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

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I. Introduction

The single greatest time constraint on the process of drug discovery has been the practice of synthesizing and evaluating candidate molecules one at a time. One vivid illustration of this approach was the global effort expended over a 30 year period in which thousands of β-lactam compounds were serially synthesized and screened for antibiotic activity.[1,2] During the 1970s, a more detailed appreciation of enzyme mechanism heralded the advent of rational strategies for drug design,[3−5] culminating in the creation of the antihypertensive drug captopril as one notable example.[6,7] The utilization of structural information derived from crystallographic and NMR studies of target biomolecules, together with modeling and computational methods, has further increased the sophistication of the rational design approach, and collectively these techniques have somewhat reduced the number of compounds needed to be made in order to find and optimize a drug lead.

Although dramatic technological advances in modern biology have uncovered many new biochemical pathways and targets for pharmaceutical intervention, the rate of discovery of new drugs has not increased commensurately. Recent revolutionary changes in the health-care sector have compounded the problem by increasing pressure to shorten drug discovery and development time lines. The pharmaceutical industry has reacted by urgently seeking new schemes for increasing productivity and for more quickly capitalizing on the steady stream of potential new drug targets (fueled, in part, by the fruits of the human genome project). Combinatorial chemistry (for historical background, see ref 8) has emerged as a long-term solution to the high costs of serial organic synthesis of drug leads. Around 1990, the idea was crystallizing in the minds of a number of researchers that if sound synthetic strategies could be devised, the necessary chemical tools might already exist to efficiently generate large collections of molecules for biological evaluation.[10−19] The newly-formed chemistry group at Affymax became excited by the power inherent in creating many small, nonpolymeric organic molecules simultaneously.[8,9] Strategies were defined so that this wealth of molecular diversity not only would contain the familiar pharmacophores of the past but also could lead to the pharmacophores of the future.[20]

II. Strategy in Combinatorial Synthesis

Comparison of the conventional approaches to drug discovery with emerging combinatorial methods reveals an apparent discontinuity in strategies and tactics which must be brought to bear. Though the principles underlying chemical reactions are of course invariant, the practice of combinatorial organic chemistry diverges markedly from serial compound synthesis. Whereas synthesis in medicinal chemistry conventionally serves the goal (usually) of producing a single product of previously specified structure for bioassay, the goal of combinatorial chemistry is to

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create searchable *populations* of molecules. Combinatorial chemistry is a type of synthetic strategy which leads to collections of differing molecules, i.e., "chemical libraries". The process proceeds by the systematic interconnection of a set, or sets, of chemical building blocks. This approach has clear parallels in Nature, where the polymerization of small monomeric units (amino acids, monosaccharides, nucleosides, etc.) produces the various classes of key biological macromolecules. Building blocks (BBs) are small, reactive molecules which may be intercombined with each other, or with members of other BB families (e.g., amines, carboxylic acids, aldehydes, etc.) in varied ways to eventually produce a combinatorial library containing many different products. Because the acquisition of BBs is a resource-intensive activity, these collections, once formed, should be fully leveraged by their integration into many varied synthetic schemes and into the production of numerous libraries.

In drug discovery, at least two major types of combinatorial libraries are apparent: (1) primary libraries for use in random screening, where *a priori* no specific structure or substructure is obligatory as a starting point; and (2) directed, biased, or focused libraries where the library is purposefully constructed around a known starting structure having biological activity (i.e., a lead compound), to be used in optimization of the activity or other properties of the lead molecule. Approaches based on a specific structure can often be assisted by a *retrocombinatorial analysis* of the parent compound, in which comparative evaluation of various retrosynthetic dissections is accompanied by an assessment of whether an adequate variety of the required building blocks are readily available, and whether chemistry in the forward direction is sufficiently reliable to drive library production. While primary and focused library approaches demand rather different strategies for library construction, both must permit the extraction of information made available by library screening, i.e., the ability to know or deduce the structure of active compounds. From the perspective of drug discovery, library members should usually conform to predetermined criteria, among which are chemical stability, conformational flexibility/inflexibility, molecular weight constraints <750, etc.

Combinatorial approaches often rely on the so-called "split-synthesis" method first introduced by Furka, in which compounds are generated by solid phase synthesis on polymeric resin beads, and each synthesis cycle potentially results in an exponential increase in library size.[21] With the exception of peptide or oligonucleotide synthesis, and some notable earlier work (for reviews, see refs 22 and 23), polymer-supported organic synthesis has not historically been an area of widespread interest. This necessitated, on our part, a period of experimentation during which we sought to develop solid phase adaptations of known solution phase syntheses. This process required examination of several factors, including determining which available solid supports met the demands of organic synthesis, splitting and pooling, and potential use in automated instruments (*vide infra*). Synthesis on a solid support greatly simplifies the problem of product isolation and, in contrast to solution phase synthesis, easily permits the use of large reaction component and reagent excesses to drive reactions to completion. We frequently observe that solid phase synthesis provides products in higher yield and purity than the corresponding chemistry in solution! Since multistep syntheses on solid supports are carried out without liberation of the intermediate products, the standard spectroscopic and analytical techniques of solution phase organic synthesis were, at least initially, inapplicable to our work. A considerable effort was made to apply NMR and mass spectroscopy to the characterization of resin-tethered small molecules, and these have become important tools for validating our solid phase chemistry (*vide infra*).

An analysis of typical lead structures pursued in medicinal chemistry clearly suggests that combinatorial syntheses of these molecules can be very short! For example, the interconnection of three to five building blocks of MW = 150 may require just two to four synthetic steps, or even fewer in cases of multicomponent condensation reactions. Since combinatorial chemical reactions must proceed reliably in the face of a diverse range of chemical functionality, an early emphasis was placed upon well-established synthetic chemistry which operates "generically" on a broad array of substrates. Many cycloaddition reactions appeared to be especially useful starting points, as structurally complex molecules could be simply built up in a few chemical steps from readily available BBs. We also wished to ensure that our investment in adapting specific chemistry for solid phase synthesis was maximized by avoiding "dead-end" resin-bound products. Rather, versatile intermediates were specifically identified that could be routinely used in multiple syntheses. 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 BBs or scaffolds (see, for example, the pyrrolidine case outlined below).

