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## COMMUNICATION

## Diastereoselective reduction of the tricarbonyl moiety in bicyclic tetramates giving pyroglutamates

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Accepted 00th January 20xxLaia Josa-Culleré,<sup>a</sup> Kirsten E. Christensen<sup>a</sup> and Mark G. Moloney<sup>a</sup>

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The reduction of C(6)-acyl bicyclic tetramic acids has been achieved with complete diastereoselectivity via catalytic hydrogenation using PtO<sub>2</sub>. The resulting pyroglutamates had potent antibacterial and anticancer properties and will allow the preparation of simple mimics of pyroglutamate-containing natural products.

## Introduction

With the threatening emergence of resistance to known antibiotics, the need for novel drugs with new mechanisms of action is critical. The privileged structures of natural products offer a valuable source of diversity as well as an inspiration for the design of new drugs that could cover wider chemical matter.<sup>1</sup> However, the use of such structures is often limited by long and low yielding synthetic routes, which limits their availability and the access to analogues.

A possible solution is to prepare simpler mimics of such natural products, which retain biological activity while being more synthetically accessible.<sup>2–4</sup> Our group has had some success in this area, and for instance bicyclic tetramate **1** (Figure 1) is easily accessible and serves as a mimic of natural tetramic acids.<sup>5,6</sup> While the tetramate core itself has no biological activity, its functionalisation, in particular via acylation of the C(6) position to **2**, can provide analogues with potent antibacterial properties.<sup>7–9</sup>

We have earlier successfully reduced the C(7)-ketone of tetramates like **1**, but only in C(6)-unsubstituted systems;<sup>10</sup> we have not investigated substituted C(6)-systems, which are in any case heavily tautomerised.<sup>11</sup> To extend this work, we were interested in accessing C(6)-substituted pyroglutamates **3**. Pyroglutamates with varying oxidation patterns can be found in several natural products with various biological activities, such as pramanicin,<sup>12</sup> virgarcin<sup>13</sup> and lactacystin.<sup>14</sup>

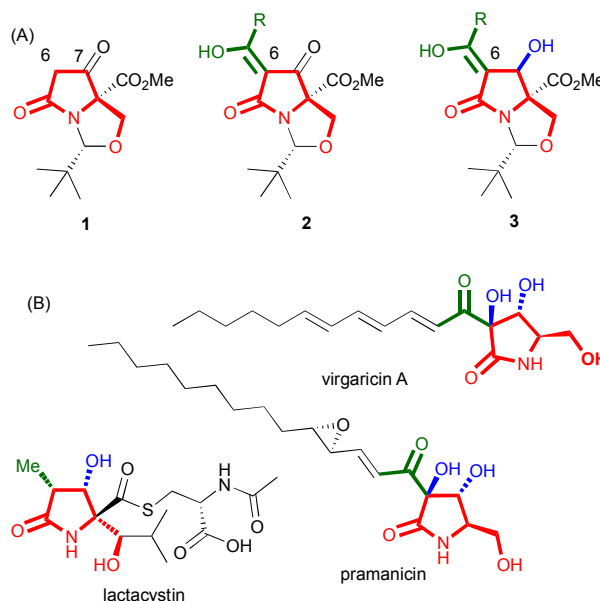


Figure 1. Synthetic (A) and natural (B) tetramates and pyroglutamates.

## Results and Discussion

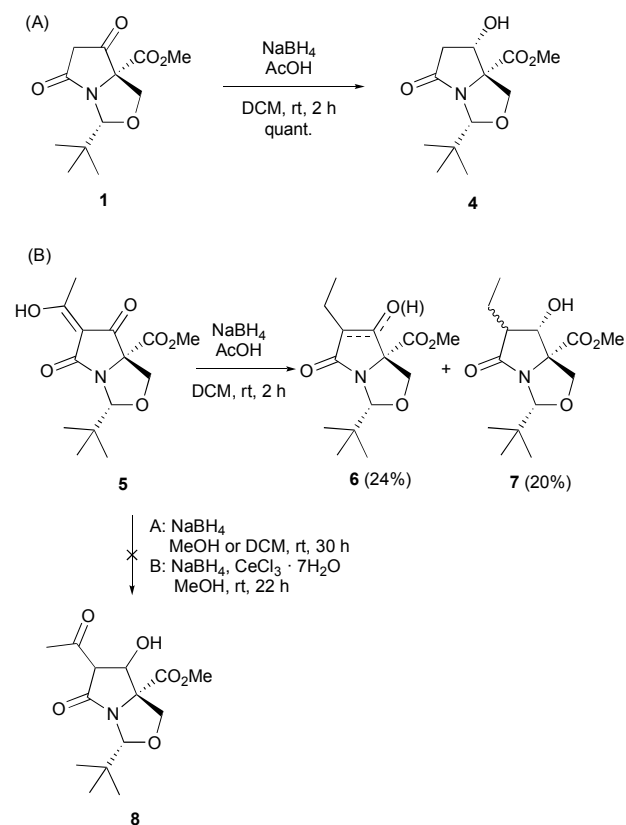
We have previously reported that tetramic acid **1** undergoes diastereoselective reduction to alcohol **4** when treated with sodium acetoxyborohydride (Scheme 1 A).<sup>15,10</sup> However, when we applied the same conditions to acetyl **5**,<sup>8,9</sup> the more accessible *exo*-carbonyl group underwent reduction, followed by elimination and further reduction to analogues **6** and **7** (Scheme 1 B). Other attempted conditions were unsuccessful in providing alcohol **8**.

