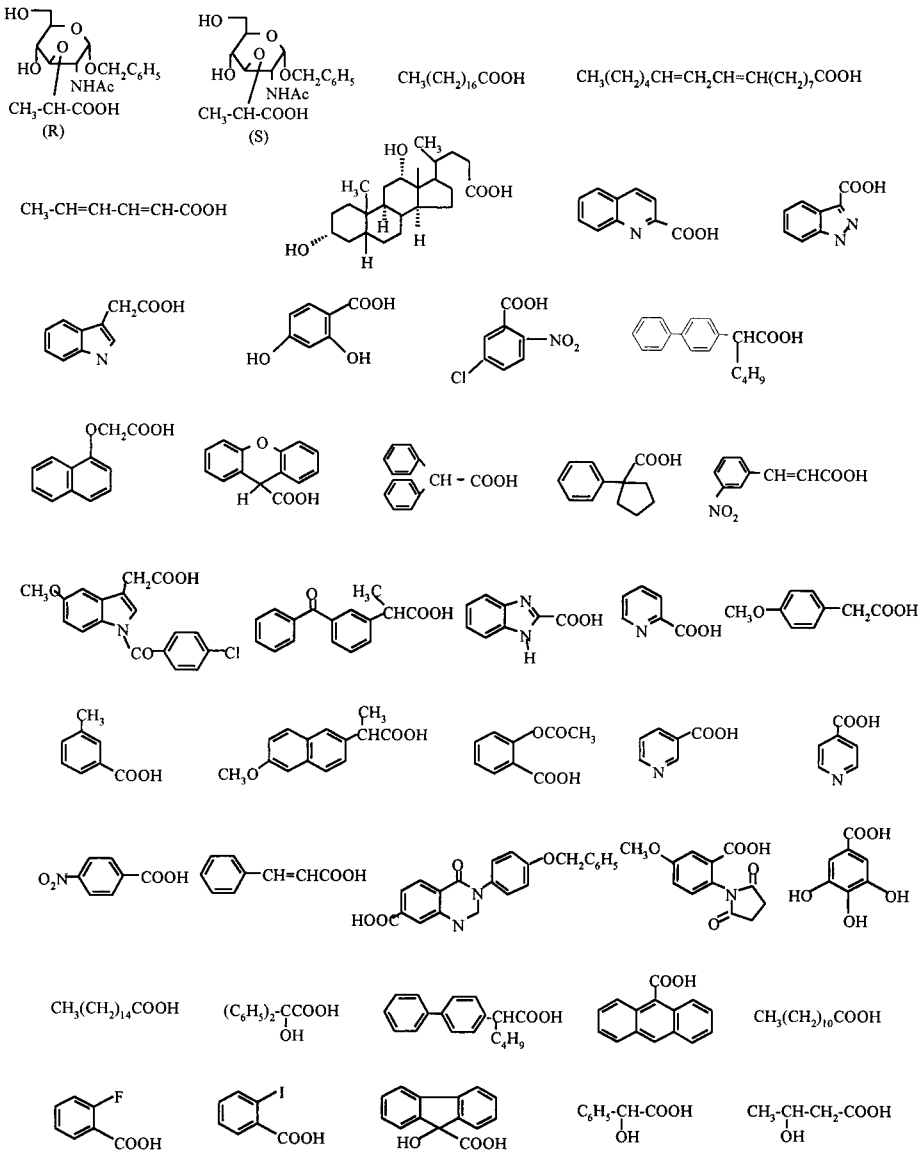


Figure 5. Selected organic acid building blocks for the synthesis of muramyl dipeptide scaffold library.

