Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 5426–5430, June 1995
Pharmacology

Library of libraries: Approach to synthetic combinatorial library design and screening of "pharmacophore" motifs

NIKOLAI F. SEPETOV*, VIKTOR KRCHŇÁK*, MAGDA STANKOVÁ*, SHELLY WADE*, KIT S. LAM†, AND MICHAL LEBL*

*Selectide Corporation, 1580 East Hanley Boulevard, Tucson, AZ 85737; and †Arizona Cancer Center and Department of Medicine, University of Arizona College of Medicine, Tucson, AZ 85724

Communicated by Avram Goldstein, Stanford University, Stanford, CA, January 17, 1995 (received for review October 19, 1994)

ABSTRACT **Construction of synthetic combinatorial libraries is described that allows for the generation of a library of motifs rather than a library of compounds. Peptide libraries based on this strategy were synthesized and screened with model targets streptavidin and anti-β-endorphin antibody. The screens resulted in observation of expected motifs providing evidence of the effectiveness of the suggested approach.**

The development of methods for generation of large combinatorial libraries has provided pharmacologists a tool in the search for new drug candidates. The most well-developed and highly used combinatorial libraries have been, until recently, oligomeric (peptide and oligonucleotide) in nature. Leads from these libraries tend to have poor pharmacological properties and generally must be modified to become useful pharmaceuticals. Therefore, the principal objective of screening oligomeric libraries should not be to identify a single active compound but to determine structural features critical for biological activity—i.e., to find the motif, or motifs, that can be used in the design of a drug candidate.

There are several strategies for the discovery of lead compounds that are based on the synthetic design of the combinatorial library used (for a review, see, for instance, refs. 1–3). The iterative (or serial) approach (4–6) is based on the synthesis of a number of libraries with defined but different amino acids at one or two adjacent positions. The positional scanning approach uses (7) n groups of peptide mixtures (n = the number of residues in the sequence) with a different amino acid defined at a certain position in each group.

Both of these methods include screening of libraries in which the target of interest is exposed to a pool of peptides. The concentration of an individual peptide in a pool can be very low; therefore, activity may be observed only if the total concentration of peptides, which are different but share the motif required for exhibition of activity, is high enough. In the case in which an interaction of peptide with a target requires a few residues that are not necessarily adjacent, such concentrations are not always achieved with pools of peptides having defined amino acids at only one or two adjacent positions. Thus, a high probability for successful screening with either the iterative or positional scanning approach can be expected only if the motif required for biological activity is short or continuous, or both.

Lam *et al.* (8) introduced the "parallel" approach based on the principle of generating a complete (or as complete as possible) representation of structures in a single library with one entity on each solid-phase support, which is then screened, and the identity of the positive test compound is determined from information on the solid-phase support. Table 1 shows the number of compounds and the amount of solid-phase resin (130-μm diameter beads) necessary to synthesize a library when 20 subunits are randomized at each position.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

We have designed and developed a method for the generation and screening of an innovative type of combinatorial library combining principles of iterative and parallel approach. This library type is free of the limitations of the methods described above. The new "library of libraries" method described in this manuscript allows the exploration of tremendous structural and chemical diversity through the use of a one motif–one bead approach rather than a one compound–one bead approach.

MATERIALS AND METHODS

General. Sequencing by Edman degradation was performed on an ABI 4778 protein sequencer (Applied Biosystems). Fluorenylmethoxycarbonyl (Fmoc)-protected amino acids with standard side-chain protecting groups were obtained from Propeptide (Vert-le-Petit, France). 2-Bromo-3-chloroindol phosphate was obtained from Ameresco (Solon, OH). Anti-β-endorphin monoclonal antibodies (clone 3-E7) were obtained from Boehringer Mannheim. Streptavidin conjugated to alkaline phosphatase was purchased from Pierce.