From our experiences, a recurring pattern has developed in defining a stepwise process for preparing combinatorial libraries. A combinatorial experiment is initiated by design of a library, followed by BB selection and acquisition. In the most time- and labor-intensive step, selected BBs are then "rehearsed" individually through reactions in the solid phase format, under conditions mimicking those that will ultimately be used in library synthesis. The rehearsal process builds confidence that the library will be faithfully produced as expected. Since it will often be impractical to examine every member of the finished library to certify its presence, BB combinations that are anticipated to represent worst case scenarios (e.g., with respect to steric and/or electronic factors) are studied and optimized, with problematic BBs being excluded from the library construction. The "auditioning" of BBs is monitored by "cleaving and characterizing" products from resin, as well as by using the newly developed solid phase spectroscopic techniques noted below. Split−pool library synthesis is then performed either manually or under automated

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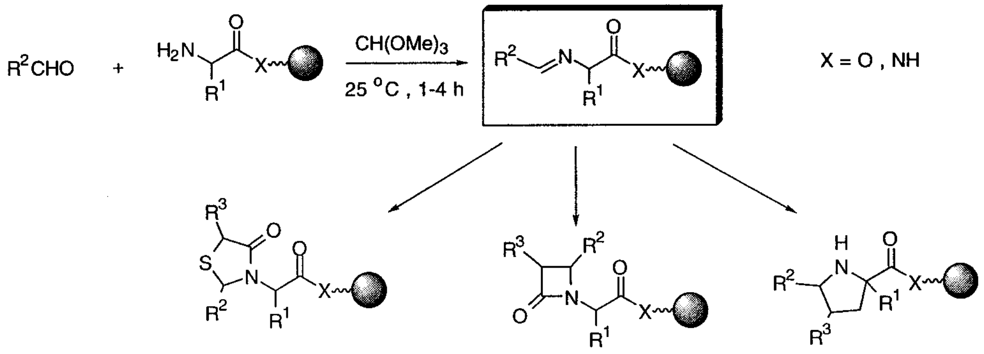

Figure 1. The imine as a versatile intermediate for solid phase synthesis.

instrumental control; the library is screened in an appropriate format (in solution or tethered to a support; see ref 9), and any “hits” are independently confirmed by separate synthesis. The interpretation of the resulting library screening data then drives the design of a new (secondary, tertiary, etc.) library, creating an iterative cycle of combinatorial library preparation and evaluation. Other strategic considerations are discussed by Gordon *et al.*[9]

To reduce to practice the creation of useful small-molecule libraries, we have sought to unify the needs of drug discovery chemistry with factors suggested by a strategic analysis of combinatorial synthesis. An important near-term goal in our program was to intersect historical medicinal chemistry in terms of generating 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.

III. Case Studies in Combinatorial Organic Synthesis

A. Approaches to Heterocyclic Diversity: 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. In evaluating the characteristics of chemical transformations that would be useful in combinatorial synthesis, a high degree of reaction generality and reliability became obvious requirements and those transformations allowing mild conditions were considered especially valuable.

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 solid phase synthetic approach wherein the amino group of an immobilized amino acid is a reactive center (see Figure 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.

Generation of resin-immobilized imines via the condensation of tethered amino acids with an excess of a soluble aldehyde component has precedent in the work of Sasaki and Coy, who studied the reductive alkylation of amino acids in the solid phase format.[24,25] It rapidly became clear from our early investigations that establishing optimal reaction conditions for imine formation would greatly benefit from the development of convenient approaches to nondestructively monitoring the progress of chemical transformations on resins. Previous workers had demonstrated that high-quality gel phase ^{13}C NMR spectra could be obtained on resin slurries, particularly poly(ethylene glycol)-grafted polystyrene, though prolonged spectral acquisition was typically required.[10,26] By incorporating selectively ^{13}C-enriched building blocks (i.e., [^{13}CHO]aldehydes or α-^{13}C-labeled amino acids) into the synthesis, we could easily obtain high-resolution spectra at the center of interest within several minutes of acquisition using a conventional NMR probe, without interference from unenriched peaks and solvent.[27] For example, Figure 2 shows the gel phase ^{13}C NMR spectrum of the ^{13}C-labeled benzylidene imine of glycine and its clean reduction to the secondary amine by sodium cyanoborohydride. Significantly, the imine resin had undergone multiple washings and filtrations in the course of this experiment, demonstrating that tethered aryl imines are stable species that can be readily manipulated.

Our group routinely makes use of fast ^{13}C gel phase NMR analysis in the course of optimizing solid phase reaction conditions. Although the commercial availability of labeled starting materials is somewhat limited, the judicious choice of ^{13}C-enriched building blocks (e.g., [2-^{13}C]valine, [^{13}CHO]anisaldehyde) permits both sterically or electronically disfavored reactions to be assessed. One recent exciting development in solid phase NMR analysis is the finding that high-resolution ^{1}H NMR spectra of resin-bound molecules

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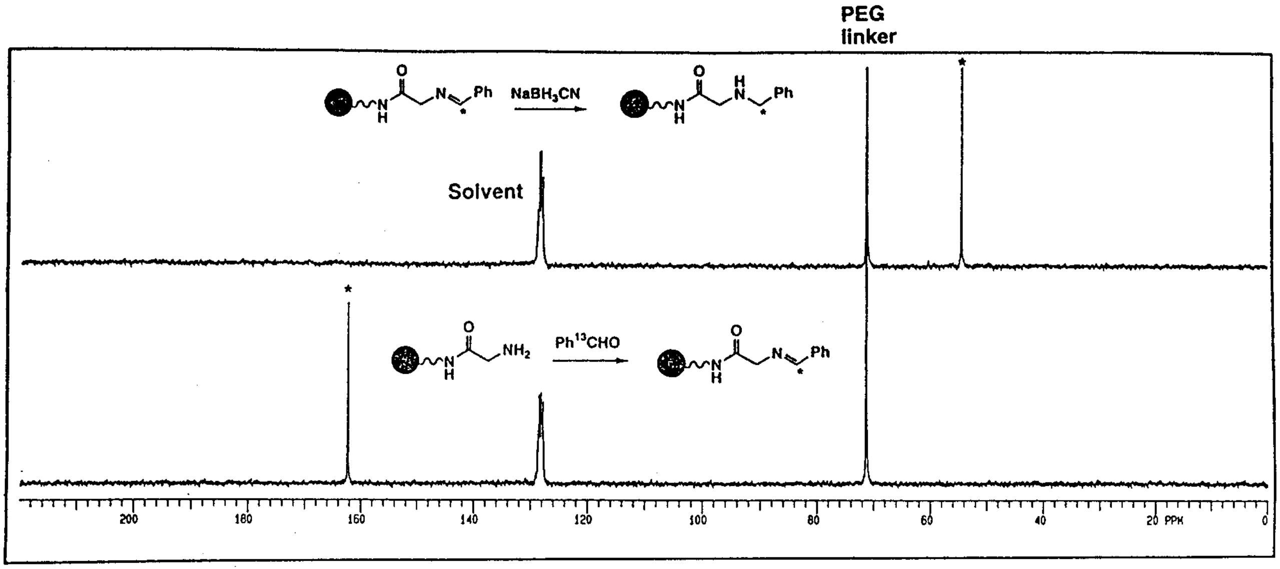

