A R T I C L E S

Combinatorial Organic Synthesis Using Parke-Davis's DIVERSOMER Method

SHEILA HOBBS DEWITT* AND ANTHONY W. CZARNIK*

BioOrganic Chemistry Section, Department of Chemistry, Parke-Davis Pharmaceutical Research Division of Warner-Lambert Company, Ann Arbor, Michigan 48105

Received October 16, 1995

The most fundamental and lasting objective of synthesis is not production of new compounds, but production of properties.

George S. Hammond[1]

Norris Award Lecture, 1968

At the cornerstone of organic chemistry is an ability to synthesize molecules of interest from other, more available molecules. Whether trained on a natural product, a conducting polymer, a semisynthetic enzyme, or a biodegradable polymer, the *science* of organic synthesis empowers us to understand our physical world by recreating it, one molecule at a time. Simultaneously, the *art* of organic synthesis stimulates human creativity and raises our collective spirit each time another molecular pinnacle falls to the artist's hand. Along the way, the practice of organic synthesis creates *utility* that can enhance our quality of life. Inasmuch as the utility of a molecule is discovered by measuring its properties and not so much by viewing its structure, chemical discovery distills to a search for properties.

In the pharmaceutical industry, the route to discovery takes many forms. The isolation of biologically active components from natural sources, modification of drugs with known activity, and pure happenstance are all well-trodden paths. The advent of genetic engineering techniques in the early 1970s afforded macroscopic quantities of many proteins for the first time, and with them two new routes to the discovery of drugs. Crystallization of target proteins and their cocrystallization with drug candidates provide the starting point with which to predict compounds that may be better drug candidates. A quite different approach involves the high-volume, *in vitro* screening of many compounds against target proteins of interest. By performing binding experiments in microtiter plates and employing both special pooling strategies and automation, it is now reasonable to screen 50 000 compounds against a given target in a single month. That rate will only increase. Screening represents the ultimate in empiricism: it is a brute force search for properties that has been characterized as "Edisonian".[2] On its surface, screening techniques may appear to be essentially nonintellectual exercises. In fact, screening represents instead a paradigm shift in the way chemists use their intellect. "How can I best design this experiment so that it is possible to test 50 000 compounds this month with a good level of reliability?" "Is there a way I can improve the method so that I don't have to stand here all day watching this robot transfer solutions?" And, most importantly to the focus of this dedicated issue of *Accounts*, "What compounds will I feed into this biological screen once all the compounds in my sample archive have been tested?" The present and likely future success of screening methodology fairly dictates that organic chemists address this question, if they are to remain integral to the search for useful properties.

Combinatorial chemistry is one response to this challenge. Those involved earliest in a new field typically bear the responsibility of helping to define it, knowing that it means different things to different people. As one of us has stated elsewhere,[3] combinatorial chemistry is the science of efficient *divergent synthesis*. If one starting material is converted to a product in three addition or substitution steps, then employment of one example of each reagent type will yield one product. Assuming generality in all steps, employment of 10 examples of each reagent will yield 10^3, or 1000, products. Employment of 100 examples of each reagent will yield 100^3, or 1 000 000, products. The resulting collection of products is called a library in part because there are so many components that

Sheila Hobbs DeWitt was born and raised in Gasport, NY. She received her B.A. from Cornell University in 1981. She obtained her Ph.D. in synthetic organic chemisty from Duke University in 1986, working with D. Sternbach. Following two years in process research at FMC Corporation, she joined the Medicinal Chemistry Department at Parke-Davis. In 1991, she was a founding member of the BioOrganic Group, which initiated the DIVERSOMER project. She is currently the Vice President of Technical Development for Diversomer Technologies, Inc., a start-up company formed to commercialize its DIVERSOMER system for small-molecule automated organic synthesis and combinatorial chemistry. Her primary interests are to develop and implement automation and enabling technologies for scientific discovery efforts.

Anthony W. Czarnik was a graduate student at the University of Illinois at Urbana/Champaign with Nelson J. Leonard, an NIH postdoctoral fellow at Columbia University with Ronald Breslow, and a member of the faculty at Ohio State University for 10 years, and is currently Director of BioOrganic Chemistry at Parke-Davis Pharmaceutical Research. He serves on the Editorial Board of *Organic Reactions*, on the Editorial Advisory Board of *Accounts of Chemical Research*, and as an Editor of both the *Journal of Molecular Recognition* and the Fluorosensor WWW Database [http://www.cursci.co.uk/fluoro/]. Dr. Czarnik holds adjunct appointments in the Departments of Chemistry at the University of Michigan, Ohio State University, the University of Toledo, and Wayne State University and has been an Eli Lilly awardee, a Fellow of the Alfred P. Sloan Foundation, and a Teacher-Scholar Fellow of the Camille and Henry Dreyfus Foundation. His current research interests include combinatorial chemistry as a tool for drug discovery, nucleic acids as targets for small molecule intervention, and fluorescent chemosensors of ion and molecule recognition.

(1) Hirschmann, R. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1278.
(2) (a) Pirrung, M. C. *ChemTracts: Org. Chem.* **1995**, *8*, 5. (b) Mitscher, L. A. *ChemTracts: Org. Chem.* **1995**, *8*, 19.
(3) Czarnik, A. W. *ChemTracts: Org. Chem.* **1995**, *8*, 13.

0001-4842/96/0129-0114$12.00/0

special methods of cataloging and storage are required to use the collection efficiently.

The rules of engagement change when combinatorial synthesis, rather than total synthesis, is the goal; this point cannot be overemphasized. For example, while the compounds made should be of a general type known to be useful toward a given application, emphasis must be given to the reactions that can get you there rather than just on the specific products one might make. Experience is teaching us that a very wide range of chemistries are amenable to library generation, but also that the development of high-yielding, clean reaction sequences requires the investment of time and effort. Library generation utilizing a mediocre set of individual reactions guarantees an exercise in frustration. Furthermore, library generation requires efficient methods for the isolation of intermediate reaction products from the reagents that created them. Whether the goal is lead generation (10^3-10^6 compounds) or lead optimization (10^1-10^3 compounds), neither project will succeed if each step requires purification and the removal of solvents by evaporation. Our group at Parke-Davis has been engaged in the development of methods and tools for lead optimization. We refer to our resulting method as the DIVERSOMER[31] approach. The term "diversomer" is derived from the Latin *divertere* meaning "to divert" and the Greek *meros* meaning "parts". In this Account, we will provide a survey of how the DIVERSOMER method evolved and of those nonproprietary projects to which it has been applied. While the authors of this Account are current group members engaged in combinatorial chemistry, many Parke-Davis colleagues have contributed to this program over the past several years; their work is cited in the references to the original literature. Also, while we will focus on our group's approach, it is only fair to emphasize that other methods for library creation have been invented elsewhere, many of which are presented in this issue of *Accounts*.