Library Synthesis. Libraries of peptides were constructed on TentaGel Resin S Amino-NH_2 (Rapp Polymere, Tubingen, Germany). Standard solid-phase peptide synthesis chemistry (Fmoc chemistry) was used. The mixture of protected amino acids with molar ratios adjusted according to the results from the pilot experiment was used in steps in which the amino acids in the mixture were coupled. The randomization steps were performed in accordance with the split-synthesis methodology (4, 8, 9). Finally, the Fmoc groups were removed with 20% (vol/vol) piperidine in dimethylformamide, and the side-chain protecting groups were removed with a mixture of trifluoroacetic acid/phenol/anisole/ethanedithiol, 94:2:2:2; vol/wt/vol/vol, or with reagent K [trifluoroacetic acid/phenol/water/thiophenol/ethanedithiol, 82.5:5:5:5:2.5 (vol/wt/vol/wt/vol)] (10). The resin was then washed thoroughly in dimethylformamide, neutralized with 10% (vol/vol) *N*,*N*-diisopropylethylamine (DIEA) in dimethylformamide, thoroughly washed again, hydrated, and stored in 0.01% HCl at 4°C.

Hexapeptide Library of Libraries with Three Randomized Positions. The resin (90-μm particle size, 0.29 mmol/g, 10 g) was swollen in dimethylformamide, and Fmoc-glycine, Fmoc-β-alanine, Fmoc-glycine, and Fmoc-β-alanine were coupled by using diisopropylcarbodiimide (DIC) and *N*-hydroxybenzotriazole (HOBt). The Fmoc groups were removed and synthesis proceeded according to the scheme in Fig. 1.

Library of Libraries of Variable Length. After the assembly of the linker (as above), this library was synthesized according to the algorithm described later in the text.

RESULTS AND DISCUSSION

Our approach is based on the assumption that only a few subunits (amino acids) of a compound or motif are crucial for interaction of the compound with the target molecule, and we call these "pharmacophores"; the remaining components of

Table 1. Numbers of beads (N_b) and weight of resin (W_r) with 130-μm bead size required for complete libraries of different lengths using the one bead–one peptide and the library of libraries approaches

	One bead–one peptide		Library of libraries	
Length, aa	$N_b \times 10^{-3}$	W_r, g	$N_b \times 10^{-3}$	W_r, g
3	8	0.008	8	0.008
4	160	0.160	32	0.032
5	3,200	3.20	80	0.080
6	64,000	64.0	160	0.160
7	1,280,000	1.28×10^3	280	0.280
8	25,600,000	25.6×10^3	448	0.448
9	512,000,000	512×10^3	672	0.672
10	10,240,000,000	10.2×10^6	960	0.960
11	204,800,000,000	204×10^6	1320	1.32
12	4,096,000,000,000		1760	1.76
13	81,920,000,000,000		2288	2.29
14	1,638,000,000,000,000		2912	2.91
15	32,770,000,000,000,000		3640	3.64

Calculations were performed assuming 20 amino acids (aa) will be used for randomization and that the libraries of libraries contain three-pharmacophore motifs.

the compound serve to present these pharmacophores in the proper position and orientation. Thus, amino acids within active peptides can be considered either as pharmacophores or as structural units. The basic idea of our approach is to synthesize on solid-phase support, a library consisting of compounds having only a limited number of pharmacophores in each compound spaced with structural units. Two problems are encountered with this scenario that do not occur with standard library approaches: (*i*) which amino acids should be used as structural units, and (*ii*) how many pharmacophores in each library compound are necessary to exhibit biological activity. Unfortunately, amino acids known as structural units do not exist in nature. By postulating that the side chain of the amino acid used as a structural unit is not involved in an interaction with a target, any amino acid could be suitable as a structural unit. However, one cannot ignore the possibility that a particular amino acid used as a structural unit within a peptide may have some negative effect on its interaction with a particular target. Moreover, peptides in a library synthesized with pharmacophore residues and any particular amino acid chosen to be the structural units may have some characteristics (secondary structure, propensity for aggregation, etc.) associated with properties of the structural-unit amino acid. For example, alanine, which is a very good candidate for the role of structural unit, can cause problems connected with the insolubility of oligoalanines (11). These issues may be avoided by using a mixture of different amino acids as the structural unit in the library in much the same way as a mixture of the colors of the spectrum form white light.

Each solid-phase support of a library utilizing a mixture of amino acids as the structural units will consist of many different compounds. For example, 400 different peptides will be synthesized on a single bead when a mixture of 20 amino acids is used in two structural-unit positions—8000 peptides with three positions, 160,000 with four positions, etc. However, all of these compounds will be characterized by a certain motif—i.e., they will contain certain pharmacophores at certain positions.

