Design, Synthesis, and Evaluation of Small-Molecule Libraries

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Introduction

One of the major goals in chemical and biological research is the identification of high affinity and specific ligands to receptors and enzymes. The identification of such ligands is a fundamental step in the development of molecular probes for the study of receptor and enzyme function,[1] as well as for the eventual development of therapeutic agents.

An initial lead compound that has modest binding or inhibitory activity is generally identified by screening large numbers of compounds or biological extracts. Many analogs of the lead compound 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 biological targets that continue to be identified through modern molecular biology methods.[2]

To address this demand, very powerful chemical and biological methods have been developed for the generation of large combinatorial libraries of peptides[3] and oligonucleotides,[4] which 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* clearing times, and therefore their utility as pharmacological probes or therapeutic agents is often limited.[5] Although many small organic compounds (<600−700 molecular weight) have favorable pharmacokinetic properties,[6] they have until recently been synthesized one at a time, thus dramatically limiting the number of derivatives that can be studied. Over the past several years, an increasing number of researchers have worked to overcome this limitation by developing methods for the synthesis of libraries of small molecules. In this Account we review our own efforts in the design, synthesis, and evaluation of small-molecule libraries.[7]

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

Compound Selection and Synthesis

A number of factors may be considered in the selection of a compound class for library synthesis. One strategy that we have employed is to select "privileged" structures, where the display of different functionality upon the core structure has previously provided a number of potent and specific drugs or drug candidates for different therapeutic targets.[8] A second strategy that we have employed is to design a scaffold or template for compound synthesis that is based upon an important molecular recognition motif in biological systems, such as a scaffold that mimics a key secondary structural motif of proteins or that is based upon a stable mimetic of the transition state or intermediate of a reaction that is catalyzed by an important enzyme class.

For both privileged structure libraries and libraries based on designed templates, we use three criteria in the design and development of the library synthesis sequence. First, several different sets of building

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(4) For reviews on oligonucleotide libraries, see: (a) Gold, L.; Polisky, B.; Uhlenbeck, O.; Yarus, M. *Annu. Rev. Biochem.* **1995**, *64*, 763−797. (b) Ecker, D. J.; Vickers, T. A.; Hanecak, R.; Driver, V.; Anderson, K. *Nucleic Acids Res.* **1993**, *21*, 1853−1856. (c) Bock, L. C.; Griffin, L. C.; Latham, J. A.; Vermaas, E. H.; Toole, J. J. *Nature* **1992**, *355*, 564−566.

(5) Smith, A. B.; Hirschmann, R.; Pasternak, A.; Akaishi, R.; Guzman, M. C.; Jones, D. R.; Keenan, T. P.; Sprengeler, P. A.; Darke, P. L.; Emini, E. A.; Holloway, M. K.; Schleif, W. A. *J. Med. Chem.* **1994**, *37*, 215−218.

(6) Kim, E. E.; Baker, C. T.; Dwyer, M. D.; Murcko, M. A.; Rao, B. G.; Tung, R. D.; Navia, M. A. *J. Am. Chem. Soc.* **1995**, *117*, 1181−1182.

(7) For reviews on small-molecule libraries, see: (a) Ellman, J. A.; Thompson, L. A. *Chem. Rev.* **1996**, *96*, 555−600. (b) Gordon, E. M.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gallop, M. A. *J. Med. Chem.* **1994**, *37*, 1385−1401. (c) Terrett, N. K.; Gardner, M.; Gordon, D. W.; Kobylecki, R. J.; Steele, J. *Tetrahedron* **1995**, *51*, 8135−8173. (d) Blondelle, S. E.; Perezpaya, E.; Dooley, C. T.; Pinella, C.; Houghten, R. A. *Trends Anal. Chem.* **1995**, *14*, 83−92. (e) Lebl, M.; Krchnak, V.; Sepetov, N. F.; Seligmann, B.; Strop, P.; Felder, S.; Lam, K. S. *Biopolymers (Pept. Sci.)* **1995**, *37*, 177−198. (f) Desai, M. C.; Zuckermann, R. N.; Moos, W. H. *Drug. Dev. Res.* **1994**, *33*, 174−188. (g) Janda, K. D. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 10779−10785. (h) Moos, W. H.; Green, G. D.; Pavia, M. R. *Annu. Rep. Med. Chem.* **1993**, *28*, 315−324.

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blocks should be incorporated to provide rapid access to a large number of diverse compounds. Second, the chemistry should be compatible with the display of as much functionality as possible, including reactive functionality commonly found in drugs, such as alcohols, phenols, amines, guanidines, imidazoles, indoles, carboxylic acids, amides, nitriles, nitro groups, and halides. Finally, for rapid library synthesis, the building blocks used in the synthesis of the library must be commercially available or at least readily accessible.

In our library synthesis efforts, we employ solid-phase synthesis strategies for the same reason that it has been employed for all of the chemical approaches for the synthesis and evaluation of peptide libraries: the isolation of support-bound reaction products is accomplished simply by washing reagents away from the support thereby making it straightforward to drive reactions to completion by the use of excess reagents. In order to achieve the goals described above, new chemistry must often be developed and adapted to the solid support. For some compound classes the chemistry that is developed is based upon analogous chemistry in solution; however, for many compound classes much of the chemistry that is developed does not have a direct solution-phase counterpart.

Library Synthesis Strategy

A number of methods for library synthesis and evaluation are currently available. We have always elected to cleave compounds from the support for biological evaluation in solution, since the solid support or linkage functionality can complicate or interfere with receptor binding. We have also chosen to synthesize libraries of discrete compounds in a spatially separate fashion rather than libraries of compound mixtures to allow for rigorous analytical characterization of selected compounds in the library. Since new solid-phase synthesis methods are being developed for each library synthesis project, it is important to be able to determine the chemical integrity of the derivatives that are prepared in order to demonstrate the quality of the library synthesis chemistry. Of course, compounds that have been synthesized in a spatially separate fashion can be assayed either separately or after first pooling the compounds.[9] With either assay strategy we avoid the time-consuming resynthesis of compounds that is necessary when compounds are synthesized as mixtures.

On the basis of the above criteria, for our initial library synthesis efforts we chose to employ a Chiron Mimotopes pin apparatus developed for peptide epitope mapping.[10,11] The Chiron Mimotopes apparatus is configured such that 96 polyethylene pins are attached to a supporting block so that each pin fits into a well of a 96-well microtiter plate. The pins are prederivatized with aminoalkyl groups providing sites for substrate attachment. Even though each well of the microtiter plate serves as a distinct reaction vessel for performing chemical reactions, each synthesis step can rapidly and efficiently be performed on multiple pins simultaneously by employing preexisting microtiter plate based instrumentation, developed predominantly for screening procedures. Pin loading levels from 100 nmol up to 50 μmol are available. 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.

1,4-Benzodiazepines

In order to demonstrate the feasibility of small-molecule library synthesis and evaluation strategies we first selected the 1,4-benzodiazepine class of privileged structures, which have widespread biological activities and are one of the most important classes of bioavailable therapeutic agents. In addition to 1,4-benzodiazepines with anxiolytic activity such as Valium,[12] derivatives have been identified that are highly selective cholecystokinin receptor subtype A antagonists, highly selective cholecystokinin receptor subtype B antagonists,[13] κ-selective opioids,[14] platelet-activation factor antagonists,[15] HIV Tat antagonists,[16] reverse transcriptase inhibitors,[17] gpIIbIIIa inhibitors,[18] and ras farnesyltransferase inhibitors.[19]

We chose to focus on the 1,4-benzodiazepin-2-one structure as our first target, since a majority of the biologically active benzodiazepines that have been identified to date fall within this subclass. The 1,4-benzodiazepin-2-one derivatives were initially constructed from three components: 2-aminobenzophenones, amino acids, and alkylating agents.[20,21] Employing solution chemistry, substituted 2-*N*-Fmoc aminobenzophenones are coupled to the acid-cleavable (4-(hydroxymethyl)phenoxy)acetic acid (HMP) linker.[22] As shown in Scheme 1, the linker may be attached through a hydroxyl or carboxyl group located on either aromatic ring of the 2-aminobenzophenone. The linker-derivatized aminobenzophenones **1a** or **1b** are then coupled to the solid support employing standard amide bond forming methods.