Solid-Phase Organic Synthesis (SPOS)

High-volume synthesis strategies will invariably require that intermediate reaction products be isolable from reaction solutions without resort to crystallization, chromatography, or solvent evaporation. The fastest and simplest method of isolating a substance from a liquid, such as a solution of reactants, is filtration.[4] Of course, filtration is possible only when the substance is a solid. This, then, is the rationale for growing organic compounds on an insoluble polymer matrix.[5] It is a motivating force sufficient to overcome even the inherent, potent aversion most chemists have to conducting reactions with heterogeneous reactants, and it was precisely the motivation for the development of solid-phase peptide synthesis 30 years ago by Merrifield.[6] Because polypeptide synthesis can involve hundreds of reactions in series, simple product isolation is as necessary as for combinatorial synthesis that involves hundreds of reactions in parallel. The ease of intermediate product isolation brings two important benefits to each type of synthesis. First, excess reagent can be used with the frequent effect of driving recalcitrant reactions toward completion. Second, the advantages of automation can now be brought to bear on the repetitive aspects of the synthesis. Polypeptides and polynucleotides are today synthesized almost exclusively via automated systems employing solid-supported chemistries. Although presently less developed, combinatorial chemistry already benefits from automation, and it is a certainty that this will increase.

Technical challenges inherent to SPOS remain, despite its introduction by Rapoport[7] and Leznoff[8] over 20 years ago. It is not possible to purify resin-bound intermediate products from each other. For this reason, it is crucial that reaction conditions be optimized so that most reactions proceed to near completion. Even accomplishing this is made more difficult by the simple fact that one cannot monitor reaction progress using a benchtop technique such as TLC. Gel-phase ^{13}C NMR spectroscopy has proven a very useful tool in our hands. While this method has found application in the characterization of solid-supported biopolymers for some time,[9] its use in monitoring SPOS was first reported by our group in 1993.[10] In many (but not all) cases, we find that this NMR method gives resonances sufficiently sharp and with chemical shifts expected for analogous homogeneous samples. A useful refinement was described recently by the Affymax group, in which gel-phase ^{13}C NMR spectra are enhanced by use of labeled starting materials or reagents.[11] Magic angle spinning HMQC and TOCSY NMR methods have been applied to the ^1H NMR and ^{13}C NMR of Wang resin-bound lysine.[12] IR has been used to characterize SPOS reaction intermediates,[13] and a recent report from the Sandoz group refines this method for the analysis of individual reaction beads.[14] Direct monitoring of reaction products by MALDI mass spectrometry has been described.[15] Necessity is the mother of invention, and as the need dictates we will learn how to monitor SPOS reactions conveniently.

In general, the idea behind SPOS is that an insoluble polymer bearing chemically reactive functional groups is "charged" (chemically functionalized) with a reagent that can serve as starting material for a multistep synthetic sequence. Schematically, this is depicted as addition of BB_1 in step one of Scheme 1. Subsequent chemical reactions then modify this starting material. While Scheme 1 depicts this functionalization as linear, the actual mode of compound construction depends entirely on the sequence. Cleavage of the final product from the solid support prior to biological screening is highly desirable, as many

(4) In principle, the removal of either a solid or a liquid from a gas is even simpler, but we have not examined library generation schemes based on gaseous reagent delivery.

(5) SPOS can also be useful to selectively protect one end of a bifunctional starting material.

(6) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149.

(7) Crowley, J. I.; Rapoport, H. *Acc. Chem. Res.* **1976**, *9*, 135.

(8) (a) Leznoff, C. C. *Chem. Soc. Rev.* **1974**, *3*, 65. (b) Leznoff, C. C. *Acc. Chem. Res.* **1978**, *11*, 327.

(9) For an early example, see: Epton, R.; Goddard, P.; Ivin, K. J. *Polymer* **1980**, *21*, 1367.

(10) (a) DeWitt, S. H.; Kiely, J. S.; Stankovic, C. J.; Schroeder, M. C.; Cody, D. M. R.; Pavia, M. R. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 6909. (b) DeWitt, S H.; Schroeder, M. C.; Stankovic, C. J.; Strode, J. E.; Czarnik, A. W. *Drug Dev. Res.* **1994**, *33*, 116. (c) DeWitt, S. H.; Kiely, J. S.; Pavia, M. R.; Schroeder, M. C.; Stankovic, C. J. Apparatus for Multiple Simultaneous Synthesis. US 5,324,483, 1994.

(11) Look, G. C.; Holmes, C. P.; Chinn, J. P.; Gallop, M. A. *J. Org. Chem.* **1994**, *59*, 7588.

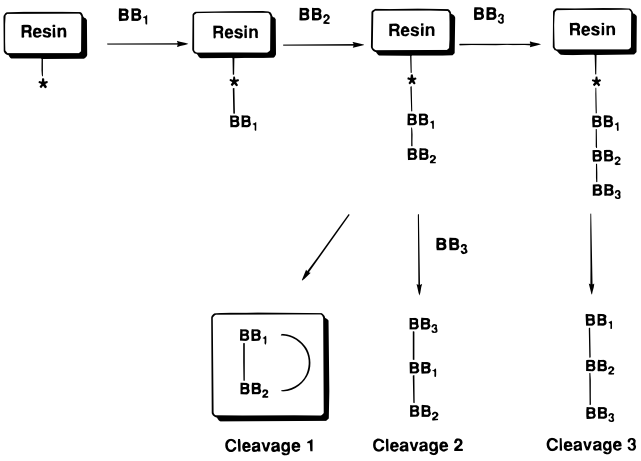
(12) Anderson, R. C.; Stokes, J. P.; Shapiro, M. J. *Tetrahedron Lett.* **1995**, *36*, 5311.

(13) Crowley, J. I.; Rapoport, H. *J. Org. Chem.* **1980**, *45*, 3215.

(14) Yan, B.; Kumaravel, G.; Anjaria, H.; Wu, A.; Petter, R. C.; Jewell, C. F., Jr.; Wareing, J. R. *J. Org. Chem.*, in press.

(15) Egner, B. J.; Langley, G. J.; Bradley, M. *J. Org. Chem.* **1995**, *60*, 2652.

