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Synthesis of a conformationally constrained threonine–valine dipeptide mimetic: design of a potential inhibitor of plasminogen activator inhibitor-1

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Abstract—The stereoselective synthesis of a conformationally restricted threonine–valine dipeptide mimetic and its incorporation into a tetrapeptide is described.

Peptides and proteins have wide-ranging biological functions and are important targets for drug discovery. However, the use of peptides as drugs has generally been limited due to their metabolic instability and limited oral bioavailability. One strategy to overcome the inherent instability of the amide bond in peptides is to design peptide mimetics that alter and stabilize the peptide backbone but retain the essential three dimensional pharmacophores necessary to retain receptor recognition and desired biological activity.[1] One common strategy in peptide mimetic design is to restrict the conformational freedom available to the peptide and thus probe the bioactive conformation of the endogenous peptide.[2] In many instances, stabilization of a biologically active conformer can lead to improved selectivity and potency.

Plasminogen activator inhibitor-1 (PAI-1) is an endogenous serine protease inhibitor of both tissue-type plasminogen activator (t-PA) and urokinase plasminogen activator and is an important regulator of vascular fibrinolysis.[3] We previously reported an X-ray structure of a complex of a glucosylated mutant of PAI-1 and two molecules of the inhibitory reactive-center loop peptide N-Ac-TVASS-NH_2 (amino acid residues 333–337).[4] During the course of a program to develop therapeutically useful inhibitors of (PAI-1), we found that the corresponding tetrapeptide, N-Ac-TVAS-NH_2, retained the inhibitory potency of the pentapeptide.[5] Using the X-ray structural information, we designed conformationally restricted peptide mimetics in which the spatial orientation of the side-chains (particularly those oriented towards the interior of the protein, i.e. P14Thr, P12Ala and P10Ser) closely resembled those of the biologically active conformation.

In this paper, we report the synthesis of a conformationally constrained threonine–valine dipeptide mimetic and its incorporation into a tetrapeptide. We chose to design an asymmetric synthesis of **1**, a lactam containing dipeptide mimetic with an appropriate protection scheme that would resemble a threonine containing dipeptide (Fig. 1). Lactams have been shown to be a useful type of conformational constraint in peptide mimetics.[6] Generic dipeptide mimetic **1** contains three

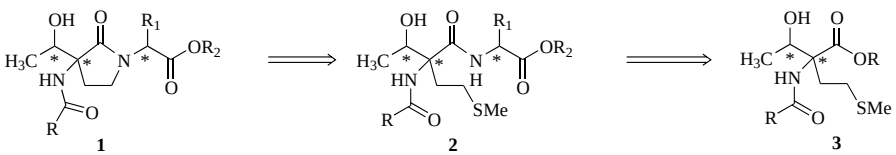

Figure 1.

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stereocenters, one of which is an asymmetric quaternary carbon, a feature which presents a challenge for its synthesis.

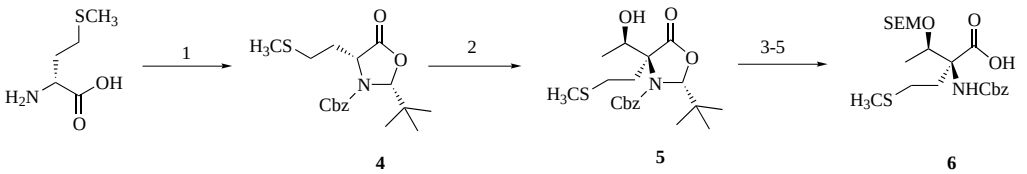
We envisioned lactam **1** being constructed from dipeptide **2** utilizing the lactamization methodology originally developed by Freidinger.[6a] Dipeptide **2** could be prepared from unusual amino acid **3** and presumably any other suitably protected amino acid using standard peptide coupling procedures. Seebach's 'chiral self reproduction' methodology[7] appeared to be well suited for the preparation of the quaternary carbon containing amino acid **3** needed for our synthesis.

D-Methionine was converted to oxazolidinone **4** following a variation of Seebach's original method[7b] (Scheme 1). Seebach's original method incorporated a benzenamide protecting group for the amino acid nitrogen atom; however, the Cbz group was found to be more readily removed in later functional group transformations. Condensation of the lithium enolate of oxazolidinone **4** with acetaldehyde gave **5** as the only diastereomer isolated. The stereochemical assignment of **5** is based upon previous assignments in analogous systems.[7a] Notably aldol product **5** has the same stereochemical configuration as natural threonine. Alcohol **5** was protected as a SEM ether to prevent a retro-aldol reaction in the subsequent base hydrolysis. Unnatural amino acid **6** was obtained after base hydrolysis and careful acidification in 63% yield.

