Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron 64 (2008) 4363–4369
Tetrahedron
www.elsevier.com/locate/tet

The 'unexpected' epimerization on bicyclic thiazolidine γ-lactam scaffolds

Ruzhang Liu [a], Yang Wu [a], Qiang Li [a], Wensheng Liao [a,*], Shu-Hui Chen [a], Ge Li [a], Juan M. Betancort [b], David T. Winn [b], David A. Campbell [b]

[a] *WuXi PharmaTech Co., Ltd, No. 1 Building, 288 FuTe ZhongLu, WaiGaoQiao Free Trade Zone, Shanghai, 200131, PR China*
[b] *Phenomix Corporation, 5871 Oberlin Drive, Suite 200, San Diego, CA 92121, United States*

Received 28 November 2007; received in revised form 4 February 2008; accepted 22 February 2008
Available online 4 March 2008

Abstract

Preliminary findings related to the base induced cyclization and spontaneous epimerization leading to stereochemically defined bicyclic thiazolidine γ-lactam systems are reported.

1. Introduction

The past decade has witnessed a great deal of research activities directed toward the design, synthesis, and evaluation of peptide and peptidomimetic based therapeutic agents.[1] These efforts have culminated in the discovery of many important peptidomimetics endowed with desirable biological activity and toxicological profiles such as renin inhibitors (hypertension),[2] HIV protease inhibitors (AIDS),[3] rhino virus 3C protease inhibitors (common cold),[4] HCV NS3 protease inhibitors (HCV infections),[5] beta-secretase inhibitors (Alzheimer's disease), and cathepsin K inhibitors (osteoclasts).[6,7]

Despite the impressive successes achieved by the agents described above, peptide based therapeutic agents suffer from poor ADME properties such as poor absorption and extensive metabolism resulting in low oral bioavailability. This is due in part to the presence of numerous amide bonds and an overall lack of structural rigidity.[8] To circumvent these deficiencies, many approaches have been investigated including incorporation of conformationally constrained peptidomimetics such as substituted bicyclic thiazolidine lactams. This motif can be found in melanocyte-stimulating hormone release-inhibiting factor analogs **2a** and **2b** and angiotensin II analog **4** as shown in Figure 1.[9–11] The potential use of the conformationally restricted Phe–Pro surrogate **2b** in the design of HIV protease inhibitors has also been postulated by Baldwin et al. in 1992.[12]

Prompted by their diverse biological activities and unique structural features (rigidity imposed by the bicyclic framework and complexity due to the presence of three stereo centers),[13] we became interested in the synthesis of the four stereoisomers of bicyclic thiazolidine γ-lactams **9a–9d** as shown in Scheme 1 via a base induced final lactam bond formation reaction. We were also interested in optimizing conditions for the production of **9a** because of its unique structure (possessing the same relative stereochemistry to that seen in **2b** as shown in Fig. 1).

In this paper, we describe some of the 'unexpected' base induced epimerization reactions that we encountered while synthesizing these systems and how we use them to our advantage for the synthesis of the desired target compounds. In addition, we present herein some preliminary molecular modeling calculations performed on these bicyclic γ-lactam frameworks.

2. Result and discussion

2.1. Synthesis of the key cyclization precursors

The synthetic route used for the preparation of the target molecules **9a** through **9d** is shown in Scheme 1. The requisite

* Corresponding author. Tel.: +86 21 50363913; fax: +86 21 50463718.
E-mail address: liao_wensheng@pharmatechs.com (W. Liao).

0040-4020/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2008.02.070

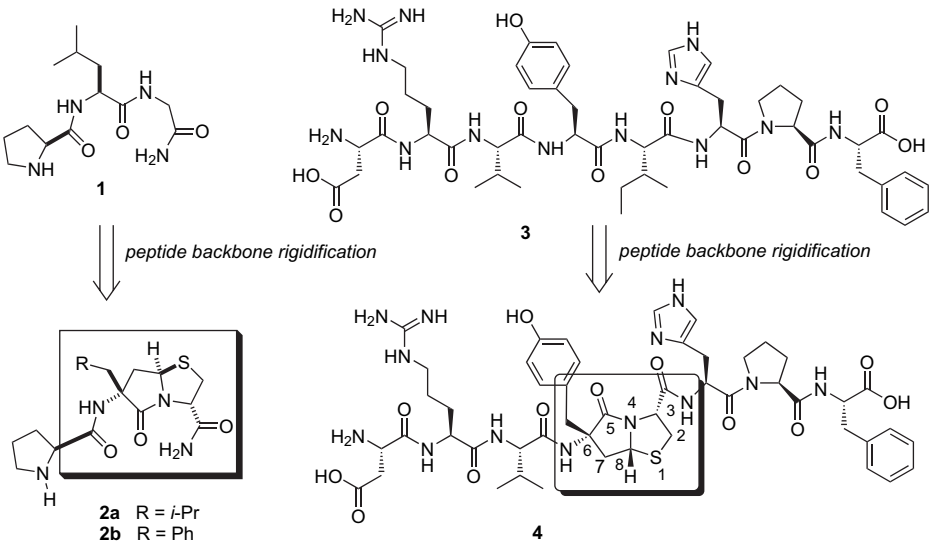
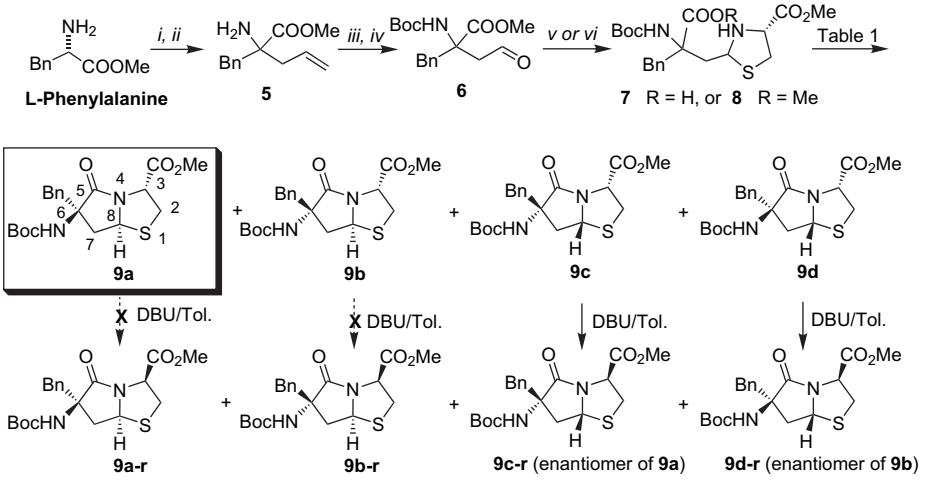


