Pure Appl. Chem., Vol. 73, No. 7, pp. 1033–1039, 2001.

Integrating natural product synthesis and combinatorial chemistry*,†

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Abstract: The fields of natural product total synthesis and combinatorial chemistry have major differences as well as much in common. Unique to combinatorial chemistry is the need to devise rapid and efficient methods for parallel synthesis and purification, while an area of overlap is the targeting of natural product scaffolds for combinatorial libraries. Both these aspects are illustrated with examples from our research.

THE CHALLENGE OF PARALLEL PROCESSING

Organic synthesis, including the total synthesis of natural products, has made tremendous strides over the last century. We are now at the enviable stage whereby a plausible route can be written down for *any* natural product—at least on paper! It is worth reflecting that this is a recent and hard-earned right: less than 50 years ago, R. B. Woodward pronounced [1] "Erythromycin, with all our advantages, looks at present quite hopelessly complex, particularly in view of its plethora of asymmetric centers." Despite the wonderful advances in organic synthesis, we were blissfully unaware of one limitation until recently. That is, the field is dominated by the art of making single molecules one at a time. The exponential growth of combinatorial chemistry has highlighted this deficiency in stark relief. The combinatorial chemist cannot obsess about maximizing the yield of a single reaction, but must develop methodology that is applicable across a broad range of substrates. Equally important is the ability to effect rapid and efficient purification. Indeed, the combinatorial chemist would happily sacrifice some gains in absolute yield if this simplifies reaction workup and results in higher product purity.

The synthesis of combinatorial libraries can be achieved in two fundamentally different ways. In the early days, solid-phase synthesis was the preferred approach and is particularly suited for very large libraries, especially those featuring the repetition of a few reliable carbon-heteroatom bond-forming reactions. Recently, solution-phase library synthesis is increasingly popular. Here, the entire cornucopia of organic reactions is available, but high-throughput workup does require more creative solutions than simple filtration as in solid-phase synthesis. I am often asked which method we favor. The answer is neither—the key is to be flexible and equally adept at both, and work out what is best for a given project.

SOLUTION-PHASE LIBRARY SYNTHESIS

Some of our libraries illustrate different paradigms for parallel solution-phase purification. Unusually, the β-amino alcohol library [2] (Fig. 1a) required no purification. Epoxides were activated by lithium perchlorate and reacted with a slight excess of amines. Afterwards, the solvent was simply evaporated off. While this certainly does not yield analytically pure material, the compounds were sufficiently

*Lecture presented at the 38th IUPAC Congress/World Chemistry Congress 2001, Brisbane, Australia, 1–6 July 2001. Other presentations are published in this issue, pp. 1033–1145.

†Dedicated to the memory of my father, S. T. Arasu (1928–2001).

clean for biological screening. Previous control experiments established that the lithium perchlorate present did not interfere in enzyme, receptor, or cell-based assays. This was our first foray into combinatorial chemistry, and Bee Lee Chng prepared >5,000 pooled samples containing ~30 000 compounds.

The thiohydantoin library [3] (Fig. 1b) is a typical combinatorial heterocycle synthesis with three points of diversity introduced from readily available building blocks. α-Amino acid esters were reductively alkylated with aromatic aldehydes to give the secondary amine. Such alkylations, by the way, are one of the workhorses of combinatorial chemistry as the reaction tolerates a large variety of amines and aldehydes. In this case, the resulting amine is then treated with an isothiocyanate to give a transient thiourea, cyclizing under the reaction conditions to afford the thiohydantoin. Both the reductive alkylation and isothiocyanate addition are high-yielding reactions that do not require a large excess of reagents. At the end of this sequence, an excess of glycine was added as a scavenger. This reacts with any residual aldehyde or isothiocyanate to give water-soluble products, removed by a final aqueous wash. Initially, Mui Mui Sim made a library of ~600 discrete thiohydantoins, followed by several hundred more while optimizing leads discovered by screening.

