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Synthesis and Management of Chemical Library

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Today, screening large number of chemicals obtained from natural products, combinatorial chemistry, and in-house collection of previously synthesized compounds has become a mainstay in the drug discovery stage. Success of high throughput screening (HTS) is depending on the diversity and the number of the chemical libraries. Consequently, a proper management of the chemical library to support HTS has become a critical task in the drug discovery process. In this symposium, we will discuss the development of chemical library on the solid phase and general overview on the chemical library management.

1. Synthesis of Chemical Library; Efficient Solid Phase Synthesis of Benzodiazepine Library

Recently, the demand for compounds for drug discovery efforts has increased dramatically. This is due in large part to recent technological advances in screening procedures, HTS, for many therapeutic targets. To address this demand, very powerful chemical and biological methods have been developed for the generation of large combinatorial libraries. Combinatorial chemistry is one response to this challenge and the process that a numbers of compounds can be produced from a limited selection of starting materials.[1] Over the past several years, an increasing number of researchers have worked to generation of small molecule libraries, such as benzodiazepine, isoquinoline, pyrazole, and hydantoins.[2]

Derivatives of 1,4-benzodiazepines have widespread biological activitis and one of the most important class of bioavailable therapeutic agents as β-turn peptide minetics. In one of the first article to address the synthesis and evaluation of small molecule combinatorial libraries, Ellman's group reported the solid phase synthesis of 1,4-benzodiazepine derivatives.[3] However, this method has several problems. Firstly, functionalized 2-aminobenzophenones, specially having hydroxy or carboxy acid group as a tether to polymer support, are not readily accessible. Secondly, very unstable and moisture sensitive *N*-Fmoc amino acid fluorides must be prepared from the corresponding amino acids for coupling with 2-aminobenzophenones. Finally, after

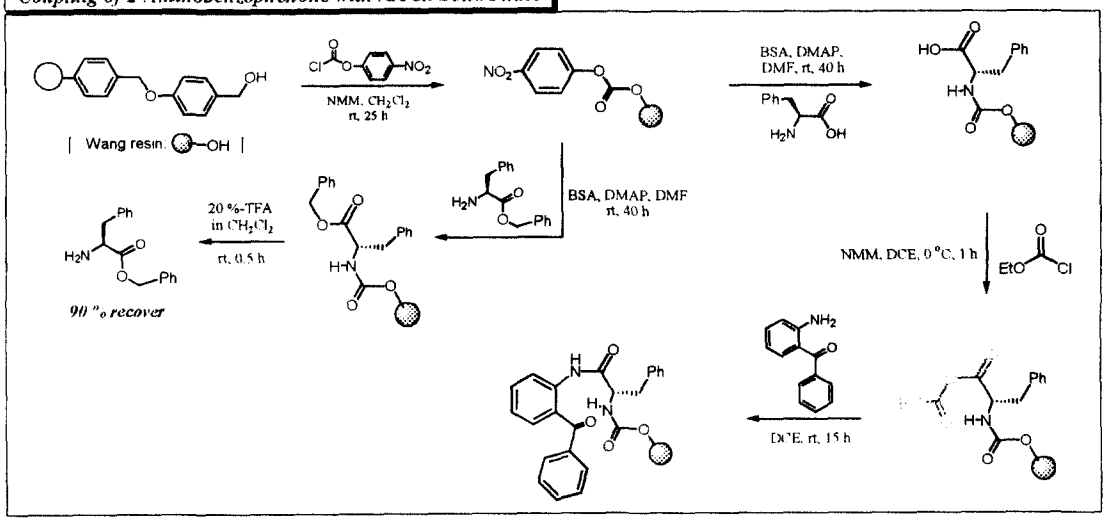
cleavage from the polymer support. would leave trace of the linkage site, may give negative effect on the biological activity of the target molecule. These problems were originated from a tether to the polymeric support at benzene ring and cleavage of this support after formation of benzodiazepine ring system.

Now, we do like to introduce a new method for high-throughput combinatorial library of 1,4-benzodiazepin-2-ones. We chose to develop new method that would construct the benzodiazepine ring system utilizing a traceless cleavage-cyclization step. The central feature of new strategy is a cleavage of the polymer support by treatment with TFA, and *in-situ* cyclization of generated amine with ketone to release desired 1,4-benzodiazepin-2-ones.

