ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1996, p. 1067–1071 Vol. 40, No. 5
0066-4804/96/$04.00+0

MINIREVIEW

Synthetic Combinatorial Libraries: Novel Discovery Strategy for Identification of Antimicrobial Agents

SYLVIE E. BLONDELLE,* ENRIQUE PÉREZ-PAYÁ,† AND RICHARD A. HOUGHTEN

Torrey Pines Institute for Molecular Studies, San Diego, California

Bacterial resistance to existing drugs is a constantly growing process that is reaching alarming levels (for reviews, see references 16 and 35). This, combined with the recent decline in the development of new antibiotics, can be anticipated to lead to the appearance of infectious diseases lacking a ready treatment regimen. For instance, methicillin-resistant *Staphylococcus aureus* (MRSA) is resistant to all β-lactams, including penicillins, carbapenems, and penems, because of the production of a penicillin-binding protein that has a low affinity for β-lactam antibiotics (31). This resistance positions MRSA as an increasingly dangerous pathogen in the 1990s (10). Vancomycin is currently the agent of choice against MRSA (for a review, see reference 9). However, its widespread use, as expected, is leading to new resistant strains, as has been observed in enterococcal species (43).

Classical sources for novel antimicrobial agents include biological sources (e.g., marine waters, insects, mammals, plants, and soil samples [1, 8, 33, 34, 39]), as well as large collections of individual compounds gathered from various laboratories. While these compounds have diverse skeletal and functional arrays, the isolation and characterization of such natural compounds or the synthesis of large collections of individual compounds can be cumbersome and can delay the discovery process. The recent development of synthetic combinatorial libraries (SCLs) represents a major advancement in the discovery of new lead compounds for drug development (for reviews, see references 4, 21, and 37). Although its use for the identification of novel antimicrobial agents has not been fully exploited, the findings reported so far indicate that combinatorial techniques will play an important role in the infectious disease area.

SCL APPROACHES

A library is commonly defined as any collection of compounds ranging in number from tens to millions that can be prepared either separately by parallel synthesis methods or by combinatorial chemistry. In contrast, combinatorial libraries represent vast numbers (thousands to millions) of compounds prepared simultaneously in a highly systematic manner. These libraries consist of all possible combinations of a class of compounds. Each of these compounds can be physically separated from one another by being attached to a solid support (20, 22, 30) or can be combined in mixtures of compounds free in solution (28). Combinatorial libraries enable the simultaneous screening of thousands to millions of compounds and the rapid identification of novel, biologically active compounds without any prior structural or sequence knowledge. Several techniques have been developed to generate combinatorial libraries (i.e., using molecular biology or chemical processes [for reviews, see references 4, 21, and 37]). To date, only one of the existing SCL techniques has been used to identify antimicrobial agents (4–7, 25, 28, 36). This involves the use of soluble SCLs made up of mixtures of thousands of peptides or peptidomimetic compounds that are free to interact with microorganisms in solution (i.e., the compounds are not attached to a solid support). For example, because of the solubility properties of these mixtures, standard microdilution assays have been used for the identification of antimicrobial compounds. In contrast, because of their heterogeneity and the presence of a secondary insoluble support, difficulties are encountered in the use of libraries that are chemically bound to a solid support (i.e., plastic pins [22], resin beads [30], silica surface [20], etc.) in cell-based assays.

Soluble SCLs are prepared by a solid-phase, simultaneous multiple synthesis methodology (24); this is followed by cleavage of the mixtures from their support (26). The simultaneous multiple synthesis technology enables mixtures of compounds to be synthesized in separate groups, each being defined by individual residues or building blocks at one or more given positions (25, 28). The following are soluble hexapeptide SCLs: OOXXXX-NH_2, XXOOXX-NH_2, XXXXOO-NH_2, OXXXXX-NH_2, XOXXXX-NH_2, XXOXXX-NH_2, XXXOXX-NH_2, XXXXOX-NH_2, and XXXXXO-NH_2, where X is a mixture of 20 L-amino acids and O is one of the 20 L-amino acids. Such segregation permits the straightforward identification of the active compound(s) present in a pool of millions of other compounds without carrying out additional analytical analyses. Two deconvolution processes have been proposed for the identification of the active compound(s) in these mixtures: (i) an iterative process involving three consecutive steps of screening, selecting the most active mixture(s), and synthesizing new mixtures composed of a smaller number of individual compounds (28) and (ii) a positional scanning process in which the most important amino acid(s) or building block(s) is determined for every position in a single screening assay (25). As illustrated in Table 1, the iterative process results in the successive identification of one residue or building block at a time. For example, following the screening of 400 separate hexapeptide mixtures defined as Ac-OOXXXX-NH_2 (where Ac is N-acetylated), Ac-RRXXXX-NH_2 was found to show the highest degree of anticandidal activity (4). The next iterative step involved the synthesis and screening of 20 new peptide mixtures defined by the formula Ac-RROXXX-NH_2. By defining an additional position with a single amino acid, the number of individual compounds within a mixture decreases (20 times in the example