An extension [4] of the thiohydantoin synthesis (Fig. 1c) featured β-amino acid esters in the same reaction sequence. The intermediate thiourea was now stable, as the heterocyclization involves a 6- rather than a 5-membered ring closure, and required heating with triethylamine to promote formation of the thioxodihydropyrimidinone. In this manner, Mui Mui Sim and Cheng Leng Lee prepared an array of 125 discrete compounds. Instead of the glycine scavenger, we switched to aminomethyl polystyrene as an insoluble equivalent, thus avoiding the need for an aqueous workup. Polymer-supported reagents and scavengers are now in routine use and enable solution-phase reactions to be driven forward by employing a large excess of one of the reagents.

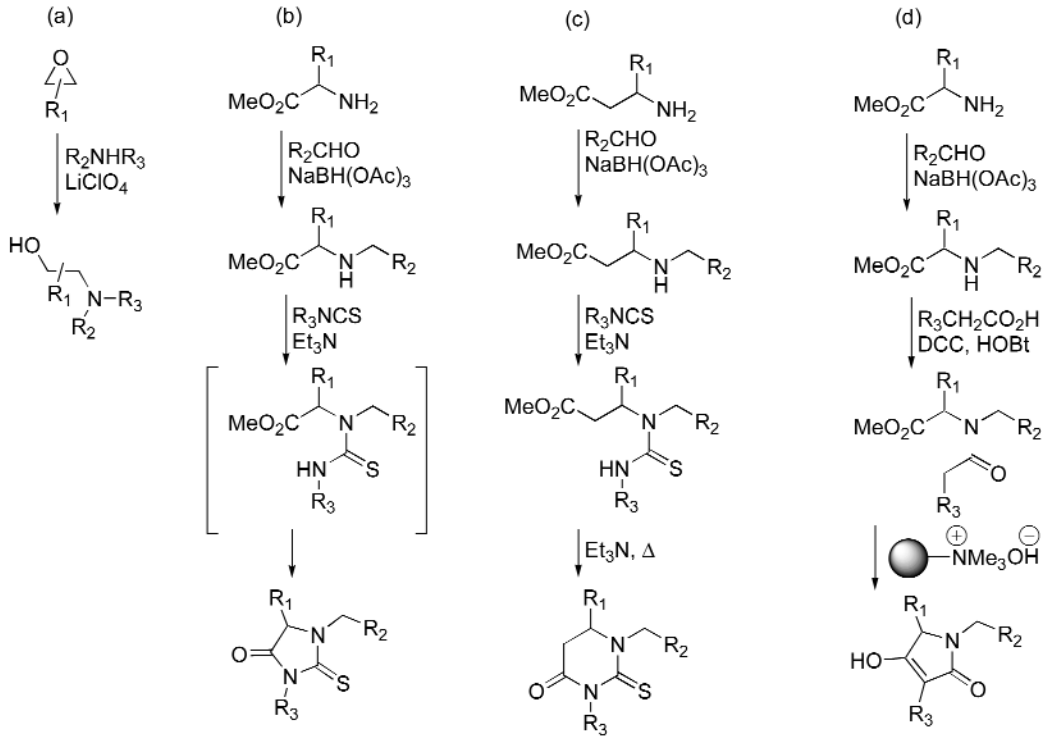

Fig. 1 Libraries prepared by solution-phase synthesis.

Ion-exchange resins are another useful aid in library purification. They are cheap, come with a high loading of functionality, and in macroporous format are compatible with many solvents. Although mainly used as reaction scavengers, we were interested in exploiting their potential as catalysts. In a tetramic acid library [5] (Fig. 1d), Bheemashankar Kulkarni followed reductive alkylation of an α-amino acid ester by acylation with a substituted acetic acid bearing an electron-withdrawing group. The crude product is treated with an ion-exchange resin (OH^- form), catalyzing the intramolecular Claisen condensation to yield the tetramic acid. As a reagent, the ion-exchange resin has significant advantages. Firstly, it is readily removed by filtration. More importantly, the product tetramic acid is sufficiently acidic to exchange with OH^- to become the noncovalent counterion on the resin. Because of this positive and selective *sequestration* (as opposed to negative scavenging), we could carry out the entire 3-step sequence without purifying any intermediates. At the end, simple filtration removes all impurities together with the supernatant while the tetramic acid remains bound to the ion-exchange resin. Adjustment of the pH effects product release in very high purity. Similarly, Kulkarni prepared libraries of hydroxyquinolinones [6] and aryloxazoles [7] by the same principle, using the OH^- resin.