Figure 1. Representative bicyclic thiazolidine lactam bearing molecules.



Scheme 1. Synthesis of the bicyclic thiazolidine framework. Conditions: (i) PhCHO, $MgSO_4$; (ii) KO^{t}Bu, allyl bromide, then 2 N HCl, 88% (two-step); (iii) Boc_2O; (iv) O_3, PPh_3, 61% (two-step); (v) LiOH, then aq $NaHCO_3$, EtOH, L-Cys–OMe, 72%; (vi) NaOAc, AcOH, aq EtOH, L-Cys–OMe, 84%.

aldehyde precursor **6** was prepared from L-phenylalanine via a four-step sequence consisting of imine formation, C-allylation, *N*-Boc protection, and ozonolysis of the resulting olefin according to a modified protocol from Takahashi et al.[12–15] Treatment of the aldehyde **6** with LiOH, followed by L-cysteine in the presence of sodium bicarbonate in aqueous ethanol afforded the methyl ester **7**. Alternatively, reacting **6** with L-cysteine in conjunction with NaOAc and acetic acid yielded the diester **8**.

2.2. Construction of the bicyclic ring system via 7 (acid) or 8 (ester)

The conversion of the acid **7** to the target molecules **9a** through **9d** was carried out under various conditions as shown in Table 1. According to the procedure of Khalil and Johnson,[9] Mukaiyama reagent (entry A) was used to affect cyclization reaction and provided four respective isomers with similar ratio in a total yield of 82%. While isomers **9a** (17%) and **9d** (26%) were easily isolated after silica gel column chromatography, isomers **9b** (28%) and **9c** (11%) were obtained via preparative HPLC separation. The structures of **9a** through **9d** were confirmed on the basis of extensive NOE experiments (see Fig. 2 for details).

In order to increase the yield of isomer **9a**, additional coupling conditions were evaluated and the cyclization results are listed in Table 1. The use of DCC/DMAP (entry B) or HOBt/EDCI/Et_3N (entry D) produced similar ratios and yields for **9a** (16–19%). To our disappointment, a lower yield of **9a** (up to 9%) was obtained when $ClCO_2Bu$-*i*/Et_3N (entry C) or PyBOP/Et_3N (entry E) or TBTU/Et_3N (entry F) was used as the coupling reagents.

In light of the poor yields obtained under basic coupling conditions, we resorted to the use of TsOH as the coupling reagent (entry G). To our satisfaction, a much cleaner product distribution (only **9a** and **9b**) and higher yield of **9a** (38%)

Table 1
Bicyclic γ-lactam isomer distribution under different coupling conditions

Entry	Substrate	Conditions	Isolated product ratio (%)	Total yield (%)
A	**7**	Mukaiyama reagent/TEA/DCM[a]	17 (**9a**); 28 (**9b**); 11 (**9c**); 26 (**9d**)[b]	82
B	**7**	DCC/cat. DMAP/DCM[c]	19 (**9a**); 28 (**9b**); 11 (**9c**); 19 (**9d**)[d]	77
C	**7**	$ClCO_2Bu$-*i*/Et_3N/DCM[c]	9 (**9a**); 4 (**9b**); 12 (**9c**); 13 (**9d**)[d]	38
D	**7**	HOBt/EDCI/Et_3N/DCM[c]	16 (**9a**); 20 (**9b**); 18 (**9c**); 17 (**9d**)[d]	71
E	**7**	PyBOP/Et_3N/DCM[c]	1 (**9a**); 6 (**9b**); 12 (**9c**); 12 (**9d**)[d]	31
F	**7**	TBTU/Et_3N/DCM[c]	3 (**9a**); 11 (**9b**); 6 (**9c**); 15 (**9d**)[d]	35
G	**7**	cat. TsOH/Tol.[a]	38 (**9a**); 35 (**9b**)	73
H	**8**	cat. TsOH/Tol.[a]	49 (**9a**); 11 (**9b**)	60
I	**8**	DBU/Tol.[e]	33 (**9a**); 24 (**9b**); 9 (**9c-r**); 3 (**9d-r**)[d]	69

[a] Refluxed overnight.
[b] The yield after separation.
[c] Stirred overnight.
[d] The yields of **9b**, **9c**, **9c-r**, and **9d-r** were calculated on the basis of chiral HPLC trace.
[e] Base (3 equiv) in refluxing toluene for 2 h, the reaction was conducted in anoxic conditions.