Figure 2. Fast ^{13}C gel phase NMR monitoring of a stepwise reductive amination sequence on TentaGel resin. Asterisk indicates ^{13}C-enriched center. Spectrum acquired in C_6D_6.

can be rapidly acquired on a conventional spectrometer using a Varian Nano-NMR probe, which was specifically designed to exploit magic-angle spinning (MAS) for very small (40 μL) liquid samples.[28] MAS removes line broadening caused both by chemical-shift anisotropy and bulk magnetic susceptibility discontinuities, and the quality of spectra attainable by this method is evidenced by Figure 3. These spectra, each obtained from several milligrams of TentaGel resin with suppression of the large poly(ethylene glycol) resonance at ~3.6 ppm, readily allows one to monitor the conversion of a photolabile hydroxy resin to its bromide counterpart (methine quartet at ~6.0 ppm) and the subsequent displacement of this benzylic bromide by phenethylamine (methine quartet at ~4.4 ppm).[29] MAS NMR spectroscopy clearly promises to be a valuable analytical method for the solid phase organic chemist.

Returning to the problem of generalizing reaction conditions for the formation of resin-tethered imines, investigation of the classical methods for Schiff base formation (e.g., azeotropic removal of water or reaction in the presence of molecular sieves) proved useful, although the requirements for elevated reaction temperatures or an additional solid component that would ultimately need to be removed from the resin were seen as liabilities. We have found trimethyl orthoformate (TMOF) to be a convenient solvent with the dehydrating power to drive imine formation on solid supports.[30] Condensations are typically performed at room temperature over 1–2 h using 10–20 equiv of soluble aldehyde, though most unhindered imines are formed in less than 10 min. Imines may also be formed by treating immobilized aldehydes with excess amine in solution, the reaction being promoted by weak acid.[31] Cosolvents (e.g., THF, MeCN) can also be used when the solubility of the reactant in neat TMOF is poor. In certain cases, subsequent transformations of the tethered imine intermediate can also be advantageously conducted in TMOF as solvent (*vide infra*).

1. Additions of Mercapto Acids to Imines: Solid Phase Synthesis of 4-Thiazolidinones. The condensation of a primary amine, an aldehyde, and a mercaptoacetic acid affords the 4-thiazolidinone nucleus in a reaction that proceeds via thiolate addition to an imine intermediate. The chemistry of thiazolidinones has been extensively investigated, and a variety of biological activities, frequently mediated through interaction with G-protein coupled receptors, have been described for this interesting family of heterocycles.[32] We have found that condensation of α-mercapto carboxylic acids with resin-bound arylidene imine derivatives of amino acids provides 4-thiazolidinone products in high yield and good purity after cleavage from solid supports equipped with either acid labile or photochemically labile linkers (Scheme 1).[33]

While additions to imines derived from α-amino acids with small substituents (e.g., glycine, alanine) proceed readily under ambient conditions, the reaction is sensitive to the steric bulk of the reactants, with condensations of β-branched amino acids (e.g., valine) requiring elevated temperatures (70 °C) to achieve complete conversion. Thiazolidinone formation is favored by the use of a high concentration of the mercapto acid in large molar excess with respect to imine, the product being easily separated from unreacted reagent by filtration and washing of the resin. It is also possible to run this reaction as a one-pot condensation in which the aldehyde and mercapto acid are heated together with the amino acid resin. This protocol is particularly useful with aliphatic or other enolizable aldehydes where the stability of the imine is limited by tautomerization to the corresponding enamine.

Thiazolidinone adducts derived from chiral amino acids are formed as diastereomeric mixtures, with moderate levels of stereoinduction being observed in the solid phase syntheses (typically, major:minor

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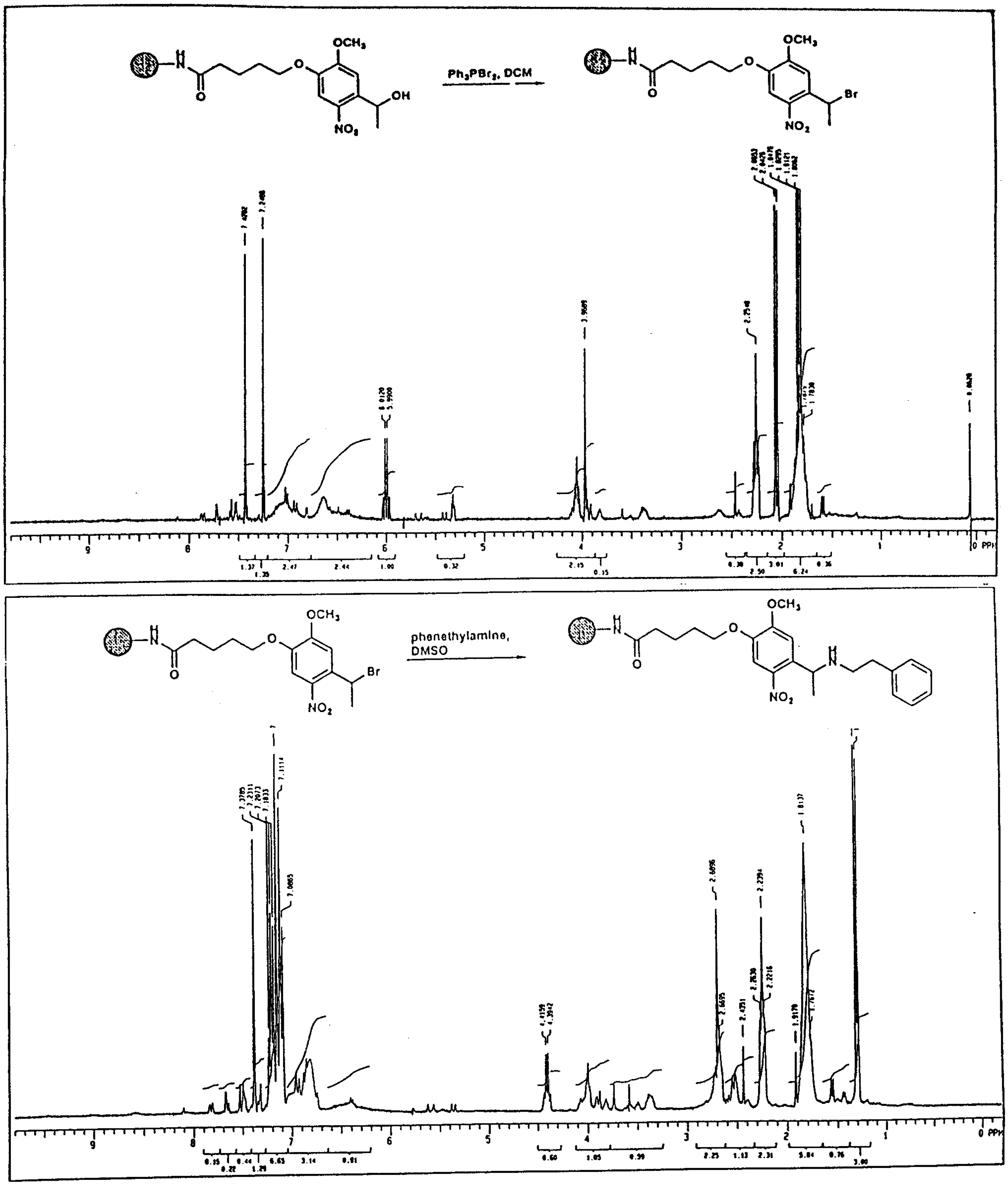
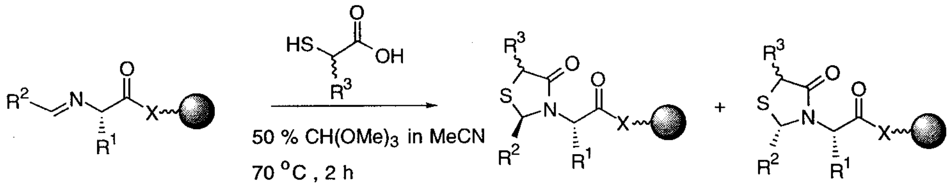


