Serine and Threonine β-Lactones: A New Class of Hepatitis A Virus 3C Cysteine Proteinase Inhibitors

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Hepatitis A virus (HAV) 3C enzyme is a cysteine proteinase essential for viral replication and infectivity and represents a target for the development of antiviral drugs. A number of serine and threonine β-lactones were synthesized and tested against HAV 3C proteinase. The D-*N*-Cbz-serine β-lactone **5a** displays competitive reversible inhibition with a K_i value of 1.50×10^{-6} M. Its enantiomer, L-*N*-Cbz-serine β-lactone **5b** is an irreversible inactivator with $k_{inact} = 0.70$ min^{-1}, $K_I = 1.84 \times 10^{-4}$ M and $k_{inact}/K_I = 3800$ M^{-1} min^{-1}. Mass spectrometry and HMQC NMR studies using ^{13}C-labeled **5b** show that inactivation of the enzyme occurs by nucleophilic attack of the cysteine thiol (Cys-172) at the β-position of the oxetanone ring. Although the *N*-Cbz-serine β-lactones **5a** and **5b** display potent inhibition, other related analogues with an *N*-Cbz side chain, such as the five-membered ring homoserine γ-lactones **14a** and **14b**, the four-membered ring β-lactam **33**, 2-methylene oxetane **34**, cyclobutanone **36**, and 3-azetidinone **39**, fail to give significant inhibition of HAV 3C proteinase, thus demonstrating the importance of the β-lactone ring for binding.

Introduction

Cysteine proteinases are potentially interesting chemotherapeutic targets because of their involvement in many diseases such as viral infections, parasitic ailments, arthritis, cancer, and osteoporosis.[1] Such enzymes play a key role in the family of picornaviruses (Picornaviridae) which contains more than 200 members, including pathogens such as poliovirus (PV), human rhinovirus (HRV), encephalomyocarditis virus, foot-and-mouth disease virus, and hepatitis A virus (HAV). HAV is most commonly found in developing countries, although occasional cases still arise elsewhere, and it is usually spread through the contamination of drinking water and food.[2,3] The picornavirus genome consists of a positive-sense single strand RNA which generates a single ∼250 kDa protein that subsequently undergoes multiple cleavages by the 2A or 3C proteinases to produce the mature viral proteins. In HAV, the 3C proteinase is the key enzyme involved in the co- and posttranslational processing of the polyprotein and is therefore of prime importance for viral maturation and infectivity.[4,5] Hence it represents an attractive target for the development of new antiviral agents. Crystal structures for HAV 3C,[6] HRV 3C,[7] and PV 3C[8] proteinases have been reported. Interestingly, these cysteine proteinases have three-dimensional structures that are analogous to those of the chymotrypsin family of serine proteinases with the active site serine being replaced with a cysteine.[4,9] The nucleophilicity of the active site cysteine (Cys-172) thiol of the HAV 3C proteinase is enhanced through deprotonation by a histidine residue (His-44) which acts as a general acid−base catalyst.[6] The enzyme requires amino acids from P_5 to P_3' in peptide substrates for optimally efficient cleavage and has a recognition site for a glutamine at P_1. It also displays some preference for leucine at P_4 and phenylalanine at P_2'.[4] For an octapeptide substrate (Ac-ELRTQSFS−NH_2) corresponding to the truncated viral polyprotein 2B/2C junction, the k_{cat} is about 1.8 s^{-1} with a K_m of 2.1 mM at pH 7.5.[4] Various classes of inhibitors have been studied for HAV and HRV 3C proteinases as potential therapeutic leads, and these include compounds such as peptide

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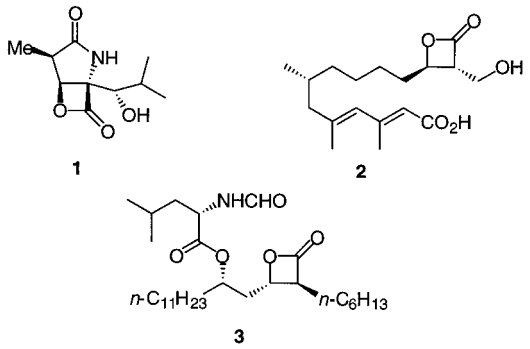

Figure 1. Some important β-lactone inhibitors.

aldehydes,[10,11] peptide fluoromethyl ketones,[12] β-lactams,[13] isatins,[14] homophthalimides,[15] vinylogous esters and sulfones,[16−18] halomethylacetamides,[19,20] azapeptides,[9,21,22] and azodicarboxamides.[23] Some of these inhibitors display in vitro antiviral activity in cell culture,[12,14−18] but these are often limited by high toxicity due to the reactive nature of these compounds, especially toward thiols.[14]

Over the last 20 years, much attention has been focused on β-lactones, because of their occurrence in many biologically active natural products.[24] Among the most widely studied β-lactones are the *clasto*-lactacystin β-lactone (Omuralide) **1** (Figure 1), a potent inhibitor of the threonine proteinase 20S proteasome,[25] the antibiotic F-244 (1233 A) **2**, an inhibitor of HMG-CoA synthase,[26] and the antiobesity drug tetrahydrolipstatin (Orlistat) **3**, an inhibitor of the serine pancreatic lipase.[27] For each of these enzymes, the proposed mode of inactivation is by acylation of the active site nucleophile residue by the β-lactone as opposed to alkylation through attack at C-3 of the oxetanone.[28] In a recent preliminary communication we reported on inhibition of HAV 3C proteinase by *N*-Cbz-serine β-lactone.[29] We now describe additional studies on the interaction of this cysteine proteinase with enantiomerically pure serine and threonine β-lactones, other four-membered ring derivatives with an *N*-Cbz side chain, and γ-lactones. A variety of inhibitory modes (competitive and irreversible) are observed. HMQC NMR spectroscopic analysis of the enzyme−inhibitor complex of HAV 3C proteinase with β-lactone **5b** (β-^{13}C) demonstrates that *alkylation* of the active site thiol (attack at C-3 of the oxetanone) can occur to cause irreversible inactivation of the enzyme.

Results and Discussion

Serine β-Lactones. D- and L-*N*-Cbz-serine-β-lactones **5a** and **5b** were initially chosen as the targets because they can readily be obtained by Mitsunobu cyclization of *N*-Cbz-serine[28] and the presence of a benzyl group may mimic the P_2' phenylalanine side chain of the HAV 3C substrates.[20,30] The general synthetic protocol for the preparation of serine β-lactones **5a** is outlined in Scheme 1. The amino group of D-serine **4a** is protected with benzyloxycarbonyl (Cbz) group by treatment with benzyl chloroformate and aqueous sodium bicarbonate. The hydroxy acid is subsequently cyclized via a modified Mitsunobu reaction using the preformed adduct of dimethyl azodicarboxylate (DMAD) and triphenylphosphine to afford D-*N*-Cbz-serine-β-lactone **5a**.[28a,c] Its enantiomer, the L-*N*-Cbz-serine-β-lactone **5b** is synthesized in a similar manner. It is known that α-amino-β-lactones bearing no β-substituent display low stability in basic aqueous media and are readily hydrolyzed to the corresponding hydroxy acids.[28] However, the half-life for hydrolysis of **5a** in phosphate buffer at pH 7.5 is 76 min, which is sufficiently stable for inhibition studies. Enzyme assays employed an overexpressed C24S mutant in which the nonessential surface cysteine was replaced with serine and which displays catalytic properties indistin-

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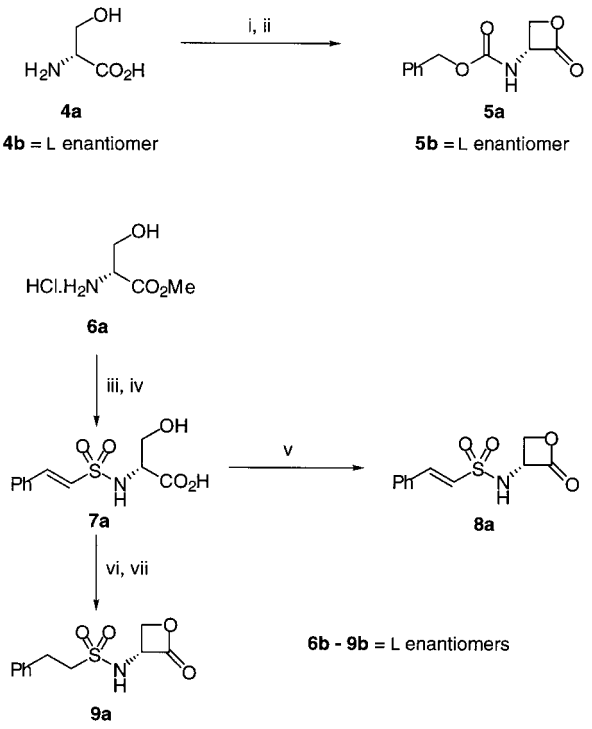
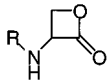
Scheme 1[a]



[a] Conditions: (i) $NaHCO_3$, BnOCOCl, THF/H_2O, 82%; (ii) DMAD, PPh_3, THF, −78 °C to RT, 40%; (iii) *(E)*-$Ph(CH)_2SO_2Cl$, NEt_3, CH_2Cl_2, 37%; (iv) LiOH, THF/H_2O, 91%; (v) DMAD, PPh_3, THF, −78 °C to RT, 14% (unoptimized yield) (vi) H_2, Pd/C, MeOH, 87%; (vii) DMAD, PPh_3, THF, −78 °C to RT, 32%.

guishable from the native proteinase.[31] This mutant is convenient because it avoids enzyme dimerization via intermolecular disulfide bond formation. The enzyme activity is conveniently monitored using a fluorometric assay at an enzyme concentration of 0.1 μM and a fluorogenic peptide substrate Dabcyl-GLRTQSFS-Edans. IC_{50} values were generally measured without enzyme−inhibitor preincubation. Despite the absence of the P_1 glutamine side chain important for HAV 3C substrate/inhibitor recognition, β-lactones **5a** and **5b** are potent inhibitors of HAV 3C proteinase with IC_{50} values of 6 and 35 μM, respectively (Table 1). Further kinetic studies reveal that **5a** is a competitive reversible inhibitor of HAV 3C ($K_i = 1.50 \times 10^{-6}$ M).[32] Surprisingly, the L-enantiomer **5b** displays a different mode of inhibition under the same conditions and acts as a time-dependent irreversible inhibitor ($k_{inact} = 0.70$ min^{-1}, $K_I = 1.84 \times 10^{-4}$ M and $k_{inact}/K_I = 3800$ M^{-1} min^{-1}).[33] To determine whether the D-enantiomer **5a** could possibly act as a time-dependent inhibitor under different conditions, a variety of pH conditions and widely varying concentrations of inhibitor **5a** were examined (pH 6, 7, 7.5 and [**5a**] 0.2−50 μM). In each case, only competitive behavior is observed with the D-enantiomer. The inhibitory activities of **5a** and **5b** are not affected by the presence of a 10-fold molar excess concentration of dithiothreitol (DTT, 100−500 μM, pH 7.5), suggesting that these β-lactones are not dramatically thiophilic and could therefore be specific

Table 1



compd	R	α config	HAV 3C IC_{50} (μM)[a]	aq hydrolysis $t_{1/2}$ (min)[b]
5a	R = CO_2Bn	*R*	6	76
5b	R = CO_2Bn	*S*	35	76
8a	R = (*E*)-$SO_2(CH)_2Ph$	*R*	3	64
9a	R = $SO_2(CH_2)_2Ph$	*R*	4	32
8b	R = (*E*)-$SO_2(CH)_2Ph$	*S*	38	64
9b	R = $SO_2(CH_2)_2Ph$	*S*	25	32
10	R = SO_2Me	*S*	>100	15
11	R = Boc	*S*	>100	285
12	R = Phthalimido	*R*	27	67

[a] IC_{50} conditions: 0.1 μM HAV 3C C24S, 10 μM Dabcyl-GLRTQSFS-Edans, 2 mM EDTA, 0.1 mg/mL BSA, 100 mM KH_2PO_4/K_2HPO_4 at pH 7.5, 1% DMF. [b] β-Lactone hydrolysis half-life in phosphate buffer pH 7.5

enzyme inhibitors that would not react randomly with free biological thiols (e.g., glutathione). The corresponding hydroxy acids (hydrolysis products of β-lactones), D- and L-*N*-Cbz-serine **4a** and **4b** and also their lithium carboxylate salts show no significant inhibition of HAV 3C at a concentration of 100 μM.

To examine the structural influences and to potentially identify better inhibitors, both the saturated and unsaturated sulfonamide β-lactones **8** and **9** (Table 1) were prepared. The amino group of D-serine methyl ester couples readily with *trans*-β-styrenesulfonyl chloride (Scheme 1) to afford the sulfonamide ester. Hydrolysis of the methyl ester with lithium hydroxide generates the hydroxy acid **7a**. This can either be cyclized directly to afford the β-lactone **8a**, or the side chain double bond can first be reduced by hydrogenation over palladium on charcoal[34] followed by ring closure of the intermediate hydroxy acid to give β-lactone **9a**. The L enantiomers **8b** and **9b** (Table 1) are available via a similar route. Testing with HAV 3C proteinase shows that β-lactones **8a**, **8b**, **9a**, and **9b** are potent inhibitors with IC_{50} values in the range of 3−38 μM (Table 1), although that there is no improvement in half-life for nonenzymatic hydrolysis. The electron-withdrawing sulfonamido group enhances somewhat the rate of attack by water on the oxetanone and decreases stability in aqueous solution. Interestingly, the modes of inhibition observed with L- *N*-Cbz β-lactones **5a** and **5b** are not repeated with the *N*-sulfonamido derivatives. Compounds **8a**, **8b**, and **9b** are reversible inhibitors, whereas **9a** is a time dependent irreversible inactivator of HAV 3C proteinase. Apparently, the HAV 3C enzyme shows quite different modes of binding for the different β-lactones, but the factors governing these effects are not understood. Further insight into this problem could potentially be obtained from crystallographic analysis of enzyme−inhibitor complexes;[6b] however, attempts to accomplish this with the β-lactone inhibitors have thus far been unsuccessful.

