Analytical Methods in Combinatorial Chemistry

Second Edition

Bing Yan
Bin Zhang

CRC Press
Taylor & Francis Group

Analytical Methods in Combinatorial Chemistry

Second Edition

CRITICAL REVIEWS IN COMBINATORIAL CHEMISTRY

Series Editors

BING YAN
School of Chemistry and Chemical Engineering
Shandong University, China

ANTHONY W. CZARNIK
Department of Chemistry
University of Nevada–Reno, U.S.A.

A series of monographs in molecular diversity and combinatorial chemistry, high-throughput discovery, and associated technologies.

Analytical Methods in Combinatorial Chemistry, Second Edition
Bing Yan and Bin Zhang

Combinatorial and High-Throughput Discovery and Optimization of Catalysts and Materials
Edited by Radislav A. Potyrailo and Wilhelm F. Maier

Combinatorial Synthesis of Natural Product-Based Libraries
Edited by Armen M. Boldi

High-Throughput Lead Optimization in Drug Discovery
Edited by Tushar Kshirsagar

High-Throughput Analysis in the Pharmaceutical Industry
Edited by Perry G. Wang

A Practical Guide to Assay Development and High-Throughput Screening in Drug Discovery
Edited by Taosheng Chen

Analytical Methods in Combinatorial Chemistry

Second Edition

Bing Yan

Bin Zhang

CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

Version Date: 20140514

International Standard Book Number-13: 978-1-4200-0352-9 (eBook - PDF)

Contents

Preface

During the preparation of this edition, we were as excited as when we were working on the first edition of this book. After 11 years, chemical library and combinatorial chemistry methods have been developed into mature technologies and merged into various cutting-edge approaches, such as chemical biology, chemical genetics, drug discovery, and nanotechnology. The concepts and technologies have also been integrated into the discovery processes of many industries and scientific disciplines.

Over the past decade, combinatorial chemistry has been gaining strength. It has become an active research field worldwide after its beginnings in the United States. Combinatorial chemistry publications are now mostly from academia, whereas a decade ago they were mostly from industry. All pharmaceutical companies have accepted compound libraries as a main source of screening compounds, although they are now almost entirely outsourced to countries such as China, Russia, and India. Internally they rely heavily on small library synthesis to optimize hit compounds. And applications of combinatorial chemistry approaches in other disciplines, such as those concerned with catalysts, material, and agricultural products, are very successful.

Over the years, there have been significant shifts in emphasis in combinatorial synthesis. This edition reflects such shifts. First, the demand for quality control of every library member stimulated the development of many high-throughput analytical techniques and the associated informatics. We have thus significantly modified and enriched Chapter 6, on high-throughput analytical methods. Second, the chromatographic purification of every compound in a library has become a must. We therefore added a new Chapter 7, focused entirely on high-throughput purification

methods. Third, we updated all other chapters with new data, and the new Chapter 8 was added to describe future directions and the associated analytical challenges. We are very optimistic about the next wave of progress in this field in the coming decade.

Bing Yan

Member, St. Jude Children's Research Hospital, Memphis, Tennessee, U.S.A.
Professor, Shandong University, Jinan, China

Bin Zhang

Associate Professor, Shandong University, Jinan, China

Authors

Bing Yan received his Ph.D. from Columbia University with Koji Nakanishi in 1990. He was a postdoctoral fellow at the University of Cambridge from 1990 to 1991, and at University of Texas Medical School in Houston from 1991 to 1993. From 1993 to 1999, he worked at Novartis in the area of Core Drug Discovery Technologies as assistant fellow, associate fellow, and senior associate fellow. From 1999 to 2004, he worked at Discovery Partners International as the director of analytical sciences. From 2004 to 2005, he worked at Bristol-Myers Squibb as a senior principal scientist. Now he is a faculty member at the Department of Chemical Biology and Therapeutics at St. Jude Children's Research Hospital in Memphis, Tennessee, and an adjunct professor at Shandong University. He has published or edited eight books and more than 105 peer-reviewed papers. He is the editor of the book series *Critical Reviews in Combinatorial Chemistry* published by the Taylor & Francis Group. He also serves as the associate editor for the *Journal of Combinatorial Chemistry* published by the American Chemical Society.

