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Stereoselective synthesis of optically active bicyclic β-lactam carboxylic acids that target pilus biogenesis in pathogenic bacteria †

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Optically active bicyclic β-lactams were synthesized, starting from 2-H-Δ^2-thiazolines and Meldrum's acid derivatives. Several methods to accomplish an ester hydrolysis without damaging the β-lactam framework were investigated. A rapid CsOH saponification of the β-lactam methyl esters was developed and protonation of the Cs-carboxylates by Amberlite (IR-120 H^+) afforded a series of bicyclic β-lactam carboxylic acids. Moreover, a convenient method for the synthesis of 2-H-Δ^2-thiazolinecarboxylic acid methyl ester **2** was developed. Bicyclic β-lactam carboxylic acids **7a**–**g** and aldehydes **4a**–**d** were screened for their affinity to the bacterial periplasmic chaperone PapD using a surface plasmon resonance technique. β-Lactams substituted with large acyl substituents showed better binding to the chaperone than the native C-terminal peptide PapG **8**, demonstrating that bicyclic β-lactams constitute a new class of potential bacterial chaperone inhibitors.

Introduction

β-Lactams have gained substantial interest in the scientific community not only due to their antibiotic properties[1–3] but also as reactive intermediates and as starting materials in a wide range of synthetic settings.[4,5] The heavy use of antibiotics during the second half of the last century has resulted in an increasing number of bacteria being resistant to the drugs available today.[6,7] One way of overcoming this problem involves development of antibacterial agents against novel targets in the microorganism.[8]

A large number of infectious Gram negative bacteria produce pili, which are a family of extracellular, supramolecular protein organelles that mediate attachment to host tissue.[9] Pilus assembly is performed by periplasmic chaperones that fold and transport the subunits to the outer cell membrane where they are incorporated into a growing pilus.[10] Thus, if the chaperone-subunit complex can be prevented from being formed, colonization of host tissue cannot occur.[11,12] Moreover, periplasmic chaperones constitute a highly conserved structure, which can be found in a large number of pathogenic bacteria such as *B. pertussis, S. typhimurium* and *H. influenzae* responsible for the diseases whooping cough, gastrointestinal disorder and meningitis respectively.[9,13] In addition, the structures of the chaperone-subunit complexes are well studied and it has been shown, both by NMR[14,15] and X-ray crystallography,[16,17] that the pili-subunits bind to the chaperone by anchoring of the carboxy terminus to the side chains of Arg 8 and Lys 112. Previously, we reported a stereoselective synthesis of optically active β-lactams, which were designed to be a suitable scaffold for the development of compounds (termed pilicides) that inhibit pilus formation in uropathogenic *E. coli*.[18] Bicyclic β-lactam methyl esters **3** were synthesized in yields as high as 93% by reacting the two key intermediates, the acyl Meldrum's acids **1** and 2-H-Δ^2-thiazoline methyl ester **2** (Fig. 1). These esters could then be selectively reduced to the corresponding aldehydes **4** by DIBAL-H, which would have potential to form imines with Lys 112.[18]

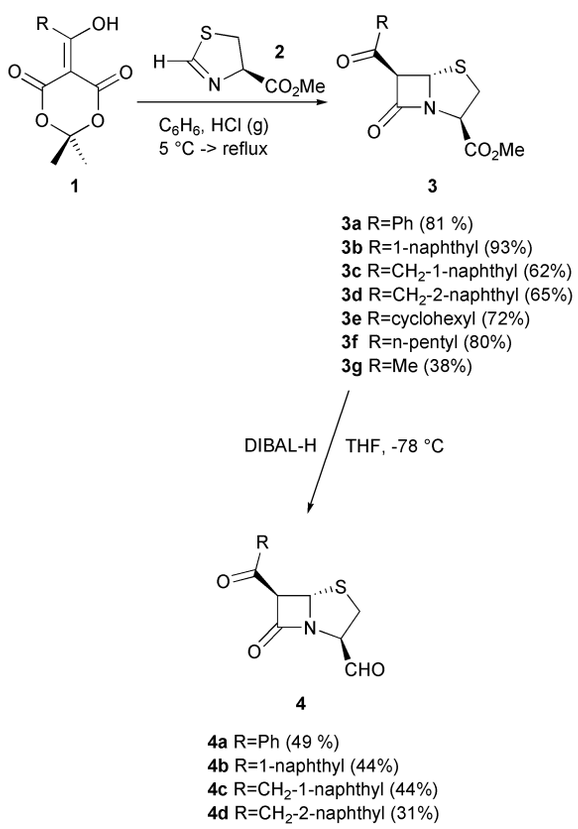

Fig. 1 Original procedure for the formation of the β-lactam methyl esters **3** and their corresponding aldehydes **4**.

The stereochemistry of the β-lactams synthesized by this method is different compared to the original penicillin's, thus having a chance to withstand enzymatic degradation by penicillin-resistant bacteria. Other compounds such as bicyclic 2-pyridinones **5** and amino acid derivatives **6** (Fig. 2) are known

† Electronic supplementary information (ESI) available: ^{13}C NMR spectra of **7(a–g)**, **10**–**14**, **16(a and d)** and **17(a–b)**. See http://www.rsc.org/suppdata/ob/b2/b210551a/

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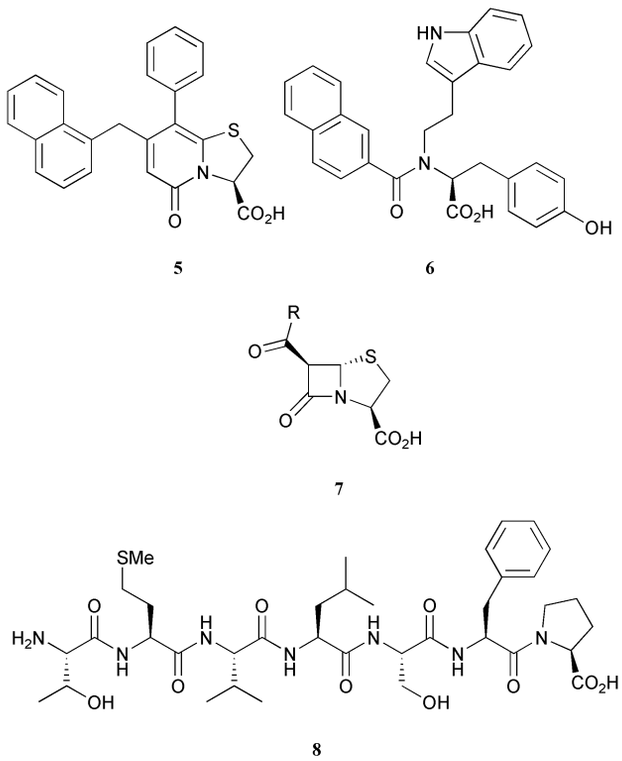


