



*“DOS, like combinatorial chemistry, attaches different appendages to a common molecular skeleton, but DOS also adds diversity to the molecular skeleton and molecular stereochemistry.”*

# Drug Discovery

## A Shotgun Approach

By Hai Xi Li

The process of discovering a drug is critical to our society's progress in medical care and quality of life. Traditional drug discovery starts with discovering a lead compound, a potential drug that bears the desired physical and biological features. Scientists will then try to refine the lead compound through chemical means to make it suitable for human use. In the past, most drugs were discovered either serendipitously or by analysis of traditional remedies. Scientists could either screen for a compound that exhibits desired biological effects or design a compound based on known biological targets. This process took time and often meant at least a decade of research before practical treatments were possible. In the last two decades, advancements in genomic studies have allowed scientists to identify biological targets more rapidly and developments in biological technology have increased drug screening efficiency. As the probability of finding an effective molecule is proportional to the number of molecules subjected to the screening process, new techniques such as combinatorial chemistry and diversity-oriented synthesis (DOS) attempt to increase the number of compounds that can be screened. These

two techniques both focus on creating a compound library of enormous diversity by making many molecules at a time (1). Combinatorial chemistry and DOS increase the probability of finding something meaningful and thereby lower the cost and time needed.

Conventionally, scientists used target-oriented synthesis (TOS) to discover new drugs. With the hope of finding a molecule that could affect known biological targets, scientists analyzed traditional remedies and used nature as a guide to drug discovery. This process was time consuming and challenging for chemists because it is often hard to synthesize natural products in test tubes. Combinatorial chemistry changed the field and its development was inspired by the development of Merrifield's solid-phase synthesis of peptides in 1963 and parallel solid phase peptide synthesis in the mid-1980s (2). Solid-phase peptide synthesis stabilizes the reaction conditions by anchoring the starting materials to supporting beads, providing stability that allows undesired materials to be washed away after synthesis. This technique allows synthesis of unnatural peptides and incorporation of unnatural building blocks into the target peptide. In addition, solid phase

synthesis allows automation, which speeds up the process dramatically. Parallel solid phase peptide synthesis, similar in principle, has multiple assembly lines while conventional synthesis proceeds in a one-compound-at-a-time linear fashion. For example, conventional synthesis combines reagents A and B to produce AB. Parallel synthesis follows the idea of combining reagent A with multiple reactants, B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub> ... B<sub>n</sub>, to produce a compound library of n individual products AB<sub>1</sub>, AB<sub>2</sub>, AB<sub>3</sub> ... AB<sub>n</sub>. These ideas of automation and compound libraries led to the coining of a new chemistry term – combinatorial chemistry – in the early 1990s. Combinatorial chemistry generates products with a common molecular skeleton and different appendages.

In 1998, the Schreiber group at Harvard University used combinatorial chemistry to synthesize over two million small molecules that have structural features of natural products (3). Small molecules are considered the most promising class of drugs because of their ability to interfere with macromolecules such as enzymes that are important to living systems. However, the two million products were not shown to have biological effects after

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screening. The Schreiber group's focus on small molecules did not benefit from the limited variation in chemical structures generated by combinatorial chemistry. Thus, the Schreiber group introduced a new technique: Diversity Oriented Synthesis (DOS). DOS, like combinatorial chemistry, attaches different appendages to a common molecular skeleton, but DOS also adds diversity to the molecular skeleton and molecular stereochemistry. Differences in stereochemistry – the spatial arrangement of atoms – can cause very different biological reactions in living systems. For instance, enantiomers are chiral, or handed, molecules of the same chemical composition but are mirror images of each other, much like our left hand and right hand. Most biological molecules such as enzymes only recognize and interact with one of the two enantiomers. Therefore, it is important to generate stereo-diversity and search for the particular stereoisomer (spatial variant) that is linked to significant biological effects. Diversity in the molecular skeleton further increases the diversity of the compound library and the possibility of discovering a lead compound.

**“Absorbing the central feature of combinatorial chemistry, attaching different appendages to a common molecular skeleton, DOS adds diversity in stereochemistry and the molecular skeleton itself.”**

To achieve stereochemical and skeletal diversity, the Schreiber group both transforms chiral starting materials into its stereoisomers and creates a collection of products having distinct skeletons (4).

The development of DOS has led the Schreiber lab to discover a variety of molecules, such as macrocyclic biaryl and tubacin (5). The synthesis of macrocyclic biaryl demonstrates the efficiency of DOS in the generation of a stereochemically diverse compound library. The biaryl moiety is the core structure in many natural products and has been used as a basis for drug design. Biaryl synthesis has been developed for about a century. DOS has allowed the generation of a theoretical 1,412-member library of biaryl-containing molecules. Through biological screening, it has been discovered that macrocyclic

biaryl affects the cardiovascular system during zebrafish development. The enantiomer of biaryl has been shown to possess no biological effect, justifying that this new method generates stereoisomer diversity (6). This has outlined a pathway that is now known as chemical genetics – the process of exploring biology through “a comprehensive and systematic chemical approach.”