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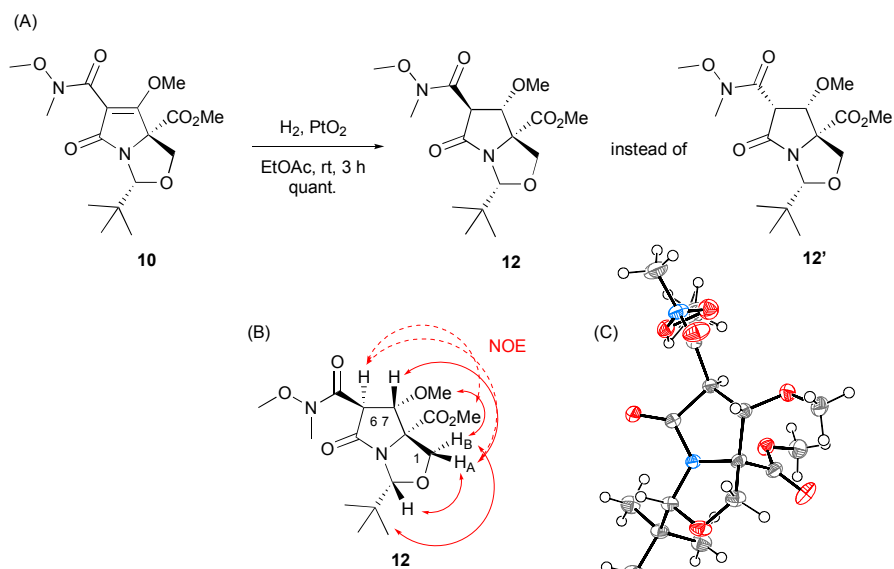
Scheme 1. Reported reduction of tetramic acid **1** (A) and unsuccessful formation of **8** (B).

Reduction of Weinreb amide **9** (Table 1),<sup>9</sup> which can give C(6)-acyl products via Grignard reaction, was also unsuccessful and only starting material was recovered. This lack of reactivity could be a result of the steric hindrance of the bicyclic system, but also of the significant stability of the highly conjugated tricarbonyl system. In fact, there are only a handful of literature examples of the reduction of tricarbonyl groups. Thus, where reduction with NaBH<sub>4</sub> was successful, the ketone was in the keto form, and in a less sterically demanding compound.<sup>16</sup> In other cases, reduction was achieved via hydrogenation at high pressure.<sup>17,18</sup> However, treatment of Weinreb amide **9** under the reported conditions also resulted in no conversion (Table 1, Entries 3 and 4).

Table 1. Attempted conditions for the reduction of Weinreb amides **9** and **10**.