The synthesis of benzodiazepine derivatives on solid support is initiated by removal of the Fmoc protecting group from **2** by treatment with piperidine in DMF followed by coupling of an α-*N*-Fmoc amino acid to the resulting unprotected 2-aminobenzophenone (Scheme

(9) Deprez, B.; Williard, X.; Bourel, L.; Coste, H.; Hyafil, F.; Tartar, A. *J. Am. Chem. Soc.* **1995**, *117*, 5405−5406.
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(20) Bunin, B. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1992**, *114*, 10997−10998.
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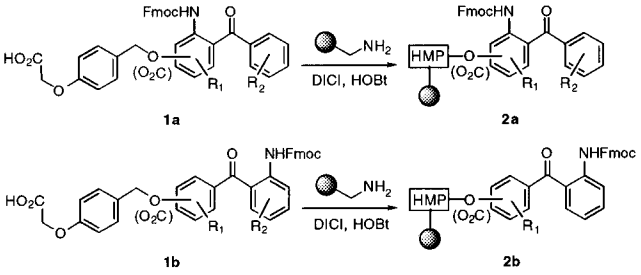
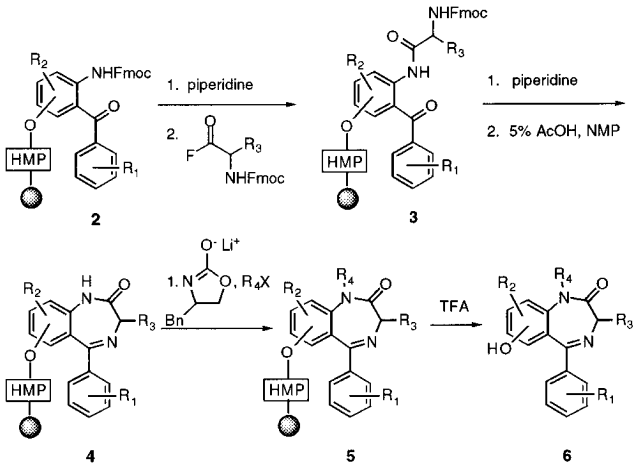
Scheme 1



Scheme 2



2). Standard activation methods for solid-phase peptide synthesis were not successful for this coupling step due to the poor basicity and nucleophilicity of 2-aminobenzophenones. However, the activated α-*N*-Fmoc amino acid fluorides developed by Carpino[23] couple efficiently to provide the amide products **3** even for electron-deficient 2-aminobenzophenone derivatives. The Fmoc protecting group is then removed via treatment with piperidine in DMF, and the resulting free amine is treated with 5% acetic acid in *N*-methylpyrrolidinone (NMP) at 60 °C to provide the benzodiazepine derivatives **4**, which incorporate two of the three components for introducing diversity.

Alkylation of the anilide of **4** provides the fully derivatized 1,4-benzodiazepines **5**. In order to ensure complete reaction on solid support, excess reagent is generally employed. We therefore employ either lithiated acetanilide or lithiated 5-(phenylmethyl)-2-oxazolidinone[24] as the base since they are basic enough to completely deprotonate the anilide of **4**, but will not deprotonate other functionality that may be present in the benzodiazepine structure such as amide, carbamate, or ester functionality (entries 6 and 7, Table 1). Treatment with the volatile acid cleavage cocktail trifluoroacetic acid/dimethyl sulfide/H_2O (95:5:5)[25] then affords the benzodiazepine products **6**, which after chromatography are obtained in high yield (85−100%) based on the support-bound starting material **2**. Finally, no racemization of representative benzodiazepines was detected (<1%) as determined by chiral HPLC.

(23) Carpino, L. A.; Sadataalaee, D.; Chao, H. G.; Deselms, R. H. *J. Am. Chem. Soc.* **1990**, *112*, 9651−9652.

(24) The pK_a of 5-(phenylmethyl)-2-oxazolidone is 20.5 in DMSO as determined by Bordwell. Evans, D. A.; Britton, T. C.; Ellman, J. A.; Dorow, R. L. *J. Am. Chem. Soc.* **1990**, *112*, 4011−4030. Lithiated 5-(phenylmethyl)-2-oxazolidinone is employed rather than unsubstituted 2-oxazolidinone due to its greater solubility in THF.

(25) Dimethyl sulfide and H_2O are added to scavenge cations generated in the deprotection step.

Table 1. Structures and Yields of 1,4-Benzodiazepine Derivatives 6 (Scheme 2)

	derivative				
entry	R_1	R_2	R_3	R_4	yield (%)[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_2CHCH_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	$CH_2C_6H_4$-4-OH	CH_2CH_3	98
9	H	4-CO_2H-5-Cl	CH_2Ph	CH_3	100
10	H	4-CO_2H-5-Cl	CH_3	CH_2Ph	93

[a] Mass balance yields of purified and fully characterized compounds are based on support-bound starting material **2**.

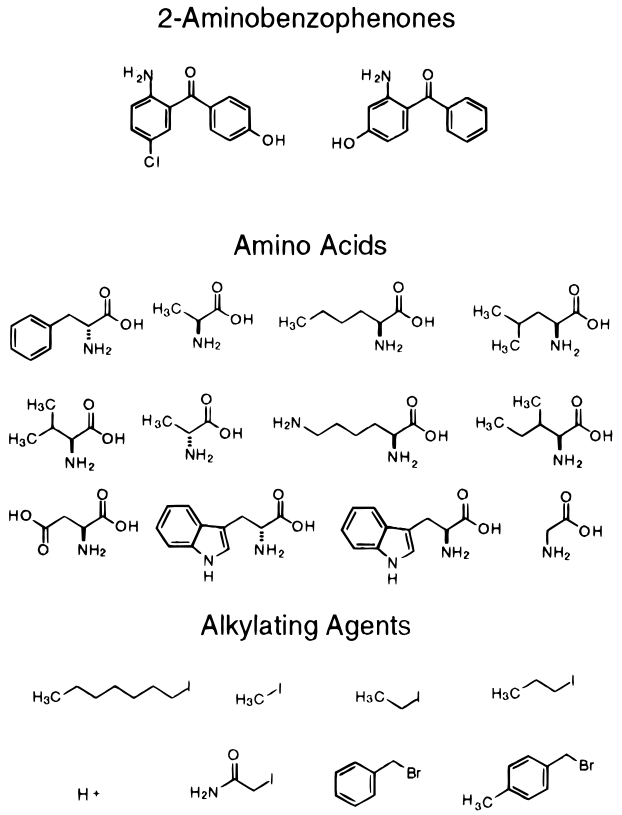


Figure 1. Building blocks used in the synthesis of the 192-member benzodiazepine library.

A small library of 192 benzodiazepines was prepared in order to evaluate the spatially separate synthesis of a library of these compounds using Chiron Mimotopes pin support.[26] 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 1).

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. Yields were also determined for 20 derivatives, where each of the 2-aminobenzophenones, amino acids, and alkylating agents was

(26) Bunin, B. A.; Plunkett, M. J.; Ellman, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *6*, 4708−4712.

incorporated in 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 for the benzodiazepine derivatives was observed as calculated from the experimentally determined extinction coefficients of the selected derivatives.

