A Mild and Convenient Base-Catalysed Approach to Disubstituted Epidithiodiketopiperazines

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Abstract: A novel base-catalysed approach to disubstituted epidithiodiketopiperazines (ETP) is described. The synthetic route involves a multicomponent reaction of a diacetoxyacetamide with a range of different amines and *p*-methoxybenzylmercaptan to generate the protected ETP core.

Key words: epidithiodiketopiperazine, multicomponent reactions, chaetocin, gliotoxin, chetomin

The epidithiodiketopiperazines (ETPs) are a structurally diverse group of natural products that are classified by the presence of a disulfide bridged diketopiperazine core **1**.[1,2] All members of this class of compound display potent biological activity, ranging from nanomolar anticancer activity through to antiviral and antibacterial activity.[1–3] There has been widespread interest in these molecules focusing on chetomin (**2**) and chaetocin (**3**) in particular, due to their structural complexity and reported ability to act on epigenetic targets and to inhibit protein–protein interactions (Figure 1).[4] As part of an ongoing research programme into the biological activity and mechanism of action of this class of compound, we needed to be able to access highly substituted derivatives with diverse functionality. In our previous approach towards the ETP core,[1,5] we had described an acid-catalysed cyclisation approach, but had found that it proved to be intolerant of a number of functional groups. We therefore decided to explore whether we could carry out the cyclisation under milder conditions. Herein, we now report our initial results towards functionalised derivatives of the ETP core.

Most reported approaches towards either the ETP core or the parent natural product have relied on formation of the diketopiperazine ring prior to addition of sulfur either via displacement of a leaving group, or addition of sulfur to an intermediate iminium ion.[6] As an example, recent approaches described by both Nicolaou and Reisman have shown that simple ETP derivatives can be obtained by reaction of the diketopiperazine ring with sulfur or sulfur electrophiles under basic conditions.[7] However, one main drawback to these approaches, is the need to form the diketopiperazine ring prior to incorporation of sulfur. In our research programme, we have developed a novel alternative approach, whereby the diketopiperazine ring is formed at the same time as sulfur is incorporated. This simple yet effective approach therefore reduces the overall number of steps required to access the ETP core whilst simultaneously avoiding the notorious solubility problems associated with diketopiperazine formation. The differing strategies are outlined below (Scheme 1).

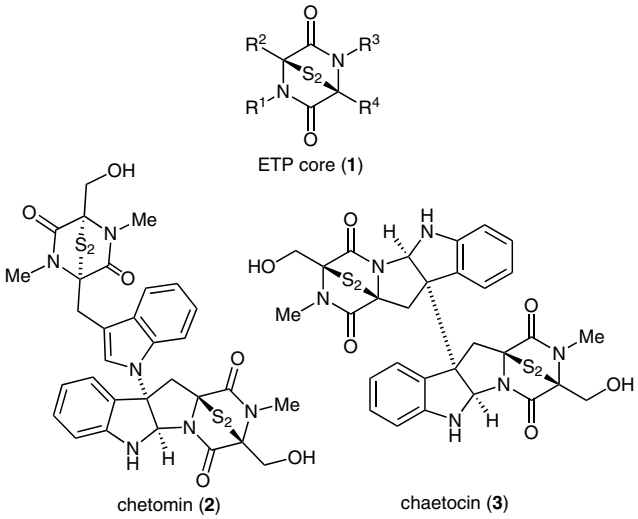
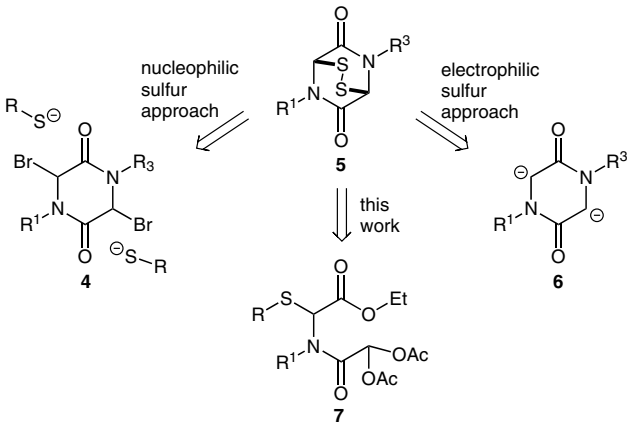


Figure 1 Examples of ETP natural products



Scheme 1 Retrosynthetic approaches to the ETP core

In our previous reports, cyclisation of the diacetate precursor **7** to the protected ETP core **8** required trifluoroacetic acid (TFA) to be used as a catalyst in the presence of a number of amines and *p*-methoxybenzylmercaptan as shown below (Scheme 2).

