Synthesis and Applications of Small Molecule Libraries

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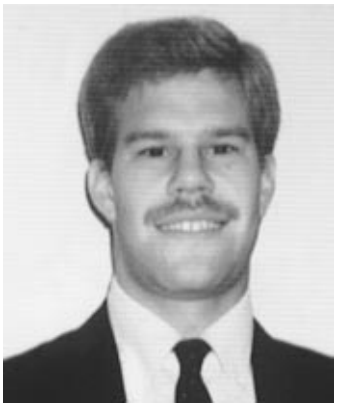

Lorin A. Thompson was born in Lexington, KY, in 1970. He received the Bachelor of Science degree from the University of North Carolina, Chapel Hill, in 1992 where he worked under the guidance of Joseph Desimone. He is currently pursuing his doctorate in the laboratory of Jonathan Ellman at UC Berkeley where he is the 1994 Glaxo-Wellcome fellow. His research interests include the development of synthetic methodology for organic library construction.

Jonathan Ellman was born in California in 1962. He received his S.B. degree from M.I.T. in 1984 where he worked in the laboratory of K. Barry Sharpless. He received his Ph.D. degree with David A. Evans at Harvard University in 1989, where he worked on the synthesis of enantiomerically pure nonproteinogenic amino acids, cyclopeptide alkaloids, and vancomycin. After an NSF postdoctoral fellowship with Peter G. Schultz at the University of California at Berkeley on the incorporation of unnatural amino acids into proteins, he joined the faculty at the University of California at Berkeley in 1992 as an assistant professor. His laboratory is engaged in the development of new chemistry for the synthesis of organic compound libraries, and the application of organic compound libraries to different research problems in chemistry and biology.

I. Introduction

One of the initial steps in the development of therapeutic agents is the identification of lead compounds that bind to the receptor or enzyme target of interest. Many analogs of these lead compounds are then synthesized to define the key recognition elements for maximal activity. In general, many compounds must be evaluated in both the lead identification and optimization steps. Increasing burdens have been placed on these efforts due to the large number of new therapeutic targets that continue to be identified thorough modern molecular biology methods.[1]

To address this demand, very powerful chemical and biological methods have been developed for the generation of large combinatorial libraries of peptides[2] and oligonucleotides[3] that are then screened against a receptor or enzyme to identify high-affinity ligands or potent inhibitors, respectively. While these studies have clearly demonstrated the power of library synthesis and screening strategies, peptides and oligonucleotides generally have poor oral activities and rapid *in vivo* clearance;[4] therefore their utility as bioavailable therapeutic agents is often limited. Due to the favorable pharmacokinetic properties of many small organic molecules (<600−700 molecular weight),[5] the design, synthesis, and evaluation of libraries of these compounds[6] has rapidly become a major frontier in organic chemistry.

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In addition to the development of therapeutic agents, many other applications of organic compound libraries are currently being pursued. These include important advances in molecular recognition, as well as recent efforts in materials and catalysis.

This review focuses on efforts toward the synthesis and evaluation of small organic molecule libraries. Peptide and oligonucleotide libraries will not be described, since a number of very thorough articles have recently reviewed this subject.[2,3] In addition, greater emphasis will be placed upon library synthesis methods than upon assay results because this *Chemical Reviews* issue is devoted to frontiers in organic synthesis.

II. Libraries Synthesized on a Solid Support

The majority of the compound libraries that have been synthesized to date have been synthesized on a solid support (a solid support is a insoluble material to which compounds are covalently attached during a synthesis sequence). There are two advantages to solid-phase synthesis strategies. First, isolation of support-bound reaction products is accomplished simply by washing away reagents from the support-bound material, and therefore reactions can be driven to completion by the use of excess reagents. Second, innovative methods are available for the manipulation of discrete compounds and for "tracking" the identity of compounds when compounds are attached to a solid support.

A number of general strategies have been developed for the synthesis and evaluation of compound libraries synthesized on solid supports. Although most of these strategies were initially demonstrated with peptide libraries, many of these approaches have now been applied to other compound classes. Many of these strategies have been reviewed recently,[2] and therefore only an overview of these methods will be provided with an emphasis on recent advances.

A. Library Synthesis and Evaluation Strategies

1. Discrete Compounds

a. Spatially Separate Synthesis. Conceptually, the most straightforward method for the preparation of a compound library is to synthesize many compounds in parallel and to keep each compound in a separate reaction vessel. When the final compounds are spatially separate, the identity of a compound at a particular location is known and can be confirmed by analytical methods. In addition, biological evaluation of the library can provide specific information about each compound in the library. Of course, the compounds can still be pooled (a pool refers to a mixture of compounds) when the assay of interest is not sufficiently high-throughput. Recently reported pooling strategies may be particularly useful for this purpose.[7]

A number of approaches for the parallel synthesis of organic compounds have been reported.[8] The first method was originally developed by Geysen for peptide epitope mapping.[9] Since that time, several improvements have been introduced.[10] In this method,

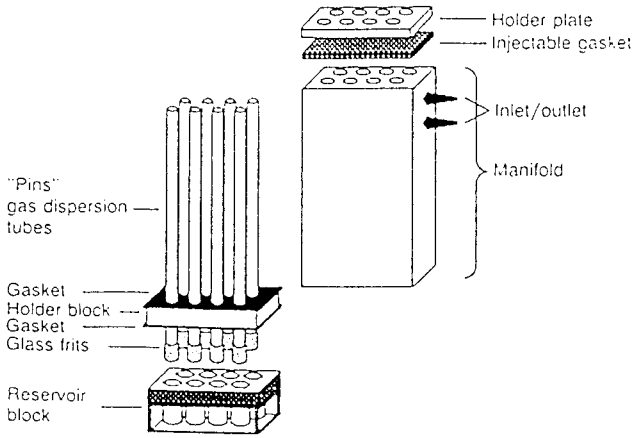


Figure 1. Diversomer™ apparatus. (Reprinted from ref 12a. Copyright the National Academy of Sciences of the United States of America.)

96 polyethylene pins are placed into a supporting block so that each pin fits into a separate well of a 96-well microtiter plate. Each pin is coated with a polymeric material that is amenable to solid-phase synthesis such as poly *N*,*N*-dimethylacrylamide. The polymeric material is derivatized with aminoalkyl groups to provide sites for substrate attachment. During a synthesis sequence each pin is placed in a separate well of the microtiter plate so that each well serves as a distinct reaction vessel. With this approach, on the order of 10 000 spatially separate compounds have been prepared[11] in parallel by employing inexpensive labware, instrumentation, and automation developed predominantly for high-throughput microtiter-based screening efforts. Currently, pin loading levels range from 100 nmol to 50 μmol of material per pin. Even 100 nmol of material is sufficient for multiple biological assays, as well as for analytical evaluation of the purity and chemical integrity of the individual compounds.

DeWitt and co-workers have reported the Diversomer™ apparatus, which is one of the first reaction apparatus designed expressly for the parallel synthesis of small organic molecules (Figure 1).[12] This apparatus is based on the use of porous gas dispersion tubes that serve as containers for resin beads. Reagents and solvents are placed in up to 40 vials that are located in a reservoir block, and the ends of the gas dispersion tubes containing resin are placed into the vials allowing the reagents to diffuse into the tubes and contact the support. The temperature of the reaction solutions can be controlled by heating and cooling the block. The apparatus is also enclosed in a manifold with an injectable gasket employed for reagent and solvent additions so that reactions can be maintained under an inert atmosphere.

Meyers and co-workers have recently reported a conceptually related approach (Figure 2).[13] The reaction apparatus is prepared by drilling a hole into each well of a deep-well polypropylene microtiter plate. Porous frits are then placed at the bottom of each well. The solid support and reaction solutions are placed into the wells of the apparatus. During a reaction step, the plate is clamped against a Viton gasket in order to seal the hole at the bottom of the well. In between steps in the synthesis sequence, the reaction solution can be drained and the resin can

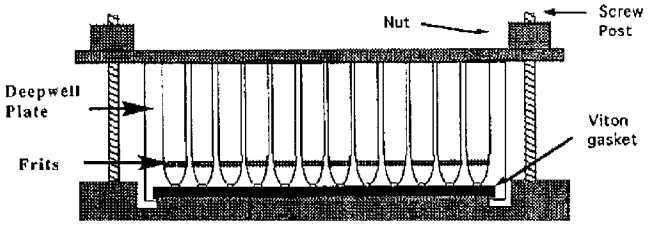


Figure 2. Multiple synthesis apparatus of Meyers and co-workers. (Reprinted from ref 13. Copyright ESCOM Scientific publishers.)

then be rinsed by removal of the Viton gasket.

The reaction apparatus employed in the three strategies described above are relatively inexpensive. This can be an important consideration, since the parallel synthesis of hundreds to thousands of spatially separate compounds will generally require that multiple reaction apparatus be used simultaneously. Clearly, when compounds are synthesized in pools, a single parallel synthesis apparatus is usually sufficient for the synthesis of large numbers of compounds (*vide infra*).

Commercial apparatus for parallel organic synthesis have also recently been developed. As one example, Advanced Chemtech has developed the model 496 multiple organic synthesizer instrument for automated chemical synthesis.[14] The instrument is designed to produce 96 different compounds. A range of temperatures, mix times, and speeds can be employed. The instrument is also compatible with a wide range of reaction conditions. Finally, reactions can be performed under an inert atmosphere.

b. Light-Directed, Spatially Addressable Parallel Chemical Synthesis. While libraries of greater than 10 000 compounds based upon a single structural type have been prepared by parallel synthesis using separate reaction vessels, the synthesis of libraries of hundreds of thousands to millions of compounds per structural type would not be practical by this approach. Fodor and co-workers have developed a strategy based upon photolithographic methods that can be used to synthesize libraries containing more than 100 000 spatially separate compounds.[15] In this method a silica wafer (borosilicate glass microscope slide) serves as the solid support (Figure 3). Aminoalkyl groups or other reactive functionalities that are attached to the surface of the wafer are blocked with photolabile protecting groups. The photolabile protecting groups are cleaved at specific regions on the silica wafer by site-specific illumination using masks and instrumentation initially developed for computer microchip construction. The silica wafer is then exposed to a reaction solution with reactions occurring only at the regions that were deprotected by illumination. Only a single photolabile group is necessary for the preparation of oligomeric compounds, since a monomer that is protected with the same photolabile group is introduced at each step in oligomer synthesis. However, orthogonal photolabile protecting groups that are cleaved at different wavelengths of light would likely be required in order to differentiate between different sites in nonoligomeric structures. The structure of a final compound at a specific location on the silica wafer is dependent upon the masking scheme and

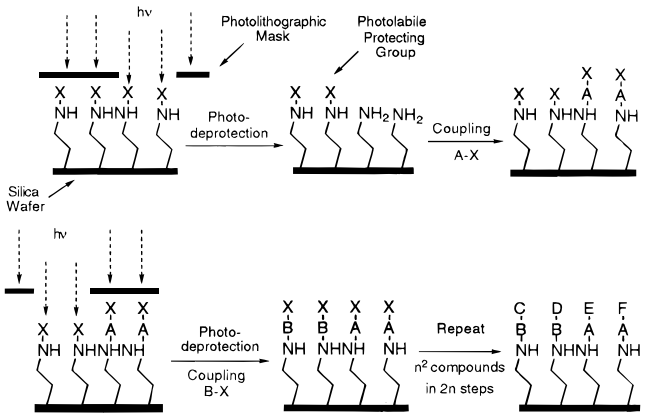


Figure 3. Light-directed, spatially addressable chemical synthesis.

upon the order of addition of the reagents. Large numbers of spatially addressable compounds can be prepared due to the exceptional spatial resolution of photolithography techniques. For example, Fodor and co-workers have assembled peptide libraries with each peptide localized to a 50 μm square.[15e] This density allows the synthesis of 40 000 oligomers per centimeter squared, and the current limits of photolithography should allow synthesis to be performed at much higher densities.[16]

In this spatially addressable array, the location of the compound on the silica wafer provides the structure of the compound. The compounds in a library therefore must be assayed while still tethered to the wafer. Although this requirement imposes constraints on the range of feasible biological assays, successful strategies have been developed for this purpose. One strategy is to measure the percent binding of a soluble fluorescently labeled receptor to different locations on the silica wafer as monitored by epifluorescence microscopy. The silica support or the linker that serves to attach the compound to the support can, however, have a pronounced effect on the binding affinity.[17,15a]

2. Split Synthesis

In general, large libraries of compounds are synthesized by employing pooling strategies. The most direct method is to employ equimolar mixtures of reactants in each synthesis step. In fact, Geysen employed this strategy in one of the early peptide library synthesis efforts.[18] One problem with this approach is that equimolar quantities of the final compounds in the library will only be obtained if all of the reactants in the mixture have comparable reactivity, and for most reaction classes, reactivity is highly dependent upon the structure of the reactants. For the purpose of synthesizing peptide libraries, several researchers have demonstrated that modest differences in reactivity can be corrected by adjusting the relative concentrations of the activated amino acids in the mixture.[19] It is also possible to synthesize approximately equimolar mixtures of products by using a total of 1 equiv of a mixture of reagents in a coupling step.[20] This strategy, however, requires that the concentration of each reagent in the mixture is fairly low and therefore is restricted to reactions that are very efficient.

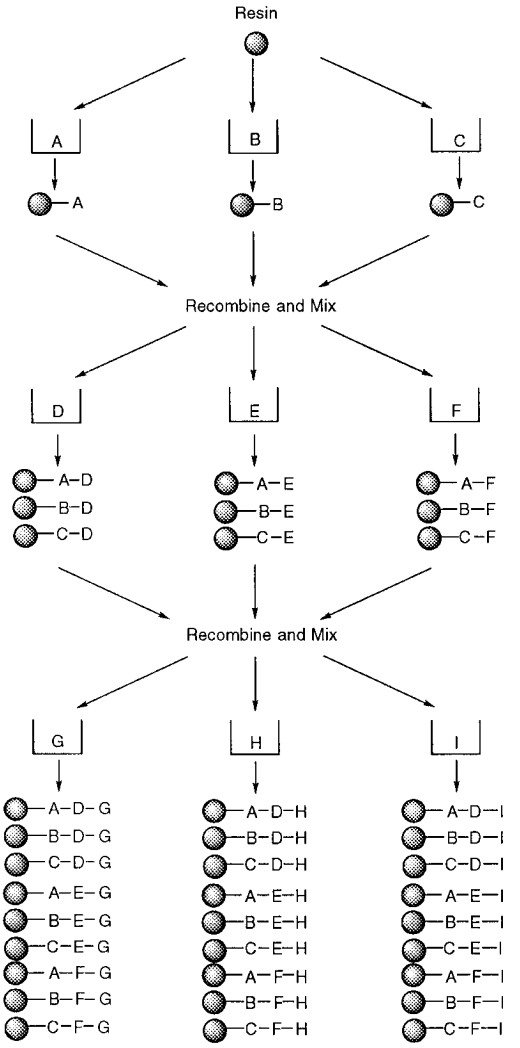


Figure 4. Split synthesis method.

Furka developed the "split synthesis" strategy for the synthesis of libraries of equimolar mixtures of compounds in order to overcome the complications that result from using mixtures of reagents that have different reactivities.[21] This strategy is currently the most popular method for the synthesis of compound mixtures. As illustrated in Figure 4, a quantity of resin is split into equal sized portions that are placed into separate reaction vessels. Excess of each building block is employed to ensure that all of the reactions are driven to completion. The resin from all of the vessels is then recombined, mixed thoroughly, and reapportioned into the requisite number of reaction vessels to perform the second synthesis step that adds diversity. The second reaction provides compounds that incorporate all of the possible combinations of the two sets of building blocks. By repeating the split, react, and mix operations compounds incorporating all possible combinations of the sets of different building blocks are theoretically generated, given that enough beads are used.[22] The total number of compounds that are theoretically synthesized by this method is easily determined by multiplying together the number of building blocks that are used in each synthesis step. For oligomers the number of compounds equals X^n where X is the number of compounds in the basis set of monomers and n is the length of the oligomer. Several automated systems have also been developed that greatly expedite the repetitive split and mix operations.[6d,23] The instrument reported by Zuckermann was one of the first to be developed for this purpose.

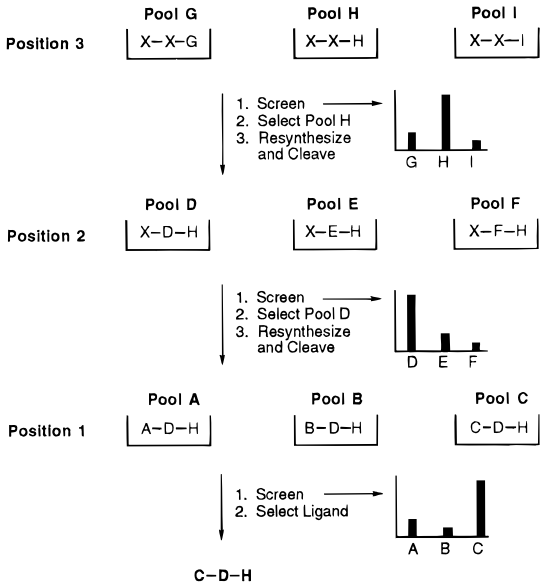


Figure 5. Deconvolution where X corresponds to an undefined position.

A number of different strategies have been developed for evaluating libraries that are prepared by the split synthesis procedure due to the considerable challenge of correct structural elucidation of the molecules having the greatest biological activity. These strategies are described in the subsequent sections.

3. Deconvolution of Soluble Libraries

Houghten pioneered one of the most popular strategies for "deconvoluting" a soluble library after cleavage from the support.[24] Although this strategy was first employed for the evaluation of peptide libraries, it has also been used by many researchers for the evaluation of small molecule libraries. In this approach (Figure 5), pools of compounds are prepared such that each separate pool has defined building blocks at either one or two positions, and at the remaining positions all combinations of building blocks are incorporated. The optimal building block(s) at the defined position(s) is selected by determining which pool(s) has the greatest biological activity. A second round of synthesis is then performed with the selected building block(s) in place at the initial defined position(s) in order to prepare pools where the next defined position is introduced. Each pool is evaluated for biological activity in order to select the optimal building block at the additional defined position. This deconvolution process of iterative resynthesis and evaluation is then repeated until all of the positions are defined.

For example, in the deconvolution sequence illustrated in Figure 5, three pools are produced that each contain a defined building block at the last position (G, H, or I) and all possible combinations of building blocks at the first and second positions. For deconvolution, the pools are assayed and building block H is selected at the last position since the pool

with H at the last position has the greatest activity. Three pools are now prepared in the second round of synthesis that incorporate building block mixtures at the first position, a defined building block at the second position (D, E, or F), and the selected building block H at the last position. Each pool is evaluated for biological activity and building block D is selected for the second to last position. Three final pools are synthesized with defined building blocks at each position; building blocks A, B, or C at the first position, building block D at the second position, and building block H at the last position. Evaluation of these pools results in the identification of compound C−D−H.

The deconvolution process described above has been applied successfully to identify high-affinity ligands, but several issues should be considered. First, as the number of compounds in a pool increases, lower concentrations of each compound must often be used in order to maintain the solubility of all of the compounds in the pool. Compounds that have modest activity therefore may not be detected. Second, since the biological activity observed for a given pool of compounds *depends on both the activity and abundance of the active compounds in each pool*, the pool that shows the greatest biological activity does not necessarily contain the most potent compound(s). In fact, for several peptide library studies the most active peptide was not found in the most active mixture.[25] Third, the iterative resynthesis and evaluation of compounds can be a time-consuming and laborious process.

A number of modifications have been reported in order to address these complications. Pharmagenics has recently reported an affinity selection process that provides the affinity of the most potent compounds in a pool rather than a sum of the affinity of all of the compounds in the pool.[26] Janda has noted that in split synthesis, resin can be saved at each step immediately prior to resin mixing.[27] This resin can later be used as an intermediate in the iterative resynthesis and deconvolution process resulting in considerable savings in time and effort. Alternative library synthesis strategies have also been reported that provide direct determination of high affinity compounds *without* iterative resynthesis and evaluation steps, for example, the positional scanning method of Houghten[25c,28] and the "orthogonal" library method of Tartar.[7]

In the positional scanning library approach developed by Houghten, separate positional libraries are prepared, each of which contains a single defined building block. For example, if a library were prepared from three building block sets, three positional libraries would be prepared. These may be denoted as OXX, XOX, and XXO, where O corresponds to the defined position in each of the pools, and X corresponds to a randomized position. Each positional library is divided into separate sublibraries with a unique building block at the defined position in each sublibrary. Each positional library is then screened to directly determine the building block(s) at each defined position that contribute the most to biological activity. Although active compounds are identified without iterative resynthesis using this approach, there is an increased likelihood that the most potent compound(s) will not be identified.[29] In addition, the number of split and mix operations that would be required for library synthesis is sufficiently high that building block mixtures are used in several of the synthesis steps in order to reduce the number of operations.

Tartar demonstrated the orthogonal library method by the cosynthesis of two libraries that contain "orthogonal" pools.[7a] Using this strategy, any of the pools in the first library will have only one compound in common with any of the pools in the second library. During an assay, any highly active compound will cause a signal in only one pool in each library. By examining which two pools are active, the compound causing the activity can immediately be identified without resynthesis. This method was demonstrated using 125 pools of 125 compounds each, or a total of 15 625 trimers of natural and unnatural amino acids, and allowed the identification of a 63 nM inhibitor of vasopressin binding to LLCPK1 cells. However, as for the positional scanning method, the authors found it necessary to employ building block mixtures in each of the synthesis steps in order to reduce the number of split and mix operations.

4. Structural Determination by Analytical Methods

a. When Assays Are Performed on Support-Bound Compounds. Methods have also been developed to assay libraries with compounds still attached to resin beads.[30] This approach is possible because the "split and mix" operations result in a single compound structure being prepared on each bead. Lam and co-workers were the first to take advantage of the one compound per bead result of the split synthesis process.[6d,21f,31] In this approach, the resin-bound library is treated with a labeled soluble receptor. For many studies a fluorescent label has been employed due to the sensitivity of fluorescence detection. The labeled receptor binds to those beads that are derivatized with molecules that have the highest affinity to the receptor. The labeled beads are then selected followed by structural determination of the support-bound compound. Very efficient, automated methods have been developed to select the labeled beads, for example, by use of a fluorescence activated cell sorting (FACS) instrument.[30b,d]

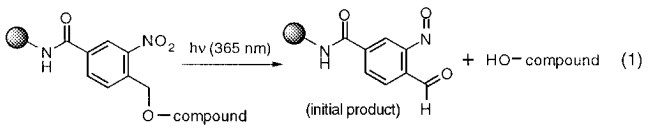
In order for this approach to be successful, analytical methods must be available for the complete structural determination of minute quantities of the compound that are present on a single resin bead (*vide infra*). For this reason, this approach has only been employed to screen peptide and oligonucleotide libraries where very sensitive and efficient sequencing methods have already been developed, e.g. Edman degradation and DNA sequencing, respectively.

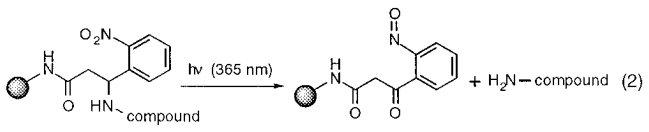
A number of groups have demonstrated that mass spectrometry is sufficiently sensitive (electron impact,[32] electrospray,[31,33] matrix-assisted laser desorption,[34] imaging time of flight secondary ion MS[35]) to observe molecular ions of compounds that are cleaved from single resin beads. However, for a large library of molecules that are of low molecular weight, many different compounds will have the same molecular

weights severely complicating structural determination.[36]

Youngquist has developed a novel strategy that should be applicable to all oligomer and some nonoligomer libraries.[34a,c] In his strategy, a small percentage of a capping reagent is added at each monomer addition step so that approximately 5−10% of the oligomer is capped at each step. After cleavage of the final oligomer product from the solid support, the oligomer sequence can be read directly by mass spectrometric analysis from the molecular ions corresponding to the truncated and capped sequences that are present in addition to the full oligomer sequence. Youngquist has demonstrated this strategy in the synthesis and evaluation of peptide libraries with acetic anhydride as the capping reagent.

b. When Assays are Performed in Solution. As mentioned earlier, the linker or resin can interfere with the interaction of a support-bound ligand with a receptor.[15a,17] To avoid these interactions, strategies have been developed for dividing the beads into separate reservoirs and then cleaving a portion of the compound on each bead into solution for biological evaluation. The collection of beads that results in the greatest biological activity is then redistributed in smaller pools. A second portion of compound is cleaved from each bead and biological evaluation is repeated to identify a progressively smaller set of beads. This process can theoretically be repeated until the beads are rearrayed singly for direct identification of the compounds responsible for activity. The sequence of the remaining compound on the bead can then be determined by the analytical methods described above. In order to carry out this strategy a method must be available for the sequential cleavage of a portion of the compound from the resin bead. Lebl and co-workers have developed multiple release linkers for peptide synthesis.[37] One serious limitation to the generality of this approach is that the reaction conditions that are employed in library synthesis must not result in cleavage of any of the linkers. Baldwin and co-workers have reported that a *single* photolabile linker may also be utilized for partial release of compounds into solution by controlled irradiation to dial in the percentage of photocleavage (eq 1).[38] The Geysen group has also reported a photolabile linker that allows controlled release and produces a terminal carboxamide (eq 2).[33]





5. Encoding Strategies

An alternative strategy to structural determination of compounds that are synthesized on single beads is to employ an encoding strategy. In this approach, readable tags that record the reaction sequence are attached to the resin bead concurrent with the synthesis of the compound on the bead. The tags may be added to provide an encoding sequence whereby the structure of the tag encodes for the building block and the location in the sequence encodes for the library synthesis step (method A, Figure 6). Alternatively, each tag may be added individually to the resin (method B, Figure 6). In this case the tags must encode for both building block structure and the step in the library synthesis. Approaches have been developed to use encoding strategies for the identification of compounds where assays are performed on support-bound compounds or on compounds in solution after cleavage from the support.

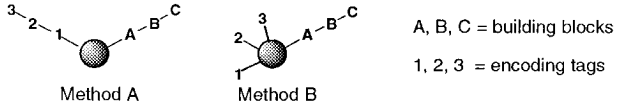


Figure 6. Encoding strategies.

a. DNA Encoding Strategies. The encoding concept was first proposed by Lerner and Brenner where an oligonucleotide was selected as the coding sequence.[39] This encoding strategy relies on two straightforward but powerful tools commonly employed in molecular biology research. Only a minute quantity of the encoding oligonucleotide sequence is required because it can be amplified employing the polymerase chain reaction, and the oligonucleotide sequence can rapidly be read by DNA sequencing methods. A large number of building blocks may be encoded by this strategy simply by employing multiple bases to encode for each building block.

Needels and co-workers[30d] and Janda and co-workers[40] have independently demonstrated this method for the synthesis of peptide libraries. In both approaches orthogonal protecting group strategies were necessary in order to differentiate between the DNA tag synthesis steps and the peptide synthesis steps. In particular, the base-labile Fmoc group was employed for amine protection for peptide synthesis while the mildly acid-labile dimethoxytrityl (DMT) group was employed for hydroxyl protection for oligonucleotide synthesis. Needels employed a 20:1 ratio of the "binding" peptide strand to the "encoding" DNA strand in order to minimize the effect of unwanted DNA−receptor interactions in binding studies. Janda introduced a linkage strategy to cleave the peptide from support while still covalently linked to the oligonucleotide in a 1:1 ratio for biological evaluation in solution.

For application of these strategies to organic compound libraries, the compatibility of the encoding DNA strand to the reaction conditions necessary to prepare the organic structure should be considered and may preclude many synthesis approaches or limit the accessibility of certain compound classes. In addition, the synthesis strategy for library preparation must accommodate a protecting group scheme that allows synthesis of the coding DNA sequence to be differentiated from the synthesis of the organic compound.

b. Peptide Encoding Strategies. An alternative encoding strategy relies on the use of peptides to code for nonnatural oligomers or organic com-

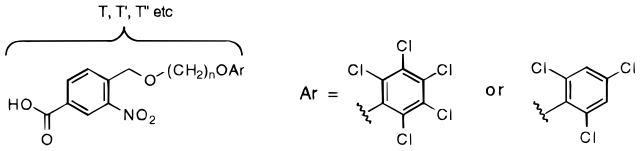


Figure 7. Haloaromatic tags attached through amide bond formation.

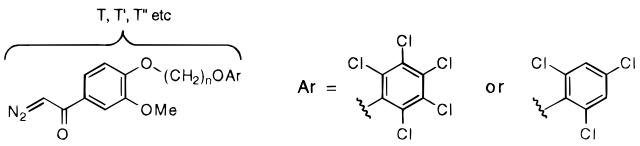


Figure 8. Haloaromatic tags attached through carbene insertion.

pounds. In this strategy first demonstrated by Zuckermann and co-workers, a coding peptide strand was employed to encode a "binding" peptide-based oligomer strand.[41] Orthogonal protecting group strategies were necessary in order to differentiate the synthesis of the "binding" strand from the synthesis of the "coding" strand. In Zuckermann's strategy the base-labile Fmoc group was employed for the binding strand and the mildly acid-labile *N*-[(4-methoxybenzyl)oxy] (Moz) or *N*-[[2-(3,5-dimethoxyphenyl)prop-2-yl]oxy] (Ddz) groups were used for the coding strand. The binding strand, linked to the coding strand, can be cleaved from the support by treatment with strong acid so that the resulting complex can be assayed in solution. Subsequent Edman degradation of the coding strand then provides the binding oligomer sequence. One potential complication with this approach is that the coding strand could also interact with the receptor, although this was not observed in the reported study.

A related peptide encoding strategy has been reported by Nikolaiev and co-workers.[42] In this study Fmoc protection was used for the binding strand and Boc protection was used for the coding strand. At the end of the synthesis sequence the binding strand could be released from the resin for biological evaluation, while the encoding strand is retained on the bead for later structural elucidation. This procedure requires that the solution containing the binding strand be spatially addressable to the bead from which it originated to allow decoding. As for DNA encoding, the application of either of these strategies to the synthesis of organic compounds requires a protecting group strategy that allows the synthesis of the coding peptide sequence to be differentiated from the synthesis of the organic compound.

c. Haloaromatic Tag Encoding Strategies. Still has developed an encoding strategy that employs molecular tags that are highly stable to a majority of reaction conditions and therefore should introduce few constraints upon compound synthesis.[44] Still employs haloaromatic compounds as molecular tags that can be detected at levels of less than 0.1 pmol using electron-capture GC. Due to the sensitivity of analytical evaluation, each tag is attached to the support at less than 1% of the loading level of the synthesized compound. This minimizes the chance of complications in library evaluation resulting from tag−receptor interactions when support-bound assays are used. The tags are synthesized by alkylation of a commercially available halophenol with an ω-bromo-1-alkanol, followed by attachment of a linker with a release site. In the initial reports of this strategy,[44b] a photolabile *o*-nitrobenzyl alcohol linker served as the release site, and the tags were attached to the solid support through amide bond formation with a support-bound reactive amine (Figure 7).