Scheme 1



assays would be inhibited or completely untenable under heterogeneous conditions. Chemically, the removal can be viewed as resulting from several different cleavage strategies. Cleavage types 2 and 3 have been used effectively here and elsewhere. However, cleavage type 1 can bring unique advantage that makes it preferable when possible. Type 1 cleavage, or "cyclative cleavage", occurs when heating or reagent treatment induces a functionality on the growing product to "bite" into BB_1 with resulting breakage of the BB_1–polymer bond. When this occurs, the cyclized product is released from the resin for purification or direct assay. Because only those polymer sites that successfully reacted with BB_2 possess the (presumably) nucleophilic site required for cyclization, truncated sites are not expected to cleave from the support. In this way, some measure of purification can be achieved.

The obvious inability to purify resin-bound intermediate products from each other is a major source of discontentment for organic chemists, and high-yielding schemes will prove valuable as they are discovered. Sequences that provide final products not requiring purification greatly facilitate rapid synthesis. However, practitioners in this field are finding that some valuable products are not sufficiently homogeneous for reliable assay and require purification. Indeed, we have observed that even a proton NMR spectrum demonstrating homogeneity is not sufficient to guarantee reliable biological assay. It is proving important to evaluate the activities of several reference compounds placed intentionally into an array synthesis. When required, parallel purification methods will no doubt prove useful and will continue to be developed.[16]

The DIVERSOMER Apparatus

While it is certainly possible to run 40 reactions at one time in a hood, in practice it would be a cumbersome exercise without some form of miniaturization, a method to physically contain all 40 reactions in a common unit, and an ability to purify intermediates from solvents and excess reactants without resort to chromatography. The Geysen approach of synthesis on a rack of polypropylene rods (i.e., "pins") fulfills all three imperatives and indeed has been applied early as a solution by Ellman.[17] In our setting, there is advantage in preparing more material than possible on the tip of such a pin. The method of Houghton,[18] in which polypeptide synthesis is accomplished on several hundred milligrams of resin beads, seemed more appropriate for our needs. In the Houghton method, individual collections of beads are contained in teabags, in which chemical reactions can be accomplished directly. This approach serves polypeptide synthesis surprisingly well, but clearly could not be used under the wide range of conditions employed in organic synthesis. It is apparent that the ideal reactor would be porous glass, as the conditions employed by the synthetic chemist are already validated for glass vessels. The common gas dispersion tube (a "PIN") serves this role well and became our group's reaction vessel of choice.

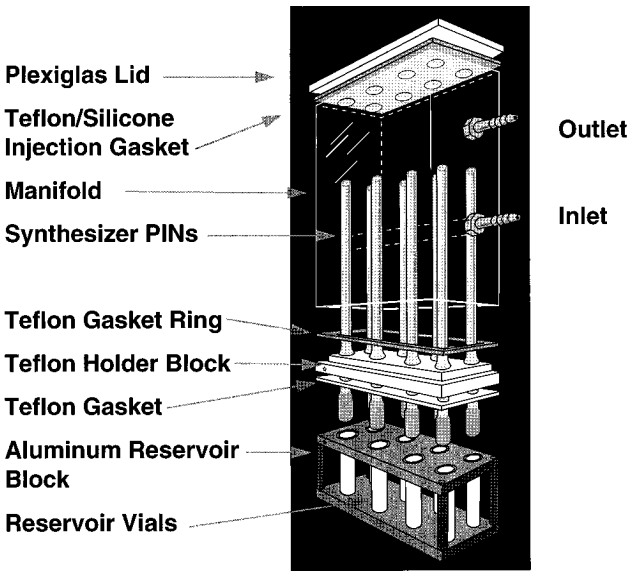


Figure 1. Schematic representation of an eight-PIN synthesizer. The apparatus consists of an array of gas dispersion tubes (PINS), a reservoir block with multiple reaction wells, a holder block, a manifold, and gaskets. Clamps are not depicted.

The design of an apparatus capable of organizing this set of gas sparge tubes was likewise required. A functional prototype capable of organizing a 4×2 reaction array is shown in Figure 1. Resin beads, from 100 to 800 mg depending on the size of the apparatus, reside in the fritted portion at the bottom of each tube. The set of PINs is held in place by a block of chemically resistant material, normally Teflon. The fritted portions of the tubes can be inserted into glass reservoir vials, themselves organized by a reservoir block. This lower portion of the apparatus can be immersed in a heated or cooled ultrasound bath to provide temperature control, thus increasing the range of reaction types that can be used. While a much more sophisticated design would provide for individual temperature control, we have chosen instead (and been successful in using) a single-temperature approach in which the choice of temperature has been optimized. Above the holder block, a Plexiglas manifold is used

(16) (a) MacDonald, A.; Halim, N.; DeWitt, S. H.; Hogan, E.; Kieras, L.; Ghosh, S.; Ramage, R. Parallel Purification and Resin By-Products in Solid Phase Synthesis. Presented at the 4th International Symposium on Solid Phase Synthesis, Edinburgh, Scotland, Sept 12, 1995. (b) Halim, N.; DeWitt, S. H.; Hogan, E.; Kieras, L.; Ghosh, S.; MacDonald, A.; Ramage, R. Parallel Purification and Resin By-Products in Solid Phase Synthesis. *Abstracts of Papers*, 210th National Meeting of the American Chemical Society; American Chemical Society: Washington, DC, 1995; ORGN 267.

(17) (a) Bunin, B. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1992**, *114*, 10997. (b) Bunin, B. A.; Plunkett, M. J.; Ellman, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4708.

(18) Houghten, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 5131.

Figure 2. A now-dated photograph of the 40-array apparatus interfaced to the *x,y,z* robot used for liquid delivery (September 1993).

to permit maintenance of an inert atmosphere. Reagents may be introduced through an injectable gasket at the top. If a chilled inert gas such as nitrogen or argon is passed through the gas inlet port, reflux conditions may be used in which the reaction solvent condenses on the inner walls of individual gas dispersion tubes.

The eight-unit array pictured in Figure 1 is now available commercially.[19] In practice it functions well with only a few caveats. First, the equalization of pressure between the dissolved reagents in the reaction vial and the resin in the filter is effectively achieved with three small release holes in the glass, above the filter but below the holder block. However, due to variations in resin mesh size, the copious washing of resin-bound intermediates sometimes results in the loss of a small amount of resin through these holes. This is most often observed with solvents exhibiting high swelling capacity. While dichloromethane is a serious culprit, use of other solvents largely avoids this problem. Second, the containment of volatile solvents at reflux (e.g., dichloromethane) over long periods of time is still imperfect. For this reason, we prefer to employ solvents with similar swelling properties but lower volatility. Third, gasket materials have finite lifetimes and must be replaced after long periods of solvent exposure, extremes in temperature, or repeated injection. This, however, is not unlike a chemist discarding a septum after repeated use.

