Combinatorial Organic Synthesis of Highly Functionalized Pyrrolidines: Identification of a Potent Angiotensin Converting Enzyme Inhibitor from a Mercaptoacyl Proline Library

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Applications of combinatorial chemistry and other multiple synthesis technologies promise to revolutionize the way in which new medicinally active compounds are discovered and developed. Recently, particularly intense interest has been directed toward methods for generating libraries of nonpolymeric, small organic molecules by solid-phase synthesis techniques.[1] Here we describe the adaptation of a versatile synthesis of functionalized pyrrolidines, exploiting a 1,3-dipolar cycloaddition reaction of resin-bound azomethine ylides, to the preparation of a combinatorial library of ~500 mercaptoacyl prolines. By screening this library against angiotensin converting enzyme (ACE), we have identified an unusually potent inhibitor of this therapeutically significant metalloprotease.

Azomethine ylides are 1,3-dipoles that undergo regio- and stereoselective cycloadditions to olefin and acetylene dipolarophiles to afford pyrrolidine and pyrroline derivatives in typically excellent yields.[2] The Lewis acid promoted ionization of α-amino acid ester aldimines represents one of the mildest routes to azomethine ylides, and the particularly facile additions of these N-metalated dipoles to electron-deficient olefins[3] (e.g., acrylates, cinnamates, conjugated enones, maleimides, etc.) make this an attractive reaction sequence to explore using resin-supported substrates.[4]

Several commercially available polystyrene peptide synthesis resins preloaded with Fmoc-protected amino acids were investigated as supports for solid-phase pyrrolidine synthesis. Piperidine deprotection provided the α-amino esters, which smoothly underwent room temperature condensation reactions with aromatic and heteroaromatic aldehydes in neat trimethyl orthoformate as solvent[5] to afford the resin-bound aryl imines in almost quantitative yields (see Figure 1). The most satisfactory conditions for conducting solid-phase cycloadditions of metalloazomethine ylides to electron-poor olefins were found to be those favored by Grigg and Tsuge for the corresponding reactions in homogeneous solution.[3] Optimization of this synthetic scheme was greatly facilitated by the use of our recently disclosed technique of rapid gel-phase ^{13}C NMR analysis, which permits the convenient and nondestructive monitoring of the progress of reactions on resins by employing selected ^{13}C-enriched building blocks in the synthesis.[6] For example, Schiff bases formed from the reaction of various resin-

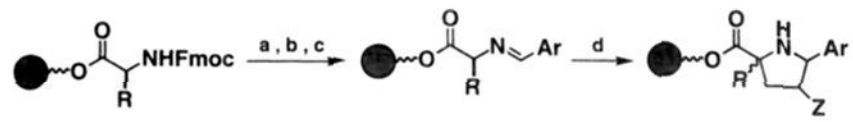

Figure 1. Solid-phase synthesis of pyrrolidines. Resin is TentaGel AC or Sasrin: (a) 20% piperidine in DMF, 20 min; (b) 1 M ArCHO in $CH(OMe)_3$, 4 h; (c) Ac_2O, NEt^iPr_2, 15 min; (d) 1 M olefin, 1 M $AgNO_3$, 1 M NEt_3 in MeCN, 8 h.

bound amino acids with $Ph^{13}CHO$ showed singlet resonances for the imine carbon around 160−165 ppm, which on cycloaddition were replaced by resonances for C-5 of the pyrrolidine ring at ~60−70 ppm.

Characterization of representative product prolines was achieved by conventional means after TFA-mediated cleavage from the resins. For six representative pyrrolidines prepared by silver-catalyzed cycloadditions, yields of purified material ranged from 50 to 80% and diastereoselectivities from 2.5:1 to greater than 10:1 (see supporting information); e.g., 2-benzyl-5-(2′-methoxyphenyl)-2,4-pyrrolidinedicarboxylic acid 4-methyl ester was formed from phenylalanine, *o*-methoxybenzaldehyde, and methyl acrylate as a 4:1 mixture of diastereomers in 54% isolated yield. Products arising from endo-selective cycloadditions to the W-configured (syn) azomethine ylides predominate from the solid-phase reactions, presumably as a consequence of chelation control in the transition states.[2a] As with solution-phase additions, poorer selectivity is seen in cycloadditions to dipolarophiles lacking a carbonyl substituent (e.g., acrylonitrile).[7]

Since functionalized prolines and proline analogs are frequently found as the C-terminal residues in numerous ACE inhibitors,[8] we were interested in applying our solid-phase chemistry to generate a combinatorial library of mercaptoacyl prolines as analogs of the clinically important antihypertensive agent, captopril. The library was prepared by the split synthesis method[9] using four amino acids, four aldehydes, five olefins, and three mercaptoacyl chlorides as shown in Figure 2. The 240 possible building block combinations were expected to yield more than 480 distinct products since the cycloaddition chemistry does not proceed with complete regio- and stereospecificity, and pyrrolidines derived from reaction of achiral aldehydes and olefins with homochiral amino acids are racemic as the α-carbon stereochemistry is scrambled in the metallodipole intermediate.[10] After cleavage from the resin (10% TFA in CH_2Cl_2) and deacetylation of the protected mercaptoacyl proline products with ethylenediamine, the library was screened for *in vitro* inhibition of ACE[11] as soluble compound pools through four iterations of assay and sublibrary resynthesis. At each step of this deconvolution analysis, the building block affording the most inhibitory pool was selected for the subsequent sublibrary resynthesis (summarized in Figure 3).

