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General method for the synthesis of cyclic peptidomimetic compounds

Michael A. Walker* and Timothy Johnson

Bristol-Myers Squibb Pharmaceutical Research Inst., 5 Research Parkway, Wallingford, CT 06492, USA

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Abstract—A number of cyclic peptides, wherein the *i* and *i*+1 residue side chains are joined by an alkyl linker and a Weinreb amide is present at the C-terminus, were synthesized. Using LiCl to solubilize these peptides in THF the C-terminus can be readily converted to an activated carbonyl such as an aldehyde or ketone. © 2001 Elsevier Science Ltd. All rights reserved.

Cyclic peptides have been exploited as exploratory tools and potential drug candidates in medicinal chemistry because they provide rigid scaffolds for locking short peptides into specific configurations.[1,2] Therefore, it is not surprising to find that this approach has been used in the area of protease inhibitor design since it is generally accepted that substrate binding to the enzyme takes the form of a β-sheet. However, not only does cyclization provide conformational rigidity, in certain cases cyclization can have an effect on metabolic stability, bioavailability and pharmacokinetics.[3]

We became interested in cyclic peptides as potential trypsin/chymotrypsin based serine protease inhibitors. Published X-ray structures of peptides (or peptidomimetics) bound to the substrate binding domain of serine proteases clearly show that a tether between the P_1 and P_3 side chains can easily be accommodated. For example, the X-ray structure of turkey ovomucoid inhibitor complexed to elastase clearly shows that the P_1 and P_3 side chains (Schecter and Berger[4] nomenclature) are separated by no more than 7.5 Å and that the approach is unimpeded by enzyme residues. In addition, P_2 and P_4 are oriented on the same face of the 'substrate' β-strand, facing away from the binding groove of the enzyme.

To be useful, and to further extend the current state-of-the-art we needed a synthesis, which was not only convenient but also flexible enough to allow the rapid assembly of a diverse set of structures. Of the available methods for synthesizing cyclic peptides we focused on the method of Mosberg, wherein the cyclic peptide is formed by linking two cysteines via a linker (cf. Fig. 1).[5] This methodology has been examined by others[6] in the area of aspartyl protease inhibitors; however, to apply this to the synthesis of a serine protease inhibitor

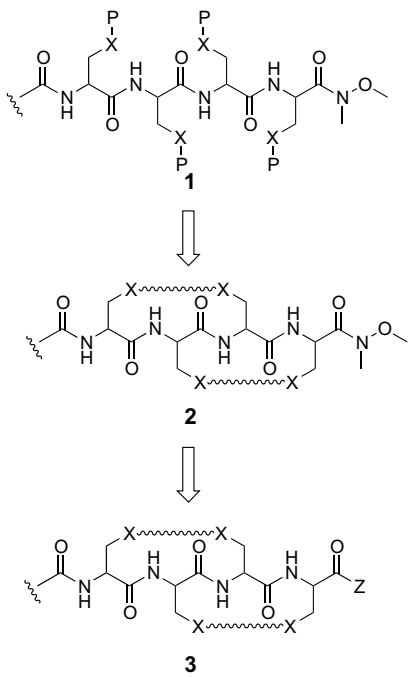

Figure 1. General plan for peptide and cyclic peptide serine protease inhibitors (X = S, P = protecting group and Z = H or heterocycle).

Keywords: cyclic peptide; Weinreb amide; peptidomimetic, thiazole, cysteine and aldehyde.

* Corresponding author. Fax: 203-677-7702; e-mail: michael.a.walker@bms.com

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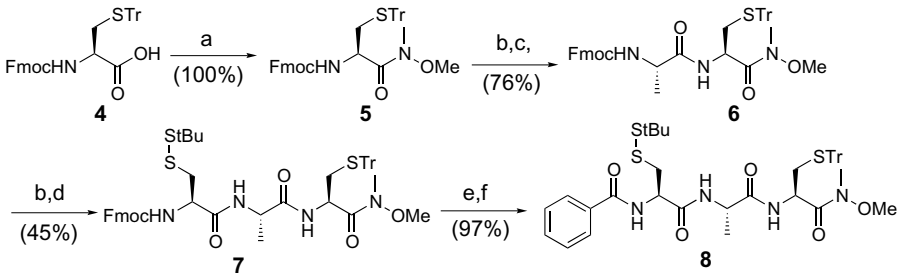
we needed to modify the method to allow the introduction of a 'serine-trap' at the C-terminus of the peptide. To complicate matters further we desired a synthesis which would save the C-terminal modification until the end of the sequence in order to allow us the convenience of examining SAR at this site without having to repeat the whole route. To the best of our knowledge this sequence has never been disclosed before presumably, as we were to learn, due to the poor solubility of peptides in THF. Therefore, this paper not only describes the synthesis of a number of cyclic peptides but includes new methodology for their subsequent transformation into potential serine protease inhibitors.

