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Novel enantiopure δ-thiolactones: synthesis, structural characterization, and reactivity studies†

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A new series of chiral δ-thiolactone derivatives have been prepared. These compounds exemplify the acetalic N–C–S reversibility of fused thiazolidines toward the thermodynamic product. The stereochemistry of the synthesized compounds was elucidated using X-ray crystallography, NOESY spectroscopy, and DFT calculations. The aminolysis reaction of the δ-thiolactone was studied with various alkyl amines, which can open the thioester to yield amido thiols in a single step. This reaction has the potential to be applied in the synthesis of bioactive compounds, polymer chemistry, and dynamic combinatorial chemistry, among others fields.

Introduction

Chemically, thioesters exhibit distinctive characteristics attributed to their thermodynamic stability. Positioned at the center of the nucleophilic addition reactivity scale, thioesters surpass unreactive amides and poorly reactive esters but fall below anhydrides and acyl chlorides. They also display higher reactivity compared to oxoesters. One contributing factor for this behaviour is the C–S bond's reduced tendency to participate in electron delocalization through the carbonyl group, when compared to the C–O bond. The reduced orbital overlap between the sulfur's 3p orbital and the carbon's 2p orbital, contributes to the weaker C–S bond, resulting in lower stability and greater susceptibility to nucleophilic attack.[1]

Due to their ability to react under low energetic barriers, thioesters play a central role in various biological processes as acylation intermediates in biochemical reactions, making them essential intermediates in biomolecular synthesis and potentially vital to the origin of life. They remain a part of the synthesis of essential cellular components such as peptides, fatty acids, sterols, and others.[2]

The thioester-amide exchange is one of the predominant processes in which this motif is involved, as in the acetyl-CoA mediated *S*-acylation of amino acid residues,[3] and peptide synthesis *via* native chemical ligation.[4]

Thiolactones, cyclic thioesters, named analogously to lactams, display relevant biological functions. For example, the γ-thiolactone of homocysteine (Hcy-thiolactone), is involved in the post-translational modification of proteins, and acts as an allosteric dopamine D2 receptor antagonist (Fig. 1).[5] Additionally, γ-thiolactones have also been suggested as precursors of life, owing to their substantial involvement in prebiotic chemistry.[5]

Thiolactones are also present in several natural products such as the antibiotic thiolactomycin, a γ-thiolactone with antimycobacterial activity. Thiolactomycin exerts its activity by selectively inhibiting fatty acid and mycolic acid biosynthesis.[6] This motif could also be found in isothiochroman-3-ones, described as metallo-amino-peptidase inhibitors,[7] and agrochemical fungicides and herbicides.[8,9] Lastly, thiosters are used in prodrug strategies, masking the thiol moiety and preventing disulfide formation. Examples of this strategy are the δ-thiolactone described as a glutamate carboxypeptidase II (GCPII) inhibitor,[10] and the ε-thiolactone described as Angiotensin-Converting Enzyme (ACE) inhibitor[11] (Fig. 1).

Indeed, the ability of thiolactones to undergo ring-opening *via* reaction with nucleophiles, such as amines, is the key to their use as reagents in synthetic chemistry and polymer science.[12,13] For example, the one-pot aminolysis of thiolactones, followed by thiol–ene conjugation, leads to double functionalized diverse polyamines and polyurethanes with high atom efficiency. With the development of post-polymerization modification and iterative protocols based on solid supports, multimers and oligomers of defined sequence could be

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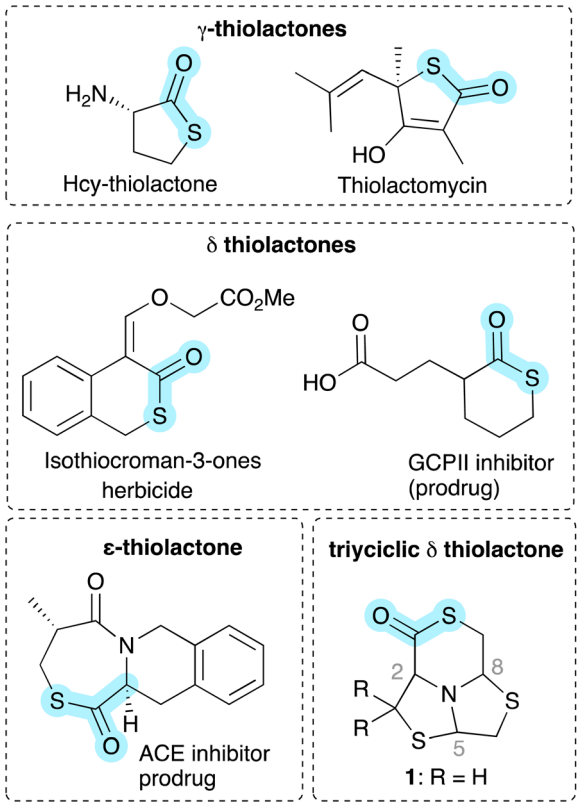


Fig. 1 Biologically relevant γ, δ and ε-thiolactones.

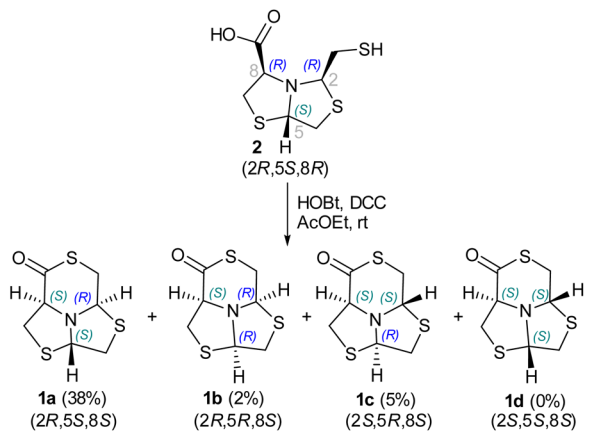


Fig. 2 Synthesis of δ-thiolactones **1a–c** starting from bisthiazolidine **2**, method A.

obtained. The versatility of thiolactones as functionalization tools, led to potential applications in biomedical[14,15] and materials fields.[16,17] Thiolactone chemistry has also been employed to overcome specific synthetic challenges, as enabling the coupling of sterically hindered peptide sites in native chemical ligation,[18] or synthesizing disulfide-linked side-chain-to-tail cyclic peptides in a straightforward, high-yield strategy.[19]

In this work, we report the preparation, structural characterization, and reactivity of different enantiopure tricyclic δ-thiolactones **1**, Fig. 1. Interestingly, thiolactones could serve as a prodrug of bisthiazolidine **2**, which was reported as a potent cross-class metallo-β-lactamase inhibitor, since the highly reactive thiol is protected.[20,21]

Results and discussion

Thioester can be prepared using different methodologies like base-initiated thioesterification of amides with various thiols.[22] During the preparation of analogs of the bisthiazolidine **2**, the formation of three tricyclic δ-thiolactones **1a–c** was noticed. This occurred when employing peptide coupling reagent DCC, which was able to activate the carboxylic acid, and lead to the formation of δ-thiolactones **1**, Fig. 2. We observed the formation of three δ-thiolactones, out of the four possible compounds, namely (2*R*,5*S*,8*S*)-**1a** (38%), (2*R*,5*R*,8*S*)-**1b** (2%) and (2*R*,5*R*,8*S*)-**1c** (5%). All these compounds retain the same chiral configuration at C8 as bisthiazolidine (2*R*,5*S*,8*R*)-**2**, but because of the thioester formation the relative priority between substituents at C8 makes the configuration change from *R* to *S*. Given the unusual and attractive biological and chemical properties of thiolactones previously mentioned, we decided to focus on their structural and chemical characterization.

Determination of the absolute configuration of δ-thiolactones 1a–c

First, we aimed to characterize these three isolated δ-thiolactones **1a–c** from the range of possible isomers. The absolute configuration of the isolated δ-thiolactones **1a–c** was assessed based on a combination of methodologies using crystal structures, two-dimensional Nuclear Overhauser Effect (NOESY) NMR experiments and theoretical calculations.

X-ray diffraction data of thiolactones 1a and 1b

X-ray diffraction data was used to determine the crystal structures of compounds **1a** and **1b**, Fig. 3. The results confirmed the absolute configurations of the isolated δ-thiolactones (2*R*,5*S*,8*S*)-**1a** and (2*R*,5*R*,8*S*)-**1b**, Fig. 2. Both compounds crystallized from enantiomerically pure solutions, and their crystals were resolved in the monoclinic and orthorhombic Sohncke space groups $P2_1$ and $P2_12_12_1$, for **1a** and **1b**, respectively. These spaces groups lack of mirror or inversion symmetry which is characteristic of enantiomerically pure compounds.[23,24] The crystal structure of **1a** presents two independent molecules due to conformational effects. The five membered rings S3–C2–N1–C5–C4 and S6–C5–N1–C8–C7 present envelope conformations on S3 ($Q = 0.518(4) - \varphi = 174.3(5)°$) and C7 ($Q = 0.465(4) - \varphi = 323.6(5)°$) for one of the independent molecules while these same rings present twisted (on C4–S3; $Q = 0.481(5) - \varphi = 169.2(6)°$) and envelope (on C7; $Q = 0.436(5) - \varphi = 318.7(6)°$) conformations for the other independent molecule. The six membered rings S10–C9–C8–N1–C2–C11 in both independent molecules have different conformations, with puckering amplitude (Q), theta (θ) and phi (φ) values of $Q = 0.597(4)$, $\theta = 37.4(4)°$, $\varphi = 194.8(8)°$, and $Q = 0.619(5)$, $\theta = 48.5(4)°$, $\varphi = 198.8(6)°$, respectively (Fig. 3a).[25,26] In the case of compound **1b**, only one independent molecule is detected. For this molecule,