* Corresponding author. Mailing address: 3550 General Atomics Ct., San Diego, CA 92121. Phone: (619) 455-3803. Fax: (619) 455-3804. Electronic mail address: sylvieb@cerf.net.

† Present address: Departament de Bioquimica i Biologia Molecular, Universitat de València, E-46100 Burjassot, València, Spain.

TABLE 1. Identification of antifungal hexapeptides by an iterative deconvolution process[a]

Process	Sequence[b]	No. of peptides per mixture	IC_{50} (μg/ml)[c]
Synthesis	Ac-OOXXXX-NH_2	160,000	
Screening and selection	Ac-RRXXXX-NH_2	160,000	1,704
Synthesis	Ac-RROXXX-NH_2	8,000	
Screening and selection	Ac-RRWXXX-NH_2	8,000	405
Synthesis	Ac-RRWOXX-NH_2	400	
Screening and selection	Ac-RRWCXX-NH_2	400	127
Synthesis	Ac-RRWCOX-NH_2	20	
Screening and selection	Ac-RRWCKX-NH_2	20	74
Synthesis	Ac-RRWCKO-NH_2	1	
Screening and selection	Ac-RRWCKR-NH_2	1	67

[a] From reference 4.
[b] X and O are as defined in the text, Ac is N-acetylated, and one-letter amino acid codes are used and are defined as follows: C, cysteine; K, lysine; R, arginine; W, tryptophan.
[c] IC_{50}, peptide mixture concentration that inhibits 50% of yeast growth (42). The activity was determined against 10^5 CFU of *C. albicans* ATCC 10231 per ml following a 48-h incubation at 30°C.

presented here) each time that the iterative process is repeated. The selection criteria at each iterative step are based on the biological activity of interest, as well as on the chemical and structural natures of the most active mixtures. Thus, one can select a number of mixtures showing similar potent activities but different chemical characteristics or a single mixture if the most active ones are characterized by amino acids or building blocks with similar chemical natures. This iterative SCL approach requires 6 to 12 weeks to complete.

The use of the positional scanning deconvolution process, on the other hand, requires only a single screening step in order to identify active individual compounds. This process was used to identify antistaphylococcal N-permethylated hexapeptidomimetic compounds (Table 2) (36). This process involves the screening of separate positional SCLs which differ by the position of the defined amino acid(s) or building block(s), with the number of positional SCLs depending on the length of the sequence and/or the number of positions defined (38). For instance, a positional scanning hexapeptide SCL can be composed of six separate SCLs in which a single position is defined (total of 120 mixtures if one considers the 20 L-amino acids) or of three separate SCLs in which two positions are defined (total of 1,200 mixtures) (see the building blocks listed above). When used in concert, the data derived from each positional SCL yield information about the most important amino acid(s) or building block(s) for every position. Individual compounds that represent all possible combinations of the selected amino acids or building blocks at each position are then synthesized in order to confirm the screening data and determine their relative activities. In addition to the selection criteria described above, an activity cutoff is necessary to minimize the number of compounds that one must ultimately synthesize.

TABLE 2. Identification of antistaphylococcal permethylated hexapeptides by a positional scanning deconvolution process[a]

Process	Sequences[b]	No. of compounds per mixture	IC_{50} (μg/ml)[c]
Synthesis	pm[OXXXXX]	1,889,568	
	pm[XOXXXX]	1,889,568	
	pm[XXOXXX]	1,889,568	
	pm[XXXOXX]	1,889,568	
	pm[XXXXOX]	1,889,568	
	pm[XXXXXO]	1,889,568	
Screening and selection	pm[LXXXXX]	1,889,568	189
	pm[XFXXXX]	1,889,568	94
	pm[XXIXXX]	1,889,568	93
	pm[XXXFXX]	1,889,568	93
	pm[XXXXFX]	1,889,568	95
	pm[XXXXXF]	1,889,568	94
Synthesis and screening	pm[LFIFFF]	1	6