Bin Zhang received his Ph.D. from the Institute of Inorganic and Analytical Chemistry and Radiochemistry at Saarland University (Germany) with Professor Doctor Horst P. Beck in 2003. He is currently an associate professor of analytical chemistry in the School of Chemistry and Chemical Engineering at Shandong University, China. His scientific interests include the development of analytical methods in combinatorial chemistry and the characterization and analysis of surface chemically functionalized nanoparticles and their interaction with biological molecules.

chapter one

Analytical issues in combinatorial chemistry

1.1 Combinatorial chemistry

Combinatorial chemistry research has undergone enormous development since the 1990s (Figure 1.1), and the practice of combinatorial chemistry has spread worldwide. These developments have been the subject of many reviews and books (Reviews: Jung and Beck-Sickinger, 1992; Pavia et al. 1993; Felder 1994; Janda 1994; Gordon et al. 1994; Czarnik 1995; Lyttle 1995; Thompson and Ellman 1996; DeWitt and Czarnik 1996; Still 1996; Balkenhohl et al. 1996; Lam et al. 1997; Fenniri 1996; Trautwein et al. 1997; Wentworth Jr. and Janda 1998; Dolle 2002 to 2005; Dolle et al. 2006; Schmatloch et al. 2003; Kenseth and Coldiron 2004; Zhou 2008; Moos et al. 2009. Books: Bannwarth and Hinzen 2006; Jung 2000; Bunin 1998; Gordon and Kerwin 1998; Obrecht and Villalgordo 1998; Terrett 1998; Czarnik and DeWitt 1997; Devlin 1997; Moos et al. 1997; Wilson and Czarnik 1997; Abelson 1996; Chaiken and Janda 1996; Cortese 1996; Epton 1996; Jung 1996; Geysen et al. 1995; Houghten 1995; Merrifield 1993).

Combinatorial chemistry can be defined (Czarnik 1998) as a subfield of chemistry that aims to combine a small number of chemical reagents, in all the combinations determined by a given reaction scheme, to yield a large number of well-defined products in a form that is easy to screen for properties of interest. It can be considered an umbrella term that covers a wide array of topics, including the combinatorial and high-throughput parallel synthesis and all the associated technologies for handling, analyzing, and screening these libraries and obtaining useful molecules from them. Combinatorial chemistry has come to be regarded as a tool rather than an end in itself. Its success is measured in terms of the lead compounds and new chemical entities discovered in various areas, such as molecular recognition, catalysis, drug discovery, materials science, and agrochemical applications.

The integration of combinatorial chemistry and high-throughput (HT) screening has provided a versatile and powerful tool in the drug discovery process. In drug discovery, several factors have catalyzed the dramatic growth of combinatorial chemistry. First, the rapid

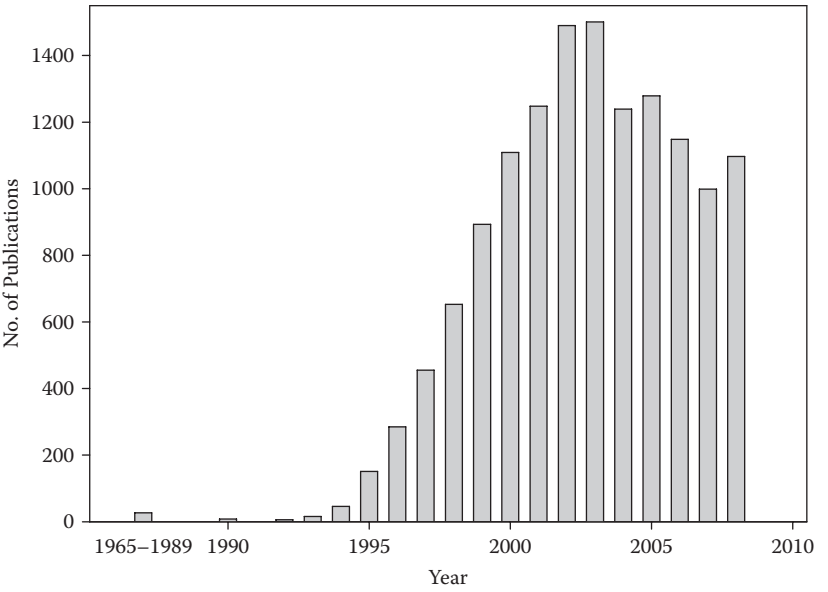

Figure 1.1 Number of publications in combinatorial chemistry based on a search result in SciFinder for the term *combinatorial chemistry*.