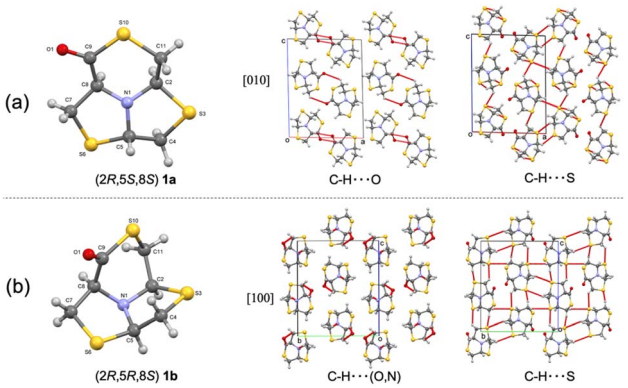
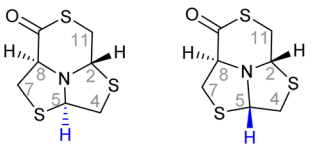


Fig. 3 Molecular and ORTEP diagram of δ-thiolactones: (a) **1a** and (b) **1b** showing the conformations of the respective enantiomers and the C–H···O, C–H···N and C–H···S hydrogen interactions. Atom color code: C (gray), H (white), O (red), N (purple), S (yellow).

Table 1 Chemical shift (δ) correlation of experimental and calculated ^{1}H-RMN for compounds **1c** (2*S*,5*R*,8*S*) and **1d** (2*S*,5*S*,8*S*)



H	Experimental δ (ppm)	Calculated δ (ppm) **1c** (2*S*,5*R*,8*S*)	$\lvert\Delta\delta\rvert$	**1d** (2*S*,5*S*,8*S*)	$\lvert\Delta\delta\rvert$
H2	4.41	4.50	0.09	5.08	0.67
H4a	3.31	3.83	0.12	3.02	0.68
H4b	3.73	3.28	0.05	3.01	0.32
H5	5.27	5.34	0.07	4.79	0.48
H7a	3.45	3.37	0.02	3.36	0.09
H7b	3.35	3.33	0.12	3.49	0.14
H8	4.09	4.05	0.04	4.11	0.02
H11a	3.22	3.58	0.36	3.59	0.37
H11b	3.58	3.20	0.38	3.30	0.28
MAE[a]	—	—	0.14	—	0.34
R^{2b}	—	—	0.91	—	0.68

[a] MAE = mean absolute error. [b] R^2 = correlation coefficient; $\Delta\delta = \delta_{experimental} - \delta_{calculated}$.

the five membered rings S3–C2–N1–C5–C4 and S6–C5–N1–C8–C7 present twisted (on C5–C4; $Q = 0.401(4) - \varphi = 304.5(6)°$) and envelope (on C8; $Q = 0.372(4) - \varphi = 102.8(6)°$) conformations (Fig. 3b), showing different conformations compared to the same five membered rings in compound **1a**. In fact, the strongest conformational difference is observed in the six membered ring S10–C9–C8–N1–C2–C11, which in compound **1b** has puckering parameters of $Q = 0.754(4)$, $\theta = 95.3(3)°$, $\varphi = 115.5(3)°$. The conformational differences in the S10–C9–C8–N1–C2–C11 ring are also detected in the C2–C11–S10–C9 torsion angle with values of 24.3° and 16.8° for the two independent molecules in compound **1a**, and 50.8° for compound **1b**. Due to the diverse conformations, the supramolecular structures are assembled differently. In the case of compound **1a**, the two independent molecules form separate molecular chains connected by C8–H8···O1 (H···O = 2.72 Å) and C11–H11···O1 (H···O = 2.85 Å) hydrogen interactions along [010] direction (Fig. 3a). In **1b**, these chains are assembled along [100] directions through C8–H8···O1 (H···O = 2.64 Å), C7–H7···O1 (H···O = 2.55 Å), and C4–H4···N1 (H···O = 2.74 Å) hydrogen interactions (Fig. 3b). In both compounds, C–H···S hydrogen interactions contribute to the formation of the crystals with H···S distances between 2.86–3.20 Å (Fig. 3b), which are values that seem long but in the case of C–H···S contacts, such values have already been reported in literature.[27]

DFT calculation and NOESY-NMR to determine δ-thiolactone 1c structure

Once the absolute configuration of δ-thiolactones **1a** and **1b** was determined based on the crystal structures, we aimed to determine if the absolute configuration of the remaining diastereomer, which could not be crystallized, was either (2*S*,5*R*,8*S*)-**1c** or (2*S*,5*S*,8*S*)-**1d**.

To assist in the assignment, DFT calculations of ^{1}H-RMN shifts were performed with GIAO (Gauge Including Atomic Orbitals) method and compared with the experimental data. The method was validated using the X-ray-confirmed assignment of the other two isomers **1a–b**. Based on the correlation coefficients (R^2) between the calculated and the experimental data, and the mean absolute error (MAE), the best-fitting configuration (lowest MAE, highest R^2) was stablished.

The comparison of the predicted ^{1}H-NMR spectra of the two δ-thiolactones **1c** and **1d** is shown in Table 1. According to the results, the configuration (2*S*,5*R*,8*S*)-**1c** fits better the experimental data, with the lowest MAE between the experimental and calculated values (0.14) and best R^2 (0.95).

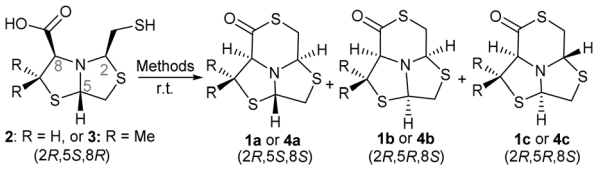
To confirm this assignment, the NOESY correlation between H2, H5 and H8 protons for each compound was studied. The assignment of the structures of **1a** and **1b** were confirmed based on the crystal structure and were used to set up the conditions for the NOESY experiment, Fig. S14–S16.†

The NOESY-NMR experiment of the non-assigned compound revealed the existence of a NOE interaction between H5 and H8 protons. No interactions were observed between H5–H2 and H2–H8, (Fig. S1c†). Considering all these results collectively, it becomes apparent that the data aligns more closely with (2*S*,5*R*,8*S*) as the absolute configuration of the third diastereomer. Overall considering the X-ray diffraction techniques, DFT theoretical calculations and NOESY-NMR, it was possible to assign the absolute configuration of the three δ-thiolactones obtained from L-**2**: (2*R*,5*S*,8*S*)-**1a**, (2*R*,5*R*,8*S*)-**1c** and (2*S*,5*R*,8*S*)-**1b**.

Optimization of the preparation of δ-thiolactones

After establishing the configuration of the thiolactones, we proceeded to optimize their preparation. To identify the best conditions for the synthesis of δ-thiolactone **1**, we conducted an

Table 2 Reaction conditions for the synthesis of thiolactones **1a–c** and **4a–b**[a]



			Isomer, yield[b] (%)		
	Thiolactone preparation (TP) method	[SM]: (mM)	2*S*,5*S*,8*S* **1a**	2*R*,5*R*,8*S* **1b**	2*S*,5*R*,8*S* **1c**
1	TP-A: DCC, HOBT	[**2**]: 0.20	**1a** (38)	**1b** (2)	**1c** (5)
2	TP-B: HATU, DIPEA	[**2**]: 0.08	—	—	**1c** (14)
3	TP-C: EDCI, HOBT	[**2**]: 0.14	**1a** (40)	**1b** (8)	**1c** (10)
4	TP-D: EDCI, DIPEA	[**2**]: 0.14	—	—	**1c** (32)
5	TP-E: COMU, DIPEA	[**2**]: 0.20	—	—	**1c** (37)
6	TP-F: EDCI, 4-DMAP	[**2**]: 0.28	—	—	**1c** (38)
7	TP-F	[**2**]: 0.20	—	—	**1c** (32)
8	TP-E: COMU, DIPEA	[**2**]: 0.20	—	**1b** (8)	**1c** (30)
9	TP-B	[**3**]: 0.08	**4a** (22)	**4c** (2)	—
10	TP-D	[**3**]: 0.14	**4a** (75)	—	—

[a] Methods conditions, A: DCC, HOBt, AcOEt, 24 h; B: HATU, DIPEA, 4-DMAP, DCM, 24 h; C: EDCI, HOBt, DCM, 24 h; D: EDCI, DIPEA, DCM, 24 h; E: COMU, DIPEA, DCM, 24 h; F: EDCI, 4-DMAP, DCM, 24 h. [b] Isolated yields.

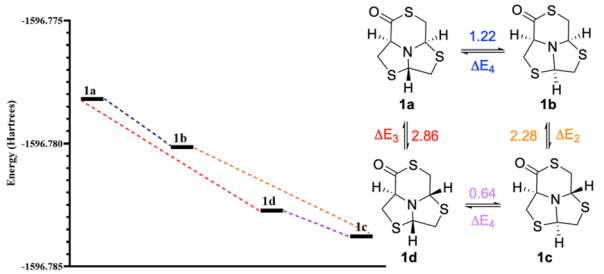


Fig. 4 Hartree free energy of compounds **1a–d** and their interconversion calculated using DFT calculations (B3LYP 311+G(d,p) level of theory).

