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# Observations on the reactivity of thiyl radicals derived from 3,6-epidithiodiketopiperazine-2,5-diones and related congeners

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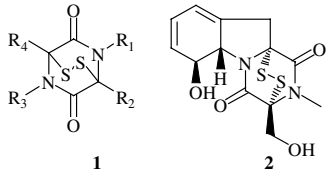
**Abstract**—A range of thiyl radicals derived from the reduced form of epidithiodiketopiperazines (ETPs) act as polarity reversal catalysts for the hydrosilylation of an enol lactone but not for H-atom abstraction from a model ribose ester.

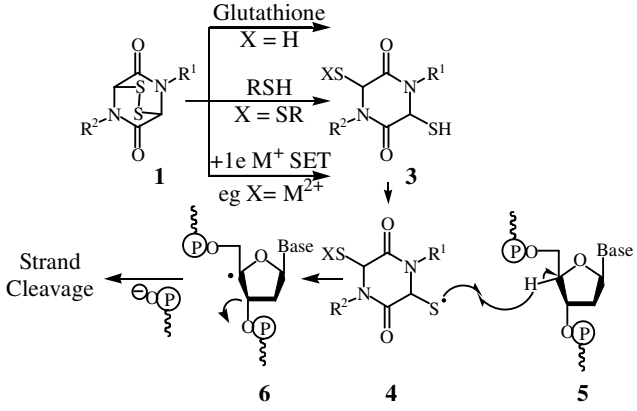

The 3,6-epidithiopiperazine-2,5-dione (ETP) family of natural products, formally derived by the incorporation of a disulfide bridge into a cyclic dipeptide to give the core structure (**1**), exhibit a wealth of structural diversity and a fascinating range of biological activities (Fig. 1).[1] The fungal metabolite gliotoxin (**2**), for example, has been shown to cleave DNA in vitro and exhibits significant antitumour, antiviral, antibacterial and immunosuppressive properties.[1,2] Although the exact mechanism by which this class of natural products operates has yet to be fully established, the bridgehead disulfide moiety, which exists in an atypical conformation with significant torsional strain,[3] is known to be essential for biological activity. Elegant studies by Waring and Chai have confirmed the unusual reactivity of these disulfides towards one electron reduction overall and also established the equilibrium constant for the glutathione–gliotoxin pair formed by thiol disulfide interchange.[1,4,5] In consequence, two mechanisms by which gliotoxin induces apoptosis have thus far been proposed, with both redox cycling to produce active oxygen species, and protein interactions via thiol–disulfide interchange implicated.[1,4]




1 2

**Figure 1.**

In light of the above studies on the nature and chemical reactivity of the disulfide bridge, we therefore elected to examine the possibility outlined in Scheme 1, for DNA strand cleavage under anaerobic conditions such as the oxygen deficient environment present in many types of




1 3 6 4 5

**Scheme 1.** Proposed mechanism for DNA strand cleavage under anaerobic conditions.

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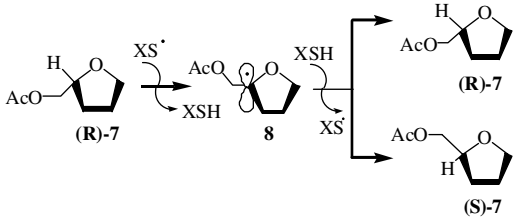
tumour cell.[6] We reasoned that if the thiyl radical (**4**) derived from ETP (**1**) via one of the indicated ring opening sequences, were sufficiently electrophilic in character to abstract the 4′-hydrogen atom from the ribose ring (**5**), then strand cleavage of DNA could be initiated via elimination of phosphate from (**6**) and subsequent nucleophilic capture of the resultant radical cation as established in a beautiful series of mechanistic studies both by Giese and co-workers[7] and Crich and Mao.[8]

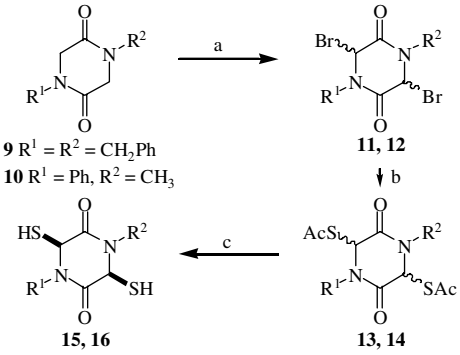
Although the reactivity of thiyl radicals of type (**4**) possessing such adjacent functionality was unknown, we were particularly encouraged by an elegant study by Roberts who demonstrated that sufficiently electrophilic thiols such as triphenylsilanethiol and 1-thio-β-D-glucopyranose tetraacetate could act as polarity reversal catalysts to promote the radical chain racemisation of (*R*)-tetrahydrofurfuryl acetate (**7**) via the planar radical (**8**) (Scheme 2).[9] The substrate tetrahydrofurfuryl acetate can of course be considered as a simple model for the ribose ring of DNA in this sequence. In a conceptually similar vein, von Sonntag had also demonstrated that thiyl radicals derived from 1,4-dithiothreitol are capable of effecting epimerisation of *cis* 2,5-dimethyltetrahydrofuran.[10]