The spatially separate library of benzodiazepines was 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. Although a detailed description of the structure−activity relationships is outside the scope of this review, the data provided by screening the library was confirmed by synthesizing a number of the derivatives on a large scale followed by purification and IC_{50} determinations. The most potent compound ($IC_{50} = 0.08\ \mu M$) 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.[27]

Employing the synthesis strategy described above, we have prepared a second library designed to give 1680 discrete benzodiazepine derivatives from three 2-aminobenzophenones, 35 amino acids, and 16 alkylating agents (structures not shown). A subset of the library was analyzed by HPLC as described above, and yields were found to range from 61% to 87% (average 72%). In addition, 48 of the compounds (randomly selected, incorporating each of the building-block derivatives at least twice) were analyzed by matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) using α-cyano-4-hydroxycinnamic acid as the matrix. For 46 (95%) of the derivatives the expected molecular ion was observed.

Additional Benzodiazepine Diversity

The original benzodiazepine synthesis sequence was based upon the combination of three different building-block sets: 2-aminobenzophenones, *N*-Fmoc amino acid fluorides, and alkylating agents. 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, we utilized the Stille coupling reaction to synthesize a variety of 2-aminoaryl ketones on solid support.[28] The Stille reaction is particularly appealing for this purpose since it proceeds under mild conditions, it is tolerant of a wide range of functionality, and well over 300 structurally diverse acid chloride building blocks are commercially available that are compatible with the 1,4-benzodiazepine synthesis sequence.

The support-bound 2-(4-biphenylyl)isopropyloxycarbonyl (Bpoc) protected (aminoaryl)stannane **7** (Scheme 3) is prepared in five steps from commercially available material. Coupling to support is again accomplished employing the HMP linker. The Stille coupling reaction can be carried out with a range of different acid chlorides and the catalyst $Pd_2(dba)_3{\cdot}CHCl_3$. Because excess acid chloride is employed in the Stille coupling step, diisopropylethylamine and K_2CO_3 are added as acid scavengers to minimize protodestannylation. The Bpoc group is cleaved by brief treatment (5 min) with 3% TFA in CH_2Cl_2, and the resulting support-bound 2-aminoaryl ketone **8** is then incorporated directly into 1,4-benzodiazepine derivatives according to the previously described synthesis sequence.

Scheme 3

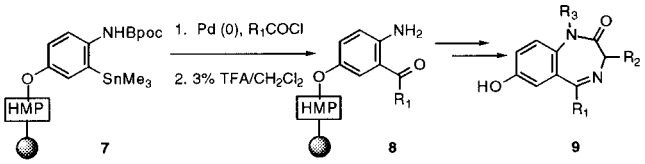


Table 2. Structures and Yields of 1,4-Benzodiazepin-2-one Derivatives 9 (Scheme 3)

	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_4-3-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_8-1-C_6H_4-4-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	(3,4-methylene-dioxy)phenyl	$CH_2C_6H_4$-4-OH	CH_2CONH_2	82
15	2-naphthyl	CH_3	CH_2CH_3	81

[a] Mass balance yields of purified and fully characterized compounds are based on the initial aminomethyl loading level of the resin.

Using this strategy, diverse acid chlorides were employed to prepare support-bound 2-aminoaryl ketones **8**, which were further incorporated into 1,4-benzodiazepines **9**, including aromatic acid chlorides that are electron rich, electron poor, alkyl substituted, polyaromatic, heterocyclic and ortho substituted and aliphatic acid chlorides that are sterically hindered. The desired benzodiazepines were isolated after the eight-step synthesis sequence in >85% purity by ^1H NMR analysis of the crude products. Yields of purified benzodiazepine products varied from 52% to 82% (Table 2) based on the initial aminomethyl loading of the polystyrene resin used.[29]

Employing this synthesis sequence, we have prepared a library designed to give 11 200 discrete 1,4-benzodiazepines[30] from 20 acid chlorides, 35 amino acids, and 16 alkylating agents, all of which were

(27) Evans, B. E.; Rittle, K. E.; Bock, M. G.; DiPardo, R. M.; Freidinger, R. M.; Whitter, W. L.; Gould, N. P.; Lundell, G. F.; Homnick, C. F.; Veber, D. F.; Anderson, P. S.; Chang, R. S. L.; Lotti, V. J.; Cerino, D. J.; Chen, T. B.; King, P. J.; Kunkel, K. A.; Springer, J. P.; Hirschfield, J. *J. Med. Chem.* **1987**, *30*, 1229−1239.

(28) Plunkett, M. J.; Ellman, J. A. *J. Am. Chem. Soc.* **1995**, *117*, 3306−3307.

(29) These yields represent the lower limit of the reaction efficiency, as when compound **7** is deprotected, acetylated, and cleaved, 4-hydroxyacetanilide is isolated in only 92% yield based upon the aminomethyl loading level of the resin, indicating that acylation to provide **7** did not proceed to completion.

(30) Bunin, B. A.; Plunkett, M. J.; Ellman, J. A. *Methods Enzymol.*, in press.

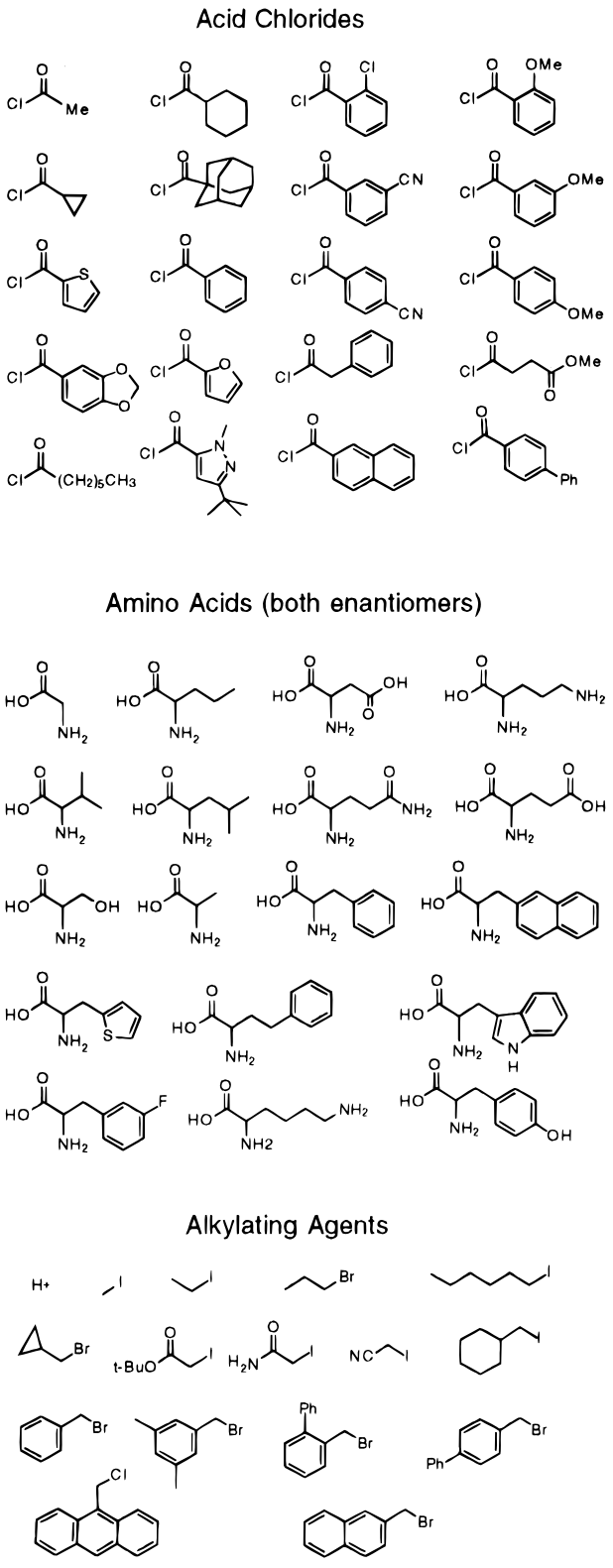


Figure 2. Building blocks used in the synthesis of the 11 200-member benzodiazepine library (Scheme 3).

commercially available (Figure 2). The 20 acid chlorides were selected to maximize diversity using a similarity grouping procedure developed by Dr. Steven Muskal at MDL Information Systems. A set of over 300 commercially available acid chlorides which are compatible with the synthesis sequence were employed in the selection process. The alkylating agents and amino acids also displayed a range of functionality.

