Applications of small-molecule combinatorial chemistry to drug discovery

Dinesh V. Patel and Eric M. Gordon*

The field of drug discovery stands on the threshold of a new era; more new synthetic molecules will come into existence in the next few years than have ever previously existed. The preparation of huge numbers of small organic molecules relies on the new techniques and strategies of combinatorial chemistry. The libraries of compounds that emerge from combinatorial chemical syntheses may be used to search for novel leads or to optimize the activity and properties of a known compound. Although the first successes with small-molecule chemical library approaches are only a few years old, most research groups now believe that the widespread use of combinatorial and related synthetic technologies will revolutionize drug discovery.

New lead structures for drug discovery have historically emerged from the screening of microbial fermentation broths, plant extracts, compound collections and, more recently, by mechanism- and structure-based design approaches, especially when the biological target is an enzyme. Very recently, combinatorial organic synthesis of small molecules has attracted attention as a means of cutting the time and high costs associated with serial organic synthesis, and has consequently become an important additional source of molecular diversity for drug discovery screens. Enthusiasm quickly mounted for the idea that, if one could create large numbers of nonpolymeric small molecules simultaneously, great leverage would be in hand for discovering new and useful molecules within an accelerated time-frame. In the early 1990s, it became apparent (Refs 1–4 and references therein) that, if sound synthetic strategies could be devised, the necessary chemical tools might already exist to create large collections of molecules efficiently. The architects of this new-found wealth of molecular diversity hoped that the portfolio of small-molecule libraries they produced would contain not only the familiar pharmacophores of historical successes[5–9] but, by inclusion of novel systems, would also permit the discovery of the pharmacophores of the future[10].

The scope of this overview is confined to recent solid-phase synthesis. Although there has been an explosion of published work in this field, space for this article is limited. This review thus can not be comprehensive, but is, rather, a reflection of the authors' own work and current interests, and much excellent, newly published organic and medicinal chemistry is not reviewed here[11–26].

Design and synthesis of small-molecule combinatorial libraries

Peptides, N*-substituted glycines and polycarbamates*

The chemical simplicity of peptide bond construction on a solid support and the commercial availability of several hundred natural and unnatural amino acid building blocks (BBs) fueled activity in creating polymeric structures during the early development of combinatorial chemistry. The fact that a collection of just 100 amino acids offers the possibility

Dinesh V. Patel and **Eric M. Gordon***, Affymax Research Institute, 4001 Miranda Avenue, Palo Alto, CA 94304, USA. *tel: +1 415 812 8728, fax: +1 415 424 9860, e-mail: eric_gordon@qmgates.affymax.com

  1359-6446/96/$15.00 S1359-6446(96)10013-1

of creating a library of a million trimer analogs (100^3) or 100 million tetramer analogs (100^4) potentially offers powerful leverage for lead discovery. Furka's bead-based 'split-pool' method[27–29], Houghten's tea-bag approach[30,31], Geysen's spatially addressable multipin synthesis[32–34] and Affymax's light-directed VLSIPS synthesis[35,36] are good examples of the various creative approaches that have been used for performing peptide-based combinatorial chemistry. Since peptides often have limited usefulness as drugs because of their poor bioavailability, libraries based on unnatural polymeric backbones have been built up in the hope that these might lead to chemical entities with enhanced proteolytic stability, absorption and pharmacokinetic properties. Two examples of such chemical libraries are the polycarbamates[37] and *N*-substituted glycines[38–40] (Figure 1).

Linear sequential coupling and deprotection of stable *N*-protected, *p*-nitrophenylamino carbonate building blocks derived from chiral amino alcohols have been utilized to construct oligocarbamates[37] (Figure 2). A spatially addressable 256-member oligocarbamate library was constructed using Affymax's VLSIPS technology by employing monomeric units with photolabile amine protecting groups. Alternating rounds of acylation of resin-bound amines with bromoacetic acid followed by alkylation of the bromide intermediate on resin with primary amines leads to an oligomeric assembly of *N*-substituted glycines (peptoids)[38]. Peptoid oligomers mimic the spatial arrangement of normal peptide side chains, but the amide groups are *N*-substituted, resulting in unnatural