SOLID-PHASE LIBRARY SYNTHESIS

Peishan Lin made a set of phosphoramidite monomers, which were combined [8] with near-quantitative yields by standard DNA coupling conditions on an automated DNA synthesizer. The resulting "unnatural biopolymer" has the phosphodiester backbone of oligonucleotides but the side-chains found in amino acids instead of a nitrogen heterocycle. Peishan then made [9] a pooled library of 1,000 trimers (Fig. 2a), each sample containing 100 phosphodiesters. For ease of synthesis and screening, the trimers were capped with deoxythymidine at both ends.

Peishan's second library [10] (Fig. 2b) starts with an α-amino acid loaded on the Rink AM resin. Reaction with *p*-nitrophenyl chloroformate gives an activated carbamate, which is displaced by *S*-methyl isothiourea. The free amine is then acylated, followed by Hg(II)-mediated displacement of the

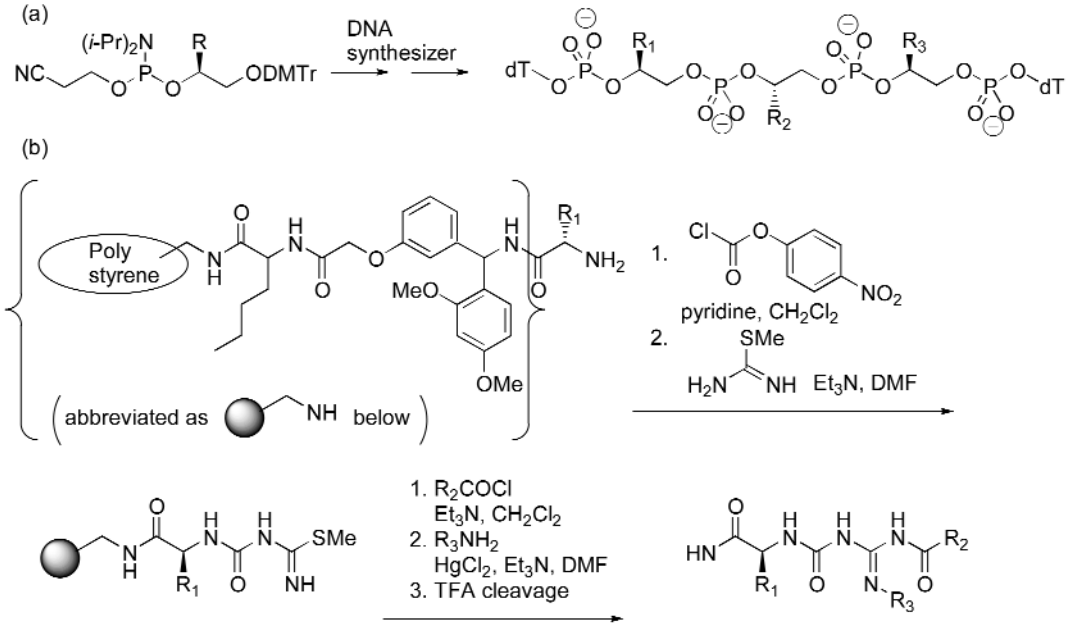

Fig. 2 Solid-phase libraries with C–X bond formation only.

thiomethyl group by primary or secondary amines. Finally, resin cleavage liberates the unsymmetrically trisubstituted guanidines. Peishan prepared an array of 600 discrete guanidines, which were evaluated for antimicrobial activity.

The next two examples feature carbon–carbon bond formation on solid-phase. Multicomponent reactions are often employed in combinatorial syntheses, as a large change in structural complexity is achieved in a single step. The most popular of these is the Ugi 4-component condensation, applied [11] by Zhengong Li to make phosphonic acid diamides (Fig. 3a). The difluoromethylene phosphonate serves as a nonhydrolyzable mimic of phosphotyrosine, and a library of 108 compounds was screened against several protein tyrosine phosphatases.

Bheemashankar Kulkarni adapted [12] the tetramic acid synthesis (*vide supra*) to solid-phase conditions as well (Fig. 3b). Since heterogeneous reagents are not suitable for solid-phase synthesis, we could not use the ion-exchange resin for the cyclization. Instead, a soluble homogeneous equivalent—tetrabutylammonium hydroxide—was employed. The Claisen condensation now results in the product tetramic acid being cleaved off the resin. After this "cyclative cleavage", the only remaining task is separation of the tetramic acid from the tetrabutylammonium hydroxide base, conveniently achieved by stirring the solution with an acidic ion-exchange resin. Over 100 tetramic acids were made, and for preparing large numbers in small quantities, we found the solid-phase route preferable to solution-phase.