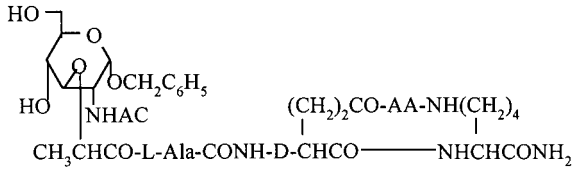


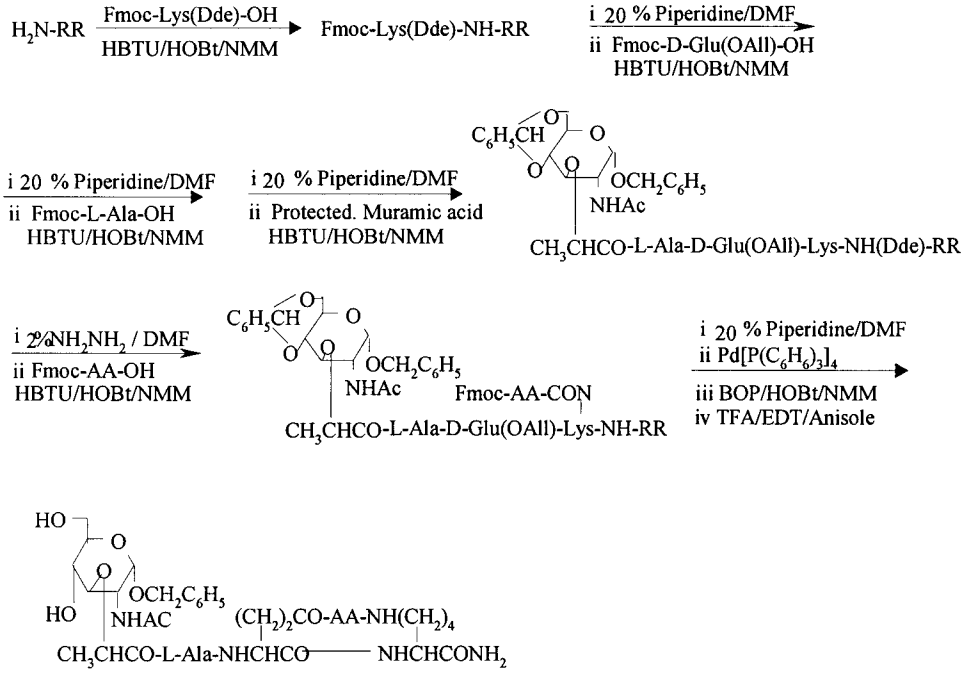
Figure 6. Scaffold of cyclic MDP mimetic.

with EtOAc (4 × 500 mL). The ethyl acetate fractions were dried over $MgSO_4$, then evaporated under reduced pressure to give 110 g of Bz-D-Glu.

Bz-D-Glu (12.46 g) was dissolved in anhydrous THF (100 mL). With stirring, DCC (10.98 g) was added, and the reaction solution was allowed to stand for 7 h at room temperature. The precipitate was filtered and washed with the minimum volume of THF, then the ice-cooled filtrate was treated with ammonia gas for 30 min while stirring vigorously. The resulting white solid was filtered under vacuum and recrystallized from methanol and ether. Then it was ground and shaken with ethyl acetate and 1 M HCl. The ethyl acetate solution was dried over $MgSO_4$ and evaporated. The residue was recrystallized from ethyl acetate and cyclohexane to yield 6.2 g of Bz-D-isoGln, mp 174−176 °C.

Bz-D-isoGln(14.0 g) was added to a three-necked flask containing 5% Pd/C (1.0 g) and distilled water (300 mL). With stirring, Bz-D-isoGln was hydrogenated for 2 days at room temperature. Then Pd/C was filtered off and washed with distilled water. The filtrate was concentrated under reduced pressure to about 200 mL. $NaHCO_3$ (4.2 g) and acetone (200 mL) were then added sequentially. The reaction solution was cooled to 0 °C using an ice−water bath, and then Fmoc-OSu (16.85 g) was added in small portions. The resulting mixture was stirred for further 3 days at room temperature. The reaction solution was acidified to pH 3.0 with stirring using 2 N HCl and then was evaporated under reduced pressure. The residue was extracted with ethyl acetate (5 × 500 mL), dried over $MgSO_4$, evaporated under reduced pressure, and then recrystallized from methanol to yield 15.35 g of Fmoc-D-isoGln; mp 204−205 °C, yield (over three steps) 41.8%. MS (FAB+) *m*/*z* (%): 369.1 [M + H^+] (60), 179.1 [$C_{14}H_{11}^+$] (100). ^{1}H NMR (300 MHz, [D_6]DMSO, 25 °C, TMS): δ 1.75−1.89 (m, 2H, CH_2), 2.24 (t, ^3J(H,H)

Scheme 1. Synthetic Route for Cyclic MDP Derivatives[a]



[a] RR = Rink amide resin.