[a] From reference 36.
[b] X represents a mixture of 18 permethylated L-amino acids, O represents 1 of 20 permethylated L-amino acids, pm is N-permethylated, and one-letter amino acid codes are used and are defined as follows: F, phenylalanine; I, isoleucine; L, leucine.
[c] IC_{50}, peptide mixture concentration that inhibits 50% of bacterial growth (42). The activity was determined against 10^5 CFU of *S. aureus* ATCC 29213 per ml following a 21-h incubation at 37°C.

In practice, there is not typically one and only one active sequence present in a mixture, but there are many acceptable conservative substitutions for most peptides and peptidomimetic compounds (e.g., valine for isoleucine and serine for threonine). This greatly increases the "effective" concentration of an active sequence and thus the "signal" seen in the assay. In the case of the identified anticandidal peptide from the hexapeptide SCL illustrated in Table 1, the concentration of each peptide was approximately 62 ng/ml (64 nM) at a mixture concentration of 10 mg/ml. The activity of 67 μg/ml found for the selected individual peptide indicates the presence of numerous other peptides with similar or lower levels of activity within the initial Ac-RRXXXX-NH_2 mixture acting in an additive or positive synergistic manner.

NOVEL ANTIMICROBIAL COMPOUNDS DERIVED FROM SCLs

L-Amino acid-containing peptides. On the basis of well-established synthetic procedures (32), peptide SCLs represent the first class of libraries that have been developed. A number of these SCLs were assayed to identify novel antimicrobial agents. For instance, series of L-amino acid hexapeptides were found to show activity at micromolar levels against both gram-positive and gram-negative bacteria and the yeast *Candida albicans*, with little or no lytic effect on erythrocyte (Table 3). Kinetic studies indicated bacteriocidal effects for these peptides (5). Also, these newly identified hexapeptides had activities similar to or greater than those of longer, naturally occurring antimicrobial peptides such as magainin and cecropin (5).

The major drawbacks in considering L-amino acid-containing peptides as therapeutic agents are their lack of oral bioavailability and their rapid enzymatic degradation. However, a number of peptide antibiotics have been shown to be of great value as topical therapeutics (e.g., polymyxin and bacitracin [29, 44]), and it appears that insects and mammals utilize peptides as a primary host defense system to counter bacterial infections (1, 8, 33, 45). In contrast to classical antibiotics, peptides generally exert their activity by altering the permeability properties of bacterial plasma membranes (40). This different mechanism of action adds value to L-amino acid-containing peptides as alternative agents in the fight against new drug-resistant strains.

D-Amino acid-and unnatural amino acid-containing peptides. While not directly derived from a deconvolution process, it was found that the all-D analogs of L-amino acid-containing hexapeptides identified from SCL screenings showed antimi-

TABLE 3. L-Amino acid-containing peptides derived from generic SCLs

Series sequences[a]	Most active compounds[a]	IC_{50} (μg/ml) for[b]:			% Hemolysis at 100 μg/ml[c]	Reference
		S. aureus	*E. coli*	*C. albicans*		
Ac-RRWWCO-NH_2	Ac-RRWWCR-NH_2	11–12	12–18	100–101	0	5
	Ac-RRWWCF-NH_2	10–14	20–21	70–97	3–4	5
Ac-RRWWRO-NH_2	Ac-RRWWRF-NH_2	5–10	8–16	39–80	0	37
	Ac-RRWWRH-NH_2	19–38	11–22	30–60	0–1	37
	Ac-RRWWRR-NH_2	16–32	16–32	28–56	1–2	4,37
Ac-FRWLLO-NH_2	Ac-FRWLLF-NH_2	11–22	ND[d]	>500	3–6	25,37
	Ac-FRWLLR-NH_2	13–26	ND	54–108	20–30	25,37
Ac-FRWWHO-NH_2	Ac-FRWWHR-NH_2	18–36	13–26	ND	6–10	27,37
	Ac-FRWWHW-NH_2	23–46	27–54	ND	4–6	27,37
Ac-RRWCKO-NH_2	Ac-RRWCKR-NH_2	ND	ND	67–130	ND	4
	Ac-RRWCKH-NH_2	ND	ND	70–140	ND	4