This strategy led to the identification of 1-(3′-mercapto-2′-(*S*)-methyl-1′-oxopropyl)-5-phenyl-2,4-pyrrolidinedicarboxylic acid 4-methyl ester (**1**) as a potent ACE inhibitor that incorporates the mercaptoisobutyryl side chain found in captopril. An HPLC analysis of an independent preparative solid-

(1) For a recent review in two parts, see: (a) Gallop, M. A.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gordon, E. M. *J. Med. Chem.* **1994**, *37*, 1233−1251. (b) Gordon, E. M.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gallop, M. A. *J. Med. Chem.* **1994**, *37*, 1385−1401.

(2) For reviews, see: (a) Tsuge, O.; Kanemasa, S. *Adv. Heterocycl. Chem.* **1989**, *45*, 231−349. (b) Grigg, R. *Chem. Soc. Rev.* **1987**, *16*, 89−121.

(3) (a) Barr, D. A.; Grigg, R.; Gunaratne, H. Q. N.; Kemp, J.; McMeekin, P.; Sridharan, V. *Tetrahedron* **1988**, *44*, 557−570. (b) Tsuge, O.; Kanemasa, S.; Yoshioka, M. *J. Org. Chem.* **1988**, *53*, 1384−1391.

(4) 1,3-Cycloaddition reactions between nitrile oxides and olefins or acetylenes have been successfully conducted on polymer supports using either the ylide or dipolarophile as the immobilized reagent: (a) Beebe, X.; Schore, N. E.; Kurth, M. J. *J. Am. Chem. Soc.* **1992**, *114*, 10061−10062. (b) Pei, Y.; Moos, W. H. *Tetrahedron Lett.* **1994**, *32*, 5825−5828.

(5) Look, G. C.; Murphy, M. M.; Campbell, D. A.; Gallop, M. A. *Tetrahedron Lett.* **1995**, *36*, 2937−2940.

(6) Look, G. C.; Holmes, C. P.; Chinn, J. P.; Gallop, M. A. *J. Org. Chem.* **1994**, *59*, 7588−7590.

(7) Interestingly, the distribution of pyrrolidine diastereomers is sensitive to the nature of the resin support and to both the identity and concentration of the Lewis acid catalyst used. Importantly, the product distribution is highly reproducible for a given set of conditions, and we routinely run reactions on TentaGel supports using 1 M $AgNO_3$ in MeCN or 2 M LiBr in THF as catalyst.

(8) (a) Petrillo, E. W.; Ondetti, M. A. *Med. Res. Rev.* **1982**, *2*, 1−41. (b) *Drugs Future* **1989**, *14*, 336−341.

(9) Furka, A.; Sebestyen, F.; Asgedom, M.; Dibo, G. *Int. J. Pept. Protein Res.* **1991**, *37*, 487−493.

(10) From an extensive series of optimization experiments it was concluded that the solid-phase cycloaddition reaction proceeds satisfactorily for all but the most sterically hindered coupling partners in this library (e.g., a pyrrolidine product was not isolated from reaction of leucine + 2-((*tert*-butyldimethylsilyl)oxy)benzaldehyde + methyl methacrylate).

(11) Cheung, H. S.; Cushman, D. W. *Biochim. Biophys. Acta* **1973**, *293*, 451−463.

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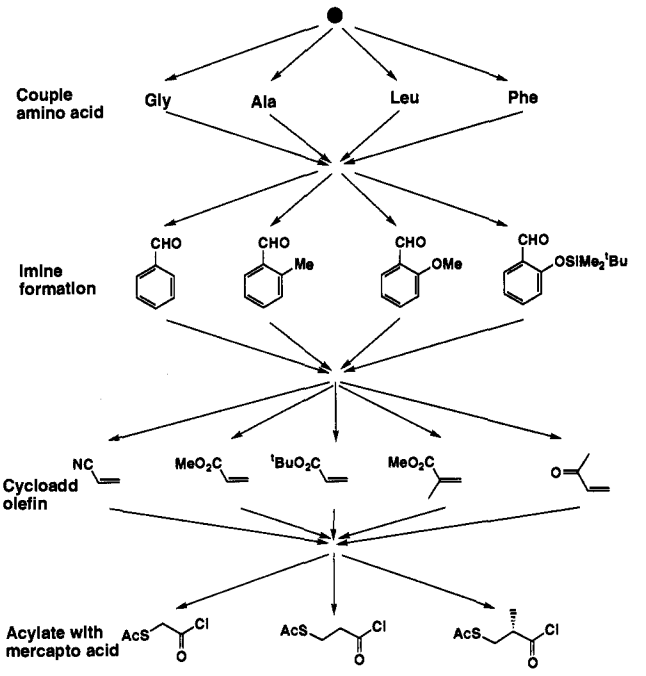

Figure 2. Split/pool combinatorial synthesis of a mercaptoacyl proline library.