Table 1. Physical Data of Cyclic Muramyl Dipeptide Mimetics

AA	MW,[a] found/calcd	purity(%)	AA	MW,[a] found/calcd	purity(%)
Gly	750.3/749.8	30.2	L-Ala-D-isoGln	892.5/892.0	20.1
β-Ala	764.2/763.8	40.8	D-Ala-D-isoGln	892.4/892.0	60.7
$NH_2(CH_2)_3COOH$	778.2/777.9	70.1	L-Ala-D-Glu(OBzl)	983.4/983.5	80.3
L-Ala	764.5/763.8	70.5	L-Ala-L-isoGln	892.5/892.0	30.5
D-Ala	764.1/763.8	85.6	D-Ala-L-Gln	892.1/892.0	60.4

[a] The molecular weight is determined by FAB-MS.

= 7.8 Hz, 2H, CH_2), 3.92 (m, 1H, CH), 4.19−4.27 (m, 3H, $CHCH_2$), 7.02 (s, 1H, CONH), 7.28−7.88 (m, 10H, 8ϕH + $CONH_2$), 12.07 (s, 1H, COOH).

Synthesis of Muramyl Dipeptide and Tuftsin Conjugates by Application of "Meshed-Bag Gather-Bunch" Technology. A total of 10 mg of Wang resin (from AUSPEP Company, 100−200 mesh, 0.85 mmol/g) was put into a MBGB apparatus. A total of 438 mg of Fmoc-Lys(Boc)-OH, 96 mg of DCC, and 11 mg of DMAP were dissolved in 3.3 mL of DMF in a container, and 10 MBGB units were suspended in this activated Fmoc-Lys(Boc)-OH solution for 1 h at 0 °C. The MBGB units were then washed in batches thoroughly with DMF and DCM. After MBGB units were transferred in batches into a reaction vessel containing 10 mL of 10% acetic anhydride in DCM, the remaining free hydroxyl group on the resin was blocked for an additional 30 min at room temperature. The MBGB units were then washed thoroughly again with DMF. Similarly, the MBGB units were treated with a 20% piperidine/DMF solution and were washed with DMF, CH_3OH, and DMF sequentially. Individual peptide assembly in each "MBGB" was finished by the following conditions: 5 equiv of protected amino acid, HOBt, HBTU, and 7.5 equiv of NMM reacting for 4 h at room temperature. Finally, the MBGB units were washed in a gathered-bunch approach with DMF, methanol, DMF, and DCM. Then, the MBGB units were dried completely in vacuo. The peptides were released off the polymer by treatment of MBGB individually with 0.5 mL of cleavage solution [TFA/EDT/anisole = 38:1:1 (v/v/v)] for 3 h at room temperature. The MBGB units each were then washed with 0.5 mL of TFA for another 10 min. TFA combination of each peptide was gently concentrated by nitrogen flow. The residue was washed with 10 mL of a mixture anhydrous diethyl ether/petroleum (30−60 °C) = 1:2 (v/v) two times and was air-dried for 1 h. The crude peptides were dissolved by 60% acetonitrile in water and were analyzed by HPLC and mass spectrometry.