[a] O is as defined in the text, Ac is N-acetylated, and one-letter amino acid codes are used and are defined as follows: R, arginine; W, tryptophan; C, cysteine; F, phenylalanine; H, histidine; L, leucine; K, lysine.
[b] See note *c* of Table 2. The activity was determined against 10^5 CFU of *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 per ml after a 21-h incubation at 37°C and against *C. albicans* ATCC 10231 after a 48-h incubation at 30°C.
[c] Percent hemolysis was determined against 0.25% human erythrocytes after a 1-h incubation at 37°C (5).
[d] ND, not determined.

crobial activities similar to those of the L-amino acid-containing hexapeptides (5). These results suggest that chirality is not a driving feature in the antimicrobial activities of these hexapeptides and that a surface active-type mechanism is most likely responsible for their lytic effects.

In a first attempt to circumvent the drawbacks inherent to L-amino acids, a tetrapeptide SCL was generated from mixtures of L-, D-, and unnatural amino acids (7). The insertion of such amino acids was anticipated to enhance the stability of the peptides toward proteolysis. Following an iterative deconvolution process, four series of antimicrobial tetrapeptides were identified (Table 4). A number of these tetrapeptides exhibited similar activities against MRSA and a susceptible strain of *S. aureus* (Table 4). In contrast to the hexapeptides described above, these tetrapeptides appeared to be bacteriostatic. However, lower activity in the presence of serum indicated the occurrence of heat-stable protease degradation (7). In addition, these peptides showed higher hemolytic activities than the L-amino acid-containing hexapeptides described above (Tables 3 and 4).

Peptidomimetic compounds. Recent advances in synthesis technologies and chemistries have led to the generation of collections of peptidomimetic and nonpeptide compounds (for reviews, see references 4, 18, and 23). Such compounds are expected to be more suitable than peptides as oral drug candidates. One approach to the development of peptidomimetic compound and chemical SCLs involved the chemical transformation of existing peptide SCLs (this was termed the "libraries from libraries" approach [15, 19, 36]). As an example of this approach, the chemical transformation of a resin-bound peptide library was carried out by permethylation of the amide nitrogens of the mixtures (36). A number of N-permethylated hexapeptides were derived from this SCL that had selective antimicrobial activity against gram-positive bacteria (Table 5) (36). All of these compounds exhibited little effect on erythrocyte (<2% hemolysis at 100 μg/ml and 0 to 16% hemolysis

TABLE 4. L-, D-, and unnatural amino acid-containing peptides derived from SCLs[a]

Series sequences[b]	Most active compounds[b]	MIC (μg/ml)[c]				% Hemolysis at 125 μg/ml[d]
		S. aureus	MRSA	*E. coli*	*C. albicans*	
(αFmoc-εεlys)WfU-NH_2	(αFmoc-εlys)WfI-NH_2	3–4	4–8	62–125	250–500	18–26
	(αFmoc-εlys)Wfw-NH_2	3–4	3–4	62–125	250–500	100
(αFmoc-εlys)WKU-NH_2	(αFmoc-εlys)WKW-NH_2	5–8	>250	16–31	250–500	80–82
	(αFmoc-εlys)WKC-NH_2	5–8	62–125	31–62	>500	56–77
(αFmoc-εlys)WYU-NH_2	(αFmoc-εlys)WYr-NH_2	5–8	62–125	31–62	250–500	100
	(αFmoc-εlys)WY(aABA)-NH_2	5–8	8–16	62–125	250–500	27–51
(αFmoc-εlys)ciU-NH_2	(αFmoc-εlys)cir-NH_2	4–8	>250	31–62	125–250	89–94
	(αFmoc-εlys)ciR-NH_2	8–16	>250	31–62	250–500	100

[a] From reference 7.
[b] U is 1 of 20 L-, 19 D-, and 19 unnatural amino acids; lowercase letters represent D-amino acids; one-letter amino acid codes are used and defined as follows: W, tryptophan; F, phenylalanine; I, isoleucine; K, lysine; C, cysteine; Y, tyrosine; R, arginine; αFmoc-εlys, N_α-fluorenylmethoxycarbonyl-N_ε-lysine; aABA, α-aminobutyric acid.
[c] The MIC is the peptide concentration that inhibits 100% of bacterial or yeast growth. The activity was determined against 10^5 CFU of *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 per ml after a 21-h incubation at 37°C and against *C. albicans* ATCC 10231 after a 48-h incubation at 30°C.
[d] See footnote *c* of Table 3.