In subsequent work, Nestler and co-workers have also employed an oxidatively labile linker that is cleaved with ceric ammonium nitrate, and the tags are directly attached to the resin beads through rhodium-catalyzed acylcarbene insertion (Figure 8).[44a] With this approach, in contrast to all of the other encoding strategies, a protection scheme is not necessary to differentiate the incorporation of the encoding tags from compound synthesis. The carbene may react with both the support and the compound attached to the support; however, because the reaction is not very selective, the carbene inserts predominantly into the support due to the greater proportion of the support. Furthermore, because the tag loading level is only ~1% of the compound loading level, compound modification should be minimal. The tags must encode for both the building block and for the reaction step in the synthesis sequence. This is accomplished by employing a binary code format. Multiple tags are used for each building block with the presence or absence of a tag corresponding to 1 or 0, respectively, in a binary sequence. This strategy has been employed both for support-bound and solution-based assays.

After the submission of this manuscript, an innovative method for encoding based on radiofrequency transponders was independently reported by two research groups. The transponders are encased in glass and are completely solvent, reagent, and temperature resistant. They can easily be scanned after every reaction step to record the identity of a compound on a batch of resin.[43]

B. Synthesis of Organic Compound Libraries

1. Introduction to Solid Supports

Two types of supports were used in a large majority of the studies that are described in this review. Polystyrene cross-linked with 1−2% divinylbenzene, and a polystyrene-polyethylene glycol copolymer (PEG-PS). Although other supports have been employed in solid-phase organic synthesis, and new supports are currently being developed, an overview of only these two most commonly used resins is provided.[45] In addition, unless specifically stated otherwise, all synthesis sequences detailed in this review were carried out on polystyrene resin.

Polystyrene cross-linked with 2% divinylbenzene was demonstrated by Merrifield as a useful support for solid-phase peptide synthesis in 1963,[46] and polystyrene cross-linked with 1−2% divinylbenzene continues to be one of the most commonly used supports for solid-phase organic synthesis. Polystyrene resin is advantageous in that it is the least expensive of the resins, high loading levels can be achieved, and it is relatively mechanically stable. One major limitation of polystyrene resin is that the resin beads are not well solvated in protic solvents result-

Table 1. Side-Chain Modifications of Amino Acids in Permethylated Peptides

parent chain	side-chain modification	nonmethylated purity,[a] %	methylated purity,[a,b] %
CGGFL-NH_2	methyl thioether	86	50
DGGFL-NH_2	methyl ester	97	60
EGGFL-NH_2	methyl ester	90	75
HGGFL-NH_2	methyl imidazole	98	40
KGGFL-NH_2	quaternary salt	99	30
LGGFL-NH_2	unmodified	99	81
M[O]GGFL-NH_2	unmodified	98	70
NGGFL-NH_2	dimethyl amide	85	86
QGGFL-NH_2	dimethyl amide	85	80
RGGFL-NH_2	trimethyl guanidine	88	75
WGGFL-NH_2	methyl indole	98	70
YGGFL-NH_2	methyl ether	99	81

[a] Estimated purity as determined by HPLC. [b] Sum of the mono-, di-, and trimethylated α-amine products.

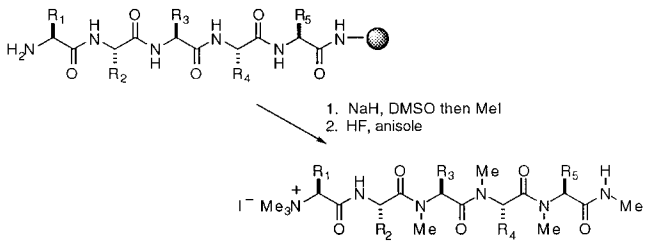
ing in poor reaction site accessibility and diminished reaction rates.

There are two forms of PEG-PS resin. Rapp and Bayer have prepared a PEG-PS resin by means of anionic polymerization of ethylene oxide to attach PEG chains of controlled lengths on cross-linked polystyrene beads containing hydroxyl groups (the resin is marketed as Tentagel resin).[47] In contrast, Millipore has prepared PEG-PS by attaching preformed polyethylene glycol onto the polystyrene bead. Both of these resins are well solvated in protic solvents, in contrast to the polystyrene resin. In addition, because the polyethylene glycol chains are not cross-linked, it has been argued that the reaction sites are more highly accessible resulting in greater reaction rates.[47a] Due to the lack of cross-linking in the polyethylene glycol chains, in combination with good aqueous solvation characteristics, PEG-PS has been the favored resin for support-bound assays. Limitations of PEG-PS relative to polystyrene include much higher cost, reduced loading levels, and significant mechanical instability (stirring or vigorous shaking results in significant loss of material from the resin).

2. Post-Synthesis Peptide Modification

Houghten has expanded upon his seminal work in the area of peptide libraries[2b] to include the post-synthesis modification of peptides in order to extend the diversity of the compounds that can be accessed and to provide oligomers that have improved pharmacokinetic properties. In his first study in this area, Houghten permethylated protected peptides while they were still attached to the solid support.[48] Permethylation was accomplished by treating the peptide libraries with excess sodium hydride in DMSO followed by addition of methyl iodide (Scheme 1). Under optimized conditions, permethylation of the peptide AGGFL-NH_2, which does not contain reactive side chain functionality, provided the permethylated product in >90% yield and purity. Permethylation of peptides that incorporate many of the different side chain functionalized amino acids was also investigated. As shown in Table 1, methylation of the functionalized side chains generally was observed, as were lower levels of purity of the permethylated products. For each of the peptides, as much as 15% of the terminal amino group had not been fully quaternized in the permethylation product. Longer reaction times could be employed to drive the quaternization to completion, but also resulted in appreciable degradation of the product. Houghten demonstrated that minimal epimerization occurs in the deprotonation step by treatment of all four possible stereoisomers of GGFL-NH_2 with NaH in DMSO followed by a water quench. Subsequent cleavage from the support and HPLC analysis showed that less than 0.75% epimerization had occurred.

Scheme 1



Employing the above post-synthesis methylation strategy, Houghten performed the synthesis and evaluation of a library that theoretically contained 37 791 360 unique permethylated hexapeptides using the positional scanning approach (see section II.A.3). In particular, the library was screened for the ability to inhibit the growth of five different strains of bacteria or yeast. A number of permethylated peptides that incorporated multiple phenylalanines were identified that had significant activity against *Staphylococcus aureus*, methicillin-resistant *S. aureus*, and *Staphylococcus sanguis*, with IC_{50} values in the 1−10 μM range.

A number of researchers have also reported on the post-synthesis modification of individual residues of peptides within a library, such as acylation or reductive amination of the N-terminus, or functionalization of reactive side chain functionality. However, these studies are outside the scope of this review and will not be discussed here.

3. Biopolymer-Mimetic Libraries

Although peptides generally have poor pharmacokinetic properties that limit their utility as drugs, the high level of success that has been achieved in identifying high-affinity ligands to diverse receptors and enzymes through the synthesis and evaluation of peptide libraries is well documented.[2] Many researchers have therefore focused on the synthesis and evaluation of biopolymer mimetics that although based upon the peptide structure, incorporate backbones that may have improved pharmacokinetic

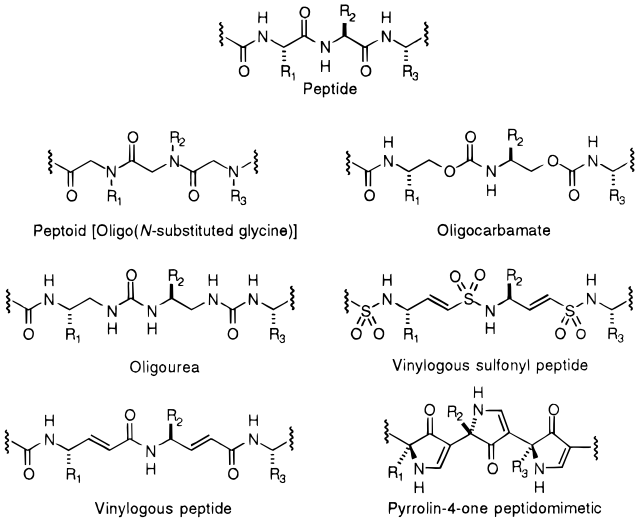


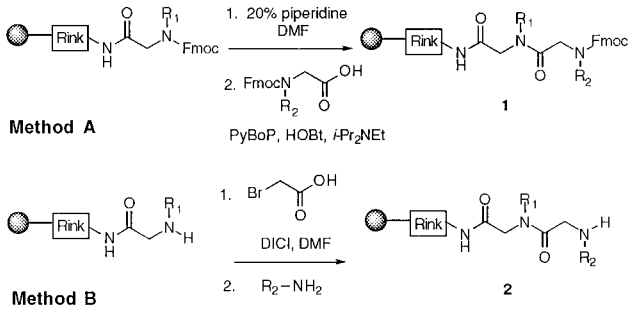
Figure 9. Unnatural biopolymers.

properties as a result of proteolytic stability, more favorable solubility characteristics, or unique structural and/or hydrogen bonding motifs. Biopolymer mimetics are also of considerable interest to other research areas, including the design of two- and three-dimensional unnatural biopolymer frameworks with novel properties, including the design of molecular receptors (*vide infra*).

Peptoids. Simon and co-workers considered a number of criteria for design of a new scaffold including simple synthesis of monomers, increased resistance to hydrolytic enzymes, the ability to display a wide range of functionality, high-yielding coupling steps amenable to automation, and the use of achiral monomers.[49] Oligo(*N*-substituted)glycines, or "peptoids" (Figure 9) were proposed to meet these requirements. The side chains of peptoids are displayed from the amide nitrogen of an oligoglycine backbone instead of the α-carbon atom, providing a protease-resistant[50] and achiral tertiary amine linkage.

In the original synthesis of peptoids, standard solid-phase peptide synthesis methods were employed with Fmoc-protected *N*-alkylglycines serving as the monomer components (method A, Scheme 2).[49]

Scheme 2



(Benzotriazol-1-yloxy)tris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP) or bromotris(pyrrolidino)phosphonium hexafluorophosphate (PyBroP) were found to be the optimal coupling agents, allowing the synthesis of up to 25-mers in high yield and excellent purity as judged by MS and RP-HPLC analysis. Subsequently, a simplified approach was reported by

Table 2. Pentamer Peptoids Prepared by the Submonomer Method (Scheme 2, Method B)

pentamer side chain (R)	characterization of unpurified product	
	purity,[a] %	mass recovery,[b] %
$(CH_2)CH_3$	>85	90
$(CH_2)Ph$	>85	74
$(CH_2)Chx$	>85	79
$(CH_2)CH(Ph)_2$	>85	70
Ph	>85	83
c-C_3H_5	>85	83
$CH_2)_2$indole	>60	52
$(CH_2)_3NH_2$	>85	63[c]

[a] Purity of unpurified compounds as determined by RP-HPLC. [b] Determined from dry weight. [c] Prepared with Boc-NH-$(CH_2)_3NH_2$.

Zuckermann and co-workers. In the submonomer approach (method B, Scheme 2),[51] each cycle of synthesis involves a two-step procedure: (1) amide bond formation with α-bromo acetic acid employing 1,3-diisopropylcarbodiimide (DICI) as the activating agent and (2) bromide displacement with a suitable primary amine to provide the secondary *N*-alkylglycine (**2**) ready for the next coupling step. This approach allows for the direct incorporation of commercially available amines as building blocks; thereby eliminating costly, time-consuming monomer synthesis and obviating the need for α-amine protection. As shown in Table 2, the mass balance and purity of unpurified material is high for the synthesis of pentamers that incorporate a range of amine nucleophiles, including α-branched amines and unreactive aniline derivatives. In addition, a nonomer with five consecutive propylamine side chains followed by four consecutive butyl side chains (not listed in table) was obtained in greater than 65% purity as determined by RP-HPLC and in 86% crude mass balance, demonstrating the efficiency of the synthesis sequence.

Using the submonomer approach, Zuckermann and co-workers assembled a library on Merrifield beads using the split-synthesis approach that was biased toward the 7-transmembrane G-protein coupled class of receptors.[52] The Chiron group synthesized the library of approximately 5000 dimer and trimer peptoids employing 23 different monomers as well as three terminal amine capping reagents (Figure 10). Seven monomers were selected based upon the structures of known ligands to this class of receptors, while 17 monomers were selected to be as diverse as possible. The Chiron group has recently described their experimental design to maximize molecular diversity for a given library or to target a library with key features for a given receptor.[53]

Initial studies were performed to demonstrate that all of the amines were fully incorporated into a test oligomer. The library was then synthesized using a Zymark robot to carry out resin manipulations and micropipetting[54] and assayed as 18 pools of 216, 255, or 272 compounds for the ability to inhibit [^{3}H]-prosazin binding to an α_1-adrenergic receptor preparation. By using the iterative resynthesis and evaluation strategy, a 5 nM inhibitor was identified (**3**, Figure 11). Similarly, a 6 nM inhibitor of [^{3}H]-DAMGO binding to a μ-specific opioid receptor preparation was identified (**4**, Figure 11). The peptoid products are completely stable to proteolysis as

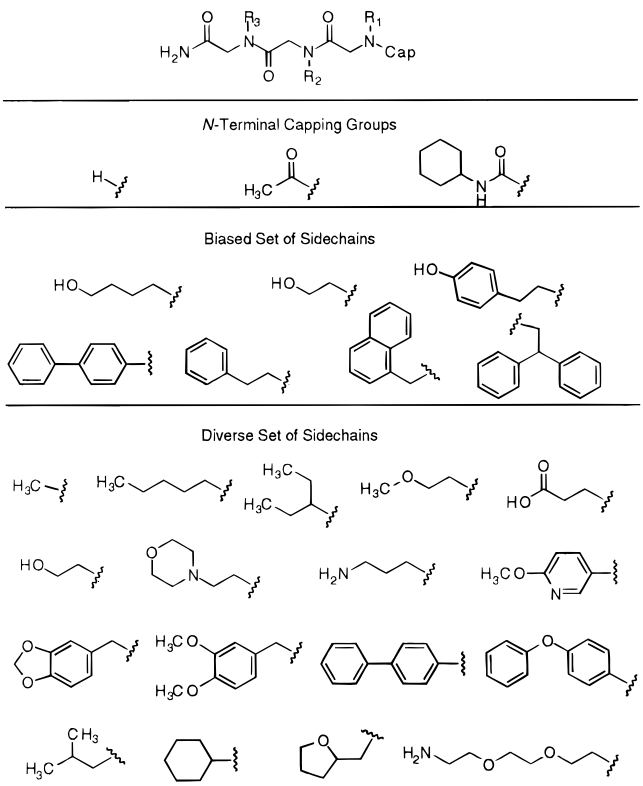


Figure 10. Monomer side chains and capping reagents used in peptoid library synthesis.

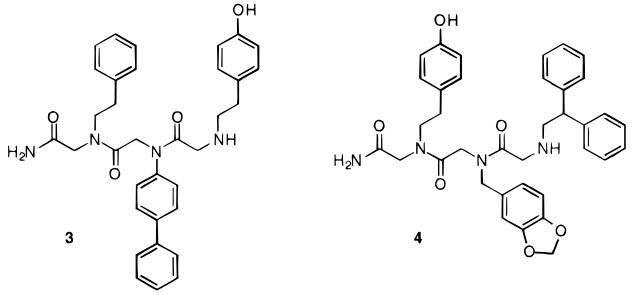


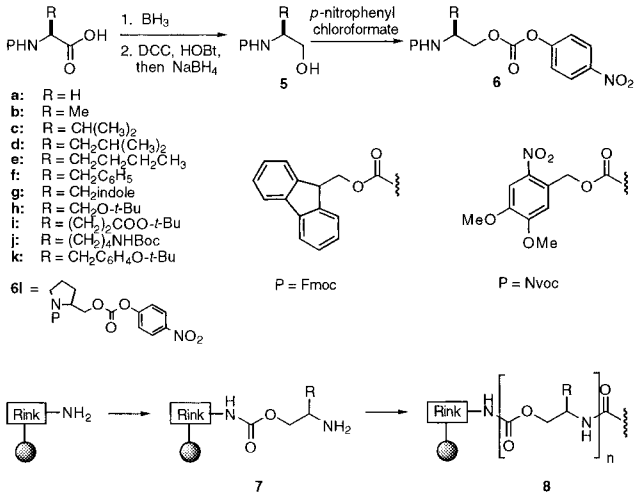
Figure 11. Peptoid ligands.

determined by incubating a number of peptoid derivatives with carboxypeptidase A, chymotrypsin, elastase, papain, pepsin and thermolysin.[50b]

Additional diversity has also been achieved through modification of peptoid side chains. Pei and Moos have described a procedure for modification of peptoids that have alkenyl or alkynyl side chains by a [3 + 2] cycloaddition reaction with nitrile oxides to produce support-bound isoxazoles and isoxazolines.[55] The products are formed with high regioselectivity in >80% purity by HPLC analysis, and the authors conclude by the lack of byproducts that the chemical yields are similar, although only one mass balance (72%) of an unpurified product is provided. The Chiron group has also reported multistep modification of peptoid structures to provide six- and seven-membered ring heterocycles. These studies are described in later sections.

Oligocarbamates. Another unnatural biopolymer-based library was reported by Cho and coworkers.[17,56] Their method involves the use of amino acids as precursors to chiral building blocks for the synthesis of a library of oligocarbamates. *N*-Protected *p*-nitrophenyl carbonate monomers **6** were prepared by reduction of the corresponding protected amino acids followed by treatment of the resulting alcohols **5** with *p*-nitrophenyl chloroformate (Scheme 3). Oligomers were initially assembled on polystyrene resin using the Fmoc group as the amine protecting group and a standard Rink amide linker. Two reactions were performed in each coupling cycle: (1) removal of the Fmoc group by treatment with 20% piperidine in *N*-methylpyrrolidinone (NMP) and (2) coupling with an *N*-Fmoc carbonate monomer employing hydroxybenzotriazole (HOBt) as an additive. Coupling yields were determined to be >99% by RP-HPLC and quantitative ninhydrin tests,[57] and oligocarbamates were characterized by FAB-MS and ^{1}H NMR spectroscopy. Two representative oligocarbamates were also incubated with trypsin and porcine pepsin and were found to be stable to proteolysis.

Scheme 3

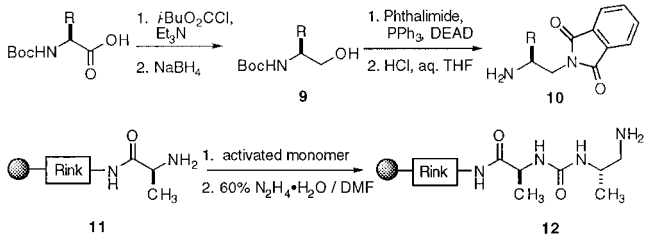


The photolithography technique developed by Fodor and co-workers (section II.A.1.b) was then used to prepare a library of oligocarbamates.[17] In order to introduce a protecting group scheme that was compatible with photolithography, the carbamate monomers were prepared with the photolabile [(nitroveratryl)oxyl]carbonyl (Nvoc) group in place of the Fmoc group. An eight-step binary masking scheme was then employed to synthesize a library of 256 oligocarbamates around the parent sequence Ac$Y^cF^cA^c$-$S^cK^cI^cF^cL^c$ (where X^c refers to the carbamate monomer formed from the amino acid X) such that the library contained all of the possible deletion sequences for the parent oligomer. The library was assayed for the ability to bind the monoclonal antibody (mAb) 20D6.3, which was raised to the keyhole limpet hemocyanin conjugate of oligocarbamate Ac-$Y^cK^cF^cL^c$G-OH (G-OH is a terminal glycine residue). Binders were identified by scanning epifluorescent microscopy using a goat α-mouse fluorescein-conjugated secondary antibody. Five out of the 10 highest affinity oligocarbamates, Ac$K^cF^cL^c$G-OH, Ac$F^cK^cF^cL^c$G-OH, Ac$Y^cK^cF^cL^c$G-OH, Ac$A^cK^cF^cL^c$G-OH, and Ac$I^cF^cL^c$G-OH, were resynthesized and purified on large scale using Fmoc chemistry. The IC_{50} values of all five ligands determined in solution were in the 60−180 nM range. These studies also revealed that the support or linker can interfere with receptor interactions, since the ligand Ac$Y^cF^cL^c$G-OH was also

prepared on large scale and assayed in solution and found to have an IC_{50} value of approximately 160 nM, even though this ligand ranked in the bottom 30% of ligands in the support-bound assay.

Oligoureas. Burgess has reported on initial studies toward the solid-phase synthesis of oligoureas with the goal of synthesizing oligourea libraries (Scheme 4).[58] The monomers **10** for oligourea syn-

Scheme 4



thesis were prepared on average in 50−60% overall yield by the three step process of reduction of the corresponding *N*-Boc-protected amino acid followed by converting the resulting primary alcohol **9** to the phthalimide under Mitsunobu reaction conditions and final removal of the Boc protecting group. In the solid-phase synthesis of the oligourea, two reactions are performed in each coupling cycle: (1) removal of the phthaloyl group by treatment with 60% hydrazine hydrate in DMF and (2) coupling with a monomer that is activated in situ as the isocyanate by treatment with triphosgene. Two oligourea/peptide hybrids, **13** and **14** (Figure 12), were prepared by this general approach in unoptimized 46% and 17% isolated yield, respectively.

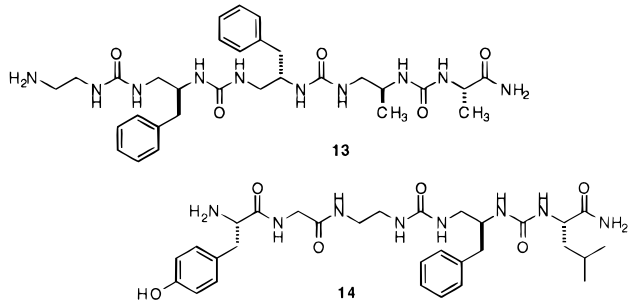
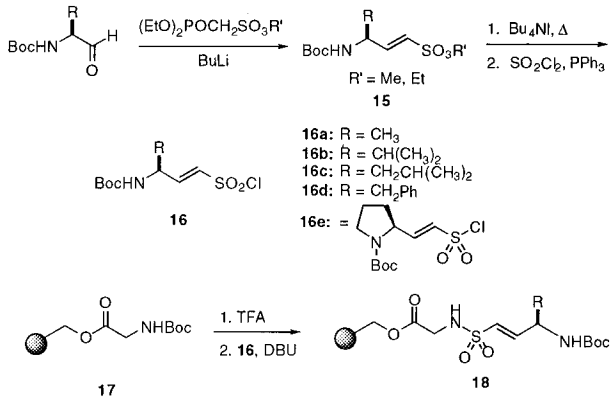


Figure 12. Oligurea/peptide hybrids prepared on solid support.

Vinylogous Sulfonyl Peptides. Gennari and Still have reported on the solid-phase synthesis of vinylogous sulfonyl peptides with the goal of making sulfonyl peptide libraries as well as in employing these biopolymer mimetics as synthetic receptors.[59] The authors focused upon the vinylogous sulfonamide structure because the sulfonamide may mimic the tetrahedral geometry of amide hydrolysis in the protease cleavage of peptide bonds thereby serving as an interesting pharmacophore for protease inhibition. In addition, the stronger polarization of the sulfonamide bond compared to a regular peptide bond favors the formation of hydrogen bonds to potentially provide a more distinct preorganization of the sulfonyl peptides. The monomers **16** for oligomer synthesis were prepared on average in 65−75% overall yield by a three step process from the corresponding *N*-Boc-

Scheme 5



protected amino aldehyde (Scheme 5). A Wittig-Horner-Emmons reaction provides the α,β-unsaturated sulfonate **15**, which is then converted into the sulfonyl chloride by deprotection with tetrabutylammonium iodide followed by activation with SO_2Cl_2 and PPh_3.[60] In the solid-phase synthesis of the sulfonyl peptides, two reactions are performed in each coupling sequence: (1) removal of the Boc group by treatment with trifluoroacetic acid (TFA) and (2) four cycles of coupling with 1 equiv of vinylsulfonyl chloride **16** followed by slow addition of 1,8-diazabicyclo[5.4.0]undecane (DBU). Four cycles of coupling and slow addition of DBU is necessary, since excess DBU results in decomposition of the vinylsulfonyl chloride monomers, but DBU is the only base that was studied that provides clean conversion to product. By using Tentagel resin, all five monomers, **16a** to **16e**, were coupled to the support-bound Gly ester followed by cleavage from the support by treatment with 10% triethylamine in methanol. The methyl ester products were isolated in good overall yield, 60−70%. In addition, two sulfonyl dipeptides were prepared to demonstrate oligomer synthesis by the above method. Sulfonyl dipeptides **19** and **20** were obtained in 57% and 52% isolated yields, respectively (Figure 13). In addition, only one diastereomer of **19** was detected indicating that no racemization occurred either in monomer synthesis or in the coupling steps.

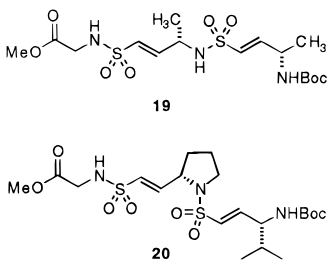


Figure 13. Vinylogous sulfonyl peptides.

Vinylogous Peptides. In one of the earliest studies on unnatural biopolymers, Schreiber and coworkers reported on the synthesis of vinylogous peptides and demonstrated that these structures can adopt specific secondary as well as tertiary structures (Figure 14).[61] The vinylogous peptide **21** was observed to adopt a stacked array of parallel sheets as determined by X-ray crystallography, while the pep-

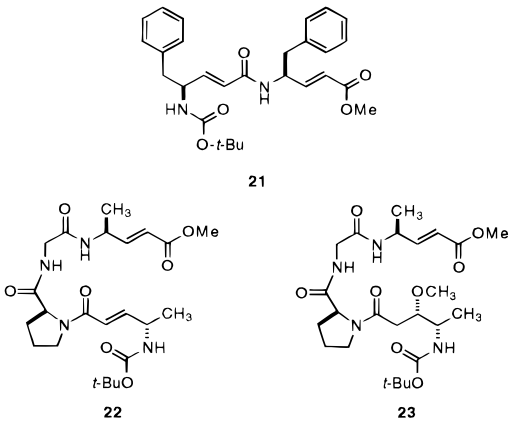


Figure 14. Vinylogous peptide and vinylogous peptide/amide hybrids.

tide/vinylogous amide hybrids **22** and **23** were determined to adopt structures corresponding to antiparallel sheets and helical conformations, respectively, with turns about the Pro−Gly bonds as determined by NMR. In this study, the compounds were prepared in solution for the purpose of structural evaluation; however, the monomer synthesis is expedient and solid-phase synthesis strategies for the purpose of library construction should be straightforward using standard peptide synthesis methods.

Smith and Hirschmann have developed an unnatural biopolymer based upon linked pyrrolin-4-ones that also incorporates a vinylogous amide into its structure (Figure 9).[62] Although these oligomers have not been employed for library synthesis, they do adopt a secondary structure that mimics a β-strand[62c] and have been utilized in the synthesis of potent protease inhibitors.[62b]

4. Nonoligomeric Compound Libraries

Nonoligomeric molecules, which are nonpeptidic in nature and are below 600−700 in molecular weight, have become the major focus of library synthesis efforts for the development of medicinal agents. Libraries of small nonoligomeric molecules have been prepared both for the identification of lead compounds and for the optimization of lead compounds that have been identified through either library screening efforts or alternative methods.

While the compound class for library synthesis toward lead optimization is predetermined, a number of factors must be considered in the selection of a compound class for the purpose of lead identification. To varying degrees, three general strategies for compound selection have been used for the library syntheses outlined in this review.[63] The first strategy is to select "privileged" structures,[64] where the display of different functionality upon the structure has previously provided a number of potent and specific therapeutic agents or candidates toward different therapeutic targets. The second strategy is to design compound scaffolds based on important recognition elements of biological receptors. The final strategy is to select stable compounds upon which few therapeutic agents or candidates have been based, but which are straightforward to prepare with multiple sites available for the display of functionality.

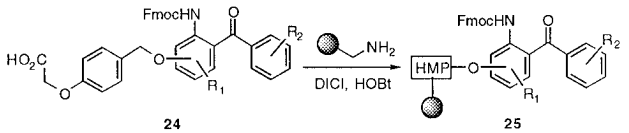
In designing a synthesis scheme to access a library based upon a specific compound class three criteria should be considered: (1) The synthesis scheme should provide the majority, if not all, of products in the library in high yield and purity. (2) The chemistry should be compatible with the display of as much diverse functionality as possible including heteroatom functionality that is commonly found in drugs such as alcohols, phenols, amines, indoles, guanidines, carboxylic acids, amides, nitriles, imidazoles, nitro groups, and halides. (3) The building blocks for synthesis of the library should be commercially available or at least readily accessible, since a library cannot be made rapidly and efficiently if many of the building blocks must be prepared.[65]

Finally, it should be emphasized that for a given compound class, we and others have found that developing a high-yielding and general synthesis sequence followed by rigorously establishing that a diverse array of functionality can be displayed can require significant effort. In contrast, using the optimized synthesis sequence libraries can be constructed rapidly and efficiently as long as the building blocks are commercially available or readily accessible.

a. Heterocycle Libraries. *1. Seven-membered Rings.* All of the efforts toward the synthesis of libraries of seven-membered ring heterocycles have focused upon the 1,4-benzodiazepine structure. The 1,4-benzodiazepine class of compounds have widespread biological activities and are one of the most important classes of bioavailable therapeutic agents. In addition to 1,4-benzodiazepines such as Valium that have anxiolytic activity,[66] there are also derivatives that are highly selective cholecystokinin (CCK) receptor subtype A antagonists, highly selective CCK receptor subtype B antagonists, [67] κ-selective opioids,[68] platelet-activation factor antagonists,[69] HIV Tat antagonists,[70] reverse transcriptase inhibitors,[71a] gpIIbIIIa inhibitors,[71b] and ras farnesyltransferase inhibitors.[72]

1,4-Benzodiazepin-2-ones. In one of the first articles to address the synthesis and evaluation of small molecule combinatorial libraries, Bunin and Ellman reported the solid-phase synthesis of 1,4-benzodiazepine derivatives.[73] In this initial report, benzodiazepine derivatives were constructed from three components: 2-aminobenzophenones, amino acids, and alkylating agents. By employing solution chemistry, a hydroxyl-substituted 2-*N*-Fmoc-aminobenzophenone is coupled to the [4-(hydroxymethyl)phenoxy]acetic acid (HMP) linker.[74] The linker-derivatized aminobenzophenone **24** (Scheme 6) is

Scheme 6



then coupled to the solid support by employing standard amide bond-forming methods (the linker may be attached to either ring of the 2-aminobenzophenone).