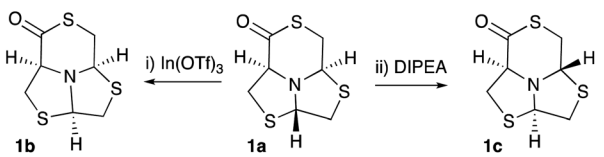


Fig. 5 (i) $In(OTf)_3$ (10% molar), AcOEt, MW, 40 °C, 10 min, 54%; (ii) DIPEA (1.1 eq.) AcOEt, MW, 65 °C, 35 min, 60%.

initial analytical-scale screening based on recommendations from a previous report on thioester preparation.[28] For the intramolecular cyclization, we tested five coupling agents with their respective additives: two second-generation carbodiimides, DIC and the water-soluble EDCI, as alternatives to DCC, which forms a hard-to-remove by-product precipitate; CDI, useful for ester and thioester formation; HATU, a more reactive iminium salt than carbodiimides;[29] and COMU, a third-generation derivative with enhanced solubility and safety.[30] We chose diethyl carbonate (DEC) and acetonitrile (ACN) for their sustainability and performance, using dichloromethane (DCM) as a reference solvent due to its effectiveness with the selected coupling agents.[31] Out of the 15 tested conditions, the results indicated that the highest yields were achieved using combinations of EDCI/4-DMAP and COMU/DIPEA, as shown in ESI Table S1.† The best results were obtained using DCM as solvent and EDCI (80%) or COMU (40%) as coupling reagents.

Based on these results the reaction was scaled up using DCM as solvent and EDCI or COMU as coupling reagents. The additions of base or not were also explored. The results were compared with the previously obtained using HATU and DCC, as shown in Table 2, entries 1–7.

Interestingly, different distribution of diastereomers **1** were observed depending on the conditions employed for its preparation. When a base like DIPEA or 4-DMAP was used as additive **1c** was the main isolated product, with yields from 14 to 38% (Table 2, entries 2, 4–8). In contrast, when HOBt was used as carbodiimide additive,[29] the diastereomer **1a** was isolated as the main isomer, together with minor proportions of **1b** and **1c** (Table 2, entries 1 and 3). All together those results underscore the crucial role of the basic medium in the isomerization process of thiolactones **1**.

The intramolecular cyclization was also studied for analogous bisthiazolidine **3**, derived from penicillamine,[21] giving place to the δ-thiolactones **4**. Using HATU and DIPEA compound **4a** (22%) was obtained as the main product together with a minor proportion of compound **4c** (2%) (Table 2 entry 9). The stereochemistry assignment was done based on 2D NOESY NMR, Fig. S17 and S18.† When using EDCI and DIPEA the only

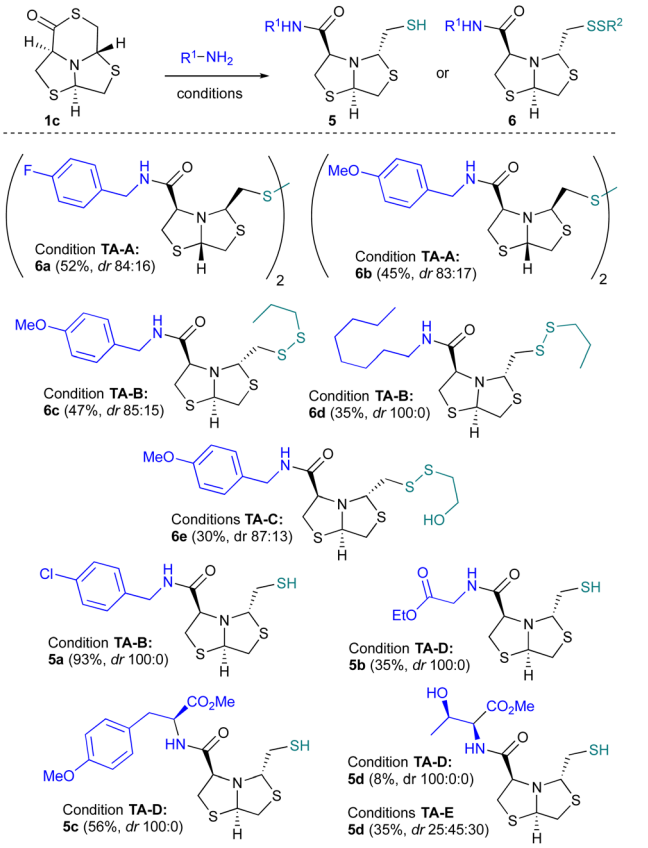


Fig. 6 Thiolactone amine ring opening conditions: (TA-A) R^1NH_2 (2eq.), MeCN AgOTf (1 eq.) DABCO (1.2 eq.); (TA-B) R^1NH_2 (1.1 eq.), THF, *n*-PropSH (30 eq.); (TA-C) R^1NH_2 (1.2 eq.), MeCN, β-ME (2 eq.); (TA-D) R^1NH_2 (1.2 eq.), 4-DMAP (5%) *n*-PropSH (30 eq.) MeCN; (TA-E) R^1NH_2 (1.2 eq.), DTT (2 eq.), MeCN.

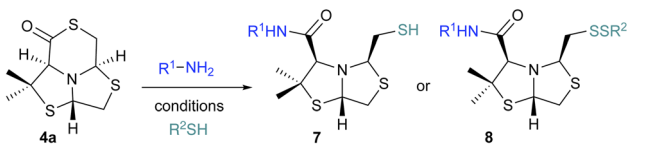


Fig. 7 Thiolactone amine ring opening conditions: (TA-B) R^1NH_2: *p*-Cl-Bn-NH_2 (1.1 eq.), THF, *n*-PropSH (30 eq.), **8a** (34%); (TA-D) R^1NH_2: L-H_2N-Gly-OEt (1.2 eq.), 4-DMAP (5%) *n*-PropSH (30 eq.) MeCN, no reaction.

product was the kinetic product **4a**, in good yield (75%, Table 2, entry 10). The different behavior observed for the thiolactone bearing a *gem*-dimethyl group at C7, could be due to a high isomerization barrier between **4a** and **4c**, resulting in a difference in energetic profile of the possible isomers.

Isomerization studies of δ-thiolactones 1

Bisthiazolidines bear two fused thiazolidine rings with acetal bonds at N–C2–S and N–C5–S. Thiazolidines are recognized for the reversible nature of their acetalic bonds, demonstrating a capacity to accommodate substituents under specific conditions and promoting the formation of the most stable product.[32]

Despite the potential of tricyclic δ-thiolactones **1** to generate four diastereomers (**1a–d**), only three isomers **1a–c** were detected in ^{1}H-NMR analysis of the crude reactions. In order to explain this results, we performed *ab initio* calculations for the free energy of the four possible δ-thiolactones **1a–d**, Fig. 4.

The highest energy enantiomer, **1a**, bearing the same configuration in carbons 2, 5 and 8 as the starting material **2**, is the kinetic product, the first being formed. The isomerization process towards the thermodynamically more stable product, **1c**, with lower energy, may encompass the diastereomer **1b**, as outlined in the proposed isomerization mechanisms (Fig. 4). Interestingly, the free energy difference (ΔE_2) between **1b** and **1c** is −2.28 kcal mol^{-1}, enabling the isolation of both isomers. On the other hand, the energy gap between **1d** and **1c** (ΔE_4) is −0.64 kcal mol^{-1}, however, despite this, the presence of **1d** was not detected. It is plausible to hypothesize that the transient state between **1c** and **1d** is energetically unfavorable and therefore not attainable at room temperature.

The thiolactones interconversion was experimentally investigated, as outlined in Fig. 5. The kinetic thiolactone **1a** could be converted to **1b** (54% yield) using Lewis acid $In(OTf)_3$ at 40 °C using microwave heating for 10 min. Alternatively, when DIPEA was used, **1a** was isomerized to the thermodynamic product **1c** (60% yield) by heating at 65 °C in microwave for 40 min (Fig. S19†).

We proposed a mechanism for the interconversion of **1a** to **1c** *via* thiolactone **1b**, with the consecutively ring opening and closing towards the most stable compound, **1c** (Fig. S20†). Anew, when a base is used, the interconversion between **1b** and **1c** is accomplished quickly and the last is the only product observed.

Aminolysis of δ-thiolactones

Bearing in mind the characteristic reactivity of this motif, we studied the ring opening reaction of **1c** by using simple nucleophiles like primary amines or amino acid esters to get amide analogs of bisthiazolidine **2** bearing a different stereochemistry from the previously prepared analogs.[33]

First, the ring opening was studied using benzyl amines in presence of DABCO as a base and AgOTf to prevent thiol dimerization, as described recently for the ring opening of γ-thiolactones.[34] Compound **1c** was opened smoothly with *p*-F-Bn-NH_2 or *p*-OMe-Bn-NH_2 but the isolated products **6a** and **6b** (Fig. 6), obtained in 52% and 45% respectively, were the result of isomerization in C2 and C5 and homodimer formation. Based on these results, to prevent thiol dimerization we incorporate *n*-propanethiol in large excess to act as a scavenger.[35] For *p*-OMe-benzyl and *n*-octyl amines, the obtained product resulted in a disulfide formation (–S–S–) between propanethiol and the mercapthomethyl-bisthiazolidine, compounds **6c–d** (47% and 35% yields respectively). Otherwise, when *p*-Cl-benzylamine was used the free thiol was obtained as the main product **5a** in 56% yield. We also focus on the use of aminoacidic derivatives like L-H_2N-Gly-OEt, L-H_2N-Phe-OMe and L-NH_2-Thr-OMe as nucleophiles to open the δ-thiolactone **1c**. The free thiol products **5b** (35%), **5c** (56%) and **5d** (8%) were obtained in moderate

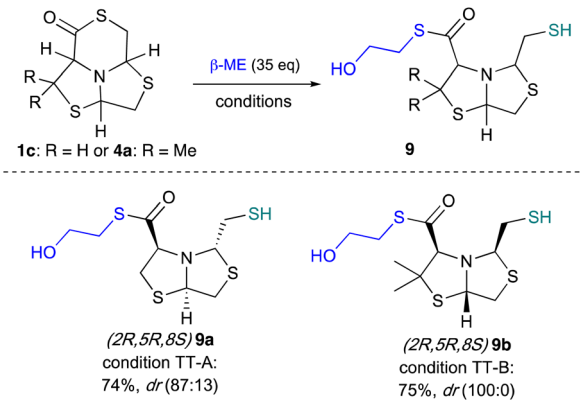


Fig. 8 Thiolysis of δ-thiolactones **1c** and **4a**. Condition TT-A: Gly (1.2 eq.), MeCN : DMF (3 : 1), gave: **9a** 74%, dr (87 : 13); condition TT-B: Gly (1.2 eq.), H_2O : AcOEt (1 : 1), gave: **9b** 75%, dr (100 : 0).