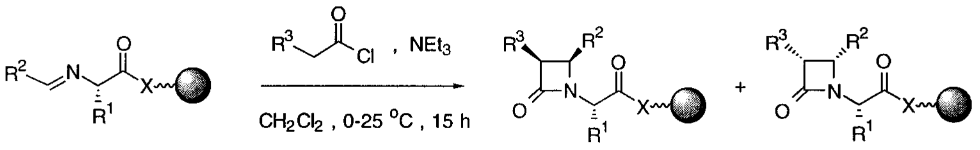
Figure 3. MAS ^{1}H NMR monitoring of the bromination and aminolysis of a photolabile linker group attached to TentaGel resin. Spectrum acquired in $CDCl_3$.

Scheme 1

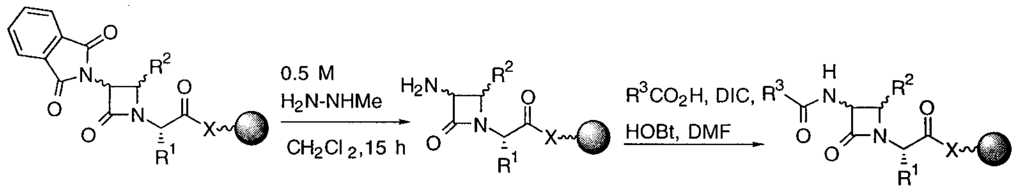


isomer ratios < 4:1). Racemic α-mercapto acids have typically been used in these studies, and thus two diastereomeric pairs of thiazolidinone products are obtained. Similar levels of induction are seen for the condensation performed in homogeneous solution, and it has been our general experience that the innate

Scheme 2



Scheme 3



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stereochemical preferences of most reactions are not significantly perturbed by tethering one of the reactants to a resin support.

This chemistry has been successfully used at Affymax to generate a number of combinatorial thiazolidinone libraries following the split-synthesis protocol. Moreover, using synthesis automation hardware designed and constructed by our engineering group, it has been possible to make thiazolidinone library production a particularly efficient process. Applications of these libraries in cases of both random and directed biological screening have provided a significant number of novel lead structures, including inhibitors of cyclooxygenase (COX-1) and antagonists of CCK and κ-opioid receptors.[34]

2. Cycloaddition Reactions of Resin-Bound Imines: Synthesis of β-Lactams. 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.[35] 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 versatile approaches to structurally diverse β-lactams.[36] 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 (Scheme 2).[37] Reaction of imines derived from aromatic or α,β-unsaturated aldehydes with achiral Bose–Evans or Sheehan ketenes[36] are typically highly cis-selective in the solid phase (as in solution), affording nearly equimolar mixtures of the α and β cis 3,4-disubstituted 2-azetidinones in very good overall yields. By contrast, the ketene derived from optically pure (4-phenyloxazolidyl)acetyl chloride[38] undergoes enantiospecific cycloaddition to a variety of resin-supported imines, yielding single cis β-lactams upon cleavage from resin. Interestingly, the quality of crude β-lactam products obtained by solid phase synthesis is frequently superior to that obtained under classical solution synthesis conditions. Likely this is largely due to the profound excess of ketene that can be used in the solid phase syntheses, with byproducts (e.g., dimerized ketene) being simply removed by washing of the resin.

3-Amino-2-azetidinones are valuable precursors to α-amido-β-lactams including many important β-lactam antibiotics. These compounds are readily accessible from cycloadditions with the ketene derived from phthalimidoacetyl chloride. Treatment of resin-bound α-phthalimido-β-lactam derivatives with methylhydrazine in CH_2Cl_2 affords the corresponding 3-amino-2-azetidinones. The chemical versatility of the 3-amino functionality promises to be useful for incorporating additional elements of diversity within the β-lactam scaffold. For example, acylation of **1** with a variety of amino acids using standard peptide coupling reagents proceeds smoothly to afford the desired 3-amido-2-azetidinones in excellent yield and purity upon cleavage from the resin (Scheme 3).

Combinatorial libraries of monocyclic β-lactam compounds have been generated from amino acids, aldehydes, and various ketene precursors following the split synthesis paradigm and are presently undergoing biological evaluation in a range of *in vitro* and cell-based bioassays. Representative members of one early library (see Figure 4), designed to contain 1760 different β-lactams, illustrate the types of functionality that have been appended to the 2-azetidinone scaffold.