Synthesis of the benzodiazepine derivative on solid support then proceeds by removal of the Fmoc

Scheme 7

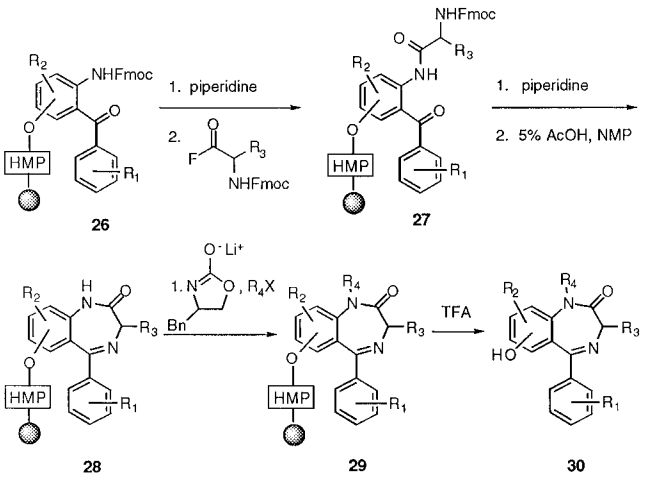


Table 3. 1,4-Benzodiazepine Derivatives 30 (Scheme 7)

	derivative				yield
entry	R^1	R^2	R^3	R^4	(%)[a]
1	4′-OH	5-Cl	CH_3	H	95
2	4′-OH	5-Cl	CH_3	CH_3	100
3	4′-OH	5-Cl	CH_3	CH_2CH_3	97
4	4′-OH	5-Cl	CH_3	$CH_2CH{=}CH_2$	90
5	4′-OH	5-Cl	$CH(CH_3)_2$	CH_2CH_3	85
6	4′-OH	5-Cl	CH_2CO_2H	CH_2CH_3	95
7	4′-OH	5-Cl	$(CH_2)_4NH_2$	CH_2CH_3	95
8	4′-OH	5-Cl	CH_2Ph(4-OH)	CH_2CH_3	98
9		4-CO_2H,5-Cl	CH_2Ph	CH_3	100
10		4-CO_2H,5-Cl	CH_3	CH_2Ph	93

[a] Yields of purified material based on support-bound starting material **26** (Scheme 7).

protecting group from **26** by treatment with piperidine in DMF followed by coupling an α-*N*-Fmoc amino acid fluoride to the resulting unprotected 2-aminobenzophenone (Scheme 7). The activated α-*N*-Fmoc amino acid fluoride[75] is employed in order to achieve complete conversion to the amide product **27** even for electron-deficient 2-aminobenzophenone derivatives. The Fmoc protecting group is then removed, and the resulting free amine is treated with 5% acetic acid in NMP to provide the initial benzodiazepine derivative **28**.

Alkylation of the anilide of **28** then provides the fully derivatized 1,4-benzodiazepine **29**. To maximize synthesis generality, lithiated 5-(phenylmethyl)-2-oxazolidinone[76] or lithiated acetanilide is employed as the base since it is basic enough to completely deprotonate the anilide of **28**, but not basic enough to deprotonate amide, carbamate, or ester functionality. By employing these conditions 1,4-benzodiazepine derivatives containing esters and carbamates were alkylated in high yield on solid support with no overalkylation observed (entries 6 and 7, Table 3). Treatment with standard trifluoroacetic acid cleavage reagents affords the benzodiazepine products **30** in high yield (85−100%, 95% av after purification based on support-bound starting material **26**). Finally, no racemization of the amino acid component is detected (<1%) as determined by chiral HPLC.

A small library of 192 benzodiazepines was then prepared in order to demonstrate spatially separate

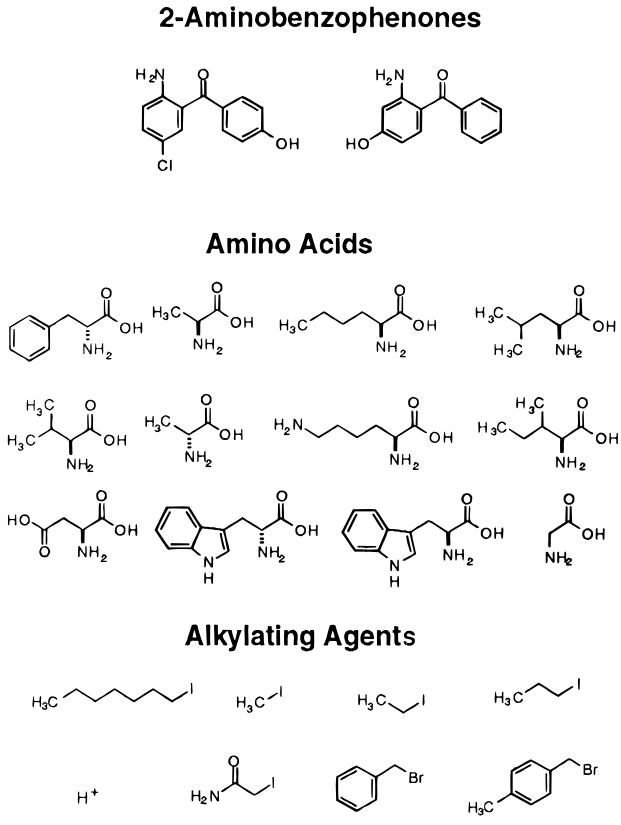


Figure 15. Building blocks used in the synthesis of a 192-member benzodiazepine library.

library synthesis of these compounds by employing the Chiron mimotopes pin support (see section II.A.1.a).[77] The library of 192 compounds was assembled using all combinations of two 2-aminobenzophenones, 12 amino acids, and eight alkylating agents with a variety of functionality being displayed (Figure 15).

The chemical integrity and yield of many of the compounds in the library were determined by two analytical methods. For 28 of the structurally diverse benzodiazepine derivatives, FAB mass spectrometry confirmed the structure of the compound corresponding to the major peak (in almost all cases the only peak) observed by HPLC. Each of the 2-aminobenzophenones, amino acids, and alkylating agents was incorporated into at least one of the 28 derivatives, indicating that all of the building blocks were compatible with the general solid-phase synthesis sequence. Yields were also determined for 20 derivatives, where again each of the 2-aminobenzophenones, amino acids, and alkylating agents was incorporated into at least one of the derivatives. This was accomplished by addition of a stock solution containing fluorenone as an internal standard followed by HPLC analysis. An 86% average yield was then calculated on the basis of the extinction coefficients of the 1,4-benzodiazepine derivatives and the relative peak area of the benzodiazepines to the peak area of the internal standard.

The spatially separate library of benzodiazepines was then screened to identify ligands to the cholecystokinin A receptor using a competitive radioligand binding assay. Detailed structure versus activity information was obtained toward this receptor target.

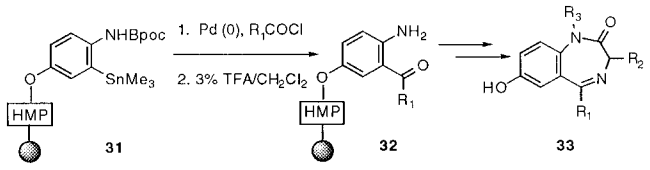
The data provided by screening the library was confirmed by synthesizing a number of the derivatives on large scale followed by purification and IC_{50} determinations. The most potent compound (IC_{50} = 0.01 μM) incorporated 2-amino-4-hydroxybenzophenone, D-tryptophan, and methyl iodide as the alkylating agent. In addition, the structure activity data correlated closely with Merck's data on structurally related benzodiazepine derivatives.[78]

By employing the synthesis strategy described above, a second library of 1680 benzodiazepine derivatives has also been prepared from three 2-aminobenzophenones, 35 amino acids, and 16 alkylating agents (structures not shown). The library has been screened against a number of receptor and enzyme targets. Inhibitors of pp60$^{s\text{-}src}$ tyrosine kinase[79] and ligands that block an autoimmune DNA−antibody interaction implicated in systemic lupus erythematosus[80] have been identified.

The original benzodiazepine synthesis sequence was based upon the combination of three different building block sets: a 2-aminobenzophenone, an Fmoc amino acid fluoride, and an alkylating agent. While alkylating agents are commercially available, and *N*-Fmoc amino acid fluorides can be prepared in a single step without purification from the corresponding *N*-Fmoc amino acids, few appropriately functionalized 2-aminobenzophenones are readily accessible. To increase the diversity of 1,4-benzodiazepin-2-ones available through solid-phase synthesis, Plunkett and Ellman utilized the Stille coupling reaction to synthesize a variety of 2-aminoaryl ketones on solid support.[81] The Stille reaction is particularly appealing for this purpose since it proceeds under mild conditions, is tolerant of a wide range of functionality, and well over 300 structurally diverse acid chloride building blocks are commercially available.

A [[2-(4-biphenyl)isopropyl]oxy]carbonyl (Bpoc)-protected (aminoaryl)stannane, which is prepared in four steps in solution, is coupled to the solid support through the HMP linker (Scheme 8). Stille coupling

Scheme 8



can then be carried out with a range of different acid chlorides and the catalyst $Pd_2(dba)_3{\cdot}CHCl_3$. The Bpoc group is cleaved by brief treatment (5 min) with 3% TFA in CH_2Cl_2. The support-bound 2-aminoaryl ketones are then incorporated directly into 1,4-benzodiazepine derivatives according to the previously described synthesis sequence.

Using this strategy, diverse acid chlorides were employed to prepare support-bound 2-aminoaryl ketones **32** that were further incorporated into 1,4-benzodiazepines **33**, including aromatic acid chlorides that are electron-rich, electron-poor, alkyl-substituted, polyaromatic, heterocyclic, and ortho-substituted and aliphatic acid chlorides that can be sterically hindered (Table 4). The desired benzodiazepines were isolated after the eight-step synthesis sequence

Table 4. 1,4-Benzodiazepin-2-one Derivatives 33 (Scheme 8)

	derivative			yield
entry	R^1	R^2	R^3	(%)[a]
1	C_6H_4-2-OCH_3	CH_3	CH_2CH_3	67
2	C_6H_4-3-OCH_3	CH_3	CH_2CH_3	79
3	C_6H_4-4-OCH_3	CH_3	CH_2CH_3	73
4	*c*-C_6H_{11}	CH_3	CH_2CH_3	70
5	C_6H_4-2-CH_3-3-CN	CH_3	CH_2CH_3	64
6	C_6H_4-3-CF_3	CH_3	CH_2CH_3	74
7	2-thienyl	CH_3	CH_2CH_3	52
8	2-furyl	CH_3	CH_2CH_3	59
9	adamantyl	CH_3	CH_2CH_3	80
10	C_6H_4-2-Cl	CH_3	CH_2CH_3	52
11	*c*-C_5H_{10}-1-C_6H_5-4-Cl	$(CH_2)_2CO_2H$	CH_2CN	63
12	$(CH_2)_2CO_2CH_3$	CH_3	CH_2CH_3	58
13	C_6H_4-4-$C(CH_3)_3$	CH_3	CH_2CH_3	75
14	5-(1,3-benzodioxolyl)	$CH_2C_6H_5$-4-OH	CH_2CONH_2	82
15	3-naphthyl	CH_3	CH_2CH_3	81

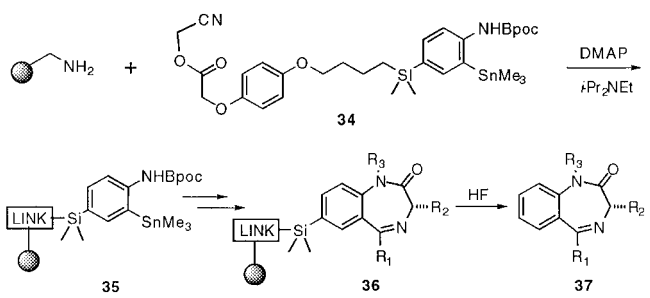
[a] Yields of purified material are based on the initial aminomethyl loading level of the resin.

in >85% purity by ^{1}H NMR analysis of crude products. Yields of purified benzodiazepine products varied from 46% to 72% (av 61%, 15 compounds) based on the initial aminomethyl loading of the polystyrene resin used.[82]

Bunin and co-workers have reported the preparation of a library of 11 200 discrete 1,4-benzodiazepines[11] from 20 acid chlorides, 35 amino acids, and 16 alkylating agents, all of which were commercially available (Figure 16). The acid chlorides were selected from a set of over 300 commercially available acid chlorides that are compatible with the synthesis sequence using a similarity grouping procedure developed by Dr. Steven Muskal at MDL Information Systems to select as diverse a set as possible. The alkylating agents and amino acids also displayed a range of functionality. The library is currently being screened by a number of industrial and academic collaborators.

Plunkett and Ellman have also demonstrated a silyl linkage strategy for the synthesis of benzodiazepine derivatives.[83] Linkage of aromatic compounds to solid supports through silyl groups is likely to become an important and general strategy, since cleavage from the resin is accomplished by protodesilation to leave no trace of the linkage site. For the synthesis of 1,4-benzodiazepine derivatives, the silyl-substituted (aminoaryl)stannane derivative **34**, which is synthesized in five steps in solution, is coupled to aminomethylated polystyrene (Scheme 9). The syn-

Scheme 9



thesis of support-bound 1,4-benzodiazepines then proceeds as described previously. Treatment of the

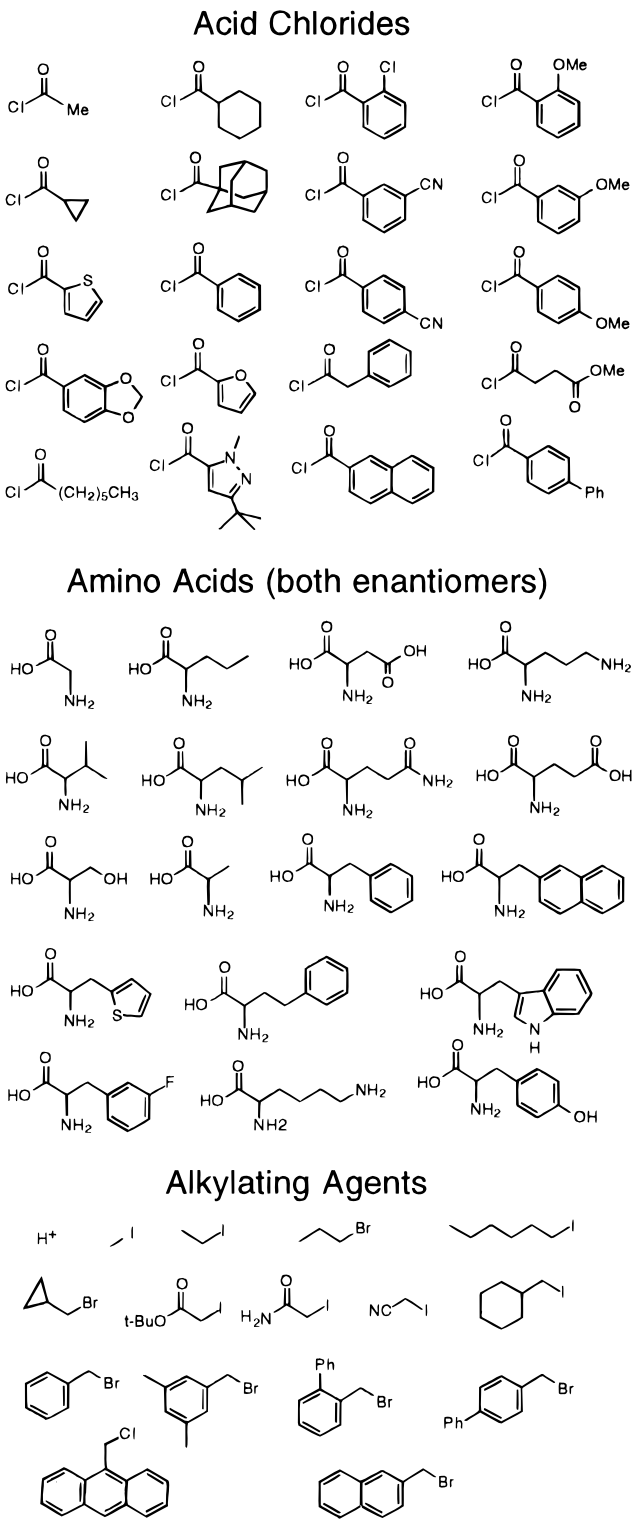


Figure 16. Building blocks used to prepare a 11 200-member benzodiazepine library.

support-bound benzodiazepine **36** with anhydrous HF then provides the benzodiazepine product **37**. Good purity of the crude product is observed, >85% by ^{1}H NMR, and the isolated yield is 50−68% (av 61%, four compounds) after purification based on the initial aminomethyl loading of the polystyrene resin.

In one of the early reports of small molecule library synthesis, DeWitt and co-workers described an alternative strategy for the synthesis of 1,4-benzodiazepin-2-ones.[12] The synthesis is based on a two-step procedure of trans-imidation followed by a cyclative cleavage (Scheme 10). The resins were purchased as Boc-protected Merrifield resins and deprotected with 1:1 TFA/CH_2Cl_2. The 2-aminobenzophenone imine derivatives **38** were then added to form the support-bound imines, followed by heating to 60 °C in TFA to provide the desired benzodiazepine product. The cyclative nature of the cleavage was designed to enhance the purity of the cyclized product, since incomplete products would be expected to stay bound

Scheme 10

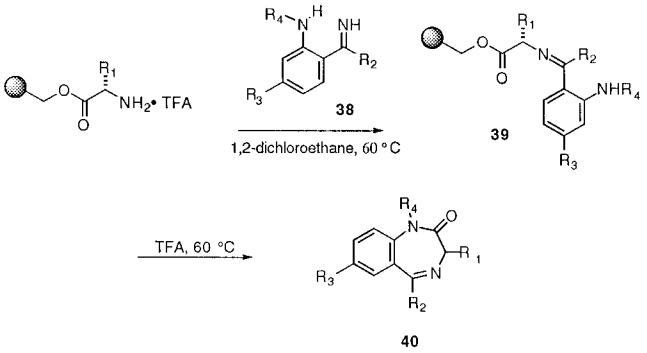


Table 5. 1,4-Benzodiazepin-2-one Derivatives 40 (Scheme 10)

	derivative				mass balance
entry	R_1	R_2	R_3	R_4	(%)[a]
1	CH_3	C_6H_5	H	H	40
2	CH_3	C_6H_5	Cl	H	56
3	CH_3	C_6H_5-4-OCH_3	H	H	34
4	CH_3	C_6H_5	NO_2	H	28
5	CH_3	see *b* below	see *b* below	H	63
6	CH_3	C_6H_5	Cl	Me	18
7	CH_3	*c*-C_6H_{11}	H	H	41
8	CH_3	2-thienyl	H	H	47
9	H	C_6H_5	H	H	44
10	H	C_6H_5	Cl	H	55
11	H	C_6H_5-4-OCH_3	H	H	23
12	H	C_6H_5	NO_2	H	31
13	H	see *b* below	see *b* below	H	16
14	H	C_6H_5	Cl	Me	20
15	H	*c*-C_6H_{11}	H	H	32
16	H	2-thienyl	H	H	41
17	$CH_2C_6H_5$	C_6H_5	H	H	52
18	$CH_2C_6H_5$	C_6H_5	Cl	H	46
19	$CH_2C_6H_5$	C_6H_5-4-OCH_3	H	H	41
20	$CH_2C_6H_5$	C_6H_5	NO_2	H	26
21	$CH_2C_6H_5$	see *b* below	see *b* below	H	52
22	$CH_2C_6H_5$	C_6H_5	Cl	Me	13
23	$CH_2C_6H_5$	*c*-C_6H_{11}	H	H	39
24	$CH_2C_6H_5$	2-thienyl	H	H	48
25	3-CH_2indolyl	C_6H_5	H	H	43
26	3-CH_2indolyl	C_6H_5	Cl	H	33
27	3-CH_2indolyl	C_6H_5-4-OCH_3	H	H	31
28	3-CH_2indolyl	C_6H_5	NO_2	H	23
29	3-CH_2indolyl	see *b* below	see *b* below	H	23
30	3-CH_2indolyl	C_6H_5	Cl	Me	10
31	3-CH_2indolyl	*c*-C_6H_{11}	H	H	34
32	3-CH_2indolyl	2-thienyl	H	H	40
33	$CH(CH_3)_2$	C_6H_5	H	H	31
34	$CH(CH_3)_2$	C_6H_5	Cl	H	28
35	$CH(CH_3)_2$	C_6H_5-4-OCH_3	H	H	29
36	$CH(CH_3)_2$	C_6H_5	NO_2	H	9
37	$CH(CH_3)_2$	see *b* below	see *b* below	H	29
38	$CH(CH_3)_2$	C_6H_5	Cl	Me	11
39	$CH(CH_3)_2$	*c*-C_6H_{11}	H	H	27
40	$CH(CH_3)_2$	2-thienyl	H	H	37

[a] Mass balance of crude material cleaved from the resin based on amino acid loading level. [b] Prepared from 2-amino-9-fluorenone rather than a 2-aminobenzophenone.

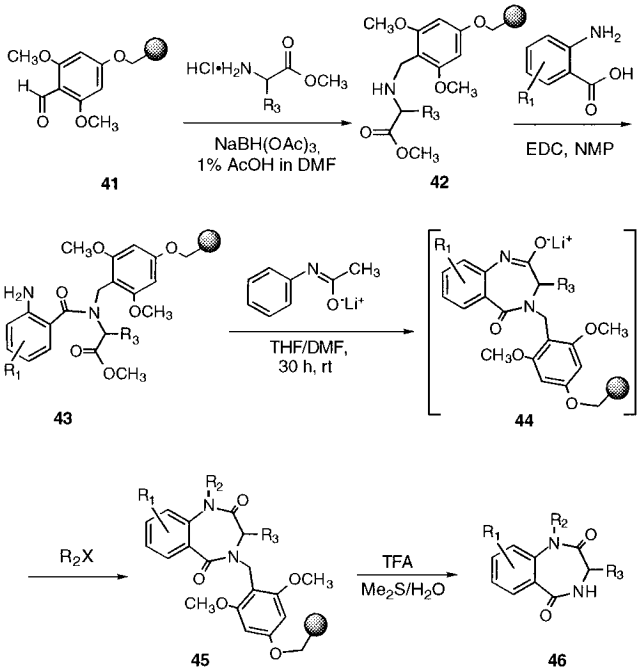
Figure 17. Structures of 1,4-benzodiazepin-2-one and 1,4-benzodiapine-2,5-dione.

to the support. The desired benzodiazepine derivatives were purified by extraction and isolated in crude mass balances of 9−63% (av 34%, 40 compounds) in >90% purity as measured by ^{1}H NMR (Table 5).

1,4-Benzodiazepine-2,5-diones. Boojamra and co-workers have also reported a general and high-yielding method for the solid-phase synthesis of 1,4-benzodiazepine-2,5-diones (Figure 17).[84] The synthesis strategy complements the previously described 1,4-benzodiazepin-2-one synthesis sequence described by Bunin and co-workers, since a wide range of functionality can be directly introduced onto the aromatic core of the benzodiazepine structure (R^1) from the greater than 40 commercially available anthranilic acids or related heterocyclic structures. The other two sites of diversity are introduced with α-amino esters and alkylating agents, of which there are also a number of derivatives that are commercially available.

The synthesis of 1,4-benzodiazepine-2,5-diones is initiated by loading an α-amino ester onto the aldehyde-derivatized support by reductive amination employing $NaBH(OAc)_3$ in DMF with 1% AcOH (Scheme 11).[85] Racemization is not observed if the

Scheme 11



imine resulting from condensation of the α-amino ester and aldehyde **41** is reduced immediately upon its formation. Acylation of the resulting secondary amine **42** with a commercially available unprotected anthranilic acid then provides the support-bound tertiary amide **43**. The optimal conditions for effecting this transformation are to employ a carbodiimide in conjunction with the hydrochloride salt of a tertiary amine. Ethyl-3-[3-(dimethylamino)propyl]-

Table 6. Synthesis of 1,4-Benzodiazepine-2,5-diones 46 (Scheme 11)

entry	R_1	R_2	R_3	yield (%)[a]
1	8-Cl	CH_2CH_3	$CH_2CH(CH_3)_2$	75
2	7-Cl	$CH_2CH{=}CH_2$	$CH_2C_6H_5$	89
3	7-Br	CH_2CH_3	$CH_2CH(CH_3)_2$	71
4	8-NO_2	CH_2CH_3	$CH_2CH(CH_3)_2$	92
5	6-F	CH_2CONH_2	$CH_2C_6H_5$	62
6	8-OCH_3	CH_2c-C_3H_5	$CH_2CH(CH_3)_2$	79
7[b]	7-(C_6H_4)-*p*-OCH_3	CH_2CH_3	$CH_2CH(CH_3)_2$	62
8[b]	8-$(CH_2)_5CH_3$	$CH_2C_6H_4$-*p*-Ph	$CH_2C_6H_4OH$	77
9	7-Cl	$CH_2CH{=}CH_2$	$(CH_2)_4NH_2$	63
10	8-Cl	H	$CH_2CH(CH_3)_2$	89
11	7-Cl	H	$CH_2C_6H_5$	89

[a] Yields of purified materials are based on the loading levels of leucine and phenylalanine ester derived resins. [b] Suzuki cross-coupling products.

carbodiimide (EDC) is the most convenient activating agent since the tertiary amine hydrochloride is present in the carbodiimide structure. Cyclization and then subsequent alkylation of the support-bound anilide anion **44** generated in situ is next accomplished in a single step by treatment of amide **43** with the lithium salt of acetanilide in DMF/THF (1:1) for 30 h, followed by addition of an appropriate alkylating agent. Additional diversity may also be introduced onto the benzodiazepine through the Suzuki cross-coupling reaction as is exemplified in entry 7 (Table 6), where a cross-coupling reaction was carried out with *p*-methoxybenzeneboronic acid, and in entry 8 (Table 6) where a Suzuki cross-coupling reaction was performed using *B*-hexyl-9-BBN. The benzodiazepine products are cleaved from the support by treatment with TFA/Me_2S/H_2O (90:5:5). Good yields were obtained for a range of different derivatives including benzodiazepines that incorporate amino acids with side-chain functionality such as tyrosine and lysine, entries 8 and 9 in Table 6, respectively. In addition, no detectable racemization, <3%, is observed throughout the synthesis sequence as determined by chiral HPLC analysis and derivatization studies.

A synthetic route to the 1,4-benzodiazepine-2,5-dione class was also reported by Zuckermann and co-workers at Chiron.[86] In this study the 1,4-benzodiazepine-2,5-dione was synthesized from the *N*-terminus of a support-bound peptoid intermediate **47** (Scheme 12). Support-bound peptoid intermediate **47** is acylated with bromoacetic acid followed by displacement of the bromide with an α-amino ester.

Scheme 12

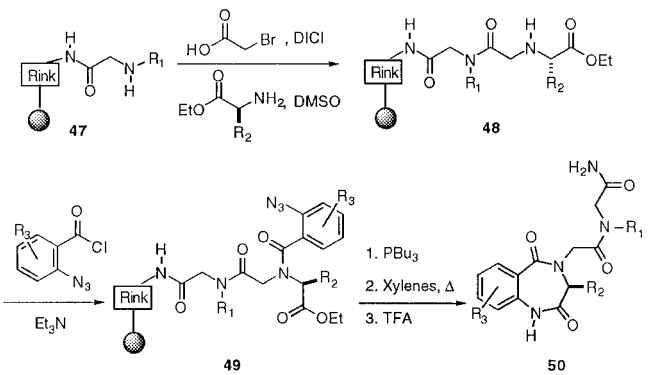


Table 7. 1,4-Benzodiazepine-2,5-dione Derivatives 50 (Scheme 12)

	derivative				
entry	R_1	R_2	R_3	yield (%)[a]	purity (%)[b]
1	$CH_2CH(CH_3)_2$	H	H	55	>65
2	$CH_2CH(CH_3)_2$	CH_3	H	55	80
3	$CH_2CH(CH_3)_2$	CH_3	9-Cl	>90	79
4	$CH_2CH(CH_3)_2$	$CH_2C_6H_5$	H	41	92
5	$CH_2CH(CH_3)_2$	C_6H_5	H	53	>95
6	$CH_2CH(CH_3)_2$	CH_2OH	H	34	80
7	$CH_2CH(CH_3)_2$	$CH_2C_6H_5$-4-OH	H	68	61
8	$CH_2CH(CH_3)_2$	$CH(CH_3)_2$	H	41	72
9	$CH_2CH(CH_3)_2$	CH_2CO_2H	H	52	69
10	$CH_2CH(CH_3)_2$	$(CH_2)_2CO_2H$	H	60	70
11	$CH_2CH(CH_3)_2$	$(CH_2)_4NH_2$	H	50	97
12	$CH_2CH(CH_3)_2$	$(CH_2)_3NH_2$	H	90	63
13	$CH_2CH(CH_3)_2$	CH_3	10-CH_3	37	61
14	$CH_2CH(CH_3)_2$	CH_3	8-OTf	nd	88
15	$CH_2CH(CH_3)_2$	CH_3	8-NO_2	nd	93
16	$CH_2CH(CH_3)_2$	$CH(OH)CH_3$	H	50	59
17	myrtanyl[c]	CH_3	H	75	93
18	myrtanyl[c]	$CH_2C_6H_5$	H	55	84
19	9-fluorenyl	CH_3	H	41	85
20	5-indanyl[d]	CH_3	H	58	83
21	$CH(CO_2CH_2CH_3)CH_2C_6H_5$	$CH_2C_6H_5$	H	49	64

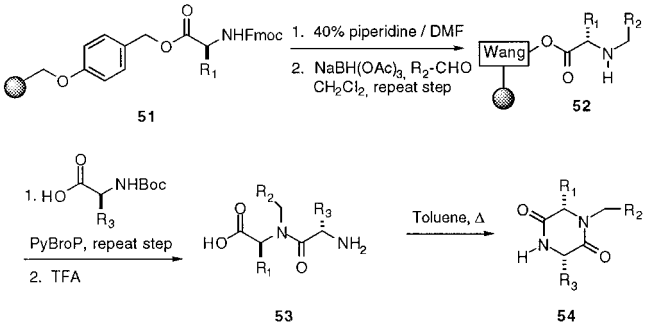
[a] Yields of unpurified product based on the loading level of the starting resin. [b] Purity as estimated by HPLC. [c] Derived from myrtanylamine. [d] Derived from 5-aminoindan. nd = not determined.

The resulting amine **48** is then acylated with a substituted *o*-azidobenzoyl chloride. Reaction with tributylphosphine affords the iminophosphorane, which cyclizes upon heating to 125 °C to afford the support-bound benzodiazepine. Cleavage with TFA provides the 1,4-benzodiazepine-2,5-dione **50**, which is lyophilized twice from acetic acid. A number of amino acids were evaluated for compatibility with the synthesis sequence (entries 1−12, Table 7). The study includes the synthesis of 21 compounds that were isolated in crude mass balances of 34−90% (av 55%) and HPLC purities estimated at 59−97% (av 78%). Racemization was examined for one model benzodiazepine (entry 18). The diastereomeric excess of this product was 87%, and further studies are reported to be in progress.

A model study was also performed to demonstrate that the synthesis sequence could be performed in a split synthesis format for library synthesis. An equimolar mixture of eight monopeptoid resins were pooled and transformed into 1,4-benzodiazepine-2,5-diones using L-Phe methyl ester and 2-azidobenzoyl chloride. Cleavage of the resulting products from the resin provided a sample pool of eight compounds that was evaluated by Electrospray MS and HPLC. All eight desired benzodiazepines were present in addition to uncyclized material. The authors report that further functionalization of the benzodiazepin-2,5-diones can be accomplished by Suzuki reaction of triflate substituted derivatives or a nitro reduction and acylation sequence (data not provided).