Solid-phase synthesis necessarily requires a functional group for immobilization, which is then revealed upon resin cleavage. With nucleotides (e.g., Fig. 2a) and peptides, this "dangling" functional group is naturally part of the oligomer, but it may be less desirable in small-molecule synthesis. Cyclative cleavage strategies (e.g., Fig. 3b) are one solution, in which the point of resin attachment undergoes a cyclization reaction in the last step. This serves to "mask" the functional group used for immobilization, and ensures a high degree of product purity. Any failed intermediates that did not undergo the desired reactions earlier in the sequence would be unable to cyclize, and remain behind on the resin.

A second method for avoiding functional groups due to resin attachment is the "traceless synthesis" introduced by Jon Ellman where cleavage yields an innocuous C–H bond, essentially leaving no history of the immobilization. This can be achieved by creating an unstable functional group that is then converted to a C–H bond, or by the direct formation of a C–H bond during cleavage. The first route is

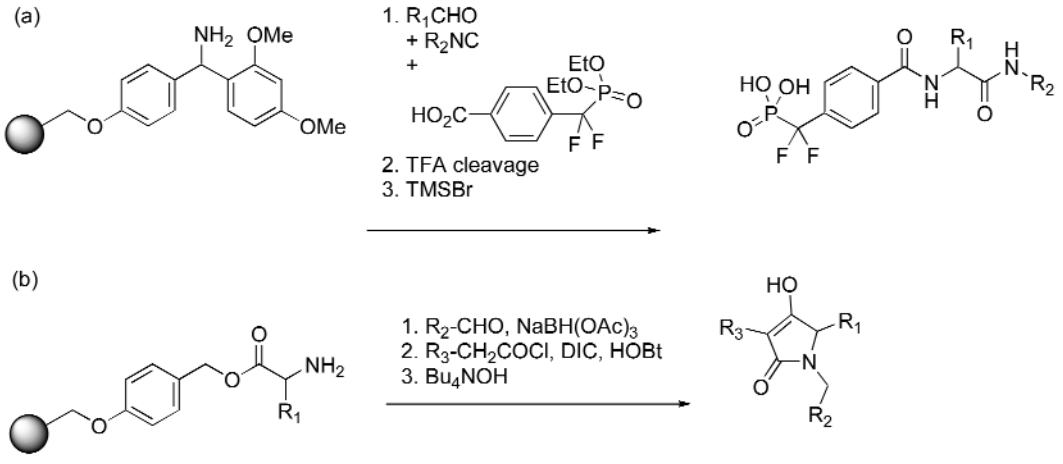

Fig. 3 Solid-phase library synthesis with C–X and C–C bond formation.

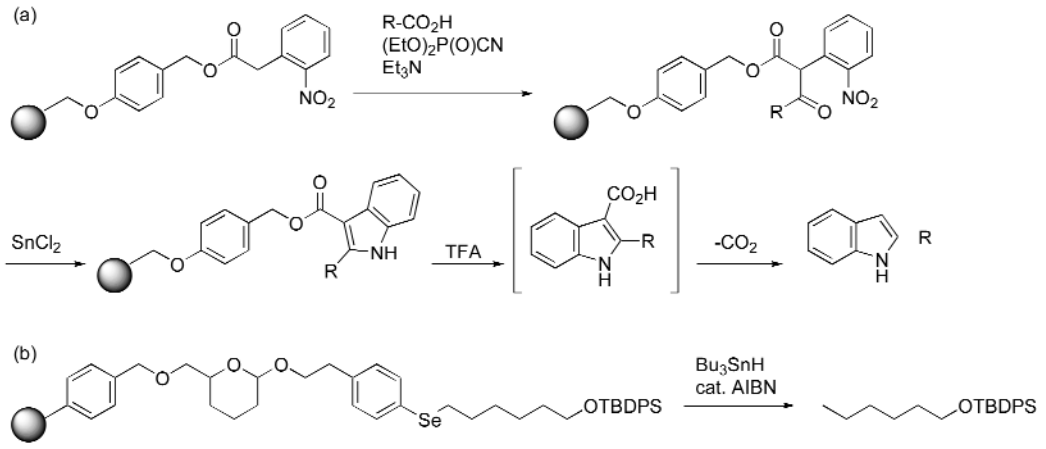

Fig. 4 Two examples of 'traceless' solid-phase synthesis.