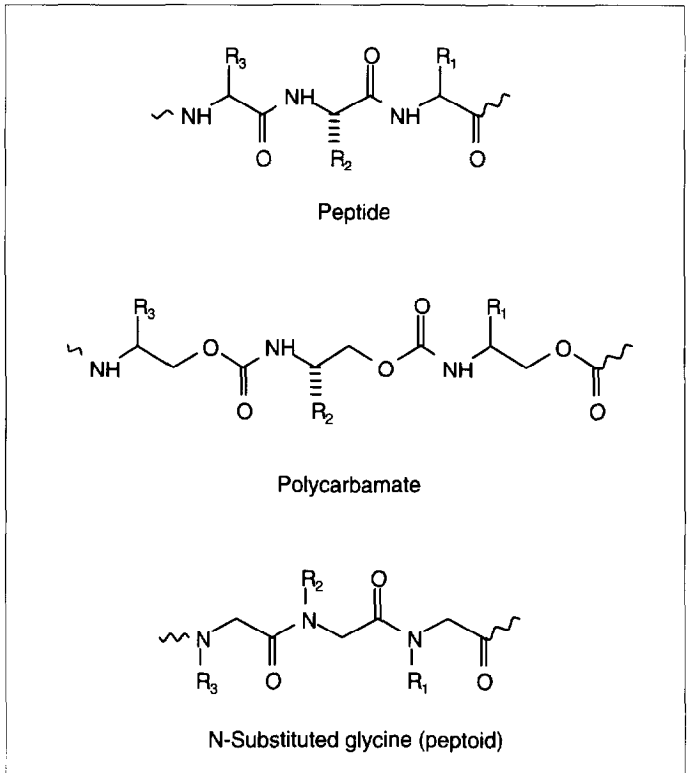

Figure 1. *Examples of polymeric molecular diversity.*

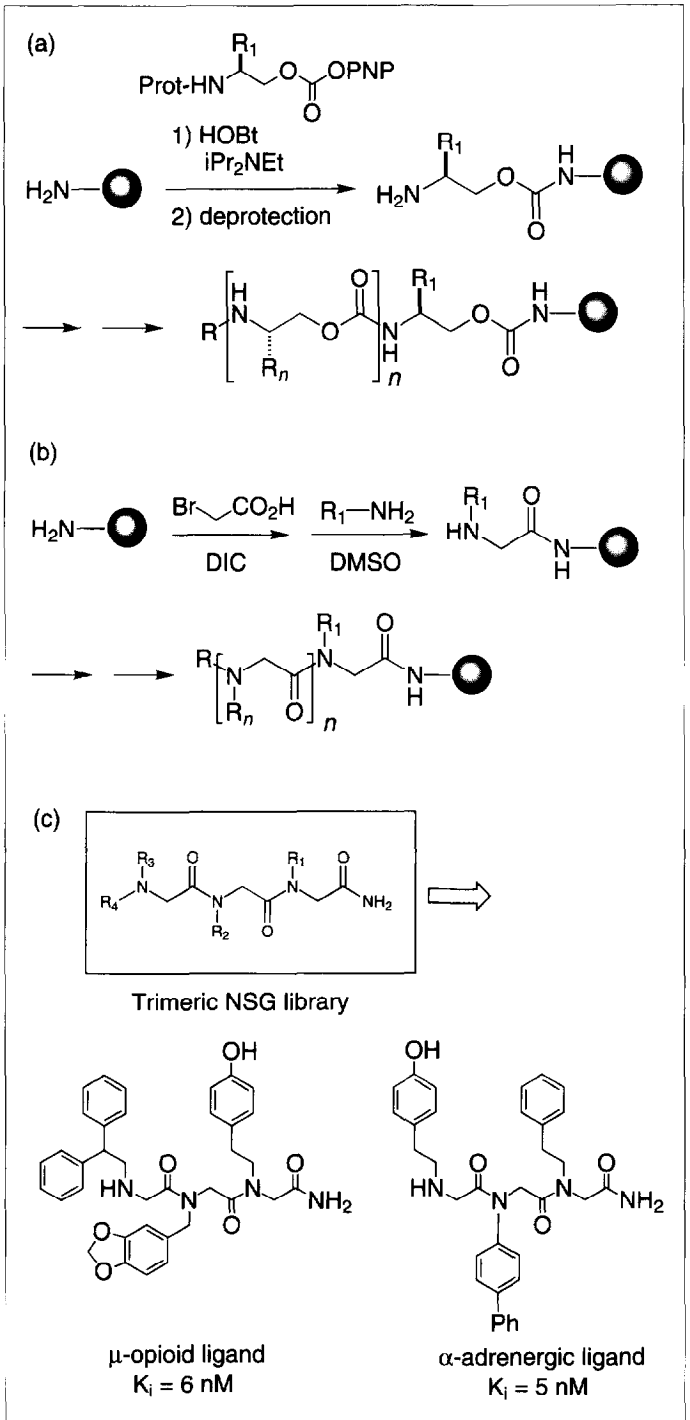

Figure 2. *Solid-phase synthesis of (a) polycarbamates and (b) N-substituted glycines (NSGs, peptoids). (c) Synthesis and screening of an NSG (peptoid) library.*

amide oligomers that can be expected to be resistant to proteolytic degradation[39]. As an example, screening of 18 pools of 204 peptoid trimers in which the choice of side chains was biased to resemble known ligands of the 7-transmembrane G protein-coupled receptor (7-TM/GPCR) family, led to the identification of nanomolar ligands for μ-opioid receptors and α-adrenoceptors (Figure 2)[40].

Peptidylphosphonic acids: transition-state analog libraries

One of the first examples of combinatorial chemistry to incorporate the elements of rational drug design came in the form of tripeptidylphosphonic acid analog libraries. Phosphonyl acids are transition-state analogs and metal chelators that have historically been used to design potent inhibitors of metalloproteases such as thermolysin and angiotensin-converting enzyme (ACE)[41–43]. Construction of libraries bearing this pharmacophoric unit requires the nontrivial task of forming a phosphorus–oxygen (P–O) bond on a solid support. This was accomplished by adaptation of Mitsunobu-type coupling chemistry between phosphonic acids and bead-bound secondary alcohols. Specifically, the method utilizes an alcohol on a solid support, a phosphonic acid, DIAD, tris(4-chlorophenyl)phosphine (which is a more electron-deficient phosphine than the typically used triphenylphosphine) and an exogenous base, such as triethylamine, as a general base catalyst.

This procedure, which is a significant departure from normal Mitsunobu conditions, is versatile in scope and yields satisfactory results even with most hindered coupling partners[44–46]. These optimized conditions have been effectively utilized for the construction of a transition-state analog inhibitor combinatorial library of trimer peptidylphosphonates[47]. The split-pool method was used to prepare a 540-member Cbz-$X_{(6)}$P-$OY_{(5)}$-$Z_{(18)}$-NH resin library on a non-cleavable solid support, which was screened for thermolysin inhibition by rank ordering of pools of compounds in an iterative fashion employing a depletion assay. Besides identifying the most potent peptidylphosphonate reported in the literature (Figure 3), the experiment also led to the identification of a number of additional active sequences composed of basic (P_2' = Arg- and His-like) and neutral H-bonding (P_2' = Glu-like) side chains. The discovery of P_2' Arg, His and Gln analogs was unexpected since all peptidylphosphonate inhibitors of thermolysin that have previously been reported invariably possess hydrophobic residues at that position.

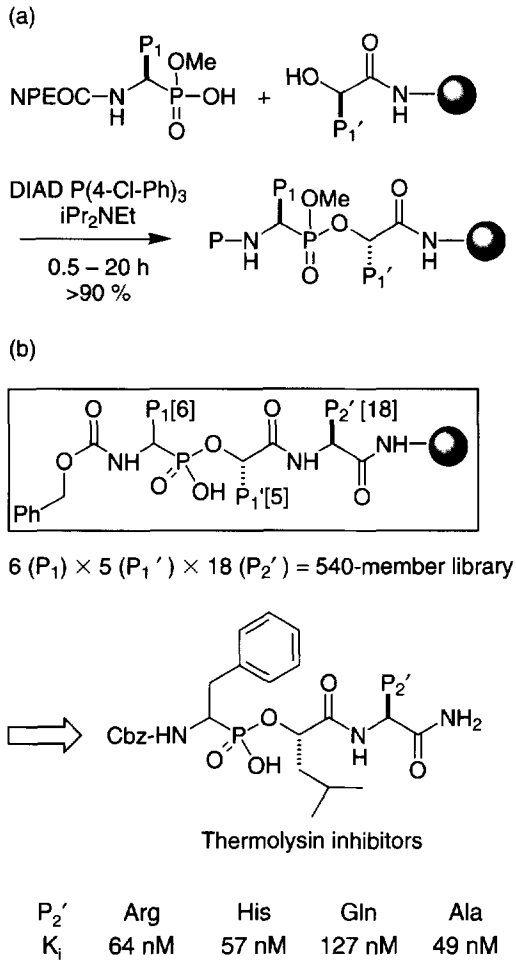

Figure 3. *Solid-phase peptidylphosphonate synthesis (a), and rational design, synthesis and screening of a tripeptidylphosphonate library (b).*

This study serves to illustrate several important aspects of combinatorial chemistry-based drug discovery. First, it highlights the fact that combinatorial chemistry imposes a different set of demands on medicinal and organic chemists than those traditionally encountered. In this case, the desire to create a library of rationally designed phosphonic acid pharmacophore-based metalloprotease inhibitors triggered the necessity for new solid-phase chemistry that would permit the assembly of the core unit on a solid support. This requirement, in turn, led to the discovery of a modified Mitsunobu-type P–O bond-forming reaction for both solution- and solid-phase chemistry. Thus, in general, a consequence of the preparation of combinatorial libraries of nonpeptidic small molecules will be the discovery and development of new solid-phase chemistries. Second, enzyme inhibitor libraries used as drug discovery tools can be expected to provide powerful leverage, as illustrated by the successful identification of the previously reported tripeptidylphosphonate Cbz-PheP-OLeu-Ala-NH_2 (K_i = 49 nM), which is a good thermolysin inhibitor. Third, the identification of unexpectedly potent analogs possessing polar P_2' side chains demonstrates an

important advantage of the combinatorial drug discovery approach over the classical medicinal chemistry approach. Individual serial analog syntheses may lead one to form biases based on initial findings (e.g. study of thermolysin inhibitors with only hydrophobic P_2' side chains), whereas the combinatorial chemistry approach imposes less bias, is more versatile and efficient, and provides more extensive SAR data at a faster rate. Thus, with more biologically active molecules to choose from, compounds with preferred pharmacological and pharmacokinetic profiles should emerge more readily.

Benzodiazepines

Since most current drug molecules are heterocyclic entities, this area has attracted a great deal of attention in the development of combinatorial chemical approaches. Unlike the synthesis of the linear oligomeric, peptidic and nonpeptidic libraries described so far, these nonpolymeric scaffolds are constructed from an interlocking assembly of diverse BBs employing nonrepetitive chemistry.

One of the first examples of this type was provided by Ellman and coworkers in their preparation of libraries of benzodiazepines, an important class of heterocycles that have long found widespread use in the pharmaceutical industry[48]. Their solid-phase synthesis commenced with the attachment of an hydroxyl or carboxyl group of a 2-aminobenzophenone BB onto a solid support via an acid-cleavable linker. The limitation of scarce availability of these BBs was overcome by developing a facile method for constructing 2-aminoaryl ketone derivatives on a solid support using a palladium-mediated Stille coupling between 2-aminoarylstannane (on solid support) and an acid chloride (the solution coupling partner)[49,50]. Thus, the need for aminobenzophenone BBs in the overall library synthesis is met by on-support synthesis using readily available acid chlorides.

