

# Mechanistic and Chiroptical Studies on the Desulfurization of Epidithiodioxopiperazines Reveal Universal Retention of Configuration at the Bridgehead Carbon Atoms

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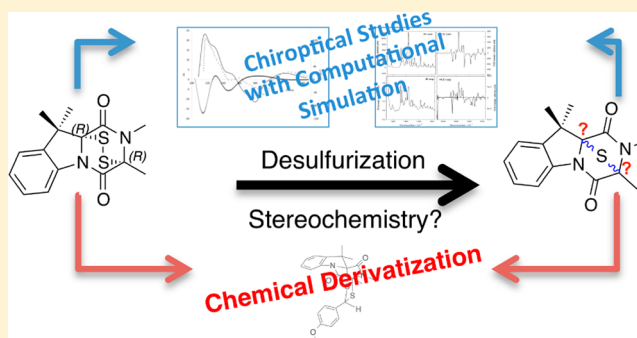
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**ABSTRACT:** The stereochemistry of the desulfurization products of chiral natural and synthetic 3,6-epidithiodiketopiperazines (ETPs) is specified inconsistently in the literature. Qualitative mechanisms have been put forward to explain apparently divergent stereochemical pathways, but the quantitative feasibility of such mechanistic pathways has not been assessed. We report a computational study revealing that desulfurization of ETPs should occur universally with retention of configuration. While the majority of stereochemically assigned and reassigned cases fit this model, until now desulfurization of the synthetic gliotoxin analogue shown has remained assigned as proceeding via inversion of configuration. Through detailed chiroptical studies comparing experimentally obtained optical rotation values, electronic circular dichroism spectra, and vibrational circular dichroism spectra to their computationally simulated counterparts as well as chemical derivatization studies, we have unambiguously demonstrated that contrary to its current assignment in the literature, the desulfurization of this synthetic ETP also proceeds with retention of configuration.



## INTRODUCTION

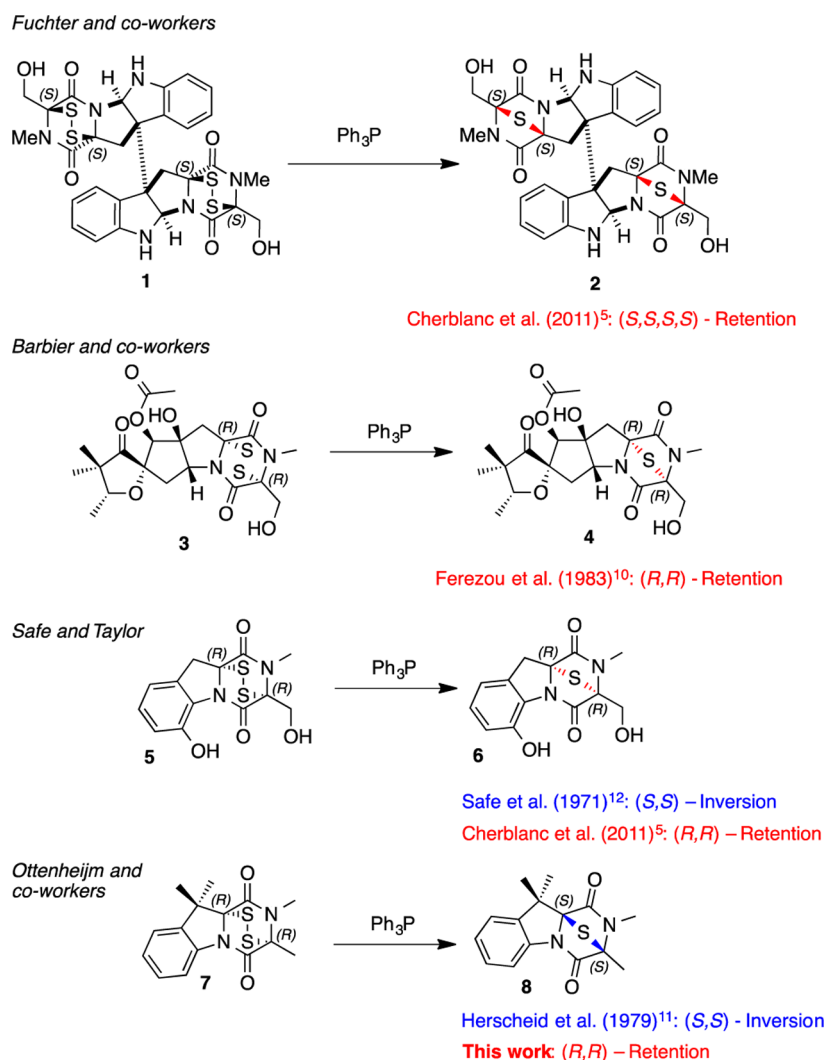
Unambiguously assigning the absolute configuration of chiral molecules, particularly of complex natural products, is often a significantly challenging endeavor. Such assignments not only identify the correct three-dimensional architecture of a given molecule but are also often instrumental in the understanding of reaction mechanisms for stereoselectivity. The pioneering work of Kirkwood in the 1950s enabled the use of quantum mechanics to help determine absolute configuration on the basis of the predicted sign of a given optical rotation.<sup>1</sup> With modern-day computational power, we are now able to simulate a range of chiroptical spectra [optical rotatory dispersion (ORD), electronic circular dichroism (ECD), vibrational circular dichroism (VCD), Raman optical activity (ROA)], offering a straightforward way of assigning the absolute configuration of a given molecule through comparison with corresponding experimental data.<sup>2–4</sup>

We have previously employed this strategy to unambiguously determine the absolute configuration of a desulfurized analogue (2) of the 3,6-epidithiodiketopiperazine (ETP) natural product chaetocin (1) (Figure 1),<sup>5</sup> a nonspecific histone lysine

methyltransferase inhibitor.<sup>6–8</sup> While the desulfurization of ETPs with triphenylphosphine has been known for decades,<sup>9</sup> the stereochemical course of this reaction has been contested in several papers (e.g., see Figure 1).<sup>9–12</sup> In order to assign the stereochemistry of analogue 2, we compared the experimentally obtained and computationally simulated optical rotation values and ECD spectra. On the basis of these data, we concluded that chaetocin (1) is desulfurized with retention of configuration (Figure 1). Barbier and co-workers reached the same conclusion in their work on the desulfurization of sirodesmin PL (3) into monosulfide 4 (Figure 1)<sup>10</sup> using chemical derivatization studies and X-ray analysis of a diacetyl derivative. Conversely, on the basis of the observation that the ECD curve of product 6 exhibited an opposite sign of the Cotton effect compared with dehydrogliotoxin (5), Safe and Taylor had previously reported the desulfurization of dehydrogliotoxin (5) (Figure 1) to proceed with inversion of configuration.<sup>12</sup> Through simulation of the ECD spectra of the two possible enantiomers of