Synthesis of Dipeptide Derivatives at Both N-Terminus and C-Terminus. A total of 5.6 g of Wang resin (100−200 mesh, 0.8 mmol/g) was put inside a big MBGB unit. After that, the resin was swelled in DMF for 30 min and the MBGB unit was suspended in 20 mL of Fmoc-Ala-OH·H_2O (2.999 g) and HOBt (1.215 g) solution in DMF. After the above solution was cooled completely by an ice−water bath, 111 mg of DMAP was added. After that, the protected amino acid was activated by 2.04 g of DCC for 30 min. The reaction was continued for an additional 48 h at room temperature. The MBGB unit was washed thoroughly with methanol and DCM. After being dried completely in vacuo, 12.0 mg of resin was weighed out and treated with 4.0 mL of 20% piperidine in DMF for 20 min. A total of 200.0 μL of this resulting solution was transferred into a container and further

diluted with 2.8 mL of 20% piperidine in DMF. The OD value was detected under 290 nm wavelength by a control of 20% piperidine in DMF solution. The loading capacity of the resin was then calculated as 0.64 mmol/g as previously described (instructions of the Ultrospec II apparatus from Pharmacia LKB Biochrom). The remaining hydroxyl group on the Wang resin was blocked by a reaction with 10% acetic anhydride in DCM for 30 min at room temperature. The MBGB unit was washed thoroughly with DMF and DCM sequentially. The following deprotection steps of Fmoc, washing, coupling, washing steps, were the same as above. Fmoc-Lys(Dde)-OH was assembled onto the peptide by the following: DMF 40 mL, Fmoc-Lys(Dde)-OH 4.8 g, HOBt 1.215 g, HBTU 3.411 g, NMM 1.49 mL, coupling for 4 h. The following amino acids and other building block coupling steps were carried out with the same procedure as above. Finally, 10 mg of Fmoc-removed dipeptide or its mimetic preloaded dry resin was divided into our MBGB units with tags corresponding to acid building blocks. Acylation of acid building blocks was performed under the following conditions: 10 equiv of acid building blocks, HOBt, and HBTU were dissolved by a suitable solvent with corresponding labels on the side-wall of the container. Then, 15 equiv of NMM was added into each reaction container, the reaction was allowed to stand overnight at room temperature. All MBGB units collected as a gathered bunch were thoroughly washed with various solvents, treated with 2% of NH_2NH_2/DMF for 3 min two times, and washed with DMF again. Tagged "MBGB" units were then distributed into a designed acid building block container for a second acylation. After gathered-bunch washing with various solvents, the individual MBGB unit was treated with 0.5 mL of cleavage solution in the same manner above for 2 h at room temperature. The final products were analyzed by HPLC and mass spectrometry after being washed and dried as above.

Synthesis of Cyclic MDP Derivatives. A total of 0.5 g of Fmoc−Rink resin (100−200 mesh, 0.7 mmol/g) in our MBGB was treated with 20% piperidine/DMF for the removal of Fmoc. After being thoroughly washed with DMF, the MBGB unit was then reacted with 0.4 equiv of Fmoc-Lys(Dde)-OBt in DMF for 1 h at room temperature. The remaining free amino group was blocked by treatment with 10% acetic anhydride in DCM for an additional 30 min. After it was washed with dried DCM completely, the substitution of resin was decreased to about 0.28 mmol/g. Then, 30 mg of substitution-decreased resin was distributed into each tagged MBGB unit. The peptide assembly was performed under the following conditions: 6.0 equiv of protected amino acid, HBTU, HOBt, and 9.0 equiv of NMM for 4 h at room temperature.

Side Chain Deprotection. The −OAll protecting group of Fmoc-D-Glu(OAll)-OH was removed by suspension of the MBGB unit in 0.65 mL of $Sn(But)_3$, 0.18 mL of acetic acid, and 150 mg of $Pd[P(C_6H_6)_3]_4$ in 10 mL of dried DCM for 3 h at room temperature. The Dde group of Fmoc-Lys-(Dde)-OH was removed by treatment with 2% of NH_2NH_2/DMF for 5 min two times.

In Situ Cyclization. The MBGB unit was suspended in 10 equiv of BOP, HOBt, and 15 equiv of NMM in DMF overnight.

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References and Notes

(1) Geysen, H. M.; Barteling, S. J.; Meloen, R. H. Small peptides induce antibodies with a sequence and structural requirement for binding antigen comparable to antibodies raised against the native protein. *Proc. Natl. Acad. Sci U.S.A.* **1985**, *82* (1), 178−182.