Proton NMR of selected compounds in the library showed the expected benzodiazepine as the major product in each case. Twenty derivatives were analyzed by reverse phase HPLC with UV detection at 315 nm for benzodiazepines derived from aromatic acid chlorides and 285 nm for those derived from aliphatic acid chlorides. One major product (with retention time identical to that of authentic material prepared on a large scale) was observed in all cases. To further confirm that the expected compounds had been synthesized, a number of the compounds in the library were analyzed by MALDI-MS. The expected molecular ion was observed for 67 of 72 randomly selected derivatives, where each building-block derivative was incorporated into at least two of the compounds where a molecular ion was observed.

The previously described 1,4-benzodiazepine libraries are currently being screened against a number of receptor and enzyme targets, for example, to identify inhibitors of tyrosine kinases implicated in cancer[31] and ligands that block an autoimmune DNA−antibody interaction implicated in systemic lupus erythematosus[32] as well as ligands to SH3 domains, 7-transmembrane G-protein coupled receptors,[33] and transcriptional regulatory factors.

Alternative Linkage Strategies

In the above synthesis strategy the benzodiazepine is attached to the solid support through phenolic functionality. After cleavage from the support the residual hydroxyl group may have a negligible, positive, or negative effect on the biological or chemical activity of the target molecule. For certain applications it would be desirable to have a linkage strategy that after cleavage from the support would leave behind no trace or "memory" of the solid-phase synthesis sequence. For 1,4-benzodiazepines and more generally for aromatic-containing compounds, we envisioned that linkage through a silicon−aryl bond would fulfill these requirements. This type of bond is often cleaved using acidic conditions, and the effect of aromatic substitution[34] and silicon substituents[35] on reaction rate has been studied extensively. The lability of aryl−silicon bonds to fluoride ion is also well documented.[36]

As the first demonstration of a silicon-based linkage strategy for the traceless synthesis of aromatic compounds on solid supports, we reported the solid-phase synthesis of 1,4-benzodiazepines by a silyl linkage strategy.[37] The silyl-substituted (aminoaryl)stannane derivative **10** (Scheme 4), which is synthesized in five steps in solution, is first coupled to aminomethylated polystyrene. The synthesis of support-bound 1,4-benzodiazepines then proceeds as described previously. Treatment of the support-bound benzodiazepine **12** with anhydrous HF provides the benzodiazepine product **13**. Good purity of the crude

(31) Buddie, R. A.; Levin, V. Anderson Cancer Center, Houston, TX, unpublished results, 1995.
(32) Glick, G. University of Michigan, unpublished results, 1995.
(33) Morrissey, M.; Snider, M. Berlex, unpublished results, 1995.
(34) Deans, F. B.; Earborn, C. *J. Chem. Soc.* **1959**, 2299−2303.
(35) Bott, R. W.; Earborn, C.; Jacson, P. M. *J. Organomet. Chem.* **1967**, *7*, 79−83.
(36) Mills, R. J.; Taylor, N. J.; Snieckus, V. *J. Org. Chem.* **1989**, *54*, 4372−4385.
(37) Plunkett, M. J.; Ellman, J. A. *J. Org. Chem.* **1995**, *60*, 6006−6007.

Scheme 4

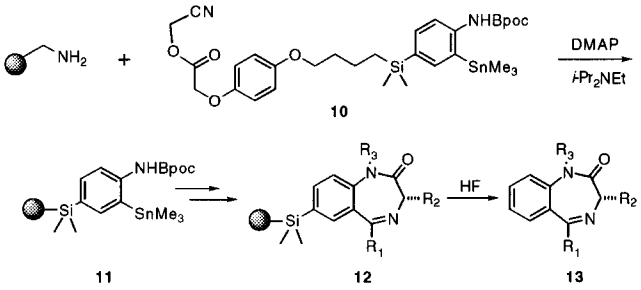


Table 3. Structures and Yields of 1,4-Benzodiazepine Derivatives 13 (Scheme 4)

	derivative			
entry	R_1	R_2	R_3	yield (%)[a]
1	C_6H_4-3-OCH_3	$CH_2CH(CH_3)_2$	CH_2CH_3	66
2	1-adamantyl	$CH_2CH(CH_3)_2$	CH_2CH_3	60
3	C_6H_5	$CH_2C_6H_4$-4-OH	$CH_2C_6H_5$	50[b]
4	C_6H_4-4-OCH_3	CH_3	H	68

[a] Mass balance yields of purified and fully characterized compounds are based on the initial aminomethyl loading level of the resin. [b] The debenzylated product was also obtained in 11% yield.

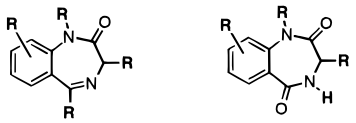


Figure 3. Structures of 1,4-benzodiazepin-2-one and 1,4-benzodiapine-2,5-dione.

product is observed, >85% by ^{1}H NMR, and the isolated yield after purification ranged from 50% to 68% for four representative compounds, based on the initial aminomethyl loading of the polystyrene resin (Table 3).

1,4-Benzodiazepine-2,5-dione

We have also reported a general and high-yielding method for the solid-phase synthesis of 1,4-benzodiazepine-2,5-diones (Figure 3).[38,39] The synthesis strategy complements the previously described 1,4-benzodiazepin-2-one synthesis sequence, since a wider range of functionality can be directly introduced onto the aromatic core of the benzodiazepine structure from the over 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 also are 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 5).[40] Racemization is not observed if the imine resulting from condensation of the α-amino ester and aldehyde **14** is reduced immediately upon its formation. Acylation of the resulting secondary amine **15** with a commercially available unprotected anthranilic acid then provides the support-bound tertiary amide **16**. The optimal conditions for effecting this transformation are to employ a carbodiimide in conjunction with the hydrochloride salt of a tertiary amine. 1-Ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDC) is the most convenient activating agent since the tertiary amine hydrochloride is present in the carbodiimide structure.

Scheme 5

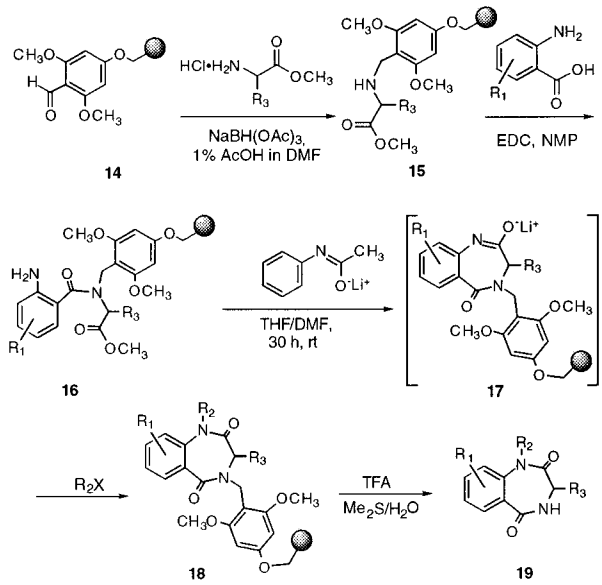


Table 4. Structures and Yields of 1,4-Benzodiazepine-2,5-diones 19 (Scheme 5)

	derivative			yield
entry	R_1	R_2	R_3	(%)[a]
1	8-Cl	CH_2CH_3	$CH_2CH(CH_3)_2$	75
2	7-Cl	CH_2CHCH_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_2-c-C_3H_5	$CH_2CH(CH_3)_2$	79
7[b]	7-C_6H_4-4-OCH_3	CH_2CH_3	$CH_2CH(CH_3)_2$	62
8[b]	8-$(CH_2)_5CH_3$	$CH_2C_6H_4$-4-Ph	$CH_2C_6H_4$-4-OH	77
9	7-Cl	CH_2CHCH_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
12	7-Cl	$(CH_2)_4CH_3$	$CH_2CH_2CO_2H$	50

[a] Mass balance yields of purified and fully characterized compounds are based on the loading levels of leucine and phenylalanine ester derived resins. [b] Suzuki cross-coupling products.

