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Enantioselective synthesis of new oxazolidinylthiazolidines as enzyme inhibitors

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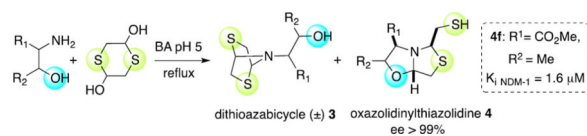
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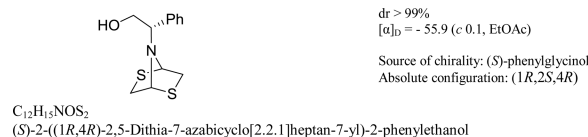
Abstract

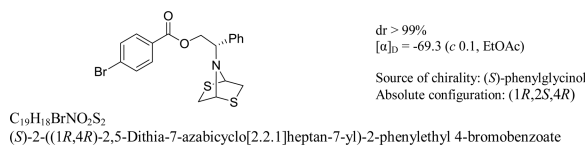
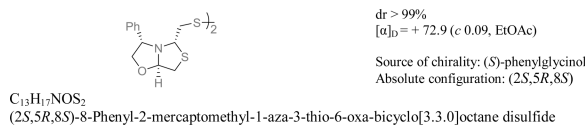
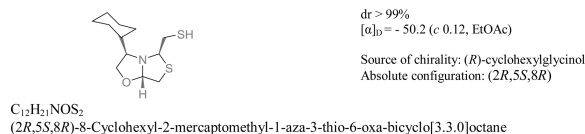
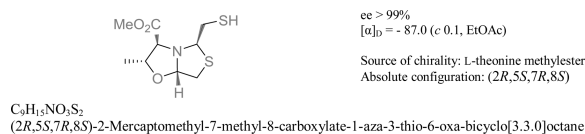
The synthesis of new oxazolidinylthiazolidines bicycles, oxygen analogues of bithiazolidines, also known as metallo- β -lactamase inhibitors is described. The reaction of β -aminoalcohols and 2,5-dihydroxy-1,4-dithiane led to oxazolidinylthiazolidines and/or dithia-azabicycles as the main products. The distribution pattern depends mainly on the aminoalcohol substituents. In a one-pot reaction, four new bonds are formed in good yields and with high atom efficiency. When the oxazolidinylthiazolidines are formed, two stereogenic centres are generated with high enantiospecificity. The reaction mechanism is discussed based on crystallographic data and interconversion studies. Two oxazolidinylthiazolidines were evaluated as inhibitors of the potent lactamase NDM-1 and compound **4f** displayed competitive inhibition with $K_i = 1.6 \pm 0.6 \mu\text{M}$.

Graphical Abstract



Stereochemistry Abstract





1. Introduction

Novel fused heterocyclic systems are important scaffolds in medicinal chemistry due to their ability to generate complex structures.^{1,2} In particular, fused five-membered 1,3-heterocycles containing oxygen, nitrogen, or sulfur are interesting scaffolds in the preparation of complex chiral molecules such as natural products, pharmaceuticals or catalysts.³

In this context, our group has explored the preparation of new bicycles by taking advantage of the ring-chain tautomerism, a process that involves the reversible movement of a proton followed by a change from an open structure to form a new heterocycle.^{4,5} By using this strategy, we have been able to prepare new heterocycles, including the synthesis of 5,5-fused heterocycles thiazolo[3,4-*c*]oxazoles⁴ and the sulfur analogues thiazolo[4,3-*b*]thiazoles,⁶ present different connectivity (Figure 1). The thiazolo[4,3-*b*]oxazole system is the oxygen analogue of thiazolo[4,3-*b*]thiazoles and attracted our interest (Figure 1).

In an earlier approach, we prepared heterocycles thiazolo[3,4-*c*]oxazoles and thiazolo[4,3-*b*]thiazoles via simple synthetic pathways and with high diastereoselectivity, using low cost and readily available starting materials. The new bithiazolidine scaffolds thiazolo[4,3-*b*]thiazoles showed diverse chemical and biological properties. We have recently constructed a dynamic combinatorial library suitable for disulfide exchange, using thiazolo[4,3-*b*]thiazole scaffolds. The dynamic library was responsive to the enzyme thioredoxin glutathione reductase, a flavoprotein from *Echinococcus granulosus*, and new inhibitors were identified.⁷ The prepared bithiazolidines were also evaluated as metallo- β -lactamase inhibitors, enzymes that hydrolyze almost all β -lactam antibiotics and are unaffected by clinically available β -lactamase inhibitors. The thiol-bearing thiazolo[4,3-*b*]thiazole derivatives are competitive β -lactamase inhibitors of all metallo- β -lactamase subclasses, with K_i values in the low micromolar range against a broad range of metallo- β -lactamases enzymes such as NDM-1,⁸ VIM-2,⁹ Sfh-I, L1, IMP-1, GOB-13 and BcII.¹⁰

Due to our interest in the preparation and evaluation of new thiazolo[4,3-*b*]thiazole analogues, we were prompted to prepare fused oxazolidinythiazolidines following the same methodology as used for thiazolo[4,3-*b*]thiazoles. There are few reports in the literature for the synthesis of the thiazolo[4,3-*b*]oxazole system (Figure 2). Different intermediates were used for thiazolo[4,3-*b*]oxazole preparation: azomethine ylides for (A)^{11,12,13} vinylogous *N*-acyliminium ions for (B),¹⁴ *S*-benzylthiocarbamates for (C)¹⁵ or CS₂ for (D)¹⁶ (figure 2).

Herein we focus our attention on the development of a new strategy for the preparation of new substituted thiazolo[4,3-*b*]oxazole, and report our findings on the synthesis of new thiazolo[4,3-*b*]oxazole and/or dithia-azabicycles through iminium ion formation by the condensation of β -aminoalcohols and mercaptoacetaldehyde.

2. Results and discussion

In order to synthesize thiazolo[4,3-*b*]oxazole via an efficient route, we used the same methodology we described before for the thiazolo[4,3-*b*]thiazole preparation.⁶ The strategy is based on a cascade reaction between mercaptoacetaldehyde and β -aminoalcohols. The preliminary results of heating linear β -aminoalcohols (X = O) and 2,5-dihydroxy-1,4-dithiane (mercaptoacetaldehyde dimer) in EtOH and catalytic *p*-TsOH acid led to dithia-azabicycles instead of the desired thiazolo[4,3-*b*]oxazole bicycle (Figure 3).

Crystallographic data allowed us to elucidate the correct structure of dithioazabicyclo, previously misassigned as bistiiranes.⁶ A reaction mechanism is proposed based on the results of a double iminium cyclization starting from an amine and two equivalents of mercaptoacetaldehyde, see Figure 4.

Intrigued by the structural requirements for thiazolo[4,3-*b*]oxazole formation, we used (*S*)-phenylglycinol **1a** as the starting material, in order to study the influence of the substituent α to the nitrogen. The reaction of **1a** and dithiane **2** catalyzed by *p*-TsOH acid in EtOH at reflux revealed the presence of 5% of the desired thiazolo[4,3-*b*]oxazole (*S*)-**4a** and dithioazabicyclo **3a** as the main product in 90% yield (entry 1, Table 1). We attributed the thiazolo[4,3-*b*]oxazole formation to the presence of a bulky substituent adjacent to the nitrogen in **1a**.

In order to optimize the ratio of thiazolo[4,3-*b*]oxazole formation, we assayed different reaction conditions. Reflux in toluene gave dithia-azabicyclo **3a**/oxazolidine **4a** in a 7:3 ratio (entry 2, Table 1). It is noteworthy that a higher temperature in toluene favoured the formation of the oxazolidine **4a**, but longer reaction times increased decomposition of the products (entry 3, Table 1). The reaction was also performed in a buffer acetate at pH = 5, which led to the same distribution pattern, but higher yields than the reaction in toluene (entries 4 and 5, Table 1).

We isolated dithia-azabicyclo (*R*)-**3a** by flash-column chromatography, but diastereomer (*S*)-**3a** co-eluted with oxazolidinythiazolidine (*S*)-**4a**. This result suggested that both compounds could be in tautomeric equilibrium. We performed different experiments to understand a possible interconversion mechanism. We first assigned the absolute configuration of the products after derivatization and crystallization. Fractions containing the

mixture of diastereomer (*S*)-**3a** and bicycle (*S*)-**4a** in a 3:1 ratio were oxidized in basic media ($K_2CO_3/MeOH/air$) to give dimer (*S*)-**5a** as a unique product (91% yield) (Scheme 1). The formation of (*S*)-**5a** occurred at the expense of (*S*)-**3a**, through (*S*)-**4a**, triggered by blocking the reactivity of the thiol group to form the disulfide. This result supports our hypothesis about the tautomeric equilibria between **3a** and **4a**.

The crystal structure of (*S*)-**5a** was obtained after slow evaporation of CH_2Cl_2/n -hexanes (1:6) at room temperature and then determined by single-crystal X-ray diffraction methods. The molecular structure allowed us to confirm the absolute stereochemistry of the precursor oxazolidinylthiazolidine (*S*)-**4a**. The ORTEP view of compound is shown in Figure 5.