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.[39] Azomethine ylides represent a synthetically versatile class of 1,3-dipolar species that react with olefin and acetylene dipolarophiles to afford pyrrolidine and pyrroline derivatives in typically excellent yields.[40] The pyrrolidine nucleus appears in numerous bioactive molecules of natural and synthetic origin, and the regio- and stereochemical predictability of the azomethine

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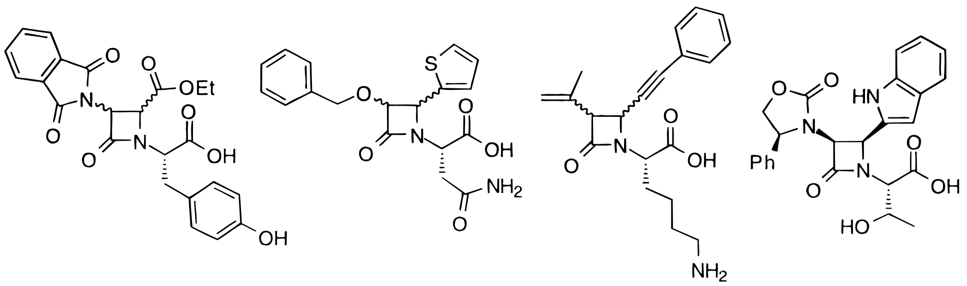
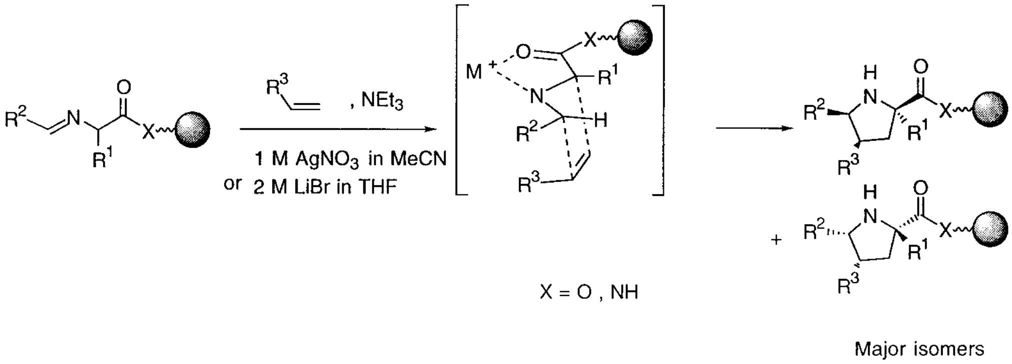


Figure 4. Representative members of a *β*-lactam library.

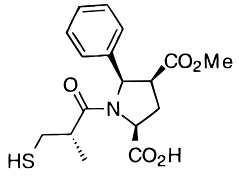
Scheme 4



ylide cycloaddition route has made it a popular approach within natural products and medicinal chemistry. From a combinatorial chemist's perspective, we were particularly attracted by the mild reaction conditions afforded by Lewis acid catalysis of this cycloaddition[41,42] and by the prospect of converting our solid-supported amino ester imines into collections of highly functionalized proline derivatives. As amino acids, prolines offer many opportunities for further synthetic elaboration by solid phase methods, and their unique structural characteristics are frequently exploited for conformational control in peptidomimetic design.

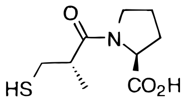
Our successful implementation of the 1,3-dipolar cycloaddition reaction of resin-bound azomethine ylides to electron-deficient olefins has recently been reported.[43] Treatment of imine resins with concentrated solutions of the dipolarophile in the presence of NEt$_3$ (or other weak base) and AgNO$_3$ (in MeCN) or LiBr (in THF) as catalyst provide pyrrolidine products in good yield and purity (see Scheme 4). Regio- and stereochemical control in the solid phase reactions again track that seen for their solution phase counterparts, although the concentration of catalyst employed can signficantly impact the product isomer distribution. The relative stereochemistry seen for the 4,5 and 2-carboxyalkyl substituents in the major isomers (all syn) is consistent with the reactions proceeding via endo olefin addition to the *E*,*E*- (i.e., *W*-) configuration of the tethered N-metalated azomethine ylide intermediates.[42] It should be noted that the proline products formed in this reaction are racemic mixtures since the original stereochemistry at the α-carbon in the amino acid component is scrambled in the 1,3-dipole intermediate.

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 relationships around an existing bioactive lead compound.[6,7] The split-synthesis protocol was used to prepare a library of pyrrolidines that were acylated with a set of mercapto acid chlorides (see Figure 5), producing a collection of analogs of the angiotensin converting enzyme (ACE) inhibitor, captopril. After cleavage from the resin and deprotection of the mercapto functionality, this ~500 compound library was screened for *in vitro* inhibition of ACE as soluble compound pools through four iterations of assay and sublibrary resynthesis (i.e., deconvolution). At each step of this analysis, the building block affording the most inhibitory pool was selected for the subsequent sublibrary resynthesis. Upon chromatographic separation and bioassay of the two anticipated diastereomeric mercaptoacyl proline adducts from the final step of the deconvolution, compound **2** was identified as an unusually potent inhibitor of ACE (K_i = 160 pM; cf. captopril K_i = 500 pM). Perhaps the most remarkable feature of this experiment was that, upon development of the solid phase synthetic methodology, the process of manually synthesizing and assaying this library and confirming the structure of the most active inhibitor was completed by two talented individuals (a chemist and a biochemist) within 2 months.



(2)