Cyclization and then subsequent alkylation of the support-bound anilide anion **17** generated *in situ* is next accomplished in a single step by treatment of amide **16** 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 support-bound benzodiazepine using the Suzuki cross-coupling reaction as is exemplified in entry 7 (Table 4), where a cross-coupling reaction was carried out with (*p*-methoxyphenyl)boronic acid, and in entry 8 (Table 4), where a Suzuki cross-coupling reaction was performed using *B*-hexyl-9-BBN. The benzodiazepine-2,5-dione 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 benzodiazepines **19** including derivatives that incorporate amino acids with side-chain functionality such as tyrosine, lysine, and glutamic acid. In addition, no detectable racemization,

(38) Boojamara, C. G.; Burow, K. M.; Ellman, J. A. *J. Org. Chem.* **1995**, *60*, 5742−5743.
(39) Goff, D. A.; Zuckermann, R. N. *J. Org. Chem.* **1995**, *60*, 5744−5745. Goff has also reported a solid-phase method for the preparation of 1,4-benzodiazepine-2,5-diones.
(40) Landi, J. J.; Ramig, K. *Synth. Commun.* **1991**, *21*, 167−171.

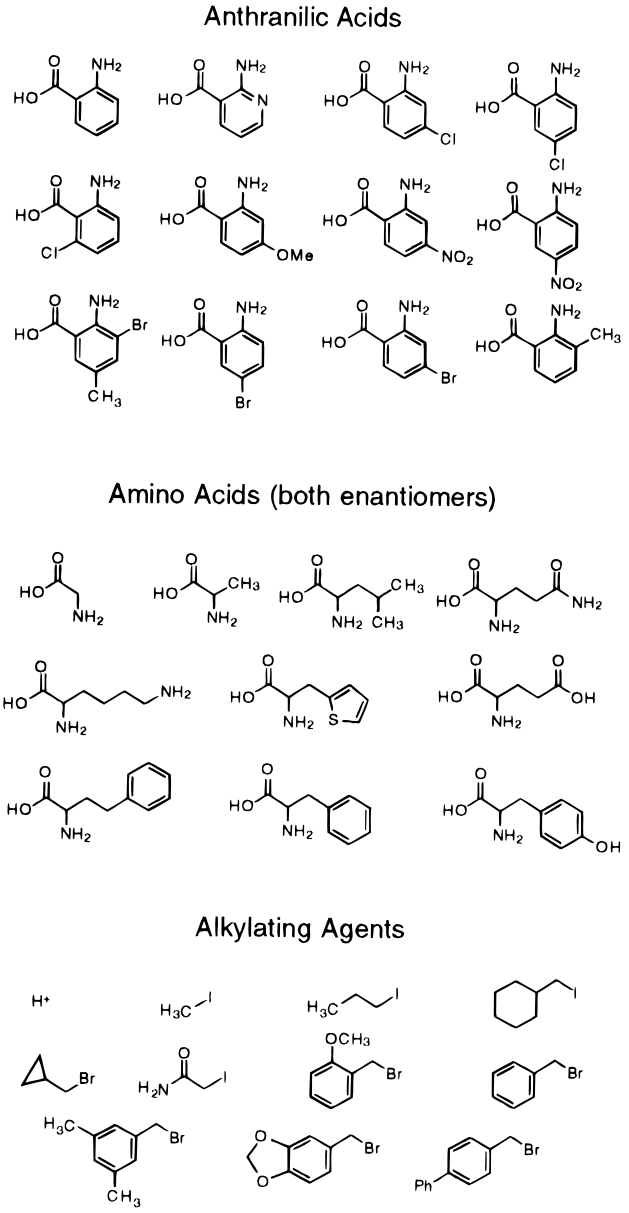


Figure 4. Building blocks employed to synthesize a 2508-member 1,4-benzodiazepine-2,5-dione library (Scheme 5).

<3%, occurs during the synthesis sequence as determined by chiral HPLC analysis and derivatization studies.

Employing the above synthesis sequence, we have recently prepared a library of spatially separate derivatives designed to give 2508 1,4-benzodiazepine-2,5-diones from 12 anthranilic acids, 19 amino acids, and 12 alkylating agents (Figure 4). The Chiron Mimotopes pin apparatus was employed for library synthesis, and library synthesis was completed in $1^1/_2$ weeks.[41]

β-Turn Mimetics

In addition to developing libraries based upon privileged structures, we have also focused on the design of new templates for library synthesis. One of our efforts in this area is the design, synthesis, and evaluation of libraries of *β*-turn mimetics. *β*-Turns, **20** (Figure 5), are a key structural motif in peptides and proteins which often play a key role in molecular

(41) Boojamra, D.; Burow, K. Unpublished results, 1995.

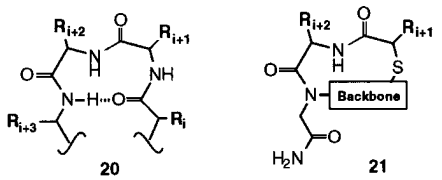


Figure 5. Structures of *β*-turns and *β*-turn mimetics.

recognition events in biological systems.[42] Therefore, a great deal of effort has been focused on the design of small non-peptide mimetics of the turn structure which have improved affinity, specificity, and/or pharmacokinetic properties relative to the corresponding peptide ligands.[43,44] 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 we developed a general method for the solid-phase synthesis of *β*-turn mimetics, **21**, for the rapid identification of ligands that are based upon the *β*-turn structure.[45]

Turn mimetics **21** are prepared 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 and the relative orientations of the side chains can be modulated by introducing different backbone linkages to provide 9- or 10-membered rings. In addition, different side chain orientations can be 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.

In order to demonstrate the fidelity of the synthesis sequence for turn mimetics **21**, *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 (Scheme 6). α-Bromoacetic acid was then coupled to the support-bound *p*-nitrophenylalanine by activation with diisopropylcarbodiimide. Subsequent treatment with the aminoalkyl thiol protected as the *tert*-butyl mixed disulfide provided the secondary amine **23**.[46] The secondary amine was then coupled with an *N*-Fmoc α-amino acid employing *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) to provide **24**.[47] Treatment with 20% piperidine to remove the Fmoc group followed by reaction with the symmetric anhydride of the appropriate α-bromo acid provided acyclic intermediate **25**, which incorporates both the $i + 1$ and the $i + 2$ side chain residues. Cleavage of the mixed disulfide was then accomplished by treatment of the cyclization precursor **25** with tributylphosphine in a 5:3:2 pro-

(42) Rose, G. D.; Gierasch, L. M.; Smith, J. A. *Adv. Protein Chem.* **1985**, *37*, 1−109.
(43) Rizo, J.; Gierasch, L. M. *Annu. Rev. Biochem.* **1992**, *61*, 387−418.
(44) For reviews on *β*-turn mimetics, see: (a) Ball, J. B.; Alewood, P. F. *J. Mol. Recognit.* **1990**, *3*, 55−64. (b) Kahn, M. (guest ed.) *Tetrahedron* **1993**, *49*, Symp. 50, 3433−3677.
(45) Virgilio, A. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1994**, *116*, 11580−11581.
(46) Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. *J. Am. Chem. Soc.* **1992**, *114*, 10646−10647.
(47) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397−4398.

