Pergamon

Tetrahedron Letters 42 (2001) 1305–1308

TETRAHEDRON LETTERS

The synthesis of β-strand mimetic templates via regioselective 1,3-dipolar cycloaddition with vinylsulfone

Nobuhiro Fuchi,[a] Takayuki Doi,[a] Takeo Harada,[a] Jan Urban,[b] Bolong Cao,[b] Michael Kahn[c] and Takashi Takahashi[a,*]

[a]*Department of Applied Chemistry, Graduate School of Science and Engineering, Tokyo Institute of Technology, 2-12-1 Ookayama, Meguro, Tokyo 152-8552, Japan*

[b]*Molecumetics Ltd, 2023 120th Avenue NE, Bellevue, WA 98005-2199, USA*

[c]*University of Washington, Department of Pathobiology, SC-38, Seattle, WA 98195, USA*

Received 9 November 2000; revised 29 November 2000; accepted 1 December 2000

Abstract—We have developed a practical synthetic route to constrained β-strand mimetic templates by regioselective 1,3-dipolar cycloaddition of azomethine imines with vinyl sulfone. A small library of β-strand mimetic templates was generated which include potent and selective inhibitors of serine proteases.

The β-strand is a basic protein secondary structure unit and together with helixes and reverse turns constitutes greater than 90% of all protein structure. These secondary structures[1] provide the elements required to delineate ligand–receptor and enzyme substrate/inhibitor interactions for a multitude of processes. The cleavage site of chymotrypsin-like serine proteases and its substrates uniformly adopt an antiparallel β-sheet. The β-strand template **1** reproduces the three-dimensional orientation of the peptide-serine protease substrate, and its hydrogen bond patterns.[2] By attaching a functional group, which forms a tetrahedral intermediate with the hydroxyl group of the corresponding Ser195 residue of the target enzyme (i.e. keto amide, chloromethyl ketone) to the template, these compounds become potent serine protease inhibitors (Fig. 1).

We designed the bicyclic template **1** as a mimetic of the D-Phe-Pro orientation of PPACK[3] (D-Phe-Pro-ArgCH_2Cl) and converted these into a number of potent inhibitors of serine proteases.[4,5] We expected that R^1 substituted phenyl ring and/or the R^2 group on the template could generate selective inhibitory activity for serine proteases. In this paper, we report the synthesis and inhibitory activity of the bicyclic templates[6] **1** having various substituents R^1 on the benzene ring and

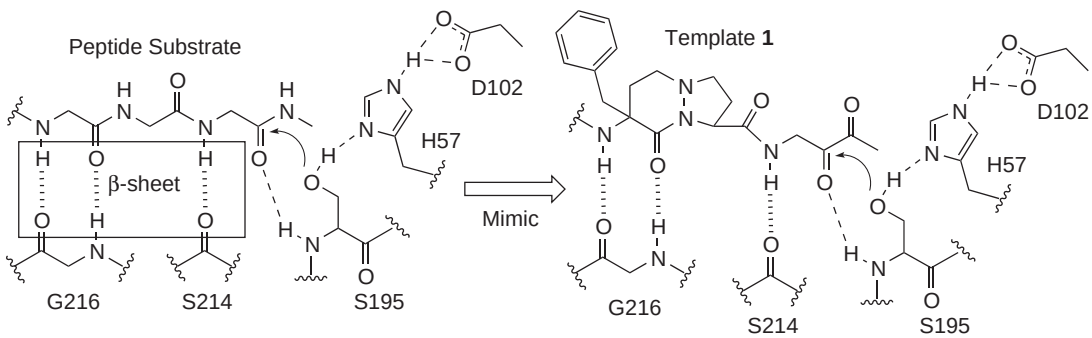


Figure 1.

Keywords: 1,3-dipolar cycloaddition; vinyl sulfone; peptidomimetics; serine protease inhibitor.

* Corresponding author.