On the other hand, esterification of pure compound (*R*)-**3a** was performed and single crystals of (*R*)-**6a** were obtained (Scheme 1). Compound (*R*)-**6a** was elucidated by X-ray experiments and the absolute configuration of this dithia-azabicycle was determined (Figure 5). Once the stereochemistry of the products was assigned, we studied the mechanism of the interconversion. We performed two parallel experiments: isolated diastereomers were dissolved in buffer solutions (pH 2, 5, 7 and 9) and stirred for 24h at room temperature. The product distribution was analyzed by 1H NMR (see Figure 6).

Interconversion of diastereomer (*S*)-**3a** to (*R*)-**3a** was observed, especially at pH 2. We also observed the inverse conversion from (*R*)-**3a** to tautomer (*S*)-**4a** and (*S*)-**3a** (data shown in Supplementary material).

These results indicate that a dynamic interconversion between diastereomers occurs mainly in acidic media. It is known that thiazolidines can undergo ring-chain tautomerism in solution.¹⁷ Our group has also reported this type of transformation for thiazolidines and oxazolidinylthiazolidines.^{4,5} The interconversion mechanism between dithiaazabicycles (*R*)-**3a** and (*S*)-**3a** could be explained by the formation of oxazolidine (*S*)-**4a** and (*R*)-**4a** intermediates (Scheme 2).

When (*S*)-**3a** equilibrates to (*R*)-**3a**, two stereogenic centers should be inverted. If we commence with dithia-azabicycle (*S*)-**3a**, the acetal bond could break, forming the iminium ion (*S*)-**Ia** with the loss of a stereogenic center. The intermediate could evolve to oxazolidinylthiazolidine (*S*)-**4a**, as observed by 1H -NMR. Compound (*S*)-**4a** could open and form the iminium ion **Ib** and then lead to the unstable oxazolidinylthiazolidine (*R*)-**4a**. This intermediate could then in turn open to form the iminium ion (*R*)-**Ia** followed by a ring closure to finally afford dithia-azabicycle (*R*)-**3a** (Scheme 2).

The interconversion between (*S*)-**3a** into (*S*)-**4a** was also observed in the Michael addition when a mixture of **3a:4a** (95:5) in presence of DMAD led to the thiazolo[4,3-*b*]oxazole **7** in 53% yield (Scheme 3).

This finding reinforces the concept of an interconversion between dithioazabicycle **3a** and thiazolo[4,3-*b*]oxazole **4a** as we started with a mixture of 4% of thiazolo[4,3-*b*]oxazole **4a** and finally obtained the Michael adduct thiazolo[4,3-*b*]oxazole **7** in 53%.

On the other hand we explored the scope of the reaction using other β -aminoalcohols. Starting with methylthreonine.HCl **1b** in PhMe we found erratic product distribution and yields. At this point, we suspected that the pH could be playing a crucial role in the reaction. We assayed different buffer solutions, from pH 2 to 6 (see Table 2). The best yield for the preparation of oxazolidinylthiazolidine **4b** was obtained using buffer pH 5 (entry 4, Table 2). Lower and higher pH values led to decomposition products, probably due to lower reaction rates or dithiane decomposition.

Having established the optimum pH for the thiazolo[4,3-*b*]oxazole formation, we explored the reaction using different β -aminoalcohols. We subjected aminoalcohols **1c–h** to the same cyclization process in buffer acetic acid/acetate sodium pH 5 or an organic solvent, in order to study the influence of the solvent in the product distribution. Depending on the β -aminoalcohol substituents, we obtained dithia-azabicyclo **3** and/or oxazolidinylthiazolidine **4** (see Table 3). The results showed that when the reaction was performed in an aqueous medium, it gave better yields than the same reaction in organic solvents. However the product distribution depended mainly on the aminoalcohol substituents R¹ and R². We observed that aminoalcohols with small or linear substituents at R¹ only led to dithia-azabicyclo **3** (entries 1–5, Table 3), but when R¹ was bulky, an increased proportion of **4** was observed (entries 9 and 10, Table 3). The same result was observed for glycinol **1a** and threonine **1b** (see entry 4, Tables 1 and 2 respectively).

The reaction with methylserine **1f** mainly gave dithioazabicyclo **3f** and traces of OTZ **4f** (entry 7, Table 3), but when we started with methylthreonine **1b**, we observed thiazolo[4,3-*b*]oxazole **4b** as the main product (entry 4, Table 2). This clearly indicates that the R² substituents affect the product distribution. This could be explained by the Thorpe-Ingold effect, where the substituents increase the rate or equilibrium constant for ring forming reactions.¹⁸ In summary, both substituents at R¹ and R² are important: bulky substituents in R¹ increase the formation of thiazolo[4,3-*b*]oxazole **4** vs dithioazabicyclo **3** while the methyl group in R² also shifts the equilibrium to the formation of thiazolo[4,3-*b*]oxazole.

In general, the absolute configuration of the oxazolidinylthiazolidines was assigned based on different experiments. The relative stereochemistry of compound **4b** was established as *syn* by NMR using 1D gradient NOESY (Table 4). The configuration of compound **5a** was determined by X-ray experiments; hence the same configuration for precursor **4a** was proposed. Finally, the configuration of compound **4h** was established as *syn* in analogy to **4b** and **5a**. This assignment was also supported by the analysis of the coupling constants observed for the oxazolidinylthiazolidines where the values for H¹, H² and H³ are in the same order of magnitude, see table 4.

Enzymatic activity

Recently we reported that bithiazolidine compound, L-**CS319** is able to inhibit metallo- β -lactamases from all subclasses,¹⁰ particularly the clinically relevant New Delhi metallo- β -Lactamase NDM-1.⁸ The molecular basis for the inhibition was based on the crystal structure of NDM-1:L-**CS319**. This complex indicated the importance of the thiol moiety

that bridges the two Zn (II) ions, while the carboxylate interacts with Lys224 in the active site of the enzyme.

Two of the prepared compounds **4f** and **4h** are oxygen analogues of heterocycle L-**CS319**, which also bears a free thiol, but with different substituents at the carboxylate position.

We envisioned that thiazolo[4,3-*b*]oxazoles **4f** and **4h** could be evaluated as NDM-1 inhibitors and therefore we studied the effect on the initial rates of imipenem hydrolysis by NDM-1. Progress curves revealed a competitive inhibition model for **4f** with an inhibition constant of $K_i = 1.6 \pm 0.6 \mu\text{M}$, a good value for this type of enzymes. This compound is four times more potent than the sulfur analogue L-**CS319**, (see Figure 7). It is noteworthy that the isosteric replacement of the oxygen vs. sulphur is well tolerated. Neither the ester nor the methyl group present in **4f** are deleterious to the inhibitor activity. Based on our previous results, we expected similar interactions at the active site with the thiol coordinating both Zn (II) ions and the ester interacting with a Lys residue of the active site. The methyl group probably has additional hydrophobic interactions leading to a lower K_i value than L-**CS319**. In contrast, compound **4h** is less potent than **4f** and L-**CS319** and therefore was only characterized for IC_{50} . The bulky cyclohexyl substitution present in **4h** is counterproductive and probably responsible for the low inhibitory activity shown.

3. Conclusion

In conclusion we have enantiospecifically prepared oxazolidinylthiazolidine **4** bicycles with very high ee and good yields. The best yields were obtained when the reaction was performed in buffer acetate at pH 5 while the distribution pattern was independent of the solvent used. In general, the product distribution for this reaction depends on the aminoalcohol substituents (R^1 and R^2). Experimental data supported the idea that the products of the phenylglycinol cyclization **3a** and **4a** were in tautomeric equilibrium and an interconversion mechanism was proposed. Compound **4f** was evaluated against the metallo- β -lactamase NDM-1, an oxygen analogue of known metallo- β -lactamase inhibitors bithiazolidines and resulted in a good inhibitor.

4. Experimental

General

Reactions were monitored by analytical thin layer chromatography (TLC) 0.25 mm Silica gel plastic sheets (Macherey-Nagel, Polygram® SIL G/UV 254). Flash chromatography on Silica gel 60 (J. T. Baker, 40 μm average particle diameter) was used to purify the crude reaction mixtures. NMR spectra were recorded at 400 MHz and 100 MHz (^1H -NMR, ^{13}C -NMR) using a Bruker AVANCE at 21 °C. Chemical shifts (δ) are reported as follows: chemical shift (multiplicity, coupling constant, integration). High-resolution mass spectrometry experiments were measured on a VG AutoSpect spectrometer (EIS mode). Crystallographic data was collected on a Bruker D8 Venture Single Crystal diffractometer. Melting points were determined using a Laboratory Devices Gallenkamp apparatus. Optical rotations were measured using a Kruss Optronic GmbH P8000 polarimeter with a 0.5-mL cell, $[\alpha]_D$ values are given in $0.1 \text{ deg}\cdot\text{cm}^2\cdot\text{g}^{-1}$ (concentration *c* given as g/100 mL). All

solvents were purified according to literature procedures. All reactions were carried out in dry, freshly distilled solvents under anhydrous conditions unless otherwise stated. All yields refer to isolated compounds after the final purification process, unless otherwise stated. Relative stereochemistry for **4b** was determined using 1D gradient NOESY experiments, pulse sequence selnpgp, D8 at 300 and 800 ms. Enantiomeric excess was determined using HPLC Chiral OD column 250 × 4.6 mm (L × I.D.). Diastereomeric excess was calculated based on ¹H NMR spectra.