(19) ChemGlass, Inc., Vineland, NJ 08360.

Automation

While the eight-array apparatus can be easily operated by a single chemist, the repetitive liquid dispersements required by use of the larger 40-array apparatus (the DIVERSOMER unit shown in Figure 2) can lead to operator fatigue and disenchantment. There is an obvious value to employment of automated solvent dispersement techniques, among which valve-driven fluidic and motor-driven robotic approaches are the most obvious.[20] Our group has focused on the application of robotic methods, largely because of concern that the extensive tubing and valving needs of fluidic methods would not be compatible with the wide range of caustic and/or environment-sensitive reagents employed in organic synthesis. Additionally, we believe that the automation approach affords the kinds of "hand-on" interface most consistent with the character of synthetic chemists.

Laboratory automation, although not historically used in synthetic chemistry laboratories, has been implemented in analytical chemistry, clinical chemistry, and process development laboratories.[21] Most synthetic chemistry automation applications have focused on sequential solution-phase synthesis,[22−24] albeit the number of simultaneous reactions carried out have been limited. Recently, major developments

(20) DeWitt, S. H.; Czarnik, A. W. *Curr. Opin. Biotechnol.* **1995**, *6*, 640.

(21) (a) Fawzi, M. B. In *Advances in Laboratory Automation*; Strimaitis, J. R., Little, J. N., Ed.; Zymark Corporation: Hopkington, MA, 1991; Vol. 7, pp 19−35. (b) Strimaitis, J. R. *J. Chem. Educ.* **1989**, *66*, A13−A17.

in multiple, simultaneous solid-phase peptide synthesis (SPPS) have been reported.[25] Although SPPS reactions are often fully optimized, the scope of this automation effort is limited due to ambient and neutral reaction conditions and the repetitive nature of the chemistry. The DIVERSOMER technology fully exploits a wide repertoire of chemistry and reaction conditions while relying heavily on a flexible automation system.

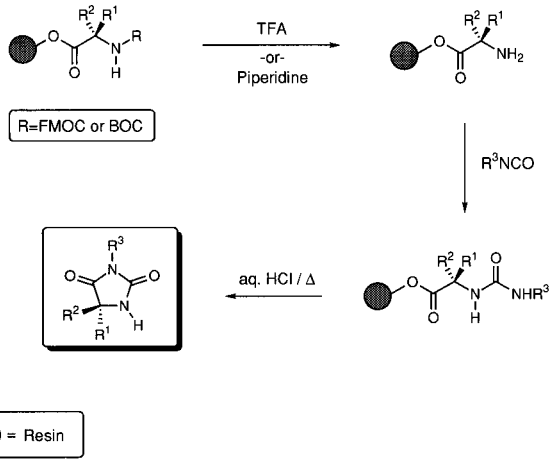
All liquid sample handling in the DIVERSOMER approach is achieved using a cartesian liquid handling robot (LHR). The hardware and the software of the LHR has been modified suitably to interface with the DIVERSOMER apparatus and to perform a wide variety of manipulations common to organic synthesis. Modifications to the LHR include the use of custom sample racks, removal of the platform deck, use of an elongated probe, and use of a needle as a probe. The robot is currently being used for various tasks involved in the DIVERSOMER approach including resin loading, reaction cycle monitoring, wash cycles, and parallel purification by solid-phase extraction (SPE) methods.[26]

Examples of DIVERSOMER Syntheses

All of the following recapitulations of nonproprietary synthetic schemes have been disclosed previously, as indicated by the identifying references. Some additional editorializing is included here given the wisdom of multiple successes and failures since the original reports. It is important to note that all of these syntheses afforded sufficient product amount for characterization using proton NMR and mass spectrometry of each successful reaction product. In general, resin-bound intermediates were characterized using gel-phase ^{13}C NMR spectroscopy.[4a]

Members of the hydantoin class of compounds (e.g., Dilantin) are useful in controlling the symptoms of epilepsy, making targeted libraries of hydantoins of interest. Our hydantoin library synthesis, carried out with Ms. Donna Reynolds Cody, is shown in Scheme 2.[10] Any parallel synthesis benefits by starting with a set of reactions that work efficiently with a wide range of reagents. In this scheme, those reactions are amine deprotection (step 1), reaction of an amine with an isocyanate to give a urea (step 2), and acid-catalyzed cyclization of the urea to give a hydantoin (step 3). Each of these reactions is known to be general and typically high yielding in solution, although one cannot predict how such reactions will translate to chemistry on a solid support. Thus, one or two reactions are carried out on a solid-supported starting material before running a set of 40 compounds, to ensure that appropriate conditions can be found. The actual synthesis of hydantoins was carried out as follows. Eight resins containing different protected amino acids were each placed into five gas dispersion tubes, affording a total of 40 reaction vessels. Deprotection as shown in step one afforded

Scheme 2

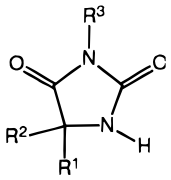


the corresponding amino acid charged resins, now possessing free amine (NH_2) groups. Each resin-bound amino acid was then reacted with five different isocyanates (step two), to afford a total of 40 different ureas. In the final step, treatment of all reaction tubes with 6 M HCl and heat resulted in cyclative cleavage and release of the product hydantoins from the resin, which were dissolved individually in methanol, concentrated, and analyzed. Products and weight yields are shown in Table 1; it should be noted that we now determine mole yields instead using ^1H NMR with an internal standard and/or quantitative HPLC. The reader will note that one reaction (no. 32) yielded no product. One accepts this deletion as the cost of making larger numbers of compounds; if the trend indicates that it should be made, it will be synthesized individually. Each of the 39 product hydantoins in this library was then available for biological testing.

