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HIGHLY EFFICIENT AND VERSATILE SYNTHESIS OF LIBRARIES OF CONSTRAINED β-STRAND MIMETICS

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Abstract: The general approach of using a bicyclic template to produce inhibitors of the protease superfamily of enzymes has been investigated. The Diels–Alder cycloaddition reaction on solid support has been found to be highly efficient for the synthesis of libraries of compounds that mimic the β-strand secondary structure of proteins. Several potent and selective inhibitors of proteases have been discovered.

In recent years there has been much effort focused on improving combinatorial chemistry methods and expanding the scope of the techniques to include a wide array of structural diversity.[1] The strengths of combinatorial chemistry as a research and discovery tool are becoming more apparent. One of the driving forces behind the explosion of new combinatorial methods is the potential for the rapid discovery and development of new bioactive compounds.[2] Nature, through the utilization of twenty amino acid side chains, displayed on a limited array of topological templates (i.e., common secondary structure motifs; reverse turns, β-strands, and α-helices) provides the elements required to delineate ligand-receptor and enzyme substrate/inhibitor interactions for a multitude of processes. We have been involved in a program to extend combinatorial methods for the production of nonpeptide compounds that mimic these peptide secondary structures.[3] The ability to rapidly produce numerous compounds that incorporate not only the twenty common amino acid side chains but also novel side chain moieties displayed on a template that mimics the secondary structures of peptides has been the focus of our efforts. In this communication, we describe methods used to produce libraries of compounds that mimic an extended β-strand structural motif.[4]

Limited proteolysis is an important regulatory event which is controlled in vivo by the constant interplay between proteolytic enzymes and their endogenous proteolytic inhibitors (i.e., serpins, Kunitz domain inhibitors). Examination of numerous X-ray crystal structures has highlighted the fact that an extended strand motif is uniformly adopted by the inhibitor/pseudo-substrate in the active site.[5] We have developed a series of templates that mimic the extended β-strand secondary structure of peptide substrates bound to their cognate enzymes within the protease superfamily. These templates were designed with the goal of developing a versatile scaffold on which a wide variety of pharmacophore units could be rapidly introduced. Our initial work produced a series of thrombin inhibitors of the general structure shown below (Figure 1). Although these inhibitors were

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both potent and selective, their synthesis did not allow for the rapid introduction of diverse functionality.

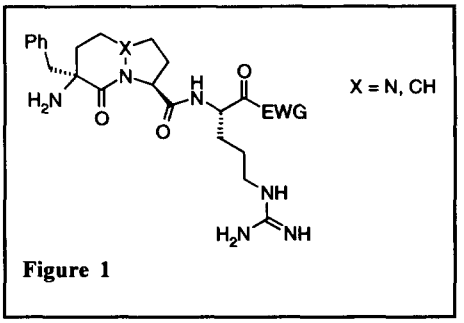


Figure 1

The template above, however, served to demonstrate the concept of utilizing a compound with a bicyclic structure to mimic an extended strand. Through comparison of molecular models of a variety of constrained templates with the peptide backbone of an idealized β-strand, we concluded that other bicyclic structures could meet the spatial requirements of a dipeptide mimetic.[6] Monte Carlo conformational searches were carried out for the template above as well as the bicyclic structures shown in Figure 2 using BATCHMIN version 5.5 (Columbia University, 1997) with MMFFs/GBSA water force field parameters. The results were compared with an ideal anti-parallel β-strand at seven back-bone atom position (N^1, $C_\alpha{}^1$, C^1, O^1, N^2, $C_\alpha{}^2$, C^2) or four hydrogen bonding atoms (N^1, C^1–O^1, N^3) which are found in many X–ray crystal structures to form three critical hydrogen bonds at the active site of serine proteases in antiparallel fashion. The best conformers for the β–strand were found within 1.2 kJ/mol of the global energy minimum for all of the templates. RMSD values from the ideal β–strands are 0.40 (0.29) Å, 0.37 (0.24) Å, and 0.51 (0.36) Å at 7 (4) atom positions for the templates **1**, **2**, and **3**, respectively.