K$_i$ (ACE) ~ 160 pM



Captopril

K$_i$ (ACE) ~ 500 pM

4. Dihydropyridines and Pyridines. The condensation of amines and carbonyls to produce resin-

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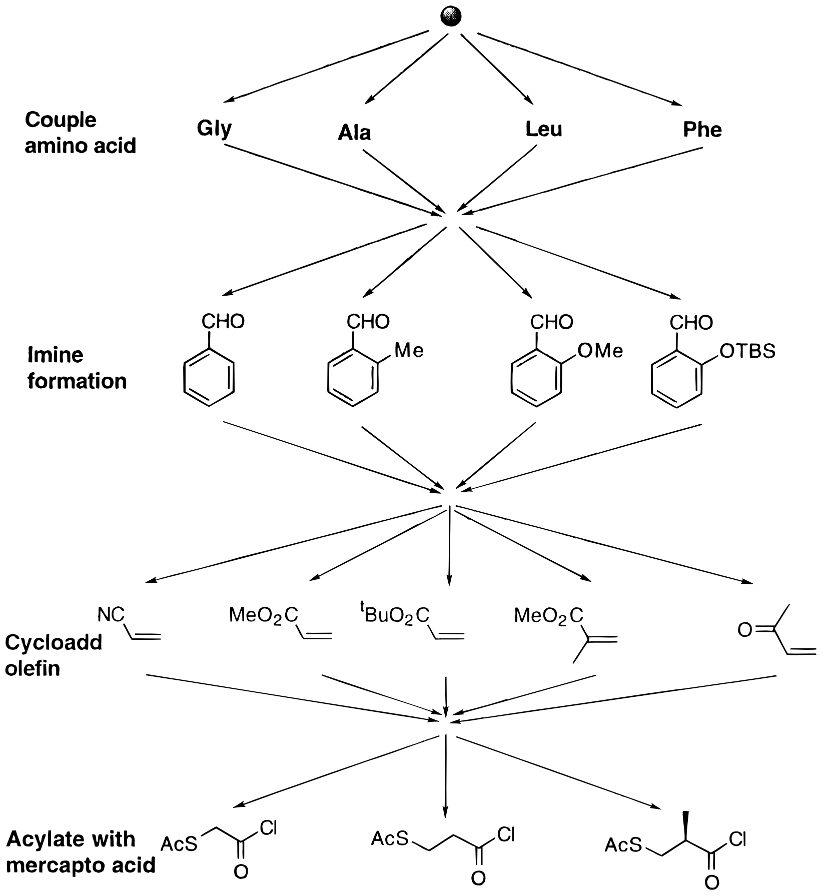
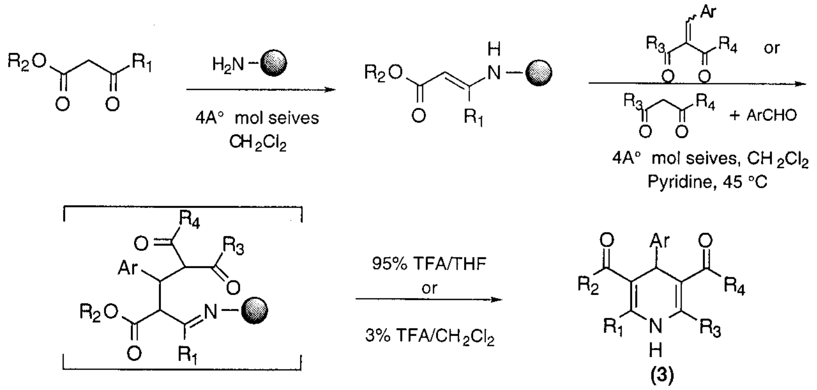


Figure 5. Split–pool synthesis of an *N*-mercaptoacyl pyrrolidine library.

Scheme 5



bound imines can be modified to lead to other heterocyclic systems. The dihydropyridine (DHP) nucleus is common to numerous bioactive compounds which include various vasodilator, antihypertensive, bronchodilator, antiatherosclerotic, hepatoprotective, antitumor, antimutagenic, geroprotective, and antidiabetic agents.[44] DHPs have found commercial utility as calcium channel blockers, as exemplified by therapeutic agents such as Nifedipine (**3a**), Nitrendipine (**3b**), and Nimodipine (**3c**).

We recently developed a general method for solid phase synthesis of 1,4-dihydropyridines, based on a two- or three-component cyclocondensation of enamino esters with 2-arylidene β-keto esters, or β-keto esters and aldehydes, respectively.[45] The general procedure typically consists of (i) preparation of the immobilized N-tethered enamino component, (ii) condensation of the latter with a preformed 2-benzylidene β-keto ester or a mixture of β-keto ester and aldehyde, and (iii) TFA cleavage to afford the desired DHP **3** (Scheme 5, Table 1). Our synthetic protocol was developed using model studies directed at solid phase synthesis of the calcium channel blocker drug Nifedipine (**3a**). Thus, methyl acetoacetate was reacted with polystyrene-based acid-cleavable PAL or Rink amine resins to afford methyl aminocrotonate immobilized on a solid support (R_1 = R_2 = Me). Next, the N-tethered enamino ester (R_1 = R_2 = Me) was reacted with preformed methyl 2-(2-nitrobenzylidene)acetoacetic

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(44) (a) Godfraind, T.; Miller, R.; Wibo, M. *Pharmacol. Rev.* **1986**, *38*, 321. (b) Janis, R. A.; Silver, P. J.; Triggle, D. J. *Adv. Drug Res.* **1987**, *16*, 309. (c) Sausins, A.; Duburs, G. *Heterocycles* **1988**, *27*, 269. (d) Mager, P. P.; Coburn, R. A.; Solo, A. J.; Triggle, D. J.; Rothe, H. *Drug Des. Discovery* **1992**, *8*, 273. (e) Mannhold, R.; Jablonka, B.; Voigdt, W.; Schoenafinger, K.; Schraven, E. *J. Med. Chem.* **1992**, *27*, 229.

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Table 1. 1,4-Dihydropyridine Derivatives 3

compd no.	derivative					yield (%)[a]
	Ar	R_1	R_2	R_3	R_4	
3a[b]	*o*-$NO_2C_6H_4$	Me	Me	Me	OMe	65[c] (70)[d]
3b[e]	*m*-$NO_2C_6H_4$	Me	Me	Me	OEt	75[d]
3c[f]	*m*-$NO_2C_6H_4$	Me	^{i}Pr	Me	$O(CH_2)_2OMe$	78[d]
3d	*p*-$NO_2C_6H_4$	Me	Me	Me	Me	75[d]
3e[g]	*p*-$NO_2C_6H_4$	$^{13}CH_3$	Et	Me	OMe	70[c]
3f	*p*-$NO_2C_6H_4$	Et	Me	Ph	OEt	70[d]
3g	*p*-NCC_6H_4	Me	Me	Me	OMe	74[d]
3h	Ph	Me	Me	Me	OAll	72[h]
3i	4-Py	Me	Me	Me	OMe	75[d]

[a] Yields are based on loading of the starting resin. [b] Nifedipine. [c] Prepared by two-component condensation with $ArCH{=}C(COR_3)CO_2R_4$ using PAL resin. [d] Prepared by three-component condensation using [e] Rink resin. [f] Nitrendipine. [g] Nimodipine. [h] Contains ^{13}C C-3 labeling. [i] Prepared by two-component condensation with $PhCH{=}C(COMe)CO_2All$ using Rink resin.

ester, or directly with 2-nitrobenzaldehyde and methyl acetoacetate in pyridine, followed by cleavage of the final product **3a** from resin with trifluoroacetic acid.