Benzodiazepines are well-known β-turn peptide mimetics, and structural variation leads to a wide range of biological activities. Our method for the synthesis of a combinatorial benzodiazepine array, accomplished by Mel Schroeder, Charles Stankovic, and John Kiely (all BioOrganic Group members at the time), is shown in Scheme 3.[10] Once again starting with lots of five different amino acid charged Merrifield resins, transimination using eight different benzophenone imines produced a set of 40 resin-bound imines. Cyclization of the aromatic amine onto the ester carbonyl group led to cyclative cleavage and release of the 40 benzodiazepine products in weight yields ranging from 9% to 63% (Table 2). These products were tested without purification for activity in their ability to displace radiolabeled fluoronitrazepam from bovine cortical membranes. The results, which have been reported previously,[10] demonstrate the same trends in activity that have been identified previously in this series. This important experiment indicated that the products of DIVERSOMER syntheses could be used directly for property testing in at least one example. Our subsequent work has shown this to be the rule rather than the exception, although we have experienced the exceptions as well in our group. (We note that several other groups have now reported alternate SPOS routes for benzodiazepine synthesis).[17,27]

(22) Kramer, G. W.; Fuchs, P. L. In *Advances in Laboratory Automation—Robotics 1985*; Strimaitis, J. R., Hawk, G. L., Eds.; Zymark Corporation: Hopkington, MA, 1985; pp 417–429.
(23) Lindsey, J. S. *Chemom. Intell. Lab. Syst.* **1992**, *17*, 15–45.
(24) Metivier, P.; Josses, P.; Bulliot, H.; Corbet, J. P.; Joux, B. *Chemom. Intell. Lab. Syst.* **1992**, *17*, 137–43.
(25) Jung, G.; Beck-Sickinger, A. G. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 367–383.
(26) DeWitt, S. H.; Czarnik, A. W. *Curr. Opin. Biotechnol.* **1995**, *6*, 640.

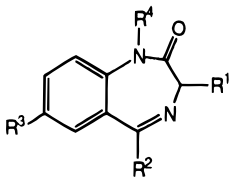
Table 1. Hydantoins Generated in Array



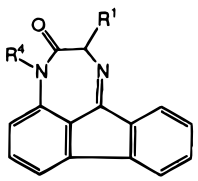
no.	R^1	$R^{2\,a}$	$R^{3\,a}$	yield[b] mg	yield[b] %
1	H	Me	H	4.1	67
2	H	Bn	H	2.5	38
3	H	H	H	3.3	65
4	H	*sec*-Bu	H	3.1	42
5	H	*i*-Bu	H	4.9	61
6	H	*i*-Pr	H	4.9	58
7	H	2-MeInd	H	5.0	35
8	Ph	Ph	H	1.4	5
9	H	Me	Bu	1.6	17
10	H	Bn	Bu	3.9	47
11	H	H	Bu	1.0	13
12	H	*sec*-Bu	Bu	5.3	48
13	H	*i*-Bu	Bu	0.7	7
14	H	*i*-Pr	Bu	0.9	8
15	H	2-MeInd	Bu	0.9	5
16	Ph	Ph	Bu	1.6	5
17	H	Me	allyl	0.3	4
18	H	Bn	allyl	2.4	29
19	H	H	allyl	3.7	48
20	H	*sec*-Bu	allyl	3.6	36
21	H	*i*-Bu	allyl	5.0	54
22	H	*i*-Pr	allyl	1.6	14
23	H	2-MeInd	allyl	1.9	11
24	Ph	Ph	allyl	2.1	7
25	H	Me	2-$CF_3C_6H_4$	2.6	23
26	H	Bn	2-$CF_3C_6H_4$	2.2	23
27	H	H	2-$CF_3C_6H_4$	2.9	28
28	H	*sec*-Bu	2-$CF_3C_6H_4$	5.7	46
29	H	*i*-Bu	2-$CF_3C_6H_4$	4.7	37
30	H	*i*-Pr	2-$CF_3C_6H_4$	4.9	33
31	H	2-MeInd	2-$CF_3C_6H_4$	3.0	15
32	Ph	Ph	2-$CF_3C_6H_4$	0.0	0
33	H	Me	4-$MeOC_6H_4$	3.1	22
34	H	Bn	4-$MeOC_6H_4$	3.5	32
35	H	H	4-$MeOC_6H_4$	5.6	46
36	H	*sec*-Bu	4-$MeOC_6H_4$	11.5	81
37	H	*i*-Bu	4-$MeOC_6H_4$	3.2	21
38	H	*i*-Pr	4-$MeOC_6H_4$	4.1	24
39	H	2-MeInd	4-$MeOC_6H_4$	4.9	22
40	Ph	Ph	4-$MeOC_6H_4$	3.0	7

[a] Benzyl (Bn), 2-methylindolyl (2-MeInd), 2-trifluorotolyl (2-$CF_3C_6H_4$), 4-methoxyphenyl (4-$MeOC_6H_4$). [b] Yields based upon reported loading of commerically available functionalized resins (0.34−1.04 mequiv g).

Table 2. Benzodiazepines Generated in Array



Structure A



Structure B

no.	$R^{1\,a}$	$R^{2\,a}$	R^3	R^4	yield[b] mg	yield[b] %	IC-50[c] nM
1	Me	Ph	H	H	6.1	40	1700
2	Me	Ph	Cl	H	9.6	56	200
3	Me	4-$MeOC_6H_4$	H	H	5.8	34	69000
4	Me	Ph	NO_2	H	4.9	28	91
5	Me	see structure **B**		H	9.6	63	29000
6	Me	Ph	Cl	Me	3.2	18	160
7	Me	Chx	H	H	6.4	41	31000
8	Me	2-Thn	H	H	7.4	47	5500
9	H	Ph	H	H	9.4	44	1100
10	H	Ph	Cl	H	13.7	55	19
11	H	4-$MeOC_6H_4$	H	H	5.5	23	33000
12	H	Ph	NO_2	H	8.0	31	16
13	H	see structure **B**		H	3.4	16	44000
14	H	Ph	Cl	Me	5.2	20	21
15	H	Chx	H	H	7.0	32	6100
16	H	2-Thn	H	H	8.8	41	940
17	Bn	Ph	H	H	8.6	52	19000
18	Bn	Ph	Cl	H	8.8	46	1800
19	Bn	4-$MeOC_6H_4$	H	H	7.3	41	>100 μM
20	Bn	Ph	NO_2	H	4.9	26	2400
21	Bn	see structure **B**		H	8.6	52	>100 μM
22	Bn	Ph	Cl	Me	2.5	13	5000
23	Bn	Chx	H	H	6.5	39	>100 μM
24	Bn	2-Thn	H	H	8.4	48	47000
25	3-MeInd	Ph	H	H	9.5	43	69000
26	3-MeInd	Ph	Cl	H	8.0	33	16000
27	3-MeInd	4-$MeOC_6H_4$	H	H	7.4	31	>100 μM
28	3-MeInd	Ph	NO_2	H	5.8	23	12000
29	3-MeInd	see structure **B**		H	5.2	23	>100 μM
30	3-MeInd	Ph	Cl	Me	2.5	10	14000
31	3-MeInd	Chx	H	H	7.8	34	>100 μM
32	3-MeInd	2-Thn	H	H	9.2	40	71000
33	*i*-Pr	Ph	H	H	7.1	31	>100 μM
34	*i*-Pr	Ph	Cl	H	7.0	28	>100 μM
35	*i*-Pr	4-$MeOC_6H_4$	H	H	7.1	29	>100 μM
36	*i*-Pr	Ph	NO_2	H	2.2	9	>100 μM
37	*i*-Pr	see structure **B**		H	6.4	29	>100 μM
38	*i*-Pr	Ph	Cl	Me	3.0	11	82000
39	*i*-Pr	Chx	H	H	6.0	27	>100 μM
40	*i*-Pr	2-Thn	H	H	8.4	37	>100 μM