The next step involves acylation of the newly formed aromatic amine with Fmoc-protected amino acid fluorides. Removal of the Fmoc group is followed by mild acid treatment (5% acetic acid) to facilitate the desired cyclization reaction. Further functionalization of the benzodiazepine ring skeleton is achieved by *N*-alkylation of the lactam with alkyl halides and lithiated 5-(phenylmethyl)-2-oxazolidinone. Treatment with trifluoroacetic acid (TFA) effects cleavage from the solid support to afford the molecule of interest in adequate levels of yield and purity (Figure 4, Method a).

This methodology has been successfully utilized in the parallel synthesis of a 192-member 1,4-benzodiazepine library comprising analogs bearing such diverse substituent functionalities as amides, amines, phenols, carboxylic acids and indoles[51]. Upon screening for binding to the cholecystokinin A (CCK) receptor using a competitive radioligand binding assay, detailed SAR data were acquired from which the indole analog was identified as a potent CCK ligand. More recently, the Ellman group has employed this methodology to generate a 11,200-analog library from 20 acid chlorides, 35 amino acids and 16 alkylating agents[49,50]. An alternate synthetic strategy for benzodiazepines has also been described, which proceeds by condensation of a resin-bound α-amino ester with

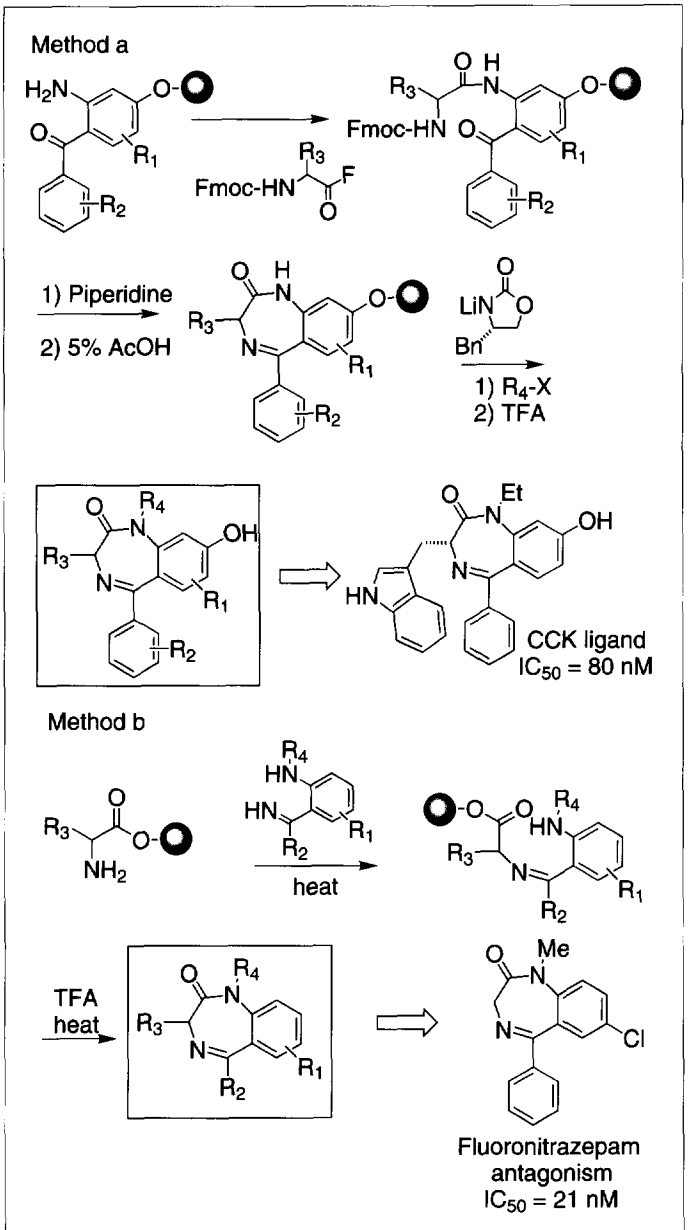

Figure 4. *Synthesis and screening of a benzodiazepine library.*

2-aminobenzophenone imines followed by TFA treatment of the intermediate to effect cleavage and cyclization[52]. In this work, no vestige of the solid-support linker is retained in the cleaved products. Parallel synthesis of 40 discrete analogs related to valium was performed, and the expected SAR was observed in a radioligand binding assay based on antagonism of fluoronitrazepam (Figure 4, Method b).

Pyrrolidines and mercaptoacylprolines

(3 + 2)-cycloaddition of 1,3-dipoles such as azomethine ylides with olefin dipolarophiles results in a facile assembly of five-membered nitrogen ring systems. An adaptation of this chemistry to solid-phase synthesis of complex pyrrolidines has recently been described[53]. Resin-bound α-amino esters reacted cleanly with aromatic and heteroaromatic aldehydes at room temperature in neat trimethyl orthoformate as a solvent to afford the corresponding imines. Lewis acid-mediated ionization of α-amino aldimines promotes chelation-controlled regio- and stereoselective cycloaddition with a wide variety of electron-deficient olefins to yield pyrrolidines (Figure 5, a). Rapid gel-phase ^{13}C-NMR analysis of resin-bound intermediates and final products provided a convenient method for optimizing reaction parameters[54].

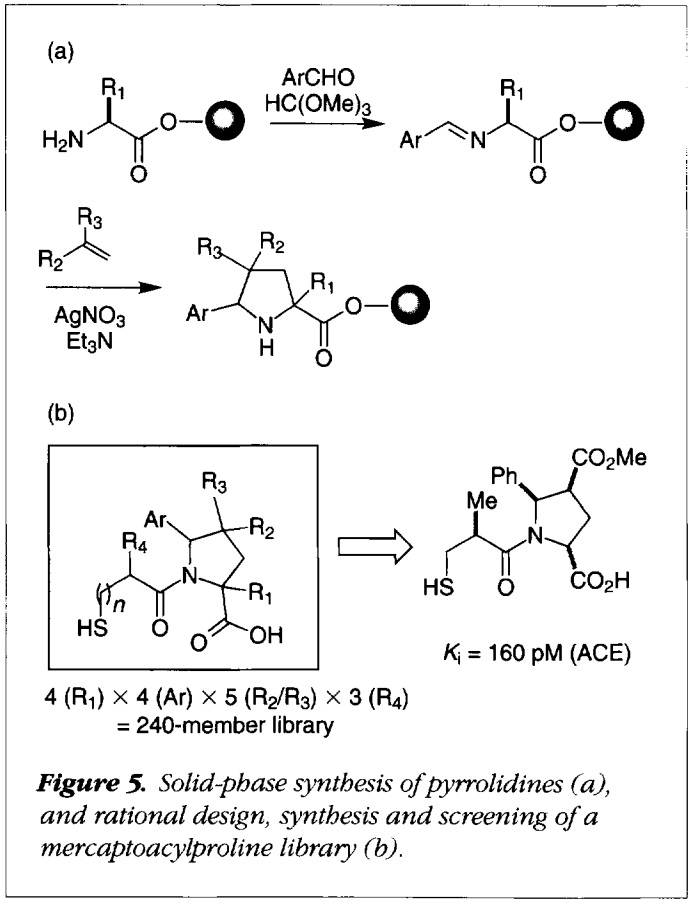

Figure 5. *Solid-phase synthesis of pyrrolidines (a), and rational design, synthesis and screening of a mercaptoacylproline library (b).*

Immobilized pyrrolidines derived from this process can themselves be viewed as BBs and subjected to further derivatization. Thus, a pool of pyrrolidines prepared combinatorially from four α-amino acids, four aromatic aldehydes and five olefins was acylated with three different mercaptoacyl chlorides to provide, after appropriate deprotection and TFA cleavage from resin, a library of >480 mercaptoacylproline analogs. The resulting library members are analogs of the antihypertensive drug captopril, an ACE inhibitor. When screened for activity against ACE as soluble compound pools, serial deconvolutions of this library led to the identification of one of the most potent thiol-based ACE inhibitors (K_i = 160 pM, three times more potent than captopril) yet described (Figure 5, b).