TABLE 5. Permethylated hexapeptides derived from SCLs[a]

Series sequences[b]	Most active compounds[b]	MIC (μg/ml)[c]		*C. albicans*	% Hemolysis at 330 μg/ml[d]
		S. aureus	*E. coli*		
pm[OOOOOO]	pm[LFIFFF]	11–15	NA[e]	NA	12
	pm[FFIFFF]	11–15	NA	NA	0
	pm[FFFFFF]	11–15	NA	NA	16
	pm[LFFFFF]	21–31	NA	NA	10
pm[OOOOOOO]	pm[FFFFFFF]	3–4	NA	NA	ND[f]

[a] From reference 36.
[b] O is as defined in the text, and one-letter amino acid codes are used and are defined as follows: F, phenylalanine; I, isoleucine; L, leucine.
[c] The MIC is the peptide concentration that inhibits 100% of bacterial or yeast growth. The activity was determined against 10^5 CFU of *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 per ml after a 21-h incubation at 37°C and *C. albicans* ATCC 10231 after a 48-h incubation at 30°C.
[d] See footnote *c* of Table 3.
[e] NA, nonactive.
[f] ND, not determined.

at 330 μg/ml [Table 5] [36]). These compounds were stable to proteolysis following overnight exposure. Interestingly, the quaternary trimethyl ammonium salt of the nonpermethylated FFFFFF-NH_2 (where F is phenylalanine) did not show any activity against the microorganism tested (50% inhibitory concentration, >250 μg/ml) (36). These results indicate that the activities found for the permethylated compounds are not solely due to their highly hydrophobic nature.

Extensive studies on the generation of peptidomimetic and organic compound SCLs are ongoing in order to extend the available diversity of compounds to be assayed. The difficulty in generating such libraries derives from the lack of solid-phase methods for the straightforward synthesis of organic compounds. Peptidomimetic and organic SCLs include those composed of polyamines (15), amide functionalized polyglycines (41), benzodiazepine derivatives (11), oligocarbamates (14), hydantoins (17), β-mercaptoketones (13), or cubane derivatives (12). However, their use in antimicrobial assays has yet to be reported.

SCLs AS RAPID ALTERNATIVE TO ANALOGING METHODS

While the examples described above refer to generic SCLs, which are not targeted to a specific assay system, the SCL technology was also used to improve the antimicrobial activities of existing compounds. Once an active compound has been isolated either from natural sources or from structure-activity relationship studies, a number of analogs are usually prepared to improve the compound's activity. Such procedures require a large number of individual compounds to be synthesized and often become lengthy and laborious. The SCL approach combined with the simultaneous multiple synthesis methodology (24) allows the requisite large number of individual analogs to be generated and screened simultaneously and relies on a "natural" selection process that is driven by the assay to lead to analogs having greater activities. Furthermore, prior knowledge of the structure-activity relationship of the antimicrobial peptide being studied was shown to permit the optimization of the design of the combinatorial library positions. Thus, an 18-mer amphipathic antimicrobial peptide (YKLLKKLLKKL KKLLKKL-NH_2, the termed [YLK] peptide) was selected as a scaffold to build SCLs in which four positions were randomized (Table 6) (6). This peptide was found in earlier studies to adopt an α-helical conformation in lipidic environments (3), which was correlated to its antimicrobial activity (2). Surprisingly, the most active analogs of the [YLK] peptide identified following the screening of such SCLs contained the helix-breaker residues proline and/or glycine (6). These analogs exhibited antimicrobial activities 1 order of magnitude higher than that of the original [YLK] sequence against *S. aureus* (Table 4). These peptides were found to undergo conformational changes in the presence of liposomes or polysialic acids. The occurrence of such a lipid-peptide association may perturb the lipid packing of bacterial cell membranes and ultimately may result in cell lysis. The lower level of activity found against the fungus *Candida albicans* may be due to the structural differences between bacterial and fungi cell walls. One can

TABLE 6. SCLs as analoging methods[a]