To explore the significance of the benzyl group as a potential mimic of the peptide substrate P_2' phenylalanine side chain, the methyl sulfonamide **10**,[35] *N*-Boc **11**,[28c] and *N*-phthalimido **12** serine β-lactones were prepared

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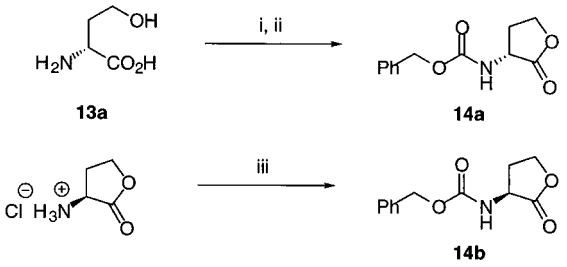
Scheme 2[a]



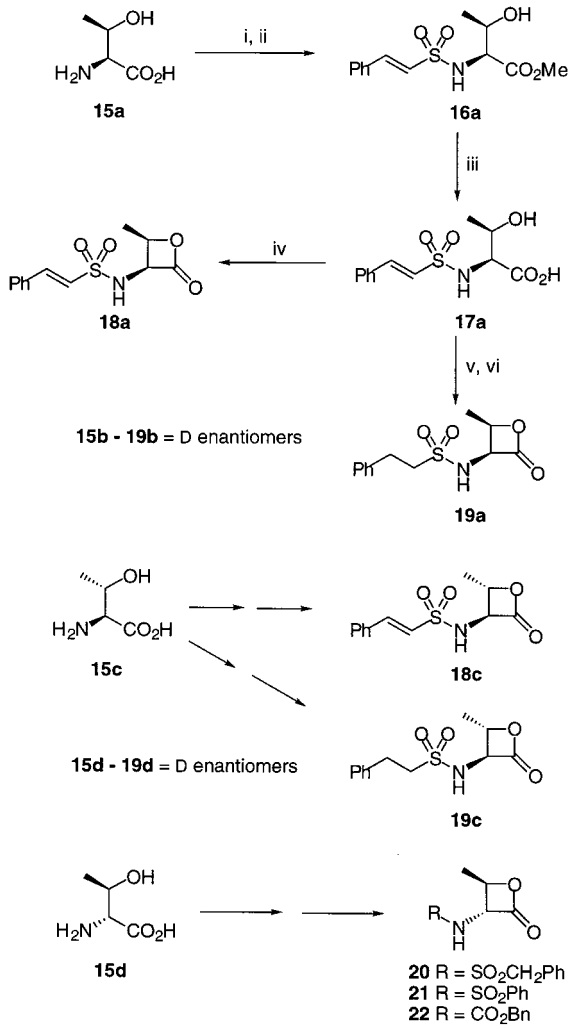
[a] Conditions: (i) $NaHCO_3$, BnOCOCl, THF/H_2O; (ii) toluene, Δ, 50% (over two steps); (iii) BnOCOCl, NEt_3, CH_2Cl_2, 23%.

by Mitsunobu cyclization of the corresponding N-protected serine amino acids as described for **5a**. Enzyme assay of **10** and **11** gives weak time-dependent inhibition (IC_{50} >100 μM, Table 1). However, the *N*-phthalimido compound **12** which contains a phenyl ring on the side chain, although in a quite different geometrical location compared to the *N*-Cbz group of **5**, displays time independent (reversible) inhibition with an IC_{50} of 27 μM. These results indicate that a phenyl side chain is needed for effective inhibition of HAV 3C proteinase.

To probe the importance of the β-lactone ring for inhibition, D- and L-*N*-Cbz-homoserine-γ-lactones **14a** and **14b** were prepared. γ-Lactones are well-known to undergo ring opening as a result of nucleophilic attack at the carbonyl,[36] and some show biological activity against serine proteinases[37] as well as thiol-containing enzymes.[38] Furthermore, nucleophilic attack by thiol[39] and hydroxyl[40] groups at the γ-position of the γ-lactone ring have also been reported. Treatment of D-homoserine **13a** with benzyl chloroformate in the presence of aqueous sodium bicarbonate (Scheme 2) followed by heating under reflux to effect cyclization affords the D-*N*-Cbz-homoserine-γ-lactone **14a**. The L-enantiomer **14b** is prepared by reaction of the commercially available L-homoserine-γ-lactone with benzyl chloroformate in the presence of triethylamine. However, these γ-lactones **14a** and **14b** show no significant inhibition of HAV 3C proteinase at concentrations of 100 μM. Clearly the five-membered lactone ring cannot substitute for the four-membered β-lactone as a successful inhibitor of this enzyme.

Threonine β-Lactones. Our next objective was to examine D- and L-stereoisomers of threonine and *allo*-threonine β-lactones. The presence of a methyl substituent at the β-position of these β-lactones could improve hydrolytic stability in basic media and should provide insight into substitution patterns potentially acceptable to the HAV 3C enzyme. In contrast to the serine analogues, cyclization of β-alkyl-substituted β-hydroxy-α-amino acids, e.g., **17a** (Scheme 3) requires carboxyl

Scheme 3[a]



[a] Conditions: (i) $SOCl_2$, MeOH, Δ, 98%; (ii) *(E)*-$Ph(CH)_2SO_2Cl$, NEt_3, CH_2Cl_2, 80%; (iii) LiOH, THF/H_2O, 99%; (iv) BOP, NEt_3, CH_2Cl_2, 61%; (v) H_2, Pd/C, MeOH, 93%; (vi) BOP, NEt_3, CH_2Cl_2, 75%.

group activation as opposed to hydroxyl group activation in order to avoid decarboxylative elimination to form the corresponding enamines.[28b] Both the saturated and unsaturated β-lactone analogues **18a**−**d** and **19a**−**d** were targeted for testing with HAV 3C proteinase. Treatment of L-threonine **15a** with thionyl chloride in methanol (Scheme 3) affords the methyl ester hydrochloride salt which is subsequently coupled to *trans*-β-styrenesulfonyl chloride to yield the sulfonamide ester **16a**. The methyl ester **16a** is conveniently hydrolyzed with lithium hydroxide to the acid **17a**. Cyclization of the hydroxy acid **17a** to the unsaturated β-lactone **18a** proceeds with benzotriazole-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP)[41] reagent in the presence of triethylamine. For the preparation of **19a**, the side chain double bond is first reduced by hydrogenation over palladium on charcoal to afford the intermediate saturated hydroxy acid, which is then cyclized with BOP. The β-lactones **18b**−**d** and **19b**−**d** can be prepared analogously. Enzymatic testing of β-lactones **18a**−**d** and **19a**−**d** gives time-dependent inhibition with IC_{50} values

(36) For general discussions of ring opening of γ-lactones, see: (a) Aldridge, D. C.; Nicholson, S.; Taylor, P. J. *J. Chem. Soc., Perkin Trans. 2* **1997**, 1929−1938. (b) Huisgen, R.; Ott, H. *Tetrahedron* **1959**, *6*, 253−267.

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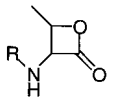
Table 2



compd	R	config α	config β	HAV 3C IC_{50} (μM)[a]	aq hydrolysis $t_{1/2}$ (min)[b]
18a	R = (*E*)-$SO_2(CH)_2Ph$	*S*	*R*	131	595
19a	R = $SO_2(CH_2)_2Ph$	*S*	*R*	168	358
18b	R = (*E*)-$SO_2(CH)_2Ph$	*R*	*S*	86	595
19b	R = $SO_2(CH_2)_2Ph$	*R*	*S*	136	358
18c	R = (*E*)-$SO_2(CH)_2Ph$	*S*	*S*	20	135
19c	R = $SO_2(CH_2)_2Ph$	*S*	*S*	32	136
18d	R = (*E*)-$SO_2(CH)_2Ph$	*R*	*R*	5	135
19d	R = $SO_2(CH_2)_2Ph$	*R*	*R*	12	136
20	R = SO_2CH_2Ph	*R*	*R*	6	96
21	R = SO_2Ph	*R*	*R*	25	135
22	R = CO_2Bn	*R*	*R*	13	647

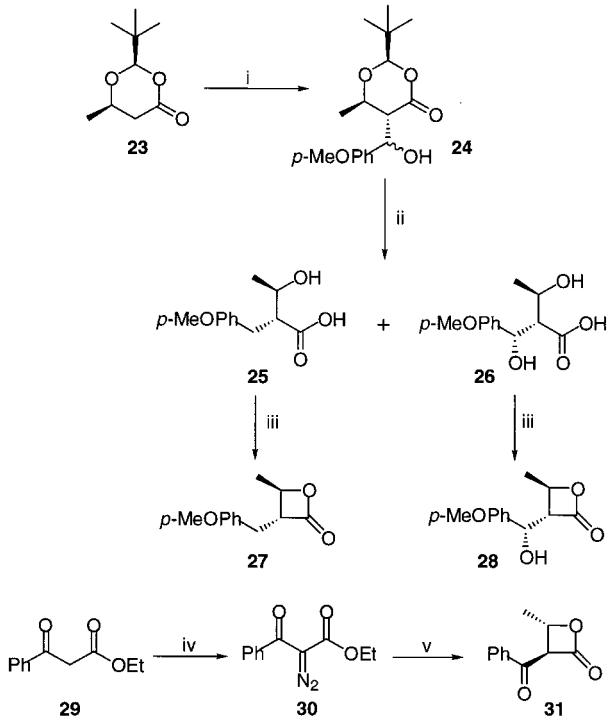
[a] IC_{50} conditions: 0.1 μM HAV 3C C24S, 10 μM Dabcyl-GLRTQSFS-Edans, 2 mM EDTA, 0.1 mg/mL BSA, 100 mM KH_2PO_4/K_2HPO_4 at pH 7.5, 1% DMF. [b] β-Lactone hydrolysis half-life in phosphate buffer pH 7.5

in the range of 5 to 168 μM (Table 2). The *trans*-β-lactones **18c**, **19c**, **18d**, and **19d** are generally more potent (IC_{50} 5−32 μM) than the *cis*-β-lactones **18a**, **19a**, **18b**, and **19b** (IC_{50} 86−168 μM). As expected, these β-methyl-substituted β-lactones show enhanced stability in basic aqueous media ($t_{1/2}$ 135−595 min) compared to their serine β-lactone counterparts (Table 1).

Since the trans-substituted D-*allo*-threonine β-lactones **18d** and **19d** are the most potent inhibitors in this series, β-lactones **20**, **21**, and **22** with varying side chain length and functionality were prepared (Table 2).[42] Compounds **20**−**22** give potent time-dependent inhibition with IC_{50} values in the range of 6−25 μM. Although the effects are not dramatic, shortening of the side chain by *one* carbon (cf. **19d** and **20**) results in a 2-fold increase in inhibition. However, reducing the same side chain by *two* carbons (cf. **19d** and **21**) leads to a 2-fold decrease in inhibition. One notable feature is that the aqueous half-life of the β-lactones (except for **22**) seems to decrease with increasing potency for enzyme inactivation, presumably because enhanced reactivity of the β-lactone ring system to enzyme nucleophiles parallels susceptibility to attack by water.

***β*-Lactone Analogues.** To examine whether the α-nitrogen functionality in the amino acid derived β-lactones is important for inhibitory activity, analogues **27**, **28**, and **31** which contain a methylene or a carbonyl group at the α-position were synthesized. Treatment of dioxanone[43] **23** with *tert*-butyllithium followed by trapping of the enolate with *p*-anisaldehyde at −78 °C (Scheme 4) gives a 1:1 mixture of diastereoisomers **24**. Hydrogenolysis using palladium on charcoal cleaves the *R* benzylic hydroxyl group preferentially with concomitant removal of the pivaloyl group to generate **25** and **26** in a 1:1 ratio. The β-hydroxy acids **25** and **26** readily cyclize with BOP to furnish the β-lactones **27** and **28**,[44] respectively. The

Scheme 4[a]



[a] Conditions (i) *t*-BuLi, THF, −78 °C then *p*-anisaldehyde −78 °C to RT, 66%; (ii) H_2, 10% Pd/C, MeOH, 40% for **25** and 35% for **26;** (iii) BOP, NEt_3, CH_2Cl_2, 73% for **27** and 70% for **28**; (iv) TsN_3, NEt_3, CH_3CN, 93%; (v) $Rh_2(OAc)_4$, CH_2Cl_2, Δ, 52%.

known β-lactone **31** can be synthesized using a modified literature procedure.[45] Reaction of the β-keto ester **29** with tosyl azide in the presence of triethylamine generates the diazoester **30**, which on treatment with a refluxing solution of rhodium (II) acetate dimer affords the racemic *trans*-β-lactone **31**. The reaction proceeds through insertion of the intermediate carbenoid species into the carbon−hydrogen bond of the methylene group of the ethyl ester. Testing of **27**, **28**, and **31** against HAV 3C proteinase shows poor inhibition by these compounds at a concentration of 100 μM (IC_{50}'s ≫100 μM). The phenyl-bearing side chain in these compounds is one atom shorter than in the β-lactones **12** and **21**, which may account for the poorer inhibition, but the α-nitrogen functionality may also play an important role in enzyme recognition.