0040-4039/01/$ - see front matter

PII: S0040-4039(00)02229-2

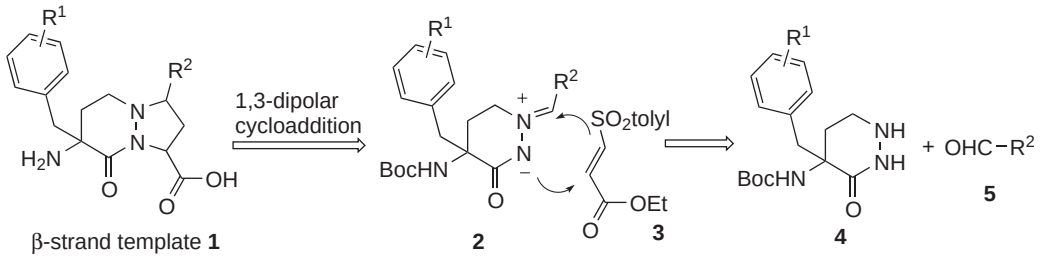


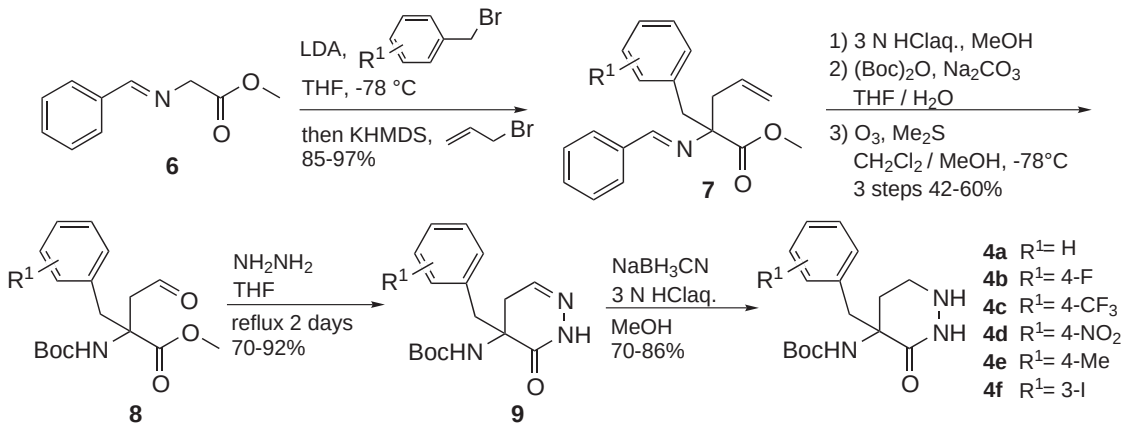
Figure 2. Synthetic strategy of β-strand mimetic template.

R^2 on the bicyclic template. The bicyclic ring can be prepared by regioselective 1,3-dipolar cycloaddition[7–9] of azomethine imines **2** with vinylsulfone **3**. The former can be prepared from hydrazides **4** and aldehydes **5**. The combination of various functionalities R^1 and R^2 on **4** and **5**, respectively, provides various templates **1** (Fig. 2).

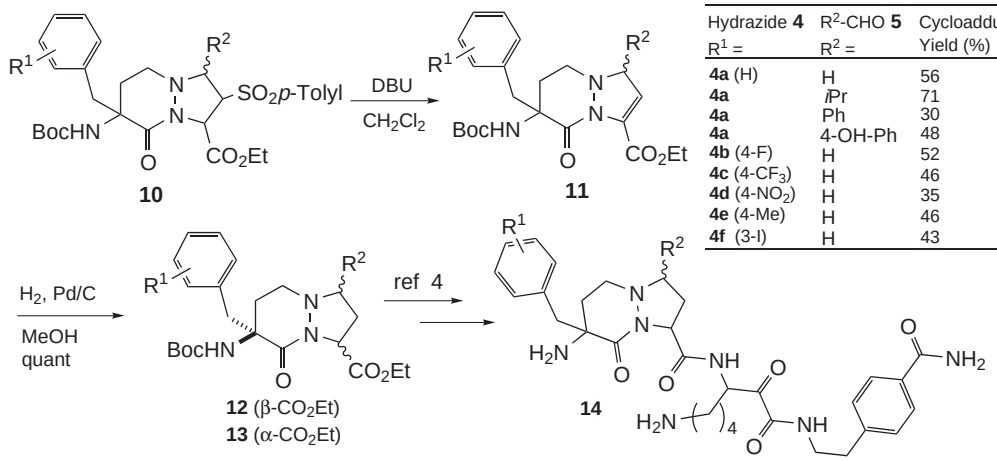
The synthesis of racemic six-membered cyclic hydrazides **4** is shown in Scheme 1. The benzaldimine of glycine methyl ester **6** was treated with LDA and alkylated with substituted benzyl bromides (R^1 = H, 4-fluoro, 4-trifluoromethyl, 4-nitro, 4-methyl, and 3-iodo). The subsequent alkylation was carried out by addition of KHMDS and allyl bromide to the above reaction flask without work-up.[10] α-Allylphenylalanine derivatives **7** were generated in 85–97% yields. After hydrolysis of imine **7** and protection of the resulting amines by a Boc group, ozonolysis of the olefin followed by reductive work-up gave the aldehydes **8**. Heating of aldehydes **8** with hydrazine for 2 days afforded the cyclic hydrazones **9** in good yield. Reduction by sodium cyanoborohydride with hydrochloric acid yielded the tetrahydropyridazinones **4a**–**f**, respectively.