Through this new chemical genetic approach, tubacin, which inhibits histone deacetylase 6 (HDAC6), was identified from a library of 7,200 compounds. Nine human histone deacetylases (HDACs) have been found to participate in cellular pathways that control cell shape and differentiation. HDAC6 participates in breaking down misfolded proteins through a mechanism termed aggresome. It has been shown that an HDAC inhibitor is involved in treating cancer. Therefore, the identification of HDAC inhibitors is of interest to researchers. However, due to the lack of deacetylase selectivity against biological molecules, it has been difficult to develop small molecules that target or inhibit any one member of the HDACs. With a view toward solving this

problem, the Schreiber group prepared thousands of small molecule inhibitors of HDACs using DOS (7), an effort that has led to the identification of tubacin.

In collaboration with the Anderson lab at Harvard Medical School, the Schreiber group has shown that tubacin induces cytotoxicity in multiple myeloma cells while no toxicity is observed in normal peripheral blood mononuclear cells (8). Tubacin inhibits HDAC6 thereby inhibiting the degradation of misfolded proteins. This induces cell cytotoxicity which then triggers apoptosis

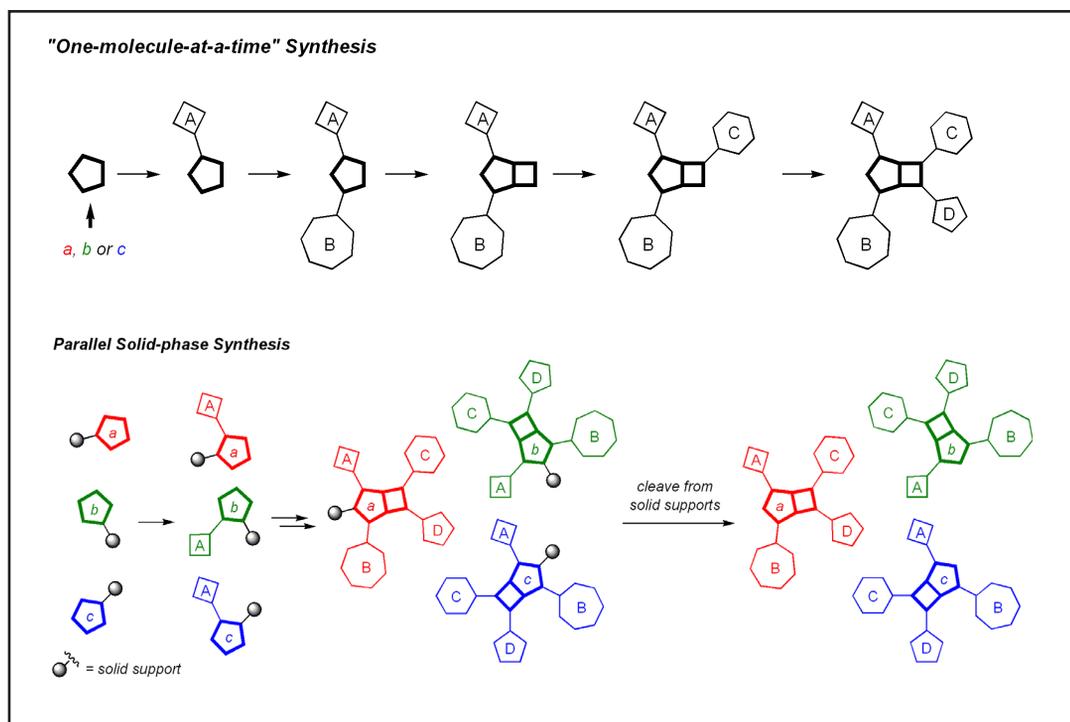


Figure 1. Illustration of linear “one molecule at a time” synthesis and parallel synthesis.