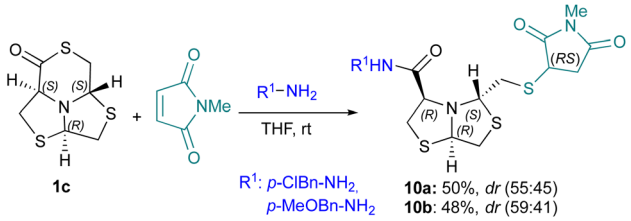


Fig. 9 Three component reaction of δ-thiolactones **1c**, benzylamines and, methylmaleimide.

to poor yields. Due to the intense odor of propanethiol, we explored its substitution by β-mercaptoethanol (βME), a less volatile thiol. The ring opening of **1c** using *p*-OMe-benzylamine and this scavenger, gave the heterodisulfide of βME, compound **6e** (52% yield). These results show neither βME nor propanethiol prevent thiol oxidation. The disulfide formation seems to be influenced in greater extent by the used amine. Additionally, when dithiothreitol, a potent thiol scavenger broadly used in biology,[36] was used with *p*-OMe-Bn-NH_2 and L-NH_2-Thr-OMe as nucleophiles, surprisingly **1c** undergo ring opening and free thiol products were obtained. The ^{1}H-NMR analysis of the reaction crude showed a varied mixture of diastereomers (Fig. S64–S66†). In case of L-NH_2-Thr-OMe three diastereomers were identified and product maintaining the configuration of the starting material **5d** was the minor product, Fig. 6 and S66.†

In most of the cases, under the assayed conditions, the main product retain the stereochemistry of the starting material. However, for compound **5b** in $CDCl_3$ we observed the interconversion towards the most stable compound (2*R*,5*R*,8*S*)-**5b**, as previously reported for similar systems,[37] achieving a 1 : 1 ratio after 24 h.

In summary the opening of δ-thiolactone **1c** was successfully achieved using eight amines under different conditions. When a base, AgOTf and benzylic amines were employed, the primary product was the dimeric amides **6a** and **6b**. When the base was omitted and an excess of thiol scavenger was used, the predominant isolated products were **6c–e**, resulting from the ring opening by the amine and subsequent disulfide formation with the scavenger. The isolation of the free thiol was observed in products **5a–d**, suggesting a possible influence of the substituent on the reduction potential of the formed products.

When δ-thiolactone **4a** was assayed for ring opening using conditions TA-B: *p*-Cl-benzylamine as nucleophile the disulfide **8a** was obtained in 34% yield, see Fig. 7. When glycine ethyl ester was used as nucleophile conditions TA-D, no opening product was observed, and the starting material was recovered, see Fig. 7 conditions TA-D.

Thiolysis of δ-thiolactones

Thiol-thioester exchange is a well know reaction used in the preparation of dynamic covalent libraries.[38] We further explore the ring opening of **1c** and **4a** using β-mercaptoethanol in large excess (35 equivalents) and Gly (1.2 equivalents) across different solvent mixtures. Under these conditions, it was observed the ring opening by the thiol of the β-mercaptoethanol, instead of the amino acid, and products **9a–b** were obtained in good yields. These results can be explained by the fact that amino acids are in a zwitterionic form and therefore are weaker nucleophiles than the previously used amines, Fig. 8.

Products obtained by the ring opening reaction are prime examples of another widely exploited chemoselective reaction, namely the *S*-to-*N* acyl transfer reaction,[39] which forms the basis of the native chemical ligation methodology, which revolutionized peptide and protein synthesis.

Multicomponent reaction of δ-thiolactones

Taking advantage of the high reactivity of the thiol, we explored the potential of three component reaction using δ-thiolactone **1c**, amines as nucleophiles and *N*-methylmaleimide as a Michael acceptor,[40] Fig. 9.

The addition product was obtained in both cases in moderate yields **10a** (50%, dr 55 : 45) or **10b** (48%, dr 59 : 41) as diastereomeric mixtures. These results evidence the versatility of the δ-thiolactone **1c** towards amine ring opening and thiol–ene conjugation.

Conclusions

We have described the preparation of five new δ-thiolactones. Its structural elucidation was achieved using a combination of X-ray crystallography, NMR spectroscopy and *ab initio* calculations.

The ring opening of the new tricyclic δ-thiolactones was investigated, using amines and thiols as nucleophiles. Compound **1c** exhibited a high reactivity against both types of nucleophiles, and the ring opening products strongly depended on the presence or not of a thiol scavenger.

The δ-thiolactones are useful scaffolds for the preparation of new structurally diverse bisthiazolidines bearing different substituents and stereochemistry in the bicycle core from previously reported ones. Together, these results demonstrate the versatility of thiolactone's chemistry and its usefulness in addressing synthetic challenges.

Experimental

Synthesis

Unless otherwise stated, all reagents and solvents were purchased at the highest quality from commercial suppliers and used as received without further purification. Experiments involving moisture and/or air-sensitive compounds were performed in oven or flame dried glassware with rubber septa under a positive pressure of nitrogen. Nonaqueous reagents were transferred by hypodermic syringe. Heating was accomplished by silicon oil bath using a temperature controller. Organic solutions were concentrated under reduced pressure at 30 °C (water bath temperature) using a Büchi rotary evaporator, unless otherwise noted. Reactions were magnetically stirred and monitored by thin-layer chromatography (TLC). Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous (>95%) materials, unless otherwise stated. TLC was performed on E. Merck 60-F254 precoated plates (0.25 mm), and spots were visualized by UV fluorescence (254 nm) quenching, iodine vapor, ninhydrin. Flash chromatography was carried out with Merck silica gel 60 (200–400 mesh) according to the procedure of Still *et al.*[41]

Analytical data of compounds

Melting points were measured using a Fisher-Johns apparatus and are uncorrected. Optical rotation was measured using a Jasco p-2000 polarimeter with a 2.0 mL cell (3.5 × 100 mm), optical path length of 100 mm, and sodium lamp (λ = 589 nm) at 20 °C. The concentration c is given as g/100 mL, α_D values are given in 10^{-1} deg cm^2 g^{-1}. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance Neo 400 (400 MHz and 101 MHz, respectively) spectrometer. Chemical shifts of the ^{1}H NMR ($CDCl_3$: 7.26 ppm, MeOD: 4.78 ppm, DMSO-d_6: 2.50 ppm) and ^{13}C NMR ($CDCl_3$: 77.00 ppm, MeOD: 49.15 ppm, DMSO-d_6: 39.50 ppm) spectra were referenced to residual solvent peaks or Me_4Si (0.00 ppm) as an internal standard. ^{1}H NMR spectra are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quarter, quin = quintet, sext = sextet, sept = septet, m = multiplet, br = broad), coupling constant (J) in Hz and integration. High-resolution mass spectra were acquired at Institut Pasteur Montevideo, in a Q Exactive Plus mass spectrometer (Thermo Scientific, USA) by direct injection (10 μL min^{-1}) using 100% methanol HPLC grade as solvent and an Ion Max API source with a HESI-II probe. The mass spectrometer was operated in a positive mode, ion spray voltage was set at 3.5 kV and capillary temperature at 250 °C.

Synthetic procedures

Compounds L-**2** and L-**3** were prepared as previously reported.[21]

Conditions for δ-thiolactone preparation (TP)

Condition TP-A. To a stirred solution of L-**2** (250 mg, 2.1 mmol) in EtOAc (15 mL) were added HOBt (337 mg, 2.5 mmol) and DCC (950 mg, 2.5 mmol). The mixture was stirred overnight at room temperature and the solvent evaporated under vacuum. The reaction crude was suspended in $CHCl_3$, filtered under vacuum and purified by column chromatography (*n*Hex : AcOEt 90 : 10), to give δ-thiolactones **1a** (92 mg, 0.42 mmol, 20% yield) and **1c** (82 mg, 0.38 mmol, 18%).

Condition TP-B. To a stirred solution of L-**2** (100 mg, 0.4 mmol) in DCM (5 mL) cooled to 0 °C were added HATU (194 mg, 0.5 mmol), DIPEA (0.16 m, 0.9 mmol) and 4-DMAP (1%). The mixture was stirred overnight at room temperature and the solvent evaporated under vacuum. The crude was extracted with 5% HCl (aq., 10 mL) and AcOEt (3 × 20 mL), the organic layer was dried over Na_2SO_4 and concentrated under vacuum. The crude was purified by column chromatography (*n*-Hex : AcOEt 90 : 10) to give **1c** (12 mg, 0.056 mmol, 14% yield).