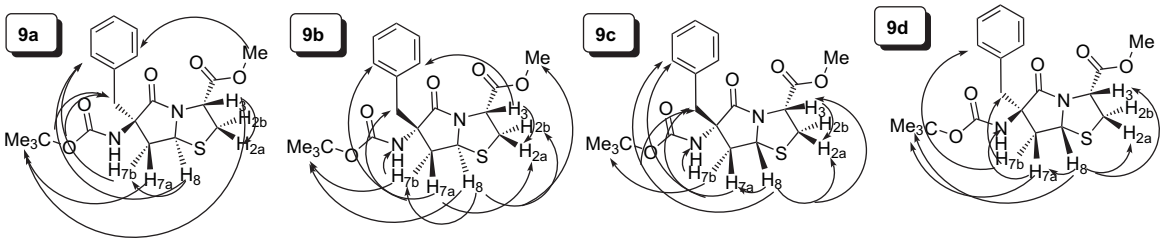


Figure 2. Graphical summary of the NOEs observed for the bicyclic thiazolidine lactams **9a** through **9d**.

were observed. Encouraged by this success, the methyl ester **8** was treated with TsOH in toluene under reflux for 2 h. Once again, the desired isomer **9a** was obtained in 49% yield along with 11% of its epimer **9b** (entry H).

2.3. *In situ C3 epimerization*

Prompted by the improved yield of **9a** obtained when the cyclization reaction was performed under elevated temperature, we decided to carry out the γ-lactam ring formation reaction using DBU in refluxing toluene. However, in this event, two peaks corresponding to isomers '**9a**' (42%) and '**9b**' (27%) were detected by HPLC analysis, respectively. Upon careful inspection by chiral HPLC analysis, compound '**9a**' was separated into two peaks with identical mass units. Likewise, compound '**9b**' was also a mixture of two compounds with equal mass.

After extensive analysis, we concluded that '**9a**' was indeed a mixture of **9a** with its enantiomer **9c-r** in a 3.6:1 ratio favoring the former. Similarly, compound '**9b**' was found to be a mixture of **9b** and **9d-r** in a ratio of 8:1 favoring **9b**. Furthermore, two C-6 isopropyl analogs of '**9a**' and '**9b**', namely '**11a**' (57%) and '**11b**' (40%) were obtained using condition I in Table 1. Both of these bicyclo-products were subjected to crystallization and solid state structure analysis. As shown below, we found that the crystal obtained from '**11b**' was a single compound **11b**. In contrast, a pair of enantiomers (**11a** and **11c-r**) was obtained from '**11a**'. Evidently, the corresponding stereochemistry (at C-3, C-6, and C-8) found with these crystals was in good agreement with that deduced from NOE experiments for **9a** and **9b** (Fig. 3).

We further reasoned that isomers **9c-r** and **9d-r** might arise from base induced C3 epimerization of **9c** and **9d**, respectively. Consistent with the results revealed by chiral HPLC analysis, we discovered that the optical rotation value ($[\alpha]_D$) observed for '**9a**' was −76.4 (*c* 1.0, MeOH), which was lower than that measured for the optically pure **9a** ($[\alpha]_D$ −102.8 (*c* 1.0, MeOH)). The same trend was also found with '**9b**' ($[\alpha]_D$ −131.3 (*c* 1.0, MeOH)) and the optically pure sample **9b** ($[\alpha]_D$ −170.7 (*c* 1.0, MeOH)).

To verify the in situ C3 epimerization event, isomers **9c** and **9d** were treated with DBU in refluxing toluene. Indeed, these two isomers were epimerized to their C-3 isomers **9c-r** and **9d-r** in 93 and 97% yield, respectively. When compounds **9a** and **9b** were treated under identical reaction conditions, no C3 epimerization took place. The difference in behavior observed for the four isomers (**9a**–**9d**) under basic treatment was later rationalized by computational analysis as outlined in the following section.

Intrigued by this interesting in situ base induced C3 epimerization event, we further investigated the aminolysis of **9a**–**9d** under elevated temperature.[9] We discovered that C3 epimerization did occur and gave rise to the corresponding amide derivatives **10c-r** and **10d-r**, which were confirmed by ^{1}H NMR and chiral HPLC analysis, to be the enantiomers of **10a** and **10b** obtained via aminolysis of **9a** and **9b**. (Scheme 2).

2.4. *Molecular modeling calculations*

To further understand this base mediated in situ C3 epimerization observed with **9a**–**9d**, we carried out molecular modeling calculations using the Cerius2 4.10 software