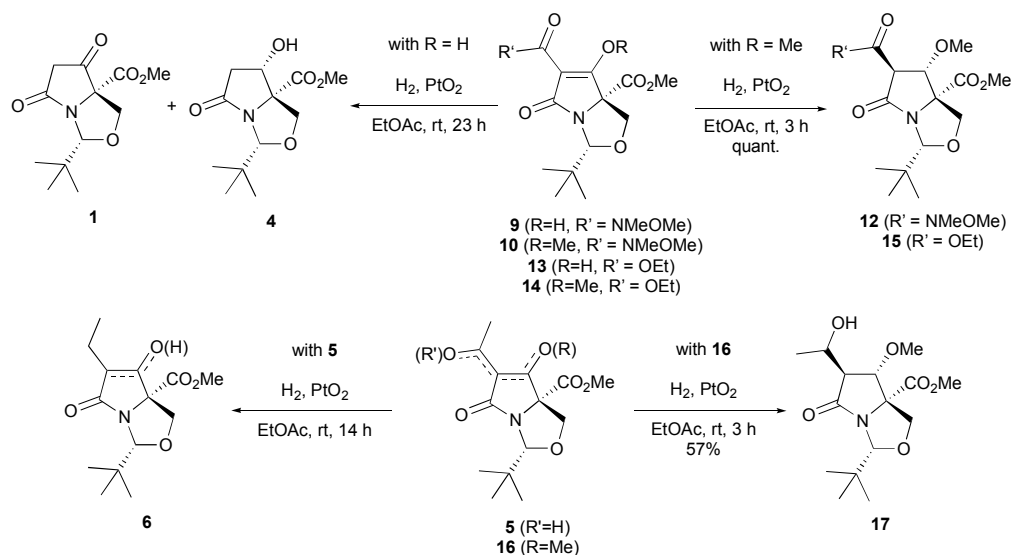
Entry	R	Conditions	Outcome
1	H or Me	NaBH <sub>4</sub> (2.2 eq) DCM or MeOH, rt, 24 h	Recovered starting material
2	H or Me	NaBH <sub>4</sub> (2.2 eq), AcOH (9 eq) <sup>15,10</sup> DCM, rt, 24 h	Recovered starting material
3	Me	NaBH <sub>4</sub> (4 eq), AcOH (2 eq), 2.5% Pd/C <sup>19</sup> toluene, rt, 24 h	Recovered starting material
4	H or Me	H <sub>2</sub> , 10% Rh/C <sup>18</sup> AcOH, rt, 3.5 bar, 21 h	Recovered starting material
5	Me	H <sub>2</sub> , 10% Pd/C EtOAc, rt, 2 bar, 15 h	20% conversion to <b>12</b>
6	Me	H <sub>2</sub> , 20% PtO <sub>2</sub> EtOAc, rt, 3 h	<b>12</b> isolated as a single diastereomer in quantitative yield

In an attempt to decrease the delocalisation of the system, we hypothesised that reduction of enol ether **10**, accessible from the enol analogue under Mitsunobu-like conditions (diisopropyl azodicarboxylate, triphenylphosphine),<sup>9</sup> should have enhanced reactivity to give ether **12**. While hydrogenation in the presence of Rh/C or Pd/C at 2-3.5 bar gave almost no conversion, we were pleased to find that with PtO<sub>2</sub>, the starting material was consumed after 3 h at atmospheric pressure. Remarkably, the product was isolated in quantitative yield after purification, and as a single diastereomer. We have previously shown that in the reduction of bicyclic tetramate **1**, hydride attack occurs from the less hindered *endo*-face, away from the methyl ester, the bulky *tert*-butyl group and the nitrogen lone pair. Hence, we expected the product to be diastereomer **12'** (Scheme 2), resulting from *syn*-addition as expected for a metal-based hydrogenation to the less hindered *endo*-face. NOE analysis indicated that while the OMe was in the *exo*-face as expected, the stereochemistry of C(6) was less conclusive since only a weak enhancement of the methyl ester, but also of C(1)H<sub>ax</sub>, was observed. Therefore, crystals were grown and analysed by single crystal X-ray diffraction and we were surprised to find that the product was in fact a C(6)-epimer of the expected structure.<sup>20</sup> We explain the stereochemical outcome by initial formation of **12'**, which undergoes isomerisation under the reaction conditions to give **12**. Presumably, steric interactions with the methoxy, *tert*-butyl and methyl ester groups favour the epimerisation to give the less hindered *trans*-6,7-arrangement.

Scheme 2. Successful reduction of Weinreb amide **10** (A); NOE interactions (B) and X-ray crystal structure (C) of **12**.

The same conditions could be applied to the stereoselective hydrogenation of ethyl ester **14** to give **15** (Scheme 3). Interestingly, when unprotected Weinreb amide **9** or ethyl ester **13** were subjected to the same hydrogenation conditions, a mixture was obtained, comprising tetramic acid **1**, presumably arising from hydrolysis and subsequent decarboxylation at C(6), along with **4**, arising from further diastereoselective hydrogenation. With the ketone derivative **5** (Scheme 3), the crude product was mainly C(6)-ethyl **6**, together with trace amounts of the alcohols, analogous to those observed for the reduction with  $NaBH(OAc)_3$ . Instead, with methylated **16**, hydrogenation occurred alongside ketone reduction, to give alcohol **17** as a single diastereomer.

