Combinatorial Chemistry: A New Paradigm for Drug Discovery

H. Mario Geysen*

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Strategy and Tactics in Combinatorial Organic Synthesis. Applications to Drug Discovery

Eric M. Gordon*, Dinesh V. Patel, Jeffrey W. Jacobs, Mikhail F. Gordeev, and Joseph Zhou

Abstract. A strategic analysis of various issues which pertain to the enablement of combinatorial organic synthesis to produce libraries of non-polymeric organic molecules is given. Methods and examples of the development of solid-phase organic chemistry and its subsequent application to combinatorial library synthesis for drug discovery is illustrated with successful case studies. The synthetic versatility of resin-bound amino-acid-derived imine intermediates to produces β-sultams and pyridines is shown. Use of natural products as key components for creation of combinatorial libraries is presented using *Rauwolfia* alkaloids and the cephalosporin nucleus as examples.

1. Strategy in Combinatorial Synthesis

A comparison between conventional and combinatorial approaches to drug discovery reveals an apparent discontinuity in the strategies and tactics brought to bear by these respective techniques [1][2]. Though the principles underlying chemical reactions are of course invariant, the practice of combinatorial organic chemistry as it relates to lead discovery diverges markedly from serial compound synthesis. For example, in a conventional medicinal chemistry approach, single compounds of previously specified structure are iteratively synthesized and subjected to biological evaluation. In contrast, the goal of combinatorial chemistry is to create screenable *populations* of molecules. Thus, the potential success of the combinatorial approach is leveraged since extremely large numbers of analogs of a previously specified *substructure* are prepared and screened. The mechanics of developing a combinatorial synthesis also affords distinct advantages. Combinatorial synthesis on solid support greatly simplifies the problem of product isolation, and in contrast to solution-phase synthesis, easily permits use of large numbers of building blocks (BB) and reagent excesses to drive reactions to completion. These factors frequently result in solid-phase synthesis (SPS) providing products in higher yield and purity than the corresponding chemistry in solution! Combinatorial chemical reactions must proceed reliably in the face

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of a diverse range of chemical functionality. Hence an early emphasis on well-established synthetic chemistry which operates 'generically' on a broad array of substrates was indicated. Significantly, an analysis of typical lead structures identified in medicinal chemistry clearly suggests that combinatorial syntheses of such molecules can be very short! For example, the interconnection of three to five building blocks of MW = 150 could require as few as two to four synthetic steps. Experimentally simple reactions such as many cycloadditions, which utilize readily available BB's to quickly give rise to structurally complex molecules, seemed especially useful as starting points. We also wished to ensure that our investment in adapting specific chemistry for SPS was maximized

by avoiding 'dead-end' resin-bound intermediates. Rather, versatile intermediates were identified that could be routinely used in multiple varieties of products. Early on, we recognized that libraries could themselves serve as starting materials for construction of other libraries, and that combinatorial synthesis could be used to generate key BB's .

In pursuing recent research, we have attempted to unify the needs of drug discovery chemistry with those demanded by a strategic combinatorial approach to small-molecule library synthesis. An important near term goal in our program was to intersect historical medicinal chemistry in terms of being able to generate combinatorially the same types of molecules traditionally considered valuable in drug

discovery. The following case studies in small-molecule library construction exemplify the stratagems outlined above.

2. Case Studies in Combinatorial Organic Synthesis

### 2.1. *The Imine as a Versatile Synthetic Intermediate*

An essential prerequisite for performing solid-phase synthesis is the ability to immobilize an initial building block to a resin support for subsequent elaboration. In the course of extensive work with peptide and peptidomimetic libraries, we have amassed a substantial collection of natural and unnatural amino acids whose side chains reflect a broad array of physicochemical properties. This amino-acid building-block set provided a convenient starting point for exploring the scope of solid-phase organic synthesis, since conventional peptide activation chemistries could be used for the derivatization of various synthesis resins.