Series sequences[b]	Most active compounds[b]	MIC (μg/ml)[c]			HD_{50} (μg/ml)[d]
		Staphylococcus aureus	*Pseudomonas aeruginosa*	*Candida albicans*	
YKLLKOLLOKLKOLLOKL-NH_2	YKLLKLLLPKLKGLLFKL-NH_2	2–3	6–12	120–185	24
	YKLLKLLLPKLKPLLFKL-NH_2	3–5	7–14	130–175	55
	YKLLKLLLPKLKGLLIKL-NH_2	4–6	14–28	130–206	28
	YKLLKLLLPKLKPLLIKL-NH_2	4–6	8–16	120–174	69
[YLK]	YKLLKKLLKKLKKLLKKL-NH_2	30–55	10–20	50–78	6.1

[a] From reference 6.
[b] O is as defined in the text, and one-letter amino acid codes are used and are defined as follows: F, phenylalanine; G, glycine; I, isoleucine; K, lysine; L, leucine; P, proline; Y, tyrosine.
[c] The MIC is the peptide concentration that inhibits 100% of bacterial or yeast growth. The activity was determined against 10^5 CFU of *S. aureus* ATCC 29213 and *P. aeruginosa* ATCC 27853 per ml after a 21-h incubation at 37°C and *C. albicans* ATCC 10231 after a 48-h incubation at 30°C.
[d] HD_{50}, peptide mixture concentration that lyses 50% of erythrocytes.

envision weaker effects on the lipid packing of the fungal cell wall because of its multilayered nature and high degree of compactness compared with the bacterial cell wall.

CONCLUSION

Although not fully exploited, the examples described above show that novel antimicrobial compounds, as well as analogs having activities equal to or greater than those of existing antimicrobial agents, can be rapidly identified by using the SCL technology. While most of these studies have focused on the development of antimicrobial peptides, the recent successes in generating peptidomimetic and organic compound libraries make the SCL technology a valuable tool in the search for novel antimicrobial agents. These developments take on increased significance because of the alarming emergence of strains resistant to existing therapeutic regimens.