Four-Membered Ring Analogues. Since five-membered ring *N*-Cbz-γ-lactones (**14a** and **14b**) are poor inhibitors compared to their four-membered ring counterparts *N*-Cbz-β-lactones (**5a** and **5b**), analogues of **5b** with a four-membered ring scaffold and an *N*-Cbz side chain group were investigated. The targets chosen were β-lactam **33**, 2-methylene oxetane **34**, cyclobutanone **36**, and 3-azetidinone **39**. Monocyclic β-lactams analogous to **33** are potent inhibitors of the serine human cytomegalovirus (HCMV) proteinase.[46] The β-lactam **33** is readily available by cyclization of the *N*-Cbz-L-diaminopropionate tosylate salt **32** with mesyl chloride[46a] (Scheme 5). Treatment of L-*N*-Cbz-serine β-lactone **5b** with dimeth-

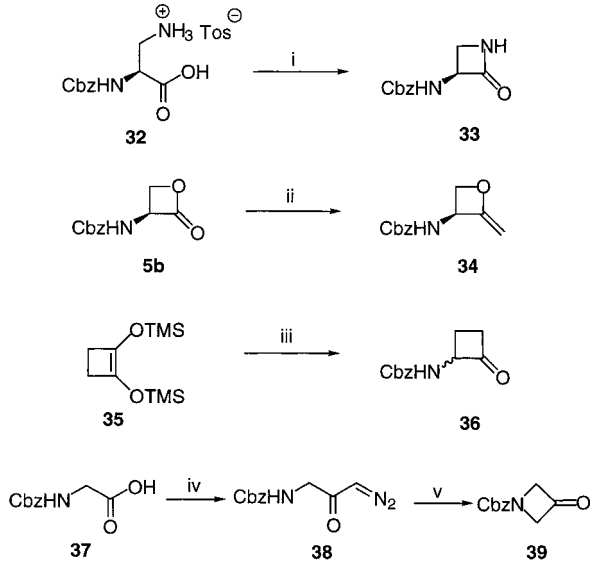
(42) In contrast to β-lactones **18a**−**d** and **19a**−**d**, the *N*-sulfonamide β-hydroxy acids of **20** and **21** were prepared by direct coupling of D-*allo*-threonine to the corresponding sulfonyl chloride, see Experimental Section.

(43) (a) Amberg, W.; Seebach, D. *Chem. Ber.* **1990**, *123*, 2413−2428. (b) Gautschi, M.; Schweizer, W. B.; Seebach, D. *Chem. Ber.* **1994**, *127*, 565−579. (c) Murer, P.; Seebach, D. *Helv. Chim. Acta* **1998**, *81*, 603−631.

(44) The relative configuration of **27** was determined by NOE studies and that of **28** by its X-ray crystal structure.

(45) (a) Box, V. G. S.; Marinovic, N.; Yiannikouros, G., P. *Heterocycles* **1991**, *32*, 245−251. (b) Lee, E.; Jung, K. W.; Kim, Y. S. *Tetrahedron Lett.* **1990**, *31*, 1023−1026. (c) Balaji, B. S.; Chanda, B. M. *Tetrahedron Lett.* **1998**, *39*, 6381−6382.

Scheme 5[a]

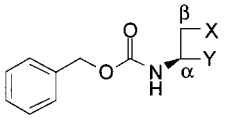


[a] Conditions: (i) CH_3SO_2Cl, $NaHCO_3$, CH_3CN, Δ, 49%; (ii) Cp_2TiMe_2, toluene, Δ, 24%; (iii) $CbzNH_2$, HCl, Et_2O, Δ, 81%; (iv) (a) EtOCOCl, NEt_3, THF; (b) CH_2N_2, Et_2O, 60%; (v) $Rh_2(OAc)_4$, CH_2Cl_2, Δ, 28%.

yltitanocene[47] generates the 2-methylene oxetane **34**. The cyclobutanone **36** is obtained by the reaction of 1,2-bis-(trimethylsiloxy)cyclobutene **35** with benzyl carbamate in the presence of ethereal hydrogen chloride.[48] Reaction of *N*-Cbz-glycine with ethyl chloroformate followed by trapping of the mixed anhydride with diazomethane gives the α-diazoketone **38**.[12] Treatment of **38** with rhodium(II) acetate dimer affords 3-azetidinone **39**.[49] Testing of **33**, **34**, **36**, and **39** against HAV 3C proteinase shows no significant inhibition at a concentration of 100 μM. These results clearly demonstrate the importance of the β-lactone functionality for inhibitory activity of four-membered ring analogues, either in the reversible competitive mode (e.g., **5a**) or as irreversible inactivators (e.g., **5b**).

Mechanism of Inhibition. The kinetic studies with β-lactone **5b** are consistent with rapid covalent bond formation leading to inactivation of HAV 3C proteinase. To confirm this hypothesis, HAV 3C proteinase was incubated with β-lactone **5b** in phosphate buffer at pH 7.5, and after dialysis, the enzyme−inhibitor complex was subjected to electrospray mass spectrometry. The mass difference obtained between the enzyme−inhibitor complex (24101 Da) and the enzyme alone (23882 Da) is 219 Da, which is within the experimental error for the calculated mass of the inhibitor (221 Da).[29] This supports the proposal that **5b** forms a covalent complex with HAV 3C enzyme. In accord with this idea, extensive dialysis of the HAV 3C enzyme-**5b** complex showed no recovery of activity under conditions (e.g., 8 h, 4 °C) where the noninhibited enzyme retained full activity.

Table 3



compound[a]	X	Y	β-carbon (ppm)
5b(β-^{13}C)◆[b]	O—CO		68
4b(β-^{13}C)◆[b]	OH	CO_2H	62
40[c]	SMe	CO_2H	37
41[c]	OH	COSEt	64
42[c]	OH	CO_2Me	62
43[c,d]	OMe	CO_2H	73

[a] ◆ = ^{13}C-labeled at β-position. [b] ^{1}H/^{13}C HMQC conditions: D_2O at pD 5.0, 6% DMSO-d_6. [c] ^{1}H/^{13}C HMQC conditions: 20 mM Na_2PO_4/D_2O at pD 7.5, 6% DMSO-d_6. [d] Racemic.

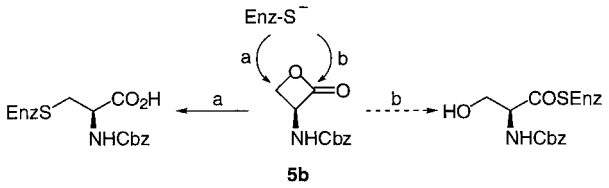


Figure 2. Possible modes of nucleophilic attack on L-*N*-Cbz-serine β-lactone.

Although in previous studies serine or threonine residues in proteins were shown to attack β-lactones at the carbonyl to give acylated products,[25,50] alkylation of enzyme nucleophiles had not been observed. However, serine β-lactones are also known to react with nucleophiles at the β-position, and thiols are thereby able to generate thioethers under appropriate conditions.[28] To determine the type of adduct formed between **5b** and HAV 3C proteinase, β-lactone **5b**(β-^{13}C) was synthesized with ^{13}C (99% isotopic purity) from the corresponding commercially available labeled (β-^{13}C) L-serine as described earlier for **5b**. In addition, model serine thioether **40** and thioester **41** analogues were prepared[29] (Table 3) to assist in ascertaining whether enzymatic thiolate attack on **5b** proceeds at the β-position (Figure 2, pathway a) or at the carbonyl position (pathway b). The methyl ester **42** and methyl ether **43** were also made as reference compounds to examine the possibility of ring opening of **5b** by serine or threonine residues of HAV 3C proteinase. The HMQC spectra in Figure 3 show that **5b**(β-^{13}C) in the absence of enzyme has a cross-peak at δ_c 68 ppm for the labeled methylene carbon, and that upon addition of HAV 3C proteinase this signal disappears and a new cross-peak emerges at δ_c 40 ppm which corresponds to thioether formation (cf. **40**). These NMR results demonstrate that inactivation of HAV 3C enzyme by **5b** occurs primarily by alkylation of the active site cysteine residue at the β-carbon of **5b** as opposed to acylation. Thus, this cysteine proteinase displays a quite different pattern of reactivity compared to the serine- or threonine-containing lipases and proteases which are O-acylated by β-lactones.[26,27]

(46) (a) Yoakim, C.; Ogilive, W. W.; Cameron, D. R.; Chabot, C.; Guse, I.; Hache, B.; Naud, J.; O'Meara, J. A.; Plante, R.; Deziel, R. *J. Med. Chem.* **1998**, *41*, 2882−2891. (b) Deziel, R.; Malenfant, E. *Bioorg Med. Chem. Lett.* **1998**, *8*, 1437−1442. (c) Hanessian, S.; Sahoo, S.; Couture, C.; Wyss, H. *Bull. Soc. Chim. Belg.* **1984**, *93*, 571−578.

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(48) Bisel, P.; Breitling, E.; Frahm, W. *Eur. J. Org. Chem.* **1998**, 729−733.

(49) (a) Sengupta, S.; Das, D. *Synth. Commun.* **1998**, *3*, 403−408. (b) For a review on organic synthesis with α-diazocarbonyl compounds, see: Ye, T.; McKervey, M. A.; *Chem. Rev.* **1994**, *94*, 1091−1160. (c) Wang, J.; Hou, Y.; Wu, P. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2277−2280.

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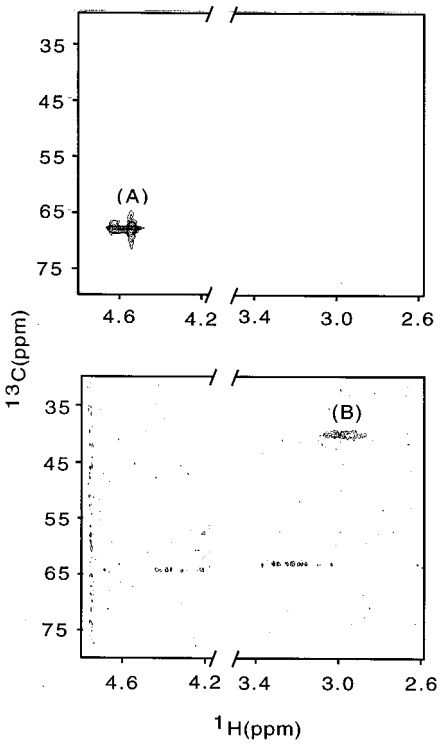

Figure 3. ^{1}H/^{13}C 600 MHz HMQC spectra of **5b**(β-^{13}C) inhibitor alone (top spectrum) and in complex with HAV 3C proteinase (1.2 mM HAV 3C/1.2 mM **5b**(β-^{13}C) (bottom spectrum) at 25 °C in D_2O at pD 5.0 and 20 mM Na_3PO_4/D_2O at pD 7.5, respectively. Cross-peak A shows the proton−carbon correlation of the unreacted inhibitor **5b**(β-^{13}C) and peak B is the cross-peak for the β-thioalkylated adduct, i.e., HAV 3C-**5b**(β-^{13}C) enzyme−inhibitor complex.

Summary

In conclusion, *N*-substituted serine and threonine β-lactones have been identified as potent inhibitors of HAV 3C proteinase. β-Lactone **5a** is a competitive (reversible) inhibitor of HAV 3C proteinase. In contrast, its enantiomer **5b** displays time-dependent irreversible inhibition and inactivates the enzyme through reaction of the cysteine thiolate at the β-carbon of the oxetanone ring, as shown by mass spectrometry and NMR HMQC experiments. The different mode of inhibition observed is probably due to the orientation of the β-methylene carbon of **5a** and **5b** with respect to the required trajectory of the incoming cysteine thiolate nucleophile. Interestingly, for unknown reasons this pattern of inhibition is not maintained with the *N*-sulfonamido derivatives, as **8a**, **8b**, and **9b** are reversible inhibitors, whereas **9a** is an irreversible inactivator. The threonine β-lactones showed enhanced stability in basic aqueous media, with the trans isomers being slightly better time-dependent inhibitors of HAV 3C proteinase than the corresponding cis isomers. The D-*allo*-threonine β-lactones **18d** and **19d** are the most potent in this series. The poor inhibitory results obtained for the γ-lactones and various modified four-membered ring analogues further substantiate the importance of the β-lactone functionality. The nonpeptidic nature of the serine and threonine β-lactones makes them an interesting motif that warrants further studies for the development of new antiviral agents. This is especially true as the enzyme inhibition is not affected by a modest excess (10-fold) of extraneous thiols such a dithiothreitol. Preliminary studies on HRV-14 3C proteinase (data not shown), an enzyme with similar substrate specificity to HAV 3C at P_1,[30] shows that β-lactones **5a** and **5b** have comparable inhibitory activity. Further studies on the inhibition of other picornaviral cysteine proteinases with serine and threonine β-lactones are in progress.