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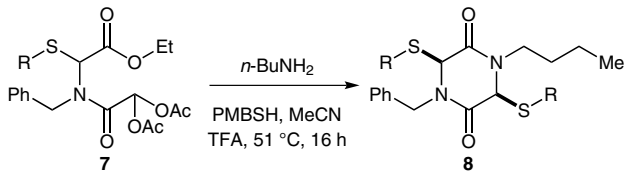
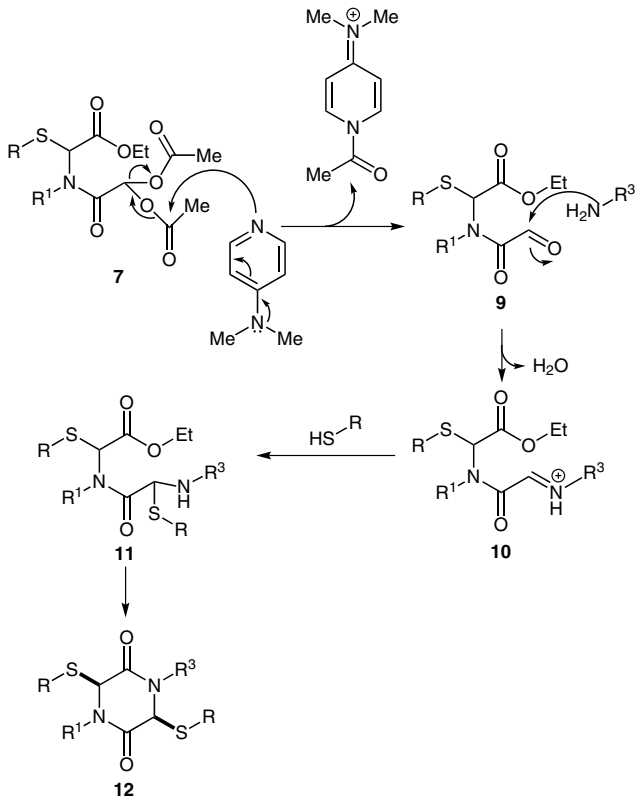


Scheme 2 Formation of the protected ETP core

However, in our hands, TFA proved to be incompatible with amines containing either tethered esters and amines or aromatic rings with carbamate groups attached. In our previous proposed mechanism of cyclisation, we envisaged that TFA is able to catalyse the reaction by unmasking the protected glyoxamide **7** in situ, which can then react with an equivalent of amine and thiol prior to cyclisation to form the ETP core (Scheme 3). We therefore decided to investigate whether 4-*N*,*N*-dimethylaminopyridine (DMAP) could also catalyse the cyclisation of the diacetate **7** to the protected dithiodiketopiperazine **8** for the development of a novel milder synthetic strategy for ETP production.



Scheme 3 Proposed mechanism of deprotection and conversion to the ETP core

We anticipated that DMAP would similarly be able to unmask the glyoxamide **9** by nucleophilic attack at the carbonyl group of one of the acetate groups of the diacetate **7**, resulting in concomitant removal of the second acetate group and formation of the intermediate glyoxamide. This would then facilitate reaction with the nucleophilic amine and lead to formation of an iminium ion **10** where the nucleophilic sulfur of *p*-methoxybenzylmercaptan can react, followed by cyclisation to produce the ETP precursor **12** analogous to the mechanism with TFA (Scheme 3).

In order to investigate cyclisation of the diacetate using these newly developed conditions, a series of reactions were carried out towards the synthesis of the *n*-butyl ETP precursor **8** as our reaction standard. We initially decided to investigate the effects of both conventional and microwave heating conditions in order to try to reduce the length of time that it took to carry out the reaction and a preliminary optimization study was carried with the results shown below (Table 1).

Table 1 Initial Screen of Reaction Conditions using DMAP[a]

Entry	Method	Time	Temp (°C)	Yield (%)
1	A	16 h	51	80
2	B	15 min	130	38
3	B	20 min	130	40
4	B	30 min	130	37
5	B	30 min	160	19

[a] Reaction conditions: (A) MeCN, *p*-methoxybenzylmercaptan, DMAP, reflux conditions; (B) MeCN, *p*-methoxybenzylmercaptan, DMAP, microwave conditions. Yields are based on isolation of **8** after purification.