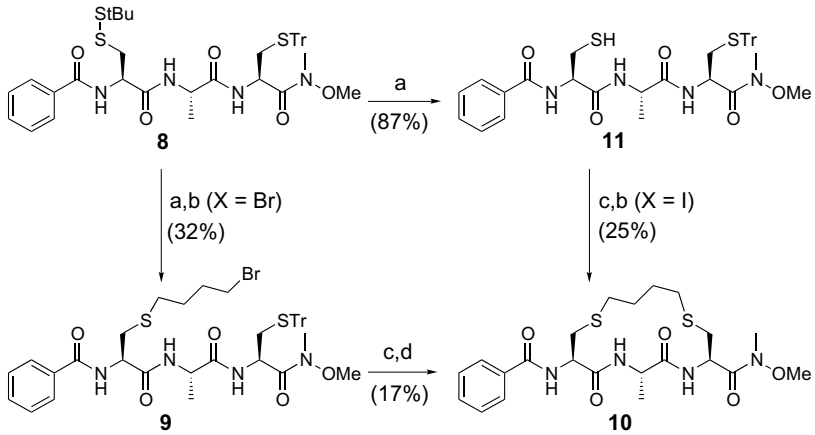
A representative synthesis of the peptide cyclization precursor is shown in Scheme 1. The synthesis commenced with the introduction of a Weinreb amine in order to set up the C-terminal modification envisioned later in the sequence. Standard Fmoc peptide synthesis procedures were then used to introduce the remaining amino acids in the sequence. The less than optimal yield encountered in the transformation of **6** to **7** is due principally to the formation of the piperizane byproduct during the deprotection reaction. This side reaction was eliminated when piperidine/DMA was used to remove the Fmoc group during the synthesis of subsequent analogues. The N-terminus was capped as the corresponding benzoyl amide.

The synthesis of the macrocycle is demonstrated for compound **10** (Scheme 2). In our first attempt at forming this ring we intended to take advantage of the orthogonal cysteine protecting groups by carrying out the cyclization in a two-step sequence. Thus, the *t*-butyl dissulfide group was removed with Bu_3P and the resulting thiol reacted with 1,4-dibromobutane to yield **9**. The yield of this alkylation reaction was much lower than expected due to the formation of the bis-alkylated byproduct. Nonetheless this intermediate was carried on to cyclic peptide **10** by first treating with TFA/Et_3SiH to remove the trityl protecting group followed by DBU to effect the cyclization. Although unoptimized, the yield of this step was also disappointingly low. In the end we found that we could achieve a slightly better yield by removing both protecting groups prior to forming the macrocycle. This latter method was used to introduce a wide variety of linkers as shown in Fig. 2.

Modification of the C-terminus is shown is Scheme 3 for the synthesis of **12a** and **12b**.[7] As mentioned previously, we found that the cyclic tripeptide was insoluble in THF, the solvent of choice for air and moisture sensitive organometallic reagents. This turned out to be a problem with all of the peptides longer than three residues, requiring a general solution. Fortunately, we found that the addition of LiCl, according to the



Scheme 1. (a) 1.25 equiv. $(MeO)MeNH_2Cl$, 3.0 equiv. iPr_2NEt, 1.5 equiv. HBTU, 1.0 equiv. $BtOH{\cdot}H_2O$ DMA; (b) 1:1 Et_2NH/CH_2Cl_2; (c) 1.5 equiv. Fmoc-Ala-OH·H_2O, 3.0 equiv. iPr_2NEt, 1.5 equiv. HBTU, 1.0 equiv. $BtOH{\cdot}H_2O$, DMA; (d) 1.25 equiv. Fmoc-Cys(StBu)-OH, 3.0 equiv. iPr_2Net, 1.5 equiv. HBTU, 1.0 equiv. $BtOH{\cdot}H_2O$, DMA; (e) 50 equiv. piperidine, DMA; (f) 1.2 equiv. BnCOCl, CH_2Cl_2, satd $NaHCO_3$ (aq.).