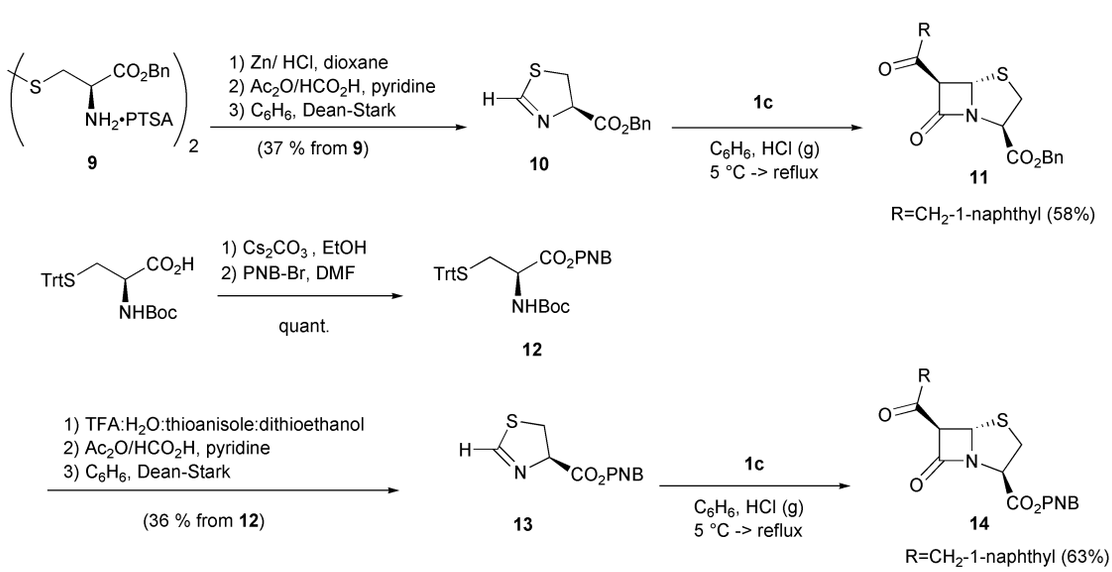
Fig. 2 Bicyclic 2-pyridinone **5**, amino acid derivative **6** and the heptapeptide **8**, corresponding to the *C*-terminus of the pilus adhesion protein PapG, have shown chaperone binding and chaperone/subunit inhibitor activity.[19] Novel bicyclic β-lactam carboxylic acids **7** targeted in this article are also designed to have corresponding biological activity.

to bind to chaperones as free carboxylic acids[19] or as their corresponding Li-salts.[20] This article describes the synthesis of β-lactam carboxylic acids and the study of their binding affinity to the periplasmic chaperone PapD by the surface plasmon resonance technique. Moreover, a new practical and efficient procedure for the synthesis of the important intermediate, 2-H-Δ^2-thiazolinecarboxylic acid methyl ester **2**, was developed.

Results and discussion

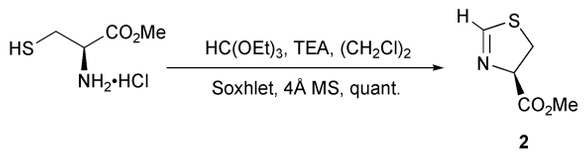
The first approach was to synthesize the desired β-lactam carboxylic acids from the 2-H-Δ^2-thiazoline carboxylic acid and a Meldrum's acid derivative. Following a procedure developed by Walker,[21] the Δ^2-thiazoline carboxylic acid hydrochloride was constructed. Unfortunately, it turned out to be almost insoluble in solvents suitable for the following β-lactam synthesis (benzene or 1,2-dichloroethane), and no β-lactam was formed. Therefore the strategy was to synthesize the β-lactam esters, which then have to be hydrolyzed to generate the carboxylic acids. Thus, with the methyl esters **3** in hand,[18] (Fig. 1) attempts to hydrolyze **3a** by mild saponification (*e.g.* K_2CO_3 in MeOH–H_2O 9 : 1) gave in the best case a 1 : 1 mixture of starting material and product, which was difficult to purify. Prolonged reaction time resulted in decomposition of the β-lactam ring. In addition, attempts to hydrolyze **3a** with $(Bu_3Sn)_2O$ in benzene,[22,23] or **3c** with pig liver esterase[24,25] were also unsuccessful. Tsuji *et al.* developed a method for the hydrolysis of benzyl esters of cephalosporin analogs using $AlCl_3$,[26] which seemed attractive. Thus, the cystein benzyl ester was prepared[27] and the 2-H-Δ^2-thiazoline was synthesized based on the same protocol as for the methyl ester[28] giving the benzyl ester **10** in 37% overall yield in three steps (Scheme 1). Allowing **10** to react with the Meldrum's acid derivative **1c** gave the β-lactam **11** in 58% yield but all attempts of using $AlCl_3$,[26] $TiCl_4$[26] or hydrogenation with Pd/C[29] to cleave the benzyl-ester were unsuccessful.