Following the synthesis of Nifedipine (**3a**), a set of related compounds **3b**–**i** were prepared using an analogous synthetic protocol (Table 1). Evidently, the solid-phase synthesis appears to tolerate variations of both ester (substituents R_1, R_2, R_3, R_4) and aromatic (or heteroaromatic) groups in the β-keto esters or aldehydes, respectively. β-Diketones can also be successfully employed at the second step of these Hantzsch-type reactions on solid supports, as exemplified by the synthesis of compound **3d** from the Rink resin N-tethered methyl aminocrotonate ($R_1 = R_2 =$ Me) and acetylacetone (Table 1). The presence of pyridine, which probably facilitates isomerization of intermediate imines into the thermodynamically more stable conjugated enamines, is essential for successful transformation of immobilized enamino esters into DHPs **3**. Final cleavage and isolation of products is typically performed under an inert atmosphere to avoid the conversion of readily oxidizable DHPs **3** into the corresponding pyridines.

Our route to 1,4-dihydropyridines is based on nonamide heterocyclic chemistry and does not require the use of amino acid building blocks. Significantly, this synthesis enables preparation of diverse 1,4-dihydropyridine derivatives **3** which encompass various compounds reported in connection with structure–activity relationship studies of calcium channel blockers.[44] Additionally, this method is well suited for use in combinatorial "split–pool" protocols and has been employed for the preparation of dihydropyridine libraries.[46] In addition, the solid-supported functionalized enamines of Scheme 5 are also convenient precursors for combinatorial syntheses of other bioactive heterocyclic molecules.[46]

B. Approaches to Protease Inhibitor Libraries: Peptidyl Phosphonates. Combinatorial libraries composed of known classes of enzyme inhibitors may prove to be important tools in rapidly profiling novel protease drug targets and for determining which pharmacophores are most effective at their inhibition. Using information derived from generic primary libraries, secondary libraries may probe specific subsite specificities and rapidly point the way to finding high-affinity and selective drug leads. In addition to the imine-derived mercaptoacyl prolines noted above, we have undertaken the syntheses of phosphonyl acid-based pharmacophore libraries to facilitate the discovery of novel metalloprotease inhibitors of medicinal interest.

Phosphinyl (onyl) acids are competent transition state analogs (tetrahedral geometry) and metal chelators and have found use as inhibitors of metalloproteases such as thermolysin and angiotensin converting enzyme.[47,48] An early appreciation that existing solution methods were not robust enough to meet the diverse structural and electronic requirements of a combinatorial synthesis prompted Campbell to develop a general and efficient synthesis of phosphonate esters utilizing a Mitsunobu-type coupling between phosphonic acids and alcohols.[49] This study employed a stoichiometric excess of alcohol and Mitsunobu reagents compared to the phosphonic acid. Further refinements enabled phosphorus–oxygen bond formation under alcohol limiting conditions, a situation encountered during library synthesis where the reactive hydroxyl is attached to the solid support. This was achieved by performing the coupling with an equimolar excess of phosphonic acid, DIAD, and tris(4-chlorophenyl)phosphine, a more electron deficient phosphine than triphenylphosphine, and by employing an exogenous base such as triethylamine as a general base catalyst (Figure 6). This method is versatile and yields satisfactory results even with very hindered coupling partners.[50] The latter conditions were effectively utilized for solid phase peptidylphosphonate synthesis (SPPPS), as demonstrated by preparation of several trimeric peptidylphosphonate inhibitors ($\text{Cbz-X}^{p}\text{-}{}^{o}\text{Y-Z-OH}$) of thermolysin.[51] While the remainder of the chemistry was straightforward and was based on the familiar Fmoc/*t*-Bu protecting group strategy, an important observation made during the course of this study was that the betaine formed during Mitsunobu coupling was basic enough to cause β-elimination of the Fmoc group. This necessitated use of the more stable (4-nitrophenethyl)oxycarbonyl (NPEOC) group in the protection of the α-amino phosphonic acid building blocks. This chemistry has now been successfully extended to construction of a transition state analog inhibitor combinatorial library of trimer peptidyl phosphonates.[52] The split–pool method was used to prepare a 570-member $\text{Cbz-X}_{(6)}{}^{p}\text{-}{}^{o}\text{Y}_{(5)}\text{-Z}_{(18)}\text{-NH-resin}$ library on a noncleavable solid support (Figure 7). The library consisted of six pools of 90 members, with each pool bearing a discrete Cbz-X^{p} residue. It was assayed for thermolysin inhibition by rank ordering of pools of compounds in an iterative fashion employing a depletion assay. The rank ordering indicated the following preference for X^{p} (P_1): $\text{Phe}^{p} > \text{Leu}^{p} > \text{Ile}^{p}, \text{Ala}^{p} > \text{Val}^{p} > \text{Gly}^{p}$, a result that is in agreement with the literature finding.[47] In the next step of deconvolution, the best P_1 residue (Cbz-Phe)

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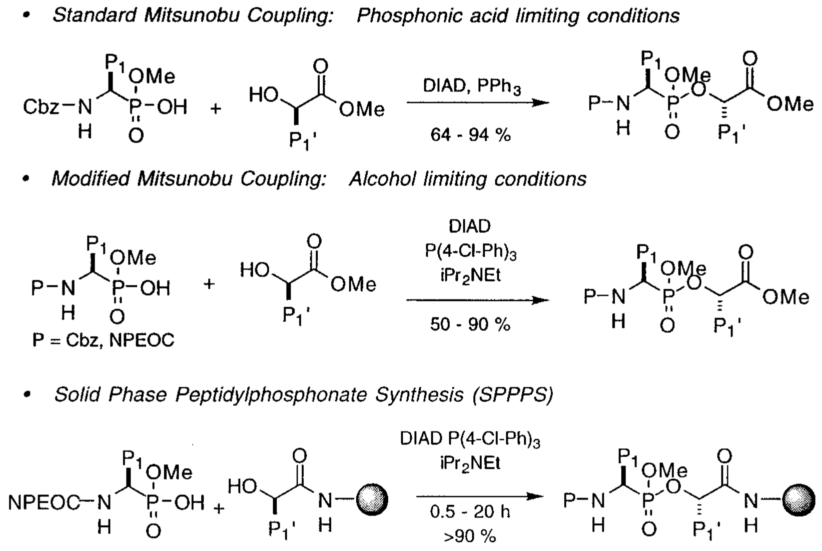


Figure 6. Synthetic methodology for the preparation of peptidylphosphonate libraries.