The formation of imines *via* condensation of amines with aldehydes or ketones appeared as one particularly appropriate reaction. The imine is a highly versatile intermediate in organic synthesis, providing a conduit into diverse nitrogen heterocycles through cycloadditions, condensation reactions, and other nucleophilic additions. We have found that several of these classical transformations can be adapted to a SPS approach wherein the imine is derived from the amino group of an immobilized amino acid to become a reactive center (see *Scheme 1*). In addition, the commercial availability of thousands of reactive carbonyl and amine components provides access to theoretically millions of imine intermediates, underscoring the considerable opportunities for generating large combinatorial heterocyclic libraries.

2.2. *Cycloaddition Reactions of Resin-Bound Imines: Synthesis of β-Lactams and β-Sultams*

Cycloaddition chemistry can provide efficient approaches to constructing densely functionalized molecular scaffolds through reactions that frequently proceed with a high degree of regio- and stereochemical control [3]. Imines are well-known as two-electron partners in a variety of [2+2], [3+2], and [4+2] cycloadditions, and we have found these to be excellent reactions for exploiting in combinatorial chemistry using solid-phase synthesis techniques. The *Staudinger* addition of ketenes to imines offers one of the most

Scheme 1. *The Imine as a Versatile Intermediate for Solid-Phase Synthesis*
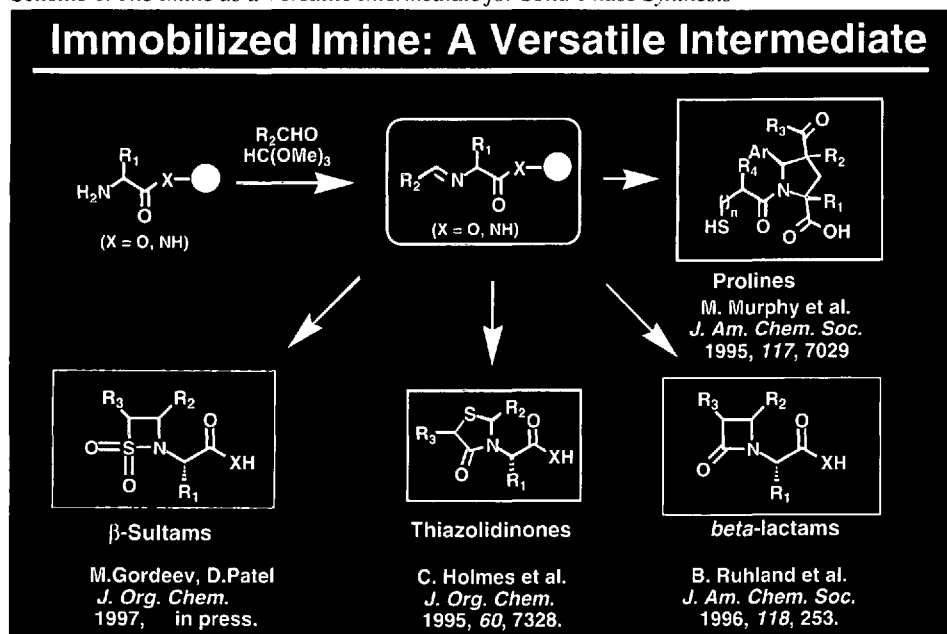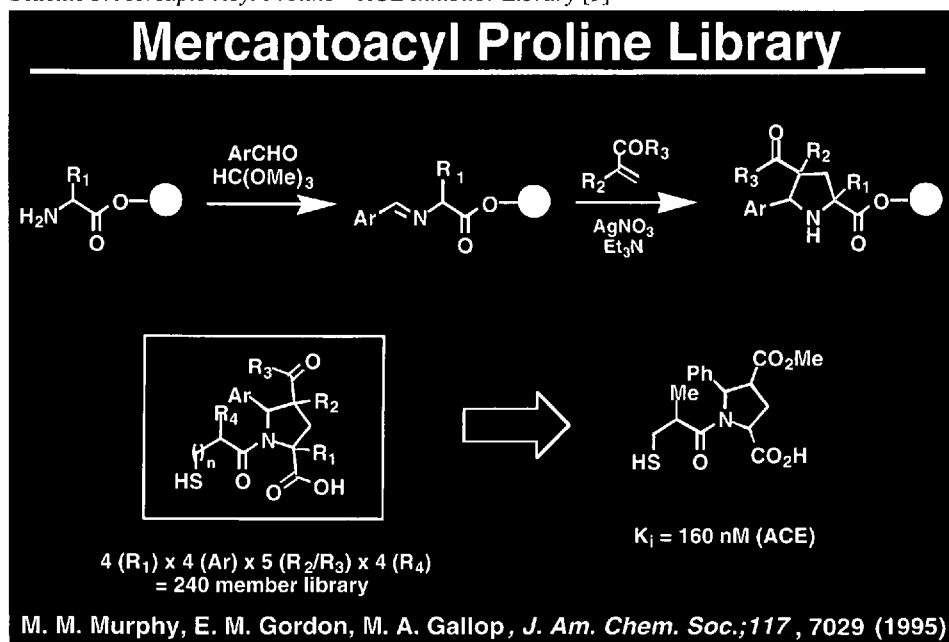