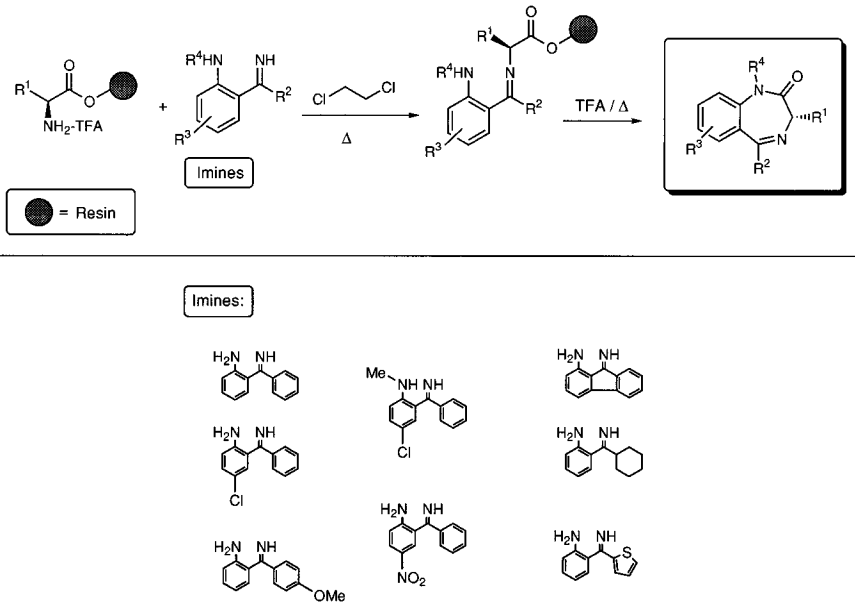
[a] Benzyl (Bn), 3-methylindolyl (3-MeInd), 4-methoxyphenyl (4-$MeOC_6H_4$), cyclohexyl (Chx), 2-thienyl (2-Thn). [b] Yields based on indicated loading of commerically available functionalized resins (0.50−0.89 mequiv/g). [c] Approximate IC-50 values based on three-point fit. Values were also obtained for the commerically available Diazepam (1.46 nM), Nordiazepam (0.2 nM), and Nitrazepam (0.67 nM) corresponding to sample numbers 14, 10, and 12, respectively.

A third array synthesis, that of benzisothiazolones, was accomplished by Mel Schroeder and is shown in Scheme 4.[28] Reaction of the chloromethyl Merrifield resin with 2-carboxythiophenol afforded the corresponding sulfide-linked starting material. BOP activation of the carboxylic acid group in the presence of 40 different amines/hydrazides yielded the corresponding amides. Oxidation of the sulfide link to the corresponding sulfoxide provided an interesting problem. In solution, this oxidation can be accomplished using a stoichiometric amount of oxidant such that an undesirable overoxidation to the sulfone is avoided. In practice, it is not possible to control the stoichiometry of reagent to starting material in SPOS. In general, the ability to use a large excess of reagent and to remove it easily is considered an advantage of the SPOS method. In this case, it became necessary to find a kinetic solution to the overoxidation problem. Fortunately, sulfide oxidation is typically faster than sulfoxide oxidation. After a survey of several potential oxidants, *N*-(phenylsulfonyl)-3-phenyloxaziridine was selected as the reagent of choice. Selective monooxidation was achieved by limiting the reaction time and removal of the reagent excess. Activation of the resulting sulfoxide-bound products with trichloroacetic

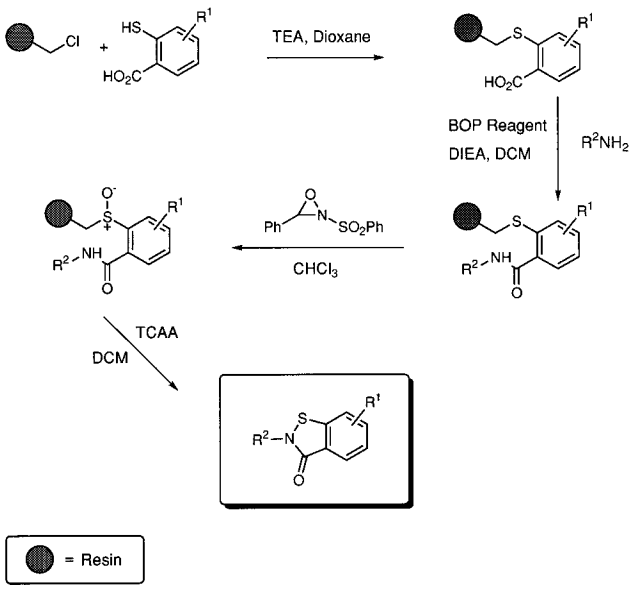
(27) (a) Boojamra, C. G.; Burow, K. M.; Ellman, J. A. *J. Org. Chem.* **1995**, *60*, 5742. (b) Goff, D. A.; Zuckermann, R. N. *J. Org. Chem.* **1995**, *60*, 5744. (c) Luth, J.; Rudolph-Bohner, S.; Moroder, L.; Kolbeck, W.; Osapay, G.; Goodman, M. A novel and facile route to benzodiazepine diversomers. Presented at the 14th American Peptide Symposium, Columbus, OH, June 1995.

(28) Schroeder, M. C.; Kraker, A. J.; Moore, C. W.; Kiely, J. S.; DeWitt, S. H.; Czarnik, A. W. DIVERSOMER Technology: The Synthesis of Benzisothiazolones as Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors. *Abstracts of Papers*, 208th National Meeting of the American Chemical Society; American Chemical Society: Washington, DC, 1994; MEDI 239.

Scheme 3



Scheme 4



anhydride led to the expected cyclative ring closure with release of the product benzisothiazolones in up to 60% yield. All yields were determined here by use of the NMR/internal standard method, which indicated that four of the reactions gave less than 1% yield.