Using condition TP-B starting from L-3 (105 mg, 0.4 mmol), and purified by column chromatography (*n*Hex : EtOAc 80 : 20) the thiolactones **4a** (22 mg, 0.08 mmol, 22% yield) and **4c** (2 mg, 0.008 mmol, 2% yield) were obtained.

Condition TP-C. To a stirred solution of L-2 (500 mg, 2.10 mmol) in DCM (15 mL) were added EDCI (358 mg, 2.3 mmol) and HOBt (310 mg, 2.3 mmol). The mixture was stirred 24 h at room temperature. The crude was extracted with 5% aq. HCl (20 mL) and DCM (3 × 20 mL), the organic layer was dried over Na_2SO_4 and concentrated under vacuum. The crude was purified by column chromatography (*n*Hex : AcOEt 90 : 10 to 70 : 30) to give **1a** (184 mg, 0.84 mmol, 40% yield), **1b** (37 mg, 0.17 mmol, 8% yield) and **1c** (46 mg, 0.21 mmol, 10% yield).

Using condition TP-C starting from L-3 (105 mg, 0.4 mmol) and purified by column chromatography (*n*Hex : EtOAc 80 : 20) **4a** (148 mg, 0.60 mmol, 75% yield) was obtained.

Condition TP-D. To a stirred solution of L-**2** (500 mg, 2.10 mmol) in DCM (15 mL) were added EDCI (358 mg, 2.3 mmol) and DIPEA (360 μL, 2.1 mmol) the mixture was stirred 24 h at room temperature. The crude was extracted with 5% aq. HCl (20 mL) and DCM (3 × 20 mL), the organic layer was dried over Na_2SO_4 and concentrated under vacuum. The crude was purified by column chromatography (*n*Hex : AcOEt 90 : 10) to give **1c** (147 mg, 0.67 mmol, 32%).

Condition TP-E. To a stirred solution of L-**2** (250 mg, 1.10 mmol) in DCM (5 mL) were added COMU (541 mg, 1.26 mmol) and DIPEA (135 mg, 1.0 mmol) and the mixture was stirred 24 h at room temperature. The crude was extracted with 5% aq. HCl (20 mL) and DCM (3 × 20 mL), the organic layer was dried over Na_2SO_4 and concentrated under vacuum. The crude was purified by column chromatography (*n*Hex : AcOEt 90 : 10) to give **1c** (89 mg, 0.41 mmol, 37% yield).

Condition TP-F. To a stirred solution of L-**2** (200 mg, 0.8 mmol) in DCM (5 mL) were added EDCI (140 mg, 0.9 mmol) and 4-DMAP (1%). The mixture was stirred 24 h at room temperature. The crude was extracted with 5% aq. HCl (20 mL) and DCM (3 × 20 mL), the organic layer was dried over Na_2SO_4 and concentrated under vacuum. The crude was purified by column chromatography (*n*Hex : AcOEt 90 : 10) to give **1c** (67 mg, 0.30 mmol, 38% yield).

(2*R*,5*S*,8*S*)-9-One-1-aza-3,6,10-trithiotricycle[3.3.5.0]eleventh (1a). Prepared using conditions TP-A or TP-C gave **1a** as a white solid, mp: 105–106 °C; $[\alpha]_D^{21} = -89.7°$ (c 0.345, $CHCl_3$); ^{1}H-RMN ($CDCl_3$; Me_4Si) δ 5.17 (dd, J = 6.7, 8.5, 1H), 4.56 (dd, J = 3.1,

11.1 Hz, 1H), 3.94 (dd, J = 5.3, 11.5 Hz, 1H), 3.69 (dd, J = 9.6, 11.5 Hz, 1H), 3.60 (t, J = 11.0 Hz, 1H), 3.57 (dd, J = 5.3, 11.5 Hz, 1H), 3.30 (dd, J = 3.1, 10.7 Hz, 1H), 3.20 (m, 2H); ^{13}C-RMN ($CDCl_3$) δ: 196.8, 73.7, 70.2, 65.3, 38.9, 38.8, 37.7; HRMS calculated for $C_7H_{10}NOS_3$ $[M + H]^+$: 219.9919, found: 219.9927.

(2*R*,5*R*,8*S*)-9-One-7-dimethyl-1-aza-3,6,10-trithiotricycle [3.3.5.0]eleventh (1b). Prepared using method TP-A, gave **1b** as a white solid, mp: 135–136 °C; $[\alpha]_D^{20} = -68.8°$ (c 0.335, $CHCl_3$); ^{1}H-RMN ($CDCl_3$) δ 5.19 (dd, J = 2.6, 5.2 Hz, 1H), 4.89 (dd, J = 5.0, 7.4 Hz, 1H), 4.06 (dd, J = 3.7, 6.5 Hz, 1H), 3.70 (dd, J = 3.7, 10.8 Hz, 1H), 3.64 (dd, J = 1.8, 14.4 Hz, 1H), 3.40 (dd, J = 6.5, 10.8 Hz, 1H), 3.35 (dd, J = 5.2, 14.4 Hz, 1H), 3.21 (dd, J = 5.0, 11.0 Hz, 1H), 2.83 (dd, J = 7.4, 11.2 Hz, 1H); ^{13}C-RMN ($CDCl_3$) δ 198.5, 73.9, 69.1, 63.1, 39.0, 35.1,34.6. $[M + H]^+$: 219.9919, found: 219.9927. Molecular structure of **1b** was obtained by X-ray crystallography.

(2*S*,5*R*,8*S*)-9-One-1-aza-3,6,10-trithiotricycle[3.3.5.0]eleventh (1c). Prepared using method TP-A, TP-B, TP-C, TP-D or TP-E gave white solid, mp: 124–125 °C; $[\alpha]_D^{20} = +86.8°$ (c 0.235, $CHCl_3$); ^{1}H-RMN ($CDCl_3$) δ 5.24 (d, J = 6.1 Hz, 1H), 4.39 (dd, J = 10.9, 2.3 Hz, 1H), 4.07 (dd, J = 10.2, 8.0 Hz, 1H), 3.69 (dd, J = 11.2, 6.2 Hz, 1H), 3.56 (t, J = 11.1 Hz, 1H), 3.42 (dd, J = 10.5, 8.1 Hz, 1H), 3.37–3.28 (m, 2H), 3.20 (dd, J = 11.4, 2.3 Hz, 1H); ^{13}C-RMN ($CDCl_3$) δ 197.4, 76.8, 73.9, 59.32, 35.5, 34.7, 34.5. $[M + H]^+$: 219.9919, found: 219.9927.

(2*R*,5*S*,8*S*)-9-One-7-dimethyl-1-aza-3,6,10-trithiotricycle [3.3.5.0]eleventh (4a). Prepared using conditions TP-B or TP-C gave **4a** as a white solid: mp = 76–77 °C; $[\alpha]_D^{20} = -222.5°$ (c 0.385, $CHCl_3$); ^{1}H-RMN ($CDCl_3$) δ 5.19 (dd, J = 5.7, 9.5 Hz, 1H), 4.52 (dd, J = 2.8, 11.5 Hz, 1H), 3.72 (s, 1H), 3.56 (dd, J = 10.5, 11.4 Hz, 1H), 3.24 (m, 3H), 1.65 (s, 3H), 1.63 (s, 3H); ^{13}C-RMN ($CDCl_3$) δ 197.0, 82.7, 67.7, 67.4, 62.6, 39.1, 37.3, 26.9, 25.3; HRMS calculated for $C_9H_{13}NNaOS$ $[M + H]^+$: 248.0232, found: 248.0222.

(2*S*,5*R*,8*S*)-9-One-7-dimethyl-1-aza-3,6,10-trithiotricycle [3.3.5.0] eleventh (4c). Prepared using condition TP-C gave **4c** as an oil; $[\alpha]_D^{21} = 105°$ (c 0.083, $CHCl_3$); ^{1}H-RMN ($CDCl_3$) δ: 5.27 (d, J = 6.1 Hz, 1H), 5.02 (dd, J = 2.2, 11.1 Hz, 1H), 3.84 (s, 1H), 3.63 (dd, J = 6.4, 11.2 Hz, 1H), 3.54 (t, J = 11.2 Hz, 1H), 3.25 (d, J = 11.3 Hz, 1H), 3.21 (dd, J = 2.4, 11.4 Hz, 1H), 1.67 (s, 3H), 1.60 (s, 3H); ^{13}C-RMN ($CDCl_3$) δ: 197.2, 81.4, 74.1, 62.7, 59.1, 34.9, 34.5, 33.6, 29.8; HRMS calculated for $C_9H_{13}NNaOS$ $[M + Na]^+$: 270,0051, found: 270, 0053.