Experimental Section

General Methods and Enzyme Assays. Most general procedures and instrumentation have been previously described.[22,51] Recombinant C24S HAV 3C proteinase in which the nonessential surface cysteine was replaced by serine proteinase was expressed in *Escherichia coli* and purified as reported previously[31] (the C24S mutant is more convenient to use because it avoids intermolecular disulfide bond formation between two enzyme molecules).[6a] Purity of the enzyme samples was greater than 90% as determined by SDS−PAGE analysis (data not shown). Proteinase concentrations were determined spectrophotometrically $\epsilon_{280} = 1.2$ mg/mL. Enzyme was dialyzed against reaction buffer to remove DTT immediately prior to use. Cleavage reactions (700 μL) were performed at 25 °C in a solution containing 100 mM KH_2PO_4/K_2HPO_4 at pH 7.5, 2 mM EDTA, 0.1 mg/mL BSA, 10 μM fluorogenic substrate Dabcyl-GLRTQSFS-Edans (Bachem), 0.1 μM 3C proteinase and 1% DMF.[52] Enzyme assay, sensitivity of inhibitors **5a** and **5b** to DTT, dialysis experiments with **5a**, the rate of hydrolysis of β-lactones in phosphate buffer (the $t_{1/2}$ data reported are within ±20% error), mass spectrometry of HAV 3C-**5b** inhibitor complex, ^{1}H/^{13}C HMQC of HAV 3C-**5b**(β-^{13}C) inhibitor complex and X-ray crystallographic data for **5a**, and synthesis of compounds **4b**(β-^{13}C), **5b**(β-^{13}C), **14a**, **14b**, **40**, **41**, **42**, and **43** have been reported previously.[29]

***N*-(Benzyloxycarbonyl)-D-serine.** This material was prepared from a modified procedure of Greenstein and Winitz.[53] To a suspension of $NaHCO_3$ (10.0 g, 119.35 mmol) in THF (25 mL) and water (50 mL) at room temperature was carefully added D-serine **4a** (5.0 g, 47.65 mmol). After cessation of gas evolution, benzyl chloroformate (7.5 mL, 52.5 mmol) was added dropwise over a 30 min period and stirring was continued for a further 1 h. The reaction mixture was washed with diethyl ether (2 × 25 mL) and acidified to pH 2 by careful addition of 1 N HCl. The slurry was extracted with ethyl acetate (2 × 25 mL), and the organic phases were combined and washed with 1 N HCl (2 × 25 mL) and brine (50 mL), dried over $MgSO_4$, filtered, and evaporated in vacuo to give the title product as a white solid after recrystallization from $CHCl_3$/hexane (9.38 g, 82%): mp 117−119 °C, lit.[53b] mp 117−119 °C; ^{1}H NMR (360 MHz, CD_3OD) δ 7.45−7.20 (m, 5H), 5.08 (br s, 2H), 4.27 (dd, 1H, J = 4.4, 4.2 Hz), 3.88 (dd, 1H, J = 11.4, 4.9 Hz), 3.83 (dd, 1H, J = 11.4, 4.9 Hz); HRMS (EI, M^+) Calcd for $C_{11}H_{13}NO_5$ 239.0793, found 239.0788.

***N*-(Benzyloxycarbonyl)-D-serine-β-lactone (5a).**[28a,c] Triphenylphosphine (5.50 g, 20.0 mmol) was dissolved in THF (143 mL), and the flask was cooled to −78 °C. Dimethyl azodicarboxylate (2.30 mL, 20.0 mmol) was added dropwise over 10 min. A solution of *N*-Cbz-D-serine (5.0 g, 20.0 mmol) in THF (31 mL) was added dropwise to the mixture over 30 min. The mixture was then stirred at −78 °C for 20 min, the cooling bath was removed, and the mixture was slowly warmed with stirring to room temperature over 2.5 h. The solvent was

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removed in vacuo, and the residual pale yellow syrup was purified by flash column chromatography (hexane/ethyl acetate, 4:1) which gave **5a** (1.82 g, 40%) as a white solid after recrystallization from ($CHCl_3$/hexane): mp 133−134 °C, lit.[28c] mp 133−134 °C; $[\alpha]_D^{26}$ +34.2° (*c* 0.5, CH_3CN); ^1H NMR (360 MHz, CD_2Cl_2) δ 7.48−7.31 (m, 5H), 5.49 (br s, 1H), 5.14 (br s, 2H), 5.10−5.00 (m, 1H), 4.50−4.35 (m, 2H); HRMS (EI, M^+) Calcd for $C_{11}H_{11}NO_4$ 221.0688, found 221.069.

***N*-(*trans*-β-Styrenesulfonyl)-D-serine Methyl Ester.** To a solution of D-serine methyl ester hydrochloride **6a** (4.50 g, 28.92 mmol) in CH_2Cl_2 (40 mL) at room temperature was added triethylamine (10.0 mL, 72.31 mmol). The solution was stirred for 5 min, and then *trans*-β-styrenesulfonyl chloride (7.8 g, 38.57 mmol) dissolved in CH_2Cl_2 (20 mL) was added dropwise over 10 min. The resulting solution was stirred overnight, and the reaction mixture was extracted with 1 N HCl (3 × 20 mL), washed with brine (20 mL), dried over $MgSO_4$, filtered, and evaporated in vacuo to give a crude yellow oil. Purification by flash column chromatography (ethyl acetate/hexane, 1:1) gave the title compound (3.21 g, 40%) as a pale yellow solid after recrystallization from $CHCl_3$/hexane: mp 63−65 °C; $[\alpha]_D^{26}$ −8.8° (*c* 1.4, $CHCl_3$); IR ($CHCl_3$ cast) 3503, 3280, 2954, 1741, 1616, 1436, 1216, 1072, 747, 690 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.52−7.28 (m, 6H), 6.82 (d, 1H, *J* = 15.0 Hz), 5.86 (d, 1H, *J* = 8.5 Hz), 4.11 (ddd, 1H, *J* = 12.3, 7.8, 3.8 Hz), 3.89 (br d, 2H, *J* = 3.7 Hz), 3.72 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9, 141.9, 132.5, 131.0, 129.1, 128.4, 125.1, 63.8, 57.8, 53.1; HRMS (ES, MNa^+) Calcd for $C_{12}H_{15}NO_5SNa$ 308.0569, found 308.0566. Anal. Calcd for $C_{12}H_{15}NO_5S$: C, 50.51; H, 5.29; N, 4.91. Found: C, 50.24; H, 5.20; N, 4.85.

***N*-(*trans*-β-Styrenesulfonyl)-L-serine Methyl Ester.** Reaction of L-serine methyl ester hydrochloride (5.0 g, 32.14 mmol), triethylamine (11.14 mL, 80.34 mmol), and *trans*-β-styrenesulfonyl chloride (7.8 g, 38.57 mmol) as described above gave the title product (3.37 g, 37%) as a pale yellow solid: mp 76−78 °C; $[\alpha]_D^{26}$ +14.4° (*c* 1.0, $CHCl_3$). Anal. Calcd for $C_{12}H_{15}NO_5S$: C, 50.51; H, 5.29; N, 4.91. Found: C, 50.44; H, 5.21; N, 4.85.

***N*-(*trans*-β-Styrenesulfonyl)-D-serine (7a).** To a solution of *N*-(*trans*-β-styrenesulfonyl)-D-serine methyl ester (2.10 g, 7.36 mmol) in THF and water (1:1, 100 mL) at room temperature was added lithium hydroxide monohydrate (0.62 g, 14.72 mmol). The resulting solution was stirred for 2 h. The solvent was removed in vacuo, the residue dissolved in saturated aqueous $NaHCO_3$ (20 mL), washed with diethyl ether (3 × 15 mL), acidified to pH 2 with 1 N HCl, extracted with ethyl acetate (3 × 15 mL), washed with brine (15 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to give **7a** (1.7 g, 85%) as a white solid after recrystallization from $CHCl_3$/hexane: mp 187−188 °C; $[\alpha]_D^{26}$ −3.8° (*c* 0.2, MeOH); IR (CH_3CN cast) 3305, 3050, 2960, 1727, 1612, 1449, 1145, 1064, 746, 689 cm^{-1}; ^1H NMR (360 MHz, CD_3CN) δ 7.65−7.40 (m, 6H), 6.92 (d, 1H, *J* = 15.6 Hz), 5.84 (d, 1H, *J* = 8.4 Hz), 3.99 (ddd, 1H, *J* = 12.8, 8.6, 4.3 Hz), 3.82 (dd, 1H, *J* = 11.1, 4.2 Hz), 3.75 (dd, 1H, *J* = 11.1, 4.2 Hz); ^{13}C NMR (75 MHz, CD_3CN) δ 171.9, 141.4, 134.0, 131.6, 130.0, 129.3, 127.3, 63.9, 58.6; HRMS (EI, M^+) Calcd for $C_{11}H_{13}NO_5S$ 271.0515, found 271.0508. Anal. Calcd for $C_{11}H_{13}NO_5S$: C, 48.70; H, 4.83; N, 5.16. Found: C, 48.58; H, 4.74; N, 5.05.

***N*-(*trans*-β-Styrenesulfonyl)-L-serine.** Reaction of *N*-(*trans*-β-styrenesulfonyl)-L-serine methyl ester (3.16 g, 11.08 mmol) and lithium hydroxide monohydrate (0.93 g, 22.15 mmol) as described for **7a** gave the title product (2.73 g, 91%) as a white solid: mp 187−188 °C; $[\alpha]_D^{26}$ +3.3° (*c* 0.15, CH_3CN). Anal. Calcd for $C_{11}H_{13}NO_5S$: C, 48.70; H, 4.83; N, 5.16. Found: C, 48.71; H, 4.83; N, 5.13.

***N*-(*trans*-β-Styrenesulfonyl)-D-serine-β-lactone (8a).** Cyclization of *N*-(*trans*-β−styrenesulfonyl)-D-serine **7a** (0.45 g, 1.67 mmol) by Mitsunobu procedure[28a] using dimethyl azodicarboxylate (0.18 mL, 1.67 mmol) and triphenylphosphine (0.44 g, 1.67 mmol) as described for **5a** gave β-lactone **8a** (60.0 mg, 14%) after recrystallization from $CHCl_3$/hexane: mp 113−114 °C; $[\alpha]_D^{26}$ +6.4° (*c* 0.1 $CHCl_3$); IR ($CHCl_3$ cast) 3285, 3059, 2950, 1831, 1576, 1322, 1145, 745, 689 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.59−7.35 (m, 6H), 6.90 (d, 1H, *J* = 15.4 Hz), 5.27 (d, 1H, *J* = 8.4 Hz), 5.14 (ddd, 1H, *J* = 8.6, 6.4, 4.6 Hz), 4.58 (dd, 1H, *J* = 12.2, 5.8 Hz), 4.39 (dd, 1H, *J* = 12.5, 5.7 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.4, 143.7, 132.7, 132.1, 129.9, 129.3, 125.4, 68.3, 61.4; HRMS (ES, MNa^+) Calcd for $C_{11}H_{11}NO_4SNa$ 276.0307, found 276.0305.

***N*-(*trans*-β-Styrenesulfonyl)-L-serine-β-lactone (8b).** Cyclization of *N*-(*trans*-β-styrenesulfonyl)-L-serine (0.50 g, 1.85 mmol) by Mitsunobu procedure[28a] using dimethyl azodicarboxylate (0.20 mL, 1.85 mmol) and triphenylphosphine (0.49 g, 1.85 mmol) as described for **5a** gave β-lactone **8b** (70.1 mg, 15%): mp 113−114 °C; $[\alpha]_D^{26}$ −4.4° (*c* 0.1, $CHCl_3$).

***N*-(Phenethylsulfonyl)-D-serine.** To a solution of *N*-(*trans*-β-styrenesulfonyl)-D-serine **7a** (1.0 g, 3.67 mmol) in methanol (50 mL) under argon was added 10% palladium on carbon (0.25 g) at room temperature. The flask was evacuated and flushed with hydrogen and then stirred at room temperature under hydrogen overnight. The mixture was then filtered through a pad of Celite and evaporated in vacuo. This procedure was repeated three times to yield a pale white solid which was recrystallized from (MeOH/$CHCl_3$/hexane) to give the title compound (0.91 g, 90%) as a white solid: mp 168−170 °C; $[\alpha]_D^{26}$ +8.5° (*c* 1.0, MeOH); IR ($CHCl_3$ cast) 3438, 3302, 2946, 1727, 1498, 1322, 1151, 1064, 736, 697 cm^{-1}; ^1H NMR (360 MHz, CD_3CN) δ 7.40−7.15 (m, 5H), 5.85 (d, 1H, *J* = 8.7 Hz), 4.11 (ddd, 1H, *J* = 12.9, 8.6, 4.3, Hz), 3.85 (dd, 1H, *J* = 11.2, 4.6 Hz), 3.75 (dd, 1H, *J* = 11.2, 4.6 Hz), 3.40−3.15 (m, 2H), 3.11−2.08 (m, 2H); ^{13}C NMR (125 MHz, CD_3CN) δ 172.3, 139.7, 129.6, 129.5, 127.6, 64.1, 58.8, 55.2, 30.5; HRMS (ES, MNa^+) Calcd for $C_{11}H_{15}NO_5SNa$ 296.0569, found 296.0570.

***N*-(Phenethylsulfonyl)-L-serine.** Reaction of *N*-(*trans*-β-styrenesulfonyl)-L-serine (1.93 g, 7.11 mmol) and 10% palladium on carbon (0.5 g) as described above afforded the title product (1.68 g, 87%) as a white solid: mp 168−170 °C; $[\alpha]_D^{26}$ −8.4° (*c* 1.0, MeOH).