p-Nitrobenzyl (PNB) esters have shown to be hydrolyzable by using Zn in a phosphate buffer.[30] Consequently, the orthogonally protected cysteine-PNB ester **12** was synthesized (Scheme 1). After simultaneous acidic removal of the *N*- and *S*-protecting groups, the corresponding cysteine-PNB ester was converted to the 2-H-Δ^2-thiazoline-PNB ester **13** in 36% overall yield (from **12** in three steps) and reaction of this intermediate with the Meldrum's acid derivative **1c** gave the β-lactam-PNB ester **14** in 63% yield (Scheme 1). However, this ester also proved to be difficult to hydrolyze using procedures such as Zn in phosphate buffer, Zn in HCl (6 M),[27] Na_2S in THF–H_2O[31] or hydrogenation with different catalysts (*e.g.* Pd/C or PtO_2). At this stage, to be able to find a productive route to the desired β-lactam carboxylic acids, the need for a practical procedure to synthesize different 2-H-Δ^2-thiazoline esters became critical. In the literature there are a wide range of mild methods for the hydrolysis of different carboxylic esters[32] and the strategy to prepare cysteine esters *via* the previous two routes was time consuming. Thus, we wanted to prepare the 2-H-Δ^2-thiazoline **2** on a large scale and then perform transesterifications to the desired esters. Scaling up the previous method[28] was not straightforward since flash chromatography had to be conducted to remove unreacted formylated amino acid and the 2-Me-Δ^2-thiazoline methyl ester, which occasionally appeared in the synthesis. By using a modified procedure, previously used to prepare optically active 2-oxazolines,[33] the



Scheme 1 Two different routes to furnish the benzyl- or the *p*-nitrobenzyl ester **11** and **14** respectively.

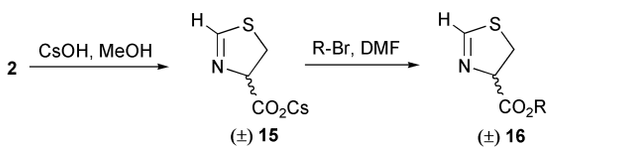
optically active 2-H-Δ^2-thiazoline methyl ester **2** could be prepared in quantitative yield without the need for chromatography (Scheme 2). The enantiomeric ratio (er) was established by ^{1}H NMR (in the presence of Eu(hfc)$_3$) to be 9 : 1 (corresponding to 80% ee), which also is an improvement compared to the former procedure.[28]



2

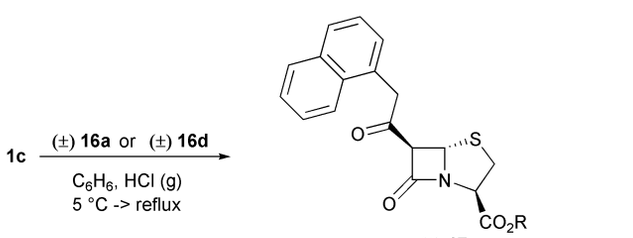
Scheme 2 A convenient method for the synthesis of optically active 2-H-Δ^2-thiazoline methyl ester **2**.

The transesterification was performed by first generating the Cs-carboxylate (±)-**15** *via* a CsOH (0.1 M aq.) saponification. Thereafter, (±)-**15** was sequentially alkylated to furnish the new esters (±)-**16** in moderate to excellent overall yields 57–98% (Scheme 3).



(±) **15** (±) **16**

16a R=heptyl (57 %)
16b R=Bn (60 %)
16c R=pNO_2Bn (70 %)
16d R=propargyl (98 %)



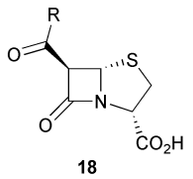
(±) **17**

17a R=heptyl (70 %)
17b R=propargyl (91 %)

Scheme 3 Synthesis of different 2-H-Δ^2-thiazoline esters (±)-**16** and their corresponding β-lactam esters (±)-**17a** and (±)-**17b**.

Thus, with these new 2-H-Δ^2-thiazolines in hand the corresponding heptyl and propargyl β-lactam esters (±)-**17a** and (±)-**17b** were synthesized in 70 and 91% yields respectively. Others had shown that many heptyl esters can be easily hydrolyzed by enzymes under relatively mild conditions (acetone–phosphate buffer pH = 7 at 37 °C)[34–36] but treating the β-lactam heptyl ester (±)-**17a** with a variety of lipases gave no desired carboxylic acid. Another mild alternative was to use the propargyl ester, which should be possible to hydrolyze by treatment with $Co_2(CO)_8$ and TFA in CH_2Cl_2.[37] Disappointingly, the propargylic ester (±)-**17b** could not be hydrolysed under these conditions and increasing the temperature or the amount of $Co_2(CO)_8$ gave no improvement and most of the staring material could be recovered.

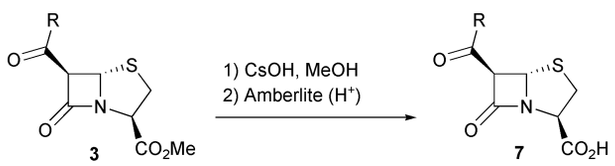
Since the CsOH hydrolysis was so successful in the 2-H-Δ^2-thiazoline case, we anticipated that it could be fruitful also for the β-lactam methyl esters provided that the reaction time could be kept short. Thus, 1 eq. of aqueous CsOH (0.1 M) was added to a solution of the β-lactam methyl ester **3** in MeOH and the resulting solution was immediately concentrated under reduced pressure. After being co-concentrated twice from dry EtOH followed by dilution in dry EtOH, the β-lactam carboxylic acids were obtained by protonation with Amberlite (IR-120 H^+) (Fig. 3).



18

Fig. 3 Epimerization of the α-carbon would result in the diastereomers **18**.

Although this method had caused racemization in the hydrolysis of **2**, the ^{1}H NMR spectrum of the crude product showed no undesired diastereomers **18** and the β-lactam moiety was still intact. Purification by flash chromatography turned out to be difficult and gave decomposition of the β-lactam framework. Fortunately, trituration with acetone resulted in precipitation of the β-lactam carboxylic acids. A series of optically active bicyclic β-lactam carboxylic acids **7a**–**g** were synthesized with this method, all in excellent yields >98% except for the pentyl derivative **7f**, which was problematic to triturate resulting in a moderate yield of 60% (Scheme 4).