developments in genetics and molecular biology can now provide an ever-increasing number of therapeutic target proteins for testing. Second, to meet the need for screening biological targets, significant progress in high-throughput screening technologies has been made since the beginning of the 1990s. It is possible to test more than 100,000 compounds in a period of months. This has made it clear that the synthesis of molecules has become the rate-limiting step in the drug-discovery process. Third, the huge cost and the lengthy process of developing a marketable drug have become formidable, so that all pharmaceutical companies are eagerly searching for more efficient and cost-effective ways of drug discovery. Figure 1.1 shows a literature search using SciFinder for the term *combinatorial chemistry*. Although the use of this term has had a 3% drop in recent years, publications on *parallel synthesis*, *high-throughput synthesis*, and *compound library synthesis* have increased by the same percentage. The combined research and publications have kept steady for the past eight years. Although no longer in the spotlight or heralded as the savior of the drug industry, combinatorial chemistry is alive and well: actually, combinatorial chemistry science is more prevalent and widespread than ever before (Kennedy et al. 2008).

Combinatorial synthesis is a technology that allows the rapid synthesis of large numbers of compounds with diverse features (structural and physicochemical properties). The term *combinatorial chemistry* used to be

more accurately reserved for the synthesis of compound libraries containing an extremely large number of compounds with all possible combination of substituents, to distinguish it from *parallel synthesis*, where the compound libraries consist of a smaller number of individual or discrete compounds; but it is now used indiscriminately to embrace both methodologies. Combinatorial libraries can be made on solid supports, on soluble polymer supports, or in solution.

From the vast new sources of organic compounds, efficient screening machines identify active compounds for defined targets—a process known as *lead generation*. Once active compounds are found, more focused libraries based on those chemical structures provide valuable structure-activity relationship data by way of what is known as *lead optimization*. Note that current lead generation and lead optimization are based only on *in vitro* activity. Physicochemical and other properties are optimized during a later developmental stage. Ideally, compounds with good physicochemical properties should also be selected as lead compounds, and their *in vitro* activity can be optimized in lead optimization.

In the lead identification phase, the goal is to make the most diverse organic compound libraries in the shortest time. The molecular diversity of a compound library means the greatest variation in molecular structure, size, shape, polarity, and polarizability—therefore, with a wide variation in *in vitro* activity and physicochemical properties such as LogP, solubility, permeability, metabolic stability, and bioavailability. The lead selection should be based on all of these criteria. Unfortunately, for technical and philosophical reasons, lead compounds are currently selected on the basis of *in vitro* activity alone.

1.2 Synthesis methods

Few advances in chemistry have had as profound an effect on biotechnology as solid-phase-supported synthesis. First developed by Merrifield (1963) for peptide synthesis, the method has already been adapted to small organic molecules (Fridkin et al. 1966; Leznoff 1974; Crowley and Rapoport 1976; Leznoff 1978; Frechet 1981; Hodge 1988; Brown 1997) and is now routinely used in laboratories worldwide. Although SPOS was continuously studied by several academicians in the 1970s and 1980s, the technology flourished only after the seminal publications of Bunin and Ellman (1992) and of De Witt et al. (1993).

Solid-phase chemistry has advantages that make it an extremely important combinatorial methodology. Since the synthesis is conducted on a solid support, the removal of excess reagents and byproducts is accomplished easily by a copious washing of the polymer. A large excess of reagents can be added and then washed away after the reaction terminates. This allows each reaction step to be driven very close to completion. If, on the other

hand, the reaction efficiency were to be much less than 100%, then a fraction of unreacted chains would remain incomplete at the end of each reaction step. Multiply this by any reasonable number of steps, and the resulting combinatorial library will contain a large portion of undesired products.