Scheme 2. *Solid-Phase Synthesis of β-Sultams*
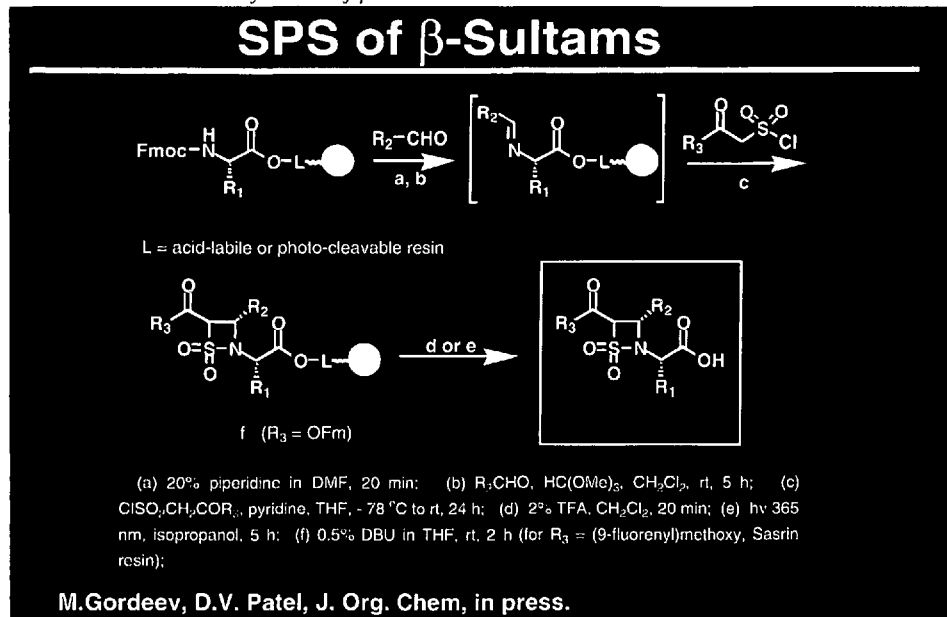
versatile approaches to structurally diverse β-lactams [4]. A solid-phase variant of this [2+2] cycloaddition has also proven to be a robust reaction, wherein tethered imines react with ketene formed *in situ* by dehydrohalogenation of an acyl halide. Combinatorial libraries of monocyclic β-lactam compounds have thus been generated from amino acids, aldehydes, and various ketene precursors following the split synthesis paradigm [5].