Grignard addition to Weinreb amide **12** with 1 equivalent of methylmagnesium bromide gave the desired acetyl derivative **18** (Table 2), with no observed addition to the methyl ester or over-addition to the amide. However, the crude product also contained small amounts of **23**, resulting from elimination. Unfortunately, purification on (slightly acidic) silica gel favoured formation of the alkene, which prevented the successful isolation of the methoxy analogue **18**. The same was observed when **18** was stored in  $CDCl_3$ , and this elimination could also be accelerated by stirring in PTSA. Weinreb amide **12** could also be reacted with different Grignard reagents, from which enones **24-27** were readily isolated after elimination.

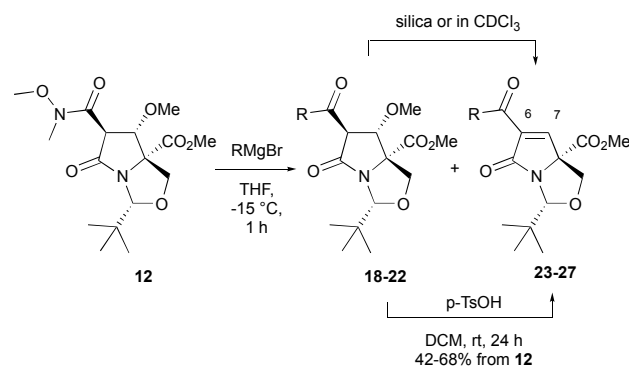


Scheme 3. Hydrogenation of several C(6)-substituted tetramates.

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Table 2. Grignard addition to Weinreb amide **12**, followed by elimination.

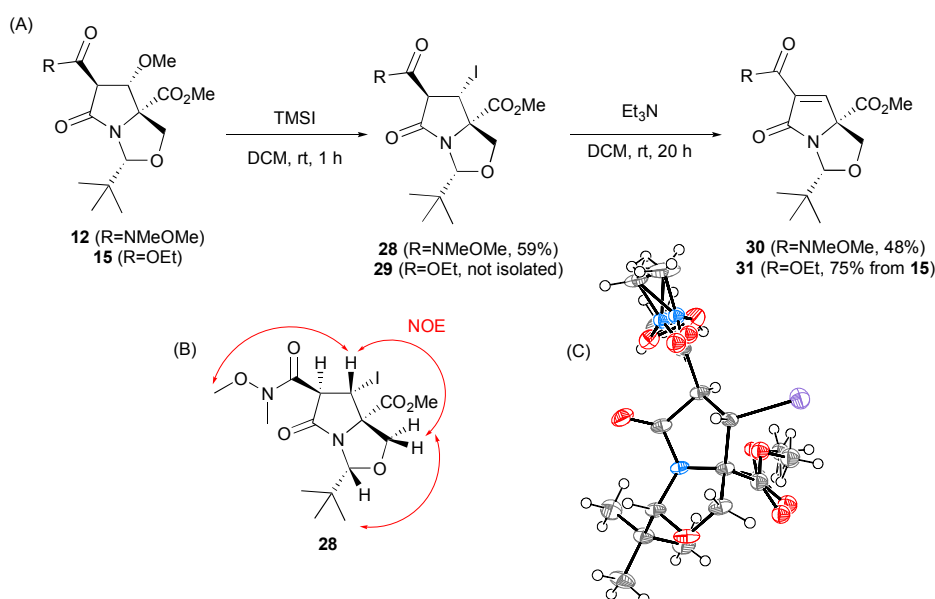


Compound	R	Yield from <b>12</b> [%]
<b>23</b>	Me	48
<b>24</b>	Ph	68
<b>25</b>		50
<b>26</b>		49
<b>27</b>		42

Of interest is that direct elimination of Weinreb amide **12** was less efficient, probably owing to the weaker electron withdrawing ability of the amide. Treatment with an excess of PTSA led to a mixture of products. However, with TMSI, iodide **28** was isolated as a single diastereomer (Scheme 4 A), seemingly via initial elimination followed by nucleophilic attack of the iodide. The stereochemistry was confirmed by X-ray

single crystal diffraction (Scheme 4 C).<sup>20</sup> Enone **30** was obtained upon treatment with triethylamine. The ethyl ester derivative **15** gave a similar outcome, although in this case purification of the iodide intermediate was not possible due to elimination during silica gel purification. Isolation of these enones opens the door to further functionalisation of these systems. Thus, following introduction of a range of acyl groups at C(6), the alkene could be hydroxylated, dihydroxylated and epoxidised to mimic core structures of known natural products, using general modes of reactivity shown to be efficient in related systems.<sup>21–23</sup> It would also be possible to manipulate the enones via nucleophilic conjugate addition at C(7), and the isolation of iodide **28** suggests that such addition is possible.