exemplified by Mui Mui Sim's "traceless" indole synthesis [13] (Fig. 4a). *o*-Nitrobenzylacetic acid, immobilized on the Wang linker, was *C*-acylated under Shioiri conditions. The nitro group was then reduced, resulting in intramolecular cyclization to the indole. TFA cleavage gives the 3-indole carboxylic acid, which decarboxylates under these conditions. In the final product, the initial solid-phase attachment is not intuitively obvious.

Zhengong Li and Bheemashankar Kulkarni developed [14] a linker that shows potential for the direct formation of a C–H bond during resin cleavage. The linker contains a selenide functional group that can be used to load substrates such as alkyl halides. Tributyltin hydride-mediated reduction of the C–Se bond (Fig. 4b) accomplishes a "traceless" cleavage. This comes at a price, however, as the product has to be separated from tributyltin impurities. Generally speaking, this is the main disadvantage of the "traceless" approach. Although a number of systems are available that produce C–H bonds upon resin cleavage, the reaction conditions are rather "traceful". Linkers that can be cleaved with high efficiency under mild and neutral conditions, which are compatible with a wide variety of functional groups, are unfortunately still a rarity.

COMBINATORIAL CHEMISTRY DRIVING NATURAL PRODUCT SYNTHESIS

How does one select a scaffold around which to build a library for drug discovery? One solution is to choose a biologically active natural product, whose structure is already the result of combinatorial experimentation by nature. I believe this interface between natural product and combinatorial chemistry will become increasingly important in the 21st century. In such programs, the completion of a total synthesis is only the starting point, the real objective being the search for novel biologically active analogs. Often, an existing route might be acceptable for total synthesis of the natural product, but simply too impractical for library preparation. Thus, combinatorial motivations are a powerful driver for optimal and general solutions, and we have found them to be an inspirational source for significantly improved syntheses and the development of new methodology.

The indole alkaloid demethoxyfumitremorgin C (Fig. 5a) is of interest as a selective inhibitor of mammalian cell cycle control as well as the breast cancer resistance protein, a multidrug efflux transporter. A novel *N*-acyliminium Pictet-Spengler reaction [15] led Haishan Wang to a 3-step synthesis. Since the target is a diketopiperazine containing one additional C–N and C–C bond each, it is hard to imagine a shorter route. Furthermore, the aldehyde and amino acid inputs were readily varied to pro-