Treatment of hydrazide **4** with formaldehyde (**5**) (R^2 = H) formed the azomethine imine intermediate **2** (R^2 = H), which underwent 1,3-dipolar cycloaddition upon heating with ethyl (*E*)-3-(*p*-tolylsulfonyl)acrylate[11] (**3**) in refluxing 1,2-dichloroethane for 36 h (see Fig. 2). The desired cycloadducts **10** (R^2 = H) were regioselectively formed regardless of the R^1 substituent. Regiochemistry and stereoselectivity of the cyclization were determined at the stage of the α,β-unsaturated ester **11** (R^2 = H) obtained by elimination of the *p*-tolylsulfonyl group (DBU, 0°C) (Scheme 2). The ^{1}H NMR spectra[12] at the vinyl position (triplet, J = 2.6 Hz) in **11** showed that the 1,3-dipolar cycloaddition proceeds with the regioselection depicted in Fig. 2. The 1,3-dipolar cycloaddition, followed by elimination of a *p*-tolylsulfonyl group was also performed in the same manner to provide **11** as a 1:1 stereoisomeric mixture of the other R^2 substituent (i.e. Ph, *p*-hydroxyphenyl, and isopropyl). Hydrogenation with Pd/C resulted in a 3:1 diastereomeric mixture of **12** and **13** and the structure of the former was unequivocally determined by X-ray crystallography (R^1 = H, R^2 = H). The products obtained in our hands were used for the next several steps as a stereoisomeric mixture in order to examine inhibitory activity. After hydrolysis of the ethyl ester, the carboxylic acid was treated according to the same procedure previously reported[4] to yield **14**.

The selectivity and potency of in vitro assays toward various serine proteases (thrombin, trypsin, factor VIIa, tryptase, etc.) are summarized in Table 1.[13] It is clear that the template can effectively mimic the peptide bound conformation in the protease active site. The thrombin and trypsin inhibitory activity are nearly identical to that of PPACK (1.5 nM inhibition of thrombin). In addition, substituents introduced on the template played an important role in defining selectivity. For example, by introducing an isopropyl group with R^2 substituent, the trypsin selectivity over thrombin increased about 15 times. By introducing a



Scheme 1.



Hydrazide **4** R^1 =	R^2-CHO **5** R^2 =	Cycloadduct **11** Yield (%)
4a (H)	H	56
4a	*i*Pr	71
4a	Ph	30
4a	4-OH-Ph	48
4b (4-F)	H	52
4c (4-CF_3)	H	46
4d (4-NO_2)	H	35
4e (4-Me)	H	46
4f (3-I)	H	43

Scheme 2.

Table 1. Inhibition and selectivity of coagulation versus anticoagulation enzymes

Compd.	R^1	R^2	Thrombin *Ki* (nM)	Trypsin *Ki* (nM)	Factor VIIa *Ki* (nM)	Tryptase *Ki* (nM)	Selectivity Ki_{thr}/Ki_{trp}
14a	4-F	H	41.2	26.2	9277	164.4	1.57
14b	4-CF_3	H	17.7	11.3	8277	62.6	1.57
14c	4-Me	H	6.5	7.8	2308	15.8	0.83
14d	H	*i*-Pr	61.6	4.3	1096	15.4	14.3

halogen group (4-F, 4-CF_3) as the R^1 substituent, the tryptase inhibition activity decreased. These results demonstrate the versatility of this template to inhibit a wide range of serine proteases and importantly, that specificity can be introduced using combinatorial introduction of substituents. In summary, we have demonstrated a practical synthetic route to a constrained β-strand mimetic template via the regioselective 1,3-dipolar cycloaddition of azomethine imines with vinyl sulfone. The solid-phase version of this approach is now underway in our laboratory and will be reported in due course.