2. Six-membered Rings. ***Diketopiperazines.*** Gordon and Steele have reported a general strategy for the solid-phase synthesis of diketopiperazines employing readily available amino acids and aldehydes to introduce diversity.[87] Reductive amination of a support-bound amino acid was first accomplished by treatment with an aldehyde and sodium triacetoxyborohydride in CH_2Cl_2 (Scheme 13). The reductive amination step was performed twice to improve conversions to 85−95%, except for sterically hindered

Scheme 13



amino acids and/or electronically deactivated aldehydes which gave lower conversions. In fact, for the most difficult case where Val and an electronically deactivated aldehyde was employed, only 20% conversion was observed. Partial racemization was also noted for some amino acids (Phe gave approximately 10% racemization by chiral HPLC). Both aromatic and aliphatic aldehydes were employed in the reductive amination step, although aliphatic aldehydes provided noticeable dialkylation (1−10%). An *N*-Boc amino acid was then coupled to the secondary amine employing PyBroP as the activating agent (double coupling was used to drive the reaction to completion). The Boc protecting group was then removed with concomitant cleavage from the support with TFA. The resulting dipeptide acids were then dissolved in toluene and heated at reflux for 5 h to provide the desired diketopiperazine products **54**. The yields of two purified diketopiperazines were reported, 42% yield when $R_1 = CH_2C_6H_5$, $R_2 = CH_2C_6H_4$-4-OCH_3, and $R_3 = CH_3$, and 24% yield when $R_1 = CH(CH_3)_2$, $R_2 = CH_2C_6H_2$-2,4,6-$(OCH_3)_3$ and $R_3 = CH_2CH(CH_3)_2$.

A library of 1000 DKP's was then prepared using the split synthesis approach from 10 *N*-Fmoc amino acid-derivatized resins, 10 aldehydes, and 10 *N*-Boc amino acids (Figure 18). The 10 *N*-Fmoc amino acid-derivatized resins were deprotected, pooled, and

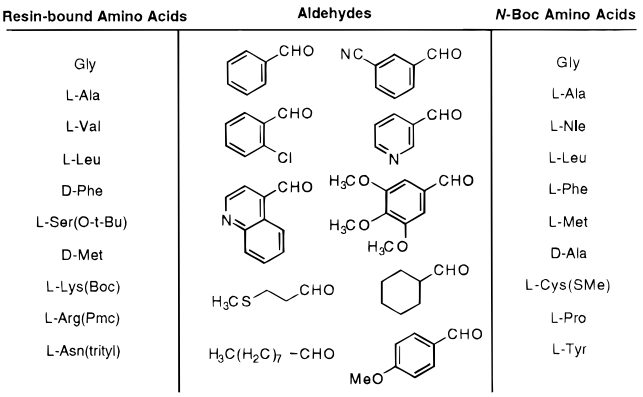


Figure 18. Building blocks used to prepare a 1000-member diketopiperazine library.

reductively alkylated with 10 different aldehydes to generate 10 mixtures of 10 support-bound *N*-alkyl amino acids **52**. These mixtures were characterized using HPLC-MS and MS-MS after cleavage from the support to confirm the presence of 96 out of the 100 expected *N*-alkyl amino acids. The resulting resins were then repooled, mixed, and divided into 10 separate pools. Each pool was coupled with a unique *N*-Boc amino acid, followed by cleavage from the resin and cyclization to provide a library of 1000 compounds as 10 mixtures of 100 compounds each. In a later publication, Terrett reported that biological evaluation of this library resulted in the identification of a number of active diketopiperazines including a ligand to the neurokinin-2 receptor ($IC_{50} = 313$ nM).[6b]

Isoquinolinones. Zuckermann and Goff have reported a method for the solid-phase synthesis of isoquinoline derivatives, which grew from a desire to increase the structural rigidity, complexity, and diversity of their existing peptoid libraries.[88] Coupling of *trans*-4-bromo-2-butenoic acid to the support through the Rink linker is accomplished with DICI (Scheme 14). Bromide displacement with a primary amine is followed by acylation of the resulting secondary amine with a 2-iodobenzoyl chloride derivative to provide the support-bound amide **57**. Palladium-mediated cyclization by an intramolecular Heck reaction using $Pd(PPh_3)_4$ followed by cleavage with TFA then provides the (2*H*)-isoquinoline **58**. However, when a 2-iodobenzoyl chloride derivative was employed that also contained a substituent in the 3-position (entries 4 and 8, Table 8), a mixture of product isomers **58** and **59** was seen, which could favor isomer **59** (entry 4, Table 8). Further experiments lead the authors to conclude that isomer **59** is produced first, with equilibration to isoquinoline **58** under the reaction conditions, probably through a mechanism of readdition and elimination of Pd−H. Presumably, an ortho substituent hinders the readdition of Pd−H. Mass balances after cleavage and lyophilization are reported for eight compounds (65−92%, av 77%), which are obtained in good to purity as estimated by HPLC (70−95%, av 87%).

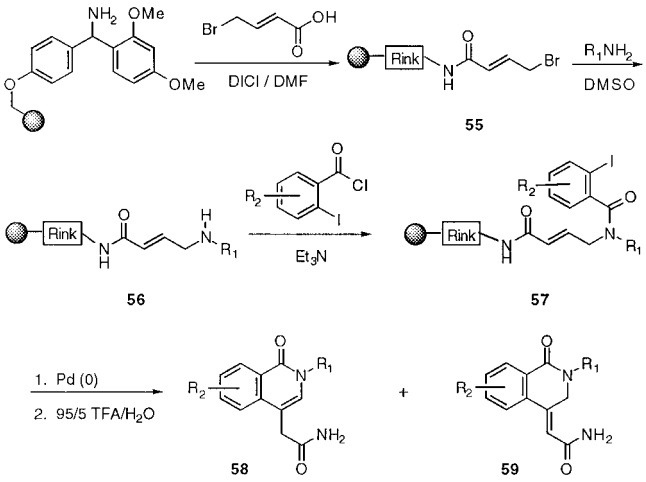
A pooling strategy was also investigated, where seven of the different support-bound intermediates **56** (Scheme 14) were mixed and then converted to the isoquinoline products. Cleavage and HPLC showed the presence of all seven desired products and the structures were verified by ES-MS.

Table 8. 2-Substituted 1-(2H)-Isoquinolinones 58 (Scheme 14)

	derivative		yield	purity
entry	R_1	R_2	(%)[a]	(%)[b]
1	$CH_2CH(CH_3)_2$	H	69	83
2	$CH_2CH_2C_6H_5$	H	65	80
3	C_6H_5	H	85	>70
4	$CH_2CH(CH_3)_2$	5-CH_3	92 (1/3.2)[c]	94
5	$CH_2CH(CH_3)_2$	8-F	80	90
6	$CH_2CH(CH_3)_2$	6,7-$(OCH_3)_2$	77	95
7	$CH_2CH(CH_3)_2$	7-Cl	79	90
8	$CH_2CH(CH_3)_2$	5-OCH_3	69 (1.7/1)[c]	93

[a] Yields of unpurified product after lyophilization from acetic acid. [b] Purity as estimated by HPLC. [c] Values in parentheses are ratios of **58** to **59**, otherwise only **58** was observed.

Scheme 14



1,4-Dihydropyridines. Gordeev and co-workers at Affymax have utilized a Hantzsch-type reaction to provide 1,4-dihydropyridines, which have served as the nucleus of numerous bioactive compounds.[89] Condensation of a β-keto ester with the free amine from either the Rink (shown) or the PAL linker gives the support-bound enamino ester **60** (Scheme 15). Treatment with an aldehyde and a second β-keto ester, which forms a 2-arylidene β-keto ester in situ (this reagent can also be preformed), provides the resin-bound intermediate **61**. Cleavage with TFA results in cyclization to give 1,4-dihydropyridines **62** (Table 9). The intermediacy of a support-bound cyclized 1,4-dihydropyridine is also possible; however, in a model study using ^{13}C-enriched ethyl acetoacetate ($R_1 = {}^{13}C$ methyl), the ^{13}C NMR of the support-bound intermediate prior to cyclization showed two signals for the R_1 methyl, tentatively assigned to the

Scheme 15

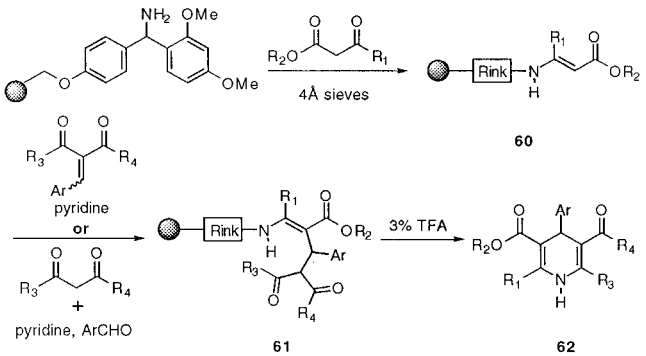


Table 9. 1,4-Dihydropyridine Derivatives 62 (Scheme 15)

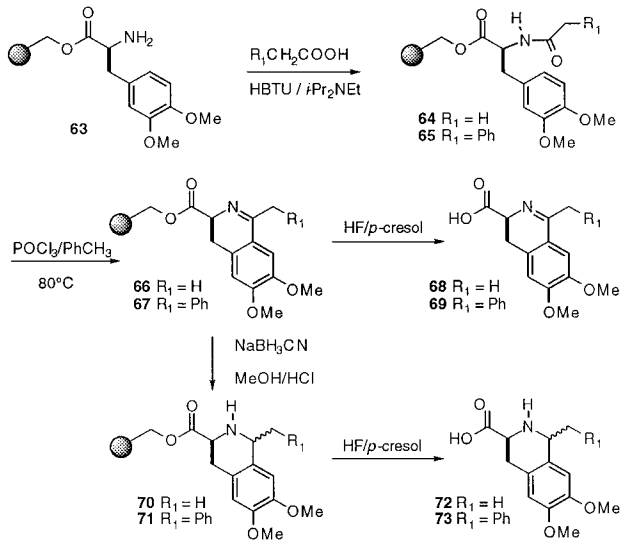
	derivative					
entry	Ar	R_1	R_2	R_3	R_4	yield (%)[a]
1	C_6H_4-2-NO_2	CH_3	CH_3	CH_3	OCH_3	65[b] (70)[c]
2	C_6H_4-3-NO_2	CH_3	CH_3	CH_3	OCH_2CH_3	75[c]
3	C_6H_4-3-NO_2	CH_3	$CH(CH_3)_2$	CH_3	$O(CH_2)_2OCH_3$	78[c]
4	C_6H_4-4-NO_2	CH_3	CH_3	CH_3	CH_3	75[c]
5	C_6H_4-4-NO_2	CH_3	CH_2CH_3	CH_3	OCH_3	70[c]
6	C_6H_4-4-NO_2	CH_2CH_3	CH_3	C_6H_5	OCH_2CH_3	70[c]
7	C_6H_4-4-CN	CH_3	CH_3	CH_3	OCH_3	74[c]
8	C_6H_5	CH_3	CH_3	CH_3	$OCH_2CH{=}CH_2$	72[b]
9	4-pyridyl	CH_3	CH_3	CH_3	OCH_3	75[c]

[a] Yields are based on the loading level of the starting resin. [b] Prepared by two-component condensation with $ArCH{=}C(COR_3)CO_2R_4$ using PAL resin. [c] Prepared by three-component condensation using Rink resin, ArCHO, and $R_3COCH_2COR_4$.

E and *Z* isomers of intermediate **61**. The IR of the support-bound intermediate also showed an absorbance at 1735 cm^{-1} while the cyclized product showed the expected absorbance at 1705 cm^{-1}. Interestingly, pyridine was also necessary in the condensation step to facilitate isomerization of the imine to the enamine, as undesired cycloaddition byproducts were observed without it. The synthesis sequence is notable because the reaction conditions are mild, the synthesis proceeds in good overall yields (av 73%, nine compounds), and a variety of functionality could potentially be displayed about the structure from commercially available starting materials.

Dihydro- and Tetrahydroisoquinolines. Meutermans and Alewood have demonstrated the synthesis of dihydro- and tetrahydroisoquinolines on support.[90] Acylation of support-bound dimethoxyphenylalanine with either acetic acid or phenylacetic acid produced the intermediate amide **64** or **65** (Scheme 16). The support-bound dihydroisoquinolines **66** and **67** were then obtained by treatment of amides **64** and **65** with $POCl_3$ at 80 °C to effect a Bischler−Napieralski cyclization reaction. The dihydroisoquinolines **68** and **69** were released from the resin by treatment with HF/*p*-cresol. The support-bound dihydroisoquinolines **66** and **67** were also converted to the tetrahydroquinolines **70** and **71** by treatment with $NaBH_3CN$ before cleavage from the support. All four products were obtained in high purity as estimated by HPLC analysis. The isolated

Scheme 16

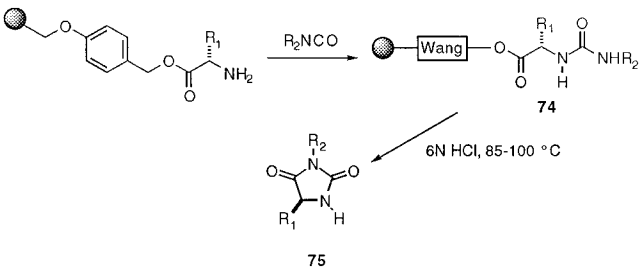


yield after HPLC purification for each of the dihydroisoquinolines **68** and **69** was ∼40%, and the yields of the tetrahydroisoquinolines **72** and **73** were 25% and 30%, respectively, as a 6:1 mixture of diastereomers for both compounds.

In a separate experiment an equimolar mixture of eight acetic acid derivatives (R_1 = H, phenyl, *t*-Bu, naphthyl, *p*-methoxyphenyl, 3,4-dimethoxyphenyl, *p*-nitrophenyl, and *p*-hydroxyphenyl) was coupled to support-bound amino ester **63** and then submitted to the cyclization and cleavage steps. The parent ions of all eight dihydro- and tetrahydroisoquinolines were identified by ionspray MS, but no other characterization of the mixtures was provided.

3. Five-Membered Rings. ***Hydantoins.*** In one of the earliest studies of organic compound library synthesis, DeWitt and co-workers reported a method for the synthesis of hydantoin derivatives employing the Diversomer™ apparatus.[12] Eight resin-bound amino acids (Phe, Gly, Ile, Leu, Ala, Val, Trp, and diphenylglycine) were condensed with five different isocyanates (structures not provided) in DMF to provide resin-bound ureas **74** (Scheme 17). Heating

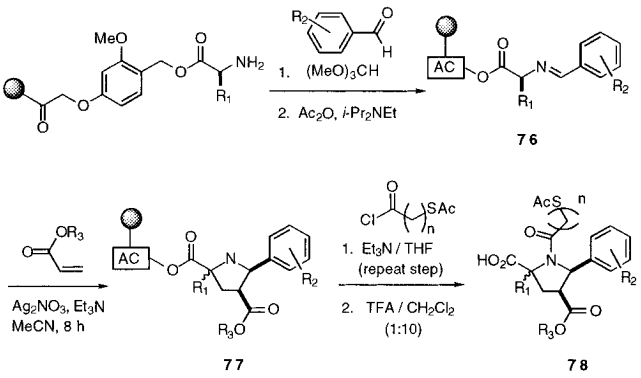
Scheme 17



the resin-bound ureas **74** at 85−100 °C in 6 M HCl for 2 h resulted in cyclative cleavage to provide the hydantoin products **75** in crude mass balances of 4−81%, (av 30%, 40 compounds) with characterization by TLC, MS, and ^1H NMR.

Pyrrolidines. Gallop and co-workers have developed a solid-phase synthesis of mercaptoacyl proline derivatives with a metalloazomethine ylide cycloaddition reaction serving as the key step for introducing diversity.[91] Condensation of an amino acid-derivatized resin with an aromatic aldehyde in neat trimethylorthoformate provided the Schiff base **76**, and any unreacted amines were capped with acetic anhydride (Scheme 18). The dehydrating conditions that were employed to effect Schiff base formation

Scheme 18



had previously been reported by the authors.[92] The 1,3-dipolar cycloaddition was then performed with the azomethine ylide derived from the support-bound Schiff base **76** and an acrylate or acrylonitrile as the dipolarophile under Lewis acid-mediated conditions that are analogous to those initially reported by Grigg and Tsuge for the corresponding solution-phase reaction.[93] The resulting racemic substituted proline derivatives were then acylated with various ω-mercaptoacyl chlorides followed by acidolytic cleavage from the support to provide the desired (mercaptoacyl)proline product **78** (Scheme 18). The isolated yields for six representative products ranged from 50 to 80% and the diastereoselectivities ranged from 2.5:1 to 10:1. The authors reported that the products typically arose from an endo-selective cycloaddition with the syn configuration of the support-bound azomethine ylide. Dipolarophiles lacking a carbonyl substituent showed decreased stereoselectivity. In addition, the diastereoselectivity was dependent upon the nature of the resin support and the concentration of the Lewis acid catalyst.

The authors prepared a small library of (mercaptoacyl)proline derivatives **78**, employing the optimized synthesis sequence and the split synthesis procedure. The library was prepared from four amino acids, four aromatic aldehydes, five olefins, and three mercaptoacyl chlorides (Figure 19). Because the library was targeted toward the identification of angiotensin-converting enzyme (ACE) inhibitors, the building blocks were selected in part upon the structure activity relationships of known (mercaptoacyl)proline-based inhibitors such as captopril. Although the total number of combinations of the building blocks in the library was 240; because the reactions were not completely regio- and stereospecific, approximately 500 compounds were prepared.

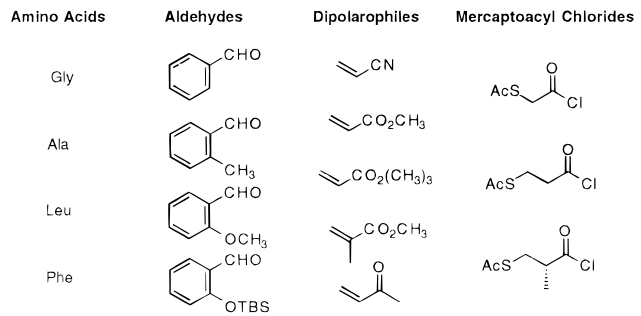


Figure 19. Building blocks used to prepare an ~500-member mercaptoacyl proline library.

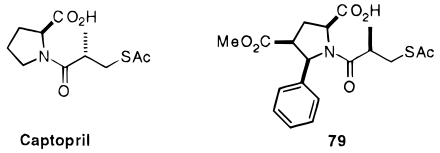
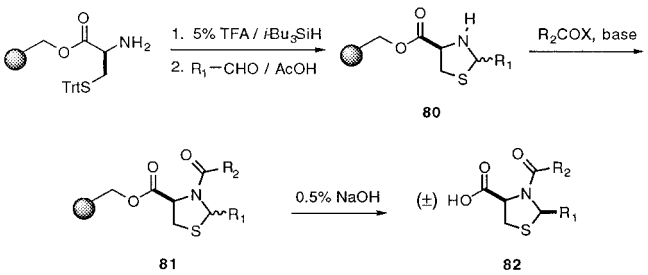


Figure 20. Captopril and the identified mercaptoacyl proline ACE inhibitor, **79**.

The library was deacetylated by treatment with ethylenediamine and then screened for inhibition of ACE. Deconvolution using the standard iterative resynthesis and evaluation protocol resulted in the identification of a potent new inhibitor, **79** (Figure 20), with a K_i of 160 pM. Inhibitor **79** is 3-fold more potent than captopril and is among the most potent thiol-containing ACE inhibitors yet described.

Thiazolidine-4-carboxylic Acids. Utilizing Fmoc-Cys(Trt)-OH as a starting point, the Selectide group has synthesized a number of *N*-acylthiazolidines on PEG-PS resin.[94] Fmoc-Cys(Trt)-OH was coupled directly onto PEG-PS-OH resin (Scheme 19). The

Scheme 19



Fmoc and side chain trityl protecting groups were then cleaved under standard conditions. Addition of an aldehyde in acetic acid resulted in imine formation followed by cyclization to provide the support-bound thiazolidine **80**. Acylation of the thiazolidine **80** in pyridine with either acetic anhydride, benzoyl chloride, or an isocyanate followed by cleavage with NaOH provided the racemic *N*-acylthiazolidine **82** as a single diastereomer (epimerization occurs upon hydrolysis). The products were isolated in <10−94% yield after extractive workup. Purity was estimated at >97% by HPLC analysis (Table 10). The authors also report that *N*-acylthiazolidines prepared from electron-rich aromatic aldehydes are not stable in 60−100% TFA/CH_2Cl_2, conditions that are necessary to cleave many of the side-chain protecting groups

Table 10. Thiazolidine-4-carboxylic Acid Derivatives (Scheme 19)

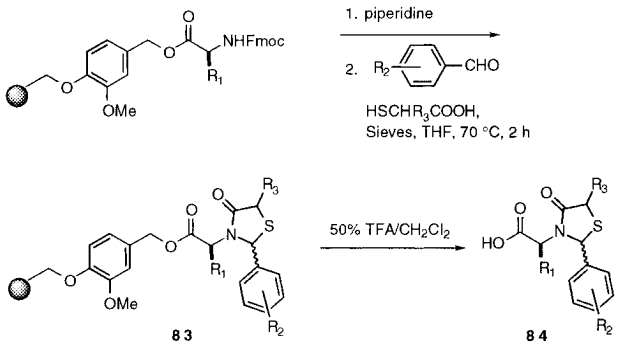
	derivative		
entry	R_1	R_2	yield (%)[a]
1	C_6H_4-4-OCH_3	CH_3	90
2	C_6H_4-4-NO_2	CH_3	0
3	C_6H_4-4-OCH_3	$NHCH_3$	69
4	H	CH_3	61
5	H	C_6H_5	81
6	$CH(CH_3)_2$	CH_3	40
7	$CH(CH_3)_2$	C_6H_5	79
8	$CH(CH_3)_2$	NHC_6H_5	58
9	$CH_2CH(CH_3)_2$	CH_3	75
10	2-thienyl	CH_3	63

[a] Crude mass balance after extractive workup.

that are commonly used in solid-phase synthesis. To address this problem, oxidation of the thioether to the sulfoxide was accomplished in good yield using MCPBA, providing acid-stable products. A variety of conditions, however, failed to provide the corresponding sulfone in good yield.

4-Thiazolidinones and Related 4-Metathiazanones. Holmes and co-workers at Affymax have reported the synthesis of 4-thiazolidinones and related metathiazanones from a support-bound amino acid, an aromatic aldehyde and a mercaptoacetic acid derivative (Scheme 20).[95] After Fmoc deprotection

Scheme 20



of commercially available amino acid-loaded resins (polystyrene, PEG-PS, and a polydimethylacrylamide/polyhipe support were all used with equal success), a one-pot reaction was performed with an aryl aldehyde and a mercaptoacetic acid (mercaptoacetic acid and thiolactic acid were used) with heating at 70 °C and with 3 Å molecular sieves as a dehydrating agent. Cleavage from the support with 50% TFA in CH_2Cl_2 provided the thiazolidinone derivatives in good purity as estimated by HPLC (84−98%, av 94%, Table 11). Diastereomeric thiazolidinones are formed from chiral amino acids, and bulky substituents were shown to provide modest diastereoselectivity (≤4:1 major/minor). Attempts to use β-mercaptopropionic acid to form the analogous six-membered ring metathiazanones were not as successful. A derivative of glycine was isolated in good purity, but extension to Ala and Phe led to the formation of <10% of the desired product.

Table 11. 4-Thiazolidinones 84 and Related 4-Metathiazanones Derivatives (Scheme 20)

	derivative			purity	yield
entry	R_1	R_2	R_3	(%)[a]	(%)[b]
1	H	C_6H_5	H	60	98
2	H	C_6H_5	CH_3	97	96
3	H	C_6H_4-2-CH_3	H	92	98
4	H	C_6H_4-3-CH_3	H	91	97
5	H	C_6H_4-4-CH_3	H	100	95
6	H	3-pyridyl	H	55	99
7	CH_3	C_6H_5	H	82	92
8	see *c* below	C_6H_5	H	94	96
9	$CH_2C_6H_5$	C_6H_5	H	86	84
10	$CH(CH_3)_2$	C_6H_5	H	99	88
11[d]	H	3-pyridyl	see *d* below	93	64
12[d]	CH_3	C_6H_5	see *d* below	78	<10
13[d]	$CH_2C_6H_5$	C_6H_5	see *d* below	67	<10

[a] Purity as estimated by HPLC. [c] Prepared from β-aminopropionic acid. [d] Synthesis of 4-metathiazanones from β-mercaptopropionic acid.

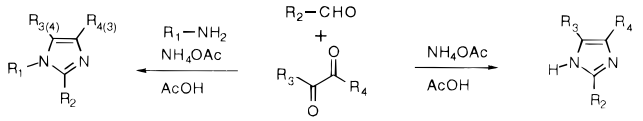
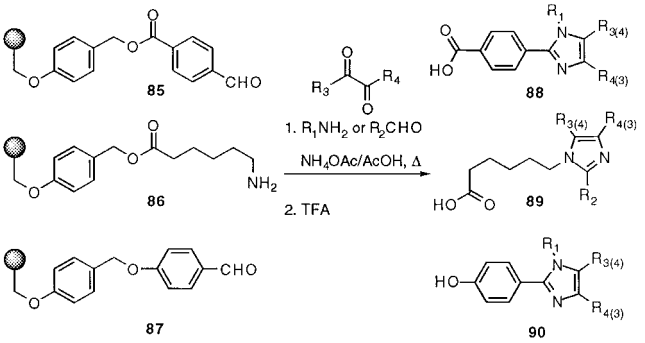


Figure 21. Preparation of imidazoles.

Imidazoles. Sarshar and co-workers at Ontogen corporation have developed a method for the solid-phase synthesis of imidazole derivatives based on a one-pot procedure.[96] The imidazole derivatives may be prepared by either a three-component or a four-component process (Figure 21). In the three-component process NH_4OAc, a 1,2-dione, and an aldehyde are heated in AcOH to provide the imidazole product, and in the four-component process NH_4OAc, a primary amine, a 1,2-dione, and an aldehyde are submitted to the same reaction conditions (the relative stoichiometry of the reagents is used to control primary amine introduction).

The authors perform the synthesis on solid support by linking either the amine or aldehyde component to the support (Wang resin) as shown in Scheme 21.

Scheme 21



The support-bound component is then heated with the remaining reagents in AcOH at 100 °C for 4 h. The derivatized resins **85**−**87** were prepared by either simple carbodiimide-mediated ester formation, or a solid-phase Mitsunobu protocol originally reported by Richter and Gadek.[97] Rink resin was also examined in addition to Wang resin, but it was found to be too unstable under the acidic reaction conditions.

The imidazoles **88**−**90** were isolated in 90−95% purity and excellent yield (71−99%) as judged by ^1H NMR after acidolytic cleavage from the resin followed by chromatography (Table 12). The authors also reported an extension of the protocol to the preparation of unsymmetrical bis-imidazoles (Scheme 22).

Scheme 22

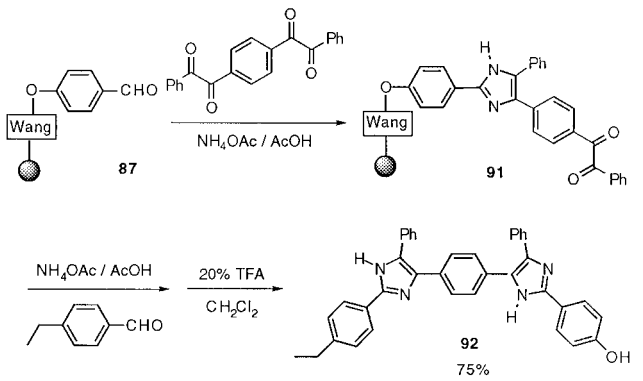


Table 12. Imidazoles 88−90 (Scheme 21)

	derivative				
entry	R_1 or Linker	R_2 or Linker	R_3	R_4	yield (%)[a]
1	C_6H_4-4-CO_2H	H	C_6H_5	$C_6(CH_3)_5$	98
2	C_6H_4-4-CO_2H	C_6H_5	C_6H_5	C_6H_5	99
3	C_6H_4-4-CO_2H	c-C_5H_9	C_6H_4-4-F	C_6H_4-4-F	93
4	C_6H_4-4-OH	H	C_6H_5	CH_3	82
5	C_6H_4-4-OH	$CH_2C_6H_5$	C_6H_4-4-OCH_3	C_6H_4-4-OCH_3	95
6	C_6H_4-4-OH	$(CH_2)_5CH_3$	2-furyl	2-furyl	71
7	C_6H_3-3,5-$(OCH_3)_2$	$(CH_2)_5CO_2H$	C_6H_4-2-Cl	C_6H_4-2-Cl	96
8	C_9H_{19}	$(CH_2)_5CO_2H$	C_6H_4-3-OCH_3	C_6H_4-3-OCH_3	95
9	c-C_6H_{11}	$(CH_2)_5CO_2H$	C_6H_4-4-Br-2-OH	C_6H_4-4-Br-2-OH	95

[a] Yields of chromatographed product are based on the loading level of the starting resin.

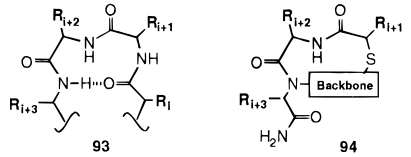


Figure 22. Structures of β-turn and β-turn mimetic.

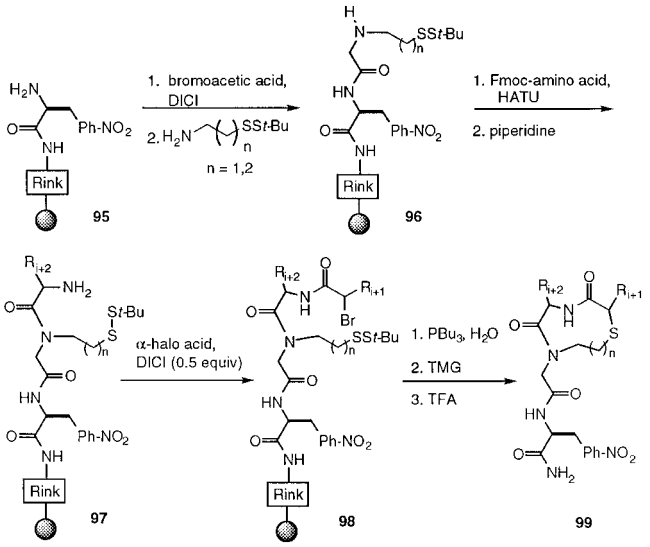
Treatment of aldehyde functionalized resin **87** with NH_4OAc and 1,4-bis-benzil (20 equiv) under standard conditions provided the support-bound and 1,2-dione-substituted imidazole product **91**. The support-bound dione intermediate **91** was then treated with NH_4OAc and 4-ethylbenzaldehyde under standard conditions followed by cleavage from the support to provide the unsymmetrical bis-imidazole **92** in 75% mass recovery and 92% purity.

b. Targeted Libraries. 1. *β-Turn Mimetics.* β-Turns, **93** (Figure 22), are a key structural motif in peptides and proteins and often play a key role in molecular recognition events in biological systems.[98] A great deal of effort therefore has been focused on the design of small constrained mimetics of turn structure in order to identify high affinity and specific ligands to receptor and enzyme targets.[99] However, these efforts have met with only limited success due to difficulties in identifying the key turn residues and the relative orientations of those residues in the receptor bound conformation. To address these issues Virgilio and Ellman have developed a method for the solid-phase synthesis of a library of β-turn mimetics, **94**, for the rapid identification of ligands that are based upon the β-turn structure.[100]

The turn mimetic **94** is constructed from three readily available components. The $i + 1$ side chain is derived from an α-halo acid and the $i + 2$ side chain is derived from an α-amino acid. The mimetic is constrained in a turn structure by replacing the hydrogen bond between the i and $i + 3$ residues with a covalent backbone linkage. The flexibility of the turn mimetic as well as the relative orientations of the side chains can be varied by introducing different backbone linkages to provide nine- or 10-membered rings. In addition, different side-chain orientations are obtained by introducing different absolute configurations at each of the stereocenters introduced by the $i + 1$ and $i + 2$ side chains of the turn mimetic.