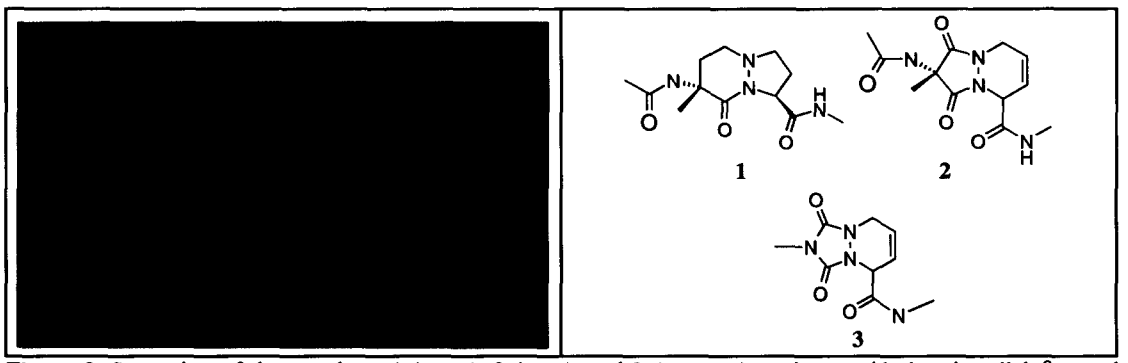
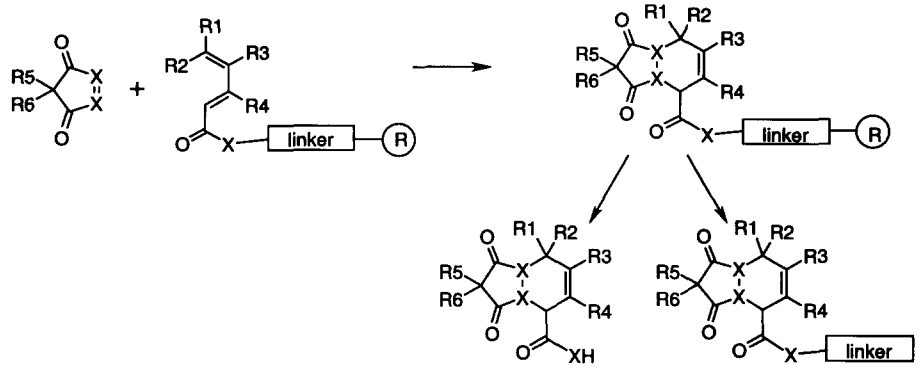


Figure 2. Stereoview of the templates **1** (green), **2** (cyan), and **3** (magenta) against an ideal antiparallel β-strand (red).

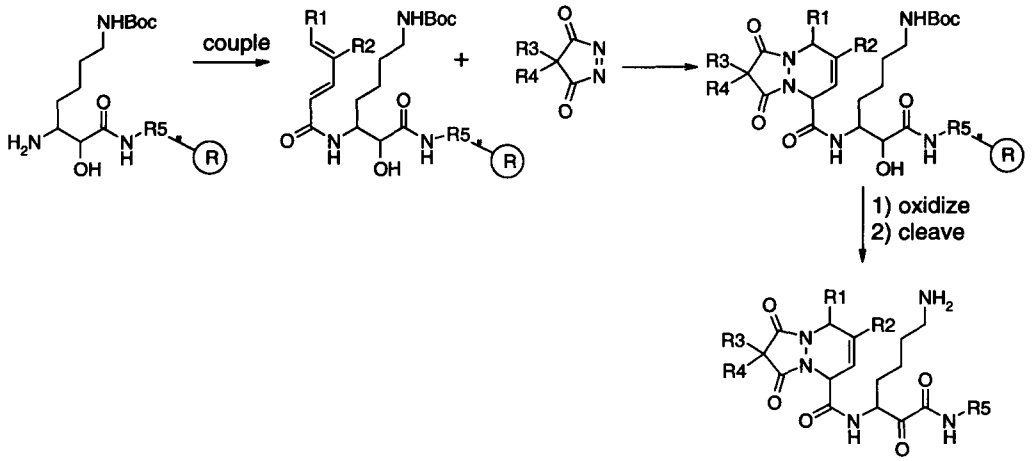
We next turned our attention to the synthetic aspects of template formation. With the goal of taking full advantage of the benefits of solid-phase organic synthesis while using a modular synthetic approach, we began a search for new bicyclic templates that could be constructed rapidly on solid support. Perhaps, the most efficient method for the synthesis of bicyclic compounds is the Diels–Alder (D–A) cycloaddition reaction, however, the use of this method on solid support had not been extensively investigated.[7] We sought to incorporate D–A partners that would allow for a high degree of structural diversity in the synthesized templates as well as high efficiency and reproducibility for use on solid phase. To this end, we recently reported the results of a study of the solid-phase synthesis of dienes.[8] Scheme 1 illustrates the general approach that we have implemented. The attractive features of this approach are the ability to rapidly introduce several different diversity elements

(R1–R6) within the template as well as the ability to link the template to a variety of components. Additionally, any of the groups attached to the template may include a chemically reactive functional group that can be further elaborated.