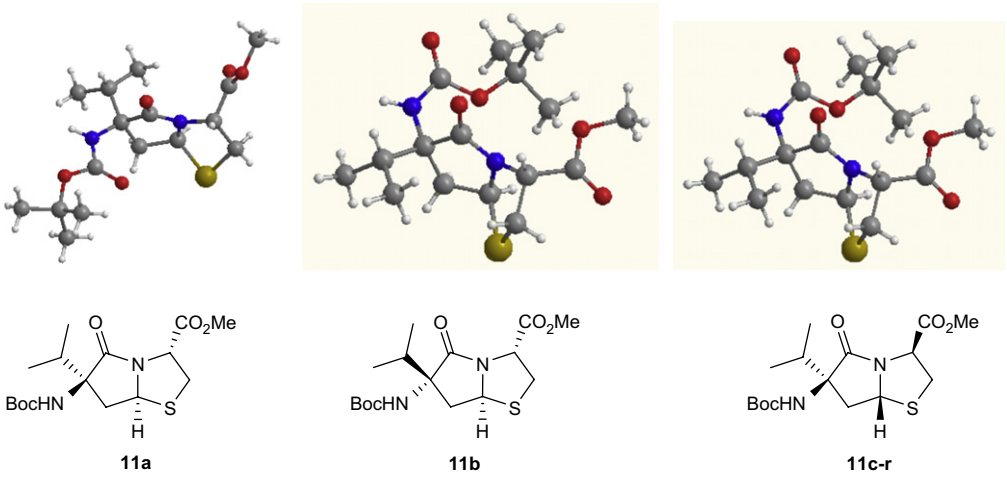
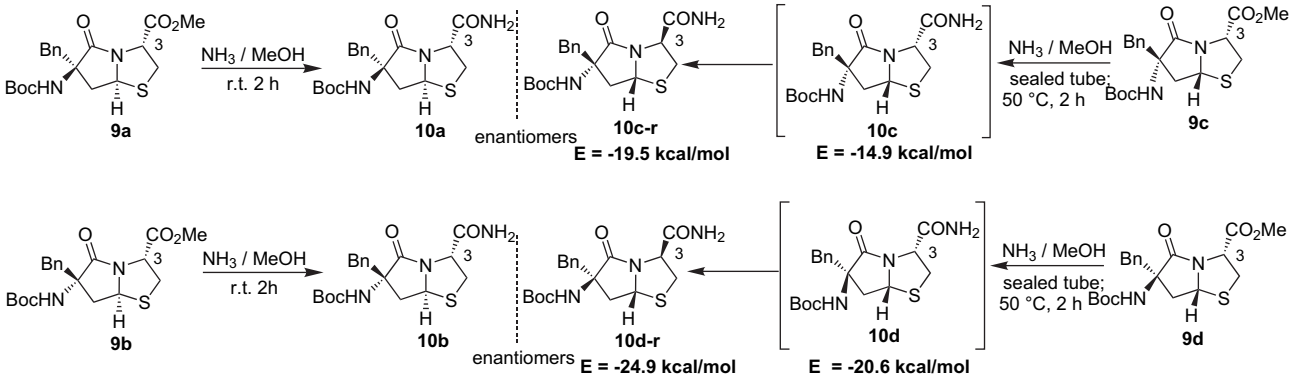


Figure 3. ORTEP drawings of compounds **11a**, **11b**, and **11c-r**.



Scheme 2. Base induced epimerization of **10c** and **10d**.

package,[16] which utilizes molecular mechanics methods to obtain minimized energy values. The molecular structures were built by Cerius2 3D-Sketcher Module, from which CFF[17] force field was selected for energy calculations.

According to the energy calculations shown in Table 2, isomers **9a** and **9b** were found to be thermodynamically more stable than their corresponding C3 epimers **9a-r** (by 5.3 kcal/mol) and **9b-r** (by 1.4 kcal/mol). Contrary to this finding, isomers **9c** and **9d** had relatively higher energy than their corresponding C3 epimers **9c-r** (by 5.0 kcal/mol) and **9d-r** (by 1.4 kcal/mol), suggesting that **9c** and **9d** could be converted to **9c-r** and **9d-r** under DBU treatment. The chemical reactivity toward C3 epimerization predicted by the molecular modeling calculations is in agreement with the experimental findings outlined in Table 1 and Scheme 1.

Table 2
Minimum energy calculation for thiazolidine γ-lactam isomers

Isomer	**9a**	**9b**	**9c**	**9d**
E (kcal/mol)	9.011	7.102	11.953	11.386
Isomer	**9a-r**	**9b-r**	**9c-r**	**9d-r**
E (kcal/mol)	14.257	8.507	6.921	9.978

The minimized energies for **10c**, **10c-r**, **10d**, and **10d-r** were also calculated using the same molecular modeling program. In light of the results highlighted in Scheme 2, it became evident that compounds **10c** and **10d** could undergo C3 epimerization to yield their corresponding lower energy C3 epimers **10c-r** and **10d-r** under the reaction conditions previously outlined.

3. Conclusion

We describe herein a detailed investigation into the thiazolidine γ-lactam isomer distribution (**9a**–**9d**) obtained under various cyclization conditions. As a result of this effort, we found that isomer **9a** could be prepared in close to 50% yield (highest theoretical yield) by treatment of methyl ester **8** with TsOH in toluene. Furthermore, we discovered a C3 epimerization event occurred to **9c**, **9d** as well as to **10c**, **10d** upon basic treatment under thermodynamic conditions (e.g., DBU or ammonia in methanol). This 'unexpected' C3 epimerization is further rationalized and supported by molecular modeling calculations as well as confirmed by crystal structure analyses on **11a**, **11b**, and **11c-r**.

4. Experimental

4.1. General

Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 or 400 spectrometer at ambient temperature. Chemical shifts are reported relative to residual solvents. Abbreviations for ^{1}H NMR: s=singlet, d=doublet, t=triplet, m=multiplet, br=broad. Mass spectra were obtained on a Finnigan LCQ mass spectrometer with ESI source. Melting points were obtained on a WRR melting point apparatus and are uncorrected. Optical rotations were measured on a PerkinElmer model 341LC polarimeter at the 589-nm Na D-line. X-ray diffraction was performed on BRUKER SMART APEX-CCD.