Thiolactone aminolysis (TA)

Thiolactone aminolysis using base DABCO (TA-A): disulfide of (2*S*,5*S*,8*R*)-8-*N*-(4-fluorobenzyl)carbamoyl-2-(mercaptomethyl)-1-aza-dithiobicilo[3.3.0]octane (6a). To a stirred solution of **1c** (40 mg, 0.18 mmol) dissolved in degassed MeCN (0.5 mL), was added AgOTf (47 mg, 0.18 mmol), a solution of *p*-F-benzylamine (45 mg, 0.36 mmol), and dropwise a solution of DABCO (33 mg, 0.22 mmol) in degassed MeCN (0.5 mL). The reaction mixture was stirred for 24 h at rt. The solvent was evaporated under vacuum. The crude was extracted with 1 M HCl (10 mL) and AcOEt (3 × 15 mL), the organic layer was dried over Na_2SO_4 and concentrated under vacuum. The crude was purified by column chromatography (*n*-Hex : AcOEt 80 : 20–30 : 70) to give **6a** (64 mg, 0.09 mmol, 52%, dr: 84 : 16) as a yellow to brown solid; mp: 168–170 °C; ^{1}H-NMR ($CDCl_3$) δ: 7.26–7.18 (m, 2H), 7.08–6.90 (m, 2H), 4.87 (dt, J = 6.0, 3.8 Hz, 1H), 4.62–4.44 (m, 2H), 4.27 (dd, J = 14.9, 5.3 Hz, 1H), 4.07 (dd, J = 7.2, 3.1 Hz, 1H), 3.50 (ddd, J = 12.0, 7.4, 4.4 Hz, 2H), 3.34–3.26 (m, 1H), 3.10 (dd, J = 11.8, 4.4 Hz, 1H), 2.96–2.85 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 170.17, 160.97, 129.94, 129.35, 115.73, 71.42, 71.23, 70.93, 46.97, 43.42, 39.76, 33.61; HRMS calculated for $C_{28}H_{32}F_2N_4NaO_2S_6^+$ $[M + Na]^+$: 709.0710, found 709.0704.

Conditions TA-A: disulfide of (2*S*,5*S*,8*R*)-8-*N*-(4-methoxybenzyl)carbamoyl-2-(mercaptomethyl)-1-aza-dithiobicilo[3.3.0]octane (6b). Prepared using the same conditions as for **6a**, starting with **1c** (55 mg, 0.25 mmol) and *p*-MeO-benzylamine (69 mg, 0.25 mmol) and purified by column chromatography (*n*Hex : EtOAc 80 : 20) to give dimer **6b** (80 mg, 0.11 mmol, 45%, dr (83 : 17)) as a brown oil: ^{1}H-NMR ($CDCl_3$) δ: 7.19 (t, J = 8.6 Hz, 2H), 6.89–6.74 (m, 2H), 4.91–4.79 (m, 1H), 4.58–4.36 (m, 2H), 4.25 (dd, J = 14.6, 5.1 Hz, 1H), 4.06 (dd, J = 7.1, 3.3 Hz, 1H), 3.79 (s, 3H), 3.57–3.39 (m, 2H), 3.29 (dd, J = 11.1, 7.3 Hz, 1H), 3.09 (dd, J = 11.9, 4.2 Hz, 1H), 2.96–2.77 (m, 1H); ^{13}C-NMR ($CDCl_3$) δ 169.93, 159.07, 130.05, 129.04, 114.15, 73.06, 72.50, 70.94, 55.35, 47.10, 42.99, 39.73, 33.63; HRMS calculated for $C_{30}H_{38}N_4NaO_4S_6^+$ $[M + Na]^+$: 733.1110, found: 733.1089.

Thiolactone aminolysis using propanethiol as thiol scavenger (TA-B): (2*S*,5*R*,8*R*)-8-*N*(4-chlorobenzyl)carbamoyl-2-mercaptomethyl-1-aza-3,6-dithiobicycle[3.3.0]octane (5a). To a stirred solution of **1c** (40 mg, 0.18 mmol) in THF (2 mL) was added, propanethiol (0.3 mL, 3.3 mmol) and *p*-Cl-benzylamine (28 mg, 0.20 mmol). The reaction mixture was stirred for 24 hours at rt, and the solvent was evaporated under vacuum. The crude was extracted with HCl (5% aq., 10 mL) and AcOEt (3 × 15 mL), the organic layer was dried over Na_2SO_4 and concentrated under vacuum. The crude was purified by column chromatography (*n*-Hex : AcOEt 80 : 20) to give **5a** (60 mg, 0.17 mmol, 93%, dr (100 : 0)) as a white solid: mp: 148–150 °C; $[\alpha]_D^{20} = -46$ (c 0.59 $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ: 7.33 (d, J = 8.4 Hz, 1H), 7.27–7.22 (m, 4H), 6.41 (s, 1H), 5.19 (dd, J = 4.4, 1.8 Hz, 1H), 4.50 (dd, J = 6.7, 4.8 Hz, 1H), 4.44 (t, J = 5.7 Hz, 1H), 3.93 (dd, J = 9.5, 5.5 Hz, 1H), 3.57 (ddd, J = 11.8, 4.4, 1.1 Hz, 1H), 3.37 (dd, J = 10.9, 9.5 Hz, 1H), 3.20–3.10 (m, 1H), 3.01 (dd, J = 11.9, 1.9 Hz, 1H), 2.54 (ddd, J = 8.4, 5.8, 3.3 Hz, 1H), 1.62 (dd, J = 8.8, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$) δ: 165.50, 133.35, 131.44, 127.10, 126.69, 74.11, 68.65, 66.05, 40.84, 34.95, 32.17, 31.41. HRMS calculated for $C_{14}H_{17}ClN_2NaOS_3^+$ $[M + Na]^+$: 383.0084, found: 383.0078.

Conditions TA-B: (2*S*,5*R*,8*R*)-8-*N*-(4-methoxybenzyl) carbamoyl-2-(propyldisulfaneyl)methyl-1-aza-3,6-dithiobicycle [3.3.0]octane (6c). Prepared using the same methodology as for **5a**, starting with **1c** (40 mg, 0.18 mmol), propanethiol (0.3 mL, 3.3 mmol) and *p*-OMe-benzylamine (27 mg, 0.20 mmol). Purified by column chromatography (*n*Hex : EtOAc 80 : 20) to give **6c** (34 mg, 0.08 mmol, 47%, dr (85 : 15)) as a yellow solid: mp 120–122 °C; ^{1}H-NMR ($CDCl_3$) δ: 7.25 (d, J = 8.8 Hz, 2H), 6.88 (d, J = 8.7 Hz, 2H), 6.36 (s, 1H), 5.17 (dd, J = 4.3, 2.1 Hz, 1H), 4.64 (dd, J = 6.7, 4.8 Hz, 1H), 4.51 (dd, J = 14.3, 6.0 Hz, 1H), 4.35 (dd, J = 14.3, 5.0 Hz, 1H), 3.93 (dd, J = 9.4, 5.6 Hz, 1H), 3.81 (s, 3H), 3.57

(ddd, J = 11.9, 4.3, 1.0 Hz, 1H), 3.39 (dd, J = 10.9, 9.4 Hz, 1H), 3.16 (dd, J = 10.8, 5.6 Hz, 1H), 3.04 (dd, J = 11.8, 2.1 Hz, 1H), 2.92 (dd, J = 11.3, 5.8 Hz, 2H), 2.67 (t, J = 7.2 Hz, 2H), 1.77–1.63 (m, 2H), 0.99 (t, J = 7.3 Hz, 3H); ^{13}C-NMR ($CDCl_3$) δ: 166.79, 158.46, 128.71, 113.43, 75.55, 70.28, 64.74, 54.50, 49.16, 42.69, 40.45, 37.11, 33.76, 21.59, 12.30. HRMS calculated for $C_{18}H_{27}N_2O_2S_4^+$ $[M + H]^+$: 431.0950, found: 431.0942.

Conditions TA-B: (2*S*,5*R*,8*R*)-8-*N*-octylcarbamoyl-2-((propyldisulfaneyl)methyl)-1-aza-3,6-dithiobicycle[3.3.0]octane (6d). Prepared using the same methodology as for **5a**, starting with **1c** (40 mg, 0.18 mmol), propanethiol (0.3 mL, 3.3 mmol), and octylamine (25 mg, 0.20 mmol) and purified by column chromatography (*n*Hex : EtOAc 80 : 20) to give **6d** (23 mg, 0.05 mmol, 30%, dr (100 : 0)) as a brown solid: mp 120–122 °C; $[\alpha]_D^{20} = -7$ (c 0.29 $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ: 6.12 (s, 1H), 5.19 (dd, J = 4.3, 2.1 Hz, 1H), 4.63 (dd, J = 6.7, 4.9 Hz, 1H), 3.91 (dd, J = 9.4, 5.6 Hz, 1H), 3.65–3.56 (m, 1H), 3.42–3.33 (m, 2H), 3.26 (dt, J = 13.3, 6.6 Hz, 1H), 3.15 (dd, J = 10.9, 5.6 Hz, 1H), 3.06 (dd, J = 11.8, 2.1 Hz, 1H), 2.99–2.93 (m, 2H), 2.69 (t, J = 7.2 Hz, 2H), 1.71 (q, J = 7.3 Hz, 2H), 1.39–1.17 (m, 14H), 0.99 (t, J = 7.3 Hz, 3H), 0.93–0.85 (m, 3H); ^{13}C-NMR ($CDCl_3$) δ: 167.81, 76.42, 71.14, 65.55, 50.04, 41.29, 39.92, 37.83, 34.66, 31.79, 29.36, 29.20, 27.05, 22.65, 22.44, 14.10, 13.13. HRMS calculated for $C_{18}H_{34}N_2NaO_2S_4^+$ $[M + Na]^+$: 445.1446, found: 445.1427.