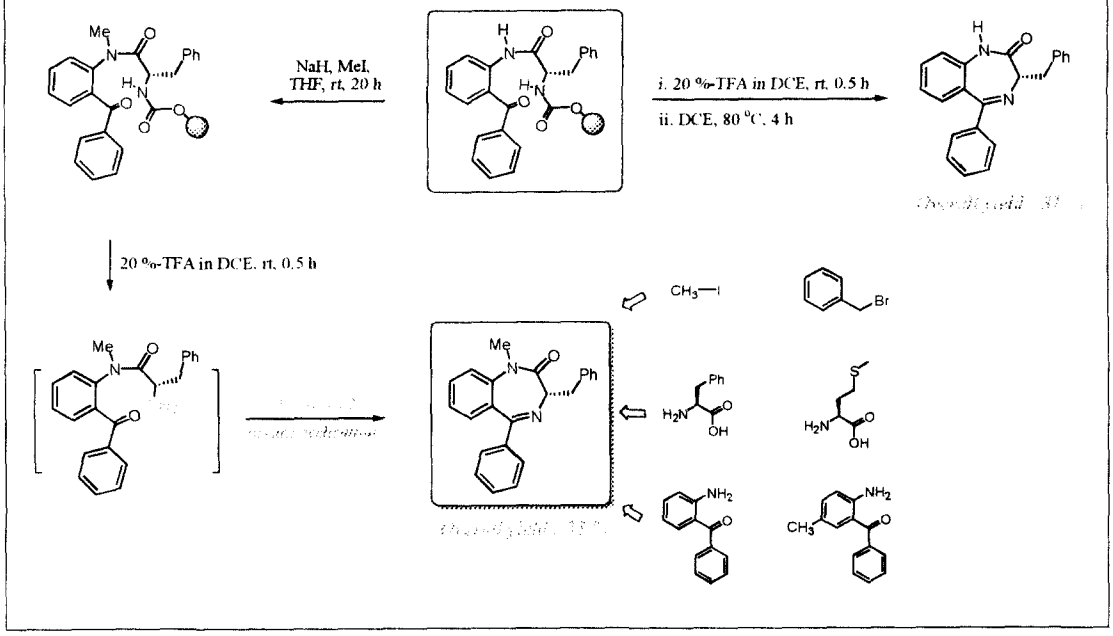
Our first goal is to explore the coupling of 2-aminobenzophenones with amino acids on the polymer support under mild condition. Because of the poor basicity and nucleophilisty of 2-aminobenzophenone, amide bond formation does not occur when the standard activation methods were employed. However, treatment of support bound acid intermediate with ethyl chloroformate using *N*-methylmorpholine as base in 1,2-dichloroethane at 0 °C for 1h to generate activated mixed anhydride, and then addition of 2-aminobenzophenone resulted in complete coupling to provide amide after stirring for 15 h at rt. Alkylation at 1-position of the support bound amide intermediate provides the fully derivatized 1,4-benzodiazepin-2-ones. Cleavage of polymer support and *in-situ* cyclization of intermediate was done under mild conditions, 20%-TFA in 1,2-dichloroethane at rt for 0.5 h and then 80 °C for 2 h. 3-Benzyl-1-methyl- 1,4-benzodiazepin-2-one was isolated in 35% overall yield based on support bound active carbonate. In addition, racemization almost does not occur during the reaction sequence as determined by chiral HPLC analysis of the benzodiazepin derivatives prepared from both (*L*) and (*D*) phenylalanine. The Enantiomeric integrity was accessed by HPLC on a chiral OJ colum using IPA/*n*-hexane=1:9 solvent system.

We point out that a variety of free amino acids and 2-aminobenzophenone are commercially available and very cheap for building general library using present method. With the employment of this general and expedient solid-phase synthetic methodology, the construction of small size combinatorial library of benzodiazepine derivatives was carried out. The library of 250 compounds was developed using all combinations of seven 2-aminophenones, six free amino acids, and seven alkylating agents, with a variety of functionality being displayed.

Coupling of 2-Aminobenzophenone with AA on Solid Phase



Efficient Solid Phase Synthesis of 1,4-Benzodiazepin-2-one



2. Management of Chemical Library[4,5]

Recent advances in genomics and proteomics have provided large numbers of novel biological targets. In the past decade pharmaceutical companies have been through a remarkable transition in their efforts to identify novel compounds that interact with new biological targets and to pave their ways to new therapeutic agents. HTS was risen to the surface and quickly became an important source for finding initial "hits" prerequisite for the generation of new leads. When proceeding a screening program for a novel therapeutic target, large libraries of compounds must first be screened in order to identify chemical leads. A library of over 50,000 structurally defined compounds is a good starting point for the identification of active lead chemical entities for a novel target.

The limiting factor to success, the discovery of new and marketable bioactive molecules, has become the ability to access large numbers of chemically diverse compounds for HTS. Both synthetic and naturally derived substances are often sources for this diversity through a combination of external and internal supports. Most pharmaceutical companies have large libraries of pure compounds that have been built up over decades. Most of the compounds may have come from directed medicinal chemistry efforts around particular pharmacophores. Thus, many of the compounds in a library will fall into a number of related series.