Received: June 19, 2013

Published: September 27, 2013



**Figure 1.** Stereochemical assignments of desulfurized ETP-containing compounds.

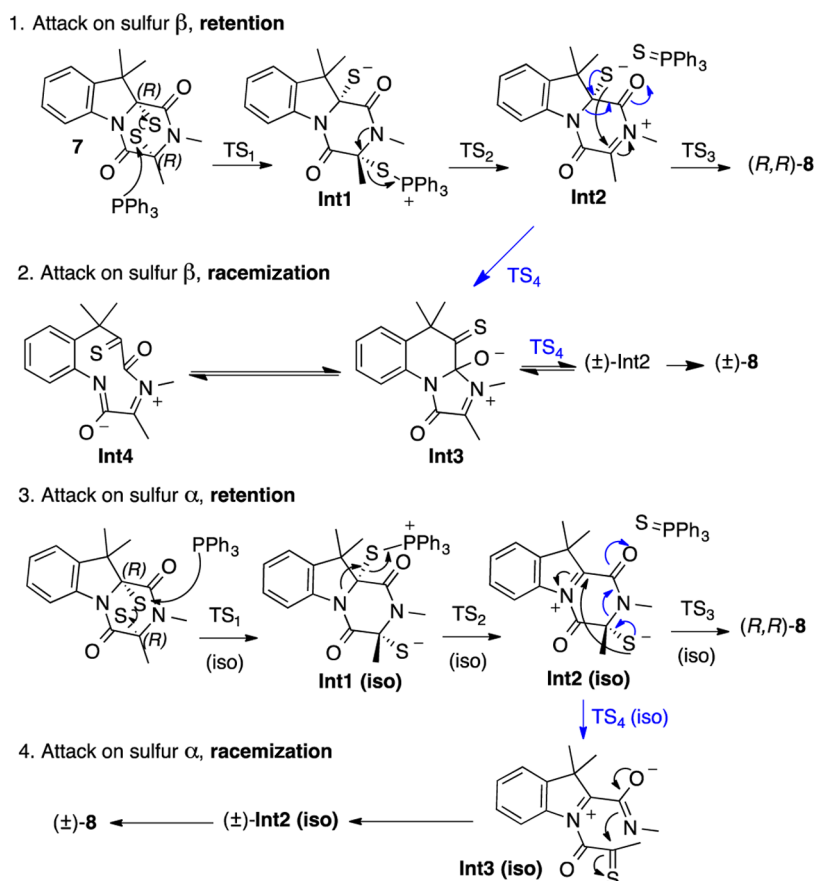
**6** (*R,R* and *S,S*), we found the assignment by Safe and Taylor to be erroneous and therefore reassigned this desulfurization reaction to also occur with retention of configuration.<sup>5</sup>

In light of the apparent common stereochemical course of ETP desulfurization, with retention at the bridgehead carbons, it was clear to us that only one example remained in the literature where ETP desulfurization was reported to occur with inversion of configuration. In 1979, Ottenheijm and co-workers reported the desulfurization of gliotoxin analogue **7** into monosulfide **8** (Figure 1).<sup>11</sup> The stereochemical assignment of **8** was obtained by anomalous-dispersion X-ray crystallography (vide infra). While qualitative mechanistic scenarios have been reported to account for the apparent inversion of configuration for this isolated case,<sup>5,11</sup> we decided to computationally study the mechanistic course of this reaction in order to determine the feasibility of an inversion mechanism. In this paper, we report our results on this mechanistic study as well as the use of chiroptical methods and chemical derivatization to characterize the stereochemistry of compounds **7** and **8**. Taken together, our results reveal this desulfurization reaction to proceed similarly via retention of configuration (Figure 1). This study not only unambiguously defines a common mechanistic course for ETP desulfurization but also has strong implications for the use of chiroptical spectroscopy in stereochemical assignment.

## RESULTS AND DISCUSSION

Intrigued by the inversion of stereochemistry reported for the desulfurization of compound **7** by Ottenheijm and co-workers,<sup>11</sup> we decided to calculate the free energies of the transition states along the most probable mechanism leading to retention of configuration as well as plausible pathways that would alter the stereochemistry of the bridgehead chiral centers. The selected pathways were derived from previous mechanistic proposals for this transformation.<sup>5</sup> It is worth noting that the calculations can only address preselected pathways; a stochastic exploration of all possible pathways is currently not feasible (although such a stochastic approach has been attempted in an exploration of all possible structures for a given molecular formula<sup>13</sup>). All of the pathways explored are detailed in Figure 2 and Interactivity Box 1. Importantly, in addition to the retention pathway, we were able to identify only (higher energy) alternative pathways that would result in product racemization, not stereospecific inversion (vide supra).

Calculations were performed at the  $\omega$ B97XD/6-311G(d,p) level including a solvent model for THF, a representative solvent for these reactions.<sup>12</sup> The initial bimolecular step involves attack by the phosphorus nucleophile on either of the two sulfur atoms of ETP **7**, sulfur  $\beta$  (TS1) or sulfur  $\alpha$  [TS1 (iso)]. The former (TS1) has a calculated barrier  $\Delta G_{298}^\ddagger$  (for a standard state of 1



**Figure 2.** Mechanistic scheme for desulfurization of **7** to give **8**. Indicated transition states were all located at the  $\omega\text{B97XD}/6\text{-311G(d,p)}$  level with inclusion of a continuum solvent model for THF, and the free energies were obtained using the computed vibrational frequencies. Triphenylphosphine sulfide ( $\text{SPPH}_3$ ) was included in the energy calculation throughout. Data and data DOIs associated with this figure are available via Interactivity Box 1 and the DOI for this figure: 10.6084/m9.figshare.797484.

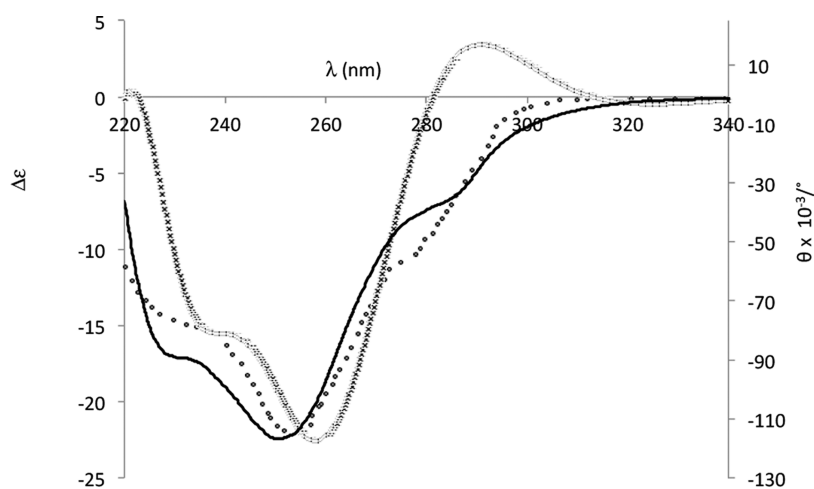
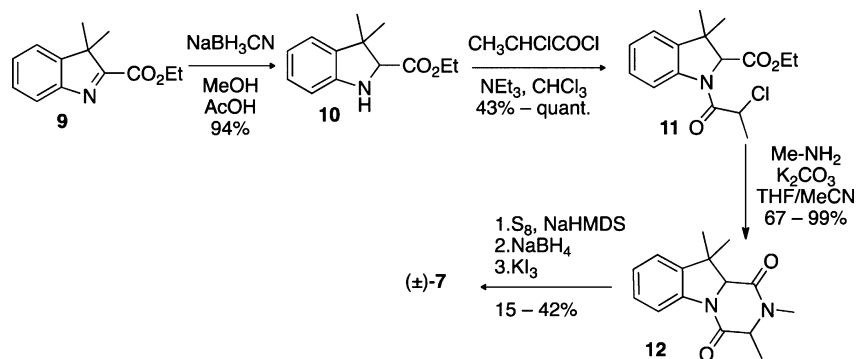
atm, 0.041 M) of 19.6 kcal/mol relative to **7**, while the latter [ $\text{TS1 (iso)}$ ] has a higher barrier of 27.7 kcal/mol because of hindrance from the methyl groups (these bimolecular barriers are reduced by  $-1.89$  kcal/mol for a standard state of 1 M; unimolecular barriers are not affected by this correction). Only the former corresponds to a viable room-temperature reaction. Indeed, this regiochemical outcome is consistent with the experimental work of Ottenheijm and co-workers through trapping of a key intermediate (**Int2**) with a methanol nucleophile.<sup>11</sup> The second unimolecular step via  $\text{TS2}$  [or  $\text{TS2 (iso)}$ ] corresponds to elimination of triphenylphosphine sulfide from **Int1** to create the zwitterionic intermediate **Int2**. Computational modeling of the geometry of such ionic intermediates has historically been difficult. A correction for solvation energy is absolutely essential, and this in turn requires evaluation of first and second solvated-energy derivatives to accurately locate and characterize the geometry of the transition state. The first complex mechanism to be so studied was reported only recently.<sup>14</sup> The barrier for elimination of  $\text{Ph}_3\text{PS}$  from **Int1** is very small:  $\Delta G_{298}^\ddagger = 2.2$  ( $\text{TS2}$ ) or 4.8 [ $\text{TS2 (iso)}$ ] kcal/mol relative to **Int1**, corresponding to 16.7 ( $\text{TS2}$ ) or 21.0 [ $\text{TS2 (iso)}$ ] kcal/mol relative to **7**. These energies are lower than those for  $\text{TS1}$ , and hence, this second step is not rate-determining. It is worth noting that the inversion mechanism proposed by Ottenheijm and co-workers<sup>11</sup> involved diketopiperazine ring opening, bond rotation, and ring closure prior to elimination of  $\text{Ph}_3\text{PS}$ . The low activation barrier for  $\text{Ph}_3\text{PS}$  elimination from **Int1** (2.2 kcal/mol) renders this

intermediate highly transient, and thus, the previously proposed mechanism is unlikely. **Int2** itself is computed to be 4.5 kcal/mol lower than **Int1**.