Diketopiperazines

Diketopiperazines (DKPs) are a class of rigid cyclic scaffolds that possess a rich history as bioactive materials. Recently, a three-step solid-phase synthesis leading to a 1000-member DKP library employing two sets of α-amino acids and one set of aldehyde BBs has been reported[55] (Figure 6). The first critical step involves reductive alkylation of the amino group of resin-bound amino esters with aldehydes and is best accomplished using sodium triacetoxyborohydride as the reducing agent. While this reaction can be further optimized because it suffers from problems of partial racemization, poor yields (≈20%) with hindered amines and electronically deactivated aromatic aldehydes and slight overalkylation (≈10%) with aliphatic aldehydes, 96 of the 100 components resulting from combinatorial reaction of ten amines with ten aldehydes were correctly identified by LC-MS. PyBrop-mediated double coupling provided a solution to the sterically demanding acylation of the hindered secondary amine with the next set of Boc-protected amino acids. Boc removal with TFA treatment followed by brief reflux in toluene to induce cyclization afforded the desired molecules in solution as ten pools with 100 members in each pool. Iterative screening and resynthesis of this library has reportedly led to several bioactive molecules, as exemplified by the identification of a neurokinin-2 receptor ligand (I_{50} = 313 nM)[3].

Transition-state analog HIV protease inhibitors

Extensive efforts directed towards the rational design of aspartylprotease inhibitors such as renin and human immunodeficiency virus (HIV) have led to the discovery of several successful transition-state analog design elements. In

an appropriate library format these functionalities can serve as the central unit around which molecular diversity can be generated by application of known solution chemistries.

Recently, solid-phase synthesis of hydroxyethylamine and 1,2-diol transition-state analogs leading to libraries of HIV protease inhibitors have been reported by two different groups[56,57]. In the first instance, a diamino alcohol or diamino diol BB is attached to the solid support through their hydroxyl groups in the form of an acetal or ketal, respectively (Figure 7, Method a). This core unit is arranged to support a solid-phase synthesis strategy for preparing C-2 symmetric HIV protease inhibitors. A library of >300 discrete analogs was prepared and screened against HIV protease to identify several potent inhibitors (I_{50} < 100 nM)[56]. In the other method, a masked amino diol pharmacophoric unit is attached through its hydroxyl group onto a dihydropyran functionalized polystyrene support (Figure 7, Method b). The tosyl and azido groups of this BB unit provide convenient handles for orthogonal derivatization. The tosyl group can be displaced with primary amines, and the resulting secondary amine can be converted to ureas or acylated to give amides. The azido group of the pharmacophore can be reduced to an amine, which thus becomes available for further functionalization. The versatility of this approach was demonstrated by the synthesis of various known HIV protease inhibitors in good yields (47–86%)[57].

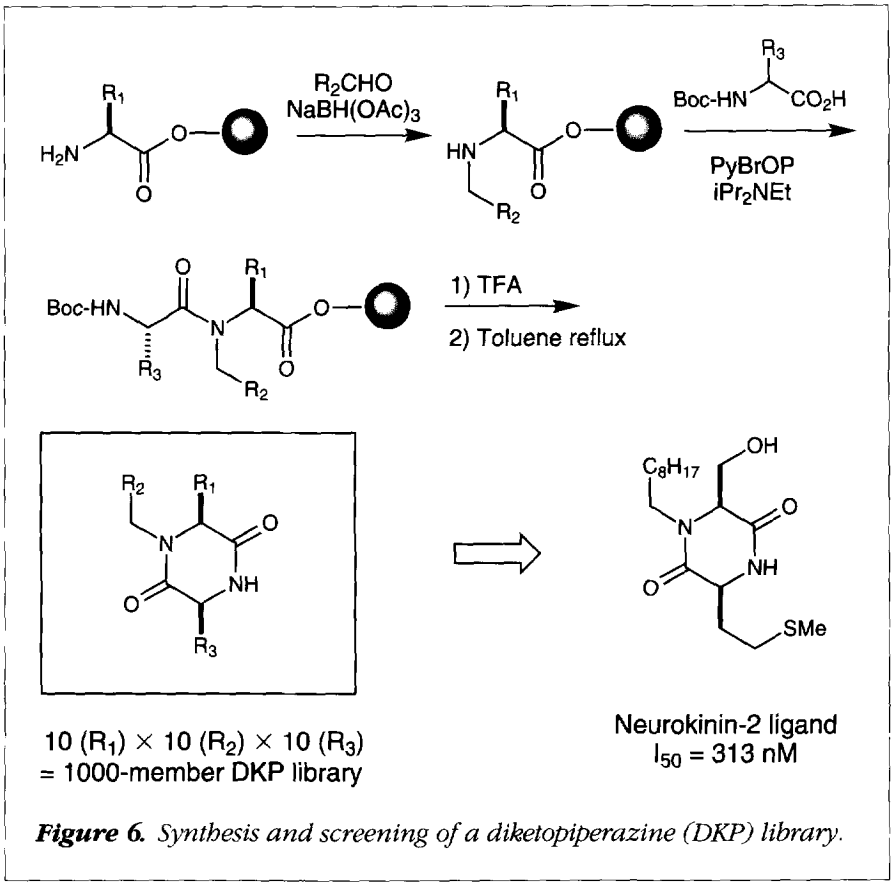

Figure 6. *Synthesis and screening of a diketopiperazine (DKP) library.*

Other new methods

Aldol condensation of zinc enolates of resin-bound alkyl esters with aromatic aldehydes or ketones forms β-hydroxy esters, which upon DIBAL-H treatment lead to simultaneous reduction and cleavage of the ester moiety from the resin to give soluble 1,3-diols[58]. Parallel synthesis commencing with three ester and nine carbonyl BBs afforded a library of 27 analogs, which were screened for their antioxidative efficiency using a ferric thiocyanate assay (Figure 8).

A 24-analog hydroxystilbene library was constructed by Horner–Emmons olefination of four resin-bound hydroxybenzaldehyde BBs and six different benzylphosphonate anions[59]. Screening for activity in a cell-based estrogenic assay identified three analogs with I_{50} values in the range 5–15 μM (Figure 9).

Solid-phase synthesis of small molecules of pharmaceutical interest

The explosive use of combinatorial libraries as tools to discover new ligands and optimize known ones has dramatically heightened interest in the development of solid-phase synthesis of historically 'privileged' chemical classes. In the near future, this field can certainly be expected to expand its scope both in the degree of chemical sophistication performed and in the complexity of the products synthesized on solid phase. Nonoligomeric and air- or moisture-sensitive chemistries, such as cycloaddition, cyclization and carbanion condensation, have already been successfully performed on solid supports, and some recent examples are discussed below. Each of these studies lays the groundwork for further exploitation of these chemistries in library format.

Resin-bound α-amino esters, besides being traditionally used for making peptides, have recently been utilized for the construction of various heterocyclic scaffolds. Thus, they react smoothly with isocyanates to form ureas, which in turn cyclize to form hydantoins upon heating under acidic conditions[52] (Figure 10). A one-pot, three-component condensation of resin-bound α-amino esters with aldehydes and α-mercapto acids affords 4-thiazolidinones in good yields[60] (Figure 10). In most