The organic soluble *cis* dithiols (**15** and **16**) (Scheme 3) required as thiyl radical precursors were accordingly prepared from the corresponding diketopiperazines (**9** and **10**) using the classical methodology developed by Trown and Kishi involving sequential radical bromination with *N*-bromosuccinimide, followed by thioacetate displacement and acid catalysed thiol ester hydrolysis.[11,12]

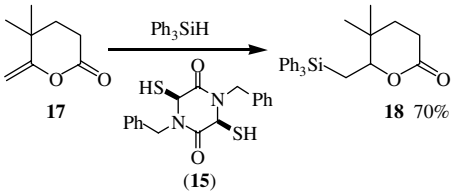
With the necessary *cis* dithiols in hand, we then elected in the first instance to establish the probable intermediacy of the derived thiyl radical through its involvement once again, as a polarity reversal catalyst for the free radical addition of triphenylsilane to the enol lactone (**17**) (Scheme 4).[9]




**Scheme 2.**



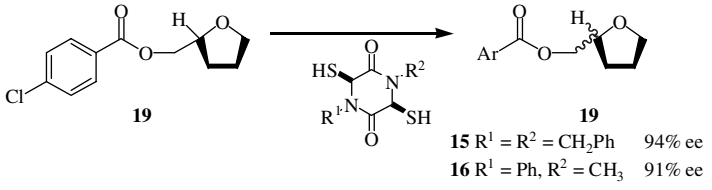

**Scheme 3.** Reagents and conditions: (a) NBS, $(PhCO)_2O_2$, $CCl_4$, reflux; (b) KSAc, $CH_2Cl_2$, 0 °C—(**13**) 53% from **9**; (**14**) 44% from **10**; (c) HCl, EtOH, reflux—(**15**) 68% from **13**; (**16**) 94% from **14**.




**Scheme 4.** Regents and conditions: Thiol (**15**) (2.5 mol %), $Ph_3SiH$, TBHN (*tert*-butyl hyponitrite) (10 mol %), dioxane 60 °C, 3 h, 70%.

The successful obtention of the desired hydrosilylated product, which requires the derived thiyl radical to abstract a hydrogen atom from triphenylsilane as a key propagation step was very encouraging and provided strong presumptive evidence for the required electrophilic character of the intermediate thiyl radicals derived from the piperazine-2,5-dione 3,6-dithiol framework.

In order to explore the potential reactivity of such thiyl radicals for hydrogen atom abstraction from the 4′-position of DNA under anaerobic conditions, we then directed our attention to the model study outlined in Scheme 5, which is of course based on the seminal work of Cai and Roberts (vide supra Scheme 2).[9] The *para*-chlorobenzoate ester of (*R*)-tetrahydrofurfuryl alcohol (**19**), readily prepared in essentially quantitative yield (94% and 99% ee) by reaction of the alcohol with the acid chloride in the presence of triethylamine, was selected since the two enantiomers were readily separated on chiral HPLC using a Chiralcel© OD column. Following the protocol developed by Cai and Roberts,[9] appropriate control experiments using 5 mol % of both triphenylsilanethiol and ethylthioglycolate (vide infra) as the



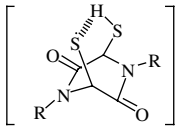

**Scheme 5.** Reagents and conditions: Thiol (2.5 mol %), TBHN (10 mol %), PhH, 60 °C, 3 h.

catalysts and *tert*-butylhyponitrite (10 mol %) as initiator in benzene at 60 °C for 3 h led to essentially complete racemisation for the former (1.5% ee) and partial racemisation (65% ee) for the latter, thereby confirming the validity of the *para*-chlorobenzoate ester as a model substrate.

In the event however, extremely limited racemisation was observed when either of the two organic soluble *cis* dithiols (**15** or **16**) was employed as the racemisation catalyst. Whilst it could be argued for the case of the dibenzyl derivative (**15**) that intramolecular hydrogen abstraction from the benzylic position by the thiyl radical might well be a competitive process, this is certainly not feasible for (**16**). Since a possible explanation for the lack of reactivity might involve thiyl radical stabilisation via intramolecular hydrogen bonding as implied in Figure 2, we therefore decided to focus on the more immediate electronic environment surrounding the thiol through progressive study of a range of monothiol derivatives.

Those compounds selected are shown in Figure 3, and range from the three α-mercaptodiketopiperazines (**21**, **22** and **23**) in which the basic heterocyclic core has been retained to the acyclic thiols (**24**, **25** and **26**) whose electrophilic character varies as a function of the electron withdrawing substituents attached to the carbon atom bearing the thiol.