3 **7**

7a R=Ph (quant.)
7b R=1-naphthyl (quant.)
7c R=CH_2-1-naphthyl (quant.)
7d R=CH_2-2-naphthyl (quant.)
7e R=cyclohexyl (98%)
7f R=n-pentyl (60%)
7g R=Me (quant.)

Scheme 4 Rapid saponification of the methyl esters **3** with CsOH followed by protonation to give the β-lactam carboxylic acids **7**.

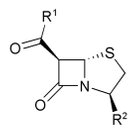
Biological evaluation

The β-lactam carboxylic acids **7(a–g)** and the β-lactam aldehydes **4(a–d)** were tested for their capability of binding to the periplasmic chaperone PapD with surface plasmon resonance on a Biacore 3000 instrument. This method has earlier proven to be a good screening device for pilicides, since the chaperone binding properties have been shown to correlate to the capability of disrupting a preformed chaperone-subunit complex.[19] Normalized responses were calculated using the 2-pyridinone **5** as reference since this was the most efficient pilicide in the earlier study.[19] In this screening β-lactams with a fairly large acyl substituent showed substantial binding to the chaperone PapD (Table 1). Interestingly, this was true also for the aldehyde derivatives **4(a–d)**. The aldehydes came off the chaperone as easily as the carboxylic acids in the washing step, which indicates that no imine was formed. Although not as good binders as the reference compound **5**, **7(a–g)** still bound better than the native C-terminal peptide PapG **7** which previously had showed inhibition of PapD in an ELISA.[16] Furthermore, **8(e–f)** showed almost the same affinity as the peptide PapG but the β-lactam bearing a small methyl substituent **7g** showed very weak binding.

Conclusions

A new practical synthesis of the key building block 2-H-Δ^2-2-thiazoline methyl ester **2** was developed which gave excellent yield and limited racemization. Then, a series of β-lactam esters was synthesized in good to excellent yields and different methods for ester hydrolysis were investigated. Among these the only fruitful protocol was a very fast saponification procedure, developed in our laboratory, resulting in a series of optically active β-lactam carboxylic acids. A surface plasmon resonance

Table 1 Affinities of compounds **4(a–d)** and **7(a–g)** for the PapD chaperone. Peptide PapG **8** and 2-pyridinone **5** are included for comparison



Compound	R^1	R^2	Norm. response for PapD[a]
7a	Ph	CO_2H	35
7b	1-Naphthyl	CO_2H	29
7c	CH_2-1-naphthyl	CO_2H	35
7d	CH_2-2-naphthyl	CO_2H	46
7e	Cyclohexyl	CO_2H	18
7f	*n*-Pentyl	CO_2H	22
7g	Me	CO_2H	2
4a	Ph	CHO	31
4b	1-Naphthyl	CHO	21
4c	CH_2-1-naphthyl	CHO	34
4d	CH_2-2-naphthyl	CHO	25
5	—	—	100
8	—	—	25

[a] Determined by surface plasmon resonance at a fixed pilicide concentration (30 μM) using a Biacore 3000 instrument. The normalized responses were calculated using **5** as standard (100 % norm. response) and all compounds were tested in triplicate in random order.

screening for the capability of binding to a periplasmic chaperone, PapD, revealed that β-lactams substituted with fairly large acyl substituents bound stronger to the chaperone than the native *C*-terminal peptide PapG **8**. Consequently, the bicyclic β-lactams can be considered as a new family with chaperone binding properties and they will be further evaluated as pilicides in the near future.

Experimental

General

All reactions were carried out under an inert atmosphere with dry solvents under anhydrous conditions, unless otherwise stated. 1,2-Dichloroethane and CH_2Cl_2 were distilled from calcium hydride (and THF from potassium, respectively). Benzene and toluene were distilled from sodium. TLC was performed on Silica Gel 60 F_{254} (Merck) with detection by UV light and staining with a solution of anisaldehyde (26 mL), glacial acetic acid (11 mL) and concentrated sulfuric acid (35 mL) in 95% ethanol (960 mL). Flash column chromatography (eluents given in brackets) was performed on silica gel (Matrix, 60 Å, 35–70 μm, Grace Amicon). Centrifugal preparative TLC was performed using rotors coated with silica gel 60 PF_{254} containing $CaSO_4$ (Merck). The moving bands were visualized using UV light. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-400 for solutions in $CDCl_3$ [residual $CHCl_3$ (δ_H 7.26 ppm) or $CDCl_3$ (δ_C 77.0 ppm) as internal standard] or in DMSO-d_6 [residual DMSO-d_5 (δ_H 2.50 ppm) or DMSO-d_6 (δ_C 40.0 ppm) as internal standard] at 298 K. First-order chemical shifts and coupling constants were obtained from one-dimensional spectra. IR spectra were recorded on an ATI Mattson Genesis Series FTIR™ spectrometer. Optical rotations were measured with a Perkin-Elmer 343 polarimeter and are given in 10^{-1} deg cm^2 g^{-1}. High resolution mass spectra were recorded on a JEOL SX102 A mass spectrometer. Ions for FABMS were produced by a beam of Xenon atoms (6 keV) from a matrix of glycerol and thioglycerol. Mass spectra on the β-lactam carboxylic acids **7** were recorded on a Waters micromassZQ using positive electrospray (ES+).

(4*R*)-4,5-Dihydrothiazole-4-carboxylic acid methyl ester (2)

TEA (2.9 mL, 20.6 mmol) was added dropwise to a suspension of L-cystein methyl ester hydrochloride (4000 mg, 23.3 mmol) in dry 1,2-dichloroethane (23 mL) at 0 °C. After stirring for 10 min the mixture was diluted with 1,2-dichloroethane (30 mL) and triethyl orthoformate (5.7 mL, 34.6 mmol) was added. After refluxing the mixture using a Soxhlet with molecular sieves 4 Å for 2 h a catalytic amount of PTSA was added, and the mixture was refluxed for another 18 h before reaching rt. Toluene was added and the mixture was cooled to −18 °C. Precipitated TEA–HCl was filtrated off and the filtrate was concentrated yielding **2** as an oil in quantitative yield. $[a]_D^{20}$ 130 (*c* 1.18, $CHCl_3$); IR (neat) 1741, 1572, 1437, 1340, 1219, 1039, 810 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, J = 2.29 Hz, 1H), 5.01–5.11 (m, 1H), 3.78 (s, 3H), 3.39–3.53 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 159.6, 77.7, 52.9, 33.3; HRMS (EI+) Calcd. for $C_5H_7NO_2S$: 145.0197 Obsd. 145.0186.