The β-turn mimetics are prepared with PEG-PS as the support and using the Rink amide linker. In the initial study, *p*-nitrophenylalanine was loaded onto the support before the synthesis was initiated to serve as a convenient UV tag for accurate determination of the overall purity of the turn mimetic by HPLC (*vide infra*). α-Bromoacetic acid is first coupled to the support-bound *p*-nitrophenylalanine by activation with DICI (Scheme 23).[51] Subsequent treatment with the aminoalkyl mercaptan protected as the *tert*-butyl mixed disulfide provides the secondary amine **96**. The secondary amine is then coupled with an *N*-Fmoc α-amino acid employing *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) to provide amide **97**.[101] Treatment with 20% piperidine in DMF followed by reaction with the symmetric anhydride of the appropriate α-bromo acid provides acyclic intermediate **98** that incorporates both the $i + 1$ and the $i + 2$ side-chain residues. Cleavage of the mixed disulfide is then accomplished by treatment of the cyclization precursor **98** with tributylphosphine in a 5:3:2 propanol/DMF/water comixture. It is necessary to use PEG-PS as the solid support, which is well solvated under the aqueous reaction conditions, in order for clean reduction of the disulfide bond to occur without side reactions. Cyclization to provide the nine- or 10-membered thioether is accomplished by treatment with tetramethylguanidine (TMG) in a DMF/H_2O comixture.[102] Cleavage of the turn mimetic from the support by treatment with 95:5:5 TFA/DMS/H_2O then provides mimetic **99** (Table 13).

Scheme 23



Employing this synthesis sequence, turn mimetics **99** were obtained with an average purity of 75% (11 compounds) over the eight-step process as determined by HPLC analysis (Table 13). Any side

Table 13. β-Turn mimetics 99 (Scheme 23)

entry	derivative[a] R^{i+1}	derivative[a] R^{i+2}	backbone (n)	purity (%)[b] PEG-PS	purity (%)[b] pins
1	CH_3	$CH_2C_6H_5$	2	90	79
2	CH_3[c]	$CH_2C_6H_5$	2	59	90
3	$CH(CH_3)_2$	$CH_2C_6H_5$	2	81	86
4	CH_2CO_2H	$CH_2C_6H_5$	2	65	79
5	CH_3	$(CH_2)_4NH_2$	2	72	87
6	CH_3	CH_2CO_2H	2	63	86
7	H	$CH_2C_6H_5$	2	85	91
8	H	CH_2OH	2	82	93
9	CH_3	$CH_2C_6H_4$-4-OH	2	74	88
10	CH_3	$CH_2C_6H_5$	1	77	75
11	$CH_2C_6H_4$-4-OH	CH_3	1	81	90

[a] The stereochemical configuration at the $i + 1$ site is R and at the $i + 2$ site is S unless otherwise specified. [b] Purity by HPLC. [c] The stereocenter has the S configuration.

products that were produced during the synthesis of the turn mimetic would be detected by HPLC analysis since the UV tag, *p*-nitrophenylalanine, was introduced before the synthesis of the mimetic was initiated. For all of the turn mimetics synthesized, cyclization provided the desired cyclic monomer with no cyclic dimer detected (<5%).[103] This includes mimetics incorporating both (*R*) and (*S*) α-bromo acids, α-bromoisovaleric acid which corresponds to the sterically hindered amino acid, valine, and α-chloroacetic acid which corresponds to the least sterically hindered amino acid, glycine, at the $i + 1$ site. A variety of side-chain functionality could be incorporated successfully into the turn mimetics including alcohol, phenol, carboxylic acid, and amine functionality. In addition, less than 3% racemization was observed in the synthesis sequence.

To demonstrate the utility of the synthesis sequence for the rapid construction of a library of turn mimetics **99**, the 11 mimetics were synthesized simultaneously employing Chiron Mimotopes pin apparatus (section II.A.1.a). All 11 derivatives were obtained in a very high level of purity as determined by HPLC analysis (Table 13). On the basis of these results, a library of 1292 β-turn mimetics has been prepared using the 19 α-bromo acids, 34 α-amino acids, and two backbone elements (Figure 23).[104] A modified library has also been prepared from the initial library by oxidation of the thioethers to sulfoxides with hydrogen peroxide in DMF/H_2O comixture.[105] Dr. Andrew Bray from Chiron Mimotopes evaluated 7% of the library by mass spectrometry using electrospray ionization, and for all of the derivatives that were tested the expected molecular ion was observed. The library has successfully been evaluated for the identification of several specific ligands to rat somatostatin receptor subtypes I−IV by researchers at Genentech, and screening by Berlex identified novel ligands to the f-MLP receptor.

2. Protease Inhibitor Libraries. Several groups have worked toward the identification of protease inhibitors by the preparation of libraries of compounds that incorporate isosteres that mimic the tetrahedral intermediate for peptide hydrolysis.[106] Statine, hydroxyethylamine, hydroxylethylurea, the diamino diol core and the diamino alcohol core, have been used to target proteases of the aspartic acid class, while peptidylphosphonates have been used to target the metalloprotease class (Figure 24).

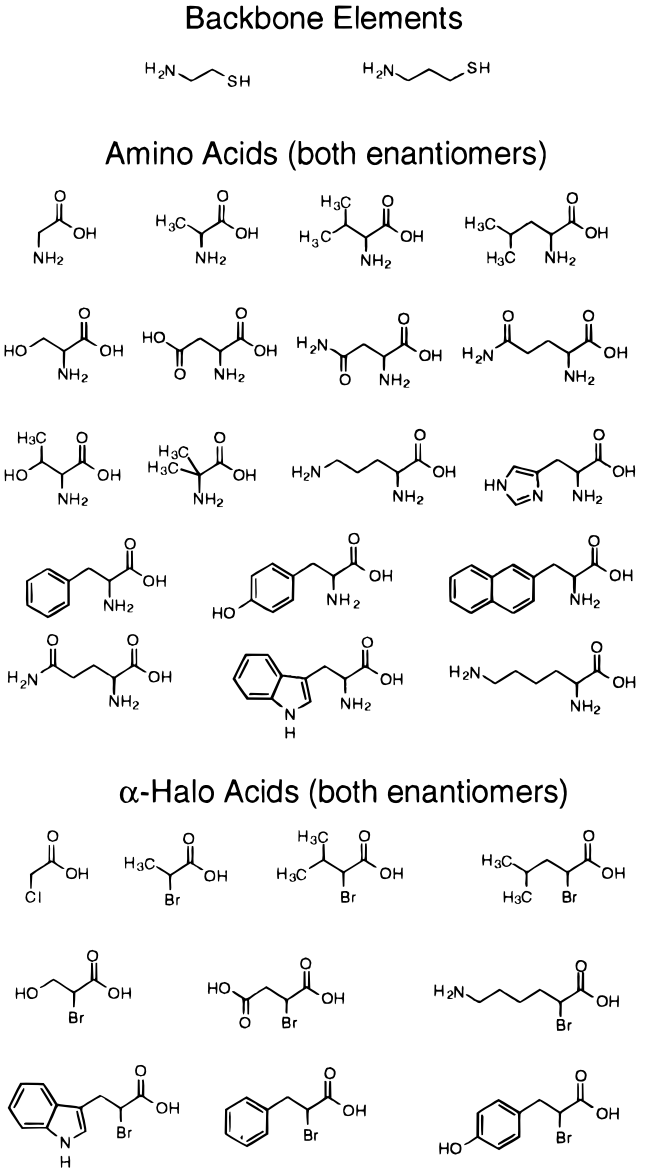


Figure 23. Building blocks for the synthesis of a β-turn library.

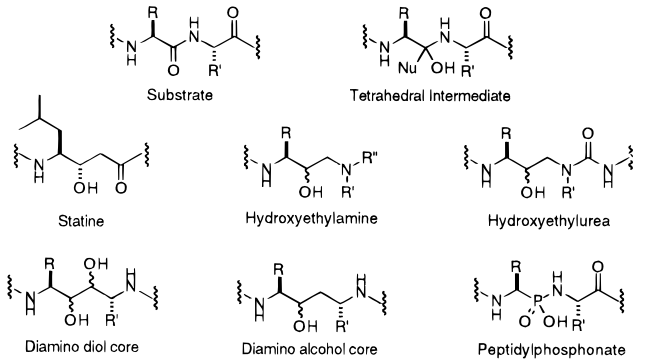


Figure 24. Isosteres that have been employed for the synthesis of protease inhibitor libraries.

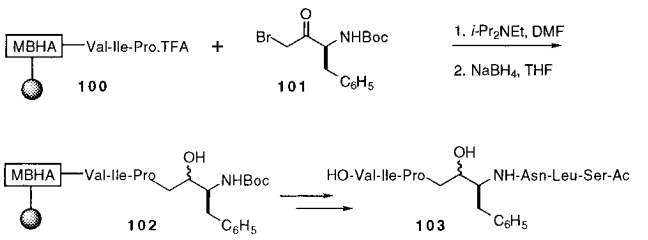
Two distinct strategies have been employed. The first approach is to incorporate the isostere directly into a peptide chain. Large numbers of compounds can thereby be synthesized rapidly and efficiently using standard peptide synthesis methods. Alternatively, the isostere can be attached to the support through the functionality that corresponds to the site of peptide hydrolysis, such as the secondary hydroxyl

group(s) in the hydroxyethylamine, diamino diol, or diamino alcohol, thereby allowing the display of diverse functionality including nonpeptide functionality from *both* ends of the molecule.

a. Aspartic Acid Proteases. ***Peptide Library Incorporating Statine.*** In 1991 Owens and co-workers incorporated statine (Figure 24), a known transition-state analog for the aspartic acid proteases, into a peptide library for the purpose of identifying inhibitors of HIV-1 Protease (HIV-PR).[21d] This report was the first example of the synthesis and evaluation of a biased peptide library that was designed to target a protease by the incorporation of a transition state isostere. In this effort, Owens synthesized a tetrapeptide library by the split synthesis method using Boc chemistry and 22 amino acids of both D and L stereochemistry. At the second position from the resin, the same mixture was employed with the addition of statine. The split synthesis protocol was then continued to give a final library of acetylated tetrapeptide amides, which were assayed in solution for the ability to inhibit the cleavage of a known substrate by HIV-PR. Active inhibitors were identified by iterative resynthesis and evaluation. Not surprisingly, statine was found at the second site in the most active tetrapeptide identified, Ac-Trp-Val-Sta-D-Leu-NH_2 (IC_{50} = 200 nM). On the basis of the data from the first library, subsequent work resulted in the identification of a compound (structure not provided) with an IC_{50} of 5 nM.

Peptides Incorporating the Hydroxyethylamine Isostere. Although not explicitly developed for the purpose of compound library synthesis, Alewood and co-workers have developed an expedient solid-phase method for the incorporation of the hydroxyethylamine isostere into peptides.[107] As shown in Scheme 24, the key steps in the synthesis were to treat the

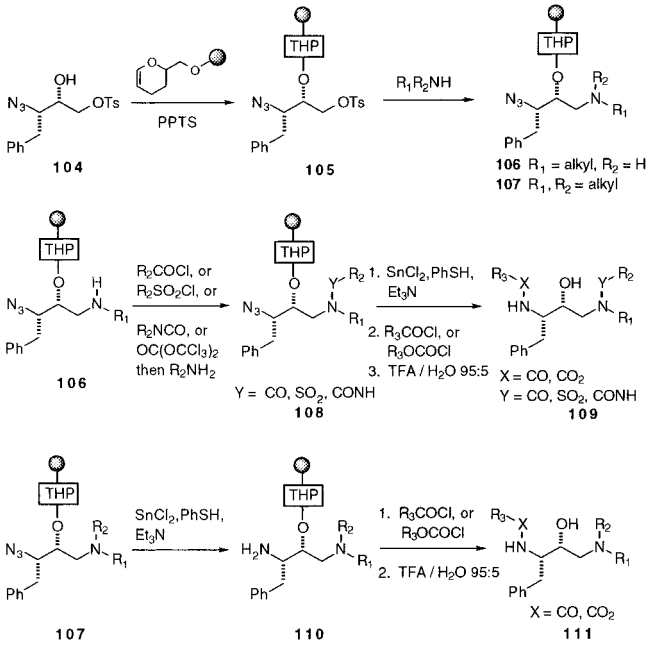
Scheme 24



free amine of resin-bound peptide **100** with excess of an α-bromo ketone **101**, in the presence of *i*-Pr_2NEt in DMF. Reduction of the resulting ketone product with $NaBH_4$ in THF then provided the support-bound hydroxyethylamine isostere **102**.[108] Three additional amino acids were incorporated into the peptide, and after cleavage with HF/*p*-cresol, the hexapeptide product was isolated by HPLC in 52% overall yield as a 6:4 ratio of (*R*) to (*S*) alcohol epimers.

Nonpeptide Inhibitors. Kick and Ellman have reported a solid-phase method for displaying functionality from the (hydroxyethyl)amine and (hydroxyethyl)urea isosteres for the synthesis of a library of potential aspartic protease inhibitors.[109] Using dihydropyran-functionalized resin,[110] the scaffold **104** is coupled to support using pyridinium *p*-toluenesulfonate (PPTS) (Scheme 25). The primary tosyl alcohol **105** is then displaced with either func-

Scheme 25



tionalized or unfunctionalized primary or secondary amines. After coupling of the primary amines, the resulting secondary amine products **106** can be converted to ureas by reaction with isocyanates or by stepwise treatment with triphosgene followed by amine addition.[111] The stepwise procedure provides access to a wide range of ureas from the large pool of commercially available primary amines. The secondary amine may also be cleanly acylated with acid chlorides and sulfonyl chlorides employing standard methods.

The synthesis about the P_1 site of the inhibitor is initiated by efficient reduction of azides **107** or **108** using thiophenol/Et_3N/$SnCl_2$ (4:5:1) according to the procedure described by Bartra for the corresponding solution-phase reaction.[112] The resulting primary amine is then acylated to provide carbamate or amide products. If the amine is acylated with an Fmoc amino acid, then protecting group cleavage and further functionalization is possible. The concomitant removal of the side-chain protecting groups and cleavage of the material from the solid support is then accomplished by treatment with 95:5 TFA/water. Complete cleavage is observed in less than 1 h without decomposition.

By employing the previously described synthesis method a number of different compounds were prepared (Figure 25). In order to demonstrate the versatility of the synthesis sequence, a particular emphasis was placed on incorporating functionality that is present in known nonpeptide-based HIV-1 protease inhibitors,[113] including *N-tert*-butylpipecolamide (**120**), piperazine derivatives (**116**), *N-tert*-butyl ureas (**112**−**114**, **117**, **118**), quinaldic amides (**112**−**114**, **121**), and 3(*S*)-hydroxytetrahydrofuranyl carbamates (**115**−**120**). The derivatives were isolated after four to six steps in 47%-86% yield (av 74%, nine compounds) after chromatography to provide analytically pure material. The only unacceptable yield, 47%, was for compound **120** that resulted from slow tosyl alcohol displacement by the hindered

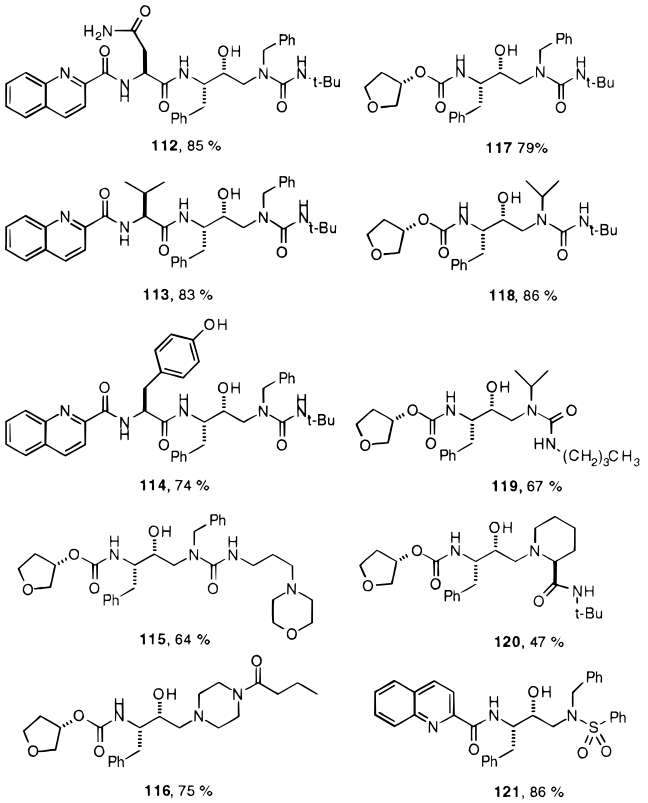


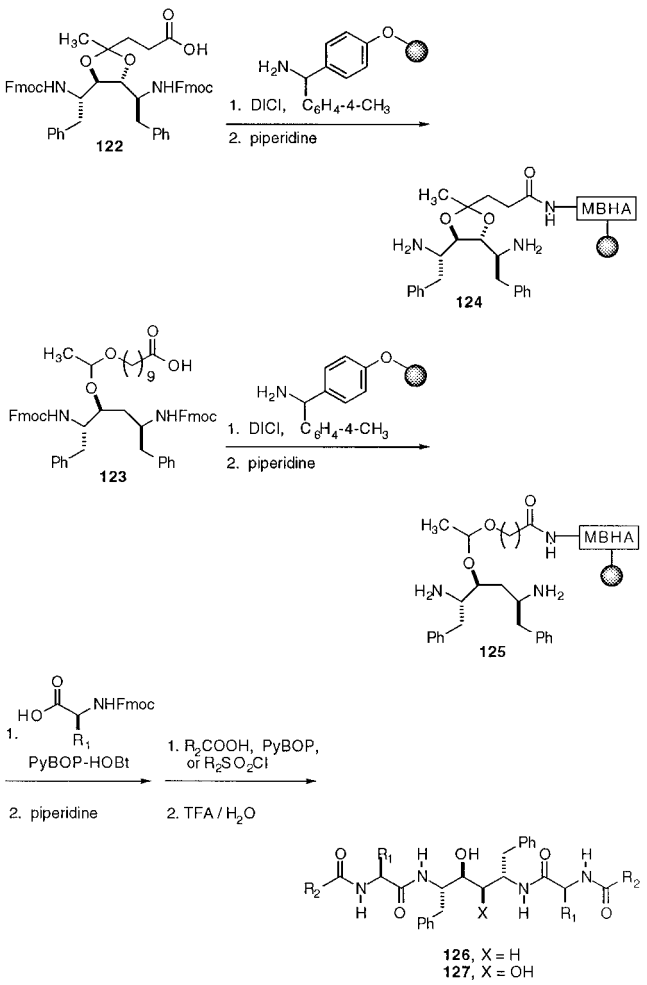
Figure 25. Potential protease inhibitors prepared according to Scheme 25.

N-tert-butylpipecolamide. In current library synthesis efforts toward the identification of cathepsin D inhibitors, we now employ the *p*-nitrobenzenesulfonyl alcohol, which is an order of magnitude more reactive that the corresponding tosyl alcohol and provides the desired reaction products under milder conditions and for less reactive amine nucleophiles, in higher yields (e.g. compound **120** was obtained in 65% overall yield).

Wang and co-workers at Abbott have developed a solid-phase synthesis method to display functionality about two classes of C_2 symmetric transition state isosteres that target HIV-1 protease.[114] The linker-derivatized isosteres **122** and **123** were prepared in solution and then coupled to 4-methylbenzhydrylamine (MBHA) resin (Scheme 26). After Fmoc deprotection, the same *N*-Fmoc amino acid is simultaneously coupled to both amines of the support-bound isostere **124** and **125**. The Fmoc protecting groups are then removed, and the amines are capped either as an amide or sulfonamide. The monoalcohol linker could be cleaved with 30% TFA/H_2O for 3 h; however, the diol linker required more stringent conditions: TFA/H_2O (95:5 overnight).

The synthesis sequence was evaluated by the preparation of 12 model compounds employing the two support-bound isosteres **124** and **125**, the amino acid Val, and six different acylating agents (Figure 26). The crude mass balance was close to theoretical; however, the purity was assessed at 30−70% for the mono-ol series **126** and 20−50% for the diol series **127** as determined by either TLC or HPLC. Mass spectrometry indicated that the lower purity of the diol products was in part due to the presence of a side product whereby the linker had remained at-

Scheme 26



tached to the diol product. The crude compounds were assayed for inhibition of HIV-1 protease providing IC_{50} values (<1 nM to 6.4 mM) that were consistent with previous work in the area. The authors report that a library of 300 derivatives (structures not provided) has been prepared in parallel using the above chemistry and employing an Abimed synthesizer, and that the library is currently being evaluated for HIV-1 protease inhibition.

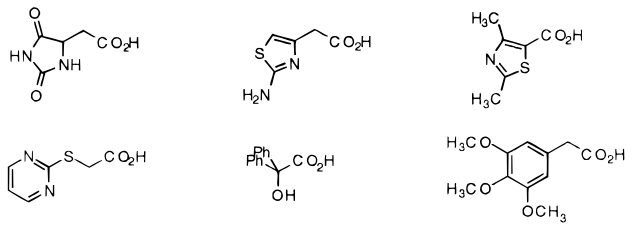
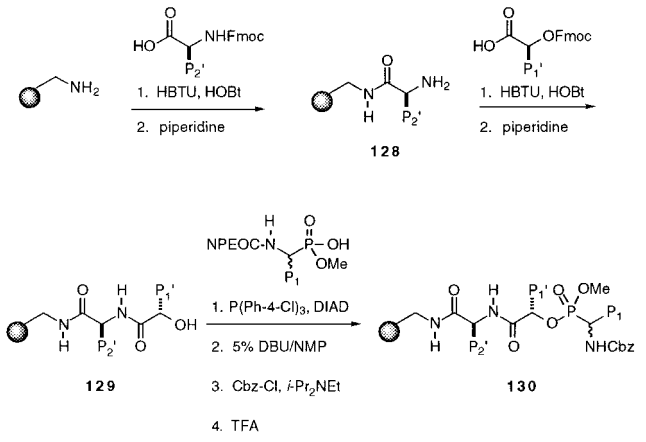


Figure 26. Carboxylic acid components.

b. Zinc Protease Library. Campbell and co-workers at Affymax have reported the synthesis of a peptide library that incorporates the phosphonic acid transition state isostere for the purpose of targeting metalloproteases.[30a] The library was synthesized on Tentagel-S-NH_2. A cleavable linker was not used, since a support-bound assay had been developed for biological evaluation. However, the fidelity of the synthesis sequence (Scheme 27) had previously been demonstrated by the solid-phase synthesis of several

Scheme 27



peptidylphosphonates.[115] For both the *N*-Fmoc amino acid and *O*-Fmoc α-hydroxy acid coupling steps, double coupling is performed first with HOBt/HBTU and then with PyBroP in order to ensure complete reaction conversion. The secondary alcohols **129** are then coupled with [(nitrophenyl)ethoxy]carbonyl (NPEOC)-protected (α-aminoalkyl)phosphonic acids under modified Mitsunobu reaction conditions with tris(4-chlorophenyl)phosphine and DIAD.[115] The NPEOC group is removed with 5% DBU in NMP and the resulting free amines are capped with Cbz-Cl. All of the reactions can be monitored by UV quantitation of the dibenzofulvene−piperidine adduct or 4-nitrostyrene released upon protecting group cleavage. The coupling reactions routinely proceed with >90% conversion. Side-chain deprotection with TFA then provides the support-bound peptidylphosphonates.

By employing this synthesis sequence, a library was prepared by the split synthesis method that contained all combinations of 18 of the naturally occurring amino acids at the P_2'-position (Cys and Asn were excluded), five α-hydroxy acids at the P_1'-position (glycolic acid, (*R*)-lactic acid, (*R*)-mandelic acid, 3(*R*)-phenyllactic acid, and 2(*R*)-hydroxyisocaproic acid), and six (α-aminoalkyl)phosphonic acids at the P_1-position (the (aminoalkyl)phosphonic acids corresponded to Gly, D,L-Ala, D,L-Val, D,L-Leu, D,L-Ile, D,L-Phe).

The library was evaluated employing a depletion assay whereby resin-bound mixtures were incubated with thermolysin and then filtered to remove resin-bound enzyme/inhibitor complex. Proteolytic activity of the filtrates were then assayed to rank order the library mixtures. Iterative resynthesis and evaluation provided qualitative structure vs activity relationships as well as resulting in the identification of a number of potent inhibitors of thermolysin, including the expected Cbz-Phe^{p}-^{o}Leu-Ala, which was resynthesized as the terminal amide (K_i = 49 nM) and as the carboxylate (K_i = 122 nM). More significantly, novel thermolysin inhibitors (synthesized as terminal amides) Cbz-Phe^{p}-^{o}Leu-His (K_i = 57 nM), Cbz-Phe^{p}-^{o}Leu-Arg (K_i = 64 nM), and Cbz-Phe^{p}-^{o}Leu-Gln (K_i = 127 nM) were identified that were unexpected on the basis of literature precedent for hydrophobic residues at the P_2' position.

3. Carbonic Anhydrase Inhibitors. Baldwin and co-workers have synthesized a library of 6727 acylpiperidines utilizing the split synthesis method and the haloaromatic tag encoding strategy, with the tags attached to the polymer support using the rhodium-catalyzed carbene insertion method (section II.A.5.c).[38a,116b] The synthesis was initiated by coupling four amino acids and three amino alcohols to the PEG-PS solid support using a 4-carboxy-2-nitrobenzyl alcohol linker through ester or carbonate

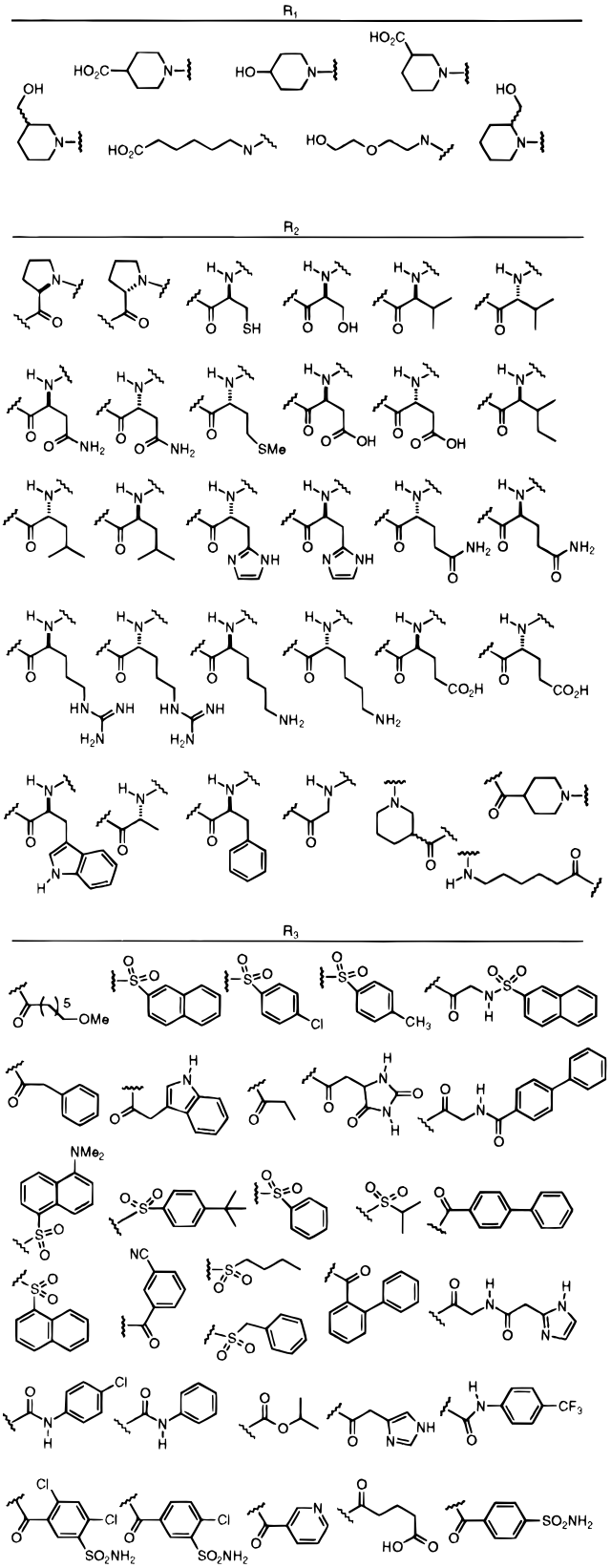
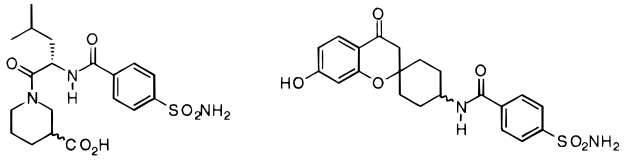


Figure 27. Building blocks used in the synthesis of a library of carbonic anyhdrase inhibitors.



131 132

Figure 28. Carbonic anhydrase inhibitors.

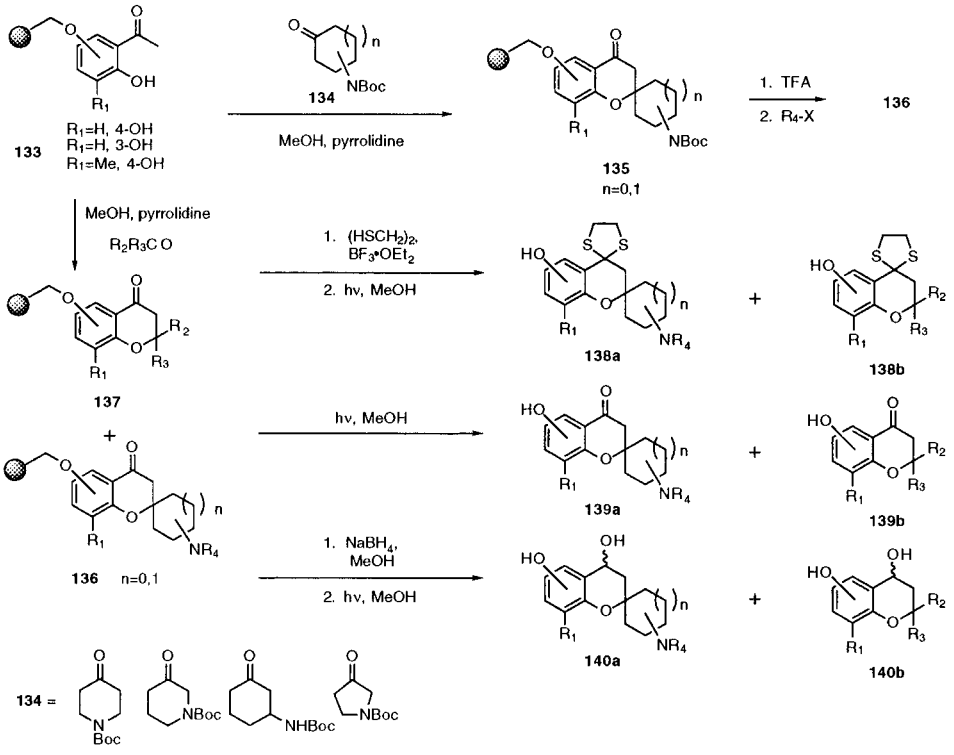
formation, respectively. Thirty-one natural and unnatural *N*-Fmoc amino acids were then coupled to the support-bound amines by employing standard solid-phase amide bond forming methods. After removal of the Fmoc group, the resulting free amine was acylated with a set of 31 sulfonyl chlorides, isocyanates, carboxylic acids, and chloroformates (Figure 27). Thirteen tags were sufficient to encode the 6727 compounds that were synthesized. No compound characterization was reported, although the synthesis sequence involved amide bond formation and was therefore well precedented. The library was biased by the inclusion of some arylsulfonamide derivatives which are known pharmacophores to the enzyme carbonic anhydrase (CA).[117]

Screening was accomplished by photoeluting compounds from either single beads or 10 bead mixtures into the wells of a microtiter plate and by using a fluorescence-based ligand-displacement assay (controlled release allows repeated use of the same bead, see section II.A.4.c). The structures of the active compounds were identified by reading the tag sequences and verified by resynthesis and independent evaluation. Although a detailed description of the assay protocol and results are beyond the scope of this review, some general findings warrant comment.