The mechanism now bifurcates into two pathways, one involving retention of configuration and the other racemization. The retentive pathway involves reclosure of the zwitterion to reform the sulfur bridge, with barriers of 10.6 ( $\text{TS3}$ ) or 15.3 [ $\text{TS3 (iso)}$ ] kcal/mol. Once again, these are significantly lower in energy than  $\text{TS1}$ . This suggests that for product retention, attack by triphenylphosphine ( $\text{TS1}$ ) is rate-determining, with the remainder of the pathway being energetically downhill.

We identified a number of higher-energy fates for **Int2/Int2 (iso)**.  $\text{TS4}$  corresponds to formation of a  $\text{C}=\text{S}$  double bond with  $\text{N}-\text{C}$  bond migration to give **Int3**. This can undergo further ring opening to give an intermediate **Int4** that can in principle atropisomerise. Such a process would result in racemization of the original carbon stereogenic centers. Alternatively,  $\text{TS4 (iso)}$  corresponds to the formation of a  $\text{C}=\text{S}$  double bond with diketopiperazine ring opening. Bond rotation and ring closure would also result in racemization of the original carbon stereogenic centers. The barriers to these racemization processes are 35.3 ( $\text{TS4}$ ) and 30.9 [ $\text{TS4 (iso)}$ ] kcal/mol, which are significantly higher than that for the rate-limiting step on the pathway to retention of configuration.

The energy profiles of these reaction sequences strongly suggest that desulfurization of ETP compound **7** occurs with retention of configuration rather than racemization. It is difficult

Scheme 1. Synthesis of ( $\pm$ )-7

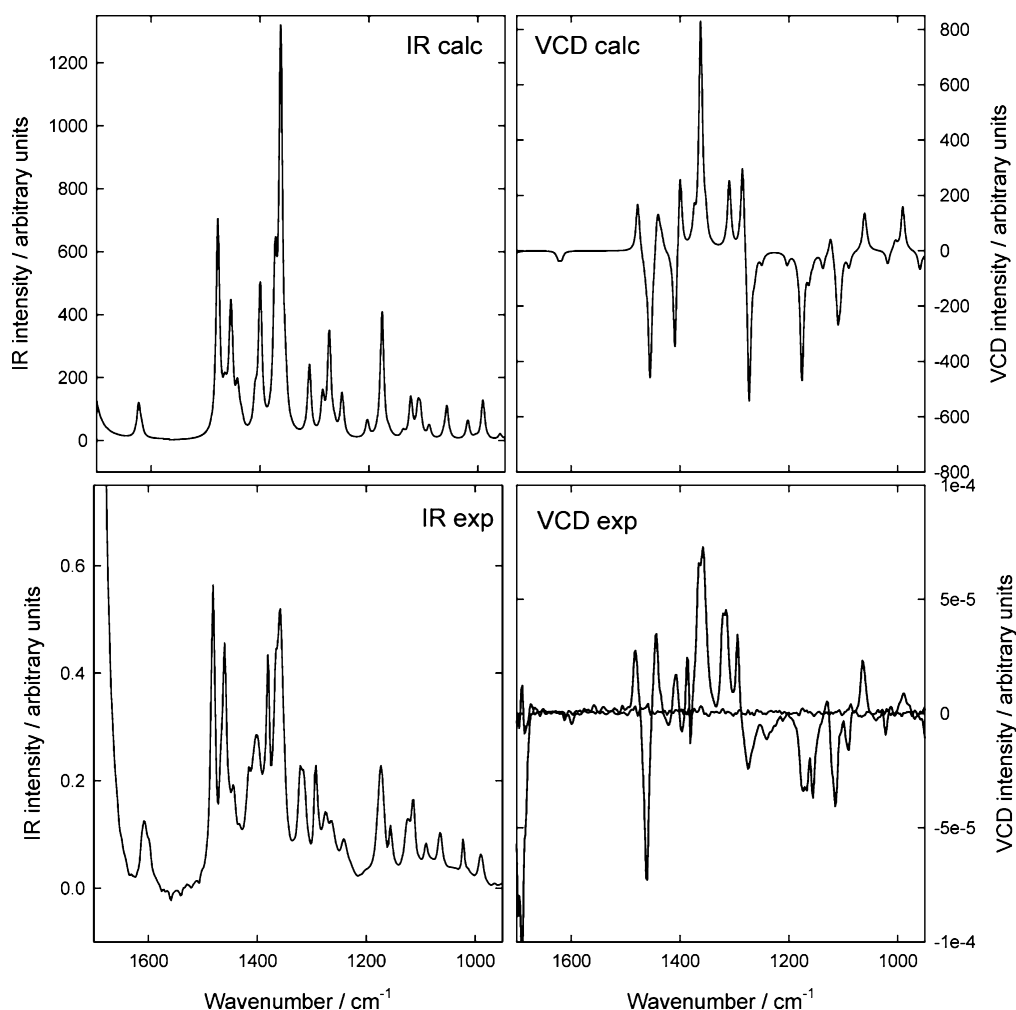
**Figure 3.** ECD spectra of gliotoxin analogue 7: (solid line) measured data for 7 (right y axis) in dichloromethane; (●) data from Ottenheim and co-workers<sup>20</sup> for 7 (left y axis); (x) data for (*R,R*)-7 calculated at the M06-2X/6-311++G(d,p) scrf(cpcm, solvent=dichloromethane) level (left y axis, rescaled by 0.385, shifted by +25 nm, and convoluted with a line width of 0.24 eV).

to envisage a mechanistic pathway (not involving entropically disfavored intervention of other molecules such as solvent) that could result in highly stereospecific inversion of the both stereogenic centers, as claimed by Ottenheim and co-workers.<sup>11</sup>

Since this computational study brought into question the stereochemical assignment of product 8, we proceeded to prepare gliotoxin analogue 7 in order to study the stereochemical course of its desulfurization. Racemic ETP 7 was prepared in seven steps from commercially available material. Indolenine 9<sup>15</sup> was reduced<sup>16</sup> to indoline 10 in excellent yield, and subsequent treatment with 2-chloropropanoyl chloride afforded the acylated product 11 (Scheme 1).<sup>17</sup> Diketopiperazine 12 was obtained by refluxing acylated indoline 11 with methylamine in THF/MeCN.<sup>17</sup> The sulfonylation of diketopiperazine 12 was performed using the method recently described by Nicolaou and co-workers<sup>18,19</sup> and afforded gliotoxin analogue ( $\pm$ )-7. The enantiomers were separated using semipreparative HPLC on a chiral stationary phase (see the Experimental Section for details).