β-Lactam methyl esters 3a–g and β-lactam aldehydes 4a–d

Prepared according to published procedures.[18]

(4*R*)-4,5-Dihydrothiazole-4-carboxylic acid benzyl ester (10)

To the suspension of L-cystine dibenzyl ester di-tosylate (1640 mg, 2.14 mmol) in dioxane (7.0 mL) and conc. HCl (1.0 mL) was added zinc dust (600 mg, 9.17 mmol) in many portions over a period of 15 minutes with vigorous stirring. After stirring at rt for 1.5 h, undissolved zinc was filtered off and washed with dioxane and the filtrate was concentrated. The residue was freeze-dried from water. The residue was dissolved in pyridine (10 mL) and a previously prepared solution of acetic anhydride (1.05 eq.) and formic acid (1.2 eq.) was added. After stirring for 2 h at rt the solution was concentrated. The residue was dissolved in $CHCl_3$ and the pH was adjusted to neutral with 6 M HCl and the solution was co-concentrated from $CHCl_3$ (3×). The residue was suspended in benzene (100 mL), a catalytic amount of PTSA was added, and the solution was refluxed overnight using a Dean Stark apparatus. The organic phase was washed with saturated $NaHCO_3$, water and brine. The water phase was extracted twice with CH_2Cl_2 and the combined organic extracts were dried with Na_2SO_4, filtered and concentrated. Flash chromatography (heptane–ethyl acetate 5 : 1–1 : 1) gave **10** (350 mg, 37%). $[a]_D^{20}$ 16 (*c* 4.40, $CHCl_3$); IR (neat) 1747, 1662, 1513, 1392, 1344, 1172, 964, 775, 734 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, J = 2.38 Hz, 1H), 7.31–7.41 (m, 5H), 5.19–5.30 (m, 2H), 5.10–5.17 (m, 1H), 3.42–3.56 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1, 159.6, 135.2, 128.5, 128.4, 128.2, 77.7, 67.3, 33.2 HRMS (FAB+) Calcd. For (M + 1) $C_{11}H_{12}NO_2S$: 222.0589 Obsd. 222.0591.

Boc-Cys(Trt)-PNB, (12)

Boc-Cys(Trt)-CO_2H (6000 mg, 12.9 mmol) was dissolved in 80% aqueous EtOH (100 mL), and the pH was adjusted to pH 10 with 30% aqueous Cs_2CO_3, concentrated at reduced pressure, then diluted with EtOH (absolute), and concentrated at high vaccum. The resulting powder was dissolved in DMF (50 mL) and cooled to 0 °C, after which 4-nitrobenzyl bromide (3300 mg, 15.3 mmol) was added dropwise. After 12 h at rt the reaction mixture was diluted with water and extracted with Et_2O. The organic phase was dried with Na_2SO_4, filtered and concentrated. Flash chromatography (heptane–ethyl acetate 8 : 2–7 : 3) gave **12** (7900 mg, 100%). $[a]_D^{20}$ 11 (*c* 1.7, $CHCl_3$); IR (neat) 3374, 2966, 1745, 1712, 1606, 1511, 1444, 1346, 1261, 1157, 1012, 844, 738, 698 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (d, J = 8.69 Hz, 2H), 7.46 (d, J = 8.69 Hz, 2H), 7.33–7.38 (m, 5H), 7.19–7.30 (m, 10H), 5.15–5.30 (m, 2H), 5.04 (d, J = 8.23 Hz, 1H), 4.26–4.38 (m, 1H), 2.52–2.73 (m, 2H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 155.0, 147.7,

144.1, 142.5, 129.4, 128.4, 128.0, 127.0, 123.7, 80.2, 66.9, 65.6, 52.6, 34.0, 28.3 HRMS (FAB+) Calcd. for (M + Na) $C_{34}H_{34}N_2O_6SNa$: 621.2035 Obsd. 621.2030.

(4*R*)-4,5-Dihydrothiazole-4-carboxylic acid 4-nitrobenzyl ester (13)

A solution of TFA–H_2O–thioanisole–ethanedithiol 175 : 10 : 10 : 5 (200 mL) was added to **12** (7900 mg, 13.2 mmol) and after stirring for 3 h, AcOH (100 mL) was added and the solution was concentrated. To the residue was added AcOH (100 mL) and the white precipitate was filtrated off. The filtrate was concentrated and the residue was triturated with Et_2O which gave a solid, crude product which was dissolved in a solution of AcOH and water and freeze-dried yielding Cys-PNB (3200 mg) as a powder which was used in the next step without further purification. To a solution of Cys-PNB (1450 mg) in pyridine (7.5 mL) was added a previously prepared solution of acetic anhydride (1.05 eq.) and formic acid (1.2 eq.) and after stirring for 2 h at room temperature the solution was concentrated. The residue was dissolved in $CHCl_3$ and pH was adjusted to neutral with 6 M HCl and the solution was co-concentrated from $CHCl_3$ (3×). The residue was suspended in benzene (180 mL), a catalytic amount of PTSA was added, and the solution was refluxed overnight using a Dean Stark apparatus. The organic phase was washed with saturated $NaHCO_3$, water and brine. The water phase was extracted twice with CH_2Cl_2 and the combined organic extracts were dried with Na_2SO_4, filtered and concentrated. Flash chromatography (heptane–ethyl acetate 3 : 1–1 : 2) gave **13** (550 mg, 35%). $[\alpha]_D^{20}$ 43 (*c* 1.40 $CHCl_3$); IR (neat) 3066, 1749, 1680, 1602, 1512, 1342, 1315, 1180 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, J = 8.60 Hz, 2H), 8.06 (d, J = 2.38 Hz, 1H), 7.54 (d, J = 8.78 Hz), 5.33 (s, 1H), 5.15–5.22 (m, 1H), 3.45–3.60 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1, 160.2, 147.9, 142.5, 128.6, 124.0, 77.7, 65.9, 33.3; HRMS (CI+) Calcd. for $C_{11}H_{11}N_2O_4S$: 267.0440 Obsd. 267.0443.