As might be expected, a high percentage of those compounds incorporating the sulfonamide structure showed significant activity, while no active compounds lacking sulfonamide functionality were identified. Acylpiperidine **131** (Figure 28) was one of the most potent compounds to be identified with a K_D of 4 nM for bovine CA(II). Further evaluation of a 217 member focused acylpiperidine sublibrary vs human CA(I) and CA(II) subforms also showed interesting SAR data. Almost all of the compounds that were assayed were slightly to moderately selective for CA-(II) over CA(I), suggesting that CA(II) selectivity may be a property of the entire class of compounds.

The Pharmacopeia group has also synthesized a library of dihydrobenzopyrans, which has also been screened against CA, with the 15 nM inhibitor **132** being identified.[116] Although few details of library synthesis or analytical evaluation were provided, an overview of the synthesis sequence is provided in Scheme 28. Three dihydroxyacetophenones were first coupled to the (4-carboxy-2-nitrobenzyl)oxy photocleavable linker through an ether bond, and the resulting linker-derived acetophenones were then coupled to PEG-PS support employing standard amide bond-forming methods. Condensation of the resulting support-bound dihydroxyacetone derivatives with seven ketones gave the support-bound dihydrobenzopyrans. Four of the ketones (**134**, Scheme 28) contained a Boc-protected amine for further functionalization (dihydrobenzopyrans **135**) while three ketones (acetone, cyclohexanone, and tetrahydro-4*H*-pyran-4-one) did not (dihydropyrans **137**). After *N*-Boc deprotection of compounds **135** with TFA, the amines were functionalized to provide amides, ureas, thioureas, amines, carbamates, and sulfonamides **136** (Scheme 28). The resin batches without amines, **137**, were pooled with the functionalized amine resins, **136**, and split into three batches. One batch was treated with ethanedithiol and $BF_3 \cdot OEt_2$ to form the dithioacetals **138a** and **138b**; the

Scheme 28



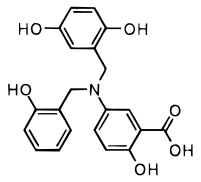
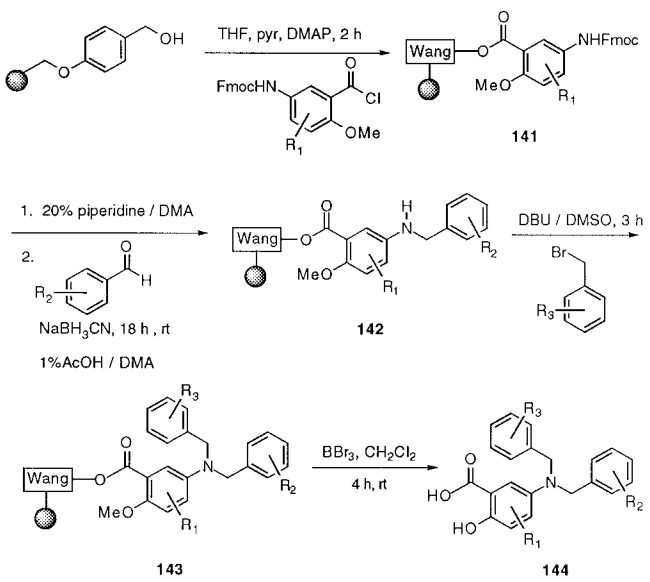


Figure 29. Lavendustin A.

second was directly cleaved, producing compounds **139a** and **139b**; and the third was reduced with $NaBH_4$, producing compounds **140a** and **140b**. The chemistry was encoded with the haloaromatic tag strategy and resulted in the synthesis of 1143 compounds. A representative set of these compounds was reportedly prepared on beads for characterization, and the authors report that in general the dihydrobenzopyrans were isolated with purities of >80%; however, the exact number of compounds and the structures of the compounds that were examined were not provided. The inhibitor **132** (Figure 28) was resynthesized and isolated in 95% purity as estimated by HPLC analysis.

4. Tyrosine Kinase Inhibitors. ***Method I.*** Green at Ariad Pharmaceuticals has carried out a parallel synthesis of a 60-member designed library that is based upon the known tyrosine kinase inhibitor lavendustin A (Figure 29). The library was synthesized by reductive amination of a resin-bound aniline with a substituted benzaldehyde followed by alkylation with a benzylic bromide.[118] The initial studies were performed on Rink amide resin, but after working out the reaction conditions, library synthesis was carried out on Wang resin. The substituted benzoyl chloride is esterified onto the Wang resin with DMAP as a catalyst. The Fmoc group of the support-bound aniline **141** is then removed followed by reductive amination employing substituted benzaldehyde derivatives to provide the resin-bound secondary amine **142** (Scheme 29). Alkylation with a benzyl bromide derivative followed by cleavage from the resin with TFA or BBr_3 when Rink or Wang resin is employed, respectively, provides the desired tertiary amine products **144**. Cleavage with BBr_3 has the added advantage that methyl phenyl ethers are

Scheme 29



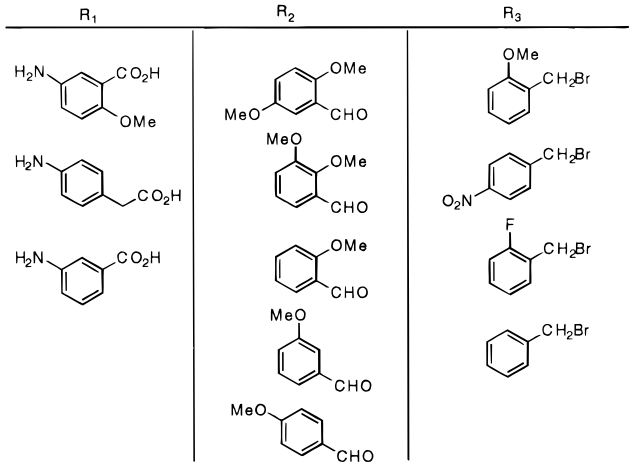


Figure 30. Building blocks used in the synthesis of a library of potential tyrosine kinase inhibitors.

concomitantly cleaved to provide the corresponding free phenols.

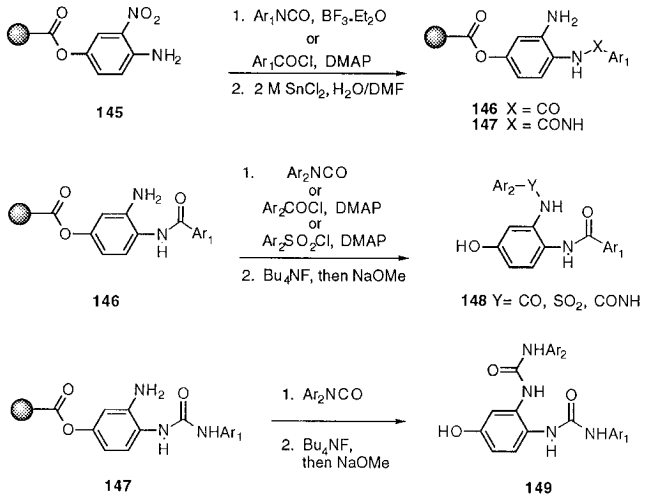
Several lavendustin A derivatives were synthesized in high isolated yields. The primary amide of tetramethyllavendustin A was synthesized on Rink resin in 98% yield after chromatographic purification, while tetramethyllavendustin A was synthesized on Wang resin with TFA cleavage in 78% yield after purification (the crude material was of 95% purity as estimated by HPLC). Finally, lavendustin A was synthesized on hydroxymethyl polystyrene with concomitant methyl ether deprotection and cleavage from the resin in 90% yield (the crude material was of 87% purity as estimated by HPLC).

A series of 60 spatially separate derivatives were synthesized by employing the building blocks in Figure 30 on Wang resin by employing the BBr_3-mediated cleavage and deprotection sequence. The purity of each of the compounds was assessed by HPLC (30−97%, av 69%), and the yield for each compound was determined by ^1H NMR using maleic acid as an internal standard (range from 10% to 83%, av 55%).

Method II. Researchers at Sphinx Pharmaceuticals have designed and synthesized a library of phenol derivatives based upon the structures of lavendustin A and balanol, a serine/threonine kinase inhibitor, which share the phenol as a recognition element.[13] 4-Amino-3-nitrophenol is first coupled to carboxylate-functionalized polystyrene resin[119] employing DICI (Scheme 30). The support-bound aniline **145** is then coupled with an acid chloride or an isocyanate (hydroxyl and carboxyl groups are protected as *tert*-butyldimethylsilyl ethers). Reduction of the nitro group is then accomplished with $SnCl_2$ in DMF, and the resulting aniline is coupled with an acid chloride or isocyanate derivative to introduce functionality at the second position of diversity. Removal of the silyl groups with Bu_4NF followed by cleavage from the support by treatment with sodium methoxide provides the substituted phenolic derivatives.

A small library of these derivatives was synthesized using the previously described microtiter-based synthesis apparatus (Figure 2). Benzamide-derivatized resins **146** (17 unique resins were prepared)

Scheme 30



were loaded into the microtiter plate and then acylated as previously described. Four microtiter plates (384 compounds) of bis-amides were synthesized, one plate each (96 compounds) of amide−sulfonamide and amide−ureas were synthesized, and one plate of bis-ureas **149** were synthesized. Relevant compounds were deprotected with Bu_4NF and the resins cleaved to afford 3−9 mg (av weight per microtiter plate, 15−45% based on an av molecular weight of 400) of the desired compounds. Characterization by positive ion EI MS resulted in the observation of molecular ion peaks for a row of 12 bis-amides. HPLC analysis of two selected polyhydroxylated phenols gave purities of 87% and 63%. All 672 compounds were analyzed by TLC against purified standards to get a qualitative assessment of product purity. When mixtures were observed, the compounds in the wells were chromatographed and the major components were isolated and characterized by ^1H NMR, allowing side products to be identified. The estimated purity for the amide−urea sublibrary was 60% and the estimated purity for the bis-urea sublibrary was 80%.

5. Estrogen Receptor Ligands. Williard and coworkers have reported the synthesis of 23 hydroxystilbene derivatives targeting the estrogen receptor.[120] Four hydroxybenzaldehydes that had been prederivatized with the HMP linker[74] were coupled to (aminomethyl)polystyrene followed by a Horner−Emmons olefination with six different benzylphosphonate anions (Scheme 31).[121] Cleavage of the resulting stilbene derivatives from support with TFA provided a 7−85% yield of the desired products after chromatography on the basis of the loading level of

Scheme 31

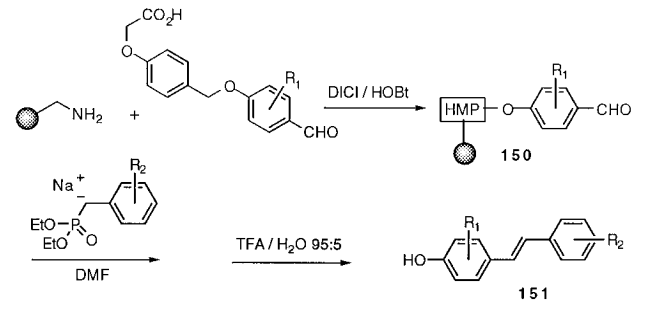


Table 14. Hydroxystilbene Derivatives 151 (Scheme 31)

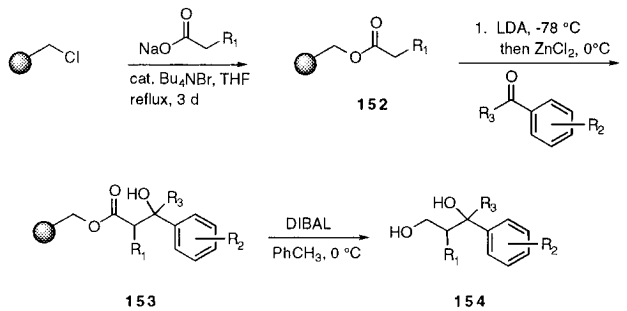
	derivative		
entry	R_1	R_2	yield (%)[a]
1	3-OH	H	44
2	3-OH	4-NO_2	85
3	3-OH	4-Br	45
4	3-OH	3,5-OCH_3	41
5	3-OH	4-F	40
6	3-OH	3-F	35
7	3-OH,4-OCH_3	H	8
8	3-OH,4-OCH_3	4-NO_2	28
9	3-OH,4-OCH_3	4-Br	20
10	3-OH,4-OCH_3	3,5-OCH_3	25
11	3-OH,4-OCH_3	3-F	16
12	3-NO_2,4-OH	H	12
13	3-NO_2,4-OH	4-NO_2	8
14	3-NO_2,4-OH	4-Br	7
15	3-NO_2,4-OH	3,5-OCH_3	7
16	3-NO_2,4-OH	4-F	10
17	3-NO_2,4-OH	3-F	14
18	2-Cl,4-OH	H	55
19	2-Cl,4-OH	4-NO_2	67
20	2-Cl,4-OH	4-Br	57
21	2-Cl,4-OH	3,5-OCH_3	46
22	2-Cl,4-OH	4-F	40
23	2-Cl,4-OH	3-F	62

[a] Yields of purified material are based on resin-bound hydroxybenzaldehyde starting material as the limiting reagent.

the aldehyde on support (Table 14). Although many of the yields were modest, enough material was obtained for biological screening in a cell-culture assay for activation of the estrogen receptor. Three derivatives based on 2-chloro-4-hydroxybenzaldehyde showed the highest activity, and dose−response curves were generated that provided EC_{50} values of approximately 5−15 μM for the three most active derivatives. The biological response was likely mediated by the estrogen receptor, since the estrogenic activity of all active compounds was effectively inhibited by the antiestrogenic steroid ICI 164384.

6. Antioxidants. Kurth has reported the synthesis and evaluation of nine pools of three antioxidants by the split synthesis approach.[122] Alkylation of Merrifield resin with the sodium salt of a carboxylic acid provided support-bound ester **152** (Scheme 32). Treat-

Scheme 32



ment of the ester with LDA at −78 °C provided the lithium enolate, which was transmetalated to the zinc enolate using anhydrous zinc chloride at 0 °C. Addition of an aldehyde or ketone resulted in aldol condensation to provide the β-hydroxy ester. The zinc enolate was employed instead of the lithium enolate in order to minimize retroaldolization. Cleavage from the solid support was effected by DIBAL

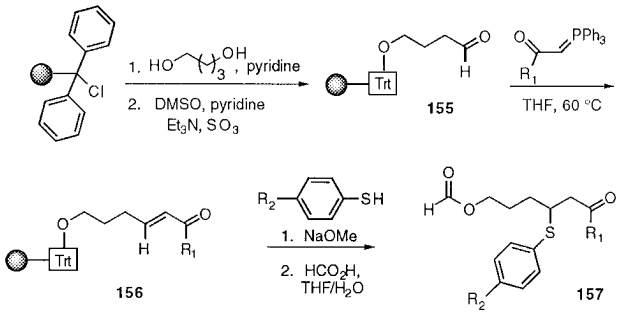
reduction to the 1,3-diol **154**. The yield was reported for one compound, 26% after preparative TLC (R_1 = CH_2Ph, $R_2 = OCH_3$).

A library was then assembled using three carboxylic acids and nine aldehydes to provide 27 compounds which were purified as nine pools of three compounds using preparative TLC. Characterization by GC−MS identified all 27 desired compounds in the library. The pools were then assayed for their ability to inhibit oxidation using the ferric thiocyanate method.[123] The most active pools were deconvoluted. The three compounds incorporating electron-rich 3,4,5-trimethoxybenzaldehyde were determined to have the greatest activity.

c. Solid-phase Synthesis Methods. A number of significant studies have been reported on the solid-phase synthesis of organic molecules; however, only those studies that were expressly carried out for the purpose of establishing reaction generality for library synthesis will be described in this review. A number of thorough reviews have been published that describe the early work on solid-phase organic synthesis.[124] In addition, several recent reports from Kurth and Schore are worthy of note.[125]

1. β-Mercapto Ketones and Esters. In one of the early reports in the area of organic compound library synthesis, Kurth reported the synthesis of nine β-mercapto ketones in three pools of three employing the split synthesis approach and demonstrated that each compound could be identified by GC−MS methods.[32] Condensation of commercially available trityl chloride resin with 1,4-butanediol followed by oxidation of the pendant primary alcohol with SO_3−pyridine in DMSO provides the resin-bound aldehyde **155** (Scheme 33).[126] Condensation with a substituted

Scheme 33

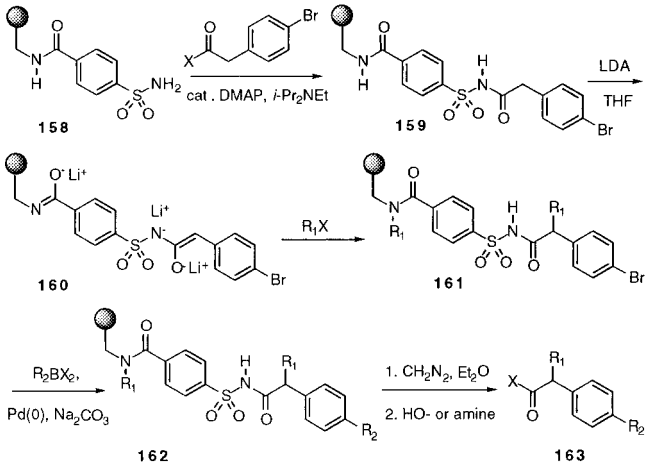


Horner−Emmons reagent introduces the first element of diversity. Treatment of the resulting enone **156** with a substituted thiophenol provides the target β-mercapto ketone, which is cleaved from the solid support using formic acid to provide the formate ester.

By employing this procedure, an array of nine compounds was synthesized as three pools of three compounds. Pools were characterized by GC and GC−MS and all nine compounds were determined to be of high purity by GC−MS. Structural elucidation of a compound that had been cleaved from one synthesis bead was also demonstrated using GC−MS. The isolation of each compound from the three pools was carried out by preparative TLC. The derivatives were isolated in 7−27% overall yield based on the initial trityl chloride loading level of the solid support.

2. Arylacetic Acids. Backes and Ellman have reported a method for the solid-phase synthesis of arylacetic acid derivatives **163** (Scheme 34).[127] Al-

Scheme 34



though these compounds represent an important class of cyclooxygenase inhibitors, the method was predominantly developed for the purpose of evaluating two important carbon−carbon bond-forming reactions on solid support; enolate alkylation and palladium-mediated Suzuki cross-coupling. A carboxylic acid linker was chosen that would be stable to the basic reaction conditions of the enolate alkylation step and Suzuki cross-coupling process, but that could also be activated for nucleophilic cleavage to complete the synthesis sequence. A variant of Kenner's "safety-catch" linker was developed for this purpose.[128] Commercially available 4-carboxybenzensulfonamide was coupled to the aminomethylated polystyrene by treatment with DICI and HOBt. The pentafluorophenyl ester or the symmetric anhydride of 4-(bromophenyl)acetic acid was then loaded onto the resin employing catalytic DMAP and *i*-Pr_2NEt. Treatment of the acylsulfonamide **159** with excess LDA in THF at 0 °C results in rapid deprotonation to give the trianion. Subsequent addition of activated or unactivated alkyl halides results in rapid alkylation of the enolate trianion to provide **161**. In contrast to ester[129] or carboximide[130] enolate alkylations, ketene formation is not observed even when employing the unreactive alkylating agent isopropyl iodide (entry 6, Table 15), since ketene formation would require that the sulfonamide dianion be the leaving group. In addition, minimal overalkylation is observed (<4%).

The Suzuki reaction of acylsulfonamide **161** is then performed according to standard conditions using Pd(PPh_3)$_4$ as the catalyst, 2 M aqueous Na_2CO_3 as the base, and THF as the solvent at reflux. Deprotonation of the acylsulfonamide under the basic reaction conditions again prevents any hydrolysis from occurring. Good conversion is observed both for *B*-alkyl-9-BBN derivatives that are prepared by in situ hydroboration of primary alkenes and for arylboronic acids that are electron poor or electron rich as well as ortho-substituted.

Table 15. Substituted Arylacetic Acid Derivatives 163 (Scheme 34)

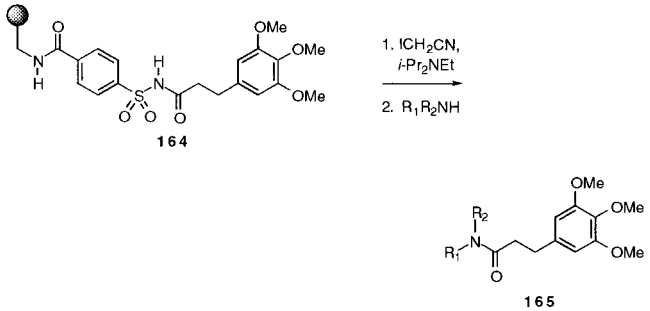
	derivative			
entry	R_1	R_2	nucleophile	yield (%)[a]
1	H	$CH_2CH(CH_3)_2$	H_2O	100
2	CH_3	$CH_2CH(CH_3)_2$	H_2O	96
3	CH_3	$CH_2CH(CH_3)_2$	$BnNH_2$	96
4	$CH_2C_6H_5$	$CH_2CH(CH_3)_2$	$BnNH_2$	98
5	CH_2CH_3	$CH_2CH(CH_3)_2$	$BnNH_2$	92
6	$CH(CH_3)_2$	$CH_2CH(CH_3)_2$	$BnNH_2$	91
7	CH_3	$CH_2CH(CH_3)_2$	piperidine	96
8	CH_3	$CH_2CH(CH_3)_2$	aniline	0[b]
9	H	C_6H_5	H_2O	93
10	CH_3	C_6H_5	$BnNH_2$	95
11	CH_3	C_6H_4-4-CF_3	$BnNH_2$	87
12	CH_3	C_6H_4-4-OCH_3	$BnNH_2$	88
13	CH_3	C_6H_3-2,4-$C1_2$	$BnNH_2$	88

[a] Yields of analytically pure material are based on the loading level of support-bound starting material **159**. [b] No cleavage of material from the resin was observed with aniline as the nucleophile.

The final step in the synthesis is nucleophile-mediated cleavage of the material from the support. Acylsulfonamide activation is accomplished by treatment with CH_2N_2 in Et_2O. Addition of hydroxide or amine nucleophiles provides the corresponding carboxylic acid or amide products **163** in high yield (av 93%, 12 compounds) on the basis of analytically pure material after filtration through silica (Table 15). Although both primary and secondary amines resulted in efficient cleavage, attempted cleavage of the material from the resin with aniline did not provide any anilide product and defines the level of reactivity of the activated 1-methylacylsulfonamide linkage (entry 8).

Backes and co-workers have recently reported a new method that provides a significantly more labile activated acylsulfonamide linker.[131] Activation is accomplished by treatment of the support-bound acylsulfonamide with iodoacetonitrile and *i*-Pr_2NEt in DMSO or NMP at ambient temperature (Scheme 35). The resulting cyanomethylated derivative is

Scheme 35



>150-fold more labile to nucleophilic displacement than the corresponding *N*-methyl derivative (the $t_{1/2}$ for displacement with 0.007 M benzylamine in DMSO is <5 min). Both nonbasic amines and sterically hindered amines efficiently react with the support-bound *N*-(cyanomethyl)acylsulfonamide. For example, activation and nucleophilic displacement of acylsulfonamide **164**, with *tert*-butylamine and aniline provides the corresponding analytically pure amide products in 92% and 96% yield, respectively, on the basis of the initial amine loading level of the resin. In addition, treatment of the activated acylsulfonamide with *limiting* amounts of an amine nucleophile results in complete consumption of the amine to provide the amide product in pure form, uncontaminated with excess amine. This allows novel pooling strategies to be employed whereby a limiting amount of an equimolar mixture of amines are added to the *N*-(cyanomethyl)acylsulfonamide resin to provide an equimolar mixture of amide products in pure form. As illustrated in Figure 31, an equimolar mixture of amide products was obtained by activation of acylsulfonamide **164** followed by addition of limiting amounts of an equimolar mixture of piperidine, cyclohexylamine, morpholine, benzylamine, and 4-(3-aminopropyl)morpholine.

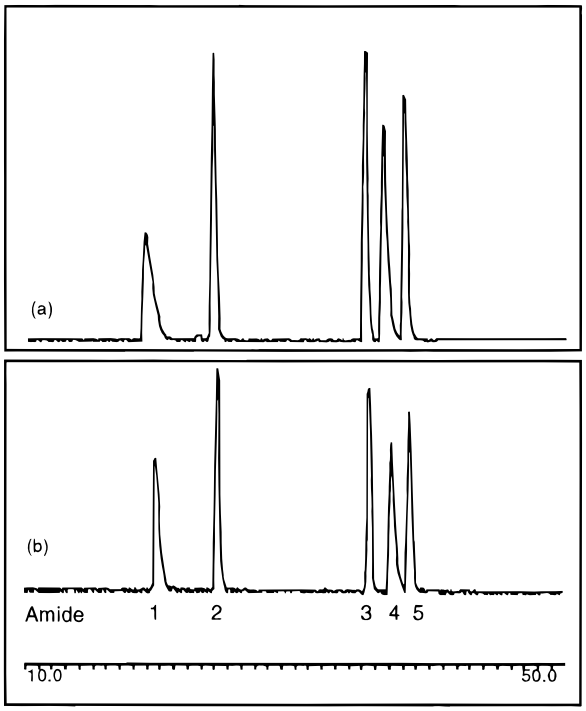


Figure 31. HPLC traces of (a) addition of limiting amounts of five amines to acylsulfonamide resin **164** resulted in equimolar amounts (± 2%) of the five amide products prepared from (**1**) 4-(3-aminopropyl)morpholine, (**2**) morpholine, (**3**) benzylamine, (**4**) piperidine, and (**5**) cyclohexylamine and (b) standard containing an equimolar mixture of the five amide products.

3. Palladium-Mediated Processes. In addition to the palladium-mediated processes described previously (Stille reactions and Suzuki coupling reactions reported by Ellman, and Heck reactions reported by Zuckermann), the utility of Pd(0)-mediated carbon−carbon bond-forming processes on solid-support has also been amply demonstrated by a number of researchers.

Suzuki Reaction. Frenette and Friesen have examined the Suzuki reaction on support-bound halo-substituted benzoic acids (Scheme 36).[132] A number

Scheme 36

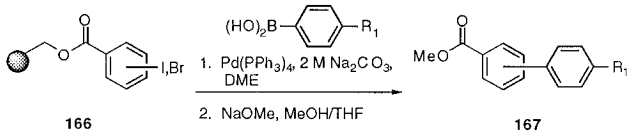


Table 16. Biaryl Derivatives 167 (Scheme 36)

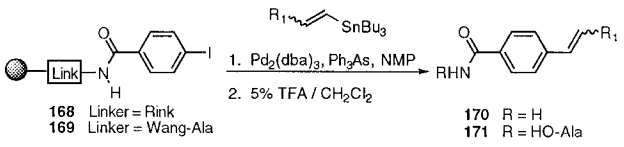
entry	aryl halide R_1	boronic acid R_2	yield (%)[a]
1	4-I	4-OCH_3	>95
2	4-Br	4-OCH_3	>95
3	4-Br	H	95
4	4-Br	4-CH_3	95
5	4-Br	4-NO_2	95
6	4-Br	4-F	91
7	3-Br	H	90
8	2-Br	H	>95
9	3-Br,4-OCH_3	H	>95
10	3-Br,4-CH_3	H	>95

[a] Yields of unpurified material are based on resin-bound aryl halide as the limiting reagent. All derivatives were of >90% purity as determined by ^{1}H NMR and HPLC analysis.

of iodo- or bromo substituted benzoic acids were loaded onto Merrifield resin (chloromethylated polystyrene) by esterification under standard alkylation conditions (Cs_2CO_3, KI, DMF). Coupling reactions were performed with a variety of palladium catalysts and boronic acids to produce the biphenyl derivatives **167**, which were cleaved from the support using catalytic NaOMe in MeOH/THF. Frenette found that the optimal reaction conditions were 5 mol % Pd-(PPh_3)$_4$ in DME with 2 M Na_2CO_3 as a base. Crude mass balances for the reaction sequence employing a variety of substituted aryl boronic acids and halobenzoic acids were generally greater than 95%, and the compounds were obtained in greater than 90% purity as estimated by HPLC analysis (Table 16).

Stille Reaction. Deshpande has examined the Stille coupling reaction on support in the synthesis of 4-substituted benzamides.[133] 4-Iodobenzoic acid was coupled onto Rink resin or Ala-derivatized Wang resin to provide the support-bound aryl iodide (Scheme 37). Stille reaction using a number of stannanes was

Scheme 37



then performed with 5 mol % $Pd_2(dba)_3$ and added Ph_3As to afford the support-bound styrene and biaryl products. Cleavage from support using 5% TFA in CH_2Cl_2 (Rink) or 90% TFA in CH_2Cl_2 (Wang) provided the products **170** or **171** in crude mass balances of 85−92% and in greater than 90% purity as estimated by HPLC (Table 17).

Table 17. Stille Reaction Products 170 and 171 (Scheme 37)

entry	product[a]	vinyl stannane	yield (%)[b]
1	**170**	CH=CH_2	89
2	**170**	(*Z*)-CH=CH(CH_3)	91
3	**170**	CH=C(CH_3)$_2$	85
4	**170**	(*E*)-CH=CH(Ph)	89
5	**170**	(*E*)-CH=CH(Ph-3,4-di-OCH_3)	90
6	**171**	CH=CH_2	92
7	**171**	(*Z*)-CH=CH(CH_3)	88

[a] Compounds **170** were synthesized on Rink resin and were isolated as the primary amide. Compounds **171** were synthesized on Wang-Ala resin. [b] Yields are based on resin-bound 4-iodobenzoic acid as the limiting reagent.