Scheme 1

Our initial templates in this series were derived from D–A reactions between 4,4-disubstituted-3,5-pyrazolinediones, generated in situ by oxidation of the corresponding pyrazolidinediones, and substituted pentadienoic acids (Scheme 2). This system incorporates as many as five diversity elements within the template



Scheme 2

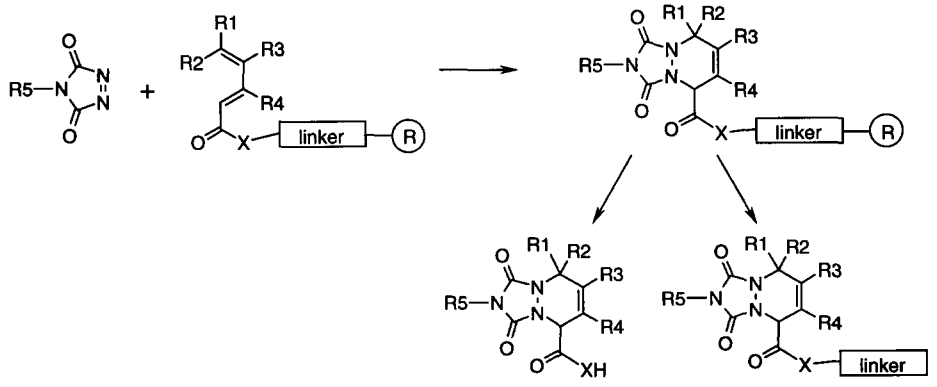
itself and two peripheral attachment points for the incorporation of additional diversity. The pyrazolidinediones were synthesized in solution[9] and oxidized in the presence of the dienoic amide resin with bis(trifluoroacetoxy)iodobenzene.[10] The D–A reaction gives the expected *cis* isomer but is nonstereoselective with respect to the lysine side chain. The synthetic products were evaluated as mixtures of diastereomers. The example presented in Scheme 2 demonstrates utilization of this methodology for the production of a 100 member library that produced six compounds with thrombin $K_i < 20$ nM (Table 1).

Table 1

R1	R2	R3	R4	R5	Thrombin K_i (nM)
H	CH_3	$PhCH_2$	$PhCH_2$	4-$H_2NCOPhCH_2CH_2$	1.89
H	CH_3	$(CH_3)CHCH_2$	$(CH_3)CHCH_2$	4-$H_2NCOPhCH_2CH_2$	5.09
H	CH_3	$PhCH_2$	NH_2	4-$H_2NCOPhCH_2$	1.58
$PhCH_2$	H	CH_3CH_2	NH_2	H	4.33
H	CH_3	*n*-butyl	*n*-butyl	4-$H_2NCOPhCH_2CH_2$	0.2
H	CH_3	CH_2=$CHCH_2$	CH_2=$CHCH_2$	4-$H_2NCOPhCH_2CH_2$	18

One of the major drawbacks in this approach is that a rather lengthy and labor intensive synthesis of the pyrazolidinedione component is required. Although a high degree of diversity can be achieved with this template, we have investigated several variations of this Diels–Alder cycloaddition in order to simplify both the chemistry and structure of the templates.

Among the most reactive dienophiles known for the D–A reaction are the 1,2,4-triazolinediones.[11] This dienophile is normally generated in situ by oxidation of the corresponding urazole.[12] Although most of the accounts of 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) reacting with dienoic esters and amides have employed high temperature,[13] we have found that 1,2,4-triazolinediones generally undergo highly efficient D–A cycloadditions at room temperature or below. In fact, the solution-phase reaction of PTAD with pentadienoic acid proceeds at room temperature in 3h to give consistently greater than 90% yields of the cycloadduct. The solid-phase version of this chemistry is also very efficient (Scheme 3). A typical synthetic sequence involving attachment of the linker, coupling of the dienoic acid, D–A cycloaddition and cleavage yields products that are consistenly greater than 90% pure and in high chemical yield.[14] The efficiency of this process allows for the rapid production of high numbers of diverse compounds and has the same advantages as the pyrazolinedione template. The synthesis of urazoles is a two step process and several hundred unique structures may be employed in this synthesis starting with commercially available isocyanates or amines.[15]

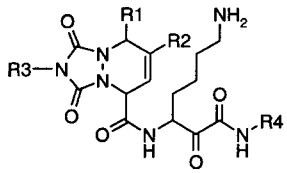


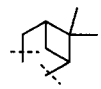
Scheme 3

An alternative to the solution-phase synthesis of urazoles has also been developed. The D–A reaction of 1,2,4-triazolinedione, produced from commercially available urazole, with dienoic amides is also an efficient reaction and has been applied to solid phase (Scheme 3, R5 = H). Alkylation of the resulting unsubstituted urazole template with alkyl halides is capricious, however, Mitsunobu reaction is effective in introducing alkyl groups to the urazole nitrogen.[16] This method circumvents the necessity of synthesizing the urazoles in solution and allows for the introduction of diversity late in the synthetic scheme and on solid support.