4,5-Dihydrothiazole-4-carboxylic acid heptyl ester ((±)-16a)

Aqueous CsOH (0.1 M, 68.5 mL) was added dropwise to a stirred solution of thiazoline methyl ester **2** (1000 mg, 6.85 mmol) in MeOH (200 ml) at rt. The mixture was immediately concentrated and the residue was co-concentrated four times from dry EtOH giving the thiazoline Cs-salt as a white foam. A solution of heptyl bromide (1.50 mL, 9.50 mmol) in DMF (6 ml) was added dropwise to a solution of the Cs-salt (±)-**15** (1390 mg, 5.28 mmol) in DMF (16 ml) at 0 °C. After stirring for 2 h the cooling bath was removed and the reaction was allowed to reach rt overnight. The reaction mixture was diluted with Et_2O and washed with ice cooled water (7×) and the organic extract was dried with Na_2SO_4, filtered and concentrated. Flash chromatography (heptane–ethyl acetate 1 : 1), spinning centrifugal preparative TLC (heptane–ethyl acetate 3 : 7), spinning centrifugal preparative TLC (heptane–ethyl acetate 7 : 3) gave (±)-**16a** (692 mg, 57%) as an oil. $[\alpha]_D^{20}$ 0 (*c* 1.05 $CHCl_3$); IR (neat) 2954, 2929, 2858, 2360, 1741, 1571, 1465, 1186 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, J = 2.2 Hz, 1H), 5.09 (dt, J = 9.51, 2.38 Hz, 1H), 4.15–4.21 (m, 2H), 3.43–3.55 (m, 2H), 1.61–1.71 (m, 2H), 1.18–1.38 (m, 10H), 0.81–0.89 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 159.7, 77.6, 66.0, 33.2, 31.6, 28.8, 28.4, 25.6, 22.4, 14.0; HRMS (EI+) Calcd. for $C_{11}H_{19}NO_2S$: 229.1136, Obsd. 229.11337.

4,5-Dihydrothiazole-4-carboxylic acid benzyl ester ((±)-16b)

By following the procedure described for the preparation of (±)-**16a** from (±)-**15**, (±)-**15** (188 mg, 1.30 mmol) and benzyl bromide (400 mg, 2.33 mmol) gave (±)-**16b** (180 mg, 62%) as a an oil. $[\alpha]_{20}^{D}$ 0 (*c* 1.00, $CHCl_3$), all spectroscopic data were in agreement with **10**.

4,5-Dihydrothiazole-4-carboxylic acid 4-nitrobenzyl ester ((±)-16c)

By following the procedure described for the preparation of (±)-**16a** from (±)-**15**, (±)-**15** (188 mg, 1.30 mmol) and 4-nitrobenzyl bromide (504 mg, 2.33 mmol) gave (±)-**16c** (240 mg, 70%) as a white powder. $[\alpha]_D^{20}$ 0 (*c* 1.23, $CHCl_3$), all spectroscopic data were in agreement with **13**.

4,5-Dihydrothiazole-4-carboxylic acid prop-2-ynyl ester ((±)16d)

By following the procedure described for the preparation of (±)-**16a** from (±)-**15**, (±)-**15** (800 mg, 3.04 mmol) and propargyl bromide (0.60 ml, 5.55 mmol) gave (±)-**16d** (504 mg, 98%) as an oil. $[\alpha]_D^{20}$ 0 (*c* 0.92, $CHCl_3$); IR (neat) 3282, 1745, 1570, 1176, 1038, 810 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, 2.47 Hz, 1H), 5.11–5.18 (m, 1H), 4.80 (dq, J = 15.55, 2.47 Hz, 2H), 3.46–3.58 (m, 2H), 2.51 (t, J = 2.38 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.7, 160.1, 77.6, 77.3, 75.7, 53.2, 33.3; HRMS (EI+) Calcd. for $C_7H_7NO_2S$: 169.07975 Obsd. 169.0274.

6-(2-Naphthalen-1-ylacetyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]-heptane-2-carboxylic acid heptyl ester ((±)17a)

By following the published procedure described for the preparation of **3**,[18] (±)-**16a** (810 mg, 3.53 mmol) and **1c** (1440 mg, 4.59 mmol) gave (±)-**17a** (1100 mg, 70%) as a foam. $[\alpha]_D^{20}$ 0 (*c* 1.0, $CHCl_3$); IR (neat) 2972, 2656, 1741, 1676, 1402, 1199, 968 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.88 (t, J = 6.95 Hz, 2H), 7.81 (d, J = 8.14 Hz, 1H), 7.34–7.56 (m, 4H), 6.54 (s, 1H), 5.26 (d, J = 6.31 Hz, 1H), 5.10 (s, 1H), 4.14 (s, J = 6.68 Hz, 2H), 4.01 (s, 1H), 3.46–3.53 (m, 1H), 3.21 (d, J = 11.16 Hz, 1H), 1.57–1.67 (m, 2H), 1.20–1.37 (m, 10H), 0.84–0.92 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.1, 168.8, 160.7, 133.9, 131.8, 130.5, 128.8, 128.3, 128.1, 126.5, 125.9, 125.4, 123.4, 101.2, 94.1, 66.3, 60.8, 36.7, 32.0, 31.6, 28.7, 28.4, 25.6, 22.5, 14.0; HRMS (EI+) Calcd. for $C_{25}H_{29}NO_4S$: 439.18173 Obsd. 440.18897.