From our studies, the results clearly showed that optimum conditions were achieved when using conventional heating with 80% of the protected ETP obtained after heating the reaction overnight at 51 °C (Table 1, entry 1). Surprisingly, when the reaction was carried out in the microwave, only 40% of the protected ETP **8** was obtained. Prolonged heating of the reaction led to reduced yields and associated product degradation. Under conventional heating, DMAP proved to be an effective catalyst for the deprotection of diacetate **7** and formation of the ETP core as shown above (Table 1). Following the success of our initial screen, the synthesis of a number of different protected dithiodiketopiperazines containing functionality that was previously impossible was attempted using this approach (Table 2).

From the results obtained, it was noticeable that heating the reaction at reflux to obtain the protected ETP was generally far superior to that of microwave-accelerated reactions in terms of reaction yield. This was in contrast to the acid-catalysed reaction, where heating the reaction under microwave conditions did not lead to product degradation. This may be attributed to the stability of the intermediate glyoxamide **9**, which is unstable and cannot be isolated. During our studies, we also investigated the effects of microwave heating on the cyclisation and its effects on various functional groups. As predicted from our initial screen with butylamine, reaction yields were higher when the reaction was heated under conventional conditions with DMAP as catalyst. Only the allylamine derivative (Table

Table 2 Reaction of Amines with Diacetate **7** using DMAP[a]

Entry	Amine	Method	Yield (%)
1	3-chlorobenzylamine	A	75
2	3-chlorobenzylamine	B	68
3	benzylamine	A	68
4	allylamine	A	53
5	allylamine	B	76
6	ethyl 4-aminobutanoate	A	70
7	ethyl 4-aminobutanoate	C	28
8	methyl 6-aminohexanoate	A	76
9	methyl 6-aminohexanoate	C	46
10	ethyl 3-aminopropanoate	A	61
11	methyl 5-aminopentanoate	A	78

[a] Reaction conditions: (A) MeCN, *p*-methoxybenzylmercaptan, DMAP, reflux; (B) MeCN, *p*-methoxybenzylmercaptan, DMAP, microwave (5 min, 150 ºC); (C) MeCN, *p*-methoxybenzylmercaptan, DMAP, microwave (30 min, 130 ºC). Yields refer to isolation of the corresponding protected ETP **12** after purification.

2, entries 4 and 5) gave a higher yield when microwave conditions were used. This may be due to the fact that when the reaction is heated in a sealed vessel in this manner, allylamine cannot be lost by evaporation. The successful obtention of the protected ETP core compounds containing ester moieties (Table 2, entries 6–11) clearly highlights the importance of DMAP as an alternative catalyst for the cyclisation of ETP intermediates. Using this approach, we were able to introduce different carbon length ester groups in the ETP core which had previously not been possible.

The final step of the reaction sequence involved a combined deprotection/oxidation step for conversion of the protected derivatives to the ETP core (Table 3).[1]

All compounds underwent clean conversion to the ETP core **1** in good yield (Table 3). Pleasingly, protected ETPs incorporating ester groups (Table 3, entries 7–10) were readily deprotected and oxidised to the ETP core **1** also in good yield, clearly highlighting the effectiveness of DMAP as a catalyst.

In summary, DMAP proved to be an effective catalyst for the synthesis of novel ETP derivatives.[8] It is clearly superior to the use of TFA which had proven to be incompatible with a number of functional groups and further investigations into the scope of the DMAP-catalysed reaction are under active investigation in our laboratories.

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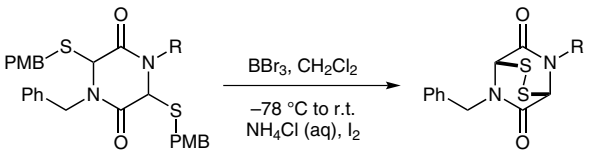
Table 3 Deprotection/Oxidation and Synthesis of the ETP Core



Entry	Amine (R^1)	Yield (%)
1	3-chlorobenzylamine	60
3	benzylamine	85
5	allylamine	60
7	ethyl 4-aminobutanoate	64
9	methyl 6-aminohexanoate	75
10	ethyl 3-aminopropanoate	61
11	methyl 5-aminopentanoate	59

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(8) **General Procedures**

Method A: *n*-Butylamine (0.18 mL, 1.82 mmol) was added to a solution of diacetate **7** (0.31 g, 0.61 mmol) in MeCN (30.0 mL) followed by addition of *p*-methoxybenzylmercaptan (0.13 mL, 0.91 mmol) and the resulting mixture was stirred for 2 min. DMAP (0.04 g, 0.30 mmol) was added in a single portion and the resulting mixture was heated at reflux for 16 h, cooled to r.t. and the solvent was removed under reduced pressure. Purification via Biotage™ Horizon [petroleum spirit (40–60 °C)–EtOAc, 3:1; snap 25 g] gave protected ETP **8** (0.28 g, 80%) as a colourless solid (see ref. 1).