REFERENCES

1. **Bevins, C. L., and M. Zasloff.** 1990. Peptides from frog skin. Annu. Rev. Biochem. **59:**395–414.
2. **Blondelle, S. E., and R. A. Houghten.** 1992. Design of model peptides having potent antimicrobial activities. Biochemistry **31:**12688–12694.
3. **Blondelle, S. E., J. M. Ostresh, R. A. Houghten, and E. Pérez-Payá.** 1995. Induced conformational states of amphipathic peptides in aqueous/lipid environments. Biophys. J. **68:**351–359.
4. **Blondelle, S. E., E. Pérez-Payá, C. T. Dooley, C. Pinilla, and R. A. Houghten.** 1995. Chemical combinatorial libraries, peptidomimetics and peptide diversity. Trends Anal. Chem. **14:**83–92.
5. **Blondelle, S. E., E. Takahashi, K. T. Dinh, and R. A. Houghten.** 1995. The antimicrobial activity of hexapeptides derived from synthetic combinatorial libraries. J. Appl. Bacteriol. **78:**39–46.
6. **Blondelle, S. E., E. Takahashi, R. A. Houghten, and E. Pérez-Payá.** 1996. Rapid identification of compounds having enhanced antimicrobial activity using conformationally defined combinatorial libraries. Biochem. J. **313:**141–147.
7. **Blondelle, S. E., E. Takahashi, P. A. Weber, and R. A. Houghten.** 1994. Identification of antimicrobial peptides using combinatorial libraries made up of unnatural amino acids. Antimicrob. Agents Chemother. **38:**2280–2286.
8. **Boman, H. G., and D. Hultmark.** 1987. Cell-free immunity in insects. Annu. Rev. Microbiol. **41:**103–126.
9. **Brighty, K. E., W. Kohlbrenner, and P. R. McGuirk.** 1993. Recent developments in antibacterial resistance mechanisms. Annu. Rep. Med. Chem. **28:** 141–150.
10. **Brumfitt, W., and J. Hamilton-Miller.** 1989. Methicillin-resistance *Staphylococcus aureus*. N. Engl. J. Med. **320:**1188–1196.
11. **Bunin, B. A., and J. A. Ellman.** 1992. A general and expedient method for the solid-phase synthesis of 1,4-benzodiazepine derivatives. J. Am. Chem. Soc. **114:**10997–10998.
12. **Carell, T., E. A. Wintner, A. Bashir-Hashemi, and J. Rebek, Jr.** 1994. A novel procedure for the synthesis of libraries containing small organic molecules. Angew. Chem. Int. Ed. Engl. **33:**2059–2061.
13. **Chen, C., L. A. Ahlberg-Randall, R. B. Miller, A. D. Jones, and M. J. Kurth.** 1994. "Analogous" organic synthesis of small-compound libraries: validation of combinatorial chemistry in small molecule synthesis. J. Am. Chem. Soc. **116:**2661–2662.
14. **Cho, Y. C., E. J. Moran, S. R. Cherry, J. C. Stephans, S. P. A. Fodor, C. L. Adams, A. Sundaram, J. W. Jacobs, and P. G. Schultz.** 1993. An unnatural biopolymer. Science **261:**1303–1305.
15. **Cuervo, J. H., F. Weitl, J. M. Ostresh, V. T. Hamashin, A. L. Hannah, and R. A. Houghten.** 1995. Polyalkylamine chemical combinatorial libraries, p. 465–466. *In* H. L. S. Maia (ed.), Peptides 94: Proceedings of the 23rd European Peptide Symposium. ESCOM, Leiden, The Netherlands.
16. **Davies, J.** 1994. Inactivation of antibiotics and the dissemination of resistance genes. Science **264:**375–382.
17. **DeWitt, S. H., J. S. Kiely, C. J. Stankovic, M. C. Schroeder, D. M. R. Cody, and M. R. Pavia.** 1993. "Diversomers": an approach to nonpeptide, nonoligomeric chemical diversity. Proc. Natl. Acad. Sci. USA **90:**6909–6913.
18. **Dörner, B., S. E. Blondelle, C. Pinilla, J. Appel, C. T. Dooley, J. Eichler, J. M. Ostresh, E. Pérez-Payá, and R. A. Houghten.** 1996. Soluble synthetic combinatorial libraries: the use of molecular diversities for drug discovery, p. 1–25. *In* R. Cortese (ed.), Combinatorial libraries: synthesis, screening and application. Walter de Gruyter, Berlin.
19. **Dörner, B., J. M. Ostresh, G. M. Husar, and R. A. Houghten.** 1995. Extending the range of molecular diversity through amide alkylation of peptide libraries, p. 463–464. *In* H. L. S. Maia (ed.), Peptides 94: Proceedings of the 23rd European Peptide Symposium. ESCOM, Leiden, The Netherlands.
20. **Fodor, S. P. A., J. L. Read, M. C. Pirrung, L. Stryer, A. T. Lu, and D. Solas.** 1991. Light-directed, spatially addressable parallel chemical synthesis. Science **251:**767–773.
21. **Gallop, M. A., R. W. Barrett, W. J. Dower, S. P. A. Fodor, and E. M. Gordon.** 1994. Applications of combinatorial technologies to drug discovery. 1. Background and peptide combinatorial libraries. J. Med. Chem. **37:**1233–1251.
22. **Geysen, H. M., S. J. Rodda, and T. J. Mason.** 1986. *A priori* delineation of a peptide which mimics a discontinuous antigenic determinant. Mol. Immunol. **23:**709–715.
23. **Gordon, E. M., R. W. Barrett, W. J. Dower, S. P. A. Fodor, and M. A. Gallop.** 1994. Applications of combinatorial technologies to drug discovery. 2. Combinatorial organic synthesis, library screening strategies, and future directions. J. Med. Chem. **37:**1385–1401.