Extension of the above synthetic strategy to the preparation of β-sultams relies on the feasibility of performing efficent [2+2] cycloaddition of sensitive and reactive intermediates generated *in situ*, namely the imine and sulfene, at low temperatures (–78°) on solid support. As part of the effort to arrive at the optimal set of reaction parameters and choice of appropriate sulfonyl reagent, we undertook a gel-phase ^{13}C-NMR study employing ^{13}C-labeled benzaldehyde to monitor the formation of β-sultam moiety on solid support [6]. Following the synthetic protocol, in *Scheme 2*, several β-sultams were obtained in good yield and purity. While the initial studies employed polystyrene-based Sasrin resin possessing the highly acid-labile alcohol linker (1% TFA (= trifluoroacetic acid) cleavable; 58–90% yield, 70–95% purity), the synthetic sequence is robust enough to tolerate other types of linkers and cleavage conditions. Thus, we have successfully synthesized β-sultams using polyethyleneglycol (PEG)-based TentaGel resin derivatized with the *Holmes* [7] α-methyl-6-nitroveratrylalcohol-based photolabile linker. The final products were released from the polymeric support upon photolysis in propan-2-ol at 365 nm. In most cases, β-sultam products were isolated in good yield and purity (19–39% yield, 70–94% purity).
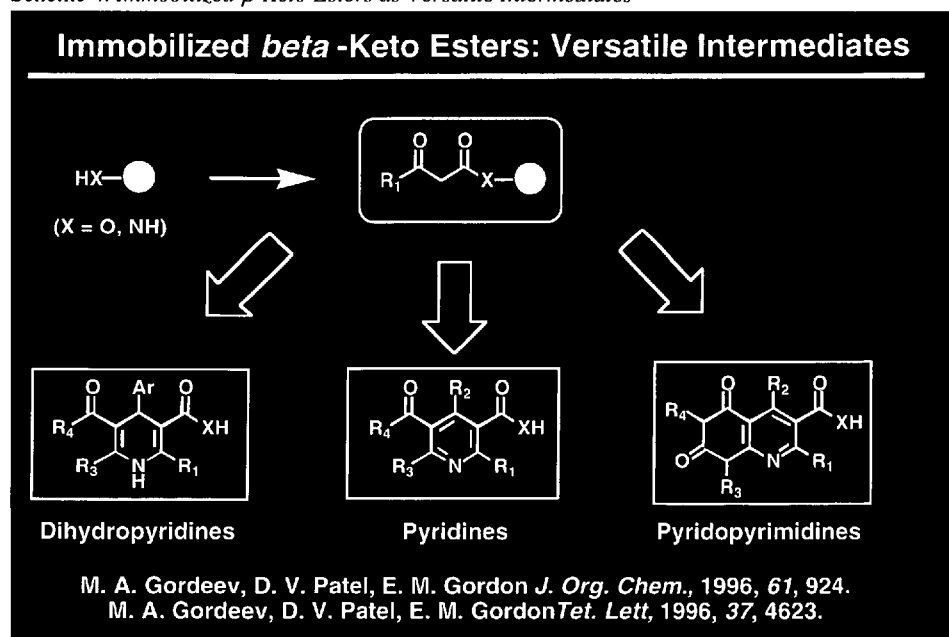
2.3. Cycloaddition Reactions of Resin-Bound Imines: Synthesis of Pyrrolidines via 1,3-Dipolar Additions

Imines appropriately activated by adjacent electron-withdrawing groups can undergo 1,2-prototropic rearrangements to form azomethine ylides, a reaction first observed in 1978 by *Grigg* and coworkers for α-amino ester aldimines [8]. From a combinatorial chemist's perspective, we were particularly attracted by the mild reaction conditions afforded by *Lewis*-acid catalysis of this cycloaddition and by the prospect of converting our solid-supported amino ester imines into collections of highly functionalized proline derivatives. Our successful implementation of the 1,3-dipolar cycloaddition reaction of resin-bound azomethine ylides to elec-

tron-deficient olefins has recently been reported (*Scheme 3*) [9].