***N*-(Phenethylsulfonyl)-D-serine-β-lactone (9a).** Cyclization of *N*-(phenethylsulfonyl)-D-serine (0.50 g, 1.85 mmol) by Mitsunobu procedure[28a] using dimethyl azodicarboxylate (0.20 mL, 1.85 mmol) and triphenylphosphine (0.49 g, 1.85 mmol) as described for **5a** gave β-lactone **9a** (0.17 g, 37%): mp 119−120 °C; $[\alpha]_D^{26}$ +29.2° (*c* 0.14, $CHCl_3$); IR ($CHCl_3$ cast) 3281, 3050, 2990, 1829, 1496, 1320, 1150, 743, 699 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.43−7.22 (m, 5H), 5.29 (d, 1H, *J* = 9.2 Hz), 5.16 (ddd, 1H, *J* = 9.3, 6.6, 4.7 Hz), 4.58 (dd, 1H, *J* = 12.6, 5.7 Hz), 4.31 (dd, 1H, *J* = 12.5, 5.4 Hz), 3.60−3.41 (m, 2H), 3.28−3.18 (m, 2H,); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.9, 137.9, 129.7, 129.3, 127.8, 68.2, 61.4, 56.7, 30.6; HRMS (EI, M^+) Calcd for $C_{11}H_{13}NO_4S$ 255.0565, found 255.0546. Anal. Calcd for $C_{11}H_{13}NO_4S$: C, 51.75; H, 5.13; N, 5.49. Found: C, 51.42; H, 5.37; N, 5.81.

***N*-(Phenethylsulfonyl)-L-serine-β-lactone (9b).** Cyclization of *N*-(phenethylsulfonyl)-L-serine (0.50 g, 1.85 mmol) by Mitsunobu procedure[28a] using dimethyl azodicarboxylate (0.20 mL, 1.85 mmol) and triphenylphosphine (0.49 g, 1.85 mmol) as described for **5a** gave β-lactone **9b** (0.15 g, 32%): mp 119−120 °C; $[\alpha]_D^{26}$ −35.2° (*c* 0.15, $CHCl_3$). Anal. Calcd for $C_{11}H_{13}NO_4S$: C, 51.75; H, 5.13; N, 5.49. Found: C, 51.63; H, 5.04; N, 5.42.

***N*-(Methylsulfonyl)-L-serine Benzyl Ester.** To a solution of L-serine benzyl ester (2.5 g, 10.79 mmol) in CH_2Cl_2 (50 mL) at 0 °C was added triethylamine (3.6 mL, 25.9 mmol) and then methylsulfonyl chloride (1.0 mL, 12.95 mmol) dropwise over 10 min. The mixture was stirred and warmed to room temperature over 1 h. The solvent was removed in vacuo and the residue purified by flash column chromatography (ethyl acetate/hexane, 1:1) to yield the title compound (0.88 g, 30%) as a white solid: mp 68−70 °C; $[\alpha]_D^{26}$ −17.4° (*c* 1.1, $CHCl_3$); IR ($CHCl_3$ cast) 3501, 3033, 2938, 1739, 1498, 1326, 1153, 1066, 753, 699 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.48−7.30 (m, 5H), 5.62 (d, 1H, *J* = 6.8 Hz), 5.24 (d, 1H, *J* = 13.0 Hz), 5.21 (d, 1H, *J* = 13.0 Hz), 4.25 (ddd, 1H, *J* = 11.9, 7.8, 3.7 Hz), 4.05 (dd, 1H, *J* = 11.3, 4.1 Hz), 3.95 (dd, 1H, *J* = 11.3, 4.1 Hz), 2.98 (s, 3H), 2.24 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ

170.3, 134.9, 128.8, 128.4, 68.0, 63.8, 58.1, 41.6; HRMS (EI, M^+) Calcd for $C_{11}H_{15}NO_5S$ 273.0671, found 273.0668. Anal. Calcd for $C_{11}H_{15}NO_5S$: C, 48.34; H, 5.53; N, 5.12. Found: C, 48.26; H, 5.61; N, 5.03.

***N*-(Methylsulfonyl)-L-serine.** Hydrogenation of *N*-(methylsulfonyl)-L-serine benzyl ester (0.68 g, 2.48 mmol) in methanol (10 mL) with 10% palladium on charcoal (7.0 mg) as described for *N*-(phenethyl)-D-serine furnished the title product (0.37 g, 82%) as a white solid: mp 159−161 °C; $[\alpha]_D^{26}$ −17.3° (*c* 1.0, $CHCl_3$); IR (*µ*scope) 3431, 2947, 1741, 1462, 1326, 1168, 1026 cm^{-1}; ^1H NMR (360 MHz, CD_3OD) *δ* 4.36−4.18 (m, 1H), 3.79 (dd, 1H, *J* = 13.1, 4.9 Hz), 3.72 (dd, 1H, *J* = 13.1, 6.8 Hz), 2.81 (s, 3H); ^{13}C NMR (75 MHz, CD_3OD) *δ* 173.5, 64.3, 59.6, 41.5; MS (CI) *m*/*z* (relative intensity) 201.4 (MNH_4^+, 100%). Anal. Calcd for $C_4H_9NO_5S$: C, 26.23; H, 4.95; N, 7.65. Found: C, 26.34; H, 4.74; N, 7.33.

***N*-(Methylsulfonyl)-L-serine-*β*-lactone (10).** Cyclization of *N*-methylsulfonyl-L-serine (0.20 g, 1.09 mmol) by Mitsunobu procedure[28a] using dimethyl azodicarboxylate (0.12 mL, 1.09 mmol) and triphenylphosphine (0.29 g, 1.09 mmol) as described for **5a** gave *β*-lactone **10** (6.4 mg, 4%): mp 107−109 °C; $[\alpha]_D^{26}$ −17.1° (*c* 0.1, $CHCl_3$); IR (*µ*scope) 3283, 2934, 1828, 1348, 1151, 1071 cm^{-1}; ^1H NMR (360 MHz, CD_3CN) 6.41 (br s, 1H), 5.15 (ddd, 1H, *J* = 18.5, 8.6, 7.8 Hz), 4.53 (dd, 1H, *J* = 6.8, 5.4 Hz), 4.28 (dd, 1H, *J* = 5.4, 4.9 Hz), 3.03 (s, 3H); ^{13}C NMR (125 MHz, CD_3CN) *δ* 170.4, 68.4, 61.7, 42.6; HRMS (EI, M^+) Calcd for $C_4H_7NO_4S$ 165.0096, found 165.0097.

***N*-(Phthalimido)-D-serine-*β*-lactone (12).** Cyclization of *N*-(phthalimido)-D-serine (0.40 g, 1.70 mmol) by Mitsunobu procedure[28a] using dimethyl azodicarboxylate (0.22 mL, 2.04 mmol) and triphenylphosphine (0.49 g, 1.87 mmol) as described for **5a** gave *β*-lactone **12** as a white solid (0.22 g, 60%) after recrystallization from CH_3CN/Et_2O: mp 240−241 °C; $[\alpha]_D^{26}$ +17.1° (*c* 0.1, CH_3CN); IR (*µ*scope) 3345, 2917, 1823, 1775, 1712, 1611 cm^{-1}; ^1H NMR (300 MHz, CD_3CN) 7.92−7.76 (m, 4H), 5.88 (dd, 1H, *J* = 7.0, 5.0 Hz), 4.62−4.53 (m, 2H); ^{13}C NMR (75 MHz, CD_3CN) *δ* 168.5, 167.6, 135.8, 132.6, 124.6, 65.6, 56.2; HRMS (EI, M^+) Calcd for $C_{11}H_7NO_4$ 217.0375, found 217.0371. Anal. Calcd for $C_{11}H_7NO_4$ C, 60.83; H, 3.23; N, 6.59. Found: C, 60.76; H, 3.24; N, 6.59.

L-Threonine Methyl Ester Hydrochloride.[54] Methanol (25 mL) was cooled to 0 °C, and thionyl chloride (1.80 mL, 25.20 mmol) was added dropwise. To the resultant solution of HCl in methanol was added L-threonine (3.0 g, 25.20 mmol), and the reaction mixture was heated under reflux for 1 h. The solvent was removed in vacuo, another 25 mL of a 2 M solution of HCl in methanol, prepared in the same manner as before, was added, and the reaction mixture was heated under reflux for another 1 h. The solvent was removed in vacuo to yield the title compound (4.2 g, 98%) as a white solid: mp 160−163 °C; $[\alpha]_D^{26}$ −10.0° (*c* 0.1, MeOH); IR (*µ*scope) 3366, 3050, 2958, 1746, 1442, 1047 cm^{-1}; ^1H NMR (360 MHz, CD_3OD) *δ* 4.27 (dq, 1H, *J* = 6.5, 4.2 Hz), 3.92 (d, 1H, *J* = 4.2 Hz), 3.84 (s, 3H), 1.31 (d, 3H, *J* = 6.5 Hz); ^{13}C NMR (125 MHz, CD_3OD) *δ* 169.6, 66.3, 59.9, 53.7, 20.5; HRMS (EI, M^+) Calcd for $C_5H_{12}NO_3$ 134.0817, found 134.0810.

D-Threonine Methyl Ester Hydrochloride.[54] Reaction of D-threonine (5.0 g, 41.14 mmol) and thionyl chloride (3.0 mL, 41.14 mmol) in methanol (100 mL) as described for L-threonine methyl ester hydrochloride gave the title compound (7.1 g, 99%) as a white solid: mp 159−162 °C; $[\alpha]_D^{26}$ +7.8° (*c* 0.1, MeOH). Anal. Calcd for $C_5H_{12}ClNO_3$: C, 35.41; H, 7.13; N, 8.26. Found: C, 35.07; H, 7.42; N, 8.01.

L-*allo*-Threonine Methyl Ester Hydrochloride.[54] Reaction of L-*allo*-threonine (3.0 g, 25.20 mmol) and thionyl chloride (1.80 mL, 25.20 mmol) in methanol (25 mL) as described for L-threonine methyl ester hydrochloride gave the title compound (4.22 g, 99%) as a white solid: mp 95−97 °C; $[\alpha]_D^{26}$ +28.0° (*c* 0.1, MeOH); IR (*µ*scope) 3354, 3050, 2895, 1735, 1440, 1059 cm^{-1}; ^1H NMR (360 MHz, CD_3OD) *δ* 4.24 (dq, 1H, *J* = 6.6, 3.5 Hz), 4.08 (d, 1H, *J* = 3.5 Hz), 3.83 (s, 3H), 1.26 (d, 3H, *J* = 6.6 Hz,; ^{13}C NMR (125 MHz, CD_3OD) *δ* 168.8, 66.5, 59.4, 53.5, 18.7; MS (ES) *m*/*z* (relative intensity) 134 (MH^+, 100%). Anal. Calcd for $C_5H_{12}ClNO_3$: C, 35.41; H, 7.13; N, 8.26. Found: C, 35.13; H, 7.24; N, 8.13.

D-*allo*-Threonine Methyl Ester Hydrochloride.[54] Reaction of D-*allo*-threonine (3.0 g, 25.20 mmol) and thionyl chloride (1.80 mL, 25.20 mmol) in methanol (25 mL) as described for L-threonine methyl ester hydrochloride gave the title compound (4.20 g, 98%) as a white solid: mp 95−97 °C; $[\alpha]_D^{26}$ −26.0° (*c* 0.15, MeOH).

***N*-(*trans*-*β*-Styrenesulfonyl)-L-threonine Methyl Ester (16a).** Reaction of L-threonine methyl ester hydrochloride (4.32 g, 25.47 mmol), triethylamine (8.73 mL, 62.98 mmol), and *trans*-*β*-styrenesulfonyl chloride (6.20 g, 30.59 mmol) as described for *N*-(*trans*-*β*-Styrenesulfonyl)-D-serine methyl ester gave the title product **16a** (6.08 g, 80%) as a white solid: mp 100−102 °C; $[\alpha]_D^{26}$ +15.0° (*c* 1.0, $CHCl_3$); IR (*µ*scope) 3526, 3275, 3025, 2981, 1738, 1387, 1149, 1082, 742, 687 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) *δ* 7.51−7.35 (m, 6H), 6.79 (d, 1H, *J* = 15.5 Hz), 5.32 (d, 1H, *J* = 9.7 Hz), 4.11 (dq, 1H, *J* = 6.3, 2.7 Hz), 3.88 (dd, 1H, *J* = 9.6, 2.8 Hz), 3.68 (s, 3H), 1.95 (br s, 1H), 1.37 (d, 3H, *J* = 6.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) *δ* 171.4, 141.9, 132.5, 131.0, 129.2, 128.3, 125.2, 68.3, 60.9, 52.9, 20.1; HRMS (EI, M^+) Calcd for $C_{13}H_{18}NO_5S$ 300.0906, found 300.0893. Anal. Calcd for $C_{13}H_{17}NO_5S$: C, 52.16; H, 5.72; N, 4.68. Found: C, 51.76; H, 5.70; N, 4.64.

***N*-(*trans*-*β*-Styrenesulfonyl)-D-threonine Methyl Ester.** Reaction of D-threonine methyl ester hydrochloride (4.94 g, 29.13 mmol), triethylamine (10.0 mL, 72.83 mmol), and *trans*-*β*-styrenesulfonyl chloride (7.08 g, 34.96 mmol) as described for *N*-(*trans*-*β*-styrenesulfonyl)-D-serine methyl ester gave the title product (5.29 g, 61%) as a white solid: mp 100−102 °C; $[\alpha]_D^{26}$ −15.3° (*c* 1.0, $CHCl_3$). Anal. Calcd for $C_{13}H_{17}NO_5S$: C, 52.16; H, 5.72; N, 4.68. Found: C, 52.40; H, 5.74; N, 4.67.