7-(4-Fluorobenzyl)-2,5-dithia-7-aza-bicyclo[2.2.1]heptane DTA-1

To a suspension of 1,4-dithiane-2,5-diol **2** (0.73 g, 4.8 mmol) in ethanol (10 mL) were added *p*-F-benzylamine (0.5 g, 4.0 mmol) and catalytic *p*-TsOH acid (15 mg). The resulting mixture was heated at reflux for 2h. The solvent was removed under reduced pressure; the crude was poured into water and extracted with EtOAc (3 × 80 mL). The combined organic layers were dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the crude was purified by column chromatography on SiO₂ flash (1:5 EtOAc/hexanes) to afford pure **DTA-1** as a white solid (0.9 g, 93% yield). The crude was recrystallized from a mixture of hexanes/CH₂Cl₂ to afford single crystals: mp 77–79 °C. ¹H NMR (CDCl₃) δ 3.24 (d, *J* = 9.5 Hz, 2H), 3.37 (dd, *J* = 9.5, 3.1 Hz, 2H), 3.56 (s, 2H), 4.77 (d, *J* = 3.1 Hz, 2H), 7.03 (t, *J* = 8.5 Hz, 2H), 7.37 (dd, *J* = 8.5, 5.5 Hz, 2H). ¹³C NMR (CDCl₃) δ 44.0, 52.3, 69.7, 77.2, 115.4, 115.7, 130.5, 130.5, 133.3, 162.4 (C–F, *J* = 244.3 Hz); HRMS calcd for C₁₁H₁₄FNS₂ [M+H]⁺ 242.0473, found: 242.0498.

General procedure for the synthesis of dithia-azabicyclo **3** and oxazolidinylthiazolidine **4**

Route A (organic solvent)

To a stirred solution of aminoalcohol **1** (3.6 mmol) in toluene (25 mL) were added 1,4-dithiane-2,5-dithiol **2** (4.3 mmol) and catalytic *p*-TsOH acid (0.025 g, 0.12 mmol). The mixture was heated at reflux for 3 h. The solvent was then removed under reduced pressure. The crude was poured into water (30 mL), extracted with EtOAc (3 × 50 mL), dried (Na₂SO₄) and filtered. The organic layer was evaporated under reduced pressure. The crude was purified by column chromatography (n-hexanes/EtOAc) to obtain compounds **3** and/or **4**.

Route B (buffer acetate)

To a stirred suspension of aminoalcohol **1** (0.63 mmol) in acetic acid/acetate sodium [1M] pH = 5 (9 mL), was added 1,4-dithiane-2,5-dithiol **2** (0.750 mmol). The mixture was heated at reflux for 1h, cooled and extracted with EtOAc (4 × 30 mL), then dried (Na₂SO₄) and filtered. The organic layer was evaporated under reduced pressure. The crude was purified by column chromatography using (n-hexanes/EtOAc) to obtain compounds **3** and/or **4**.

(2S)-2-((1S/R,4S/R)-2,5-dithia-7-azabicyclo[2.2.1]heptan-7'-yl)-2-phenylethanol (S,R)-3a and (2S,5S,8S)-8-phenyl-2-mercaptomethyl-1-aza-3-thio-6-oxabicyclo[3.3.0]octane (S)-4a

Prepared according to Route B, using (*S*)-phenylglycinol **1a** as the starting aminoalcohol. The crude was purified by column chromatography on SiO₂ flash (1:5 EtOAc/hexanes) to afford pure (*R*)-**3a** as a transparent oil and a mixture of (*S*)-**3a** and (*S*)-**4a** as an oil (0.585 g, total yield = 64%, (*S/R*)-**3a**/*S*-**4a** 7:3).

(2S)-2-((1R,4R)-2,5-Dithia-7-azabicyclo[2.2.1]heptan-7'-yl)-2-phenylethanol (R)-3a

$[\alpha]_D = -55.9$ (*c* 0.1, EtOAc); ¹H NMR (CDCl₃) δ 3.19 (d, *J* = 9.5 Hz, 2H), 3.40 (dd, *J* = 9.5, 3.0 Hz, 2H), 3.46 (t, *J* = 5.2, 1H), 3.91 (d, *J* = 5.2 Hz, 2H), 5.00 (d, *J* = 3.0 Hz, 2H), 7.39 (m, 5 H); ¹³C NMR (CDCl₃) δ 43.9, 63.8, 66.9, 68.4, 128.3, 128.6, 129.0, 138.7; HRMS Calcd for C₆H₁₂NS₃ [M+H]⁺ 254.0698; found 254.0673.

Mixture of (S)-3a and (S)-4a. (S)-3a

¹H NMR (CDCl₃) δ 3.20 (d, *J* = 9.5 Hz, 2H), 3.40 (dd, *J* = 9.5, 3.1 Hz, 2H), 3.46 (t, *J* = 5.2 Hz, 1H), 3.90 (m, 2H), 4.96 (d, *J* = 3.1 Hz, 2H), 7.36 (m, 3H), 7.43 (m, 2H); ¹³C NMR (CDCl₃) δ 43.4, 63.7, 66.1, 68.5, 127.8, 128.1, 128.8, 129.0, 139.7. (*S*)-**4a**: ¹H NMR (CDCl₃) δ 1.68 (dd, *J* = 8.8, 7.5 Hz, 1H_{SH}), 2.55 (ddd, *J* = 13.6, 8.8, 6.7 Hz, 1H), 2.71 (dt, *J* = 13.6, 7.5 Hz, 1H), 3.12 (dd, *J* = 12.5, 1.8 Hz, 1H), 3.33 (dd, *J* = 12.5, 4.9 Hz, 1H), 3.62 (dd, *J* = 9.0, 8.2 Hz, 1H), 4.14 (dd, *J* = 9.2, 6.5 Hz, 1H), 4.28 (m, 2H), 5.40 (dd, *J* = 5.0, 1.8 Hz, 1H), 7.35 (m, 3H), 7.40 (m, 2H); ¹³C NMR (CDCl₃) δ 32.8, 38.3, 69.2, 74.1, 100.1, 127.8, 128.2, 128.4, 129.0, 139.1.

(2R,5S,7R,8S)-2-Mercaptomethyl-7-methyl-8-carboxylate-1-aza-3-thio-6-oxa-bicyclo [3.3.0]octane 4b

Prepared following Route B, using L-threonine-OMe.HCl **1b** as starting aminoalcohol. The crude was purified by chromatography (1:5, EtOAc:hexanes) to give **4b** as oil (118 mg, 79 %, 99% ee): $[\alpha]_D = -87.0$ (*c* 0.1, EtOAc); ¹H NMR (CDCl₃) δ 1.43 (d, *J* = 6.0 Hz, 1H), 2.04 (dd, *J* = 9.3, 7.0 Hz, 1H), 2.56 (ddd, *J* = 13.6, 9.3, 6.1 Hz, 1H), 2.77 (ddd, *J* = 13.6, 8.2, 7.0 Hz, 1H), 3.09 (d, *J* = 12.9 Hz, 1H), 3.26 (dd, *J* = 12.9, 4.4 Hz, 1H), 3.31 (d, *J* = 8.5, 1H), 3.79 (s, 3H), 4.08 (dd, *J* = 8.5, 6.0 Hz, 1H), 4.26 (dd, *J* = 8.2, 6.1 Hz, 1H), 5.22 (d, *J* = 4.4, 1H); ¹³C NMR (CDCl₃) δ 18.1, 32.7, 37.3, 52.5, 73.9, 76.8, 78.5, 99.3, 171.4; HRMS calcd for C₉H₁₅NO₃S₂Na [M+Na]⁺ 272.0386, found 272.0491. The ee value was determined by Chiral HPLC analysis with a Chiralcel OD column [(hexane/2-propanol = 90/10, 1 mL/min, 220 nm); retention time: *t*_R = 5.33 min (L), *t*_R = 5.96 min].

Compounds **3c** and **3d** are described in literature.⁶

2-(2,5-Dithia-7-azabicyclo[2.2.1]heptan-7-yl)-2-(hydroxymethyl) propane-1,3-diol 3e

prepared according to Route A, using tris(hydroxyl-methyl)aminomethane **1e** as starting aminoalcohol. The solvent was removed under reduced pressure, the residue was dissolved in EtOH (10 mL) and after the addition of Et₂O (30 mL) the product precipitated as a white solid (0.906 g, 97%): mp = 129.8 – 130.0 °C (decomposition, from Et₂O). ¹H NMR (CDCl₃)

δ 2.83 (dd, J = 13.6, 7.2 Hz, 2H), 3.23 (dd, J = 13.6, 2.2 Hz, 2H), 3.47 (s, 6 H), 4.82 (m, 2H), 5.22 (s, 3H), 6.20 (s, 1H); ^{13}C NMR (CDCl_3) δ 35.1, 59.2, 61.1, 69.0.