We have used this solid-phase pyrrolidine chemistry in a model system designed to illustrate how 'focused' (or 'biased') combinatorial libraries can be used to explore structure-activity relationship (SAR) around an existing bioactive lead compound. The split synthesis protocol was used to prepare a library of pyrrolidines that were acylated with a set of mercapto acid chlorides producing a collection of analogs of the angiotensin-converting enzyme (ACE) inhibitor, captopril. After cleavage and deprotection of this ~500-compound library, screening through four iterations of assay and sublibrary resynthesis (*i.e.* deconvolution) led to the identification of an unusually potent

inhibitor of ACE ($K_i = 160$ pM; *cf.* captopril $K_i = 500$ pM).

2.4. The β-Keto Ester as a Versatile Synthetic Intermediate: Synthesis of Dihydropyridines, Pyridines, and Pyridopyrimidines

The dihydropyridine (DHP) nucleus is common to numerous bioactive compounds which include various vasodilator, antihypertensive, bronchodilator, antiatherosclerotic, hepatoprotective, antitumor, antimutagenic, geroprotective, and antidiabetic agents. We recently developed a general method for SPS of DHPs, based on a two- or three-component cyclocondensation of immobilized enamino esters with 2-arylidene β-keto esters, or β-keto esters and aldehydes, respectively

Scheme 3. *Mercapto Acyl Proline – ACE Inhibitor Library* [9]

Mercaptoacyl Proline Library

4 (R_1) x 4 (Ar) x 5 (R_2/R_3) x 4 (R_4) = 240 member library

$K_i = 160$ nM (ACE)

M. M. Murphy, E. M. Gordon, M. A. Gallop, *J. Am. Chem. Soc.;117*, 7029 (1995)

Scheme 4. *Immobilized β-Keto Esters as Versatile Intermediates*

Immobilized *beta*-Keto Esters: Versatile Intermediates

(X = O, NH)

Dihydropyridines   Pyridines   Pyridopyrimidines

M. A. Gordeev, D. V. Patel, E. M. Gordon *J. Org. Chem.,* 1996, *61*, 924.
M. A. Gordeev, D. V. Patel, E. M. Gordon *Tet. Lett,* 1996, *37*, 4623.

[10]. This method is well suited for use in combinatorial 'split/pool' protocols and has been employed for the preparation of dihydropyridine libraries [11]. An alternate strategy for preparing DHPs commences with immobilized β-keto esters shown in *Scheme 4*, and offers the added advantage that these intermediates are amenable to combinatorial syntheses of other bioactive heterocyclic molecules such as pyridines and pyridopyrimidines [12].