Scheme 6

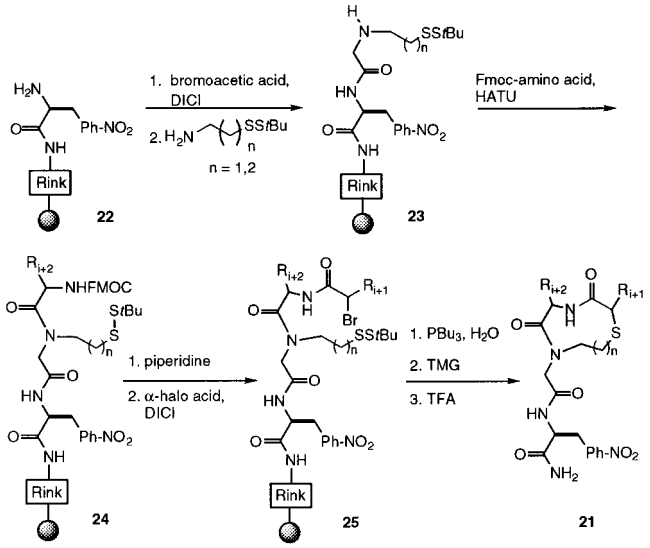


Table 5. Structures and Yields of β-Turn Mimetics 21 (Scheme 6)

	derivative[a]			purity (%)[b]	
entry	R_{i+1}	R_{i+2}	backbone *n*	PEG-PS	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 had the *S* configuration.

panol/DMF/H_2O comixture. It is necessary to use PEG-PS as the solid support, which is well solvated under the aqueous reaction conditions, in order to obtain clean reduction of the disulfide bond without side reactions. Cyclization to provide the 9- or 10-membered thioether was accomplished by treatment with tetramethylguanidine in a DMF/H_2O comixture.[48] Cleavage of the turn mimetic from the support by treatment with 95:5:5 TFA/DMS/H_2O then provided mimetic **21**.

Employing this synthesis sequence, turn mimetics **21** were obtained with an average purity of 75% (11 compounds) over the eight-step process as determined by HPLC analysis (Table 5). For all of the turn mimetics synthesized, cyclization provided the desired cyclic monomer with no cyclic dimer detected (<5%).[49] A variety of side chain functionality could be incorporated successfully into the turn mimetics, including alcohol, phenol, carboxylic acid, and amine functionality.

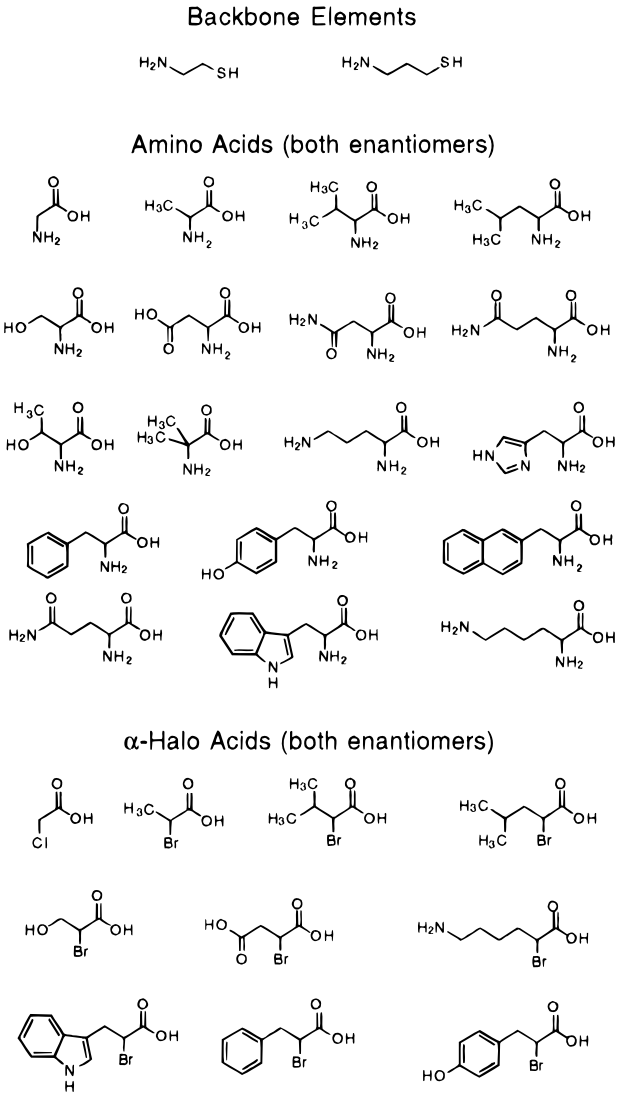


Figure 6. Building blocks employed to synthesize a β-turn mimetic library (Scheme 6). Only the natural enantiomer of the amino acid threonine was employed.

To demonstrate the utility of the synthesis sequence for the rapid construction of a library of turn mimetics **21**, the 11 mimetics were synthesized simultaneously employing a Chiron Mimotopes pin apparatus. All 11 derivatives were obtained in a high level of purity as determined by HPLC analysis (Table 5). On the basis of these results, a library of 1292 β-turn mimetics has been prepared using 19 α-halo acids, 34 α-amino acids, and the two backbone elements (Figure 6).[50,51] Dr. Andrew Bray from Chiron Mimotopes analyzed 7% of the compounds in the library by mass spectrometry using electrospray ionization. For all of the derivatives that were tested, the expected molecular ion was observed. In collaboration with Dan Fitzpatrick at Genentech, a subset of the library was assayed to identify several specific ligands with low micromolar

(48) Researchers at Genentech have reported the synthesis of 15-membered-ring RGD mimetics by thioalkylation: Barker, P. L.; Bullens, S.; Bunting, S.; Burdick, D. J.; Chan, K. S.; Deisher, T.; Eigenbrot, C.; Gadek, T. R.; Gantzos, R.; Lipari, M. T.; Muir, C. D.; Napier, M. A.; Pitti, R. M.; Padua, A.; Quan, C.; Stanley, M.; Struble, M.; Tom, J. Y. K.; Burnier, J. P. *J. Med. Chem.* **1992**, *35*, 2040−2048.

(49) Cyclization to provide **2** was initially attempted by macrolactamization between the $i + 1$ and $i + 2$ residues rather than by thioalkylation; however, cyclization with a range of activating agents and solid supports provided significant amounts of cyclic dimer. These results are in accord with the well-precedented difficulties in macrolactamization to provide 9- and 10-membered-ring structures. (a) Story, S. C.; Aldrich, J. V. *Int. J. Pept. Protein Res.* **1994**, *43*, 292−296. (b) Kemp, D. S.; Stites, W. E. *Tetrahedron Lett.* **1988**, *29*, 5057−5060.

(50) Virgilio, A. A.; Ellman, J. A. In *Combinatorial Chemistry and Molecular Diversity in Drug Discovery*; Gordon, E. M., Kerwin, J. F., Jr., Eds.; Wiley: New York, in press.

(51) Fifty percent of each compound in the library was employed to prepare a second library of sulfoxide containing turn mimetics by oxidation of the thioether with hydrogen peroxide in aqueous DMF. Unpublished results.

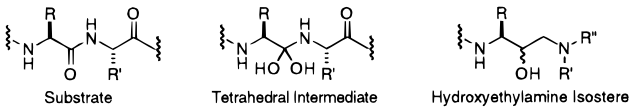


Figure 7. Hydroxyethylamine isostere for inhibition of the aspartic acid protease class.