(2S)-Methyl-2-(2,5-dithia-7-azabicyclo[2.2.1]heptan-7-yl)-3-hydroxypropanoate (S/R)-3f

Prepared according to Route B, using L-serine-OMe.HCl **1f** as the starting aminoalcohol. The residue was purified by chromatography (EtOAc/hexanes 1:5) to give **3f** (dr 1:1) as white solid and (*S*)-**4f** (**3f/4f** ratio 9:1, 45 mg, total yield = 32%). (*S/R*)-**3f**: ^1H NMR (CDCl_3) δ 3.25 (m, 3H), 3.37 (td, J = 10.0, 3.2 Hz, 2H), 3.80 (s, 3H), 3.97 (m, 2H), 5.15 (t, J = 2.7 Hz, 2H); ^{13}C NMR (CDCl_3) δ 43.5, 43.8, 52.7, 61.9, 62.1, 62.2, 62.8, 68.0, 68.2, 170.8, 170.9; HRMS calcd for $\text{C}_8\text{H}_{13}\text{NNaO}_3\text{S}_2$ [$\text{M}+\text{Na}$] $^+$ 258.0019, found 258.0018.

2-(2,5-Dithia-7-azabicyclo[2.2.1]heptan-7-yl)phenol 3g

Prepared according to Route A, using *o*-aminophenol **1g** as starting aminoalcohol. The residue was purified by column chromatography (EtOAc/hexanes 1:5) to give **3g** as red foam and *rac*-**4g** (**3g/4g** ratio 8.5:1.5, 130 mg, total yield = 74%). **3g**: ^1H NMR (CDCl_3) δ 3.34 (d, J = 9.4 Hz, 1H), 3.38 (dd, J = 9.4, 2.5 Hz, 1H), 5.26 (d, J = 2.5 Hz, 1H), 6.84 (m, 1H), 6.95 (dd, J = 8.0, 1.4 Hz, 1H), 7.09 (td, J = 8.0, 1.5 Hz, 1H), 7.62 (dd, J = 8.0, 1.5 Hz, 1H); ^{13}C NMR (CDCl_3) δ 45.2, 68.5, 76.8, 77.2, 77.5, 115.6, 120.6, 120.9, 127.0, 130.5, 150.6; HRMS calcd for $\text{C}_{10}\text{H}_{11}\text{NOS}_2$ [$\text{M}-\text{nH}$] $^-$ 224.0198, found 224.0281. *rac*-**4g**: ^1H NMR (CDCl_3) δ 1.94 (dd, J = 10.3, 6.6 Hz, 1H_{SH}), 2.70 (ddd, J = 13.7, 10.3, 4.9 Hz, 1H), 2.89 (ddd, J = 13.7, 9.0, 6.6 Hz, 1H), 3.19 (dd, J = 12.3, 3.7 Hz, 1H), 3.46 (dd, J = 12.3, 4.9 Hz, 1H), 4.74 (dd, J = 9.0, 4.9 Hz, 1H), 6.06 (m, 1H), 6.75 (m, 2H), 6.88 (m, 2H); ^{13}C NMR (CDCl_3) δ 33.5, 37.9, 75.6, 102.1, 108.7, 112.6, 121.9, 123.3, 135.5, 144.3, 144.9.

(2R,5S,8R)-8-Cyclohexyl-2-mercaptomethyl-1-aza-3-thio-6-oxa-bicyclo [3.3.0]octane 4h

Prepared following Route B, starting from (*R*)-cyclohexylglycinol **1h** as the starting aminoalcohol. The residue was purified by column chromatography on flash SiO_2 (EtOAc/hexanes 1:9) to afford **4h** (0.114 g, 89%, 99% ee) as an oil: $[\alpha]_{\text{D}} = -50.2$ (*c* 0.12, EtOAc); ^1H NMR (CDCl_3) δ 0.97 (m, 2H), 1.20 (m, 3H), 1.41 (m, 1H), 1.75 (m, 4H), 1.81 (dd, J = 8.8, 7.8 Hz, 1H), 1.90 (m, 1H), 2.62 (dt, J = 13.6, 7.8 Hz, 1H), 2.76 (ddd, J = 13.6, 8.8, 5.9 Hz, 1H), 2.91 (q, J = 7.1 Hz, 1H), 3.05 (dd, J = 12.5, 0.9 Hz, 1H), 3.24 (dd, J = 12.5, 4.2 Hz, 1H), 3.53 (dd, J = 8.4, 7.0 Hz, 1H), 4.00 (dd, J = 8.4, 7.0 Hz, 1H), 4.23 (dd, J = 7.8, 5.9 Hz, 1H), 5.04 (dd, J = 4.2, 0.9 Hz, 1H); ^{13}C NMR (CDCl_3) δ 26.1, 26.3, 26.6, 29.2, 30.7, 32.8, 37.1, 41.8, 68.4, 71.6, 79.0, 99.5; HRMS calcd for $\text{C}_{12}\text{H}_{21}\text{NOS}_2$ [$\text{M}+\text{Na}$] $^+$ 282.0957, found 282.1025.

(2S,5R,8S)-8-Phenyl-2-mercaptomethyl-1-aza-3-thio-6-oxa-bicyclo[3.3.0]octane disulfide (S)-5a

To a stirred solution of a mixture of (*S*)-**3a**/*(S)*-**4a** (0.1 g, 0.36 mmol) in MeOH (8 mL) and CH_2Cl_2 (2 mL) was added K_2CO_3 (0.1 g, 0.98 mmol). The mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and the residue was purified by column chromatography (1:8 EtOAc:hexanes) to afford compound (*S*)-**5a** as a white solid (82 mg, 91%, 99% ee). The product obtained was recrystallized from CH_2Cl_2 /hexanes to afford single crystals: mp = 60.0 – 60.1 °C; $[\alpha]_{\text{D}} = +72.9$ (*c* 0.09, EtOAc); ^1H

NMR (CDCl₃) δ 2.70 (dd, *J* = 13.5, 6.1 Hz, 1H), 2.83 (dd, *J* = 13.5, 8.5 Hz, 1H), 3.09 (dd, *J* = 12.4, 2.0 Hz, 1H), 3.25 (dd, *J* = 12.4, 5.0 Hz, 1H), 3.58 (dd, *J* = 9.1, 8.4 Hz, 1H), 4.08 (dd, *J* = 9.2, 6.5 Hz, 1H), 4.25 (dd, *J* = 8.3, 6.5 Hz, 1H), 4.33 (dd, *J* = 8.4, 6.1 Hz, 1H), 5.32 (dd, *J* = 4.9, 1.9 Hz, 2H), 7.33 (m, 6H), 7.41 (m, 4H); ¹³C NMR (CDCl₃) δ 38.6, 47.6, 68.6, 72.9, 74.1, 100.0, 127.9, 128.1, 128.6, 138.7; HRMS calcd for C₂₄H₂₈N₂O₂S₄ [M+H]⁺ 505.1106, found 505.1112.

**(S)-2-((1*R*,4*R*)-2,5-Dithia-7-azabicyclo[2.2.1]heptan-7-yl)-2-phenylethyl-4-bromo benzoate
(*R*)-6a**

To a stirred solution of (*R*)-**3a** (0.1 g, 0.4 mmol) in CH₂Cl₂ (5 mL) were added *p*-BrPhCOCl (0.1 g, 0.43 mmol), DIPEA (0.081 g, 0.63 mmol) and DMAP (0.002 g, 0.02 mmol). The mixture was stirred at room temperature for 3 h. After the addition of water (10 mL), the product was extracted with CH₂Cl₂ (3×15 mL), dried (Na₂SO₄) and filtered. The solvent was removed under reduced pressure and the residue was purified by chromatography (EtOAc/hexanes 1:5) to afford (*R*)-**6a** as a crystalline solid (135 mg, 77%). The product was recrystallized from CH₂Cl₂/hexanes to afford single crystals: mp 132.9 – 135.4 °C; [α]_D = – 69.3 (*c* 0.1, EtOAc). ¹H NMR (CDCl₃) δ 3.21 (d, *J* = 9.6 Hz, 2H), 3.35 (dd, *J* = 9.6, 3.0 Hz, 2H), 3.70 (t, *J* = 5.1 Hz, 1H), 4.48 (dd, *J* = 11.5, 5.8 Hz, 1H), 4.63 (dd, *J* = 11.5, 4.4 Hz, 1H), 4.99 (d, *J* = 3.0 Hz, 2H), 7.38 (m, 3H), 7.46 (m, 2H), 7.59 (d, *J* = 8.6, 2H), 7.83 (d, *J* = 8.6 Hz, 2H); ¹³C NMR (CDCl₃) δ 43.9, 61.4, 68.5, 68.4, 128.2, 128.5, 128.7, 128.8, 129.1, 131.2, 132.0, 138.4, 165.5; HRMS calcd for C₁₉H₁₈BrNO₂S₂ [M+Na]⁺ 457.9855, found 457.9916.