(2) Bunin, B. A.; Ellman, J. A. A general and expedient method for the solid-phase synthesis of 1,4-benzodiazepine derivatives. *J. Am. Chem. Soc*. **1992**, *114*, 11997−10998.

(3) Moos, W. H. *Combinatorial Chemistry and Molecular Diversity in Drug Discovery*; Gordon, Eric M., Kerwin, James F., Jr., Eds.; Wiley-Liss: New York, 1998; p ix.

(4) Ellman, J.; Stoddard, B.; Wells, J. Combinatorial thinking in chemistry and biology. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94* (7), 2779−2782.

(5) Furka, A.; Sebestyen, F.; Asgedom, M.; Dibo, G. In *Highlights of Modern Biochemistry, Proceeding of the 14th International Congress of Biochemistry*; VSP: Utrecht, The Netherlands, 1988; Vol. 5, p 47.

(6) (a)Scott, J. K.; Smith, G. P. Protein, nucleotide searching for peptide ligands with an epitope library. *Science* **1990**, *249* (4967), 386−390. (b) Cwirla, S.; Peters, E. A.; Barrett, R. W.; Dower, W. J. Peptides on phage: a vast library of peptides for identifying ligands. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 6378. (c) Devlin, J. J.; Panganiban, L. C.; Devlin, P. E. Random peptide libraries: a source of specific protein binding molecules. *Science* **1990**, *249*, 404.

(7) (a) Lam, K. S.; Salmom, S. E.; Hersh, E. M.; Hruby, V. J.; Kazmierski, W. M.; Knapp, R. J. A new type of synthetic peptide library for identifying ligand-binding activity. *Nature* **1991**, *354*, 82. (b) Houghten, R. A.; Pinlla, C.; Blondelle, S. E.; Appel, J. R.; Dooley, C. T.; Cuervo, J. H. Generation and use of synthetic peptide combinatorial libraries for basic research and drug discovery. *Nature* **1991**, *354*, 84.

(8) Service, R. F. Combinatorial Chemistry: High-Speed Materials Design. *Science* **1997**, *277*, 474−475.

(9) (a) GlaxoWellcome. Redesigning drug discovery. *Nature* **1996**, *384* (Suppl.), 1−5. (b) Radioman, J.; Jung, G. Integrating combinatorial chemistry and bioassays. *Science* **2000**, *287*, 1947.

(10) Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. Efficient method for the preparation of peptoids [oligo-(N-substituted glycines)] by submonomer solid-phase synthesis. *J. Am. Chem. Soc*. **1992**, *114*, 10646−10647.

(11) Songyang, Z.; Shoelson, S. E.; Chaudhuri, M.; Gish, G.; Pawson, T.; Haser, W. G.; King, F.; Roberts, T.; Ratnofsky, S.; Lechleider, R. J.; et al. SH2 domains recognize specific phosphopeptide sequences. *Cell* **1993**, *72* (5), 767−778.

(12) Songyang, Z.; Fanning, A. S.; Fu, C.; Xu, J.; Marfatia, S. M.; Chishti, A. H.; Crompton, A.; Chan, A. C.; Anderson, J. M.; Cantley, L. C. Recognition of unique carboxyl-terminal motifs by distinct PDZ domains. *Science* **1997**, *275* (5296), 73−77.