The first ETP enantiomer eluted from the HPLC column had an optical rotation and an ECD spectrum that matched those of the starting material used by Ottenheim and co-workers in their desulfurization studies.<sup>11</sup> Ottenheim and co-workers had assigned this stereoisomer to have (*R,R*) stereochemistry by qualitative comparison with the ECD spectra of the closely related gliotoxin.<sup>20</sup> Since correlative methods, which compare experimental chiroptical spectra of a given molecule to those acquired from related molecular frameworks, can lead to a

significant chance of error<sup>5,21</sup> and ECD quantum-chemical simulations are generally less reliable than, for example, those for VCD and ROA,<sup>22</sup> we sought to confirm this assignment. The optical rotation of (*R,R*)-7 was calculated and compared to the experimental values. At the M06-2X/6-311++G(d,p) level with a solvent model for chloroform, the optical rotation of (*R,R*)-7 was predicted to be  $-498$ . Ottenheim et al. had measured a value of  $-502$  ( $c$  2.345, CHCl<sub>3</sub>),<sup>20</sup> and we obtained a value of  $-335$  ( $c$  2.00, CHCl<sub>3</sub>). While the strong negative sign of the optical rotation suggested the configuration of this stereoisomer to be (*R,R*), we sought further confirmation, particularly since computational optical rotational values are often only qualitatively similar to their experimental counterparts.<sup>23</sup> The ECD spectra were experimentally measured and computationally simulated for (*R,R*)-7. Figure 3 compares the experimental ECD spectra obtained by us and those reported by Ottenheim et al.<sup>20</sup> with the computationally simulated one. The two experimental spectra are analogous, with three negative Cotton effects around 230, 250, and 280 nm. Curiously, while the higher-energy (230 and 250 nm) bands are reproduced in the simulated spectrum, the Cotton effect at around 280 nm seems to be of the wrong sign (positive). Therefore, while the ECD simulation of (*R,R*)-7 is clearly closer to the experimentally obtained spectra than the simulated spectrum for (*S,S*)-7 (the mirror image of that shown in Figure 3), this level of theory is unable to correctly predict all three negative Cotton effects. We therefore felt that the assignment would benefit from additional characterization.



**Figure 4.** IR and VCD Spectra of Gliotoxin analogue 7. The top panels show the calculated IR and VCD spectra for (*R,R*)-7 obtained at M06-2X/6-311++G(d,p) scrf(cpcm, solvent=chloroform). The bottom panels show the experimental IR and VCD spectra for 7 obtained for a solution in CDCl<sub>3</sub>. The theoretical spectra were obtained by using a scale factor of 0.968. The thin black line given in the bottom right panel refers to the noise spectrum supplementing the measured VCD data.

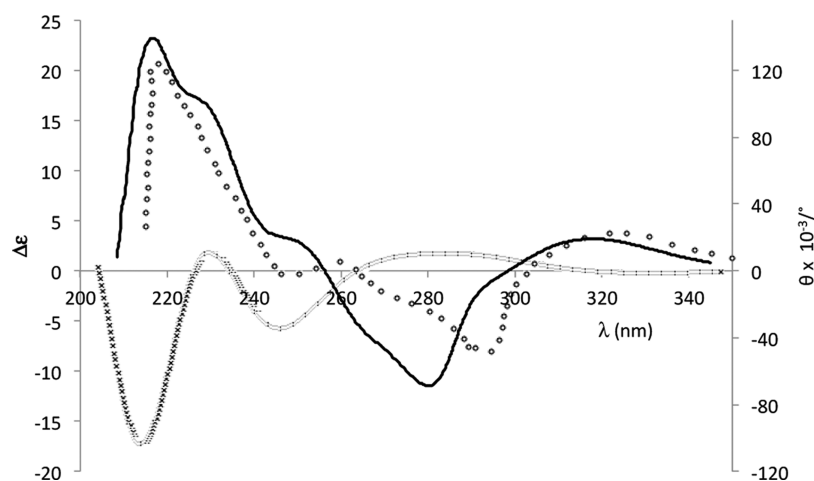
The experimentally measured and computationally simulated IR and VCD spectra for (*R,R*)-7 are depicted in Figure 4. Whereas ECD is based on the relatively small number of electronic transitions, VCD has the important advantage of addressing all  $3N - 6$  vibrational modes (where  $N$  is the number of atoms), thereby offering more information-rich spectra. The factor of 0.968 used to rescale the calculated wavenumbers was derived from the IR similarity measure  $S_{fg}$  describing the agreement of the experimental and calculated IR spectra.<sup>24</sup> Inspection of the data presented shows that good agreement is found between theory and experiment, as almost all of the bands observed in the IR and/or VCD spectra are neatly reproduced by the calculations. Two features observed in the experimental spectrum that are not reproduced by the calculations are the ones at 1441 and 1084 cm<sup>-1</sup>. The reason for these discrepancies is most probably related to the appearance of additional, stronger VCD features with opposite sign in the immediate vicinity. The good agreement is confirmed by the similarity measures  $\Sigma_{fg}$  and  $\Sigma_{fg}^-$ ,<sup>24</sup> which describe the level of agreement between the experimental VCD spectra and the calculated data derived for the (*R,R*) and (*S,S*) enantiomers, respectively. The resulting values  $\Sigma_{fg}$  and  $\Sigma_{fg}^-$  are 75.5% and 6.9%. Combination of the  $\Sigma_{fg}$  values, the enantiomeric similarity index  $ESI = |\Sigma_{fg} - \Sigma_{fg}^-|$ , and the spectral

database in the CompareVOA program<sup>24</sup> leads to a confidence level of 100% for the (*R,R*) stereochemistry. Taken together, the comparative optical rotation, ECD, and VCD evidence confirms the (*R,R*) assignment of the ETP 7 used in the desulfurization chemistry, as suggested by Ottenheijm and co-workers.<sup>11,20</sup>

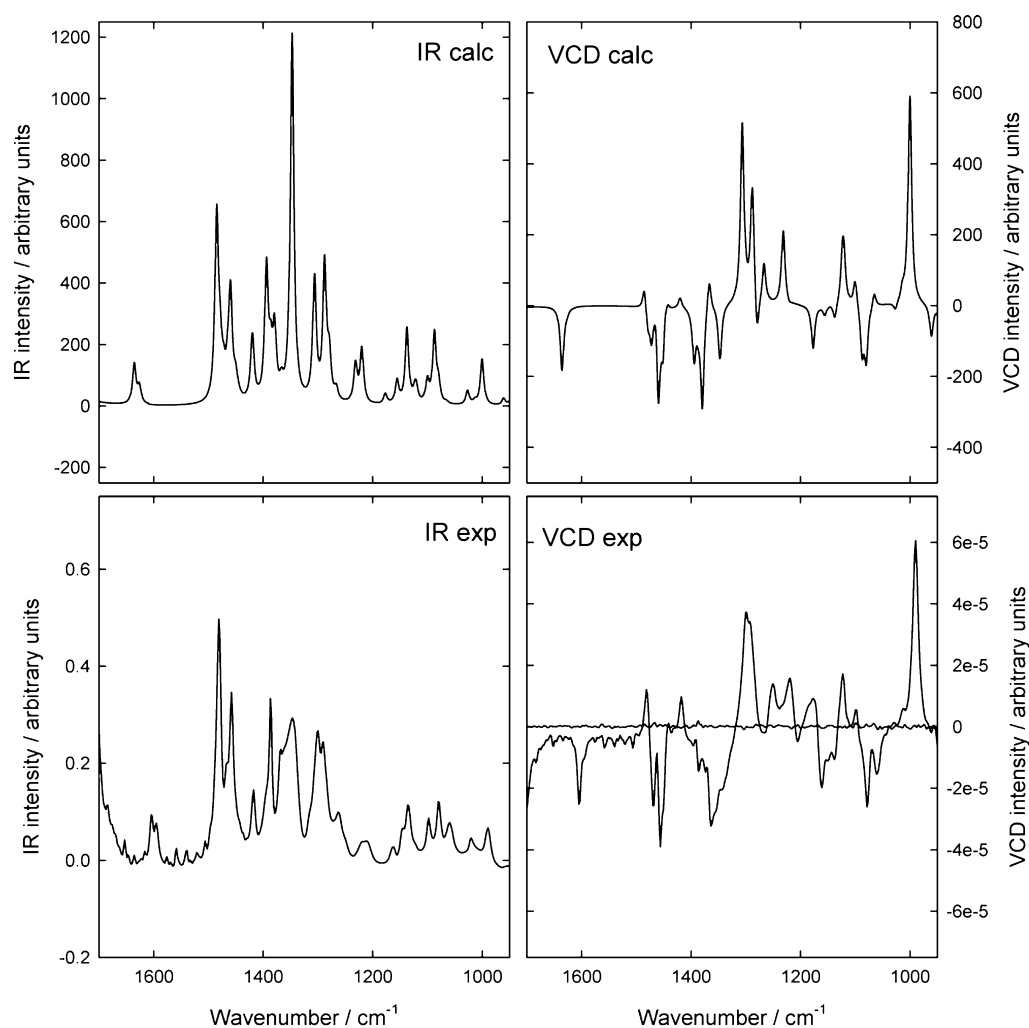
ETP (*R,R*)-7 was desulfurized with triphenylphosphine in dioxane.<sup>11</sup> Chiral HPLC analysis gave a 93:7 enantiomeric ratio (e.r.) of the product 8. Similarly, (*S,S*)-7 gave 2:98 e.r. of the product 8, the major isomer being enantiomeric to that obtained with (*R,R*)-7. In order to access larger and enantiopure quantities of 8, semipreparative chiral HPLC was also employed on a racemic mixture of product 8 to enable full stereochemical assignment.