***N*-(*trans*-*β*-Styrenesulfonyl)-L-*allo*-threonine Methyl Ester.** Reaction of L-*allo*-threonine methyl ester hydrochloride (4.37 g, 25.79 mmol), triethylamine (8.85 mL, 64.48 mmol), and *trans*-*β*-styrenesulfonyl chloride (6.27 g, 30.95 mmol) as described for *N*-(*trans*-*β*-styrenesulfonyl)-D-serine methyl ester furnished the title product (4.46 g, 58%) as a white solid: mp 94−96 °C; $[\alpha]_D^{26}$ +24.8° (*c* 1.0, $CHCl_3$); IR (*µ*scope) 3431, 3264, 3021, 2974, 1737, 1496, 1381, 1154, 1031, 746, 690 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) *δ* 7.48−7.35 (m, 6H), 6.78 (d, 1H, *J* = 15.5 Hz), 5.49 (d, 1H, *J* = 8.9 Hz), 4.18 (dq, 1H, *J* = 6.3, 4.0 Hz), 4.02 (dd, 1H, *J* = 9.0, 3.9 Hz), 3.67 (s, 3H), 2.23 (br s, 1H), 1.22 (d, 3H, *J* = 6 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) *δ* 170.7, 142.5, 132.3, 131.1, 129.1, 128.3, 124.6, 68.6, 60.9, 52.9, 19.0; HRMS (EI, MNa^+) Calcd for $C_{13}H_{18}NO_5S$ 300.0906, found 300.0896. Anal. Calcd for $C_{13}H_{17}NO_5S$: C, 52.16; H, 5.72; N, 4.68. Found: C, 52.02; H, 5.72; N, 4.58.

***N*-(*trans*-*β*-Styrenesulfonyl)-D-*allo*-threonine Methyl Ester.** Reaction of D-*allo*-threonine methyl ester hydrochloride (4.0 g, 23.62 mmol), triethylamine (8.18 mL, 59.04 mmol), and *trans*-*β*-styrenesulfonyl chloride (5.74 g, 28.34 mmol) as described for *N*-(*trans*-*β*-styrenesulfonyl)-D-serine methyl ester afforded the title product (4.51 g, 64%) as a white solid: mp 94−96 °C; $[\alpha]_D^{26}$ −23.8° (*c* 1.0, $CHCl_3$). Anal. Calcd for $C_{13}H_{17}NO_5S$: C, 52.16; H, 5.72; N, 4.68. Found: C, 52.21; H, 5.61; N, 4.58.

***N*-(*trans*-*β*-Styrenesulfonyl)-L-threonine (17a).** Reaction of *N*-(*trans*-*β*-styrenesulfonyl)-L-threonine methyl ester **16a** (3.0 g, 10.02 mmol) and lithium hydroxide monohydrate (0.84 g, 20.04 mmol) as described for **7a** afforded **17a** (2.83 g, 99%) as a white solid: mp 145−147 °C; $[\alpha]_D^{26}$ +39.9° (*c* 1.0, MeOH); IR (*µ*scope) 3447, 3304, 3026, 2982, 1720, 1576, 1449, 1372, 1132, 1030, 746, 690 cm^{-1}; ^1H NMR (360 MHz, CD_3CN) *δ* 7.61−7.42 (m, 6H), 6.91 (d, 1H, *J* = 15.5 Hz), 5.69 (d, 1H, *J* = 9.5 Hz), 4.19 (dq, 1H, *J* = 6.4, 3.1 Hz), 3.81 (dd, 1H, *J* = 9.5, 3.1 Hz), 2.25 (br s, 1H), 1.21 (d, 3H, *J* = 6.4 Hz); ^{13}C NMR (125 MHz, CD_3CN) *δ* 172.2, 141.3, 133.9, 131.6, 130.0, 129.3, 127.4, 68.5, 61.9, 20.3; HRMS (EI, M^+) Calcd for $C_{12}H_{16}NO_5S$ 286.0749, found 286.0739. Anal. Calcd for $C_{12}H_{15}NO_5S$: C, 50.52; H, 5.30; N, 4.91. Found: C, 50.24; H, 5.25; N, 4.88.

(54) Anderson, P. G.; Guijarro, D.; Tanner, D. *J. Org. Chem.* **1997**, *62*, 2, 7364−7375.

***N*-(*trans*-β-Styrenesulfonyl)-D-threonine.** Reaction of *N*-(*trans*-β-styrenesulfonyl)-D-threonine methyl ester (3.0 g, 10.02 mmol) and lithium hydroxide monohydrate (0.84 g, 20.04 mmol) as described for **7a** gave the title product (2.74 g, 96%) as a white solid: mp 145−147 °C; $[\alpha]_D^{26}$ −38.8° (*c* 1.0, MeOH). Anal. Calcd for $C_{12}H_{15}NO_5S$: C, 50.52; H, 5.30; N, 4.91. Found: C, 50.45; H, 5.37; N, 4.82.

***N*-(*trans*-β-Styrenesulfonyl)-L-*allo*-threonine.** Reaction of *N*-(*trans*-β-styrenesulfonyl)-L-*allo*-threonine methyl ester (3.0 g, 10.02 mmol) and lithium hydroxide monohydrate (0.84 g, 20.04 mmol) as described for **7a** gave the title product (2.74 g, 96%) as a white solid: mp 140−142 °C; $[\alpha]_D^{26}$ +33.6° (*c* 1.0, MeOH); IR (μscope) 3430, 3255, 3050, 2982, 1719, 1576, 1449, 1327, 1135, 1031, 745, 690 cm^{-1}; ^1H NMR (360 MHz, CD_3CN) δ 7.59−7.54 (m, 2H), 7.46−7.38 (m, 4H), 6.90 (d, 1H, *J* = 15.5 Hz), 5.83 (d, 1H, *J* = 9.1 Hz), 4.00 (quintet, 1H, *J* = 6.3 Hz), 3.84 (dd, 1H, *J* = 9.1, 6.1 Hz), 1.18 (d, 3H, *J* = 6.4 Hz); ^{13}C NMR (75 MHz, CD_3CN) δ 171.8, 141.6, 134.0, 131.7, 130.0, 129.3, 127.3, 68.8, 62.3, 19.6; HRMS (ES, MH^+) Calcd for $C_{12}H_{16}NO_5S$ 286.0749, found 286.0747.

***N*-(*trans*-β-Styrenesulfonyl)-D-*allo*-threonine.** Reaction of *N*-(*trans*-β-styrenesulfonyl)-D-*allo*-threonine methyl ester (3.0 g, 10.02 mmol) and lithium hydroxide monohydrate (0.84 g, 20.04 mmol) as described for **7a** gave the title product (2.80 g, 98%) as a white solid: mp 140−142 °C; $[\alpha]_D^{26}$ −31.6° (*c* 1.0, MeOH). Anal. Calcd for $C_{12}H_{15}NO_5S$: C, 50.52; H, 5.30; N, 4.91. Found: C, 50.77; H, 5.23; N, 4.84.

***N*-(*trans*-β-Styrenesulfonyl)-L-threonine-β-lactone (18a).** A suspension of *N*-(*trans*-β-phenethylsulfonyl)-L-threonine **17a** (0.5 g, 1.74 mmol) in CH_2Cl_2 (40 mL) was cooled to 0 °C and treated with triethylamine (0.72 mL, 5.22 mmol) followed by benzotriazole-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP)[41] (0.92 g, 2.11 mmol). The cooling bath was removed, and the reaction mixture was stirred at room temperature for 3 h. The solvent was removed in vacuo, and the residue purified by flash column chromatography (ethyl acetate/hexane, 1:1), followed by recrystallization from ($CHCl_3$/hexane) to give β-lactone **18a** (0.29 g, 61%) as a white solid: mp 128−129 °C; $[\alpha]_D^{26}$ −19.5° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$ cast) 2983, 1824, 1614, 1576, 1449 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.32−7.58 (m, 6H), 6.83 (d, 1H, *J* = 15.4 Hz), 5.64−5.80 (m, 1H), 5.10 (dd, 1H, *J* = 9.4, 6.0 Hz), 4.88 (quintet, 1H, *J* = 6.3 Hz), 1.52 (d, 3H, *J* = 9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.4, 142.9, 132.1, 131.4, 129.2, 128.6, 124.5, 75.0, 61.4, 15.4; HRMS (EI, M^+) Calcd for $C_{12}H_{13}NO_4S$ 267.0565, found 267.0559.

***N*-(*trans*-β-Styrenesulfonyl)-D-threonine-β-lactone (18b).** Cyclization of *N*-(*trans*-β-phenethylsulfonyl)-D-threonine (0.50 g, 1.74 mmol) using BOP (0.92 g, 2.11 mmol) and triethylamine (0.72 mL, 5.22 mmol) as described for **18a** gave β-lactone **18b** (0.33 g, 72%) as a white solid: mp 129−130 °C; $[\alpha]_D^{26}$ +16.0° (*c* 1.0, $CHCl_3$).

***N*-(*trans*-β-Styrenesulfonyl)-L-*allo*-threonine-β-lactone (18c).** Cyclization of *N*-(*trans*-β-phenethylsulfonyl)-L-*allo*-threonine (0.50 g, 1.74 mmol) using BOP (0.92 g, 2.11 mmol) and triethylamine (0.72 mL, 5.22 mmol) as described for **18a** gave β-lactone **18c** (0.39 g, 61%) as a white solid: mp 130−131 °C; $[\alpha]_D^{26}$ −56.0° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$ cast) 3278, 2977, 1831, 1614, 1495, 1449 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.40−7.55 (m, 6H), 6.85 (d, 1H, *J* = 15.4 Hz), 5.94 (d, 1H, *J* = 8.2 Hz), 4.70 (dt, 1H, *J* = 19.5, 6 Hz), 4.50 (dd, 1H, *J* = 8.5, 4.0 Hz), 1.78 (d, 3H, *J* = 7.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.9, 143.0, 132.1, 131.4, 129.3, 128.6, 124.6, 78.3, 65.2, 18.7; HRMS (EI, M^+) Calcd for $C_{12}H_{13}NO_4S$ 267.0565, found 267.0557.

***N*-(*trans*-β-Styrenesulfonyl)-D-*allo*-threonine-β-lactone (18d).** Cyclization of *N*-(*trans*-β-phenethylsulfonyl)-D-*allo*-threonine (0.50 g, 1.74 mmol) using BOP (0.92 g, 2.11 mmol) and triethylamine (0.72 mL, 5.22 mmol) as described for **18a** gave β-lactone **18d** (0.29 g, 63%) as a white solid: mp 129−130 °C; $[\alpha]_D^{26}$ +51.7° (*c* 1.0, $CHCl_3$).

***N*-(Phenethylsulfonyl)-L-threonine.** Hydrogenation of *N*-(*trans*-β-styrenesulfonyl)-L-threonine **17a** (2.0 g, 7.01 mmol) as described for *N*-(phenethylsulfonyl)-D-serine furnished the title product (1.88 g, 93%) as a white solid: mp 157−159 °C; $[\alpha]_D^{26}$ −10.1° (*c* 1.0, MeOH); IR (μscope) 3470, 3303, 2979, 1718, 1335, 1152, 1068, 750, 690 cm^{-1}; ^1H NMR (360 MHz, CD_3CN) δ 7.38−7.15 (m, 5H), 5.65 (d, 1H, *J* = 9.2 Hz), 4.23 (dq, 1H, *J* = 6.3, 2.9 Hz), 3.93 (dd, 1H, *J* = 9.4, 2.9 Hz), 3.32−3.15 (m, 2H), 3.12−2.95 (m, 2H), 1.21 (d, 3H, *J* = 6.3 Hz); ^{13}C NMR (125 MHz, CD_3CN) δ 172.6, 139.7, 129.6, 129.5, 127.6, 68.5, 62.1, 55.2, 30.5, 20.3; HRMS (EI, M^+) Calcd for $C_{12}H_{18}NO_5S$ 288.0906, found 288.0898. Anal. Calcd for $C_{12}H_{18}NO_5S$: C, 50.16; H, 5.96; N, 4.87. Found: C, 49.88; H, 5.97; N, 4.90.

***N*-(Phenethylsulfonyl)-D-threonine.** Reaction of *N*-(*trans*-β-styrenesulfonyl)-D-threonine (1.97 g, 6.90 mmol) and 10% palladium on carbon (0.5 g) as described for *N*-(phenethylsulfonyl)-D-serine gave the title product (1.61 g, 81%) as a white solid: mp 157−159 °C; $[\alpha]_D^{26}$ +10.0° (*c* 1.0, MeOH). Anal. Calcd for $C_{12}H_{18}NO_5S$: C, 50.16; H, 5.96; N, 4.87. Found: C, 49.84; H, 6.14; N, 4.92.

***N*-(Phenethylsulfonyl)-L-*allo*-threonine.** Reaction of *N*-(*trans*-β-styrenesulfonyl)-L-*allo*-threonine (1.79 g, 6.28 mmol) and 10% palladium on carbon (0.5 g) as described for *N*-(phenethylsulfonyl)-D-serine gave the title product (1.57 g, 87%) as a white solid: mp 122−124 °C; $[\alpha]_D^{26}$ +4.3° (*c* 4.6, CO-$(CH_3)_2$); IR (μscope) 3482, 3291, 2973, 1726, 1350, 1127, 1087, 745, 697 cm^{-1}; ^1H NMR (360 MHz, CD_3CN) δ 7.35−7.17 (m, 5H), 5.74 (d, 1H, *J* = 8.7 Hz), 3.95 (quintet, 1H, *J* = 6.3, 4.9 Hz), 4.01 (dd, 1H, *J* = 8.9, 4.9 Hz), 3.38−3.22 (m, 2H), 3.15−3.00 (m, 2H), 1.18 (d, 3H, *J* = 6.3 Hz); ^{13}C NMR (125 MHz, CD_3CN) δ 171.9, 139.6, 129.5, 129.4, 127.5, 68.6, 62.3, 55.1, 30.4, 19.1; HRMS (EI, M^+) Calcd for $C_{12}H_{18}NO_5S$ 288.0906, found 288.0880.