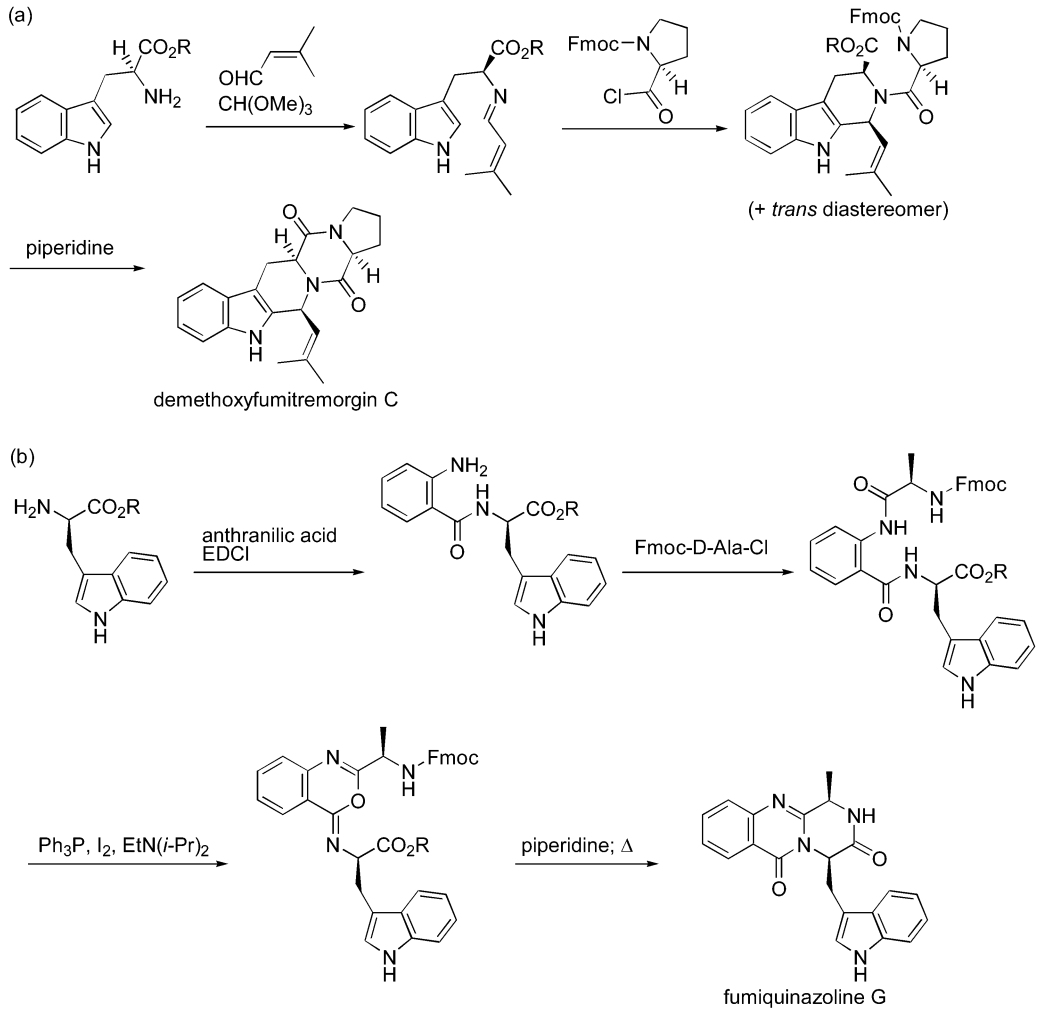

Fig. 5 Combinatorial natural product synthesis.

duce analogs. By using the Wang resin as the ester group R, Haishan performed this synthesis in both solution- and solid-phase, [16] the latter being another example of "cyclative cleavage" at the last step. Screening these compounds by the National Cancer Institute (NCI) yielded [17] a lead nearly tenfold more potent than the natural product.

Previously, natural products such as fumiquinazoline G (Fig. 5b) were made by Staudinger (aza-Wittig) annulation of diketopiperazines, requiring rather lengthy sequences. Methodology for the more logical dehydration of a linear diamide was lacking, until realized by Haishan using the triphenylphosphine/iodine reagent combination. Haishan's solution enabled [18,19] the total synthesis of fumiquinazoline G in only four steps, without significant epimerization of either chiral center. The desired diamides are readily prepared from a range of commercial anthranilic acids and α-amino acids, and a family of these quinazolines was then made [20] on solid-phase. Once again, the Wang resin as the ester group R served in a cyclative cleavage, furnishing the final compounds in high purity. In terms of overall efficiency and generality, this fumiquinazoline synthesis can be considered nearly ideal.

CONCLUSIONS

The debt owed by combinatorial chemistry to organic synthesis is obvious and widely recognized by practitioners of either field. As combinatorial chemistry matures, it is becoming clear that combinatorial chemistry is also fuelling major improvements in organic synthesis. The scope and limitations of a number of venerable reactions is now much better understood with their incorporation into combinatorial sequences for the generation of compound libraries. Many of the techniques developed by combinatorial chemists for parallel synthesis and purification can be profitably employed even when making one compound at a time. Combinatorial chemistry will also have a growing impact on natural product total synthesis, an area that has primarily focused on a target molecule as an end in itself rather than an exciting avenue for discovering new compounds with new properties.

ACKNOWLEDGMENTS

I have been blessed with a talented and enthusiastic group of coworkers, who carried out the work described here at the Centre for Natural Product Research (1994–1996, funded by Glaxo Wellcome and the Economic Development Board) and the Institute of Molecular and Cell Biology (1996–1999, funded by the National Science and Technology Board) in Singapore.

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