Scheme 38

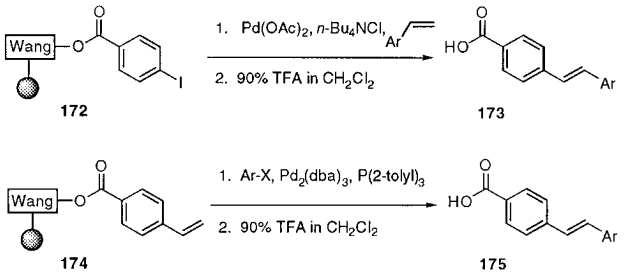


Table 18. Compounds Synthesized by a Heck Reaction (Scheme 38)

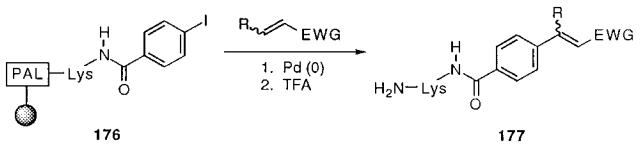
entry	reactant	product	mass balance (%)[a]
1	(4-carbomethoxy)styrene	**175**	90
2	phenylacetylene	**175**	90
3	ethyl acrylate	**175**	91
4	ethyl propenoate	see *b* below	see *b* below
5	Ph-I	**173**	81
6	3-bromonaphthyl	**173**	64
7	2-bromothienyl	**173**	76
8	3-bromopyridyl	**173**	87

[a] Mass balances are based on resin-bound 4-vinyl- or 4-iodobenzoic acid as the limiting reagent. The products are >90% pure as determined by ^{1}H NMR and HPLC analysis. [b] A mixture of products was obtained.

Heck Reaction. Yu and co-workers have examined the generality of the Heck reaction on both support-bound iodides and support-bound alkenes under a variety of reaction conditions (Scheme 38).[134] Yields of the final products **173** were excellent when the support-bound aryl iodide was coupled with different alkene components in solution using Pd-($OAc)_2$ in DMF at 80−90 °C. The polymer-bound alkene was also coupled with four different aryl halides using $Pd_2(dba)_3$ and P(2-tolyl)$_3$ in DMF at 100 °C to give good yields of the final product **175**, although Heck reactions with aryl triflates were not successful (Table 18).

Zhou and co-workers have extended the scope of the Heck reaction on solid support to include reactions that proceed under mild conditions (Scheme 39).[135] Using a phase-transfer system developed by

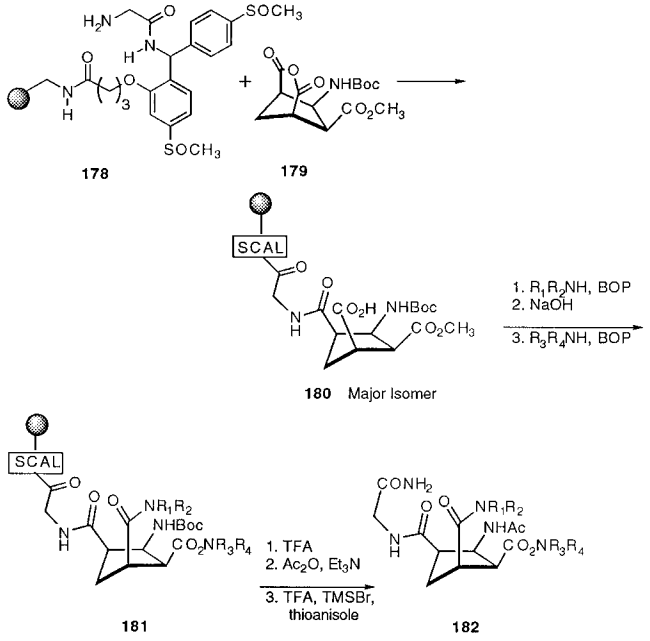
Scheme 39



Jeffery[136] and PEG-PS resin from Millipore, the authors have shown that Heck reactions can be performed on support-bound 4-iodobenzoic acid in aqueous solvent comixtures (DMF/H_2O/Et_3N, 1:1:1) using $Pd(OAc)_2$, PPh_3, and Bu_4NCl at 37 °C. Yields of Heck products range from fair to excellent (54−95%, av 79%, 6 compounds) for a variety of vinylic reagents.

4. Amide Bond Formation on a Cyclic Template. Lebl and co-workers have synthesized an *all-cis*-cyclopentane template for the display of amide-based functionality.[137] Compound **179** is synthesized in solution by a multistep route starting from com-

Scheme 40



mercially available *cis*-5-norbornene-*endo*-2,3-dicarboxylic anhydride (Scheme 40). The anhydride is opened with a resin-bound secondary amine of SCAL[138]-derivatized PEG-PS resin **178** to give a 9:1 mixture of the regioisomeric carboxylic acids (only the major diastereomer is shown). After coupling the resulting free carboxylic acid to an amine using (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP), the methyl ester is saponified with 0.5% NaOH. The resulting carboxylic acid is then coupled with either a primary or secondary amine and BOP. The *N*-Boc group is then removed by treatment with TFA, and the resulting amine is acetylated. Final cleavage from the SCAL linker is accomplished by treatment with 1 M TMSBr and 1 M thioanisole in TFA. The authors report that several derivatives with different side chains were obtained in high yield and purity employing the described sequence, but no structures or analytical data were provided. The Selectide group has recently extended this chemistry to include a related scaffold, 1,3,5-trimethyl-1,3,5-cyclohexanetricarboxylic acid (Kemp's triacid).[139]

5. Ether Formation. Rano and Chapman have optimized the Mitsunobu coupling reaction on solid support for the synthesis of aryl ethers with either the phenol or alcohol serving as the support-bound component (Scheme 41).[140] Either 4-(hydroxymethyl)benzoic acid or 3-(4-hydroxyphenyl)propionic acid was coupled to Rink-derivatized PEG-PS resin with EDC to give the support-bound alcohol or phenol.

Scheme 41

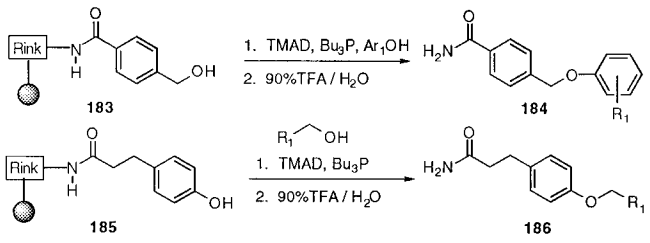


Table 19. Biaryl Derivatives 184 or 186 (Scheme 41)

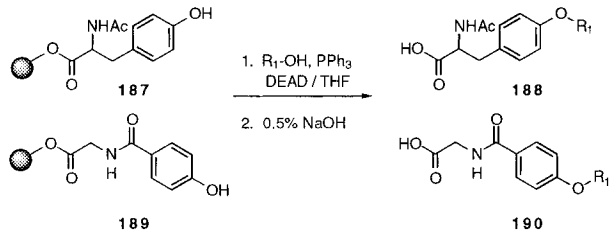
entry	alcohol or phenol R^1	product	yield (%)[a]	purity (%)[b]
1	H	**184**	75	92
2	4-CH_3	**184**	79	90
3	4-OCH_3	**184**	75	92
4	4-OPh	**184**	81	89
5	4-Br	**184**	90	93
6	4-CO_2CH_3	**184**	92	96
7	4-CN	**184**	99	95
8	2-CH_3	**184**	72	90
9	2-Ph	**184**	99	97
10	2-CH_3, 4-CHO	**184**	80	94
11	C_6H_4-4-Br	**186**	87	92
12	C_6H_4-4-CO_2CH_3	**186**	77	97
13	see *c* below	**186**	94	98
14	Bu	**186**	66	88
15	(3-C_6H_5)-C_3H_6	**186**	68	81

[a] Yields of unpurified material are based on resin-bound aryl halide as the limiting reagent. [b] Purities were estimated by HPLC analysis. [c] 4-[(2-aminomethyl)phenyl]phenyl.

Mitsunobu reaction with 5 equiv of both *N*,*N*,*N*′,*N*′-tetramethylazodicarboxamide (TMAD) and Bu_3P and excess of the phenol or alcohol followed by cleavage with aqueous TFA provided Mitsunobu products **184** or **186.** Crude mass balances ranged from 66−99% (av 82%, 15 compounds) and purities ranged from 81−99% (av 92%) as estimated by HPLC (Table 19). Mitsunobu reactions using diethyl or diisopropyl azodicarboxylate were also successful, but resulted in slightly lower purities and yields of the final products.

Krchnak and co-workers have also reported a method for the modification of a resin-bound phenol using the Mitsunobu reaction (Scheme 42).[141] *O*-

Scheme 42



Alkylation of Ac-Tyr-OH and 4-(hydroxybenzoyl)-glycine bound to PEG-PS resin was accomplished using PPh_3 and DEAD with a variety of primary and secondary alcohols. The Mitsunobu reaction is exothermic; however, reaction mixture warming promotes decomposition of DEAD, liberating ethyl alcohol and producing the corresponding ethyl ether as a byproduct. Accordingly, the reaction is performed by premixing the resin, alcohol, and PPh_3, and then adding a solution of DEAD in THF portionwise over the period of 20 min. DIAD was also found to give better results in problem cases, however, the reaction time is longer, typically 3 h instead of 1 h. The product purities by HPLC range from fair to excellent, although yields were not reported (Table 20). This chemistry has been used to synthesize a library of 4200 compounds using split synthesis. Twenty amino acids were coupled to PEG-PS resin, a variety of 10 aromatic hydroxy acids were coupled under standard conditions, and a Mitsunobu reaction was

Table 20. Alkyl Aryl Ethers 188 or 190 (Scheme 42)

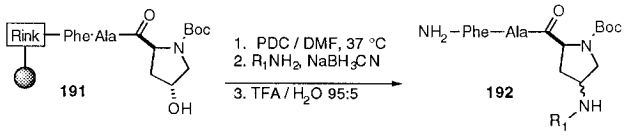
entry	alcohol R^1	product purity (%)[a,b]		ethyl ether (%)[a,b]	
		188	**190**	**188**	**189**
1	methanol	95	99	<1	<1
2	ethanol	96	99	main product	
3	2-propanol	93	99	<1	<1
4	1-butanol	75	80	23	20
5	allyl alcohol	95	99	<1	<1
6	1,3-propanediol	94	99	<1	<1
7	benzyl alcohol	99	93	<1	<1
8	4-methoxybenzyl alcohol	98	99	<1	<1
9	4-(methylthio)benzyl alcohol	78	90	<1	<1
10	2-(hydroxymethyl)furan	66	74	9	8
11	3-(hydroxymethyl)furan	98	99	<1	<1
12	2-(hydroxymethyl)thiophene	94	93	2	<1
13	4-methyl-5-(2-hydroxyethyl)thiazole	59	85 (96)	2	13 (<1)
14	2-(hydroxymethyl)pyridine	84	99	6	<1
15	3-(hydroxymethyl)pyridine	86	99	6	<1
16	4-(hydroxymethyl)pyridine	57	92	6	<1
17	2,6-bis(hydroxymethyl)pyridine	82	98	9	<1
18	1-(2-hydroxyethyl)pyrrolidine	43 (60)	53 (82)	5 (<1)	8 (<1)
19	1-(2-hydroxyethyl)-2-pyrrolidinone	42	47 (85)	10	47 (9)
20	1-(Boc-amino)ethyl alcohol	39 (52)	51 (82)	46 (29)	45 (15)
21	3-(Fmoc-amino)propyl alcohol	90	69	5	30

[a] Purities were estimated by HPLC analysis. [b] Values in parentheses refer to the analogous reaction performed with DIAD.

performed with 21 alcohols. Structural determination and screening are reported to be in progress.

6. Amine Alkylation. Bray and co-workers at Chiron Mimotopes have demonstrated a useful application of the Chiron Mimotopes pin method (see section II.A.1.a) for the rapid optimization of reactions performed on solid support.[142] In specific, multiple reactions are performed in parallel in a spatially separate format in order to rapidly identify the optimal reaction conditions for the transformation of interest. Bray demonstrated this approach for the two-step sequence of converting a support-bound 4-hydroxyproline derivative to a support-bound 4-aminoproline derivative (Scheme 43). A peptidic system

Scheme 43



was chosen to evaluate the reaction sequence because both reaction conversion and product purity have previously been reliably monitored for peptides by ion spray MS methods.[10a] To show the power of the multipin approach, five reaction parameters were simultaneously varied, including the amine structure, the amine concentration, the $NaBH_3CN$ concentration, the pH, and the reaction solvent. Fifty-six reactions were run in parallel followed by cleavage and ion spray MS analysis. On the basis of the trends provided by the data, the optimal reaction conditions were identified: 2.0 M [amine], 0.05 M [$NaBH_3CN$], methanol as the solvent, and pH = 5 for nonaromatic amines and pH = 7 for aromatic amines. A set of eight amines with widely different structures were surveyed using the optimized conditions and representative examples of percent conversion were determined by both HPLC and MS (Table 21). Good correlation was generally observed between the two analytical methods, although the MS data tended to give a low value for amines with two

Table 21. Reductive Amination Products 192 (Scheme 43)

entry	amine	conversion (%) HPLC	MS
1	β-alanine	95	80
2	5-amino-2-methoxypyridine	92	74
3	2-amino-1-propanol	90	88
4	4-bromoaniline	92	72
5	3,4-dihydroxybenzylamine	93	86
6	2,2-diphenylethylamine	94	86
7	3-methoxyaniline	95	91
8	piperidine	88	88

basic sites. HPLC conversions ranged from 88−95% and MS conversions ranged from 72−91%.

7. Urea Formation. Hutchins and Chapman have reported a straightforward method for the synthesis of ureas on PEG-PS resin (Scheme 44).[143] *N*-Fmoc-L-glutamic acid α-allyl ester was attached to support with the γ-carboxylic acid employing the 4-[4-(hydroxymethyl)-3-methoxyphenoxy]butyric acid (HMPB) handle. After cleavage of the Fmoc group under standard conditions, the resulting free amine was acylated with *p*-nitrophenylchloroformate and with *i*-Pr_2NEt as a base. The activated carbamate **194** was then treated with excess of an amine in DMF at ambient temperature to provide the amide **195**.

Scheme 44

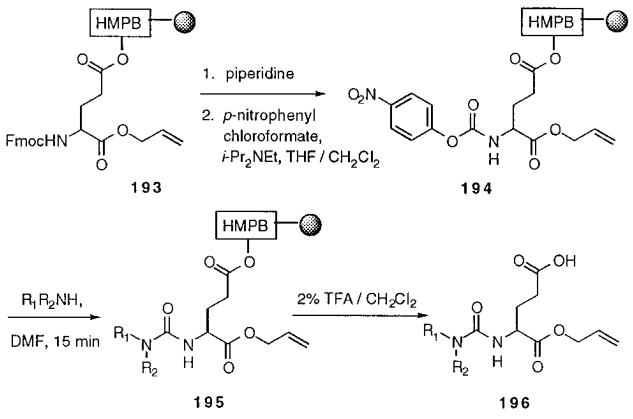


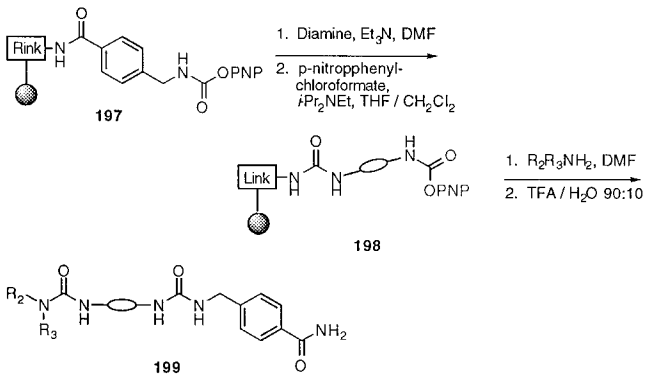
Table 22. Urea Products 196 (Scheme 44)

entry	amine	purity (%)[a]
1	benzylamine	96
2	adamantylamine	92
3	*N*-(2-chlorophenyl)piperazine	95
4	*N*-benzylpiperazine	96
5	1,2,3,4-tetrahydroisoquinoline	98
6	aniline	96
7	1,2,3,4-tetrahydroquinoline	90
8	4-nitroaniline	no reaction

[a] Estimated purity as determined by RP-HPLC. The expected molecular ions were observed by electrospray MS for all of the products.

Final cleavage from the support was accomplished by treatment with 2% TFA in CH_2Cl_2 to provide products **196** (Table 22). The authors have extended this work to the preparation of urea-linked diamines

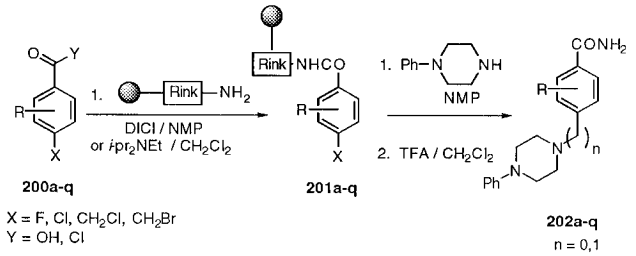
Scheme 45



for library synthesis.[144] As shown in Scheme 45, 4-(aminomethyl)benzoic acid is linked to PEG-PS resin, followed by activation of the amine as a *p*-nitrophenyl carbamate as described previously (Scheme 44) to provide **197**. Reaction with a primary diamine followed by repeating the carbamate synthesis sequence produces compound **198**. Urea formation with a variety of primary or secondary amines followed by cleavage produces urea-linked diamines **199** in excellent purity by RP-HPLC analysis (Table 23), although yields were not reported.

8. Aryl Substitution. Dankwardt and co-workers have synthesized a variety of aryl- and benzylpiperazines on solid support.[145] Haloaromatic compounds **200a**−**q** were coupled to support and evaluated for the ability to react with phenylpiperazine (Scheme 46). As shown in Table 24, fluorobenzoic

Scheme 46



acids activated by an ortho or *p*-nitro group reacted efficiently, while less highly activated aryl derivatives did not undergo reaction. Several benzylic piperazines were also formed from the corresponding support-bound benzylic halides. On the basis of these results, the five core structures **200a**−**e** were chosen for library synthesis. These cores were coupled to support and the resins mixed. The resulting resin mixture was reacted with one of 38 different substituted piperazines (structures not provided) to provide 38 pools of five compounds each. The piperazines had

Table 23. Urea-Linked Diamines 199 (Scheme 45)

entry	diamine	amine ($R_1R_2NH_2$)	purity (%)[a]
1	1,4-phenylenediamine	1,2,3,4-tetrahydroisoquinoline	99
2	1,4-phenylenediamine	aniline	89
3	1,4-phenylenediamine	benzylamine	99
4	*p*-xylenediamine	1,2,3,4-tetrahydroisoquinoline	90
5	1,3-cyclohexyldiamine	2,2-diphenylethylamine	95

[a] Estimated purity as determined by RP-HPLC.

Table 24. Benzamides 202 (Scheme 46)

entry	aryl starting material (**200a**−**q**)	product	purity (%)[a]
1	4-fluoro-3-nitrobenzoic acid	**202a**	82
2	2-fluoro-5-nirtobenzoic acid	**202b**	81
3	[4-(bromophenyl)phenyl]acetic acid	**202c**	86
4	3-(chloromethyl)benzoyl chloride	**202d**	84
5	4-(chloromethyl)benzoic acid	**202e**	85
6	2-fluoro-3-(trifluoromethyl)benzoic acid	**202f**	NR
7	2-fluoro-5-(trifluoromethyl)benzoic acid	**202g**	63 + 23% SM
8	4-fluoro-2-(trifluoromethyl)benzoic acid	**202h**	NR
9	4-fluoro-3-(trifluoromethyl)benzoic acid	**202i**	NR
10	4-fluoro-1-naphthoic acid	**202j**	NR
11	2-chloro-4-flourobenzenesulfonyl chloride	**202k**	MP
12	3-chloro-4-flourobenzenesulfonyl chloride	**202l**	NR
13	2-chloro-6-methylpyridine-4-carboxylic acid	**202m**	MP
14	6-chloronicotinic acid	**202n**	NR
15	2-chloro-3-nitrobenzoic acid	**202o**	NR
16	3-chloro-2-nitrobenzoic acid	**202p**	NR
17	5-chloro-2-nitrobenzoic acid	**202q**	NR

[a] Estimated purity as determined by RP-HPLC. NR = No Reaction. MP = multiple products observed.

been independently shown to react with support-bound benzamide **201a** and to give products of >70% purity by HPLC. These pools are being screened for biological activity.

5. Molecular Recognition in Designed Receptor Systems

The molecular recognition of ligands by biological receptors plays a critical role in virtually all biological processes. To better understand the underlying noncovalent interactions that are responsible for the selectivity and specificity of large natural receptors, many researchers have focused on characterizing the specificity and affinity of small, well-defined host molecules. Combinatorial chemistry has rapidly become a very powerful tool for accomplishing this goal. Two general approaches may be envisioned. In one approach, a library of host molecules is screened against a defined ligand or small set of ligands in order to identify the key elements of the host structure that are necessary to provide affinity or selectivity. In the second approach, a defined host molecule or set of host molecules is screened against a library of ligands. Both of these strategies have been implemented and are described below.

Although outside the scope of this review, peptide and oligonucleotide library methods are certainly valuable tools for studying natural receptor−ligand recognition. As a particularly significant example, Schreiber has described the application of biased peptide libraries to define the recognition motifs of SH3 domains, which mediate many essential protein−protein interactions in biological systems.[146]

Libraries of Host Molecules. Still has explored combinatorial strategies to develop synthetic receptors by the display of diverse peptides from a steroid scaffold.[147] In this work, the steroid cheno(12-deoxy)-cholic acid was coupled to (aminomethyl)polystyrene beads by amide bond formation. The support-bound steroid was then selectively acylated with Fmoc-glycyl fluoride at the less hindered C3 position (Figure 32). All combinations of a dipeptide were then synthesized from a basis set of 10 Fmoc amino acids using the split synthesis method and haloaromatic tag strategy to record the synthesis steps. The free amino terminus was then capped with acetic anhydride and the more hindered C7 hydroxyl was acylated again using Fmoc-glycyl fluoride, but with DMAP as a catalyst. The dipeptide synthesis and capping sequence was then repeated to produce a 10^4 member library of potential receptors.

The library of support-bound steroidal receptors was screened for binding to four different enkephalin-like peptides that were each labeled at the amine terminus with the dye Disperse Red 1 (Figure 32). The support-bound receptor library was incubated with the dye-linked peptide in $CHCl_3$ with the concentration of the peptide adjusted such that approximately 1% of the beads were stained bright red (100−150 μM depending upon the structure of the dye-linked peptide). For each binding study, approximately 50 of the darkly stained beads were selected and the tags read to identify the structures of the tight-binding host molecules. Significant amino acid preferences were observed for each of the peptide ligands. Receptors that bound ligands **206** and **207** have a strong preference for Pro at the AA_2 and AA_3 sites, while fewer than 10% of receptors that were selective for ligands **204** and **205** contained Pro at those sites.

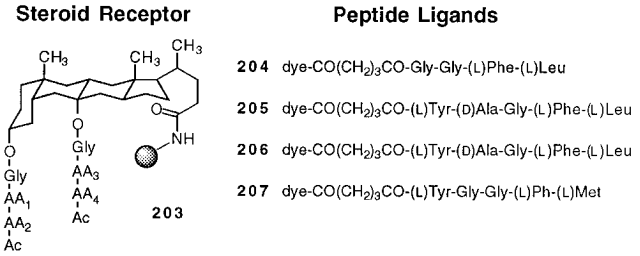


Figure 32. Steroid receptor.

In order to select which host molecules bind selectively as well as tightly to a given ligand, competition experiments were performed with peptide ligands **205** and **206**, but while **205** was still labeled with Disperse Red 1, **206** was now labeled with Disperse Blue 3. Selectivity was readily observed since a bead containing a nonselective host would turn purple, while a bead that contains a selective host would retain the red or blue color of the respective dye. Several receptor beads were found to turn bright blue. Upon decoding the blue beads, two predominant consensus sequences were observed, AA_1−AA_4 = Phe,Pro,Pro,Leu and Asp,Pro,-Pro,Val. Resynthesis of these receptors followed by HPLC-based binding studies confirmed that these receptors were indeed very selective, with $(\Delta\Delta G)$ = −1.0 and −1.6 kcal/mol, respectively. None of the receptors screened, however, were able to selectively bind Leu enkephalin, (L)Tyr-Gly-Gly-(L)Phe-(L)Leu, over Met enkephalin, (L)Tyr-Gly-Gly-(L)Phe-(L)Met, with the most selective receptor identified showing a $(\Delta\Delta G)$ = −0.2 kcal/mol.

Libraries of Ligands. Still has utilized the haloaromatic tag encoding strategy for the synthesis and evaluation of libraries of tripeptides in order to define the key structural elements responsible for recognition by a number of host molecules. Two generalized classes of host molecules have been investigated, a class of C_3-symmetric hosts (Figure 33)[148] and a class of hosts of either D_2- or tetrahedral symmetry that are prepared by the cyclooligomerization of 1,3,5-benzenetricarboxylic acid (trimesic acid) and the 1,2-diamines **216**-**219** (Figure 34).[149] Both host molecule classes show a high level of selectivity for specific acylated tripeptide sequences. In addition, despite their large structures, compounds in both host molecule classes can be prepared very efficiently allowing the rapid synthesis of modified structures for further study.

For all of the host molecules except for the water-soluble host **213**, a library of ~50 000 acyl tripeptides was screened for binding. The library was prepared by split synthesis using the haloaromatic tag strategy. The acyl tripeptides were coupled to the aminomethylated polystyrene support through an ω-aminohexanoic acid linker. Fifteen amino acids were employed for each of the three positions in the tripeptide, and 15 acylating agents were employed to cap the tripeptide to provide a theoretical number

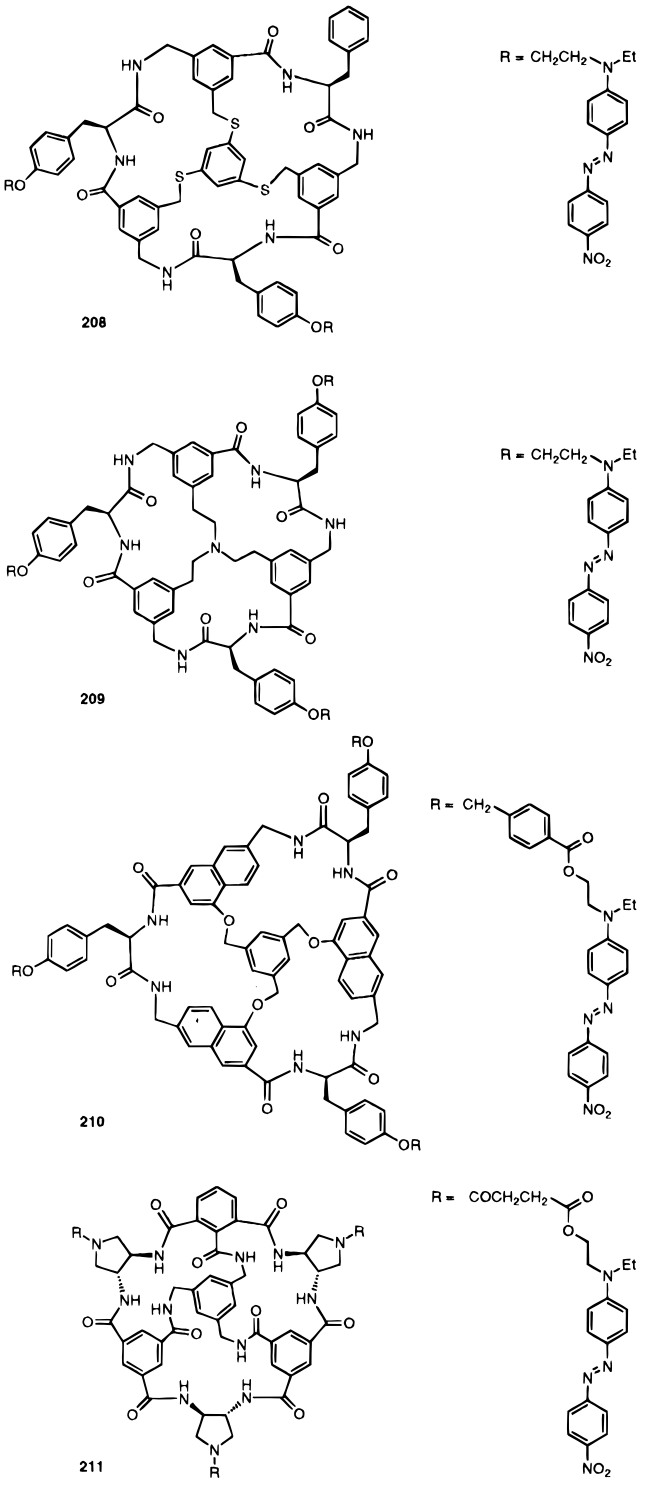


Figure 33. C_3-Symmetric hosts.

of 50 625 unique compounds. The acyl tripeptide library was screened either in side chain-protected form or after side chain deprotection by trifluoroacetic acid treatment. For water soluble host **213**, a tripeptide library was screened that had the general structure AA3-AA2-AA1-NH$(CH_2)_2$-support. This library was prepared on PEG-PS due to its favorable solvation characteristics in aqueous solvents. All possible combinations of 29 amino acids were introduced at each position using the split synthesis process to provide a theoretical library size of 24 389 tripeptides. The library was screened both in completely deprotected form and with the side chains

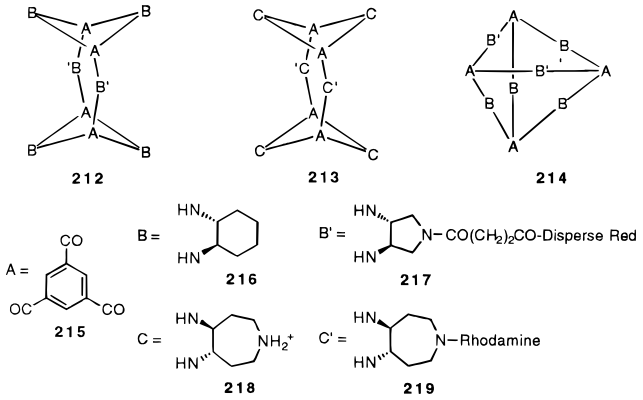


Figure 34. Hosts prepared by the cyclooligomerization of trimesic acid and 1,2-diamines.

protected and with the *N*-terminus acetylated.

The general screening procedure was to mix the support-bound peptide library with the dye-labeled host molecule at a set concentration (usually between 1 and 100 μM). Chloroform was employed as the solvent for the polystyrene-bound libraries and the organic soluble hosts, and water was employed as the solvent for the PEG-PS-bound libraries and the water-soluble host **213**. After letting the mixture stand for a minimum of 24 h, a percentage of the beads became darkly stained, with the percentage of beads that were stained depending upon the selectivity and affinity of the host molecule as well as the host molecule concentration. Multiple darkly stained beads were picked (usually between 30 and 100) and the tags read to provide the structures of the tight-binding tripeptide ligands.