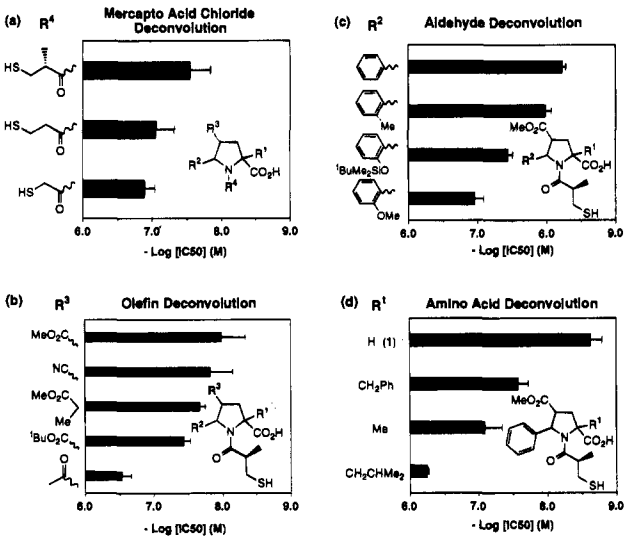

Figure 3. Identification of a potent ACE inhibitor by deconvolution analysis. The inhibitory activity of pools of decreasing complexity (a–d) produced by iterative resynthesis is shown, where IC_{50} refers to the total concentration of the pool (determined as free thiol by Ellman assay) giving 50% inhibition of hydrolysis of Hip-His-Leu (1 mM) by rabbit kidney ACE (2 nM).

phase synthesis of the S-acetylated precursor (**2**) (Figure 4) indicated that the crude product predominantly consisted of an equimolar mixture of two components, characterized as diastereomers derived from a racemic proline intermediate. These isomers were purified, and the relative stereochemical relationships between the 2-, 4-, and 5-pyrrolidine substituents (all syn)

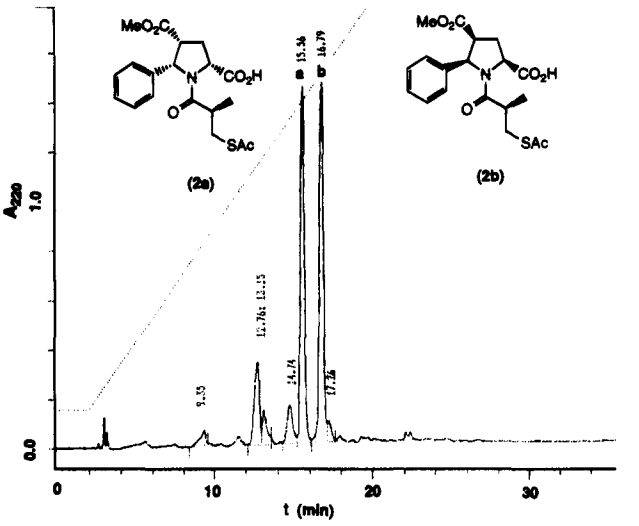

Figure 4. HPLC analysis of ACE-inhibitory mercaptoacyl proline precursor **2** from preparative solid-phase synthesis.

were established by correlation with literature ^{1}H NMR data,[3b] confirming that the proline ring arose through an endo-cycloaddition reaction. Biological assay of the individual diastereomers after deacetylation facilitated assignment of the absolute configurations at C-2 of the prolines in these compounds. The thiol derived from the earlier eluting isomer (**2a**) displayed very weak ACE-inhibitory activity ($K_i > 1$ μM) and, on the basis of extensive structure–activity data,[8a] is consistent with a 2-*R* configuration in this diastereomer. By contrast, the later eluting 2-*S* pyrrolidine isomer (**2b**) provided an exceedingly potent ACE inhibitor ($K_i \sim 160$ pM), approximately 3-fold more active than captopril in this assay[12] and among the highest affinity thiol-containing ACE inhibitors yet described.

In summary, we have demonstrated the use of combinatorial organic synthesis to efficiently prepare numerous analogs of a known pharmacophoric structure, and that highly active enzyme inhibitors can be identified from libraries of modest diversity by a deconvolution analysis. Though successful, the inherent limitations of this assay strategy (discussed in ref 1b) together with the idiosyncracies of the cycloaddition product distribution make it quite possible that the most potent ACE inhibitor in this library remains to be discovered. Screening strategies designed to address these uncertainties will be reported soon.

Acknowledgment. We thank Steven Ferla for assistance in the synthesis and characterization of several pyrrolidines.

Supporting Information Available: Detailed experimental procedures for solid-phase pyrrolidine synthesis, library construction and biological assay (21 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instruction.

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(12) Bull, H. G.; Thornberry, N. A.; Cordes, M. H. J.; Patchett, A. A.; Cordes, E. H. *J. Biol. Chem.* **1985**, *260*, 2952–2962.