The synthetic strategy for our preparation of cyclic dinucleotides, accomplished in conjunction with Shomir Ghosh and Houng-Yau Mei, is illustrated in Scheme 5.[29] Solid-phase phosphotriester methods for oligonucleotide synthesis were adapted for this work. Commercially available aminomethyl polystyrene resin was selected as the solid support for the preparation of an eight-unit array of cyclic dinucleotides. The resin was first functionalized with a succinyl linker to enable attachment of the first nucleotide through the exocyclic amino group of each base. For convenience, cytidine was chosen as the first nucleotide to be attached to the solid support through the exocyclic amino group of the base. Following immobilization and deprotection, a set of eight linear dinucleotides were constructed using both natural and modified nucleotides as the second building blocks. The 5′- and 3′-ends of the linear dimers were then deprotected and cyclized to afford the penultimate resin-bound intermediates. Final cleavage of the cyclic compounds with concurrent removal of the chlorophenyl protecting group was anticipated to afford a set of eight cyclic dinucleotides. A large-scale (retaining up to 800 mg of resin in each PIN), eight-unit DIVERSOMER apparatus was used for the parallel synthesis of eight cyclic dinucleotides. After synthesis and complete deprotection, the eight cyclic dinucleotides were further purified in parallel utilizing automation and solid-phase extraction (SPE) technology. SPE cartridges prepacked with C-18 silica were attached to a customized vacuum box at the LHR workstation. A LHR was programmed to dispense solvent to condition the cartridges, to load the crude products onto cartridges, and then to dispense the selected solvent to elute the product. Milligrams of the purified products were obtained over the eight-step synthesis starting with aminomethyl polystyrene and after final purification. The yields of the eight cyclic oligonucleotides were all quite low, ranging from 0 (one case) to 5%. The overall low yields for the eight products may be attributable to the inefficient cyclization step in the reaction sequence and/or lack of optimization of the SPE purification procedures.

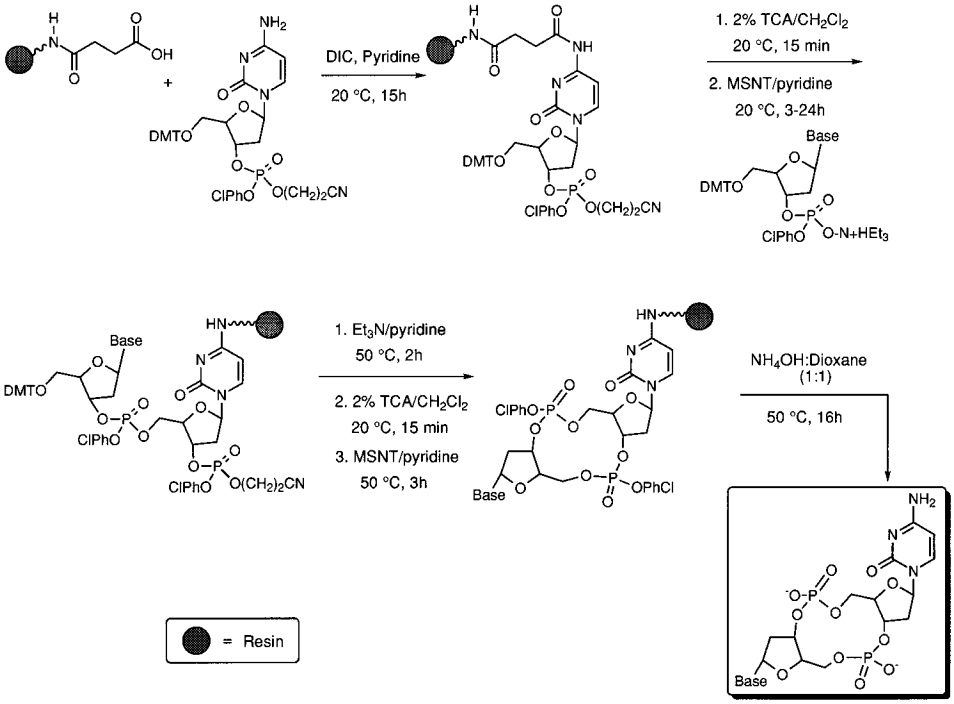
In collaboration with Alasdair MacDonald and Prof. Robert Ramage (both of the University of Edinburgh), we have conducted a parallel synthesis of quinolones related to ciprofloxacin, a broad-spectrum antibacterial agent.[30] As shown in Scheme 6, transesterification of two ethyl benzoylacetates with Wang resin gave the corresponding resin-bound starting materials. Condensation with DMF acetal followed by displacement with a set of four primary amines afforded eight different resin-bound intermediates varying at R^1 and R^2 positions. Cyclization under basic conditions fol-

(29) Ghosh, S.; DeWitt, S.; Hogan, E.; Mei, H.; Sanders, K.; Czarnik, A. W. Parallel Synthesis and Purification of Cyclic Dinucleotides Using Large-Scale, Semiautomated DIVERSOMER Technology. Submitted to *J. Am. Chem. Soc.*

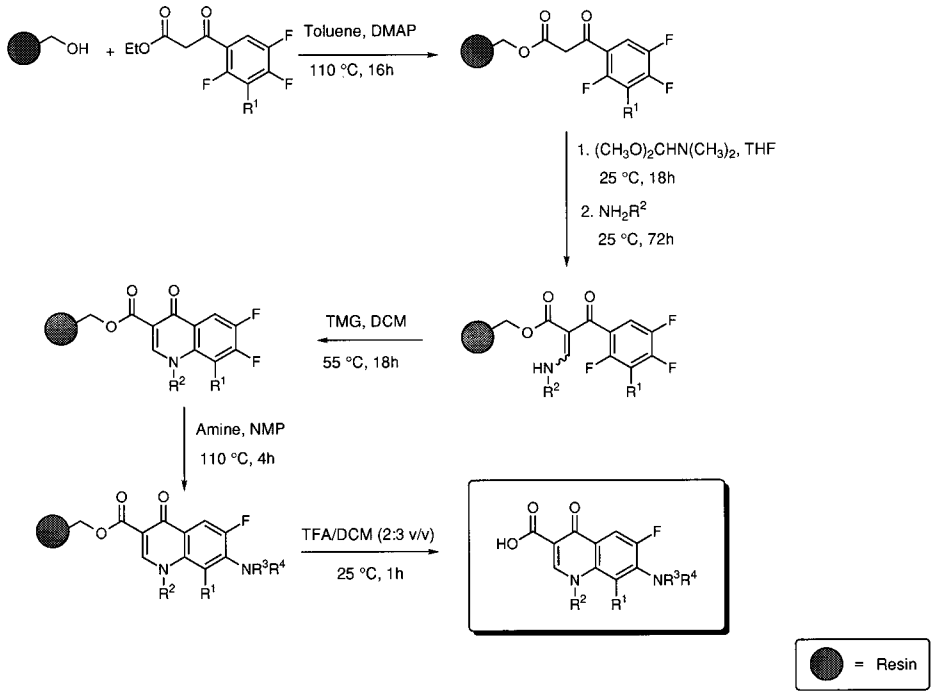
(30) MacDonald, A.; DeWitt, S. H.; Hogan, E.; Ramage, R. Synthesis of Quinolone Antibiotics by DIVERSOMER Technology. Combinatorial Synthesis Symposium, Exeter, UK, July 20, 1995.