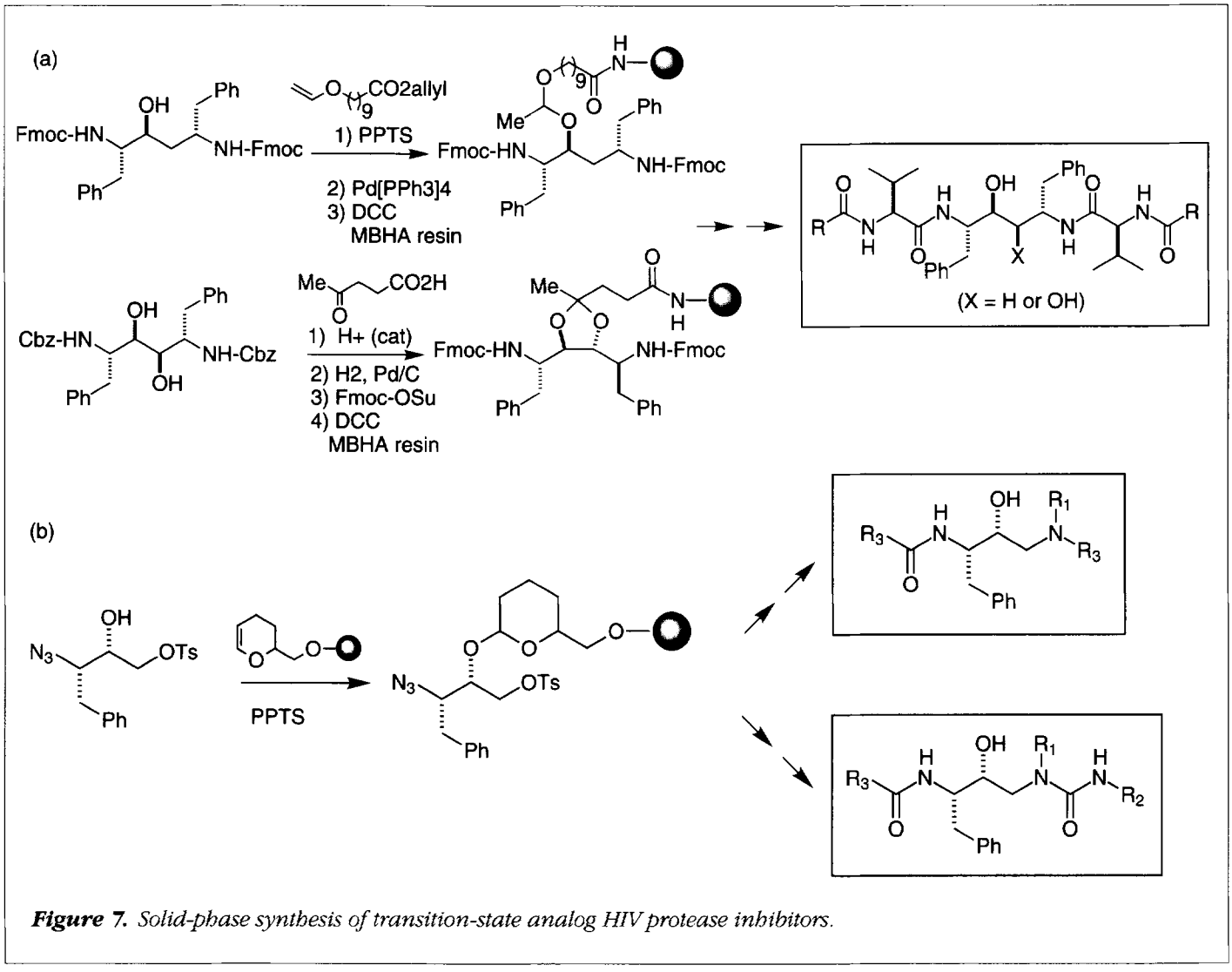

Figure 7. *Solid-phase synthesis of transition-state analog HIV protease inhibitors.*

instances, further purification of cleaved compounds is not required, highlighting one advantage of solid-phase synthesis compared with solution chemistry. The reaction proceeds through imine intermediates, as evidenced by the successful conversion of stable imines derived from aromatic aldehydes to the cyclized products upon treatment with mercapto acids. Such imines provide a convenient starting point for further generation of heterocyclic diversity. Besides their reaction with azomethine ylides to form pyrrolidines, as discussed previously, they also undergo smooth (2 + 2)-cycloaddition with ketenes derived from acid chlorides to provide β-lactams[61] (Figure 10). Heterocycles such as hydantoins, thiazolidinones and β-lactams possess a wide range of biological activities, and

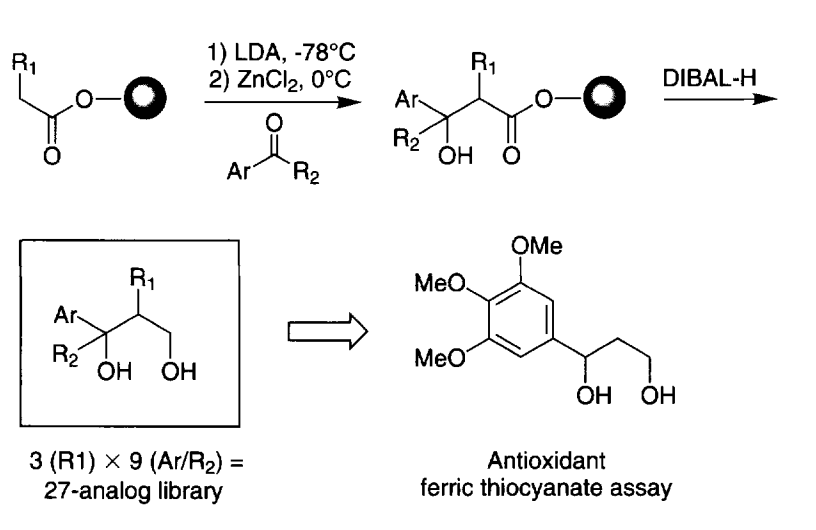

Figure 8. *Parallel synthesis and screening of a 1,3-diol library.*

generic libraries of such molecules will likely provide new leads from primary screening. Alternatively, these structures can be embedded as peptidomimetic fragments within larger molecules of designed libraries.

Two different routes have been reported for the solid-phase synthesis of 1,4-benzodiazepine-2,5-diones[62,63]. In the first (Figure 11, Method a), Merrifield's chloromethyl-polystyrene resin is derivatized to afford an aromatic aldehyde linker. Reductive amination with α-amino esters forms a secondary amine, which is acylated by double coupling of anthranilic acid BBs with EDC. The tertiary amide is a critical requirement for efficient lactamization, presumably by favoring a *cis* conformation for the acyclic precursor. Cyclization and alkylation are effected by formation of an anilide anion by treatment with the lithium salt of *p*-methoxyacetanilide, which after 30 h is followed by addition of the appropriate alkylating agent. Cleavage from the resin by TFA treatment affords the final products in high yield and purity[62]. The second method (Figure 11, Method b) effects ring closure on a solid support via an intramolecular aza-Wittig (Staudinger) reaction to construct a benzodiazepinedione scaffold embedded on an *N*-substituted glycine scaffold. A monopeptoid unit is acylated with bromoacetic acid, and the product halide is alkylated with an α-amino ester instead of a simple primary amine employed in a typical peptoid synthesis. The resulting secondary amine is acylated with *o*-azidobenzoyl chloride and then