In general, it is impossible to predict *a priori* for a particular target how many residues in a peptide must be defined to observe activity in a particular screening assay. According to published data, only two to four residues in the ligand are essential for biological activity for many different targets. Thus, it can be surmised, peptide libraries displaying two, three, or four pharmacophore residues will be most applicable. These pharmacophores may be arranged within compounds in different ways. In the case of peptides, pharmacophores may be located at the N terminus or C terminus, or within a peptide they may be adjacent or separated by structural units, and so on. For example, three pharmacophore positions within the hexapeptide framework may be placed in 20 different arrangements or positional motifs (Table 2). Several of these motifs are similar (e.g., $R_1R_2R_3$, patterns 1, 4, 10, and 20; $R_1R_2XR_3$, patterns 2, 8, and 15; etc.), differing only in the distance from

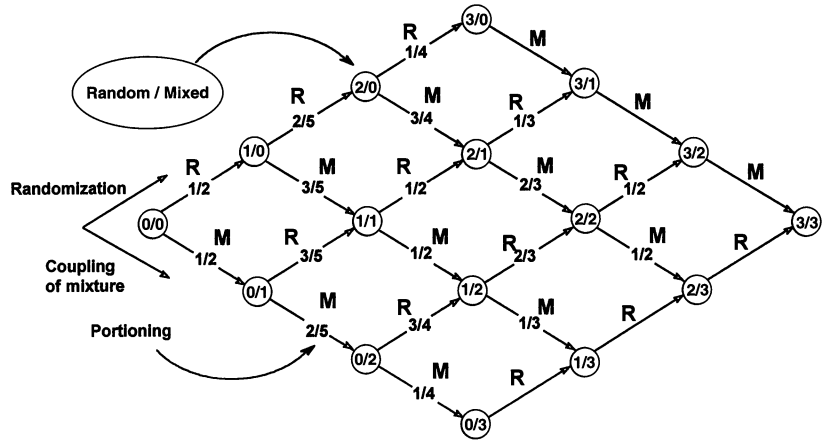

FIG. 1. Scheme of the synthesis of hexapeptide library of libraries with a three-amino-acid motif. Arrows carrying the symbol R represent performed randomization, and arrows carrying the symbol M means that mixture of amino acids was coupled.

Table 2. All of the possible arrangements for three pharmacophores (R_1-R_2-R_3) that can be obtained within a hexapeptide structure in which X denotes a structural unit

1	$R_1R_2R_3XXX$	11	$R_1R_2XXXR_3$
2	$R_1R_2XR_3XX$	12	$R_1XR_2XXR_3$
3	$R_1XR_2R_3XX$	13	$R_1XXR_2XR_3$
4	$XR_1R_2R_3XX$	14	$XR_1R_2XXR_3$
5	$R_1R_2XXR_3X$	15	$XXR_1R_2XR_3$
6	$R_1XR_2XR_3X$	16	$XR_1XR_2XR_3$
7	$R_1XXR_2R_3X$	17	$R_1XXXR_2R_3$
8	$XR_1R_2XR_3X$	18	$XR_1XXR_2R_3$
9	$XR_1XR_2R_3X$	19	$XXR_1XR_2R_3$
10	$XXR_1R_2R_3X$	20	$XXXR_1R_2R_3$

the N or C terminus, thereby possibly affecting binding of the target molecule. In general, the number of possible positional motifs (N_{pm}) in a library is described by the formula: $N_{pm} = (r + m)!/r!{\cdot}m!$, where r is the number of pharmacophore positions, m is the number of structural unit positions, and $r + m$ is the number of residues in the peptide.

Randomization at pharmacophore positions within one positional motif creates a single library in which the number of permutations is determined by the number of pharmacophore residues (a^r, where a is the number of pharmacophore amino acids used during each step of randomization and r is the number of pharmacophore positions) rather than the total number of residues in a peptide. Randomization at pharmacophore positions within all positional motifs will create a library of libraries. Therefore, a peptidic library of libraries should be considered more as a library of motifs than a library of peptides. Though the library of libraries approach does not utilize the one bead–one peptide principle, it is clearly a parallel approach, since all possible motifs can be presented to the target at the same time and characterized only after they are proven to be functionally relevant.