Dimethyl 2-((((3*S*,5*S*,7*aR*)-3-phenyltetrahydro-2H-thiazolo[4,3-*b*]oxazol-5-yl)methyl)thio)but-2-enedioate **7**

To a stirred solution of a mixture of **3a/4a** (95:5) (0.098 g, 0.39 mmol) in CH₂Cl₂ (9 mL) was added dimethylacetylenedicarboxylate (0.085g, 0.5 mmol). The mixture was stirred overnight at room temperature. The crude was poured into water (20 mL), extracted with CH₂Cl₂ (3 × 20 mL), dried (Na₂SO₄) and filtered. The solvent was removed under reduced pressure and the residue was purified by column chromatography (EtOAc/hexanes 1:9) to afford **7** as a mixture of isomers 6:1 (81 mg, 53% total yield). Major isomer: [α]_D = + 141.8 (*c* 0.1, EtOAc); ¹H NMR (CDCl₃) δ 2.92 (m, 2H), 3.16 (dd, *J* = 12.6, 1.8 Hz, 1H), 3.35 (dd, *J* = 12.6, 4.8 Hz, 1H), 3.60 (dd, *J* = 9.2, 8.5 Hz, 1H), 3.68 (s, 3H), 3.75 (s, 3H), 4.10 (dd, *J* = 9.2, 6.5 Hz, 1H), 4.26 (dd, *J* = 8.5, 6.5 Hz, 1H), 4.37 (dd, *J* = 8.3, 6.4 Hz, 1H), 5.41 (dd, *J* = 4.8, 1.8 Hz, 1H), 5.62 (s, 1H), 7.33 (m, 3H), 7.45 (m, 2H); ¹³C NMR (CDCl₃) δ 38.6, 39.9, 52.0, 53.1, 69.2, 72.7, 74.3, 100.1, 114.7, 127.9, 128.2, 128.7, 138.5, 149.0, 164.1, 165.9. HRMS calcd for C₁₈H₂₁NO₅S₂ [M+H]⁺ 396.0934, found 396.1043. Minor isomer: ¹H NMR (CDCl₃) δ 2.93 (dd, *J* = 14.3, 6.0 Hz, 1H), 3.09 (dd, *J* = 12.4, 2.1 Hz, 1H), 3.29 (dd, *J* = 12.4, 5.0 Hz, 1H), 3.30 (dd, *J* = 14.3, 8.6 Hz, 1H), 3.62 (dd, *J* = 9.5, 8.4 Hz, 1H), 3.74 (s, 3H), 3.76 (s, 3H), 4.05 (dd, *J* = 9.5, 6.5 Hz, 1H), 4.24 (dd, *J* = 8.4, 6.5 Hz, 1H), 4.31 (dd, *J* = 8.6, 6.0 Hz, 1H), 5.33 (dd, *J* = 5.0, 2.1 Hz, 1H), 6.39 (s, 1H), 7.3 (m, 3 H), 7.41 (dd, *J* = 7.9, 1.3 Hz, 2H); ¹³C NMR (CDCl₃) δ 38.6, 39.4, 51.8, 53.2, 68.7, 74.1, 74.2, 99.9, 120.6, 128.0, 128.2, 128.6, 138.0, 148.0, 164.5, 165.4.

Materials and methods

Interconversion studies

A mixture of (*S*)-**3a**/*S*)-**4a** (3:2) (30 mg, 0.12 mmol) was stirred in an appropriated buffer solution [1M] (8 mL) and acetonitrile (2 mL) at room temperature for 24 h. The solution was then neutralized by the addition of NaHCO₃ saturated solution and extracted with EtOAc (3 × 30 mL). The combined organic layers were dried (Na₂SO₄), filtered and the solvent was removed under reduced pressure. The mass balance was quantitative and the product distribution was determined by ¹H NMR. The experiment was performed using different buffer solutions (pH 2, 5, 7 and 9). The same experiments were repeated starting from pure (*R*)-**3a** diastereomer.

Inhibition assays on NDM-1

Hydrolysis of imipenem by NDM-1 was monitored using a Jasco V-670 spectrophotometer by following the changes in absorbance at 300 nm using a $De_{300} = -9000 \text{ M}^{-1} \text{ cm}^{-1}$. The reaction medium employed was 10 mM HEPES pH 7.5, 200 mM NaCl, 50 µg/mL BSA and 20 µM ZnSO₄ at 30 °C. Reactions were carried out in 0.1-cm path length quartz cuvettes in a final volume of 300 µL, with a final enzyme concentration of 1 nM.

Oxazolidinylthiazolidines were dissolved in DMSO to a final concentration of 100 mM, and then diluted 10-fold (to 10 mM) in 10 mM HEPES pH 7.5, 200 mM NaCl. Appropriate volumes of the 10 mM stock solutions were used to achieve the desired final concentrations. The final DMSO concentration in the reaction mixture was then maintained between 0.01 to 0.07%, which did not alter the enzyme activity (data not shown). The assay was initiated by the addition of NDM-1 to the mixture of substrate and inhibitor. In the presence of inhibitor, the initial phase of the time courses was linear but showed a decreased rate of hydrolysis with respect to the reaction in the absence of inhibitor. The initial rate of reaction for each substrate or substrate-inhibitor concentration, under steady state conditions (< 5% of substrate consumed), was calculated from the slope of the initial linear phase of the respective time course. Inhibition constants (K_i) were evaluated by nonlinear fitting of the initial velocities, at various concentrations of the substrates and inhibitors, with the equations for different inhibition models as implemented in GraphPad 5.0. Best fits were obtained with the Competitive Inhibition Model.

Crystallography

Single-crystal X-ray diffraction experiments on **DTA-1**, (*S*)-**5a** and (*R*)-**6a** were performed with a Bruker D8 Venture diffractometer operating with a sealed-tube Mo $K\alpha$ radiation ($\lambda = 0.71069 \text{ \AA}$) and a PHOTON100 CMOS area detector, at room temperature.

Crystallographic data for compounds **DTA-1**, (*S*)-**5a** and (*R*)-**6a** have been deposited with the accession number CCDC 1451797, 1451798 and 1451799 respectively and can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/structures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Notes and references

1. Marson CM. Chem. Soc. Rev. 2011; 40:5514–5533. [PubMed: 21837344]
2. Walters WP, Green J, Weiss JR, Murcko MA. J. Med. Chem. 2011; 54:6405–6416. [PubMed: 21755928]
3. Royer J, Bonin M, Micouin L. Chem. Rev. 2004; 104:2311–2352. [PubMed: 15137793]
4. Saiz C, Wipf P, Mahler G. J. Org. Chem. 2011; 76:5738. [PubMed: 21634420]
5. Saiz C, Manta E, Wipf P, Mahler G. Org. Lett. 2009; 11:3170. [PubMed: 19585999]
6. Saiz C, Castillo V, Mahler G. Synlett. 2012; 23:1090.
7. Saiz C, Castillo V, Fontán P, Bonilla M, Salinas G, Rodríguez A, Mahler G. Mol. div. 2014; 18:1.
8. González M, Kosmopoulou M, Mojica MF, Castillo V, Hinchliffe P, Pettinati I, Brem J, Schofield CJ, Mahler G, Bonomo RA, Llarrull LI, Spencer J, Vila AJ. ACS Infect. Dis. 2015; 1:544. [PubMed: 27623409]
9. Mojica MF, Mahler G, Bethel CR, Taracila MA, Kosmopoulou M, Papp-Wallace KM, Llarrull LI, Wilson BM, Marshall SH, Wallace CJ, Villegas MV, Harris ME, Vila AJ, Spencer J, Bonomo RA. Biochem. 2015; 54:3183–3196. [PubMed: 25915520]
10. Hinchliffe P, González MM, Mojica MF, González JM, Castillo V, Saiz C, Kosmopoulou M, Tooke CL, Llarrull LI, Mahler G, Bonomo RA, Vila AJ, Spencer J. Proc. Natl. Acad. Sci. USA. 2016; 113:E3745. [PubMed: 27303030]
11. Kanemasa S, Doi K, Wada E. Bull. Chem. Soc. Jpn. 1990; 63:2866.
12. Cardoso AL, Kaczor A, Silva A, Fausto R, Pinho e Melo T, Rocha Gonsalves A. Tetrahedron. 2006; 62:9861.
13. Purushothaman S, Raghunathan R. Tetrahedron Lett. 2009; 50:6848.
14. Stojanovic M, Markovic R, Kleinpeter E, Baranac-Stojanovic M. Tetrahedron. 2011; 67:9541.
15. Stratmann O, Hoppe D, Fröhlich R. J. Prakt. Chem. 2000; 8:342.
16. Stalling T, Pauly J, Schmidtman M, Martens J. Eur. J. Org. Chem. 2014; 4:833.
17. Fulöp F, Mattinenand J, Pihlaja K. Tetrahedron. 1990; 46:6545.
18. Beesley R, Ingold C, Thorpe JJ. Chem. Soc. Trans. 1915; 107:1080.