Two formats for library synthesis are high-throughput parallel synthesis on polymer supports or in solution, and combinatorial synthesis on polymer support using methods such as "split-and-pool" synthesis (also known as *split synthesis*). Parallel synthesis makes discrete compounds and, if they are pure, provides substances for unambiguous testing. The number of compounds and the diversity made through this method are, however, limited. Split synthesis, on the other hand, is a promising technique for covering much more chemical diversity. It is capable of making a library of millions of compounds, with each bead containing only one compound. In a sense, split synthesis also makes discrete compounds at a single-bead level. Because of the huge number of compounds, it is not practical at present to assay discrete compounds released from every bead. Future development in the miniaturization of HT screening will enforce this format. At present, compounds from split libraries are usually assayed in the form of mixtures. The screening of pooled compounds in solution, however, may give ambiguous results. On-bead screening has also been combined with tagging schemes in identifying lead compounds.

After years of widespread practice of SPOS, difficulties associated with this technology became more evident. First, the synthetic methods required to make organic molecules on a polymer support have not been optimized. The shortage of SPOS literature, the insufficient knowledge of solid support and its effect on SPOS, and the lack of effective ways of monitoring many diverse organic reactions on support have significantly prolonged the optimization of combinatorial reactions. Further problems include the following:

- Reactions are frequently thought to be retarded on solid phase owing to the lack of understanding of solid-phase reaction kinetics and fundamental effects of support on organic reactions.
- Heterogeneous reagents are typically less effective.
- Resin supports display significant solvent-dependent swelling and shrinking properties, which can severely affect reaction rates and site accessibility.
- The optimal solvent for resin swelling may not be the optimal solvent for the desired reaction.
- There is an attachment step and a cleavage step.

For these reasons, the size of the typical library has been decreasing since the end of the last century, while there has been an upswing of solution-phase synthesis.

Even though a lot of work is needed to perfect the SPOS methodology, SPOS is still the foundation of the now most widely practiced form of combinatorial chemistry and remains the method of choice for most combinatorial library synthesis. In addition to solid-phase synthesis, the synthesis of small-molecule combinatorial libraries has also been pursued using both liquid-phase (on soluble polymers see Bayer and Mutter 1972; Bonora et al. 1990; Han et al. 1995) and solution-phase chemistry (Storer 1996; Garr et al. 1996; special issue of *Tetrahedron* 1998).

In liquid-phase combinatorial synthesis (LPCS), the key is a soluble, linear homopolymer (polyethylene glycol monomethyl ether (MeO-PEG)), which also serves as a terminal protecting group for the library of compounds synthesized. Two properties inherent in this homopolymer's structure provide the necessary elements for it to be attractive in a combinatorial format. First, with its helical structure, MeO-PEG has a strong propensity to crystallize; thus, as long as the polymer remains unaltered during the construction of the library, purification by crystallization can be accomplished at each stage of the synthesis. Second, MeO-PEG has remarkable solubilizing effects in a variety of aqueous and organic solvents. This property can be used to advantage if the homopolymer is treated as a reagent and used in large excess. Another virtue of the polymer's good solubility is that all manipulations in the LPCS, including split synthesis, can be carried out under homogeneous conditions. Furthermore, reaction progress can be monitored by NMR. The drawback is that intermediate separation and purification are not as simple as in solid-phase synthesis.

Solution-phase combinatorial synthesis has undergone some recent progress. This field is basically an amalgam of synthetic/medicinal chemistry, parallel processing, and laboratory automation. A wide range of reactions is potentially available for solution-phase library generation. In contrast to solid-phase synthesis, no additional steps for attachment to or detachment from supports are required, and thus only the particular reaction of interest needs be developed. The reactions and techniques to be employed in solution-phase library generation are already familiar to all organic chemists. Because the chromatographic purification is impossible in high-throughput synthesis, intermediate purification and removal of excess reagent and solvent are the major hurdles. These make the multistep synthesis nearly impossible. In "split-and-pool" libraries, each bead contains a compound that is physically separated, making tagging to code for the active bead possible, something not possible for solution-pooled compounds.