The optical rotation of the first eluted enantiomer of 8 was  $-47.5$  ( $c$  1.12, CH<sub>2</sub>Cl<sub>2</sub>), in good agreement with the value of  $-53$  ( $c$  1.13, CH<sub>2</sub>Cl<sub>2</sub>) for the compound isolated from the desulfurization reaction of (*R,R*)-7 by Ottenheijm and co-workers.<sup>11</sup> At the M06-2X/6-311++G(d,p) level with a solvent model for dichloromethane, the optical rotation of (*R,R*)-8 was  $-169$ . Although the quantitative magnitude of this predicted value is different, the strong negative sign of the rotation is suggestive that the first eluted enantiomer has the (*R,R*) configuration. This enantiomer corresponds to the product of





**Figure 5.** ECD spectra of desulfurized gliotoxin analogue **8**: (solid line) measured data for **8** (enantiomer **1**) in dichloromethane (right y axis); (O) data from Ottenheijm and co-workers<sup>11</sup> for **8** (left y axis); (x) data for (*R,R*)-**8** calculated at the M06-2X/6-311++G(d,p) scrf(cpcm, solvent=dichloromethane) level (left y axis, rescaled by 0.167, shifted by +25 nm, and convoluted with a line width of 0.24 eV).

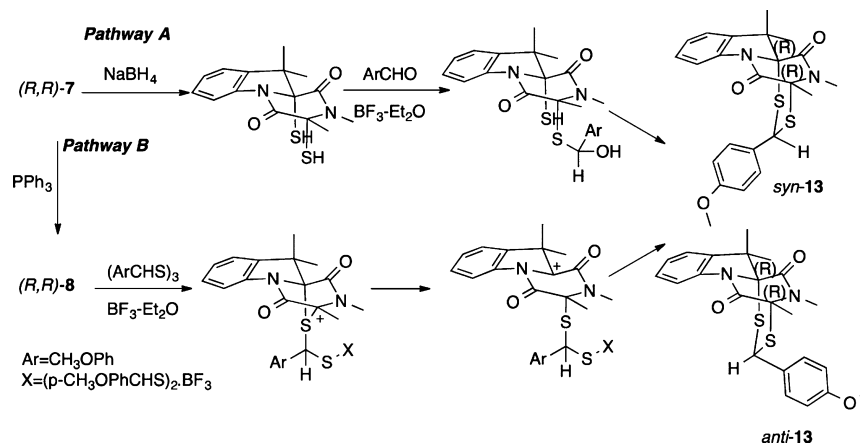


**Figure 6.** IR and VCD spectra of desulfurized gliotoxin analogue **8**. The top panels show the calculated IR and VCD spectra for (*R,R*)-**8** obtained at the M06-2X/6-311++G(d,p) scrf(cpcm, solvent=chloroform) level. The bottom panels show the experimental IR and VCD spectra for **8** obtained for a solution in CDCl<sub>3</sub>. The theoretical spectra were obtained by using a scale factor of 0.976. The thin black line given in the bottom right panel refers to the noise spectrum supplementing the measured VCD data.

the desulfurization of (*R,R*)-**7**, suggesting that the reaction proceeded with retention of configuration.

The ECD spectrum of the putative (*R,R*)-**8** was also recorded (Figure 5). This too was compared to the data obtained by

Scheme 2. Synthesis of 13



Ottenheijm and co-workers.<sup>11</sup> Once again, the data were in good agreement, with two positive Cotton effects (around 220 and 250–260 nm) followed by a negative one (280–290 nm), and finally a positive one (320 nm). The ECD spectrum of  $(R,R)$ -8 was simulated and compared to the experimental data. The comparison was poor, however, both qualitatively and quantitatively. In particular, the strong predicted negative Cotton effect at around 210 nm was apparently of the wrong sign. It was apparent to us that the  $(S,S)$  stereochemistry (mirror image of the spectrum depicted in Figure 5) would be a similarly poor fit.

In light of the fact that the ECD spectral comparison was inconclusive, we again employed comparative VCD. The IR and VCD spectra of the putative  $(R,R)$ -8 dissolved in CDCl<sub>3</sub> and the corresponding simulated spectra, rescaled by using a uniform wavenumber scaling factor of 0.976, are given in Figure 6. Comparison of the experimental and calculated data shows that also for the desulfurized gliotoxin analogue 8 an excellent agreement is found between theory and experiment. The observed one-to-one correlation results in similarity measures  $\Sigma_{\text{fg}} = 66.5\%$  and  $\Sigma_{\text{fg}} = 4.2\%$  for  $(R,R)$  and  $(S,S)$ , respectively; in combination with the spectral database in CompareVOA,<sup>24</sup> these values lead to a confidence level of 99% for the  $(R,R)$  stereochemistry of 8. Since this enantiomer corresponds to the product of the desulfurization of  $(R,R)$ -7, this result, in combination with the optical rotation comparison, strongly suggests that the reaction proceeds with retention of configuration.

Although comparison of the experimental and simulated values for optical rotation and VCD gave a high statistical probability of the  $(R,R)$  assignment for our product enantiomer 8, the inconclusive result for the ECD assignment remained. We therefore sought an additional and, more particularly, non-spectroscopic method of assignment. Previously, Barbier and co-workers determined the stereochemistry of the desulfurization of Sirodesmin PL (3) by chemical derivatization.<sup>10</sup>  $(R,R)$ -3 (Figure 1), the configuration of which was determined by X-ray crystallographic analysis, was stereospecifically converted to a dithioacetal. Concurrently, the isolated desulfurized product 4 was converted to the same key intermediate. Thus, if the stereochemistries of the bridgehead chiral centers in the intermediates isolated from the starting ETP and the isolated monosulfide are the same, then the desulfurization reaction proceeds with retention of configuration. This approach has been validated by Barbier and co-worker by X-ray crystallography of an acetylated derivative of monosulfide 4 and is thus a reliable

method for determining the relative stereochemistry of the parent ETP and its monosulfide. Scheme 2 presents a summary of this strategy as applied to the substrates of interest, 7 and 8.

We used this derivatization protocol on ETP  $(R,R)$ -7 and product 8 of proposed  $(R,R)$  stereochemistry. ETP  $(R,R)$ -7 was reduced to the dithiol by treatment with sodium borohydride and then converted into thioacetal 13 by treatment with anisaldehyde in the presence of boron trifluoride. Thioacetal 13 was obtained as a single diastereoisomer (*syn* with respect to the anisaldehyde and polycyclic residues<sup>10</sup>). Concurrently, enantiopure monosulfide 8 [proposed to have  $(R,R)$  stereochemistry] was treated with the trithiane derivative of anisaldehyde. The product thioacetal 13 was obtained as a mixture of diastereoisomers that was readily separable by column chromatography. The second diastereoisomer isolated had <sup>1</sup>H NMR, <sup>13</sup>C NMR, HSQC, and NOESY spectra identical to the ones obtained from the reaction of  $(R,R)$ -7. Importantly, analytical HPLC on a chiral stationary phase indicated both thioacetal products to be the same enantiomer (see the Supporting Information). As the same enantiomer of thioacetal 13 was obtained via either pathway, we concluded that the stereochemistry of both ETP 7 and monosulfide 8 under study was  $(R,R)$ . Therefore, contrary to the original report,<sup>11</sup> desulfurization of ETP  $(R,R)$ -7 occurs with retention of stereochemistry at the bridgehead carbon atoms.