3. Combinatorial Modification of Natural Products

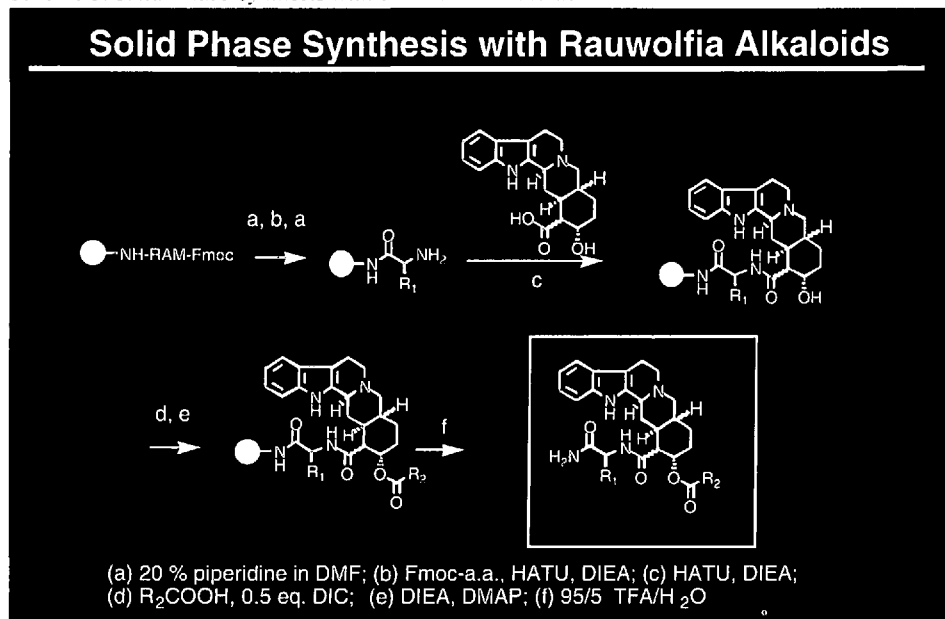Historically, the most prolific source of biologically active lead compounds and novel pharmacophores has been natural products. In our opinion, such secondary metabolites (and relevant substructures) could also serve as excellent starting points for combinatorial modification. The structural complexity of most natural products deter analog approaches relying upon 'total synthesis'. Instead, traditional serial synthesis methods have shown that simple modifications to existing functionality on a natural product skeleton can often afford derivatives with dramatically altered biological activity. In many ways this strategy parallels the combinatorial chemistry paradigm, where simple and reliable synthetic protocols consisting of a limited number of chemical steps are employed to introduce diversity elements into a substructure of interest. We have applied this strategy to indole alkaloids from the *Rauwolfia genus* as well as 7-aminocephalosporanic acid – an extremely important precursor to the cephalosporin family of antibiotics.
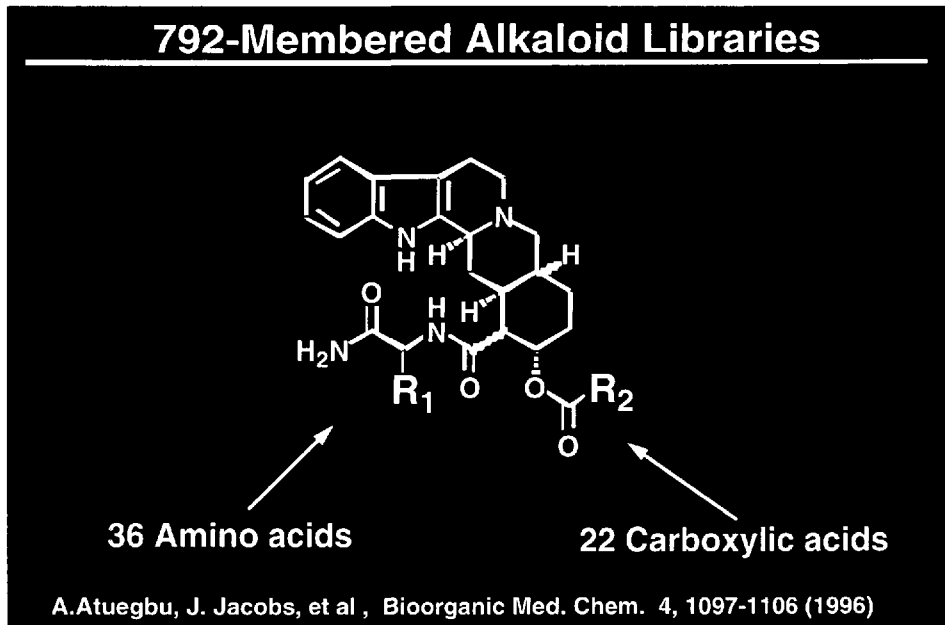
3.1. Rauwolfia *Alkaloid Libraries*