The antibacterial properties of the synthesised pyroglutamates against Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* were evaluated using the hole-plate method.<sup>24</sup> The results for the tested analogues are shown in Figure 2, where a larger zone size illustrates a more active compound. It was interesting to find that, while tetramic acids **2** had been previously found to have selective Gram-positive activity,<sup>7–9</sup> the analogues synthesised herein displayed antibacterial activity against both strains, and in particular enones **24–27** were more potent against Gram-negative *E. coli*. Another striking observation is that the trends in activity of the enones for both strains differed - amongst the keto-derivatives, acetyl **23**, the most active compound against *E. coli*, was the least active against *S. aureus*, and the opposite was true for alkynyl **27**. Acetyl **23** is 2-fold more active than the standard (Cephalosporin C) against *E. coli*, and alkenyl **26** and alkynyl **27** are 4-fold more active than CephC against *S. aureus*.



Scheme 4. Elimination of Weinreb amide **12** and ester **15** (A); NOE interactions (B) and X-ray crystal structure (C) of iodide **28**.

The activity of these pyroglutamates and enones against both strains of bacteria, in contrast to the results from the tetramate systems, might indicate that either they act via a different mechanism of action, especially since their pKa behaviour is very different from tetramates. Moreover, their activity in Gram negative species indicates that they possess appropriate physicochemical properties to effectively cross the bacterial cell membrane.

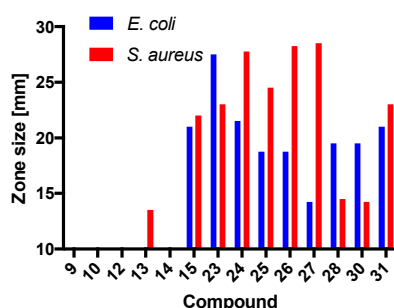


Figure 2. Antibacterial properties of synthesised pyroglutamates.

We also examined the anticancer properties of enones **23–27** in two cancer cell lines: MCF7 (breast cancer) and H460 (non-small cell lung cancer). The most active analogue, alkenyl **26**, had EC<sub>50</sub> values in the mid-nanomolar range.

Table 3. Anticancer properties of enones **23–27**

Compound	MCF7 IC <sub>50</sub> [μM]	H460 IC <sub>50</sub> [μM]
<b>23</b>	6 ± 3	13 ± 6
<b>24</b>	1.8 ± 0.6	4.4 ± 0.9
<b>25</b>	1.0 ± 0.3	3.5 ± 0.9
<b>26</b>	0.5 ± 0.2	0.6 ± 0.2
<b>27</b>	12 ± 4	12 ± 3

## Conclusions

With this work, we have shown that C(6)-substituted bicyclic tetramates can be reduced via hydrogenation with PtO<sub>2</sub> with high diastereoselectivity. Given the low reactivity of such tricarbonyl systems, and the lack of reported conditions for their successful reduction under mild conditions, we believe that our results will find wider application.

The introduction of a range of acyl groups was possible via Grignard addition, and the resulting methoxy products easily eliminated to enones **23–27**. Further functionalisation of these systems will enable the preparation of closer analogues to pyroglutamate-containing natural products.

Some of the synthesised compounds had antibacterial activity against Gram-positive and Gram-negative bacteria, as well as anticancer properties against 2 cell lines. The potent activity of some of the analogues against *E. coli*, in contrast to related

tetramates,<sup>9</sup> suggests a different mechanism of action, and that proper functionalisation of these systems might lead to different classes of active compounds. Importantly, this bioactivity supports the value of using natural products as starting points for drug discovery, as well as the suitability of bicyclic tetramates and pyroglutamates as 3D templates to prepare novel antibiotics and anticancer agents.

## Conflicts of interest

There are no conflicts to declare.

## Acknowledgements

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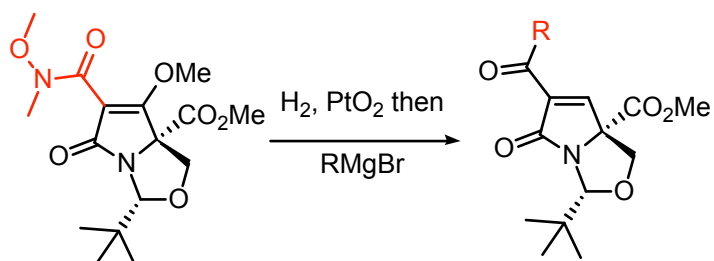
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# Diastereoselective reduction of the tricarbonyl moiety in bicyclic tetramates giving pyroglutamates

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The formation of densely functionalised bicyclic tetramic acids by both stereoselective reduction and Grignard displacement of a Weinreb amide gives bioactive small molecules, with antibacterial activity along with some cancer-cell line inhibitory activity.