## CONCLUSIONS

Through comparison of experimental and simulated chiroptical spectra as well as derivatization studies, we have established that the desulfurization of ETP  $(R,R)$ -7 with triphenylphosphine occurs with retention of stereochemistry. To the best of our knowledge, in combination with our previous paper,<sup>5</sup> we have now demonstrated that all chiral ETP compounds in the literature (with suitable chiroptical information available) are desulfurized with retention of stereochemistry at the bridgehead carbons, likely via the mechanism depicted in Figure 2. Unfortunately, we are unable to unambiguously determine the origin of the erroneous stereochemical assignment of compound 8 by Ottenheijm and co-workers. They did after all determine a sample of compound 8 to have  $(S,S)$  stereochemistry using X-ray crystallography (anomalous dispersion) when the desulfurization of  $(R,R)$ -7 should have given  $(R,R)$ -8. While this assignment was prior to the publication of the commonly used Flack parameter,<sup>25</sup> Ottenheijm and co-workers employed the *R*-factor test, which remains a simple and powerful approach for the assignment of absolute stereochemistry. In addition, the authors

stated that they manually examined the Bijvoet pairs and came to the same conclusion in terms of stereochemical assignment. We see no reason to doubt these results, and in the absence of the original intensity data, we have no way of verifying them for ourselves. Despite this, it may be that the crystal structure assignment may need to be revised. There may be an alternative reason that the reported structure does not represent the product of the reaction. Two scenarios may account for this possibility: (1) Since they had access to both enantiomeric series of synthetic compound **7**, a mislabeling may have led to the accidental use of (*S,S*)-**7** in the desulfurization reaction. (2) Since we observed a minor amount of racemization in our own study, one could imagine homochiral crystallization of the desulfurization reaction product **8** (~94% e.e.) and accidental picking of an (*S,S*) crystal for crystallographic analysis. It appears that reanalysis (optical rotation or ECD) of the crystal used for the crystallographic studies was not performed to ensure that its absolute configuration matched that of the bulk material. We have been unable to crystallize our samples of (*R,R*)-**8** or enantiomerically pure **7**. This is perhaps unsurprising, since Ottenheim and co-workers also commented that “after numerous attempts one suitable crystal could be prepared for the X-ray analysis”.<sup>11</sup>

In general terms, the erroneous stereochemical assignment of the ETP natural and unnatural products in the literature stemmed from the attempted use of correlative methods, particularly using ECD, comparing experimental chiroptical spectra of a given molecule to those acquired from related molecular frameworks. In our study, we have determined that the sign of a given Cotton effect is not characteristic for ETP or desulfurized ETP stereochemistry. In this instance, despite the fact that the two compounds differ only by one sulfur atom, the ECD spectrum of an ETP compound is not necessarily comparable with that of its desulfurized analogue and vice versa because different molecular orbitals are involved for the transitions in each scaffold (see Interactivity Box 2). We recommend that such experimental techniques should always be accompanied by suitably accurate quantum-chemical simulations of chiroptical properties for assignment and, if appropriate, by validation of proposed mechanisms using quantum-mechanical procedures. In practice, this means at least two different chiroptical techniques should be used to increase the level of confidence in the assignment. Indeed, the poor correlation of the simulated and experimental ECD spectrum for compound **8** in this study is a testament to the dangers of using a single technique in isolation.

## EXPERIMENTAL SECTION

**Computational Procedures. Mechanistic Exploration.** The  $\omega$ B97XD/6-311G(d,p) procedure with continuum solvation model for THF as implemented in Gaussian 09 (revision C.01)<sup>26</sup> was used. All transition states were characterized with one negative root of the Hessian force constant matrix, and thermal corrections (including entropy) were included to give free energies at 298 K and 1 atm (0.041 M). A correction of -1.89 kcal/mol for a standard state of 1 M is required for a bimolecular reaction only.<sup>27,28</sup>

Full details of all calculations are available via the individual digital repository entries associated with Interactivity Boxes 1 and 2 (Web-enhanced objects) available with this article or directly by the following DOI resolvers: TS1, <http://doi.org/10042/to-13699>; TS1 (iso), <http://doi.org/10042/to-13247>; Int1, <http://doi.org/10042/24695>; Int1 (iso), <http://doi.org/10042/24698>; TS2, <http://doi.org/10042/to-13795>; TS2 (iso), <http://doi.org/10042/to-13603>; Int2, <http://doi.org/10042/to-13896>; Int2 (iso), <http://doi.org/10042/24702>; TS3, <http://doi.org/10042/to-13894>; TS3 (iso), [13568; Int3, <http://doi.org/10042/to-13902>; Int3 \(iso\), <http://doi.org/10042/to-13909>; TS4, <http://doi.org/10042/to-13570>; TS4 \(iso\), <http://doi.org/10042/to-13893>.](http://doi.org/10042/to-</a></p></div><div data-bbox=)

**Chiroptical Calculations.** Optical rotations (589 nm) were calculated at the M06-2X/6-311++G(d,p) level of theory with a continuum solvent model for the indicated solvent (chloroform or dichloromethane). Electronic circular dichroism spectra were simulated using time-dependent density functional (TDDFT) calculations at the M06-2X/6-311++G(d,p) level (dichloromethane continuum solvent model) for 50 states (extending the number of states to 100 was shown to have a negligible effect on the predicted spectra for wavelengths greater than 190 nm). Vibrational circular dichroism spectra were also computed at the M06-2X/6-311++G(d,p) level with a continuum solvent model for chloroform using the standard methods implemented in Gaussian 09.

The DOIs for the chiroptical calculations are as follows: Compound **7**: optical rotation, <http://doi.org/10.6084/m9.figshare.777752>; ECD, <http://doi.org/10042/24715>; VCD, <http://doi.org/10.6084/m9.figshare.779754>. Compound **8**: optical rotation, <http://doi.org/10042/24713>; ECD, <http://doi.org/10.6084/m9.figshare.676012> (50 states) and <http://doi.org/10042/24716> (100 states); VCD, <http://doi.org/10.6084/m9.figshare.778538>.

**Compound Synthesis. Ethyl 3,3-Dimethylindoline-2-carboxylate (10).** Compound **10** was prepared as previously reported.<sup>15,16</sup> The measured spectroscopic and physical data were in agreement with the published data.<sup>29</sup>

**Ethyl 1-(2-Chloropropanoyl)-3,3-dimethylindoline-2-carboxylate (11).** 2-Chloropropionyl chloride (0.35 mL, 3.56 mmol) was added dropwise to a solution of triethylamine (0.46 mL, 3.29 mmol) and indoline **10** (600 mg, 2.70 mmol) in chloroform at 0 °C. The cooling bath was then removed, and after 4 h, the reaction mixture was poured into ice–water and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 50 mL). The combined organic extracts were washed with brine, dried (MgSO<sub>4</sub>), and filtered, and the solvents were removed under reduced pressure. The residue was then purified by column chromatography (PE/EtOAc 95:5) to afford a 1:1 mixture of two diastereoisomers as a colorless oil (combined mass 731 mg, 87%). IR (neat) 2972, 1747, 1673, 1598, 1483, 1409, 1199, 752 cm<sup>-1</sup>. Diastereoisomer 1: <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  8.22 (br s, 1H), 7.28–7.24 (m, 1H), 7.10–7.08 (m, 2H), 4.86 (s, 1H), 4.26–4.11 (m, 2H + 1H), 1.73 (br d, *J* = 6.4 Hz, 3H), 1.42 (s, 3H), 1.40 (s, 3H), 1.26 (t, *J* = 7.2 Hz, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  169.8, 166.1, 141.2, 138.7, 128.3, 124.9, 121.6, 117.5, 72.0, 61.7, 51.6, 45.1, 32.0, 22.7, 20.3, 14.2. Diastereoisomer 2: <sup>1</sup>H NMR (500 MHz, DMSO)  $\delta$  8.05 (br d, *J* = 7.8 Hz, 1H), 7.28–7.24 (m, 2H), 7.10 (br t, *J* = 7.5 Hz, 1H), 5.09 (br s, 1H), 5.02 (br q, *J* = 6.2 Hz, 1H), 4.10 (q, *J* = 7.0 Hz, 2H), 1.78 (br d, *J* = 6.2 Hz, 3H), 1.33 (br s, 3H), 1.31 (br s, 3H), 1.17 (t, *J* = 7.0 Hz, 3H); <sup>13</sup>C NMR (100 MHz, DMSO)  $\delta$  169.2, 166.8, 141.4, 139.6, 127.6, 124.6, 122.1, 116.7, 71.0, 60.9, 51.5, 44.6, 31.2, 21.8, 21.2, 13.9. MS (CI) *m/z* 310 [(*M* + *H*)<sup>+</sup>]; HRMS (ESI) *m/z* calcd for C<sub>16</sub>H<sub>21</sub>NO<sub>3</sub>Cl [(*M* + *H*)<sup>+</sup>] 310.1210, found 310.1213.