The size of a library of libraries (the total number of possible motifs) is determined by the number of permutations in a library with one positional motif and by the number of possible positional motifs: $N_m = a^r N_{pm}$. This number is much less than the number of possible compounds in classical one bead–one compound libraries ($N_c = a^n$, where n is the number of residues in a peptide). Table 1 summarizes the calculations of the number of beads required for complete libraries using the one bead–one peptide and library of libraries approaches.

Synthesis of Libraries of Libraries. Synthesis of a library of libraries is accomplished through two diverse synthetic steps corresponding to pharmacophore (motif) positions and structural unit positions. The structural unit positions are filled by using a mixture of amino acids for coupling, while the randomization procedure (the split and mix method described by Furka *et al.* (9) and others; refs. 4 and 8) is utilized to fill pharmacophore positions. In filling structural unit positions, it is important to achieve approximately equimolar ratios of coupled amino acids, otherwise some structures will be over- or underrepresented in the pool of compounds synthesized on the solid-phase particle. This can be done by acylation with a coupling mixture comprising disproportionate molar quantities of each component, determined inversely according to each subunit's reactivity (5, 12) or by allowing subequimolar amounts of the mixture to react, followed by coupling with an excess of the mixture to assure complete coupling (13–15). The first procedure was used in our library of libraries synthesis.

Three different methods for synthesis of libraries of libraries can be considered. The first method is the routine synthesis of individual sublibraries corresponding to each positional motif. For synthesis of a library of libraries by this method, the resin must be split into N_{pm} individual batches, which are then carried through the synthesis by using the randomization procedure at the motif positions and allowing the resins to react with a mixture of amino acids at the structural unit positions, and at the end of synthesis all batches are combined. The method is simple but laborious. For example, the synthesis of a hexapeptide library of libraries with a three-amino-acid motif requires 60 randomization steps for the synthesis of the 20 sublibraries.

The second method is more complicated but allows for a significant reduction in the number of randomization steps. The synthesis of a hexapeptide library of libraries with a three-amino-acid motif in which only 12 randomization steps are required serves as an illustration of this method. The analysis of Table 2 shows that 10 of the 20 sublibraries display peptide populations with C-terminal positioning for randomization, while the other 10 reveal positions for coupling a mixture of amino acids. This demonstrates that in the first step of synthesis, it is not necessary to divide the resin into 20 batches. The resin needs only to be divided into two equal parts: one part to be coupled with a mixture of amino acids and the other to be randomized. According to the scheme in Fig. 1, 6 of 10 sublibraries with amino acid mixtures at the C terminus of the peptide population are randomized at the second position from the C terminus, while the other four have the mixture of amino acids at that position. Hence, after initial coupling, the portion of resin with a peptide population having a mixture of amino acids coupled at the C terminus needs to be divided in the ratio of 6:4 or 3:2 for randomization and coupling with the mixture, respectively. The same logic can be applied to the portion of the resin initially randomized. Further analysis of Table 2 allows us to create an algorithmic diagram for synthesis of a hexamer library of libraries with a three-amino-acid motif (Fig. 1). After the second library synthetic step, it is possible to recombine parts of the resin on which one randomization coupling and one coupling of the mixture was performed. Splitting and recombination of the resin for next synthetic steps are repeated until three steps of randomization and three mixed couplings are performed on all resin particles. The ratios for splitting the resin at each divergence point of the diagram, given in Fig. 1, are necessary for presentation of all individual sublibraries in equal quantities within a library of libraries.

An analogous diagram for synthesis can be created for any library of libraries with r positions for randomization and with m positions for coupling a mixture of amino acids. The number of randomization steps is equal to $r{\cdot}(m + 1)$. The ratio for splitting the resin at each divergence point in any synthesis scheme, based on this method, will be determined by the number of randomization and mixture coupling steps necessary to complete synthesis from this point in the scheme.