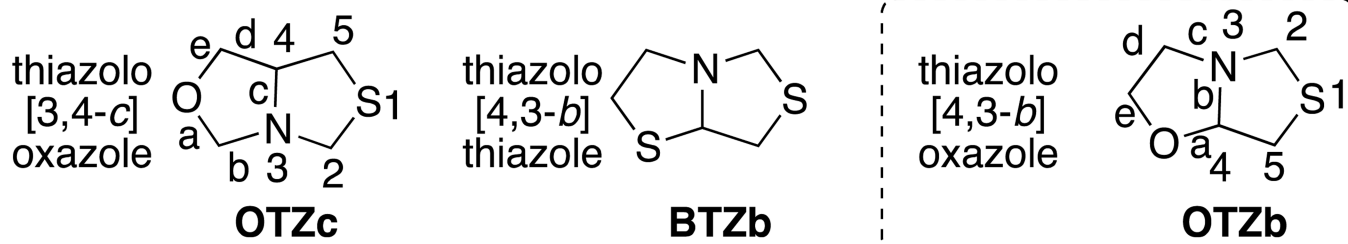


Figure 1.

Previously prepared heterocycles thiazolo[3,4-*c*]oxazoles⁴ and thiazolo[4,3-*b*]thiazoles.⁶

Compound of synthetic interest: thiazolo[4,3-*b*]oxazole.

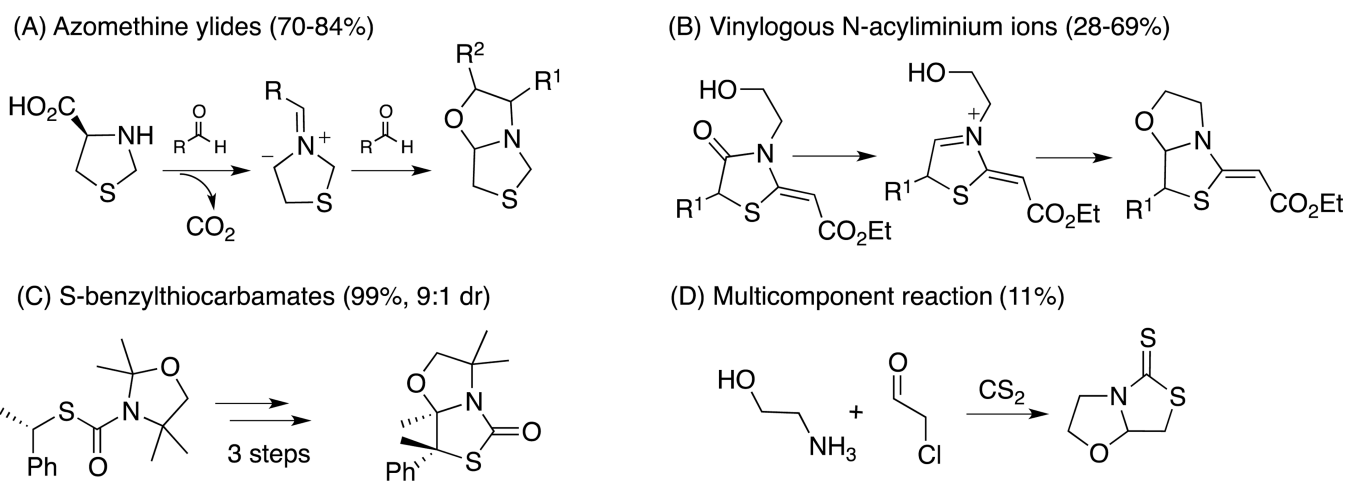


Figure 2.
Different synthetic methodologies for thiazolo[4,3-b]oxazole structures reported in literature.

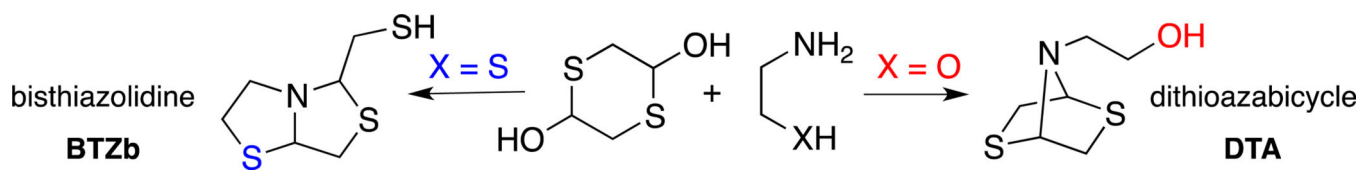


Figure 3.
Synthesis of bithiazolidine or dithioazabicyclo starting from linear aminothiols or aminoalcohols.

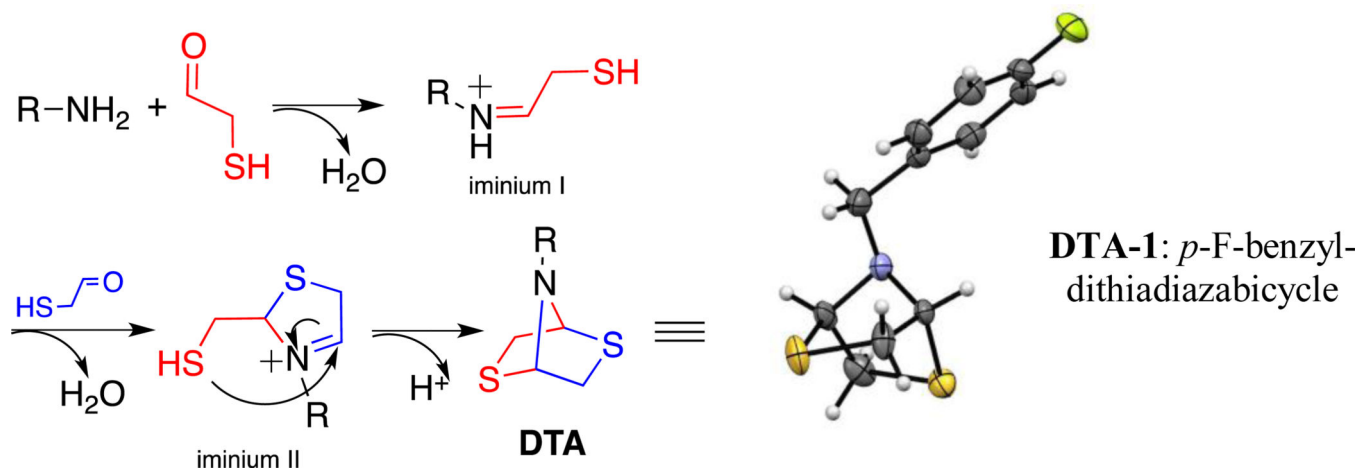


Figure 4. Suggested mechanism for the synthesis of dithioazabicyclo. ORTEP diagram of **DTA-1** (R = *p*-F-Bn).

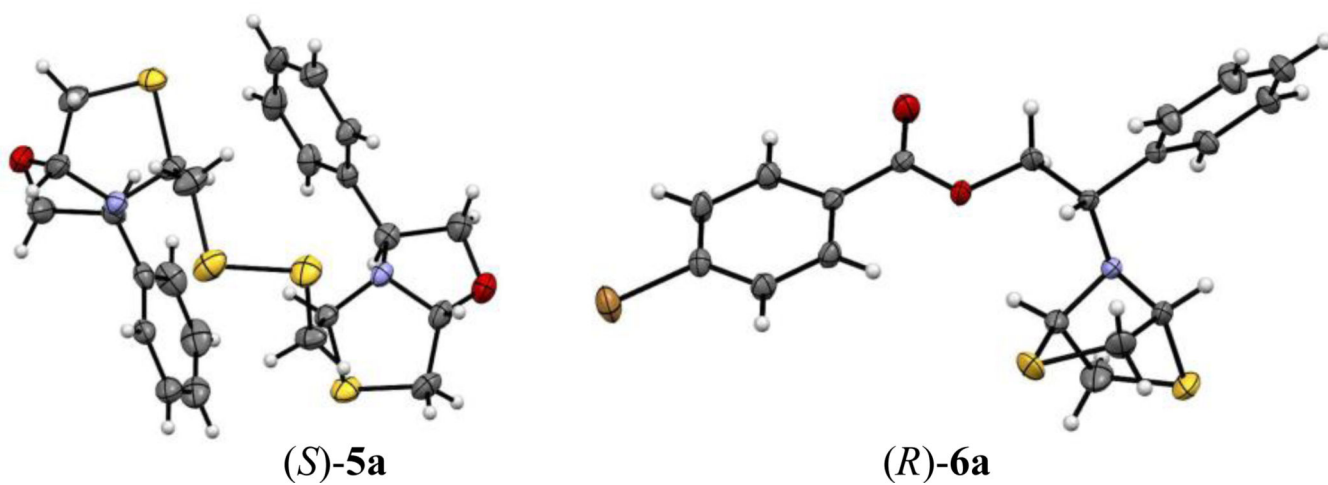


Figure 5.
ORTEP plots of compounds (*S*)-5a and (*R*)-6a.