There are over 50 known, characteristic alkaloids which belong to the *genus Rauwolfia*, and these compounds have been shown to have a broad range of biological activities. Such a large family of related natural products is an ideal starting point for combinatorial modification, since the effort expended to develop solid-phase protocols for one compound should presumably be applicable to other members of this extended class. Thus, solid-phase library synthesis protocols were developed for the *Rauwolfia* alkaloids yohimbine and rauwolscine. Much of the prior work on this family of compounds has focussed on modifications of the E-ring carboxylate and hydroxy groups in efforts to obtain derivatives with altered biological properties, and esterification of these functional groups is the most common modification. We therefore pursued a similar strategy for their modification on a solid support (*Scheme 5*). The alkaloids were first converted to their free acids by saponification in methanolic potassium hydroxide. SPS Beads derivatized with amino acids could then be acylated by these alkaloids using HATU activation in the presence of DIEA. Protection of the E-ring hydroxy group during this step was found to be unnecessary, as polymerization products were not observed for either compound. Subsequent acylation of the hydroxy group at C(17) was performed using a large excess of the symmetrical anhydride of the desired carboxylic acid. This procedure afforded functionalized alkaloids in moderate to good isolated yield and was used to prepare 792-membered libraries of both yohimbine and rauwolscine (*Scheme 6*) [13].
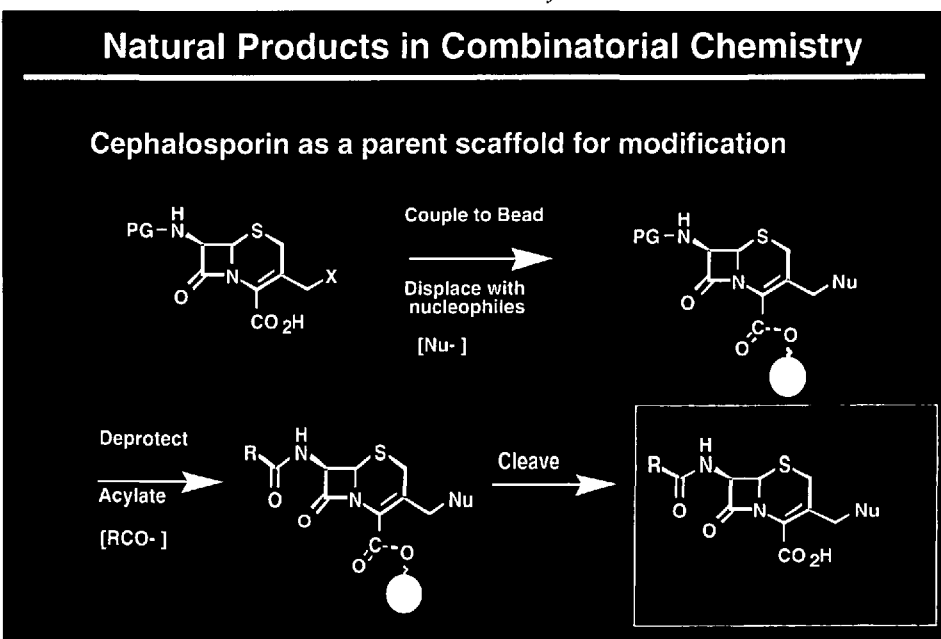
3.2. *Solid-Phase semi-Synthesis of Cephalosporin Derivatives*

7-Aminocephalosporanic acid (7-ACA) is an essential intermediate for the semi-synthesis of cephalosporin antibiotics, and over 70 drugs derived from 7-ACA are marketed worldwide [14][15]. The 7-ACA nucleus has been subjected to chemical modification for over 30 years, and these chemical modifications have in general focussed on only two positions of the core molecule. We reasoned that a combinatorial approach to the modification of 7-ACA would have the highest likelihood of successfully discovering

Scheme 5. *Solid-Phase Synthesis with* Rauwolfia *Alkaloids*

Solid Phase Synthesis with Rauwolfia Alkaloids

(a) 20 % piperidine in DMF; (b) Fmoc-a.a., HATU, DIEA; (c) HATU, DIEA; (d) R₂COOH, 0.5 eq. DIC; (e) DIEA, DMAP; (f) 95/5 TFA/H₂O