While combinatorial chemistry matured, new synthetic strategies and techniques, such as multicomponent reaction (MCR) (Hulme and Gore 2003; Habashita et al. 2006; Nishizawa et al. 2007; Liddle et al. 2008), microwave synthesis (Gedye et al. 1986), fluorous synthesis (Studer et al. 1997), click chemistry (Kolb et al. 2001), diversity-oriented synthesis (DOS)

(Schreiber 2000), and flow chemistry (Jas and Kirschning 2003) were born, fine-tuned, and eventually incorporated into combinatorial chemistry to expand the synthesis of novel chemical entities and further explore the realm of molecular diversity. This book addresses both solid-phase and solution-phase methods and compound libraries generated from any method.

1.3 Analytical challenges

The rapid evolution of combinatorial chemistry and the steady state activities in this field constantly pose challenges for analytical technologies and methodologies. Challenges facing analytical chemists involve at least four major areas.

1.3.1 Properties of solid supports

Although there are many advantages to carrying out organic synthesis on solid support, the effects range from basic swelling and solvent compatibility to site-site interaction in the matrix and alteration of reaction kinetics and the entrapped impurities.

Many of these effects have been inadequately studied. The prolonged reaction optimization time, the recent trends towards reduced library size, and a moving away from SPOS can partially be attributed to the lack of knowledge on the effects of solid supports.

1.3.2 Reaction optimization stage

Synthetic route development in combinatorial synthesis remains a great hurdle and a time-limiting factor in most library synthesis projects. It is estimated that reaction development generally can take months, whereas the library synthesis likely takes only weeks. It is not feasible to purify intermediates in multistep synthesis and library products if the libraries are large, so investing work at the route development stage becomes essential. Although it is impractical to characterize all of the products formed during a combinatorial synthesis, significant credibility could be gained by using the same chemical manipulations to synthesize a single representative compound and small trial libraries to measure the success rate. A good quality control (QC) of building blocks and reagents and an analysis of yield, byproducts, and minimization of side reactions are also crucial.

On-support analytical methods are ideal for monitoring reactions at this stage because the chemical changes and the reaction yield on solid support supply the most relevant information. To “cleave and analyze” is time-consuming, laborious, and destructive. Some synthetic intermediates are not even stable enough under cleavage conditions.

1.3.3 Library synthesis stage

After reactions are optimized and trial libraries are rehearsed, a final large library will be generated on polymer supports or in solution. The quality control of this final collection of compounds is a major challenge due to the large number of compounds that are each present in very small amounts. Should QC be done on support or after cleavage? Because most *in vitro* assays are performed in solution, it is advantageous and more relevant to characterize the library in solution. The high-throughput parallel or fast analytical methods are needed for such characterization. Furthermore, the high-throughput methods unavoidably require automatic data interpretation capability, especially for high-throughput NMR methods. It is highly desirable, at least for discrete libraries, to obtain the concentration and purity as the structure is confirmed. In order to make compounds of the same quality as compounds made by conventional methods, all compounds need to be purified and stored for future screenings. High-throughput purification of hundreds of thousands of compounds within a short time posed another serious challenge to analytical chemists.

1.3.4 Lead selection and optimization stage

Should the selection assay be done on support or in solution? Routine HT screening methodology is still the main method for screening discrete compound libraries in solution. If the target biomolecule is soluble, then on-bead screening can be more efficient, and this has been in use. Unfortunately, the utility of on-bead screening is limited by the fact that not all targets are soluble. Additionally, attachment to a solid support can distort the structure of the protein. In-solution screening is clearly more favorable and unambiguous. Another issue is how to balance the unambiguity in single-compound evaluation with the time-efficient, but more complicated, mixture evaluation. The split synthesis actually makes discrete compounds at the level of a single bead. Fewer than one-third of all compounds made on a single low-loading TentaGel bead are enough for a current screening assay (96-well format). As the screening methods become more miniaturized, significantly less material will be needed for evaluation.