4.2. Methyl 2-amino-2-benzylpent-4-enoate (**5**)

To a suspension of L-phenylalanine hydrochloride (110.0 g, 0.5 mol) in dichloromethane (1000 mL) was added triethylamine (83.5 mL, 0.6 mol). After stirring for 0.5 h, benzaldehyde (53 g, 0.5 mol) was added dropwise to the mixture, followed by the addition of magnesium sulfate (120 g, 0.5 mol). The mixture was stirred overnight. Dichloromethane was removed under vacuo and ethyl acetate (1000 mL) was added. After filtration, the filtrate was concentrated to afford the corresponding imine (133.72 g, 100%). To a solution of potassium *tert*-butoxide (72.8 g, 0.65 mol) in THF (1000 mL) at room temperature was added a solution of the above imine (133.72 g, 0.5 mol) in THF (300 mL). After stirring for 0.5 h, allyl bromide (84 g, 0.7 mol) was added dropwise to the resulting mixture. The mixture was stirred overnight at room temperature. THF was removed under vacuo and ethyl acetate (1000 mL) was added. After filtration, the filtrate was concentrated to afford the alkylated imine (153.66 g, 100%). To a solution of the alkylated imine (153.66 g, 0.5 mol) in THF (500 mL) was added 3 M HCl (500 mL, 1.5 mol) slowly at room temperature. After stirring for 1 h, THF was removed in vacuo, and ethyl acetate (500 mL) and 3 M HCl (150 mL) were added. After separation of both phases, 300 mL of ethyl acetate were used to extract away the impurity and then the aqueous layer was adjusted to pH=10–12. The aqueous phase was extracted with dichloromethane (2×250 mL). The combined organic layers were washed with brine (100 mL) and concentrated to afford compound **5** (96.75 g, 88%) as a colorless oil, which was used directly to the next step without further purification. ^{1}H NMR ($CDCl_3$) δ 2.36 (dd, J=8.0 and 12.8 Hz, 1H), 2.50–2.75 (br, 2H), 2.79 (dd, J=6.0 and 12.8 Hz, 1H), 2.82 (d, J=12.8 Hz, 1H), 3.19 (d, J=12.8 Hz, 1H), 3.70 (s, 3H), 5.15–5.21 (m, 2H), 5.68–5.74 (m, 1H), 7.13–7.17 (m, 2H), 7.22–7.29 (m, 3H).

4.3. Methyl 2-benzyl-2-(tert-butoxycarbonylamino)-4-oxobutanoate (**6**)

A solution of compound **5** (50.0 g, 0.228 mol) and Boc anhydride (78.0 g, 0.342 mol) in THF (300 mL) was heated to reflux. After refluxing overnight, THF was removed in vacuo. The residue was dissolved in dichloromethane (300 mL) and cooled to −78 °C. Then ozone in oxygen (5–10%) was bubbled through until the color turned gray. Nitrogen was bubbled through the reaction mixture to remove extra ozone and triphenyl phosphine (78.0 g, 0.3 mol) was added slowly. The reaction was stirred for 1 h. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography to afford compound **6** (45.0 g, 61%). ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H), 2.96 (d, J=13.2 Hz, 1H), 3.06 (d, J=13.2 Hz, 1H), 3.61 (d, J=12.6 Hz, 1H), 3.73 (s, 3H), 3.85 (d, J=12.6 Hz, 1H), 5.55 (br, 1H), 7.00–7.04 (m, 2H), 7.25–7.26 (m, 3H), 9.67 (s, 1H).

4.4. 2-Benzyl-2-(tert-butoxycarbonylamino)-3-((4R)-4-(methoxycarbonyl)thiazolidin-2-yl)propanoic acid (**7**)

To a solution of compound **6** (8.0 g, 25 mmol) in methanol (120 mL) and water (40 mL), lithium hydroxide hydrate (4.2 g, 100 mmol) was added. After stirring overnight, the resulting mixture was adjusted to pH=5–6 using 1 M HCl, and extracted with ethyl acetate. The combined organic phases were concentrated to afford the corresponding acid (7.58 g, 99%). To a solution of the above acid (7.58 g, 24.8 mmol) in EtOH (100 mL) and water (100 mL) was added $NaHCO_3$ (2.5 g, 30 mmol), followed by the addition of L-cysteine hydrochloride (4.76 g, 28 mmol). The pH value of the resulting mixture was adjusted to 6.5 with 1 M $NaHCO_3$. After stirring overnight, the mixture was concentrated to about one-half volume, and the pH was adjusted to 6 with 1 M HCl. The mixture was poured into water (100 mL) and extracted with ethyl acetate (200 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated to afford compound **7** (9.12 g, 72%). MS (ESI): 425 (M^++H).

4.5. (4R)-Methyl 2-(2-benzyl-2-(tert-butoxycarbonyl amino)-3-methoxy-3-oxopropyl)thiazolidine-4-carboxylate (**8**)

To a solution of compound **6** (9.6 g, 20 mmol), AcOH (1.25 mL, 20.9 mmol), and AcONa (3.43 g, 41.8 mmol) in an ethanol (50 mL) and water (5 mL) mixture was added L-cysteine hydrochloride (3.93 g, 23.0 mmol). After stirring overnight, ethyl acetate (150 mL) was added and the organic layer was washed with brine (50 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated to afford compound **8** (7.33 g, 84%). MS (ESI): 439 (M^++H).

