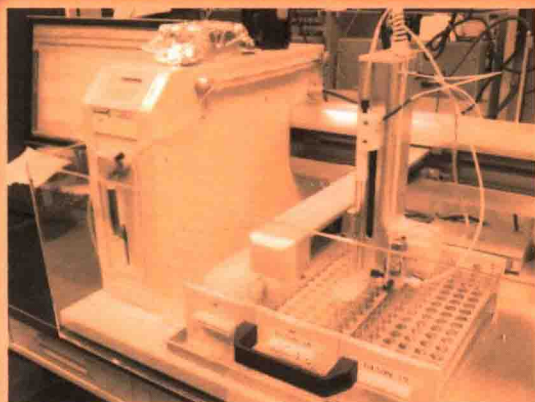


# ANALYTICAL METHODS IN COMBINATORIAL CHEMISTRY

SECOND EDITION



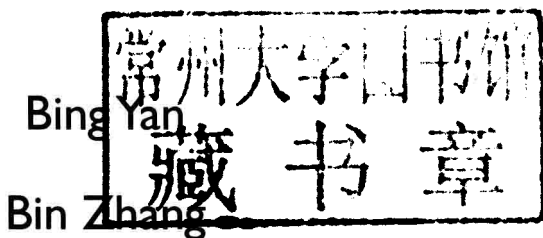
Bing Yan  
Bin Zhang



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# **ANALYTICAL METHODS IN COMBINATORIAL CHEMISTRY**

**SECOND EDITION**

# CRITICAL REVIEWS IN COMBINATORIAL CHEMISTRY

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## *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.

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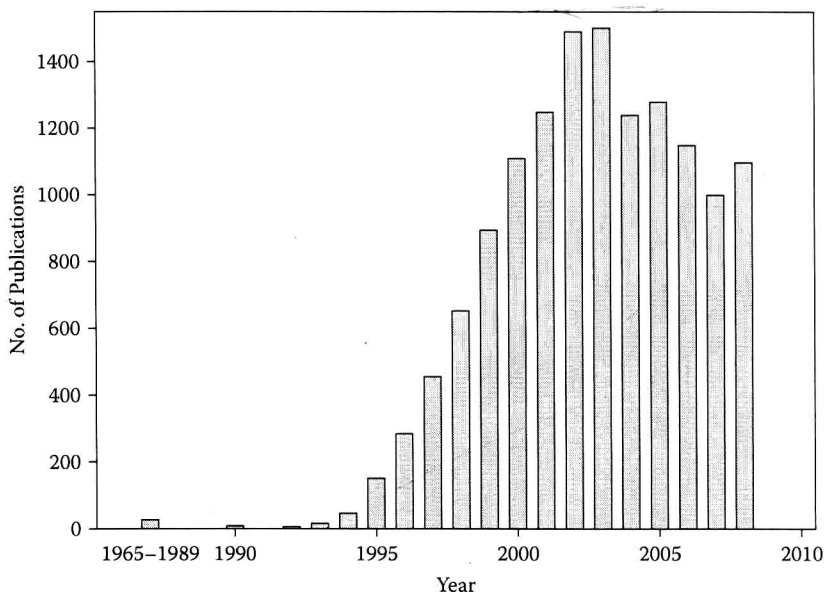
# *Analytical issues in combinatorial chemistry*

## *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; Lytle 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.