The third way of constructing a library of libraries is very simple but results in a library with peptides of different lengths, which can be considered as a disadvantage of this method. The synthesis of a library with a three-amino-acid motif is performed as follows. At the beginning of synthesis and after each randomization step, one-quarter of the resin is separated and the mixture of amino acids is coupled to the remaining part. After this coupling, one-third of the resin is separated, and the remainder undergoes coupling with the mixture of amino acids. The next coupling is performed with half of the resin from the previous coupling. All portions of the resin are then combined and a randomization is performed. Synthesis of a library of libraries with a three-amino-acid motif by this method consists of three randomization steps and four stages of multiple couplings of amino acid mixtures. As a result of the described procedure, each solid-phase particle of the library went through three mandatory randomization steps and as many as 12 acylations with the mixture of amino acids. This library, containing peptides of lengths from 3 to 15 residues, consists of 256 positional motif sublibraries. Among sublibraries of peptides up to hexapeptides, all positional motifs are presented. However, because this synthetic scheme does not

allow more than three successive acylations with the amino acid mixture, motifs in which pharmacophore positions are separated by more than three adjacent structural unit positions are not represented.

We have synthesized both of the above described versions of the library of libraries with a three-amino-acid motif: the hexapeptide library (**I**) and the 3- to 15-mer library (**II**). Nineteen proteinogenic amino acids (no cysteine) were used for each randomization step, and a mixture of the same amino acids were coupled to fill structural unit positions.

Screening Libraries of Libraries. Two model screening systems (binding with streptavidin and anti-β-endorphin monoclonal antibody) were used to test the library of libraries approach. The motifs from peptide libraries for both targets are well known. Peptides containing the three-residue motif His-Pro-(Gln or Met) [HP(Q/M)] bind streptavidin with affinities in high micromolar range. The N-terminal tetrapeptide motif Tyr-Gly-(Gly or Ala)-(Phe or Trp) [YG(G/A)(F/W)] is crucial for binding anti-β-endorphin monoclonal antibodies. Binding of peptides with this motif to antibodies is very tight and has nanomolar affinity. Screening with both targets was performed by using a chromogenic assay according to a published protocol (16). Colorized beads from the screens were selected, destained, and incubated with the same concentration of the target molecule in the presence of a known specific competitor (biotin or [Leu^5]enkephalin) to prove specificity of binding. Beads that did not stain in this experiment (specific binders) were washed and stained again in the absence of competitor. The peptides on positive beads after this staining were sequenced by Edman degradation. Positions in the peptide population occupied by pharmacophores are clearly marked by a single amino acid signal corresponding to the total amount of peptide on the bead (≈50–150 pmol), whereas those positions occupied by a mixture of amino acids yield all amino acids used for coupling in amounts ≈20 times lower (2–7 pmol).

Antibody screening confirmed earlier findings of the N-terminal YG(G/A)(F/W) as a very strong binding motif (Table 3). This table shows that even hits (identified sequences) not containing tyrosine very clearly indicate the importance of positioning of the Gly-(Gly or Ala)-Phe [G(G/A)F] triad, placed one amino acid from the N terminus.

Screening with streptavidin (Table 4) revealed the known motif HP(Q/M). However, sequences containing tryptophan were also identified, in which the residue is separated by as many as three amino acid residues from the HP or P(Q/M) sequences. The sequences found may represent the longer motif WXX[HP(Q/M)]. This motif was not and could not be observed in pentapeptide libraries, but the motif was displayed in 8 of 20 sequences determined with the use of phage libraries (17).

While the protocol used for screening the library of libraries is identical to that of standard one bead–one peptide libraries,

Table 3. Sequences obtained from screening hexamer and variable-length libraries of libraries (5×10^5 beads) with anti-β-endorphin antibodies

Library **I**		Library **II**
YGXFXX	**YXAFXX**	X**GAF**XXX
YGXFXX	**YGG**XXX	X**GAF**XXX
YGXFXX	**YGA**XXX	X**GAF**XXX
YGXFXX	X**GGF**XX	X**GGF**
YGXFXX	X**GGF**XX	
YGXWXX	X**GAF**XX	
YXGFXX	X**GAF**XX	
YXGFXX	X**GAF**XX	
YXGFXX	X**GAF**XX	

The single-letter amino acid code is used in which X is a position reflecting a mixture of amino acids.