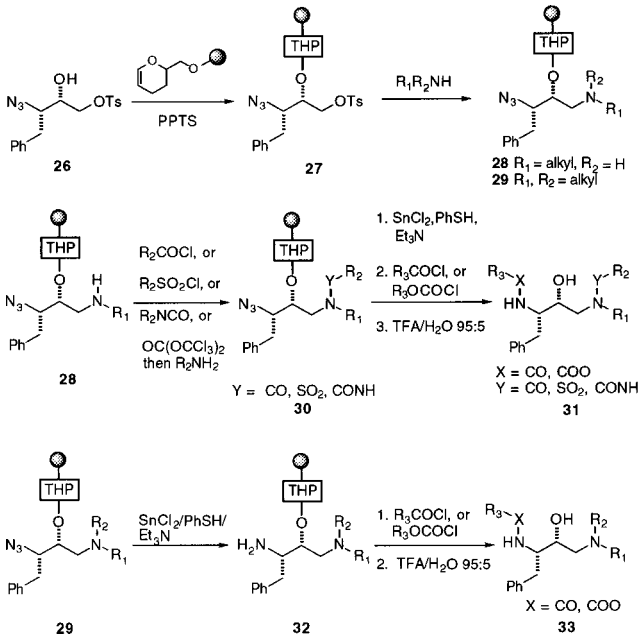
binding affinities to rat somatostatin receptor subtypes I−III.[52] In collaboration with researchers at Berlex, low micromolar to high nanomolar ligands to the NK1 and f-MLP receptors have also been identified.[33] The binding affinities of the active compounds were confirmed by evaluation of purified compounds prepared on a large scale.

Protease Inhibitor Libraries

In addition to designing libraries based upon important elements of protein secondary structure, we have also focused on the preparation of libraries by the display of functionality about isosteres that mimic transition states or intermediates of reactions catalyzed by important enzyme classes. One example is the display of non-peptide functionality about the hydroxyethylamine isostere, which mimics the tetrahedral intermediate for peptide hydrolysis as catalyzed by the aspartic acid class of proteases (Figure 7).[53,54] A number of important therapeutic targets are members of this protease class, including renin, HIV-1 protease, *Candida albicans* protease, and cathepsin D. Of the possible isosteres upon which to construct a library of potential non-peptide inhibitors, we selected the (hydroxyethyl)amine and (hydroxyethyl)-urea isosteres for two reasons. First, several orally available HIV-1 protease inhibitors that incorporate these isosteres have been identified, including several compounds that have been approved or are in the late stages of clinical trials for the treatment of HIV infection. Second, we believed that solid-phase methods could be developed to display a wide range of diverse functionality about these isosteres.[55]

Initially we chose to display functionality from scaffold **26** (Scheme 7), which incorporates the hydrophobic benzyl side chain (the phenylalanine side chain) at the P_1 site, since large hydrophobic side chains are generally preferred at this site by the aspartic acid proteases. In addition, scaffold **26** provides access to known HIV-1 protease inhibitors. In studies which are in progress, we are developing general methods to introduce a variety of side chains at the P_1 position on solid support in order to introduce an added level of diversity in library synthesis. Using dihydropyran-functionalized resin,[56] the scaffold **26** is coupled to support using PPTS. The primary tosyl alcohol **27** is then displaced with either functionalized or unfunctionalized primary or secondary amines to provide **28** and **29**, respectively. After coupling of the primary amines, the resulting secondary amine products **28** can be converted to ureas by reaction with isocyanates or by stepwise treatment with triphosgene followed by amine addition (Scheme 7).[57] 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 carboxylic acids and sulfonyl chlorides under standard conditions.

Scheme 7



The synthesis about the opposite side of the inhibitor is initiated by efficient reduction of azides **29** or **30** using thiophenol/Et_3N/$SnCl_2$ (4:5:1) according to the procedure described by Bartra for the corresponding solution-phase reaction (Scheme 7).[58] 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 are 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.

The previously described synthesis method was employed to prepare a number of different compounds (Figure 8). In order to demonstrate the versatility of the synthesis sequence, a particular emphasis was placed on incorporating functionality that is present in known non-peptide-based HIV-1 protease inhibitors,[59] including *N*-*tert*-butyl pipecolamines, piperazine derivatives, *N*-*tert*-butylureas, quinaldic amides, and 3(*S*)-hydroxytetrahydrofuranyl carbamates. The derivatives were isolated after four to six steps in 65−86% yield after chromatography to provide analytically pure material. In current library synthesis efforts toward the identification of cathepsin D inhibitors, we now employ the *p*-nitrobenzenesulfonate, which is an order of magnitude more reactive that the corresponding tosyl alcohol, and provides the desired reaction

(52) Dan Fitpatrick, Genentech, unpublished results, 1995.
(53) Kick, E. K.; Ellman, J. A. *J. Med. Chem.* **1995**, *38*, 1427−1430.
(54) Wang, G. T.; Li, S.; Wideburg, N.; Krafft, G. A.; Kempf, D. J. *J. Med. Chem.* **1995**, *38*, 2995−3003. Wang and co-workers have also reported a strategy for displaying nonpeptide functionality from C_2-symmetric isosteres.
(55) Alewood, P. F.; Brinkworth, R. I.; Dancer, R. J.; Garnham, B.; Jones, A.; Kent, S. B. H. *Tetrahedron Lett.* **1992**, *33*, 977−980. Alewood and co-workers have reported an efficient solid-phase method for incorporating the hydroxyethylamine isostere into peptides.
(56) Thompson, L. A.; Ellman, J. A. *Tetrahedron Lett.* **1994**, *35*, 9333−9336.
(57) Majer, P.; Randad, R. S. *J. Org. Chem.* **1994**, *59*, 1937−1938.
(58) Bartra, M.; Romea, P.; Urpi, F.; Vilarrasa, J. *Tetrahedron* **1990**, *46*, 587−594.
(59) West, M. L.; Fairlie, D. P. *Trends Pharmacol. Sci.* **1995**, *16*, 67−75.

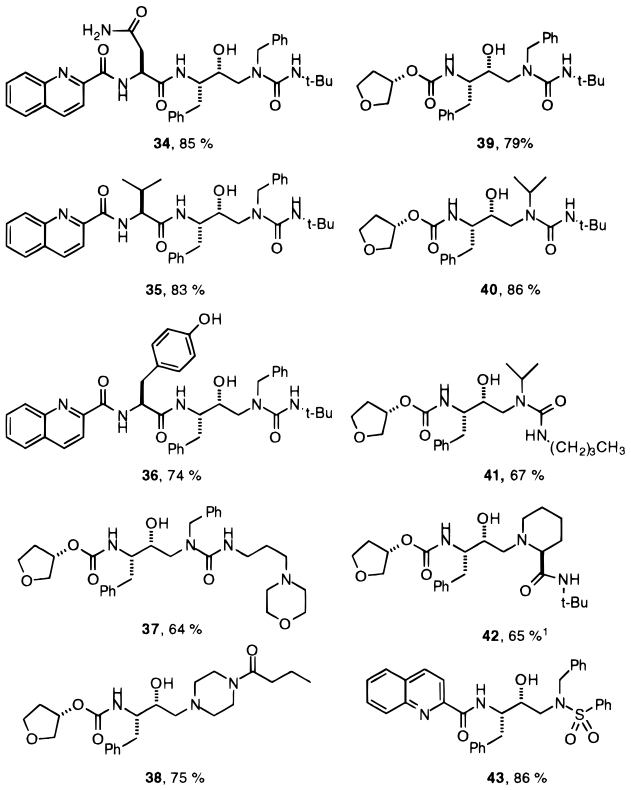


Figure 8. Protease inhibitors prepared according to Scheme 7. The reported yields are of purified material based upon the loading level of **26**. Compound **42** was prepared employing the *p*-nitrobenzenesulfonate rather than the tosylate as a leaving group.

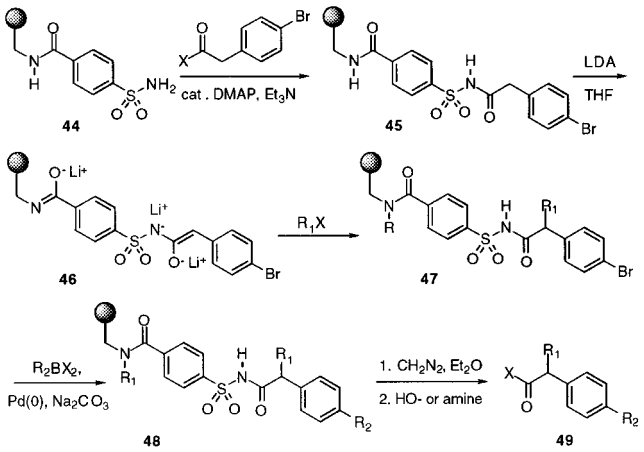
products under milder conditions and for less reactive amine nucleophiles, in higher yields (e.g., compound **42**, Figure 8).