(13) Buchardt, J.; Schiodt, C. B.; Krog-Jensen, C.; Delaisse, J.-M.; Foged, N. T.; Meldal, M.. Solid-phase combinatorial library of phosphinic peptides for discovery of matrix metalloproteinase inhibitors. *J. Comb. Chem.* **2000**, *2* (6), 624−638.
(14) Nicolaou, K. C.; Xiao, X. Y.; Parandoosh, Z.; Senyei, A.; Nova, M. P. Radiofrequency encoded combinatorial chemistry. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2289.
(15) Xiao, X. Y.; Zhao, C.; Potash, H.; Nova, M. P. Combinatorial chemistry with laser optical encoding. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 780.
(16) Brenner, S.; Lerner, R. A. Encoded combinatorial chemistry. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89* (12), 5381−5383.
(17) Needels, M. C.; Jones, D. G.; Tate, E. H.; Heinkel, G. L.; Kochersperger, L. M.; Dower, W. J.; Barrett, R. W.; Gallop, M. A. Generation and screening of an oligonucleotide-encoded synthetic peptide library. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90* (22), 10700−10704.
(18) Ohlmeyer, M. H.; Swanson, R. N.; Dillard, L. W.; Reader, J. C.; Asouline, G.; Kobayashi, R.; Wigler, M.; Still, W. C. Complex synthetic chemical libraries indexed with molecular tags. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90* (23), 10922−10926.
(19) Borchard, A.; Still, W. C. Synthetic receptor binding elucidated with an encoded combinatorial library. *J. Am. Chem. Soc.* **1994**, *116*, 373.
(20) Geysen, H. M.; Rodda, S. J.; Mason, T. J. A priori delineation of a peptide which mimics a discontinuous antigenic determinant. *Mol. Immunol.* **1986**, *23*, 709−715.
(21) Pinilla, C.; Appel, J. R.; Blanc, P.; Houghten, R. A. Rapid identification of high affinity peptide ligands using positional scanning synthetic peptide combinatorial libraries. *Biotechniques* **1992**, *13* (6), 901−905.
(22) Dooley, C. T.; Houghten, R. A. Synthesis and screening of positional scanning combinatorial libraries. *Methods Mol. Biol.* **1998**, *87*, 13−24.
(23) Deprez, B.; Williard, X.; Bourel, L.; Coste, H.; Hyafil, F.; Tartar, A. Orthogonal combinatorial chemical libraries. *J. Am. Chem. Soc.* **1995**, *117*, 5405−5406.
(24) Erb, E.; Janda, K. D.; Brenner, S. Recursive deconvolution of combinatorial chemical libraries. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91* (24), 11422−11426.
(25) Pinilla, C.; Appel, J. R.; Houghten, R. A. Tea bag synthesis of positional scanning synthetic combinatorial libraries and their use for mapping antigenic determinants. *Methods Mol. Biol.* **1996**, *66*, 171−179.
(26) Pabst, M. J.; Beranova-Giorgianni, S.; Krueger, J. M. Effects of muramyl peptides on macrophages, monokines, and sleep. *NeuroImmunoModulation* **1999**, *6*, 261.
(27) Liu, G.; Zhang, S. D.; Xia, S. Q.; Ding, Z. K. Solid-Phase Synthesis of Muramyl Dipeptide (MDP) Derivatives Using a Multipin Method. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1361−1363.
(28) Siemion, I. Z.; Kluczyk, A. Tuftsin: on the 30-year anniversary of Victor Najjar's discovery. *Peptide* **1999**, *20* (5), 645−674.
(29) Johannsen, L.; Obal, F., Jr.; Kapas, L. Somnogenic activity of muramyl peptide-derived immune adjuvants. *Int. J. Immunopharmacol*. **1994**, *16* (2), 109.
(30) Lefrancier, P.; Lederer, E. Muramyl-peptides. *Pure Appl. Chem.* **1987**, *59*, 449.
(31) Kikelj, D.; Povsic, L.; Stalc, A.; Pristovsek, P.; Kidric, J. Novel immunologically-active conformationally restricted carbocyclic muramyl dipeptide analogs. *Med. Chem. Res*. **1996**, *6*, 118.
(32) Kikelj, D.; Pecar, S.; Kotnik, V.; Stalc, A.; Wraber-Herzog, B.; Simcic, S.; Ihan, A.; Klamfer, L.; Povsic, L.; Grahek, R.; Suhadolc, E.; Hocevar, M.; Honig, H.; Rogi-Kohlenprath, R. *N*-[*trans*-2-[[2′-(acetylamino)cyclohexyl]oxy]acetyl]-L-alanyl-D-glutamic acid: a novel immunologically active carbocyclic muramyl dipeptide analogue. *J Med Chem.* **1998**, *41* (4), 530−539.

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