Method B: 3-Chlorobenzylamine (0.13 mL, 1.07 mmol) was added to a solution of diacetate **7** (0.18 g, 0.36 mmol) in MeCN (5.00 mL) followed by addition of *p*-methoxybenzylmercaptan (0.07 mL, 0.53 mmol) and the resulting mixture was stirred for 2 min. DMAP (0.02 g, 0.18 mmol) was added and the mixture was heated in the microwave at 150 °C for 5 min, allowed to cool to r.t. and the solvent was removed under reduced pressure. Purification via Biotage™ Horizon [petroleum spirit (40–60 °C)–EtOAc, 3:1; snap 25 g] gave 1-benzyl-4-(3-chlorobenzyl)-3,6-bis[(4-methoxybenzyl)thio]-piperazine-2,5-dione (0.15 g, 68%) as a colourless solid (see ref. 1).

Method C: Ethyl 4-aminobutanoate hydrochloride (0.10 g, 0.61 mmol) was added to a solution of diacetate **7** (0.24 g, 0.47 mmol) in MeCN (3 mL) followed by addition of Et_3N (0.34 mL, 2.45 mmol) and *p*-methoxybenzylmercaptan (0.10 mL, 0.73 mmol) and the resulting mixture was stirred for 2 min. DMAP (0.04 g, 0.31 mmol) was added and the mixture was heated in the microwave at 130 °C for 30 min, allowed to cool to r.t. and the solvent was removed under reduced pressure. Purification via Biotage™ Horizon [petroleum spirit (40–60 °C)–EtOAc, 3:1; snap 25 g] gave ethyl 4-{4-benzyl-2,5-bis[(4-methoxybenzyl)thio]-3,6-dioxopiperazin-1-yl}butanoate (0.11 g, 28%) as a colourless solid; mp 105–107 °C. IR: 2931, 1730, 1674 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.13 (t, *J* = 7.1 Hz, 3 H, OCH_2CH_3), 1.31–1.51 (m, 2 H, $CH_2CO_2CH_2CH_3$), 1.93–2.02 (m, 2 H, NCH_2CH_2), 2.54–2.66 (m, 1 H, NCH_2CH_2), 3.41–3.54 (m, 2 H, NCH_2Ph), 3.70 (d, *J* = 12.2 Hz, 1 H, SCH_2), 3.73 (s, 3 H, OMe), 3.76 (s, 3 H, OMe), 3.78–3.83 (m, 1 H, SCH_2), 3.85 (d, *J* = 13.9 Hz, 1 H, SCH_2), 3.90–4.01 (m, 3 H, $CO_2CH_2CH_3$, SCH_2), 4.39 (s, 1 H, CHS), 4.21 (s, 1 H, CHS), 5.08 (d, *J* = 14.5 Hz, 1 H, NCH_2), 6.61–6.65 (m, 2 H, ArH), 6.77–6.81 (m, 2 H, ArH), 6.80–6.84 (m, 2 H, ArH), 7.03–7.09 (m, 2 H, ArH), 7.11–7.13 (m, 1 H, ArH), 7.23–7.27 (m, 2 H, ArH), 7.30–7.34 (m, 2 H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.04 (CO_2Et), 21.95 (CH_2), 31.29 (CH_2), 36.10 (SCH_2), 36.80 (SCH_2), 43.13 (CH_2), 46.00 (CH_2), 55.14 (OMe), 55.15 (OMe), 57.23 (CHS), 59.26 (CHS), 60.24 (CO_2Et), 113.96 (ArH), 114.01 (ArH), 127.72 (ArH), 128.46 (ArH), 128.52 (ArH), 128.56 (C), 128.73 (C), 130.60 (ArH), 130.67 (ArH), 134.84 (C), 159.01 (C), 159.03 (C), 164.92 (C), 165.07 (C), 172.26 (C). MS: *m/z* = 645 (100%) $[M + Na]^+$. HRMS: *m/z* $[M + Na]^+$ calcd for $C_{33}H_{38}N_2O_6S_2Na$: 645.2069; found: 645.2069.