4.6. Methyl 6-benzyl-6-(tert-butoxycarbonylamino)-5-oxohexahydropyrrolo[2,1-b]thiazole-3-carboxylate (**9**)

4.6.1. Method A

To a solution of the thiazolidine mixture **7** (4.24 g, 10 mmol) in dichloromethane (400 mL) was added 2-chloro-1-methylpyridinium iodide (Mukaiyama reagent) (3.06 g, 12 mmol), followed by the addition of triethylamine (2.75 g, 25 mmol). This resulting mixture was heated to reflux overnight. The reaction mixture was allowed to cool to room temperature and

then washed with 10% citric acid (50 mL), 1 M $NaHCO_3$ (50 mL), and brine (50 mL). The concentrated mixture was purified by silica gel column chromatography and preparative HPLC to afford compounds **9a** (0.68 g, 17%), **9b** (1.13 g, 28%), **9c** (0.43 g, 11%), and **9d** (1.05 g, 26%). [Note: compounds **9b** and **9c** were purified by HPLC. Analytic HPLC conditions: YMC 150 mm×4.6 mm, 5 μm; mobile phase: 50% MeOH+50% H_2O (0.05% TFA); flow rate: 1 mL/min; detector: PDA 207 nm. Retention times for **9a**, **9b**, **9c**, and **9d** were 50.15, 55.89, 45.61, 52.44 min, respectively. Preparative HPLC conditions: AS-V, 250 mm×77 mm, 20 μM; mobile phase: 4% ethanol+96% *n*-hexane; flow rate: 250 mL/min; detector: PDA 220 nm. Retention times for **9b** and **9c** were 16.5 and 12.3 min, respectively.]

*4.6.1.1. (3R,6R,8S)-Methyl 6-benzyl-6-(tert-butoxycarbonylamino)-5-oxohexahydropyrrolo[2,1-b]thiazole-3-carboxylate (**9a**).* White solid; TLC R_f=0.6 (PE/EA=5:1, twice); mp 121.5−122.2 °C; $[\alpha]_D$ −102.8 (*c* 1.0, MeOH); ^{1}H NMR (MeOD) δ 1.42 (s, 9H, $C(CH_3)_3$), 2.56 (dd, *J*=7.5 and 12.9 Hz, 1H, H_{7a}), 2.79 (dd, *J*=6.6 and 12.9 Hz, 1H, H_{7b}), 2.91 (d, *J*=12.9 Hz, 1H, C*H*HPh), 3.07 (d, *J*=12.9 Hz, 1H, CH*H*Ph), 3.19−3.22 (m, 1H, H_{2b}), 3.35−3.39 (m, 1H, H_{2a}), 3.66 (s, 3H, $COOCH_3$), 3.97 (br, 1H, H_8), 5.05 (br, 1H, H_3), 7.22−7.29 (m, 5H, Ph); ^{13}C NMR (MeOD) δ 26.5, 33.0, 39.5, 40.8, 50.8, 57.7, 59.5, 64.8, 78.4, 126.1, 127.2, 129.3, 133.2, 154.1, 168.5, 173.1; HRMS (ESI) calcd for $C_{20}H_{27}N_2O_5S$: 407.1641 (M^++H), found: 407.1631.

*4.6.1.2. (3R,6S,8S)-Methyl 6-benzyl-6-(tert-butoxycarbonylamino)-5-oxohexahydropyrrolo[2,1-b]thiazole-3-carboxylate (**9b**).* White solid; TLC R_f=0.53 (PE/EA=5:1, twice); mp 107.0−108.8 °C; $[\alpha]_D$ −170.7 (*c* 1.0, MeOH); ^{1}H NMR (CD_3CN) δ 1.39 (s, 9H, $C(CH_3)_3$), 2.42 (dd, *J*=2.7 and 14.4 Hz, 1H, H_{7a}), 2.64 (dd, *J*=7.8 and 14.4 Hz, 1H, H_{7b}), 2.93 (d, *J*=13.5 Hz, 1H, C*H*HPh), 3.03 (d, *J*=13.5 Hz, 1H, CH*H*Ph), 3.07 (dd, *J*=7.8 and 11.1 Hz, 1H, H_{2a}), 3.22 (dd, *J*=3.3 and 11.1 Hz, 1H, H_{2a}), 3.71 (s, 3H, $COOCH_3$), 4.96 (dd, *J*=3.3 and 7.8 Hz, 1H, H_3), 5.16 (dd, *J*=2.7 and 7.8 Hz, 1H, H_8), 5.50 (br, 1H, NH), 7.22−7.35 (m, 5H, Ph); ^{13}C NMR (CD_3CN) δ 26.7, 33.0, 34.7, 41.3, 51.5, 57.6, 61.5, 62.1, 78.9, 126.4, 127.6, 129.9, 134.4, 153.5, 169.1, 173.9; HRMS (ESI) calcd for $C_{20}H_{27}N_2O_5S$: 407.1641 (M^++H), found: 407.1635.