An important issue is that the "lead" selection criteria need to be redefined. A new chemical entity must possess a combination of several optimal properties. Taking a drug as an example, a combination of optimal potency, bioavailability, permeability, and physicochemical properties (not to mention stability and feasibility for synthesis and formulation) is required. It would be improper to select leads based only on *in vitro* activity and to disregard compounds with good physicochemical characteristics and bioavailability, but with low scores in the *in vitro* activity assay.

References

Abelson, J. N. *Combinatorial Chemistry*. 1996. Academic Press, San Diego.

Balkenhohl, F., Bussche-Hunnefeld, C., Lansky, A., Zechel, C. 1996. Combinatorial synthesis of small organic molecules. *Angew. Chem. Int. Ed. Engl*. 35, 2288.

Balkenhohl, C., von dem Bussche-Hunnefeld, F., Lansky, A., Zechel, C. 1997. Combinatorial chemistry. *Fresenius J. Anal. Chem*. 359, 3.

Bannwarth, W., Hinzen, B. *Combinatorial Chemistry*. 2006. 2nd edition. Wiley-VCH, Weinheim.

Bayer, E., Mutter, M. 1972. Liquid phase synthesis of peptides. *Nature* 273, 512.

Bonora, G. M., Scremin, C. L., Colonna, F. P., Garbesi, A. 1990. HELP (high efficiency liquid phase) new oligonucleotide synthesis on soluble polymeric support. *Nucleic Acids Res*. 18, 3155.

Brown, R. Recent developments in solid-phase organic synthesis. 1997. *Contemp. Org. Syn.* 4, 216.

Bunin, B. A. 1998. *The Combinatorial Index*. Academic Press, San Diego.

Bunin, B. A., Ellman, J. A. 1992. A general and expedient method for the solid-phase synthesis of 1,4-benzodiazepine derivatives. *J. Am. Chem. Soc*. 114, 10997.

Chaiken, I. M., Janda, K. D. 1996. *Molecular Diversity and Combinatorial Chemistry: Libraries and Drug Discovery*. American Chemical Society, Washington, DC.

Cortese, R. 1996. *Combinatorial Libraries: Synthesis, Screening and Application Potential*. Walter de Gruyter, New York.

Crowley, J. I., Rapoport, H. 1976. Solid-phase organic synthesis: Novelty or fundamental concept? *Acc. Chem Res.* 9, 135.

Czarnik, A. W. 1995. Combinatorial organic synthesis using Parke-Davis's Diversomer method. *Chemtracts – Org. Chem*. 8, 13.

Czarnik, A. W. 1998. Combinatorial chemistry: What's in it for analytical chemists? *Anal. Chem*. 378A.

Czarnik, A. W., DeWitt, S. H. 1997. *A Practical Guide to Combinatorial Chemistry*. American Chemical Society, Washington, DC.

Devlin, J. P. 1997. *High Throughput Screening: The Discovery of Bioactive Substances*. Marcel Dekker, Inc., New York.

DeWitt, S. H., Czarnik, A. W. 1996. Combinatorial organic synthesis using Parke-Davis's Diversomer method. *Acc. Chem. Res*. 29, 114.

DeWitt, S. H., Kiely, J. S., Stankovic, C. J., Schroeder, M. C., Reynolds, D. M., Ravia, M. R. 1993. "Diversomers": An approach to nonpeptide, nonoligomeric chemical diversity. *Proc. Natl. Acad. Sci. U.S.A.* 90, 6909.

Dolle, R. E. 2002. Comprehensive survey of combinatorial library synthesis: 2001. *J. Comb. Chem.* 4, 369.

———. 2003. Comprehensive survey of combinatorial library synthesis: 2002. *J. Comb. Chem*. 5, 693.

———. 2004. Comprehensive survey of combinatorial library synthesis: 2003. *J. Comb. Chem.* 6, 623.

———. 2005. Comprehensive survey of combinatorial library synthesis: 2004. *J. Comb. Chem.* 7, 739.

Dolle, R. E., Le Bourdonnec, B., Morales, G. A., Moriarty, K. J., Salvino, J. M. 2006. Comprehensive survey of combinatorial library synthesis: 2005. *J. Comb. Chem.* 8, 597.