One option to increase the diversity of an in-house library is to purchase commercially available compounds. There are over thirty companies offering chemicals and natural products for sale. Most of the chemical libraries have been assembled by collecting the archives of academic chemists worldwide. There is a variable degree of quality assessment done on these libraries by the vendors and there can be a significant overlap between the libraries. However, with the ability to evaluate whether purchased compounds would increase the diversity of the in-house library, these commercial libraries offer a cost effective method to build or expand a screening library. Prices vary depending on the source of compounds and the number of compounds purchased but generally at the level of $1-10 per milligram(mg)

Natural products are the richest resource for chemical diversity. Theoretically, their efficient use can provide the optimum opportunity for the discovery of new bioactive compounds in HTS program. Natural extracts are a proven source for pharmaceutical agents. However, considerable time, cost and specialized skills in natural products chemistry are required to duplicate hits and elucidate the structures of the active components. For this reason it is usually advantageous for the start-up company to focus initially on screening compounds of known structure.

During the past decade, a new source for generating compounds has arisen and that is through the rapid combinatorial chemical synthesis. Many companies have initiated in-house efforts in combinatorial chemistry. The combinatorial technologies being developed to offer the potential for the rapid and inexpensive synthesis of very large libraries. Despite of early expectation that the ability to make and screen very large libraries would allow researchers to find hits quickly, it has not yielded more useful hits. More important factors are in the types of compounds in the library, their diversity and physical properties, molecular weight, substructure, and ease of incorporation, *etc.* Researchers are now looking for compounds clustered in certain areas of library space. The compounds in certain clusters have a better chance to be found as new leads.

A collection of compounds dissolved in solution has a finite lifetime. Ideally, 25 mg of a compound can be used for 2,000 assays. In reality, however, each compound can be used for only about 250 assays by manual methods. This is due to errors in handling and distribution of samples and due to the researcher's tendency to request more amount of samples than they need. Assuming 50 assays per year, these compounds can last only for 5 years. An immediate concern is then to conserve compounds in collections. Furthermore, a growing trend among synthetic chemists is synthesizing and submitting compounds in a smaller quantity. This reduction in amount of compounds ultimately limits the compounds available for the HTS. The depletion of a compound from the compound collection limits the utility and diversity of the collection. Few mechanisms exist for preventing the depletion of compounds in corporate compound collections or the recovery of unused compounds from HTS laboratories.

Therefore, efforts to encourage the synthesis and submission compounds in larger quantities and to establish more efficient data management, storage, and distribution systems are warranted. Automated compound management systems have begun to emerge to address issues of HTS, compound conservation, and more efficient data and compound handling. There are several semi-automated systems available for weighing, dissolution, and aliquoting processes. It has become the standard to construct working libraries solubilized in DMSO, aliquoted into 96 or 384-well plates and stored in refrigerators or freezers.

3. Conclusions

Pharmaceutical companies are under tremendous pressure to increase the productivity of their drug discovery efforts, to decrease R&D time to market, and to reduce the cost of drug discovery. In the early '90s, the internal and external cost

pressures threatened profitability of pharmaceuticals, forcing them to find more efficient drug discovery methods such as HTS. As HTS improves, the rate determining steps in drug discovery becomes the processes before and after the screening step. To cope with this tremendous increase in throughput, innovations in all aspects of the HTS process are required; diverse compound libraries, combinatorial chemistry, assay development, detection systems, robotics, and data management software. Because of the potential importance of compound collections as a source for finding new leads, new companies have been created to serve as drug discovery partners and service providers.

For every compound that reaches market, around 10,000 compounds should have been synthesized. By using chemical library and HTS, 500,000-1,000,000 compounds would be tested for every marketed drug in the future. With a chemical library management system for handling diverse chemical collections and the HTS system for assaying biological activities more effectively, pharmaceutical companies can increase the probability of finding new leads and optimizing leads, thus minimize the time and cost for bringing new drugs to the market.

4. References and Notes

(1) Borman, S. *C&EN*, **1999**, 77, 10, 33. Kaldor, S. W.; Siegel, M. G. *Comb. Chem. Mol. Diversity Drug. Disc.* **1998**, 307.

(2) Booth, S.; Gravert, D. J.; Janda, K. D. *Chem. Rev.* **1997**, 97, 489. Bunin, B. A. In *The Combinatorial Index*; Academic Press: Sandiego, 1988.

(3) Ellman, J. A. *Acc. Chem. Res.* **1996**, 29, 132 and references sited therein.

(4) Kim, S. S; Gong, Y.-D.; Yoo, S.-e. *Korean J. of Med. Chem.*, **2000**, 10, 22.

(5) Devlin J. P. Eds. *High Throughput Screening*; MARCEL DEKKER: New York, 1997