Scheme 6. *792-Membered Alkaloid Libraries*

792-Membered Alkaloid Libraries

36 Amino acids   22 Carboxylic acids

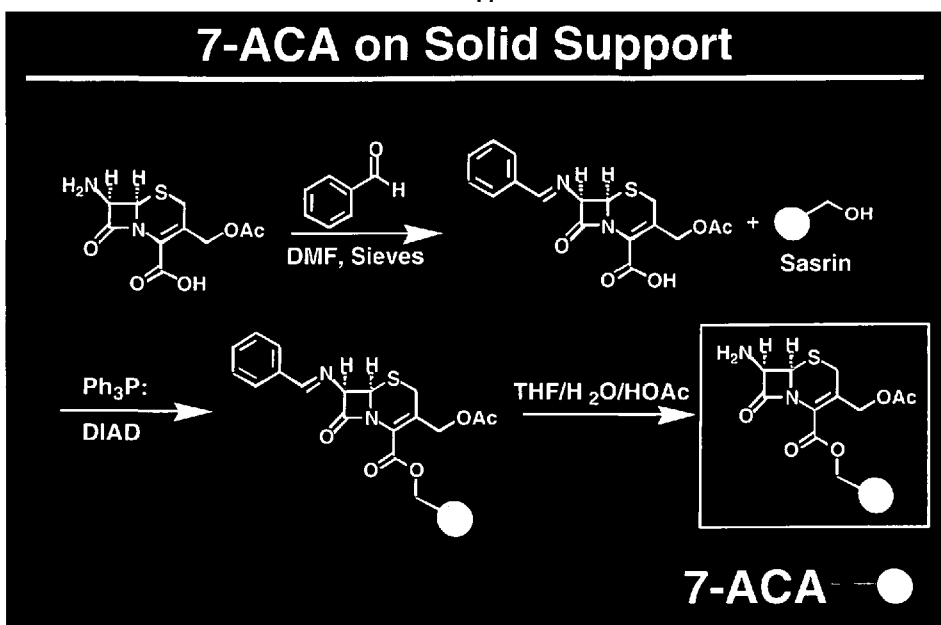A.Atuegbu, J. Jacobs, et al , Bioorganic Med. Chem. 4, 1097-1106 (1996)

novel cephalosporin derivatives with new activities (*Scheme 7*).

7-ACA is a relatively sensitive molecule and required the development of extremely mild chemistries to adapt it to solid-phase synthesis. To suppress the base and acid lability of this heterocycle, a mild imine-protecting group strategy was devised for N(7), and *Mitsunobu* conditions were utilized to esterify the β-lactam nucleus to a resin derivatized with a super-acid-sensitive cleavable linker (*Scheme 8*). Hydrolysis of the imine was performed with a mildly acidic aqueous/organic solvent mixture. The resulting resin-bound 7-ACA could then be acylated and cleaved from the solid support. This procedure successfully minimized isomerization of the olefin to the inactive delta-2 isomer, and afforded N-substituted cephalosporin derivatives in moderate to good yields [16].

4. Conclusions and Future Prospects

The combinatorial preparation of small-molecule libraries has been accomplished through the adaptation of synthetic organic chemistry to the solid-phase format. Successful implementation of a combinatorial library approach has required a highly interdisciplinary research environment-involving chemistry, biology, automated instrumentation, and informatics. The field of small-molecule combinatorial chemistry is but a few years old, and certainly much additional technology development will be required before these approaches are routinely and widely practised. Looking forward, we can expect that combinatorial chemistry will be integrated with the other main tools of drug discovery, including structure- and mechanism-based design, molecular modelling and computational chemistry to ultimately define a more powerful discovery paradigm. Finally and significantly, combinatorial technologies may ultimately find use in surmounting some of the typical hurdles encountered in drug development (*e.g.* cell penetration, metabolism, bioavailability, pharmacokinetics – *combinatorial drug development*).

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Scheme 7. *Natural Products in Combinatorial Chemistry*

Scheme 8. *7-ACA as an Intermediate on Solid Support*

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