*4.6.1.3. (3R,6S,8R)-Methyl 6-benzyl-6-(tert-butoxycarbonylamino)-5-oxohexahydropyrrolo[2,1-b]thiazole-3-carboxylate (**9c**).* White solid; TLC R_f=0.53 (PE/EA=5:1, twice); mp 157.3−158.7 °C; $[\alpha]_D$ −25.2 (*c* 1.0, MeOH); ^{1}H NMR (CD_3CN) δ 1.44 (s, 9H, $C(CH_3)_3$), 2.50 (dd, *J*=8.7 and 12.6 Hz, 1H, H_{7b}), 2.98 (dd, *J*=5.7 and 12.6 Hz, 1H, H_{7a}), 3.06 (d, *J*=13.5 Hz, 1H, C*H*HPh), 3.16 (d, *J*=13.5 Hz, 1H, CH*H*Ph), 3.32 (dd, *J*=2.4 and 12.6 Hz, 1H, H_{2b}), 3.52 (dd, *J*=7.5 and 12.6 Hz, 1H, H_{2a}), 3.69 (s, 3H, $COOCH_3$), 4.14 (dd, *J*=2.4 and 7.5 Hz, 1H, H_3), 4.55 (dd, *J*=5.7 and 8.7 Hz, 1H, H_8), 5.26 (br, 1H, NH), 7.15−7.17 (m, 2H, Ph), 7.25−7.31 (m, 3H, Ph); ^{13}C NMR (CD_3CN) δ 26.7, 37.1, 40.0, 40.2, 51.2, 55.6, 62.7, 65.3, 78.3, 126.2, 127.2, 129.4, 134.6, 153.2, 167.6, 169.8; HRMS (ESI) calcd for $C_{20}H_{27}N_2O_5S$: 407.1641 (M^++H), found: 407.1629.

*4.6.1.4. (3R,6R,8R)-Methyl 6-benzyl-6-(tert-butoxycarbonylamino)-5-oxohexahydropyrrolo[2,1-b]thiazole-3-carboxylate (**9d**).* White solid; TLC R_f=0.4 (PE/EA=5:1, twice); mp 71.1−73.2 °C; $[\alpha]_D$ +15.1 (*c* 1.0, MeOH); ^{1}H NMR (CD_3CN) δ 1.32 (s, 9H, $C(CH_3)_3$), 2.37 (dd, *J*=5.4 and 13.8 Hz, 1H, H_{7b}), 2.98 (dd, *J*=7.2 and 13.8 Hz, 1H, H_{7a}), 3.00 (d, *J*=14.1 Hz, 1H, C*H*HPh), 3.11 (d, *J*=14.1 Hz, 1H, CH*H*Ph), 3.31 (dd, *J*=3.0 and 12.3 Hz, 1H, H_{2b}), 3.59 (dd, *J*=7.5 and 12.3 Hz, 1H, H_{2a}), 3.73 (s, 3H, $COOCH_3$), 4.23 (dd, *J*=3.0 and 7.5 Hz, 1H, H_3), 5.18 (dd, *J*=5.4 and 7.2 Hz, 1H, H_8), 5.45 (br, 1H, NH), 7.26−7.37 (m, 5H, Ph); ^{13}C NMR (CD_3CN) δ 26.7, 34.2, 37.1, 41.3, 51.2, 56.1, 63.3, 63.8, 78.6, 126.2, 127.6, 129.8, 134.8, 153.7, 167.9, 170.8; HRMS (ESI) calcd for $C_{20}H_{27}N_2O_5S$: 407.1641 (M^++H), found: 407.1625.

4.6.2. Method G

A solution of compound **7** (4.24 g, 10 mmol) and *p*-toluenesulfonic acid monohydrate (0.19 g, 1.0 mmol) in toluene (300 mL) was heated to reflux overnight. The solution was allowed to cool down to room temperature, washed with $NaHCO_3$ solution (50 mL) and brine (50 mL), and dried over Na_2SO_4. After filtration and removal of solvents under reduced pressure, the residue was purified by silica gel column chromatography to give compounds **9a** (1.56 g, 38%) and **9b** (1.41 g, 35%).

4.6.3. Method H

Similar procedure to Method G.

4.6.4. Method I

A solution of compound **8** (3.13 g, 7.1 mmol) and DBU (3.3 g, 22 mmol) in toluene (60 mL) was heated to reflux overnight. The reaction mixture was allowed to cool down to room temperature and then neutralized with acetic acid. The solution was washed with brine (20 mL), dried over Na_2SO_4, and filtered. After concentration, the residue was purified by silica gel column chromatography to give compounds '**9a**' (1.21 g, 42%; **9a**: 33%, **9c-r**: 9%) and '**9b**' (0.80 g, 27%; **9b**, 24%, **9d-r**, 3%). [Chiral HPLC conditions: AD-H 250 mm×4.6 mm, 5 μm; mobile phase: 15% EtOH (0.1% TFA)+85% *n*-hexane; flow rate: 0.5 mL/min; detector: PDA 207 nm. Retention times detected for **9a**, **9b**, **9c**, **9d**, **9c-r**, and **9d-r** were 18.51, 34.85, 27.63, 17.24, 25.43, and 23.52 min.]

4.7. Epimerization experiment

A solution of compound **9c** (81 mg, 0.2 mmol) and DBU (90 mg, 0.6 mmol) in toluene (5 mL) was heated to reflux overnight. The reaction mixture was allowed to cool down to room temperature and then neutralized with acetic acid. The solution was washed with brine (2 mL), dried over

Na_2SO_4, and filtered. After concentration, the residue was purified by column chromatography on silica gel to give compound **9c-r** (75 mg, 93%). The same experiment is carried out for **9a**, **9b**, and **9d** to give **9a** (95%), **9b** (94%), and **9d-r** (97%).