A detailed description of the results from each binding experiment is outside the scope of this review; however, a great deal of information was obtained about the structural features of the tripeptides that were important for binding. Distinct selectivities were observed for different host molecules depending upon the size, shape, hydrophobicity or hydrophilicity, and hydrogen-bonding character of the host. The screening protocol provided the positions in the acyl tripeptide that are important for binding. For those positions that are important determinants for binding, both the side-chain structure and the stereochemistry of the amino acids were also provided. In addition, for several of the hosts, different families of tight-binding sequences were identified. This large body of information would have been difficult if not impossible to obtain by alternative nonlibrary methods.

6. Analytical Techniques

Because such a large variety of analytical techniques have been used to characterize solid-phase chemistry or compounds bound to solid supports, only a summary of the available methods will be presented here. A number of methods are routinely used for monitoring reactions. This includes the use of traditional methods for functional group titration, many of which can be quantitative. Some examples are ninhydrin,[57] picric acid,[150] trinitrobenzenesulfonic acid,[151] and bromophenol blue[152] tests for free amines, and Ellman's test for free thiols.[153] In addition, the cleavage of many protecting groups gives stable UV-

active byproducts which can be quantified. This includes the piperidine−dibenzofulvene adduct that results from Fmoc group cleavage with piperidine,[154,155] the highly colored cation that results from acid-mediated dimethoxytrityl ether cleavage,[156,157] and 4-nitrostyrene that results from deprotection of the (nitrophenyl)ethyloxy group.[115] Additional methods continue to be developed such as the recent report of Reich and co-workers on a new reagent, nitrophenyl *O*-tritylisothiocyanate (NPIT), that is effective for quantitation of sterically hindered or nonbasic amines.[158] Other methods that have typically been used to calculate resin loading include Volhard titration for chloride,[159] and elemental analysis for a variety of atoms.

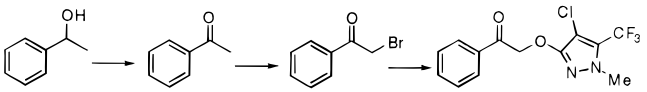
Mass spectroscopy has already been mentioned as one of the few available methods sensitive enough to characterize reaction products from a single synthesis bead;[32−35] however, MS has many other useful applications in library analysis. MS has a short sampling time, allowing rapid MS characterization of a large number of samples.[142] Deletion products and side products often give MS signals that can be used to optimize synthesis sequences.[31,34a−c] MS characterization of mixtures is possible,[87,160,161] and judicious choice of compounds that are pooled can provide pools where all compounds have molecular ions that are separable.[161b] Finally, the use of MS in conjunction with other analytical techniques (HPLC−MS, GC−MS, MS−MS) is well documented and has been successfully employed in library characterization.[31,32,87]

Many NMR and IR spectroscopic techniques have been used for the characterization of compounds attached to solid supports. Using PEG-PS resins, ^{13}C NMR spectra of compounds attached to support can often be collected.[162] ^{13}C NMR of compounds on standard polystyrene resins can be useful, but the resolution can be poor relative to the spectra obtained on PEG-PS resin and usually require thousands of transients. The use of ^{13}C-enriched compounds has been reported to greatly expedite acquisition of data and to provide greatly improved signal to noise.[163] Other techniques have also been reported including magic-angle-spinning (MAS) solid-state ^{1}H NMR,[164] MAS $^{13}C-^{1}H$ correlation experiments,[165] and recently MAS HMQC and TOCSY experiments.[166] IR is one of the most convenient methods for evaluating support-bound compounds and has also been employed by many researchers, including a recent report of FT-IR microspectroscopy allowing data collection from a single resin bead.[167]

III. Libraries Synthesized in Solution

While the majority of published work on small molecule library synthesis has been performed using solid supports, solution-phase strategies have been applied successfully by a number of researchers. A successful solution-phase approach can have significant advantages over a comparable solid-phase approach. In particular, a method does not need to be developed to attach the initial starting material onto support or to cleave the final product from the support. One step or short reaction sequences that proceed in high yield with stoichiometric reagents are amenable to solution-phase strategies, since purification or isolation is not required. Quite complex structures can be accessed in a single step by employing multicomponent reactions, such as the Ugi reaction (*vide infra).* In addition, numerous support-bound reagents and catalysts are available that greatly facilitate the isolation of the products in solution and thereby may provide access to multistep reaction sequences for the purposes of library synthesis.[168] Parlow has provided a recent impressive example of the potential application of polymer-bound reagents to organic synthesis.[169] In his study *sec*-phenethyl alcohol was treated *simultaneously* with three different polymer-supported reagents that would not be compatible if they were in solution; the oxidant poly(4-vinylpyridinium dichromate), the brominating agent perbromide on Amberlyst A-26, and the alkylation agent Amberlite IRA 900 (4-chloro-1-methyl-5-(trifluoromethyl)-1*H*-pyrazol-3-ol) (Scheme 47). After filtration of the polymer reagents, the desired α-alkoxyketone was obtained in 48% isolated yield.

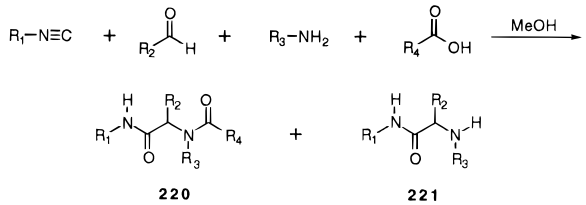
Scheme 47



A. Spatially Separate Synthesis

Few reports have appeared in the literature on the simultaneous solution-phase synthesis of libraries in a spatially separate manner, although a number of research groups in academics and industry have presented on this strategy.[170] Several researchers have focused on solution-phase synthesis using the Ugi reaction to provide libraries of small molecules. The Ugi reaction uses four different components, an isocyanide, an aldehyde, an amine, and a carboxylic acid, to provide one major reaction product (**220**, Scheme 48).[171] Ugi has recently published an overview of potential strategies for best applying multicomponent reactions to library synthesis and evaluation.[172]

Scheme 48



Weber and co-workers have employed the Ugi reaction to synthesize molecules in a spatially separate and parallel format.[173] A structurally diverse and commercially available set of 10 isocyanides, 40 aldehydes, 10 amines, and 40 carboxylic acids were chosen to provide a virtual library of 160 000 compounds. Reaction conditions were optimized so that a major side product was observed only when weakly nucleophilic amines, such as 4-aminobenzamidine, were used in the coupling process resulting in appreciable amounts of the amine side product **221** that does not incorporate the carboxylic acid component.

An iterative search and synthesis procedure was then employed to identify a moderately potent throm-

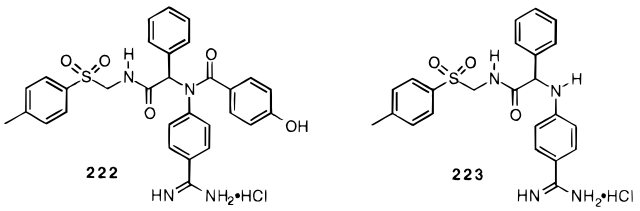


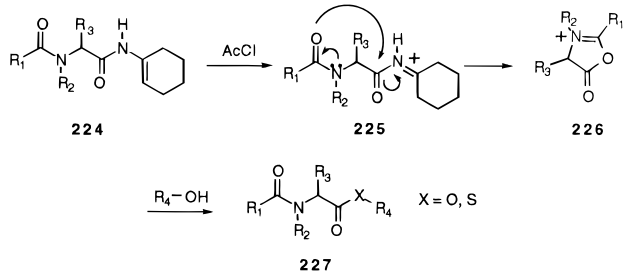
Figure 35. Ugi products selected by screening with a genetic algorithm.

bin inhibitor by synthesizing and evaluating only a small fraction of the virtual library of 160 000 possible compounds. By assigning a binary code to the individual components, a genetic algorithm was employed to select for increased inhibitory activity of the compounds in a thrombin assay. The potential application of genetic algorithms to combinatorial synthesis has previously been modeled.[174] Weber developed the genetic algorithm that was employed for the search and synthesis procedure by screening the virtual library against different test functions corresponding to compounds with arbitrary biological activities. The population size and building block interchanges that correspond to mutation rates were adjusted so that the most active candidate would be discovered in 10−50 cycles of synthesis and evaluation.

Weber performed 20 synthesis and evaluation cycles of 20 Ugi products that were synthesized in parallel. The first set of Ugi products were selected randomly from the available set of reagents. The remaining Ugi products for each synthesis cycle were selected using the developed genetic algorithm, with building block interchanges corresponding to crossovers and random mutations. From the first cycle to the last cycle, the average inhibitory activity of the set of 20 Ugi products in the thrombin assay increased from approximately 1 mM to less than 1 μM. The most active compounds, **222** and **223** (Figure 35), had IC_{50} values of 1.4 μM and 0.22 μM, respectively. Interestingly, compound **223** did not incorporate the carboxylic acid functionality and demonstrates the importance of fully characterizing reaction efficiency. It is also significant that the application of genetic algorithms is in no way limited to solution-phase library approaches.

Keating and Armstrong have also investigated the Ugi reaction for the purpose of library synthesis.[175]

Scheme 49



Having noted the limited commercial availability of isocyanides, they developed 1-isocyanocyclohexene as a "universal" isocyanide, which upon incorporation into an Ugi product (**224**, Scheme 49) can be activated to introduce an additional level of diversity. (Ugi had previously demonstrated that Ugi products could in fact be obtained employing isocyanocyclohexene.[176]) Armstrong has shown that upon exposure to HCl, Ugi products that have incorporated isocyanocyclohexene become activated for nucleophilic displacement presumably through cyclization to form the activated münchone **226**, which then reacts with a nucleophile to provide product **227**. Treatment with H_2O, alcohol, or mercaptan provides the carboxylic acid, ester, or thioester, respectively, in 45−100% isolated yield of purified material (Table 25, average 70%, 13 compounds). This method provides access to a variety of esters, including the sterically hindered *tert*-butyl ester (64% yield), significantly increasing the number of Ugi products that are accessible. Although amides are not directly available using this procedure, they could be synthesized in one step from the corresponding acid.

B. Synthesis in Pools

1. A Library of Amides and Esters

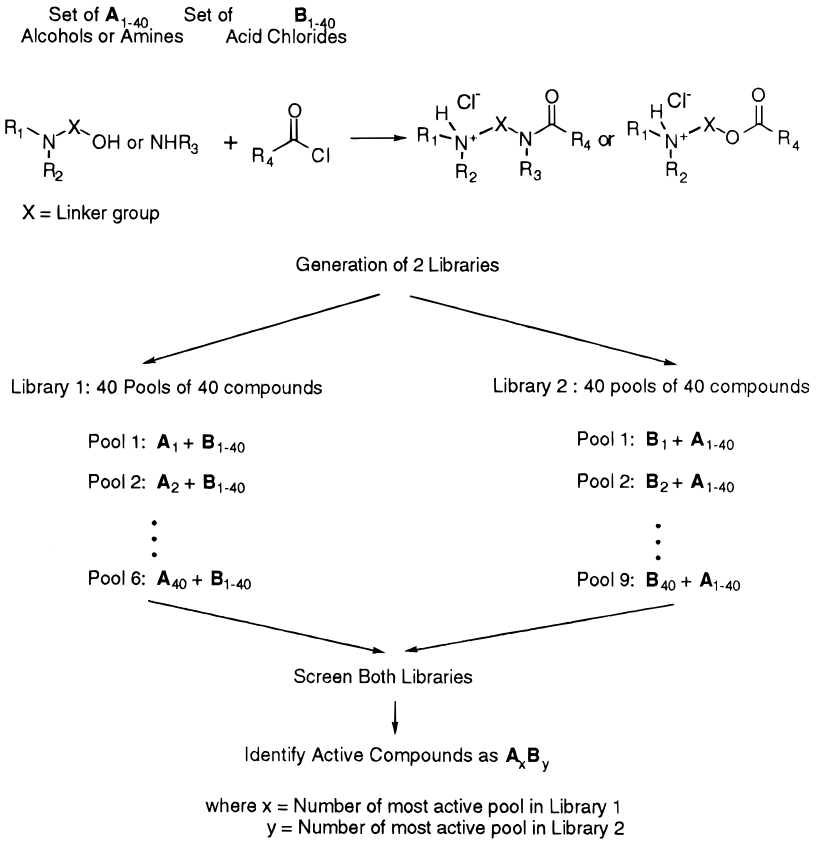
Two reports have appeared in the literature on pooling strategies for the rapid synthesis and evaluation of organic compound libraries in solution.[177] Smith has synthesized a library of potentially 1600 esters or amides from the reaction of 40 alcohols and amines with 40 acid chlorides (Scheme 50).[178] Two separate libraries were prepared that each contain amides and esters synthesized from all 1600 combi-

Table 25. Compounds 227 Synthesized via the Universal Isocyanide Route (Scheme 49)

	Ugi Product				
entry	R_1	R_2	R_3	XR_4	yield (%)[a]
1	CH_3	$CH_2C_6H_4$-4-OCH_3	$CH(CH_3)_2$	OH	56[b]
2	CH_3	$CH_2C_6H_4$-4-OCH_3	C_6H_5	OH	83[b]
3	CH_3	$CH_2C_6H_4$-4-OCH_3	C_6H_5	OCH_3	100[b]
4	CH_3	$CH_2C_6H_4$-4-OCH_3	C_6H_5	OCH_2CH_3	57[b]
5	CH_3	$CH_2C_6H_4$-4-OCH_3	C_6H_5	$OCH_2C_6H_5$	75[b]
6	CH_3	$CH_2C_6H_4$-4-OCH_3	$CH(CH_3)_2$	SCH_2CH_3	68[b]
7	CH_3	$CH_2C_6H_4$-4-OCH_3	1,5-*c*-C_5H_{11}	$OC(CH_3)_3$	64[b]
8	$(CH_2)_{10}CH_3$	$CH_2C_6H_4$-4-OCH_3	C_6H_5	OCH_3	65[c]
9	CH_3	$CH_2C_6H_4$-4-OCH_3	C_6H_5	OCH_3	79[c]
10	CH_3	$(CH_2)_9CH_3$	C_6H_5	OCH_3	99[c]
11	H	$CH_2C_6H_4$-4-OCH_3	C_6H_5	NH_2	45[c]
12	$CH_2C_6H_5$	*c*-C_6H_{11}	$CH(CH_3)_2$	OCH_3	67[c]
13	CH_3	$CH_2C_6H_4$-4-OCH_3	1,5-*c*-C_5H_{11}	OCH_3	55[c]

[a] Isolated yield of purified compound. [b] Yield based upon conversion of purified Ugi product **227**. [c] Yield based upon intial starting materials.

Scheme 50



nations of the 40 alcohol or amine reagents and the 40 acid chloride reagents. The first library is composed of 40 separate pools of 40 compounds with each pool containing compounds prepared from a single alcohol or amine and each of the different acid chlorides. The second library is composed of 40 pools of 40 compounds with each pool containing compounds prepared from a single acid chloride and each of the 40 different alcohols or amines. Screening the first library set for binding to a receptor provides the alcohol or amine component that putatively contributes the most to the binding affinity, while screening the second library provides the acid chloride component that contributes most to the binding affinity.

Stoichiometric quantities of the reagents were used, and all of the nucleophiles contained an internal tertiary amine moiety in order to neutralize HCl liberated in the reaction so that no byproducts were produced. Extended reaction times (48 h) were also employed in order to achieve reaction completion. Analytical determination of all of the compounds in the libraries was not attempted, but GC and GC−MS was performed on two of the pools of 40 compounds. In one pool molecular ions that corresponded to 25 out of 40 compounds were observed by electron impact ionization while in the second pool 30 out of 40 were observed. The libraries were screened in a number of assays, and two active compounds were identified. After synthesis on large scale and purification, amide **229** (Figure 36) was determined to have an IC_{50} value of 60 μM for the NK_3 receptor, and amide **230** was determined to have an IC_{50} value of 55 μM for inhibition of the matrix metalloproteinase-1 (MMP-1).

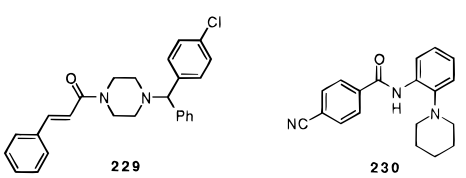


Figure 36. Novel ligand to the NK_3 receptor and novel MMP-1 inhibitor.

2. Acetylcholinesterase Inhibitors

Pirrung has reported a general pooling strategy for performing solution-based library synthesis and evaluation that he has defined as "indexed" combinatorial libraries.[179] Pirrung demonstrates this method with the solution-phase synthesis of 54 carbamates from the combination of nine alcohols with six isocyanates (Figure 37). Two libraries are again prepared as pools where each alcohol is combined with all isocyanates (library 1) or all alcohols are combined with each isocyanate (library 2). Screening the two libraries directly provides the structure of the alcohol and

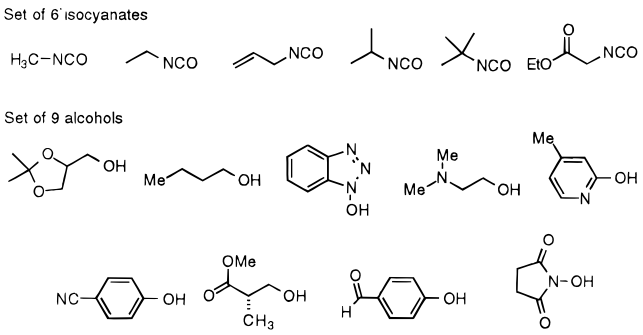
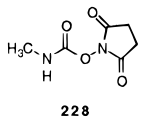


Figure 37. Alcohols and isocyanates used by Pirrung in library synthesis.



228

Figure 38. Novel acetylcholinesterase inhibitor.

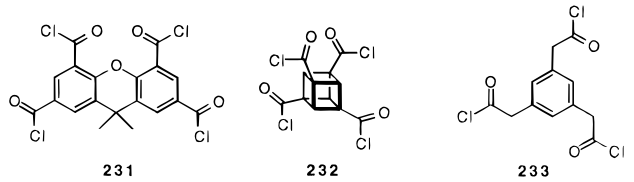
isocyanate that contribute most to inhibitory activity. Equimolar quantities of each compound in the individual pools are prepared by employing stoichiometric quantities of the respective alcohols and isocyanates and by ensuring complete reaction conversion for all starting materials by heating the reaction mixture in a sealed pressure tube. Pirrung comments that the approach could be extended to compounds that are prepared from more than two components, with *n* different components represented as an *n*-dimensional matrix. He also points out that assay precision will put constraints on the pool size for accurate library evaluation. The compounds are screened for inhibition of acetylcholinesterase to identify a novel inhibitor, **228** (Figure 38), that has modest inhibitory activity ($IC_{50} = 700\ \mu M$ for acetylcholinesterase inhibition).

3. Amides Displayed from a Core Molecule

An alternative strategy for preparing libraries in pools has been reported by Rebek.[20,161,180] In this approach, libraries are synthesized by treating a core molecule that incorporates acid chloride moieties with an equimolar mixture of a variety of protected amines. The amines are chosen from a group of amino acids and small heterocyclic amines, with the main criteria for selection being comparable reactivity of the amines toward the acid chlorides on the core molecule. One tri- and two tetraacid chloride core molecules have been employed (Figure 39). A key feature in the selection of the different core molecules is that the tri- and tetraamide products display functionality in different spatial arrays in order to achieve maximal diversity.

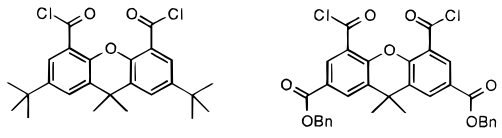
For a set number of amine components, the theoretical number of molecules produced per core is dependent upon the number of reactive acid chloride sites and on the symmetry of the core element. Therefore, for the same set of amines, the higher symmetry of the cubane core **232** will result in a smaller theoretical number of molecules than for the xanthene core **231**. Rebek prepared three separate libraries from a combination of 19 diverse amines and the three different core elements. The theoretical number of compounds per core were 65 341 molecules for xanthene core **231**, 11 191 molecules for cubane core **232**, and 1330 molecules for benzene triacid core **233**.

To ensure that all of the amine building blocks reacted in high yield, four equivalents of each amine were treated with xanthene core **231** in a spatially separate manner. All of the expected tetramide products were obtained in high yield with complete conversion in less than 30 min. In addition, although complete analytical evaluation of the three large libraries was not possible, the chemistry that was employed to prepare the libraries was tested using both HPLC and ESI-MS analysis on smaller sublibraries designed to cover different amine combinations. For example, in one study, xanthene derivative **234** (Figure 40) that contained two acid chlorides was employed to prepare six model libraries employing three sets of eight amines, two sets of nine amines, and one set of 10 amines to provide libraries of 36, 45, and 55 compounds, respectively. The amine building blocks were grouped in sets such that each product in the model library would contain a different molecular weight. Analytical evaluation by electrospray ionization mass spectrometry demonstrated that >80% of the compounds for each sublibrary were observed.[161b]



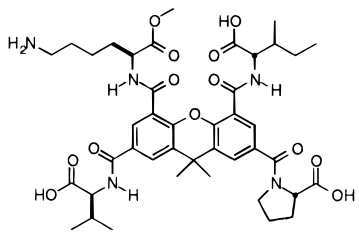
231 232 233

Figure 39. Core structures utilized by Rebek for library synthesis.



234 235

Figure 40. Modified cores utilized by Rebek in model studies and in deconvolution efforts.



236

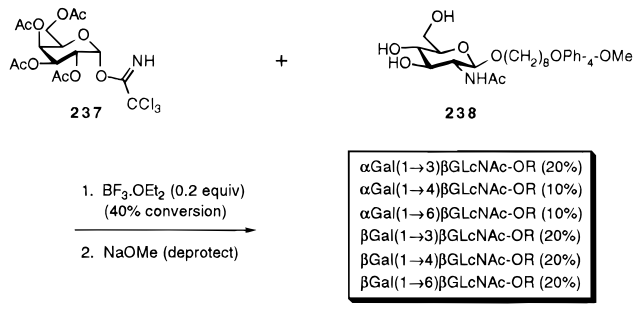
Figure 41. Novel trypsin inhibitor.

The three large libraries were screened in solution for inhibition of trypsin catalyzed hydrolysis of *N*-α-benzoyl-D,L-arginine-*p*-nitroanilide (BAPA).[180] Although inhibition was not observed for the libraries based upon cores **232** and **233**, the library based on core **231** did inhibit the enzyme. Deconvolution was performed by making pools missing three amines and reassaying to determine which set of amines was necessary for inhibitory activity. Nine amines were identified, and then nine sublibraries each missing one amine were synthesized, and the five most important amines were identified. The modified core **235**, in which the two bottom carboxylic acids are protected as benzyl esters, was then utilized to differentiate the top and bottom positions. Amines were first coupled to the top carboxylic acids, and the benzyl esters were then cleaved by hydrogenation followed by coupling amines to the bottom carboxylic acids. The deconvolution procedure resulted in the identification of an inhibitor of moderate activity ($K_i = 9 \pm 2\ \mu M$), **236** (Figure 41).

4. Oligosaccharide Libraries

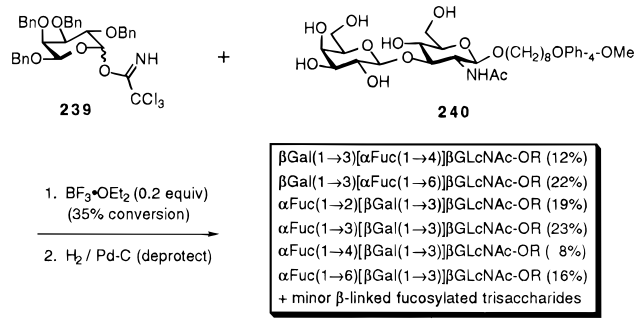
Hindsgaul has made considerable progress toward utilizing solution-based pooling strategies to synthesize and evaluate di- and trisaccharide combinatorial libraries for the purpose of identifying novel biologi-

Scheme 51



cally active oligosaccharide ligands.[181] Because each monosaccharide carries at least three hydroxyl groups, the development of an orthogonal protecting group strategy that would provide access to all possible monosaccharide combinations as well as all possible connectivities and both α- and *β*-isomers would be extremely challenging, particularly for trisaccharides. Hindsgaul therefore chose to investigate a random glycosylation approach whereby libraries of small pools of di- or trisaccharides would be generated that include all possible connectivities between the monosaccharide starting materials (≤112 compounds). Literature precedent suggests that random glycosylation would be difficult to achieve since glycosylation rate is highly dependent on the steric and electronic environment of the alcohol acceptor. However, two thoroughly characterized examples demonstrate the potential utility of this approach. As shown in Scheme 51, random glycosylation of *N*-acetylglucosamine (GlcNAc) derivative **238** with the peracetylated galactosyl (Gal) donor **237** provided a significant amount of each of the six possible disaccharide products (three different connectivities with both α- and *β*-isomers). The reaction was terminated at 40% conversion to minimize the formation of tri- and higher order oligosaccharide derivatives. The hydrophobic *p*-methoxyphenoxyoctyl aglycon of acceptor **238** facilitated separation of the disaccharide products from reaction byproducts using reverse-phase HPLC and also provided a chromophore for accurate isomer quantitation. Similarly, random fucosylation of *β*Gal(1→3)*β*GlcNAc-OR **240** provided all six possible α-fucosylated products (8−23%), although only minor quantities of the *β*-fucosylated isomers were observed (Scheme 52). In these two studies, all of the isomeric products were rigorously characterized by NMR, MS, and methylation analysis. Whether or not this strategy can be applied to the majority of the other mammalian monosaccharides remains to be demonstrated.

Scheme 52



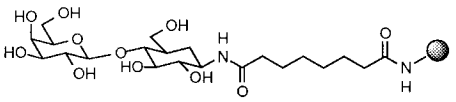


Figure 42. Lactosylamine linked to Tentagel resin.

Several researchers have reported very elegant approaches for the solid-phase synthesis of oligosaccharides and peptide−oligosaccharide hydrids.[182] Although these methods were developed for important applications independent of library synthesis, they provide the groundwork for any future efforts in the solid-phase synthesis of oligosaccharide libraries.

Vetter and Gallop have also developed a solution-phase synthesis of glycosylamines. By using a procedure described by Kochetkov,[183] commercially available reducing sugars are treated with a saturated ammonium carbonate solution to convert the anomeric hydroxyl to an amine.[184a] This simple, one-step reaction provides the *β*-anomer in >95% isomeric purity in almost all cases, and was demonstrated on 54 charged, neutral, and di- and oligosaccharides. Monoacylation of the glycosylamine with excess disuccimidyl suberate provides an active carbohydrate conjugate which can be purified by precipitation and linked to aminomethyl Tentagel resin for screening purposes[30b] (Figure 42). The recognition of carbohydrate-conjugated beads by a lectin was demonstrated. Preparation of seven carbohydrate−bead conjugates from the glycosylamines of maltose, chitobiose, lactose, sialyllactose, LacNAc, GlcNAc6SO_3, and GlcNAcPO_3^{2-} was followed by mixing the beads. Incubation with fluorescently labeled lectin (wheat germ agglutinin or ricinus communis agglutinin) followed by sorting using a FACS instrument showed selective binding of the lectin to different fractions of the pool. Although the structures of the sugars bound from the pool were not directly determined, chitobisylamide-derivatized beads bound to wheat germ agglutinin and lactosylamide beads bound ricinus communis agglutinin, as determined by assaying each sugar-bead conjugate individually. These disaccharides are known ligands for the corresponding lectins.

This work has been extended to the synthesis of *N*-linked glycopeptides.[185] By incorporating allyl ester protection of Glu or Asp into a peptide library, these side chains can be selectively deprotected with palladium and activated with pentafluorophenyl trifluoroacetate. Condensation with a variety of glycosylamines gives the support-bound glycopeptide, which can be deprotected and cleaved from the support. Glycopeptides were synthesized from 18 different mono- and oligosaccharidrylamines and five support-bound peptides, which were derivatives of the Leu-enkephalin sequence (Tyr-Gly-Gly-Phe-Leu) with an Asp or Glu added in various places to bind the sugar. Mono- and most disaccharide-derived glycopeptides were isolated in quantitative yields, and uncharged oligosaccharides gave yields in the 50−80% range. Charged sugars gave considerably lower conversions (30−50%).

IV. Future Directions

A number of important new advances continue to be made in the synthesis and applications of com-

pound libraries. Although much of the chemistryperformed on solid support will continue to be based upon analogous chemistry in solution, new chemistry that does not have a current solution-phase counterpart will also be developed. There is also considerable enthusiasm for combinatorial biosynthesis strategies for achieving libraries of diverse and complex structures, particularly through genetic manipulation of polyketide biosynthetic pathways.[186]

The synthesis and evaluation of libraries will increasingly be used not only to study ligand−receptor interactions, but in any area of chemistry where the identification of the optimal chemical structure for a particular application typically requires the synthesis and evaluation of many different compounds. Two examples where reports have already appeared are the evaluation of asymmetric catalysts for the synthesis of optically active compounds[187] and the development of new materials.[188]

V. Acknowledgments

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VI. Glossary

AC	acid cleavable linker, [4-(hydroxymethyl)-3-methoxyphenoxy]acetic acid
BOP	(benzotriazol-1-yloxy) tris(dimethylamino)phosphonium hexafluorophosphate
Bpoc	[[2-(4-biphenyl)isopropyl]oxy]carbonyl
CA	carbonic anhydrase
DCC	1,3-Dicyclohexylcarbodiimide
DEAD	diethyl azodicarboxylate
DIAD	diisopropyl azodicarboxylate
DICI	1,3-diisopropylcarbodiimide
DBU	1,8-diazabicyclo[5.4.0]undecane
EDC	ethyl-3-[3-(dimethylamino)propyl]carbodiimide
Fmoc	[(fluorenylmethyl)oxy]carbonyl
HATU	*O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HMPB	4-[4-(hydroxymethyl)-3-methoxyphenoxy]butyric acid
HMP	linker derived from [4-(hydroxymethyl)phenoxy]-acetic acid
HOBt	hydroxybenzotriazole
MBHA	4-methylbenzhydrylamine
NMP	*N*-methylpyrrolidinone
NPEOC	[(nitrophenyl)ethoxy]carbonyl
Nvoc	[(nitroveritryl)oxy]carbonyl
PAL	peptide amide linker derived from 5-[4-(aminomethyl)-3,5-dimethoxyphenoxy]valeric acid
PEG-PS	polystyrene-polyethylene glycol graft copolymer
PPTS	pyridinium *p*-toluenesulfonate
PyBOP	(benzotriazol-1-yloxy)tris(pyrrolidino)phosphonium hexafluorophosphate
PyBrOP	bromotris(pyrrolidino)phosphonium hexafluorophosphate
Rink	linker derived from 4-[[(2,4-dimethoxyphenyl)-amino]methyl]phenol
SCAL	safety catch amide linker, 4-[4,4′-bis(methylsulfinyl)-2-oxy-(9-fluorenylmethyloxycarbonyl] benzhydrylamino]butanoic acid
Tentagel	PEG-PS polymer marketed by Rapp Polymere
TFA	trifluoroacetic acid
THP	linker derived from 6-(hydroxymethyl)-3,4-dihydro-2*H*-pyran
TMAD	*N*,*N*,*N*′,*N*′-tetramethylazodicarboxamide
TMG	tetramethylguanidine
Trt	resin derived with a trityl chloride
Wang	linker derived from (hydroxymethyl)phenol

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