Table 4. Sequences obtained from screening hexamer and variable-length libraries of libraries (5×10^5 beads) with streptavidin

Library **I**		Library **II**
XXX**HPQ**	**HPQ**XXX	X**W**XXX**PQ**XXX
XXX**HPQ**	**HPQ**XXX	XX**W**XXX**HP**XXX
XXX**HPQ**	**W**XX**HPX**	XX**N**X**P**X**F**XXX
XXX**HPQ**	**W**XXX**PM**	
XXX**HPQ**	X**W**X**HP**X	
XXX**HPQ**	XX**WHP**X	
XXX**HPM**	X**R**X**HP**X	
XX**HPQ**X	**HP**X**F**XX	
XX**HPM**X	XX**PQF**X	
X**HPQ**XX		

The single-letter amino acid code is used in which X is a position reflecting a mixture of amino acids.

the outcome of the screens have two differences worth noting. First, we found higher specificity of the library of libraries' hits in comparison with hits from standard libraries. In our experience with streptavidin and anti-β-endorphin antibodies, approximately half of the hits selected in the primary screen from a standard library are due to nonspecific interactions, evidenced by the lack of competition with a specific inhibitor. In the case of the library of libraries, almost all of the beads made it through the competition step. One explanation for this occurrence can be found when considering properties of the pool of peptides on the surface of solid-phase particles in the one bead–one peptide and one bead–one motif libraries. In a one bead–one peptide library, there are beads displaying peptides with highly expressed properties—they can be extremely charged, hydrophobic, hydrophilic, etc. Some of these characteristics may promote nonspecific binding of a target with such beads. This situation cannot occur in a library of libraries, since the presence of a mixture of amino acids in structural unit positions generates on each bead a pool of peptides with diverse properties.

The second observed difference in the outcome of the screens of standard libraries and libraries of libraries is that the total number of specific positive beads obtained from screening one bead–one peptide libraries is larger than that from one bead–one motif libraries, when equal amounts of the libraries were screened. The explanation for this phenomenon is based on the recognition that the sequence of all peptides on each bead in a one bead–one peptide library fits more than one motif; thus, the redundancy of motifs in standard libraries is higher.

Interpretation of Results from Screening the Library of Libraries. To identify a motif using the one bead–one peptide library approach, it is often necessary to sequence many beads, especially with long peptides, before consensus can be observed. In contrast, sequencing even a single hit from a library of libraries may result in identification of a motif. It should be mentioned that utilizing a mixture of amino acids instead of an "ideal" structural unit (the side chain of which has absolutely no interaction with the target) in a library of libraries may complicate the interpretation of screening results. For example, the N-terminal tetrapeptide YG(G/A)(F/W) motif was easily composed from sequences obtained from a screen with anti-β-endorphin antibodies. However, because the screened libraries have only three positions for randomization, it was not possible to expose all four residues important for binding in a single sequence, and in this case the motif cannot be identified from a single hit. For instance, the importance of the tyrosine residue for binding cannot be elucidated from the XGGF and XGAF sequences found in the screen (Table 3). Binding of these peptides with anti-β-endorphin antibodies might be observed even if tyrosine were absolutely essential, because the mixture of amino acids used as structural units at the N

terminus includes tyrosine. Thus, the portion of the peptide pool with N-terminal tyrosine may be sufficient for colorization of the beads during the screening procedure because of the high affinity of peptides with the YG(G/A)F motif for anti-β-endorphin antibodies. (It should be noted that library **II** was screened incompletely—only about 30% of the library of 1,755,904 mixtures (motifs) was submitted to the test—and structure with a defined N-terminal tyrosine may not even have been present in the sample. In the hexapeptide library, screened in 3-fold redundancy, the N-terminal tyrosine was clearly detected.)

On the other hand, the presence of a mixture of amino acids at structural unit positions can be considered as an advantage. In the case where the number of pharmacophore residues required to produce biological effect is bigger than the number of positions for randomization, there is still a chance that activity will be detected based on the added interaction of particular amino acids in the structural unit positions. Both issues can be illustrated by screening with anti-β-endorphin antibodies.