6-(2-Naphthalen-1-ylacetyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]-heptane-2-carboxylic acid prop-2-ynyl ester ((±)-17b)

By following the published procedure described for the preparation of **3**,[18] (±)-**16d** (340 mg, 2.01 mmol) and **1c** (816 mg, 2.61 mmol) gave (±)-**17b** (693 mg, 91%). $[\alpha]_D^{20}$ 0 (*c* 1.05, $CHCl_3$); IR (neat) 3288, 1753, 1666, 1398, 1184, 968, 752 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.78–7.91 (m, 3H), 7.34–7.57 (m, 4H), 6.57 (s, 1H), 5.30 (d, J = 6.31 Hz, 1H), 5.11 (s, 1H), 4.74 (d, J = 2.38 Hz, 2H), 4.01 (s, 2H), 3.52 (dd, J = 11.71, 6.40 Hz, 1H), 3.25 (d, J = 11.07 Hz, 1H), 2.49 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.2, 168.5, 160.9, 134.0, 131.9, 130.6, 129.0, 128.5, 128.2, 126.7, 126.0, 125.6, 123.6, 101.2, 94.2, 76.8, 76.0, 60.8, 53.6, 36.9, 31.9; HRMS (EI+) Calcd. for $C_{21}H_{17}NO_4S$ 379.0900 Obsd. 379.0962.

(2*R*,5*S*,6*R*)-6-(Naphthalen-1-ylacetyl)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]heptane-2-carboxylic acid benzyl ester (11)

By following the published procedure described for the preparation of **3**,[18] **10** (536 mg, 2.42 mmol) and **1c** (904 mg, 2.89 mmol) gave **11** (606 mg, 58%). $[\alpha]_D^{20}$ 7 (*c* 0.70, $CHCl_3$); IR (neat) 2973, 2900, 1739, 1662, 1515, 1394, 1180, 1066, 962, 773, 732, 694 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.78–7.92 (m, 3H), 7.28–7.57 (m, 9H), 6.55 (s, 1H), 5.32 (d, J = 6.22 Hz, 1H), 5.18 (s, 2H), 5.09 (s, 1H), 4.00 (s, 2H), 3.49 (dd, J = 11.25, 6.22 Hz, 1H), 3.23 (d, J = 11.25 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.00, 168.98, 160.8, 135.0, 133.9, 131.8, 130.5, 128.9, 128.6, 128.5, 128.4, 128.2, 128.1, 126.5, 125.9, 125.5, 123.5, 101.2, 94.1, 67.8, 60.9, 36.8, 31.9; HRMS (FAB+) Calcd. for (M + 1) $C_{25}H_{22}NO_4S$ 432.1270 Obsd. 432.1270.

(2*R*,5*S*,6*R*)-6-(Naphthalen-1-ylacetyl)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]heptane-2-carboxylic acid 4-nitrobenzyl ester (14)

By following the published procedure described for the preparation of **3**,[18] **13** (1060 mg, 3.95 mmol) and **1c** (1570 mg, 5.03 mmol) gave **14** (1200 mg, 63%). $[\alpha]_{20}^{D}$ 8 (*c* 0.60, $CHCl_3$); IR (neat) 3363, 2971, 2902, 1513, 1392, 1342, 1249, 1056, 734, 696 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.21 (d, J = 8.69 Hz, 2H), 7.85–7.91 (m, 2H), 7.82 (d, J = 8.14 Hz, 1H), 7.40–7.56 (m, 5H), 7.37 (d, J = 6.31 Hz, 1H), 6.52 (s, 1H), 5.34 (d, J = 6.22 Hz, 1H), 5.22–5.32 (m, 2H), 5.11 (s, 1H), 4.02 (s, 2H), 3.54 (dd, J = 11.25, 6.22 Hz, 1H), 3.25 (d, J = 11.25 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.3, 168.9, 160.8, 147.8, 142.2, 133.9, 131.8, 130.4, 128.9, 128.4, 128.2, 126.6, 125.9, 125.5, 123.9, 123.4, 101.1, 94.0, 66.1, 60.8, 36.8, 31.9; HRMS (FAB+) Calcd. for (M + 1) $C_{25}H_{21}N_2O_6S$ 477.1120 Obsd. 477.1120.

(2*R*,5*S*,6*R*)-6-Benzoyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (7a)

Aqueous CsOH (0.1 M, 10 mL) was added to a stirred solution of β-lactam methyl ester **3a** (300 mg, 1.03 mmol) in MeOH (40 mL) at room temperature and the solution was immediately concentrated. The residue was co-concentrated twice from dry EtOH giving a yellow oil. The resulting yellow oil was dissolved in EtOH and pH was adjusted to pH = 4 with Amberlite (IR-120 H^+). The amberlyst was filtrated off and the filtrate was concentrated and the residue was triturated with acetone to give **7a** as a powder in quantitative yield. $[\alpha]_D^{20}$ 57 (*c* 1.48, DMSO); IR (neat) 3365, 1639, 1593, 1408, 1373, 1273 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.72–7.77 (m, 2H), 7.42–7.52 (m, 3H), 6.63 (s, 1H), 6.05 (s, 1H), 4.73 (d, J = 6.10 Hz, 1H), 3.28–3.34 (m, 1H), 3.19 (d, J = 9.80 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.3, 162.3, 160.9, 131.7, 129.3, 126.7, 99.0, 95.4, 63.6, 33.3; MS (ES+) Calcd. for (M + 1) $C_{13}H_{12}NO_4S$: 278.05 Obsd. 277.93.

(2*R*,5*S*,6*R*)-6-Naphthalenyl-7-oxo-4-thia-1-azabicyclo[3.2.0]-heptane-2-carboxylic acid (7b)

Compound **7b** was prepared from **3b** (300 mg, 0.88 mmol) as described above for the synthesis of **7a** from **3a**. After trituration with acetone **7b** was obtained as a powder in quantitative yield. $[\alpha]_D^{20}$ 16 (*c* 0.9, DMSO); IR (neat) 1563, 1508, 1338 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.96–8.18 (m, 3H), 7.51–7.73 (m, 4H), 6.94 (s, 1H), 5.7 (s, 1H), 4.74 (d, J = 5.85 Hz, 1H), 3.17–3.37 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.1, 164.0, 160.6, 133.7, 131.6, 130.6, 130.6, 129.3, 128.6, 127.8, 126.9, 125.7, 125.5, 104.3, 95.6, 63.1, 33.4; MS (ES+) Calcd. for (M+1) $C_{18}H_{16}NO_4S$: 328.06 Obsd. 327.88.