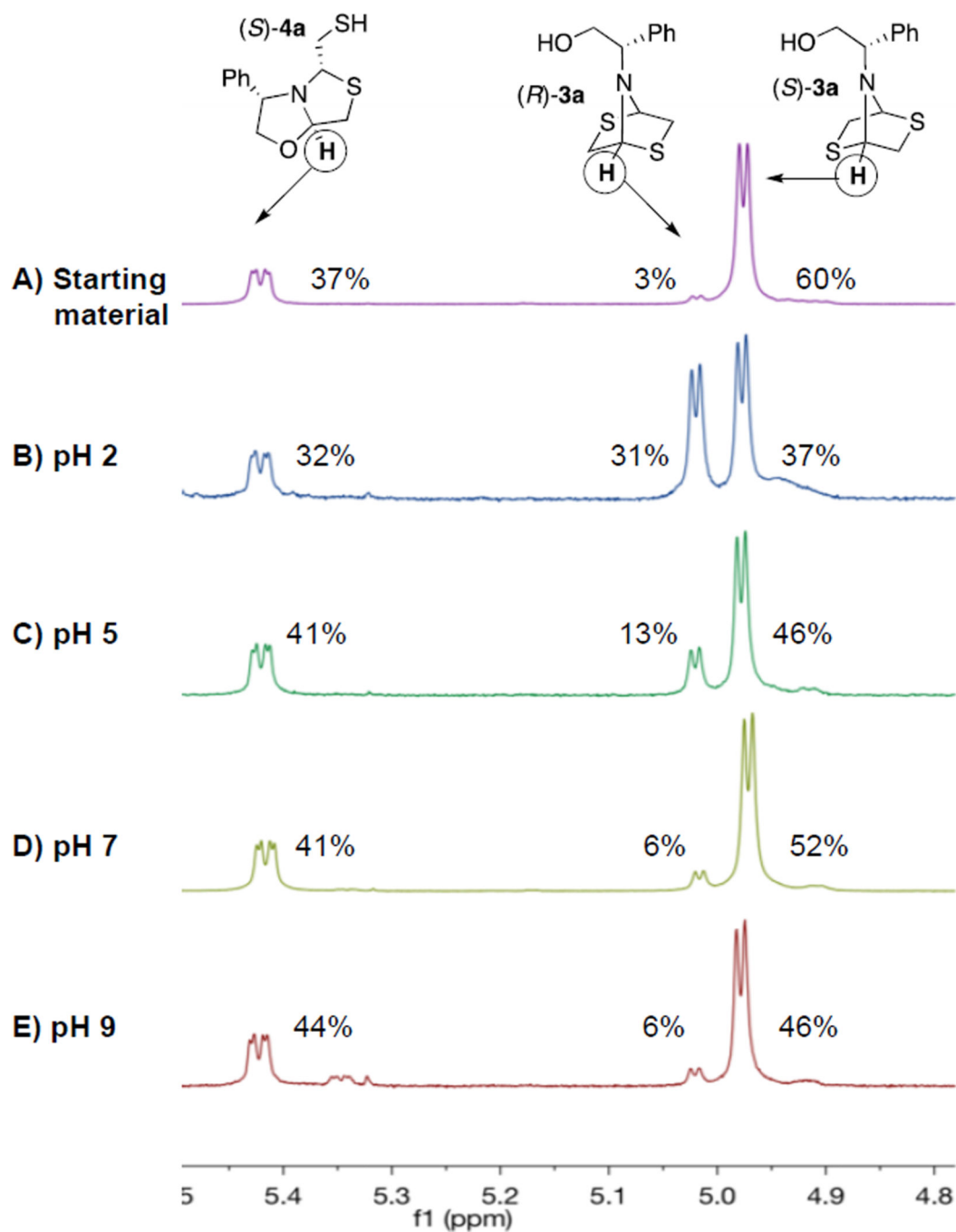


Figure 6. Interconversion studies. (A): Starting material: mixture of (S)-3a and (S)-4a (2:1). (B): reaction mixture after stirring starting material (A) for 24 h in a buffer solution at pH 2. (C): Same procedure than (B) but at pH 5. (D): pH 7. (E): pH 9.

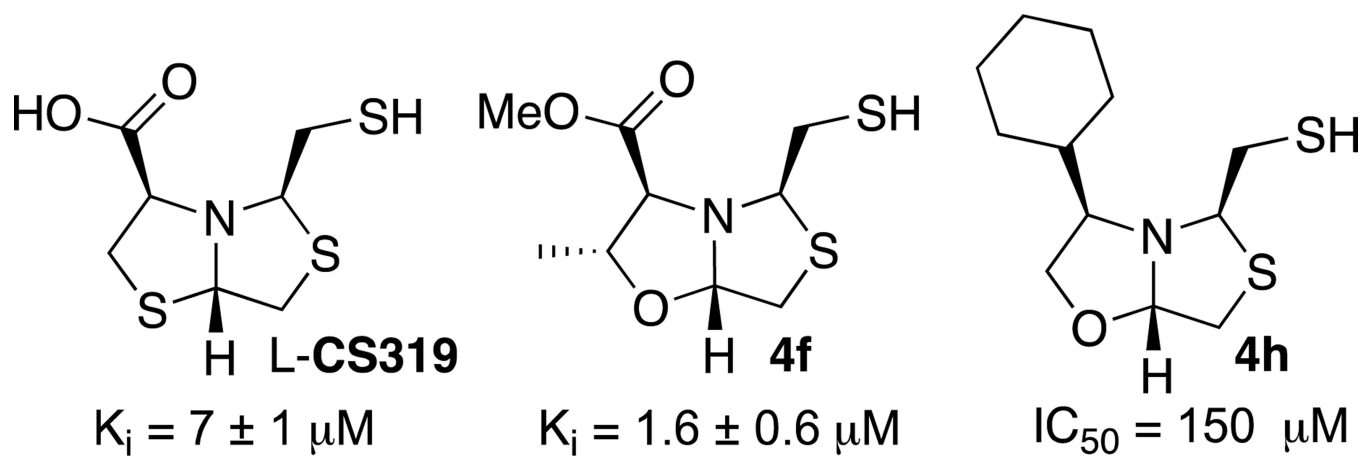
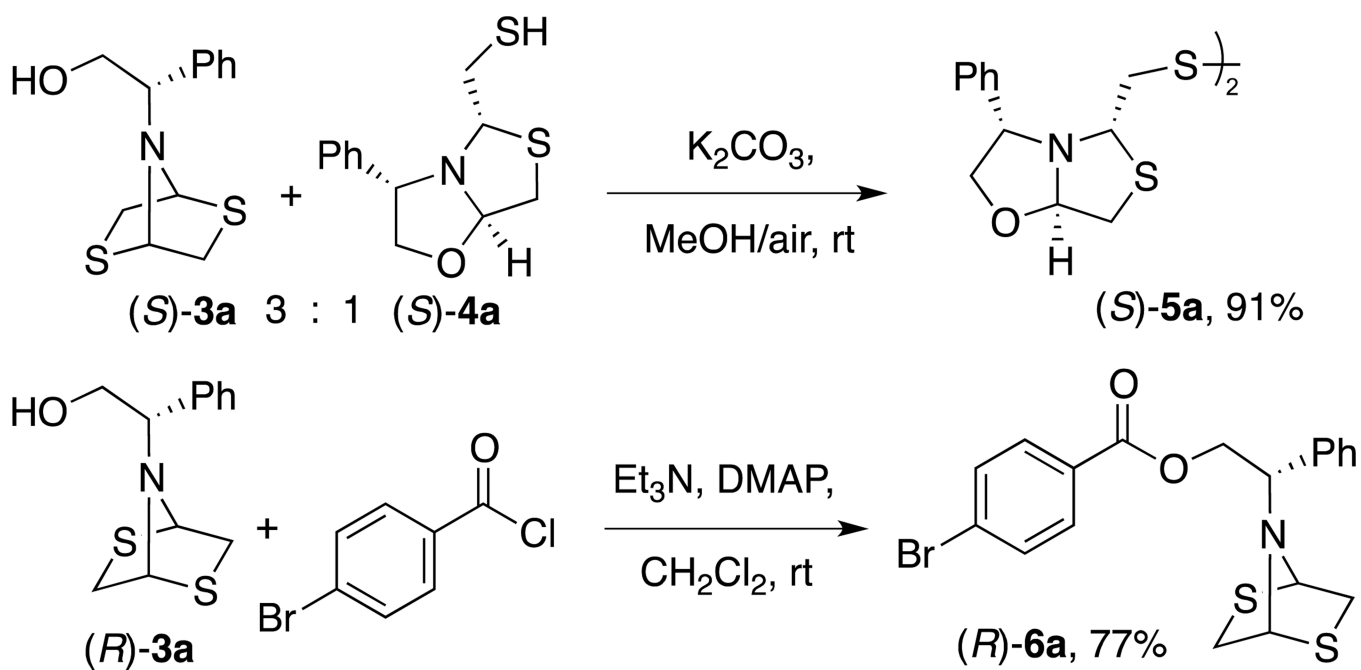
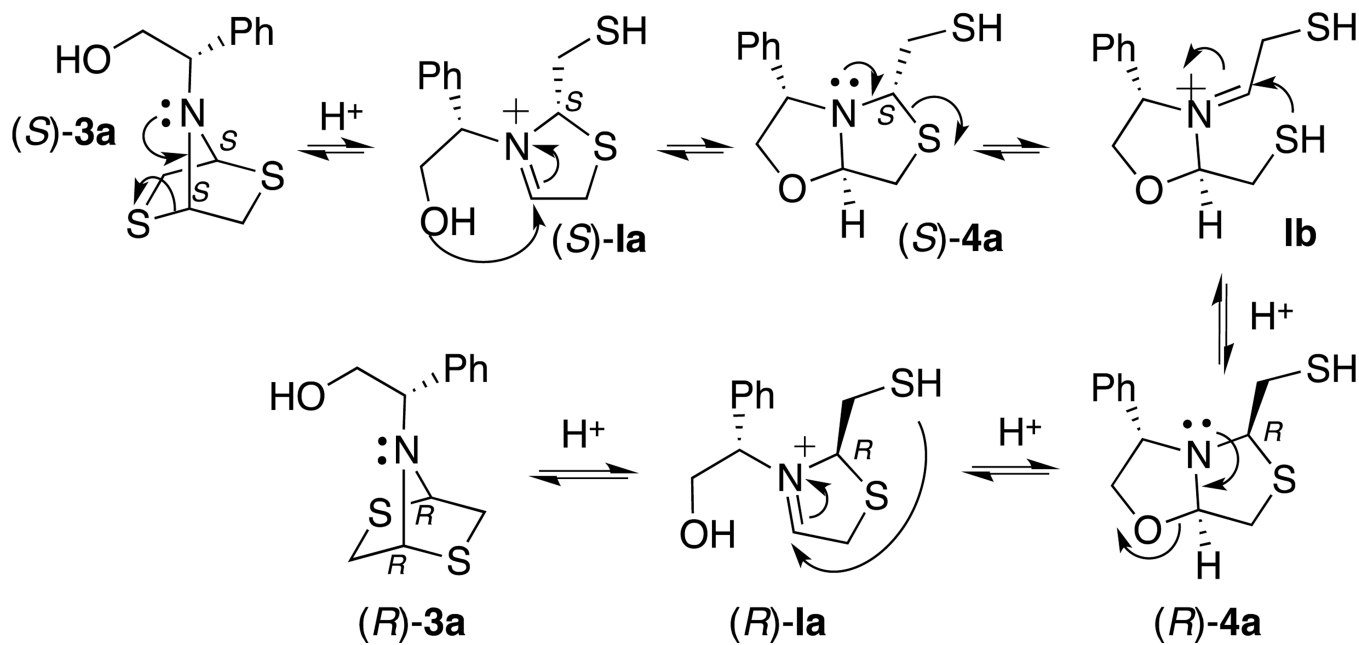