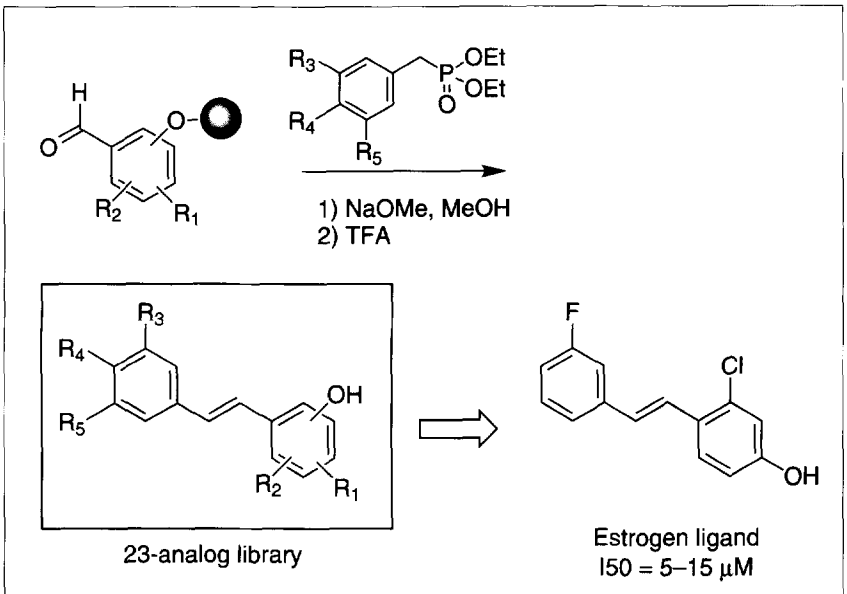

Figure 9. *Parallel synthesis and screening of a hydroxystilbene library.*

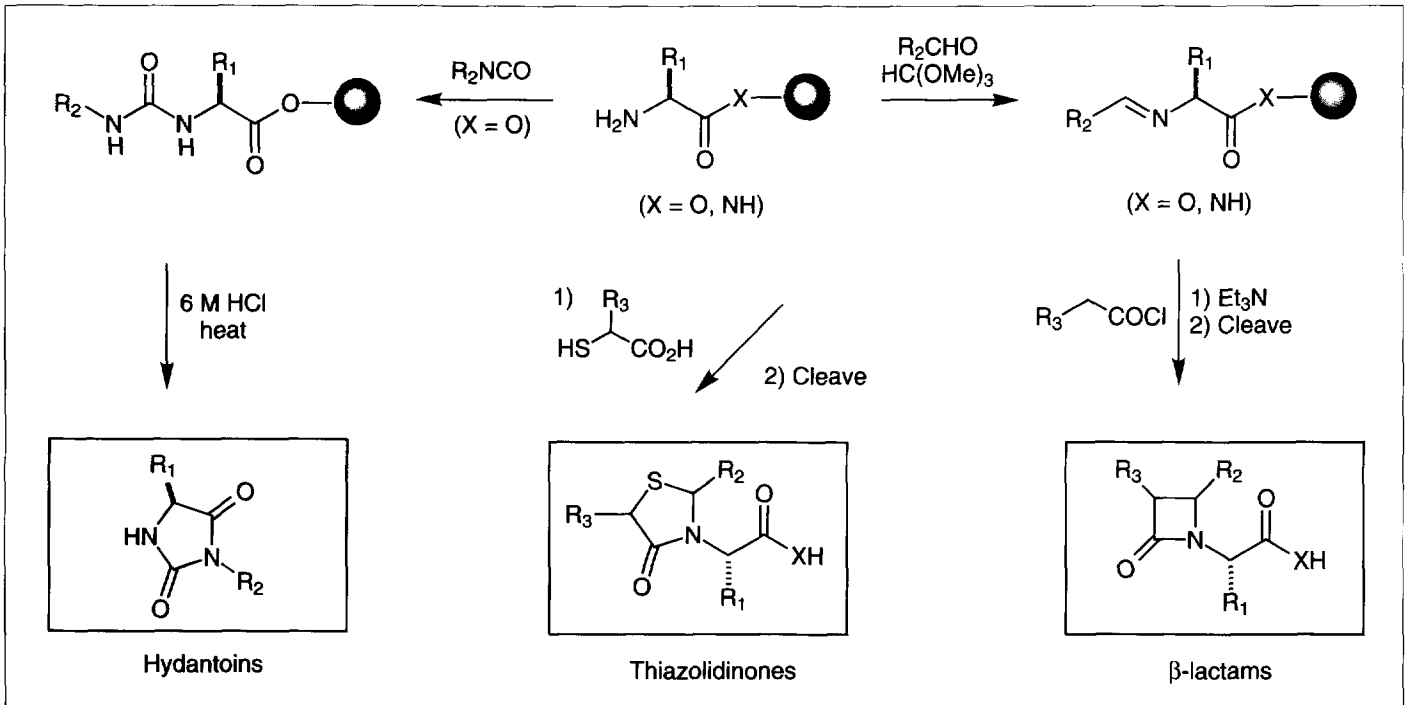

Figure 10. *Solid-phase synthesis of hydantoins, thiazolidinones and β-lactams.*

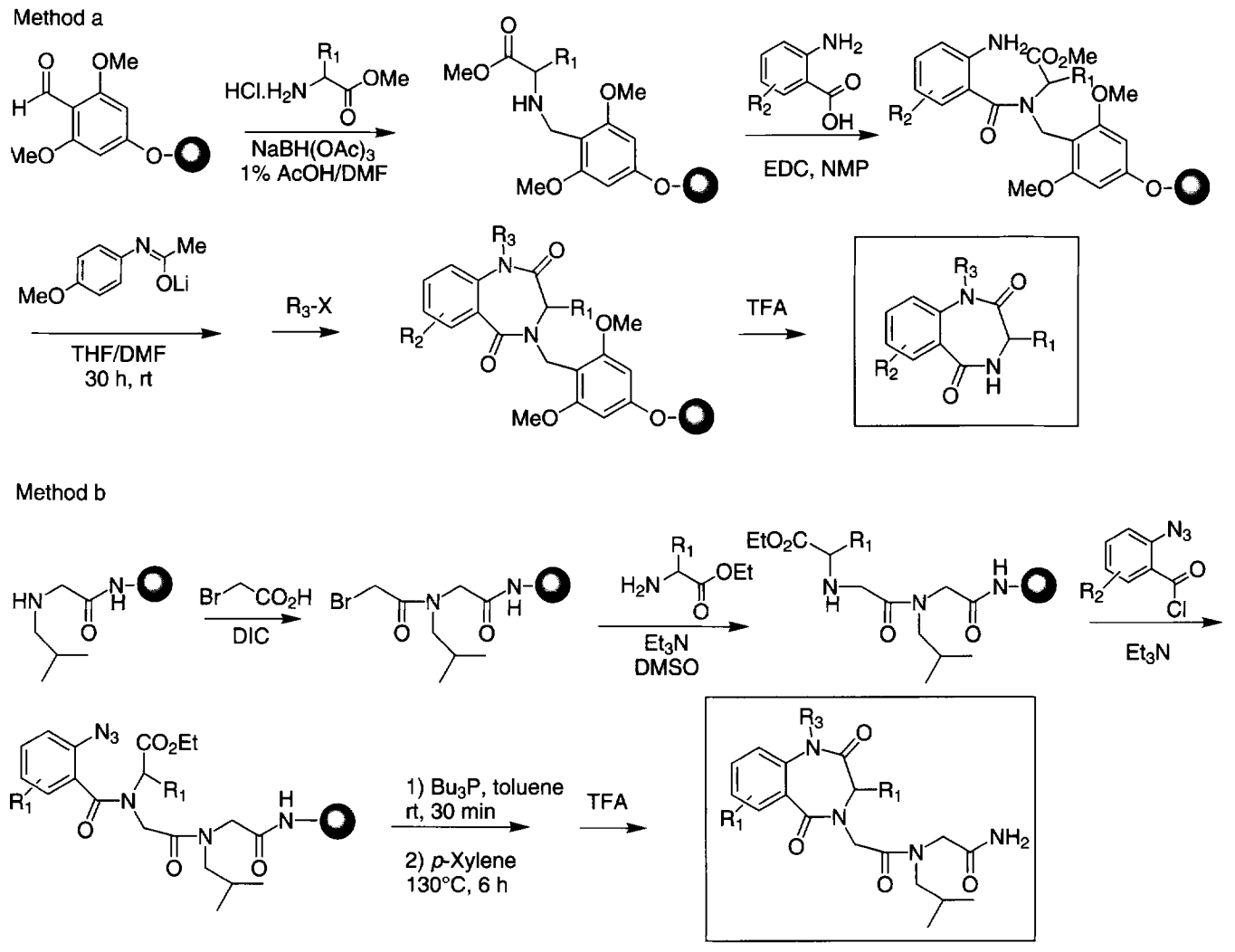

Figure 11. *Solid-phase synthesis of 1,4-benzodiazepine-2,5-diones.*

treated with Bu_3P in toluene at room temperature to form the iminophosphorane. Heating at 130°C for several hours leads to cyclization, and TFA cleavage produces the desired product, which is typically obtained in adequate yield (34–90%) and good purity (60–95%). A mixture of eight monopeptoids, when subjected to this reaction sequence utilizing *o*-azidobenzoyl chloride, gave a crude mixture in which all eight products were identified by MS and HPLC (Ref. 63).