(31) DIVERSOMER is a registered trademark.

Scheme 5



Scheme 6



lowed by displacement with a final set of seven amines afforded the resulting library of resin-bound quinolones. Type 3 cleavage from the resin afforded the free quinolones in yields of 7−90% (weight-based yields). The six-step quinolone synthesis described here represents a relatively long SPOS sequence and attests to the fact that multistep reaction sequences need not be avoided. The biological activity of these samples is not accurately determined using the unpurified products, and approaches to overcome this problem are currently in progress.

Information Management

The planning of 40 simultaneous syntheses generates a great deal of information, a fact one cannot really appreciate before going through the exercise. Likewise, after making 40 new compounds the last thing one wants to do is fill out 40 analytical submission forms. For that reason, we have been developing spreadsheet-based information management systems tightly integrated with the DIVERSOMER synthesis method.

Customized Microsoft Excel spreadsheets have been developed to track the multistep syntheses from design and planning through the submission of products for biological testing. Current laboratory operations implement a "master" spreadsheet using Microsoft Excel in combination with DIVERS, a proprietary structural database (MDL software), to track and document the final compounds generated in the

DIVERSOMER array, calculate molecular weights, monitor reaction rates, and calculate product yields. "Reaction cycle" spreadsheets are generated in association with the product spreadsheet for each reaction in the synthetic sequence. These spreadsheets include reagent information such as molecular formula, molecular weight, equivalents, targeted reagent weights and volumes, final reagent concentration, reaction parameters, and wash protocols.

Summary and Conclusions

The DIVERSOMER approach to the generation of focused libraries for lead optimization integrates several technologies: solid-phase organic synthesis, a patented apparatus, novel applications of robotic automation, and information management tools. Today, the application of some of these methods to combinatorial synthesis is already becoming commonplace. This is truly quite astonishing, given the incredulity some of our earliest presentations elicited only two years ago. One of the very first symposia on the generation of chemical libraries was sponsored by CHI in January 1994. The first five contributors to this special issue of *Accounts* presented an invited talk at that meeting, which included sessions on peptide, antibody, oligosaccharide, and oligonucleotide libraries. The immediate relevance of our "chemicals" session to drug discovery was immediately grasped, such that an entire meeting was soon devoted to "Small Molecule Libraries for Drug Discovery" with over 500 attendees (January 1995). This year, an entirely separate meeting has been added on the subject of "Solid Phase Synthesis: Developing Small Molecule Libraries". Combinatorial organic chemistry would seem to be a field whose time came upon the community suddenly and with unavoidable logic.

Despite the eagerness with which combinatorial chemistry has now been embraced, it is very important that we not fall prey to hyperbole and view this field as anything but the continuously evolving science that it is. One does not simply buy a bucket of beads and prepare a million compounds in a few days. Indeed, our experience at Parke-Davis is that one does not even make 40 compounds in a few days unless reaction conditions optimized to the solid-phase have been predetermined. There is now value in the creation of synthetic sequences that work well on solid support, are sufficiently general to permit the use of diverse building blocks (which must be available commercially), and lead to unbound products of interesting structure. Several such examples are contained in this Account. Academic advisors would be well advised to encourage graduate students to undertake such projects with an eye toward support by industry; whether that support is sought before or after the scheme is created remains to be tested. Very important issues lie ahead in the development of new resins (higher loading, more easily cleaved, more resistant to electrophilic reagents), a broad range of automation and software development topics, and the creation of screening methods that permit a bead to serve as assay vessel as well as compound storage vial.

What might be the logical end point of this research thrust? In the best case scenario, we may soon find integrated technologies capable of making and screening millions of compounds per month for activity. When that becomes a reality, it will be crucial that a combinatorial chemistry effort not simply provide the medicinal chemist with several orders of magnitude more "hits" to follow up on. Instead, screens will have to be developed "closer to the bone" so that the compounds delivered to the medicinal chemist are of greater value than hits delivered today.

One thing is certain: when it becomes routine to screen such millions of samples and interesting compounds are thus identified, the position of the chemist will be different from what it has been to date using an in-house chemical library. Today, when an active compound is discovered from our library, the chemist can (a) obtain larger (i.e., several milligrams) amounts from the archived library to confirm that the activity is real and (b) do a computer search of the library to get samples of structurally related molecules. When such "hits" are discovered from a library of beads, neither option (a) nor (b) will be available. The chemist will accomplish option (a) by synthesizing the compound in the traditional way. It seems likely that the chemist will best accomplish option (b) by using a tool for parallel organic synthesis such as the DIVERSOMER apparatus.

This work could not have been carried out in an environment that did not support adventurous science, even if aimed at a practical end point. Much of the early phase planning benefitted from the guidance of Drs. Walter Moos, Robert Root-Bernstein, and Michael Pavia, immediate past director of the BioOrganic Group. The stalwart support and advice of Dr. James Bristol, John Topliss, Michael Taylor, Christine Humblet, John Domagala, Bruce Roth, Jack Hodges, Michael Rafferty, and Annette Doherty is specifically acknowledged. A great many people have made contributions to this project. They include (Chemistry, current) Mark Bush, Ela Hogan, Laura Kieras, Alasdair MacDonald, and Robert Ramage; (Chemistry, former) Donna Reynolds Cody, Jeffrey Cohen, John Kiely, John Topliss, Robert Root-Bernstein, Noah Rosenberg, Mel Schroeder, Charles Stankovic, John Strode, Shomir Ghosh, and Nadia Halim; (Automation) Hsiou Ping Chang, Tammy Grant, Laurie Schumer, Mark Duffield, Yansong Shan, Robert Fijan, and David Nickell; (Engineering) Joe Bauer, Ralph Harms, Mike Hopkins, Joe Greenfelder, David Von Hofe, and John Peterson; (Analytical) Dana DeJohn, Carmen Faraianu, Chris Kibbey, Joe Loo, Leslie McMacken, Bob Short, Tracy Stevenson, Brian Tobias, Steve Werness, and Kathy Zisek. Ms. Joanne Rasmussen has provided expert support in several areas.

AR950209V