Thiolactone aminolysis using β-mercaptoethanol as thiol scavenger (TA-C): (2*S*,5*R*,8*R*)-8-(*N*-*p*-methoxybenzyl)carbamoyl-2-(2-hydroxyethyl)disulfaneylmethyl-1-aza-3,6-dithiobicycle [3.3.0]octane (6e). To a stirred solution of **1c** (40 mg, 0.18 mmol) dissolved in MeCN (2 mL), β-mercaptoethanol (25 μL, 0.36 mmol) and 4-OMe-benzylamine (30 mg, 0.21 mmol) were added. The reaction mixture was stirred overnight at room temperature, and the solvent was evaporated under vacuum. The crude was extracted with 5% aq. $NaHCO_3$ (10 mL) and AcOEt (3 × 15 mL), the organic layer was concentrated under vacuum, dissolved in DCM (20 mL) and extracted with $NaHCO_3$ 5% aq. (3 × 15 mL) to eliminate the remaining β-mercaptoethanol, the organic layer was dried over Na_2SO_4 and concentrated under vacuum. The crude was purified by silica gel flash column chromatography (*n*-Hex : AcOEt 60 : 40–30 : 70) to give **6e** (23 mg, 0.05 mmol, 30%, dr (87 : 13)) as a brown solid: mp 100–103 °C; ^{1}H-NMR ($CDCl_3$) δ: 7.25 (d, J = 8.7 Hz, 2H), 6.88 (d, J = 8.6 Hz, 2H), 6.48 (s, 1H), 5.19 (dd, J = 4.2, 1.7 Hz, 1H), 4.70 (dd, J = 7.7, 4.1 Hz, 1H), 4.48 (dd, J = 14.3, 5.8 Hz, 1H), 4.39 (dd, J = 14.3, 5.4 Hz, 1H), 3.97–3.89 (m, 1H), 3.85 (dt, J = 8.1, 5.7 Hz, 2H), 3.81 (s, 3H), 3.57 (ddd, J = 12.0, 4.3, 1.1 Hz, 1H), 3.40 (t, J = 10.3 Hz, 1H), 3.13 (dd, J = 10.8, 5.5 Hz, 1H), 3.07–2.98 (m, 2H), 2.90–2.85 (m, 2H), 2.81 (dd, J = 13.6, 7.6 Hz, 1H); ^{13}C-NMR ($CDCl_3$) δ: 165.37, 157.01, 127.25, 126.76, 111.96, 74.50, 68.66, 63.35, 57.70, 53.02, 52.98, 47.30, 41.30, 40.26, 34.84, 32.06. HRMS calculated for $C_{17}H_{24}N_2NaO_2S_4^+$ $[M + Na]^+$: 455.0562, found: 455.0553.

Thiolactone aminolysis using propanethiol as thiol scavenger (TA-D): (2*S*,5*R*,8*R*)-8-(*N*-ethylglycinate)carbamoyl-2-mercaptomethyl-1-aza-3,6-dithiobicycle[3.3.0]octane (5b). To a stirred solution of **1c** (40 mg, 0.18 mmol) dissolved in MeCN (1 mL) was added, propanethiol (0.5 mL, 5.51 mmol), 4-dimethylaminopyridine (5%) and dropwise a solution of Gly-OEt (34 mg, 0.22 mmol) in MeCN (0.5 mL). The reaction mixture was stirred for 24 h at rt, and the solvent evaporated under vacuum. The crude was extracted with HCl (5% aq. 10 mL) and AcOEt (3 × 15 mL), the organic layer was dried over Na_2SO_4 and concentrated under vacuum. The crude was purified by column chromatography (*n*-Hex : AcOEt 60 : 40) to give **5b** (20 mg, 0.06 mmol, 35%, dr (100 : 0)) as an oil: ^{1}H-NMR ($CDCl_3$) δ: 6.59 (s, 1H), 5.22 (dd, J = 4.3, 1.9 Hz, 1H), 4.52 (dd, J = 7.3, 4.4 Hz, 1H), 4.25 (q, J = 7.1 Hz, 2H), 4.08 (dd, J = 11.9, 5.3 Hz, 2H), 4.01–3.96 (m, 1H), 3.34 (dd, J = 10.8, 9.5 Hz, 1H), 3.17 (dd, J = 10.9, 5.6 Hz, 1H), 3.06 (dd, J = 11.9, 2.0 Hz, 1H), 2.77 (td, J = 9.0, 4.5 Hz, 1H), 2.68–2.61 (m, 1H), 1.71 (dd, J = 9.1, 8.0 Hz, 1H), 1.30 (t, J = 7.2 Hz, 3H); ^{13}C-NMR ($CDCl_3$) δ: 172.04, 169.68, 75.36, 74.25, 62.54, 61.89, 61.65, 41.14, 39.44, 35.29, 26.29, 14.17. HRMS calculated for $C_{11}H_{18}N_2NaO_3S_3^+$ $[M + Na]^+$: 345.0372, found: 345.0363. After 12 h in $CDCl_3$ the diasteromeric ratio changed to 50 : 50.

Condition TA-D: (2*S*,5*R*,8*R*)-8-(*N*-methylphenylalaninate) carbamoyl-2-mercaptomethyl-1-aza-3,6-dithiobicycle[3.3.0] octane (5c). Prepared using the same methodology as for **5b**, starting with **1c** (40 mg, 0.18 mmol) and L-Phe-OMe (39 mg, 0.22 mmol) and purified by column chromatography (*n*Hex : EtOAc 70 : 30) to give **5c** (40 mg, 0.10 mmol), 56%, dr (100 : 0) as a white solid: mp 150–153 °C; $[\alpha]_D^{20} = -10$ (c 0.51 $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ: 7.30 (ddd, J = 11.6, 7.7, 5.9 Hz, 2H), 7.13–7.08 (m, 2H), 6.47–6.40 (m, 1H), 5.16 (dd, J = 4.2, 1.8 Hz, 1H), 4.89 (dt, J = 7.6, 6.0 Hz, 1H), 4.36 (dd, J = 7.6, 4.2 Hz, 1H), 3.85 (dd, J = 9.7, 5.4 Hz, 1H), 3.78 (s, 3H), 3.55 (ddd, J = 12.0, 4.2, 1.1 Hz, 1H), 3.29–3.19 (m, 2H), 3.16–3.06 (m, 2H), 2.99 (dd, J = 11.9, 1.9 Hz, 1H), 2.66 (ddd, J = 13.5, 9.2, 4.2 Hz, 1H), 2.55 (dt, J = 13.7, 7.8 Hz, 1H), 1.65 (dd, J = 9.2, 8.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$) δ: 170.22, 166.41, 134.15, 127.90, 127.76, 127.59, 126.20, 75.18, 69.91, 67.79, 51.97, 51.42, 36.42, 36.30, 33.36, 32.64. HRMS calculated for $C_{17}H_{21}N_2NaO_3S_3^+$ $[M + Na]^+$: 421.0685, found: 421.0671.

Condition TA-D: (2*S*,5*R*,8*R*,12′*R*)-8-(*N*-methylthreoninate) carbamoyl-2-mercaptomethyl-1-aza-3,6-dithiobicycle[3.3.0] octane (5d). Prepared using conditions TA-D, same as for **5b**, starting with **1c** (40 mg, 0.18 mmol) and L-Thr-OMe (30 mg, 0.22 mmol) and purified by column chromatography (*n*Hex : EtOAc 40 : 60) to give **5d** (5 mg, 0.014 mmol), 8%, dr (100 : 0) as a brown oil: ^{1}H-NMR ($CDCl_3$) δ: 6.79 (d, J = 8.4 Hz, 1H), 5.24 (d, J = 4.1 Hz, 1H), 4.59 (dd, J = 8.6, 2.3 Hz, 1H), 4.46 (dd, J = 8.0, 4.0 Hz, 2H), 4.04 (dd, J = 9.7, 5.3 Hz, 1H), 3.80 (s, 3H), 3.62 (dd, J = 12.1, 4.2 Hz, 1H), 3.34 (t, J = 10.3 Hz, 1H), 3.16 (dd, J = 11.0, 5.4 Hz, 1H), 2.85 (ddd, J = 13.3, 9.3, 4.1 Hz, 1H), 2.67 (dd, J = 13.8, 7.8 Hz, 1H), 1.73 (dd, J = 9.2, 8.0 Hz, 1H), 1.31–1.23 (m, 3H); ^{13}C-NMR ($CDCl_3$) δ: 170.92, 168.55, 77.23, 71.51, 69.48, 67.67, 57.35, 52.92, 37.78, 34.54, 34.03, 20.23. HRMS calculated for $C_{12}H_{20}N_2NaO_4S_3^+$ $[M + Na]^+$: 375.0477, found: 375.0470.

Conditions TA-B: (2*S*,5*R*,8*R*)-8-*N*(4-chlorobenzyl)carbamoyl-2-mercaptomethyl-7-dimethyl-1-aza-3,6-dithiobicycle[3.3.0] octane (8a). Prepared using the same methodology as for **5a**, starting with **4a** (50 mg, 0.20 mmol), propanethiol (0.32 mL, 3.3 mmol) and *p*-Cl-benzylamine (50 mg, 0.22 mmol). Purified by column chromatography (nHex : EtOAc 80 : 20) to give **8a** (26 mg, 0.068 mmol, 34%, dr (100 : 0)) as a yellow oil; $[\alpha]_D^{20} = -140$ (c 0.32 $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ: 7.28 (t, J = 2.6 Hz, 4H),

4.86 (dd, J = 6.8, 5.6 Hz, 1H), 4.71 (dd, J = 14.5, 7.7 Hz, 1H), 4.57 (dd, J = 10.0, 4.5 Hz, 1H), 4.19 (dd, J = 14.4, 4.5 Hz, 1H), 3.64 (s, 1H), 3.41 (dd, J = 11.5, 6.9 Hz, 1H), 3.06 (dd, J = 11.6, 5.6 Hz, 1H), 2.99–2.84 (m, 2H), 2.67 (td, J = 6.9, 3.2 Hz, 2H), 1.68 (qd, J = 7.1, 2.1 Hz, 2H), 1.31 (s, 3H), 1.26 (s, 3H), 0.99 (t, J = 7.4 Hz, 3H); ^{13}C-NMR ($CDCl_3$) δ: 168.43, 136.71, 133.30, 129.82, 128.82, 128.77, 78.48, 71.91, 68.50, 55.30, 46.57, 42.63, 41.23, 40.71, 29.71, 29.67, 28.32, 22.43, 13.07. HRMS calculated for $C_{19}H_{28}ClN_2OS_4^+$ $[M + H]^+$: 463.0768 found: 436.0751.