Reaction of amine functionalized acid-labile resins with β-keto esters leads to versatile enamino ester intermediates which can be used to perform a wide range of heterocyclic chemistry. For example, when treated with preformed arylidene β-keto esters, or with a mixture of aromatic aldehydes and β-diketones in pyridine, cyclization occurs to generate dihydropyridines (DHPs) after acidic cleavage[64] (Figure 12, a). Following this method, several DHPs, including the commercial calcium channel blocker nifedipine, have been synthesized. The method is well suited to the preparation of DHP libraries (M.F. Gordeev, B. England and D.V. Patel, unpublished).

A three-component Biginelli cyclization of ureas on resin with a solution mixture of aldehydes and β-keto esters provides dihydropyrimidines (DHPMs) in high yield and purity[65] (Figure 12, b). Heterocycles such as dihydropyridines and dihydropyrimidines have historically proven to be a rich source of antimicrobial, antitumor, antiviral and cardiovascular agents.

Solid-phase Heck reactions between resin-bound aryl iodides or styrene derivatives with olefins or aryl halides, respectively, have been described to generate 1,2-disubstituted olefins[66], and an intramolecular version was recently employed to prepare peptoid isoquinolinones[67].

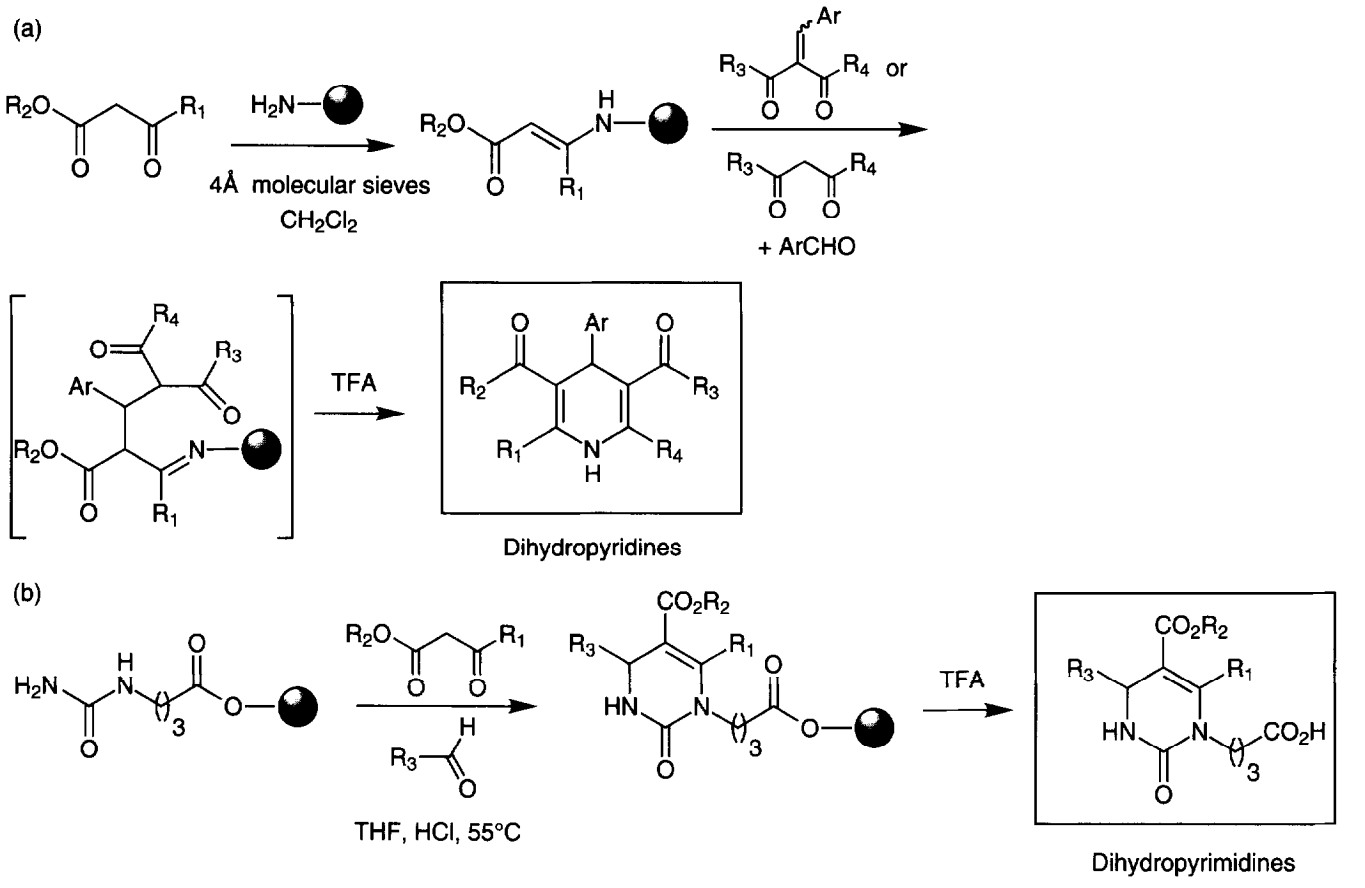

Figure 12. *Solid-phase synthesis of (a) dihydropyridines and (b) dihydropyrimidines.*

Conclusions and future issues for drug discovery

The intriguing possibility of creating huge libraries of small, nonpolymeric molecules has been transformed in a matter of months from dream to reality. Traditionally successful paradigms that focused on serial organic syntheses to find and propagate drug discovery leads will be markedly accelerated by the additional capacity to apply combinatorial chemistry technologies to mass-produce and evaluate novel lead molecules. The opportunity to challenge numerous emerging new drug targets with huge numbers of potential ligands should increase the rate at which new leads are found and ultimately promote the efficiency of drug discovery. The requirement of combinatorial chemistry to routinely intercombine large numbers of BBs by repetitive interconnecting chemistries will stimulate the design and production of new generations of automated instrumentation to perform organic syntheses and create libraries largely under computer control. These libraries may be prepared either by split-pool synthesis or by parallel synthesis of discrete compounds. Progress in inventing novel, higher-throughput screening methods, along with cost-control measures for conserving recombinant target proteins and synthetic BBs, will continue to drive combinatorial technologies and instrumentation towards miniaturization and nanotechnology.

Considerable effort will continue to be invested in developing new types of synthetic strategies which utilize the strengths of chemical library and combinatorial technologies[2,10–26]. For example, combinatorial library approaches directed towards broad screening differ substantially from focused library approaches which are aimed at optimizing a known lead. Advances in adapting historical solution chemistry to solid-phase organic synthesis will continue on a broad front. In order to be generally applicable, these efforts will necessitate the creation of a collection of reliable, cleavable linkers which will tether specific common functional groups to supports and enhance flexibility in planning new synthetic schemes. Much more investigation into identifying viable solid synthesis supports and beads will be needed to improve reaction yields and product loadings. The general concept

of 'encoding' of libraries will continue to be employed and improved upon[1].

Major new initiatives in designing libraries will be tested. Selections of BBs for library synthesis may be driven by quantitative diversity measurements, computational approaches – possibly involving virtual libraries – and molecular modeling. Combinatorial library approaches will not generally displace older methods, but will need to be integrated with them. The unification of structure- and mechanism-based inhibitor design with library design should prove to be a potent combination. Creation of so much chemical and biological data will demand new data-handling tools to process, monitor and interpret results. Efforts at meeting this challenge are already well under way.

Taken *in toto*, implementation of combinatorial technologies demands a broad, well-integrated, interdisciplinary approach to drug discovery, in which chemistry, engineering, biochemistry, screening technology and data-processing expertise must all be coordinated for the best results. With intense worldwide interest and a firm commitment by the pharmaceutical industry to exploit combinatorial chemistry techniques aggressively, we stand on the threshold of a new era in drug discovery. More new synthetic molecules will come into existence in the next few years than have ever previously existed! Discovering what novel properties they possess should reap rich rewards and should be an exciting adventure!