(2*R*,5*S*,6*R*)-6-(Naphthalen-1-ylacetyl)-7-oxo-4-thia-1-azabi-cyclo[3.2.0]heptane-2-carboxylic acid (7c)

Compound **7c** was prepared from **3c** (300 mg, 0.84 mmol) as described above for the synthesis of **7a** from **3a**. After trituration with acetone **7c** was obtained as a powder in quantitative yield. $[\alpha]_D^{20}$ −1 (*c* 0.1, DMSO); IR (neat) 3313br, 3056, 2923, 1714, 1596, 1373, 1095, 779 cm^{-1}; ^1H NMR(400 MHz, DMSO-d_6) δ 8.06 (d, J = 8.13 Hz, 1H), 7.83–7.98 (m, 2H), 7.41–7.61 (m, 4H), 6.51 (s, 1H), 5.01 (s, 1H), 4.71 (d, J = 6.73 Hz, 1H), 3.99–4.14 (m, 2H), 3.22–3.29 (m, 1H), 3.16 (d, J = 10.27 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.8, 167.9, 160.5, 133.9, 132.2, 132.0, 129.0, 128.3, 128.2, 126.9, 126.4, 126.2, 124.5, 101.5, 95.0, 62.9, 36.2, 32.9; MS (ES+) Calcd. for (M + 1) $C_{18}H_{16}NO_4S$: 342.08 Obsd. 341.86.

(2*R*,5*S*,6*R*)-6-(Naphthalen-2-ylacetyl)-7-oxo-4-thia-1-azabi-cyclo[3.2.0]heptane-2-carboxylic acid (7d)

Compound **7d** was prepared from **3d** (200 mg, 0.56 mmol) as described above for the synthesis of **7a** from **3a**. After trituration with acetone **7d** was obtained as a powder in quantitative yield. $[\alpha]_D^{20}$ 1 (*c* 0.2, DMSO); IR (neat) 2987, 2902, 1708, 1629, 1394, 1220, 1058, 968, 757 cm^{-1}; ^1H NMR(400 MHz, DMSO-d_6) δ 7.72–7.94 (m, 4H), 7.34–7.53 (m, 3H), 6.47 (s, 1H), 5.24 (s, 1H), 4.71 (d, J = 5.35 Hz, 1H), 3.73 (s, 2H), 3.09–3.27 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.9, 167.7, 160.6, 134.2, 133.6, 132.5, 128.6, 128.1, 128.0 (2×), 127.9, 126.8, 126.4, 101.7, 63.1, 95.0, 39.1, 32.9; MS (ES+) Calcd. for (M + 1) $C_{18}H_{16}NO_4S$: 342.08 Obsd. 341.86.

(2*R*,5*S*,6*R*)-6-Cyclohexanecarbonyl-7-oxo-4-thia-1-azabicyclo-[3.2.0]heptane-2-carboxylic acid (7e)

Compound **7e** was prepared from **3e** (130 mg, 0.44 mmol) as described above for the synthesis of **7a** from **3a**. After trituration with acetone **7e** was obtained as a powder (121 mg, 98%). $[\alpha]_D^{20}$ 27 (*c* 0.95, DMSO); IR (neat) 3319, 2927, 2852, 1647, 1595, 1414, 1371, 960, 752 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 6.40 (s, 1H), 5.14 (s, 1H), 4.67 (d, J = 6.13 Hz, 1H), 3.21–3.27 (m, 1H), 3.10–3.16 (m, 1H), 2.07–2.17 (m, 1H), 1.59–1.80 (m, 5H), 1.11–1.28 (m, 5H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.4, 170.5, 160.8, 98.5, 94.8, 63.1, 41.2, 33.0, 30.2, 30.0, 25.9, 25.8, 25.7; MS (ES+) Calcd. for (M + 1) $C_{13}H_{18}NO_4S$: 284.10 Obsd. 283.95.

(2*R*,5*S*,6*R*)-6-Hexanoyl-7-oxo-4-thia-1-azabicyclo[3.2.0]-heptane-2-carboxylic acid (7f)

Compound **7f** was prepared from **3f** (160 mg, 0.56 mmol) as described above for the synthesis of **7a** from **3a**. After trituration with acetone **7f** was obtained as a powder (91 mg, 60%). $[\alpha]_D^{20}$ 13 (*c* 0.36, DMSO); IR (neat) 3278, 2924, 2854, 1645, 1593, 1414, 1373, 1273, 968 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 6.50 (s, 1H), 5.28 (s, 1H), 5.00 (d, J = 6.40 Hz, 1H), 3.41 (dd, J = 10.98, 6.40 Hz, 1H), 3.17 (d, J = 10.98 Hz, 1H), 2.21 (t, J = 7.50 Hz, 2H), 1.42–1.50 (m, 2H), 1.20–1.31 (m, 4H), 0.83–0.89 (t, J = 6.68 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ171.2, 170.1, 160.6, 99.9, 94.3, 61.4, 32.7, 32.2, 31.0, 25.8, 22.2, 14.3: MS (ES+) Calcd. for (M + 1) $C_{12}H_{18}NO_4S$: 272.10 Obsd. 272.07.

(2*R*,5*S*,6*R*)-6-Acetyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (7g)

Compound **7g** was prepared from **3g** (190 mg, 0.83 mmol) as described above for the synthesis of **7a** from **3a**. After trituration with acetone **7g** was obtained as a powder in quantitative yield. $[\alpha]_D^{20}$ 24 (*c* 1.0, DMSO); IR (neat) 3294, 2949, 1645, 1593, 1412, 1367, 964, 748 cm^{-1}; ^1H NMR(400 MHz, DMSO-d_6) δ 6.46 (s, 1H), 5.22 (s, 1H), 4.69 (d, J = 6.04 Hz, 1H), 3.18–3.27 (m, 1H), 3.12–3.17 (m, 1H), 1.92 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.8, 165.7, 160.6, 101.0, 94.5, 63.2, 33.0, 19.3; MS (ES+) Calcd. for (M + 1) $C_8H_{10}NO_4S$: 216.03 Obsd. 215.95.

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