The synthesis of the monothiodiketopiperazines (**21**, **22** and **23**) was carried out in a similar manner to that illustrated for the dithiodiketopiperazines (**15** and **16**) save that only 1 equiv of *N*-bromosuccinimide was used in the bromination step. The synthesis of the two mercaptans (**24** and **26**) is outlined below (Scheme 6).[13,14]



**20**

**Figure 2.** Possible hydrogen bonding stabilisation of the thiyl radical (**20**).

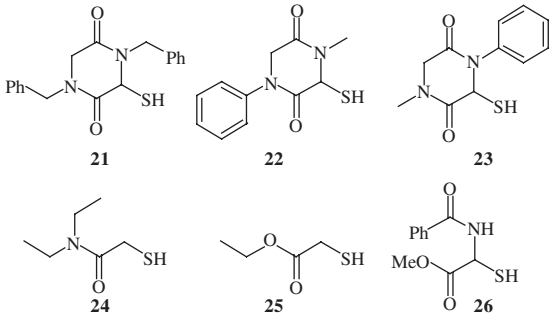
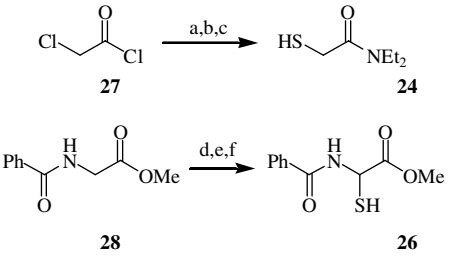


**Figure 3.**



**Scheme 6.** Reagents and conditions: (a) $HNEt_2$, $NEt_3$, $CH_2Cl_2$, 0 °C, 99%; (b) KSAc, $CH_2Cl_2$, 0 °C, 96%; (c) HCl, MeOH, 43%; (d) $^tBuOCl$, MeOH, NaOMe, 64%; (e) $BF_3{\cdot}OEt_2$, $CH_2Cl_2$, AcSH, 59%; (f) NaOMe, MeOH, 83%.

The results for a series of experiments using thiols (**21**–**26**) as polarity reversal catalysts for racemisation of the chiral ester (**19**) are shown in Table 1 and reveal that, save for the case of the ester (**25**) where racemisation had occurred to a significant extent (65%) all other mercaptans were virtually without effect. From these observations it is clear that the presence of the second thiol moiety in *cis* dithiols (**15** and **16**) did not have a detrimental effect on reactivity. The requirements for efficient hydrogen atom abstraction from (**19**) by a thiyl radical XS· are clearly related to the electron withdrawing nature of X, probably because the S–H bond is stronger, and the thiyl radical more electrophilic. The most striking observation in this respect can be seen through a comparison of the simple acyclic mercapto ester (**25**) with the corresponding diethyl amide (**24**) in which the reduced electrophilic character of the amide carbonyl group relative to the ester has effectively led to an inert system. Given that the electron withdrawing nature of the hemithioacetal unit in carbohydrate thiols such as 2,3,4,6-tetra-*O*-acetyl-1-β-D-glucopyranose contributes to their efficiency as polarity reversal catalysts,[9] it was anticipated that the further inclusion of the additional amidic nitrogen atom on the α-carbon of the mercaptan would have contributed to increased reactivity in the three α-mercapto piperazinediones (**21**, **22** and **23**) and in the acyclic model (**26**) when compared to the diethyl amide (**24**). In the event however, even with incorporation of this additional electron withdrawing amide unit a sufficiently reactive catalytic entity has not been produced. It should be noted however that **26**, unlike **21**–**23**, is prone to elimination of hydrogen sulfide and hence loss of potential activity.

**Table 1.** Reaction of the monothiols (Fig. 3) in the racemisation studies

| Entry | Thiol | Ee (%) |
|---|---|---|
| **1** | **21** | 96 |
| **2** | **22** | 98 |
| **3** | **23** | 98 |
| **4** | **24** | 93 |
| **5** | **25** | 65 |
| **6** | **26** | 95 |

Reagents and conditions: Thiol (5 mol %), TBHN (10 mol %), PhH, 60 °C, 3 h.

Recent studies by Waring and co-workers[15] have suggested that intracellular levels of gliotoxin exist almost exclusively in the reduced form and that when glutathione levels fall following induction of apoptotic cell death, the oxidised disulfide is then released. In this manner, the toxin can act further on neighbouring cells in a pseudocatalytic way. The above studies have demonstrated however, that under the anaerobic conditions found in many types of tumour cell, the thiyl radicals derived from the reduced form of epidithiodiketopiperazines are not sufficiently reactive per se in *intermolecular* hydrogen atom abstraction from the model ribose ester (**19**). However, as noted by Robins in a model study of ribonucleotide reductase,[16] significant rate enhancements are observed in the *intramolecular* mode, even when the thiyl radical does not possess additional electron withdrawing groups. For the ETP family of natural products, the possibility therefore exists that one of the sulfur atoms could be involved either in covalent modification through thiol disulfide interchange whilst the second thiol can then be available for radical generation.

## Acknowledgements

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