**2,3,10,10-Tetramethyl-2,3,10,10a-tetrahydropyrazino[1,2-*a*]indole-1,4-dione (12).** Potassium carbonate (218 mg, 1.65 mmol) and an excess of methylamine (2 M in THF, 5.5 mL, 11 mmol) were added to a solution of acylated indoline **11** (340 mg, 1.10 mmol) in MeCN (10 mL) in a sealed tube. After refluxing for 18 h, the reaction mixture was filtered and concentrated, and the residue was purified by column chromatography (PE/EtOAc 1:1) to afford the product as a mixture of nonseparable diastereoisomers as a colorless oil (190 mg, 67%). IR (neat) 2969, 1666, 1602, 1483, 1426, 1402, 1290, 755 cm<sup>-1</sup>. Diastereoisomer 1: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.03 (app d, *J* = 7.6 Hz, 1H), 7.28–7.13 (m, 3H), 4.38 (d, *J* = 1.6 Hz, 1H), 4.16 (qd, *J* = 7.1, 1.6 Hz, 1H), 3.05 (s, 3H), 1.70 (s, 3H), 1.67 (d, *J* = 7.1 Hz, 3H), 1.16 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  164.6, 163.8, 140.4, 138.8, 127.5, 125.4, 121.6, 116.9, 68.5, 57.3, 44.5, 30.9, 24.0, 23.1, 18.0. Diastereoisomer 2 (*trans*): <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.04 (app d, *J* = 7.8 Hz, 1H), 7.28–7.13 (m, 3H), 4.34 (s, 1H), 4.02 (q, *J* = 7.1 Hz, 1H), 3.01 (s, 3H), 1.70 (s, 3H), 1.54 (d, *J* = 7.1 Hz, 3H), 1.19 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  165.1, 164.8, 140.8, 138.7, 127.9, 125.6, 121.9, 116.9, 68.2, 60.2, 45.1, 31.7, 24.9, 24.3, 17.4. MS (ESI) *m/z* 259



$[(M + H)^+]$ ; HRMS (ESI)  $m/z$  calcd for  $C_{15}H_{19}N_2O_2$   $[(M + H)^+]$  259.1447, found 259.1444.

**2,3,10,10-Tetramethyl-2,3-dihydro-1H-3,10a-epithiopyrazino[1,2-a]indole-1,4(10H)-dione 11-Sulfide (7).**<sup>30</sup> To a solution of elemental sulfur (328 mg, 10.24 mmol) in dry THF (40 mL) was added dropwise NaHMDS (0.6 M in PhMe, 6.4 mL, 3.84 mmol) under a nitrogen atmosphere at room temperature. The solution was stirred for 1 min. Diketopiperazine **12** (330 mg, 1.28 mmol) in THF (6 mL) was then added dropwise. After the solution was stirred for 1 min, additional NaHMDS (0.6 M in PhMe, 4.3 mL, 2.56 mmol) was added, and the resulting orange/light-brown mixture was stirred for 30 min at room temperature. The solution was quenched with aq. sat.  $NH_4Cl$  and extracted with  $CH_2Cl_2$  ( $3 \times 50$  mL). The combined organic layers were dried ( $MgSO_4$ ), filtered, and concentrated to give a dark-green residue, which was dissolved in degassed THF/EtOH (1:1, 26 mL) and cooled to 0 °C. Sodium borohydride (1.210 g, 32 mmol) was added in small proportions to the solution. The resulting mixture was stirred for 45 min under  $N_2$ , and the ice bath was removed. Next, the solution was cooled again to 0 °C and quenched by addition of aq. sat.  $NH_4Cl$ . The mixture was extracted with EtOAc ( $3 \times 50$  mL), and the combined organic extracts were then treated with an aqueous solution of  $KI_3$  (1.4 M) (until the dark-purple coloration persisted). The mixture was stirred for 10 min and quenched with 5% aq.  $Na_2S_2O_3$  solution until disappearance of the dark-purple coloration. The resulting mixture was extracted with EtOAc ( $3 \times 50$  mL), and the combined organic layers were dried ( $MgSO_4$ ), filtered, and concentrated. The residue was purified by column chromatography (PE/EtOAc 90:10) to afford a yellow solid (100 mg, 24%). The solid was recrystallized from  $CH_2Cl_2$  to afford white crystals: mp 104–106 °C; IR (neat) 1690, 1460, 1357, 1174  $cm^{-1}$ ;  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  8.02 (d,  $J = 8.0$  Hz, 1H), 7.37–7.33 (m, 1H), 7.28–7.25 (m, 2H), 3.12 (s, 3H), 2.10 (s, 3H), 1.79 (s, 3H), 1.59 (s, 3H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  164.3, 162.4, 139.1, 136.1, 128.8, 126.1, 121.3, 115.9, 82.6, 74.3, 49.2, 28.7, 27.3, 20.5, 18.2; MS (CI)  $m/z$  321  $[(M + H)^+]$ , 257  $[(M - S_2 + H)^+]$ ; HRMS (CI)  $m/z$  calcd for  $C_{15}H_{17}N_2O_2S_2$   $[(M + H)^+]$  321.0731, found 321.0745. The obtained enantiomers could be separated by chiral HPLC (OD+ semiprep column, hexane/isopropanol 1:1): first peak,  $[\alpha]_D^{25} -335$  (c 2.00,  $CHCl_3$ ); second peak,  $[\alpha]_D^{25} +394$  (c 2.05,  $CHCl_3$ ).

**2,3,10,10-Tetramethyl-2,3-dihydro-1H-3,10a-epithiopyrazino[1,2-a]indole-1,4(10H)-dione (8).**<sup>30</sup> To a solution of gliotoxin analogue **7** (33 mg, 0.10 mmol) in dioxane (8 mL) was added  $PPh_3$  (33 mg, 0.16 mmol), and the resulting mixture was stirred overnight at room temperature. The solvent was then removed under reduced pressure, and the pink residue was purified by column chromatography (PE/EtOAc 100:0 to 95:5) to afford a colorless oil (19 mg, 64%), which was recrystallized from  $CH_2Cl_2$  to give a white solid: mp 58–60 °C; IR (neat) 1720, 1456, 1387, 1288, 1134  $cm^{-1}$ ;  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  8.54 (app d,  $J = 7.8$  Hz, 1H), 7.25 (td,  $J = 7.8, 1.0$  Hz, 1H), 7.20 (dd,  $J = 7.8, 1.0$  Hz, 1H), 7.13 (td,  $J = 7.8, 1.0$  Hz, 1H), 2.96 (s, 3H), 1.83 (s, 3H), 1.75 (s, 3H), 1.48 (s, 3H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  172.5, 172.0, 139.7, 138.1, 128.1, 124.7, 122.4, 113.6, 86.6, 75.1, 43.5, 27.2, 26.3, 25.7, 13.3; MS (CI)  $m/z$  289  $[(M + H)^+]$ , 306  $[(M + NH_4)^+]$ ; HRMS (CI)  $m/z$  calcd for  $C_{15}H_{17}N_2O_2S$   $[(M + H)^+]$  289.1011, found 289.1026. The obtained enantiomers could be separated by chiral HPLC (OD+ semiprep column, hexane/isopropanol 90:10): first peak,  $[\alpha]_D^{25} -47.5$  (c 1.12,  $CH_2Cl_2$ ); second peak,  $[\alpha]_D^{25} +34.4$  (c 1.12,  $CH_2Cl_2$ ).<sup>31</sup>