CONCLUSION

The library of libraries approach for construction of synthetic combinatorial libraries offers an additional tool for the medicinal chemist in the search for lead compounds for drug development. By enabling the identification of key information regarding the groups responsible for ligand–target interaction in a single screening process, this approach overcomes many potential disadvantages of other synthetic strategies for oligomeric libraries. Although we are generally not interested in large oligomers as a starting point for lead optimization, the discovery of a small set of active residues within a larger framework may provide information useful for the synthesis of nonoligomeric mimics, or for the design of nonoligomeric small molecule libraries. Also, one can consider the minimization of nonspecific binding during the one-bead screening process as an important advantage of the library of libraries approach.

While we have demonstrated the library of libraries approach using peptide libraries synthesized on resin, it is equally applicable to nonpeptide structures and is not limited to the use of solid-phase particles but can be used with other library and multiple-synthesis formats. For example, both the Mimotopes (5) and Affymax (18) approaches are easily adapted to this approach. In this respect, the technique described can be considered enabling in that it converts these multiple synthesis formats to true library formats, since synthesis of a complete library of libraries would require thousands of pins or surface sites rather than the millions otherwise necessary.

The authors are indebted to their colleagues at Selectide Corp. for their technical help, encouragement, and constructive criticism. This work was supported in part by National Cooperative Drug Discovery Group (NCDDG) cooperative agreement CA-57723.

1. Gordon, E. M., Barrett, R. W., Dower, W. J., Fodor, S. P. A. & Gallop, M. A. (1994) *J. Med. Chem.* **37,** 1385–1401.
2. Gallop, M. A., Barrett, R. W., Dower, W. J., Fodor, S. P. A. & Gordon, E. M. (1994) *J. Med. Chem.* **37,** 1233–1251.
3. Lebl, M., Krchňák, V., Sepetov, N. F., Seligmann, B., Štrop, P., Felder, S. & Lam, K. S. (1995) *Biopolymers* **37,** 177–198.
4. Houghten, R. A., Pinilla, C., Blondelle, S. E., Appel, J. R., Dooley, C. T. & Cuervo, J. H. (1991) *Nature (London)* **354,** 84–86.
5. Geysen, H. M., Rodda, S. J. & Mason, T. J. (1986) *Mol. Immunol.* **23,** 709–715.
6. Owens, R. A., Gesellchen, P. D., Houchins, B. J. & DiMarchi, R. D. (1991) *Biochem. Biophys. Res. Commun.* **181,** 402–408.
7. Dooley, C. T. & Houghten, R. A. (1993) *Life Sci.* **52,** 1509–1517.
8. Lam, K. S., Salmon, S. E., Hersh, E. M., Hruby, V. J., Kazmierski, W. M. & Knapp, R. J. (1991) *Nature (London)* **354,** 82–84.
9. Furka, A., Sebestyén, F., Asgedom, M. & Dibó, G. (1991) *Int. J. Pept. Protein Res.* **37,** 487–493.
10. King, D. S., Fields, C. G. & Fields, G. B. (1990) *Int. J. Pept. Protein Res.* **36,** 255–266.
11. Bedford, J., Johnson, T., Jun, W. & Sheppard, R. C. (1992) in *Innovation and Perspectives in Solid Phase Peptide Synthesis*, ed. Epton, R. (Intercept, Andover, MA), pp. 213–219.
12. Pinilla, C., Appel, J. R., Blanc, P. & Houghten, R. A. (1992) *BioTechniques* **13,** 901–905.
13. Frank, R. (1994) in *Innovation and Perspectives in Solid Phase Synthesis*, ed. Epton, R. (Mayflower, Birmingham, U.K.), pp. 509–512.
14. Kramer, A., Volkmer-Engert, R., Malin, R., Reineke, U. & Schneider-Mergener, J. (1993) *Pept. Res.* **6,** 314–319.
15. Andrews, P. C., Boyd, J., Ogorzalek, Loo, R., Zhao, R., Zhu, C. Q., Grant, K. & Williams, S. (1994) in *Techniques in Protein Chemistry V*, ed. Crabb, J. W. (Academic, San Diego), pp. 485–492.
16. Lam, K. S. & Lebl, M. (1992) *Immunomethods* **1,** 11–15.
17. Devlin, J. J., Panganiban, L. C. & Devlin, P. E. (1990) *Science* **249,** 404–406.
18. Fodor, S. P. A., Read, J. L., Pirrung, M. C., Stryer, L., Lu, A. T. & Solas, D. (1991) *Science* **251,** 767–773.