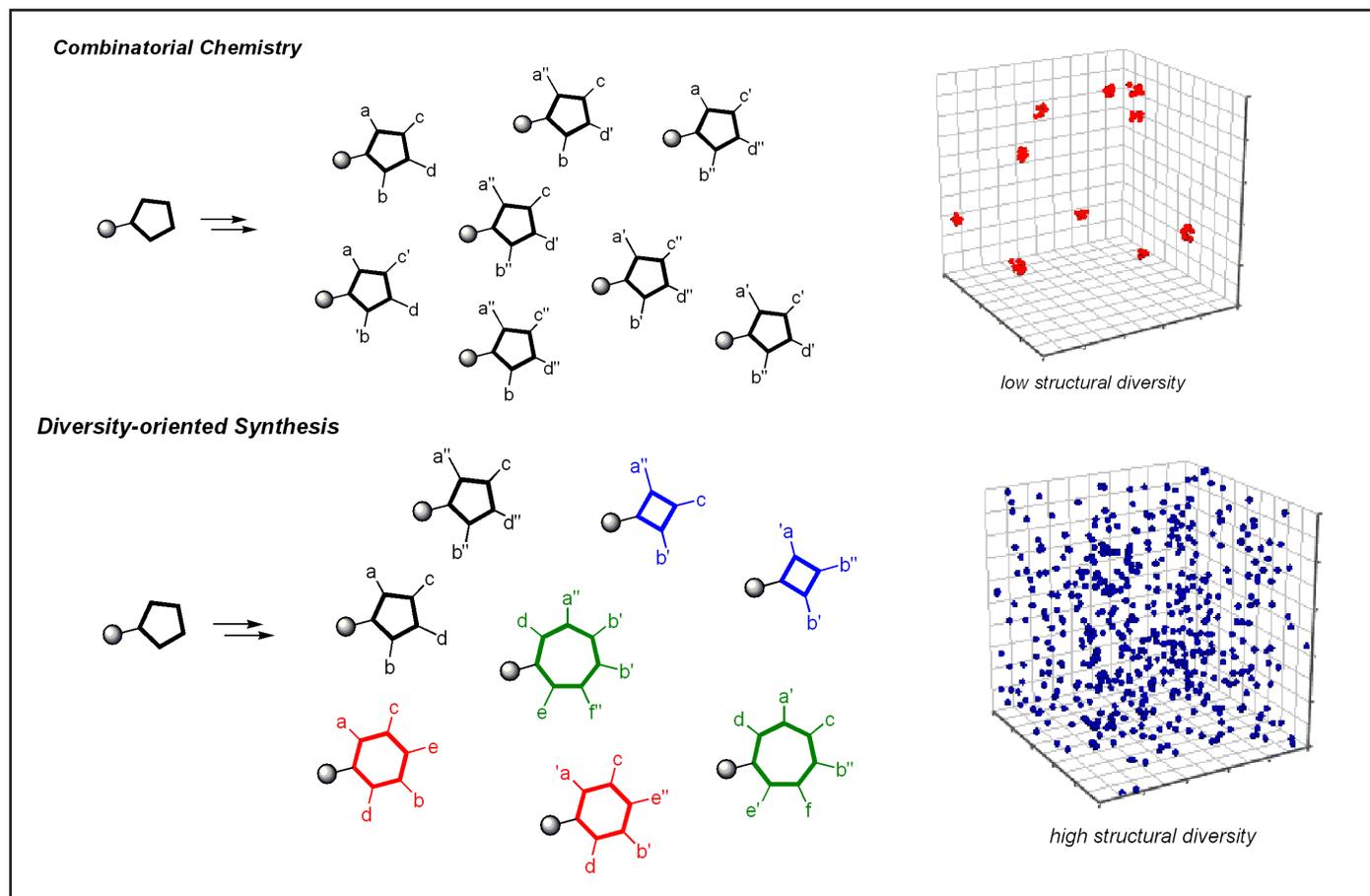


Figure 2. Comparison between Combinatorial chemistry and DOS.

– programmed cell death – in these cells. Through this pathway, tubacin induces cell death in cells with misfolded proteins. Both groups have also treated multiple myeloma cells with a combination of tubacin and bortezomib, a known molecule that induces cytotoxicity by inhibiting proteasomes. The results have brought to the field a new framework for the treatment of multiple myeloma: tubacin, in combination with bortezomib, mediates significant anti-multiple myeloma activity, inducing toxicity in cell lines that have been shown to be drug resistant.

Using the method of DOS, the Shair group of Harvard University synthesized a 10,000-member library of molecules resembling carpanone, a natural product. Among these molecules, the Shair group discovered a series of molecules that act as vesicular traffic inhibitors by inhibiting exocytosis from the Golgi apparatus (9). In an offshoot from the basic idea of combinatorial

chemistry, DOS has shown efficiency in small molecule discovery and promises the discovery of interesting, new compounds for drug development.

Combinatorial chemistry is thus useful for generating compounds with significant potential in designing materials or catalysts, starting from specific building blocks. More importantly, DOS combined with phenotype-based screening has emerged as a powerful tool to study biological systems and to discover new drugs (1). Continued improvements in library design and in the computational assessment of structural diversity in the starting materials will be conducive to further development of DOS. The increasing number of biological targets being identified in this post-genomic era will also accelerate drug discovery in academia and the pharmaceutical industry. **H**

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