ACKNOWLEDGMENTS

We thank Drs Alex Zaffaroni, Mark Gallop, Jeff Jacobs, Chris Holmes, David Campbell, Bill Fitch, Jeff Sugarman and our coworkers at Affymax for their contributions to understanding this new field. We acknowledge the help of Mrs Terri Nebozuk in the preparation of the manuscript.

REFERENCES

1 Gallop, M.A. *et al.* (1994) *J. Med. Chem.* 37, 1233–1251
2 Gordon, E.M. *et al.* (1994) *J. Med. Chem.* 37, 1385–1401
3 Terrett, N.K. *et al.* (1995) *Tetrahedron* 51, 8135–8173
4 Gordon, E.M. (1995) *Curr. Opin. Biotechnol.* 6, 624–631
5 Morin, R.B. and Gorman, M. (eds) (1982) *Beta-Lactam Antibiotics, Chemistry and Biology, Vols 1–3.* Academic Press, New York
6 Hirschmann, R. (1991) *Angew. Chem.* 1278–1301
7 Patchett, A.A. (1993) *J. Med. Chem.* 36, 2051–2058
8 Petrillo, E.W. and Ondetti, M.A. (1982) *Med. Res. Rev.* 2, 1–41
9 Wiley, R.A. and Rich, D.H. (1993) *Med. Res. Rev.* 13, 327–384
10 Gordon, E.M., Gallop, M.A. and Patel, D.V. (1995) *Account. Chem. Res.* (in press)
11 Baldwin, J.J. *et al.* (1995) *J. Am. Chem. Soc.* 117, 5588–5589
12 Pei, Y. and Moos, W.H. (1994) *Tetrahedron Lett.* 35, 5825–5828
13 Forman, F.W. and Sucholeiki, I. (1995) *J. Org. Chem.* 60, 523–528
14 Chen, C. *et al.* (1994) *J. Am. Chem. Soc.* 116, 2661–2662
15 Carell, T., Wintner, E.A. and Rebek, Jr, J. (1994) *Angew. Chem. Int. Ed.* 33, 2061–2064
16 Carell, T. *et al.* (1994) *Angew. Chem. Int. Ed.* 33, 2059–2061
17 Carell, T. *et al.* (1995) *Chem. Biol.* 2, 171–183
18 Terrett, N.K. *et al.* (1995) *Bioorg. Medicinal Chem. Lett.* 5, 917–922
19 Pirrung, M.C. and Chen, J. (1995) *J. Am. Chem. Soc.* 117, 1240–1245
20 Moran, E.J. *et al.* (1995) *Biopolymers* 37, 213–220
21 Green, J. (1995) *J. Org. Chem.* 60, 4287–4290
22 Sundram, U.N. and Griffin, J.H. (1995) *J. Org. Chem.* 60, 1102–1103
23 Rano, T.A. and Chapman, K.T. (1995) *Tetrahedron Lett.* 36, 3789–3792
24 Burbaum, J.J. *et al.* (1995) *Proc. Natl Acad. Sci. USA* 92, 6027
25 Beebe, X., Schore, N.E. and Kurth, M.J. (1995) *J. Org. Chem.* 60, 4196–4203
26 Beebe, X. *et al.* (1995) *J. Org. Chem.* 60, 4204–4212
27 Furka, A. *et al.* (1988) Abstracts 14th International Congress Biochemistry, Prague, Vol. 5, p. 47
28 Furka, A. *et al.* (1988) Abstracts 10th International Symposium Med. Chemistry, Budapest, p. 288
29 Furka, A. *et al.* (1991) *Int. J. Peptide Protein Res.* 37, 487–493
30 Houghten, R.A. (1985) *Proc. Natl Acad. Sci. USA* 82, 5131–5135
31 Houghten, R.A. *et al.* (1986) *Int. J. Peptide Protein Res.* 27, 673–678
32 Geysen, H.M., Meloen, R.H. and Barteling, S.J. (1984) *Proc. Natl Acad. Sci. USA* 81, 3998–4002
33 Valerio, R.M. *et al.* (1993) *Int. J. Peptide Protein Res.* 42, 1–9
34 Bray, A.M., Maeji, N.J. and Geysen, H.M. (1990) *Tetrahedron Lett.* 31, 5811–5814
35 Fodor, S.P.A. *et al.* (1991) *Science* 251, 767
36 Jacobs, J.W. and Fodor, S.P.A. (1994) *Trends Biotechnol.* 12, 19–26
37 Cho, C.Y. *et al.* (1993) *Science* 261, 1303–1305
38 Simon, R.J. *et al.* (1992) *Proc. Natl Acad. Sci. USA* 89, 9367–9371
39 Zuckermann, R.N. *et al.* (1992) *J. Am. Chem. Soc.* 114, 10646–10647
40 Zuckermann, R.N. *et al.* (1994) *J. Med. Chem.* 37, 2678–2685
41 Bartlett, P.A. and Marlowe, C.K. (1987) *Biochemistry* 26, 8553–8561
42 Morgan, B.P. *et al.* (1991) *J. Am. Chem. Soc.* 113, 297–307
43 Karanewsky, D.S. *et al.* (1988) *J. Med. Chem.* 31, 204
44 Campbell, D.A. (1992) *J. Org. Chem.* 57, 6331–6335
45 Campbell, D.A. and Bermak, J.C. (1994) *J. Org. Chem.* 59, 658–660
46 Campbell, D.A. and Bermak, J.C. (1994) *J. Am. Chem. Soc.* 116, 6039–6040
47 Campbell, D.A. *et al.* (1995) *J. Am. Chem. Soc.* 117, 5381–5382
48 Bunin, B.A. and Ellman, J.A. (1992) *J. Am. Chem. Soc.* 114, 10997–10998
49 Bunin, B.A., Plunkett, M.J. and Ellman, J.A. (1996) *Methods Enzymol.* (in press)
50 Plunkett, M.J. and Ellman, J.A. (1995) *J. Am. Chem. Soc.* 117, 3306–3307
51 Bunin, B.A., Plunkett, M.J. and Ellman, J.A. (1994) *Proc. Natl Acad. Sci. USA* 91, 4708–4712
52 DeWitt, S.H. *et al.* (1993) *Proc. Natl Acad. Sci. USA* 90, 6909–6913
53 Murphy, M.M. *et al.* (1995) *J. Am. Chem. Soc.* 117, 7029–7030
54 Look, G.C. *et al.* (1994) *J. Org. Chem.* 59, 7588–7590
55 Gordon, D.W. and Steele, J. (1995) *Bioorg. Medicinal Chem. Lett.* 5, 47–50
56 Wang, G.T. *et al.* (1995) *J. Med. Chem.* 38, 2995–3002
57 Kick, E.K. and Ellman, J.A. (1995) *J. Med. Chem.* 38, 1427–1430
58 Kurth, M.J. *et al.* (1994) *J. Org. Chem.* 59, 5862–5864
59 Williard, R. *et al.* (1995) *Chem. Biol.* 2, 45–51
60 Holmes, C.P. *et al.* (1995) *J. Org. Chem.* 60, 7328–7333
61 Ruhland, B. *et al.* (1995) *J. Am. Chem. Soc.* (in press)
62 Boojamra, C.G., Burow, K.M. and Ellman, J.A. (1995) *J. Org. Chem.* 60, 5742–5743
63 Goff, D.A. and Zuckermann, R.N. (1995) *J. Org. Chem.* 60, 5744–5745
64 Gordcev, M.F., Patel, D.V. and Gordon, E.M. (1995) *J. Org. Chem.* (in press)
65 Wipf, P. and Cunningham, A. (1995) *Tetradedron. Lett.* 36, 7819–7822
66 Yu, K L., Deshpande, M.S. and Vyas, D.M. (1994) *Tetrahedron Lett.* 35, 8919–8972
67 Goff, D.A. and Zuckermann, R.N. (1995) *J. Org. Chem.* 60, 5748–5749