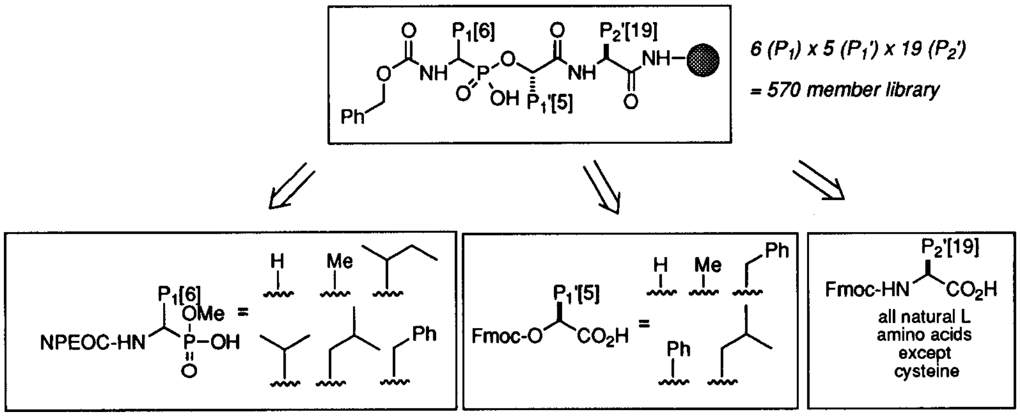
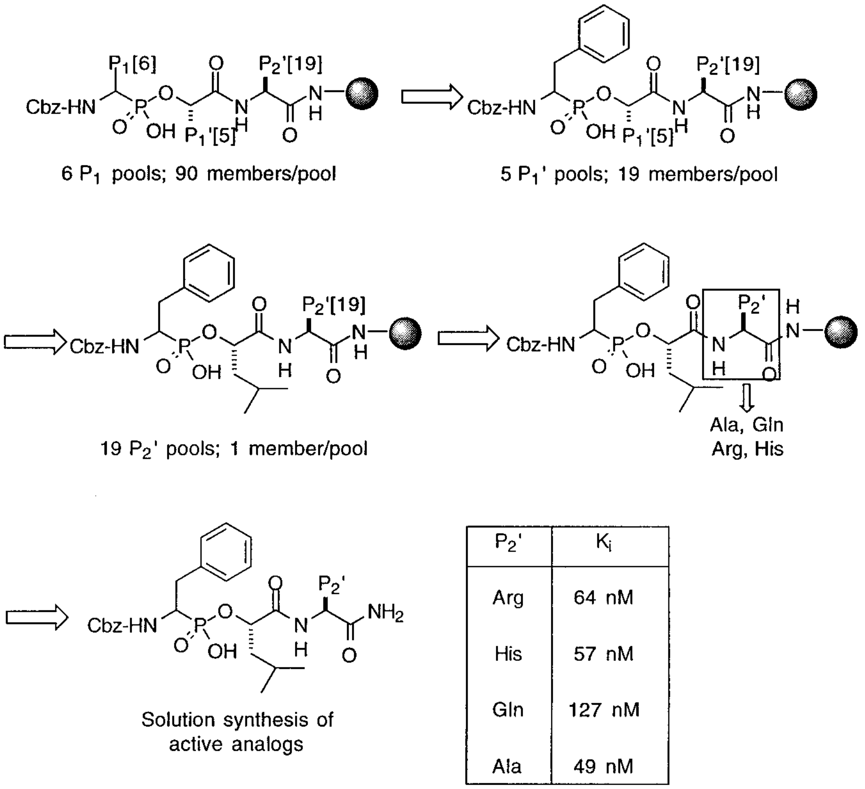


Figure 7. Design and construction of a solid phase peptidylphosphonate library.

was held constant and a Cbz-Phe^{P}-^{o}Y$_{(5)}$-Z$_{(18)}$-NH-resin sublibrary was assayed, now as five pools with a distinct ^{o}Y component, and with 18 members in each pool. This led to identification of the leucine analog as the optimal ^{o}Y(P$_1'$) component, a result that is once again consistent with the literature data. The final Z(P$_2'$) deconvolution involved 18 individual Cbz-Phe^{P}-^{o}Leu-Z$_{(18)}$-NH-resin peptidylphosphonates. Besides identifying the most potent peptidylphosphonate reported in the literature [Z(P$_2'$) = Ala], a number of additional active sequences composed of basic [Z(P$_2'$) = arginine and histidine] and neutral H-bonding [Z(P$_2'$) = glutamine] residues were also uncovered (Figure 8). These results were verified by solution synthesis and *in vitro* thermolysin I_{50} determinations of these inhibitors and were found to be consistent with the solid phase depletion assay data.

Conclusions and Future Prospects

The preparation of small-molecule libraries at Affymax Research Institute has been accomplished through the adaptation of synthetic organic chemistry to the solid phase format, guided by a strategic analysis of the principles underlying combinatorial drug discovery, and with integration of new technology tools as appropriate. Successful implementation of a combinatorial library approach has required a highly interdisciplinary research environment involving chemistry, biology, engineering, instrumentation, optics, informatics, and other themes. 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 practiced. Looking forward, we can expect that solid phase organic chemistry will become increasingly more sophisticated, offering greater synthetic flexibility and expanding the scope of structures accessible through combinatorial synthesis. Adaptation of solid phase combinatorial chemistries to run under automated instrumental control will soon become routine, and the historically distant relationship between instrumental engineering/automation and organic synthesis will narrow with increased demands for hardware to automate more complex synthetic schemes. New screening technologies emphasizing assay minaturization will be developed to capitalize on the ready availability of screenable molecules and to cope with higher throughput. Combinatorial chemistry will be integrated with the other main tools of drug discovery, including structure and mechanism based design, molecular modeling, and computational chemistry to ultimately define a more powerful discovery paradigm. The huge numbers of molecules which will be made and screened will demand a new generation of database management tools. The most effective data analysis strategies will make use of information about both active and inactive molecules, to help construct hypothetical active sites/



P2'	Ki
Arg	64 nM
His	57 nM
Gln	127 nM
Ala	49 nM

Figure 8. Screening and deconvolution of a solid phase peptidylphosphonate library against thermolysin.

binding sites of potential drug targets. Analysis of library screening results in turn will drive the next round of library synthesis. 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*).

Though some chemists may see combinatorial approaches as “replacing them”, in actuality the growth of combinatorial chemistry marks a new level of empowerment for the chemist. Large numbers of molecules which in the recent past might have taken a year or a career to synthesize may now be prepared in a single experiment. The availability of such numbers of molecules and their ability to be screened for particular criteria allows researchers to expand the scope of questions they may ask and to direct efforts to more demanding tasks. The answers to these questions will certainly form the basis for new understandings of molecular behavior.

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