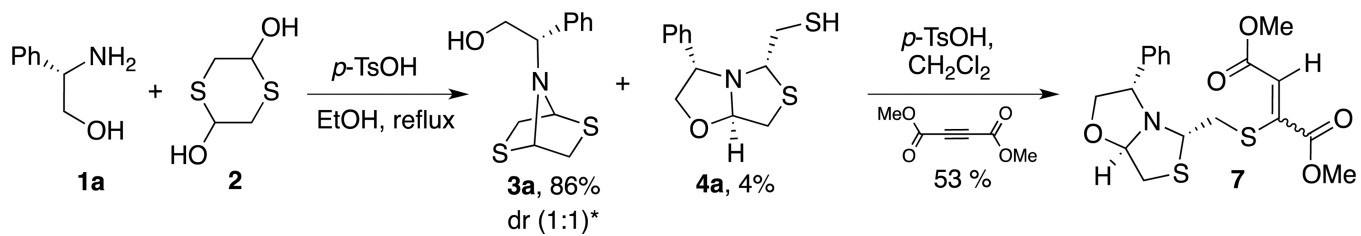
Figure 7.
Inhibition constant (K_i) of NDM-1 by L-CS319, **4f** and half inhibitory concentration (IC_{50}) of NDM-1 by **4h**.



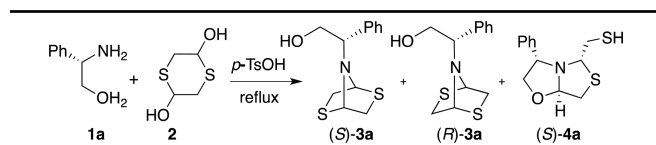
Scheme 1.
Derivatization of compounds *(S)*-4a and *(R)*-3a.

**Scheme 2.**

Suggested mechanism for the reversible interconversion of dithia-azabicycles (*S*)-3a into (*R*)-3a.

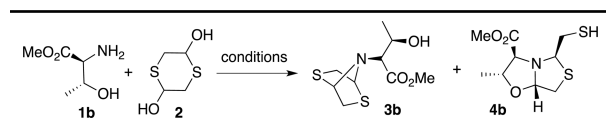
**Scheme 3.**

Diastereomeric resolution via 1,4-Michael addition of oxazolidinylthiazolidine **4a** to DMAD. *dr = diastereomeric ratio.

Table 1Optimization of reaction conditions starting from glycinol **1a**.

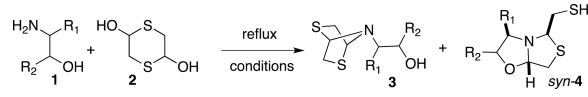
Entry	Solvent	Time	Ratio 3a:4a [*]	Total yield (%)
1	EtOH, [1a] = 0.4 M	2h	9.5:0.5	90
2	PhMe, [1a] = 0.1 M	3h	7:3	64
3	PhMe, [1a] = 0.1 M	7h	7:3	51
4	BA pH5, [1a] = 0.07 M	30'	7:3	83
5	BA pH5, [1a] = 0.07 M	4h	7:3	80



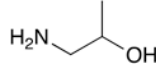
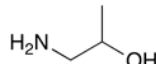
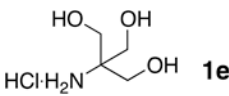
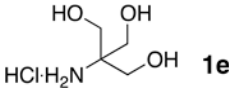
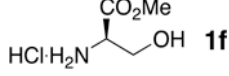
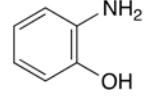
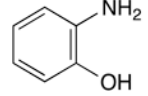
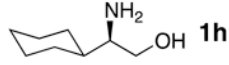
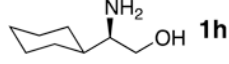
* diastereomeric ratio 1:1. BA = acetic acid/acetate sodium, 1M.

Table 2Product distribution for **3b/4b** formation at different pH.

Entry	Conditions*	Yield (%)	Ratio 3:4
1	Buffer pH 2, 1 h	Decomp	-
2	Buffer pH 3, 1 h	Decomp	-
3	Buffer pH 4, 1 h	40 %	2:8
4	Buffer pH 5, 1 h	79 %	1:9
5	Buffer pH 6, 1 h	53 %	3:7

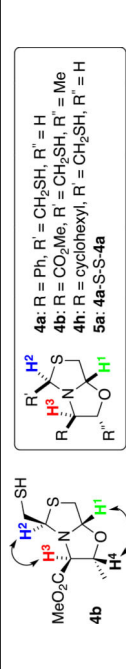
* [1b] = 0.07M, Buffer pH 2 (HCl/KCl 1M), Buffer pH 3 (citric acid/citrate sodium 1M), Buffer pH 4 (BA: acetic acid/acetate sodium 1M), Buffer pH 5 (BA, 1M), Buffer pH 6 (NaH₂PO₄/NaOH 1M).

Table 3Reaction of aminothiols **1c-1h** with dithiane **2**.


Entry	Starting material	Conditions	Ratio 3:4	Yield (%)
1	 1c	BA pH5, 1h	10:0	3c , 75%
2	 1c	MeCN, <i>p</i> -TsOH ac, 2h	10:0	3c , 65%
3	 1d	BA pH 5, 1 h	10:0	3d *, 80%
4	 1d	EtOH, <i>p</i> -TsOH ac, 2h	10:0	3d * 100%
5	 1e	BA pH5, 1 h	-	NR
6	 1e	PhMe, <i>p</i> -TsOH ac., 3h	10:0	3e , 97%
7	 1f	BA pH5, 30min	9:1	3f *+ 4f , 32%
8	 1g	BA pH5, 30min	10:0	3g , 74%
9	 1g	PhMe, 3h	8.5:1.5	3g + 4g , 42%
10	 1h	BA pH5, 1h	0:10	4h , 85%
11	 1h	PhMe, 1h	0:10	4h , 78%

* diastereomeric ratio 1:1. NR = no reaction. BA = acetic acid/acetate sodium

Table 4
NOE for **4b** and coupling constants of oxazolidinylthiazolidines **4a**, **4b**, **4h** and dimer **5a**.



	H ¹		H ²		H ³	
	δ (ppm)	J^1	δ (ppm)	J^1	δ (ppm)	J^1
4a	5.42	4.9	4.30	7.5	4.16	9.2
4b	5.24	4.7	4.27	8.3	3.32	8.5
4h	5.05	4.3	4.23	7.8	4.01	8.5
5a	5.35	5.0	4.36	8.4	4.11	9.2