**2-(4-Methoxyphenyl)-4,11,11,13-tetramethyl-4H-4,11a-(epiminomethano)[1,3,5]dithiazepino[5,4-a]indole-5,12(11H)-dione (13).** From Gliotoxin Analogue **7**.  $NaBH_4$  (4.5 mg, 0.117 mmol) was added to a solution of racemic gliotoxin analogue **7** (15 mg, 0.047 mmol) in degassed MeOH/THF (1:1, 5 mL) at 0 °C. The reaction progress was monitored by TLC. Once the reaction was complete, 2–3 drops of 0.1 N aq. HCl were added, and the solvent was removed under reduced pressure. The residue was taken up with  $CH_2Cl_2$  (5 mL) and dried ( $MgSO_4$ ), and the solvent was removed under reduced pressure. The residue obtained was dried under vacuum and then dissolved in dry  $CH_2Cl_2$  (5 mL). *p*-Anisaldehyde (7  $\mu L$ , 0.056 mmol) and boron trifluoride etherate (9  $\mu L$ , 0.073 mmol) were added, and the reaction mixture was stirred for 16 h under  $N_2$ . The solvent was then removed under reduced pressure, and the residue was purified by column

chromatography (PE/EtOAc 80:20) to afford a colorless oil (9 mg, 43%) as a single diastereoisomer: IR (neat) 1682, 1606, 1511, 1483, 1372, 1257  $cm^{-1}$ ;  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  8.18 (d,  $J = 7.9$  Hz, 1H), 7.35–7.31 (m, 1H), 7.28 (app d,  $J = 8.8$  Hz, 2H), 7.19 (d,  $J = 4.2$  Hz, 2H), 6.80 (app d,  $J = 8.8$  Hz, 2H), 5.10 (s, 1H), 3.76 (s, 3H), 3.27 (s, 3H), 1.98 (s, 3H), 1.72 (s, 3H), 1.34 (s, 3H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  165.5, 162.2, 160.4, 139.5, 137.5, 130.6 (2C), 128.4, 126.8, 125.9, 121.5, 117.4, 114.2 (2C), 82.1, 68.8, 55.3, 52.0, 49.2, 28.0, 27.6, 22.3, 21.8. A NOESY experiment showed a correlation between the signal at 5.1 ppm (CH of the thioacetal carbon) and the signal at 3.27 ppm ( $CH_3$  on the nitrogen atom of the ETP ring), thus suggesting that the isolated diastereoisomer is *syn* with respect to the anisaldehyde and polycyclic residues<sup>10</sup> (see Scheme 2 for a representation). MS (ES)  $m/z$  441  $[(M + H)^+]$ ; HRMS (ES)  $m/z$  calcd for  $C_{23}H_{25}N_2O_3S_2$   $[(M + H)^+]$  441.1307, found 441.1316. For racemic starting material **7**, the two enantiomers of product **13** were separable by chiral HPLC (OD+ analytical column, hexane/isopropanol 98:2, 1 mL/min) with retention times of 60 and 100 min. The reaction was repeated on a single enantiomer, (*R,R*)-**7**, and the product was also obtained as a single enantiomer eluting with a comparable retention time (around 60 min):  $[\alpha]_D^{25} -66$  (c 0.20,  $CHCl_3$ ).

**From Gliotoxin Analogue Monosulfide 8.** The trithiane derivative of *p*-anysaldehyde was prepared following the literature procedure.<sup>32,33</sup> A solution of the racemic gliotoxin analogue monosulfide **8** (21 mg, 0.073 mmol) in  $CH_2Cl_2$  (10 mL) was treated with the trithiane derivative of *p*-anysaldehyde (16.6 mg, 0.036 mmol) and boron trifluoride etherate (15  $\mu L$ , 0.117 mmol). The mixture was stirred at 40 °C for 5 h, and then extra boron trifluoride etherate (2  $\times$  30  $\mu L$ , 0.234 mmol) was added. The mixture was then adsorbed on silica gel and purified by column chromatography (PE/EtOAc 85:15), and the two diastereoisomers were obtained as a colorless oil in medium yields (56% for diastereoisomer **1** and 25% for diastereoisomer **2**, 7:3 d.r.). Diastereoisomer **1**: IR (neat) 1682, 1606, 1510, 1481, 1459, 1372, 1254  $cm^{-1}$ ;  $^1H$  NMR (500 MHz,  $CDCl_3$ )  $\delta$  8.23 (d,  $J = 8.0$  Hz, 1H), 7.37–7.33 (m, 1H), 7.24 (app d,  $J = 8.7$  Hz, 2H), 7.23 (m 2H), 6.78 (app d,  $J = 8.7$  Hz, 2H), 4.98 (s, 1H), 3.76 (s, 3H), 3.14 (s, 3H), 2.00 (s, 3H), 1.74 (s, 3H), 1.33 (s, 3H);  $^{13}C$  NMR (125 MHz,  $CDCl_3$ )  $\delta$  166.0, 161.7, 160.4, 140.0, 136.6, 130.6 (2C), 128.2, 126.7, 126.5, 122.2, 116.8, 114.2 (2C), 77.8, 71.3, 55.3, 51.6, 50.4, 28.5, 27.6, 22.7, 19.5. A NOESY experiment did not show any correlation between the signal at 5.1 ppm (CH of the thioacetal carbon) and the signal at 3.37 ppm ( $CH_3$  on the nitrogen atom of the ETP ring), thus suggesting that this diastereoisomer is *trans* with respect to the anisaldehyde and polycyclic residues<sup>10</sup> (see Scheme 2 for a representation). MS (ES)  $m/z$  441  $[(M + H)^+]$ ; HRMS (ES)  $m/z$  calcd for  $C_{23}H_{25}N_2O_3S_2$   $[(M + H)^+]$  441.1307, found 441.1308;  $[\alpha]_D^{25} -49.4$  (c 0.20,  $CHCl_3$ ). The data for diastereoisomer **2** (IR,  $^1H$ ,  $^{13}C$ , HSQC, and NOESY) matched those for the compound obtained by reaction of gliotoxin analogue **7**. MS (ES)  $m/z$  441  $[(M + H)^+]$ ; HRMS (ES)  $m/z$  calcd for  $C_{23}H_{25}N_2O_3S_2$   $[(M + H)^+]$  441.1307, found 441.1318. For racemic starting material **8**, the two enantiomers of product **13** were separable by chiral HPLC (OD+ analytical column, hexane/isopropanol 98:2, 1 mL/min) with retention times of 52 and 80 min. The reaction was repeated on a single enantiomer, (*R,R*)-**8**, and the product was obtained as a single enantiomer eluting with a comparable retention time (around 60 min):  $[\alpha]_D^{25} -40$  (c 0.20,  $CHCl_3$ ).

**Chiroptical Measurements.** The experimental ECD spectra were recorded on an Applied Photophysics Chirascan spectrometer in dichloromethane (temperature, 22 °C; wavelength, 180–260 nm; step, 0.5 nm; bandwidth, 1 nm; time per point, 1 s). For VCD measurements, solutions of **7** and **8** and of the corresponding racemates were prepared in  $CDCl_3$  (99.98%, Aldrich). All spectra were recorded using a demountable liquid cell equipped with  $BaF_2$  windows and 100  $\mu m$  spacers. All spectra were recorded at 4  $cm^{-1}$  resolution for approximately 13 h, accumulating 40 000 scans. The spectra shown were obtained using solutions of 5.2 mg of both samples dissolved in 115 mL. Background corrections for VCD were introduced by subtracting the spectra for **7** and **8** and those obtained for the corresponding racemates.

## ■ ASSOCIATED CONTENT

### ■ Supporting Information

Copies of NMR spectra and HPLC data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

### ■ Web-Enhanced Features

Interactivity Boxes 1 and 2 are available in the HTML version of the paper.

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### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

We acknowledge Cancer Research UK (studentship for F.C.) for funding (Grant C21484/A6944) and Frank Trundle (Biotools) for assistance with VCD. This article follows the principles espoused in the Amsterdam Manifesto (<http://www.force11.org/AmsterdamManifesto>).

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