Thiolactone thiolysis by β-mercaptoethanol (TT)

(2*S*,5*R*,8*R*)-8-(*S*-Hydroxyethyl)thioate-2-mercaptomethyl-1-aza-3,6-dithiobicycle[3.3.0]octane (9a). To a stirred solution of **1c** (40 mg, 0.18 mmol) dissolved in a mixture of MeCN (1.5 mL) and DMF (0.5 mL), β-mercaptoethanol (0.5 mL, 7.04 mmol) and Gly (15 mg, 0.20 mmol) were added. The reaction mixture was stirred 24 h at rt, and the solvent was evaporated under vacuum. The crude was extracted with HCl (5% aq., 10 mL) and AcOEt (3 × 15 mL), dried over Na_2SO_4 and concentrated under vacuum. The crude was purified by column chromatography using silica gel (*n*-Hex : AcOEt 60 : 40) to give **9a** (40 mg, 0.13 mmol), 74%, dr (87 : 13) as a pale oil; ^{1}H-NMR ($CDCl_3$) δ: 5.15 (dd, J = 5.2, 3.0 Hz, 1H), 4.35 (dd, J = 6.6, 2.5 Hz, 1H), 4.31 (t, J = 6.5 Hz, 1H), 3.78 (t, J = 6.1 Hz, 2H), 3.65–3.59 (m, 1H), 3.42 (dd, J = 11.0, 2.6 Hz, 1H), 3.21 (dd, J = 11.0, 6.6 Hz, 1H), 3.14–3.05 (m, 3H), 2.98 (ddd, J = 13.8, 8.3, 6.8 Hz, 1H), 2.79–2.71 (m, 1H), 2.06 (dd, J = 9.1, 8.3 Hz, 1H); ^{13}C-NMR ($CDCl_3$) δ: 200.75, 78.57, 76.77, 76.46, 76.14, 75.50, 73.73, 60.95, 38.73, 33.38, 33.23, 31.50. HRMS calculated for $C_9H_{15}NNaO_2S_4^+$ $[M + Na]^+$: 319.9878, found: 319.9876.

(2*S*,5*R*,8*R*)-8-(*S*-Hydroxyethyl)thioate-7-dimethyl-2-mercaptomethyl-1-aza-3,6-dithiobicycle[3.3.0]octane (9b). Prepared using the same methodology as for **9a**, starting with **4a** (50 mg, 0.20 mmol) dissolved in AcOEt (2 mL), β-mercaptoethanol (0.5 mL, 7.04 mmol), 4-dimethylaminopyridine (5%) and Gly (18 mg, 0.24 mmol). The crude was purified using (*n*-Hex : AcOEt 90 : 10–70 : 30) to give **9b** (50 mg, 0.15 mmol, 75%) as a pale oil; ^{1}H-NMR ($CDCl_3$) δ: 5.14 (dd, J = 6.4, 3.5 Hz, 1H), 4.30 (dd, J = 9.5, 4.4 Hz, 1H), 3.81–3.76 (m, 3H), 3.44 (dd, J = 12.1, 6.4 Hz, 1H), 3.16 (dt, J = 13.9, 6.1 Hz, 1H), 3.10–3.02 (m, 2H), 3.02–2.98 (m, 1H), 2.70 (ddd, J = 13.6, 9.5, 6.8 Hz, 1H), 1.86 (dd, J = 10.2, 6.8 Hz, 1H), 1.57 (s, 3H), 1.46 (s, 3H); ^{13}C-NMR ($CDCl_3$) δ: 199.37, 84.80, 78.43, 69.86, 61.04, 54.61, 38.42, 31.05, 30.95, 30.61, 27.40, 26.67; HRMS calculated for $C_{11}H_{20}N_2O_2S_4^+$ $[M + H]^+$: 326,0371, found: 326.0361.

Thiolactone multicomponent reaction (TM)

(2*S*,5*R*,8*R*,3′*SR*)-8-(*N*-4-Chlorobenzyl)carbamoyl-2-(((1′-methyl-2′,5′-dioxopyrrolidin-3′-yl)thio)methyl)-1-aza-3,6-dithiobicycle[3.3.0]octane (10a). To a stirred solution of **1c** (40 mg, 0.18 mmol) dissolved in THF (2 mL), *N*-methyl maleimide (40 mg, 0.36 mmol) and *p*-Cl-benzylamine (30 mg, 0.22 mmol) were added. The reaction mixture was stirred for 2 hours at room temperature, and the solvent was evaporated under vacuum. The crude was extracted with HCl (5% aq. 10 mL) and AcOEt (3 × 15 mL), the organic layer was dried over Na_2SO_4 and concentrated under vacuum. The crude was purified by silica gel flash column chromatography (*n*-Hex : AcOEt 70 : 30–30 : 70) to give **10a** as a mayor *syn* diastereomer (43 mg, 0.09 mmol, 50%, dr: 55 : 45) as a white solid: mp = 149–152 °C; ^{1}H-NMR ($CDCl_3$) δ: 7.32 (d, J = 8.57 Hz, 2H), 7.28 (d, J = 8.66 Hz, 2H), 6.61 (t, J = 5.75 Hz, 1H), 5.18 (dd, J = 4.35 Hz, 1.71 Hz, 1H), 4.68 (dd, J = 7.46 Hz, 4.15 Hz, 1H), 4.52 (dd, J = 14.62 Hz, 6.16 Hz, 1H), 4.41 (dd, J = 14.61 Hz, 5.26 Hz, 1H), 3.90 (dd, J = 9.61 Hz, 5.55 Hz, 1H), 3.68 (dd, J = 9.17 Hz, 3.86 Hz, 1H), 3.60 (dd, J = 11.91 Hz, 4.30 Hz, 1H), 3.40 (dd, J = 10.83 Hz, 9.65 Hz, 1H), 3.22 (dd, J = 13.89 Hz, 4.15 Hz, 1H), 3.03 (dd, J = 11.88 Hz, 1.77 Hz, 1H), 2.99 (s, 3H), 2.85 (dd, J = 13.88 Hz, 7.48 Hz, 1H), 2.45 (dd, J = 18.76 Hz, 3.83 Hz, 1H); ^{13}C-NMR ($CDCl_3$) δ: 177.41, 174.58, 167.86, 136.06, 133.73, 129.71, 128.99, 76.91, 70.85, 65.45, 43.34, 41.91, 38.76, 37.33, 35.92, 34.43, 25.19. HRMS calculated for $C_{19}H_{23}ClN_3O_3S_3^+$ $[M + H]^+$: 472.0405, found: 472.0585.

(2*S*,5*R*,8*R*,3′*SR*)-8-(*N*-4-Methoxybenzyl)carbamoyl-2-((((*S*)-1-methyl-2,5-dioxopyrrolidin-3-yl)thio)methyl)-1-aza-3,6-dithiobicycle[3.3.0]octane (10b). Prepared using the same methodology as for **10a**, starting with **1c** (40 mg, 0.18 mmol) and 4-OMe-benzylamine (30 mg, 0.22 mmol) and purified by column chromatography (*n*Hex : EtOAc 60 : 40–40 : 60) to give **10b** as major *syn* diastereomer (40 mg, 0.09 mmol, 48%, dr: 59 : 41) as a white solid; mp = 170–172 °C; ^{1}H-NMR ($CDCl_3$) δ: 7.26 (d, J = 8.6 Hz, 2H), 6.87 (d, J = 8.6 Hz, 2H), 6.43 (s, 1H), 5.19 (dd, J = 4.2, 1.7 Hz, 1H), 4.69 (dd, J = 7.7, 4.0 Hz, 1H), 4.43 (t, J = 5.8 Hz, 2H), 3.87 (dd, J = 9.9, 5.5 Hz, 1H), 3.80 (s, 3H), 3.66 (dd, J = 9.2, 3.9 Hz, 1H), 3.60 (dd, J = 11.8, 4.3 Hz, 1H), 3.41 (t, J = 10.2 Hz, 1H), 3.23 (dd, J = 13.9, 4.0 Hz, 1H), 3.15–3.06 (m, 2H), 3.04 (dd, J = 11.8, 1.7 Hz, 1H), 3.00 (s, 3H), 2.84 (dd, J = 14.0, 7.6 Hz, 1H), 2.45 (dd, J = 18.7, 3.9 Hz, 1H); ^{13}C-NMR ($CDCl_3$) δ: 175.11, 172.41, 165.25, 157.09, 127.53, 127.31, 127.01, 111.97, 75.00, 68.76, 63.23, 53.12, 41.33, 39.76, 36.60, 35.00, 33.82, 32.11, 22.95. HRMS calculated for $C_{20}H_{26}N_3O_4S_3^+$ $[M + H]^+$: 468.1080, found: 468.1585.

Data availability

The data supporting this article have been included as part of the ESI.† Crystallographic data was deposited in Cambridge Crystallographic Data Centre. CCDC 2387412–2387413.

Author contributions

MR and VM contributed to experiments and product characterization. MM, FV and DD contributed to product characterization. GM conceived the project. FV run the DFT calculations. GH run the NOESY experiments and analysis. MR, FV, GH and GM, prepared the manuscript, and contributed to discussions.

Conflicts of interest

There are no conflicts to declare.

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