The viability of the urazole based templates to function as mimics for the β-strand secondary structure has been demonstrated in numerous enzyme assays of compounds produced from combinatorial libraries employing the methodology described above as well as by X-ray crystallography.[17] A library of 1500 compounds has been constructed and screened against several proteases and a number of potent and selective inhibitors have been discovered. Some representative examples are listed in Table 2.[18]

Table 2



A: R1-R2 

R1	R2	R3	R4	Enzyme	K_i (nM)
H	CH_3	Ph_2CHCH_2	4-$H_2NCOPhCH_2CH_2$	thrombin	0.035
H	H	Ph_2CHCH_2	4-$H_2NCOPhCH_2CH_2$	thrombin	0.3
$(CH_3)_2CHCH_2$	H	$(CH_3)_2CH(CH_2)_2$	4-$H_2NCOPhCH_2CH_2$	trypsin	0.43
$(CH_3)_2CHCH_2$	H	4-MeOPh	4-$H_2NCOPhCH_2CH_2$	trypsin	0.28
H	CH_3	Ph_2CH	4-$H_2NCOPhCH_2CH_2$	tryptase	5.9
MeO_2C	$PhCH_2CH_2$	2,5-di-FPhCH_2	4-$H_2NCOPhCH_2CH_2$	kallikrein	21
A	A	3,4-di-ClPhCH_2	4-$H_2NCOPhCH_2CH_2$	kallikrein	31

In order to take further advantage of the synthetic potential of the D–A approach to the synthesis of bicyclic templates on solid phase, we have also investigated the use of maleimides. These are considerably less reactive than the dienophiles mentioned above and require heating (80–100 °C) for several hours to achieve complete reaction. We are currently investigating this use of maleimides in the solid-phase D–A reaction in more detail in order to determine the scope and limitations more specifically.

The use of the D–A cycloaddition in solid-phase organic synthesis is an important addition to the current techniques available for combinatorial chemistry. We have used the methods described above to produce thousands of compounds of high purity in parallel combinatorial synthesis on solid phase. From these libraries of compounds, several potent and selective inhibitors of a wide range of serine proteases (factors VIIa, Xa and XIa, uPA, and tryptase) as well as cysteine, metallo and aspartyl proteases have been discovered. That minor variation in the chemical structure of these compounds produces inhibitors with specificity for distinct enzymes, validates the generic approach of using a common template for a broad superfamily of enzymes. We

are currently using this technology in 96-well format to produce thousands of inhibitors in a high throughput fashion to discover inhibitors of a variety of proteolytic enzymes as well as members of other superfamilies (protein kinases, etc.) which recognize their substrates in extended strand conformations.

References and Notes

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10. We have found bis(trifluoroacetoxy)iodobenzene to be an excellent oxidant for diacylhydrazides, in general. Oxidation using iodobenzene diacetate and lead tetraoxide (ref 9) were slower and and gave lower yields of D–A adduct. For oxidation of diacylhydrazides using iodobenzene diacetate see: Moriarty, R. M.; Prakash, R. M. I.; Penmasta, R. *Synth. Comm.* **1987**, 409.
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14. The linker and aminoacid analogues were coupled using standard Fmoc technology and the dienoic acid coupled with PyBOP/HOBt. For 60–90 mg of such resin with ca. 0.4 mmol/g loading the remaining synthetic manipulations are as follows: A solution of urazole in DMF (0.5 mL of a 0.4 *M* solution) is treated with a solution of [Bis(trifluoroacetoxy)iodo]benzene in DMF (0.5 mL of a 0.4 *M* solution). The resin is treated with this solution and the resulting mixture is shaken for 2 h. The mixture is filtered and the resin washed with DMF (6 × 1 mL) and CH_2Cl_2 (6 × 1 mL). The CH_2Cl_2 wet resin is treated with a solution of the Dess-Martin reagent in DMSO (1 mL of a 0.25 *M* solution) and the mixture is shaken for 90 min. The mixture is then filtered and the resin washed with DMSO (3 × 1 mL) and CH_2Cl_2 (6 × 1 mL). The CH_2Cl_2 wet resin is treated with 95:5 TFA:H_2O (1 mL) and the mixture is agitated periodically. After 90 min the mixture is filtered and the supernatant is collected. The resin is washed with additional TFA (2 × 0.5 mL) and the combined filtrates are concentrated in vacuo. The residue is then reconstituted with a few drops of TFA and Et_2O is added to precipitate the product. The mixture is cooled (ice bath or freezer) briefly and then centrifuged. The supernatant is decanted and discarded. The product is reconstituted with 1:1 CH_3CN:H_2O (ca. 3 mL), frozen and lyophilized.
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18. All of the inhibitors listed in Table 2 have >5-fold selectivity for the enzyme listed over the others except for the kallikrein inhibitors which were slightly more potent for inhibition of trypsin.