References

1. Kahn, M. *Synlett* **1993**, *3*, 821–826.
2. Bode, W.; Huber, R. *Eur. J. Biochem.* **1992**, *204*, 2554–2566.
3. Kettner, C.; Shaw, E. *Thromb. Res.* **1979**, *14*, 969.
4. Boatman, P. D.; Ogbu, C. O.; Eguchi, M.; Kim, H.; Nakanishi, H.; Cao, B.; Shea, J. P.; Kahn, M. *J. Med. Chem.* **1999**, *42*, 1367–1375.
5. Ogbu, C. O.; Qabar, M. N.; Boatman, P. D.; Urban, J.; Meara, J. P.; Ferguson, M. D.; Tulinsky, J.; Lum, C.; Babu, S.; Blaskovich, M. A.; Nakanishi, H.; Ruan, F.; Cao, B.; Minarik, R.; Little, T.; Nelson, S.; Nguyen, M.; Gall, A.; Kahn, M. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2321–2326.
6. Hanessian, S.; McNaughton-Smith, G.; Lombart, H. G.; Lubell, W. D. *Tetrahedron* **1997**, *53*, 12789–12854.
7. Jungheim, L. N.; Barnett, C. J.; Gray, J. E.; Horcher, L. H.; Shepherd, T. A.; Sigmund, S. K. *Tetrahedron* **1988**, *44*, 3119–3126.
8. Caramella, P.; Albini, E.; Bandiera, T.; Corsico Coda, A.; Grunanger, P.; Albini, F. M. *Tetrahedron* **1983**, *39*, 689–699.
9. Shimizu, T.; Hayashi, Y.; Miki, M.; Teramura, K. *J. Org. Chem.* **1985**, *50*, 904–907.
10. Stork, G.; Leong, A. Y. W.; Touzin, A. M. *J. Org. Chem.* **1976**, *41*, 3491–3493.
11. Najera, C.; Baldo, B.; Yus, M. *J. Chem. Soc., Perkin Trans. 1* **1988**, 1029–1033.
12. Compound **11a** (R^1=H, R^2=H) ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm): 7.18–7.26 (m, 5H), 5.81 (t, 1H, J=2.6 Hz), 5.58 (brs., 1H), 4.21–4.38 (m, 3H), 3.92 (dd, 1H, J=2.6, 14.9 Hz), 3.49 (d, 1H, J=13.5 Hz), 3.37 (d, 1H, J=13.5 Hz), 2.95–3.05 (m, 2H), 2.54–2.68 (m, 1H), 2.40–2.50 (m, 1H), 1.44 (s, 9H), 1.33 (t, 3H, J=7.3 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ (ppm): 165.6, 160.6, 154.5, 135.9, 132.7, 130.5, 129.1, 127.0, 114.5, 79.4, 62.0, 60.9, 58.6, 53.5, 43.2, 34.2, 28.4, 14.1.
13. All protease assays were performed at room temperature in 96-well microplates using Bio-Rad microplate reader (Model 3550), Molecular Devices SpectroMax 250, Labsystems Fluoroskan Ascent, or Wallac Victor^2 fluorescence plate reader. Either 1 mM solutions of testing compounds in water or 10 mM solutions of testing compounds in DMSO served as the stock solutions for the inhibition assays. For thrombin and trypsin assays, the hydrolysis of chromogenic substrate, *N*-*p*-tosyl-Gly-Pro-Arg-pNA (Sigma) or fluorogenic substrate *N*-*p*-tosyl-Gly-Pro-Arg-AMC (Sigma) was monitored at 405 nm or at Ex: 355 nm, Em: 460 nm, respectively. For tryptase assay, the release of pNA from chromogenic substrate S-2366, pyroGlu-Pro-Arg-pNA (Chromogenix) was monitored at 405 nm. In Factor VIIa assays, the hydrolysis of fluorogenic substrate (D)Phe-Pro-Arg-AMC was moni-

tored at Ex: 355 nm, Em: 460 nm. The reaction progress curves were recorded by reading the plates, typically 80 times with 24 second intervals. Initial rates were determined by unweighted nonlinear least square fitting to a first-order reaction in either GraFit (Erithacus Software Limited, London, England) or GraphPad Prism (GraphPad Software, Inc., San Diego, USA). The determined initial velocities were then nonlinear least square fitted against the concentrations of a tested compound using GraFit or Prism to obtain *K*i. The general format of these assays were: 100 μl of inhibitor solution and 50 μl of enzyme solution were added in a microplate well, incubated at room temperature for 30 min, then 100 μl of a substrate solution was added to initiate the reaction.