***N*-(Phenethylsulfonyl)-D-*allo*-threonine.** Reaction of *N*-(*trans*-β-styrenesulfonyl)-D-*allo*-threonine (2.0 g, 7.01 mmol) and 10% palladium on carbon (0.5 g) as described for *N*-(phenethylsulfonyl)-D-serine gave the title product (1.74 g, 87%) as a white solid: mp 122−124 °C; $[\alpha]_D^{26}$ −3.8° (*c* 5.3, CO-$(CH_3)_2$).

***N*-(Phenethylsulfonyl)-L-threonine-β-lactone (19a).** Cyclization of *N*-(phenethylsulfonyl)-L-threonine (0.50 g, 1.74 mmol) using BOP (0.92 g, 2.11 mmol) and triethylamine (0.72 mL, 5.22 mmol) as described for **18a** gave β-lactone **19a** (0.33 g, 72%) as a white solid: mp 139−141 °C; $[\alpha]_D^{26}$ −23.5° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$ cast) 3319, 3050, 2980, 1813, 1497, 1338, 1150, 750, 698 cm^{-1}; ^1H NMR (360 MHz, CD_2Cl_2) δ 7.44−7.25 (m, 5H), 5.19 (dd, 1H, *J* = 10.1, 6.3 Hz), 5.65 (d, 1H, *J* = 10.2 Hz), 4.23 (quintet, 1H, *J* = 6.3 Hz), 3.54−3.38 (m, 2H), 3.28−3.09 (m, 2H), 1.47 (d, 3H, *J* = 6.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.0, 137.8, 129.7, 129.3, 128.0, 75.0, 62.3, 56.4, 30.7, 16.0; HRMS (EI, M^+) Calcd for $C_{12}H_{15}NO_4S$ 269.0722, found 269.0719. Anal. Calcd for $C_{12}H_{15}NO_4S$: C, 53.52; H, 5.61; N, 5.20. Found: C, 53.53; H, 5.52; N, 5.16.

***N*-(Phenethylsulfonyl)-D-threonine-β-lactone (19b).** Cyclization of *N*-(phenethylsulfonyl)-D-threonine (0.50 g, 1.74 mmol) using BOP (0.92 g, 2.11 mmol) and triethylamine (0.72 mL, 5.22 mmol) as described for **18a** gave β-lactone **19b** (0.35 g, 75%) as a white solid: mp 139−141 °C; $[\alpha]_D^{26}$ −23.6° (*c* 1.0, $CHCl_3$). Anal. Calcd for $C_{12}H_{15}NO_4S$: C, 53.52; H, 5.61; N, 5.20. Found: C, 53.46; H, 5.48; N, 5.12.

***N*-(Phenethylsulfonyl)-L-*allo*-threonine-β-lactone (19c).** Cyclization of *N*-(phenethylsulfonyl)-L-*allo*-threonine (0.50 g, 1.74 mmol) using BOP (0.92 g, 2.11 mmol) and triethylamine (0.72 mL, 5.22 mmol) as described for **18a** gave β-lactone **19c** (0.36 g, 78%) as a white solid: mp 130−132 °C; $[\alpha]_D^{26}$ −71.3° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$ cast) 3302, 3030, 2971, 1828, 1496, 1360, 1147, 760, 696 cm^{-1}; ^1H NMR (360 MHz, CD_2Cl_2) δ 7.44−7.26 (m, 5H), 5.01 (d, 1H, *J* = 8.3 Hz), 4.63 (dq, 1H, *J* = 6.1, 4.3 Hz), 4.54 (dd, 1H, *J* = 8.8, 4.3 Hz), 3.54−3.88 (m, 2H), 3.32−3.08 (m, 2H), 1.59 (d, 3H, *J* = 6.1 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.8, 137.9, 129.7, 129.2, 127.9, 78.6, 66.0, 56.6, 30.7, 19.3; HRMS (ES, MNa^+) Calcd for $C_{12}H_{15}NO_4SNa$ 292.0611, found 292.0623. Anal. Calcd for $C_{12}H_{15}NO_4S$: C, 53.52; H, 5.61; N, 5.20. Found: C, 53.16; H, 5.44; N, 5.05.

***N*-(Phenethylsulfonyl)-D-*allo*-threonine-β-lactone (19d).** Cyclization of *N*-(phenethylsulfonyl)-D-*allo*-threonine (0.50 g, 1.74 mmol) using BOP (0.92 g, 2.11 mmol) and triethylamine (0.72 mL, 5.22 mmol) as described for **18a** gave β-lactone **19d** (0.42 g, 89%) as a white solid: mp 130−132 °C; $[\alpha]_D^{26}$ +65.4° (*c*

1.0, $CHCl_3$). Anal. Calcd for $C_{12}H_{15}NO_4S$: C, 53.52; H, 5.61; N, 5.20. Found: C, 53.14; H, 5.49; N, 5.09.

***N*-(Phenylmethanesulfonyl)-D-*allo*-threonine.**[55] D-*allo*-Threonine (0.30 g, 2.52 mmol) was dissolved in water (15 mL) and treated with Na_2CO_3 (1.07 g, 10.1 mmol) followed by α-toluenesulfonyl chloride (0.28 g, 3.02 mmol). The mixture was stirred for 24 h. The reaction mixture was then washed with diethyl ether (2 × 10 mL), acidified to pH 2 with 1 N HCl, and then extracted with ethyl acetate (3 × 10 mL). The combined organic layers were dried over $MgSO_4$ and evaporation of the volatiles in vacuo followed by recrystallization of the crude product from MeOH/Et_2O/hexane furnished a white solid (0.95 g, 28%): mp 138−139 °C; IR (μscope) 3489, 3207, 2974, 1732, 1696, 1456 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.44−7.32 (m, 5H), 4.38 (d, 1H, J = 13.7 Hz), 4.34 (d, 1H, J = 13.7 Hz), 4.0 (quintet, J = 5.2 Hz, 1H), 3.90 (d, 1H, J = 5 Hz), 1.18 (d, 3H, J = 7.5 Hz); ^{13}C NMR (75 MHz, CD_3OD) δ 173.4, 132.1, 131.1, 129.4, 129.3, 69.3, 63.5, 60.5, 19.1; HRMS (EI, M^+) Calcd for $C_{11}H_{15}NO_5S$ 273.0671, found 273.0658.

***N*-(Phenylmethanesulfonyl)-D-*allo*-threonine-β-lactone (20).** Cyclization of *N*-(phenylmethanesulfonyl)-D-*allo*-threonine (0.10 g, 0.37 mmol) using BOP (0.20 g, 0.45 mmol) and triethylamine (0.16 mL, 1.12 mmol) as described for **18a** gave β-lactone **20** (0.06 g, 64%) as a white solid after recrystallization from $CHCl_3$/hexane: mp 150−151 °C; $[\alpha]_D^{26}$ +47.5° (*c* 0.2, MeOH); IR ($CHCl_3$ cast) 3278, 2926, 1825, 1759, 1495, 1386, 1331 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.52−7.38 (m, 5H), 4.98 (d, 1H, J = 8.3 Hz), 4.63 (dq, 1H, J = 6.1, 4.3 Hz), 4.40 (d, 2H, J = 14 Hz), 4.30 (dd, 1H, J = 8.4, 4.2 Hz), 1.42 (d, 3H, J = 6.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.9, 131.0, 129.3, 129.1, 128.4, 78.3, 65.6, 60.51, 18.5; HRMS (EI, M^+) Calcd for $C_{11}H_{13}NO_4S$ 255.0572, found 255.0565. Anal. Calcd for $C_{11}H_{13}NO_4S$: C, 51.75; H, 5.13; N, 5.49. Found: C, 51.69; H, 5.07; N, 5.38.

***N*-(Phenylsulfonyl)-D-*allo*-threonine.** Reaction of D-*allo*-threonine (0.44 g, 3.69 mmol) with benzenesulfonyl chloride (0.56 g, 4.43 mmol) and Na_2CO_3 (1.57 g, 14.7 mmol) as described for *N*-(phenylmethanesulfonyl)-D-*allo*-threonine gave the title product as a white solid (0.46 g, 48.5%): mp 171−173 °C; $[\alpha]_D^{26}$ −17.2° (*c* 0.3, MeOH), lit.[55] $[\alpha]_D^{26}$ −16.8° (*c* 1.6, MeOH); ^1H NMR (300 MHz, CD_3OD) δ 7.84 (d, 2H, J = 7.5 Hz), 7.60−7.44 (m, 3H), 3.85 (quintet, 1H, J = 6.4 Hz), 3.78 (d, 1H, J = 5.9 Hz), 1.38 (d, 3H, J = 6.3 Hz; HRMS (ES, MNa^+) Calcd for $C_{10}H_{13}NO_4SNa$ 282.0412, found 282.0405.

***N*-(Phenylsulfonyl)-D-*allo*-threonine-β-lactone (21).** Cyclization of *N*-(benzenesulfonyl)-D-*allo*-threonine (0.44 g, 1.69 mmol) using BOP (0.89 g, 2.03 mmol) and triethylamine (0.71 mL, 5.07 mmol) as described for **18a** gave β-lactone **21** (0.26 g, 64%) as a white solid: mp 110−111 °C; $[\alpha]_D^{26}$ +9.2° (*c* 0.3, $CHCl_3$); IR ($CHCl_3$ cast) 3265, 3069, 2916, 1832, 1447, 1335 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.85 (d, 2H, J = 10.0 Hz), 7.64−7.52 (m, 3H), 5.50 (br d, 1H, J = 5.5 Hz), 4.68−4.59 (m, 1H), 4.42 (dd, 1H, J = 7.5, 4.0 Hz), 1.58 (d, 3H, J = 6.3 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.1, 139.2, 133.6, 129.6, 127.2, 78.3, 65.2, 18.7; HRMS (ES, MNa^+) Calcd for $C_{10}H_{11}NO_4SNa$ 264.0307, found 264.0305. Anal. Calcd for $C_{10}H_{11}NO_4S$; C, 49.78; H, 4.60; N, 5.81. Found: C, 49.52; H, 4.41; N, 5.73.

***N*-(Benzyloxycarbonyl)-D-*allo*-threonine-β-lactone (22).** Cyclization of *N*-Cbz-D-*allo*-threonine (0.32 g, 1.27 mmol) using BOP (0.67 g, 1.52 mmol) and triethylamine (0.53 mL, 3.79 mmol) as described for **18a** gave β-lactone **22** (0.23 g, 68%) as a white solid: mp 136−137 °C; $[\alpha]_D^{26}$ +61.0° (*c* 0.4, MeOH); IR (μscope) 3312, 2972, 1830, 1535, 1382 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.40 (br s, 5H), 5.40 (br s, 1H), 5.14 (br s, 2H), 4.80−4.68 (m, 1H), 4.65−4.58 (m, 1H), 1.60 (d, 3H, J = 6.3 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.5, 155.3, 135.6, 128.7, 128.6, 128.4, 128.2, 76.7, 67.8, 64.5, 18.9; HRMS (EI, M^+) Calcd for $C_{12}H_{13}NO_4$ 235.0845, found 235.0835. Anal. Calcd for $C_{12}H_{13}NO_4$: C, 61.27; H, 5.57; N, 5.95. Found: C, 61.16; H, 5.73; N, 5.99.

(1′*RS*,2*R*,5*R*,6*R*)-5-[α-*p*-Methoxybenzyl]-2-(*tert*-butyl)-6-methyl-1,3-dioxan-4-one (24). This material was prepared from a modified procedure of Gautschi et al.[43b] To a solution of *t*-BuLi (1.88 mL, 1.7 M in pentane, 3.19 mmol) in THF (10 mL) at −78 °C under an atmosphere of argon was added a precooled solution of dioxanone **23** (0.5 g, 2.90 mmol) in THF (3 mL) via cannula. The reaction mixture was stirred for 45 min after which it was treated dropwise with *p*-anisaldehyde (0.40 mL, 3.77 mmol). The mixture was stirred for a further 24 h at −78 °C after which it was quenched with sat. NH_4Cl (10 mL) and then extracted with EtOAc (3 × 5 mL) and dried over $MgSO_4$. The solvent was evaporated in vacuo, the crude product was purified by flash column chromatography (hexane/Et_2O, 3:1) and the product was recrystallized from (Et_2O/hexane) to give a white solid (0.59 g, 66%); mp 157−158 °C IR (μscope) 3408, 2960, 2918, 1795, 1720, 1611, 1511 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$)(1:1 mixture of diastereomers) δ 7.25 (dd, 2H, J = 8.9, 4.2 Hz), 6.90 (d, 2H, J = 8.8 Hz), 5.32 (d, 1H, J = 3.4 Hz), 4.83 (s, 1H) 3.98−3.88 (m, 1H), 3.80 (s, 3H), 2.78 (dd, 1H, J = 9.2, 3.4 Hz,), 0.98 (s, 9H), 0.85 (d, 3H, J = 7.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.9, 171.4, 159.4, 132.9, 128.8, 128.2, 127.8, 126.9, 114.5, 114.1, 107.7, 102.0, 74.2, 72.5, 71.2, 69.3, 55.4, 55.3, 46.9, 35.1, 34.9, 24.7, 24.4, 23.9, 21.1, 19.2; HRMS (EI, M^+) Calcd for $C_{17}H_{24}O_5$ 235.0845, found 235.0835. Anal. Calcd for $C_{17}H_{24}O_5$: C, 66.21; H, 7.84; Found: C, 65.99; H, 7.79.