Scheme 2. (a) 4.0 equiv. Bu_3P, THF/H_2O; (b) $X\text{-}(CH_2)_4\text{-}X$, DBU, PhH; (c) 2.0 equiv. Et_3SiH, 3:1 CH_2Cl_2/TFA; (d) DBU, PhH.

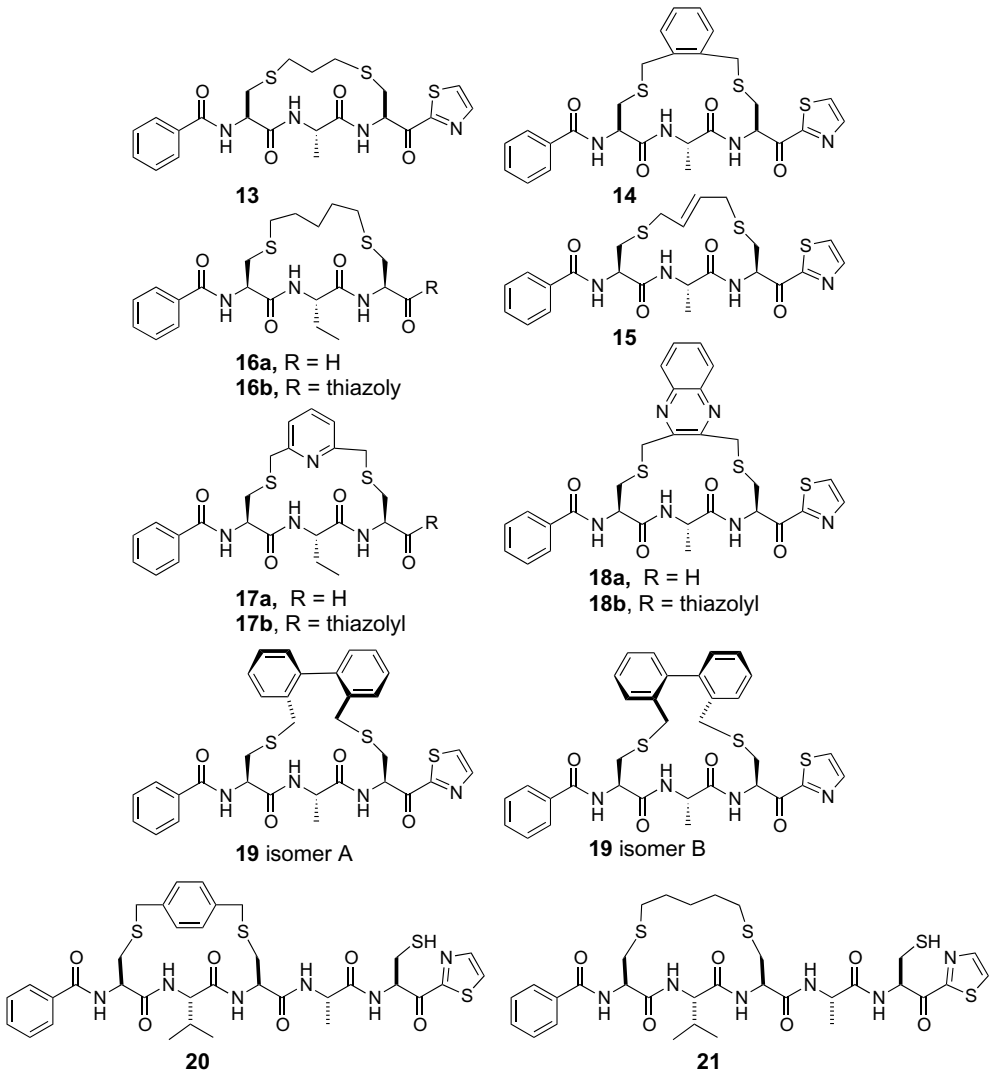
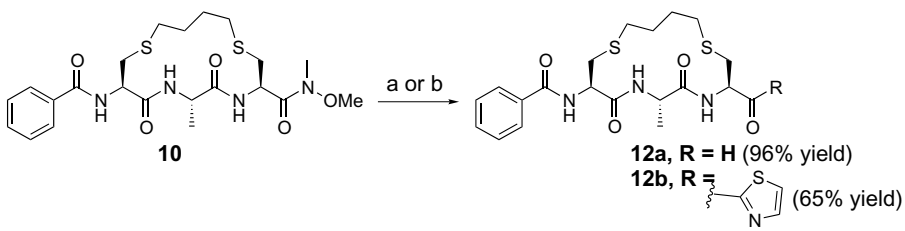