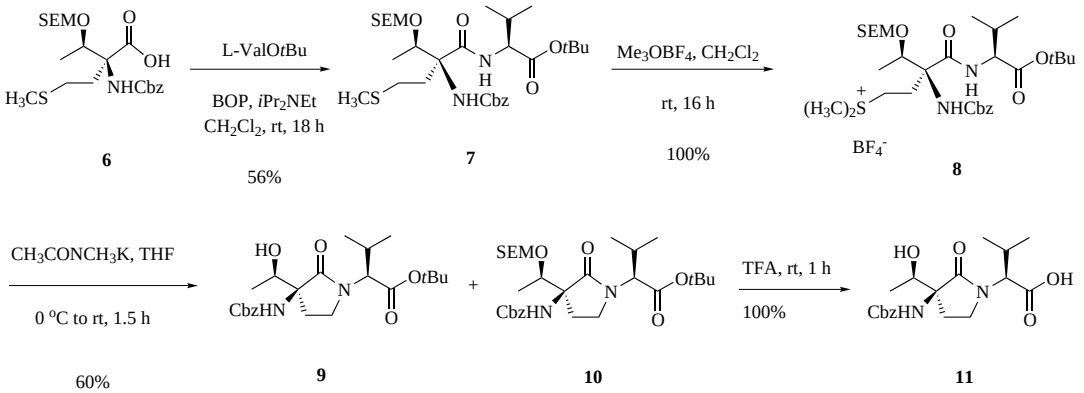
Condensation of quaternary amino acid **6** with L-valine *t*-butylester utilizing the BOP reagent provided dipeptide **7** in 56% yield (Scheme 2). Conversion of **7** to the methylsulfonium salt **8** required for lactamization was unsuccessful after the standard treatment with methyl iodide. The formation of sulfonium salts can be reversible when strong nucleophiles (e.g. I^-) are present in the reaction. However, reaction of **7** with trimethyloxonium tetrafluoroborate at room temperature gave quantitative conversion to sulfonium salt **8**.[8] Cyclization of sulfonium salt **8** was induced by treatment with the potassium salt of *N*-methylacetamide as base to give lactams **9** and **10** without any observable epimerization.[6c] The partial removal of the SEM ether was unexpected but of no consequence in the synthesis. Both **9** and **10** were converted to acid **11** by treatment with TFA.

Dipeptide mimetic **11** was coupled to dipeptide **12** utilizing EEDQ[9] to afford protected tetrapeptide **13** in 67% yield (Scheme 3). Ammonolysis of **13** followed by hydrogenation and acetylation gave **14**, an analog of the lead peptide (TVAS) for biological testing.[10] Unfortunately, **14** possessed an order of magnitude less affinity for PAI-1 than the lead peptide (TVAS).

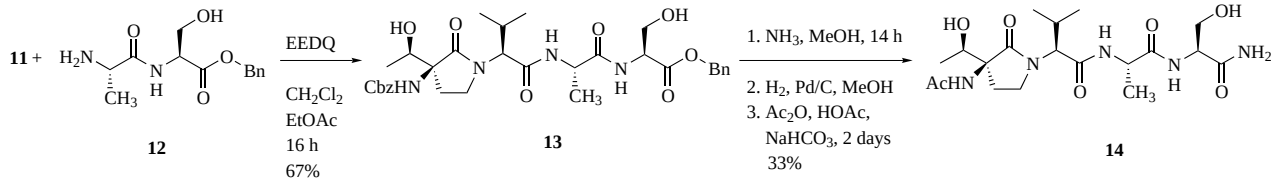
In conclusion, dipeptide mimetic **11** is a versatile conformationally constrained intermediate which can be incorporated into peptides to help define the important components for binding affinity to a receptor. Importantly, using the methodology described in this report, all of the stereocenters in **11** can be controlled by the choice of the starting amino acids.



Scheme 1. *Reagents and conditions*: (1) (i) NaOH, EtOH, rt, evaporate, (ii) pivalaldehyde (1.4 equiv.), CH_2Cl_2, reflux, 5 h, (iii) CbzCl, 0°C to rt, 24 h, 37%; (2) (i) LDA, THF, –78°C, (ii) CH_3CHO (4 equiv.), –78°C, 1 h, 53%; (3) SEMCl, iPr_2NEt, DMAP (cat.), CH_2Cl_2, rt, 24 h; (4) NaOH, MeOH, reflux, 24 h; (5) HCl, pH 2, 63%.



Scheme 2.



Scheme 3.

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9. EEDQ=*N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline. See: Belleau, B.; Malek, G. *J. Am. Chem. Soc.* **1968**, *90*, 1651.
10. Characterization data for key compounds: Compound **9**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (s, 5H), 5.73 (s, 1H), 5.05 (s, 2H), 4.37 (d, J=10.2 Hz, 1H), 4.09 (s, 1H), 3.91 (q, J=6.2 Hz, 1H), 3.66 (m, 1H), 3.57 (m, 1H), 2.45 (m, 1H), 2.1–2.3 (m, 2H), 1.46 (s, 9H), 1.19 (d, J=6.5 Hz, 3H), 1.03 (d, J=6.7 Hz, 3H), 0.98 (d, J=6.7 Hz, 3H); CIMS m/z=435 $(M+H)^+$, 379 $(m-t\text{butyl})^+$. Compound **11**: (300 MHz, $CDCl_3$) δ 7.34 (s, 5H), 6.79 (s, 2H), 5.88 (s, 1H), 5.06 (s, 2H), 4.45 (d, J=10.2 Hz, 1H), 4.01 (s, 1H), 3.60 (m, 2H), 2.50 (m, 1H), 2.25 (m, 2H), 1.21 (d, 3H), 1.07 (m, 6H); CIMS m/z=379 $(M+H)^+$. Compound **14**: ^{1}H NMR (300 MHz, D_2O) δ 4.40 (m, 1H), 4.34 (q, J=7.2 Hz, 1H), 4.24 (d, J=11.1 Hz, 1H), 4.09 (q, J=6.7 Hz, 1H), 3.88 (m, 2H), 3.69 (t, J=9.9 Hz, 1H), 3.58 (dd, J=7.7, 16.5 Hz, 1H), 2.66 (dd, J=7.5, 14.5 Hz, 1H), 2.2 (m, 3H), 1.96 (s, 3H), 1.39 (d, J=7.2 Hz, 3H), 1.13 (d, J=6.4 Hz, 3H), 0.97 (d, J=6.2 Hz, 3H), 0.91 (d, J=6.5 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 175.7, 175.4, 172.2, 171.4, 69.7, 66.2, 63.2, 62.2, 56.3, 50.8, 43.2, 27.3, 24.4, 19.2, 19.0, 17.4, 17.1; FABMS m/z=466 $[M+Na]^+$.