(2*R*,3*R*)-2-(*p*-Methoxybenzyl)-3-hydroxybutanoic acid (25) and (1′*S*,2*R*,3*R*)-2-(1′-Hydroxy-*p*-methoxybenzyl)-3-hydroxybutanoic Acid (26). Reaction of dioxanone **24** (0.4 g, 1.95 mmol) and 10% palladium on carbon (0.04 g) for 24 h as described for *N*-(phenethylsulfonyl)-D-serine gave **25** (0.13 g, 40%) and **26** (0.14 g, 35%) as white solids after purification by flash column chromatography (EtOAc/hexane, 2:1 and 1% AcOH). These materials were recrystallized from ($CHCl_3$/hexane). Data for **25**: mp 107−108 °C; $[\alpha]_D^{26}$ −39.7° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$ cast) 3259, 3059, 2936, 2835, 1696, 1610, 1582, 1512 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.11 (d, 2H, J = 8.6 Hz), 6.79 (d, 2H, J = 8.6 Hz), 3.85 (dq, 1H, J = 6.4, 1.2 Hz), 3.75 (s, 3H), 2.92 (d, 2H, J = 8.6 Hz), 2.62 (dt, 1H,, J = 5.1, 1.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.2, 158.4, 130.3, 129.9, 114.0, 67.3, 55.3, 54.3, 34.4, 21.8; HRMS (EI, M^+) Calcd for $C_{12}H_{16}O_5$ 240.0998, found 240.0997. Anal. Calcd for $C_{12}H_{16}O_5$: C, 64.85; H, 6.35; Found: C, 64.66; H, 6.34.

Data for **26**: mp 128−129 °C; $[\alpha]_D^{26}$ −5.8° (*c* 0.9, $CHCl_3$); IR ($CHCl_3$ cast) 3387, 2934, 1708, 1612, 1586, 1456 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.22 (d, 2H, J = 11.3 Hz), 6.84 (d, 2H, J = 11.3 Hz), 5.18 (d, 1H, J = 6.0 Hz), 4.80 (br q, 1H, J = 4.5 Hz), 3.78 (s, 3H), 2.70 (dd, 1H, J = 6.0, 2.3 Hz), 1.24 (d, 3H, J = 6.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.0, 159.2, 133.2, 127.3, 113.9, 172.9, 65.7, 57.2, 55.3, 21.4; HRMS (EI, M^+) Calcd for $C_{12}H_{16}O_4$ 224.1049, found 224.1053.

(2*R*,3*R*)-3-(*p*-Methoxybenzyl)-4-methyloxetan-2-one (27). Cyclization of hydroxy acid **25** (0.14 g, 0.63 mmol) using BOP (0.33 g, 0.75 mmol) and triethylamine (0.26 mL, 1.87 mmol) as described for **18a** gave β-lactone **27** (0.09 g, 73%) as a white solid after recrystallization from Et_2O/petroleum ether: mp 34−35 °C; $[\alpha]_D^{26}$ −28.7° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$ cast) 2975, 2932, 1816, 1612, 1583, 1442, 1385 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.18 (d, 2H, J = 10 Hz), 6.82 (d, 2H, J = 10 Hz), 4.42 (dq, 1H, J = 6.1, 4.1 Hz), 3.78 (s, 3H), 3.40 (ddd, 1H, J = 7.2, 5.8, 4.0 Hz), 3.0 (ddd, 2H, J = 23.5, 14.6, 8.9 Hz), 1.42 (d, 3H, J = 10 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 158.7, 129.6, 128.9, 114.3, 74.0, 58.7, 55.3, 32.6, 20.1; HRMS (EI, M^+) Calcd for $C_{12}H_{14}O_3$ 206.0943, found 206.0941.

(1′*S*,2*R*,3*R*)-2-(1′-Hydroxy-*p*-methoxybenzyl)-4-methyloxetan-2-one (28). Cyclization of hydroxy acid **26** (0.08 g, 0.34 mmol) using BOP (0.19 g, 0.43 mmol) and triethylamine (0.15 mL, 1.02 mmol) as described for **18a** gave β-lactone **28** (0.05 g, 70%) as a white solid after recrystallization from CH_2Cl_2/hexane: mp 88−89 °C; $[\alpha]_D^{26}$ −85.5° (*c* 0.3, $CHCl_3$); IR ($CHCl_3$ cast) 3411, 2984, 2960, 1807, 1612, 1514, 1448 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.24 (d, 2H, J = 11.3 Hz), 6.90 (d, 2H, J = 11.3 Hz), 5.18 (d, 1H, J = 4.4 Hz), 4.96−4.85 (m, 1H), 3.80 (s, 3H,), 3.52 (t, 1H, J = 4.4 Hz), 1.42 (d, 3H, J = 10 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.6, 132.6, 126.7, 114.3, 70.6, 69.3, 64.8, 55.4, 20.1; HRMS (EI, M^+) Calcd for $C_{12}H_{14}O_2$

(55) Maurer, P. J.; Takahata, H.; Rapoport, H. *J. Am. Chem. Soc.* **1984**, *106*, 1095−1098.

222.0892, found 222.0887. Anal. Calcd for $C_{12}H_{14}O_2$: C, 64.85; H, 6.35; Found: C, 64.66; H, 6.34.

2-Diazo-3-oxo-3-phenylpropionic Acid Ethyl Ester (30).[45] To a solution of ethyl benzoyl acetate **29** (2.5 g, 13.0 mmol) and triethylamine (1.98 mL, 14.3 mmol) in acetonitrile (25 mL) was added tosyl azide (2.56 g, 13.0 mmol). The reaction mixture was stirred for 16 h after which the solvent was removed in vacuo and the residue purified directly by flash column chromatography (Et_2O/hexane, 1:2) to give a light greenish oil (2.63 g, 93%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40−7.65 (m, 5H), 4.25 (q, 2H, J = 10.0 Hz), 1.25 (t, 3H, J = 11.3 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 189.9, 161.0, 137.2, 132.3, 128.4, 127.9, 76.2, 61.6, 14.2; HRMS (EI, M^+) Calcd for $C_{11}H_{10}O_3N_2$ 218.0689, found 218.0691.

(3*RS*,4*RS*)-3-Benzoyl-4-methyloxetan-2-one (31). A solution of diazo-ketoester **30** (0.8 g, 3.67 mmol) in CH_2Cl_2 (10 mL) was added dropwise over 2 h to a refluxing solution of rhodium(II) acetate dimer (8.0 mg, 0.04 mmol) in CH_2Cl_2 (40 mL). The reaction mixture was heated under reflux for a further 1 h and the solvent evaporated in vacuo. The residue was diluted with Et_2O (30 mL) and filtered through a pad of Celite. Concentration of the filtrate followed by purification of the crude product by flash column chromatography (Et_2O/hexane, 1:2) afforded **31** which was recrystallized from Et_2O/hexane to give a white solid (0.36 g, 52%): mp 64−65 °C; IR ($CHCl_3$ cast) 3024, 2977, 2916, 1817, 1681, 1597, 1449 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 (d, 2H, J = 11.3 Hz), 7.65−7.50 (m, 3H), 5.38 (dq, 1H, J = 4.2, 1.2 Hz), 4.94 (d, 1H, J = 4.3 Hz), 1.75 (d, 3H, J = 6.3 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.23, 163.6, 134.9, 134.9, 134.5, 129.3, 129.0, 70.2, 66.5, 19.7; HRMS (ES, MNa^+) Calcd for $C_{11}H_{10}O_3Na$ 213.0528, found 213.0527. Anal. Calcd for $C_{11}H_{10}O_3$: C, 69.46; H, 5.30; Found: C, 69.36; H, 5.28.

(3*S*)-3-Benzyloxycarbonylamino-2-azetidinone (33).[46a] A suspension of $NaHCO_3$ (0.33 g, 3.90 mmol) in CH_3CN (40 mL) was stirred and heated to reflux. To this was added methanesulfonyl chloride (0.08 mL, 0.97 mmol) followed by portionwise addition of the amino acid **32** (0.2 g, 0.84 mmol) over 4 h. The reaction mixture was heated under reflux overnight and the solid removed by filtration at 60 °C. Concentration of the filtrate in vacuo followed by purification of the crude product by flash column chromatography (100% EtOAc) afforded **33** which was recrystallized from $CHCl_3$/hexane to give a white powder (0.05 g, 49%): mp 164−165 °C, lit.[46c] mp 164−165 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (br s, 5H), 5.80 (br s, 1H), 5.38 (d, 1H, J = 6.1 Hz), 5.10 (br s, 2H), 3.60 (dd, 1H, J = 10.6, 5.1 Hz), 3.39 (br s, 1H); HRMS (EI, M^+) Calcd for $C_{11}H_{12}O_3N_2$ 220.0848, found 220.0826.

(*S*)-*N*-(Benzyloxycarbonyl)-3-amino-2-methyleneoxetane (34). Dimethyltitanocene[47b] (2.87 mL, 0.5M in toluene, 1.44 mmol) and β-lactone **5b** (0.15 g, 0.72 mmol) were stirred at 80 °C under an atmosphere of argon in the dark. The reaction was monitored by TLC, and after the disappearance of the starting material (2 h), the reaction mixture was allowed to cool and treated with an equal volume of petroleum ether. The reaction mixture was stirred for a further 30 min, and the yellow precipitate was filtered through a pad of Celite and washed with more petroleum ether until the filtrate was colorless. The solvent was removed in vacuo, and the residue purified by flash column chromatography (EtOAc/hexane, 1:4) to afford **34** which was recrystallized from Et_2O/pentane to give a white crystalline solid (0.04 g, 24%): mp 76−77 °C; $[\alpha]_D^{26}$ +30.6° (*c* 0.3, $CHCl_3$); IR ($CHCl_3$ cast) 3323, 3033, 2961, 1695, 1595, 1528, 1454 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 (br s, 5H), 5.25 (br s, 1H), 5.10 (s, 1H), 4.84−4.75 (m, 1H), 4.42 (dd, 1H, J = 5.2, 2.8 Hz), 4.22 (d, 1H, J = 4.2 Hz), 4.0 (d, 1H, J = 4.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.5, 155.3, 135.9, 128.7, 128.4, 128.3, 81.2, 77.2, 67.3, 50.8; HRMS (ES, MNa^+) Calcd for $C_{12}H_{13}O_3NNa$ 242.0793, found 242.0793.

(*R*,*S*)-*N*-(Benzyloxycarbonyl)cyclobutanone (36). A mixture of benzyl carbamate (0.24 g, 1.56 mmol) and HCl-saturated diethyl ether (3 mL) was cooled to 0 °C and 1,2-bis(dimethylsilyoxy)cyclobutene **35**[48] (0.3 g, 1.30 mmol) was added dropwise with stirring under argon. The reaction mixture was then heated to 80 °C for 4 h. The solvent was removed in vacuo and the residue purified by flash column chromatography (Et_2O/hexane, 1:1) to afford **36** as a colorless oil (0.23 g, 81%): IR ($CHCl_3$ cast) 3336, 3064, 2960, 1790, 1709, 1526 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (br s, 5H), 5.40 (br s, 1H), 5.15 (br s, 2H), 4.82 (q, 1H, J = 8.8 Hz), 2.94−2.79 (m, 2H), 2.48−2.32 (m, 1H), 2.00 (dt, 1H, J = 19.2, 9.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 205.3, 156.8, 156.4, 136.3, 136.0, 128.6, 128.3, 128.2, 128.1, 67.2, 66.9, 65.2, 41.6, 19.8; HRMS (ES, MNa^+) Calcd for $C_{12}H_{13}O_3NNa$ 242.0793, found 242.0792. Anal. Calcd for $C_{12}H_{13}O_3N$: C, 65.74; H, 5.98; N, 6.39; Found: C, 65.49; H, 6.02; N, 6.44.

Diazo-(*N*-benzyloxycarbonyl-glycyl)methane (38). This known compound was prepared from a modified procedure of Wang *et al.*[49c] To a 0 °C solution of acid **37** (5.0 g, 23.9 mmol) in THF (125 mL) was added triethylamine (3.65 mL, 26.3 mmol) followed by ethyl chloroformate (2.52 mL, 26.3 mmol). The reaction mixture was stirred for 30 min, and the mixture was filtered into an ethereal solution of diazomethane (200 mL, ca. 96 mmol) at 0 °C. After stirring for a further 4 h, the solvent was evaporated and the crude product purified by flash column chromatography (100% EtOAc) to afford **38** as an oil (3.32 g, 60%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 (br s, 5H), 5.55 (br s, 1H), 5.35 (s, 1H), 5.15 (br s, 2H), 3.95 (br s, 2H); HRMS (ES, MNa^+) Calcd for $C_{11}H_{11}O_3N_3Na$ 256.0698, found 256.0704.

***N*-(Benzyloxycarbonyl)azetidinone-3-one (39).** This known compound was prepared from a modified procedure of Wang et al.[49c] Reaction of the diazoketone **38** (0.3 g, 1.28 mmol) with rhodium(II) acetate dimer (3.0 mg, 0.01 mmol) as described for **31** afforded the 3-azetidinone **39** (0.08 g, 28%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (br s, 5H), 5.14 (d, 1H, J = 10.3 Hz), 5.16 (d, 1H, J = 8.7 Hz), 4.78 (d, 4H, J = 1 Hz; LRMS (CI) for $C_{11}H_{11}O_3N$ *m*/*z* (relative intensity) 223.2 (MNH_4^+, 100%).

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of selected compounds and crystallographic data for compound **28**. This material is available free of charge via the Internet http://pubs.acs.org.

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