Arylacetic Acids

In the previously described library synthesis efforts, we based the selection of a library template in large part upon the potential biological activity of the compounds that would be generated. We have also focused on the synthesis of some compound classes predominately to explore and develop chemistry on the support. For example, we developed a solid-phase synthesis of arylacetic acid derivatives, which represent an important class of cyclooxygenase inhibitors, predominately to evaluate two important carbon−carbon bond-forming reactions on solid support: enolate alkylation, one of the most fundamental of carbon−carbon bond forming methods, and palladium-mediated Suzuki cross coupling,[60] a method that is extensively employed in natural product synthesis and in medicinal chemistry.[61]

Cyclooxygenase inhibitors that are built upon the phenylacetic acid core typically incorporate three elements of variability: an α-alkyl group, R^1; alkyl, aryl, or heteroaryl substitution on the phenyl ring, R^2; and acid or amide functionality, X (structure **49**, Scheme 8). The appropriate choice of a linker to attach the phenylacetic acid to the solid support is central to the successful synthesis of these compounds. The linkage must be compatible with the basic enolate

Scheme 8



alkylation and Suzuki reaction conditions, yet at the end of the synthesis be labile for nucleophilic cleavage of the final product from the solid support. The seldom used acylsulfonamide linker developed by Kenner for peptide synthesis fulfills these requirements.[62] Under basic conditions the acylsulfonamide ($pK_a \sim 2.5$) is deprotonated, preventing nucleophilic cleavage; however, once solid-support synthesis is complete, treatment with diazomethane results in the formation of the *N*-methylated derivative that is activated for nucleophilic displacement (*vide infra*).

The sulfonamide-derivatized support **44** (Scheme 8) can readily be prepared by treating aminomethylated resin with 4-carboxybenzenesulfonamide, *N,N*-diisopropylcarbodiimide, and hydroxybenzotriazole. Bromophenylacetic acid, activated as the pentafluorophenyl ester or the symmetric anhydride, is then loaded onto the resin employing catalytic DMAP and *i*-Pr_2NEt. Treatment of the acylsulfonamide **45** with excess LDA in THF at 0 °C results in rapid deprotonation presumably to give the trianion **46**. Subsequent addition of activated or unactivated alkyl halides results in rapid alkylation of the enolate trianion to provide **47**. Notably, *N*-alkylation of the acylsulfonamide is not observed under the reaction conditions due to the highly stabilized nature of the acylsulfonamide anion. In addition, in contrast to ester[63] or carboximide[64] enolate alkylations, ketene formation is not observed even when the unreactive alkylating agent isopropyl iodide is employed (entry 6, Table 6), since ketene formation would require that the sulfonamide dianion be the leaving group.

The Suzuki reaction of acylsulfonamide **47** 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 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.

The final step in the synthesis is nucleophile-mediated cleavage of the material from the support.

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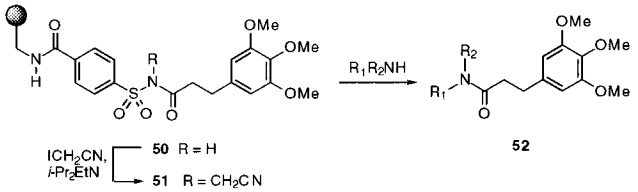
Table 6. Structures and Yields of Substituted Arylacetic Acid Derivatives 49 (Scheme 8)

	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-Cl_2	$BnNH_2$	88

[a] Mass balance yields of analytically pure material are based on the loading level of support-bound starting material **45**. [b] No cleavage of material from the resin was observed with aniline as the nucleophile.

Scheme 9



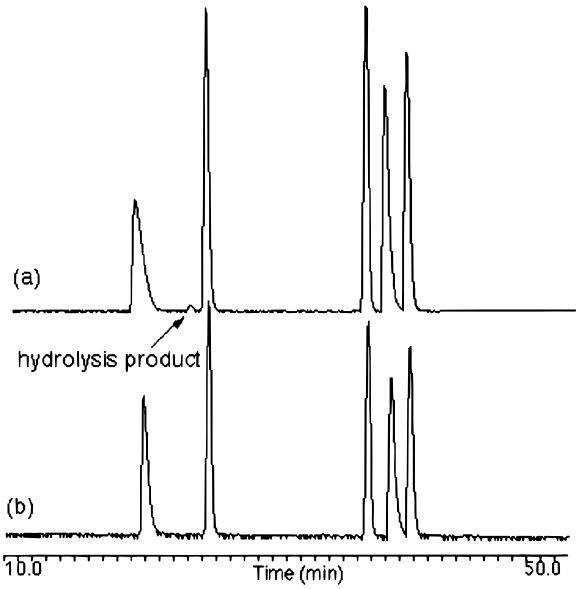


Figure 9. (a) HPLC trace of addition of limiting amounts of five amines to acylsulfonamide resin **51** (Scheme 9) resulting in equimolar amounts (±3%) of the five amide products listed in order of elution (relative peak area): 4-(3-aminopropyl)-morpholine (0.97), morpholine (1.00), benzylamine (1.00), piperidine (1.01), cyclohexylamine (1.01). (b) HPLC trace of standard containing an equimolar mixture of the five amide products.

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 **49**, which are obtained in high yield based upon analytically pure material after filtration through silica. 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 *N*-methyl acylsulfonamide linkage (entry 8, Table 6).

Kenner's safety-catch linker proved to be a useful linkage method due to its stability to basic or strong nucleophilic conditions. However, the poor reactivity of the *N*-methyl acylsulfonamide limits the utility of this safety catch method for small-molecule library synthesis as well as for peptide synthesis. Non-nucleophilic amines do not cleave the material from the *N*-methylated acylsulfonamide, and even for nucleophilic amines, excess reagent is usually employed, which can complicate product isolation. We therefore developed a method for providing an activated acylsulfonamide linkage with greatly enhanced reactivity toward nucleophilic displacement.[65] In particular, we hypothesized that alkylation to introduce an electron-withdrawing *N*-alkyl group would provide enhanced reactivity toward nucleophilic displacement.

Treatment of acylsulfonamide **50** with bromoacetonitrile or iodoacetonitrile and *i*-Pr_2EtN in DMSO or NMP provides the *N*-cyanomethyl acylsulfonamide **51** (Scheme 9). The cyanomethylated derivative **51** is 130-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 activated acyl sulfonamide. For example, activation followed by nucleophilic cleavage of acylsulfonamide **50** with *tert*-butylamine and aniline provides the corresponding analytically pure amide products **52** in 98% and 92% yield, respectively, based upon the initial amine loading level of the resin.

Due to the high level of reactivity of *N*-cyanomethyl acylsulfonamides, treatment 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. The high efficiency of this process provides the opportunity to apply novel pooling strategies, whereby equimolar quantities of highly pure amide products are obtained by employing a limiting amount of an equimolar mixture of several amines. When support-bound *N*-cyanomethyl acylsulfonamide **58** is treated with a limiting amount (0.5 equiv of total amine) of an equimolar mixture of the five amines 4-(3-aminopropyl)morpholine, morpholine, benzylamine, piperidine, and cyclohexylamine, a pool of the five amide products is obtained (Figure 9). Equal amounts of the five products (±3%) are observed by HPLC analysis, and the free acid (<0.5% of combined amide products) resulting from acylsulfonamide hydrolysis is the only side product observed.

Conclusion

A number of important new advances continue to be made in the synthesis and applications of compound libraries. Although much of the chemistry performed on solid support will continue to be based upon analogous chemistry in solution, new chemistry

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that does not have a current solution-phase counterpart will also be developed.

The synthesis and evaluation of libraries will increasingly be used not only to study ligand−receptor interactions but also 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.

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