Figure 2. Cyclic peptiditomimetics.



Scheme 3. (a) 5.0 equiv. $LiAlH_4$, 5.0 equiv. LiCl; (b) 8.0 equiv. thiazole, 8.0 equiv. *n*-BuLi, 5.0 equiv. LiCl.

procedure first described by Seebach,[8] resulted in the dissolution of the tripeptide Weinreb intermediates. This appears to be one of the few instances we are aware of where formation of an activated carbonyl has been accomplished as the last step in the synthesis of a peptide-based serine protease inhibitor.[9] Normally, the activated carbonyl functionality is introduced prior to the synthesis of the peptidic portion of the molecule. Formation of the activated carbonyl at such an early stage can potentially be problematic due to epimerization at the corresponding α-position. In our case, only one diastereomer was observed in the synthesis of **12a** and **12b**. Moreover, the current methodology is more versatile since a variety of C-terminal modifications can be rapidly explored.

The same chemistry was applied to the synthesis of the related tripeptide analogues shown in Fig. 2. In our view the linker not only functions as a device for conformational control but also serves as a platform for probing regions adjacent to the substrate-binding pocket. Thus, in the current study we have employed linkers which contain aryl functionality such as phenyl (**14**), pyridyl (**17**) and quinazolinyl (**18**). The cyclic

compounds **19** (isomers A and B) were formed as separable diasteromers derived from a single reaction. We believe these compounds to be atropisomers due to rotational hindrance about the bond connecting the two phenyl groups. Lastly we have been able to apply the same chemistry to the synthesis of pentapeptide analogues **20** and **21**.

In conclusion we have presented a method for the rapid synthesis of a diverse set of cyclipeptides as potential inhibitors of serine protease. Moreover, we have successfully applied the peptide solubilizing method of Seebach in the introduction of the C-terminal activated carbonyl late in the synthetic sequence. It is interesting to note that shortly after we completed our investigation a polymer bound version of the Weinreb amine became commercially available (Novabiochem). This could be of use in the synthesis of libraries based on our methodology.

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7. (a) Procedure for the synthesis of **12a**: Thiazole (23 μL, 0.32 mmol) was dissolved in 1.0 of THF under N_2. The resulting solution was cooled to −78°C and 130 μL of 2.5 M *n*BuLi (THF) added dropwise to form a slurry. In a separate flask lithium chloride (8.0 mg, 0.19 mmol), dried overnight at 150°C under vacuum, was combined with peptide **10** (20.0 mg, 40.3 mmol) and dissolved in 0.5 mL of THF. The peptide solution was added to the thiazole-anion mixture by cannula and the resulting mixture stirred for 45 min. Saturated NH_4Cl was added to quench the reaction. EtOAc was added and the mixture transferred to a separatory funnel. The organic layer was separated, dried over Na_2SO_4, filtered and solvent removed under vacuum. The crude product was purified by flash column chromatography (SiO_2, 96:4 CH_2Cl_2/EtOH) to yield 13 mg (65% yield) of **12a**. (b) Procedure for the synthesis of **12b**: Peptide **10** (30 mg, 60 mmol) was combined with lithium chloride (13 mg, 310 mmol) which had been dried overnight at 150°C under vacuum, and the mixture dissolved in 1.0 mL of THF. The solution was cooled to −78°C and 300 μL of 1 M LiAlH (THF) added dropwise. The resulting mixture was stirred for 0.5 h, then quenched with 1 M $KHSO_4$. The reaction was worked up as above to yield 25 mg (96% yield) of **12b**.
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