Egner, B. J., Bradley, M. 1997. Analytical techniques for solid-phase organic and combinatorial synthesis. *Drug Disc. Today* 2, 102.

Epton, R. 1996. *Innovation and Perspectives in Solid Phase Synthesis and Combinatorial Libraries*. Mayflower Scientific Limited, Birmingham.

Felder, E. R. 1994. The challenge of preparing and testing combinatorial compound libraries in the fast lane, at the front end of drug development. *Chimia* 48, 531.

Fenniri, H. 1996. Recent advances at the interface of medicinal and combinatorial chemistry: Views on methodologies for the generation and evaluation of diversity and application to molecular recognition and catalysis. *Curr. Med. Chem*. 3, 343.

Fitch, W. L. 1998. Analytical methods for quality control of combinatorial libraries. *Annual Reports in Combinatorial Chemistry and Molecular Diversity* 1, 59.

Fitch, W. L. 1999. Analytical methods for quality control of combinatorial libraries. *Mol. Diversity* 4, 39.

Frechet, J. M. J. 1981. Synthesis and applications of organic polymers as supports and protecting groups. *Tetrahedron* 37, 663.

Fridkin, M., Patchornik, A., Katchalski, E. 1966. Use of polymers as chemical reagents. *J. Am. Chem. Soc*. 88, 3164.

Gallop, M. A., Fitch, W. L. 1997. New methods for analyzing compounds on polymeric supports. *Curr. Opin. Chem. Biol*. 1, 94.

Garr, C. D., Peterson, J. R., Schultz, L., Oliver, A. R., Underiner, T. L., Cramer, R. D., Ferguson, A. M., Lawless, M. S., Patterson, D. E. 1996. Solution phase synthesis of chemical libraries for lead discovery. *J. Biomol. Screening* 1, 179.

Gedye, R., Smith, F., Westaway, K., Ali, H., Baldisera, L., Laberge, L., Rousell, J. 1986. The use of microwave ovens for rapid organic synthesis. *Tetra. Lett.* 27, 279.

Geysen, H. M., Armstrong, R. W., Kay, B. K. 1995. *Chemical Diversity: The Synthetic Techniques of Combinatorial Chemistry*. American Chemical Society, Washington, DC.

Gordon, E. M., Barrett, R. W., Dower, W. J., Fodor, S. P. A., Gallop, M. A. 1994. Applications of combinatorial techniques to drug discovery. 2. Combinatorial organic synthesis, library screening strategies, and future directions. *J. Med. Chem.* 37, 1385.

Gordon, E. M., Kerwin, J. F. J. 1998. *Combinatorial Chemistry and Molecular Diversity in Drug Discovery*. John Wiley & Sons, New York.

Habashita, H., Kokubo, M., Hamano, S.-I., Hamanaka, N., Toda, M., Shibayama, S., Tada, H., Sagawa, K., Fukushima, D., Maeda, K., Mitsuya, H. 2006. Design, synthesis, and biological evaluation of the combinatorial library with a new spirodiketopiperazine scaffold: Discovery of novel potent and selective low-molecular-weight CCR5 antagonists. *J. Med. Chem.* 49, 4140.

Han, H., Wolfe, M. M., Brenner, S., Janda, K. D. 1995. Liquid-phase combinatorial synthesis. *Proc. Natl. Acad. Sci. U.S.A.* 92, 6419.

Hodge, P. 1988. In *Synthesis and Separations Using Functional Polymers*. Sherrington, D. D., Hodge, P., eds. Chapter 2. Wiley, Chichester.

Houghten, R. A. 1995. *Combinatorial Libraries: The Theory and Practice of Combinatorial Chemistry*. ESCOM, Leiden.

Hulme, C., Gore, V. 2003. Multi-component reactions: Emerging chemistry in drug discovery from xylocain to crixivan. *Curr. Med. Chem*. 10, 51.

Janda, K. D. 1994. Tagged versus untagged libraries: Methods for the generation and screening of combinatorial chemical libraries. *Proc. Natl. Acad. Sci. U.S.A.* 91, 10779.