24. **Houghten, R. A.** 1985. General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids. Proc. Natl. Acad. Sci. USA **82:**5131–5135.
25. **Houghten, R. A., J. R. Appel, S. E. Blondelle, J. H. Cuervo, C. T. Dooley, and C. Pinilla.** 1992. The use of synthetic peptide combinatorial libraries for the identification of bioactive peptides. BioTechniques **13:**412–421.
26. **Houghten, R. A., M. K. Bray, S. T. De Graw, and C. J. Kirby.** 1986. Simplified procedure for carrying out simultaneous multiple hydrogen fluoride cleavages of protected peptide resins. Int. J. Peptide Protein Res. **27:**673–678.
27. **Houghten, R. A., K. T. Dinh, D. E. Burcin, and S. E. Blondelle.** 1993. The systematic development of peptides having potent antimicrobial activity against *E. coli* through the use of synthetic peptide combinatorial libraries, p. 249–256. *In* R. H. Angeletti (ed.), Techniques in protein chemistry IV. Academic Press, Inc., Orlando, Fla.
28. **Houghten, R. A., C. Pinilla, S. E. Blondelle, J. R. Appel, C. T. Dooley, and J. H. Cuervo.** 1991. Generation and use of synthetic peptide combinatorial libraries for basic research and drug discovery. Nature (London) **354:**84–86.
29. **Jawetz, E.** 1961. Polymoxin, colistin and bacitracin. Pediatr. Clin. N. Am. **8:**1057–1071.
30. **Lam, K. S., S. E. Salmon, E. M. Hersh, V. J. Hruby, W. M. Kazmierski, and R. J. Knapp.** 1991. A new type of synthetic peptide library for identifying ligand-binding activity. Nature (London) **354:**82–84.
31. **Lyon, B. R., and R. Skurray.** 1987. Antimicrobial resistance of *Staphylococcus aureus*: genetic basis. Microbiol. Rev. **51:**88–134.
32. **Merrifield, R. B.** 1963. Peptide synthesis. I. The synthesis of a tetrapeptide. J. Am. Chem. Soc. **85:**2149–2154.
33. **Moore, K. S., C. L. Bevins, M. M. Brasseur, N. Tomassini, K. Turner, H. Eck, and M. Zasloff.** 1991. Antimicrobial peptides in the stomach of *Xenopus laevis*. J. Biol. Chem. **266:**19851–19857.
34. **Moore, K. S., S. Wehrli, H. Roder, M. Rogers, J. N. J. Forrest, D. McCrimmon, and M. Zasloff.** 1993. Squalamine: an aminosterol antibiotic from the shark. Proc. Natl. Acad. Sci. USA **90:**1354–1358.
35. **Neu, H. C.** 1992. The crisis in antibiotic resistance. Science **257:**1064–1078.
36. **Ostresh, J. M., G. M. Husar, S. E. Blondelle, B. Dörner, P. A. Weber, and R. A. Houghten.** 1994. "Libraries from libraries": chemical transformation of combinatorial libraries to extend the range and repertoire of chemical diversity. Proc. Natl. Acad. Sci. USA **91:**11138–11142.
37. **Pinilla, C., J. Appel, S. E. Blondelle, C. T. Dooley, B. Dörner, J. Eichler, J. M. Ostresh, and R. A. Houghten.** 1995. A review of the utility of peptide combinatorial libraries. Biopolymers (Peptide Sci.) **37:**221–240.
38. **Pinilla, C., J. R. Appel, S. E. Blondelle, C. T. Dooley, J. Eichler, J. M. Ostresh, and R. A. Houghten.** 1994. Versatility of positional scanning synthetic combinatorial libraries for the identification of individual compounds. Drug Dev. Res. **33:**133–145.
39. **Rammelsberg, M., and F. Radler.** 1990. Antibacterial polypeptides of *Lactobacillus* species. J. Appl. Bacteriol. **69:**177–184.
40. **Saberwal, G., and R. Nagaraj.** 1994. Cell-lytic and antibacterial peptides that act by perturbing the barrier function of membranes: facets of their conformational features, structure-function correlations and membrane-perturbing abilities. Biochim. Biophys. Acta Rev. Biomembr. **1197:**109–131.
41. **Simon, R. J., R. S. Kania, R. N. Zuckermann, V. D. Huebner, D. A. Jewell, S. Banville, S. Ng, L. Wang, S. Rosenberg, C. K. Marlowe, D. C. Spellmeyer, R. Tan, A. D. Frankel, D. V. Santi, F. E. Cohen, and P. A. Bartlett.** 1992. Peptoids: a modular approach to drug discovery. Proc. Natl. Acad. Sci. USA **89:**9367–9371.
42. **Soothill, J. S., R. Ward, and A. J. Girling.** 1992. The IC_{50}: an exactly defined measure of antibiotic sensitivity. J. Antimicrob. Chemother. **29:**137–139.
43. **Uttley, A. H. C., C. H. Collins, J. Naidoo, and R. C. George.** 1988. Vancomycin-resistant enterococci. Lancet **i:**57–58.
44. **Verhoef, J. C., H. E. Bodde, A. G. DeBoer, J. A. Bouwstra, H. E. Junginger, F. W. H. M. Merkus, and D. D. Breimer.** 1990. Transport of peptide and protein drugs across biological membranes. Eur. J. Drug Metab. Pharmacokinet. **15:**83–93.
45. **Zasloff, M.** 1992. Antiobiotic peptides as mediators of innate immunity. Curr. Opin. Immunol. **4:**3–7.