4.8. Aminolysis experiment

A round-bottom flask was charged with compound **9a** (500 mg, 1.2 mmol) and 10 M ammonia solution in methanol (50 mL), and the mixture was stirred overnight. The solvent was distilled out to afford compound **10a** (465 mg, 99%). ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 9H), 2.64–2.70 (m, 1H), 3.03–3.09 (m, 2H), 3.19–3.25 (m, 2H), 3.54 (dd, J=4.5 and 11.4 Hz, 1H), 4.01 (t, J=9.6 Hz, 1H), 4.80–4.83 (m, 1H), 5.27 (br, 1H), 5.74 (br, 1H), 7.25–7.36 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 28.3, 31.5, 40.9, 42.7, 57.8, 60.6, 66.5, 80.8, 127.6, 128.7, 129.6, 133.5, 154.5, 171.1, 172.0; HRMS (ESI) calcd for $C_{19}H_{26}N_3O_4S$: 392.1644 (M^++H), found: 392.1635.

The same experiment is carried out for **9b** to give **10b** (98%). ^{1}H NMR ($CDCl_3$) δ 1.38 (s, 9H), 2.48–2.55 (m, 2H), 2.94 (d, J=12.9 Hz, 1H), 3.13 (d, J=12.9 Hz, 1H), 3.31 (dd, J=8.9 and 11.2 Hz, 1H), 3.53 (dd, J=4.0 and 11.2 Hz, 1H), 4.81–4.83 (m, 1H), 5.08 (br, 1H), 5.14–5.17 (m, 1H), 5.45 (br, 1H), 7.21–7.25 (m, 2H), 7.30–7.36 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 28.5, 31.4, 36.3, 43.4, 57.7, 62.6, 64.1, 81.2, 128.0, 129.2, 130.6, 134.4, 155.1, 171.7, 172.7; HRMS (ESI) calcd for $C_{19}H_{26}N_3O_4S$: 392.1644 (M^++H), found: 392.1626.

A round-bottom flask was charged with compound **9c** (500 mg, 1.2 mmol) and 10 M ammonia solution in methanol (50 mL) and sealed. It was heated to 60 °C for 2 h. After cooling to room temperature, the solvent was distilled out to afford compound **10c-r** (460 mg, 98%).

The same experiment is carried out for **9d** to give **10d-r** (97%).

Acknowledgements

We shall thank Mr. Qusheng Xu and Yujun Bai for their help with synthetic work, Ms. Wei Ji for her help with NMR analysis. We are also indebted to the chemists from Analytic Department at WuXi PharmaTech for their support.

References and notes

1. Martin-Martinez, M.; Patino-Molina, R.; Garcia-Lopez, M. T.; Gonzalez-Muniz, R. *Mini Rev. Med. Chem.* **2004**, *4*, 669–680.
2. Review: Greenlee, W. J. *Med. Res. Rev.* **1990**, *10*, 173–236.
3. Review: Abdel-Rahman, H. M.; Al-karamany, G. S.; El-Koussi, N. A.; Youssef, A. F.; Kiso, Y. *Curr. Med. Chem.* **2002**, *9*, 1905–1922.
4. Review: Wang, Q. M.; Chen, S. H. *Frontiers of Biotechnology & Pharmaceuticals*; Reiner, Z., Chen, G., Eds.; Science: USA, 2004; Vol. 4, pp 30–49.
5. Reviews: (a) De Francesco, R.; Tomei, L.; Altamura, S.; Summa, V.; Migliaccio, G. *Antiviral Res.* **2003**, *58*, 1–16; (b) Chen, S. H.; Tan, S.-L. *Curr. Med. Chem.* **2005**, *12*, 2317–2342.
6. Review: John, V.; Beck, J. P.; Bienkowski, M. J.; Sinha, S.; Heinrikson, B. L. *J. Med. Chem.* **2003**, *46*, 4625–4630.
7. Barrett, D. G.; Catalano, J. G.; Deaton, D. N.; Hassell, A. M.; Long, S. T.; Miller, L. R.; Shewchuk, L. M.; Wells-Knecht, K. J.; Willard, D. H.; Wright, L. L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4897–4902.
8. (a) Gante, J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1699–1720; (b) Giannis, A.; Kolter, T. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1244–1267.
9. Khalil, E. M.; Pradhan, A.; Ojala, W. H.; Gleason, W. B.; Mishra, R. K.; Johnson, R. L. *J. Med. Chem.* **1999**, *42*, 2977–2987.
10. Johannesson, P.; Erdelyi, M.; Lindeberg, G.; Frandberg, P.-A.; Nyberg, F.; Karlen, A.; Hallberg, A. *J. Med. Chem.* **2004**, *47*, 6009–6019.
11. Genin, M. J.; Johnson, R. L. *J. Am. Chem. Soc.* **1992**, *114*, 8778–8783.
12. Baldwin, J. E.; Lee, V.; Schofield, C. J. *Heterocycles* **1992**, *34*, 903–906.
13. Campbell, D.; Betancort, J.; Winn, D. WO2006017292, 2006.
14. Fuchi, N.; Doi, T.; Harada, T.; Urban, J.; Cao, B.; Kahn, M.; Takahashi, T. *Tetrahedron Lett.* **2001**, *42*, 1305–1308.
15. Dolbeare, K.; Pontoriero, G. F.; Gupta, S. K.; Mishra, R. K.; Johnson, R. L. *J. Med. Chem.* **2003**, *46*, 727–733.
16. *Cerius2 Modeling Environment, Release 4.10*; Accelrys: San Diego, CA, USA, 2005.
17. Hagler, A. T.; Ewig, C. S. *Comput. Phys. Commun.* **1994**, *84*, 131–155.